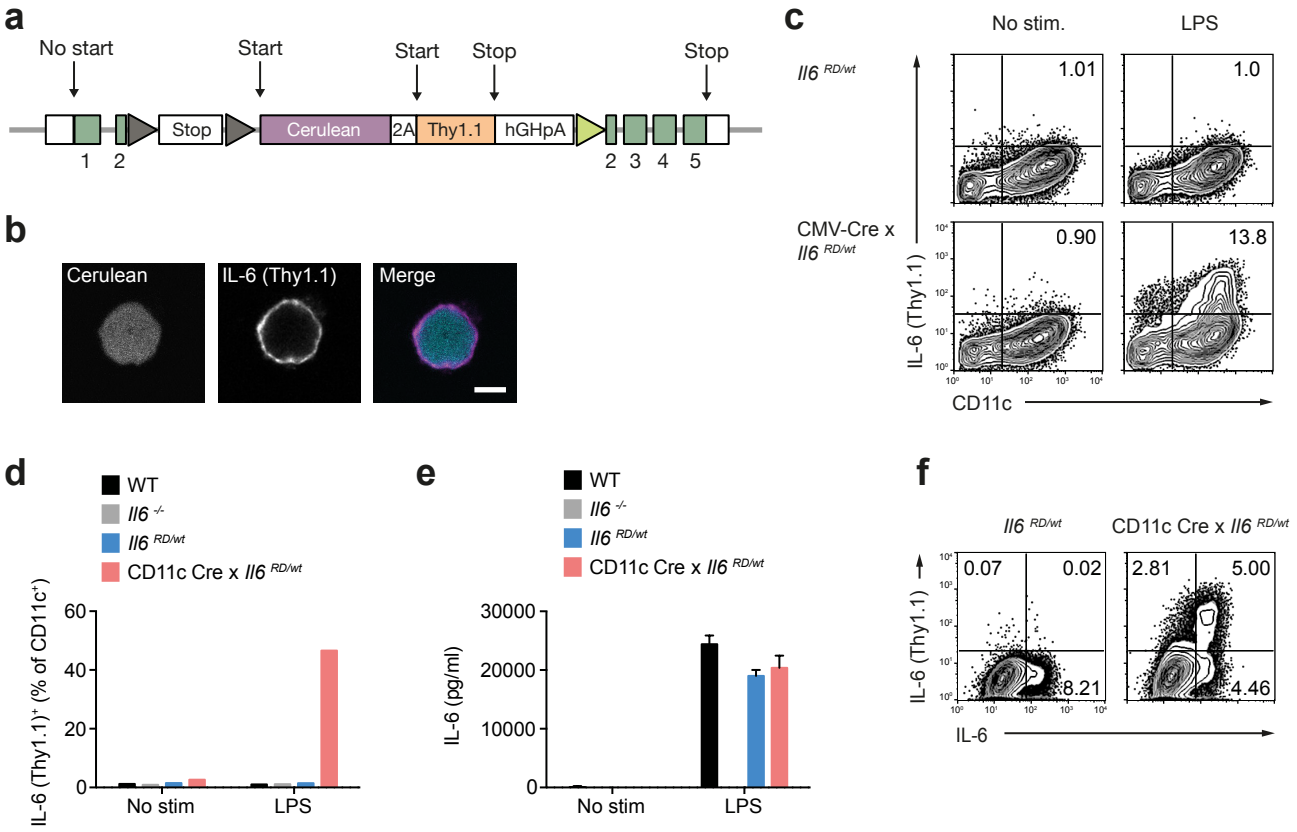
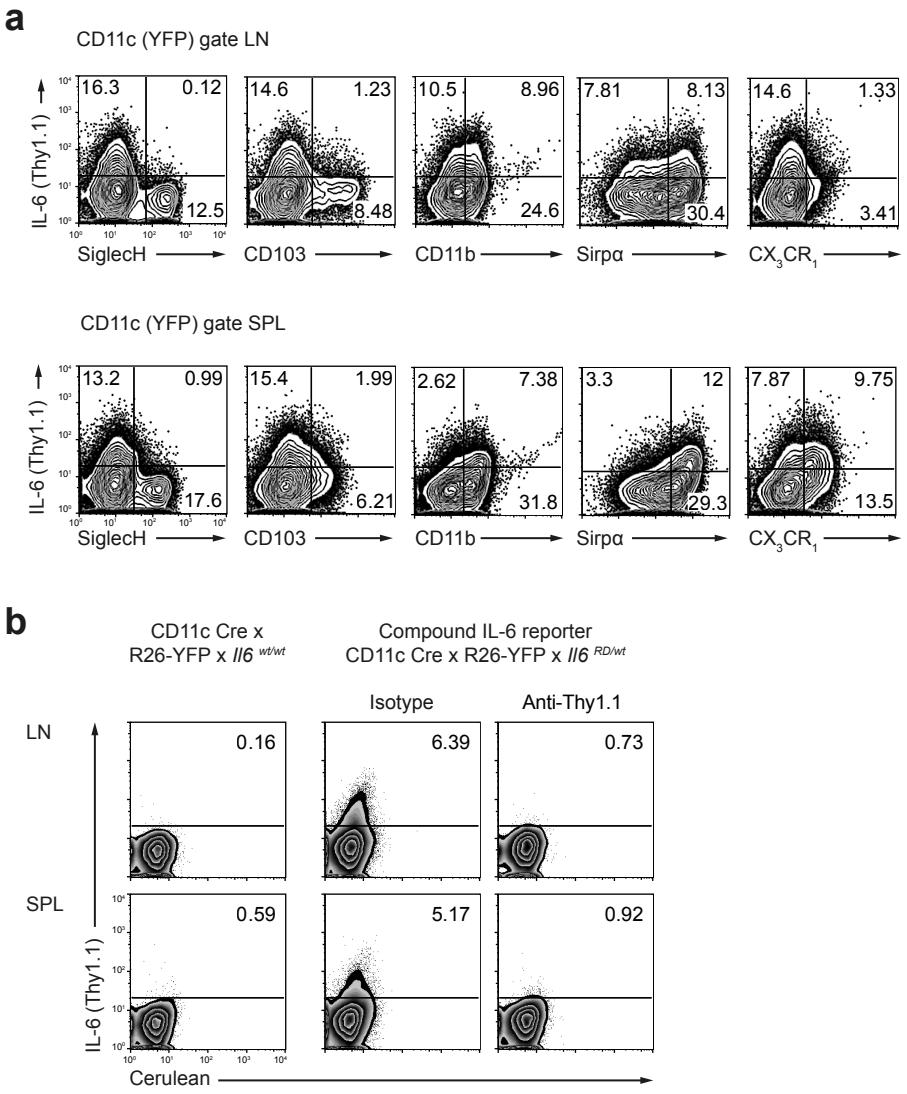
Supplementary Figure 1



