Supporting Information

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SI Materials and Methods

Mice. CD45.1$^+$ congenic mice were obtained from the European Mouse Mutant Archive (Centre de Développement des Techniques Avancées pour l'expérimentation animale Centre National de la Recherche Scientifique, Orleans, Cedax2, France).

Antibodies and Flow Cytometry. The antibodies used for the analysis are as follows: biotin, FITC or PE anti-CD3ε (145-2C11), PE-Cy7 or biotin anti-CD4 (RM4-5), anti-CD16/CD32 (2.4G2), anti-CD28 (37.51), biotin anti-CD45R (B220), biotin anti-erythroid cells (Ter119), biotin Gr.1 (anti-Ly-6G and anti-Ly-6C) (RB6-8C5), biotin anti-NK1.1 (PK136), biotin anti-CD11b (M1/70), biotin anti-CD11c (HL3), FITC or PE anti-CD44 (IM7), PE anti-CD8b (Ly3.2, 53.5.8), FITC anti-CD69 (H1.2F3), FITC anti-CD62L (MEL-14), PE anti-Va2 (B20.1), PE anti-CD25 (PC61.5), and Horizon V450 anti-TCRβ (H57-597) from BD Biosciences; Pacific blue anti-CD3ε (500A2) and APC anti-CD8α (53.6.7) from eBioscience; PE-Cy7 anti-CD4 (RM4-5) from Biolegend. Samples were acquired on FACSCalibur and LSRII (BD Biosciences) and data were analyzed with FlowJo software (Treestar).

Isolation of Splenic T Cells and CFSE-labeling of T Cells. CD8$^+$ T cells from the various *Cd8* enhancer-deficient mice were purified by negative depletion. Cell suspensions of spleens (after red blood cell lysis) were incubated with biotinylated (bio)–anti-CD4 (final concentration: 3 μg/mL), bio–anti-CD45R (4 μg/mL), bio–anti-Gr1 (4 μg/mL), bio–anti-CD11b (1 μg /mL), bio–anti-CD11c (1 μg/mL), bio–anti-NK1.1 (1 μg /mL), bio–anti-Ter119 (1 μg/mL) for 30 min in 1 mL PBS/2% FBS. The cells were washed once, and subsequently the CD8$^+$ T cells were purified by negative depletion using streptavidin beads (BD Biosciences) according to the manufacturer's instructions. The purity of the cells was assessed by flow cytometry and was routinely > 90%. Purified CD8$^+$ splenic T cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes) by incubating 1×10^7 cells/mL in PBS with 10 mM CFSE for 10 min at 37°. The CFSE-labeling reaction was stopped by adding 5 mL FCS. CD8$^+$ T cells were washed twice with 10 mL of T-cell medium.

Runx3$^{F/F}$ and *Cbfb*$^{F/F}$ T cells (with or without Cre) were purified by FACS sorting after initial negative depletion of splenic cells using bio–anti-CD45R (4 μg/mL), bio–anti-Gr1 (4 μg/mL), bio–anti-CD11b (1 μg /mL), bio–anti-CD11c (1 μg/mL), bio–anti-NK1.1 (1 μg /mL), bio–anti-Ter119 (1 μg/mL). For the experiment described in Fig. 5*A*, *Runx3*$^{F/F}$ x *Cd4*-Cre and *Runx3*$^{F/F}$ CD8$^+$ T cells were purified by positive selection using CD8 MACS beads according to the manufacturer's protocol (Miltenyi).

Differentiation of Th1 Cells. Purified CD4$^+$ T cells were stimulated (day 0) with plate-bound anti-CD3ε (1 μg/mL) and anti-CD28 (3 μg/mL) on 48-well plates (0.5×10^6 cells per well) in 1 mL T-cell medium (RPMI Glutamax-I supplemented with 10% FCS, antibiotics and 2-mercaptoethanol; all from Invitrogen) in the presence of rhIL-2 (20 U/mL), IL-12 (5 ng/mL), and anti–IL-4 (3 μg/mL). Cells were split 1:2 after 48 h, transferred into new 48-well plates without stimulation, and further cultured for an additional 3 d in the presence of rhIL-2 (20 U/mL) and anti–IL-4. The generation of Th1 cells was confirmed by IFN-γ induction upon 4 h of PMA/ionomycin stimulation.

CD8$^+$ T-Cell Stimulation. Purified CD8$^+$ T cells were stimulated with plate-bound anti-CD3ε (1 μg/mL) and anti-CD28 (2 μg/mL) on 48-well plates (0.5×10^6 cells per well) in 500 μL T-cell medium [RPMI Glutamax-I supplemented with 10% FCS, antibiotics, 50 μM β-2-mercaptoethanol, 1 mM sodium pyruvate (1 mM) and 1 mM nonessential amino acids] in the presence of recombinant human interleukin 2 (20 U/mL rhIL-2, a kind gift from W. Held, Ludwig Institute for Cancer Research, Lausanne, Switzerland). The cells were split 1:2 after 2 d and cultured on 48-well plates in 1 mL T-cell medium in the presence of 100 U/mL rhIL-2 for a period of up to 14 d. The cells were split (1:2) every 2 to 3 d. Cells were analyzed for surface marker expression at various time points during the culture period.

