Supplementary Table 1.

Peptide matrix systems

Peptide matrix system 1 contains overlapping peptides spanning the H5N1 internal proteins and the H3N2 HA and NA.
Peptide matrix system 2 contains overlapping peptides spanning the H3N2 internal proteins with amino acid sequences different from the corresponding regions of the H5N1 internal proteins.
Peptide matrix system 3 contains overlapping peptides spanning the H5N1 HA and NA.

In summary, 1102 overlapping peptides arranged in Peptide matrix systems 1-3 covered the full proteome of the H5N1 and H3N2.

Peptide matrix system 1[A]

	Peptide Pools	Source Protein	Amino Acid Number[D]
1st Dimension	1	H3N2 HA	1-208
	2		198-390
	3		381-566
	4	H3N2 NA	1-168
	5		159-328
	6		319-469
	7	H5N1 PB1	1-195
	8		185-380
	9		371-563
	10		554-750
	11	H5N1 PB2	1-198
	12		189-384
	13		375-577
	14		568-759
	15[E]	H5N1 M1,M2	1-132,1-57
	16[E]		123-252,49-97
	17	H5N1 NP	1-179
	18		170-346
	19		337-497
	20	H5N1 NS1,NS2	1-123,1-58
	21		117-230,50-121
	22	H5N1 PA	1-201
	23		192-370
	24		361-551
	25		542-707
	26	-[F]	
2nd Dimension	27-52	All peptides above arranged non-sequentially[G]	
3rd Dimension	53-78	All peptides above arranged non-sequentially[G]	

Peptide matrix system 2[B]

	Peptide Pools	Source Protein	Amino Acid Number[D]
1st Dimension	1	H3N2 M1	1-213
	2[E]	H3N2 M1,M2	204-250,1-97
	3	H3N2 NP	1-194
	4		185-366
	5		356-498
	6	H3N2 NS1	6-119
	7		110-230
	8	H3N2 NS2	6-121
	9	H3N2 PA	1-192
	10		183-326
	11		317-443
	12		434-716
	13	H3N2 PB1	1-396
	14		417-753
	15	H3N2 PB2	1-151
	16		142-368
	17		359-570
	18		561-759
	19	-[F]	
2nd Dimension	20-38	All peptides above arranged non-sequentially[G]	

Peptide matrix system 3[C]			
	Peptide Pools	Source Protein	Amino Acid Number[D]
1st Dimension	1	H5N1 HA	1-89
	2		80-164
	3		155-242
	4		233-323
	5		314-405
	6		396-487
	7		478-565
	8	H5N1 NA	1-104
	9		95-176
	10		167-249
	11		240-320
	12		309-393
	13		384-477
2nd Dimension	14-24	All peptides above arranged non-sequentially	
	25-26	-[F]	

[A] Peptide matrix system 1 consisted of 3 sets (or “Dimensions") of 26 peptide pools.

[B] Peptide matrix system 2 consisted of 2 sets (or “Dimensions”) of 19 peptide pools.

[C] Peptide matrix system 3 consisted of 2 sets (or “Dimensions”) of 13 peptide pools.

