Supplemental Data

Figure S1 – Th17 generation is promoted with unactivated *Ifnar1*$^{-/-}$ DC under nonpolarizing conditions.

Antigen-specific T cell activation using MOG-specific 2D2 TCR Tg CD4$^+$ T cells. 2D2 T cells were activated by BM-derived DC (*Ifnar1*$^{+/+}$ vs. *Ifnar1*$^{-/-}$) with 1 μg/ml MOG peptide. The proportion of IL-17$^+$ CD4$^+$ and IFNγ$^+$ CD4$^+$ T cells, determined by flow cytometry, is shown. Tissue culture conditions were not polarized. DC were pre-treated with LPS. Data are representative of two experiments.

Figure S2 – IFNAR on T cells does not elicit major effects on Th17 generation.

Non-transgenic CD4$^+$ T cells from the indicated IFNAR genotype were activated by soluble CD3ε Ab (0.5 μg/ml) with DC (10^5 DC/well) before gating on CD4$^+$ cells. (**A**) Co-cultures of CD4$^+$ T cells (2×10^5 T cells/well) and DC were non-polarized or polarized with the indicated reagents [rIL-23 (100 ng/ml) and IL-4 + FNγ Abs (10 μg/ml each) or rIL-6 (20 ng/ml) and rTGFβ (3 ng/ml)]. DC were derived from spleens and not activated. Data shown in the top two panels were obtained under non-polarized conditions; the bottom four panels show Th17-polarizing conditions with the indicated reagents. Data are representative of two similar experiments. (**B**) rIFNα was added to the first three sets of T-cell + DC co-cultures with the indicated final concentrations. The last two sets of samples, as indicated, included DC pre-treated with either CpG (0.2 μg/ml) or LPS (1 μg/ml) for 3 hrs before setting up co-cultures. Cell culture conditions were skewed to Th17 with rIL-23 (10 ng/ml), rIL-6 (20 ng/ml), rTGFβ (1 ng/ml), IL-4 Ab (10 μg/ml) and IFNγ Ab (10 μg/ml). Data are representative of two similar experiments.

Figure S3 – *Ifnar1*$^{-/-}$ DC do not activate T cells better than *Ifnar1*$^{+/+}$ DC.

(**A**) Splenocytes were harvested from age- and sex-matched naïve mice and stained with Abs for flow cytometry. T, B, NK cells, and dead cells were gated out as CD3ε-CD19-NK1.1-7-AAD- prior to gating on DC as CD11c$^+$. Histograms shown denote CD11c$^+$ DC. (**B**) BM-derived DC (cultured with Flt-3L) were harvested on day 6. Cells were gated on CD11c$^+$ and stained with indicated Abs for flow cytometry. (**C**) OT-2 cell proliferation to OT-2 peptide-pulsed BM-derived DC (*Ifnar1*$^{+/+}$ vs. *Ifnar1*$^{-/-}$). Tritiated thymidine (1 μCi/well) was added to DC-T cell co-cultures on day 3 and cells were harvested 18 hrs later. Culture conditions were either non-polarized or Th17-polarized with the indicated conditions. Results from triplicate wells are shown as mean ± SEM.

Fig. S4 – Opn expression in DC induces Th17 generation without altering Treg generation and T cell proliferation. (**A**) 2D2 T cells were activated by DC with 1 μg/ml of MOG peptide. T cells were re-stimulated with 1 μg/ml of CD3ε Ab on day 6. DC were either immature or pre-activated with CpG before co-culture set-up with 2D2 $CD4^+$ T cells. Cells were harvested for intracellular Foxp3 staining 24 hrs after re-stimulation. (**B**) 2D2 cells were cultured with BM-derived DC with the indicated concentrations of MOG peptide. T cell proliferation was assessed by tritium uptake from day 3 in an 18-hr time window. Th17 conditions are indicated in the figure. DC were untreated.

Fig. S5 – IL-10 levels in DC-T cell co-culture supernatants.

