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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Open source software: µManager 1.48v (MM core version 8.4.0; MM studio version 1.4.22) for acquisition of mitochondrial transport; Seahorse XFe96 Wave, Seahorse Bioscience, Inc., (version 2.3.0.19.) for oxygen consumption measurements. Commercial software: for Q-Exactive Tune (2.8 SP1 build 2806) & XCalibur (version 3.1.66.10), Thermo Fisher Scientific for mass spectrometry; for Q-Exactive HF Tume 2.8 (SP1 build 2806) & XCalibur (version 4.0.27.19), Thermo Fisher Scientific for mass spectrometry; Summit software, Dako for flow cytometry; FUSION-CAPT Advance FX7, Vilber Lourmat (version 16.12) for Western blot development; FV10-ASW, Olympus (version 4.2) for confocal microscopy; FV31S-SW, Olympus for confocal microscopy; TEM Center, Jeol Tokyo for electron microscopy; CLARIOstar (software version 5.20 R5) for calcium uptake assay.
Data analysis	Open source software: ImageJ/Fiji (version 1.51u) for processing of images; Seahorse XFe96 Wave, Seahorse Bioscience, Inc., (version 2.3.0.19.) for processing of oxygen consumption measurements; GraphPad (version 7) for statistics and figure representation; Microsoft Excel 2016 (version 16.0.6741.2048)for statistics; Image Studio Lite, Licor (version 5.2.5) for Western blot analysis; MaxQuant (version 1.5.1.1; version 1.5.5.1) for processing of mass spectrometry; Perseus (version 1.6.1.1.) for analysis of proteomic data and statistics. Open source online tools: WebGestalt 2017 (update 1/27/2017) for analysis of proteomic data; Kegg mapper (version v3.1 released October 1, 2017) for analysis of proteomic data; Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) for figure representation. Commercial software: FlowJo software (version 10.5), TreeStar (version 10) for analysis of flow cytometry data; Adobe Photoshop CS5, (version 12.0) for image processing; Adobe Illustrator CS5, (version 15.0.0) for figure representation.

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. 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 proteomic data generated within this study are deposited to the ProteomeXchange Consortium via the PRIDE44 partner repository with the dataset identifiers: PXD010772, PXD010774, PXD010781 and PXD013380. Additionally, Supplementary Data Table 1 provides information on the proteomics data analysis used for the conclusions in the main and supplementary figures.
The MitoTag mouse line will be available from The Jackson Laboratory as JAX#032675 (Rosa26-CAG-LSL-GFP-OMM). The McuFL/FL and the Rbp4:Cre mouse strain are protected under a material transfer agreement with the MMRRC (C57BL/6N-Mcutm1a(EUCOMM)Hmgu/H; Tg(Rbp4-cre)KL100Gsat/Mmucd), and the Rmdn3 -/- mouse strain is protected under a material transfer agreement with the KOMP Repository (KO mouse project 3U01HG004080).

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	No statistical methods were used to pre-determine sample sizes but the chosen sample sizes are similar to those reported in previous publications (e.g. Brill et al. Neuron 2016; Baughman et al., Nature 2011 ; Pagliarini et al. Cell, 2008).
Data exclusions	Exclusion of data points was only performed in oxygen consumption measurements for technical replicates in which one of the multiple injections failed during the experiment; no other data points were excluded.
Replication	All attempts to replicate were successful. To ensure reproducibility experiments were independently repeat at least three times with the following exceptions: Figure 7b: data is representative for two biological replicates with N=160/133/120 mitochondria from N=10/72/16 individual cells. Supplementary Figure 3d: data is representative for four biological replicates with N=88/83 NMJ areas and N=56/54 AChR density measurements.
Randomization	Samples were not randomized during data collection and animals assigned to experimental groups according to genotype. Randomization was not possible due to constraints in animal availability, and determination of experiment by given genotype.
Blinding	Blinding was applied to the following experiments: Supplementary Figure 3a: Mitochondrial transport measurements were analyzed by blinded investigator. Figure 1g & Supplementary Figure 3e: Isolation were performed by C.F. with information on genotype. Further oxygen consumption measurements and analysis were performed by L.T. in a blinded way. Figure 7b and 7e: Data were acquired by a blinded investigator with no information on genotypes. For analysis, it was not possible to blind the investigator for the different cell types (PC, GC, A) due to unique structural features.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Name : Supplier , Catalog # , clone (lot); Dilution:

Acads : Abcam , ab156571 , EPR10862(B) (lot: GR117677-4); Dilution: IF - 1: 200 ;
Ak3 : Santa Cruz , sc-398571 , E-5 (lot: E1616); Dilution: IF - 1: 100 ;
Ak4 : Santa Cruz , sc-271161 , A-9 (lot: A0512); Dilution: WB - 1: 100 ; IF - 1: 100 ;
Amyloid-β (1-16)-AF647 : BioLegend , 803020 , 6E10 (lot: B250539); Dilution: IF - 1: 500 ;
Amyloid-β (17-24) : BioLegend , 800712 , 4G8 (lot: NaN); Dilution: IF - 1: 1000 ;
Amyloid-β (8-17) : Dako , M0872 , 6F/3D (lot: 00086092); Dilution: IF - 1: 100 ;
Anti-chicken IgY-Alexa Fluor 488 : ThermoScientific , A-11039 , - (lot: 1599396, 1899514); Dilution: IF - 1: 1000 ;
Anti-chicken IgY-Alexa Fluor 647 : ThermoScientific , A-21449 , - (lot: 1622573); Dilution: IF - 1: 1000 ;
Anti-mouse IgG1-Alexa Fluor 594 : ThermoScientific , A-21125 , - (lot: 41652A); Dilution: IF - 1: 1000 ;
Anti-mouse IgG-Alexa Fluor 647 : Jackson Immunoresearch , 715-605-151 , - (lot: 127051); Dilution: IF - 1: 2000 ;
Anti-mouse IgG-HRP : BioRad , 1706516 , - (lot: NaN); Dilution: WB - 1: 5000 ;
Anti-rabbit IgG-Alexa Fluor 555 : ThermoScientific , A-31572 , - (lot: 1806147); Dilution: IF - 1: 2000 ;
Anti-rabbit IgG-Alexa Fluor 568 : ThermoScientific , A-11011 , - (lot: NaN); Dilution: IF - 1: 1000 ;
Anti-rabbit IgG-Alexa Fluor 594 : ThermoScientific , A-11012 , - (lot: 1310680); Dilution: IF - 1: 1000 ;
Anti-rabbit IgG-HRP : BioRad , 1706515 , - (lot: 64170140); Dilution: WB - 1: 5000 ;
ATP5a : Abcam , ab14748 , 15H4C4 (lot: GR303070-4); Dilution: WB - 1: 10000 ;
Beta-Actin : Sigma , A2228 , AC-74 (lot: 058M4808V); Dilution: WB - 1: 4000 ;
Calreticulin : CellSignaling , #12238 , D3E6 (lot: 0004); Dilution: WB - 1: 1000 ;
Catalase : Rockland , 100-4151 , - (lot: 18043); Dilution: IF - 1: 200 ;
ChAT : Sigma , AB144P , - (lot: 3167107); Dilution: IF - 1: 500 ;
Cox4i1 : Abgent , AP22111a , RB55992 (lot: SA160128HA); Dilution: WB - 1: 1000 ; IF - 1: 200 ;
Cpt1a : ProteinTech , 15184-1-AP , - (lot: 00052905); Dilution: IF - 1: 50 ;
CypD : Abcam , ab110324 , E11AE12BD4 (lot: GR316345-2); Dilution: WB - 1: 5000 ;
Emre (C22orf32) : Santa Curz , sc-86337 , C-12 (lot: K0415); Dilution: WB - 1: 500 ;
Gfap : Abcam , ab4674 , - (lot: GR1473-13); Dilution: IF - 1: 2000 ;
GFP : Abcam , ab13970 , - (lot: GR236651-23); Dilution: IF - 1: 1000 ;
GFP : Santa Cruz , sc-9996 , B-2 (lot: K1815); Dilution: WB - 1: 500 ;
