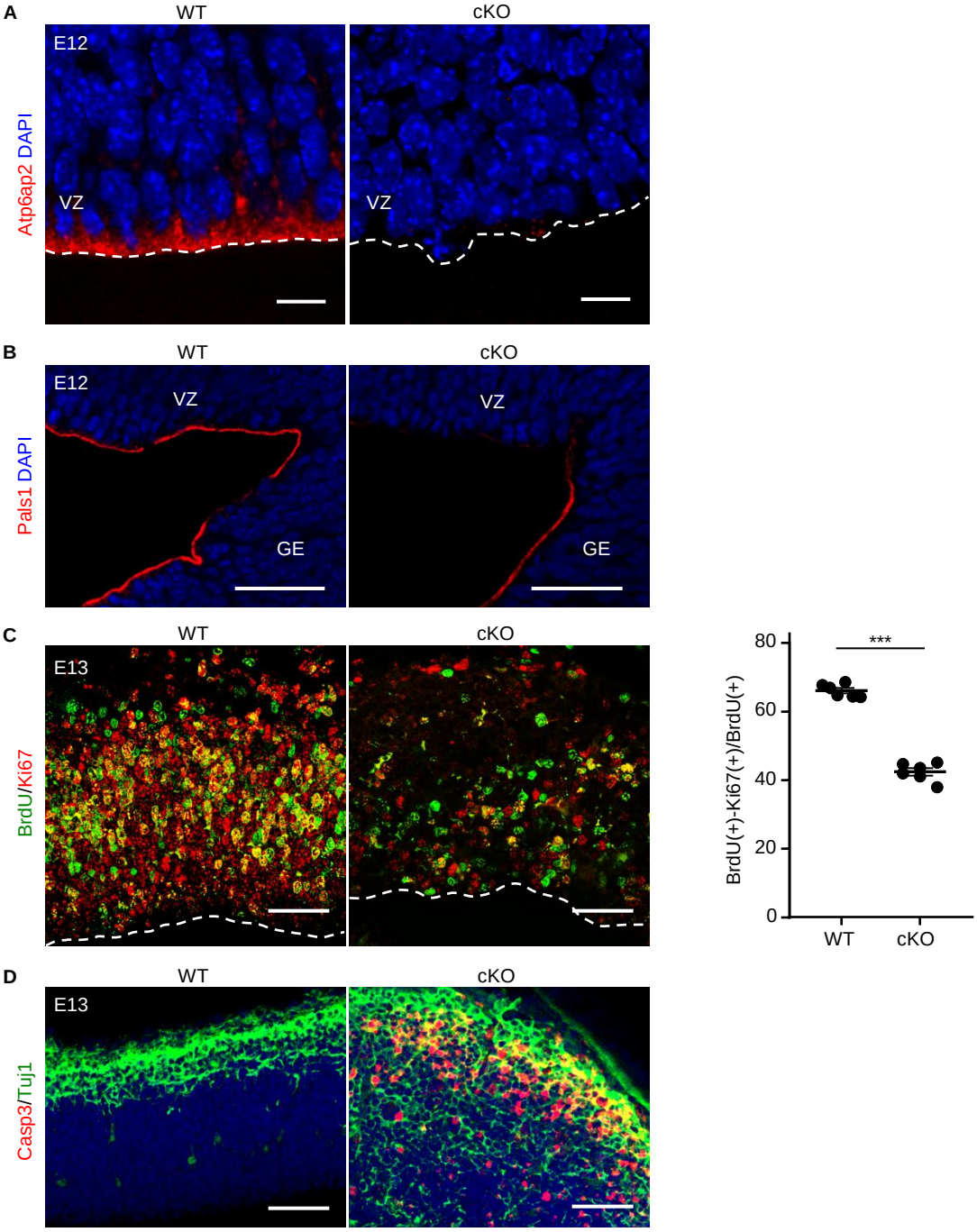
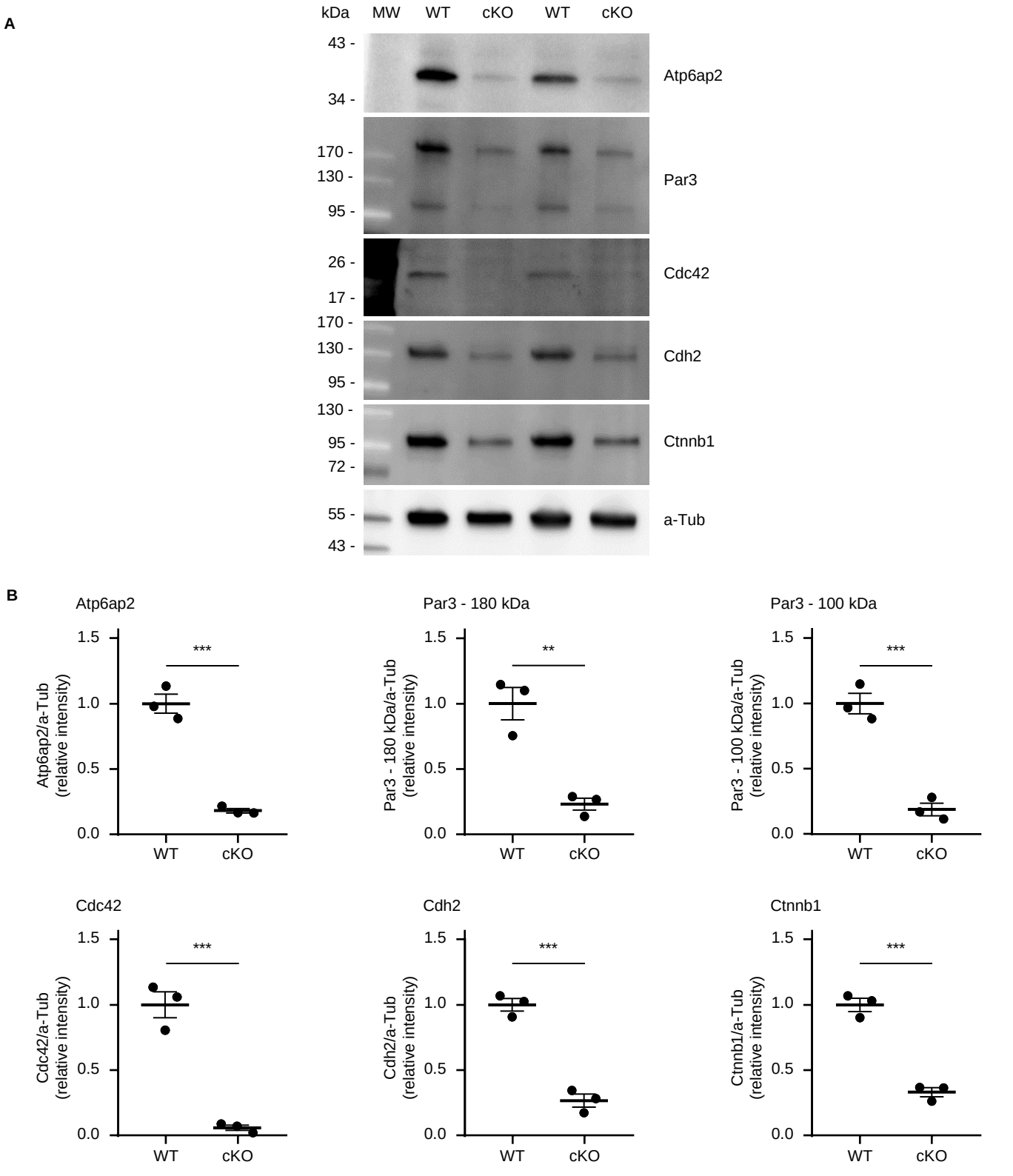
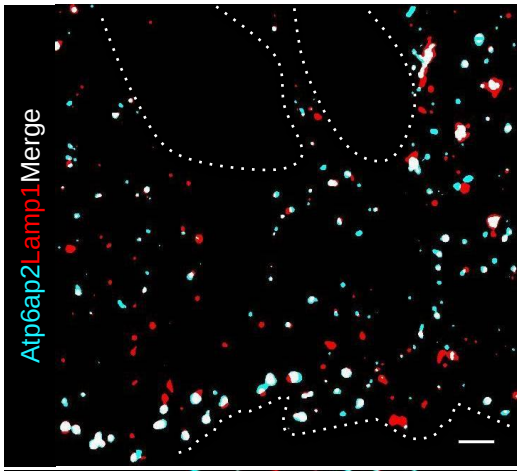
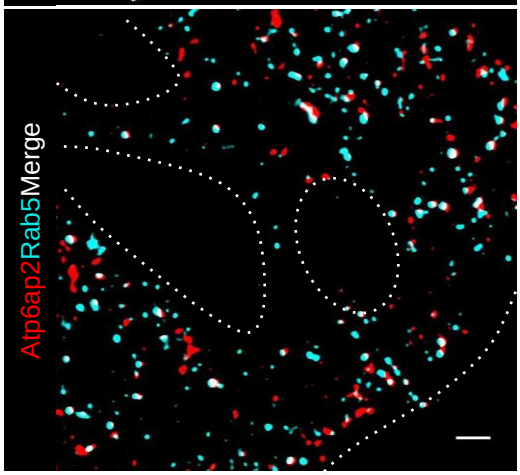


