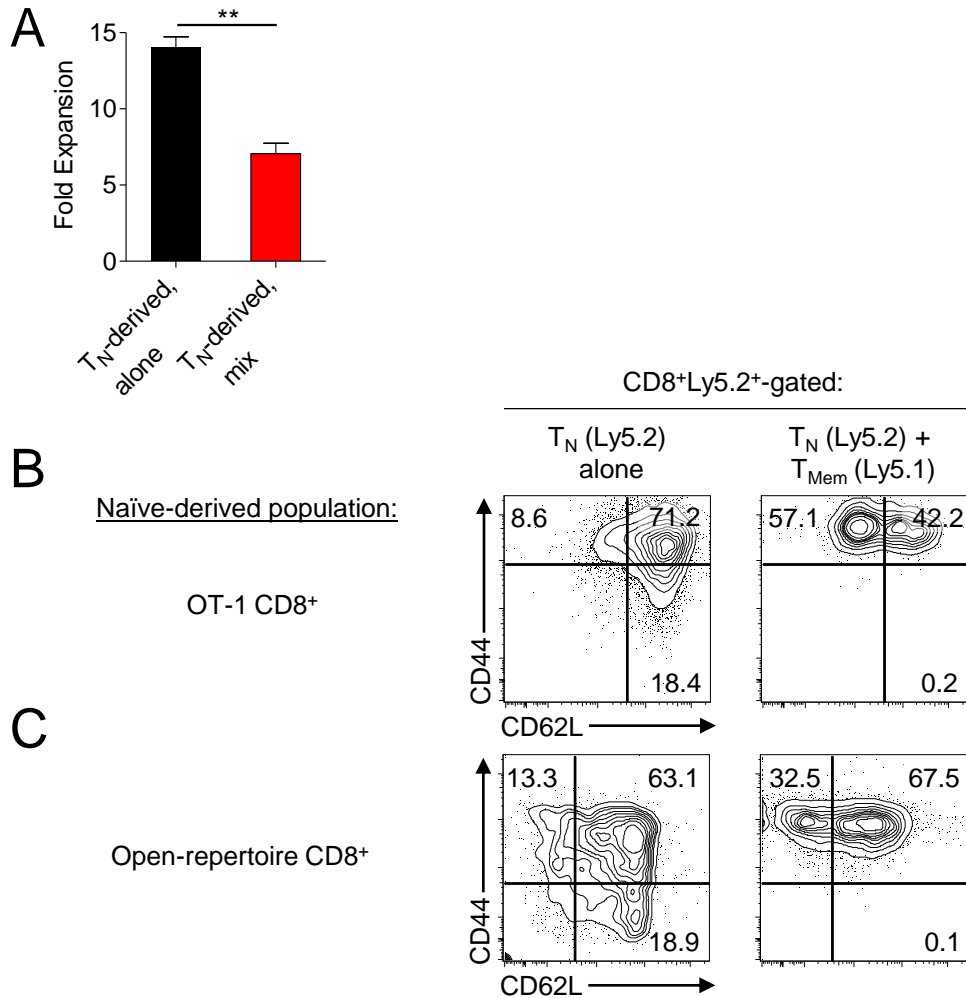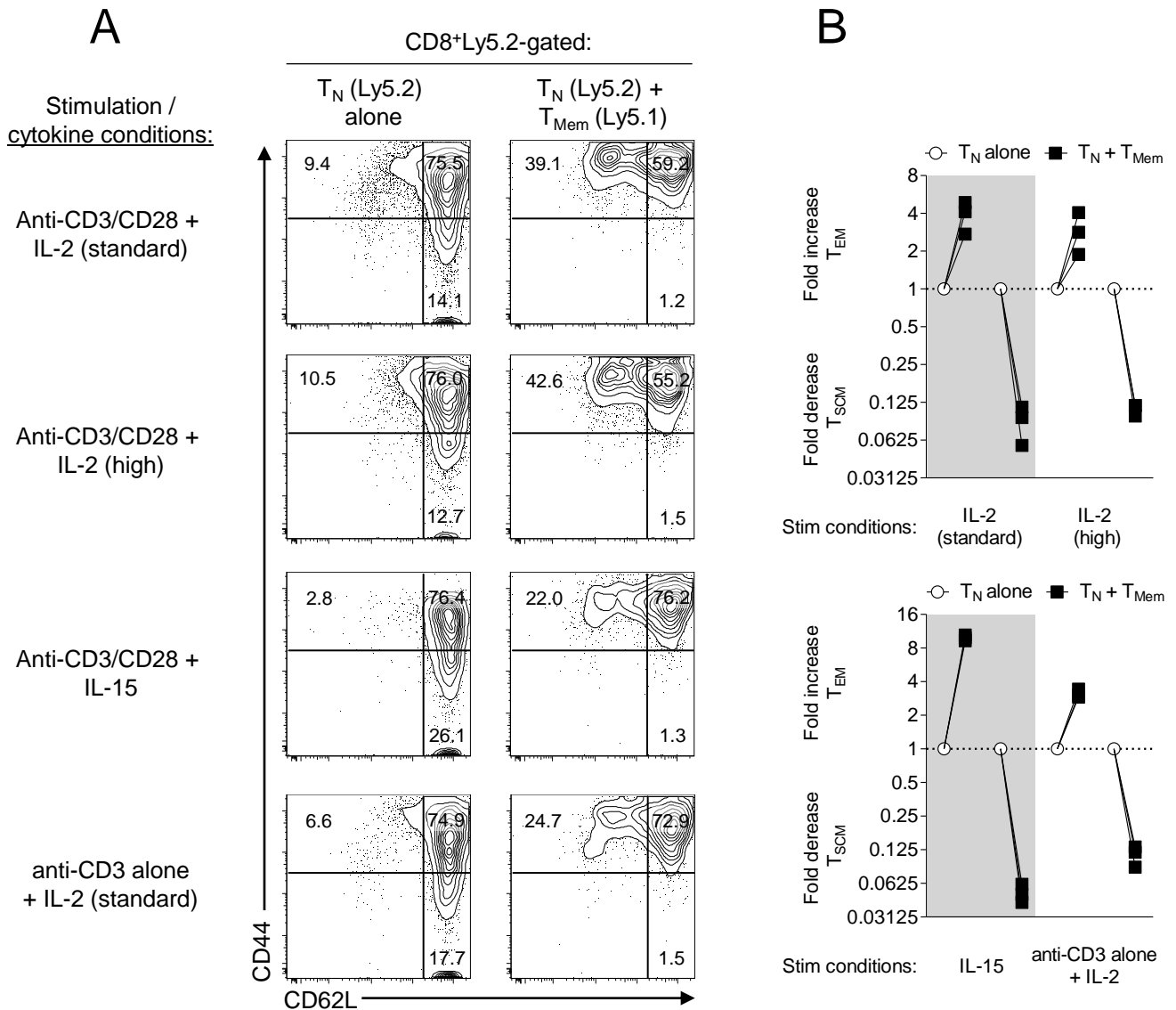**Supplemental Figure 1. Precocious differentiation causes attrition of the T_{SCM} population**. (**A**) Representative FACS plots showing the gating strategy for T_N/T_{SCM} and T_{Mem} populations. (**B**) Representative FACS plots and (**C**) scatter plots showing Sca-1 and CD122 expression on resting T_N CD8+ T cells and T_N- or T_{Mem}-derived CD8+ T cells expanded alone for 6d with CD3/CD28-specific antibodies and IL-2. Data shown are representative of two independently performed experiments (n = 2-3 independent mice or cultures per condition) with results shown as mean ± SEM. Statistical comparisons performed using an unpaired 2-tailed Student's t test corrected for multiple comparisons by a Bonferroni adjustment (***P<0.001; **P<0.01).

