

## Supplementary Methods.

**Mice.** 4 to 6-week-old C57BL/6, BALB/c, OTII, DO11.10, *Rag1*<sup>-/-</sup>, *Foxp3-RFP* and Thy1.1 mice were purchased from Jackson Laboratories. DO11.10 or OTII *Nfat1*<sup>-/-</sup> mice were generated by crossing *Nfat1*<sup>-/-</sup> mice [15] (in a BALB/c or C57BL6 background) with DO11.10 or OTII mice, respectively. Transgenic B6 mice expressing a dominant-negative form of Ikaros [37] were obtained from K. Georgopoulos (Harvard Medical School, Boston, MA). Mice were maintained in pathogen-free conditions. All animal work was performed according to the guidelines set by the Institutional Animal Care Committee of the Albert Einstein College of Medicine.

***In vitro* Th1 differentiation.** CD4<sup>+</sup> T cells were stimulated with 0.5 µg/ml immobilized anti-CD3 (145-2C11. eBioscience) and anti-CD28 (37.51. eBioscience) or irradiated T-cell depleted APCs loaded with 10 µM of the OVA OVA in the presence of recombinant IL2 (10U/ml) and anti-IL4 (10 µg/ml) (11B.11) (National Cancer Institute Biological Resources Branch), IL12 (10 ng/ml) (Cell Signal) for one week.

**ELISA.** 25 to 50 x10<sup>3</sup> T cells were stimulated in 96 well plates. Supernatants were collected 24-72 hours after stimulation and IL2 levels were measured with a sandwich ELISA kit following the manufacturer's recommendations (BD).

**Immunofluorescence.** Thy1.2 Th1 cells were 1  $\mu$ M ionomycin or stimulated in the presence or absence of Thy1.1 Tregs for 4-6 hours. Cells were then fixed and stained with anti-NFAT1 (MA1-25; Pierce) or anti-phospho-cJun (Ser63. Cell Signaling). Nuclei were stained with DAPI. Effector T cells were identified using anti-Thy1.2 antibodies (30-H12; eBioscience).

Total RNA was isolated using Trizol reagent (Ambion) and cDNA was synthesized using qScript cDNA SuperMix (Quanta) according to manufacturer's recommendations. Realtime PCR reactions were conducted in a StepOne Plus thermocycler (Applied Biosystems) using Power SYBR Green PCR mix (Applied Biosystems) and primers for Ikaros (forward: 5'-GCTGGCTCTCAAGGAGGAG-3'; reverse: 5'-CGCACTTGTACACCTTCAGC-3') or b-Actin (forward: 5'-GGCTGTATTCCCCTCCATCG-3'; reverse: 5'-CCAGTTGGTAACAATGCCATGT-3'). Messenger RNA induction was calculated at  $2^{-\Delta\Delta C_t}$  using actin as control.

**Chromatin Immunoprecipitation (ChIP) assays.** OT-II Th1 cells were stimulated for 24 hours with T-cell depleted splenocytes loaded with 1 $\mu$ M OVA in the presence or absence of pre-activated Tregs isolated from *FoxP3-RFP* mice. Following a 24-hour stimulation, responder Th1 cells were recovered by sorting as  $V\alpha 2^+$  RFP $^-$  cells. Histone H3 acetylation was assayed using a ChIP assay kit (Upstate Biotechnology), following manufacturer's protocol. Briefly,  $1-2 \times 10^6$  cells were subjected to immunoprecipitation overnight at 4C with anti-acetylated H3 antibodies (17-615. Millipore). Specific primer pairs were designed to amplify the *Il2* promoter (forward: 5'-TAAGTGTGGGCTAACCCGA-3'; reverse: 5'-TTGAGGTCCTGTGAGGAGT-3').

Purified DNA was subjected to quantitative PCR analysis. Specific enrichment was corrected for background using a control IgG and reported as percent recovery on input.