In Vivo Cytotoxic T-Lymphocyte Assay. $E8_I^{+/+}$ and $E8_I^{-/-}$ mice were immunized into each footpad with 50 μL ovalbumen (OVA) peptide (SIINFEKL; 2 mg/mL in PBS)/adjuvant (CpG ODN1668; 1 mg/mL) or with 50 μL PBS as control. Six days postimmunization, target cells (3×10^7) were iontravenously injected into the immunized mice. Target cells consisted of a 1:1:1 mixture of CD45.1 splenocytes. Splenocytes were either pulsed without peptide, with irrelevant peptide (VYDFFVWL, derived from murine tyrosine-related protein-2; Bachem) or with relevant OVA peptide (SIINFEKL). Splenocytes (1×10^7 cells in 1 mL PBS) were pulsed with peptide (10 μg/mL) at 37° for 2 h. Subsequently, "empty" splenocytes, or splenocytes pulsed with irrelevant or relevant peptide were labeled with 0.05, 0.5, and 5 μM CFSE, respectively, as described above. Eight hours after target cell injection, immunized $E8_I^{+/+}$ and $E8_I^{-/-}$ mice were killed and the percentage of CFSE^{low}, CSFE^{med}, and CSFE^{hi} target cells in the draining lymph node was determined by flow cytometry. Specific killing was calculated as follows: % of specific killing = [1 – (% of CFSE^{hi} cells/% of CFSE^{low} cells] × 100.

cDNA Synthesis, Quantitative Real-Time PCR, and Semiquantitative RT-PCR. Total RNA was isolated from sorted splenic naive or activated (day 5) CD8$^+$ T cells (5×10^5 cells) using TRI reagent (Sigma) and cDNA synthesis was performed using oligo-dT primers and SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's protocol. SYBR Green and CFX96 Real-Time PCR system (Bio-Rad) were used for quantitative real-time PCR. Primers to detect the expression of *Hprt1* and *Runx3* are listed in Table S1. Cycling conditions for quantitative real-time PCR were 3 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 62 °C, and 30 s at 72 °C. Cycling conditions for RT-PCR were 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 40 s at 58 °C, and 40 s at 72 °C.

Propidium Iodide and Annexin V Staining. Propidium iodide and Annexin V-eFluor 450 Apoptosis Detection Kit (eBioscience) were used for the analysis of cell death rate according to the manufacturer's protocol.

Retroviral Constructs and Virus Production. Phoenix-E packaging cells were grown in DMEM supplemented with 10% of FCS and antibiotics. A murine stem-cell virus (M-SCV)-based retroviral constructs (15 μg) containing CRE recombinase (M-SCV-CRE-pgk-GFP; kindly provided by Dan Littman, New York University Medical Center, New York, NY) and control vector (M-SCV-pgk-GFP) were transiently transfected into Phoenix-E packaging cells plated in a 10-cm culture dish by using standard calcium phosphate precipitation. One day after transfection, the medium was changed into T-cell medium. Viral supernatants were collected on the following day, filtrated through 0.45-μm filter, and used for the infection of CD8$^+$ T cells.

Retroviral Infection of CD8$^+$ T Cells. Retroviral infection was performed according to published protocols (1), with minor modification. Briefly, 1 d after anti-CD3/28 stimulation, 5×10^5 CD8$^+$ T cells were suspended in 1 mL of viral supernatants containing 10 μg of polybrene (Sigma; H9268), and centrifuged at $600 \times g$ for 1 h at 32 °C. After spin infection, cells were placed into fresh media containing 20 U/mL rhIL-2, and further cultured as described above. Five days after activation, surface marker expression was analyzed by flow cytometry.

Adoptive Transfer Experiments. Purified CD8$^+$ T cells isolated from $E8I^{+/+}$,OT-I and $E8I^{-/-}$,OT-I mice were injected (2×10^6 cells in 200 μL PBS) intravenously into C57BL/6 mice. After 2 d, the recipients were immunized with 50 μL OVA peptide (SIINFEKL; 1 mg/mL in PBS) into each footpad of mice. Immunized mice were killed at day 5 and draining lymph node T cells were analyzed for CD8α expression by flow cytometry.

Immunoblot Analysis to Detect Loss of Core-Binding Factor-β Expression. Purified $Cbfb^{F/F}$ CD8$^+$ T cells that were transduced with M-SCV-pgk-GFP or M-SCV-CRE-pgk-GFP were sorted at day 5 in GFP$^-$ and GFP$^+$ populations. Sorted CD8$^+$ T cells (1×10^5 cells) were directly lysed in 20 μL of sample buffer (20% β-Mercaptoethanol, 8% SDS, 43.5% Glycerin, 0.25M Tris-HCl pH 6.8 and bromophenol blue) and heated at 95 °C for 10 min, and separated on a 12% SDS-polyacrylamide gel. Resolved proteins were transferred onto a PVDF membrane (Immobilon-P; Millipore). The membrane was incubated with a rabbit anti–core-binding factor-β (CBFβ) antibody (2) or with rabbit anti–β-actin (Sigma-Aldrich), followed by HRP-conjugated goat anti-rabbit (Pierce Biotechnology). Detection was performed with the chemiluminescent substrate SuperSignal West-Dura (Pierce Biotechnology). The signal was measured on Fuji Film (LAS 4000).

