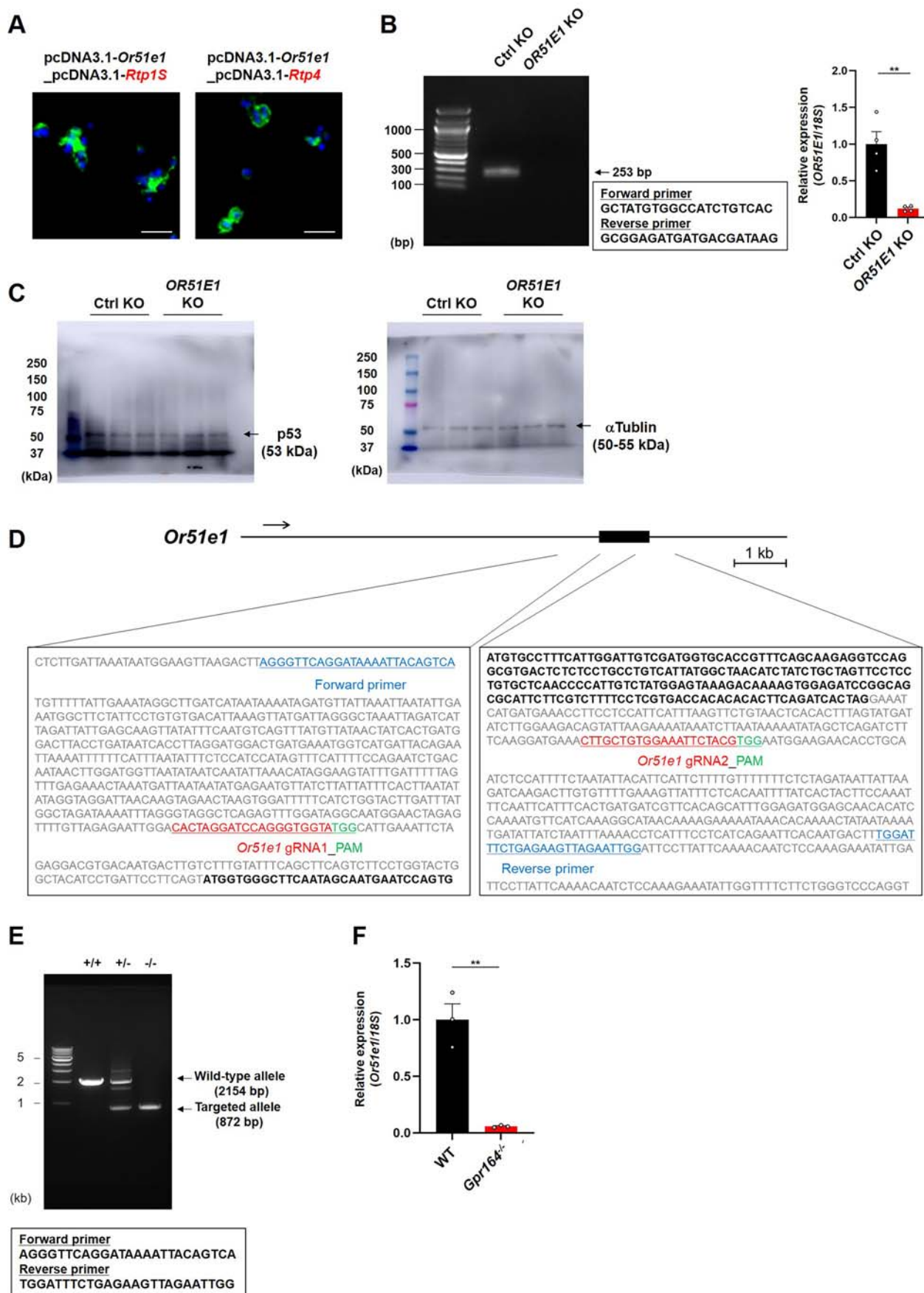
