UNIVERSITY of HOUSTON

Division of Research

May 23, 2016 **CONFIDENTIAL**

To: Dr. Mousa Abkhezr
Teaching Assistant
Department of Biology and Biochemistry
University of Houston
mabkhezr@central.uh.edu
m_abkhezr@yahoo.com

Dr. Stuart Dryer
Professor
Department of Biology and Biochemistry
University of Houston
sdryer@uh.edu

SUBJECT: Final Determination: Research Misconduct

Dear Drs. Mousa and Dryer,

The purpose of this communication is to provide you, as named respondents, with the University's final determination regarding research misconduct allegations (Appendix A) received by the Division of Research (DOR). The proceeding adhered to the Division of Research Responding to Allegations of Research Misconduct policy:

http://www.uh.edu/research/compliance/res-misconduct/

The delegated Deciding Official (DO)[1] has reviewed the investigation report and has determined that the institution agrees with the investigation committee and the Research Integrity Officer's (RIO's) findings and recommended actions. Specifically:

The following were found, based on the preponderance of the evidence, to be **substantiated instances of research misconduct**:

- **Allegation 1. a. Substantiated Finding of Research Misconduct: *Falsification***

[1] Chancellor/President Dr. Renu Khator serves as the DO for the University of Houston, and has delegated sanctioning authority to Dr. Paula Myrick Short, Senior Vice President for Academic Affairs and Provost.

The STAT3 blots in Figures 2D, 4A and 5A are identical to blots previously published in [Dr. Dryer's] article in the journal *Molecular Pharmacology* (*Mol Pharmacol* 87:231–239, February 2015) as Figures 1B and 1D.

- **Allegation 1. b. Substantiated Finding of Research Misconduct: *Falsification***
  The β-actin blots in Figures 3A, 3B and 9B are duplicates, as are the β-actin blots in Figures 4A, 4B and 5A

Person responsible for these findings of misconduct: Dr. Mousa Abkhezr

Summary, Allegations 1.a. and 1.b.:

Based on the detailed analysis conducted by the American Physiological Society using DHHS Office of Research Integrity (ORI) image review tools, review of the images by the investigation committee, and written concurrence by both Dr. Dryer and Dr. Abkhezr, these findings are determined to be substantiated. The STAT3 blots are irrefutably the same as those previously published in the *Molecular Pharmacology* paper and are improperly represented in the AJP manuscript as a separate set of experiments conducted with different treatments[2]. In addition, the β-actin blots are duplicates as alleged. This represents a significant departure from accepted practices in the scientific research community.

During both the Inquiry and Investigation phases of the misconduct proceeding, Dr. Abkhezr maintained that using the controls from the *Molecular Pharmacology* paper was a mistake; that he "lost track of all the bands" and did not always label them. The committee carefully considered Dr. Abkhezr's statements that using these control bands was an honest error and has no strong evidence to support the falsification as intentional, however when combined with the fact that similar errors occurred in nine separate figures within the manuscript, the committee believes that the misconduct rises to the level of having been committed ***recklessly[3].***

Dr. Abkhezr stated that in a previous lab position (under a different principal investigator) he had been trained to properly label data, however as data management was not emphasized or checked within the Dryer laboratory, he did not keep up good practices when he became overwhelmed with the pressures of both teaching and multiple research projects.

In his response to the committee, Dr. Abkhezr provided the committee with .pdf versions of two additional blots, labeled "WB DATA" and "DATA." He explained that these bands were from different experiments and not duplicated, and should be reviewed in response to allegations 1.b. and 1.c. Unfortunately, these images have no dates on them, are not labeled, and do not in any way identify the experiment run or the context under which they were run. For this reason the committee is unable to consider these blots as additional information related to this investigation. This further exemplifies the poor data management practices of Dr. Abkhezr and the reckless nature of the misconduct.

---

[2] In the case of figure 4A, the treatment was the same as in the previous paper, however the experiment was run on a different date and looking at different consequences, which requires a new positive control.

[3] **Reckless**: 1. Marked by lack of proper caution, careless of consequences. 2. Irresponsible. http://www.merriam-webster.com/dictionary/reckless

Required actions:

- Due to substantiated findings of research misconduct, the following publication must be retracted:

  Abkhezr, et al., (2015). Pleiotropic Signaling Evoked by Tumor Necrosis Factor in Podocytes. *American Journal of Physiology – Renal Physiology,* 309, F98-F108. doi: 10.1152/ajprenal.00146.2015

  This process has been initiated. All authors on this publication must be notified of these findings by being provided a copy of this determination letter. Notification to DOR is required once retraction is complete and authors have been notified. Associated institutions will be notified by DOR.

- According to information provided during the investigation interviews, Dr. Abkhezr's doctoral dissertation is partially based on research conducted for the paper currently requiring retraction. The dissertation cannot be published with the inclusion of any data included in the retracted manuscript.[4]

- While there have been no allegations of misconduct with respect to the Mol Pharm paper (*Mol Pharmacol* 87:231–239, February 2015), due to the pattern of data management and disordered documentation represented by the above findings, it is highly recommended that Dr. Dryer thoroughly review the original data for this paper to determine if it, too, should be retracted. He may wish to seek independent assistance from others and/or request guidance from the Editor-in-Chief of *Molecular Pharmacology*.

- Based on descriptions provided by all interviewees regarding data management practices in the laboratory (e.g. lack of lab notebooks, lack of lab meetings, and no overarching standards for data storage or backup), corresponding author review of data and co-author review of manuscripts, the committee feels that the culture of the lab was likely a contributing factor in the misconduct.

  Dr. Dryer has initiated the following corrective actions to address these issues:
  - Digital laboratory archiving has been adopted by the lab. A system from Lab Archives (digital lab notebooks with date stamped Digital Object Identifiers (DOI)) has been purchased. The PI states that "it is not the most user-friendly but we will continue with it for the time being at least."
  - The PI has also insisted on new standards for preservation of original x-ray films and annotation of experiments in hard-bound laboratory notebooks. He will carry out random notebook inspections to make sure that nothing becomes lax.

[4] Publication should be linked to successful completion of all degree requirements; this remains the purview of the College of Natural Sciences and Mathematics and/or the Provost. A Department of Biology and Biochemistry academic honesty hearing is currently pending the outcome of the DOR investigation; a copy of this letter and the full investigation report will be provided to the Department Chair and the Associate Chair for Graduate Affairs for consideration in this hearing.

