SUPPLEMENTAL MATERIAL

Peterson et al., https://doi.org/10.1084/jem.20170347

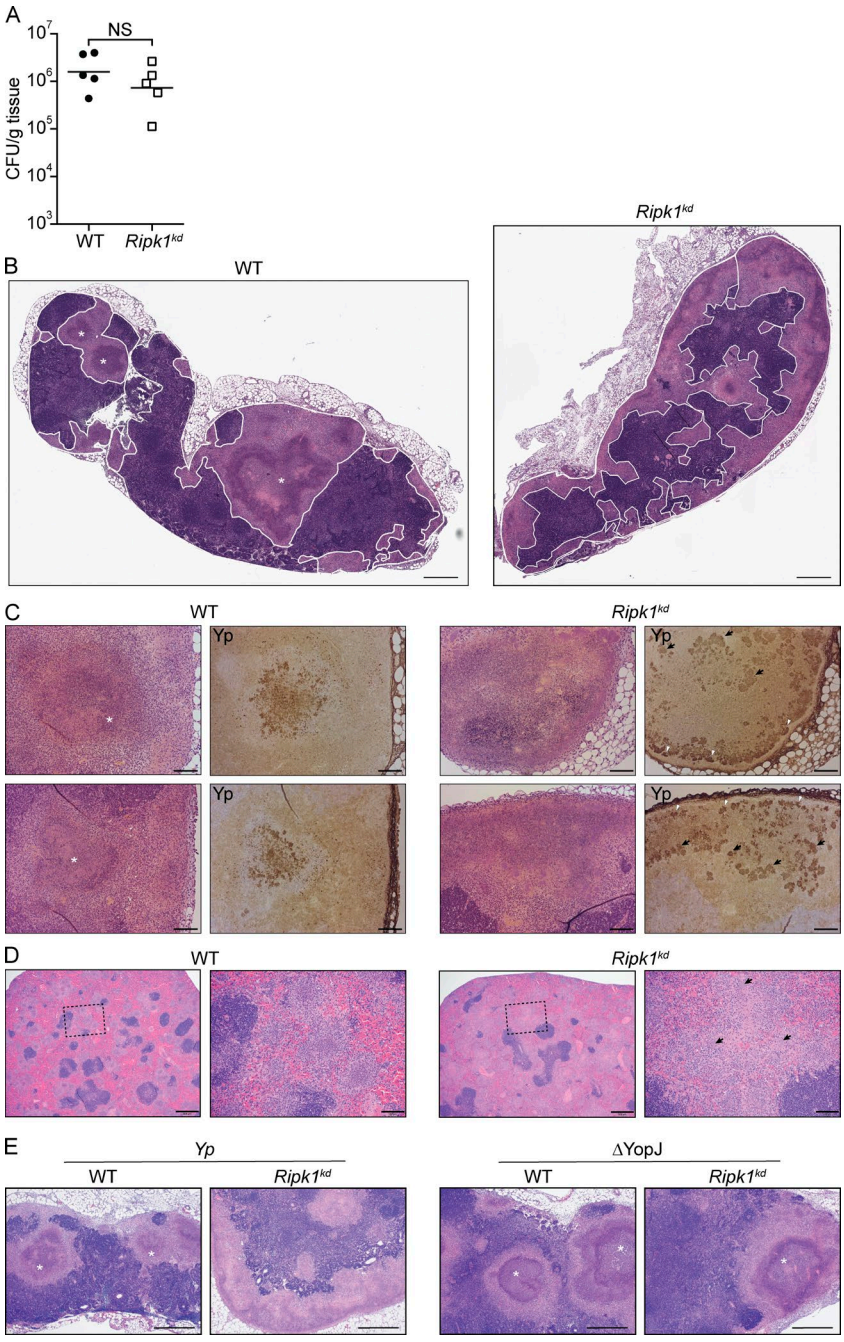

Figure S1. **RIPK1 kinase activity limits tissue pathology during in vivo *Yersinia* infection.** (A) WT and *Ripk1*^{kd} mice were infected with 2 × 10^4 CFUs *Yp* by intraperitoneal injection and splenic bacterial burdens were measured on day 3 postinfection. (B) H&E staining of MLN sections from WT and *Ripk1*^{kd} mice infected with *Y. pseudotuberculosis* (*Yp*) were used to quantify percentage effacement. Bars, 500 µm. Total area and effaced areas were traced on ImageJ software by a pathologist blinded to sample identification. Results are shown in Fig. 2 G. (C) Representative H&E and immunohistochemical staining for *Yersinia* from WT and *Ripk1*^{kd} MLNs. In WT mice (left), bacteria are primarily located in pyogranulomas, characterized by central cores of cell debris surrounded by dense cuffs of neutrophils and epithelioid macrophages (asterisks). In *Ripk1*^{kd} mice (right), many bacterial colonies are scattered throughout areas lymphoid tissue effacement (arrows) and border the subcapsular sinus (arrowheads). Bars, 100 µm. (D) Representative H&E staining of sections from WT and *Ripk1*^{kd} spleens, with increased numbers of bacterial colonies present in infected *Ripk1*^{kd} spleens (arrows). Bars: (left) 500 µm; (right, magnified image from area designated by dotted box) 100 µm. (E) Representative H&E staining of sections from MLN of WT and *Ripk1*^{kd} mice infected with wild-type (*Yp*) or YopJ-deficient (ΔYopJ) *Yersinia*. While WT mice infected with either *Yp* or ΔYopJ and *Ripk1*^{kd} mice infected with ΔYopJ contain bacteria within pyogranulomas (white asterisks), *Ripk1*^{kd} mice infected with *Yp* fail to contain bacteria and have scattered bacterial colonies throughout the MLN. Bars, 500 µm. Data in all panels are representative of two independent experiments. NS, not significant by Student's *t* test.

