## Supplemental Information

### Supplemental Figures

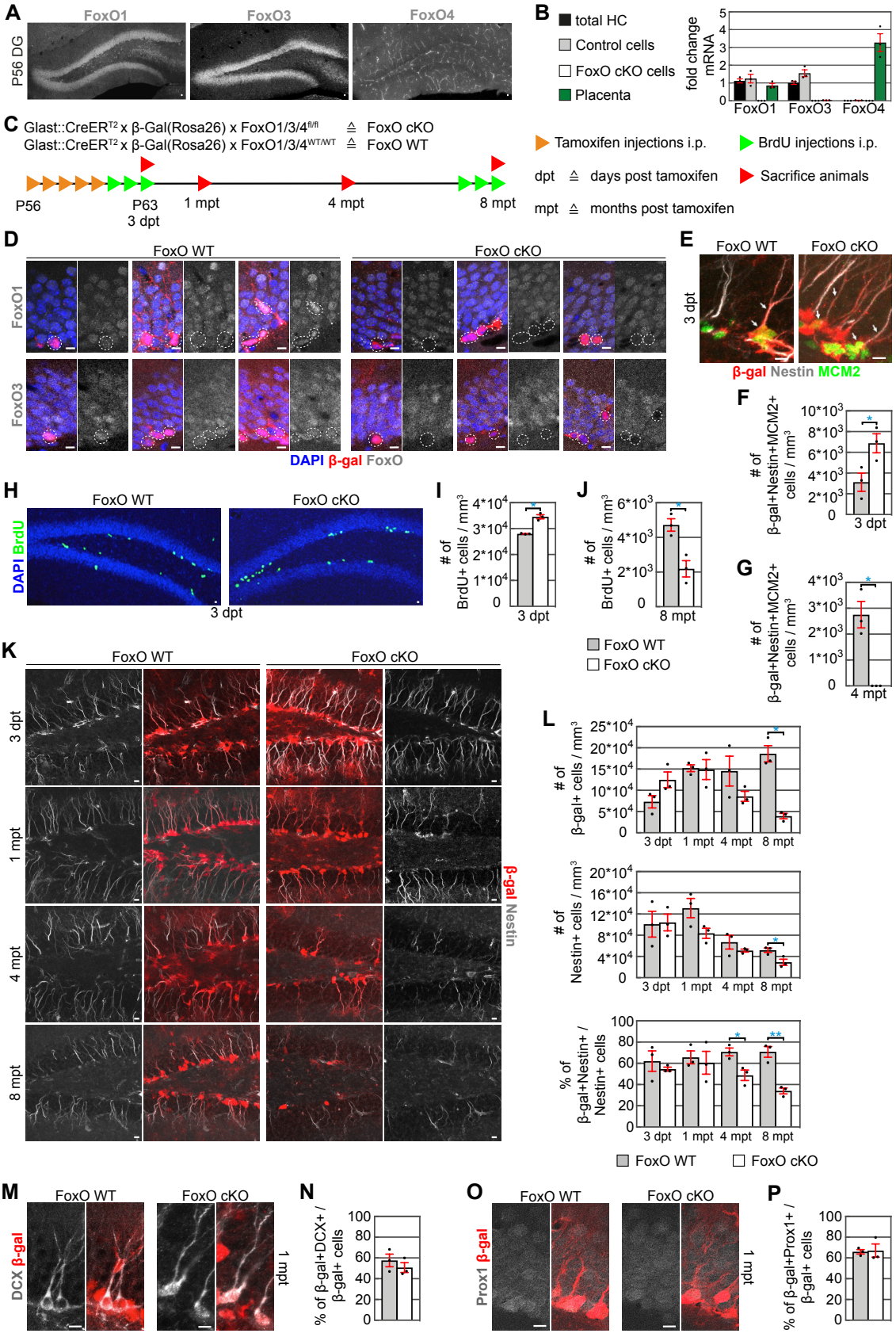


**Figure S1.** Conditional deletion of FoxOs results in progressive loss of radial-glia like NSPCs in the adult dentate gyrus. Related to Figure 1.

**A** FoxO1, FoxO3 and FoxO4 (all in grey) immunoreactivity in the adult dentate gyrus.

**B** FoxO1, FoxO3 and FoxO4 mRNA expression in tissue of the hippocampus (total HC), aNSPCs isolated from $FoxO^{WT/WT}$ mice (control cells) and $FoxO^{fl/fl}$ mice (FoxO cKO cells; KO induced by virus mediated Cre recombinase expression), and placenta (positive control for FoxO4 mRNA expression). mRNA levels were normalized to total HC FoxO3. Expression levels of FoxO1 and FoxO3 were comparable in the sample of the total HC and the control cells. No expression of FoxO1 or FoxO3 was detected in FoxO cKO cells. High FoxO4 expression levels were only detected in the placenta (positive control). n=3 biological replicates / group.

**C** Experimental scheme of tamoxifen and BrdU paradigms used in D – P. Adult Glast::$CreER^{T2}$xβ-Gal(Rosa26) mice with either wildtype or floxed alleles for FoxOs received i.p. tamoxifen injections for five consecutive days twice per day to induce recombination. Animals were sacrificed 3 days post tamoxifen (dpt), 1 month post tamoxifen (mpt), 4 mpt, or 8 mpt. Animals sacrificed 3 dpt and 1 mpt received daily BrdU injections for 3 consecutive days after tamoxifen injections. Animals sacrificed 8 mpt received daily BrdU injections for 3 consecutive days whereby the last injection took place three hours before sacrifice.

