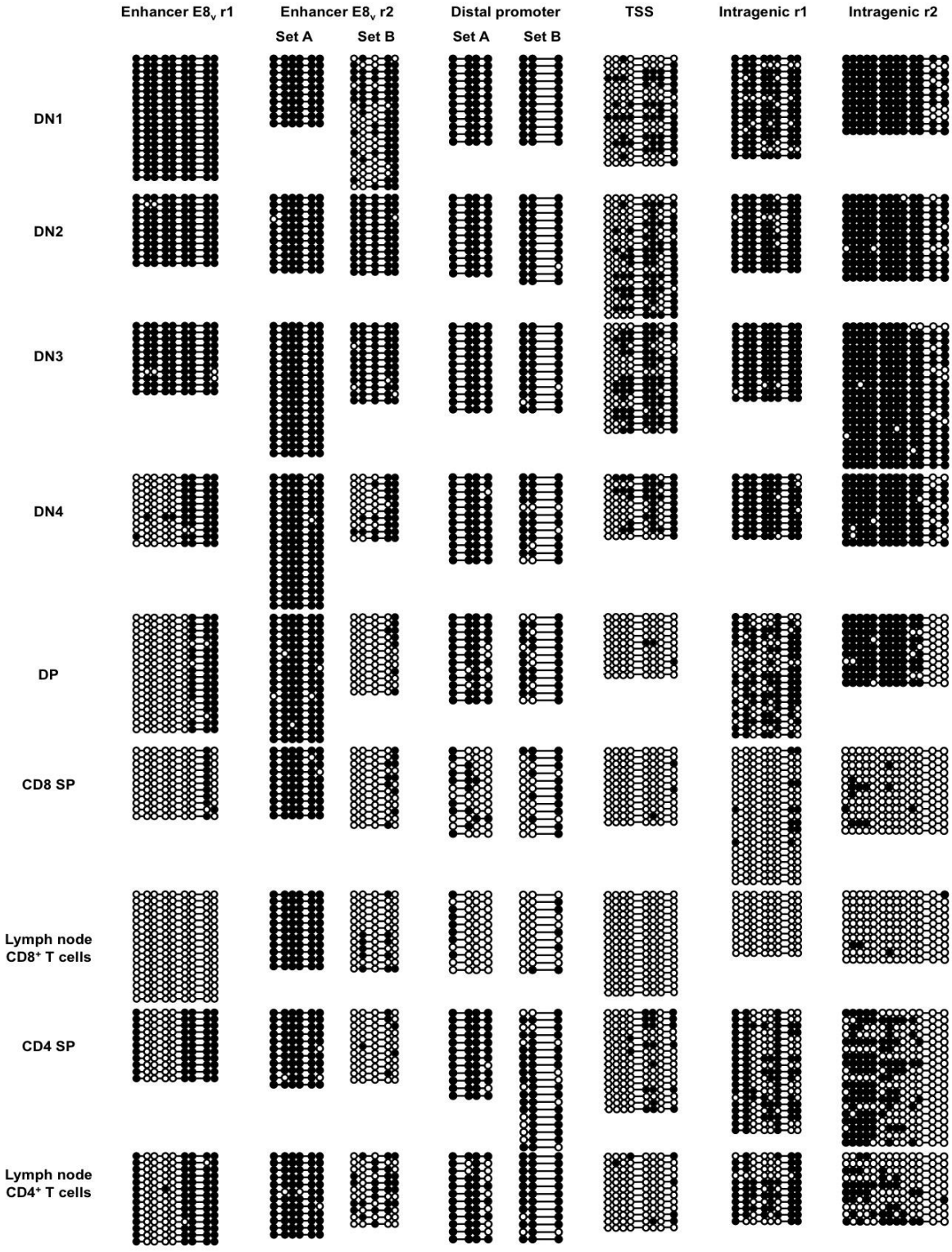
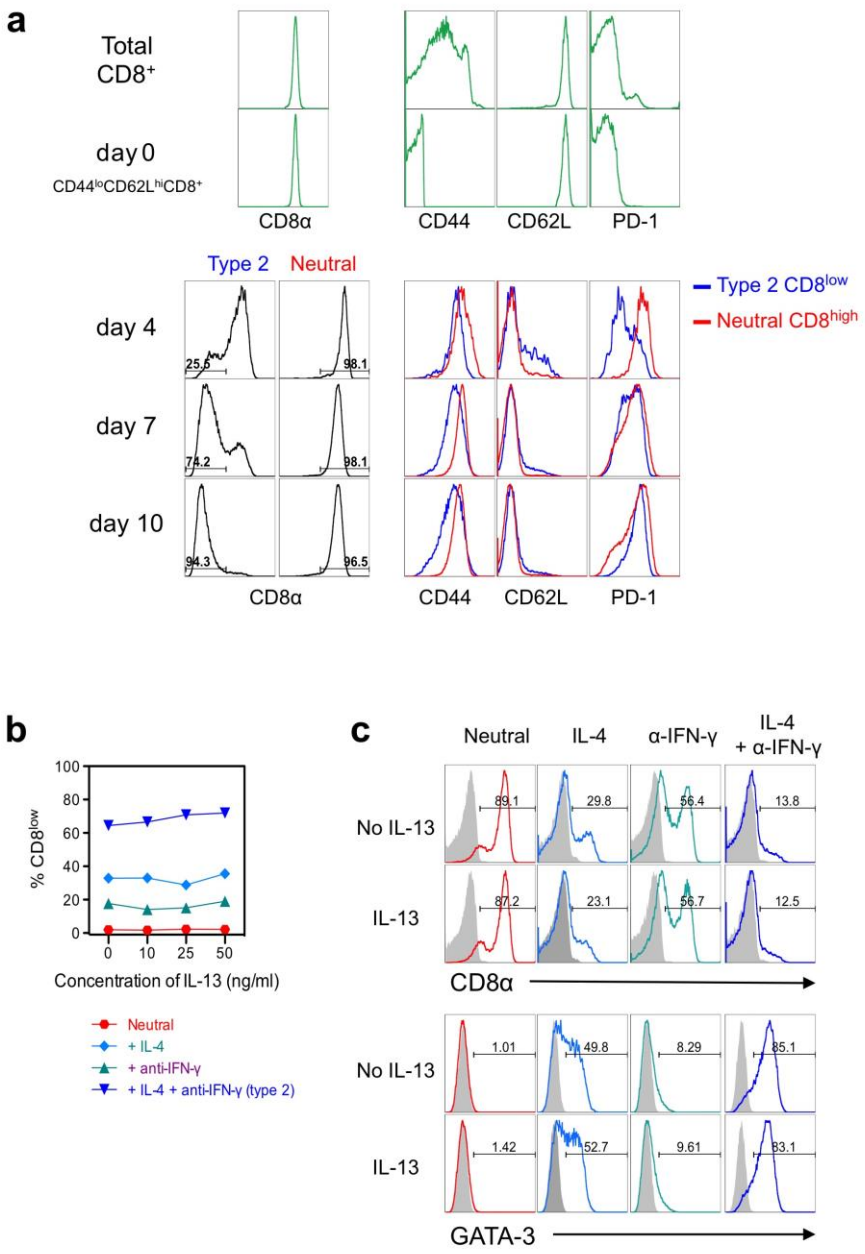
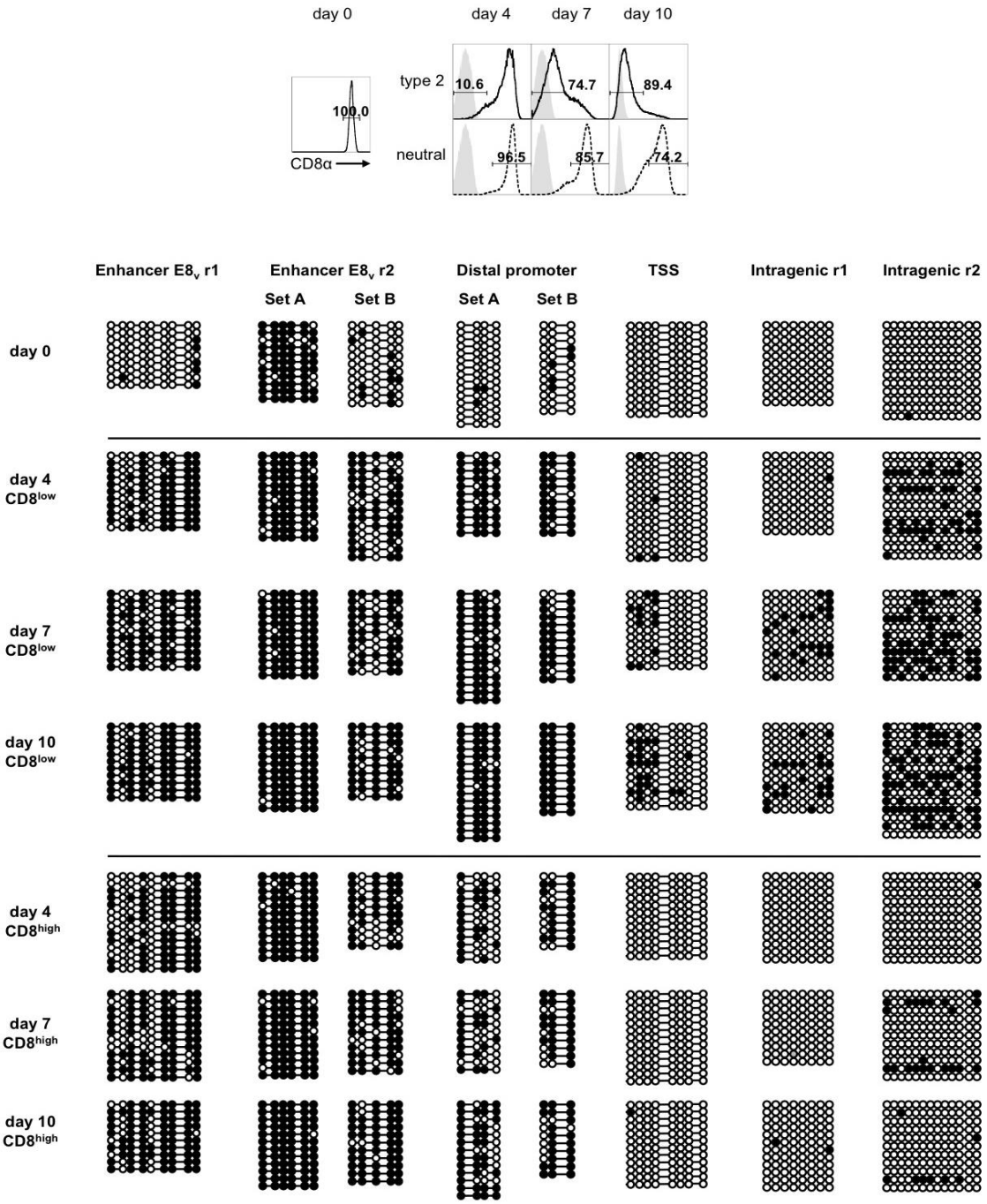


