Supplemental material

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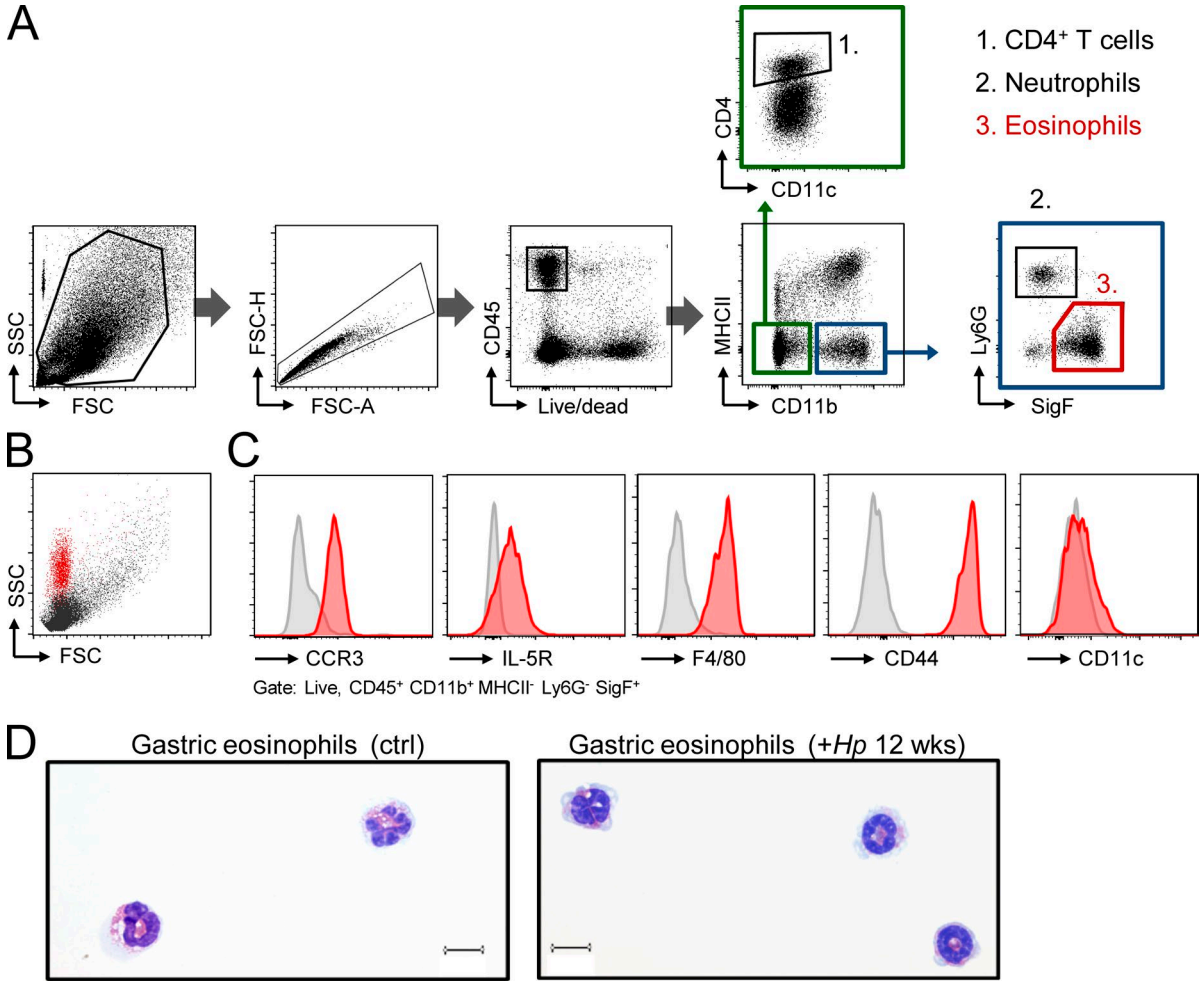

Figure S1. **Eosinophils are identified as CD45⁺CD11b⁺MHCII⁻Ly6G⁻SiglecF⁺ cells in single-cell preparations of the gastric LP. (A)** Gating strategy used for the identification and quantification of eosinophils, neutrophils, and CD4⁺ T cells among gastric LP leukocytes that were isolated by enzymatic digestion and percoll gradient centrifugation and analyzed by flow cytometry. Forward and sideward scatter (FSC and SSC) and forward scatter height versus area (FSC-H and FSC-A) as well as CD45 and live/dead staining were used to eliminate doublets and identify live leukocytes among all cells; the myeloid marker CD11b along with MHCII was used to discriminate granulocytes from mononuclear phagocytes. Granulocytes were further differentiated based on their Ly6G and SiglecF (SigF) expression as Ly6G-positive neutrophils and SigF-positive eosinophils. CD4⁺ T cells were identified as being MHCII-, CD11b-, and CD11c-negative and CD4-positive. **(B and C)** Gastric LP eosinophils are characterized by high side-scatter (SSC) and express CCR3, IL-5R, F4/80 and CD44, but no or only barely detectable CD11c. Negative peaks are shown in gray. **(D)** Cytospin images, stained using the Microscopy Hemacolor staining set, of gastric eosinophils sorted from LP preparations of a control and an infected mouse (12 wk after infection) using the gating strategy outlined in A.