**D** Immunohistochemistry for β-galactosidase (red) and FoxO1 (grey) or FoxO3 (grey) in the dentate gyrus verified the deletion of FoxO1 and FoxO3 in recombined cells of mice with floxed alleles for FoxOs. DAPI in blue. The specificity of the FoxO3 antibody was previously validated on FoxO3 knockout tissue (Webb et al., 2013).

**E** Confocal images of the dentate gyrus of FoxO WT and FoxO cKO animals 3 dpt stained for β-galactosidase (red), Nestin (grey) and MCM2 (green).

**F, G** Quantification of β-galactosidase, Nestin and MCM2 triple positive cells in the dentate gyrus showed an increase in activated recombined NSPCs 3 dpt followed by a loss at 4 mpt in FoxO cKO animals. n=3 mice / group.

**H** Confocal images of BrdU-labeled cells (green) in FoxO WT and FoxO cKO 3 dpt. DAPI in blue.

**I, J** Quantification of $BrdU^+$ cells at 3 dpt and 8 mpt indicated an initial increase in proliferation followed by a strong decline in aged FoxO cKO animals. n=3 mice / group.

**K, L** Confocal images of β-galactosidase (red) and Nestin (grey) positive cells in the dentate gyrus 3 dpt, 1 mpt, 4 mpt and 8 mpt. Quantifications show a pronounced age-dependent loss of recombined cells and of NSPCs in FoxO cKO mice. n=3 mice / group.

**M - P** Confocal images of β-galactosidase positive cells (red) expressing the neuronal markers DCX (grey, M) or Prox1 (grey, O) at 1 mpt. Quantification indicated no differences in neuronal differentiation between FoxO WT and FoxO cKO animals. n=3 mice / group.

Data represented as mean ± SEM; t-test was used to determine significance; all scale bars = 10 µm.

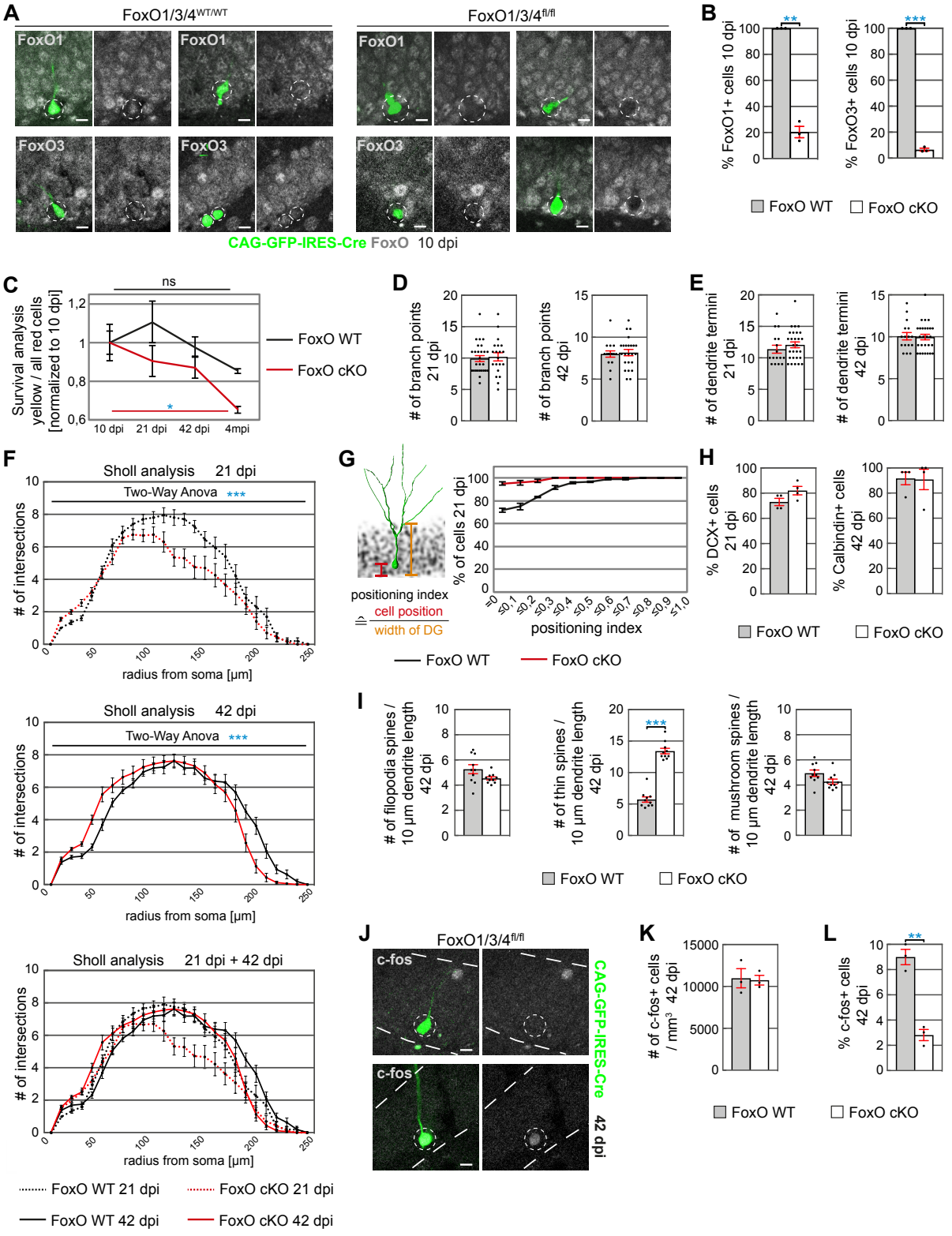


**Figure S2. Related to Figure 1.**

**A, B** Immunohistochemistry and quantified recombination efficiencies for FoxO1 (grey) and FoxO3 (grey) indicate efficient deletion of FoxO1 and FoxO3 in CAG-GFP-IRES-Cre transduced cells (green) of animals harboring conditional knockout alleles for FoxOs. The specificity of the FoxO3 antibody was previously validated on FoxO3 knockout tissue (Webb et al., 2013). Graphs in B depict % FoxO+ cells amongst transduced cells. n=3 mice / group.

