

SUPPLEMENTARY INFORMATION DATA

Mice with a diverse human T cell receptor repertoire selected on multiple HLA class I molecules

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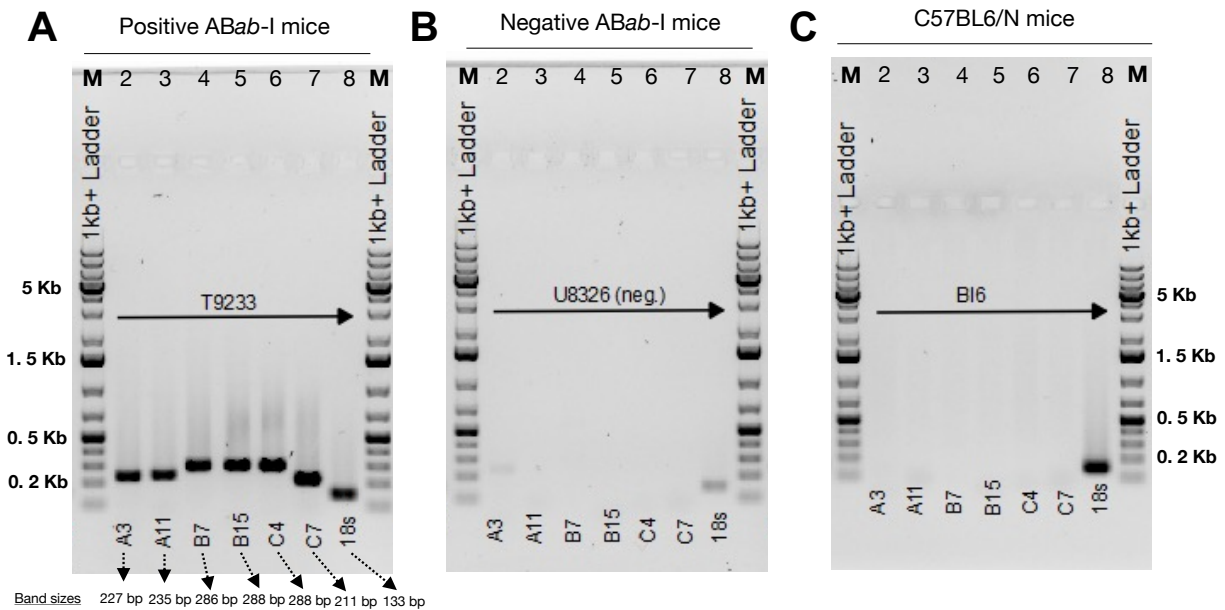
