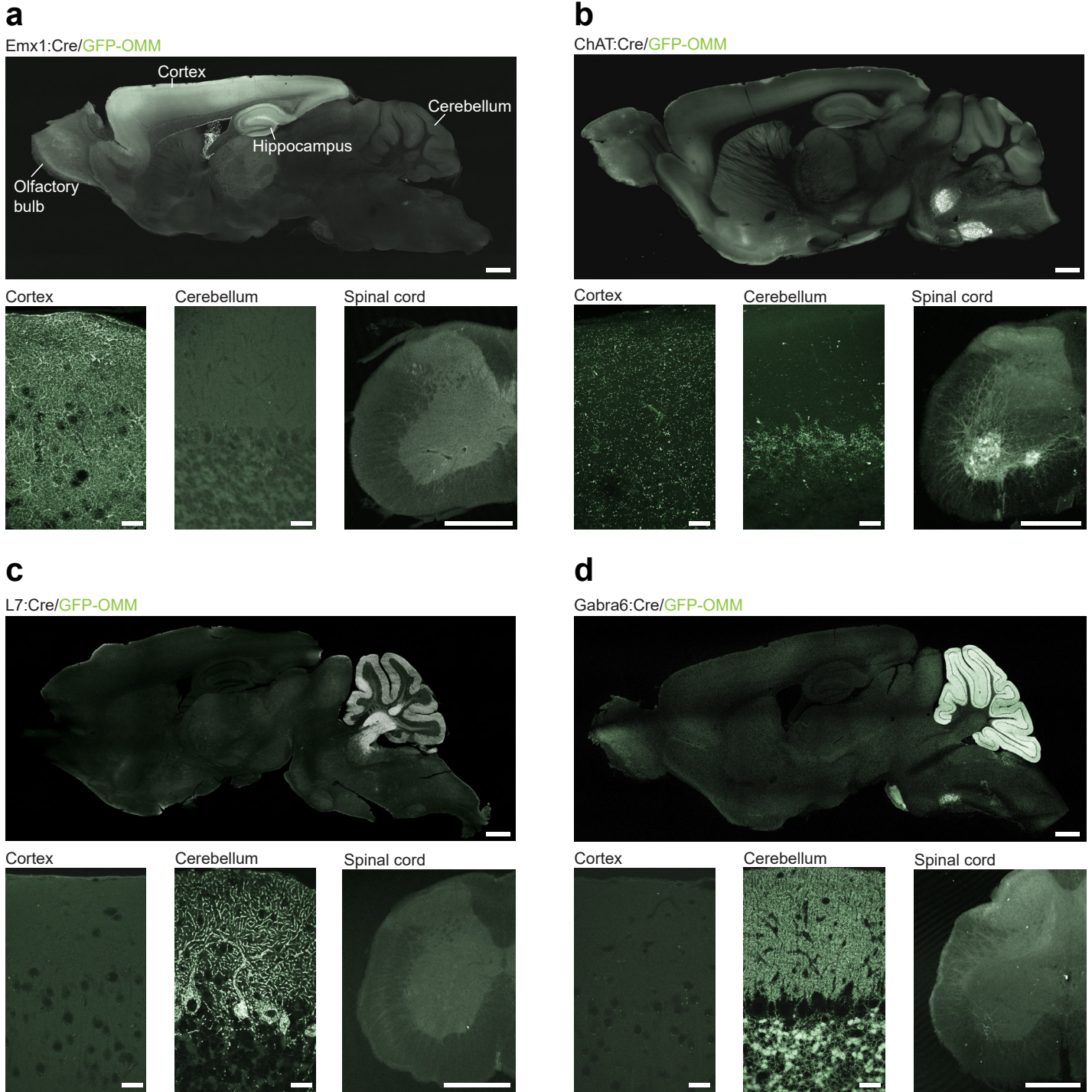


In the format provided by the authors and unedited.

Cell-type-specific profiling of brain mitochondria reveals functional and molecular diversity

Caroline Fecher^{1,2,3,18}, Laura Trovò^{1,2,18}, Stephan A. Müller^{2,4}, Nicolas Snaidero^{1,2}, Jennifer Wettmarshausen^{5,6}, Sylvia Heink⁷, Oskar Ortiz^{8,9,15}, Ingrid Wagner¹⁰, Ralf Kühn^{8,9,16,17}, Jana Hartmann¹¹, Rosa Maria Karl¹¹, Arthur Konnerth^{11,12,13}, Thomas Korn^{7,12}, Wolfgang Wurst^{2,8,9,12}, Doron Merkler^{10,14}, Stefan F. Lichtenthaler^{2,4,12}, Fabiana Perocchi^{5,6,12} and Thomas Misgeld^{1,2,12,13*}

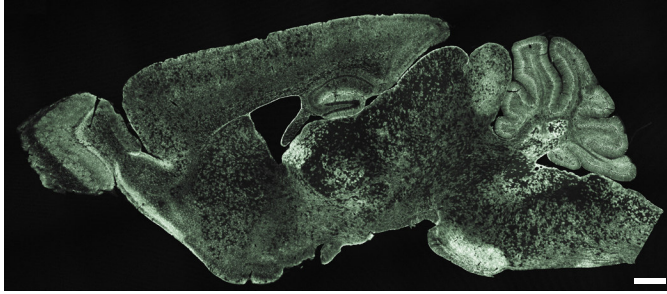
¹Institute of Neuronal Cell Biology, Technical University of Munich, Munich, Germany. ²German Center for Neurodegenerative Diseases, Munich, Germany. ³Graduate School of Systemic Neurosciences, Ludwig-Maximilians University, Munich, Germany. ⁴Neuroproteomics, School of Medicine, Klinikum rechts der Isar and Institute for Advanced Study, Technical University of Munich, Munich, Germany. ⁵Gene Center Munich, Ludwig-Maximilians University, Munich, Germany. ⁶Institute for Diabetes and Obesity, Helmholtz Zentrum München, Munich, Germany. ⁷Experimental Neuroimmunology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany. ⁸Institute of Developmental Genetics, Helmholtz Zentrum München, Munich, Germany. ⁹Institute of Developmental Genetics, Technical University of Munich, Munich, Germany. ¹⁰Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland. ¹¹Institute of Neuroscience, Technical University of Munich, Munich, Germany. ¹²Munich Cluster for Systems Neurology, Munich, Germany. ¹³Center of Integrated Protein Science, Munich, Germany. ¹⁴Division of Clinical Pathology, Geneva University Hospital, Geneva, Switzerland. ¹⁵Present address: Merck KGaA, Darmstadt, Germany. ¹⁶Present address: Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany. ¹⁷Present address: Berlin Institute of Health, Berlin, Germany. ¹⁸These authors contributed equally: Caroline Fecher, Laura Trovò. *e-mail: thomas.misgeld@tum.de



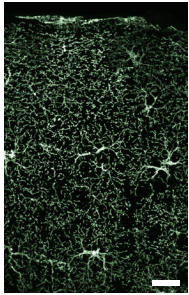
Supplementary Fig. 1 | *MitoTag* mice express GFP-OMM when crossed to several Cre-driver lines in the CNS. a-f, Sagittal brain and spinal cord overview (left), detail of cortex and cerebellum from *MitoTag* mice (GFP-OMM, green) crossed to the following Cre-driver lines: (a) Emx1:Cre, excitatory neurons and glia in the forebrain; (b) ChAT:Cre, cholinergic neurons including motor neurons; (c) L7:Cre, Purkinje cells; (d) Gabra6:Cre, cerebellar granule cells; (e) Gfap:Cre, astrocytes (including cerebellar Bergmann glia); or (f) virally transduced via adeno-associated virus delivering Cre under the human synapsin promoter, AAV9-hSyn:iCre. Images are representative for $n = 5$ independent experiments. Scale bars: 1 mm for sagittal brain; 50 μm for cortex and cerebellum; 500 μm for spinal cord.

e

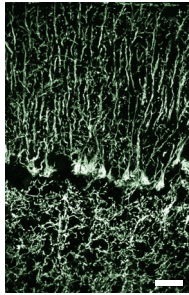
Gfap:Cre/GFP-OMM



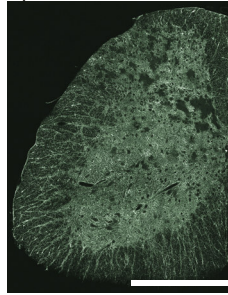
Cortex



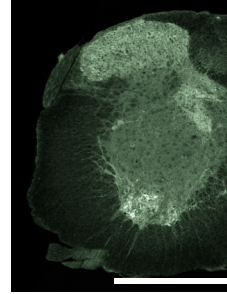
Cerebellum



Spinal cord

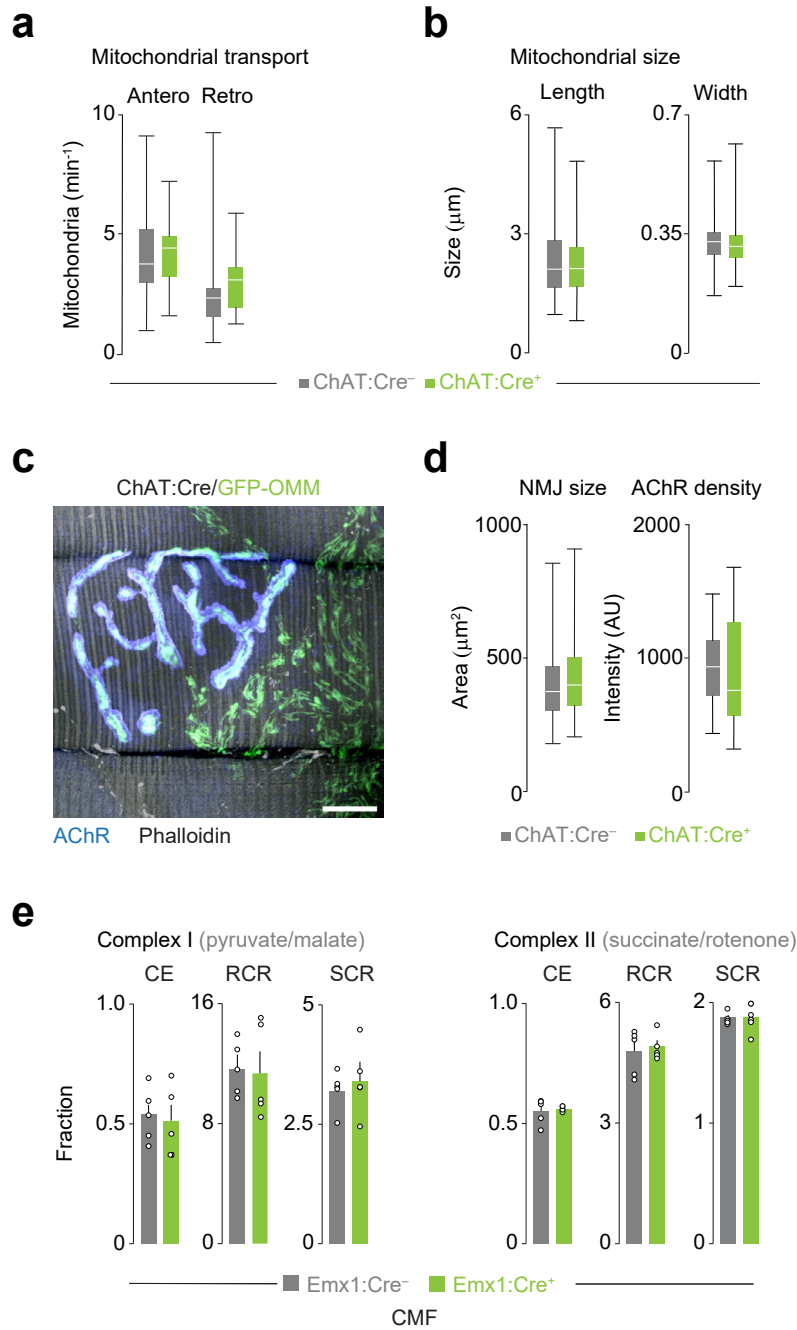
**f**

AAV9-hSyn:iCre/GFP-OMM



Spinal cord

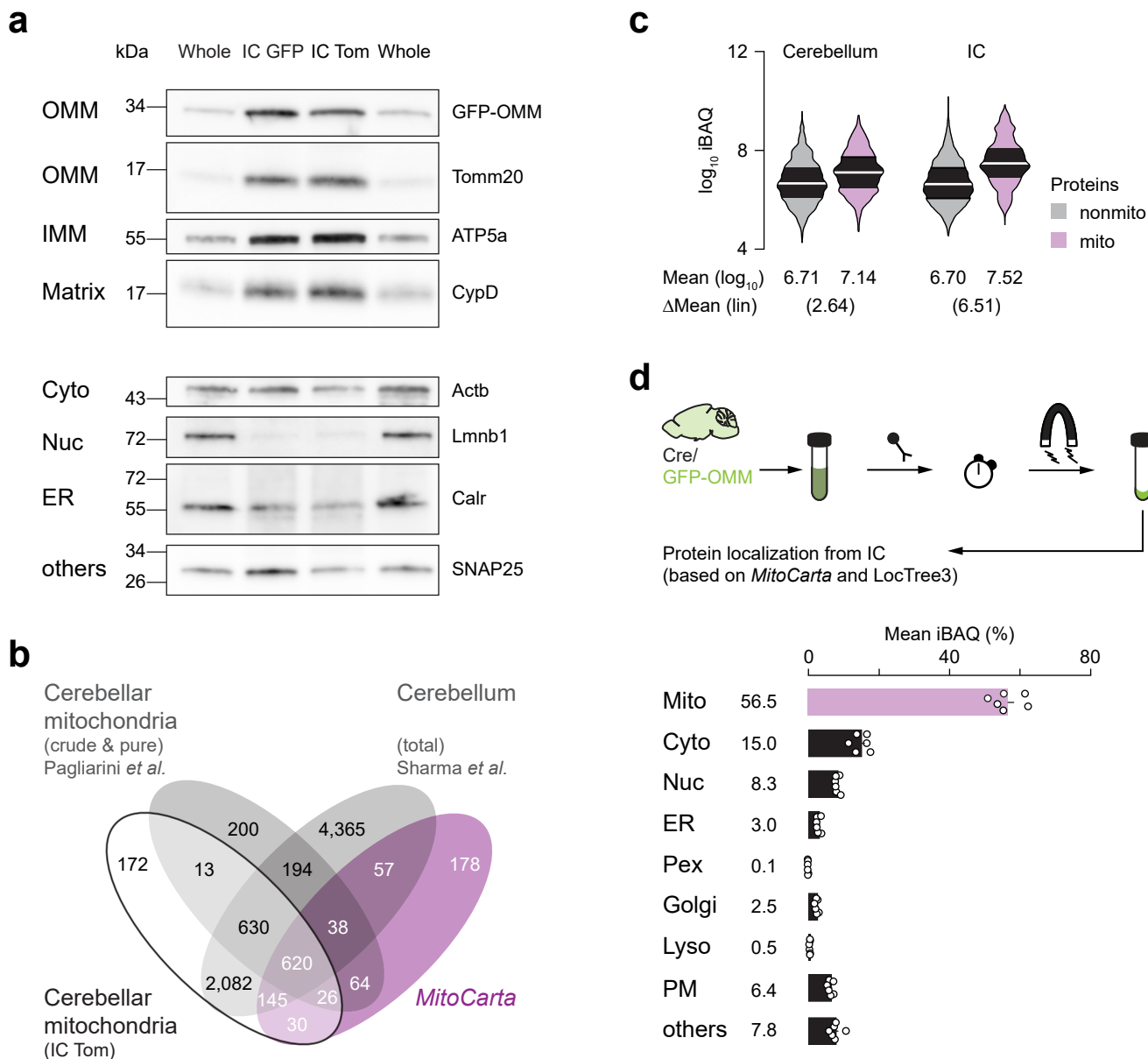
Supplementary Fig. 1 | *MitoTag* mice express GFP-OMM when crossed to several Cre-driver lines in the CNS. a-f, Sagittal brain and spinal cord overview (left), detail of cortex and cerebellum from *MitoTag* mice (GFP-OMM, green) crossed to the following Cre-driver lines: (a) Emx1:Cre, excitatory neurons and glia in the forebrain; (b) ChAT:Cre, cholinergic neurons including motor neurons; (c) L7:Cre, Purkinje cells; (d) Gabra6:Cre, cerebellar granule cells; (e) Gfap:Cre, astrocytes (including cerebellar Bergmann glia); or (f) virally transduced via adeno-associated virus delivering Cre under the human synapsin promoter, AAV9-hSyn:iCre. Images are representative for $n = 5$ independent experiments. Scale bars: 1 mm for sagittal brain; 50 μm for cortex and cerebellum; 500 μm for spinal cord.