ChIP Assays. Naive or activated CD8$^+$ T cells ($5 \times 10^6 - 10^7$ cells) were resuspended in 10 mL PBS containing 0.6% formaldehyde and incubated for 5 min at 37 °C, followed by addition of 1 mL 2.5 M glycine. After washing twice with PBS, cells were lysed with lysis buffer (50 mM Tris pH8.0, 10 mM EDTA, 1% SDS,) on ice and sonicated to obtain 0.5- to 1-kb chromatin fragments. ChIP assays were performed according the protocol from Upstate Biotechnology. Five microliters (5 μg) of anti-AcH3 (#06–599; Upstate Biotechnology), 5 μL (6.6 μg) of anti-H3K4me3 (#pAb-003–050; Diagenode), 5 μL (5 μg) of anti-H3K27me3 (a kind gift from Meinrad Busslinger, Research Institute of Molecular Pathology, Vienna, Austria), and 10 μL (5 μg) of anti-CBFβ and 12.5 μL (5 μg) of control rabbit-IgG (sc-2027; Santa Cruz) were used per reaction, which was performed in a total volume of 2 mL. The presence of DNA target sequences was assessed by PCR. The PCR was carried out for 3 min at 95 °C, followed by 34 cycles of 30 s at 95 °C, 40 s at 58 °C, and 40 s at 72 °C. PCR products were resolved on a 2% agarose gel. PCR products were quantified using Multiguage software (Fuji). Signal intensities at 1:5 input dilutions were used to calculate relative signal intensities for each amplicon. The relative values (arbitrary units) for each amplicon are shown below the PCR blots and were calculated according to Sato et al. (3): [(intensity antibody precipitates) – (intensity mock precipitates)]/(intensity input). The primers used for the various PCR reactions are shown in Table S1.

1. Jenkinson SR, et al. (2007) Expression of the transcription factor cKrox in peripheral CD8 T cells reveals substantial postthymic plasticity in CD4-CD8 lineage differentiation. *J Exp Med* 204:267–272.
2. Naoe Y, et al. (2007) Repression of interleukin-4 in T helper type 1 cells by Runx/Cbf beta binding to the Il4 silencer. *J Exp Med* 204:1749–1755.
3. Sato T, et al. (2005) Dual functions of Runx proteins for reactivating CD8 and silencing CD4 at the commitment process into CD8 thymocytes. *Immunity* 22: 317–328.

