UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLUMBIA

IVANA FRECH, Plaintiff, v. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, *et al.* Defendants.	Civ. No. 1:23-cv-02530-CRC

MOTION FOR TEMPORARY RESTRAINING ORDER OR PRELIMINARY INJUNCTION

For more than a decade, Plaintiff Dr. Ivana Frech ("Dr. Frech" or "Plaintiff"), has gone about her life following an unfortunate, and misguided, decision by the University of Utah ("UU") to terminate her employment following an investigation it conducted on the behalf of the Office of Research Integrity ("ORI") at the U.S. Department of Health and Human Services ("HHS") into allegations of research misconduct that arose in 2011. Settling down into a small rural Iowa community and having left the world of federally-funded research behind her, Dr. Frech continued to pursue her passion for science, eventually taking on an administrative role at the local hospital. Though displeased with how her time at UU had ended, life was otherwise well in Iowa for Dr. Frech.

In July 2023, approximately ten (10) years after the UU investigation had concluded and been provided to ORI for its action, ORI appeared seemingly out of nowhere and informed Dr. Frech that it agreed with UU's decade-old findings and that it was debarring her from participating in certain transactions involving the federal government. Because Dr. Frech's employment did

not involve any such transactions at the time, nor did Dr. Frech have any intent (or interest) in becoming involved in such covered transactions in the future, ORI's improper debarment action could be legally challenged in due course. Now, however, circumstances have dramatically changed and immediate action is necessary to protect Dr. Frech from irreparable harm.

On January 31, 2024, ownership of the community hospital where Dr. Frech worked was to change hands, with the University of Iowa ("UI") taking ownership of the hospital. Based on Dr. Frech's invaluable contribution to the hospital under its previous ownership, UI wished to retain Dr. Frech in the same role and offered to employ her, contingent on passing UI's background screening. During that screening, UI learned that Dr. Frech had been debarred by ORI (a fact that she has not tried to hide, and which she informed UI she is in the process of challenging) and rescinded her employment offer, thus rendering Dr. Frech unemployed with little to no prospects of other employment in her trained field due to the limited number of related employers within a reasonable distance from her home, where she had already become woven into the fabric of the community.

To be sure, Dr. Frech has been told by UI that, in the absence of a debarment, she would be warmly welcomed back to her job—but time is of the essence, lest the job be permanently filled by another individual. In short, after more than a decade of inaction by the Defendants, Dr. Frech was debarred, despite having long since abandoned any federally-funded research and having changed careers entirely (leaving scientific research behind for an administrative position at a community hospital). As a foreseeable result of the governments substantially delayed and improper debarment, on January 30, 2024, Dr. Frech was constructively terminated from her job—based solely on the debarment—even though her duties have no relation to covered transactions or federal procurement activities.

Dr. Frech therefore moves to enjoin the government-wide debarment of her from participating in covered transactions and federal procurement activities as imposed by defendant, the Department of Health and Human Services ("HHS"), acting by and through defendants, the Secretary of Health and Human Services, the Office of Research Integrity ("ORI"), ORI's Director, and HHS's Deputy Assistant Secretary for Acquisitions and Suspension and Debarment Official ("SDO"). The debarment of Dr. Frech constitutes a final agency action that is so lacking in supporting evidence that it is arbitrary, capricious, an abuse of discretion, contrary to law, regulation, and public policy, and in violation of the Administrative Procedure Act ("APA"), 5 U.S.C. §§ 701 *et seq*. For purposes of this motion, Dr. Frech relies on the Complaint filed in this matter and the below. Dr. Frech requests that an emergency hearing be set at the Court's earliest convenience.

I. INTRODUCTION

Dr. Frech (then known as Dr. De Domenico) arrived in Salt Lake City as an exchange student from Italy to attend the University of Utah ("UU") in 2004. From approximately 2005 to 2008, Dr. Frech was a member of Dr. Jerry Kaplan's laboratory at UU, until she was promoted in or about April 2008 to become a tenure-track assistant professor and independent investigator at UU. While a member of Dr. Kaplan's lab, Dr. Frech's scientific research was supervised by both Dr. Kaplan and by Dr. Diane Ward, who managed Dr. Kaplan's lab. Dr. Ward was responsible for overseeing the experiments conducted by lab members such as Dr. Frech, writing manuscripts, and generating figures for publication.

When Dr. Frech left Dr. Kaplan's lab, she sought to redirect her own research focus to pursue lines of research other than those she had engaged in during her time in Dr. Kaplan's lab. Nevertheless, due to a lack of senior research presence in Dr. Kaplan's and Dr. Ward's lab, Dr. Frech was occasionally asked to guide their technicians on some experiments and, if necessary, to assist in assembling figures for publication. During those times when Dr. Frech assisted in figure assembly, she necessarily relied on the data generated and provided by Drs. Kaplan and Ward's technicians. Drs. Kaplan and Ward had ultimate responsibility for any final manuscripts and figures, which they submitted to journals.

Dr. Frech's employment at UU was terminated in or about June 2013. After leaving UU, Dr. Frech sought and obtained an MBA, and eventually returned to scientific research – though ***not*** Public Health Service ("PHS") supported research – on or about September 29, 2015, when she joined the lab of Dr. Fenghuang Zhan at the University of Iowa ("UI"). When Dr. Zhan left UI to join the University of Arkansas, Dr. Frech declined to follow. Dr. Frech's last day in Dr. Zhan's lab was on or about December 18, 2019, and Dr. Frech resigned from UI entirely as of May

1, 2020, when no suitable new position at UI could be identified for her. Since 2020, Dr. Frech had been working as an administrator at a community hospital in Iowa City. This employment was abruptly brought to an end on January 30, 2024, due to the Defendants' improper debarment of Dr. Frech. Nevertheless, UI has represented to Dr. Frech that, should the debarment be lifted, it would like to have her in the same position, with the same job duties, that she had been in for years before the community hospital's sale. However, no job can remain unstaffed indefinitely, and UI has already made an interim hire. Absent immediate injunctive relief, Dr. Frech will forever lose the opportunity to reclaim her employment.

As described in greater detail herein, every factor in determining whether preliminary injunctive relief should be granted weighs in Dr. Frech's favor. Moreover, the Defendants have entirely failed to identify any evidence demonstrating that she is not presently responsible given that the purported conduct upon which the debarment action is based took place ***more than a decade ago***, about which the Defendants have been fully informed for ***at least*** nine years, if not more. The debarment is, on its face, astoundingly untimely, purely punitive, unjustified, and improper—and, thus, arbitrary and capricious.

II. STATEMENT OF RELEVANT FACTS FOR PURPOSES OF THE MOTION[1]

A. The UU Research Misconduct Process

In or about October 2011, allegations of research misconduct arose involving multiple papers coauthored by Dr. Frech that were published from Dr. Kaplan's lab. Pursuant to the applicable federal regulations (42 C.F.R. Part 93) and UU's corresponding research misconduct policy, UU conducted an inquiry and an investigation into the allegations. The allegations involved research published between 2007 and 2011 (and, therefore, involved data that had been

[1] For a full discussion of facts, see Dr. Frech's complaint.

generated even earlier) that was supported by PHS funding. ORI, a component of HHS, acts on behalf of HHS as the office responsible for overseeing and directing PHS research integrity activities. ORI does not itself conduct research misconduct inquiries and investigations. Instead, ORI refers allegations to the relevant institution where the research was conducted for inquiry and investigation. *See* 42 C.F.R. §§ 93.400(a), 93.402. ORI then conducts an oversight review of an institution's findings and process, and may then make a federal finding of research misconduct and propose HHS administrative actions. *See* 42 C.F.R. §§ 93.400(a)-(c), 93.403. Thus, when allegations in this matter arose, ORI relied upon UU to conduct the inquiry and investigation.

Despite the fact that UU's inquiry concluded that it "did not uncover evidence of intent to deceive or misrepresent the data from the experiments in question," UU nevertheless determined that an investigation was necessary and appointed an investigation committee. *See* Exhibit 1, Feb. 16, 2012 UU Inquiry Report, AR 612-619 at AR 615; Exhibit 2, Mar. 7, 2012 RIO Report, AR 633-635 at AR 634-635. The investigation committee reviewed allegations of research misconduct in 11 papers, but expressly disclaimed any review of publications arising from Dr. Kaplan's lab for which Dr. Frech was not a coauthor. *See* Exhibit 3, Dec. 14, 2012 Final UU Investigation Report, AR 962-1058 at AR 968.

UU's investigation report concluded that Dr. Frech had engaged in research misconduct. The basis for this determination was a purported "pattern of recklessness," which the investigation committee defined as "actions that are marked by lack of proper caution and care with respect to the consequences of the actions, and are therefore negligent and a breach of expected professional responsibility." *See* Exhibit 3, Dec. 14, 2012 Final UU Investigation Report at AR 966. Furthermore, the investigation committee found that the alleged research misconduct "was committed in reckless disregard of accepted practices." *See id.* UU's finding as to Dr. Frech's

level of intent was thus that her actions were reckless, rather than intentional or knowing. Even a finding of recklessness by UU, however, was erroneous because (1) UU utilized so broad a definition of recklessness as to include negligence and carelessness (neither of which rise to the level of recklessness), and (2) UU conflated two separate elements of research misconduct (a finding of intent and a significant departure from accepted practices of the research community).

Additionally, although UU made research misconduct findings against Dr. Frech, it also determined that she was not solely responsible for the errors, and that Dr. Kaplan, Dr. Ward, and others also had varying degrees of responsibility. *See* Exhibit 3, Dec. 14, 2012 Final UU Investigation Report at AR 967-969. Indeed, the investigation committee recommended that UU "relieve Dr. Kaplan of his senior leadership duties" based on his role in the errors and the "systematic" issues in his lab. *See id*. at AR 967-968.

After receiving UU's final investigation report in or about December 2012, Dr. Frech appealed UU's findings and recommendations to a Consolidated Hearing Committee ("CHC") at UU. The CHC heard Dr. Frech's appeal on April 29, 2013 and issued its report on May 8, 2013. Despite also finding that Dr. Frech engaged in research misconduct "based on 'reckless disregard of accepted practices'[,]" the CHC – similarly to the UU investigation – found that "[t]here was complicity in this misconduct within the laboratory that goes substantially beyond Dr. De Domenico." *See* Exhibit 4, May 8, 2013 CHC Report, AR 4045-4053 at AR 4050. Notably, the CHC found that responsibility was shared with Dr. Kaplan and Dr. Ward (and, potentially, others) and therefore recommended "further investigation of the laboratory procedures uncovered in this investigation." *See id*.; *see also id.* at AR 4045, 4048-4049.

B. ORI's Charge Letter

After UU's investigation concluded in early 2013, UU sent the records of its inquiry and investigation to ORI for ORI's oversight review and to determine what findings and actions, if any, should be made by HHS. ORI and HHS have thus been considering this matter for more than 10 full years. Furthermore, on September 19, 2014, Dr. Frech—through counsel—wrote to ORI to express concerns that UU may not have provided ORI with the entirety of the record, and, in an effort to mitigate that possibility, provided ORI with relevant materials. *See* Exhibit 5, Sept. 19, 2014 Letter to ORI and Index of Enclosed Documents, AR 4078-4081. In that letter, Dr. Frech offered to provide any other materials that ORI determined were missing. *See id*. at AR 4080. ORI never responded nor did it request any additional information from Dr. Frech or UU, indicating that ORI had all of the information it needed to conduct its oversight review by no later than September 19, 2014. On or about July 19, 2023, ***more than a decade*** after the process at UU concluded (and approximately nine years after the latest possible date that ORI received relevant materials), Dr. Frech received a Charge Letter from ORI identifying its findings and administrative actions. Despite the numerous allegations involving 11 papers at issue during UU's investigation, ORI made six research misconduct findings with respect to only three papers.

In each of ORI's six findings of research misconduct, ORI states that Dr. Frech acted "intentionally, knowingly, or recklessly" and that she "falsified and/or fabricated" figures in the three papers. *See* Exhibit 6, July 18, 2023 HHS Charge Letter, AR 4783-4803 at AR 4791-4801. ORI also states that Dr. Frech's "actions were knowing and intentional" and that she "solely planned, initiated, and carried out the wrongdoing." *See id*. at AR 4802. These conclusions are not only erroneous, but are also directly contradicted by the evidence, including UU's findings upon which ORI necessarily relied. Additionally, these conclusions do not represent a legitimate

finding as required by the regulations since they only name all of the possible outcomes, without concluding by a preponderance of the evidence as to a particular finding. In addition to describing ORI's findings, the Charge Letter also informs Dr. Frech that she will be debarred from participation in covered transactions for a three-year period as an administrative action imposed by HHS. Significantly, since her employment at UU was terminated in 2013, Dr. Frech has voluntarily excluded herself from participation in covered transactions. Specifically, Dr. Frech has refrained from engaging in any scientific research supported by PHS funds. Thus, had ORI imposed its debarment of Dr. Frech contemporaneously with UU's findings, its imposed debarment would have been fully satisfied ***three times over*** by the time the Charge Letter was prepared.

Following receipt of ORI's Charge Letter, Dr. Frech requested copies of the exhibits to the Charge Letter, which were not provided concurrently with the Charge Letter itself. ORI provided those exhibits on August 10, 2023. After receiving the exhibits, beginning on August 15, 2023, Dr. Frech sought in good faith to negotiate with ORI to resolve this matter in a mutually acceptable manner. Through August 18, 2023, Dr. Frech and ORI exchanged correspondence that made it clear that, although Dr. Frech disagreed with and contested ORI's findings, she was willing to reach a settlement. Although ORI rejected the specific proposals by Dr. Frech during this time, it did not foreclose the possibility of reaching an agreement.

On August 18, 2023, following ORI's rejection of Dr. Frech's most recent proposal, Dr. Frech, through counsel, informed ORI that she would convey a settlement proposal to ORI the following week. ORI did not respond. Consistent with Dr. Frech's representation, on August 22, 2023, Dr. Frech contacted ORI to engage in further discussion. Only then did ORI respond, taking the position that the deadline to contest ORI's findings had passed and that the findings and

administrative actions (including debarment) had therefore become final. Dr. Frech promptly responded, noting that her communications seeking to resolve the matter amicably had made it abundantly clear that she contested the findings.

The following day, August 23, 2023, Dr. Frech and ORI exchanged further communications, both via email and telephone. Despite Dr. Frech's shock at being sandbagged by ORI's silence until it asserted the deadline had already passed, ORI insisted that it could neither continue to negotiate nor could Dr. Frech now request a hearing, despite her communications with ORI detailing her disagreement with the findings. ORI repeatedly expressed that any hearing request now would be denied. Dr. Frech therefore filed suit in this court on September 5, 2023.

C. Dr. Frech's Employment Is Terminated

On or about November 6, 2023, Mercy Iowa City—the community hospital that Dr. Frech had been working for since 2020—was approved by a bankruptcy court for a sale to UI. Ownership of the hospital was set to officially change to UI as of January 31, 2024. Like other employees of Mercy Iowa City, Dr. Frech received an offer of employment by UI, formalized through a transition agreement. However, on January 30, 2024—hours before the ownership change—UI rescinded Dr. Frech's transition agreement based solely on Dr. Frech's debarment. This decision was reached despite the facts that (1) the hospital will remain a community hospital; (2) nothing in Dr. Frech's job description or duties would change with the ownership transition; and (3) Dr. Frech's debarment has no impact on her ability to fulfill the duties of that position. Instead, the decision was reached based on a UI Policy that precludes UI from employing anyone who has been excluded or debarred from participation in federal programs. Thus, the Defendants' improper and grossly untimely debarment of Dr. Frech has caused her actual and substantial harm. Dr. Frech

therefore moves to enjoin Defendants from enforcing the debarment and seeks to have the debarment lifted pending the resolution of this action.

III. ARGUMENT

A. Standard of Review

1. Temporary Restraining Order or Preliminary Injunction

Federal Rule of Civil Procedure 65 authorizes federal courts to issue temporary restraining orders ("TROs") and preliminary injunctions. To obtain a TRO or a preliminary injunction, the moving party must establish that she "is likely to succeed on the merits, that [she] is likely to suffer irreparable harm in the absence of preliminary relief, that the balance of equities tips in [her] favor, and that an injunction is in the public interest." *Sherley v. Sebelius*, 644 F.3d 388, 392 (D.C. Cir. 2011) (quoting *Winter v. Natural Res. Def. Council, Inc.*, 555 U.S. 7, 20 (2008)); *see also Chef Time 1520 LLC v. Small Bus. Admin.*, 646 F. Supp. 3d 101, 109 (D.D.C. Dec. 20, 2022) (explaining that the same factors applicable to analysis of whether to grant a preliminary injunction apply to consideration of a TRO).

As this court has noted, prior to *Winter*, "courts in this circuit applied a 'sliding-scale' approach under which a 'strong showing on one factor could make up for a weaker showing on another'" and that "[s]ince *Winter*, the D.C. Circuit has hinted on several occasions that 'a likelihood of success is an independent, free-standing requirement,' but it 'has not yet needed to decide the issue.'" *Chef Time 1520 LLC*, 646 F. Supp. 3d at 109 (quoting *Sherley*, 644 F.3d at 392-393 and *League of Women Voters of U.S. v. Newby*, 838 F.3d 1, 7 (D.C. Cir. 2016)); *see also Brady Campaign to Prevent Gun Violence v. Salazar*, 612 F. Supp. 2d 1, 12 (D.D.C. 2009). However, when pursuing a TRO or preliminary injunction, "the movant has the burden to show

that all four factors, taken together, weigh in favor of the injunction." *Chef Time 1520 LLC*, 646 F. Supp. 3d at 109 (quoting *Abdullah v. Obama*, 753 F.3d 193, 197 (D.C. Cir. 2014)).

2. Administrative Procedure Act

Pursuant to the Administrative Procedure Act ("APA"), 5 U.S.C. § 706(2)(A), the Court has the authority to "'hold unlawful and set aside' agency actions[, findings, and conclusions] that are [found to be] 'arbitrary, capricious, an abuse of discretion or otherwise not in accordance with law.'" *Oceana, Inc. v. Ross*, 363 F. Supp. 3d 67, 76 (D.D.C. 2019). "[T]he Supreme Court 'insists that an agency examine the relevant data and articulate a satisfactory explanation for its action." *Bois v. U.S. Dept. of Health and Human Services*, Civ. No. 11-1563, 2012 WL 13042904 at *4 (D.D.C. Mar. 2, 2012) (quoting *F.C.C. v. Fox Television Stations, Inc.*, 556 U.S. 502, 513). "Such a review 'is not merely perfunctory. We are to engage in a searching and careful inquiry, the keystone of which is to ensure that the agency engaged in reasoned decisionmaking.'" *See id.* (quoting *Int'l Ladies' Garment Workers' Union v. Donovan*, 722 F.2d 795, 815 (D.C. Cir. 1983).

B. Dr. Frech Is Entitled To Injunctive Relief

1. Dr. Frech Has A Substantial Likelihood Of Success On The Merits

A party seeking a TRO or a preliminary injunction must show clearly that it is likely to succeed on the merits. *Tate v. Pompeo*, 513 F.Supp.3d 132, 140-141 (D.D.C. 2021). The party "need not establish an absolute certainty of success on the merits" but must "raise[] serious legal questions going to the merits, so serious, substantial, difficult as to make them a fair ground of litigation and thus for more deliberative investigation." *Akiachak Native Community v. Jewell*, 995 F. Supp. 2d 7, 13 (D.D.C. 2014) (quoting *Population Inst. v. McPherson*, 797 F.2d 1062, 1078 (D.C. Cir. 1986)). For the reasons set forth below, Dr. Frech will likely succeed on the merits of her challenge to HHS's and ORI's debarment action.

a. The Federal Research Misconduct Regulations

In imposing its debarment, the Defendants relied on the general proposition that research misconduct is, at times, a cause for debarment. *See* Exhibit 6, July 18, 2023 HHS Charge Letter, AR 4783-4803 at AR 4803. While this may be true, the circumstances here demonstrate that the Defendants' imposition of debarment against Dr. Frech is entirely arbitrary and capricious, given HHS' mere formulaic recitations of the elements of research misconduct and the ***vast*** amount of time that passed between when Defendants became aware of the conduct and when debarment was imposed.

The purpose of the federal regulations applicable to PHS-funded research for which ORI has oversight and pursuant to which ORI and HHS make findings and institute administrative actions is remedial in nature. Indeed, the regulations are intended to "[p]rotect the health and safety of the public, promote the integrity of PHS supported research and the research process, and conserve public funds." 42 C.F.R. § 93.101(e). "Any interpretation of this part must further the policy and purpose of the HHS and the Federal government to protect the health and safety of the public, to promote the integrity of research, and to conserve public funds." 42 C.F.R. § 93.107.

Research misconduct is defined as "fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results." *See* 42 C.F.R. § 93.103. "Fabrication" is defined as "making up data or results and recording or reporting them" whereas "falsification" is defined as "manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record." *See id*. at (a) and (b).[2] Honest error is expressly excluded from the definition of research misconduct. *See id*. at (d).

[2] There is neither an allegation nor any purported finding of plagiarism in this matter.

A finding of research misconduct requires more than simply finding that the definition of research misconduct has been met through fabrication, falsification, or plagiarism. Specifically, a finding of research misconduct requires that "(a) [t]here be a significant departure from accepted practices of the relevant research community; and (b) [t]he misconduct be committed intentionally, knowingly, or recklessly; and (c) [t]he allegation be proven by a preponderance of the evidence." *See* 42 C.F.R. § 93.104. Each of these elements is separate and must be met for each allegation to support a research misconduct finding.

ORI does not itself conduct research misconduct inquiries and investigations, instead referring allegations to the relevant institution (or HHS component) where the research was conducted for inquiry and, should one be found warranted, investigation. *See* 42 C.F.R. §§ 93.400(a), 93.402. ORI conducts an oversight review of an institution's findings and process, and may then make a federal finding of research misconduct and propose HHS administrative actions. *See* 42 C.F.R. §§ 93.400(a)-(c), 93.403. ORI thus relies heavily on the institutional reports and record developed during the institutional inquiry and investigation.

b. The Research Misconduct Findings Violate the APA

Under the APA, agency findings and actions may be held "unlawful and set aside . . . if . . . [they are] arbitrary, capricious, an abuse of discretion, or otherwise not in accordance with law . . . or if [they are] unsupported by substantial evidence." *Chef Time 1520 LLC*, 646 F. Supp. 3d at 109 (internal quotation marks and citations omitted). Agency action may be considered arbitrary and capricious where, *inter alia*, the agency "entirely failed to consider an important aspect of the problem, offered an explanation for its decision that runs counter to the evidence before the agency, or is so implausible that it could not be ascribed to a difference in view or the product of agency expertise." *Motor Vehicle Mfrs. Ass'n of U.S., Inc. v. State Farm Mut. Auto. Ins. Co.*, 463 U.S.

29, 43 (1983). A court "may not supply a reasoned basis for the agency's action that the agency itself has not given." *Id.* (quoting *SEC v. Chenery Corp.*, 332 U.S. 194, 196 (1947)). Similarly, "[a]gency fact-finding warrants considerable deference" only when "it is supported by substantial evidence." *Chef Time 1520 LLC*, 646 F. Supp. 3d at 110 (internal quotation marks and citation omitted).

Here, the findings described in the Charge Letter are contradicted outright by the UU investigation and CHC report. The Defendants have not provided – and cannot provide – any explanation for finding that Dr. Frech solely engaged in research misconduct, when the evidence plainly demonstrates that there were systemic problems in Dr. Kaplan's lab and that responsibility for the errors at issue in the allegations went substantially beyond Dr. Frech, including to Dr. Kaplan, Dr. Ward, and others. ORI's finding in contradiction to the UU investigation upon which it necessarily relied – and in contradiction to a preponderance of the evidence – is arbitrary and capricious, an abuse of discretion, unsupported by substantial evidence, and contrary to law and regulation and thus violates the APA.

Similarly, the defendants cannot provide any evidence for declaring that Dr. Frech's actions were intentional and knowing, when the available evidence led the CHC to conclude that, with respect to the only manipulation that appeared intentional, there is insufficient evidence to conclude "that it was done by Dr. De Domenico as opposed to an unknown third person." Instead, both the UU investigation and the CHC found, based on a preponderance of the evidence, that – at most – Dr. Frech was reckless.

Furthermore, both the UU investigation and the CHC utilized a broad definition of recklessness that included ordinary negligence and mere carelessness, neither of which rise to the level necessary to support a research misconduct finding. Indeed, a HHS Administrative Law

Judge ("ALJ") considering research misconduct findings by ORI has previously adopted the Black's Law Dictionary definitions as "the common definitions for intentional, knowing, and reckless and their adverb forms." *In re Decision of Kreipke*, Recommended Decision, Docket No. C-16-402, Decision No. CR5109 (May 31, 2018) at p. 14. The definition for "reckless" in Black's Law Dictionary and adopted by the ALJ is "[c]haracterized by the creation of a substantial and unjustifiable risk of harm to others and by a conscious (and sometimes deliberate) disregard for or indifference to that risk; heedless; rash. Reckless conduct is much more than mere negligence: it is a gross deviation from what a reasonable person would do." *See* Black's Law Dictionary (emphasis added); *see also Kreipke* at p. 14. The UU investigation expressly based its finding that Dr. Frech was reckless on its conclusion that recklessness "mean[s] actions that are marked by lack of proper caution and care with respect to the consequences of the actions, and are therefore negligent and a breach of expected professional responsibility." This improper inclusion of negligence and carelessness in the definition of recklessness suggests that, if anything, ORI's findings should be that Dr. Frech lacked any requisite level of intent to engage in research misconduct. Regardless, a finding that Dr. Frech acted intentionally or knowingly is entirely unsupported by the evidence and, thus, is arbitrary and capricious, an abuse of discretion, unsupported by substantial evidence, and contrary to law and regulation in violation of the APA. "Reasoned decisionmaking . . . precludes the agency from offering an explanation that runs counter to the evidence before the agency." *Sierra Club v. Salazar*, 177 F. Supp. 3d 512, 533 (internal quotation marks and citations omitted). But ORI's findings here are counter to the evidence.

In each of its six research misconduct findings against Dr. Frech, ORI purports to find that Dr. Frech acted "intentionally, knowingly, or recklessly" and that she "falsified and/or fabricated" figures in the three papers. By simply listing all possible outcomes, ORI treats multiple elements

of finding research misconduct as a mere box-checking exercise without identifying substantial evidence to support its conclusions. The first of these statements, repeated as to each finding, abdicates responsibility for finding a single level of intent for each allegation by a preponderance of the evidence in favor of simply regurgitating the requirement and concluding that an element has been satisfied. In the second statement, ORI abandons its burden for determining whether data were made up or altered. If ORI cannot make a determination as to Dr. Frech's specific level of intent or the specific type of research misconduct she purportedly engaged in, it cannot plausibly satisfy its burden of making a research misconduct finding by a preponderance of the evidence. In essence, these conclusory statements that merely claim that Dr. Frech possessed any requisite level of intent and engaged in any form of research misconduct (other than plagiarism) are a "mere assertion" that does not "articulate a satisfactory explanation" for the agency's findings. *See, e.g.*, *Water Quality Ins. Syndicate v. U.S.*, 225 F. Supp. 3d 41, 68 (listing cases where agency findings were contradicted by evidence and agency's unsupported assertions were insufficient).

Thus, Dr. Frech is likely to succeed on the merits that the research misconduct findings by HHS and ORI violate the APA. Accordingly, use of those findings in support of debarment should be enjoined pending Dr. Frech's challenge of those findings in this litigation.

c. Imposing Debarment Is Purely Punitive And Violates The APA

The purpose of the federal research misconduct regulations is remedial. It is not intended to be punitive, but rather to safeguard science and allow for the rehabilitation of even those scientists who engage in intentional research misconduct. *See* 42 C.F.R. §§ 93.100(a), 93.101(e), 93.408. Indeed, the regulations expressly state that "[t]he purpose of HHS administrative actions is remedial." 42 C.F.R. § 93.408 (additionally identifying mitigating and aggravating factors that

HHS considers in determining administrative actions). Debarment is the most severe potential administrative action enumerated in the regulations. *See* 42 C.F.R. § 93.407(a).

The purpose of debarment is to protect the public interest by ensuring that the Federal Government conducts business only with responsible persons, and utilizes the suspension and debarment system "to exclude from Federal Programs persons who are not *presently* responsible." 2 C.F.R. § 180.125 (emphasis added). In recognition of the potentially financially-crippling consequence and irreparable reputational stigma that attaches to a person from being debarred, the regulations permit HHS to only debar a person in order to protect the public interest; HHS cannot use debarment as means of punishment.

The Charge Letter asserts that, with respect to each research misconduct finding, Dr. Frech acted intentionally, knowingly, or recklessly. However, this fails to explain which of these three levels of intent the evidence shows Dr. Frech acted with in carrying out the alleged research misconduct. As described above, this is itself contradicted by the UU investigation report, which concluded only that Dr. Frech acted "in reckless disregard of accepted practices." Furthermore, although the Charge Letter repeatedly states that Dr. Frech acted with any of the three levels of intent, it inexplicably claims in its consideration of mitigating and aggravating factors that Dr. Frech's "actions were knowing and intentional." Defendants provide no explanation for this discrepancy, nor for how they reached a conclusion contrary to UU's findings and thus inconsistent with the evidence before the agency. Acting recklessly is not the equivalent of acting knowingly or intentionally, and the SDO fails to explain under which level of intent the cause for debarment was considered, the aggravating and mitigating factors were analyzed, and the length of debarment evaluated. Indeed, whether a respondent acted recklessly as opposed to knowingly or intentionally is one of the mitigating factors that should be considered. *See* 42 C.F.R. § 93.408(a).

Similarly, the extent to which a respondent planned, initiated, or carried out the wrong doing is another aggravating or mitigating factor that should be considered. *See* 2 C.F.R. § 180.860(f). As with the inexplicable jump from UU's finding that Dr. Frech acted, at most, with reckless intent, HHS contradicts the evidence before it by finding that she "solely planned, initiated, and carried out the wrongdoing." This is directly contradicted by extensive evidence of systematic problems in Dr. Kaplan's lab, leading both the UU investigation and the CHC to conclude that responsibility and complicity in any wrongdoing went "substantially beyond" Dr. Frech. These failures render the SDO's debarment decision arbitrary and capricious and unsupported by substantial evidence.

Moreover, simply repeating over and over that Dr. Frech acted "intentionally, knowingly, or recklessly" is insufficient to establish that research misconduct is cause for debarment, particularly where, as here, Dr. Frech has voluntarily excluded herself from participating in PHS-funded research for more than a decade. The SDO fails to explain why the alleged misconduct here is so serious and compelling as to impact Dr. Frech's present responsibility, particularly after having waited for more than 10 full years to act on the record before HHS. Indeed, HHS's very failure to take action in the span of time it has had the evidence in this matter vitiates any contention that the alleged misconduct is so serious or compelling that the Federal Government's interests must be protected from Dr. Frech today by debarring her for what is, in essence, an additional three-year term that has already been completed three times. *See, e.g.*, *Inchcape Shipping Services Holdings Ltd. v. U.S.*, No. 13-953C, 2014 WL 12838793 (Fed. Cl. Jan. 2, 2014). As *Inchcape* made clear, even when there is adequate evidence to support a suspension or debarment, the lack of an immediate need to do so to protect governmental interests can preclude such severe sanctions. *See id.* at *2. In that case, the Navy's delay of just over a year (from November 21, 2012 to

November 26, 2013) to suspend the plaintiff "cast[] serious doubt on the government's claim that immediate action was necessary." *See id.* As with *Inchcape*, here there is no explanation as to why HHS waited nine years—or more—to debar Dr. Frech, nor is there any "evidence of an ongoing threat against which the Government need[s] to be protected." *See id.*

As with the research misconduct findings themselves, the Charge Letter's conclusory statements that merely claim that the SDO considered the appropriate aggravating and mitigating factors are a "mere assertion" that does not "articulate a satisfactory explanation" for the agency's findings. *Water Quality Ins. Syndicate*, 225 F. Supp. 3d at 68. As with the research misconduct findings, the debarment itself and the SDO's scant analysis provide no basis for the Defendants to reach conclusions directly contradicted by the evidentiary record, as reflected in the UU investigation and the CHC report. Because HHS has failed to demonstrate by a preponderance of the evidence that Dr. Frech engaged in research misconduct so serious or compelling in nature that it affects her present responsibility to conduct business with the Federal Government, Dr. Frech is likely to succeed on the merits of her APA claim challenging the debarment. Thus, as with *Inchcape*, "the suspension looks like a punishment more than a protective measure." *See id.*; *see also Lion Raisins, Inc. v. U.S.*, 51 Fed. Cl. 238, 247 (2001) (finding that the USDA SDO abused his discretion in determining that "evidence of plaintiff's lack of integrity in April 1998, which was known to the agency as of May 1999, 'seriously and directly' affected plaintiff's 'present responsibility' as a Government contractor in February of 2001").

2. Dr. Frech Will Suffer Irreparable Harm If The Defendants Are Not Enjoined From Enforcing The Debarment

More than a decade ago, Dr. Frech was terminated from UU as a result of this matter, and voluntarily abandoned any federally-funded research. When it became clear to Dr. Frech that continuing to work as a scientific researcher in academia without utilizing federal funds would

require her to uproot her life and her family yet again, she left science entirely. Since 2020, Dr. Frech has been entirely out of scientific research, working in an administrative position at a community hospital. As a direct result of the debarment that the Defendants waited ***nine years or more*** to impose, Dr. Frech has now been terminated once again, and is once again faced with the prospect of needing to try to start yet another new career or trying to find a similar job in another city or state, should one even be available anywhere. Dr. Frech has a limited window of time to lift the debarment and reclaim her job at the community hospital she has worked for without incident for years. Without injunctive relief, that opportunity will vanish.

"The cornerstone of preliminary injunctive relief is irreparable harm that injures the moving party in a manner that cannot be remedied through other types of relief." *Clevinger v. Advocacy Holdings, Inc.*, Civ. Nos. 23-1159 & 23-1176, Slip Op., 2023 WL 4560839 at *4 (D.D.C. Jul. 15, 2023) (citing *Sampson v. Murray*, 415 U.S. 61, 88 (1974)). Reputational damage is a valid, non-economic harm upon which to grant a TRO or preliminary injunction. *See Luokong Tech. Corp. v. Dept. of Defense*, 538 F. Supp. 3d 174, 194 (D.D.C. 2021); *see also Celsis in Virto, Inc. v. CellzDirect, Inc.*, 664 F.3d 922, 930 (Fed. Cir. 2012) ("Price erosion, loss of goodwill, damage to reputation, and loss of business opportunities are all valid grounds for finding irreparable harm" (emphasis added)).

This Court has noted that "harm to reputation has been recognized repeatedly as a type of irreparable injury[.]" *Brodie v. U.S. Dept. of Health and Human Services*, 715 F. Supp. 2d 74, 84 (D.D.C. 2010) (citation omitted). In *Brodie*, this Court nevertheless declined to grant the preliminary injunction because "there is no indication . . . that Dr. Brodie's employer or any particular colleagues are likely to learn of the ALJ's decision in the near future . . . . The generalized risk that individuals in the scientific community may learn of the ALJ's findings

against Dr. Brodie during the pendency of this matter is not the sort of threat that can be neutralized by court order." *Id*. In part, this conclusion was based on the fact that a summary of the ALJ's decision against Dr. Brodie had already been published. *See id*. at 84-85. In contrast, here the publication of ORI's and HHS's erroneous findings has not only occurred, but the fact of those findings and the institution of debarment resulted in Dr. Frech losing her job ***outside*** of science. Dr. Frech may no longer work in scientific research, but has already suffered reputational and economic harm due to the allegations in this matter (which resulted in the termination of her employment by UU, and, now, the rescission of her transition agreement by UI). Dr. Frech already voluntarily left scientific research, and has now been prevented from continuing her chosen career outside of science. Dr. Frech lives in Coralville, Iowa, with limited opportunities for work similar to the role she held at Mercy Iowa City. UI has represented that, were it not for the debarment, it would be interested in having Dr. Frech to continue to work in the role she had been in up through January 30, 2024. However, UI has already filled the role on an interim basis. Without immediate injunctive relief, the opportunity for Dr. Frech to reclaim her job will pass.

Therefore, the irreparable harm to Dr. Frech weighs in favor of granting injunctive relief.

3. <u>The Balance Of Harms And Equities Weighs In Favor Of Dr. Frech</u>

"The balance the equities weighs the harm to Plaintiffs absent a TRO against the harm to the agency if the Court grants the motion." *Chef Time 1520 LLC*, 646 F. Supp. 3d at 116 (citing *Pursuing America's Greatness v. FEC*, 831 F.3d 500, 511 (D.C. Cir. 2016)). Here, ORI has waited for more than a decade to make its findings and take administrative actions against Dr. Frech. In that vast amount of time, Dr. Frech has not conducted any PHS-funded research. For more than three full years, Dr. Frech has not conducted any scientific research whatsoever. There is therefore no harm whatsoever to the Defendants if they are enjoined from enforcing debarment against Dr.

Frech pending adjudication of this matter. In contrast, Dr. Frech will suffer immediate, irreparable harm if the debarment is not lifted. The balance of the equities thus plainly weighs in favor of Dr. Frech and in favor of granting injunctive relief.

4. The Public Interest Favors Issuance Of A TRO Or An Injunction

Where, as here, the non-moving party is the government, "the government's interest *is* the public interest" making the balance of equities "and the public interest factor . . . one and the same[.]" *Chef Time 1520 LLC*, 646 F. Supp. 3d at 116 (quoting *Pursuing America's Greatness*, 831 F.3d at 511). Because neither the Defendants nor the public would be harmed by the issuance of a TRO or preliminary injunction in this matter pending adjudication of the merits, the public interest weighs in favor of granting Dr. Frech such relief. *See, e.g.*, *N. Mariana Islands v. United States*, 686 F. Supp. 2d 7, 21 (D.D.C. 2009) (noting that "[t]he public interest is served when administrative agencies comply with their obligations under the APA"). If anything, this factor additionally weighs in favor of granting injunctive relief to Dr. Frech due to "the public's interest to ensure that the government's suspension and debarment process is administered in a fair manner." *See Inchcape*, 2014 WL 12838793 at *3 (further stating that "the limited scope of a temporary injunction is no great threat to the government's concern in" protecting from any potential wrongdoing by plaintiff).

CONCLUSION

For the foregoing reasons, Dr. Frech respectfully requests that the Court grant her Order for a Temporary Restraining Order or Preliminary Injunction and award Dr. Frech all relief the Court deems appropriate, including her reasonable attorneys' fees and costs under the Equal Access to Justice Act. Dr. Frech further asks that an emergency hearing be set at the Court's earliest convenience.

Dated: February 9, 2024 COHEN SEGLIAS PALLAS GREENHALL & FURMAN PC

By: */s/ Jackson S. Nichols*
Jackson S. Nichols (D.C. Bar #975511)
Stephen D. Tobin (D.C. Bar #90020495)
(D.D.C. *pro hac vice*)
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STATEMENT OF CONFERRAL

On February 7, 2024, counsel for Dr. Frech informed counsel for Defendants of Dr. Frech's intent to seek preliminary injunctive relief. Counsel for Defendants replied that Defendants oppose this motion.

CERTIFICATE OF SERVICE

I, the undersigned, hereby certify that on February 9, 2024, a true and accurate copy of the foregoing document was filed via the Court's CM/ECF system and notification of such filing was sent to all counsel of record.

/s/ Jackson S. Nichols
Jackson S. Nichols

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLUMBIA

IVANA FRECH, Plaintiff, v. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, *et al.* Defendants.	Civ. No. 1:23-cv-02530-CRC

[PROPOSED] ORDER GRANTING PLAINTIFF'S MOTION FOR A TEMPORARY RESTRAINING ORDER/PRELIMINARY INJUNCTION

Upon consideration of Plaintiff Dr. Ivana Frech's Motion for a Temporary Restraining Order/Preliminary Injunction, the relevant legal authorities, and the record of this case as a whole;

The Court finds that Plaintiff is likely to succeed on the merits of her claims. The Court further concludes that Plaintiff has shown a likelihood of irreparable injury in the absence of injunctive relief, that the balance of equities favors Plaintiff, and that an injunction would be in the public interest. Given these considerations, the Court finds that injunctive relief is warranted. It is, therefore,

ORDERED that Plaintiff's motion for a Temporary Restraining Order/Preliminary Injunction is GRANTED and the Defendants' debarment of Plaintiff is LIFTED.

Defendants and their officers, agents, employees, attorneys, as well as any other persons who are in active concert or participation with the foregoing, are ENJOINED from enforcing

debarment of Plaintiff and are ORDERED to remove her name from the System for Award Management available at www.sam.gov.

DATE: _____

_____
Hon.
United States District Judge

Exhibits

Exhibit 1 - Feb. 16, 2012 UU Inquiry Report

Exhibit 2 - Mar. 7, 2012 RIO Report

Exhibit 3 - Dec. 14, 2012 Final UU Investigation Report

Exhibit 4 - May 8, 2013 CHC Report

Exibit 5 - Sept. 19, 2014 Letter to ORI and Index of Enclosed Documents

Exhibit 6 - July 18, 2023 HHS Charge Letter

EXHIBIT 1

Inquiry Committee for Allegations of Scientific Misconduct by Drs. De Domenico and Kaplan
16 February 2012
Dana Carroll, Ph.D., Professor of Biochemistry
Gary Schoenwolf, Ph.D., Professor of Neurobiology and Anatomy
Carl Thummel, Ph.D., Professor of Human Genetics

The committee met with Dr. Jeff Botkin on December 22, 2011 to hear their responsibilities and to discuss the allegations against Drs. De Domenico and Kaplan. We decided to schedule two meetings – one with Dr. De Domenico on January 6, 2012 and one with Dr. Kaplan on January 17, 2012. Based on our findings, a second meeting with Dr. De Domenico was held on February 7, 2012.

Meeting 1: January 6, 2012
This meeting focused on the allegations. We asked Dr. De Domenico to go through each of the figures in question, show us the primary data on the autoradiographs, and explain how the scanned images were used to make the final figures. She stated that in all cases, one person did the experiment, a second person scanned the data, and she used the scanned images to make the figures.
Fig 1C in Nov 2, 2011 Cell Metabolism paper: Dr. De Domenico did not know who did the original experiment. The plasma membrane (PM) and flow through (FT) samples were run on separate gels in opposite order such that one set of data would need to be flipped around the vertical axis to be aligned with the other in the final figure. As a result, two scans were made, with one an inverse image of the other. Dr. De Domenico used the same data from each scan (from the FT samples) to create the erroneous figure – i.e., the same 4 lanes were used in both tiers of the figure. The fact that the correct data were available and looked quite convincing, but were not used, indicates that this error was due to carelessness rather than an intention to deceive. The committee also noted that the central two lanes (36 h FAC; 0 h DFX) are not distinguished from each other in the figure – they are labeled identically. Yet the left lane (lane 2 in the gel) represents a sample without iron while the right lane (lane 3) represents a sample with iron. There would be no way to know this from the legend or figure.
Fig 2Aii in Nov 2, 2011 Cell Metabolism paper ("IP:Strepavidin" data on left): Dr. De Domenico confirmed that the same scanned image was used twice – in this figure and in panel B – as alleged. Looking at the film, however, revealed that the image was flipped around the vertical axis from the original data and was taken from samples in the FT, not the PM as labeled. It thus has no relation to what is labeled in either panel A or B. In addition, upon looking at the lower two panels in this figure (FT, α-GFP), the data for the right panel (36 hr, α-GFP) were found to be flipped from what was on the original film, and taken from PM samples rather than FT as labeled. It thus has no relation to what is labeled in the figure. The data for the lower left panel could not be found on the film at that time (although we returned to this later). There are thus two problems with this figure in addition to the alleged duplication of data. Neither of these problems could have been detected without looking at the original film.
Fig 4A and 3B in March 10, 2009 PNAS paper: The original data behind this allegation could not be found and may have been lost with the notebooks that were taken from the lab.

Fig 2A in PNAS paper and Fig 4A in 2009 Blood paper: Dr. De Domenico indicated that the labeling in the PNAS paper is correct. The "control media" sample in the Blood paper is thus treated with siRNA for human Jak2 and does not represent a control as labeled.
Fig. 1B in 2007 Blood paper: The images of zebrafish were all captured blindly to avoid any bias in interpretation. The use of a control image to represent the Fpn-GFP N144H mutant sample was an accident according to Dr. De Domenico.

We had no way to confirm or deny the allegation that the phospho-specific anti-Fpn antiserum does not exist. Dr. De Domeico (and Dr. Kaplan later) asserted its existence and stated that no one had requested it from them. It is true that the company (Cell Signaling) never offered it commercially.

Meeting 2: January 17, 2012
The second meeting, with Dr. Kaplan, was relatively brief. Dr. Kaplan expressed deep regret at the errors that were made but emphasized that an independent person in the lab had duplicated all the experiments in question. The committee did not look over data from those experiments. He told us that the replicated experiments, in all cases, look similar to the data that were published and thus the overall conclusions of those papers are sound. Dr. Kaplan expressed strong support for Dr. De Domenico as an excellent research scientist, both within and outside his group. He stated the intention of replacing each of the erroneous figures with corrected data in a published erratum.

Neither Dr. De Domenico nor Dr. Kaplan disputed any of the allegations, except those regarding the existence of the phospho-specific antiserum and the size of the error bars in various histograms. Dr. Kaplan asserted that the latter were similar to those in other papers in the field and that the statistical analyses were correct.

Meeting 3: February 7, 2012
The committee asked to meet with Dr. De Domenico again in order to follow up on discussions from the first meeting. In particular, the discovery of two additional errors in Fig 2Aii in the Nov 2, 2011 Cell Metabolism paper raised the possibility that more errors might be revealed if the original films were compared with the published data. Accordingly, the committee asked Dr. De Domenico to present the original data for all of the autoradiographs published in the Nov 2, 2011 Cell Metabolism paper. In addition, we asked Dr. De Domenico to present the original data for all the autoradiographs in the Jan 5, 2011 Cell Metabolism paper, for which no allegations had been raised. Because of excellent preparations on the part of Dr. De Domenico, we were able to cover all of the January paper and part of the November paper in the allotted two-hour time. All published data in the Jan 5, 2011 paper matched the data on the original films with the following exceptions. Dr. De Domenico emphasized that all of the figures for this paper were prepared by her.
Fig 4B in Jan. 5, 2011 Cell Metabolism paper – lower two panels (+Fe): The left panel is flipped around the vertical axis compared to the original film and thus does not represent the lanes as published. The data for the right lower panel were not on the same film and could not be found. These were the only data in the paper that could not be found.
Fig 5A in Jan. 5, 2011 Cell Metabolism paper: The right-most band in the upper panel is shifted to the right from its proper location, under the heading "65". The two bands in the lower panel

should correspond to the lanes "65" and "75", not "75" and "85" as indicated. There is a blank lane to the right of the lower band in the original data for this panel that is not shown in the figure (corresponding to lane "85"). The figure thus misrepresents the original data.
Fig 5B in Jan. 5, 2011 Cell Metabolism paper: The published data are flipped from the original film and thus do not represent what is shown.
Fig 7B in Jan. 5, 2011 Cell Metabolism paper: The upper left panel is flipped from the original film and thus does not represent what is shown. The lower panel shows loading controls that were run on a separate gel and thus cannot be directly compared to the experimental samples shown above. In addition, one loading control has not been included and they are not aligned with the upper panel. There are 16 lanes depicted in the upper panel and only 15 in the lower. The last sample on the right of the lower panel was cut off in making the figure.
Fig 1A in Nov. 2, 2011 Cell Metabolism paper: The films for the autoradiographs could not be found.
Fig 1C in Nov. 2, 2011 Cell Metabolism paper: Allegation discussed above.
Fig 1D in Nov. 2, 2011 Cell Metabolism paper: It was unclear whether the data in the upper right panel (+DFX, α-ubiquitin) are the same as the bands that were presented on the film. The data in the lower left panel (-DFX, α-GFP) are flipped from the original film and thus do not represent what is shown. The data for the lower right panel (which is alleged to be duplicated in Fig. 3) could not be found.
Fig 2Aii in Nov. 2, 2011 Cell Metabolism paper: We returned to these panels in which problems had been discovered during our first meeting, as described above. The original data for the lower left panel (18 hr, FT), which could not be found during our first meeting with Dr. De Domenico, were found on the film. They appear to match the data that were used for the two right lanes in the upper panel in Fig. 5 of this paper. The film used for Fig. 5 is a darker exposure from what was used in Fig. 2Aii, and the data were flipped and inverted from that shown in Fig. 5. An overlay of these images in Photoshop is attached. The data in Fig. 2Aii thus have no relation to what is labeled in the figure.
Fig 3 in Nov. 2, 2011 Cell Metabolism paper: The original data could not be found.
Fig 5 in Nov. 2, 2011 Cell Metabolism paper: The Western blot data in the upper panel (α-GFP) are flipped from the original film and thus do not represent what is shown.
Figs 4 & 7 in Nov. 2, 2011 Cell Metabolism paper: There was no time to discuss these figures.

All of the errors described above, aside from the original allegations, were identified by the committee and not by Dr. De Domenico.

Additional analysis
We asked Greg Stoddard to reanalyze some of the primary data from the Nov. 2, 2011 Cell Metabolism paper. His report is attached. He concludes that the error bars for the graph in Fig. 7B, as published, faithfully represent the primary data. He raises a question about the overall design of the experiment, but we did not discuss this issue with either Drs. De Domenico or Kaplan. He also notes that "in Figure 7B, the two top y-axis labels are shown as 300 and 350, but should be 250 and 300, to correctly reflect the data." He assumes that this is a typo. Given the frequency of errors cited above, however, the committee believes that a more complete assessment of the accuracy of the graphical data is warranted.

During the interviews with Dr. De Domenico the committee was made aware of several uses of poor laboratory practice that, taken together, contributed to the problems listed above.

1. Dr. De Domenico informed us that all of the lanes and size markers were properly marked on the original autoradiographs immediately after developing the film. Instead of then scanning those films, however, she or a technician would remove all the markings with alcohol, scan the images, and then relabel the films. In addition, a number of exposures that were used to make figures were entirely unlabeled. Some of these films had no date or other information to relate them to lab notes.
2. Western blot detection of loading controls (anti-tubulin) was done using a separate gel, rather than stripping and re-probing the blot with the experimental samples.
3. Data from different experiments were combined to make a figure rather than re-doing the experiments and loading them onto one gel.
4. Some experiments were not repeated at the time of the initial discovery, but were repeated over a long period of time – on occasion over more than a year after the initial work was done.

Conclusions

The Committee did not uncover evidence of intent to deceive or misrepresent the data from the experiments in question. Rather, there was extreme carelessness on the part of Dr. De Domenico in preparing the figures and little oversight from Dr. Kaplan in ensuring that the figures in the papers accurately represented the primary data. The arrangement and approximate intensity of the bands was symmetrical in all cases where the data were erroneously flipped, and thus careful attention was needed. In addition, most of the data were binary – either a band or no band – and thus care was needed to select the correct bands that represented the corresponding experiment. The fact that acceptable and accurate data were frequently present on the film, but not used, argues against any intention to deceive. In addition, because of the symmetry of the flipped panels, the overall conclusions from these figures are likely to be correct. However, given the multiple errors discovered in the Jan. 5, 2011 Cell Metabolism paper – for which no allegations had been raised – and the additional errors discovered in the Nov. 2 Cell Metabolism paper, the committee concludes that each of these papers, and perhaps others from these combined authors, contain misrepresentations of the primary results and thus deviate significantly from accepted scientific practice. We recommend that the authors fully disclose the errors discovered during the course of this inquiry to the editors of the journals involved. In addition, we recommend that all papers jointly authored by Drs. De Domenico and Kaplan be independently reevaluated, and if additional errors are found, that these be fully disclosed to the editors of the journals involved.

Comparison of Figs 2Aii and 5 in Nov. 2, 2011 Cell Metabolism paper

Fig. 2Aii aligned with Fig. 5, which is flipped, inverted, and shifted over one lane

Fig. 2Aii

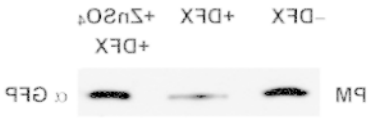
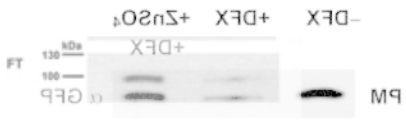


Fig. 5

Above with Fig. 2Aii aligned



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* Confidential *

I was asked to check the calculations of the error bars in the article,

> De Domenica I, Lo E, Yang B, Korolnek T, Hamza I, Ward DM, Kaplan J. The role of ubiquitination in hepcidin-independent and hepcidin-dependent degradation of ferroportin. *Cell Metabolism* 2011;14:635-646.

In an e-mail correspondence with [redacted], the concern raised was that the error bars reported in the figures appeared to be much two small, not honestly reflecting the variability that should have been seen the investigators.

Here is the e-mail stream showing the details:

1) ----- From [redacted] to Jeff Botkin -----

Dear Jeff, I am enclosing an extract from the methods article on the ferritin assay referenced by De Domenico et al. that will be of interest to the statistician. The coefficient of interassay variability is around 11%. Of course, he should contact me with any questions. Bes[redacted]

Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein,and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr. 2004 Nov;134(11):3127-32.PubMed PMID: 15514286.

TABLE 2 *Intra- and interassay variability (% CV) for the sandwich ELISA methods for a control sample with a concentration of the measured proteins in a medium range*

	Intra-assay variability, CV % (n = 5)	Interassay variability, CV % (n = 8)
Ferritin	6.2	11.4
sTfR	5.6	7.5
RBP	7.3	12.9
CRP	8.5	14.3

2) ----- From Greg Stoddard t[redacted] -----

Dear [redacted]
I am the biostatistician that is investigating this inquiry. I very much appreciate your willingness to talk to me. Perhaps I have what I need. From the email stream below, it appears that your concern was that we should be seeing error bars that reflect inter-assay variability of 11% in an article that claims to be reporting the results from "independent" experiments. Do I understand the concern correctly, or do we need to discuss it further, such as with a phone call.
Thanks, Greg

3) ----- From [redacted] Greg Stoddard -----

Dear Greg,
Yes, that was a part of what I wanted to communicate. Even these numbers (e.g. 11%) tend to underestimate the real world variability of this assay because they are obtained with identical samples so the chemical confounders in the sample are the same. The error bars of other investigators for the same type of studies and measurements are substantially larger. If this is sufficient for your assessment, there is no need to discuss this any further. Feel free to contact me if I can help with the process. Best,
[redacted]

I was then provided the data from the laboratory notebooks from the study reported in the *Cell Metabolism* article.

From the data in the laboratory notebooks, the figures, both the height of the bars in the bar charts, representing the mean, as well as the length of the error bars, representing the standard errors, were correctly drawn.

The concern raised by [redacted], however, is clearly present.

Statistics for Figure 7B ELISA to analyze cytosolic ferritin
(N=3 per displayed bar)

Sample: bars in graph from left to right	Mean*	SEM*	SD*	CV*
+FAC empty vector	224.3	1.8	3.1	0.01
+/- FAC empty vector	189.7	6.1	10.5	0.06
+FAC mFpn	241.0	2.3	4.0	0.02
+/- FAC mFpn	46.0	2.1	3.6	0.08
+FAC cFpn	224.3	2.7	4.7	0.02
+/- FAC cFpn	62.0	1.5	2.6	0.04

*Mean = arithmetic mean, which is height of error bar
SEM = standard error of the mean, which is the displayed error bar
SD = standard deviation
CV = coefficient of variation = SD/Mean

(For completeness, in Figure 7B, the two top y-axis labels are shown as 300 and 350, but should be 250 and 300, to correctly reflect the data That is just a typo, however, and is not pertinent to the concern that was raised.)

We see that the coefficient of variation (CV) is less than 0.1 for every test condition. Dr [redacted] had pointed out that these should be at least 11.0 for independent experiments. In the Cell Metabolism article, these data are reported as having come from independent experiments.

I have seen something like this before at the U of Utah. A graduate student had well-to-well variability that was very small, similar to these data. It arose from all wells being filled from a flask holding the solution that contained the cells, so essentially there was only one experiment. The faculty mentor informed me she had a flawed experiment, as she had not introduced sufficient experiment-to-experiment variability.

Perhaps something like that occurred, so that the data actually are not from “independent” experiments. However, according to the Erhardt paper Table 2 shown above, there should have been a CV of at least 5, simply from the variabilitity in the ELISA method, using samples from the same experiment (as least as far as my naïve understanding of the problem).

EXHIBIT 2

THE UNIVERSITY OF UTAH

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Research Integrity Officer Report
University of Utah Case: 02-2011
Federal Office of Research Integrity Case: DIO 4554
Revised: March 7, 2012

Summary

I, the Research Integrity Officer, Jeffrey Botkin, was initially contacted by Dr. Kaplan and, shortly thereafter, by several journals regarding concerns identified in several publications jointly authored by Drs. De Domenico, Kaplan and others. Dr. De Domenico is an Assistant Professor in Hematology and Dr. Kaplan is Professor of Pathology and Associate Dean for Research in the School of Medicine. The concerns involved multiple instances of figures that appeared to be duplicated or otherwise manipulated. A detailed set of PowerPoint slides were submitted by an outside investigator who reviewed a number of recent publications by the Utah group and identified several instances of duplication of western blots and other concerns.

Federal grants were used for some of the work in question including: NIH grants DK030534, DK070947, and SP30 DK072437 to Dr. Kaplan, and DK090257 to Dr. De Domenico.

