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Supplemental information

HLA class-I-peptide stability mediates CD8$^+$ T cell immunodominance hierarchies and facilitates HLA-associated immune control of HIV

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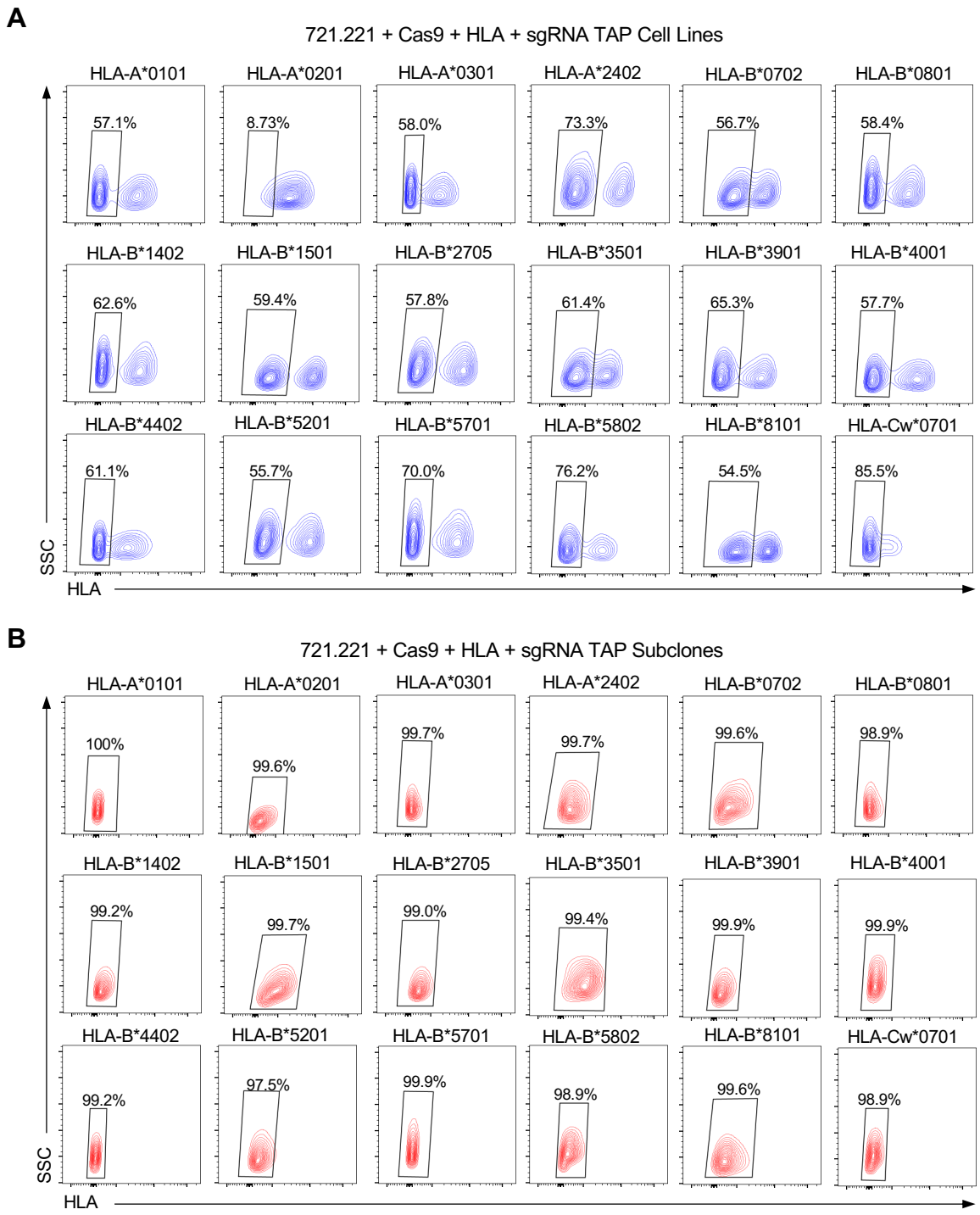

