Supplementary Information

Repression of the DNA-binding inhibitor Id3 by Blimp1 limits CD8$^+$ T cell memory formation

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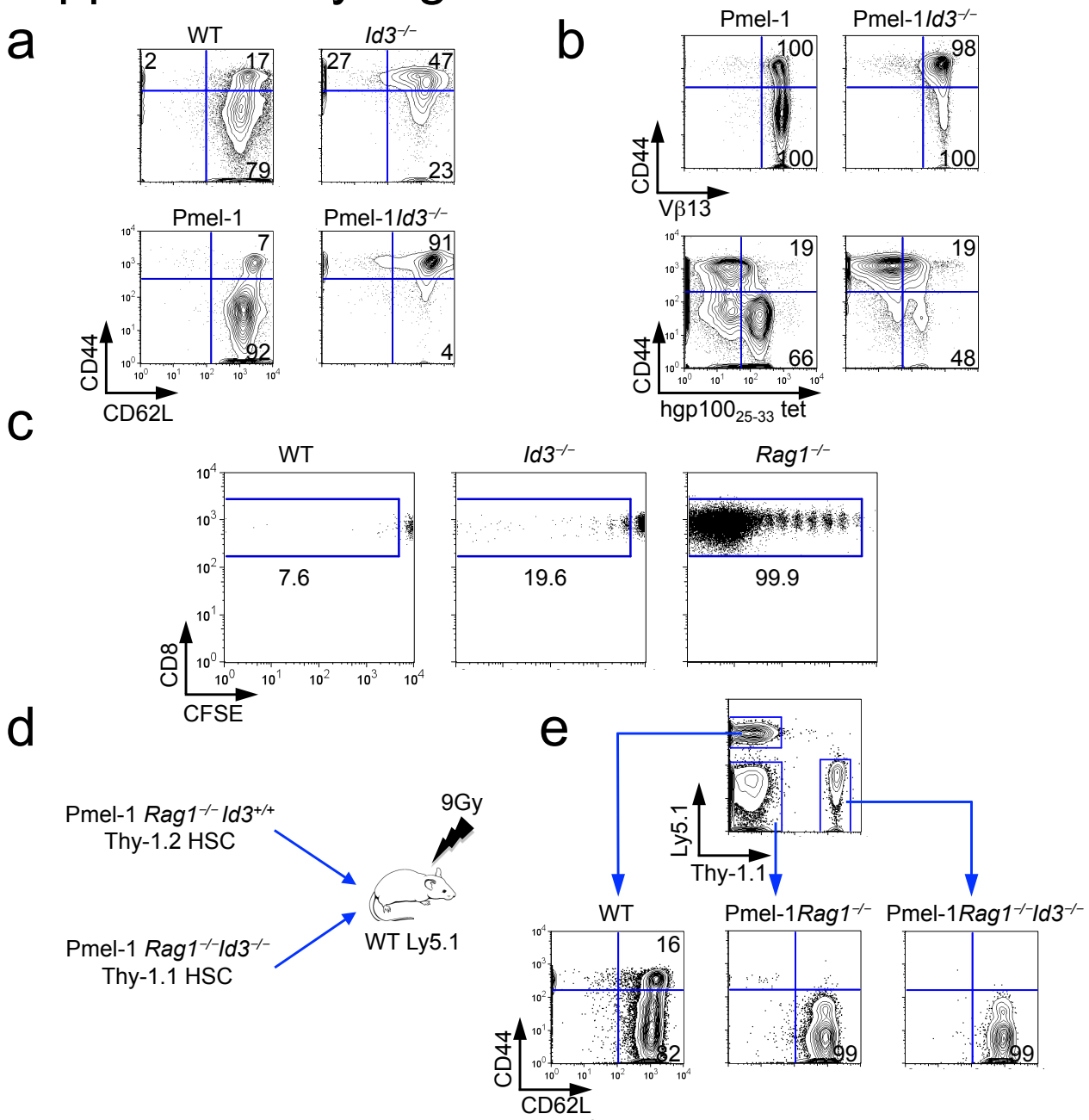
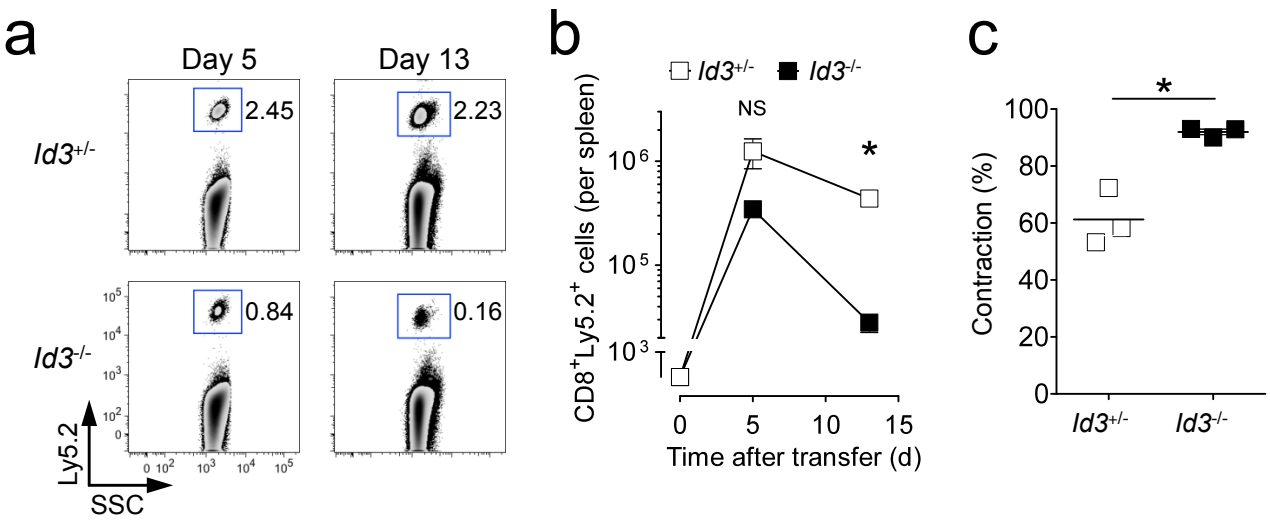
Supplementary Fig. 1