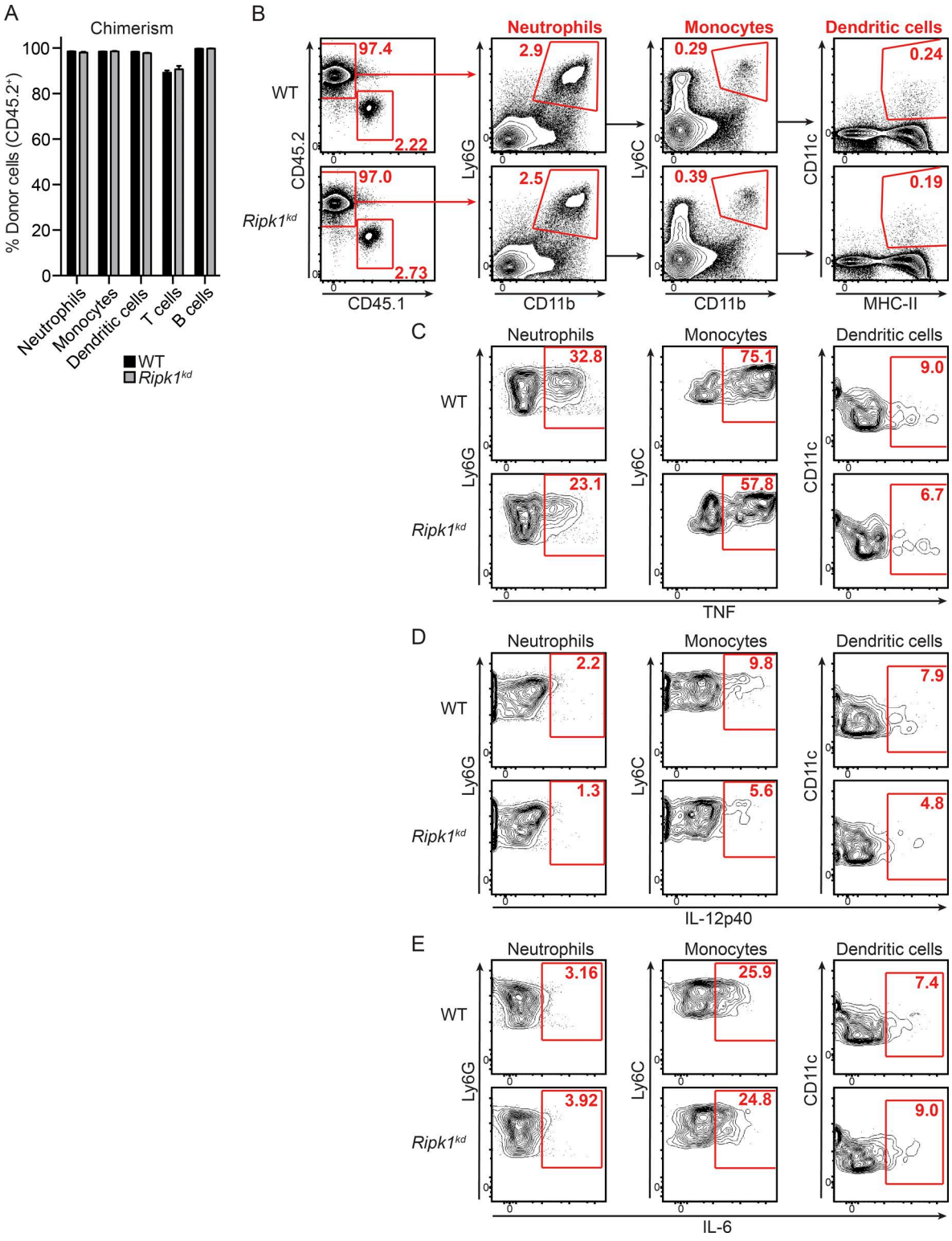

Figure S2. **Gating strategy for flow cytometry and BM chimerism.** Flow cytometry was performed on MLN samples from WT and *Ripk1*^{kd} BM chimeras on day 5 postinfection by oral gavage with 1–2 × 10^8 CFUs *Y. pseudotuberculosis*. (A and B) Singlet, live cells were gated to identify the percentage of donor BM-derived (CD45.2$^+$) neutrophils (CD11b^{hi}Ly6G^{hi}), monocytes (CD11b^{hi}Ly6C^{hi}Ly6G$^-$), and DCs (MHC-II$^+$CD11c$^+$). Data are representative of three independent experiments (n = 7 mice per group). (C–E) TNF (C), IL-12p40 (D), and IL-6 (E) production by gated cell populations was measured by intracellular cytokine staining. Flow plots are representative of data shown in Fig. 3 (E–G) and consistent across three independent experiments.

