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Supplemental Information

An IL-27-Driven Transcriptional Network Identifies Regulators of IL-10 Expression across T Helper Cell Subsets

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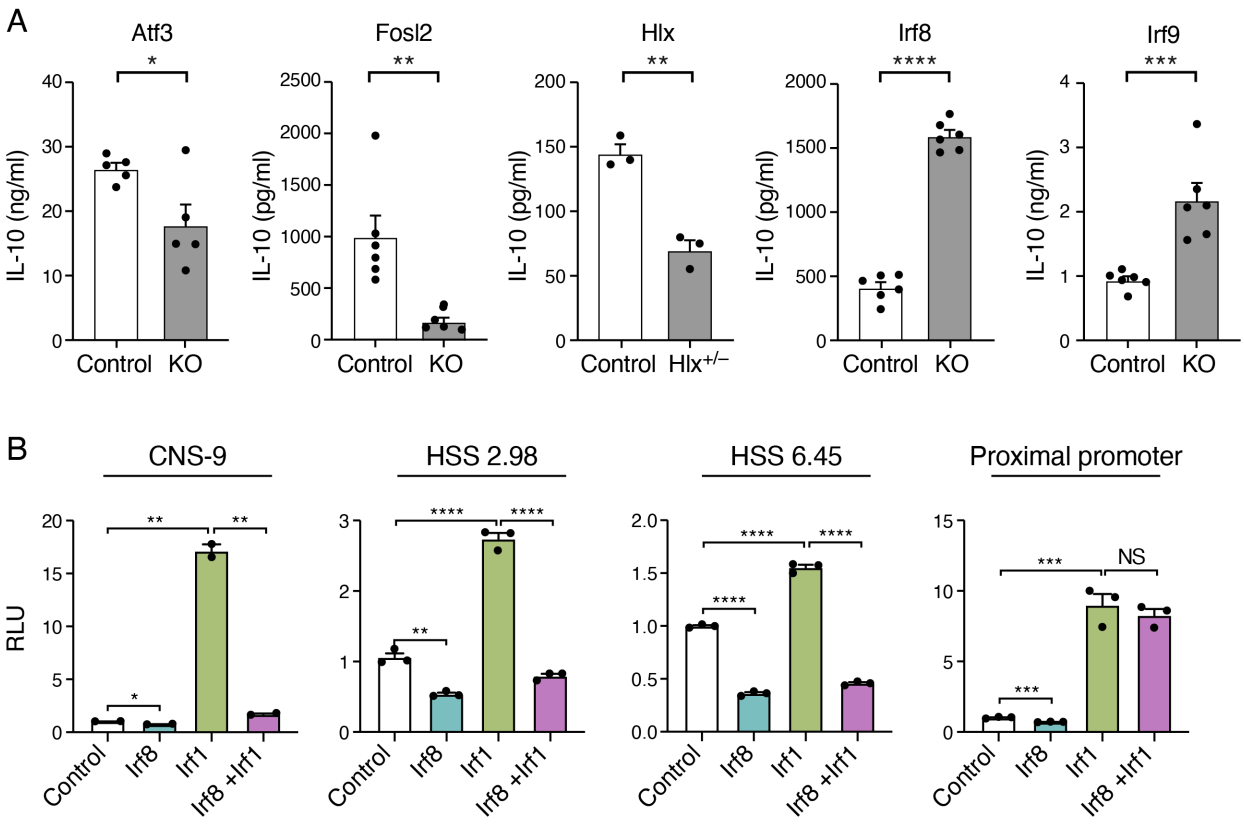
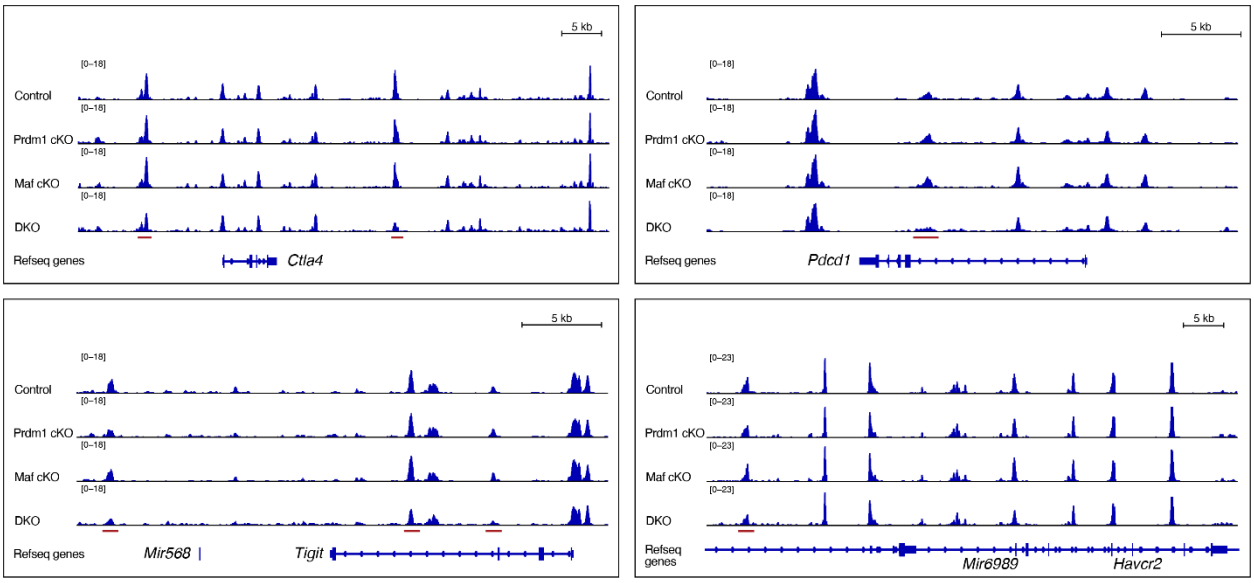


