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Supplemental Information

Myeloid-Derived Suppressor Cells Produce IL-10 to Elicit DNMT3b-Dependent IRF8 Silencing to Promote Colitis-Associated Colon Tumorigenesis

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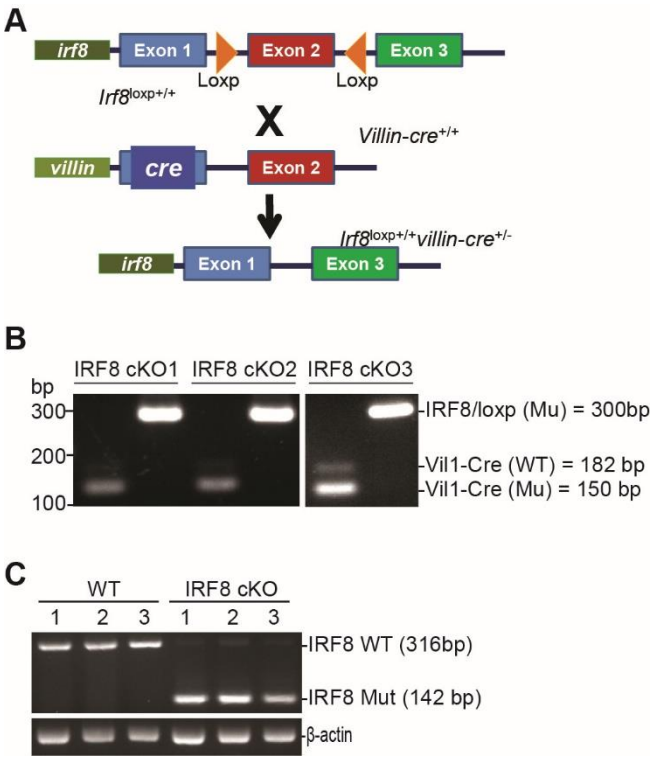

Figure S1. Creation of the IRF8 cKO mouse. **Related to Figures 1 and 2.** (A) Diagram of creation of mice with IRF8 deficiency in the colonic epithelial cells (termed IRF8 cKO mouse). (B) DNA was extracted from mouse tail snaps and analyzed by PCR. The genotypes of 3 homozygous IRF8 cKO mice are shown. (C) RNA was extracted from colon tissues of WT (n=3) and IRF8 cKO (n=3) mice, and analyzed by RT-PCR using a PCR primer pair flanking the exon 2 of *irf8* cDNA. The WT IRF8 (IRF8 WT) and truncated mutant IRF8 (IRF8 Mut) are indicated at the right.