Supplemental Figure 2. Precocious differentiation is not attributable to differences in cell expansion and is not TCR-specific. (**A**) Expansion of T_N-derived cells 6d after priming alone or in the presence of a 1:1 mixture T_{Mem} using CD3/CD28-specific antibodies and IL-2. Results shown as mean ± SEM from n = 3 independently maintained cultures per condition. Statistical comparison performed using an unpaired 2-tailed Student's t-test (**P<0.01). Representative flow cytometry analyses showing the distribution of Ly5.2⁺ T_N-derived CD8⁺ subsets from either (**B**) OT-1 TCR transgenic mice or (**C**) polyclonal WT mice 6d following priming alone or in the presence of a 1:1 mixture with Ly5.1⁺ T_{Mem} using CD3/CD28-specific antibodies and IL-2. Cells gated on live⁺CD8⁺Ly5.2⁺ T cells. Numbers represent the percentage of cells in each quadrant. All experiments shown were repeated at least twice with similar results.

Supplemental Figure 3. Precocious differentiation occurs independently of cytokine and priming conditions. (**A**) Representative flow cytometry analyses showing the distribution of Ly5.2^{+} T_N-derived CD8^{+} subsets 6d following priming alone or in the presence of a 1:3 mixture with Ly5.1^{+} T_{Mem} using CD3/CD28-specific antibodies or anti-CD3 alone and either standard dose IL-2 (4.4ng/mL), high dose IL-2 (10ng/mL), or IL-15 (10ng/mL). Cells gated on live^{+}CD8^{+}Ly5.2^{+} T cells. Numbers represent the percentage of cells in each quadrant. (**B**) Summary plot showing the fold change in T_{EM} or T_{SCM} populations under different cytokine and stimulation conditions. ($n = 3$ independent cultures per condition). Experiments shown were repeated twice with similar results.
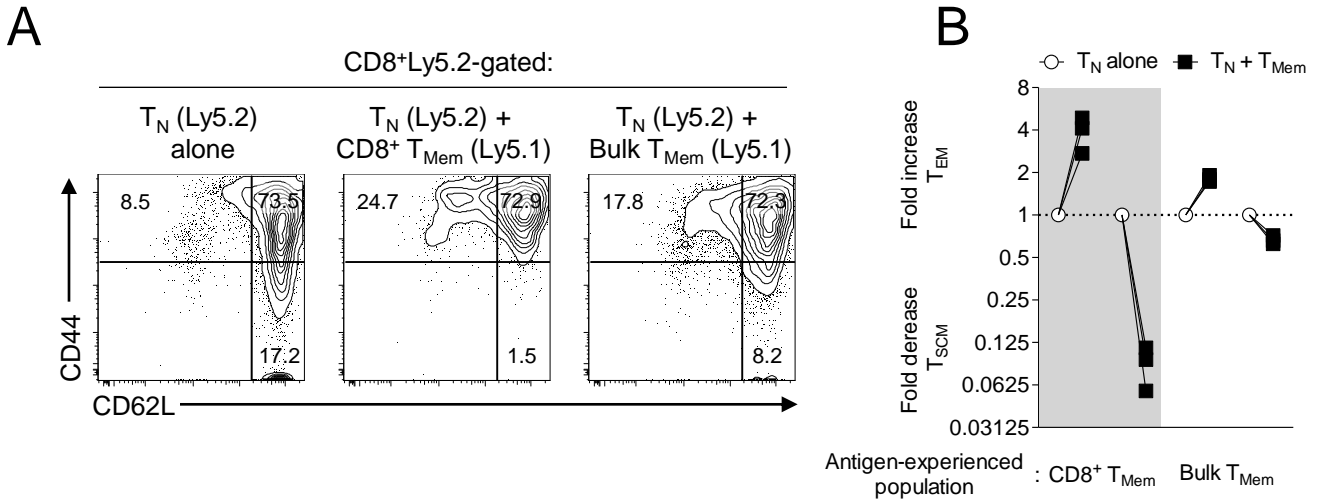
Supplemental Figure 4. Evaluation of precocious differentiation using CD8$^+$ T$_{Mem}$ versus a bulk population of T$_{Mem}$. (**A**) Representative flow cytometry analyses showing the distribution of Ly5.2$^+$ T$_N$-derived CD8$^+$ subsets 6d following priming alone or in the presence of a 1:3 mixture with CD8+ or bulk Ly5.1$^+$ T$_{Mem}$ using CD3/CD28-specific antibodies and standard dose IL-2 (4.4ng/mL). The bulk T$_{Mem}$ population was ~2:1 CD4$^+$:CD8$^+$ T cells. Cells gated on live$^+$CD8$^+$Ly5.2$^+$ T cells. Numbers represent the percentage of cells in each quadrant. (**B**) Summary plot showing the fold change in T$_{EM}$ or T$_{SCM}$ populations using CD8$^+$ versus bulk T$_{Mem}$ populations (n = 3 independent cultures per condition). Experiments shown were repeated twice with similar results.
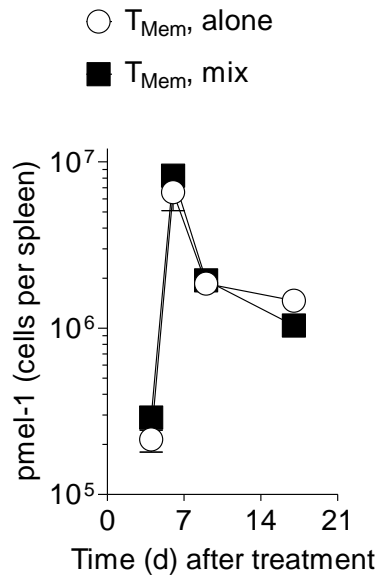
Supplemental Figure 5. T_{Mem} expansion is not altered by the co-transfer of T_N. In vivo expansion and persistence of $3x10^5$ Ly5.1+ CD8+ T_{Mem} injected alone or in combination with $1x10^5$ adoptively transferred Thy1.1+ T_N cells into Ly5.2+ WT mice bearing 10d established B16 melanomas. All treated mice received 6Gy irradiation, i.v. rVV-gp100 ($2x10^7$ pfu), and 3d of i.p. IL-2 (10μg/dose twice daily). $n = 3$ mice per group per time point. Results are displayed as mean ± SEM. Data shown are representative of 2 independently performed experiments.

A

CD8+TN gated:

TN alone, no stim	TN alone, αCD3/αCD28 stim	TN mix, αCD3/αCD28 stim	TN mix, no stim	TN mix, transwell αCD3/αCD28 stim

CD44

CD62L

Panel values:
- TN alone, no stim: 0.000 | 0.000 / 3.448 | 96.552
- TN alone, αCD3/αCD28 stim: 4.645 | 26.026 / 1.563 | 67.766
- TN mix, αCD3/αCD28 stim: 57.213 | 16.094 / 12.758 | 13.935
- TN mix, no stim: 0.000 | 0.000 / 5.000 | 95.000
- TN mix, transwell αCD3/αCD28 stim: 3.835 | 10.347 / 2.388 | 83.430

B

CD8+Ly5.1 gated:

TN (Ly5.1) + media control	TN (Ly5.1) + TMem supernatant	TN (Ly5.1) + TMem (Ly5.2)

Day 0:
- media control: 0 | 2 / 3 | 95
- TMem supernatant: 0 | 2 / 3 | 95
- TMem (Ly5.2): 0 | 2 / 3 | 95

Day 4:
- media control: 1 | 36 / 1 | 62
- TMem supernatant: 1 | 20 / 1 | 78
- TMem (Ly5.2): 31 | 63 / 1 | 5