- Regular training sessions will be held on how to properly document biochemical and physiological experiments.
- Backup of digital data will be emphasized on an ongoing basis.
- The PI has decided to hold regular laboratory meetings
- All students in the PI's laboratory will be required to take the Ethical Conduct of Research course that will be taught by Dr. Sater each Fall.

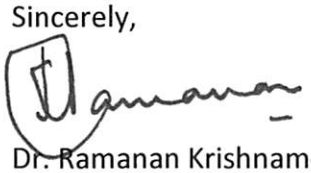
A communication will be provided under separate cover to the Vice President for Research and Technology Transfer regarding the need for follow-up of this issue.

Appeals

Within 15 calendar days of receipt of the findings, the respondent may appeal the decision in writing to the Deciding Official. The Deciding Official may request that the RIO reconvene the investigation committee to review the appeal, or may require that a separate committee be convened to reopen the matter. Appeals must contain new information not previously considered by the investigation committee.

Please let me know if you have any questions regarding this determination; you may also contact the Responsible Conduct of Research (RCR) Coordinator, Ms. Kirstin Rochford, at kmrochfo@central.uh.edu.

Sincerely,



Dr. Ramanan Krishnamoorti
Interim Vice Chancellor/Vice President for Research and Technology Transfer
Research Integrity Officer
University of Houston
316 E. Cullen Building
Houston, TX 77204-2015
RKrsihna@Central.UH.EDU

Cc: Dr. Renu Khator
Dr. Paula Myrick Short
Dona Cornell
Dr. Amy Sater
Dr. Ricardo Acevedo
Kirstin Rochford

Appendix A

The American Physiological Society

9650 Rockville Pike • Bethesda, Maryland 20814-3991 USA • Tel: 301.634.7070 • Fax: 301.634.7245
E-mail: publications@the-aps.org • Web: www.physiology.org

August 13, 2015

Stuart E. Dryer, Ph.D., F.A.S.N.
University of Houston
Biology and Biochemistry
4800 Calhoun
Houston, TX 77204-5001

RE: Request for clarification

Dear Dr. Dryer,

I am writing in regard to your manuscript, "Pleiotropic signaling evoked by tumor necrosis factor in podocytes" (F-00146-2015), accepted for publication in *AJP – Renal Physiology*. While your manuscript was being processed, several irregularities were detected within the Figures; please see the attached figure file for details. Specifically:

- The STAT3 blots in Figures 2D, 4A and 5A appear to be identical to blots previously published in your article in the journal *Molecular Pharmacology* (*Mol Pharmacol* 87:231–239, February 2015) as Figures 1B and 1D

- The β-actin blots in Figures 3A, 3B and 9B appear to be duplicates, as are the β-actin blots in Figures 4A, 4B and 5A

- The P-STAT3 image in Figure 4B shows signs of selective editing

- The lane labels on the figures indicate that they are from completely different experiments, with different treatments, yet appear to be identical gels

I am writing, therefore, to request an explanation for these image irregularities and also ask that you provide us copies of all original captures for the images in question. Note that further

processing of your manuscript is on hold until this matter has been resolved, so I urge you to respond as soon as possible.

Sincerely,

Rita Scheman
APS Director of Publications and Executive Editor

Cc: Curt Sigmund, Chair, APS Publications Committee
Hershel Raff, Vice Chair for Ethics, APS Publications Committee
P. Darwin Bell, Editor in Chief, *AJP – Renal Physiology*

Pleiotropic signaling evoked by tumor necrosis factor in podocytes

MOUSA ABKHEZR[1], EUN YOUNG KIM[1], HILA ROSHANRAVAN[1], FOTIS NIKOLOS[1,3], CHRISTOFOROS THOMAS[1,3] HENNING HAGMANN[2], THOMAS BENZING[2], AND STUART E, DRYER[1,4]

1. Department of Biology and Biochemistry, University of Houston, Houston, Texas USA.

2. Department of Internal Medicine, University of Cologne, Cologne, Germany.

3. Center for Nuclear Receptors and Cell Signaling, University of Houston, Houston Texas, USA.

4. Division of Nephrology, Baylor College of Medicine, Houston, Texas, USA.

Running head: TNF activates STAT3, NFAT and TRPC6

Address for correspondence:

Stuart E. Dryer, Ph.D., F.A.S.N.
sdryer@uh.edu
+1-713-743-2697 (tel)
+1-713-743-2632 (FAX)

Figure 2D - AJP-Renal: F-00146-2015

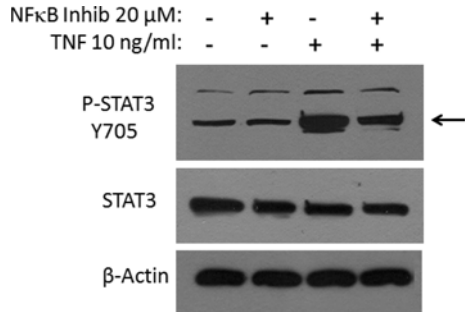


Figure 1B - Mol Pharm: 2015; Abkhezr - 231-9

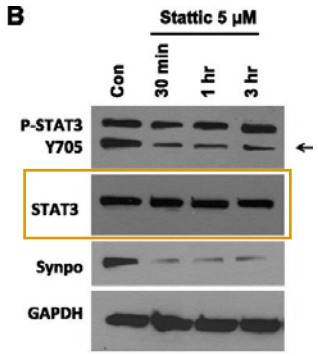


Figure 2D - 1B - Gel/Image - Duplication

Figure 2D - AJP-Renal: F-00146-2015

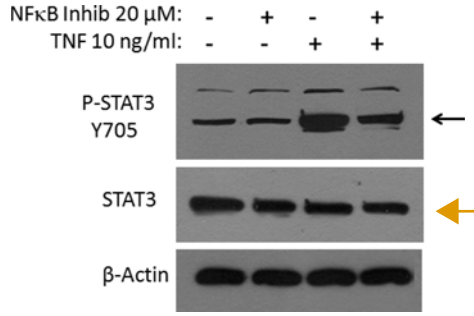
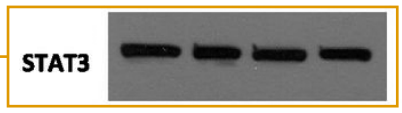
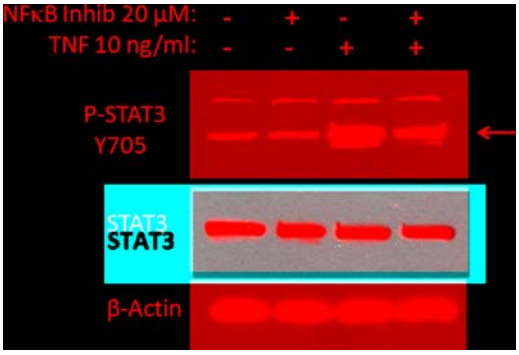


Figure 1B - Mol Pharm: 2015; Abkhezr - 231-9





Images compared using ORI image review tools.