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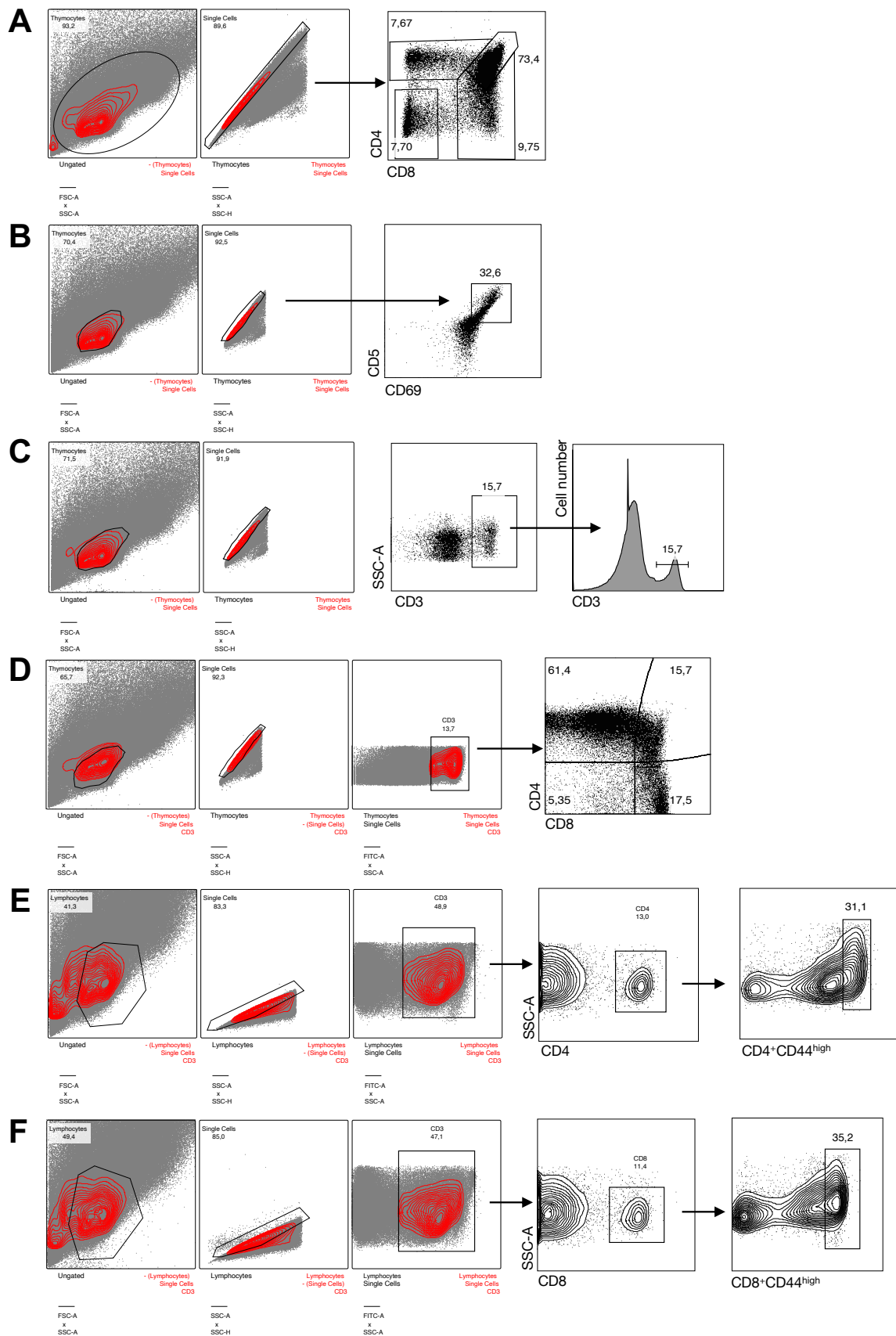
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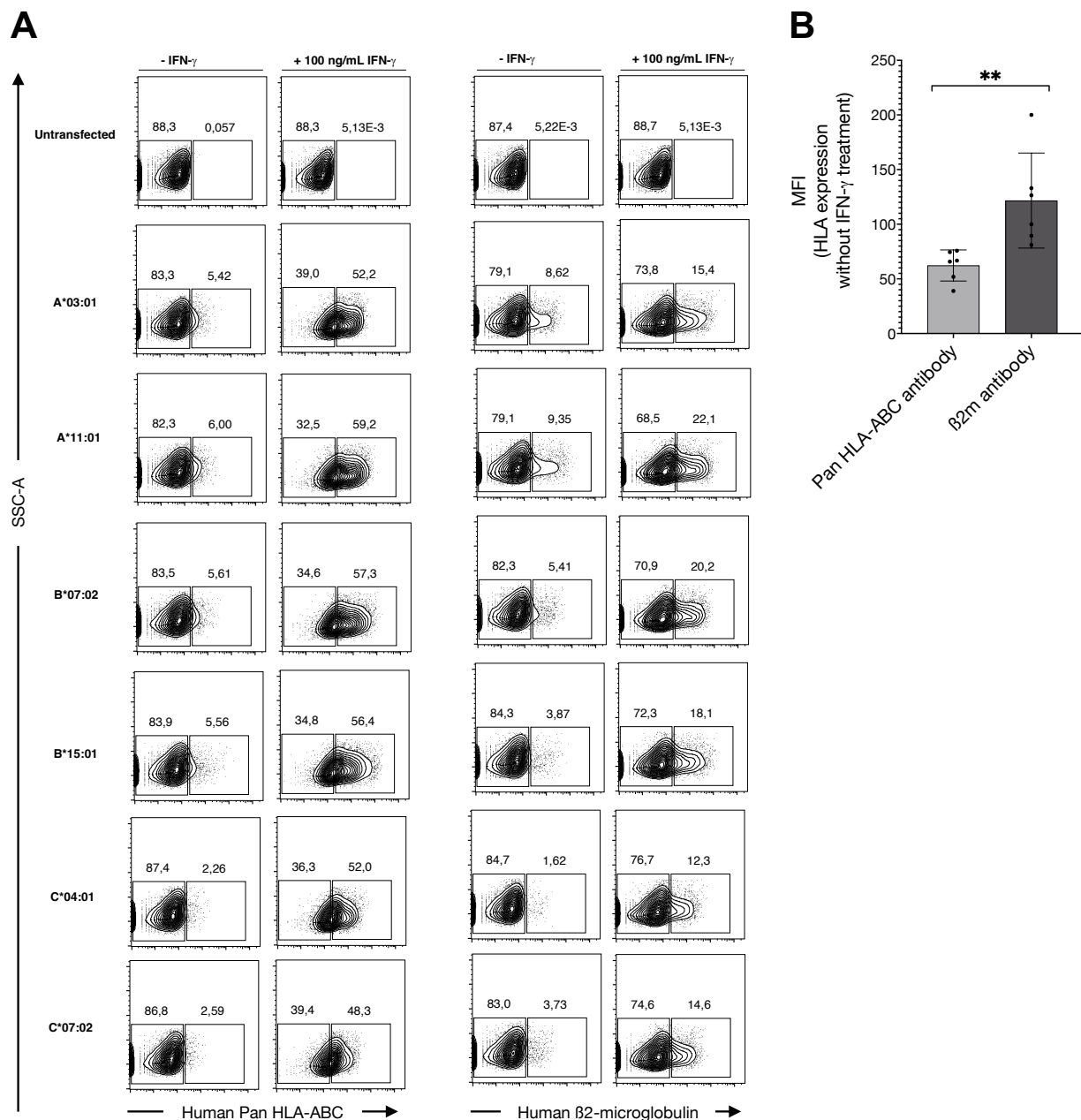


