nature research

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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.

   No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications.

2. Data exclusions

   Describe any data exclusions.

   Four of the 28 sequencing libraries were computationally down-sampled (a random fraction of reads was removed) to match sequencing depths across samples (see methods).
   Cell doublets were removed. The criteria is consistent with other papers and is reported in the methods.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   The experiment was carried out across 28 independent samples, derived from 23 animals across 20 sequencing runs and 7 batches (separate days on which cells were collected and libraries processed). All data was pooled and analyzed together. We used multiple methods to confirm key findings (scRNAseq, FISH).

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Mice were randomly assigned to the 0h, 1h and 4h cohorts. No other randomization was used.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Only cell encapsulation and transcriptomic alignment were performed in a blinded fashion. Knowledge of experimental conditions was necessary during other parts of data collection. Analysis was conducted using automated scripts.

Note: all studies involving animals and/or human research participants 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.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ 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 central tendency (e.g. median, mean) and variation (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.

Matlab 2016b, R 3.3.1, Limma 3.26.9, edgeR 3.12.1, Python 2.7.6, matplotlib 2.0.2. study. For details see methods.

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.

There are no restrictions

9. Antibodies

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

NA

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

NA

b. Describe the method of cell line authentication used.

NA

c. Report whether the cell lines were tested for mycoplasma contamination.

NA

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.

NA

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.

Animal experiments were approved by the National Institute of Health and Harvard Medical School Institutional Animal Care and Use Committee. The experiments used adult (6–8 weeks old) C57BL/6J virgin male mice (The Jackson Laboratory).

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.	NA

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Editorial Policy Checklist

This form is used to ensure compliance with Nature Research editorial policies related to research ethics and reproducibility in the life sciences. For further information, please see our Authors & Referees site. All questions on the form must be answered.

Data availability

Policy information about availability of data

Data availability statement

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 source data
- A description of any restrictions on data availability

☒ A full data availability statement is included in the manuscript.

Required accession codes

Data deposition is mandated for certain types of data.

Confirm that all relevant data have been deposited into a public repository and that all accession codes are provided.

☒ Accession codes will be available before publication ☐ No data with mandated deposition ☐ All relevant accession codes are provided

Data presentation

Image integrity

☒ Confirm that all images comply with our image integrity policy.

Unprocessed data must be provided upon request. Please double-check figure assembly to ensure that all panels are accurate (e.g. all labels are correct, no inadvertent duplications have occurred during preparation, etc.).

Data distribution

Data should be presented in a format that shows data distribution (dot-plots or box-and-whisker plots), with all box-plot elements (e.g. center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers) defined. If bar graphs are used, the corresponding dot plots must be overlaid.

☒ Confirm that all data presentation meets these requirements.

☒ Confirm that in all cases where the number of data points is <10, individual data points are shown.

Structural data

Policy information about special considerations for specific types of data

☒ If this study did not involve data of these types, check here and skip the rest of this section.

Electron microscopy

☐ For all electron microscopy work, confirm that you have deposited any density maps and coordinate data in EMDB.

Macromolecular structures

☐ For all macromolecular structures studied, confirm that you have provided an official validation report from wwPDB.

Code availability

Policy information about availability of computer code

Code availability statement

For all studies using custom code, the Methods section must include a statement under the heading "Code availability" describing how readers can access the code, including any access restrictions.

☒ A full code availability statement is included in the manuscript ☐ No custom code used

Research animals

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

☐ If this study did not use animals and/or animal-derived materials for which ethical approval is required, check here and skip the rest of this section.

Ethical compliance

☒ Confirm that you have complied with all relevant ethical regulations and that a statement affirming this is included in the manuscript.

Ethics committee

☒ Confirm that you have stated the name(s) of the board and institution that approved the study protocol in the manuscript.

Human research participants

Policy information about studies involving human research participants

☒ If this study did not involve any human research participants, check here and skip the rest of this section.

Ethical compliance

☐ Confirm that you have complied with all relevant ethical regulations and that a statement affirming this is included in the manuscript.

Ethics committee

☐ Confirm that you have stated the name(s) of the board and institution that approved the study protocol in the manuscript.

Informed consent

☐ Confirm that informed consent was obtained from all participants.

Identifiable images

For publication of identifiable images of research participants, confirm that consent to publish was obtained and is noted in the Methods.

Authors must ensure that consent meets the conditions set out in the Nature Research participant release form.

☐ Yes ☐ No identifiable images of human research participants

Clinical studies

Policy information about clinical studies

☒ If this study was not a clinical trial, check here and skip the rest of this section.

Clinical trial registration

☐ Confirm that you have provided the trial registration number from ClinicalTrials.gov or an equivalent agency in the manuscript.

Phase 2 and 3 randomized controlled trials

Confirm that you have provided the CONSORT checklist with your submission.

☐ Yes ☐ No ☐ Not a phase 2/3 randomized controlled trial

Tumor marker prognostic studies

Did you follow the REMARK reporting guidelines?

☐ Yes ☐ No ☐ Not a tumor marker prognostic study

Methods reporting

Nature Research wishes to improve the reproducibility of the work we publish. As part of this effort, all life science manuscripts require a reporting summary; certain types of research require specialized modules in addition to this form.

☒ Confirm that you have provided a complete and accurate reporting summary.

n/a	Confirmed
☐	☐ For MRI studies, confirm that you have completed the additional MRI module.
☐	☐ For flow cytometry studies, confirm that you have completed the additional flow cytometry module.
☐	☐ For ChIP-seq studies, confirm that you have completed the additional ChIP-seq module.

I certify that all the above information is complete and correct.

Typed signature Sinisa Hrvatin Date Oct 9, 2017