Supporting Information

Clambey *et al.* 10.1073/pnas.0805465105

SI Text

SI Materials and Methods. Antibodies used in this study included the following clones and sources. Antibodies from BD PharMingen included (with clone name in parentheses): CD8α (53–6.7), CD25 (PC61), integrin α4 [9C10(MFR4.B)], CD62L (MEL-14), IE^k (14–4-4S), KLRG1 (2F1), Ly49A (A1), Ly49C/I (SW5E6), Ly49F (HBF-719), Ly49I (YLI-90), NK1.1 (PK136), TCR Vβ2 (B20.6), Vβ3 (KJ25), Vβ4 (KT4), Vβ5.1/5.2 (MR9–4), Vβ6 (RR4–7), Vβ8.1/8.2 (MR5–2), Vβ8.3 (1B3.3), Vβ8.1/8.2/8.3 (F23.1), Vβ9 (MR10–2), Vβ14 (14–2), VCAM1 [429(MVCAM.A)], and streptavidin-APC. Antibodies from eBioscience included: integrin α4 (R1–2), a rat IgG2b isotype control for R1–2, and PD-1 (J43). Antibodies from BioLegend were CD29 (HMβ1–1) and CD61 [2C9.G2(Hmb3–1)]. Antibodies grown in our laboratory and/or conjugated with AlexaFluor647, Phycoerythrin, or PacificBlue (MolecularProbes) were: CD5 (53–7.313), CD44 (IM7.8.1), CD122 (TM-β1.4), CD127 (A7R34), MHC class II (Y3P), or TCR Cβ (H57–597).

RNA Purification and Microarray Analysis. All samples collected for microarray analysis were frozen as dry-cell pellets on dry ice immediately after sorting and stored at −80°C. Total RNA was purified from all samples in parallel by using the PicoPure RNA Isolation Kit (Arcturus). To obtain enough RNA for microarray analysis, all samples were subjected to double RNA amplification by using the RiboAmp OA RNA amplification kit (Arcturus). Amplified RNA samples were then labeled with biotin by using the Affymetrix GeneChip IVT labeling kit. Labeled antisense RNA samples were purified (by using OA amplification kit purification columns) and fragmented. Fragmented, labeled RNA samples were then hybridized overnight onto Affymetrix mouse genome 430 2.0 microarray, containing 45,101 probe sets.

Analysis of Microarray Data. Analysis of microarray results was done by using GeneSpring 6.2 (Agilent Technologies). For all analyses, data were imported into GeneSpring and subjected to: (i) data transformation (set measurements <0.01 to 0.01), and (ii) per chip normalization (normalized to the 50th percentile). Data were not subjected to per gene normalization. To identify genes altered in monoclonal expansions of CD8 memory T cells (TCEs) relative to polyclonal CD8 memory-phenotype (MP) T cells, data were separated into two groups: (i) TCEs (samples 2A, 4A, and 5A in Table S1) and (ii) polyclonal CD8 MP T cells (samples 2B, 4B, and 5B in Table S1). Genes whose expression was increased within TCEs were determined by three sequential analyses applied within GeneSpring: (i) samples were filtered on flags, to include only genes expressed (i.e., "present") in three of three TCEs; (ii) samples were then subjected to one-way statistical test, comparing TCEs versus polyclonal CD8 MP T cells by using a Welch t test (parametric test, variances not assumed equal), with a P value cutoff of 0.05; and (iii) samples were filtered on fold change (threshold = 2.0) for genes increased in TCEs relative to polyclonal CD8 MP T cells. Samples were not subjected to multiple testing correction. For genes decreased in TCEs, the same set of criteria was used, except the polyclonal CD8 MP T cell group was used in step (i).

