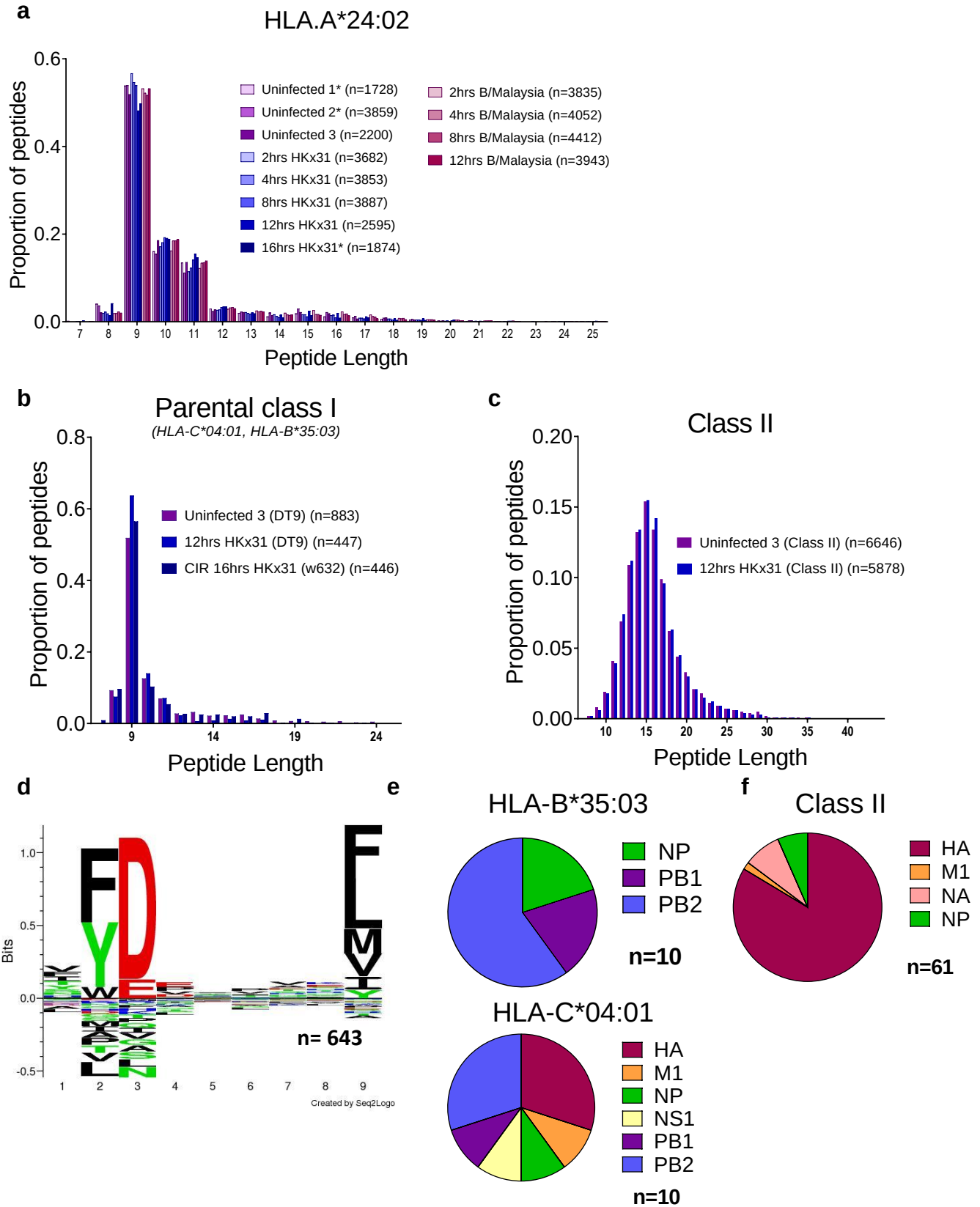


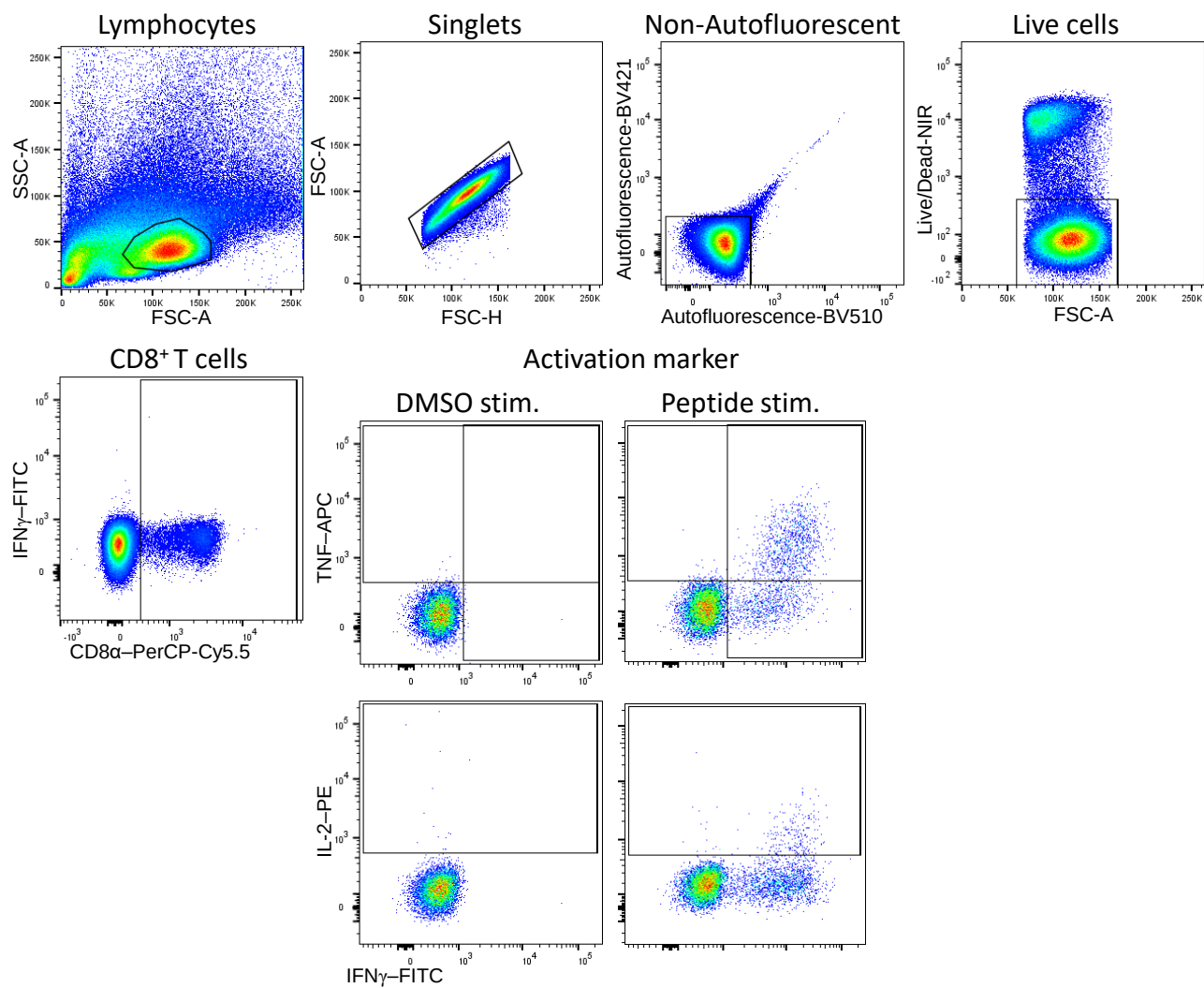
SUPPLEMENTARY INFORMATION

CD8⁺ T-cell landscape in Indigenous and non-Indigenous people restricted by influenza mortality-associated HLA-A*24:02 allomorph

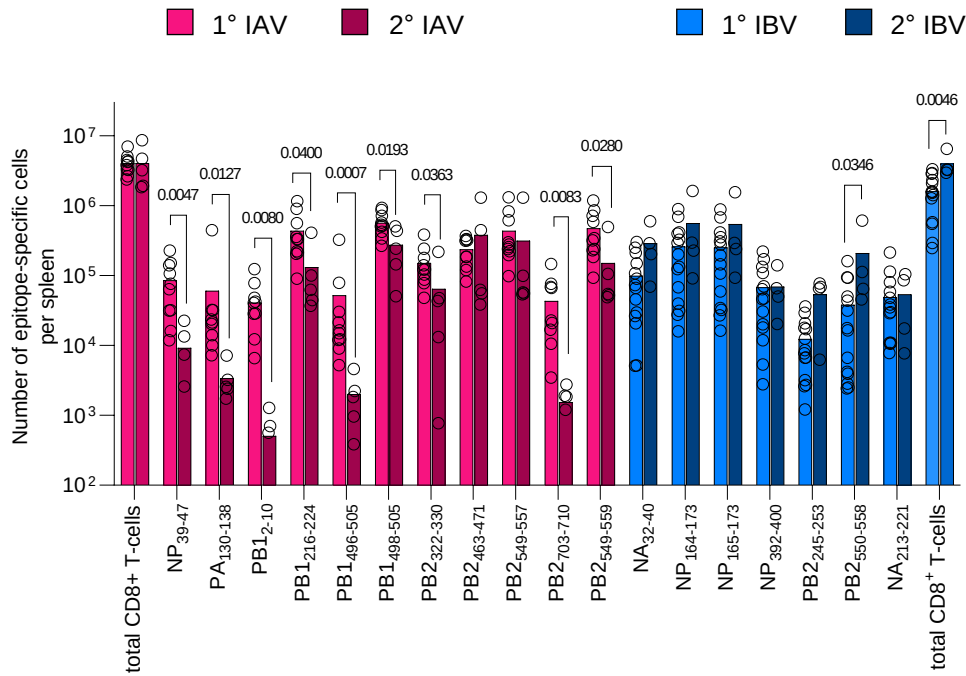
Luca Hensen^{1*}, Patricia T. Illing^{2*}, E. Bridie Clemens^{1*}, Thi H.O. Nguyen¹, Marios Koutsakos¹, Carolien E. van de Sandt^{1,3}, Nicole A. Mifsud², Andrea T. Nguyen², Christopher Szeto², Brendon Y. Chua¹, Hanim Halim², Simone Rizzetto⁴, Fabio Luciani⁴, Liyen Loh¹, Emma J. Grant^{1,2}, Phillipa M. Saunders¹, Andrew G. Brooks¹, Steve Rockman^{1,5}, Tom C. Kotsimbos^{6,7}, Allen C. Cheng^{8,9}, Michael Richards¹⁰, Glen P. Westall¹¹, Linda M. Wakim¹, Thomas Loudovaris¹², Stuart I. Mannering¹², Michael Elliott^{13,14}, Stuart G. Tangye^{15,16}, David C Jackson¹, Katie L Flanagan^{17,18,19,20}, Jamie Rossjohn^{2,21,22}, Stephanie Gras^{2,22,23}, Jane Davies²⁴, Adrian Miller²⁵, Steven Y.C. Tong^{10,24†}, Anthony W. Purcell^{2†} and Katherine Kedzierska^{†1#}



