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Supplemental Information

Distinct Polymorphisms in HLA Class I Molecules Govern Their Susceptibility to Peptide Editing by TAPBPR

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Supplementary information

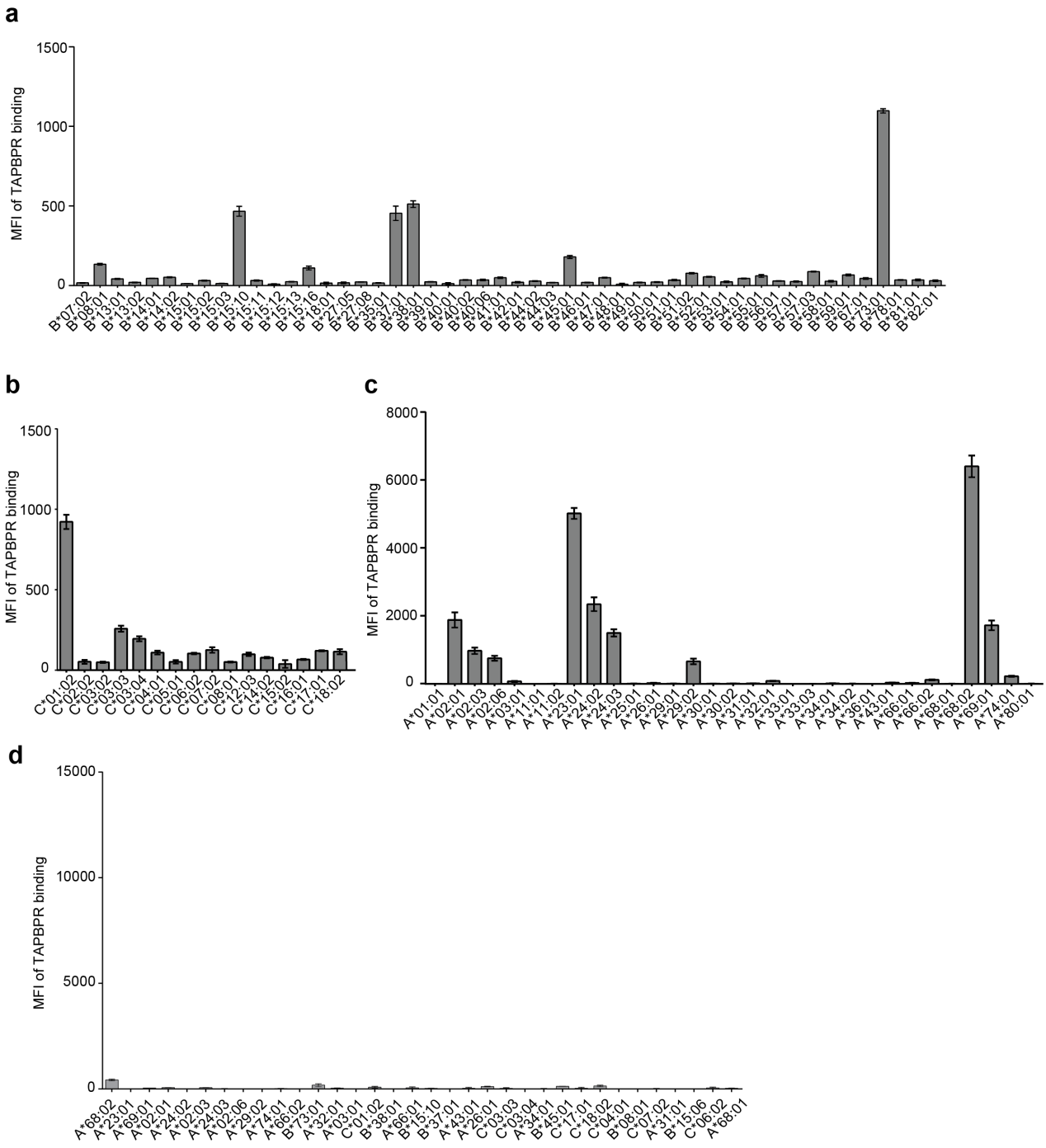

Figure S1. TAPBPR binding to HLA-B and -C molecules on the single HLA beads, Related to Figure 1. Bar graphs showing soluble TAPBPR binding to (**a**) HLA-B and (**b**) HLA-C molecules, upon treatment with 1 μM TAPBPR, to (**c**) HLA-A molecules treated with 100 nM TAPBPR and (**d**) with 1μM TN5 TAPBPR mutant to the top 34 HLA I binders to WT TAPBPR, as shown in **figure 1b**, using the SAB library. The SABs were treated with TAPBPR for 1 h at 22°C. The data including error bars was generated based on triplicates within one experiment. This is a representative example of three independent experiments.