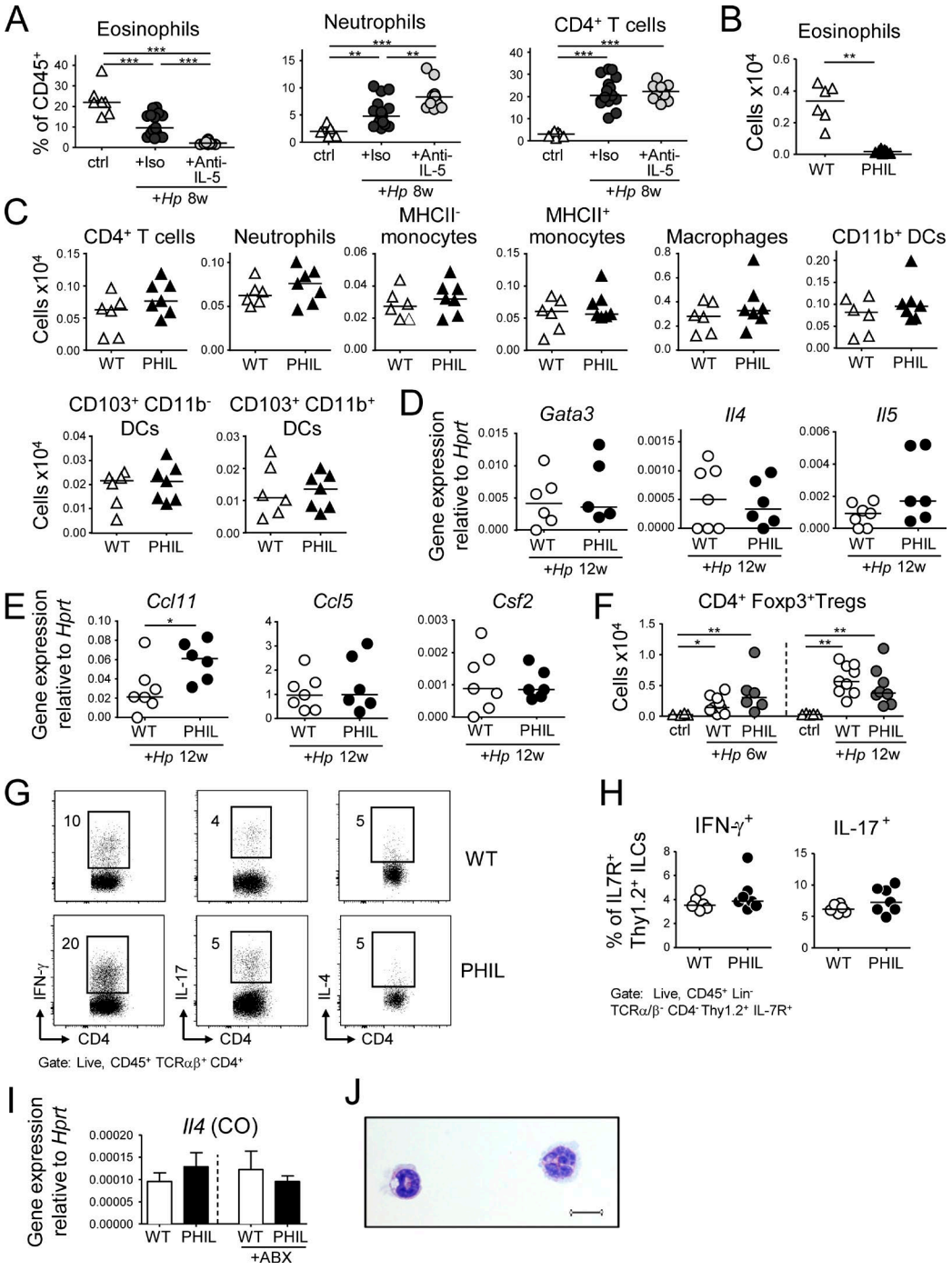

Figure S2. **The depletion of eosinophils does not affect the frequencies of major GI leukocyte populations in the steady-state but increases Th1 and Th17 responses in the steady-state and during *H. pylori*–induced inflammation. (A)** Mice were treated with either anti–IL-5 or isotype control antibody for 8 wk and infected with *H. pylori* for the same time frame. Gastric LP leukocytes were prepared and analyzed by multi-color FACS. Anti–IL-5 treatment efficiently depletes eosinophils, but increases the frequencies of neutrophils. $CD4^+$ T cells frequencies do not change significantly upon anti–IL-5 treatment. Data are pooled from two independent studies. **(B and C)** In the steady-state, PHIL mice exhibit strongly diminished gastric eosinophil populations, whereas the other indicated major gastric leukocyte populations are normal. **(D and E)** Eosinophil-deficient PHIL mice were infected with *H. pylori* for 12 wk and analyzed with respect to their gastric mucosal chemokine, transcription factor and eosinophil growth factor expression, as determined