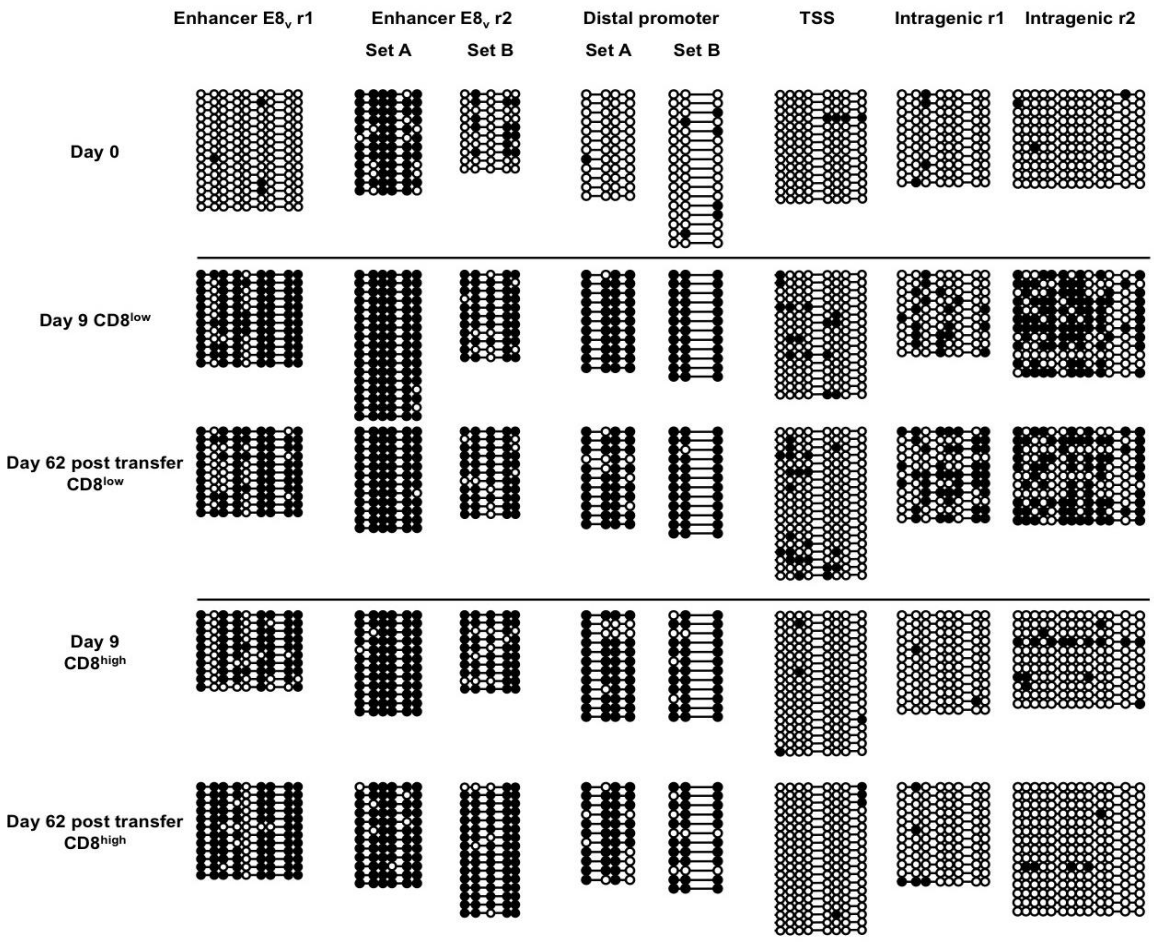
Supplementary Figure 1. DNA methylation patterns at *Cd8a* correlate with changes in CD8 expression levels during T cell development. Genomic DNA from thymocyte populations and lymph node T cells was treated with sodium bisulfite. Eight regions spanning the *Cd8a* locus were amplified by PCR. Individual PCR products were then cloned, and at least 10 randomly selected clones per lymphocyte population were sequenced. Each row of circles represents one clone and circles correspond to single unmethylated (open) or methylated (filled) CpG sites. Data are representative of two independent experiments.



