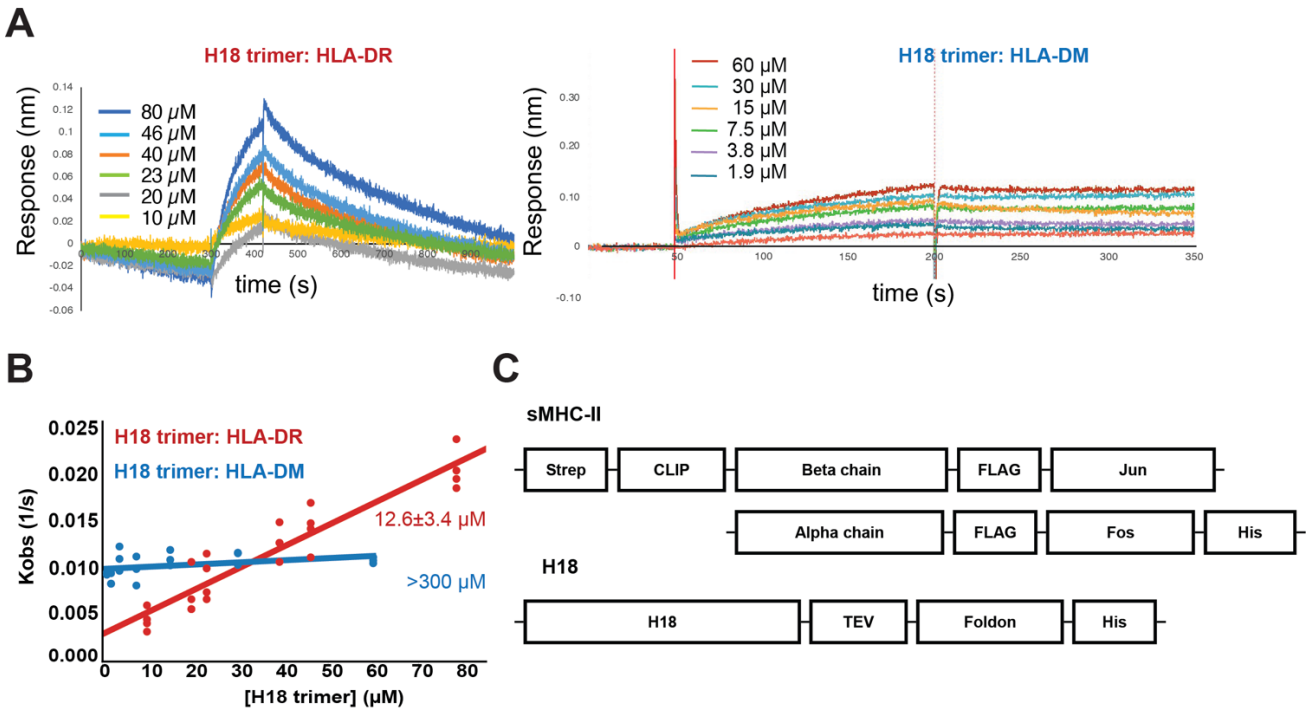
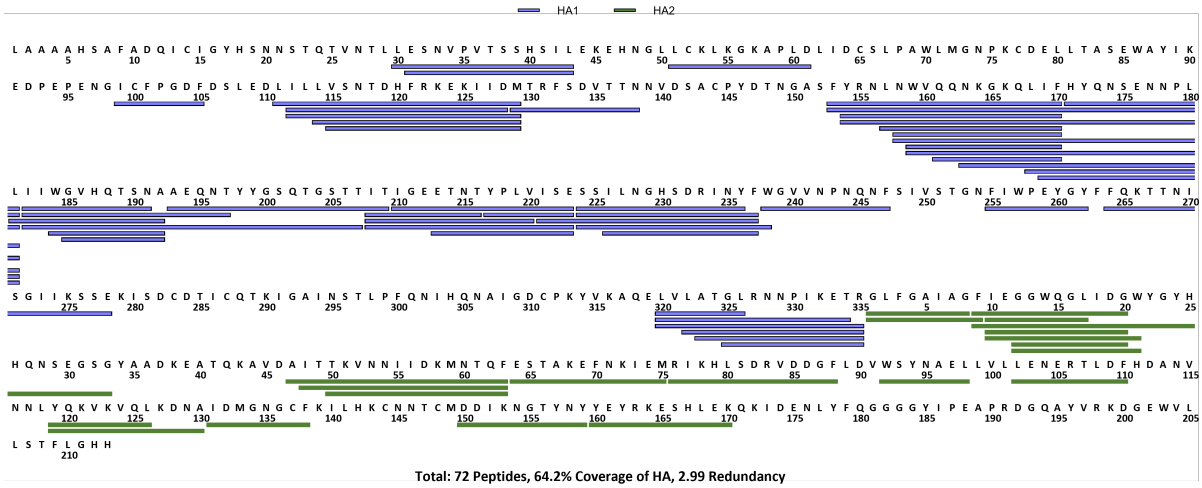
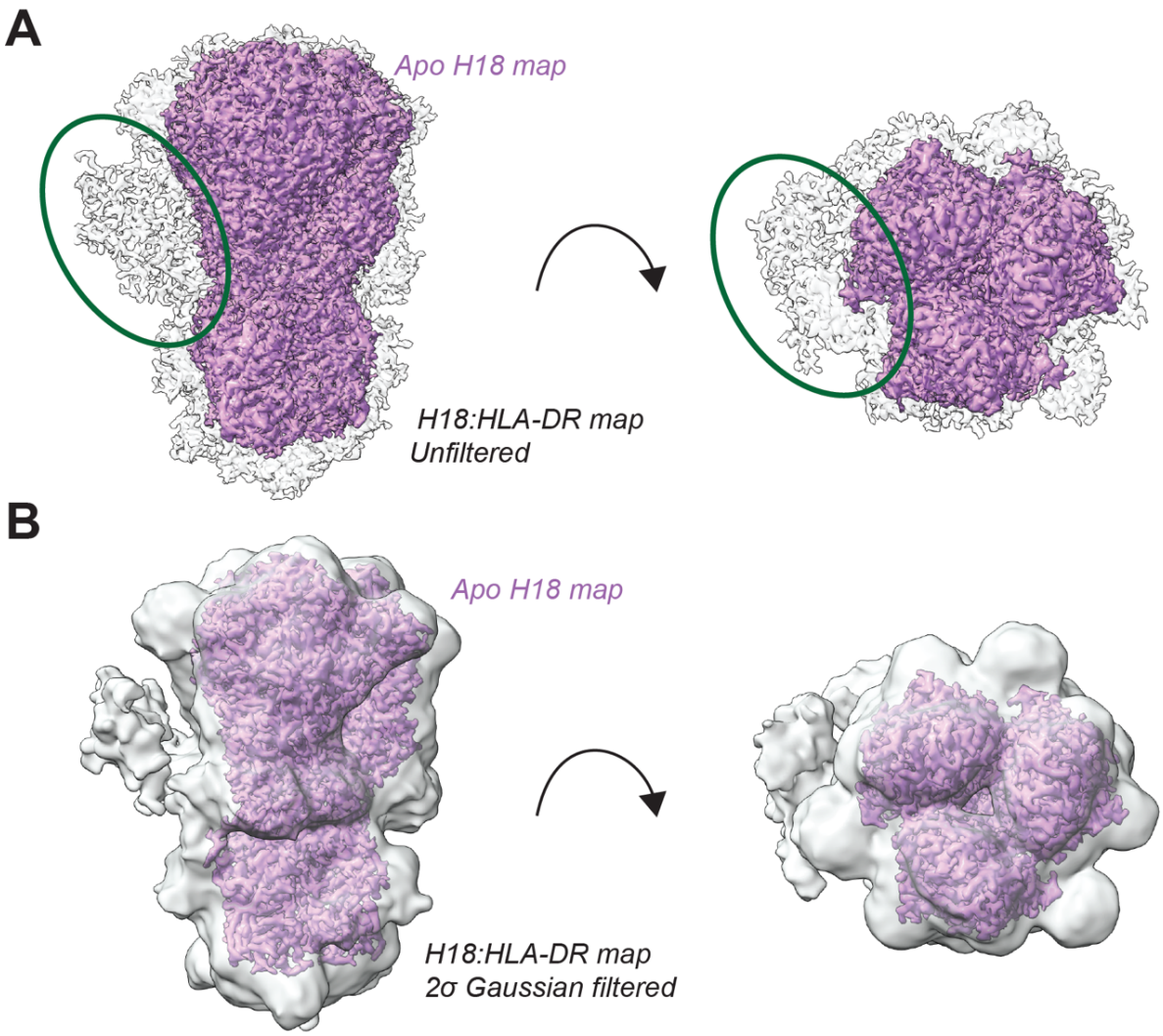


