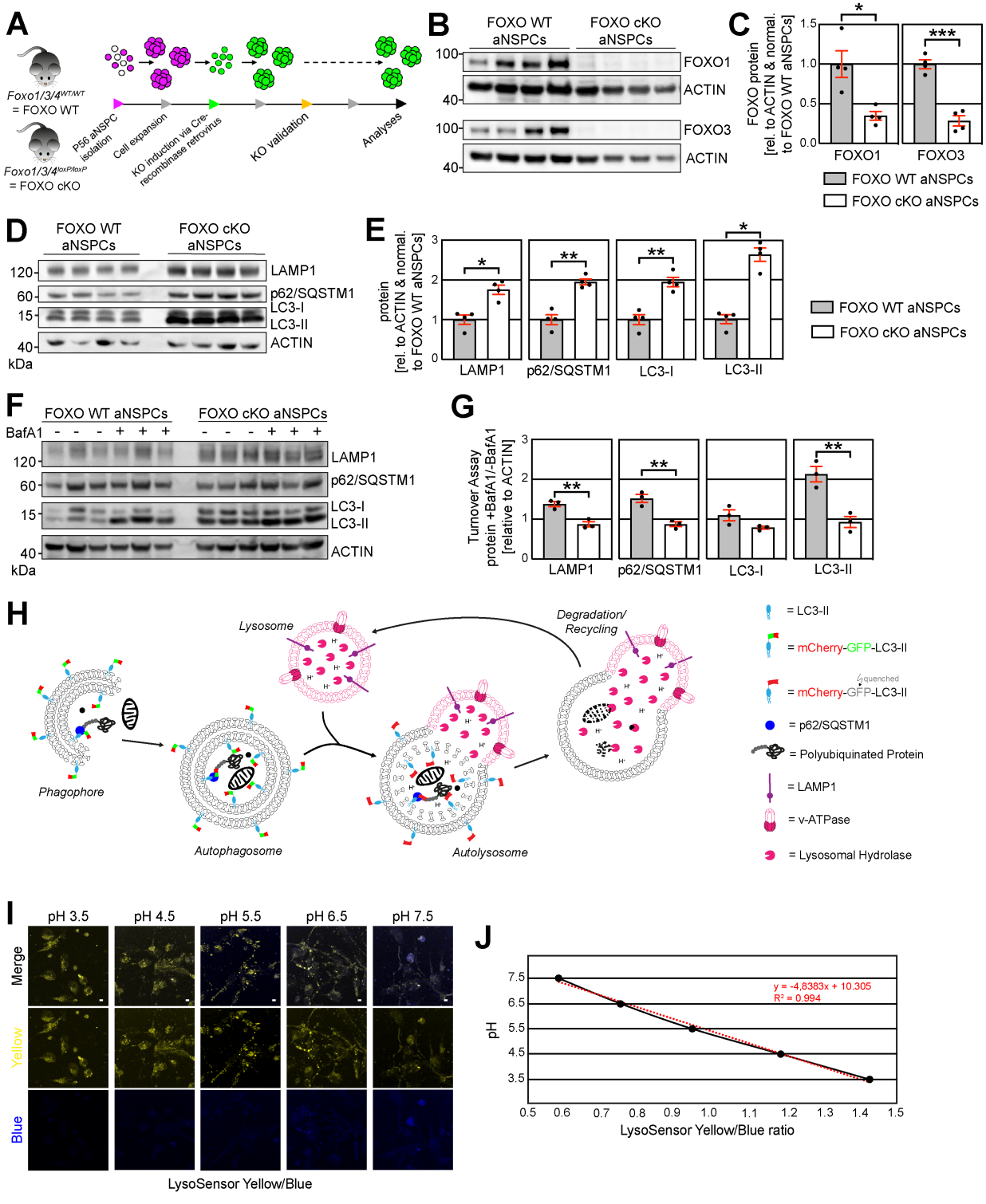
Supplemental Figures