Mol Pharm STAT3 image is shown superimposed over the AJP-Renal image of 2D. All bands matched up.

DUPLICATION

STAT3 image from 2D and STAT3 image from 1B appear to be duplicates.

Please request original captures and explanation of submitted composition.

Figure 3A- As Submitted to AJP-Renal

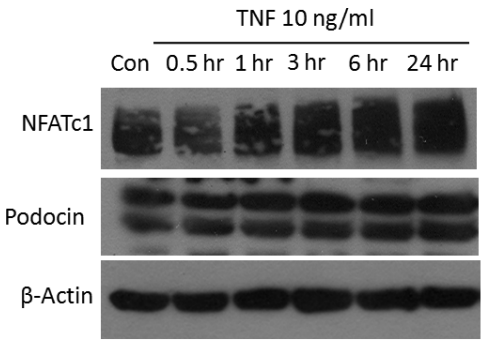


Figure 3A - Gel(s) in Question

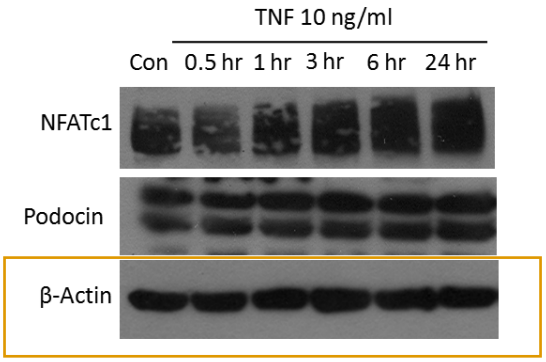


Figure 3B- As Submitted to AJP-Renal

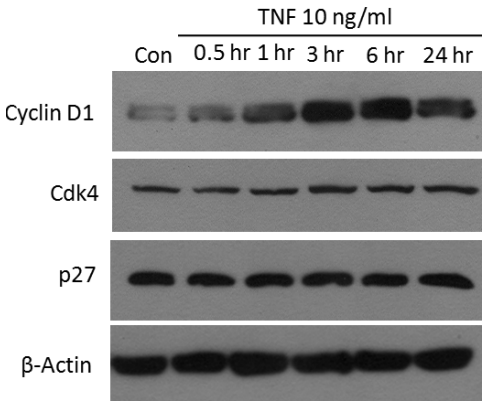


Figure 3B - Gel(s) in Question

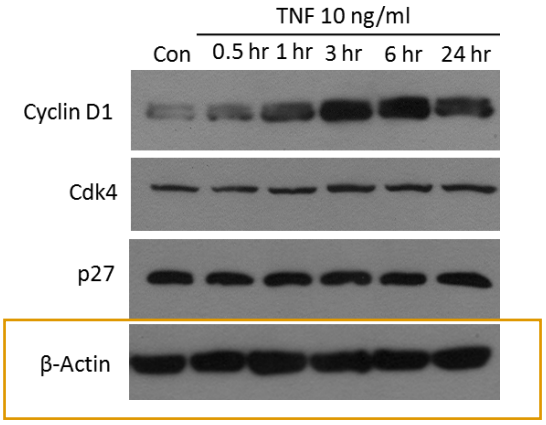


Figure 9B - As Submitted to AJP-Renal

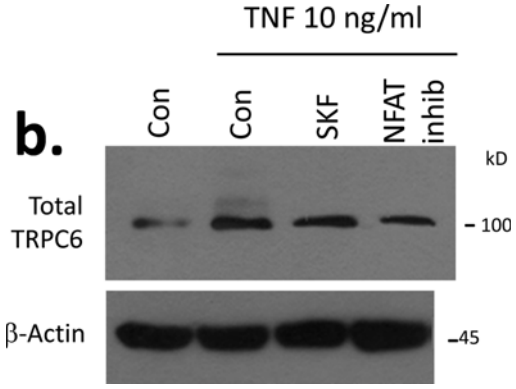


Figure 9B - Gel(s) in Question

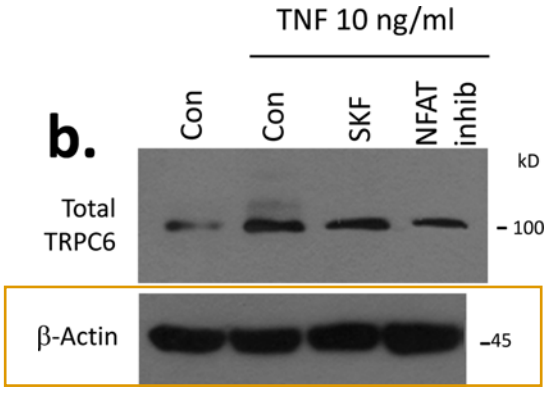
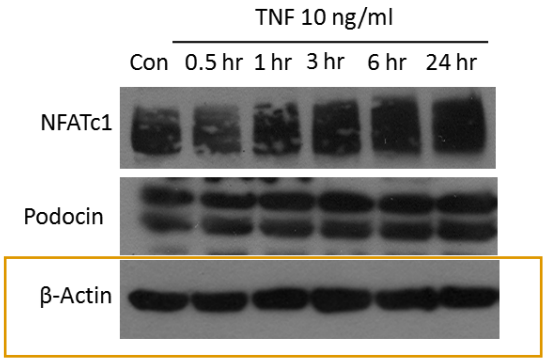
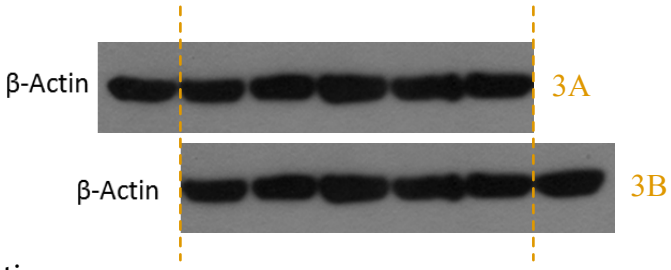
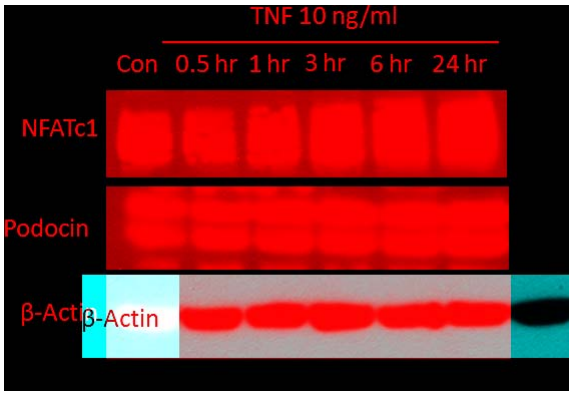