CD44

CD62L

Supplemental Figure 6. Precocious differentiation is contact-dependent and is not transmissible through transfer of T$_{Mem}$ supernatant. (**A**) Representative flow cytometry analysis showing the distribution of Ly5.1$^+$ T$_N$-derived CD8$^+$ T cell subsets 6d following priming alone or with Ly5.2$^+$ T$_{Mem}$ either in physical contact or separated by a 0.4 μM semi-permeable membrane. (**B**) Representative FACS analysis and plots summarizing the distribution of CD8$^+$ T cell subsets on Ly5.1$^+$ T$_N$-derived subsets primed alone or in the presence or absence of Ly5.2$^+$ T$_{Mem}$ cells or supernatant derived from stimulated T$_{Mem}$ cells. For FACS plots, numbers represent the percentage of cells in each quadrant.
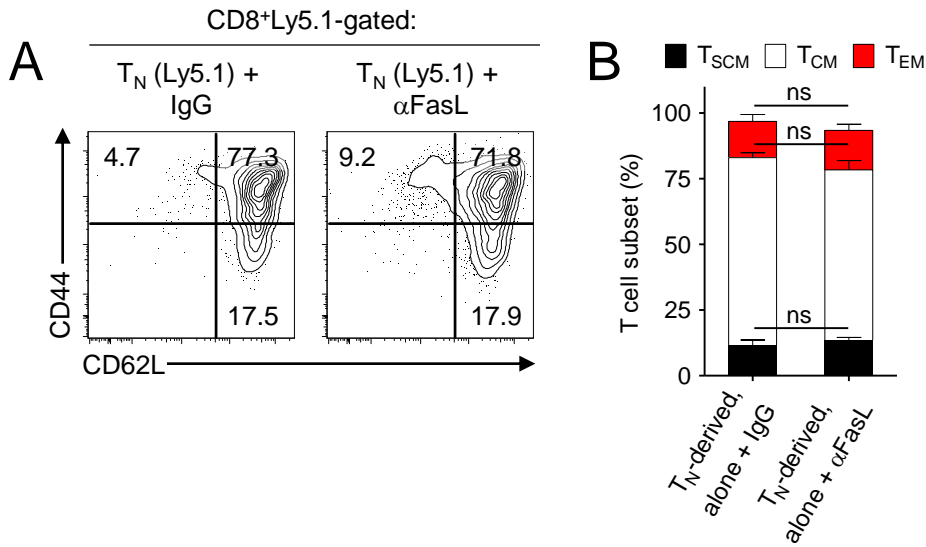
Supplemental Figure 7. Blockade of FasL on T_N CD8$^+$ T cells primed in the absence of T_{Mem} not alter the distribution of T-cell subsets. (**A**) Representative FACS plot and (**B**) summary bar graph showing the distribution of T cell subsets 6d after Ly5.1$^+$ T_N (CD44^{low}CD62L$^+$) pmel-1 CD8$^+$ T cells were primed alone with CD3/CD28-specific antibodies and IL-2 in the presence of 5ug/mL of αFasL or IgG control antibody. Numbers represent the percentage of cells in each quadrant for FACS plots after gating on live$^+$CD8$^+$Ly5.1$^+$ cells; graphs represents the mean $\pm$ SEM of $n = 6$ independently maintained cultures per condition from 2 pooled experiments. unpaired 2-tailed Student's t test corrected for multiple comparisons by a Bonferroni adjustment (ns, not significant).
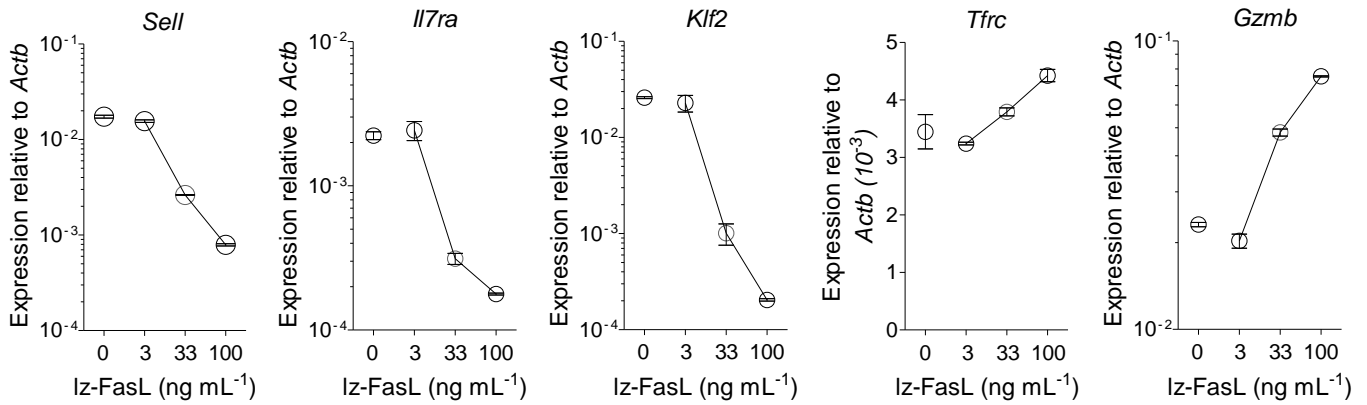
Supplemental Figure 8. Agonizing Fas-signaling in T_N in the absence of T_{Mem} causes dose-dependent changes in differentiation-associated genes. Quantitative PCR analysis of *Sell*, *Il7ra*, *Klf2*, *Tfrc*, and *Gzmb* in CD8$^+$ T_N-derived cells primed with CD3/CD28-specific antibodies and IL-2 alone or in the presence of titrated concentrations of Iz-FasL. Data shown as mean ± SEM of $n = 3$ independent cultures for each condition.

A

lz-FasL
(50 ng mL^{-1}):

	T$_{SCM}$	T$_{CM}$	T$_{EM}$

(-)

| 0.0 | 0.1 | 4.9 |
| 99.6 | 0.2 | 98.8 | 0.9 | 79.2 | 15.1 |

(+)

| 16.0 | 2.3 | 7.8 |
| 77.8 | 6.2 | 81.3 | 16.1 | 40.5 | 51.5 |

PI

Annexin V ⟶

B

☐ viable 🟥 early apoptotic ⬛ necrotic

T$_{SCM}$ T$_{CM}$ T$_{EM}$

population (%)

100
75
50
25
0

lz-FasL: (-) (+)   lz-FasL: (-) (+)   lz-FasL: (-) (+)
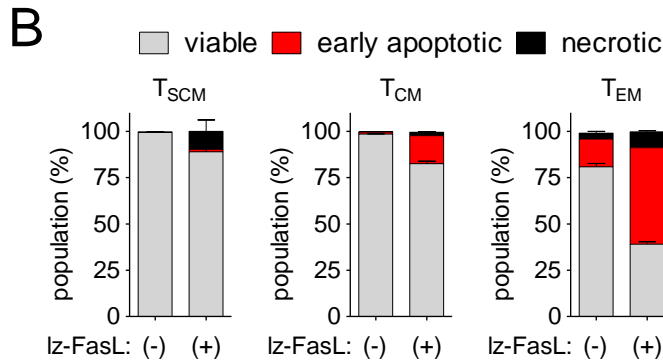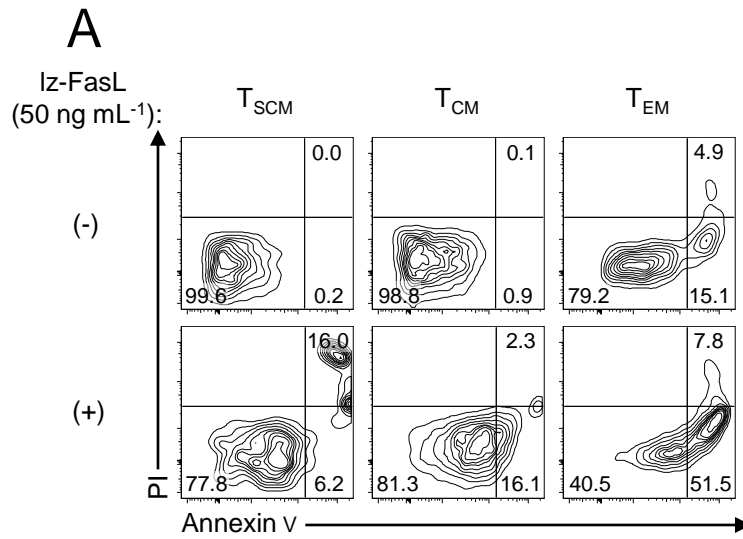
Supplemental Figure 9. **T$_{EM}$ CD8^{+} T cells have a higher level of baseline and lz-FasL induced apoptosis relative to T$_{SCM}$ and T$_{CM}$ subsets**. (**A**) Representative FACS plots and (**B**) summary bar graphs demonstrating annexin/PI staining at baseline or 6h following exposure to 50 ng/mL of lz-FasL (+) or vehicle control (-) in T$_{SCM}$, T$_{CM}$, and T$_{EM}$ subsets. Bar graphs represents the mean ± SEM of n = 3 independently maintained cultures. Results from one of two representative experiments is shown.

