

Supplemental Figure 1, Related to Figure 1

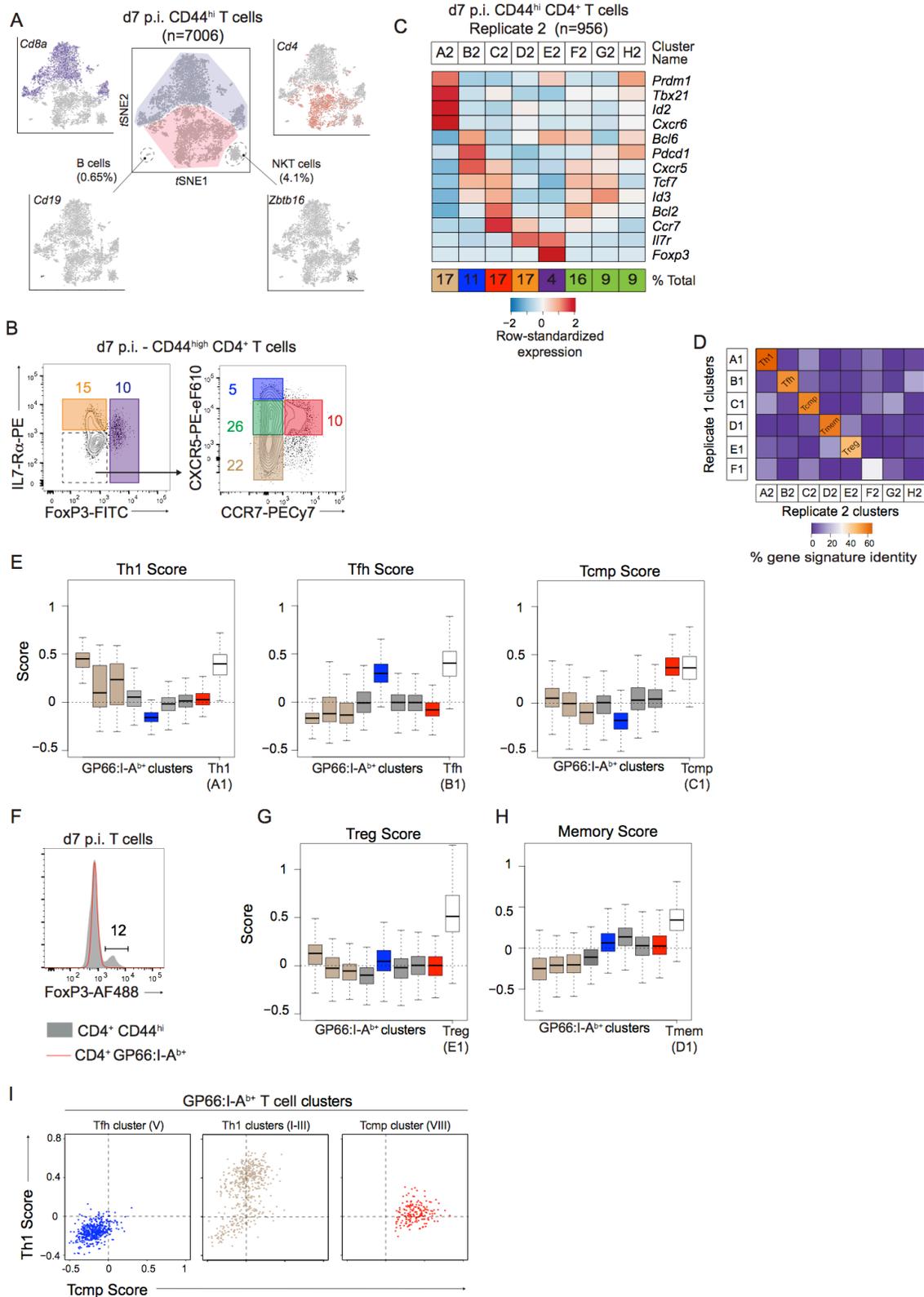


Figure S1: Identification and validation of CD4⁺ signatures by scRNAseq.

(A) CD44^{hi} spleen T cells from WT mice at d7 p.i. with LCMV Arm were analyzed by scRNAseq. Plot displays single CD44^{hi} T cells from experimental replicate 1, visualized with the t-SNE algorithm. Blue and red areas highlight the *Cd8a* and *Cd4* expressing cells respectively. Circles show B cells and iNK T cells, expressing *Cd19* and *Zbtb16* (encoding PLZF), respectively.

(B) Plots show the expression of CCR7 and CXCR5 on Foxp3⁻ IL-7R α ^{lo} spleen CD44^{hi} CD4⁺ T cells at d7 p.i. with LCMV Arm. Gates are colored according the code used for cell clusters defined in Fig. 1B, and numbers show the percentage of each subsets relative to the total population.

(C) Second replicate scRNAseq analysis of spleen WT CD44^{hi} T cells at d7 p.i. with LCMV Arm. Heatmap shows the relative expression of selected genes on the 8 unsupervised CD4⁺ clusters.

(D) Correlation matrix shows the percentages of identical genes between the gene signatures among the clusters of CD4⁺ T cells detected in Replicate 1 (Clusters A1 to F1, as shown in Fig. 1B, C) and in Replicate 2 (Clusters A2 to H2, Fig. S1C).