OT-2 cell proliferation with unstimulated BM-derived DC (*Ifnar1*$^{+/+}$ vs. *Ifnar1*$^{-/-}$). Culture supernatants were harvested 24 hrs after T cell re-stimulation with soluble CD3 antibody and levels of IL-10 were analyzed by ELISA in triplicate wells shown as mean ± SEM. Data are representative of 3 similar experiments. Th17 polarizing conditions used were rIL-23, IL-4 and IFNγ Ab.

Fig. S6.

(A) Regulation of Th17 differentiation by an IFNAR–Opn-i pathway in DC. Engagement of IFNAR suppresses Opn-i, resulting in upregulation of IL-27 and suppression of Th17 cell generation.

(B) The effects of IL-27 Ab on the Th17 response in cultures containing Opn wt DC.

2D2 transgenic T cells were incubated with Opn wt BM-DC with 1 μg/ml of MOG peptide. On day 0, 20 μg/ml of IL-27 Ab or isotype IgG was added to cultures. T cells were re-stimulated with soluble CD3 Ab (1 μg/ml) on day 6 and culture supernatants and cells were harvested 24 hrs after re-stimulation. Two different Th17 skewing conditions were tested as indicated.

Fig. S7 – Intracellular expression of IL-27p28 in splenic DC 5 days after EAE induction.

Spleen cells harvested from mice on day 5 were treated immediately with PMA and ionomycin before incubation with IL-27p28 Ab. Levels of IL-27p28 in DC ($CD11b^+CD11c$) from Opn wt and Opn-deficient *Rag2*$^{-/-}$ hosts given $2D2^+$ CD4 cells on day 0 were evaluated by flow cytometry and compared with splenic DC from Opn-deficient *Rag2*$^{-/-}$ mice as a control (shaded histogram).