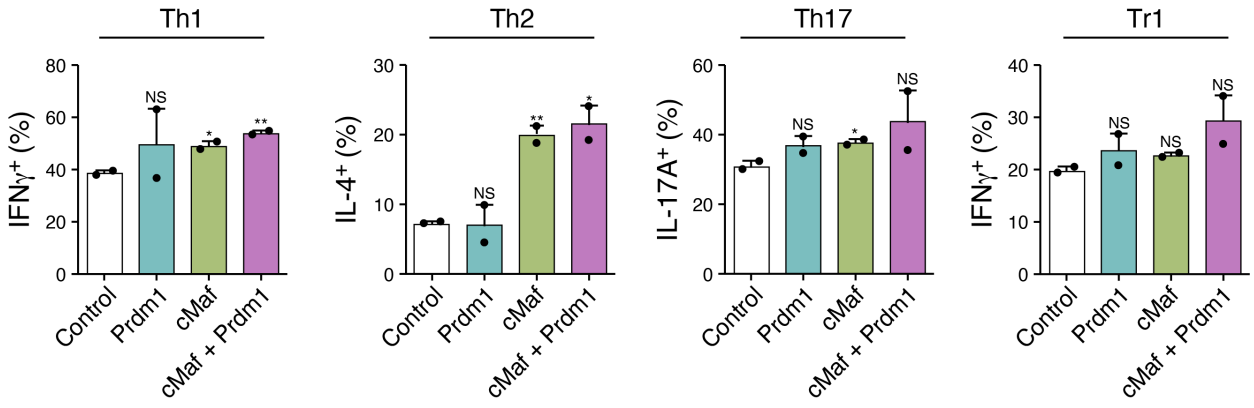
Supplementary Figure 1. Validation of *Il10* regulators predicted by IL-27 network. Related to Figure 2.

(A) Naïve CD4 T cells from indicated mice were differentiated *in vitro* into Tr1 cells. Supernatant were collected at 72h and IL-10 protein was measured by ELISA or Legendplex. (**B)** Luciferase activity in 293T cells transfected with *Il10* luciferase reporters along with empty vector (control) or constructs encoding Irf8, Irf1 or both.



