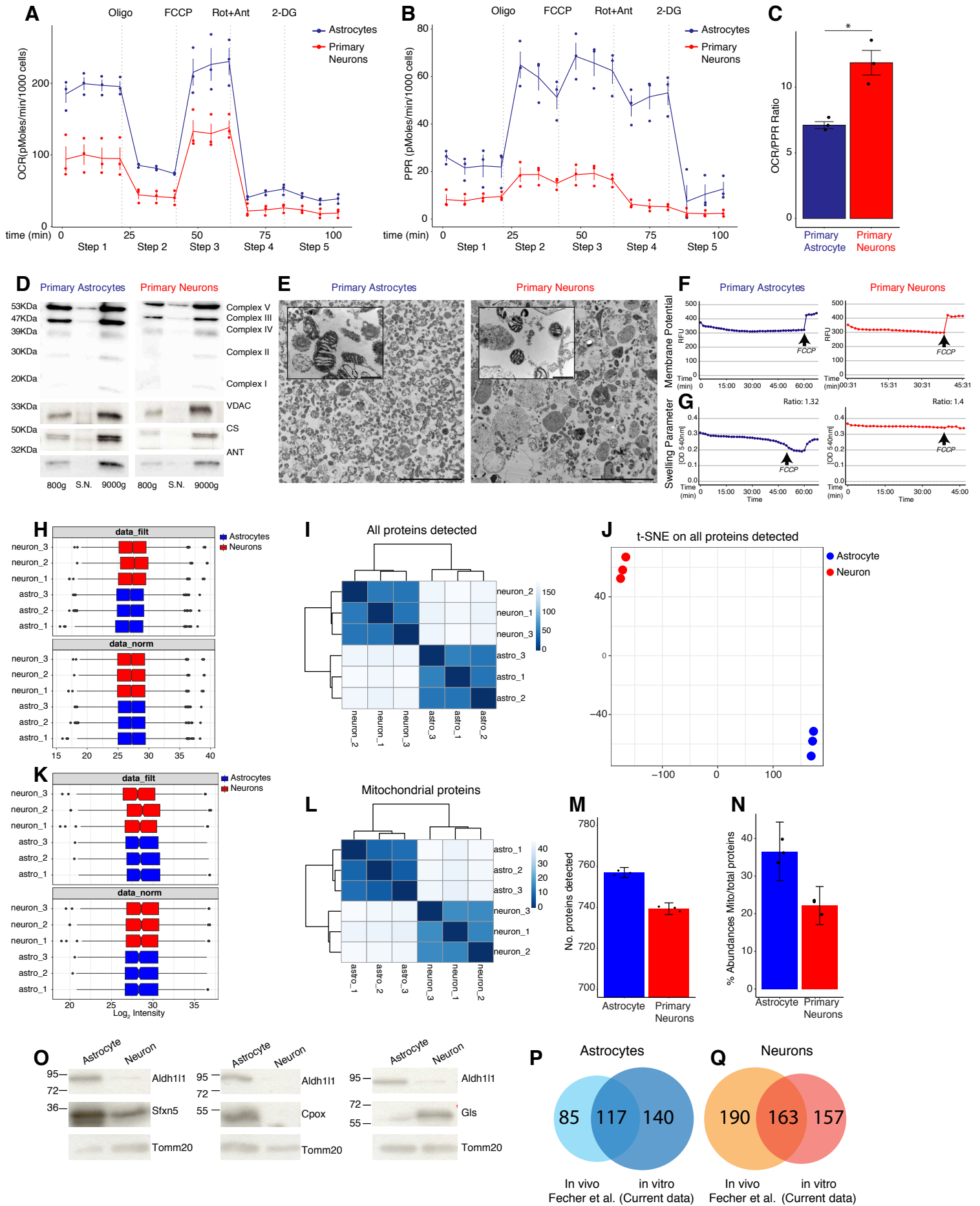


Supplemental Information

CRISPR-Mediated Induction of Neuron-Enriched Mitochondrial Proteins Boosts Direct Glia-to-Neuron Conversion

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Figure S1



SUPPLEMENTAL MATERIAL

Figure S1, related to Figure 1. Astrocytes and neurons from cerebral cortex differ in mitochondrial function