Supplementary Fig. 2 | GFP-OMM expression does not alter mitochondrial and synaptic health.

a, Mitochondrial transport measurements in the intercostal nerve of ChAT:Cre/GFP-OMMxThy1:mito-RFP mice that were either Cre⁺ (ChAT:Cre⁺, GFP-OMM expression in cholinergic motor neurons), or Cre⁻ littermates (ChAT:Cre⁻; $n \geq 29$ axons from five mice). Box plot: median, Q1-Q3, max./min. value. **b**, Mitochondrial size in intercostal axons of motor neurons used for measurements in **(a)** ($n \geq 170$ mitochondria from five mice). Box plot: median, Q1-Q3, max./min. value. **c**, Confocal micrograph of neuromuscular junction (NMJ) in a ChAT:Cre/GFP-OMM muscle showing normal postsynaptic morphology judged by visualizing the acetylcholine receptor (AChR; stained with α -bungarotoxin, blue) and presynaptic and axonal GFP-OMM-labeled mitochondria (green). Muscle actin labeled with phalloidin (gray). **d**, Motor neurons expressing GFP-OMM have normal postsynaptic areas and AChR densities in the triangularis sterni muscle (NMJs areas, $n \geq 83$; AChR intensities, $n \geq 54$ NMJs, from four mice; genotypes as in **a**). Box plot: median, Q1-Q3, max./min. value. **e**, Bioenergetic parameters from oxygen consumption measurements of cortical mitochondria isolated by differential centrifugation (crude mitochondrial fraction, CMF) from *MitoTag* mice that either express Cre under the Emx1 promoter (Emx1:Cre⁺)

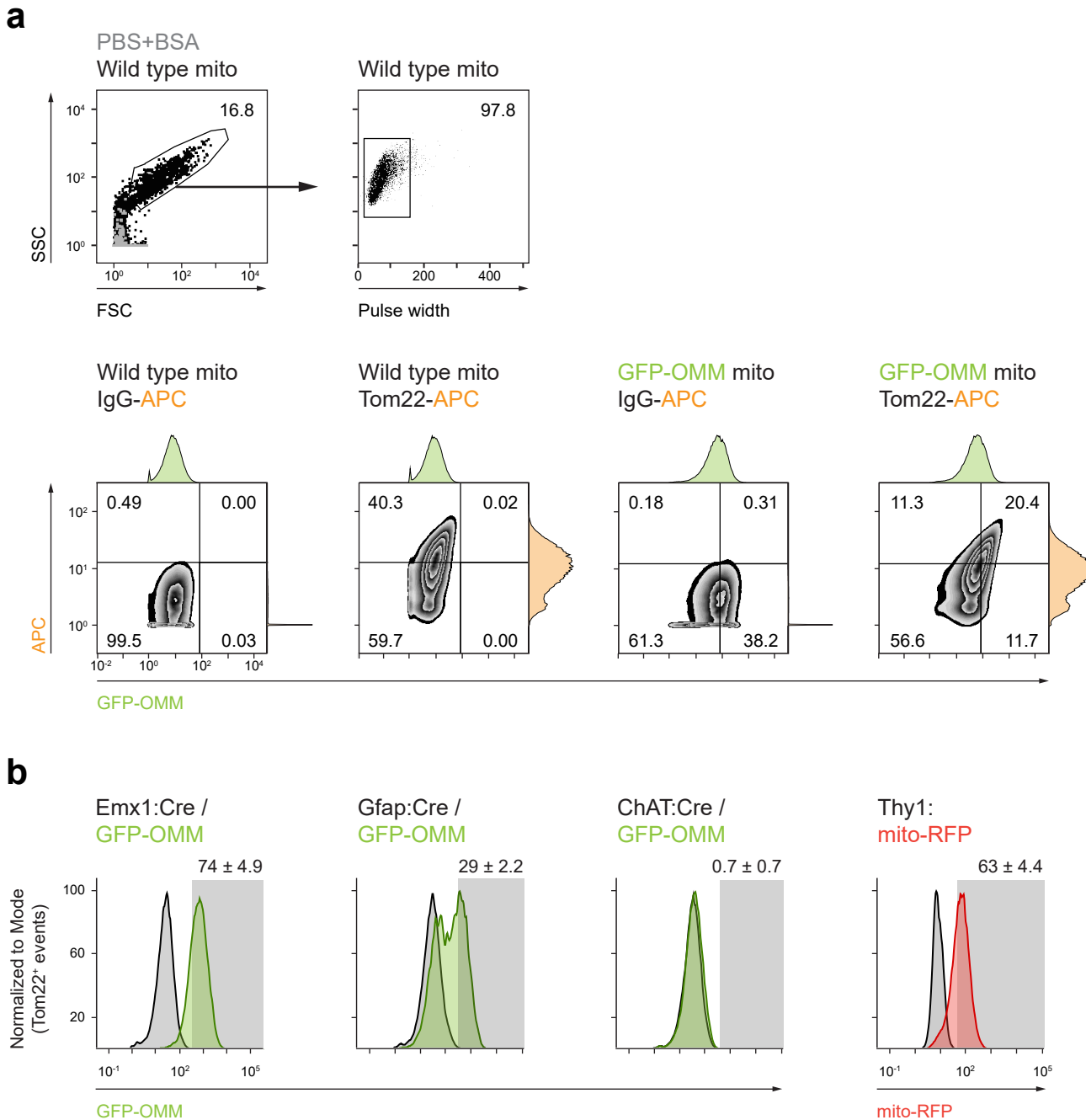
or are Cre⁻ littermates (Emx1:Cre⁻), in the presence of either complex I substrates (left, pyruvate/malate; $n = 5$ mice from three independent experiments) or complex II substrates (right, succinate/rotenone). No significant differences between genotypes. Dot plot: mean $\pm$ s.e.m. Scale bar: 20 μ m (**c**). CE, coupling efficiency; RCR, respiratory capacity ratio; SCR, spare capacity ratio.



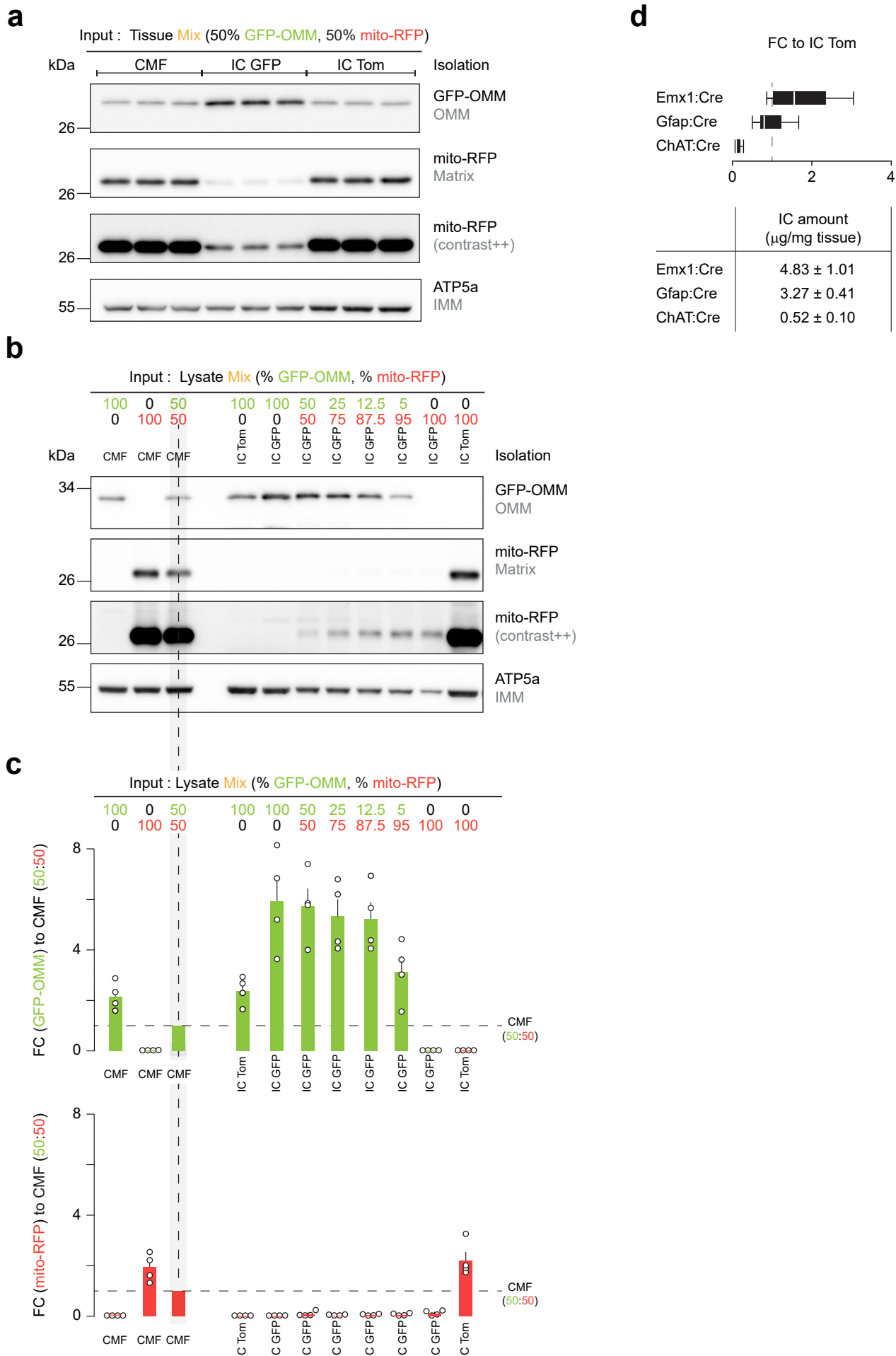
Supplementary Fig. 3 | Composition of immunocaptured mitochondria. **a**, Enrichment of mitochondrial proteins after immunocapture via GFP-OMM (IC GFP) and Tom22 (IC Tom) in comparison with whole tissue lysate obtained from Emx1:Cre/GFP-OMM cortex. Western blot probing for mitochondrial proteins: GFP-OMM, Tomm20, ATP5a and CypD; and nonmitochondrial proteins: Actb (Cyto, cytosol), Lmnb1 (Nuc, nucleus), Calr (ER, endoplasmic reticulum) and SNAP25 (others); representative experiment ($n = 3$). **b**, Venn diagram comparing the proteome of IC Tom from mouse cerebellum (white) with the proteome of cerebellar mitochondria isolated by Pagliarini, et al.¹ and to the whole cerebellum proteome by Sharma, et al.². Mitochondrial proteins are annotated via MitoCarta³ (magenta). **c**, Violin plot depicting protein abundance of mitochondrial (magenta) and nonmitochondrial (gray) proteins from whole cerebellum ($n = 4$ mice from one experiment) and immunocaptured mitochondria ($n = 34$ isolations from three independent experiments) quantified by mass spectrometry as mean intensity-based absolute quantification (iBAQ). Annotation is based on MitoCarta³ and LocTree3⁴ and the population mean (log₁₀) as well as the fold change (linear) between protein groups is indicated below. Violin plot: Median, black area: Q1-Q3, distribution with max./min. value. **d**, Protein abundance per subcellular localization after immunocapture (schematic) of cerebellar mitochondria given as iBAQ value. Mean value of at least five mice per group ($n = 6$ isolations from three independent experiments).

(Pex, peroxisome; Golgi, Golgi apparatus; Lyso, lysosome; PM, plasma membrane). Dot plot: mean $\pm$ s.e.m.

1. Pagliarini, D.J. et al. A mitochondrial protein compendium elucidates complex I disease biology. *Cell* 134, 112-123 (2008).
2. Sharma, K. et al. Cell type- and brain region-resolved mouse brain proteome. *Nat Neurosci* 18, 1819-1831 (2015).
3. Calvo, S.E., Clauser, K.R. & Mootha, V.K. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res* 44, D1251-1257 (2016).
4. Goldberg, T. et al. LocTree3 prediction of localization. *Nucleic Acids Res* 42, W350-355 (2014).



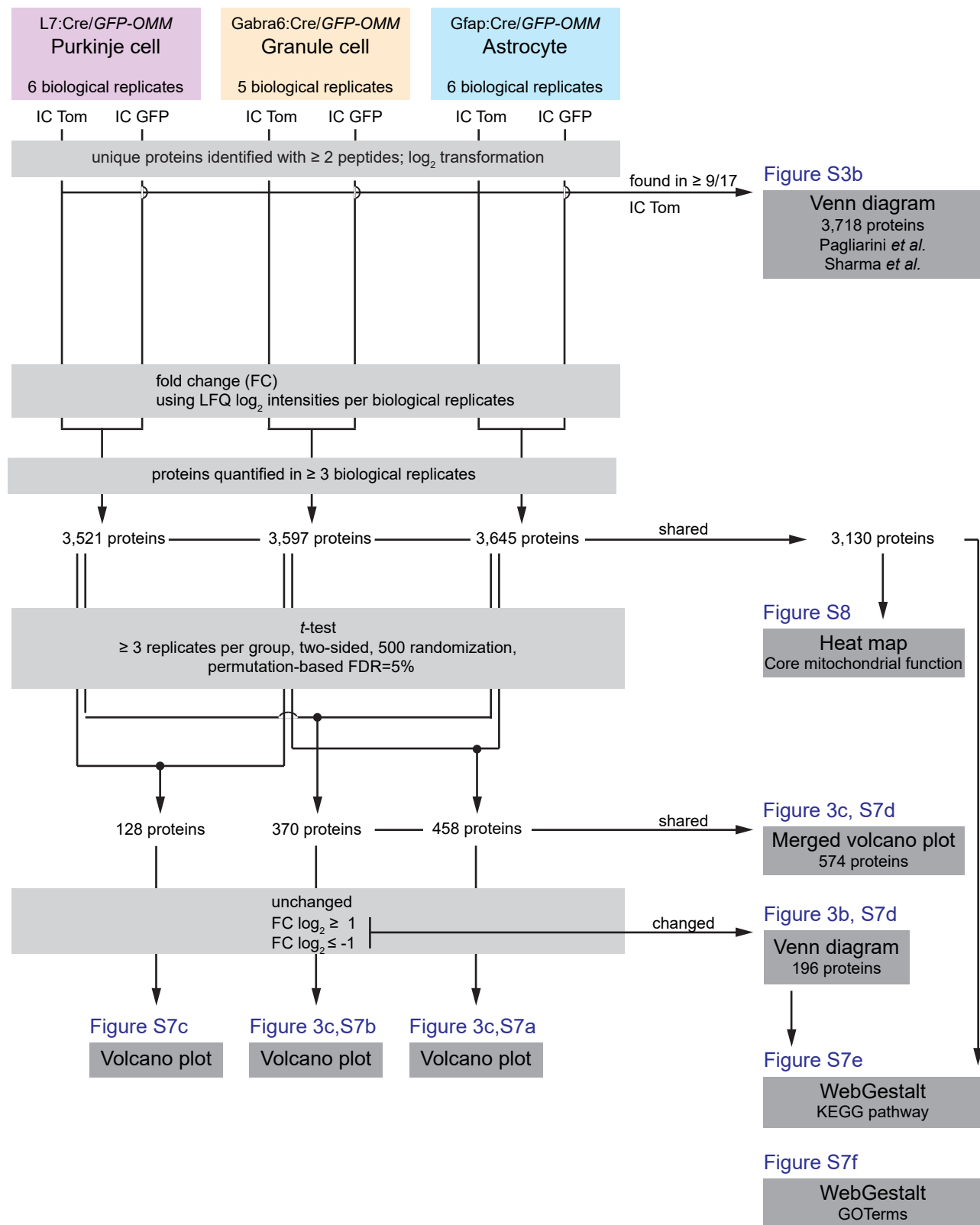
Supplementary Fig. 4 | Quantification of GFP-OMM positive cortical mitochondria in Cre-driver lines by flow cytometry. **a**, Gating strategy to quantify GFP-OMM positive mitochondria in a given Cre driver line by flow cytometry. Cortical mitochondria were isolated by differential centrifugation and further labeled against Tom22 (pan-mitochondrial marker, OMM; APC-Tom22) or isotype control (APC-IgG₁). First, events were gated by light-scattering properties (top row; forward scatter, FSC; side-ward scatter, SSC; pulse width to exclude duplicates). From this population, Tom22 (APC channel) and GFP-OMM (FITC channel) signals were manually gated with appropriate controls (IgG₁ control; wild-type mitochondria). **b**, Histogram showing the fraction (%) of GFP-OMM-positive mitochondria (green) in the following Cre driver lines: Emx1:Cre ($n = 5$ mice), Gfap:Cre ($n = 4$ mice), ChAT:Cre ($n = 4$ mice), Thy1:mito-RFP ($n = 5$ mice). Gray, wild-type mitochondria. Mitochondria derived from cortex and gated according to (a). Histogram shows sample closest to mean; mean $\pm$ s.e.m.