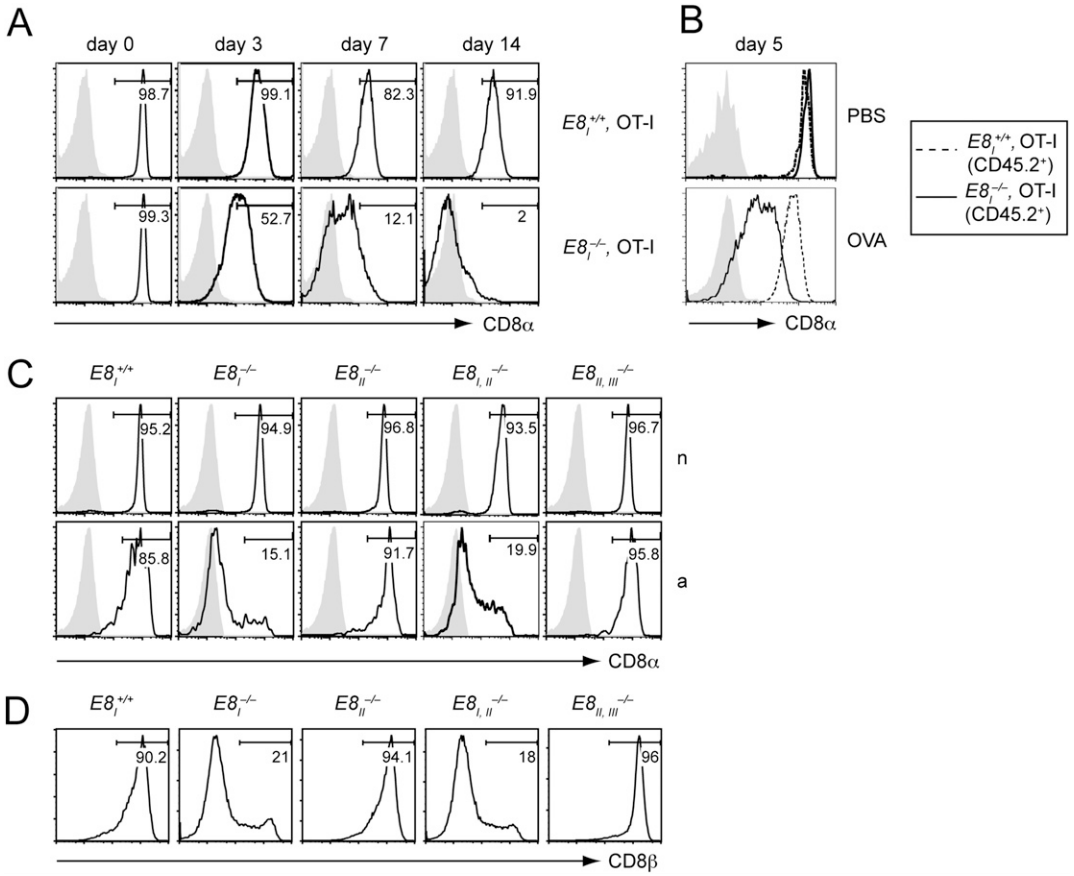

Fig. S1. (*A*) CD8$^+$ T cells isolated from *E8I*$^{+/+}$,OT-I and *E8I*$^{-/-}$,OT-I mice were activated with irradiated APC loaded with OVA peptide and CD8α expression was assessed at days 3, 7, and 14. Numbers indicate the percentage of cells in the indicated region. Filled histograms indicate CD8α expression levels on naive nonactivated CD4$^+$ T cells. Data are representative of three independent experiments. (*B*) *E8I*$^{+/+}$,OT-I and *E8I*$^{-/-}$,OT-I CD8$^+$ splenic T cells (both CD45.2$^+$) were isolated and adoptively transferred into CD45.1$^+$ C57BL/6 mice. Two days after the transfer, recipient mice were immunized with OVA peptide (SIINFEKL) /Alum (OVA) or with PBS/Alum (PBS). CD8α expression on CD45.2$^+$ *E8I*$^{+/+}$,OT-I (dotted line) and on CD45.2$^+$ *E8I*$^{-/-}$,OT-I (solid line) T cells isolated from the draining lymph node at day 5 postimmunization is shown. Filled areas indicate CD8α expression levels on naive nonactivated CD4$^+$ T cells. Data are representative of three independent experiments. (*C*) CD8$^+$ T cells isolated from mice of the indicated genotypes were activated with anti-CD3/anti-CD28. At day 14, CD8α expression was assessed. Numbers indicate percentages of cells in the respective region. Filled areas indicate CD8α expression levels on naive nonactivated CD4$^+$ T cells. Data are representative of three independent experiments. (*D*) CD8$^+$ T cells isolated from mice of the indicated genotypes were activated with anti-CD3/anti-CD28. At day 14, CD8β expression was assessed. Numbers indicate percentages of cells in the respective region. Data are representative of three independent experiments.

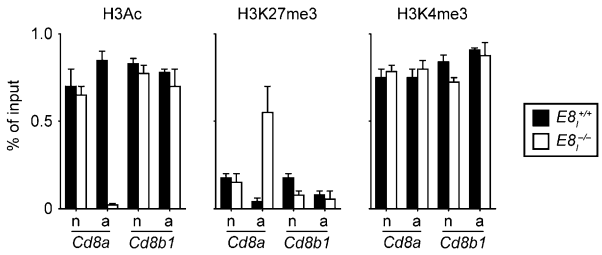

Fig. S2. PCR products from the experiments described in Fig. 3 were quantified. The relative values (arbitrary units) for each amplicon were calculated according as described in *SI Materials and Methods*. Diagrams show the summary of two independent experiments (mean ± SD).

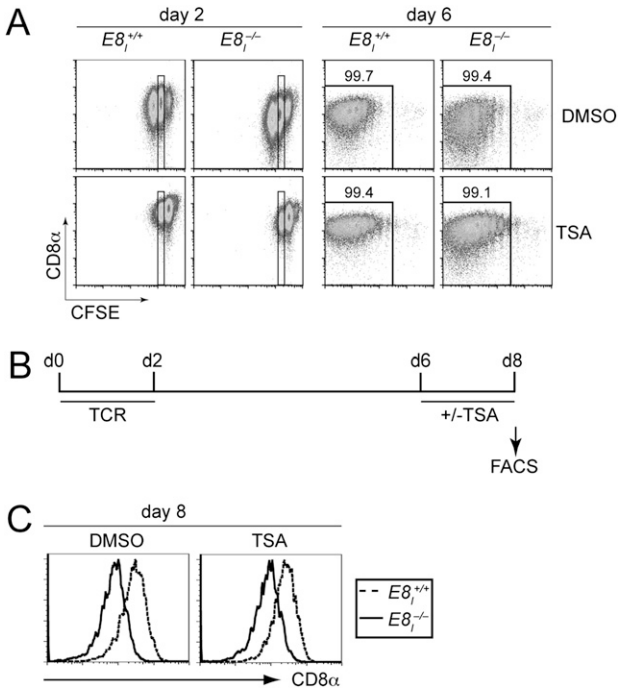

Fig. S3. (*A*) Description of gating regions. $E8_I^{+/+}$ and $E8_I^{-/-}$ $CD8^+$ T cells were labeled with CFSE and stimulated with anti-CD3/anti-CD28 in the presence of trichostatin A (TSA) or DMSO (as carrier control) as described in Fig. 4*A*. Dot plots show CD8α expression vs. CFSE at days 2 and 6 after stimulation. Data are representative for two independent experiments. (*B*) Experimental outline: $E8_I^{+/+}$ and $E8_I^{-/-}$ $CD8^+$ T cells were stimulated with anti-CD3/anti-CD28 for 2 d, and cultured for additional 4 d without TSA, as described in *Materials and Methods*. At day 6, $E8_I^{+/+}$ and $E8_I^{-/-}$ $CD8^+$ T cells were treated with TSA for 2 d. CD8α expression was assessed at day 8. (*C*) Histograms show CD8α expression at day 8 on $E8_I^{+/+}$ and $E8_I^{-/-}$ $CD8^+$ T cells that were treated as described in *B*. Data are representative of two independent experiments.