I interviewed Drs. De Domenico and Kaplan separately about the concerns. Original films and notebooks were sequestered. One set of films was discarded by a former laboratory employee and the loss of these data were documented in emails that pre-date the current allegations.

The respondents acknowledged multiple errors in the published articles in question but ascribed those to mistakes in preparation of the manuscripts. They contend that the original data, including repeated runs at the time of the relevant experiments, illustrate that there was no rationale for data fabrication or falsification and that the conclusions of the papers remain valid.

An Ad Hoc Inquiry Committee was seated including:

- Dr. Carl Thummel, Ph.D. Professor of Human Genetics,
- Dr. Gary Schoenwolf, Distinguished Professor of Neurobiology and Anatomy
- Dr. Dana Carroll, Ph.D., Professor of Biochemistry

All three are senior members of the University faculty who have expertise in the experimental techniques in question. Committee members also were chosen who would be able to review the work of a senior individual such as Dr. Kaplan.

The Inquiry Committee reviewed the primary data and interviewed Drs. De Domenico and Kaplan over the course of three meetings. Their report was submitted on 2/16/12. The Inquiry Committee identified several additional

irregularities not in the original allegations. Their primary finding was that multiple errors were due to poor laboratory methods and procedures. The Inquiry Committee did not identify instances of intentional data fabrication or falsification.

Although the Committee was concerned about the number of errors identified, it did not recommend that a formal research misconduct investigation be conducted. The Committee did recommend that a thorough review be conducted of all publications by the respondents to identify any additional irregularities. A response was received from Drs. De Domenico and Kaplan on 2/22/12. The respondents acknowledge multiple errors and intend to work with the journals in question to address these concerns.

My assessment was that the Inquiry Committee had done a thorough job in reviewing the primary data and interviewing the respondents. The respondents have been fully cooperative through the inquiry and they acknowledge the multiple errors made in the preparation of manuscripts. The Committee's findings, and my acceptance of their findings, were based on several considerations: 1) a lack of evidence that the irregularities were intentional, 2) plausible explanations about how the irregularities occurred through poor laboratory practices, 3) evidence that the correct figures existed and could have been used by the respondents without changing the scientific findings of the papers, 4) a lack of evidence that the errors in the figures led to false conclusions in the scientific literature, and 5) the admission of error for all allegations (except the error bar issue and the existence of the antibody) and for all the new irregularities identified by the Inquiry Committee. Given the admission of error, it was considered unlikely that an additional evaluation would uncover evidence of an intent to deceive. The review of other publications might trigger an inquiry or investigation if additional irregularities were identified.

The initial decision not to pursue an investigation was communicated to the respondents, the complainant, and the relevant journals.

A new communication was received on 3/4/12 from the complainant. Additional irregularities were identified in the literature, specifically western blots in two publications:

- De Domenico et al. The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol Biol Cell. 2007 Jul;18(7):2569-78 (NIH Grants DK-070947 and HL- 26922 (to J.K.) and AI-051174 (to W.I.S.)); and
- De Domenico et al. Evidence for the multimeric structure of ferroportin. Blood. 2007 Mar 1;109(5):2205-9 (NIH Grant DK070 947 (J.K.)).

The complainant sent PowerPoint slides that appear to indicate that a western blot in each of these publications were spliced together from separate images. Given the complexity of the original allegations and a new set of information suggesting deliberate alterations of images, the decision was made on 3/5/12 to conduct a full investigation of the irregularities.

I am recruiting a 5 member Investigation Committee that will review all irregularities, including the new and the previously reviewed allegations. The Investigation Committee also will review other publications by the respondents for evidence of additional irregularities.

Jeffrey R Botkin, MD, MPH
Research Integrity Officer
University of Utah

EXHIBIT 3

INVESTIGATION COMMITTEE REPORT
University of Utah Case: 02-2011
Federal Office of Research Integrity Case: DIO 4554
Final Report December 14, 2012

MASTER SUMMARY

OVERVIEW

This report summarizes the work done by the Committee charged with investigating allegations of research misconduct at the University of Utah, with Dr. Ivana De Domenico and Dr. Jerry Kaplan named as respondents. The allegations were based on multiple irregularities found in 11 papers published by the respondents over the years 2007 to 2011, as well as reports from the complainant that some of the results in these papers could not be reproduced in other laboratories. The majority of alleged irregularities involved figures representing autoradiograph films of western blot experiments, such as the re-use of the same image in multiple figures in the same or different papers. Other allegations concerned unreasonably small error estimates reported for quantitative assays. In the course of the investigation, the Committee identified additional areas of concern and investigated these as well. The investigation included examination of the published papers and the corresponding research notes and data, including autoradiograph films and computer files; and interviews with the respondents and other parties, conducted either in person, by telephone or via e-mail.

From its investigation, the Committee has concluded that the irregularities identified by the complainant constitute misrepresentations of the primary data and reflect a serious breakdown in the processes required to ensure that the scientific literature is a faithful record of research results. The large number of instances, over a period of several years, indicates a reckless disregard for the integrity of the research record, as opposed to the occasional lapses that might occur in any laboratory, and the resulting record is now nearly impossible to reconstruct. Furthermore, the Committee found evidence that this disregard for integrity extended beyond negligence to instances of intentional falsification (including the duplication of western blot bands in the same image to represent different samples, and false published statements regarding the calculation of error estimates) and the fabrication of data (circular dichroism spectra). In some cases, primary data could not be found. Finally, there is strong evidence that the research record has been altered so as to hide misconduct or shift responsibility to others. The Committee concludes that the nature and pattern of these irregularities constitute research misconduct of a highly serious degree.

IMPACT ON SCIENTIFIC COMMUNITY

The Committee stresses that its investigation and report have focused on the relationship between the published papers and the research record maintained by the respondents. The Committee has not attempted to determine whether or not the conclusions of the published papers are valid. It is entirely possible, as claimed by the respondents, that many or all of the results are reproducible. However, subsequent reproducibility of a scientific observation by the same research group or by other groups does not address the question of whether or not the original experimental data were accurately represented in the published papers. The reckless or deliberate misrepresentation of research results is unacceptable, even if other data may support the conclusions. Research misconduct can occur through faulty practices and processes that lead to a published conclusion, even if that conclusion eventually is found to be correct. Conversely, the findings of misconduct do not directly bear on whether the conclusions are valid. Indeed, the very ambiguity that has been introduced into an important body of work is perhaps the major cost of the breakdown of scientific integrity described in this report. The Committee holds that professional ethics and standard practices in the field require that the process of accurately representing scientific results and primary data are just as important as the conclusions found in a publication.

SUMMARY OF PROCESS

An Inquiry Committee of three senior faculty submitted their report on 02/16/2012. Their finding was that multiple errors were due to poor laboratory methods and procedures and did not identify instances of data fabrication or falsification. New allegations concerning two additional publications were received by Dr. Botkin in the Research Integrity Office (RIO) on 03/04/2012, and a decision was made on 03/05/12 to open a full investigation. A committee of five senior faculty members was recruited to examine previous allegations and newly arising allegations and irregularities. After the formation of the Investigation Committee, two other allegations concerning additional publications were received by the RIO.

This Investigation Committee examined the report by the Inquiry Committee, the primary data that consisted predominantly of western blot films and accompanying notes, as well as electronic data on hard drives and computer printout data, all sequestered by Dr. Botkin's office. Eleven publications were examined, nine with Dr. De Domenico as first author, one as middle (collaborating) author and one as last (corresponding) author. In the eleven papers, the Committee found irregularities in numerous (over 20) figures. Some irregularities were identified by the complainant (Papers 1- 6), some additional irregularities by the Inquiry Committee (Papers 1, 6), some in subsequent communications with Dr. Tomas Ganz (03/04/12, Papers 7- 9; 6/28/12, Paper 11) and some by the Investigation Committee (Paper 10). The Investigation Committee reconstructed the published versions of figures from primary data and films from notebooks, compared primary data and scanned films found on computer hard drives with published data, and reconstructed and analyzed some of the primary quantitative data found on hard drives. For ease of reference, each paper was assigned a number (see list below), and each paper is discussed in the Summary below. Additional data are available on the accompanying DVD; see "Summary of Hard Drives and DVD".

The Investigation Committee conducted in-person interviews with Dr. De Domenico three times (7/02/2012, 7/17/2012, and 7/19/2012), Dr. Kaplan once (7/03/2012) and Dr. Diane Ward, a co-author once (7/16/2012). Dr. Ganz initially brought forward the allegations and self-identified to this Committee and allowed this Committee to convey this information to Drs. De Domenico and Dr. Kaplan during the investigation. Dr. Ganz was interviewed by telephone conference call (6/19/12). All interviews were audio-recorded, and written summaries were provided by Dr. Botkin's office and were made available to the interviewees for corrections and comments. In addition, the committee corresponded by email through Dr. Botkin's office with questions to Drs. De Domenico and Kaplan, as well as other collaborators in some of the studies. The interviewees fully cooperated with the investigation, and promptly provided written responses to questions conveyed through Dr. Botkin's office.

The committee provided numerous opportunities for Dr. De Domenico to provide supporting data for the papers being investigated, through three interviews and multiple email exchanges via Dr. Botkin's office. In response to a draft of this report, Dr. De Domenico expressed concern that she was not interviewed for a fourth time. In this instance, however, the Committee had decided that email exchange was a more efficient and appropriate venue through which Dr. De Domenico could identify specific computer files and other supporting documentation, and these email exchanges occurred (summarized in the reviews of papers 6 and 10). Given the requirement that researchers maintain the integrity of research data and records pertinent to publications, the absence of primary data can be seen as evidence for practices outside the standards in the field. Similarly, Dr. De Domenico expressed concern that she did not have an adequate opportunity to review the accompanying DVD that collated some of the files from her computer hard drive. Nevertheless, the existence of the summary DVD was clearly stated in the draft report (10/10/12) and Dr. De Domenico obtained it with sufficient time before her final response to the draft report, which she submitted on 12/10/12.

PAPERS EXAMINED

Paper 1: De Domenico I, Lo E, Yang B, Korolnek T, Hamza I, Ward DM, Kaplan J (2011). The role of ubiquitination in hepcidin-Independent and hepcidin-dependent degradation of ferroportin. *Cell Metabolism* Nov 2;14(5):635-46. Corresponding author: Jerry Kaplan.

Retracted: *Cell Metabolism* 15:927, 6 June 2012 "We, the authors, wish to retract [ ] "The Role of Ubiquitination in Hepcidin-Independent and Hepcidin-Dependent Degradation of Ferroportin" by De Domenico et al. (*Cell Metabolism 14*, 635–646; November 2, 2011; 10.1016/j.cmet.2011.09.008) because a number of errors have been detected in the assembly of the figures, and some of the original data were inappropriately removed from the laboratory. We stand by the validity of our studies; the data are reproducible, and the conclusions were not affected by the errors. However, we believe that the most responsible course of action is to retract the paper. We are preparing a new expanded version of this study for future submission. We deeply apologize to the community for the inconvenience".

Paper 2: De Domenico I, Lo E, Ward DM, Kaplan J (2009). Hepcidin-induced internalization of ferroportin requires binding and cooperative interaction with JAK2.
*PNAS,*106: 3800-3805. Corresponding author: Jerry Kaplan.
Correction: *PNAS* (2012), 109:7583-7586. One panel replaced in each of three figures: Figure 1; Figure 2A; Figure 3.

Paper 3: De Domenico I, Zhang TI, Koenig CL, Branch RW, London N, Lo E, Daynes RA, Kushner JP, Li D, Ward DM, Kaplan J (2010). Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. *Journal of Clinical Investigation* 20:2395-2405. Corresponding authors: Jerry Kaplan and Ivana De Domenico.
Corrigendum: *Journal of Clinical Investigation* (2012),122: 2326-2326 doi: 10.1171/JCI63977 stating that the anti-Fpn and anti-Jak2 rows in Figure 2A of Paper 3 were reprinted from Paper 2 without attribution. "The anti-Fpn row in Figure 2A was reprinted from I. De Domenico et al. (reference 10) without attribution. It represents the same row as was present in Figure 3B of that manuscript. The authors regret the error."

Paper 4: Koenig CL, Miller JC, Nelson JM, Ward DM, Kushner JP, Bockenstedt LK, Weis JJ, Kaplan J, De Domenico I (2009). Toll-like receptors mediate induction of hepcidin in mice infected with *Borrelia borgdorferi*. *Blood*,114:19 13-1918. Submitted March 6, 2009, accepted June 13, 2009. Corresponding author: Ivana De Domenico.

Paper 5: De Domenico, Vaughn MB, Yoon D, Kushner JP, Ward DM, Kaplan J (2007). Zebrafish as a model for defining the functional impact of mammalian ferroportin mutations. *Blood* 110:3780-3783. Corresponding author: Jerry Kaplan.
Corrected: "On page 3781 of the 15 November 2007 issue, there is an error in Figure 1. The panel depicting Fpn-GFP N144H in Figure 1B was inadvertently duplicated from another panel in the original published figure. The authors have replaced the panel with the correct image. The findings of the paper have not been affected by the error. The authors apologize to the editors and readers for this mistake. The corrected figure is shown."

Paper 6: De Domenico I., Vaughn, M.B, Paradkar, P.N., Lo, E., Ward, D.M. and Kaplan, J. (2011). Decoupling ferritin synthesis from free cytosolic iron results in ferritin secretion. *Cell Metabolism* 13, 57-67. Corresponding author: Jerry Kaplan
Retracted: "We, the authors, wish to retract "Decoupling Ferritin Synthesis from Free Cytosolic Iron Results in Ferritin Secretion" by De Domenico et al. (*Cell Metab.*, 13 (2011) 57–67,http://dx.doi.org/10.1016/j.cmet.2010.12.003 and "The Role of Ubiquitination in Hepcidin-Independent and Hepcidin-Dependent Degradation of Ferroportin" by De Domenico et al. (*Cell Metab.*, 14 (2011) 635–646,http://dx.doi.org/10.1016/j.cmet.2011.09.008) because a number of errors have been detected in the assembly of the figures, and some of the original data were inappropriately removed from the laboratory. We stand by the validity of our studies; the data are reproducible, and the conclusions were not affected by the errors. However, we believe that the most responsible course of action is to retract the paper. We are preparing a new expanded version of this study for future submission. We deeply apologize to the community for the inconvenience".

Paper 7: De Domenico, I., D. M. Ward, M. C. di Patti, S. Y. Jeong, S. David, G. Musci & J. Kaplan.(2007). Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. *EMBO J*, 26(12):2823-2831. Corresponding author: Jerry Kaplan

Paper 8: De Domenico, I., D. M. Ward, C. Langelier, M. B. Vaughn, E. Nemeth, W. I. Sundquist, T. Ganz, G. Musci and J. Kaplan (2007). "The molecular mechanism of hepcidin-mediated ferroportin down-regulation." *Mol Biol Cell* 18(7):2569-2578. Corresponding author: Jerry Kaplan

Paper 9: De Domenico, Ivana Diane McVey Ward, Givoanni Musci and Jerry Kaplan (2007). Evidence for the multimeric structure of ferroportin. *Blood* Mar 1;109(5):2205-9. Corresponding author: Jerry Kaplan

Paper 10: De Domenico I., Nemeth, E, Nelson, JM, Philips, JD, Ajioka, RS, Kay, MS, Kushner, JP, Ganz, T, Ward, DM and Kaplan, J (2008). The hepcidin-binding site on ferroportin is evolutionarily conserved. *Cell Metabolism* 8:146-156. Corresponding author: Jerry Kaplan

Paper 11: Kieffer, C., Skalicky, JJ, Morita, E, De Domenico, I, Ward, DM, Kaplan, J and Sundquist, W I (2008). Two distinct modes of ESCRT-III recognition are required for VPS4 functions in lysosomal protein targeting and HIV-1 budding. *Dev Cell* 15: 62-73. Corresponding author: Wesley Sundquist

SUMMARY OF FINDINGS

The Investigation Committee found multiple irregularities in ten of eleven publications. In the eleventh paper (Paper 4) a figure was inappropriately reproduced in Paper 3, but this issue was resolved with the journal by a published clarification. The Committee has categorized the various irregularities as: 1) extensive carelessness in figure preparation; 2) falsification and misrepresentation of data; 3) fabrication of data; and 4) alteration of the primary record. These irregularities are described in the following sections. The Committee does not feel that it can address the question regarding the reproducibility of the peptide-binding assay for hepcidin in Paper 10 from the information available, and a resolution will most likely require additional experiments. Please note that the points listed here are summaries, the evidence and figures for each point can be found in the subsequent sections, organized by the assigned paper number. The Committee is also providing a DVD with copies of relevant electronic files; see “Summary of Hard Drives and DVD”.

1. Extensive carelessness and negligence in data reporting: The publications in question exhibit a *systematic pattern of carelessness and reckless disregard for the fidelity of the primary data*, which in some cases led to misrepresentation of data in publications. Errors in figures containing images of western blots were especially common. There are multiple instances in which bands utilized in the generation of figures for publication were inverted, taken out of order, and spliced into other images. In some cases, the same western blot band images were utilized in multiple figures for different experimental conditions either in the same paper or in two different papers. In a few cases, other data were found or subsequently provided by Dr. De Domenico to support the conclusions of the figure, but it was unclear to the Committee why these data were not used in the publication instead of the erroneous, manipulated images. Because many of the films have undergone extensive relabeling, acknowledged by Dr. De Domenico, reconstructing the research record is now nearly impossible. In addition to the western blot examples, images of cells or embryos were reused and indicated as different treatments in different papers. The result of these errors was pervasive misrepresentation of the primary data in the published figures. Given the large number of such errors, the Committee concluded that there was a persistent pattern of reckless disregard for appropriate representation of primary data in the published papers. The Committee is also struck by the conspicuous failure of all coauthors, particularly Drs. Ward and Kaplan, to catch and correct these mistakes before publication.

2. Falsification and misrepresentation of data: In one instance, Fig. 5B of Paper 11, a set of three western blot bands were duplicated within the same panel to represent another set of samples. Unlike the examples described above, this manipulation is not easily explained as simple carelessness or systematic negligence, and the Committee concludes that it represents falsification of data. The Committee also investigated a general allegation that the error bars in numerous graphs were unusually small, given the experimental techniques used. The Committee carefully examined 12 such figures and, where possible, compared the published figures with computer files from which they appeared to be derived. In four examples, the error bars were found to be calculated from only two values, clearly contradicting statements in the figure legends and text indicating that they were calculated from a minimum of three or five measurements.

3. Fabrication of data: Circular dichroism (CD) spectra published in Fig. 4 of Paper 10 were found to be extensively and inappropriately manipulated. For two panels of the figure (inset to panel A and panel C), the Committee concludes that the published data were most likely fabricated by manipulation of data also used in panel A. In her response to the initial draft of this report, Dr. De Domenico provided CD spectra recently recorded by an independent laboratory. Though these data are apparently meant to refute the conclusions of the Investigation Committee, they are, in fact, significantly different from the published data and contradict the conclusions drawn in the paper.

4. Alteration of the primary record: As noted elsewhere, there are numerous inconsistencies among the published figures, labeled films and hand written research notes, making it difficult or impossible to reconstruct the research record. In some cases, however, the Committee found evidence that the record has been deliberately altered to hide misconduct or direct responsibility to others. In one instance, a western blot film for figures in Paper 1 appears to have been relabeled in such a way as to artificially create patterns of bands consistent with published figures. For another paper, Paper 6, the dates found on films and handwritten notes are several months after the corresponding figures were submitted for publication. The most serious of these instances is a set of handwritten notes related to the CD spectra in Paper 10 and provided to the Committee late in the investigation (on 9/11/2012). In these notes, Dr. De Domenico states that Dr. Ganz provided guidance, via communication through Dr. Kaplan, in the processing and analysis of the CD data. Dr. Ganz has directly contradicted this account and has provided an e-mail message (from Dr. Kaplan) that supports Dr. Ganz's contention that he first became aware of the CD data after the dates shown on the notes and after the figures were completed. Dr. Kaplan has confirmed the date and content of the e-mail message and has stated that he does not recall Dr. Ganz playing a role in analyzing the CD data. Dr. De Domenico has not provided any supporting documentation for her account, leaving the Committee to conclude that the notebook pages were most likely created to shift responsibility for misconduct to Dr. Ganz. These findings are summarized in the section titled "Alterations of the Research Record."

CONCLUSIONS AND RECOMMENDATIONS FOR SANCTIONS

Dr. Ivana De Domenico

Dr. De Domenico was the lead or corresponding author on 10 of the 11 publications examined in this investigation. The earliest of these papers was published in 2007 when Dr. De Domenico was a postdoctoral associate in Dr. Kaplan's laboratory, and the irregularities continued to 2011 after her promotion to Assistant Professor. The number and nature of errors are such that they - in aggregate - deviate from expected scientific practice in a fashion that is reckless. This pattern of recklessness is the primary basis on which we find the conclusion of scientific misconduct. The Investigation Committee takes "recklessness" to mean actions that are marked by lack of proper caution and care with respect to the consequences of the actions, and are therefore negligent and a breach of expected professional responsibility.

According to University policy (6.1.1 Rev 2, section 6)

> *"6. A finding of misconduct requires that:*
> *a. There is a significant departure from accepted practices of the research community for maintaining the integrity of the research record.*
> *b. The misconduct be committed intentionally, or knowingly, or in reckless disregard of accepted practices and*
> *c. The allegation is proven by a preponderance of evidence."*

This committee concludes that misconduct was committed by Dr. De Domenico, based on findings of:
a. Significant departures from accepted practices of the research community for maintaining the integrity of the research record.
b. The misconduct was committed in reckless disregard of accepted practices and
c. The allegation is proven by a preponderance of evidence.

The Committee recognizes from testimonies of Drs. Kaplan and Ward that Dr. De Domenico worked hard and is dedicated to science. The Committee also recognizes that external forces, deficits in training, and the enticements of rapid success in a highly competitive field might have led to hasty work and shortcuts. Nonetheless, the long and extensive record of reckless data manipulation uncovered in this investigation, as well as her minimization of the severity of the "irregularities" and lack of remorse for the errors during interactions with the Committee, indicate that Dr. De Domenico is unaware of, or indifferent to, the requirements for ethical representation of primary research data in publication. These patterns raised concerns about Dr. De Domenico's training and qualifications to be an independent principal investigator, without further supervision and other mechanisms in place to address these deficits and to ensure that such irregularities do not occur again.

Sanctions: Because diligent and ethical attention to the conduct of research and publication of results is central to the scientific endeavor, and the Committee found numerous instances of recklessness and significant deviation from accepted scientific practices, the Committee recommends that the University of Utah either immediately revoke or decline to renew Dr. De Domenico's present appointment as a tenure-track faculty member.

Dr. Jerry Kaplan

Dr. Kaplan was the corresponding author on most of the papers that were examined and was responsible for the training of Dr. De Domenico, who was a fellow until 2009. The Committee found no evidence that Dr. Kaplan directly contributed to the misrepresentation or manipulation of data. Nevertheless, there appears to have been a systematic lack of attention to detail on Dr. Kaplan's part, coupled with inappropriate and unacceptable distance from primary findings and their reduction to figures and other displays for publication that led to ignoring data or inconsistencies that were contrary to the preconceptions or conclusions of a paper.

While the Committee recognizes that a certain level of trust is essential among co-workers and supervised laboratory personnel in a collegial research environment, the Committee is concerned that Dr. Kaplan missed opportunities to recognize concerns about irregularities in Dr. De Domenico's laboratory practices, and to repair her training in a timely fashion. Perhaps most notably, on 3/13/2008, Dr. Ganz, who was then collaborating with the Kaplan lab, notified Dr. Kaplan that a figure sent to him by Dr. De Domenico contained inappropriately spliced and duplicated western blot lanes. Strikingly, a similarly falsified western blot image was included in another paper submitted for publication less than one month later (Paper 11, submitted 4/11/2008). Dr. Kaplan apparently failed to convey the gravity of this issue, and a lesson in professional ethics, to Dr. De Domenico. The Committee is concerned that this lax oversight reinforced a pattern of misconduct established during Dr. De Domenico's training period, which then continued as she moved toward an independent position.

It is important for Dr. Kaplan, as principle investigator, to take full responsibility for issues raised by the irregularities in the published data and systematic failure of supervision of Dr. De Domenico. In his response to the initial draft of this report, Dr. Kaplan states that he is "mortified by the errors", but the majority of his short response is devoted to his efforts to confirm the scientific conclusions of the papers, as opposed to the question of misconduct. Drs. Kaplan and De Domenico stated, in their response to the Inquiry Committee report, that several irregularities identified by that committee (involving Paper 6) were resolved by examination of research notes. The Investigation Committee reexamined these irregularities and concluded that they were not, in fact, so easily resolved. When asked about them by the Investigation Committee, Dr. Kaplan acknowledged that he had not examined the films and notes himself. We thus remain deeply concerned about Dr. Kaplan's responses to serious allegations of misconduct in his laboratory.

Given that the extensive irregularities of data in the published record have significant impact on the field, it would be appropriate for Dr. Kaplan to review the details, reliability and reproducibility of the experimental data in these papers, and if at all possible repeat key experiments for the scientific community. Dr. Kaplan has stated that he and Dr. Ward have spent substantial effort over the last year repeating the experiments in question.

The Committee recognizes that Dr. Kaplan is a leader in his field, and that the irregularities outlined in this report appear to be isolated to publications generated predominantly by Dr. De Domenico's work. It should be noted, however, that the Investigation Committee did not examine any reports on which Dr. De Domenico was not a co-author. In addition, the Committee recognizes that Dr. Kaplan has made substantial, selfless contributions, in leadership positions, to the enhancement of the training and research environment at the University of Utah. These activities are to be highly commended. Perhaps this high level of involvement, time and energy commitment to the research and training community led to distractions from the intricacies of laboratory supervision of Dr. De Domenico's work.

Sanctions: The extensive pattern of irregularities in the published data, and the failure of appropriate supervision of Dr. De Domenico, undermine the credibility of a senior leader and the reputation of the University. The Committee recommends that the University relieve Dr. Kaplan of his senior leadership duties. The Committee recommends that Dr. Kaplan's laboratory repeat the research experiments that have been brought into question, which Drs. Kaplan and Ward indicate is ongoing. It will be important for the University to provide careful scrutiny of the funded research that is specifically related to these projects. The Committee recommends that the University establish a scientific panel to review the newly repeated experimental results generated by Dr. Kaplan and Dr. Ward, including examination of the primary data and its appropriate documentation, and that this panel provide a scientific review of new papers or other publishable communications that might be submitted to either substantiate, modify or retract the findings in question.

Dr. Diane Ward

Dr. Ward was not a respondent of this investigation. Dr. Ward was directly involved in making figures for Dr. De Domenico's publications up to 2007, but after 2007, she relinquished her role in figure preparation. Dr. Ward was a coauthor on all the papers examined in this investigation, and was responsible for day-to-day training of Dr. De Domenico. During this period, Dr. Ward had a high level of trust in Dr. De Domenico's work. In generating figures, Dr. Ward performed unacknowledged splicing of western blot images, some of which were obvious in the figures. The Committee does not understand how Dr. Ward could have built the figures with bands upside-down and other obvious errors. The large number of irregularities and mistakes suggests that there was a lack of attention to detail, failure to examine the raw data, inadequate mentorship in protecting the fidelity of the data, and failure to insist on rigorous and clear assurance of the integrity of the data. The Committee found no evidence that there was intent to deceive or manipulate data on the part of Dr. Ward. Nevertheless, the sloppiness and carelessness in the production of the figures, and laxness in the examination of all of the primary data were disconcerting to the Committee and suggest a dereliction of responsibility to assure the accurate representation of primary data.

Recommendations: The Committee recommends that Dr. Ward examine the work flow and processes in the lab in order to ensure that mistakes in data representation do not occur in the future. Greater systematic efforts and mechanisms should be enacted to carefully and permanently mark, track and evaluate primary data, to clearly record multiple replications of experiments, and to assure that lab personnel are adequately trained and supervised in these practices. The Committee further recommends that Dr. Ward participate with Dr. Kaplan in the repetition of research experiments that have been brought into question, as outlined above. It will be important for the University to provide careful scrutiny of the funded research that is specifically related to these projects. The Committee recommends that the University establish a scientific panel to review the newly repeated experimental results generated by Dr. Kaplan and Dr. Ward, including examination of the primary data and its appropriate documentation, and that this panel provide a scientific review of new papers or other publishable communications that might be submitted to either substantiate, modify or retract the findings in question.

Dr. Tomas Ganz

Dr. Ganz was co-author on two publications in this case, Papers 8 and 10. Dr. Ganz voluntarily included these two papers in the broader group of allegations that he and his colleagues brought forward to the attention of journal editors and the University of Utah. As a co-author on two of the papers, and with areas of expertise that are complementary to Drs. De Domenico and Kaplan, it is disappointing that Dr. Ganz did not catch inconsistencies in the process of collaboratively assembling the papers for publication.

Dr. Wes Sundquist and Dr. Colin Kieffer

Dr. Sundquist was the corresponding author and Dr. Kieffer, a graduate student at the time, was the primary author on Paper 11. The Committee has determined that the altered and manipulated mages in Figure 5B were provided by Dr. De Domenico for this paper. The loading controls were inappropriately manipulated in the figure. The splicing and image duplication were subtle, and the Committee found no evidence that Dr. Sundquist and Dr. Kieffer were aware of the manipulations in advance of recent notification by Dr. Botkin's office. Given the subtlety of the image manipulation, the Committee finds that Dr. Sundquist and Dr. Kieffer might not be expected to have noticed the manipulations, and that they are not culpable for these problems in their paper. The Committee recommends that Dr. Sundquist repeat the experiment, and recently has been informed that repeat experiments are in progress, in collaboration with Dr. Ward. Regardless of the outcome, Dr. Sundquist should submit an erratum to the journal.

Dr. Michael Kay

Dr. Kay was included as an author on Paper 10 predominantly for his collaboration in providing the equipment and training required for the generation of circular dichroism (CD) data. Given that the process of generating the CD figures was one of the most grievous examples of scientific misconduct found in this case, the Committee is concerned that Dr. Kay did not sufficiently examine the purported primary data and purported multiple replications of raw data, which would have uncovered the inconsistencies and data manipulation. As the co-author specifically associated with the CD experiments, Dr. Kay must accept responsibility for not catching the falsification and fabrication that is apparent in this set of analyses. There appears to have been minimal discussion among the coauthors on the appropriate use of this experimental approach. In addition, as discussed in detail in the Committee's analysis of Paper 10, there appears to have been little or no discussion among the various collaborators in the structural analysis, especially between Drs. Kay and Skalicky, regarding possible inconsistencies that might be apparent in comparing CD data and NMR data. Given the expertise of these two individuals, it is unlikely that the contradiction between the CD and NMR data would have gone unnoticed if they each had been fully aware of both sets of results. The circumstances leading to the publication of falsified and fabricated data indicate flaws in the management of the laboratory practices and collaborations.

The Committee recommends that in the future Dr. Kay exercise adequate oversight of collaborative projects, that he fully evaluate the raw data and results for which he has cognizant scientific expertise, and that he provide assurances that the data are appropriately represented in publication.

Journal Reviewers

The Committee takes this opportunity to recognize that the manuscript review and editorial process in some of these publications were deeply flawed. In retrospect, it is astonishing that reviewers of *Cell Metabolism* Paper 6 did not recognize some of the very obvious errors in the western blot figures, for example, an unequal number of lanes in a row of experimental samples compared to a row of loading controls immediately below the experimental samples (Fig. 7B) and mislabeled molecular weight standards (Fig. 4B).

Procedural Concerns

Lastly, the Committee is obligated to point out some aspects of the process that hampered our investigation. First, there appears to be a gap between the time when an editor of the journal *Cell Metabolism* contacted Dr. Kaplan (October 28, 2011) and when Dr. Botkin's office sequestered data notebooks (November 9, 2011). Some of the primary data may have been altered during this period in response to allegations. Second, some of the electronic data available on computer hard drives was not sequestered until July. The Committee requested that Information Technology (IT) experts evaluate the hard drive data for deleted or altered files, but their evaluation indicated that this was not possible. Third, additional allegations arrived late in the investigation process (June 28, 2012), which prompted further investigation and delayed the process and the date of this final report. In spite of these procedural concerns, the Committee found numerous and compelling examples of manipulated images, data fabrication, a lack of experimental repetitions, and other substantive errors in the publications in question.

OTHER RECOMMENDED ACTIONS

1. The data in Figure 4A in Paper 4 appear to be correct as labeled. The duplication and misrepresentation were in Paper 3, the PNAS article. This conclusion should be communicated to Dr. Cynthia Dunbar, the editor of *Blood*.

2. Regarding Paper 9, the Committee could not conclude that the flow-through samples were processed at the same time as the input/eluate samples, as requested by Dr. Dunbar the editor of Blood. The films were not labeled with dates and there were inconsistencies in the hand labeling of films and scans. Therefore, the Committee finds that the conclusions for this experiment are not substantiated in a manner consistent with accepted practices in the field. This conclusion should be communicated to Dr. Dunbar. Dr. Kaplan has requested that this action wait for repetition of the experiments. In this case, if the results are consistent with the published conclusion, the published figure should be replaced by a figure generated by accepted practices in the field.

3. The Committee recommends that Paper 10, which appears to contain falsified and fabricated data, be retracted from the journal *Cell Metabolism.*

4. The Committee finds that Fig. 5 of Paper 11 (*Developmental Cell*) contains several instances of image manipulation, data fabrication and/or misrepresentation, and recommends that the experiments in this figure be repeated and the figure formally corrected or retracted in the journal. These data were collaboratively provided to Dr. Wes Sundquist and Dr. Collin Kieffer, who were the primary authors of the paper. The Committee recommends that Dr. Sundquist take the lead in contacting the journal in a timely fashion, coordinate with Dr. Botkin in notifying the journal of the conclusions (confined to this paper) of this Committee investigation, negotiate an appropriate resolution with the journal to extirpate the inappropriate figure from the published record, and keep Dr. Botkin and the other authors informed of the outcome. Beyond those actions, Dr. Sundquist and Dr. Kieffer are acknowledged for their rapid response to queries from this Committee.

5. The Inquiry Committee recommended that all of the publications co-authored by De Domenico and Kaplan be reviewed independently for irregularities. The Investigation Committee has reviewed some of these publications. The Investigation Committee recommends that Dr. Kaplan and Dr. Ward thoroughly examine all of the publications co-authored with Dr. De Domenico, searching for misrepresentations and other irregularities. The Investigation Committee further recommends that Dr. Kaplan and Dr. Ward inform the Office of Associate Vice President for Research Integrity and the relevant journals if additional irregularities are found.

PAPER 1

Citation: De Domenico I, Lo E, Yang B, Korolnek T, Hamza I, Ward DM, Kaplan J. (2011). The Role of Ubiquitination in Hepcidin-Independent and Hepcidin-Dependent Degradation of Ferroportin. *Cell Metabolism* Nov 2;14(5):635-46

Background
The original allegation identified by the complainant included inappropriately manipulated images (Figure 1), reuse of images in the same publication (Figure 1C, Figure 2Aii and Figure 2B, Figure 1D and Figure 3) and errors bars in a ferritin ELISA (Figure 7B) that appear too small to reflect the error of the technique and stated number of replicates. The Inquiry Committee met with Dr. De Domenico on 6 January 2012 and Dr. Kaplan on 17 January 2012 to discuss these concerns. A second meeting with Dr. De Domenico was held on 7 February 2012. Dr. De Domenico was asked to provide the primary data (gel scans and films) for the figures in question and explain how the scanned images were used to prepare the published figures. Dr. De Domenico stated that one person did the experiment, a second person scanned the data and she used the scanned images to make the figures. On further analysis of the original data, the Inquiry Committee discovered additional errors, which included inappropriately manipulated and reused images (Figure 2Aii and Figure 5), flipped data panels (Figure 5) and missing original data (Figure 1A and Figure 3). Dr. De Domenico stated that all of the figures for this paper were prepared by her.

In response to the allegations, Drs. De Domenico and Kaplan stated that they "do not dispute any of the allegations" except those regarding the size of the error bars in the ferritin assays". They further stated that "the binary nature of the data (band or no band) and the symmetry of the panel lead to erroneous figures but did not lead to misrepresentation of the data. These mistakes were made in the assembly of the figures for publication, the conclusions of the figures are correct. All the previous publication and discoveries by Dr. De Domenico and Dr. Kaplan were validated by publications from others".

Paper 1 was retracted in Cell Metabolism 15:927, 6 June 2012 with the statement: "We, the authors, wish to retract [ ] "The Role of Ubiquitination in Hepcidin-Independent and Hepcidin-Dependent Degradation of Ferroportin" by De Domenico et al. (Cell Metabolism *14*, 635–646; November 2, 2011; 10.1016/j.cmet.2011.09.008) because a number of errors have been detected in the assembly of the figures, and some of the original data were inappropriately removed from the laboratory. We stand by the validity of our studies; the data are reproducible, and the conclusions were not affected by the errors. However, we believe that the most responsible course of action is to retract the paper. We are preparing a new expanded version of this study for future submission. We deeply apologize to the community for the inconvenience".

The Investigation Committee decided to reexamine the original gel films and scans in Dr. De Domenico's notebooks and computer hard drive to determine the source of errors in the published figures.

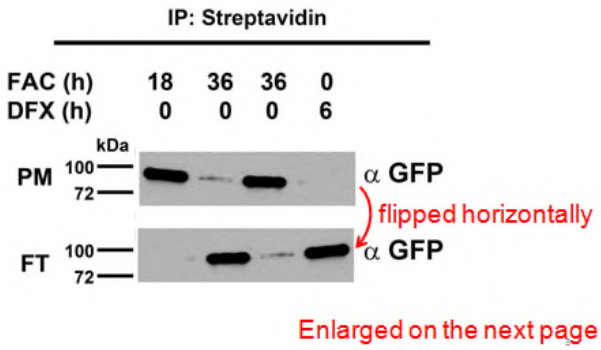
Figure 1A Allegation
The Inquiry and the Investigation Committees could not find the original data for this figure.

Figure 1C Allegation

Gel scans were duplicated in PM and FT samples. The PM scan was flipped horizontally and used for the FT sample. Figure 1C is an annotated copy from the Inquiry Committee report.

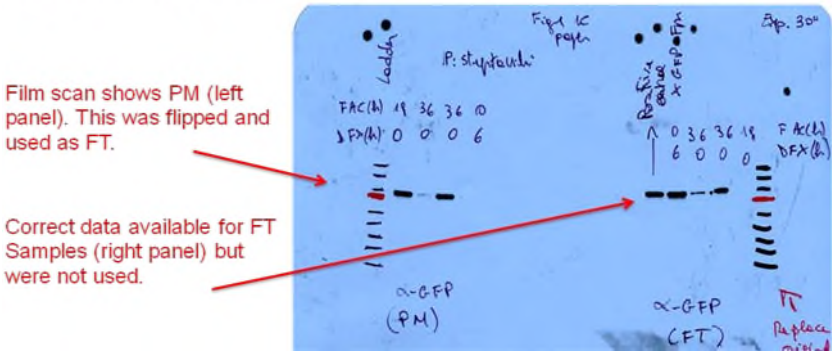
Figure 1C

• Top panel reused for the bottom panel



Enlarged on the next page

Fig. 1C from original data in Dr. De Domenico's notebook

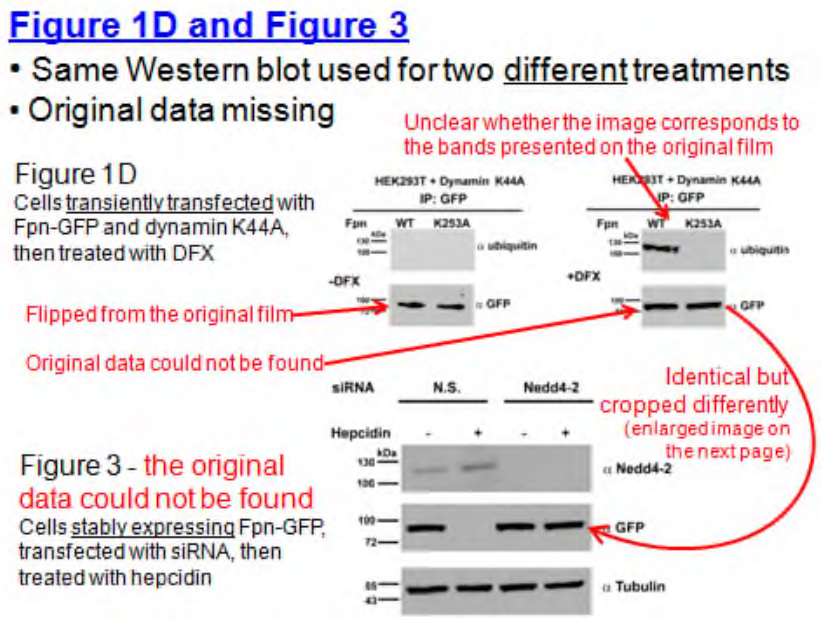


Investigation Findings: A film was found in the laboratory notebook that contains the PM samples used for the published figure. The film also contained FT samples that appear convincing.

Dr. De Domenico's Response: Dr. De Domenico presented the original gel film for Fig. 3 during an interview on 17 July, 2012. She stated in a PowerPoint document that "I now realize that the film was flipped during figure preparation. Three different individuals participated in these experiments. Unfortunately, the film was not scanned by me or who ran the experiment".

Conclusion: The Investigation Committee concurs with the Inquiry Committee that the PM sample was flipped and reused for the FT samples. It is unclear why the FT samples on the original film were not used for publication. The error in this figure suggests carelessness in figure preparation.

Figure 1D and Figure 3 Allegations
The same GFP Western blots in Figs. 1D and 3 are used for different experimental treatments. The GFP blot in Fig. 1D (right panel) is identical to two lanes in the middle panel in Figure 3. In addition, the GFP blot in Fig. 1D (left panel) is flipped from the original film. Figures 1D and 3 are annotated copies from the Inquiry Committee report.

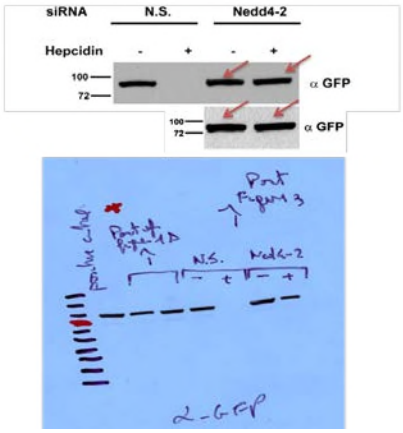


Investigation Findings: The Investigation Committee concurs with the Inquiry Committee's report that the same Western blot was used for two different treatments in Figures 1D and 3, and that the published data have no relationship to the original data. The Investigation Committee also could not find the primary data for Figs. 1D and 3.

Dr. De Domenico Response: In an interview with Dr. De Domenico on 17 July 2012, she presented a PowerPoint document to explain how the Western blot lanes were duplicated in Figs. 1D and 3. Dr. De Domenico showed a gel scan containing data for both Fig. 1D and Fig. 3 (see below). The scan is labeled "Part of figure 1D" and "Part Figure 3". In this document she stated "As shown in the original blot (left bottom) the bands belonging to Fig. 1D were next to the bands belonging to Fig. 3 (see red asterisk on the film). The gel was scanned before the lanes were clarified. **"**I now realize that the film was flipped during figure preparation. Three different individuals participated in these experiments. Unfortunately, the film was not scanned by me or who ran the experiment".

Figure 3

Figure 1D



Investigation Conclusion: The lanes labeled "Part Figure 3" in the gel scan appear to be a repeat of the published experiment, but it is not the data used for publication. Similarly, Figure 1D data are not the data used for publication. Given that the original data for Figs. 1D and 3 could not be found, this cast doubt on the accuracy of the published data.

Dr. De Domenico's Response to Missing Original Data in Figures 1A, 1D and 3: In a PowerPoint document presented to the Committee on 17July 2012, Dr. De Domenico stated that "I have provided to the committee some repeats of all experiments and most importantly of the experiments were the original films are missing because were kept in the books that the technician Mrs. Nazzicone threw away". I want to pint [point] out that the lost of these laboratory's notebooks was reported to the Human Resource Office before these concerns were communicated to us. These emails were given to Dr. Botkin"**.**

Figure 2 Allegations

There are several irregularities in this figure. First, the same Western blots are used for different experimental treatments. The top panels in Fig. 2Aii are duplicated and used for the top panels of Fig. 2B. The original data for these panels could not be found. Second, the lower left panels in Fig. 2Aii match the two right lanes in the upper panel in Fig. 5. Figure 5 panels are cropped and flipped and a lighter exposure is used for Fig. 2Aii. Third, the top panels in each figure are matched to different bottom panels unrelated to the experiment. The data in Figs.2Aii, 2B and 5 have no relationship to what is labeled in the figure. Figures 2Aii, 2B and 5 below are annotated copies from the Inquiry Committee report.

Figure 2Aii and 2B

- Published images do not correspond to original films
- Same Western blots used for different treatments

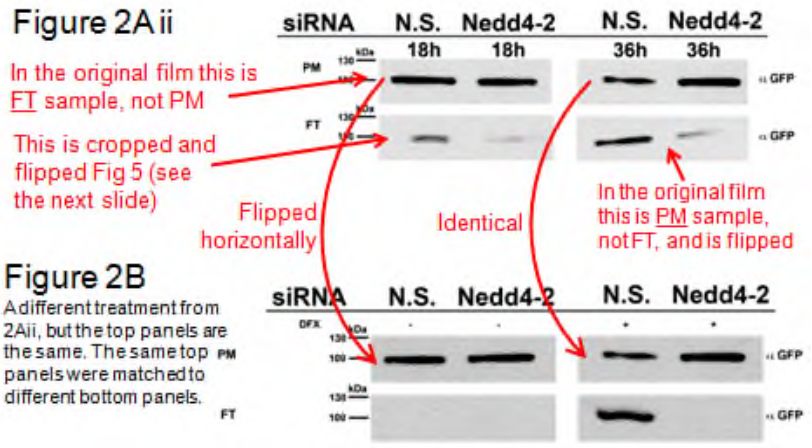
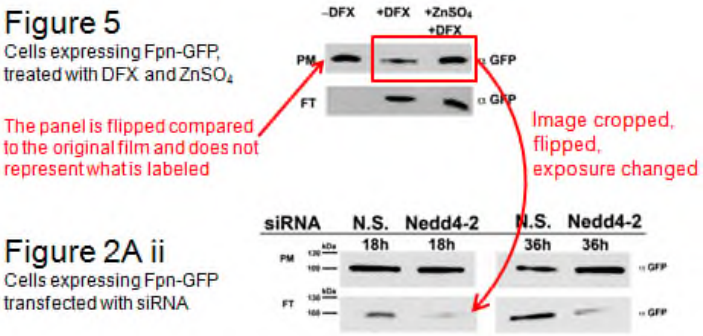


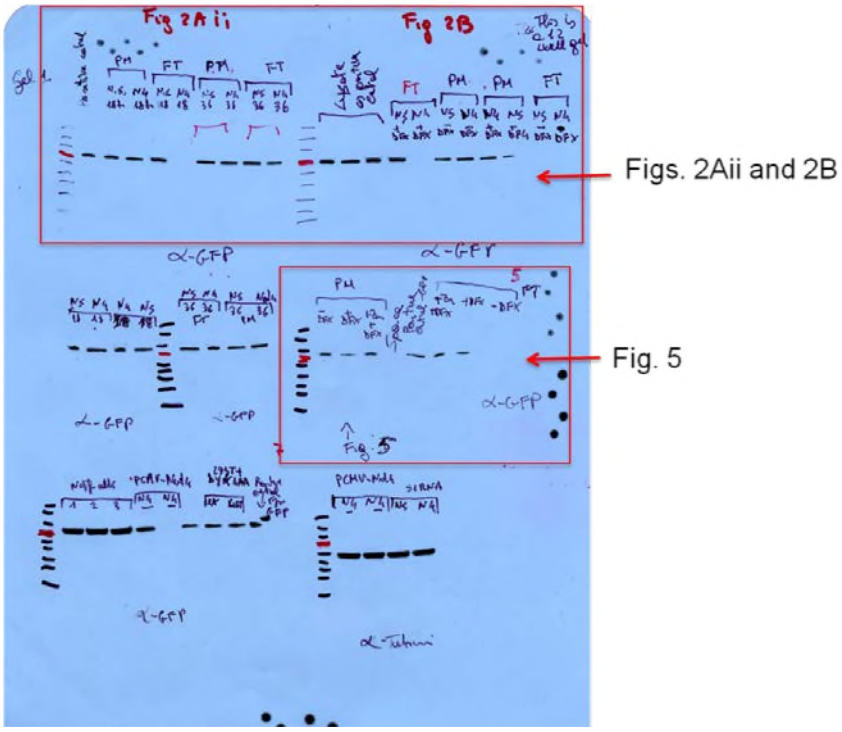
Figure 2A ii vs Figure 5

- Published image does not correspond to original film
- Same Western blots used for different treatments



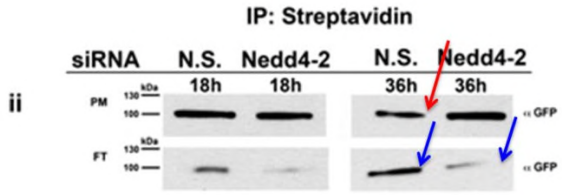
Investigation Findings: In a document to the Inquiry Committee, Dr. De Domenico provided a gel scan containing data for Figures 2Aii, 2B and 5 (large film below labeled “Gel scan provided by Dr. De Domenico”). To evaluate the data in Figure 2Aii, the Investigation Committee reconstructed the data labeled “Fig 2Aii” in the film and compared it to the published Fig. 2Aii (below). A reconstruction of the original data for Figure 2Aii reveals that the reconstructed figure differs from the published figure. The reconstructed figure does not show a reduction in the biotin-labeled Fpn-GFP in the 36 h "N.S." sample (red arrows) and a much smaller difference between the 36 h flow-through (FT) "N.S." and "Nedd4-2" fractions (blue arrows). The result stated in the paper “In cells treated with control oligonucleotides, Fpn-GFP was degraded 36 hr post expression (Fig. 2A, panel ii)” is thus not supported by the original film scan. The published data shows a greater effect compared to the original data. Figure 5 was found on the film (large film below), and corresponds to the published figure with the exception that the PM upper panel was flipped horizontally compared to the original film and does not represent the published figure.

Gel scan provided by Dr. De Domenico

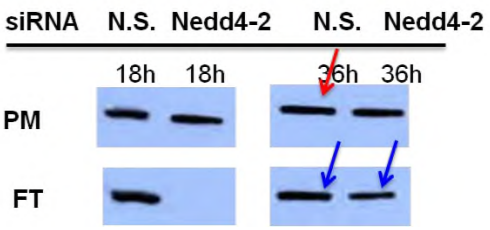


Committee's reconstruction of Figure 2Aii from the original data

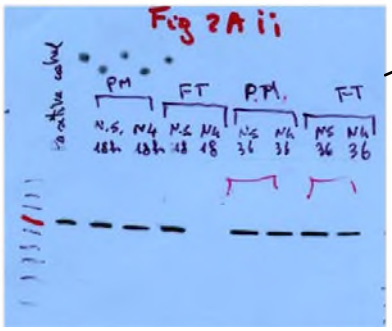
Published Figure 2Aii



Committee's reconstruction of Fig. 2Aii from the original scan below



Original notebook scan for Fig. 2Aii cropped from large film above



Dr. De Domenico's Response to Figure 2 Allegations: In response to the errors in these figures, Dr. De Domenico provided a PowerPoint document to the Committee on 17 July 2012 that stated "Again, three different individuals participated in these experiments. The order of the gel loading was not "linear" and I did not keep the labels clear when generating the figure and the scan was flipped."

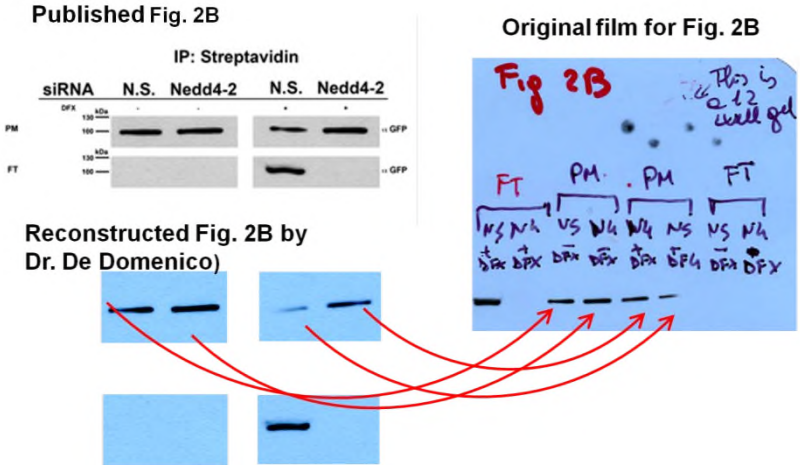
The Committee questioned Dr. De Domenico further regarding the inconsistencies found in Fig. 2Aii. Dr. De Domenico was shown the Committee's reconstructed version of the original notebook scan for Figure 2Aii. Dr. De Domenico asked "where the reconstructed figure came from" and the Committee replied "that it was made from the gel scan that she provided to the Inquiry Committee". The Committee stated that there is a "pronounced difference in the intensity in the publication [compared with the original data] and "that the published version shows a bigger effect than what was in the original gel". Dr. De Domenico was asked if this "is a misrepresentation" and she "agreed on this point". The Committee also noted that Fig. 2Aii was flipped and that Fig. 2B has the same lanes, and so it misrepresents the text. More importantly, "the primary data do not represent a strong case". Dr. De Domenico said "that she went to the repeats and is sure that it has been validated".

In response to the Committee's reconstruction of Fig. 2Aii, Dr. De Domenico subsequently showed the Committee's reconstructed Fig. 2Aii and compared it to the original gel scan (PowerPoint file provided on 17 July). She stated that "one Committee member reconstructed Fig. 2Aii using a higher exposure film" as compared to the published experiment". (The higher exposure is most likely due to Dr. De Domenico's photographing of the Committee's paper copy of the reconstructed gel scan). Even though the figure is a higher exposure, the published version still shows a better outcome than the original gel.

Dr. De Domenico's revised her response in the 2 July 2012 meeting minutes to indicate that she did not agree with her response to the question as to whether Figure 2Aii "is a misrepresentation". She revised the text to indicate that ""she does not think [so] I agree on this [is a misrepresentation] because Dr. Goldenberg reconstructed figures was generated with a different film".

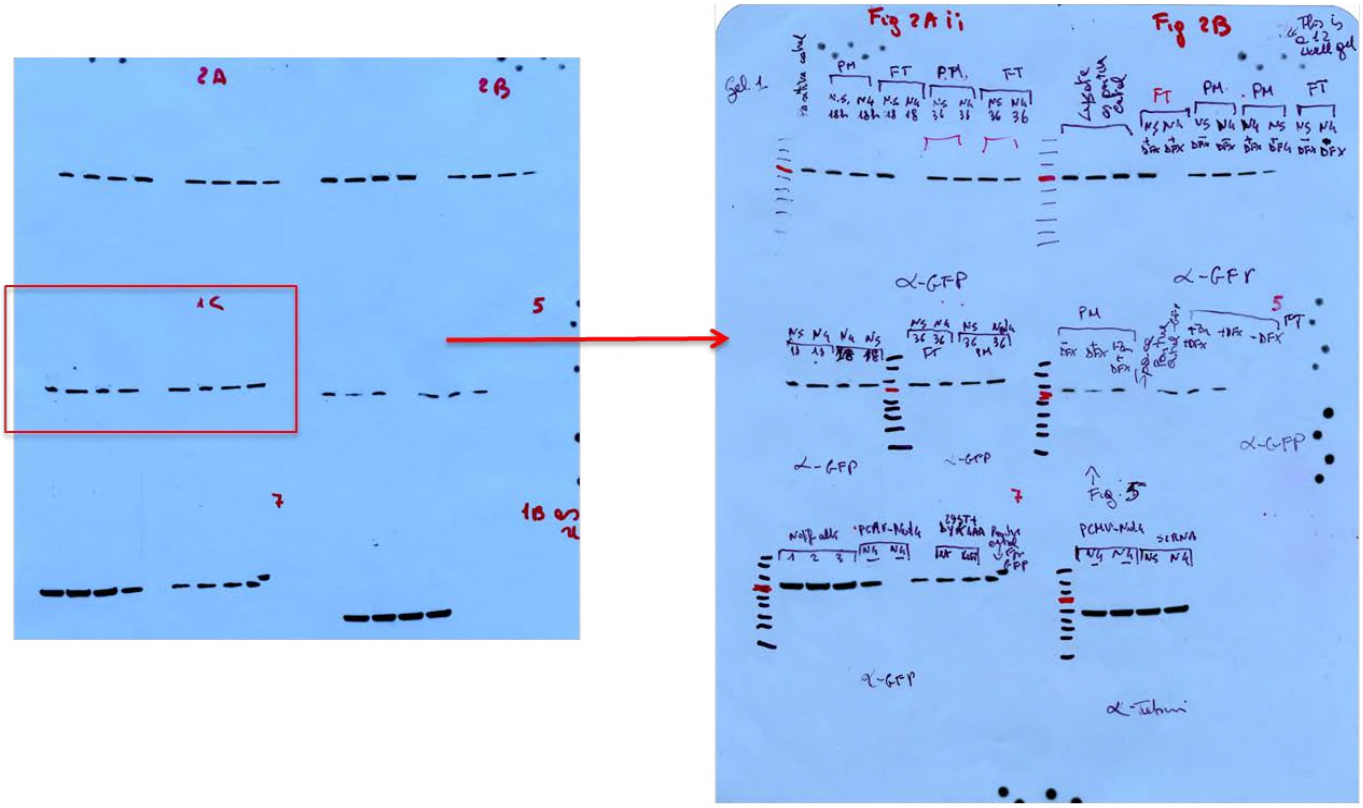
Regarding irregularities in Fig. 2B, Dr. De Domenico provided a film containing an experiment labeled Fig. 2B (cropped from the large film previously shown) and reconstructed the data for comparison to published Fig. 2B (below). Dr. De Domenico stated in a PowerPoint document that the reconstructed figure shows that "Not [a] misrepresentation of the data". In other words, Fig. 2B shows the same result as the published data. It is unclear to the Committee why this data was not used for publication.

