RESEARCH ARTICLE

Figures and figure supplements

Interrogating the recognition landscape of a conserved HIV-specific TCR reveals distinct bacterial peptide cross-reactivity

Juan L Mendoza *et al*

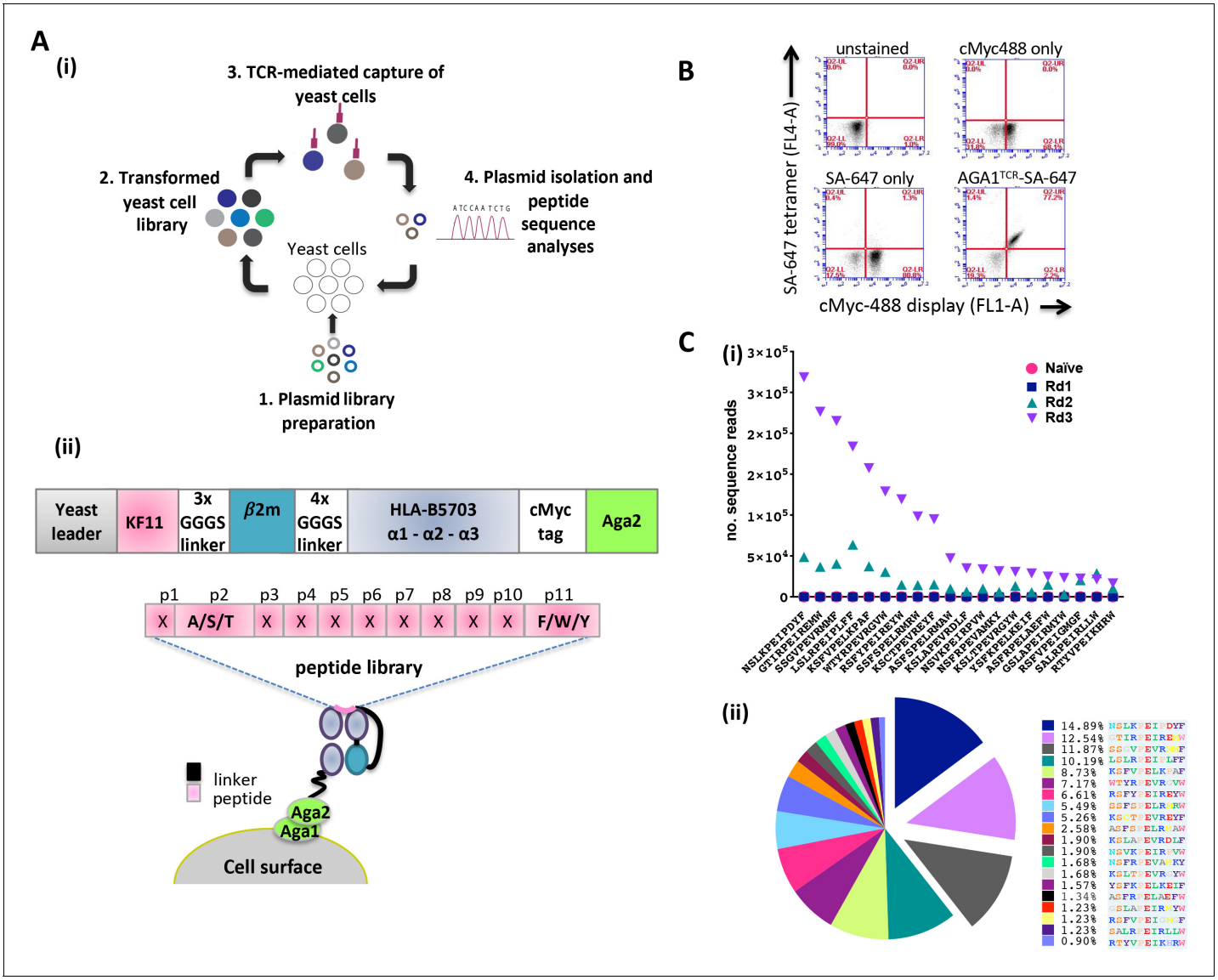

Figure 1. Schematic overview of the peptide-β2m-HLA-B*57:03 yeast display platform. (**A**) (i) Plasmid libraries encoding semi-randomized peptide sequences generated by polymerase chain reaction amplification using rationally designed degenerate oligos and linked to the HLA-B*57:03 heavy chain and β2m were (1) transfected into yeast cells to generate libraries (2). Biotinylated AGA1 TCR reagents conjugated to streptavidin-coated magnetic beads were subsequently used to purify TCR-reactive peptide-MHC complexes expressed on yeast cells (3). The identities of peptides allowing TCR binding were confirmed via plasmid extraction and next generation sequencing analyses (4). This process was repeated up to four times under successively stringent rounds of selection. (ii) Outline of the peptide-β2m-HLA-B*57:03-Aga2 yeast single chain construct illustrating the peptide-β2m-HLA-B*57:03 fusion chain, interspersed with repeating Gly-Ser linker sequence motifs (upper image). HLA-B*57:03-preferred anchor binding residues were fixed at position 2 (p2) to Ala/Ser/Thr and position 11 (p11, CΩ) to Phe/Trp/Tyr, whereas non-anchor residues were allowed to express any amino acid (X) throughout the selection process (lower image). (**B**) Staining of a KF11-β2m-HLA-B*57:03 yeast display test platform with AGA1 TCR fluorescent tetramers. cMyc staining denotes cell-surface expression of the KF11-β2m-HLA-B*57:03 construct, with SA-647 staining employed to monitor non-specific binding of the fluorescent label to transfected yeast cells. (**C**) Frequency of top 20 Round 3 peptide sequences, pre-(naïve) and post-AGA1 TCR-mediated selection. Individual peptide sequences (X-axis) versus their frequencies (number of sequence reads) in the naïve, Round 1, Round 2 and Round 3 AGA1 TCR-selected libraries are denoted (Y-axis) in (i), with the three dominant peptides following Round 3 enrichment illustrated in (ii) as exploded pie chart slices. Percentage (%) peptide frequencies are indicated. The corresponding peptide legend is color-coded according to RasMol amino schema.