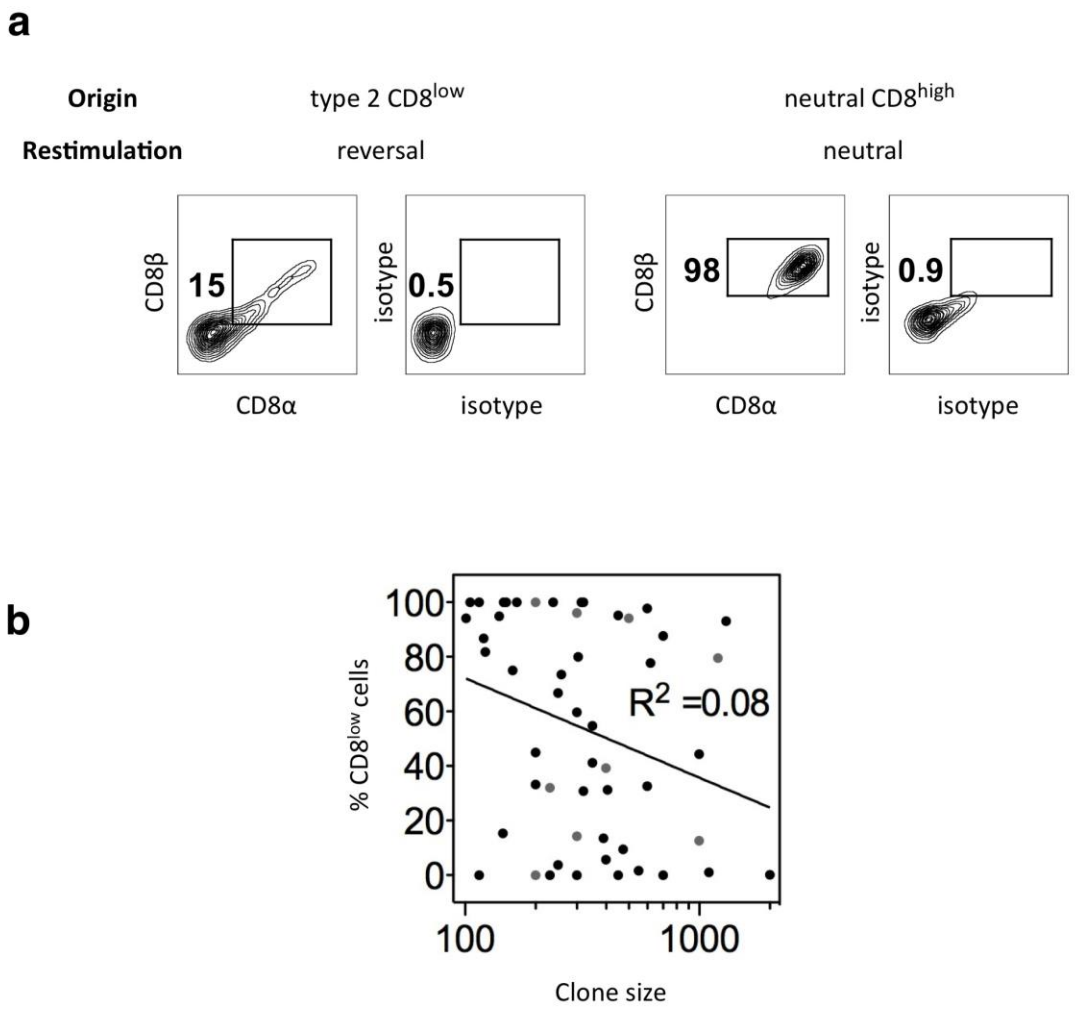
Supplementary Figure 2. IL-4 but not IL-13 modulates CD8$^+$ T cell phenotype. (**a**) Surface marker expression by unfractionated or naïve (CD44^{lo}CD62L^{hi}) CD8$^+$ T cells at day 0 (green) and purified naive CD8$^+$ T cells after activation with anti-receptor Ab in the presence of IL-2, IL-4 and anti-IFN-γ Ab (type 2 conditions) or IL-2 alone (neutral conditions) for 4, 7 or 10 days. CD8α, CD44 and CD62L Ab were conjugated to different fluorochromes in the top and bottom panels. (**b**) CD8 expression after activation of naïve CD8$^+$ cells with anti-receptor Ab and IL-2 alone (neutral) or IL-2, IL-4, IL-13 and/or anti-IFN-γ Ab as indicated for 5 days (1x10^3 cells per well in 24-well plates). (**c**) CD8 and intracellular GATA-3 expression after activation of naïve CD8$^+$ T cells as in (b) with or without 25 ng ml^{-1} IL-13 (gray, isotype control). Biological activity of IL-13 was confirmed by measuring enhancement of survival of purified BALB/c splenic B (CD19$^+$B220$^+$CD4$^-$CD8$^-$) cells *in vitro* with 10 ng ml^{-1} anti-CD40 Ab with and without 5 ng ml^{-1} IL-4 or 20 ng ml^{-1} IL-13 for 3 days[1].