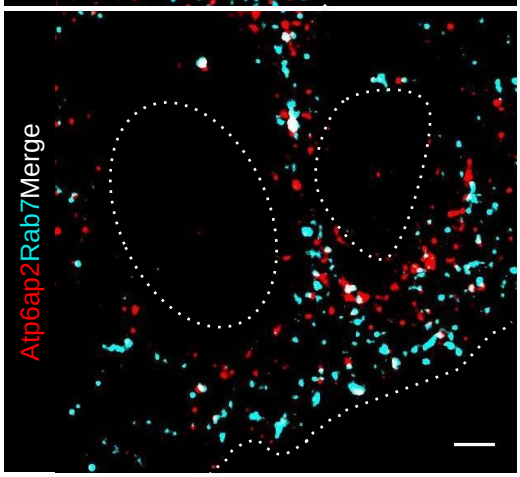
Supplemental Figure 1. *Atp6ap2* deletion in embryonic cortical stem/progenitors cells impairs self-renewal and induces cell cycle exit. (**A**) Staining of Atp6ap2 in E12 embryonic cortex of *wild type* (WT) and *cKO* mice. VZ: ventricular zone. Dotted lines: ventricular surface, Scale bars: 10 µm. (**B**) Pals1 immunostaining at cortical-subcortical boundary at E12. The reduced expression of Pals1 is restricted to the dorsal cortex (VZ) comprising the area of conditional Atp6ap2 deletion in *cKO*s, in ganglionic eminences (GE) in mutants Pals1 is normal. Scale bars: 50 µm. (**C**) S-phase cells transiently labelled by BrdU and M-phase cells stained with Ki67 are significantly reduced in *cKO*. BrdU/Ki67 double-staining following BrdU pulse labelling 24 h earlier show increase rate of cell cycle exit in *cKO* indicated by reduced ratio of BrdU+/Ki67+ double positive cells. Dotted lines: ventricular surface, Scale bars: 50 µm. Graph: Individual values and Mean±SEM. n=6 sections from 3 different litters. ****P*<0.001, Student's t-test (unpaired, two-tailed). (**D**) Double immunostaining Tuj1 and activated caspase3 shows pronounced cell death of young neurons. Scale bars: 100 µm.

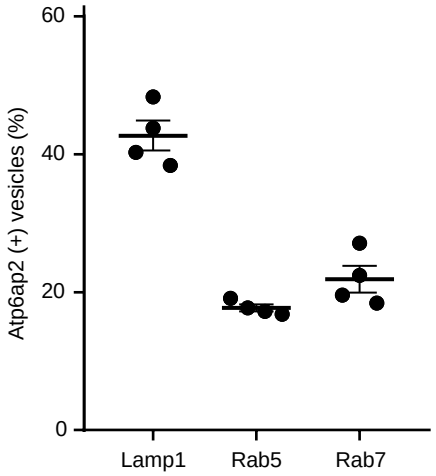


Supplemental Figure 2. Western blots of E12 cortex tissue. (**A**) Reduction of apical complex and adherens junction proteins in E12 *cKO*s. (**B**) Quantification of Western blots. Data shows individual values and Mean±SEM. n=3 different animals/group. ***P*<0.01; ****P*<0.001, Student's t-test (unpaired, two tailed).

