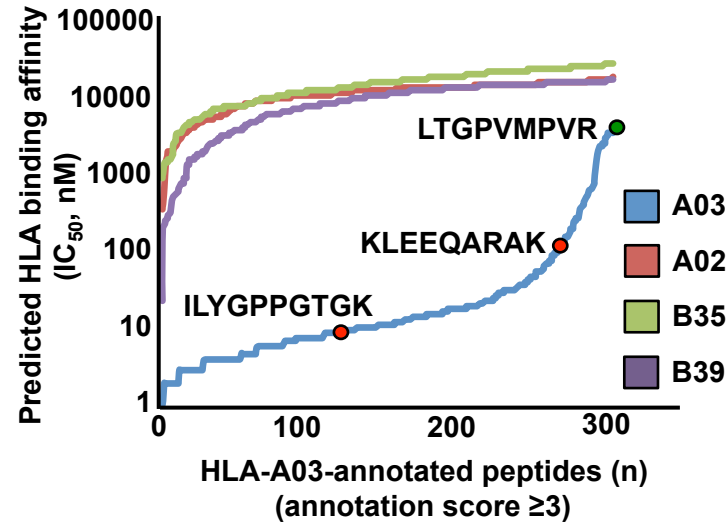


A

Peptide	Predicted HLA binding affinity (IC ₅₀ , nM)				Annotation score	HLA allele
	A02	A03	B35	B39		
ILYGPPGTGK	21158	12	33079	21637	1763	A03
KLEEQARAK	26350	278	36233	21400	77	A03
LTGPVMPVR	25402	6839	31339	21741	3	A03
TGGANLGRIG	28787	23561	35417	22213	1	Non-annotated

B



C

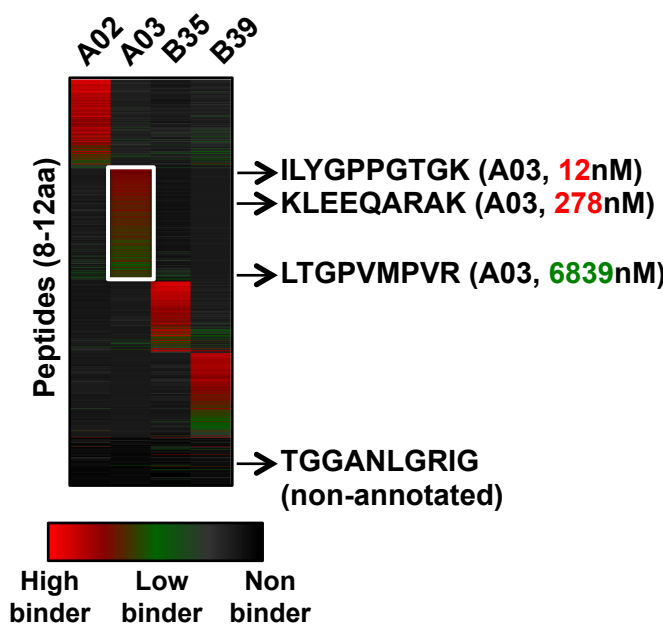


Figure 2—figure supplement 1. Automated NetMHC-based method for annotating and visualizing HLA allele-specific peptides. PBMC#2 was typed positive for HLA-A02, -A03, -B35, -B39, and is shown here as a representative sample. **(A)** The stand-alone software package of the HLA binding prediction algorithm NetMHC 3.4 was used to predict the binding affinity of all identified peptides to HLA-A02, -A03, -B35 and -B39 (four peptides are shown for simplicity). For each peptide, an annotation score was calculated by dividing the second lowest IC₅₀ value (second best predicted allele) by the lowest IC₅₀ value (best predicted allele). Peptides with a score ≥3 were annotated to the HLA allele predicted to bind best. Peptides with a score below 3 were considered as non-annotated. Non-annotated peptides were curated in the output files in Figure 2—source data 2 and correspond to 1) non-HLA peptides/contaminants, 2) peptides predicted to strongly bind more than one HLA allele (supertype peptides), 3) peptides predicted to bind HLA-C alleles, 4) exceptional HLA peptides with no known binding motifs. Annotation scores of all eluted peptides are shown in Figure 2—source data 2. Additional information is provided in Supplementary file 1. **(B)** Curves showing the distribution of the predicted HLA binding affinities for all HLA-A03-annotated peptides with a score ≥3. Overall, 91% of all HLA-A03-annotated peptides are predicted to have a binding affinity below 500 nM for the HLA-A03 molecule (see also Figure 2—figure supplement 4 and Figure 2—figure supplement 5). The same peptides are predicted to be non-binders for the other alleles – i.e., HLA-A02, -B35 and -B39. **(C)** Heat map visualization following clustering of predicted HLA binding affinity values. The white box highlights HLA-A03-annotated peptides. The four peptides in the table in (a) are indicated by arrows and their respective predicted binding affinity for the HLA-A03 molecule is indicated in parenthesis.