SUPPLEMENTAL MATERIAL

Batten et al., http://www.jem.org/cgi/content/full/jem.20100064/DC1

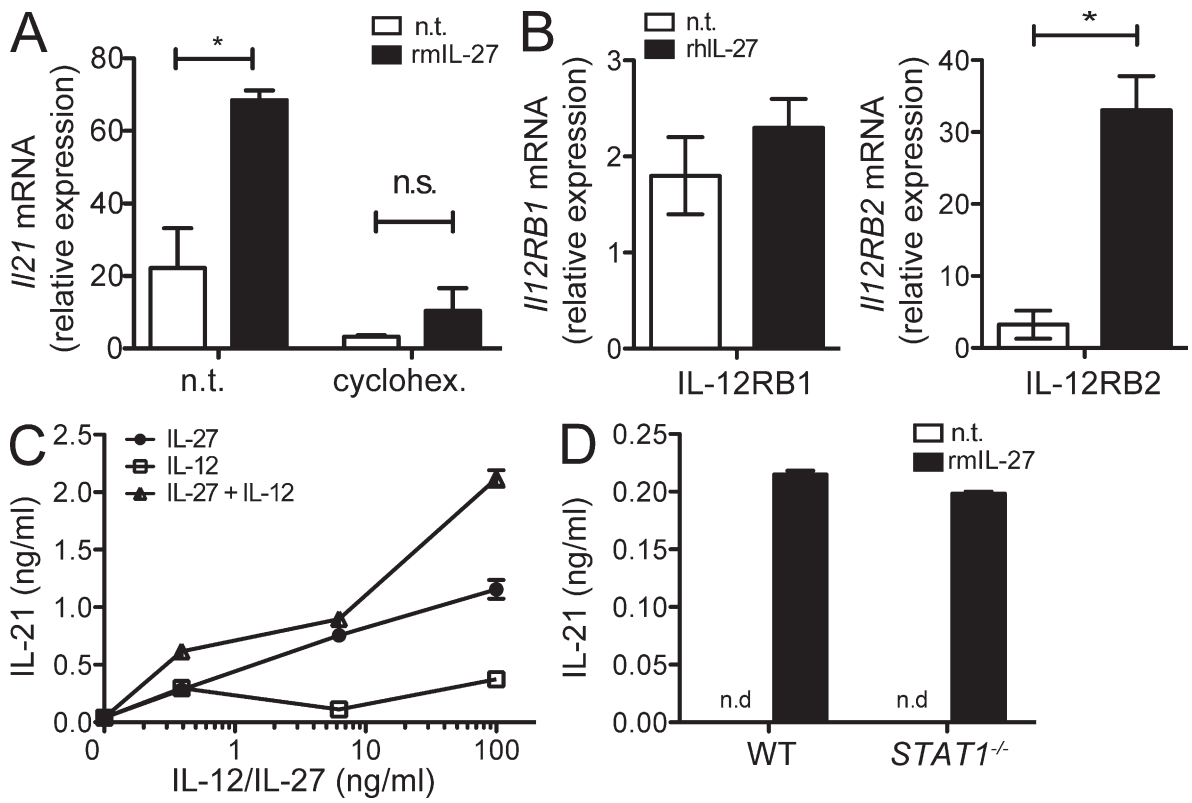

Figure S1. Regulation of IL-21 by IL-27. CD4+ T cells from C57BL6 mice were stimulated with plate-bound anti-CD3 and soluble anti-CD28 under TH0 polarizing conditions in the presence (filled symbols) or absence (open symbols) of rmIL-27 and in the presence or absence of cycloheximide for 5 h. The mean of triplicate experimental samples is given ± SD. Data are representative of three independent experiments. (B) Naive CD4+ T cells isolated from human PBMC were stimulated with T cell activation beads in the presence (filled bars) or absence (open bars) of rhIL-27 for 3 d. The expression of *IL12RB1* (left) and *IL-12RB2* (right) mRNA expression was measured by real-time RT-PCR and is given relative to human *gapdh*. Combined data from three independent donors and experiments is shown ±SD. (C) CD4+ T cells enriched from C57BL6 splenocytes were stimulated with plate-bound anti-CD3 and soluble anti-CD28 in the presence of varying concentrations of rmIL-27, rmIL-12, or both rmIL-27 and rmIL-12 for 72 h. IL-21 in the culture supernatant was measured by ELISA and the mean ± SD of triplicate experimental samples is shown. (D) CD4+ T cells from STAT1$^{+/+}$ (SvEv) or STAT1$^{-/-}$ mice were stimulated as in A for 72 h. IL-21 in the culture supernatant was measure by ELISA. Data in D are representative of two experiments. n.t., no treatment; n.d., not detectable. *, $P < 0.05$ (unpaired Student's *t* test). Bars indicate mean ± SEM.