Fig. 2B from original data in Dr. De Domenico's notebook compared to published Fig. 2B



Additional Irregularities in Figure 2 Identified by the Investigation Committee

An earlier image of the film for Figures 2Aii, 2B and 5 (file 1-2-1, dated 20 July 2011) was found on the hard drive of Dr. De Domenico and is shown below alongside the version presented to the Inquiry Committee:



Create Date: 2011:07:20 12:09:34-06:00

Create Date: 2011:10:29 13:23:02-06:00

The labels on the earlier version suggest that the blots were to be used for Figs. 1B, 1C, 2A, 2B, 5 and 7. While the original labeling suggests that the middle blot on the left-hand side was for the experiment shown in Fig. 1C, the later labeling indicates that it is a repeat of the experiment shown in Fig. 2Aii. The later labeling also indicates that roughly equal amounts of ferroportin were detected in both the plasma membrane and cytoplasmic fractions. This is not consistent with the results shown in the published versions of either Fig. 1C or 2Aii. In addition, Fig. 7 has no relationship to the published figure, and lane 7 in this figure is unlabeled.

Examination of the film for Figures 2Aii, 2B and 5 also raised concerns regarding possible alteration of the research record. Particular attention was focused on the blot for Figure 2B, which is labeled as coming from a 12-lane gel. The Committee noted that the spacing of lanes in this blot is indistinguishable from those of the other blots on this film, which are purported to represent 10-lane gels. The Committee questioned Dr. De Domenico on this point and was told that the discrepancy was due swelling of the 12-lane gel, but not the 10-lane gel. As discussed in detail elsewhere in this report (see Section on Post-Allegation Irregularities), the Committee is unconvinced by this explanation and believes it likely that the film was deliberately mislabeled in order to show the results desired by the authors.

Conclusion

The Committee concurs with the Inquiry Committee report that Western blot images were reused for different experimental treatments and that the published figures have no relation to the primary data.

The Committee also identified irregularities in the film provided by Dr. De Domenico as the primary data for Figures 2Aii, 2B and 5. The labeling of the film for Fig. 2B is of particular concern. As discussed in detail above, the spacing on the film matches closely that of a 10-well comb, but the spacing of a 12-lane gel must be expanded by a full 20% in order to match the film. The Committee concludes that these labels most likely represent a falsification of data and that the labels on other blots (e.g. Figs. 1C and 7) on this film may have been falsified as well. Specifically, we suggest that the labels were configured to place the ones indicating reduced protein concentrations in the flow-through fractions at positions where either no sample or molecular weight markers were loaded.

It should be noted that the relabeled film was originally provided by Dr. De Domenico to the Inquiry Committee as the primary data for the experiments shown in Figs. 2Aii, 2B and 5, and to demonstrate that the correct data were consistent with those published.

The earlier scans of the film found on Dr. De Domenico's computer (files 1-2-1 and 1-2-2) also raise doubts about the original explanation provided for the appearance of the same gel bands in different figures (Figs. 2Aii, 2B and 5). This and other cases of mislabeled gel images were explained by Dr. De Domenico as arising because the initial markings on the films were removed before scanning. In this case, however, the scanned image is clearly labeled by the figure numbers for which the individual blots are to be used. While the individual lanes might have been mixed up, it is very difficult to understand how the experiments could have been, unless Dr. De Domenico deliberately chose to reuse the images that best fit the expected outcomes. If, as suggested above, the labeled film represents a fabrication, then we must also conclude that Dr. De Domenico deliberately misled both the Inquiry and Investigation Committees. When the issues outlined above were raised during interviews with the Committee, Dr. De Domenico adamantly denied that there had been any relabeling of the films. Dr. De Domenico was specifically asked to state when the hand labeling was applied to the films, and she indicated that the labels were placed contemporaneously with the generation of the data and the use of the films for generation of figures for publication, not after concerns or allegations were brought forward.

Figure 7B Allegation

The original allegation regarded errors bars in a ferritin ELISA (Fig. 7B and in other publications) that appear too small to reflect the error of the technique and stated replicates. The Inquiry Committee report stated that they asked Dr. Greg Stoddard (Co-Director of the University of Utah Biostatistics Center) to analyze the primary data in Figure 7B. He concluded that "data in the laboratory notebooks, the figures, both the height of the bars in the bar charts, representing the mean, as well as the length of the error bars, representing the standard errors, were correctly drawn". However, he also questioned whether the data are actually from "independent" experiments as reported in the paper. The committee recommended that a "more complete assessment of the accuracy of the graphical data is warranted".

Investigation Findings: The Committee examined Dr. De Domenico's computer hard drive for files related to Figure 7B. One SigmaPlot file (filename: Notebook1.JNB, December 17, 2010) was found for this figure that includes the error bars, but no replicate data or any indication of where the error bars come from. The description of data analysis for the ELISA in Figure 7B in the published paper states that "Ferritin levels were determined by ELISA as described previously (De Domenico et al.

2007a). All experiments were performed a minimum of three times. Data are represented as mean standard deviation (SD)".

Conclusion: The Committee could not determine whether the data originated from "independent replicates" as reported in the paper or from replicate measurements from single experiments.

Summary

The Investigation Committee concurs with the Inquiry Committee report that found duplication of data in Figure 2Aii and Figure 2B, Figure 2Aii and Figure 5, and Figure 1D and Figure 3. We conclude that the published data have do not represent the original data. The Committee also found that the data in published Figure 2Aii shows a better outcome compared with the primary data.

The Committee concludes that the film for Figures 2Aii, 2B and 5 was deliberately mislabeled in order to show the results desired by the authors. This suggests that the primary record was altered and that the labeled film is falsified.

The original allegation regarding the small size of error bars in an ELISA could not be addressed from the Committee's investigation because files containing replicate data from additional experiments could not be found Dr. De Domenico's computer hard drive.

The Committee's findings support the retraction of this manuscript.

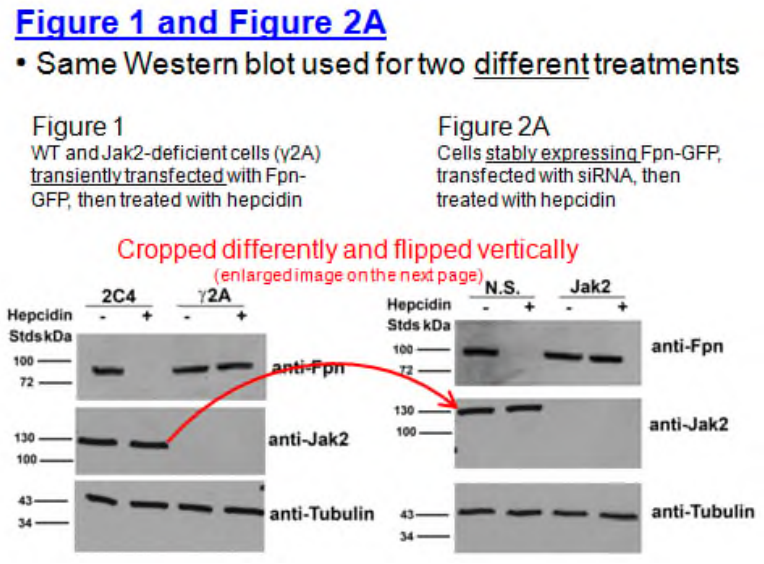
PAPER 2

Citation: De Domenico I, Lo E, Ward DM, Kaplan J. Hepcidin-induced internalization of ferroportin requires binding and cooperative interaction with JAK2. *Proceedings of the National Academy of Sciences USA* 2009; 106: 3800-3805
Submitted December 31, 2008, accepted January 14, 2009. Corresponding author, Jerry Kaplan.

Background: The allegations specifically related to Paper 2 involve Figures 1, 2A, 3B, and 4A and potential duplication and misrepresentation of data, and were previously examined by the Inquiry Committee. The Inquiry Committee's final report was reviewed by the Investigation Committee. Additional allegations of duplication of data in Paper 2 and in Papers 3 and 4 were also raised and were examined by the Inquiry Committee; these issues are discussed separately in summaries of Papers 3 and 4.

The Investigation Committee interviewed Dr. De Domenico on 7/02/2012, 7/17/2012, and 7/19/2012 and specifically reviewed allegations and details related to Paper 2 with her in the meetings on 7/02/2012 and 7/19/2012. In addition, the Investigation Committee discussed allegations and details related to Paper 2 in interviews with Dr. Kaplan (7/03/2012) and Dr. Ward (7/16/2012).

Individual Figure Irregularities
Figures 1 and Figures 2A in Paper 2: The Inquiry Committee concluded that the same western blot image was used in different figures indicating two different treatments, and were cropped differently and flipped vertically in Figures 1 and 2A. Annotated copies of these figures with arrows indicating the irregularities and enlarged versions of some of the blots were included with their final report. Figures from the Inquiry Committee report are reproduced here (see below). After review of the Inquiry Committee's findings and interviews of Dr. De Domenico, Dr. Kaplan, and Dr. Ward, the Investigation Committee agrees with the conclusion that the same blot was shown in panels in Figure 1 and 2A of Paper 2 and was identified as resulting from analysis of lysates from 2 different cell lines. **This represents duplication of data in the same report.**



(Reproduced from the Inquiry Committee report)

Responses from the authors to the findings regarding Figures 1 and 2A: The Inquiry Committee's conclusion was not contested or rebutted by Dr. Kaplan and Dr. De Domenico in their response letter on 2/23/2012. In a meeting with the Investigation Committee on 7/02/2012 Dr. De Domenico stated that she believes that the duplication occurred when the gels were scanned. In interviews with the Investigation Committee Dr. Kaplan (7/03/2012) and Dr. Ward (7/16/20120) provided no additional insights on how the irregularities may have occurred. In a powerpoint document provided to the Investigation Committee by Dr. De

Domenico in conjunction with her meeting with the Committee on 7/17/2012, she offered the defense that "I believe that the film was flipped during figure preparation. Both experiments are in the same film next to each other (see arrow). The film was not scanned by me." A photocopy identified as a copy of the original film with red arrows pointing to different blots on the film was included. No date or other identification can be seen on this photocopy.

A correction was published in the journal (*PNAS* 2012; 109: 7583-7586.) The correction states that "The authors note that several figures appeared incorrectly." They further state that "In Figure 1, the anti-Jak2 panel was replaced due to errors in preparing the figure for publication." A similar statement was made for Figure 2A. In the meeting with Dr. De Domenico on 7/19/2012, the Investigation Committee inquired about the data and new figures used in the published correction to Paper 2. Dr. De Domenico stated that the journal asked them just to replace the panels that displayed erroneous data in the original publication, and outlined how this was done. Nevertheless, she also subsequently included a copy of an email with instructions for correction from the *PNAS* editorial office (from Daniel Salsbury; 9 March 2012) in a powerpoint file (7/23/2012). These instructions say that "…the correction will reproduce the entire figures, not just the panel(s) in question." Dr. De Domenico's 7/23/2012 powerpoint file also contained a list saying that the corrected versions of Figures 1 and 2A were generated from frozen samples that were the same as those used in the original figures. It appears from inspection of the original and published corrected figures that only the middle panels of the western blot portions of figures 1 and 2A, labeled anti-Jak2 in each case, were replaced. In her interview with the Investigation Committee on 7/16/2012, Dr. Ward stated that she did not construct the figures for the *PNAS* correction, but that she saw the data and that she and Dr. Kaplan made sure that Dr. De Domenico had replicates.

Figure 3B and 4A in Paper 2: The Inquiry Committee concluded that the same western blots were used in two different treatments with two different antibodies, and that panels in Figures 3B and 4A were identical but cropped differently. The Inquiry Committee report noted that the original data could not be found and may have been lost with the notebooks that were taken from the lab. Annotated copies of the figures with arrows indicating the irregularities and enlarged copies of blots were included. Figures from the Inquiry Committee's report are reproduced here (see below). After reviewing the findings of the Inquiry Committee, meeting with Dr. De Domenico on 7/02/2012 and 7/19/2012, and reviewing powerpoint files that Dr. De Domenico supplied, the Investigation Committee agrees that the same western blots were identified as resulting from detection with two different antibodies (anti-Jak2, anti-Fpn) and displayed as outcomes of two different experiments in Figures 3B and 4A. **This represents duplication of data in the same report and misrepresentation of data.**

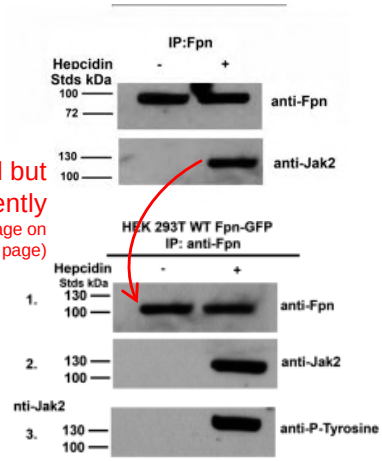
Figure 3B and Figure 4A

• Same Western blot used in two different treatments for two different antibodies

Figure 3B
Primary mouse bone marrow derived macrophages, treated with hepcidin
Blotted for Jak2

Identical but cropped differently
(enlarged image on the next page)

Figure 4A
HEK293 cells transiently transfected with Fpn-GFP and Dynamin K44A then treated with hepcidin
Blotted for Fpn



(Reproduced from the Inquiry Committee report)

Responses from the authors: The original allegations and the Inquiry Committee's conclusion were not contested by Drs. Kaplan and De Domenico in the 2/23/2012 response letter. In her meeting with the Investigation Committee on 7/02/2012, Dr. De Domenico said that she thinks Figure 4A is wrong and Figure 3B is correct. She was asked how this happened and replied that she thinks this was "a crop of the same band". No additional insight into this irregularity was provided by Dr. Kaplan in his meeting with the Investigation Committee on 7/03/2012 or by Dr. Ward in her meeting with the Committee on 7/16/2012. No additional defense or comments were included in the powerpoint document provided to the Investigation Committee by Dr. De Domenico in conjunction with her meeting with the committee on 7/17/2012.

A published correction (*PNAS* 2012; 109:7583-7586), mentioned above, stated that "Fig. 3B was replaced due to errors in preparing the figures for publication." A similar statement was made for Figure 4A. In her interview with the Investigation Committee on 7/19/2012, Dr. De Domenico stated that both the anti-Jak2 and anti-Fpn panels were replaced in Figure 3B; it is unclear from the written summary of the meeting that she stated that more than one panel was reproduced in Figure 4A. Inspection of the published corrected figure indicates that both panels in Figure 3B were replaced and that the top panel, labeled anti-Fpn, was replaced in Figure 4A. In the 7/23/2012 powerpoint file supplied to the Investigation Committee by Dr. De Domenico, a list indicates that "a repeat available in the lab was used to generate all the panels" in Figure 3B and that a gel was run using frozen samples that were the same as those used in the original figure to generate corrected 4A. Dr. Ward commented on the figures used for the correction in her meeting with the committee on 7/16/2012, as noted above.

Conclusions from the Investigation Committee's findings: In summary regarding the allegations related to Figures 1, 2A, 3B, and 4A in Paper 2, the Investigation Committee concludes that there was duplication and misrepresentation of data in this report. We were unable to establish a cogent explanation for these irregularities aside from the possibilities of carelessness, negligence, capriciousness with flagrant or reckless disregard for fidelity of the published report of the original data, and/or intent. The versions of the figures in Paper 2 published as corrections may need to be further revised or amended because of apparent discrepancies between the published corrected figures and instructions from the *PNAS* (substitutions of panels instead of completely new figures).

PAPER 3

Citation: De Domenico I, Zhang TI, Koenig CL, Branch RW, London N, Lo E, Daynes RA, Kushner JP, Li D, Ward DM, Kaplan J. Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. *Journal of Clinical Investigation* 2010; 120: 2395-2405. Submitted December 11, 2009, accepted in revised form April 7, 2010. Corresponding authors Jerry Kaplan and Ivana De Domenico.

Background: The allegation related to this report was that Figure 3B in Paper 2 (PNAS, 2009; see previous summary of allegations related to Paper 2) was reproduced as a new data figure, Figure 2A, in Paper 3. The Inquiry Committee examined this issue. The final report of the Inquiry Committee was reviewed by the Investigation Committee. In addition, the Investigation Committee specifically discussed papers 2 and 3 with Dr. De Domenico in meetings on 7/02/2012, and 7/19/2012. Papers 2 and 3 were briefly discussed in interviews of Dr. Kaplan (7/03/2012) and Dr. Ward (7/16/2012).

Individual Figure Irregularities: From their analysis, the Inquiry Committee concluded that the same western blots were used in the two different figures. An annotated photocopy of panels from the two figures with arrows indicating duplications are included with Inquiry Committee's final report and is reproduced here (see below). The Investigation Committee agrees with the conclusion of the Inquiry Committee after review of their findings and the Investigation Committee's interviews of Dr. De Domenico, Dr. Kaplan, and Dr. Ward. **Therefore this represents duplication of data in two separate publications.**

Responses from the Authors: In a letter to Jeff Botkin (November 28, 2011), Dr. Kaplan explained that the data in Papers 2 and 3 were originally included in one large manuscript that was later separated into two. He stated that the panels in question in the two reports were relevant to both papers and that the "inclusion of those panels did not alter the results of the manuscripts…". He further stated that in the JCI report they referred to the PNAS manuscript and to findings illustrated by the duplicated figure. Subsequently, the conclusion of the Inquiry Committee was not contested in the February 23, 2012 letter from Drs. Kaplan and De Domenico in response to the Inquiry Committee report. Drs. Kaplan and De Domenico then published a corrigendum in the *Journal of Clinical Investigation* stating that the anti-Fpn and anti-Jak2 rows in Figure 2A of Paper 3 were reprinted from Paper 2 without attribution (*JCI* 2012; 122: 2326-2326 doi: 10.1171/JCI63977). The Investigation Committee reviewed the correction with Dr. De Domenico in the meeting with her on 7/02/2012 and again in the meeting on 7/19/2012. In an explanationin the powerpoint document provided to the Investigation Committee by Dr. De Domenico in conjunction with her meeting with the committee on 7/17/2012, she again outlined that the data in Papers 2 and 3 were originally included in one manuscript that was later divided into two reports. She stated "In the course of dividing the manuscript into two papers, the same panels were included into the two separate manuscripts." This suggest that the figure was inappropriately constructed in the original manuscript before it was divided. She further stated that "we also have provided to the committee repeats of the experiments…" and included a photocopy of a page from a notebook showing western blot data and labeled "2-20-2008 (repeat of Fig. 3 PNAS)". No additional insights into how this irregularity occurred emerged from the Investigation Committee's interviews of Dr. Kaplan and Dr. Ward.

Conclusions from the Investigation Committee's Findings: In summary regarding the allegation related to Paper 3, the Investigation Committee concludes that there was duplication of data in two separate published reports. The findings are most consistent with carelessness, but the Committee could not exclude negligence, capriciousness with flagrant or reckless disregard for fidelity of the original data, or intent. The correction in the *Journal of Clinical Investigation* appears to have satisfied the requirements of the journal.

PNAS Figure 3B vs JCI Figure 2A

• Same Western blots used in two different papers

Figure 3B PNAS 2009

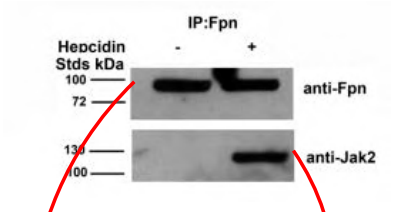
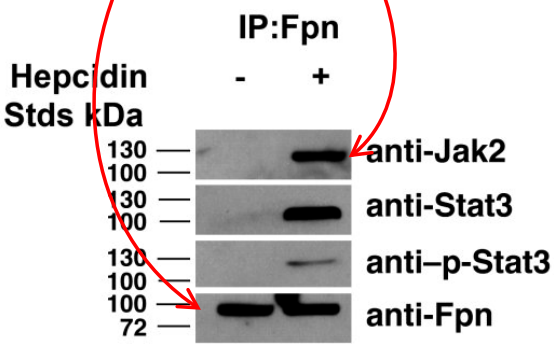


Figure 2A JCI 2010



(Reproduced from the Inquiry Committee report)

PAPER 4

Citation: Koenig CL, Miller JC, Nelson JM, Ward DM, Kushner JP, Bockenstedt LK, Weis JJ, Kaplan J, De Domenico I. Toll-like receptors mediate induction of hepcidin in mice infected with Borrelia borgdorferi. *Blood* 2009; 114: 1913-1918.
Submitted March 6, 2009, accepted June 13, 2009. Corresponding author, Ivana De Domenico.

Background: The allegation related to Paper 4 was that a microscopy image in Figure 4A in this report was the same image as published in Figure 2A in Paper 2 (PNAS, 2009; see previous summary of allegations related to Paper 2). This issue was examined by the Inquiry Committee. The Investigation Committee reviewed the Inquiry Committee's final report, and also interviewed Dr. De Domenico, Dr. Kaplan, and Dr. Ward as outlined in the summary of Paper 2.

Individual Figure Irregularities: The conclusion from the analysis by the Inquiry Committee was that the same microscopy image was used in Papers 2 and 4, and that the images were identified as resulting from different treatments in the two published articles. An annotated copy of some of the panels from the two figures with markers identifying the duplicated images is included in their final report. This illustration from the Inquiry Committee report is reproduced here (see below). After review of the Inquiry Committee's findings and interview of Dr. De Domenico, Dr. Kaplan, and Dr. Ward the Investigation Committee agrees that the same image was published in Figure 4A in Paper 4 labeled as Fpn-GFP detected in control cells, and in Figure 2A in Paper 2 labeled as human Jak2 detected in cells transfected with Jak2 siRNA. **Therefore, this represents duplication of data in two separate publications.** Because the two images are labeled as resulting from different cellular manipulations (siRNA for human Jak2; incubation with control media), **there is misrepresentation of data**.

PNAS Fig 2A vs Blood Fig 4A

- Same microscopy image used in different papers for different treatments

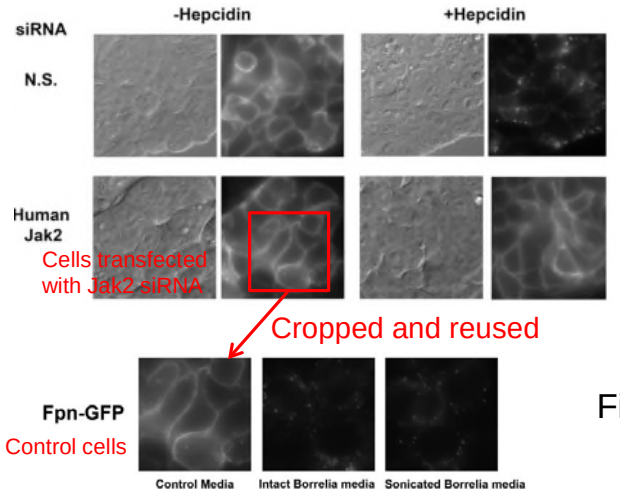


Figure 2A PNAS

Figure 4A Blood

(Reproduced from the Inquiry Committee report)

Responses from the Authors: The report by the Inquiry Committee included this statement: "Figure 2A in PNAS paper and Figure 4A in 2009 Blood paper: Dr. De Domenico indicated that the labeling in the PNAS paper is correct. The "control media" sample in the Blood paper is thus treated with siRNA for human Jak2 and

does not represent a control as labeled." (Top of p.2 in the Inquiry Committee report.) In their response letter (2-23-2012), Drs. Kaplan and De Domenico stated "It could be a misunderstanding during the meeting but from the laboratory notes it appears that the figure belongs to the Blood paper, therefore it is the figure in PNAS that needs to be replaced." As a specific detail, the Investigation Committee subsequently noted that the *PNAS* paper was submitted and accepted before the *Blood* paper was submitted.

In the published correction of Paper 2 mentioned previously (*PNAS* 2012; 109: 7538-7586; discussed in the summary of Paper 2), a new image was substituted in Fig. 2A and it is stated that the original differential interference contrast figure was replaced due to errors in preparing the figures for publication. Inspection of the published corrected Figure 2A reveals that the two left hand panels of the second row of micrographs have been replaced. The left most panel displays cells that have different morphology from those in the original figure, and the second panel is of much poorer quality than that published in the original figure. The other six micrograph panels of corrected Figure 2A appear to be the same as those in the original figure.

In the powerpoint document provided to the Investigation Committee by Dr. De Domenico in conjunction with her meeting with the Committee on 7/17/2012, she outlined a defense if the original publication of the same data in Papers 2 and 4 in which she stated that the two experiments were conducted on the same day and the microscopy images were saved on the same CD. She stated "The technician that took the data did not notice the comment in Dr. De Domenico's notebook…that the same CD has two sets of experiments. This is the reason why the same images were shown in two different publications." No additional insights into how the same figure was published in Paper 2 and Paper 4 were provided by Dr. Kaplan in his meeting with the Investigation Committee on 7/03/2012 or by Dr. Ward in her interview on 7/16/2012.

Conclusions from the Investigation Committee's findings: In summary regarding the allegation related to Paper 4, the Investigation Committee concludes that there was duplication and misrepresentation of data in the two separate published papers. The data in Figure 4A in Paper 4 appear to be correct as labeled, based on the statements of Drs. Kaplan and De Domenico. Therefore, the duplication and misrepresentation were in Paper 3, the PNAS article. **This conclusion should be communicated to Dr. Cynthia Dunbar, the editor of *Blood,* in response to her inquiry to Jeff Botkin regarding Paper 4 (email note from Anna Trudgett at *Blood* on March 27, 2012).**

PAPER 5

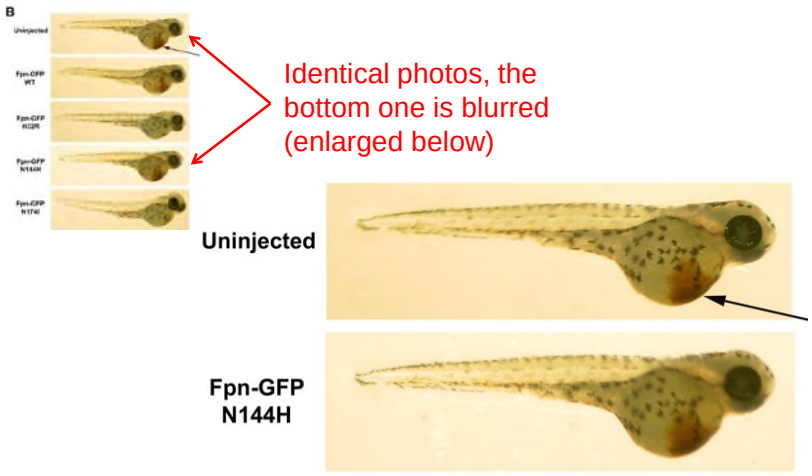
Citation: De Domenico, Vaughn MB, Yoon D, Kushner JP, Ward DM, Kaplan J. Zebrafish as a model for defining the functional impact of mammalian ferroportin mutations. *Blood* 2007; 110: 3780-3783. Submitted July 9, 2007, accepted August 22, 2007. Corresponding author, Jerry Kaplan.

Background: The allegation regarding this paper was that the same photomicrograph was used to indicate a zebrafish phenotype resulting from two different conditions. This allegation was examined by the Inquiry Committee. The Investigation Committee reviewed the final report of the Inquiry Committee and interviewed Dr. De Domenico, Dr. Kaplan, and Dr. Ward as outlined in the summary of Paper 2.

Individual Figure Irregularities: The Inquiry Committee concluded that the images labeled as "uninjected" and "Fpn-GFP N1444H" in Figure 1B show the same zebrafish larva and were manipulated differently. An annotated copy of the figure with arrows indicating the images in question is included in the Inquiry Committee's final report and is reproduced here (see below). After review of the findings of the Inquiry Committee, the Investigation Committee agrees that the same larva was shown in the top-most panel labeled as uninjected and in the fourth panel labeled as Fpn-GFP N144H. **This represents duplication of data in the same publication**.

Figure 1B

• Same microphotograph used for two different conditions and manipulated



(Reproduced from Inquiry Committee report)

Responses from the Authors: The report by the Inquiry Committee stated "Figure 1B in 2007 Blood paper: the images of zebrafish were all captured blindly to avoid any bias in interpretation. The use of a control image to represent the Fpn-GPP N144H mutant sample was *an accident according to Dr. De Domenico*." (Top of p.2 in the Inquiry Committee report; italics added.) No further comments or rebuttal related to this paper were made in the 2-23-2012 response letter by Drs. Kaplan and De Domenico. In the powerpoint document provided to the Investigation Committee by Dr. De Domenico in conjunction with her meeting with the committee on 7/17/2012, she included detailed comments regarding this figure. She stated that the data were transferred to a portable hard drive and the code was kept in a notebook that was one of the four notebooks illicitly discarded by the dismissed technician. Dr. Domenico further stated that the same image was shown twice but "…there was not image manipulation or image misrepresentation… the individual that captured all the images for each slide took three different exposure times." She also indicated that they did not notice that the same image was being included twice because the specific mutation (Fpn-GFP N1444H) did not give an obvious phenotype different from control.

Conclusion from the Investigation Committee's findings: In summary regarding this allegation, the Investigation Committee concludes that there was duplication of data in this publication. The findings are suggestive of carelessness, but the Committee cannot exclude negligence, extreme capriciousness, or intent.

PAPER 6

Citation: De Domenico I., Vaughn, M.B, Paradkar, P.N., Lo, E., Ward, D.M. and Kaplan, J. (2011) Decoupling ferritin synthesis from free cytosolic iron results in ferritin secretion. *Cell Metabolism*. **13**, 57-67.

Background: In the original allegations of November 2011, figures from this paper were identified as containing examples of unreasonably small error bars, especially for ELISA assays. No irregularities concerning Western blots or other images were identified. However, the Inquiry Committee asked Dr. De Domenico to present other figures in the paper and the corresponding primary data. During this interview, the Inquiry Committee identified four instances in which there appeared to be a discrepancy between Western blot autoradiographs and the corresponding published figures. In their response to the Inquiry Committee report, Drs. De Domenico and Kaplan stated that examination of laboratory notes corresponding to the Western blots indicated that, in most cases, the handwritten labels on the films were incorrect and that the published figures were correct. None the less, this paper and Paper 1 were publically retracted in the 6 June 2012 issue of Cell Metabolism, with the statement that "We, the authors, wish to retract [. . .] because a number of errors have been detected in the assembly of the figures, and some of the original data were inappropriately removed from the laboratory. We stand by the validity of our studies; the data are reproducible, and the conclusions were not affected by the errors."

Despite the retraction, the Investigation Committee chose to consider Paper 6 further in order to better understand the origin of the multiple discrepancies between the laboratory notes, the films and the published figures, and how these discrepancies might reflect on general laboratory practices and the integrity of other data published by the respondents.

Error Bars in Graphs

Allegation: The original allegations presented by Prof. Tomas Ganz who indicated he was conveying concerns from other colleagues as well, stated that "In all of the papers where De Domenico is the first author, bar graphs have error bars that are too small for the techniques involved and the number of replicates." The complainants identified three specific figures in Paper 6: Figure 2, Figure 3 and Supplemental Figure 2, which are reproduced below.

Figure 2.

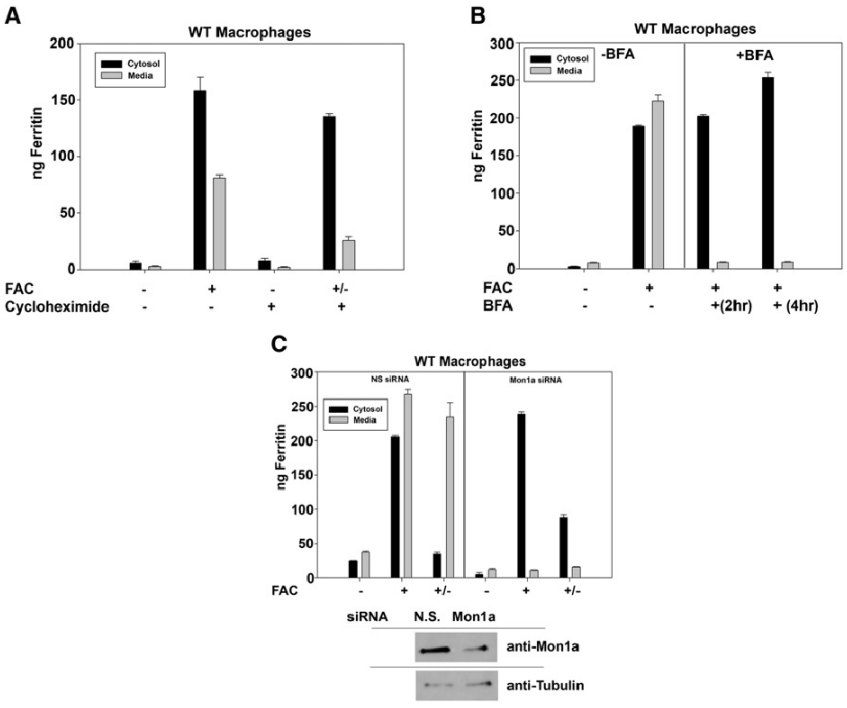
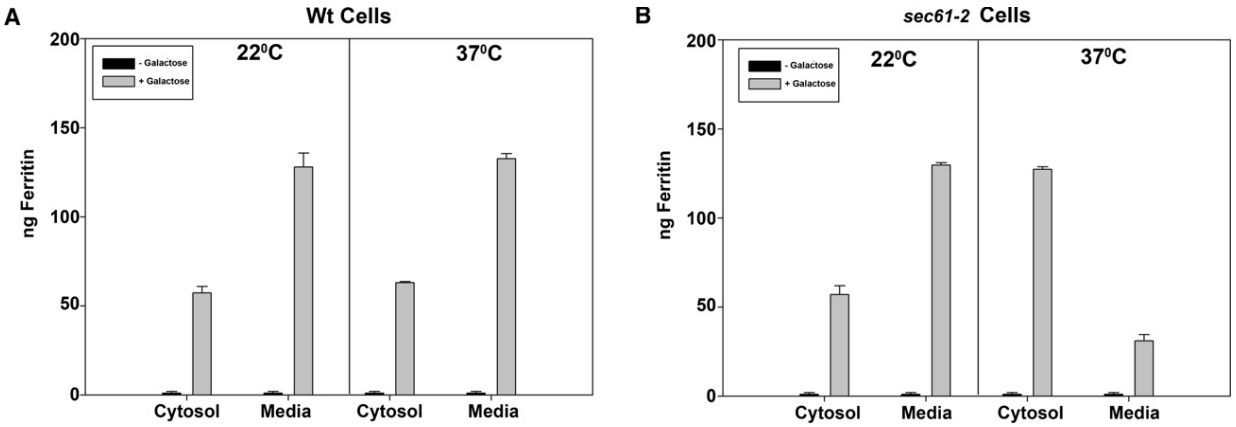
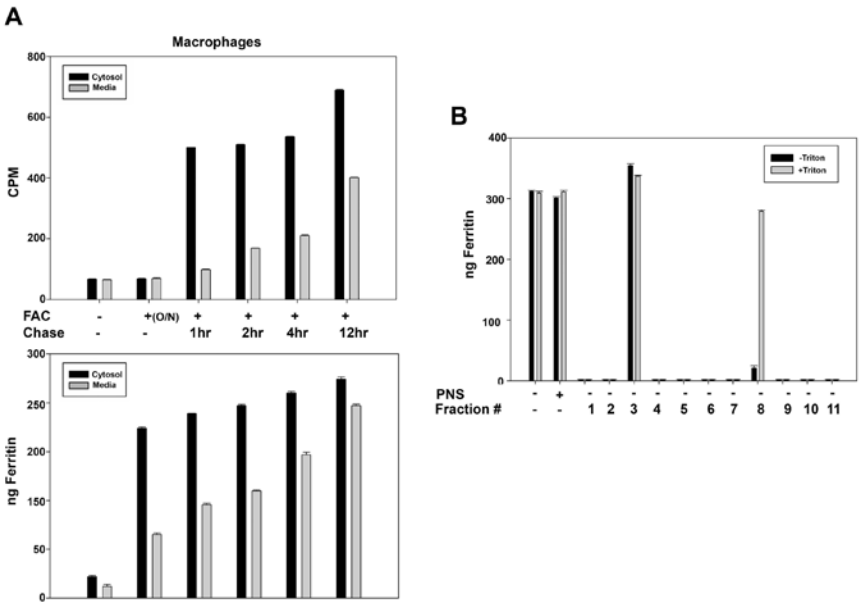


Figure 3.



Supplemental Figure 2.



The legend to each of these figures includes the statement “Error bars represent the standard error of the mean of three independent experiments.”

To address the issue of error bars, the Inquiry Committee asked a statistician, Dr. Gregory Stoddard (Co-Director of the Study Design and Biostatistics Center at the University of Utah) to examine the data from similar plots in Paper 1. Using primary data provided by Dr. De Domenico, Dr. Stoddard re-calculated the means and standard errors and concluded that “From the data in the laboratory notebooks, the figures, both the height of the bars in the bar charts, representing the mean, as well as the length of the error bars, representing the standard errors, were correctly drawn.”

Dr. Stoddard also noted that “The concern raised by Tomas Ganz, however, is clearly present.”, and went on to discuss the very low coefficients of variance (the standard deviation divided by the mean) for the data. Dr. Stoddard suggested that the low values might indicate that the data do not represent truly independent replicates, but rather separate measurements of the same samples. The Inquiry Committee did not discuss this issue further with Drs. De Domenico or Kaplan.

In their response to the Inquiry Committee report, Drs. De Domenico and Kaplan stated: "Regarding the correspondence between Dr. Stoddard, Dr. Botkin and the reader. Dr. Stoddard is correct that for these experiments we used cells that came from the same flasks. The biological variability is due to different transfections each time. The Erhardt paper analyzed serum samples and not cell lysates or culture media. Furthermore, that paper used a self-made Elisa. In Dr. Kaplan's laboratory a commercial Elisa was used for most of the experiments. Each Elisa run in the laboratory has lower "CV" that the Erhardt s paper."

"The Nemeth et al, Science 2004 was the first paper where this assay was used, the error bars are also low and this paper was not considered in the allegation."

Investigation Findings: After obtaining copies of Dr. De Domenico's computer drives, the Committee attempted to trace the analysis leading to the error bars shown in this and other papers. Towards this end, the Committee asked Dr. De Domenico (via an E-mail exchange on 7-26-12) to identify the relevant files on the drives. Although she did provide lists of files for Papers 7 and 10, she did not for Paper 6,explaining that she did not have time before leaving on a previously-scheduled overseas trip. Dr. De Domenico also provided (in the form of a PowerPoint file labeled "CD_fromIvana_31July.ppt") a description of the procedure used to calculate the means and errors. According to the PowerPoint file, the data from individual experiments were entered into Excel spreadsheets and the program's statistical functions were used to calculate the means and errors. The results were then transferred to the program SigmaPlot for graphing.

Thorough examination of the computer drives did not reveal any Excel files in the directories containing other data for Paper 6 (the directories labeled "documents/Ivana's Documents/secret ferritin" and "documents/Ivana's Documents/secreted ferritin revision")." We did find numerous SigmaPlot files (identified by the .JNB file name extension) in these directories. While many of the graphs in these files appear to be related to the published figures, it was quite difficult to link the final figures to specific files. In addition, many of the files did not contain error information at all, and only two contained replicate data from which the errors were calculated. Based on the data column labels, the two files (6-1B-1 and 6-2C-1) containing replicate data appeared to be closely related to the graphs show in Figs. 1B and 2C, respectively, but the plotted values were different from those in the published figures. For each of these files, the error values were calculated from only two data sets.

The legends for the graphs in this and the other papers we examined specifically state that the error bars represent standard errors of the mean (SEM). However, in the cases noted above (and those related to other papers for which we found more than one data set), the errors were all calculated as standard deviations (SD). Also, in the Excel spreadsheet that Dr. De Domenico provided as an example (in the form of a computer screen capture), it appears that the built-in SD function was used. Indeed, the standard Excel function library does not include the SEM, which is defined as:

$$SEM = SD/\sqrt{n}$$

where *n* is the number of observations. The situation is further confused by the statement in the Methods section that ". . . the error bars, calculated using Student's t test, represent the standard error of the mean". Though related to the standard deviation, the t-test does not define a calculation of the standard error. It seems most likely that all of the error bars are, in fact, standard deviations. This makes the small magnitudes of the reported errors even more problematic, as the SD should always be greater than the SEM.

Response from Dr. De Domenico: As noted above, Dr. De Domenico provided information about the procedures used to generate the final figures, but did not identify the specific files used for the figures in this paper. The issue of error bars was not discussed further with her or Dr. Kaplan in the interviews.

Conclusions: As noted in the original allegations, the error bars shown in many of the figures in this and other papers are very small, generally less than 10% of the measured values. Since the measurements are presumed to involve multiple pipetting operations (with errors of a few percent each), along with an enzyme assay with finite precision, the sample standard deviation for the overall measurement is unlikely to be less than 5% for repeated measurements of the same sample, under ideal circumstances. (See Hayashi, Y.,

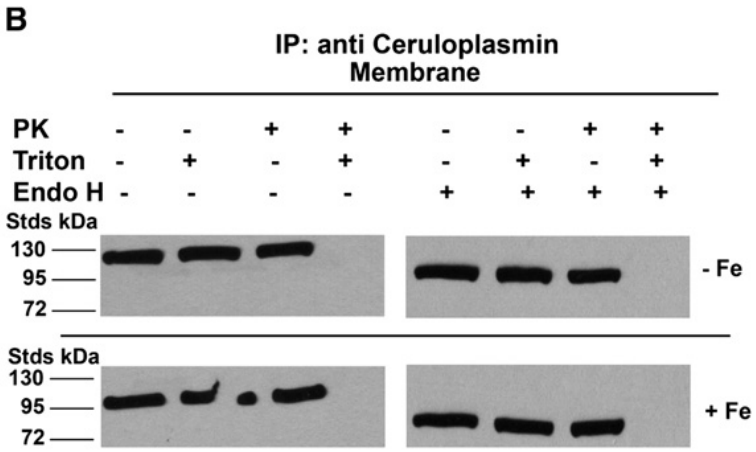
Matsuda, R. & Maitani, T. (2004). Precision, limit of detection and quantitation in competitive ELISA. *Anal. Chem.*, 76, 1295–1301. http://dx.doi.org/10.1021/ac0302859.) The additional variability from different biological experiments is expected to increase the errors substantially.

We thus share the concern expressed by the complainant, and by the previously-consulted statistician. This concern is heightened by the absence of computer files containing the replicate data and calculations. The issue of error bars is also addressed in the report for papers 1 and 10.

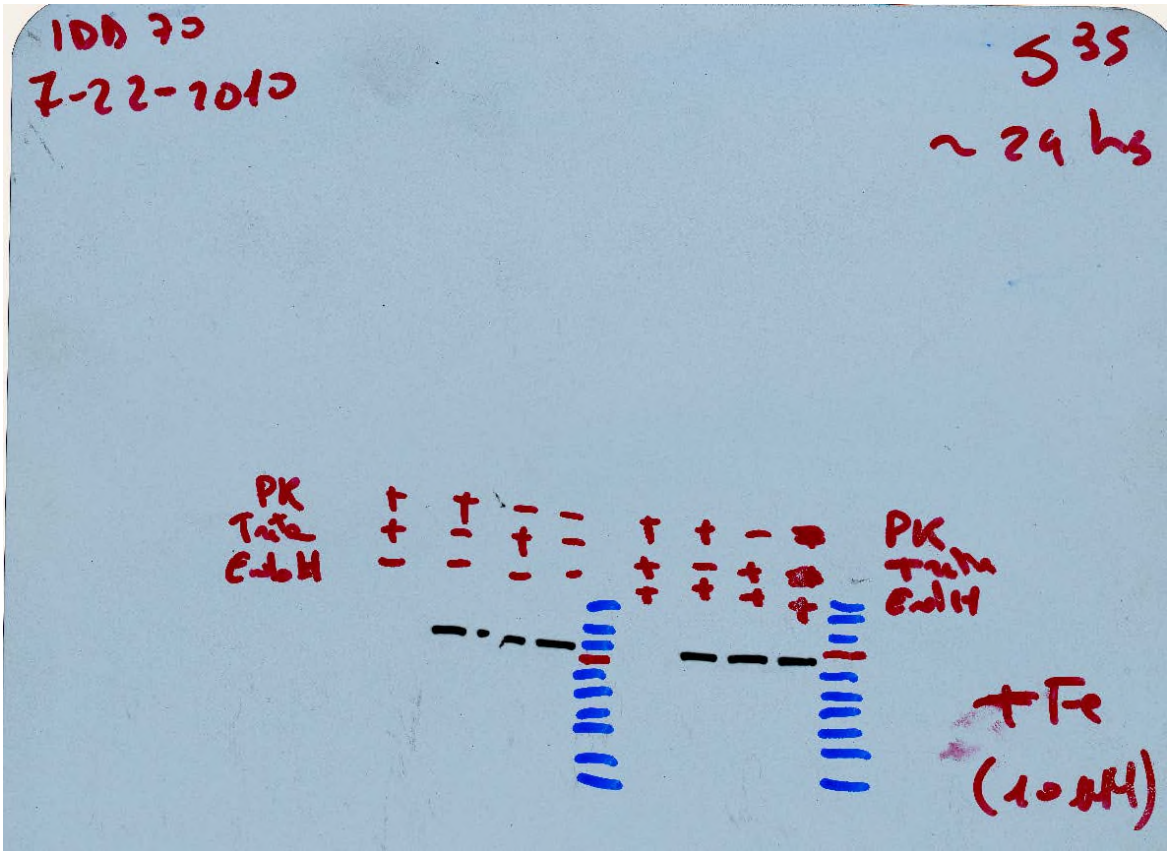
Figure 4B

Allegation: During a meeting of the Inquiry Committee with Dr. De Domenico, the Committee noted a discrepancy between this figure and a labeled autoradiography film provided by Dr. De Domenico. Referring to the lower two panels of the figure, the Committee reported that "The left panel is flipped around the vertical axis compared to the original film and thus does not represent the lanes as published. The data for the right lower panel were not on the same film and could not be found. These were the only data in the paper that could not be found."

The figure in question is shown below:



The following is a scan of the film shown to the Inquiry Committee, confirming that the image for the lower-left panel is rotated:



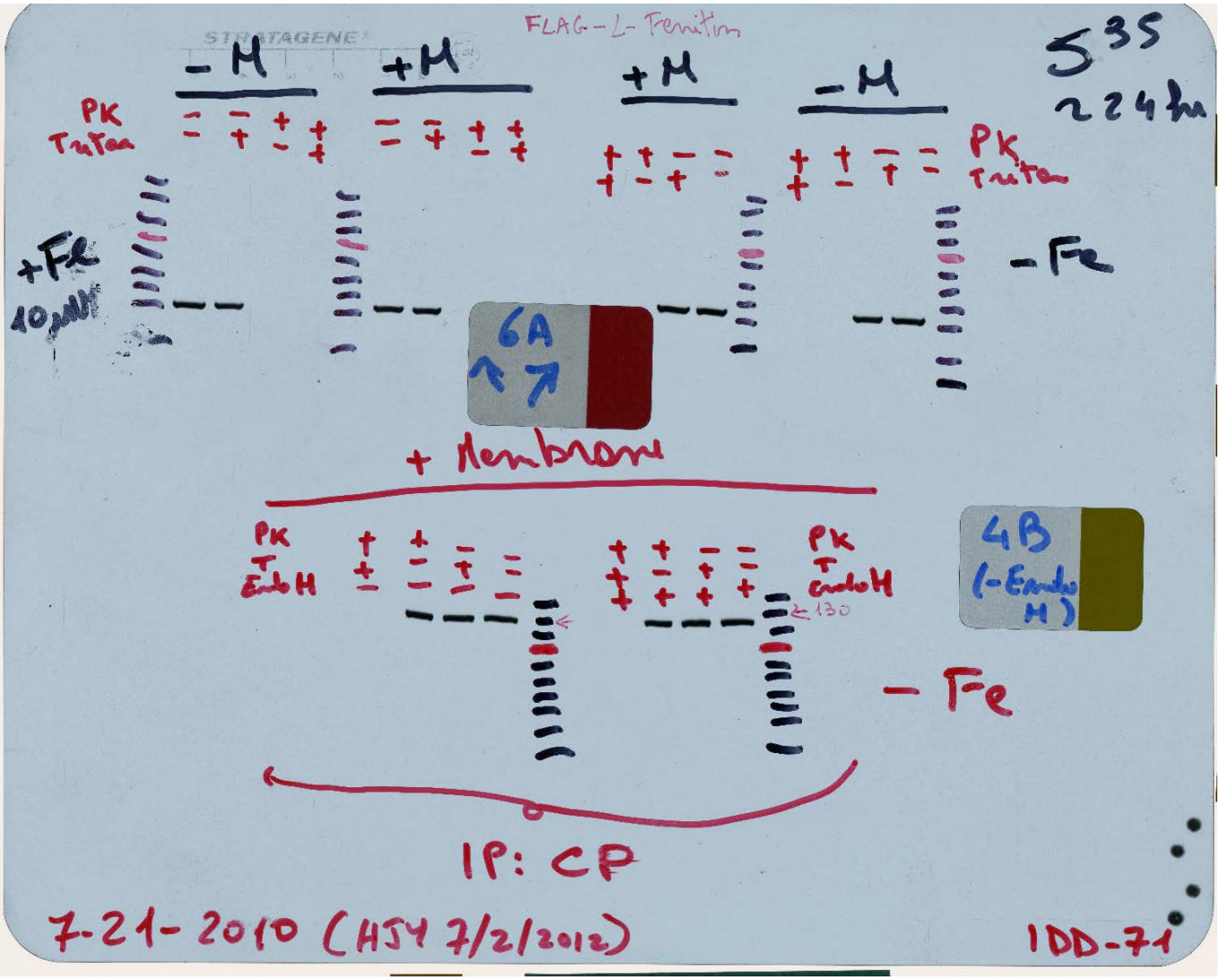
It is also apparent that the lanes on the right-hand side of this film do not correspond to those in the lower left panel in the published figure.

In their response to the Inquiry Committee report, the respondents stated that “The committee is correct that the panel was flipped around the vertical axis BUT represents the lanes as published.”

While this response suggests that the inversion of the image does not affect the interpretation of the data, it should be noted that the vertical positions of the bands are critically important. One of the key results conveyed in the published figure is the shift in electrophoretic mobility of ceruloplasmin upon treatment with endoglycosidase H (Endo H), an indication that the polypeptide had entered microsomes and been glycosylated. The apparent molecular weights, as indicated by the positions of the bands relative to the molecular weight markers, thus represent critical information. The inversion makes this comparison with the markers meaningless.

Investigation Findings: Further examination of the films for this experiment and files from Dr. De Domenico’s computer revealed additional discrepancies between the data records and the published figures, particularly with regard to molecular weight markers.

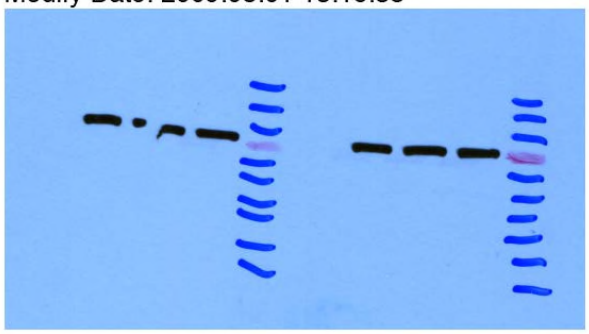
In addition to the labeled film shown above, the Committee examined a similar film in the notebooks, with labels indicating that it represented the upper panels of Fig. 4B (-Fe):



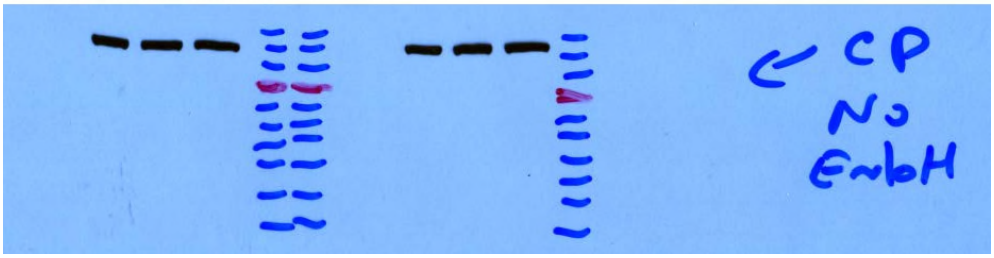
The Investigation Committee's initial concern with this film was that the difference in electrophoretic mobilities of the samples treated with and without Endo H appear to be much smaller than indicated in the published figure.

Further inconsistencies were discovered when computer files (6-4B-1 and -2) containing scans of these films were found:

File 6-4B-1
Modify Date: 2009:08:01 13:16:35



File 6-4B-2
Modify Date: 2009:08:01 13:14:22

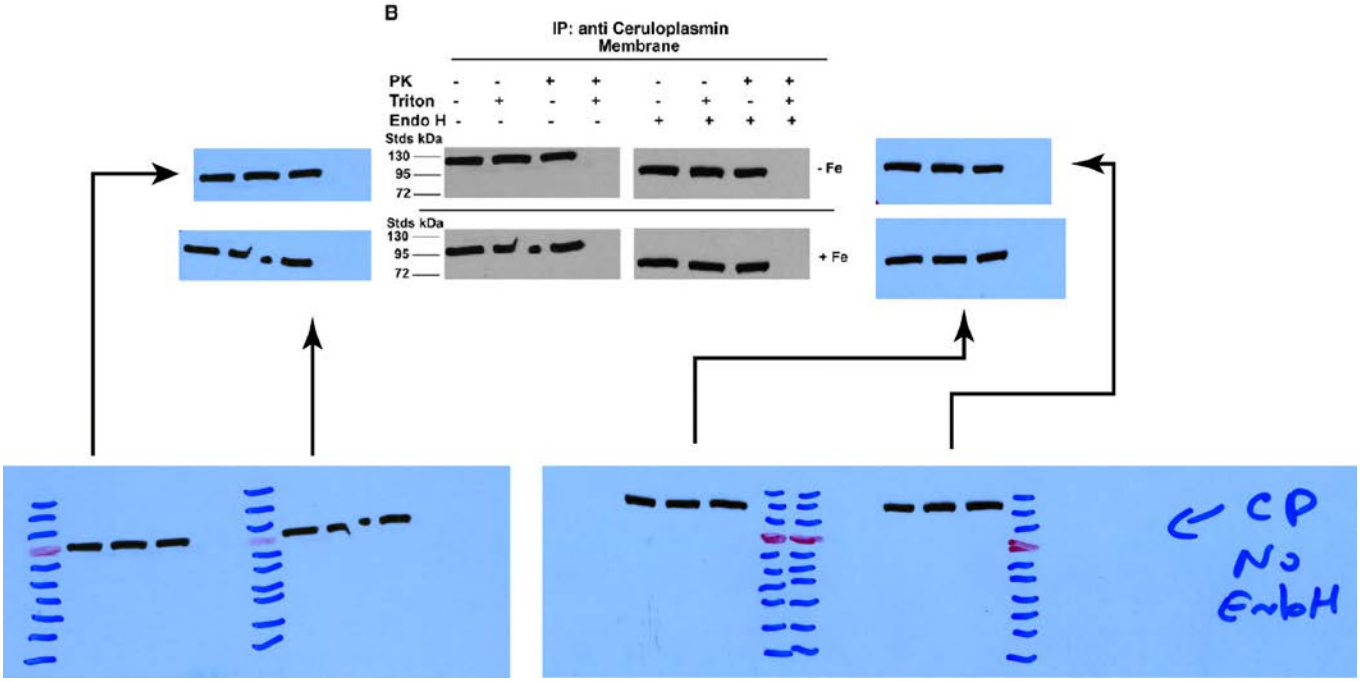


Other than the marking “cp No Endo H”, neither of these images contained any labeling of the specific lanes.

Comparison of the films (with their current labeling) and the computer images reveals the following inconsistencies:

1. The dates of the computer files (August 2009) precede those on the films currently in the notebooks (7-21-2010 and 7-22-2010) by nearly one year.
2. The dates currently on the films are after the date the manuscript was submitted to Cell Metabolism (with Figure 4 in its final form). There are no dates on the notebook pages corresponding to these films.
3. The marks indicating the positions of the molecular weight markers in the earlier images have been changed on the films, in some cases shifted to indicate different molecular weights.
4. The MW marks in the earlier images indicate that all of the bands on each blot displayed the same mobilities, consistent with one blot representing the treatment with Endo H and the other without. The lane labels currently on the films indicate that one film represents –Fe and the other +Fe, with the right-hand lanes treated with Endo H and the left-hand lanes untreated. The current MW markers appear to have been placed to indicate differences in MW among bands on the same blot.