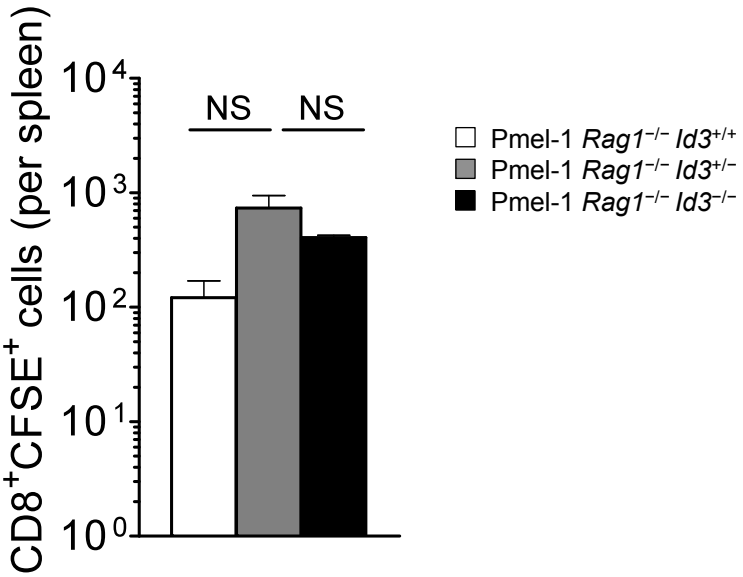
Supplementary Figure 1. The activated phenotype of *Id3*$^{-/-}$ CD8$^+$ T cells is not CD8$^+$ T cell intrinsic. (**a**) Representative flow cytometry analysis of *Id3*$^{-/-}$ CD8$^+$ T cells from WT as well as from pmel-1 mice. Contour plots show CD44 and CD62L expression after gating on CD8$^+$ cells. (**b**) Representative flow cytometry analysis of the Vβ13 (upper panel) and hgp100$_{25\text{-}33}$ tetramer(lower panel) binding efficiency of pmel-1 and pmel-1 *Id3*$^{-/-}$ CD8$^+$ T cells. Contour plots show CD44 and Vβ13 expression and hgp100$_{25\text{-}33}$ tetramer binding efficiency after gating on CD8$^+$ cells. Numbers indicate the percentage of Vβ13$^+$ or tet$^+$ cells relative to CD44 high or low cells. (**c**) Representative flow cytometry analysis of CFSE dilution profiles of adoptively transferred CFSE-labeled Ly5.1CD8$^+$ T cells into wild type, *Id3*$^{-/-}$, and *Rag1*$^{-/-}$ hosts 5 weeks after transfer. (**d**) Generation of bone marrow chimeras. Hematopoietic stem cells (HSC) from pmel-1 *Rag1*$^{-/-}$ *Id3*$^{-/-}$ Thy-1.1 and pmel-1 *Rag1*$^{-/-}$ Thy-1.2 mice were adoptively transferred into 9 Gy irradiated wild type Ly5.1 host at 1: 1 ratio. Eight weeks later CD8$^+$ T cells from the host were analyzed for the expression of CD44 and CD62L gated on the congenic markers. (**e**) Representative flow cytometry analysis of CD8$^+$ T cells derived from bone marrow chimeras. Contour plots show CD44 and CD62L expression after gating on CD8$^+$ and indicated congenic markers.