Figure 3A - Gel(s) in Question



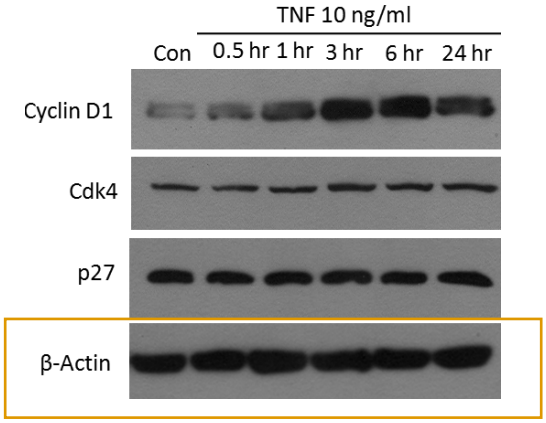


Figures 3A & 3B - "β-actin" Gel/Image - Duplication



β-actin gels from 3A & 3B have been superimposed with ORI compare tool. Lanes and bands match up.

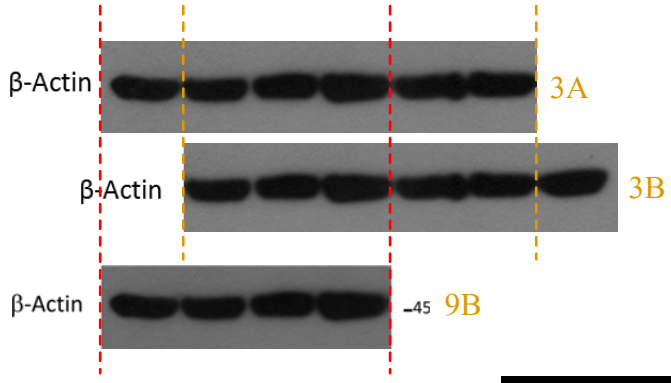
Figure 3B - Gel(s) in Question



DUPLICATION

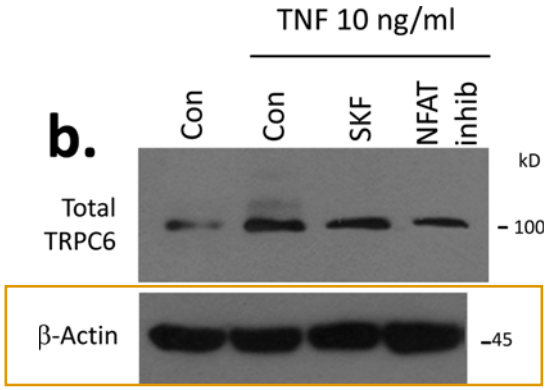
β-Actin images in figures 3A, 3B and 9B appear to be duplicates.

Please request original captures and explanation of submitted composition.



β-actin gels from 3A & 9B have been superimposed with ORI compare tool. Lanes and bands match up.