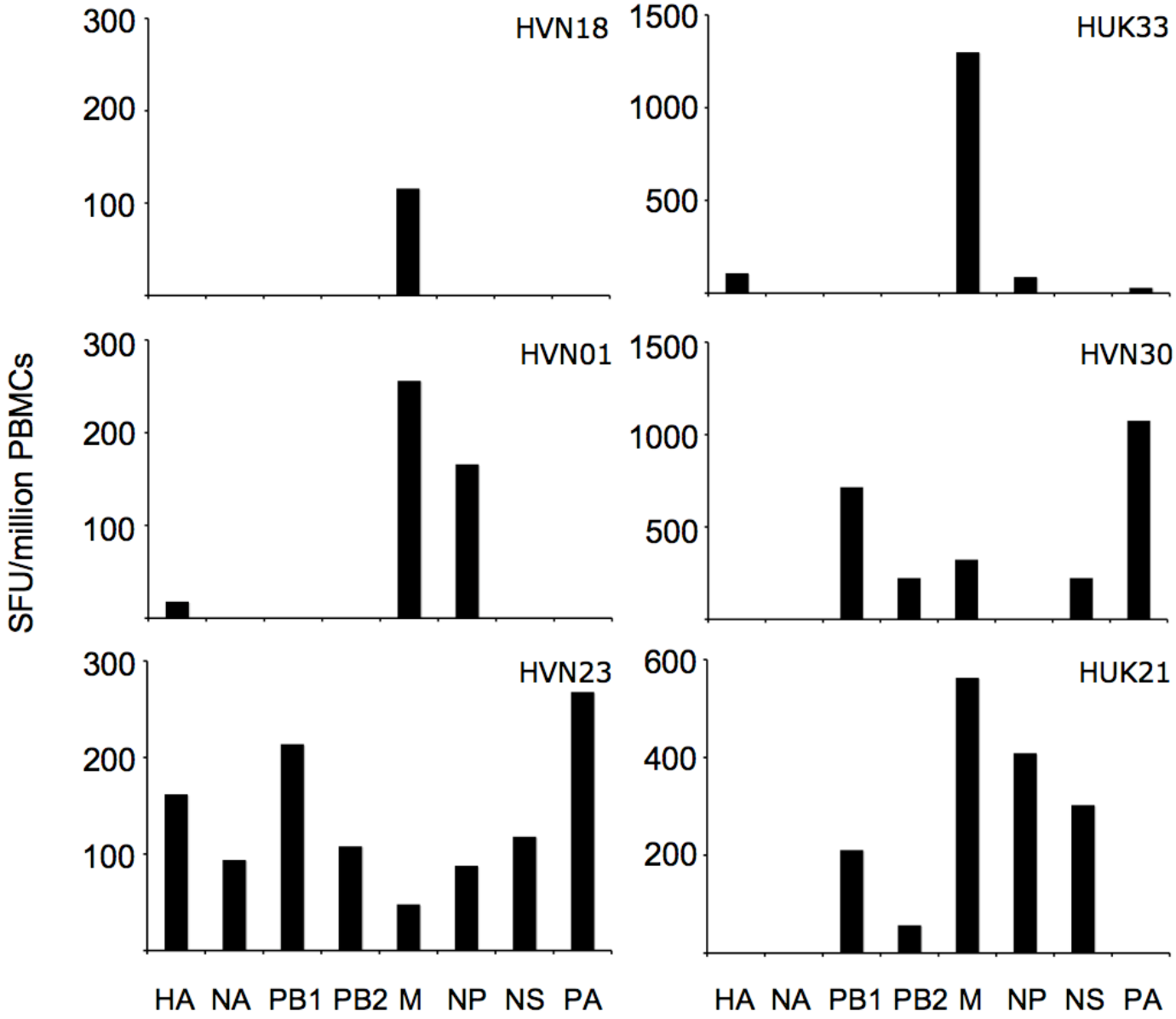
[D] Protein region covered by the corresponding overlapping peptides.

[E] Protein region covered by the corresponding overlapping peptides.

[F] The peptide pools 15-16 of the Peptide matrix system 1 and the peptide pool 2 of the Peptide matrix system 2 contained a mixture of overlapping peptides of the M1 and M2. However, the responses detected against these pools were determined to be predominantly targeted to the M1 when the pool responses were confirmed at the single peptide level.

[G] The optimal peptide arrangement for the 2nd and 3rd dimensions of the Peptide matrix system 1 was determined by a series of software (“Deconvolute-This” (1)) simulations with a goal to minimize the number of subsequent assays required to determine individual peptides containing T-cell epitopes.

1. Roederer, M., and Koup, R.A. 2003. Optimized determination of T cell epitope responses. *Journal of Immunological Methods* 274:221-228.



Supplementary Figure 1.

Examples of various influenza specific memory T-cell recognition patterns.
Considerable variation in the breadth and magnitude of the T-cell recognition was observed between different individuals. Examples of various T-cell response patterns against the overlapping peptides spanning the H3N2 full proteome and the H5N1 internal proteins are provided.

Supplementary Table 2

Peptides containing CD4+ and CD8+T-cell epitope regions across the full proteome of the H3N2 influenza A virus and the internal proteins of the H5N1 influenza A virus.

Positive responses detected during the peptide pool screening of the UK volunteers were subsequently confirmed at the single peptide level in a second round of *ex vivo* ELISpot assays.

We confirmed 136 peptides containing influenza A specific T-cell epitopes across the full proteome of the H3N2 strain and the internal proteins of the H5N1 strain. Previously identified epitope regions are highlighted in bold.

