

1.4

0.9

65.0

10<sup>3</sup>

103

9.8

0.7

5.5

10

. 10<sup>0</sup> 10

27.9

102

100

10

10<sup>0</sup>

CD3

♠

100

26.9

+ CD8

►B220

(A) The structures of the *Entpd7* gene (top), the targeting vector (middle) and the predicted disrupted gene (bottom). Black box, coding exon. (B) Southern blot analysis of offspring from the heterozygote intercrosses. Genomic DNA was extracted from mouse tail tissue, digested with Scal, separated by electrophoresis and hybridized with the radiolabeled probe shown in (A). Southern blot analysis detected a single 8.5-kb band for wild-type mice (+/+), a 5.5kb band for homozygous *Entpd7*-deficient mice (-/-), and both bands for heterozygous mice (+/-). (C) Northern blot analysis of offspring from the heterozygote intercrosses. Total RNA was extracted from the small intestine, separated by electrophoresis and hybridized with the radiolabeled *Entpd7* cDNA probe. Northern blot analysis detected *Entpd7* mRNA bands for wild-type mice (+/+) and no *Entpd7* mRNA bands for homozygous Entpd7-deficient mice (-/-). Bottom, the same amount of mRNA extracts hybridized with the radiolabeled  $\beta$ -actin (*Actb*) cDNA probe to monitor mRNA extraction. (D) Representative FACS dot plots of surface markers of splenic lymphocytes. Spleens were collected from wild-type and *Entpd7*<sup>-/-</sup> mice and single-cell suspensions were prepared. The cells were stained for CD4/CD8 or CD3/B220 and analyzed by flow cytometry.



#### Supplemental Figure 2. Lymphocyte composition in the intestine of *Entpd7*<sup>-/-</sup> mice

Intraepithelial lymphocytes and lamina propria lymphocytes were isolated from the small and large intestines of wild-type and *Entpd7*-/- mice, stained for CD3/TCR $\beta$ /TCR $\gamma\delta$ , TCR $\beta$ /CD4/CD8 $\alpha$ /CD8 $\beta$  and TCR $\gamma\delta$ /CD4/CD8 $\alpha$ /CD8 $\beta$ , and analyzed by flow cytometry. Total numbers of CD3<sup>+</sup> cells and proportion of each cellular subset were shown. Data are shown as means + SD of four mice.

# **Supplemental Figure 3**



#### Supplemental Figure 3. Establishment of small intestinal epithelial cell lines

To establish small intestinal epithelial cell lines, wild-type and  $Entpd7^{-/-}$  mice were crossed with H-2Kb-tsA58 transgenic mice. Crypts isolated from the small intestine were cultured at 33° C for 3 weeks, and growing cells were cloned and expanded to establish the epithelial cell lines. The cell lines were stained with anti-cytokeratin, and visualized with diaminobenzidine (**A**). The expression of *Krt7*, encoding cytokeratin-7, and *Entpd7*, was analyzed by real-time RT-PCR. ND; not detected (**B**).

## **Supplemental Figure 4**



Supplemental Figure 4. Cytokine production of CD4<sup>+</sup> T cells in several lymphoid organs Representative FACS dot plots of intracellular cytokine profiles of CD4<sup>+</sup> T cells and total numbers of IL17producing CD4<sup>+</sup> cells in the MLNs, Peyer's patches, and spleen. Cells were isolated from the indicated lymphoid organs of wild-type and *Entpd7*<sup>-/-</sup> mice, stimulated, and permeabilized to stain intracellular IL-17 and IFN- $\gamma$ . Data are shown as means + SD of four mice.

### **MLNs**