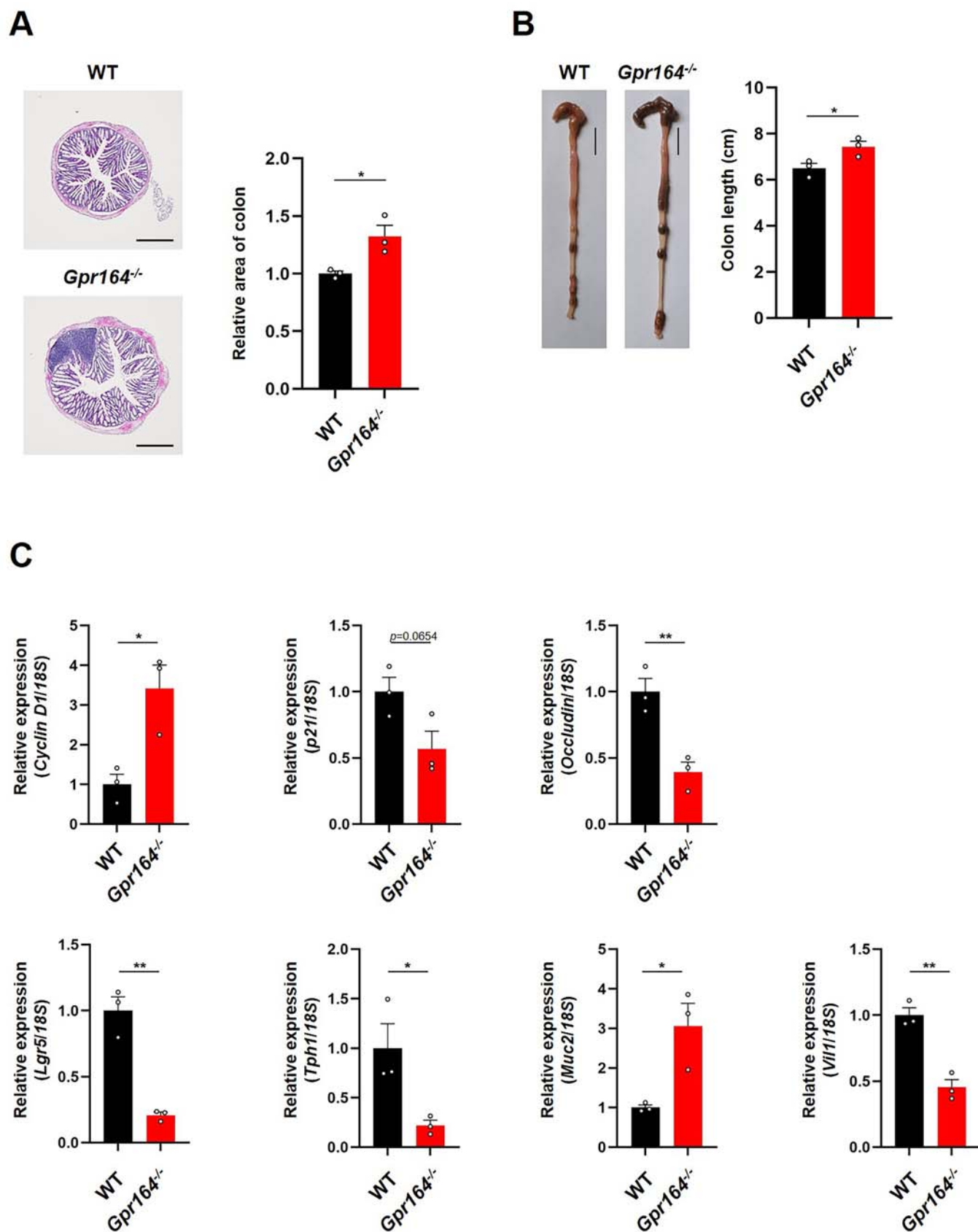


