

Supplemental Data

Acquisition of a functional T cell receptor during T lymphocyte development is enforced by HEB and E2A transcription factors

Mary Elizabeth Jones and Yuan Zhuang

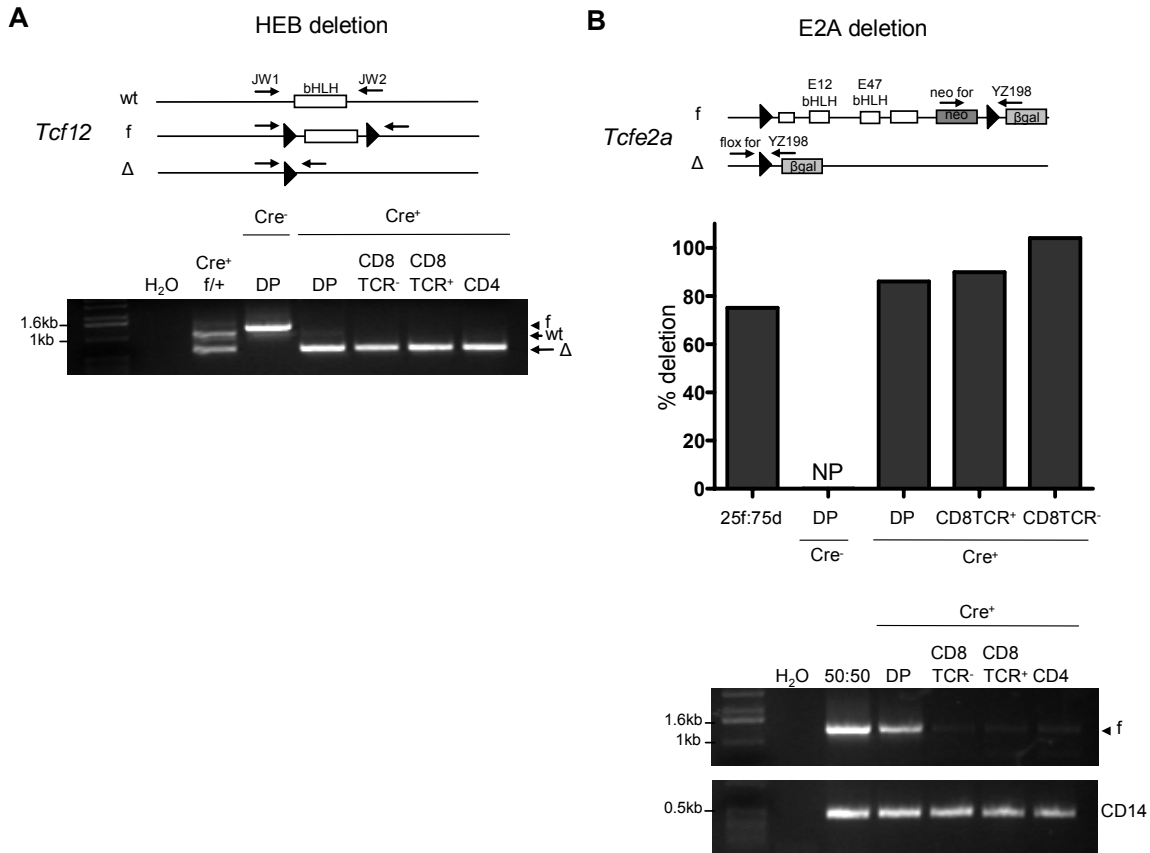


Figure S1. HEB and E2A are efficiently deleted in DP stage of development. Analysis of deletion efficiency in sorted populations (DP cells from thymus, CD8 and CD4 cells from LN) from *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* and *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁻* control mice. Primer design designated in diagrams for wild-type (wt), flox (f), and deleted (Δ) alleles (Wojciechowski et al., 2007). CD4 LN cells (Cre⁺ f/+) from a *Tcf12^{fl/fl}CD4Cre⁺* mouse were used as a control for 50% deletion. (B) E2A deletion. Primers neo for and YZ198 detect the flox allele, and primers flox for and YZ198 detect the deleted allele (Lazorchak et al., 2006). E2A deletion is analyzed by two independent methods because the difference in product size renders quantification of deletion difficult from a single PCR reaction. First, a qPCR method to detect %deletion is used with primers flox for and YZ198 as described previously (Lazorchak et al., 2006). A 25:75 mix of flox and deleted alleles, respectively, is used as a control for 75% deletion. Second, PCR for the flox allele only, using primers neo for and YZ198, at 40 cycles verifies qPCR results. A 50:50 mix of flox and deleted alleles is used as a control for 50% deletion. CD14 is used as a loading control. NP (no product).

