

SUPPORTING INFORMATION:

MHC-I ligand discovery using targeted database searches of mass spectrometry data: Implications for T cell immunotherapies

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Supplementary Figure 1. Decoy database comparisons. (A) Distribution of all target and decoy PSMs for MHC IPs from mouse EL4 cells (by Sequest XCorr) where MHC-DB decoy databases formed by either reversing all mouse proteins in Parent-DB then predicting by NetMHC (Decoy Then Predict) or by reversing the NetMHC-predicted peptides (Predict Then Decoy). (B) Distribution of H-2 D^b and H-2 K^b–specific decoy and target PSMs for each allotype-specific IP, comparing the “Decoy Then Predict” and “Predict Then Decoy” strategies. (C) Total unique peptides for each allotype-specific IP, comparing the “Decoy Then Predict” and “Predict Then Decoy” strategies at 5% FDR. (D) Same as C, at 1% FDR.

Supplementary Figure 2. Search results at 5% FDR. (A) Parent-DB search results (Sequest and Mascot) for mouse EL4 cells at 5% FDR, showing purity and allotype specificity of the B22.249 and Y3 antibodies. Shown separately for each allele-specific IP (and the total of both) are the number of unique peptides considered H-2 D^b (NetMHC ≤ 2% rank for H-2 D^b), H-2 K^b (NetMHC ≤ 2% rank for H-2 K^b) binders, or non-binders (NetMHC > 2% rank, grey). (B) Allotype-specific increases in MHC-I peptides (NetMHC ≤ 2% rank) achieved with the Sequest and Mascot MHC-DB searches (5% FDR).

Supplementary Figure 3. Search results by NetMHC % rank cutoff. (A) Total unique H-2 D^b and H-2 K^b peptides for each allotype specific IP identified by varying the % rank cutoff (1%, 2%, 5% and 10%) used to create the mouse MHC-DB. Peptides were identified using Mascot searches at 1% Percolator FDR (B) Same as (A), but using Mascot searches at 5% Percolator FDR.

Supplementary Figure 4. Predicted and Identified NetMHC % ranks. (A) Shown are the distributions, across peptide lengths, of the NetMHC % rank values for all predicted H-2 D^b and H-2 K^b peptides in the MHC-DB. (B) Distribution of NetMHC % ranks for the unique H-2 D^b and H-2 K^b peptides for each allotype-specific IP based on Mascot searches at 1% Percolator FDR.

Supplementary Figure 5. Percolator PEP statistics (A) Percolator PEP for each peptide identified in Mascot searches of both Parent-DB and MHC-DB search types are plotted in numerical order according to their q-value in the Parent-DB search. Percentages are the peptides for which the PEP of the MHC-DB search was less than that of the Parent-DB search. (B) As in A, the distribution and mean values of the PEP for each MHC-I IP search result, comparing Parent-DB and MHC-DB search types.

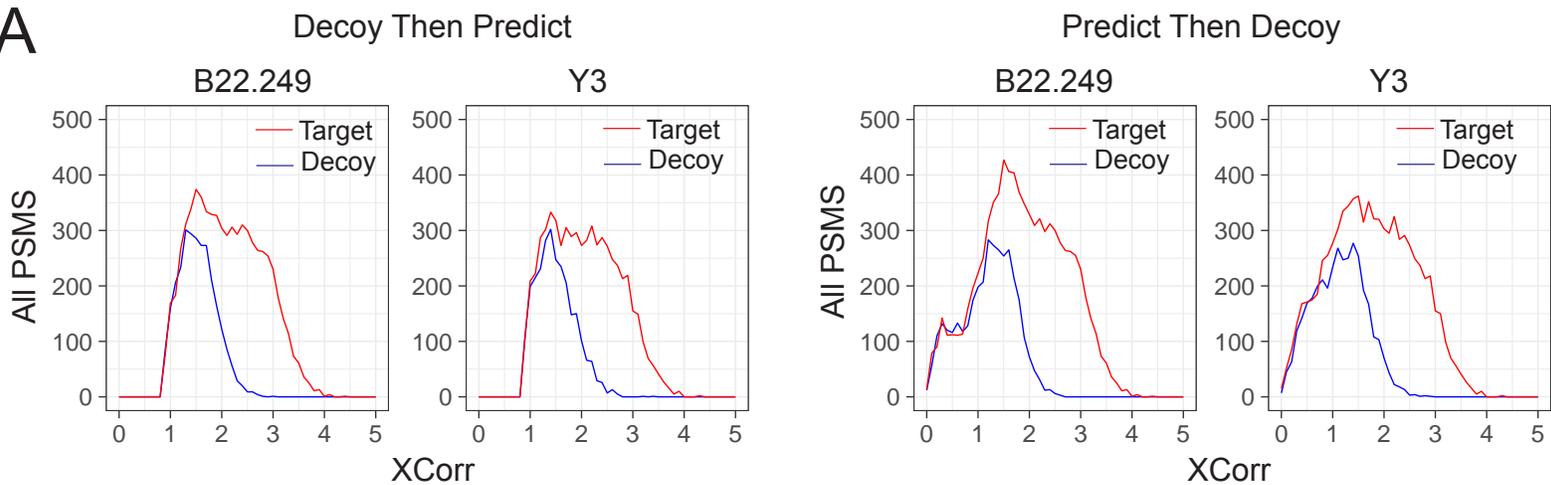
Supplementary Figure 6. Mascot ions score distributions. Distribution and mean values of Mascot ions scores among identified peptides (5% FDR) for the Parent-DB and MHC-DB search types for the B22.249 and Y3 IPs.

Supplementary Figure 7. Predicted and Identified HLA peptides (A) NetMHC-predicted peptides for eight PBMC HLA IP datasets based on the patient allotype. Shown for each PBMC is the total number of predicted peptides (≤ 2% rank) by peptide length. (B) Identified peptides (Mascot, 1% FDR) from published PBMC ligandome datasets using a reference human proteome database (Parent-DB) showing the purity and peptide distribution among alleles for each patient allotype. Based on NetMHC, peptides were considered binders to the patient allotypes (≤ 2% rank for a specific allele) or non-binders (> 2% rank).