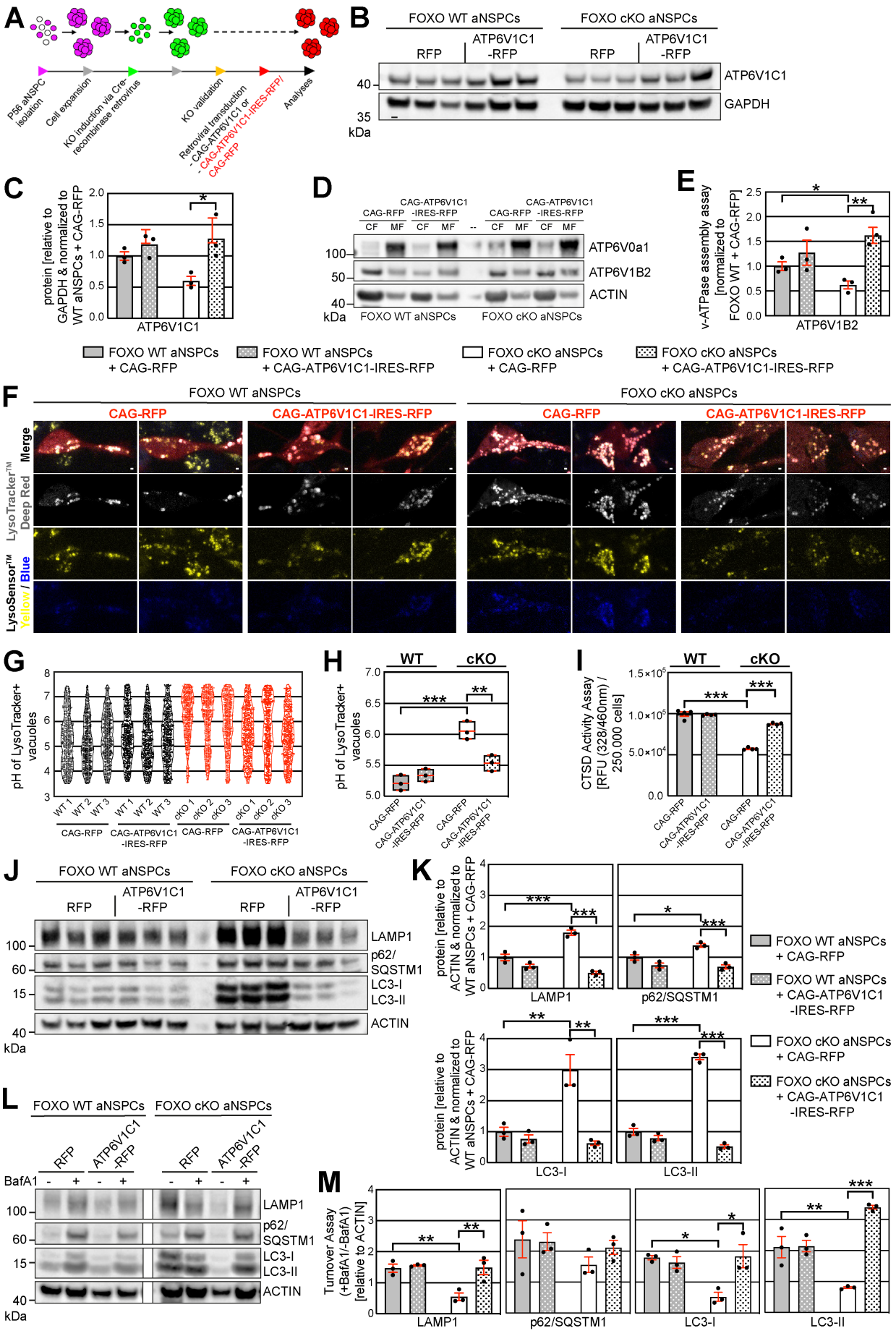
Supplemental Figure 1. Related to Figure 1.