**C** The ratio of CAG-IRES-mito-dsRed / CAG-GFP-IRES-Cre double transduced cells (yellow cells) over all CAG-IRES-mito-dsRed transduced cells (all red cells) was significantly reduced in FoxO cKO animals at 4 mpi indicating increased cell death of FoxO cKO cells. Significance was determined by Two-Way-ANOVA. n=3 mice / group.

**D, E** Quantifications of the number of branch points and dendrite termini showed no differences between FoxO WT and FoxO cKO cells. n=27/16 WT and n=20/24 cKO cells (21/42 dpi) from 6 mice / group.

**F** Consistent with shortened apical dendrite and decreased total dendritic length Sholl analysis of 21 dpi neurons shows differences between FoxO cKO and FoxO WT neurons. Consistent with the shortened apical dendrite, Sholl analysis of 42 dpi neurons shows a left shift of the curve in FoxO cKO cells. n=20 (21 dpi) and n=16 (42 dpi) cells from 6 mice / group. Two-Way-Anova was used to determine significance.

**G** Analysis of the position of the transduced cells relative to the width of the DG showed no differences between FoxO WT and FoxO cKO cells indicating that loss of FoxOs did not have major effects on positioning of the cell body. n=3 animals / group.

**H** Analysis of the neuronal differentiation with DCX as a marker for immature neurons and Calbindin as a marker for mature neurons did not show any significant differences between FoxO WT and cKO cells. Graphs depict marker positive cells amongst transduced cells. n=3 animals / group.

**I** 42 dpi FoxO WT and cKO neurons showed comparable numbers of filopodia and mushroom spines. The number of thin spines was significantly elevated in FoxO cKO neurons. n=10 cells from 4 animals / group.

**J – L** Confocal images of c-fos labeled cells (grey) and retrovirally labeled FoxO cKO cells (green) 42 dpi. The total number of c-fos+ cells per volume was similar in animals with FoxO WT or floxed alleles. The percentage of c-fos+ cells was significantly reduced amongst the FoxO cKO cells compared to FoxO WT cells. Graph in L depict % of c-fos+ cells amongst transduced cells. n=3 mice / group.

Data represented as mean ± SEM; t-test was used to determine significance if not indicated otherwise; all scale bars = 10 µm.