by qRT-PCR and normalized to *Hprt*. **(F)** Eosinophil-deficient PHIL mice were infected with *H. pylori* for 6 or 12 wk and analyzed with respect to their gastric regulatory T cell ($Foxp3^+$ Treg) compartment. (**G and H**) Colonic LP preparations of 6-wk-old WT and PHIL mice were analyzed at steady-state. **(G)** Representative FACS plots showing the identification of Th1, Th17, and Th2 cells. **(H)** IFN-γ and IL-17 production as assessed by FACS, of $CD45^+$ Lin^- $TCR\alpha/\beta^-$ $CD4^-$ $Th1.2^+$ and $IL\text{-}7R^+$ ILCs. The lineage markers used were B220, DX5, CD11b, CD11c, and GR1. **(I)** Colonic mucosal IL-4 expression, as assessed by qRT-PCR, of PHIL and WT mice that were subjected to a 7-wk regimen of bacterial eradication by quadruple antibiotic therapy relative to untreated controls. **(J)** Cytospin image of colonic eosinophils, stained using the Microscopy Hemacolor staining set, that were sorted based on the gating strategy outlined in Fig. S1 A. Data in B–I are representative of two independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

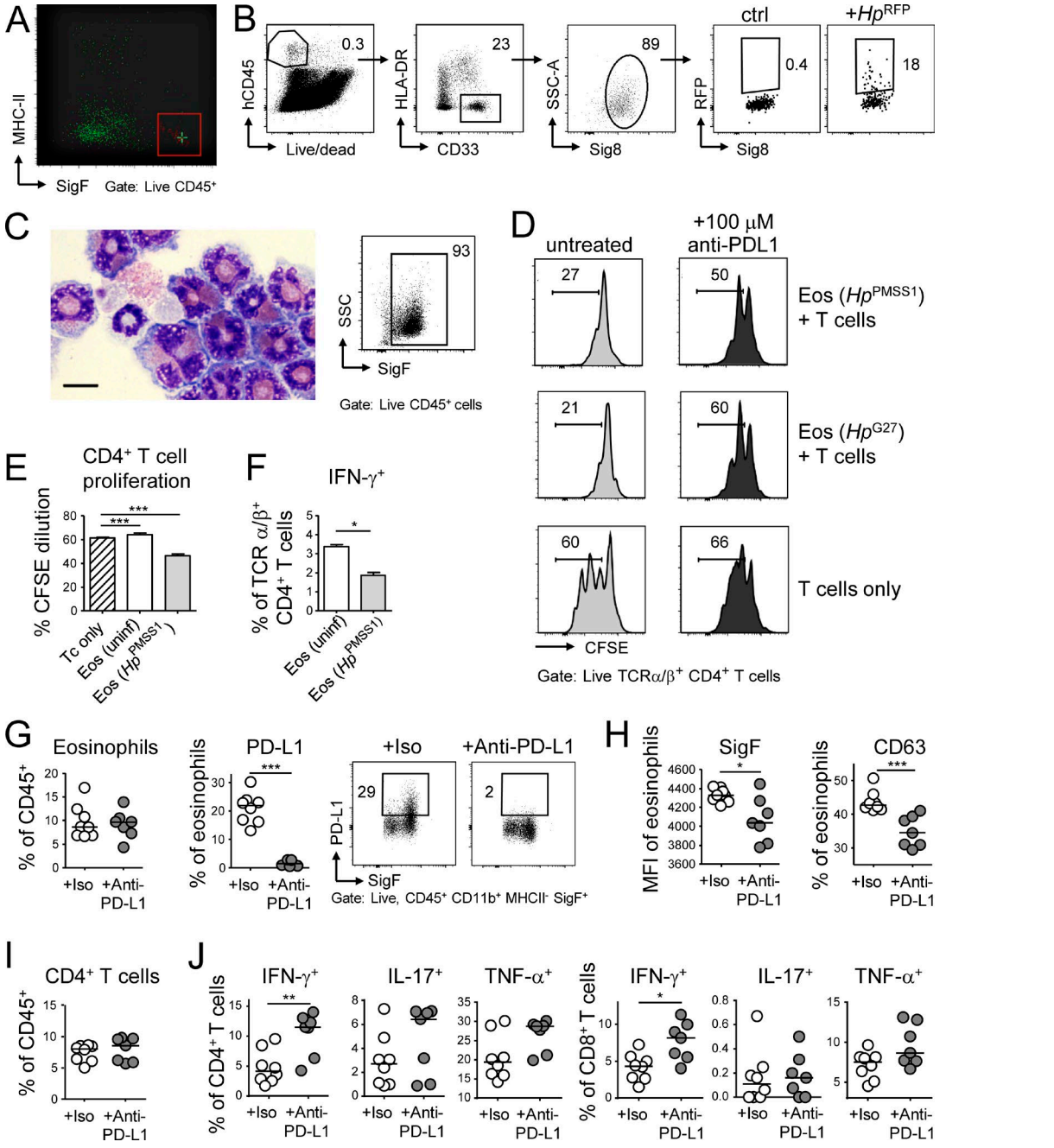