(A) Experimental paradigm. aNSPCs were isolated from the hippocampus of *Foxo1/3/4*$^{loxP/loxP}$ conditional knockout mice (FOXO cKO) or *Foxo1/3/4*$^{wt/wt}$ mice (FOXO WT) at P56. The KO of FOXOs was induced via Cre-recombinase. Following confirmation of FOXO knockout, cells were used for analyses.
(B) Western blots of whole cell lysates of FOXO WT and FOXO cKO aNSPCs. Antibodies for FOXO1 and FOXO3 were applied. ACTIN was used as endogenous loading control.
(C) Quantifications of FOXO1 and FOXO3 protein levels normalized to ACTIN in FOXO WT and FOXO cKO aNSPCs. Data is presented relative to FOXO WT aNSPCs. n=4 biological replicates/group.
(D) Western blot of whole cell lysates of FOXO WT and FOXO cKO aNSPCs. Antibodies against LAMP1 (marker for lysosomes) and p62/SQSTM1 and LC3I/II (markers for the autophagic system) were applied. ACTIN was used as endogenous loading control. For quantifications in (E), protein amounts were normalized to ACTIN and subsequently compared to FOXO WT aNSPCs.
(E) FOXO cKO aNSPCs showed increased protein levels for LAMP1, p62/SQSTM1 and LC3-I/II compared to FOXO WT aNSPCs. n=4 biological replicates/group.
(F) Western blot of whole cell lysates of FOXO WT and FOXO cKO aNSPCs with or without Bafilomycin A1 (BafA1) treatment. Antibodies against LAMP1 (marker for lysosomes), p62/SQSTM1 and LC3I/II (markers for the autophagic system) were applied. ACTIN was used as endogenous loading control. For quantifications of BafA1 autophago-lysosomal turnover assays in (G), protein amounts were normalized to ACTIN and subsequently presented as +BafA1/-BafA1 ratios.
(G) FOXO cKO aNSPCs showed decreased autophago-lysosomal turnover compared to FOXO WT aNSPCs. n=3 biological replicates/group.
(H) Schematic representation of the functional principle of the mCherry-GFP-LC3 tandem reporter. Upon induction of autophagy, cytosolic mCherry-GFP-LC3 is built into the membrane of the forming phagophore, which matures to the autophagosome. The retrovirally transduced mCherry-GFP-LC3 generates red and green puncta. Autophagosomes fuse with lysosomes to generate autolysosomes. The acidic environment of the lysosome, generated via the v-ATPase, quenches the GFP

fluorescence but not the mCherry fluorescence of the tandem mCherry-GFP-LC3 reporter.

(I) Representative confocal pictures of WT aNSPCs labeled with the live-cell imaging probe LysoSensor™ Yellow/Blue (to measure ratiometrically the lysosomal pH) under pH defined clamped buffers. Scale bars 10 µm.

(J) Determined standard curve for the live-cell imaging probe LysoSensor™ Yellow/Blue for the pH 3.5, 4.5, 5.5, 6.5 and 7.5.



Supplemental Figure 2. Related to Figure 2.

(A) Experimental paradigm. aNSPCs were isolated from the hippocampus of *Foxo1/3/4*$^{loxP/loxP}$ conditional knockout mice (FOXO cKO) or *Foxo1/3/4*$^{wt/wt}$ mice (FOXO WT) at P56. The KO of FOXOs was induced via Cre-recombinase. ATP6V1C1 overexpression was mediated via a retrovirus either encoding for ATP6V1C1 alone or bicistronically for RFP. An RFP-encoding retrovirus was used as control. Cells were analyzed three days later.