Figure 9B - Gel(s) in Question



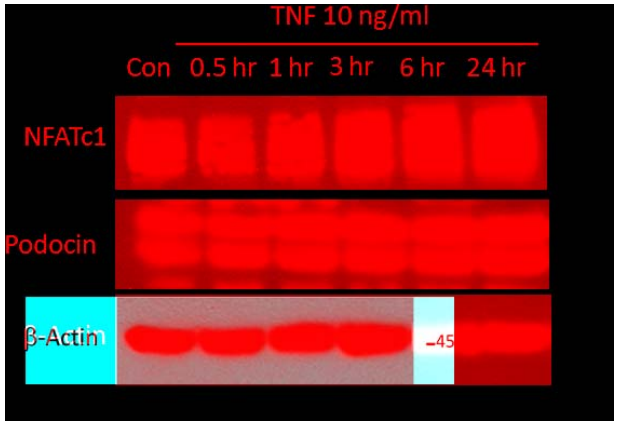


Figure 4A - AJP-Renal

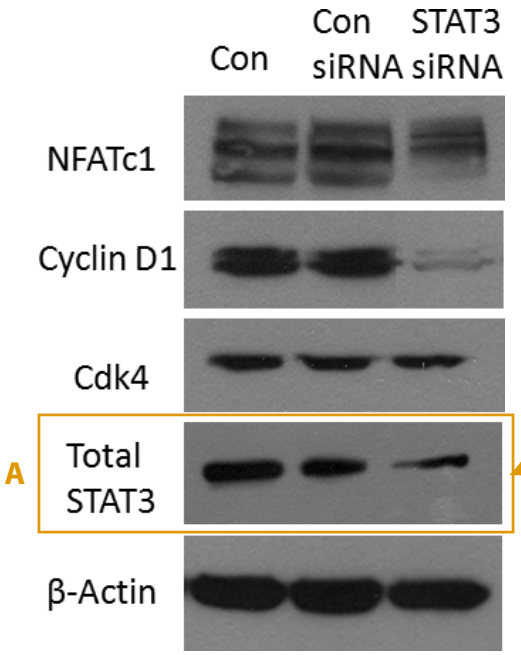


Figure 1D - Mol Pharm; 2015; Abkhezr - 231-9

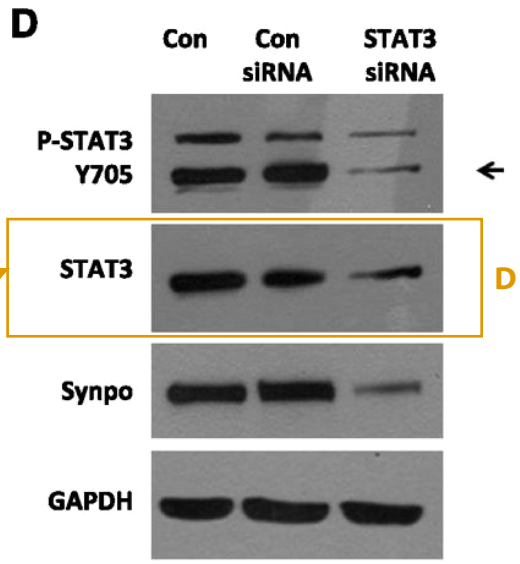
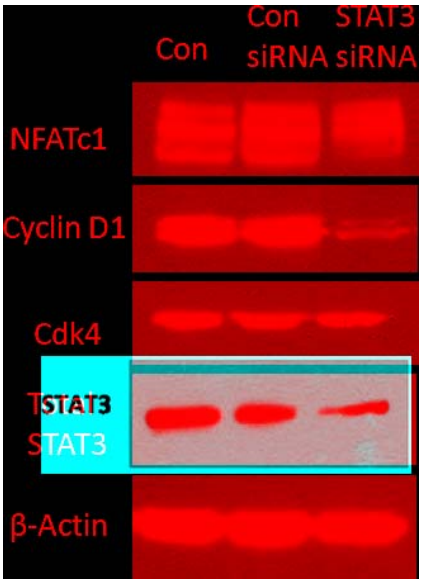


Figure 4A (AJP) & 1D (Mol Pharm) - z Gel/Image - Duplication

"Total STAT3" gel from AJP-Renal and "STAT3" gel from Mol Pharm have been superimposed with ORI compare tool. Lanes and bands match up.

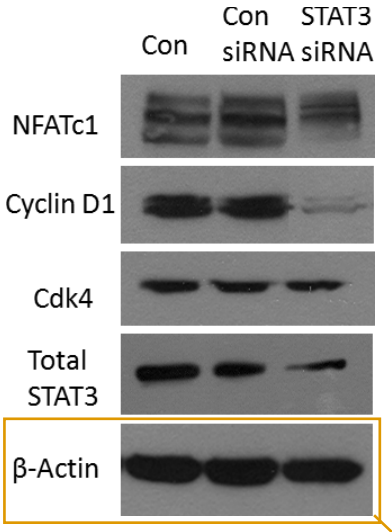


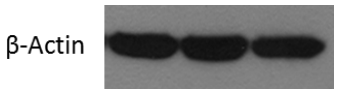
DUPLICATION

Image "A" and Image "D" appear to be duplicates.

Please request original captures and explanation of submitted composition.

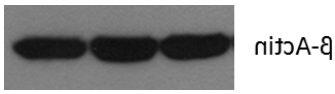
Figure 4A - As Submitted

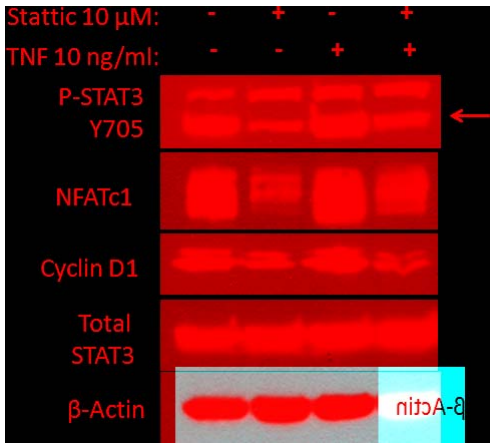




4A

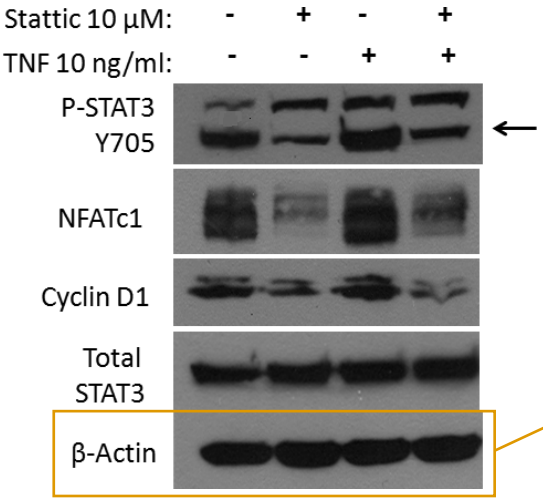
Gel has been flipped on its horizontal axis.

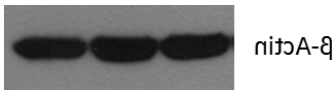




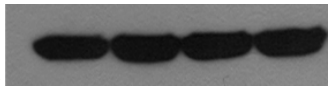
β-actin gels from 4A & 4B have been superimposed with ORI compare tool. Lanes and bands match up.

Figure 4B - As Submitted

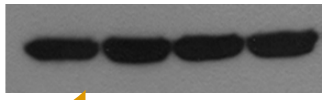




4A

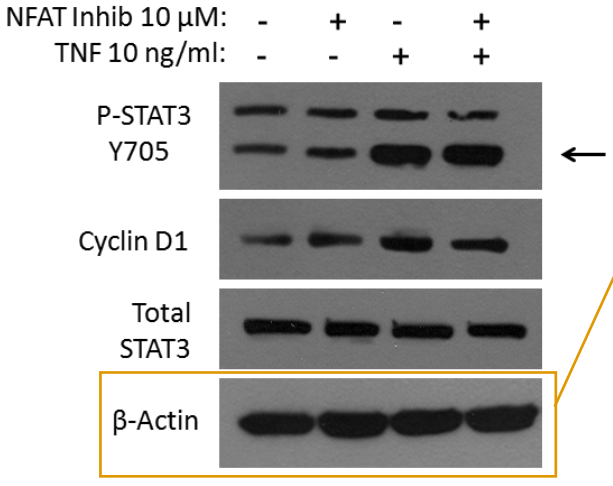


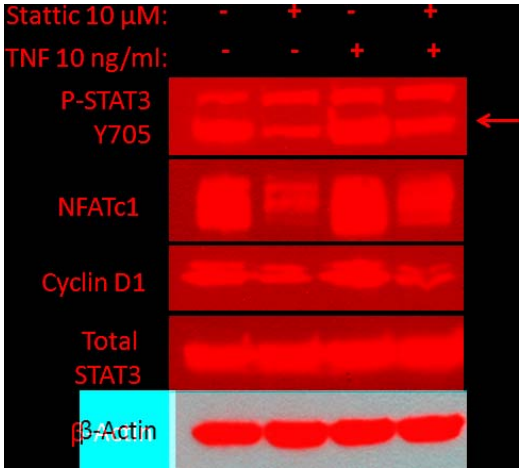
4B

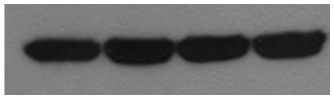


5A

Figure 5A - As Submitted







5A

β-actin gels from 4B & 5A have been superimposed with ORI compare tool. Lanes and bands match up.

