Supplemental Information

Supplemental Figures

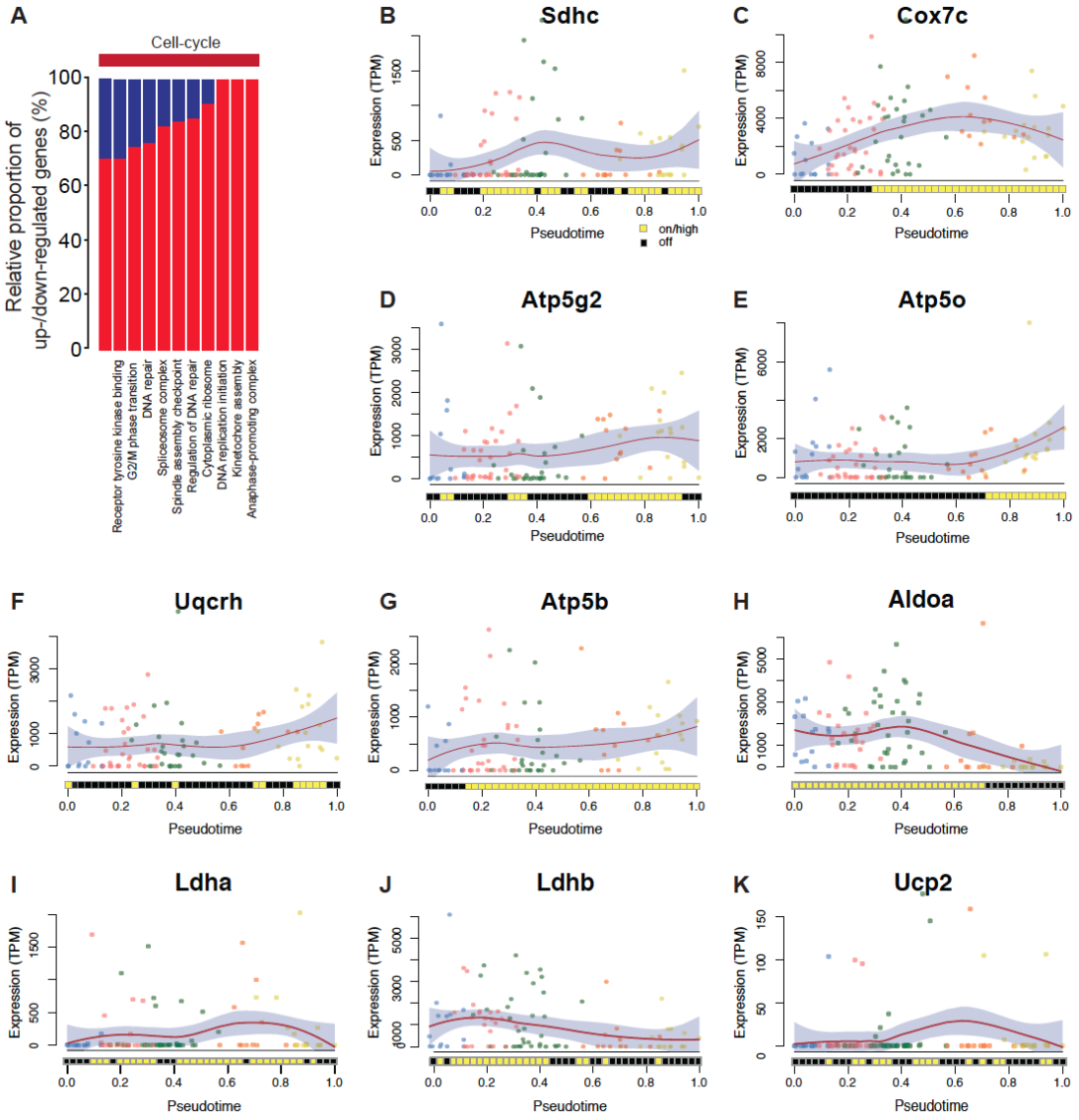

Figure S1: Biological GO entity enrichment test for upregulated and downregulated genes over early neurogenesis, related to Figure 1

(A) Proportion of upregulated and downregulated genes related to cell cycle. (B-K) Single cell resolution gene expression profile throughout pseudotime of six representative ETC and oxPhos genes (B-G), three genes related to glycolysis (H-J), and a mitochondrial uncoupling protein (K). Each data point represents the gene expression level (transcript per million reads or TPM) of each single cell. The trend line represents the linear regression curve with 95% confidence shown as gray area.

