SUPPLEMENTAL MATERIAL

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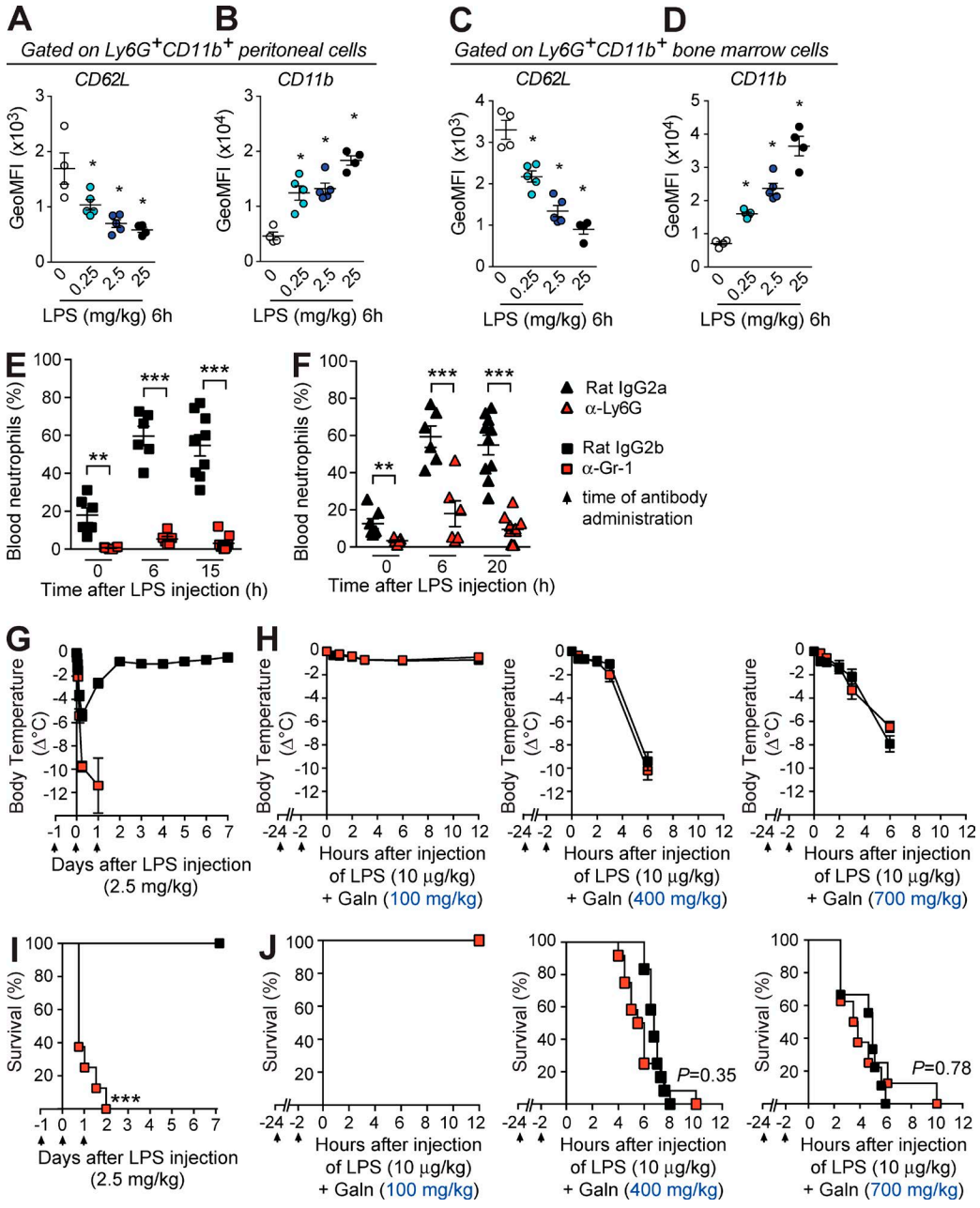

Figure S1. **Phenotype of neutrophils in the peritoneum and BM after LPS injection, and effect of treatment with neutrophil-depleting antibodies in models of LPS- or LPS/D-galactosamine–induced endotoxemia.** (A–D) Levels of CD62L (A and C) and CD11b (B and D; depicted as geometric mean fluorescence intensity [GeoMFI]) on Ly6G$^+$ CD11b$^+$ peritoneal (A and B) or BM (C and D) neutrophils 6 h after injection of various concentrations of LPS (as indicated). Results in A–D show values from individual mice; bars indicate means ± SEM pooled from two independent experiments (total n = 4–5/group). *, $P < 0.05$ versus control 0 group by two-tailed Mann–Whitney U test. (E and F) Percentage of Gr-1^{high} CD11b$^+$ blood neutrophils at the indicated time point after injection of 25 mg/kg LPS in C57BL/6J mice treated i.p. with an anti-Ly6G neutrophil-depleting antibody (clone 1A8; 500 µg/injection) or an isotype control antibody (rat IgG2a; E) and in C57BL/6J mice treated i.p. with an anti-Gr-1 neutrophil-depleting antibody (clone RB6-8C5; 300 µg/injection) or an isotype control antibody (rat IgG2b; F). Data in E and F are pooled from three independent experiments (total n = 10–12/group). **, $P < 0.01$; ***, $P < 0.001$ versus the corresponding isotype control group by two-tailed Mann–Whitney U test. (G–J) Changes in body temperature (Δ°C [mean ± SEM]; G and H) and survival (percentage of live animals; I and J) after injection of 2.5 mg/kg LPS (G and I) or 10 µg/kg LPS together with the indicated concentration of