Supplementary information

The adaptor TRAF5 limits the differentiation of inflammatory CD4$^+$ T cells by antagonizing signaling via the receptor for IL-6

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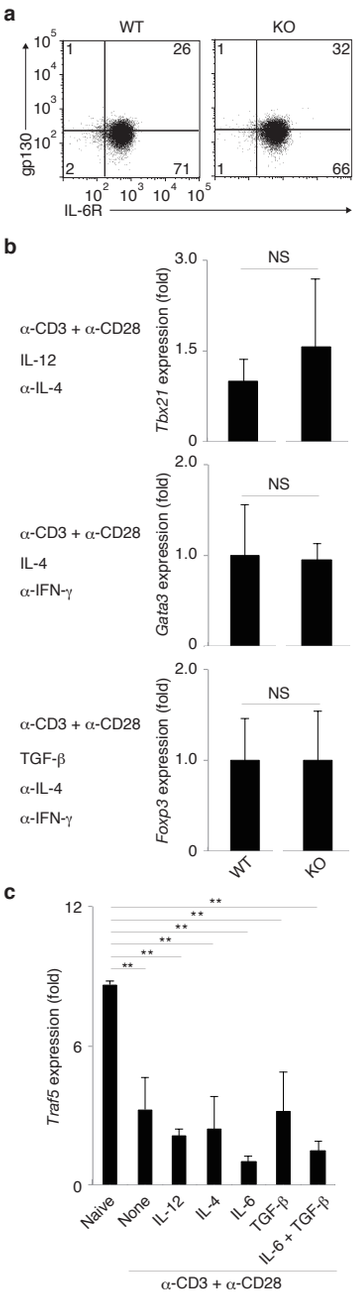
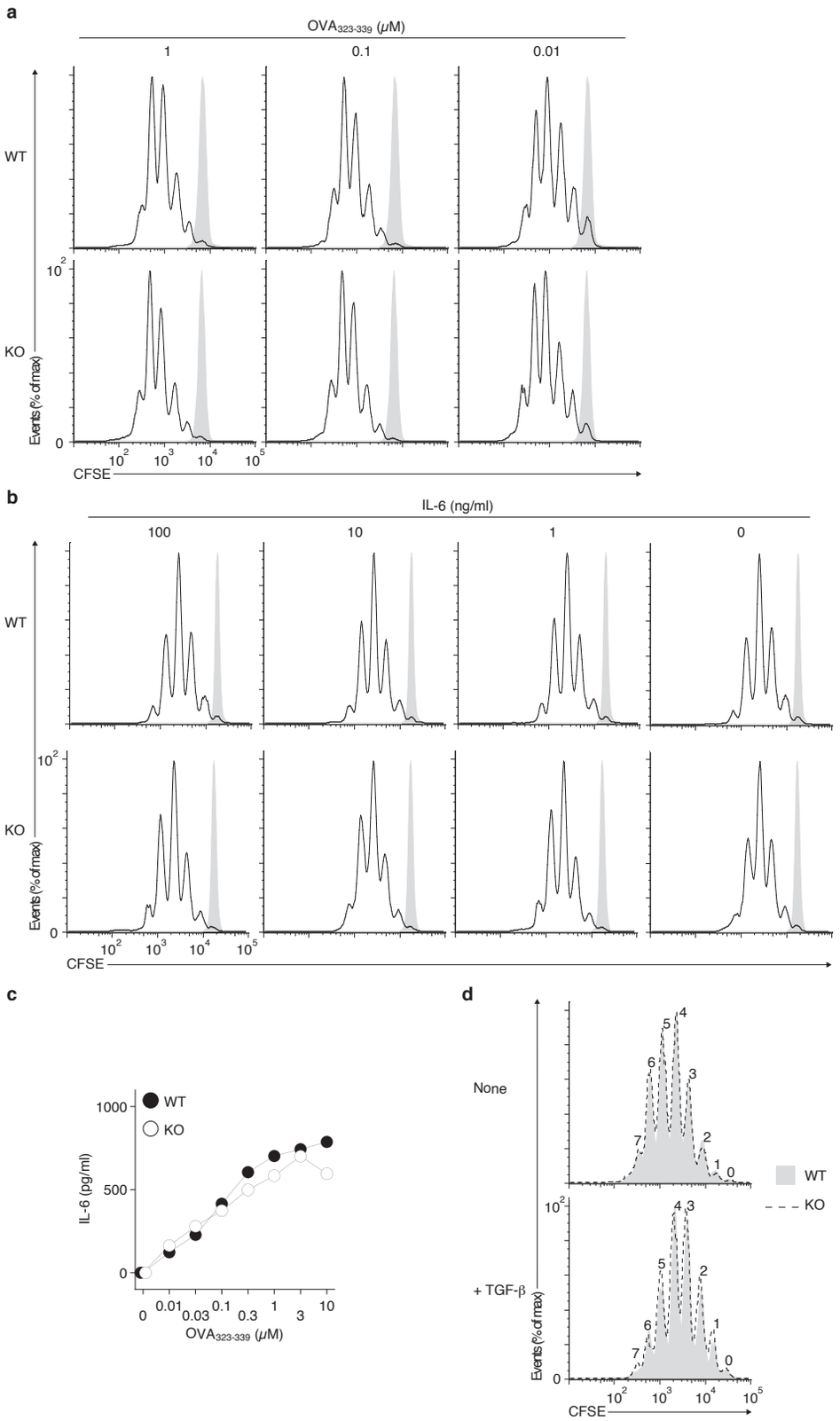


