

## Supplemental Figure Legends

### Figure S1, related to Figure 1. Phenotype of mononuclear phagocyte subsets

(A) Gating scheme used to identify intestinal MNP subsets. Lamina propria mononuclear cells were isolated from small intestines of WT and CX3CR1-GFP mice. Numbers reflect percentage of cells within the corresponding gate. The gates identifying the four mononuclear phagocyte subsets are color-coded.

(B) Expression of surface markers by small intestinal lamina propria mononuclear phagocyte subsets (black line) identified as in (A), compared to a negative cell subset in the same sample (shaded histogram)

### Figure S2, related to Figure 1. DP DCs are not required for SFB-induced Th17 cell responses

(A) Defect in DP DC development in CD11c-Cre/Notch2-flox mice (CD11c<sup>ΔNotch2</sup> mice)

(B) SFB colonization induces normal levels of Th17 cells in CD11c<sup>ΔNotch2</sup> mice. Plots gated on TCRβ<sup>+</sup>CD4<sup>+</sup> cells. Data from one of two independent experiments with similar results

### Figure S3, related to Figure 2. CD103<sup>+</sup>CD11b<sup>-</sup> (CD103 SP) DCs are dispensable for commensal Th17 cell induction

(A,B) CD11c<sup>+</sup>MHCII<sup>+</sup> MNP subsets in SI LP of *Batf3*<sup>-/-</sup> mice and control heterozygous littermates. (A) FACS plots gated on CD11c<sup>+</sup>MHCII<sup>+</sup> cells. Numbers represent percentage of cells in the corresponding gate. (B) total number of cells in individual MNP subsets represented as percentage of total live single cells

(C,D) Total number and individual DC subsets in the migratory fraction from MLN of *Batf3*<sup>-/-</sup> mice and control heterozygous littermates. Plots gated as in Figure S2. Cell numbers in (D) are presented as percentage of total live single cells

(E-G) Induction of RORγt<sup>+</sup> Th17 cells by SFB in SI LP. Plots gated on TCRβ<sup>+</sup>CD4<sup>+</sup> cells

(H-K) Induction of IL-17<sup>+</sup> Th17 cells by SFB in SI LP. Plots gated on TCRβ<sup>+</sup>CD4<sup>+</sup> cells.

(L) Response to SFB antigens of purified SI LP CD4 T cells from *Batf3*<sup>-/-</sup> and *Batf3*<sup>+/-</sup> littermates. SI LP CD4 T cells were isolated before (No SFB) and after (+SFB) colonization with SFB and incubated with CD11c<sup>+</sup> splenic DCs for 72 hours in the presence of SFB antigens.

(M) Proliferation of purified SI LP CD4 T cells in response to various antigens. LP CD4 T cells were isolated and cultured as in F in the presence of SFB or control SFB-negative bacterial antigens (Jax antigens) as described in Methods. Open circles in bar graphs represent data from individual animals. Data from one of three experiments with similar results.

### Figure S4, related to Figure 4. Cell subsets in CCR2-DTR mice following DT treatment

(A) CCR2 expression on total CD4 T cells in WT and CCR2-DTR mice treated with DT for 12 days. Plots gated on TCRβ<sup>+</sup>CD4<sup>+</sup> cells

(B) CCR2 expression on Th17 cells in WT and CCR2-DTR mice treated with DT for 12 days. Plots gated on TCRβ<sup>+</sup>CD4<sup>+</sup>IL-17<sup>+</sup> cells

(C) Effects of DT treatment on Th17 cells in CCR2-DTR mice. WT and CCR2-DTR mice were colonized with SFB for several weeks to induce Th17 cell differentiation and then treated with DT every 48 hours for 12 days. DT treatment led to ablation of CD64 Mfs in CCR2-DTR mice (not shown), but did not lead to decrease in the numbers of SFB-induced Th17 cells compared to WT mice

(D) Kinetics of Mf depletion in CCR2-DTR mice. CCR2-DTR and WT mice received a single injection of DT and SI LP MNP subsets were examined 24 or 72 hours later. Plots gated on CD11c<sup>+</sup>MHCII<sup>+</sup> cells.