Live$^+$CD8$^+$-gated:

T$_N$ Vs. T$_{CM}$ Vs. T$_{EM}$

CD95 ⟶
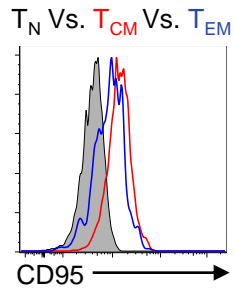
Supplemental Figure 10. T$_{CM}$ and T$_{EM}$ CD8$^+$ T cell subsets express similar amounts of surface Fas. Fas expression on naïve (CD44^{low}CD62L$^+$), T$_{CM}$ (CD44^{high}CD62L$^+$), and T$_{EM}$ (CD44^{high}CD62L$^-$) CD8$^+$ T cells subsets. This experiment has been repeated >3 times with similar results.
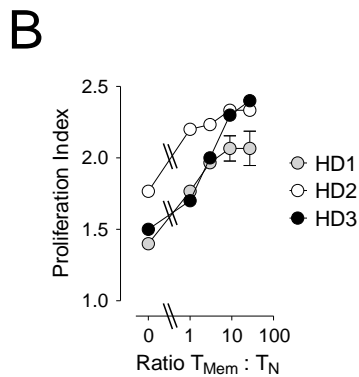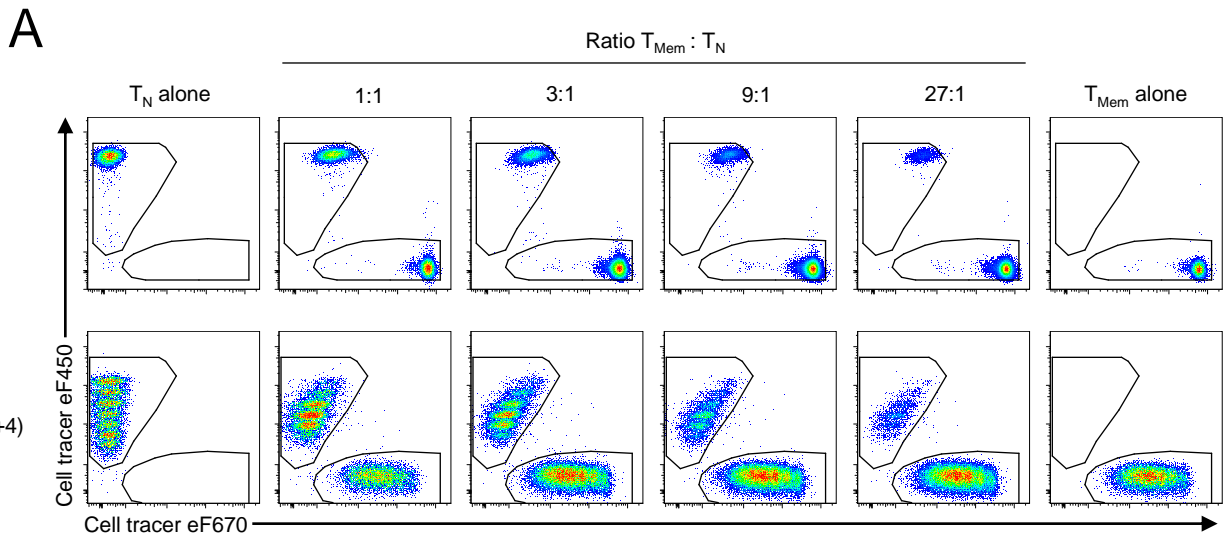
Supplemental Figure 11. Gating strategy to assess the phenotype and proliferation of mixed cultures containing human T_N and T_{Mem} cells using alternative membrane-associated dyes. Human T_N and T_{Mem} CD8+ T cells subsets were obtained by magnetic bead isolation and labeled with cell tracer eF450 or eF670 dyes, respectively. Naïve CD8+ T cells were subsequently stimulated with CD3/CD28-specific antibodies and IL-2 alone or in the presence of indicated ratios with T_{Mem}. (**A**) Representative FACS plots demonstrating the gating strategy used to distinguish T_N-derived from T_{Mem} cells immediately after membrane dye labeling and mixing (top) or 4d after stimulation (bottom). Data is shown after gating on viable CD8+ lymphocytes and is representative of 3 independently performed experiments. (**B**) Assessment of the proliferation index (PI) of T_N-derived cells 4d after expansion alone or in the presence of indicated ratios with T_{Mem} cells. Results shown as mean ± SEM of n = 3 independent cultures for each condition for all three donors.
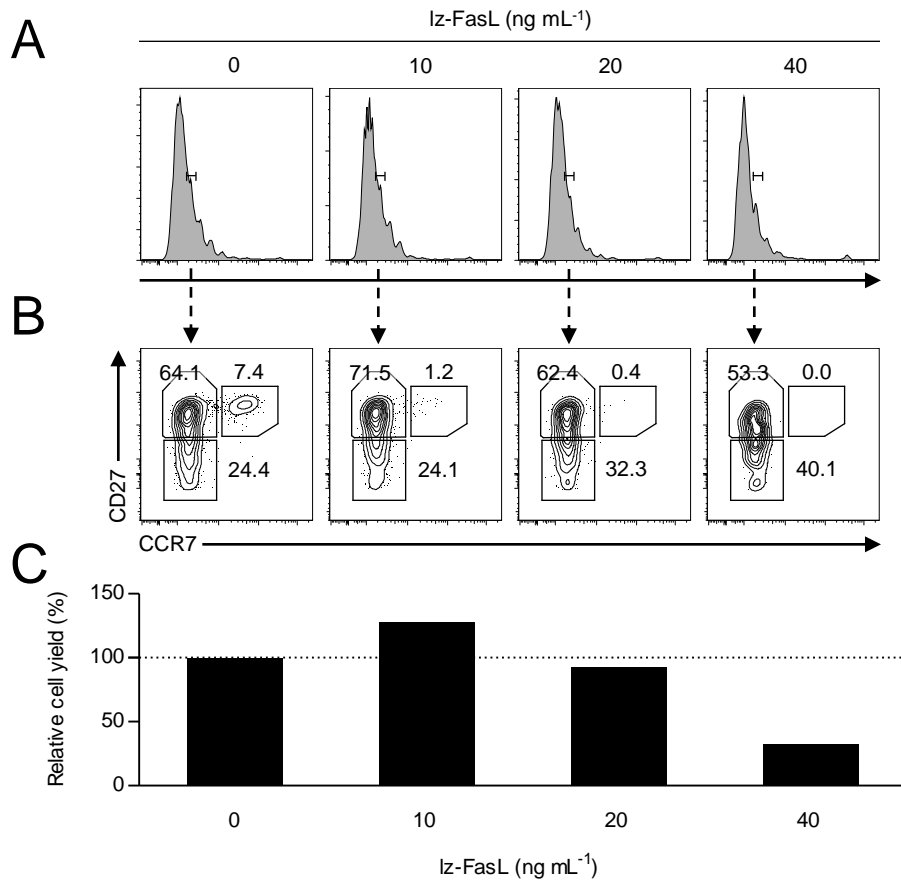
Supplemental Figure 12. Proliferation, expansion, and division-normalized differentiation of human CD8+ T cells expanded in the presence of titrated amounts of Iz-FasL. (**A**) Representative FACS plots demonstrating the proliferation of human CD8+ T cells labeled with Cell tracer eF450 and expanded for 6d with CD3/CD28-specific antibodies and IL-2 alone or in the presence of indicated concentrations of Iz-FasL. Similar results were obtained in 2 independent experiments after gating on live+CD8+ T cell that had undergone at least one cell division. (**B**) Representative FACS plots demonstrating the division-normalized surface phenotype for CD27 and CCR7 expression of expanded CD8+ T cells that had diluted an equivalent amount of Cell tracer eF450 dye. The arrows indicate the gated cell division analyzed and numbers represent the percentage of cells in each quadrant. (**C**) Relative overall cell yield of CD8+ T cells expanded in indicated concentrations of Iz-FasL. All values normalized to the vehicle only control group. Similar results were obtained in 2 independent experiments.
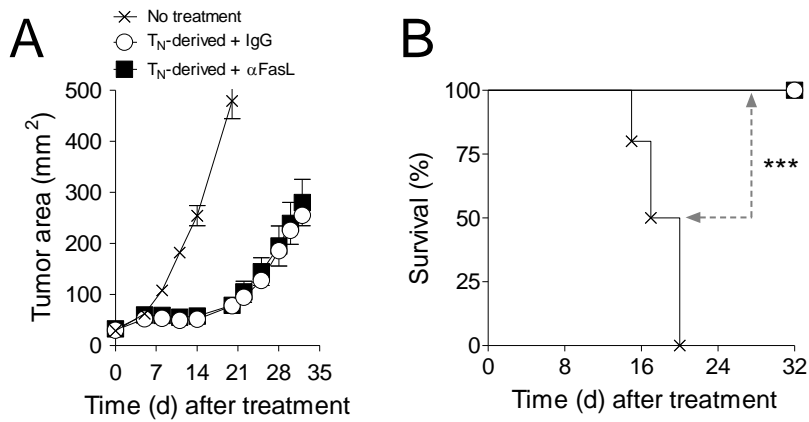
Supplemental Figure 13. **Blockade of FasL during *ex vivo* cell expansion does not influence the *in vivo* antitumor activity of isolated T$_N$ cells**. (**A**) Tumor regression and (**B**) animal survival of mice bearing 10d established s.c. B16 melanomas left untreated or treated with 2.5x10^5 T$_N$-derived pmel-1 cells primed alone in the presence of αFasL or IgG control. All mice received 6Gy irradiation prior to cell infusion in addition to i.v. rVV-gp100 (2x10^7 pfu) and 3d of i.p. IL-2 (10μg/dose twice daily). Data shown are representative of 2 independent experiments with results displayed as mean ± SEM. Statistical comparisons performed using the log-rank test for animal survival (***P<0.01).