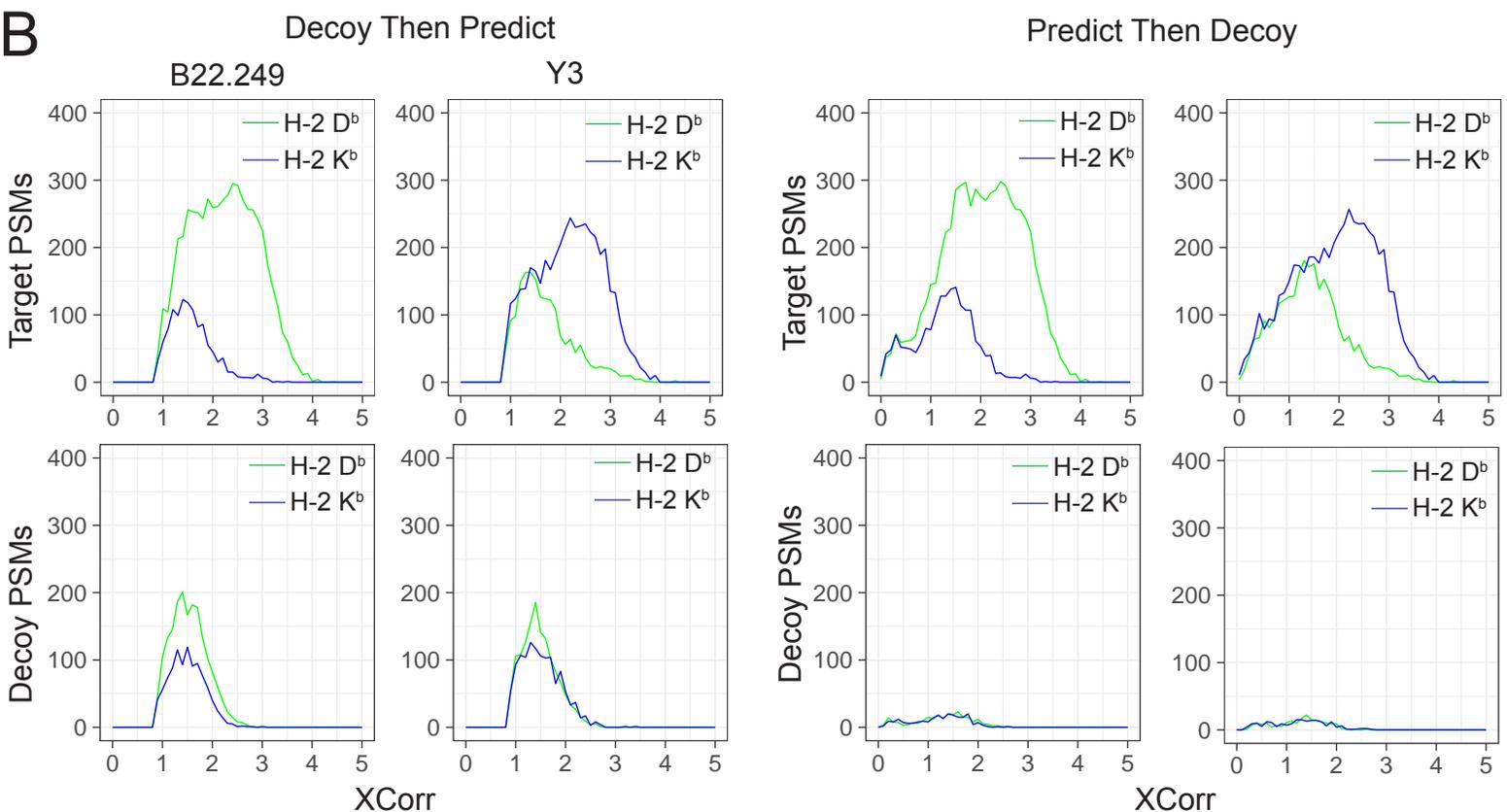
Supplementary Figure 8. Database overlap. Overlap of PSMs identified in the Parent-DB and MHC-DB for each mouse IP using searches by Mascot at (A) 1% FDR and (B) 5% FDR.

Supplementary Figure 1

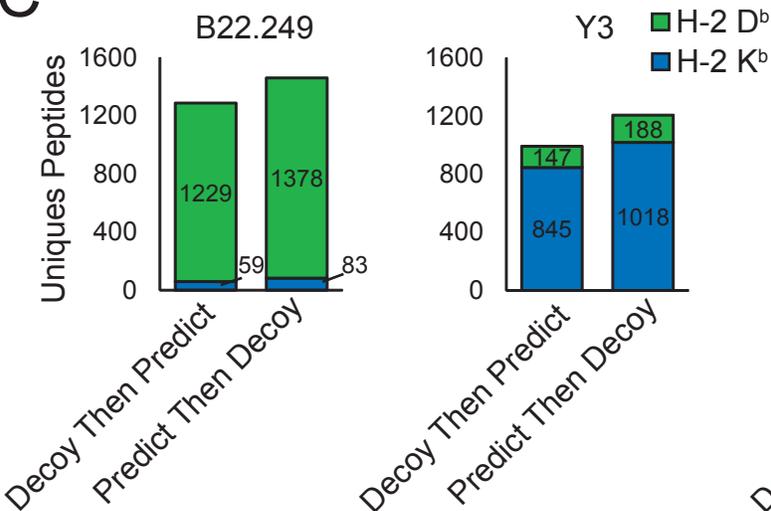
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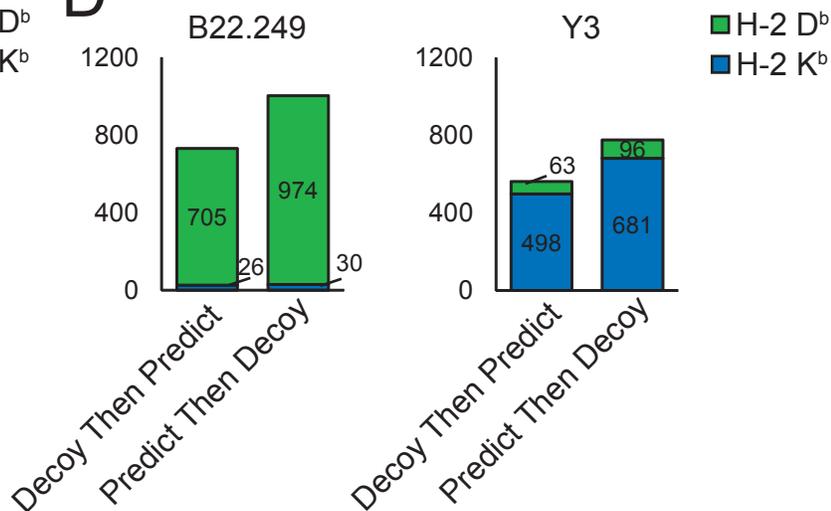
B



