nature portfolio

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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	For RNAseq studies, data was collected from GENCODE for gene annotation (https://www.gencodegenes.org/).
Data analysis	All open source softwares were used for RNAseq analysis. Gene read count table by featureCount and edgeR software. DAVID, v6.8 was used for gene functions. Venn diagram generator ((http://bioinformatics.psb.ugent.be/webtools/Venn/) . For immune repertoire analysis MIXCR was performed and the results were imported to VDJ tools to plot clonotypes ((https://github.com/mikessh/vdjtools). Graphpad Prism for data analysis, statistics and plotting graphs.

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

The datasets generated and/or analysed during the current study are available from the corresponding author on 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	This is a pilot study in which effect size and variance are unknown. We had 8 treatment groups - 3 IUT, 1 uninjected control, each with or without maternal DC depletion, and we planned four timepoints for harvest and analyses. We estimated that treated groups would have an mean chimerism of 4% while untreated groups would have a mean chimerism of 2% (based on best expected performance). At a reasonable effect size of d=0.6, conventional alpha=0.05 and 1-beta=0.8., a priori calculations suggested 45 pups per groups (DC depletion, DC undepleted). Thus we planned to inject 100 pups / 12-15 dams to achieve this target. This took into consideration a pregnancy losses through resorption or cannibalism. This worked out to 3 pups per treatment group, per timepoint.
Data exclusions	No data was excluded from the study
Replication	All our experiments were repeated to get reproducibility. Experiments were repeated to get more samples for the harvesting time point for the long term analysis. For RNAseq experiments, maternal trafficked cells from each neonate were not sufficient for RNA-seq analysis as individual samples. We pooled the samples from a group of neonates to generate sufficient number of cells for sorting and performed RNA-seq. Thus we do not have replicates for RNAseq samples that were analysed, but each sample represents a certain number of harvested animals, as we have specified in Methods and Results.
Randomization	All our experiments were conducted on E-13 and E-14 pregnant transgenic mice for IUT. When performing animal experiments, pregnant mice were randomly chosen for IUT and for downstream experiments, as the probability of getting pregnant dams in a group of breeding mice occur by chance.
Blinding	Data collection and analysis were performed by separate team of staffs. Investigators were blinded for the current study.

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

Antibodies used in our flow cytometry experiments were provided with the details on the Extended data table S1.

Antibodies Clone Fluorescence Catalogue # Company
B220 RA3-6B2 APC 103212 Biolegend
CD117 (c-kit) 2B8 BV605 563146 BD Biosciences
CD11b M1/70 Alexa Fluor 700 557960 BD Biosciences
CD11c N418 BV650 117339 Biolegend
CD127 SB/199 BV510 563353 BD Biosciences
CD150 TC15-12F12.2 BV650 115932 Biolegend
CD172a P84 Alexa Fluor 700 144021 Biolegend
CD19 6D5 FITC 115506 Biolegend
CD25 PC61 BV650 564021 BD Biosciences
CD3 17A2 BV421 564008 BD Biosciences
CD4 GK1.5 Alexa Fluor 700 100430 Biolegend

CD44 IM7 FITC 561859 BD Biosciences
CD45.1 A20 APC 110714 Biolegend
CD45.2 104 PE 109808 Biolegend
CD62L MEL-14 BV605 563252 BD Biosciences
CD8a 53-6.7 BV605 563152 BD Biosciences
FC block 2.4G2 553142 BD Biosciences
FOXP3 R16-715 PerCP Cy5.5 563902 BD Biosciences
GR1 RB6-8C5 PE-Cy7 25593182 Thermofisher Scientific
H-2Kb AF6-88.5 PerCP-Cy5.5 562831 BD Biosciences
H-2Kd SF1-1.11 BV510 742432 BD Biosciences
H2K-k 36-7-5 BUV395 750340 BD Biosciences
IgG Poly4053 APC 405308 Biolegend
IgM RMM-1 BV421 406517 Biolegend
MHC-II M5/114.15.2 PE-Cy7 25532182 Thermofisher Scientific
NK1.1 PK136 APC-Cy7 560618 BD Biosciences
Sca1 D7 PerCP 108121 Biolegend
TER119 TER-119 APC-Cy7 560509 BD Biosciences
XCR1 ZET APC-Cy7 148223 Biolegend

Validation

All the antibodies listed above were used for flow cytometry experiments. Each antibody was validated with respective positive control as per manufacturer's instructions.

Animals and other organisms

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

Laboratory animals	Four strains of mice were used in our studies. CD45.2 BALB/c (H-2Kd), C3H/HeNTac (H-2Kk), CD45.1 C57BL/6, CD11c-DTR female mice (B6.FVB-1700016L21RikTg(Itgax-DTR/EGFP)57Lan/J).
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All mouse experiments were performed according to IACUC (Institutional Animal Care and Use Committee) approved protocols (BR16-1203 and R16-1200) at the National University of Singapore (NUS), Singapore.