(E, G, H) Cells clusters from d7 p.i. GP66:I-A^{b+} WT spleen T cells (defined in Fig. 1F, G) are scored for the cell signatures. Boxplots show the centered distribution of cell scores for each cell signature. Clusters with high scores for Th1- (Clusters I-III), Tfh- (Cluster V) and Tcmp- (Cluster VIII) cell signatures are colored in brown, blue and red respectively. As a reference, the rightmost white box in each plot indicates the score of cells from the corresponding, cell signature-defining cluster in Replicate 1 (Cluster A1-E1, Fig. 1B, C).

(F) Plot shows Foxp3 expression on spleen CD4⁺ CD44^{hi} and CD4⁺ GP66:I-A^{b+} T cells from WT mice at d7 p.i. with LCMV Arm. The percentage of Foxp3⁺ among CD4⁺ CD44^{hi} T cells is shown.

(I) Dot plots show centered scores for Tcmp (x-axis) vs. Th1 (y-axis) signatures on cells from Cluster V (Tfh), Clusters I-III (Th1) and Cluster VIII (Tcmp) defined in Fig. 1F, G. Each dot represents a cell.

Supplemental Figure 2, Related to Figures 2 and 3

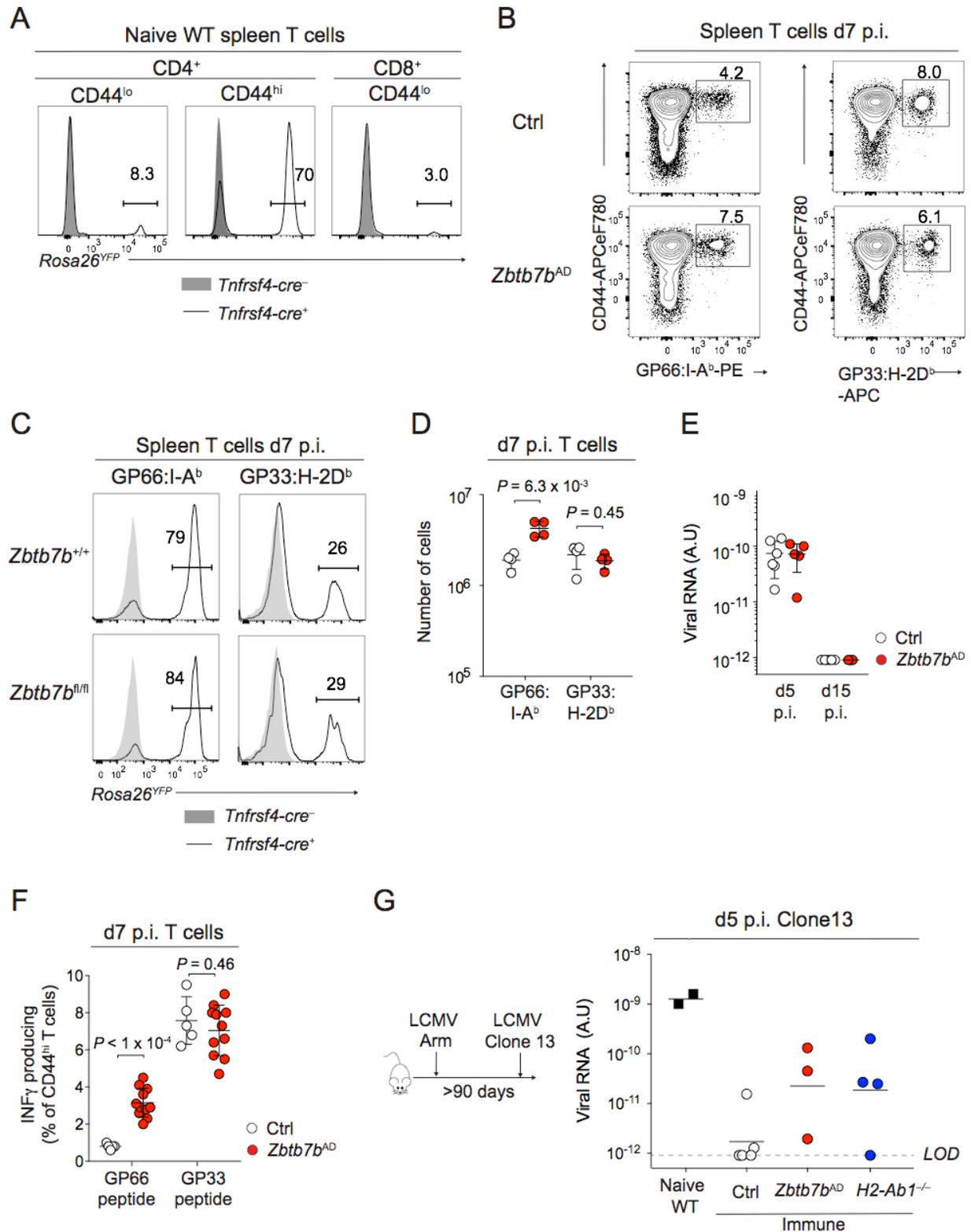


Figure S2: Post-activation disruption of *Zbtb7b* in LCMV-infected *Zbtb7b*^{AD} mice.

(A) Plots show Cre activity as reported by *Rosa26*^{YFP} expression on the indicated spleen T cell populations from *Tnfrsf4-cre*⁺ (black line) or *Tnfrsf4-cre*⁻ (grey histogram) uninfected animals. Data are representative of two independent experiments.

(B) Plots show GP66:I-A^b and GP33:H-2D^b staining at d7 p.i. with LCMV Arm on total spleen T cells from the indicated mice.

(C) Cre activity as reported by YFP expression was measured on spleen GP66:I-A^{b+} and GP33:H-2D^{b+} T cells from *Tnfrsf4-cre⁻ Rosa26^{YFP}* (grey histogram) or *Tnfrsf4-cre⁺ Rosa26^{YFP}* (black line) mice of the indicated genotype at d7 p.i.