Supplemental table 1:

Differentially expressed genes in naïve-derived T cells primed alone or with memory T cells at 18h.

Gene Symbol	RefSeq	Fold-Change (TN alone 18h vs. TN mix 18h)	p-value (TN alone 18h vs. TN mix 18h)
Akr1c18	NM_134066	3.3344	2.32E-05
Il7r	NM_008372	3.04414	3.94E-07
Kif21b	NM_001039472	2.89206	1.06E-07
Cd7	NM_009854	2.89146	2.92E-06
Slc12a7	NM_011390	2.85561	1.54E-07
Pde2a	NM_001143848	2.80423	8.15E-09
Tmem71	NM_172514	2.66872	7.24E-08
A430078G23Rik	NM_001033378	2.6661	6.89E-10
Cdc25b	NM_023117	2.64557	4.31E-07
Fam65b	NM_029679	2.6438	1.35E-07
H2-Ob	NM_010389	2.63008	2.12E-08
Fam78a	BC137951	2.60344	1.86E-09
B3gnt5	NM_001159407	2.58188	6.01E-05
Slc28a2	AF079853	2.57312	3.62E-06
Rapgef4	NM_019688	2.57178	8.15E-07
Itga4	NM_010576	2.56569	3.60E-07
Cyp2s1	NM_028775	2.53737	1.14E-06
Arhgef18	NM_133962	2.52123	4.66E-08
Gm10673	ENSMUST00000098753	2.51418	0.000269357
Zfp831	NM_001099328	2.46711	1.63E-06
Card6	ENSMUST00000118365	2.46405	5.72E-07
Rasgrp2	NM_011242	2.45711	4.72E-08
Hmha1	NM_001142701	2.45457	1.29E-07
Slc6a19	NM_028878	2.4506	1.04E-09
St6gal1	NM_145933	2.4478	2.41E-07
Il6ra	NM_010559	2.44118	2.20E-08
Acss2	NM_019811	2.43518	1.05E-07
Gria3	NM_016886	2.4156	6.60E-06
Sepp1	NM_009155	2.41462	3.72E-05
A130014H13Rik	AK079474	2.40552	0.00250053
Cmah	NM_001111110	2.39935	3.88E-06
Klrd1	NM_010654	2.38248	1.44E-06
Scml4	NM_172938	2.37409	3.40E-07
Orai2	NM_178751	2.36985	4.91E-07
Cd55	NM_010016	2.33387	4.06E-05
Sh2d3c	NM_013781	2.33357	1.22E-06
Pik3ip1	NM_178149	2.32701	1.09E-07

Dzip1	NM_025943	2.31535	4.58E-07
Ctdsp2	NM_001113470	2.30992	1.33E-07
Fam101b	ENSMUST00000021207	2.30794	1.27E-06
Zfp652	NM_201609	2.30333	7.83E-06
S1pr1	NM_007901	2.29882	5.91E-08
St6gal1	NM_145933	2.29331	3.48E-05
Itgb7	NM_013566	2.29115	1.30E-07
St8sia1	NM_011374	2.28522	3.40E-05
Ssh2	ENSMUST00000068342	2.26867	0.00184068
Klf13	NM_021366	2.26205	1.08E-07
Acss1	NM_080575	2.2577	5.43E-07
Lypd6b	NM_027990	2.23677	6.61E-05
Rasa3	NM_009025	2.23531	4.93E-08
Zbtb20	ENSMUST00000033479	2.22817	8.40E-05
Apol7e	NM_001134802	2.2186	7.46E-06
Smpdl3a	NM_020561	2.21137	2.02E-05
1810034E14Rik	ENSMUST00000099440	2.2113	8.20E-07
Adcy7	NM_007406	2.21121	3.67E-07
Acp5	NM_001102404	2.21016	2.58E-06
Kbtbd11	NM_029116	2.19833	1.60E-05
Bcl6	NM_009744	2.19462	2.20E-05
Fam102a	NM_153560	2.18717	6.42E-08
Myo1f	NM_053214	2.17507	9.48E-07
Dnahc8	NM_013811	2.16587	2.81E-06
Apol7b	NM_001024848	2.16027	6.74E-07
2410066E13Rik	BC042507	2.14243	9.45E-05
Thra	NM_178060	2.12371	7.05E-06
Arrb1	NM_177231	2.12219	3.39E-06
Actn1	NM_134156	2.11522	2.30E-08
Cdkn1b	NM_009875	2.10808	0.000261243
Ttyh3	NM_175274	2.10648	1.05E-05
C630004H02Rik	BC024617	2.0995	2.64E-05
Slc12a6	NM_133649	2.0992	9.39E-07
Klf3	NM_008453	2.09875	8.65E-06
Rnasel	NM_011882	2.09686	5.31E-05
Map4k2	NM_009006	2.09316	3.52E-06
Tmem63a	NM_144794	2.08932	2.31E-06
Gramd1a	NM_027898	2.07825	8.43E-09
Abcd1	NM_007435	2.07709	1.48E-06
Itgae	NM_008399	2.07442	1.63E-06