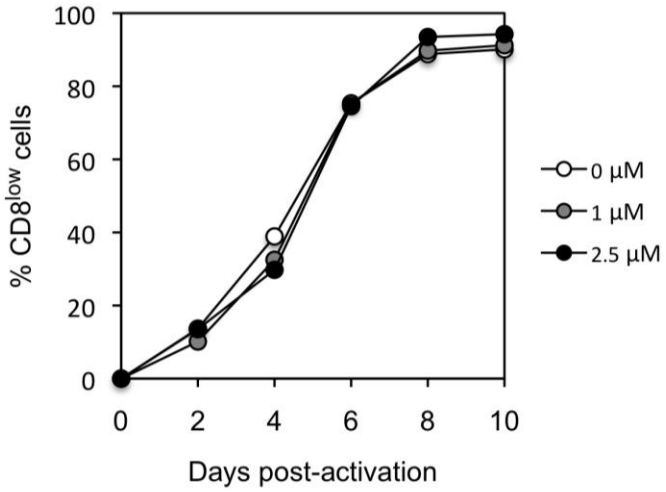
Supplementary Figure 3. IL-4-induced loss of CD8 expression is associated with increased DNA methylation at *Cd8a.* Naïve $CD8^+$ T cells were activated *in vitro* with anti-receptor Ab in the presence of IL-2, IL-4 and anti-IFN-γ Ab (type 2 conditions) or IL-2 alone (neutral conditions). After 4, 7 and 10 days, $CD8^{low}$ cells from type 2 cultures and $CD8^{high}$ cells from neutral cultures were purified and genomic DNA was isolated. Genomic DNA was also isolated from the original population of naïve $CD8^+$ T cells (day 0). DNA was subjected to CpG methylation analysis at the *Cd8a* locus. Each row of circles represents one clone and circles correspond to single unmethylated (open) or methylated (filled) CpG sites. Data are representative of at least two independent experiments.