Supplementary Fig. 2



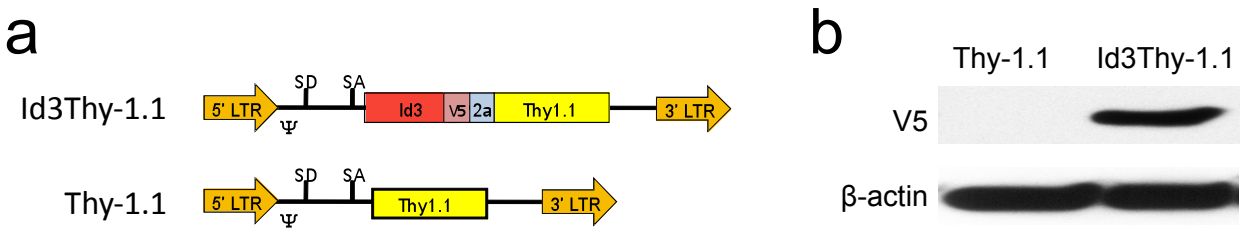
Supplementary Figure 2. Id3 regulates the contraction of effector CD8⁺ T cells. (**a**, **b**)Representative flow cytometry analysis (**a**) and numbers (**b**) of Ly5.2⁺CD8⁺ T cells following adoptive transfer of 5 x 10^3 *Id3*$^{-/-}$ or *Id3*$^{+/-}$ pmel-1 *Rag1*$^{-/-}$ CD8⁺ T cells from littermates in conjunction with gp100-VV infection at indicated time points. (**c**) Percentage contraction of *Id3*$^{-/-}$ or *Id3*$^{+/-}$pmel-1 *Rag1* $^{-/-}$ CD8⁺ T cells relative to the peak of immune response (d5). *= $P < 0.01$.

Supplementary Fig. 3



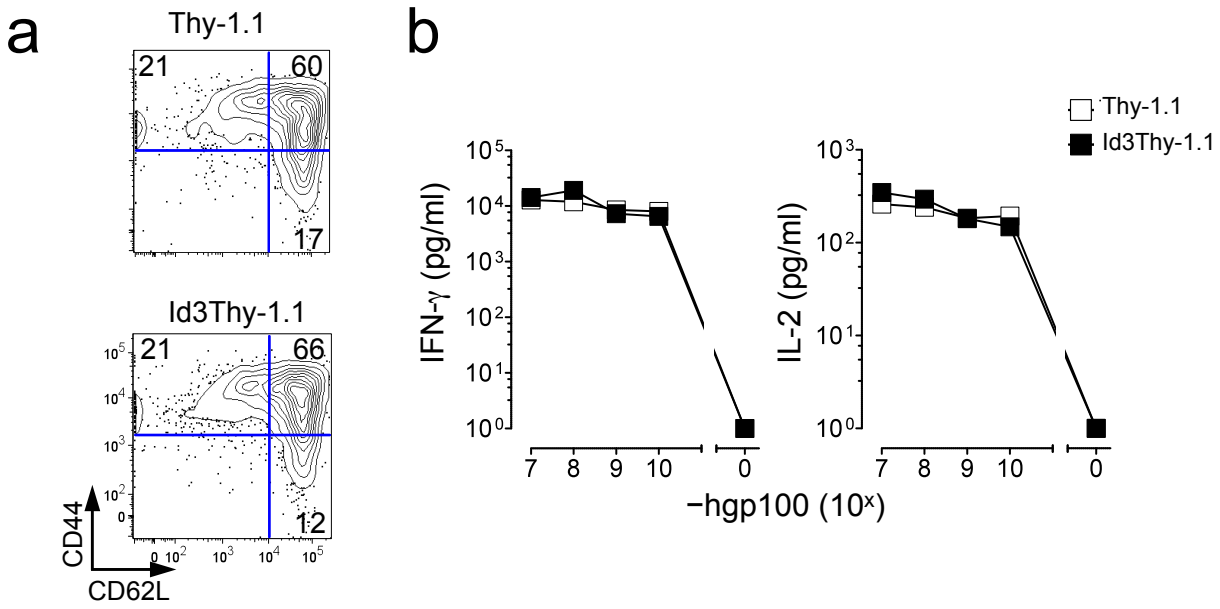
Supplementary Figure 3. Id3 was not required for the long-term survival of naïve T cells. Numbers of $CD8^+CFSE^+$ T cells 30 days following adoptive transfer of 9 X 10^4 CFSE-labeled *Id3*$^{+/+}$, *Id3*$^{+/-}$, or *Id3*$^{-/-}$ pmel-1 *Rag1*$^{-/-}$ $CD8^+$ T cells into WT hosts. Data are represented as mean ± s.d.

