

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☐ ☒ An indication of 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 statistics 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
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Bio-Rad CFX Manager 3.1, BD FACSDiva 8.0.2, PHERAstar 5.41, Seahorse XFe96 Analyzer, Xcalibur 4.0

Data analysis

FlowJo 10.5.2, Graphpad Prism Version 8.4.3, Microsoft Excel Version 16.16.25, Bio-Rad CFX Manager 3.1, ImageJ 1.50i, SCIEX 'variable window calculator' algorithm (<https://sciex.com/support/knowledge-base-articles/how-to-use-the-swath-variable-calculator-excel-sheet>), Analyst software 1.5.1 (for quantification of tryptophan) and 1.7 (for proteomics), ProteinPilot 5.0 (Revision 4769, Paragon Algorithm 5.0.0.0, 4767), Spectronaut v12 (12.020491.3.1543) software, Image J 1.50i, Tracefinder 2.1, MARS 3.32

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/reviewers upon request. 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

All data, materials, and protocols are available upon request.

Tabula Muris Senis database is accessible to the public at this link: <https://tabula-muris-senis.ds.czbiohub.org>

Proteomics data can be downloaded using the following link: <ftp://massive.ucsd.edu/MSV000083726> (MassIVE ID number: MSV000083726)

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All experiments were performed with a minimum of 3-4 biological replicates to perform statistical testing and to take into account biological variability. Animal experiments were performed using age-matched inbred male and female C57BL/6J mice or the indicated KO mice bred on a C57BL/6J background. For in vivo experiments sample sizes for each experiment is listed in the figure legends and methods section of the manuscript. Power analysis was not performed to determine cohort size, instead cohort size was determined by experiment type, availability of animals, and using standard cohort sizes in our field ranging from 4-12 mice/group for in vivo experiments, with the exception of Figure 7g which used 3 mice/group since this was a small pilot study to determine optimal kinetics of CD38 expression in response to LPS dose.
Data exclusions	A Grubbs' test was used to remove significant statistical outliers, $P < 0.05$, in the adipose and liver tissue data from Fig. 7i,j,k,l and Extended Data Fig. 7e,g,h (which are all derived from the same cohorts of mice), this criteria was pre-established and did not change the conclusion or interpretation of the results. Otherwise no data was excluded.
Replication	The majority of experiments were repeated at least three times and all attempts to replicate key findings were successful.
Randomization	In all in vivo experiments age-matched mice were randomized into control or treated groups. Randomization was not required for other experiments.
Blinding	Blinding was not performed since analysis of data was not impacted by investigator bias

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Antibodies

Antibodies used

The following primary antibodies were purchased from Cell Signaling and were used for Western blots: Tubulin (Clone:9F3, Cat# 2128, diluted 1:3000), PARP1 (Clone: 46D11, Cat#9532, diluted 1:1000), SIRT1 (Clone:D1D7, Cat# 9475, diluted 1:1000), SIRT2 (Clone:D4O5O, Cat# 12650, diluted 1:1000), SIRT3 (Clone:D22A3, Cat# 5490, diluted 1:1000), SIRT5 (Clone:D8C3, Cat# 8782, diluted 1:1000), SIRT6 (Clone:D8D12, Cat# 1248, diluted 1:1000), SIRT7 (Clone:D3K5A, Cat# 5360, diluted 1:1000), SOD2

(Clone:D9V9C, Cat# 13194, diluted 1:1000), Poly/Mono ADP ribosylated proteins, PARylation (Clone:E6F6A, Cat# 83732, diluted 1:1000) and H2AX (Clone:20E3, Cat#9718, diluted 1:1000). CD38 (Cat#AF4947, diluted 1:1000) and CD157 (Cat#AF4710, diluted 1:1000) from R&D Systems. Actin (Cat#A5441, diluted 1:3000) was from Sigma and NAMPT (Cat#A700-058-T, diluted 1:1000) from Bethyl Laboratories.