Comparison of the computer files and the published figure enabled us to identify the source of all four panels in Fig. 4B, as illustrated in the diagram below:



If the MW markers in the original scans are correct, then the panels on the left- and right-hand side of the published figure are reversed: The panels indicating treatment with Endo H, and a higher apparent molecular weight, are actually those from the treatment without Endo H. Furthermore, there are inconsistencies between the MW markers indicated in the published figure and those in the original scans.

The Committee also found what appeared to be traces of another set of MW marks on the film labeled “cp No Endo H” in the scan. These marks do not appear to be the same as those in the scan.

Responses from Dr. De Domenico: Figure 4B was discussed during two of the interviews with Dr. De Domenico (on 7-2-12 and 7-19-12). In the first interview, before the scan files were found, the discussion focused on the apparent discrepancies between the molecular weight markers in the labeled films in the notebooks and the published figure. In the second, the scan images were also discussed. In neither interview, was Dr. De Domenico able to provide a clear explanation of how the figure was generated or the origins of the multiple discrepancies. The discrepancies in dates was discovered after the 7-19-12 interview, and the Committee did not have an opportunity to discuss this with Dr. De Domenico.

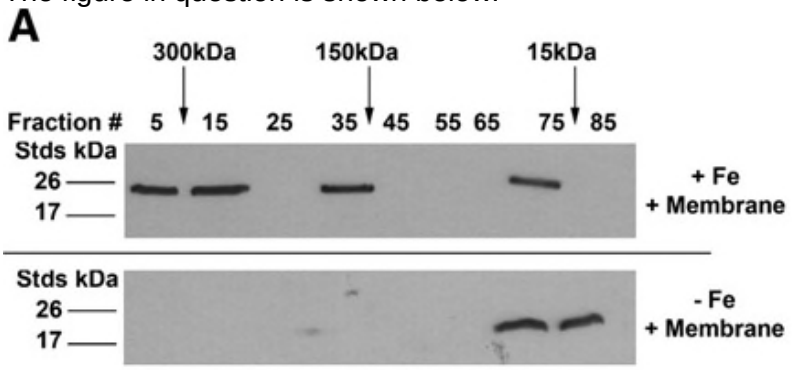
Conclusions: The original allegation (from the Inquiry Committee) concerned the inversion of the lower-left panel of Figure 4B, and the failure to find the film for the lower-right panel. Our investigation confirmed the inversion, but also revealed additional discrepancies, as well the source of the lower-right panel. Given all of the discrepancies, it is difficult or impossible to conclude whether or not the published figure accurately represents the experimental results. From the limited labels present in the original scanned images, it is possible that the results are as described, but with the +Endo H and –Endo H panels switched.

Our major conclusion is that the path from the primary data to the publication has been so poorly documented and corrupted by relabeling that no confidence can be placed on the published figure.

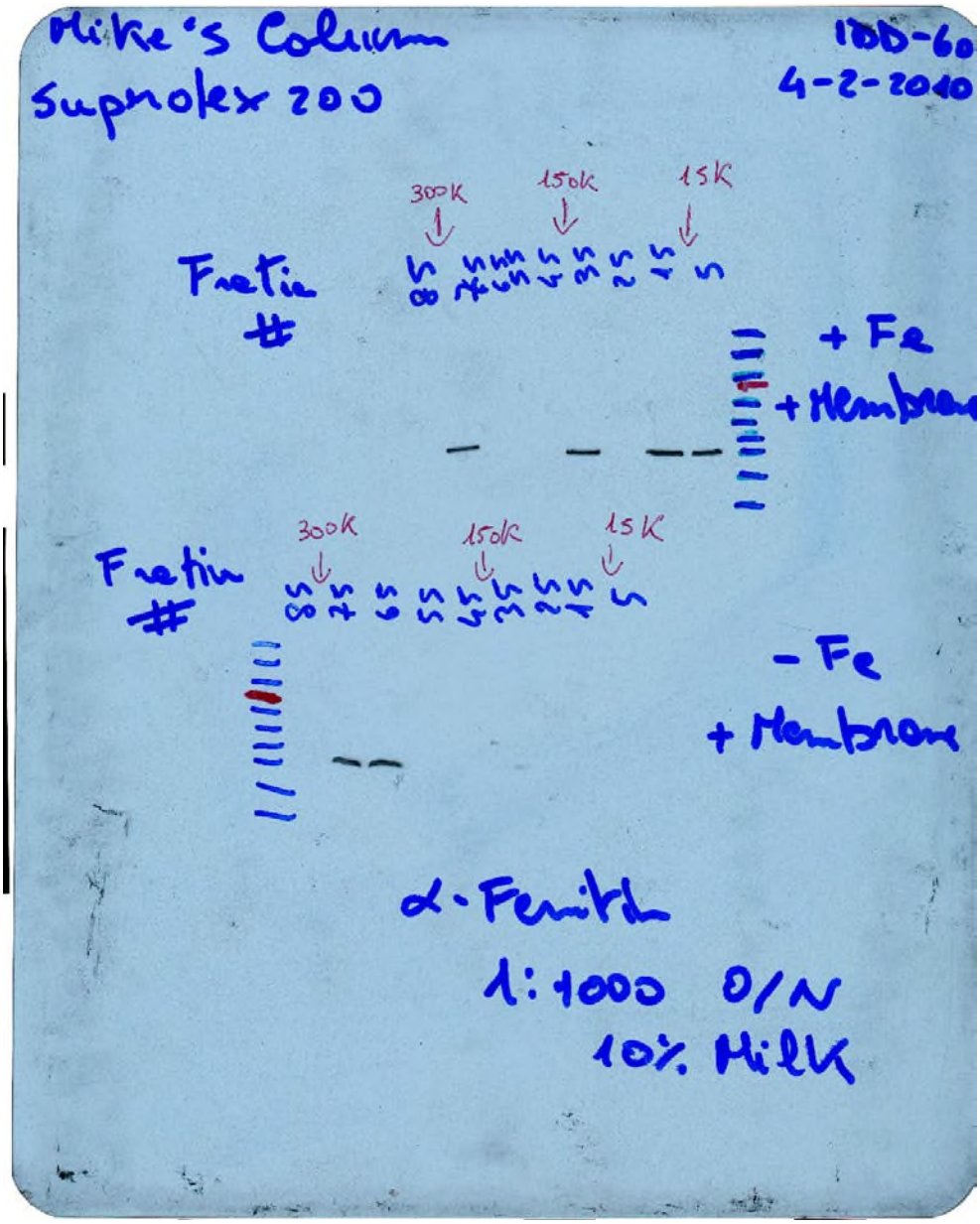
Figure 5A

Allegation: During a meeting of the Inquiry Committee with Dr. De Domenico, the Committee noted a discrepancy between this figure and a labeled autoradiography film provided by Dr. De Domenico. The Committee reported that “The right-most band in the upper panel is shifted to the right from its proper location, under the heading ‘65’. The two bands in the lower panel should correspond to the lanes ’65’ and ‘75’, not ‘75’ and ’85’ as indicated. There is a blank lane to the right of the lower band in the original data for this panel that is not shown in the figure (corresponding to lane ‘85’). The figure thus misrepresents the original data.”

The figure in question is shown below:



A scan of the film shown to the Inquiry Committee is shown below:



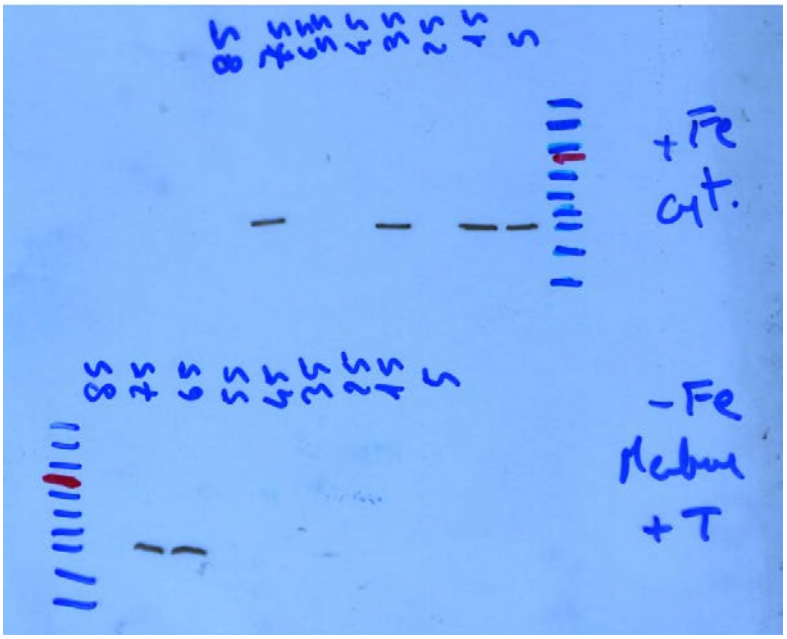
In their response to the Inquiry Committee report, the respondents stated that “During the meeting the committee suggested we examine the notes regarding these experiments. This was done in Dr. Botkin’s office. The notes reveal that the data represented in the figures are correct. The notes show that the order of the samples in the figure was correct. Furthermore, repeats of this experiment agree with the presented data. Therefore there is no misrepresentation of the original data. Please see the original notes, which are attached to this letter.”

Investigation Findings: Comparison of the published figure and the film do not confirm the claim in the response, that “the data represented in the figures are correct”, and raise additional questions:

1. In the upper panel, the band in question is located between the labels on the film for fractions 75 and 85, not between 65 and 75, as indicated in the published figure. In this respect, the Inquiry Committee appears to have been mistaken in stating that the “proper location” was under 65, but it also the case that the location is different in the film and the published figure.
2. The Inquiry Committee appears to have been correct in stating that, in the lower panel, the two bands below the labels 75 and 85 in the published figure are labeled 65 and 75 on the film.
3. For the upper panel, the labeling of both the film and the published figure is ambiguous. In both, the spacing of the labels is uneven and a band is located between two labels.
4. If the left-most band (as oriented in the film) in the upper panel corresponds to fraction 75, there are three lanes indicated between this and the lane for fraction 35. The space available for these lanes may not be sufficient. More generally, it is not clear how the lanes could be unambiguously assigned, since there are no traces of bands to the left of the one positioned between the labels for fractions 75 and 85. These issues are discussed further below.

On the disk copied from Dr. De Domenico’s computer, a file (6-5A-1) containing a scanned image of this film was found:

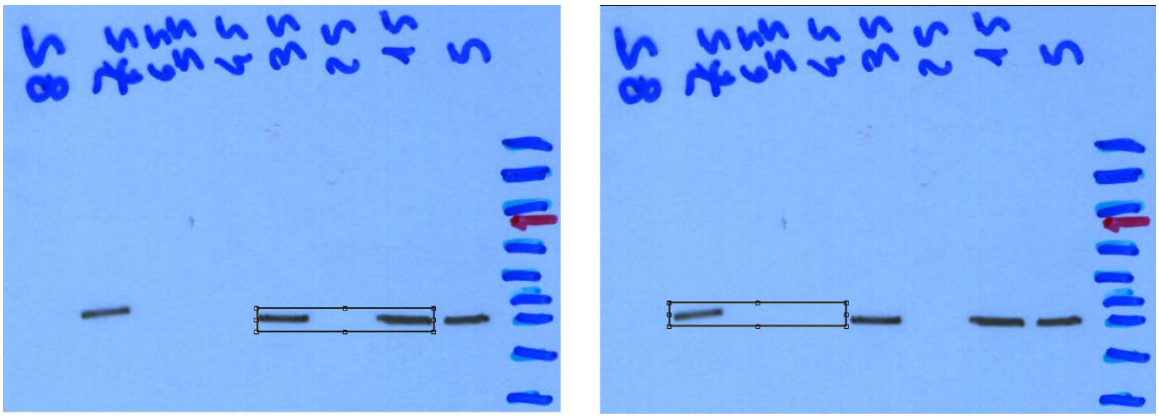
File 6-5A-1
Modify Date: 2009:08:03 13:50:16



The MW markers and the lane labels appear to be the same as those currently present on the film, but the labels on the right hand side of the gel are different.

The date indicated for the computer file is eight months earlier than the date indicated on the labeled film in the notebook and the hand-written notes for this experiment. (The notes are dated “3-29-2010”.) The manuscript was submitted on 4-20-10 (with Fig. 5), making the earlier date indicated on the computer file more likely.

The scan file was used to analyze further the spacing of the lanes. In the left-hand image below, a box has been drawn around the space identified with fractions 15, 25 and 35. In the right-hand image, this box is shifted to the left of the lane labeled 35, where it covers the space shown for fractions 45, 55, 65 and 75.



This comparison suggests that is unlikely that three lanes could fit between the lanes labeled 35 and 75, that the labels are incorrect and that the left-most band corresponds to fraction 65

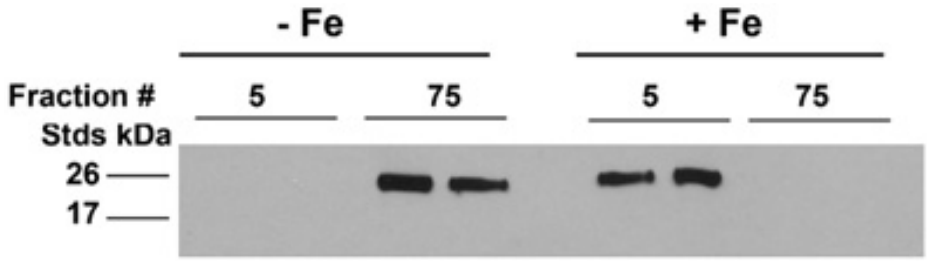
Responses from Dr. De Domenico: As noted earlier, in their response to the Inquiry Committee report, Drs. De Domenico and Kaplan indicated that "the data represented in the figures are correct". This figure was not discussed further with the respondents by the Investigation Committee.

Conclusions: The labeling of the gel lanes in the published figure (and the film) is, at best, ambiguous. Given the absence of reference points other than the MW markers, on only one side of each gel, it is not clear how the lanes could have been accurately labeled, and the spacing suggests that the upper panel was mislabeled. The available records are inadequate to resolve these questions and do not support the respondents previous response to the Inquiry Committee report. The inconsistencies between the dates of the computer files, the film labels and the notes raise additional doubts about the reliability of the notes.

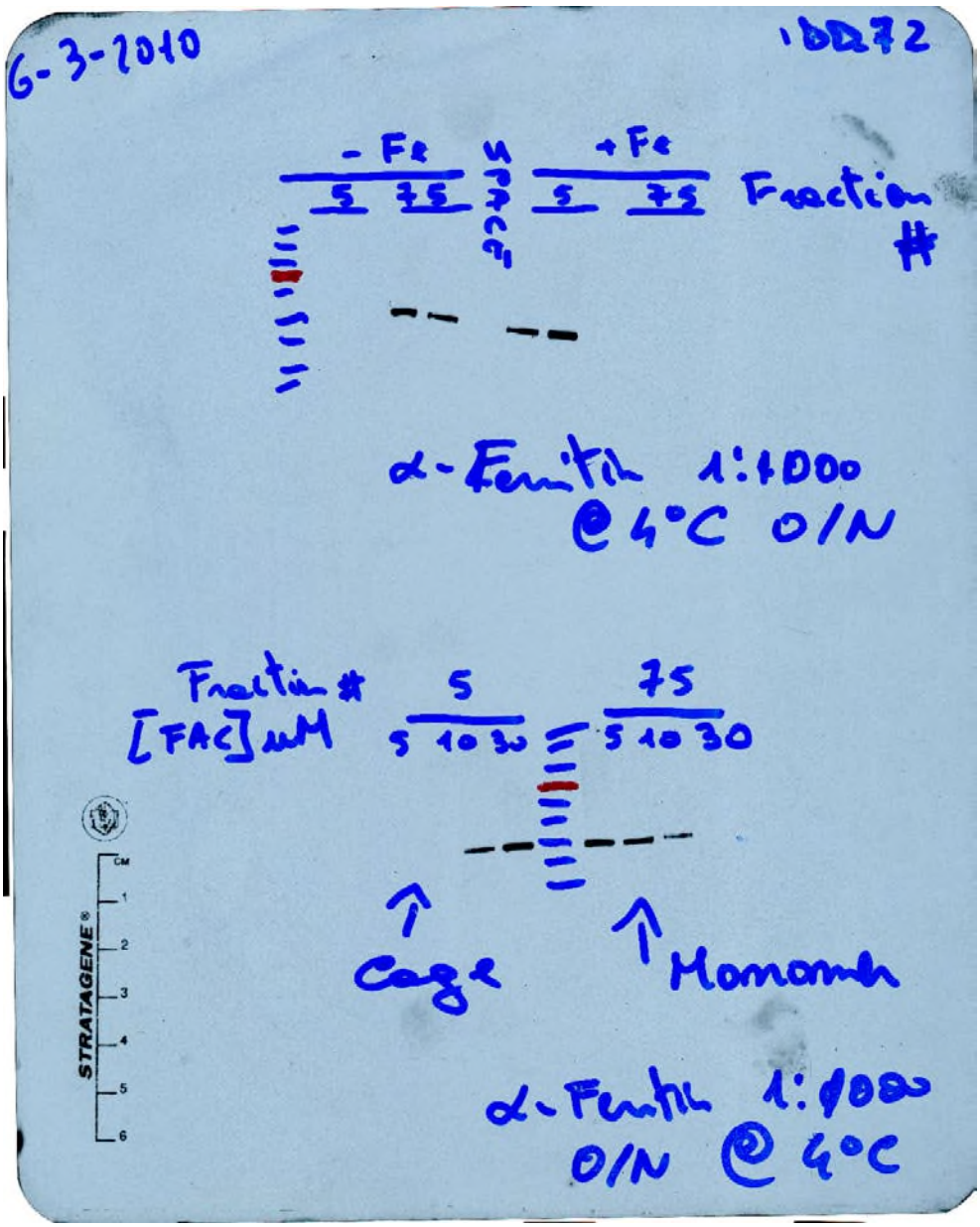
Figure 5B

Allegation: During a meeting of the Inquiry Committee with Dr. De Domenico, that Committee noted a discrepancy between this figure and a labeled autoradiography film provided by Dr. De Domenico. The Committee reported that "The published data are flipped from the original film and thus do not represent what is shown."

The figure in question is shown below:



The following is a scan of the film shown to the Inquiry Committee and currently in the notebook:



As noted by the Inquiry committee, the bands labeled fraction 75 in the published figure are labeled 5 on the film, and vice versa.

In their response to the Inquiry Committee report, the respondents stated that "Again the committee suggested analyzing the notes for this experiment and this has been done. The film was flipped because the original labeling of the film was different. The notes reveal that the data represented in the figures are correct. The notes were followed to generate the figure. Furthermore, repeats of this experiment agree with the presented data. Therefore there is no misrepresentation of the original data. Please see the original notes, which are attached."

Investigation Findings: For this figure, the committee was not able to find computer files for the film scans, which might have helped clarify the history. The notebook pages provided by Dr. De Domenico do indicate the order of samples indicated in the published figure, rather than that indicated by the labels on the film. It is still not clear, however, why there is a discrepancy or whether the notes or labeled film is correct. Furthermore, the dates shown on the notebook page (5-20-2010) and the film (6-3-2010) raise doubts about the reliability of

either, as both are later than the date the manuscript was submitted to Cell Metabolism (4-20-10), with this figure included.

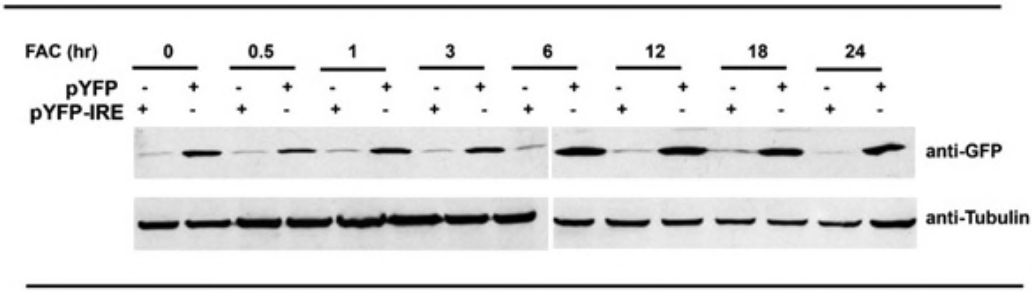
Responses from Dr. De Domenico: As noted earlier, in their response to the Inquiry Committee report, Drs. De Domenico and Kaplan indicated that "the data represented in the figures are correct". This figure was not discussed further with the respondents by the Investigation Committee.

Conclusions: The available records are inadequate to determine whether the published figure was correctly labeled. The inconsistencies between the dates shown on the film, the notes and the submission date raise additional doubts about the reliability of the notes.

Figure 7B

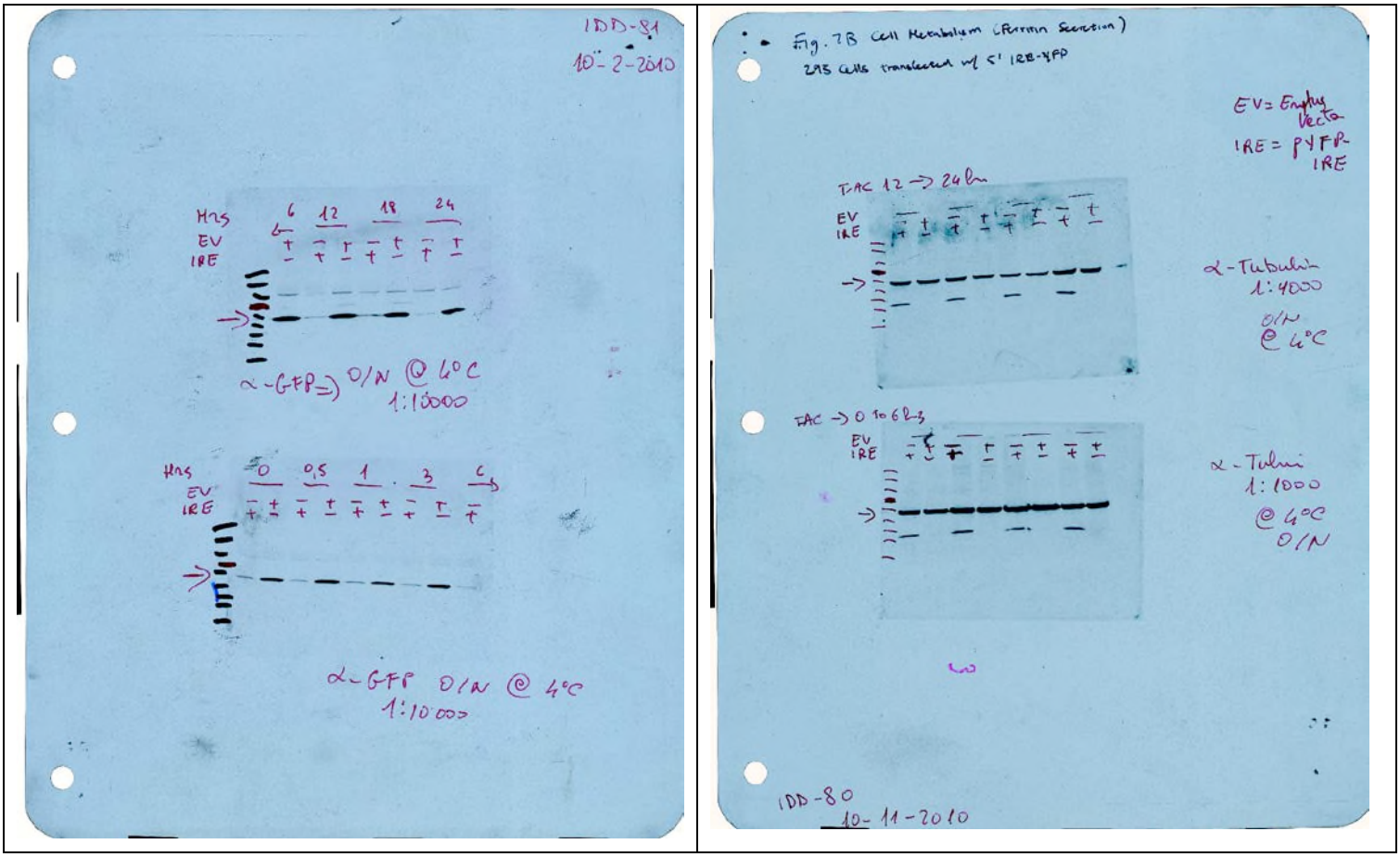
Allegation: During a meeting of the Inquiry Committee with Dr. De Domenico, the Committee noted a discrepancy between this figure and a labeled autoradiography film provided by Dr. De Domenico. The Committee reported that "The upper left panel is flipped from the original film and thus does not represent what is shown. The lower panel shows loading controls that were run on a separate gel and thus cannot be directly compared to the experimental samples shown above. In addition, one loading control has not been included and they are not aligned with the upper panel. There are 16 lanes depicted in the upper panel and only 15 in the lower. The last sample on the right of the lower panel was cut off in making the figure."

The figure in question is shown below:



As noted by the Inquiry Committee, the upper left panel contains 9 lanes, while the panel below it contains only 8.

The films presented to the Inquiry Committee are shown below:



In their response to the Inquiry Committee report, the respondents stated that “The upper panel was not flipped and the notes were followed to generate the figure therefore there is no misrepresentation of the results. However, the committee is correct regarding one band that was cut off for the lower panel for the loading control. This will be corrected.”

Investigation Findings: The notes provided by Dr. De Domenico are consistent with the order of lanes indicated in the upper left panel of the published figure, rather than that shown on the film. However, further examination of the films and notes by the Investigation Committee raised additional concerns regarding this figure:

1. This panel was not included in the original manuscript submission, but was in the revised version submitted on 9-10-10. The notebook page is dated 9-19-2010, and the films are dated 10-2-2010 and 10-11-2010. Thus, both the notes and the film labels are of questionable reliability.
2. The blot used to make the lower left panel of the figure is labeled “0 to 5 hr”, but only contains eight lanes, rather than the ten necessary for samples corresponding to 0, 0.5, 1, 3 and 6 hr. On the other hand, the blot used to make the lower right panel is labeled “12 ⇒ 24 hr”, but contains eight lanes, rather than six corresponding to 12, 18 and 24 hours. Without more specific labeling, it is not possible to tell which lanes actually correspond with the different time points and treatments.
3. The anti-tubulin blots show a second prominent band with a higher electrophoretic mobility, which was cropped out of the published figure. This band is present only in the lanes labeled +IRE/-EV on the film and thus appears to be correlated with the experimental treatment.

During the interview of Dr. Diane Ward, anti-tubulin blots were discussed in another context and Dr. Ward noted that these blots are usually very clean. When shown the blots described above, she was surprised by the presence of a second band and had no explanation for either its appearance or its correlation with the experimental treatment.

For this figure, the committee was unable to find computer files for the film scans.

Responses from Dr. De Domenico: During the interview of 2 July 2012 Dr. De Domenico was asked about the second band described above, but did not offer any explanation for its origin the correlation with the experimental treatment.

Conclusions: The relationship between the films and published figure remains unclear. While the response of Drs. De Domenico and Kaplan to the Inquiry Committee report suggests that only a minor correction to the figure is necessary (the addition of a missing lane), the records make it difficult to know just what lane was omitted or whether there may be additional labeling errors.

The presence of a second band in the anti-tubulin blot is very troubling. Given all of the other inconsistencies, we believe that the published blot may have been probed with a different antibody, or may have come from another experiment entirely.

Summary of findings for Paper 6.

The original allegation regarding this paper, concerning the sizes of reported uncertainties, is difficult to address conclusively, given the limited records and near complete absence of computer files containing the relevant data and calculations. None the less, we concur with the complainants in finding that the reported errors are unreasonably small given the methods and sample sizes.

With regard to the irregularities identified by the Inquiry Committee, the results of our investigations fail to support some of the assertions by the respondents (in their responses to the Inquiry Committee Report), which tended to minimize the discrepancies between the labeled Western blot films and the published figures. Indeed, comparisons of the films with computer files on Dr. De Domenico's computer revealed additional discrepancies, which added to the uncertainties regarding the relationships between the published figures and the primary data. For some of the figures under question, the computer files could not be found at all.

This investigation also revealed significant discrepancies among the dates on notebook pages, on films, on computer files and the manuscript submission dates. The earliest date found on any of the written notes or films in the notebook compiled by Dr. De Domenico was 3-5-2010, whereas most of the computer files had dates several months earlier. Most of the dates on the films and notes are later than the manuscript date of submission (4-20-10). The original manuscript included all of the figures discussed here, except for Figure 7B, which was added to the revised manuscript submitted on 9-10-10. The films and notes for this figure have dates of 9-19-2010 or later.

The discrepancies in dates greatly undermine any confidence in the notebook records. In her interviews with the Investigation Committee, Dr. De Domenico repeatedly emphasized that the written notes were reliable and were used to make the final figures. She attributed discrepancies between the published figures and films to errors made when re-labeling the films after they were digitized. The dates on the notebook pages and films suggest that the notes (or, at least the, dates) were written or altered at some point after the experiments were completed.

The findings regarding this paper are also troubling with regard to the responses of Drs. De Domenico and Kaplan to the Inquiry Committee report, in which they stated that examination of the records confirmed, in most cases, the published figures. It appears that these responses were made without careful re-examination of the films, and likely no examination of the computer files. In his interview with the Committee, Dr. Kaplan indicated that he did not examine the notes or films himself and relied on Dr. De Domenico's report. Given the

seriousness of the allegations and their implications for the integrity of his research, this omission is difficult to understand.

Finally, our findings fully support the decision to retract this paper, but they also call into question the reassuring statements from the authors regarding the fundamental reliability of the results.

In his response to the initial draft of this report, the attorney representing Dr. De Domenico, Mr. Ryan B. Bell, challenges both the procedures used by the Committee to investigate the allegations related to this paper and the Committee's conclusions. One of Mr. Bell's major criticisms is that the Committee interpreted the absence of computer files and other records supporting the published data as evidence of possible misconduct. The federal ORI policy on this question (policy 93.106, Evidentiary standards) states:

> The destruction, absence of, or respondent's failure to provide research records adequately documenting the questioned research is evidence of research misconduct where the institution or HHS establishes by a preponderance of the evidence that the respondent intentionally, knowingly, or recklessly had research records and destroyed them, had the opportunity to maintain the records but did not do so, or maintained the records and failed to produce them in a timely manner and that the respondent's conduct constitutes a significant departure from accepted practices of the relevant research community.

The failure of Dr. De Domenico to provide the computer files documenting the data analysis in the published paper (and the failure of the Committee to find such files through a systematic search) is evidence of recklessness in maintaining the research record. Dr. De Domenico was specifically asked to identify the files associated with the error bar calculations for this and other papers. This request was made on 7/26/12, allowing more than two months to respond before the Committee submitted its report. Although she did provide (incomplete) information for the other papers, she did not identify any files for this one.

Mr. Ryan also suggests that the absence of the relevant files on Dr. De Domenico's computer is explained by the transfer of data from a Windows computer to a Macintosh, the latter being incompatible with the SigmaPlot program used for much of the data analysis. In fact, the files that are most conspicuously missing are the Microsoft Excel files in which, according to Dr. De Domenico, the error calculations were carried out. Excel files are compatible with both the Windows and Macintosh systems and are routinely transferred between the two. Furthermore, numerous SigmaPlot files were found on Dr. De Domenico's computer, and she explained in one of her interviews that her Macintosh computer was equipped with emulation software that allowed her to work with the SigmaPlot files.

PAPER 7

De Domenico, I., D. M. Ward, M. C. di Patti, S. Y. Jeong, S. David, G. Musci & J. Kaplan (2007). "Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin" *EMBO J* **26**(12): 2823-2831.

PAPER 8:

De Domenico, I., D. M. Ward, C. Langelier, M. B. Vaughn, E. Nemeth, W. I. Sundquist, T. Ganz, G. Musci and J. Kaplan (2007). "The molecular mechanism of hepcidin-mediated ferroportin down-regulation." *Mol Biol Cell* **18**(7): 2569-2578.

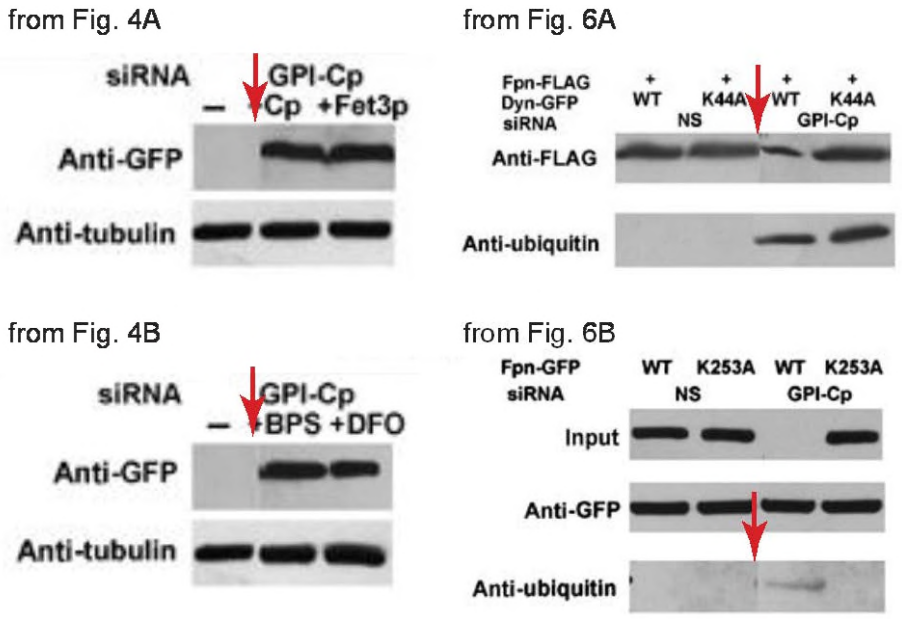
ALLEGATIONS

The allegations concerning these two publications are related and fall into two categories: (1) spliced gel images and (2) re-use and misrepresentation of western blot data.

Allegation 1: spliced gel images

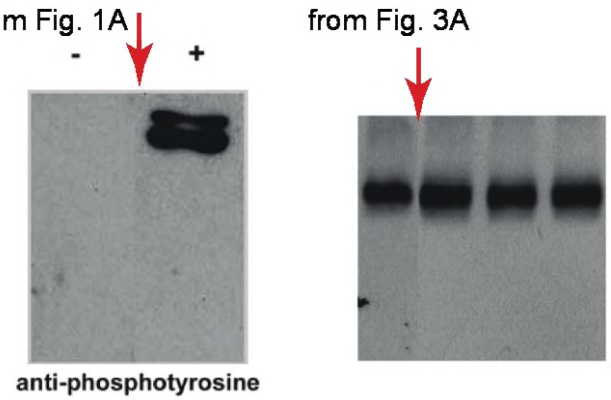
In paper #7 (Fig. 4A, 4B, 6A & 6B) western blot gel images are spliced, but not those of the loading controls [see figure below].

Images from Fig. 4A, 4B, 6A & 6B of paper #7. (Arrows indicate splice sites).



In paper #8 (Fig. 1A & 3A) images are spliced from two different blots [see figure below]

Images from Fig. 1A & 3A of paper #8. NB: brightness, contrast, and exposure levels were adjusted to emphasize the splice sites (indicated by arrows).



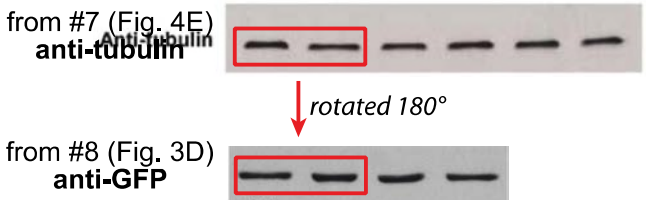
COMMITTEE FINDINGS REGARDING ALLEGATION 1

In discussions with the Investigation Committee and in rebuttal PowerPoint presentations, Dr. De Domenico acknowledged that the gel images in question had been spliced, but that "at the time those images were made (2005-2007) there was no policy prohibiting that practice in the journals in which those studies were published." Further, "they were not done in an attempt to misrepresent the data."

Allegation 2: reuse and misrepresentation of data

In paper #7 (Fig. 4E) two gel bands, labeled as an anti-tubulin western blot, appear again in paper #8 (Fig. 3D) as bands detected with anti-GFP [see figure below].

Duplicated gel bands from paper #7 (Fig. 4E) and paper #8 (Fig. 3D).



COMMITTEE FINDINGS REGARDING ALLEGATION 2

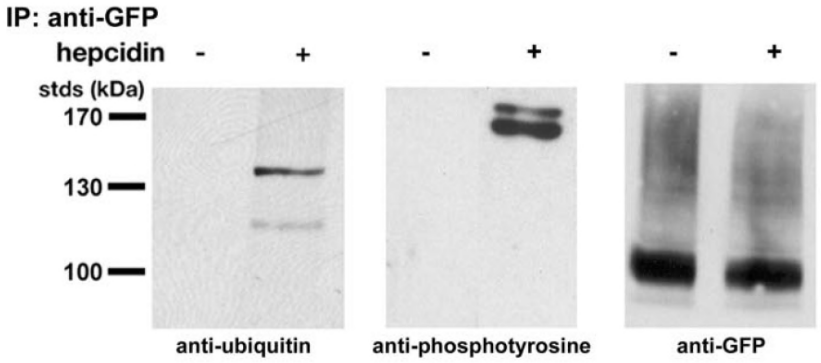
The gel bands of Fig. 4E in paper #7 are upside-down and at least one of these gel images must be incorrect, since the same bands are labeled as representative of anti-tubulin in one case and anti-GFP in the other case. Thus, **these figures misrepresent the primary data**. In discussions with the Investigation Committee, Dr. De Domenico stated that she doesn't know how these mistakes occurred, but that she is certain that the GFP gel (paper #8, Fig. 3D) is not correct, effectively acknowledging that the figures misrepresent the actual data.

Dr. De Domenico further stated that she did not assemble the figures and does not have digital files for these figures. According to Dr. De Domenico, the gel figures for paper #7 were assembled ("nondigitally") by Eric Lo, her technician at the time. The figures for paper #8 were assembled in PhotoShop by Dr. Diane Ward, a coauthor on many of the challenged publications. Discussions with Dr. Ward confirmed that she assembled figures for publications through 2007, at which time Dr. De Domenico began assembling the final figures for her own publications. The general procedure was for Drs. Ward and De Domenico to sit together and assemble the figures from image files made by Dr. De Domenico by scanning developed films. According to Dr. Ward, the digitized films often weren't explicitly or clearly labeled, in which case Dr. De Domenico would identify the lanes and labels to use in the figure.

ADDITIONAL FINDINGS

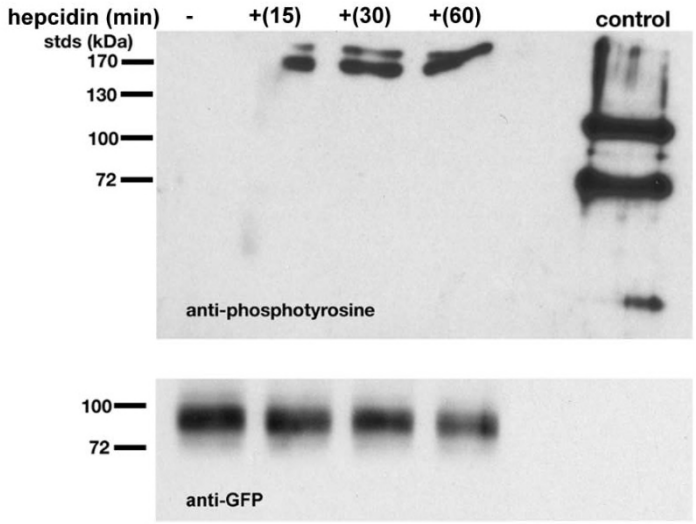
Several Committee findings raise additional concerns about Fig. 1A in paper #8 [see below]. The figure legend states that the hepicidin-treated sample in the anti-phosphotyrosine western was a 15-minute incubation. Note also the appearance of the anti-GFP bands, which purportedly represent the immunoprecipitated samples analyzed in the other panels of Fig. 1A.

Fig. 1A (published version in paper #8)



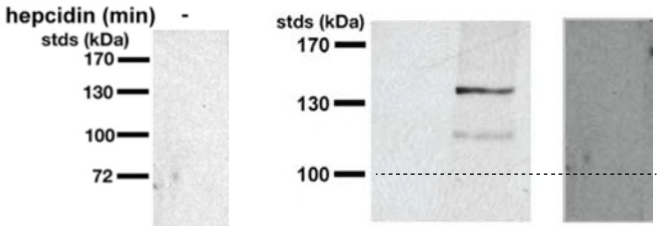
A PhotoShop file (8-1A-1), found on Dr. Ward's computer and dated 12/16/2005, appears to be an earlier version of the published figure [see below].

File 8-1A-1 from Dr. Ward's computer:



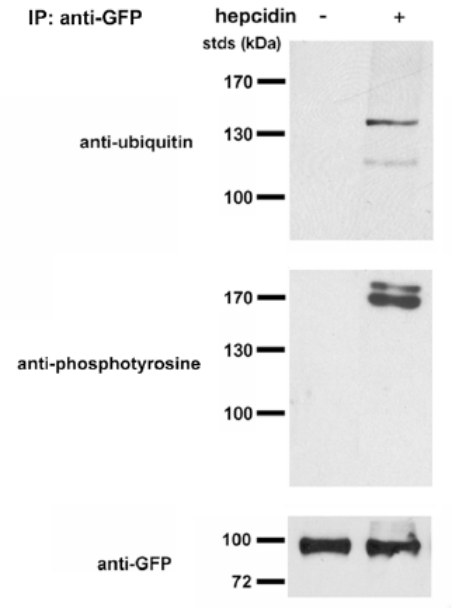
In this draft figure the anti-phosphotyrosine bands corresponding to those in the published figure are labeled as a 30-minute sample. Moreover, the accompanying anti-GFP immunoprecipitated samples do not match those in the published figure. Finally, although the "-" lane in the draft figure corresponds to the one in the published figure, it is positioned or scaled differently relative to the size standards in the two figure versions [see below].

Control lanes and accompanying size standard positions in "Figure 3a" from Dr. Ward's computer (left) and in published Fig. 1A of paper #8 (right). Note positions of dark spots in each gel image.

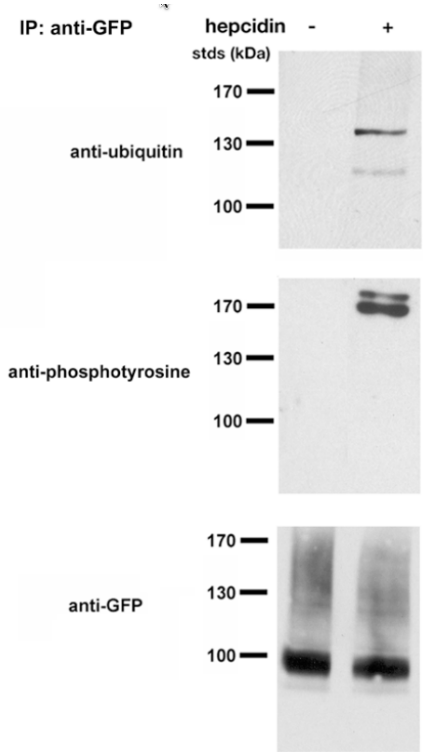


This last discrepancy illustrates the uncertainties introduced by splicing gel lanes: The MW markers on at least one of these figures are incorrect and misrepresent the data. Which, if either, of the images is the correct one? Can we be certain that the MW markers are correct for the antiphosphotyrosine bands in the published figure?

Two later draft versions of Fig. 1A of paper #8 were found on Dr. De Domenico's hard drive [files 8-1A-2 and -3, see below].



File 8-1A-2, dated 7-29-2006; author = Jerry Kaplan



File 8-1A-3, dated 12/28/2006; author = dianeward

The anti-ubiquitin and antiphosphotyrosine panels in these figures are identical to one another and to those in the published figure. However, the anti-GFP blots are different from one another and from those in the earlier "Fig. 3A" version mentioned above. The image dated 12/28/2006 contains the published anti-GFP blot. The Committee was unable to determine whether the three anti-GFP blots represented repeats with the same immunoprecipitated samples purportedly analyzed in the other panels or whether some or all of them came from unrelated experiments.

CONCLUSIONS

The act of splicing gel lanes in digital images is a matter best left to the two journals in question. The splices in the figures of paper #7 would be readily apparent to casual readers and should have been to reviewers and journal editors at the time. The splices in the figures of paper #8 are more difficult to discern and could conceivably have gone unnoticed during the review and production process. However, if this practice was explicitly proscribed by the journals' instructions to authors at the time, the journals and their reviewers should have been more vigilant.

The larger issue raised by these splicing episodes, particularly those involving negative control lanes, is whether the images presented are accurate representations of the experimental results. Dr. De Domenico asserted that the controls were run at the same time, but for reasons unclear to the Committee, on different gels from the matching experimental samples. Moreover, samples from the same experiment were not necessarily run on the same gel. This explanation defies common sense and accepted scientific practice, but the Committee cannot discount the possibility that it could be true. Nevertheless, the fact that negative control lanes in other gel figures of the same papers were not spliced does cast reasonable doubt on Dr. De Domenico's explanation and, consequently, on the validity of the experimental results reported in these two papers.

The Committee found multiple errors and discrepancies in the figures of papers #7 and #8 that cast doubt on the accuracy of the representation of data in both papers. In Fig. 4E of paper #7, gel bands labeled as an anti-tubulin western were published upside-down. In Fig. 3D of paper #8, several of the same band images were

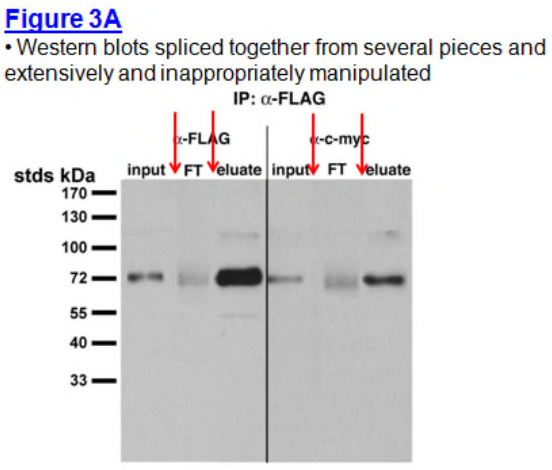
published in correct orientation and labeled as an anti-GFP western. In addition, Fig. 1A of paper #8 contained several gel images of dubious provenance and inconsistent labeling, when compared to draft versions of the figures found on Drs. De Domenico's and Ward's computer drives. It appears that Drs. De Domenico and Ward had full control over presentation of the experimental data and both of them must be held responsible for the misrepresentations that have come to light. The Investigation Committee considers it unlikely that these errors were due to simple carelessness during the preparation of the figures for publication. The Committee is also struck by the conspicuous failure of all coauthors, particularly Drs. Ward and Kaplan, to catch and correct these mistakes before publication.

Paper 9

Citation: De Domenico, I, McVey Ward, D, Musci, G and Jerry Kaplan
Evidence for the multimeric structure of ferroportin. *Blood* 2007 Mar 1;109(5):2205-9

Background
After completion of the Inquiry Investigation, the University received a new communication on 3-4-2012 from the complainant regarding an irregularity in Dr. De Domenico's publication in Blood 2007 109(5):2205-9, "*Evidence for the multimeric structure of ferroportin*". The concern regarded a Western blot in Figure 3A that was spliced together from separates images. In a communication to Dr. Dunbar the Editor of Blood, Drs. De Domenico and Kaplan acknowledged that the lanes were digitally spliced into the blots, but stated that the lanes were added to show the efficacy of the immunoprecipitation and that there was no intent to mislead the reader. They further stated that they were not aware of the splicing rules at that time and thought that splicing was an accepted practice at the time the paper was submitted. The University Research Integrity Office asked Blood for clarification of the editorial policy regarding splicing in 2006. The Editorial Director noted "Our instructions for authors at that time were not as detailed as they are now, but they were clear that no specific feature within an image was allowed to be moved or enhanced, and Blood was one of the first biomedical journals to have any such policy at the time. That policy would have applied to cutting out a gel lane or replacing any part of the original gel without indicating it clearly in the image itself (by vertical black lines) as well as in the legend". Drs. De Domenico and Kaplan clarified the experimental protocol stating that "two different gels were run BUT the samples used were from the same experiment". "The figure /experiment did not violate the splicing policy at the time as the samples were from the exact same experiment, probed with the same antibodies at the same dilution". Dr. Dunbar questioned why the samples were "not simply run on the same gel in the first place, if they were part of the same experiment". In the end, Dr. Dunbar decided not to post a Notice of Concern until the University Investigation Committee completes its examination of the notebooks and original films describing these experiments.

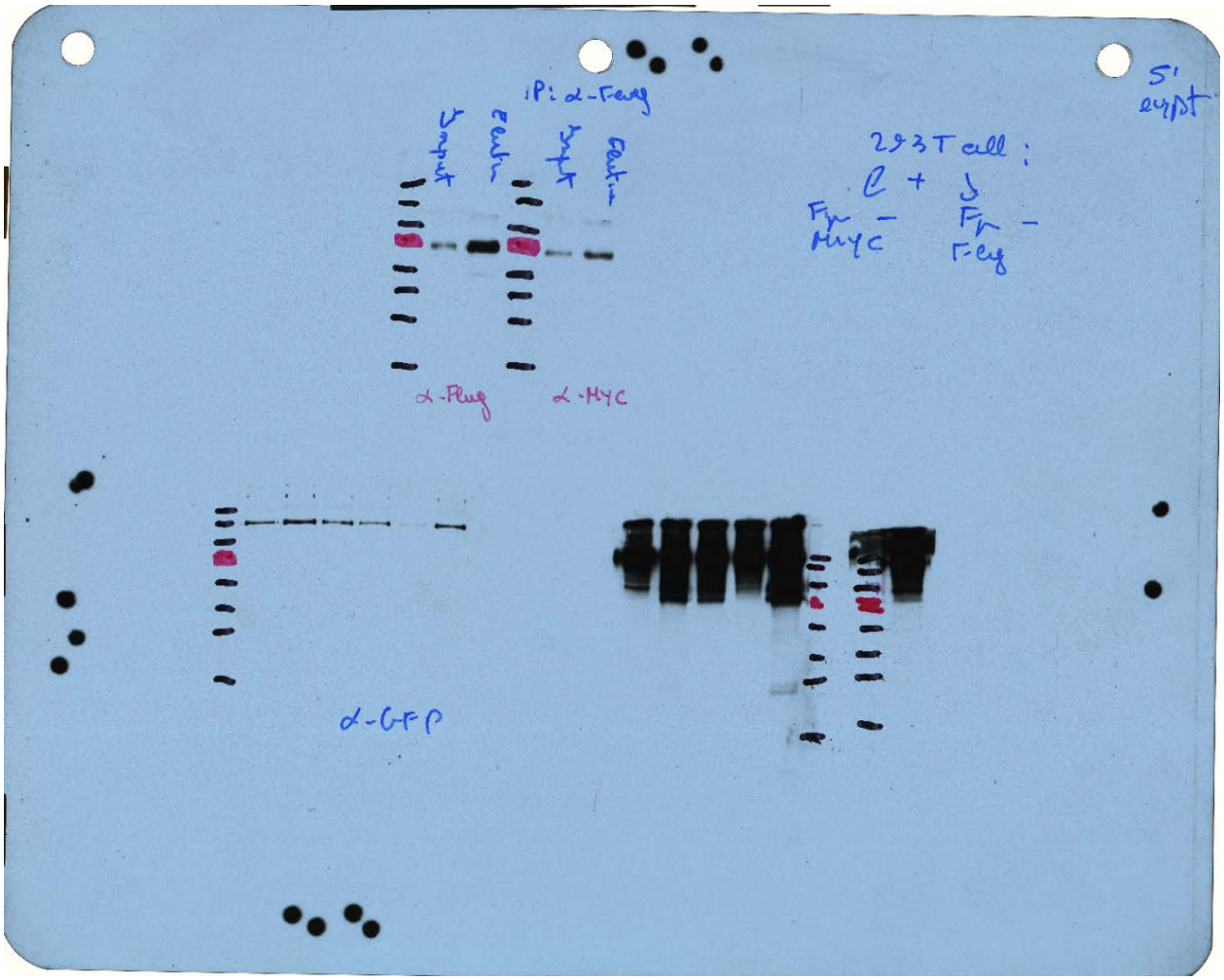
Figure 3A Allegation
The center lanes in the Western blots in Figure 3A are digitally spliced together from separate gels and inappropriately manipulated. An annotated copy of Figure 3A from the complainant with arrows indicating spliced lanes is shown below.



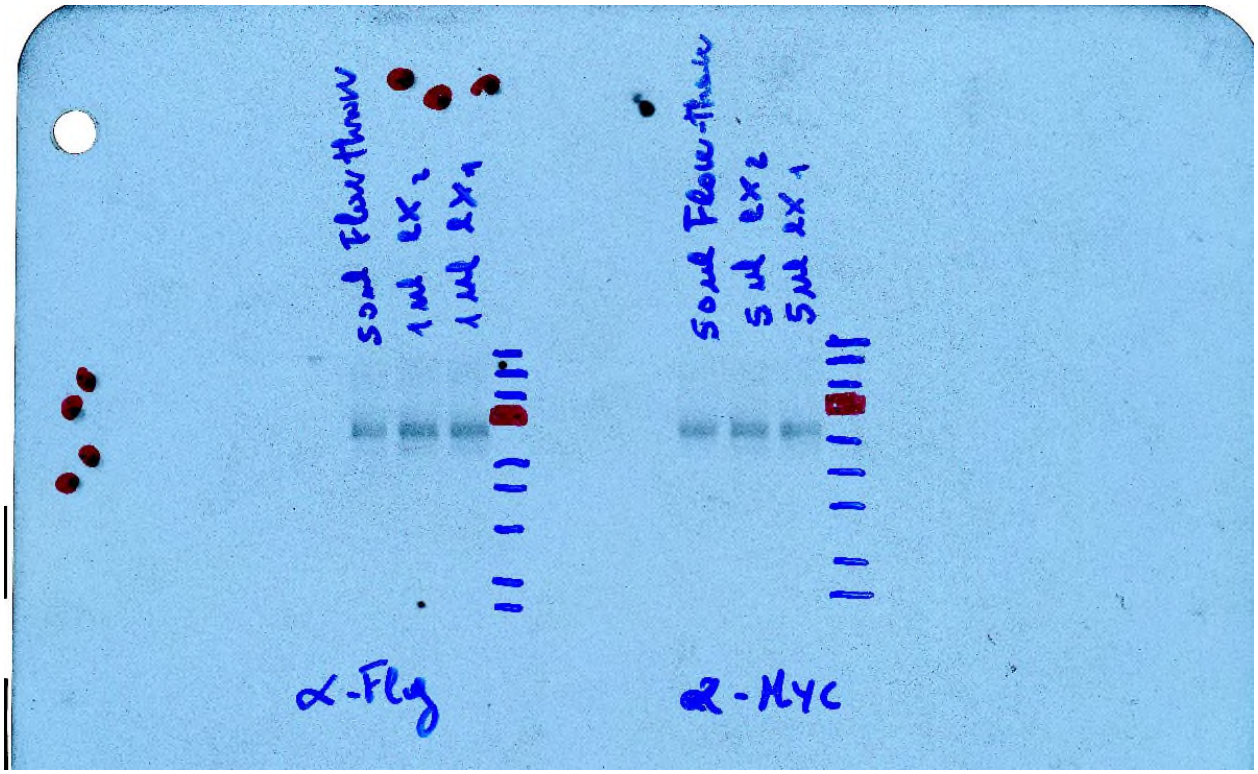
Investigation Committee Findings: The Investigation Committee examined the original films, scans and notes for Figure 3A found in Dr. De Domenico's notebook and computer hard drive. Dr. De Domenico also provided the original films used to assemble Figure 3A. The Investigation Committee focused on determining whether the evidence supports Drs. De Domenico and Kaplan assertion that the flow-through (FT) samples originated from the same experiment as the input and eluate samples, and that the samples were processed at the same time.

The original notebook films for the input and eluate samples (below, top left image) and flow-through samples (below, Flow-through film) are shown below. The input/eluate film shows that the α-Flag and α-Myc blots were processed at the same time and correspond to the published figure. The notes associated with this experiment were found in Dr. De Domenico's notebook. The flow-through film shows the published flow-through samples, but it is unclear which lanes were spliced into the published figure.