(E-I) CCR2-DTR and WT littermates (LM) were treated with DT and colonized with SFB as described in Figure 4A

(E) SFB levels on Day 12 assessed by Q-PCR for SFB 16S rRNA gene normalized to total 16S rRNA (UNI)

(F) DC subsets in the migratory fraction of MLN from WT and CCR2-DTR mice. Cell numbers are presented as percentage of total live single cells

(G-I) Induction of IL-17<sup>+</sup> Th17 cells by SFB in SI LP. Plots gated on TCRβ<sup>+</sup>CD4<sup>+</sup> cells

**Figure S5, related to Figure 5. Recovery of intestinal Mfs by monocyte transfer**

CCR2-DTR and WT littermates (LM) were treated with DT and colonized with SFB as described in Figure 5A. Shortly before SFB colonization some mice received an adoptive transfer of 5-10 X 10<sup>6</sup> bone marrow monocytes (+ Mono) from CD45.1<sup>+</sup> congenic mice (Figure 5A)

(A,B) DC subsets in the migratory DC fraction of MLN from DT-treated SFB-colonized WT and CCR2-DTR mice with and without transfer of 5-10 X 10<sup>6</sup> bone marrow monocytes (+ Mono)

(C) MNP subsets in small intestinal LP of CCR2-DTR mice with and without monocyte transfer. MNP plots (far left) are gated on all CD11c<sup>+</sup>MHCII<sup>+</sup> cells. Donor-derived cells were identified as CD45.1<sup>+</sup> and were found only in the CD64<sup>+</sup>CD24<sup>-</sup> Mf fraction, but not in the CD64<sup>-</sup>CD24<sup>+</sup> DC fraction (right plots)

(D) Donor-derived cells are absent in the migratory DC fraction of MLN. Plots gated as in Figure S2

(E) SFB levels on Day 12 assessed by Q-PCR for SFB 16S rRNA gene normalized to total 16S rRNA (UNI)

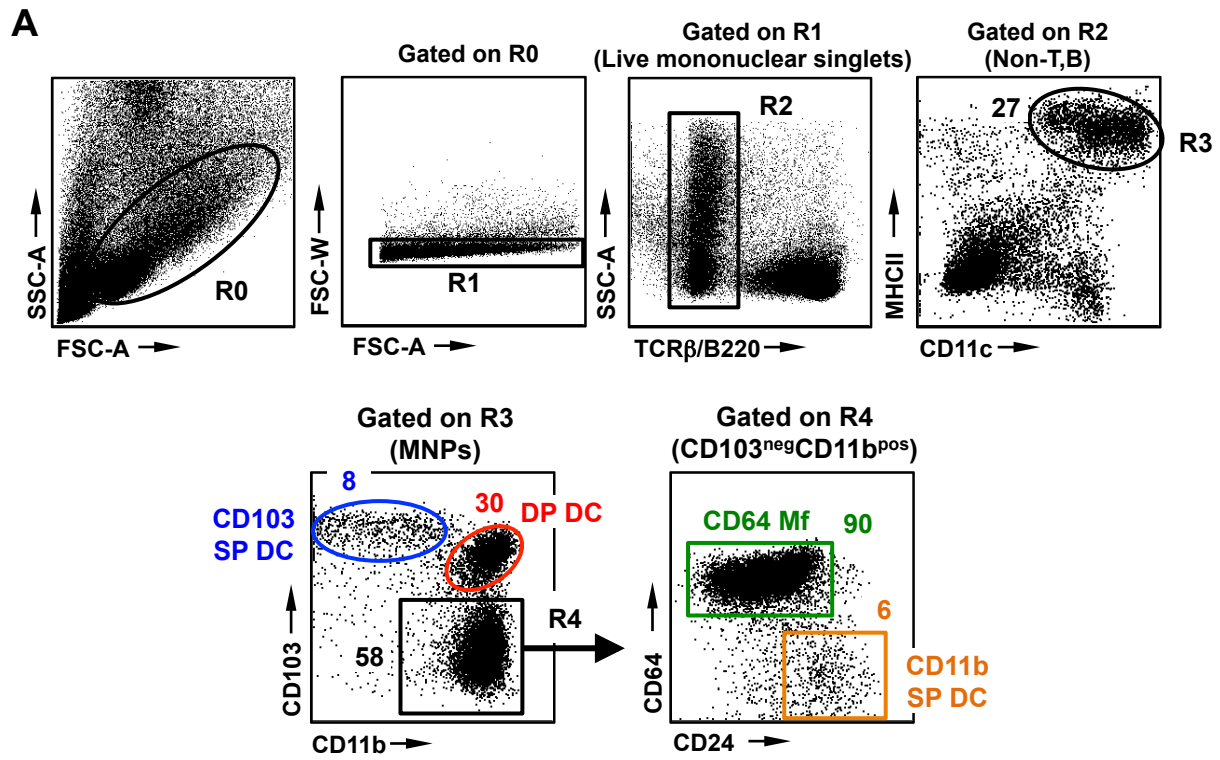
**Figure S6, related to Figure 6. CCR2 expression on lamina propria MNP subsets**

(A) CCR2<sup>+</sup> cells in small intestinal LP MNP subsets from WT and CCR2-DTR mice treated with DT for 12 days. Plots gated on MNP subsets as outlined in Figure S1.

