EMBO Molecular Medicine

Inhibition of Stat3-mediated astrogliosis ameliorates pathology in an Alzheimer's disease model

Nicole Reichenbach, Andrea Delekate, Monika Plescher, Franziska Schmitt, Sybille Krauss, Nelli Blank, Annett Halle and Gabor C. Petzold

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 12 September 2018

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript.

You will see from the set of comments pasted below that the referees are supportive, still several questions need to be answered, details and clarification provided and additional experiments performed. In particular some mechanism would be required to better understand the downstream effect and devise an informed translational application. Further, a better documentation of the therapeutic effect is equally needed.

We would welcome the submission of a revised version within three months for further consideration and would like to encourage you to address all the criticisms raised as suggested to improve conclusiveness and clarity. Please note that EMBO Molecular Medicine strongly supports a single round of revision and that, as acceptance or rejection of the manuscript will depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

Please also contact us as soon as possible if similar work is published elsewhere. If other work is published we may not be able to extend the revision period beyond three months.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript.

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***** Reviewer's comments *****

Referee #1 (Remarks for Author):

In this work Reichenbach and coworkers investigate the role of STAT3 signaling in astrocytes during AD. To this aim they generated an inducible conditional Ko mice driven by a Cx43-CreERT, backcrossed onto the APP/PS1 mouse model. In their studies the authors found a significant amelioration of the AD phenotype, as indicated by the decreased A-beta levels and plaque burden. Moreover, they detected an improvement in microglia phagocytic activity. These molecular findings were linked with an improvement in spatial learning and memory. These are interesting studies, properly controlled and described. However, it is my opinion that these studies fall short of providing a detailed understanding of the mechanisms through which STAT3 deletion in astrocytes ameliorates AD. Is this a direct effect? If that is the case, can the authors detect improved astrocyte phagocytic activity? Is it due to the increased phagocytic activity of microglia? What are the mechanisms involved?

Referee #2 (Remarks for Author):

Reichenbach and colleagues present a comprehensive study of the role of Stat3 signalling in reactive astrocytes in the context of mouse models of Alzheimer's disease (AD). Using the APP/PS1 amyloidosis model of AD, and extensive genetic ablation/silencing of largely astrocyte-specific Stat3 signalling, they report beneficial effects in memory and learning, along with changes in morphology of astrocytes and microglia, and an overall decrease in amyloid plaque load. With growing interest in the field of non-neuronal interactions in neurodegenerative diseases like AD, this study is likely to be of broad interest to the glia, degeneration, and broader neuroscience communities.

A few points would benefit from clarification:
1. does the APP/PS1 mouse have STAT3+ reactive astrocytes in the same location as human patients? Though some human post-mortem staining was provided (Fig 1G), it was unclear if this was also a peri-plaque region like that shown for mouse staining. This information is important for the reader to be able to ascertain the appropriateness of the mouse model.
2. Similarly, other groups have shown in recent years that STAT3-mediated reactive astrocytes are highly proliferative and produce a scar (in the context of acute injury) - was the same true in this mouse amyloidosis model?
3. What possible effects would the ~10% non-astrocyte specific targeting of the Cx43 mouse have on interpretation of these results? With around 5% of cells being non-astrocytes/non-neurons, if Stat3 signalling is sufficiently highly blocked in microglia this could account for the microglia-specific effects reported (e.g. increased phagocytosis of amyloid)
4. page 6, section titled 'Stat3 regulates plaque-associated...' the final sentence suggests that 'these data indicate that Stat3 signaling mediates an astrocyte-microglia crosstalk that may 'shield' the peri-plaque tissue...' there is no data for this conclusion, the conclusion to be drawn is that the astrocytes have an altered morphology in the peri-plaque region. Such prospective statements should not be included in the data section of the manuscript - please remove or move to the conclusions as a prediction to be further tested
5. Ca2+ imaging - did MRS2179 (P2Y1R inhibitor) alter the individual spontaneous events, or where there changes in the network-wide propagating Ca2+ transients?
6. End of Ca2+ imaging section - the conclusion that Ca2+ transient changes can drive astrocyte reactivity has not been shown. MRS2179 can decrease Ca2+ transients AND decrease pSTAT3 immunofluorescence, but these data give no indication of a direct causative effect of calcium transients driving a reactive phenotype. Could it also not be that a decrease in Stat3 is driving Ca2+ changes? This conclusion is similarly easily drawn from these data and suggests that a decrease in STAT3 (ie. a decrease in reactivity) is driving calcium changes.

