

Figure S1. Characterization of peripheral CD8⁺ T cells from Runx3-deficient mice. (A) CD8⁺ T cells were positively selected from *Runx3*^{-/-} mice or WT (*Runx3*^{+/+}) littermate controls (ICR background); the *Runx3*^{-/-} (KO) cells were separated into CD8⁺CD4⁻ SP or CD8⁺CD4⁺ DP cells by positive selection of CD4⁺ cells. CD8 and CD4 staining are shown for total pooled cells from the spleen and lymph nodes (after red blood cell lysis) before and after fractionation. The percentage of cells in each quadrant is shown. (B) The isolated cells were stimulated for 2 d with anti-CD3 and anti-CD28; CD25, CD69, and CD44 staining are shown for *Runx3*^{+/+} (continuous line) or *Runx3*^{-/-} (shaded) CD8⁺CD4⁻ SP cells. (C) Accumulation of *Runx3*^{+/+} versus *Runx3*^{-/-} SP cells during in vitro clonal expansion in 100 U/ml rhIL-2 after 2 d of stimulation with plate-bound anti-CD3 and anti-CD28. Total viable cell numbers are based on a starting population of 10⁶ cells for each sample and the fold expansion observed at each subsequent time point. The inset shows data for the first 3 d of culture on an expanded scale. The results are representative of at least two independent differentiations of *Runx3*^{+/+} versus *Runx3*^{-/-} cells.

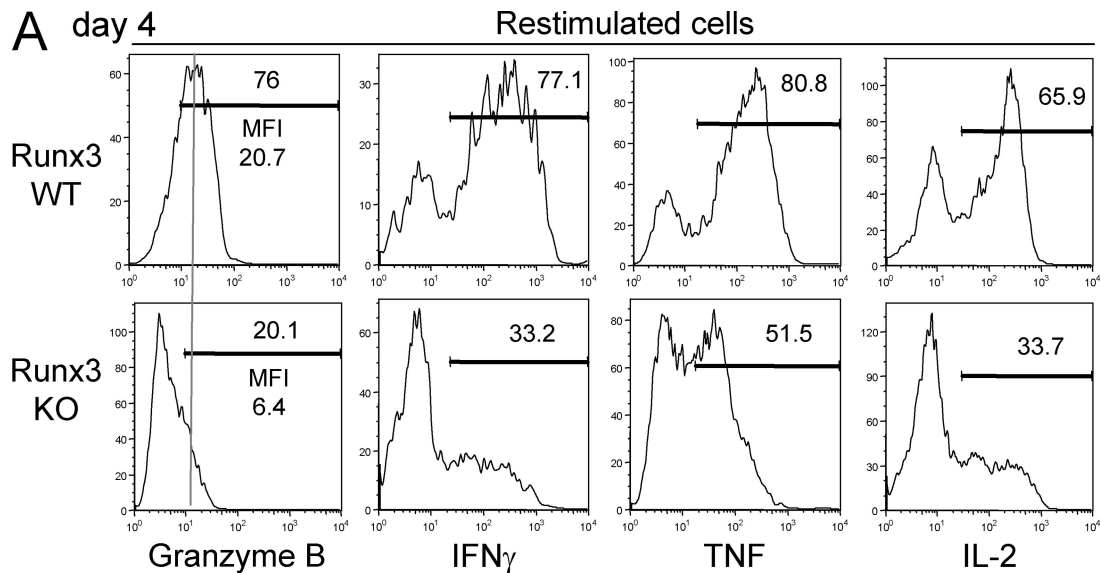


Figure S2. Granzyme B, IFN- γ , TNF, and IL-2 protein expression are substantially diminished in Runx3-deficient CD8⁺ T cells at day 4 of in vitro culture. *Runx3*^{+/+} and *Runx3*^{-/-} CD8⁺CD4⁻ SP T cells were activated and cultured for 4 d, and restimulated for 4 h with PMA and ionomycin. Expression of CTL effector proteins was determined by intracellular staining. The percentage of positively stained cells is shown above the gate in each histogram, and the MFI of granzyme B staining for the total population is presented in the leftmost column. Results are representative of at least two independent differentiations of *Runx3*^{+/+} versus *Runx3*^{-/-} cells.

Table S1. Primer sets used for ChIP experiments

Target region sequence	Forward primer (5' to 3')	Reverse primer (5' to 3')
Promoter regions (TSS)		
<i>Hprt</i>	CCTAAATCTTGAGGAATCACATCA	TCCTTCTGAAGAAAATGGTACTGG
<i>Ifng</i>	ACTCTAACATGCCACAAAACCATAG	CTCCAGTTTTATACCTGATCGAAG
<i>Il4</i>	GAGTTAAAGTGCTGAAACCAAGG	ATTTCCAATTGGTCTGATTTCAC
<i>Il2rb</i>	AACATCTCGACACAACTCCATGT	TACAAAGGGAACCAAGTTAGAGATG
<i>Gzmb</i>	TGATGACGTCTCTGAGTACTGTG	ACTCTGATACCATAGGCTACAAAAC
<i>Prf1</i> transcription unit		
-2 kb	CTATCTCAGGCACACCAAAGAG	AAAATAAGAATAAGTTGGGCTGGAG
-1 kb	AAAAGCTGTAAAAGTGTGTTCTGA	AAAGCTCAGAGACATTGAGTCTTA
TSS	CAGGGCAGGAAGTAGTAATGATATG	CTCCTCCTCTTACCTGAAGTC