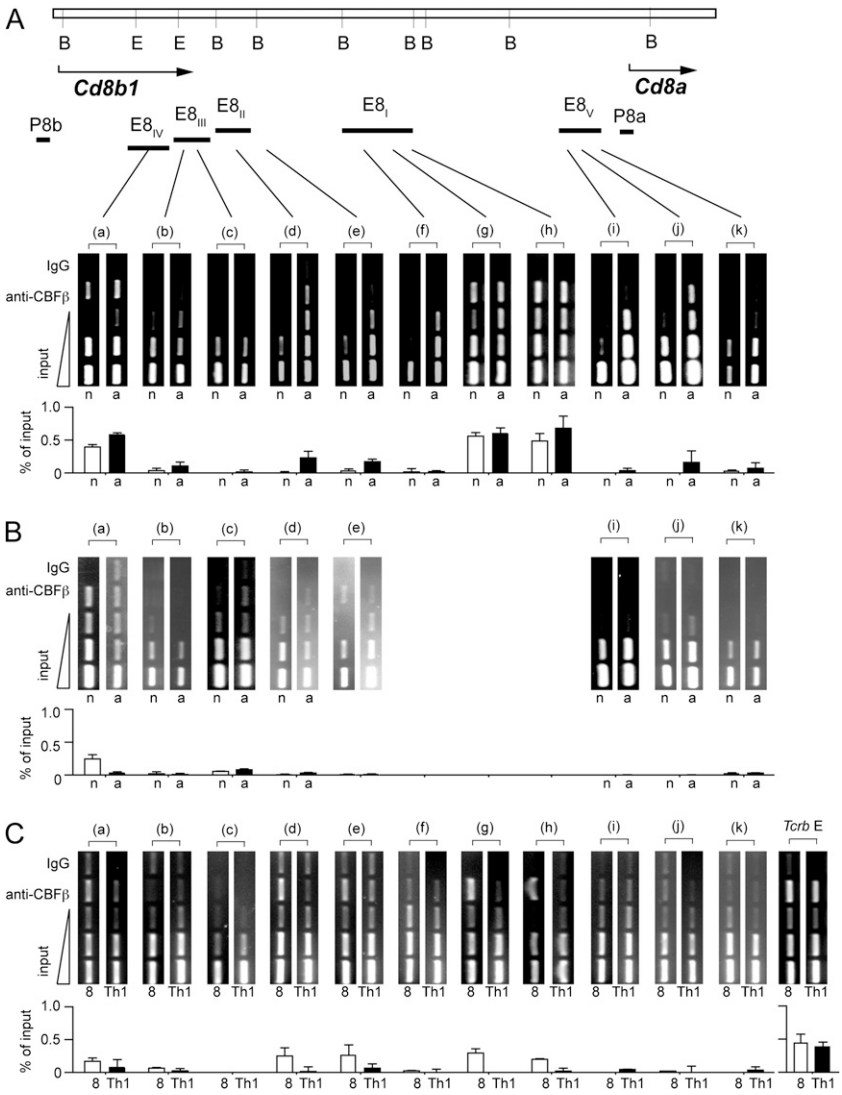

Fig. S4. (*A*) Wild-type CD8$^+$ T cells were isolated, activated for 5 d and subjected to ChIP assays using anti-CBFβ antibodies. (*Top*) Map of the *Cd8ab* gene complex indicating the location of the *Cd8a* and *Cd8b1* genes and of the five different enhancers $E8_I$-$E8_V$. (*Middle*) Semiquantitative PCR analysis of ChIP with anti-CBFβ from wild-type naive and anti-CD3/anti-CD28 activated (5 d) CD8$^+$ T cells. a, activated; n, naive. (*B*) $E8_I$-deficient cells CD8$^+$ T cells were isolated, activated for 5 d, and subjected to ChIP assays using anti-CBFβ antibodies. The panel shows the semiquantitative PCR analysis of ChIP with anti-CBFβ from wild-type naive and anti-CD3/anti-CD28 activated (5 d) CD8$^+$ T cells. The locations of the regions a to e and I to k are shown in *A*. The 7.6-kb genomic region containing $E8_I$ (as shown in *A*) is deleted in $E8_I$-deficient cells. a, activated; n, naive. (*C*) Wild-type CD4$^+$ T cells were isolated, polarized into Th1 cells, and subjected to ChIP assays using anti-CBFβ antibodies. The panel shows the semiquantitative PCR analysis of ChIP with anti-CBFβ from wild-type Th1 cells (Th1) and as control from naive CD8$^+$ T cells. The locations of the regions a to k are shown in *A*. The *Tcrb* enhancer (E) was used as a positive control for CBFβ binding (1). Data are representative of two independent experiments. PCR products were quantified as described in *SI Materials and Methods* and the relative intensity is shown in the lower part of each panel. Diagram shows the summary of two independent experiments (mean ± SD).