(D) Graph shows the numbers of spleen GP66:I-A^{b+} and GP33:H-2D^{b+} T cells in *Zbtb7b^{AD}* (n=4) and control (n=5) mice analyzed as in (B). P-values from unpaired two-sided Welch's *t*-test are indicated. Data (B-D) are representative of two independent experiments.

(E) Serum virus titer at d5 and d15 p.i. of *Zbtb7b^{AD}* (n=5) and control (n=6) mice with LCMV Arm. Data are representative of two independent experiments.

(F) Percentage of IFN γ ⁺ cells among *Zbtb7b^{AD}* (n=11) and control (n=5) CD44^{hi} spleen T cells after GP66 (MHC-II restricted) and GP33 (MHC-I restricted) peptide stimulation. P-values from unpaired two-sided Welch's *t*-test are indicated. Data are representative of two independent experiments.

(G) *Zbtb7b^{AD}*, *H2-Ab1^{-/-}* (MHC-II-deficient) or control mice were infected with LCMV Arm and re-challenged with LCMV Clone 13 more than 90 days later. Graph shows serum virus titers in animals of the indicated genotype (red, blue and empty circles) 5 days after Clone 13 infection; black squares show titers in naive WT mice as controls. Dashed line shows the limit of detection (LOD) of the assay. Data are representative of two independent experiments.

Supplemental Figure 3, Related to Figure 4

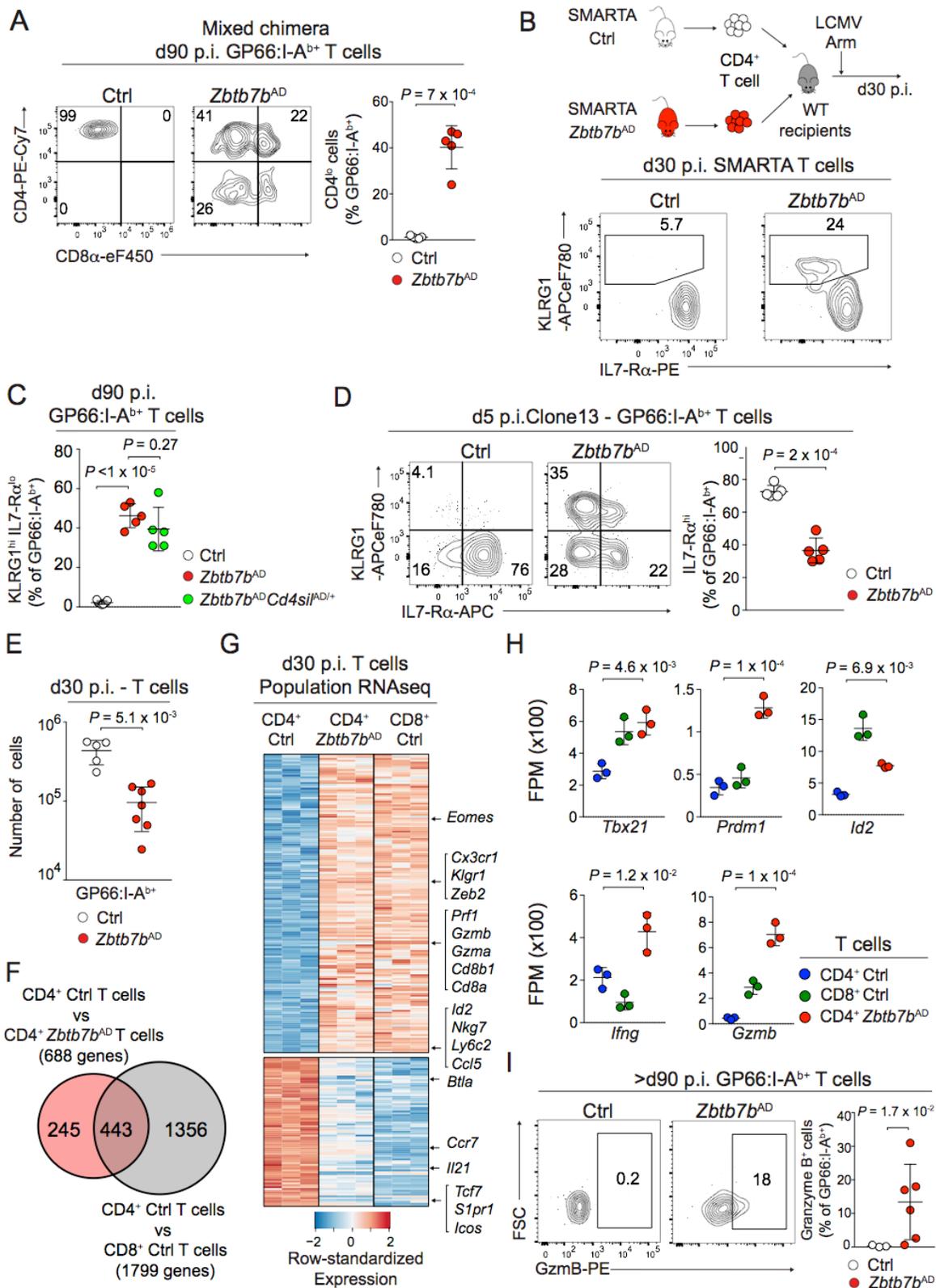


Figure S3: Phenotype and gene expression of Thpok-deficient memory CD4⁺ T cells.