Supplementary Fig. 1. Allele-specific PCR analysis of PiggyBac integrated six-HLA gene cassette in ABab-I mice.

(A) Positive ABab-I mice. PCR-amplified DNA electrophoresis bands show the complete HLA-I haplotype, encompassing six alleles: HLA-A*03:01, -A*11:01, -B*07:02, -B*15:01, -C*04:01, and -C*07:02 (Lanes 2-7), with internal control from a stable housekeeping gene, 18S rRNA (Lane 8). (B) Negative ABab-I mice and (C) C57BL6/N mice. No bands were seen for HLA-I haplotype (Lanes 2-7), but DNA bands were shown for internal control, 18S rRNA reaction (Lane 8). 'M' lanes show O'GeneRuler™ 1 kb plus DNA ladder run. One representative genotyping gel image is shown here from several genotyping assays from over several years of continuous breeding.



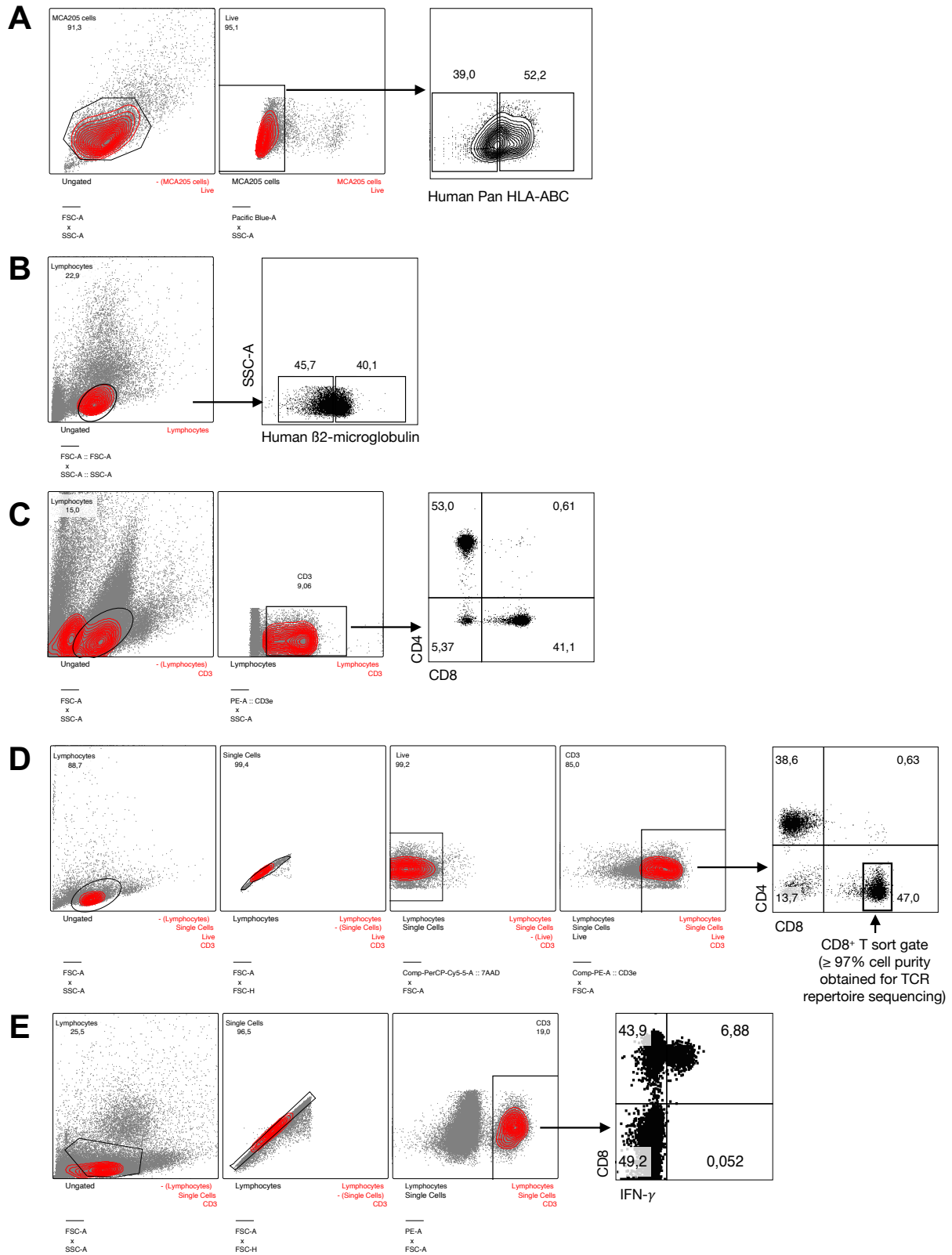
Supplementary Fig. 2: Gating Strategies used for T cell development analysis in ABab-I, ABab-A2, and C57BL6/N mice. (A) Flow cytometry gating strategy to delineate CD4⁺ and CD8⁺ single- and double-positive thymocyte populations, as presented in the first panel of Fig. 2A. **(B)** Gating strategy to define CD5⁺CD69⁺ thymocyte populations, corresponding to the second panel of Fig. 2A. **(C)** Gating strategy to quantify CD3⁺ thymocyte populations, shown in the third panel of Fig. 2A. **(D)** Gating strategy to distinguish CD3⁺CD4⁺ and CD3⁺CD8⁺ single- and double-positive thymocytes, as depicted in the fourth panel of Fig. 2A. **(E)** Gating strategy used to quantify CD4⁺CD44^{high} splenocytes, corresponding to the second panel of Fig. 2D. **(F)** Gating strategy used to quantify CD8⁺CD44^{high} splenocytes, shown in the first panel of Fig. 2D.



Supplementary Fig. 3. Human HLA class I haplotype expression in murine MCA205 cells.

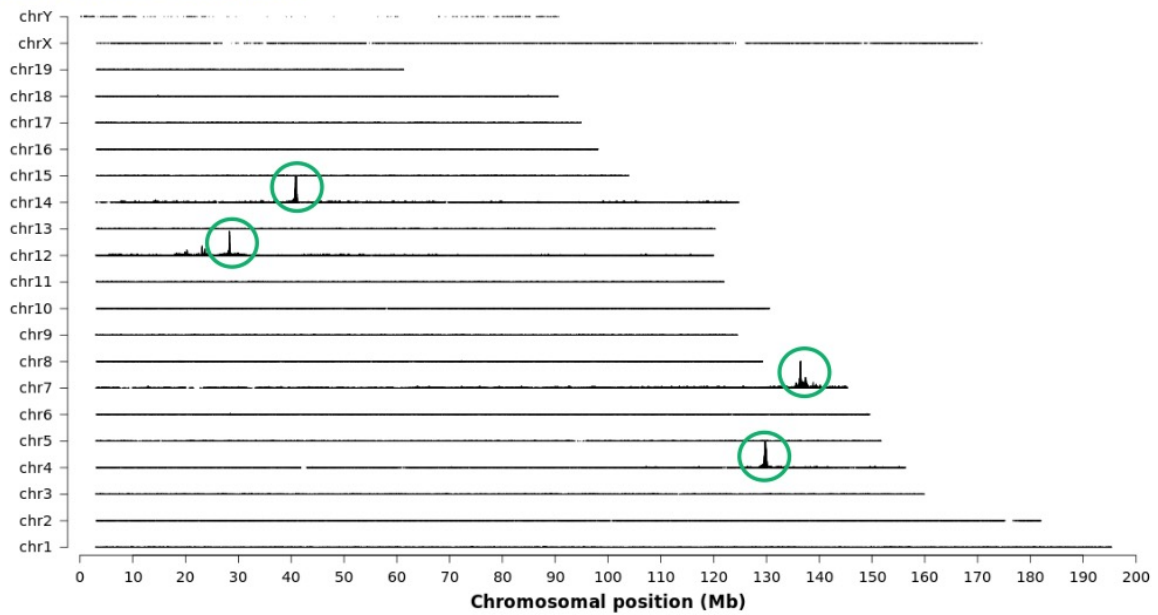
(A) Flow cytometry analysis of HLA expression in MCA205 cells transiently transfected with six individual HLA monochains, stained with pan HLA-ABC and β 2m antibodies, with or without 48 hours of IFN- γ treatment. Gated on live cells. One representative plot from multiple independent experiments is shown ($n = 3$). **(B)** Mean fluorescence intensity (MFI) of HLA expression in transfected MCA205 cells without IFN- γ treatment, stained with pan HLA-ABC or β 2m antibodies ($n = 6$). Each data point represents an independently transfected and analyzed

sample. Statistical comparisons were performed between two groups using a two-tailed unpaired Student's *t*-test. Data in Panel (B) are presented as mean \pm SD: **P < 0.01. Source data are available in the Source Data file.