Supplementary Figure 1 Expression of gp130 and IL-6R protein and *Tbx21*, *Gata3*, *Foxp3* and *Traf5* mRNA in wild-type and *Traf5*$^{-/-}$ CD4^{+} T cells. (**a**) Expression of IL-6R–gp130 on purified wild-type and *Traf5*$^{-/-}$ naive CD4^{+} T cells. (**b**) Quantitative RT-PCR analysis of the expression of *Tbx21*, *Gata3*, and *Foxp3* mRNAs in activated CD4^{+} T cells generated from naive wild-type or *Traf5*$^{-/-}$ B6 CD4^{+} T cells cultured for 48 h with anti-CD3 and anti-CD28 in various polarizing conditions (left margin) and presented relative to the expression of the gene encoding β-Actin (average and s.d. of triplicate wells). (**c**) Quantitative RT-PCR analysis of the expression of *Traf5* mRNA in naive and activated wild-type CD4^{+} T cells cultured for 48 h with anti-CD3 and anti-CD28 in various cytokine conditions (below graph) and presented relative to the expression of the gene encoding β-Actin (average and s.d. of triplicate wells). NS, not significant; $^{**}P < 0.01$ (Student *t*-test).



Supplementary Figure 2 Wild-type and *Traf5*$^{-/-}$ OT-II T cells proliferate and produce IL-6 in response to antigen similarly. (**a**,**b**) Carboxylfluorescein diacetate, succinimidyl ester (CFSE)-dilution in antigen-responding CD4^{+} T cells generated from naive wild-type or *Traf5*$^{-/-}$ OT-II CD4^{+} T cells stimulated for 3 d with wild-type B6 splenic APCs (after depletion of T cells) and indicated concentrations (above lanes) of OVA peptide (amino acids 323-339) (**a**) or 0.1 μM OVA peptide in the presence of various concentration (above lanes) of IL-6 (**b**). (**c**) Primary IL-6 in supernatants of activated CD4^{+} T cells generated from naive wild-type or *Traf5*$^{-/-}$ OT-II CD4^{+} T cells stimulated for 3 d with indicated concentrations of OVA peptide (horizontal axis) and wild-type B6 APCs. (**d**) TGF-β-mediated proliferation arrest evaluated by CFSE-dilution in antigen-responding CD4^{+} T cells generated from naive wild-type or *Traf5*$^{-/-}$ OT-II CD4^{+} T cells stimulated for 3 d with wild-type B6 APCs and 0.1 μM OVA peptide with or without 10 ng/ml TGF-β.