D-galactosamine (Galn; H and J) in C57BL/6J mice treated i.p. with an anti-Gr-1 neutrophil-depleting antibody (clone RB6-8C5; 300 µg/injection) or an isotype control antibody (rat IgG2b). Data in G–J are pooled from two or three independent experiments (total n = 8–12/group). ***, $P < 0.001$ versus the corresponding isotype control group by Mantel–Cox log-rank test. Arrows in G–J indicate time of i.p. injection of the neutrophil-depleting or isotype control antibodies.

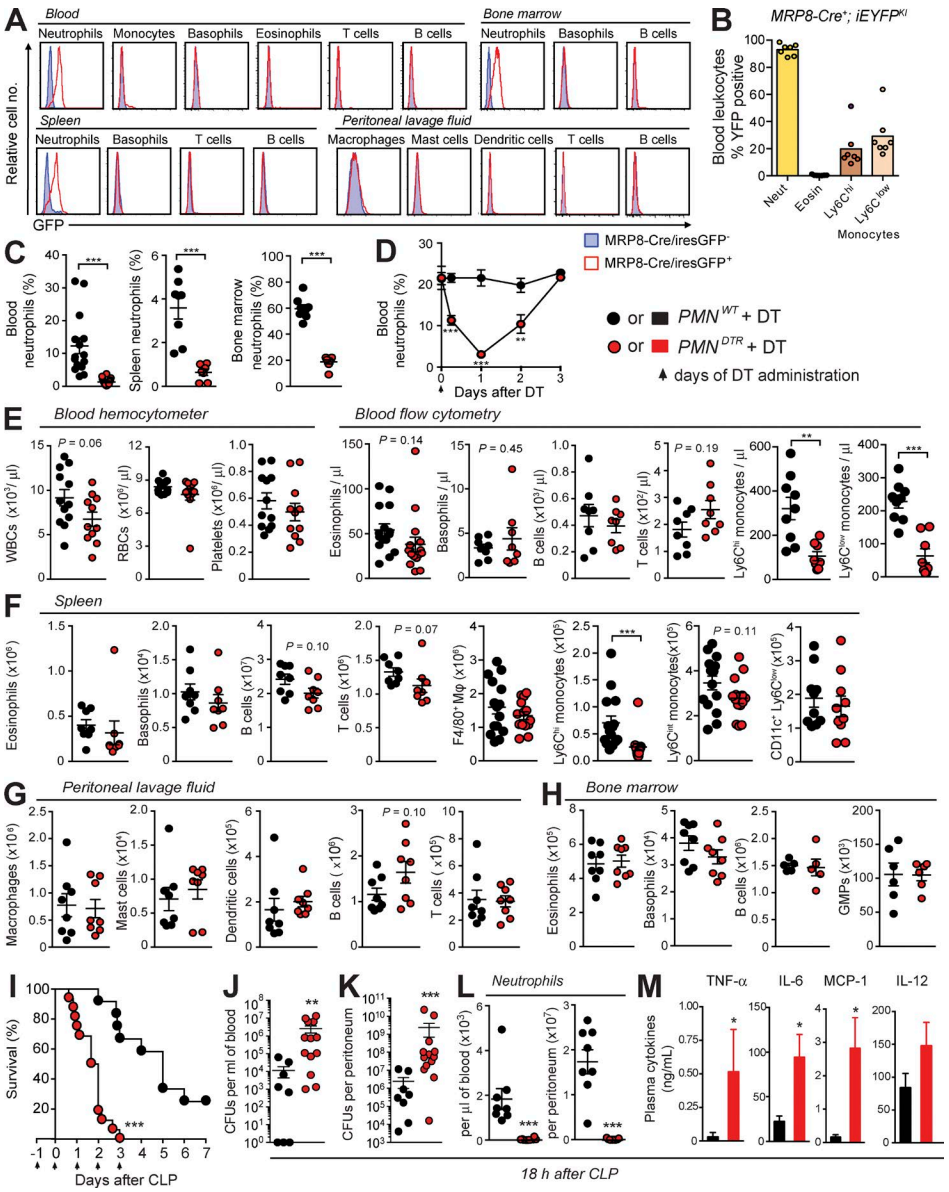

Figure S2. **Analysis of GFP expression in various immune cell types from *MRP8-Cre/IRES-GFP* mice, levels of various cell populations 24 h after DT injection into *PMN^{DTR}* mice, and responses of *PMN^{DTR}* mice in the CLP model of polymicrobial sepsis.** (A) Analysis of GFP expression (mean fluorescence intensity) in the indicated cell populations in *MRP8-Cre/IRES-GFP*$^-$ and *MRP8-Cre/IRES-GFP*$^+$ mice. Results are representative of three independent experiments. (B) Analysis of YFP expression in myeloid leukocytes from the blood of *MRP8-Cre/IRES-GFP*$^+$ × ROSA-iEYFP^{KI} reporter mice, graphed as percentage YFP$^+$ of the indicated populations. Values from individual mice are shown; bars indicate means pooled from two independent experiments (total $n = 7$). Neut, neutrophils; Eosin, eosinophils. (C) Percentage of blood, spleen, and BM neutrophils 24 h after i.p. injection of 500 ng DT into *PMN^{DTR}* mice and *PMN^{WT}* littermate control mice. (D) Percentage of blood neutrophils at the indicated time points after i.p. injection of DT on day 0. Results in D are means ± SEM from $n = 3$–8 mice per group pooled