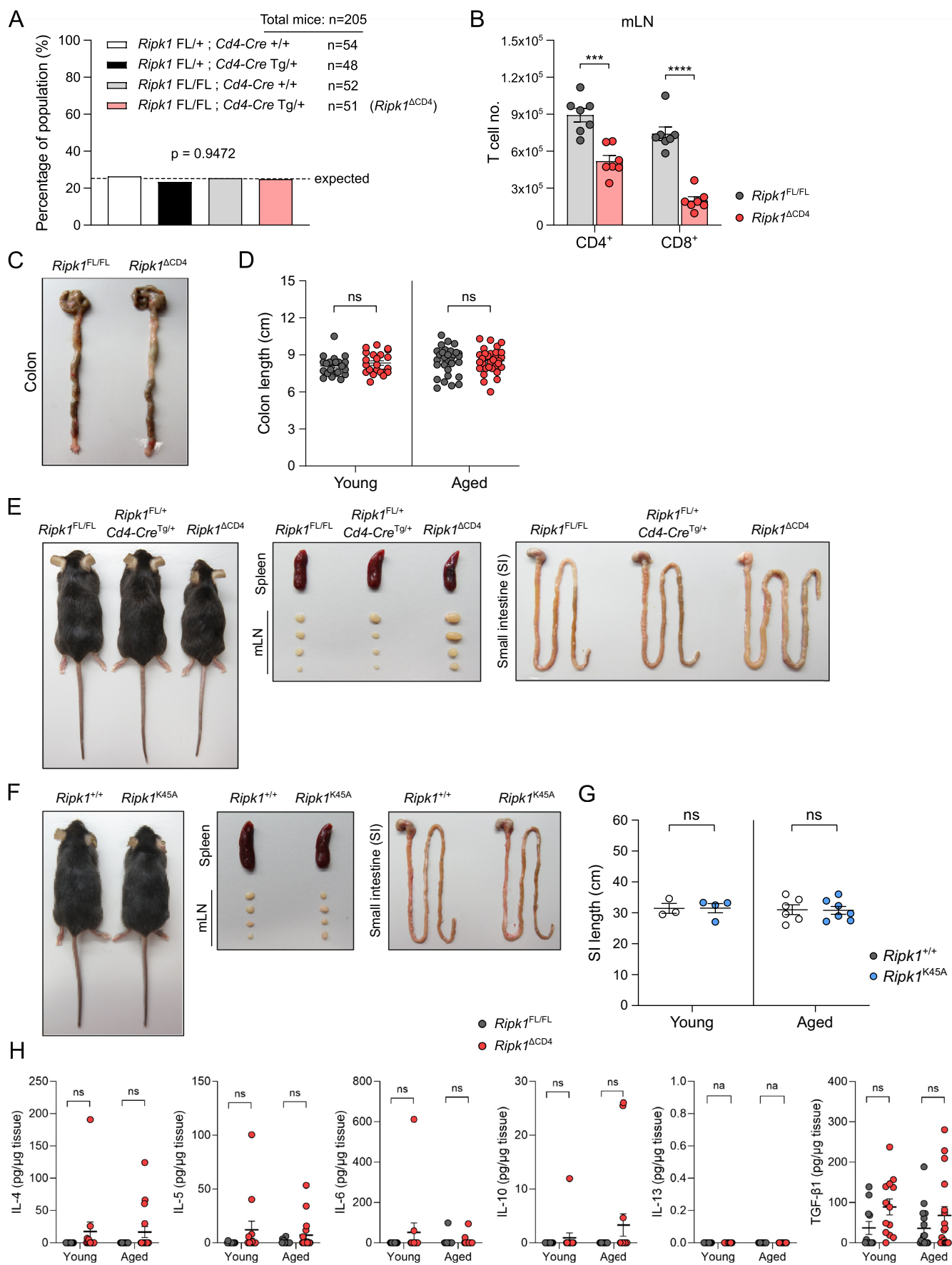
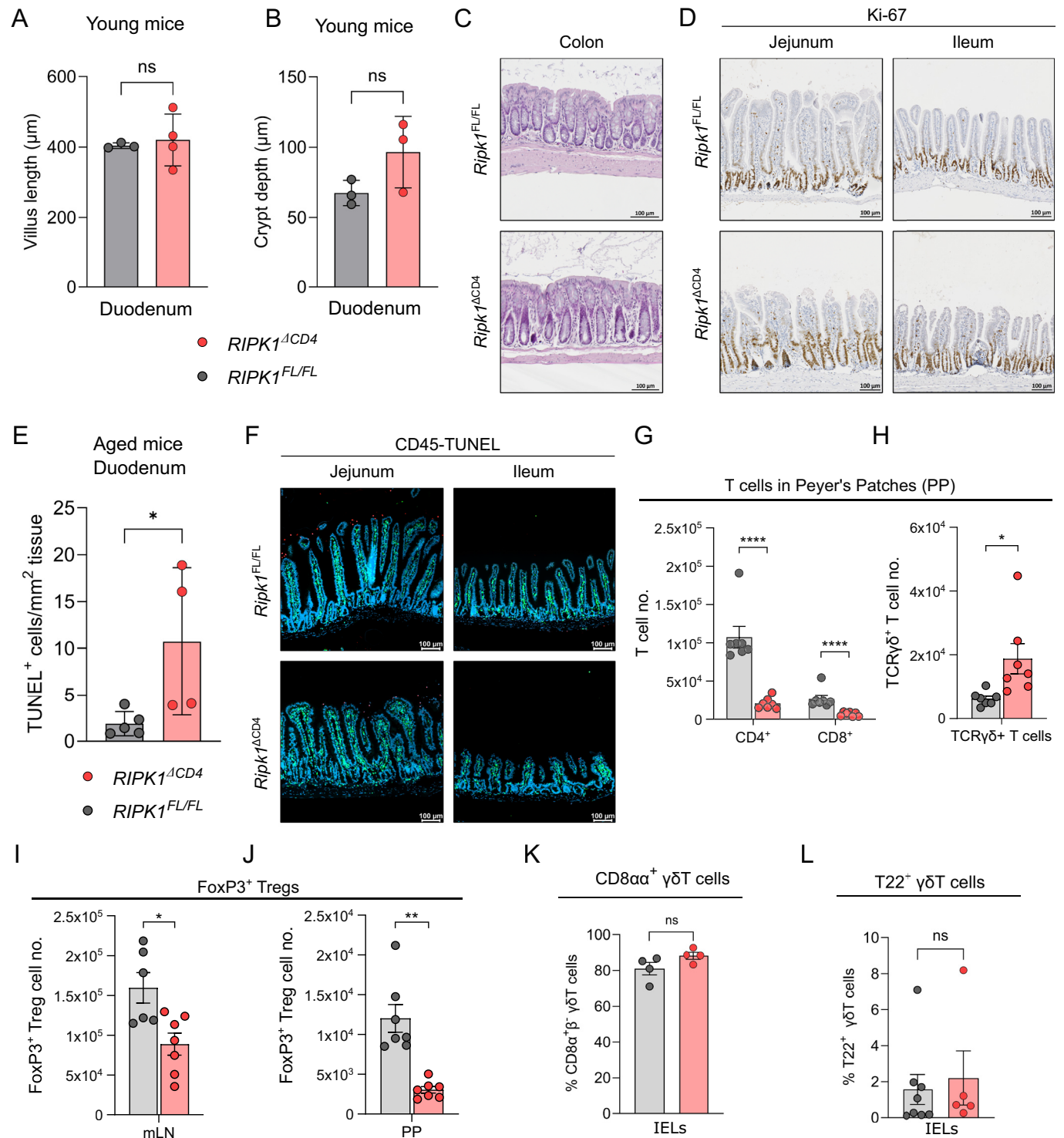


Expanded View Figures

Figure EV1. Selective deletion of *Ripk1* in conventional T cells results in small intestinal inflammation and elongation.