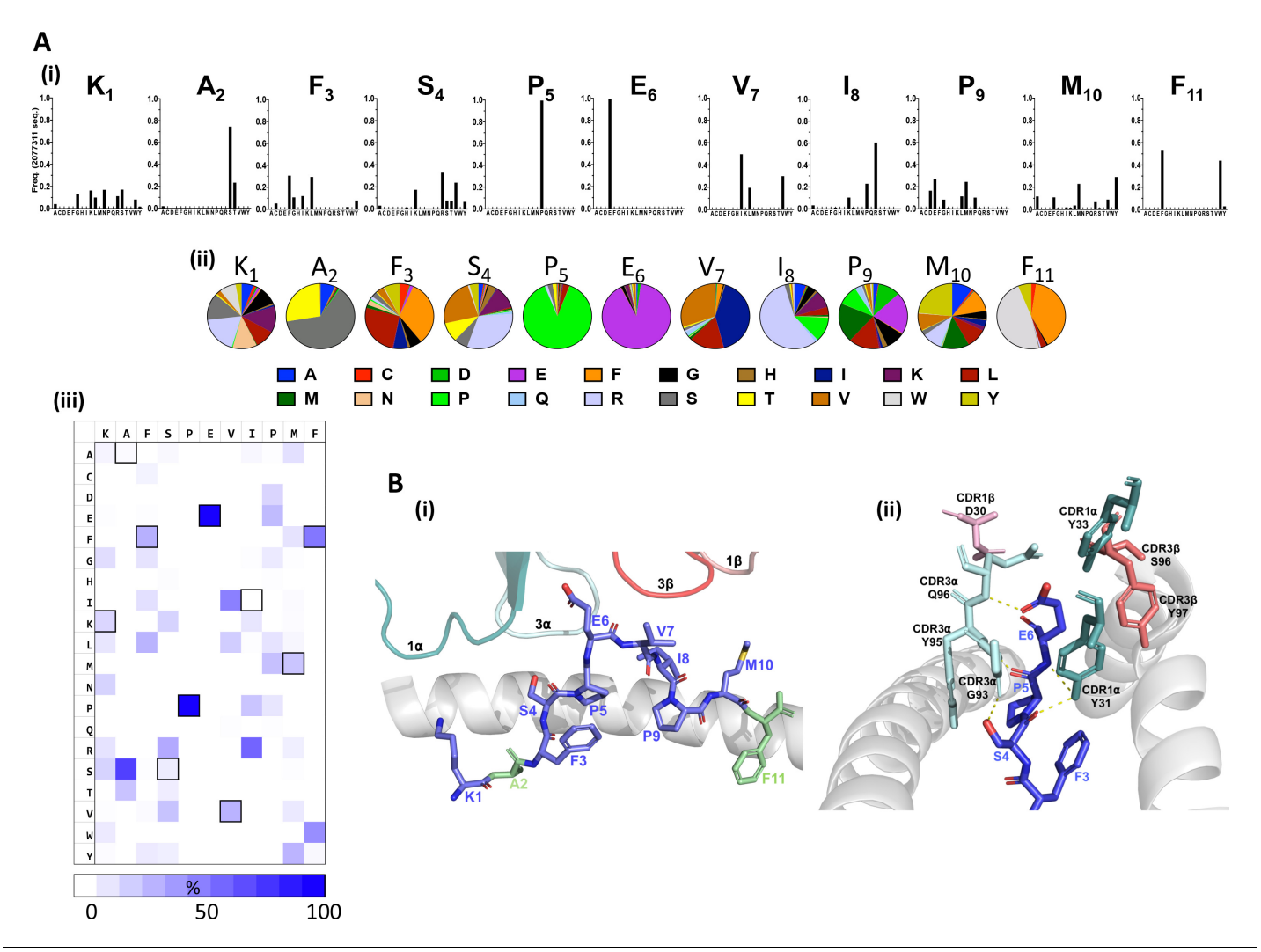

Figure 2. Fixation of specific peptide amino acids residues following Round 3 AGA1 TCR–mediated selection. (**A**) Amino acid enrichment along the length of AGA1 TCR selected 11mer peptides are illustrated (i) in bar chart format for approximately 2.0 × 10^6 selected peptide sequences, with individual amino acid enrichment at each position of the 11mer peptide represented on the X-axis and sequence frequency on the Y-axis. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 epitope which is included for reference. Enrichment data for pre-(naïve) and post-AGA1 TCR selected peptides from all libraries are provided in ***Figure 2—figure supplement 1***). (ii) The top 1000 peptide sequences are reported in pie-chart format, with the original KF11 (KAFSPEVIPMF) peptide amino acids included above the relevant pie-charts for reference. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 peptide sequence. (iii) A Heat map of the entire Round 3 peptide sequence dataset (~2.0×10^6 peptides) demonstrating the near absolute dominance of Pro and Glu at positions 5 and 6, respectively, in AGA1 TCR-selected peptide datasets. The amino acid residues corresponding to the KF11 peptide are outlined in black. Heatmap scale = 0 – 100%, 10% increments. (**B**) Structural overview of the primary contacts formed between the AGA1 TCR and KF11 when restricted by HLA-B*57:03. (i) Slide view of the HLA-B*57:03 alpha 1 (α1) helix in cartoon form (grey), with the alpha 2 (α2) helix removed for clarity. The peptide is depicted in stick format (blue) with the KF11 position 2 Ala (A2) and Position 11 Phe (F11) anchor resides highlighted in green. The TCR CDR1α (deep teal), CDR3α (pale cyan), CDR3β (deep salmon) and CDR1β (light pink) loops that form the main contacts with HLA-B*57:03-KF11 are illustrated. (ii) Barrel view of HLA-B*57:03-KF11, oriented from the peptide's N terminus, with HLA-B*57:03 α1 and α2 helices display in cartoon format (grey) and KF11 amino acids 3–6 illustrated in stick format (blue). The remainder of the KF11 peptide sequence is omitted for clarity. The primary polar contacts (~3 ångströms (Å)) between TCR CDR1α (deep teal) and CDR3α (pale cyan) amino acids and the KF11 peptide at positions 4 to 6 are illustrated (yellow dash lines). CDR1β residue D30 (light pink) and CDR3β amino acids S96 and Y97 (deep salmon) that reportedly form weaker peptides mediated contacts are also displayed. TCR amino acids positions are number according to Arden nomenclature (see Ref 37). Structural images were generated in PyMOL four using the Protein Data Bank coordinates 2YPL.