Figure S3. **Eosinophils acquire T cell–suppressive properties upon encountering *H. pylori*.** **(A)** ImageStream plot of the RFP$^+$ CD45$^+$ MHCII$^-$ SigF$^+$ SSC^{hi} eosinophil shown in Fig. 4 C; the cross indicates the SigF and MHCII expression of that cell. **(B)** Gating strategy used for the identification and quantification of human eosinophils in single-cell LP leukocyte preparations from mice reconstituted with human cord blood–derived hematopoietic stem and progenitor cells. Human eosinophils are hCD45$^+$HLA-DR$^-$CD33$^+$Sig8$^+$ cells; upon RFP$^+$ *H. pylori* infection, a fraction of human eosinophils becomes RFP-positive. **(C)** In vitro differentiated eosinophils derived from murine BM by differentiation with mouse stem cell factor, and FLT3-ligand followed by IL-5 were cytospun and visualized by staining with the Microscopy Hemacolor staining set. The FACS plot on the right shows the purity of in vitro differentiated SigF$^+$ eosinophils. **(D)** Representative FACS plots of the CFSE dilution of pure anti-CD3/CD28 bead-activated T cells relative to T cell co-cultures with *H. pylori*–experienced eosinophils; co-culturing was performed in the presence or absence of 100 µM anti–PD-L1 blocking antibody. The CFSE dilution was quantified after 4 d in (co)-culture. **(E)** Naive or *H. pylori*–experienced splenic eosinophils were co-cultured at a 1:1 ratio with CSFE-labeled immunomagnetically isolated splenic CD4$^+$ T cells in