Overall this manuscript is well written, the data and figures are carefully prepared and easy to follow. I would imagine the study would be well-received by a broad readership. Aside from the few clarifications outlined above, I have no reservations about recommending this manuscript.

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Referee #3 (Comments on Novelty/Model System for Author):

The authors used a complex way to generate their model and delete Stat3 in APPPS1 mice that does not confer full but partial Stat3 ablation. They should comment on this. Nonetheless, it is the first time Stat3 is deleted on a mouse model of AD and the novelty of the study is high. They also treated the mice with a Stat inhibitor so medical impact is potentially high. Technical quality is medium to high in most data of the MS.

Referee #3 (Remarks for Author):

By partially deleting Stat3 in astrocytes, Reichenbach et al. found decreased amyloid-beta load and neuritic dystrophy in the APPPS1 mouse model of AD, associated with several microglial alterations. Microglia become hypertrophic and increases phagocytosis and amyloid clearance pathways. A decrease of proinflammatory cytokines is also found. In addition, both Stat3 genetic deficiency and pharmacological inhibition decrease calcium hyperactivity in astrocytes and neurons and enhance learning and memory.
This work uses a variety of technical approaches to tackle an important and timely topic related to the astroglia-microglia crosstalk and the emerging essential role of these glial cells in the pathogenesis and progression of AD. However, deep revision needs to be done before being considered for publication in this journal.

1. The authors claim that the majority of reactive astrocytes in APPPS1-Stat3WT mice were Stat3+ while Stat3 activation was reduced by 80% in APPPS1-Stat3KO astrocytes, confirming its strong deletion.
Can the authors give exact numbers? Figure 1 shows that 50% of GFAP+ astrocytes are Stat3+ in the cortex and only 40% in hippocampus in APPPS1 mice and does not justify the sentence claiming that the majority of reactive astrocytes are Stat3+. Same applies for postmortem samples in which again only 40% of astrocytes are Stat3+. Moreover, around 10% of GFAP+ astrocytes are Stat3+ in both cortex and hippocampus in APPPS1-Stat3KO mice. This is neither an 80% reduction nor a strong deletion. I would also recommend adding a sentence discussing how such partial deletion of Stat3 in astrocytes leads to significant changes in AD pathology.

2. Figure 2: can authors show higher magnification images of astrocytes and microglia far from the plaques? Are there morphological differences between APPPS1-Stat3wt and KO in far areas? Is there any change in the number of cells?

3. Figure 4: the authors found a significant reduction of ApoE levels in APPPS1-Stat3 ko but it is not evident on the blots. Can they provide more representative images?

4. Whole brain levels of TNFa and IL1b are reduced in APPPS1-Stat3 ko. Why the authors assume these proinflammatory cytokines are secreted by microglia? Astrocytes might contribute as well.

5. Stat3 pharmacological inhibition decreases hyperactivity and improved learning and memory. To which extent Stat3 was inhibited? Can the authors provide images and some quantifications? Is SH4-54 treatment having any effect on AB burden, neuritic dystrophy and astroglia and microglia morphology? Can they add data on these?

6. The authors do not mention in the text the time-points at which these characterizations were performed. Were the phenotypes more or less pronounced at different timepoints? Is there any variation over time?

