nature research

Corresponding author(s): Nancy M. Bonini

Last updated by author(s): 04/07/2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No custom software or analyses were used. See methods section for full description of packages implemented.
Data analysis	No custom software or analyses were used. For differential expression analysis of dTBI, single-end reads were aligned to the fly genome using HISAT2 (v2.1.0). The HISAT2 index was built from FlyBase's Drosophila melanogaster reference genome r6.17. Alignment sorted BAM files (samtools v.15) for each sample were merged across sequencing runs (picard). Reads that uniquely aligned to exonic regions were counted with HTSeq (v0.9.1) with the union setting to produce a count matrix for differential expression analysis using the DESeq2 package in the R environment as follows. Principal component analysis was used to determine batch effects due to library preparation as follows. First, variance stabilized count data for all samples was visualized with plotPCA(). When samples were colored by library preparation, PC1 was associated with library preparation. Removing library effects with limmaRemoveBatchEffect() restored PC1 and PC2 to injury status and/or time post-injury. Thus, library was included in the DESeq2 design. Libraries prepared twice (0, 1d) did not cluster by condition after batch effect correction thus were treated as biological, not technical, replicates. To identify differentially expressed genes at each time post-injury, samples were reclassified into groups based on injury condition and post-injury time (i.e: TBI_15dpi, TBI_1dpi). The design model formula was "~library + group". Pairwise comparisons were made between sham and dTBI samples at each time using "contrast=c("group"), with an alpha cutoff of 0.05 with lfcShrink() applied. De novo motif enrichment analysis was performed with HOMER's (v.4.11.1) findMotifs.pl using the provided fly promoter set. All default parameters were used with the following exceptions: promoter region (-1000, 300 bp from TSS) and background promoter frequency. Background promoter frequency was derived from our RNAseq data and consisted of all expressed genes (non-zero read count; n = 16,201). Enrichment for de novo motifs was performed on significantly up or down-regulated genes at each time post-injury. Motifs passing a false positive cutoff (p < 1.0e-10) were matched to the best-known Drosophila motif or motif binding factor. Motif target genes were obtained using HOMER's annotatePeaks.pl. Enrichment for KEGG and Reactome pathways was determined using the interactive online tool FlyMine97. Curated gene sets were analyzed for pathway enrichment with Benjamini-Hochberg correction, using the same RNAseq defined background

gene set.

For differential expression analysis of human TBI, RNAseq data was obtained as aligned BAM files from the Allen Institute for Brain Science. Exonic read counts were calculated using the R package Rsubread (GTF file was GRCh38.p2). Differential gene expression analysis (FDR < 0.10) was performed in R using DESeq2 as above, with the following modifications. Model design (injury + brain region) was determined after principal component analysis. PCA revealed extreme outliers without an obvious pattern (n = 37; see Extended Data Fig. 7B). Removal resulted in all samples clustering by brain region along PC1 and PC2, allowing for inclusion of samples from distinct brain regions from the same donor (n = 161; 80 non-TBI and 81 TBI samples). Motif enrichment analysis against known motifs was performed using HOMER. Enriched molecular signature data base (mSigdB) gene sets and gene ontology terms were obtained from HOMER's hypergeometric comparison (p < 1.0e-7).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The differential gene expression data generated in this study are included in this published article (Supplementary Table 5 and 6). Sequencing data (fastq files and HTseq counts) that support the findings of this study have been deposited in Gene Expression Omnibus (GEO accession GSE171185). Additional data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined based on precedent by our lab and others, specific experimental parameters and readout, and considering downstream statistical tests. Sample size cutoff of n<15 was generally used for non-parametric tests and n>15 for parametric tests, along with testing for homogeneity of variance (Levene's test) and normality (Shapiro-Wilks test).
Data exclusions	No data were excluded.
Replication	All data shown are the result of a minimum of two independent experiments, based on positive findings from an initial pilot study. All replications were successful.
Randomization	Male sibling flies were randomly assigned to experimental conditions. For experiments with non-sibling flies (i.e: different genetic background), flies were age-matched and handled in parallel.
Blinding	All tissue samples (paraffin, whole mount, protein, RNA, etc) were given non-identifying sample ID's so that data acquisition and quantification was always performed blind to sample identity.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies (supplier, catalog number, lot number):
aTubulin (DHSB, AA4.3, 11/5/15-51ug/ml)
AT100 (Invitrogen, MN1060, UH2803903)
AT8 (Invitrogen, MN1020, UH2805521)
dsRed (Thermofisher, MA515257, TE264883)
elav (DSHB, Rat-Elav-7E8A10, 12/27/18-23ug/ml)
ERK (Sigma, 5670, 21190726)
GFP (Invitrogen, A11122, 1:500)
Tau (DAKO, A0024, 20024929)
JNK (Santa Cruz Biotechnology, sc571 (discontinued now), J209)
pERK (Cell Signaling, 4370, 24)
pJNK (Promega, V7931, L019)
repo (DSHB, 8D12, 12/6/19-54ug/ml)