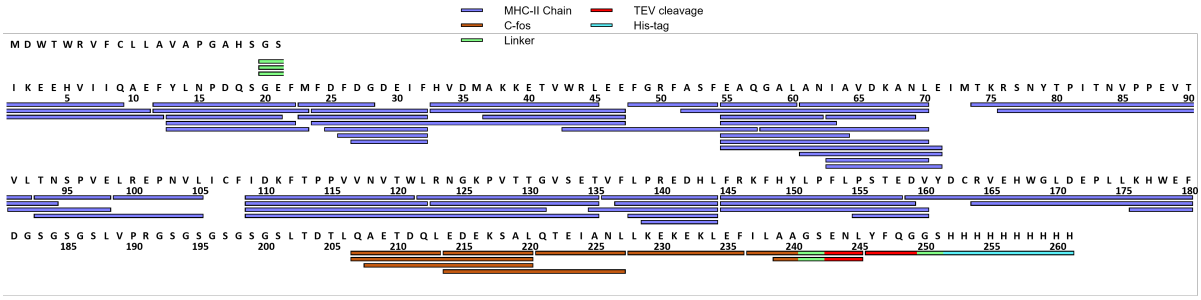
Extended Data Fig. S1. Binding of H18 trimer measured by biolayer interferometry. (A) Representative sensorgrams showing H18 trimer in mobile phase binding to immobilised MHC-II molecules (B) The corresponding kinetic analysis of the data. The shallow concentration dependence for HLA-DM produced an apparent dissociation constant of 430 μM but did not permit precise interpretation. Hence, binding is reported as > 300 μM, in line with the results obtained for the interaction between HLA-DR and the H18 head domain (Fig. 1C). (C) Schematic representation of the domain arrangements of the soluble MHC-II (sMHC-II) HLA-DR molecule and the trimeric H18 used in this study. The zipper motifs at the C-termini of HLA-DRA and HLA-DRB, Fos and Jun are used to enhance dimerization, the His- and Strep-tag are used for purification, the CLIP peptide is included for enhanced stability. The H18 has C-terminal foldon domain for enhanced trimerization.