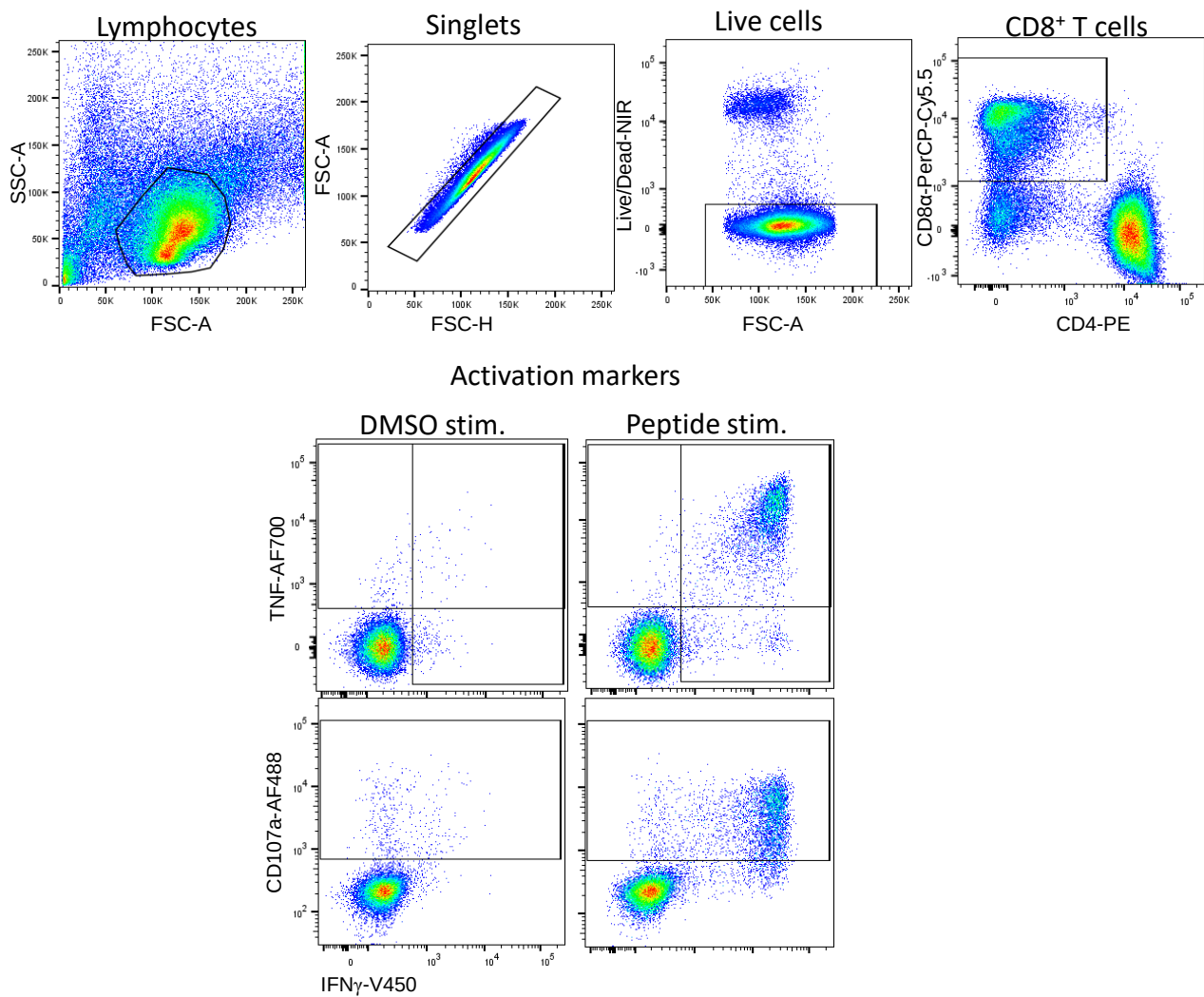
Supplementary Figure 1. Further analysis of LC-MS/MS data sets. (a-c) Peptide length distributions identified at a 5% FDR in each HLA isolation performed shown as a proportion of the peptides identified in the data set (n=number of peptides in the data set). For uninfected and HKx31 samples, data shown are based on searches against the human proteome, HKx31 proteome and 6 reading frame HKx31 genome translation. For B/Malaysia samples, data are based on searches against the human proteome, B/Malaysia proteome and 6 reading frame B/Malaysia genome translation. **(a)** Peptides from 12 data sets isolated from CIR.A24 using W6/32 (pan class I antibody) at different timepoints of infection with IAV or IBV (or uninfected). Asterisks represent data sets where DT9 was not used to deplete HLA-C*04:01 prior to W6/32 isolation and may contain increased levels of HLA-C*04:01 ligands. All data sets contain low levels of peptides presented by HLA-B*35:03 of CIR. **(b)** 3 data sets containing peptides isolated from the endogenous HLA class I of the CIR cell line, either through isolation from non-transfected CIR using W6/32 (CIR 16hrs HKx31 (W6/32)), hence containing HLA-C*04:01 and HLA-B*35:03, or through specific isolation of HLA-C*04:01 from CIR.A24 using the DT9 antibody. **(c)** HLA class II peptide ligands from 2 data sets isolated from CIR.A24 cell line using LB3.1 (HLA-DR), SPV-L3 (HLA-DQ), and B721 (HLA-DP) antibodies. **(d)** The sequence logo generated from human-derived 9mer peptides isolated in (b) (non-redundant by sequence, 5% FDR), and filtered for peptides identified in HLA class II isolations (c). **(e,f)** Pie charts showing the distribution of IAV (HKx31) derived peptides across the viral proteome potentially bound to HLA-B*35:03 and HLA-C*04:01 (e) and HLA-II of CIR cells (f). Details of HLA isolation antibodies, influenza peptide sequences, their confidence of sequence assignment, and potential binding assignment to the HLA molecules expressed by CIR.A24 are provided in **Supplementary Data 1**.



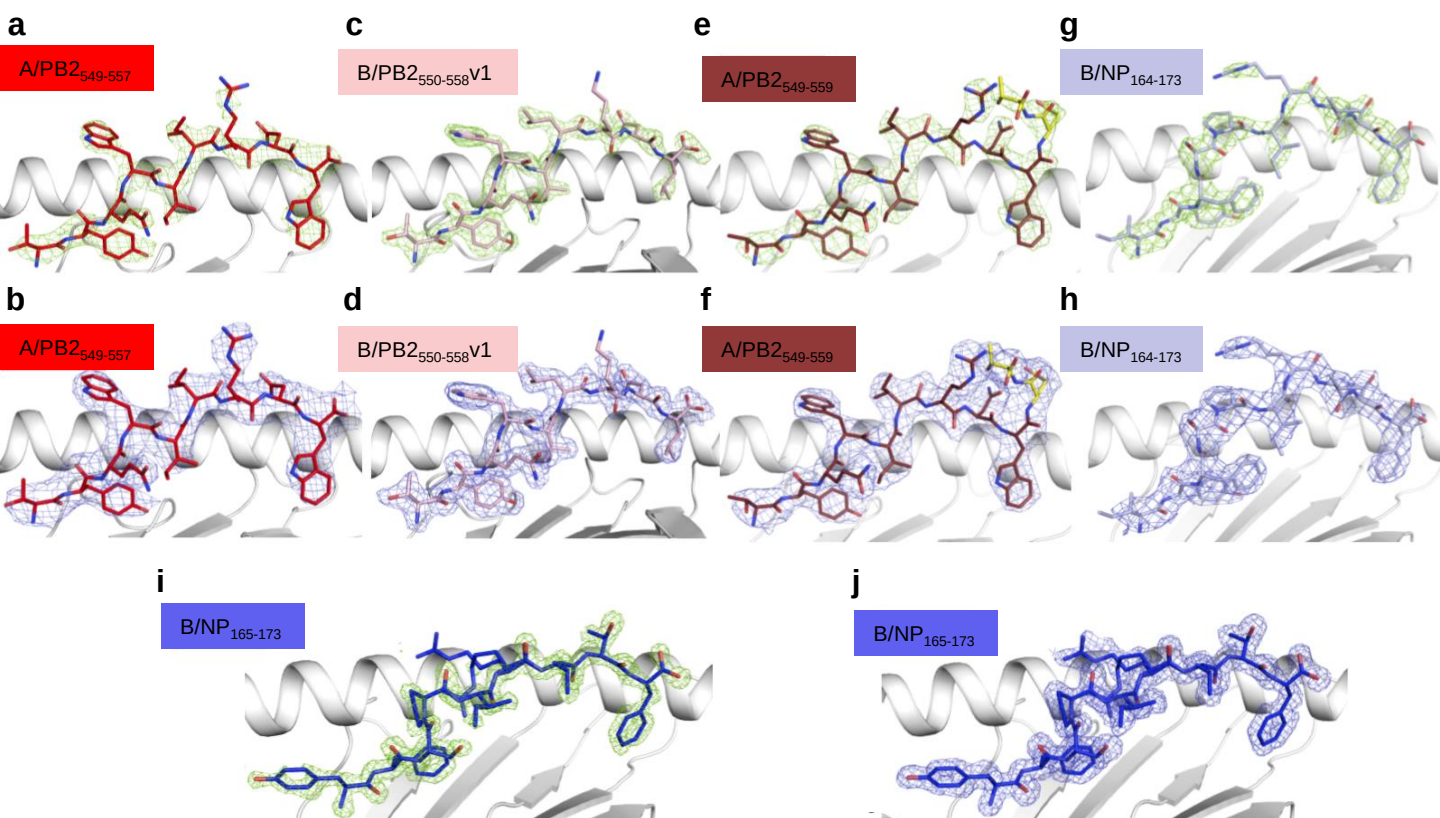
Supplementary Figure 2. Gating of mouse ICS results. Single cell suspensions were gated on live, lymphocytes without autofluorescence to assess expression of IFN- γ , TNF and IL-2 on CD8⁺ T cells after peptide stimulation.