(B) WT C57BL/6 mice were treated with a single injection of blocking anti-CSF1R antibody (AFS98) or isotype control (IgG). Small intestinal LP MNP subsets were examined a week later

**Table S1, related to Figure 7. MNP subsets in various mouse models used in this study**

Comparison of the size of individual MNP subsets in different mouse models. Total cell numbers in each fraction in the small intestinal lamina propria (x10<sup>4</sup>). Main numbers represent mean (x10<sup>4</sup>) and secondary numbers – standard deviation of the mean. Statistically significant differences from WT littermates are marked in red. Significance values are reported on the corresponding figures.



**B**

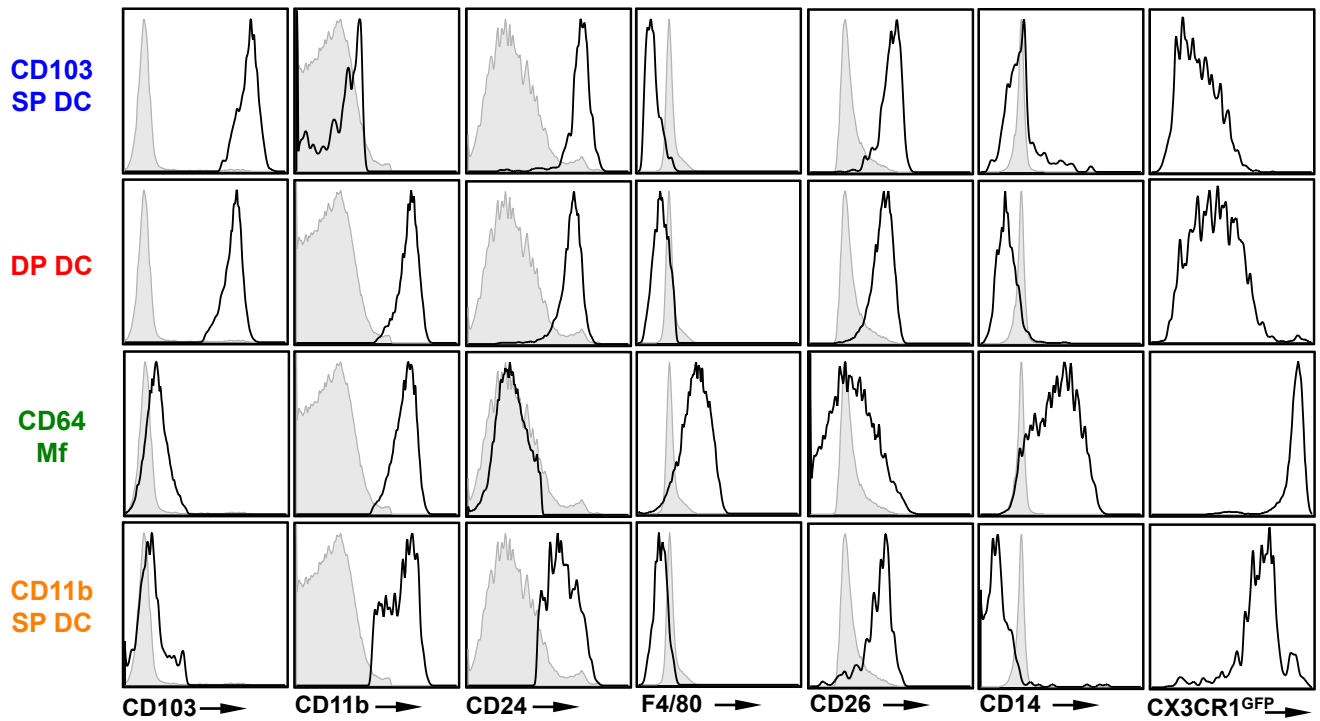
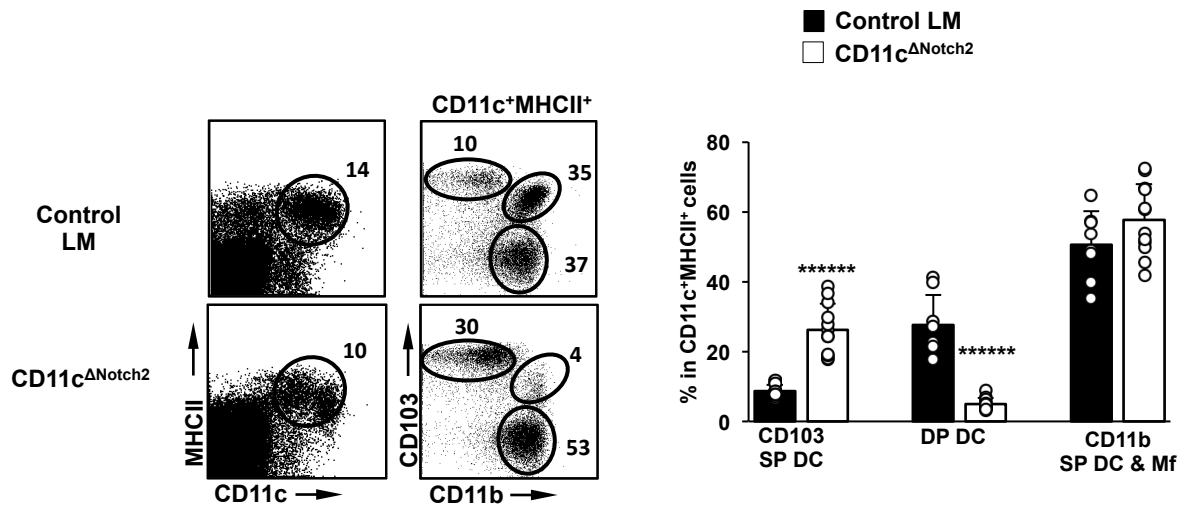
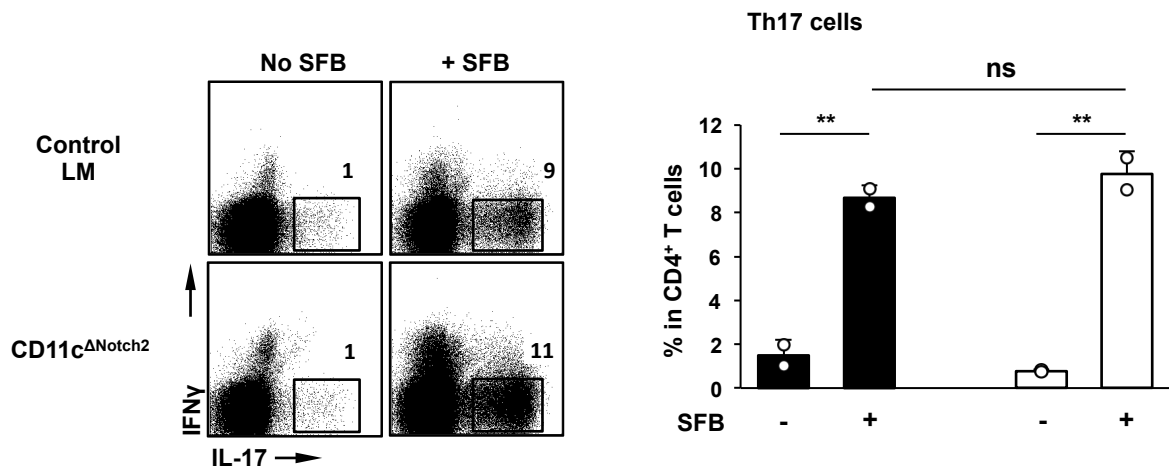