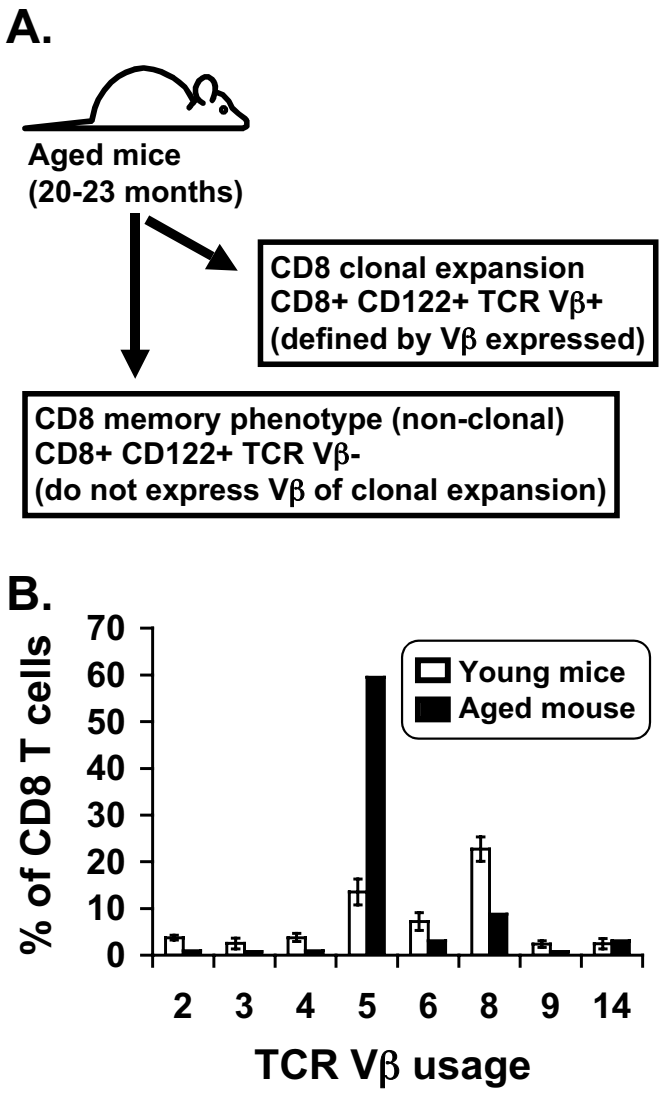

Fig. S1. Purification and identification of TCEs. (*A*) Purification of TCEs from aged mice. Mice with large TCEs were identified, as in *B*, and cells were purified as described. (*B*) Example of a large Vβ5+ TCE in a 19.5-month-old female C57BL/6J mouse (solid bars) compared to the average Vβ usage in a group of 10, 3.5-month-old female C57BL/6J mice (open bars). Vβ distribution defined as the percentage of peripheral blood CD8α+ cells expressing each Vβ. Error bars indicate 3 SD of mean Vβ usage in young mice.

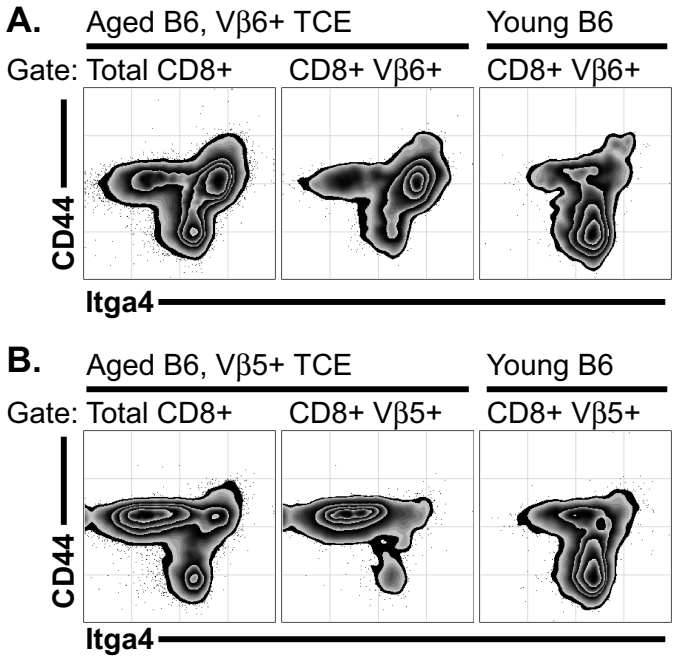

Fig. S2. itga4^{high} and itga4^{low} TCEs are CD44^{high}. Shown are CD44 expression levels in an itga4^{high} (*A*) and itga4^{low} (*B*) TCE. In each example, data include the itga4 profile on bulk CD8α+ events (*Left*) within the aged mouse with the TCE, the itga4 profile of the TCE in the same aged mouse defined by the indicated Vβ (*Center*), and the itga4 profile of CD8α+ events within the same Vβ in a young mouse (*Right*).