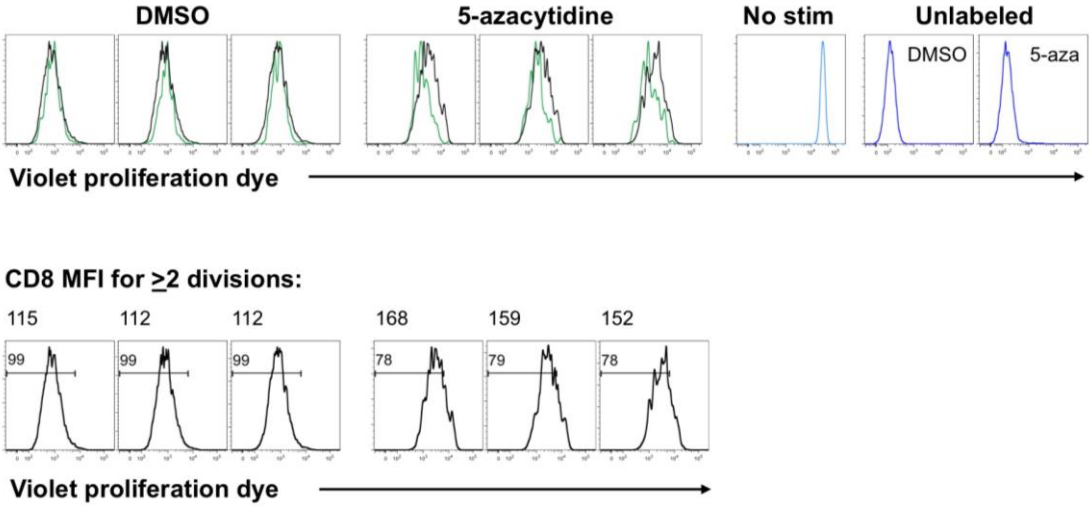
Supplementary Figure 4. Increased DNA methylation at *Cd8a* in CD8^{low} cells is maintained long term *in vivo*. Naïve CD8^{+} T cells from CD45.1^{+} C57BL/6 mice were activated with anti-receptor Ab in type 2 or neutral conditions for 7 days, then washed and re-cultured in IL-2 alone for 2 days. CD8^{low} cells from type 2 cultures and CD8^{high} cells from neutral cultures were purified and adoptively transferred into CD45.2^{+} congenic mice. CD45.1^{+} cells were recovered after 62 days *in vivo*. At each time-point, CD8^{low} cells generated in type 2 cultures and CD8^{high} cells generated in neutral cultures were purified and genomic DNA was isolated. Genomic DNA was also isolated from the original population of naïve CD8^{+} T cells (Day 0). DNA methylation patterns at the *Cd8a* locus were examined. Each row of circles represents one clone and circles correspond to single unmethylated (open) or methylated (filled) CpG sites. Data are representative of two independent experiments.