Figure S1

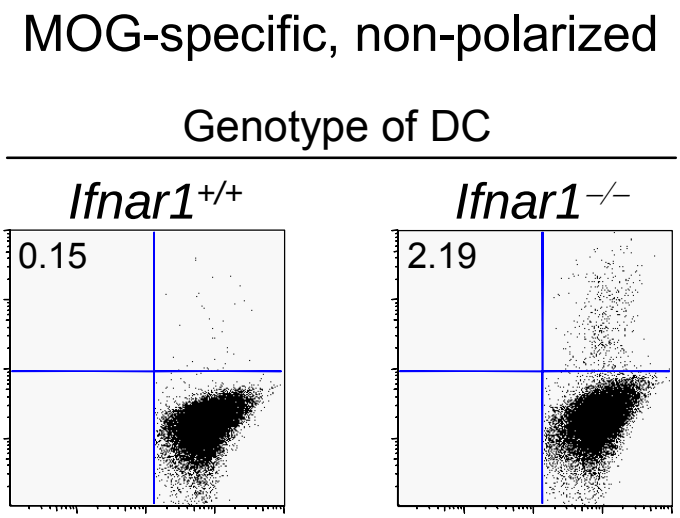

MOG-specific, non-polarized
Genotype of DC
Ifnar1+/+
Ifnar1−/−
0.15
2.19

Figure S2

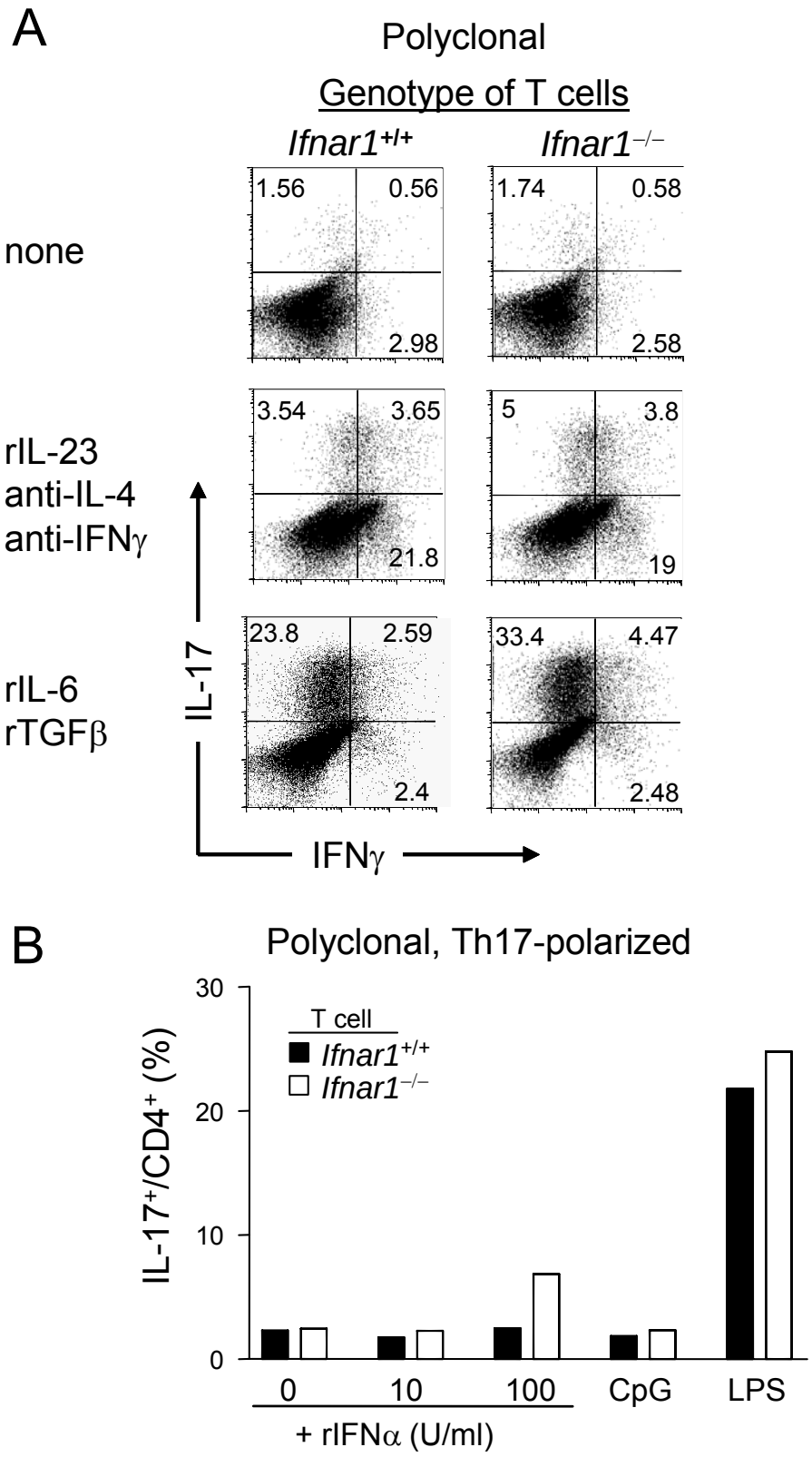
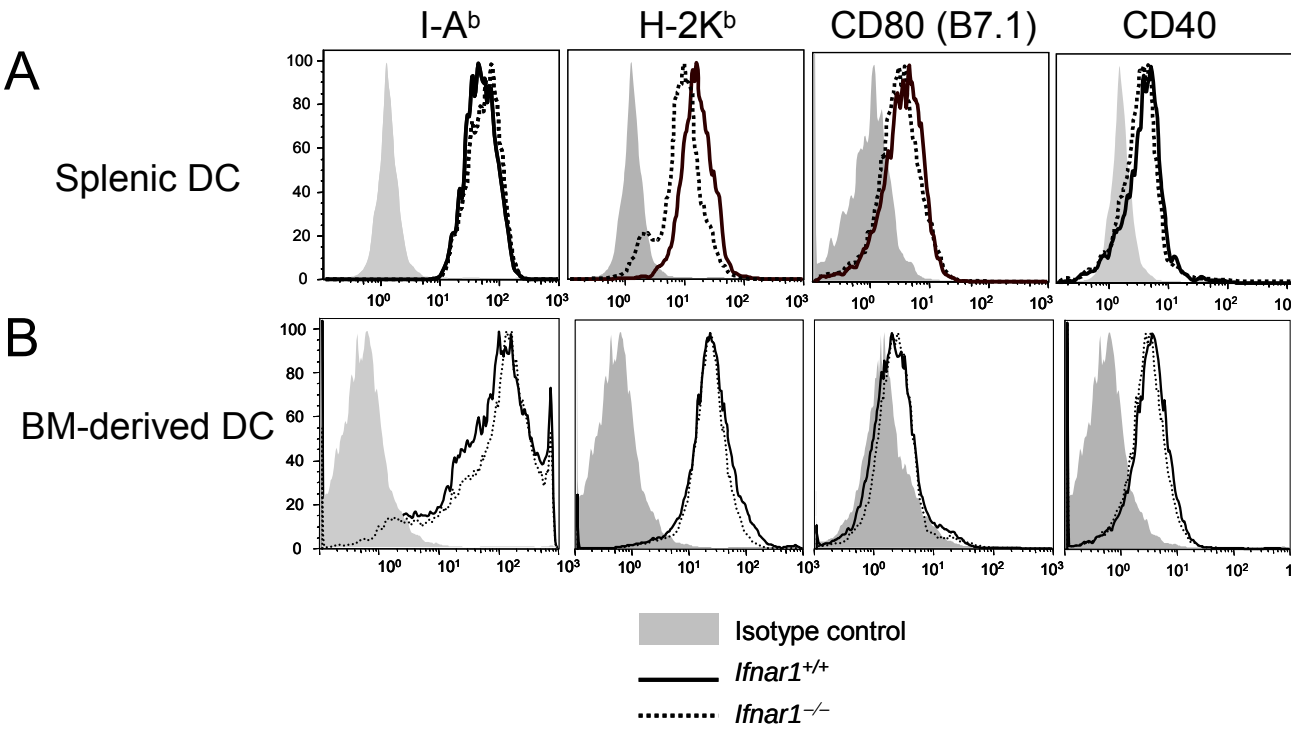