C

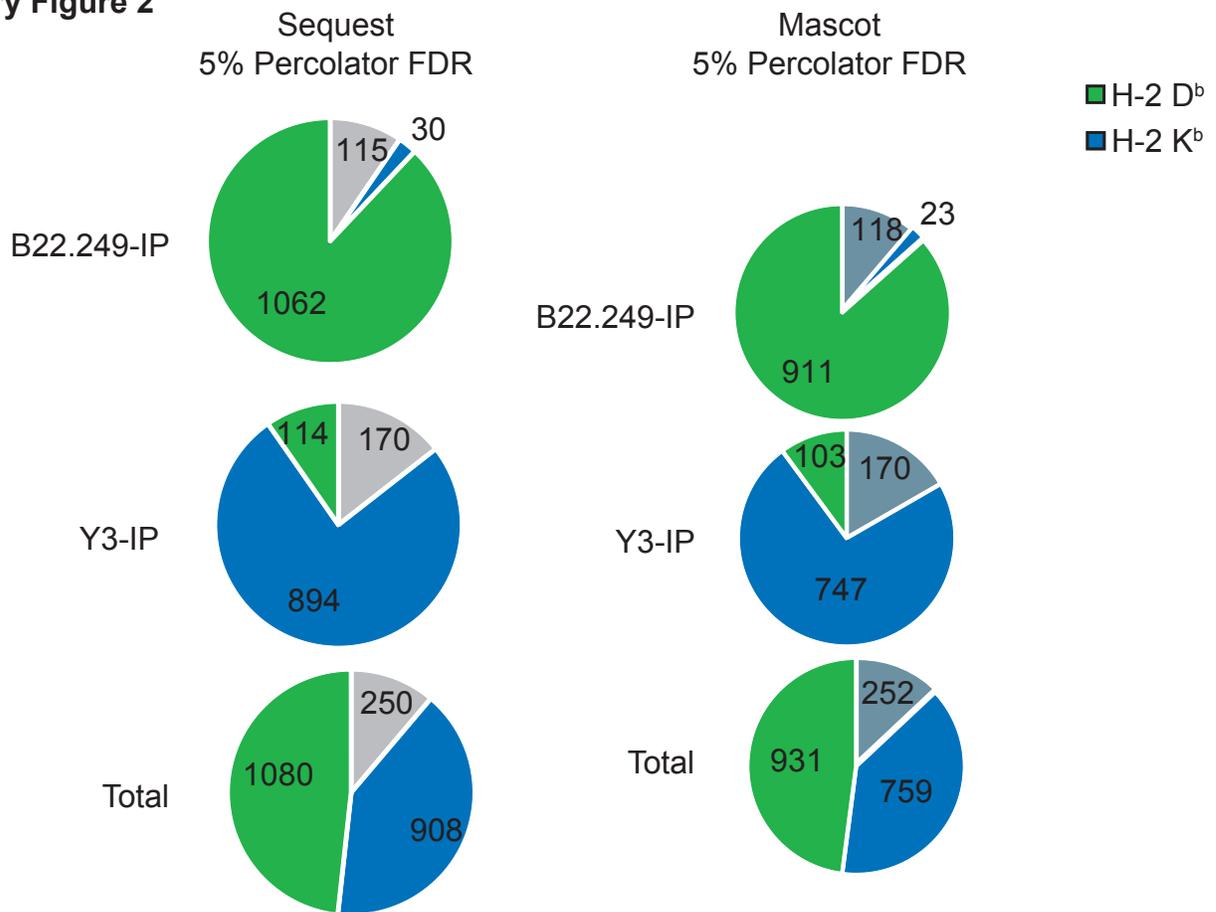


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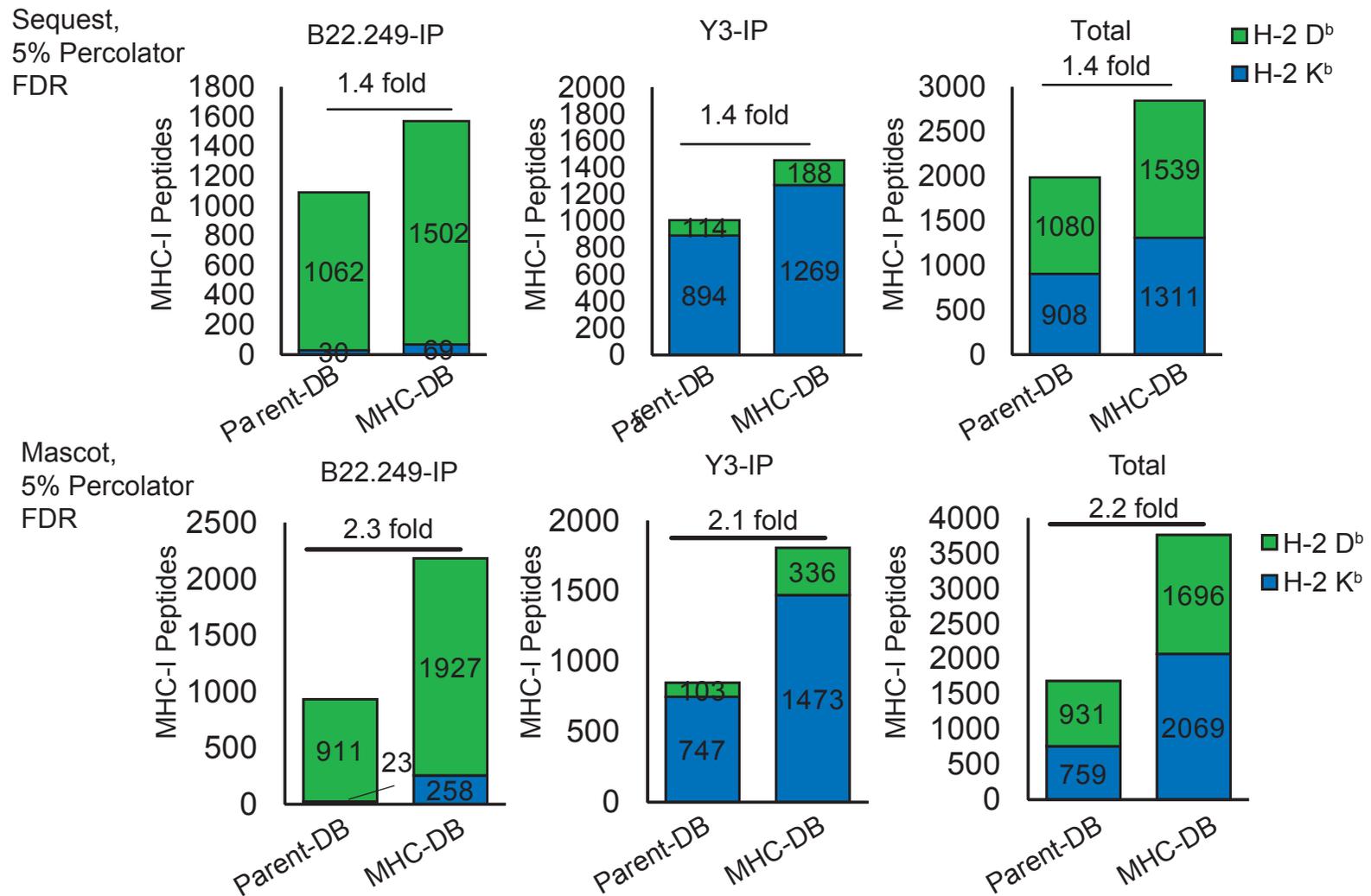


