Supplementary Materials for

Commensal bacteria *Bifidobacterium* stimulates anti-tumor response via T cell cross-reactivity

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This PDF file includes:

Supplementary Text

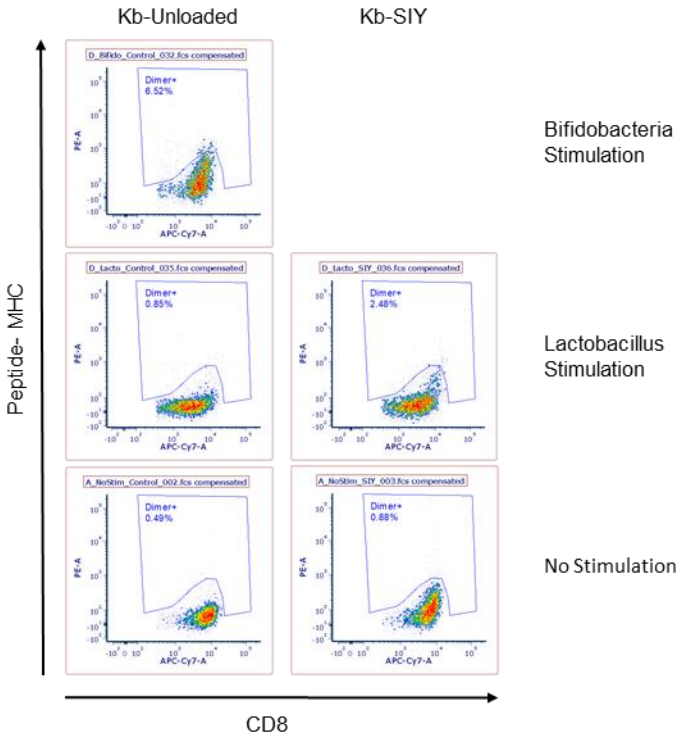

Figure S1.

Representative flow cytometry of antigen specificity of CD8+T cells from Jackson C57BL/6 mice splenocytes that were pulsed with heat-killed *Bifidobacterium* bacteria and tested for antigen cross-reactive specificity by staining with SIY peptide loaded into Kb-Ig dimer on day 11. Cells gated on lymphocytes, live, and CD8+T cells. Antigen specificity calculated by subtracting frequency in the unloaded Kb-Ig peptide staining control.

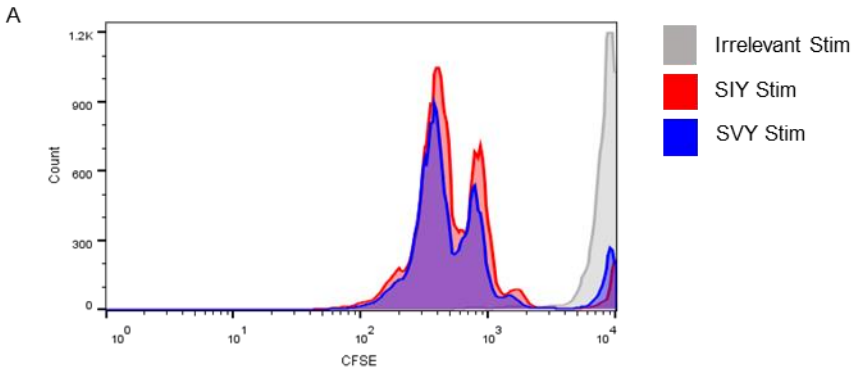

Figure S2.

2C CD8+ T cells are cross-reactive with by both SIY and SVY antigens. 2C splenic cells were stained with CFSE and pulsed with SIY, SVY or OVA peptide and harvested on D3 and analyzed for CFSE dilution. Cells gated on CD8+ T cells. 2C CD8+T cells can be stimulated by either cognate SIY peptide or cross-reactive SVY peptide.

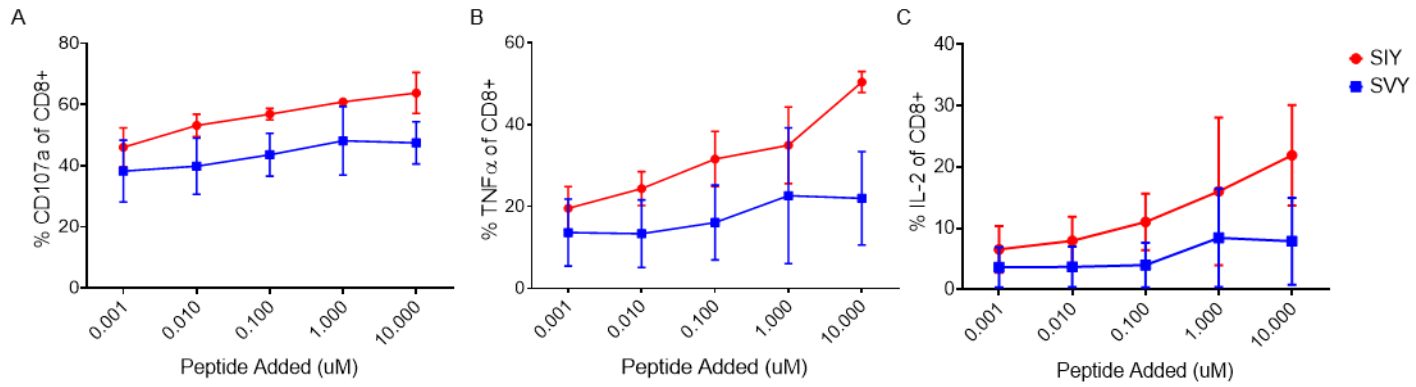

Figure S3.