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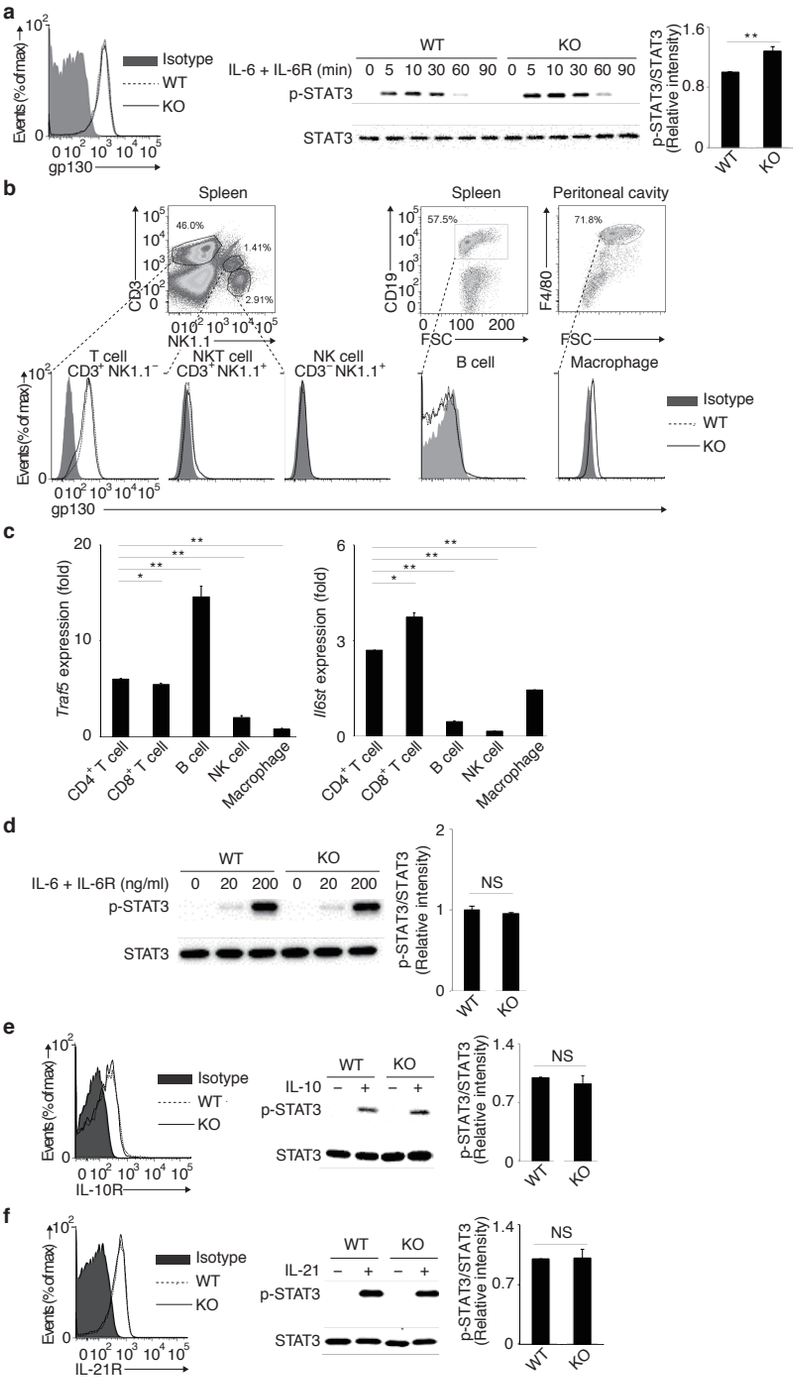