1. Djuretic IM, et al. (2007) Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. *Nat Immunol* 8:145–153.

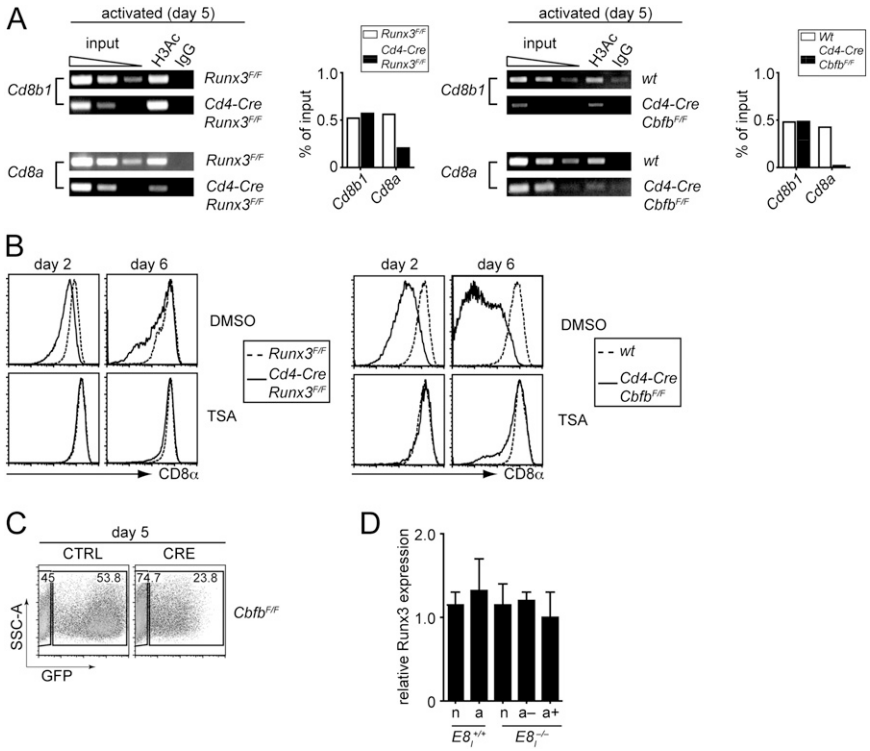

Fig. S5. (*A*) ChIP analysis of the *Cd8a* and *Cd8b1* promoter region. Chromatin from anti-CD3/anti-CD28 activated (day 5) *Runx3*$^{F/F}$ and *Cd4-Cre* × *Runx3*$^{F/F}$ CD8$^+$ T cells (*Left*) or from activated wild-type (wt) and *Cd4-Cre* × *Cbfb*$^{F/F}$ (*Right*) was immunoprecipitated with anti-H3Ac or control IgG followed by PCR with primers specific for the *Cd8a* and *Cd8b1* promoter region. Sorted CD8α$^-$ cells were used from activated Runx3-deficient or CBFβ-deficient cells. Input DNA was PCR amplified undiluted or at a dilution of 1:5 or 1:25 (wedges) to ensure PCR quantification in a nonsaturated amplification range. Diagram shows the relative intensity of PCR products calculated as described in *SI Materials and Methods*. (*B*) *Runx3*$^{F/F}$ and *Cd4-Cre* × *Runx3*$^{F/F}$ (*Left*) or wild-type (wt) and *Cd4-Cre* × *Cbfb*$^{F/F}$ (*Right*) CD8$^+$ T cells were stimulated with anti-CD3/anti-CD28 in the presence of TSA (or DMSO as a control) for 2 d, and cultured for additional 4 d without TSA, as described in *Materials and Methods*. Histograms show CD8α expression on CD8$^+$ T cells. Data for each genotype are representative of two independent experiments. (*C*) Dot plot shows side scatter (SSC-A) vs. GFP expression on day 5 of *Cbfb*$^{F/F}$ CD8$^+$ T cells that have been transduced with M-SCV-pgk-GFP (CTRL) and M-SCV-CRE-pgk-GFP (CRE) retrovirus. Gating regions for GFP$^+$ T cells are shown. (*D*) Quantitative real-time PCR expression analysis of distal promoter-derived *Runx3* in naive (n) and activated (a) (day 5) *E8*$_I^{+/+}$ and *E8*$_I^{-/-}$ CD8$^+$ T cells. Activated *E8*$_I^{-/-}$ T cells were sorted into CD8α$^-$ (a–) and CD8α$^+$ (a+) subsets. *Runx3* expression relative to *Hprt1* expression is shown. Data show summary of two independent experiments (mean ± SEM).