- (A-B) Longitudinal traces of extracellular flux analysis measured by Seahorse XF analyzer, comparing the Oxygen Consumption Rate (A) and the Proton Production Rate (B) of astrocytes (blue) versus neurons (red) over time, after challenging the cells with different ETC inhibitors. Values are normalized per 1000 cells. Each time point is shown as mean $\pm$ SD. n=3 experimental batches for each group.
- (C) Barplot showing OCR/PPR ratio in cultures of primary astrocytes versus neurons, as measured by Seahorse XF analyzer. * $p \leq 0.05$. n=3 for each group.
- (D) Immunoblot detection of mitochondrial proteins in different fractions (800g, nuclear; S.N., cytosolic; 9000g, mitochondria and other organelles) isolated from astrocytes or neurons.
- (E) Electron Microscopy images of mitochondria isolated from astrocyte and neuron cultures. Scale bar: 5 μ m. Magnifications scale bar: 500nm.
- (F) Graphs showing the membrane potential of mitochondria isolated from astrocytes (*upper panel*) and neurons (*lower panel*) measured by Rhodamine 123 assay, indicating their healthy functional state.
- (G) Graphs showing the swelling parameter of mitochondria isolated from astrocytes (*upper panel*) and neurons (*lower panel*) by absorbance at 540nm indicating their healthy functional state.
- (H) Boxplot depicting abundances of all proteins before (*upper panel*) and after (*lower panel*) normalization.
- (I) Unsupervised cluster analysis of the samples considering all quantified proteins.
- (J) *t*-SNE of the samples, based on all proteins after normalization.
- (K) Boxplot depicting abundances of mitochondrial proteins selected according to mitoCarta before (*upper panel*) and after (*lower panel*) normalization.
- (L) Unsupervised cluster analysis of the samples considering only mitochondrial proteins.
- (M) Barplot showing the number of mitochondrial proteins identified by mass spec in astrocytes (blue) and neurons (red). Each dot represents a biological replicate.
- (N) Barplot depicting the percentage of mitochondrial protein abundance over total protein abundance in astrocytes (blue) and neurons (red). Each dot represents a biological replicate.
- (O) Western blots of total lysates from cultured astrocytes or neurons confirming the selective cell enrichment by high amounts of Adlh111 in astrocyte lysates, equal mitochondrial protein loading by Tomm20 and the higher amount of Sfxn5 and CpoX in astrocytes and Glis in neurons.
- (P,Q) Venn Diagrams of all astrocyte-enriched (I) and neuron-enriched (J) mitochondrial proteins detected by Fecher et al. and our analysis showing a high degree of overlap given that mitochondrial proteins were isolated at different stages (adult versus postnatal), from different regions (cerebellum versus cortex) and in different conditions (*in vivo* versus *in vitro*).