Supplementary Figure 1. IL-6 reporter mice.

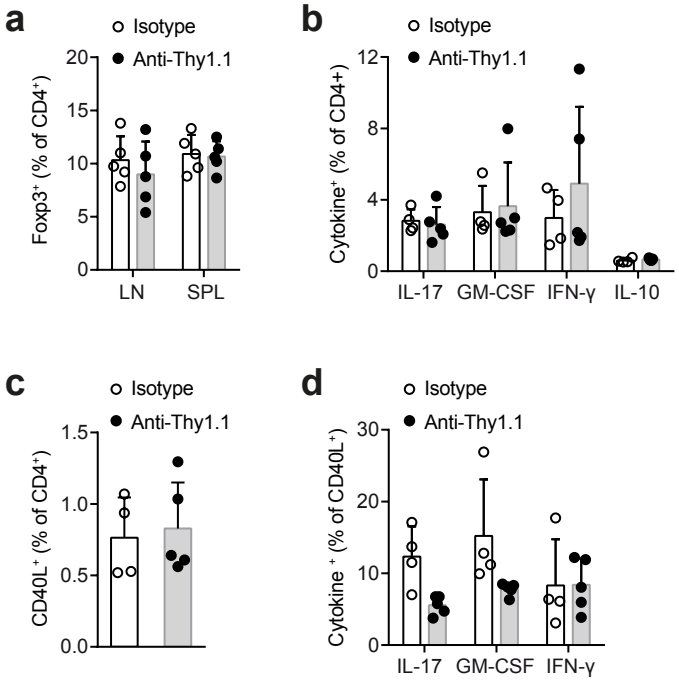
(**a**) Targeted Il6 locus. The reporter cassette including a floxed stop cassette was introduced into exon 2 of the *Il6* locus. Since the locus is disrupted by this knock-in construct, IL-6 reporter mice were bred heterozygously and compared with *Il6*$^{+/-}$ mice since *Il6* produces a gene dose effect (data not shown). (**b**) Bone marrow derived dendritic cells (BMDCs) were prepared from CMV Cre x *Il6*$^{RD/wt}$ mice and stimulated *in vitro* with CpG. Cerulean was expressed in the cytoplasm (left) and Thy1.1 at the cell surface (middle, merge right) as expected. Confocal microphotographs, Scale bar 10 µm. (**c**) *Il6*$^{RD/wt}$ control BMDCs or BMDCs prepared from IL-6 reporter mice (CMV Cre x *Il6*$^{RD/wt}$) were stimulated with LPS followed by flow cytometric assessment of Thy1.1 expression. (**d-f**) Correlation of Thy1.1 expression and IL-6 expression in BMDCs. BMDCs were prepared from different mouse strains as indicated and stimulated with LPS followed by analysis of Thy1.1 expression (**d**) and IL-6 production as measured by ELISA (**e**). (**f**) Co-expression of IL-6 and Thy1.1. Control BMDCs (*Il6*$^{RD/wt}$) and IL-6 reporter BMDCs (CD11c Cre x *Il6*$^{RD/wt}$) were stimulated with CpG for 6 h in the presence of Brefeldin A for the last 2 h followed by combined surface staining for Thy1.1 and intracellular staining for IL-6.