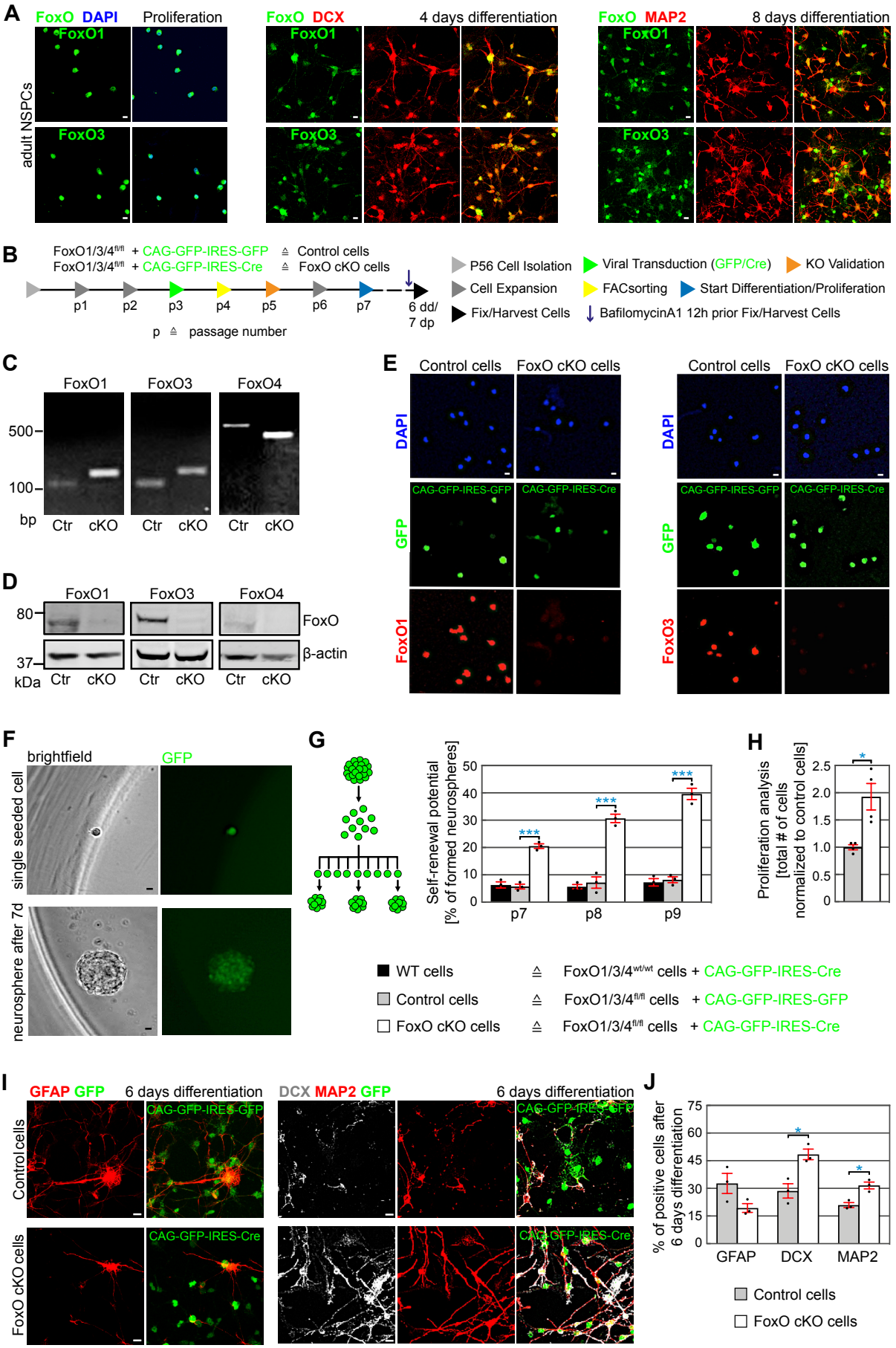


**Figure S3. Related to Figure 2.**

**A** Immunocytochemistry shows expression of FoxO1 (upper row, in green) and FoxO3 (lower row, in green) in proliferative and differentiated adult NSPCs *in vitro*. Co-expression of FoxO1 and 3 with DCX (middle panels, red) and MAP2 (right panels, red) demonstrates that FoxOs are expressed in differentiating neurons.

**B** Experimental paradigm used to analyze FoxOs in adult NSPCs *in vitro*. Adult NSPCs were isolated from FoxO conditional knockout mice at P56. Cells were expanded and transduced with either a GFP-encoding control retrovirus or a GFP-IRES-Cre encoding retrovirus. Transduced cells were isolated by FAC-sorting based on expression of the GFP-reporter. Following confirmation of FoxO knockout, cells were analyzed for their proliferation and differentiation behavior. If applicable, cells received a 12 h pulse of BafilomycinA1 (BafA1) prior fixation or harvest.

**C-E** Validation of the FoxO cKO in adult NSPCs via genotyping PCR (C), western blot (D), and immunocytochemistry (E).

**F** Fluorescence images of a single seeded adult NSPC and a neurosphere 7 days after single cell seeding onto miniwells.

**G** Experimental scheme and quantifications of the single cell neurosphere assay to assess the self-renewal potential of adult NSPCs. FoxO-deficient cells showed elevated self-renewal. n=3 biological replicates / group.

**H** Quantification of cell numbers 7 days after seeding indicated increased proliferation in FoxO cKO cells compared to control cells. n=4 biological replicates / group.

**I** Confocal images of 6 days differentiated control and FoxO cKO NSPCs stained for astrocytic (GFAP, in red) and neuronal markers (DCX, in grey; MAP2, in red).

**J** Quantification of the percentage of neurons and astrocytes after 6 days of differentiation. FoxO-deficient cells exhibited increased neuronal differentiation *in vitro*. n=3 biological replicates / group.

Data represented as mean ± SEM; t-test was used to determine significance; all scale bars = 10 µm.