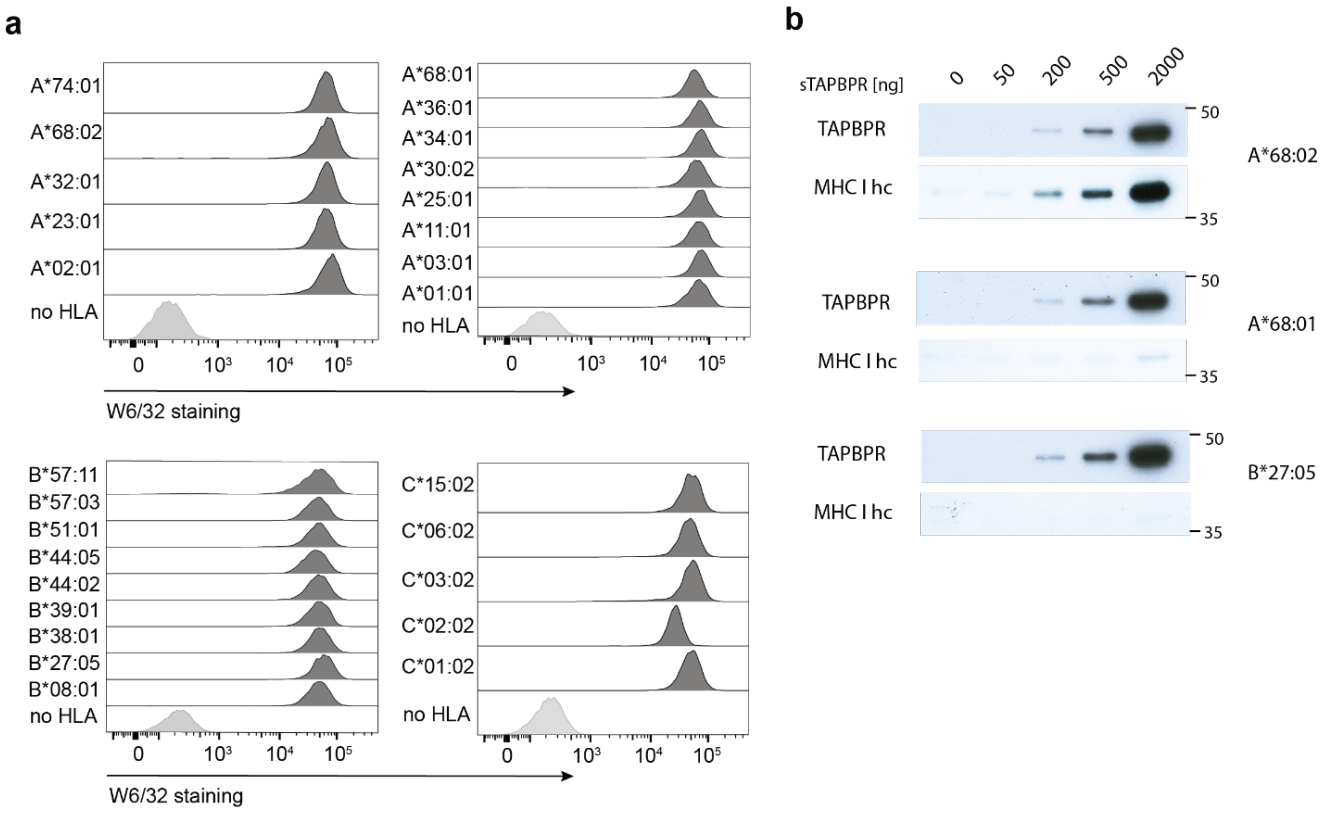

Figure S2. Levels of cell surface MHC class I molecules for individual HLA I alleles, Related to Figure 2 and Figure 3. (**a**) Histograms showing MHC class I levels detected with W6/32 antibody for each HLA I molecule transduced into HeLaM-HLA-ABC^{KO} cells (dark grey filled histograms). A light grey filled histogram was included for non-transduced HeLaM-HLA-ABC^{KO} cells. This is a representative example of three independent experiments. (**b**) Western blot analysis on recombinant TAPBPR pull-downs, when recombinant TAPBPR was titrated, on cells expressing A*68:02, A*68:01 or B*27:05. Membranes were probed for MHC I heavy chain (using HC10) and TAPBPR, as indicated. This is a representative experiment of two independent repeats.