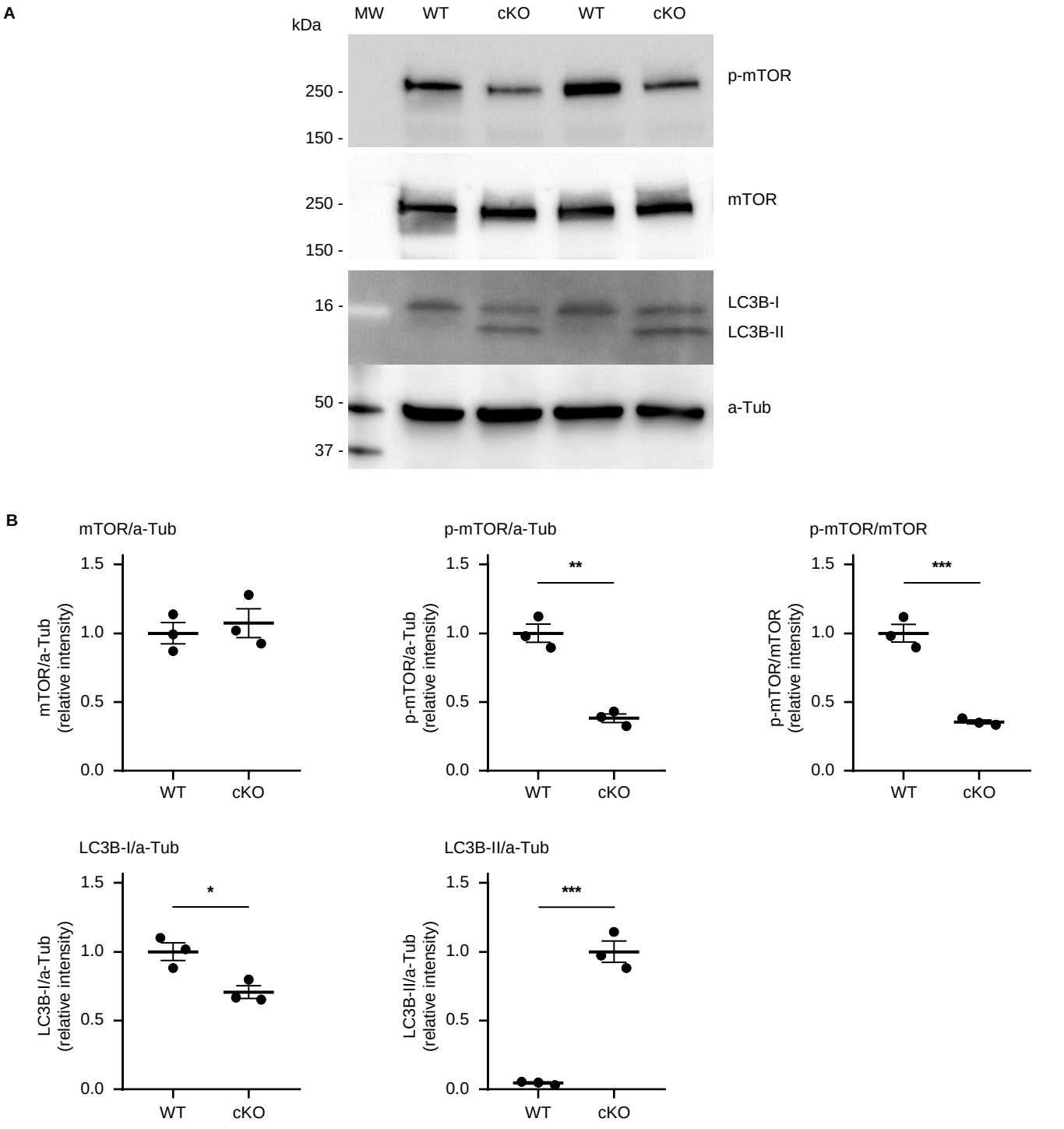




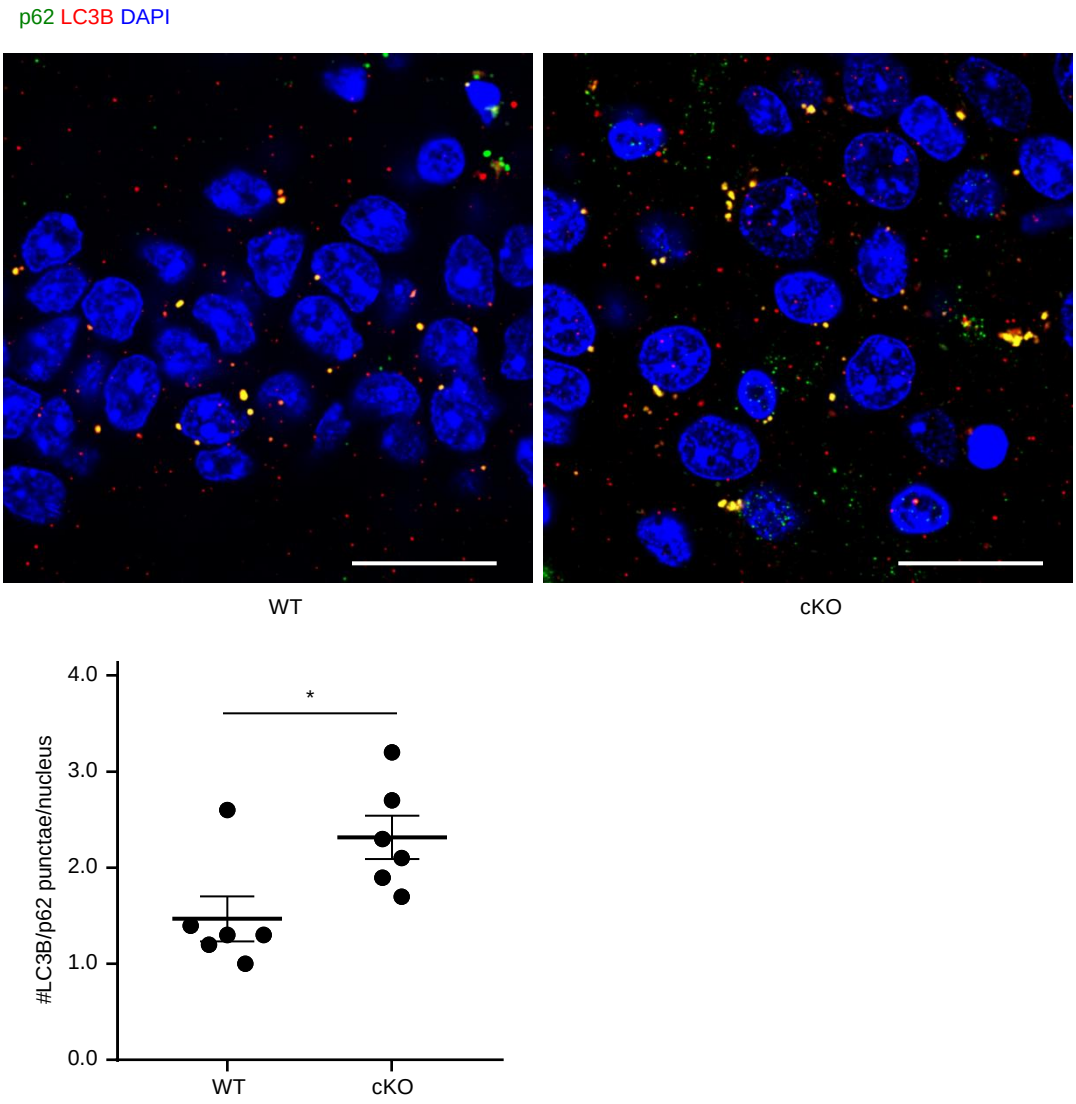




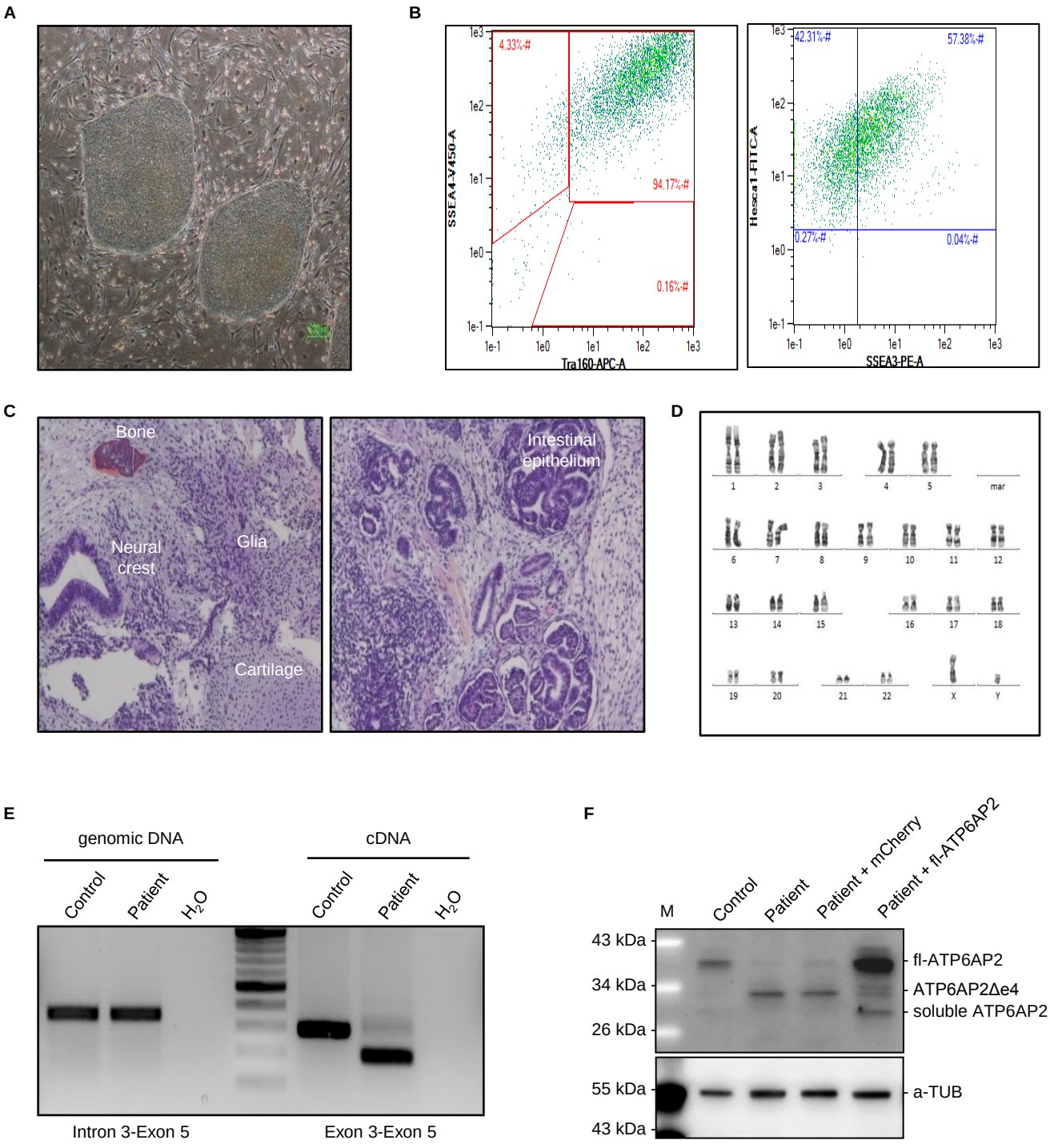
Supplemental Figure 3. Subcellular localization of Atp6ap2 in E12 radial glial cells analyzed by super-resolution Stimulated Emission Depletion (STED) imaging. Cortices from *wild type* E12 were stained with anti-Atp6ap2, anti-Lamp1, anti-Rab5 and anti-Rab7 and analyzed by STED. Atp6ap2 is largely undetectable at the plasma membrane and predominantly found in intracellular vesicles, lysosomes, early and late endosomes stained with anti-Lamp1, anti-Rab5 and anti-Rab7, respectively. Dotted lines: ventricular surface and nuclei, Scale bars: 1.0 µm. Graph displays the percentage of double positive vesicles (individual values and Mean±SEM). n=4 sections from 2 different litters.