(A) Distribution of pups with indicated genotypes from crosses between *Ripk1^{FL/FL}; Cd4-Cre^{+/-}* and *Ripk1^{FL/+}; Cd4-Cre^{Tg/+}* parents. Bars represent the observed percentage of mice with each genotype within a total population of $n = 205$ mice. The expected percentage is denoted by the horizontal dotted line, and the p -value resulting from a chi-square test are indicated. (B) Absolute numbers of CD4⁺ and CD8⁺ T cells in the mesenteric lymph nodes (mLN) of *Ripk1^{ΔCD4}* mice and *Ripk1^{FL/FL}* littermates, measured by flow cytometry. (C) Representative image of the full colon and caecum of an aged *Ripk1^{ΔCD4}* mouse and *Ripk1^{FL/FL}* littermate. (D) Quantification of the absolute length of the colon of young *Ripk1^{ΔCD4}* mice ($n = 22$) and *Ripk1^{FL/FL}* littermates ($n = 22$) and aged *Ripk1^{ΔCD4}* mice ($n = 25$) and *Ripk1^{FL/FL}* littermates ($n = 22$). (E) Representative images of an aged homozygous *Ripk1^{ΔCD4}* mouse, heterozygous *Ripk1^{FL/+}; Cd4-Cre^{Tg/+}* mouse and *Ripk1^{FL/FL}* littermate with respective spleen, mLN and small intestine (SI). (F) Representative images of an aged *Ripk1^{K45A}* mouse and *Ripk1^{+/-}* littermate with respective spleen, mLN and small intestine (SI). (G) Quantification of the absolute SI length of young *Ripk1^{K45A}* mice ($n = 4$) and *Ripk1^{+/-}* littermates ($n = 3$) and aged *Ripk1^{K45A}* mice ($n = 7$) and *Ripk1^{+/-}* littermates ($n = 6$). (H) Tissue concentrations of IL-4, IL-5, IL-6, IL-10, IL-13, and TGF- β in the SI of young *Ripk1^{ΔCD4}* mice ($n = 13$) and *Ripk1^{FL/FL}* littermates ($n = 11$), and aged *Ripk1^{ΔCD4}* mice ($n = 17$) and *Ripk1^{FL/FL}* littermates ($n = 16$), were measured using multiplex assays (Meso Scale Discovery). Data are shown as mean $\pm$ SEM, with means represented by bars or horizontal lines and each dot representing an individual mouse. Data are representative of at least three independent repeats (C, F), or are combined from two independent repeats (B). Statistical significance was calculated in Graphpad Prism by (A) chi-square test, (B) Fisher's LSD two-way ANOVA on Log₂-transformed data CD4⁺: $p = 0.0006$, CD8 β ⁺: $p < 0.0001$, (D, G) Fisher's LSD two-way ANOVA on absolute values, or (H) two-sided unpaired T test with Welch's correction. ns non-significant, na not applicable, *** $p < 0.001$, **** $p < 0.0001$. Young mice: 8–12 weeks old. Aged mice: >6 months old. Source data are available online for this figure.





◀ **Figure EV2. The duodenum of *Ripk1^{ΔCD4}* mice displays villus atrophy, crypt hyperplasia, and immune cell infiltration.**