A
Polyclonal
Genotype of T cells
Ifnar1+/+
Ifnar1−/−
none
rIL-23
anti-IL-4
anti-IFNγ
rIL-6
rTGFβ
1.56
0.56
2.98
1.74
0.58
2.58
3.54
3.65
21.8
5
3.8
19
23.8
2.59
2.4
33.4
4.47
2.48
IL-17
IFNγ
B
Polyclonal, Th17-polarized
T cell
Ifnar1+/+
Ifnar1−/−
IL-17+/CD4+ (%)
30
20
10
0
0
10
100
CpG
LPS
+ rIFNα (U/ml)

Figure S3


A
Splenic DC
I-Ab
H-2Kb
CD80 (B7.1)
CD40
B
BM-derived DC
Isotype control
Ifnar1+/+
Ifnar1−/−

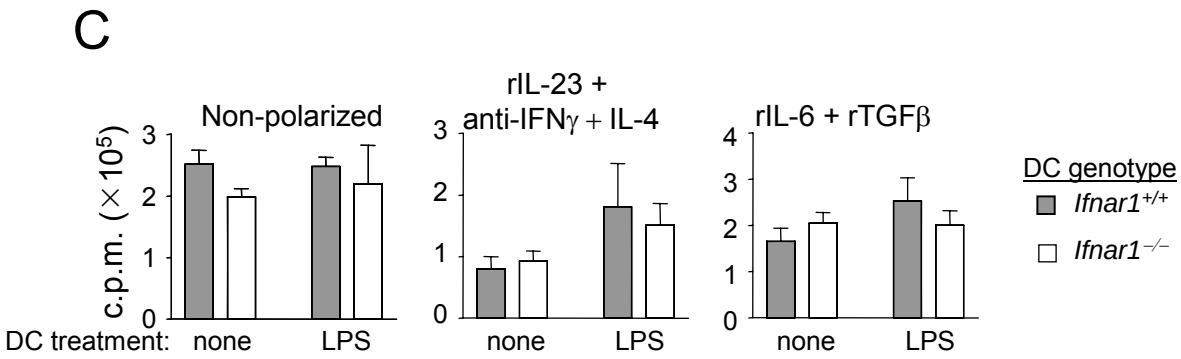

C
Non-polarized
rIL-23 + anti-IFNγ + IL-4
rIL-6 + rTGFβ
c.p.m. (×10^5)
DC treatment: none LPS
DC genotype
Ifnar1+/+
Ifnar1−/−