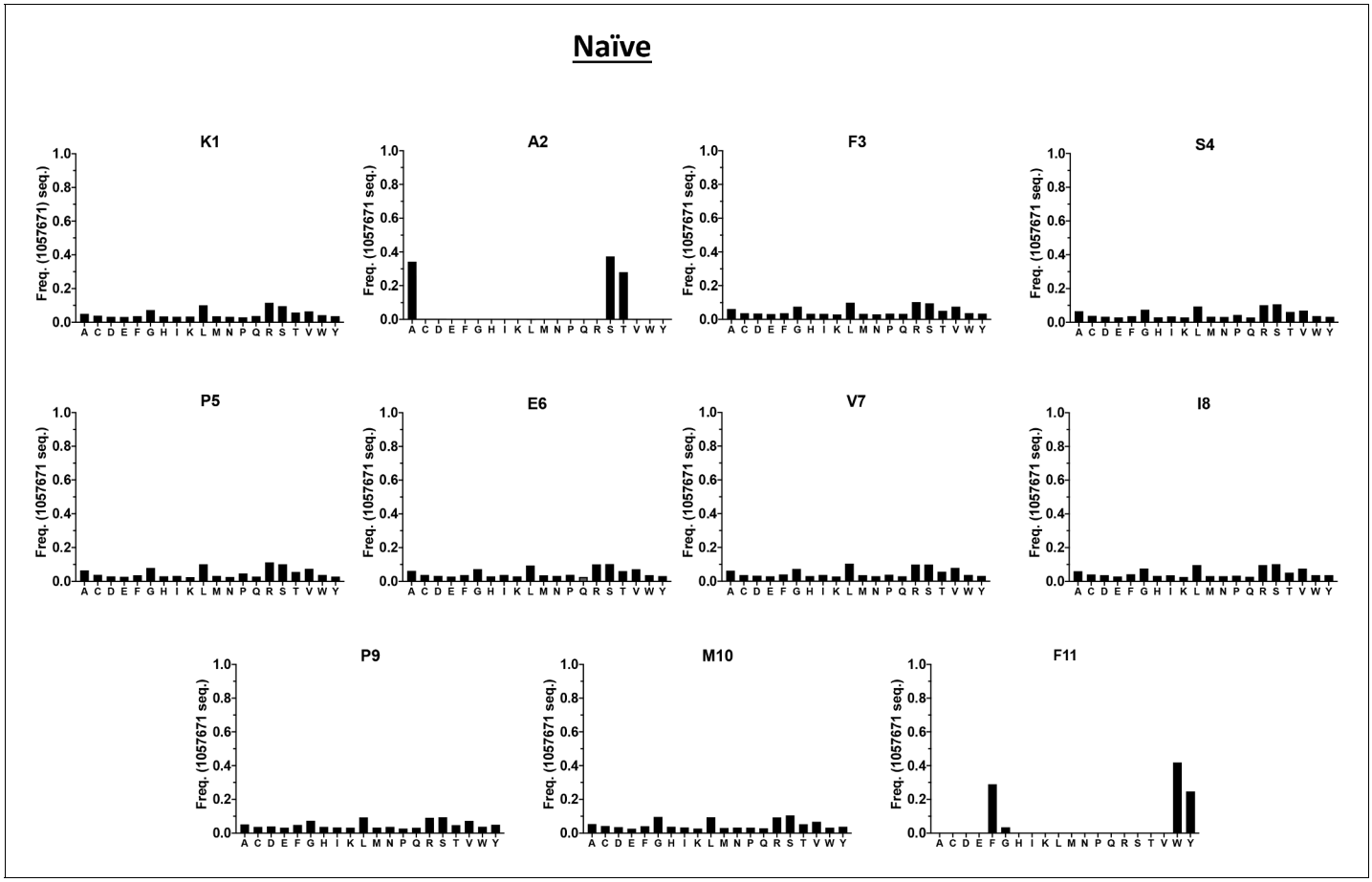

Figure 2—figure supplement 1. Individual amino acid frequencies in the naïve (pre-AGA1 TCR-selected) HLA-B*57:03-restricted yeast display peptide repertoire. Individual amino acid frequencies along the length of the 11 amino acid peptide are illustrated in bar chart format, with the frequencies of peptide sequence reads represented on the Y axis. Individual amino acids at each position of the 11mer peptide are represented on the X-axis. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 peptide sequence, which is included for reference.