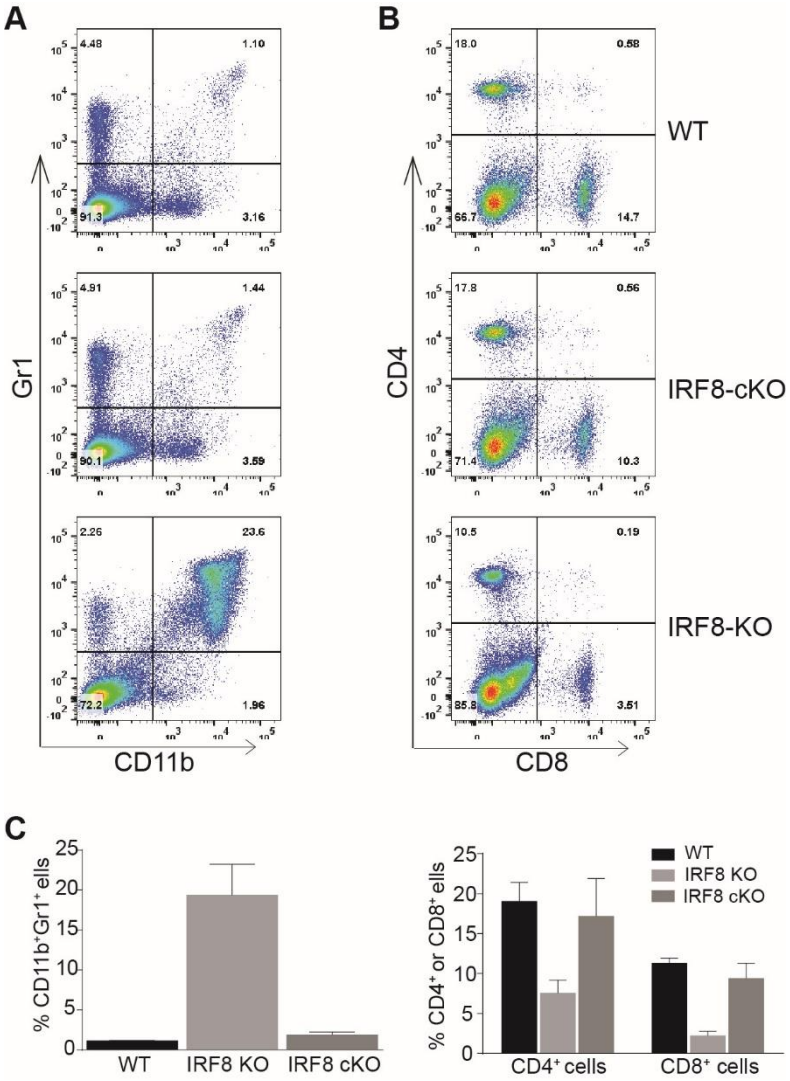

Figure S2. MDSC and T cell profiles in WT and IRF8-deficient mice. **Related to Figure 1.** (A & B). Cells were prepared from spleens of aged matched WT (n=3), IRF8KO (n=3) and IRF8cKO (n=3) mice. Cells were stained with CD11b- and Gr1-specific (A), and CD4- and CD8-specific (B) antibodies and analyzed by flow cytometry. Shown are representative flow cytometry plots. (C) Quantification of percentages of $CD11b^+Gr1^+$, $CD4^+$ and $CD8^+$ cells as shown in A and B. Column: mean; Bar: SD.

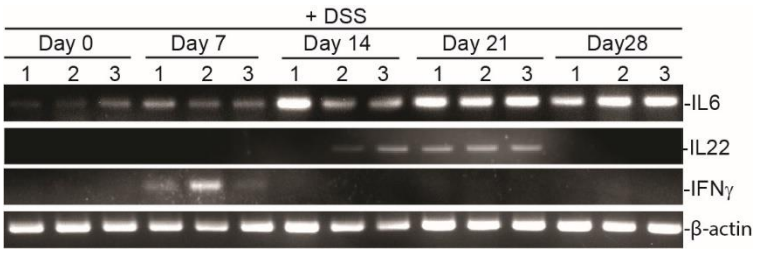

Figure S3. Cytokine expression levels in DSS-treated mouse colon. Related to Figures 4 and 5. WT C57BL/6 mice were treated with the 2% DSS-water cycle as described in the experimental procedures. Colon tissues were collected from mice at the indicated time points and analyzed by RT-PCR for IL6, IL22 and IFNγ mRNA levels. β-actin was used as normalization control.

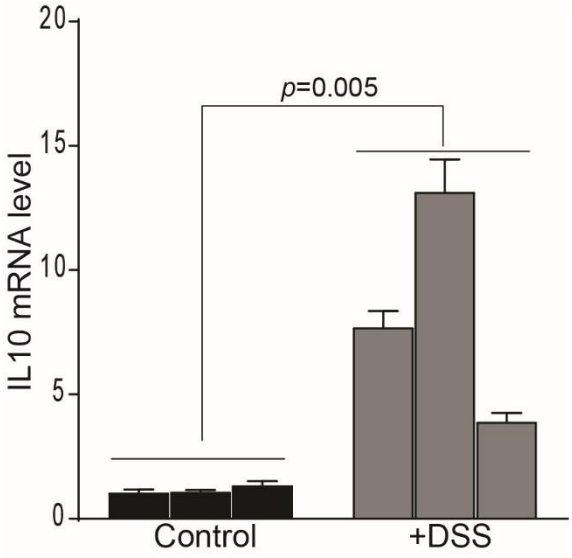

Figure S4. Inflammation induces IL10 up-regulation in colon. Related to Figure 5. C57BL/6 mice were treated with 2% DSS as described in the experimental procedures. $CD11b^{+}Gr1^{+}$ MDSCs were purified from mouse spleens at day 21 and analyzed by qPCR for IL10 mRNA level with β-action as internal control.