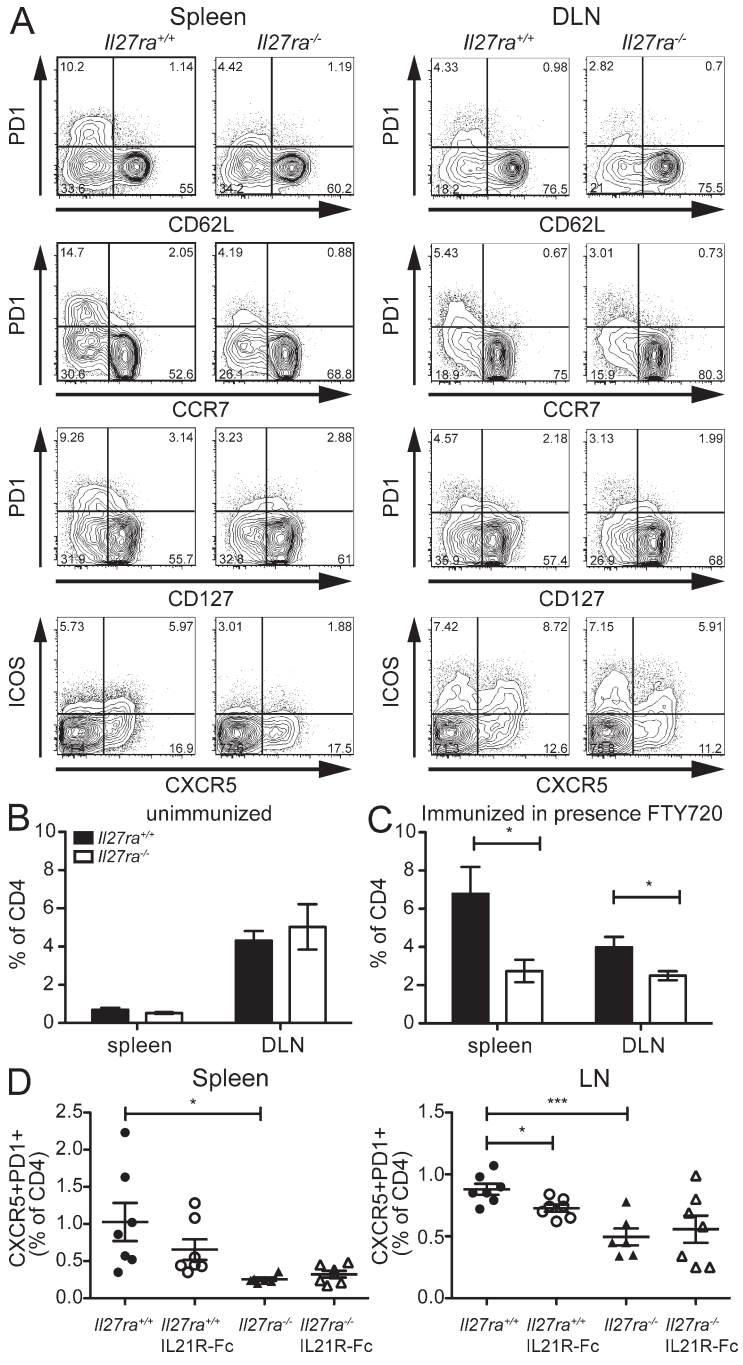

Figure S2. Detailed analysis of T_{FH} cell reduction in *Il27ra*$^{-/-}$ mice. (A) Groups of *Il27ra*$^{+/+}$ and *Il27ra*$^{-/-}$ mice were immunized twice with TNP-OVA in adjuvant and, 7 d after the second immunization, tissue was collected for analysis. Representative flow cytometric analysis for T_{FH} marker expression in the spleen (left) and DLN (right). For all plots the CD4$^+$B220$^-$ gate is shown. (B) The percentage of CXCR5$^+$PD1$^+$ cells in the CD4$^+$B220$^-$ gate in the spleen and DLN of unimmunized age-matched *Il27ra*$^{+/+}$ and *Il27ra*$^{-/-}$ mice. Data are representative of three independent experiments. (C) Groups of *Il27ra*$^{+/+}$ and *Il27ra*$^{-/-}$ mice were immunized once with TNP-OVA in CFA and FTY720 was administered i.p. every second day beginning on the day of immunization. Efficacy of the FTY720 treatment was confirmed by a >90% reduction in normal T cell number in the blood. 7 d after the immunization, tissue was collected for analysis. The percentage of CXCR5$^+$PD1$^+$ cells in the CD4$^+$B220$^-$ gate in the spleen and DLN is given. (D) Groups of *Il27ra*$^{+/+}$ and *Il27ra*$^{-/-}$ mice were immunized once with TNP-OVA in CFA. Where indicated, 20 µg/mouse of IL-21R-Fc was administered i.v. every second day beginning 1 d before immunization. 7 d after immunization, tissue was collected for analysis. The percentage of CXCR5$^+$PD1$^+$ cells in the CD4$^+$B220$^-$ gate in the spleen and DLN is given. For C and D, a single experiment with seven animals per group is represented and error bars indicate SEM. *, $P < 0.05$; ***, $P < 0.001$ (unpaired Student's *t* test). Bars indicate mean ± SEM.