The yellow-black heatmaps under each graph represent hidden Markov model predicted binomial gene expression states throughout pseudotime.

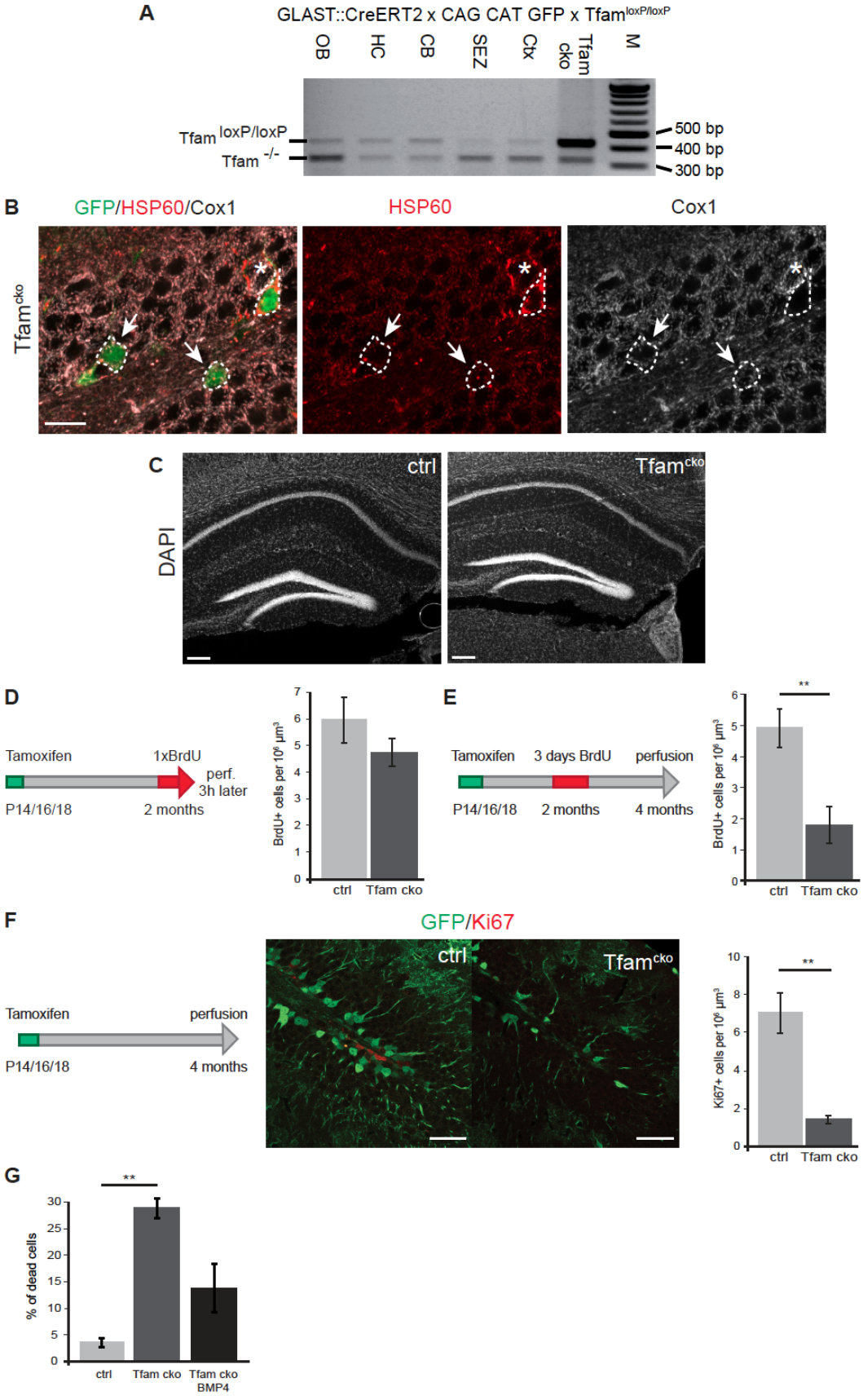

Figure S2: Tfam deficiency impairs proliferation in adult DG, related to Figure 3