Figure S1

**A**



**B**



**Figure S2**

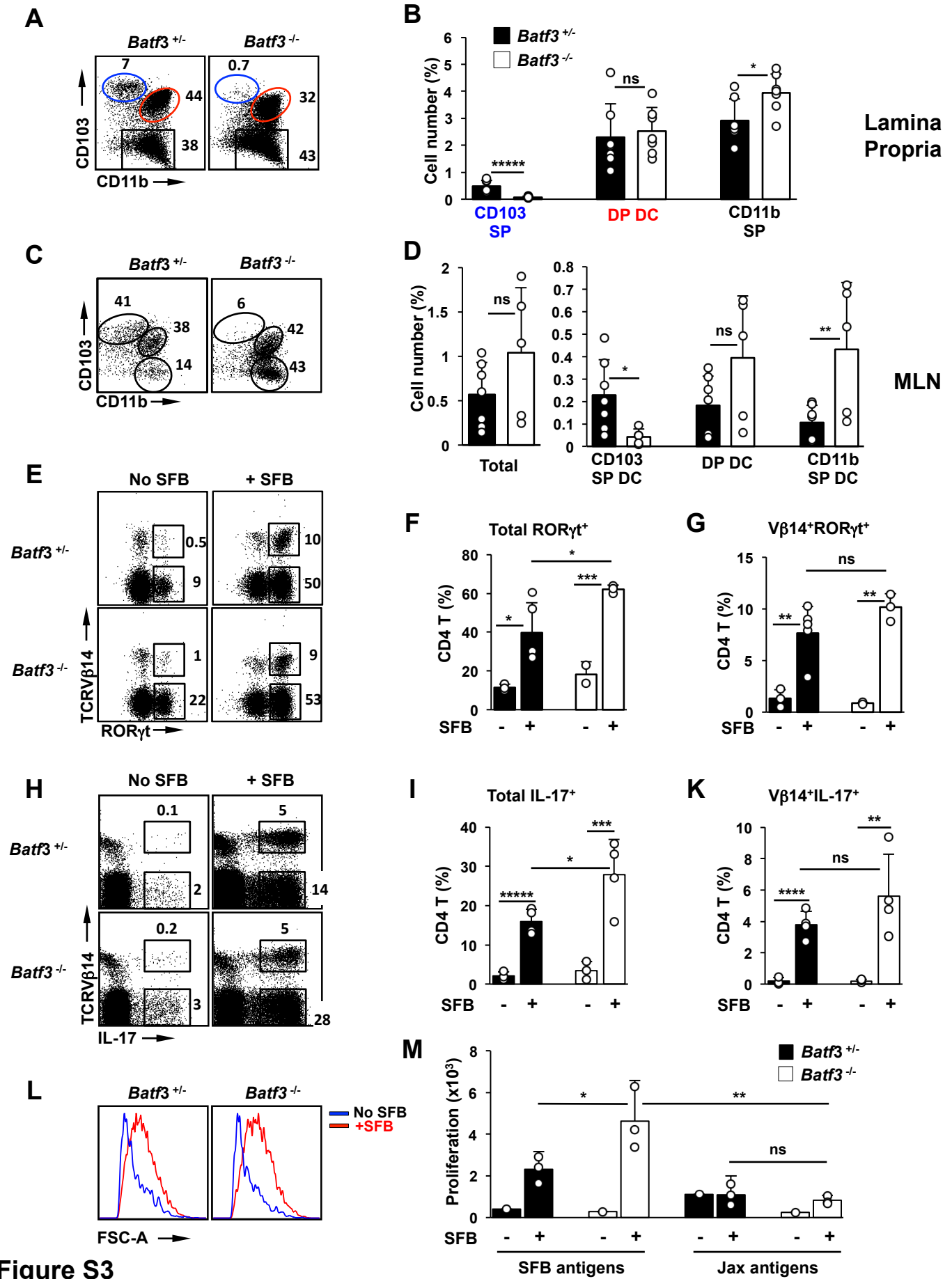


Figure S3

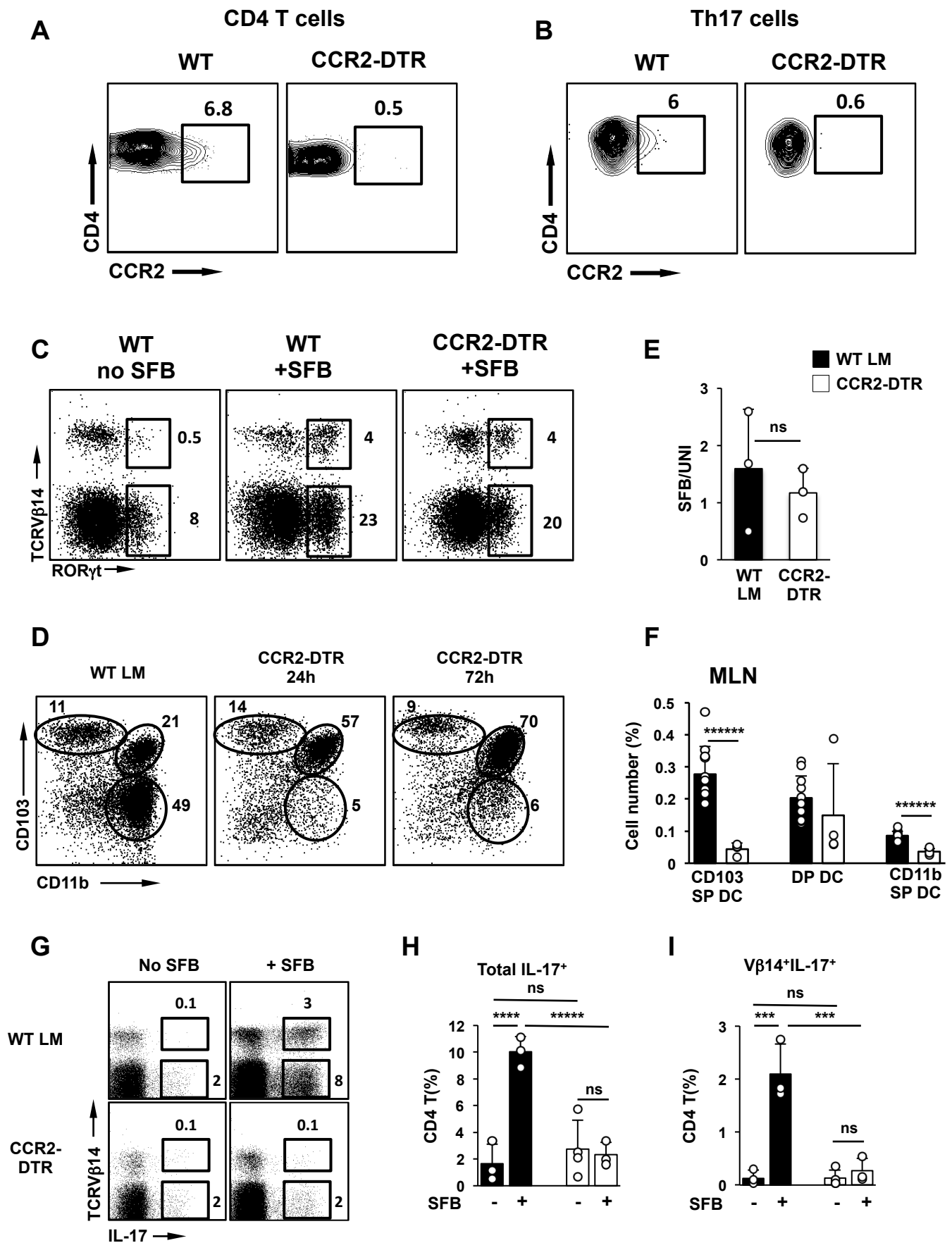


Figure S4

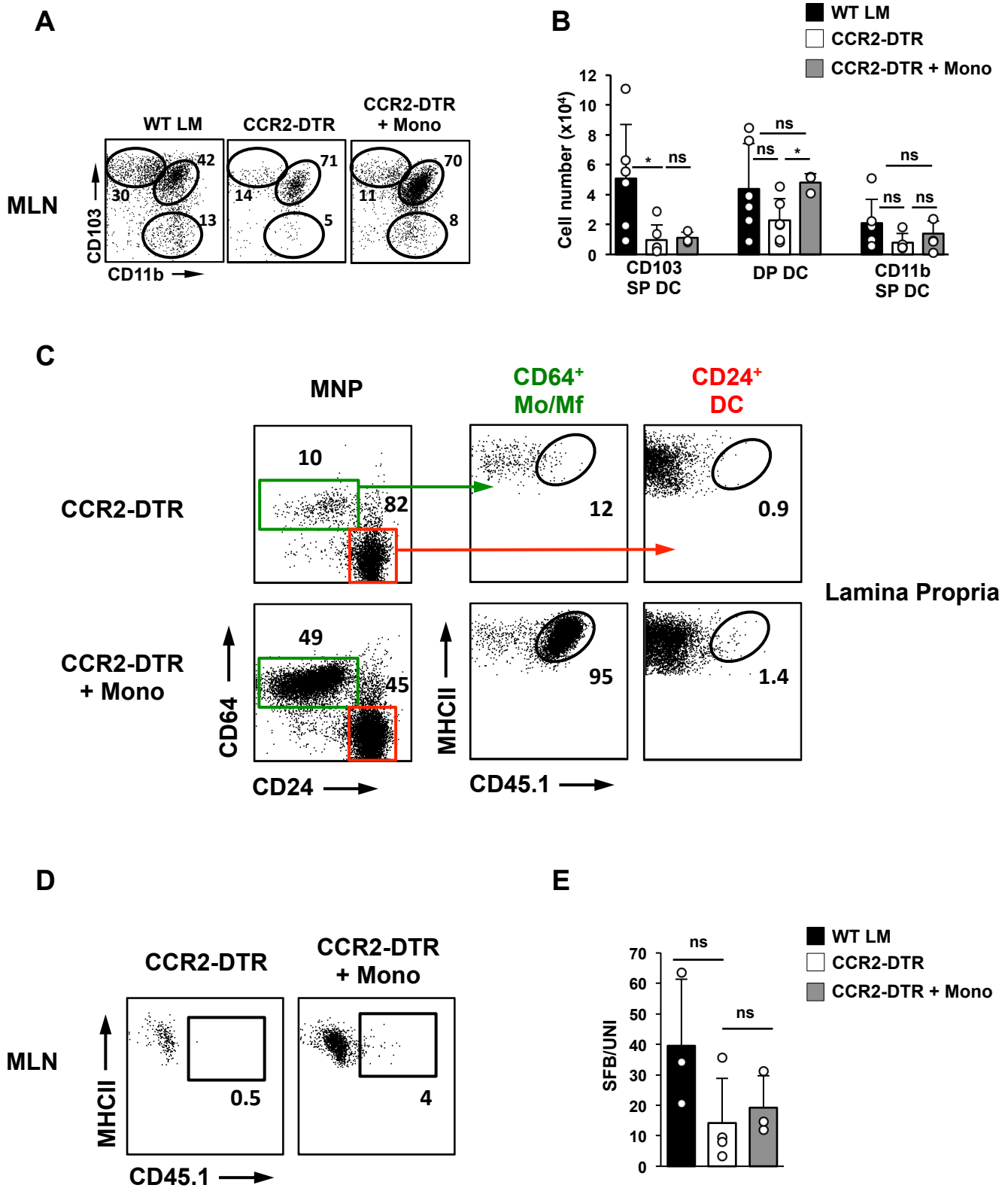


Figure S5

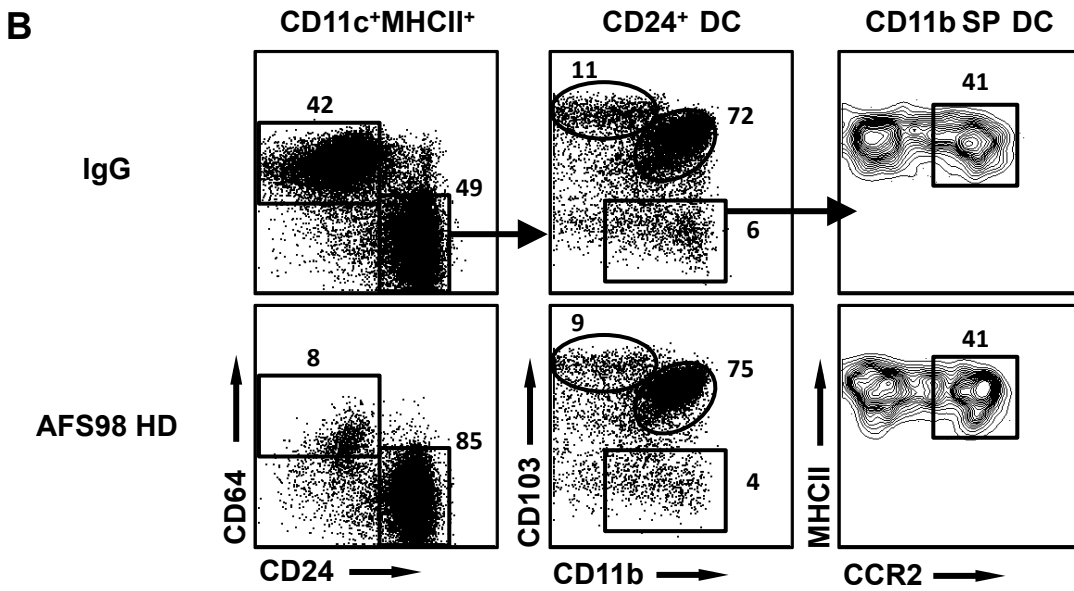