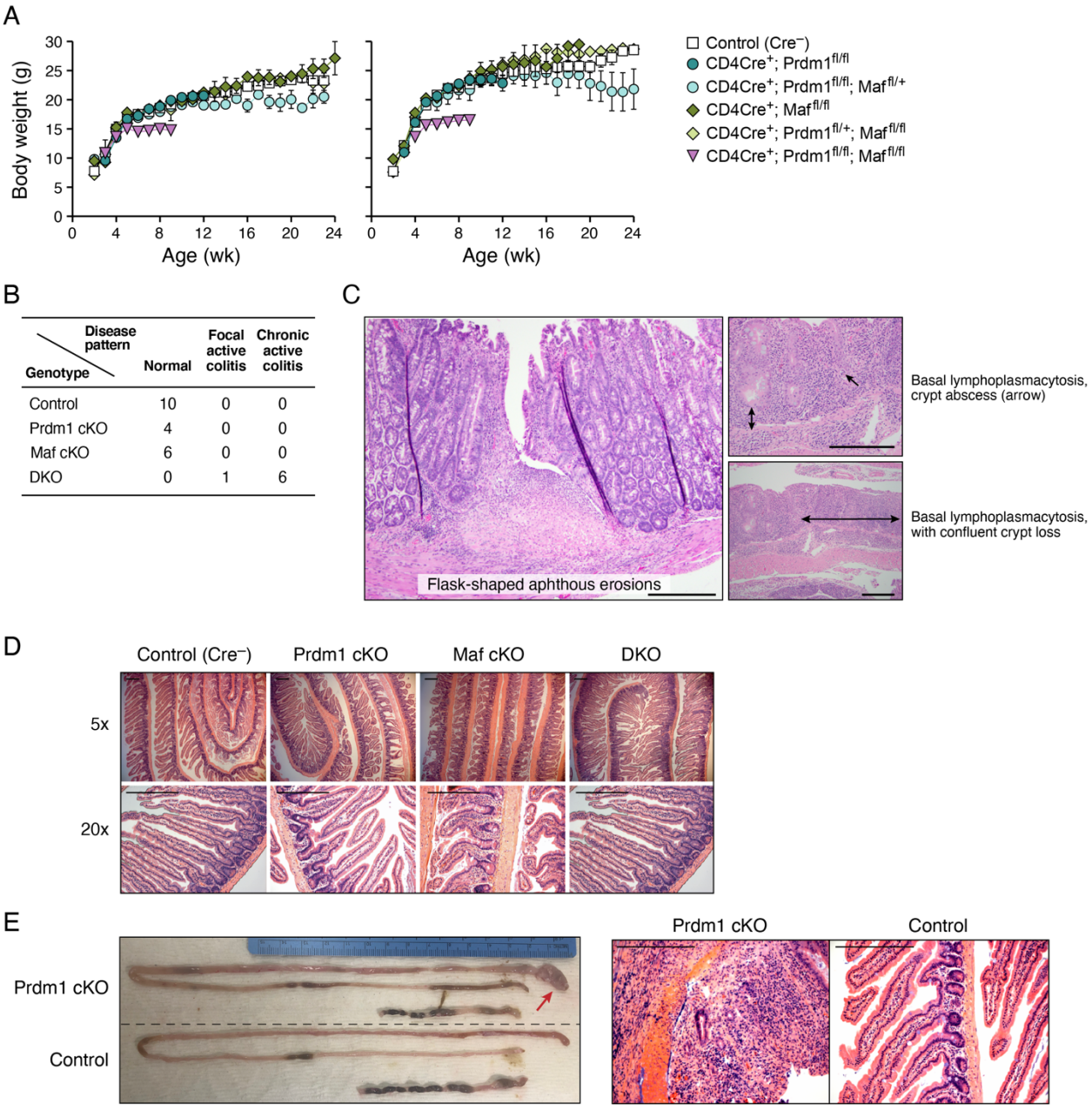
Supplementary Figure 2. Prdm1 and cMaf cooperatively regulate chromatin accessibility of co-inhibitory receptors. Related to Figure 4C.

Control, Prdm1 cKO, c-Maf cKO and Prdm1/c-Maf DKO Tr1 cells were differentiated *in vitro* for 72h and analyzed by ATAC-seq. Chromatin accessibility in the *Ctla4*, *Pdcd1*, *Tigit*, and *Havcr2* loci are shown. Red bars regions showing differential chromatin accessibility in DKO mice.