Supplementary Fig. 4



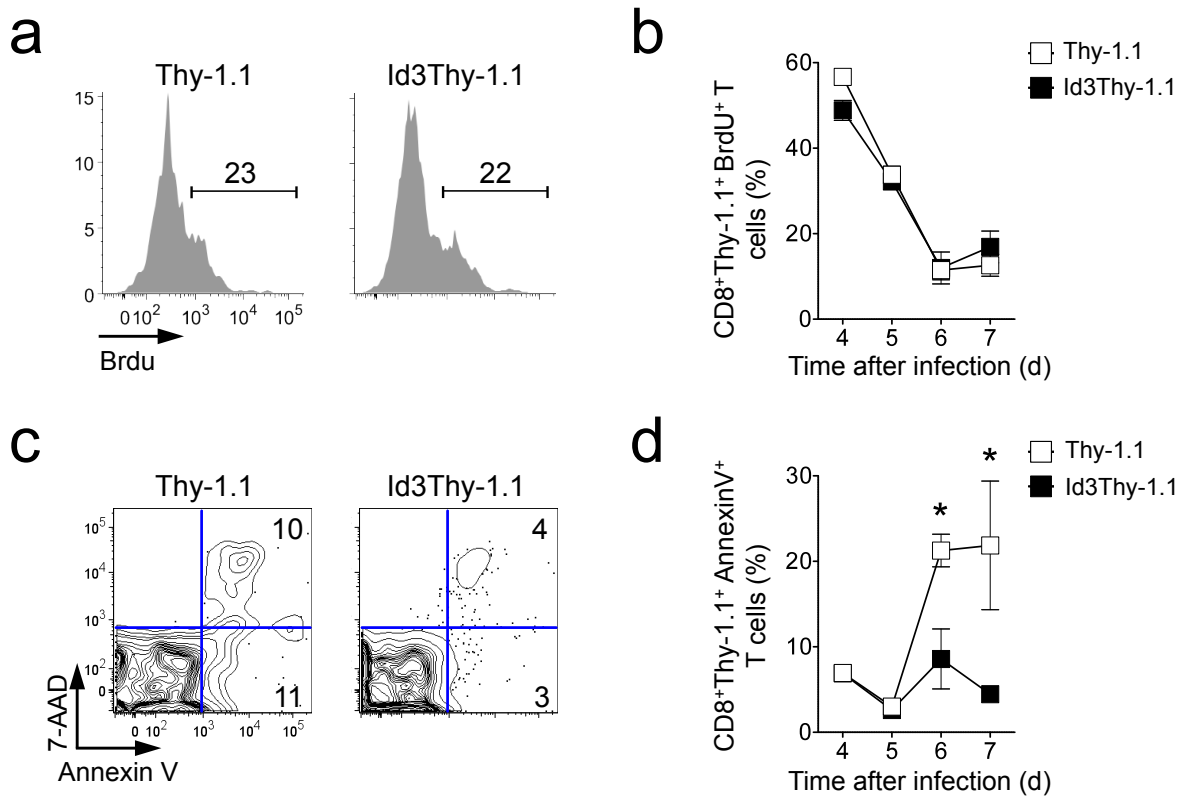
Supplementary Figure 4. Id3Thy-1.1 retroviral vector induces constitutive expression of Id3 in CD8$^+$ T cells. (**a**) Graphical representation of Id3Thy-1.1 and Thy-1.1 retro-viral constructs. (**b**) Ectopic expression levels of Id3 in Thy-1.1 and Id3Thy-1.1 pmel-1 CD8$^+$ T cells were detected by anti-V5 immunoblotting.

Supplementary Fig. 5



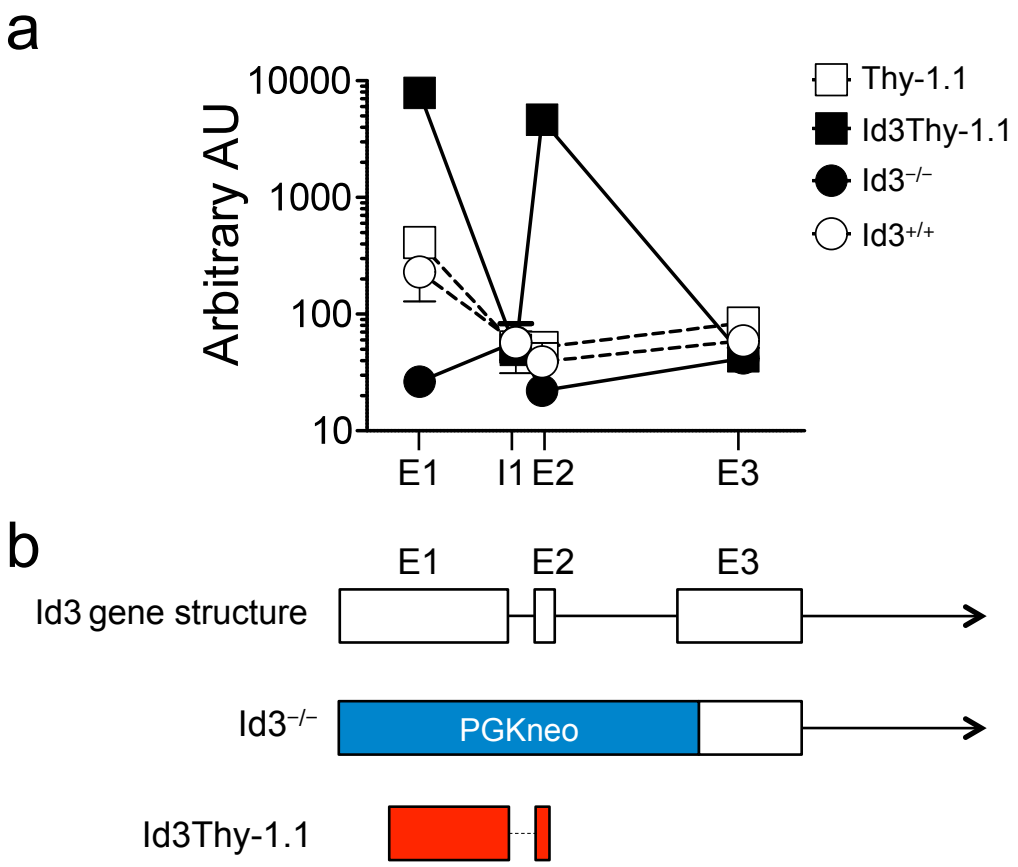
Supplementary Figure 5. Enforced expression of Id3 does not alter phenotype and function of pmel-1 CD8⁺ T cells prior to transfer. (**a**) Representative flow cytometry analysis of Id3Thy-1.1 and Thy-1.1 pmel-1 CD8⁺ T cells. Contour plots show CD62L and CD44 expression after gating on CD8⁺ Thy-1.1⁺ cells. (**b**) IFN-γ (left panel) and IL-2 (right panel) release by Id3Thy-1.1 and Thy-1.1 pmel-1 CD8⁺ T cells after 16-hour co-culture with MCA205 tumor targets pulsed with indicated concentration of hgp100 peptide. Data represented as mean ± s.e.m. of 3 samples.