(B) Western blot of whole cell lysates of FOXO WT and FOXO cKO aNSPCs transduced either with a CAG-RFP control retrovirus or a ATP6V1C2-overexpression retrovirus bicistronically encoding for RFP (CAG-ATP6V1C1-IRES-RFP). An antibody against ATP6V1C1 was applied to validate the ATP6V1C1 overexpression. GAPDH was used as endogenous control.

(C) Quantifications validated the ATP6V1C1 overexpression in FOXO WT and FOXO cKO aNSPCs. Data is represented relative to FOXO WT aNSPCs transduced with the CAG-RFP control retrovirus. n=3 replicates/group.

(D) Western blots of cytosolic (CF) and membrane (MF) protein fractions of FOXO WT and cKO aNSPCs transduced with either an RFP control retrovirus (CAG-RFP) or an ATP6V1C1-overexpression retrovirus (CAG-ATP6V1C1-IRES-RFP). Antibodies against ATP6V1B2, ATP6V0a1 and ACTIN were applied. For quantifications in (E) protein amounts of the ATP6V1B2 subunit in the MF (normalized to ATP6V0a1 in the MF) were presented as ratio relative to the ATP6V1B2 protein amounts in the CF (normalized to GAPDH in the CF) as proxy for v-ATPase assembly. Data is presented as relative to FOXO WT aNSPCs transduced with the CAG-RFP control retrovirus.

(E) ATP6V1C1 overexpression rescued the decreased v-ATPase assembly in the FOXO cKO aNSPCs. n=3 biological replicates/group.

(F) Representative confocal pictures of FOXO WT and cKO aNSPCs, each transduced either with the ATP6V1C1-overexpression retrovirus (CAG-ATP6V1C1-IRES-RFP) or the RFP control retrovirus (CAG-RFP) and labeled with the live-cell imaging probes LysoTracker™ deep red (to label lysosomes) and LysoSensor™ Yellow/Blue (to measure ratiometrically the lysosomal pH). Scale bars 1 µm.

(G and H) Measurements of the pH of LysoTracker™ deep red+ vacuoles via the LysoSensor™ Yellow/Blue signals (G) and quantification of the average lysosomal pH

(H) in FOXO WT and cKO aNSPCs. Overexpression of ATP6V1C1 reduced the increased lysosomal pH in FOXO cKO aNSPCs. n=3 biological replicates/group.

(I) ATP6V1C1 overexpression rescued the decreased CathepsinD (CTSD) activity in FOXO cKO aNSPCs. n=4 biological replicates/group.

(J) Western blot of whole cell lysates of FOXO WT and FOXO cKO aNSPCs each transduced either with the ATP6V1C1-overexpression retrovirus (CAG-ATP6V1C1-IRES-RFP) or the RFP control retrovirus. Antibodies against LAMP1 (lysosomal marker), p62/SQSTM1 and LC3-I/II (autophagic markers) were applied. ACTIN was used as endogenous loading control. For quantifications in (K), protein amounts were normalized to ACTIN and subsequently compared to FOXO WT aNSPCs transduced with the RFP control retrovirus.

(K) ATP6V1C1 overexpression rescued the increased protein levels of LAMP1, p62/SQSTM1 and LC3I/II in FOXO cKO aNSPCs. n=3 biological replicates/group.

(L) Western blots of whole cell lysates of FOXO WT and FOXO cKO aNSPCs each transduced either with the ATP6V1C1-overexpression retrovirus (CAG-ATP6V1C1-IRES-RFP) or the RFP control retrovirus and treated either with or without BafA1. Antibodies against LAMP1 (lysosomal marker), p62/SQSTM1 and LC3-I/II (autophagic markers) were applied. ACTIN was used as endogenous loading control. For quantifications of BafA1 autophago-lysosomal turnover assays in (M), protein amounts were normalized to ACTIN and subsequently presented as +BafA1/-BafA1 ratios.

(M) ATP6V1C1 overexpression rescued the decreased autophago-lysosomal turnover in FOXO cKO aNSPCs. n=3 biological replicates/group.