Interleukin-27 acts on dendritic cells to suppress the T cell response and autoimmunity by inducing their expression of ENTPD1 (CD39)

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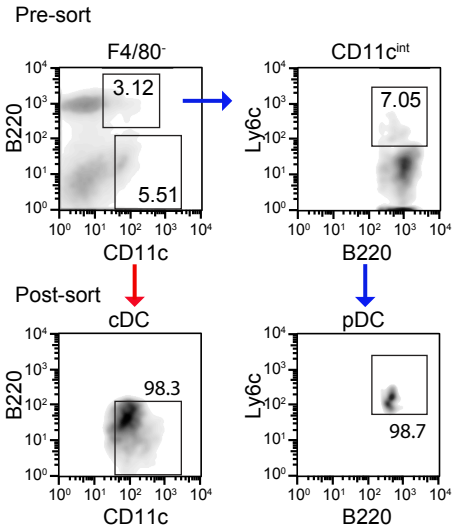
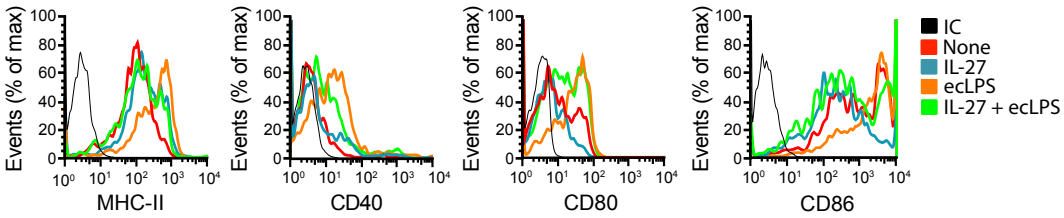
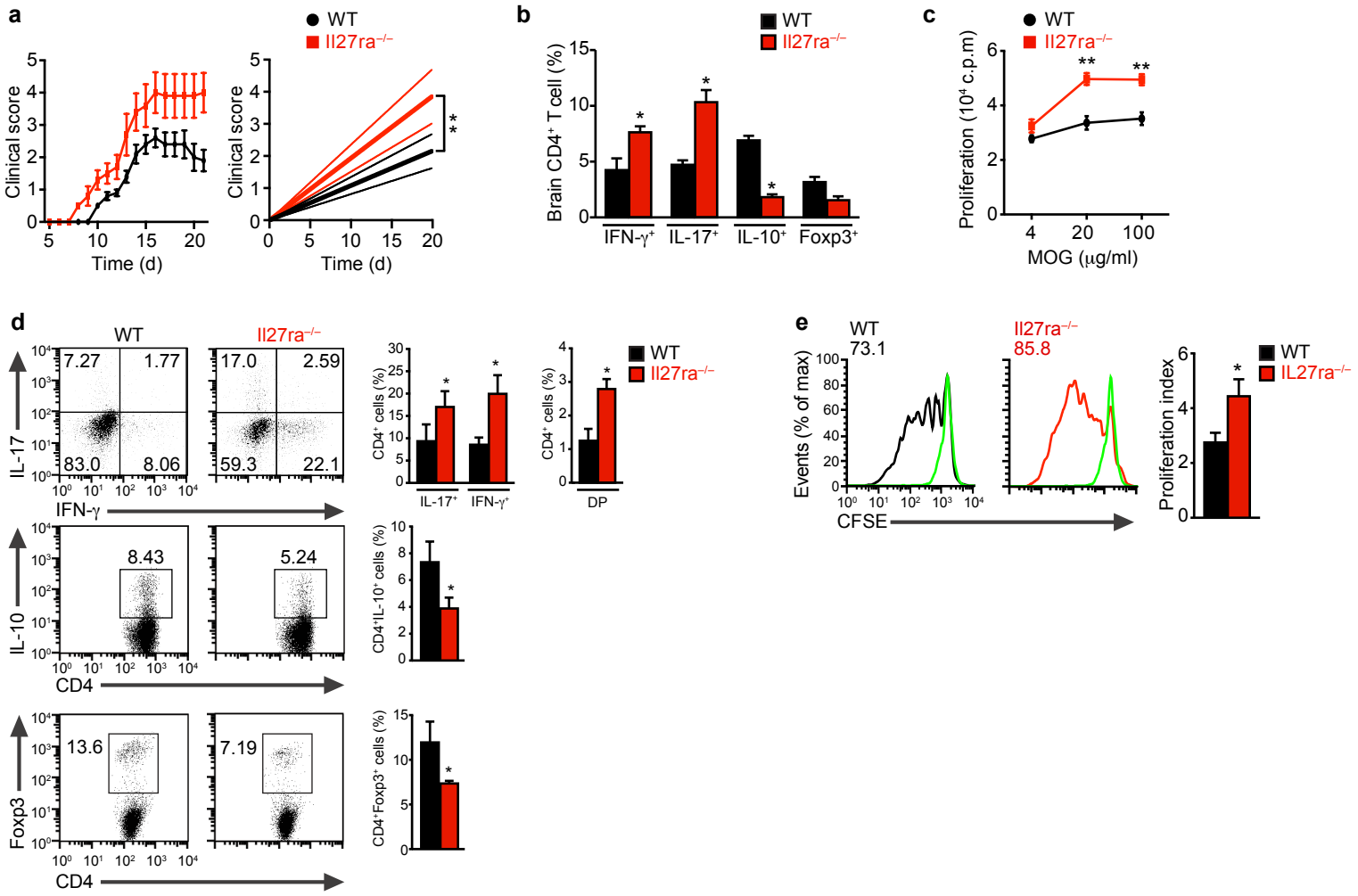