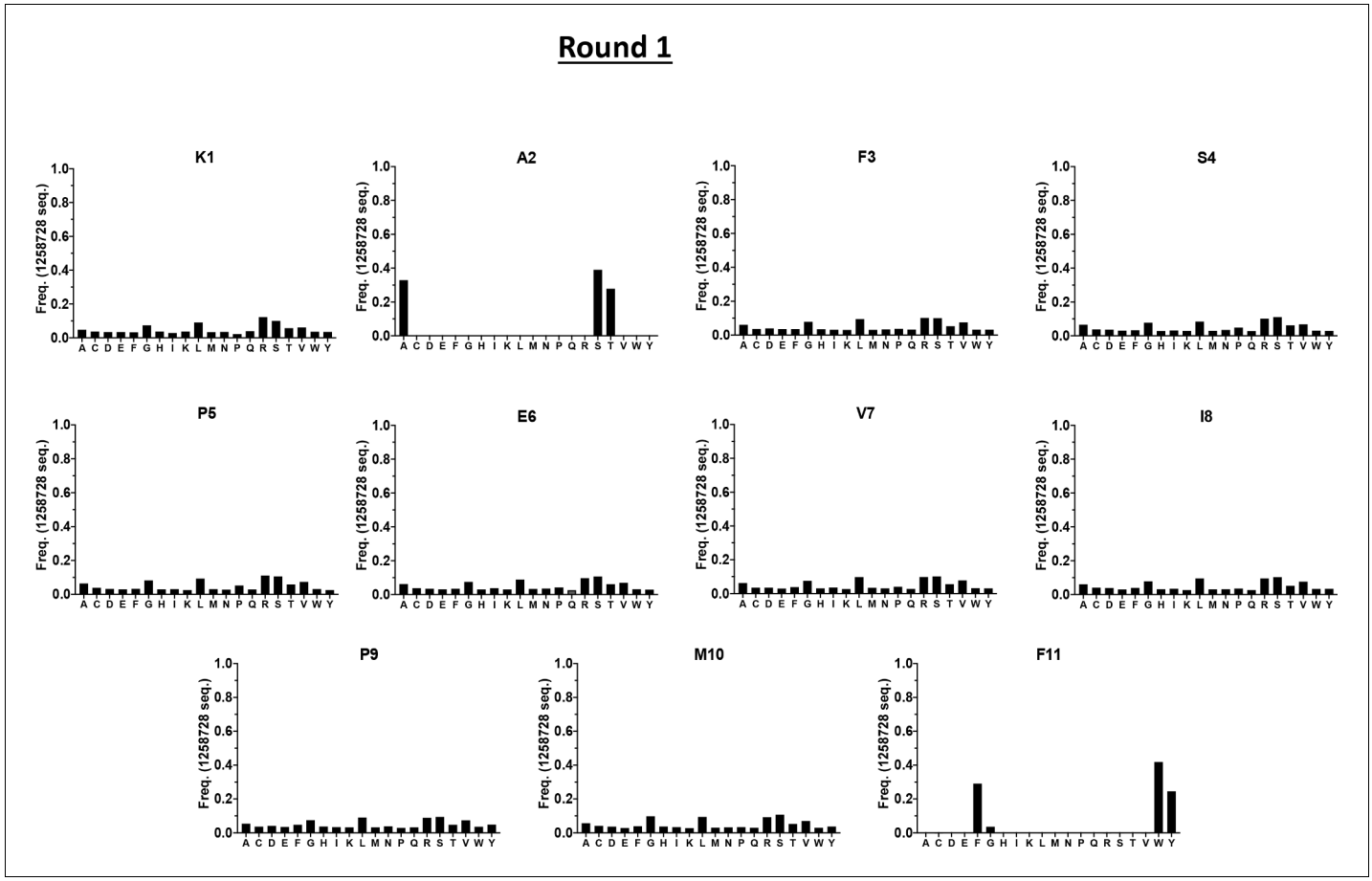

Figure 2—figure supplement 2. Individual amino acid frequencies of the AGA1 TCR-selected, HLA-B*57:03-restricted yeast display peptide repertoire following Round 1 selection. Individual amino acid frequencies along the length of the 11 amino acid peptide are illustrated in bar chart format, with the frequencies of peptide sequence reads represented on the Y axis. Individual amino acids at each position of the 11mer peptide are represented on the X-axis. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 peptide sequence, which is included for reference.

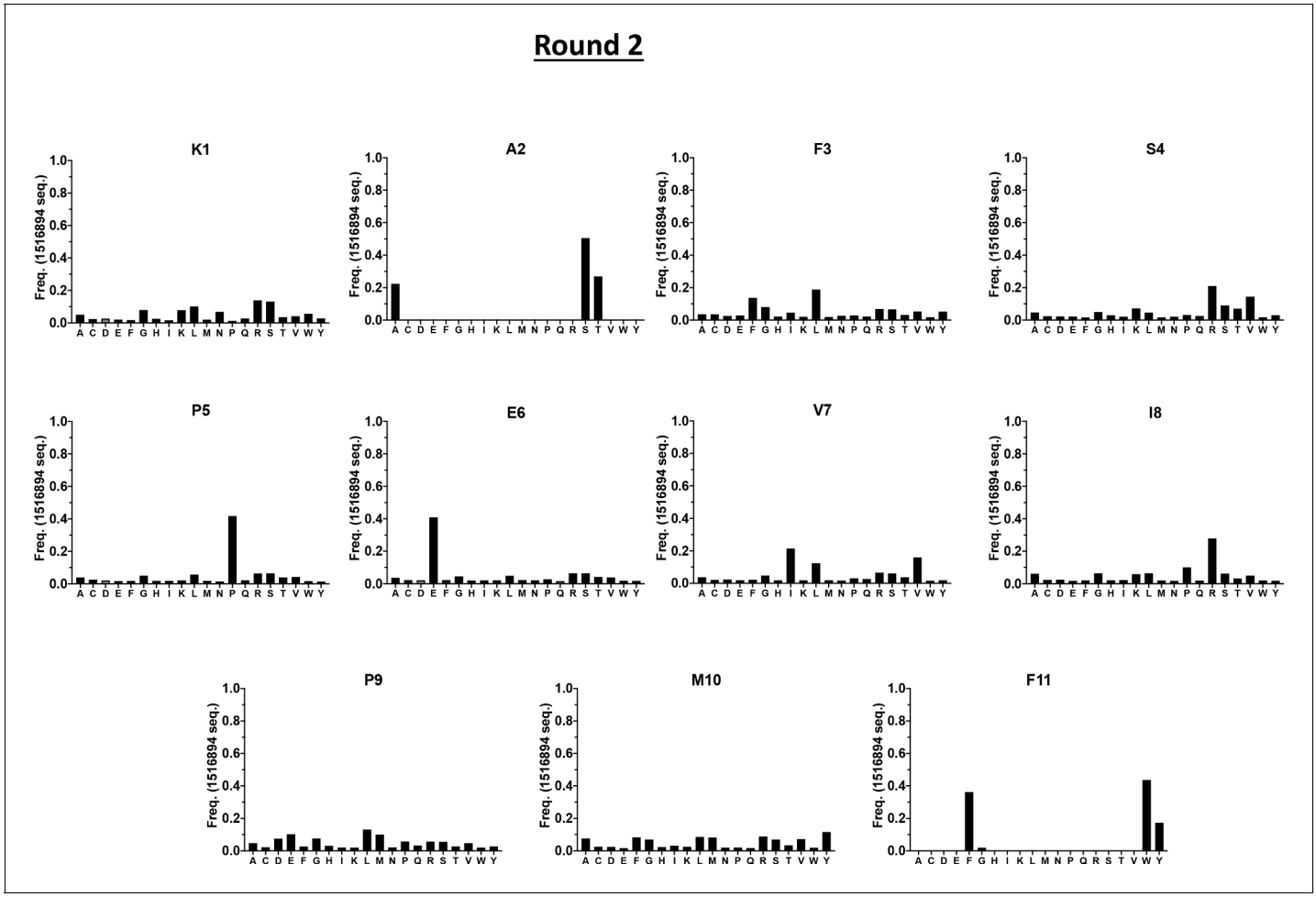

Figure 2—figure supplement 3. Individual amino acid frequencies of the AGA1 TCR-selected, HLA-B*57:03-restricted yeast display peptide repertoire following Round 2 selection. Individual amino acid frequencies along the length of the 11 amino acid peptide are illustrated in bar chart format, with the frequencies of peptide sequence reads represented on the Y axis. Individual amino acids at each position of the 11mer peptide are represented on the X-axis. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 peptide sequence, which is included for reference.