Input/Eluate film:



Flow-Through film:

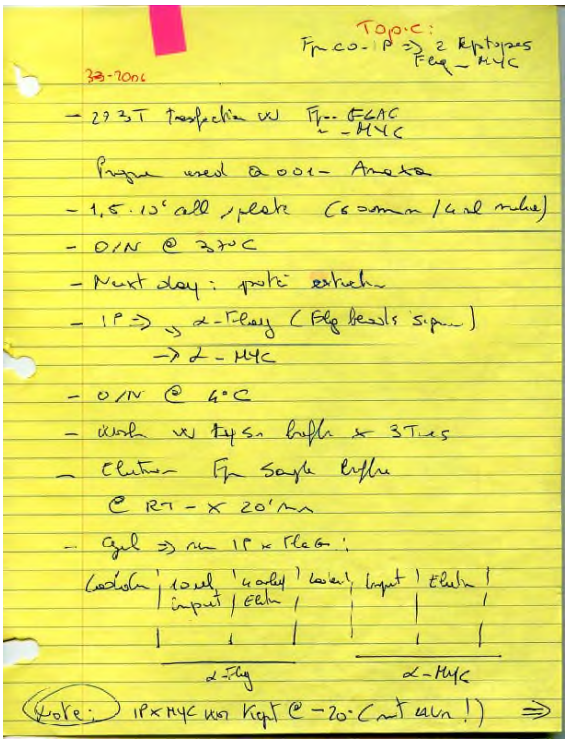


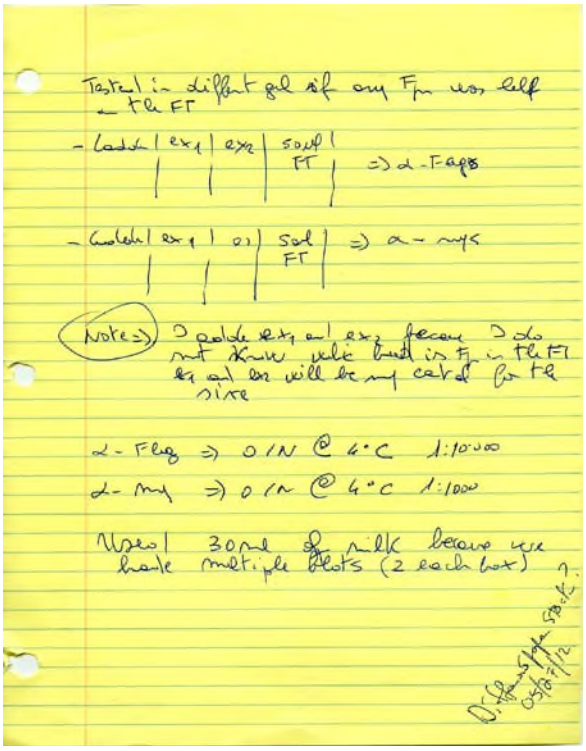
Comparison of the original films and notebook notes shows the following inconsistencies:

1. The input/eluate film is marked with an exposure time of 5 minutes (top right corner of film), whereas no exposure time is marked on the flow-through film. Without an exposure time on the flow-through film, it is impossible to compare band intensities with samples on the input/eluate film.

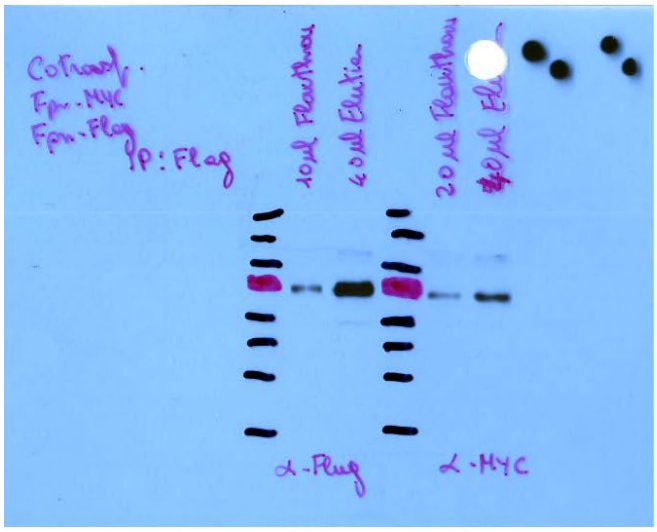
2. The notes for the input/eluate experiment indicate that it was carried out on 3-3-2006, but the film itself is not dated (below, left). The notes for the flow-through experiment are on a separate notebook page following the input/elate notes and are not dated (below, right). The notes for the flow-through experiment indicate that "30 ml of milk [were used] because we have multiple blots (2 each box)". This level of detail is not observed in the notes for other Western blots, and appears to echo the *Blood* Editor's request for confirmation that the blots were processed at the same time. This is also in contrast to discussions during interviews with Dr. De Domenico, in which she explained that her general approach was to not write specific details for each experiment in the notes that accompany films, because these details are to be found in standard protocols that are kept in a separate binder in the lab.

3. The handwritten markings on the films for the flow-through and input/eluate samples are different. The markings for α-Flag and α-Myc on the input/eluate film are red and molecular weight markers are black, while markings for α-Flag, α-Myc and the molecular markers on the flow-through film are blue. If the blots were processed at the same time, it would be expected that the labels on the films would be the same.

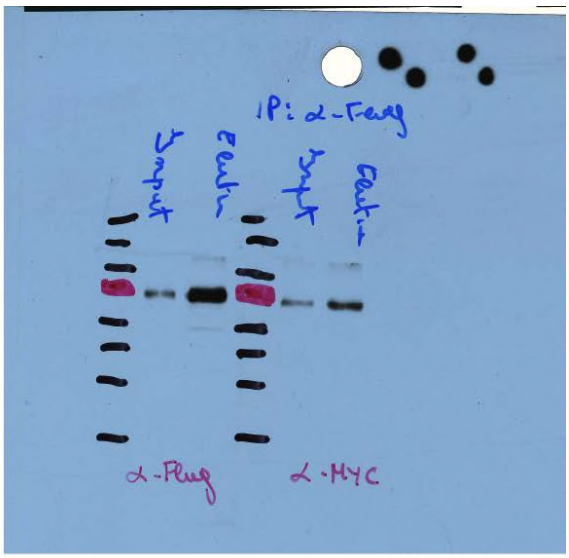




The Investigation Committee also examined a scan (file 9-3A-1) of the film found on Dr. De Domenico's computer hard drive (below,left scan), and compared it to the film in the notebook (below, right film). The scan has a create date of 4-28-2006. As mentioned above, the original film in the notebook does not have a date, but the film is associated with notes dated 3-3-2006.



Computer version



Notebook version

Examination of the scan with the film currently in the notebook used for publication show two inconsistencies:

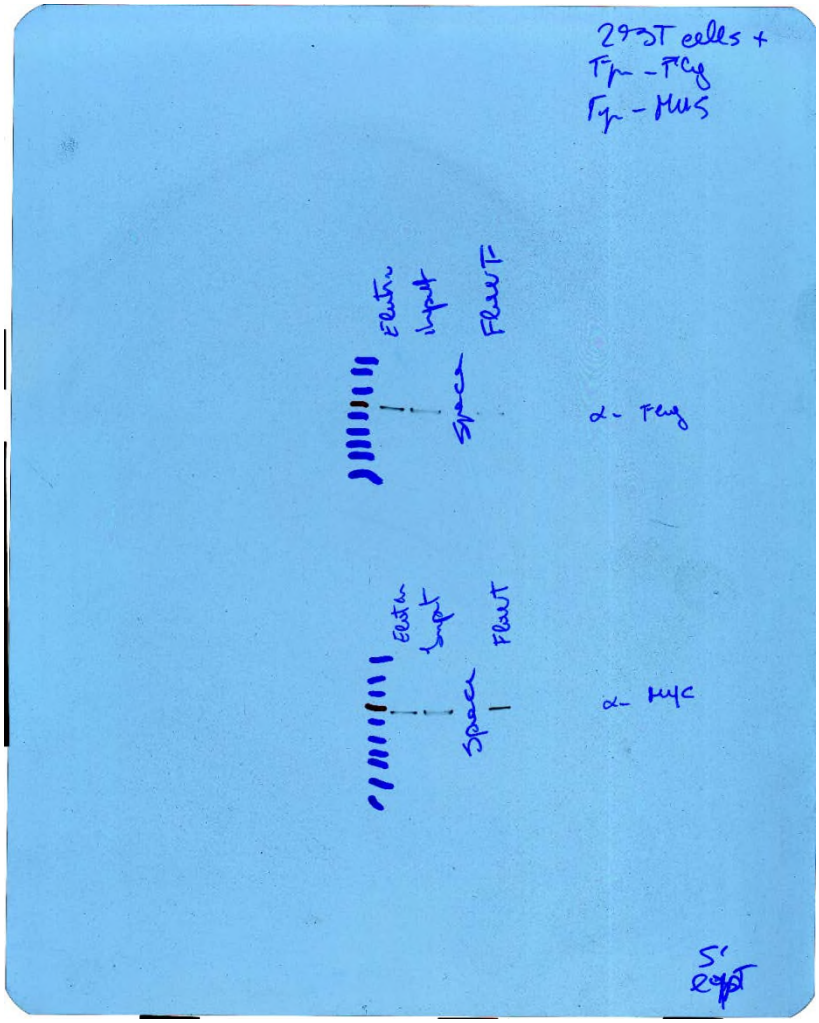
1. The lanes on the scan from the hard drive and the film are differently labeled. In the scan from 4-28-06 (left film scan), the two lanes are labeled "flow through" and "elution", while the handwritten labels now on the film indicate that the first lane contained the input sample for the column. In the published figure, this lane is also labeled "input".

2. The legend in the published figure indicates that “The volumes of the input and flow-through analyzed were twice that of the eluate “. The labeling in the scan (left) indicates that the α-Flag “Flowthrou” sample is 4-fold less than the eluate sample.

Responses from the Authors: The Investigation Committee interviewed Dr. De Domenico on July 17, 2012 to discuss the allegation regarding Figure 3A. In a PowerPoint document, Dr. De Domenico stated that she “did not make the figures for this publication, she has not digital images that belong to this publication. Second, the gels were run at the same time on 3-3-2006 according to the notes. Furthermore, the notes reported that the gels were incubated in the same container with the antibodies. Therefore, we can be sure that the antibodies had the same activity and that protein degradation due to storage was not an issue. Since the gels were run on the same day, from the same experiment and probed at the same time with the antibodies we felt comfortable generalizing about the amounts. Dr. Ward found two version[s] of this image one which the Flow Through was not shown and a second one with the addition of the Flow Through. This was added to show the efficacy of the immunoprecipitation”.

The Investigation Committee questioned Dr. De Domenico further regarding experimental protocols related to Figure 3A. Dr. De Domenico stated that “she did not make the figures”, but that she ran the gels, and that she or the technician scanned the gels. The films were then given to Dr. Ward for assembly of the final figures. The Committee asked Dr. De Domenico whether the flow-through and input/eluate samples, which were run on separate gels, were processed at the same time. “She said yes”. Dr. De Domenico stated that the Western blots were exposed for 30 sec, 2 min and 5 min. The Committee found one exposure (5 min) for the input/eluate film in the notebook. The flow-through film did not contain an exposure time. No other exposures were found in the notebook for either blot. The Committee asked Dr. De Domenico whether the films had dates. “She said there are some do and some don’t, but if they don’t have dates they are [from] the technicians.” She explained “that now she puts the date on [the films] when the technician gives her the film”. The The Committee asked Dr. De Domenico to explain why the scan found on the hard drive was labeled differently than the original film found in the notebook. She said the “flow through label [in the hard drive version] was a mistake, and that it should be input”. The Committee also questioned why the volumes of samples indicated in the hard drive scan and notebook films differ. Dr. De Domenico “agreed that this did not make sense”, and said that “she does not have any comment on this”.

Dr. De Domenico showed a replicate experiment dated 3-5-2006 in a PowerPoint file. This experiment contained input, flow-through and eluate samples on one gel. The slides in the PowerPoint slide were made from photographs of the notebook page and film, but a scan of the film was prepared later by a Committee member and is shown below:



As the notes do not indicate the amount of protein in each sample that was loaded on the gel, it is difficult to compare this result with the published one. It should be noted, however, that the band intensities for the eluate samples, relative to the input and flow through samples, are much smaller than indicated in the published figure. For the lower blot on the film above (labeled anti-myc), the band for the flow-through sample appears to be at least at least as intense as that for either the input of eulate.

The Committee interviewed Dr. Ward on July 2, 2012 to get a better understanding of how the figures were assembled for publication. The Committee asked Dr. Ward to provide an overview of her role in the lab. Dr. Ward said that she and Dr Kaplan design and analyze the experiments, and that historically her primary role is to design figures and edit manuscripts. Dr. Ward was shown a list of the papers provided by Dr. De Domenico in a previous meeting that highlighted papers in which Dr. Ward made figures for. Dr. Ward was asked if she made the figures for the four publications published in 2007. She replied “yes, this would be true”. Further, she stated “that she performed none of the science”. Dr. Ward said that she discontinued making figures for Dr. De Domenico after 2007. Dr. Ward was shown a Figure 3A and was asked if she made it. She said “she thinks so”. Further she said “the figure would have been made on what Ivana provided to her”.

Conclusion

The original allegation concerned the inappropriate splicing of lanes from one gel into another gel. The respondents acknowledged the splicing and stated that the intent was not to mislead the reader. The Committee noted that the splicing is obvious, and that the respondents were not attempting to hide the splice. The more important concern was whether the input and eluate samples were from the same experiment as the

flow-through samples and whether the samples were processed at the same time. This is critical in order to compare the relative amounts of protein in each sample.

The Committee examined notes and original data from Dr. De Domenico's notebook and computer hard drive. Several inconsistencies were identified.

1. The Committee found dated notes associated with the input/eluate samples, but the film is not dated. The flow-through notes and film are not dated. In addition, there is no exposure time on the flow-through film making it impossible to compare the band intensities in the two experiments.

2. The relabeling of the hard drive and notebook versions of the film calls into questions the actual identity of the sample in the published figure.

3. These inconsistencies do not allow the Committee to conclude that *the input/eluate and flow-through samples were from the same experiment and processed at the same time.* The relabeling of the samples corresponding to "flow through" in the initial scan to "input" for the published figure raises further doubts about the actual identity of these samples. Based on the inconsistencies in the execution of the experiments and in the preparation of the figures, little confidence can be placed on the accuracy of the published data.

PAPER 10

Citation: De Domenico I., Nemeth, E., Nelson, J.M., Philips, J.D., Ajioka, R.S., Kay, M.S., Kushner, J.P., Ganz, T, Ward, D.M. and Kaplan, J. (2008) The hepcidin-binding site on ferroportin is evolutionarily conserved. *Cell Metabolism*. **8**, 146-156.

Background: This paper was not mentioned in either the original allegations of November 2011 or the expanded ones submitted by Dr. Thomas Ganz on 3-4-12. However, Dr. Ganz submitted an additional allegation questioning the integrity of this paper on 5-25-12. No specific irregularities in the published paper (of which Dr. Ganz was a co-author) were identified, but Dr. Ganz stated that important results could not be repeated in other laboratories, including his own. In particular, Dr. Ganz reported that a biochemical assay for hepcidin, based on binding to a peptide fragment of its receptor, was not reproducible. After consulting with the NIH Office of Research Integrity, Dr. Botkin instructed the Investigation Committee to examine this paper along with those identified in the earlier allegations.

After examining the paper and allegations, the Committee initially concluded that it would be extremely difficult to assess the validity of the hepcidin assay from the review of existing data and that this was issue that was best resolved through additional experiments in other laboratories. None the less, the Committee noted that the error bars in many of the graphs were very small, and chose to investigate these further as part of its investigation of the more general question of error bars. In the course of examining the data files, the Committee identified additional problems concerning circular dichroism (CD) spectra in one of the figures and NMR data not included in the published paper.

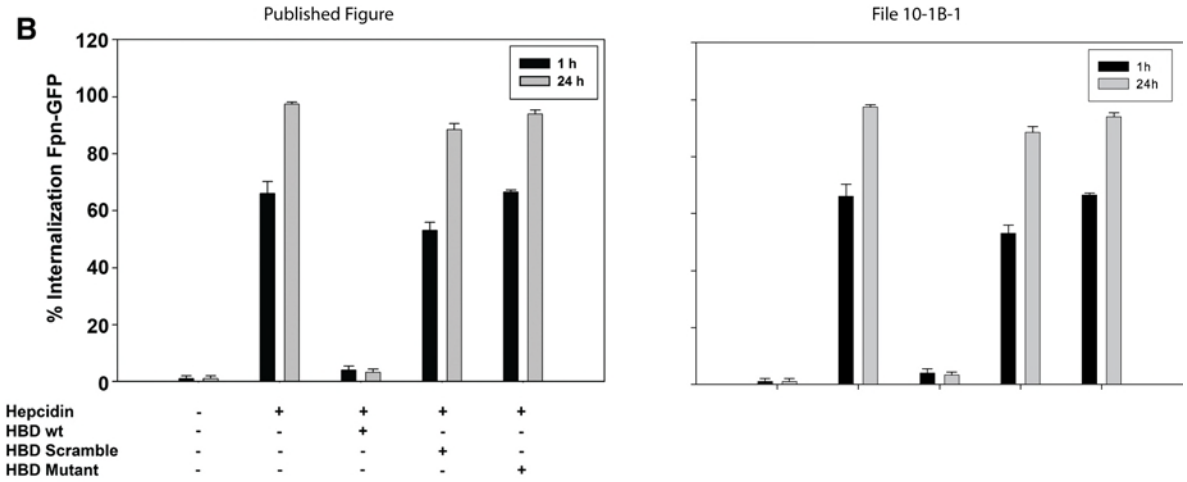
Error Bars in Graphs

Allegation: The error bars in several of the published figures are very small, often a few percent, or less, of the measured values. Errors of 10% or greater are typically reported for biochemical assays. Except for Fig. 1B, discussed below, the legend for each of the data-containing figures of the paper contains the statement “All experiments were repeated a minimum of five times, and the error bars represent the standard error of the mean.”

Investigation Findings: After obtaining copies of Dr. De Domenico’s computer drives, the Committee attempted to trace the analysis leading to the error bars shown in this and other papers. Towards this end, the Committee asked Dr. De Domenico (via an E-mail exchange on 7-26-12) to identify the relevant files on the drives. For the six data-containing figures in paper 10, Dr. De Domenico identified a total of 37 files (either by file name or by the enclosing folder names).

One of the Investigation Committee members systematically examined all of the files listed by Dr. De Domenico and attempted to identify those from which the published figures were derived and to determine how the error estimates were calculated. All of these files were generated with the program SigmaPlot and typically contained a single data table and a graph of the data. Although Dr. De Domenico explained to the Committee that the means and errors were usually calculated using Excel spreadsheets, none of the files that she identified were from Excel, and the Committee was unable to find such files.

Figure 1B: This graph represents manual counting of cells and the figure legend states that “The data are reported as the standard error of the mean and were determined by counting ten fields containing 20–30 cells/field.” No files associated with this figure were identified by Dr. De Domenico, but the Committee found a SigmaPlot file (10-1B-1) on her computer that matches the published figure, as shown below:



The data from this file are listed below:

VAR1	1h	VAR3	24h	VAR5
-Hep	1.000000	1.000000	1.000000	1.000000
+Hep	66.000000	4.242641	97.500000	0.707107
+Hep+HBD wt	4.000000	1.414214	3.250000	1.060660
+Hep+HBD Scramble	53.000000	2.828427	88.500000	2.121320
+Hep+HBD Mutant	66.500000	0.707107	94.000000	1.414214

The numbers in the columns labeled “1h” and “24h” correspond to the values plotted in the graph, and those in the columns “VAR3 and VAR5” correspond to the error bars. Note that all but one of the values are exact multiples of 0.5, as expected for the average of two integers. The one exception to this pattern is a multiple of 0.25.

Though it is conceivable that the values were rounded in this way after calculating the average, all but one of the error estimates also appear to have been generated from two integers. For a sample of just two values, x_1 and x_2, the expression used to calculate the sample standard deviation reduces to:

$$SD = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}} = \sqrt{\frac{(x_1-x_2)^2}{2}} = \frac{|x_1-x_2|}{\sqrt{2}}$$

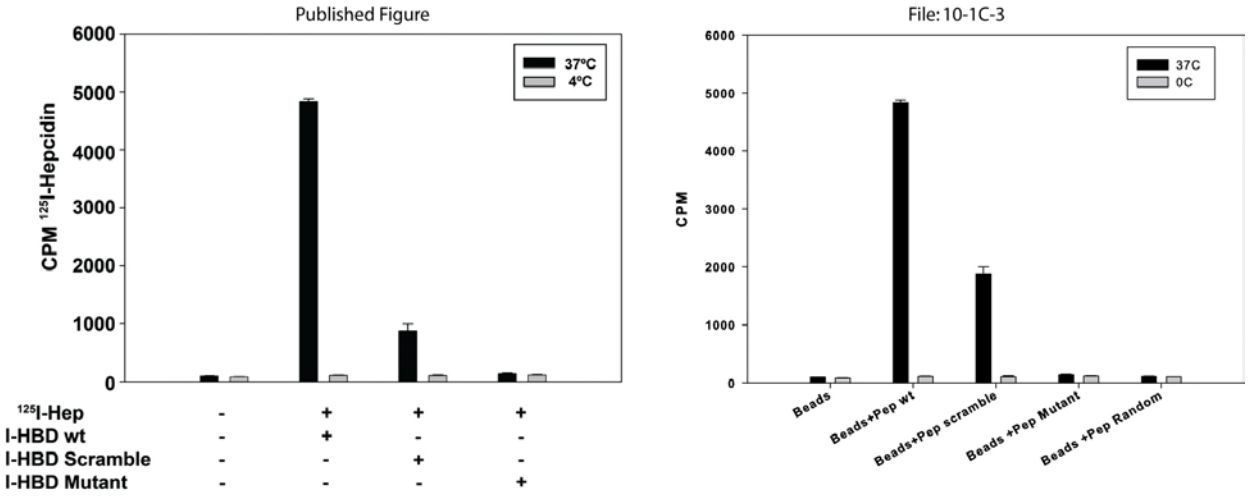
For two integers, the square of the standard deviation will always be an integer multiple of 0.5. The errors found in the SigmaPlot file and their squares are listed below:

VAR3	VAR3^2	VAR5	VAR3^2
1.000000	1.000000	1.000000	1.000000
4.242641	18.000000	0.707107	0.500000
1.414214	2.000000	1.060660	1.125000
2.828427	8.000000	2.121320	4.500000
0.707107	0.500000	1.414214	2.000000

All but one of the means and errors represented in Figure 1B appear to have been calculated from two integral values, in clear contradiction to statement in the legend.

Figure 1C. Two files (10-1C-1 and -2) were identified by Dr. De Domenico, but neither of these corresponded to the published figure. Another file (10-1C-3) that appeared to be closely related to the figure was, however,

found by the Committee. This was one of the few files containing any replicate data and the graph from it is reproduced below along with the published figure:



The data and error bars for the control sample, the wt hepcidin and mutant hepcidin appear to be similar or identical in the two plots. However, the signal for the "scrambled" hepcidin is more than two-fold smaller in the published figure. There is also an additional pair of values for "Beads +Pep Random" in the unpublished figure.

The data from the SigmaPlot file are reproduced below:

VAR1	37C	VAR3	0C	VAR5
Beads	95	2.828427125	81	4.242640687
Beads+Pep wt	4829.5	47.37615434	108	5.656854249
Beads+Pep scramble	1879.5	118.0868325	106	11.3137085
Beads +Pep Mutant	139	11.3137085	113.5	9.192388155
Beads +Pep Random	110.5	3.535533906	101.5	0.707106781
Beads	93	97	84	78
Beads+Pep wt	4796	4863	112	104
Beads+Pep scramble	1963	1796	98	114
Beads +Pep Mutant	147	131	120	107
Beads +Pep Random	113	108	101	102

The lower group of rows appears to contain data for two replicates, with the second and third columns containing the data for 37° C and the last two columns data for 0° C (as labeled in the unpublished graph). The upper lines appear to be the means and errors calculated from the two data sets. These values were reproduced using the AVERAGE and STDEV functions of Excel.

It thus appears that error bars in this figure represent the standard deviation of two values, rather than a standard error of the mean of a minimum of five experiments, as indicated in the figure legend. In addition, it is not clear how the value shown in the published figure for the scrambled peptide is related to the data in this file, if at all.

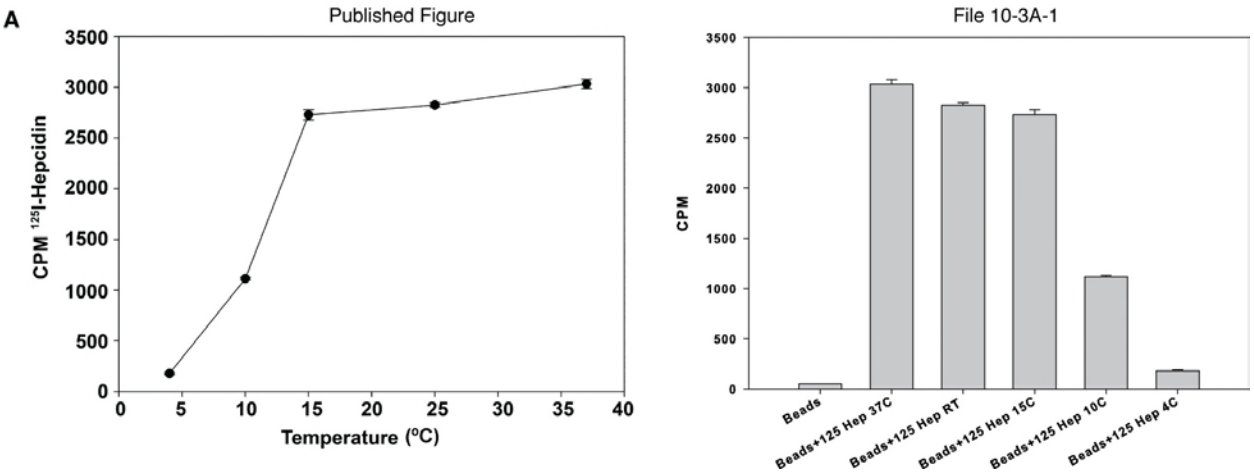
The errors shown in both the published and unpublished graphs are very small, ranging from less than 1% to 10.7% of the measured values. Especially since these data represent raw counts per minute, without any normalization, it is very unlikely that such small variations could be obtained from independent experiments. Simply aliquoting individual samples of the derivitized beads is expected to generate greater variation.

Figure 1D. Two files (10-1D-1 and -2) were identified by Dr. De Domenico. Both appeared to be related to the published figure but contained fewer data points, which were expressed in counts per minute (CPM), rather than as percentages in the published figure. Neither file included replicate data or error estimates.

Figure 2B. Two files were identified by Dr. De Domenico, but only one (10-2B-1) appeared to be related to the published figure, but was not identical to it. The file contains two data sets, but no calculations of means or errors. Another file (10-2B-3), identified by Dr. De Domenico as that for Figure 2D was found to contain data that appeared to be closed related to this figure, as indicated by the labeling related to peptides. The data did not match the published figure exactly, however. The file included error estimates, but no replicate data.

Figure 2D. One file (10-2B-3) was identified by Dr. De Domenico, but the data and graph in this file resemble that of Figure 2B, as noted above. The Committee did not find any other files related to this figure.

Figure 3A. No files associated with this figure were identified by Dr. De Domenico, but two related files were found by the Committee. One of these (10-3A-1)) contained data that matches very closely that in the published figure, though the form of the graph is different, as shown below:

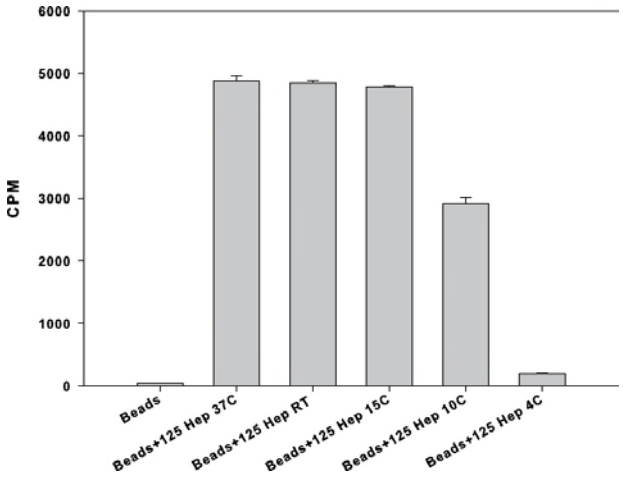


The data from the SigmaPlot file are shown below:

Beads	45	49
Beads+125 Hep 37C	3065	3001
Beads+125 Hep RT	2841	2811
Beads+125 Hep 15C	2693	2763
Beads+125 Hep 10C	1123	1104
Beads+125 Hep 4C	186	172
Beads	47	2.828427125
Beads+125 Hep 37C	3033	45.254834
Beads+125 Hep RT	2826	21.21320344
Beads+125 Hep 15C	2728	49.49747468
Beads+125 Hep 10C	1113.5	13.43502884
Beads+125 Hep 4C	179	9.899494937

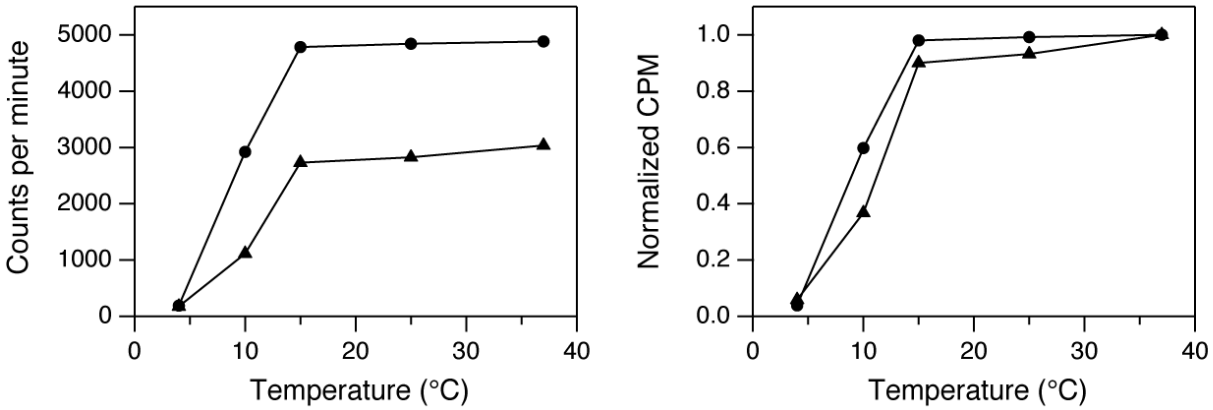
The upper rows appear to contain two data sets, and the lower rows the average and errors calculated from the two sets. The values in the lower rows were reproduced using the AVERAGE and STDEV functions in Excel.

A second file (10-3A-2) contained a very similar graph:



This file also contained data organized as described above, with two data sets, and means and errors calculated from these. Though the labeling and pattern of the data are very similar in the two graphs, the plotted values differ significantly. This difference would reasonably be expected for two fully independent experiments, but are clearly not consistent with the very small error bars shown in the published figure.

The two sets of data are plotted together in the figure below:



The raw CPM are plotted on the left, while the values on the right are normalized to the values measured at 37° C for each experiment. After normalization, there is a reasonable degree of reproducibility for this type of experiment, but the differences are still significantly greater than indicated by the error bars in the published figure.

Figure 3B. Two files (10-3B-1 and -2) were identified by Dr. De Domenico. These files contain data that appeared to be similar to that shown for 4° C in the figure. Another file (10-3B-3) found by the Committee contained the full set of data and a plot similar to the published figure, the major difference being that the results were expressed as raw counts per minute, rather than percent binding. The data table included what appear to be two data sets, with means and errors calculated from these. Yet another file (10-3B-4) found by the Committee contained a graph that matched the published figure, with error bars but no replicate data.

Figure 3C. Two files (10-3B-1 and -2) were identified by Dr. De Domenico. These files each contain a single data set expressed as raw CPM, rather than "% Binding" as shown in the figure. Both of these data sets more closely match the curve for "HBD+NEM" than the one labeled "Complex+NEM" in the published figure, but do not match it exactly. There were no replicate data sets or error estimates found in these files. The Committee identified another SigmaPlot file (10-3C-3) that did contain a graph that matched the published figure, with error bars, but this file did not contain replicate data sets.

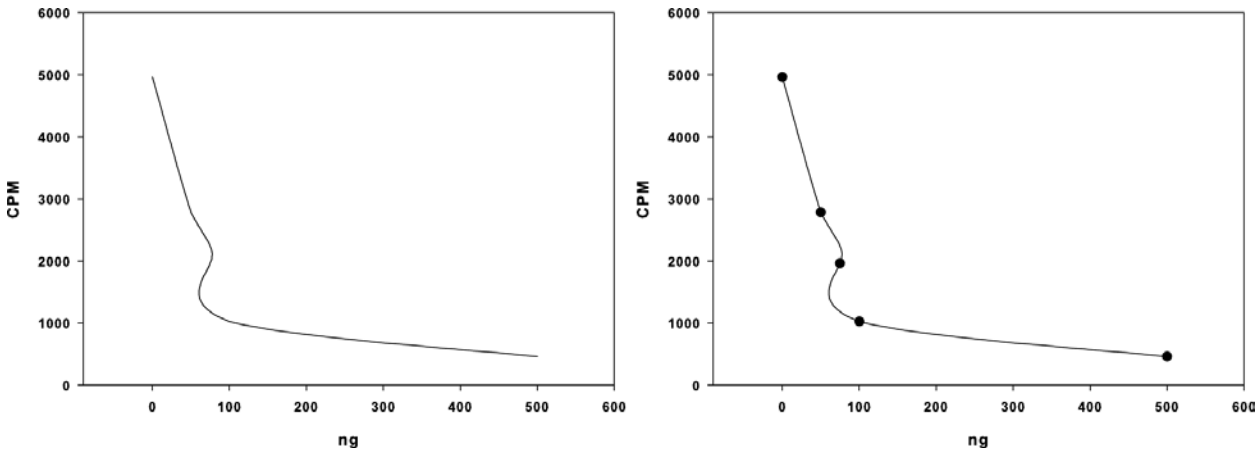
Figure 4. Panels A and C of this figure are CD spectra, for which error bars would not usually be expected, and Panel B represents ^{125}I counts for column fractions, with no error bars shown. Dr. De Domenico identified one file for this panel, which contained data and a graph closely matching the published figure. The data table included only one data set and no error estimates. That there are no errors for the CD spectra, which are discussed further below, or the column profile is not surprising for these types of data, except in light of the statement in the legend that "All experiments were repeated a minimum of five times . . ."

Figure 5C. Dr. De Domenico identified three files and a directory containing another five files. One of the files (10-5C-1) contains a graph identical to the published figure. Another (10-5C-2) is very similar except that it does not include the data for *Xenopus*. Both of these files include error estimates, but no data replicates or indication of how the errors were calculated. The other files contain what appear to be data for individual experiments, but are not clearly identified. In none of the files did we find a compilation of replicate experiments or statistical calculations.

Figure 5D. Two files were identified by Dr. De Domenico. One of these (10-5D-1) closely matches the published figure. This file includes error estimates, but no replicate data or statistical calculations. The other file (10-5D-2) appears to be related to this experiment, but the vertical axis of the graph is labeled "CPM", while the primary data in this experiment was colony counts. This file does not contain any error estimates.

Figure 6A. For this figure, Dr. De Domenico identified a directory (labeled "Standard curves") containing seven SigmaPlot files. As suggested by the directory name, each of these files contained data and a graph apparently representing CPM for ^{125}I-labeled hepcidin bound to beads as a function of unlabeled hepcidin concentration. The seven files were labeled with monthly dates from June 2007 to June 2008. It is not known which of these curves was used to convert raw CPM data to hepcidin concentrations for specific figures in the paper, but the original manuscript was submitted on 2-25-08, and a revised version on 6-2-08. This directory did not contain any files corresponding to the graph actually shown in Figure 6A, but the Committee did find a file matching the published figure (10-6A-8). This file included error estimates, but no replicate data.

Though the standard curve files do not directly represent the final graphs shown in the figure, they were examined by the Committee as a means to assess the general features of the assay and its variability. One of the standard curves (from file 10-6A-4, June 2007.JNB) is shown below:

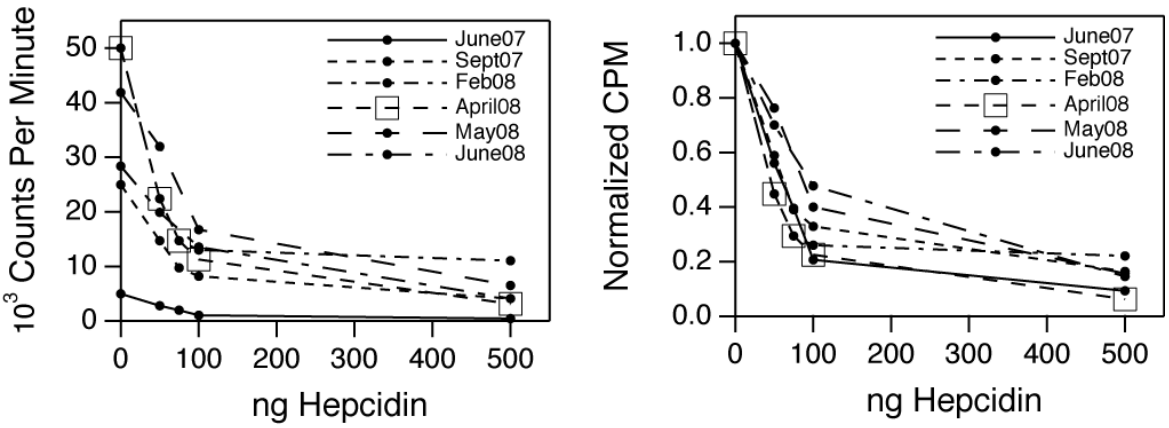


The graph as it appears in the SigmaPlot file is shown on the left. The curve was generated from 5 points (for 0, 50, 75, 100 and 500 ng) which have been added to the plot on the right. The curve appears to be a spline fit though the points. Similar spline curves was found for all but one (10-6A-7) of the seven files. If this form of the data was actually used for converting CPM to hepcidin concentration, interpolation would obviously be problematic.

Each of the standard curve files contained similar data, though some contained data for only 4 points: 0, 50, 100 and 500 ng. Although hepcidin quantities are expressed as only "ng" in the file, a protocol provided by Dr.

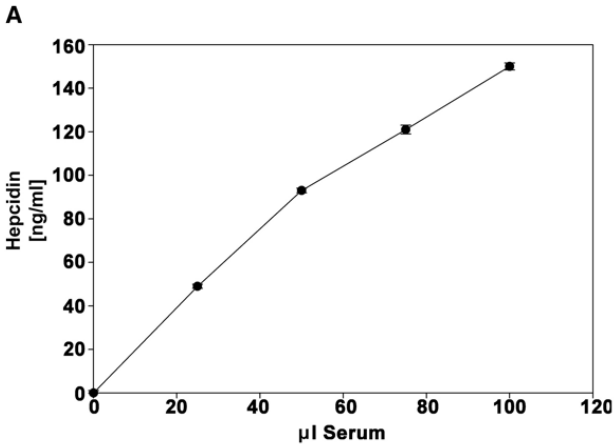
De Domenico specifies that the standard curves are made using stock solutions containing 0 to 500 ng/mL hepcidin, and we presume that the quantities shown in the files represent concentrations in these units.

The data from all seven files are plotted below:



The plots on the left are the raw CPM data found in the SigmaPlot files, while the plot on the right shows the same data normalized relative to the zero-hepcidin sample for each set. The following points may be relevant to the precision of the assay:

1. Even after normalization, there is considerable variation in the standard curves. This variation, seen using what are presumed to be well-defined, purified samples, does not appear to be consistent with the very small error bars shown in the published figures, especially those in Figure 6A, shown below:



2. The range over which the standard curves provide useful information is limited to values less than 100 ng. The reported concentrations of hepcidin plotted in Figure 6A extend to approximately 150 ng/mL.

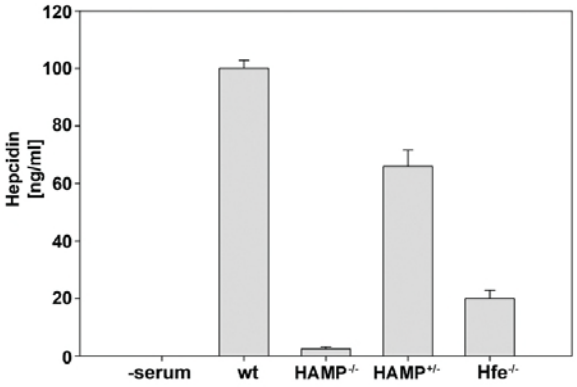
3. Two of the calibration curves, for February and April 2008, include identical values for 0, 50 and 75 ng, but not for the higher values, as listed below:

ng	Feb. 2008	April 2008
0	50000	50000
50	22369	22369
75	14698	14698
100	13001	11236
500	11023	3140

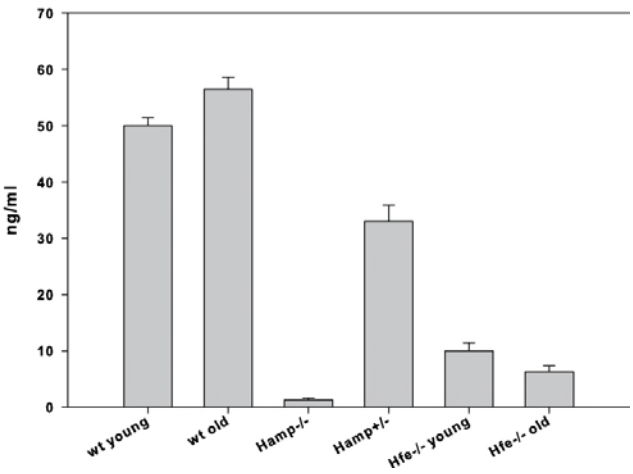
It is impossible to know the origins of these files, but one is clearly a manipulation of the other.

Figure 6C. Dr. De Domenico identified five files associated with this figure. Of these, one (10-6C-1) contained a graph similar to the published figure, as shown below:

Published Figure



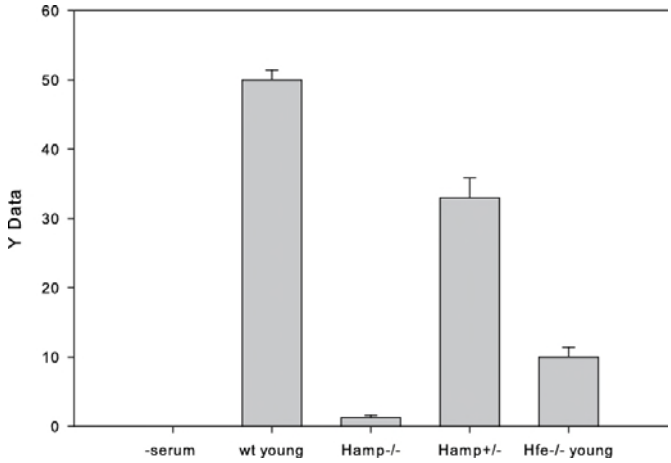
File 10-6C-1



There are two major difference between these two graphs:

1. The unpublished contains two more values than does the published one. It appears that the values for "wt" and "HFE-/-" in the published figure correspond to those labeled "wt young" and "HFE-/-" in the SigmaPlot file.
2. The values plotted in the published figure are very nearly twice those in the file.

Another file (10-6C-4) found by the Committee more closely matches the published figure, except that, again, the maximum plotted value is 50 rather than 100 ng/mL:

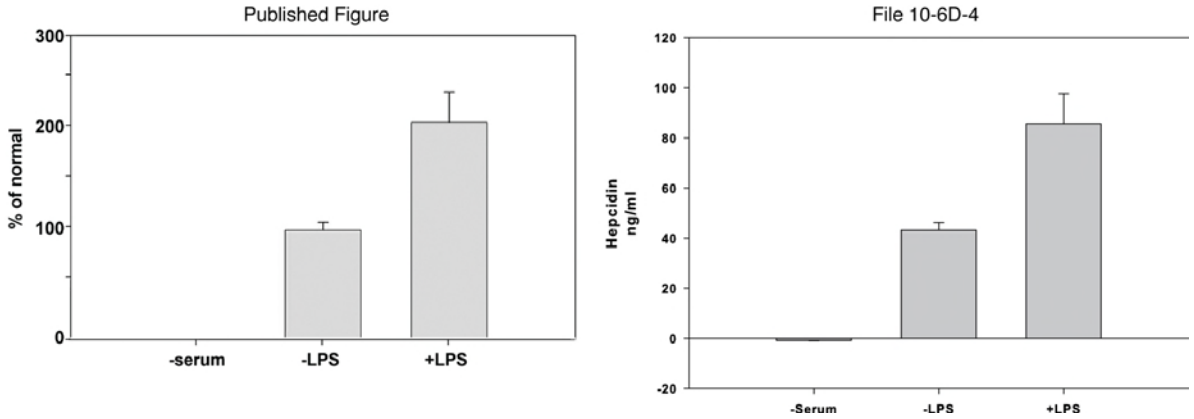


This file and the one identified by Dr. De Domenico contain the same numerical data, consisting of two data sets. The plotted means and errors were calculated from the two data sets.

Of the other four files identified by Dr. De Domenico, two (10-6C-2 and -3) were found to contain data related to different mice strains, but the relationship to the published figure was not clear, and the other two were found to contain data more closely related to Figure 6D (see below).

Figure 6D. Dr. De Domenico identified one file (10-6D-1) associated with this figure. This file was found to contain raw CPM data for what appeared to be a single experiment with two samples identified as "+LPS". As noted above, two other files (10-6D-2 and -3) were identified by Dr. De Domenico for Figure 6C, but were found to contain data similar to that found in file 10-6D-1, with CPM data for one –LPS and two +LPS samples. The numerical values in the three files were different, however, suggesting that they came from different experiments.

Another file (10-6D-4) was identified by the Committee and contains a graph similar to the published figure, except that the values are expressed as ng/mL, rather than "% of normal":



Adjusted for the normalization in the published figure, the data plotted in the two graphs appear to be identical. The SigmaPlot file contained only the values and errors, but these were found to be identical to those in file 10-6D-2, which included additional data from which the plotted values were derived. The data from file 10-6D-2 is listed below:

ng/ml	No Serum	VAR3	-LPS	VAR5	+LPS	VAR7
0	-0.913	0.086267027	43.106	3.122583546	85.70425	11.97025715
50						
75						
100						
500						

-0.913	0.086267027
43.106	3.122583546
77.24	2.709633186
94.1685	3.990203566

0	4923
50	2845
75	1982
100	1073
500	968

4881	Hep125	-0.852	-0.974
4327	-LPS	40.898	45.314
1844	+LPS	75.324	79.156
1344	+LPS	96.99	91.347

The boxes in the table above have been added to help clarify the inferred relationships among the various data:

1. The data in the top box matches exactly that in file 10-6D-4, which, in turn appears to correspond to the data shown in the published figure, before normalization.
2. The data in the lower left box may correspond to a calibration curve for the hepcidin assay.
3. The lower right box contains two data sets that were used to calculate averages and standard deviations in the middle box.
4. For "No Serum" and "-LPS", the values and errors in the top row (and the graph) are those from the top two rows of the middle box.

5. For "+LPS", the value and error were calculated from the two bottom average values in the middle box. Thus, the error bars in the final graph appear to have been calculated from two averages of two values each.
6. The significance of the data between the two boxes in the bottom section of the table is unclear. They could be raw CPM data used to calculate some of the data on the right, but not all of the values appear to be consistent with this.

It thus appears that the error bars in Figure 6D were calculated from two values for –LPS and, in an inappropriate way, from four values for +LPS. From the labels in the table it seems likely that the –LPS value came from a single sample, perhaps assayed or counted twice, while the +LPS value came from two samples. In any case, the evidence contradicts the statement in the legend that "All experiments were repeated a minimum of five times, and the error bars represent the standard error of the mean."

Response from Dr. De Domenico: As noted above, Dr. De Domenico provided information regarding the procedures for calculating the published mean values and errors, using Excel spreadsheets, and a list of files for the graphs in this paper. The issue of error bars was not discussed further with her during the interviews.

After submitting her responses to the initial draft of this report and being provided with the files used by the Committee, Dr. De Domenico was invited (on 11/26/2012) to respond to the allegations above regarding the error bars. Her response (submitted on 12/10/2012) included the following explanation of the error calculations (referring specifically to the example of Fig. 1C in Paper 10):

> "Each single value came from two-– - pulled independent experiments. In a technical terminology in a same tube for this particular experiment (or in the same well in case of an ELISA) two independent samples were pulled together. Therefore, the presence of four values is really 8 independent experiments."

The meaning of this statement is not entirely clear to the Committee, but it suggests that individual measurements were pooled and averaged, and then the errors were calculated from the averaged values. This is appears to be what was done for some of the data in Fig. 6D, described above. **This is an entirely inappropriate method for calculating error estimates and will, more often than not, lead to significantly reduced error estimates.** This calculation is also inconsistent with the statements in the paper.

Dr. De Domenico also indicated that many of the experiments were conducted by other lab members:

> "In cases where others in Dr. Kaplan's laboratory ran the experiment, Dr. De Domenico stored the data already analyzed by the individual who ran the experiment. However, she always saw the raw data generated by each individual. It is important to point out that Dr. De Domenico was not involved in the writing of each paper during her training. Drs. Kaplan and Ward piloted this."
>
> "It is also true that Dr. Kaplan never put all the repeats together when he was writing a manuscript but he used to analyze each experiment, daily, when it was carried out."

These statements leave a great deal of ambiguity as to who actually compiled data from repeated experiments and calculated averages and errors.

Conclusions regarding error bars: In total, the Committee analyzed computer files for 12 figure panels containing graphs with error bars. The findings can be summarized as follows:

1. Although Dr. De Domenico stated that the averages and errors were calculated using the program Excel, no files from this program containing data relevant to these figures were identified by her, or found by the Committee.
2. For seven of the figure panels, the origins of the error estimates could not be found in any of the SigmaPlot files identified by Dr. De Domenico or found by the Committee.

3. For four of the figures (1C, 3A, 6C and 6D), replicate data were found in SigmaPlot files and appear to have been used to calculate the averages and error bars. In each of these cases, two data sets were used (rather than the minimum of five specified in the figure legends), and the errors were calculated as standard deviations, rather than standard errors of the means, as indicated in the paper. It may be, as indicated by Dr. De Domenico, that the individual values are, themselves, averages of multiple measurements, a procedure that would tend to reduced the reported errors. These approaches are outside the standard practices of the field.
4. For the cases where two data sets were found, the nature of the replicates is not clear. For two of these (Figures 1C and 3A), the plotted values are raw CPM, making it very unlikely that such small deviations could arise from independent experiments. In one case (Fig. 3A) a file containing what appears to be a truly independent experiment shows much larger deviations than indicated in the published figure.
5. For one figure (1B), analysis of the averages and errors enabled us to infer that all but one of the data points (and errors) was calculated from two integer values each. The figure legend states that "The data are reported as the standard error of the mean and were determined by counting ten fields containing 20–30 cells/field."
6. Standard curve data examined by the Committee suggest that variability in the hepcidin assay is significantly greater than implied by the error bars.

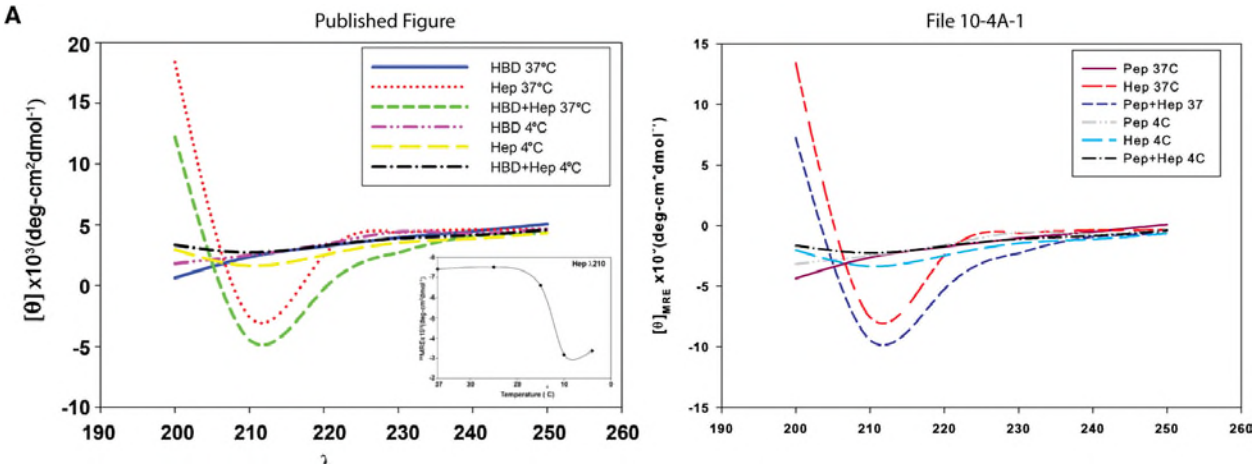
Collectively, the evidence supports the allegation that the published error bars do not accurately reflect the uncertainties in the measurements and that the calculations of the averages and errors were misrepresented in the paper. In addition, the published claim that the errors were calculated from a minimum of five experiments cannot be substantiated, and in some cases appears to be a clear **falsification**.

Circular Dichroism Spectra (Figure 4)

In the course of examining other data associated with this paper, the Committee discovered that the plots of CD spectra in panel A of Figure 4 were generated from data that had been extensively and inappropriately manipulated, and that those in panel C were likely fabricated.

Investigation Findings:

Figure 4A (main plot): Data and a plot corresponding closely to the spectra in panel A were found in a SigmaPlot file (10-4A-1) on Dr. De Domenico's computer. The published figure and the graph from the file are shown below:

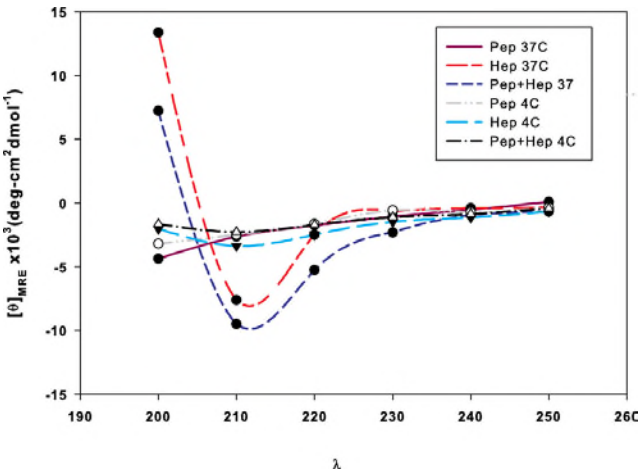


Aside from the colors of the curves, the major difference between this graph and the published figure is that the values in the published figure appear to be displaced upwards along the y-axis. The total range of the data appears to be approximately the same. No files were found that more closely match the published figure.

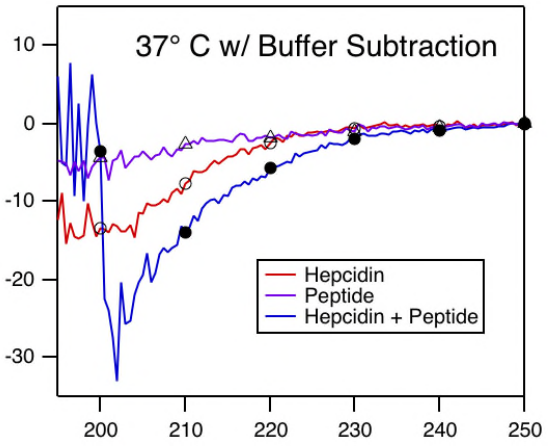
The data table in this file was discovered to contain data spaced at 10 nm intervals, as listed below:

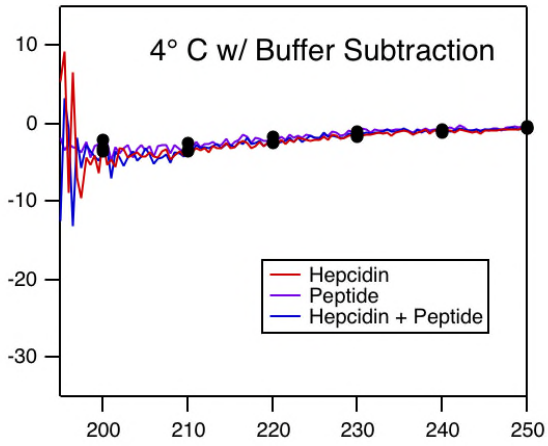
VAR1	Pep 37C	Hep 37	Pep+Hep 37	Pep 4C	Hep 4C	Pep+Hep 4C
200	-4.371	13.385	7.232	-3.184	-2.032	-1.66
210	-2.635	-7.6	-9.477	-2.489	-3.37	-2.277
220	-1.755	-2.471	-5.26	-1.615	-2.485	-1.715
230	-1.05	-0.659	-2.314	-0.574	-1.478	-1.111
240	-0.524	-0.391	-0.932	-0.72	-1.129	-0.859
250	0.084	-0.335	-0.656	-0.335	-0.656	-0.416

The curves in the graph appear to have been generated using a spline function in SigmaPlot. The curves shown above are reproduced below with markers for the individual points added:



This is a highly unusual way of presenting CD data, which are typically recorded at intervals of 1 nm or less. Further examination of the drive copied from Dr. De Domenico's computer identified files containing the primary data for this graph, both raw text files from the spectropolarimeter (files 10-4A-4 to -11) and Excel files (10-4A-12 to -16) in which background-subtraction calculations had been performed. As would be expected for CD spectra, these files contained closely-spaced data, with 0.5 nm intervals. These data were used by the Committee to generate the plots shown below, with the 4° and 37° C data plotted separately for clarity:



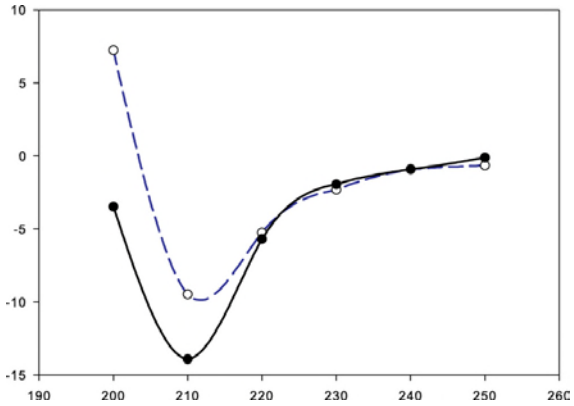


The data points corresponding to those used to generate the spline fit in the published figure are highlighted with circles..