Supplementary Figure 3. Comparison of epitope-specific CD8⁺ T-cells in primary and secondary IAV and IBV infection. Total number of epitope-specific CD8⁺ T-cells calculated by the frequency of IFN γ ⁺CD8⁺ T-cells in the INF- γ ICS assay multiplied by the total number of splenocytes per spleen (IAV primary n=10 mice from two independent experiments; IAV secondary n=5 mice from 1 experiment; IBV primary n=14 mice from three independent experiments; IBV secondary n=4 mice from 1 experiment). Statistical analysis was performed with a two-tailed Mann Whitney test. P values are indicated when <0.05.

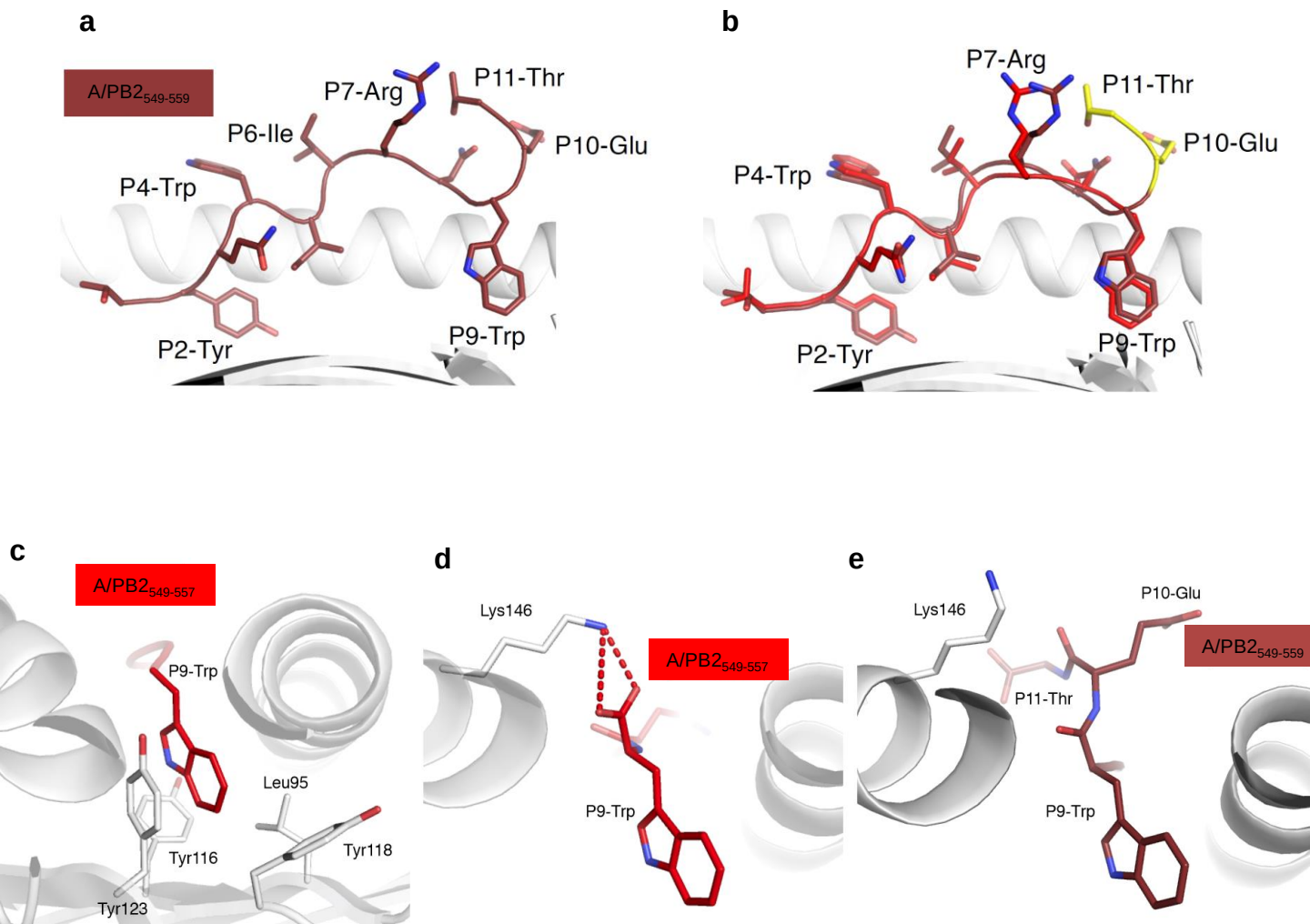


Supplementary Figure 4. Gating of human CD8⁺ T cell ICS. Single cell suspensions were gated on live, lymphocytes to assess expression of IFN- γ , TNF and CD107a on CD8⁺ T cells after peptide stimulation.

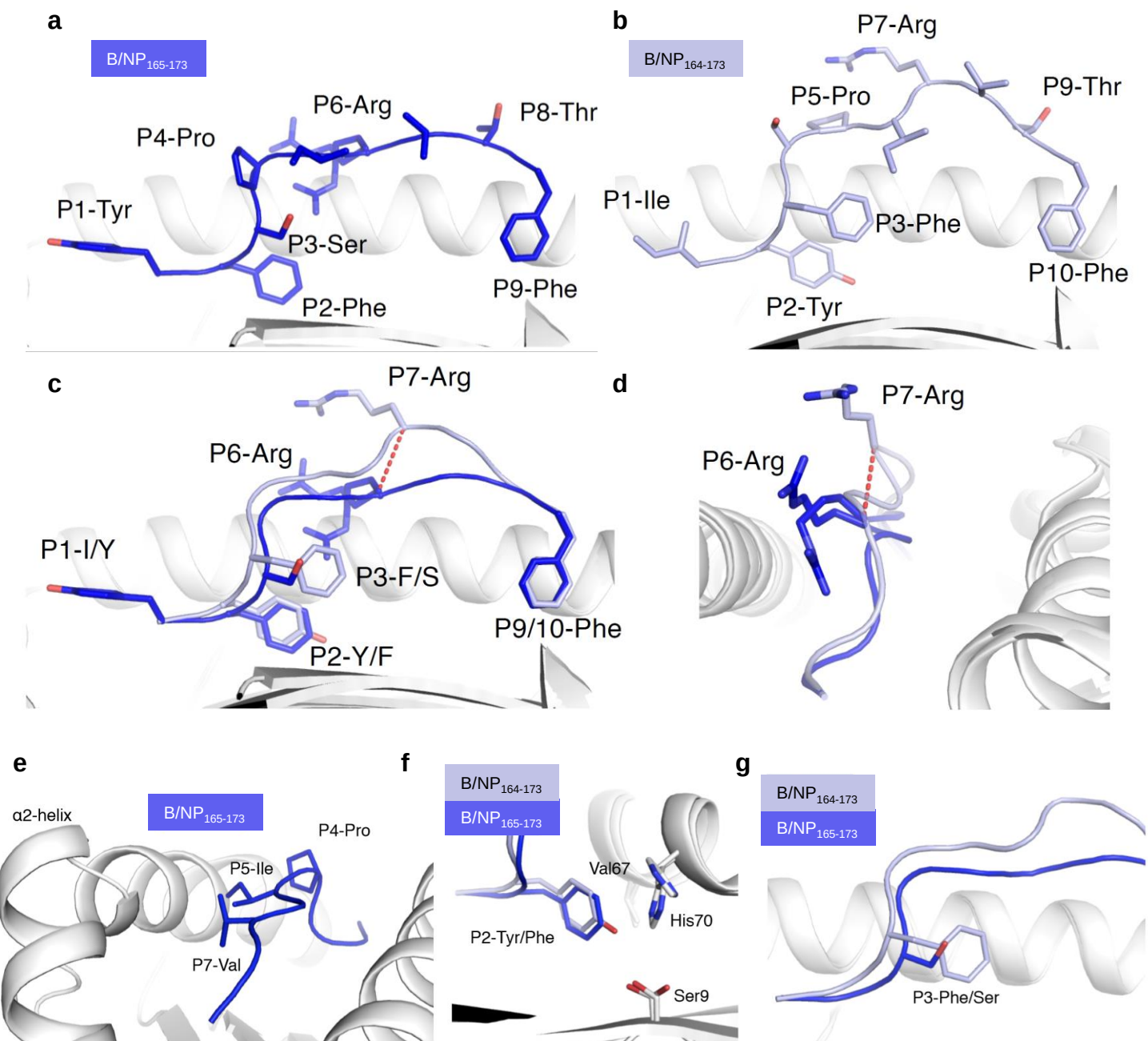


Supplementary Figure 5. Electron density maps of the peptide-HLA-A*24:02 structures.

