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Supplemental Information

Innate Sensing through Mesenchymal TLR4/MyD88 Signals Promotes Spontaneous Intestinal Tumorigenesis

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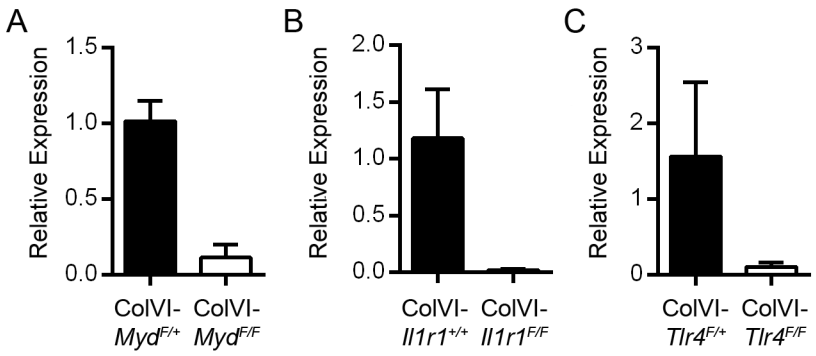

Figure S1. Deletion efficiency of *Myd88*, *Il1r1* and *Tlr4*. Related to Figures 1 and 3. qRT-PCR analysis of isolated GFP$^+$ cells from (A) ColVIcre-*Myd88*$^{F/+}$-mTmG and ColVIcre-*Myd88*$^{F/F}$-mTmG, (B) ColVIcre-*Tlr4*$^{F/+}$-mTmG and ColVIcre-*Tlr4*$^{F/F}$-mTmG and (C) ColVIcre-*Il1r1*$^{+/+}$-mTmG and ColVIcre-*Tlr4*$^{F/F}$-mTmG. B2m was used for normalization (n=3).

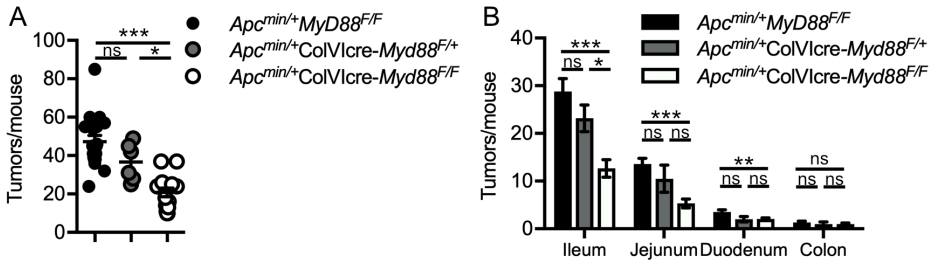

Figure S2. Cre in the *Apc*$^{min/+}$-*Myd88*^{IMCko} does not affect tumor development. Related to Figure 1. (A) Total number of tumors per mouse and (B) number of tumors per intestinal part in 4-month old *Apc*$^{min/+}$ColVIcre-*Myd88*$^{F/F}$ (n=15), *Apc*$^{min/+}$ColVIcre-*Myd88*$^{F/+}$ (n=6) mice and their littermate controls (n=18). Data represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ns = not significant.