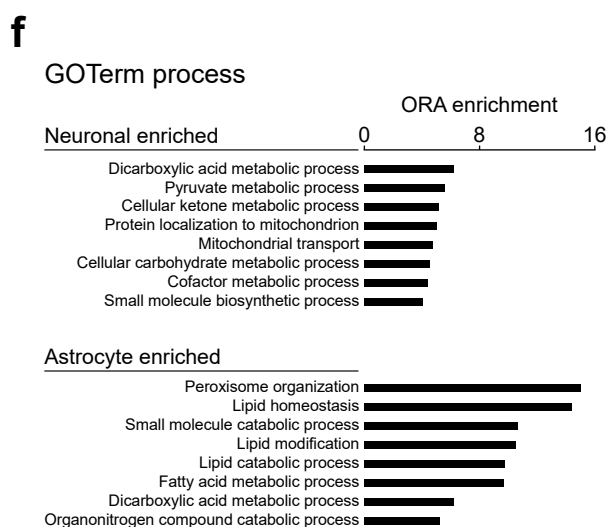
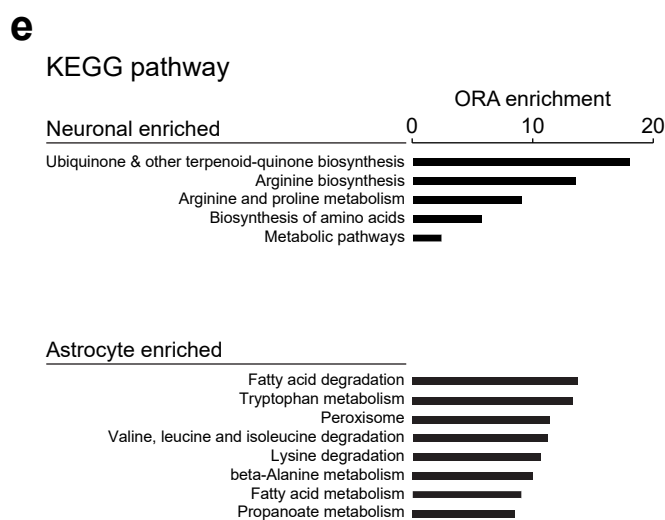
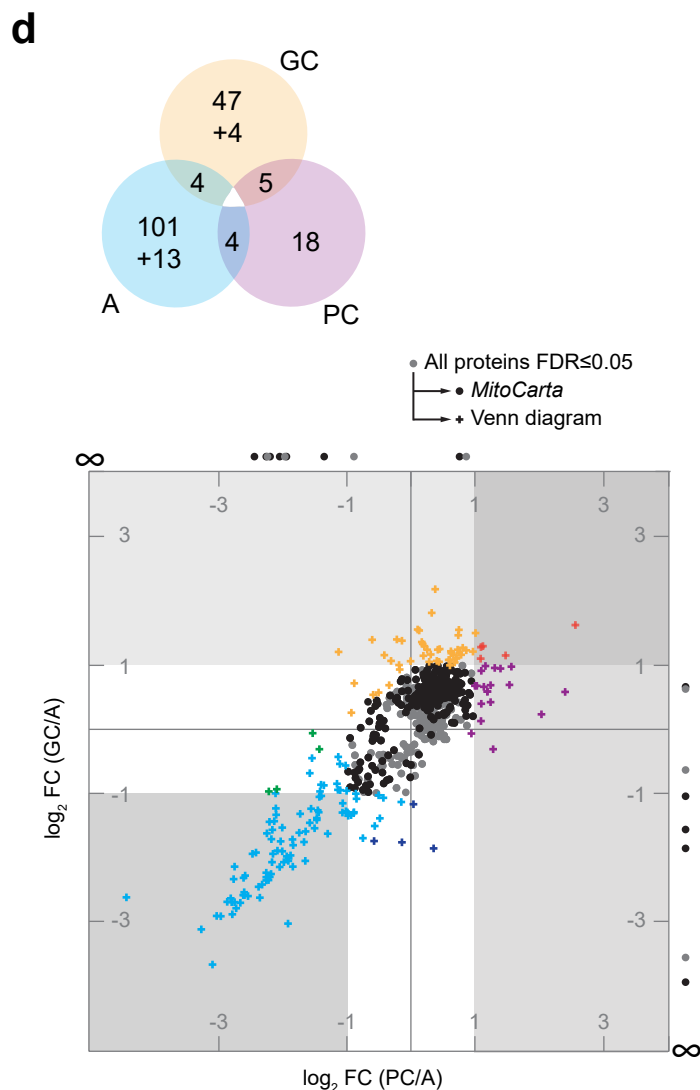
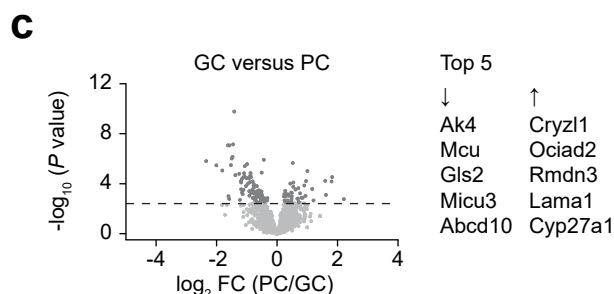
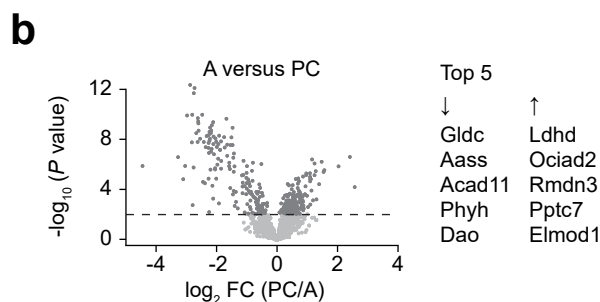
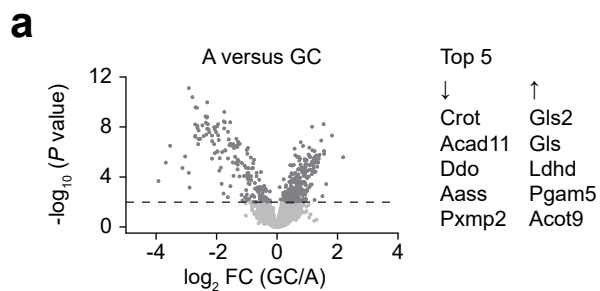
Supplementary Figure 5

Supplementary Fig. 5 | Enrichment of mitochondrial populations using the *MitoTag* approach.

a, Representative western blot related to 'spike-in' experiment quantified in Fig. 2c shows little mito-RFP contamination in IC GFP. **b, c**, For small mitochondrial populations, 'spike in' experiments were used to determine enrichment of mitochondrial populations with 100-5% GFP-OMM positive mitochondria. Cortical mitochondria from two different populations (Emx1:Cre/GFP-OMM, GFP-OMM; Thy1:mito-RFP, mito-RFP) were separately isolated. The postnuclear tissue fractions (lysate) were mixed in decreasing ratios (100-5% of lysate) based on mg tissue/ml lysate estimates and subjected to isolation via differential centrifugation (CMF, crude mitochondrial fraction) and immunocapture via Tom22 (IC Tom) or GFP-OMM (IC GFP). **(b)** Samples were probed for GFP-OMM, mito-RFP and mitochondrial proteins (Tomm20, ATP5a, CypD). Representative experiment with quantification in **c**. **(c)** Dot plots (mean $\pm$ s.e.m.) depict quantification of GFP-OMM (upper graph) and mito-RFP (lower graph) from isolations with different starting lysates ($n = 4$ experiments). Densitometry was normalized to total protein amount (SyproRuby, see Supplementary Fig. 18f) and mitochondrial content (Tom20, ATP5a, CypD). Note uneven amounts among samples because of protein amount limitations. Graph is normalized to condition: CMF, 50:50%. FC, fold change. **d**, Protein yield after immunocapture using cortex from different Cre-driver lines: Emx1:Cre/GFP-OMM ($n = 10$ mice), Gfap:Cre/GFP-OMM ($n = 12$ mice), ChAT:Cre/GFP-OMM ($n = 8$ mice). Total protein amounts are shown as fold change (FC) to IC Tom (box plot: median, Q1-Q3, max./min. value) and as absolute μ g/mg tissue (table: mean $\pm$ s.e.m.).



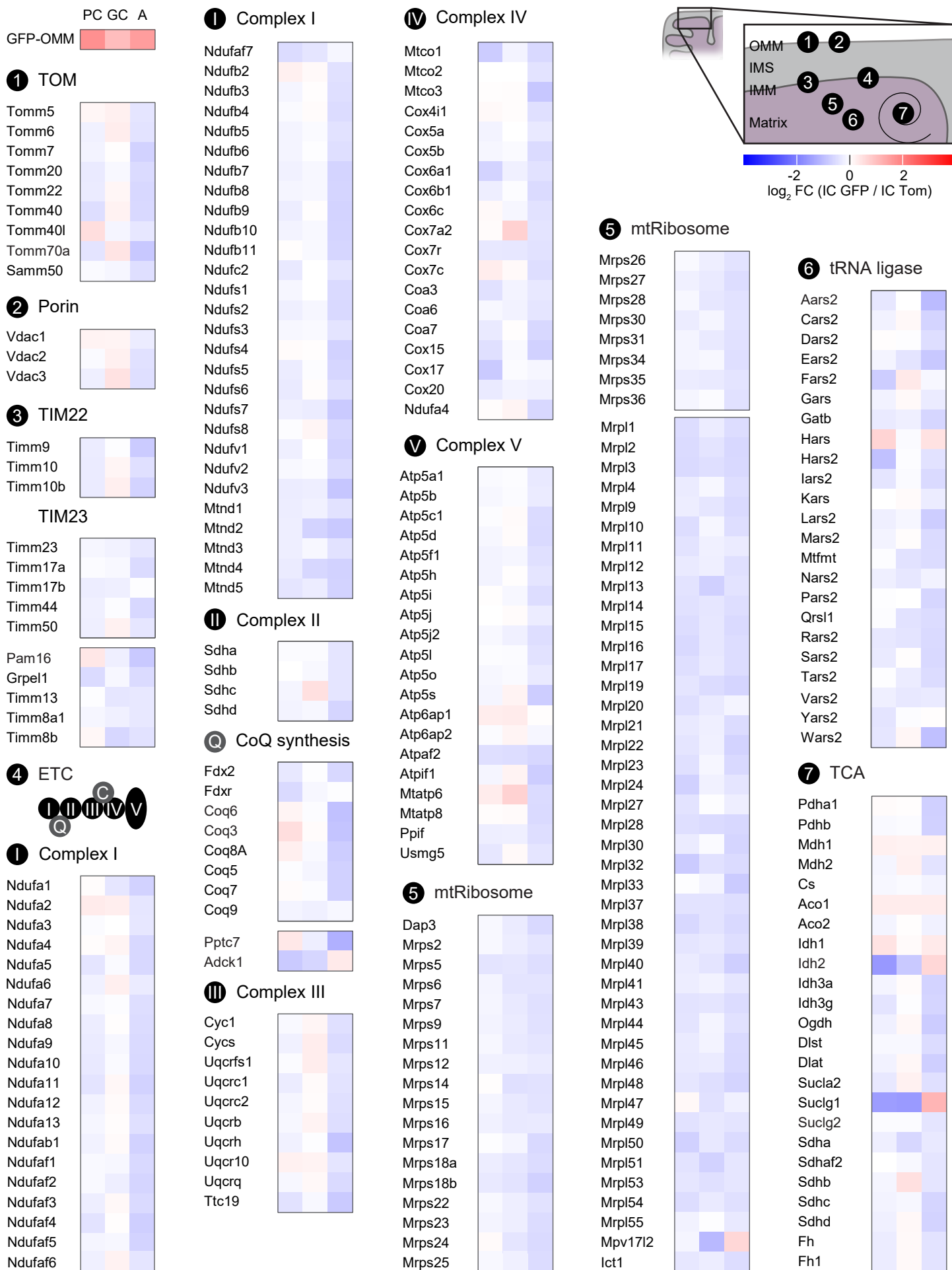
Supplementary Fig. 6 | Proteomics and bioinformatics analysis workflow. Diagram depicting the processing of mitochondrial proteomes in this study, as well as KEGG and GO Term process analysis and their comparison to other studies. See Methods for additional information. $n \geq 5$ mice per genotype group with each IC Tom and IC GFP ($n = 34$ isolations) from three independent experiments.



Supplementary Figure 7

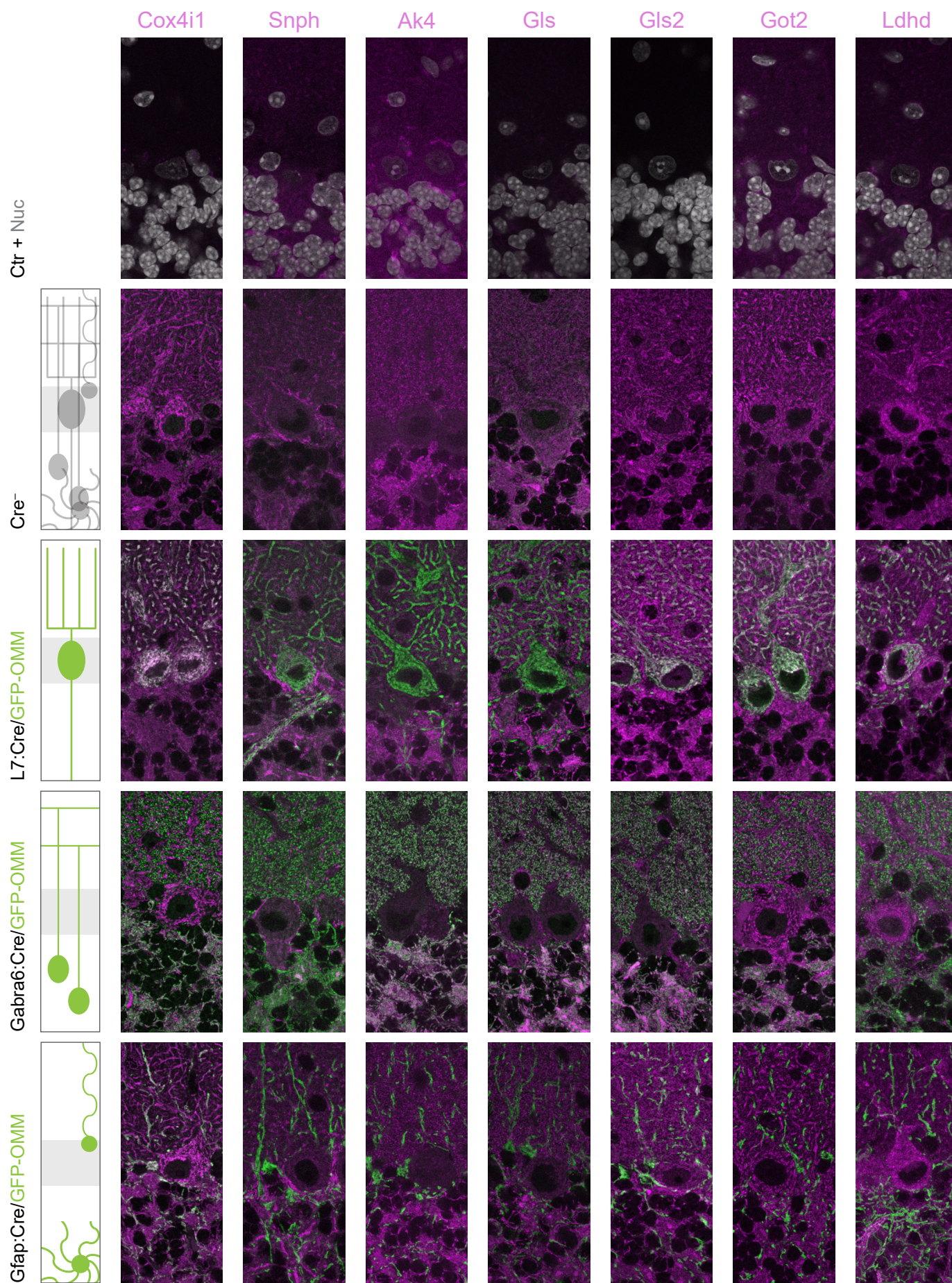
Supplementary Fig. 7 | Analysis of mitochondrial proteomes obtained from cerebellar cell types.