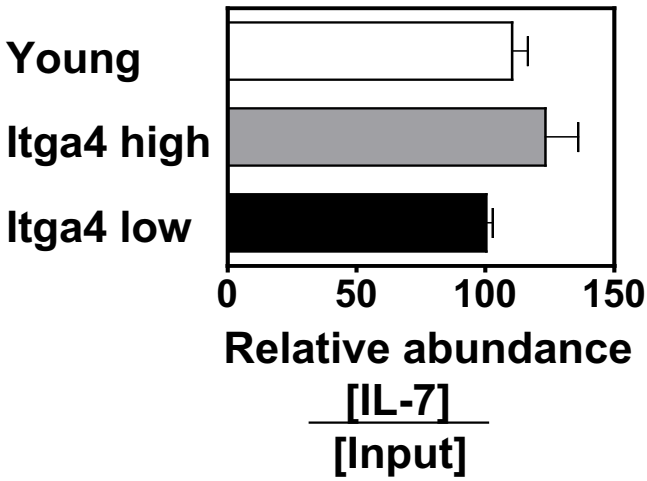

Fig. S3. itga4^{high} and itga4^{low} TCEs have equivalent short-term survival when cultured in low concentrations of IL-7. Bulk splenocytes were harvested from mice with itga4^{high} ($n = 3$) or itga4^{low} ($n = 4$) TCEs, and cells were cultured in 1 ng/ml IL-7 (R&D Systems), a condition that keeps cells alive but induces minimal proliferation in a naïve CD8 T cell. After 50 h, cultures were harvested and analyzed for the percentage of CD8 T cells that expressed the Vβ used for each individual TCE. Data represent (percentage of CD8+Vβ+ cells in IL-7)/(percentage of CD8+Vβ+ cells at time 0) $\times$ 100. All TCE samples were done in triplicate and data are plotted as mean $\pm$ SEM for multiple TCEs. Samples were subjected to two-tailed, paired *t* test analysis, to determine samples that were significantly different from 100 (i.e., no advantage or disadvantage between two conditions). No sample had a statistically significant difference from 100.

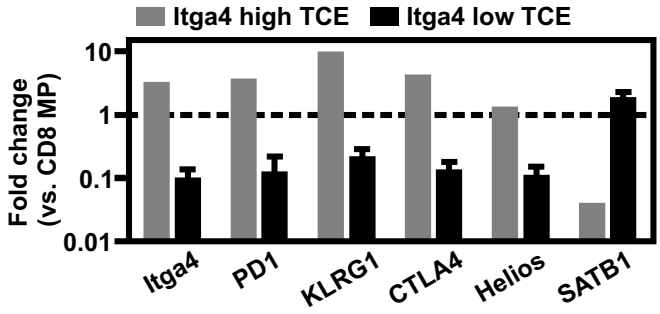

Fig. S4. itga4^{high} and itga4^{low} TCEs have divergent expression of two transcriptional regulators. Data show relative mRNA expression of the indicated genes in TCEs versus nonclonal CD8 MP T cells isolated from the same aged mice, calculated as (mRNA signal in the TCE)/(mRNA signal in the matched, nonclonal CD8 MP T cells). For these analyses, itga4 phenotype was inferred based on relative itga4 mRNA, with itga4^{high} TCE (TCE 1) (Table S1) in gray ($n = 1$), and itga4^{low} TCEs (TCE 2, 4, 5) Table S1) in black ($n = 3$, except $n = 2$ for PD1). For itga4^{low} TCEs, fold change for PD1, KLRG1, and CTLA4 was approximate, given that some TCEs had no detectable mRNA for these genes. Genes decreased in TCEs relative to nonclonal cells from the same mouse have a value <1. Data was based on normalized microarray data, and for the itga4^{low} TCEs plot mean $\pm$ SEM.