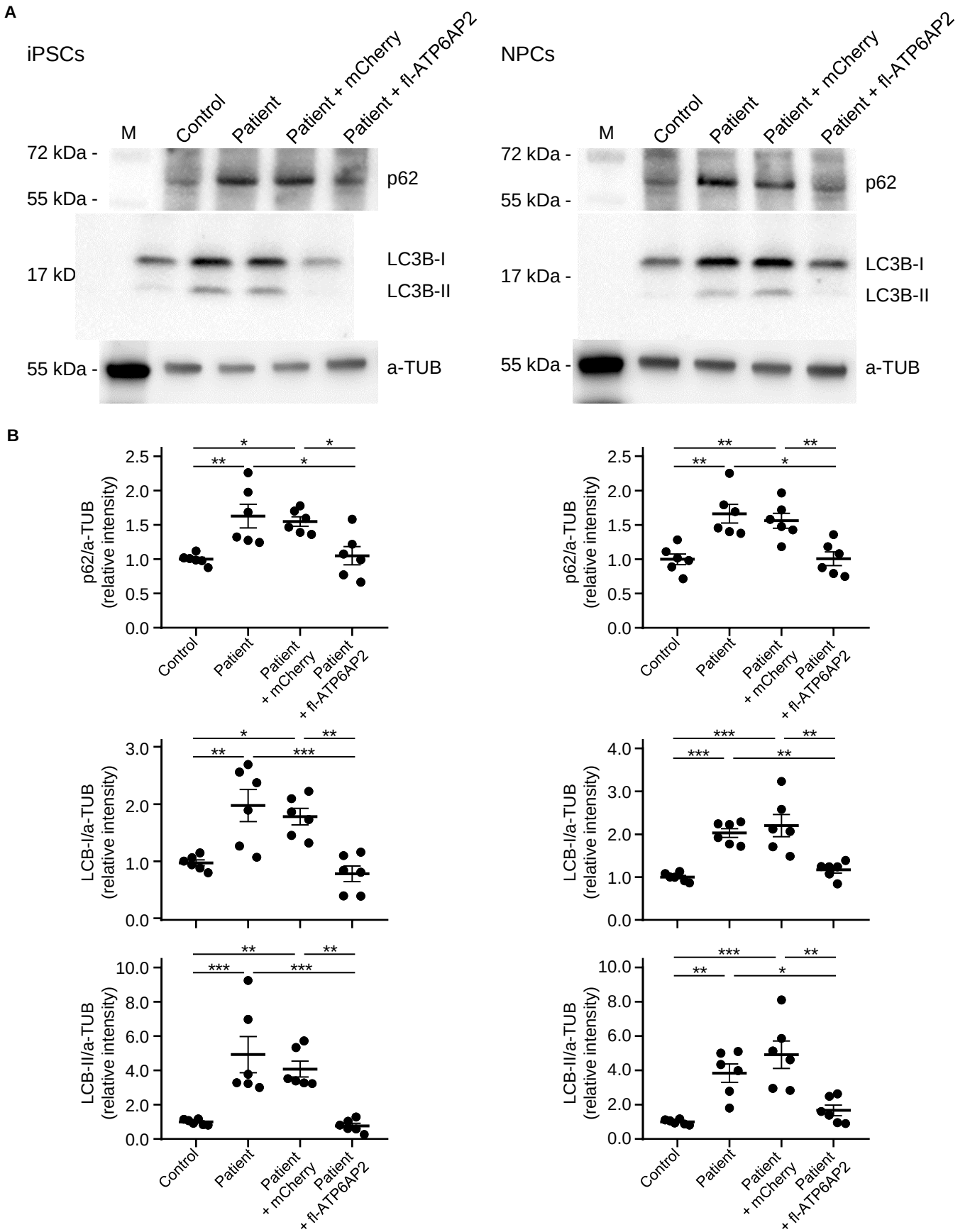
Supplemental Figure 4. Western blots of E12 cortex tissue. (**A**) Reduction of phospho-mTOR and increased LC3B-II in *cKO*s. (**B**) Quantification of Western blots. Data shows individual values and Mean±SEM. n=3 different animals/group. **P*<0.05; ***P*<0.01; ****P*<0.001, Student's t-test (unpaired, two tailed).