Minor: there are very long sentences in the abstract and introduction that are difficult to understand. Can the authors split the information on separate sentences?
The images in Figure 3D seem to be upside down

1st Revision - authors' response 22 November 2018

Reviewer #1
In this work Reichenbach and coworkers investigate the role of STAT3 signaling in astrocytes

during AD. To this aim they generated an inducible conditional Ko mice driven by a Cx43-CreERT, backcrossed onto the APP/PS1 mouse model. In their studies the authors found a significant amelioration of the AD phenotype, as indicated by the decreased A-beta levels and plaque burden. Moreover, they detected an improvement in microglia phagocytic activity. These molecular findings were linked with an improvement in spatial learning and memory. These are interesting studies, properly controlled and described. However, it is my opinion that these studies fall short of providing a detailed understanding of the mechanisms through which STAT3 deletion in astrocytes ameliorates AD. Is this a direct effect? If that is the case, can the authors detect improved astrocyte phagocytic activity? Is it due to the increased phagocytic activity of microglia? What are the mechanisms involved?

RESPONSE: We thank the referee for the positive comments. We now provide the following new data strongly indicating that the underlying mechanism involves astrocytes directing microglia to increase their phagocytic capacity:

- We show in Figure 4 (reported on page 7) that deletion of astrocytic Stat3 increases the amount of Aβ phagocytosed by microglia, but not by astrocytes, indicating a mechanism that is initiated by astrocytes but executed by microglia.
- Along these lines, we now show in Figure 4 (and reported on page 7-8) that the microglia-specific Aβ-degrading proteins CD10/neprilysin and CD36 are strongly modulated by deletion of astrocytic Stat3, again indicating that the observed effects are mediated by modified astroglia acting on microglia. Similarly, ApoE expression was also reduced in APP/PS1Stat3KO mice. We have also examined TREM2 expression, but did not find major differences induced by Stat3 deletion.
- We now provide new qPCR and immunohistochemistry data, reported in the new Figure 5 and on page 8-9. These data show that deletion of astrocytic Stat3 reduces astroglial mRNA transcripts associated with the neurotoxic astrocytic phenotype termed 'A1' (Liddelow et al., Nature 2017), while increasing transcripts associated with the neuroprotective astrocytic 'A2' phenotype. We confirm these qPCR data by western blotting and immunohistochemistry against the important astroglial effector protein C3d, demonstrating that deletion of astrocytic Stat3 reduces the fraction of peri-plaque C3d-positive reactive astrocytes. Interestingly, this is in line with a very recent paper showing that C3-receptor deletion rescues tau pathology and attenuates neuroinflammation in a tau model of AD (Litvinchuk et al., Neuron 2018), and an earlier report that C3-deficient mice are protected from AD pathology (Shi et al., Sci Transl Med 2017). This is now discussed on pages 13 and 15.

Together, our data now strongly imply that the genetic modulation of reactive astrocytes, by inducing a phenotypical switch, directs microglia to increase their phagocytic capacity to better clear Aβ.

Reviewer #2

Reichenbach and colleagues present a comprehensive study of the role of Stat3 signalling in reactive astrocytes in the context of mouse models of Alzheimer's disease (AD). Using the APP/PS1 amyloidosis model of AD, and extensive genetic ablation/silencing of largely astrocyte-specific Stat3 signalling, they report beneficial effects in memory and learning, along with changes in morphology of astrocytes and microglia, and an overall decrease in amyloid plaque load. With growing interest in the field of non-neuronal interactions in neurodegenerative diseases like AD, this study is likely to be of broad interest to the glia, degeneration, and broader neuroscience communities. A few points would benefit from clarification:

1. does the APP/PS1 mouse have STAT3+ reactive astrocytes in the same location as human patients? Though some human post-mortem staining was provided (Fig 1G), it was unclear if this was also a peri-plaque region like that shown for mouse staining. This information is important for the reader to be able to ascertain the appropriateness of the mouse model.

RESPONSE: We now provide new stainings of human brain sections (shown and quantified in Figure 1 and described on page 6), which show that a significant number of pStat3-positive reactive astrocytes cluster around Aβ plaques (stained with methoxy-XO4) in human brain tissue from AD patients, very similar to what we have observed in APP/PS1 mouse brain. We now describe on page 23 that this analysis was specifically carried out in peri-plaque astrocytes.