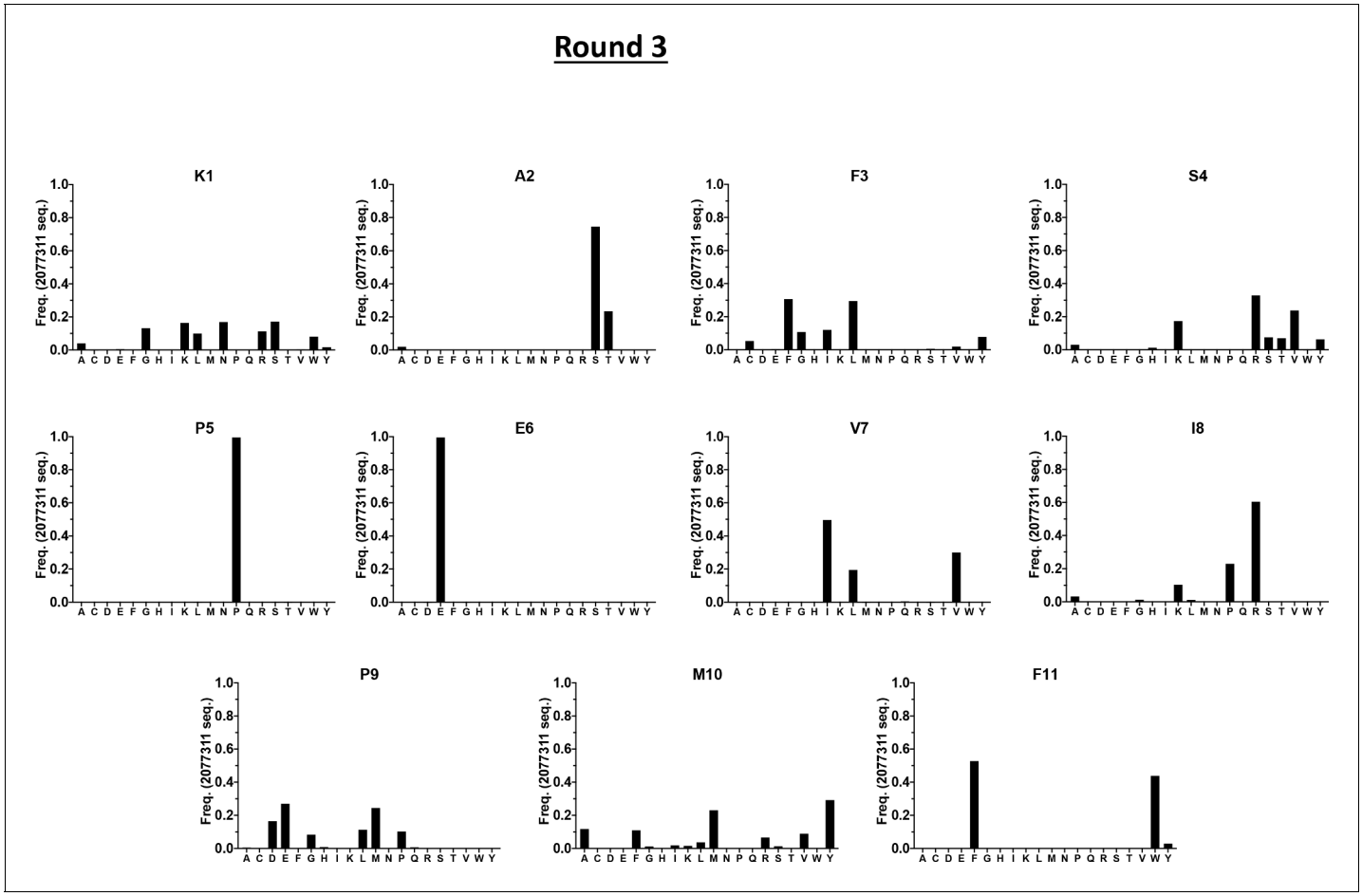

Figure 2—figure supplement 4. Individual amino acid frequencies of the AGA1 TCR-selected, HLA-B*57:03-restricted yeast display peptide repertoire following Round 3 selection. Individual amino acid frequencies along the length of the 11 amino acid peptide are illustrated in bar chart format, with the frequencies of peptide sequence reads represented on the Y axis. Individual amino acids at each position of the 11mer peptide are represented on the X-axis. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 peptide sequence, which is included for reference.