Expanded View Figures

Figure EV1. Generation of Or51e1-overexpressing cells, OR51E1-knockout cells, and Or51e1-knockout (*Gpr164*^{-/-}) mice.

(A) Representative images of immunofluorescent staining for mouse Or51e1 in HEK293 cells. Cells were co-transfected with HA-tagged Or51e1 and receptor-transporting protein (left; RTP1S, right; RTP4), and stained with anti-HA antibody (green). DAPI was used for nuclei staining. Scale bar, 50 μ m. (B) *OR51E1* expression in Caco-2 cells. *OR51E1*-deficient (*OR51E1* KO) cells were generated using the CRISPR/Cas9 system. For the detection of *OR51E1* expression, PCR amplification was done with the indicated primers, and the PCR products were separated on 1% agarose gels (left). The *OR51E1* mRNA expression level was determined by qRT-PCR ($n = 4$) (right). Error bars represent the mean $\pm$ SEM. $**P = 0.0021$ (Student's *t*-test). (C) Representative image of p53 protein expression. Cell extracts from Control KO or *OR51E1* KO cells were subjected to immunoblot analysis using an anti-p53 or anti- α Tubulin antibody ($n = 3$). (D) Schematic representation of CRISPR/Cas9 targeting sites in *Or51e1* gene. *Or51e1* gene knockout (*Gpr164*^{-/-}) mice were generated by using the CRISPR/Cas9 system in wild-type C57BL/6J zygotes. Bold letters indicate the coding region of *Or51e1* gene. Red or Green letters indicate guide RNA (gRNA) and protospacer adjacent motif (PAM), respectively. (E) For the detection of the wild-type and mutant alleles, PCR amplification was done with the indicated primers, and the PCR products were separated on 1% agarose gels. (F) The *Or51e1* mRNA expression level in colon was determined by qRT-PCR ($n = 3$). Error bars represent the mean $\pm$ SEM. $**P = 0.0025$ (Student's *t*-test). Source data are available online for this figure.





◀ **Figure EV2. Phenotypes of colonic hyperplasia in female *Gpr164*^{-/-} mice.**

(A) Representative images of colon sections stained with hematoxylin-eosin (HE). The cross-sections of colon obtained from female WT and female *Gpr164*^{-/-} mice were stained with HE, and the area of colon was measured using ImageJ software ($n = 3$). Scale bar, 500 μm . Error bars represent the mean $\pm$ SEM. $*P = 0.029$ (Student's *t*-test). (B) Representative images of colon ($n = 3$). Scale bar, 1 cm. Error bars represent the mean $\pm$ SEM. $*P = 0.0405$ (Student's *t*-test). (C) The expression levels of genes related to cell cycle, intestinal barrier and epithelial lineage were determined by qRT-PCR. Total RNA was extracted from colon tissue of female WT and female *Gpr164*^{-/-} mice ($n = 3$). Error bars represent the mean $\pm$ SEM. $*P = 0.0194$; *Cyclin D1* gene, $P = 0.0654$; *p21* gene, $**P = 0.0083$; *Occludin* gene, $**P = 0.0018$; *Lgr5* gene, $*P = 0.0366$; *Tph1* gene, $*P = 0.023$; *Muc2* gene, $**P = 0.0024$; *Vil1* gene (*Tph-1* gene; Mann-Whitney *U*-test, other genes; Student's *t*-test). Source data are available online for this figure.