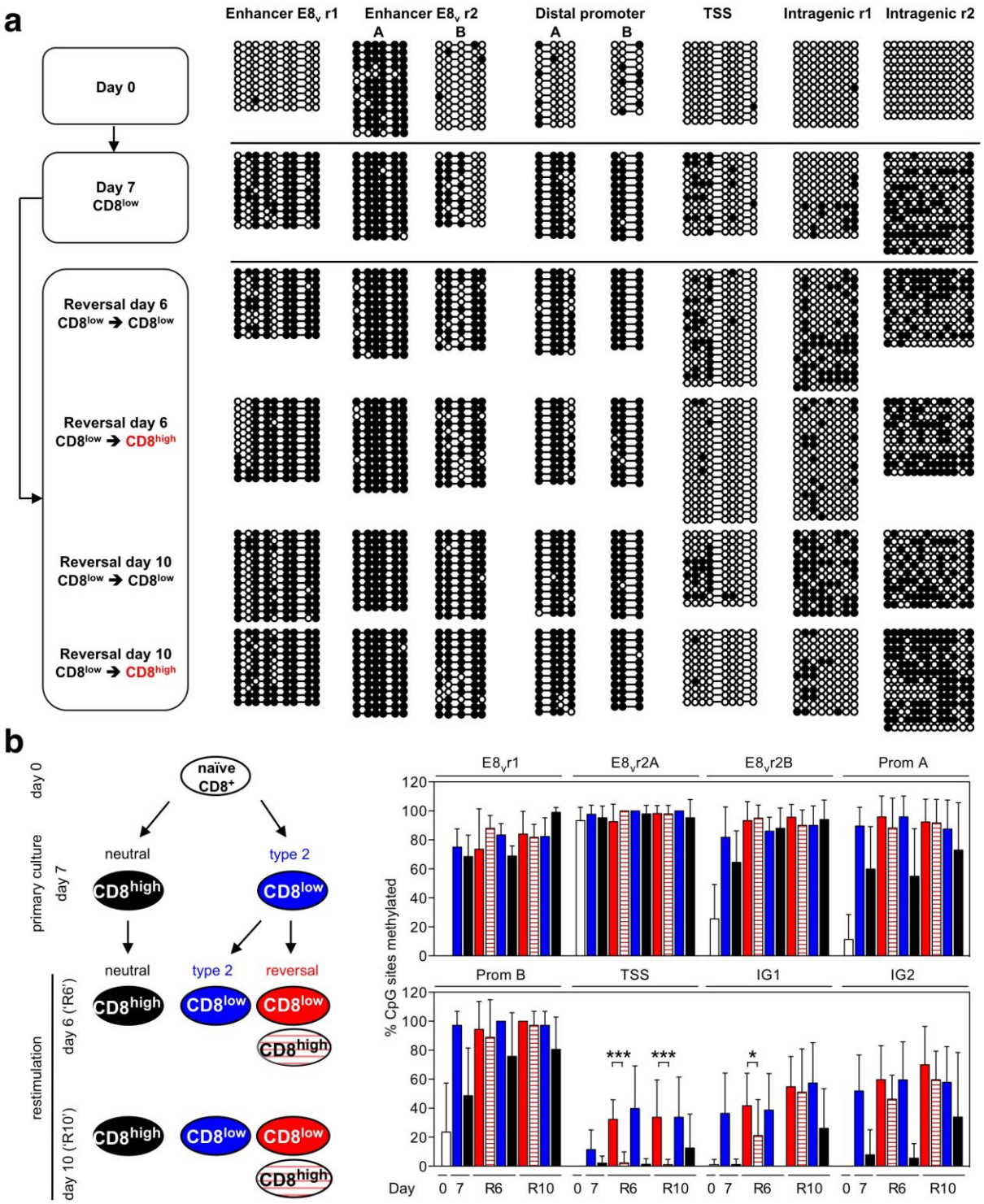
Supplementary Figure 5. Some CD8^{low} cells re-express the CD8αβ heterodimer in reversal culture. (**a**) Naïve CD8^{+} T cells were activated with anti-receptor Ab in type 2 or neutral conditions. After 7 days, CD8^{low} cells from type 2 cultures were purified and restimulated in reversal conditions (IL-2, IFN-γ and anti-IL-4 Ab) and CD8^{high} cells from neutral cultures were purified and restimulated in neutral conditions (IL-2 alone). Six days later, cells were collected, washed and incubated with fluorochrome-conjugated Ab to CD8α and CD8β (or the appropriate isotype control Ab) for analysis by flow cytometry. Data are representative of two independent experiments. (**b**) Relationship between clone size and CD8 expression in reversal cultures. Single twice-purified 7-day type 2 CD8^{low} cells were cultured in reversal conditions for 13 days (see Fig. 5). Clones estimated to contain ≥100 cells were collected for analysis by flow cytometry. Data are pooled from two independent experiments (●, ●).