the presence of anti–CD3/CD28-coated beads. The CFSE dilution was quantified after 4 d in (co)-culture. **(F)** Naive or *H. pylori*–experienced splenic eosinophils were co-cultured with immunomagnetically isolated splenic ovalbumin-specific (OT2) T cells in the presence of BM APCs pulsed with ovalbumin. T cellular cytokine production was quantified by intracellular cytokine staining for IFN-γ. **(G–J)** Mice were treated with two doses of anti–PD-L1 blocking or isotype control antibody with a 3 d interval, sacrificed 2 d after the second dose, and subjected to colon LP preparation and flow cytometry. **(G)** Frequencies of eosinophils among all colonic LP leukocytes and of PD-L1$^+$ cells among all eosinophils. Representative FACS plots are shown on the right. **(H)** Siglec-F and CD63 expression on eosinophils. **(I)** Frequency of colonic CD4$^+$ T cells. **(J)** Frequencies of colonic IFN-γ$^+$, TNFα$^+$, and IL-17$^+$ CD4$^+$ and CD8$^+$ T cells. Statistical analysis was performed by Mann-Whitney test or by one-way ANOVA with Bonferroni's correction (in case of multiple comparisons). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

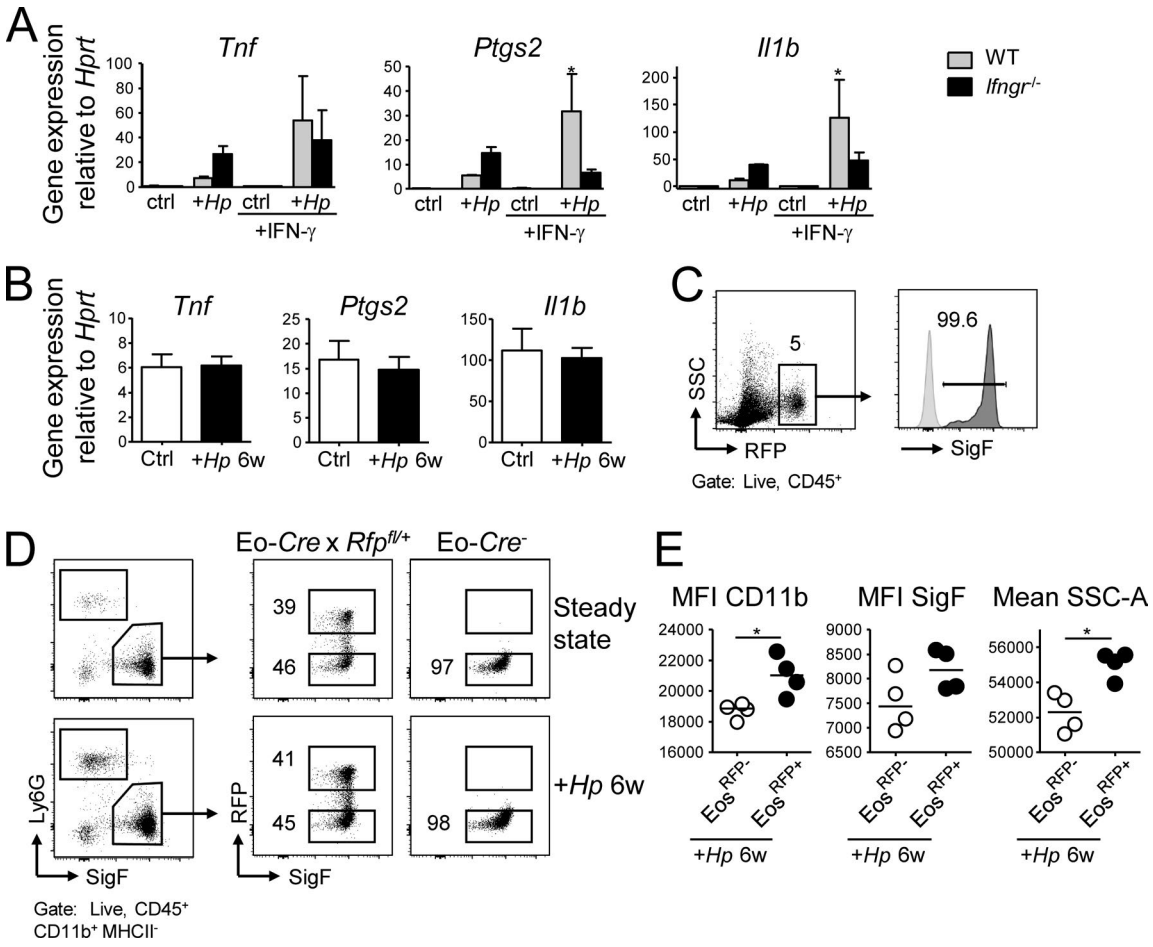