Supplementary Figure 3 Expression of gp130, STAT3 and phosphorylated STAT3 protein and *Il6st* and *Traf5* mRNA in various cells from wild-type and *Traf5*$^{-/-}$ B6 mice. (**a**) Expression of gp130 (left panel) and immunoblot analysis of phosphorylated STAT3 and total STAT3 after stimulation for various times (above lanes) with 200 ng/ml of IL-6–IL-6R (middle panel) and ratio of phosphorylated STAT3 to total STAT3 after stimulation for 10 min with 200 ng/ml IL-6–IL-6R (average and s.d. of triplicate wells, right panel) in purified splenic wild-type and *Traf5*$^{-/-}$ polyclonal CD8$^+$ T cells. Isotype, isotype-matched control antibody. (**b**) Expression of gp130 on T cells, NKT cells, NK cells, B cells, and macrophages from wild-type and *Traf5*$^{-/-}$ B6 mice. (**c**) Quantitative RT-PCR analysis of the expression of *Traf5* and *Il6st* mRNAs in different cell populations described above and presented relative to the expression of the gene encoding β-Actin (average and s.d. of triplicate wells). (**d**) Immunoblot analysis of phosphorylated STAT3 and total STAT3 in wild-type and *Traf5*$^{-/-}$ macrophages stimulated for 10 min with various concentrations (above lanes) of IL-6–IL-6R (left panel). Ratio of phosphorylated STAT3 to total STAT3 in wild-type and *Traf5*$^{-/-}$ macrophages stimulated for 10 min with 200 ng/ml of IL-6–IL-6R (average and s.d. of triplicate wells, right panel). (**e**) Expression of IL-10R (left panel) and immunoblot analysis of phosphorylated STAT3 and total STAT3 (middle panel) and ratio of phosphorylated STAT3 to total STAT3 (average and s.d. of triplicate wells, right panel) after stimulation for 10 min with 50 ng/ml of IL-10 in purified splenic wild-type and *Traf5*$^{-/-}$ polyclonal CD4$^+$ T cells. (**f**) Expression of IL-21R (left panel) and immunoblot analysis of phosphorylated STAT3 and total STAT3 (middle panel) and ratio of phosphorylated STAT3 to total STAT3 (average and s.d. of triplicate wells, right panel) after stimulation for 10 min with 10 ng/ml of IL-21 in purified splenic wild-type and *Traf5*$^{-/-}$ polyclonal CD4$^+$ T cells. NS, not significant; **P* < 0.05 and ***P* < 0.01 (Student *t*-test).

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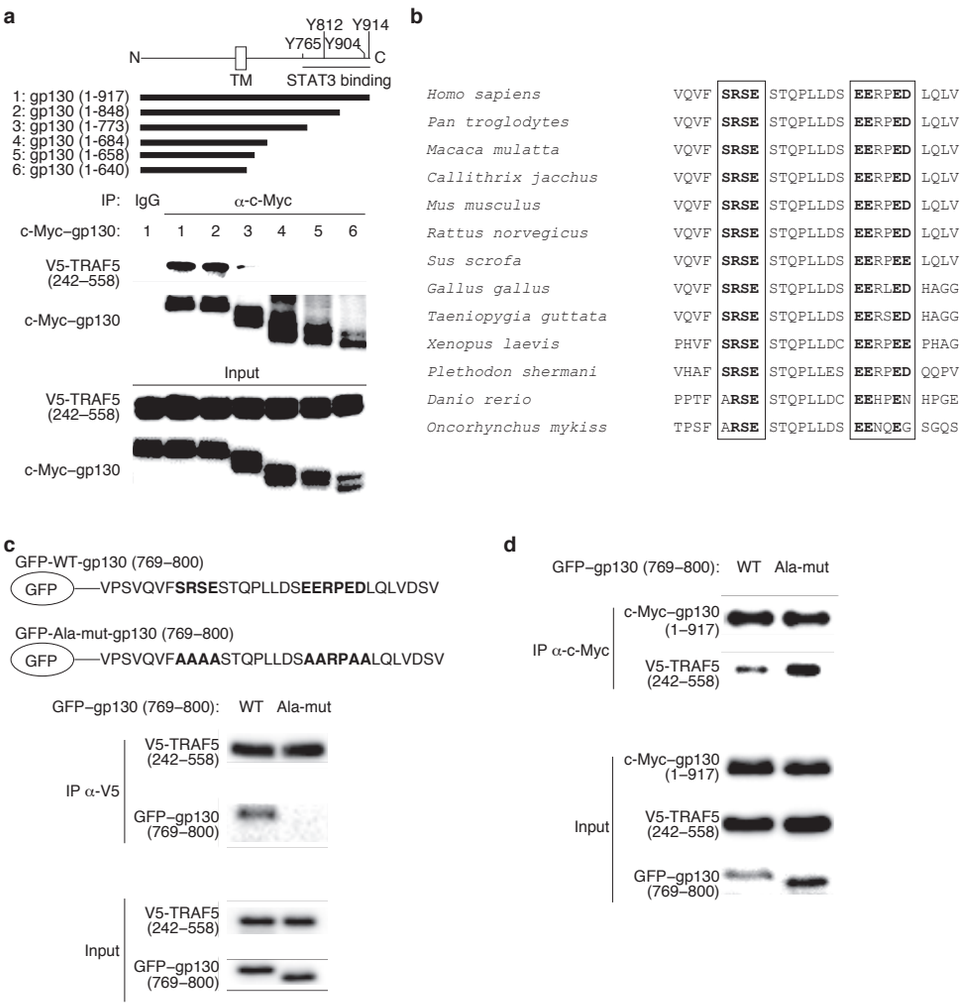
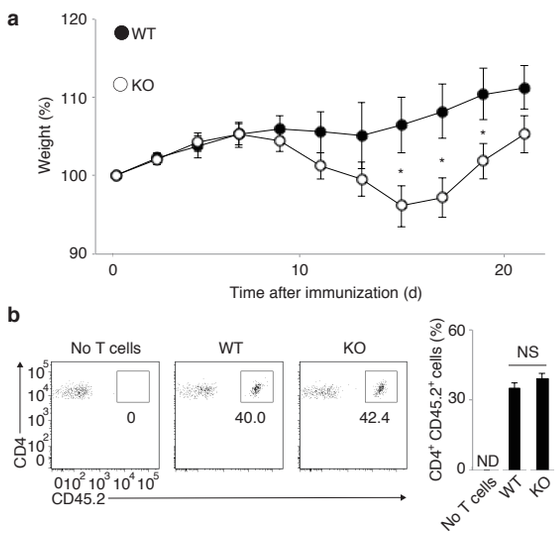