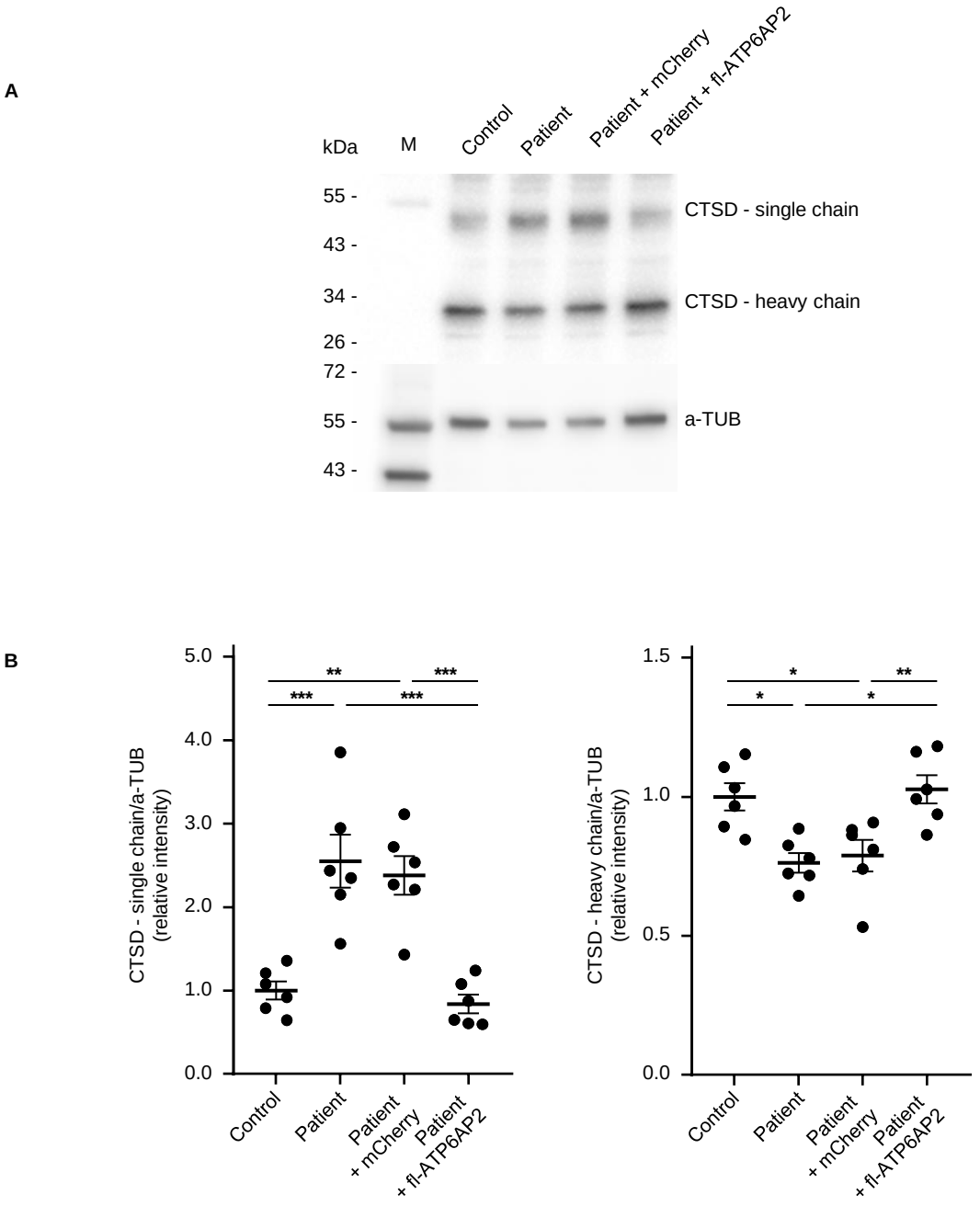
Supplemental Figure 5. Postnatal *Atp6ap2* deletion in postmitotic neurons. Immunostaining in *cKOs* shows enlarged lysosomes and accumulation of LC3B and p62 double positive aggregates in the hippocampus (CA1). Data shows individual values and Mean±SEM. n=6 different animals/group. *P<0.05, Student's t-test (unpaired, two tailed). Scale bars: 20 µm.



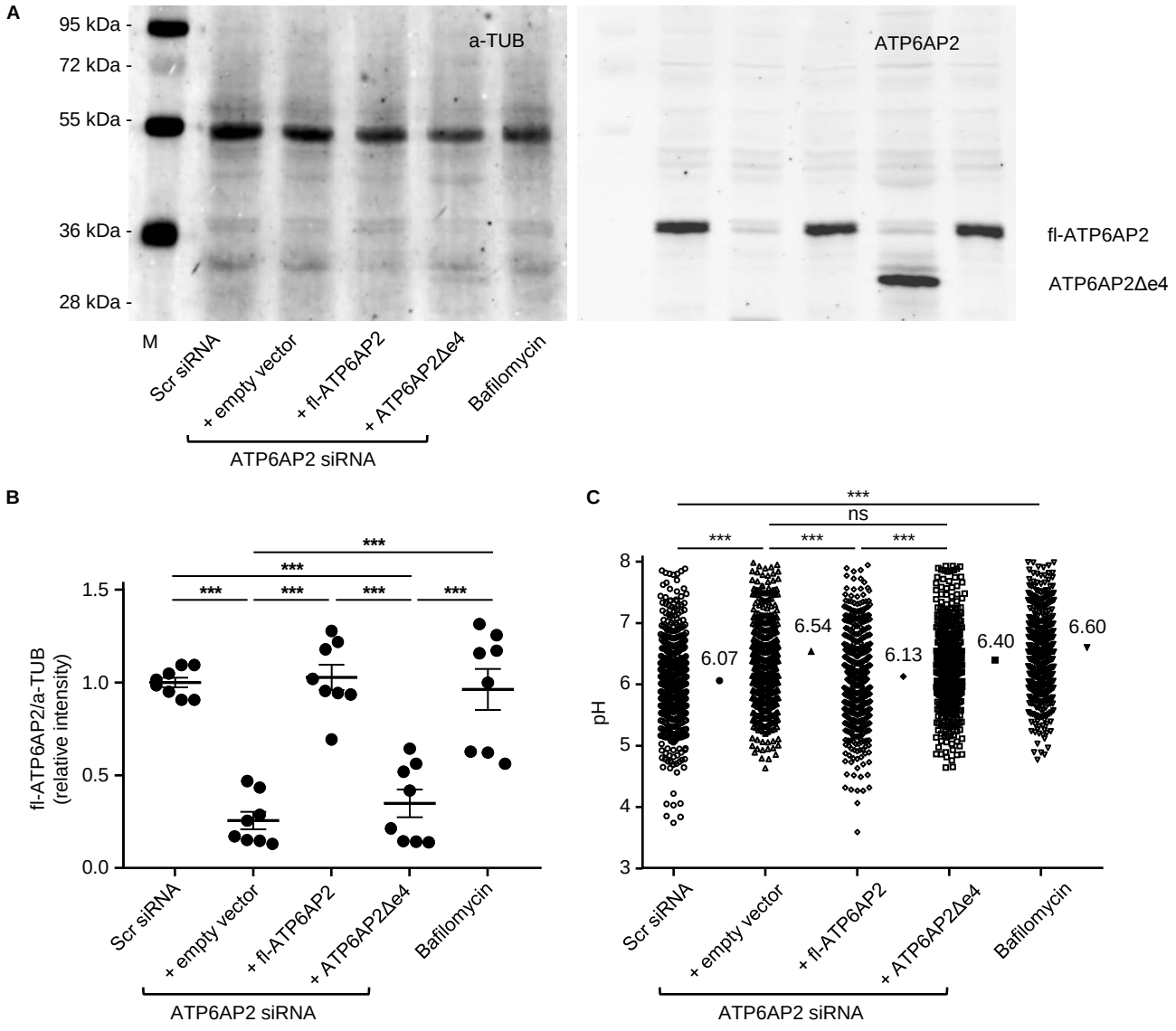
Supplemental Figure 6. Generation of iPSCs and assessment of pluripotency. (**A**) Morphology of iPSC clones generated from the patient carrying *ATP6AP2* [c.301-11_301-10delTT] variant. (**B**) Flow cytometry analysis of co-expression of stem-cell-specific surface proteins SSEA4, TRA-1-60 and SSEA3 and stem cell antigen human early stem cell antigen-1 (HESCA-1). (**C**) Hematoxylin/eosin staining of teratomas derived from patient iPSC showing generation of tissues derived from all germlayers. (**D**) Normal karyotype of patient derived iPSC line. (**E**) PCR analysis of the patient iPSCs using primers in intron 3 and exon 5 for genomic DNA and in exon 3 and exon 5 for cDNA. (**F**) ATP6AP2 Western blot of iPSC lines. The patient cell lines (with or without lentiviral vector control expressing mCherry) expressing the ATP6AP2Δ4 protein product. The cell line 'patient + fl-ATP6AP2' carries a lentiviral vector for the re-expression of full-length ATP6AP2.