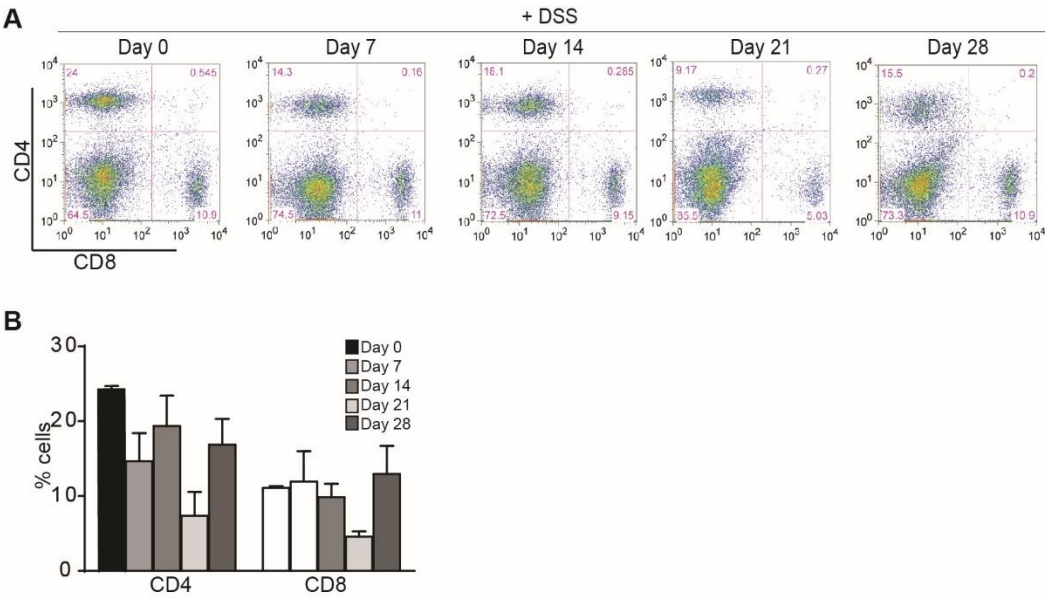

Figure S5. Effects of chronic inflammation on T cell profile. **A. Related to Figure 5**. (A) WT mice were treated with DSS as described in the experimental procedures and analyzed for $CD4^+$ and $CD8^+$ T cells in the spleens at the indicated time. Shown are representative results of one of three mice. (B) Quantification of percentages of $CD4^+$ and $CD8^+$ cells as shown in A. Column: mean; Bar: SD.