Supplementary Fig. 4: Gating Strategies used for HLA-I expression analysis, phenotypic characterization of T cells, and functional assessment of CD8⁺ T cell responses in AB*ab*-I mice. (A) Gating strategy used to quantify individual HLA-I monochains in MCA205 cells stained with pan-HLA-ABC or anti-human β 2m antibodies, as shown in Supplementary Fig. 3A. (B) Flow cytometry gating strategy to quantify β 2m-associated HLA-I molecules, as shown in the second panel of Fig. 3A. (C) Gating strategy to identify CD3⁺CD8⁺ and CD3⁺CD4⁺ T cell subsets in peripheral blood, as presented in Fig. 4A and Supplementary Fig. 6A,B. (D) Gating strategy to delineate CD3⁺CD8⁺ and CD3⁺CD4⁺ T cells in lymphoid organs, as shown in Fig. 4E. The same gating strategy was used to sort CD8⁺ T cells at $\geq 97\%$ purity for ImmunoSEQ TCR repertoire sequencing, with deep sequencing data presented in Figs. 5–8 and Supplementary Figs. 7–9. (E) Gating strategy to assess intracellular IFN- γ expression by CD3⁺CD8⁺ T cells in peripheral blood of responder mice, as shown in Fig. 10.

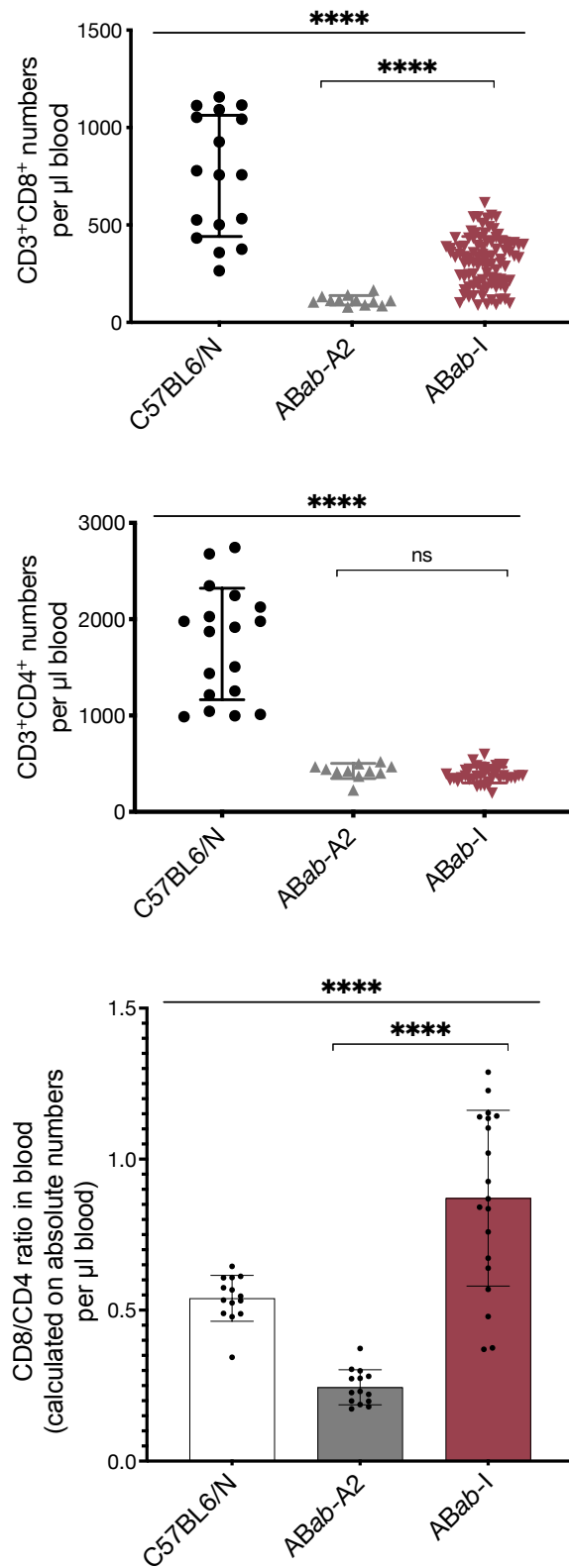
Whole genome coverage plot



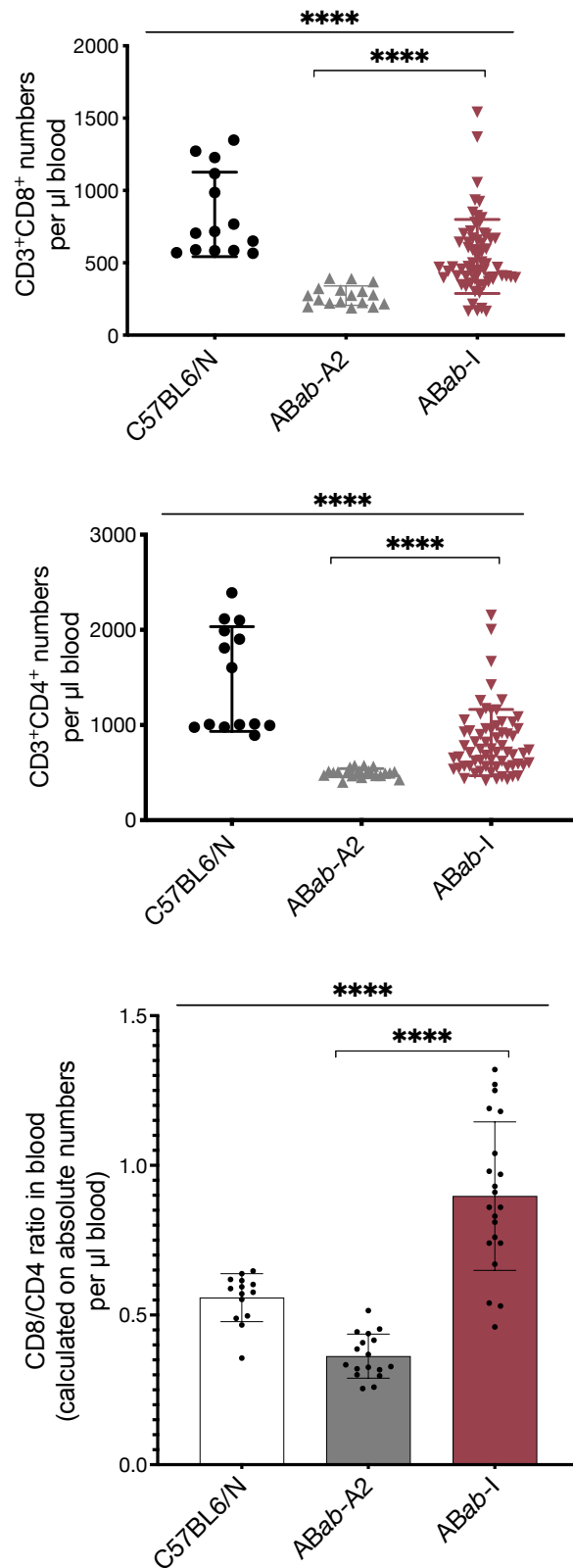
Supplementary Fig. 5. TLA analysis of PiggyBac integrated six-HLA gene cassette in *ABab-I* mice.

