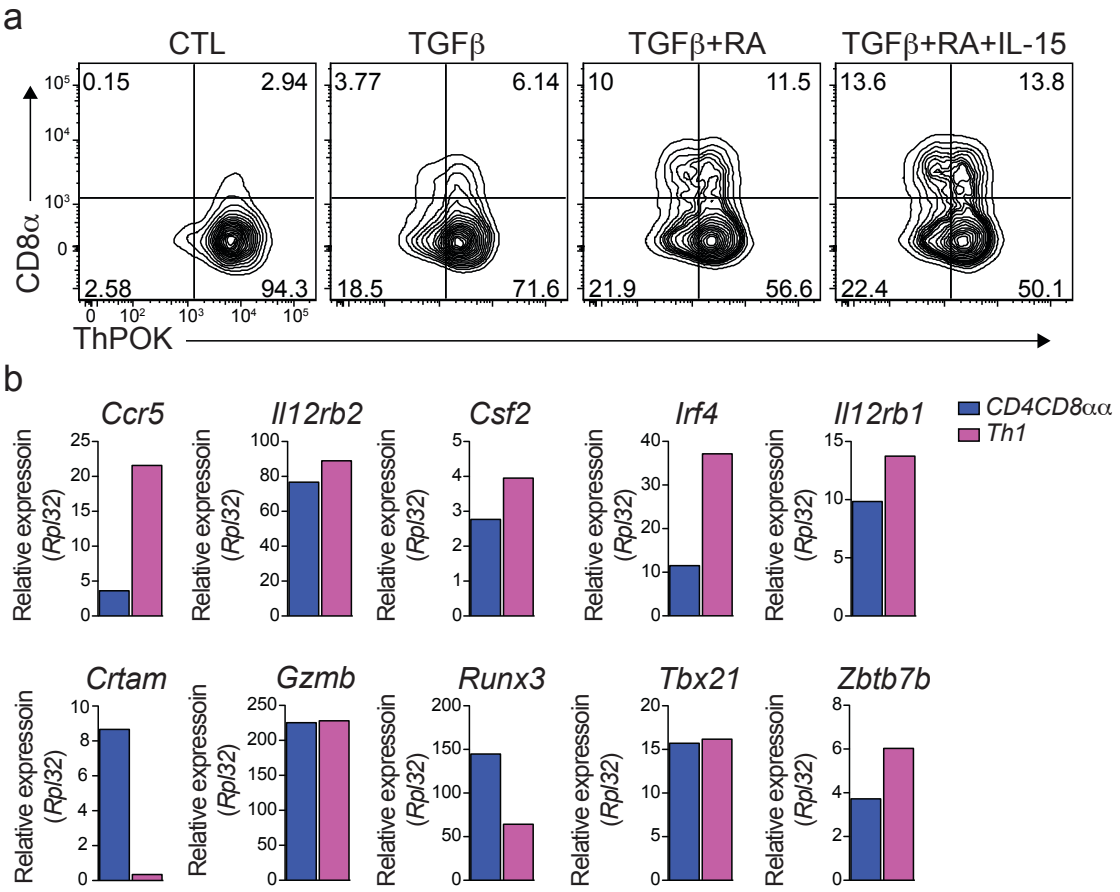

Supplemental Figure S1 (related to Figure 1). (**a**,**b**) - Sorted naïve Vα2$^+$CD4$^+$ T cells isolated from OTII *Zbtb7b*-GFP were *in vitro* co-cultured for 4.5 days with spleen DCs and 1μM OVA peptide and indicated cytokines (**a**) or differentiated to Th1 cells (IL-12+anti-IL-4), or to CD4$^+$CD8αα$^+$ (TGF-β+RA+IFN-γ) (**b**). (**a**) CD8αα and ThPOK expression. (**b**) Cells from pooled wells for each condition were sorted as ThPOK^{hi}CD8β–CD8α– (Th1) and ThPOK^{lo}CD8β–CD8α+ (CD4$^+$CD8αα+) and RNA extracted by Trizol (Invitrogen, USA). Gene expression level was determined by qPCR (AB 7500HT) and normalized by housekeeping gene *Rpl32* (which encodes the ribosomal protein L32). Data are representative of two independent experiments with similar results.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Antibodies and flow cytometry analysis. Fluorescent-dye-conjugated antibodies were purchased from BD-Pharmingen (anti-CD4, 550954; anti-CD25, 553866; anti-CD90, 561401; anti-CD103, 557495; anti-CD244.2, 553306 anti-IL-17a, 559502; anti-T-bet, 561312; anti-CD45R, 557683; anti-CD233, 552380;) or eBioscience (anti-CD8α, 56-0081; anti-CD44, 56-0441; anti-CD45.1, 25-0453; anti-CD45.2, 47-0454; anti-CD62L, 48-0621; anti-TCR-β, 47-5961; anti-IFN-γ, 25-7311; anti-Foxp3, 17-5773; anti-Vα2, 48-5812; anti-TCR$\gamma\delta$, 46-5711; anti-CD8β, 46-0083; anti-Rorγ-t, 12-6981). TL-tetramer was kindly provided by H. Cheroutre (LIAI, USA). Flow cytometry data were acquired on an LSR-II flow cytometer (Becton Dickinson) and analyzed using FlowJo software (Tree Star). Intracellular staining of T-bet, Foxp3 and Granzyme B was conducted using Foxp3 Mouse Regulatory T cell Staining Kit (eBioscience). For Rorγ-t intracellular staining, cells were incubated with rat serum prior to antibody staining. For flow cytometric analysis of cytokine-secreting cells, cells were incubated in the presence of 100ng/ml PMA (Sigma), 500ng/ml Ionomycin (Sigma) for 4.5h and 10μg/ml brefeldin A (BFA) (Sigma) for the last 2.5h prior staning. Cell populations were first stained with antibodies against the indicated cell surface markers, followed by permeabilization in Fix/Perm buffer and intracellular staining in Perm/Wash buffer (BD Pharmingen).