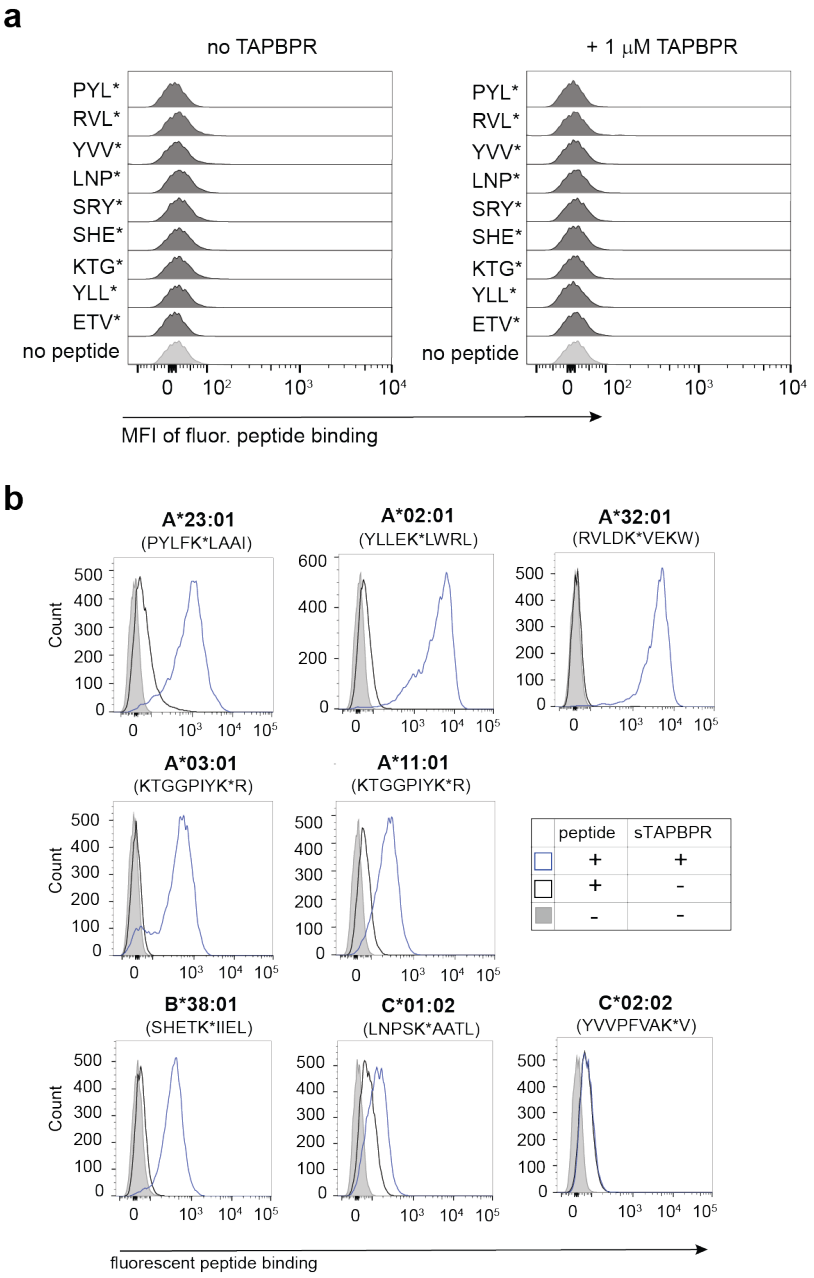

Figure S3. None of the peptides bind to HeLaM cells lacking classical HLA I molecules, Related to Figure 4. (**a)** Histograms showing binding levels of the fluorescent peptides used in **figure 4c** to HeLaM-HLA-ABC^{KO} cells, in the presence (right) or absence (left) of 1 μM TAPBPR. Cells were treated with TAPBPR for 15 min at 37°C, then peptide was added for 1 h at 37°C. The peptides tested were: ETVSK*QSNV (ETV*), YLLEK*LWRL (YLL*), KTGGPIYK*R (KTG*), SHETK*IIEL (SHE*), SRYWK*IRTR (SRY*), LNPSK*AATL (LNP*), YVVPFVAK*V (YVV*), RVLDK*VEKW (RVL*) and PYLFK*LAAI (PYL*). (**b**) Histograms showing the level of fluorescent peptide bound to individual HLA I-expressing cells, additional to the ones depicted in **figure 4b**, either untreated (filled grey line), treated with peptide alone (black line) or with peptide and TAPBPR (blue line). These histograms are representative of three independent experiments.