Figure S1. HLA class I surface expression of CRISPR/Cas9-edited TAP-deficient cell lines and subclones, Related to Figure 1. (A) Flow cytometry analysis of surface HLA expression, as determined by staining with pan-HLA antibody W6/32, for all 18 mono-allelic HLA class I-expressing 721.221 cells following transduction with sgRNA TAP and selection with G418. Cells with negative HLA class I surface expression ranged from 8.7-85.5%. (B) Flow cytometry analysis of surface HLA expression for all 18 mono-allelic HLA class I-expressing 721.221 cells following limiting dilution subcloning. Cells with negative HLA class I surface expression ranged from 97.5-100%.

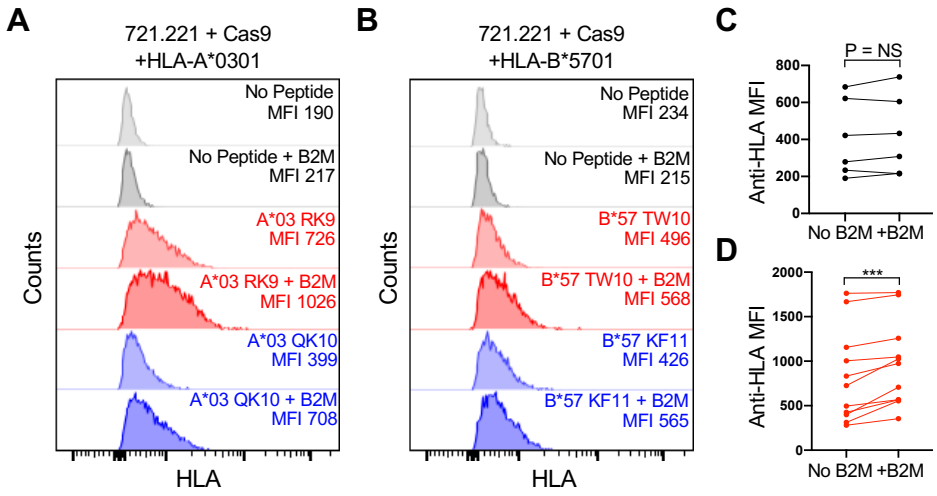

Figure S2. Addition of soluble β2m enhances peptide-mediated stabilization of HLA class I molecules, Related to Figure 1. (A) Representative surface stabilization of HLA-A*0301 molecules with no peptide, RK9 peptide (Gag p17 20-28) or QK10 peptide (Nef 73-82) in the presence or absence of β2m (3 μg/mL) or (B) HLA-B*5701 molecules with no peptide, TW10 peptide (Gag p24 108-117) or KF11 peptide (Gag p24 30-40) in the presence or absence of β2m (3 μg/mL). (C, D) Comparison of surface HLA class I expression in the presence or absence of β2m for no peptide and peptide conditions, respectively. Statistical comparisons were made using Wilcoxon matched-pairs signed rank test. Calculated P values were as follows: NS = non-significant, *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