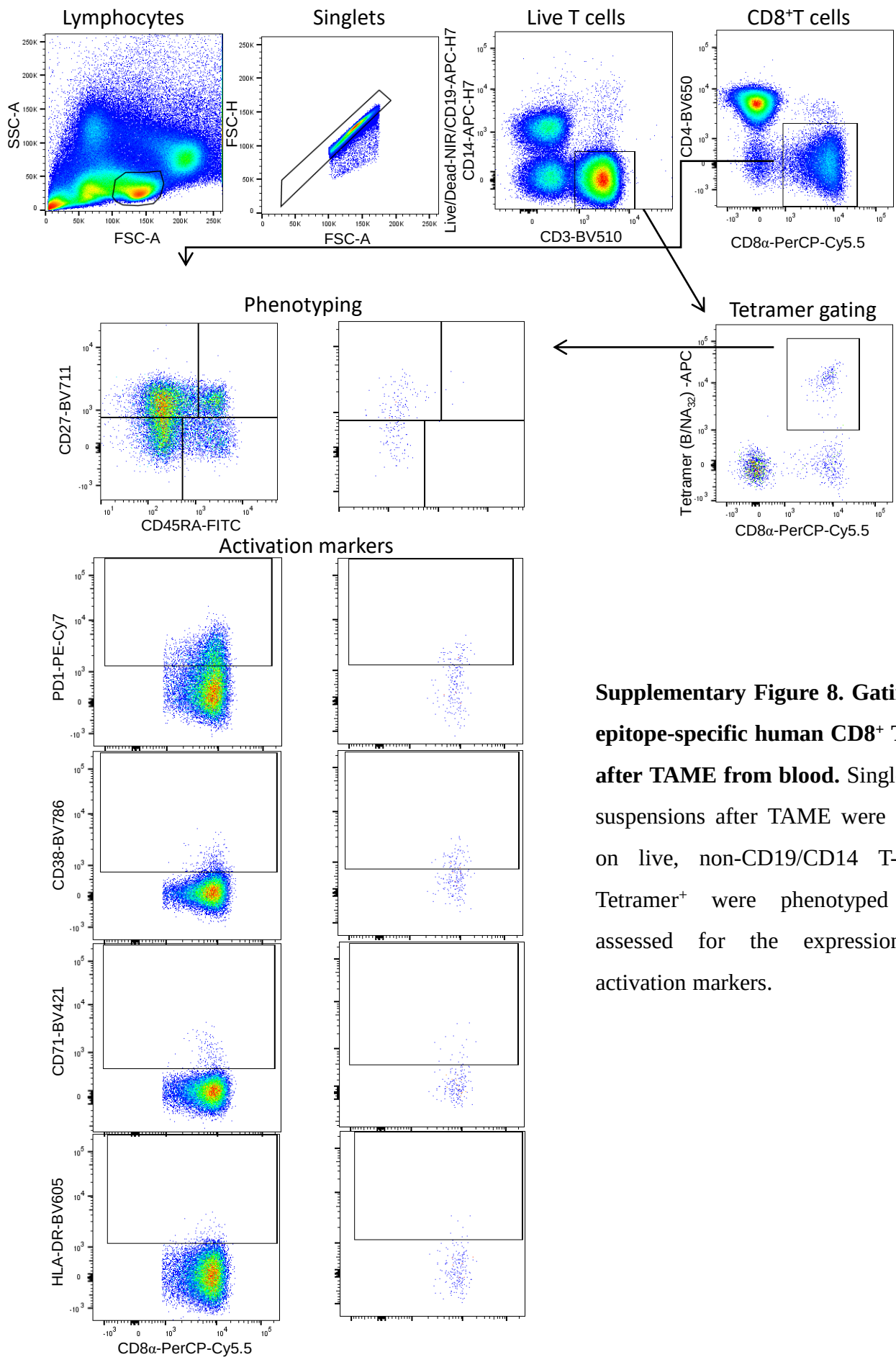
Peptide presentation in the antigen binding cleft of HLA-A*24:02 showing electron density omit maps (Fo-Fc) at 3.0σ (green) and electron density maps (2Fo-Fc) at 1.0σ (blue), for **a-d** and **i** and **e-h** and **j** respectively. The HLA-A*24:02 is represented as white cartoon, while the peptides are represented in sticks and coloured in red (A/PB2₅₄₉₋₅₅₇), pink (B/PB2₅₄₉₋₅₅₇v1), maroon (A/PB2₅₄₉₋₅₅₉), blue (B/NP₁₆₅₋₁₇₃), and light blue (B/NP₁₆₄₋₁₇₃).



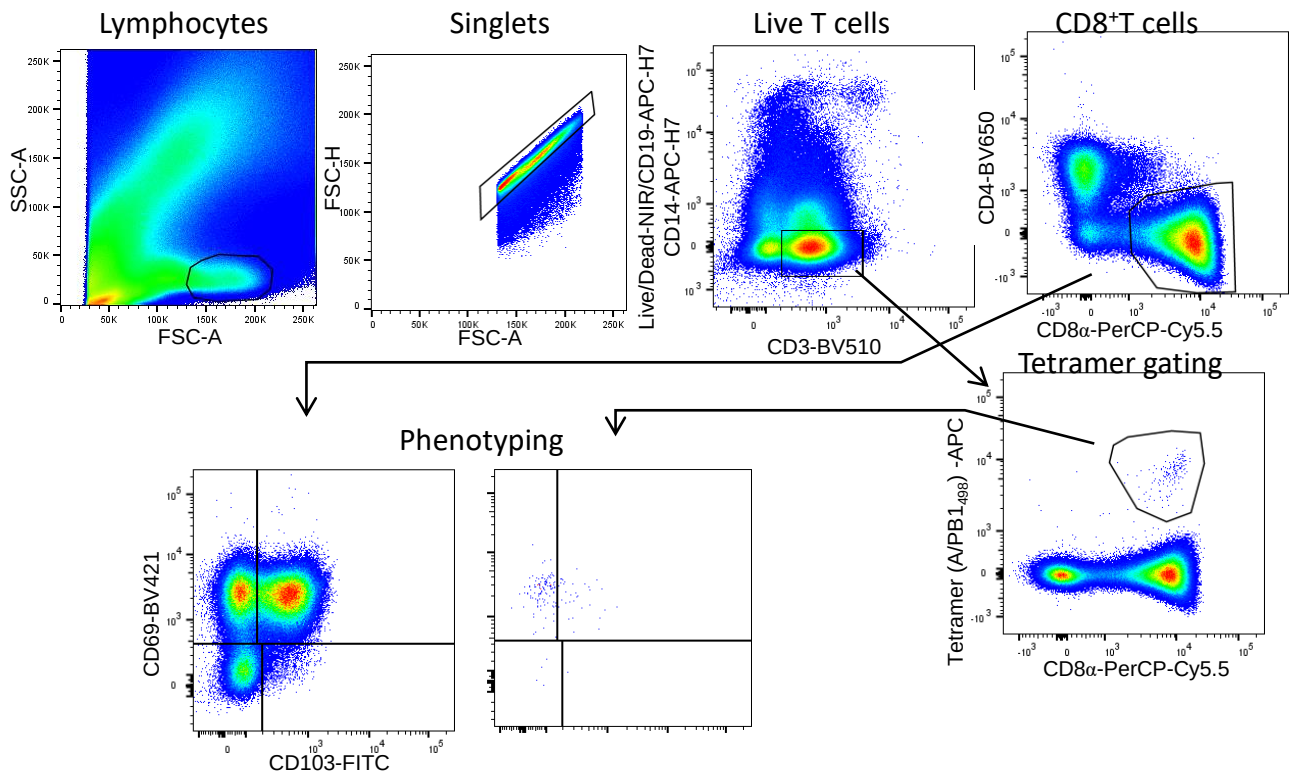
Supplementary Figure 6. Crystal structures of HLA-A*24:02 presenting the A/PB2₅₄₉₋₅₅₉ peptide. **(a)** Depiction of the PB2₅₄₉₋₅₅₉ peptide presented by HLA-A*24:02. **(b)** Overlay of A/PB2₅₄₉₋₅₅₇ (red) and PB2₅₄₉₋₅₅₉ (maroon) peptides presented by HLA-A*24:02. The two extra residues of the A/PB2₅₄₉₋₅₅₇ peptide are shown in yellow. **(c)** The P9-Trp of the A/PB2₅₄₉₋₅₅₇ peptide (red) is forming a network of interactions with Tyr at positions 116, 118 and 123 and Leu95 within the peptide-binding cleft. **(d)** Lys146 of the α 2-helix of HLA-A*24:02 also interacts with the carboxylic group of the P Ω residue of the A/PB2₅₄₉₋₅₅₇ 9mer peptide (red) but **(e)** faces outside of the cleft and does not interact with the P9-Trp of the A/PB2₅₄₉₋₅₅₉ 11mer peptide (maroon).



Supplementary Figure 7. Crystal structures of HLA-A*24:02 presenting B/NP₁₆₄₋₁₇₃ and B/NP₁₆₅₋₁₇₃ peptides. (a-c) Structures of B/NP₁₆₅₋₁₇₃ (a, blue stick), B/NP₁₆₄₋₁₇₃ (b, light blue stick) and (c) an overlay of B/NP₁₆₅₋₁₇₃ (blue) and B/NP₁₆₄₋₁₇₃ (light blue) presented on the HLA-A*24:02 molecule. (d) N-terminal side view overlay of B/NP₁₆₅₋₁₇₃ (blue) and B/NP₁₆₄₋₁₇₃ (light blue) peptides showing the largest structural difference at the C α atom of the P6/7-Arg shown by the red dashed line between B/NP₁₆₅₋₁₇₃ (blue) and B/NP₁₆₄₋₁₇₃ (light blue), respectively. (e) The P5-Ile and P7-Val of the B/NP₁₆₅₋₁₇₃ 9mer peptide (blue) are half-buried between the peptide backbone and the HLA α 2-helix, and together with the P4-Pro, form a hydrophobic patch at the centre of the peptide. (f) The substitution of P2-Tyr (B/NP₁₆₄₋₁₇₃) (light blue) for P2-Phe (B/NP₁₆₅₋₁₇₃) (blue) occurs without major structural rearrangement of the B pocket residues (white sticks), as both residues are large and aromatic. (g) The additional residue of B/NP₁₆₄₋₁₇₃ (light blue) changes the secondary anchor residue at P3 of the peptides from a small P3-Ser (B/NP₁₆₅₋₁₇₃) to a large P3-Phe (B/NP₁₆₄₋₁₇₃).



Supplementary Figure 8. Gating of epitope-specific human CD8⁺ T cell after TAME from blood. Single cell suspensions after TAME were gated on live, non-CD19/CD14 T-cells. Tetramer⁺ were phenotyped and assessed for the expression of activation markers.



Supplementary Figure 9. Gating of epitope-specific human CD8⁺ T cell after TAME from tissues. Single cell suspensions after TAME were gated on live, non-CD19/CD14 T-cells. Tetramer⁺ were phenotyped.

