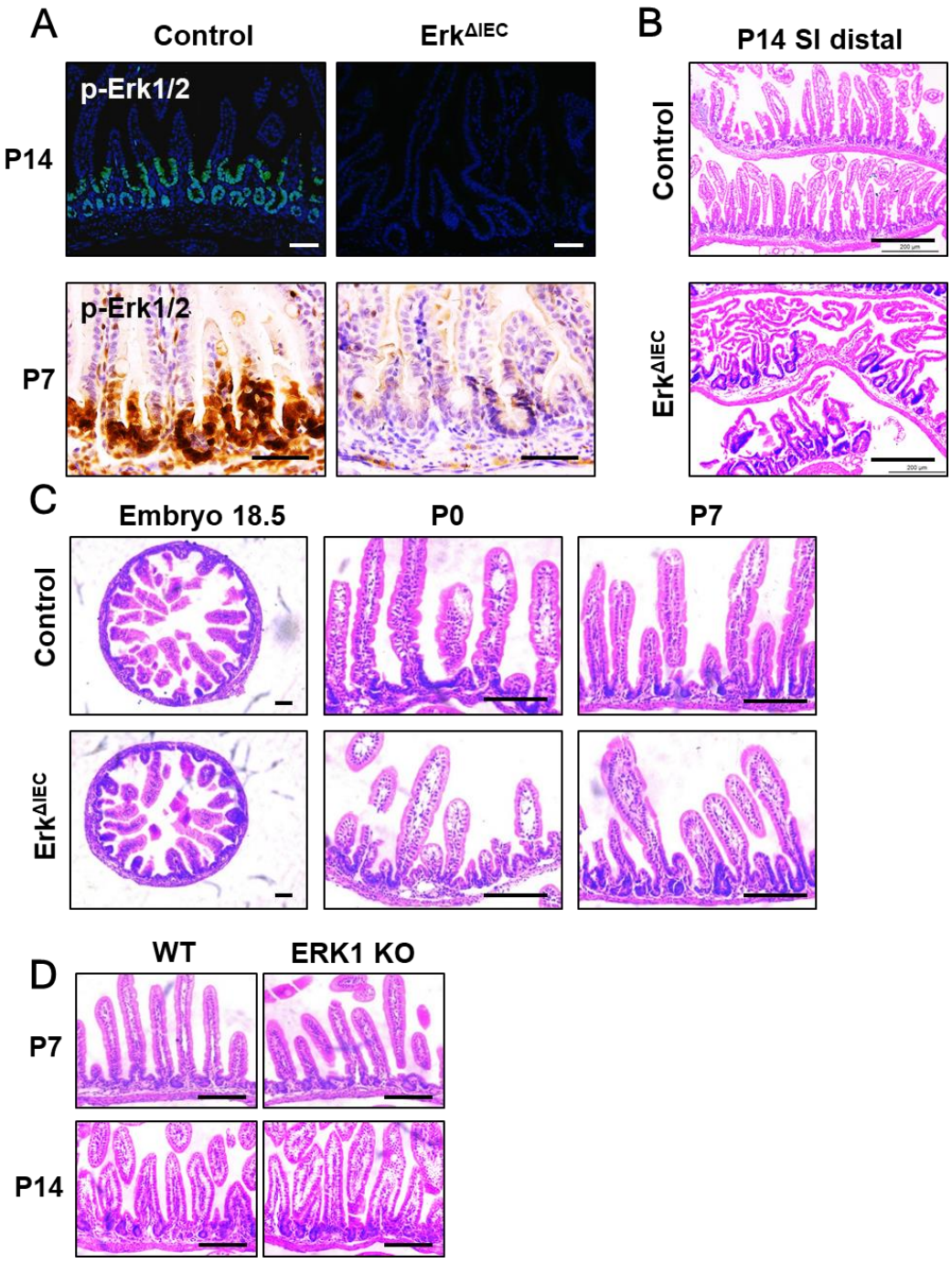

Figure S1: Analysis of intestinal morphology at different time points.

(**A**) Immunostaining for p-Erk1/2 in intestines of control and Erk$^{\Delta IEC}$ littermates at P14 and P7. (**B**) H&E staining of small intestine distal part from control and Erk$^{\Delta IEC}$ littermates at P14. (**C**) H&E staining of small intestine from control and Erk$^{\Delta IEC}$ littermates at Embryo day 18.5 and postnatal day 0 and day 7. (**D**) H&E staining of small intestine (SI) from WT and Erk1 KO littermates at P7 and P14.