Figure 4B - As Submitted

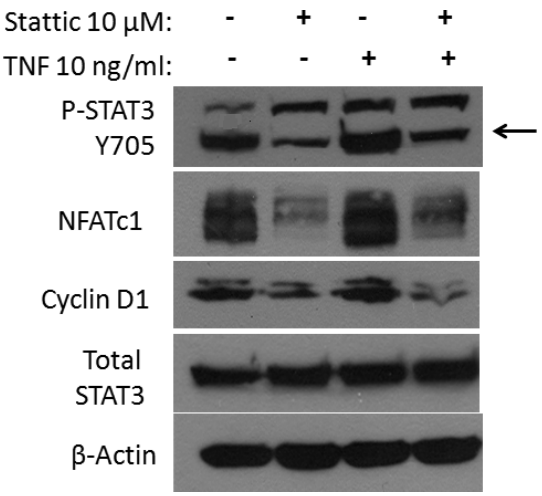


Figure 4B - Gel in Question

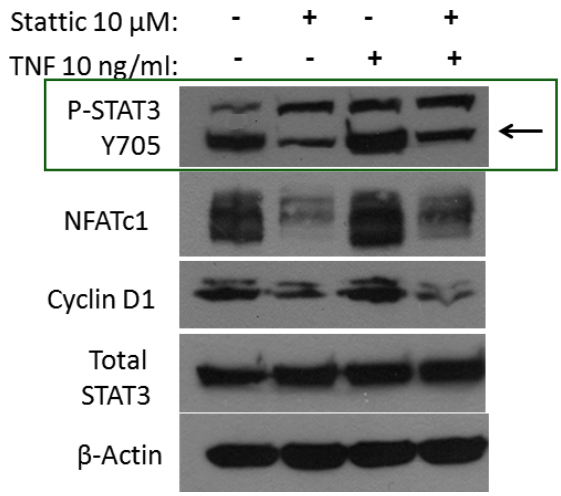
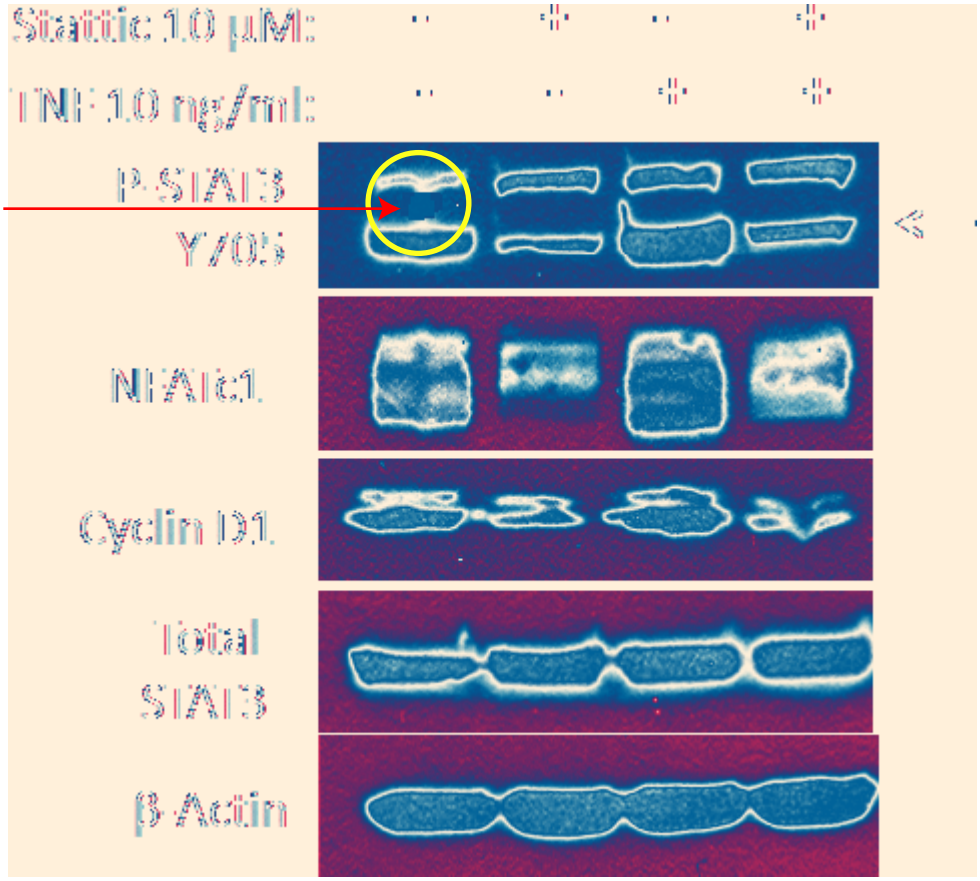


Figure 4B - "P-STAT3/Y705" Gel - Selective Editing

Original captured data appears to be "painted" over.



SELECTIVE EDITING

The indicated area(s) of this image may have been selectively edited/ manipulated with image editing software to remove/obscure data.

Please request original captures and explanation of submitted composition.