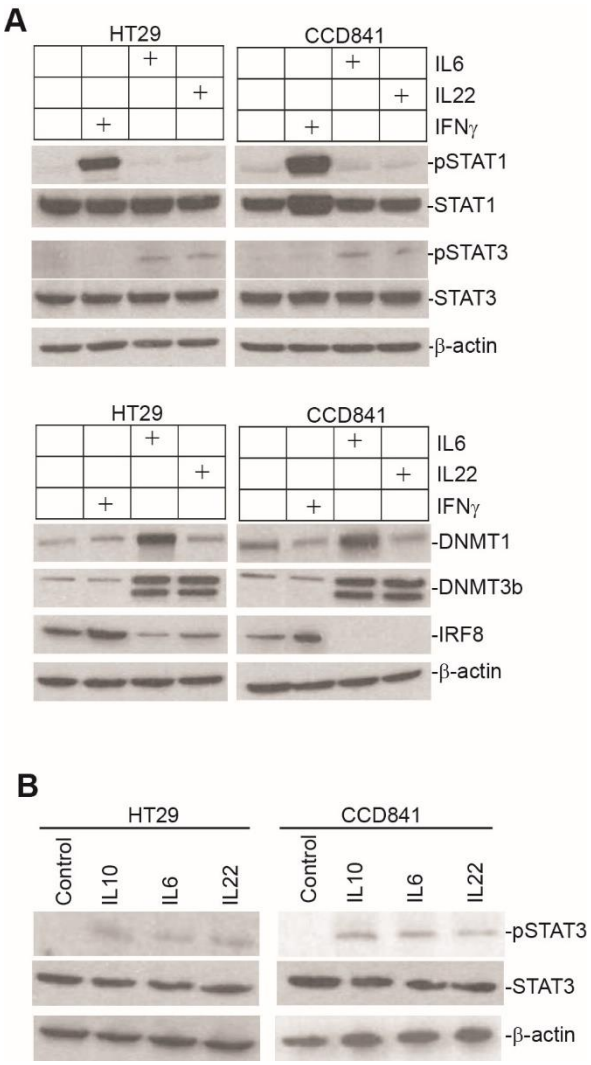

Figure S6. Cytokine regulation of gene expression in normal colon epithelial and colon carcinoma cells in vitro. Related to Figures 6 and 7. (A) HT29 and CCD841 cells were treated with recombinant IL6, IL22 and IFNγ for 24 hours and analyzed for the indicated proteins by Western blotting. (B) HT29 and CCD841 cells were treated with recombinant IL10, IL6, and IL22 for 2 hours and analyzed for the indicated proteins by Western blotting.

Table S1. Oligonucleotide Sequences*. Related to Figures 1, 2, 3, 4, 5, 6, and 7.