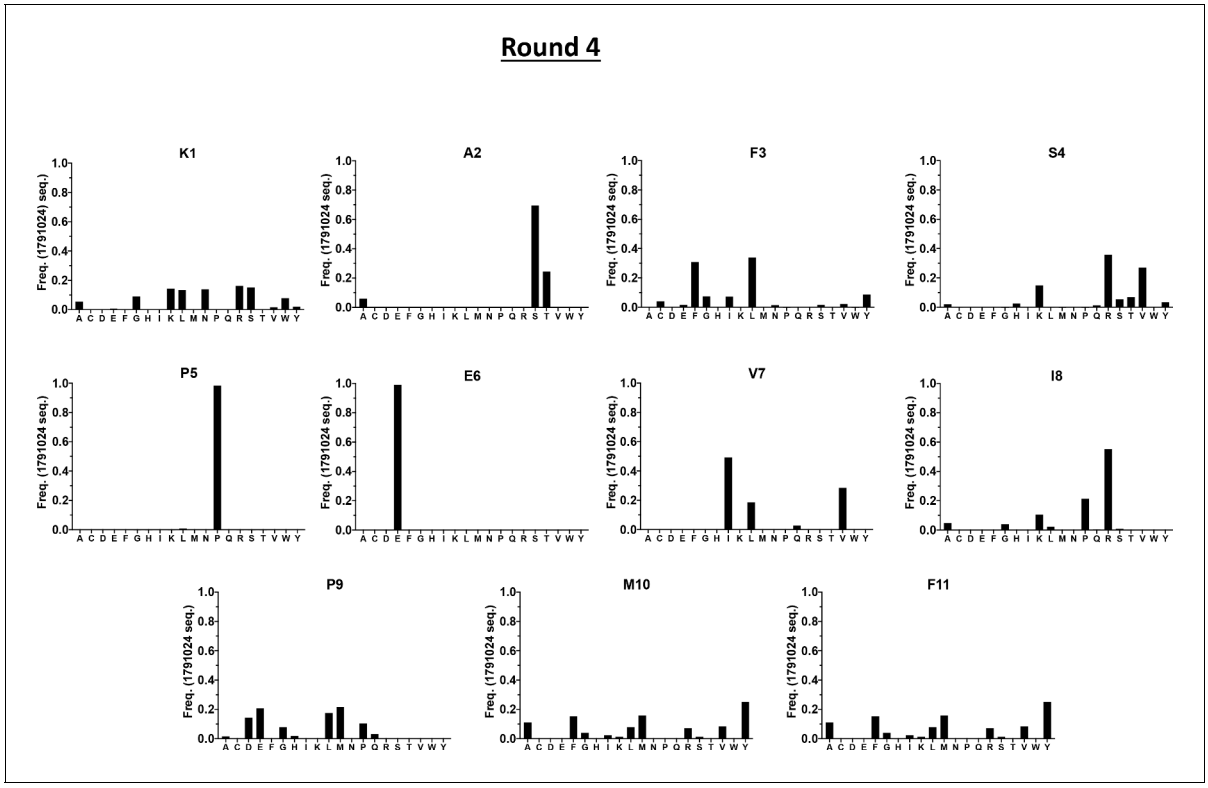

Figure 2—figure supplement 5. Individual amino acid frequencies of the AGA1 TCR-selected, HLA-B*57:03-restricted yeast display peptide repertoire following Round 4 selection. Individual amino acid frequencies along the length of the 11 amino acid peptide are illustrated in bar chart format, with the frequencies of peptide sequence reads represented on the Y axis. Individual amino acids at each position of the 11mer peptide are represented on the X-axis. K1, A2, F3 refers to amino acid positions 1, 2, 3, etc, of the HIV KF11 peptide sequence, which is included for reference.

