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Supplemental information

CD115^{+} monocytes protect microbially experienced mice against *E. coli*-induced sepsis

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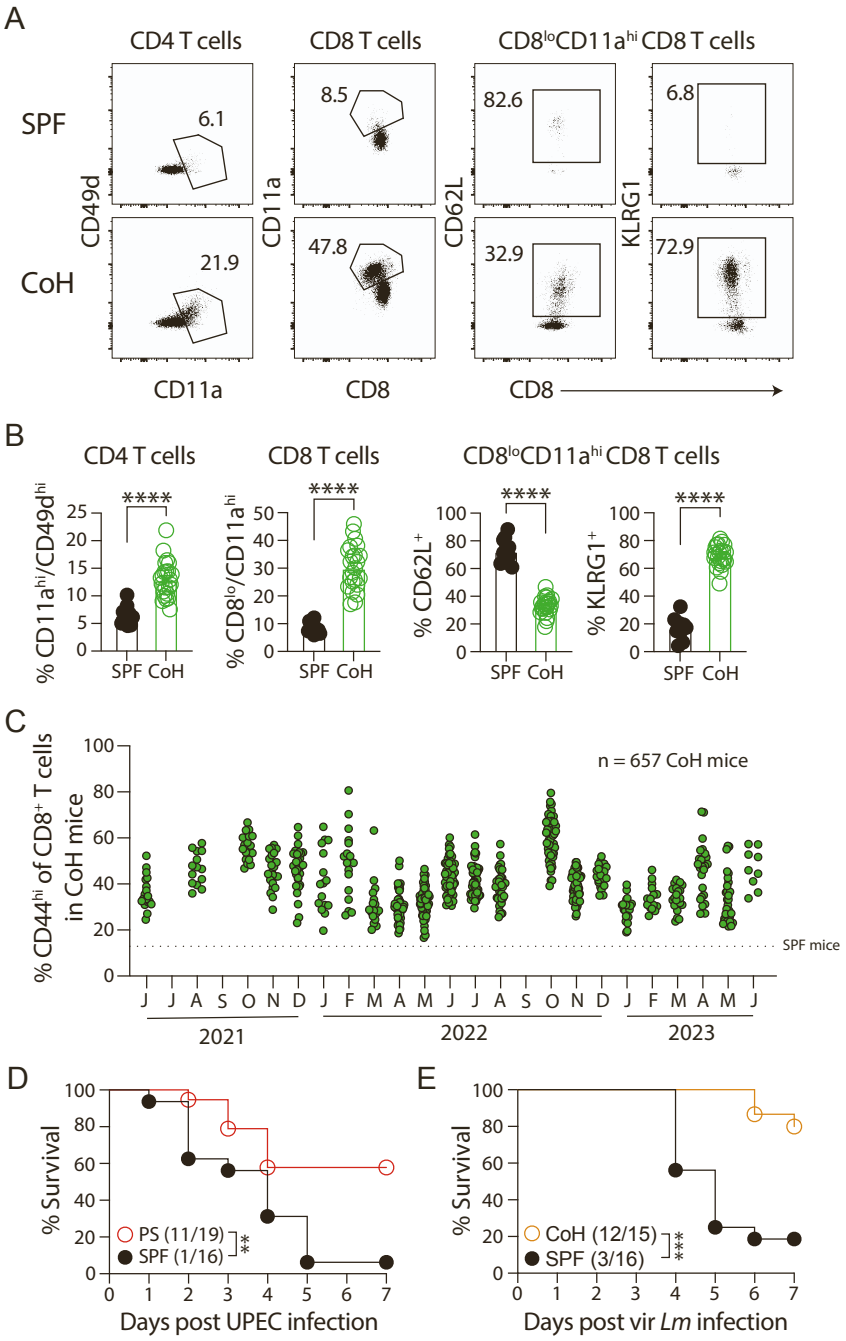
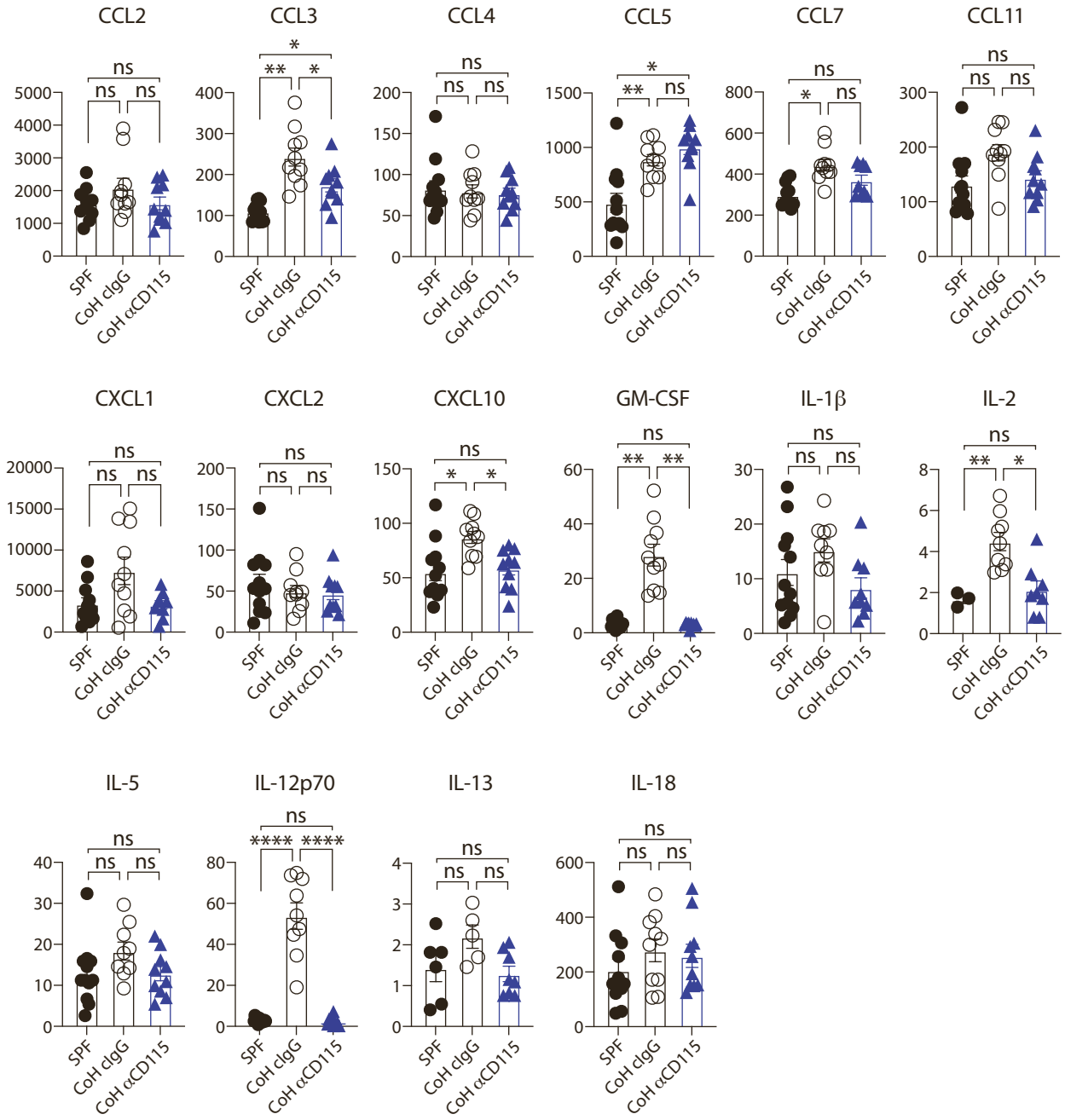
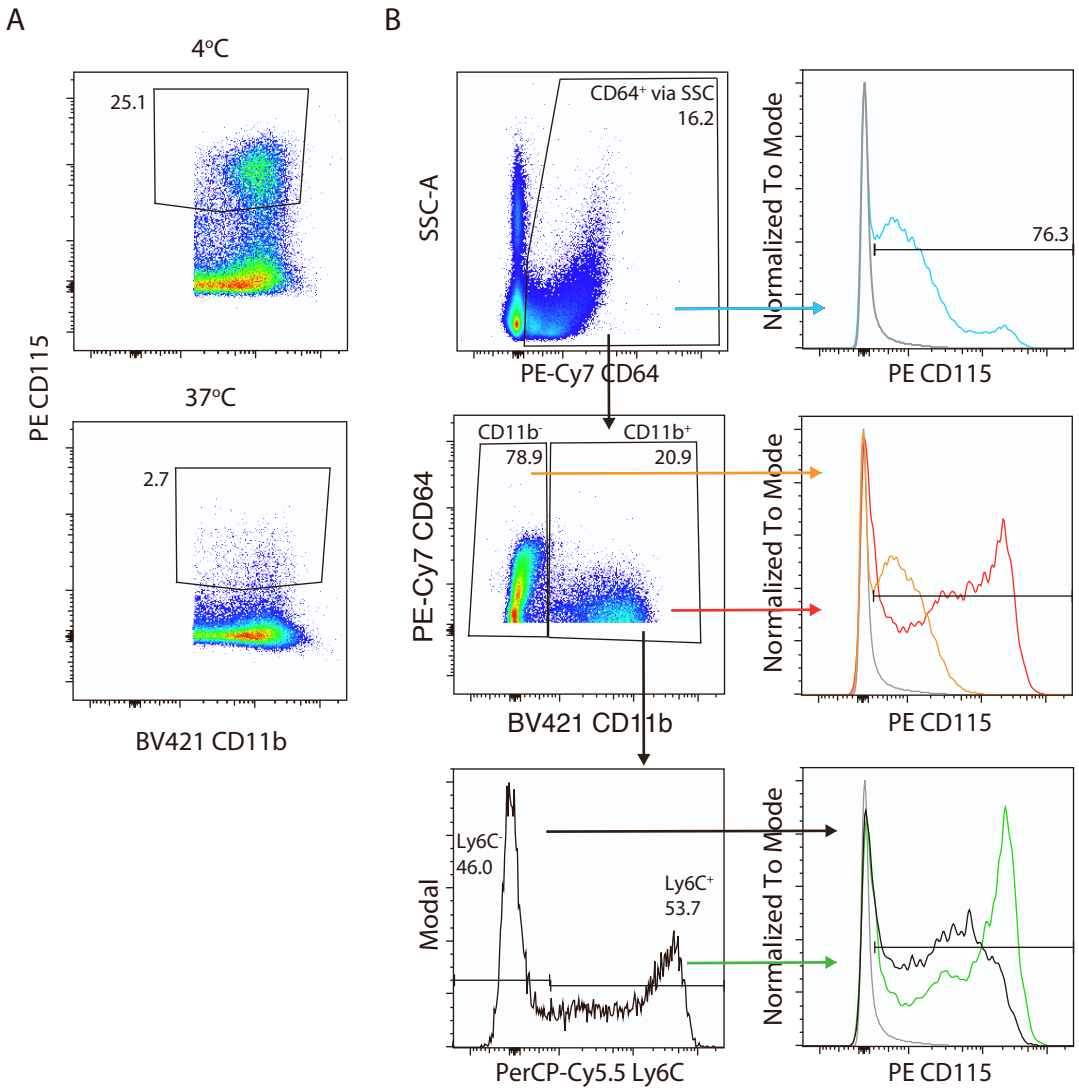


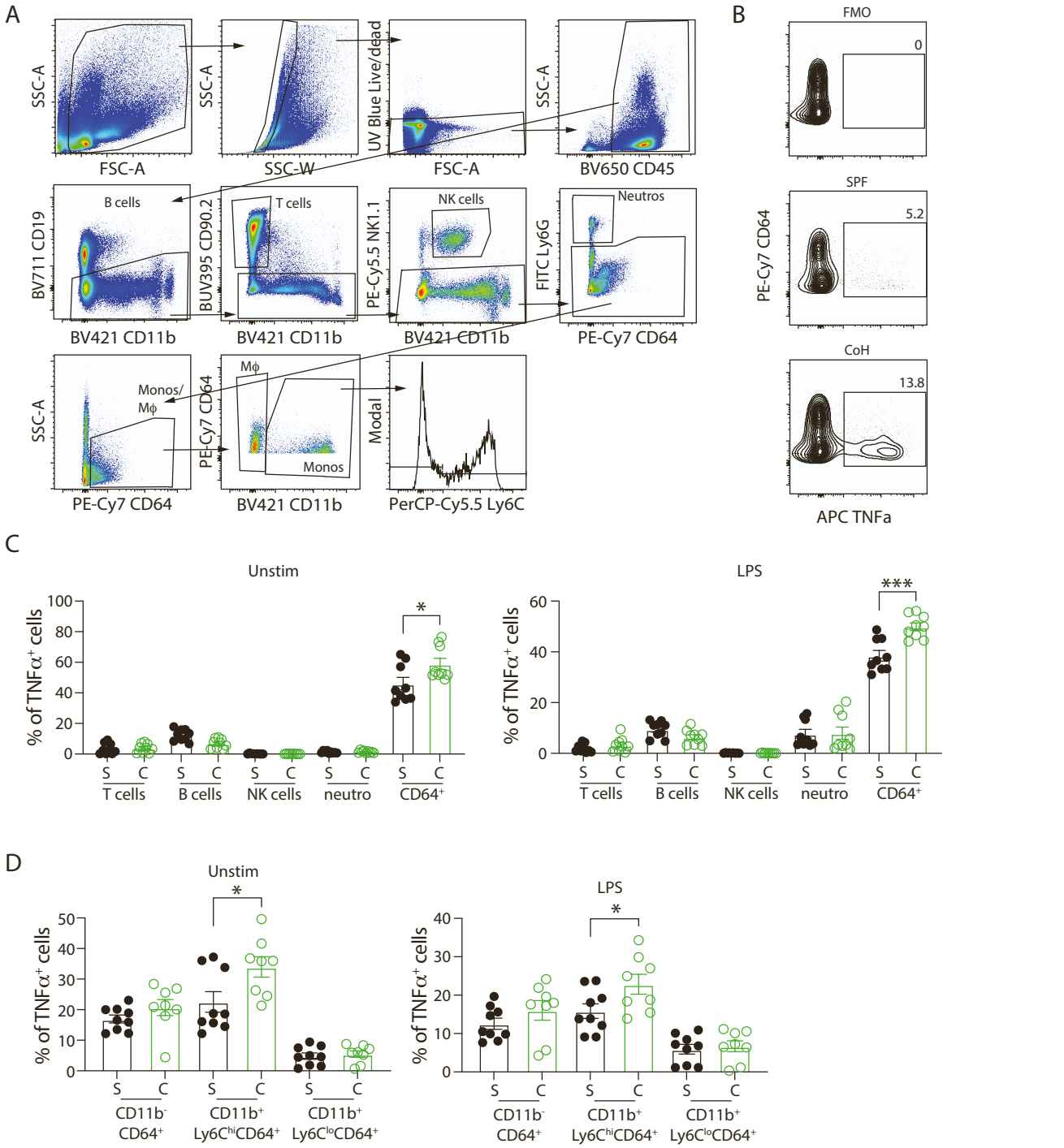
Supplemental Figure 1. Microbe-experienced mice have increased frequency of Ag-experienced T cells and resistance to systemic bacterial infection. (A) Representative plots show CD11a^{hi}CD49d^{hi} Ag-experienced CD4 T