Table S1. Purified populations used for microarray analyses

Sample*	Mouse Strain	Mouse ID #	Purified population	Age, months	Clonal expansion size†	Sort criteria‡	Purity§, %
1A¶	C57BL/10SnJ	33	CD8 clonal expansion ($CD8^{low}$)	20	31.1% Vβ3	CD8 + CD122 + Vβ3+	90.9
1B	C57BL/10SnJ	33	Polyclonal CD8 memory	20	n/a	CD8 + CD122 + $V\beta3^{neg}$	96.8
2A	C57BL/10SnJ	19	CD8 clonal expansion	20	79.6% Vβ8.1/8.2	CD8 + CD122 + Vβ8.1/8.2+	94.9
2B	C57BL/10SnJ	19	Polyclonal CD8 memory	20	n/a	CD8 + CD122 + $V\beta8.1/8.2^{neg}$	74.7
4A	C57BL/10SnJ	28	CD8 clonal expansion	23	63.2% Vβ8.3	CD8 + CD122 + Vβ8.3+	95.8
4B	C57BL/10SnJ	28	Polyclonal CD8 memory	23	n/a	CD8 + CD122 + $V\beta8.3^{neg}$	65.7
5A	B10.BR	9	CD8 clonal expansion	23	50.4% Vβ14	CD8 + CD122 + Vβ14+	98.1
5B	B10.BR	9	Polyclonal CD8 memory	23	n/a	CD8 + CD122 + $V\beta14^{neg}$	97.2

*Unique identifier for clonal expansions used throughout manuscript.
†Percentage of CD8 + T cells that express the affected TCR Vβ.
‡All samples were sorted on live cells, that were MHC class II negative. Additional sort criteria used as indicated.
§Percentage of live cells with phenotype listed in ''Sort Criteria''.
¶Sample 1A was a $CD8^{low}$ clonal expansion. All other clonal expansions were $CD8^{normal}$.

Table S2. Genes increased at least 2-fold in expression in three, independent CD8 clonal expansions (with normal CD8 levels) compared to polyclonal, aged matched CD8 memory phenotype T cells

Fold Change*	Gene Symbol†	Gene Title	Affymetrix Probe Set ID‡	UniGene ID‡
14.56	Slc35f3	solute carrier family 35, member F3	1456764_at	Mm.324992
3.88§	Qser1	glutamine and serine rich 1	1452331_s_at	Mm.274314
3.66	2610305J24Rik	RIKEN cDNA 2610305J24 gene	1426936_at	Mm.410872
	BC005512	cDNA sequence BC005512		
	LOC215866	hypothetical protein LOC215866		
	LOC629242	hypothetical protein LOC629242		
	LOC630153	similar to melanoma antigen		
	LOC630525	similar to melanoma antigen		
	LOC631386	similar to melanoma antigen		
	LOC637482	similar to melanoma antigen		
	LOC641366	hypothetical ENV polyprotein		
	LOC668904	similar to melanoma antigen		
	LOC669019	hypothetical protein LOC669019		
	LOC669231	similar to melanoma antigen		
	LOC669436	hypothetical protein LOC669436		
	LOC670270	hypothetical protein LOC670270		
	LOC670719	similar to melanoma antigen		
	LOC672061	similar to melanoma antigen		
	LOC672196	similar to melanoma antigen		
	LOC672939	similar to melanoma antigen		
	LOC673876	similar to melanoma antigen		
	LOC674912	similar to melanoma antigen		
	LOC674927	similar to melanoma antigen		
	LOC675168	similar to melanoma antigen		
	LOC675410	similar to melanoma antigen		
	LOC675706	similar to melanoma antigen		
	LOC676247	similar to melanoma antigen		
	LOC676718	similar to melanoma antigen		
	LOC677506	similar to melanoma antigen		
3.34	**6720489N17Rik**	RIKEN cDNA 6720489N17 gene	1440869_x_at	Mm.247448
3.22	**1500010J02Rik**	RIKEN cDNA 1500010J02 gene	1437713_x_at	Mm.285785
3.03	Gprasp1	G protein-coupled receptor associated sorting protein 1	1447689_at	Mm.271980
3.03	Tmed2	Transmembrane emp24 domain trafficking protein 2	1460128_at	Mm.325307
2.95	**D11Ertd333e**	DNA segment, Chr 11, ERATO Doi 333, expressed	1447783_x_at	Mm.44226
2.90	**Kntc1**	kinetochore associated 1	1435575_at	Mm.332684
2.68	1110004B13Rik	RIKEN cDNA 1110004B13 gene	1429058_at	Mm.33964
2.57	Gins4	GINS complex subunit 4 (Sld5 homolog)	1457981_x_at	Mm.271603
2.56	Qser1	glutamine and serine rich 1	1427151_at	Mm.274314
2.44	Atxn2l	ataxin 2-like	1438668_x_at	Mm.231450
2.36	Ptcd1	pentatricopeptide repeat domain 1	1416391_at	Mm.332840
2.34	Tlr1	toll-like receptor 1	1449049_at	Mm.273024
2.33	**Mtx1**	Metaxin 1	1447361_at	Mm.280943
2.31	Rab2	RAB2, member RAS oncogene family	1419946_s_at	Mm.397471
2.30	Aspm	asp (abnormal spindle)-like, microcephaly associated (Drosophila)	1452459_at	Mm.168523
2.20	4631422O05Rik	RIKEN cDNA 4631422O05 gene	1428861_at	Mm.328360
	LOC667770	similar to down-regulated in ovarian cancer 1 isoform 2		
	LOC676704	similar to down-regulated in ovarian cancer 1 isoform 2		
2.17	Orc3l	origin recognition complex, subunit 3-like (S. cerevisiae)	1416116_at	Mm.260931
2.13	Mcm7	minichromosome maintenance deficient 7 (S. cerevisiae)	1439269_x_at	Mm.241714
	LOC433027	similar to minichromosome maintenance protein 7		
2.13	Asf1a	ASF1 anti-silencing function 1 homolog A (S. cerevisiae)	1459882_at	Mm.272989
2.09	Pard6b	par-6 (partitioning defective 6) homolog beta (C. elegans)	1423175_s_at	Mm.292834
2.08	C030039L03Rik	RIKEN cDNA C030039L03 gene	1453649_at	Mm.145362
2.07	Mapk6	mitogen-activated protein kinase 6	1459146_at	Mm.18856