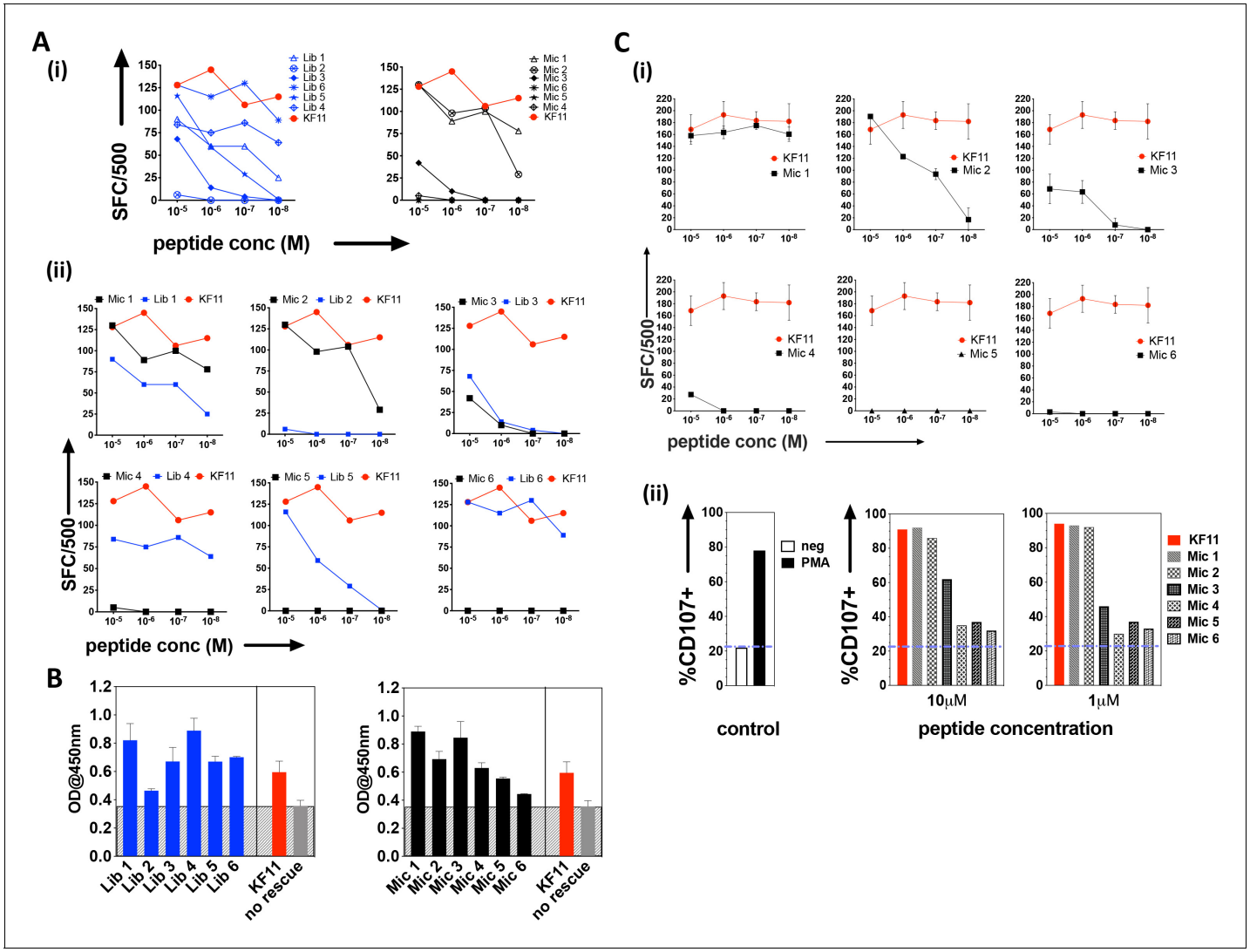

Figure 3. Recognition of library-derived peptides and their closest peptide sequence matches identified from the 'nr' database by AGA1-expressing T cell clones. (**A**) Recognition of six library peptides (Lib) and their closest 'nr'-derived microbial peptides (Mic) matches were compared to the index KF11 in IFN-γ based ELISpot assay screens using an AGA1-expressing T cell clone, summarized in (i) with individual Lib and Mic peptide responses plus comparison to KF11 denoted in (ii). Peptide concentration (Molar (M)) is denoted on the X-axis, with numbers of Spot Forming Cells (SFC) per 500 cell input on the Y-axis. Initial screens were performed with one replica per screen, and were screened on two separate occasions following re-stimulation of T cell clone 1.1 (biological repeat, n = 2), with one representative shown here. Test peptide IDs, their origins and sequence identities are specified in Table 1. (**B**) Binding of library derived and the sequence-mined microbial peptides to HLA-B*57:01, assessed by UV-mediated peptide exchange sandwich ELISA. The Y-axis denotes average absorbance readings at 450 nm, with the peptides tested reported on the X-axis. The background corresponding to the no peptide rescue (nr) control is denoted in grey (also illustrated across the samples by grey hatching). Assays were performed in duplicate (technical repeats, n = 2) on two separate occasions using different peptide stock dilutions (biological repeats, n = 2), with one representative shown here. Error bars corresponding to the Standard Error of the Mean (SEM) are reported. Test peptide IDs, their origins and sequence identities are specified in Table 1. (**C**) Recognition of the six nr-derived microbial peptides (Mic 1–6) by the closely related AGA1-like T cell clone 1.2, assessed by IFN-γ based ELISpot assay and summarized in (**i**) with comparison to the KF11 index peptide reported. Peptide concentration (Molar (M)) is denoted on the X-axis with the numbers of Spot Forming Cells (SFC) per 500 cell input on the Y axis. Assays were performed in duplicate (technical repeats, n = 2) on two separate occasions using different peptide stock dilutions and following re-stimulation and resting of T cell clone 1.2 (biological repeats, n = 2), with one representative shown here. Error bars corresponding to the Standard Error of the Mean (SEM) are reported. Test peptide IDs, origins and sequences are specified in Table 1. (ii) Up-regulation of CD107 on T cell clones in response to 1 and 10 μM Mic peptides compared to the index KF11 epitope was assessed by flow cytometry. Negative (no added peptide - purple dashed line) and positive controls (10 ng/mL PMA stimulation) are reported for reference.