Comparison of the published figure with the plot of the full data shows that the manipulations used lead to substantial changes in the data and their interpretation:

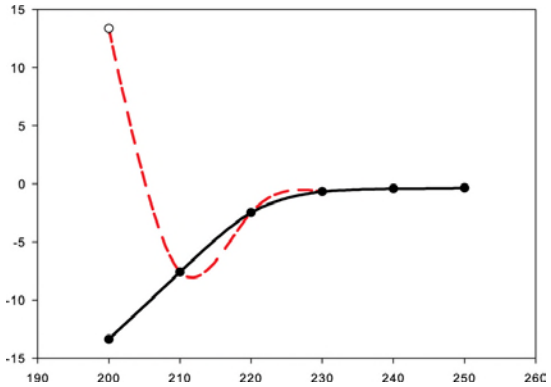
1. The noise in the primary data is completely hidden, making it impossible for the reader to assess the quality of the data. CD spectra are, by nature, rather noisy, but these are more so than typically found in published spectra.
2. The shapes of the spectra are significantly altered. In particular, the location and intensity of the minima, which provide information about the type and extent of secondary structure, are altered. Most significantly, the curves shown in the published figure for the peptide and peptide-hepcidin mixture at 37° C have very similar shapes, while the actual spectra are quite different. (The change in shape of the 37° C hepcidin curve is also due in part to the additional manipulation of the data point for 200 nm, described below.)

After the Committee discovered this manipulation of the data, Dr. Michael Kay, who provided the instrumentation and expertise for the CD measurements, was contacted through Dr. Botkin's office. In addition to confirming that the manipulation was inappropriate, Dr. Kay noted additional irregularities in the data. The first of these is that the background subtraction for the samples containing peptide+hepcidin was not carried out correctly. Instead of simply subtracting the buffer signal from the sample signal, the signals from the peptide-only and hepcidin-only samples were, in addition to the buffer, also subtracted from that of the peptide+hepcidin sample. Although this caused only small changes to the peptide+hepcidin spectrum at 4° C (because all of the spectra were essentially flat at this temperature), this manipulation had a significant effect on the 37° C spectrum as shown below:

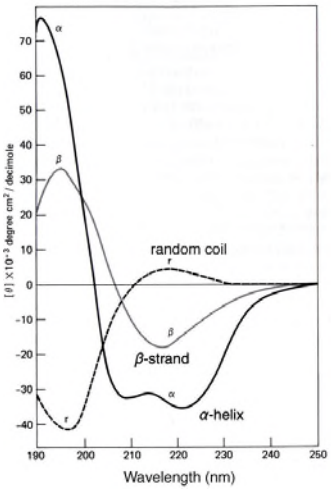


The blue dashed curve is the one in the published figure, and the black curve is a spline fit to the correctly processed data. While the subtraction of the signals from the individual components might be a useful analysis (provided the buffer contribution was correctly accounted for), it is not the spectrum described in the text and legend.

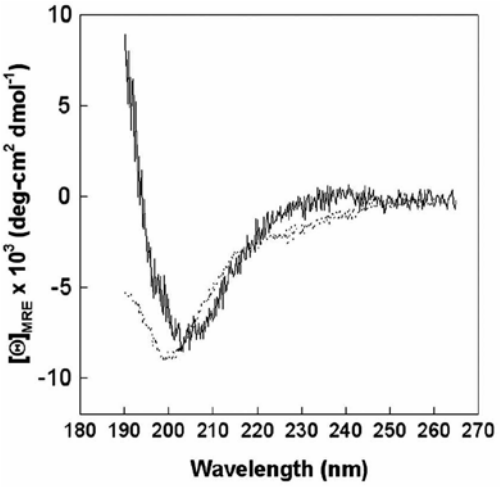
The second irregularity noted by Dr. Kay is that the sign of the signal for hepcidin at 200 nm and 37° C has been changed. This leads to a dramatic change in the shape of the spline curve used to represent the data:



The red dashed curve is the one in the published figure, with the sign of the first point reversed, while the solid black curve represents a spline fit to the correct raw data. This change has important consequences for the interpretation of the curves. The figure below (adapted from Creighton, 1993, *Proteins: Structures and Molecular Properties*, W.H. Freeman, p. 191) shows stereotypical CD spectra for peptides in different conformational states.



Together the reduction of the data to just 6 points and altering the sign of the 200 nm point change the spectrum from one typical of a disordered (random coil) polypeptide to the curve expected for a mixture of alpha-helical and beta-strand structure. Furthermore, the change makes the curve shape match more closely that of CD spectra (recorded at 25°C) previously published for hepcidin. The figure below is taken from a publication (Nemeth et al., 2006, Blood, 107, 328-333) from the laboratory of Dr. Thomas Ganz, who is both the complainant and a co-author of the disputed paper.



The darker curve is the spectrum for wild-type hepcidin, while the lighter one is for a mutant. Comparison of this spectrum with the unmodified data of De Domenico et al. suggest that the hepcidin samples used in the two studies were not equivalent: The spectrum in paper 10 indicates a more disordered conformation. This raises further doubts about the significance of the reported temperature dependence on the hepcidin conformation.

Another issue raised by Dr. Kay was the scaling of the spectral intensities to units of degree-cm^2/dmol, the standard (if rather arcane) units for protein CD spectra. The data from the spectropolarimeter (files 10-4A-4 to -11) are expressed as ellipticity with units of millidegrees. The correct scaling requires dividing the measured ellipticity by the concentration (expressed as amino acid residues in units of dmol/cm^3) and by the cell path length (in units of cm). The values in the Excel files and the SigmaPlot files all matched those in the raw data

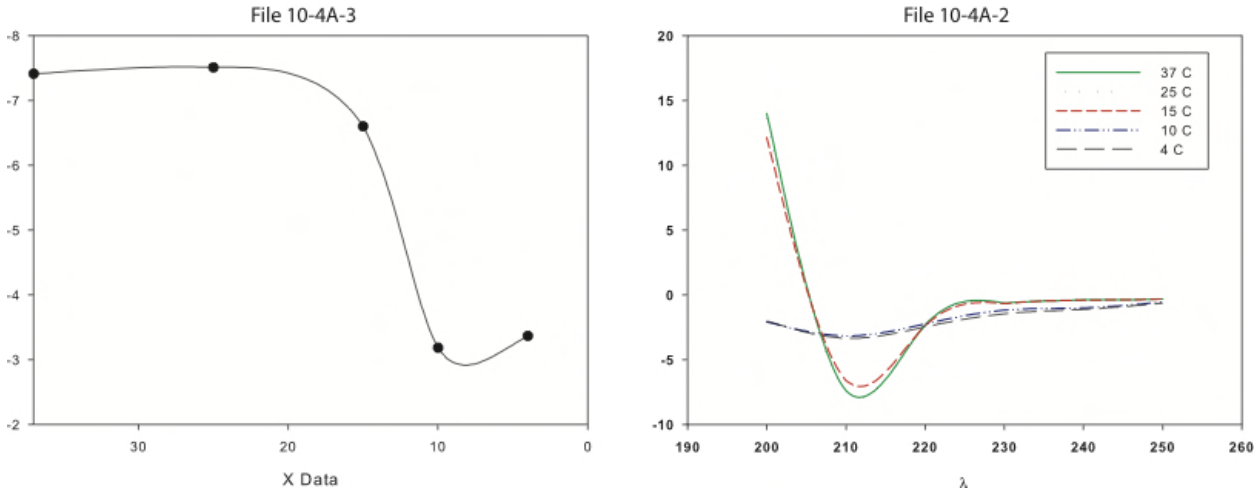
files, indicating that no correction for concentration and path length had been made. As noted above, the values shown in the published figure are all shifted upwards by about 5 units, relative to those in the computer files, but do not appear to be scaled. It thus appears that the data have not been converted to the units indicated by the axis label. The significance of this is that the spectra from different samples can only be compared if properly normalized for concentration.

In summary, the spectra in Figure 4A appear to have undergone the following manipulations:

1. Data at 10 nm intervals have been selected and used to draw a spline curve, essentially discarding 95% of the experimental data points and replacing them with a smooth curve.
2. The numerical values have all been sifted upwards by about 5 millidegrees.
3. The sign on a data point (200 nm for hepcidin at 37° C) has been changed, leading to a dramatic change in the shape of the published curve.
4. The spectra for the hepcidin + peptide have been modified by subtracting the signals for the individual components.
5. The plotted values are represented as mean residue ellipticities (degree-cm^2/dmol), but have not been properly scaled for protein concentration and cell path length.

The net result of these manipulations is to create curves that are more aesthetically pleasing and hide all of the noise in the data. In addition, the final curve for hepcidin much more closely matches a published CD spectrum for this protein.

Figure 4A (inset): The inset shows the ellipticity at 210 nm as a function of temperature. The Committee identified two related SigmaPlot files on Dr. De Domenico's computer, containing the graphs shown below:



The graph on the left (from file 10-4A-3) matches the published figure, while the one the right (file 10-4A-2) appears to represent the corresponding spectra recorded at the indicated temperatures. Like the spectra in Figure 4A, the curves in the right-hand panel were generated from six data points each (at 10 nm intervals) and a spline function. The data for the right-hand graph are listed below:

VAR1	Hep 37	Hep 25	Hep 15	Hep 10C	Hep 4C
200	14.001	13.587	12.147	-2.1	-2.032
210	-7.41	-7.51	-6.6	-3.18	-3.363
220	-2.324	-2.403	-2.39	-2.254	-2.481
230	0.6413	- 0.6486	- -0.691	-1.196	-1.471
240	0.3978	- 0.3947	- -0.403	-1.017	-1.128
250	-	-	-0.334	-0.559	-0.646

0.3214 0.3307

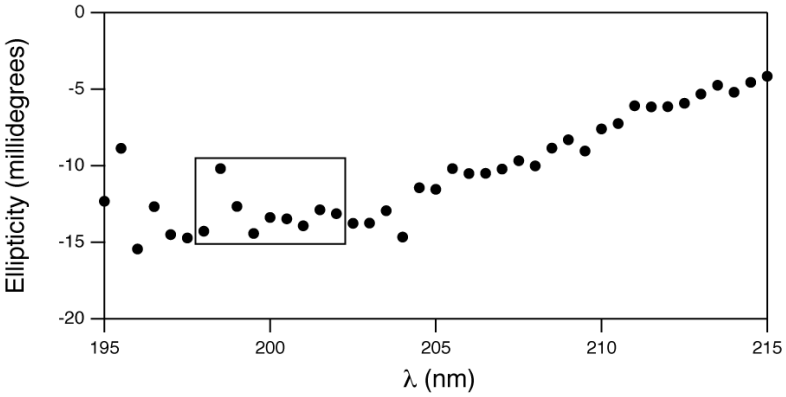
The values for 210 nm in this file match those found in the file for the final figure.

The values in the first two columns (37 and 25° C), in particular, are remarkable for their consistency: The maximum deviation between the corresponding values is 3.4%. The values for 15° C are also close to those for the higher temperatures. The values for 37 and 25° C also match closely, but not exactly, those for hepcidin at 37° C in the file (10-4A-1) used for the main graph of the figure, which are reproduced here again, along with the values for 4° C:

VAR1	Hep 37	Hep 4C
200	13.385	-2.032
210	-7.6	-3.37
220	-2.471	-2.485
230	-0.659	-1.478
240	-0.391	-1.129
250	-0.335	-0.656

The values for 4° C in the two data sets are also very similar, but not identical. According to the laboratory notes provided by Dr. De Domenico, the experiment at five temperatures was performed on or after 8-10-07, one week after the initial measurements at 4° and 37° C.

That some of the spectra recorded at different temperatures are similar is not surprising, especially if the same sample was used and the spectra were recorded sequentially, but the near identical values at each of the selected wavelengths is inconsistent with the substantial noise seen in the data for the full spectra. The scatter is particularly large in the region near 200 nm, as shown below for the 37° C hepcidin data:



For the data between 198 and 202 nm (enclosed in the box), the values cover a range of 4.2 mdeg (with a sample standard deviation of 1.26 mdeg), as compared to the difference of 0.4 mdeg between the values found in file 10-4A-2 for 25 and 37° C.

It should also be noted that the incorrect sign for the ellipticity at 200 nm (used in the curve for hepcidin 37° C in the main figure) is also found in all of the data sets for 15, 25 and 37 ° C.

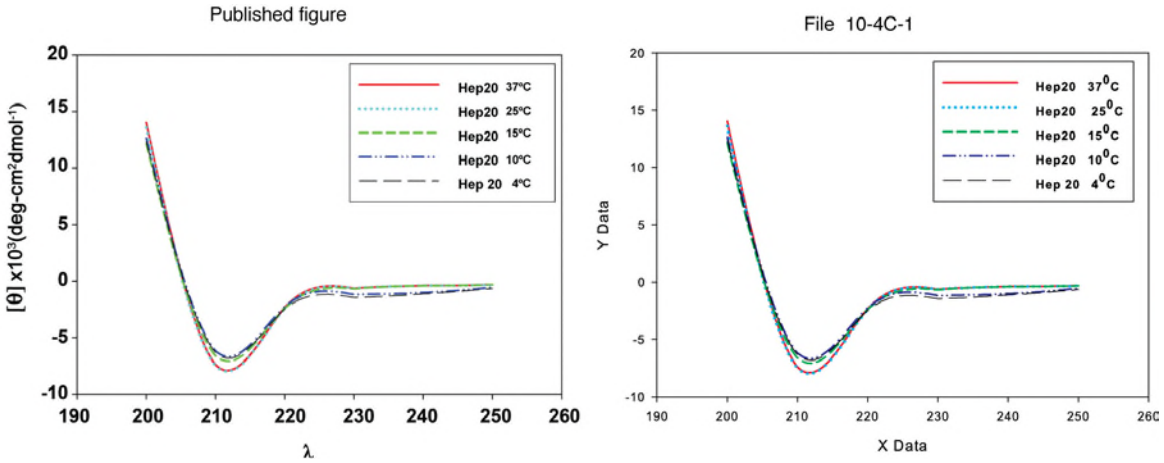
In contrast to the main panel of Figure 4A, no primary data for spectra recorded at the intermediate temperatures shown in the inset could be found on Dr. De Domenico's computer. The very small variations in the data found in SigmaPlot file 10-4A-2 and the incorrect sign for the 200 nm data lead us to believe that these data were likely fabricated by making small adjustments to the data used in the main panel of the figure.

Although the comments above focus on the data for 200 nm, which were not included in the published figure, we believe that they are important in establishing the origin and integrity of the published inset. An image file (10-4A-20) containing the full spectra (with the name "Fig 4B.tif" and matching the graph in SigmaPlot file 10-4A-2) was found on Dr. De Domenico's computer. Another file (10-4A-21) in the same directory is named "Figure 4A insert.tif" and, as indicated by the name, contains the inset graph. The date on "Fig4B.tif" is 8-26-07,

and that for "Figure 4A insert.tif" is 8-31-07, which is also the date on the SigmaPlot file (10-4A-3) used to make the inset. It thus appears that the authors decided, at some time between the two dates, to present only the graph of ellipticities at 210 nm. If, as suggested above, the data in the earlier version were fabricated, those shown in the final figure are presumed to be as well.

If the CD data shown for 10, 15 and 25° C are, in fact, manipulations of the curves from 4 and 37° C (the latter of which was manipulated also), then the conclusion of a temperature-dependent structural change is based on very little solid data.

Figure 4C: This panel shows CD spectra of a 20-residue fragment of hepcidin (hep-20) recorded at different temperatures, showing no significant temperature dependence. No data files corresponding to this figure were initially found by the Committee, but Dr. De Domenico, upon request, identified a SigmaPlot file (10-4-C1, on the hard-drive copy already in the Committee's possession) that matched the published figure, shown below:



In examining the various files associated with Figure 4, the Committee discovered that three of the curves shown in panel C match closely those found in the SigmaPlot file (10-4A-2) identified as the data source for the inset to panel A. This was confirmed by comparing the data tables in the two SigmaPlot files:

File 10-4C-1 (used for Figure 4C)

VAR1	Hep 37	Hep 25	Hep 15	Hep 10C	Hep 4C
200	14.001	13.587	12.147	12.651	12.369
210	-7.41	-7.51	-6.6	-6.18	-6.31
220	-2.324	-2.403	-2.39	-2.254	-2.481
230	0.6413	0.6486	-0.691	-1.196	-1.471
240	0.3978	0.3947	-0.403	-1.017	-1.128
250	0.3214	0.3307	-0.334	-0.559	-0.646

File 10-4A-2 (used for precursor to Figure 4A inset)

VAR1	Hep 37	Hep 25	Hep 15	Hep 10C	Hep 4C
200	14.001	13.587	12.147	-2.1	-2.032
210	-7.41	-7.51	-6.6	-3.18	-3.363
220	-2.324	-2.403	-2.39	-2.254	-2.481
230	0.6413	0.6486	-0.691	-1.196	-1.471

240	-0.3978	-0.3947	-0.403	-1.017	-1.128
250	-0.3214	-0.3307	-0.334	-0.559	-0.646

The values in the two tables are identical, except for those enclosed in boxes.

This comparison leaves little doubt that the published curves purported to represent spectra of hep20 at 37, 25 and 15° C, are the ones from the file containing data for the full length protein (and the source of the data for the inset to Figure 4A). As noted above, these curves are, themselves, suspected of being fabrications. The curves for hep20 at 4 and 10° C appear to have been generated by manipulation of two values each in the hepcidin file. These manipulations convert a set of curves meant to show a temperature effect on hepcidin into curves showing the absence of such an effect on hep20. (Though, the manipulation could have taken place in the opposite direction, the file dates are consistent with the hep20 data being derived from the other.)

CD Data Provided by Dr. De Domenico:

As noted earlier, no primary data could be found on Dr. De Domenico's computer for either panel C or the inset to panel A. On 9-5-12, Drs. De Domenico and Kay were each asked (via correspondence through Dr. Botkin's office) to provide a summary of their respective understanding of the steps leading to the published CD data, and also to provide any additional computer files they might have access to. They were asked specifically for data related to panel C and the inset to panel A. Neither author was able to provide any electronic files, and both have noted that the instrument and its associated computer were retired some years ago. Dr. De Domenico, however, stated:

> "Dr. De Domenico stored the printouts from these experiment as row data. These data are in the laboratory notebooks; one of these books is in Dr. Botkin' office the others still in Dr. Kaplan's laboratory."

She was then asked to locate the data in the notebook already in Dr. Botkin's office and to provide the other printouts. She promptly complied with the request, and the Committee carefully examined the material she provided. Although both notebooks contained printouts of CD data, all of it appeared to be derived from computer files already identified by the Committee. In particular, all of the high resolution data was the same as found in the Excel files (10-4A-12 to -16), corresponding to panel A of the published figure. There were no primary data corresponding to panel C or the inset to panel A.

The notebooks also included several handwritten pages of notes for the CD experiments. The notes already in Dr. Botkin's office were undated. Those provided on 9-11-12 had dates from 8-10-07 and 9-1-07. The notes indicate that, other than a first set of experiments, all of the spectra were recorded by a technician, Mr. Eric Lo. There a one page printout of data, attributed to Mr. Lo, that matches that found in the SigmaPlot file 10-4A-2. The notes also indicate that there were discussions between Drs. Kaplan and Dr. Ganz regarding the CD data. As discussed elsewhere in this report, the Committee believes that these notes were likely written after Dr. De Domenico was informed of concerns regarding the CD spectra.

The Committee is left to conclude that there are no existing primary CD data other than the single set of measurements represented in panel A. As detailed above, these data were extensively and inappropriately manipulated to generate panel A, and were further used to fabricate data shown in panel C and the inset of panel A.

Responses from Dr. De Domenico: Following the initial discovery that the published CD curves had been generated from data spaced at 10 nm intervals, Drs. De Domenico and Kay were asked (near the end of July 2012) to review their notes and existing data and to provide an account of their involvement in producing the figure. Later, near the end of the Committee's deliberations, on 9-10-12, the authors were each asked to provide a summary of the history and any additional data files they might have (see above). Their responses to these requests are summarized here.

Dr. De Domenico's initial response was in the form of a PowerPoint file (provided on 7-31-12), which included the following list of points (copied verbatim):

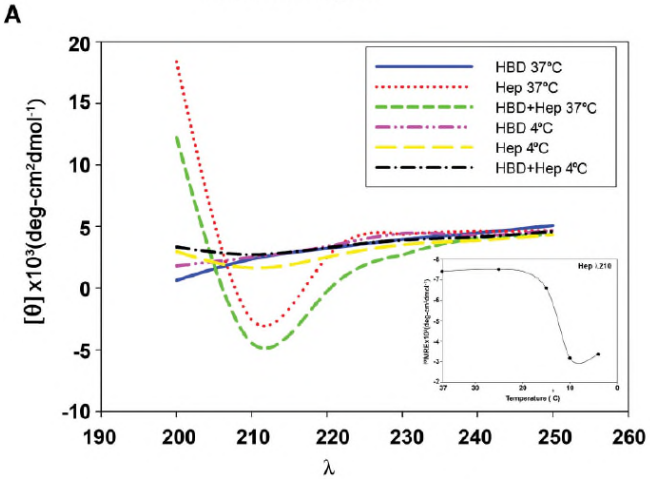
1. *The CD analysis was run in Dr. Kay laboratory from February 2007 to August 2007, according to email stored by Dr. De Domenico sent to reserve the instrument. Couple experiments were run by Dr. De Domenico and others by technician.*
2. *The raw data were given to Dr. De Domenico or the technician by floppy disk or print out. The machine used was attached to a very old PC computer.*
3. *The CD analysis also was done in collaboration with Dr. Nemeth and Ganz because they had published it for hepcidin in 2006. Nemeth et al, 2006 Blood. Dr. Ganz analyzed and approved the data and final figures. They provided all the peptides for these analysis.*
4. *Dr. De Domenico does not recall who decided to pick every 10nm for the figure generation. However, most of the lines were dotted to show discontinuity.*
5. *Dr. De Domenico sent one set of the data to Dr. Kay for analysis (see email next page). Dr. De Domenico was not involved in the communication with Dr. Kay during manuscript submission.*
6. *On July 26, 2012 Dr. Kay told (by phone) Dr. De Domenico that during paper submission he received a copy of the paper for approval from Dr. Ward (this needs to be confirmed).*

Most of these points are reiterated in Dr. De Domenico's statement of 9 September, which includes the following:

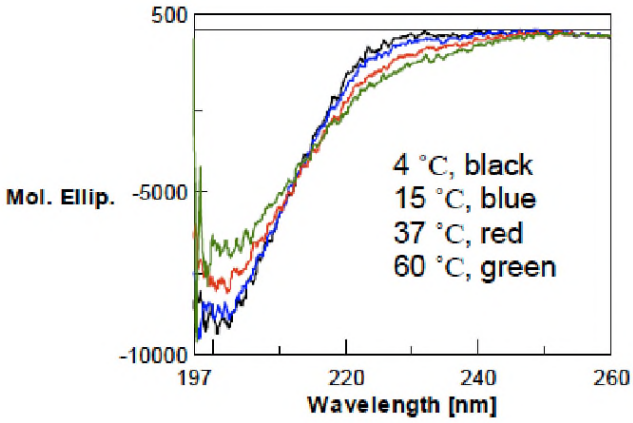
> "Once the data were converted in August 2007 sigma plot graphs were generated most of the time according to Dr. Kaplan and Dr. Ganz's instructions. Dr. Ganz was at the time consulted on CD and NMR analysis because he published couple years before the same experiments. After the sigma plots were generated were sent to Dr. Ganz for approval. Only one experiment (the first one run by Dr. De Domenico) was sent to Dr. Kay and he made his comments by emails (emails are available)."
>
> "Someone made the suggestion to plot the data as they are presented to Dr. De Domenico. Nobody in Dr. Kaplan's laboratory is CD expert. The laboratory notebooks reveal that Dr. Kay analyzed only the Excel file and later Dr. Ganz and Dr. Kaplan discussed CD and NMR data. Dr. De Domenico laboratory notes reported that Dr. Kaplan request to generated the insert in Figure 4A, this is why the file is named "Figure 4A jerry". Dr. De Domenico does not remember any further communication with Dr. Kay regarding how to plot the data. The last communications in August 2007 were regarding reservation of the instruments for the technicians to run new spectra. Dr. De Domenico was not in charge/involve to communicate with the other authors for final comments before the submission of the paper. However, Dr. De Domenico stored in the laboratory notebook an email sent from Dr. Kay to Dr. Kaplan with comments on the manuscript and figures. Suggesting that he saw a semifinal version of the manuscript. Dr. Kay remembers this email."
>
> "We also want to point out that in the Experimental procedures not details were given regarding how often the spectra were recorder. However, we understand that will be appropriate specify in the figure legends that the dotted lines represent a reading every 10nm." (underlining in the original).

In her responses to the initial draft of this report, Dr. De Domenico supplied (as Appendix 17) CD spectra of hepcidin recorded by an independent laboratory, Alliance Protein Laboratories (APL). These spectra, which were recorded at temperatures from 4° to 60° C, are reproduced below (right panel), along with Fig. 4A from the original publication.

Published Figure



From Alliance Protein Laboratories



Although Dr. De Domenico submitted the new spectra as evidence that "hepcidin goes to a temperature-dependent conformational change" (from page 75 of the file "Detailed response by Dr. De Domenico.pdf"), these spectra are very different from those published and contradict the conclusion drawn by the authors. Specifically:

1. The minima in the APL spectra are located at approximately 200 nm, as compared to 210 nm in the published spectra. As discussed above, the minima at 210 nm in the published spectra are due to the change in sign of the 200 nm points and the application of a spline curve.
2. The temperature dependence of the APL spectra indicate a small increase in secondary structure upon lowering the temperature from 37° to 4° C, while the published data indicate a major decrease in structure at the lower temperature.
3. The inset to the published figure indicates a temperature-dependent change of approximately two-fold in the ellipticity at 210 nm, with most of that change occurring between 10 and 15° C. In contrast, the APL spectra recorded at 4°, 15° and 37° C display almost no difference at this wavelength,

Thus, the spectra provided by Dr. De Domenico fail to support the authenticity of the published data or the conclusions drawn from those data.

In a subsequent response (submitted 12/10/2012), Dr. De Domenico stated that the experiments reported in Fig. 4C were, in fact, conducted:

> The experiment in Figure 4C was not run by her. However, she has no doubt that the experiment was run and the technicians gave to her the final results as shown in the file identified in "Ivana's Documents/Peptide Fpn from ivana v1/Paper folder/". To support this, recently Dr. De Domenico has queried one of the technicians (Mr. Lo) that were involved in that experiment. This technician, who still works in Dr. Kaplan's laboratory, remembers that he went to Dr. Kay's laboratory and ran the experiment. He, also, remembers that the room where the experiment was performed, because it was "very cold," since each individual performing the experiment had to sit next to a nitrogen tank. However, he does not remember where the raw data were stored because in the past years he has changed 3 computers and desks in the laboratory multiple times.

That a technician remembers doing an experiment does not offer any evidence that the published data actually came from that experiment, and Dr. De Domenico has not addressed the evidence (based on analysis of the data used to prepare the figure) indicating that the published figure was fabricated.

Responses from Dr. Kay: Dr. Kay provided initial responses to questions from the Investigation Committee via e-mail exchanges on 30 and 31 July 2012. As noted earlier, he pointed out additional inconsistencies in the data processing, including the change of sign for one of points in the 37° C hepcidin spectrum, though he did not address the significance of this change. Upon request from the Committee, he subsequently provided a

single-document summary of his role in the CD experiments and preparation of the manuscript, which included the following statements:

> "My role in this paper was to design the CD experiments, teach Ivana how to use the CD instrument, and to assist Ivana with data interpretation of her first set of CD data. Ivana (or technicians working under her direction) collected all data using our lab's CD instrument. I met and corresponded with Ivana (and sometimes Jerry Kaplan) during August 2007 to discuss data processing and analysis. I recall discussing the initial conclusions being drawn from the first set of CD data and agreeing that the results supported the conclusion that hepcidin's structure is temperature sensitive. Ivana sent me an initial set of CD data (0.5 nm spacing, unsmoothed) for my review. I suggested the acquisition of repeat data and a correction relating to blank subtraction (Excel files sent on 8/6/07). We never discussed removing x-axis points or curve smoothing, and I do not know when or why this improper processing occurred. As I suggested, additional CD data was acquired soon afterwards, but I have no record of ever seeing this data."
>
> "In November 2007, Jerry Kaplan handed me a late-stage printed manuscript draft (including figures) for my review. On 11/16, I e-mailed Jerry a list of comments and asked for an electronic file to allow me to make more detailed comments using "track changes" mode. I received an electronic version of the manuscript text from Diane Ward (Peptide manuscript v6doc.doc) that did not include figures. On 11/25, I sent annotated manuscript corrections to Diane. On 11/26, I answered a followup question from Diane (about the y-axis of CD plots).
>
> "I do not recall (and have no record of) seeing the final submitted Cell Metabolism manuscript (submitted in Feb. 2008) or proofs. I cannot recall if the paper draft I saw in 11/07 contained the final Figure 4. If it did, then I failed to notice the improper data processing, which I certainly would have corrected if I had known about it."

Responses from Dr. Kaplan: As described above, Dr. De Domenico indicated in her responses that Dr. Ganz played a role in the analysis of the CD data, with Dr. Kaplan serving as an intermediary. Following receipt of responses to the initial draft of this report, the Committee sought clarification on this point from Dr. Kaplan. The questions asked of Dr. Kaplan and his responses are provided in the section on "Alterations of the Research Record". In brief, Dr. Kaplan stated that, based on his recollections and e-mail correspondence, he did not believe that Dr. Ganz had played a role in analyzing the CD data and had not been aware of those data prior to 9/13/07, after the figures were essentially complete.

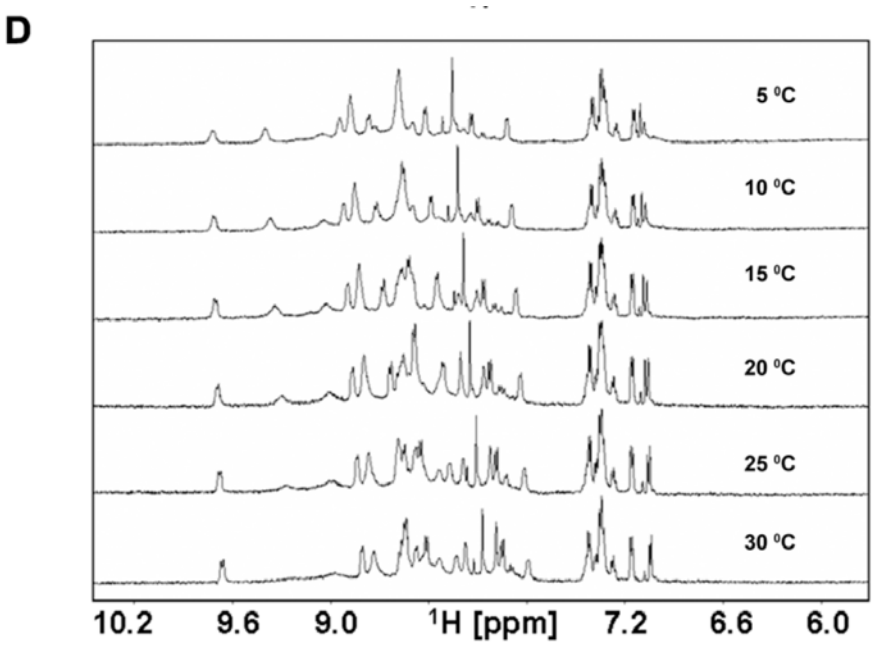
Responses from Dr. Thomas Ganz: Because Dr. De Domenico indicated in her responses that Dr. Ganz played a role in the analysis of the CD data, the Committee asked Dr. Ganz, via e-mail through Dr. Botkin's office, to explain his role in this aspect of the paper, as well as in the analysis of the NMR data described below. In his response, Dr. Ganz stated that his role in the paper was in providing samples, including the hepcidin forms used. He also he stated that he was aware of the CD and NMR data, but does not indicate that he played any role in the analysis of these data.

Conclusions regarding circular dichroism spectra: The data published in the main panel of Fig. 4A have been extensively and inappropriately manipulated, while that in panel C and the inset to panel of A were most likely fabricated. So far as the Committee can determine, there currently exists only one set of primary CD data, corresponding to that shown in the main graph of panel A. Analysis of the computer files from which the other two graphs were drawn indicates that they were generated by manipulation of the same data.

The manipulation of the data panel A not only hid the natural noise in the spectra but dramatically changed the shape of the spectrum for hepcidin at 37° C. Examination of the unmanipulated data indicates that this sample was probably not hepcidin in its native conformation, and the conclusion that hepcidin undergoes a major temperature-dependent conformational change is most likely incorrect, as indicated by NMR studies (discussed below).

Unpublished NMR data

While tracing the origins of the CD spectra shown in Figure 4, the Committee discovered what appears to be a preliminary version of the published figure (file 10-4A-22, dated 9-5-07), with the name "Figure 4 final2.tif". This figure is nearly identical to the published version, except that it includes a fourth panel containing a series of one-dimensional NMR spectra:



The Committee also discovered, on the copy of Dr. De Domenico's computer drive, two documents containing text apparently related to this figure. The first of these (file 10-X-1, dated 9-5-07) contained a description of NMR methods and a figure legend that appears to correspond to the figure above:

> Figure. Stacked plots of one dimensional NMR spectra recorded at 5 to 30 °C. The similar positions for most of the proton signals suggest Hepcidin does not undergo significant structural change over this temperature range. There are smaller changes in some of the signals that may reflect a local change in structure and/or oligomerization state.

From the file metadata, the creator is Dr. Jack Skalicky, the Director of the Biomolecular NMR Center at the University of Utah.

The second file (10-X-2) is a draft manuscript for paper 10, dated 26 September 2007. Dr. Skalicky is listed as a co-author, and the manuscript includes place holders in the Methods section (page 4) and the legend to Figure 4 (page 13) for NMR-related material. In addition, the Results section (page 8) contains the following text:

> These results suggest that the temperature-dependent changes in hepcidin structure requires the presence of the amino terminal domain of hepcidin. We used NMR to assess temperature-dependent structural changes in hepcidin. At temperatures ranging from 37ºC to 15ºC hepcidin showed the predicted ……(Figure 4D).

(The red highlighting is in the original.) This text suggests that the NMR data were to be included in the paper to support the conclusion that hepcidin undergoes a substantial temperature-dependent structural change.

From the spectra and the draft figure legend, it appeared to the Committee that the NMR results might contradict the CD data and that there may have been a deliberate decision to omit the former from the paper to hide this contradiction. The Committee therefore asked (through emails from Dr. Botkin's office) Drs. De Domenico and Kaplan, and subsequently, Drs. Ganz and Skalicky, for their recollections regarding the NMR data. In making these inquiries, the Committee did not inform any of the individuals of the evidence regarding

falsification of the CD spectra, though Dr. De Domenico had previously been questioned about the use of only the data spaced at 10 nm intervals.

Responses from Drs. De Domenico and Kaplan: Drs. De Domenico and Kaplan were sent excerpts of the material above and were asked the following specific questions about the decision to remove the NMR data from the paper:

1. Is it a correct interpretation that the NMR data in the draft figure and figure legend contradicts the CD data and the model in the published paper?
2. Why was it decided to exclude the apparently contradictory NMR data and to remove Dr. Skalicky from the list of contributing authors?
3. What authors participated in the decision to exclude the NMR data?
4. Specifically, was Dr. Michael Kay, who collaborated on the CD studies, aware of the NMR data and the decision to exclude those data from the paper?

On 9-10-12, Dr. Kaplan responded to these questions, via e-mail to Dr. Botkin, as follows:

1. I have attached an email from Jack Skalicky. Please note his comment….. "Figure. Stacked plots of one dimensional NMR spectra recorded at 5 to 30 °C. The similar positions for most of the proton signals suggest Hepcidin does not undergo significant structural change over this temperature range. There are smaller changes in some of the signals that may reflect a local change in structure and/or oligomerization state. "
2. As stated by Dr. Skalicky (see forwarded email)- "There are subtle changes in the NMR spectra with temperature that I will show you - but their interpretation is not straight-forward."
3. The reason that we did not include NMR data was that we could not assign NMR changes that we did see to specific amino acids. There were two reasons for this, first the author of the published structure could not find the assignments. I think he might have told he told me that by phone but he did tell Jack Skalicky (see forwarded email). The second reason is that Tom Ganz, who was a co-author on that paper- told us that the published structure was wrong and that a new structure would be coming out from his colleagues at Amgen. Since the NMR changes we saw could not be assigned to specific amino acids, and we could not rely on the published data, we did not include the data,
4. I do not recall if Michael Kay saw the NMR data or not. It was sent to Tom Ganz.

Dr. Kaplan also provided copies of e-mail exchanges between Drs. Skalicky and De Domenico, during the period from 8-22-07 to 10-16-07, which included a forwarded message from Dr. Hans Vogel of the University of Calgary. Dr. Vogel is the senior author of an earlier NMR study of hepcidin, and the e-mail exchanges indicate that Dr. Skalicky was attempting to obtain NMR resonance assignments, consistent with Dr. Kaplan's statements above.

Dr. De Domenico responded via e-mail on 9-11-12. Her response did not include direct answers to all of the questions asked by the Committee, but instead listed the following points:

1. Dr. De Domenico was not involved in the experimental procedure that generated the data.
2. NMR is outside of Dr. De Domenico's expertise. She did not analyzed the data or made any decision regarding.
3. Dr. De Domenico was not the corresponding author in this paper (as well in all the papers under the committee's evaluation) therefore she did not make decision regarding insertion or exclusion of data or authors.
4. Dr. De Domenico was not in charge to communicate with Dr. Kay regarding NMR data or manuscript's files.

Dr. De Domenico's response also provided copies of e-mail exchanges with Dr. Skalicky, including the one in which he provided the text for the figure legend, with these comments:

> "Attached is a first attempt at the NMR Spectroscopy description and Figure Legend. I'm sure more can be added but I will need to read a draft of your text before elaborating much more. What I wrote is very conservative.
>
> If you/Jerry get the chemical shifts for Hepcidin I'd be happy to generate a surface coloring map illustrating regions of the Hepcidin that are likely changing in structure with temperature."

Dr. De Domenico also describes her recollections of communications between Drs. Kaplan and Ganz and states that "Therefore, Dr. De Domenico believes that any further action taken by Dr. Kaplan to exclude the NMR data was suggested by and in agreement with Dr. Ganz."

Responses from Dr. Ganz: After receiving the responses quoted above from Drs. De Domenico and Kaplan, the Committee contacted Dr. Ganz via e-mail (through Dr. Botkin's office) and asked the following specific questions:

1. What, if any, was your role in the analysis of the CD or NMR data?
2. Who else was involved in the analysis of the data?
3. What authors participated in the decision to exclude the NMR data?

Dr. Ganz's response included the following key statements:

> "In general, my participation in this paper was very limited except that our group provided key reagents for the study, including the hepcidin forms that are one of the subjects of this paper."
>
> "The first version of the paper, which I received by e-mail on October 28, 2007, did not contain the NMR data, only the CD spectra. I first became aware of the NMR/CD data on Sept. 13, 2007 as a result of the e-mail quoted below" [from Dr. Kaplan].
>
> "In response to this e-mail, I arranged for Hans Vogel, an NMR expert from Alberta, Canada, to provide whatever NMR data he generated several years before on hepcidin peptides we synthesized or purified. My intention was to get Dr. Vogel to help Drs. Kaplan and DeDomenico interpret their NMR data."
>
> "I do not recall examining any of the NMR data myself, mostly because I am not an expert, and trusted Hans Vogel to take care of it."
>
> "So, in summary, I do not recall seeing a version of the manuscript that had NMR data in it but I was aware that such data existed and tried to help indirectly by connecting Dr. Skalicky and Dr. Vogel so that they could interpret the data."
>
> "I do not know how the decision not to include the NMR data was reached and by whom, and I did not know that the NMR data contradicted the CD data."
>
> "If indeed the NMR data do contradict the hypothesis that hepcidin undergoes a temperature-dependent conformational change between 4 and 37C, the omission is important. The conclusion that hepcidin undergoes a conformational change was important enough for the paper that it was included in the abstract."

Responses from Dr. Skalicky: Dr. Skalicky was asked the following questions:

1. What is your memory of why it was decided to not include the NMR data in this paper, and to remove your name as a contributing author?
2. Do you recall any discussions concerning the relationship between the NMR data and the CD data that appeared in the published paper?
3. What authors participated in the decision to exclude the NMR data?

His responses included the following:

"Dr. Kaplan asked me in 2007 to investigate the temperature dependence of human hepcidin using NMR. This was done by myself and presented to the Kaplan research group (my memory is sketchy about the meeting but I recall that Jerry Kaplan, Ivana De Domenico, John Phillips, and Diane Ward were present). The data were discussed, however I don't recall a followup meeting nor did I see a version of the manuscript with my name included as coauthor, although authorship was discussed early in the process."

"I don't recall details regarding discussion of CD and NMR but I assume we talked about both. My interpretation of the NMR data is the same between then (2007) and now: The NMR is consistent with globally-similar structures over the entire temperature range of 4 to 37C and inconsistent with a folded-unfolded transition."

"The following manuscript published in 2009 is quite useful in interpreting the pertinent De Domenico, 2008 results (Jordan et al, 2009 'Hepcidin Revisited, Disulfide Connectivity, Dynamics, and Structure' JBC, 284 pp 24155-24167). The major finding of Jordan, 2009 is that hepcidin exists in a low and high temperature form (the NMR structures are nearly identical to one another with exception of a flexible loop and cystine side chain rotamers; Figures 6 and 7 in Jordon, 2009)."

"It is quite likely that Figure 4A in De Domenico, 2008 is also showing the interconversion of the low and high temperature structures (low temperature form, 4 °C, dashed yellow line and high temperature form, 37 °C, dotted red line). I suggest the differences in CD spectra are NOT the result of a folding-unfolding transition but instead a folded(low T) – folded(high T) transition."

"The summary of De Domenico states "The increased dissociation rate is due to temperature dependent changes in hepcidin structure", in fact they probably got this right in general but not in detail. The NMR data may have allowed them to eventually hypothesize that the temperature dependent changes are subtle and within the context of a folded peptide, however I don't believe the major conclusions will change."

"I was not involved in the decision to exclude the NMR data and I can only assume the decision was made by the primary authors."

Conclusions regarding unpublished NMR data: Initial analysis by the Investigation Committee suggested that the NMR data may have been removed from the manuscript because it contradicted the CD data and the authors' model. The omission of data that could change significantly the conclusions of a paper is, potentially, a form of data falsification, as stipulated in both University of Utah Policies and the NIH Rules and Regulations. There is, however, an obvious need for judgment, as it is rarely appropriate to publish all of the data generated in a study.

Dr. Kaplan's responses to the Committee's questions indicate that the NMR data were removed from the manuscript because amino-acid specific assignments of the peaks were not available and because data from another group indicated that the published three-dimensional structure was incorrect. The absence of a correct three-dimensional structure would certainly be good reason to refrain from publishing a figure in which the resonance shifts were mapped onto a structural representation. On the other hand, neither the resonance assignments or a three-dimensional structure would be necessary to conclude from the spectra that the protein did not undergo a major structural change, as indicated in the draft figure legend and Dr. Skalicky's comments

In retrospect, the NMR data were probably a more accurate measure of the temperature-dependent conformational properties of hepcidin than were the deeply flawed CD data discussed in the previous section of this report. Although Dr. Skalicky's response suggests that the published CD data are consistent with the NMR data subsequently published by Jordan et al., those authors state:

"The observed temperature dependent structural change in hepcidin is intriguing and of particular interest in the context of ferroportin binding. It seems, however, that the temperature-dependent activity observed in previously published ferroportin internalization assays (52) does not correlate with the temperature dependence of its loop conformation; in the range from 15 to 4 °C, the observed 90% drop in activity (52) is significantly larger than the 15% change in the population distribution (Fig. 3C) between the two limiting structures. Moreover, the observed loss of secondary structure at 4 °C in the CD spectra (52) and

multimerization of hepcidin (16) are inconsistent with the current observations of identically folded and monomeric hepcidin within the 20 to 53 °C temperature range."

(Reference 52 is Paper 10 under consideration here.)

From the responses of Drs. De Domenico, Kaplan, Ganz and Skalicky, the authors appear not to have considered the NMR and CD data to be contradictory, and it is not known whether or not this question was ever discussed explicitly. Thus, there is no evidence to indicate that the removal of the NMR data from the paper was a conscious attempt to hide or obscure such a contradiction. On the other hand, the final outcome was the publication of flawed data and the omission of data that was subsequently confirmed and expanded upon. The process leading to this outcome raises questions about the management of the research, as discussed below.

Summary of findings for Paper 10.

As noted in the introduction to this section of the report, the initial allegations regarding paper 10 concerned the reproducibility of the peptide-binding assay for hepcidin. The Committee does not feel that it can address this question from the information available, and a resolution will most likely require additional experiments. However, our examination of the data for this paper has revealed evidence for the following instances of scientific misconduct:

1. Misrepresentation of experimental uncertainties in the error bars for many of the graphs.
2. Manipulation of data so as to change a significant conclusion (the CD spectra in the main panel of Fig. 4A).
3. Fabrication of data (in the inset of Fig. 4A and in Fig. 4C).

In his response to the initial draft of this report, the attorney representing Dr. De Domenico, Mr. Ryan B. Bell, challenges the Committee's conclusions regarding this paper, particularly conclusions based on the absence of computer files associated with the error bar calculations. As noted in the section on Paper 6, federal ORI policies specifically state that the absence of data in the research record can be interpreted as evidence of misconduct. Furthermore, the conclusions of misconduct with respect to the error bars are based on those computer files that were found and showed that the published error estimates were based on fewer values than specifically stated in the papers. The Committee did not attempt to draw conclusions regarding the figures for which the calculations could not be found.

With respect to the CD data, Mr. Ryan states that "The Committee has failed to point to a single piece of evidence suggesting that the falsifications of CD spectra were carried out by Dr. De Domenico." and suggests that they might have been carried out by Dr. Kay or Dr. Ganz. By Dr. De Domenico's own account, Dr. Kay saw only the initial set of data, in their raw form, and the final figures. Her statements that Dr. Ganz played a role in the analysis have been contradicted not only by Dr. Ganz but by Dr. Kaplan, who, according to Dr De Domenico, served as an intermediary in Dr. Ganz's involvement in this aspect of the paper. The statements of Dr. Ganz and Kaplan are supported by an e-mail message indicating that Dr. Ganz was not aware of the CD data until after the figures had been completed. In support of her statements, Dr. De Domenico has provided an e-mail exchange (Appendix 24 of her response to the draft report), which she indicates shows Dr. Ganz's involvement in analyzing the CD data. In fact, however, this e-mail refers only to NMR data, is dated well after the CD figures were completed, and is entirely consistent with Dr. Ganz's account. The issue of Dr. Ganz's involvement in the CD data is discussed further in the section on alterations of the research record.

Mr. Bell also argues that "Dr. De Domenico knows very little about CD analysis-too little, in fact, to have attempted or carried off a manipulation of this complex data." Dr. De Domenico makes a similar statement in her "detailed response." Without reference to Dr. De Domenico's specific expertise, the Committee notes only that the manipulations were not, in fact, very complicated. All that was required was a rough idea of what a CD spectrum of hepcidin should look like (provided by Dr. Ganz's previous publication), and the ability to manipulate a few data points and draw spline curves with the SigmaPlot program.

In addition to the specific details of these breaches of scientific standards, the Committee's investigation revealed very troubling aspects of the mode of collaboration among the paper's authors. In her responses to our questions, Dr. De Domenico stressed that she and others in the Kaplan lab lacked expertise in the CD and NMR methods used, making them dependent on their collaborators for interpretation of the data. However, the

communication among the authors appears to have been very limited, likely contributing to the publication of falsified and fabricated data.

In the case of the CD data, the collaborator was Dr. Michael Kay, who appears to have been minimally involved in the data processing or analysis. From his account and that of Dr. De Domenico, Dr. Kay saw only the raw data for the first set of data recorded, on the basis of which he recommended that Dr. De Domenico repeat the experiments with samples of known protein concentration so that the data could be properly scaled. If such experiments were performed, no data from them can be found, and the published figures are based on the initial, unscaled and manipulated data. Dr. Kay states that he did review two versions of the manuscript, but was unaware of Dr. De Domenico's use of only a small fraction of the data points to draw a spline curves. As the author of the paper specifically associated with the CD experiments, Dr. Kay must be bear some of the responsibility for the falsification and fabrication.

Dr. De Domenico's responses to questions regarding the CD and NMR data, in which she repeatedly shifts responsibility to her collaborators, are also troubling. For instance, she states "Dr. De Domenico does not recall who decided to pick every 10nm for the figure generation." It is extremely unlikely that Dr. Kay would have suggested this, and Dr. Ganz indicates, in contradiction to Dr. De Domenico's account, that he played no role in analyzing the CD data. Even if Dr. Ganz did play a role, the CD spectra in his laboratory's earlier publication were not manipulated in this way, and there is no reason to believe that he would suggest it. With regard to the NMR data, Dr. De Domenico indicates that she played no role in interpreting the spectra or the decision not to include them in the paper, even though she was the first author.

If, as she indicates, Dr. De Domenico did not participate in decisions regarding authorship or the inclusion of data, this raises serious questions regarding Dr. Kaplan's role as principal investigator and mentor. At the very least, the decision to exclude data is one that Dr. De Domenico, as a trainee and first author, should have been part of. In addition, there appears to have been little or no discussion among the various collaborators, especially Drs. Kay and Skalicky. Given the expertise of these two individuals, it is unlikely that the contradiction between the CD and NMR data would have gone unnoticed if they each been fully aware of both sets of results. Particularly in light of his own lack of expertise in these techniques, Dr. Kaplan should have ensured that all of the data were shared among the participants.

In summary, the Committee concludes that this paper contains instances of data falsification and data fabrication and that this paper should be retracted. The circumstances leading to the publication of falsified and fabricated data indicate flaws in the management of the laboratory and collaboration.

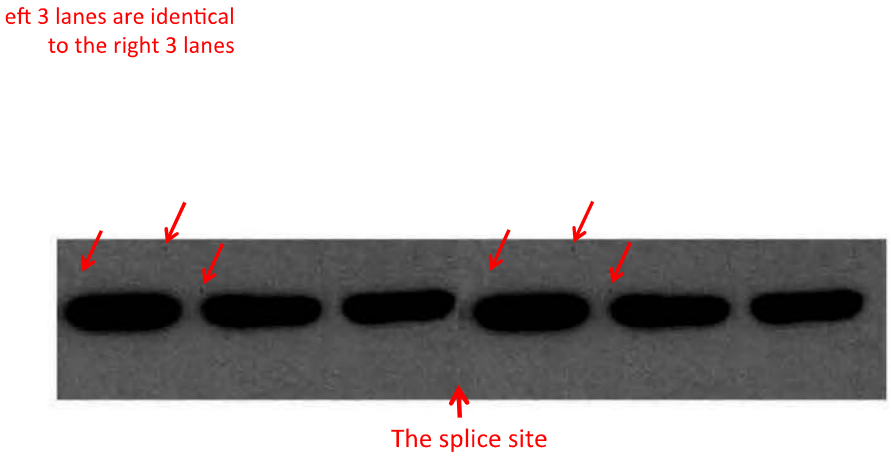
PAPER 11

Citation: Kieffer, C., Skalicky, J. J., Morita, E., De Domenico, I., Ward, D. M., Kaplan, J. and Sundquist, W. I. (2008). Two distinct modes of ESCRT-III recognition are required for VPS4 functions in lysosomal protein targeting and HIV-1 budding. *Dev Cell* **15**: 62-73.

ALLEGATION

In Fig. 5B of paper #11, a set of western blot bands was "intentionally duplicated, inserted and the splice was patched up." [see figure below]

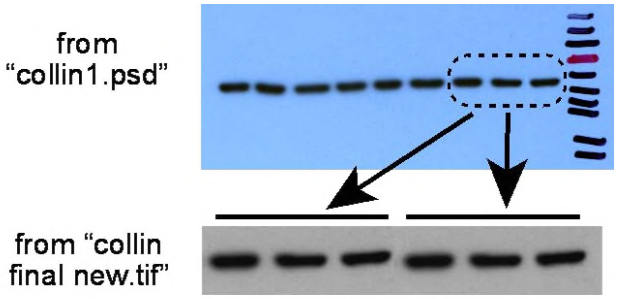
Figure 5B



COMMITTEE FINDINGS

In follow-up correspondence and/or discussions with Drs. De Domenico, Kaplan, Sundquist and Kieffer, all agreed that the published gel image had been inappropriately manipulated. The preponderance of evidence indicates that the manipulation was done by Dr. De Domenico:

(a) The files that were evidently used to prepare the manipulated image were found on Dr. De Domenico's hard drive. These include "collin1.psd" (file 11-5-1, a scan of a developed film) and "collin final new.tif" (file 11-5-5, created in PhotoShop) [see figure on right].

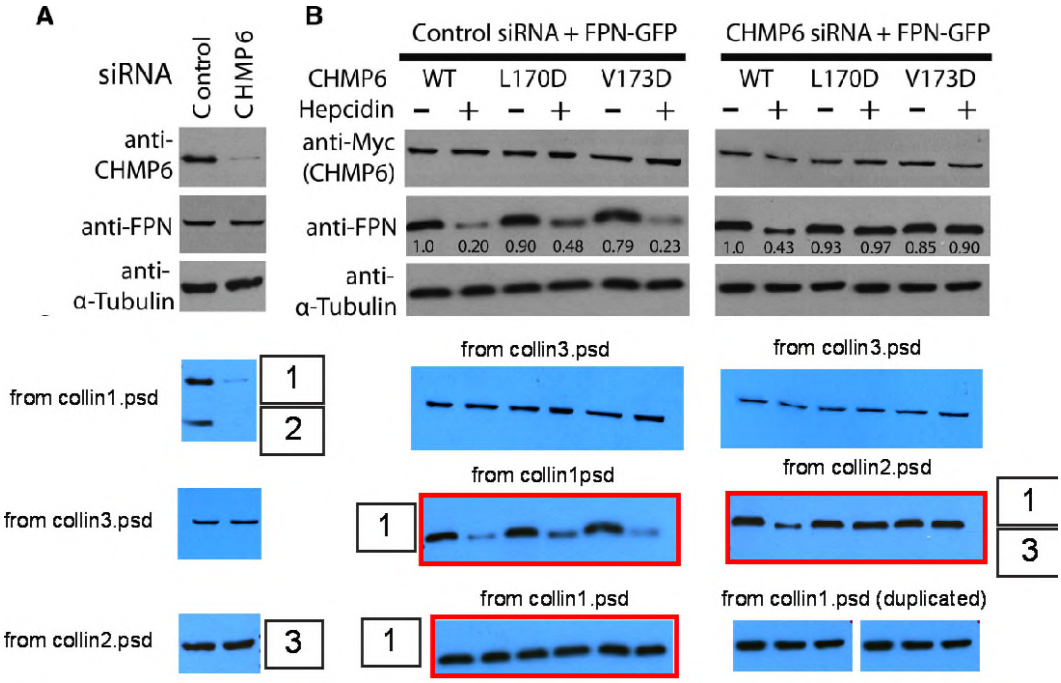


(b) The final version of Fig. 5 was assembled by Dr. Kieffer (the first author of paper #11) from images supplied by Dr. De Domenico. [see appended letter from Drs. Sundquist & Kieffer]

(c) In discussions with the Investigation Committee, Dr. De Domenico did not deny that she created the manipulated image.

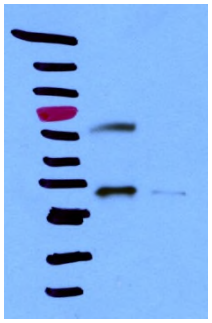
ADDITIONAL FINDINGS

The Investigation Committee identified other problems with Fig. 5A and 5B in this paper. The images used to assemble panels A and B were found in three files on Dr. De Domenico's hard drive: “collin1.psd”, “collin2.psd”, and “collin3.psd”, which are scans of developed western blot films. Comparisons of the film images and the published figure raised several new concerns. These problems were all present in the assembled image file “collin final new.tif” prepared by Dr. De Domenico [refer to boxed numbers in the figure below].

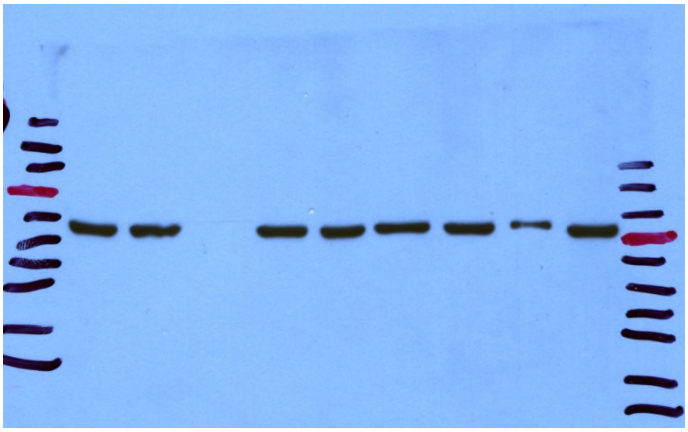


1. These four image panels in the published figure are oriented upside-down relative to the original film image and MW standard ladders marked on the films (file 11-5-1;file 11-5-2). In the three panels boxed in red, the band shapes indicate that the orientation is incorrect in the published figure. In the fourth (unboxed) panel, the orientation of the MW labels on the film is incorrect. Given the location of the computer files, Dr. De Domenico must have committed these errors and manipulations.