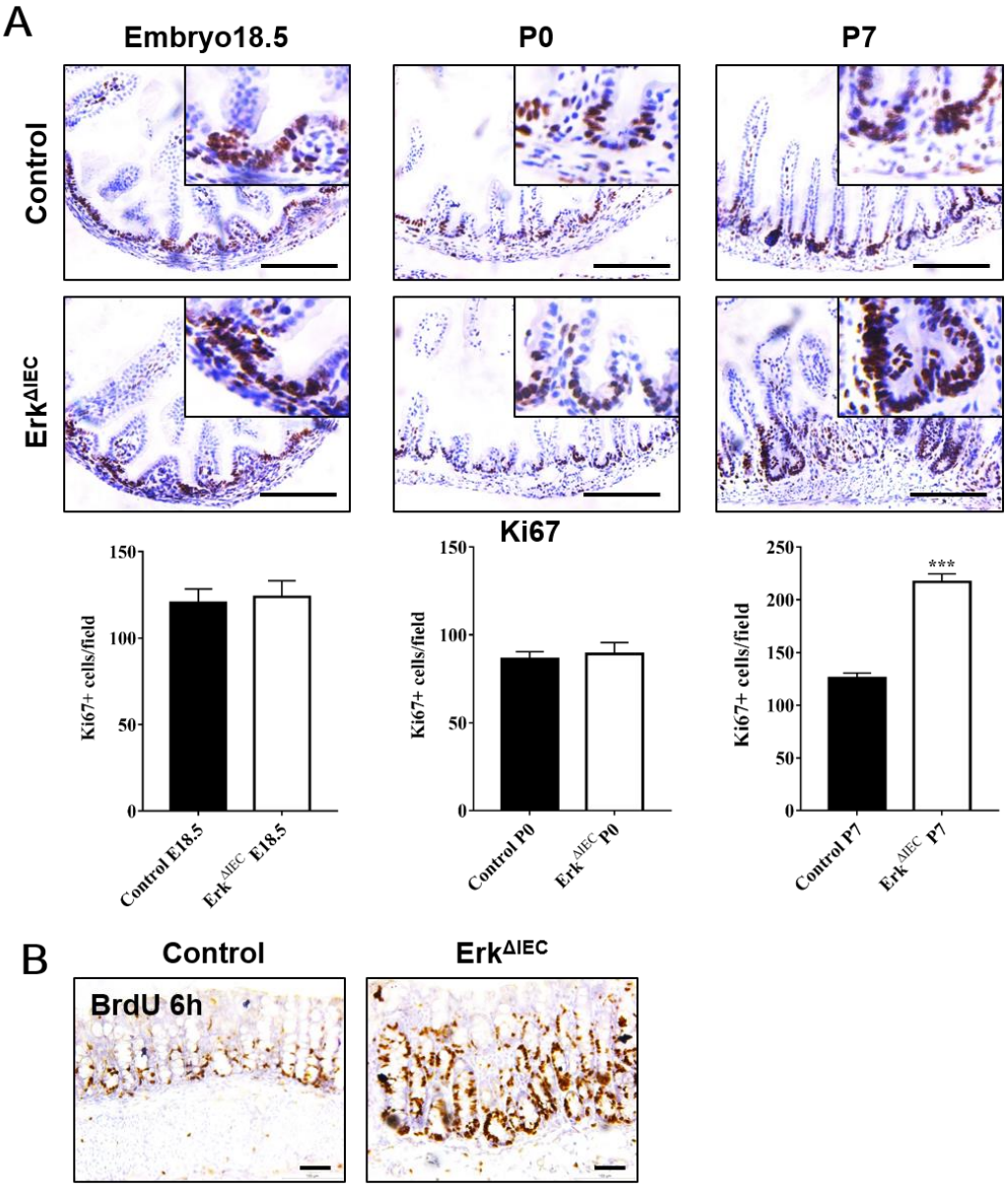

Figure S2: Analysis of cell proliferation at different time points. **(A)** Immunohistochemistry staining with antibody against Ki67 and quantification in small intestines of control and Erk$^{\Delta IEC}$ littermates at Embryo day 18.5 and postnatal day 0 and day7 (n=3). (B) BrdU was injected into two-week old control and Erk$^{\Delta IEC}$ littermates, and the intestinal tissues were harvested 6 hours after injection. BrdU incorporated cells were visualized via immunohistochemical staining. (Scale bars: 100μm)