Supplementary Figure 2

A

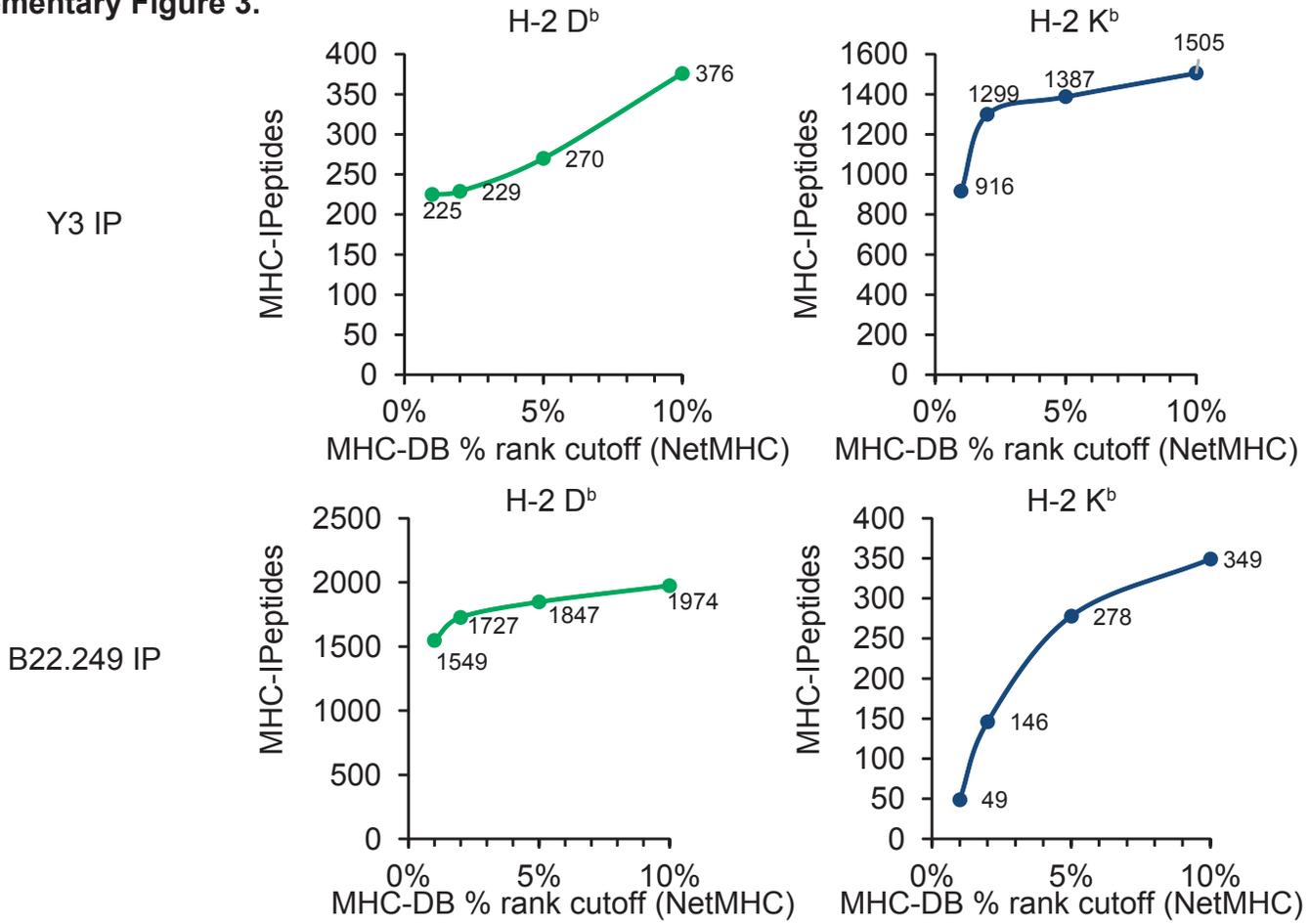


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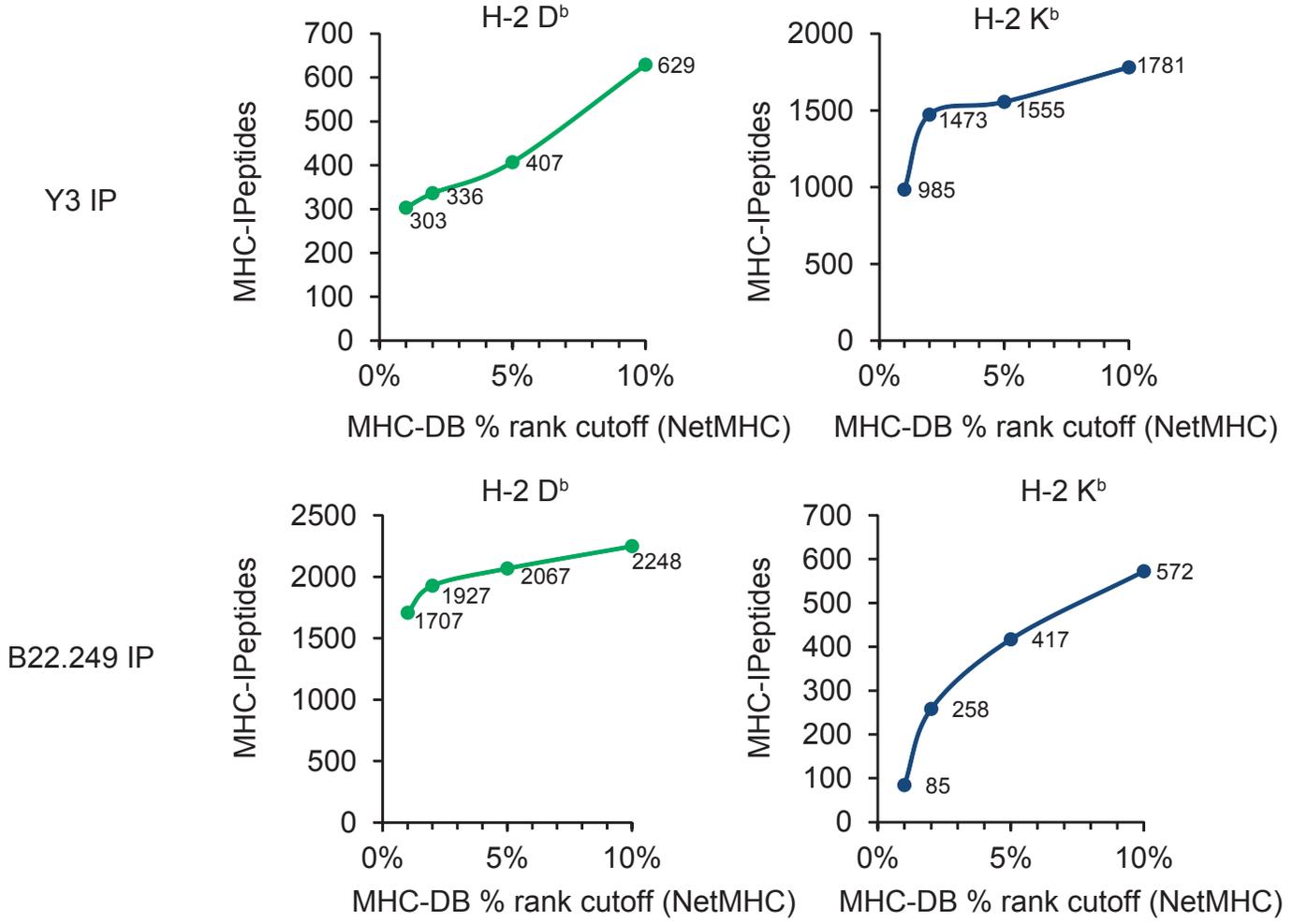


Supplementary Figure 3.

