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Confidential
"Impairment of TrkB-PSD-95 Signaling in Angelman Syndrome"

Dear Roland,

I am writing to you concerning the PLOS Biology publication "Impairment of TrkB-PSD-95 Signaling in Angelman Syndrome".

Last we spoke, Brown University (Clyde Briant, VP of Research communicated to us that a thorough investigation had been undertaken and had concluded that no misconduct had occurred. I brought suit against Dr. Marshall 'MARSHALL' and Dr. Goebel 'GOEBEL' in US Federal District court in Massachusetts for abuse of my copyright. I have uncovered extensive evidence of misconduct in the paper as I alleged in my initial letters. I have discovered that MARSHALL was aware of the misconduct before the paper was published and that he tried to conceal it.

We have seen no evidence of the investigation by Brown University and MARSHALL testified under oath that no investigation of scientific misconduct occurred. Marshall testified that only the issue of authorship was investigated.

I appreciate your patience and integrity in addressing these allegations and I am delighted to now fully substantiate them. Also, I thank you for bringing my allegations about the Conflict of Interest to the attention of readers in the correction. In summary, the evidence shows Dr, Marshall and Dr. Goebel conspired to manipulate their results by scientific misconduct (fabrication and falsification). Dr. Cao extensively fabricated and falsified results. Dr. Pedotti and Ms. Yu fabricated the electrophysiology result.

1. **The PLOS Biology paper was reviewed and finally accepted based upon fabricated results.**

Very Obvious Fabrication in the paper was discovered by MARSHALL (12 January 2013) after the manuscript had been received (27 July 2012), reviewed and officially accepted (2 January 2013) for publication (12 February 2013). During the 'proofing

stage', changes were made without editorial approval to hide fabricated data. Other fabrications that were identified remained published.

Appendix A is the final approved manuscript that was submitted, reviewed and accepted by PLOS Biology. Turn to page 66 of 74. Please inspect the TUBULIN blot in Figure 2A (see below). Upon inspection, you can see that the WT panel (left) is a **fabrication** that was constructed by cutting the left hand columns from the AS panel (right). The reviewers would have relied upon this crucial band, unaware that it was a fabrication and not realizing it was later secretly removed in the January 2013 proofing window.

2. **Emails between MARSHALL and CONG CAO detailing discussion of the fabricated and manipulated data.**

During the proofing stage the above Figure 2 TUBULIN blot and other very obvious mistakes were discovered by MARSHALL. The TUBULIN blot was removed without disclosing the fabrication, misconduct or asking for editorial approval. At this time, MARSHALL and CONG CAO also inserted language about 'sister gels' that was proposed because it came to light that many of the Western Blot figures in the paper are fabricated from different experiments. Therefore, interpretation of relative levels is not in the same experiment but between results from different experiments, undermining the conclusions fatally.

This is substantiated by MARSHALL and CONG CAO: Appendices 'B' through 'N' are emails admitting these fabrications and others. Excerpts:

Appendix H Marshall to Cong Cao (Jan 12, 2013)
"*Are you an idiot or just lazy or are you fabricating the data. Can you give me an explanation. This is an important paper and you are still doing this shit*"

Appendix J Cong Cao to Marshall (Jan 12, 2013):
"*I understand the tubulin thing is a huge mistake that will result a retraction*"

Appendix K Cong Cao to Marshall (Jan 12, 2013)
"*When I group them, I only consider them being same samples but not from same gels*…"

Appendix L Cong Cao to Marshall (Jan 12, 2013)
"*If I want to "make" some data, I can make some really nice data!!! Not the data like this!!!"*
"*I will accept this paper not being published, or publish without my name !! Even though that will ruin everything here*."

Appendix L Marshall to Cong Cao (12 Jan 2013)

"*Dear Mike- why did you do this? After all the things that have happened to you you are still not making the westerns correctly? You must have known that the blots were from different gels when you made the figs?- hence you group the blots from the same gel together*."