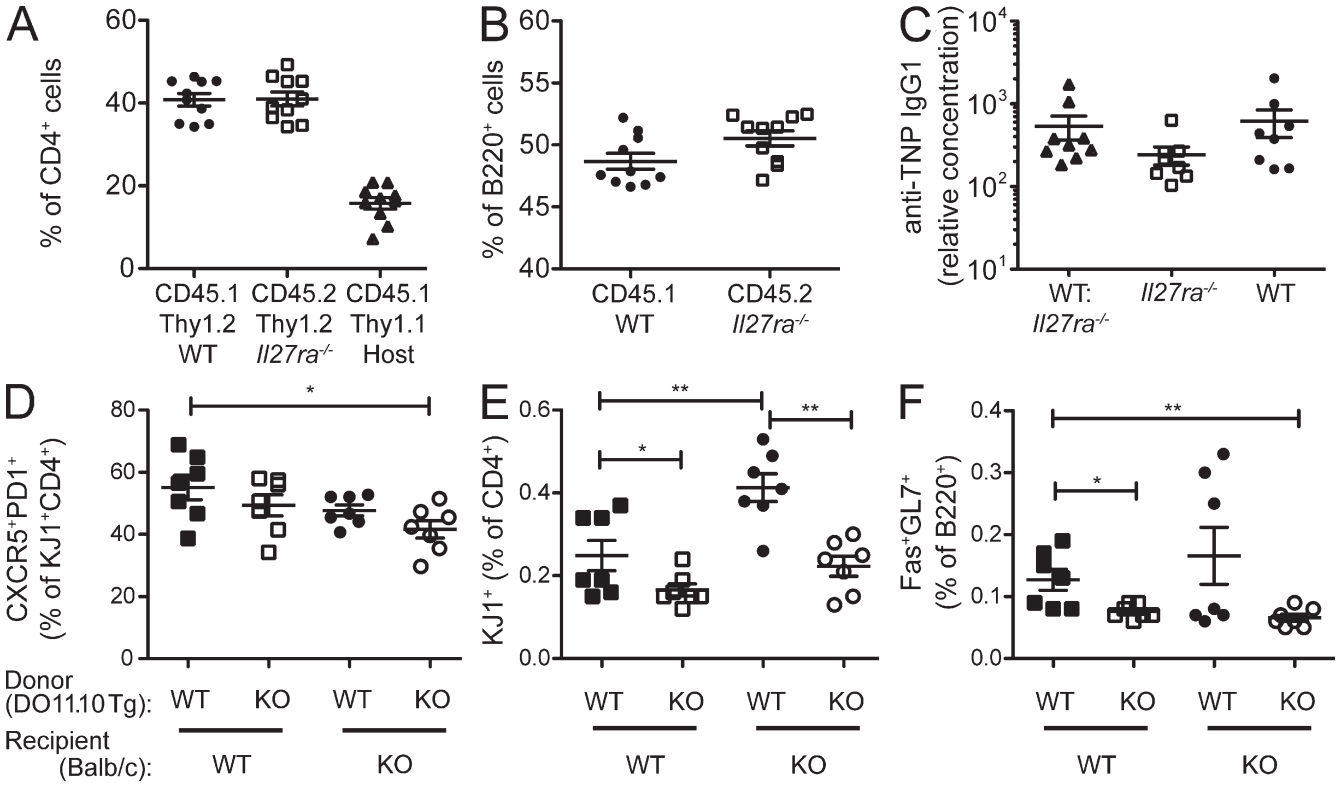

Figure S3. T cell–intrinsic defect in GC activity in *Il27ra*$^{-/-}$ mice (A–C) TCM mice (CD45.1 and Thy1.1) were lethally irradiated and reconstituted with a 50:50% mix of BM from WT (CD45.1) and *Il27ra*$^{-/-}$ (CD45.2) mice (A and B). 6 wk after BM transfer, the mice were bled to assess reconstitution by flow cytometry. (A) The percentage of WT (filled circles), *Il27ra*$^{-/-}$ (open squares), and host (filled triangles) CD4$^+$ T cells in the mixed chimeras. (B) The percentage of WT (CD45.1$^+$ host plus donor; filled circles) and *Il27ra*$^{-/-}$ (open squares) in the B220$^+$ B cell gate in the mixed chimeras. (C) Serum IgG1 levels as determined by ELISA using TNP_2-BSA coating in the mixed chimera (triangles) compared with chimeric mice reconstituted with only *Il27ra*$^{-/-}$ (squares) or *Il27ra*$^{+/+}$ (WT; circles) BM. (D–F) DO11.10Tg.*Il27ra*$^{+/+}$ (WT) or DO11.10Tg.*Il27ra*$^{-/-}$ (KO) CD4$^+$ T cells were adoptively transferred into either *Il27ra*$^{+/+}$ (WT) *or Il27ra*$^{-/-}$ (KO) hosts (as indicated) on the BALB/c background. The mice were immunized with OVA in CFA and the response analyzed 7 d later. Flow cytometric analysis was used to determine the percentage of CD4$^+$KJ1$^+$ donor cells that became CXCR5$^+$PD1$^+$ (D), KJ1$^+$ donor cells as a percentage of total CD4$^+$ cells (E), and the percentage of Fas$^+$GL7$^+$ GC B cells (F) in the spleen. Bars indicate the mean ± SEM. Each dataset is representative of two independent experiments. *, $P < 0.05$; **, $P < 0.01$ (unpaired Student's *t* test).

