nature portfolio

Corresponding author(s): Morgan Huse

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Reporting Summary

Nature Portfolio 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 Portfolio 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	Zen Blue (Zeiss), NIS-Elements (Nikon), CytExpert software(version 2.3), LasX (Leica)
Data analysis	FIJI 2.0, Imaris 9.9.0 (Oxford Instruments), Flowjo (10.10), Prism 9 (GraphPad), MATLAB (2022b). Custom MATLAB code used for analysis can be found on the Zenodo repository (https://zenodo.org/records/13313112).

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw proteomic LC/MS data is available on ProteomXchange (PXD049434, link to be provided). Source Data are provided for this paper in the Source Data file. Raw flow cytometry output has been deposited on the Zenodo Repository (https://zenodo.org/records/13351483). Imaging files are available upon request from the lead author (Morgan Huse, husem@mskcc.org).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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	We did not predetermine a selected sample size. Sample sizes were determined based prior experience and pilot studies.
Data exclusions	We did not exclude any data.
Replication	All findings and statistical analyses described in the manuscript are the result of at least 2 experimental replicates, or show data pooled from independent biological replicates. When relevant each replicate used biological material from a unique animal. Otherwise each replicate reflected a unique iteration of sample preparation.
Randomization	Experimental groups were defined by genotype, pharmacological treatment, or target identity. In all experiments, appropriate controls were taken such that individual variables were isolated. Samples were not randomized during processing or data collection.
Blinding	During image analysis where manual input was required, samples were blinded using a custom MATLAB script to present the data in a random order without labels. For all other analyses, blinding was not relevant because data capture and analysis was automated and applied equally to all samples.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

anti-CD18-AF647: Clone M18/2, BioLegend 101414
anti-CD11b-APC: Clone M1/70, Invitrogen # 17-0112-82
anti-CD11c-FITC: Clone N418, Invitrogen #11-0114-82
anti-F4/80-BV421: Clone T45-2342, BD Biosciences 565411
anti-Ly6G-BV650: Clone 1A8, BD Biosciences 740554
anti-Talin: Clone: 8D4, Abcam ab157808
anti-Vinculin: Polyclonal, Abcam ab91459
anti-actin: clone AC-15, Sigma A2066
anti-GAPDH: clone 14C10, Cell Signaling Technology 3683
Goat anti-Mouse 800CW, LI-COR 925-32210
Goat Anti-Rabbit 680RD, LI-COR 926-68071

Validation

All antibodies were commercially validated using company-specific method:

BioLegend (https://www.biolegend.com/en-us/quality/quality-control)
Specificity testing of 1-3 target cell types with either single- or multi-color analysis (including positive and negative cell types). Once specificity is confirmed, each new lot must perform with similar intensity to the in-date reference lot. Brightness (MFI) is evaluated from both positive and negative populations. Each lot product is validated by QC testing with a series of titration dilutions.

BD Biosciences (https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/quality-and-reproducibility):
The specificity is confirmed using multiple methodologies that may include a combination of flow cytometry, immunofluorescence, immunohistochemistry or western blot to test staining on a combination of primary cells, cell lines or transfectant models. All flow cytometry reagents are titrated on the relevant positive or negative cells

Invitrogen (https://www.thermofisher.com/us/en/home/life-science/antibodies/invitrogen-antibody-validation.html):
Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. [...] Using gating techniques, verification of antibody binding can be determined by analyzing expression in unique cell types.

Abcam (https://www.abcam.com/primary-antibodies/how-we-validate-our-antibodies):
Antibodies are validated in western blot using lysates from cells or tissues that we have identified to express the protein of interest. Once we have determined the right lysates to use, western blots are run and the band size is checked for the expected molecular weight. We will always run several controls in the same western blot experiment, including positive lysate and negative lysate

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

E0771 cells (female) were acquired from ATCC (CRL-3461 ™), and the MRTF-A expression lines were derived from E0771 cells as previously described (Tello La Foz et al, Immunity 2021, DOI: 10.1016/j.immuni.2021.02.020).
L929 cells (male) were acquired from ATCC (CCL-1™)
HoxB8-ER condtionally-immortalized cells were generated, as described in the manuscript, from the bone marrow of male mice from C57BL/6J (Jackson Labs Strain #:000664) or B6J.129(Cg)-Gt(ROSA)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J (Jackson Strain #: 026179)
Human macrophages were differentiated from 731.2B-iPSCs, which were generated from human male fibroblasts (GM00731) purchased from the Coriell Institute. Use of the 731.2B-iPSC cell line was approved by the MSKCC Institutional Review Board.

Authentication

731.2B-iPSCs were authenticated by the Stem Cell Research Facility at MSKCC. They express the expected levels of OCT4, SOX2, and NANOG, and have a normal karyotype. E0771 cell lines were not authenticated. L929 cells were authenticated by their capacity to stimulate BMDM differentiation.

Mycoplasma contamination

731.2B-iPSCs and HoxB8-ER conditionally immortalized cells were regularly tested for mycoplasma contamination and received negative results. E0771 cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

Mouse (M. Musculus) Strains:
C57BL/6J (Jackson Labs Strain #:000664), Ages 6-24 weeks
B6J.129(Cg)-Gt(ROSA)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J (Jackson Labs Strain #: 026179), Ages 6-8 weeks
B6.129S7-Itgb2tm2Bay/J (Jackson Labs Strain #:003329), Ages 6-12 weeks
Mice were housed in an SPF facility under standard conditions (12 h light, 12 h dark, room temperature, ambient humidity).

Wild animals	The study did not involve wild animals
Reporting on sex	For generation of HoxB8-ER cell lines and subsequent experiments, only male subjects were used because the maintenance of the cells require the absence of endogenous estrogen signaling. For all other experiments, both male and female mice were used.
Field-collected samples	The study did not involve field-collected samples
Ethics oversight	The animal protocols used for this study were approved by the Institutional Animal Care and Use Committee of Memorial Sloan Kettering Cancer Center.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For in-vitro phagocytosis assays, macrophages were incubated with FITC/LRB labeled particles and then harvested by trypsinization. Samples were collected into 96-well plates and washed by centrifugation (500x g, 2min) in PBS. For labeling, cells were first incubated with Fc-block (CD16/32) for 30 minutes followed by primary antibody for 30 minutes at 4°C. Cells were incubated in 3µM DAPI 5 minutes prior to measurement. For in vivo phagocytosis assays, cells were first harvested by peritoneal lavage with PBS and collected into 96-well plates and stained as described above.
Instrument	The Beckman Coulter CytoFLEX LX flow cytometer was used to collect flow cytometry data in this study.
Software	CytExpert software(version 2.3) was used to collect flow cytometry data. FlowJo software (version 10.10) was used to analyze flow cytometry data.
Cell population abundance	For in-vitro macrophage cultures, >95% of cells were macrophages For in vivo peritoneal macrophages, 1-5% of cells were macrophages, as determined by CD11b/F480 staining, see Fig 2F
Gating strategy	To determine the LRBhi/FITClo population, unconjugated beads were used as a negative control to determine the distribution of FITC/LRB signal in the particles. Gates were drawn at the same LRB level with FITC florescence below the measured range of unconjugated particles.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.