cells and CD62L^{hi} or KLRG1^{hi} Ag-experienced CD8 T cells (CD8a^{lo}CD11a^{hi}) in SPF and CoH mice. **(B)** Percentage of CD11a^{hi}CD49d^{hi} CD4 T cells of all CD4 T cells, CD8a^{lo}CD11a^{hi} CD8 T cells of all CD8 T cells, and CD62L^{hi} or KLRG1^{hi} cells of CD8a^{lo}CD11a^{hi} CD8 T cells in SPF or CoH mice. **** $P < 0.0001$ as determined by nonparametric Mann-Whitney test. Each symbol represents a mouse and bars indicate means with SEM. (**C**) Percentage of CD44^{hi} cells of CD8^{+} T cells in the blood of CoH mice screened prior to use in experiments. Letters indicate the months of the year. Dotted line indicates the mean frequency (12.9%) CD44^{hi} cells of CD8^{+} T cells in the blood of 10 SPF mice. **(D)** Female SPF B6 mice and pet store (PS) mice were infected with 4x10^{7} colony forming units (CFU) uropathogenic *E. coli* (UPEC) i.v.n. ** $P \leq 0.01$ as determined by Log-rank test. Data were combined from two experiments using a total of 16-19 mice per group. **(E)** Female SPF and CoH B6 mice were infected with 10^{4} CFU virulent *L. monocytogenes* i.v.. *** $P \leq 0.001$ as determined by Log-rank test. Data were combined from two experiments using a total of 15-16 mice per group.



