

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 |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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	<p>Data collection were performed with the specific instrument softwares installed on the instruments, as detailed in the methods and below.</p> <p>Instruments for data collection:</p> <p>BD LSRFortessa X-20 Cell Analyzer,, BD Bioscience</p> <p>ABL800 FLEX blood gas analyzer, Radiometer America</p> <p>PhenoMaster, TSE Systems</p> <p>LF90II Minispec Body Composition Analyzer, Bruker</p> <p>Zeiss LSM Confocal Microscope with FLIM, Carl Zeiss</p> <p>Azure 600 Imaging System, Azure Biosystems</p> <p>Nipro TRUEtrack Blood Glucose Meter ,Amazon Cat# B0727V3XQX</p> <p>VictorX4 luminescence detector, Perkin Elmer Cat# 2030-0040</p> <p>MultiScan LFER 150 large bore research PET-CT, Mediso</p> <p>LC-ESI-MS/MS, 5500 QTRAP, ABI Sciex</p> <p>LS6500 Scintillation Counter, Beckman Coulter</p> <p>Z-Theta, Applied Biophysics</p> <p>Tecan M1000, Tecan</p> <p>CFX Opus 96 Real-Time PCR Detection System, BioRad</p> <p>Seahorse XFe24 Analyzer, Agilent Technologies</p> <p>T100 PCR thermal cycler, BioRad</p> <p>MicroBeta2 plate counter ,Perkin Elmer</p>
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Aquisition and data collection/analysis software:

ConSurf Server¹, Ashkenazy et al., 2016 <https://consurf.tau.ac.il/>
 CHARMM-GUI Membrane Builder Jo et al., 2008 <http://www.charmm-gui.org/>
 AutoDock Vina ,Trott & Olson, 2010 <http://vina.scripps.edu/>
 VMD, Humphrey et al., 1996 <https://www.ks.uiuc.edu/Research/vmd/>
 PyMOL Molecular Graphics System, Schrödinger <https://pymol.org/>
 NMAD 3.0 Simulation Package,
 GraphPad Prism 10 ,GraphPad Software <https://www.graphpad.com/>
 FlowJo v10.8.1 BD, Biosciences <https://www.flowjo.com/>
 InterView FUSION Software ,Mediso <https://www.mediso.com/>
 CalR Metabolic Analysis Tool, Mina et al., Cell Metab. 2018 <https://calrapp.org/>
 Applied BioPhysics – ECIS Software v1.2.186.0 ,PC Applied Biophysics
 BD FACSDiva 8.0, BD BioScience
 Analyst 1.6 software, ABI Sciex

Data analysis

Data analysis was performed with software described in the methods section and above. For statistical analysis and data representation GraphPad Prism 10 (Graph pad) was used.

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 data is available in the main text or the supplementary materials. MD simulation trajectory files were deposited at <https://zenodo.org/records/18765542>. Source data are provided with this paper. The structure of SPNS2 in Figure 6g was created using 3D Protein Imager. Plasmids and cell lines can be requested from, and requests will be fulfilled by Dr. Cynthia Weigel.

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](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

All n numbers as biological replicates or individual animals are given in the figure legend. Sample size estimation was based on previous experience or are standard in the field.

Data exclusions

No data was excluded from analysis except data for one animal for metabolic phenotyping (Food pellets were displaced into the cage. Therefore, analysis of food consumption, locomotor activity etc. was not reliable)

Replication	All experiments were repeated and the number of independent repeats is given in the figure legend of each figure panel. The number of independent repeats is given as N.
Randomization	In all experiments mice were randomly allocated according to their genotype.
Blinding	Investigators were blinded during data collection with blood gas analyzer and in metabolic chamber experiments, as well as experiments to determine body weights and blood glucose, triglycerides, cholesterol, phospholipids and non-esterified fatty acids, insulin and glucagon after oral gavage of glucose, analyzing mass spectrometry data and qPCR analysis in cells. Genotypes of mice or cell identity were uncovered after final results were obtained. In remaining cell-based and in vitro assays the cell type and treatments or protein identities were known during data acquisition as this was essential for proper execution of the experiments.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Rabbit anti-SPNS2, Proteintech Cat# 25642-1-AP
 Anti-SPNS2 antibody, Abcam Cat# ab82629
 Primary antibody: SPNS2, Abcam Cat# ab59972
 Primary antibody: SPNS2, FabGennix Cat# SPNS2-101AP
 Anti-SPNS2 antibody, MyBioSource Cat# MBS5400801
 SPNS2 blocking peptide, MyBioSource Cat# MBS5400654
 Mouse anti-β-actin, Sigma-Aldrich Cat# A5441
 Rabbit anti-phospho-ERK1/2 (Thr202/Tyr204), Cell Signaling Technology Cat# 9101
 Rabbit anti-ERK1/2, Cell Signaling Technology Cat# 9102
 Rabbit anti-GLUT1 (FITC conjugated), Abcam Cat# ab40084
 Goat anti-rabbit IgG-HRP, Jackson ImmunoResearch Cat# 111-035-003
 Goat anti-mouse IgG-HRP, Jackson ImmunoResearch Cat# 115-035-003
 Anti-TurboGFP Nanobody, ChromoTek Cat# tbtak-20
 Anti-TurboGFP Nanobody VHH-rabbit Fc fusion, ChromoTek Cat# tbfbrb-100
 Anti-SPNS2 antibody, Abcam Cat# ab82629
 Anti-TurboGFP antibody, Origene Cat# TA150041
 Alexa Fluor 555 secondary antibody, Invitrogen Cat# A21428
 Alexa Fluor 488 secondary antibody, Invitrogen Cat# A11001
 anti-GLUT1 (extracellular)-FITC, Alomone Labs Cat# AGT-041-F
 rabbit IgG Isotype Control-FITC, Alomone Labs Cat# RIC-001-F
 anti-CD3ε, BioLegend Cat# 100301
 Anti-CD28, BioLegend Cat# 102101
 Anti-PP2A Antibody, C subunit, clone 1D6, Sigma Aldrich Cat# 05-421

Validation

All antibodies were validated by the supplier for the intended usage (Western Blot, Immunofluorescence, Flow cytometry). For SPA and thermal shift assays, the specificity of the anti-Turbo GFP nanobody ((#tbfbrb-100, ChromoTek) was validated by negative controls (buffer only) and positive controls (Vector only, TurboGFP-Glut1).