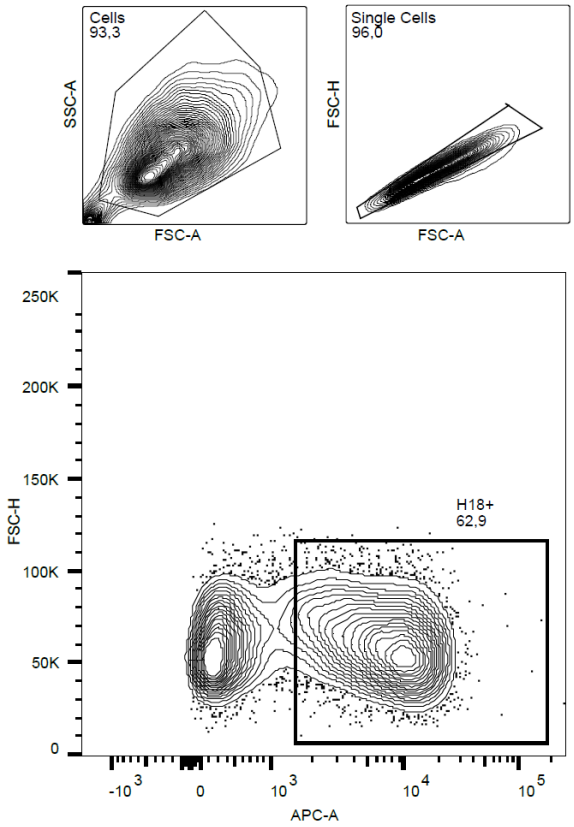
Extended Data Fig. S2. HDX sequence coverage of HA. The bars represent the peptides whose HDX was followed for HA1 (blue) and HA2 (green).



