SUPPLEMENTAL MATERIAL

Zhang et al., http://www.jem.org/cgi/content/full/jem.20092136/DC1

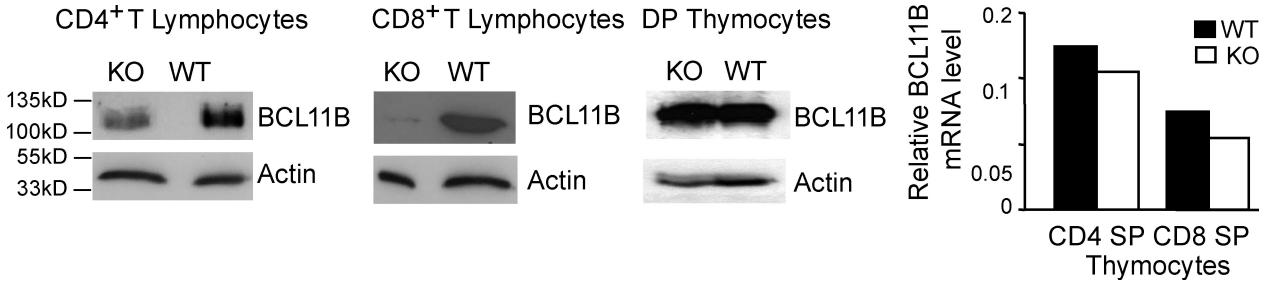

Figure S1. Conditional removal of Bcl11b in mature T cells with the use of the dLck-iCre system. CD4+ and CD8+ T lymphocytes and DP thymocytes from Bcl11b$^{F/F}$/dLck-iCre and control mice were purified, cytoplasmic and nuclear proteins were extracted as previously described (Cismasiu et al., 2005), and Bcl11b was determined by Western blot analysis or qRT-PCR. Data are representative of three pairs of mice.

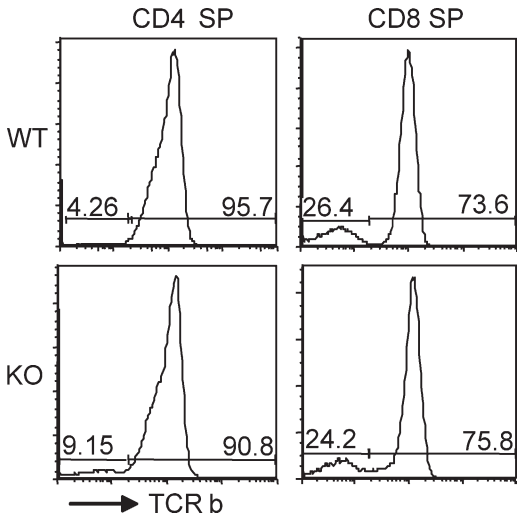

Figure S2. TCR-β on CD4 and CD8 SP thymocytes of Bcl11b$^{F/F}$/dLck-iCre mice. Thymocytes of Bcl11b$^{F/F}$/dLck-iCre and control mice were stained for CD4, CD8, and TCR-β. Histograms show expression of TCR-β on gated CD4 and CD8 SP thymocytes. Data are representative of three independent experiments.

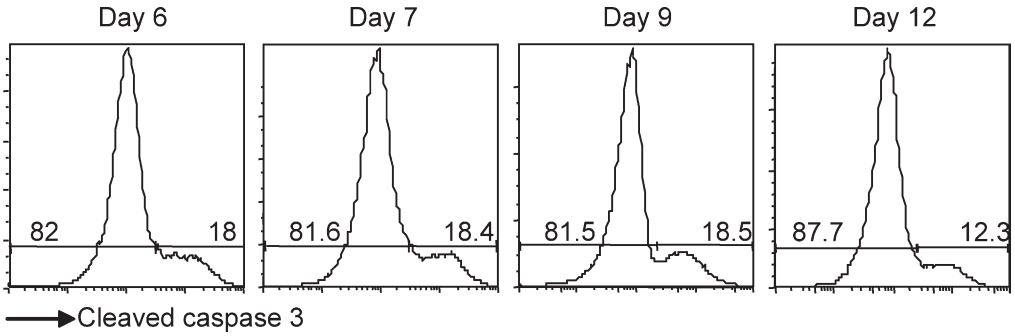

Figure S3. Histograms showing cleaved caspase-3 levels on gated CD45.1 recipient CD8⁺ T cell populations. Levels of cleaved caspase-3 in CD45.1 CD8⁺ T cells of recipient mice during the course of infection (compare to Fig. 3 C, where cleaved caspase-3 levels are indicated in the donor cells).