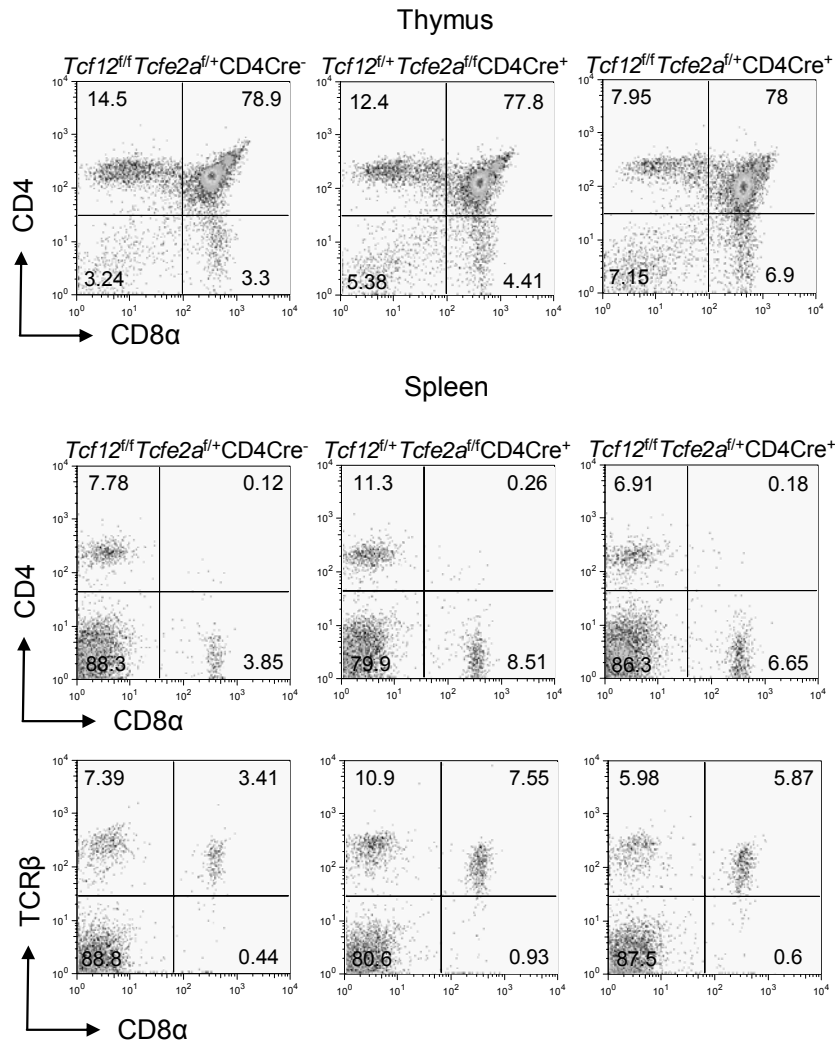


Figure S2. CD8⁺TCR⁻ cells are not detected in mice retaining one HEB or E2A wild-type allele. Representative staining of indicated tissues from *Tcf12^{fl/fl} Tcfe2a^{fl/+} CD4Cre⁻* control, *Tcf12^{fl/+} Tcfe2a^{fl/fl} CD4Cre⁺*, and *Tcf12^{fl/fl} Tcfe2a^{fl/+} CD4Cre⁺* mice for CD4, CD8α, and TCRβ. Percentages in each quadrant are displayed.

