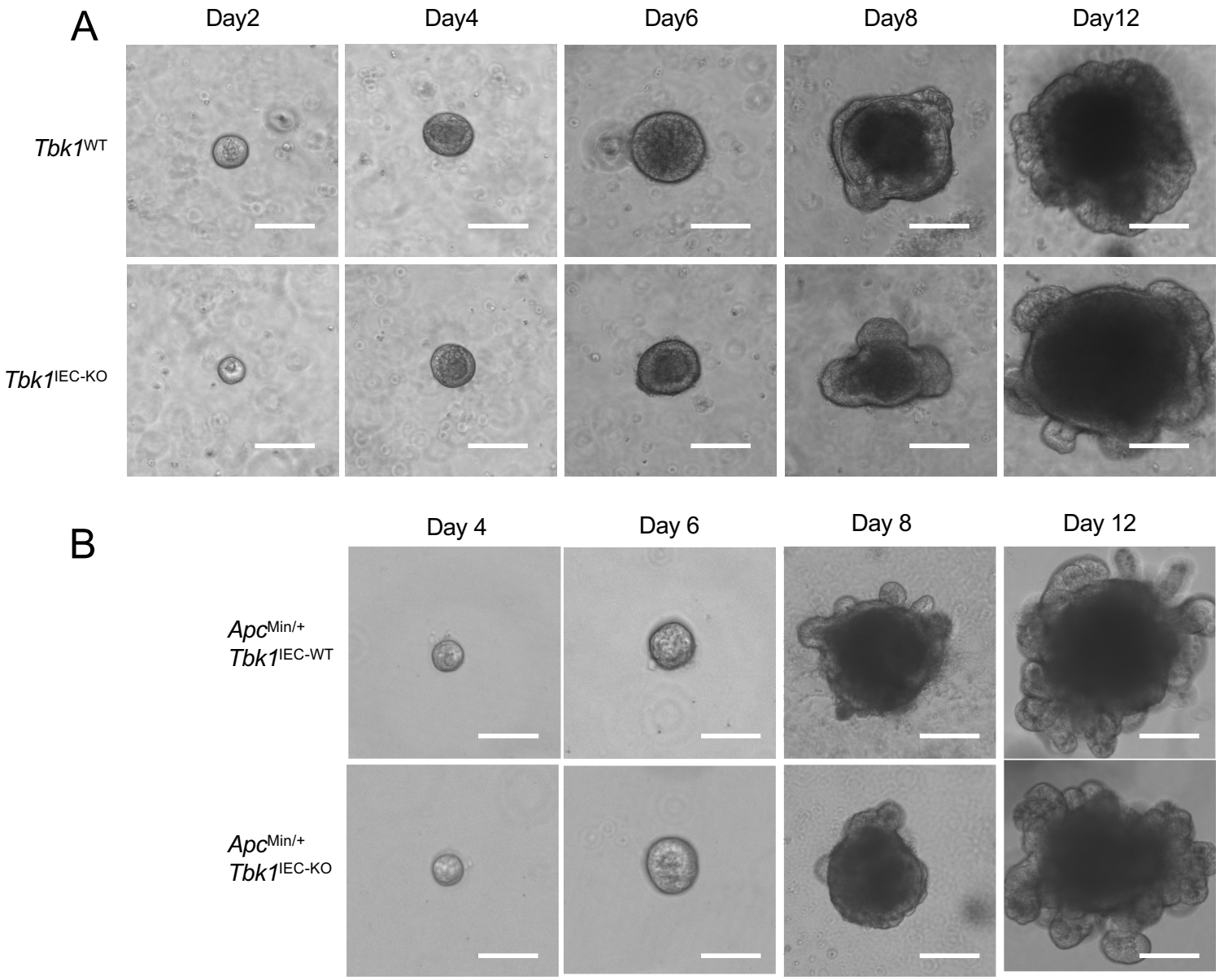
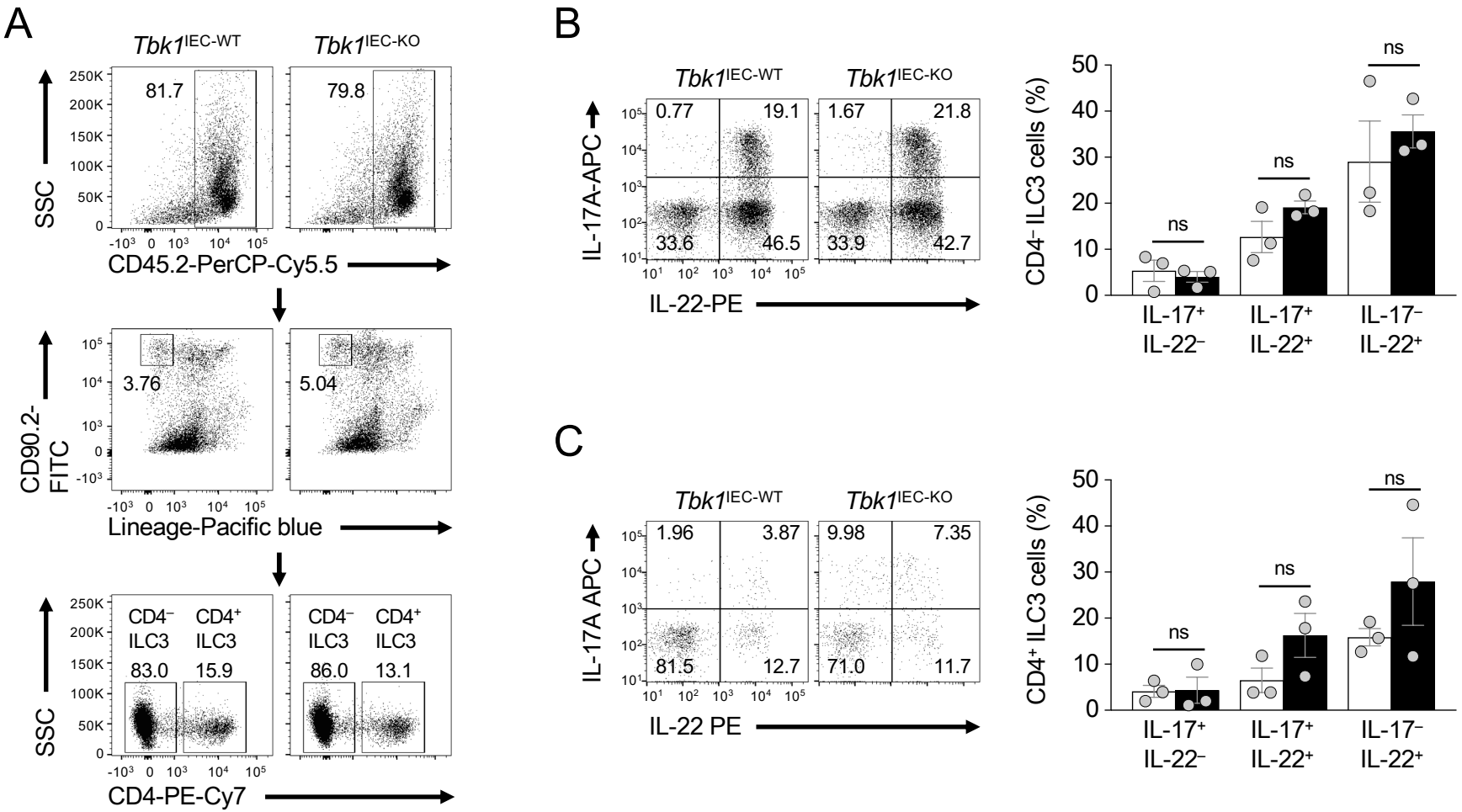
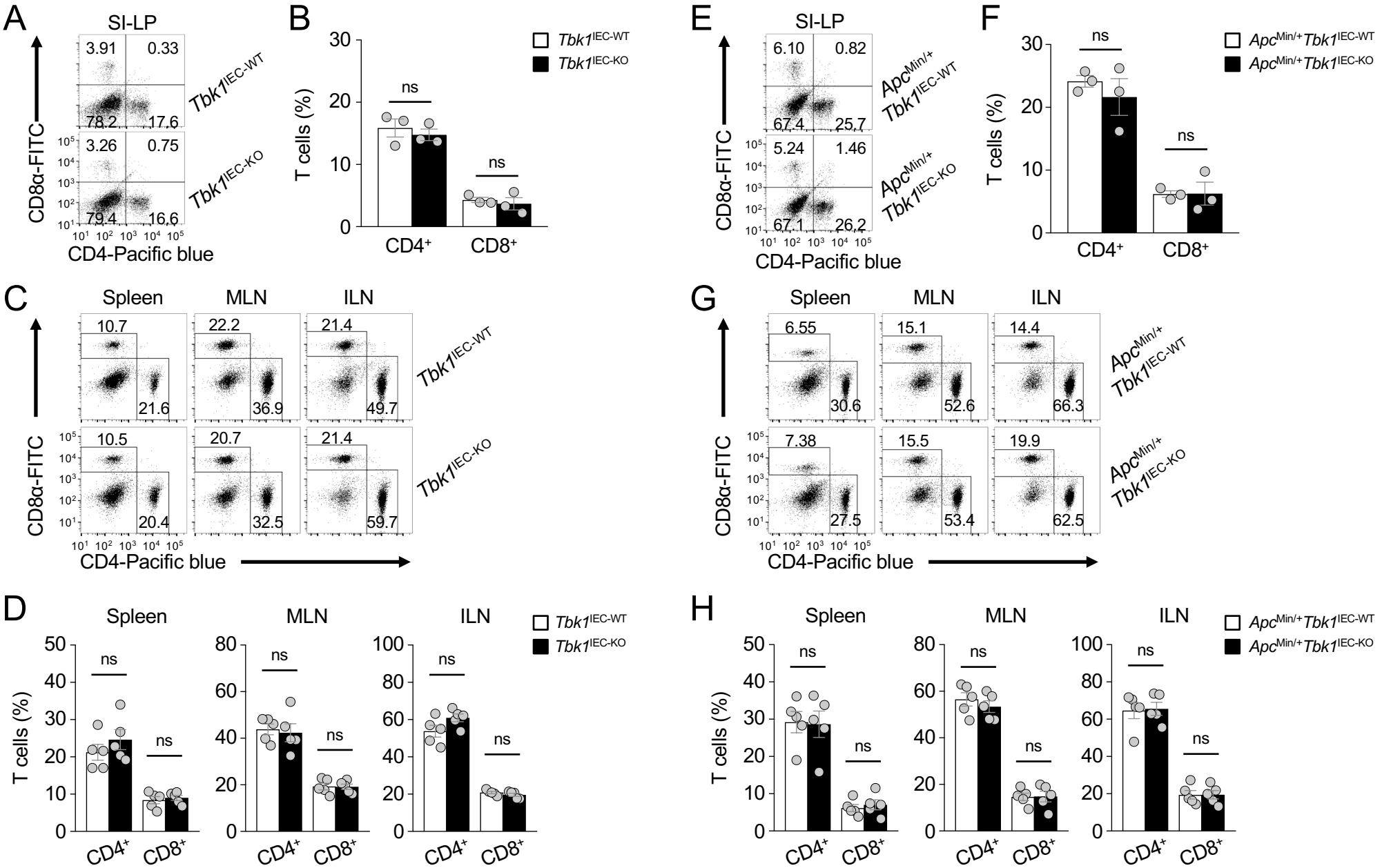


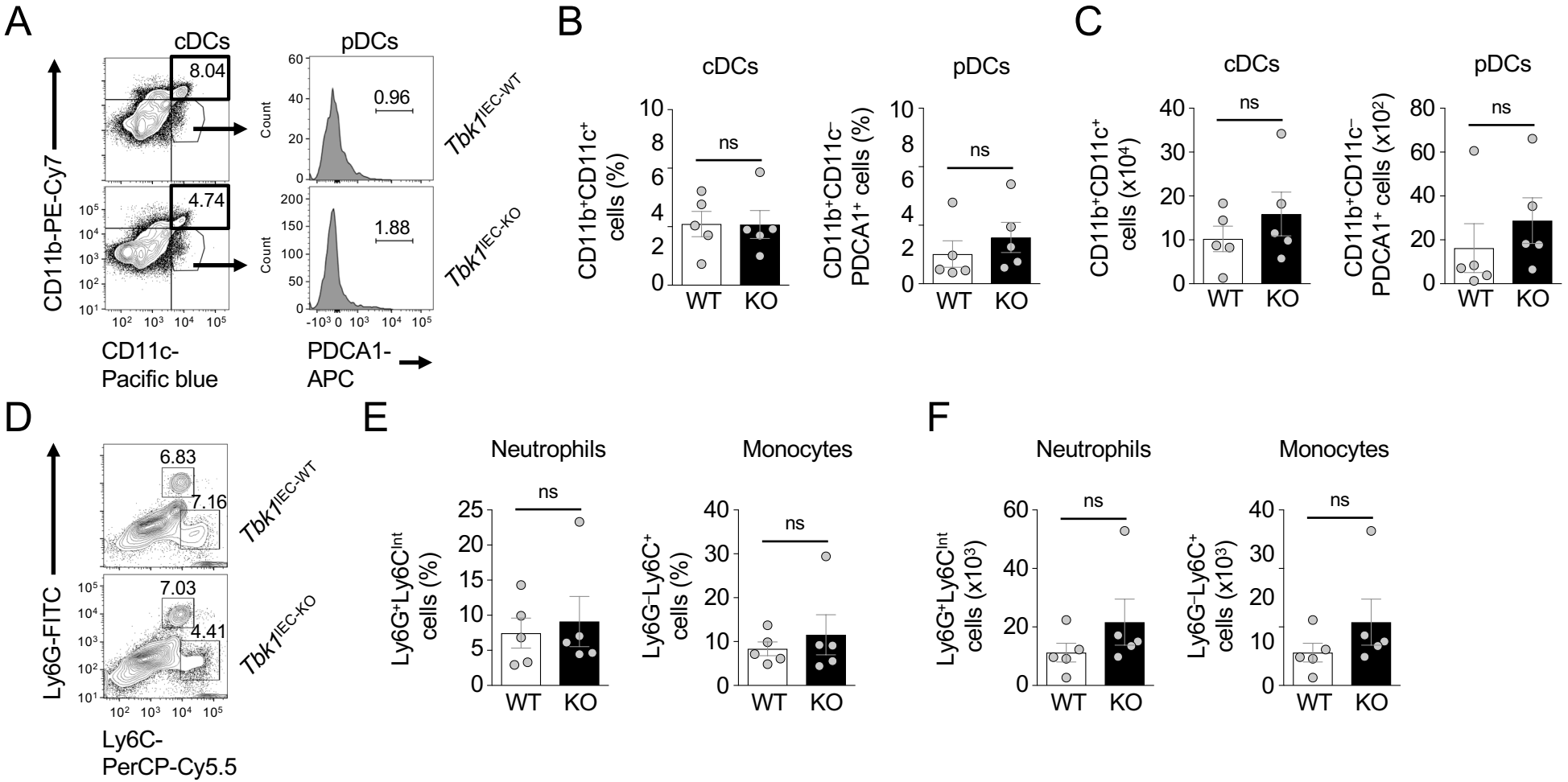
Supplementary Figure 1. IEC-specific deletion of TBK1 has no obvious effect on the propagation of intestinal stem cells and tumor cells. (*A*) Small intestinal organoids derived from 6-week-old *Tbk1*^{WT} and *Tbk1*$^{IEC\text{-}KO}$ mice were monitored for the indicated time points. (*B*) Small intestinal polyp tumoroids derived from 5-month-old *Apc*$^{Min/+}$*Tbk1*^{WT} or *Apc*$^{Min/+}$*Tbk1*$^{IEC\text{-}KO}$ mice were monitored from growth for the indicated time points. Scale bar = 100 μm. Data are representative of 2-3 independent experiments.