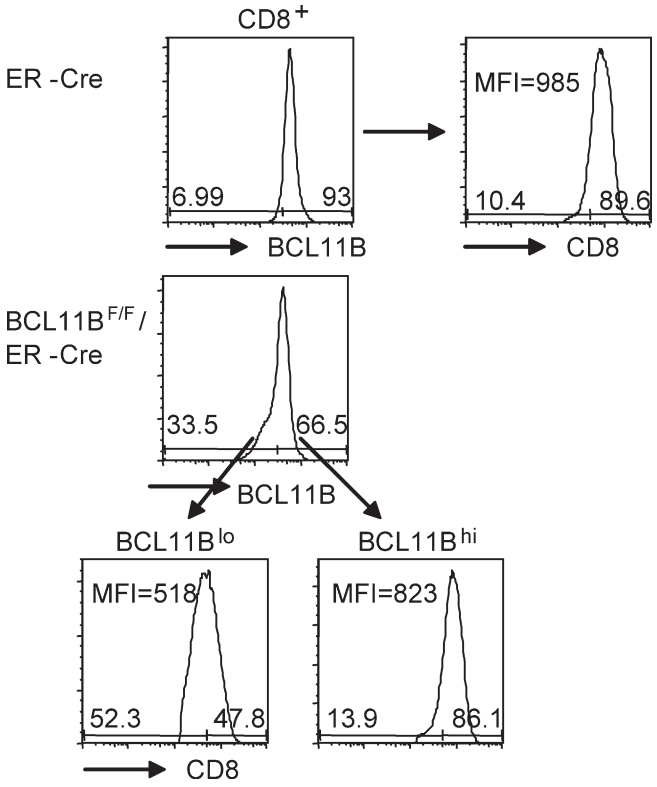

Figure S4. Acute removal of Bcl11b in vivo with the use of ER-Cre deleter results in reduced CD8 coreceptor levels. Purified CD8⁺ T lymphocytes from Bcl11b$^{F/F}$ERcre/CD45.2 and ER-Cre/CD45.1 were transferred (1:1; total 10×10^6) in Rag2γc recipient mice, which were i.p. injected with 2 mg tamoxifen/day for 5 d. Splenocytes were stained for CD8, CD45.1, CD45.2, and Bcl11b. Histogram shows the CD8 levels on gated Bcl11b^{lo} and Bcl11b^{hi} populations within the CD45.2 CD8⁺ T cell populations. This is representative data from three independent experiments.

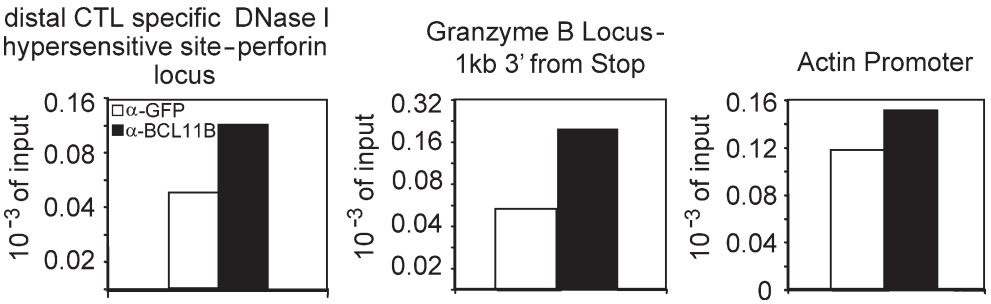

Figure S5. Bcl11b does not bind actin promoter, the distal CTL-specific DNase I hypersensitive site in the perforin locus, or a genomic region located 1 kb downstream of the granzyme B stop codon. ChIPs were conducted as described in the Materials and methods, and the eluted DNA was assayed by q-PCR using primers corresponding to the indicated genomic regions. Binding of IgG or Bcl11b was evaluated after normalization to the input as described in Materials and methods. Data are representative of three independent experiments.

Table S1. CD8 MFI in SP thymocytes and CD8+ T lymphocytes of Bcl11b$^{F/F}$/dLck-iCre and Bcl11b$^{F/F}$ mice

Mice	CD8 SP thymocytes	CD8+ T lymphocytes	
		Lymph nodes	Spleen
Bcl11b$^{F/F}$ (n = 5)	582 ± 48.8	629 ± 29.3	557 ± 59.7
Bcl11b$^{F/F}$/dLck-iCre (n = 5)	475 ± 63.0	368 ± 27.4	301 ± 17.5

CD8 SP thymocytes, P = 0.21; CD8+ T lymph node lymphocytes, P = 2.87E-05; CD8+ T spleen lymphocytes, P = 0.001.

Table S2. Ratios of CD8 MFI of Bcl11b$^{F/F}$/dLck-iCre and Bcl11b$^{F/F}$ CD8+ T lymphocytes

CD8+ T lymphocyte	Time	Ratio
Fresh CD8+ T lymphocytes	0	0.58[a]
CD8+ T lymphocytes activated with α-CD3/CD28	30 s	0.61[b]
	60 s	0.64[b]
	120 s	0.6[b]
	300 s	0.59[b]
CD8+ T lymphocytes from Lm-Ova–infected mice	7 d	0.67[c]

[a]Representative of numerous pairs of mice
[b]Representative of two independent experiments
[c]Representative of three independent experiments