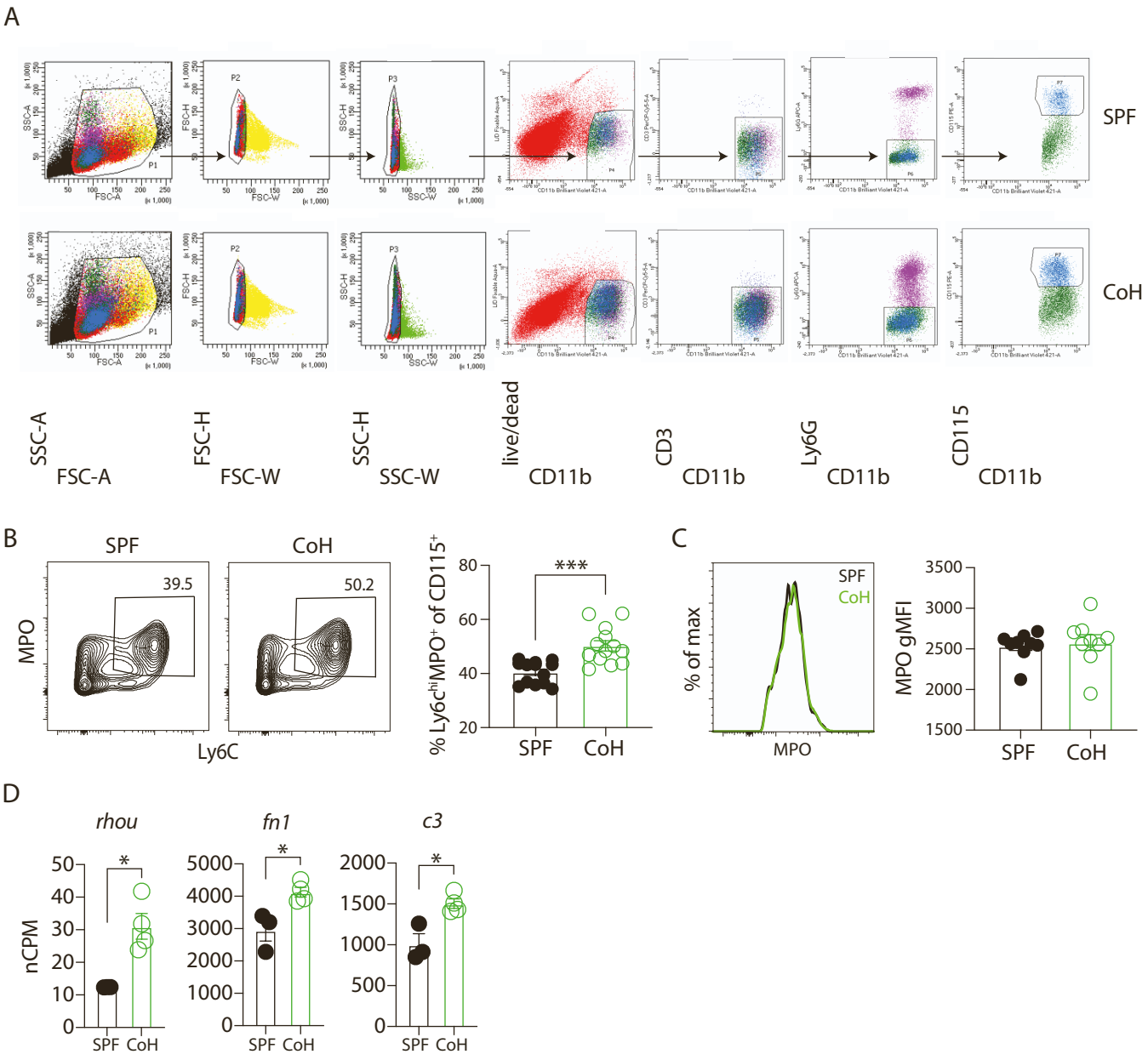
Supplemental Figure 2. CoH mice show increased inflammatory response to systemic UPEC infection, and depletion of CD115⁺ cells reduces this inflammation. Serum CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CXCL1, CXCL2, CXCL10, GM-CSF, IL-1β, IL-2, IL-5, IL-12p70, IL-12, and IL-18 concentrations 3 hours following UPEC infection in SPF mice, CoH mice given control IgG, or CoH mice given anti-CD115 mAb. Combined data from two experiments using a total 10-12 mice per group. ns – not significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and *** $P \leq 0.0001$, as determined by Kruskal-Wallis test, with a Dunn's post hoc test to correct for multiple comparisons.



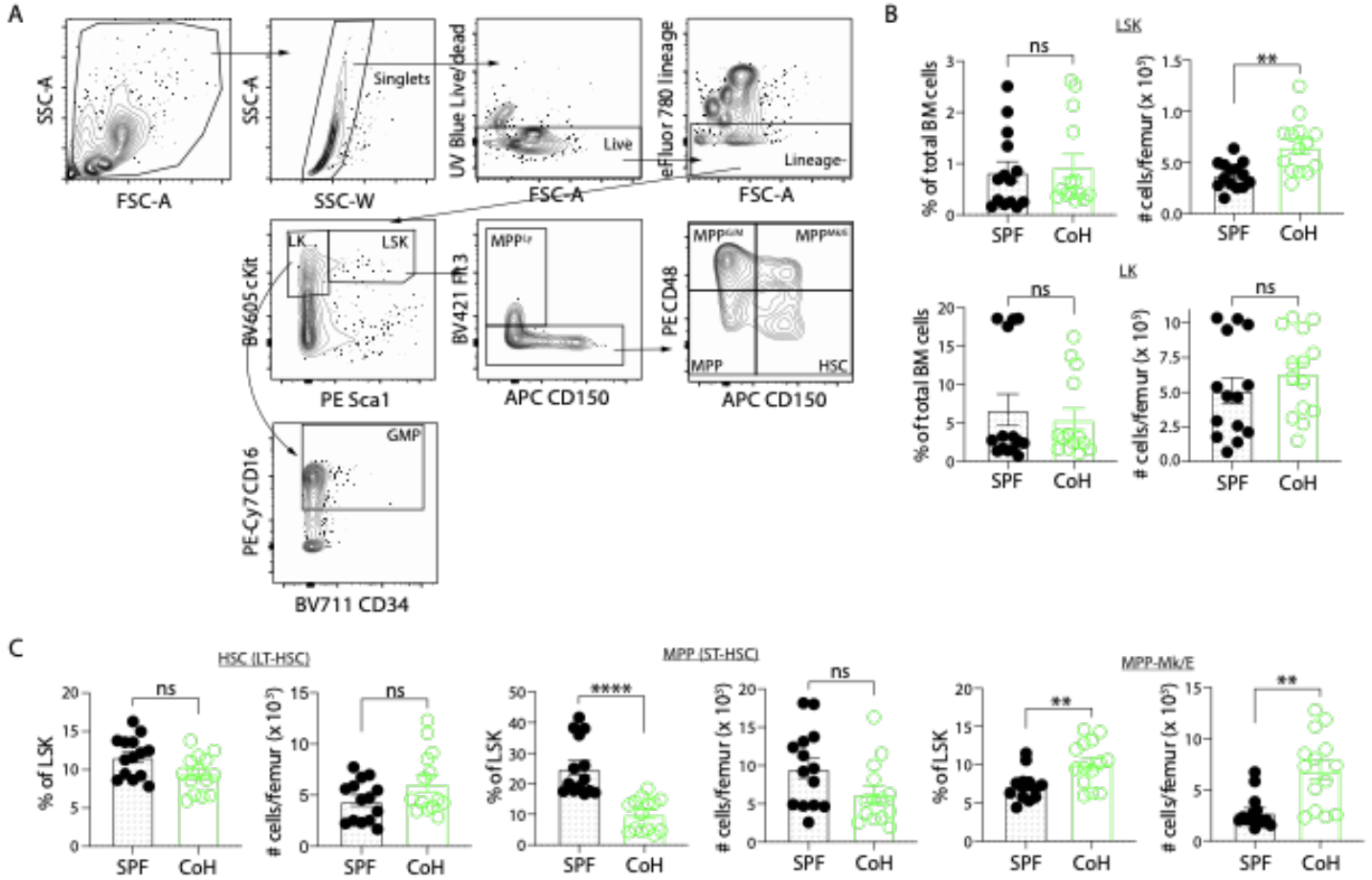
Supplemental Figure 3. Using CD115 and CD64 to identify monocytes. (A) Flow plots demonstrating CD115 is cleaved from $CD11b^+$ splenocytes with 1 hour culture at 37°C (right) vs 4°C (left). **(B)** CD115 expression on $CD64^+$ (blue), $CD11b^-CD64^+$ (orange), $CD11b^+CD64^+$ (red), $Ly6C^{hi}CD11b^+CD64^+$ (green), and $Ly6C^{lo}CD11b^+CD64^+$ (black) splenocytes compared to bulk $CD45^+$ cells (grey).