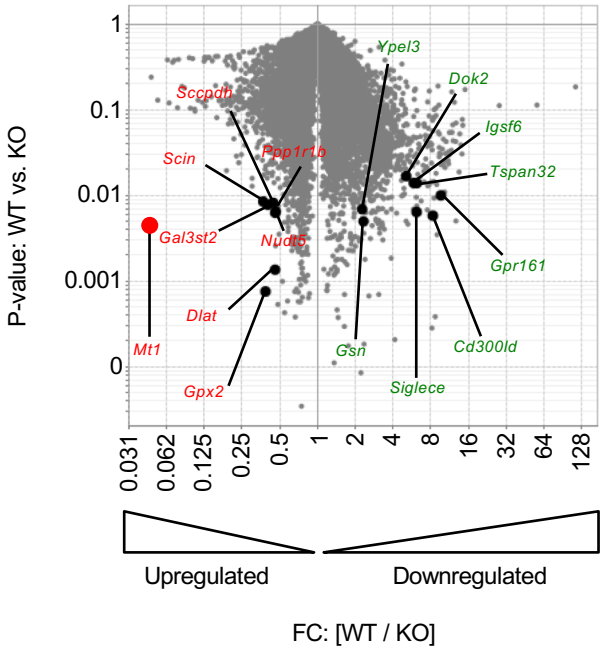
Supplementary Figure 2. IEC-specific deletion of TBK1 has no effect on the frequency of lamina propria ILC3s. (*A*) Gating strategy for flow cytometric analysis of CD4$^-$ and CD4$^+$ ILC3 populations. (*B* and *C*). Flow cytometric analysis of CD4$^-$ (*B*) and CD4$^+$ (*C*) ILC3 populations in small intestinal lamina propria of 6-week-old *Tbk1*$^{IEC\text{-}WT}$ and *Tbk1*$^{IEC\text{-}KO}$ mice (n = 3 each). Data are presented as representative FACS plots (left) and summary graphs (right, each circle represents a mouse). Data are representative of 2 independent experiments. Summary data are mean ± s.e.m. with P values being determined by unpaired two-tailed Student's *t*-test. ns, not significant.



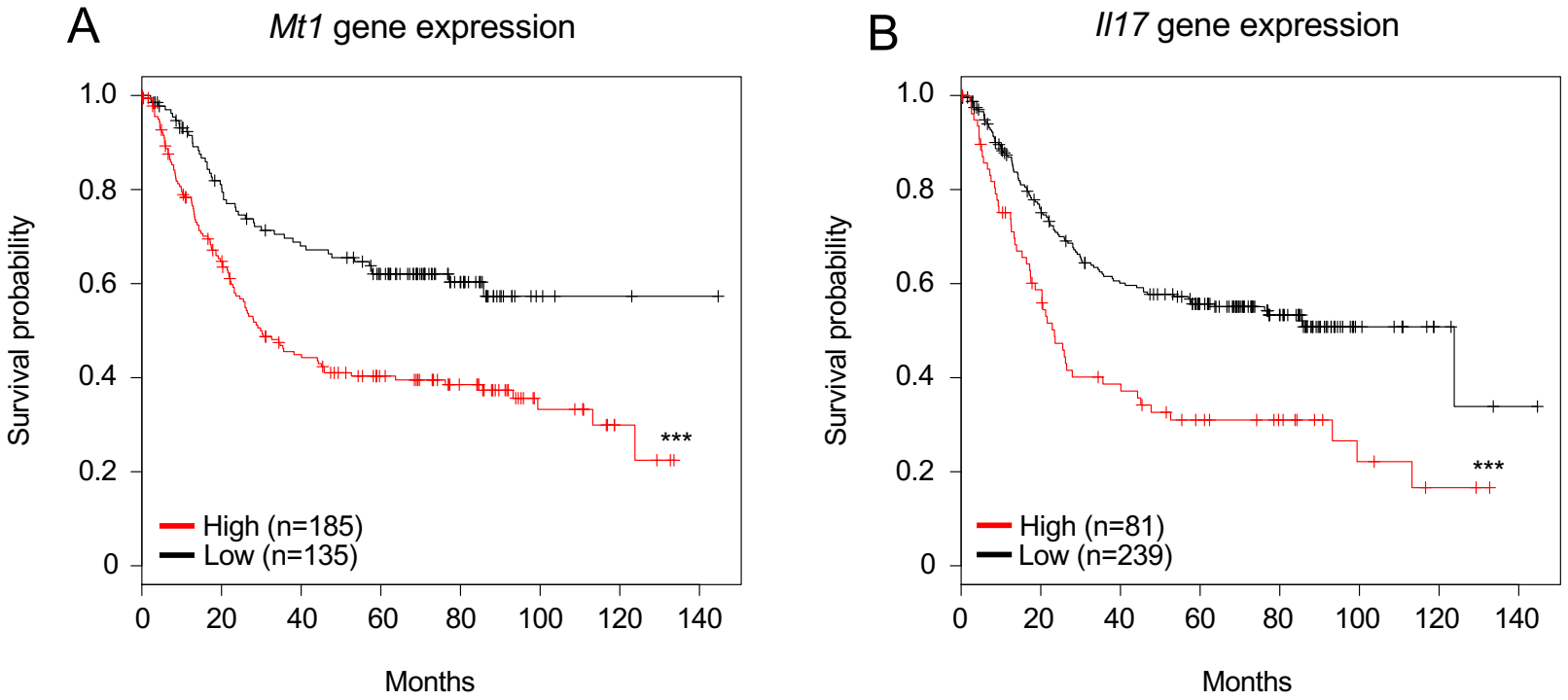
Supplementary Figure 3. IEC-specific TBK1 deficiency does not affect T cell homeostasis. (*A-D*) Flow cytometric analysis of CD4$^+$ and CD8$^+$ T cells in lymphocytes of small intestine-lamina propria (SI-LP) (*A* and *B*, n = 3) and the indicated lymphoid organs: spleen, mesenteric lymph node (MLN), and inguinal lymph node (ILN) (*C* and *D*, n = 5) of 6-week-old *Tbk1*$^{IEC\text{-}WT}$ and *Tbk1*$^{IEC\text{-}KO}$ mice. Data are presented as a representative FACS plot (*A* and *C*) or summary graphs (*B* and *D*). (*E-H*) Flow cytometric analysis of CD4$^+$ and CD8$^+$ T cells in lymphocytes of SI-LP (*E* and *F*, n = 3) and the indicated lymphoid