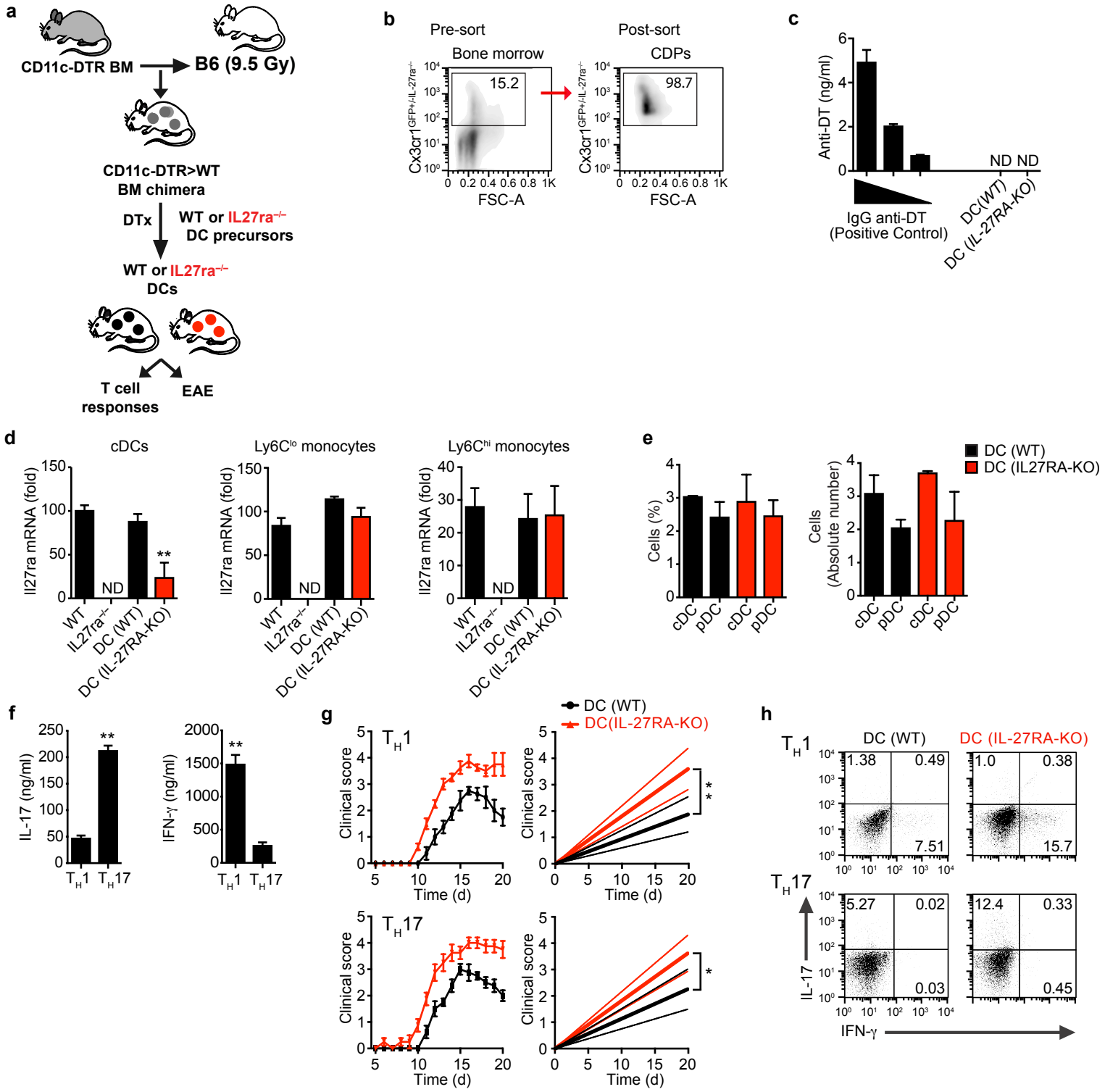
Supplementary Figure 1. Fluorescence-activated cell sorting of DCs. Splenic DCs were stained for F4/80, CD11b, CD11c, B220, MHCII and Ly6c and sorted by flow cytometry into F4/80^{-} CD11b^{-} CD11c^{low} B220^{+} MHC-II^{-} Ly6c^{+} pDCs and F4/80^{-} CD11b^{+} CD11c^{+} B220^{-} MHC-II^{+} Ly6c^{-} cDCs. Numbers adjacent to outlined areas indicate percentage of positive cells. Data are from one of more than 3 independent experiments with similar results.