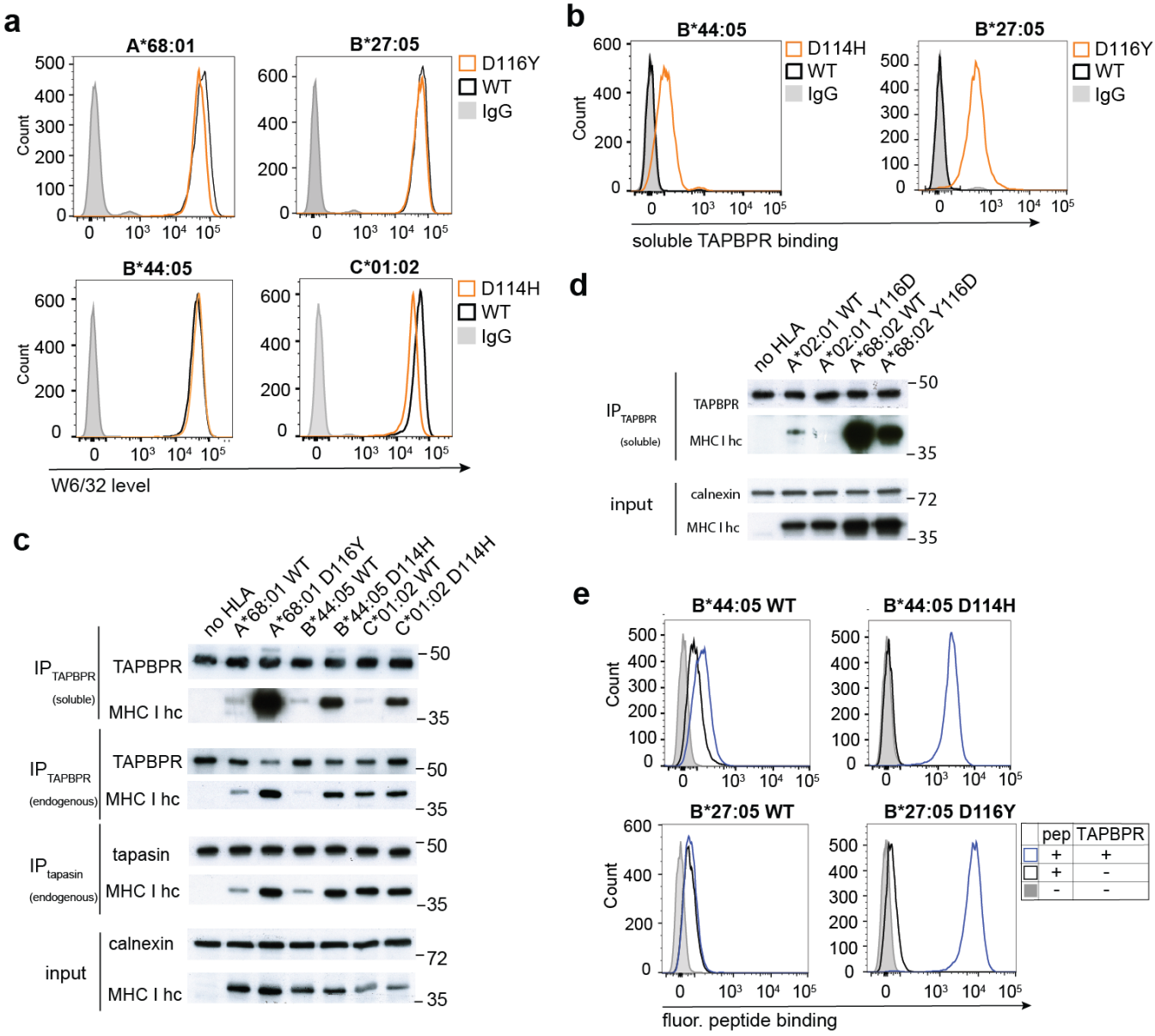

Figure S4. Disrupting the F pocket architecture impairs HLA I binding to TAPBPR, Related to Figure 6. (**a**) Histograms showing surface expression levels of A*68:01, B*27:05, B*44:05 and C*01:02 (black lines), compared to their corresponding F pocket mutants (orange lines). (**b**) Histograms showing the level of bound TAPBPR to cell surface B*44:05 or B*27:05 (black lines), as well as to their corresponding F pocket mutants (orange lines). Untreated cells are included as a negative control (solid grey line). (**c**) Western blot analysis on recombinant TAPBPR pull-downs (top) and endogenous TAPBPR and tapasin immunoprecipitates (bottom), on cells expressing A*68:01^{WT}, A*68:01^{D116Y}, B*44:05^{WT}, B*44:05^{D114H}, C*01:02^{WT} and C*01:02^{D114H} and on (**d**) recombinant TAPBPR pull-downs on cells expressing A*02:01, A*68:02, as well as their Y116D mutants. Membranes were probed for MHC I heavy chain, TAPBPR, tapasin, and calnexin, as indicated. (**e**) Histograms showing the level of bound fluorescent peptides (top) EFGK*AFSF to cells expressing either B*44:05^{WT} or B*44:05^{D114H}, and (bottom) SRYWK*IRTR to cells expressing either B*27:05^{WT} or B*27:05^{D116Y}, when cells were incubated with peptide alone (black line) or with peptide and 1 μM TAPBPR (blue line); an untreated sample was included as a negative control (solid grey line).