(A) Tfam genotyping PCR of distinct isolated brain regions of Tamoxifen treated Tfam^{cko} mice (GLAST::CreERT2; CAG CAT GFP; Tfam$^{fl/fl}$) indicate recombination throughout the brain (OB=olfactory bulb; HC=hippocampus; CB=cerebellum; SEZ=subependymal zone; Ctx=cortex; Tfam cko=whole brain). (B) At 4 months of age, *Tfam^{cko}* mice show variability in the mitochondrial phenotype of recombined cells (green; dotted white line): while some cells lack Cox1 expression (white) and display clumped mitochondria that were brightly positive for HSP60 (red; asterisk), others still express Cox1 and have normal mitochondria morphology (arrows). (C) Hippocampus of 4 month-old *Tfam^{cko}* mice does not show morphological abnormalities as revealed by DAPI staining (grey). (D) Experimental scheme of a single BrdU pulse to evaluate proliferation. Two month-old Tfam^{cko} and control mice display comparable number of BrdU^{+} cells indicating that proliferation was not affected by Tfam-deletion at this time-point. (E) Experimental scheme of BrdU pulse-chase experiment to evaluate long-term survival of cells generated in 2 month-old mice. The number of BrdU^{+} cells was greatly reduced in 4 month-old Tfam^{cko} mice indicating that survival of cells, which were generated in 2 month-old mice, was impaired by Tfam-deletion. (F) Experimental scheme, confocal images and quantification of Ki67 (red) and reporter-GFP^{+} (green) cells in 4 month-old animals showed significantly reduced number of proliferating cells in *Tfam^{cko}* mice. (G) Cell death was reduced in in BMP-silenced *Tfam^{cko}* cells compared to proliferating *Tfam^{cko}* cells. (D) n_{ctrl} = 6, n_{cko} = 6; (E) n_{ctrl} = 7, n_{cko} = 6; (F) n_{ctrl} = 7, n_{cko} = 4. Data represented as mean ± SEM; t-test was performed to check significance; scale bars (B) = 10µm, (C)= 100µm, (F) = 20µm.

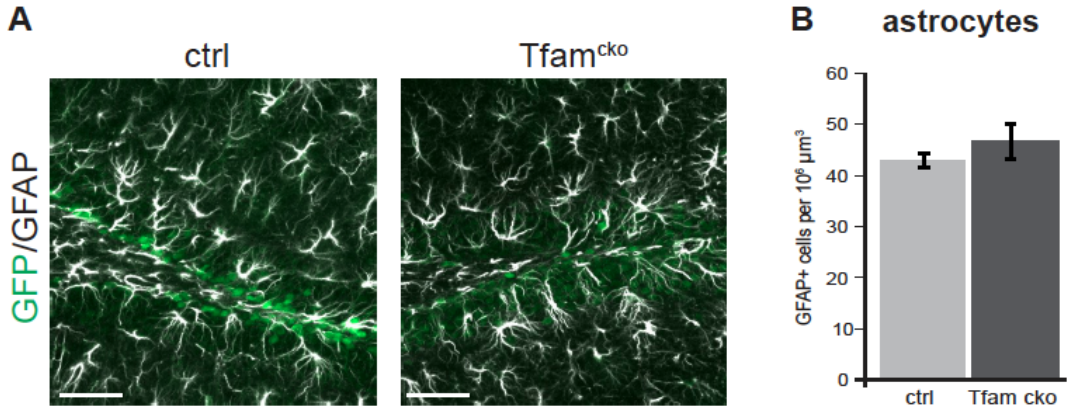

Figure S3: Deletion of Tfam does not affect astrocyte numbers in the DG, related to Fig. 3

(A, B) Confocal pictures and quantification of GFAP-expressing astrocytes (white) in the DG of ctrl and *Tfam^{cko}* mice did not reveal changes in their number and overall

appearance. GFP-reporter positive cells are shown in green. (B) n_{ctrl} = 3, n_{cko} = 4. Data represented as mean ± SEM; t-test was performed to check significance. Scale bars = 20µm.