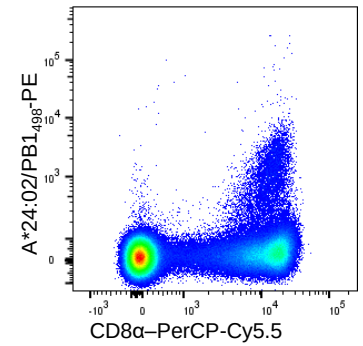
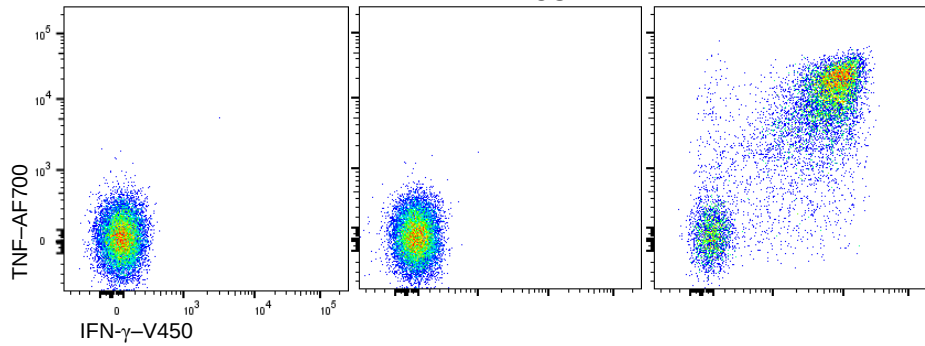
Ex vivo peptide stimulation

Ex vivo tetramer staining

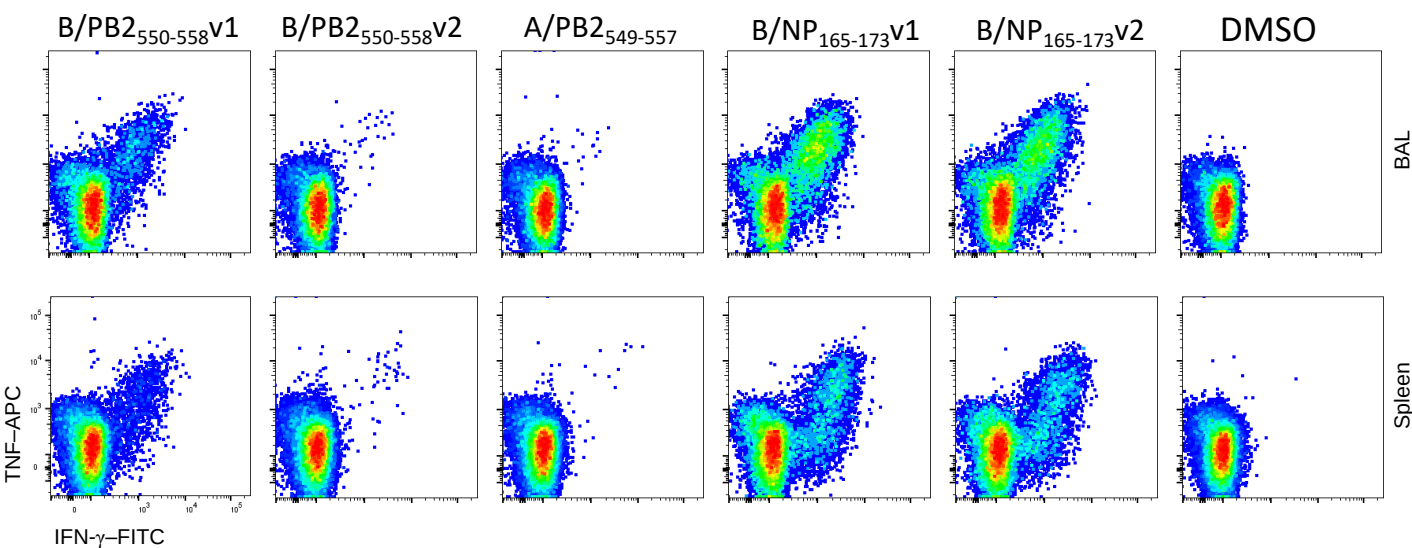
DMSO

PB1₄₉₈

PMAI



Supplementary Figure 10. Response to *ex vivo* A/PB1₄₉₈₋₅₀₅ peptide stimulation in unconventional Tetramer binding donor. PBMCs from non-LIFT8 were stimulated with A/PB1₄₉₈₋₅₀₅ peptide, DMSO (neg. control) or PMA+Ionomycin (pos. control) for a 6 h ICS to assess if unconventional *ex vivo* binding Tetramer⁺CD8⁺ T cells are peptide reactive.



Supplementary Figure 11. Cross-reactive of CD8⁺T cells in HHD-A24 after primary B/Malaysia infection. HHD-A24 were infected with B/Malaysia/2506/04 and 10 days after infection single cell suspensions stimulated in an ICS with the B/Malaysia/2506/04 derived peptide variant (V1) or with other naturally occurring variants derived by IBV or IAV.

Supplementary Data 1. Peptides identified from IAV (HKx31) and IBV (B/Malaysia)

Sequences of IAV (HKx31) and IBV (B/Malaysia)-derived peptides identified by LC-MS/MS analysis of the HLA class I and II immunopeptidomes of CIR and CIR.A24. For each peptide, the modifications with which it was identified, source protein, start site within the source protein, and the confidence of assignment for the data sets within which it was identified, are noted. For each data set, the cell line, infecting virus, antibody used (and any antibody depletion prior), time of infection and confidence cut-off for a 5% FDR are shown. Identifications above this confidence in each data set are in bold, those below are in italics and have increased likelihood of being false positive identifications. Naming of data sets match those in Supplementary Fig. 1. The predicted binding affinities (nM) and %rank for HLA-A*24:02, HLA-B*35:03 and HLA-C*04:01 for all 8-14mer peptides as calculated by NetMHCpan4.0 are shown. For HKx31, data sets derived from the sequential isolation of HLA from the same sample are noted. For B/Malaysia, previous identification in HLA isolations from B/Malaysia infected CIR and CIR.A*02:01 in Koutsakos *et al.*²⁴ are also noted. Colour fill represents isolations with w632 (blue), DT9 (yellow) and mixed class II antibodies (green). The “Best Explanation” column denotes the HLA hypothesised to present a given peptide based on appearance across the data sets and predicted binding by NetMHCpan4.0.

Supplementary Table 1. Donor demographics

Donor ID	Age group [years]	Sample	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DPB1	HLA-DQB1	IAV peptide expansion	IBV peptide expansion	IBV virus expansion	IAV virus expansion	TAME
LIFT1	40-60	PBMC	24:02, 34:01	15:21, 56:01	01:02, 04:03	04:12, 14:08	05:01, 16:01	04:02, 05:03	X				
LIFT2	40-60	PBMC	24:02	13:01, 56:01	01:02, 04:01	08:03, 14:02	04:01, 05:01	03:01, 06:01		X			
LIFT3	60-80	PBMC	24:02	13:01, 40:02	01:02, 04:01	08:03	02:01, 04:01	06:01	X				
LIFT4	60-80	PBMC	11:01, 24:02	39:01, 40:10	04:03, 12:03	08:03	02:01, 05:01	06:01	X				
LIFT5	18-40	PBMC	02:01, 24:02	15:21, 56:01	04:03, 07:02	04:05, 14:08	05:01, 14:01	04:01, 05:03		X			
LIFT6	18-40	PBMC	02:01, 24:02	07:02, 40:06	03:04, 07:02	11:01, 15:01	05:01	03:01, 06:02	X				
LIFT7	40-60	PBMC	01:01, 24:02	08:01, 15:21	04:03, 07:01	03:01, 14:07	14:01, 16:01	02:01, 05:03		X			
LIFT8	18-40	PBMC	24:02	40:01, 56:02	01:02, 03:03	08:03, 15:02	05:01	06:01		X			
LIFT9	18-40	PBMC	24:02, 34:01	07:02, 56:01	01:02, 07:02	04:12, 15:01	02:01, 04:01	04:02, 06:02		X			
LIFT10	18-40	PBMC	24:02	40:01, 40:02	01:02, 03:03	08:03, 15:02	02:01, 05:01	06:01	X				
LIFT11	18-40	PBMC	24:02, 34:01	13:01	04:01, 15:02	04:12, 12:01	05:01	03:01, 04:02		X			
LIFT12	18-40	PBMC	11:01, 24:02	13:01, 40:02	04:01, 15:02	04:05, 08:03	02:01, 04:01	03:01, 06:01		X			
LIFT13	18-40	PBMC	24:02	13:01, 56:01	01:02,03:03	14:08	04:01	05:03		X			
LIFT14	18-40	PBMC	24:02, 24:03	40:01, 52:01	03:03, 12:02	04:12, 15:02	04:01, 05:01	04:02, 06:01					X
LIFT15	18-40	PBMC	24:02	40:01, 56:02	01:02, 03:03	08:03	02:01	05:03, 06:01					X
LIFT16	18-40	PBMC	01:01, 24:02	56:01, 57:01	01:02, 06:02	07:01,15:02	04:01, 05:01	03:01, 03:02					X
LIFT17	18-40	PBMC	24:02, 24:06	40:01, 56:01	01:02, 03:03	04:12, 14:34	05:01	04:02, 05:03					X
Non-LIFT1	18-40	PBMC	01:01, 24:02	08:01, 14:02	n.D.	n.D.	n.D.	n.D.	X				
Non-LIFT2	18-40	PBMC	03:01, 24:02	35:03, 44:02	n.D.	n.D.	n.D.	n.D.	X				
Non-LIFT3	n.D.	PBMC	02:07, 24:02	46:01, 58:01	n.D.	n.D.	n.D.	n.D.	X				
Non-LIFT4	n.D.	PBMC	03:01, 24:02	15:01, 47:01	n.D.	n.D.	n.D.	n.D.	X				
Non-LIFT5	n.D.	PBMC	24:02	40:02, 51:01	n.D.	n.D.	n.D.	n.D.	X				
Non-LIFT6	40-60	PBMC	03:01, 24:02	15:01, 38:01	03:03, 12:03	13:01	n.D.	n.D.		X	X	X	
Non-LIFT7	40-60	PBMC	02:01, 24:02	15:01, 35:02	01:02, 04:01	08:01, 11:04	n.D.	n.D.		X	X	X	
Non-LIFT8	40-60	PBMC	24:02	07:02	07:02	01:01, 10:01	n.D.	n.D.		X	X	X	X
Non-LIFT9	18-40	PBMC	01:01, 24:02	35:01, 57:01	04:01, 06:02	01:01, 14:54	n.D.	n.D.		X	X	X	
Non-LIFT10	18-40	PBMC	02:07, 24:02	46:01, 67:01	01:02, 07:02	08:03, 09:01	n.D.	n.D.		X	X	X	X
Non-LIFT11	40-60	PBMC	24:02, 25:01	07:02, 18:01	n.D.	n.D.	n.D.	n.D.	X				X
Non-LIFT12	18-40	PBMC	02:03, 24:02	27:06, 40:01	03:04, 04:01	n.D.	n.D.	n.D.					X
Non-LIFT13	18-40	PBMC	01:01, 24:02	08:01, 40:01	03:04, 07:01	04:04/23, 11:01	n.D.	n.D.					X
Non-LIFT 14	18-40	PBMC	01:01, 24:02	44:02, 57:01	06:02, 16:04	04:02, 07:01	n.D.	n.D.					X
IAV-Inf 1	40-60	PBMC	01:01, 24:02	08:01, 57:01	06:02, 07:01	03:01, 07:01	01:01, 04:01	02:01, 03:03					X
IAV-Inf 2	80-99	PBMC	24:02, 29:02	18:01, 51:01	07:01, 16:02	04:03, 15:01	01:01, 04:01	03:02, 06:02					X
IAV-Inf 3	18-40	PBMC	24:02, 32:01	07:02, 56:01	01:02, 07:02	01:01	04:01, 06:01	05:01					X
IAV-Inf 4	60-80	PBMC	24:02, 74:01	07:02, 51:01	01:02, 15:02	04:03, 15:01	04:01	03:02, 06:02					X
IBV-Inf 1	40-60	PBMC	11:01, 24:02	08:01, 15:01	03:03, 07:01	04:01, 15:01	03:01	04:02					X
IBV-Inf 2	60-80	PBMC	24:02, 24:03	40:06, 51:06	12:04, 15:07	04:03, 07:01	01:01, 02:01	03:02, 03:03					X
IBV-Inf 3	60-80	PBMC	03:01, 24:02	07:02, 51:01	0x:03, 07:02	09:01, 15:01	n.D.	03:03, 06:02					X
Sp1	40-60	Spleen	02:01, 24:02	27:05, 35:01	02:01, 04:01	01:01, 12:01	n.D.	n.D.					X
Sp2	40-60	Spleen	02:01, 24:02	07:02, 15:01	03:01, 07:01	13:01, 15:01	n.D.	n.D.					X
Sp3	40-60	Spleen	02:01, 24:02	27:05, 35:01	02:01, 04:01	01:01, 11:01	n.D.	n.D.					X
Sp4	60-80	Spleen	02:01, 24:02	07:02, 15:01	03:01, 07:01	13:01, 15:01	n.D.	n.D.					X
LN1	40-60	Pancreatic Lymphnode	02:01, 24:02	27:05, 35:01	02:01, 04:01	01:01, 12:01	n.D.	n.D.					X
TN1	0-10	Tonsil	24:02, 29:02	27:05, 42:01	02:02, 17:01	04:01, 08:04	02:01	03:01, 03:02					X
TN2	40-60	Tonsil	24:02, 32:01	15:01, 44:02	03:03, 05:01	04:01, 15:01	04:01, 05:01	03:02, 06:02					X
TN3	0-10	Tonsil	24:02, 29:02	51:01, 58:01	03:03, 07:18	08:04, 15:01	04:01	04:02, 06:02					X
L1	40-60	Lung	3, 24	15, 55	3, 3	n.D	n.D	n.D					X