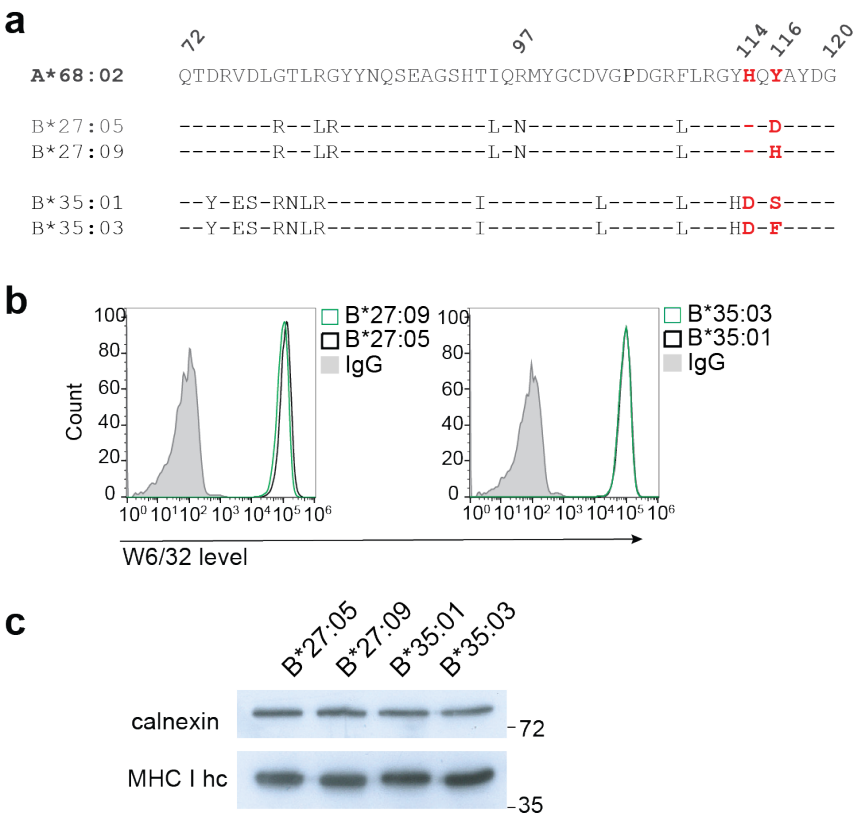

Figure S5. Expression of HLA-B molecules with natural polymorphisms at residue 116, Related to Figure 6. (**a**) Amino acid sequence alignment comparing residues 72-120 of A*68:02 with the HLA I pairs B*27:05 - B*27:09 and B*35:01 - B*35:03; residues 114 and 116 are highlighted in red. (**b**) Histograms showing the surface expression levels of the HLA-B molecules listed in (a), detected with W6/32 antibody. (**c**) Western blot analysis of the expression levels of HLA I molecules in whole cell lysates (a). Membranes were probed for MHC I heavy chain and calnexin, as indicated. These experiments are representative examples of three independent repeats.