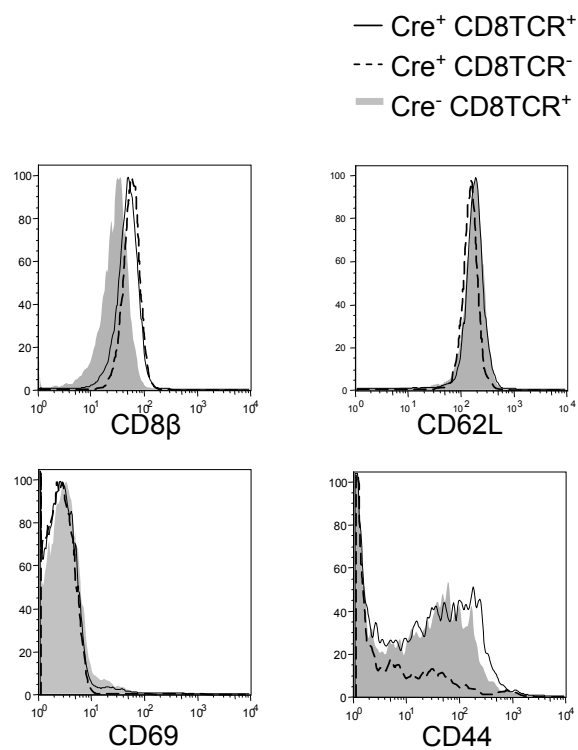


Figure S3. Peripheral CD8TCR⁻ cells demonstrate a mature resting T cell phenotype.

Representative staining of LN cells from *Tcf12^{fl/fl}Tcf2a^{fl/fl}CD4Cre⁺* and *Tcf12^{fl/fl}Tcf2a^{fl/fl}CD4Cre⁻* mice. Cells are pre-gated on CD8α and TCRβ surface expression. Histograms display surface expression of CD8β, CD69, CD62L, and CD44 for Cre⁺ CD8TCR⁺ cells (solid line), Cre⁺ CD8TCR⁻ cells (dashed line), and Cre⁻ CD8TCR⁺ control cells (shaded).

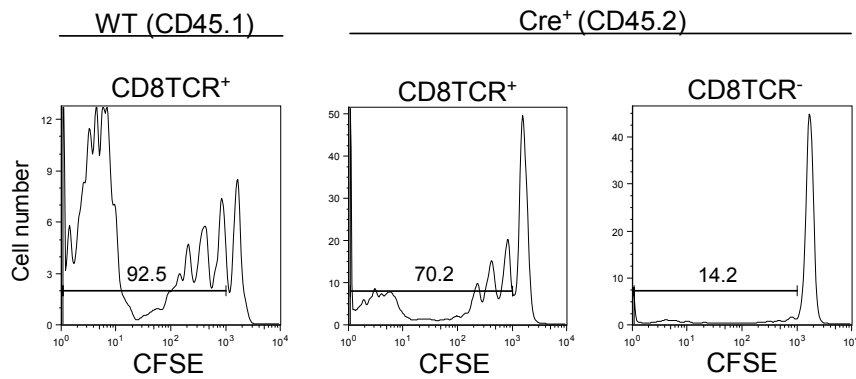


Figure S4. HEB and E2A double deficient CD8TCR⁻ cells demonstrate a defect in homeostatic proliferation when transferred to a lymphopenic host. Total LN cells isolated from wild type (CD45.1) and *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* (CD45.2) mice were mixed at a 1:3 ratio, respectively, then labeled with 5uM CFSE (Molecular Probes). Approximately 10⁷ mixed, CFSE-labeled cells were intravenously injected into *Rag2^{-/-}* deficient hosts. FACS analysis on spleen cells was performed 7 days post transfer. CFSE histograms are gated on the CD45 congenic marker, CD8 β , and TCR β as labeled. Plots are representative of 3 independent transfers. Percentages of cells undergoing one or more division are shown.