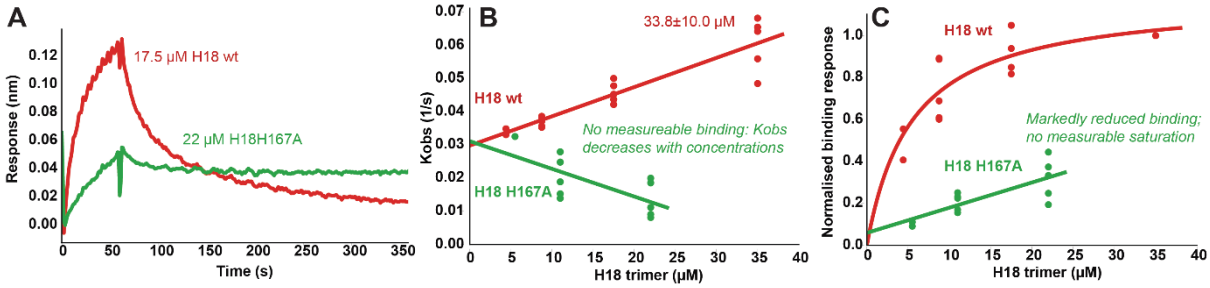
Extended Data Fig. S3. Cryo-EM maps of apo H18 and its complex with HLA-DR. (A) Unfiltered and (B) Gaussian-filtered at two-sigma maps of H18:HLA-DR complex (grey) compared to the sharpened maps of apo H18 from non-uniform refinement, without filtered applied (pink). The green oval in the top panels highlights the density not present in the apo map, which likely corresponds to HLA-DR.



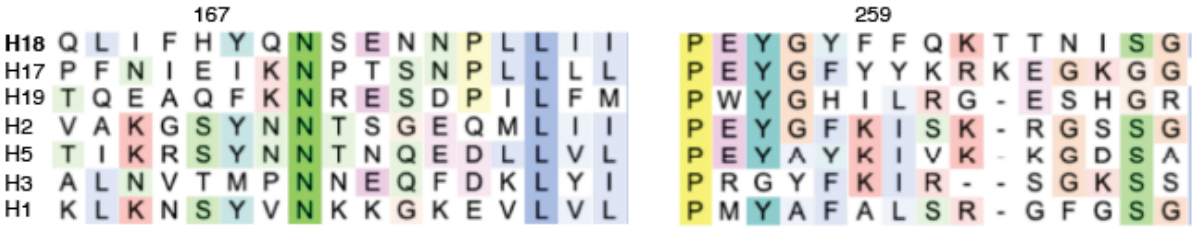
Extended Data Fig. S4. HDX sequence coverage of MHCII. The bars represent the peptides whose HDX was followed for the MHCII chains (blue) and for the additional tags and components of the protein sequence.



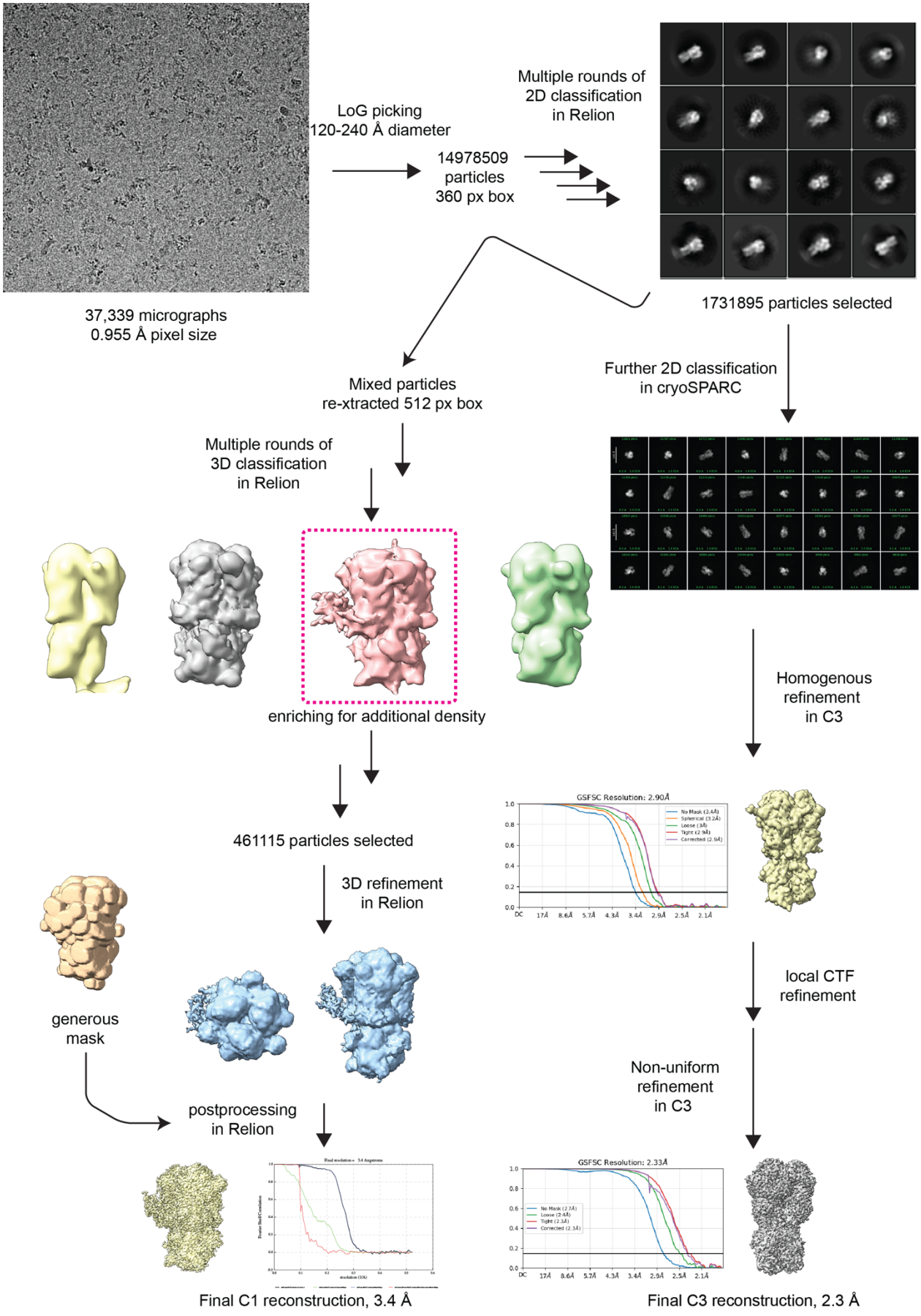
Extended data Fig. S5. Gating strategy used for flow-cytometric determination of H18 expression levels. After exclusion of doublets, H18 positive cells were quantified by fluorescent antibody staining (APC).