Supplementary Figure 2. Characterization of IL-6 (Thy1.1) expressing DCs *in vivo*.

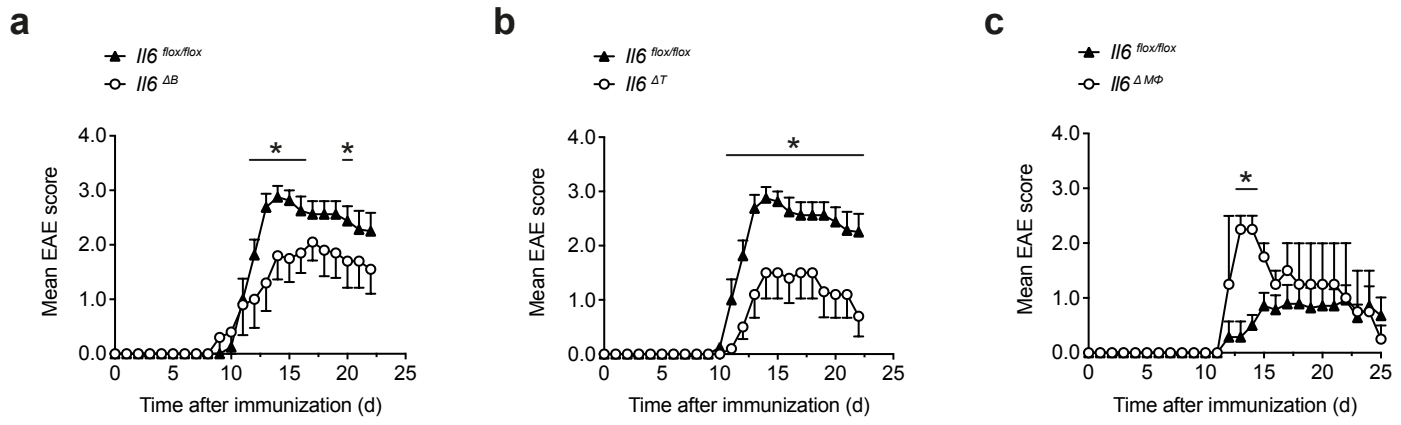
(**a**) *Il6*$^{RD/wt}$ mice were crossed to CD11c Cre and R26 *Stop*$^{flox/flox}$ YFP mice to generate compound heterozygous mice with DC conditional expression of an IL-6 reporter allele and YFP. In order to visualize large amounts of IL-6 producing DCs *ex vivo*, we injected Flt3L producing melanoma cells *s.c.* to expand DCs *in vivo* and 6 days later, treated the animals with LPS (3mg/kg LPS [E. coli 0111:B4]) *i.p.* to stimulate IL-6 production. Two days after LPS injection, lymph nodes (LN) and spleen (SPL) were prepared and stained for Thy1.1 to visualize IL-6^{+} DCs by flow cytometry directly *ex vivo*. In order to analyze whether Thy1.1 (IL-6)$^{+}$ DCs segregate into a specific DC subset, the indicated surface molecules were co-stained. (**b**) IL-6 Reporter mice allow for IL-6 conditional deletion of DCs *in vivo*. CD11c Cre x *Il6*$^{RD/wt}$ x R26 *Stop*$^{flox/flox}$ YFP mice were treated with Flt3L producing melanoma cells and LPS. The mice were then assigned to treatment with either Isotype (mouse IgG2a, C1.18.4) or anti-Thy1.1 (19E12) in order to deplete IL-6^{+} DCs. One day later, lymph nodes (LN) and spleen (SPL) were prepared and stained for Thy1.1 (OX-7) to visualize IL-6^{+} DCs by flow cytometry directly *ex vivo*. CD11c Cre x R26 *Stop*$^{flox/flox}$ YFP x *Il6*$^{wt/wt}$ mice that were treated identically as the DC conditional IL-6 reporter mice are shown as a "negative" staining control for Thy1.1 (left).