Supplementary Figure 2. IL-27 signaling in DCs modulates MHC-II and co-stimulatory molecule expression in DCs. Flow cytometry analysis of ecLPS-treated cDC in the presence or absence of IL-27. Representative histograms of three independent experiments, the staining obtained with isotype control antibodies is shown in gray.

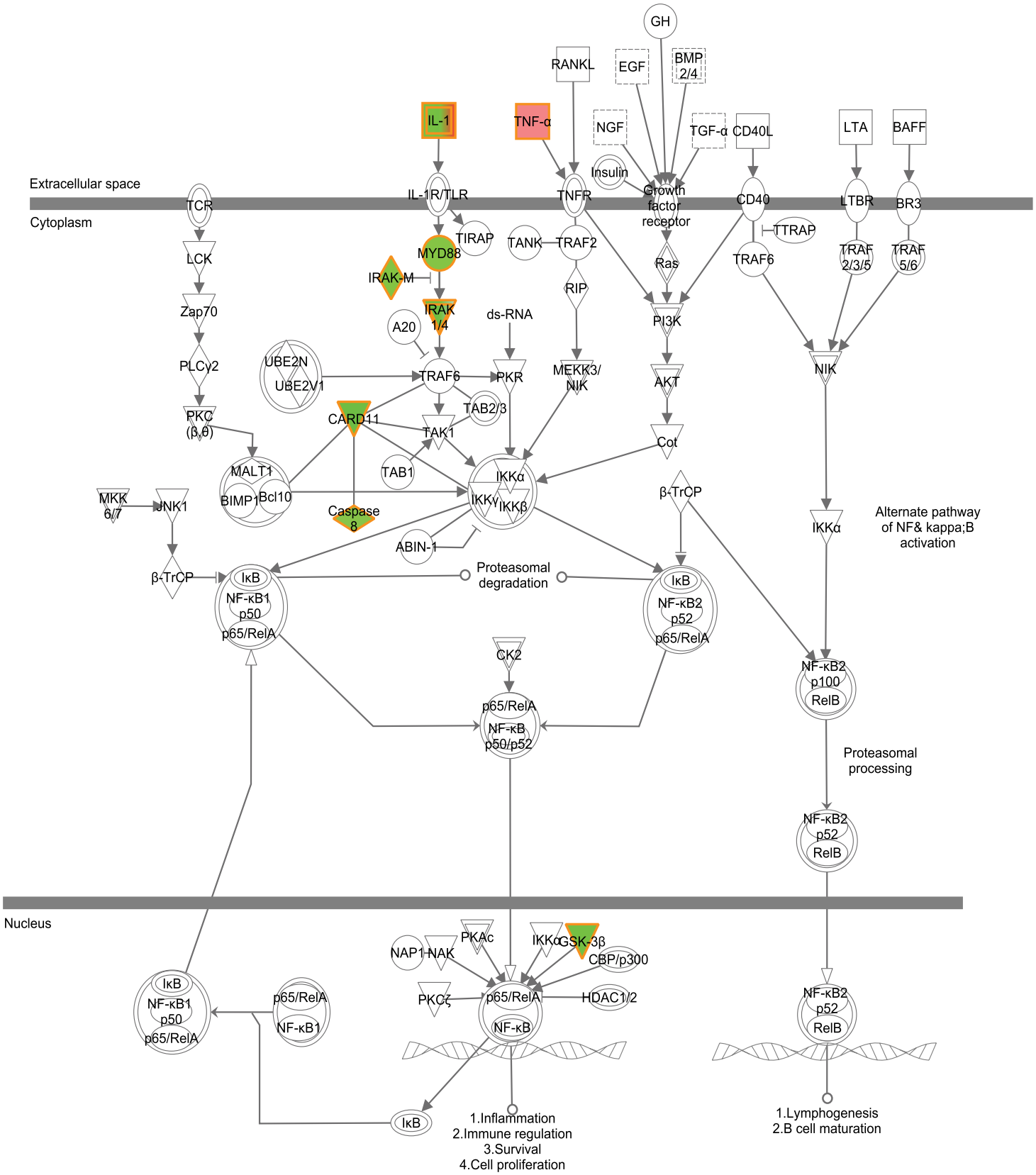


Supplementary Figure 3. IL-27 limits effector T-cell differentiation and EAE development. (a) Development of EAE in WT and Il-27ra$^{-/-}$ mice, clinical score (left panel) and linear-regression curves of disease for each group (dashed lines indicate 95% confidence intervals). **(b)** CNS-infiltrating CD4$^+$ T cells analyzed for the expression of IFN-γ, IL-17, IL-10 and Foxp3 by flow cytometry. **(c)** Recall response to MOG (35-55) in splenocytes from WT and Il-27ra$^{-/-}$ mice isolated 21 days after EAE induction. **(d)** Frequency of CD4$^+$CD44$^+$CD40Lhi splenic IFN-γ$^+$, IL-17$^+$, IFN-γ$^+$ IL-17$^+$ (DP), IL-10$^+$ and Foxp3$^+$ CD4$^+$ T cells in WT and Il-27ra$^{-/-}$ mice 21 days after EAE induction. **(e)** Naive CFSE labeled 2D2$^+$ CD4$^+$ T cells were