organs (*G* and *H*, n = 5) of 5-month-old *Apc*$^{Min/+}$*Tbk1*$^{IEC\text{-}WT}$ and *Apc*$^{Min/+}$*Tbk1*$^{IEC\text{-}KO}$ mice. Data are presented as a representative FACS plot (*E* and *G*) or summary graphs (*F* and *H*). Data are representative of 2 (*A-B* and *E-F*) or 3 (*C-D* and *G-H*) independent experiments, and summary data are mean ± s.e.m. with *P* values being determined by unpaired two-tailed Student's *t*-test. ns, not significant.



Supplementary Figure 4. IEC-specific TBK1 deficiency does not alter the frequency of intestinal innate immune cells. (*A*-*C*) Flow cytometric analysis of the frequency (*A* and *B*) and absolute number (*C*) of conventional dendritic cells (cDCs) and plasmacytoid DCs (pDCs) in the lamina propria of 6-week-old *Tbk1*$^{\text{IEC-WT}}$ and *Tbk1*$^{\text{IEC-KO}}$ mice (n = 5 each). Data are presented as a representative FACS plot (*A*) and summary graphs (*B* and *C*; each circle represents a mouse). (*D*-*F*) Flow cytometric analysis of the frequency (*D* and *E*) and absolute number (*F*) of neutrophils and monocytes in the lamina propria of 6-week-old *Tbk1*$^{\text{IEC-WT}}$ and *Tbk1*$^{\text{IEC-KO}}$ mice (n = 5 each). Data are presented as a representative FACS plot (*D*) and summary graphs (*E* and *F*). Data are representative of 3 independent experiments, and summary data are mean ± s.e.m. with *P* values being determined by two-tailed Student's *t*-test. ns, not significant.