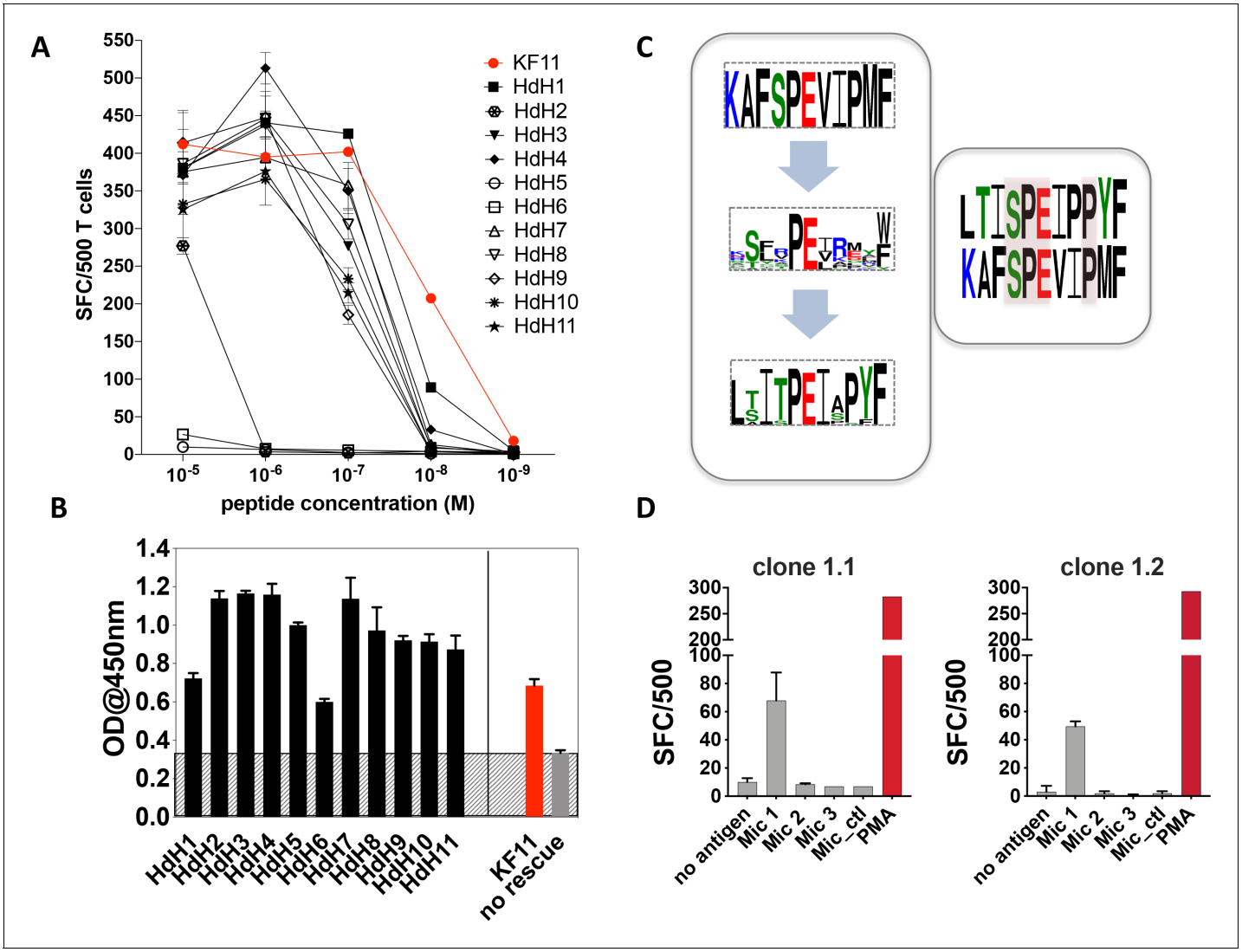

Figure 4. Recognition of haloacid dehydrogenase (HdH) peptides and bacterial lysate-derived antigen by AGA1-expressing T cell clones. (**A**) Ten non-redundant ('nr') database-mined HdH peptides with close homology to *S. newyorkensis* peptide (Mic 1/HdH1) were tested in an IFN-γ based ELISpot assays using an AGA1-expressing T cell clone. Peptide concentration (Molar (M)) is denoted on the X-axis and the numbers of Spot Forming Cells (SFC) per 500 cell input is displayed on the Y-axis. Responses to the KF11 epitope are noted in red. Assays were performed in duplicate (technical repeats) on two separate occasions using different peptide stock dilutions and following re-stimulation and resting of T cell clone 1.2 (biological repeats), with one representative shown here. Error bars corresponding to the Standard Error of the Mean (SEM) reported. Test peptide IDs, their origins and sequence identities are specified in Table 2. (**B**) Binding of the HdH peptides to HLA-B*57:01, assessed in the UV-mediated peptide exchange sandwich ELISA assay. The Y-axis denotes average absorbance readings at 450 nm and the X-axis denotes test peptides. The background corresponding to the no peptide rescue (nr) control is denoted in grey (also illustrated across the samples by grey hatching). Assays were performed in duplicate (technical repeats) on two separate occasions using different peptide stock dilutions (biological repeats, n = 2), with one representative shown here. Error bars corresponding to the Standard Error of the Mean (SEM) are reported. Test peptide IDs, their origins and sequence identities are specified in Table 2. (**C**) Summary of evolved peptide motifs recognised by the AGA1 TCR. Recognition beyond the original KAFSPEVIPMF (KF11) index motif is exemplified initially by the diverse peptide sequences retrieved during repeated rounds of AGA1 TCR-mediated peptide selection, with a Seq2Logo motif reported for the top 20 Round 3 evolved peptide libraries. Following evaluation of 'nr'-database derived peptides in T cell functional assays, peptides that elicited the strongest functional responses - in this case, a *S. newyorkensis*-derived haloacid dehydrogenase peptide -allowed further refinement of database-led search motifs and identification of related peptide that were functionally recognized by AGA1 TCR-expressing T cell clones. Amino acids shared between KF11 and the *S. newyorkensis*-derived Mic1/HdH1 peptide is illustrated in the smaller right panel (pink shading). (**D**) Recognition of bacterial cell lysates from *S. newyorkensis* (Mic 1), *C. orthopsilosis* (Mic 2), *O. uli* (Mic 3) and *R. gnavus* (control) by AGA1-expressing T cell clones 1.1 and 1.2 was tested using an IFN-γ based ELISpot assay. Bacterial cell lysates (20µg/mL) were incubated with cytokine-matured HLA-B*57:01 positive HL60 cells for 7 hours, following which T cell responses were evaluated. PMA (10ng/mL) was included as a positive control, and the background control comprised HL60 cells incubated with T cells only. Lysate identity is denoted on the X-axes and the numbers of Spot Forming Cells (SFC) per 500 cell

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input are displayed on the Y-axes. Assays were performed in duplicate (technical repeats, n = 2) on two separate occasions (biological repeats, n = 2) using fresh lysate stock dilutions and following re-stimulation and resting of T cell clones 1.1 and 1.2. One representative is shown.

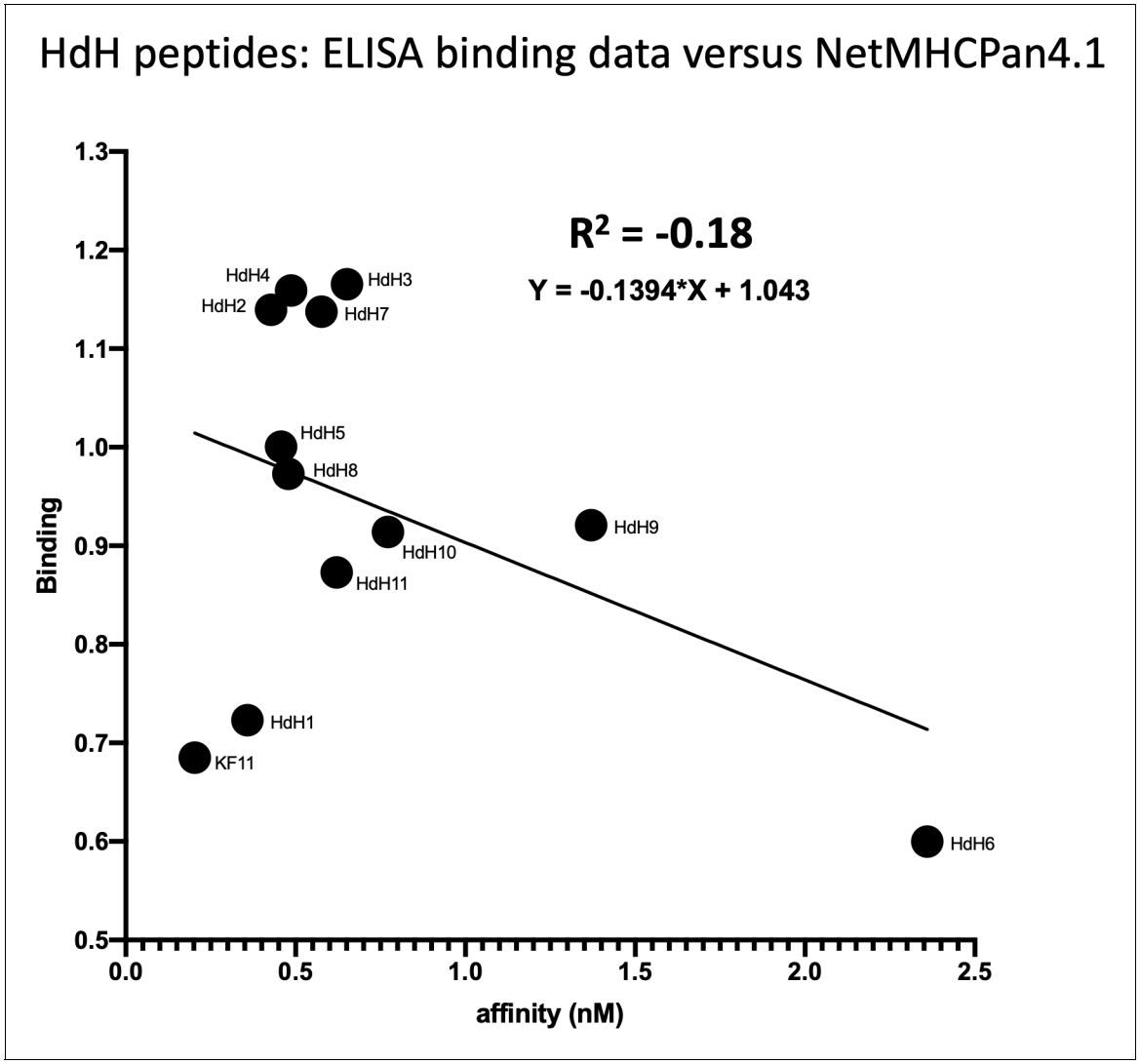

Figure 4—figure supplement 1. Weak, negative correlation between HLA-B*57:01 UV exchange-peptide binding data and Net MHCpan 4.1 predicted affinities for KF11 and the HdH peptides. UV exchange peptide binding data for HLA-B*57:01 is displayed on the Y axis and peptide binding affinities (nM) predicted using NetMHCPan4.1 (http://www.cbs.dtu.dk/services/NetMHCpan/) are reported on the X axis. Correlation coefficient (R^2) was calculated in Microsoft Excel.