9930111J21Rik	NM_173434	2.07365	2.69E-05
Sla2	NM_029983	2.06682	5.41E-06
Prkd2	NM_178900	2.05698	1.61E-06
Tmc6	NM_145439	2.04827	2.95E-06
2010016I18Rik	AK140363	2.04689	7.68E-05
Slc28a2	NM_172980	2.04527	3.34E-06
Gm10345	AK005822	2.04072	1.73E-05
Xkrx	NM_183319	2.03858	1.82E-05
Appl2	NM_145220	2.03576	2.09E-05
Nipal3	NM_028995	2.03331	1.72E-06
Mkl1	NM_001082536	2.03281	2.77E-06
Klhl6	NM_183390	2.02988	4.74E-07
Tspan32	NM_020286	2.02103	1.49E-06
Traf3ip3	NM_153137	2.01783	3.70E-06
Hexb	NM_010422	2.01759	3.03E-05
Cdkn1b	NM_009875	2.01745	3.45E-06
Zbtb20	NM_019778	2.01677	5.30E-06
Mll2	ENSMUST00000023741	2.01535	3.97E-05
Kif1b	NM_207682	2.01081	2.54E-06
Arhgap15	NM_153820	2.00845	7.32E-06
Tpcn1	NM_145853	2.00461	3.70E-06
Unc84b	NM_194342	2.00398	1.35E-08
Plaur	NM_011113	-2.00308	9.38E-06
Cks2	NM_025415	-2.00836	0.000194715
Gm5521	XR_034247	-2.01433	0.00088575
AI747699	BC052506	-2.02248	0.000830106
Idi2	NM_177197	-2.02402	8.18E-05
2010002N04Rik	NM_134133	-2.03681	9.84E-05
B3galt6	NM_080445	-2.04672	3.14E-05
Bhlhe40	NM_011498	-2.0679	1.14E-05
Rpa3	NM_026632	-2.0879	0.00176324
Pros1	NM_011173	-2.10045	5.55E-06
BC005685	BC005685	-2.11791	0.000131966
Gp49a	NM_008147	-2.14246	1.29E-05
Idi2	NM_177197	-2.1434	8.73E-05
Hexim1	NM_138753	-2.16497	1.24E-06
Mrpl47	NM_029017	-2.17326	0.000317904
Ifng	NM_008337	-2.19685	7.40E-06
Ifitm3	NM_025378	-2.20254	4.37E-05
Mphosph6	NM_026758	-2.25245	0.0073701

Gadd45g	NM_011817	-2.28497	1.06E-06
LOC641050	M11024	-2.30889	8.86E-06
Lad1	NM_133664	-2.31682	4.43E-08
Gadd45b	NM_008655	-2.33607	3.93E-08
Tnfsf4	NM_009452	-2.34152	1.54E-06
Rgs1	NM_015811	-2.36325	1.64E-06
Casp3	NM_009810	-2.41227	1.85E-06
Eomes	NM_010136	-2.4158	6.59E-07
LOC641050	M11024	-2.43212	2.58E-05
Hsf2	NM_008297	-2.43424	3.51E-06
Fam129a	NM_022018	-2.50327	1.22E-06
Gzmc	NM_010371	-2.54444	9.94E-07
LOC641050	M11024	-2.56533	4.04E-05
Serpinb6b	NM_011454	-2.56845	7.71E-08
Il2	NM_008366	-2.58201	6.95E-06
Scd2	NM_009128	-2.62031	8.65E-07
Serpinb9	NM_009256	-2.75213	3.32E-07
Cd200	NM_010818	-2.75573	4.25E-07
Cd83	NM_009856	-3.35002	1.42E-07
AA467197	ENSMUST00000047498	-3.37473	2.37E-06
Cd24a	NM_009846	-3.55811	1.61E-05
Il3	NM_010556	-4.02756	1.80E-07

Supplemental table 2:

Differentially expressed genes in naïve-derived T cells primed alone or with memory T cells at 96h.

Gene Symbol	RefSeq	Fold-Change (TN alone 96h vs. TN mix 96h)	p-value (TN alone 96h vs. TN mix 96h)
Snord116	NR_002895	8.18707	1.59E-06
H2-Aa	NM_010378	7.12535	1.31E-10
Lyz2	NM_017372	6.9125	6.48E-09
Snord116	AF241256	6.68066	3.39E-06
Gpr83	NM_010287	5.62811	1.02E-07
Snord115	AF357427	5.27641	1.84E-05
Gm3079	XM_001475615	5.24318	2.74E-05
Ifng	NM_008337	4.98197	3.50E-10
Gria3	NM_016886	4.78953	3.15E-09
Itgae	NM_008399	4.1732	3.11E-09
A130014H13Rik	AK079474	3.94944	3.95E-08
Xcl1	NM_008510	3.91683	2.37E-07
Hba-a1	NM_008218	3.84053	1.72E-07
Lgals3	NM_001145953	3.74306	3.66E-09
Cd74	NM_001042605	3.56737	1.06E-08
Hba-a2	NM_001083955	3.47265	5.09E-07
Gm3079	XM_001475615	3.36242	1.38E-05
Gm8020	ENSMUST00000095981	3.33285	0.0014538
Cpa3	NM_007753	3.30737	9.95E-05
Serpine2	NM_009255	3.25596	1.04E-06
Gm8020	ENSMUST00000095981	3.25423	0.000509083
Ccr4	NM_009916	3.24645	8.50E-07
Klrb1b	NM_030599	3.18707	1.11E-08
Ccr9	NM_001166625	3.09237	0.000895433
Cd80	NM_009855	2.82224	1.25E-07
Foxp3	NM_054039	2.80545	4.46E-09
Rbpms	NM_019733	2.79816	2.01E-08
Ifit3	NM_010501	2.77958	8.81E-07
Vmn2r96	NM_001104547	2.72041	0.000106643
Nr4a3	NM_015743	2.67466	2.61E-08
Vmn1r100	NM_001166844	2.66993	0.0051509
Vmn1r158	NM_001166841	2.64164	0.00152347
Vmn1r101	NM_001166836	2.63727	0.000539336
Vmn1r117	NM_001166743	2.63221	0.00240964
Mir186	NR_029572	2.60985	0.000590633
Ccl3	NM_011337	2.59905	3.82E-07
Gpr56	NM_018882	2.58383	6.55E-08