(A, B) Quantification of (A) the average villus length and (B) the average crypt depth in the duodenum of young *Ripk1^{ΔCD4}* and *Ripk1^{FL/FL}* littermates ($n = 3$). (C) H&E staining on sections of the colon of an aged *Ripk1^{ΔCD4}* mouse and *Ripk1^{FL/FL}* littermate. (D) Ki-67 staining on sections of the jejunum and ileum of aged *Ripk1^{ΔCD4}* mice and *Ripk1^{FL/FL}* littermates. (E) Quantification of TUNEL staining in the duodenum of aged *Ripk1^{ΔCD4}* mice ($n = 4$) and *Ripk1^{FL/FL}* littermates ($n = 5$), representing TUNEL-positive cells/mm² tissue area. (F) CD45-TUNEL staining on sections of the jejunum and ileum of aged *Ripk1^{ΔCD4}* mice ($n = 4$) and *Ripk1^{FL/FL}* littermates ($n = 5$). (C, D, F) Images are representative of *Ripk1^{ΔCD4}* mice ($n = 4$) and *Ripk1^{FL/FL}* littermates ($n = 5$). (G) Absolute numbers of CD4⁺ and CD8⁺ T cells in the Peyer's patches (PP) of young *Ripk1^{ΔCD4}* mice and *Ripk1^{FL/FL}* littermates, measured by flow cytometry. (H) Absolute numbers of TCRγδ⁺ T cells in the PP of young *Ripk1^{ΔCD4}* mice and *Ripk1^{FL/FL}* littermates, measured by flow cytometry. Data are obtained from $n = 7$ mice per group (G, H). (I, J) Absolute numbers of FoxP3⁺ Tregs in the mLN (I) and PP (J) of young *Ripk1^{ΔCD4}* mice and *Ripk1^{FL/FL}* littermates, measured by flow cytometry. (I, J) Data are obtained from *Ripk1^{ΔCD4}* mice ($n = 6$) and *Ripk1^{FL/FL}* littermates ($n = 7$). (K) Proportions of TCRγδ⁺ IELs expressing the CD8α receptor in young *Ripk1^{ΔCD4}* mice ($n = 4$) and *Ripk1^{FL/FL}* littermates ($n = 4$), measured by flow cytometry. (L) Proportions of TCRγδ⁺ IELs expressing the T22 TCR of young *Ripk1^{ΔCD4}* mice ($n = 5$) and *Ripk1^{FL/FL}* littermates ($n = 8$), measured by flow cytometry. (G, L) Data are representative of at least two independent repeats. Data are shown as mean ± SEM, with means being represented by bars or horizontal lines and each dot representing an individual mouse. Statistical significance was calculated in Graphpad Prism by Fisher's LSD two-way ANOVA on Log₂-transformed data (G) CD4⁺ and CD8⁺ T cells: $p < 0.0001$, Mann-Whitney test (A) $p = 0.6286$, (B) $p = 0.200$, and (E) $p = 0.0317$, or two-sided unpaired T test with Welch's correction (H) $p = 0.039$, (I) $p = 0.0141$, (J) $p = 0.0018$, (K) $p = 0.1345$, (L) $p = 0.7231$. ns = non-significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Young mice: 8–12 weeks old. Aged mice: >6 months old. Source data are available online for this figure.

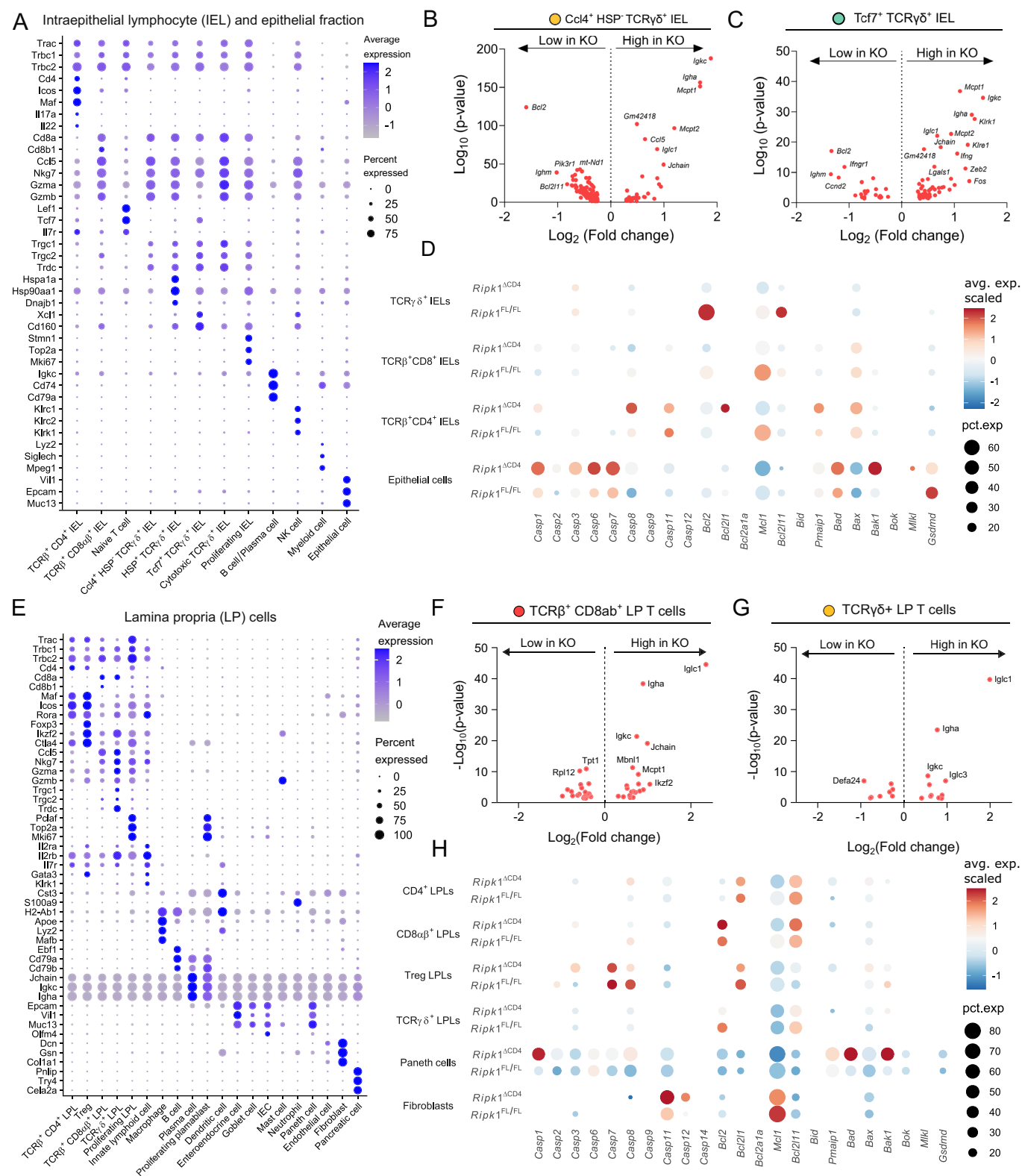


Figure EV3. *Ripk1*^{ΔCD4} mice display altered expression of cell death genes in intraepithelial and lamina propria cells.

