Supporting Information

Guo et al. 10.1073/pnas.0906988106

SI Materials and Methods

Cell Culture. CD4 T cells were purified by negative selection with FITC anti-CD8, FITC anti-B220, FITC anti-IA, FITC anti-NK1.1, FITC anti-CD24, FITC anti-CD25, and FITC anti-CD16/CD32, followed by anti-FITC microbeads (Miltenyi Biotech) using an Automacs (Miltenyi Biotech). Antigen-presenting cells (APC) were purified from splenocytes by treatment with anti-Thy1.2 and low-Tox M rabbit complement at 37 °C for 45 min and irradiated at 30 Gy. CD4 T cells from 5C.C7 transgenic $Rag2^{-/-}$ mice were primed with cytochrome C peptide (1 μM) and APC. CD4 T cells from C57BL/6, BALB/c, *Gata3*"floxed" mice, and *Stat5*"floxed" mice were primed with anti-CD3 (3 μg/mL) (Harlan Bioproducts for Science), anti-CD28 (3 μg/mL) (Harlan Bioproducts For Science), and APC. For Th1 priming, IL-2 (10 units/mL), IL-12 (10 ng/mL) (R&D), and anti-IL-4 (10 μg/mL) (Harlan Bioproducts for Science) were added; for Th2 priming, IL-2 (100 units/mL), IL-4 (1,000 units/mL), anti-IL-12 (10 μg/mL) (Harlan Bioproducts for Science) and anti-IFNγ (10 μg/mL) (Harlan Bioproducts for Science) were added; for Th17 priming, TGFβ (10 ng/mL) (R&D), IL-6 (10 ng/mL) (R&D), anti-IL-4 (10 μg/mL), anti-IL-12 (10 μg/mL), and anti-IFNγ (10 μg/mL) were added. After a 4 day-priming period, Th1 and Th2 cells were washed and put into IL-2 (10 units/mL)-containing medium; Th17 cells were put in cRPMI. For some experiments, cells were cultured under Th1 or Th2 conditions for additional rounds of 4 days each.

Cytokines and reagents used in cell culture include: IL-33 (10 ng/mL) (Alexis), TSLP (10 ng/mL) (R&D), IL-7 (1 ng/ml) (R&D), IL-23 (1 ng/mL) (R&D), IL-21 (100 ng/mL) (R&D), IL-6 (1 ng/mL) (R&D), IL-1β (1 ng/mL) (Peprotech), IL-18 (25 ng/mL) (R&D), PMA (10 ng/mL) (Calbiochem), ionomycin (1 μM) (Calbiochem), Bay 11–7082, NF-κB inhibitor, (1 μM) (Calbiochem); SB203580, p38 inhibitor, (1 μM) (Calbiochem); U0126, ERK inhibitor, (1 μM) (Calbiochem); SP600125, JNK inhibitor, (1 μM) (Calbiochem); and CsA (50 ng/mL) (Calbiochem).

Flow Cytometry. For cell surface marker staining, cells were directly stained without fixation and permeabilization. For general intracellular staining, harvested cells were fixed with 4% paraformaldehyde, washed with 0.1% BSA-containing PBS, and stored at 4 °C. Cells were then incubated with permeabilization buffer (PBS supplemented with 0.1% BSA/0.5% Triton X-100) and various antibodies for 20 min. The antibodies used were APC-anti-CD4, FITC-anti-IFNγ, PE-anti-IL-2, PE-anti-IL-4, APC-anti-IL-4, PE-anti-IL-13, and alexa 647-anti-IL13.

Retroviral Infection. CD4 T cells were purified from mice homozygous for a "floxed" *Gata3* gene or from wild-type C57BL6 mice and cultured under Th2 conditions as described above. Two days after a new round of Th2 priming, cells were incubated with GFP-Cre retrovirus-containing supernatant and polybrene (8 μg/mL) (Sigma). Cells were centrifuged at 2,500 rpm for 90 min at room temperature and maintained overnight. On the following day, fresh medium was added to the culture and the cells were continued to be cultured under Th2 conditions. Two days after infection, cells were sorted on the basis of their expression of GFP. The sorted cells went through another round of Th2 priming to dilute the existing GATA3 protein. T1ST2 mRNA was measured by QPCR immediately or the cells were cultured in IL-2-containing medium for 1 or 2 weeks. Rested cells were then cultured in medium containing IL-2 alone, IL-33 alone, or a combination of IL-2 and IL-33 for 2 days and then the *T1st2* mRNA amount was tested by QPCR.

