Supplement Figure 1

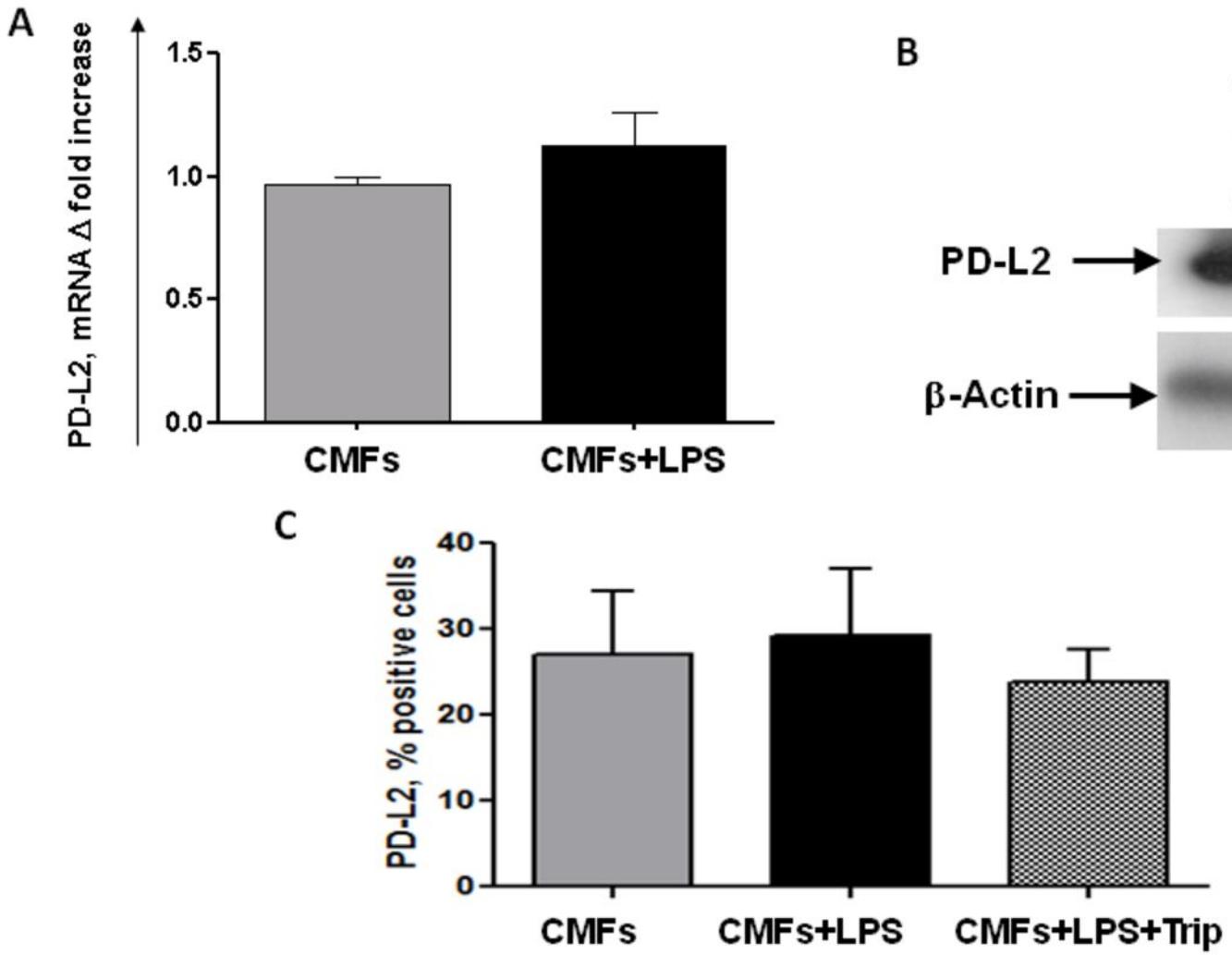

Figure S1. TLR4 stimulation on normal human CMFs did not upregulate PD-L2 expression. (A) Stimulation of cultured CMFs with 1 µg/mL of LPS (4h) does not significantly upregulate PD-L2 mRNA expression (real time RT-PCR analysis). The means ± SE are shown as the results of duplicates of four representative experiments, n=4, $p > 0.05$. (B) Western blotting using Abs specific for PD-L2 on total protein extract isolated from CMFs treated or not with LPS (1 mg/mL) in presence/absence of triptolide (Trip, 20 ng/mL). Under denaturing conditions, the PD-L2 protein migrates as subunits of ~54 kDa. Probing with anti-b-actin mAbs were used as a loading control. One representative experiment of five is shown for CMF isolates from normal mucosa. (C) Surface expression of PD-L2 on CMF treated with or without 1 µg/mL of LPS (24h)) in presence/absence of triptolide (Trip, 20 ng/mL), flow cytometry analysis values are expressed as percentage of positive cells± SE of duplicates CMF cultures isolated from four donors (n= 4), $p > 0.05$.