Gldc : Sigma , HPA002318 , - (lot: C106148); Dilution: WB - 1: 1000 ; IF - 1: 400 ;
Gls (KGA) : ProteinTech , 20170-1-AP , - (lot: 00012602); Dilution: IF - 1: 200 ;
Gls2 (C-term E513) : Abgent , AP6650d , RB20072 (lot: SA101018BU); Dilution: IF - 1: 400 ;
Got2 : Abcam , ab171739 , EPR12354(B) (lot: GR170733-1); Dilution: WB - 1: 1000 ; IF - 1: 400 (human: 1:2000) ;
ImmPRESS® HRP Anti-Rabbit IgG (Peroxidase) Polymer Detection Kit : Vector Lab , MP-7451 , - (lot: ZD0602); Dilution: IF - ready-to-use ;
Lamin B1 : Abcam , ab16048 , - (lot: 429044); Dilution: WB - 1: 1000 ;
Ldhd : Abcam , ab182146 , EPR15068(B) (lot: GR152139-1); Dilution: IF - 1: 100 ;
MaoB : Santa Cruz , sc-515354 , D-6 (lot: C0816); Dilution: IF - 1: 100 ;
Mavs : CellSignaling , #4983 , - (lot: 0003); Dilution: IF - 1: 200 ;
Mcu : Sigma , HPA016480 , - (lot: CD114474); Dilution: WB - 1: 1000 ; IF - 1: 400 (human: 1:200) ;
Mouse IgG1-APC : Miltenyi Biotec , 130-113-758 , IS5-21F5 (lot: NaN); Dilution: WB - 1: 25 ;
NeuN : Sigma , MAB377 , A60 (lot: 2742283); Dilution: IF - 1: 100 ;
NeuN-488 : Abcam , ab190195 , EPR12763 (lot: GR3233763-2); Dilution: IF - 1: 50 ;
Nipsnap1 : CellSignaling , #13226 , D1Y6S (lot: 0001); Dilution: IF - 1: 200 ;
Ociad2 : Sigma , HPA040979 , - (lot: C91827); Dilution: WB - 1: 1000 ; IF - 1: 200 (human: 1:500) ;
Ociad2 : Abcam , ab91576 , (lot: GR265190-2); Dilution: IF - 1: 200 ;
Pex14 : Denis Crane ; (lot: NaN); Dilution: IF - 1: 1000 ;
Pptc7 : Abcam , ab122548 , - (lot: GR134884-4); Dilution: IF - 1: 200 ;
Rabbit IgG zenon AlexaFluor 647 Kit : ThermoScientific , Z25308 , - (lot: 2021736); Dilution: IF - 10 microl/1 microg antibody ;
Rmdn3 (Fam82a2) : Abcam , ab189845 , - (lot: GR26796-1); Dilution: WB - 1: 2000 ; IF - 1: 400 (human: 1:2000) ;
Sfxn5 : Abcam , ab172971 , EPR9532 (lot: YK020618C5); Dilution: WB - 1: 2000 ; IF - 1: 500 ;
SNAP25 : CellSignaling , #5308 , D7B4 (lot: 0001); Dilution: WB - 1: 2000 ; ;
Snph : Abcam , ab192605 , EPR14115(2) (lot: GR300418-5); Dilution: IF - 1: 200 ;
Tom22-APC : Miltenyi Biotec , 130-107-733 , 1C9-2 (lot: NaN); Dilution: WB - 1: 25 ;
Tomm20 : Abcam , ab78547 , - (lot: GR3199811-3); Dilution: WB - 1: 1000 ;
tRFP : Evrogen , AB234 , - (lot: 23301301013); Dilution: WB - 1: 5000 ;
Tst : Abcam , ab166625 , EPR11646(B) (lot: YJ1120015); Dilution: IF - 1: 500 (human: 1:2000) .

