SUPPLEMENTAL DATA

Gut microbiota promotes hematopoiesis to control bacterial infection

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SUPPLEMENTAL FIGURES

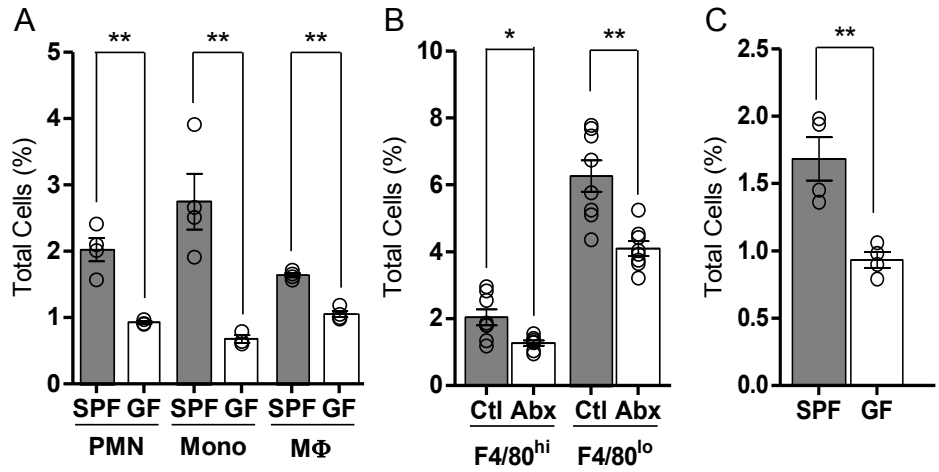

Figure S1. GF and Antibiotic-Treated Mice Have Reduced Populations of Myeloid Cells in Systemic Sites, Related to Figure 1 (A) Frequency of splenic neutrophils ($CD11b^{+}$ $GR1^{hi}$ $Ly6c^{lo}$), monocytes ($CD11b^{+}$ $Ly6c^{hi}$ $GR1^{hi}$) and macrophages ($CD11b^{+}$ $GR1^{-}$ $F4/80^{lo}$) among SPF and GF mice. (B) Frequency of splenic $CD11b^{+}$ $F4/80^{hi}$ and $CD11b^{+}$ $F4/80^{lo}$ phagocytes among untreated mice (Ctl) and SPF mice treated with oral antibiotics (Abx). (C) Frequency of liver $CD11b^{+}$ $F4/80^{hi}$ macrophages recovered from SPF or GF mice. Error bars represent SEM. Data are representative of 2-3 independent trials with n≥ 4 / group. *p<0.05, **p<0.01. PMN: polymorphonuclear cells; Mono: monocytes; MΦ : macrophages.