The following antibodies were diluted 1:200 and used for flow cytometry:CD38-FITC (Biolegend, Clone:90, Cat# 102705), CD11c-APC (Biolegend Clone:N418, Cat# 117309), CD11b-PECy7 (eBioscience, Clone: M1/70, Cat# 25-0112-82), CD45.2 PE (eBioscience, Clone:104, Cat# 12-0454-82), F4/80-BV510 (Biolegend, Clone:BM8,Cat# 123135). The following antibodies from Biolegend were diluted 1:200 and used for flow cytometry: CD4-BV650 (Clone:GK1.5, Cat# 100469); CD8α-Alexa488 (Clone:53-6.7,Cat# 100726) TCRβ-PerCP-Cy5.5 (Clone: H57-597, Cat# 109227) or TCRβ-PE (Clone: H57-597, Cat#109207); LY-6g-FITC (Clone:1A8, Cat# 127605); CD11b-PerCP-Cy5.5 (Clone:M1/70, Cat# 101227); CD62L-APC (Clone: MEL-14, Cat# 104411); CD44-APC-Cy7 (Clone:IM7, Cat# 103027); CD25-Pacific Blue (Clone:PC61, Cat# 102021); CD69-BV711 (Clone:H1.2F3, Cat# 104537); F4/80-BV510 (Clone: BM8, Cat# 123135); CD11c-Pacific Blue (Clone:N418, Cat#117321); Class II MHC (I-A/I-E)-APC-Cy7 (Clone: M5/114.15.2, Cat# 107627); NK1.1-BV650 (Clone:PK136, Cat# 108735); CD38-PE-Cy7 (Clone: 90, Cat# 102717).

For Immunofluorescence the sheep anti-CD38 polyclonal antibody (R&D Systems, Cat# AF4947, diluted 1:100), rabbit anti-F4/80 monoclonal antibody (Cell Signaling, Cat# 70076, diluted 1:100), were used for both single and co-staining. For p21 and F480+ co-staining the antibodies were: rat anti-F4/80 monoclonal antibody (Abcam, Cat# ab6640, diluted 1:200) and rabbit anti-p21 polyclonal antibody (Invitrogen, Cat# PA1-30399, diluted 1:200).

Validation

All antibodies have been validated according to the manufacturers and have been used/validated in other scientific publications. Western blot antibodies were further validated by detecting bands of the correct size and when possible using samples that lack the target protein as discussed in more detail below. Further validation of the CD38-FITC antibody (clone 90, Biolegend) was performed by comparing CD38 surface expression in WT and Cd38 KO BMDMs (See Figure 3A) as well as adipose tissue SVF cell preps from both WT and Cd38 KO mice via flow cytometry. CD38 and CD157 Western blot antibodies were validated for specificity using cell lysates from Cd38 KO and Cd157 KO mice (See Figure 3G). Western blot NAMPT antibody was validated for specificity by probing BMDMs derived from NAMPT Fl/Fl CreER mice, with partial deletion of NAMPT (Figure S2D). CD38 IF antibody was validated by probing liver tissue sections from Cd38 KO (See Figure 6B).

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

L929 cells used to make conditioned media to grow BMDMs were obtained from ATCC

Authentication

Cells were authenticated by ATCC. L929 cells were further validated by the ability of the supernatant from these cells to differentiate and grow mouse bone marrow derived macrophages.

Mycoplasma contamination

L929 stocks kept in the laboratory were analyzed for Mycoplasma contamination and tested negative

Commonly misidentified lines (See [ICLAC](#) register)

No

Animals and other organisms

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

Laboratory animals

Mice: Male and female C57BL/6J and Cd38 KO mice on a C57BL/6J background were used for in vivo studies. 6-12 week old C57BL/6J and Cd38 KO male mice were used as a source of BMDMs. Mice were maintained at the Buck Institute (though initially at UCSF-Gladstone Institute) on a standard chow diet, light cycle is 12:12 (6am-6pm), temperature throughout facility is set to be 68-72°F - the Verdin lab room (038) stays 70-72°F. The vivarium records daily humidity to make sure it stays within the allowable range of 30-70% - over the year it averages approx. 30-40% in the room used to house mice. All procedures were performed in accordance with Federal guidelines and those set forth by the Buck Institute and the UCSF Gladstone Institutional Animal Care and Use Committees (IACUC).