2C CD8+ T cells are functionally cross-reactive with by both SIY and SVY antigens. (A-C) 2C splenic cells pulsed for 5 days with SIY peptide, challenged with RMA-S cells pulsed with titrating amounts of SIY and SVY peptide and measured for CD107a, TNFα and IL-2 production upon challenge, no stimulation control subtracted for background activity. (A-C) T cells analyzed for cytokine response via 2-way ANOVA and Bonferroni post hoc test for multiple comparisons. P-value is not significant for all 3 panels. Data represents mean ± SEM. N=3.

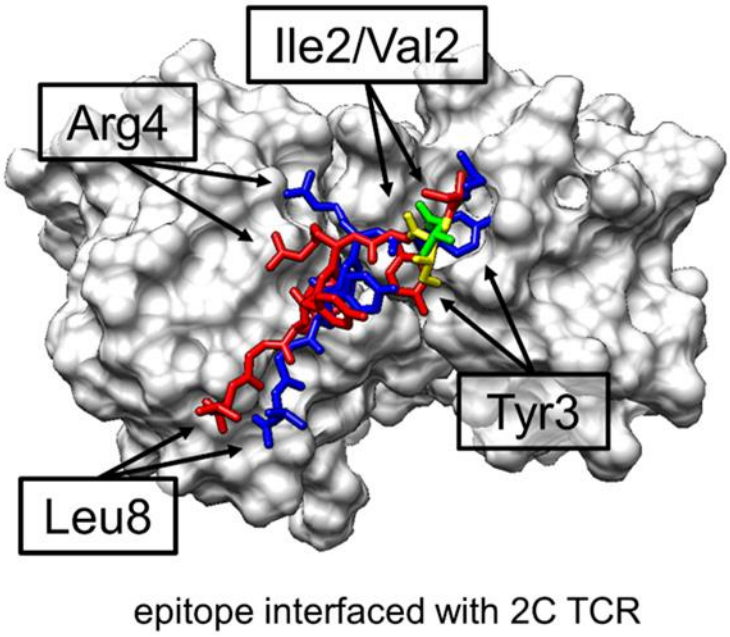

Figure S4.

The overlay of the SIY and the SVY epitopes on the TCR-epitope interface. The SIY and the SVY epitopes are represented by stick models, in red and blue, respectively. Ile2 of the SIY epitope and Val2 of the SVY epitopes are colored in yellow and green. The same colors are used for each molecule as in Figure 2B.

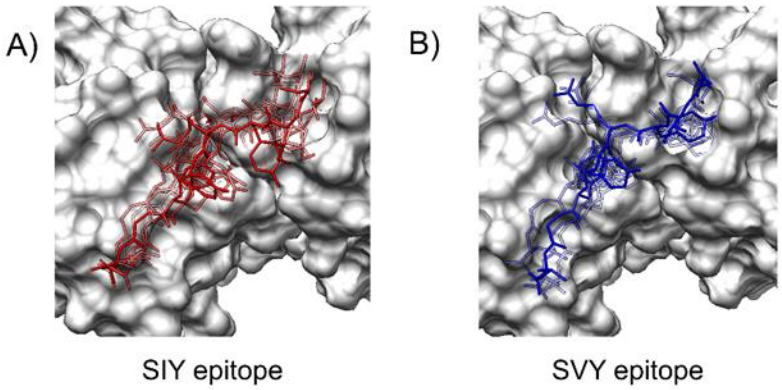

Figure S5.

SIY and SVY antigens at the TCR interface. (A) The overlay of different binding poses of the SIY epitopes at the interface with 2C TCR, taken from the frames at the centers of the five most populated clusters in the MD simulations. 2C TCR is represented by a surface model in light gray, and the SIY epitope by a stick model in red. The color of the SIY epitope gradually fades into transparency, as the epitope structure is taken from the first to the fifth ranked clusters. (B) The same figure with A, but with the SVY epitope, colored in blue.

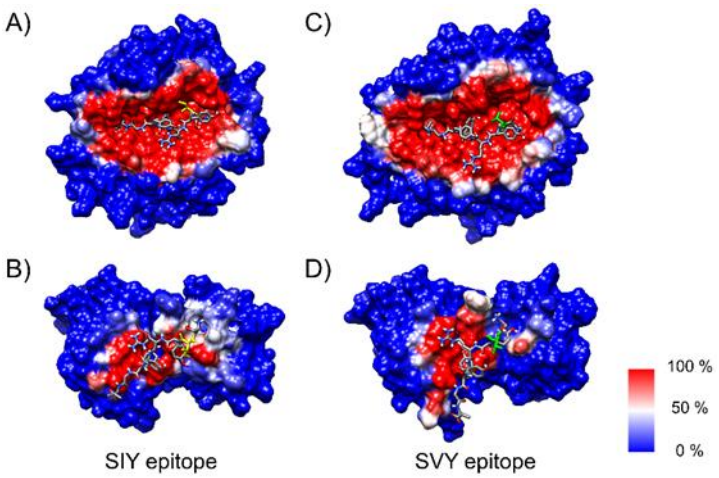

Figure S6.