Fold Change*	Gene Symbol†	Gene Title	Affymetrix Probe Set ID‡	UniGene ID‡
2.07	Zfp319	zinc finger protein 319	1455638_at	Mm.350514
2.01	1700084C06Rik	RIKEN cDNA 1700084C06 gene	1432576_at	—
2.00	0610040B10Rik	RIKEN cDNA 0610040B10 gene	1456161_at	Mm.30983
2.00	Lasp1	LIM and SH3 protein 1	1438634_x_at	Mm.271967

*Fold change indicates fold change between CD8 clonal expansions (samples 2A, 4A, and 5A) and polyclonal, CD8 memory phenotype T cells (samples 2B, 4B, and 5B), treating each group as a set of three replicates and calculating an average value for each group. Values calculated by using GeneSpring 6.2 (Agilent Technologies).

†Gene symbols in bold indicate genes whose expression was increased in all four CD8 clonal expansions (including the $CD8^{low}$, clone 1) compared to paired, aged-matched nonclonal CD8 memory T cells.

‡Affymetrix Probe Set ID numbers were used to search the NetAffx database (www.affymetrix.com) for Gene symbols/Gene Titles/UniGene IDs in October 2006. For those Probe Sets that hybridize with multiple genes, or multiple loci, all potential genes are listed.

§Values in italics indicate genes in which fold change is an approximate value. For these samples, the gene was expressed in CD8 clonal expansions but undetectable (i.e. absent) in polyclonal, CD8 memory T cells. Given that the limit of detection varies between individual genes, these values are only an estimate and, in some cases, may overestimate the difference between two samples.

Table S3. Genes decreased at least 2-fold in expression in three, independent CD8 clonal expansions (with normal CD8 levels) compared to polyclonal, aged matched CD8 memory phenotype T cells