Supplement Figure 2

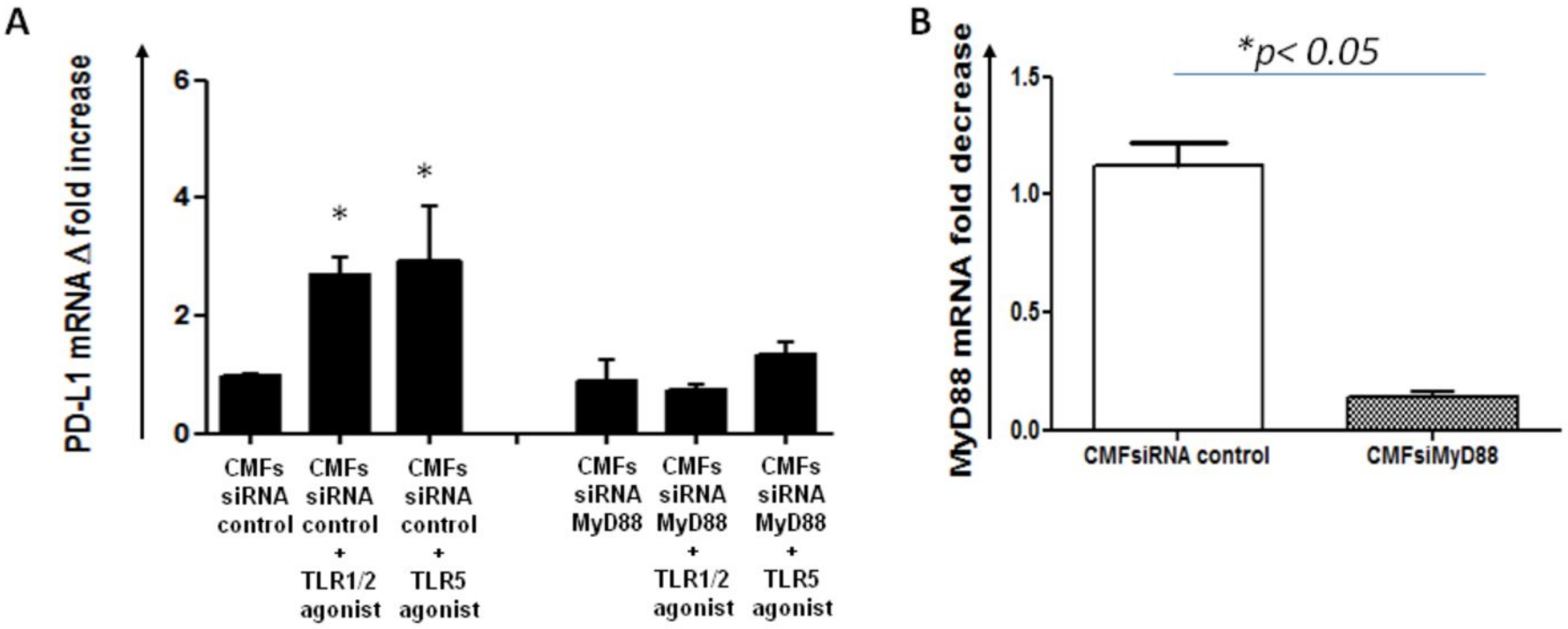

Figure S2. Silencing of the *myd88* gene in CMFs abrogates TLR1/2 and TLR5-mediated increase in PD-L1 mRNA expression. (A) Primary CMFs isolated from normal colonic mucosa were transfected with MyD88 siRNA or control siRNA seven days prior exposure to the TLR1/2 agonist (Pam3CSK4, 100 ng/mL) or TLR5 agonist (*S. typhimurium* flagellin, 100 ng/mL) for 4 h. PD-L1 expression was analyzed using real-time RT-PCR. The means ± SE, n=3; *p<0.05. (**B**) MyD88 mRNA expression was efficiently silenced by using MyD88 specific siRNA, but not siRNA control, real time RT-PCR analysis. The means ± SE are shown as the results of duplicates of four representative experiments, n=3, *p< 0.05.