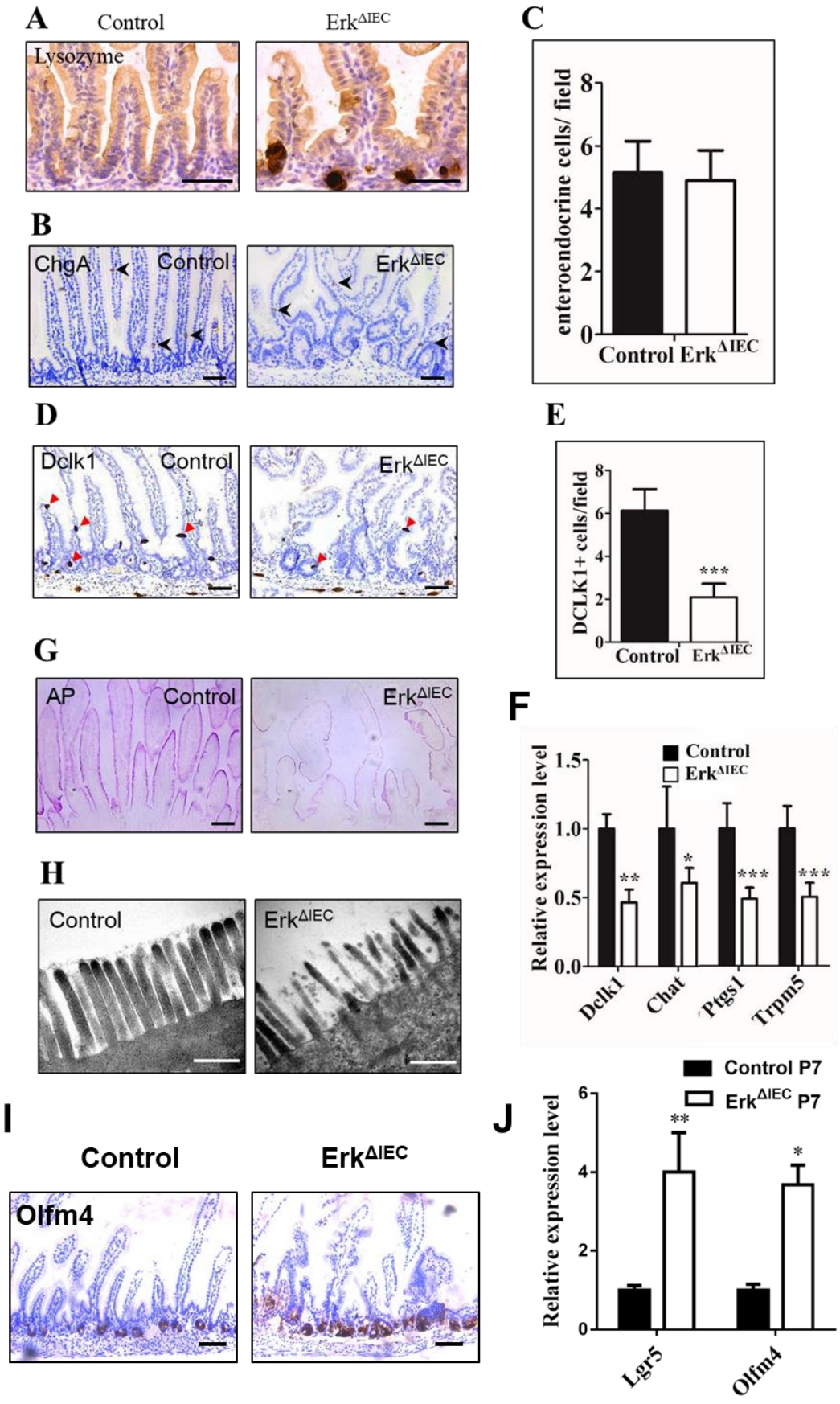

Figure S3: Erk1/2 in intestinal epithelial cells regulated cell differentiation (**A**) Paneth cells in control and Erk1/2 mutant small intestine were stained with Lysozyme specific antibody at P6. (**B**) Staining of enteroendocrine cells for Chromogranin A (ChgA) in control and Erk1/2 mutant small intestines at P14. Arrowheads: ChgA positive cells. (**C**) Quantification of Chromogranin A-positive cells in small intestines of control and Erk mutant mice at P14 (n=3). (**D**) The number of Dclk1-positive tuft cells was decreased in Erk1/2 mutant intestines compared with control intestines. Arrowheads: Dclk1 positive signals. (**E**) Quantification of Dclk1- positive cells in small intestines of control and Erk mutant mice at P14 (n=3). (**F**) qRT- PCR analysis of mRNA levels of Tuft cell specific genes in small intestines of control and Erk mutant mice at P14. Data are mean ± SEM (n=3,*P<0.05; **P<0.01). (**G**) Alkaline phosphatase (AP) positive enterocytes are immature in Erk1/2 deficient small intestines at P14. (**H**) The brush border of Erk1/2 deficient enterocytes is disrupted. The images of small intestinal tissues from control

and $Erk^{\Delta IEC}$ littermates were obtained by transmission electron microscopy. (**I**) Stem cells in control and Erk1/2 mutant small intestine were stained with Olfm4 specific antibody at P7. (**J**) qRT- PCR analysis of mRNA levels of stem cells specific genes in small intestines of control and Erk mutant mice at P7. Data are mean ± SEM (n=3,*P<0.05; **P<0.01). (Scale bars: 50μm except 0.5μm in H).

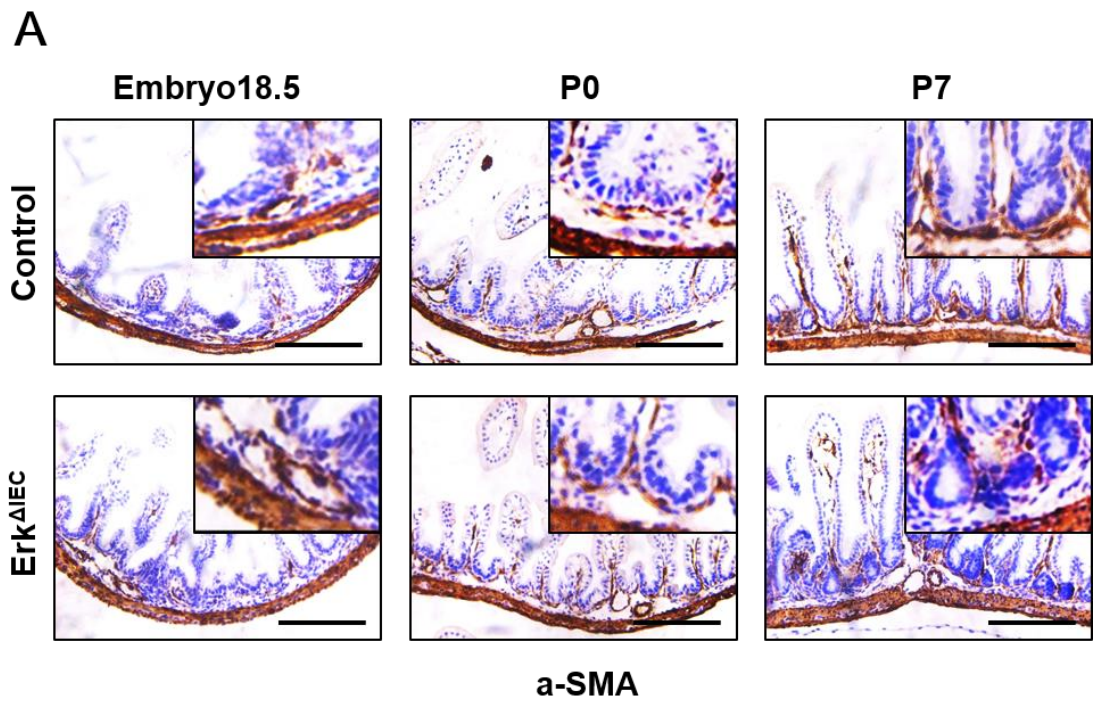

Figure S4: (**A**) Immunohistochemistry staining with antibody against a-SMA in intestines of control and $Erk^{\Delta IEC}$ littermates at Embryo day 18.5 and postnatal day 0 and day 7. (Scale bars: 100μm)