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2. Similarly, other groups have shown in recent years that STAT3-mediated reactive astrocytes are highly proliferative and produce a scar (in the context of acute injury) - was the same true in this mouse amyloidosis model?

RESPONSE: We have now tested this using immunohistochemistry against the cellular proliferation marker Ki67. Although this antibody was able to detect dividing/proliferating cells in the dentate gyrus as a positive control (now reported in the new Figure EV1), we detected few-to-none Ki67-positive (i.e. dividing/proliferating) reactive astrocytes around Aβ plaques in APP/PS1-Stat3WT or APP/PS1-Stat3KO mice (this is now reported on page 6 and in Figure EV1). This finding is in line with the current literature, given that reactive astrogliosis is a continuum that ranges from focal cellular hypertrophy to proliferation (i.e. scar formation; Sofroniew & Vinters, 2010), and that astrogliosis in Alzheimer's disease falls on the moderate end of the spectrum (Oberheim et al., J Neurosci 2008), with little-to-no astrocyte proliferation (Wang et al., Neurosci Bull 2018).

3. What possible effects would the ~10% non-astrocyte specific targeting of the Cx43 mouse have on interpretation of these results? With around 5% of cells being nonastrocytes/non-neurons, if Stat3 signalling is sufficiently highly blocked in microglia this could account for the microglia-specific effects reported (e.g. increased phagocytosis of amyloid)

RESPONSE: We have now better characterized the remaining 4.2 % (8 mo) and 6.2 % (11 mo) non-astrocytic non-neuronal cells using immunohistochemistry, and have found that these cells represent NG2 cells and a very small (<1 %) fraction of Olig2+ oligodendrocytes, but not microglia. This is now reported on page 5 and in Figure 1. Therefore, it is very unlikely that these effects were mediated by Stat3 deletion in microglia.

4. page 6, section titled 'Stat3 regulates plaque-associated...' the final sentence suggests that 'these data indicate that Stat3 signaling mediates an astrocyte-microglia crosstalk that may 'shield' the peri-plaque tissue...' there is no data for this conclusion, the conclusion to be drawn is that the astrocytes have an altered morphology in the periplaque region. Such prospective statements should not be included in the data section of the manuscript - please remove or move to the conclusions as a prediction to be further tested

RESPONSE: We agree, and have now moved this sentence to the Discussion. Other prospective statements and speculations were removed from the Results section as well.

5. Ca2+ imaging - did MRS2179 (P2Y1R inhibitor) alter the individual spontaneous events, or where there changes in the network-wide propagating Ca2+ transients?

RESPONSE: This is a good point, as we have previously shown that MRS2179 also reduces the incidence of astroglial calcium waves (Delekate et al., 2014). We now provide data in Figure 6G (mentioned on page 10) that propagating astroglial calcium transients are reduced by P2Y1R inhibition as well.

6. End of Ca2+ imaging section - the conclusion that Ca2+ transient changes can drive astrocyte reactivity has not been shown. MRS2179 can decrease Ca2+ transients AND decrease pSTAT3 immunofluorescence, but these data give no indication of a direct causative effect of calcium transients driving a reactive phenotype. Could it also not be that a decrease in Stat3 is driving Ca2+ changes? This conclusion is similarly easily drawn from these data and suggests that a decrease in STAT3 (ie. a decrease in reactivity) is driving calcium changes.

RESPONSE: We have now moved the discussion of this data to the Discussion. We agree with the referee that we have not directly shown a causative effect (this is now acknowledged on page 15). We now also discuss that a reverse sequence of events – a decrease in Stat3-mediated reactivity driving calcium changes – is also possible (page 15), but argue that this scenario may be less likely given that P2Y1R inhibition normalizes calcium hyperactivity in AD models within minutes (as shown in our earlier papers: Delekate et al., 2014 and Reichenbach et al., 2018).

Overall this manuscript is well written, the data and figures are carefully prepared and easy to follow. I would imagine the study would be well-received by a broad readership. Aside from the few clarifications outlined above, I have no reservations about recommending this manuscript.