Appendix M Marshall to Cong Cao (Jan 12, 2013)
"*Why do I find these mistakes and you see nothing. You would let the paper be published with these errors. Again your blots. You made these figs. Your mistakes. What else is wrong?... I found another major error on the blots which I did not mention. Can you tell me what it was?*"

Appendix N Cong Cao to Marshall (Jan 14, 2013)
"*I can't find the blot mistake you pointed, please advise*."
"*I grouped the blots together considering them from same set of experiments, but not same gel. We decided to run some of the old samples and replace some over-exposed blots when we are revising the paper! Now I know this probably is not the right way to do it, and its my mistake*."
"*Otherwise, I will forfeit my authorship of the paper! I have thought about this for a long-long time*."

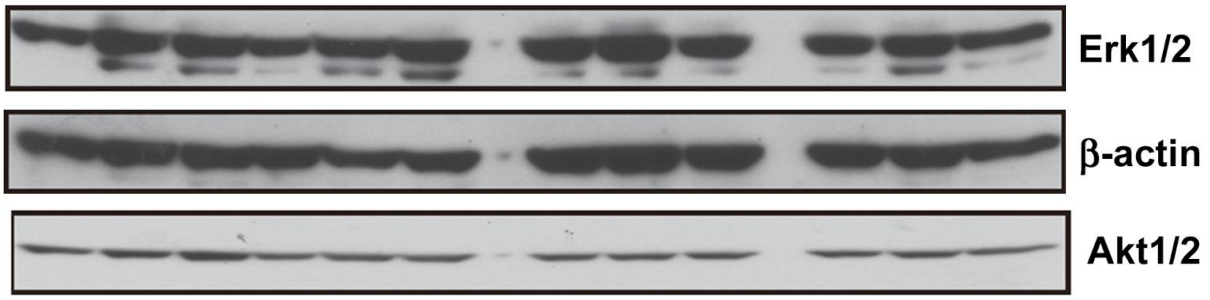
These referenced instances of fabricated data, errors and mistakes were in the reviewed and accepted manuscript and only the TUBULIN blot seems to have been deleted during proofing. I detected no other change in the paper to explain the referenced 'other' mistakes. I believe they were left in place because to remove them would have been impractical because it would have meant removing dozens of results.

Clearly, the Western Blot data set is unreliable by the admissions of the authors and the evidence of fabrication. I have discovered that the raw data autorads that substantiate the Western Blot data are not in the possession of MARSHALL and lab notebook or records relating to these CAO CONG results do not exist.

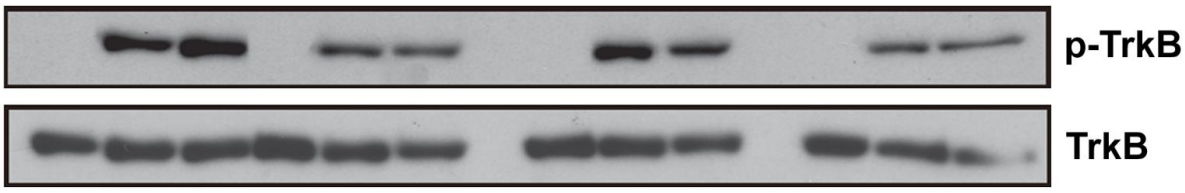
3. **Fabricated data that remains in the published paper**

Learning of the existence of fabrication in the paper and the admission that there was '"another major error on the blots", I analyzed the western blot data for instances of potential fabricated data. This uncovered extensive evidence of scientific misconduct:

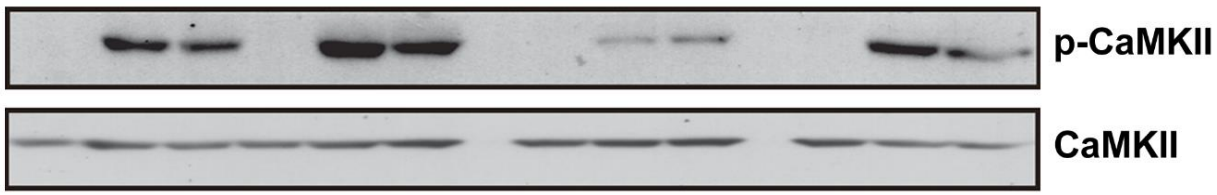
Appendix Fig 4: Please examine the Fig 4B: Akt1/2, Erk1/2 and β-Actin bands. These are identical bands from the same gel that have been exposed at different times to give the impression that they are different results. However, they overlap perfectly and are of exactly the same shape. Importantly, there is a signature imperfection in every gel between the 6^{th} and 7^{th} lane that occurs in exactly the same spot in each panel. There is also an identical 'hump' connection between the 4^{th} and 5^{th} band in the Erk1/2 and β-Actin and Akt1/2. This data seems to reappear in other figures in the paper.



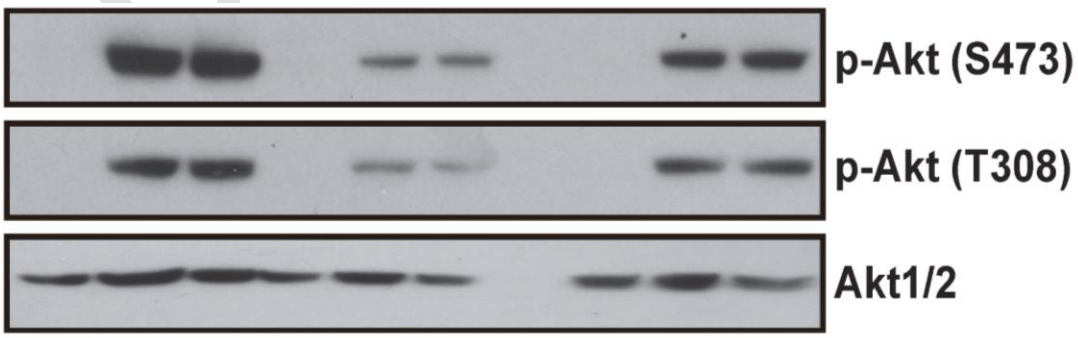
Appendix Fig 4: Please examine the blots in Fig 4B that show p-TrkB and TrkB (see below). These bands do not correspond to each other and the loading control in the final lane of TrkB suggests that there was a mistake in loading that well of that experiment. Importantly, the TrkB loading control comes from a different gel from the p-TrkB result and this data point is a fabrication.



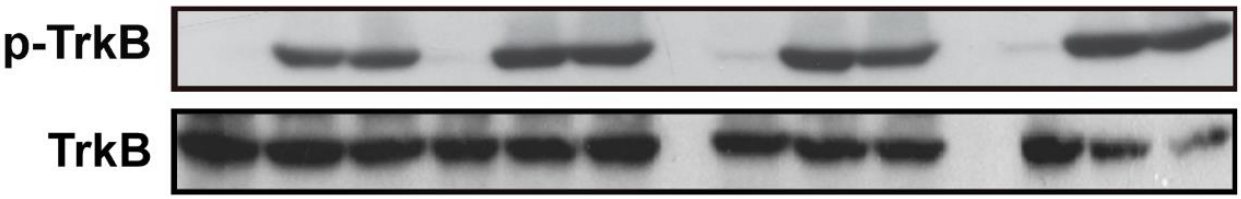
example of fabricating the result from different gels/experiments.



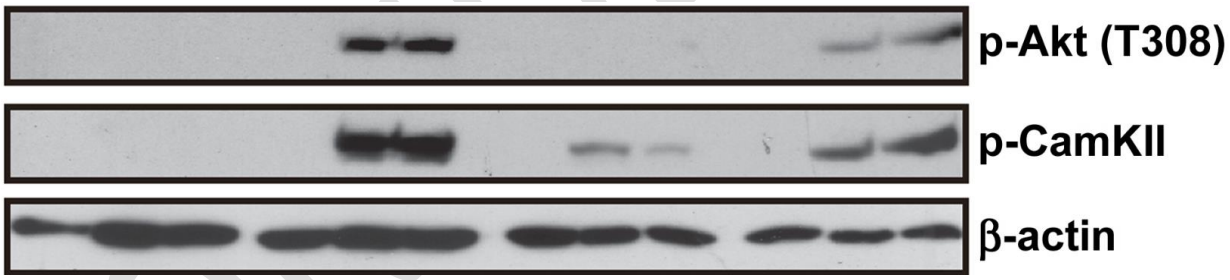
Appendix Fig S4: Please examine Figure S4-C. It is apparent that the Akt1/2 band has been taken from a separate gel/experiment. This is most obvious in lane 2, where the gel bands slope in opposite directions.