The contact frequency between the SIY epitope and either H2-Kb (A) or 2C TCR (B). H2-Kb and 2C TCR are represented by a surface model, and the SIY epitope by stick. Ile2 of the SIY epitope is colored in yellow. All atoms of H2-Kb and 2C TCR are colored by the frequency of contacting (within 6 Å) any epitope atom in the MD trajectory. The color scale represents the contact frequency ranging from 0 to 100 %. (C-D) The same figures with A-B, but with the SVY epitope. Val2 of the SVY epitope is colored in green.

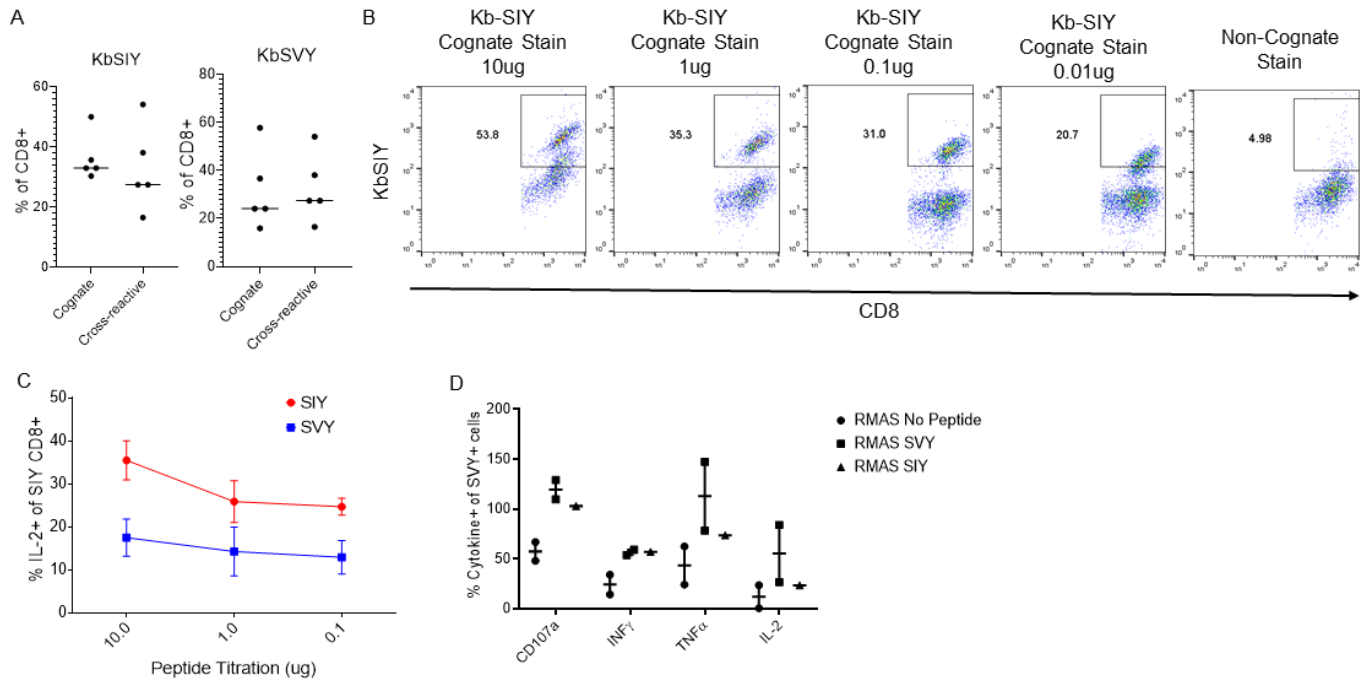

Figure S7.

Cross-reactivity of the endogenous KbSIY and KbSVY stimulated T cells. (A) Jackson C57BL/6 mice CD8+T cells isolated and E&E with KbSIY or KbSVY and α-CD28 aAPCs for 7 days. CD8+ T cells stained and run on flow cytometry with 1ug pMHC-Ig, live/dead, CD8+ T cells. Antigen specificity frequency measured by subtracting non-cognate stain from frequency in the antigen specific gate, and gated on live, CD8+ T cells. All cognate and cross-reactive stains analyzed from the same T cell expansions. N=7 for KbSIY E&E, N=5 for KbSVY E&E. There is no statistical difference between cognate and cross-reactive stain or KbSIY and KbSVY expansion from Jackson mice through pair-wise student t-test. (B) B6 CD8+ T cells are stimulated for 7 days with KbSIY particles and stained with titration of KbSIY-Ig or KbSVY-Ig as well as Non-cognate pMHC-Ig to measure decrease in antigen specificity staining ability. Since the population loses MHC-Ig binding ability the T cells can be measured for peptide-MHC TCR binding to cognate or cross-reactive antigen. (C) Day 7 KbSIY expanded T cells via aAPCs were stimulated with RMA-S pulsed with titrating amounts of peptide and IL-2 expression

measured by intercellular cytokine staining and flow cytometry, cytokine production calculated by subtracting the no stimulation control. N=3 Not statistically significant by 2-way ANOVA with Bonferroni post-test for multiple comparisons. (D) B6 CD8+ T cells stimulated with KbSVY particles, harvested on D7 and re-challenged with RMA-S cells pulsed with SIY, SVY or no peptide and measured for multiple cytokine release. KbSVY CD8+ effector cell can functionally respond to cognate and cross-reactive antigens. Data trends toward no difference. N=2. Data represents mean.

