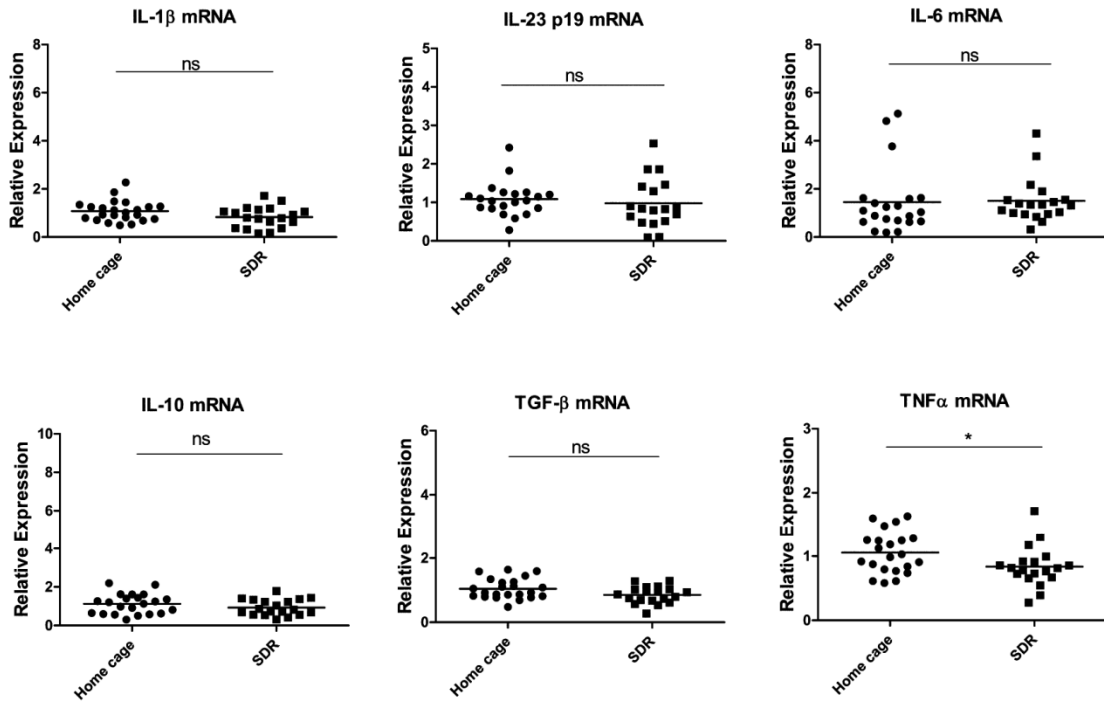


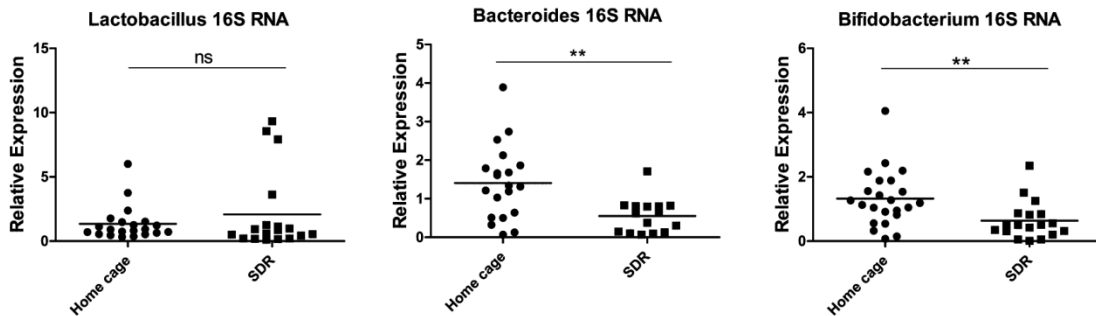
**Supplemental Figure 1. Gating strategy used to define spleen myeloid populations.**

Splenocytes were first gated using FSC and SSC (A) and then gated on the non-lymphocyte cells (B) using antibodies to T cells (CD4 and CD8), B cells (CD45R), and NK cells (NK1.1). The red pulp macrophage was then defined as CD11b<sup>-</sup> F4/80<sup>+</sup> cells (E) and the marginal zone macrophages defined as CD11b<sup>-</sup> SIGNR1<sup>+</sup> cells (F). Isotype controls for gating red pulp macrophages and marginal zone macrophages are shown in G and H. To define the monocyte and neutrophils populations, the non-lymphocyte population was first gated on CD11b<sup>+</sup> myeloid cells (C) and then Ly6C and Ly6G antibodies used to define the neutrophil ( Ly6C<sup>interm</sup> Ly6G<sup>+</sup>), classical monocytes ( Ly6C<sup>high</sup> Ly6G<sup>-</sup>) and non-classical monocytes (Ly6C<sup>low</sup> L6G<sup>-</sup>).

