

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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
<i>Give P values as exact values whenever suitable.</i> |
| ☐ | ☒ 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

Data analysis

10x Genomics Cell Ranger v3.1.0

```
python 3.7.4
doubletdeletion==2.5.2
scanorama==1.4
scanpy==1.4.5.post2
scikit-learn==0.21.3
scipy==1.3.1
scvi, commit code (a45803743d9d788b813ce5eafc30f6ab20a35411) (https://github.com/YosefLab/scvi)
seaborn==0.9.0
schpf==0.3.0a
ctpnets==0.2.5
harmonypy==0.0.4
```

```
R 3.6.2
Seurat==3.1.4
VISION=2.0.0
```

https://github.com/YosefLab/totalVI_reproducibility (v0.1)
All packages with versions are listed at https://github.com/YosefLab/totalVI_reproducibility/blob/master/requirements.txt.

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 data discussed in this manuscript (SLN-all) have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE150599 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150599>). Processed data are also available in the reproducibility GitHub repository (https://github.com/YosefLab/totalVI_reproducibility). The SLN-all dataset processed with totalVI can be explored interactively with Vision at <http://s133.cs.berkeley.edu:9000/Results.html>. Public datasets were downloaded from 10x Genomics (PBMC5k: https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.0.2/5k_pbmc_protein_v3; PBMC10k: https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.0.0/pbmc_10k_protein_v3; MALT: https://support.10xgenomics.com/single-cell-gene-expression/datasets/3.0.0/malt_10k_protein_v3). Mouse mm10 reference was downloaded from 10x Genomics (<https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/2.1/>).

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](https://www.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	No sample size calculation was performed. The sample size was chosen based on availability of materials at the time of the study
Data exclusions	No data were excluded from the analysis. Filtering and quality control of single cell sequencing data is described in the Methods.
Replication	All of the findings reported in this study are fully reproducible based on the code in the totalVI reproducibility Github repository. All experimental procedures are detailed in the Methods to ensure reproducibility. CITE-seq experiments with different antibody panels were each performed twice in biological replicate to ensure reproducibility. All attempts at replication were successful.
Randomization	Randomization was not relevant to this study. Both mice (female wildtype littermates) were treated identically as biological replicates, and each antibody panel was tested on samples from each mouse.
Blinding	Blinding was not relevant to this study since no case-control comparisons were made.

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

Methods

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

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

Antibodies

Antibodies used	All antibodies used in this study were produced by BioLegend Inc. (TotalSeq-A mouse antibody panel 1, BioLegend #900003217; TotalSeq-A mouse antibody panel 2, BioLegend #900003218) and are described in detail in the Supplementary Data.
Validation	All antibodies used in this study were validated by the manufacturer (BioLegend Inc.). Validation statements describing quality control testing of each antibody are available on the manufacturer's website searchable by catalog number. Custom conjugate antibodies were all previously validated clones (validation statements available per clone on manufacturer's website), and barcodes were validated by Sanger sequencing.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	This study used two female C57BL/6 (B6) mice at 5 weeks of age. Mice were housed in standard conditions as described in the Methods.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal care and procedures were carried out in accordance with guidelines approved by the Institutional Animal Care and Use Committee at BioLegend, Inc.

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