Overview of total coverage of TLA sequencing data showing 4 integration sites of the transgene cassette. Chromosome-specific sequencing performed using genomic DNA from the lymphoid organs of F5 generation *ABab-I* mice. 4 integration sites include Chr4, Chr7, Chr 12 and Chr 14.

A F6-F8 generation mice



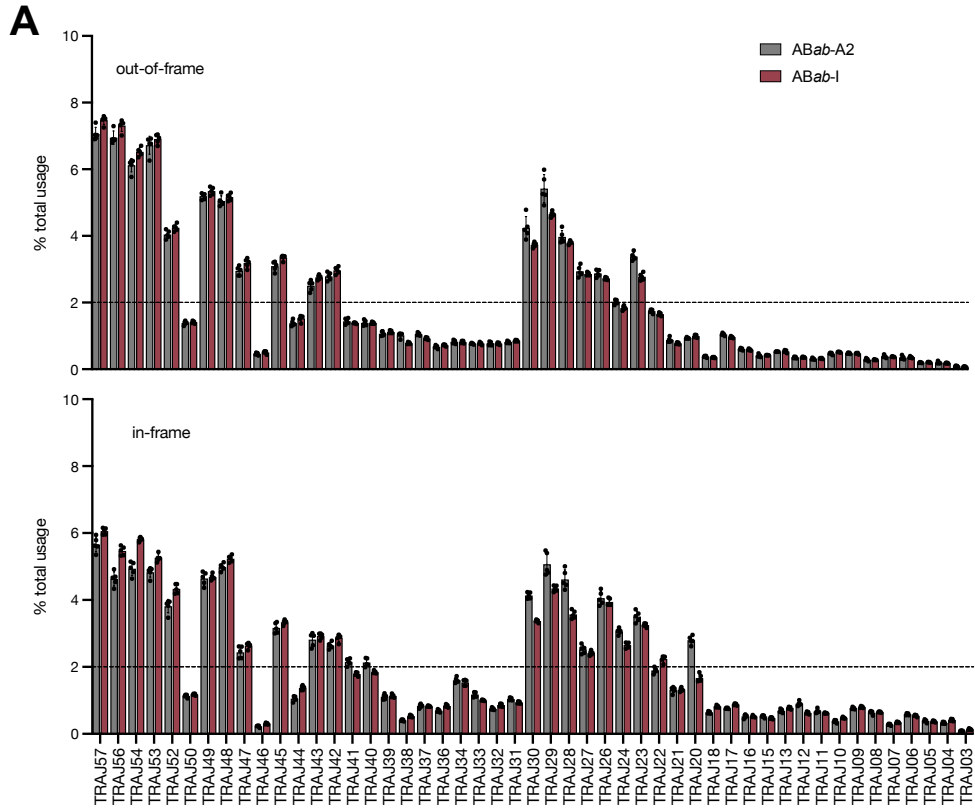
B F8-F10 generation mice



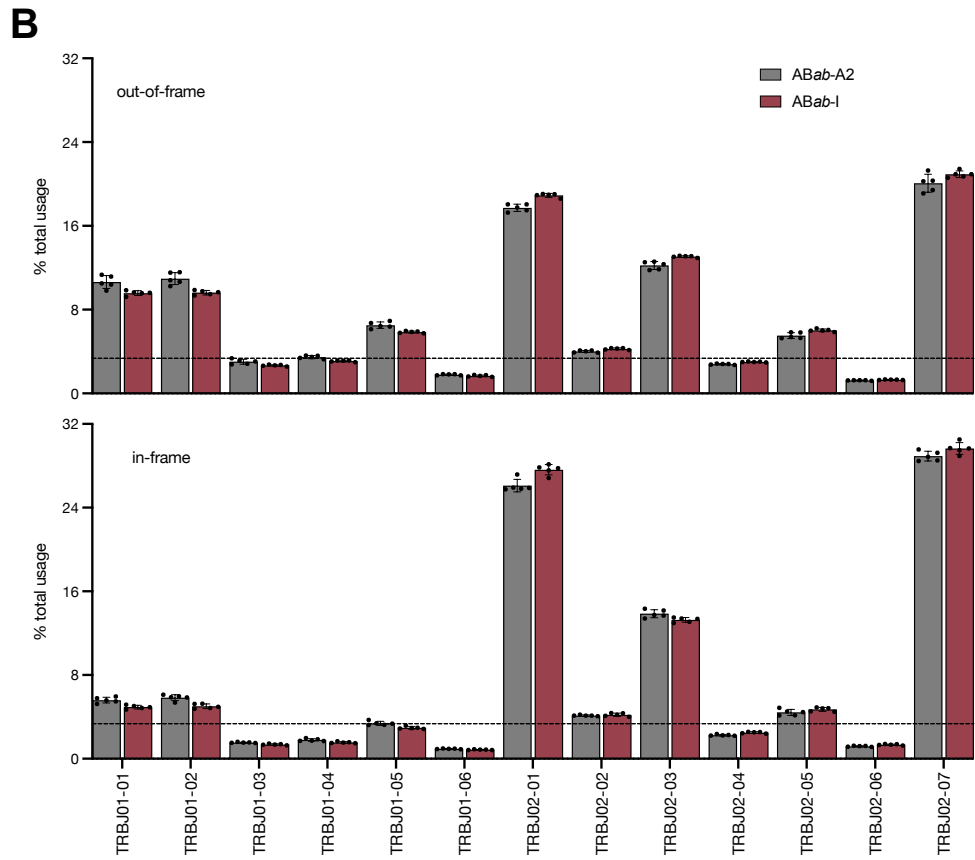
Supplementary Fig. 6. Absolute T cell counts in F6-F10 generation ABab-I mice versus ABab-A2 mice in comparison to C57BL6/N.