(A) *Zbtb7b*^{AD}:WT or Ctrl:WT mixed bone marrow chimeras were infected with LCMV Arm and analyzed at d90 p.i. Left panels show the expression of CD4 and CD8α on GP66:I-A^{b+} spleen T cells of the indicated genotype. Numbers indicate the percentage of cells in the box. Right panel shows the percentage of CD4^{lo} cells within the GP66:I-A^{b+} T cell population for each genotype (n=5 per group). P-values from unpaired two-sided Welch's *t*-test are indicated.

(B) Naive *Zbtb7b*^{AD} and control SMARTA TCR transgenic cells were adoptively transferred into CD45 congenic WT recipients one day before LCMV Arm infection. Flow cytometry plots show IL-7R α and KLRG1 expression on adoptively transferred cells of the indicated genotype isolated from the spleen at d30 p.i. The percentages of cells in the indicated gates are shown.

(C) Percentage of KLRG1^{hi} IL-7R α ^{lo} cells within the GP66:I-A^{b+} T cell population in *Zbtb7b*^{AD} (n=5), *Zbtb7b*^{AD} *Cd4sil*^{AD} (n=5) and control (n=9) mice analyzed at d90 p.i. with LCMV Arm. P-values from unpaired two-sided Welch's *t*-test are indicated. Data are from one experiment representative of two independent experiments.

(D) *Zbtb7b*^{AD} or control mice were infected with LCMV Arm and re-challenged more than 90 d p.i. with LCMV Clone 13. Left panels show the expression of KLRG1 and IL-7R α on GP66:I-A^{b+} spleen T cells of the indicated genotype. Numbers indicate the percentage of cells in the box. Right panel shows the percentage of IL-7R α ^{hi} cells among GP66:I-A^{b+} T cells of each genotype (n=5 per group). P-value from unpaired two-sided Welch's *t*-test is indicated. Data are representative of two independent experiments.

(E) Numbers of spleen GP66:I-A^{b+} T cells in *Zbtb7b*^{AD} (n=7) and control (n=5) mice 30 d p.i. with LCMV Arm. P-value from unpaired two-sided Welch's *t*-test is indicated. Data are representative of two independent experiments.

(F-H) RNAseq analyses of gene expression at d30 p.i. with LCMV Arm was performed on sorted populations of GP66:I-A^{b+} spleen T cells from *Zbtb7b*^{AD} (CD4 *Zbtb7b*^{AD}) or control (CD4 Ctrl) mice, and of GP33:H-2D^{b+} spleen T cells from WT animals (CD8 Ctrl).

(F) Venn diagrams of the numbers of overlapping genes differentially expressed between CD4 *Zbtb7b*^{AD} vs CD4 Ctrl T cells and CD4 Ctrl vs CD8 Ctrl (adjusted p-value <0.01, log2 fold change > 1).

(G) Genes differentially expressed between CD4 *Zbtb7b*^{AD} and CD4 Ctrl and also differentially expressed between CD4 Ctrl and CD8 Ctrl as shown in (F).

(H) Graphs show RNAseq-determined expression (fragments per million, FPM) of selected genes in all three populations; P-values are from one-way ANOVA. Each symbol represents a biological replicate.

(I) Left panels show Granzyme B expression on GP66:I-A^{b+} spleen T cells of the indicated genotype. Right panel shows the percentage of Granzyme B expressing cells from *Zbtb7b*^{AD} (n=6) and control mice (n=3) more than 90 days p.i. LCMV Arm. P-value is from unpaired two-sided Welch's *t*-test. Data are representative of two independent experiments.

Supplemental Figure 4, Related to Figure 4

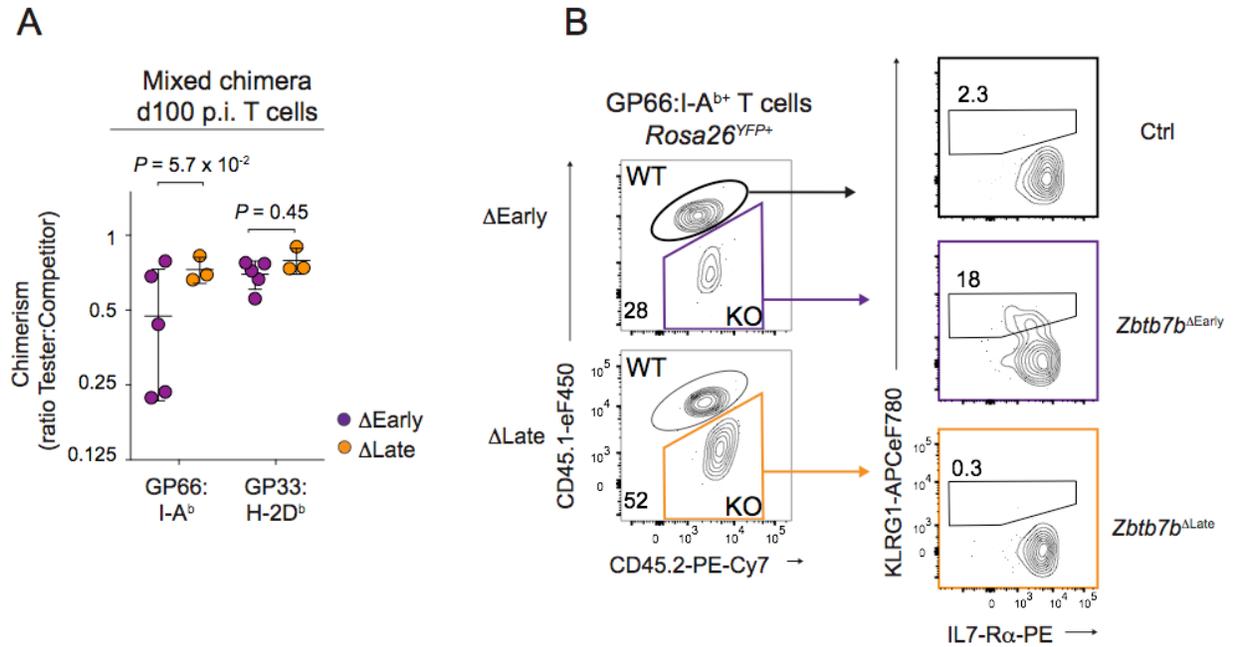


Figure S4: Early requirement for Thpok in the differentiation of memory CD4⁺ T cells.