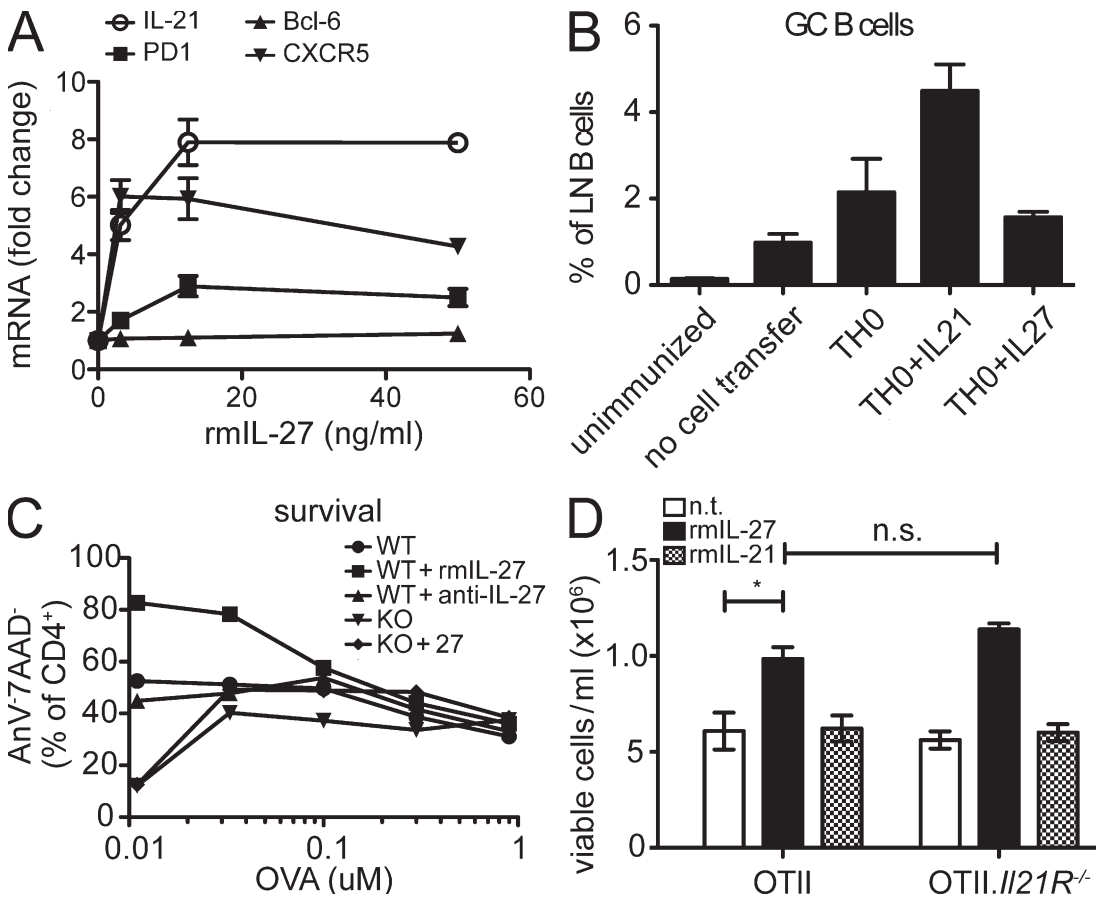

Figure S4. IL-27 does not promote T_{FH} differentiation. (A) OTII Tg CD4+ T cells were cultured with irradiated splenic APC plus 0.3 µM $OVA_{323-339}$ peptide under TH0 conditions and in the presence of either no additional cytokine or various concentrations of rmIL-27 for 5 d. After 5 d, CD4+ T cells were restimulated with anti-CD3 for 4 h and IL-21, PD1, Bcl-6, and CXCR5 mRNA levels were assessed by real-time RT-PCR. mRNA levels for each probe set were normalized to the housekeeping gene Rpl19 and the fold increase relative to samples stimulated in the absence of rmIL-27 are given. The mean ± SEM of triplicate experimental samples is shown and data are representative of two experiments. (B) Thy1.1+ OTII TCR Tg CD4+ T cells were isolated by magnetic purification and cultured with irradiated splenic APC plus $OVA_{323-339}$ peptide under TH0 conditions alone (blocking antibodies against IFN-γ and IL-4 and TGFBRII-Fc) or with the addition of 50 ng/ml rmIL-21 or 50 ng/ml rmIL-27 for 5 d. Cells were then adoptively transferred to naive Thy1.2 congenic hosts (n = 4–8 per group) before recipient mice were subcutaneously immunized with 100 µg OVA in IFA. Two additional control groups were included that did not receive cell transfers; one group was immunized as described, whereas the other group remained unimmunized. 7 d after immunization, differentiation of GC B cells in the LN were assessed by flow cytometry. The graph shows the mean percentage of $GL7^+Fas^+B220^+$ cells in the DLN. Error bars indicate SEM. (C) DO11.10tg.*rag2*$^{-/-}$ or DO11.10tg.*rag2*$^{-/-}$.