n.D. = not determined

Supplementary Table 2. Influenza A peptides identified by immunopeptidomics.

Peptide	Sequence	Virus origin	Source	Peptide Pool	Conservation ¹	Predicted binding affinity (nM) ²	Comb. CTL epitope score ³	Immunogenic in human donors (of tested)	Immunogenic in mice (of tested)	Immunogenic & presented by other HLA/MHC ⁴
PA ₄₇₋₅₃	MYSDFFHF	IAV	MS	5	1				0/10	-
PA ₄₇₋₆₀	MYSDFFHFINEQGES	IAV	MS	5	0.52	18148			0/10	-
PA ₄₈₋₅₃	YSDFHF	IAV	MS	5	1				0/10	-
PA ₁₃₀₋₁₃₈	YYLEKANKI	IAV	MS/iedb	1	1	29		1/10	1/10	A*23:01
PA ₄₅₇₋₄₆₅	EYIMKGVYI	IAV	MS	3	0.99	590	0.50		1/10	-
PA ₆₄₉₋₆₅₈	LYASPLQEGF	IAV	MS/iedb	1	0.99	59	0.81	5/10	0/10	A*11:01
PB1 ₂₋₁₀	DVNPTLLFL	IAV	MS	5	0.98	22015	0.29		4/10	-
PB1 ₁₉₋₂₆	STTFPYTG	IAV	MS	5	1	40250	0.09		0/10	-
PB1 ₂₁₆₋₂₂₂	SYLIRAL	IAV	MS	5	0.19				0/10	-
PB1 ₂₁₆₋₂₂₄	SYLIRALTL	IAV	MS	1	0.19	40	0.89	2/5	10/10	-
PB1 ₄₃₀₋₄₃₈	RYTKTTYWW	IAV	MS	1	0.15	26	0.98	2/5	0/10	-
PB1 ₄₈₂₋₄₉₀	SYINRTGTF	IAV	MS	1	0.16	23	1.03	0/5	0/10	-
PB1 ₄₈₂₋₄₉₂	SYINRTGTFEF	IAV	MS	1	0.14	20	1.07	0/5	0/10	-
PB1 ₄₉₆₋₅₀₅	FYRYGFVANF	IAV	iedb	2	0.99	62	0.90	9/10	2/10	A*23:01, A*29:02
PB1 ₄₉₈₋₅₀₅	RYGFVANF	IAV	MS/iedb	1	0.99	40	1.05	9/10	10/10	-
PB1 ₅₀₀₋₅₀₅	GFVANF	IAV	MS	5	0.99				0/10	-
PB1 ₁₋₇₄₆₋₇₅₅	QYKGLCL	IAV	MS	4		18075	0.53		0/10	-
PB1 ₂₋₈₋₁₆	EWMSIRPYF	IAV	MS	2		88	0.64	1/5	0/10	-
PB1 ₃₋₂₄₋₃₂	CYKHNFPLY	IAV	MS	3		730	0.73		0/10	-
PB1 ₃₋₆₈₂₋₆₈₉	QYGGGYGF	IAV	MS	3		1134	0.81		1/10	-
PB2 ₈₉₋₉₈	VMVSP LAVTW	IAV	MS	3	0.91	2179	0.52		0/10	-
PB2 ₁₁₀₋₁₁₉	HYPKIYKTYF	IAV	MS	1	0.11	28	0.89	3/5	0/10	-
PB2 ₁₁₂₋₁₁₉	PKIYKTYF	IAV	MS	5	0.12	39253	0.32		0/10	-
PB2 ₁₁₄₋₁₂₂	IYKTYFERV	IAV	MS	2	0.01	157	0.74	0/5	0/10	-
PB2 ₁₁₇₋₁₂₅	TYFERVERL	IAV	MS	1	0.01	57	0.69	0/10	0/5	-
PB2 ₂₀₄₋₂₁₂	AYMLERELV	IAV	MS	2	0.99	194	0.49	0/5	0/10	-
PB2 ₂₂₇₋₂₃₄	VYIEVLHL	IAV	MS	2	0.59	361	0.87	0/5	1/10	H2K ^b
PB2 ₃₂₂₋₃₃₀	SFSFGGFTF	IAV	MS	1	0.99	20	0.95	0/5	10/10	-
PB2 ₄₃₇₋₄₄₆	HFQKDAKVLF	IAV	MS	3	0.96	392	0.67		1/10	-
PB2 ₄₆₃₋₄₇₁	ILPDMTPSI	IAV	MS	3	0.01	1606	0.51		10/10	-
PB2 ₅₄₉₋₅₅₅	TYQWIIR	IAV	MS	5	0.97				0/10	-
PB2 ₅₄₉₋₅₅₇	TYQWIIRNW	IAV	MS	2	0.96	62	0.86	4/8	10/10	-
PB2 ₅₄₉₋₅₅₉	TYQWIIRNWET	IAV	MS	4	0.17	8570	0.29		10/10	-
PB2 ₅₅₂₋₅₅₉	WIIRNWET	IAV	MS	5	0.17	36284	0.04		0/10	-
PB2 ₅₉₁₋₅₉₉	QYSGFVRTL	IAV	MS	2	0.44	214	0.61	1/5	1/10	-
PB2 ₅₉₁₋₆₀₀	QYSGFVRTLF	IAV	MS	1	0.44	20	0.85	0/5	0/5	-
PB2 ₅₉₄₋₆₀₀	GFVRTLF	IAV	MS	5	0.99				0/10	-
PB2 ₇₀₃₋₇₁₀	RYGPALSI	IAV	MS	2	0.98	389	0.88	1/5	4/10	-
HA ₁₇₆₋₁₈₄	TYPVLNVMT	IAV	MS	2	0.01	351	0.70	1/5	0/10	-
HA ₂₄₈₋₂₅₉	IYWTIVKPGDVL	IAV	MS	3	0.01	958			1/10	-
HA ₅₀₆₋₅₁₆	VYRDEALNNRF	IAV	MS	3	0.40	556	0.68		0/10	-
HA ₅₀₇₋₅₁₆	YRDEALNNRF	IAV	MS	5	0.41	20902	0.28		0/10	-
HA ₅₀₈₋₅₁₆	RDEALNNRF	IAV	MS	5	0.41	28117	0.27		0/10	-
M1 ₉₉₋₁₀₉	LYRKLKREITF	IAV	iedb	3	0.48	409	0.70		0/10	-
M1 ₁₀₈₋₁₁₇	TFHGAKAISL	IAV	iedb	4	0.00	1274	0.39		0/10	-
M1 ₂₃₉₋₂₄₈	AYQKRMGVQM	IAV	iedb	4	0.66	3281			0/10	A*11:01
NP ₃₉₋₄₇	FYIQMCTEL	IAV	iedb	2	0.99	61	0.63	1/10	6/10	H2-D ^b , H2-D ^b , A*23:01, A*29:02, H2-K ^d
NP ₁₃₈₋₁₄₈	IWHSNLNDATY	IAV	MS	4	0.72	10945	0.54		0/10	-
NP ₂₅₇₋₂₆₅	TFLARSALI	IAV	MS	2	0.12	218	0.65	1/5	0/10	-
NP ₂₉₆₋₃₀₄	YSLVGIDPF	IAV	MS	3	0.9	1317	0.60		0/10	H2-D ^b
NP ₄₁₇₋₄₂₅	NLPFDRTTI	IAV	MS	4	0.01	15720	0.34		2/10	-
NP ₄₁₉₋₄₂₉	PFDRTTIMAAF	IAV	iedb	3	0.01	5016	0.51		0/10	-
NP ₄₅₆₋₄₆₄	VSFQGRGVF	IAV	MS	4	0.14	7866	0.39		1/10	-
NS2 ₉₈₋₁₀₆	TFMQALHLL	IAV	MS	1	0.01	32	0.80	3/5	0/10	-

Boldface highlights immunodominant epitopes in human donors

Underline highlights immunogenic peptides in HHD-A24 mice

¹Unique amino acid sequences of protein origin corresponding to the identified peptides from influenza A and B viruses were sourced from the NCBI database using full length sequences from human hosts isolated in Asia and/or Australia. Influenza A sequences were limited to H1N1 and H3N2 human isolate sequences. Sequence variants were identified using the *Identify short peptide in proteins* tool from the Influenza Research Database (www.fludb.org).

