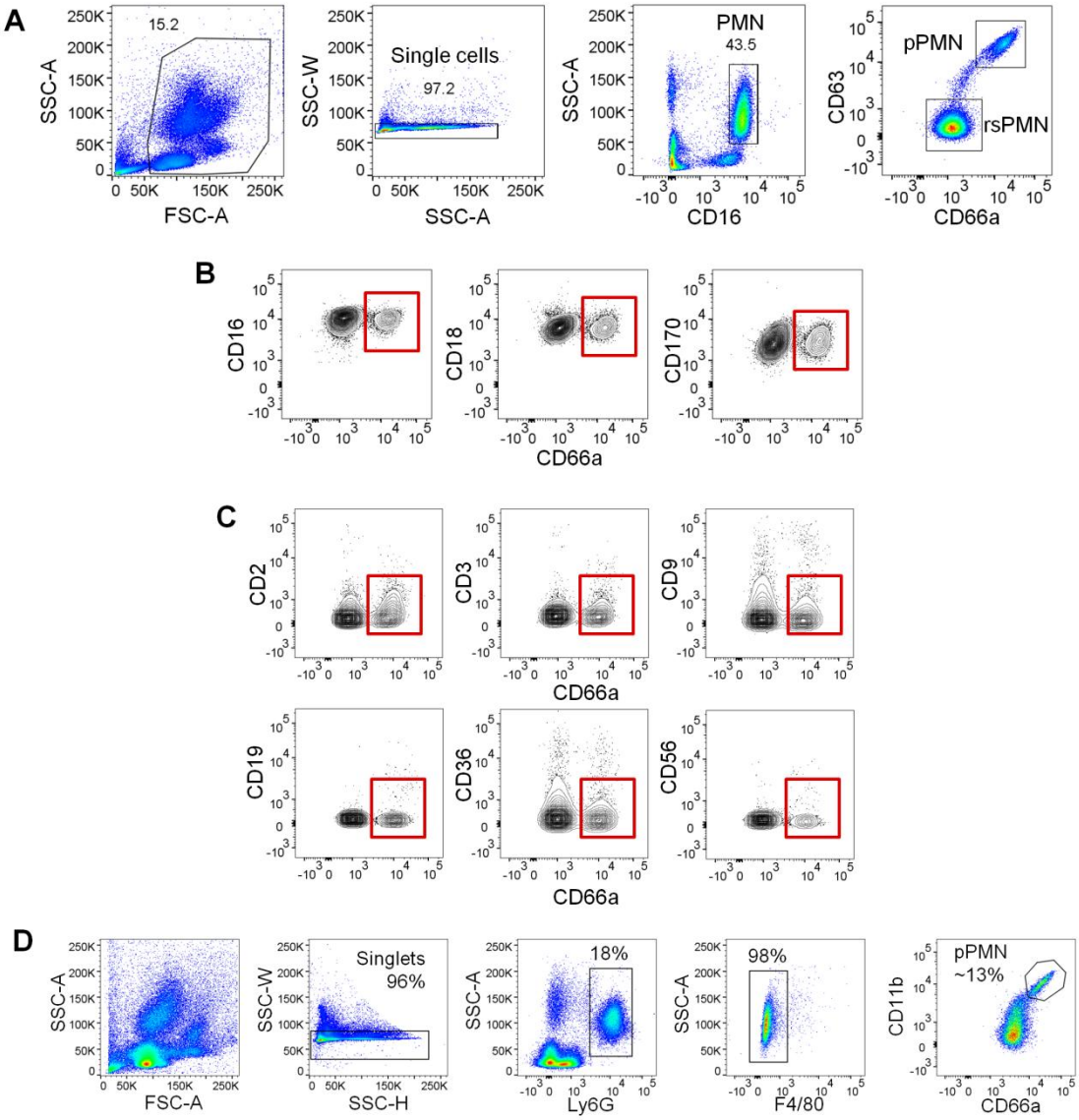
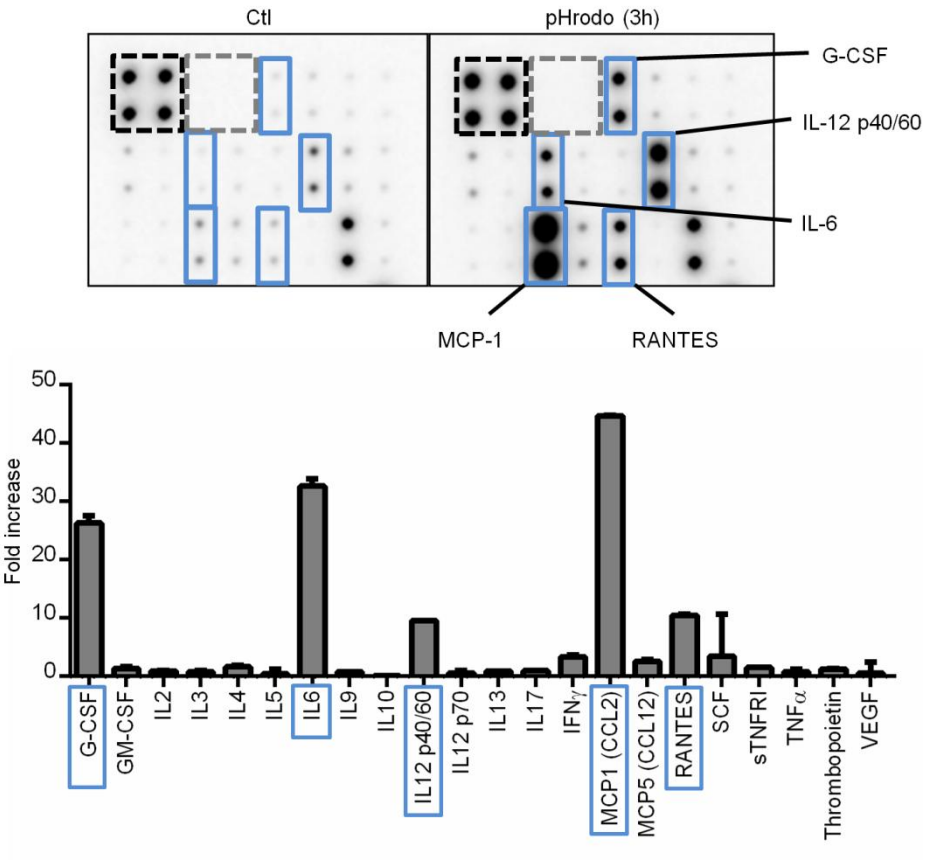
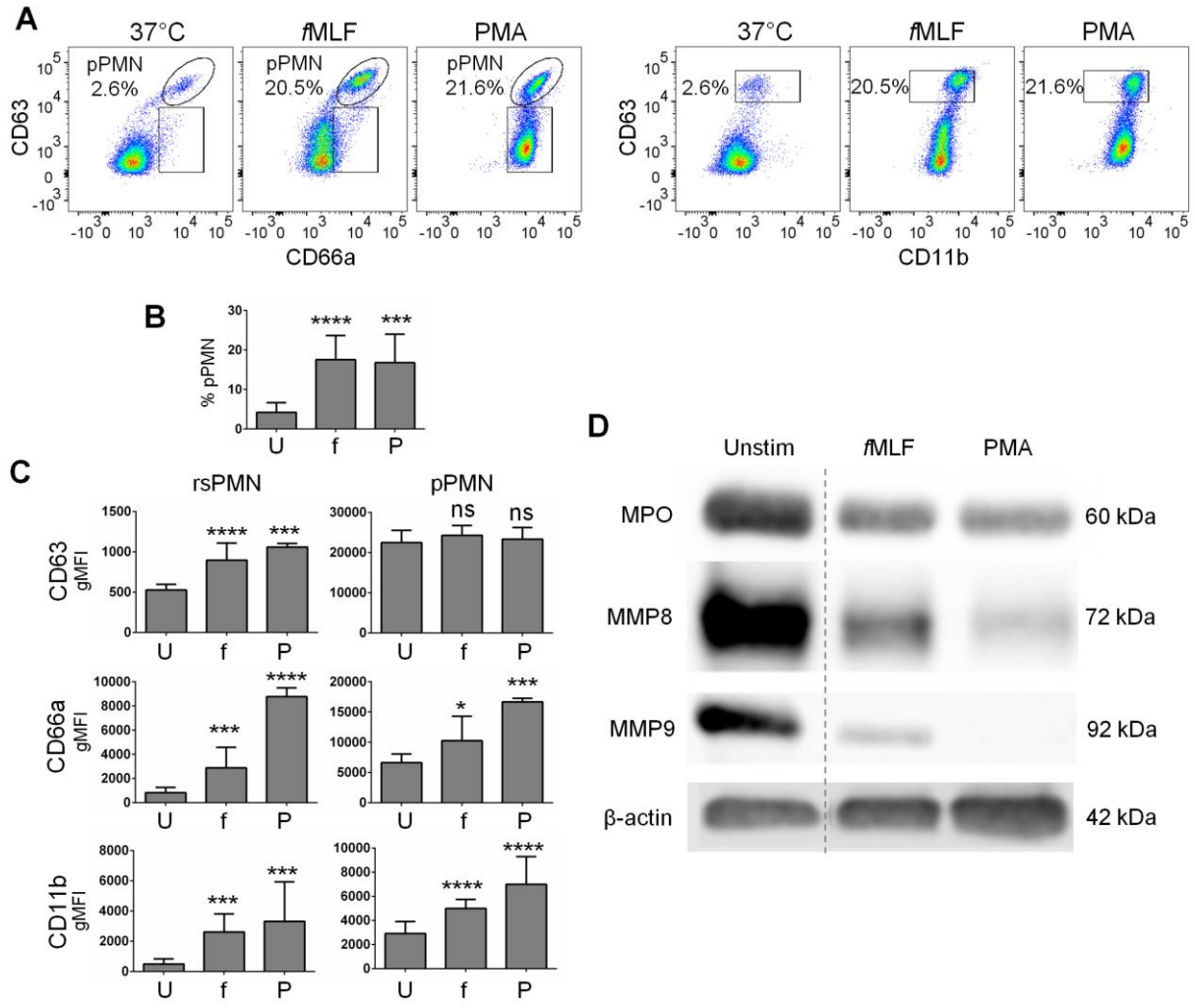
Supplemental Materials



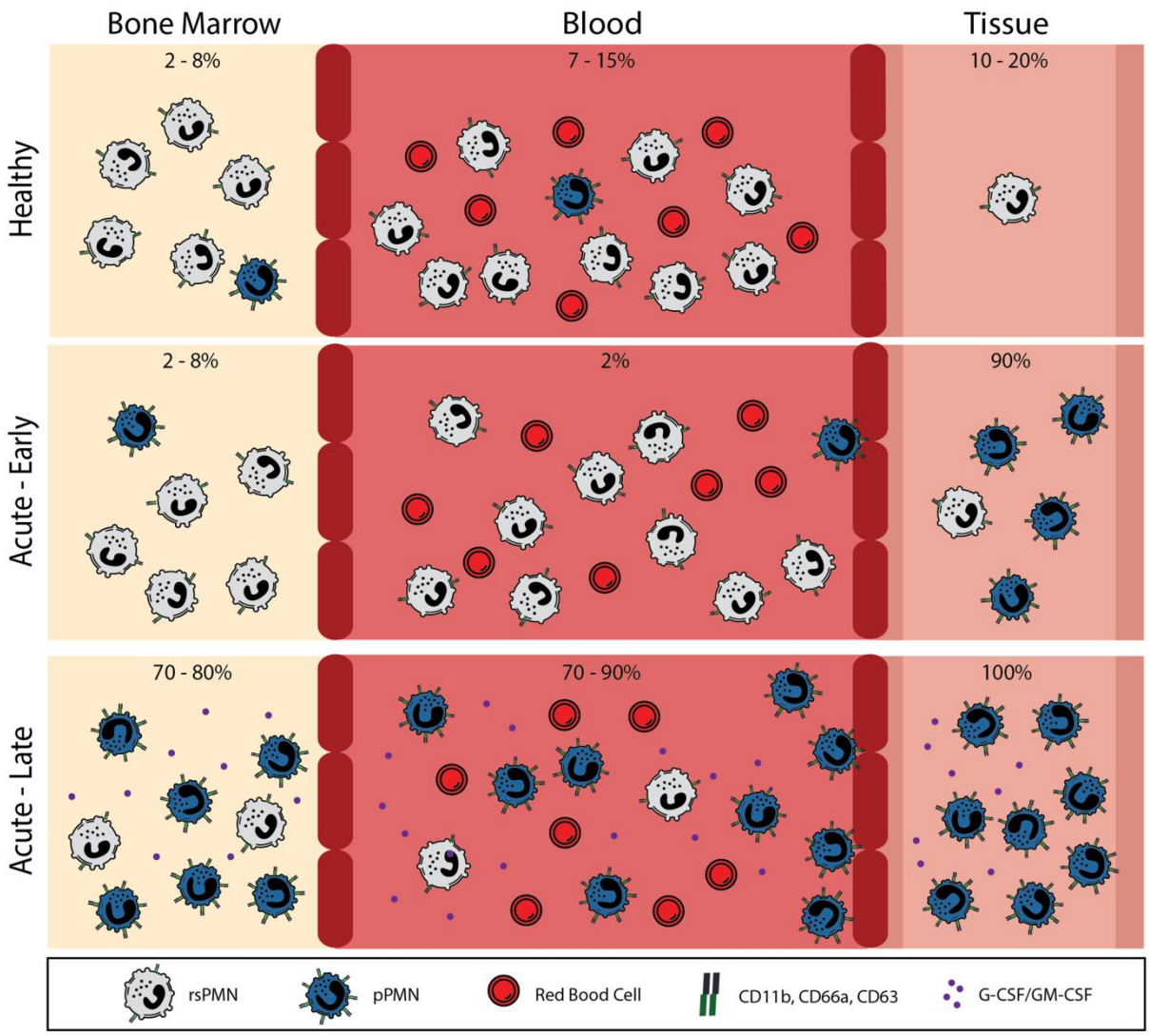
Supplementary Figure 1: Gating strategy for human and mouse blood PMNs. A) PMNs were gated in whole blood leukocyte preparations based on CD16^{high} x SSC-A^{high}. Doublets were excluded based on SSC-A x SSC-W. rsPMNs and pPMNs were gated as CD66a^{low}/CD63^{low} and CD66a^{high}/CD63^{high}, respectively. B) Representative contour plots of CD66a x CD16, CD66a x CD18 and CD66a x CD170 are shown for human blood PMNs. Red boxes indicate pPMNs (CD66a^{high}). C) Human whole blood leukocytes were labeled with markers for specific blood leukocyte subsets as follows: CD2 (T cells, NK cells), CD3 (T cells), CD9 (monocytes, eosinophils, basophils, platelets, T cells), CD19 (B cells), CD36 (monocytes, platelets) and CD56 (NK cells). Representative contour plots are shown for gated PMNs. Red boxes indicate pPMNs (CD66a^{high}). D) Mouse PMNs were gated based on Ly6G^{+ve}/F4/80^{-ve}. Doublets were excluded using SSC-W x SSC-H.