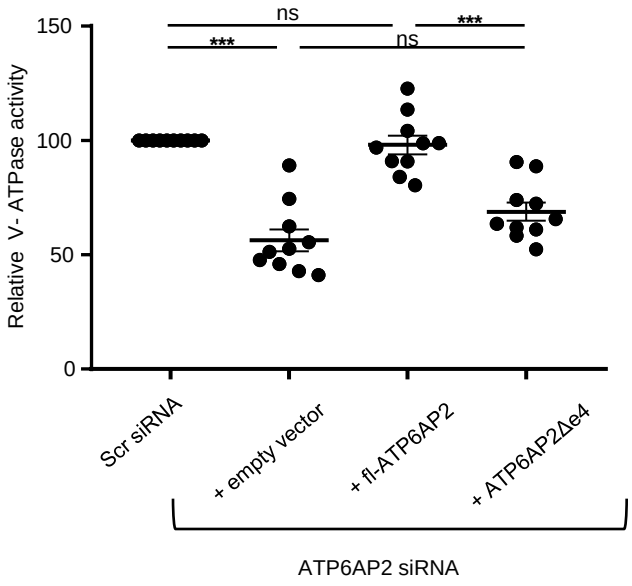
Supplemental Figure 7. Western blots iPSCs and neural progenitor cells (NPCs). (**A**) Increased p62 and LC3B in patient lines is rescued by re-expression of fl-ATP6AP2. **(B)** Quantification of Western blots. Data shows individual values and Mean±SEM. n=6/group. *P<0.05; **P<0.01; ***P<0.001, one-way ANOVA followed by Bonferroni post-test.



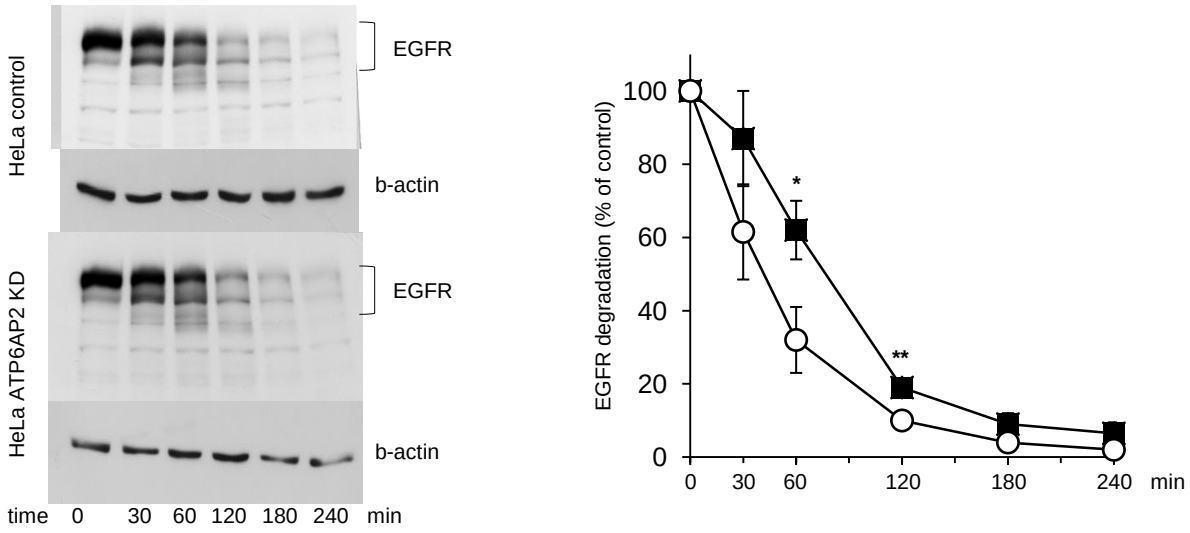
Supplemental Figure 8. Western blots of iPSC-derived neural progenitor cells (NPCs). (**A**) Impaired CathepsinD (CSTD) processing in patient lines is rescued by re-expression of fl-ATP6AP2. (**B**) Quantification of Western blots. Data shows individual values and Mean±SEM. n=6/group. **P*<0.05, ***P*<0.01, ****P*<0.001, one-way ANOVA followed by Bonferroni post-test.