Cd38 KO mice on a C57BL/6J background were from The Jackson Laboratory (Bar Harbor, ME). Nampt Fl/Fl mice were developed by the laboratory of Oberdan Leo (Université Libre de Bruxelles), and shared by the laboratory of Shin-Ichiro Imai (Washington University). Bone marrows used in experiments comparing WT, Cd157 (Bst1) KO, Cd38 KO and Cd38/Cd157 DKO mice were generously provided by the laboratory of Katsuhiko Ishihara (Kawasaki Medical School).

In vivo experiments: Age-matched Male and female C57BL/6J mice were obtained from the National Institute on Aging colony and the Jackson Laboratory (Bar Harbor, ME). Colonies of old and young male and female Cd38 KO mice were and maintained at the Buck Institute. Male and female mice aged 2 to 30 months old were used for natural aging studies. As designated in the figure legends the following age, sex, and number of animals were used in the following in vivo experiments: Fig.4a, Extended Data Fig.4a: WT young male mice (6 month) n=7 mice/group, Cd38 KO young male mice (3 month) n=5 mice/group, WT old male mice (25 month) n=10 mice/group, and Cd38 KO old male mice (26 month) n=5 mice/group; Fig.4b, Extended Data Fig.4b: 6 and 25 month-old WT male mice. (WT young male mice (6 month) n=7 mice/group, WT old male mice (25 month) n=9 mice/group); Fig.4c,d: Young (3 month) and old (30 month) WT male mice. Each lane represents one mouse (Young n=7 mice/group, Old n=4 mice/group); Extended Data Fig.4c: Young (3 Month) and old (19 month) WT male mice, each lane represents one mouse (young n=4 mice/group, old n=4 mice/group); Extended Data Fig.4d: Young (3 month) and old (19 month) WT male mice. (young n=4 mice/group, old n=4 mice/group); Fig.4e, Extended Data Fig.4f: WT male mice for the indicated ages. (2 months n=6 mice/group, 6 months n=5 mice/group, 12 months n=5 mice/group, 18 months n=5 mice/group, 25+ months n=7 mice/group); Fig.4f:

Representative image from young (4 month) and old (26 month) male and female WT mice. Representative of 4-5 mice/group; Fig. 5a: young (3 months) and old (19 months) eWAT from WT male mice; Fig. 5b: young (4 month) and old (26 month) WT male mice; Fig. 5c, Extended Data Fig. 5a: 6 month-old WT male mice (PBS n=8 mice/group, Doxo n=7 mice/group); Fig. 5d, Extended Data Fig. 5b: 6 month-old WT male mice (PBS n=8 mice/group, Doxo n=8 mice/group); Fig. 6a: Young (4 month) and old (26 month) WT male and WT female mice (old n= 6M and 6F mice/group, young n= 6M and 6F mice/group), and Cd38 KO young (3 month) and old (26 month) male and female mice (old Cd38 KO n= 5M and 5F mice/group, young Cd38 KO n= 5M and 5F mice/group); Fig. 6b: Representative image from WT young (4 month), WT old (26 month) male mice, and young (3 month) Cd38 KO male mouse. Representative of 7-8 mice/group; Fig. 6c: Analysis of WT male old and young liver slides from 6b (each dot represents one slide), n=20 slides per mouse (Young n=8 mice/group, Old n=7 mice/group). Fig. 6j: Young (3 months) and old (19 months) WT male mice. Representative images of 2 out of 4 mice per group; Fig. 6k: 4-6 month old p16-3MR male mice treated with PBS (vehicle) n=7 mice/group, DOXO (vehicle) n=4 mice/group, and DOXO (GCV) n=4 mice/group; Fig. 7c,d and Extended Data Fig. 7a,b: 2 month-old WT male mice treated with LPS or PBS (PBS n=10 mice/group, LPS n=9 mice/group); Extended Data Fig. 7c: 3 month-old WT male mice injected with PBS or LPS for 4 weeks (PBS n=4 mice/group, LPS n=5 mice/group); Fig. 7e, Extended Data Fig. 7d: 3 month-old WT male mice injected with PBS or LPS for 4 weeks. (PBS n=4 mice/group, LPS n=5 mice/group); Fig. 7f: NAD and NADP concentrations are shown in 3 month-old WT male mice i.p. injected with PBS or LPS for 4-weeks (PBS n=5 mice/group, LPS n=5 mice/group); Fig. 7g: WT male mice (4 month) treated with 1mg/kg LPS or PBS over a 24-hour period (PBS n=3 mice/group, LPS n=3 mice/group); Fig. 7i,j,k,l and Extended Data Fig. 7e,g,h: 4 month-old WT and Cd38 KO male mice injected with PBS or LPS for 12 hours. n=10 mice/group; Extended Data Fig. 7f: 4 month-old WT and Cd38 KO male mice injected with PBS or LPS for 12 hours (n=3 mice/group), each lane represents one mouse.