(A) Cells were isolated from the epithelial layer (IEL) of young *Ripk1*^{ΔCD4} and *Ripk1*^{FL/FL} littermates and subjected to single-cell RNA sequencing (scRNA-seq). (B, C) Volcano plots representing significant differentially expressed (DE) genes in the clusters of (B) *Ccl4*⁺ HSP⁺ TCRγδ⁺ IELs, and (C) *Tcf7*⁺ TCRγδ⁺ IELs of *Ripk1*^{ΔCD4} mice compared to *Ripk1*^{FL/FL} littermates. Significance ($-\log_{10}$ of the *p*-value) is indicated on the y-axis, and $\log_2$ of the fold change in gene expression is indicated on the x-axis. (D) Dot plot displaying gene expression of Caspases, intrinsic apoptosis genes, and pore-forming proteins in T cell populations and epithelial cells in the IEL fraction. Size of dots represents the fraction of cells expressing a particular marker, and color intensity indicates mean-normalized scaled expression levels. Cells were isolated from the lamina propria (LP) of young *Ripk1*^{ΔCD4} and *Ripk1*^{FL/FL} littermates and subjected to single-cell RNA sequencing (scRNA-seq). (E) Dot plot displaying expression of marker genes per cluster used for cluster annotation. The size of dots represents the fraction of cells expressing a particular marker and color intensity indicates mean-normalized scaled expression levels. (F, G) Volcano plots representing significant differentially expressed (DE) genes in the clusters of TCRβ⁺CD8⁺ LP T cells (B) and TCRγδ⁺ LP T cells (C) of *Ripk1*^{ΔCD4} mice compared to *Ripk1*^{FL/FL} littermates. Significance ($-\log_{10}$ of the *p*-value) is indicated on the y-axis, and $\log_2$ of the fold change in gene expression is indicated on the x-axis. (H) Dot plot displaying gene expression of Caspases, intrinsic apoptosis genes, and pore-forming proteins in T cell populations, Paneth cells, and Fibroblasts in the LP fraction. Size of dots represents the fraction of cells expressing a particular marker, and color intensity indicates mean-normalized scaled expression levels. Data were obtained from *n* = 3 mice per group (A–H). (B, C, F, G) Wilcoxon test was used to determine DE genes and *p*-values were adjusted using Bonferroni correction. Young mice: 8–12 weeks old. IEL intraepithelial lymphocytes, LP lamina propria. Source data are available online for this figure.