***In vitro* T cell culture.** Naïve (defined as TCRβ^+CD4$^+$CD25$^-$CD62^{hi}CD44^{lo}) T cells were sorted using a FACS Aria cell sorter (Becton Dickinson) and cultured for 4.5 days in 96-well plates pre-coated with 2μg/ml of anti-CD3ε (17A2) and 1μg/ml of soluble anti-CD28

(37.51). Cells were then stimulated with indicated cytokines (all from R&D): 10ng/ml of IL-1β, 20ng/ml of IL-6, 10ng/ml of IL-12, 10ng/ml of IL-15, 10ng/ml of IL-23, 10nM of RA, 2ng/ml of TGF-β (Treg), 0.2ng/ml of TGF-β (T_H17), 20ng/ml of IFN-γ, 20ng/ml of IL-27, 1μg/ml of anti-IL-4, 1μg/ml of anti-IFN-γ. OTII cells were co-cultured with magnetic bead-isolated (MACS, Miltenyi Biotec) $CD11c^+$ splenic DCs in the presence of 500nM OVA peptide and indicated cytokines.

Retroviral transduction of $CD4^+$ T cells. Retroviral vectors for were previously described (Muroi et al., 2008; Naoe et al., 2007). Retroviral vectors for T-bet were generously provided by L. Glimcher (Cornell) and were previously described (Lazarevic et al., 2011). Sorted naïve T cells were *in vitro* activated with 2μg/ml of plate-bound anti-CD3ε and 1μg/ml of soluble anti-CD28 for 36-48h. Cells were then spin transduced with retrovirus supernatant for 2h at 1250 x *g*. For *in vitro* T cell culture, transduced cells were incubated in 96-well plates with indicated cytokines. For adoptive transfer, transduced cells were incubated for 48 hours in RPMI complete medium and sorted for GFP (Runx3 transduced) or Thy1.1 (T-bet transduced) expression using a FACS Aria cell sorter (Becton Dickinson).

Quantitative PCR. qPCR was performed as previously described (Mucida et al., 2007). *Rpl32* housekeeping gene was used to normalize samples. Primers used were: *Zbtb7b*-forward 5'-atgggattccaatcaggtca-3', *Zbtb7b*-reverse 5'-ttcttcctacaccctgtgcc-3'; *Tbx21*-forward 5'-atcctgtaatggcttgtggg-3', *Tbx21*-reverse 5'-tcaaccagcaccagacagag-3'; *Rpl32*-forward 5'-gaaactggcggaaaccca-3', *Rpl32*-reverse 5'- caccgctaaaggcagttctc-3'; d*Runx3*-

forward 5'- acagcatctttgactccttcc-3', d*Runx3*-reverse 5'-tgttctcgcccatcttgc-3'; *Gzmb*-forward 5'-gcccacaacatcaaagaacag-3', *Gzmb*-reverse 5'-aaccagccacatagcacacat-3'; *Crtam*-forward 5'- tcaagaaacttcagatgcccc-3', *Crtam*-reverse 5'- gtcttgagtggcatgttctttc-3'; *Il12rb1*-forward 5'- atggctgctgcgttgagaa-3', *Il12rb1*-reverse 5'- agcactcatagtctgtcttgga-3'; *Il12rb2*-forward 5'- agagaatgctcattggcacttc-3', *Il12rb2*-reverse 5'-aactgggataatgtgaacagcc-3'; *Ccr5*-forward 5'- ttttcaagggtcagttccgac-3', *Ccr5*-reverse 5'-ggaagaccatcatgttacccac-3'; *Csf2*-forward 5'- ggccttggaagcatgtagagg -3', *Csf2*-reverse 5'- ggagaactcgttagagacgactt-3'; *Irf4*-forward 5'- tccgacagtggttgatcgac-3', *Irf4*-reverse 5'-cctcacgattgtagtcctgctt-3'.