Supplementary Fig. 6



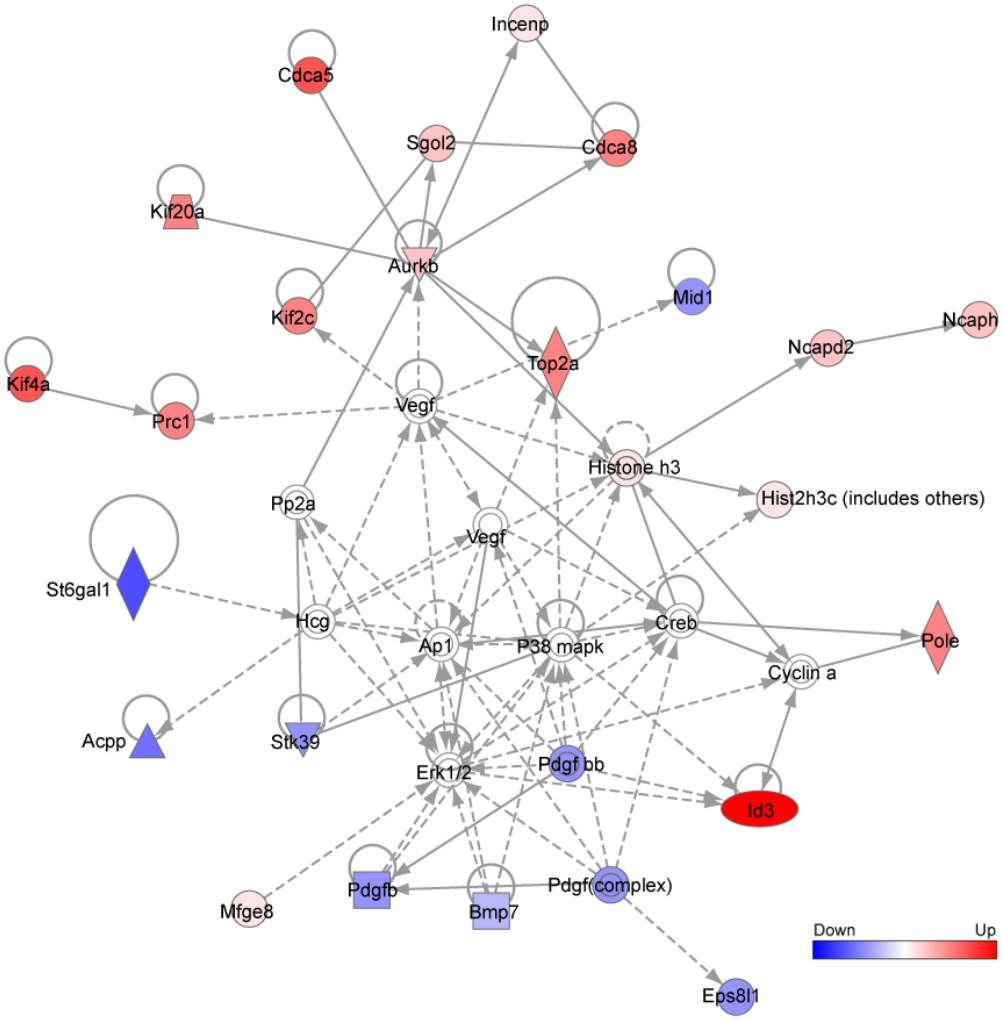
Supplementary Figure 6. Enforced expression of Id3 enhances the survival but not the proliferation of CD8⁺ T cells. (**a**) BrdU⁺ incorporation by adoptively transferred Id3Thy-1.1 and Thy-1.1 pmel-1 CD8⁺ T cells 6 days after transfer and gp100-VV infection. Infected mice received 1.5 mg BrdU 16 hours before analysis of splenocytes. Representative histograms show BrdU labeling after gating on CD8⁺Thy-1.1⁺ cells. (**b**) Percent of BrdU⁺CD8⁺Thy-1.1⁺ T cells at indicated time-points after transferring Id3Thy-1.1 and Thy-1.1 pmel-1 CD8⁺ T cells in conjunction with gp100-VV infection. Data are represented as mean ± s.e.m. of 3 samples. (**c**) Representative flow cytometry analysis of Id3Thy-1.1 and Thy-1.1 pmel-1 CD8⁺ T cells 6 days following adoptive transfer in conjunction with gp100-VV infection. Contour plots show Annexin V and 7-AAD expression after gating on CD8⁺Thy-1.1⁺ cells. (**d**) Percent of Annexin V⁺CD8⁺Thy-1.1⁺ T cells at indicated time-points after transferring Id3Thy-1.1 and Thy-1.1 pmel-1 CD8⁺ T cells in conjunction with gp100-VV infection. Data are represented as mean ± s.e.m. of 3 samples. * = $P < 0.05$.