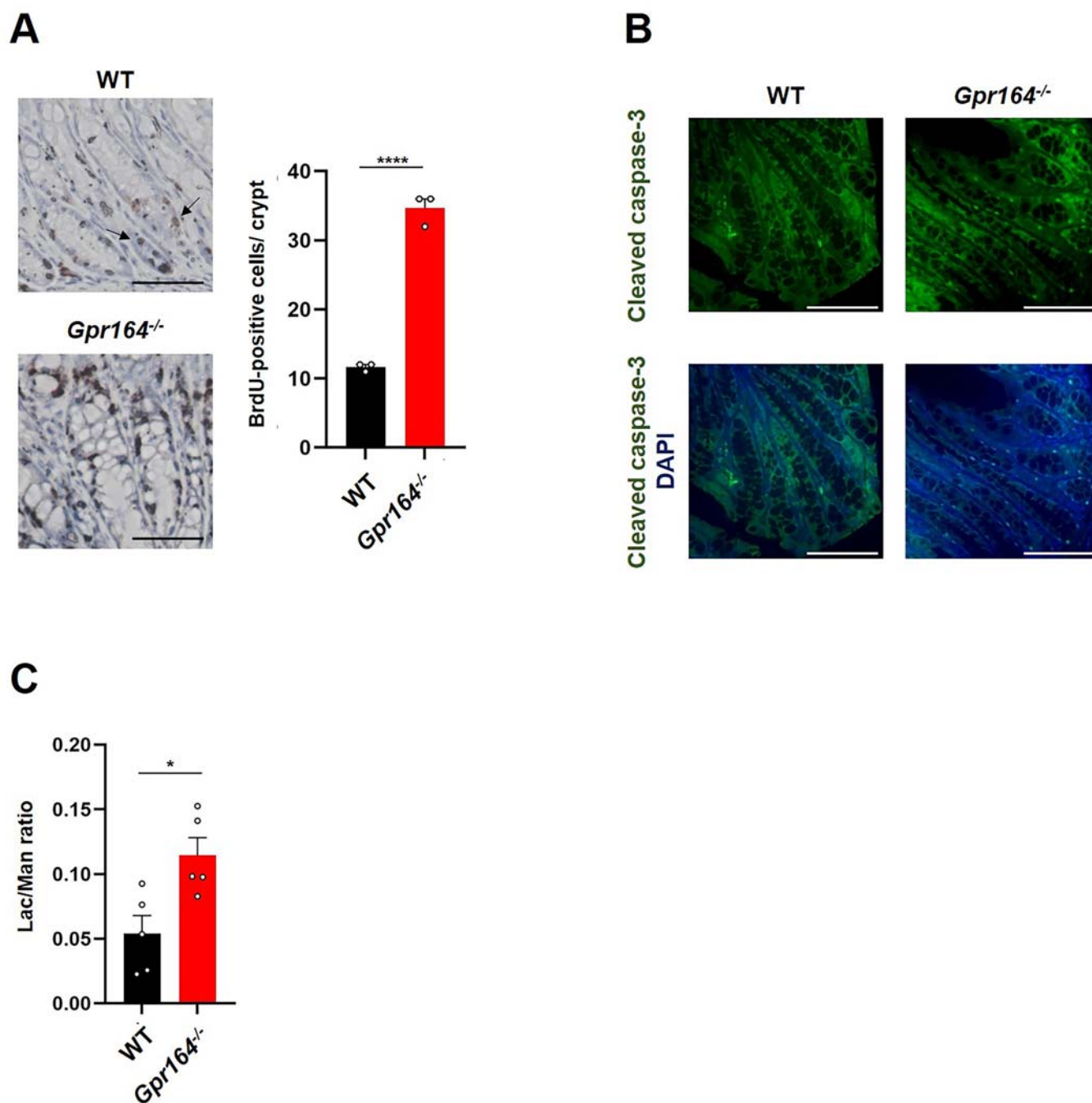
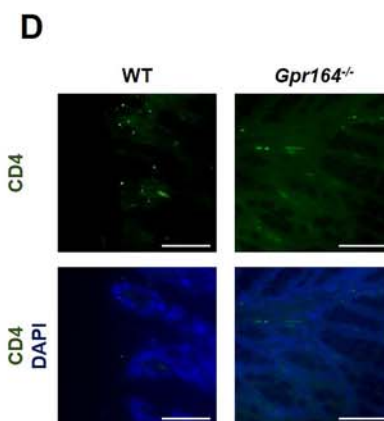
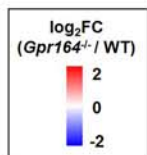
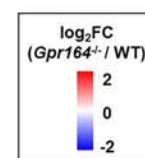


Figure EV3. Effects of *Gpr164* deletion on proliferation, apoptosis, and intestinal barrier function.

(A) Representative images of 5-bromo-2'-deoxyuridine (BrdU)-labeled cells in colon. The cross-sections of colon obtained from WT and *Gpr164*^{-/-} mice were stained with anti- BrdU antibody, and visualized with DAB ($n = 3$). Hematoxylin was used for nuclei staining. Scale bar, 50 μm . Error bars represent the mean $\pm$ SEM. **** $P < 0.0001$ (Student's t -test). (B) Representative images of immunofluorescent staining for cleaved caspase-3. The cross-sections of colon obtained from WT and *Gpr164*^{-/-} mice were stained with anti-cleaved caspase-3 antibody ($n = 3$). DAPI was used for nuclei staining. Scale bar, 50 μm . (C) Assessment of intestinal permeability by measuring urinary lactulose and mannitol. The excretion ratio of lactulose/mannitol in urine was assessed ($n = 5$). Error bars represent the mean $\pm$ SEM. * $P = 0.0143$ (Student's t -test). Source data are available online for this figure.



**Figure EV4. Genome-wide RNA sequencing of *Gpr164*^{-/-} mice.**

(A) KEGG enrichment analysis involved in the molecular function in colon of *Gpr164*^{-/-} mice ($n = 5$). P values were adjusted based on the false discovery rate (FDR). (B, C) KEGG pathway enrichment related to tight junction (B) and inflammatory bowel disease (C). Increased or decreased levels of gene expressions are shown in red or blue, respectively. (D) Representative images of immunofluorescent staining for CD4. The cross-sections of colon obtained from WT and *Gpr164*^{-/-} mice were stained with anti-CD4 antibody ($n = 3$). DAPI was used for nuclei staining. Scale bar, 50 μm . Source data are available online for this figure.