Supplementary Figure 3



Supplementary Figure 3. *In vivo* priming of T cell responses in the absence of IL-6 producing DCs.

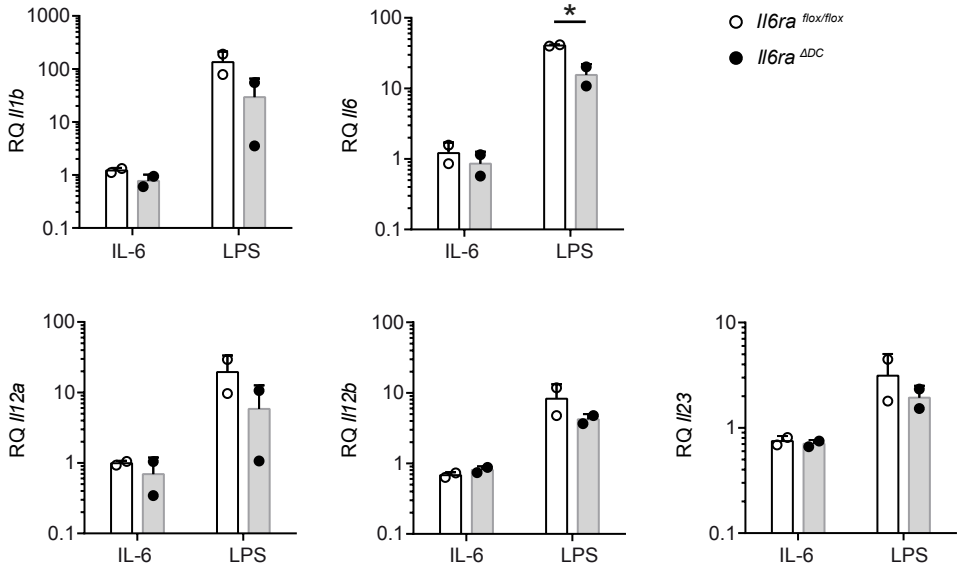
DC conditional IL-6 reporter mice (CD11c Cre x *Il6*$^{RD/wt}$) were immunized with MOG_{35-55} in CFA followed by control treatment (mouse IgG2a isotype) or anti-Thy1.1 (19E12) to deplete IL-6 (Thy1.1)$^+$ DCs. Antibody treatment was performed by *i.p.* injection of 200 µg antibody every other day starting on day 1 after immunization. On day 7 after immunization, draining lymph nodes (LN) and spleen (SPL) were prepared and stained for Foxp3 to quantify the fraction of Tregs in the CD4$^+$ T cell compartment (**a**). (**b**) Subsequent to PMA/ionomycin restimulation, LN CD4$^+$ T cells were stained intracellularly for IL-17, GM-CSF, IFN-γ, and IL-10. (**c, d**) Antigen specific T cell responses were assessed by intracellular staining of CD40L (CD154) and cytokines in splenic CD4$^+$ T cells of control treated or IL-6$^+$ DC depleted mice after recall with MOG_{35-55}. Mean + SD (n=5 mice per genotype).



Supplementary Figure 4. Ablation of *Il6* in B cells, T cells, or macrophages does not result in resistance to MOG_{35-55} induced EAE.