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

SVEC4-10, A549 were obtained from ATCC (CRL-2181, CCL-185). SV-LEC were provided by Dr. J.S. Alexander (Shreveport, LA, USA) and described previously (Ando T, et al. Isolation and characterization of a novel mouse lymphatic endothelial cell line: SV-LEC. *Lymphat Res Biol.* 2005;3(3):105-15. doi: 10.1089/lrb.2005.3.105. PMID: 16190815)

Authentication

Certificate of authentication was obtained by ATCC for SVEC4-10 and A549. SV-LEC were not authenticated.

Mycoplasma contamination	Cell lines were tested negative for mycoplasma before cryo stock preservation of early passages. No mycoplasma testing was carried out during the experiments.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell 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	<p>The generation of Spns2tm1a(KOMP)Wtsi (referred to as tm1a/tm1a), Lyve1tm1.1(EGFP/cre)Cys/J (referred to as Lyve1cre) and Spns2tm1c(KOMP)Wtsi (referred to as tm1c/tm1c) mice have been described previously. Spns2 global knockout mice, Spns2-/- or Spns2tm1d(KOMP)Wtsi (referred to as tm1d/tm1d) were derived from germline recombination generated by crossing Spns2tm1c/tm1c mice with Cre recombinase mice resulting in globally deleted Spns2tm1d/tm1d mice. Male (wt/tm1d; Cre-/-) were back-crossed with female (wt/tm1d; Cre-/-). As reported by others, the global deletion of SPNS2 led to reduced survival rates. Therefore, adhering to the 3R principles of animal welfare, the first generations of homozygous Spns2-/- and heterozygous Spns2+/- breedings were used to generate global knock-out and wild type mice for experiments (aged 12-23 weeks). Global knock-out mice derived from both homozygous and heterozygous breedings with no significant differences in the measured outcomes were allocated in equal numbers together with wild-type littermates obtained from heterozygous breedings. Mice were housed in a pathogen-free VCU vivarium under automatically controlled 12 h day-night cycles, temperature at 21 – 23 °C and 50 – 60% humidity. Mice were fed a standard diet (Control diet 7012 (Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet) and water ad libitum.</p> <p>Mus musculus Spns2tm1a(KOMP)Wtsi, KOMP Repository, RRID:MMRRC_048327-UCD Mus musculus Lyve1tm1.1(EGFP/cre)Cys/J, The Jackson Laboratory, Stock# 012601 Mus musculus Spns2tm1c(KOMP)Wtsi, KOMP Repository, RRID:MMRRC_048327-UCD Mus musculus Spns2tm1d/tm1d, This study</p>
Wild animals	This study did not involve wild animals.
Reporting on sex	Both gender were used for all experiments except metabolic chamber and PET-CT scans. For metabolic phenotyping and PET-CT scans only male mice were used due to low animal numbers in these experiments. No significant sex-based differences were observed in the measured outcomes. For individual experiments, the ages and numbers of males and females were matched to ensure consistency in the results.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All experimental procedures were approved by the VCU Institutional Animal Care and Use Committee that is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (IACUC protocol number AD10000996)

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

Single cell suspensions of Spns2 overexpressing or knock-out cells were centrifuged (300xg, 10 min) and washed with flow buffer (PBS with 2 mM EDTA and 0.5% BSA). After centrifugation, the cell pellet was resuspended in flow buffer and two vials per sample each with 10^5 cells were aliquoted. One Aliquot was stained with 5 μ g anti-GLUT1 (extracellular)-FITC, the other with 5 μ g rabbit IgG Isotype Control-FITC for 30 min at room temperature. After washing, cells were then fixed with FluoroFix Buffer. Cells were washed and resuspended in flow buffer for analysis with a BD LSRFortessa X-20 Cell Analyzer equipped with BD FACSDiva 8.0 software for acquisition. As a positive control, primary T lymphocytes from WT animals were isolated from the spleen with a combination of CD4 (L3T4) and CD8a (Ly-2) MicroBeads and magnetic-activated cell sorting. Isolated T lymphocytes were left untreated or stimulated with surface-coated monoclonal anti-CD3 ϵ (5 μ g/mL diluted in PBS, incubated for 2 h at 37°C for immobilization) and 1.7 μ g/mL monoclonal soluble anti-CD28 for 24 h. Cells were then stained and analyzed as described above.

Instrument

BD LSRFortessa X-20 Cell Analyzer (BD Bioscience)

Software

BD FACSDiva 8.0 software for acquisition. FlowJo version 10.8.1 (BD Bioscience) for analysis.

Cell population abundance

Cells of interest were first selected by FSC/SSC selection. A minimum number of 10,000 cells were collected.

Gating strategy

Cells were gated on forward/side scatter, followed by doublet exclusion in FSC-A/FSC-H, and analyzed for Glut1 FITC surface expression.

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