Supplement Figure 3

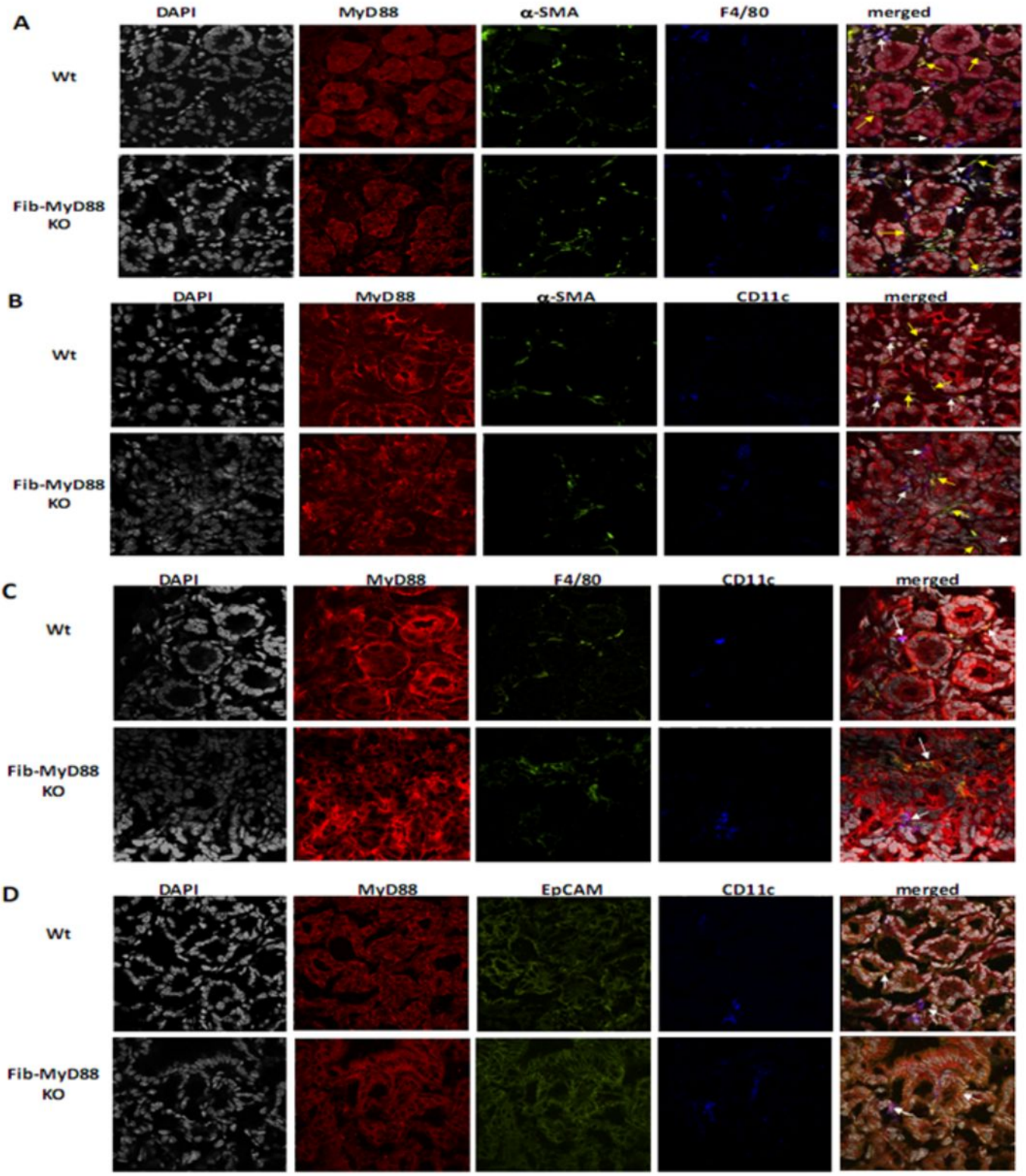

Figure S3. In colonic mucosa of Fib-MyD88 KO mice MyD88 expression is impaired in α-SMA$^+$ CMFs, but not in other TLR expressing cells. Expression of MyD88 was analyzed by immunostaining followed by confocal microscopy. Frozen mucosal colonic tissue sections from wild type (wt) and fibroblast specific MyD88 knockout (Fib-MyD88 KO) mice were analyzed for expression of MyD88 (in red) along with (**A**) α-SMA$^+$ CMFs (in green) and F4/80$^+$ macrophages (in blue); (**B**) α-SMA$^+$ CMFs (in green) and CD11c$^+$ dendritic cells (in blue); (**C**) F4/80$^+$ macrophages (in green) and CD11c$^+$ dendritic cells (in blue); (**D**) EpCAM$^+$ epithelial cells (in green) and CD11c$^+$ dendritic cells (in blue). Formation of the yellow-orange between green and red colors, as well purple-pink between blue and red colors on merged images indicates co-localization between MyD88 and studied cell population. Yellow arrows pointing out CMF and white arrow pointing out other studied TLR expressing cells on merged images. A representative sections of data from seven wild-type and eight Fib-MyD88 KO mice are shown.