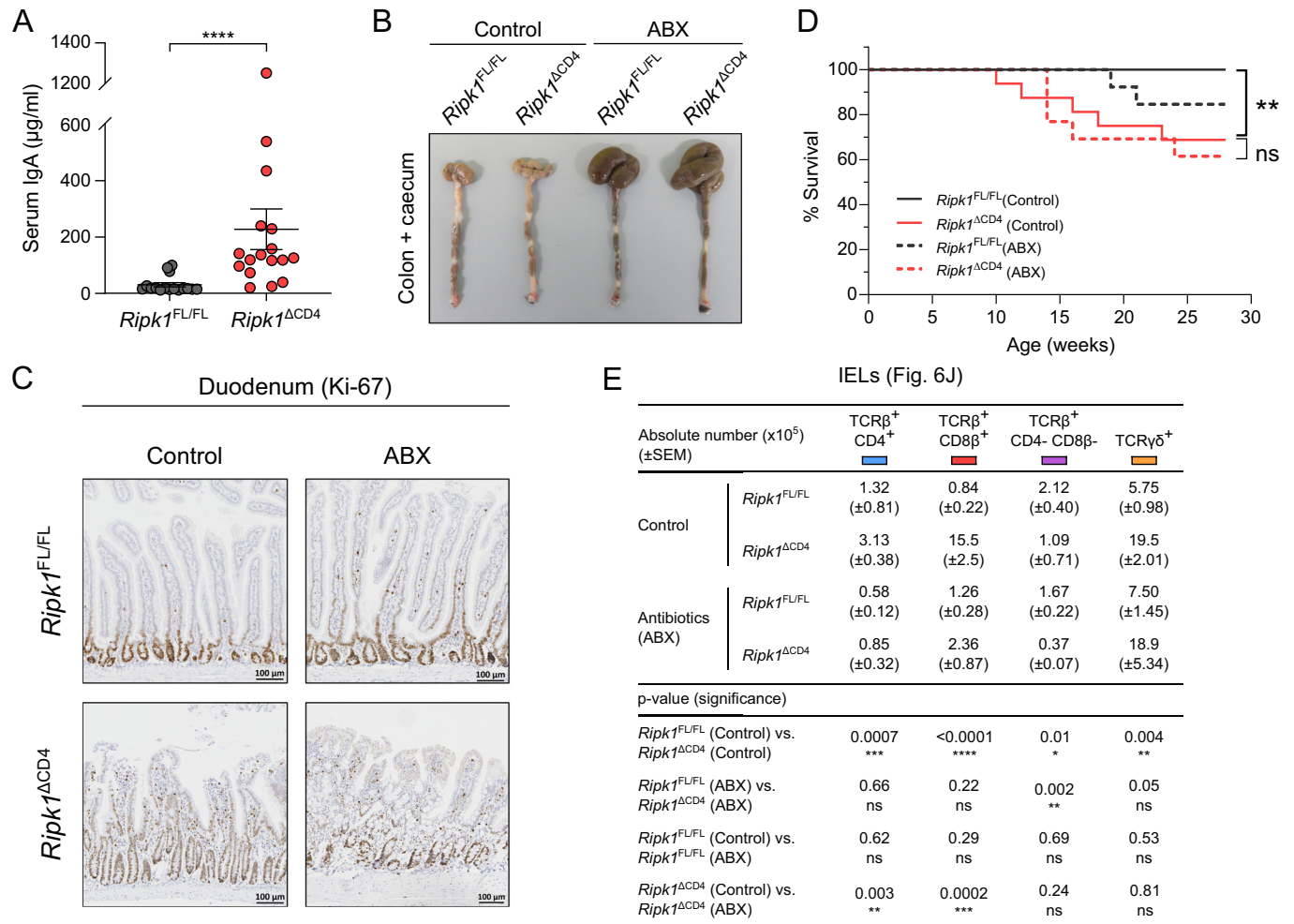


Figure EV4. SI elongation in *Ripk1*^{ΔCD4} mice is a response to the intestinal microbiome, while villus atrophy and TCR $\gamma\delta^+$ IEL expansion are not.