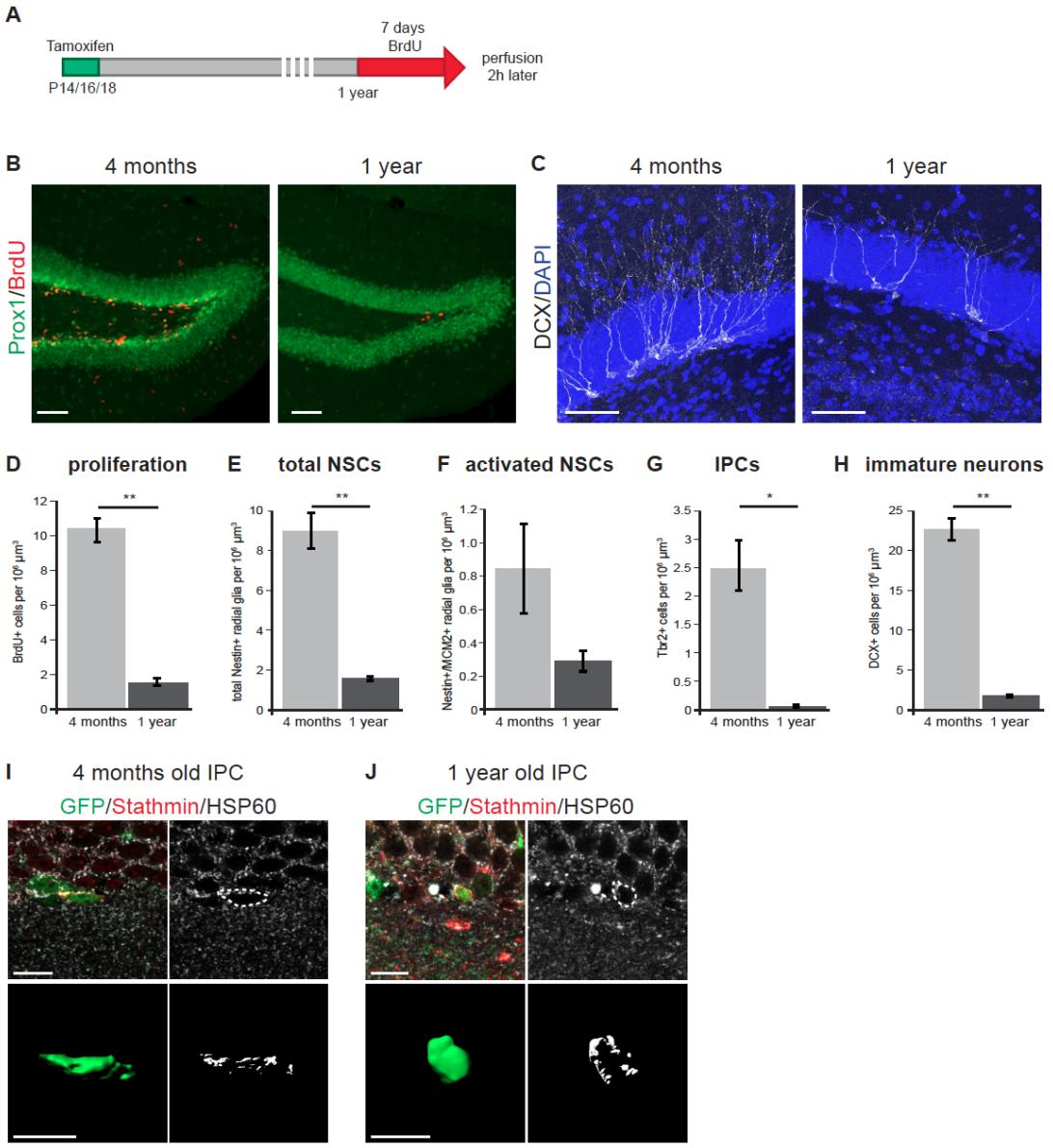

Figure S4: Impact of ageing on hippocampal neurogenesis, related to Figure 4

(A) Experimental scheme of BrdU-paradigm used in (B, D). (B, C) Confocal picture of $BrdU^+/Prox1^+$ newly generated neurons (B) and DCX expressing neuroblasts (C) in young and middle-aged mice (GLAST::CreERT2; CAG CAT GFP). (D) 1 year-old mice showed a significant reduction in the number BrdU-expressing cells. (E-H) Total number of NSCs (E), activated NSCs (F), IPCs (G), and DCX+ cells (H) were decreased in middle-aged animals. (I, J) Comparison of $HSP60^+$ (white) mitochondria in IPCs of young and middle-aged mice; IPC are GFP^+ (green; GLAST::CreERT2; CAG CAT GFP) and co-express Stathmin (red). Lower panels of each cube

represent reconstructions for GFP (green; lower left) and HSP60 (white; lower right). (D, F, H) $n_{4\ months}$ = 3, $n_{1\ year}$ = 4; (E, G) $n_{4\ months}$ = 3, $n_{1\ year}$ = 3 (Table S1). Data represented as mean ± SEM; t-test was performed to determine significance; scale bars (B) = 50µm, (C) = 20µm, (I, J) = 10µm.