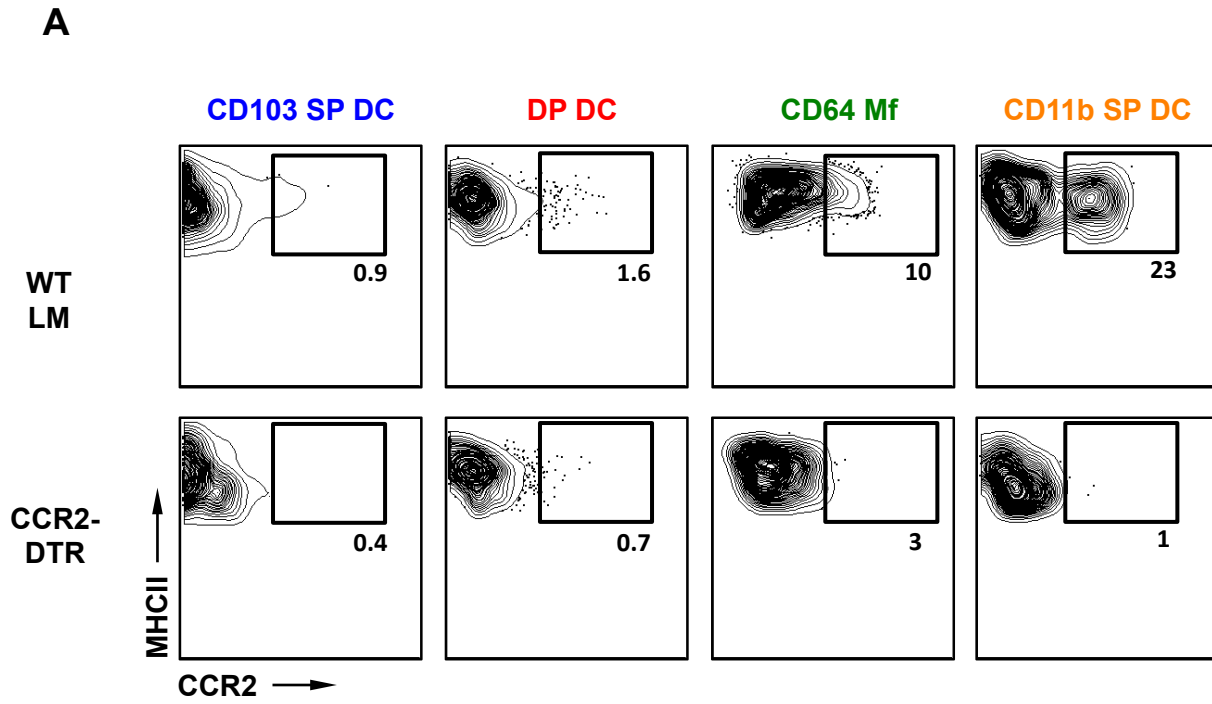


Figure S6



## Total cell numbers of SI LP MNP subsets (x10<sup>4</sup>)

		<b>CD103 SP DC</b>	<b>DP DC</b>	<b>CD64 Mf</b>	<b>CD11b SP DC</b>
Langerin- DTA	WT	6.6 ± 3.6	17 ± 5.2	33 ± 19	4.2 ± 2.3
	DTA	8.3 ± 1.5	1.7 ± 1.6	37 ± 19	4.1 ± 2.4
Batf3 <sup>-/-</sup> x Lang-DTA	WT	11 ± 4.3	33 ± 15	59 ± 20	8.8 ± 5.0
	DKO	0.24 ± 0.30	0.71 ± 0.48	37 ± 10	0.69 ± 0.22
Flt3L-KO	WT	5.9 ± 2.4	14 ± 9.1	52 ± 20	5.2 ± 2.5
	KO	0.77 ± 0.39	1.3 ± 1.0	40 ± 16	2.6 ± 1.6
CCR2- DTR	WT	1.6 ± 0.90	5.2 ± 2.7	8.5 ± 3.1	0.66 ± 0.18
	DTR	0.22 ± 0.18	8.3 ± 5.8	0.43 ± 0.22	0.94 ± 0.69
AFS98	IgG	4.6 ± 1.8	12 ± 4.6	32 ± 16	3.3 ± 1.2
	AFS98	4.6 ± 1.1	15 ± 5.2	1.8 ± 0.33	2.2 ± 0.29

**Table S1**