²Analysed with NetpanMHC 4.0 (<http://www.cbs.dtu.dk/services/NetMHCpan-4.0/>)

³Analysed with NetCTLpan 1.1 (<http://www.cbs.dtu.dk/services/NetCTLpan/>)

⁴Peptides were screened on the immune epitope database (www.iedb.org) to search for positive T cell assays.

Supplementary Table 3. Influenza B peptides identified by immunoepitidomics

Peptide	Sequence	Virus origin	Peptide pool	Conservation ¹	Predicted binding affinity (nM) ²	Comb. CTL epitope score ³	Immunogenic in human donors (of tested)	Immunogenic in mice (of tested)	Immunogenic & presented by other HLA/MHC ⁴
PA ₁₄₆₋₁₅₆	MIFSYNQDYSL	IBV	11	0.99	2146	0.38		2/10	-
PA ₄₅₃₋₄₆₁	TVMMKYVLF	IBV	11	0.99	122	0.58		0/14	-
PA₄₅₇₋₄₆₅	KYVLFHTSL	IBV	10	0.96	220	0.72	8/14	0/14	-
PB1 ₄₉₅₋₅₀₄	FYRDGFVSNF	IBV	10	0.99	312	0.90	2/14	0/14	-
PB1₅₀₃₋₅₁₁	NFAMELPSF	IBV	10	0.39	473	0.58	7/14	0/14	-
PB2 ₁₀₃₋₁₁₃	TYGPIGDTEGF	IBV	10	0.93	357	0.84	3/14	0/14	-
PB2 ₂₄₅₋₂₅₃	IYHPGGNKL	IBV	11	0.99	384	0.70		3/14	-
PB2 ₄₀₅₋₄₁₃	VFSQDTRMF	IBV	11	0.99	343	0.60		0/14	-
PB2 ₄₃₃₋₄₄₁	MYQLQRYFL	IBV	10	1	219	0.84	6/14	0/14	-
PB2 ₄₃₉₋₄₄₈	YFLNRSNDLF	IBV	10	0.65	63	0.87	5/14	0/14	-
PB2₅₅₀₋₅₅₈	TYQWVLKLN	IBV	10	0.85	335	0.80	11/14	9/14	-
HA ₂₁₉₋₂₂₈	LYGDSKPQKF	IBV	10	0.34	111	0.82	4/14	0/14	-
HA ₂₃₈₋₂₄₆	HYVSQIGGF	IBV	11	0.68	728	0.62		0/14	-
HA ₃₃₈₋₃₄₆	IWVKTPLKL	IBV	10	0.98	247	0.79	1/14	3/14	-
HA ₃₃₉₋₃₄₆	WVKTPLKL	IBV	11	0.98	15986	0.30		1/10	-
HA ₃₇₀₋₃₈₀	GFLEGGWEGMI	IBV	11	0.99	18807	0.44		1/9	-
HA₅₅₂₋₅₆₀	YYSTAASSL	IBV	10	0.99	73	0.68	9/14	0/14	H2-K ^d
HA ₅₇₁₋₅₇₉	VYMSVRDNV	IBV	11	0.89	2295	0.37		0/14	H2-K ^d
HA+3 ₂₇₆₋₂₈₄	NYLSKRYFI	IBV	10	n.d.	73	0.75	4/14	0/14	-
M1 ₁₃₁₋₁₄₀	MYLNPGNYSM	IBV	10	0.80	642	0.72	7/14	0/14	-
NA ₁₋₁₂	MLPSTIQTTLF	IBV	11	0.98	3376	n.d.		1/14	-
NA ₃₂₋₃₉	LYSDILLK	IBV	11	0.91	24963	0.35		0/10	-
NA₃₂₋₄₀	LYSDILLKF	IBV	10	0.91	44	1.04	9/14	14/14	-
NA ₂₀₉₋₂₁₇	AYTDTYHSY	IBV	11	0.99	4132	0.62		0/13	-
NA₂₁₃₋₂₂₁	TYHSYANNI	IBV	10	0.25	65	0.69	6/14	11/14	-
NA ₄₀₂₋₄₁₁	SMEEPGWYSF	IBV	11	0.63	943	0.60		1/14	-
NA ₄₅₈₋₄₆₆	TVTGVNMAL	IBV	11	0.10	23313	0.31		0/10	-
NA+3 ₄₈₀₋₄₈₉	PFVPILFEQL	IBV	11		2296	0.59		0/14	-
NP ₇₉₋₈₉	VYNMNVKLGEF	IBV	11	1	954	0.77		0/14	-
NP₁₆₄₋₁₇₃	IYFSPIRVTF	IBV	10	0.17	25	1.00	11/14	14/14	-
NP₁₆₅₋₁₇₃	YFSPIRVTF	IBV	10	0.17	65	1.05	12/14	14/14	-
NP ₁₈₁₋₁₉₁	MYKTTMGSDGF	IBV	11	0.97	2637	0.68		0/12	-
NP ₂₁₈₋₂₂₈	VGLDPSLISTF	IBV	11	1	8661	0.51		0/10	-
NP ₃₃₈₋₃₄₅	IYAKIPQL	IBV	11	1	1015	0.90		0/12	-
NP ₃₃₈₋₃₄₇	IYAKIPQLGF	IBV	10	1	9	1.01	7/14	4/14	-
NP₃₉₂₋₄₀₀	AAYEDLRVL	IBV	11	0.99	36058	0.29		13/14	-
NP ₃₉₉₋₄₀₈	VLSALTGTEF	IBV	11	0.97	3729	0.45		0/12	-
NS1 ₇₈₋₉₀	KAIGVKMMKVLLF	IBV	11	0.95	6046	n.d.		0/12	-
NS1 ₂₁₁₋₂₁₈	AYDQSGRL	IBV	11	0.89	25406	0.38		0/10	-
NS1 ₂₁₁₋₂₁₉	AYDQSGRLV	IBV	11	0.89	17951	0.29		0/10	-
NS2 ₂₈₋₃₇	VLMKDIQSQF	IBV	11	0.89	1134	0.62		0/12	-

Boldface highlights immunodominant epitopes in human donors

Underline highlights immunogenic peptides in HHD-A24 mice

¹Unique amino acid sequences of protein origins corresponding to the identified peptides from influenza A and B viruses were sourced from the NCBI database using full length sequences from human hosts isolated in Asia and/or Australia. Influenza A sequences were limited to H1N1 and H3N2 human isolate sequences. Sequence variants were identified using the *Identify short peptide in proteins* tool from the Influenza Research Database (www.fludb.org).

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³Analysed with NetCTLpan 1.1 (<http://www.cbs.dtu.dk/services/NetCTLpan/>)

⁴Peptides were screened on the immune epitope database (www.iedb.org) to search for positive T cell assays.

Supplementary Table 4. Screened IAV and IBV peptide variants

Peptide	Sequence	Origin	Conservation ¹
PB1 ₂₁₆₋₂₂₄ V1	SYLIRALTL	IAV	0.19
PB1 ₂₁₆₋₂₂₄ V2	GYLIRALTL	IAV	0.79
PB1 ₄₃₀₋₄₃₈ V1	RYTKT <u>T</u> YWW	IAV	0.15
PB1 ₄₃₀₋₄₃₈ V2	KYT <u>K</u> TIYWW	IAV	0.28
PB1 ₄₃₀₋₄₃₈ V3	KYT <u>K</u> TIYWW	IAV	0.55
PB1 ₄₈₂₋₄₉₀ V1	SYINRTGTF	IAV	0.16
PB1 ₄₈₂₋₄₉₀ V2	SYIN <u>K</u> TGTF	IAV	0.83
PB1 ₄₈₂₋₄₉₂ V1	SYINRTGTFEF	IAV	0.14
PB1 ₄₈₂₋₄₉₂ V2	SYIN <u>K</u> TGTFEF	IAV	0.83
PB2 ₁₁₀₋₁₁₉ V1	HYPKIYKTYF	IAV	0.11
PB2 ₁₁₀₋₁₁₉ V1	HYPK <u>V</u> YKTYF	IAV	0.87
PB2 ₁₁₄₋₁₂₂ V1	IYKTYFERV	IAV	0.01
PB2 ₁₁₄₋₁₂₂ V2	<u>V</u> YKTYFEK <u>V</u>	IAV	0.47
PB2 ₁₁₄₋₁₂₂ V3	IYKTYFEK <u>V</u>	IAV	0.11
PB2 ₁₁₄₋₁₂₂ V4	<u>V</u> YKTYFDK <u>V</u>	IAV	0.40
PB2 ₁₁₇₋₁₂₅ V1	TYFERVERL	IAV	0.01
PB2 ₁₁₇₋₁₂₅ V2	TYFEKVERL	IAV	0.58
PB2 ₁₁₇₋₁₂₅ V3	TYFD <u>K</u> VERL	IAV	0.40
PB2 ₂₂₇₋₂₃₄ V1	VYIEVLHL	IAV	0.59
PB2 ₂₂₇₋₂₃₄ V2	<u>I</u> YIEVLHL	IAV	0.39
PB2 ₂₂₇₋₂₃₄ V3	<u>M</u> YIEVLHL	IAV	0.01
PB2 ₄₆₃₋₄₇₁ V1	ILPDMTPSI	IAV	0.01
PB2 ₄₆₃₋₄₇₁ V2	ILPDMTP <u>S</u> T	IAV	0.57
PB2 ₄₆₃₋₄₇₁ V3	<u>V</u> LPDMTP <u>S</u> T	IAV	0.40
PB2 ₅₉₁₋₅₉₉ V1	QYSGFVRTL	IAV	0.44
PB2 ₅₉₁₋₅₉₉ V2	<u>R</u> YSGFVRTL	IAV	0.46
PB2 ₅₉₁₋₆₀₀ V1	QYSGFVRTLF	IAV	0.44
PB2 ₅₉₁₋₆₀₀ V2	<u>R</u> YSGFVRTLF	IAV	0.46
M1 ₉₉₋₁₀₉ V1	<u>L</u> YRKLKREIT <u>E</u>	IAV	0.48
M1 ₉₉₋₁₀₉ V2	<u>L</u> YKKLKREIT <u>E</u>	IAV	0.49
HA ₁₇₆₋₁₈₄ V1	TYPVLNVTM	IAV	0.01
HA ₁₇₆₋₁₈₄ V2	KYPALNVTM	IAV	0.34
HA ₁₇₆₋₁₈₄ V3	TYPALNVTV	IAV	0.05
HA ₁₇₆₋₁₈₄ V4	KYPVLNVTM	IAV	0.01
NP ₂₅₇₋₂₆₅ V1	TFLARSALI	IAV	0.12
NP ₂₅₇₋₂₆₅ V2	<u>I</u> FLARSALI	IAV	0.80
NP ₂₅₇₋₂₆₅ V3	<u>I</u> F <u>S</u> ARSALI	IAV	0.07
NS2 ₉₈₋₁₀₆ V1	TFMQALHLL	IAV	0.01
NS2 ₉₈₋₁₀₆ V2	TFMQALQLL	IAV	0.96
NP ₁₆₄₋₁₇₃ V1	IYFSPIRVTF	IBV	0.17
NP ₁₆₄₋₁₇₃ V2	IYFSPIRITF	IBV	0.79
NP ₁₆₅₋₁₇₃ V1	YFSPIRVTF	IBV	0.17
NP ₁₆₅₋₁₇₃ V2	YFSPIRITF	IBV	0.79
NA ₃₂₋₄₀ V1	LYSDILLKF	IBV	0.91
NA ₃₂₋₄₀ V2	LYSD <u>V</u> LLKF	IBV	0.01
NA ₂₁₃₋₂₂₁ V1	TYHSYANNI	IBV	0.25
NA ₂₁₃₋₂₂₁ V2	TYHSYAK <u>N</u> I	IBV	0.41
NA ₂₁₃₋₂₂₁ V3	THYSYANK <u>I</u>	IBV	0.25
NA ₂₁₃₋₂₂₁ V4	THYSYAH <u>N</u> I	IBV	0.01
PB2 ₅₅₀₋₅₅₈ V1	TYQWVLKNL	IBV	0.85
PB2 ₅₅₀₋₅₅₈ V2	TYQWVMKNL	IBV	0.14
PB2 ₄₃₉₋₄₄₈ V1	YFLNRSNDLF	IBV	0.65
PB2 ₄₃₉₋₄₄₈ V2	YFLSRNDLF	IBV	0.34
PB1 ₅₀₃₋₅₁₁ V1	NFAMELPSF	IBV	0.39
PB1 ₅₀₃₋₅₁₁ V2	NFAME <u>I</u> PSF	IBV	0.61
M1 ₁₃₁₋₁₄₀ V1	MYLNPGNYSM	IBV	0.80
M1 ₁₃₁₋₁₄₀ V2	MYLN <u>R</u> GNYSM	IBV	0.02
M1 ₁₃₁₋₁₄₀ V3	MYLN <u>P</u> ENYSM	IBV	0.02
HA ₂₁₉₋₂₂₈ V1	LYGDSKPQKF	IBV	0.34
HA ₂₁₉₋₂₂₈ V2	LYGDSNPQKF	IBV	0.61