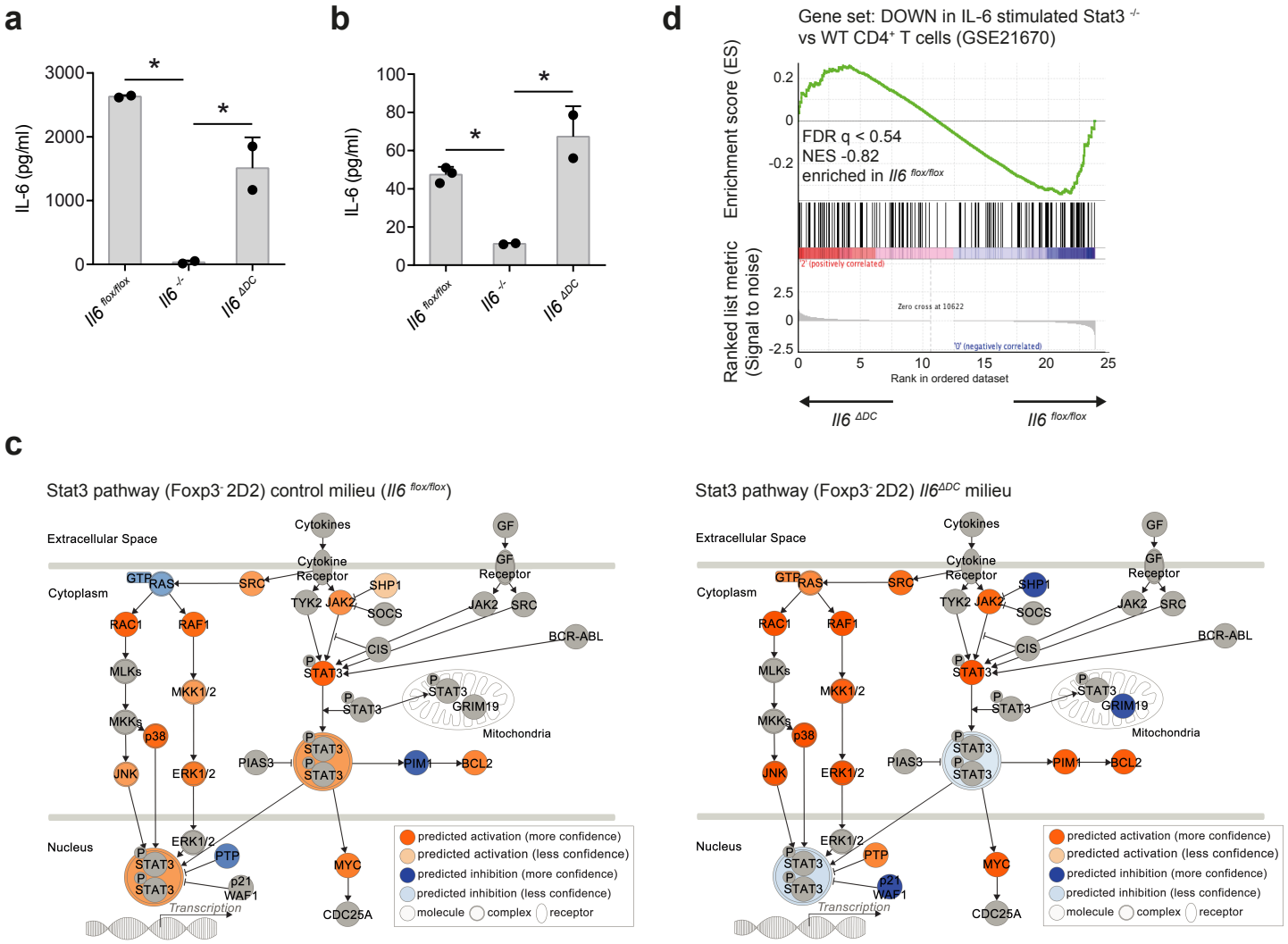
Course of MOG_{35-55} induced EAE in mouse strains with conditional ablation of *Il6* in B cells (**a**), T cells (**b**), and $LysM^+$ myeloid cells (**c**). Mice were subcutaneously immunized with MOG_{35-55} in CFA and i.v. injected with pertussis toxin on days 0 and 2. Mean clinical EAE score and SEM, n ≥ 4 per group. *P<0.05, ANOVA plus Fisher's LSD test for individual days.