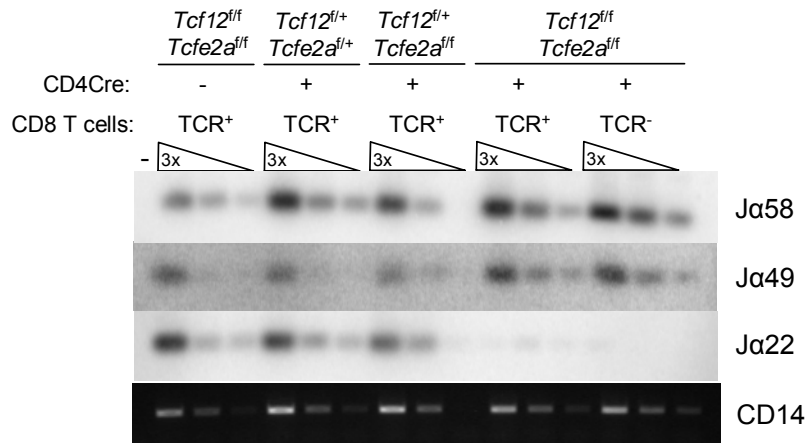


Figure S5. *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* T cells demonstrate a skewing of J α usage to the 5' end of the locus. Analysis of J α 58, 49, and 22 usage in TCR α rearrangements from *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁻*, *Tcf12^{fl/+}Tcfe2a^{fl/+}CD4Cre⁺*, *Tcf12^{fl/+}Tcfe2a^{fl/fl}CD4Cre⁺*, and *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* sorted peripheral CD8 T cells. PCR and Southern blot analysis of V α 8 to J α rearrangements were done as described (Abarrategui and Krangel, 2006). CD14 was used as a DNA loading control. Water was used as a negative control (-). Results are from one mouse per genotype. J α 58, 49, and 22 are located 5' to 3' within the J α locus.