Supplementary Figure 4 The cytoplasmic amino acid residues in gp130 responsible for TRAF5 binding. (**a**) Immunoassay of HEK cells transduced with plasmids encoding gp130 mutants (above lanes) with various deletions in the cytoplasmic region in positions 641–917 (above blot) and cotransfected to express V5-TRAF5 (242–558), followed by immunoprecipitation of proteins from lysates with control IgG or anti-c-Myc and immunoblot analysis with anti-V5 or anti-c-Myc. (**b**) Amino acid sequence alignment of the TRAF5 binding sites in gp130 from various species. (**c**) Immunoprecipitation of V5-TRAF5 (242–558) from lysates of HEK cells transiently transfected with plasmid vectors encoding V5-TRAF5 (242–558) and GFP-tagged gp130 (769–800) with wild-type sequence (WT) or alanine substitutions (Ala-mut) (above blot), followed by immunoblot analysis with anti-V5 or anti-GFP. Input (bottom), immunoblot analysis of lysates without immunoprecipitation. (**d**) Immunoprecipitation of c-Myc-gp130 (1–917) from lysates of HEK cells transiently transfected with plasmid vectors encoding c-Myc-gp130 (1–917), V5-TRAF5 (242–558), and GFP-tagged gp130 (769–800) with wild-type sequence (WT) or alanine substitutions (Ala-mut) (above blot), followed by immunoblot analysis with anti-V5, anti-c-Myc, or anti-GFP.



Supplementary Figure 5 The role of TRAF5 in active and passive EAE. (**a**) Body weight changes in wild-type and *Traf5*$^{-/-}$ mice after induction of active EAE as in **Fig. 6a–c**, monitored over 22 d (average and s.e.m. of $n = 10$ mice per genotype). (**b**) The percentages of donor CD4^{+}CD45.2^{+} cells in the peripheral blood CD4^{+} T cells from recipient CD45.1^{+} B6.SJL mice 7 days after induction of passive EAE as in **Fig. 6d** (average and s.e.m. of $n = 3$ mice (no cell transfer) or 6 mice (adoptive transfer)). NS, not significant; *$P < 0.05$ (Student *t*-test).