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RESPONSE: We thank the referee for the positive comments.

Reviewer #3

Comments on Novelty/Model System for Author:
The authors used a complex way to generate their model and delete Stat3 in APPPS1 mice that does not confer full but partial Stat3 ablation. They should comment on this. Nonetheless, it is the first time Stat3 is deleted on a mouse model of AD and the novelty of the study is high. They also treated the mice with a Stat inhibitor so medical impact is potentially high. Technical quality is medium to high in most data of the MS.

RESPONSE: Thank you for the positive comments. We now mention on pages 5 and 13 that Stat3 was deleted "in the majority of astrocytes". Moreover, we now discuss that our model does not confer full but partial ablation, but that this partial deletion is sufficient to achieve therapeutically relevant effects (page 14).

Remarks for Author:
By partially deleting Stat3 in astrocytes, Reichenbach et al. found decreased amyloid beta load and neuritic dystrophy in the APPPS1 mouse model of AD, associated with several microglial alterations. Microglia become hypertrophic and increases phagocytosis and amyloid clearance pathways. A decrease of proinflammatory cytokines is also found. In addition, both Stat3 genetic deficiency and pharmacological inhibition decrease calcium hyperactivity in astrocytes and neurons and enhance learning and memory.

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1. The authors claim that the majority of reactive astrocytes in APPPS1-Stat3WT mice were Stat3+ while Stat3 activation was reduced by 80% in APPPS1-Stat3KO astrocytes, confirming its strong deletion. Can the authors give exact numbers? Figure 1 shows that 50% of GFAP+ astrocytes are Stat3+ in the cortex and only 40% in hippocampus in APPPS1 mice and does not justify the sentence claiming that the majority of reactive astrocytes are Stat3+. Same applies for postmortem samples in which again only 40% of astrocytes are Stat3+. Moreover, around 10% of GFAP+ astrocytes are Stat3+ in both cortex and hippocampus in APPPS1-Stat3KO mice. This is neither an 80% reduction nor a strong deletion. I would also recommend adding a sentence discussing how such partial deletion of Stat3 in astrocytes leads to significant changes in AD pathology.

RESPONSE: In the original manuscript, we had indeed stated that the majority of reactive astrocytes in APP/PS1 mice were positive for Stat3. However, this statement was specifically a description of Stat3-positive astrocytes around plaques, which – as the images in Figure 1 show – is indeed the region where most of Stat3 immunoreactivity occurs. However, the graph in the original Figure 1 had reported the numbers for all astrocytes, regardless of plaque proximity, resulting in a discrepancy between what we reported in the text and what the graph showed. We now report the fraction of Stat3-positive astrocytes from all astrocytes in the text (page 5), and in addition report the number of peri-plaque astrocytes positive for Stat3 (page 5) in the new Figure 1. These data indeed confirm that the majority of peri-plaque astrocytes were Stat3-positive, and that this was reduced by ~80 % in KO mice. Nevertheless, we agree with the referee that the deletion was only partial, and we now explicitly state this on page 14.

We also report the number of peri-plaque astrocytes positive for Stat3 in human sections in the new Figure 1, confirming that the majority of these astrocytes in human AD tissue was Stat3-positive.

2. Figure 2: can authors show higher magnification images of astrocytes and microglia far from the plaques? Are there morphological differences between APPPS1-Stat3wt and KO in far areas? Is there any change in the number of cells?

RESPONSE: We now provide higher-magnification images of astrocytes and microglia remote form

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plaques in Figure EV2 (described on page 6). We also provide a quantification of morphological features and cell numbers in this figure, which shows no significant difference between the groups.

3. Figure 4: the authors found a significant reduction of ApoE levels in APPPS1-tat3 ko but it is not evident on the blots. Can they provide more representative images?

RESPONSE: We have repeated all Western Blot experiments, and now provide more representative images (Figure 4).