Supplementary Figure 5



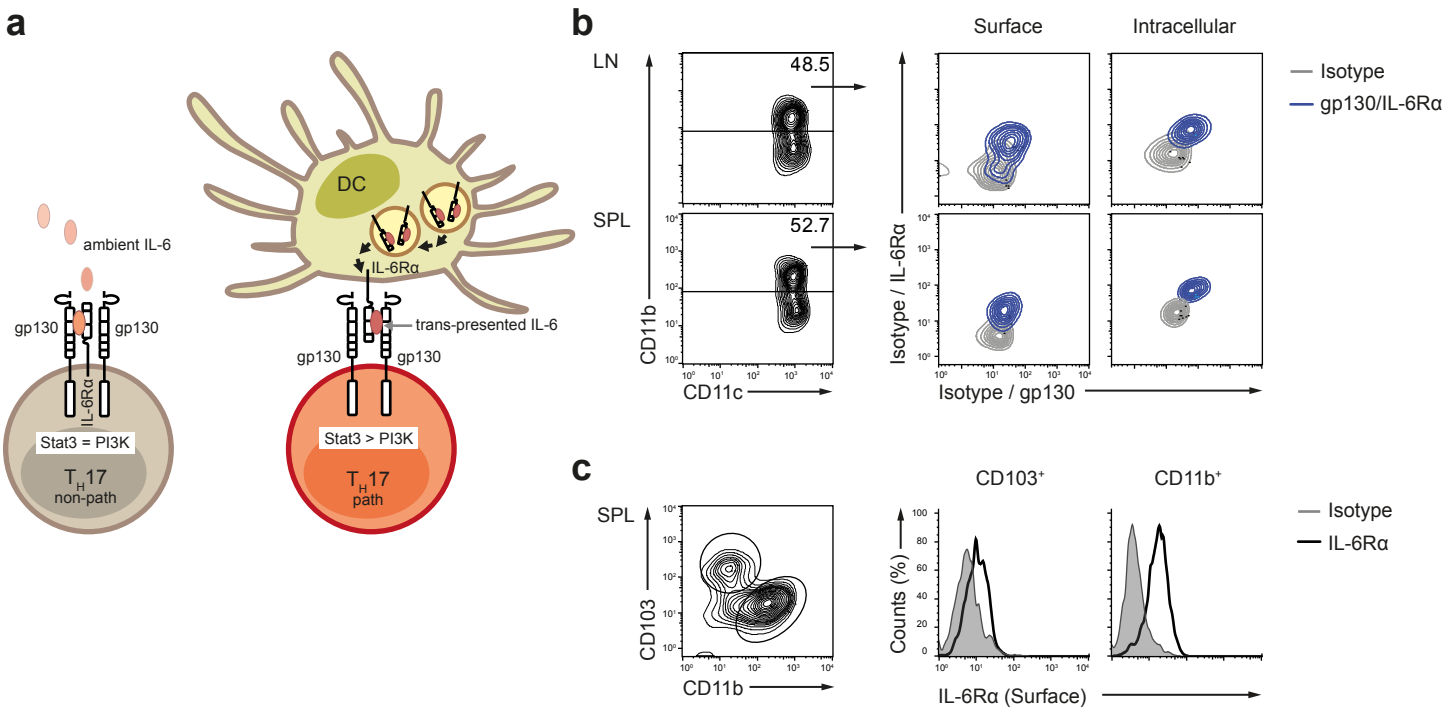
Supplementary Figure 5. *Il6ra*$^{-/-}$ BMDCs are not deficient in the production of pro-inflammatory cytokines in response to LPS stimulation.

Control *Il6ra*$^{flox/flox}$ or IL-6Rα deficient BMDCs (*Il6ra*$^{\Delta DC}$) were stimulated over night with either IL-6 or LPS followed by analysis of *Il1b, Il6, Il12a, Il12b,* and *Il23* mRNA production by quantitative RT PCR. Mean + SD of technical replicates. One out of two independent experiments. *P<0.05, ANOVA plus Sidak's multiple comparisons test.



Supplementary Figure 6. DC derived IL-6 is required for robust Stat3 activation in T cells.

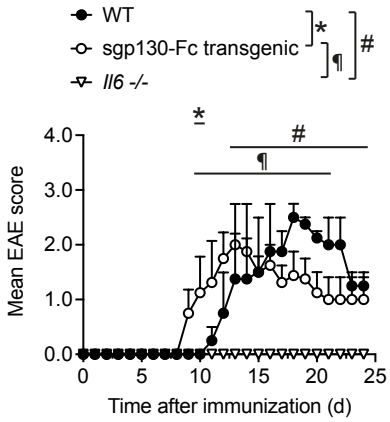
Subcutaneous immunization with a peptide antigen in CFA induces similar amounts of serum IL-6 in control mice (*Il6*$^{flox/flox}$) and *Il6*$^{\Delta DC}$ mice. Control animals (*Il6*$^{flox/flox}$), *Il6*$^{-/-}$ mice, and *Il6*$^{\Delta DC}$ mice were either injected with LPS (**a**) to induce systemic IL-6 or immunized subcutaneously with MOG_{35-55} in CFA (**b**). Serum samples were collected 5 h after LPS injection or 1 day after subcutaneous immunization for the assessment of IL-6 by ELISA (n=3, SD, *P<0.04, One-way-ANOVA plus Tukey's multiple comparisons test). (**c, d**) RNA Seq analysis was performed in 2D2 T cells re-isolated from draining lymph nodes of control hosts (*Il6*$^{flox/flox}$) or *Il6*$^{\Delta DC}$ hosts after immunization with cognate MOG peptide. (**c**) Ingenuity pathway analysis was performed to evaluate the strength of Stat3 pathway activation in control primed (left panel) or *Il6*$^{\Delta DC}$ primed (right panel) 2D2 effector T cells. (**d**) Notably, in contrast to T cells primed in a control milieu, *Il6*$^{\Delta DC}$ primed T cells exhibited a weakened "Stat3" signature when their RNA profile was directly tested for the enrichment of Stat3 dependent genes by GSEA (see also Supplementary Tables).