Supplementary Figure 6. An inhibitor of DNA methylation does not prevent CD8 down-regulation during primary CD8$^+$ T cell activation. Purified naïve CD8$^+$ T cells were activated with anti-receptor Ab in type 2 conditions in the presence of 0, 1 or 2.5 µM 5-azacytidine. Surface CD8 expression was assayed by flow cytometry at each time-point.



Supplementary Figure 7. 5-azacytidine enhances CD8 re-expression during re-programming of type 2 CD8^{low} T cells. CD8^{low} T cells from 7-day type 2 cultures were incubated with violet proliferation dye, then twice-purified and cultured with anti-receptor Ab in the presence of IFN-γ, anti-IL-4 mAb and IL-2 (reversal conditions) with 2.5 μM 5-azacytidine in DMSO or DMSO alone. CD8 expression and cell division were assayed by flow cytometry 4 days later. Top panels: CD8^{high} cells (green) and CD8^{low} cells (black); dye-labeled twice-purified CD8^{low} cells held at 4^{o}C for 4 days (No stim); unlabeled twice-purified CD8^{low} cells after type 2 culture with or without 5-azacytidine for 4 days (Unlabeled). Bottom panels: gates denote cells that had undergone two or more divisions based on dilution of dye; CD8 mean fluorescence intensity (MFI) of the gated population is shown above the panels.



Supplementary Figure 8. Distinct patterns of DNA methylation are observed at *Cd8a* in CD8^{low} cells that re-express CD8. (**a**) Naïve CD8^{+} T cells were activated with anti-receptor Ab in type 2 conditions (IL-2, IL-4 and anti-IFN-γ Ab). After 7 days, CD8^{low} cells were purified twice and restimulated in reversal conditions (IL-2, IFN-γ and anti-IL-4 Ab). After restimulation for 6 or 10 days, CD8^{low} and CD8^{high} progeny were purified. Genomic DNA from each of the above populations was isolated and subjected to DNA methylation analysis. Each row of circles represents one clone and circles correspond to single, either unmethylated (open) or methylated

(filled) CpG sites. (**b**) Repeat of the experiment in (**a**) with additional control populations: type 2 $CD8^{low}$ cells purified twice at day 7 then continued in type 2 culture, and neutral $CD8^{high}$ cells purified at day 7 and continued in neutral culture. Data show the mean percentage (± s.d.) of methylated CpG sites within a region. Mann-Whitney tests were used to compare % CpG methylation within a region between $CD8^{low}$ (solid red bars) and $CD8^{high}$ (striped red bars) cells from the same cultures following restimulation of $CD8^{low}$ cells in reversal conditions (* $P<0.05$, *** $P<0.001$).

Supplementary Table 1. Lower recovery of transferred $CD8^{high}$ cells is not due to Ab-mediated deletion *in vivo*.

	Residual anti-CD8α Ab[a]		No residual anti-CD8α Ab[b]	
	$CD8^{low}$	$CD8^{high}$	$CD8^{low}$	$CD8^{high}$
Levels of bound anti-CD8α Ab	42.6[c] (2.0)[d]	7132.0 (258.4)	12.3 (1.0)	18.9 (0.9)
% Recovery	13.43	2.52	7.24	1.46

[a] Naïve $CD45.1^+$ $CD8^+$ T cells were activated in neutral or type 2 conditions for 7 days, then re-cultured in IL-2 alone for 2 days. $CD8^{low}$ cells from type 2 cultures and $CD8^{high}$ cells from neutral cultures were purified and adoptively transferred into $CD45.2^+$ congenic mice. $CD45.1^+$ cells were recovered from the spleen and pooled lymph nodes after 32 days *in vivo*.

[b] Naïve $CD45.1^+$ $CD8^+$ T cells were activated in neutral or type 2 conditions for 7 days, then $CD8^{low}$ cells from type 2 cultures and $CD8^{high}$ cells from neutral cultures were isolated and re-cultured in IL-2 alone for 6 days to allow complete loss of bound anti-CD8α Ab (determined by flow cytometry) before adoptive transfer into $CD45.2^+$ congenic mice. $CD45.1^+$ cells were recovered from the spleen and pooled lymph nodes after 27 days *in vivo*.

[c] Mean fluorescence intensity (MFI) of bound anti-CD8α Ab at the time of adoptive transfer

[d] Ratio of anti-CD8α Ab MFI to isotype MFI at the time of adoptive transfer

Supplementary Table 2. Primers used to amplify regions of the bisulfite-modified *Cd8a* locus.