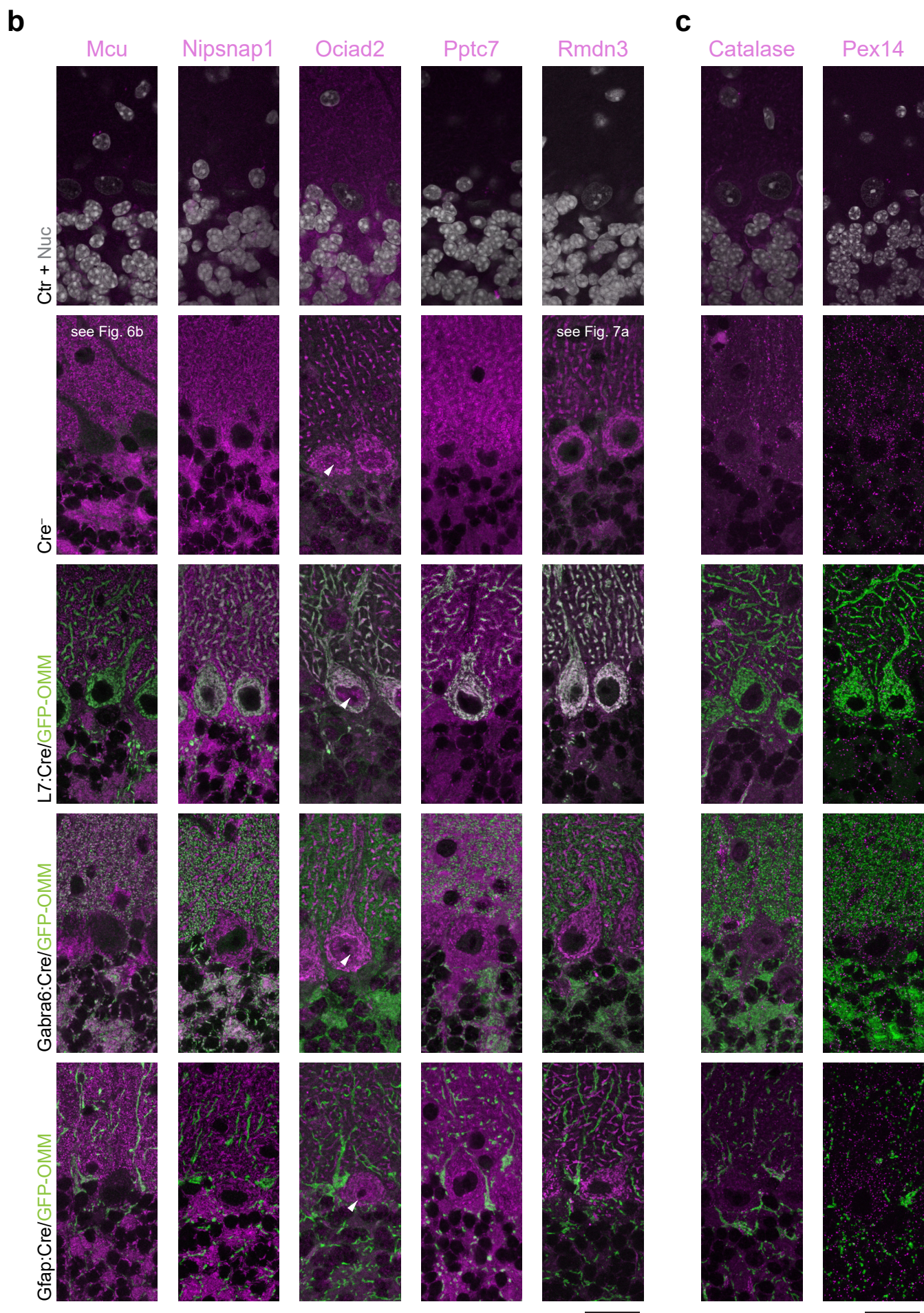
a-c, Volcano plots of cell type-specific mitochondrial proteomes ($n \geq 5$ mice per group from three independent experiments) comparing (**a**) astrocytes versus granule cells (GC/A), (**b**) astrocytes versus Purkinje cells (PC/A), and (**c**) granule cells versus Purkinje cells (PC/GC). The top 5 up-/down-regulated proteins are listed next to each graph. Dark gray circles, proteins with $\text{FDR} \leq 0.05$. A, astrocytes; FC, fold change; GC, granule cells; PC, Purkinje cells. **d**, Proteomic comparison among cell type-specific mitochondrial proteomes. Upper part: Venn diagram of differentially enriched candidates (A, blue; GC, orange; PC, magenta; see Fig. 3b). Lower part: scatterplot depicting the merged changes among cell types shown in (**a**, GC/A; y axis) and (**b**, PC/A; x axis; see Fig. 3c). Gray circles, proteins from volcano plots with $\text{FDR} \leq 0.05$ in at least one comparison; black circles, MitoCarta annotated proteins. Candidates with cell type enrichment are indicated by color (see Venn diagram). Circles outside of scatterplot show candidates which were quantified in two of three cell type-specific proteomes. **e,f**, Analysis of KEGG pathway (**e**) and gene ontology term (GOTerm) processes (**f**) of neuronal ($n = 74$ proteins) and astrocytic ($n = 114$ proteins) candidates using overrepresentation enrichment analysis (ORA, $p \leq 0.05$; see Methods for testing).



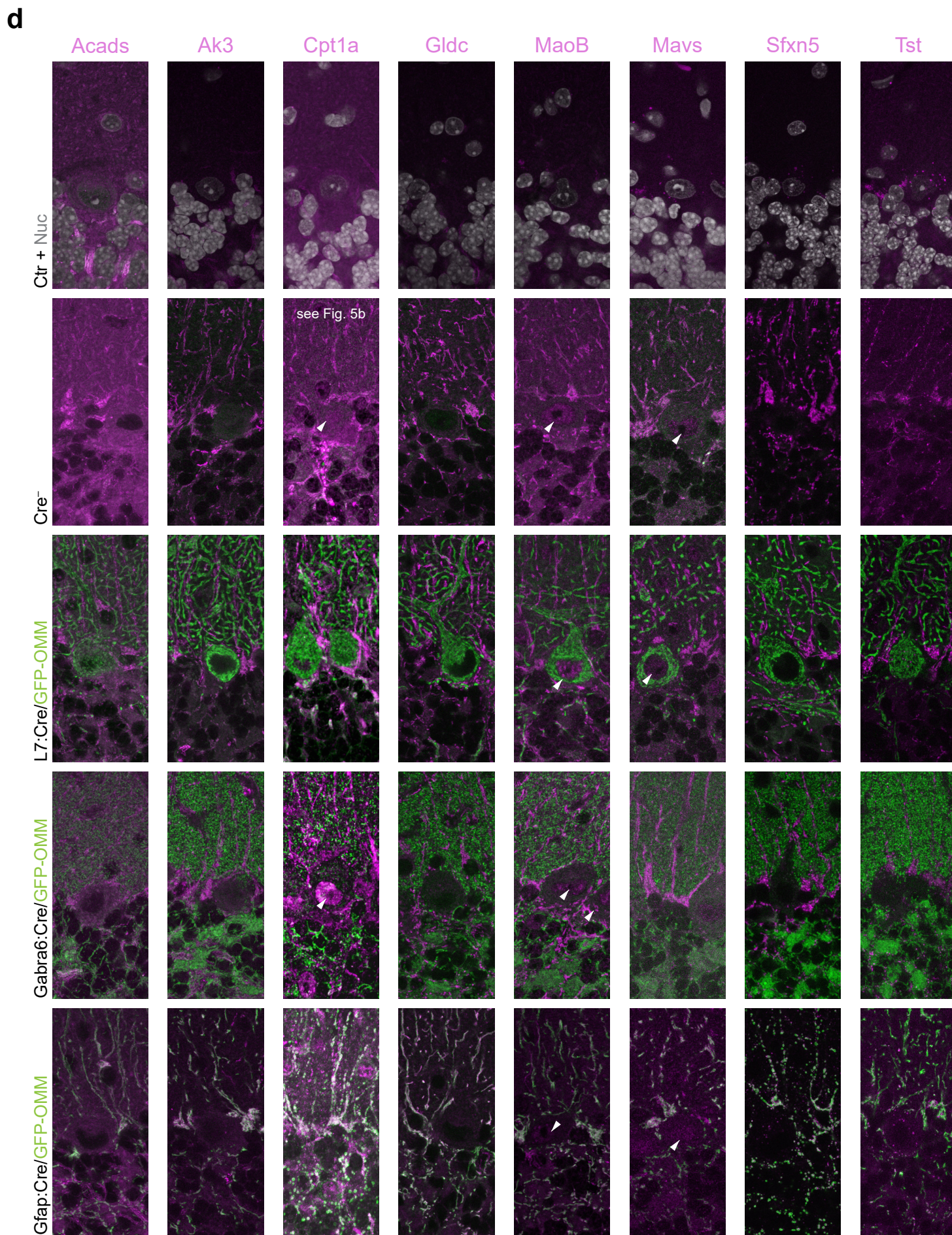
Supplementary Figure 8

Supplementary Fig. 8 | Cell type-specific comparison of core mitochondrial functions in mitochondrial proteomes. Differences are shown as average $\log_2$ FC (IC GFP/IC Tom) from LFQ intensity per protein from Purkinje cell (PC), granule cell (GC) and astrocytic (A) mitochondria of the following core mitochondrial functions (schema depicting mitochondrial localization): protein import (TOM, porin, TIM22 and TIM23), respiratory chain (complex I-V, CoQ synthesis), mitochondrial ribosome subunits (small, large), mitochondrial translation (tRNA ligases) and tricarboxylic acid cycle (TCA). The following proteins are among differentially regulated candidates: Tomm70a, Pam16, Coq6, Coq3, Pptc7 (see Fig. 3d), Adck1, Mpv17l2, Aars2, Yars2, Idh2 and Sucgl2.

a**Supplementary Figure 9**



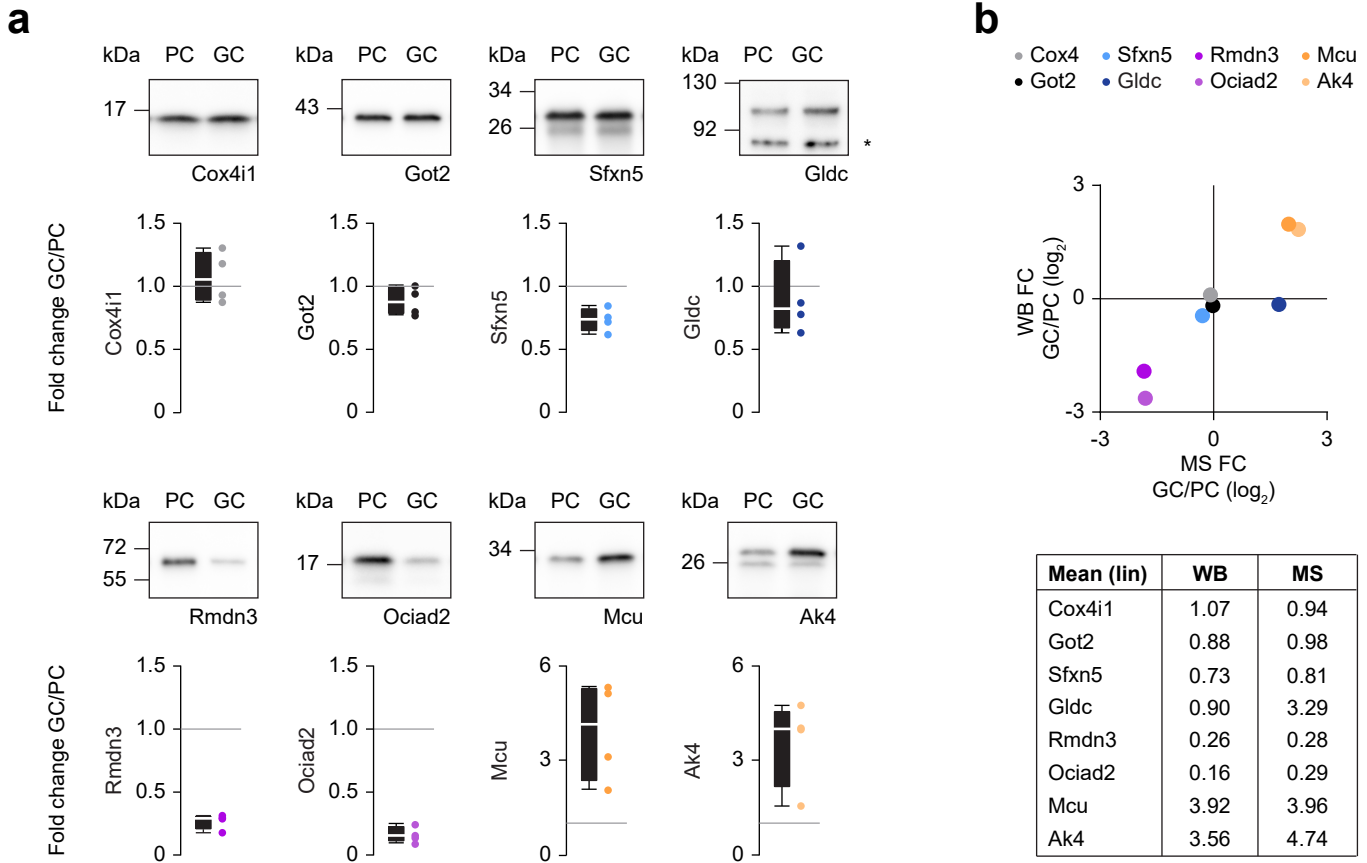
Supplementary Figure 9



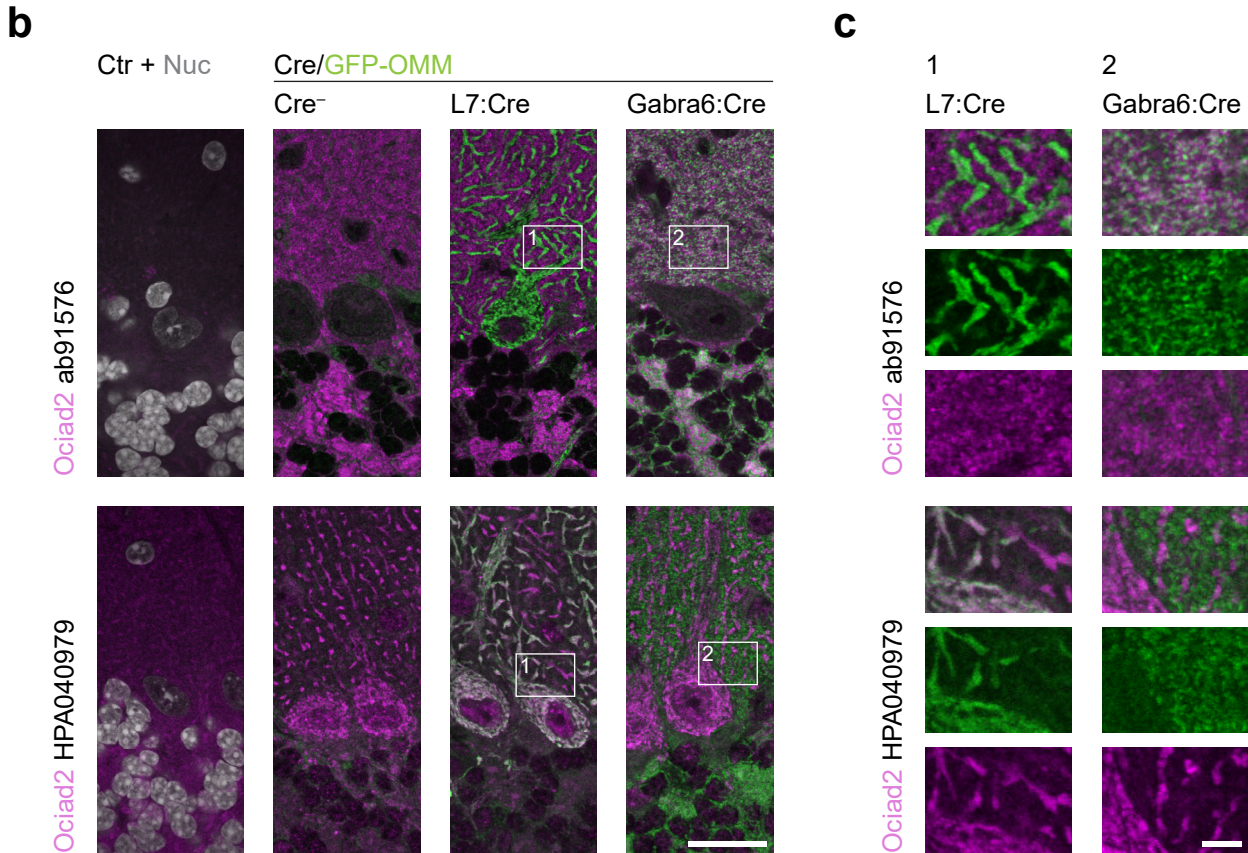
Supplementary Figure 9

Supplementary Fig. 9 | Immunofluorescence validation of candidates. Candidate proteins found differentially expressed in cell type-specific mitochondrial proteomes from cerebellum (schematic) were verified by immunofluorescence stainings (magenta). Cell type-specific mitochondria shown via GFP-OMM (green) expressed in Purkinje cells (L7:Cre/GFP-OMM), granule cells (Gabra6:Cre/GFP-OMM) and astrocytes (Gfap:Cre/GFP-OMM). Ctr + Nuc, background signals with omission of primary antibody shown in top row per condition with nuclear counterstain (Nuc). Cre⁻, antibody staining on wild type tissue shown in second row from the top. Arrowhead, nuclear signal. Schematic illustrates cell morphology and anatomical position in the cerebellum. Note differences among controls due to different tissue processing protocols (see Supplementary Table 1). Validated candidates are: **a**, pan-mitochondrial: Cox4i1, and neuronal: Snph, Ak4, Glis, Glis2, Got2, Ldhd; **b**, neuronal (continued): Mcu, Nipsnap1, Ociad2, Pptc7, Rmdn3; **c**, peroxisomal: Cat, Pex14; and **d**, astocytic: Acads, Ak3, Cpt1a, Glis, MaoB, Mavs, Sfxn, Tst. Note partial colocalization of Cat in astrocytic mitochondria according to its reported dual localization^{1, 2}, as well as tethering of individual Pex14 puncta to mitochondria. Nuclear signal of Cpt1a as previously reported³. Images are representative for $n = 3$ independent experiments. Scale bars: 25 μ m.