Mixed bone marrow chimeras (*Cd4-creER^{T2+} Zbtb7b^{fl/fl}* tester: *Cd4-creER^{T2+} Zbtb7b^{+/+}* competitor) were infected with LCMV Arm after (Δ Early) or 3 weeks before (Δ Late) tamoxifen treatment.

(A) Graph shows tester-competitor chimerism among spleen GP66:I-A^{b+} *Rosa26^{YFP+}* and GP33:H-2D^{b+} T cells at d100 p.i. (Δ Early, n=5; Δ Late, n=3). P-values from two-way ANOVA are indicated.

(B) Right panels show the expression of IL-7R α and KLRG1 on the GP66:I-A^{b+} cell populations defined in the left panels. The percentages of cells in the indicated gates are shown. Data are representative of 2 independent mixed bone marrow experiments and 3 non-chimeric experiments.

Supplemental Figure 6, Related to Figure 6

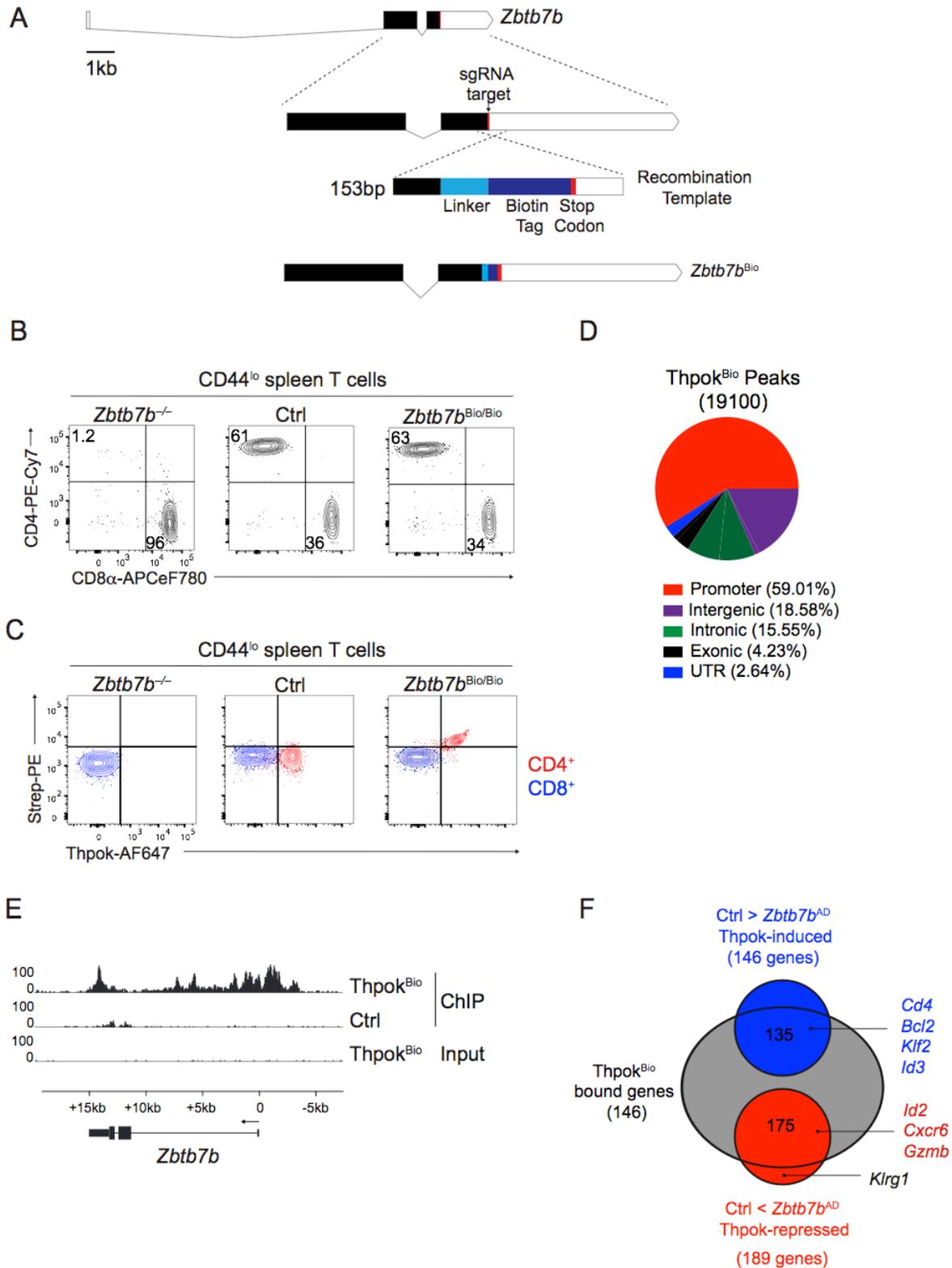


Figure S6: Generation and characterization of *Zbtb7b*^{Bio} mice.