Supplementary Fig. 7



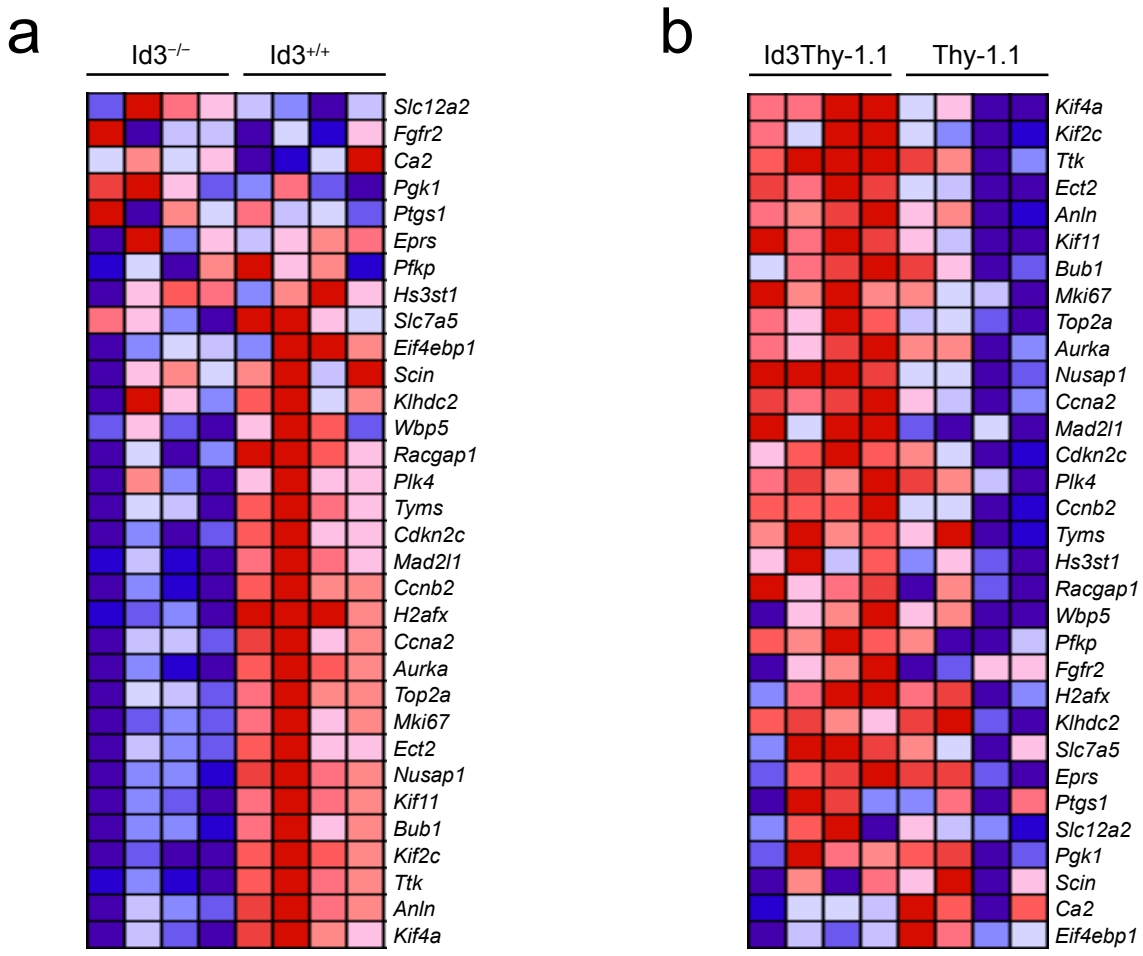
Supplementary Figure 7. Expression of Id3 exons and intron by WT Mouse Gene 1.0 ST arrays reflects differences in the structure of loss and gain-of-function constructs. (**a**) Expression levels of exon 1, 2, 3, and intron 1 of Id3 by WT Mouse Gene 1.0 ST arrays. (**b**) Graphical representation of Id3 gene structure compared to the *Id3* deletion construct targeting exon 1, 2 and the first 135 bp of exon 3[22] and the Id3Thy-1.1 overexpressing construct, which contains the coding region of *Id3* (the last 300 bp of exon 1 and the first 60 bp of exon 2).