(A) ABab-I mice from generations F6 through F8 were monitored for absolute CD3⁺ CD8⁺ and CD4⁺ T cell numbers/ μ l in peripheral blood with the CD8/CD4 ratio. (B) ABab-I mice from generations F8 through F10 were monitored for absolute CD3⁺ CD8⁺ and CD4⁺ T cell numbers/ μ l in peripheral blood with the CD8/CD4 ratio. Summarized data include C57BL6/N, ABab-A2, and ABab-I mice (n > 10 per group). Each data point represents an individual mouse. Data are presented as mean \pm SD. Overall statistical group differences by comparing multiple groups were assessed by one-way ANOVA, followed by pairwise comparisons using two-tailed unpaired Student's *t*-test. P values indicate: ****P < 0.0001; ns, not significant. Exact P values and source data are available in the Source Data file.

TRAJ



TRBJ



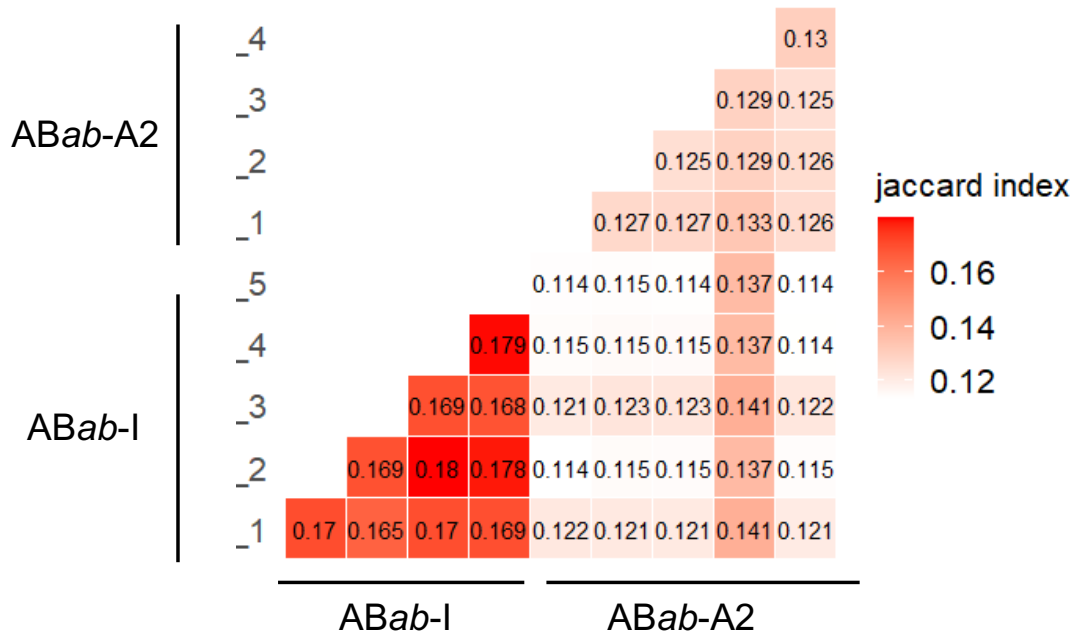
Supplementary Fig. 7. J gene usages in the α and β repertoire of ABab-I and ABab-A2 mice.

Frequencies of J $_{\alpha}$ (**A**) and J $_{\beta}$ (**B**) gene usages of unique TCR $\alpha\beta$ clonotypes in CD8⁺ T cells of ABab-A2 and ABab-I mice. The dotted line represents the frequency of random J $_{\alpha\beta}$ gene usage (α , 2.3%; β , 2.1%). Arrangement of J $_{\alpha}$ and J $_{\beta}$ gene segments mentioned on the x -axis according to their position on the human chromosome from 5' to 3'. 1st top lane: out-of-frame and 2nd bottom lane: in-frame frequencies. Data are from ABab-A2 (n = 5) and ABab-I (n = 5) mice. Data are presented as mean \pm SD. Source data are available in the Source Data file.

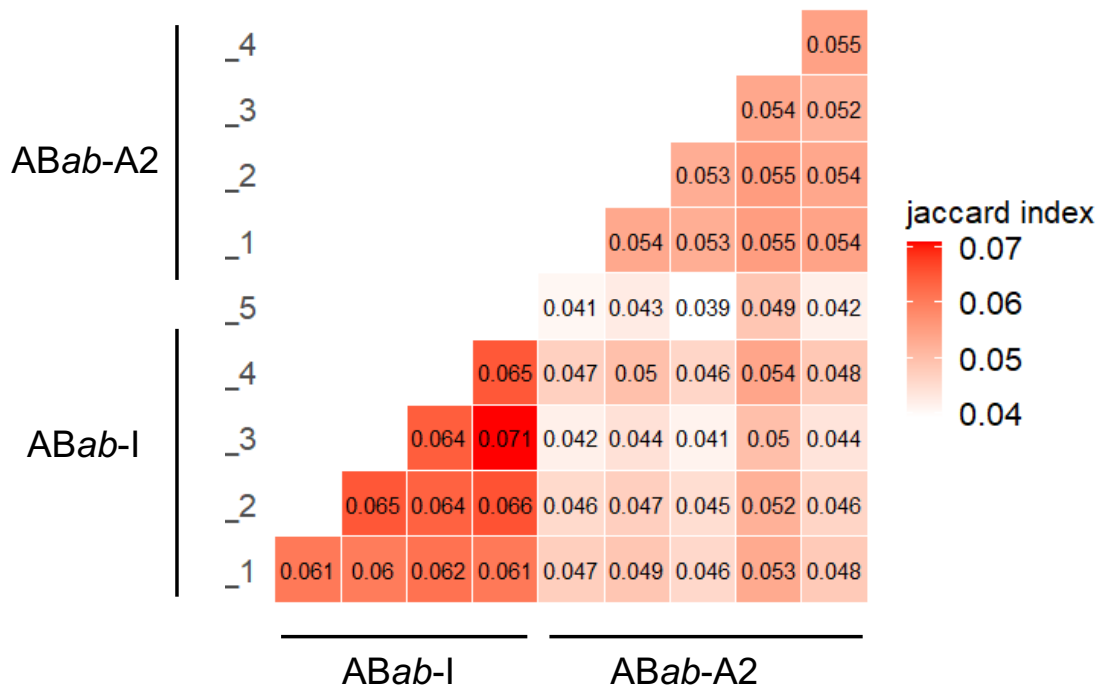
Supplementary Fig. 8. V-J pairing frequencies of TCR α and β clonotypes in ABab-I and ABab-A2 mice.

