SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. CO ameliorates histologic colitis in TCRa d **mice.** TCRa d mice were housed in ambient air or a chamber maintaining a constant concentration of CO at 250 ppm (*n*=10 each) from 12 to 16 weeks of age. Representative hematoxylin and eosin staining and depiction of histologic colitis of *TCRa* colonic tissues. Top panel, ambient air; Bottom panel, CO-treated (magnification at 40X). Colons of inflamed 16 week old TCRa mice housed in ambient air have elongated crypts, thickened mucosal and submucosal layers with transmural inflammation compared to CO exposed mice. Infiltrates are predominantly lymphocytes and monocytes with few neutrophils.

Supplemental Figure 2. Surface marker expression in mononuclear cell

populations from TCRα^{-/-}**mice.** Colonic LPMCs from ALF186 (30 mg/kg) and iALF186 (30 mg/kg) treated TCRα^{-/-}mice were labeled with antibodies against **(A)** macrophage activation markers, F4/80, CD14, CD80 in CD11b⁺ gated LPMCs **(B)** CD1d expression for B cells gated on B220⁺ LPMCs. Dead cells were excluded with propidium iodide staining. Representative staining patterns of LPMCs harvested from 4 individual mice is shown. **(C)** Splenocytes from WT and TCRα^{-/-} mice were isolated and pooled (n=3) and were labeled with antibodies against CD4 followed by intracellular cytokine staining for FoxP3. Representative pattern of splenocyte staining from 3 individual mice is shown.

Supplemental Figure 3. Hmox and II10 induction in LPMC from CORM-186

treated WT mice. LPMCs were isolated from colons of WT mice treated with inactive CO-releasing molecule 186 (iALF186, n=4, white bars) and ALF186 (n=4, black bars). LPMCs were further separated into CD11b and CD11b⁺ cells and analyzed for **(A)** *Hmox1* **(B)** and *II10* expression by real-time RT-PCR. Results were normalized to β-actin. Error bars represent mean<u>+</u>SEM triplicate cultures from pooled LPMCs from

four mice per group. p<0.05 vs. iALF186 treated CD11b⁺ LPMCs.

FIGURE 4. Regulation of IL-10 in macrophages is MyD88 dependent. WT and

MYD88^{-/-}BMMs were stimulated with LPS (100 ng/mL), CpG (1 μ M), SbLP (100 ng/mL), and flagellin (10 ng/mL) for 24 hours. IL-10 protein was analyzed by ELISA. Data is representative of 3 independent experiments. Error bars represent mean<u>+</u>SEM from 3 independent experiments. p<0.05 vs. WT simulated BMMs.



Supplemental Figure 1









В



Supplemental Figure 3



Supplemental Figure 4