1. Petrova, V.Y., Drescher, D., Kujumdzieva, A.V. & Schmitt, M.J. Dual targeting of yeast catalase A to peroxisomes and mitochondria. *Biochem J* 380, 393-400 (2004).
2. Calvo, S.E. et al. Comparative analysis of mitochondrial N-termini from mouse, human, and yeast. *Mol Cell Proteomics* 16, 512-523 (2017).
3. Mazzarelli, P. et al. Carnitine palmitoyltransferase I in human carcinomas: a novel role in histone deacetylation? *Cancer Biol Ther* 6, 1606-1613 (2007).



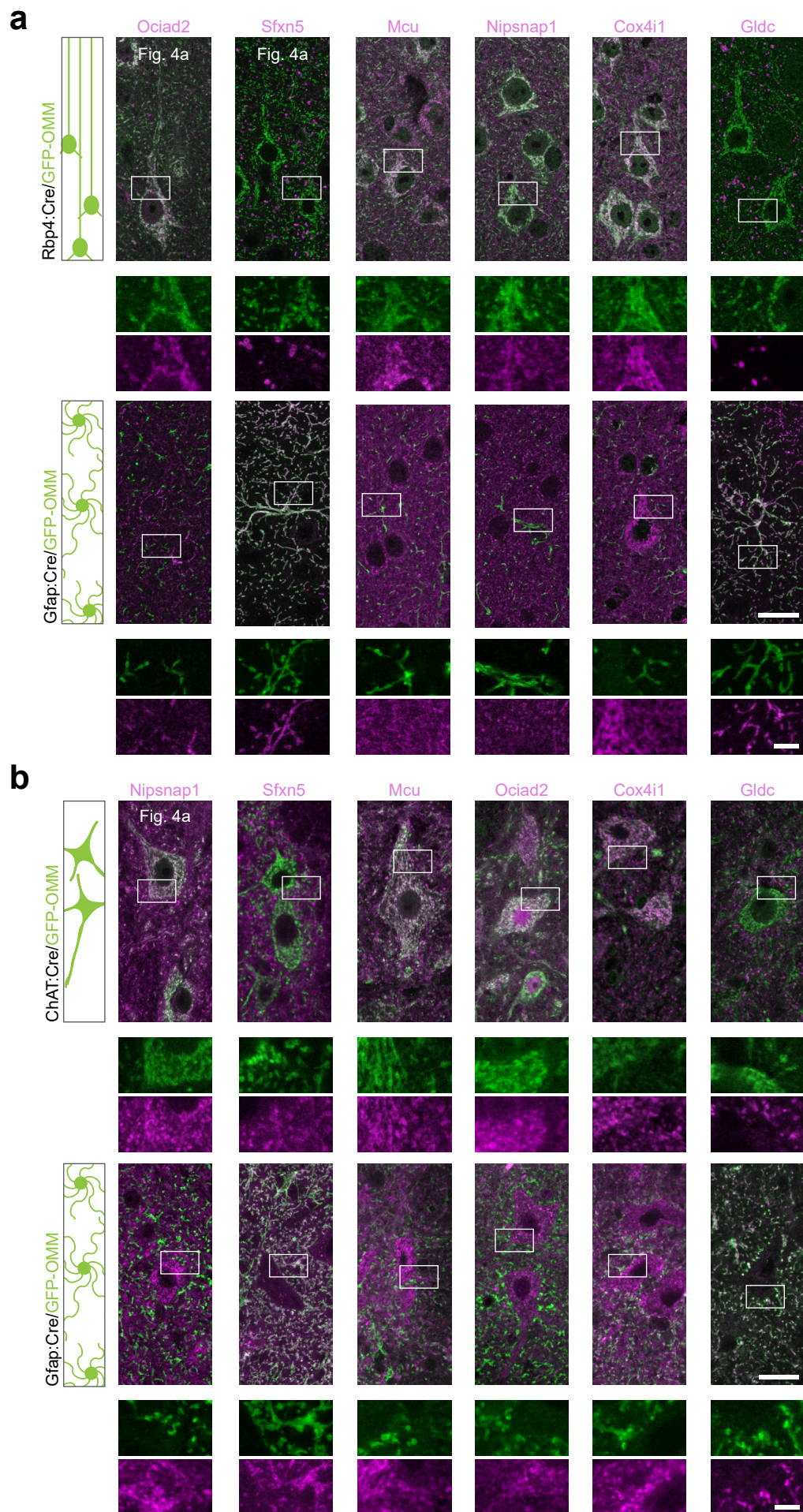
Supplementary Fig. 10 | Quantitative validation of protein fold changes observed in mass spectrometry experiments between Purkinje cell and granule cell mitochondria. **a**, Representative western blots and quantifications ($n = 4$ biological replicates from three independent experiments) for Cox4i1 (pan-mitochondrial); Got2 (pan-neuronal); Sfxn5, Gldc (astrocytic; *, unspecific band); Rmdn3, Ociad2 (Purkinje cell-specific) and Mcu, Ak4 (granule cell-specific). Box plot: median, Q1-Q3, max./min. value. **b**, These correlate with obtained mass spectrometry values (see Supplementary Fig. 7c; $n = 6$ and 5 animals from two independent experiments for Purkinje cell and granule cell mitochondria, respectively). Dot plot: mean as $\log_{10}$ value; table inset: mean as linear fold change (FC).



Supplementary Fig. 11 | Mouse Ociad2 localizes to different subcellular compartments in Purkinje cells and granule cells. a, Mouse Ociad2 protein (154 AA) structure: first depicted with its domains: OCIA domain (1-120 AA) and predicted helices (yellow; 55-76 AA, 83-103 AA)¹; second reported isoforms (light gray): one canonical (1-154 AA) and four truncated. The common region is indicated by a box (***); next, the mass spectrometry coverage of peptides (dark gray) in immunocaptured mitochondria from granule cells (GC) and Purkinje cells (PC); and finally, the epitope coverage of two antibodies used for validation (black; N-terminal, ab91576; C-terminal, HPA040979). AA, amino acids. **b**, Immunostaining for Ociad2 (magenta) using an N-terminal antibody (ab91576, upper panel) and a C-terminal antibody (HPA040979, lower panel) demonstrates two different patterns in mouse cerebellum. Staining is shown in tissue from Cre⁻, L7:Cre/GFP-OMM and Gabra6:Cre/GFP-OMM mice. Ctrl + Nuc, staining performed with the omission of primary antibody and nuclear counterstain; GFP-OMM, green. **c**, Details from (**b**, 1, L7:Cre/GFP-OMM; 2, Gabra6:Cre/GFP-OMM)

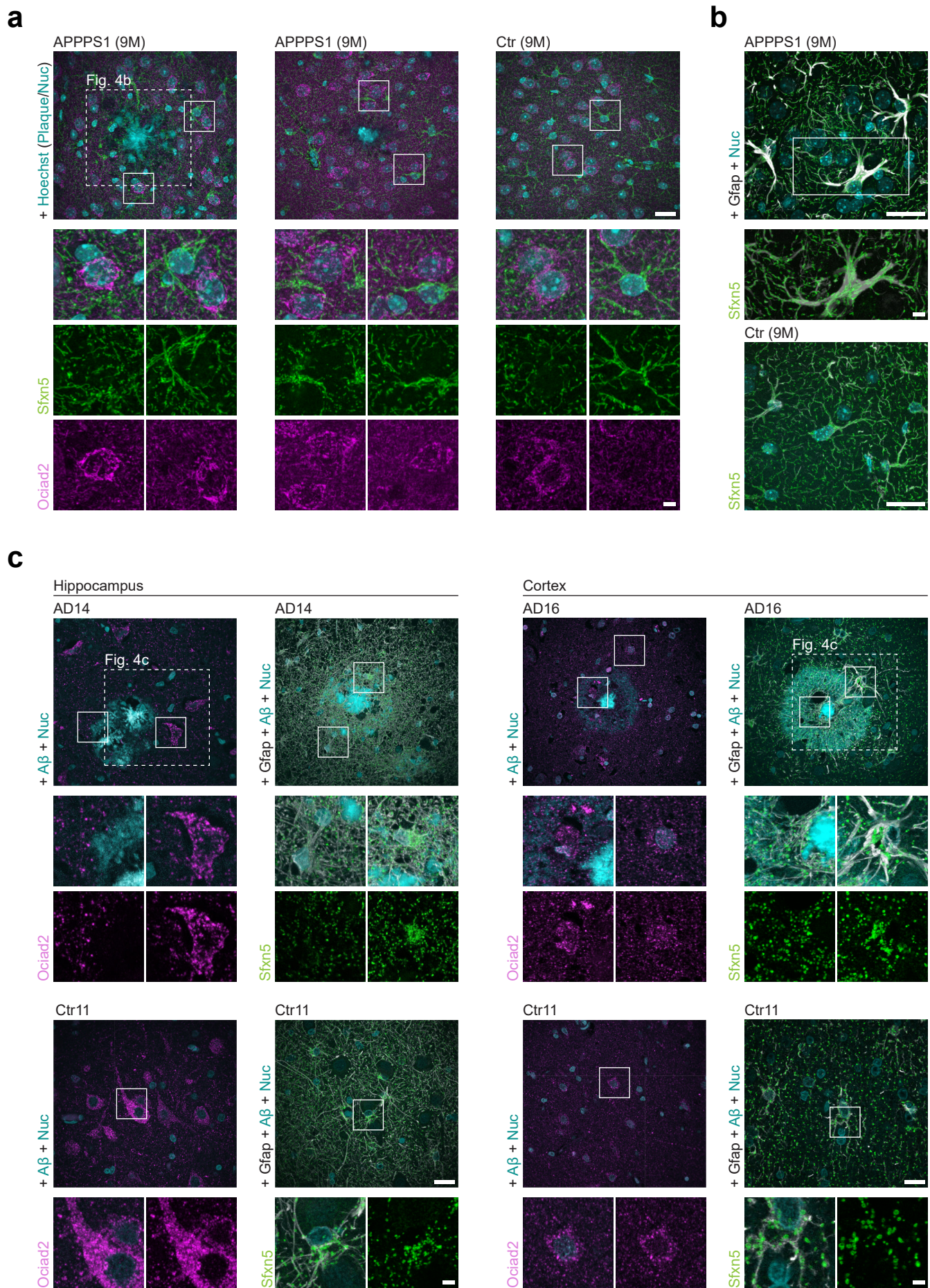
illustrate full colocalization of Ociad2 with PC mitochondria using the C-terminal antibody (lower panels), but only partial colocalization with GC mitochondria using the N-terminal antibody (upper panels). Additional signal could be localized to endosomes as recently reported¹. Scale bars: 25 μm (**b**); 5 μm (**c**).

1. Sinha, S., Bheemsetty, V.A. & Inamdar, M.S. A double helical motif in OCIAD2 is essential for its localization, interactions and STAT3 activation. *Sci Rep* 8, 7362 (2018).



Supplementary Figure 12

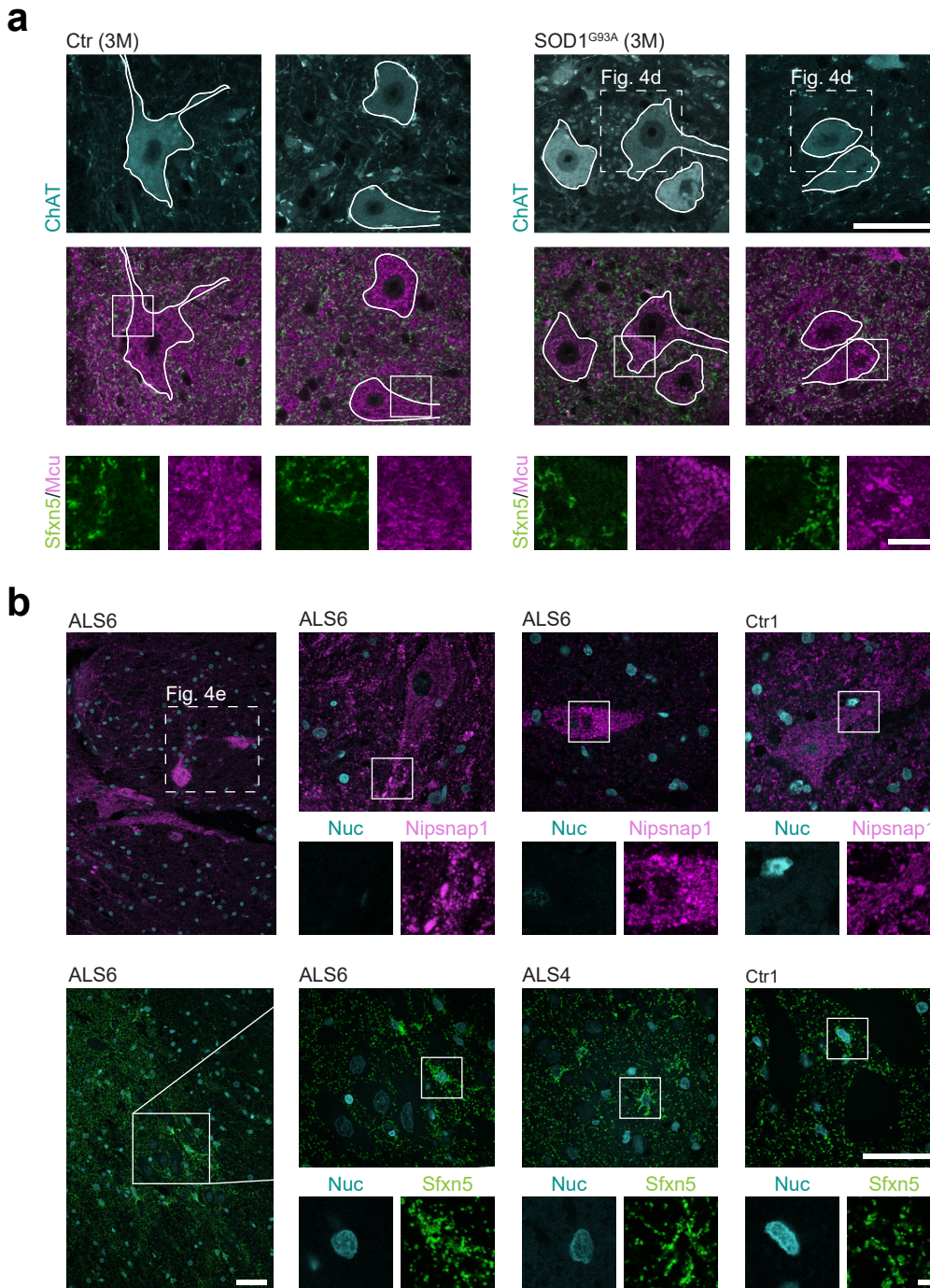
Supplementary Fig. 12 | Cell type-enriched mitochondrial markers are conserved in the CNS.
a, b, Selection of candidates tested in mouse cortex (**a**) and spinal cord (**b**) of *MitoTag* mice crossed to Rbp4:Cre (principle neurons layer 5/6) or ChAT:Cre (motor neurons) and Gfap:Cre (astrocytes) confirming the results obtained from cerebellum (see Supplementary Fig. 9). Images for Ociad2, Sfxn5 and Nipsnap1 also shown in Fig. 4a. Images are representative for $n = 2$ independent experiments. Scale bars: 20 μm (5 μm in insets).