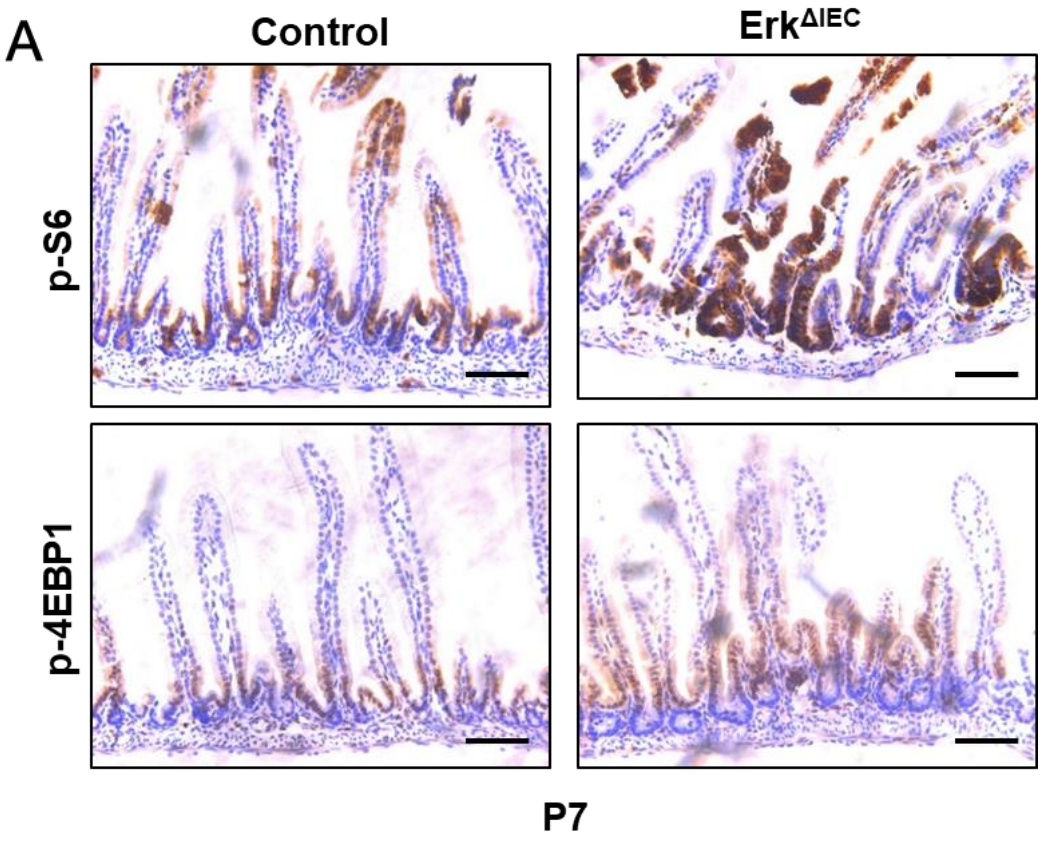

Figure S5: (**A**) Immunohistochemical analysis of p-S6 and p-4EBP1 in control and Erk mutant intestines at P7. (Scale bars: 50µm)