Validation

Name (Supplier , Catalog #): Validation ;

Acads (Abcam , ab156571): Data sheet: mouse, IF; Ref: doi: 10.1111/jcmm.12828 ;
Ak3 (Santa Cruz , sc-398571): Data sheet: mouse, IF ;
Ak4 (Santa Cruz , sc-271161): Data sheet: mouse, IF ;
Amyloid-β (1-16)-AF647 (BioLegend , 803020): Data sheet: human, Tg-mouse, IF; Ref:doi: 10.1073/pnas.0813404106; PMID: 10028915 ;
Amyloid-β (17-24) (BioLegend , 800712): Data sheet: human, Tg-mouse, IF; Ref: PMID: 10028915 ;
Amyloid-β (8-17) (Dako , M0872): Data sheet: human, IHC; Ref: PMID: 10028915 ;
Anti-chicken IgY-Alexa Fluor 488 (ThermoScientific , A-11039): RRID:AB_2534096; 168 citations ;
Anti-chicken IgY-Alexa Fluor 647 (ThermoScientific , A-21449): RRID:AB_2535866; 40 citations ;
Anti-mouse IgG1-Alexa Fluor 594 (ThermoScientific , A-21125): RRID:AB_2535767; 22 citations ;
Anti-mouse IgG-Alexa Fluor 647 (Jackson Immunoresearch , 715-605-151): RRID:AB_2340863; 26 citations ;

Anti-mouse IgG-HRP (BioRad , 1706516): RRID: AB_11125547; Ref: doi: 10.1016/j.cell.2016.07.041; WB, rat ;
Anti-rabbit IgG-Alexa Fluor 555 (ThermoScientific , A-31572): RRID:AB_162543; 115 citations ;
Anti-rabbit IgG-Alexa Fluor 568 (ThermoScientific , A-11011): RRID:AB_143157; 251 citations ;
Anti-rabbit IgG-Alexa Fluor 594 (ThermoScientific , A-11012): RRID:AB_2534079; 215 citations ;
Anti-rabbit IgG-HRP (BioRad , 1706515): RRID:AB_2617112; Ref: doi: 10.1016/j.cell.2016.07.041; WB, rat ;
ATP5a (Abcam , ab14748): Data sheet: mouse, WB; Ref: doi: 10.1111/acel.12715 ;
Beta-Actin (Sigma , A2228): Data sheet: mouse, WB ;
Calreticulin (CellSignaling , #12238): Data sheet: mouse, WB ;
Catalase (Rockland , 100-4151): Data sheet: mouse, IF ;
ChAT (Sigma , AB144P): Data sheet: mouse, WB ;
Cox4i1 (Abgent , AP22111a): Data sheet: mouse, IF ;
Cpt1a (ProteinTech , 15184-1-AP): Data sheet: mouse, IF; Ref:doi: 10.1038/emm.2017.243 ;
CypD (Abcam , ab110324): Data sheet: mouse, WB; KO validation: Ref: doi: 10.1016/j.expneurol.2009.02.015 ;
Emre (C22orf32) (Santa Curz , sc-86337): Data sheet: mouse, WB; Ref: doi: 10.1016/j.celrep.2017.02.032 ;
Gfap (Abcam , ab4674): Data sheet: mouse, human, IF; Ref: doi: 10.1177/1759091415601636 ;
GFP (Abcam , ab13970): Data sheet: mouse, IF ;
GFP (Santa Cruz , sc-9996): Data sheet: mouse, WB ;
Gldc (Sigma , HPA002318): Data sheet: mouse, IF; siRNA KD validation ;
Gls (KGA) (ProteinTech , 20170-1-AP): Data sheet: mouse, IF; Ref:doi: 10.1038/s41598-017-16327-z ;
Gls2 (Abgent , AP6650d): Data sheet: mouse, IF ;
Got2 (Abcam , ab171739): Data sheet: mouse, IF; Ref:doi: 10.1186/s13024-017-0219-3. ;
ImmPRESS® HRP Anti-Rabbit IgG (Peroxidase) Polymer Detection Kit (Vector Lab , MP-7451): RRID:AB_2336529; 7 citations;
Ref: doi: 10.1172/JCI92300.; mouse, IF ;
Lamin B1 (Abcam , ab16048): Data sheet: mouse, WB; KO validation in cells ;
Ldhd (Abcam , ab182146): Data sheet: mouse; human, WB. ;
MaoB (Santa Cruz , sc-515354): Data sheet: mouse, IF ;
Mavs (CellSignaling , #4983): Data sheet: mouse, IF ;
Mcu (Sigma , HPA016480): Data sheet: mouse, IF; KO validation: This study ;
Mouse IgG1-APC (Miltenyi Biotec , 130-113-758): RRID:AB_2733439; Ref: doi: 10.1016/j.ab.2009.02.040; isolation, FACS, mouse ;
NeuN (Sigma , MAB377): Data sheet: human, IF ;
NeuN-488 (Abcam , ab190195): Data sheet: human, IF; Ref: doi: 10.1152/ajpgi.00371.2016 ;
Nipsnap1 (CellSignaling , #13226): Data sheet: human, mouse, WB ;
Ociad2 (Sigma , HPA040979): Data sheet: human, IF; human, WB ;
Ociad2 (Abcam , ab91576): Data sheet: mouse, human, WB ;
Pex14 (Denis Crane , -): Ref: doi: 10.1242/jcs.02776: mouse, WB, IF ;
Pptc7 (Abcam , ab122548): Data sheet: mouse, IF ;
Rabbit IgG zenon AlexaFluor 647 Kit (ThermoScientific , Z25308): RRID:AB_2736962; Ref: doi: 10.1128/JVI.01457-17; mouse, IF ;
Rmdn3 (Fam82a2) (Abcam , ab189845): Data sheet: mouse, IF; KO validation: This study ;
Sfxn5 (Abcam , ab172971): Data sheet: mouse, human, WB ;
SNAP25 (CellSignaling , #5308): Data sheet: mouse, WB ;
Snph (Abcam , ab192605): Data sheet: mouse, IF ;
Tom22-APC (Miltenyi Biotec , 130-107-733): RRID:AB_2654220; Ref: doi: 10.1016/j.ab.2009.02.040; isolation, FACS, mouse ;
Tomm20 (Abcam , ab78547): Data sheet: human, WB, IF ;
tRFP (Evrogen , AB234): Data sheet: mouse, WB ;
Tst (Abcam , ab166625): Data sheet: mouse, IF .