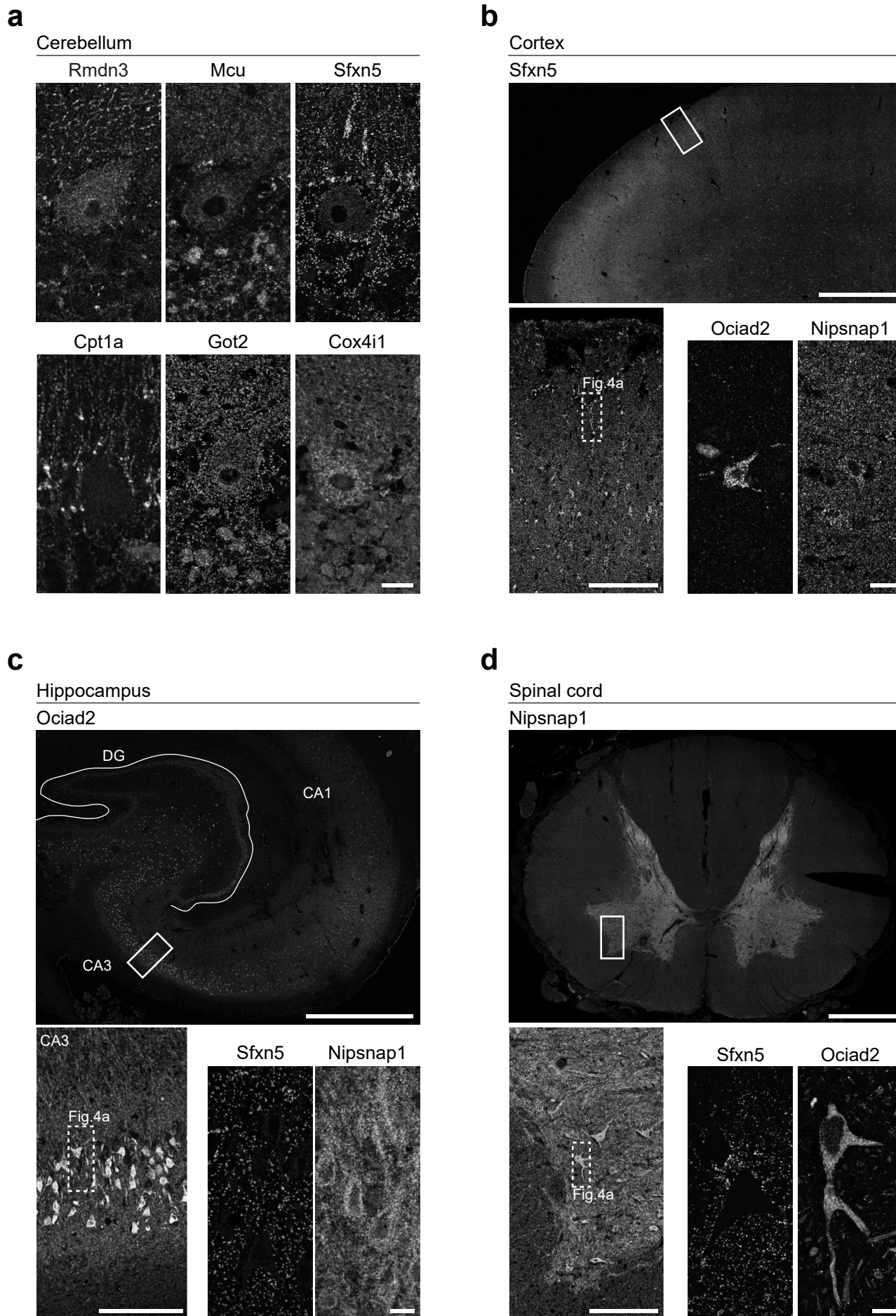
Supplementary Figure 13

Supplementary Fig. 13 | Mitochondrial markers reveal neuronal and astrocytic mitochondria in mouse models of Alzheimer's disease and post mortem AD tissue. **a, b**, In cortex from APPPS1 mice (9M, 9 month) and control (Ctr) prominent amyloid plaques (Hoechst¹, cyan) can be found surrounded by neuronal (Ociad2, magenta) and astrocytic (Sfxn5, green) mitochondria. Mitochondrial shapes are unaltered, while reactive astrocytes (**b**, Gfap) display an increased mitochondrial mass (see inset). Images are representative for $n = 3$ mice from two independent experiments. **c**, In post mortem hippocampus and cortex of AD patients the distribution of neuronal mitochondria (Ociad2, magenta) and astrocytic mitochondria (Sfxn5, green) around amyloid plaques (A β antibody; channel merged with DAPI) is similarly as in the APPPS1 mouse model. Additionally, prominent astrogliosis is observed (Gfap, gray). Details in dashed boxes are shown in Fig. 4b, c. Images are representative for four cases from one experiment. Scale bars: 20 μ m (5 μ m in insets).

1. Uchida, Y. & Takahashi, H. Rapid detection of Abeta deposits in APP transgenic mice by Hoechst 33342. *Neuroscience Letters* **448**, 279-281 (2008).

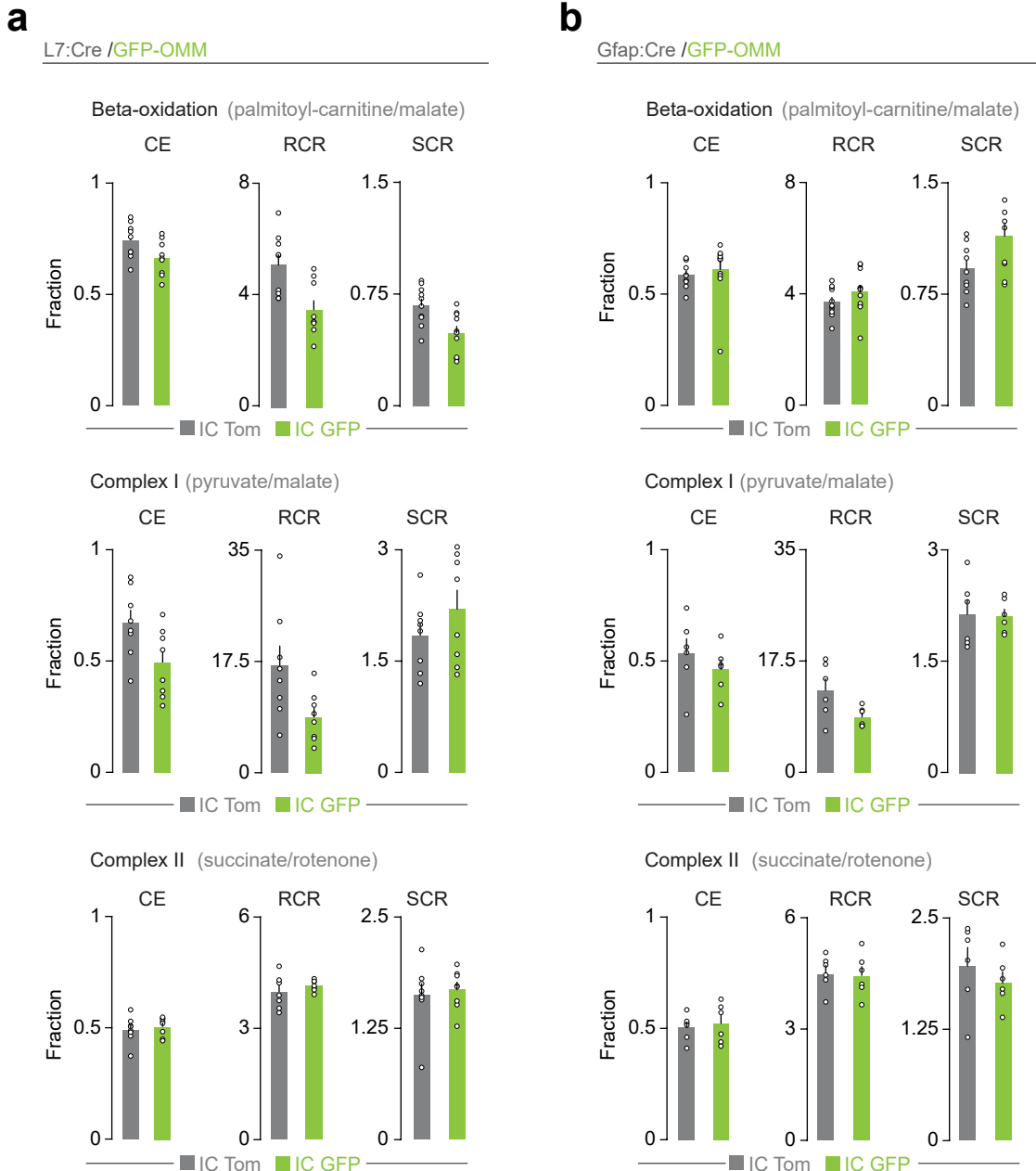


Supplementary Fig. 14 | Mitochondrial markers visualize neuronal and astrocytic mitochondria in SOD1^{G93A} mice and post mortem ALS spinal cord. **a**, In lumbar spinal cord of presymptomatic SOD1^{G93A} mice (3M, 3 month) prominent morphology alterations are found in neuronal mitochondria (Mcu, magenta) – specifically motor neurons (ChAT, cyan). Astrocytic mitochondria (Sfxn5, green) are normal in morphology. Images are representative for $n = 2$ mice from two independent experiments. **b**, In post mortem spinal cord of ALS patients few, large neurons can be found in the gray matter. In remaining neurons, mitochondria (Nipsnap1, magenta) are clustered, while astrocytic mitochondria (Sfxn5, green) appear normal. Details in dashed boxes are shown in Fig. 4d, e. Nuc, nuclear counterstain. Images are representative for four cases from one experiment. Scale bars: 50 μm (5 μm in insets).



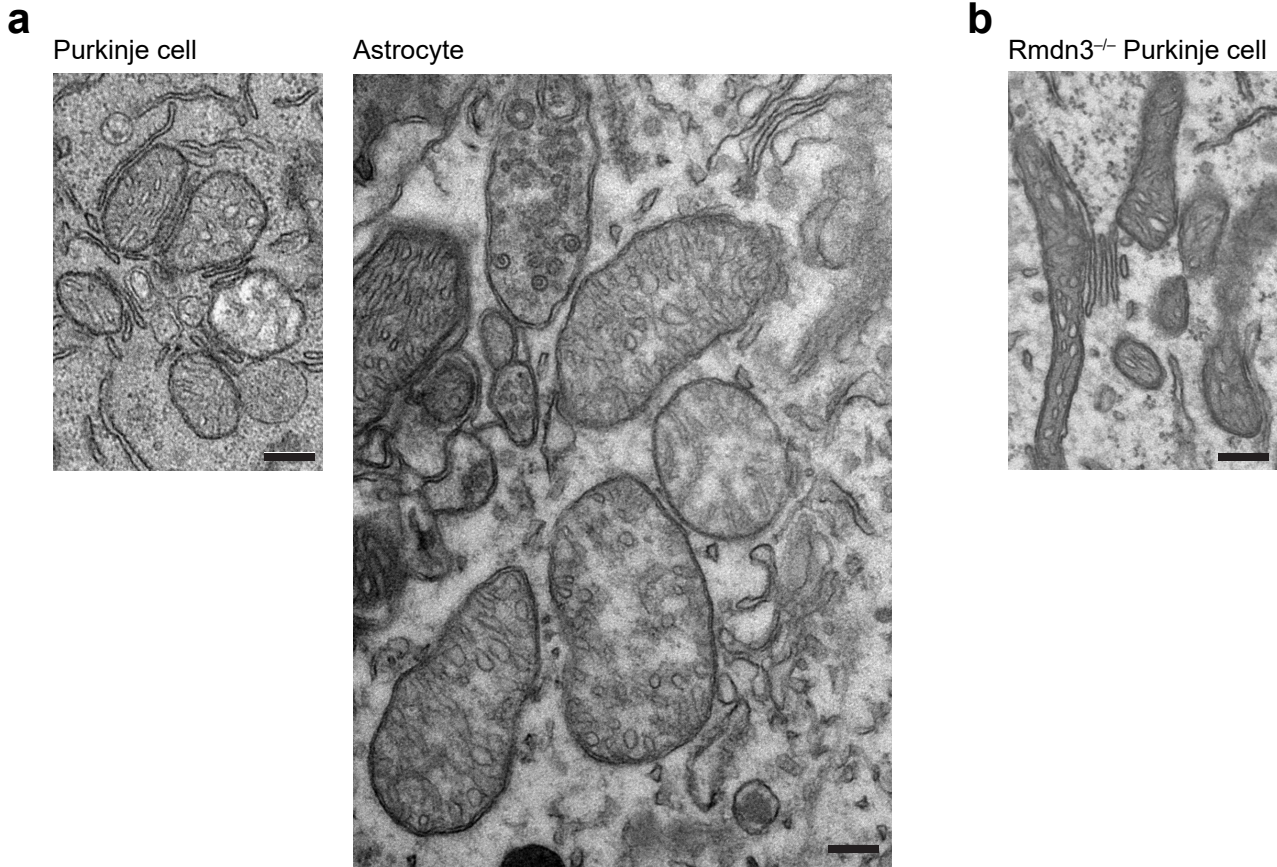
Supplementary Figure 15

Supplementary Fig. 15 | Mitochondrial markers are conserved from mouse to human and distinguish neuronal and glial mitochondria in situ. **a**, Confirmation of cell type-enriched mitochondrial pattern in human post mortem cerebellum using Rmdn3 (Purkinje cells), Mcu (granule cells), Sfxn5 and Cpt1a (astrocytes), Got2 (pan-neuronal) and Cox4i1 (pan-mitochondrial). Images are representative for one experiment. **b-d**, Neuronal and astrocytic mitochondria can further be discriminated in **(b)** human cortex, **(c)** hippocampus and **(d)** spinal cord using Sfxn5, Ociad2 and Nipsnap1, respectively. While Nipsnap1 labels the majority of neurons, we observed with Ociad2 a pattern of a neuronal subpopulation specifically present in cortical layer 5/6 and CA1/3 of the hippocampus **(c)**, tissue from AD15 case). Detail from dashed box also shown in Fig. 4a. Images are representative for three cases from one experiment. Scale bars: 20 μm **(a)**; 2 mm (top pannel in **b**, **c** and **d**); 250 μm (insets bottom left in **b**, **c** and **d**); 20 μm **(b-d)** for other markers.



Supplementary Fig. 16 | Nonnormalized bioenergetic parameters related to Figure 5c, d, e.

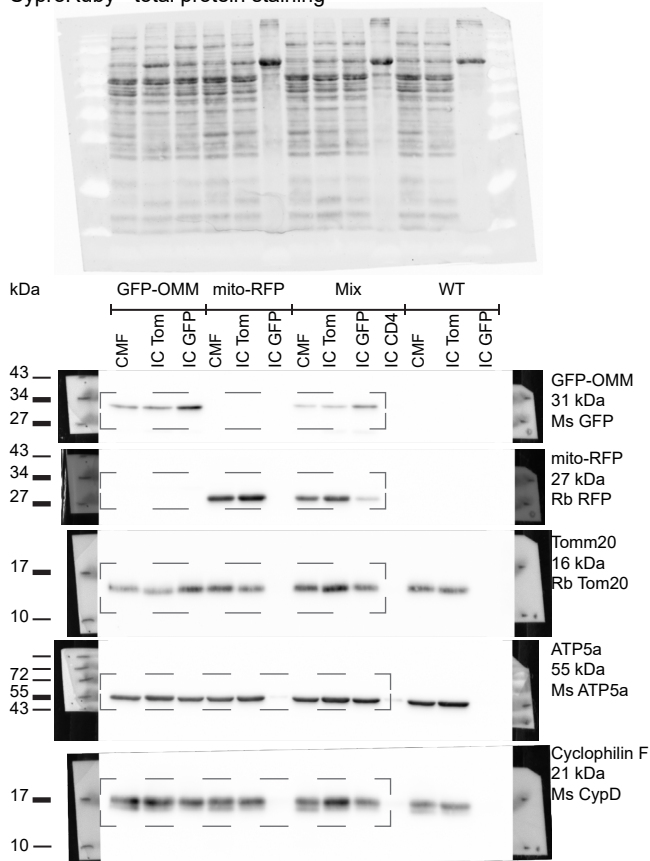
a, b, Bioenergetic parameters derived from oxygen consumption measurements of immunocaptured mitochondria from **(a)** Purkinje cells (L7:Cre/GFP-OMM) and **(b)** astrocytes (Gfap:Cre/GFP-OMM), in the presence of different substrates (top to bottom: palmitoyl-carnitine/malate, pyruvate/malate and succinate/rotenone). Data are shown for IC Tom (gray) and IC GFP (green). Note variability between genotypes and experiments, which were taken into account by normalization (IC GFP/IC Tom) in Fig. 5c-e. CE, coupling efficiency; RCR, respiratory capacity ratio; SCR, spare capacity ratio. Exact sample size and number of independent experiments are given in Supplementary Table 4. Dot plot: mean $\pm$ s.e.m.