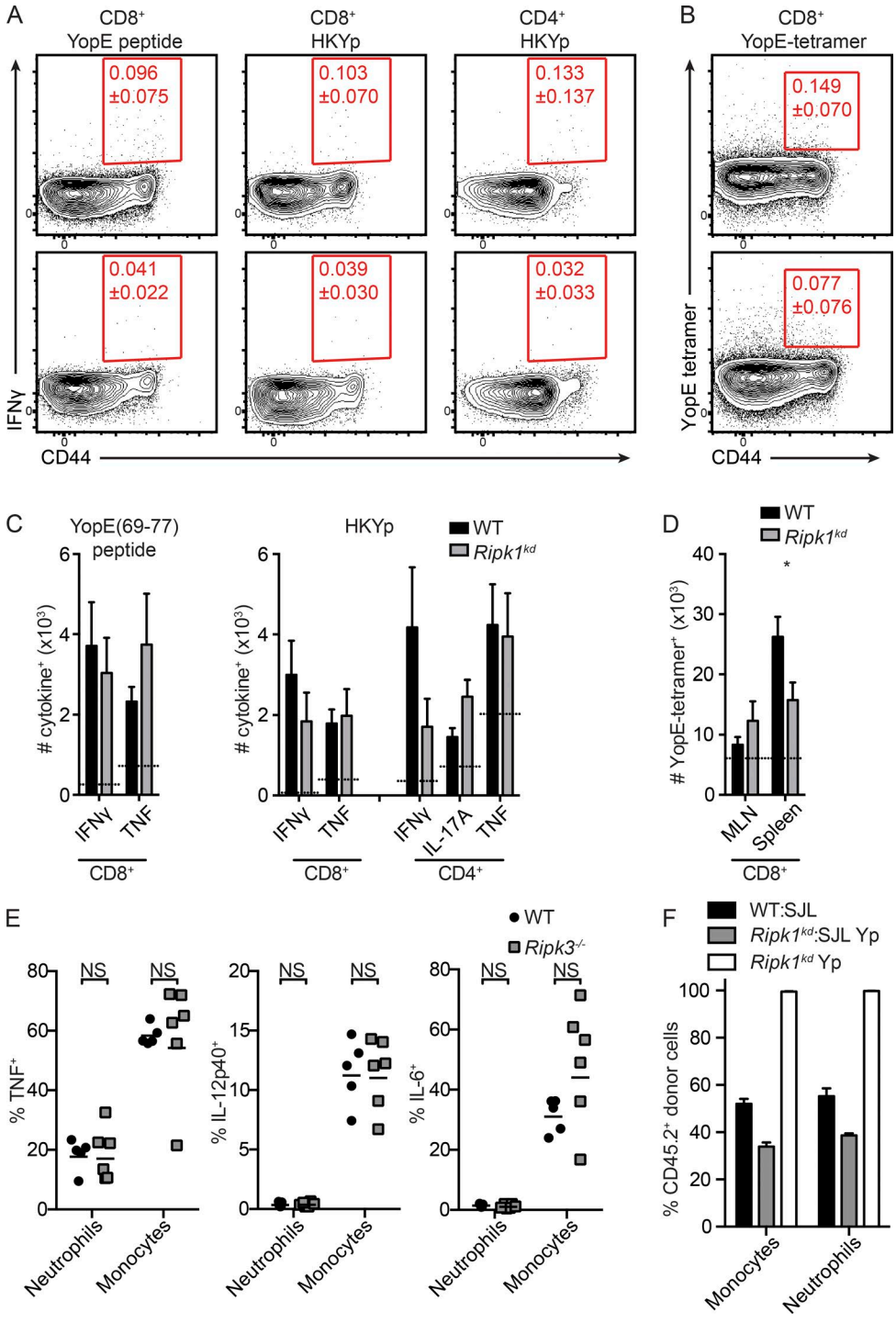

Figure S3. **RIPK1-dependent cytokine production in response to *Yp* infection is independent of adaptive immunity and RIPK3.** (A–D) T cell response on day 5 postinfection. (A) Representative flow plots showing intracellular cytokine staining for IFN-γ produced by $CD8^+$ T cells stimulated with $YopE_{69-77}$ peptide and both $CD8^+$ and $CD4^+$ T cells stimulated with heat-killed *Yp* (HKYp). (B) Representative flow plots showing $YopE_{69-77}$-tetramer (Lin et al., 2011)–positive $CD8^+$ T cells. (C and D) Total number of cells gated as in A and C and similarly for TNF or IL-17A production. Numbers in flow plots and bar graphs show mean and SD. Data are representative of two independent experiments ($n = 6–7$ animals per group). Statistical differences determined by Student's *t* test. *, $P < 0.05$. (E) WT and *Ripk3*$^{-/-}$ mice were infected with 2×10^8 CFUs *Y. pseudotuberculosis* by oral gavage, and cytokine expression by MLN neutrophils and monocytes was measured on day 5 postinfection. Line represents the mean. Data are representative of three independent experiments. NS, not significant by Student's *t* test. (F) Percentage of $CD45.2^+$ donor BM-derived neutrophils and monocytes. Data are representative of three independent experiments ($n = 7$ mice per group).

REFERENCE

Lin, J.S., F.M. Szaba, L.W. Kummer, B.A. Chromy, and S.T. Smiley. 2011. *Yersinia pestis* YopE contains a dominant CD8 T cell epitope that confers protection in a mouse model of pneumonic plague. *J. Immunol.* 187:897–904. http://dx.doi.org/10.4049/jimmunol.1100174