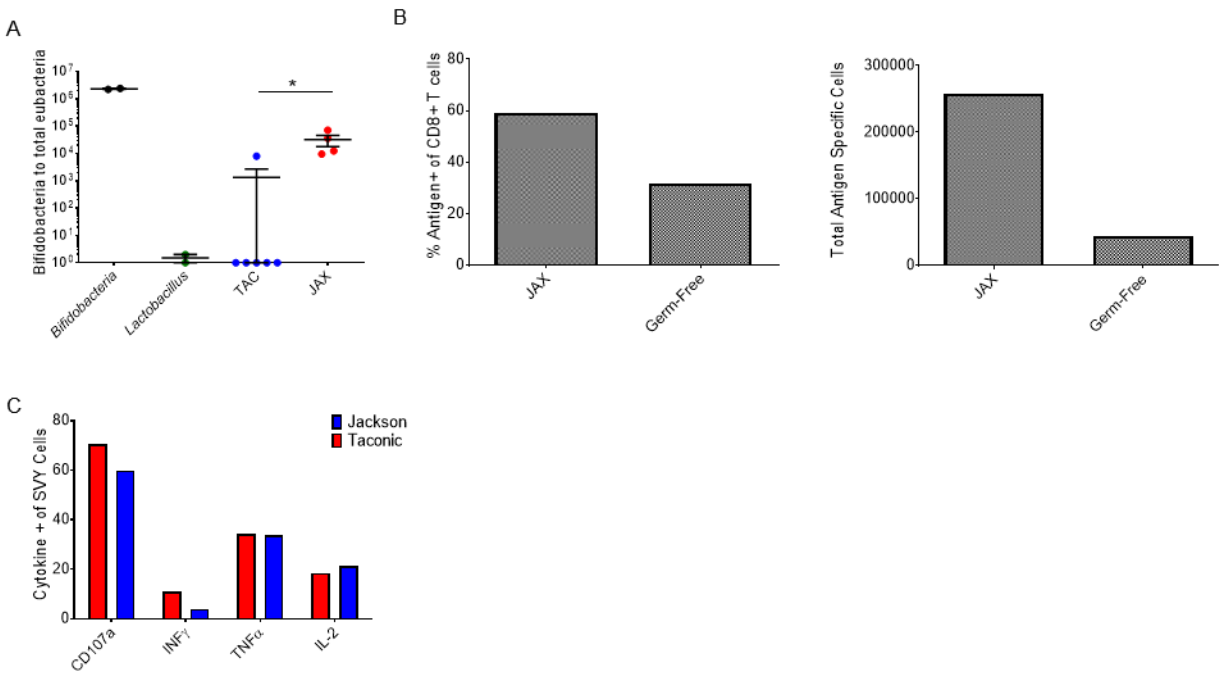

Figure S8

(A) qPCR of *Bifidobacterium* sequence from genomic DNA collected from probiotic capsules (*Bifidobacterium* and *Lactobacillus*) or murine fecal material from B6 mice housed at Taconic or Jackson facilities. P-value = 0.0265, significance measured by unpaired, 2-tailed T-test. N=4 mice/group. Data represents mean ± SEM. (B) Splenic cells from Jackson and Germ-free Mouse enriched and expanded with Kb-SVY aAPCs and stimulated for 7 days, and specificity assessed by frequency of CD8+ antigen staining subtracting non-cognate staining. N=1. (C) CD8+ T cells from Jackson and Taconic mice were stimulated for KbSVY and harvest on D7 and challenged with RMA-S cells pulsed with SVY peptide and measured for cytokine response. Jackson (blue) and Taconic (red) effector KbSVY response are similar in functional ability. N=1.