from two or three independent experiments. (E–H) Numbers of various cell populations in blood (E), spleen (F), peritoneal lavage fluid (G), and BM (H) from *PMN^{DTR}* mice and *PMN^{WT}* littermate control mice 24 h after i.p. injection of 500 ng DT, determined by hemocytometer measurements or flow cytometry. Results in C and E–H show values from individual mice; bars indicate means ± SEM pooled from two independent experiments for blood monocytes and spleen monocyte-derived DCs (moDCs; CD11b$^+$CD11c$^+$Ly6C^{low}; total $n = 8$–10/group) and BM B cells (total $n = 5$/group), four experiments for blood neutrophils (B; total $n = 15$–16/group), or three independent experiments for all other cell populations (total $n = 7$–15/group). P-values <0.3 are indicated (two-tailed Mann–Whitney *U* test). WBCs, white blood cells; RBCs, red blood cells; Mϕ, macrophages. (I–M) Responses of DT-treated *PMN^{DTR}* versus *PMN^{WT}* mice in the CLP model of polymicrobial sepsis. (I) Survival after CLP ($n = 12$–16). (J–L) Numbers of bacterial CFUs in the blood (J) and peritoneal lavage fluid (K) and numbers of blood and peritoneal neutrophils (L) 18 h after CLP ($n = 8$–13/group). (M) Levels of TNF-α, IL-6, MCP-1, and IL-12 in the plasma 18 h after CLP ($n = 7$–8/group). Results in J–L show values from individual mice; bars indicate means ± SEM; results in M are means + SEM. Data in I–M are pooled from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus *PMN^{WT}* group by Mantel–Cox log-rank test (I) or Mann–Whitney *U* test (J–M). Arrows in D and I indicate days of i.p. injection of the neutrophil-depleting or isotype control antibodies.

