Yeast display of MHC-II enables Rapid Identification of Peptide-ligands from Protein Antigen (RIPPA)

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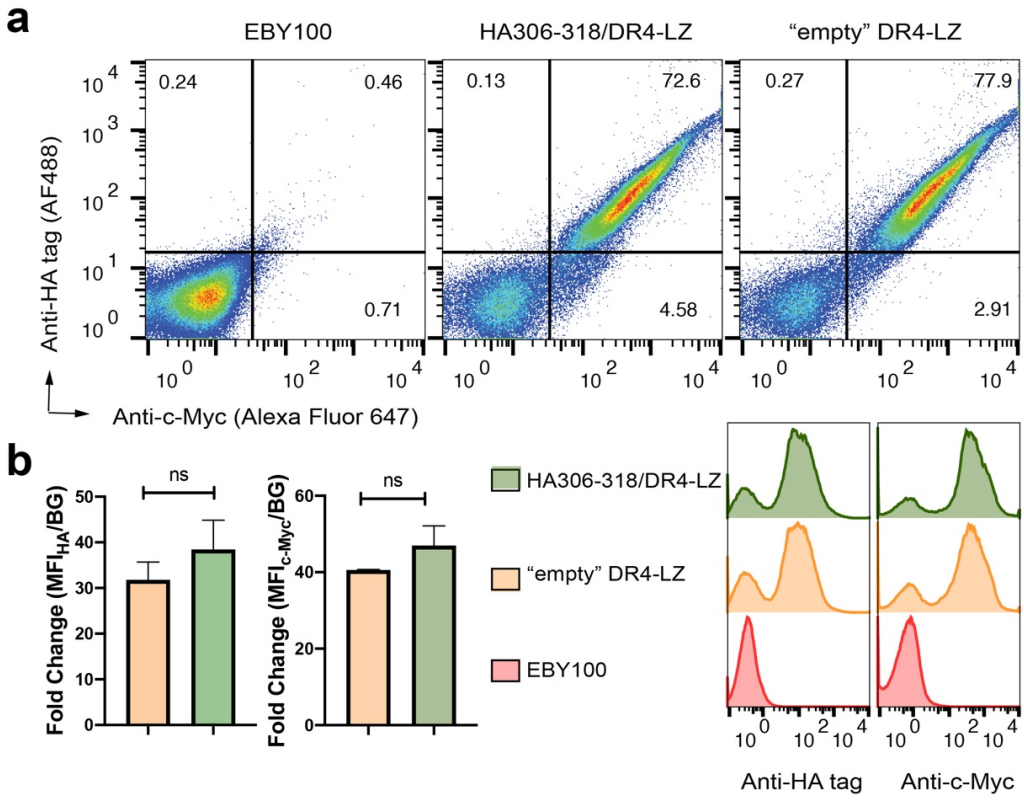
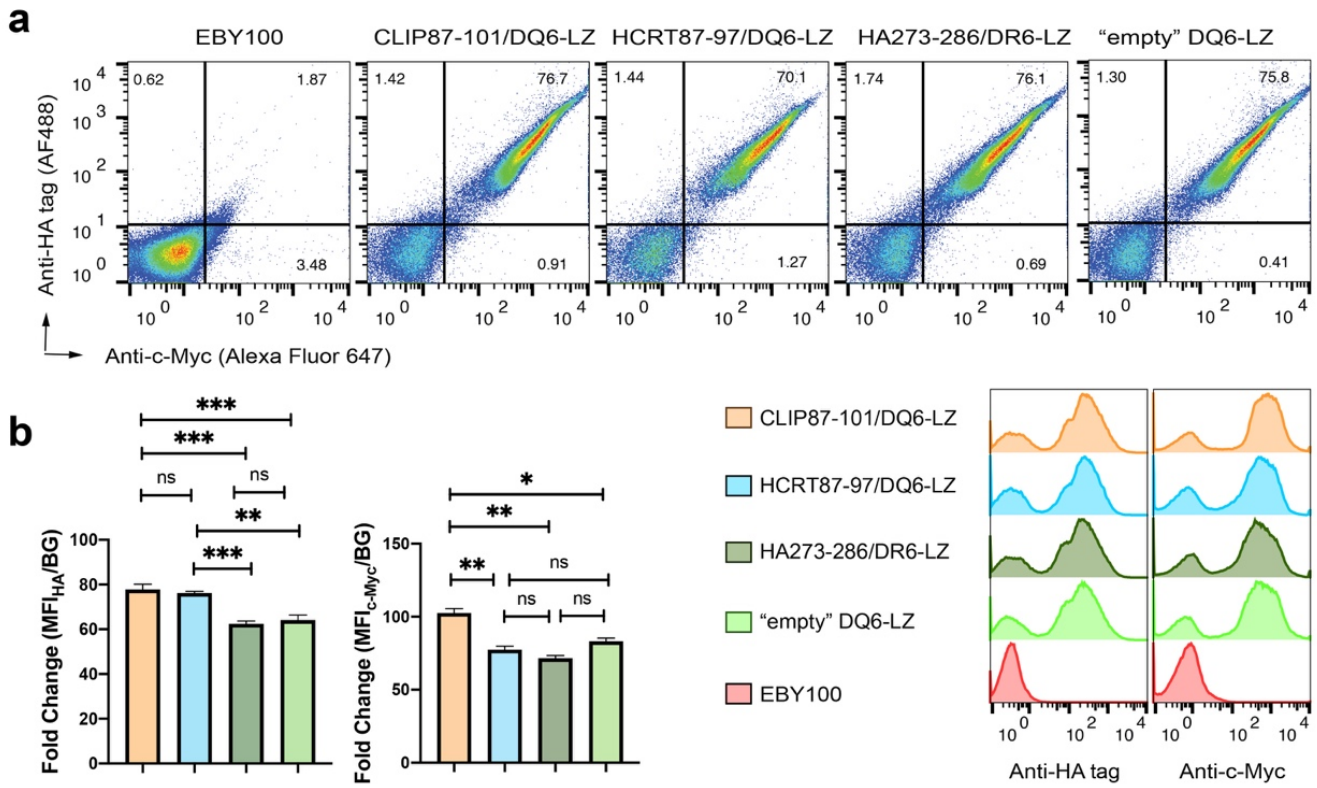
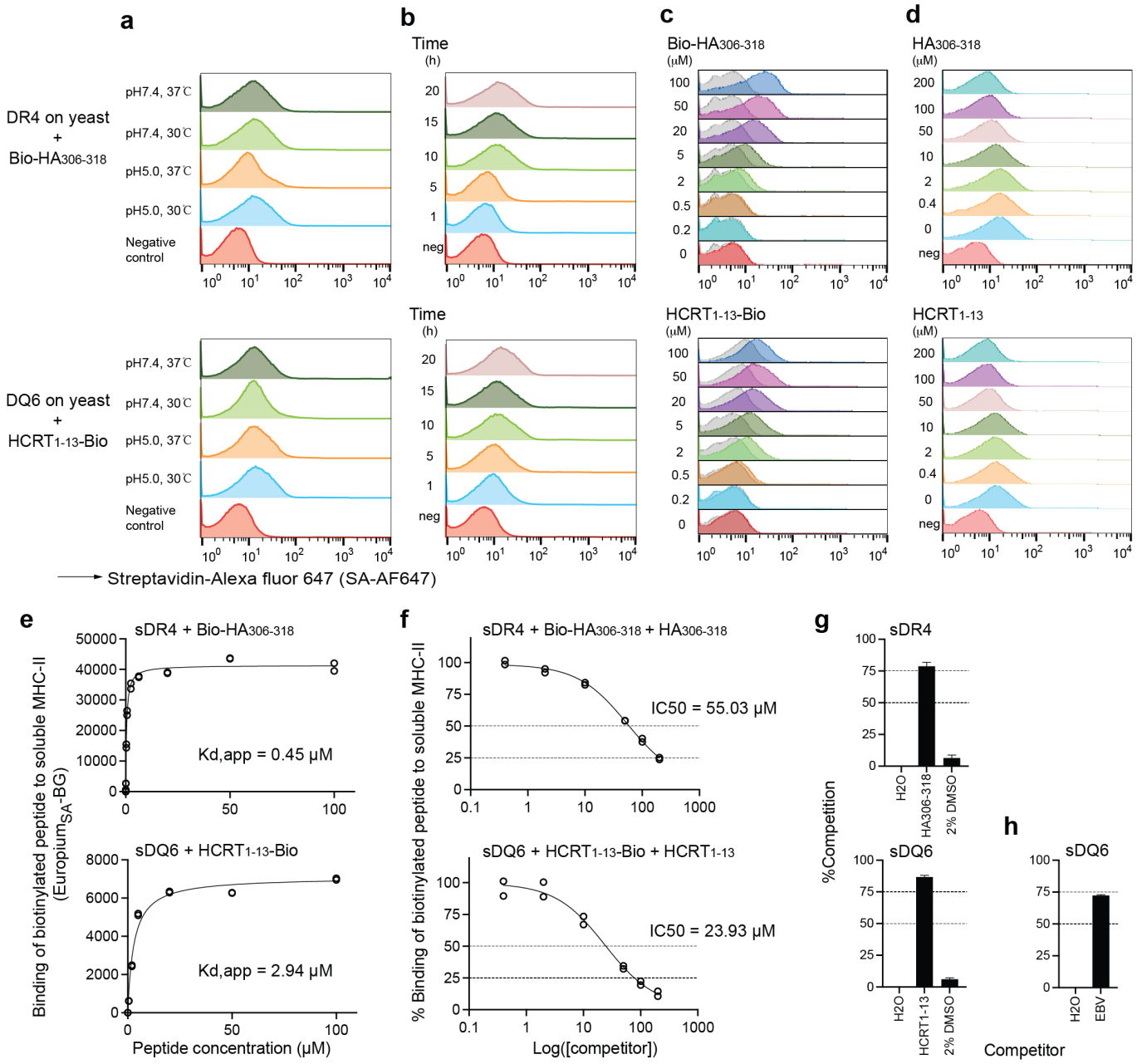


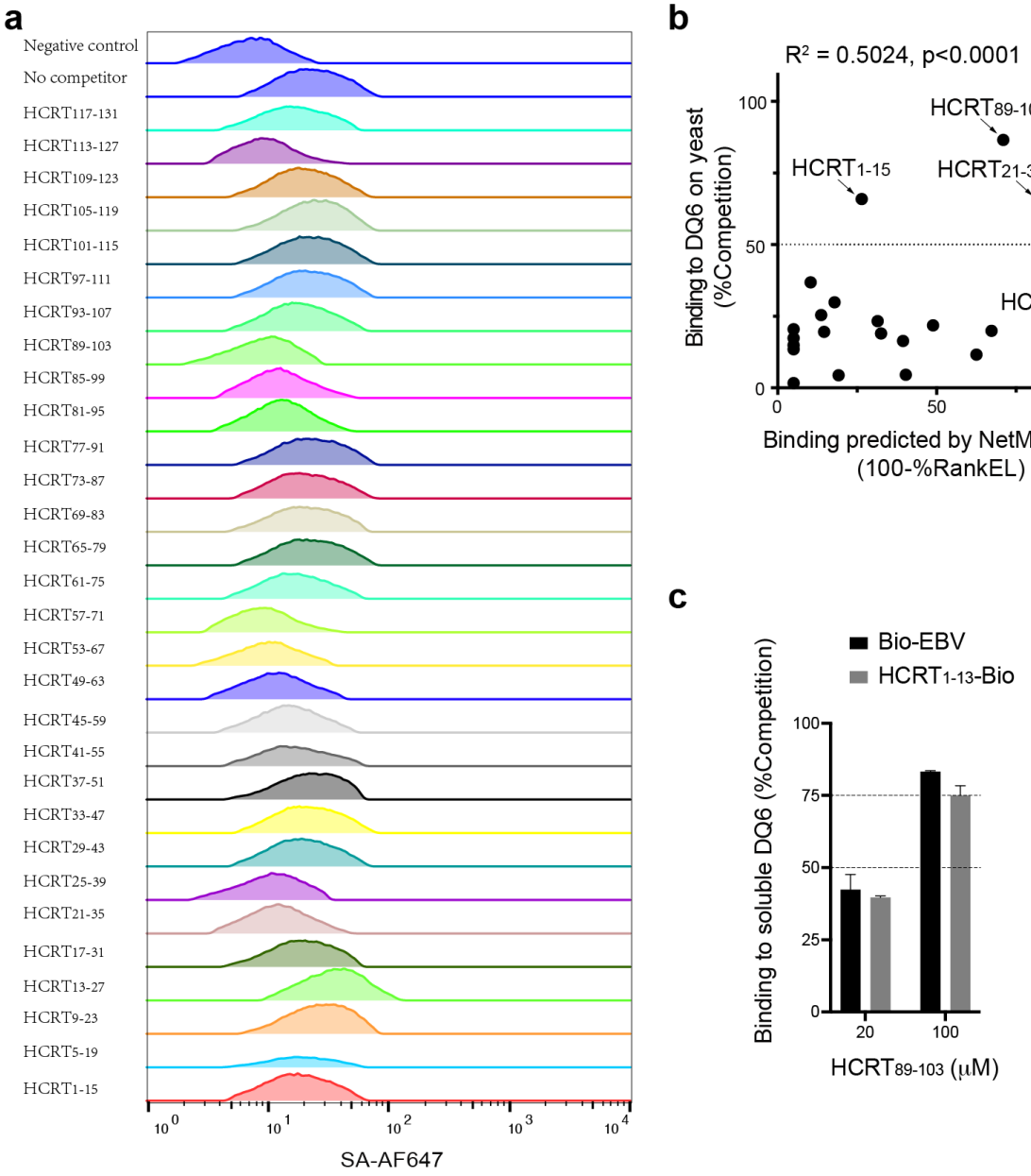
Supplementary Fig. 1 Expression of both chains of DR4 in yeast analyzed by flow cytometry. a, Yeast cells transformed with $HA_{306\text{-}318}$/DR4-LZ or "empty" DR4-LZ constructs were induced for protein expression and double-stained with anti-HA-tag and anti-c-Myc-tag antibodies to confirm that both chains of DR4 in the LZ constructs were expressed by yeast. **b**, Comparison of DRα or β expression in the two yeast transformants as in (**a**). Fold change of MFI of HA-tag or c-Myc-tag signal on the surface of transformants over background (BG) is quantified as in figure 1d. Representative histograms are shown to the right. Error bars represent standard error of the mean (SEM) from at least three independent experiments. One-way ANOVA test was used for comparison. No significant difference in expression of either chain was observed between $HA_{306\text{-}318}$/DR4-LZ and "empty" DR4-LZ (ns: $p > 0.05$).