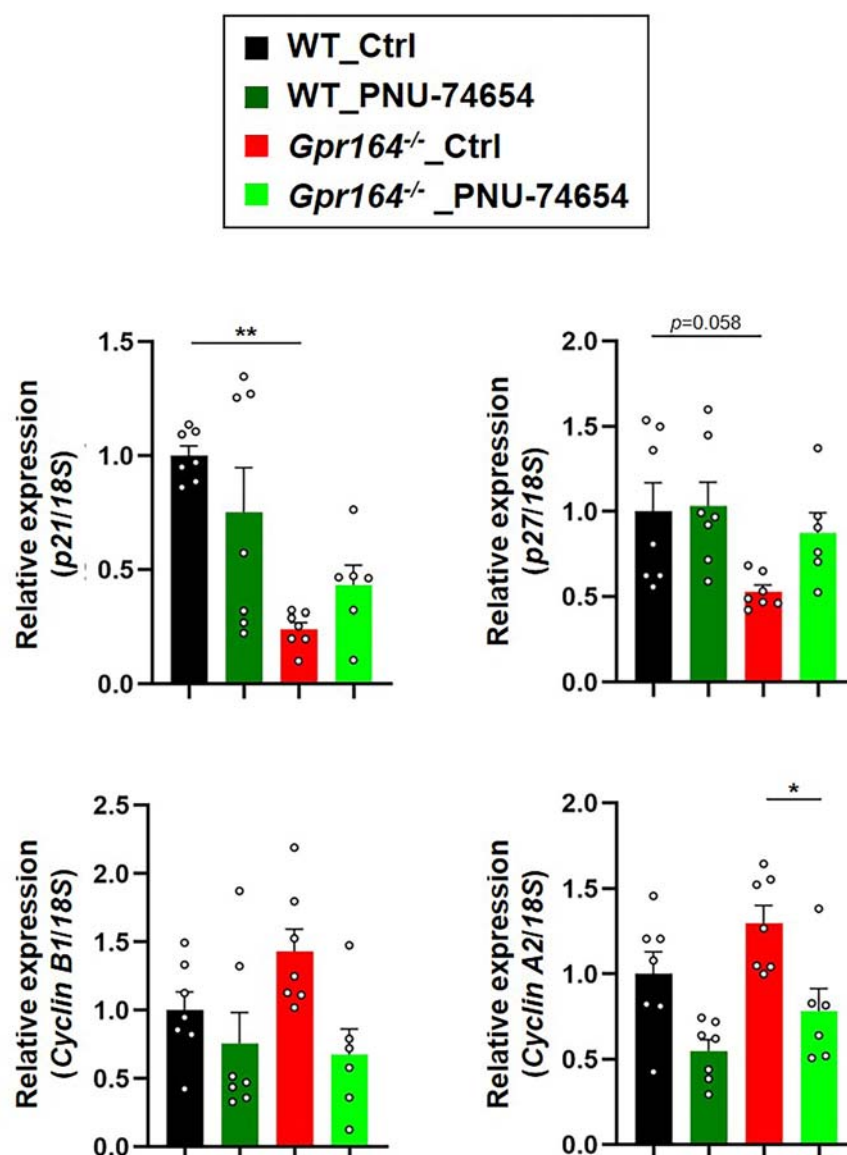


Figure EV5. Cell cycle gene expressions in PNU-74654-treated *Gpr164*^{-/-} mice.

The mRNA expression levels of cell cycle genes determined by qRT-PCR ($n = 6-7$). WT and *Gpr164*^{-/-} mice were injected intraperitoneally with PNU-74654 (15 mg/kg body weight, every 2 days for 3 weeks), and total RNA was extracted from colon tissue of WT and *Gpr164*^{-/-} mice ($n = 6-7$). Error bars represent the mean $\pm$ SEM.

** $P = 0.0028$; WT_Ctrl vs *Gpr164*^{-/-}_Ctrl of p21 gene, $P = 0.0586$; WT_Ctrl vs *Gpr164*^{-/-}_Ctrl of p27 gene, $P = 0.0173$; *Gpr164*^{-/-}_Ctrl vs *Gpr164*^{-/-}_PNU-74654 of Cyclin A2 gene (p21 and Cyclin B1 genes; Dunn's test, p27 and Cyclin A2 genes; Tukey-Kramer test). Source data are available online for this figure.