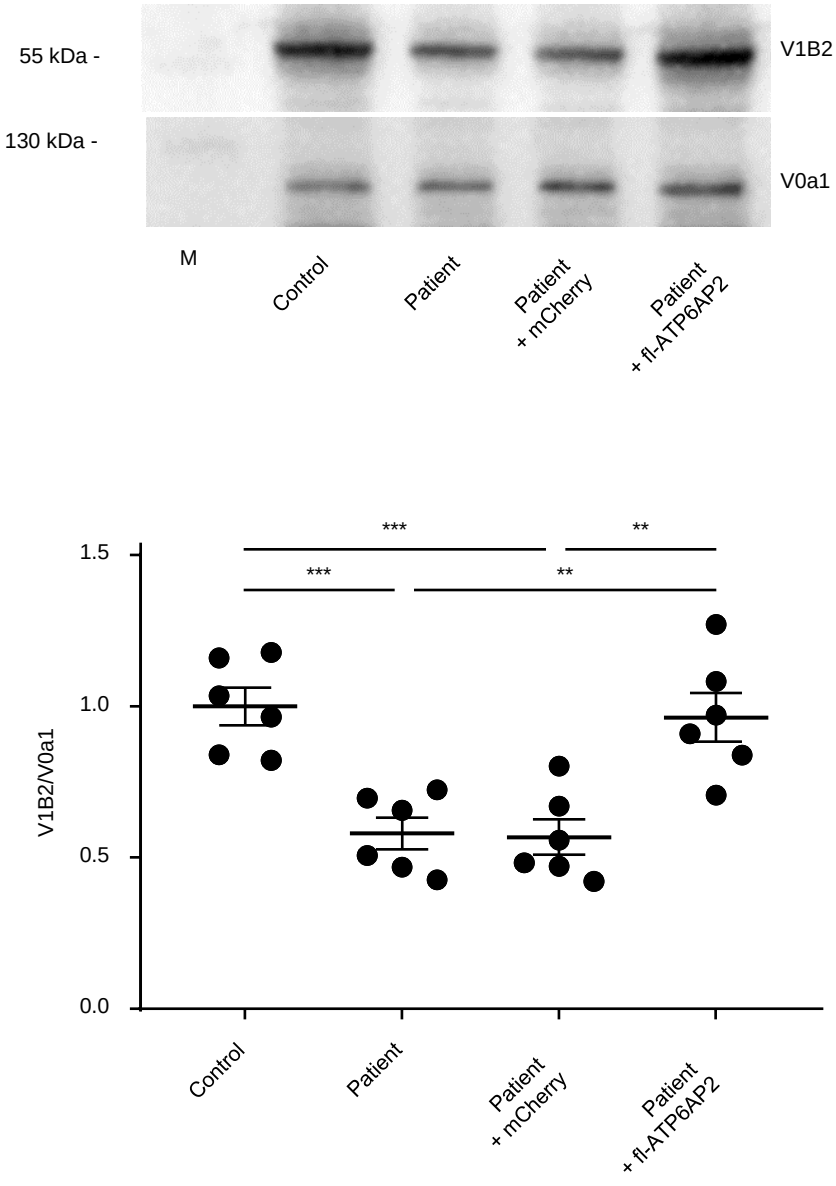
Supplemental Figure 9. *ATP6AP2* siRNA knockdown in HeLa cells impairs vesicular acidification. (**A**) HeLa cells were transfected with scramble (Scr) siRNA, 3'-UTR ATP6AP2 siRNA, or co-express 3'-UTR ATP6AP2 siRNA and fl-ATP6AP2 or co-express 3'-UTR ATP6AP2 siRNA and ATP6AP2Δe4, or treated with proton pump inhibitor bafilomycin. Cell lysate was analyzed by Western blot 24 h post transfection. Left: a-Tubulin loading control with molecular weight marker (M). Right: ATP6AP2. (**B**) Quantification of Western blots. Data shows individual values and Mean±SEM. n=8/group from four different experiments. ***P<0.001, one-way ANOVA followed by Bonferroni post-test. (**C**) Vesicular pH in HeLa cells transfected with Scr siRNA or ATP6AP2 siRNA with or without fl-ATP6AP2 and ATP6AP2Δe4, or cells treated with bafilomycin. Graph shows individual vesicular pH and the median value. n=436-553 measurements from five independent experiments. ***P<0.001; ns: P>0.05, Kruskal-Wallis test (chi-squared=174.42, df=4, P<0.001) followed by Dunn's multiple comparison test. (**D**) Overexpression of fl-ATP6AP2 or ATP6AP2Δe4 does not change vesicular pH. Graph shows individual vesicular pH and the median value. n=366-386 measurements from two independent experiments. Kruskal-Wallis test (chi-squared=0.47, df=2, P=0.79).



Supplemental Figure 10. *ATP6AP2* siRNA knockdown impairs lysosomal V–ATPase activity. HEK293T cells were transfected with scramble (Scr) siRNA, 3'-UTR ATP6AP2 siRNA, or co-express 3'-UTR ATP6AP2 siRNA and fl-ATP6AP2 or co-express 3'-UTR ATP6AP2 siRNA and ATP6AP2Δe4. Cells were analyzed 48 h post transfection. Scr siRNA serves as reference, defining 100% in each experiment. n=10/group, one-way ANOVA $F(3, 36)=33.8383$, ***$P<0.001$ by Bonferroni post-test.



Supplemental Figure 11. *ATP6AP2* siRNA knockdown in HeLa cells impairs epidermal growth factor receptor (EGFR) degradation. HeLa Silencix cells stably expressing *ATP6AP2* shRNA or scramble shRNA were incubated with recombinant human EGF and cell lysate was analyzed at 0, 30, 60, 120, 180, 240 minutes. Left: Representative Western blot. Right: Quantification of the blots. Data shows Mean±SEM. n=3/group from three independent experiments. **P*<0.05; ***P*<0.01, Mann Whitney *U* test.



Supplemental Figure 12. Western blots of iPSC-derived neural progenitor cells (NPCs). Membrane-bound V1B2 subunit of the V1 sector and V0a1 subunit of the V0 sector of the V-ATPase of were analyzed by Western blot in iPSC-derived NPCs. Quantified as ratio of V1B2 to V0a1. Data shows individual values and Mean±SEM. n=6/group. ***P*<0.01; ****P*<0.001, one-way ANOVA followed by Bonferroni post-test.