Figure S4

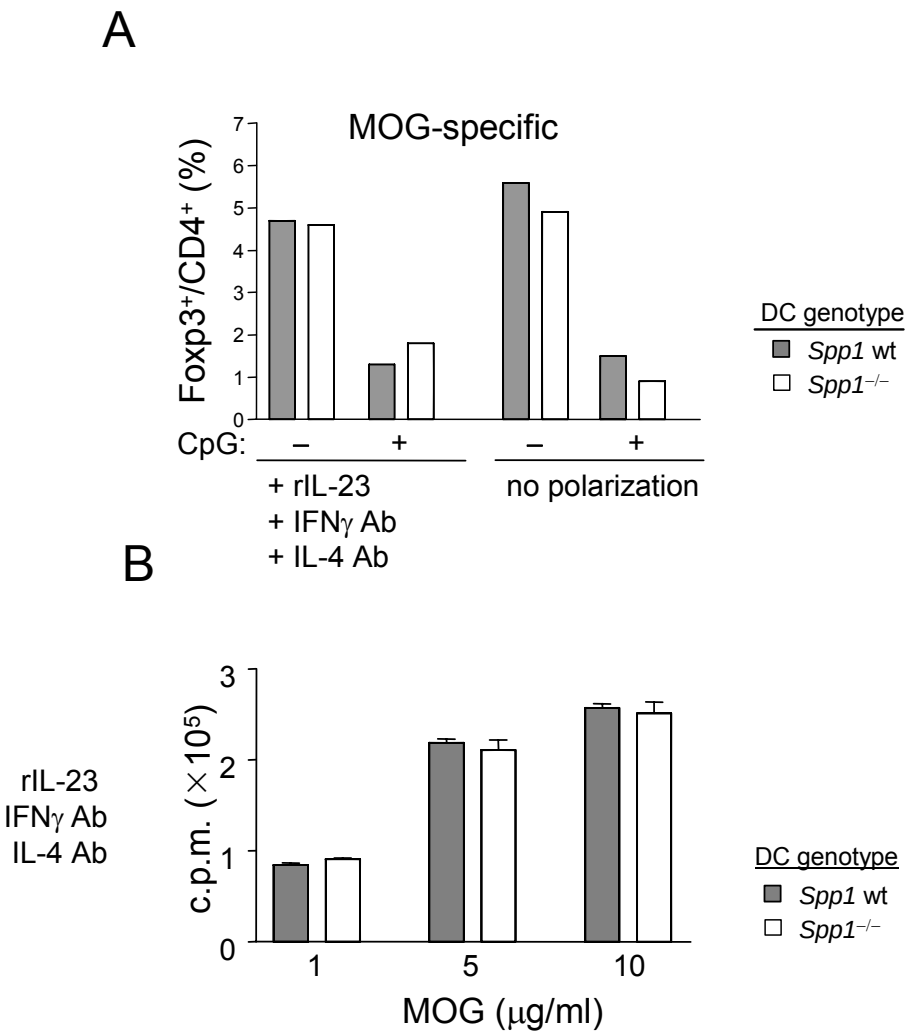

A
MOG-specific
Foxp3+/CD4+ (%)
7
6
5
4
3
2
1
0
CpG:
–
+
–
+
+ rIL-23
+ IFNγ Ab
+ IL-4 Ab
no polarization
DC genotype
Spp1 wt
Spp1–/–
B
rIL-23
IFNγ Ab
IL-4 Ab
c.p.m. (×10^5)
3
2
1
0
1
5
10
MOG (μg/ml)
DC genotype
Spp1 wt
Spp1–/–