Primer	Sequence (5'-3')	Use	PCR conditions
$E8_Vr1$ ext F	gaggtagttagttttttgagattttaaggttagt[a]	1st round PCR	Touchdown 68-53°C[b]
$E8_Vr1$ ext R	cactacttctcctacaaaaaaatattcacaa[a]		
$E8_Vr1$ int F	atagtgagggagttttaggtaagttatggt[a]	2nd round PCR	Touchdown 68-53°C[b]
$E8_Vr1$ int R	ccttcaaatatatacatcttcaaaaccaa[a]		
$E8_Vr2$ Set A ext F	gtatgtagtgttgaggtaggttagtgaatat[a]	1st round PCR	Touchdown 68-53°C[b]
$E8_Vr2$ Set A ext R	caaactttctctaaataaccctaacta		
$E8_Vr2$ Set A int F	gaaagtgttatatgtattttatgaattt[a]	2nd round PCR	Touchdown 66-48°C[c]
$E8_Vr2$ Set A int R	cctaaaactcactttataaaccaaa		
$E8_Vr2$ Set B ext F	gtatttagattatatttaagagatatagtt	1st round PCR	Touchdown 68-53°C[b]
$E8_Vr2$ Set B ext R	aatcaacaacctttataaacaacacaa		
$E8_Vr2$ Set B int F	ttatggttttatttgaagaattgagggt	2nd round PCR	Touchdown 66-48°C[c]
$E8_Vr2$ Set B int R	aacttccaaaataacttcttaaatcacaaa		
Prom Set A ext F	gagtttggtggtttaagtttgtaattttagt[a]	1st round PCR	Touchdown 68-53°C[b]
Prom Set A ext R	acccactacaatcctaaaaactaccaa		
Prom Set A int F	aatgtaattggattattatgaaattagggt[a]	2nd round PCR	Touchdown 68-53°C[b]
Prom Set A int R	attttccacttaaaaccccatacaaaata		
Prom Set B ext F	gaaagttaaaggtttaagttagatggt	1st round PCR	Touchdown 66-48°C[c]
Prom Set B ext R	accttaaataacactcttaactacta		
Prom Set B int F	gtttagtatttgttaggtagagagtagt	2nd round PCR	Touchdown 68-53°C[b]
Prom Set B int R	taacctcccacccaaaaaacaaaa		
TSS ext F	gttttgtaagggtgtatttttattttg	1st round PCR	Touchdown 65-53°C[d]
TSS ext R	atacctataacttaacttctccact		
IG1 ext F	ttgggtaaaggttaagtggaaagg	1st round PCR	Touchdown 68-53°C[b]
IG1 ext R	accaaaaatacccaaatataaatatcacaa		
IG1 int F	gtggaaaggggttggtgtatttata	2nd round PCR	Touchdown 68-53°C[b]
IG1 int R	ttaaaaaccctctaaaacaaccccc		
IG2 ext F	tagatttttttttgggtgttttgagg	1st round PCR	Touchdown 68-53°C[b]
IG2 ext R	atcacacccctactaaaacaatacaa		
IG2 int F	aagttattaaaaattgagtgtgtgtgtgtg	2nd round PCR	Touchdown 68-53°C[b]
IG2 int R	acccctactaaaacaatacaaaaaacaa		

[a] Described previously by Bilic et al. (2006)[2].

[b] Touchdown 68-53°C PCR conditions:

1 cycle	95°C, 5 min
2 cycles each	95°C, 30 sec, 68°C, 30 sec, 72°C, 45 sec

	95°C, 30 sec, 66°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 64°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 62°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 60°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 59°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 58°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 57°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 56°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 55°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 54°C, 30 sec, 72°C, 45 sec
40 cycles	95°C, 30 sec, 53°C, 30 sec, 72°C, 45 sec
1 cycle	95°C, 1 min, 53°C, 1 min, 72°C, 7 min

[c] Touchdown 66-48°C PCR conditions

1 cycle	95°C, 5 min
2 cycles each	95°C, 30 sec, 66°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 64°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 62°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 60°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 58°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 56°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 54°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 52°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 50°C, 30 sec, 72°C, 45 sec
40 cycles	95°C, 30 sec, 48°C, 30 sec, 72°C, 45 sec
1 cycle	95°C, 1 min, 48°C, 1 min, 72°C, 7 min

[d] Touchdown 65-53°C PCR conditions:

1 cycle	95°C, 5 min
2 cycles each	95°C, 30 sec, 65°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 64°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 63°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 62°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 61°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 60°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 59°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 58°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 57°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 56°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 55°C, 30 sec, 72°C, 45 sec
	95°C, 30 sec, 54°C 30 sec, 72°C, 45 sec
40 cycles	95°C, 30 sec, 53°C 30 sec, 72°C, 45 sec
1 cycle	95°C, 1 min, 53°C, 1 min, 72°C, 7 min

Supplementary References

1. Lai, Y. H. & Mosmann, T. R. Mouse IL-13 enhances antibody production in vivo and acts directly on B cells in vitro to increase survival and hence antibody production. *J. Immunol.* **162**, 78-87 (1999).
2. Bilic, I. *et al.* Negative regulation of CD8 expression via *Cd8* enhancer-mediated recruitment of the zinc finger protein MAZR. *Nat. Immunol.* **7,** 392-400 (2006).