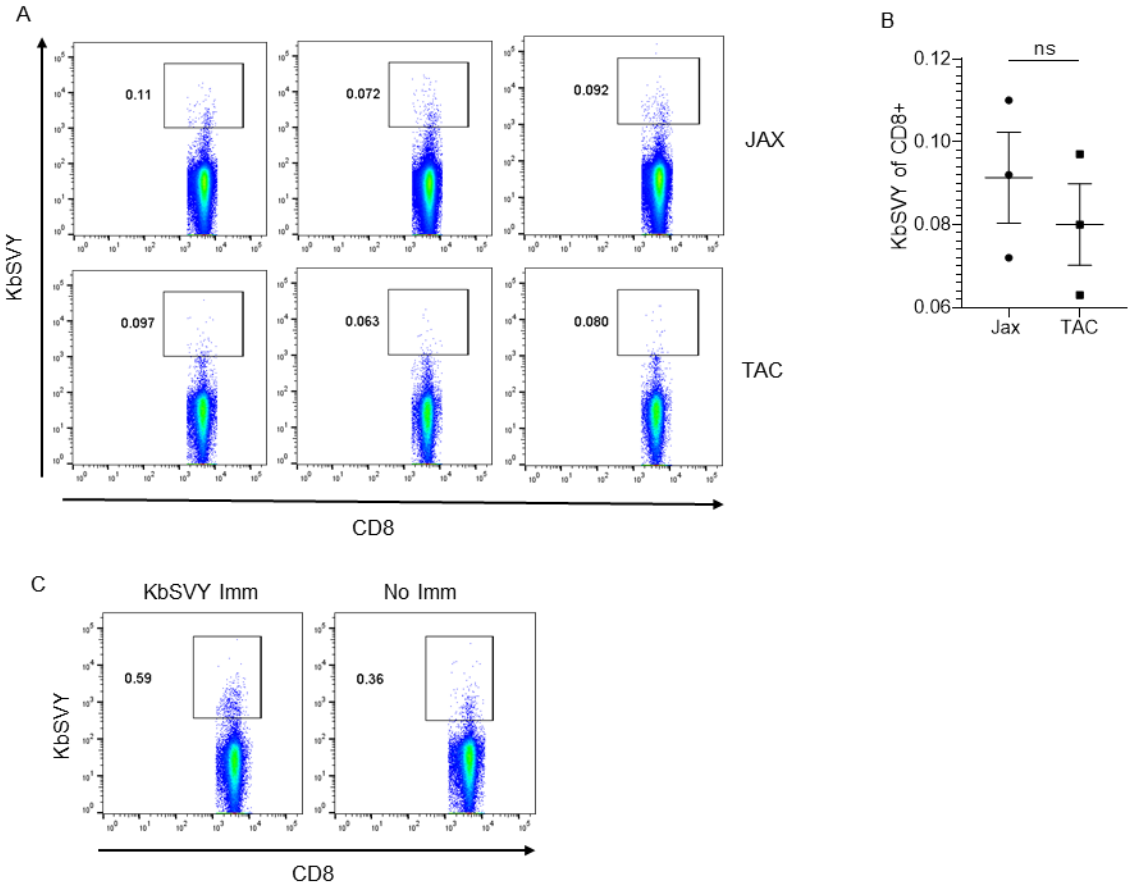

Figure S9

(A). Jackson and Taconic B6 mice splenocytes were isolated and stained with Live/Dead, CD8+ T cells, and 1ug of Kb-SVY dimer. Shown is the flow cytometry for 3 individual mice, cells gated on Live, CD8+T cells. (B). Summary of KbSVY+ CD8+ precursors in Jackson and Taconic splenocytes. (B) Representative flow cytometry for KbSVY staining in KbSVY immunized or non-immunized Taconic mice, 8 days after immunization. Experimental groups = 3/No Immunized, 6/KbSVY Immunized. Cells gated on Live, CD8+T cells.

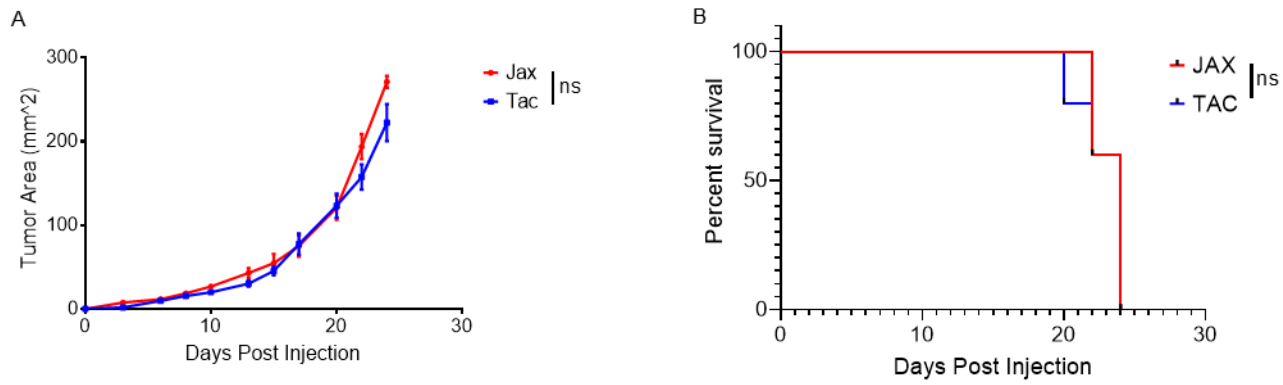

Figure S10

Jackson and Taconic B6 mice were injected subcutaneously with B16.F10 (3x105 cells) in the flank and measured for tumor growth with calipers over time and for overall survival. N=5/group for B16.F10 experiment. B16F10 repeated twice with similar results. Error bars for SEM.