Figure S5

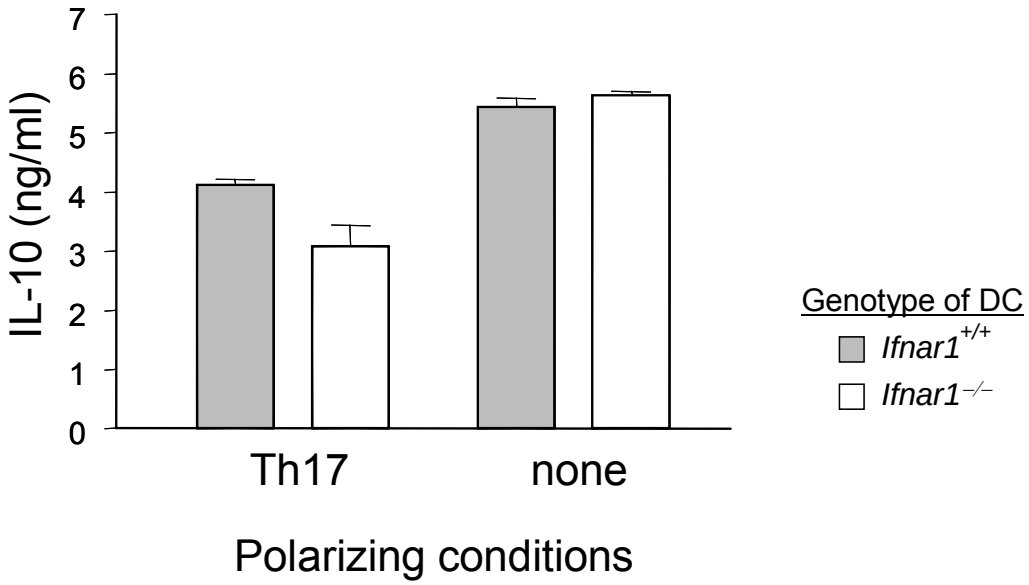

IL-10 (ng/ml)
7
6
5
4
3
2
1
0
Th17
none
Polarizing conditions
Genotype of DC
Ifnar1+/+
Ifnar1−/−