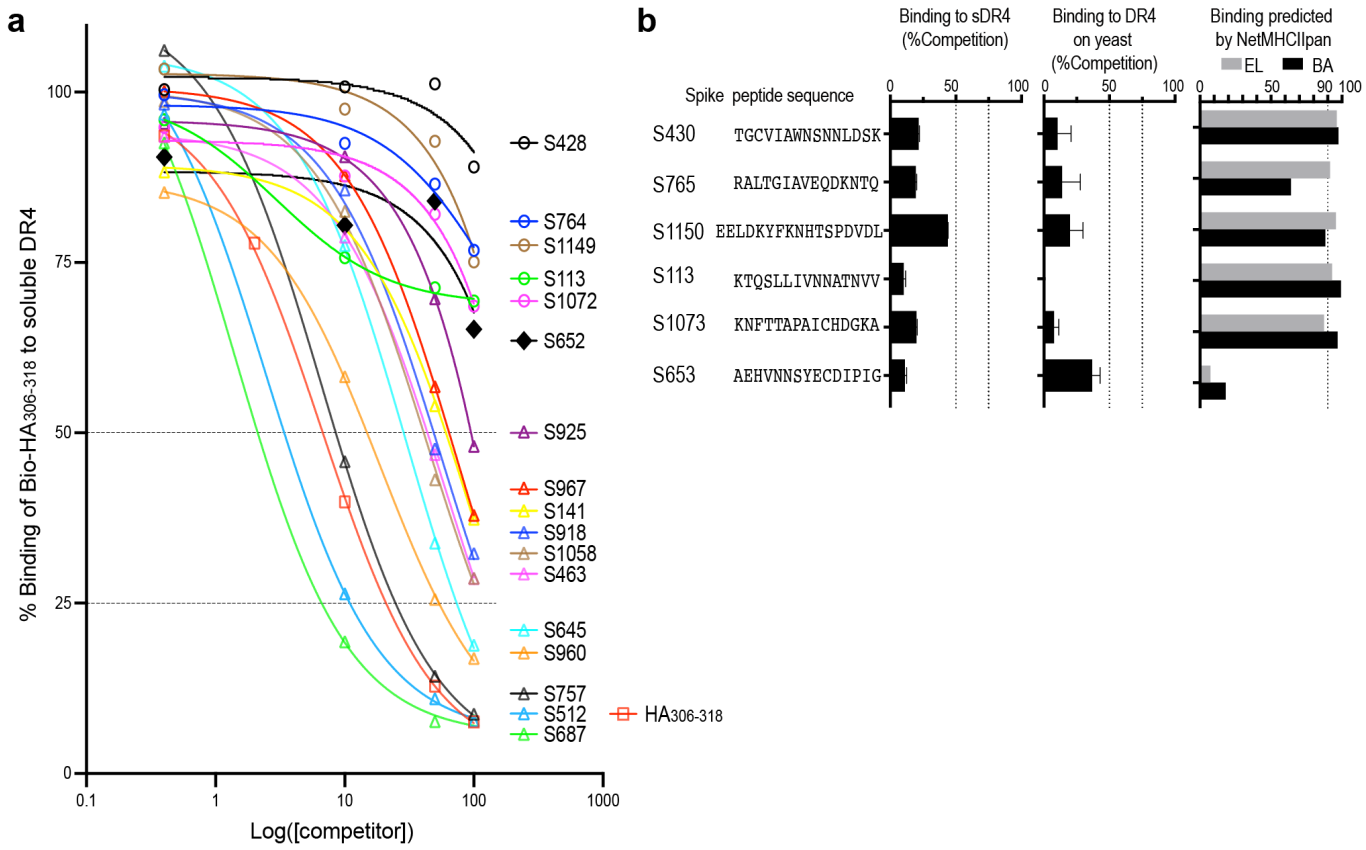
Supplementary Fig. 2 Expression of both chains of DQ6 in yeast analyzed by flow cytometry. a, Yeast cells transformed with peptide/DQ6-LZ or "empty" DQ6-LZ constructs were induced for protein expression and double-stained with anti-HA-tag and anti-c-Myc-tag antibodies to confirm that both chains of DQ6 in the LZ constructs were expressed by yeast. Background staining of untransformed yeast (EBY100) is shown. **b**. Comparison of DQα or β expression in the four yeast transformants as in (**a**). Fold change of MFI over BG is quantified (as in supplementary Fig. 1b). Representative histograms are shown to the right. Error bars represent standard error of the mean (SEM) from at least three independent experiments. The significance was determined using one-way ANOVA test. ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. The expression level of DQ6 β chain, represented by fold change of c-Myc-tag staining over background staining, was increased significantly for $CLIP_{87\text{-}101}$/DQ6 versus the other three constructs.