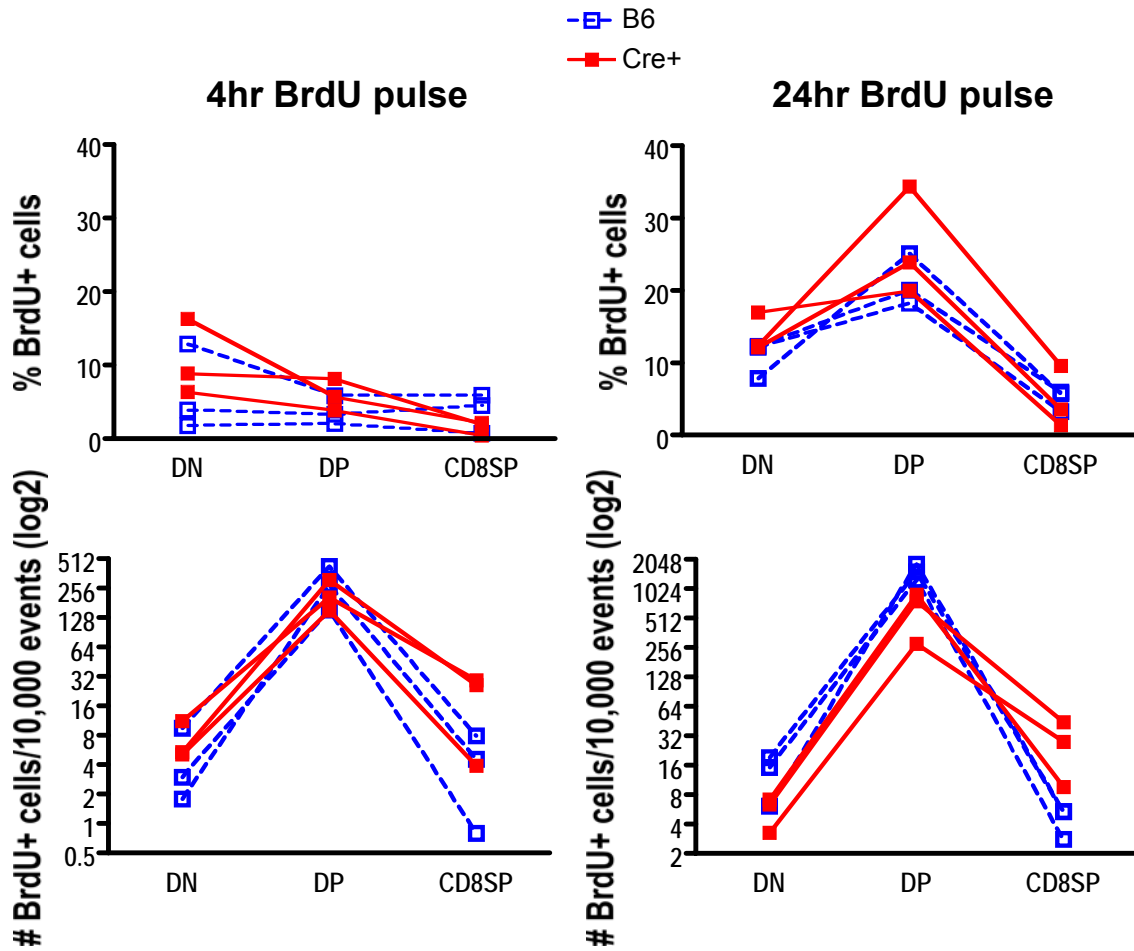


Figure S6. In vivo BrdU labeling demonstrates generation of more CD8SP cells in *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* mice. Wild-type (B6) and *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* mice were IP injected with 1mg BrdU and analyzed 4 or 24hrs after injection. Thymocytes were stained for CD4, CD8, and TCR β surface expression, then processed to analyze BrdU labeling with a FITC BrdU Flow Kit as per manufacturer's instructions (BD Pharmingen). Graphs display percent (top panel) and cell number (lower panel) of BrdU⁺ cells within each pre-gated population: DN (CD4⁻CD8⁻), DP (CD4⁺CD8⁺), and CD8SP (CD4⁻CD8⁺TCR β ⁺).