Vmn1r122	NM_001166714	2.58122	0.00268472
Fcer1g	NM_010185	2.56899	8.44E-07
Airn	NR_002853	2.56621	0.0030745
Vmn1r125	NM_001166740	2.56076	0.00656702
Gm14085	NM_001085518	2.51984	1.75E-06
Vmn1r103	NM_001166737	2.50905	0.00287627
Mcpt8	NM_008572	2.4894	0.000241069
Gm10375	NM_001098269	2.46707	0.000379168
Gm5891	NM_001034904	2.46461	0.00641827
Vmn1r93	NM_207547	2.4575	0.00315899
Gm3079	XM_001475615	2.45139	0.000219289
Vmn1r151	NM_001166712	2.44892	0.00345932
Vmn1r121	NM_001166741	2.43593	0.00425721
1700054O19Rik	ENSMUST00000079800	2.4227	3.72E-05
Vmn1r114	NM_001166837	2.41908	0.00345255
Timp2	NM_011594	2.41831	1.27E-06
Vmn1r158	NM_001166841	2.41483	0.00107066
Gm2799	NM_001168334	2.40124	0.000193617
Furin	NM_011046	2.39647	2.88E-07
Kit	NM_001122733	2.39466	9.81E-08
Rps3a	NM_016959	2.3928	0.00212091
Cybb	NM_007807	2.38473	7.01E-09
Fes	NM_010194	2.3837	1.13E-06
D18Ertd653e	BC096371	2.37982	2.12E-08
H2-Ab1	NM_207105	2.37433	1.44E-06
Folr4	NM_176807	2.36679	9.34E-06
Vmn1r-ps79	NR_030707	2.34307	0.00316584
Gm10688	ENSMUST00000098856	2.34091	0.000214528
Vmn1r-ps79	NR_030707	2.33638	0.00346481
Eng	NM_001146350	2.32923	1.60E-07
Igfbp4	NM_010517	2.32067	4.60E-07
Clec12a	NM_177686	2.31291	9.91E-08
Il6ra	NM_010559	2.31288	2.71E-06
Il7r	NM_008372	2.27708	7.06E-07
Mir15a	NR_029733	2.27318	0.00179753
Gm3994	ENSMUST00000098805	2.26878	2.09E-05
Tgfb3	NM_009368	2.25936	7.29E-06
Slc12a7	NM_011390	2.25692	2.44E-09
Gm5929	XR_032222	2.25424	0.000785038
Cd80	BC131959	2.24898	9.18E-05

Gm5111	NM_183309	2.24661	1.12E-05
4930518I15Rik	ENSMUST00000099141	2.2394	1.43E-07
Tnfsf4	NM_009452	2.2382	9.85E-05
Snora74a	NR_002905	2.23543	2.73E-07
Snora69	NR_002900	2.23425	0.000234045
S1pr1	NM_007901	2.22511	3.47E-07
Speer4e	NM_001122661	2.20885	0.000285796
Gm3994	ENSMUST00000098805	2.1963	4.27E-05
Hsd11b1	NM_008288	2.19432	3.79E-06
9330175E14Rik	BC103768	2.18679	1.64E-05
Cpd	NM_007754	2.18113	4.33E-06
Rps13	NM_026533	2.16727	2.06E-05
Boll	NM_029267	2.15948	6.47E-05
Gm2799	NM_001168334	2.15567	0.000378794
Rps13	NM_026533	2.14217	1.31E-05
Ahr	NM_013464	2.13705	4.47E-06
Gm10406	NM_001164727	2.13587	0.000115357
Gm3002	NR_033388	2.13316	0.000117514
Cd7	NM_009854	2.13239	1.26E-06
Csprs	NM_033616	2.13235	0.000176924
Pgpep1l	NM_030101	2.12733	2.50E-06
H2-Eb1	NM_010382	2.1272	2.68E-07
Epcam	NM_008532	2.12331	3.70E-05
Pisd-ps1	NR_003517	2.12037	2.65E-06
Napsa	NM_008437	2.11371	9.84E-06
Gm9282	XR_033945	2.10205	0.00344243
Gm9282	XR_033945	2.10205	0.00344243
Cd200r1	NM_021325	2.09284	2.10E-05
Tlr1	NM_030682	2.07676	2.13E-06
Gm2799	NM_001168334	2.07331	0.00380429
Adamts6	NM_001081020	2.06868	0.000861485
Gm10375	NM_001098269	2.06655	0.000475087
Lpar6	NM_175116	2.0533	5.52E-05
Speer4d	NM_025759	2.05285	0.000139811
Snord52	NR_028527	2.05039	0.00294367
Gm10673	ENSMUST00000098753	2.0494	0.00398398
Pla2g7	NM_013737	2.04171	1.14E-05
Nr4a1	NM_010444	2.03976	5.36E-08
Gm3002	NR_033388	2.03555	5.62E-05
Ikzf2	NM_011770	2.0289	0.000102254

9030617O03Rik	BC066161	2.02786	6.68E-07
Mir181b-1	NR_029820	2.0271	0.000105395
Zbtb20	ENSMUST00000033479	2.02596	0.00455857
3110052M02Rik	NM_001166497	2.02477	0.000183681
EG381936	NM_001037248	2.00256	0.000651818
Psrc1	NM_019976	2.00128	1.41E-07
Sytl2	NM_001040085	-2.01054	5.36E-05
P4ha2	NM_001136076	-2.01635	3.48E-07
Chd7	NM_001081417	-2.01985	3.10E-05
Prelid2	NM_029942	-2.02279	2.75E-08
Il10	NM_010548	-2.02888	0.000125628
Myo1f	NM_053214	-2.03352	6.06E-08
Dsc1	ENSMUST00000025187	-2.03647	1.84E-08
Fgl2	NM_008013	-2.03796	6.54E-06
Gls2	NM_001033264	-2.03835	1.45E-07
Rnf157	NM_027258	-2.04962	1.06E-06
Cercam	NM_207298	-2.06445	3.54E-07
Fads1	NM_146094	-2.06516	1.73E-07
Vcl	NM_009502	-2.06872	8.58E-07
Fam64a	NM_144526	-2.07465	2.09E-06
Endod1	NM_028013	-2.0933	5.17E-05
Tmem67	NM_177861	-2.0975	9.02E-05
Selp	NM_011347	-2.10301	8.93E-07
Slc1a4	NM_018861	-2.12185	9.39E-07
Zfp280b	NM_177475	-2.12391	1.12E-06
Gm129	BC132471	-2.12393	3.19E-06
Crmp1	NM_007765	-2.12441	1.80E-08
Lipg	NM_010720	-2.12526	2.07E-06
Ero1l	NM_015774	-2.13454	5.23E-06
Sepw1	NM_009156	-2.13535	8.77E-07
Pfkl	NM_008826	-2.14279	1.96E-07
Chsy1	NM_001081163	-2.14464	3.57E-08
Mboat2	NM_026037	-2.14634	0.000200545
Bmpr1a	NM_009758	-2.14981	4.43E-06
Smpd1	NM_011421	-2.15194	1.55E-07
Lss	NM_146006	-2.15283	2.03E-07
Sqle	NM_009270	-2.15403	1.49E-06
Arsb	NM_009712	-2.15447	6.10E-07
Fbxo44	NM_173401	-2.16351	5.93E-07
Abcb1a	NM_011076	-2.17105	1.08E-06

Gys1	NM_030678	-2.17164	1.11E-07
St3gal6	NM_018784	-2.1737	6.68E-08
Cxcr3	NM_009910	-2.17546	4.00E-08
Arsb	NM_009712	-2.19224	8.73E-07
Dgat1	NM_010046	-2.19303	2.71E-08
Pld4	NM_178911	-2.20663	9.20E-06
Arrb1	NM_177231	-2.21265	3.89E-07
Bex6	NM_001033539	-2.21285	1.10E-07
Acsl3	NM_028817	-2.2233	2.24E-05
Gmfg	NM_022024	-2.22756	3.88E-09
Chac1	NM_026929	-2.23494	1.19E-06
Serpina3f	NM_001168294	-2.23989	2.25E-06
Rgs11	NM_001081069	-2.27626	3.90E-07
Grtp1	NM_025768	-2.27859	7.15E-07
Slc7a3	NM_007515	-2.28735	6.04E-06
Ppap2c	NM_015817	-2.29995	6.33E-07
Hpse	NM_152803	-2.31971	3.87E-05
4930420K17Rik	BC147127	-2.32427	5.86E-07
Ttc39c	NM_028341	-2.35172	1.01E-05
Hemgn	NM_053149	-2.3518	2.15E-06
P4ha1	NM_011030	-2.36022	1.13E-07
Entpd1	NM_009848	-2.36387	1.94E-07
Pgm2	NM_028132	-2.37444	2.56E-09
Gzmk	NM_008196	-2.37882	7.59E-07
Cysltr2	NM_133720	-2.38542	0.000363537
Grhpr	NM_080289	-2.38565	4.68E-08
Tgm2	NM_009373	-2.39375	1.71E-06
Cyp51	NM_020010	-2.40526	6.75E-06
Ndrg1	NM_008681	-2.41432	6.93E-09
Mctp2	NM_001024703	-2.42726	2.68E-07
Slc6a9	NM_008135	-2.4307	1.67E-08
Ctla2b	NM_007797	-2.46379	0.000190099
Gpnmb	NM_053110	-2.48161	3.90E-06
Ifitm2	NM_030694	-2.48251	3.83E-05
Rundc3b	NM_198620	-2.49143	3.49E-06
E430029J22Rik	NM_001162938	-2.49411	5.95E-06
Fosb	NM_008036	-2.52681	1.09E-08
Ly86	NM_010745	-2.56145	2.35E-06
Hmgcs1	NM_145942	-2.57022	1.09E-07
Gpt2	NM_173866	-2.58294	6.10E-06

