Responses to the individual referees' comments on the manuscript entitled "Characterisation of NLRP3 pathway-related neuroinflammation in temporal lobe epilepsy" (Manuscript ID: PONE-D-21-35199)

Reviewer 1.

The individual points of the reviewer were addressed in detail as follows:

Point 1: The study and correlation of seizures with the dynamics of NLRP3-dependent transcripts and proteins would be of great interest.

Response: According to the referee's suggestions, we have added highly detailed phenotypic seizure data to the study and calculated the correlation between seizure frequency and gene expression in both models for molecules with representative expression profile changes over the time course of the epilepsy models. A direct correlation between neuronal hyperexcitability (seizure frequency) and gene expression was observed for Il1b in the KA model. Other NLRP3-related genes under study did not show similar expression changes (Representative calculation see S3 Fig and S2 Table). Direct comparability of seizure frequency, mRNA, and protein expression may be affected by time intervals between mRNA generation and protein expression in a gene-dependent manner, as the translation process is highly variable and complex (Ingolia et al., 2011).

For correlation analysis we used the median of the summarized seizure frequency of three consecutive days before mRNA analysis for every animal (day 3: seizure sum of day 1-3; day 5: sum of day 3-5; day10: seizure sum of day 8-10; day 28: sum of day 26-28). The median seizure frequency is now found in Suppl. Fig. 3A correlated to representative genes (NLRP3, TLR4, Il1b). Correlation of these three representative genes is presented in Suppl. Fig. 3B. The statistical analysis of the correlation for all genes under study is found in Suppl. Table 2.

Point 2: In addition, in one part of the Discussion (p.21 l. 6-12) there is a paragraph with a hypothesis linking seizure frequency to neuroinflammation. Hypothesis that could be supported and discussed extensively with the authors' findings.

Response: The referee addresses an important point. Our present data suggest that robust positive correlations between expression of the inflammation associated genes under study and seizure frequencies are not evident for these molecules. Thus, our study does not support immediate conclusions such as seizure activity induced inflammation or vice versa. However, given time delays in such interfering mechanisms, we can also not rule out that an interplay between seizure activity and levels of innate inflammation exist in the models under study. To study this more in detail will require selectively interfering functionally with individual molecules under study. However, such approaches clearly go beyond the framework of the present study. We discuss this issue in the revised manuscript (p. 21, l. 22 – p. 22, l. 4).

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