Supplementary Figure 5. RNA sequencing analysis of TBK1-regulated gene expression in IECs. RNA sequencing was performed using primary IECs of *Tbk1*$^{\text{IEC-WT}}$ and *Tbk1*$^{\text{IEC-KO}}$ mice (n = 3 per genotype), and up- and down-regulated genes are shown in a volcano plot. Genes included in the heatmap of Fig. 6A are indicated.



Supplementary Figure 6. Association of *Mt1* and *Il17* expression with colorectal cancer patient survival. Kaplan-Meier plots of TCGA database analysis (using UCSC Xena software) showing the association of high expression of *Mt1* (*A*) and *Il17* (*B*) with significantly reduced survival of colorectal cancer patients (n = 320).

Table S1. Gene-specific primers for genotyping

Gene Name	Primer Sequence
Tbk1	Forward 5'- GCCAGATATCCCTGTAACTCACC -3' Reverse 5'- CAGATGGTTGGAGGTGCTCTGCAT -3'
Apc	Wild-type primer 5'- GCCATCCCTTCAAGTTAG -3' Common primer 5'- TTCCACTTTGGCATAAGGC -3' Min primer 5'- TTCTGAGAAAGACAGAAGTTA -3'
Villin-Cre	Forward 5'- CATGTCCATCAGGTTCTTGC -3' Reverse 5'- TTCTCCTCTAGGCTCGTCCA -3'
Mt1	Forward 5'- TCACCAGATCTCGGAATGG -3' Reverse 5'- AAGAACCGGAATGAATCGC -3'
Mt2	Forward 5'- CGCGCTCACTGACTGCCTTC -3' Reverse 5'- CTGGGAGCACTTCGCACAGC -3'

Table S2. Gene-specific primers for qRT-PCR

Gene Name	Primer Sequence
Il17a	Forward 5'- AGCGATGGTGGATGGCTCATGGTTAG -3' Reverse 5'- AGCTTTCCCTCCGCATTGACACAG -3'
Il1β	Forward 5'- GGAGAACCAAGCAACGACAAAATA -3' Reverse 5'- TGGGGAACTCTGCAGACTCAAAC -3'
Il23	Forward 5'- CGTATCCAGTGTGAAGATGGTT -3' Reverse 5'- CTATCAGGGAGTAGAGCAGGCT -3'
Il22	Forward 5'- ATGAGTTTTTCCCTTATGGGGAC -3' Reverse 5'- GCTGGAAGTTGGACACCTCAA -3'
Il6	Forward 5'- TGAACAACGATGATGCACTTGC -3' Reverse 5'- GCTATGGTACTCCAGAAGACC -3'
Il21	Forward 5'- ATCCTGAACTTCTATCAGCTCCAC -3' Reverse 5'- GCATTTAGCTATGTGCTTCTGTTTC -3'
Il10	Forward 5'- CCAGAGCCACATGCTCCTAGA -3' Reverse 5'- GGTCCTTTGTTTGAAAGAAAGTCTTC -3'
E-cadherin	Forward 5'- CACCTGGAGAGAGGCCATGT -3' Reverse 5'- TGGGAAACATGAGCAGCTCT -3'
Claudin-2	Forward 5'- TATGTTGGTGCCAGCATTGT -3' Reverse 5'- TCATGCCCACCACAGAGATA -3'
Occludin	Forward 5'- CCTCCAATGGCAAAGTGAAT -3' Reverse 5'- CTCCCCACCTGTCGTGTAGT -3'
ZO-1	Forward 5'- CCACCTCTGTCCAGCTCTTC -3' Reverse 5'- CACCGGAGTGATGGTTTTCT -3'
Mt1	Forward 5'- AAGAGTGAGTTGGGACACCTT -3' Reverse 5'- CGAGACAATACAATGGCCTCC -3'
Actin	Forward 5'- CGTGAAAAGATGACCCAGATCA -3' Reverse 5'- CACAGCCTGGATGGCTACGT -3'