Ldlr	NM_010700	-2.59052	1.60E-10
Dennd5a	NM_021494	-2.59323	6.21E-07
Fasl	NM_010177	-2.6057	0.000249099
Scpep1	NM_029023	-2.62176	1.20E-07
Ctla2a	NM_007796	-2.6328	0.000527223
Fam183b	NM_029283	-2.63864	5.07E-07
Ccr5	NM_009917	-2.65624	1.24E-06
Ccr5	NM_009917	-2.65624	1.24E-06
Nrn1	NM_153529	-2.65718	5.38E-06
Dkkl1	NM_015789	-2.65909	3.30E-06
Ryk	NM_013649	-2.67053	3.20E-07
Tirap	NM_001177845	-2.69014	7.29E-06
Slc2a1	NM_011400	-2.69409	5.44E-09
AY036118	AY036118	-2.71105	7.59E-05
ATP6	ENSMUST00000082408	-2.71193	0.00062153
Sla	NM_001029841	-2.72314	7.93E-09
Gm129	BC132471	-2.72606	2.13E-09
Ifitm2	NM_030694	-2.74383	9.34E-07
Plek	NM_019549	-2.75117	1.88E-07
Insig1	NM_153526	-2.75719	7.69E-08
Ndrg2	NM_013864	-2.8062	6.41E-08
Plod2	NM_001142916	-2.80992	1.11E-05
Sc4mol	NM_025436	-2.82562	1.33E-05
Mapk11	NM_011161	-2.82883	6.33E-07
Gp49a	NM_008147	-2.87022	1.16E-06
Klrk1	NM_033078	-2.90361	6.39E-06
Nov	NM_010930	-2.93305	1.42E-07
Atf6	NM_001081304	-2.97307	4.14E-09
Vaultrc5	NR_027885	-2.99834	9.91E-05
Serpinb6a	NM_001164117	-2.99853	0.000121947
Serpinb6b	NM_011454	-3.01678	5.92E-07
Ifitm2	NM_030694	-3.0246	8.35E-08
Atf3	NM_007498	-3.04952	6.72E-10
H1f0	NM_008197	-3.11068	1.00E-04
Anxa2	NM_007585	-3.17521	2.18E-07
Pik3ap1	NM_031376	-3.26399	2.77E-10
Itih5	NM_172471	-3.35682	3.39E-08
Scd2	NM_009128	-3.46975	1.91E-10
4930583H14Rik	NR_028121	-3.50537	3.93E-06
Dusp6	NM_026268	-3.52973	1.41E-09

Slc2a3	NM_011401	-3.54646	4.38E-10
Kdm5d	NM_011419	-3.73923	4.68E-07
Idh1	NM_010497	-3.75899	2.51E-05
Ak3l1	NM_001177602	-3.96266	6.89E-07
Fads2	NM_019699	-3.97998	2.89E-12
Slc16a3	NM_001038653	-4.71744	1.59E-08
Bnip3	NM_009760	-4.74355	3.32E-09
Egln3	NM_028133	-4.91642	3.94E-12
Ccr2	NM_009915	-5.0435	5.52E-07
Mt1	NM_013602	-5.4243	2.64E-11
Ddx3y	NM_012008	-5.81093	1.32E-05
Uty	NM_009484	-6.03981	1.01E-07
I830127L07Rik	ENSMUST00000100541	-6.22349	4.36E-09
Aldoc	NM_009657	-6.48711	2.43E-09
Zdhhc2	NM_178395	-6.87551	2.65E-06
Asns	NM_012055	-7.02253	8.43E-11
Bnip3	NM_009760	-8.10778	2.72E-10
Mt2	NM_008630	-10.8374	5.60E-08
Eif2s3y	NM_012011	-11.1468	4.59E-08
Ak3l1	NM_001177602	-11.2109	2.11E-09
Scd1	NM_009127	-25.7879	2.62E-11

Supplemental table 3:
Differentially expressed genes in memory-derived T cells expanded alone or with naïve T cells at 96h.

Gene Symbol	RefSeq	Fold-Change (TMem alone Vs. TMem mix, 96h)	p-value (TMem alone Vs. TMem mix, 96h)
Snord14d	NR_028274	2.20137	5.88E-06
Gm11277	NM_001110555	2.12943	0.000282587
Mela	D10049	-2.0691	1.52E-05
Aldoc	NM_009657	-2.07042	3.82E-06
Nrn1	NM_153529	-2.14928	3.29E-05
Dapl1	NM_029723	-2.17512	1.37E-06
Ifit3	NM_010501	-2.20701	6.16E-06
4930583H14Rik	NR_028121	-2.31383	7.55E-05
Art2b	NM_019915	-2.54866	7.16E-07
Ctla4	NM_009843	-2.70508	4.47E-06

Supplemental table 4:

Comparative expression of TNF superfamily member ligands in memory-derived versus naïve-derived T cells 18h after stimulation.

Gene Symbol	RefSeq	Soluble only	Known expression in T cells	P-value	Fc (TMem:TN, 18h)
Lta (aka Tnfsf1)	NM_010735	yes	yes	No expression detected	No expression detected
Tnf (aka Tnfsf2)	NM_001278601	no	yes	No expression detected	No expression detected
Ltb (aka tumor necrosis factor (ligand) superfamily, member 3)	NM_008518	no	yes	0.000105737	-1.732255692
Tnfsf4 (aka Ox40l)	NM_009452	no	yes	No expression detected	No expression detected
Tnfsf5 (aka Cd40l)	NM_011616	no	yes	No expression detected	No expression detected
Tnfsf6 (aka Fasl)	NM_010177	no	yes	0.0029402	2.874390396
Tnfsf7 (aka Cd70)	NM_011617	no	yes	No expression detected	No expression detected
Tnfsf8 (aka Cd30l)	NM_009403	no	yes	No expression detected	No expression detected
Tnfsf9 (aka 41bbl)	NM_009404	no	yes	0.001312768	1.646170308
Tnfsf10 (aka Trail)	NM_009425	no	yes	0.015966499	1.055258694
Tnfsf11 (aka Rankl)	NM_011613	no	yes	0.076537174	2.101580205
Tnfsf12 (aka Dr3l, Tweak)	NM_011614	no	no	No expression detected	No expression detected
Tnfsf13 (aka Tall2, April)	NM_001159505	no	no	No expression detected	No expression detected
Tnfsf13b (aka Tall1)	NM_033622	no	no	No expression detected	No expression detected
Tnfsf14 (aka Hveml)	NM_019418	no	yes	No expression detected	No expression detected
Tnfsf15 (aka Tl1a)	NM_177371	no	yes	No expression detected	No expression detected
Tnfsf18 (aka Gitr)	NM_183391	no	yes	0.012698273	-0.241070729
Eda	NM_001177937	no	no	No expression detected	No expression detected
Btla	NM_001037719	no	yes	0.068384995	-0.902823172