Animals and other organisms

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

Laboratory animals	Male and female animals of the species Mus musculus/C57BL/6 (mixed N/J) background were used between 6 weeks to 6 month.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field-collected damples.
Ethics oversight	All animal experiments were approved by the responsible regulatory agencies (Regierung von Oberbayern).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Male and female patients, 52-91 years of age; three groups: no relevant diagnosis; Alzheimer's disease, ALS
Recruitment	Tissue selected from archival autopsy material.
Ethics oversight	Use of human samples was in accordance with institutional ethical guidelines and approved by the ethics committee of the University of Geneva (Switzerland).

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	The crude mitochondrial fraction (CMF) from 20 mg mouse cortex samples was resuspended in 100 µl 4% PFA/PBS and incubated in the dark for 5 minutes at room temperature. Samples were washed three times with PBS and resuspended in 100 µl. 20 µl fixed mitochondria were used per staining. First the sample was washed once with 0.5% BSA/PBS and then incubated with APC-conjugated antibodies: IgG1 or Tom22 (Miltenyi Biotec, 1:25) in the dark, 4°C, 60 rpm for 60 minutes. Samples were washed three times with 0.5%BSA/PBS and finally resuspended in 100 µl PBS.
Instrument	Samples were acquired on a CyAn ADP 9 flow cytometer (Beckman Coulter) with the following laser lines: 405, 488, 642 nm.
Software	Data was acquired with the Summit software (Dako) and further analyzed with FlowJo, TreeStar (version 10).
Cell population abundance	No sorting was performed.
Gating strategy	To allow for optimal identification and separation of small particles, the flow rate was adjusted accordingly and optical parameters were scaled logarithmically. The gate was adjusted to exclude a PBS+BSA probe (buffer only; negative) in each experiment. Mitochondria were gated by FSC and SSC, as well as pulse width to discriminate doublets. Then Tom22-APC staining (APC, 665/20) intensities were gated against IgG1-APC control samples to define mitochondria (on average 34.27±1.62%). From this, individual gates for GFP-OMM (FITC, 530/40) and mito-RFP (PE, 575/25) were adjusted according to wild type mitochondria (Tom22+).

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