Figure S4. **Eosinophil T cell suppressive activities are regulated by IFN-γ signaling. (A)** In vitro differentiated WT or IFN-γR$^{-/-}$ eosinophils were co-cultured for 18 h with *H. pylori* PMSS1 (multiplicity of infection of 10) in the presence or absence of 20 ng/ml recombinant IFN-γ. Expression of the indicated transcripts was analyzed by qRT-PCR and normalized to *Hprt*. Means of three technical replicates +SEM are shown for one representative of two experiments. **(B)** Gastric LP eosinophils were FACS-sorted from naive mice and mice infected with *H. pylori* for 6 wk and analyzed by qRT-PCR for their expression of the indicated transcripts. Pooled data for six to eight mice per group are shown. **(C–E)** Eo-*Cre*$^{RFP\ fl/+}$ mice and *Cre*$^{-}$ controls were subjected to single-cell preparation of gastric LP leukocytes followed by FACS. **(C)** >99% of RFP^{+} cells in the gastric LP of Eo-*Cre*$^{RFP\ fl/+}$ mice are SiglecF-positive eosinophils. Negative peak is shown in light gray. **(D)** Only approximately one half of gastric eosinophils in both the steady-state and the *H. pylori*–infected gastric mucosa are RFP^{+} in Eo-*Cre*$^{RFP\ fl/+}$ mice. **(E)** RFP^{+} eosinophils in *H. pylori*–infected Eo-*Cre*$^{RFP\ fl/+}$ mice are more activated as judged by their CD11b and Siglec-F expression and side scatter than RFP^{-} cells of the same mice. *, P < 0.05.