Oligo Name	Sequence (5'-3')	Application
mIRF8-F	GATCGAACAGATCGACAGCA	RT-PCR
mIRF8-B	GCTGGTTCAGCTTTGTCTCC	
mDNMT1-F	TTGATGGTGGCGAGAAGGTG	RT-PCR
mDNMT1-B	AATGGTAGAAGGAGGAACAGTGGTC	
mDNMT3A-F	CCAGCCAAGAAACCCAGAAAG	RT-PCR
mDNMT3A-B	AGCAATCCCATCAAAGAGAGACAG	
mDNMT3b-F	CAAACCCAACAAGAAGCAACCAG	RT-PCR
mDNMT3b-B	CCAGACACTCCACACAGAAGCATC	
mCD11b-F	GCAGTCATCTTGAGGAACCGTG	RT-PCR
mCD11b-B	AGTTGGTATTGCCATCAGCGTC	
mIL10-F	GCTGGACAACATACTGCTAACCGAC	RT-PCR
mIL10-B	CTTGCTCTTATTTTCACAGGGGAG	
mIL6-F	TCTGGGAAATCGTGGAAATGAG	RT-PCR
mIL6-B	TCTCTGAAGGACTCTGGCTTTGTC	
mIL22-F	CGTCAACCGCACCTTTATGC	RT-PCR
mIL22-B	TTCTGGATGTTCTGGTCGTCACCG	
mIFNG-F	CCATCAGCAACAACATAAGCGTC	RT-PCR
mIFNG-B	TCTCTTCCCCACCCCGAATCAGCAG	
mKi67-F	CATCATTGACCGCTCCTTTAGG	RT-PCR
mKi67-B	TGTTTCTGCCAGTGTGCTGTTCTAC	
mβ-actin-F	ATTGTTACCAACTGGGACGACATG	RT-PCR
mβ-actin-B	CTTCATGAGGTAGTCTGTCAGGTC	
mβ-actin-Q-F	CTGGCACCACACCTTCTACAATG	qPCR
mβ-actin-Q-B	GGGTCATCTTTTCACGGTTGG	
mIRF8exon1F	GCGCGGGCAGCGTGGGAACCGGCG	RT-PCR: IRF8 cKO
mIRF8exon3B	GTCACTTCTTCAAAATCTGGGCTC	
mIRF8-CpGI-F	GGGATAGAGGTTTTTTAAATTTGAA	BS-PCR of *irf8* promoter CpG island
mIRF8-CpGI-B	AACAACCAAAACAAACACCTACTAAC	
mIRF8-MSP-U-F	TTTTGGGGTAGTTTTTTTTTTTGTTGTTTTT	MS-PCR of *irf8* promoter CpG ialand
mIRF8-MSP-U-B	TCCCACACACAAAACAACAATCACACA	
mIRF8-MSP-M-F	TGGGGTAGTTTTTTTTTTCGTCGTTTTC	MS-PCR of *irf8* promoter CpG ialand
mIRF8-MSP-M-B	GCGCGCAAAACGACGATCGCGCG	
Tg-Vil1-Cre-Common	GCCTTCTCCTCTAGGCTCGT	Genotyping *villin-cre* mouse
Tg-Vil1-Cre-Wild	TATAGGGCAGAGCTGGAGGA	
Tg-Vil1-Cre-Mutant	AGGCAAATTTTGGTGTACGG	
IRF8-Floxp-F	TTGGGGATTTCCAGGCTGTTCTA	Genotyping *irf8 floxp* mouse
IRF8-Floxp-B	CACAGGGAGTCCCTCTTACAAT	
hDNMT1-XhoI-F	GTA*CTCGAG* TCCAGGCACACACTACCATTC	PGL3-Cloning
hDNMT1-HindIII-B	CAT*AAGCTT* CGGGTTCAAGCGATTCTTCTG	
hDNMT3b-KpnI-F	ACTC*GGTACC* TGTAAAGAAAGGCTGGACGGC	PGL3-Cloning
hDNMT3b-HindIII-B	CAT*AAGCTT* AAATAAACAGGTCTATGGGGAGGG	
hDNMT3b-ChIP1-F	TTTGTGCCAGAAAGCCAA	ChIP
hDNMT3b-ChIP1-B	CCAGGTGAGTAAATGAGTGAAGGG	
hDNMT3b-ChIP2-F	GTGCTGTTTTCCAGTGGTTCAATG	ChIP
hDNMT3b-ChIP2-B	TGCCTGTCATCCTGCTTTGG	
hDNMT3b-ChIP3-F	AATTACCTGGCCTCTGCCTTGG	ChIP
hDNMT3b-ChIP3-B	GCTCTTTCCTAAAGCTGGGATTG	
hDNMT1-ChIP1-F	GCCTGGGAGATAAAGGAAGACTCTG	ChIP
hDNMT1-ChIP2-B	GCGGAAATGATGGACACTACACC	
hDNMT1-CHIP3-F	GACCCCATCTCTACAAAAAACTGC	ChIP
hDNMT1-CHIP2-B	TAAGTGCCTACTGTGTGTCCACCC	
hDNMT1-ChIP3-F	TGAGCGAGGGCAAAAGGATG	ChIP
hDNMT1-ChIP3-B	AGTTGAAAGAGGAGGCGTCTGAG	
hDNMT1-F	GAAGGAGGCAGATGACGATGAG	RT-PCR
hDNMT1-B	AATAACAGAGACACAGTCCCCCAC	
hDNMT3b-F	CCAACAACAAGAGCAGCCTGG	RT-PCR
hDNMT3b-B	GCACTCCACACAGAAACACCG	
hIRF8-CpGI-F	TTTTGAAGTTGGGATTTTTTTGTTT	RT-PCR
hIRF8-CpGI-B	TAAAATCCRAACCTCTTCTAAAACC	
hIRF8-MSP-U-F	CCATCCCCATAAAATAACACACAACAAA	MS-PCR of *IRF8* promoter CpG ialand
hIRF8-MSP-U-B	GATGGTGTAGATGTGTGTTTGTGGTTT	
hIRF8-MSP-M-F	TCCCCGTAAAATAACGCGCGACGAA	MS-PCR of *IRF8* promoter CpG ialand
hIRF8-MSP-M-B	CGGTGTAGACGTGCGTTTGCGGTTT	
hIRF8-F	GCTCATCCAATCTCCCAAGTCTC	RT-PCR
hIRF8-B	CTCTATTCGCCTGTGAACTCCATC	
hβ-actin-F	GGAACGGTGAAGGTGACAGCAG	RT-PCR
hβ-actin-B	TGTGGACTTGGGAGAGGACTGG	
hβ-actin-Q-F	TGAAGGTGACAGCAGTCGGTTG	qPCR
hβ-actin-Q-B	GGCTTTTAGGATGGCAAGGGAC	

*m: mouse; h:human