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Initial submission 📃 Revised version

Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

| 1. | Sample size | |
|----|---|---|
| | Describe how sample size was determined. | Sample sizes were based on previous experiments in our lab and published data for each of the assays. |
| 2. | Data exclusions | |
| | Describe any data exclusions. | See "Exclusion of mice from analysis" in online methods. |
| 3. | Replication | |
| | Describe whether the experimental findings were reliably reproduced. | All experimental findings were made using groups of mice (for group sizes see figure legends). |
| 4. | Randomization | |
| | Describe how samples/organisms/participants were allocated into experimental groups. | Mice were randomly assigned to groups, except for ensuring equal distribution of females/males across groups. Method of randomization was based on arbitrary numbers that were assigned at the time of weaning. |
| 5. | Blinding | |
| | Describe whether the investigators were blinded to group allocation during data collection and/or analysis. | Investigators were not blinded to group allocation during data collection and/or analysis. All data collection and analysis was performed using predefined pipelines without ad-hoc alterations. |
| | Note: all studies involving animals and/or human research partici | nants must disclose whether blinding and randomization were used |

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-------------|--|
| | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| \boxtimes | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| \ge | A statement indicating how many times each experiment was replicated |
| | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| | A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| | Clearly defined error bars |
| | See the web collection on statistics for biologists for further resources and guidance. |

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

SIFT and EEGLAB plugins in MATLAB, and Actimetrics FreezeFrame software.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company. No restrictions, except that some antibodies are only available from a for-profit company.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies used are described in methods, and were validated based on the known cellular and subcellular localization of the epitopes.

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines

- 10. Eukaryotic cell lines
 - a. State the source of each eukaryotic cell line used.
 - b. Describe the method of cell line authentication used.
 - c. Report whether the cell lines were tested for mycoplasma contamination.
 - d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

eukaryotic cell lines were used. Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly misidentified cell lines were used.

describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no

Confirm that all cell lines tested negative for mycoplasma contamination OR

• Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Mice were used (see methods for details).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A

N/A

were used.