Figure S2

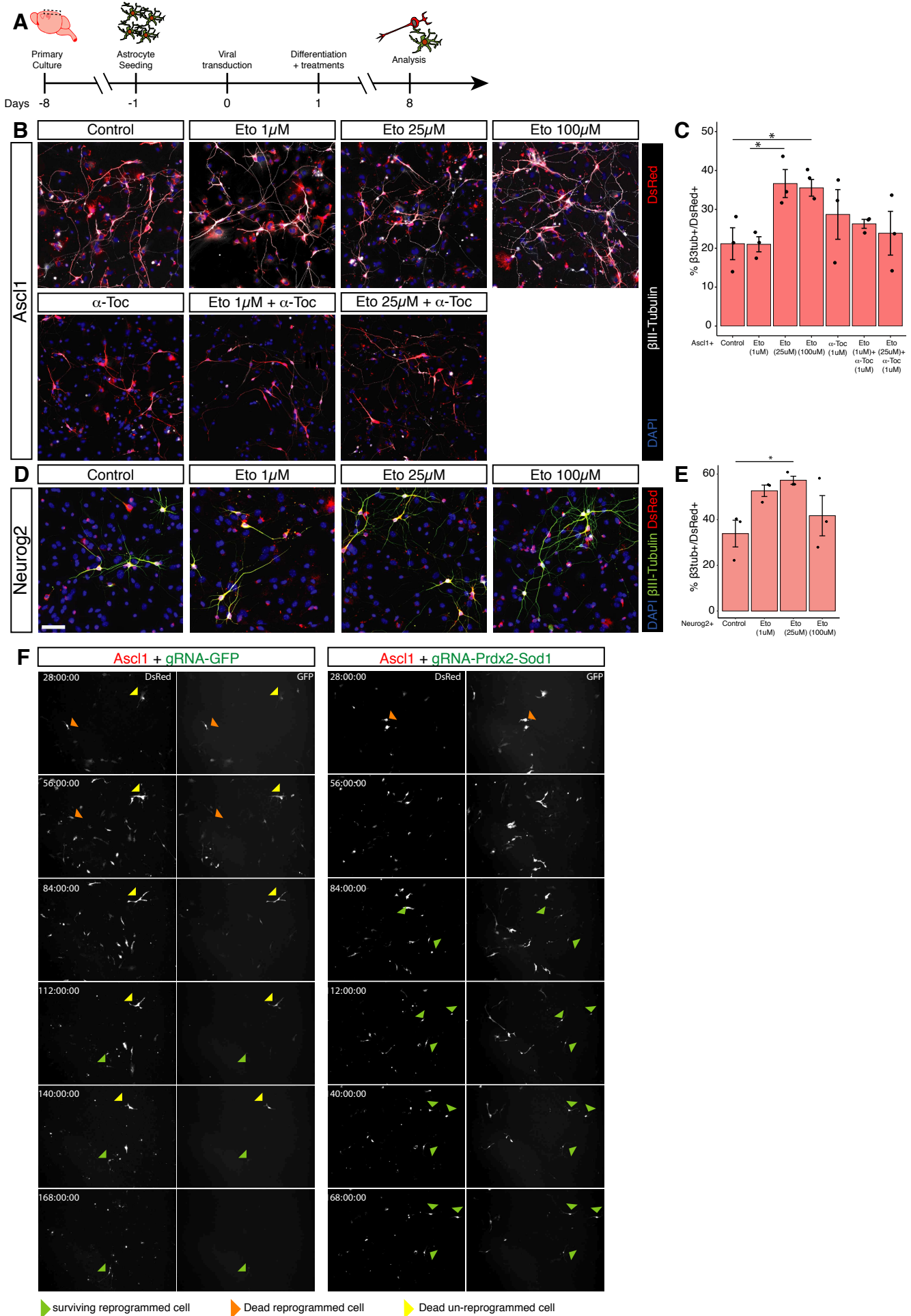


Figure S2, related to Figure 1 and 4. Etomoxir treatment improves direct neuronal reprogramming.

(A) Schematic drawing of the experimental setup.

(B) Micrographs showing the Ascl1-transduced cells (DsRed⁺) that are β III-tubulin⁺ neurons (in white), nuclei labeled in DAPI (Blue), in the treatment conditions indicated. Scale bar: 50 μ m.

(C) Histogram depicting the percent of β III-tubulin⁺ cells amongst Ascl1-transduced DsRed⁺ cells at 8 DPI. Data are shown as mean $\pm$ SEM. Each dot represents a biological replicate (n=3 for experimental condition). *p $\leq$ 0.05

(D) Micrographs showing the efficiency of neuronal conversion in upon Neurog2 expression together with treatment of different concentration of Etomoxir. Reprogrammed neurons (DsRed⁺) are β III-tubulin⁺ (in white). Nuclei labeled in DAPI (Blue). Scale bar: 50 μ m.

(E) Histogram depicting the percentage of β III-tubulin⁺ cells amongst Neurog2-transduced DsRed⁺ cells at 8 DPI. Data are shown mean $\pm$ SEM. Each dot represents a biological replicate (n=3 for experimental condition). *p $\leq$ 0.05

(F) Example of fluorescent pictures acquired during the imaging and used for analysis (related to **Figure 4**).