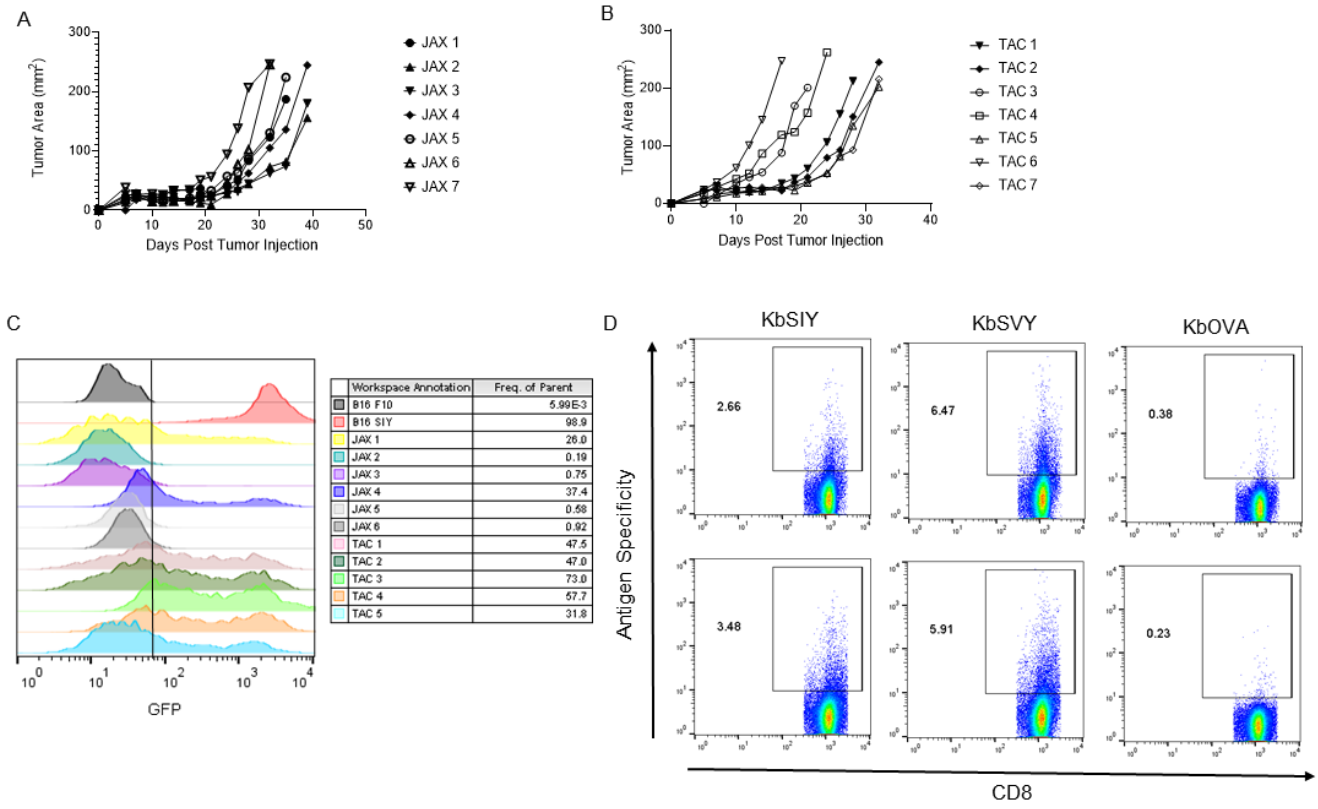

Figure S11

(A) Jackson C57BL/6 mice (n= 7) were injected with 2x106 B16.SIY cells subcutaneously on Day 0. Individual mouse tumor growth curves displayed. (B) Taconic C57BL/6 mice (n=7) were injected with 2x106 B16.SIY cells subcutaneously on Day 0. Individual mouse tumor growth curves displayed. (C) Jackson and Taconic C57BL/6 mice were injected with 2x106 B16.SIY cells subcutaneously on Day 0. Tumors were harvested at an approximate size of 200-250 mm2 and analyzed by flow cytometry for GFP expression. Histograms of all tumors displayed along with the percent GFP+ compared to B16F10 tumor cell line or the original B16.SIY tumor cell line injected into animals on Day 0. (D) Representative flow cytometry from Jackson and Taconic C57BL/6 mice injected with 2x106 B16.SIY cells subcutaneously on Day 0. Tumor infiltrating lymphocytes were harvested on Day 24 and analyzed by flow cytometry for CD8+ T cell specificity, gated on live, CD8+T cells.

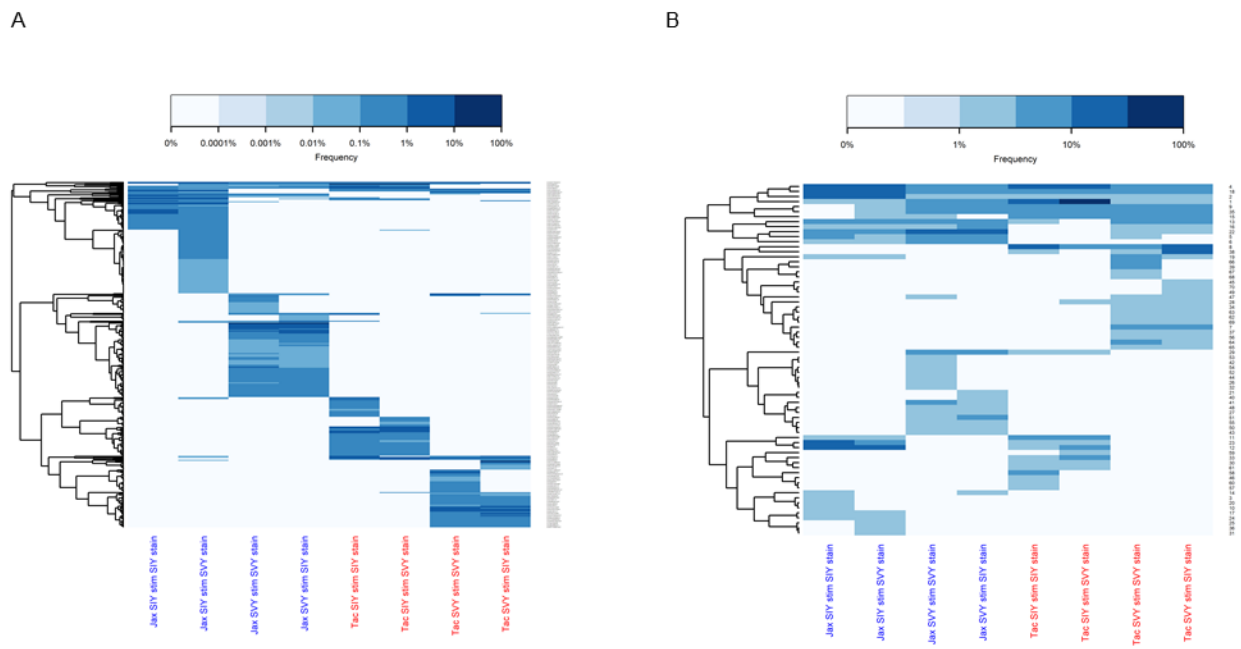

Figure S12.