4. Whole brain levels of TNFa and IL1b are reduced in APPPS1-Stat3 ko. Why the authors assume these proinflammatory cytokines are secreted by microglia? Astrocytes might contribute as well.

RESPONSE: Thank you for pointing this out. We have now changed the text to point out that microglia and astrocytes might both contribute to cytokine secretion (page 14).

5. Stat3 pharmacological inhibition decreases hyperactivity and improved learning and memory. To which extent Stat3 was inhibited? Can the authors provide images and some quantifications? Is SH4-54 treatment having any effect on AB burden, neuritic dystrophy and astroglia and microglia morphology? Can they add data on these?

RESPONSE: Heeding the referee's excellent point, we have performed new experiments now reported on page 12 and in the new Figure 9. Using immunohistochemistry, we now show that plaque size is significantly reduced after chronic treatment, while plaque load and dystrophic neurite area show nonsignificant trends towards a reduction, perhaps as expected given the relatively short treatment time. Moreover, we show that the relative number of pStat3-positive reactive astrocytes around plaques is significantly reduced in mice treated with SH-4-54 compared to controls. Finally, we find that total process length of near-plaque microglia is increased, similar to their morphology in APP/PS1-Stat3KO mice.

6. The authors do not mention in the text the time-points at which these characterizations were performed. Were the phenotypes more or less pronounced at different timepoints? Is there any variation over time?

RESPONSE: The time-points (i.e. age of the animals) are now reported in the Figure legends for all experiments and datasets. Most experiments were performed in mice aged 8-9 months old. Moreover, to investigate variation over time as requested by the referee, we now include data from 13-14 month-old mice, which is considered an advanced/late disease stage in the APP/PS1 model. These experiments, which are now reported in the new Figure EV3 and on page 10-11, show that the behavioral benefits, as well as reduced plaque load and size, persist at this later stage in APP/PS1-Stat3KO mice.

Minor: there are very long sentences in the abstract and introduction that are difficult to understand. Can the authors split the information on separate sentences?

RESPONSE: Thank you for pointing this out. This has now been corrected.

The images in Figure 3D seem to be upside down

RESPONSE: Thank you. We have corrected this.

Literature cited in reply to the referees' comments

Delekate A, Füchtemeier M, Schumacher T, Ulbrich C, Foddis M, Petzold GC. Metabotropic P2Y1 receptor signalling mediates astrocytic hyperactivity in vivo in an Alzheimer's disease mouse model. *Nat Commun* 2014 5: 5422. doi: 10.1038/ncomms6422.

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 2017 541: 481-487. doi: 10.1038/nature21029.

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Litvinchuk A, Wan YW, Swartzlander DB, Chen F, Cole A, Propson NE, Wang Q, Zhang B, Liu Z, Zheng H. Complement C3aR Inactivation Attenuates Tau Pathology and Reverses an Immune Network Deregulated in Tauopathy Models and Alzheimer's Disease. *Neuron* 2018 doi: 10.1016/j.neuron.2018.10.031.

Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M. Loss of astrocytic domain organization in the epileptic brain. *J Neurosci* 2008 28: 3264-3276. doi: 10.1523/JNEUROSCI.4980-07.2008.

Reichenbach N, Delekate A, Breithausen B, Keppler K, Poll S, Schulte T, Peter J, Plescher M, Hansen JN, Blank N, Keller A, Fuhrmann M, Henneberger C, Halle A, Petzold GC. P2Y1 receptor blockade normalizes network dysfunction and cognition in an Alzheimer's disease model. *J Exp Med* 2018 215: 1649-1663. doi: 10.1084/jem.20171487.

Shi Q, Chowdhury S, Ma R, Le KX, Hong S, Caldarone BJ, Stevens B, Lemere CA. Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice. *Sci Transl Med* 2017 9: eaaf6295. doi: 10.1126/scitranslmed.aaf6295.

Wang D, Zhang X, Wang M, Zhou D, Pan H, Shu Q, Sun B. Early Activation of Astrocytes does not Affect Amyloid Plaque Load in an Animal Model of Alzheimer's Disease. *Neurosci Bull* 2018 doi: 10.1007/s12264-018-0262-2.