Supplemental Figure 4. *In vitro* measurement of TNFα production from SPF and CoH splenocytes. (A) Gating strategy used to identify immune cell subsets from 4-hour *in vitro* splenocyte cultures. **(B)** Representative plots of intracellular TNFα staining of CD64$^+$ SPF (middle) or CoH (bottom) splenic monocytes/Mϕ compared to TNFα FMO control (top). **(C-D)** Analysis was done by gating on TNFα$^+$ cells first and then immune cell subsets to determine what percentage each cell contributes to the total TNFα$^+$ population. **(C)** Frequency of T cells, B cells, NK cells, neutrophils, and CD64$^+$ cells of the TNFα$^+$ cells from 4-hour cultures of unstimulated and 100 ng/mL LPS-stimulated splenocytes determined by flow cytometry. **(D)** Frequency of CD11b$^-$CD64$^+$ resident macrophages, CD11b$^+$Ly6C^{hi} classical monocytes, and CD11b$^+$Ly6C^{lo} non-classical monocytes of the TNFα$^+$ cells from 4-hour cultures of unstimulated and 100 ng/mL LPS-stimulated splenocytes. **C-D** were combined from 3 experiments using a total of 9 mice per group. * $P \leq 0.05$ and *** $P \leq 0.005$, and **** p<0.0001 as determined by nonparametric Mann-Whitney test. Each symbol in **C** and **D** represents a mouse and bars indicate means with SEM.