(A) Serum IgA concentrations in aged *Ripk1*^{ΔCD4} mice ($n = 17$) and *Ripk1*^{FL/FL} littermates ($n = 16$), measured by multiplex analysis (Meso Scale Discovery). Data are shown as mean $\pm$ SEM, with means represented by bars and each dot representing an individual mouse. (B) Representative images of the colon with caecum isolated from aged *Ripk1*^{ΔCD4} mice and *Ripk1*^{FL/FL} littermates, with and without ABX treatment. (C) Ki-67 staining on the duodenum of aged *Ripk1*^{ΔCD4} mice and *Ripk1*^{FL/FL} littermates, with and without ABX treatment. Images are representative of two independent repeats with ($n = 3$) *Ripk1*^{ΔCD4} mice (both ABX and control groups) or ($n = 4$) *Ripk1*^{FL/FL} littermates (both ABX and control groups). (D) Kaplan-Meier survival analysis of *Ripk1*^{ΔCD4} mice ($n = 16$) and *Ripk1*^{FL/FL} littermates ($n = 21$) without ABX control, and *Ripk1*^{ΔCD4} mice ($n = 13$) and *Ripk1*^{FL/FL} mice ($n = 13$) on ABX. (E) Table corresponding to Fig. 6J, indicating the absolute numbers of the different IEL populations and resulting p -values of the statistical analysis (Fisher's LSD two-way ANOVA), with ($n = 4$) *Ripk1*^{ΔCD4} mice (control) ($n = 3$) *Ripk1*^{ΔCD4} mice (ABX) or ($n = 5$) *Ripk1*^{FL/FL} littermates (both ABX and control groups). (A, D) Data are combined from two independent repeats, or (B, C, E) representative of two independent repeats. Statistical significance was calculated in Graphpad Prism by (A) Mann-Whitney test $p < 0.0001$, (D) Gehan-Wilcoxon test for survival, *Ripk1*^{FL/FL} vs *Ripk1*^{ΔCD4} (Control): $p = 0.0067$, Control vs ABX (*Ripk1*^{ΔCD4}): $p = 0.8301$, or (E) two-way ANOVA on Log₂-transformed data with p -values indicated in table. ns = non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Aged mice: >6 months old. IEL intraepithelial lymphocytes. Source data are available online for this figure.

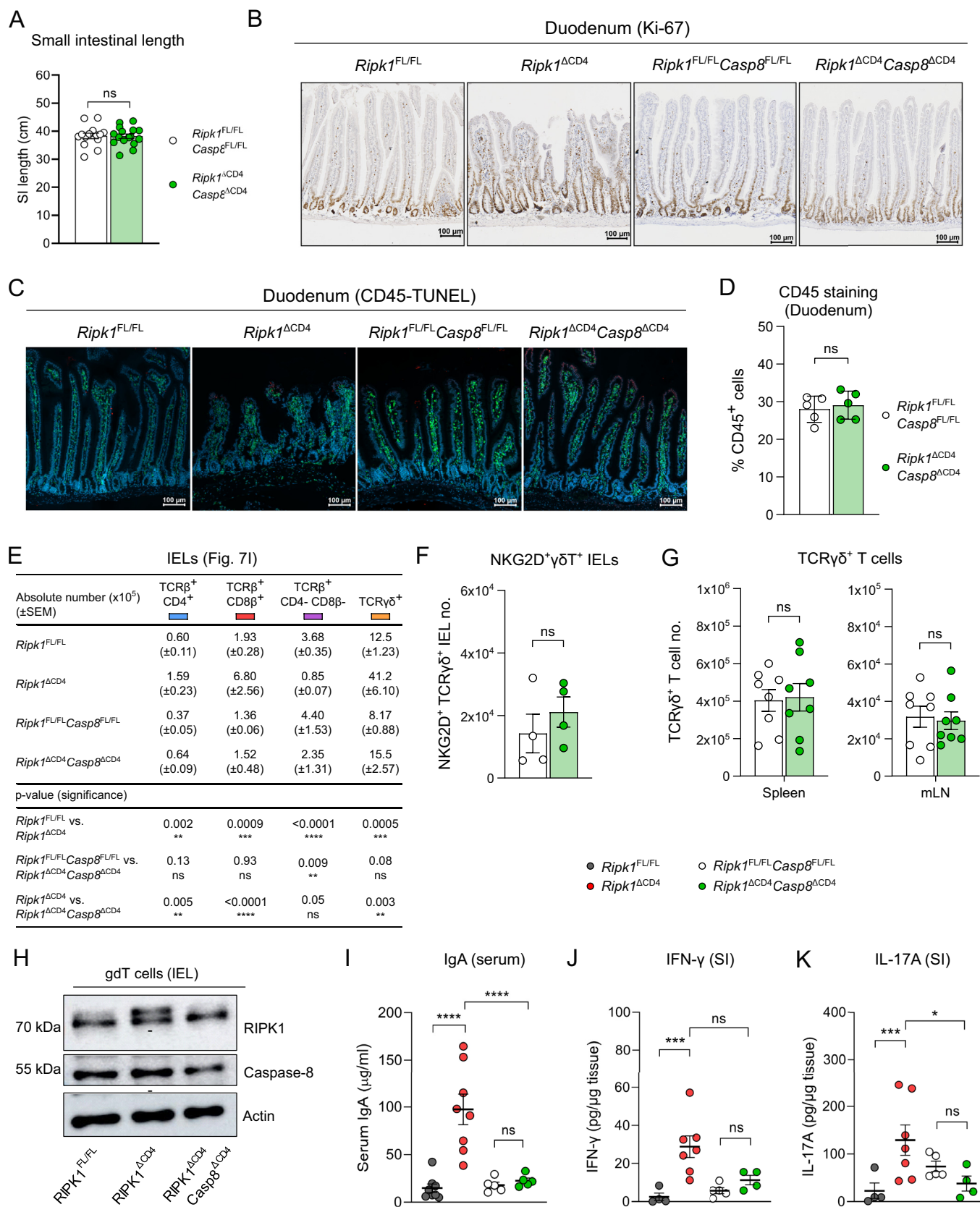


Figure EV5. Villus atrophy is TNFR1-dependent, and T cell ablation of caspase-8 prevents the development of intestinal pathology.