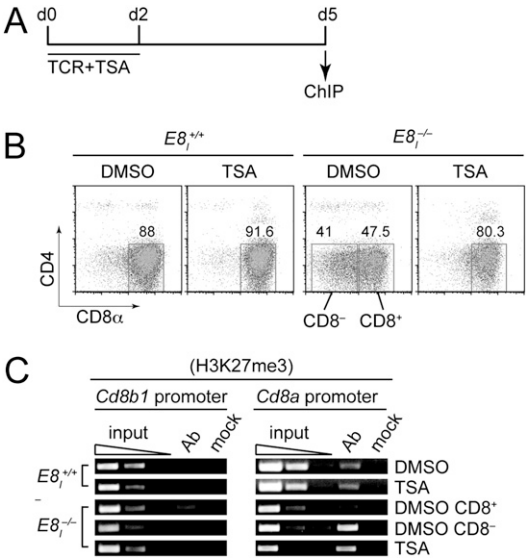

Fig. S6. (*A*) Experimental outline: $E8_I^{+/+}$ and $E8_I^{-/-}$ CD8$^+$ T cells were stimulated with anti-CD3/anti-CD28 in the presence of TSA or DMSO for 2 d, and cultured for additional 3 d without TSA. At day 5, $E8_I^{+/+}$ and $E8_I^{-/-}$ CD8$^+$ T cells were sorted. (*B*) Dot plots showing CD4 vs. CD8α expression on anti-CD3/anti-CD28 activated (day 5 treated with DMSO or TSA) $E8_I^{+/+}$ and $E8_I^{-/-}$ CD8$^+$ T cells. Rectangles indicate sorting gates for cell separation and subsequent isolation of chromatin. Activated DMSO-treated $E8_I^{-/-}$ cytotoxic T cells were sorted for CD8α$^-$ and CD8α$^+$ subsets. (*C*) Chromatin from the sorted cells as described in *B* was immunoprecipitated with anti-H3K27me3 antibody followed by PCR with primers specific for the *Cd8a* and *Cd8b1* promoter region. For the mock precipitations, no antibody was added. Input DNA was PCR amplified undiluted or at a dilution of 1:5 or 1:25 (open wedges) to ensure PCR quantification in a nonsaturated amplification range. Data are representative of two independent experiments.

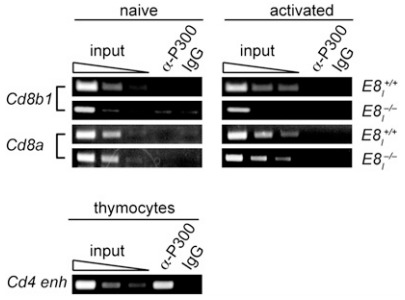

Fig. S7. (*Upper*) ChIP analysis of the *Cd8a* and *Cd8b1* promoter region. Chromatin from naive (*Left*) or anti-CD3/anti-CD28 activated (day 5, *Right*) $E8_I^{+/+}$ and $E8_I^{-/-}$ CD8$^+$ T cells (sorted CD8α$^-$ cells from activated $E8_I^{-/-}$ cells) was immunoprecipitated with an anti-p300 ChIP or IgG (as control) antibodies followed by PCR with primers specific for the *Cd8a* and *Cd8b1* promoter region. (*Lower*) Positive control showing p300 binding to the *Cd4* enhancer, as previously described (1). Chromatin from double-positive (DP) thymocytes was immunoprecipitated with an anti-p300 ChIP or IgG (as control) antibodies followed by PCR with primers specific for the *Cd4* enhancer. (*Upper* and *Lower*) Input DNA was PCR amplified undiluted or at a dilution of 1:5 or 1:25 (open wedges) to ensure PCR quantification in a nonsaturated amplification range. Data are representative of two independent experiments.

1. Yu M, et al. (2008) Nucleoprotein structure of the CD4 locus: Implications for the mechanisms underlying CD4 regulation during T cell development. *Proc Natl Acad Sci USA* 105: 3873–3878.