stimulated with MOG (35–55) and cDCs sorted from WT and Il-27ra$^{-/-}$ mice 21 days after immunization, and T-cell proliferation was analyzed. The frequency of proliferated cells is shown in the histogram and the proliferation index is shown in the right **(e)**. Numbers within histograms show the percentage of positive cells. Shown is a representative experiment (of three) with n ≥ 5 mice/group. *P < 0.05 and **P < 0.01 (Student's t-test).



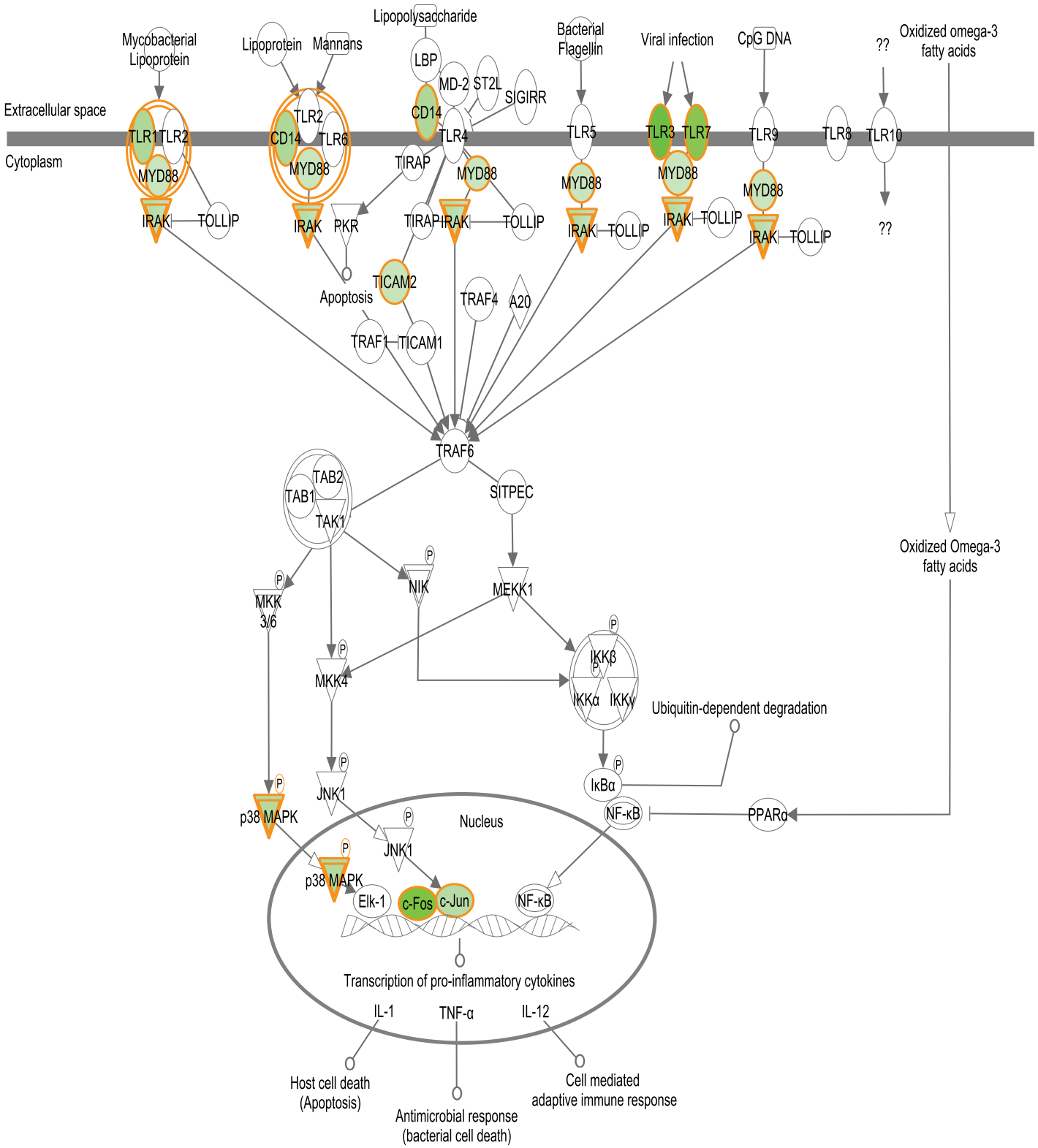
Supplementary Figure 4. Generation of mice lacking IL-27RA expression in DCs. (a) Lethally irradiated WT mice were reconstituted with bone marrow (BM) from mice expressing the diphtheria toxin receptor (DTR) under the control of the CD11c (itgax) promoter (CD11c-DTR mice). Following reconstitution, DCs were depleted by the administration of diphtheria toxin (DTx) and DCs compartment was reconstituted with DC precursors from WT (Cx3Cr1-GFP$^{+/-}$WT) or Il-27ra$^{-/-}$ (Cx3Cr1-GFP$^{+/-}$Il-27ra$^{-/-}$) mice. **(b)** Representative flow cytometry analysis of DCs precursors (CDPs). **(c)** Antibodies against Diphtheria toxin (DT) in serum from DC (WT) and DC (IL-27RA-KO) mice. **(d)** Expression of IL27ra in cDCs, Ly6Clo and Ly6Chi monocytes sorted from naive DC (WT) and DC (IL-27RA-KO) mice, analyzed by qPCR. **(e)** Frequency (left panel) and absolute numbers of cDCs and pDCs in spleens from DC (WT) and DC (IL-27RA-KO) mice. **(f-h)** Passive transfer EAE in DC (WT) and DC (IL-27RA-KO) recipients. 2D2 mice were immunized with MOG (35–55) and 7 d after immunization T cells were cultured with MOG (35–55) in the presence of IL-12 or IL-23 and 48 h after re-stimulation IL-17 and IFN-γ secreted into the cell culture medium were determined by ELISA **(f)**. Following transfer of T_H1 or T_H17 polarized T cells into in DC (WT) and DC (IL-27RA-KO) mice, the development of EAE was monitored in the recipient mice. Clinical score (left panel) and linear-regression curves of disease for each group (dashed lines indicate 95% confidence intervals) **(g)**. CNS-infiltrating CD4$^+$ T cells analyzed for the expression of IFN-γ and IL-17, IL-10 by flow cytometry (h). $^{**}P < 0.01$ (One-way ANOVA and student's t-test) versus DC (WT). Data are representative of at least three independent experiments.