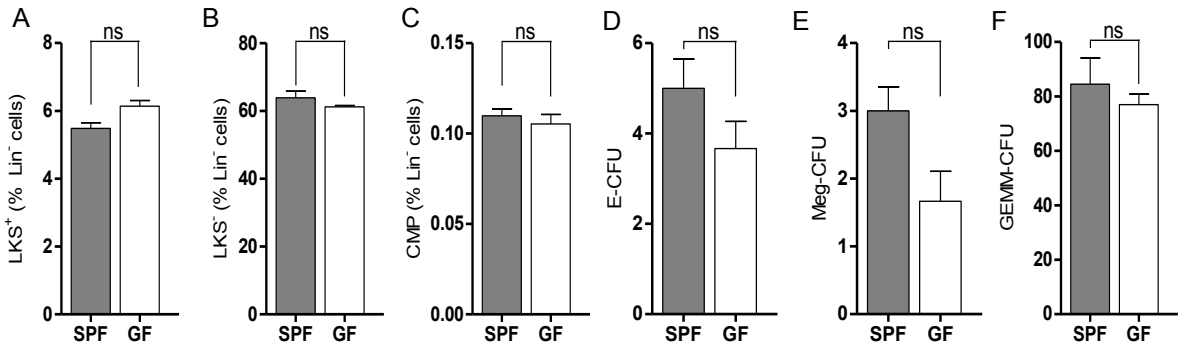

Figure S2. GF Mice Have Normal Proportions and Differentiation Potential of HSCs and Early Myeloid Progenitors in the Bone Marrow, Related to Figure 2 (A) Proportion of LKS^+ cells (Lin^- c-Kit^+ Sca-1^+; HSCs and MPPs), (B) LKS^- cells (Lin^- c-Kit^+ Sca-1^-; lineage-restricted progenitors) and (C) CMPs (LKS-$CD34^+$ $Fc\gamma R^{lo}$) among total progenitors (Lin^- cells) of SPF and GF mouse bone marrow. (D-F) Unfractionated bone marrow progenitor cells (Lin^- cells) from SPF and GF mice cultured in methylcellulose to assess the colony forming potential of progenitors. (D) E-CFU; erythrocyte colony forming units, (E) Meg-CFU; megakaryocyte CFU, (F) GEMM-CFU; Granulocyte/erythrocyte/monocyte/megakaryocyte CFU. Error bars represent SEM. Data are representative of 3 independent trials with $n \geq 4$ / group. Error bars represent SEM. ns: non-significant.

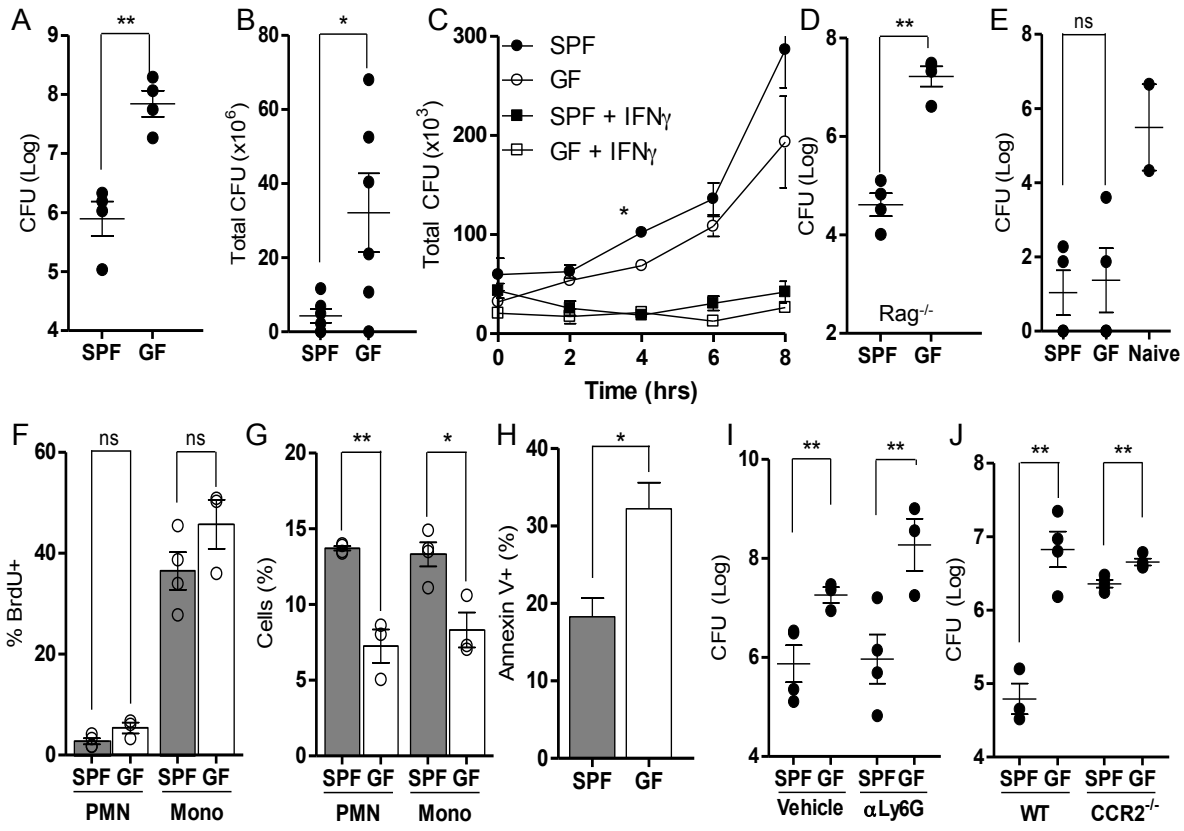

Figure S3. Resident Phagocytes Mediated Commensal-Enhanced Protection Against Infectious Disease, Related to Figure 3