Figure S3

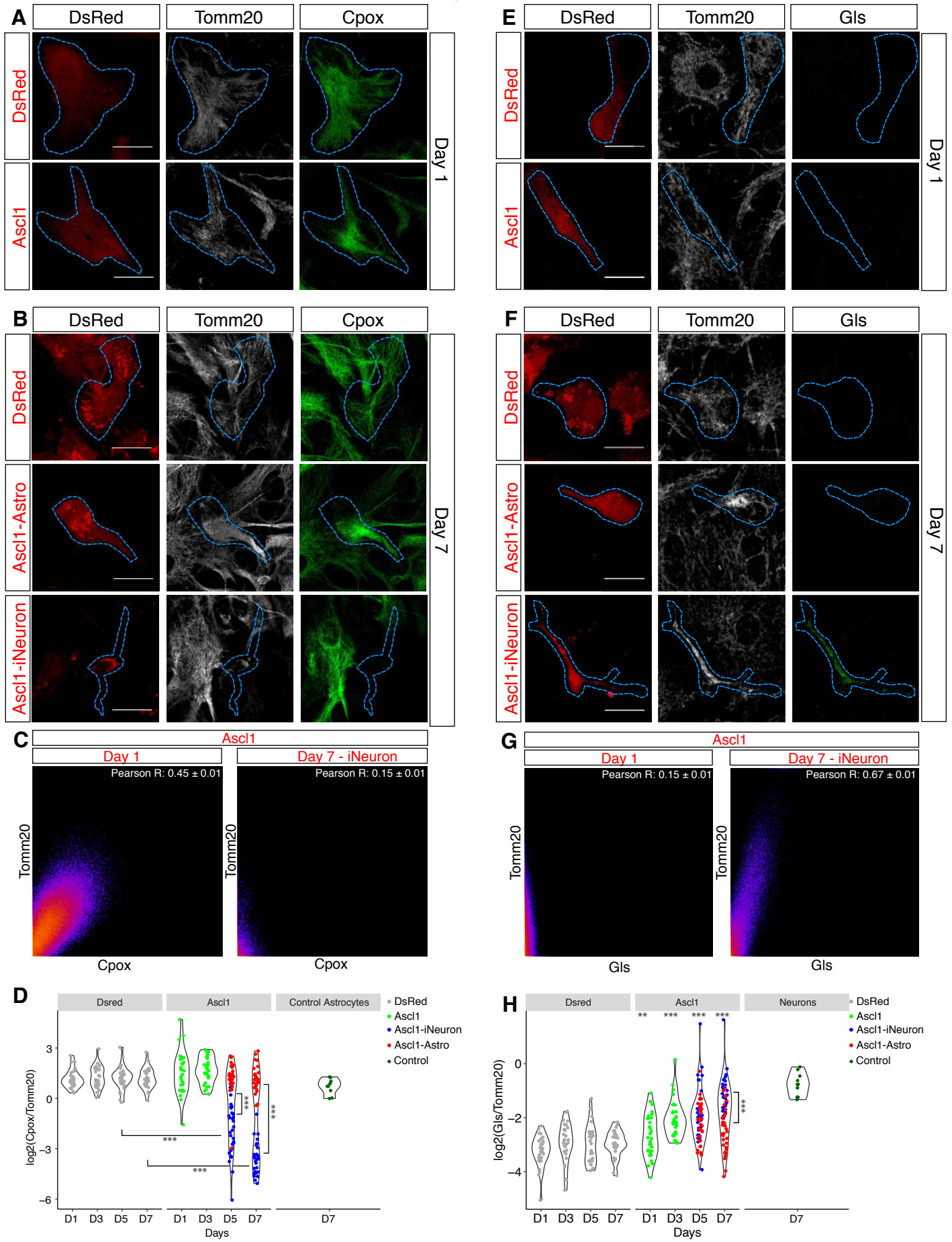


Figure S3, related to Figure 2. Mitochondrial protein changes during astrocyte-to-neuron reprogramming

(A, B) Micrographs showing immunostainings of the astrocyte-enriched mitochondrial protein Cpx in astrocytes transduced with DsRed (control) or Ascl1-ires-DsRed at 1 (A) or 7 (B) DPI as indicated. Mitochondria are identified by the expression of Tomm20. Scale bar: 20µm.

(C) Example of scatter plot of the pixel intensity correlation between Tomm20 and Cpx in Ascl1-transduced cells at 1 (*left panel*) and 7 (*right panel*) DPI. Pearson's coefficient as average of 3 cells/biological replicate; n=3 biological replicates.

(D) Violin plot depicting the log2-ratio of the intensity of the expression of Cpx versus Tomm20 over time (D1, D3, D5, D7) and in cortical astrocyte cultures at day 7. Each dot represents 1 analyzed cell. 10 cells analyzed per biological replicate, each condition. n=3 biological replicates; ***p ≤ 0.001.

(E, F) Micrographs showing the expression of the neuron-specific mitochondrial protein Prdx2 in astrocytes transduced with DsRed (control) or Ascl1-ires-DsRed at 1 (E) and 7 (F) DPI. Mitochondria are identified by the expression of Tomm20. Scale bar: 20µm.

(G) Example of scatter plot of the pixel intensity correlation between Tomm20 and Glis in Ascl1-transduced cells at 1 (*left panel*) and 7 (*right panel*) DPI. Pearson's coefficient as average of 3 cells/biological replicate; n=3 biological replicates.

(H) Violin plot depicting the log2-ratio of the intensity of the expression of Glis versus Tomm20 over time (D1, D3, D5, D7) and in E14 cortex-derived cultures at 7 days in vitro. Each dot represents 1 analyzed cell. 10 cells analyzed per biological replicate, each condition. n=3 biological replicates; ***p ≤ 0.001.