(A) Schematic representation of the generation of the *Zbtb7b*^{Bio} allele.

(B, C) *Zbtb7b*^{Bio} animals were crossed with *Rosa26*^{BirA} mice and *Zbtb7b*^{Bio/Bio} *Rosa26*^{BirA} (*Zbtb7b*^{Bio/Bio}), *Zbtb7b*^{-/-} and *Zbtb7b*^{+/+} *Rosa26*^{BirA} (Ctrl) animals were analyzed. (B) Expression of CD4 and CD8 α in the spleen T cell population from the indicated animals. The percentages of cells in the indicated gates are

shown. **(C)** Intra-cellular Thpok and biotinylated Thpok were assessed on spleen T cells by anti-Thpok antibody (x axis) and streptavidin (y-axis). Data are representative of two independent experiments. **(D-F)** Thpok DNA binding was assessed by ChIP-seq in activated CD4⁺ T cells from Thpok^{Bio/+} Rosa26^{BirA+} (Thpok^{Bio}) or Rosa26^{BirA+} (Ctrl) animals. **(D)** Pie chart shows the genome-wide distribution of Thpok binding. **(E)** IGV browser tracks show binding by Thpok^{Bio} molecules on the *Zbtb7b* locus (Thpok^{Bio} ChIP), compared to background signals from streptavidin pull-down on Ctrl cells (Ctrl ChIP), and input DNA signal from Thpok^{Bio} cells. **(F)** Gene occupancy by Thpok molecules was compared to Thpok-dependent genes defined on d7 p.i. GP66:I-A^{b+} T cells by scRNAseq.

Supplemental Figure 7, Related to Figure 7

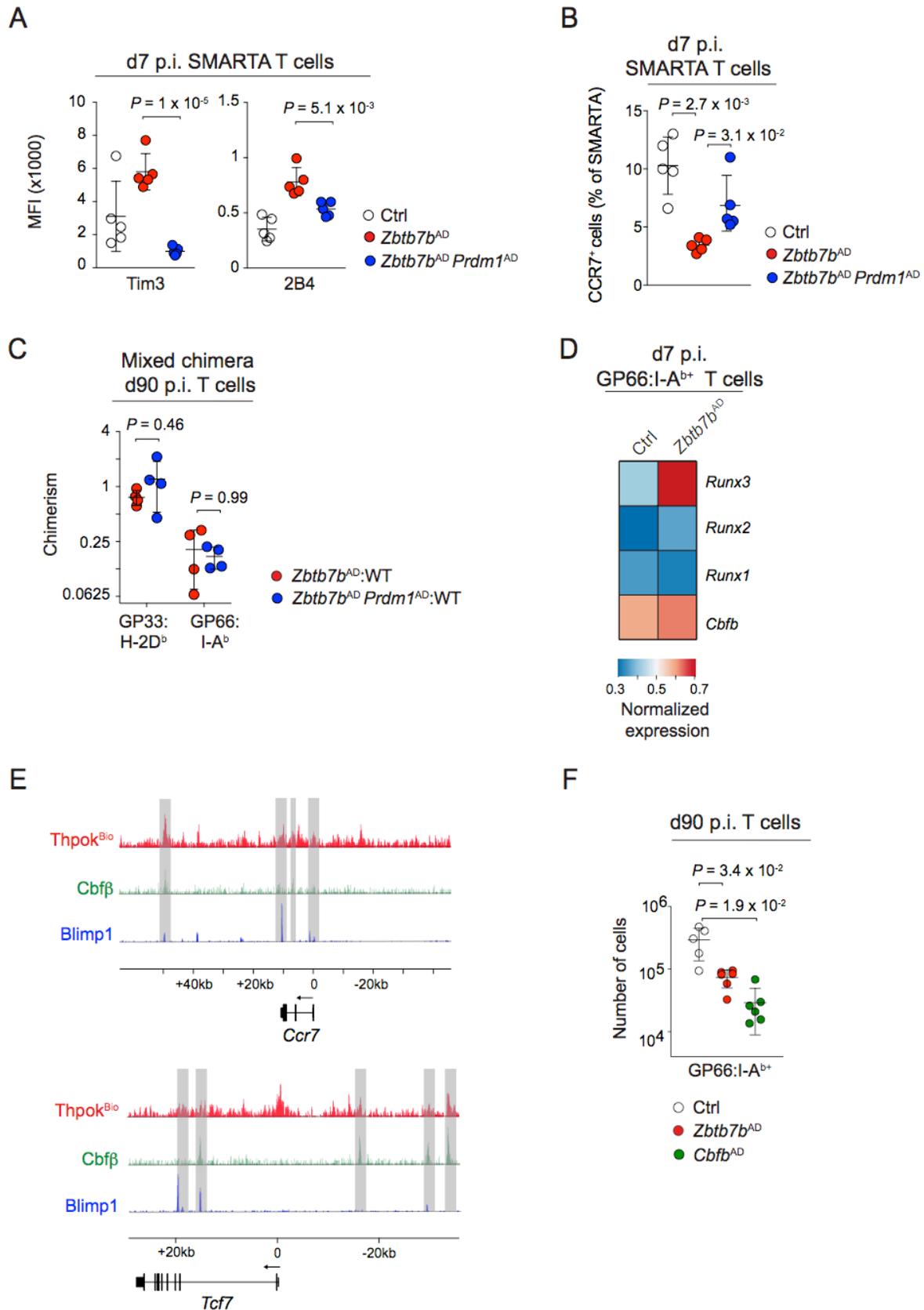


Figure S7: Thpok repression of Blimp1 and Runx3 and memory CD4⁺ T cell differentiation.