a

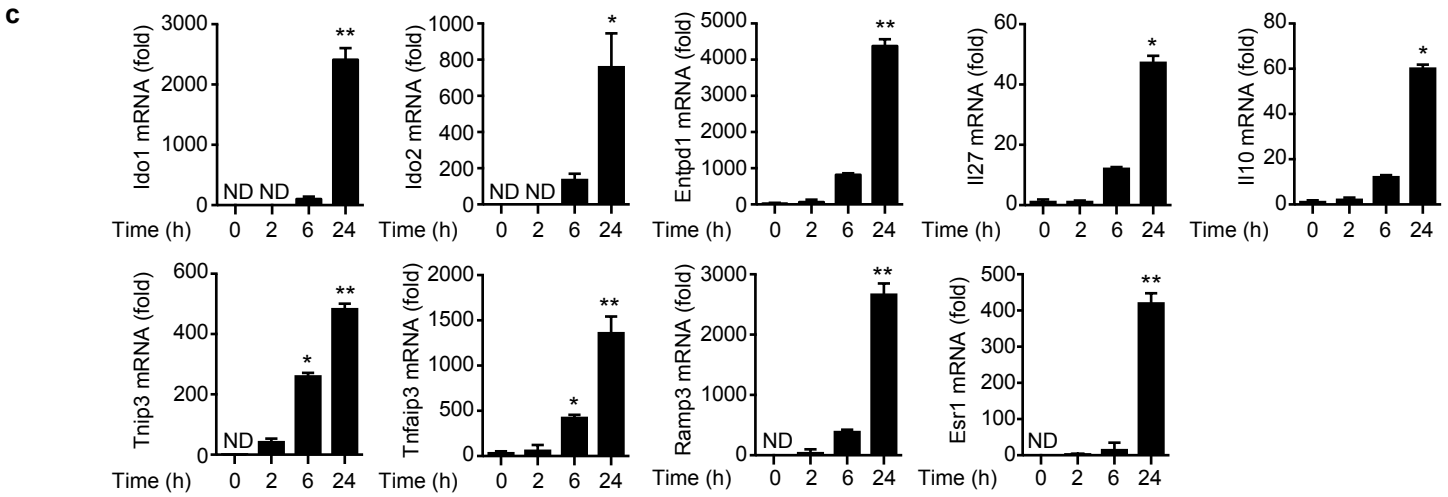


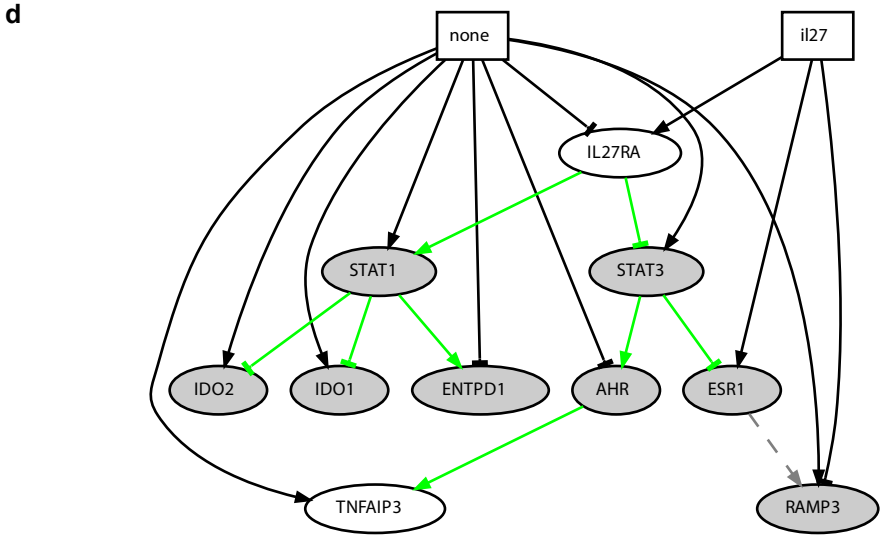
Supplementary Fig. 5

b

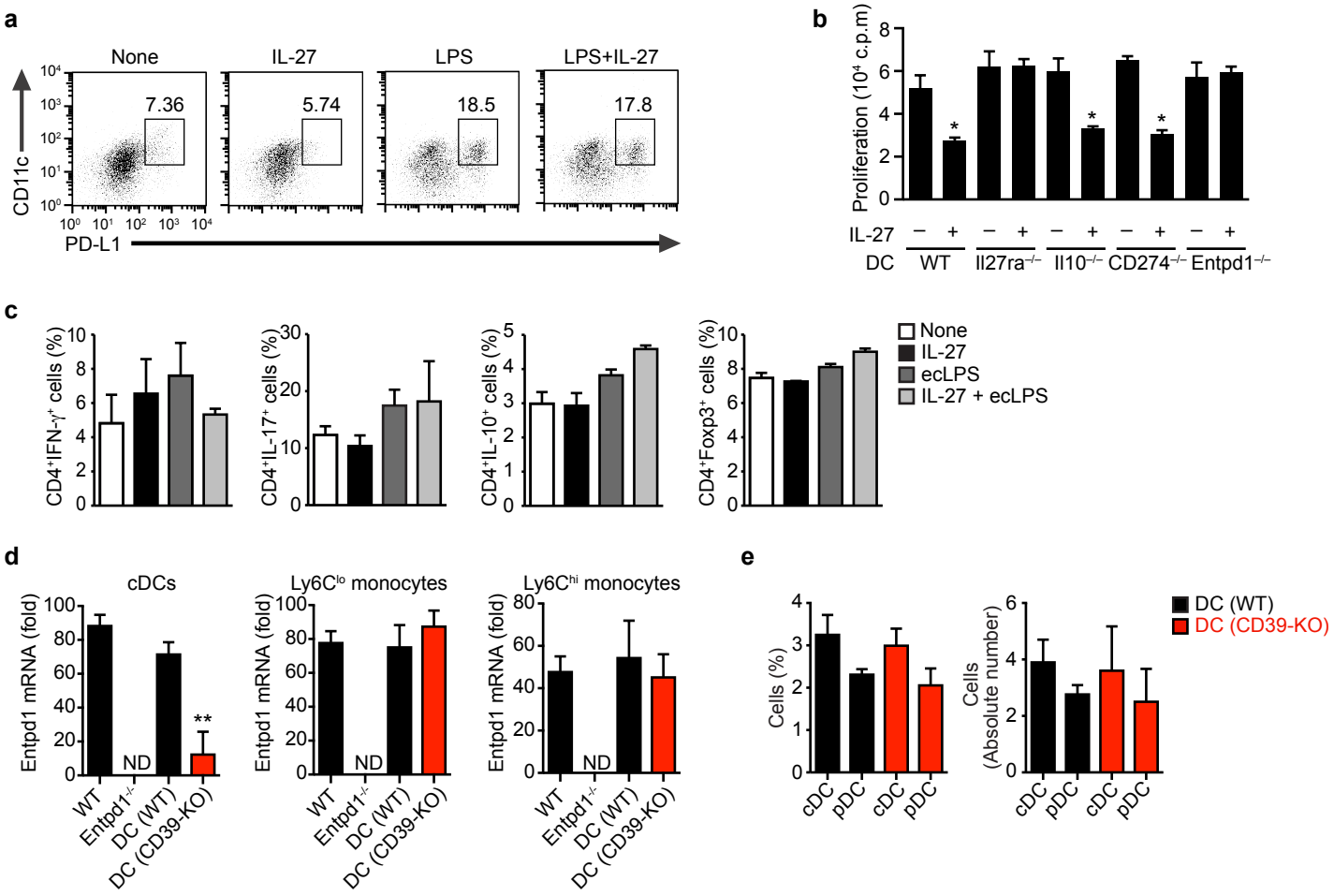


Supplementary Fig. 5

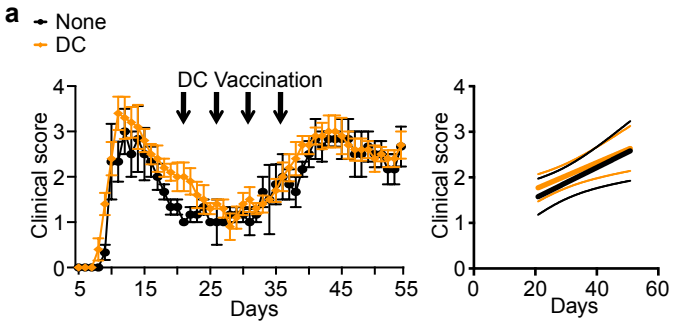


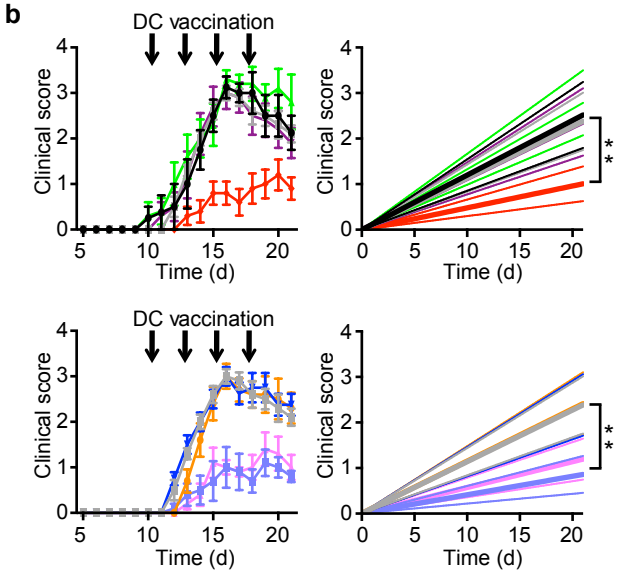


Supplementary Figure 5. Transcriptional effects of IL-27 on cDCs. **(a,b)** Ingenuity Pathway Analysis (IPA) of the transcriptional effects of IL-27 in DCs identified significant effects of IL-27 on NF-kB **(a)** and Toll-like Receptor **(b)** signaling pathways. In NF-kB and Toll-like Receptor Signaling pathways, green shaded regions indicate down-regulation and red shaded regions indicate up-regulation of genes. **(c)** Time course of Ido1 and Ido2, Entpd1, Il27, Il10, Tnip3, Tnfaip3, Ramp3 and Esr1 expression measured by quantitative real-time PCR in cDCs treated with IL-27 for 0, 2, 6, and 24 h. Results are shown relative to the expression of mRNA encoding Gapdh. **(d)** Computational model of the effects of IL-27 on DCs generated with NetGenerator. Integrated interactions in splenic IL-27-treated cDCs compared with untreated cDCs are shown. *$P < 0.05$ and **$P < 0.01$ (One-way ANOVA) compared with untreated cDCs (Time 0).