Supplementary Figure 7. IL-6 cluster signaling and surface and intracellular expression of IL-6Rα and gp130 by cDC2.

(**a**) Scheme of IL-6 cluster signaling. IL-6 is loaded onto the IL-6Rα in intracellular compartments of DCs and is brought to the cell membrane as an IL-6-IL-6Rα complex. During a cognate interaction between DCs and T cells, DCs present IL-6 via their IL-6Rα *in trans* to T cells. Trans-presentation of IL-6 leads to the engagement of gp130 on the T cell side and induces a pathogenic phenotype in sensitized T cells. (**b**) cDC2 express IL-6Rα on their cell surface. WT mice were immunized with MOG_{35-55} in CFA and on day 7 after immunization, cells from draining lymph nodes (LN) and spleen (SPL) were analyzed by flow cytometry. (**b**) IL-6Rα and gp130 expression was assessed in $CD11c^{+}$MHC class $II^{+}CD11b^{+}$ cDC2 either by surface staining (left column) or by intracellular staining (right column). Grey: isotype. Blue overlay: IL-6Rα or gp130, respectively. (**c**) Surface expression of IL-6Rα was assessed on splenic cDC1 cells ($CD103^{+}$) or cDC2 cells ($CD11b^{+}$) isolated from immunized mice on day 7 after immunization.

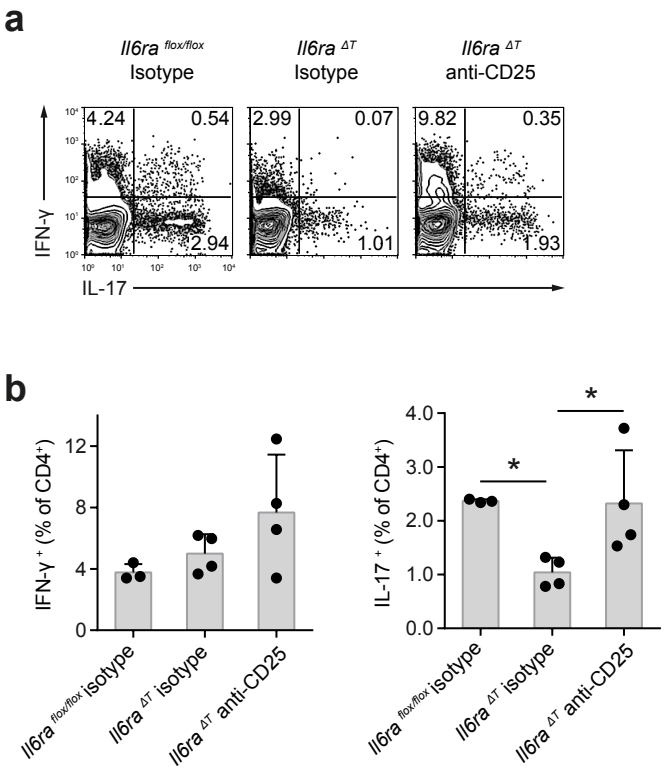
Supplementary Figure 8



Supplementary Figure 8. IL-6 trans-signaling by the soluble IL-6/IL-6Rα complex is irrelevant during MOG_{35-55} induced EAE.