2. The MW marker labels on this film imply that the gel was run in the top-down direction (because the red marker should be closer to the top of the gel) [see figure at right]. Thus, the lower bands are consistent with the size of CHMP6 (~33 kDa), purportedly the protein band represented in the image. However, the band shapes indicate that the gel was most likely run in the bottom-up direction, so the MW markers appear to be incorrect. Were the MW standards marked on the developed film so as to be consistent with the size of CHMP6? What is the second band in the "siRNA control" lane? Why is it not present in the “CHMP6 siRNA” lane? The figure legend and methods make no mention of an unrelated protein that crossreacts with anti-CHMP6 serum. These errors and ambiguities cast doubt on the provenance and true identity of the bands labeled “anti-CHMP6” in the published figure.



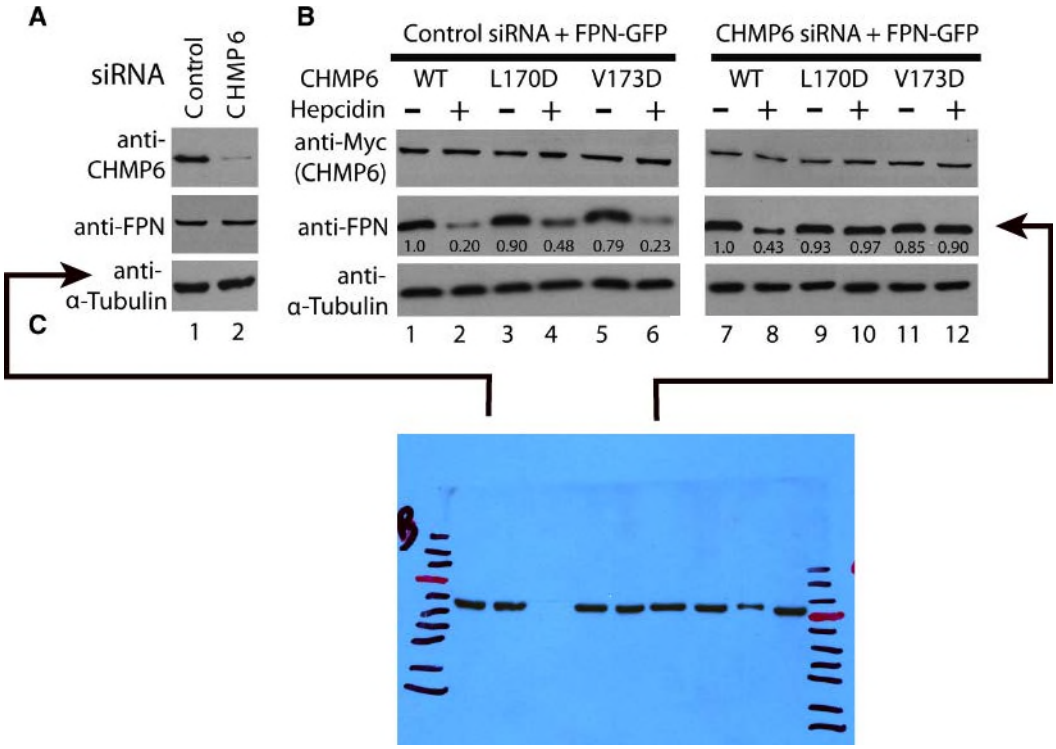
3. These two panels of the published figure supposedly represent protein bands detected with different antisera. However, they appear to originate from the same gel [see film at right]. Note that all bands run at virtually the same position. And yet, there are two different sets of MW marker labels, implying that the two bands on the left have a size consistent with tubulin, whereas the six bands on the right have a size consistent with ferroportin.



In discussions with the Investigation Committee, Dr. De Domenico explained that actually two partial membranes were placed in the cassette side-by-side and that the resulting alignment of the bands from the two gels was strictly coincidental. If this explanation were true, one would expect to detect some discontinuity in the background from the two membranes. If the membranes were derived from two different gels, one would expect that the spacing of the MW markers would be different on the two gels, because the markers would have migrated further in the gel on the right. If the membranes were derived from a blot of the same gel (at least 11 lanes), then cut apart and processed with different antibodies, the markers would have run the same distance from the gel origin. Clearly, this is not the case; the MW ladder markings exhibit identical spacing, but different distances from the gel origin. The Committee concludes that these bands are from the same gel and western blot and do not represent proteins that reacted with different antibodies, as claimed in the published figure. The true identity of the proteins represented in these panels and their relationship, if any, to the published experiment are unclear.

Statements to the Investigation Committee regarding western blots for Paper 11

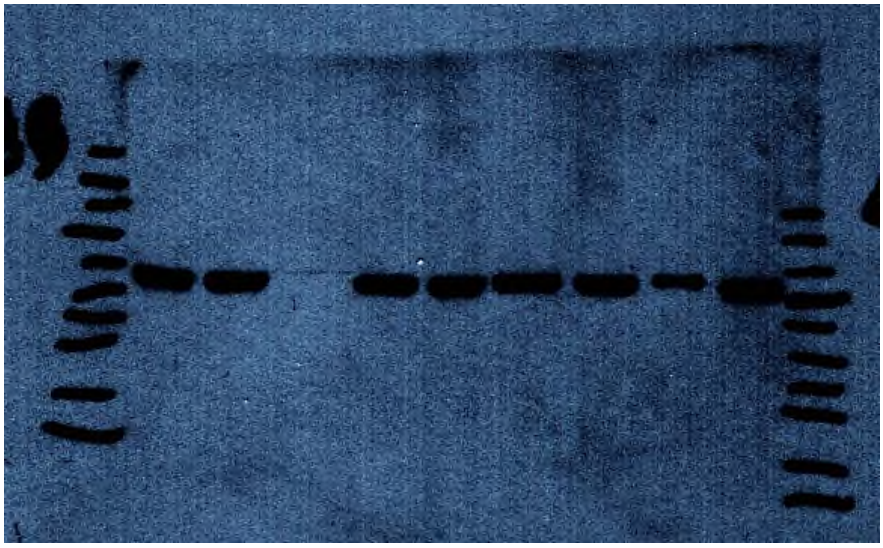
In the course of its investigation of duplicated and spliced lanes in a western blot published in Fig.5 of paper 11, the Committee reconstructed the figure in question using files found on Dr. De Domenico's computer and discovered that images of lanes from a single film were used to represent western blots with two different antibodies (anti-Fpn and anti-tubulin), as shown below:



(The lanes labeled to represent the anti-Fpn blot are inverted about the horizontal axis in the published figure.) Given the nature of the western blot procedure it is physically impossible for the same autoradiograph to represent blots with two-different antibodies.

Dr. De Domenico was presented with this evidence during the interview of 7-17-12 and responded by stating that the image shown above represented two separate blots placed next to each other in the film cassette. Members of the Committee noted that this seemed unlikely since the bands in the gel line up almost exactly and there is no obvious discontinuity between the two parts of the image. None the less, Dr. De Domenico insisted that there were two separate blots.

The image below is from the same computer file (11-5-2), but has been adjusted to increase contrast:

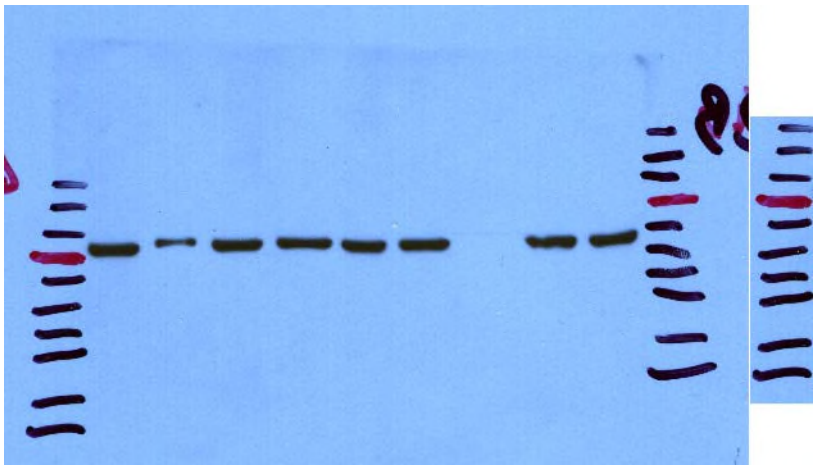


Even with greatly enhanced contrast, there is no indication that this image came from two blots. Also, with the edges of the blot more clearly delineated, there does not appear to be room for the molecular-weight marker lanes drawn on the film, especially on the left-hand side of the blot.

In order for Dr. De Domenico's explanation to be valid, the following conditions would have to be satisfied:

1. The two gels were run for different times, such that the tubulin and the Fpn-GFP fusion protein (with approximate molecular weights of 55,000 and 90,000, respectively) migrated to the same position on the blot.
2. The band intensities, for two unrelated proteins probed with different antibodies, for the two blots match almost exactly.
3. The two blots were cut and aligned with remarkable precision so as to hide any discontinuity.

If, as required by point 1 above, the gels were run for very different times, the separation of the marker bands would be expected to be quite different. In the image below, the markers on the left-hand side have been duplicated and aligned with those on the right-hand side:



There does not appear to be any significant difference in the spacing of the markers.

The Committee concludes that the image used to represent blots with two different antibodies almost certainly represents, instead, a single blot. One, at least, of the blots in the published figure must, therefore, be a falsification. Dr. De Domenico's statements to the Committee are unlikely to be true.

SUMMARY

The Committee finds that Fig. 5 of paper #11 contains several instances of data fabrication and/or misrepresentation in images manipulated by Dr. De Domenico. Thus, the experimental results reported in Fig. 5A and 5B are not supported by the data presented. This raises substantial concerns about the reliability of the other experimental data provided Dr. De Domenico and the Kaplan lab for this collaborative study. At the very least, the conclusions drawn from the experiments documented in Fig. 5 should be formally retracted.

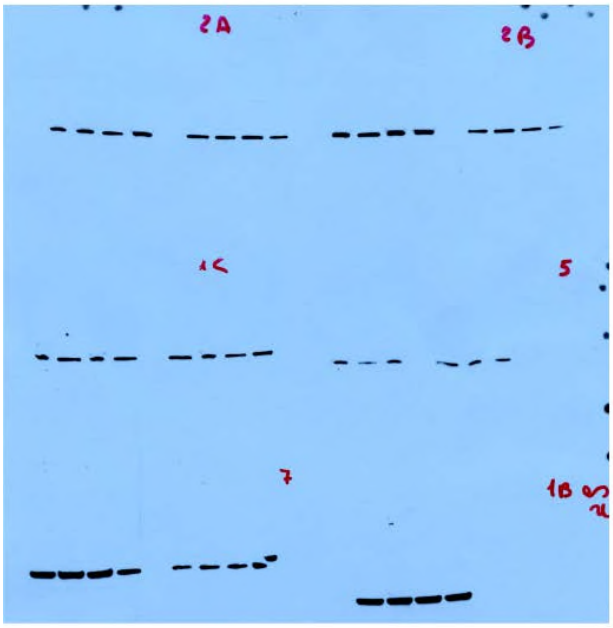
Alterations of the Research Record

Evidence for misrepresentations to the Inquiry and Investigation Committees and alteration of the research record following receipt of allegations

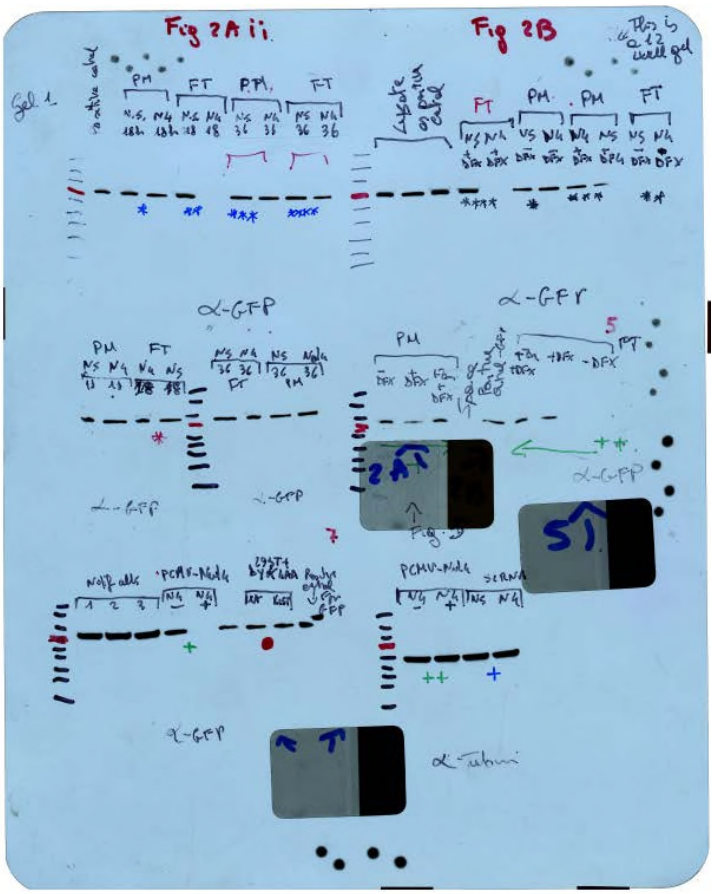
During the investigation, there were instances in which materials or information provided by Dr. De Domenico appeared to have been improperly manipulated or gave the appearance of intention to mislead the Investigation Committee. Specific examples are detailed below.

1. Manipulation of an autoradiograph film for Paper 1.

One of the original allegations made in November 2011 concerned the duplication of western blots shown in Figs. 2, 3 and 5 of Paper 1. In response to these allegations, Dr. De Domenico provided to the Inquiry Committee a labeled film purported to represent the correct results for some of these figures, specifically Figs. 2Aii, 2B and 5. Two scans of this film, the context of which is discussed in detail in the section devoted to Paper 1, are reproduced below:



File 1-2-1
From Ivana De Domenico's computer



Scan of film in sequestered notebook
(scanned 10-27-2012)

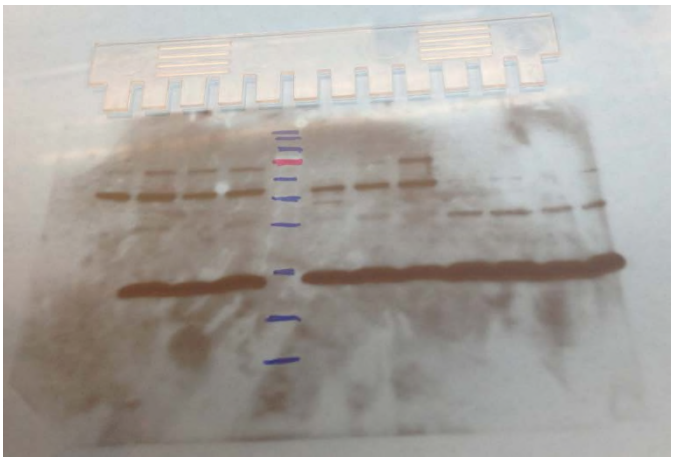
The image on the left is from a file (dated 7-20-11) found on a copy of the hard drive from Dr. De Domenico's computer. The extensively labeled image on the right is a scan of the film presented to the Inquiry Committee. The film in this form was included in notebooks sequestered by the Research Integrity Office on 11-9-11. A scan of the film with the same labeling (except for the adhesive labels visible above) was also found on Dr. De Domenico's computer drive (file 1-2-3, dated 10-29-11). Comparisons of these images led the Investigation Committee to question the fidelity of the later labels.

Viewed without the subsequent labeling, the film on the left is remarkable for the consistency and symmetry of the blots. The minimal labeling suggests that the blots were to be used for Figs. 1B, 1C, 2A, 2B, 5 and 7. The published versions of Figs. 1C, 2A, 2B and 5 all included blots showing biotin-streptavidin fractionation of plasma membrane and cytoplasmic proteins. Although Fig. 7 did not show this type of experiment, it would have been appropriate for this figure as well. Except for the one labeled 1B, each of the blots displays four lanes, each with a single clear band, separated by a single blank lane. In each blot, the regions to the left and right of the visible lanes are completely blank (as revealed in both the scan image and direct inspection of the film), as is the central lane. From the minimally-labeled film, it would appear that the blots were the results of a set of carefully planned experiments. The correspondence between the initial labels and the published figures indicate that these gels were run specifically to prepare the final figures. However, the labeled film shown above on the right indicates an almost haphazard loading of samples, with controls added in different positions and with the “PM” and “FT” and the “NS” and “N4” samples organized differently in the different gels.

The blot for Fig. 2 was of particular concern to the Investigation Committee, who discussed it with Dr. De Domenico twice, on 7-2-12 and 7-17-12. The current handwritten labels on the film indicate that this was a 12-lane gel, rather than the 10-lane gels apparently used in the majority of the films examined by the committee. During the 7-2-12 interview, the Committee questioned this point, as the width of the bands in the Fig. 2B blot is similar to those in the other 10-well gels on the film. The Committee noted that if the 10-well and 12-well gels are the same width and run in the same electrophoresis apparatus, then the well width of the 12-well gel should be smaller than that of a 10-well gel. In addition, the full width of the lanes indicated on the film is about 9.4 cm, as compared to the standard mini-gel width of 8.6 cm.

When questioned about this discrepancy on 7-2-12, Dr. De Domenico insisted that a 12-lane gel had been used and that the labels were correct. Shortly after the interview, she provided ordering information for 12-lane pre-cast gels supplied by Bio-Rad. To substantiate the use of twelve-well gels in his lab, Dr. Kaplan provided the committee with a twelve-well comb during his interview on 7-3-12.

During the 7-17-12 interview, this film was again discussed, and Dr. De Domenico stated that the discrepancy between the widths of the different gels was explained by swelling of the 12-lane gel. In support of this argument, she showed a photograph of a 12-well comb and gel, indicating that the gel had swelled beyond the width of the comb:



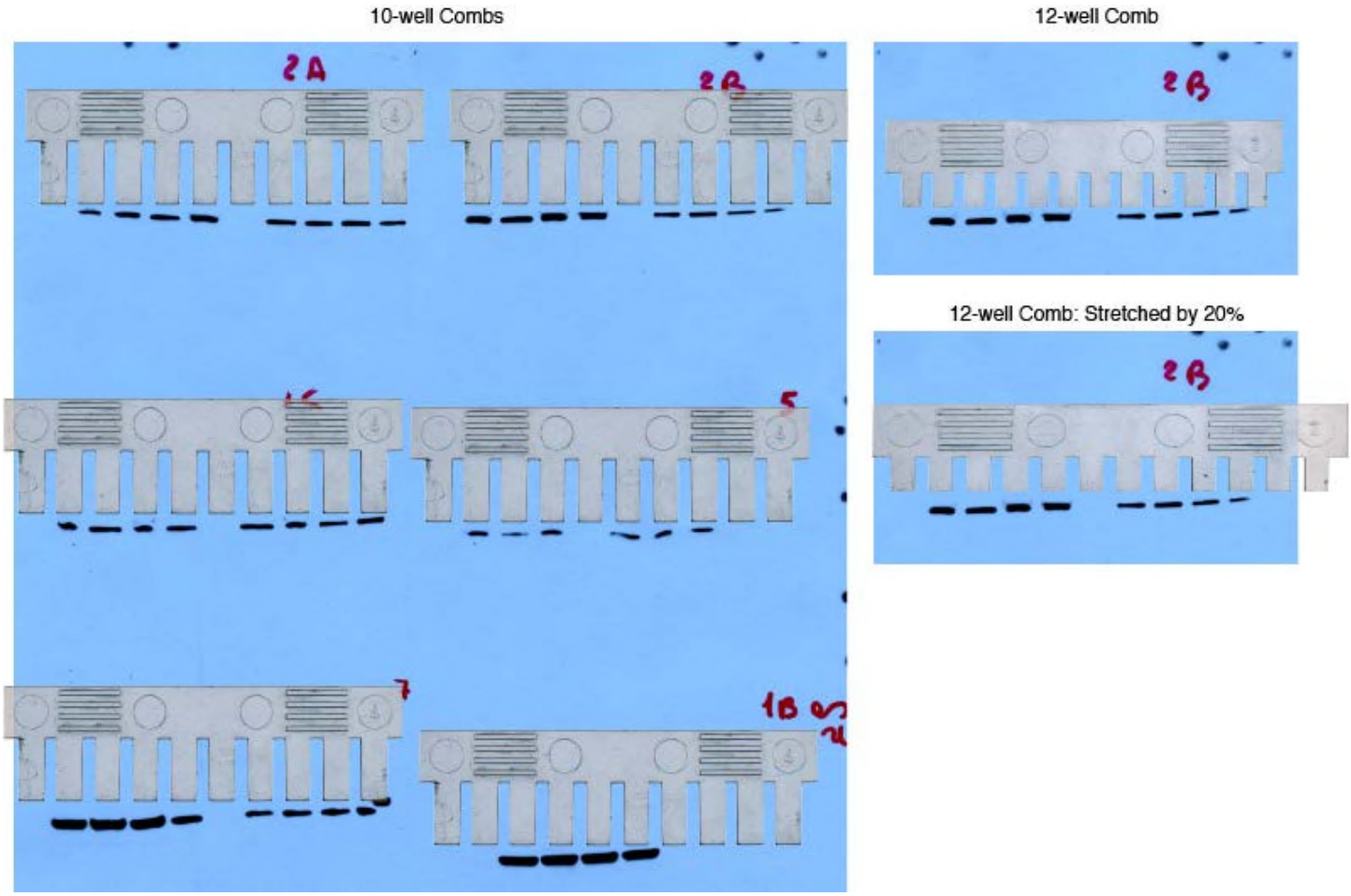
Dr. De Domenico also provided an e-mail exchange with a Bio-Rad representative. Although she asked why a 12-lane gel might swell more than a 10-lane gel, the representative did not address this question, but stated:

> “It looks like your bands/lanes seem to get wider towards the bottom of the gel. Too much salt in the sample can cause the effect of lane pinching/broadening.”

Since all the blots on the film appear to represent very similar experiments, this does seem to be a likely explanation for the different gel widths. (It also is not clear whether or not the gel has really swelled more towards the bottom, or if this is a perspective effect in the photograph.) In the 7-17-12 interview, Dr. De

Domenico also suggested that the greater swelling of the gel for Fig. 2B might be due to the use of a different gel type (Tris-HCl vs. Tris-Tricine, both of which are sold by Bio-Rad).

In considering Dr. De Domenico's assertion that a 12-lane gel was used for Fig. 2B and had swollen more than the 10-lane gels, the Committee overlaid images of 10- and 12-lane combs onto the film scan:



The 12-well comb in the images is the one provided to the Committee by Dr. Kaplan, and the 10-well comb is from the laboratory of one of the Committee members and has the same overall width (8.3 cm) as the 12-well comb. The images of the combs were made with a flatbed scanner using a resolution to match that of the film scan. As shown on the left, the 10-well comb matched all of the blots (with the possible exception of the blot labeled "1B", where the bands appear to have spread more than in the other gels). In contrast, when the 12-well comb is overlaid on the autoradiogram for Fig. 2B (upper right panel), there is a clear mismatch between the comb and bands. In order to match the bands, the image of the comb has to be stretched by 20%, as shown in the lower panel on the right. In contrast, the example of a swelled gel provided to the Committee by Dr. De Domenico appears to have swelled by about the width of one lane, or about 10%.

Having considered this issue at length, the Committee does not find Dr. De Domenico's explanations plausible, for the following reasons:

1. We are aware of no reason that a 12-lane gel would swell more than a 10-well gel.
2. The spacing of bands on the gel matches closely that of a 10-well comb, and of the other blots. It seems unlikely that a 12-lane gel would swell just enough to match the 10-well gels.

3. The required amount of swelling, 20% in a linear dimension, is quite large, and twice that shown in the example provided by Dr. De Domenico.
4. The example provided by Dr. De Domenico did not include any control, such as a 10-lane gel prepared under the same conditions.

The Committee also notes that in all of the blots represented on this film, for each of the samples for which reduced protein concentration in the cytoplasmic (flow-through) fraction is reported, the lane lies either in the center lane flanked by four lanes with detectable bands, or at the extreme sides of the lanes with bands. Furthermore, the absence of signal in these lanes appears to be absolute. This placement of the "negative" samples is dependent on the placement of control lanes, which shows no consistency among the different blots. In the interviews on 7-2-12 and 7-17-12, Dr. De Domenico was specifically asked about the three control lanes indicated on the left-hand side of the blot for Fig. 2B. In the first interview, she indicated that these were controls for this experiment, and that two or three controls were typically included in all experiments. In the second interview, however, she stated that they were actually samples from another experiment performed by a technician and that the 12-lane gel was used in order to avoid running an additional gel.

The Committee concludes that the labels on the film presented to the Inquiry Committee most likely represent a falsification of data and that the labels on other blots (e.g. Figs. 1C and 7) on this film may have been falsified as well. Specifically, it appears that the labels were configured to place the ones indicating reduced protein concentrations in the flow-through fractions at positions where either no sample or molecular weight markers were actually loaded.

It is unclear when the labels currently on the films were added. Examination of draft manuscripts and correspondence on Dr. De Domenico's computer indicates that the biotin-streptavidin experiments were performed in response to reviewers' comments on the original manuscript (submitted on 4-20-11). The scan file with minimal labels has a date of 7-20-11, consistent with the experiments having been performed between the original submission date and the submission of the final revised manuscript, on 9-10-11. A scan of the film with the more extensive labels was also found on Dr. De Domenico's computer, with a file date of 10-29-11, the day after Dr. Kaplan was notified by the journal of allegations concerning this paper, and 10 days before the notebooks were sequestered by the Research Integrity Officer.

If, as suggested above, the labeled film represents a falsification, then we must also conclude that Dr. De Domenico deliberately misled both the Inquiry and Investigation Committees. When the issues outlined above were raised during interviews with the Investigation Committee, Dr. De Domenico adamantly denied that there had been any relabeling of the film to falsify the research record.

2. Incorrect dates on films and notes for Paper 6

The notebooks currently held by the Research Integrity Office include autoradiography films and handwritten notes for experiments shown in paper 6. These films were presented by Dr. De Domenico to the Inquiry Committee, which identified several inconsistencies between the films and the published figures. In their responses to the Inquiry Committee's report, Drs. De Domenico and Kaplan stated that they had re-examined the films and corresponding notes and found, in most cases, that the notes supported the labels shown in the published figures.

The Investigation Committee revisited this issue during interviews with Drs. De Domenico and Kaplan, asking how they could be sure that the notes, rather than the labels on the film, were correct. Dr. Kaplan acknowledged uncertainty, but Dr. De Domenico repeatedly insisted that the notes were the correct record.

Following these interviews, the Committee noted that nearly all of the dates on the films and notes were subsequent to the submission date of the manuscript (or the revised manuscript for a figure added in the revision). In the one case (for Fig. 5A) for which the date on the film (4-2-10) precedes the submission date, a scan file of this film on Dr. De Domenico's computer is dated eight months earlier (8-3-2009).

It seems very unlikely that records would have been incorrectly dated in this way at the time the experiments were performed. At the least, the dates on the films and notes were most likely added several months after the work was done, and it is possible that the notes themselves were written then. This may have occurred after receipt of the allegations.

4. Notes for CD spectra in paper 10

Following the discovery of incorrect manipulations, and likely fabrication, of the circular dichroism spectra shown in Fig. 4 of paper 10, Dr. De Domenico was asked (on 9-5-12) to provide an account of the recording of these spectra and the data processing leading to the figure. Although she was aware at this time of the Committee's concern regarding the use of only the data points separated by 10 nm, she was not aware that the Committee had discovered evidence of falsification or fabrication. In response to this request, Dr. De Domenico delivered another notebook to the Research Integrity Officer's office on 9-11-12. In addition to printouts of some of the CD spectra, this notebook contained several pages of handwritten notes, with dates of 8-7-07, 8-17-07 and 9-1-07. The handwritten pages, along with relevant e-mail correspondence included in the notebook, have been scanned and are provided as a pdf file named "cd-nmr-notes.pdf".

Almost all of these notebook pages refer to instructions from "Tom" regarding the handling of the CD data, and the notes indicate that the data were sent to "Tom" for approval. These notes appear to support Dr. De Domenico's claim that Dr. Tomas Ganz played a major role in the analysis of the CD data.

In the context of the Committee's concern regarding unpublished NMR spectra, Dr. Ganz was specifically asked about his role in both the CD and NMR studies, though he was not informed of the evidence for manipulation of the CD spectra. Dr. Ganz's response is quoted in part below:

> "In general, my participation in this paper was very limited except that our group provided key reagents for the study, including the hepcidin forms that are one of the subjects of this paper."
>
> "The first version of the paper, which I received by e-mail on October 28, 2007, did not contain the NMR data, only the CD spectra. I first became aware of the NMR/CD data on Sept. 13, 2007 as a result of the e-mail quoted below."

The e-mail message, from Dr. Kaplan, is copied below:

> Dear Tom
> I tried calling you today. Just got back from vacation in San Diego- Met Pauline Lee the day after you did- she gave me your regards. We are in the final throes of sending you a manuscript. One of the punch lines is that hepcidin does not bind to Fpn at low temperature because of an increased rate of dissociation. The driving force is a change in hepcidin conformation at low temperature. We can show this by CD and by molecular sieve chromatography (at low temp- hepcidin forms a dimer). we have also done one dimensional NMR and can see changes. Our NMR guy (Jack Skalicki) send Hans Vogel an email asking him for assignments- he never answered. If we get the assignments then we can model the changes in hepcidin occurring at low temp. Is it possible for you to ask him for that data? HEP20 does not show the low temp changes- either by CD or by molecular sieve chromatography.
> The data is pretty compelling and (to me) interesting. Ivana is in Italy and except for the NMR modeling data we have everything done. Diane and I are working on the english and we hope to send you a readable version next week.
>
> Jerry

Note that this message is dated 9-13-07, twelve days after the latest date in the notes provided by Dr. De Domenico. Furthermore, a draft figure containing the CD spectra in their final form was found on Dr. De Domenico's computer, with a date of 9-3-07.

After receiving the information above from Dr. Ganz (and after receiving comments on the initial draft of this report from all of the parties), the Committee asked Dr. Kaplan (via e-mail) about the CD data. The specific questions asked of Dr. Kaplan are listed below, along with his responses:

1. Can you confirm the authorship and date of the e-mail message above [referring to the e-mail dated 9/13/07) ?

   I confirm that I wrote and sent that email to Dr Ganz

2. Do you know whether Dr. Ganz was sent or made aware of the CD data prior to 13 Sept. 2007?

   I believe that Dr. Ganz did not see the CD data prior to my email. It is, however, possible that we discussed it by phone, as we had numerous conversations about data as experiments were performed

3. Did Dr. Ganz play any role in the processing or analysis of the CD data?

   To my knowledge Dr. Ganz was not involved in the processing or analysis of the CD data. I think it is correct that he saw the data in near final form. I have found an email that indicates that we discussed a potential phone call regarding the manuscript on or about November 1. I do not remember if we had the conversation or what the conversation was about. He certainly saw the data as presented in the manuscript as he and Dr. Nemeth made comments/corrections relating to CD in the Methods, Results and Figure Legends of the manuscript prior to submission. (See attached manuscript drafts edited by Drs. Ganz and Nemeth. I received two versions of their comments V3 and V4 on the same day Nov6- I have enclosed both versions). I do not know who sent Dr. Ganz the manuscript- I cannot find an email from me to him with that attachment; I think Dr. Diane Ward sent him the manuscript

4. Did you serve as an intermediate in any communications regarding the CD data analysis, in particular passing on instructions from Dr. Ganz to Dr. De Domenico, or sending the data to Dr. Ganz for his comments?

   To my knowledge all of his comments about the CD data are reflected by his edits in the manuscript. . I do not recall passing on any comments from Dr Ganz to Dr. De Domenico regarding CD.

The responses from both Dr. Ganz and Dr. Kaplan, supported by the e-mail message of 9/13/07, clearly contradict the contents of the notebook pages provided by Dr. De Domenico to the RIO on 9/11/12 . Since Dr. Ganz is now in an adversarial relationship with Drs. De Domonico and Kaplan, the Committee recognizes that some caution is required in considering the statements from all of the parties. We note, however, that Dr. Ganz statements regarding the CD data were in response to questions concerning NMR spectra, and he was not informed of any irregularities regarding the CD at that time.

In her response to the initial draft of this report, Dr. De Domenico provided an e-mail exchange (Appendix 24 of her response) in support of her statements that Dr. Ganz was involved in the analysis of the CD data. However, this exchange (a message from Dr. Jack Skalicky to Dr. De Domenico and forwarded from her to Dr. Ganz) concerns only NMR data and is dated 10/29/07. These messages thus provide no support for the authenticity of the notebook pages.

The preponderance of evidence leads the Committee to conclude that the notebook pages provided by Dr. De Domenico on 9-11-12 were most likely created after she became aware of concerns regarding the CD spectra and were intended to mislead the Committee and shift responsibility to Dr. Ganz.

SUMMARY OF HARD DRIVES AND DVD

Notes on the computer drives and files used in RIO investigation 02-2111

Several different computer hard drives containing data from the respondents' computers were provided to the Investigation Committee. The following is a summary of the origins of these drives, as understood by the Committee, and their use in the investigations.

All of these drives were prepared, by the University Information Technology Office, from computers used by Drs. De. Domenico, Kaplan and Ward. Drives 1 and 2 were initially examined in the Research Integrity Office by one of the Committee members. Subsequently, secondary copies of these and the other drives were made available to the Committee members to use in their own offices.

Drive 1: Copied from Dr. De Domenico's computer on 9 November 2011, following the initiation of the inquiry. Examination of this drive by a Committee member on 24 June 2012 revealed that nearly all of the files on this drive were micrograph images organized in directories, most of which were named by date. Although this drive was identified as being a copy of Dr. De Domenico's computer drive, it appeared to be a copy of an auxiliary disk used specifically for image storage. Much of the material on this drive appeared to also be stored on a large number of CDs contained in notebooks sequestered by the Research Integrity Officer.

The drive also contained files that appeared to have been collected specifically for the inquiry, with the directory name "To Dr. Botkin". Most of the files in this directory appear to be related to one of the papers involving zebrafish.

Drive 2: Copied from Dr. Kaplan's computer on 5 March 2012, the day after receipt of the second set of allegations. This drive was examined by a Committee member on 24 June 2012, and was found to contain what appears to be a full copy of the system disk of Dr. Kaplan's computer. Several directories on this drive were found to contain manuscript drafts and correspondence regarding the papers under investigation. Image files containing figures for these papers were also found, but these were generally composite images for the final figures. No files containing primary data were identified on this drive.

This drive was not used further in the investigation.

Drive 3: A copy of a hard drive used for backups in the Kaplan laboratory. This copy was made in March 2012 with help from Diane Ward. This drive contained a variety of material, including laboratory data bases and backups of multiple computers, including Dr. Ward's. The most recent backup was dated 2 February 2006.

The only material found to be relevant to the investigation was a directory containing figures for paper 8, including multiple versions of the figures alleged to have been made from spliced blot images. These included both original film scans and composite images.

The files that appeared to be relevant to the figures in paper 8 were copied to a Committee member's computer for future reference.

Drive 4: After discovering that Drive 1 did not contain most of the files expected to be present on Dr. De Domenico's primary computer, the Committee requested that a full copy of her computer's drive be prepared. On 28 June 2012 the University IT office made copies of the system drives for both Dr. De Domenico's desktop computer and a new laptop computer. For these drives, the University IT office prepared forensic copies, which were retained by that office. A secondary copy was provided to the Research Integrity Office and the Committee.

The drive copied from Dr. De Domenico's laptop computer was not found to contain useful information for the investigation and was not used further.

The drive copied from Dr. De Domenico's desktop computer, however, was found to contain directories with files relevant to all of the challenged papers. These were found in either the "Desktop" or "Documents" directory of Dr. De Domenico's user directory.

Most of the investigation of this drive was carried out by one Committee member, who made a further copy for his own use, using the program Carbon Copy Cloner to insure that all of the data, including file dates, were preserved. To minimize the chances of altering the files and date information, the write permissions for all of the copied contents were turned off.

The Committee also asked the Universtiy Information Technology Office to scan the forensic copy of Dr. De Domenico's desktop computer drive, specifically asking the staff to identify Excel and SigmaPlot files that had been deleted in the previous year. The staff was unable to find evidence of such files.

Scans of Autoradiography Films and Notes in Laboratory Notebooks: Much of the research record examined by the Investigation Committee was in the form of autoradiography films of western blots, which were contained in loose-leaf notebooks sequestered by the Research Integrity Officer. These notebooks also contained handwritten notes for the experiments. To provide documentation for the Committee's report and an electronic record of key films, one of the Committee members scanned selected films and notes on 9-27-12. The scans were made in the Research Administration Office.

File Catalog: The files from Dr. De Domenico's computer relevant to the investigation were found in a number of different directories, many of which are partial duplications of others. For instance, files for paper 10, were found in directories named "Peptide", "Peptide Fpn", "Peptide Fpn from home", "Peptide Fpn from ivana" and "Peptide Fpn from ivana v1". Within these directories, there are often further duplications of directories and files. In order to keep track of the various files used in the investigation, the Committee has prepared a catalog in the form of an Excel worksheet, and this catalog, along with copies of the original files, are provided on a DVD supplement to the report.

Each file has been given an identifier code of the form:

paper number – figure number – index number

For instance, file 8-1A-1 is the first file relevant to Figure 1A of paper 8. The catalog includes the file name, its directory path on the original disk, the last date of modification, as well as brief notes regarding the file contents.

The copies of the files provided with this report have been organized into directories for each of the papers and have been renamed by prepending the original name with the identifier code. Thus, for the example above, the original file "Figure3a.tif" has been renamed "8-1A-1_Figure3a.tif". The files on the DVD should be identical, other than the added name prefixes, to those on the forensic disk copy held by the Information Technology Office.

The DVD also contains the scans of notebook pages and films, as well as other documents collected by the Committee.

INVESTIGATION COMMITTEE MEMBERS
University of Utah Case: 02-2011
Federal Office of Research Integrity Case: DIO 4554
December 14, 2012

H. Joseph Yost, Ph.D.
Investigation Committee Chairman
Professor, Department of Neurobiology & Anatomy

David Goldenberg, Ph.D.
Professor, Department of Biology

Elizabeth Leibold, Ph.D.
Professor, Molecular Medicine Program

John "Sandy" Parkinson, Ph.D.
Distinguished Professor, Department of Biology

Guy Zimmerman, M.D.
Associate Chair, Department of Internal Medicine

EXHIBIT 4

University of Utah
Consolidated Hearing Committee
Complaint – Research Misconduct

Respondent: Dr. Ivana DeDomenico

Complainants: Dr. Thomas Parks & Dr. Vivian Lee

Panel Report and Recommendations

May 8, 2013

Overview

This is a proceeding to hear charges against Assistant Professor Ivana DeDomenico in the Department of Internal Medicine, Division of Hematology. The charges in this matter were brought by the Senior Vice-President for Health Sciences and the Vice-President for Research following a report by an Investigation Committee dated December 14, 2012. A hearing panel was convened by the President of the Academic Senate and a hearing was conducted on April 29, 2013.

The CHC Panel has concluded that Dr. DeDomenico has engaged in research misconduct by "reckless disregard of accepted practices" in her area of research.

The Panel also finds that her behavior was part of a larger pattern of complicity in misconduct within the Kaplan laboratories. We emphasize that the institutional responsibility for these matters should not end with sanctions against Dr. DeDomenico.

Research Misconduct Policy and Procedure

UU Policy 7-001(IV)(H):

> "Misconduct" or "Misconduct in Research" means fabrication, falsification, plagiarism, or other practices that seriously deviate from those practices that are commonly accepted within the research community for proposing, conducting, or reporting research. It does not include honest error or honest difference in interpretations or judgments of data.

UU Policy 7-001(V)(6):

> A finding of misconduct requires that:
>
> 1. There is a significant departure from accepted practices of the research community for maintaining the integrity of the research record.
> 2. The misconduct be committed intentionally, or knowingly, or in reckless disregard of accepted practices and
> 3. The allegation is proven by a preponderance of evidence.

UU Policy 6-002(III)(10)(D)(3)(b):

> viii. Complaints alleging misconduct in sponsored research.
>
> The CHC must find, by a preponderance of the evidence, that the respondent(s) engaged in research misconduct as defined in Policy 7-001.

In summary, to find the Respondent guilty of research misconduct, the Complainants bear the burden of proof to show (1) by a preponderance of the evidence (2) that the Respondent committed fabrication, falsification, or serious deviations from accepted research practices, (3) with intent, knowledge, or reckless disregard of accepted practices.

Charges and Response

The chronology of this matter is a bit murky. Apparently, it began in the fall of 2011 with concerns expressed by editors of a journal in which one of Respondent's papers had been published. This concern was communicated to the University's Research Integrity Officer, Dr. Jeffrey Botkin, who contacted the Respondent and her mentor, Dr. Jerry Kaplan. In the meantime, additional concerns were communicated from the Federal Office of Research Integrity from an informant "who wished to remain anonymous," but who later was identified as a previous collaborator on published papers, a professor at UCLA, Dr. Tomas Ganz.

A total of 11 published papers have been examined in the course of these proceedings. Those papers are numbered 1-11 in the attached table. The numbering corresponds to the examination that was conducted by prior committees as explained here. The irregularities discussed in those examinations fall into three main categories, all dealing with figures published as if they reflected results of laboratory experiments: (1) misrepresentation of images from films of laboratory experiments (gels transferred to "western blot" images and then scanned into computer files), (2) misleading or erroneous creation of graph curves reflecting CD (circular dichroism) data, (3) and inaccurate error bars. The types of errors found in each paper are indicated in the attached table.

Dr. Botkin convened an Inquiry Committee on December 22, 2011, and charged the committee to look at alleged irregularities in three papers. The Inquiry Committee expanded its scope to six papers and submitted its report on February 16, 2012, with findings of errors with regard to several blot figures. A review by an independent statistician, however, found no significant difficulty with the error bars in those papers. The Inquiry Committee finished with these conclusions:

> The Committee did not uncover evidence of intent to deceive or misrepresent the data from the experiments in question. Rather, there was extreme carelessness on the part of Dr DeDomenico in preparing the figures and little oversight from Dr. Kaplan in ensuring that the figures accurately reflected the primary data. . . . [W]e recommend that all papers jointly authored by Drs. DeDomenico and Kaplan be independently reevaluated, and if additional errors are found, that these be fully disclosed to the editors of the journals involved.

Shortly thereafter, on March 4, 2012, Dr. Botkin received allegations again from Dr. Ganz of additional errors in two other published papers. Dr. Botkin met with Drs. DeDomenico and Kaplan on March 5, 2012, and informed them of his decision to convene an Investigation Committee with regard to all questioned papers. That committee was formed in April 2012. It examined not just the six papers initially in question but went further to examine five additional papers on which Drs. DeDomenico and Kaplan were co-authors. The committee produced a preliminary report on October 2, 2012, to which the Respondents were allowed to comment, and then produced its final report on December 14, 2012.

The Final Report of the Investigation Committee provided this summary conclusion:

> From its investigation, the Committee has concluded that the irregularities . . . constitute misrepresentations of the primary data and reflect a serious breakdown in the processes required to ensure that the scientific literature is a faithful record of research results. The large number of instances, over a period of several years, indicates a reckless disregard for the integrity of the research record, as opposed to the occasional lapses that might occur in any laboratory, and the resulting record is now nearly impossible to reconstruct.

The Investigation Committee also declared that it found instances of intentional falsification or fabrication of data. The CHC Panel has concluded, as explained below, that there likely was intentional falsification of published data or images, but we could not determine by a preponderance of the evidence that these items were the work of Dr. DeDomenico.

Thus, the CHC finding of misconduct is based on reckless disregard of accepted practice, a finding heavily influenced by the number of departures from established norms over an extended period. We recognize that the "accepted practices" in this particular laboratory and some of its collaborating laboratories may have countenanced the carelessness and obfuscation described by both prior committees, but these irregularities exceeded both accepted practice in the broader research community in which Respondent was educated as well as basic norms of right and wrong.

Evidence

This proceeding has produced a rather voluminous record. The principal items of evidence consisted of the report of the Investigation Committee (a 97 page document thoroughly examining the graphs and images in all 11 papers), detailed responses of Respondent to that analysis, testimony at the hearing by Dr. Yost explaining some of the key points in the Investigation Committee report, testimony in support of Dr. DeDomenico by Drs. Kaplan and Coulombe, and statements by Respondent herself.

To simplify understanding of the record, the CHC Panel constructed a table listing each paper by number along with the types of alleged irregularities, the Investigating Committee conclusions regarding the allegations, and some indication of the evidence behind those conclusions.

At the hearing, Dr. Yost produced a PowerPoint and oral presentation focusing on three of the papers (## 1, 10, & 11) that seemed to be the key evidence of either intentional or reckless disregard of accepted practices. To clarify our own conclusions, the Panel will likewise concentrate here on those same three papers. What we have gleaned from these and Dr. Yost's summary is that there was intentional misrepresentation of data in at least one instance but we cannot determine who did it, that Drs. DeDomenico and Kaplan as either lead author or senior author bore responsibility for spotting what seemed in at least two instances to be attention-getting misrepresentations, that Dr. Ward was probably responsible for at least one irregularity that was beyond the knowledge base of Dr. DeDomenico, and that in sum total the number of instances of irregularities in these papers shows reckless disregard for accepted practices in the research community.

We have not taken instances of missing data to count against Dr. DeDomenico except in those instances in which she was constructing figures for publication and should have had the data to support those figures.

Respondent's Procedural Concerns

Dr. DeDomenico objected to Dr. Botkin's convening of an Investigation Committee. She asserted that the procedure violated University regulations, federal law, and due process. There were two separate sets of issues raised – one set of issues regarding the original six papers and the other regarding the additional five papers. The Chair treated these objections as a motion to dismiss and issued a ruling on April 17, 2013.

With regard to the first set of papers, the argument is that the Investigation was not warranted at all because the Inquiry Committee did not recommend further investigation after reviewing the papers that were subject of the original Inquiry. DeDomenico points out that no provision of law or University policy empowers the RIO to determine independently that an investigation is warranted or to act against the finding of the Inquiry Committee. The University position was that the Inquiry Committee found "some substance" to the allegations of misconduct on the initial papers and that in the midst of determining how to proceed at that point, more allegations came in, at which point Botkin decided, with the federal RIO's blessing, to proceed with the investigation phase.

There are two important interests at stake in these proceedings, the importance of research integrity to the University and due process to the individual. The Chair agreed that the federal office could not speak for the University with regard to our procedures.

With regard to the first six papers, the Inquiry Committee did find "that each of these papers, and perhaps others from these combined authors, contain misrepresentations of the primary results and thus deviate significantly from accepted scientific practice." The committee then recommended that "all papers jointly authored by Drs. DeDemonico and Kaplan be independently reevaluated." Although not a formal recommendation for the convening of an Investigation Committee, the conclusions did encourage the RIO to proceed further in light of departures from accepted scientific practice. Indeed, the University regulations do not explicitly preclude the RIO from convening an Investigation Committee in the absence of a formal recommendation from the Inquiry Committee. Although the regulations require the convening of an Investigation Committee when the Inquiry Committee recommends it, the integrity of the University could mandate an Investigation in any event. This prospect flows from the requirement that the "inquiry record must contain sufficiently detailed documentation of the inquiry to permit a later assessment of the reasons for determining that an investigation was not warranted," and the requirement that the records be maintained in a secure manner for at least three years. All of these provisions look toward the prospect of a follow-up investigation at a later date.

Given that new allegations with respect to additional papers then surfaced, it was understandable that the RIO chose to manage procedures to ensure a thorough investigation, which resulted in charges brought before the CHC which must make its own findings on the evidence presented to it. Under these circumstances, the lack of an Inquiry Committee on the additional papers was not a sufficiently fundamental flaw as to require dismissal just to protect the integrity of University processes.

It is possible, however, with due process in mind, that Respondent was prejudiced in her ability to respond to the allegations by the timing of the two committee proceedings and the resulting methods of the Investigation. Under these circumstances, the Chair suggested that she explain any harm or prejudice that she experienced from the procedural posture of the proceedings.

In her written and oral statements, she did suggest that she would have been better prepared to respond to allegations if there had been an Inquiry Committee on the additional papers. She also pointed to a paucity of personal interaction with the Investigation Committee and to open hostility from one member of the Investigation Committee.

With regard to ability to respond, she did meet with the Committee three times and was given six weeks to respond to a preliminary report of the Committee, which she did in rather thorough fashion. With regard to personal hostility, when the Panel asked the Chair of the Investigation Committee about this, he responded that passions naturally run high in charges of misconduct and that he did not perceive any bias or prejudice on the part of the one rather outspoken member of the Committee. Respondent did not pursue this issue further during the hearing.

Findings

The hearing panel finds the following facts established by a preponderance of the evidence:

1. Paper 11 contained images that were intentionally manipulated to present false data. This was done by computer copying of information from one scanned image to another or in some instances by splicing of gel images to make one image. While the Panel agrees that this manipulation does appear to have been intentional, we cannot conclude that it was done by Dr. DeDomenico as opposed to an unknown third person. Nevertheless, the error appears so obvious that it should have been caught by a responsible lead or senior author.

2. Paper 1 contained several instances of flipped or mislabeled images. Although neither the Committee nor this Panel has found that these irregularities were intentional, the number and severity show reckless disregard to accepted practices. (Recklessness in this instance consists of proceeding with publication in the face of a very substantial risk of disseminating inaccurate information to the scientific community. The substantiality of risk stems from the number and severity of the errors.)

3. Paper 10 contained misleading graphs of CD data. The graphs were misleading by having been "smoothed out" in violation of usual practice in the field and fabricated by switching the sign of one data point so that the first half of the graph was inverted. Also significant and unlikely was that the CD data had exactly the same magnitude in different tables with a couple of exceptions; in addition, it was significant that the fabricated data were inconsistent with the conclusions of the paper. Dr. Kaplan testified that these graphs were produced by Dr. Ward and that he did not expect Dr. DeDomenico to have expertise with regard to these processes. Indeed, although Dr. DeDomenico was listed as lead author on this paper, it seems that she had little or nothing to do with the experiments leading to the contested portions of the paper. The Investigation Committee laid the blame for these errors squarely on the procedures in Dr. Kaplan's laboratory.

4. The Investigation Committee listed 3 instances of what they described as "Alteration of Research Record." These are highlighted at the end of the attached table. For the following reasons, the CHC Panel is not persuaded by a preponderance of the evidence that the alleged alterations of research records can be laid at the feet of Dr. DeDomenico:

   a. As stated above, the CHC Panel cannot find that Dr. DeDomenico was responsible for the intentional manipulation in Paper 11.

   b. Nor do we find the alleged falsification in Paper 6 to be supported by the record. The Committee stated: "At the least, the dates on the films and notes were most likely added several months after the work was done, and it is possible that the notes themselves were written then. This may have occurred after receipt of the allegations." But MAY HAVE is not enough for our purposes; the evidence that she fabricated notebook pages is confusing and not sufficiently persuasive of intentional falsification.

   c. With regard to Paper 10, the e-mail strings regarding handling of the CD data do not include Dr. Ward, on whom Dr. Kaplan stated that he relied for the CD data presentation.

5. There are multiple instances of errors in 10 of the 11 papers examined. The only one in which no errors were found was the one in which Dr. DeDomenico was the "Senior Author," meaning that other authors were responsible for the initial preparation of data, charts, and images. As first author on the other papers, she bore responsibility for checking all graphs and figures against the raw data and then maintaining an adequate record from which later researchers could replicate the experiment. Her explanations of these errors all relied on asserting that someone else did key parts of the work, but in almost all instances she was responsible for producing the actual images that were presented to the publisher. For two reasons, her explanations are inadequate:

   a. if true, then her explanations show a lack of control over data for which she was responsible as first author or preparer of the figure for publication

   b. it strains credulity that so many people could make similar errors without her catching at least some of them

6. In summary, our findings on the overall record are similar to what we found with regard to Paper 1. Although the Panel does not find that intentional irregularities can be attributed to Dr. DeDomenico, the number and severity of errors in the publications where she is first author show reckless disregard for accepted practices. Recklessness consists of proceeding with publication in the face of a very substantial risk of disseminating inaccurate information to the scientific community. The substantiality of risk stems from the number of publications affected and the severity of the errors.

Conclusions

Based on the above findings, the panel concludes

1. Dr. DeDomenico violated the Faculty Code through Research Misconduct based on "reckless disregard of accepted practices."
2. There was complicity in this misconduct within the laboratory that goes substantially beyond Dr. DeDomenico.

Recommendations

1. The Panel regretfully concurs with the Investigation Committee's recommendation that Dr. DeDomenico be terminated or allowed to resign from the University.
2. The Panel is concerned that the University take full appropriate action with regard to the institutional difficulties uncovered in this proceeding. The problems outlined by the Investigation Committee are not likely to be resolved with one termination and one retirement. Thus, we recommend further investigation of the laboratory procedures uncovered in this investigation.

Respectfully submitted,

Wayne McCormack 5/8/13

Wayne McCormack, Chair, College of Law

Richard Dorsky, Neurobiology & Anatomy

Scott Wright, Nursing

Jon Rainier, Chemistry

Darrell Davis, Medicinal Chemistry

Mikhail Skliar (Alternate), Chemical Engineering

Table of Investigation Committee Report Items Regarding Dr. DeDomenico

Paper & Date	Problem	Evidence & Invest. Comm Conclusions	Lead Author	Senior Author
1 (2011)	blot manipulation	no indication of where original film is located -Kaplan's files not examined for originals or notebooks - her computer had no files deleted - she says "three people participated in experiments" - not clear who had control of blots and films but she was primary author	DeDomenico	Kaplan
	error bars	Stoddard found no problem with calculations but doubted veracity of original data		
2 (2008)	blots flipped & duplicated	"We were unable to establish a cogent explanation for these irregularities aside from the possibilities of carelessness, negligence, capriciousness with flagrant or reckless disregard for fidelity of the published report of the original data, and/or intent." Kaplan did not rule out possibility of carelessness in lab processes.	DeDomenico	Kaplan
3 (2009)	duplication of data in two publications	"The findings are most consistent with carelessness, but the Committee could not exclude negligence, capriciousness with flagrant or reckless disregard for fidelity of the original data, or intent."	DeDomenico	Kaplan
4 (2009)	duplication of data in two publications	"The data in Figure 4A in Paper 4 appear to be correct as labeled, based on the statements of Drs. Kaplan and De Domenico. Therefore, the duplication and misrepresentation were in Paper 3, the PNAS article."	Koenig	DeDomenico
5 (2007)	same photo shown twice	"In summary regarding this allegation, the Investigation Committee concludes that there was duplication of data in this publication. The findings are suggestive of carelessness, but the Committee cannot exclude negligence, extreme capriciousness, or intent." Code in notebooks "illicitly discarded by dismissed technician"	DeDomenico	Kaplan
6 (2011)	error bars	"The issue of error bars was not discussed further with her or Dr. Kaplan in the interviews." Kaplan stated that the processes for calculating these were consistent with his scientific expertise.	DeDomenico	Kaplan
	fig. 4B blot disparities	"Our major conclusion is that the path from the primary data to the publication has been so poorly documented and corrupted by relabeling that no confidence can be placed on the published figure."		
	fig. 5B film date disparities	"The available records are inadequate to determine whether the published figure was correctly labeled. The inconsistencies between the dates shown on the film, the notes and the submission date raise additional doubts about the reliability of the notes."		

6 (cont'd)	general concerns	"The failure of Dr. De Domenico to provide the computer files documenting the data analysis in the published paper (and the failure of the Committee to find such files through a systematic search) is evidence of recklessness in maintaining the research record." Her response is that Committee was asked to look at Kaplan's records and no request was made to Kaplan for original data.		
7 & 8 (2007)	gel lane inaccuracies	Kaplan stated that cell expansion easily accounts for apparent lane violations	DeDomenico	Kaplan
	inaccurate splicing of gel images	"It appears that Drs. De Domenico and Ward had full control over presentation of the experimental data and both of them must be held responsible for the misrepresentations that have come to light. The Investigation Committee considers it unlikely that these errors were due to simple carelessness during the preparation of the figures for publication. The Committee is also struck by the conspicuous failure of all coauthors, particularly Drs. Ward and Kaplan, to catch and correct these mistakes before publication."		
9 (2007)	splicing of gels	"Based on the inconsistencies in the execution of the experiments and in the preparation of the figures, little confidence can be placed on the accuracy of the published data." No finding of intent or reckless disregard. Ward stated she was responsible for making figures.	DeDomenico	Kaplan
10 (2008)	error bars	Kaplan stated that statistical analysis was consistent with his scientific practices.	DeDomenico	Kaplan
	CD graphs	Kaplan stated that he saw no problem with "smoothing" of data and that he knew DeDomenico had no expertise in CD. No finding of intent from the Invest. Committee.		
	NMR data	Kaplan stated that he did not trust the data so omitted it.		
	general concerns	DeDomenico was not involved in these experiments. Committee stated: "Particularly in light of his own lack of expertise in these techniques, Dr. Kaplan should have ensured that all of the data were shared among the participants. In summary, the Committee concludes that this paper contains instances of data falsification and data fabrication and that this paper should be retracted. The circumstances leading to the publication of falsified and fabricated data indicate flaws in the management of the laboratory and collaboration."		
11	blot manipulation	"substantial concerns" that published images were misrepresentations of scans	Kieffer	Kaplan -- DeDomenico is contributor

"Alteration of Research Record"				
Allegation 1 (paper 1)	12 cell / 10 cell issue	Kaplan stated that he had seen "swelling" account for the apparent disparity		
Allegation 2 (paper 6)	incorrect dates on film and notebook pages	"At the least, the dates on the films and notes were most likely added several months after the work was done, and it is possible that the notes themselves were written then. This may have occurred after receipt of the allegations." No direct evidence that she altered dates and particularly not after allegations		
Allegation 3 [IC labelled 4] (paper 11)	CD data	"The preponderance of evidence leads the Committee to conclude that the notebook pages provided by Dr. De Domenico on 9-11-12 were most likely created after she became aware of concerns regarding the CD spectra and were intended to mislead the Committee and shift responsibility to Dr. Ganz." Kaplan made it clear that he did not expect CD expertise from DeDomenico. He stated that "I think Dr. Ward sent Ganz the manuscript."		