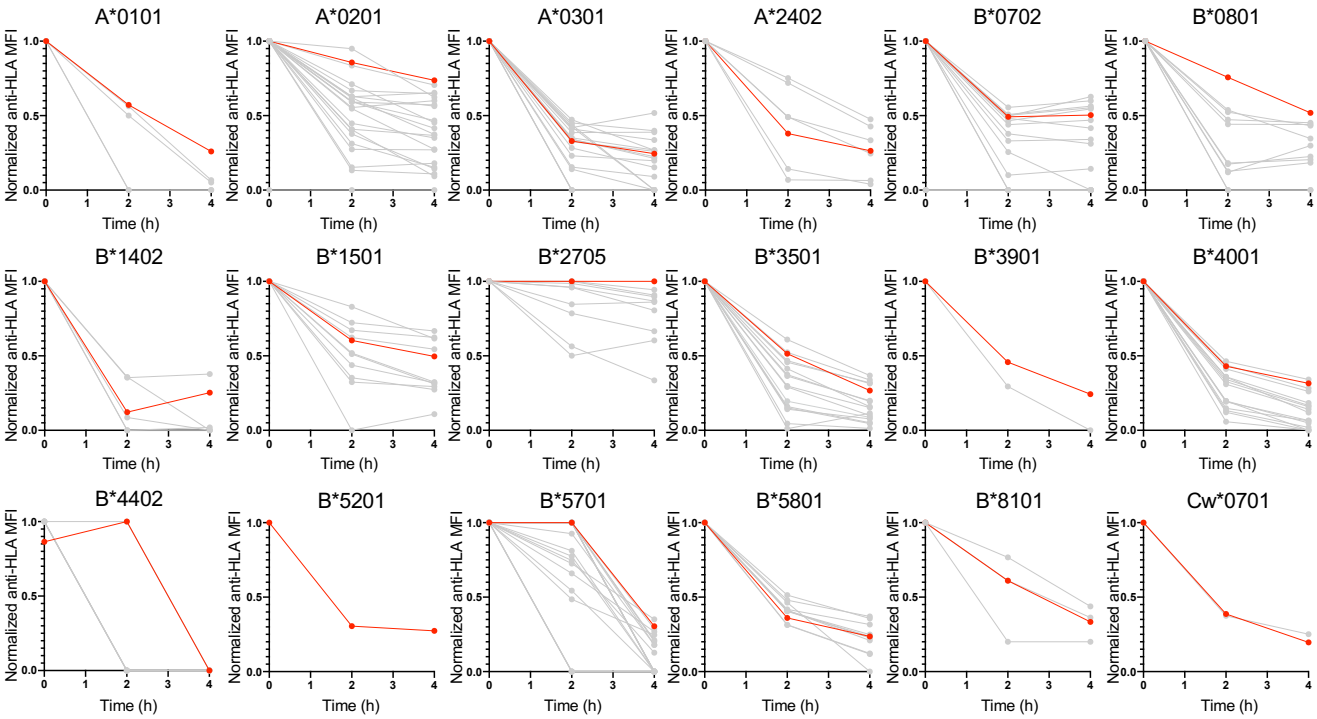

Figure S3. Time-based HLA class I-peptide stabilization of HIV epitopes, Related to Figure 2. Stabilization of HLA class I molecules with 186 known optimal HIV $CD8^+$ T cell epitopes (10μM) in the presence of BFA (5μg/mL) across 18 TAP-deficient mono-allelic HLA class I-expressing cell lines. The *y* axis depicts the anti-HLA MFI normalized to the highest value for each HLA class I allele (0-1). Known immunodominant HIV epitopes based on frequency of $CD8^+$ T cell targeting are indicated in red.