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

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	Peripheral blood and bone marrow mononuclear cells were prepared by density gradient centrifugation using Ficoll-Paque Plus density gradient media (GE Healthcare, NJ, USA). Liver MNC and uterus MNC were prepared by digesting chopped pieces with collagenase IV (0.04%, 10 minutes at 37°C), passing through 70 µm nylon mesh, centrifugation (150g × 5 minutes) to remove debris, pelleting of cells (400g × 5 minutes), resuspension of cell pellet in AutoMACS rinse solution and overlaid on Ficoll-Paque PLUS density gradient media, centrifugation (400g × 20 minutes), and harvesting MNC from the Percoll interface. Spleen was minced and mashed through a 70 µm cell strainer producing a single-cell suspension flow-through which was centrifuged (400g × 5 minutes), the cell pellet was subjected to RBC lysis and washed with PBS. LN were excised from the axillary, brachial, and inguinal regions, minced and digested with collagenase IV (0.04% × 20 minutes at 37°C), passed through a 70 µm cell strainer, centrifuged (400g × 5 minutes), cell pellets washed with PBS and resuspended in FACS buffer (AutoMACS rinse solution containing 2% bovine serum albumin (BSA)). rinse solution. Cells were enumerated using tryphan blue exclusion and stained for cell surface antibodies. For intracellular staining, cells were permeabilized using BD Cytofix/Cytoperm solution and washed with Perm/Wash buffer and stained for intracellular marker. Cells were washed 2 times, pelleted (800g x 5minutues), and resuspended in FACS buffer and read on the flow cytometry machine immediately.
Instrument	BD X-20 Fortessa™ flow cytometer (BD Biosciences, Franklin Lakes NJ, accessed at the NUS Life Science Institute)
Software	FlowJo™ software (FlowJo LLC, Ashland OR, USA).
Cell population abundance	For RNA-seq experiments, maternal trafficked cells and fetal recipient cells were sorted with distinct markers and live-dead stain. The samples were 100% pure with gating strategy. The sorted cells were directly collected in an RNA-lysis solution
Gating strategy	For immune profile: flow cytometry analysis gating strategy, the cells were first gated to forward scattering (FSC) / side scattering (SSC) to exclude debris. Remaining cells were gated to forward scattering (FSC)/ forward scattering height (FSH), to get singlets. The singlets were then subjected to live-dead staining gating to exclude dead cells. The live cells were further gated for population of maternal trafficked cells (H2K-b-PerCP Cy5.5), donor cells (CD45.1-FITC for mIUT, H2K-d-BV510 for pIUT, C3H-BUV395 for aIUT) and fetal recipient cells (H2K-b PerCP-Cy5.5+ H2K-d-BV510+). Under each population, cells were gated for CD3-BV421 (further gated to CD4-AlexaFluor 700 and CD8-BV605), CD19-APC. CD3 negative and CD19 negative population is then gated to CD11c-BV650+ and MHC-11-PE-Cy7+ population to get conventional dendritic cells (cDC). cDC were further gated to XCR-1-APC-Cy7 (cDC-1) and CD172a-Alexa Fluor700 (cDC2). CD11c-negative and MHC-II-negative population was gated to NK1.1-APC-Cy7. For hematopoietic stem cells: flow cytometry analysis gating strategy, the cells were first gated to forward scattering (FSC) / side scattering (SSC) to exclude debris. Remaining cells were gated to forward scattering (FSC)/ forward scattering height (FSH), to get singlets. The singlets were then subjected to live-dead staining gating to exclude dead cells. The live cells were further gated for population of donor cells (CD45.1-FITC for mIUT, H2K-d-BV510 for pIUT, C3H-BUV395 for aIUT) and fetal recipient cells (H2K-b PerCP-Cy5.5+ H2K-d-BV510+). Donor cells were further gated to exclude lineage markers (CD3, CD19, B220-APC), TER119-APC-C7, CD11b-Alexa Fluor700, GR-1-PE-Cy7. Lineage negative population then gated for HSC population c-Kit-BV605, Sca1-PerCP. From each of these markers, further gated for CD48-FITC (short-term HSC), CD150-BV650 (long-term HSC) population.

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

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s) *Specify: functional, structural, diffusion, perfusion.*

Field strength *Specify in Tesla*

Sequence & imaging parameters *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*

Area of acquisition *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016) *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

Correction *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity *Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*

Graph analysis *Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*

Multivariate modeling and predictive analysis *Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*