Supplemental Figure 5. Gating for sort-purified CD115⁺ monocytes and expression of myeloperoxidase. (A) Gating strategy used to sort purify CD115^{++}CD11b^{+} monocytes for transcriptional analysis using RNAseq. **(B)** Representative plots showing the percentage of Ly6C^{hi} MPO^{+} cells in SPF and CoH steady-state CD115^{+} splenocytes (left). Frequency of Ly6C^{hi}MPO^{+} amongst CD115^{+} monocytes in steady-state CoH mice (right). **(C)** Representative histogram of MPO expression by SPF and CoH MPO^{+} Ly6C^{hi}CD115^{+}monocytes (left). MPO gMFI expression among SPF and CoH MPO^{+} Ly6C^{hi}CD115^{+} monocytes on a per cell basis as measured by flow cytometry (right). **(D)** Number of *rhou, fn1, and c3* mRNA transcripts in CD115^{+} monocytes from steady-state CoH and SPF mice. Flow data in **B-C** were combined from three experiments using a total of 9-13 mice per group; Transcript (normalized copies per million; nCPM) data in **D** were obtained from 3-4 mice per group. * $P \leq 0.05$ and *** $P \leq 0.005$, and **** p<0.0001 as determined by nonparametric Mann-Whitney test. Each symbol represents a mouse and bars indicate means with SEM.



Supplemental Figure 6. Gating and analysis of bone marrow multipotent progenitor populations from SPF and CoH mice. (A) Gating scheme used to identify bone marrow multipotent progenitor (MPP) populations, as proposed by Challen et al. [1] Representative plots show gating of singlet live cells using UV blue live/dead. The lineage$^-$ population (negative for B220, CD11c, Ly6G, Ter119, and CD3) was further gated on LK (lineage$^-$Sca1$^-$cKit$^+$) and LSK (lineage$^-$Sca1$^+$cKit$^+$) populations. Granulocyte/Monocyte Progenitor (GMP) population is defined by being CD16$^+$ LK cells. The LSK population was further analyzed using Flt3 and CD150 with Flt3$^+$CD150$^-$ cells constituting MPP-Lymphocyte (Ly). The Flt3$^-$ LSK compartment was further analyzed using CD150 and CD48, with MPP-granulocyte/monocyte (G/M) defined as CD48$^+$ CD150$^-$, MPP-megakaryocyte/erythrocyte (Mk/E) defined as CD48$^+$CD150$^+$, hematopoietic stem cell (HSC) defined as CD48$^-$CD150$^+$, and MPP defined as CD48$^-$CD150$^-$. **(B)** Absolute number per femur and percentage of the LSK (top) and LK (bottom) population within total bone marrow cells. **(C)** Absolute number per femur and percentage of HSC (also known as LT-HSC, left), MMP (also known as ST-HSC, middle) and MPP-Mk/E (right) within the LSK population. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.005$, and **** $P < 0.0001$ as determined by nonparametric Mann-Whitney test. **B-C** were combined from 3 independent experiments using a total of 14 mice per group.

1. Challen, G.A., Pietras, E.M., Wallscheid, N.C., and Signer, R.A.J. (2021). Simplified murine multipotent progenitor isolation scheme: Establishing a consensus approach for multipotent progenitor identification. Exp. Hematol. *104*, 55-63. 10.1016/j.exphem.2021.09.007.