2nd Editorial Decision 3 December 2018

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending minor editorial amendments.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

The authors have addressed all my comments.

Referee #3 (Comments on Novelty/Model System for Author):

It is the first time Stat3 is deleted on a mouse model of AD and therefore the novelty is high. They also treated the mice with a Stat inhibitor so there is a potential medical impact. Technical quality is high in most data of the MS.

Referee #3 (Remarks for Author):

The authors answered all my questions. I consider that after revision the MS highly improved. It is of high interest and suitable for publication at EMBO molecular Medicine.

2nd Revision - authors' response 4 December 2018

Authors made the requested editorial changes.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Gabor C. Petzold
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EM-2018-09665

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was chosen based on a statistical power of 0.8 and pre-specified effect sizes using G*Power 3 analysis software (Faul et al, 2007) and based on previous experience.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	See above.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Data were excluded from the analysis if an animal died during or between experiments, or if it did not display any meaningful attempts or motivation to search for the hidden platform in the behavioral assessment.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	All mice were randomly assigned to experimental groups.
For animal studies, include a statement about randomization even if no randomization was used.	N/A.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	All studies were performed by investigators blinded to treatment groups and sample identity.
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A.
5. For every figure, are statistical tests justified as appropriate?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We only used non-parametric tests and hence did not test for normality.
Is there an estimate of variation within each group of data?	The coefficient of variation as an estimate of interindividual variability was calculated.
Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

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http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	rat anti-GFAP: https://www.antibodypedia.com/gene/3505/GFAP/antibody/2159075/13-0300 rabbit anti-GFAP: Reichenbach et al. J Exp Med 2018, PMID 29724785 mouse anti-Aβ: Reichenbach et al. J Exp Med 2018, PMID 29724785 rabbit anti-pStat3: https://www.antibodypedia.com/gene/660/STAT3/antibody/107831/9145 rabbit anti-Stat3: https://media.cellsignal.com/pdf/12640.pdf rabbit anti-RFP: http://evrogen.com/antibody-descriptions/AB23301291014_antibody.pdf rat anti-LAMP1: Reichenbach et al. J Exp Med 2018, PMID 29724785 mouse anti-S100β: https://www.antibodypedia.com/gene/3474/S100B/antibody/91028/S2532 rat anti-C3d: http://www.finels.com/product/up_files/A0063.pdf rabbit anti-Ki67: Figure EV1 and https://assets.thermofisher.com/TFS-Assets/APD/Specification-Sheets/D12537~.pdf mouse anti-NeuN: Delekate et al., Nat Commun 2014, PMID 25406732 rabbit anti-NG2: Viganò et al., Nat Neurosci 2013, PMID 23995069 rabbit anti-Olig2: Viganò et al., Nat Neurosci 2013, PMID 23995069 6E10 antibody: Reichenbach et al. J Exp Med 2018, PMID 29724785 C1/6.1 antibody: Reichenbach et al. J Exp Med 2018, PMID 29724785 anti-CD10: Reichenbach et al. J Exp Med 2018, PMID 29724785 anti-ApoE: https://www.antibodypedia.com/gene/3639/APOE/antibody/550618/AB947 anti-TREM2: https://www.abcam.com/trem2-antibody-n-terminal-ab175525.html#top-0 anti-CD36: https://www.abcam.com/cd36-antibody-ab124515.html anti-β-actin: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/6/a2103dat.pdf
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Mice; C57BL/6N; male and female; 8-14 months; APP/PS1 tg/wt or wt/wt, Cx43-CreERT tg/wt or wt/wt, Stat3-loxP tg/tg or wt/wt, tdTomato-loxP tg/tg; Animals were housed in groups on a 12-h light/dark cycle with food and water available ad libitum.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All applicable international, national, and institutional guidelines for the care and use of animals were followed, and all experiments were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz of North Rhine-Westphalia (Germany).
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Experiments were performed according to the ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A.

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A.

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No.