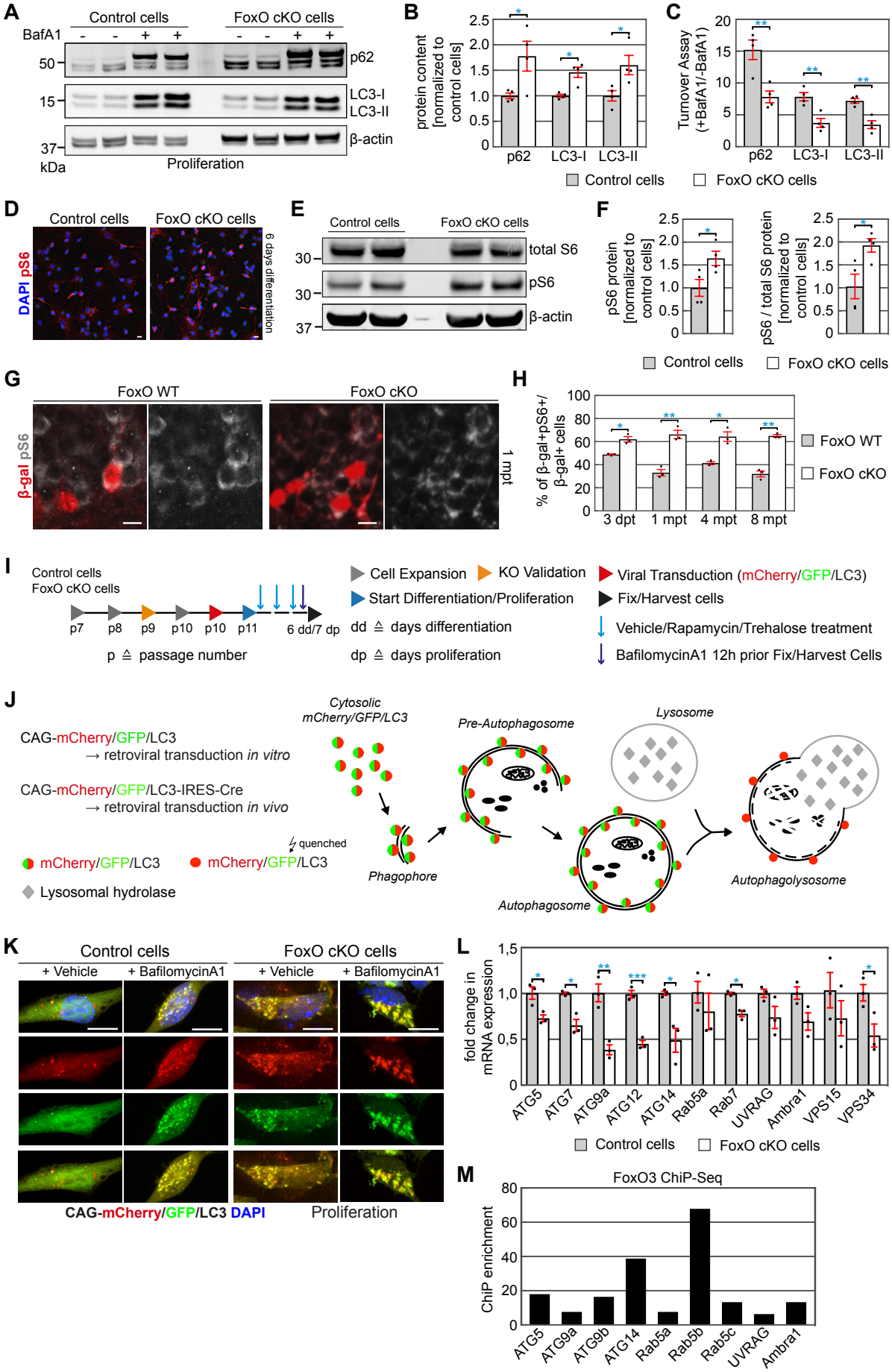


**Figure S4. Related to Figure 2 and 3.**

**A** Western blot of lysates of proliferating NSPCs with and without BafilomycinA1 (BafA1) treatment with antibodies against LC3 and p62 and β-actin as endogenous loading control. For quantification in B and C, protein amounts were normalized to β-actin and subsequently compared to control cells.

**B, C** Quantification of the western blot signal revealed increased protein content for LC3-I, LC3-II and p62 in FoxO cKO cells compared to control cells. The turnover assay analyzing the ratio of protein content with BafA1 over protein content without BafA1 revealed decreased turnover of autophagic proteins in FoxO cKO cells. n=4 biological replicates.

**D** Immunocytochemistry for pS6 (red) revealed increased expression of pS6 in differentiated FoxO-deficient cells *in vitro*. DAPI in blue.

**E** Western blot with protein lysates of control and FoxO cKO cells with antibodies against pS6, total S6 and β-actin as endogenous loading control. For quantification in F, protein amounts were normalized to β-actin and subsequently compared to control cells.

**F** Western blot quantifications showed elevated pS6 protein content and significantly increased ratio of pS6 over total S6 protein in FoxO cKO cells compared to control cells indicating elevated mTOR signaling. n=4 biological replicates.

**G, H** Confocal images and quantifications of β-galactosidase (red) and pS6 (grey) immunoreactive cells showed increased pS6 expression in FoxO-deficient cells at 3 dpt, 1mpt, 4 mpt and 8 mpt indicating elevated mTOR signaling in FoxO deficient cells *in vivo*. n=3 mice / group.

**I** Experimental scheme of *in vitro* autophagy induction paradigm. Control and FoxO cKO cells were expanded and knockout was re-validated. After further cell expansion cells were transduced with a mCherry/GFP/LC3 retrovirus. Autophagy was induced via Rapamycin or Trehalose treatment every second day during proliferation or differentiation analysis.

**J** Schematic representation of the mCherry/GFP/LC3 reporter. Upon induction of autophagy, cytosolic mCherry/GFP/LC3 is built into the membrane of the forming phagophore, which matures via the pre-autophagosome to the autophagosome. The virally transduced mCherry/GFP/LC3 generates yellow (red+green) puncta. Autophagosomes fuse with lysosomes to generate autophagolysosomes whose acidic environment quenches the GFP fluorescence but not the mCherry fluorescence of the mCherry/GFP/LC3.

**K** Confocal images of proliferating control and FoxO cKO cells transduced with the CAG-mCherry/GFP/LC3 retrovirus and treated with or without BafA1. FoxO-deficient cells showed more and larger autophagosomes [puncta in yellow (red+green)]. While in control cells autophagosomes (yellow puncta) and functional autophagolysosomes (red only puncta) were detected, functional autophagolysosomes were only rarely found in FoxO cKO cells indicating impairments in the autophagic flux in FoxO-deficient cells. BafA1 treatment, which inhibits V-ATPase-dependent acidification, resulted in the almost complete disappearance of GFP-/mCherry+ puncta.

**L** mRNA expression levels of autophagy related targets in control vs FoxO cKO cells. mRNA expression of targets involved in autophagosome formation, maturation and autophagosome-lysosome fusion were significantly reduced in FoxO cKO cells compared to control cells. n=3 biological replicates / group.

**M** Chip-Seq data extracted from (Webb et al., 2013). ChiP enrichment scores were generated with MACS (Model-based Analysis of ChIP-Seq) (Zhang et al., 2008).

Data represented as mean ± SEM; t-test was used to determine significance; all scale bars = 10 μm.