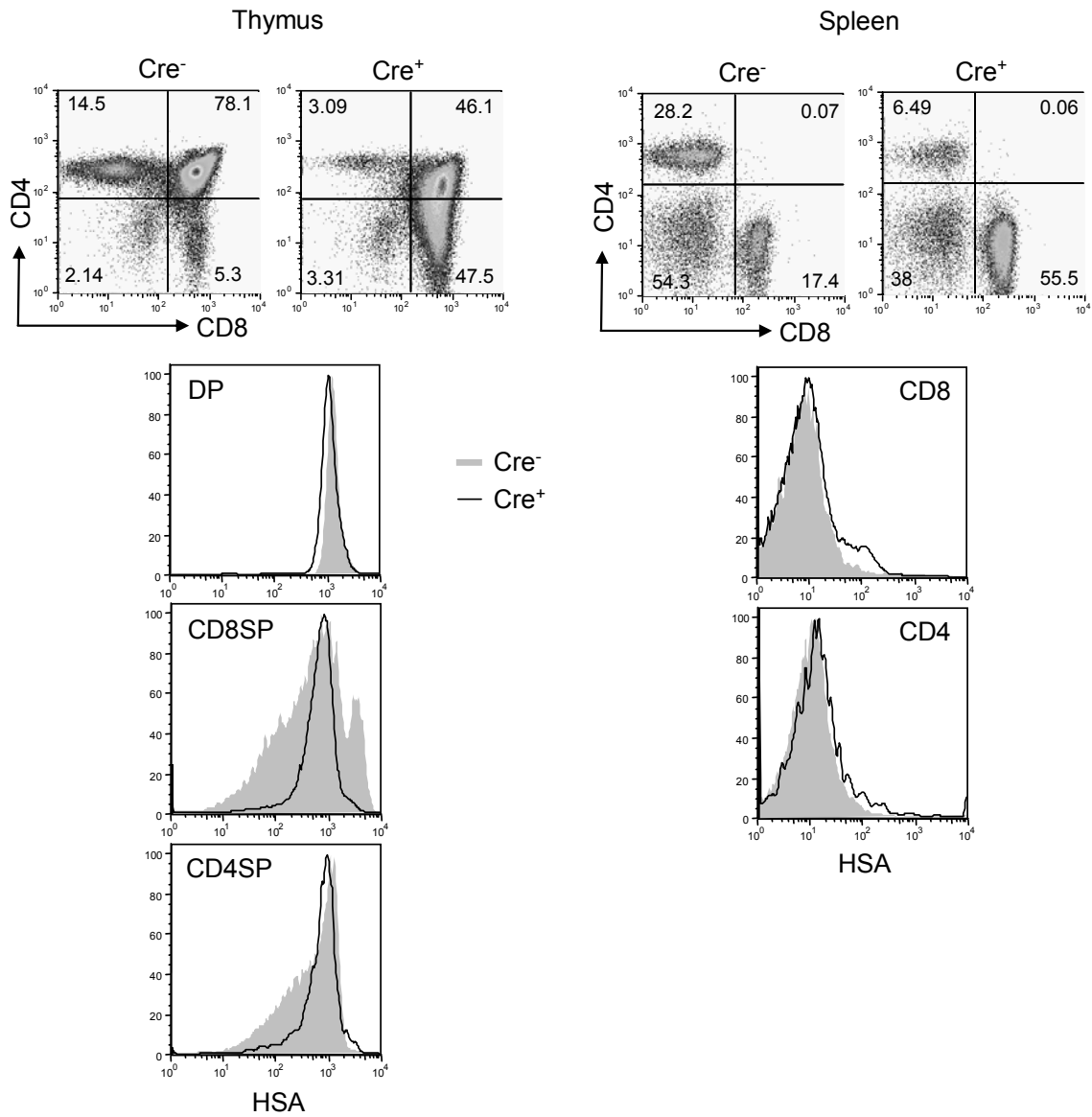


Figure S7. *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* SP thymocytes have not yet down-regulated HSA expression to the levels detected in the periphery. Staining of indicated tissues from *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁻* (shaded in histogram) and *Tcf12^{fl/fl}Tcfe2a^{fl/fl}CD4Cre⁺* (line in histogram) mice for expression of CD4, CD8, and HSA (clone M1/69). Percentages in each quadrant are displayed for CD4 vs CD8 plots. Histograms display HSA expression in gated populations from above plots: DP (CD4⁺CD8⁺), CD8SP/CD8 (CD4⁻CD8⁺), CD4SP/CD4 (CD4⁺CD8⁻).

Table S1. List of microarray identified genes downregulated greater than 3 fold in *Tcf12^{f/f}Tcfe2a^{f/f}CD4Cre⁺* DP cells with a p-value ≤ 0.05