Paper 1: De Domenico I, Lo E, Yang B, Korolnek T, Hamza I, Ward DM, Kaplan J. (2011). The Role of Ubiquitination in Hepcidin-Independent and Hepcidin-Dependent Degradation of Ferroportin. Cell Metabolism Nov 2;14(5):635-46

Paper 2: De Domenico I, Lo E, Ward DM, Kaplan J. Hepcidin-induced internalization of ferroportin requires binding and cooperative interaction with JAK2. Proceedings of the National Academy of Sciences USA 2009; 106: 3800-3805

Paper 3: De Domenico I, Zhang TI, Koenig CL, Branch RW, London N, Lo E, Daynes RA, Kushner JP, Li D, Ward DM, Kaplan J. Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. Journal of Clinical Investigation 2010; 120: 2395-2405.

Paper 4: Koenig CL, Miller JC, Nelson JM, Ward DM, Kushner JP, Bockenstedt LK, Weis JJ, Kaplan J, De Domenico I. Toll-like receptors mediate induction of hepcidin in mice infected with Borrelia borgdorferi. Blood 2009; 114: 1913-1918.

Paper 5: De Domenico, Vaughn MB, Yoon D, Kushner JP, Ward DM, Kaplan J. Zebrafish as a model for defining the functional impact of mammalian ferroportin mutations. Blood 2007; 110: 3780-3783.

Paper 6: De Domenico I., Vaughn, M.B, Paradkar, P.N., Lo, E., Ward, D.M. and Kaplan, J. (2011) Decoupling ferritin synthesis from free cytosolic iron results in ferritin secretion. Cell Metabolism. 13, 57-67.

Paper 7: De Domenico, I., D. M. Ward, M. C. di Patti, S. Y. Jeong, S. David, G. Musci & J. Kaplan (2007). "Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin" EMBO J 26(12): 2823-2831.

Paper 8: De Domenico, I., D. M. Ward, C. Langelier, M. B. Vaughn, E. Nemeth, W. I. Sundquist, T. Ganz, G. Musci and J. Kaplan (2007). "The molecular mechanism of hepcidin-mediated ferroportin down-regulation." Mol Biol Cell 18(7): 2569-2578.

Paper 9: De Domenico, I, McVey Ward, D, Musci, G and Jerry Kaplan. "Evidence for the multimeric structure of ferroportin." Blood 2007 Mar 1;109(5):2205-9

PAPER 10: De Domenico I., Nemeth, E., Nelson, J.M., Philips, J.D., Ajioka, R.S., Kay, M.S., Kushner, J.P., Ganz, T, Ward, D.M. and Kaplan, J. (2008) The hepcidin-binding site on ferroportin is evolutionarily conserved. Cell Metabolism. 8, 146-156.

PAPER 11: Kieffer, C., Skalicky, J. J., Morita, E., De Domenico, I., Ward, D. M., Kaplan, J. and Sundquist, W. I. (2008). Two distinct modes of ESCRT-III recognition are required for VPS4 functions in lysosomal protein targeting and HIV-1 budding. Dev Cell 15: 62-73.

EXHIBIT 5

RAY QUINNEY & NEBEKER

September 19, 2014 4554

John Dahlberg, Ph.D.
Deputy Director
Susan Garfinkel, Ph.D.
Director, Division of Investigative Oversight
U.S. Dep't. of Health and Human Services
Office of Research Integrity
1101 Wootton Pkwy., Ste. 750
Rockville, MD 20852

Ryan B. Bell
ATTORNEY AT LAW

PO Box 45385
Salt Lake City, Utah
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Dear. Drs. Dahlberg & Garfinkel:

This firm represents Dr. Ivana De Domenico, a former Assistant Professor at the University of Utah, Department of Internal Medicine. Dr. De Domenico was accused of research misconduct and was the subject of an inquiry and investigation process, which concluded with the issuance of a Consolidated Hearing Committee Report on May 8, 2013 recommending her termination, followed by her removal from the University. While the Hearing Committee did not find grounds to support charges of falsification and fabrication, they did find misconduct on the basis of recklessness.

Dr. De Domenico vigorously opposed the accusations made against her, and continues to maintain that her accusers were incorrect and were motivated by bad faith. She also alleges that the University's treatment of her was discriminatory, and has begun legal proceedings to litigate the merits of that position.

In connection with Dr. De Domenico's discrimination proceedings, it has come to light that the University of Utah submitted a final report to your office in June 2013 relaying the decision of the Consolidated Hearing Committee. However, I asked Dr. Jeffrey Botkin, the University's Vice President for Research Integrity, to confirm that he sent the complete file to ORI, which would include documents submitted by Dr. De Domenico in her defense. He would not offer any assurance that he had done so. During the lengthy and complicated proceedings conducted by the University of Utah, Dr. De Domenico submitted a number of papers and evidentiary items establishing her defenses and making arguments rebutting the accusations against her. Without these documents, your office's file would be incomplete and harmfully one-sided.

I write for three reasons. First, we provide herewith a set of documents that will be useful in the event that ORI undertakes an oversight review of the University's determinations. We strongly believe that any such

review would be woefully incomplete without inclusion of the materials submitted by Dr. De Domenico. In this proceeding, the University essentially acted as both judge and prosecutor, and thus a review of only the documents generated by the University would provide an extremely prejudicial account of the relevant facts. The University disregarded or misinterpreted a number of important facts Dr. De Domenico raised in her defense, and any reliance on the University's conclusions, without reference to contrary evidence, will only compound the University's mistakes. We therefore request that the materials enclosed herewith be added to ORI's official record of this proceeding, and all such materials be made part of any additional oversight review undertaken by ORI.

Secondly, given the amount of time that has passed since the University concluded its investigation, we request an update from ORI regarding its intentions with regard to Dr. De Domenico's case. While we understand that ORI follows its own internal procedures and timelines, we wish to convey that Dr. De Domenico's life and career have been thrown into disarray by the University's proceedings, and that the continuing threat of additional process continues to complicate her pursuit of new opportunities. Dr. De Domenico is entitled to finality in this process. If ORI has already determined not to make additional conclusions or review of Dr. De Domenico's actions, please advise. Alternatively, if Dr. De Domenico's case may yet be reviewed by ORI at some point, we ask that ORI convey its plans, to provide something upon which Dr. De Domenico may rely in her career planning. Being forced to operate under the threat of additional action has become a significant and ultimately prejudicial burden on Dr. De Domenico and her ability to earn a living.

Finally, we wish to communicate to you that Dr. De Domenico has discovered a number of new facts not available to her during the period of the investigation that significantly buttress her defenses. For example, it came to light after the investigation was closed that Drs. Kaplan and Ward, two senior co-authors not censured by the Consolidated Hearing Committee, made changes to one of the main papers under review without Dr. De Domenico's knowledge, after she had approved it for publication. These changes created problems in the paper for which Dr. De Domenico was later held responsible. The co-authors continue to seek and receive federal research funding even though Dr. De Domenico and others have reported substantial errors in their work. Further, the University altered its final report to protect another co-author with a vested interest in the outcome, Dr. Wes Sundquist, whom Dr. De Domenico believes was responsible for the errors in one published paper. This alteration was made specifically at the request of Dr. Sundquist, and over Dr. De Domenico's objection, further complicating the procedural improprieties that compromised the University's investigation

from the very beginning. A number of other problems have infected the University's process, several of which have come to light only recently. This provides further reason for ORI to involve Dr. De Domenico in any oversight review it performs on this case.

In summary, we ask that ORI add the attached materials to its file and include in any oversight review it undertakes, that it advise as to whether it currently intends or does not intend to conduct a review of Dr. De Domenico's case, and that it provide an opportunity for Dr. De Domenico to submit additional argument and evidence, if an additional review is initiated. If you find reference in your files to any materials that have not been sent to you, please let me know and I will be happy to provide them to you.

Please feel free to contact me with any questions regarding the above.

Sincerely,

RAY QUINNEY & NEBEKER P.C.

Ryan B. Bell

1295852

INDEX OF DOCUMENTS

1. CD containing University Audio Recording of Consolidated Hearing Committee Hearing, April 29, 2013.

2. Dr. De Domenico Response to Report of Investigation Committee, November 16, 2012.

3. Detailed In-line Response to Report of Investigation Committee, November 16, 2012.

4. Appendix of Documents Referred to in De Domenico Response, November 16, 2012.

5. Supplemental Response of Dr. De Domenico Regarding Paper 10.

6. Request for Appeal Proceeding, January 28, 2013.

7. Reply to University Response to Dr. De Domenico Submissions, March 15, 2013.

8. Dr. De Domenico Disclosures for Appellate Hearing, April 24, 2013.

9. Letter to President David Pershing, May 15, 2013.

EXHIBIT 6

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Assistant Secretary for Health
Office of Research Integrity
1101 Wootton Parkway, Suite 240
Rockville, MD, 20852

Phone: 240-453-8200
FAX: 301-594-0043

CONFIDENTIAL/SENSITIVE

Ivana Frech, Ph.D.
771 Highland Park Ave.
Coralville, IA 52241-3378

RE: ORI Case No. 2017-05

Dear Dr. Frech:

This is to notify you that the Office of Research Integrity (ORI) in the U.S. Department of Health and Human Services (HHS) is making findings of research misconduct under 42 C.F.R. Part 93 against you based on the evidence and findings of an investigation by the University of Utah (UU) and additional information obtained and analysis conducted by ORI during its oversight review of this investigation.

The following sets forth a summary of ORI's findings of research misconduct and HHS' proposed administrative actions. This notice also provides information about your opportunity to contest these findings and actions. A more detailed charging document setting forth the basis for ORI's findings of research misconduct and the HHS administrative actions is enclosed.

I. Summary of ORI's Findings of Research Misconduct

ORI Findings: ORI finds that you (Respondent) intentionally, knowingly, or recklessly engaged in research misconduct by falsifying and/or fabricating research results in three (3) published papers funded by the U.S. Public Health Service (PHS).

Specifically, ORI makes six (6) findings of research misconduct based on the preponderance of the evidence that Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot and autoradiogram images related to mechanisms of cellular iron regulation by reusing, relabeling, and manipulating images to falsely report data in three (3) published papers.

In the enclosed charging document and the attached and incorporated exhibits, ORI has provided a detailed description of the facts and circumstances supporting its findings of research misconduct.

II. Definition of Research Misconduct

42 C.F.R. Part 93 defines *research misconduct* as:

> fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results. (a) Fabrication is making up data or results and recording or reporting them. (b) Falsification is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record. (c) Plagiarism is the appropriation of another person's ideas, processes, results, or words without giving appropriate credit. (d) Research misconduct does not include honest error or differences of opinion.

III. Proposed HHS Administrative Actions

A. Debarment

The Deputy Assistant Secretary for Acquisitions (Debarring Official), the HHS official authorized to impose debarment, has reviewed these findings and finds that the research misconduct involved in this case provides a cause for debarment under 2 C.F.R. § 180.800(d). HHS adopted and gave regulatory effect to 2 C.F.R. Part 180 at 2 C.F.R. Part 376.

The Debarring Official proposes debarring you for a period of three (3) years from participating in "covered transactions" as defined in 2 C.F.R. § 180.200 and procurement transactions covered under the Federal Acquisition Regulation (48 C.F.R. chapter 1).

Some of the significant consequences of debarment are:

- You will be ineligible to be a participant in a federal agency transaction that is a covered transaction (including grants and cooperative agreements); or act as a principal of a person participating in one of those covered transactions; except as provided in 2 C.F.R. §§ 180.135, 315, and 420. *See* 2 C.F.R. §§ 180.130 and 376.995.

- You will be excluded from receiving contracts, and agencies shall not solicit offers from, award contracts to, or consent to subcontracts with you, unless the agency head determines that there is a compelling reason for such action. You also will be excluded from conducting business with the Government as an agent or representative of other contractors. *See* 48 C.F.R. §§ 9.401, 9.405.

- Your name will be placed in the "System for Award Management" (SAM), which is maintained by the General Services Administration.

B. Additional Administrative Actions

It is proposed that you be prohibited from serving in any advisory capacity to PHS, including but not limited to service on any PHS advisory committee, board, and/or peer review committee, or as a consultant for a period of three (3) years.

It also is proposed that ORI will send a notice to the pertinent journal of the following published paper that requires retraction or correction, in accordance with 42 C.F.R. § 93.407(a)(1) and § 93.411(b):

- Two Distinct Modes of ESCRT-III Recognition are Required for VPS4 Functions in Lysosomal Protein Targeting and HIV-1 Budding. *Dev Cell.* 2008 Jul;15(1):62-73. doi: 10.1016/j.devcel.2008.05.014

IV. Opportunity to Contest ORI's Findings of Research Misconduct

In accordance with the PHS Policies on Research Misconduct, Subpart E, you may contest the ORI findings of research misconduct and proposed HHS administrative actions by requesting an administrative hearing before an administrative law judge with the HHS Departmental Appeals Board (DAB). Your hearing request must be in writing, signed by you or your attorney, and sent to the DAB and ORI within 30 days of receipt of this letter. 42 C.F.R. § 93.501. If you do not request a hearing, the research misconduct findings and administrative actions set forth above will become effective 30 days from the date of receipt of this letter.

The DAB may be experiencing delays in processing documents received by mail. To avoid delay, the DAB strongly encourages parties to file hearing requests through its online e-filing system. You may register for and access the online filing system at https://dab.efile.hhs.gov/.

If you do not have access to the Internet to use the DAB's online e-filing system, you must send your hearing request by certified mail, or other equivalent (i.e., with a verified method of delivery) to:

Chief Administrative Law Judge
Departmental Appeals Board
Department of Health and Human Services
MS 6132
330 Independence Ave., S.W.,
Cohen Building, Room G-644, Washington, DC 20201

For further information, you can find the PHS Policies on Research Misconduct, 42 C.F.R. Part 93, on the ORI website at:

http://ori.hhs.gov/sites/default/files/42_cfr_parts_50_and_93_2005.pdf

Please be advised that the PHS Policies on Research Misconduct, Subpart E, provide for a hearing only to address "a genuine dispute over facts material to the findings of research misconduct or proposed administrative actions." 42 C.F.R. § 93.503(a). Thus, any request for a hearing should identify the specific material facts in the ORI findings of research misconduct that you dispute, including a statement of the reason(s) for disputing the finding(s) and proposed administrative action(s).

You also must send a complete copy of your hearing request, including enclosures, by certified mail, or other equivalent (i.e., with a verified method of delivery) to:

Sheila Garrity, JD, MPH, MBA
Director
Office of Research Integrity
1101 Wootton Parkway, Suite 240
Rockville, MD 20852-5003

If you do not request a hearing, the research misconduct findings and administrative actions set forth above will become effective 30 days from the date of receipt of this letter.

Sincerely,

Sheila R. Garrity -S
Digitally signed by Sheila R. Garrity -S
Date: 2023.06.22 16:07:24 -04'00'

Sheila Garrity, JD, MPH, MBA
Director
Office of Research Integrity

katrina brisbon (Jul 3, 2023 15:05 EDT)

H. Katrina Brisbon
Deputy Assistant Secretary for Acquisitions
and Suspension and Debarment Official

Enclosures

cc: Dr. Tara A. Schwetz, Agency Research Integrity Liaison Officer, NIH, w/o Exhibits
Erin Rothwell, Ph.D., Research Integrity Officer, University of Utah

THE OFFICE OF RESEARCH INTEGRITY'S FINDINGS OF RESEARCH MISCONDUCT AGAINST IVANA FRECH, PH.D.

The Office of Research Integrity ("ORI") makes findings of research misconduct against Ivana Frech, Ph.D. (formerly "Ivana De Domenico") ("Respondent"), former Assistant Professor in the Department of Internal Medicine in the University of Utah ("UU") School of Medicine. ORI's findings are based on evidence and findings of an investigation conducted by UU, ORI's oversight review of UU's investigation, and additional analysis conducted by ORI in its oversight review. UU submitted an investigation report to ORI (the "UU Report"), incorporated herein by reference and attached hereto as Charge Letter Exhibit ("**C.L. Ex.**") **1**.

I. JURISDICTION

A. ORI's Authority

1. ORI has the statutory and regulatory authority to make findings of research misconduct and propose administrative actions. *See* 42 U.S.C. § 289b; 42 C.F.R. Part 93.

B. Definition of Research Misconduct

2. Under 42 C.F.R. Part 93:

> *Research misconduct* means fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results.
>
> (a) Fabrication is making up data or results and recording or reporting them.
>
> (b) Falsification is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.
>
> (c) Plagiarism is the appropriation of another person's ideas, processes, results, or words without giving appropriate credit.
>
> (d) Research misconduct does not include honest error or differences of opinion.

42 C.F.R. § 93.103.

3. A finding of research misconduct made under 42 C.F.R. Part 93 requires that—

> (a) There be a significant departure from accepted practices of the relevant research community; and
>
> (b) The misconduct be committed intentionally, knowingly, or recklessly; and
>
> (c) The allegation is proven by a preponderance of the evidence.

42 C.F.R. § 93.104.

C. U.S. Public Health Service Financial Support

4. The questioned research was supported by the following U.S. Public Health Service ("PHS"), National Institutes of Health ("NIH"), grants:

   a. National Institute of Diabetes and Digestive and Kidney Diseases ("NIDDK") grant R01 DK070947, "Mechanism and Regulation of Iron Export by Ferroportin," Dr. Jerry Kaplan, Principal Investigator (P.I.), from 05/15/2005-06/30/2015. **C.L. Ex. 2**.

   b. NIDDK grant R01 DK090257, "Regulation of Iron Homeostasis," Dr. Ivana De Domenico, P.I., from 09/30/2010-06/30/2015. **C.L. Ex. 3**.

   c. NIDDK grant R01 DK030534, "Factors regulating the cellular uptake of iron," Dr. Jerry Kaplan, P.I., from 01/01/1982-01/31/2019. **C.L. Ex. 4**.

   d. National Institute of General Medical Sciences ("NIGMS") grant P50 GM082545, "Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV," Dr. Wesley I. Sundquist, P.I., from 08/27/2007-07/31/2022. **C.L. Ex. 5**.

   e. National Institute of Allergy and Infectious Diseases ("NIAID") grant R01 AI051174, "Biochemistry of HIV-1 Membrane Recognition and Budding," Dr. Wesley Sundquist, P.I., from 02/01/2002-03/31/2022. **C.L. Ex. 6**.

   f. National Heart, Lung, and Blood Institute ("NHLBI") grant R01 HL026922, "Receptor Mediated Endocytosis in Aveolar Macrophages," Dr. Jerry Kaplan, P.I., from 05/01/1981-05/31/2013. **C.L. Ex. 7**.

D. Records That Contain Falsified and/or Fabricated Research Results

5. ORI's findings of research misconduct concern the falsification and/or fabrication of figures reported in the following PHS-supported published papers:

   a. Kieffer C, Skalicky JJ, Morita E, De Domenico I, Ward DM, Kaplan J, Sundquist WI. Two Distinct Modes of ESCRT-III Recognition are Required for VPS4 Functions in Lysosomal Protein Targeting and HIV-1 Budding. *Dev Cell.* 2008 Jul;15(1):62-73. doi: 10.1016/j.devcel.2008.05.014 ("*Dev. Cell* 2008") ("Paper 11" in UU Report). **C.L. Ex. 8**.

   b. De Domenico I, Lo E, Yang B, Korolnek T, Hamza I, Ward DM, Kaplan J. The Role of Ubiquitination in Hepcidin-independent and Hepcidin-dependent Degradation of Ferroportin. *Cell Metab.* 2011 Nov 2;14(5):635-46. doi: 10.1016/j.cmet.2011.09.008. ("*Cell Met*. Nov. 2011") ("Paper 1" in UU Report). **C.L. Ex. 9**. Retracted: *Cell Met*. 2012 Jun 6;15(6):927. doi: 10.1016/j.cmet.2012.04.107. **C.L. Ex. 10**.

   c. De Domenico I, Vaughn MB, Paradkar PN, Lo E, Ward DM, Kaplan J. Decoupling Ferritin Synthesis from Free Cytosolic Iron Results in Ferritin Secretion. *Cell Metab.* 2011 Jan 5;13(1):57-67. doi: 10.1016/j.cmet.2010.12.003 ("*Cell Met*. Jan. 2011") ("Paper 6" in UU Report). **C.L. Ex. 11**. Retracted: *Cell Met.* 2012 Jun 6;15(6):927. doi: 10.1016/j.cmet.2012.04.012. **C.L. Ex. 10**.

II. SUMMARY OF ORI FINDINGS AND HHS PROPOSED ADMINISTRATIVE ACTIONS

6. ORI makes six (6) findings of research misconduct based on the preponderance of the evidence that Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot and autoradiogram images related to mechanisms of cellular iron regulation by reusing, relabeling, and manipulating images to falsely report data in eight (8) figures included in three (3) PHS-supported published papers.

7. ORI finds that these acts constitute a significant departure from accepted practices of the relevant research community. *See* 42 C.F.R. § 93.104(a).

8. ORI finds by a preponderance of the evidence that Respondent's intentional, knowing, or reckless falsification and/or fabrication of data constitutes research misconduct within the meaning of 42 C.F.R. §§ 93.103 and 93.104.

9. The Deputy Assistant Secretary for Acquisitions and Suspension and Debarment Official (Debarring Official) proposes that for a period of three (3) years, Respondent be debarred from participating in "covered transactions" as defined in 2 C.F.R. § 180.200 and procurement transactions covered under the Federal Acquisition Regulation (48 C.F.R. chapter 1).

10. HHS also proposes that for a period of three (3) years, Respondent be prohibited from serving in any advisory capacity to PHS, including but not limited to service on any PHS advisory committee, board, and/or peer review committee, or as a consultant.

11. In addition, ORI finds that *Dev. Cell* 2008 should be retracted or corrected. *Cell Met*. Jan. 2011 and *Cell Met*. Nov. 2011 already have been retracted by the journal *Cell Metabolism*.

12. In accordance with 42 C.F.R. §§ 93.407(a)(1) and 93.411(b), HHS proposes to send to the journal *Developmental Cell* a notice of ORI's findings and of the need for retraction or correction of *Dev. Cell* 2008.

III. FACTUAL BACKGROUND

A. Respondent's Background

13. Respondent received a B.S. in biological science in 2002 from the University of Messina, Italy, and a Ph.D. in 2005 from the University of Messina.

14. From 2005-2007, Respondent was a postdoctoral fellow in the laboratory of Dr. Jerry Kaplan, a professor in the UU School of Medicine's Department of Pathology, and from 2007-2008, Respondent was a research associate in the Kaplan laboratory. In 2008, Respondent was promoted to research assistant professor in the Department of Internal Medicine at UU.

15. In June 2013, UU terminated Respondent's employment.

B. Scientific Background and Respondent's Research

16. Iron is essential for life, but its levels must be tightly regulated in animals. Excess amounts of iron either within cells or in blood and other extracellular fluids are toxic. Regulation of iron homeostasis is important for normal physiological function, and its dysregulation is implicated in a number of diseases, including anemias.

17. It is well established that the regulation of iron levels in human blood is controlled by two proteins, hepcidin ("HEP") and ferroportin ("FPN"). As an iron transporter, FPN is a transmembrane protein that exports iron out of cells and into the bloodstream. When HEP binds the FPN iron transporter protein, it inactivates FPN, and as a result less iron is pumped from inside cells into the blood plasma. Once FPN is internalized by the binding of HEP, it undergoes lysosomal degradation (i.e., FPN protein is broken down in a regulated manner inside the cell) such that the total concentration of the FPN protein in the cell decreases. The downregulation of FPN is dependent on proteins that mediate cell membrane remodeling through membrane budding events.

18. Respondent's research involved studies of the molecular mechanisms of cellular iron regulation. Respondent's three papers that are the subject of ORI's findings presented seminal data on mechanisms of the regulation of FPN by HEP and ferritin, thereby adding to the body of knowledge regarding the ability of an individual to appropriately respond to changes in the environment through regulation of iron homeostasis.

C. Allegations and UU's Research-Misconduct Proceeding

19. In November 2011, a complainant contacted UU's Research Integrity Officer ("RIO") and ORI with allegations of research misconduct against Respondent, including in *Cell Met*. Nov. 2011 (referenced as "Paper 1" in the UU Report) and *Cell Met*. Jan. 2011 (referenced as "Paper 6" in the UU Report).

20. On November 8, 2011, the RIO met with Respondent and Dr. Kaplan for initial interviews. On the same day, the RIO sequestered laboratory notebooks that included autoradiography films and handwritten notes.

21. In December 2011, UU initiated an inquiry into the allegations. The inquiry committee met with Respondent and with Dr. Kaplan to discuss the allegations.

22. The complainant submitted additional allegations to the RIO in March, May, and June 2012, including allegations involving *Dev. Cell* 2008 (referenced as "Paper 11" in the UU Report).

23. In April 2012, UU initiated an investigation into all of the allegations and later added the May and June 2012 allegations to its investigation.

24. The investigation committee interviewed multiple witnesses, including Respondent, Dr. Kaplan, Dr. Diane Ward (Associate Professor in the UU Department of Pathology and a coauthor on the papers at issue), and the complainant.

25. The committee also posed questions regarding the *Dev. Cell* 2008 allegations to Dr. Collin Kieffer and Dr. Wesley Sundquist, both in the Biochemistry Department at UU School of

Medicine and coauthors of *Dev. Cell* 2008. Dr. Sundquist and Dr. Kieffer responded jointly on July 19, 2012. **C.L. Ex. 14**. On July 21, 2012, Respondent sent to the RIO an email with a letter attachment describing Respondent's responsibility for the falsification in Figure 5B of *Dev. Cell* 2008. **C.L. Ex. 13**. Drs. Sundquist and Kieffer provided a follow-up response to the investigation committee on July 26, 2012, confirming Respondent's responsibility for that falsification. **C.L. Ex. 15**.

26. After providing Respondent with the opportunity to comment on its draft investigation report (*see, e.g.*, **C.L. Ex. 12** (Detailed Response to Investigation Report)), the investigation committee issued its final investigation report (the UU Report) on December 14, 2012, recommending findings of research misconduct against Respondent and termination of employment, which UU accepted in January 2013.

27. Respondent appealed the research misconduct findings and sanctions to the UU Office of the Academic Senate. A Consolidated Hearing Committee (CHC) panel conducted a hearing in April 2013. The CHC panel issued its report and recommendations in May 2013 and concluded that Respondent had engaged in research misconduct under UU policy by "reckless disregard of accepted practices." It agreed with the investigation committee's recommendation to terminate Respondent's employment or allow Respondent to resign.

28. In June 2013, the President of UU accepted the recommendations of the CHC.

29. UU sent the investigation report to ORI on June 10, 2013.

IV. ORI'S FINDINGS OF RESEARCH MISCONDUCT

30. ORI's Division of Investigative Oversight conducted an oversight review of the UU investigation.

31. ORI made the following six (6) findings of research misconduct based on the preponderance of the evidence that Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot and autoradiogram images to falsely report data in eight (8) figures included in three (3) PHS-supported published papers.

<u>ORI FINDING #1</u>: Respondent intentionally, knowingly, or recklessly falsified and/or fabricated a western blot image in Figure 5B (right bottom panel) of *Dev. Cell* 2008 by reusing and relabeling an image of three western blot bands to represent the results of different experiments.

Figure 5 of *Dev. Cell* 2008 ("Paper 11" in the UU Report) reports on research involving charged multivesicular body protein 6 (CHMP6), which mediates FPN downregulation. Figure 5 demonstrates that "silencing" CHMP6 using a silencing RNA (siRNA) strategy prevents the hepcidin-dependent downregulation through lysosomal degradation of FPN,

whereas a control siRNA does not. **C.L. Ex. 8** (*Dev. Cell* 2008) at 7. Use of siRNA is a common method in cell and molecular biology to target and reduce a specific target protein to study its function.

32. According to *Dev. Cell* 2008, Figure 5B shows western blot analyses of HEP-induced FPN-GFP[1] downregulation in human embryonic kidney 293 (HEK 293) cells in the presence of wild type and mutant CHMP6.

33. The western blot bands in the middle panel (labeled "anti-FPN") show FPN levels in the presence of wild type ("WT") and mutant CHMP6 proteins and in the presence or absence of endogenous CHMP6.

34. The western blot image panel in the bottom row (labeled "anti-α-Tubulin") of Figure 5B, right panel, shows six α-tubulin bands, which serve as an internal loading control to ensure that any differences in FPN levels shown in the middle row (labeled "anti-FPN") are due to a biological process and not differences in total protein loaded across lanes.

35. In the bottom-right western blot image panel corresponding to the α-tubulin loading control in published Figure 5B (CHMP6 siRNA + FPN-GFP tubulin loading control), the image of the three bands in lanes 7-9 is identical to the image of the three bands in lanes 10-12. Thus, the image of α-tubulin bands in lanes 7-9 was duplicated, spliced into a composite image, and relabeled to represent different α-tubulin bands in lanes 10-12. *See* **C.L. Ex. 18** (ORI Image Analysis) at 1-2.

36. Falsification and/or fabrication of loading controls is significant because loading controls validate the amount of protein in each lane. Changes reported in the protein of interest (FPN) could result from unequal loading of sample in each lane or other technical issues, as opposed to the proposed regulatory mechanism of protein targeting and degradation.

37. Respondent created the composite image containing the duplicated α-tubulin bands. *See* **C.L. Ex. 13** (July 21, 2012, Respondent email and attached letter to Botkin (stating that "a[n] image was generated by me and sent to Dr. Kieffer and Sundquist on March 3, 2008" and that "the duplication came from me")); **C.L. Ex. 14** (July 19, 2012, Kieffer & Sundquist Letter to Botkin); **C.L. Ex. 15** (July 26, 2012, Kieffer and Sundquist Letter to Botkin); **C.L. Ex. 16** (Nov. 12, 2013 Kieffer and Sundquist letter to Dahlberg).

38. It is implausible that the reuse and relabeling of the image is attributable to honest error rather than intentional and knowing falsification and/or fabrication because the duplication occurred within an image panel and thus involved splicing two images together to make a composite image of six bands in a row.

[1]Green fluorescent protein ("GFP") is a protein tag commonly attached to a protein of interest in molecular biology. The tag allows a researcher to distinguish FPN introduced into the cell from the naturally occurring FPN and visualize FPN expression under different conditions using traditional techniques in biochemistry, molecular biology, and microscopy.

39. Moreover, the falsification and/or fabrication of an image was not an isolated event, but rather part of a pattern of image reuse, relabeling, and manipulation in multiple figures across three papers, as discussed throughout these findings. This further supports that Respondent's falsification and/or fabrication was intentional and knowing and did not result from honest error.

40. Respondent intentionally, knowingly, or recklessly falsified and/or fabricated the bottom right panel in Figure 5B of *Dev Cell* 2008.

41. Respondent's falsification and/or fabrication constitutes a significant departure from accepted practices of the relevant research community.

ORI FINDING #2: **Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images in Figure 1C (top and bottom panels) of *Cell Met.* Nov. 2011 by reusing and relabeling an image of western blot bands to represent the results of two different experiments.**

42. *Cell Met.* Nov. 2011 proposes a second mechanism of FPN inactivation that does not require the hormone HEP. **C.L. Ex. 9**. The model reported in the paper proposes that this second HEP-independent mechanism involves the direct "sensing" of internal cellular iron levels, presumably by the FPN protein itself. In the model proposed in the paper, FPN becomes internalized inside the cell when iron levels inside the cell are low (iron is required for cellular processes within the cell). This result would constitute a very important finding for the field of iron regulation and human physiology because the requirement for HEP in iron regulation is so well established.

43. Figure 1C of *Cell Met.* Nov. 2011 supports the paper's hypothesis that depletion of iron within the cell leads to HEP-independent FPN degradation. **C.L. Ex. 9** at 3. In Figure 1C, Respondent engineered HEK 293 cells to express FPN protein that was fused with a GFP tag. In the western blot results shown in Figure 1C: lane 1 indicates cells without iron (ferric ammonium citrate, or "FAC") supplementation for 18 hrs; lane 2 indicates cells without iron (FAC) supplementation for 36 hrs; and lane 3 shows cells supplemented with iron (FAC) for 36 hrs. As would be expected by the model, the amount of FPN on the plasma membrane ("PM") (therefore able to transport iron out of the cell) goes away after depriving the cells of iron for 36 hrs (lane 2). According to both the original HEP-dependent and new HEP-independent models, the FPN transporter would be expected to be internalized inside the cell when iron levels are low.

44. Figure 1C, top right panel, shows a western blot analysis of affinity-purified samples and the flow through from FPN-GFP-expressing cells in the presence of and absence of FAC or DFX.[2] The western blot bands show the amount of FPN retained in the PM (in the "PM" row) versus the amount of FPN inside the cell's cytoplasm (in the "FT," or flow

[2]Deferasirox (DFX) is a strong iron chelator, i.e., it renders nearly all iron unavailable in a biological context. Therefore, with DFX treatment, one would hypothesize that nearly all detectable FPN would be internalized (as shown in the FT subpanel lane 4), as iron levels are scarce and the cell would conserve internal supplies.

through, row) after removal (lanes 1 and 2) or supplementation (lane 3) with FAC or treatment with DFX.

45. The top row, labeled "PM," in Figure 1C of *Cell Met.* Nov. 2011 is an image of western blot bands showing the amount of FPN retained in the PM with or without FAC at certain time intervals, or without FAC but with DFX treatment (lane 4). The bottom row, labeled "FT," is an image of western blot bands showing the amount of FPN inside the cell's cytoplasm under the same experimental conditions.

46. Respondent reused and relabeled the same image of four western blot bands in the top and bottom panels. In particular, the image of the bands in lanes 1-4 in the upper panel ("PM"), representing FPN-GFP found in the PM, is a copy, flipped horizontally, of the image of the bands in lanes 1-4 in the lower panel ("FT"), representing FPN-GFP found in the flow through. *See* **C.L. Ex. 18** (ORI Image Analysis) at 3-4.

47. Respondent is the first author of *Cell Met.* Nov. 2011.

48. Respondent generated the figures in *Cell Met.* Nov. 2011 (UU Report "Paper 1"). *See* **C.L. Ex. 12** (Detailed Response to Investigation Report) at 9.

49. Respondent knowingly and intentionally duplicated and horizontally flipped the western blot bands in both PM and FT rows to show ideal results, strongly supporting Respondent's hypothesis, in which all of the FPN protein was completely accounted for in either the PM or internal cytoplasmic (FT) fractions.

50. Respondent would have understood that in a perfect experimental system, the total amount of FPN in Respondent's experiments would be expected to be: FPN (TOTAL) = FPN on PM + FPN inside the cell (FT). For example, in lanes 2 and 3, the intensity of each protein band (PM + FT) ideally would sum to account for all FPN in the cell. There are many steps in this biochemical assay, including purifying out the protein by binding it to affinity beads (leading to some protein loss) and then eluting the protein off the beads (also leading to some protein loss) for the western blot. As some protein loss is unavoidable at nearly all these steps, ideal results could not be achieved without knowing and intentional falsification and/or fabrication.

51. The manipulation of the copied western blot bands also shows that the falsification and/or fabrication is attributable to intentional and knowing conduct rather than honest error.

52. Moreover, the falsification and/or fabrication of an image was not an isolated event, but rather part of a pattern of image reuse, relabeling, and manipulation in multiple figures across three papers, as discussed throughout these findings. This further supports that Respondent's falsification and/or fabrication was intentional and knowing and did not result from honest error.

53. Respondent intentionally, knowingly, or recklessly falsified and/or fabricated Figure 1C (top right panel) of *Cell Met.* Nov. 2011.

54. Respondent's falsification and/or fabrication constitutes a significant departure from accepted practices of the relevant research community.

ORI FINDING #3: **Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images in Figures 1D and 3 of *Cell Met.* Nov. 2011 by reusing and relabeling one image to represent the results of two different experiments.**

55. According to *Cell Met.* Nov. 2011, Figure 1D shows that FPN is targeted for degradation in HEK 293 cells by modifying the amino acid lysine at position 253 through a process known as ubiquitination. **C.L. Ex. 9** at 3. Ubiquitination is a protein modification that specifically occurs on lysine (abbreviated as "K") amino acid residues in proteins that can signal that protein for targeted degradation. By reporting equal FPN-GFP levels in the western blot image of the right panel of Figure 1D, Respondent showed that lack of a ubiquitin band in the K253A (i.e., lysine substituted for an alanine (A) that cannot be modified) column was not attributed to different FPN protein expression or recovery levels, but rather that the specific lysine at amino acid 253 was modified.

56. According to *Cell Met.* Nov. 2011, Figure 3 shows that the enzyme Nedd4-2 is required for the internalization and degradation of FPN-GFP in HEK 293 cells genetically engineered to stably express the FPN-GFP gene (Figure 1D shows HEK 293 cells that do not have this genetic modification). Figure 3 represents a different experiment that claims to identify the specific ubiquitin ligase (i.e., the protein enzyme that adds the ubiquitin to the lysine) as Nedd4-2. **C.L. Ex. 9** at 5. In Figure 3, Respondent alleges that depletion of the Nedd4-2 enzyme in lane 4 prevents internalization of FPN-GFP in the presence of hepcidin.

57. Respondent reused and relabeled FPN-GFP western blot bands in Figure 1D to also represent GFP western blot bands in Figure 3 (lanes 3 and 4). In particular, the lower right blot image panel (labeled GFP and treated with DFX) in Figure 1D is identical to the blot image in the last two lanes of the middle panel (Nedd4-2 column) in Figure 3 (labeled GFP and treated with or without HEP) after cropping out lanes one and two. *See* **C.L. Ex. 18** (ORI Image Analysis) at 5-6. Therefore, Respondent reused and relabeled control data in Figure 1D to also represent key experimental results from a different experiment to claim to identify the specific enzyme (Nedd4-2) required for FPN-GFP ubiquitination, internalization, and subsequent degradation. Thus, the same western blot images in Figures 1D and 3 were used to represent results from different experiments using different cell lines -- one an unmodified cell line and one cell line genetically modified with FPN-GFP stable gene integration.

58. Respondent is the first author of *Cell Met.* Nov. 2011.

59. Respondent generated the figures in *Cell Met.* Nov. 2011 (UU Report "Paper 1"). *See* **C.L. Ex. 12** (Detailed Response to Investigation Report) at 9.

60. The image manipulation (cropping of the larger image to create the smaller image) shows that the image reuse and relabeling was not honest error but rather knowing and intentional falsification and/or fabrication.

61. Moreover, this falsification and/or fabrication of an image was not an isolated event, but rather part of a pattern of image reuse, relabeling, and manipulation in multiple figures across three papers, as discussed throughout these findings. This further supports that Respondent's falsification and/or fabrication was intentional and knowing and did not result from honest error.

62. Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images by reusing and relabeling one image to represent the results of different experiments in Figure 1D and Figure 3 of *Cell Met.* Nov. 2011.

63. Respondent's falsification and/or fabrication constitutes a significant departure from accepted practices of the relevant research community.

ORI FINDING #4: **Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images in Figures 2Aii and 2B of *Cell Met.* Nov. 2011 by reusing and relabeling one image as representing the results of two different experiments.**

64. According to *Cell Met.* Nov. 2011, Figure 2Aii purportedly shows that Nedd4-2 is the specific enzyme (ubiquitin ligase) responsible for FPN internalization and degradation. **C.L. Ex. 9** at 4. Nedd4-2 is a family member of ubiquitin ligases previously known to regulate ion channels, so Respondent hypothesized that the FPN iron ion transporter would be regulated by a similar mechanism.

65. In Figure 2Aii, Respondent purportedly shows that FPN-GFP is internalized 36 hours after expressing the protein in HEK 293 cells (compare band intensity in column 1, lane 1 (N.S. 18h) with column 3, lane 1 (N.S. 36h)). However, when the cells are depleted of the Nedd4-2 ubiquitin ligase using siRNA, FPN-GFP was not degraded (compare band intensity in column 2, lane 1 (Nedd4-2 18h) with column 4, lane 1 (Nedd4-2 36h)). Therefore, these results support that Nedd4-2 is required for FPN internalization under normal conditions (i.e., iron levels were not manipulated in this experiment).[3]

66. According to *Cell Met.* Nov. 2011, Figure 2B shows that Nedd4-2 depletion prevents cells from internalizing FPN during iron depletion (DFX treatment). **C.L. Ex. 9** at 4. Therefore, these results support that Nedd4-2 also is required for appropriate FPN regulation when iron levels are low (versus Figure 2Aii, which shows Nedd4-2 degradation under normal iron conditions).

[3]The samples purportedly cseparated into two fractions: plasma membrane (PM, or cell surface) or flow through (FT, or cytoplasmic). FPN identified in the FT fraction presumably represents internalized FPN.

67. The two panels in the upper row of Figure 2Aii purportedly represent HEK 293 cells that have expressed FPN-GFP for 18 hours (left panel) or 36 hours (right panel). These cells allegedly were treated either with a non-targeting control siRNA or Nedd4-2 specific siRNA.

68. The two panels in the upper row of Figure 2B purportedly represent HEK 293 cells that have expressed FPN-GFP and were treated with a non-targeting control siRNA or Nedd4-2 specific siRNA just as in Figure 2Aii. However, in Figure 2B the cells purportedly were treated with DFX (+, right panel) or served as controls (-, left panel). DFX strongly binds to iron and makes it unavailable to the cells. Therefore, Figure 2B supports the model that Nedd4-2 also is required for FPN degradation when cells experience low levels of iron.

69. The image of the western blot bands in the top right panel of Figure 2Aii is a copy of the image of the western blot bands in the top right panel of Figure 2B. *See* **C.L. Ex. 18** (ORI Image Analysis) at 7.

70. The image of the western blot bands in the top left panel of Figure 2Aii is a copy, flipped horizontally, of the image of the western blot bands in the top left panel of Figure 2B. *See* **C.L. Ex. 18** (ORI Image Analysis) at 8.

71. Thus, the same western blot images are used to represent different experimental treatments.

72. Respondent is the first author of *Cell Met.* Nov. 2011.

73. Respondent generated the figures in *Cell Met.* Nov. 2011 (UU Report "Paper 1"). *See* **C.L. Ex. 12** (Detailed Response to Investigation Report) at 9.

74. It was not honest error but rather knowing and intentional falsification and/or fabrication because the top left panel in Figure 2Aii was copied, flipped horizontally, and relabeled to represent a different experiment in Figure 2B. Additionally, the corresponding bottom panels for both figures are from different source images.

75. Further, the corresponding FT panels are different but support the expected outcome for the different illustrated experiments. For example, the FPN-GFP internalization shown in Figure 2B (FT) purports to demonstrate impressively that no detectable FPN is internalized during iron depletion when Nedd4-2 is absent. Also, the falsification and/or fabrication supports the authors' hypothesis that Nedd4-2 is the specific ubiquitin ligase that targets FPN for degradation. This also shows that the falsification and/or fabrication was knowing and intentional and not honest error.

76. Moreover, the falsification and/or fabrication of an image was not an isolated event, but rather part of a pattern of image reuse, relabeling, and manipulation in multiple figures across three papers, as discussed throughout these findings. This further supports that Respondent's falsification and/or fabrication was intentional and knowing and did not result from honest error.

77. Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images by reusing and relabeling one image to represent the results of different experiments in Figures 2Aii and 2B of *Cell Met.* Nov. 2011.

78. Respondent's falsification and/or fabrication constitutes a significant departure from accepted practices of the relevant research community.

ORI FINDING #5: **Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images in Figures 2Aii and 5 of *Cell Met.* Nov. 2011 by reusing and relabeling one image as representing the results of two different experiments.**

79. According to *Cell Met.* Nov. 2011, Figure 5 shows that treatment of cells with zinc maintains FPN on the PM, thereby partially blocking FPN-GFP internalization and subsequent degradation. **C.L. Ex. 9** at 7. This result supports the authors' hypothesis that FPN can transport other metal ions in addition to iron (Fe), specifically manganese (Mn) and zinc (Zn).

80. *Cell Met.* Nov. 2011 Figure 2Aii, in contrast, shows a different experiment. As previously described in Finding #4, Figure 2Aii tests the authors' hypothesis that the internalization of the FPN-GFP iron transporter is regulated by the ubiquitin ligase Nedd4-2 under normal conditions. The western blot depicted in Figure 2Aii purportedly demonstrates that depletion of Nedd4-2 in HEK 293 cells prevents the normal internalization of FPN-GFP. Therefore, the results presented in Figure 2Aii, if true, would have provided important insight into the normal regulation of the FPN iron transporter.

81. In the upper image panel in Figure 5, the top panel was labeled as FPN-GFP from the "PM," for plasma membrane. The samples purportedly were separated into two fractions: plasma membrane (PM, or cell surface) or flow through (FT, or cytoplasmic).

82. In the lower left image panel in Figure 2Aii, the bottom left panel was labeled as FPN-GFP from the "FT."

83. The first and second lanes in the lower left panel in Figure 2Aii are identical to the two right lanes (+DFX and +$ZnSO_4$/+DFX) in the upper panel in Figure 5. The image in Figure 2Aii is flipped horizontally and has a lighter contrast than the identical image in Figure 5. *See* **C.L. Ex. 18** (ORI Image Analysis) at 9.

84. Thus, the same image was used by Respondent to support both a model where FPN is degraded under normal iron conditions in Figure 2Aii and a different model in Figure 5, notably that FPN is promiscuous and also binds zinc (Zn) in addition to its well-established iron (Fe) binding partner.

85. Thus, the same western blot images were used to represent results from different, unrelated experiments.

86. The manipulation (flipping and contrast adjustment) of the duplicated images shows that the falsification and/or fabrication was knowing and intentional rather than honest error.

87. The results as presented in Figure 5 support the authors' hypothesis that FPN is promiscuous for other positive metal ions. This also shows that the falsification and/or fabrication was knowing and intentional and not honest error. The falsifications and/or fabrications provide a more-detailed understanding of the regulation of FPN, thereby increasing the overall novelty and impact of this study in the field of iron physiology.

88. Moreover, the falsification and/or fabrication of an image was not an isolated event, but rather part of a pattern of image reuse, relabeling, and manipulation in multiple figures across three papers, as discussed throughout these findings. This further supports that Respondent's falsification and/or fabrication was intentional and knowing and did not result from honest error.

89. Respondent is the first author of *Cell Met.* Nov. 2011.

90. Respondent generated the figures in *Cell Met.* Nov. 2011 (UU Report "Paper 1"). *See* **C.L. Ex. 12** (Detailed Response to Investigation Report) at 9.

91. Respondent intentionally, knowingly, or recklessly falsified and/or fabricated western blot images by reusing and relabeling one image to represent the results of different experiments in Figures 2Aii and 5 of *Cell Met.* Nov. 2011.

92. Respondent's falsification and/or fabrication constitutes a significant departure from accepted practices of the relevant research community.

ORI FINDING #6: **Respondent intentionally, knowingly, or recklessly falsified and/or fabricated images in Figure 4B (top and bottom left panels) of *Cell Met.* Jan. 2011 by reusing and relabeling an image of an autoradiogram to misrepresent the reported experimental conditions and results.**

93. *Cell Met.* Jan. 2011 ("Paper 6" in the UU Report) examined the synthesis and subsequent intracellular trafficking of ferritin in response to changes in cytosolic iron concentration. The reported experiments involved synthesizing a protein in a test tube (in vitro) and in the presence of the radioactively labeled amino acid methionine (^{35}S-methionine). The relative abundance and molecular weight of the resulting protein under different treatment conditions was determined by isolating the (radioactive) protein of interest, subjecting the sample to gel electrophoresis, and imaging by autoradiogram (X-ray film exposed to the radioactive protein samples). Like a western blot, the intensity of the resulting bands correlates with protein abundance.

94. According to *Cell Met*. Jan. 2011, the results reported in Figure 4 demonstrate that iron depletion induces ferritin secretion. **C.L. Ex. 11** at 5. Figure 4B purportedly shows

autoradiograms of ceruloplasmin[4] protein recovery under different experimental conditions, including the presence or absence of Endo H (an enzyme that removes a specific sugar modification from proteins), iron (Fe), the protease PK, and the strong detergent Triton.

95. The top left image panel of Figure 4B reported the results of experiments without treatment with Endo-H ("- Endo H") and without iron treatment ("-Fe").

96. The bottom left image panel of Figure 4B reported the results of experiments without Endo H ("- Endo H") and with iron treatment ("+Fe").

97. Respondent's laboratory notebook contained a labeled film of an autoradiogram. **C.L. Ex. 17** (nb_6_4B-1.tif). The autoradiography film is labeled with Respondent's initials ("IDD"). *See id.*

98. The autoradiography film from the laboratory notebook shows the same sets of bands that appear in the left two image panels in Figure 4B but flipped.

99. Specifically, Figure 4B's top left image panel shows bands in lanes labeled -Endo H / -Fe. The same set of bands appears on the right-hand side of the autoradiogram in lanes labeled +Endo H / +Fe but flipped horizontally. *See* **C.L. Ex. 18** (ORI Image Analysis) at 10-11.

100. Thus, identical images were labeled differently to represent different experimental conditions (with or without Endo H and with or without iron) in the Figure 4B top left panel and the autoradiogram.

101. Figure 4B's bottom left image panel shows a set of bands that also appears on the left-hand side of the autoradiogram, in lanes labeled -Endo H / +Fe, but flipped both horizontally and vertically, i.e., around both the x and y axes.

102. In response to the inquiry committee's finding that the lower left panel of Figure 4B was flipped compared to the original film, Respondent stated: "The committee is correct that the panel was flipped around the vertical axis BUT represents the lanes as published." **C.L. Ex. 1** at 33.

103. However, the protein standards or molecular weights marked on the film are critical in interpreting whether the Endo-H treatment cleaved off the hypothesized glycosylation modification. If cleaved, the band would run at a smaller molecular weight since the glycosylation modification. If not cleaved, it would add mass to the protein. Alternatively, if ceruloplasmin is not glycosylated, the band would run at the same size as a band not treated with Endo-H. Thus, inversion of the image misrepresents the molecular weight and the overall conclusion that the ceruloplasmin protein undergoes a glycosylation modification.

104. The bands as reported in Figure 4B's bottom left image panel do not match the conditions and molecular weight represented in the X-ray film for those bands. The images in Figure 4B

[4]Ceruloplasmin is an enzyme important in copper and iron transport. Low levels of ceruloplasmin in the blood can have adverse health effects and are often indicative of liver disease.

and the autoradiogram report different conditions and protein masses under those specific conditions.

105. The falsification and/or fabrication was intentional and knowing and did not result from honest error. The bands in the lower left panel of Figure 4B were flipped vertically and horizontally. Inadvertent, vertical flipping of both the right- and left-hand sets of bands in the film would not lead to the bands in the bottom left image panel to be selectively flipped horizontally.

106. Moreover, both the right- and left-hand sets of bands in the film were labeled as deriving from experiments with iron treatment. Inadvertent flipping of the images in the film around the vertical axis would not have changed that labeling. However, in Figure 4B's top left image panel, the image of bands from the autoradiogram is shown in lanes labeled "-Fe." *See* **C.L. Ex. 18** (ORI Image Analysis) at 12-13. Thus, Respondent's honest error argument is not credible.

107. If only inadvertent flipping had occurred, the molecular weight markers shown in the bottom left and right image panels would have corresponded to the markers in the film, as flipping around the vertical axis would not have changed the molecular markers' positions on the vertical axis. The bands as reported in the figure do not match the conditions and molecular weight represented in the X-ray film.

108. The paper's authors hypothesized that ceruloplasmin is modified by glycosylation. The falsified and/or fabricated images in Figure 4B support this hypothesis. This is significant, as additional insight into this regulatory process increases the understanding of iron regulation and overall impacts the field of iron physiology. However, Respondent's autoradiogram indicated no difference in the molecular weight of ceruloplasmin when treated with Endo H, which would fail to support this hypothesis and reduce the novelty of the research findings in Figure 4B.

109. Moreover, the falsification and/or fabrication of an image was not an isolated event, but rather part of a pattern of image reuse, relabeling, and manipulation in multiple figures across three papers, as discussed throughout these findings. This further supports that Respondent's falsification and/or fabrication was intentional and knowing and did not result from honest error.

110. Respondent is the first author of *Cell Met.* Jan. 2011.

111. Respondent intentionally, knowingly, or recklessly falsified and/or fabricated Figure 4B of *Cell Met.* Jan 2011 (UU Report "Paper 1").

112. Respondent's falsification and/or fabrication constitutes a significant departure from accepted practices of the relevant research community.

V. AGGRAVATING AND MITIGATING FACTORS

113. Under 42 C.F.R. § 93.408, HHS considered aggravating and mitigating factors in determining appropriate HHS administrative actions and their terms. The HHS Debarring Official also considered factors in 2 C.F.R. § 180.860 in determining to debar Respondent and the length of Respondent's debarment period. The applicable factors in this case are described below.

114. Respondent's actions were knowing and intentional. 42 C.F.R. § 93.408(a).

115. Respondent solely planned, initiated, and carried out the wrongdoing. 2 C.F.R. § 180.860(f).

116. Respondent's research misconduct was not an isolated event but rather was a pattern that occurred over the course of several years in three (3) PHS-supported papers. 42 C.F.R. § 93.408(b).

117. Respondent's research misconduct had a significant impact on the proposed and reported research record, other researchers, institutions, and the public health or welfare. 42 C.F.R. § 93.408(c); *see also* 2 C.F.R. §180.860(a). (Respondent's misconduct resulted in actual and potential harm or impact that resulted and may result from Respondent's wrongdoing.)

118. Specifically, Respondent's falsified and/or fabricated research results were included in three (3) PHS-supported published papers and utilized funds that PHS otherwise could have spent on research that was not falsified or fabricated.

119. Respondent's falsifications and/or fabrications led to retraction of two (2) published papers and ORI's finding that an additional paper should be retracted or corrected.

120. Chronic dysregulation of iron in blood plasma leads to anemia (low plasma iron) or tissue damage and serious inflammation (high plasma iron). Specifically, the HEP/FPN regulatory axis is targeted in both the diagnosis and treatment of various iron disorders. Respondent's proposed new mechanism for inactivating FPN, particularly in a HEP-independent manner, would have been considered a significant and exciting finding in the field on which other basic and clinical researchers would and/or did follow up. Thus, Respondent's fabricated and/or falsified research papers led to waste of valuable time and resources.

121. According to Web of Science™, an index of metrics based on international citation activity of science journals, books, and scientific proceedings reflects that, as of April 19, 2023, Respondent's three affected papers have been cited by others a total of 142 times in the scientific literature.

122. Respondent failed to accept responsibility for Respondent's misconduct and failed to recognize the seriousness of Respondent's misconduct. 42 C.F.R. § 93.408(e); 2 C.F.R. §180.860(g).

123. As a mitigating factor, Respondent cooperated with the institution's research-misconduct proceeding. Respondent also admitted to the creation of certain figures and the duplication of one of the images in question. 42 C.F.R. § 93.408(e).

VI. RESEARCH MISCONDUCT IS A CAUSE FOR DEBARMENT

124. Respondent's falsification and/or fabrication in three published papers constitutes research misconduct under 42 C.F.R. Part 93.

125. The purpose of debarment is to protect the federal government from an individual who has proven to be untrustworthy. *See Kimon J. Angelides*, DAB No. 1677 (1999).

126. Moreover, the policy underlying debarment is that the federal government should conduct business only with responsible contractors and grantees. *See Dr. Paul F. Langlois*, DAB No. 1409 (1993), 1993 WL 742594 (DAB May 6, 1993).

127. The HHS Departmental Appeals Board has held that "[t]he scientific community functions on trust and openness; once that trust is breached, the harm from incidents of fabrication and falsification of data is far greater than simply discrediting the work known to be false or fabricated." *Id.*

128. Respondent's intentional, knowing, or reckless research misconduct establishes a lack of trustworthiness and is so serious and compelling in nature that it demonstrates a lack of present responsibility to be a steward of federal funds. 2 C.F.R. § 180.800(d).

129. Respondent should be debarred.

VII. HHS ADMINISTRATIVE ACTIONS AGAINST RESPONDENT

130. Based on the preponderance of the evidence supporting the ORI findings of research misconduct stated herein, the HHS Debarring Official proposes that for a period of three (3) years, Respondent be debarred from participating in "covered transactions" as defined in 2 C.F.R. § 180.200 and procurement transactions covered under the Federal Acquisition Regulation (48 C.F.R. chapter 1).

131. HHS also proposes that, for a period of three (3) years, the Respondent be prohibited from serving in any advisory capacity to PHS including but not limited to service on any PHS advisory committee, board, and/or peer review committee, or as a consultant.

132. In accordance with 42 C.F.R. §§ 93.407(a)(1) and 93.411(b), HHS proposes that ORI will send to the journal *Developmental Cell* a notice of ORI's findings and the need for retraction or correction of *Dev. Cell* 2008.