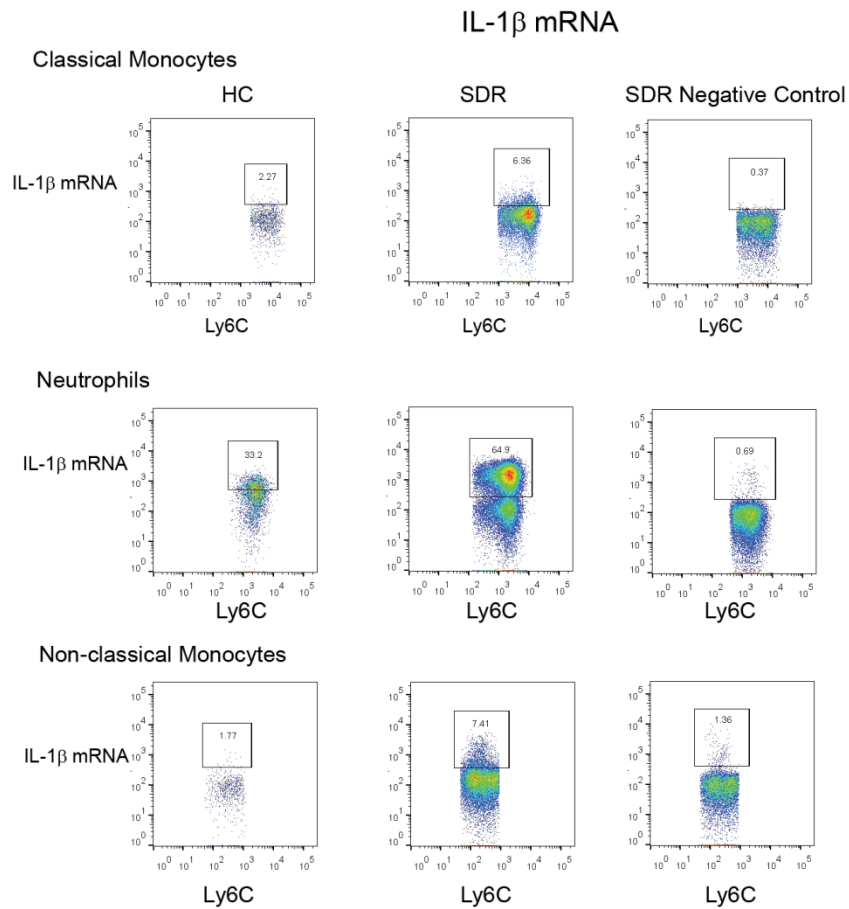
A.



B.

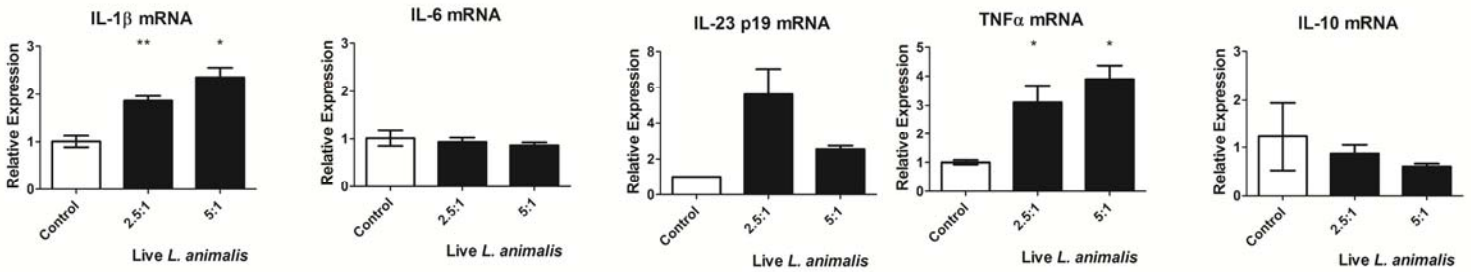


**Supplemental Figure 2. Cytokine mRNA and Bacterial 16S rRNA levels in mesenteric lymph nodes from home cage and SDR stressed mice.** RNA was isolated from spleens of SDR stressed and home cage control mice. Cytokine mRNA levels (A) and bacterial 16S rRNA levels (B) were analyzed by qRT-PCR. Relative mRNA expression was determined using GAPDH mRNA as the normalizer. Expression levels of individual home cage and SDR mice were determined relative to the mean  $\Delta$ CT of the home cage mice. Statistical analysis was performed by Student t test with Welch's correction for unequal variance. N=15-23 mice per group. \* p<0.05, \*\* p<0.01