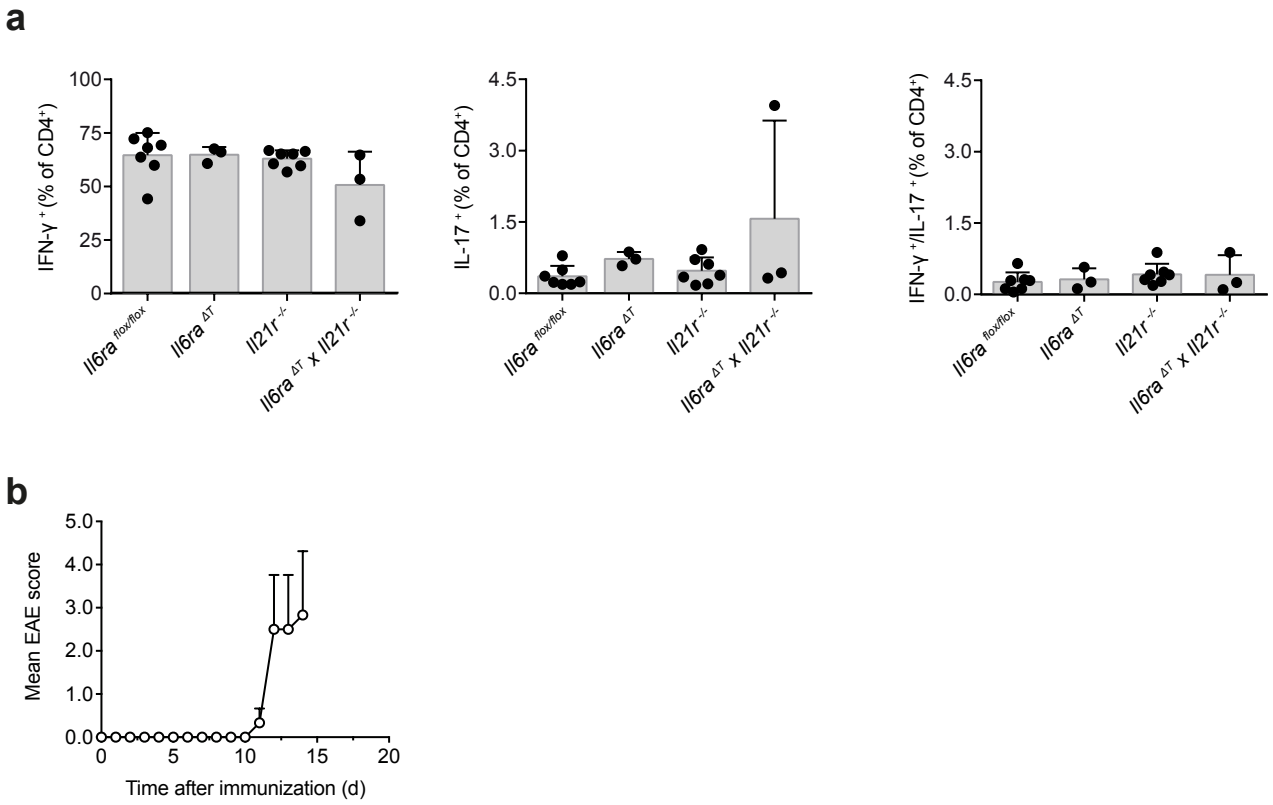
WT mice, opt_sgp130-Fc transgenic mice, and *Il6*$^{-/-}$ mice were immunized with MOG_{35-55} in CFA. Opt_sgp130-Fc transgenic mice produce large amounts of sgp130, which blocks endogenous IL-6 trans-signaling *in vivo*. Mean EAE scores + SEM, *P<0.04, ANOVA plus Tukey post test.

Supplementary Figure 9



Supplementary Figure 9. IL-6Rα deficient T cells differentiate into pathogenic T_H17 cells.

Il6ra$^{flox/flox}$ control mice and Treg sufficient or deficient (anti-CD25 treated) *Il6ra*$^{\Delta T}$ animals were immunized with MOG_{35-55} in CFA. After priming of antigen specific T cells *in vivo*, the cytokine response was assessed in $CD4^+$ T cells isolated from the spleen on day 10 after immunization after short term *ex vivo* restimulation with PMA/ionomycin and intracellular cytokine staining. (**a**) Representative cytograms of the $CD4^+$ T cell gate. (**b**) Frequency of IFN-γ producing $CD4^+$ T cells (left) and IL-17 producing $CD4^+$ T cells (right); n=3 (Ctrl), n=4 (isotype treated *Il6ra*$^{\Delta T}$) and n=4 (anti-CD25 treated *Il6ra*$^{\Delta T}$). ANOVA, Fisher's LSD post-test, *P<0.03.



Supplementary Figure 10. IL-6 cluster signaling is sufficient to induce pathogenic T_H17 cells in the simultaneous absence of classic IL-6 signaling and the IL-21 mediated alternative pathway to induce T_H17 cells.

Naive CD4+ T cells were purified from *Il6ra*$^{flox/flox}$ control mice, *Il6ra*$^{\Delta T}$ mice, *Il21r*$^{-/-}$ mice, or *Il6ra*$^{\Delta T}$ x *Il21r*$^{-/-}$ mice and transferred into *Rag1*$^{-/-}$ host animals followed by immunization with MOG_{35-55} in CFA. (**a**) Intracellular cytokine staining of T cells re-isolated from the spleen on day 14 after immunization and subjected to *ex vivo* stimulation with PMA/ionomycin. (**b**) Clinical course of EAE in *Rag1*$^{-/-}$ recipients of T cells deficient in both, IL-6Rα and IL-21R. Mean clinical score + SEM, n=3.