In doxorubicin experiments 4-6 month-old male mice were injected i.p. with a single dose of 10 mg Doxorubicin (Sigma Aldrich)/kg or vehicle (PBS) and euthanized six weeks later. To eliminate p16-positive cells, p16-3MR male mice were i.p. injected with 25 mg/kg GCV for 5 consecutive days before euthanasia. For chronic LPS experiments, 2 month-old male C57BL/6J mice from The Jackson Laboratory were injected with 0.25mg/kg LPS or vehicle (PBS) twice weekly for four weeks (LPS EK-Ultrapur, Invivogen). For acute LPS experiments, 3-4 month-old male Cd38 KO mice bred in-house and aged-matched C57BL/6J control male mice from The Jackson Laboratory were injected with a single dose of 1mg/kg LPS or vehicle (PBS) (LPS EK-Ultrapur, Invivogen). All animals were euthanized by CO₂ followed by cervical dislocation. Epididymal fat and/or liver were excised and portions of these tissues were either fixed for microscopy, flash frozen in liquid nitrogen for qPCR/Western analysis or processed for flow cytometry. Aged-matched mice, but not littermates were used for in vivo experiments including those used to compare WT and Cd38 KO mice. All procedures were approved by the Buck Institute IACUC.

Wild animals

No wild animals were used in the study

Field-collected samples

No field collected samples were used

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Peripheral blood mononuclear cells (PBMCs) were purified from the blood of anonymous healthy donors (we specifically requested blood from healthy 20-30 year old males and females). This study only involved the use of human biological samples, obtained from a blood center (see below), that did not contain personal information about the identity of the patient and is therefore exempt from IRB approval.

Recruitment

Samples from anonymous healthy adult patients were obtained from Blood Centers of the Pacific, San Francisco, CA.

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

Sample preparation is detailed in the Materials and Methods "Flow Cytometry" section. Adipose tissue SVF cells (isolated as described above), were counted and blocked with anti-mouse FC-block (BD Biosciences) per the manufacturer's protocol for 15 minutes on ice. Cells were labelled on ice for 30 minutes with the following antibodies diluted 1:200: CD38-FITC (clone 90, Biolegend), CD11c-APC (N418, Biolegend), CD11b-PECy7 (M1/70, eBioscience), CD45.2 PE (clone 104, eBioscience), F4/80-BV510 (clone BM8, Biolegend). Cells were immediately analyzed using the BD FACSAria flow cytometer and the software BD FACSDiva 8.0.2 to acquire the data. Analysis of the data was performed using the FlowJo 10.5.2 software. For cell culture experiments, activated BMDMs were lifted from non-TC treated plates after activation using cold PBS with 5mM EDTA for 15 minutes prior to blocking with FC block and staining with antibodies as described above. Click-iT Edu 488 Flow Cytometry Kit (ThermoFisher) was used to measure macrophage proliferation as per manufacturers protocol. Leukocyte suspensions were prepared from mouse spleens by crushing, dissociation of cells by pipetting, straining through 40 micron nylon mesh, and ACK lysis. After 15 minutes, treatment with Fc block, surface staining with fluorochrome-conjugated antibodies was performed for 30 minutes on ice, at 1