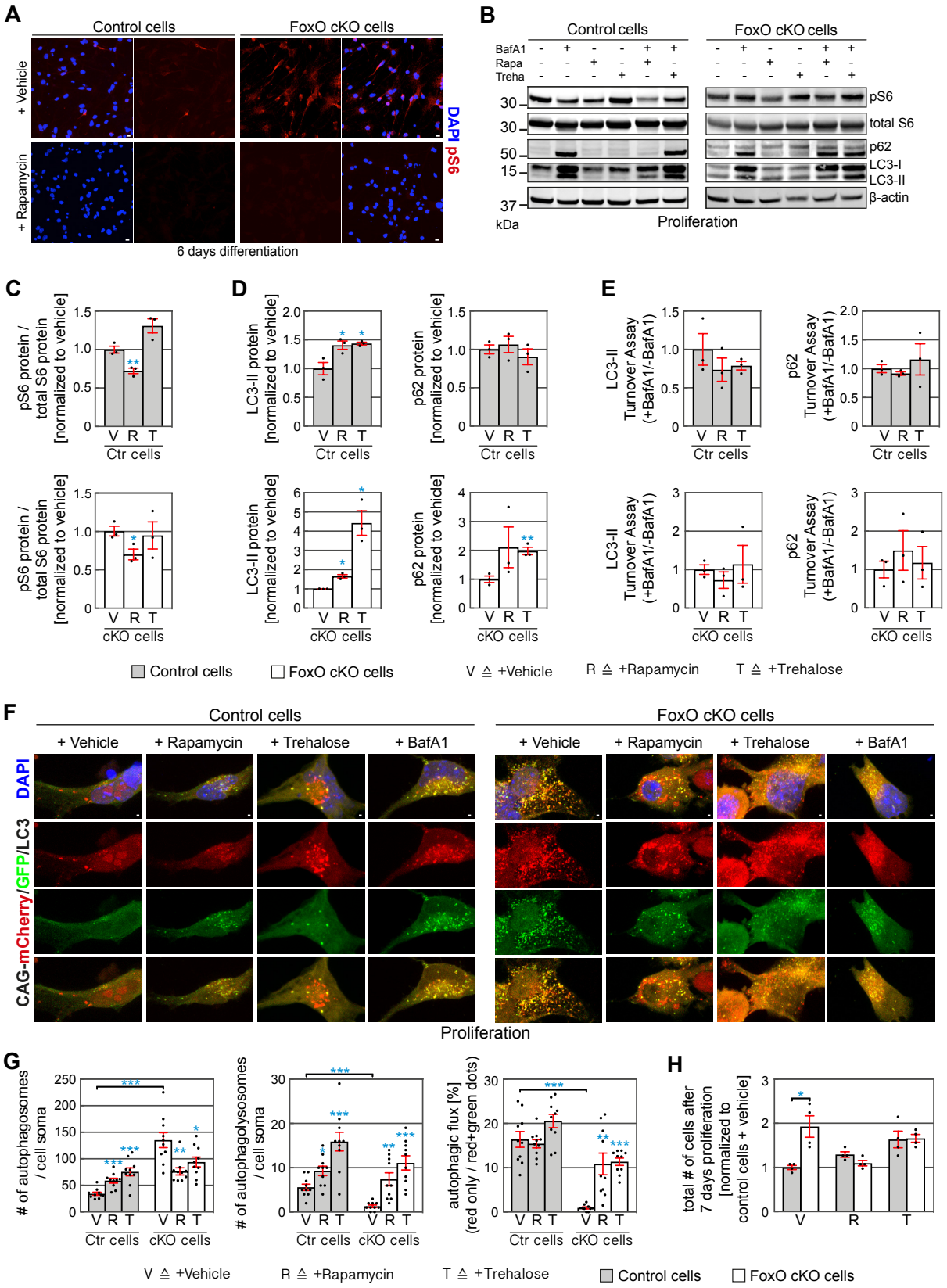


**Figure S5. Related to Figure 4.**

**A** Immunocytochemistry for pS6 of control and FoxO cKO cells revealed a strongly reduced signal for pS6 after treatment with Rapamycin *in vitro* indicating inhibition of mTOR signaling. Scale bars = 10 µm. **B** Western blots with lysates of control and FoxO cKO cells treated with vehicle, Rapamycin, Trehalose and BafilomycinA1 (BafA1). Antibodies against LC3, p62, pS6 and total S6 protein were analyzed. β-

actin was used as endogenous loading control. For quantification in C - E, protein amounts were normalized to β-actin and subsequently compared to control cells.

**C** Quantifications of the ratio of pS6 over total S6 protein showed decreased pS6 levels with Rapamycin, validating the functionality of the Rapamycin treatment. Note that pS6 levels were not altered after Trehalose treatment. n=3 biological replicates / group.

**D** Quantifications of protein content showed elevated LC3-II levels in control and FoxO cKO cells following treatment with Rapamycin and Trehalose. Treatment with Rapamycin and Trehalose also increased p62 levels in FoxO cKO. n=3 biological replicates / group.

**E** Quantification of the BafA1+/BafA1- ratio to estimate LC3-II and p62 turnover showed no significant alterations after Rapamycin or Trehalose treatment in control and FoxO cKO cells. n=3 biological replicates / group.

**F** Confocal images of proliferating control and FoxO cKO cells transduced with CAG-mCherry/GFP/LC3 retrovirus and treated with either vehicle or Rapamycin or Trehalose or BafA1. Scale bars = 1µm.

**G** Quantifications of autophagosomes (yellow puncta) and functional autophagolysosomes (red only puncta) showed an increased number of autophagosomes in FoxO cKO cells compared to control cells while rarely any functional autophagolysosomes were observed in FoxO cKO cells under vehicle conditions. Rapamycin and Trehalose treatment led to an increase of autophagosomes and functional autophagolysosomes in control cells indicating induction of autophagy. FoxO cKO cells treated with Rapamycin or Trehalose showed a reduction in the number of autophagosomes and a significant increase in the number of autophagolysosomes indicating induction of autophagy. n=10 cells from 3 biological replicates / group.

**H** FoxO cKO cells and control cells show similar levels of proliferation following treatment with Rapamycin and Trehalose. n=4 biological replicates.

Data represented as mean ± SEM; t-test was used to determine significance.