Comparisons of all TCR repertoires. (A) Exact TCR beta chain CDR3 sequence comparison shared between repertoires for all sequenced samples. (B) TCR repertoires are clustered together based off of TCR homology after sequencing alignment. Clusters are then compared between different repertoires and represented in by heat-map to show the differential expression of certain clusters between groups of repertoires. TCR exact overlap and TCR homology show four pairings of T cell repertoires: Jackson SIY stimulated, Jackson SVY stimulated, Taconic SIY stimulated, and Taconic SVY stimulated.

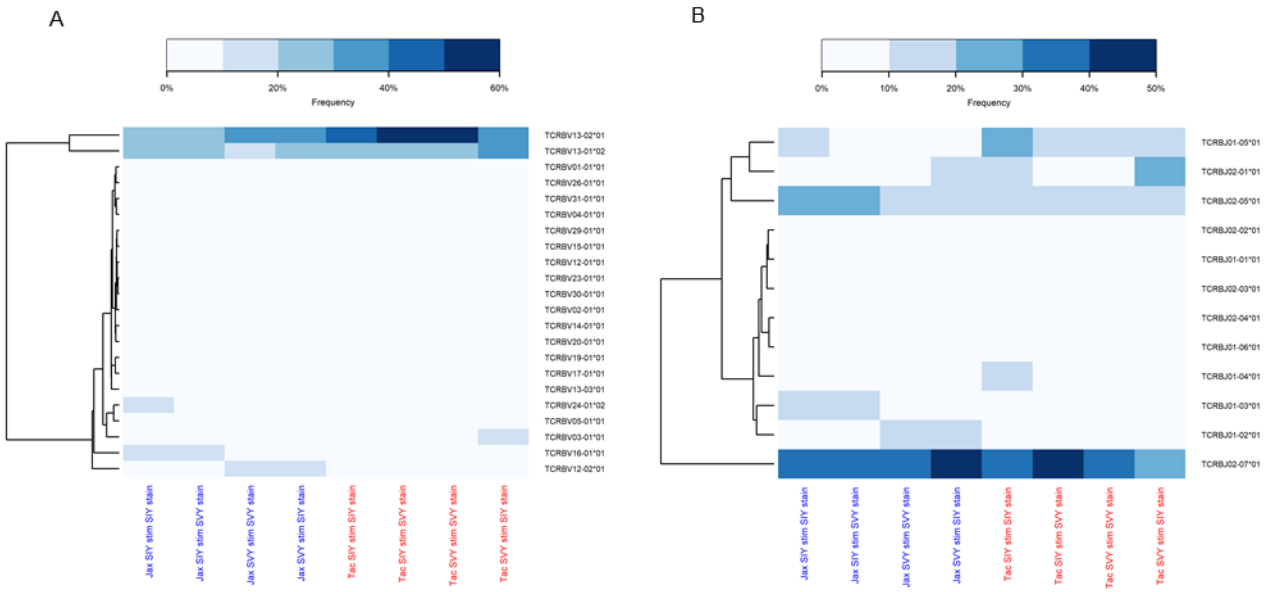

Figure S13.

V and J gene allele expression in the KbSIY and KbSVY repertoires. (A-B) V and J Gene Alleles selection for SIY and SVY stimulated and sorted T cell populations. T cell clones segmented into V and J gene alleles (A and B respectively) show distinct pairings of T cell clones based on mouse facility and antigen stimulation.