Figure S6

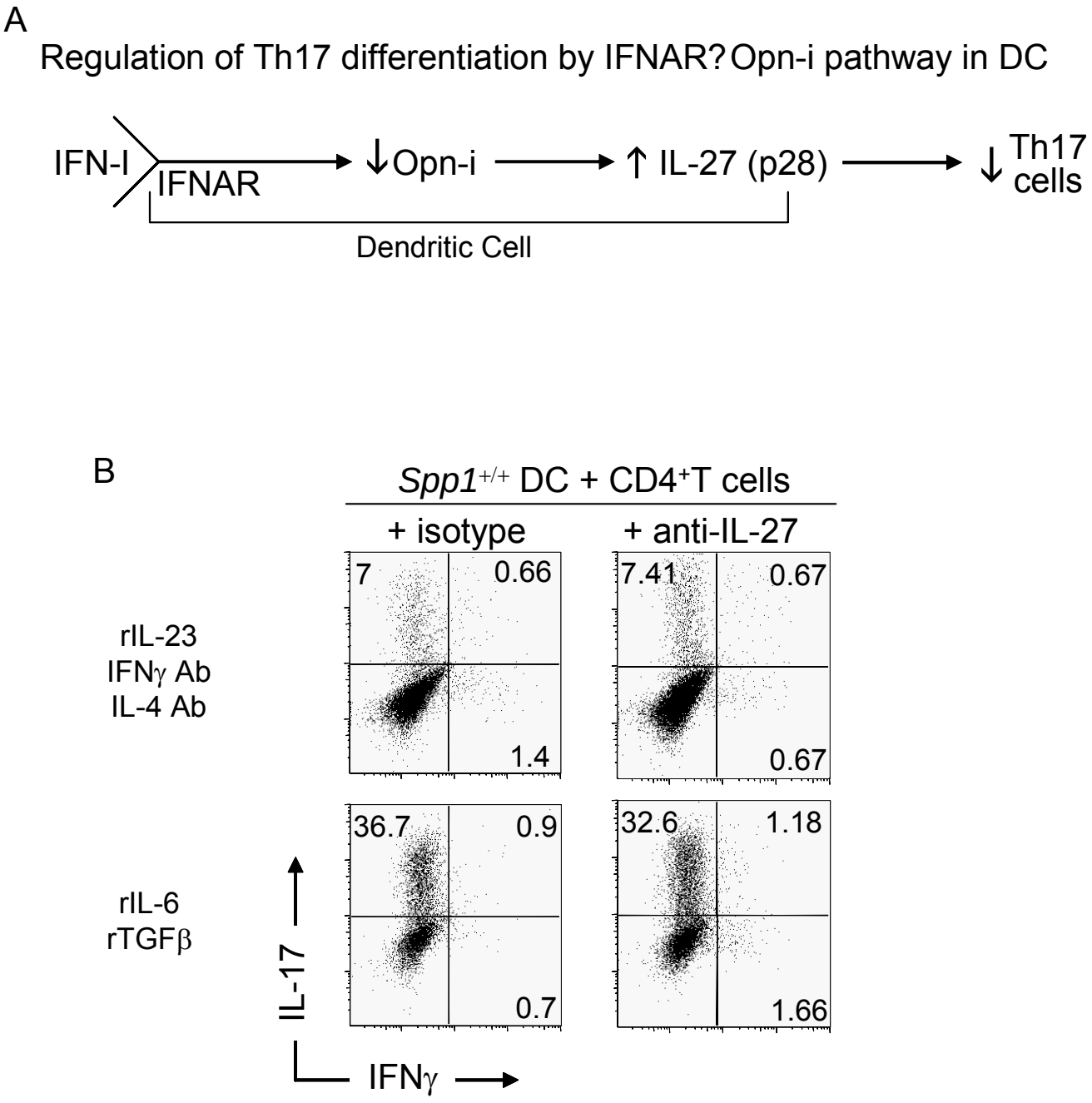
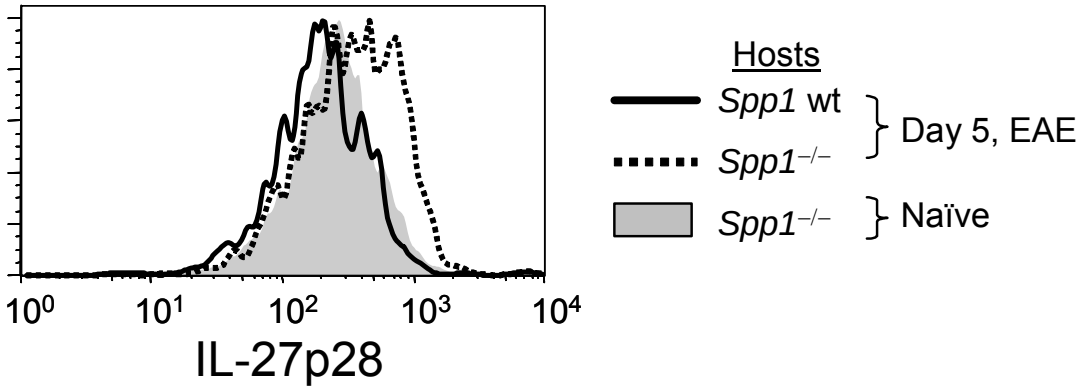

A
Regulation of Th17 differentiation by IFNAR?Opn-i pathway in DC
IFN-I
IFNAR
↓Opn-i
↑ IL-27 (p28)
↓Th17 cells
Dendritic Cell
B
Spp1+/+ DC + CD4+T cells
+ isotype
+ anti-IL-27
rIL-23
IFNγ Ab
IL-4 Ab
rIL-6
rTGFβ
7
0.66
1.4
7.41
0.67
0.67
36.7
0.9
0.7
32.6
1.18
1.66
IL-17
IFNγ

Figure S7


Hosts
Spp1 wt
Spp1−/−
Day 5, EAE
Spp1−/−
Naïve
10^0
10^1
10^2
10^3
10^4
IL-27p28