Cre ⁺ /Cre ⁻ fold change	Ensembl	Unigene	Common Name	Description
0.0459	Smoc1	Mm.273295	SRG; AI848508; 2600002F22Rik	SPARC related modular calcium binding 1
0.125		Mm.186434	C43; Mf3; Twh; Fkh5; Foxb1a; Foxb1b; Hfh-e5.1; MGC130499	forkhead box B1
0.128	Prodh			
0.13		Mm.48648	KIAA0523; MGC55022; mKIAA0523; RP23-223C15.3	cDNA sequence BC030477
0.131	Ppfbp2	Mm.2817	Cclp1; MGC117695	protein tyrosine phosphatase, receptor-type, F interacting protein, binding protein 2
0.145	Olig3	Mm.156946	Bhlhb7	oligodendrocyte transcription factor 3
0.149	Rragd			
0.158	Cbfa2t3h			
0.18	Idb3			
0.182	Car2	Mm.1186	CAII; Car-2; Ltw-5; Lvtw-5; AI131712	carbonic anhydrase 2
0.182	Tec	Mm.319581	Tsc2	cytoplasmic tyrosine kinase, Dscr28C related (Drosophila)
0.186	Egr2	Mm.290421	Egr-2; Zfp-6; Krox20; NGF1-B; Zfp-25; Krox-20	early growth response 2
0.187		Mm.330745	kr; Krml; Krml1; Kreisler	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)
0.187		Mm.28456	Pro1; Pro-1; Ym24d07	proline dehydrogenase
0.194	Pip5k1a			
0.198		Mm.314618	Zak; MLTK; AV006891; MLTKbeta; MLTKalpha	RIKEN cDNA B230120H23 gene
0.205	Pip5k1a			
0.211	Ephx2	Mm.15295	Eph2; AW106936	epoxide hydrolase 2, cytoplasmic
0.216	Lass4			
0.223	Irf6			
0.225	Plxdc1	Mm.39617	Tem7; AI848450; MGC130377; 2410003I07Rik	plexin domain containing 1
0.235	Nab2	Mm.336898	AI451907	Ngfi-A binding protein 2
0.236	Mad1l1	Mm.27250	Mad1; AI173502; AW550425	mitotic arrest deficient 1-like 1
0.236	Dusp6			
0.237	Zdhhc14			
0.247	Arl6ip2			
0.25	Egr1		egr; TIS8; Zenk; Egr-1; NGFIA; Zfp-6; ETR103; Krox-1; Krox24; NGF1-A; NGF1-A; Zif268; Krox-24; A530045N19Rik	early growth response 1
0.26	Hrb1	Mm.260869	RABR; MGC7148; MGC61203; A630095P14Rik	HIV-1 Rev binding protein-like
0.263	D10Bwg094 Oe	Mm.297971	BC013565; KIAA1357; mKIAA1357; A730096F01; D10Bwg0940e	NHS-like 1
0.269		Mm.38269	LOC666794	Similar to RNA binding motif protein 24
0.28		Mm.227912	opt; Ip3r; P400; Pcp1; IP3R1; Pcp-1; D6Pas2; InsP3R; Itpr-1	inositol 1,4,5-triphosphate receptor 1
0.282	Fbp1	Mm.423078	Fbp2; Fbp-2; FBpase 1	fructose biphosphatase 1
0.283	H2-D1		Q10; Qa10; H-2Q10	histocompatibility 2, Q region locus 10
0.293	Spg3a			
0.293	Ets2	Mm.290207	Ets-2; AU022856	E26 avian leukemia oncogene 2, 3' domain
0.308	Cdk1			
0.313	Tle6	Mm.206764	Grg6; 1810057E06Rik	transducin-like enhancer of split 6, homolog of Drosophila E(spl)
0.316	Slc35d1			
0.331	Rapgef3	Mm.24028	Epac; Epac1; MGC19192; 2310016P22Rik; 9330170P05Rik	Rap guanine nucleotide exchange factor (GEF) 3
0.332	Socs3	Mm.3468	CIS3; Cish3; EF-10; SSI-3; SOCS-3	suppressor of cytokine signaling 3

Table S2. List of microarray identified genes upregulated greater than 3 fold in *Tcf12^{f/f}Tcfe2a^{f/f}CD4Cre⁺* DP cells with a p-value ≤ 0.05