Fold Change*	Gene Symbol†	Gene Title	Affymetrix ProbeSet ID‡	UniGene ID‡
35.14§	**A430107P09Rik**	RIKEN cDNA A430107P09 gene	1441552_at	Mm.423148
26.95	Coch	coagulation factor C homolog (Limulus polyphemus)	1423285_at	Mm.21325
23.40	Gzmk	granzyme K	1422280_at	Mm.56993
22.91	**Xlr3a**	X-linked lymphocyte-regulated 3A	1420357_s_at	Mm.195091
	Xlr3b	X-linked lymphocyte-regulated 3B		
	MGC76689	hypothetical protein LOC574437		
15.49	Itga4	integrin alpha 4	1421194_at	Mm.33596
15.23	Nr4a2	Nuclear receptor subfamily 4, group A, member 2	1455034_at	Mm.3507
14.30	Cerkl	ceramide kinase-like	1436037_at	Mm.31903
14.08	Ptprj	protein tyrosine phosphatase, receptor type, J	1425452_s_at	Mm.330393
	AW125753	expressed sequence AW125753		
13.12	Zfpn1a2	zinc finger protein, subfamily 1A, 2 (Helios)	1456956_at	Mm.106343
11.86	Casp1	caspase 1	1449265_at	Mm.1051
9.54	Rab39b	RAB39B, member RAS oncogene family	1435014_at	Mm.45148
9.34	A730095J18Rik	RIKEN cDNA A730095J18 gene	1437542_at	—
8.51	Penk1	preproenkephalin 1	1427038_at	Mm.2899
8.13	Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	1444073_at	Mm.275549
7.41	Casp4	caspase 4, apoptosis-related cysteine peptidase	1449591_at	Mm.1569
7.23	Gmds	GDP-mannose 4, 6-dehydratase	1434158_at	Mm.247143
6.68	Nr4a2	nuclear receptor subfamily 4, group A, member 2	1447863_s_at	Mm.3507
6.50	AI851523	expressed sequence AI851523	1440156_s_at	Mm.381287
6.20	Klrg1	killer cell lectin-like receptor subfamily G, member 1	1420788_at	Mm.20434
6.17	Perp	PERP, TP53 apoptosis effector	1416271_at	Mm.28209
6.09	6330403K07Rik	RIKEN cDNA 6330403K07 gene	1426766_at	Mm.27768
5.39	Iigp1	interferon inducible GTPase 1	1419043_a_at	Mm.261140
5.37	**Dsg2**	desmoglein 2	1439476_at	Mm.345891
4.71	Trak1	trafficking protein, kinesin binding 1	1459666_at	Mm.305318
4.36	Tcrb-V8.2	T cell receptor beta, variable 8.2	1445895_at	—
4.05	Itga4	Integrin alpha 4	1457376_at	Mm.33596
3.67	D19Wsu12e	DNA segment, Chr 19, Wayne State University 12, expressed	1446228_at	Mm.267131
3.66	Ints7	Integrator complex subunit 7	1443045_at	Mm.338175
3.51	Trat1	T cell receptor associated transmembrane adaptor 1	1437561_at	Mm.167298
3.48	Slc4a7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	1457528_at	Mm.258893
3.42	Sp100	Nuclear antigen Sp100	1447213_at	Mm.290906
3.38	2600010E01Rik	RIKEN cDNA 2600010E01 gene	1452834_at	Mm.24138
3.36	Nab1	Ngfi-A binding protein 1	1448781_at	Mm.25903
3.19	Galnt2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2	1452182_at	Mm.33808
3.09	**Rnf149**	ring finger protein 149	1429321_at	Mm.28614
3.06	Traf5	Tnf receptor-associated factor 5	1448861_at	Mm.389227
	LOC622602	similar to TNF receptor-associated factor 5		
3.04	Art3	ADP-ribosyltransferase 3	1452474_a_at	Mm.263514
3.01	—	—	1438235_at	—
2.90	E130308A19Rik	RIKEN cDNA E130308A19 gene	1435931_at	Mm.45688
2.84	**Vamp4**	vesicle-associated membrane protein 4	1422896_at	Mm.10699
2.82	—	Transcribed locus	1440883_at	Mm.393779
2.69	Itm2c	integral membrane protein 2C	1415961_at	Mm.29870
2.69	Rapgef1	Rap guanine nucleotide exchange factor (GEF) 1	1427006_at	Mm.298274
2.62	9030227G01Rik	RIKEN cDNA 9030227G01 gene	1458233_at	Mm.152357
2.61	**Zc3h12d**	zinc finger CCCH-type containing 12D	1458504_at	Mm.140055
2.58	**Ski**	Sloan-Kettering viral oncogene homolog	1426373_at	Mm.28520
2.58	Pes1	pescadillo homolog 1, containing BRCT domain (zebrafish)	1416172_at	Mm.28659
2.57	Hnrpr	Heterogeneous nuclear ribonucleoprotein R	1438807_at	Mm.31051
2.55	Tox	Thymocyte selection-associated HMG box gene	1446950_at	Mm.87051
2.52	C130098B18Rik	RIKEN cDNA C130098B18 gene	1446354_at	—
2.50	Sh2d1a	SH2 domain protein 1A	1449393_at	Mm.235391
2.50	Rora	RAR-related orphan receptor alpha	1424034_at	Mm.267116
2.49	Tle3	transducin-like enhancer of split 3, homolog of Drosophila E(spl)	1458512_at	Mm.24255