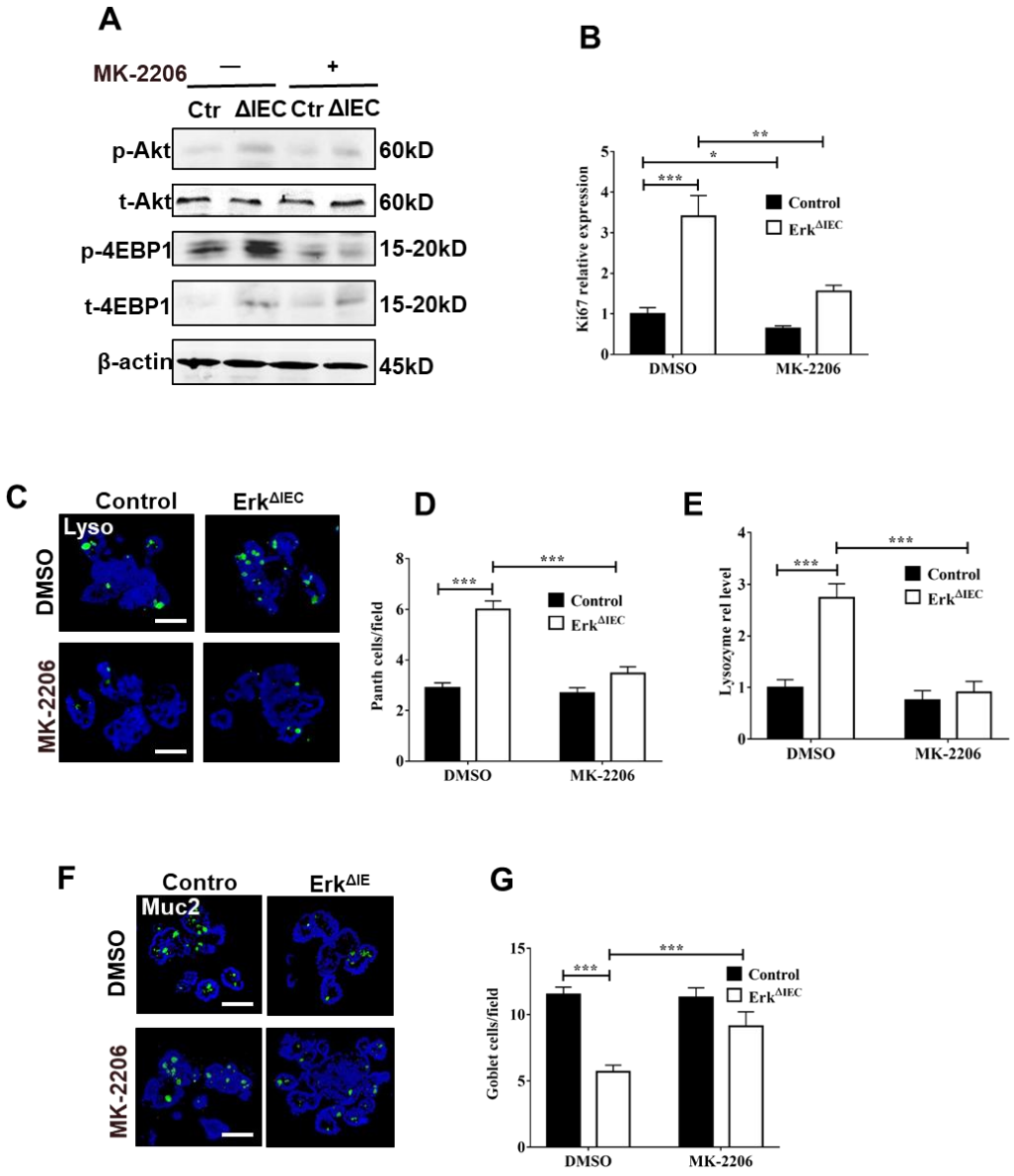

Figure S6: Akt inhibition through MK-2206 treatment rescued Erk1/2 depletion-induced cell differentiation defects ex vivo. (**A**) Treatment with MK-2206 inhibited Akt and 4EBP1 activity in Erk mutant mouse organoids. The protein level of p-Akt and p-4EBP1 was determined by Western blotting in intestinal organoid samples isolated from control and inhibitor treatment groups. β-actin was used as a loading control. (*B*) Quantitative real-time PCR analysis of Ki67 mRNA levels in intestinal organoid samples isolated from control and inhibitor treatment groups after 4 days of culture. Data are mean ± SEM (n=3,*P<0.05; **P<0.01). (**C-G**) MK-2206 treatment rescued Erk1/2 deficiency-induced cell differentiation defects in intestinal organoid culture model. Immunofluorescent analysis for Lysozyme (C) and Muc2 (F) in

intestinal organoid samples isolated from control and inhibitor treatment groups after 4 days. Quantification of Paneth cells (D) and goblet cells (G) per field (n=3 mice per group and a total of 90 fields were analyzed for each genotype). (E) Quantitative real-time PCR analysis of Lysozyme mRNA levels in intestinal organoid samples isolated from control and inhibitor treatment groups after treatment for 4 days. Data are mean ± SEM (n=3,*P<0.05; **P<0.01). (Scale bars: 100μm).