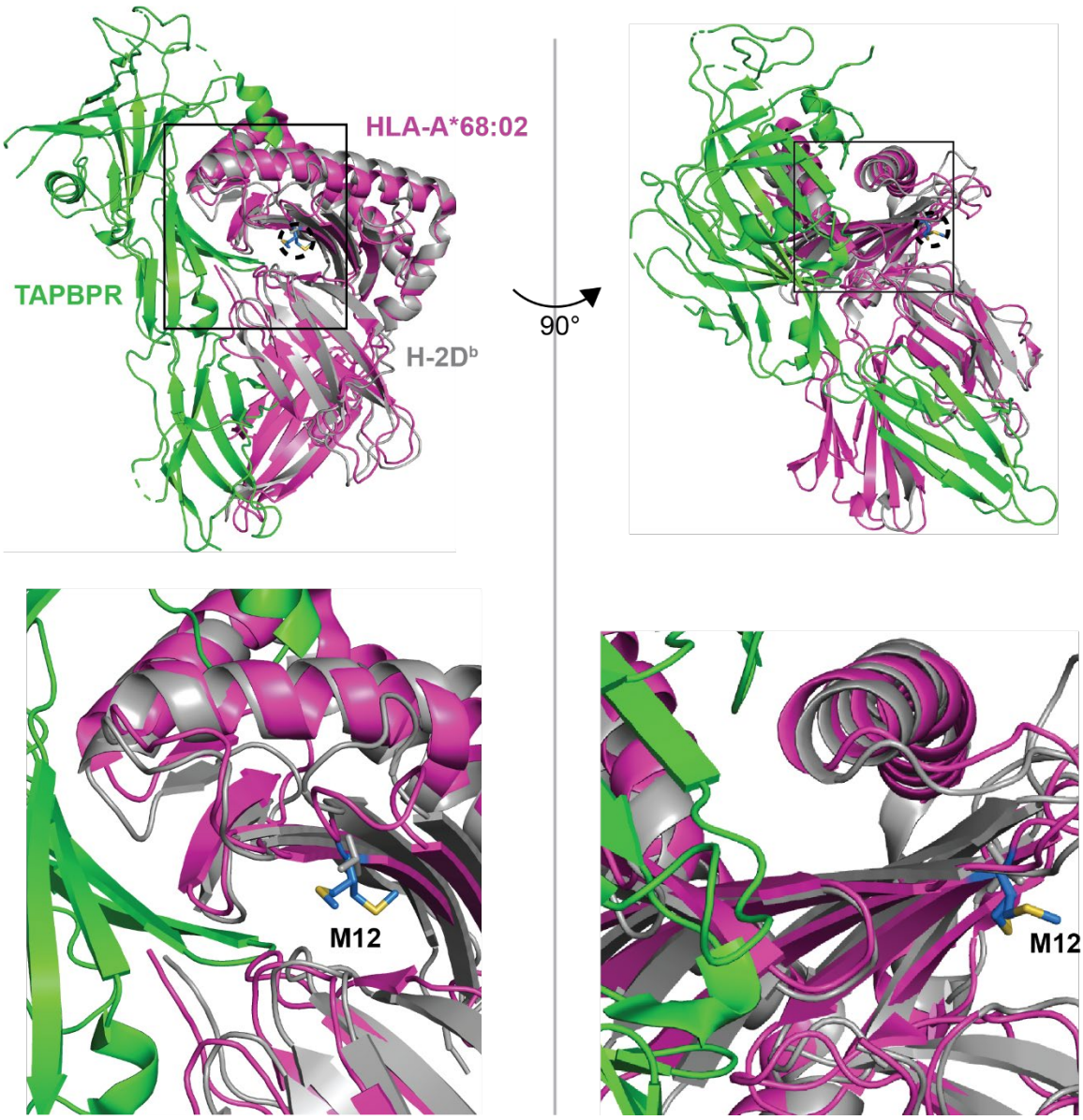

Figure S6. Residue M12 of HLA-A*68:02 does not appear to directly interact with TAPBPR, Related to Figure 7. PyMOL figure of the structure of HLA-A*68:02 folded with peptide SVYDFFVWL (pink) (PDB ID 4HX1) overlaid onto the structure of the H-2D^b:TAPBPR complex (grey and green respectively) (PDB ID 5OPI), depicted from different angles. Residue M12 of HLA-A*68:02 is colored in blue and highlighted in a dotted circle. Magnified selected areas are depicted below the whole structures. Residue M12 was captured in two different orientations in the crystal structure of HLA-A*68:02.

Table S1. MHC class I levels on single antigen HLA beads detected using W6/32, Related to Figure 1.