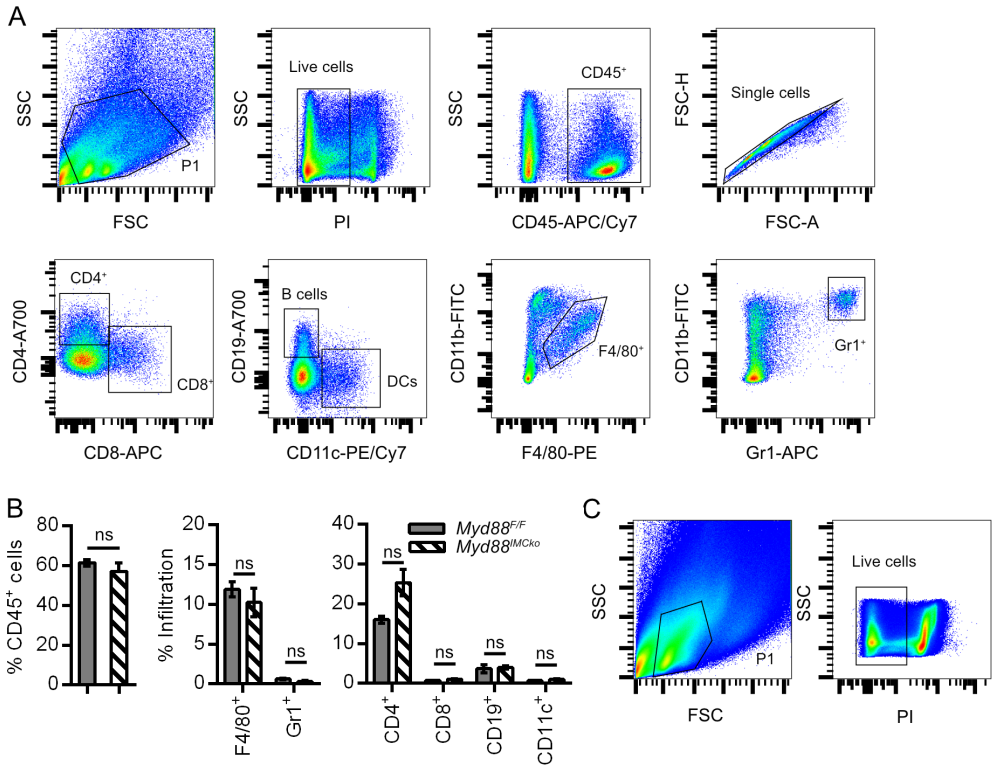

Figure S3. FACS gating strategies. Related to Figure 2. (A) FACS gating strategy for measuring inflammatory cell infiltration in the small intestine and tumors. The cell suspension was gated for live CD45$^+$. In this CD45$^+$ population, we plotted CD4 and CD8, for measuring the respective T cell populations, CD19 and CD11c for B and dendritic cell and CD11b with F4/80 and Gr1 to identify double positive CD11b$^+$F4/80$^+$ and CD11b$^+$Gr1$^+$ cells. (B) Infiltration of CD45$^+$ cells, CD11b$^+$F4/80$^+$ macrophages, CD11b$^+$Gr1$^+$ neutrophils, CD4$^+$ T cells, CD8$^+$ T cells, CD19$^+$ B cells and CD11c$^+$ dendritic cells in intestinal tissue from *Myd88*$^{F/F}$ and *Myd88*^{IMCko} mice or 3-month old *Apc*$^{min/+}$*Myd88*$^{F/F}$ and *Apc*$^{min/+}$*Myd88*^{IMCko} mice (n=4-5), quantified by FACS analysis. Data represent mean ± SEM. ns = not significant. (C) Gating strategy for Figure 2H. Single-cell suspensions from tumors were gated for live cells using Propidium Iodide (PI) (n=3 mice).

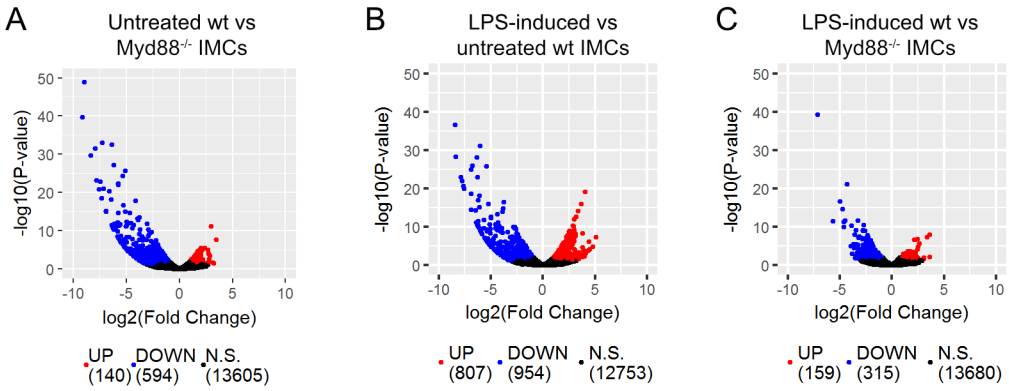

Figure S4. Comparisons between samples used for RNA sequencing. Related to Figure 4. (A) Volcano plot of deregulated genes between untreated wt and MyD88$^{-/-}$ IMCs. (B) Volcano plot of deregulated genes between untreated and LPS-treated wt IMCs. (C) Volcano plot of deregulated genes between LPS-treated wt and MyD88$^{-/-}$ IMCs. Not significant genes are depicted with black color (N.S), significant up regulated genes (p-value < 0.05, log2FC > 1) with red and significant down regulated genes (p-value < 0.05, log2FC < -1) with blue.

Table S1. List of primers for qRT-PCR. Related to STAR Methods.

Gene	Primer (5'-3')	Size (bp)	Anneal. Tem.	Reference
Ptgs2	F: TGAGCACAGGATTTGACCAG R: CCTTGAAGTGGGTCAGGATG	150	58	(Salcedo et al., 2010)
Il6	F: GTTCTCTGGGAAATCGTGGA R: TCCAGTTTGGTAGCATCCATC	138	59	(Salcedo et al., 2010)
Il11	F: AACTGTGTTTGTCGCCTGGT R: CGTCAGCTGGGAATTTGTCT	199	59	(Salcedo et al., 2010)
Cd44	F: TTATCCGGAGCACCTTGGCCAC R: TGCACTCGTTGTGGGCTCCTGAG	143	59	
Tnf	F: CACGCTCTTCTGTCTACTGA R: ATCTGAGTGTGAGGGTCTGG	110	55	
Mmp7	F: GCTGCCACCCATGAATTTGGCC R: GGACCCAGTGAGTGCAGACCG	209	59	
Cxcl1	F: CGCACGTGTTGACGCTTCCC R: TCCCGAGCGAGACGAGACCA	105	59	
Igf1	F: GGGAGATGCAAAGGCCTCCCC R: ACCAGGACTCCCAAATCCCTAGCC	142	56	
Igfbp5	F: ACGGCGAGCAAACCAAGATA R: GAGGGCTTACACTGCTTTCT	382	55	(Ding et al., 2016)
Mmp10	F: CACAAGCCCAGCTAACTTCC R: TTTGTCTGGGGTCTCAGGTC	136	59	(Salcedo et al., 2010)
Myd88	F: CTAGGACAAACGCCGGAACT R: ATTAGCTCGCTGGCAATGGA	176	60	
Tlr4	F: TTCAGAACTTCAGTGGCTGGAT R: GTCTCCACAGCCACCAGATT	177	58	
Il1r1	F: ACAACGTGAGCTTCTTCGGA R: GCTTCCCCCGGAACGTATAG	108	60	
B2m	F: TTCTGGTGCTTGTCTCACTGA R: CAGTATGTTCGGCTTCCCATTC	104	58	
Hprt	F: TGCCGAGGATTTGGAAAAAGTG R: CACAGAGGGCCACAATGTGATG	116	55	