(A) SPF and GF mice infected with *L. monocytogenes*, liver bacterial burden assessed 72 hpi. (B) SPF and GF mice infected with *S. aureus.* Kidney bacterial burden assessed 5 days post-infection. (C) Peritoneal macrophages isolated from SPF or GF mice, untreated or stimulated with interferon-γ (IFNγ), infected with *L. monocytogenes*. Recovery of intracellular bacteria measured over time. Data is non-significant for all time points measured, except where indicated (untreated SPF vs. GF, 4 hpi). (D) SPF and GF $Rag^{-/-}$ mice infected with *L. monocytogenes*, splenic bacterial burden assessed 72 hpi. (E) SPF and GF mice were immunized with *L. monocytogenes* Δ*actA*. 45 days after immunization, SPF and GF mice, as well as naïve, non-immunized SPF controls, were infected with wild-type (WT) *L. monocytogenes*. Splenic bacteria burden of the WT strain was measured at 72 hpi. Note: two of the four naïve, non-immunized SPF mice died following infection, prior to the 72 hour time point (data not shown). (F) BrdU incorporation among bone marrow neutrophils ($CD11b^{+}$ $GR1^{hi}$) and monocytes ($CD11b^{+}$ $CD115^{+}$), 72 hpi. (G) Percentage of splenic neutrophils ($Gr1^{hi}$ $Ly6C^{lo}$) and monocytes ($Gr1^{hi}$

Ly6C^{hi}) among SPF and GF mice, 72 hpi. (H) Annexin V^{+} bone marrow monocytes, 72 hpi. (I) SPF and GF mice infected with *L. monocytogenes*, following neutrophil depletion. Splenic bacterial burden assessed at 72 hpi. (J) Splenic bacterial burden of SPF and GF mice, reconstituted with bone marrow from WT or CCR2$^{-/-}$ mice, 72 hpi. SPF mice reconstituted with CCR2$^{-/-}$ bone marrow display a two-fold reduction in splenic CFUs compared to GF CCR2$^{-/-}$ mice. For all panels, data are representative of 2-3 independent trials with n≥ 4 / group. Each symbol represents data from a single animal. Error bars represent SEM. **p*<0.05, ***p*<0.01. PMN: polymorphonuclear cells; Mono: monocytes.