Extended Data Fig. S6. Binding of HEK293-expressed wt (red) vs H167A (green) H18 to HLA-DR measured by biolayer interferometry. (A) A comparison of reference-subtracted sensorgrams for the wt vs H167A mutant. (B) Kinetic analysis of the mutant vs wt H18 trimer binding to HLA-DR: points represent individual measurements; lines show fits. Mutant k_{obs}values decreased with concentration preventing affinity determination. The kinetic analysis of wt measurements on HEK293F-expressed H18 yields an apparent Kd of 33.8±10.0 µM – comparable with the ~20 µM determined for the Sf9-expressed trimers described in Fig. 1 and S1. (C) Analysis of the equilibrium response normalised to the highest wt concentration measured. The mutant produced reduced response that did not approach saturation suggesting very weak to no binding.



Extended Data Fig. S7. Comparison of sequences of MHCII-binding and non-binding influenza HAs around residue clusters key for HLA-DR binding identified in this study. Following sequences were aligned using Clustal Omega to H18 used in this study: [MHCII-binding] H17: AFC35428, H19: 0AAT8XUI6, H2: P03451 (A/Japan/305/1957), H5: Q80A28 (A/Chicken/Hong Kong/FY150/2001); H3: A0A097PF39 (A/Victoria/361/2011); and [MHCII-non-binding] H1: P03452, A/Puerto Rico/8/1934 H1N1)



Extended Data Fig. S8. Cryo-EM processing workflow.

Data Set	HA apo	HA bound to MHCII
HDX reaction details	Deuterated PBS, pH_{read} = 7.2, 80% D_2O	
HDX time course	7 s, 2 min, and 40 min at 23 °C; 5 h at 28 °C	
# of Peptides	72	
Sequence coverage	64%	
Average peptide length / Redundancy	13.53 / 2.99	
Replicates (technical)	3	2 or 3
Repeatability (average standard deviation)	0.0581	0.0455
Significant differences in HDX (98% CI)	ΔHDX ≥ 0.36 Da	

Table S1. HDX summary table for the experiment H18 trimer apo vs H18 trimer bound to MHCII.

Data Set	MHCII apo	MHCII bound to HA
HDX reaction details	Deuterated PBS, pH_{read} = 7.2, 75% D_2O	
HDX time course	6 s, 1 min, 10 min, 100 min and 7 h at 23 °C	
# of Peptides	67 for chain A; 68 for chain B	
Sequence coverage	96.7% of chain A; 92.9% of chain B	
Average peptide length / Redundancy	11.63/ 3.36 -chain A; 12.82/3.82 - chain B	
Replicates (technical)	3	3
Repeatability (average standard deviation)	0.0324	0.0290
Significant differences in HDX (99% CI)	ΔHDX ≥ 0.30 Da	

Table S2. HDX summary table for the experiment MHCII apo vs MHCII bound to H18 head domain.