Appendix Fig S3: The reviewers asked for a TrkB band to be provided in Figure S3 (See below). However, it is apparent from a copy of raw data we discovered and examination of the blot that the result provided was from a different gel/experiment. It is obvious from the pattern of bands that these are different gels and that the TrKB bands fabricate the result. This is clear in lanes 2, 3, 5, 6, 9, and 10. In addition, the TrkB band at the end indicates that a mistake was made in loading that lane. Resulting in a loss of signal that is not reflected in the p-TrkB result it is supporting.



Appendix Fig3: As is common throughout all of the Western blots, bands from other gels/experiments have been used to fabricate results that are obviously unrelated. By way of illustration, please see Fig 3E, where the β-Actin is clearly unrelated to the bands two sets of bands above it. Especially obvious in the far right band. Also, there was a mistake in loading the gel on the far left that could lead to a false negative.



4. **Appendix O**: **Misreporting of data**

This piece of raw data shows that whilst the researchers report using 1.5ug of PSD95-cDNA, they actually used 1.25ug. This also shows what I found to be the only method of recording of data when I first uncovered misconduct by Cao Cong in 2011. You can see that often, there is no dating or annotation of what the autorad refers to.

5. **Appendix P**: **AS mouse identification problems.**

In my original complaint, I also alleged that Cong Cao and MARSHALL had lost control of the Angelman Syndrome (AS) mouse colony and were not able to reliably identify wild type or AS model mice. This allegation is confirmed in this email, where the researchers are guessing the identity of the mouse based upon predicted results. Results were being generated without basic scientific standards or methodology.

6. **Appendix Q & R**: **MARSHALL, GOEBEL, CAO CONG discuss fabricating and manipulating results.**

Additional emails between MARSHALL and CAO CONG discuss sitting down together to improve figures with the intention of making bad results that do not look good, look better so as not to raise alarms with reviewers (Q). The figure they refer to improving are pieces of raw data, Western blot autorads (July 2012).

At the same time, GOEBEL writes to MARSHALL saying that "No matter how I try to improve the cut-outs, the more it looks to be not believable (i.e. fudged). With that said, I went back and tried to improve the original figure (see attached). Even though its "over-cooked" the data still tells the story, and more importantly is 100% believable."

These emails provide insight into how the data in this paper were being openly fabricated, manipulated and falsified, with consideration given to ensure reviewers and readers would be convinced by the data and not question its believability.

7. **A thorough investigation was undertaken by Brown University and no scientific misconduct occurred.**

As you will remember, when you reached out to Brown University to query my allegations you were immediately told by Professor Clyde Briant that his office and Brown University General Counsel carried out a thorough investigation and no scientific misconduct occurred. However, under oath, MARSHALL testified that the investigation was only focused upon the question of authorship and not into the allegations of wrongdoing and fraud in the results.

In conclusion, I have now provided you with independent evidence substantiating all of my initial allegations of scientific misconduct and given some insight into how the misconduct was planned and hidden.

I continue to pursue MARSHALL and GOEBEL in Federal court for their abuse of my copyright and authorship of this work.

I was removed from my paper as first author because I communicated to MARSHALL that I had lost confidence in CAO CONG's research and it could not have been published without my approval. The evidence directly implicates MARSHALL and GOEBEL in the scientific misconduct. Please retract this paper and inform the scientific community of the nature of these results and their lack of reliability.

Yours Sincerely,

Dr Andrew P Mallon,