*Il27ra*$^{-/-}$ splenocytes were activated with various concentrations of $OVA_{323-339}$ in the presence or absence of 20 ng/ml rmIL-27 or 10 µg/ml of anti-IL-27 for 72 h and the percentage of AnV-neg and 7AAD-neg (viable) cells in the CD4+ gate was determined by flow cytometry. Data are representative of three independent experiments. (D) OTII.*Il21R*$^{+/+}$ and OTII.*Il21R*$^{-/-}$ splenocytes were stimulated with $OVA_{323-339}$ peptide for 72 h in the absence of additional cytokine (open bars) or in the presence of rmIL-27 (black bars) or rmIL-21 (gray bars). $7AAD^{neg}$ CD4+ cells were assessed by flow cytometry and multiplied by total cells counts to give viable CD4+ cells per ml of culture medium. The mean of three replicate samples are shown, and these data are representative of two independent experiments. Error bars indicate SEM. *, $P < 0.05$, (unpaired Student's t test).

Table S1. Primers and probes for real-time RT-PCR

Gene	Primers	Probe
mIl21	5′-CTTCCCGTGTCAGGGATT-3′; 5′-TCACAGTTGGGCAATAAGATG-3′	5′-AGCCACAGCTTGAGAAGCACCAGA-3′
mIl27p28	5′-TCAGGTGTCATCCCAAGTGT-3′; 5′-GACAAGCTCCAGGGAGTGA-3′	5′-GGTAGGTATAGAGCAGCTGGGGCCAG-3′
mEbi3	5′-GGCCTGTCCTGAGTCTGAATA-3′; 5′-AGTCAAGTGAATTATCCAGTGCTT-3′	5′-CTTTCCATGTACTGGGCTGCTCCG-3′
mRpl19	5′-ATCCGCAAGCCTGTGACTGT-3′; 5′-TCGGGCCAGGGTGTTTTT-3′	5′-TTCCCGGGCTCGTTGCCG-3′
mBcl6	Inventoried Taqman assay Mm00477633_m1	
hGAPDH	5′-CTCTGCTCCTCCTGTTCGAC-3′; 5′-ACGACCAAATCCGTTGACTC-3′	Roche UPL probe #60
hIL12RB1	5′-CGGCTGACCCTGAAAGAG-3′; 5′-CAGCCCTTGACAGCCTTC-3′	Roche UPL probe #78
hIL12RB2	5′-TCCAGATCCAGCAAATAGCA-3′; 5′-GTCCAAGGGCAGCTGTGT-3′	Roche UPL probe #82