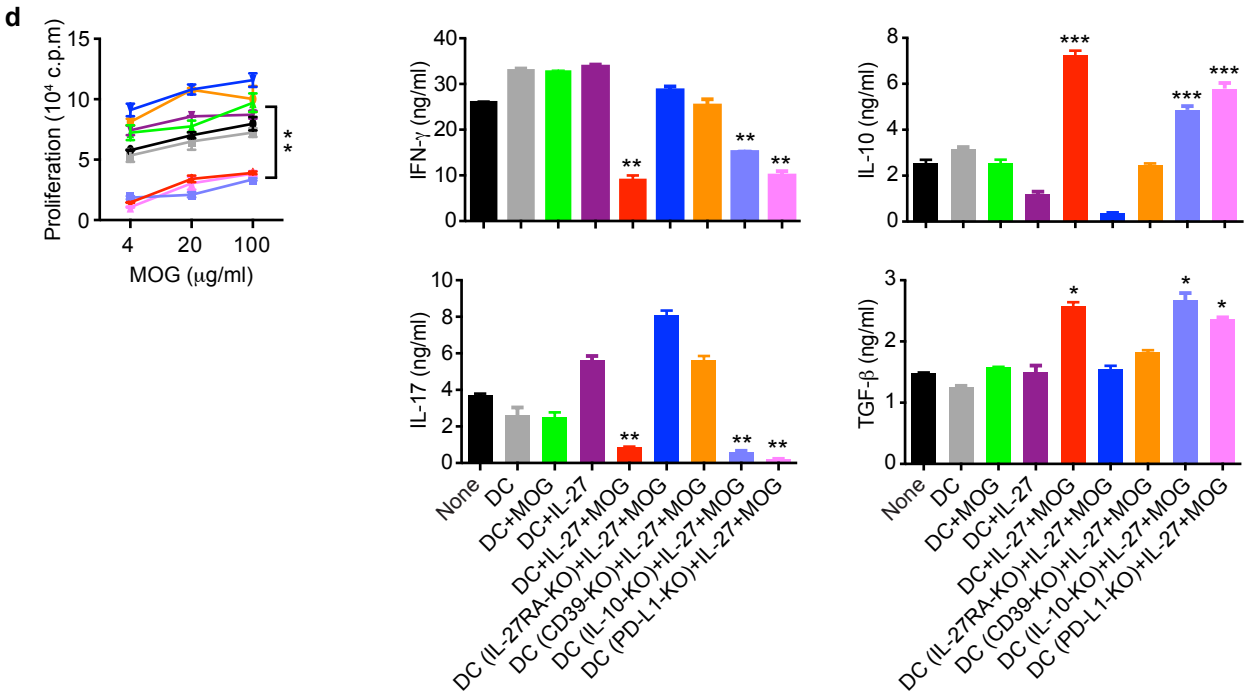
Supplementary Figure 6. ENTPD1 is required for the effects of IL-27 on DCs. (a) PD-L1 expression in IL-27-treated cDC in the presence or absence of ecLPS. Numbers adjacent to outlined areas indicate percentage of CD11c PD-L1 positive cells. **(b)** Naive $CD4^+$ T cells were stimulated with anti-CD3 and ecLPS- or ecLPS+IL-27-treated WT, Il27ra$^-$, Il10$^-$, CD274 (PD-L1)$^-$ or Entpd1 (CD39)-deficient cDCs and proliferation was analyzed. **(c)** Naive $CD4^+$ T cells were stimulated with anti-CD3 and ecLPS- or ecLPS+IL-27-treated Entpd1-deficient cDCs and the differentiation of IFNγ$^+$, IL-17$^+$, IL-10$^+$ and $Foxp3^+$ T cells was analyzed by flow cytometry. **(d)** Entpd1 expression in cDCs, Ly6Clo and Ly6Chi monocytes sorted from naive DC (WT) and DC (CD39-KO) mice, analyzed by qPCR. **(e)** Frequency (left panel) and absolute numbers of cDCs and pDCs in spleens from DC (WT) and DC (CD39-KO) mice. *P < 0.05; **P < 0.01 (One-way ANOVA). Data are representative of at least three independent experiments.





c

Treatment	n	Maximum score	*P* value
None	5	3.125 ± 0.24	—
DC	5	3.000 ± 0.16	NS
DC+MOG	5	3.300 ± 0.20	NS
DC+IL-27	5	3.000 ± 0.35	NS
DC+IL-27+MOG	5	1.200 ± 0.34	0.05
DC (IL-27RA-KO)+IL-27+MOG	5	3.000 ± 0.93	NS
DC (CD39-KO)+IL-27+MOG	5	3.000 ± 0.74	NS
DC (IL-10-KO)+IL-27+MOG	5	1.100 ± 0.29	0.01
DC (PD-L1-KO)+IL-27+MOG	5	1.400 ± 0.37	0.05



Supplementary Figure 7. Vaccination with IL-27 conditioned DCs suppresses EAE. EAE was induced by immunization of naive SJL mice with PLP (131–159), and DCs were administered i.v. 4 times, once every 4 days, starting at day 20. **(a)** The course of EAE is shown as the mean EAE score ± SEM (n = 5 mice per group) for the whole observation period (left panel), and also as the linear regression curves of the disease for each group from day 20 until the termination of the experiment. Arrows indicate DC vaccine administration. **(b-d)** EAE was induced by immunization of naive B6 mice with MOG (35-55), and DCs were administered i.v. 4 times, once every 4 days, starting at day 10 after EAE induction. **(b)** The course of EAE is shown as the mean EAE score ± SEM (n = 5 mice per group) for the whole observation period (left panel), and also as the linear regression curves of the disease for each group. Arrows indicate DC vaccine administration. **(c)** Effects of therapeutic DC vaccination on B6 EAE. **(d)** Recall proliferative and cytokine response to MOG (35-55) in splenocytes taken from DCs-treated mice 21 days after EAE induction. Data are representative of at least three independent experiments. NS, not significant. **P* < 0.05, ***P* < 0.01 and ***P* < 0.001 (One-way ANOVA) versus control mice.

Supplmentary table 1. Effect of DC vaccination on experimental autoimmune encephalomyelitis in SJL mice

Treatment	n	Maximum score	*P* value	Relapse rate	*P* value
None	5	1.790 ± 0.86	—	3.5 ± 0.50	—
DC+IL-27	5	2.063 ± 0.83	NS	3.0 ± 0.74	NS
DC+PLP	5	2.012 ± 0.68	NS	2.8 ± 0.83	NS
DC+IL-27+PLP	5	1.203 ± 0.62	0.03	1.3 ± 0.27	0.03

NS, not significant.

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