(A, B) Naive *Zbtb7b^{AD}*, *Zbtb7b^{AD} Prdm1^{AD}* or control SMARTA cells were adoptively transferred into WT recipients a day before LCMV Arm infection. Graphs show (A) Graphs show Tim3 and 2B4 surface

expression and **(B)** percentage of CCR7 positive cells on the adoptively transferred SMARTA cells of the indicated genotype at d7 p.i. (per group n=5). P-values from unpaired two-sided Welch's *t*-test are indicated. Data are representative of one adoptive transfer experiment and 2 independent experiments on *Zbtb7b*^{AD} or *Zbtb7b*^{AD} *Prdm1*^{AD} and control animals.

(C) *Zbtb7b*^{AD}:WT and *Zbtb7b*^{AD} *Prdm1*^{AD}: WT mixed bone marrow chimeras were analyzed at d90 p.i with LCMV Arm. Graph shows Tester:Competitor ratios among spleen GP33:H-2D^{b+} and GP66:I-A^{b+} T cells in *Zbtb7b*^{AD}:WT (red) and *Zbtb7b*^{AD} *Prdm1*^{AD}:WT (empty circles) chimeras (n=4 per group). Data are representative of one mixed bone marrow experiment and 3 non-chimeric experiments *Zbtb7b*^{AD}, *Zbtb7b*^{AD} *Prdm1*^{AD} and control animals.

(D) Heatmap shows the expression of Runx-related genes on GP66:I-A^{b+} T cells measured by scRNAseq at d7 p.i. with LCMV Arm.

(E) IGV browser tracks show ChIP-seq binding of Thpok, Cbfb and Blimp1 on the *Ccr7* and *Tcf7* loci. Grey shading highlights detected Thpok peaks overlapping with detected CBFβ or Blimp1 peaks

(F) *Zbtb7b*^{AD}, *Cbfb*^{AD} or control mice were infected with LCMV Arm. Graph shows the number of spleen GP66:I-A^{b+} cells in *Zbtb7b*^{AD} (n=6), *Cbfb*^{AD} (n=6) and control (n=5) animals at d90 p.i. P-values from unpaired two-sided Welch's *t*-test are indicated. Data are representative of 2 independent experiments.

Supplemental Table 1, Related to Figures 1, 2 and 5

Cell designation	Capture Session Number	Figure Panel	Timepoint	Cell Type	Genotype	Number of cell capture	Number of cells after filtering	Median Number UMI per cell	Median Number genes per cell
d7 Replicate 1	1	Fig. 1B-D Fig. S1A, D, E	d7 p.i.	CD44 ⁺ T cells	WT	7911	7006	3982	1446
d7 Replicate 2	2	Fig. 1D Fig. S1C-E	d7 p.i.	CD44 ⁺ T cells	WT	2416	2185	5039	1808
d7 GP66:I-A ^{b+} Ctrl	2	Fig. 2A, B Fig. S5C	d7 p.i.	GP66:I-A ^{b+} <i>Rosa26</i> ^{YFP+} T cells	<i>Tfnrsf4-cre</i> ⁺ <i>Zbtb7b</i> ^{+/+}	2459	2154	5277	1758
d7 GP66:I-A ^{b+} <i>Zbtb7b</i> ^{AD}	2	Fig. 2A, B Fig. S5C	d7 p.i.	GP66:I-A ^{b+} <i>Rosa26</i> ^{YFP+} T cells	<i>Tfnrsf4-cre</i> ⁺ <i>Zbtb7b</i> ^{fl/fl}	1414	1330	5889	1984
d30 GP66:I-A ^{b+} Ctrl	2	Fig. 5B, C Fig. S5C	d30 p.i.	GP66:I-A ^{b+} <i>Rosa26</i> ^{YFP+} T cells	<i>Tfnrsf4-cre</i> ⁺ <i>Zbtb7b</i> ^{+/+}	1355	723	2017	832
d30 GP66:I-A ^{b+} <i>Zbtb7b</i> ^{AD}	2	Fig. 5B, C Fig. S5C	d30 p.i.	GP66:I-A ^{b+} <i>Rosa26</i> ^{YFP+} T cells	<i>Tfnrsf4-cre</i> ⁺ <i>Zbtb7b</i> ^{fl/fl}	827	703	2385	1030
d7 GP66:I-A ^{b+} WT	3	Fig. 1E-G Fig S1E-I	d7 p.i.	GP66:I-A ^{b+} T cells	WT	2717	2213	5620	1936