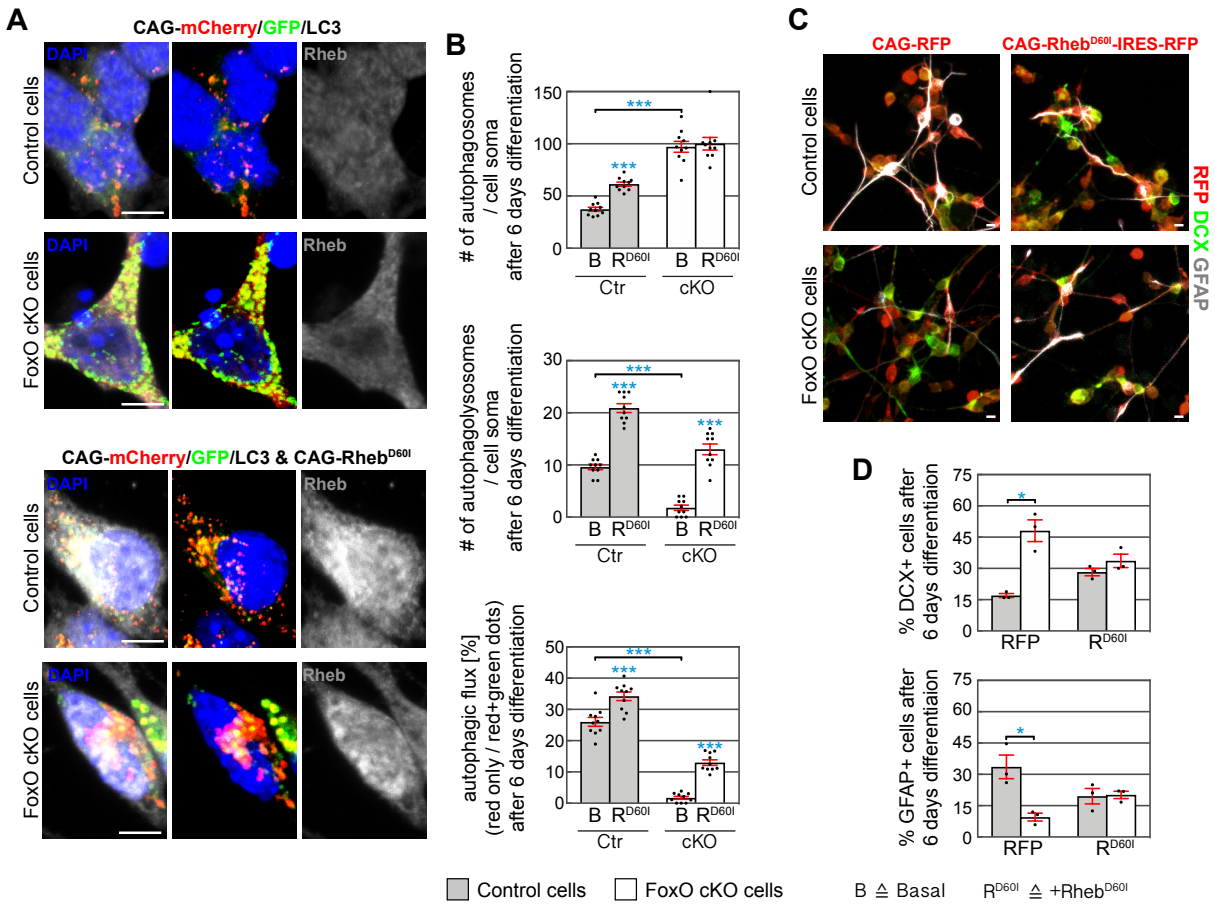


**Figure S6. Related to Figure 4**

**A** Confocal images of differentiated control and FoxO cKO cells transduced with CAG-mCherry/GFP/LC3 retrovirus or CAG-mCherry/GFP/LC3 retrovirus together with CAG-Rheb$^{D60I}$ retrovirus and stained for Rheb (grey). DAPI in blue.

**B** Quantifications showed more and larger autophagosomes [puncta in yellow (red+green)] in FoxO deficient cells. While in control cells autophagosomes (yellow puncta) and functional autophagolysosomes (red only puncta) were detected, functional autophagolysosomes were only rarely found in FoxO cKO cells indicating impairments in the autophagic flux in FoxO-deficient cells under basal conditions. Overexpressing Rheb$^{D60I}$ increased the number of autophagosomes and of autophagolysosomes in control cells. Calculation of the autophagic flux confirmed induction of autophagy via Rheb$^{D60I}$. Rheb$^{D60I}$ increased significantly the number of autophagolysosomes in FoxO cKO cells and enhanced autophagic flux in FoxO-deficient cells. n=10 cells from 3 biological replicates / group.

**C** Confocal images of differentiated control and FoxO cKO cells transduced with either a CAG-RFP retrovirus or a CAG-Rheb$^{D60I}$-IRES-RFP retrovirus and stained for astrocytic (GFAP, in grey) and neuronal markers (DCX, in green).

**D** Quantifications of RFP+ cells showed that Rheb$^{D60I}$-IRES-RFP overexpression reverted the increased neuronal and decreased astrocytic differentiation of FoxO-deficient cells. n=3 biological replicates / group.

Data represented as mean ± SEM; t-test was used to determine significance; all scale bars = 10 µm.