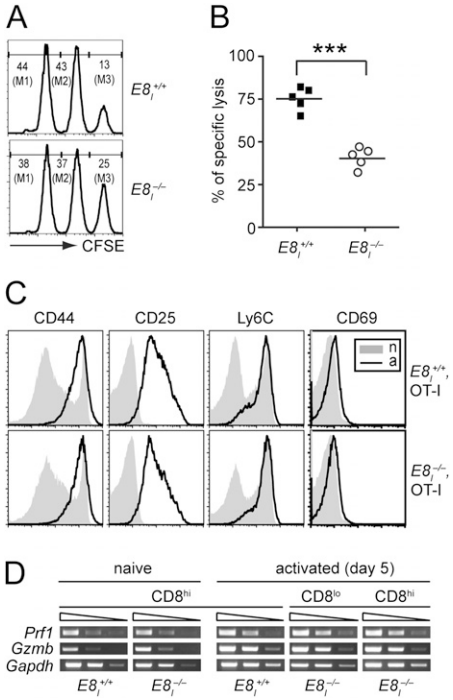

Fig. S8. (*A*) $E8I^{+/+}$ and $E8I^{-/-}$ mice were immunized with OVA peptide (SIINFEKL) plus adjuvant, and 6 d later target cells were intravenously injected. Target cells consisted of a 1:1:1 mixture of CFSE-labeled splenocytes that were either pulsed with OVA peptide ($CFSE^{hi}$), with irrelevant peptide ($CFSE^{med}$), or without peptide ($CFSE^{low}$). Eight hours after target-cell injection, the percentage of $CFSE^{low}$, $CSFE^{med}$, and $CSFE^{hi}$ target cells in the draining lymph node of $E8I^{+/+}$ and $E8I^{-/-}$ mice was determined. (*B*) Summary of all in vivo CTL experiments performed ($n = 5$). Diagram shows the percentage of specific lysis, as defined in *SI Materials and Methods*. Each symbol represents one mouse, and horizontal lines indicate average values. An unpaired Student *t* test was used for statistical analysis. $***P < 0.001$. (*C*) $CD8^+$ T cells isolated from $E8I^{+/+}$,OT-I and $E8I^{-/-}$,OT-I mice were activated with irradiated APC loaded with OVA peptide, and the expression of activation markers was determined at day 3. Filled histograms indicate surface marker expression on nonactivated $E8I^{+/+}$,OT-I and $E8I^{-/-}$,OT-I $CD8^+$ T cells. Data are representative of three independent experiments. (*D*) Naive and activated $E8I^{+/+}$ and $E8I^{-/-}$ $CD8^+$ T cells were sorted and RNA was isolated. Activated $E8I^{-/-}$ cytotoxic T cells were further sorted for $CD8\alpha^-$ and $CD8\alpha^+$ subsets. Semiquantitative RT-PCR analysis shows *Prf1* and *Gzmb* expression in the various cell subsets. *Gapdh* expression was used as loading control. The triangle indicates fivefold dilutions of input. Data are representative of two independent experiments.

Table S1. PCR primers used for ChIP assays, semiquantitative RT-PCR, and quantitative real-time PCR

Gene loci amplified	Sequence (5′ to 3′)	Ref.
Cd8b1 promoter	GCC CCG CCC TCT TAG CTC ACT TAC GCG CCT GTC TAT TCC TCC TGT TGC	(1)
Cd8a promoter	ACC TCC AGA CAA GAA GCA AAA ACA AGT GGC TGA AAG AAG AAG GAT AAA	(1)
$E8_{IV}$ (a)	TGC ATT TGC AGA AAT GGA TAC ACT TCA GAT TTT CCT CC	Site a from ref. 2
$E8_{III}$ (b)	CTA GCT GAA CCT GGT GCA CA ATG CAG GGA GGA GAG AGA AG	Site b from ref. 2
$E8_{III}$ (c)	GAC AGT ATC TCA AAG GCT AG ATG CTC TAA GAC AGA AGG TTG	Site c from ref. 2
$E8_{II}$ (d)	GAG GCC CTT TGC TTG TAT CC AGC CGC CCA CTT CCT ATC G	This study
$E8_{II}$ (e)	AGT GGG CGG CTT GTT TTC GGG TCC CGA TCT GAG TGC	Amplicon 6 from ref. 1
$E8_{I}$ (f)	TAG ACT CAA GGG AGA AGA GA TCC CTG GCT GGA GGT ACC TG	Site g from ref. 2
$E8_{I}$ (g)	GCC TCT GCT ACT TCT AGA TG CTC AGA TGC CGA TCT CAT TT	Site h from ref. 2
$E8_{I}$ (h)	CAC TTC CTT TTC TCC CAA CC ATG AAG CTT GTG AAT GGA CC	Site j from ref. 2
$E8_{V}$ (i)	GAA GCT AAC ACA GTG GAA GGT GAA GAT ACT GTT TCA GCA ACT	Site m from ref. 2
$E8_{V}$ (j)	ACA CAG AGA AAC CCT GCC TTG TGC CTG CCC ATC ACT ATA CTT	Site n from ref. 2
$E8_{V}$ (k)	CTG CAC CCA AGA TTC ACC CG ACT AGC CCT TGG ATA GGT GC	Site o from ref. 2
Cd8b1	CGC TGA TCA TTT GTG AAA CTG TTT GAG TGG CCG TCT ACT TTT ACT GTG T	(3)
Cd8a	GTT CTG TCG TGC CAG TCC TT GCC GAC AAT CTT CTG GTC TC	(3)
Prf1	TAG CCA ATT TTG CAG CTG AG CTG AGC GCC TTT TTG AAG TC	(4)
Gzmb	GGA TAT AAG GAT GGT TCA CC CAC CTG TCC TAG AGC AAT CC	(5)
Gapdh	GAA ATC CCA TCA CCA TCT TCC AGG CGC GGC CAT CAC GCC ACA GTT TCC	(6)
Runx3	GTC AGC GTG CGA CAT GGC TTC CAA CAG AGC ACG TCC ACC ATC GAG CGC ACT TCG G	(7)
Tcrb	AGA ATG GCC ACC TGC CAT AG GAT GTG GCA AGT GTG GTT CCC AAA	(8)
Cd4 enhancer	GTG GAG GCG GAT CCT GTC AGC TTT G GGG TCG GTA GCT GGG CTG GGT TAC A	(9)
Hprt1	GAT ACA GGC CAG ACT TTG TTG GGT AGG CTG GCC TAT AGG CT	(1)

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