Supplementary Figure 3. Prdm1 and cMaf do not inhibit signature cytokines of Th1, Th2, Th17 and Tr1 cells. Related to Figure 6D.

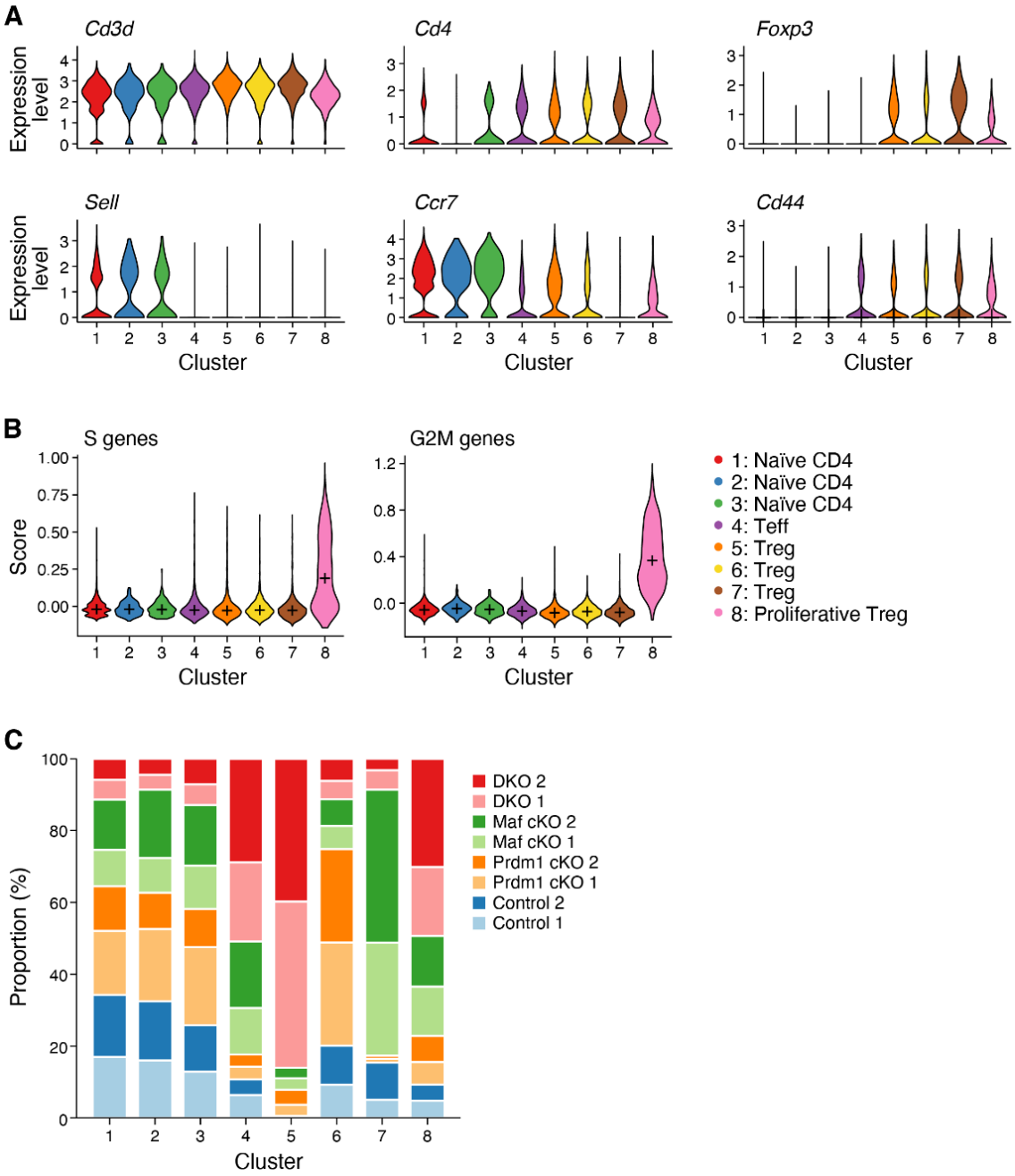
In-vitro differentiated T helper cells were transduced with retroviruses overexpressing Prdm1 and c-Maf respectively as described in Figure 6D. Production of signature cytokines of each lineage was measured by flow cytometry.