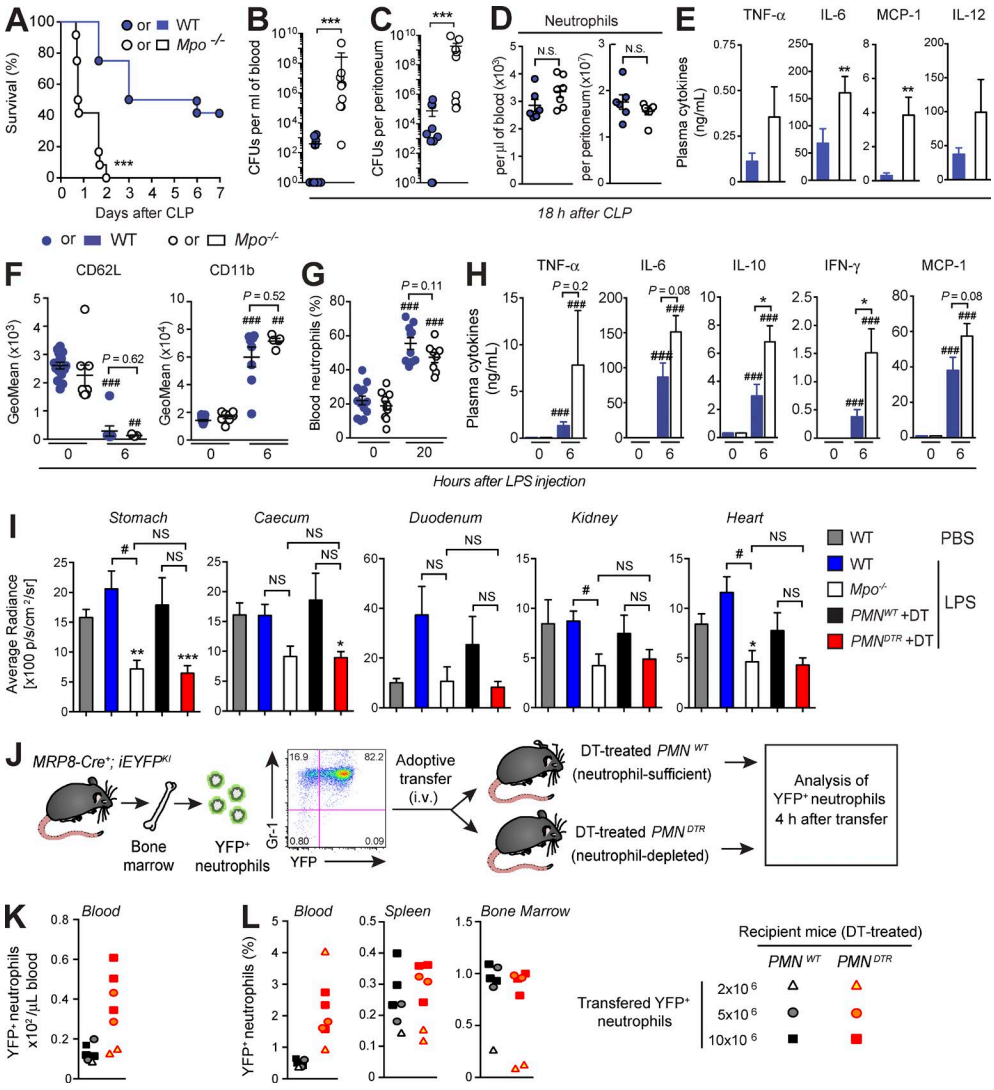

Figure S3. **Responses of MPO-deficient mice in models of polymicrobial sepsis and LPS-induced endotoxemia, systemic quantification of MPO-induced bioluminescence, and levels of neutrophils after adoptive transfer in *PMN*^{WT} versus *PMN*^{DTR} mice.** (A–E) Responses of *Mpo*$^{-/-}$ versus WT mice in the CLP model of polymicrobial sepsis. (A) Survival after CLP (n = 12). (B–E) Numbers of bacterial CFUs in the blood (B) and peritoneal lavage fluid (C; n = 9–11/group), numbers of blood and peritoneal neutrophils (D; n = 6–7/group), and levels of TNF-α, IL-6, MCP-1, and IL-12 in the plasma 18 h after CLP (n = 9–11/group). Results in B–D show values from individual mice; bars indicate means ± SEM; results in E are means + SEM. Data in A–E are pooled from two (D) or three (A–C and E) independent experiments. **, P < 0.01; ***, P < 0.001 versus WT group by Mantel–Cox log-rank test (A) or Mann–Whitney U test (B–E). (F) Quantification of CD62L and CD11b levels (geometric mean fluorescence intensity [GeoMean]) on Ly6G$^+$ CD11b$^+$ blood neutrophils before (time 0) or 6 h after injection of LPS in WT or *Mpo*$^{-/-}$ mice. (G) Percentage of blood neutrophils before (time 0) and 20 h after LPS injection (25 mg/kg) in WT and *Mpo*$^{-/-}$ mice. F and G show values from individual mice; bars indicate means ± SEM pooled from two (F) or three (G) independent experiments (total n = 5–15/group). (H) Levels of TNF-α, IL-6, IL-10, IFN-γ, and MCP-1 in the plasma of WT and *Mpo*$^{-/-}$ mice before (time 0) and 6 h after LPS injection. Data in H are means + SEM (total n = 8–12/group from three independent experiments). *, P < 