Table S1: scRNAseq captures and metrics

Supplemental Table 2, Related to Figure 1

Signature:	Th1	Th1 (continued)	Tfh	Tcmp	Tmem	Treg	Dysfuntion
	4930453N24Rik	Il18rap	2310001H17Rik	1810058I24Rik	Btg1	Bmyc	201011101Rik
	Abrac1	Il2rb	Aldoa	Ablim1	Ccnd2	Btg1	Arf6ip1
	Acot7	Itga4	Angptl2	Acot7	Eif4a2	Capg	AW112010
	Actb	Itgb2	Asap1	Acp5	Gm10073	Ccnd2	Bhlhe40
	Actg1	Itgb7	Baff	Actn1	Il7r	Cd2	Ccl3
	Actr3	Klrc1	BC021614	Adgre5	Malat1	Cd74	Ccl4
	Adgre5	Krtcap2	Bcl2a1b	Adk	Rabac1	Ctla4	Ccl5
	Agpat4	Lfng	Borcs8	Ahnak	Rpl12	Foxp3	Ccr5
	Ahnak	Lgals1	Cd160	Anp32a	Rpl13	Gbp7	Cd160
	Anxa1	Lgals3	Cd200	Anxa2	Rpl13-ps3	Gimap7	Cd27
	Anxa2	Lsp1	Cd3g	Arl5c	Rpl18a	Gpx4	Cd3g
	Anxa5	Ly6c2	Cd8g	Atp1b3	Rpl36	H2-T22	Cd7
	Anxa6	Mrp133	Cox14	Atp2b1	Rpl36a	Ifngr1	Cst7
	Ap2s1	Ms4a4b	Ctsb	Bcl2	Rpl37a	Ikzf2	Ctla2a
	Aprt	Ms4a6b	Cxcr5	Bin2	Rpl38	Il7r	Ctla4
	Arhgdib	Mtpn	Ddit4	Ccdc28b	Rpl9	Izumo1r	Ctsb
	Arf6ip5	Myl12a	Dennd2d	Ccr7	Rpl9-ps6	Ltb	Ctsw
	Arpc5	Myl6	Eea1	Cd9	Rplp1	Mbnl1	Cxcr6
	Atp1a1	Myo1f	Fam162a	Cdc25b	Rplp2	Peli1	Cyba
	Atp5c1	Myo1g	Fyn	Cdc42se1	Rps14	Rgs1	Dnaj1
	Atp5h	Ndufb7	Gapdh	Cdkn2d	Rps15	Samsn1	Efn2
	Atp5l	Nkg7	Gdi2	Crip1	Rps15a	Sdf4	Fas1
	AW112010	Nptn	Gimap5	Crip2	Rps16	Sell	Fyn
	Bhlhe40	Ostf1	Gna13	Dock2	Rps19	Seinc3	Gadd45b
	Calm1	Pgylp1	Gng2	Emb	Rps21	Shisa5	Gimap7
	Capzb	Plac8	Hif1a	Ezr	Rps23	Tnfrsf18	Gpr65
	Ccl5	Plek	Hmgb1	Fam177a	Rps24	Tnfrsf4	Gzmk
	Ccr2	Podn1	Icos	Fam65b	Rps27		H2-K1
	Cd2	Ppib	Ifi2712a	Glud1	Rps27rt		Hspa1a
	Cd47	Ppp1ca	Isg15	Hint1	Rps28		Hspa8
	Cd48	Ppp3ca	Izumo1r	Id3	Rps29		Hsph1
	Cd52	Preld1	Limd2	Il6ra	Rps5		Id2
	Cdc42	Psemb3	Lpp	Iqgap1	Rps9		Lag3
	Cdc42ep3	Pycard	Lmp	Itgb1			Lilr4b
	Cfl1	Rap1b	Maf	Itpkb			Nkg7
	Clic1	Rasgrp2	Marcks11	Jund			Nr4a2
	Ctla	Rbm3	Matk	Klf2			Pdcd1
	Coro1a	Reep5	Mif	Klf3			Plac8
	Cox17	Rnf138	Mmd	Klf6			Psemb10
	Cox5a	Rnf166	Nsg2	mt-Co1			Psemb8
	Cox5b	Rora	Nt5e	Pbxip1			Rgs1
	Crip1	Rpa2	P2rx7	Pdlim1			Rnaset2b
	Crot	Runx3	Pdcd1	Prr13			Samsn1
	Ctla2a	S100a10	Pfkl	Psma6			Serpina3g
	Ctsc	S100a11	Pfkp	Raf1			Sh2d2a
	Ctsd	S100a13	Pgam1	Rasa3			Shisa5
	Ctsw	S100a4	Pkm	Rasgrp2			Slamf7
	Cxcr6	S100a6	Ppp1r14b	Rora			Sub1
	Cyba	S1pr4	Prkca	Rpa1			Tapbp1
	Cyth4	Sec61b	Ptp4a2	S100a10			Tnfrsf1b
	Dnajc15	Sec61g	Ptpn11	S100a11			Traf4
	Dok2	Selplg	Ptprcap	S100a4			Ubb
	Ech1	Sept11	Pth1	S100a6			
	Emp3	Serpnb6b	Rab37	Sfr1			
	Eno1	Serpnb9	Rgs10	Slamf6			
	Epsti1	Sh3bgrl3	Rnaset2a	Spn			
	Esyt1	Slamf1	Rnaset2b	Srp1			
	Fam107b	Sms	Rpsa	Stk24			
	Gabarapl2	Spn	Scd2	Stk38			
	Gapdh	St3gal6	Sept7	Tagap			
	Gbp7	Stmn1	Sh2d1a	Tagln2			
	Ggh	Sub1	Smco4	Tcf7			
	Gimap7	Tagln2	Sostdc1	Tcp1112			
	Glipr2	Taldo1	Tbc1d4	Tspan13			
	Glrx	Tbx21	Tnfai8	Tuba1a			
	Gm4950	Tceb2	Tnfsf8	Vim			
	Gmfg	Thy1	Tox	Vsir			
	Gna15	Tmed2	Tox2	Xm2			
	Gramd3	Tmsb4x	Tpi1				
	Gzmb	Tpm4	Trim8				
	H2afy	Tpst2	Zap70				
	H2afz	Tspo	Zfp3611				
	Hmgb2	Txn1					
	Hsp90b1	Txndc5					
	Id2	Ube2g2					
	Idh3a	Vim					
	Ifngr1	Zyx					
	Il18r1						

Table S2: T cell gene signatures