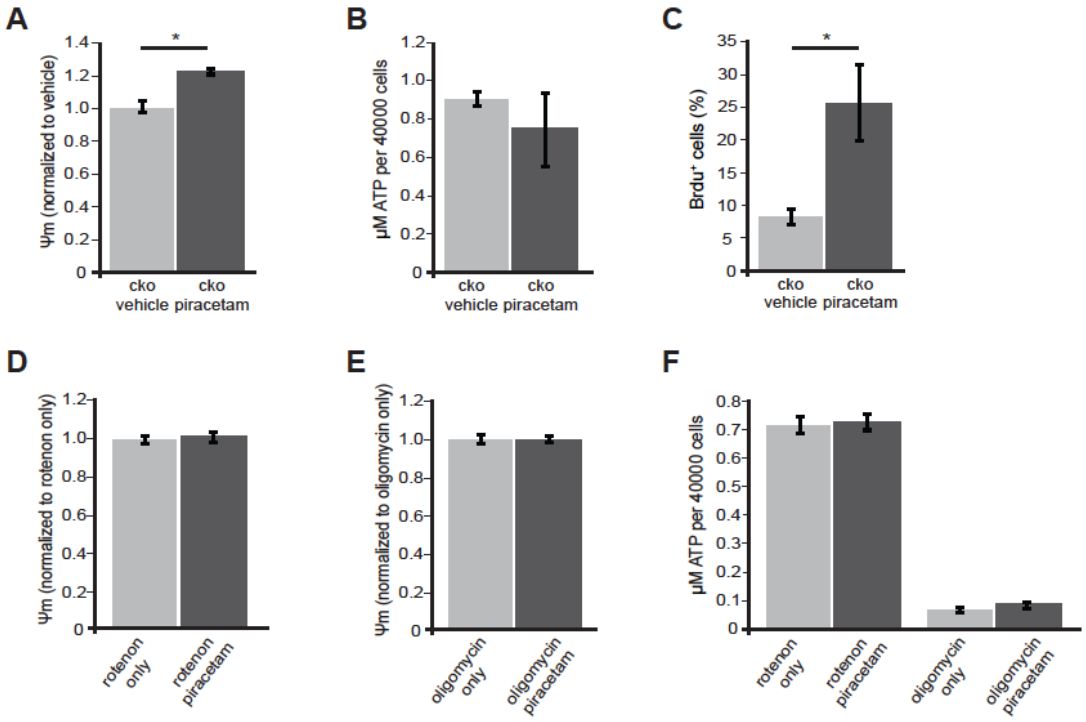

Figure S5: Effects of Piracetam on impaired mitochondria, related to Figure 5
(A-C) Treatment of *Tfam*^{cko} cells with Piracetam significantly increased membrane potential (A) and improved proliferation compared to vehicle treated *Tfam*^{cko} cells (C); ATP content was not affected (B). (D-F) Administration of Piracetam to cells treated for 24 h with either Rotenon or Oligomycin did not improve membrane potential (D, E), ATP content (F). Data represented as mean ± SEM; t-test or One-Way ANOVA were performed to check significance.

Table S1: Quantification of in vivo phenotypes, related to Fig. 3, 4, 5, S4.

Movie S1, related to Fig. 1: Serial electron micrographs (70 nm thickness) showing the primary process of a NSC of the dorsal dentate gyrus (yellow) with mitochondria marked in red, green and blue. See Figure 1A for 3D reconstruction and methods for further details on immunolabelling for movies S1-3.

Movie S2, related to Fig. 1: Serial electron micrographs (70 nm thickness) showing the primary process of an IPC of the dorsal dentate gyrus (yellow) with mitochondria marked in red, green and blue. See Fig. 1B for 3D reconstruction.

Movie S3, related to Fig. 1: Serial electron micrographs (40 nm thickness) showing the dendrite of a newborn neuron of the dorsal dentate gyrus (yellow) with mitochondria marked in red and blue. See Fig. 1C for 3D reconstruction.