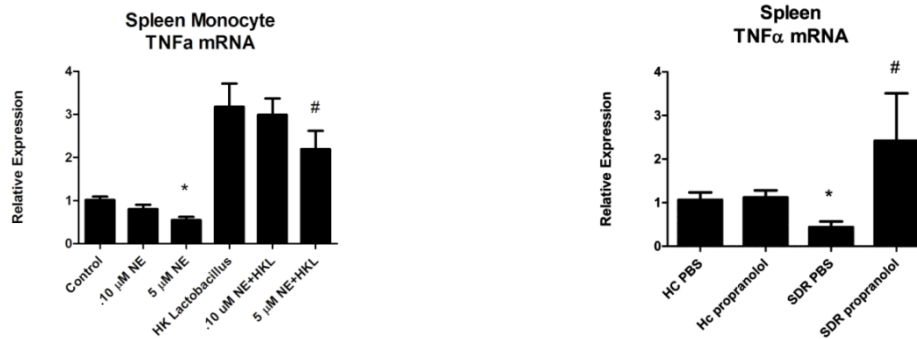


**Supplemental Figure 3. RNA flow cytometry gating strategy used to define myeloid population positive for IL-1 $\beta$  mRNA.** Cell surface staining and gating strategy described in supplemental figure 1 was used to define the classical monocytes (CD11b<sup>+</sup> Ly6C<sup>high</sup> Ly6G<sup>-</sup>), neutrophils (CD11b<sup>+</sup> Ly6C<sup>interm</sup> Ly6G<sup>+</sup>), and non-classical monocytes (CD11b<sup>+</sup> Ly6C<sup>low</sup> Ly6G<sup>-</sup>). To detect IL-1 $\beta$  mRNA, cells were hybridized with a Alexa-647 probe set specific for IL-1 $\beta$  following the protocol provided by manufacturer. Gates for positive cells were set using negative control samples (no probe). Representative gates for each of populations and negative controls are shown for home cage control and SDR mice.

A.



B.



**Supplemental Figure 4. Activation of spleen myeloid cells by *L. animalis*.**

**A.** CD11b<sup>+</sup> myeloid cells were isolated from mouse spleen cells by magnetic beads and treated with live *L. animalis* for 3 hrs. RNA was isolated from the non-adherent neutrophils and monocytes and analyzed by qRT-PCR. The relative mRNA expression was determined using  $\beta$ -actin mRNA as the normalizer. Data represents means  $\pm$ SEM of 4 replicate wells. Statistical analysis was performed by Student t test. \*  $p < 0.05$ , \*\*  $p < 0.01$

**B. (Left).** Isolated spleen monocytes were incubated with 5  $\mu$ M and 10  $\mu$ M norepinephrine (NE) for 30 minutes prior to stimulation with heat-killed *Lactobacillus animalis* at 5 bacteria/cell for 6 hrs. Cytokine mRNA levels were analyzed by qRT-PCR and the relative mRNA expression was determined using  $\beta$ -actin mRNA as the normalizer. Statistical analysis was performed by Student t test with Welch's correction for unequal variance. Data represents means  $\pm$ SEM of 6 replicate wells from two experiments. \*  $p < 0.05$ , #  $p = 0.076$ ,

**(Right).** Home cage (HC) and SDR stressed mice were injected with PBS or propranolol (10mg/kg) at 30 minutes prior to each cycle of SDR. After 6 cycles of SDR, spleens were isolated and TNF $\alpha$  mRNA levels determined by qRT-PCR. Relative mRNA expression was determined using GAPDH mRNA as the normalizer. Expression levels of individual home cage and SDR mice were determined relative to the mean  $\Delta$ CT of the home cage mice. Statistical analysis was performed by Student t test. N=5-6 mice per group \*  $p < 0.05$ , #  $p = 0.054$