A

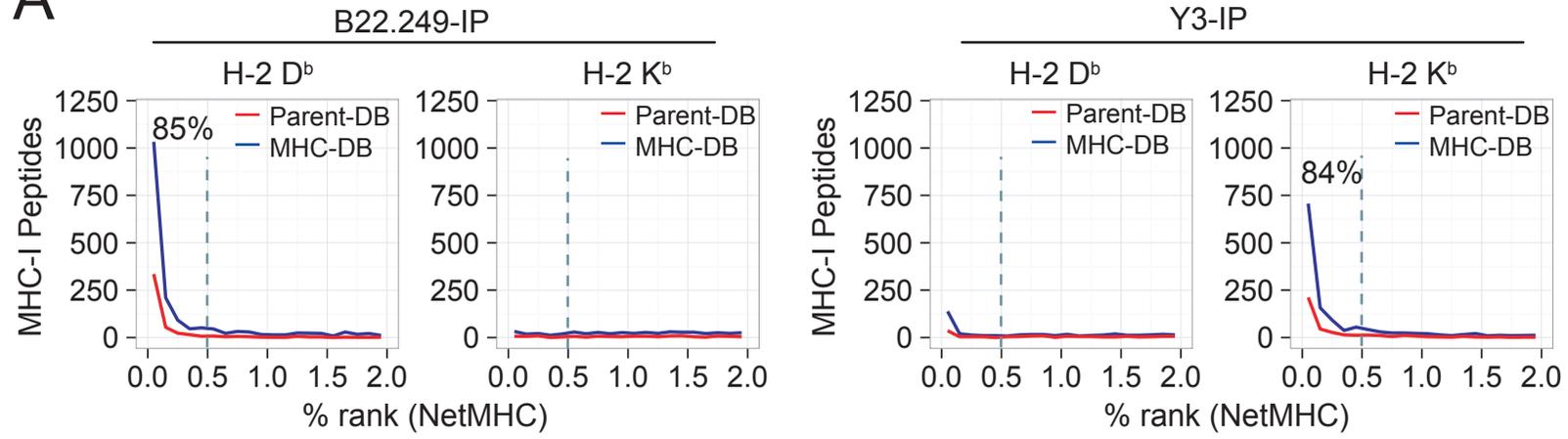


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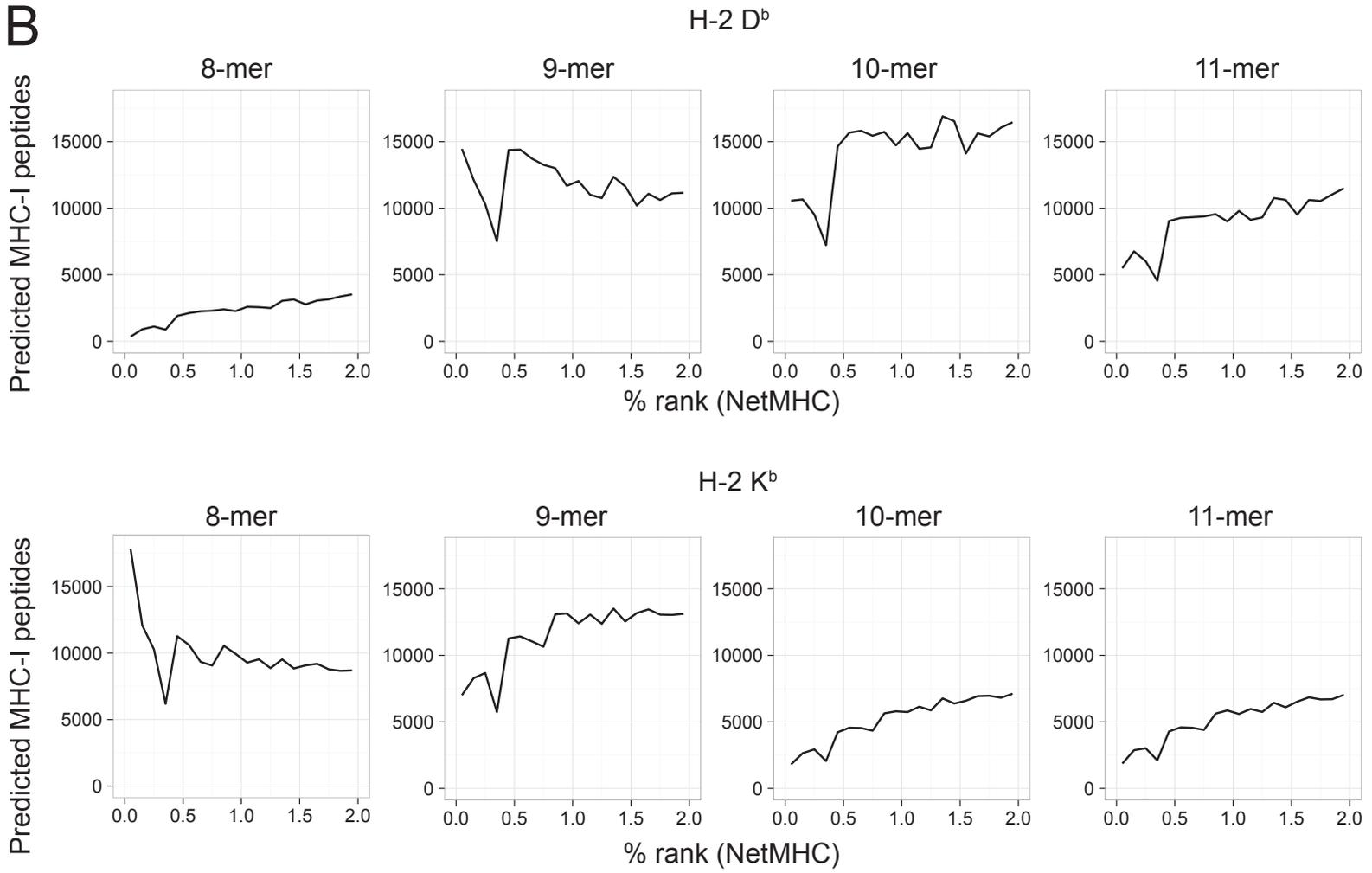


Supplementary Figure 4

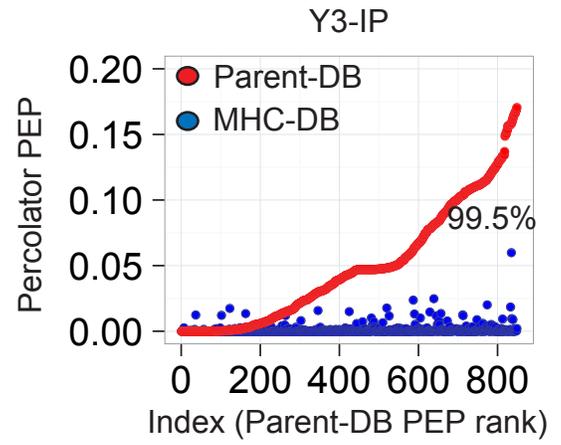
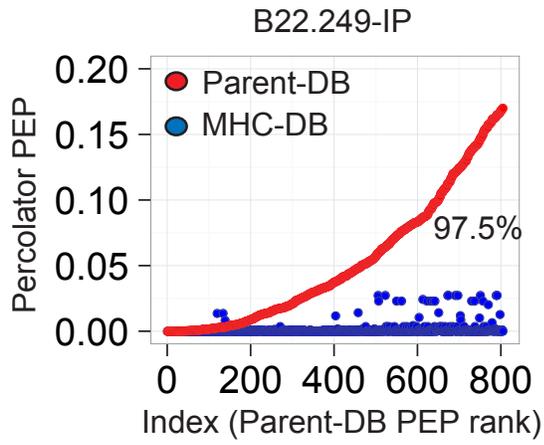