Supplementary Fig. 8



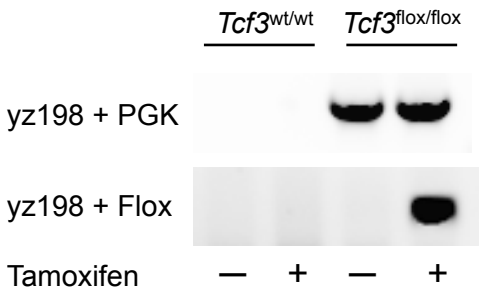
Supplementary Figure 8. DNA replication and repair network identified by Ingenuity Pathway Analysis. Genes differentially expressed in response to Id3 manipulation. Colors indicate the average expression fold-change of knockout and overexpression experiments. Solid lines indicate direct interactions; dashed lines indicate indirect interactions.

Supplementary Fig. 9



Supplementary Figure 9. A set of genes that are found upregulated in *Tcf3* deficient cells is enriched in *Id3*$^{+/+}$ and Id3Thy-1.1 cells compared to controls. (**a**) GSEA enrichment heat map of genes over-expressed in *Tcf3* deficient B cell lines compared to transcriptomes of *Id3*$^{-/-}$ or *Id3*$^{+/+}$ pmel-1 CD8^{+} T cells. (**b**) GSEA enrichment heat map of over-expressed genes compared to transcriptomes of Id3Thy-1.1 or Thy-1.1 pmel-1 CD8^{+} T cells. Red and blue indicate upregulated and downregulated genes respectively.

Supplementary Fig. 10



Supplementary Figure 10. Tamoxifen induces the deletion of *Tcf3* in CD8⁺ T cells. PCR products of Pmel-1 *Tcf3*$^{wt/wt}$ Cre-ER^{T2} and Pmel-1 *Tcf3*$^{flox/flox}$ Cre-ER^{T2} CD8⁺ T cells five days after activation in the presence of 500 nM tamoxifen or vehicle DMSO. The insertion of loxP was detected by PCR with primer pair yz198 and PGK while the deletion of loxP insertion was detected by PCR with primer pair yz198 and Flox.

Supplementary Table 1: Genes differentially expressed in $Id3^{high}$ and $Id3^{low}$ $CD8^{+}$ effector T cells

Gene Symbol	Fold change $Id3^{+/+}$ vs. $Id3^{-/-}$	Fold change Id3Thy-1.1 vs. Thy-1.1
Id3	3.85251	42.9152
Foxm1	1.88648	1.64546
Prr11	1.70871	1.5785
Nek2	1.78809	1.56293
Kif2c	1.78569	1.56269
Ncapg2	1.71622	1.5564
Dlgap5	1.69066	1.55271
Kif4	2.08204	1.55253
5730590G19Rik	1.53412	1.53955
Aspm	2.25651	1.52354
Kif23	1.57334	1.51933
Il12rb2	2.41062	1.5157
Cdca5	2.17632	1.51468
Kif11	1.61491	1.51422
Chn2	1.40237	1.5042
Ccnb1	1.58764	1.49421
Fancd2	1.91392	1.48553
Kif18b	1.98318	1.47769
Pole	1.67229	1.46888
Prc1	1.75958	1.463
Rrm2	1.63077	1.46284
Cit	1.60228	1.46041
Cenpn	1.97648	1.4604
Pif1	1.41821	1.44921
Nusap1	1.78917	1.43494
Ube2c	1.54684	1.43455
Ccnb1	1.56456	1.42432
Espl1	1.93267	1.41691
Smpdl3b	1.62733	1.41657
Brip1	1.72293	1.39648
Kif20a	1.7433	1.39588
Mki67	1.55968	1.39139
Nuf2	1.80399	1.39065
Aurkb	1.57721	1.38513
Parm1	1.61514	1.3851
Eme1	1.4501	1.37307
Mcm3	1.43416	1.37072
Mcm2	1.48113	1.3707
Rrm1	1.3998	1.36879
Top2a	1.5453	1.36621