CD4 T cells were purified from mice homozygous for a "floxed" *Stat5a* and *Stat5b* gene or wild-type C57BL6 mice and cultured under Th2 conditions as described above. Th2 cells were retrovirally infected with GFP-Cre supernatant as above and similarly sorted on the basis of GFP expression. Sorted cells were cultured in IL-4-containing cRPMI to maintain cell survival. Six days later, these rested cells were cultured in medium containing IL-2 alone, IL-33 alone, or a combination of IL-2 and IL-33 for 2 days, with the presence of IL-4 in each group, and then the *T1st2* mRNA amount was tested by QPCR and GATA3 expression by intracellular staining.

CD4 T cells were purified and cultured under Th2 conditions for 2 days and Gata3-NGFR retroviral infection was performed as above. After a total of 4 days priming under Th2 conditions, cells were washed and maintained in IL-2 containing cRPMI. T1ST2 expression was examined by cell surface staining at the end of the 4-day Th2 priming period and when the cells had been cultured in IL-2 containing medium for a week.

ChIP. Briefly, cells were treated with formaldehyde and chromatin was fragmented by sonication. This was followed by chromatin immunoprecipitation with anti-GATA3 (BD Pharmingen), anti-STAT5A (R&D), anti-STAT5B (R&D), or control IgG (Santa Cruz). The precipitated DNA was purified and quantified using real-time PCR with Sybr Green reagents (ABI). The primers for −12K *T1st2* enhancer used to test GATA3 binding are: 5′-CCA-TAA-TTG-AGG-GAA-GCC-AGG-3′ and 5′-TTG-CCT-CCA-GGT-TTC-AGA-CTG-3′. The primers for the *Gata3* 800-bp promoter used to test STAT5 binding are: 5′-tgc-gct-ccg-agt-tta-aag-gt-3′ and 5′-aat-gtt-aag-gcg-gtc-atg-agt-tg-3′. The primers for *T1st2* intron 7 used to test STAT5 binding are: 5′-acc-ctt-tca-cat-cag-gac-aca-tt-3′ and 5′-gtg-gtc-agg-gct-atg-cat-ga-3′. The primers for *T1st2* intron 4 are: 5′-cat-cta-caa-aac-aaa-agt-cta-cac-aag-agt-t-3′ and 5′-cct-ctt-aat-ttg-tgg-ctt-tta-tat-cca-3′. The primers for mitochondrial DNA-directed RNA polymerase are: 5′-CAT-CAG-CTT-CCA-TCC-ACC-ACT-T-3′ and 5′-GAC-TCG-ATT-CCC-ATG-GAT-CCT-3′. The degree of enrichment was calculated by normalization to the amount of PCR product from the non-GATA3, non-STAT5-binding mitochondrial DNA-directed RNA polymerase gene.

CFSE Labeling. Cells were extensively washed to remove serum and then resuspended in PBS containing 0.1% BSA. Two micromolars of CFSE were freshly prepared in PBS containing 0.1% BSA and mixed with the single-cell suspension and incubated at 37 °C for 8 min. Two volumes of FBS were added to stop reaction immediately and then cells were washed in cRPMI 3 times to remove excessive nonincorporated dye. The cells were cultured for 5 days to examine CFSE dilution.

Quantitative PCR. Total RNA was isolated using RNeasy mini kit (Qiagen); first strand cDNA was prepared using SuperScript III (Invitrogen). All PCR was performed on 7900HT sequence detection systems (Applied Biosystems). The TaqMan universal PCR SuperMix and the primer and probe sets for murine *Il4*, *Il13*, *Il5*, *Il2*, *Socs3*, *Tbet*, *Gata3*, *Rorc*, *Il18r1*, *T1st2*, *Il1r1*, *18s ribosome RNA*, and *Gapdh* were purchased from Applied Biosystems.