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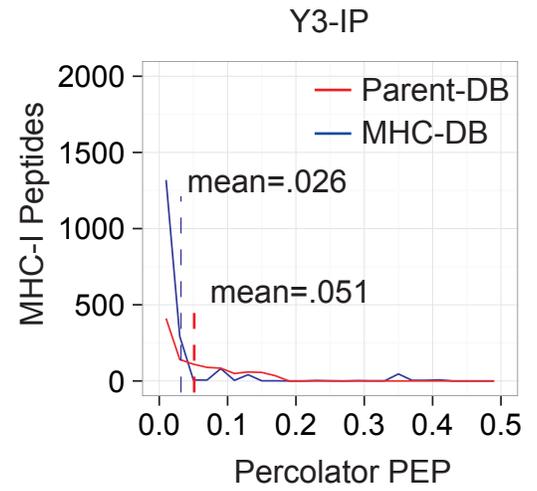
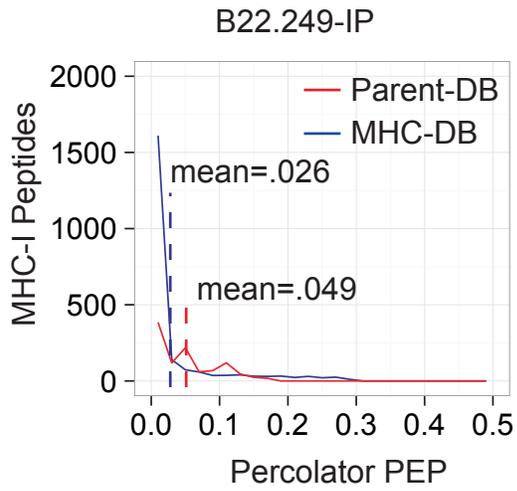
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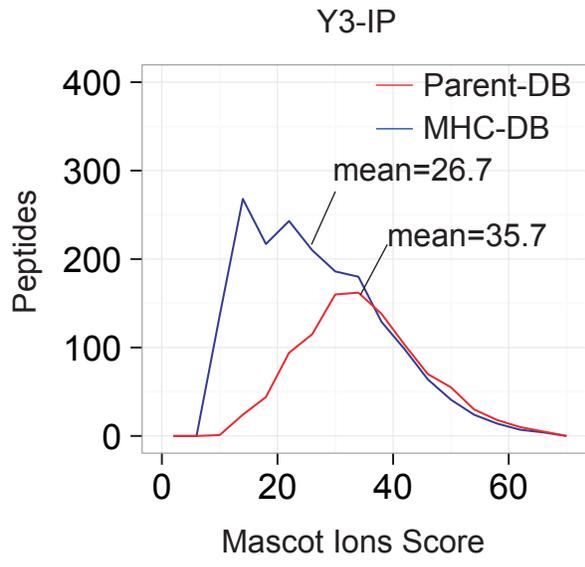
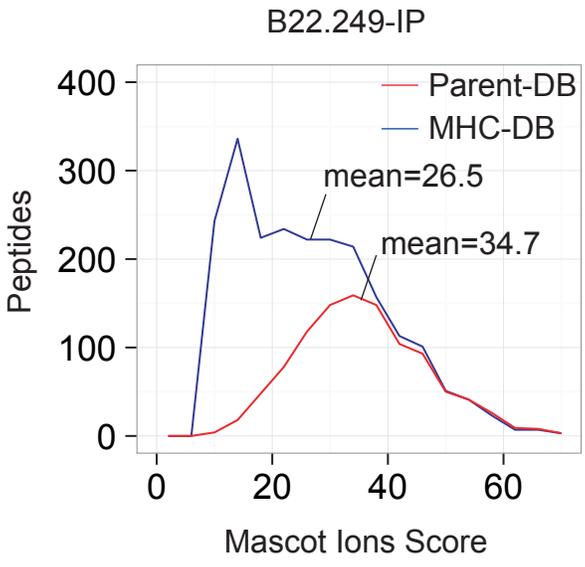
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B