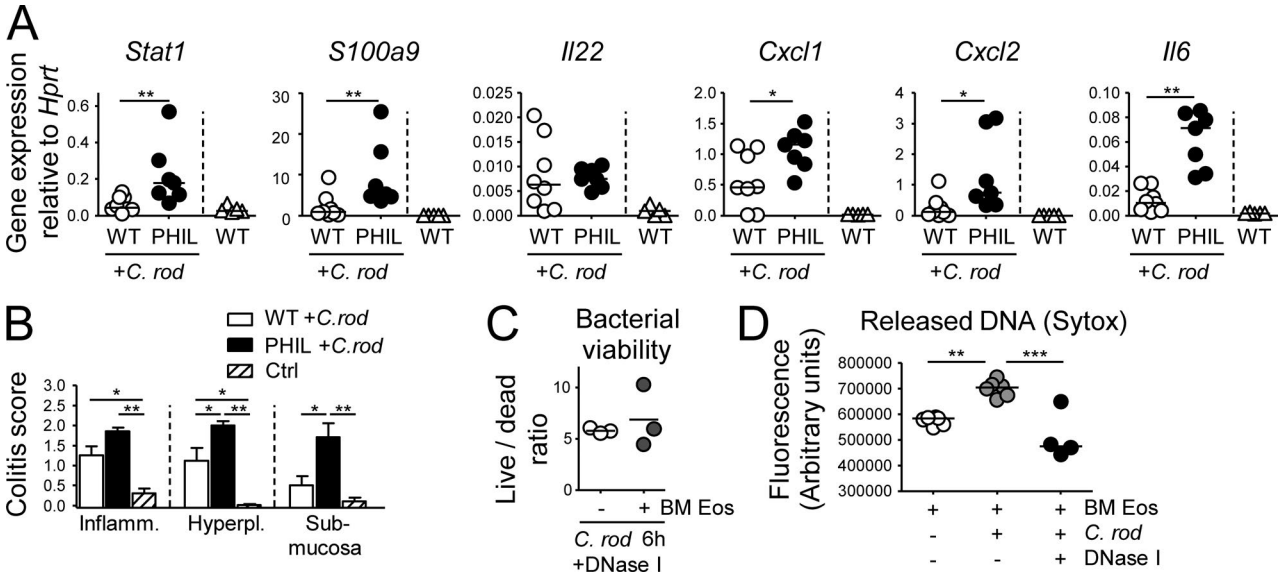

Figure S5. **Eosinophils suppress *C. rodentium*–induced colitis and contribute to bacterial clearance by extracellular trap formation. (A and B)** PHIL mice and their WT littermates were infected with *C. rodentium* for 12 d and analyzed with respect to their colonic mucosal gene expression and development of colitis. Data are representative of two independent experiments. **(A)** Expression of the indicated transcripts, as determined by qRT-PCR and normalized to *Hprt*. **(B)** Colitis scores, as assessed by histopathological examination of H&E-stained sections, of the mice shown in A. Mucosal and submucosal inflammation, as well as epithelial hyperplasia, were all assessed independently. **(C)** DNase I treatment dissolves EETs and abrogates *C. rodentium* killing in vitro. **(D)** Quantification of DNA release by BM eosinophils cultured with *C. rodentium* in the presence or absence of DNase I. For the quantification of extracellular DNA, 100,000 BM eosinophils were stained with 5 µM Sytox green for 10 min; 100 µl of culture supernatant per sample were transferred to a white, clear-bottom 96-well plate, and fluorescence emission of DNA-bound Sytox green was measured on a SpectraMax i3 fluorometer at 485 nm (excitation)/527 nm (emission). *, P < 0.05; **, P < 0.01; ***, P < 0.001.