

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

In vivo cell populations were analyzed and isolated using the BD FACS Fusion in the Georgia Institute of Technology Cellular Analysis Core. The samples were then processed as indicated in the text and library QC for deep sequencing was conducted using the Agilent Bioanalyzer 2100, qPCR, and qubit to measure DNA prior to sequencing. The final library was sequenced using a Illumina HiSeq paired-end 150-cycle run. The data were processed using zUMIs (v 2.9.7) for the RNA mapping and counting and Salmon Alevin (v1.5.2) for the DNA barcode and cell hashes. All output files were then loaded into Seurat (v 4.0.4) and data was further analyzed as indicated in the text. DoubletFinder (v3) was used to identify doublets. All code (https://github.com/Jack-Feldman/barcode_count)

Data analysis

Data were analyzed and plotted in GraphPad Prism 8. The specifics of the data analyses are described in the text. Flow cytometry data was analyzed using FlowJo v10. Sequencing data were analyzed using BBrowser2 (v2.9.23).

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](#)

All raw sequencing data is available online (GEO: GSE186395). All other data are in the main text or the supplementary materials.

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	For in vivo single cell RNA sequencing experiments: N = 4 mice / group were used for control mice (treated with PBS) and experimental mice (treated with an LNP pool). For validation in vivo experimental data shown in Supplementary Fig. 17b - f, and Supplementary Fig. 20 we used n = 2 mice / group for control mice (treated with PBS) and n = 4 mice / group for experimental mice treated with LNP pool. In this case, control mice were used for flow cytometry gating. Sample sizes were chosen to ensure accuracy of the data and to be able to obtain accurate statistics.
Data exclusions	None
Replication	Four biological replicates were used per condition for scRNA sequencing. scRNA sequencing data was validated using single LNP experiments, data for which is shown in Supplementary Fig. 17. Validation experiments showed that LNPs identified in the scRNA seq data as top performers, were able to perform well in single LNP experiments.
Randomization	When possible, mice were randomly selected and ensured to be the same age and sex.
Blinding	Some experiment participants were blinded (e.g., during mice injection and cell isolation). We did not blind the authors performing the analysis due to limited access to infrastructure.

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	Antibody clones used for staining were anti-CD31 (390, BioLegend, cat# 102427), anti-CD45.2 (104, BioLegend, cat# 109832), anti-CD68 (FA-11, ThermoFisher, cat# 46-0681-82), anti-αVHH (96A3F5, GenScript, cat# A01994), anti-ASGPR1 (8D7, Santa Cruz Biosciences, cat# sc-52623 FITC) live/dead (Thermo Fisher, cat# L34975). To allow for pooling of samples into a single device, cells were stained with a streptavidin-conjugated H-2 MHC class I (M1/42, BioLegend, cat# 125502) antibody, and biotinylated cell hash oligos were added at 0.5 μM final concentration after a single wash to remove unbound antibody. Antibodies were diluted according to the manufacturer's recommendation.
Validation	anti-CD31 (BioLegend, 390): "Anti-mouse CD31 clones 390 and MEC13.3 bind to their respective non-overlapping epitopes in IgD2 of CD31. CD31 is a 130-140 kD glycoprotein, also known as platelet endothelial cell adhesion molecule (PECAM-1) and EndoCAM". anti-CD45.2 (BioLegend, 104): "CD45.2 is an alloantigen of CD45, expressed by Ly5.2 bearing mouse strains (e.g., A, AKR, BALB/c, CBA/Ca, CBA/J, C3H/He, C57BL, C57BR, C57L, C58, DBA/1, DBA/2, NZB, SWR, 129). CD45, a member of the protein tyrosine phosphatase (PTP) family, is a 180-240 kD glycoprotein expressed on all hematopoietic cells except mature erythrocytes and platelets." anti-CD68 (FA-11, BioLegend): "CD68 is an 85-115 kD member of the lysosomal-associated membrane protein (LAMP) family. It is expressed on tissue macrophages, Langerhans cells and at low levels on dendritic cells." Anti-Camelid VHH (96A3F5, GenScript): "A Camelid VHH Antibody (also called single-domain antibody, sdAb or Nanobody by Ablynx) is a peptide chain of about 110 amino acids long, comprising one variable domain of a heavy-chain antibody, these are called VHH fragments. It is produced from a hybridoma resulting from the fusion of partner and B-lymphocytes obtained from a rabbit immunized with llama sdAb." Anti-ASGPR1 (8D7, Santa Cruz Biosciences): "The 8D7 monoclonal antibody recognizes Asialoglycoprotein receptor 1 (ASGPR 1), also known as Hepatic lectin H1 (HL-1). ASGPR 1 is an approximately 42 kDa type II integral membrane protein that is expressed on the surface of hepatic cells." H-2 MHC class I (BioLegend, M1/42): "The M1/42 antibody reacts with the H-2 MHC class I alloantigens expressed on nucleated cells from mice of the a, b, d, j, k, s, and u haplotypes (Stallcup, KC et al, 1981). MHC class I is involved in antigen presentation to T cells expressing CD3/TCR and CD8 proteins." All antibodies were validated for flow cytometry by the manufacturer.

Animals and other organisms

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

Laboratory animals	BL/6 (C57Bl/6J) mice were acquired from Jackson Laboratories. All mice used were females between 5 and 8 weeks old. The light cycle of the mouse housing room is from 7 am to 7 pm. Housing rooms are kept at ~ 70 F with ~ 30% average humidity.
Wild animals	None
Field-collected samples	None
Ethics oversight	All animal experiments were performed in accordance with the Georgia Institute of Technology's Institutional Animal Care and Use Committee (IACUC) policy.

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

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	In all cases, mice were sacrificed 1 day after administration of LNPs and immediately perfused with 20 mL of 1X PBS through the right atrium. The liver was isolated immediately following perfusion, minced with scissors, and then placed in a digestive enzyme solution with collagenase type I (Sigma Aldrich), collagenase XI (Sigma Aldrich), and hyaluronidase (Sigma Aldrich) at 37°C and 750 rpm for 45 minutes. Digested tissues were passed through a 70 µm filter and red blood cells were lysed. Cells were stained to identify specific cell populations and sorted using the BD FACS Fusion cell sorter at the Georgia Institute of Technology Cellular Analysis Core.
Instrument	BD FACS Fusion cell sorter
Software	The data were analyzed using FlowJo (BD Biosciences)
Cell population abundance	Greater than 1000.
Gating strategy	Cells were gated on the live/dead marker to identify live cells followed by FSC-A / SSC-A to identify populations. Singlets were gated on FSC-A / FSC-W. Cells types were gated by plotting CD31 against CD45.2 to identify endothelial cells and immune cells. Hepatocytes were gated as CD31-CD45-ASGPR1+ and Kupffer cells were gated as CD45+CD68+. See supporting figure 3 for more information.

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