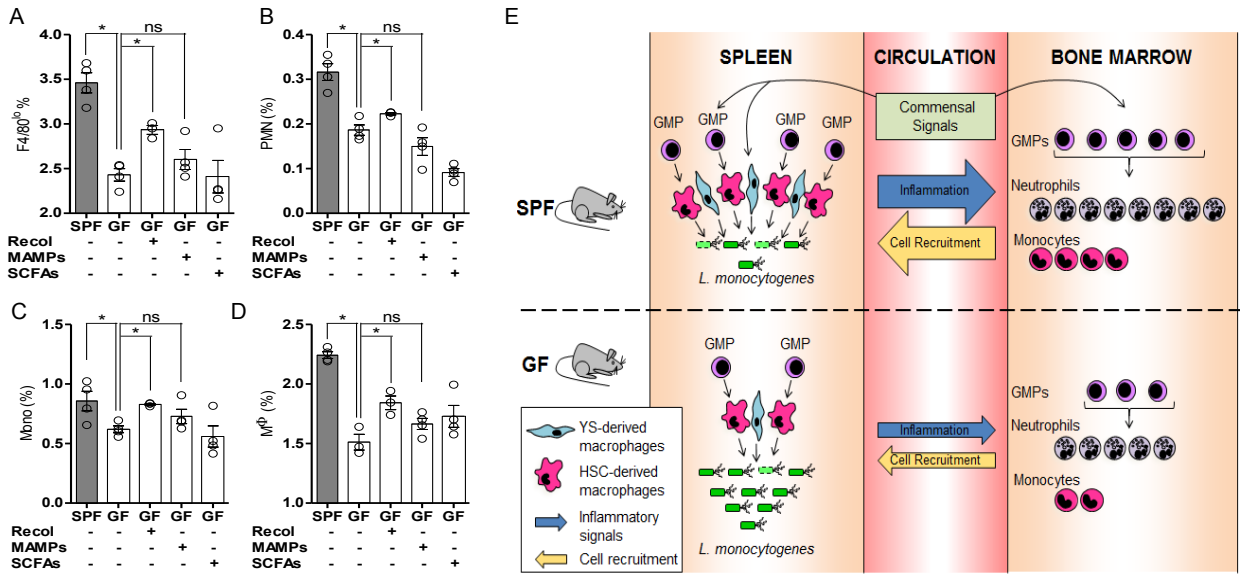

Figure S4. Re-colonization of GF Mice Rescues Tissue-Resident Phagocytes and Protects Against Systemic Infection, Related to Figure 4 Percentage of F4/80^{lo} splenocytes (A) as well as splenic neutrophils (B), monocytes (C), and F4/80^{lo} macrophages (D) among SPF, GF, re-colonized GF and GF mice treated with MAMPs or SCFAs. (E) A proposed model for how the microbiota mediates host resistance to systemic infection. Commensal microbes stimulate bone marrow and splenic myelopoiesis during naïve conditions (in the absence of infection), expanding systemic pools of mature myeloid cells in SPF mice that are essential for restricting pathogen dissemination upon acute infection. GF mice have reduced proportions and differentiation potential by GMPs during the steady state, as well as diminished expansion of yolk sac-derived macrophages, impairing the immune response to infection with *L. monocytogenes*. This model suggests that conditions in which the microbiota is disrupted may result in deficient expansion of myeloid cells, compromising host resistance to infectious disease. For all panels, data are representative of at least 2 independent trials with n≥4 / group. Error bars represent standard error of mean (SEM). *p<0.05. Recol: re-colonized; MAMPs: molecular associated molecular patterns; SCFAs: short chain fatty acids; PMN: polymorphonuclear cells; Mono: monocyte; MΦ: macrophage.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

SPF C57BL/6 and CCR2$^{-/-}$ mice were purchased from Taconic Farms and Jackson Laboratories, respectively. In some experiments, SPF and GF mice were immunized with 3x10^4 CFU *L. monocytogenes* Δ*actA* (Lara-Tejero and Pamer, 2004), and immunized mice and non-immunized controls were infected with 2x10^5 CFU of wild-type (WT) *L. monocytogenes* 45-day post immunization, with splenic bacterial burden measured 72 hpi. SPF and GF mice were infected with 1x10^7 CFU of *Staphylococcus aureus* (strain Newman) via tail vein injection and kidney bacterial burden assessed 5 days post-infection. CCR2$^{-/-}$ chimeras were generated by transferring bone marrow from WT or CCR2$^{-/-}$ donors into SPF or GF recipients that had been lethally irradiated (1000 rads) 48 hours prior. Mice were infected with 3x10^4 CFU of *L. monocytogenes* 8 weeks post reconstitution, and splenic bacterial burden was assessed 72 hpi. For neutrophil depletion, SPF and GF mice were injected *i.p.* with 0.5 mg of anti-Ly6G antibody (Bioxpress), or saline control, 24 hours prior to infection with *L. monocytogenes*. To measure cell proliferation during *Listeria* infection, mice were injected *i.p.* with 100 μg BrdU (Sigma), and BrdU incorporation among progenitor and mature myeloid cells was determined 3 hours later via a BrdU detection kit (eBioscience). Apoptosis and cell viability was assessed by staining with Annexin V (eBioscience) and 7-Aminoactinomycin-D (Invitrogen). *Listeria*-killing assays were conducted as previously described (Portnoy et al., 1989). Briefly, peritoneal macrophages were collected from naïve SPF and GF mice. Adherent cells were stimulated with 100 U/ml of interferon gamma (IFNγ) (PeproTech) or left untreated for 24 hours. Macrophages were washed and infected with *L. monocytogenes* at a multiplicity of infection (MOI) of 10. Cells were washed 30 minutes later and fresh media with 5 μg/ml of Gentamycin (Phoenix) was added. Cells were washed and lysed at various time points to quantitate intracellular *Listeria* via microbiological plating.

SUPPLEMENTAL REFERENCES

Lara-Tejero, M., and Pamer, E.G. (2004). T cell responses to Listeria monocytogenes. Curr Opin Microbiol *7*, 45-50.

Portnoy, D.A., Schreiber, R.D., Connelly, P., and Tilney, L.G. (1989). Gamma interferon limits access of Listeria monocytogenes to the macrophage cytoplasm. The Journal of Experimental Medicine *170*, 2141-2146.