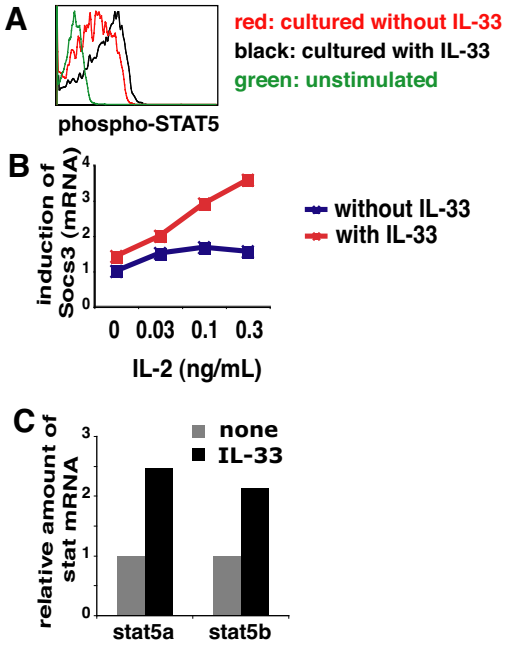

Fig. S1. IL-33 treatment enhances STAT5 phosphorylation. D1 3xTh2 cells were extensively washed and cultured in cRPMI with or without IL-33 for 20 h. (*A*) Cells were stimulated with 0.3 ng/mL IL-2 for 15 min and STAT5 phosphorylation determined by intracellular staining. (*B*) Cells were stimulated with IL-2 for 30 min and *Socs3* mRNA. (*C*) Cells were collected to measure *stat5a* and *stat5b* mRNA amount.