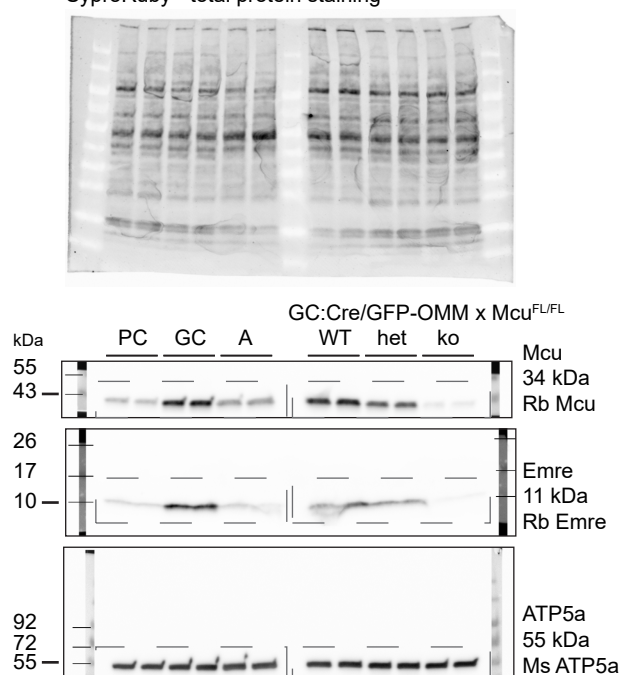
Supplementary Fig. 17 | Ultrastructural analysis of mitochondria in cerebellum related to Figure 7. a, b, Electron micrographs shown in (a) Fig. 7b and (b) 7e without pseudocolor annotation of organelle areas and contact sites. Note different micrograph size with same scale bar size due to mitochondrial size. Images are representative for $n \geq 8$ cells from (a) one independent experiment and (b) two independent experiments. Scale bars: 200 nm (a, b).

a

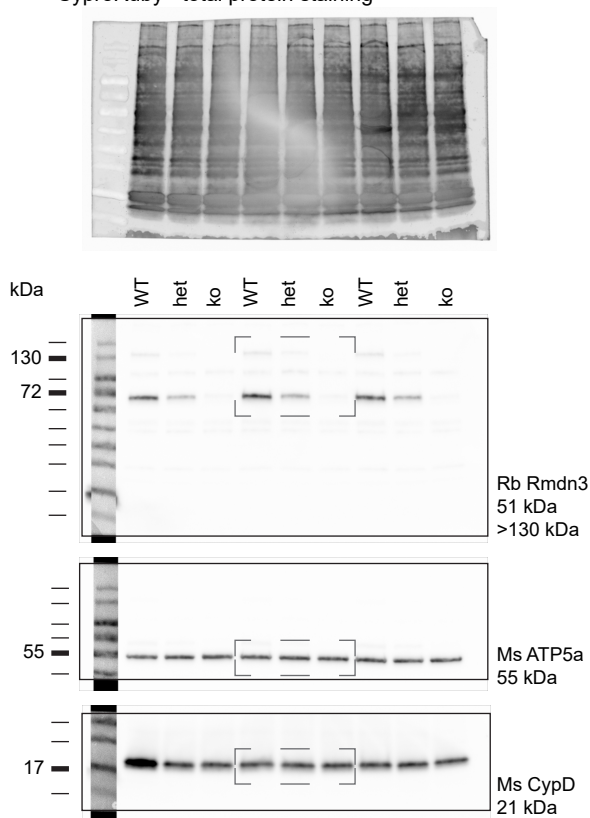
SyproRuby - total protein staining

**b**

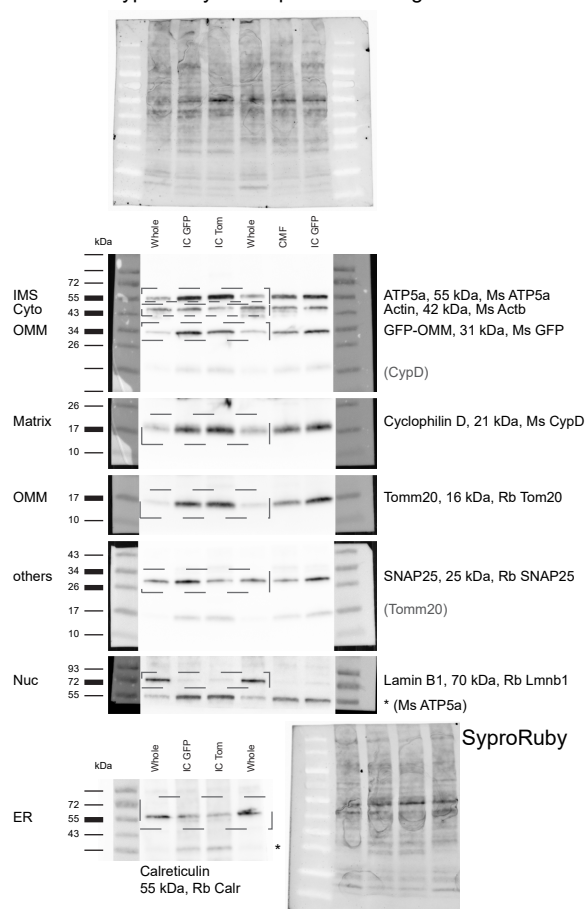
SyproRuby - total protein staining

**c**

SyproRuby - total protein staining

**d**

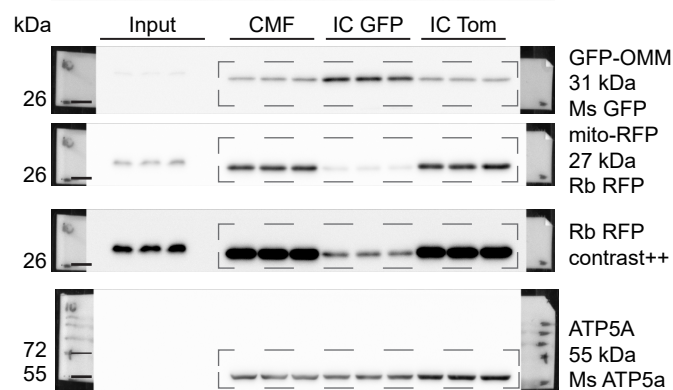
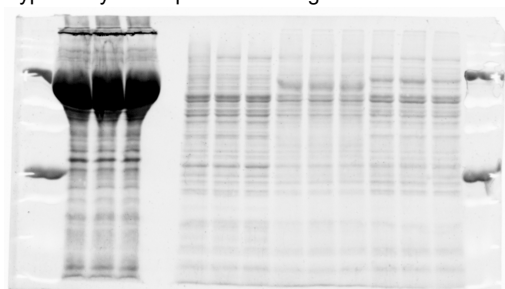
SyproRuby - total protein staining



Supplementary Figure 18

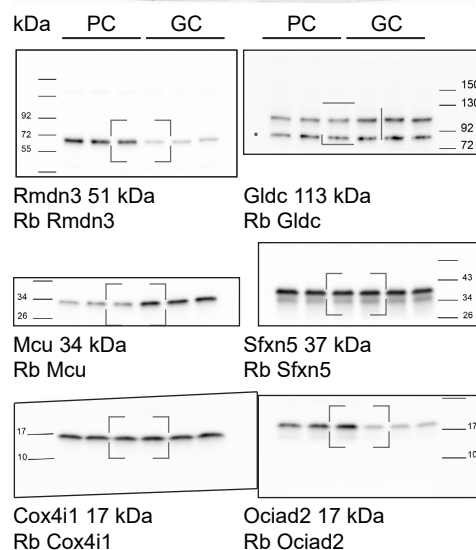
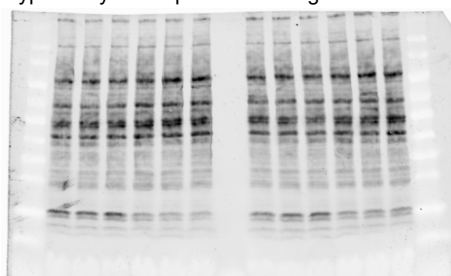
e

SyproRuby - total protein staining



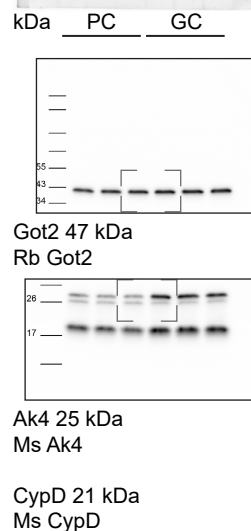
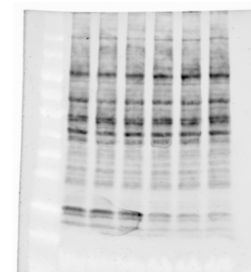
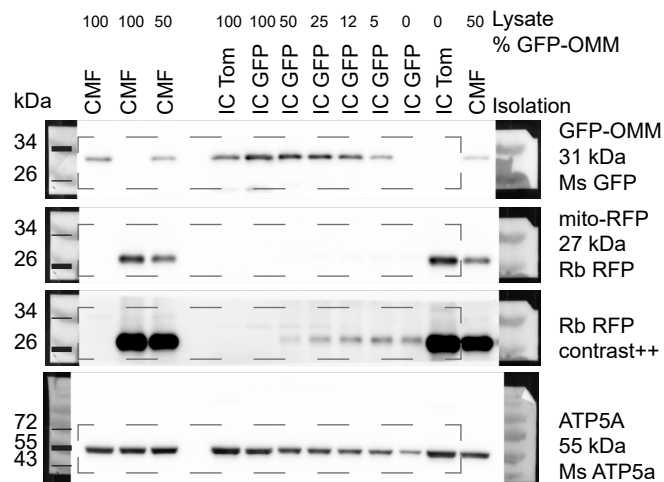
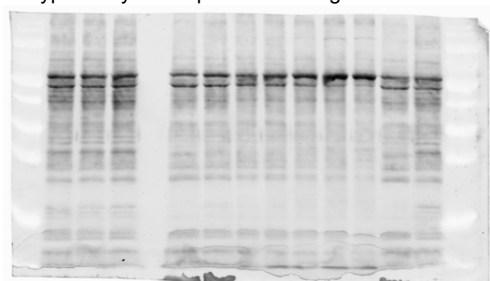
g

SyproRuby - total protein staining



f

SyproRuby - total protein staining



Supplementary Fig. 18 | Uncropped western blots. Showing first total protein staining (SyproRuby) for sample normalization; second the full membranes with indicated areas shown in main figures. **a**, Western blot shown in Fig. 2b probing for GFP-OMM, mito-RFP, Tomm20, ATP5a, CypD. IC CD4, microbeads coupled with mouse IgG₁ antibody against CD4. These microbeads were used as unspecific control beads. WT, material obtained from wild-type animals. **b**, Western blot shown in Fig. 6c, f probing for Mcu, Emre and ATP5a. **c**, Western blot from Fig. 7c probing for Rmdn3, ATP5a and CypD. Area for Rmdn3 is identical with molecular weight range (50-170 kDa) quantified in Fig. 7c. **d**, Western blot from Supplementary Fig. 3a probing for ATP5a, Actb, GFP-OMM, CypD, Tomm20, SNAP25, Lmnb1. Calr was probed on a different membrane generated with the same lysate. **e**, Western blot from Supplementary Fig. 5a probing for GFP-OMM, mito-RFP and ATP5a. Second RFP blot was oversaturated to visualize mito-RFP signal in IC GFP. Input, postnuclear fraction used for immunocapture. **f**, Western blot from Supplementary Fig. 5b probing for GFP-OMM, mito-RFP and ATP5a. Second RFP blot was oversaturated to visualize mito-RFP signal in IC GFP. **g**, Western blot from Supplementary Fig. 10a probing for Rmdn3, Gldc, Mcu, Sfxn5, Cox4i1, Ociad2, Got2 and Ak4 (21 kDa band represents CypD which was probed together with Ak4). Note that in many cases membranes were cut enabling the blotting of multiple antibodies.

Supplementary Table 1

Conditions for immunofluorescence staining of mitochondrial proteins in mouse cerebellum, cortex and spinal cord.

Epitope	Antibody				Tissue, mouse thickness (μm), treatment	
	#	Company	Species	Conc		
Acads	ab156571	Abcam	Rb mono	1:200	50	HIER Tris pH 10.0
Ak3	sc-398571	Santa Cruz	Ms mono	1:100	50	HIER Tris-EDTA pH 9.0
Ak4	sc-271161	Santa Cruz	Ms mono	1:100	50	HIER Tris-EDTA pH 9.0
Aβ (1-16)-AF647	803020	BioLegend	Ms mono	1:500	*	*
Aβ (17-24)	800712	BioLegend	Ms mono	1:1000	*	*
Catalase	100-4151	Rockland	Rb ser	1:200	50	HIER Tris pH 10.0
ChAT	AB144P	Sigma	Gt	1:500	*	*
Cox4i1	AP22111a	Abgent	Rb	1:200	50	HIER Citrate-EDTA pH 6.2
Cpt1a	15184-1-AP	ProteinTech	Rb	1:50	50	HIER TBS pH 9.0
GFP	ab13970	Abcam	Chi	1:1000	*	*
Gldc	HPA002318	Sigma	Rb	1:400	50	HIER Tris pH 10.0
Gls	20170-1-AP	ProteinTech	Rb	1:200	50	HIER Tris-EDTA pH 9.0
Gls2	AP6650D	Abgent	Rb	1:400	50	HIER Tris-EDTA pH 9.0
Got2	ab171739	Abcam	Rb mono	1:400	50	HIER Tris pH 10.0
Ldhd	ab182146	Abcam	Rb mono	1:100	50	HIER Tris pH 10.0
MaoB	sc-515354	Santa Cruz	Ms mono	1:100	50	HIER Tris pH 10.0
Mavs	#4983	CellSignaling	Rb	1:200	50	HIER EDTA pH 8.0
Mcu	HPA016480	Sigma	Rb	1:400	50	HIER EDTA pH 8.0
Nipsnap1	#13226	CellSignaling	Rb	1:200	50	HIER Tris-EDTA pH 9.0
Ociad2	HPA040979	Sigma	Rb	1:200	50	HIER Tris-EDTA pH 9.0
Ociad2	ab91576	Abcam	Rb	1:200	50	HIER Tris-EDTA pH 9.0
Pex14	-	Denis Crane	Rb	1:1000	30	0.2% Tx100/PBS
Pptc7	ab122548	Abcam	Rb	1:200	50	HIER TBS pH 9.0
Rmdn3	ab189845	Abcam	Rb	1:400	50	HIER Tris-EDTA pH 9.0
Sfxn5	ab172971	Abcam	Rb mono	1:500	30	0.2% Tx100/PBS
Snph	ab192605	Abcam	Rb mono	1:200	50	HIER Tris pH 10.0
Tst	ab166625	Abcam	Rb mono	1:500	30	0.2% Tx100/PBS

Species: Rb, rabbit; Ms, mouse; Chi, chicken; Gt, goat; Ser, serum; mono, monoclonal; *, dependent on co-staining. HIER, heat-induced antigen-retrieval: performed in a water bath at 90°C for 15 minutes (cortex and spinal cord), for 30 minutes (cerebellum). After HIER, GFP signals needed to be enhanced with anti-GFP antibody. Tx100, Triton X-100 diluted in PBS and section incubation at room temperature for 30 minutes.

Supplementary Table 2

Conditions for immunofluorescence staining of mitochondrial proteins in human cerebellum, cortex and spinal cord.

Antibody				Tissue, human			
Epitope	#	Company	Species	Concentration	Thickness (µm)	Treatment / HIER with	Detection
Aβ (8-17)	M0872	Dako	Ms mono	1:100	4	Tris-EDTA pH 9.0	AF
Cox4i1	AP22111a	Abgent	Rb	1:200	4	Tris-EDTA pH 9.0	HRP/TSA
Cpt1a	15184-1-AP	ProteinTech	Rb	1:50	4	Tris-EDTA pH 9.0	AF
Gfap	ab4674	Abcam	Chi	1:2000	4	Tris-EDTA pH 9.0	AF
Got2	ab171739	Abcam	Rb mono	1:2000	4	Tris-EDTA pH 9.0	AF
Mcu	HPA016480	Sigma	Rb	1:200	4	Tris-EDTA pH 9.0	HRP/TSA
NeuN-AF488	ab190195	Abcam	Rb	1:50	4	Tris-EDTA pH 9.0	conjugated
NeuN	MAB377	Sigma	Ms mono	1:100	4	Tris-EDTA pH 9.0	AF
Nipsnap1	#13226	CellSignaling	Rb	1:200	4	Tris-EDTA pH 9.0	AF
Ociad2	HPA040979	Sigma	Rb	1:500	4	Tris-EDTA pH 9.0	HRP/TSA
Rmdn3	ab189845	Abcam	Rb	1:2000	4	Tris-EDTA pH 9.0	AF
Sfxn5	ab172971	Abcam	Rb mono	1:2000	4	Tris-EDTA pH 9.0	AF

Species: Rb, rabbit; Ms, mouse; Chi, chicken; mono, monoclonal. HIER, heat-induced antigen-retrieval. HRP/TSA, Tyramide Amplification System with a secondary antibody coupled to HRP. AF, conventional secondary antibodies coupled to Alexa Fluor dyes.

Supplementary Table 3

Information to human tissue samples used in this study and their handling prior to the experiment according to BRISQ.