Cre ⁺ /Cre ⁻ fold change	Ensembl	Unigene	Common Name	Description
15.13	Ctla2b			
11.4	Rapgef4	Mm.196153	Epac2; KIAA4040; mKIAA4040; 1300003D15Rik; 5730402K07Rik	Rap guanine nucleotide exchange factor (GEF) 4
9.494	Sgnel			
8.523	Tesc			
8.285	Elov16	Mm.314113	FAE; LCE; C77826; MGC107467	ELOVL family member 6, elongation of long chain fatty acids (yeast)
7.35	Ctla2b	Mm.30144	Epb4.1l2	Erythrocyte protein band 4.1-like 2
6.854		Mm.21814	AU018597; 6720474K14Rik; 9430079M16Rik	tetraspanin 9
6.626	Ccr7	Mm.2932	EBI1; CD197; Ebi1h; Cdw197; Cmkbr7	chemokine (C-C motif) receptor 7
5.917	Q8K244			
5.754		Mm.333048	Ian1; IMAP4; mIAN1; AU019574; MGC11734; E430007K16Rik	GTPase, IMAP family member 4
5.499	Cd53			
5.448	Pde3b	Mm.430730	AI847709	phosphodiesterase 3B, cGMP-inhibited
5.39		Mm.35548	Clint1	RIKEN cDNA A330049M08 gene
5.323	Lix1	Mm.268018	5730466L18Rik	limb expression 1 homolog (chicken)
5.296	Ssb4			
5.296	Q9CZZ7			
5.137	Piwil2	Mm.85253	mili; Piwil1	piwi-like homolog 2 (Drosophila)
5.131	Klf2	Mm.26938	Lklf	Kruppel-like factor 2 (lung)
4.845	Dncl2b	Mm.23114	Dynlrb2	Dynein light chain roadblock-type 2
4.789	Mpra			
4.754	Gm2a	Mm.287807	AA408702; AW215435	GM2 ganglioside activator protein
4.621	Q9CZY7			
4.392		Mm.31817	EKNI; 1700010I24Rik	dyslexia susceptibility 1 candidate 1 homolog (human)
4.373	Eng	Mm.225297	CD105; AI528660; S-endoglin	endoglin
4.332	Cnn2	Mm.157770	AA408047; AI324678	calponin 2
4.321	Ank2	Mm.220242	Ank-2; AI835472; AW491075; Ankyrin-2; Ankyrin-B; ankyrin B; ankyrin>B<	ankyrin 2, brain
4.284	Add3	Mm.426080	R75380; AI463285	adducin 3 (gamma)
4.264	Rasa3			
4.2	Ggta1			
4.174	Pscd4			
4.128	Gng2			
4.012	Slc26a11			
3.991	Foxo1	Mm.29891	Afxh; FKHR; Fkhr1; Foxo1a; AI876417	forkhead box O1
3.929	Pdlim1			
3.846	Q8BJ19		AI225904; 1700108N18Rik; E430013J17Rik	RIKEN cDNA 6330500D04 gene
3.824	Scotin	Mm.196533	Scotin; 2310008D10Rik; 6430628I05Rik	scotin gene
3.772	Rtn			
3.766	Gig1		Gig1; C030026M03; C030034L19Rik	zinc finger protein 704
3.752		Mm.4065	Eeig1; AI426465; MGC38572	RIKEN cDNA C230093N12 gene
3.658	Rps6ka1	Mm.301827	rsk; Rsk1; p90rsk	ribosomal protein S6 kinase polypeptide 1
3.619	Cst7	Mm.12965	Cst3	Cystatin C
3.582	Chd3	Mm.178246	Chd7; Prp7; Prp9-1; AF020312; MGC40857; 2600010P09Rik	chromodomain helicase DNA binding protein 3
3.572	Rassf3	Mm.41265	AW212023; AW322379	Ras association (RalGDS/AF-6) domain family 3
3.506	Zfpn1a4			
3.413	Ian3			
3.382	Iitgb7			
3.305	Ly6c			
3.288	Ramp2			
3.254		Mm.393405	Clp; 1810074P22Rik; 2010004C08Rik	coactosin-like 1 (Dictyostelium)
3.236	CNC2_MOUSE			
3.204	CATW_MOUSE			
3.11	Ms4a6d	Mm.290390	AI447446	membrane-spanning 4-domains, subfamily A, member 11
3.067	Chd3			
3.064	Hexb	Mm.27816	Hbb-b1	hexosaminidase B
3.01	Tmsb4x			

Table S3.

Primers for RT-PCR: 5'-3'	
C α for	ctg cct gtt cac cga ctt tga c
C α rev	gat tcg gag tcc cat aac tga cag
Gfi1 for	gat tcc acc aga agt cag
Gfi1 rev	ggt agt gtt aca cag ctg gtg
Mad1 F1	gct gac cgg cta cca gat tg
Mad1 R1	gct cag gca cag acc gtg ag
KLF2 F4	cct aca cca aga gct cgc acc
KLF2 R4	gtc gac cca ggc tac atg
Foxo1 F1	gga ctg tga cat gga gtc c
Foxo1 R1	gga ctg ctc ctc agt tcc tg

Supplemental Data References

Abarategui, I., Krangel, M.S. (2006). Regulation of T cell receptor- α gene recombination by transcription. *Nat Immunol* 7, 1109-1115.

Lazorchak, A. S., Wojciechowski, J., Dai, M., and Zhuang, Y. (2006). E2A promotes the survival of precursor and mature B lymphocytes. *J Immunol* 177, 2495-2504.

Wojciechowski, J., Lai, A., Kondo, M., and Zhuang, Y. (2007). E2A and HEB Are Required to Block Thymocyte Proliferation Prior to Pre-TCR Expression. *J Immunol* 178, 5717-5726.