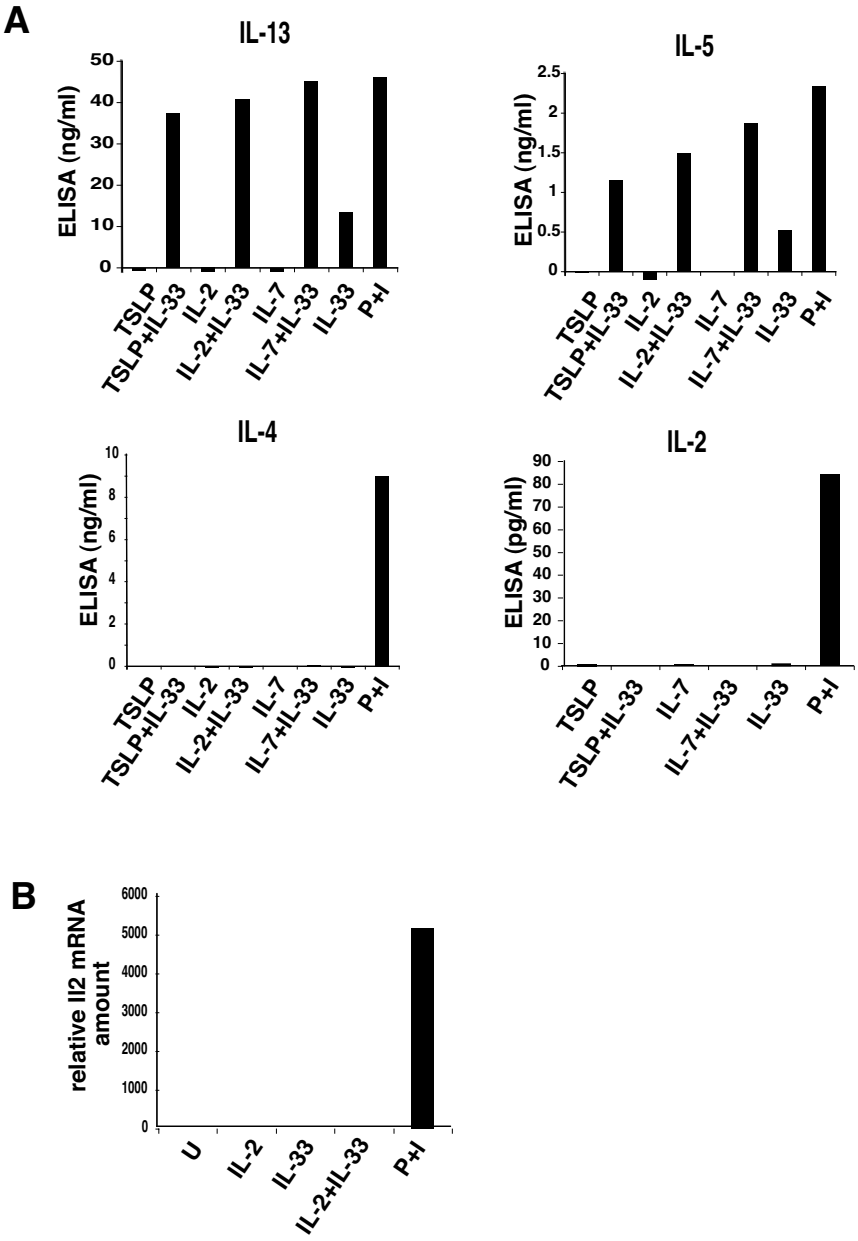

Fig. S2. IL-33 plus IL-2 or IL-33 plus IL-7 induces production of IL-13 and IL-5, but not IL-4 or IL-2. (*A*) 3xTh2 cells were cultured in medium containing indicted cytokines for 24 h and then supernatants were collected for ELISA (*A*), or (*B*) 3xTh2 cells were cultured in medium containing indicted cytokines for 2 h and then cells were collected for PCR.

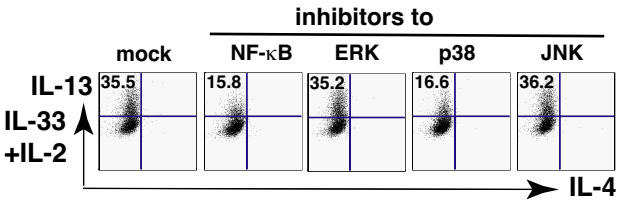

Fig. S3. IL-33/STAT5-induced-IL-13 production requires NF-κB and p38 but not Erk or JNK. Three-round primed Th (3xTh2) cells were pretreated with the indicated inhibitors for 30 min and then stimulated with IL-33 plus IL-2 or P+I for 4 h to test IL-4 and IL-13 production.

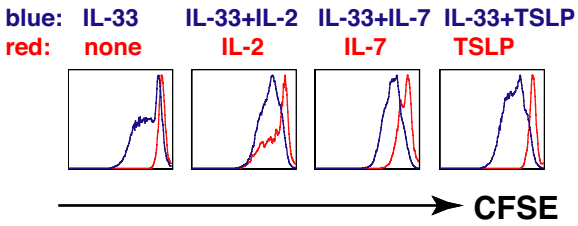

Fig. S4. IL-33/STAT5 leads to cell proliferation. D14 2xTh2 cells were CFSE labeled and then cultured in medium containing the indicated cytokines for 5 days and then CFSE dilution was examined by FACS.

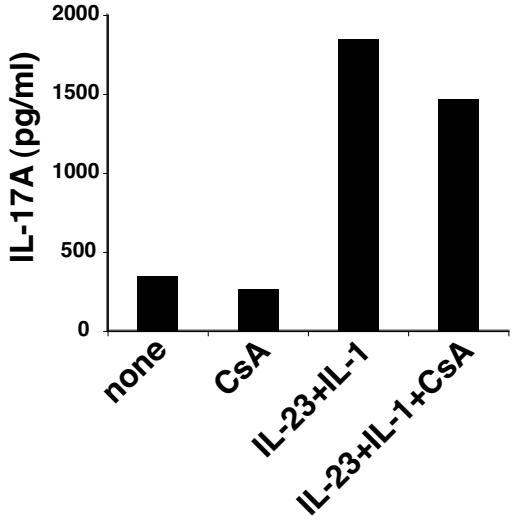

Fig. S5. IL-1/IL-23 induced IL-17A production is slightly inhibited by CsA. D1 1xTh17 cells were cultured in medium supplemented with indicated cytokines and/or inhibitor for 24 h and supernatants were collected to test IL-17A by ELISA.