Cortex				
Case	Age	Sex	Histological specimen	shown in Figure
9	52	male	normal temporal lobe (hippocampus)	Fig. 4a, Supplementary Fig. 15b
10	67	male	normal temporal lobe (hippocampus)	Supplementary Fig. 15c
11	58	male	normal temporal lobe (hippocampus)	Supplementary Fig. 13c
14	88	female	temporal lobe with Alzheimer type degenerative encephlaopathie	Fig. 4c, Supplementary Fig. 13c
15	91	female	temporal lobe with Alzheimer type degenerative encephlaopathie	Fig. 4a, Supplementary Fig. 15c
16	82	male	temporal lobe with Alzheimer type degenerative encephlaopathie	Fig. 4c, Supplementary Fig. 13c

Spinal cord				
Case	Age	Sex	Histological specimen	shown in Figure
1	56	male	spinal cord without lesion	Supplementary Fig. 14b
2	52	male	spinal cord without lesion	Fig. 4a, Supplementary Fig. 15d
4	77	female	ALS spinal cord	Supplementary Fig. 14b
6	66	male	ALS spinal cord	Fig. 4e, Supplementary Fig. 14b

BRISQ summary	
Biospecimen type	<i>Solid tissue</i>
Anatomical site	<i>Brain, Spinal cord</i>
Disease status of patients	<i>Alzheimer, Amyotrophic lateral sclerosis, Non-neurological disease (control)</i>
Clinical characteristics of patients	<i>Dementia (Alzheimer), Tetraparesis (ALS)</i>
Vital State of patients	<i>Postmortem</i>
Clinical diagnosis of patients	<i>Alzheimer, Amyotrophic lateral sclerosis, Non-neurological disease (control)</i> <i>ALS type degeneration of motoneurons; Normal tissue (control)</i>
Pathology diagnosis	<i>Alzheimer type degenerative encephalopathy (Alzheimer);</i>
Collection mechanism	<i>Autopsy</i>
Type of stabilization	<i>4 degree</i>
Type of long-term preservation	<i>Formalin Fixation</i>
Constitution of preservative	<i>10% neutral-buffered formalin</i>
Storage temperature	<i>20 to 25 °C</i>
Storage duration	<i>The time or range thereof between biospecimen acquisition and distribution or analysis.</i>
Shipping temperature	<i>20 to 25 °C</i>
Composition assessment & selection	<i>ALS: Motoneuron loss with TDP43+ inclusions (spinal cord);</i> <i>Alzheimer: Pathologique neuritiques plaques and neurofibrillary degeneration;</i> <i>control: no microscopic alterations</i>

Comprehensive information on the statistical analysis used in this publication.

Figure

Figure 1b	Expression pattern	5
Figure 1c	IF staining	5
Figure 1d	IF staining/EM	2
Figure 1f	EM	2

Figure 1g / I

CE	3	Cre-/IC Tom	4	Cre+/IC Tom	5	Cre+/IC GFP	6
RCR	3	Cre-/IC Tom	4	Cre+/IC Tom	5	Cre+/IC GFP	6
SCR	4	Cre-/IC Tom	5	Cre+/IC Tom	6	Cre+/IC GFP	7

One-way ANOVA, Tukey's <i>posthoc</i>	0.0836	2,12	0.9203	ns
One-way ANOVA, Tukey's <i>posthoc</i>	0.5447	2,12	0.5937	ns
One-way ANOVA, Tukey's <i>posthoc</i>	0.62	2,15	0.5512	ns

Figure 1g / II

CE	3	Cre-/IC Tom	4	Cre+/IC Tom	6	Cre+/IC GFP	6
RCR	3	Cre-/IC Tom	4	Cre+/IC Tom	6	Cre+/IC GFP	6
SCR	4	Cre-/IC Tom	6	Cre+/IC Tom	6	Cre+/IC GFP	6

One-way ANOVA, Tukey's <i>posthoc</i>	0.4186	2,13	0.6665	ns
One-way ANOVA, Tukey's <i>posthoc</i>	0.9171	2,13	0.4241	ns
One-way ANOVA, Tukey's <i>posthoc</i>	0.3878	2,15	0.6851	ns

Figure 2b

WB - GFP-OMM	1
WB - mito-RFP	1
WB - Mix (50:50)	5

Representative experiment for quantification in Figure 2c

Figure 2c

GFP-OMM	5	IC Tom	5	IC GFP	5	-
mito-RFP	5	IC Tom	5	IC GFP	5	-

One-tailed, ratio paired <i>t</i> -test	11.03	4	0.0002	***
One-tailed, ratio paired <i>t</i> -test	2.411	4	0.0368	*

Figure 2d

GFP-OMM	3	IC Tom	5	IC GFP	5	-
mito-RFP	3	IC Tom	5	IC GFP	5	-

One-tailed, ratio paired <i>t</i> -test	10.33	4	0.0002	***
One-tailed, ratio paired <i>t</i> -test	1.867	4	0.0677	ns

Figure 3a

IF staining

3

Figure 3c

Mass spectrometry

3

PC

6

GC

5

A

6

Figure 3d-f

IF staining

3

See Supplementary Figure 7: LC-MS/MS data analysis

Figure 4a

IF staining/Mouse

2

IF staining/Human

1

Figure 4b

IF staining

2

Figure 4c

IF staining/Human

1

Figure 4d

IF staining

2

Figure 4e

IF staining/Human

1

Figure 5b

IF staining

3

Figure 5c

CE	12	PC	10	A	11	-
RCR	12	PC	9	A	11	-
SCR	12	PC	9	A	9	-

One-tailed, unpaired Welch's test	2.092	11,48	0.0297	**
One-tailed, unpaired Welch's test	5.028	16,83	<0.0001	***
One-tailed, unpaired Welch's test	5.228	15,86	<0.0001	***

Figure 5d

CE	9	PC	8	A	6	-
RCR	9	PC	8	A	6	-
SCR	9	PC	8	A	6	-

One-tailed, unpaired Welch's test	0.8558	6,352	0.2116	ns
One-tailed, unpaired Welch's test	0.8953	8,527	0.1976	ns
One-tailed, unpaired Welch's test	1.382	10,87	0.0973	ns

Figure 5e

CE	9	PC	8	A	6	-
RCR	9	PC	7	A	6	-
SCR	9	PC	8	A	6	-

One-tailed, unpaired Welch's test	0.1861	11,21	0.4279	ns
One-tailed, unpaired Welch's test	0.6322	7,615	0.2729	ns
One-tailed, unpaired Welch's test	1.072	9,102	0.1556	ns

Figure 6b

IF staining

3

Figure 6c

Mcu	4	PC	5	GC	5	A	5
	4	PC	5	GC	5	-	
	4	PC	5	-	-	A	5
	4	-	-	GC	5	A	5

One-way Friedman test, Dunn's <i>posthoc</i>	-	-	0.0008	***
Dunn's <i>posthoc</i>	-10	-	0.0047	**
Dunn's <i>posthoc</i>	-5	-	0.3415	ns
Dunn's <i>posthoc</i>	5	-	0.3415	ns

Figure 6d

Ca2+ uptake assay

Representative experiment for quantification in Figure 6e

Figure 6e

ΔAUC	8	PC	7	GC	5	A	7
	8	PC	7	GC	5	-	
	8	PC	7	-	-	A	7
	8	-	-	GC	5	A	7

One-way ANOVA, Tukey's <i>posthoc</i>	8.881	2,16	0.0025	**
Tukey's multiple comparisons test	5.944	16	0.0018	**
Tukey's multiple comparisons test	3.108	16	0.102	ns
Tukey's multiple comparisons test	3.106	16	0.1023	ns

Figure 6f

Mcu	6	Mcu +/-	4	Mcu +/-	6	Mcu -/-	8
	6	Mcu +/-	4	Mcu +/-	6	-	
	6	Mcu +/-	4	-	-	Mcu -/-	8
	6	-	-	Mcu +/-	6	Mcu -/-	8

One-way REML, Holm-Sidak's <i>posthoc</i>	382.7	1,023, 6,14	<0.0001	***
Tukey's multiple comparisons test	11.4	5	<0.0001	***
Tukey's multiple comparisons test	115	5	<0.0001	***
Tukey's multiple comparisons test	13.74	7	<0.0001	***

Figure 6g

IF staining

3

Figure 6h

Ca2+ uptake assay

Representative experiment for quantification in Figure 6i

Figure 6i

ΔAUC	6	Mcu +/-	5	Mcu +/-	6	Mcu -/-	7
	6	Mcu +/-	5	Mcu +/-	6	-	
	6	Mcu +/-	5	-	-	Mcu -/-	7
	6	-	-	Mcu +/-	6	Mcu -/-	7

One-way ANOVA, Tukey's <i>posthoc</i>	33.99	2,15	<0.0001	***
Tukey's multiple comparisons test	2.073	15	0.3344	ns
Tukey's multiple comparisons test	10.64	15	<0.0001	***
Tukey's multiple comparisons test	8.947	15	<0.0001	***

Figure 7a

IF staining

3

Figure 7b

mitochondria	1	PC	160	GC	134	A	120
		PC	160	GC	134	-	
	1	PC	160	-	-	A	120
	1	-	-	GC	134	A	120
cells	1	PC	10	GC	74	A	16

One-way Kruskal-Wallis, Dunn's <i>posthoc</i>	-	-	<0.0001	***
Dunn's <i>posthoc</i>	215.4	-	<0.0001	***
Dunn's <i>posthoc</i>	131.5	-	<0.0001	***
Dunn's <i>posthoc</i>	-83.9	-	<0.0001	***
-	-	-	-	-

Figure 7c

Rmdn3	3	Rmdn3 +/-	4	Rmdn3 +/-	4	-
	3	Rmdn3 +/-	4	Rmdn3 -/-	4	-
	3	Rmdn3 +/-	4	Rmdn3 -/-	4	-

Two-tailed, paired <i>t</i> -test	16.05	3	0.0005	***
Two-tailed, paired <i>t</i> -test	25.63	3	0.0001	***
Two-tailed, paired <i>t</i> -test	20.72	3	0.0002	***

Figure 7d

IF staining

2

Figure 7e

PC cells	2	Rmdn3 +/-	12	Rmdn3 +/-	8	Rmdn3 -/-	14
	2	Rmdn3 +/-	12	Rmdn3 +/-	-	-	
	2	Rmdn3 +/-	12	-	-	Rmdn3 -/-	14
	2	-	-	Rmdn3 +/-	8	Rmdn3 -/-	14
A cells	2	Rmdn3 +/-	12	Rmdn3 +/-	10	Rmdn3 -/-	11
PC mitochondria	2	Rmdn3 +/-	207	Rmdn3 +/-	189	Rmdn3 -/-	306
A mitochondria	2	Rmdn3 +/-	135	Rmdn3 +/-	105	Rmdn3 -/-	119

One-way ANOVA, Tukey's <i>posthoc</i>	37.45	2,31	<0.0001	***
Tukey's multiple comparisons test	5.254	31	0.0022	**
Tukey's multiple comparisons test	12.23	31	<0.0001	***
Tukey's multiple comparisons test	5.444	31	0.0016	**
One-way ANOVA, Tukey's <i>posthoc</i>	2.567	2,30	0.0935	ns
-	-	-	-	-
-	-	-	-	-

independent experiments

Supplementary Table 4 (continued)

Comprehensive information on the statistical analysis used in this publication.

Supplementary Figure	Condition	Exp.#	A	N	B	N	C	N	Test	F/t	df	P value	
Figure 1a-f	Expression pattern	5											
Figure 2a	Antero	10	Cre -	30	Cre +	29	-		Two-tailed, unpaired Welch's test	0.2044	54.14	0.8388	ns
	Retro	10	Cre -	30	Cre +	29	-		Two-tailed, unpaired Welch's test	1.288	53.03	0.2034	ns
Figure 2b	Length	10	Cre -	171	Cre +	222	-		Two-tailed, unpaired Welch's test	0.5751	353.9	0.5658	ns
	Width	10	Cre -	170	Cre +	222	-		Two-tailed, unpaired Welch's test	1.595	343.8	0.1117	ns
Figure 2c	IF staining	1											
Figure 2d	NMJ size	8	Cre -	88	Cre +	83	-		Two-tailed, unpaired Welch's test	0.9931	168.9	0.3221	ns
	AChR density	8	Cre -	56	Cre +	54	-		Two-tailed, unpaired Welch's test	0.845	98.37	0.4002	ns
Figure 2e / I	CE	3	Cre -	5	Cre +	5	-		Two-tailed, unpaired Welch's test	0.3236	7.449	0.7551	ns
	RCR	3	Cre -	5	Cre +	5	-		Two-tailed, unpaired Welch's test	0.1959	6.403	0.8507	ns
	SCR	3	Cre -	5	Cre +	5	-		Two-tailed, unpaired Welch's test	0.5323	6.348	0.6126	ns
Figure 2e / II	CE	3	Cre -	5	Cre +	5	-		Two-tailed, unpaired Welch's test	0.5852	4.336	0.5875	ns
	RCR	3	Cre -	5	Cre +	5	-		Two-tailed, unpaired Welch's test	0.4233	6.431	0.6859	ns
	SCR	3	Cre -	5	Cre +	5	-		Two-tailed, unpaired Welch's test	0.03813	5.644	0.9709	ns
Figure 3c	Violin plot/MS data	1	Cerebellum	4	IC	34	-						
Figure 3d	iBAQ abundance	1	IC Tom/GFP	6	-		-						
Figure 5a	WB	Representative experiment for quantification in Figure 2c											
Figure 5b	WB	Representative experiment for quantification in Supplementary Figure 6c											
Figure 5c	WB quantification	4											
Figure 7a	Mass spectrometry	3	-		GC	5	A	6	See Supplementary Figure 7: LC-MS/MS data analysis				
Figure 7b	Mass spectrometry	3	PC	6	-		A	6	See Supplementary Figure 7: LC-MS/MS data analysis				
Figure 7c	Mass spectrometry	3	PC	6	GC	5	-		See Supplementary Figure 7: LC-MS/MS data analysis				
Figure 7d	Mass spectrometry	3	PC	6	GC	5	A	6	See Supplementary Figure 7: LC-MS/MS data analysis				
Figure 7e, f	ORA in WebGestalt	3	PC	6	GC	5	A	6	See LC-MS/MS data analysis				
Figure 9a-d	IF staining	3											
Figure 10a	WB	3	PC	4	GC	4	-	-					
Figure 11b, c	IF staining	2											
Figure 12a	IF staining	2											
Figure 12b	IF staining	2											
Figure 13a	IF staining	2											
Figure 13b	IF staining	2											
Figure 13c	IF staining/Human	1											
Figure 14a	IF staining	2											
Figure 14b	IF staining/Human	1											
Figure 15a-d	IF staining/Human	1											
Figure 16a / FA	CE	6	IC Tom	9	IC GFP	9	-		One-tailed, paired <i>t</i> -test	5.161	8	0.0004	***
	RCR	6	IC Tom	9	IC GFP	9	-		One-tailed, paired <i>t</i> -test	5.357	8	0.0003	***
	SCR	6	IC Tom	10	IC GFP	10	-		One-tailed, paired <i>t</i> -test	4.459	9	0.0008	***
Figure 16a / I	CE	6	IC Tom	8	IC GFP	8	-		One-tailed, paired <i>t</i> -test	2.945	7	0.0108	*
	RCR	6	IC Tom	8	IC GFP	8	-		One-tailed, paired <i>t</i> -test	2.797	7	0.0133	*
	SCR	6	IC Tom	8	IC GFP	8	-		One-tailed, paired <i>t</i> -test	2.329	7	0.0263	*
Figure 16a / II	CE	6	IC Tom	8	IC GFP	8	-		One-tailed, paired <i>t</i> -test	0.6286	7	0.2748	ns
	RCR	6	IC Tom	7	IC GFP	7	-		One-tailed, paired <i>t</i> -test	1.165	6	0.1441	ns
	SCR	6	IC Tom	8	IC GFP	8	-		One-tailed, paired <i>t</i> -test	0.3991	7	0.3509	ns
Figure 16b / FA	CE	6	IC Tom	11	IC GFP	11	-		One-tailed, paired <i>t</i> -test	0.6287	10	0.2718	ns
	RCR	6	IC Tom	11	IC GFP	11	-		One-tailed, paired <i>t</i> -test	1.576	10	0.073	*
	SCR	6	IC Tom	9	IC GFP	9	-		One-tailed, paired <i>t</i> -test	0.194	8	0.0064	**
Figure 16b / I	CE	3	IC Tom	6	IC GFP	6	-		One-tailed, paired <i>t</i> -test	0.7953	5	0.2312	ns
	RCR	3	IC Tom	6	IC GFP	6	-		One-tailed, paired <i>t</i> -test	1.95	5	0.0544	ns
	SCR	3	IC Tom	6	IC GFP	6	-		One-tailed, paired <i>t</i> -test	0.1192	5	0.4549	ns
Figure 16b / II	CE	3	IC Tom	6	IC GFP	6	-		One-tailed, paired <i>t</i> -test	0.8611	5	0.2143	ns
	RCR	3	IC Tom	6	IC GFP	6	-		One-tailed, paired <i>t</i> -test	0.1837	5	0.4307	ns
	SCR	3	IC Tom	6	IC GFP	6	-		One-tailed, paired <i>t</i> -test	1.533	5	0.0929	ns
Figure 17a	EM	1	PC		-		A						
Figure 17b	EM	2	PC Rmdn3 -/-		-		-						

independent experiments

independent experiments