¹Unique amino acid sequences of protein origins of the identified peptides of influenza A and B viruses were sourced from the NCBI database using full length sequences from human hosts isolated in Asia and/or Australia. Influenza A sequences were limited to H1N1 and H3N2 human isolate sequences. Sequence variants were identified using the *Identify short peptide in proteins* tool from the Influenza Research Database (www.fludb.org).

Supplementary Table 5. Data Collection, Refinement Statistics and Thermal stability of peptide-HLA complexes

Data Collection Statistics	HLA-A*24:02- PB2 ₅₄₉₋₅₅₉	HLA-A*24:02- PB2 ₅₄₉₋₅₅₇	HLA-A*24:02- PB2 _{549-557B}	HLA-A*24:02- NP ₁₆₅₋₁₇₃	HLA-A*24:02- NP ₁₆₄₋₁₇₃
Space group	I2	P6 ₅ 22	P2 ₁	P2 ₁ 2 ₁ 2 ₁	C222 ₁
Cell Dimensions (a,b,c) (Å)	89.68, 43.65, 236.49, β=95.56°	87.29, 87.29, 312.46	46.07, 123, 86.59 β=103.4°	62.95, 75.55, 89.91	79.92, 120.25, 187.80
Resolution (Å)	39.23 – 2.95 (3.13 – 2.95)	48.16 – 2.90 (3.08 – 2.90)	44.81 – 2.16 (2.23 – 2.16)	34.91 – 1.51 (1.54 – 1.51)	46.95 – 2.75 (2.90 – 2.75)
Total number of observations	73078 (11941)	159459 (25130)	188348 (15744)	625371 (30507)	158168 (24193)
Number of unique observations	19624 (3149)	16623 (2577)	49524 (4188)	64117 (3233)	23763 (3421)
Multiplicity	3.7 (3.8)	9.6 (9.8)	3.8 (3.8)	9.8 (9.4)	6.7 (7.1)
Data completeness (%)	99.6 (99.4)	99.9 (99.9)	99.2 (96.9)	94.0 (96.2)	99.4 (99.3)
I/σ _I	12.3 (2.0)	16.4 (2.1)	14.4 (2.6)	28.4 (4.4)	7.8 (1.9)
R _{pim} ^a (%)	6.7 (40.4)	2.9 (31.1)	4.6 (31.9)	1.4 (14.9)	7.5 (40.3)
Refinement Statistics					
Non-hydrogen atoms	6405	3177	6898	3914	6387
Protein	6328	3159	6309	3320	6295
Water	77	14	587	594	91
R _{factor} ^b (%)	20.3	24.1	17.8	18.2	18.9
R _{free} ^b (%)	27.6	27.4	22.9	20.6	26.1
Rms deviations from ideality					
Bond lengths (Å)	0.010	0.007	0.010	0.006	0.010
Bond angles (°)	1.16	0.96	1.08	0.87	1.16
Ramachandran plot (%)					
Favoured region	91.0	93.0	99.3	98.0	94.0
Allowed region	8.0	6.0	0.7	2.0	5.0
Disallowed region	1.0	1.0	0.0	0.0	0.0
Average B-factor					
Macromolecules	63.81	108.46	34.63	21.60	56.82
Water molecules	36.60	91.60	40.54	34.62	45.69
Ligand	NA	115.80	39.06	30.85	78.83
Tm (C)	57.1 ± 1.3	61.9 ± 1.3	57.1 ± 1.2	57.5 ± 0.3	64.0 ± 2.2

^aR_{p.i.m} = $\sum_{hkl} [1/(N-1)]^{1/2} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \langle I_{hkl} \rangle$, ^bR_{factor} = $\sum_{hkl} ||F_o| - |F_c|| / \sum_{hkl} |F_o|$ for all data except ≈ 5% which were used for R_{free} calculation.

Supplementary Table 6. Staining panels

Panel 1 (Mouse ICS)			
Antibody	Flouorochrome	Vendor (clone/cat. No)	Dilution
Live/Dead	NIR	Invitrogen (L34976)	1:800
CD8	PerCP-Cy 5.5	BD Pharmingen (53-6.7/551162)	1:350
CD4	PE-Cy7	eBioscience (GK1.5/25-0041-82)	1:200
IL-2	PE	BD Pharmingen (JES6-5H4/554428)	1:200
TNF	APC	BD Pharmingen (MP6-XT22/554420)	1:200
IFN γ	FITC	BioLegend (XMG1.2/505806)	1:200

Panel 2 (Human ICS)			
Antibody	Flouorochrome	Vendor (clone/cat. No)	Dilution
Live/Dead	NIR	Invitrogen (L34976)	1:800
CD3	PE-Cy7	BD Pharmingen (UCHT1/563423)	1:50
CD4	PE	BD Pharmingen (RPA-T4/555347)	1:50
CD8	PerCP-Cy5.5	BD Pharmingen (SK1/565310)	1:100
CD107a	AF488	Invitrogen (eBioH4A3/53107942)	1:200
IFN γ	BV421	BD Horizon (B27/560371)	1:100
TNF	AF700	BD Pharmingen (MAb11/557996)	1:50
MIP-1 β	APC	BD Pharmingen (D21-1351/560686)	1:40

Panel 3 (PBMC TAME)			
Antibody	Flourochrome	Vendor (clone/cat. No)	Dilution
CD71	BV421	BD Horizon (M-A712/562995)	1:50
CD3	BV510	BioLegend (OKT3/317332)	1:200
HLA-DR	BV605	BioLegend (L243/307640)	1:100
CD4	BV650	BD Horizon (SK3/563875)	1:200
CD27	BV711	BD Horizon (L128/563167)	1:200
CD38	BV786	BD Horizon (HIT2/563964)	1:100
Tetramer 1	APC	In house	
CCR7	AF700	BD Pharmingen (150503/561143)	1:50
CD14	APC-H7	BD Pharmingen (MφP9/560180)	1:100
CD19	APC-H7	BD Pharmingen (SJ25C1/560177)	1:100
Live/Dead	NIR	Invitrogen (L34976)	1:800
CD45RA	FITC	BD Pharmingen (HI100/555488)	1:200
CD8	PerCp-Cy5.5	BD Pharmingen (SK1/565310)	1:50
Tetramer 2	PE	In house	
CD95	PE-CF594	BD Horizon (DX2/562395)	1:100
PD1	PE-Cy7	BD Pharmingen (EH12.1/561272)	1:50

Panel 4 (Tissue TAME)			
Antibody	Flouorochrome	Vendor (cat. No)	Dilution
CD69	BV421	BioLegend (FN50/310930)	1:100
CD3	BV510	BioLegend (OKT3/317332)	1:200
HLA-DR	BV605	BioLegend (L243/307640)	1:100
CD4	BV650	BD Horizon (SK3/563875)	1:200
CD27	BV711	BD Horizon (L128/563167)	1:200
CD38	BV786	BD Horizon (HIT2/563964)	1:100
Tetramer 1	APC	In house	
CCR7	AF700	BD Pharmingen (150503/561143)	1:50
CD14	APC-Cy7	BD Pharmingen (MφP9/560180)	1:100
CD19	APC-Cy7	BD Pharmingen (SJ25C1/560177)	1:100
Live/Dead	NIR	Invitrogen (L34976)	1:800
CD103	FITC	BioLegend (Ber-ACT8/350203)	1:100
CD8	PerCp-Cy5.5	BD Pharmingen (SK1/565310)	1:50
Tetramer 2	PE	In house	
CD95	PE-CF594	BD Horizon (DX2/562395)	1:100
CD45RO	PE-Cy7	ThermoFisher (UCHL1/25-0457-41)	1:100

Supplementary Table 7. Primers

Primer	Sequence (5' – 3')
Mycopl-for	YGCCTGVGTAGTAYRYWCGC
Mycopl-rev	GCGGTGTGTACAARMCCCGA