Fold Change*	Gene Symbol†	Gene Title	Affymetrix ProbeSet ID‡	UniGene ID‡
2.49	Tcf4	transcription factor 4	1416723_at	Mm.392967
2.49	—	Mus musculus, clone IMAGE:3983419, mRNA	1427195_at	Mm.392140
2.45	**Mns1**	meiosis-specific nuclear structural protein 1	1419402_at	Mm.387671
2.42	Ddef1	Development and differentiation enhancing	1438301_at	Mm.277236
2.41	Smpdl3b	sphingomyelin phosphodiesterase, acid-like 3B	1417300_at	Mm.287187
2.37	Foxo1	Forkhead box O1	1459170_at	Mm.29891
2.31	Lck	Lymphocyte protein tyrosine kinase	1457917_at	Mm.293753
2.28	Necap1	NECAP endocytosis associated 1	1448106_at	Mm.288114
2.27	Mtdh	Metadherin	1456548_at	Mm.130883
2.25	St6 galnac3	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3	1440121_at	Mm.296453
2.23	Kif5c	kinesin family member 5C	1422945_a_at	Mm.256342
2.22	5430434G16Rik	RIKEN cDNA 5430434G16 gene	1432850_at	—
2.22	Tcf12	Transcription factor 12	1438762_at	Mm.171615
2.22	**9230104K21Rik**	RIKEN cDNA 9230104K21 gene	1442406_at	—
2.18	Zwint	ZW10 interactor	1429786_a_at	Mm.62876
2.18	—	—	1438814_at	—
2.17	Armet	arginine-rich, mutated in early stage tumors	1428112_at	Mm.29778
2.17	A530052I06Rik	RIKEN cDNA A530052I06 gene	1458933_at	—
2.17	Sft2d2	SFT2 domain containing 2	1425026_at	Mm.288369
2.17	Stk4	serine/threonine kinase 4	1421107_at	Mm.234472
2.16	Mical1	microtubule associated monoxygenase, calponin and LIM domain containing 1	1416759_at	Mm.290431
2.15	**Spred2**	Sprouty-related, EVH1 domain containing 2	1441415_at	Mm.266627
2.14	Rps15a	ribosomal protein S15a	1430290_at	Mm.288212
	LOC434460	similar to ribosomal protein S15a		
	LOC664903	similar to ribosomal protein S15a		
	LOC675729	similar to ribosomal protein S15a		
2.13	Gprk5	G protein-coupled receptor kinase 5	1449514_at	Mm.279400
2.13	—	Transcribed locus	1438819_at	Mm.336778
2.13	Cugbp2	CUG triplet repeat, RNA binding protein 2	1446670_at	Mm.147091
2.13	Stat1	signal transducer and activator of transcription 1	1450033_a_at	Mm.277406
2.12	BB045044	expressed sequence BB045044	1443387_at	—
2.12	Plekhb2	pleckstrin homology domain containing, family B (evectins) member 2	1460341_at	Mm.292751
2.10	Ier3	immediate early response 3	1419647_a_at	Mm.25613
2.08	Ddb1	damage specific DNA binding protein 1	1415735_at	Mm.289915
2.07	Kpna4	Karyopherin (importin) alpha 4	1442928_at	Mm.30601
2.06	Casp3	caspase 3	1449839_at	Mm.34405
2.06	9630010G10Rik	RIKEN cDNA 9630010G10 gene	1460121_at	—
2.05	Ttn	titin	1427446_s_at	Mm.373672
2.04	Ahi1	Abelson helper integration site	1455177_at	Mm.253280
2.03	Braf	Braf transforming gene	1445786_at	Mm.245513
2.02	Arih2	ariadne homolog 2 (Drosophila)	1449119_at	Mm.290447
2.02	BC028789	cDNA sequence BC028789	1460064_at	—
2.01	Ndrg3	N-myc downstream regulated gene 3	1417663_a_at	Mm.279256
2.01	LOC383103	similar to RIKEN cDNA 1810036I24	1435130_at	—

*Fold change indicates fold change between CD8 clonal expansions (samples 2A, 4A, and 5A) and polyclonal, CD8 memory phenotype T cells (samples 2B, 4B, and 5B), treating each group as a set of three replicates and calculating an average value for each group. Values calculated by using GeneSpring 6.2 (Agilent Technologies).

†Gene symbols in bold indicate genes whose expression was also decreased in all four CD8 clonal expansions (including the $CD8^{low}$, clone 1) compared to paired, aged-matched nonclonal CD8 memory T cells.