(A) Quantification of the absolute small intestine (SI) length of *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice ($n = 16$) and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates ($n = 14$). (B) Ki-67 staining on duodenum sections of aged *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates. (C) CD45-TUNEL staining on duodenum sections of aged *Ripk1^{ΔCD4}* and *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice and their respective *Ripk1^{FL/FL}* and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates (CD45 = Green; TUNEL = Red). (D) Quantification of CD45 staining in the duodenum of aged *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates ($n = 5$), represented as the percentage of CD45-positive cells within the total number of cells (determined by DAPI staining). (E) Table corresponding to Fig. 7I, indicating the absolute numbers of the different IEL populations and their resulting p -values of the statistical analysis (Fisher's LSD two-way ANOVA). (F) Absolute numbers of NKG2D⁺ TCRγδ⁺ IELs in the SI epithelial layer of young *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates ($n = 4$), measured by flow cytometry. (G) Absolute numbers of TCRγδ⁺ T cells in the spleen (left) and mLN (right) of young *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates ($n = 8$), measured by flow cytometry. (H) Western blot on FACS-sorted TCRγδ⁺ IELs, showing RIPK1, Caspase-8, and Actin in *Ripk1^{FL/FL}*, *Ripk1^{ΔCD4}* and *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice. (I) Serum IgA concentrations in young *Ripk1^{ΔCD4}* mice ($n = 8$) and *Ripk1^{FL/FL}* littermates ($n = 8$), and aged *Ripk1^{ΔCD4}Casp8^{ΔCD4}* mice ($n = 5$) and *Ripk1^{FL/FL}Casp8^{FL/FL}* littermates ($n = 5$), measured by multiplex analysis (Meso Scale Discovery). Data are representative of two (E) or three (A) independent repeats, or (G) are combined from two independent repeats. Data are shown as mean ± SEM, with means being represented by bars or horizontal lines and each dot representing an individual mouse. Statistical significance was calculated in Graphpad Prism by unpaired T-test with Welch's correction (A, D, F, G), or Fisher's LSD two-way ANOVA on Log₂-transformed data (E) with p -values indicated in table (I) *Ripk1^{FL/FL}* vs *Ripk1^{ΔCD4}*: $p < 0.0001$, *Ripk1^{ΔCD4}* vs *Ripk1^{ΔCD4}Casp8^{ΔCD4}*: $p < 0.0001$, *Ripk1^{FL/FL}Casp8^{FL/FL}* vs *Ripk1^{ΔCD4}Casp8^{ΔCD4}*: $p = 0.7831$; (J) *Ripk1^{FL/FL}* vs *Ripk1^{ΔCD4}*: $p = 0.0002$, *Ripk1^{ΔCD4}* vs *Ripk1^{ΔCD4}Casp8^{ΔCD4}*: $p = 0.1071$, *Ripk1^{FL/FL}Casp8^{FL/FL}* vs *Ripk1^{ΔCD4}Casp8^{ΔCD4}*: $p = 0.1931$; (K) *Ripk1^{FL/FL}* vs *Ripk1^{ΔCD4}*: $p = 0.0009$, *Ripk1^{ΔCD4}* vs *Ripk1^{ΔCD4}Casp8^{ΔCD4}*: $p = 0.0401$, *Ripk1^{FL/FL}Casp8^{FL/FL}* vs *Ripk1^{ΔCD4}Casp8^{ΔCD4}*: $p = 0.1621$. ns = non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Young mice: 8–12 weeks old, aged mice: >6 months old. IEL intraepithelial lymphocytes. Source data are available online for this figure.