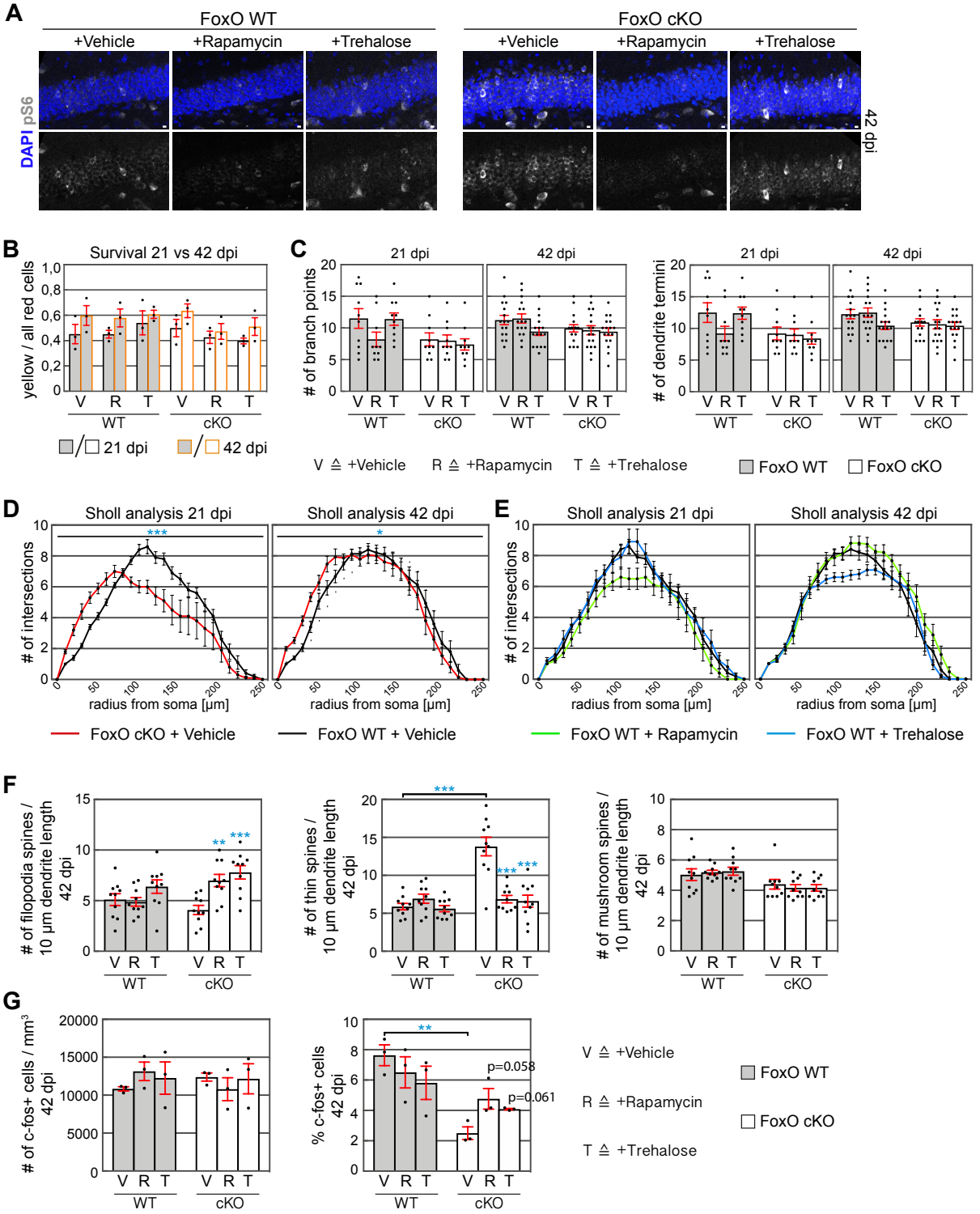


**Figure S7. Related to Figure 6 and 7.**

**A** Confocal imaging of sections stained for pS6 showed a reduction of the pS6 signal in the dentate gyrus of Rapamycin treated FoxO WT and FoxO cKO animals but not in the dentate gyrus of Trehalose treated animals. Scale bars = 10 µm.

**B** Survival analysis quantifying the ratio of double transduced cells (yellow) over all red cells revealed comparable rate of survival of FoxO WT and FoxO cKO cells between 21 dpi and 42 dpi in the context of vehicle, Rapamycin or Trehalose treatments. n=3 animals / group.

**C** FoxO WT and FoxO cKO neurons have comparable numbers of branch points and dendrite termini following vehicle, Rapamycin or Trehalose treatment. n=10 (21 dpi) and n=16-17 (42 dpi) cells from 4 mice / group.

**D** Consistent with shortened apical dendrite and decreased total dendritic length Sholl analysis of 21 dpi neurons showed differences between vehicle-treated FoxO cKO and FoxO WT neurons. Consistent with the shortened apical dendrite, Sholl analysis of 42 dpi neurons showed a left shift of the curve in vehicle-treated FoxO cKO cells. Note, that the data is consistent with the data of untreated FoxO cKO and FoxO WT neurons (Figure S2F). n=10 (21 dpi) and n=15 (42 dpi) cells from 4 mice / group. Two-Way-Anova was used to determine significance.

**E** FoxO WT neurons, treated with Rapamycin or Trehalose were comparable to vehicle treated WT neurons in Sholl analysis. n=10 (21 dpi) and n=15 (42 dpi) cells from 4 mice / group. Two-Way-Anova was used to determine significance.

**F** FoxO WT and FoxO cKO neurons have similar numbers of mushroom spines following vehicle, Rapamycin or Trehalose treatment. The numbers of filopodia spines were significantly increased after Rapamycin or Trehalose treatment in FoxO cKO neurons. Note, that the increased number of thin spines in the FoxO cKO neurons was reduced to WT levels by Rapamycin or Trehalose treatment. n=10 cells from 4 mice / group.

**G** The density of c-fos+ cells in the dentate gyrus was comparable between vehicle, Rapamycin or Trehalose treated FoxO WT and FoxO cKO animals. At 42 dpi, the percentage of c-fos+ neurons amongst FoxO-deficient neurons was reduced compared to control neurons. Treatment with Rapamycin or Trehalose resulted in a clear trend towards higher number of c-fos+ neurons amongst FoxO-deficient neurons. n=3 mice / group.

Data represented as mean ± SEM; t-test was used to determine significance if not indicated otherwise.