Mean frequencies of V_{α} - J_{α} (**A**) and V_{β} - J_{β} (**B**) gene pairings of unique TCR $\alpha\beta$ clonotypes in CD8⁺ T cells of ABab-A2 and ABab-I mice. Arrangement of gene segments (y -axis top to bottom, J and x -axis left to right, V segments) mentioned are according to their position on the human chromosome from 5' to 3'. The heat map arrangement represents frequency strength with red to grey (strongest to least). Top lane: in-frame and bottom lane: out-of-frame frequencies. Data are from ABab-A2 (n = 5) and ABab-I (n = 5) mice.

A

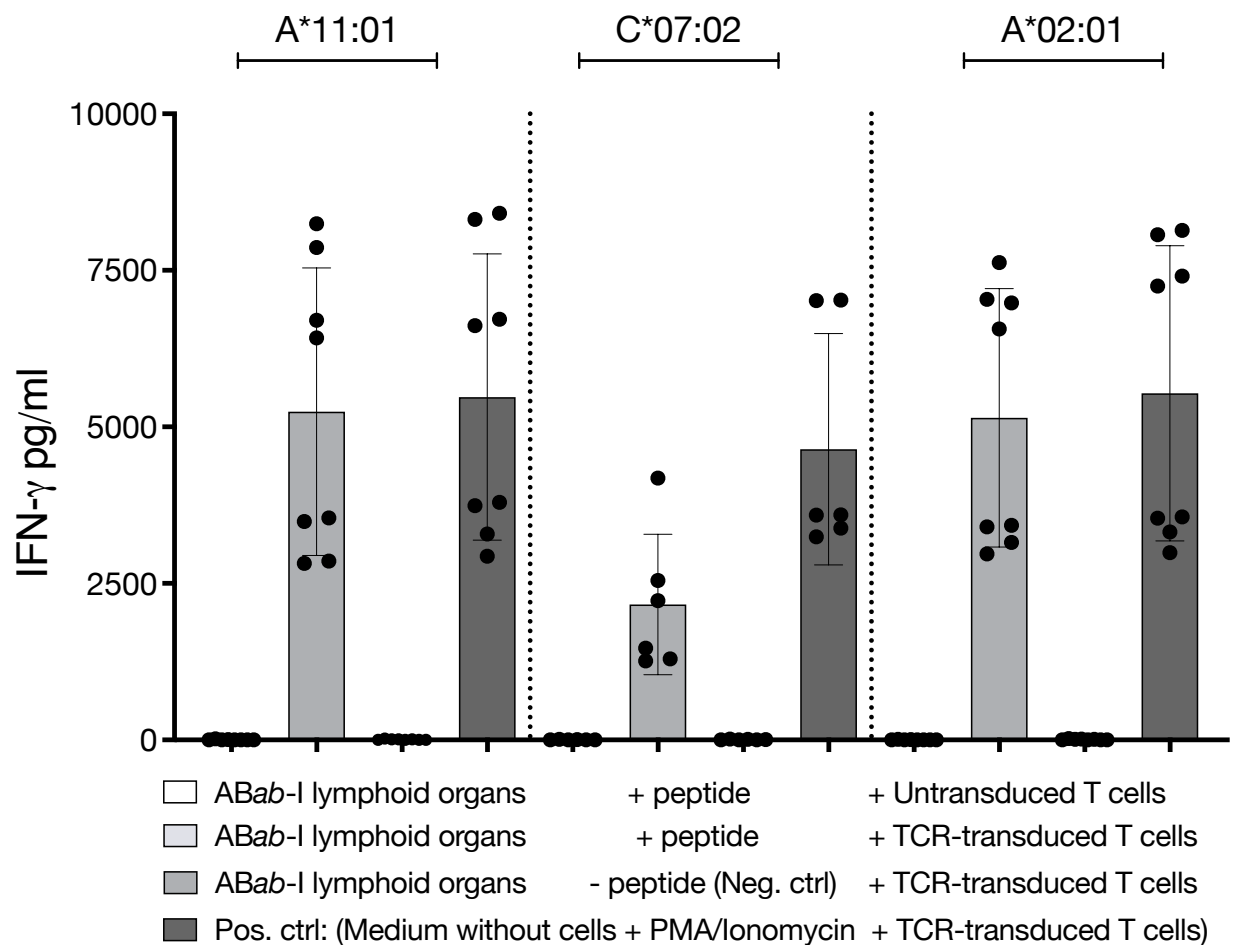


B



Supplementary Fig. 9. Shared clones among ABab-I and ABab-A2 mice.

Heat map representation of Jaccard index score based on the number of shared TCR α (**A**) and TCR β (**B**) clones within and between groups of ABab-A2 and ABab-I mice represented. The heat map arrangement represents frequency strength with red to grey (strongest to least). Five mice per strain were pooled for analysis.



Supplementary Fig. 10. TCR recognition of cognate peptide-bound HLAs in ABab-I mice *ex vivo*.

Ex vivo-isolated lymphoid organs from ABab-I mice were co-cultured with TCR-transduced or untransduced T cells, with or without the addition of cognate peptides. In addition to previously described TCRs, a new TCR specific for the HLA-A2-restricted epitope MAGE-A1₂₇₈ was also tested. Cytokine levels were measured after 16 h of co-culture using a mouse IFN- γ ELISA assay. Consolidated data from multiple TCR co-culture experiments are shown, grouped by HLA restriction as follows: A*11:01, n = 8; C*07:02, n = 6; A*02:01, n = 8. Data are presented as mean \pm SD. Source data are available in the Source Data file.

Supplementary Table 1. TCR α and TCR β diversity with in-frame and out-of-frame clonotypes.

The total numbers of in-frame and out-of-frame clonotypes detected in α and β deep sequencing among ABab-A2 and ABab-I mice with valid total reads. nt, nucleotide, and aa, amino acid clonotypes.