‡Affymetrix Probe Set ID numbers were used to search the NetAffx database (www.affymetrix.com) for Gene symbols/Gene Titles/UniGene IDs in October 2006. For those Probe Sets that hybridize with multiple genes, or multiple loci, all potential genes are listed.

§Values in italics indicate genes in which fold change is an approximate value. For these samples, the gene was expressed in polyclonal, CD8 memory T cells but undetectable (i.e. absent) in CD8 clonal expansions. Given that the limit of detection varies between individual genes, these values are only an estimate and, in some cases, may overestimate the difference between two samples.

Table S4. Characteristics of integrin α4-defined TCE subtypes

Phenotype	Integrin α4 high	Integrin α4 mid	Integrin α4 low
Prevalence*	41.4%	10.3%	48.3%
Largest clone identified†	75.1% Vβ5x‡	83.3% Vβ8x‡	84.9% Vβ8x‡
Stability§	Unstable	Stable	Stable
Age range, months¶	11.5–20.5	16.5–22.5	18.5–36
Response to stimulation‖	Impaired	Not tested	Normal
In vivo localization**	Absent from peripheral lymph nodes	Not tested	Absent from Peyer's patches
Markers of chronic stimulation††	CD8 low IL7Rα low IL2Rβ mid PD-1 + or KLRG1+	Not tested	None

*Prevalence of integrin α4-defined TCEs (excluding ambiguous/mixed phenotypes), using data from Figure 1B.

†Based on data from 31 clones (within 27 aged mice), with 13 integrin $\alpha4^{high}$, 3 integrin $\alpha4^{mid}$, 15 integrin $\alpha4^{low}$. These clonal expansions include those displayed in Figure 1*B* plus additional clones identified in subsequent analyses.

‡Clonal expansions were identified by using antibodies that recognize multiple members of the indicated TCR Vβ. Vβ8x indicates that clone could be Vβ8.1, 8.2, or 8.3. Vβ5x indicates that clone could be Vβ5.1 or 5.2. On average, young B6 mice have 13.7% CD8α+Vβ5x+ cells and 23.6% CD8α+Vβ8x+ cells in peripheral blood.

§Based on data from Figure 2.

¶Age range indicates the youngest and oldest age of mice in which CD8 clonal expansions with each of the integrin α4 phenotypes were identified. This analysis is different from Figure 2*A*, which is only a cross-sectional analysis and does not indicate the oldest age at which each subtype of clonal expansion is present.

‖Based on response to phorbol 12-myristate 13-acetate (PMA) and ionomycin stimulation, Figure 4*A*.

**Based on Figure 3.

††Based on Figure 4 *B–D* and Table S5.

Table S5. Cell surface phenotype of integrin $\alpha 4^{high}$ and integrin $\alpha 4^{low}$ CD8 clonal expansions

Cell surface protein	Integrin α4 high expansions	Integrin α4 low expansions	Function of protein
CD8α	Low	Normal	Coreceptor for TCR engagement
CD44	High	High	Adhesion, Trafficking
Integrin α4 (CD49d)	High	Low	Adhesion, Trafficking
CD29	Mid/High	Mid	Adhesion, Trafficking
CD62L	Low	Mid/High	Adhesion, Trafficking
IL-2/15Rβ (CD122)	Intermediate (>naïve CD8)	High	Cytokine receptor (IL-15, IL-2)
IL-7Rα (CD127)	Low*	High	Cytokine receptor (IL-7)
PD-1	Positive†	Negative	Inhibitory receptor
KLRG1	Positive†	Negative	Inhibitory receptor
Ly49A/C/F/I	Negative	Negative	Inhibitory receptors
NK1.1	Low	Low	Activating receptor‡

Data are compiled from two independent experiments. For all conclusions, cells were analyzed by flow cytometry, and gated on CD8+CD44^{high}Vβ+ cells (appropriate for the affected Vβ).

*The majority of each integrin $\alpha 4^{high}$ clone was IL-7Rα low. However, a minor fraction of each clone expressed normal levels of IL-7Rα.

†For PD-1 and KLRG1 expression, integrin $\alpha 4^{high}$ clonal expansions have variable expression (see Fig. 4).

‡All mice analyzed here were either C57BL/6J or B6.PL. As such, the antibody used to detect the NK1.1 antigen (PK136) detects the activating receptor gene product Klrb1c/NKR-P1C.