Figure 5A (AJP-Renal) - As Submitted

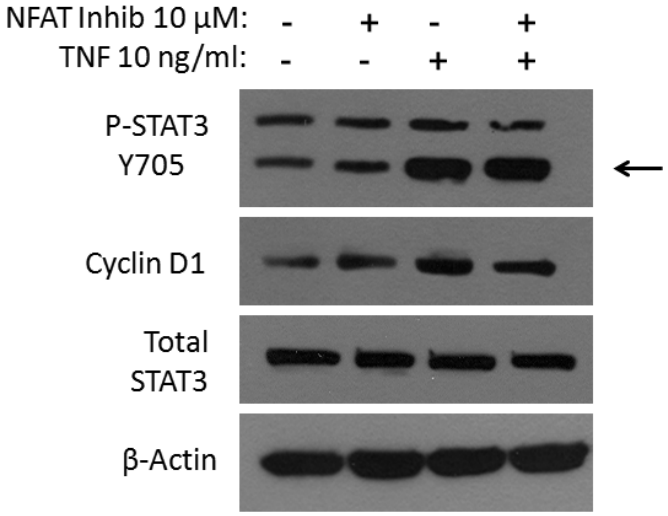


Figure 1B (Mol Pharm) - As published

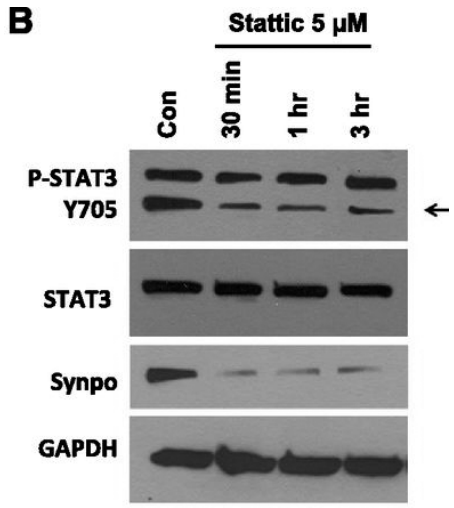


Figure 5A (AJP-Renal) & 1B (Mol Pharm) - Gel(s) in Question

Figure 5A (AJP-Renal) - "Total STAT3" gel

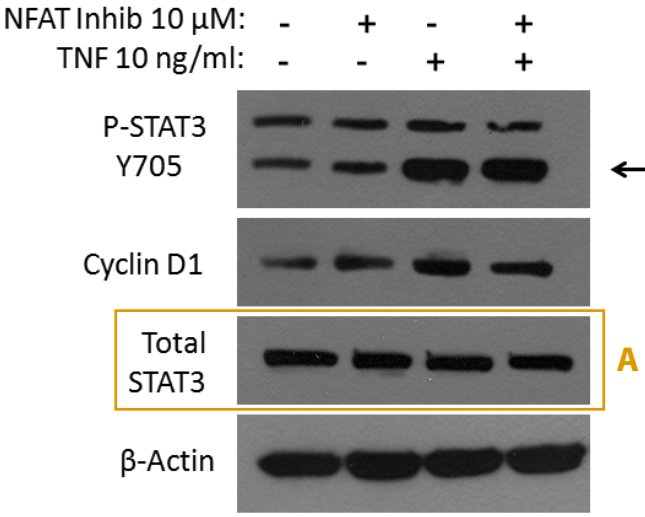


Figure 1B (Mol Pharm) - "STAT3" gel

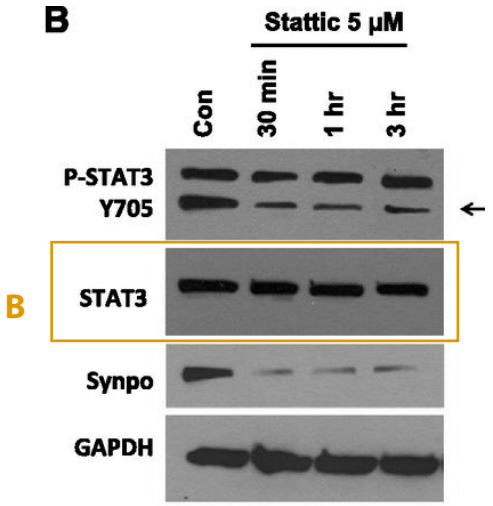


Figure 5A (AJP-Renal) & 1B (Mol Pharm) - Gel/Image - Duplication

"Total STAT3" gel from 5A (AJP) & "STAT3" gel from 1B (Mol Pharm) have been superimposed with ORI compare tool. Lanes and bands match up.

DUPLICATION

Image A and Image B appear to be duplicates.

Please request original captures and explanation of submitted composition.

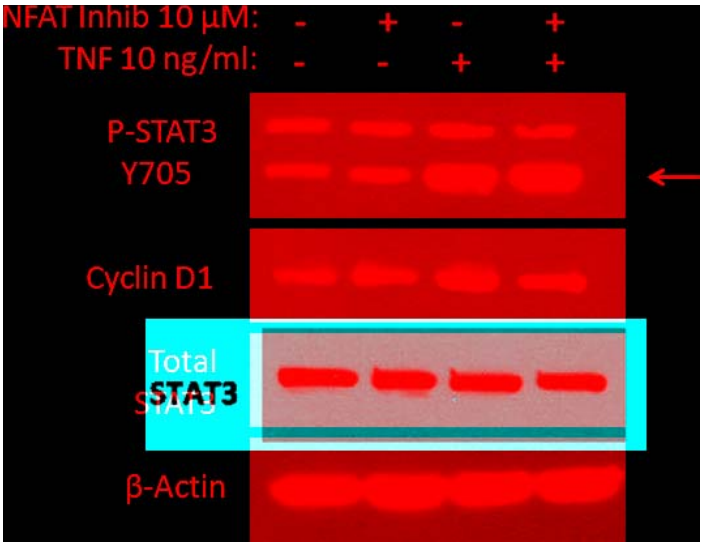


Figure Legends

Figure 1. Extrinsic regulation of TNF expression in podocytes. (**a**) Immunoblot showing that TNF expression is increased in immortalized mouse podocytes cultured for 24 hr in the presence of 10% human sera from patients with recurrent FSGS. Serum samples from patient 1 were taken before (relapse) and after remission was achieved by three LDL plasmapheresis sessions. Serum from patient 1 while he was in relapse evoked a marked increase in TNF expression in podocytes. A similar effect was produced by serum from two other recurrent FSGS patients in relapse. The bar graph to the right shows densitometric analysis of these experiments. In this and all subsequent figures, the bar graph represents mean ± s.e.m. **(b)** Representative immunoblot shows that exposing podocytes to recombinant TNF (10 ng/ml) for 24 hr causes a marked increase in TNF abundance. Bar graph to the right shows densitometric analysis of this experiment. Asterisks indicate $P < 0.05$ by unpaired *t*-test.