TCR-α	Nr. of inframe seq. (nt)	Nr. of inframe seq. (aa)	% inframe aa/inframe nt	Nr. of inframe (nt) (% of total Nr. (nt))	Nr. of out-of-frame (nt)	Total reads
ABab-A2	53319	46900	0.879611	0.38446	196246	362147
	56107	49367	0.879872	0.392271	240095	389542
	54829	48554	0.885553	0.37694	201305	339300
	77765	67065	0.862406	0.382807	253578	433140
	54534	48275	0.885228	0.382079	255208	366840
ABab-I	122573	102394	0.835372	0.398108	80612	822804
	154974	126692	0.817505	0.389378	87987	821040
	121786	101915	0.836837	0.383817	88023	674599
	157279	128529	0.817204	0.381586	126029	913362
	157803	128726	0.815739	0.38589	86786	903633

TCR-β	Nr. of inframe seq. (nt)	Nr. of inframe seq. (aa)	% inframe aa/inframe nt	Nr. of inframe (nt) (% of total Nr. (nt))	Nr. of out-of-frame (nt)	Total reads
ABab-A2	46197	42897	0.92856679	0.477582161	50534	207469
	52844	49075	0.92867686	0.450207451	64533	270263
	42987	40072	0.932188801	0.466610946	49139	201766
	67441	61911	0.918002402	0.460162801	79118	286749
	49214	45742	0.929450969	0.472094853	55032	253233

TCR-β	Nr. of inframe seq. (nt)	Nr. of inframe seq. (aa)	% inframe aa/inframe nt	Nr. of inframe (nt) (% of total Nr. (nt))	Nr. of out-of- frame (nt)	Total reads
ABab-I	90804	83512	0.919695168	0.463775518	104989	386697
	112554	102863	0.913899106	0.465901988	129029	420213
	139591	126336	0.905044021	0.475235761	154139	400974
	105319	96018	0.91168735	0.471766318	117925	369917
	170114	152425	0.896016789	0.481303969	183330	446095

Supplementary Table 2. Genotyping PCR primers used for genomic confirmation of all six HLA-I alleles in ABab-I mice.

Primers	Sequence
Primers used for HLA-A*03:01 PCR reaction (product length - 227 bp)	
GP-A3-1	ACCGACAGAGTGGACCTGG
GP-A3-2	TCCCACTTTCTCTTGGTGATCTGG
Primers used for HLA-A*11:01 PCR reaction (product length - 235 bp)	
GP-A11-3	CAAAGCTCAGTCCCAGACAGAC
GP-A11-4	CCGCTTTGTAATCTGAGCAGC
Primers used for HLA-B*07:02 PCR reaction (product length - 286 bp)	
GP-B7-5	CCCAGATCTACAAGGCTCAGGCC
GP-B7-6	TAGGCCCTTCTCTGCTCAGC
Primers used for HLA-B*15:01 PCR reaction (product length - 288 bp)	
GP-B15-7	CAACACCCAGACATACAGAGAGTCC
GP-B15-8	ACGCACAGTCCTTCCAGATAGG
Primers used for HLA-C*04:01 PCR reaction (product length - 288 bp)	
GP-C4-9	CCCAGAAGTACAAGAGACAGGCC
GP-C4-10	GATAGGCTCTGCGCTGTTCG
Primers used for HLA-C*07:02 PCR reaction (product length - 211 bp)	
GP-C7-11	GAACCTAGATTCATCAGCGTCGG
GP-C7-12	CGGACTGGTTATAGTAGCCTCTGAG
Primers used for 18s rRNA - control PCR reaction (product length - 133 bp)	
18s rRNA For	GGCCGTTCTTAGTTGGTGGAGCG
18s rRNA Rev	CTGAACGCCACTTGTCCCTC

Supplementary Table 3. Antibodies used for HLA-I expression analysis and T cell characterization assays.

Antibody	Catalog Number	Clone	Dilution	Application in this study	Validation in Peer-Reviewed Articles
Anti-mouse CD16/32 Fc receptor block	BioLegend, 101320	93	1:100	Fc receptor block for T cell staining	Poncette et al., 2019, J Clin Invest.
Anti-mouse CD28 (unconjugated for cell culture)	BioLegend, 102116	37.51	0.1 µg/ml	Used in T cell activation assays	Leisegang et al., 2016, Clin Cancer Res.; Engels et al., 2012, Mol Ther
Anti-mouse CD3 (unconjugated for cell culture)	BioLegend, 100340	OKT-3	1 µg/ml	T cell activation for transduction	Leisegang et al., 2016, Clin Cancer Res.; Engels et al., 2012, Mol Ther
Anti-mouse CD3ε (PE)	BioLegend, 100308	145-2C11	1:200	For intracellular staining of T cells	Obenaus et al., 2015, Nat Biotechnol.
Anti-mouse CD8α (APC)	BioLegend, 100712	53-6.7	1:200	For flow cytometry of CD8+ T cells	Obenaus et al., 2015, Nat Biotechnol.
Anti-mouse IFN-γ (BV421)	BioLegend, 505830	XMG1.2	1:200	For IFN-γ cytokine detection in T cells	Obenaus et al., 2015, Nat Biotechnol.
Anti-mouse CD4 (BV421)	BioLegend, 100438	GK1.5	1:200	For T cell subset characterization	Chen et al., 2017, J Exp Med.

Antibody	Catalog Number	Clone	Dilution	Application in this study	Validation in Peer-Reviewed Articles
Anti-mouse CD44 (FITC)	BioLegend, 156007	NIMR8	1:200	For T cell activation and memory markers	Chen et al., 2017, J Exp Med.
Anti-mouse CD5 (PE/Cy7)	BioLegend, 100621	53-7.3	1:200	For T cell subset analysis	Chen et al., 2017, J Exp Med.
Anti-mouse CD69 (PE)	BioLegend, 104507	H1.2F3	1:200	For early T cell activation marker	Chen et al., 2017, J Exp Med.
Anti-human β 2 microglobulin (PE)	BD Pharmingen, 551337	TÜ99	1:200	For human β 2m detection in flow cytometry	Boucherma et al., 2014, J Immunol.
Anti-human Pan HLA-ABC (PE)	BioLegend, 311406	W6/32	1:100	For human HLA-I expression analysis	Gavvovidis et al., 2018, Clin Cancer Res.; Shields and Ribaldo, 1998, Tissue Antigens)