0.05 versus WT group and $^{\#\#}$, P < 0.01; $^{\#\#\#}$, P < 0.001 versus the same group at time 0 by Mann–Whitney U test. (I) Quantification of MPO-induced bioluminescence in the stomach, cecum, duodenum, kidney, and heart 6 h after i.p. injection of PBS (in WT mice) or LPS (25 mg/kg) in WT mice, *Mpo*$^{-/-}$ mice, DT-treated *PMN*^{DTR} mice, and DT-treated *PMN*^{WT} littermate controls, and 5 min after luminol injection. Data are means + SEM from three independent experiments (total n = 6–14/group) except for *Mpo*$^{-/-}$ mice (two independent experiments with a total of three to four mice). *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus PBS-treated WT group, and $^{\#}$, P < 0.05 versus corresponding LPS-treated control group by unpaired Student's t test. N.S., not significant (P > 0.05). (J–L) Levels of neutrophils after adoptive transfer into DT-treated *PMN*^{DTR} or *PMN*^{WT} mice. (J) Experimental outline. We purified BM neutrophils by negative selection using a commercially available kit. Purified cells were >95% Gr-1$^+$ CD11b$^+$ (as shown in J) and >90% Ly6G$^+$ CD11b$^+$ (not depicted) on average for all adoptive transfer experiments (Fig. 3 and panels K and L). Cells were >80% YFP$^+$ on average for all adoptive transfer experiments (K and L). Various numbers of purified neutrophils were transferred i.v. into DT-treated *PMN*^{DTR} mice (in which endogenous YFP$^-$ neutrophils were depleted) or DT-treated *PMN*^{DTR} mice (which contained endogenous YFP$^-$ neutrophils). 4 h after adoptive transfer, levels of YFP$^+$ neutrophils (Ly6G$^+$ CD11b$^+$) were analyzed by flow cytometry in the blood (K and L), spleen and BM (L).