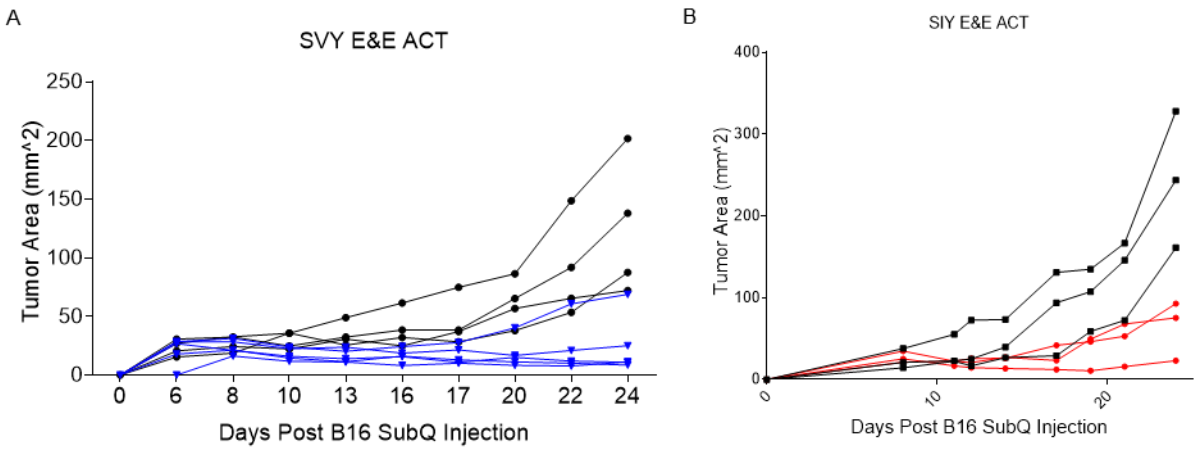

Figure S14

Commensal bacteria reactivity mediates an anti-tumor specific response. (A) Jackson C57BL/6 mice (n= 5 SVY ACT, n = 4 No Treatment) were injected with 2x106 B16.SIY cells subcutaneously on Day 0. Jackson mice CD8 T cells were harvested from spleens and stimulated with KbSVY/anti-CD28 nanoparticles intravenously injected 1.3x105 KbSVY-specific CD8+T cells on day 8. Spider plots for the individual mice in each group, Black lines: no treatment, Blue lines: KbSVY ACT. (B). Jackson C57BL/6 mice (n= 3 SIY ACT, n = 3 No Treatment) were injected with 2x106 B16.SIY cells subcutaneously on Day 0. Jackson mice CD8 T cells were harvested from spleens and stimulated with KbSVY/anti-CD28 nanoparticles intravenously injected 1.3x105 KbSVY-specific CD8+T cells on day 8. Spider plots for the individual mice in each group. Black lines: No treatment, Red lines: KbSIY ACT.

sequence	Ser1	Ile2/Val2	Tyr3	Arg4	Tyr5	Tyr6	Gly7	Leu8
RMSD (Å)	2.45	2.94	4.56	3.63	2.18	1.56	2.24	4.32

Table S1.

The RMSD per residue between the SIY and the SVY epitopes. The protein structures were taken from the frame at the center of the highest populated clusters in the MD trajectories. The alpha carbons of H2-Kb and 2C TCR were aligned between two structures, then the RMSD was calculated for each residue between the SIY and the SVY epitopes.

1) Val2 → Ile2 in state 1 (epitope only)

ΔG_{epi}	8.3 (0.3)

2) Val2 → Ile2 in state 2 (H2-Kb/epitope)

Cluster No.	Population	ΔG (kcal/mol)
1	0.62 (259)	8.2 (0.2)
2	0.36 (150)	8.2 (0.1)
3	0.01 (5)	7.7 (0.1)
4	0.005 (2)	6.8 (0.2)
5	0.005 (2)	9.4 (0.3)
$\Delta G_{K_b/epi}$		8.2 (0.2)

3) Val2 → Ile2 in state 3 (H2-Kb/epitope/2C TCR)

Cluster No.	Population	ΔG (kcal/mol)
1	0.65 (324)	7.3 (0.2)
2	0.21 (106)	6.3 (0.1)
3	0.08 (39)	5.8 (0.4)
4	0.04 (22)	5.8 (0.1)
5	0.01 (9)	10.8 (0.3)
$\Delta G_{K_b/epi/TCR}$		7.0 (0.1)

Table S2.

ΔG for the mutation from Val2 to Ile2 is calculated using the FEP method, for the protein complex in three different states: 1) epitope only (ΔG_{epi}), 2) the H2-Kb/epitope ($\Delta G_{K_b/epi}$), and 3) the H2-Kb/epitope/2C TCR complex ($\Delta G_{K_b/epi/TCR}$). ΔG_{epi} is calculated from a single trajectory, while $\Delta G_{K_b/epi}$ and $\Delta G_{K_b/epi/TCR}$ are calculated from five independent runs, each of which are started from the MD frame at the centers of the top five populated clusters. The population and the number of frames (in the parentheses) of the individual runs are shown in the second column, and ΔG and its statistical error (in the parentheses) in the third column. The error in ΔG was estimated by the block average method, by dividing the trajectory into four consecutive blocks at each λ-window. $\Delta G_{K_b/epi}$, $\Delta G_{K_b/epi/TCR}$, and their errors are averaged over the results from the five runs, each of which is weighted by the population of each cluster.

Sample	Unique Clones	Shannon Entropy	Dominant Motifs	Sequences/ Motif	Singular Response	Singular Contribution	TCR Diversity Score	Mean Hamming Distance
Jax SIY stim SIY stain	47	3.48	20	1.95	61.70	70.78	0.51	5.55
Jax SIY stim SVY stain	121	4.12	21	3.76	51.24	73.24	0.50	5.53
Jax SVY stim SVY stain	112	4.02	28	2.89	51.79	74.23	0.54	5.82
Jax SVY stim SIY stain	100	3.85	21	3.00	53.00	73.56	0.54	5.64
Tac SIY stim SIY stain	70	3.65	20	2.60	57.14	73.45	0.52	5.24
Tac SIY stim SVY stain	57	3.28	16	2.50	57.89	78.10	0.51	5.06
Tac SVY stim SVY stain	75	3.86	27	2.26	61.33	78.23	0.53	5.95
Tac SVY stim SVY stain	61	3.57	24	2.25	60.66	80.18	0.53	5.31

Table S3.

Summary of TCR clustering by homology. TCR clusters analyzed based on homology ImmunoMAP algorithm. Each repertoire is described by Shannon Entropy and TCR Diversity Score (based on ImmunoMap algorithm), show little difference between the overall diversity of each T cell population.