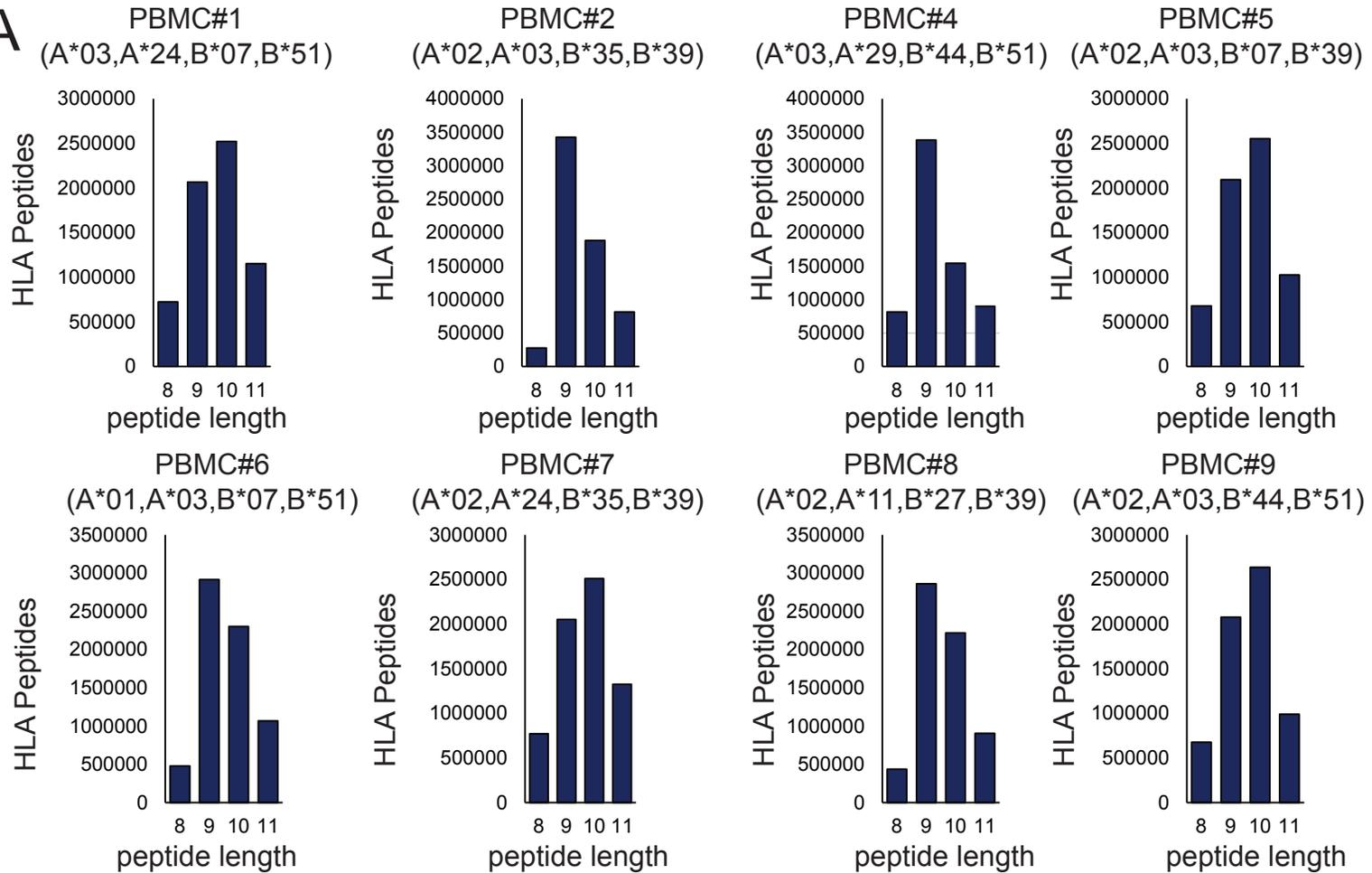


Supplementary Figure 6

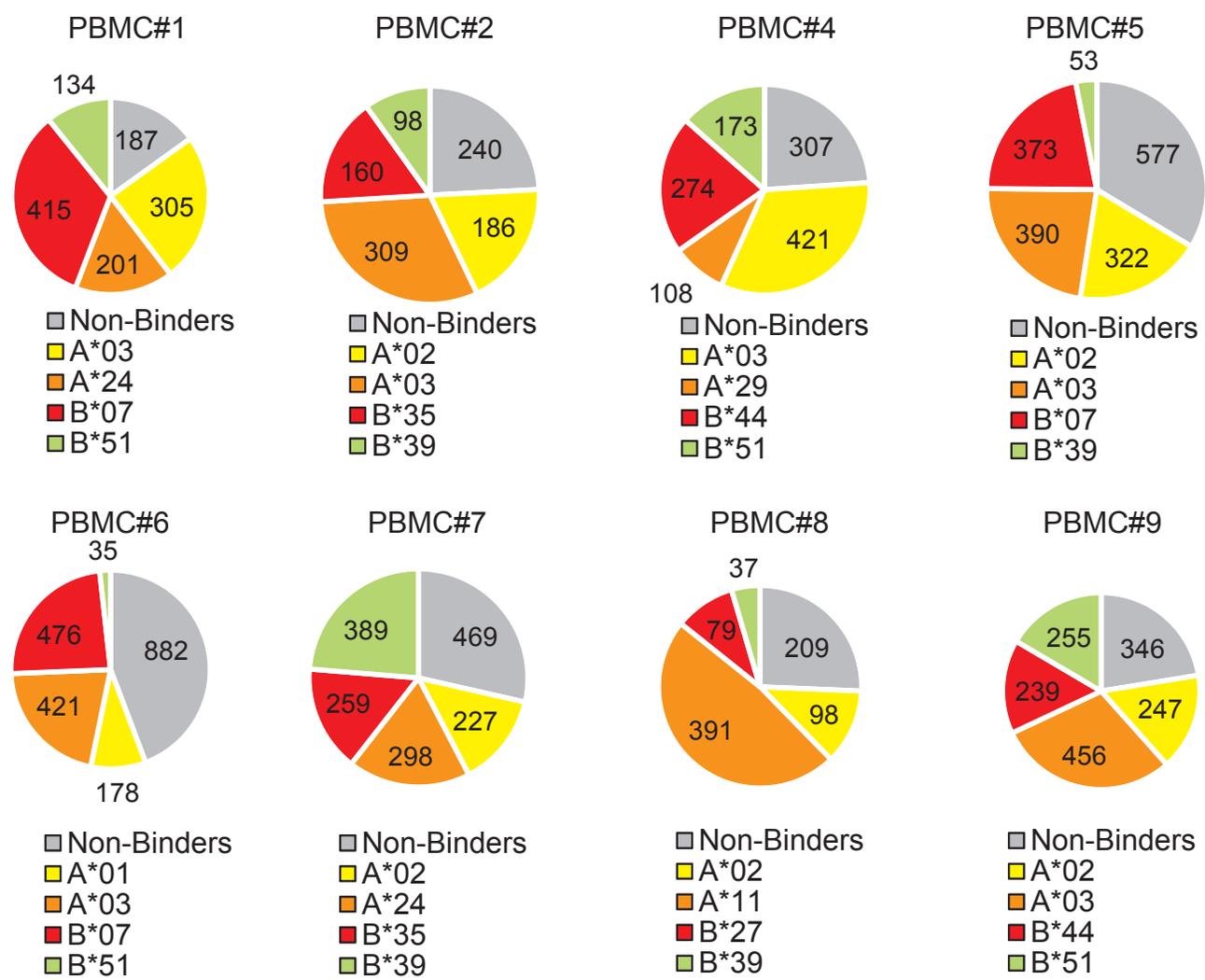