Experimental colitis model. Colitis was induced after transfer of 5 x 10^5 sorted naïve ($TCR\beta^+CD4^+CD25^-CD62^{hi}CD44^{lo}$) $CD4^+$ T cells into *Rag1*$^{-/-}$ mice. Recipient mice were monitored regularly for signs of disease, including weight loss, hunched appearance, pilo-erection of the coat and diarrhea, and analyzed at various times after the initial transfer or when they reached 80% of their initial weight.

Preparation of intraepithelial and lamina propria lymphocytes. Intraepithelial and lamina propria lymphocytes were isolated as previously described (Mucida et al., 2007). Briefly, small and large intestines were removed and placed in chilled HBSS media containing 2% FCS. The intestines were carefully cleaned from the mesentery and flushed of fecal content. Intestines were opened longitudinally and then cut into 1cm pieces. The intestinal tissue was transferred to a 50-ml Falcon tubes containing 25ml of cold HBSS complemented with 2%FCS and 5mM EDTA and shaken (2x) at 230 rpm for

20 min at 37°C. The tissue suspension was passed through a stainless steel sieve into 50-ml conical tubes and the cells were pelleted by centrifugation at 1200 rpm for 10 min at 4°C. The cell pellet was resuspended in complete HBSS, layered over a discontinuous 40/70% Percoll gradient, and centrifuged at 2000 rpm for 30 min. Cells from the 40/70% interface were collected, washed and resuspended in complete RPMI media. These purified cells constituted the intraepithelial lymphocyte (IEL) population. To isolate the lamina propria lymphocytes (LPL), the remaining intestinal tissue in the stainless steel sieve was minced and transferred to conical tubes. The minced pieces were resuspended in 6ml of complete RPMI containing 0.1mg/ml of collagenase VIII (Sigma, USA) plus 100μg/ml DNAseI (Roche) and shaken at 80 rpm for 30-60 min at 37°C in a 6-well plate. The tissue suspension was collected and passed through a 70 mm cell strainer and the cells were pelleted by centrifugation at 1200 rpm. The cells were then resuspended and layered onto a 40/70% Percoll gradient, centrifugated and processed as described above for the IEL preparation.

Chromatin immunoprecipitation qPCR (ChIP-qPCR). Chromatin was prepared from 5-10x10^6 cells isolated from wild-type (WT), *Runx3* conditional knock-out (*Cd4*Δ*Runx3*) mice, *Tbx21*$^{-/-}$ mice (negative control) or from 3x10^6 *in vitro* differentiated Th1 and CD4$^+$CD8αα$^+$ cells (naive CD4$^+$ T cells isolated from OTII mice were cultured as decribed above). In brief, cells were fixed with 1% (vol/vol) methanol-free formaldehyde for 10 min at room temperature and glycine was added to a final concentration of 0.125 M. Cell were washed with cold 1X PBS followed by a 10 min lysis with 0.5% Triton-X on ice. The chromatin was sonicated to obtain fragments around 500 bp–1 kb in length via

S220 Focused-ultrasonicator (Covaris). The chromatin was incubated with Protein A beads coupled to anti-T-bet antibody (H-210, Santa Cruz) overnight at 4°C. Beads were washed extensively and immune-precipitated chromatin was treated with Proteinase K overnight at 56°C. DNA was extracted by phenol-chloroform extraction followed by ethanol precipitation in the presence of 0.3 M sodium acetate and 20 μg of glycogen. Extracted DNA was analyzed by real-time PCR with the following primer sequences spanning the -17kb *Runx3* regulatory region (forward 5'-atcttctaaggggccgtgac-3', reverse 5'-ttgtggttctgtggttgagg -3'), -39kb *Runx3* regulatory region (forward 5'-agcccattcgtgactgctc-3', reverse 5'-agctggggtgagctcattta-3'), *Zbtb7b* regulatory binding site 1 (RBS1, forward 5'-cagaataggcgcgcagtta-3', reverse 5'-ctggctggtccaagtacaca-3') and *Zbtb7b* regulatory binding site 2 (RBS2, forward 5'-ctaaagagctgtgtgctagacc-3', reverse 5'-gtttcaggcaggtgaggttc-3')(Setoguchi et al., 2008). We used the -1kb region of *Up1* as a negative control (forward 5'-tgtttagtttggcttgagccc-3', reverse 5'-ccctcctactcgtttcaaac-3') (Setoguchi et al., 2008) and -34kb CNS region of *Ifng* (forward 5'- ggtatgcatcatcccggg-3', reverse 5'-tggcctgtcttcagaagtttgc-3') as positive control (Zhu et al., 2012).

Statistics. Statistical analysis was performed in GraphPad Prism software. Data were analyzed by applying one-way ANOVA or unpaired Student's *t*-test whenever necessary. A *P* value of less than 0.05 was considered significant.

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