Supplement Figure 4

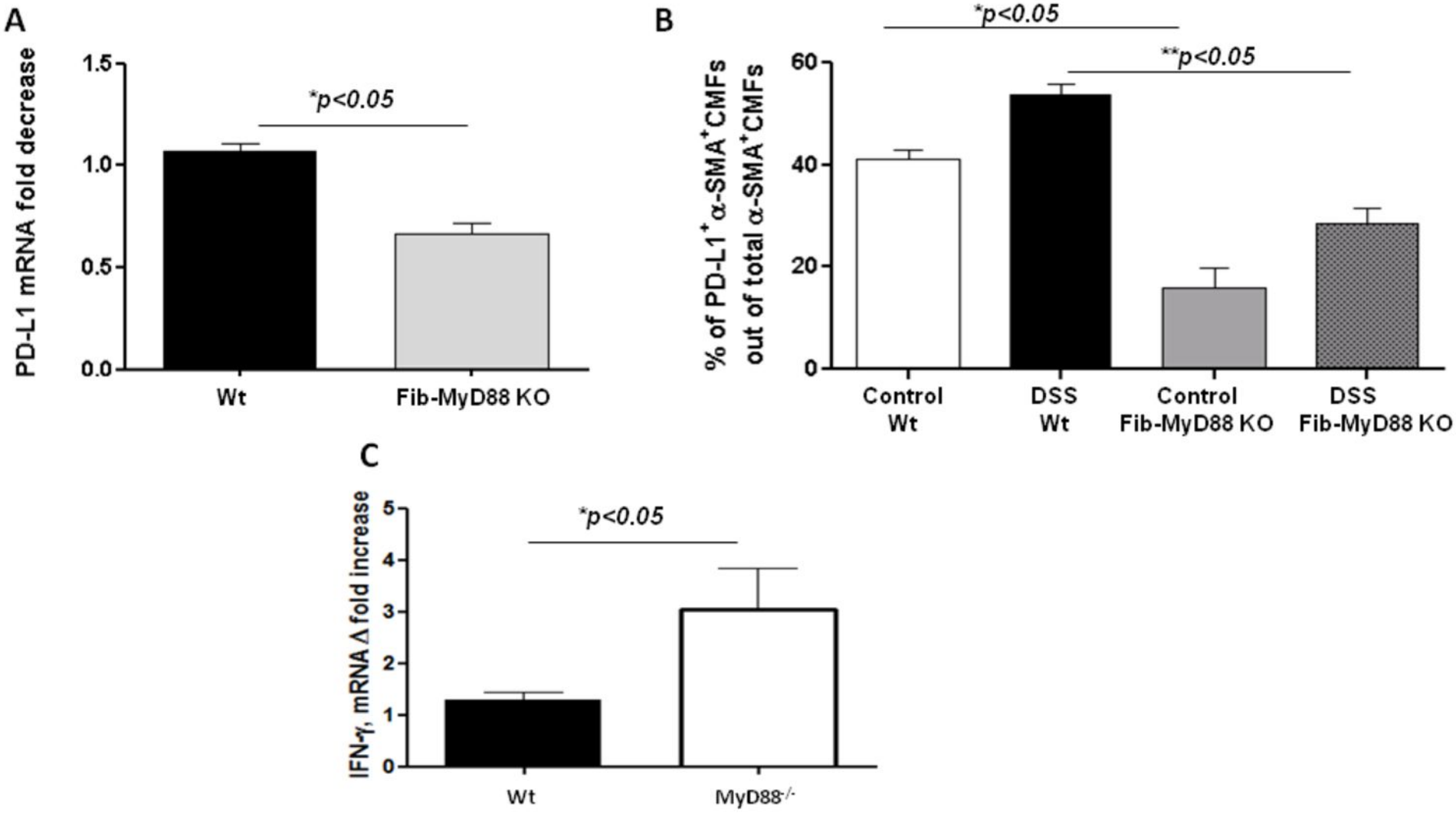

Figure S4. (A) Expression of PD-L1 is reduced in colonic mucosa from mice with impaired MyD88 signaling in CMFs (Fib-MyD88 KO). PD-L1 mRNA expression was analyzed using real-time RT-PCR. The means ± SE, n=9 animal per group, *p<0.05. (B) Freshly digested murine colonic mucosal cell preparations were stained with α-SMA and PD-L1 and were analyzed by flow cytometry. Percentages of α-SMA$^+$ PD-L1$^+$ cells (activated CMFs) out of α-SMA$^+$ CMFs were calculated. Isotype controls were included for each staining, n= 5 animals per group were analyzed, *p<0.05. (C) Expression of IFN-γ is increased in colonic mucosa from mice depleted of MyD88 signaling (MyD88$^{-/-}$). IFN-γ mRNA expression was analyzed using real-time RT-PCR. The means ± SE, n=6 animal per group, *p<0.05.