Exo1	1.49882	1.34317
Mcm10	1.50148	1.3386
Sgol2	1.73674	1.32638
Ncapd2	1.5097	1.31328
Lamc1	1.83883	1.31065
Sord	1.48253	1.30684
E2f7	1.57501	1.3041
Kif14	1.92676	1.30138
Mast2	1.37578	1.30027
Sord	1.44881	1.291
Ckap2l	1.97736	1.28835
Wdr62	1.68852	1.27232
Dna2	1.40608	1.26426
Arhgap19	1.86216	1.26309
Ncaph	1.72762	1.26156
Cdk2	1.42161	1.25941
Diap3	1.70944	1.25775
Stmn1	1.68983	1.25091
Incenp	1.37516	1.24897
Plin2	1.50337	1.24693
Hist1h2bm	1.50415	1.24686
Hist2h2aa1	1.40313	1.23866
Cdca8	2.02102	1.23498
Hjurp	1.39921	1.23043
Plk1	1.85983	1.22642
Hmgn2	1.39351	1.22637
Ccnb2	1.5223	1.20026
F630043A04Rik	1.48668	1.19108
Gtse1	1.51465	1.18775
Pik3c2b	1.54536	1.18339
unknown	2.35654	1.16945
Zwilch	1.81937	1.15949
Rps6ka5	1.47627	1.14756
Serpina3f	-2.97158	1.14462
Hist1h2bb	1.77006	1.13906
unknown	2.81317	1.13672
Swap70	2.14678	1.12593
2610002D18Rik	2.50902	1.1113
Gm7609	-4.73476	1.10811
Gm10002	-4.22895	1.09691
Mfge8	1.58426	1.09365
Igf2bp3	2.87729	1.08122
Gm3579	2.00555	1.06941
LOC641050	-1.79797	1.06313
Gm3579	2.16865	1.05273
Myo1e	2.22477	1.05228
Mid1	-2.53888	1.03114

unknown	-3.93819	1.01597
Sfn	3.23473	1.00646
Gm7609	-2.11706	-1.00455
Wdtc1	-1.77842	-1.01028
unknown	4.46894	-1.02156
Sntb1	-2.39275	-1.02965
Mela	3.94702	-1.03333
unknown	-2.14921	-1.04372
unknown	36.7392	-1.05365
Stk39	-2.50634	-1.05677
Lilrb4	-4.62989	-1.05768
Ikzf2	1.90947	-1.06322
unknown	-2.70401	-1.08357
Rab4a	-2.67804	-1.10268
Lysmd2	-1.56549	-1.1029
Ppic	-3.79314	-1.10616
Entpd4	-1.62954	-1.10683
Naip3	-3.16492	-1.14447
Cyp4v3	-1.48896	-1.14761
Gas5	-1.47852	-1.15597
Litaf	-1.71126	-1.17086
Sytl1	-2.58675	-1.18448
Dapl1	-3.07135	-1.19001
Gm4841	-1.80246	-1.19852
Mela	107.167	-1.19893
Aim2	-1.57383	-1.21085
Cnr2	-1.69614	-1.21198
Gpr25	-1.61444	-1.21644
unknown	-1.73344	-1.2242
Tlr1	-1.4902	-1.22989
Gp49a	-5.33109	-1.2312
Ctla4	-1.40996	-1.27766
Sh3d20	-1.43419	-1.27954
Tanc2	-1.39033	-1.29058
Ccr7	-1.31535	-1.29128
Bmp7	-1.45961	-1.29352
Eps8l1	-1.51043	-1.29437
unknown	-1.37162	-1.31612
F2rl1	-2.1879	-1.33099
Acpp	-2.08446	-1.33502
unknown	-1.39881	-1.34628
Atp1b1	-4.57052	-1.36184
unknown	-1.57218	-1.37047
St6gal1	-1.97331	-1.49964
Wfikkn2	-1.31748	-1.51688
Ptbp2	-1.19227	-1.51838
Pdgfb	-1.5765	-1.53815

Smyd1	-1.26593	-1.70987
Cxcr5	-2.01828	-1.71353
Slc16a5	-1.43574	-2.03152
Slc6a19	-1.61074	-2.19814

*Genes involved in DNA replication and repair are highlighted in gray

Supplementary Methods

Generation of BM chimeras. We enriched hematopoietic stem cells (HSC) obtained from femurs and tibias of pmel-1 *Rag1*$^{-/-}$*Id3*$^{-/-}$ Thy-1.1 and pmel-1 *Rag1*$^{-/-}$ Thy-1.2 mice with EasySep mouse hematopoietic progenitor cell enrichment kit (Stemcell Technologies). Ly5.1 recipients were irradiated with 9 Gy and injected i.v. with 1.2 X 10^6 HSC cells. We analyzed bone marrow chimeras 8 weeks after transplantation.

Cytokine release assays. 2.5 X 10^4 MCA205 cells were pulsed with hgp$100_{25–33}$ or irrelevant influenza nucleoprotein peptide at indicated concentrations and incubated overnight with T cells at a 1:1 ratio at 37 °C. We analyzed the supernatants using mouse IFN-γ and mouse IL-2 by ELISA (R&D Systems).

BrdU/Annexin incorporation assay. We injected mice i.p with 1.5 mg of BrdU (Sigma-Aldrich) 16 h before collecting the spleens. $CD8^+$ T cells were enriched and stained with $CD8^+$ and congenic surface makers. Annexin V/7-AAD assay and BrdU quantification were performed following the manufacturer's instruction (BD Biosciences).