HLA allele	Bead #	MHC class I levels
A*01:01	3	18527
A*02:01	4	19739
A*02:03	5	18974
A*02:06	6	18702
A*03:01	7	19116
A*11:01	8	18910
A*11:02	9	19415
A*23:01	10	19552
A*24:02	11	19068
A*24:03	12	17692
A*25:01	13	19499
A*26:01	14	18366
A*29:01	15	19014
A*29:02	17	18142
A*30:01	18	17818
A*30:02	19	17921
A*31:01	20	18716
A*32:01	21	19548
A*33:01	22	18132
A*33:03	100	18062
A*34:01	23	19320
A*34:02	24	19845
A*36:01	25	17031
A*43:01	26	18328
A*66:01	27	18555
A*66:02	28	19088
A*68:01	29	19016
A*68:02	30	17506
A*69:01	31	18749
A*74:01	32	19216
A*80:01	33	18535
B*07:02	34	20419
B*08:01	35	20346
B*13:01	97	18064
B*13:02	36	19727
B*14:01	37	19502
B*14:02	38	17233
B*15:01	40	19608
B*15:02	41	19350
B*15:03	42	19668
B*15:10	43	20197
B*15:11	98	17639
B*15:12	44	19294
B*15:13	45	19296
B*15:16	46	18461
B*18:01	47	19915
B*27:05	16	19969
B*27:08	48	19129
B*35:01	49	19066
B*37:01	50	18551
B*38:01	51	18845
B*39:01	52	20136
B*40:01	53	19119
B*40:02	54	19336
B*40:06	99	18638
B*41:01	55	20023
B*42:01	56	20116
B*44:02	57	17659
B*44:03	58	18984
B*45:01	59	19214
B*46:01	63	19173
B*47:01	61	17077
B*48:01	62	19200
B*49:01	60	19506
B*50:01	64	19141
B*51:01	65	19516
B*51:02	66	18795
B*52:01	67	17497
B*53:01	68	18690
B*54:01	69	19465
B*55:01	70	19803
B*56:01	71	19119
B*57:01	72	18956
B*57:03	73	19594
B*58:01	74	18524
B*59:01	75	18090
B*67:01	76	19989
B*73:01	77	19542
B*78:01	78	17716
B*81:01	79	18679
B*82:01	80	17708
C*01:02	81	18988
C*02:02	82	16840
C*03:02	83	18982
C*03:03	84	19000
C*03:04	85	18943
C*04:01	86	12491
C*05:01	87	19122
C*06:02	88	16573
C*07:02	89	18133
C*08:01	90	19692
C*12:03	91	20447
C*14:02	92	17786
C*15:02	93	21276
C*16:01	94	14783
C*17:01	95	14369
C*18:02	96	18683

Table S2. Characterization of HLA-A molecules subjected to TAPBPR binding using the single antigen HLA beads, Related to Figure 5.

HLA allomorph	Supertype	F pocket specificity	Residue 114	Residue 116
A*68:02	**A2**	**Hydrophobic**	**H**	**Y**
A*23:01	**A24**	**Hydrophobic**	**H**	**Y**
A*69:01	**A2**	**Hydrophobic**	**H**	**Y**
A*02:01	**A2**	**Hydrophobic**	**H**	**Y**
A*24:02	**A24**	**Hydrophobic**	**H**	**Y**
A*02:06	**A2**	**Hydrophobic**	**H**	**Y**
A*03:01	A3	Basic	R	D
A*01:01	A1	Aromatic	R	D
A*26:01	A1	Aromatic	Q	D
A*30:01	A3	Basic / Aromatic	E	H
A*30:02	A1	Aromatic	E	H
A*33:01	A3	Basic	Q	D
A*36:01	A3	Basic	R	D
A*66:01	A3	Basic	Q	D
A*68:01	A3	Basic	R	D

- strong TAPBPR binders are highlighted in bold

Table S3: Primers used for cloning the MHC class I mutants, Related to STAR Methods.

Primer name	Sequence 5'-3'
B2705_D116Y_Fwd	GTACCACCAGTACGCCTACG
B2705_D116Y_Rev	CGTAGGCGTACTGGTGGTAC
B4405_D114H_Fwd	CGCGGGTATCATCAGTACGC
B4405_D114H_Rev	GCGTACTGATGATACCCGCG
C0102_D114H_Fwd	GCGGGTATCACCAGTACGC
C0102_D114H_Rev	GCGTACTGGTGATACCCGC
A0201_V12M_Fwd	CACATCCATGTCCCGGCC
A0201_V12M_Rev	GGCCGGGACATGGATGTG
A0201_S105P_Fwd	GACGTGGGGCCGGACTGG
A0201_S105P_Rev	CCAGTCCGGCCCCACGTC
A6802_M12V_Fwd	CTACACTTCCGTGTCCCGGC
A6802_M12V_Rev	GCCGGGACACGGAAGTGTAG
A6802_P105S_Fwd	CGTGGGGTCGGACGGG
A6802_P105S_Rev	CCCGTCCGACCCCACG