Supplementary Figure 7

A

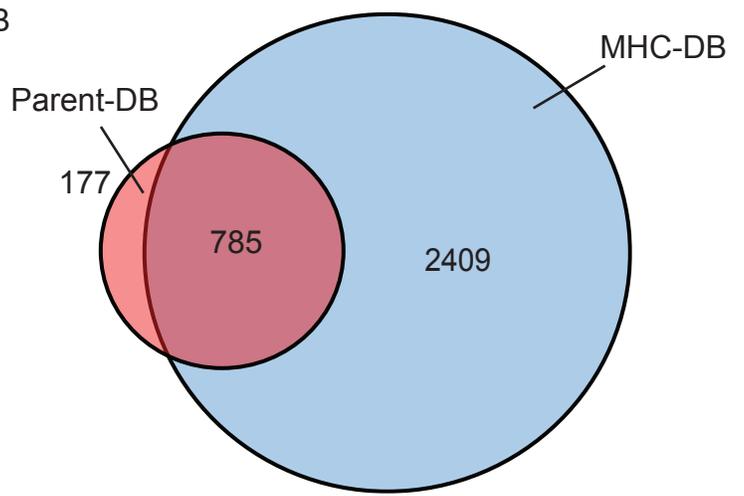
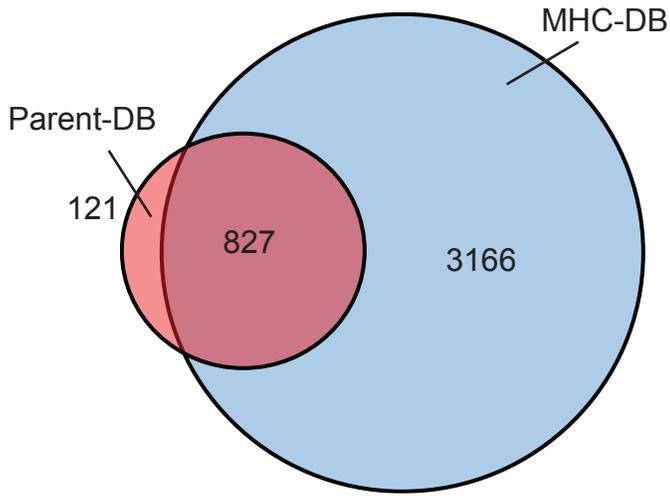


B



S-8

A



B