microgram/mL for each antibody, in PBS with 2% FCS and 2mM EDTA, followed by fixation in PBS/1% PFA and analysis on a BD LSR II flow cytometer. Compensation and gating were performed using FlowJo 10.5.2.

Instrument

BD FACSAria flow cytometer and BD LSR II flow cytometer

Software

Data collection: BD FACSDiva 8.0.2. Data Analysis: FlowJo 10.5.2

Cell population abundance

Experiments analyzing cells derived from the stromal vascular fraction (SVF) of mouse visceral adipose tissue (Fig. 4e, Extended Data Fig. 4f, Fig. 5d, and Extended Data Fig. 5b) cell population abundance was measured as % Total SVF (% of live, single, non-autofluorescent events in the total SVF, based on the gating shown in Fig. S4D), and not total cell abundance/absolute number of cells since the amount of cells recovered can vary broadly depending on the weight of the tissue (due to the age of the animal or due to animal treatments). Thus we determined composition of the SVF was more informative in terms of the inflammatory state of the tissue than absolute number of cells recovered. In experiment 8c,d extended data fig. 8A, analysis of immune cells in mouse spleens, immune cell population abundance were graphed as amount number per 200,000 live, single splenocytes, based on the FSC/SSC gating shown in extended Data Fig. 7a.

Gating strategy

Gating strategy for Figure 4e, Extended Data Fig. 4, Figure 5d, and Extended Data Fig. 5b. Flow cytometry gating strategy to identify CD45+ immune cells from the stromal vascular fraction of eWAT. Cells were first gated on forward scatter (FSCA) vs side scatter (SSCA) to discard cell debris and dead or dying cells. Next FSCH (height) vs FSCA (Area) was used to select single cells. Single cells were then gated for auto-fluorescent using the Empty(E) BV421 vs BV711 channels (not used as antibody fluorophores) to discard cells that showed auto-fluorescence in these channels. Then CD45+ cells were selected and analyzed for CD38 and macrophage markers. Flow cytometry gating strategy to identify resident and non-resident macrophages from the stromal vascular fraction of eWAT, showing representative flow plots and histograms for the indicated ages of mice. Representative gating for the splenic leukocyte populations quantified in Figure 7c,d and Extended Data Fig. 7a. Left six panels show gating for identification of B cells and different myeloid cells, as indicated, as well as gating for CD38-positive cells in all populations. Right six panels show gating for T cell subsets, as indicated. Red arrows indicate sequential gating, pointing from parent plots towards child plots. For both the myeloid/B and T cell cells analysis, cells were first gated as shown on FSC area (FSCA) vs SSC area (SSCA) to identify intact cells larger than erythrocytes, followed by gating on FSC area vs. FSC height (FSCH) to identify single cells. CD38 expression was gated for all populations as shown in the lower-leftmost panel. For the myeloid/B analysis, single cells were gated on CD19 (B cells) vs. CD11b (myeloid//NK cells), and CD11b-positive cells further gated on LY-6G (granulocytes) vs. F4/80 (macrophages). Cells expressing neither of these population markers were then gated on NK1.1 (NK cells) vs. IaE (Class II MHC, a marker of myeloid cell activation). For the T cells, single cells were gated on TCR-beta vs. SSCA to identify ab T cells, followed by CD4 vs. CD8. Each of these T cell subsets was then gated on CD44 vs. CD62L, to identify naive and memory subsets as indicated.

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