Supplementary Figure 2: Serum cytokines are induced in peritonitis. A neutrophil cytokine antibody array (abcam) was used to determine expression of cytokines and chemokines in serum of control mice (Ctl) and mice subjected to pHrodo *E. coli* BioParticle-induced peritonitis for 3h (pHrodo). Blue boxes indicate factors that were significantly induced. Black and grey boxes indicate positive and negative control wells, respectively. Representative results from two independent experiments are shown. Mean fold-increase ± SD are shown.



Supplementary Figure 3: PMN degranulation coincides with PMN priming. Anti-coagulated human blood was stimulated *in vitro* at 37°C for 30 minutes with PBS, *f*MLF (100 nM) or PMA (200 nM), fixed and analyzed by flow cytometry (A-C) and Western blot (D). A) Representative scatterplots of CD66a x CD63 and CD11b x CD63 expression on gated PMNs are shown. B) The percentage (Mean ± SD) of pPMNs is shown for unstimulated (U), *f*MLP (*f*) stimulated and PMA (P) stimulated blood. Results are compiled from stimulation of blood from at least three volunteers (n ≥ 3). C) Geometric MFI of CD63, CD66a and CD11b expression was determined on gated rsPMNs and pPMNs. Mean (± SD) gMFIs for unstimulated (U), *f*MLP (*f*) stimulated and PMA (P) stimulated rsPMNs and pPMNs are shown (n ≥ 3). Repeated measures ANOVA was performed with Fisher's LSD test for multiple comparisons. All comparisons are relative to unstimulated. *, P ≤ 0.05; ***, P ≤ 0.0005; ****, P ≤ 0.0001. ns = not significant. D) Cell lysates were blotted for markers of specific PMN granules, including MPO (primary granules), MMP8 (secondary granules) and MMP9 (tertiary granules). Lanes that were not relevant were removed from these blots. A dotted line indicates where extra lanes were removed and spliced together.



Supplementary Figure 4: Model of PMN activation subsets in health and disease. The percentage of pPMNs as a fraction of total PMNs is indicated for BM, blood and tissue, during acute inflammation. As opposed to conventional rsPMNs (grey), pPMNs (blue) are primed at steady state and display tissue level expression of several surface markers (CD11b/CD66a/CD63) that allow rapid recruitment in response to tissue injury or infection. pPMN percentages fluctuate in BM, blood and tissue during the transition from health to acute inflammation. pPMNs are initially depleted from blood upon recruitment to the peritoneum, during the early acute phase of tissue inflammation. As inflammation progresses, pPMNs become the predominant subset in BM and blood, and return to baseline levels with resolution of inflammation (not pictured).

Table S1. Mouse CD marker panel for multicolor flow cytometry.

CD marker	Protein - Function	Fluorochrome	Supplier	Clone
Ly6G	Lymphocyte antigen 6 Complex, locus G	PerCP-Cy5.5	BD	1A8
F4/80	EGF-like module-containing mucin-like hormone receptor-like 1	BV421	Biolegend	BM8
CD66a	CEACAM1–Adhesion, degranulation	APC	Ebio	CC1
CD11b	αM-integrin–Adhesion/crawling, complement receptor	Alexa700	Biolegend	M1/70
CD63	signal transduction, adhesion	PE	Biolegend	MVG-2