Genotype \ Disease pattern	Normal	Focal active colitis	Chronic active colitis
Control	10	0	0
Prdm1 cKO	4	0	0
Maf cKO	6	0	0
DKO	0	1	6

Supplementary Figure 4. Histological features of spontaneous colitis in Prdm1/cMaf DKO mice. Related to Figure 7A-7C.

(A) Body weight of indicated genotypes monitored weekly, n≥5. **(B)** HE slides of swiss rolls of colon were evaluated for disease pattern by a histopathologist in a blinded manner, the numbers in the table indicate number of mice that were evaluated for the presence of histological disease. **(C)** HE staining of swiss rolls of small intestine with no significant pathologic change. (**D)** Picture showing swelling of the proximal end of small intestine in a male Prdm1 cKO mouse and HE staining of the same tissue. (**E)** histological features in the colon of Prdm1/c-Maf DKO mice with aphthous ulcers and severe chronic active colitis that resemble human inflammatory bowel disease. (**C, D, E**) Scale bars represent 250 μ m.



Supplementary Figure 5. Genetic deficiency of both Prdm1 and cMaf but not either alone in T cells leads to human IBD-like spontaneous colitis driven by a unique cluster of Tregs. Related to scRNA-seq analysis in Figure 7D-7K.

(A) Expression of key T cell subtype marker genes across clusters depicted as log(TP10K+1). (B) Distribution of gene signature scores of Tconv cells (cluster 1-4) by cluster. "+" Indicates median. (C) Proportions of cells of different genotypes within each cluster.

Table S1. Related to Figure 4B

Condition	Reference	Description	IL-10$^+$ population	IL-10$^-$ population
Small intestine Tr1 vs Th17	Gagliani et al, 2015	Intestinal lymphocytes were isolated from small intestine after intraperitoneal treatment of anti-CD3	IL-10$^+$ Foxp3$^-$ Tr1	Th17
Small intestine Tr1ExTh17 vs ExTh17	Gagliani et al, 2015		IL-10$^+$ Foxp3$^-$ Tr1 ExTh17	ExTh17
Small intestine Treg	Gagliani et al, 2015		IL-10$^+$ Foxp3$^+$Tregs	Total Foxp3$^+$ Tregs
i.p. anti-CD3	In house generated	Lymphocytes were isolated from mesenteric lymph node after intraperitoneal treatment of anti-CD3	IL-10$^+$ Foxp3$^-$ Tr1	Teff cells
intranasal anti-CD3	Mayo et al, 2016	Lymphocytes were isolated from cervical lymph nodes and spleen of anti-CD3 mAb treated B6NODF1IL-10:GFP mice during the progressive phase of EAE	CD3$^+$ CD4$^+$ GFP (IL-10)$^+$ cells	Naïve CD4 T cells
CD28SA treated Treg	Langenhorst et al, 2012	Lymphocytes were isolated from mice treated with a superagonistic anti-mouse CD28 mAb (CD28SA) intraperitoneally	IL-10$^+$ CD4$^+$CD25$^+$ Tregs	IL-10$^-$ CD4$^+$CD25$^+$ Tregs
Th1 + Notch ligand	Neumann et al, 2014	Mouse naïve CD4 T cells were polarized in Th1 condition *in vitro* in the presence of the Notch ligand Dll4	IL-10$^+$ CD4 T cells	IL-10$^-$ CD4 Tcells
Human tDC primed CD4	Boks et al, 2016	Human CD4 T cells were cocultured with allogeneic IL-10 producing tolerogenic dendritic cells	IL-10$^+$ CD4 T cells	IFN-γ$^+$IL-10$^-$ CD4 T cells
CD8	Trandem et al, 2011	CD8 cells were sorted from brain of Coronavirus-infected mice at the peak of Coronavirus-induced encephalitis	IL-10$^+$ CD8 T cells	IL-10$^-$ CD8 T cells
CD4 tolerant to self Ag	Burton et al, 2014	Tg4 TCR transgenic mice were treated with MBP Ac1-9[4Y] peptide s.c. repeatedly to inducce tolerance to self antigen. IL-10 was increased as the number of treatment goes up. Transcritpion factors that are associated with the increase in IL-10 along the dosage escalation were identified.		