Figure S4

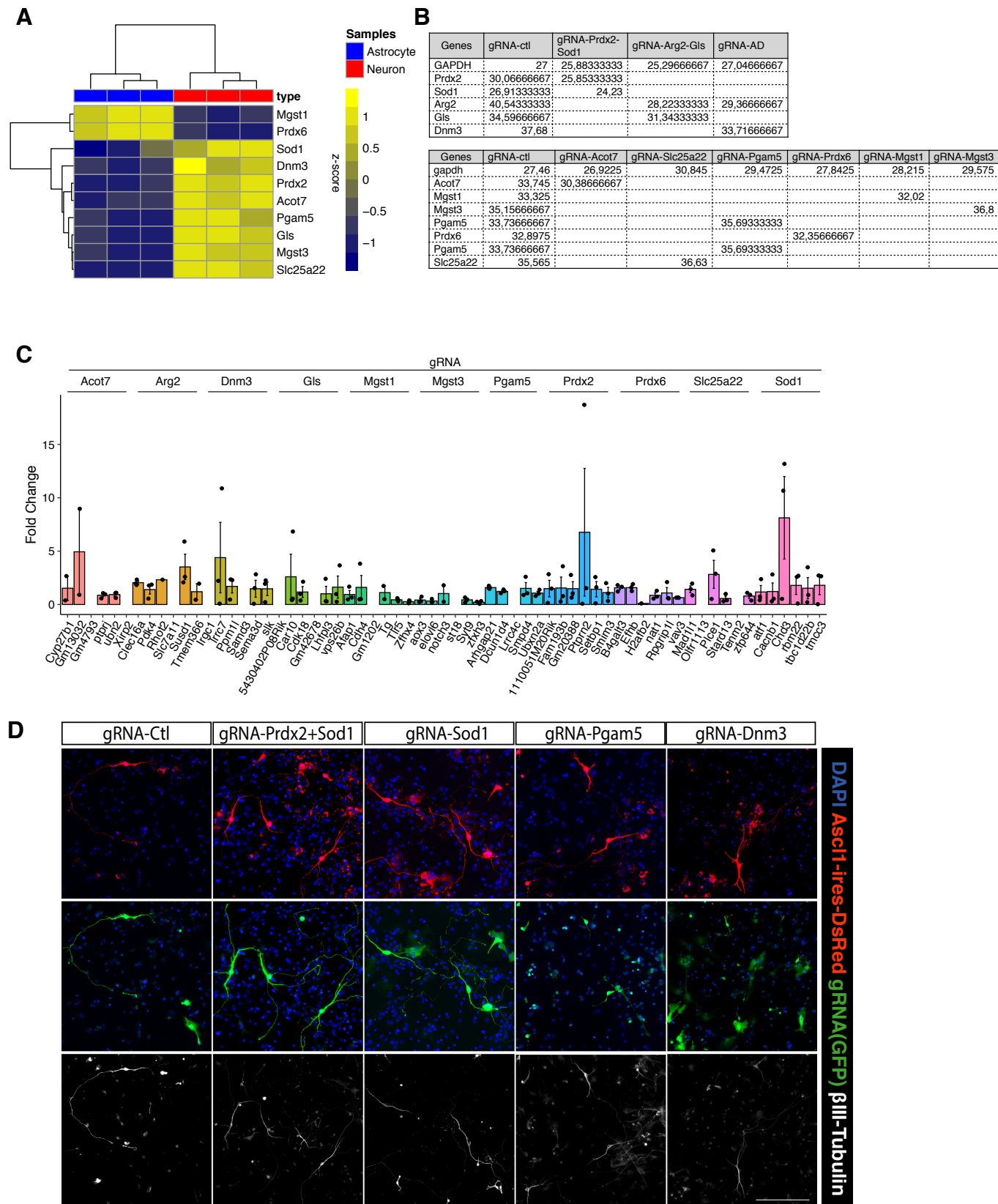


Figure S4, related to Figure 3. Characterization of selected candidates

(A) Unsupervised heatmap depicting the relative expression of the selected candidates.

(B) Graphs depicting the log₂-normalized abundance of the astrocyte-enriched (blue dots) and neuron-enriched (red dots) candidates, as analyzed in Figure 2. Each dot represents a biological replicate.

(C) Real Time quantitative PCR (RT-qPCR) showing the fold change of putative off-targets following the transfection of gene-specific gRNA. Data are shown as fold change over the gRNA-scramble control (mean $\pm$ SEM). None of this is significant over Paired t-test used. control. n=3 biological replicates for each group.

(D) Single channel immunofluorescence images showing reprogrammed neurons (β III-tubulin⁺-DsRed⁺-GFP⁺) transfected by Ascl1-ires-DsRed (red) and different STAgR constructs (green). Scale bar: 100 μ m.