Figure 2. TNF evokes activation of NFκB and STAT3 in mouse podocytes. (a) Immunoblot analysis showing abundance of phosphorylated and total p65-RelA, one of the essential subunits of NFκB, in response to 10 ng/ml TNF for various times as indicated. (**b**) TNF (10 ng/ml) evoked an increase in tyrosine phosphorylation of STAT3 (at Y705) but had no effect on abundance of serine phosphorylated STAT3 (at S727) or total STAT3. This effect was seen with exposures ≥1 hr. In this and subsequent blots on tyrosine phosphorylate STAT3,

there are typically two bands. Only the lower molecular weight band (~86 kD, indicated by the dashed box) corresponds to phospho-STAT3. (**c**) The STAT3 inhibitor stattic did not affect p65-RelA phosphorylation evoked by 24-hr exposure to 10 ng/ml TNF. (**d**) The NFκB activation inhibitor JSH-23 (20 μM) blocked STAT3 phosphorylation evoked by 24-hr exposure to 10 ng/ml TNF. Asterisks indicate $P < 0.05$ by Bonferonni *t*-test.

Figure 3. Effects of TNF on NFATc1 and cell cycle regulatory proteins in podocytes. (**a**) Immunoblot analysis showing increase in the total abundance of NFATc1 in podocytes evoked by exposure to TNF (10 ng/ml) for ≥ 1 hr. TNF treatment had no effect on podocin abundance. The multiple NFATc1 bands reflect expression of several transcript variants. (**b**) TNF also induced a marked increase in the total abundance of cyclin D1 but had no effect on cell cycle regulatory proteins Cdk4 or $p27^{kip}$. Asterisks indicate $P < 0.05$ by Bonferonni *t*-test.

Figure 4. Role of STAT3 in regulation of NFATc1 and cyclin D1 expression in podocytes. (**a**) STAT3 knockdown using siRNA resulted in reduced total abundance of NFATc1 and cyclin D1 but had no effect on Cdk4. Effectiveness of knockdown is seen from loss of total STAT3. (**b**) The STAT3 inhibitor stattic (10

μM) blocked the increase in NFATc1 and cyclin D1 evoked by 24-hr exposure to 10 ng/ml TNF. Asterisks indicate $P < 0.05$ by Bonferonni *t*-test.

Figure 5. TNF causes increased nuclear NFATc1 accumulation downstream of STAT3. (**a**) Nearly complete TNF-evoked increases in phosphorylated STAT3 and increases in cyclin D1 persist in the presence of the NFATc1 activation inhibitor 11R-VIVIT (10 μM). (**b**) Exposure to TNF causes an increase in NFATc1 detected by immunoblot of nuclei isolated from podocytes. This effect was blocked by the NFκB inhibitor JSH-23 (20 μM), and by the STAT3 inhibitor stattic (10 μM). (**c**) TNF-evoked accumulation of NFATc1 in podocyte nuclei was also blocked by the calcineurin inhibitor cyclosporine A (20 μM) but not by the pan-TRP channel inhibitor SKF-96365 (10 μM). Asterisks indicate $P < 0.05$ by Bonferonni *t*-test.

Figure 6. TNF treatment for up to 72 hr does not cause podocytes to re-enter the cell cycle. (**a**) The phases of the cell cycle were measured by FACS analysis in podocytes after TNF treatment (10 ng/ml) for 24, 48 and 72 hr, as indicated. Orange denotes cells in sub-G1 phase, green denotes cells in G0/G1 phase, red denotes cells in S phase, and blue denotes cells in G2/M phase. (**b**) Distribution of cells in various phases of the cell cycle after 24, 48, 72 hr of exposure to TNF.

Figure 7. TNF treatment causes mobilization of TRPC6 channels in podocytes. **(a**) Cell-surface biotinylation showing increased steady-state surface expression of TRPC6 channels in podocytes after 24-hr exposure to 10 ng/ml TNF. (**b**) Fluorescence based assays showing increased ROS generation in podocytes exposed to TNF for 24 hr. The TNF-evoked increase in ROS generation can be antagonized by 20 μM JSH-23 or 10 μM stattic. (**c**) The ROS quencher TEMPOL blocks TNF-evoked increase in steady-state expression of TRPC6 on the podocyte cell surface. As with effects on ROS generation, the TNF-evoked increase in surface TRPC6 is attenuated by the NFκB inhibitor JSH-23 (20 μM) (**d**) and by the STAT3 inhibitor stattic (10 μM) (**e**). Asterisks indicate $P < 0.05$ by Bonferonni *t*-test.

Figure 8. Increased cationic current in response to diacylglycerol analog in podocytes treated with TNF. (**a**) Examples of currents evoked by voltage ramps (from -80 mV to +80 mV over 2.5 sec) before and during application of 100 μM OAG, a membrane-permeable analog of diacylglycerol. These currents were completely eliminated by 50 μM La^{3+}. Note that the OAG-evoked currents were larger in cells treated with TNF. (**e**) Summary of results from $N = 10$ cells per group showing that OAG-evoked current relative to baseline currents measured at +80 mV are greater in TNF-treated cells ($P < 0.05$ by Student's unpaired *t*-test).

Figure 9. TNF evokes increase in total abundance of TRPC6 through NFATc1. (**a**) Immunoblot analysis showing increased total abundance of TRPC6 protein in podocytes after TNF treatment (10 ng/ml) for times indicated. (**b**) Effects of TNF were blocked by the NFATc1 inhibitor 11R-VIVIT (10 μM). However TNF effects were not robustly inhibited by 10 μM SKF-96365. Asterisks indicate $P < 0.05$ by Bonferonni *t*-test.

Figure 10. Schematic summary of pathways identified in the present study. TNF causes activation of NFκB in an early step in the transduction cascade in podocytes. After ~ 1 hr, this leads to tyrosine phosphorylation and activation of STAT3, which translocates to the nucleus. Active STAT3 causes an increase in the overall abundance of NFATc1, and translocation of active NFATc1 to the nucleus. There is also an increased expression of the cell cycle regulatory protein cyclin D1, but not of other key cell cycle proteins such as Cdk-4, and consequently the cells do not reenter the cell cycle. An appropriate "second hit" that increases Cdk-4 and degrades $p27^{kip}$ could result in cell-cycle dysregulation leading to glomerular collapse *in vivo*. Through calcineurin-NFATc1 signaling, TNF causes an increase abundance of TRPC6 channels, and owing to increased ROS generation the TRPC6 channels traffic to the cell surface and become active. Increases in TRPC6 surface expression and activation can over time lead to Ca^{2+} overload that drives other pathological processes.