Secondary antibodies (supplier, catalog number, lot number):
Goat anti-rabbit AlexFluor 488 (Invitrogen, A11008, 1911238)
Goat anti-mouse AlexaFluor 488 (Invitrogen, A32723, 2120125)
Goat anti-rat AlexaFluor 546 (Invitrogen, A-111081, 1921310)
Goat anti-mouse AlexaFluor 594 (Invitrogen, A11032, 1887003)
Goat anti-mouse AlexaFluor 647 (Invitrogen, A21236, 2011576)
Goat anti-rabbit HRP (Jackson ImmunoResearch, 111-035-144, 127629)
Goat anti-mouse IgG H&L HRP (Abcam, ab6789, NA)

Supplementary Table 4 details specific concentrations used for different applications.

Validation

All antibodies were validated in this study prior to use for data collection. Validation varied with experimental technique.

For whole mount antibodies:
repo: well-established glial marker in Drosophila, see https://dshb.biology.uiowa.edu/8D12-anti-Repo
elav: well-established neuronal marker in Drosophila, see https://dshb.biology.uiowa.edu/8D12-anti-Repo
GFP: no signal in non-transgenic (non-GFP expressing) animals

For western immunoblot:
aTubulin: well-established, correct band size, see https://dshb.biology.uiowa.edu/AA4-3
dsRed: single band at expected molecular weight, no signal in non-transgenic (non-RFP expressing) animals
ERK: band at expected molecular weight, previously confirmed in Drosophila, see doi: 10.1242/dev.056671 and https://www.sigmaaldrich.com/catalog/product/sigma/m5670?lang=en&region=US
pERK: confirmed in Drosophila, 7 publications listed on https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370?Ntk=Products&Ntt=4370
pJNK: confirmed in Drosophila, see 10.1128/MCB.20.9.3015-3026.2000
JNK: confirmed in Drosophila, see https://www.scbt.com/p/jnk-antibody-fl-human
tau: well-established antibody to human tau, see 10.1186/s13024-017-0229-1

For western immunoblots, antibodies were additionally validated by the presence of a single band (at the correct size) with little to no evidence of off-target labeling on a full blot.

For paraffin:
AT8: well-established antibody, see citations at https://www.thermofisher.com/antibody/product/Phospho-Tau-Ser202-Thr205-Antibody-clone-AT8-Monoclonal/MN1020
AT100: well-established antibody, see citations at ttps://www.thermofisher.com/antibody/product/Phospho-Tau-Thr212-Ser214-Antibody-clone-AT100-Monoclonal/MN1060

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Male Drosophila melanogaster were used for this study. Animal age varied by experiment. For all dTBI experiments, 3d old males

Laboratory animals	were subjected to sham or dTBI injury then collected at noted post-injury time points. See Supplementary Tables 1 and 2 for detailed genotype information.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All live animals were invertebrates, ethical approval and oversight are not required.

Note that full information on the approval of the study protocol must also be provided in the manuscript.