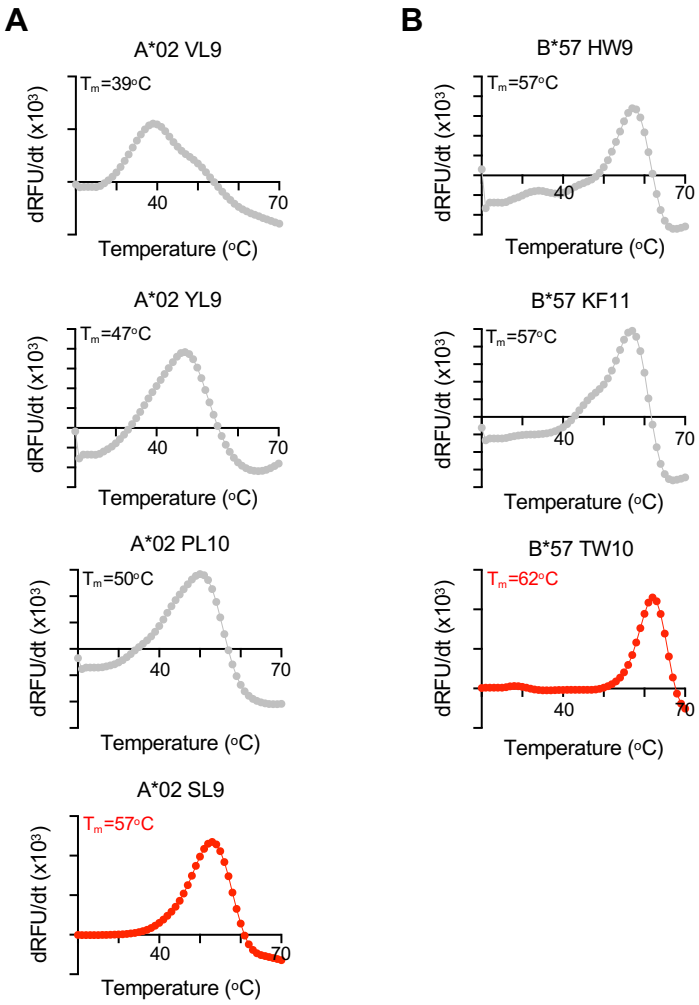

Figure S4. Thermal denaturation of soluble HLA-A*02 and HLA-B*5701 monomers in complex with immunodominant or subdominant HIV epitopes, Related to Figure 3. (A, B) Representative thermal denaturation of HLA-A*0201 and HLA-B*5701 peptide monomers for indicated HIV epitopes, respectively. The *x* axis depicts temperature (20-70°C). The *y* axis depicts the derivative of the temperature versus fluorescence (-dRFU/dT). The thermal stability (Tm) is indicated for each HLA-peptide complex. Each thermal denaturation was performed twice as technical triplicates.

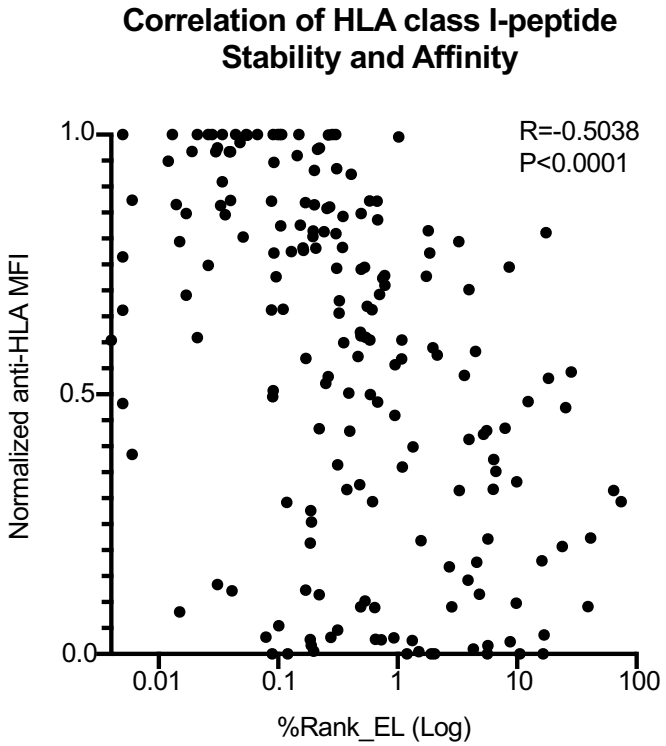

Figure S5. Correlation of relative HLA class I-peptide stability to predicted HLA affinity for all HIV epitopes, Related to Figure 4. Scatter plot of NetMHCPan 4.1 affinity values (log 10, x axis) with relative anti-HLA MFI values (y axis) for each HIV epitope evaluated in the study. Correlation was calculated by Spearman's rank correlation coefficient.