Supplementary Fig. 3 Binding of peptides to MHC-II on yeast validated using soluble MHC-II. a, **b**, **c**, and **d**, Representative flow cytometric histograms for Fig. 3a, c, d, and e, respectively. Gray shades in (**c**) show the background (BG) staining by biotinylated peptides using MHC-II negative yeast. Non-specific background (BG) staining is negligible at ≤20 μM of biotinylated peptides. **e**, soluble recombinant MHC-II was incubated with different concentrations of biotinylated peptides in the presence of soluble DM (reaching equilibrium fast) for 20 h and analyzed by capture-ELISA using time-resolved fluorescence of europium (Eu). Data approximating equilibrium binding at each peptide concentration were fitted to calculate apparent equilibrium dissociation constant ($K_{d,app}$). **f**, soluble MHC-II was incubated with 20 μM biotinylated peptides and various concentrations of non-biotinylated peptide competitors in the presence of soluble DM for 20 h. MHC-II/Bio-pep binding was quantified by capture-ELISA using time-resolved fluorescence. %Binding = (Eu-$SA_{with\ competitor}$ - BG) / (Eu-$SA_{no\ competitor}$ - BG) x 100%. **g** and **h**, soluble recombinant MHC-II was incubated with 20 μM biotinylated and 200 non-biotinylated peptides or negative controls, as indicated. %Competition = 100% - %Binding (calculated as in **f**). Tight duplicates from a representative experiment are shown (**e** and **f**). All experiments were repeated at least three times with similar results, error bars represent SEM (**g** and **h**). DR4/DM: 10 nM/50 nM; DQ6/DM: 25 nM/100 nM.



Supplementary Fig. 4 Binding of HCRT peptides to DQ6 on yeast validated using soluble DQ6. a, Representative flow cytometric histograms showing the streptavidin staining of yeast (quantified in Fig. 4a). **b**, Correlation analysis for binding data acquired using "empty" DQ6 on yeast versus NetMHCIIpan-4.0 (EL) prediction. Arrows indicate peptides that show binding in one method but not the other in the comparison. $HCRT_{117-131}$ represents a false positive in prediction, ranking top 10% using NetMHC algorithm trained mainly using EL mass spectrometry data. **c**, 25 nM soluble DQ6 was incubated with 1 μM Bio-EBV or $HCRT_{1-13}$-Bio and the indicated concentrations of non-biotinylated $HCRT_{89-100}$ peptide in the presence of 100 nM soluble DM for 20 h. DQ6-bound biotinylated peptides at each condition was quantified by capture ELISA using time-resolved fluorescence and %Competition was calculated (as in Supplementary Fig. 3g). Error bars represent SEM from three independent experiments. $HCRT_{89-103}$ represents a false negative in the ELISA-based approach, yielding <50%Competition when [competitor]:[indicator]=20.



Supplementary Fig. 5 Binding of SARS-CoV-2 spike peptides to DR4 on yeast validated using soluble DR4. a, 10 nM soluble DR4 was incubated with 1 μM Bio-$HA_{306-318}$ and various concentrations of competitor peptides in the presence of 50 nM soluble DM. DR4-bound Bio-$HA_{306-318}$ at each condition was quantified by capture ELISA using time-resolved fluorescence and %Competition was calculated (as in Supplementary Fig. 3g). IC50 values of each competitor yielding >50%Competition at the highest concentration were calculated from three independent experiments and normalized to non-biotinylated $HA_{306-318}$ and represented as mean + SEM (in Fig. 6e). **b**, Alternative peptides as indicated from the six regions that generate peptides yielding <50%Competition when [competitor]:[indicator]=100 (in **a**) were synthesized and further analyzed for binding to DR4. Left panel: competitive binding to soluble DR4 was quantified (as in **a**), [competitor]:[indicator]=100; middle panel: competitive binding to "empty" DR4 on yeast was quantified at Fig. 5's condition, [competitor]:[indicator]=10; right panel: %RankEL and %RanBA were predicted using NetMHCIIpan-4.0 and represented as 100%-%RankEL and 100%-%RankBA, respectively. Error bars represent SEM from three independent experiments. S428, S764, S1149, S113 and S1072 represent false positives in prediction, ranking top 10% using at least one of the two NetMHCIIpan-4.0 algorithms (EL and BA). S652 likely represents a false positive in RRIPA, yielding >50%Competition when [competitor]:[indicator]=10.