Source Protein	Amino Acid Number	Sequence[A]		H5N1 Cross[B]	CD4/CD8[C]	Recognition Frequency[D]	Ref[E]
		H3N2	H5N1	Recognition	Dependency		
HA	48-67	DQIEVTNATELVQSSSTGGI	EQVDTIMEKNVTVTHA	-	.	1	
	181-198	NVTMPNNEKFDKLYIWGV	IKRSYNNTNQEDLLVMW	-	.	1	
	233-250	IGSRPRVRDIPSRISIYW	VPRIATRSKVNGQSGRM	-	.	1	
	241-258	DIPSRISIYWTIVKPGDI	RMEFFWTILKPNDAINF	-	CD4	1	
	249-267	**YWTIVKPGDILLINSTGNL**	RMEFFWTILKPNDAINF	-	CD4	1	(1)
	324-339	**YVKQNTLKLATGMRNV**	YVKSNRLVLATGLRNSPQR	-	CD4	1	(1)
	338-355	NVPEKQTRGIFGAIAGFI	KKRGLFGAIAGFIEGGW	-	CD4	1	
	338-355	NVPEKQTRGIFGAIAGFI	KKRGLFGAIAGFIEGGW	+	CD4	2	
	346-363	**GIFGAIAGFIENGWEGMV**	KKRGLFGAIAGFIEGGW	-	CD4	2	(1)
	346-363	**GIFGAIAGFIENGWEGMV**	KKRGLFGAIAGFIEGGW	+	CD4	2	(1)
	416-433	**SEVEGRIQDLEKYVEDTK**	NNLERRIENLNKKMEDGF	-	CD4	1	(1)
	402-418	GKTNEKFHQIEKEFSEV	DKMNTQFEAVGREFNNL	-	.	2	
NA	259-275	EGKIVHTSTLSGSAQHV	KIFKMEKGKVVKSVELDA	-	.	1	
	308-322	KDYSIVSSYVCSGLV	NQNLEYQIGYICSGVF	-	.	1	
	319-336	SGLVGDTPRKNDSSSSSH	CSGVFGDNPRPNDGTGSC	-	CD4	1	
	394-410	RQVIVERGNRSGYSGIF	IVAITDWSGYSGSFVQH	-	.	2	
M1	1-15	MSLLTEVETYVLSIV	MSLLTEVETYVLSII	+	CD8	1	
	6-22	EVETYVLSIVPSGPLKA	EVETYVL**SIIPSGPLK**A	-	.	1	(2)
	19-35	**PLKAEIAQRLEDV**FAGK	PLKAEIAQKLEDVFAGK	-	.	1	(3)

	33-49	AGKNTDLEALMEWLKTR	*identical to H3N2*	+	CD8	1	
	40-57	EALMEWLKTRPILSPLTK	*identical to H3N2*	+	CD8	9	
	55-72	LTKGILG**FVFTLTVPSER**	*identical to H3N2*	+	CD4	1	(4)
	55-72	LTK**GILGFVFTLTV**PSER	*identical to H3N2*	+	CD8	2	(5)
	63-80	VF**TLTVPSERGLQRRRFV**	*identical to H3N2*	+	CD4	1	(6)
	71-88	ER**GLQRRRFVQNALNGNG**	*identical to H3N2*	+	CD4	2	(6)
	87-104	NGDPNNMDKAVKLYRKLK	NGDPNNMDRAVKLYKKLK	+	CD4	1	
	95-112	KA**VKLYRKLKREITFHGA**	RAVKLYKKLKREITFHGA	+	CD4	1	(6)
	95-112	KA**VKLYRKLKREITFHGA**	RAVKLYKKLKREITFHGA	-	CD4	5	(6)
	95-112	KAVKLYRKLKREITFHGA	RAVKLYKKLKREITFHGA	+	CD8	1	
	103-119	LKREITFHGAKEIALSY	**LKREITFHGAKEVALSY**	+	CD4	2	(6)
	103-119	LKREITFHGAKEIALSY	LKREITFHGAKEVALSY	-	CD4	3	
	110-125	HGAKEIALSYSAGALA	HGAKEVALSYSTGALA	+	.	1	
	110-125	HGAKEIALSYSAGALA	HGAKEVALSYSTGALA	-	.	1	
	116-132	ALSYSAGAL**ASCMGLIY**	ALSYSTGAL**ASCMGLIY**	-	CD8	1	(7)
	123-138	AL**ASCMGLIY**NRMGAV	AL**ASCMGLIY**NRMGTV	-	.	1	(7)
	137-154	AVTTEVAFGLVCATCEQI	TVTTEVAFGLVCATCEQI	-	.	1	
	152-168	EQIADSQHRSHRQMVAT	EQIADSQHRSHRQMATI	+	.	1	
	166-182	VATTNPLIKHENRMVLA	ATITNPLIRHENRMVLA	+	.	3	
	173-189	IKHEN**RMVLASTTAK**AM	IRHEN**RMVLASTTAK**AM	-	CD4	3	(2)
	173-189	IKHEN**RMVLASTTAK**AM	IRHEN**RMVLASTTAK**AM	+	CD4	4	(2, 6)
	180-199	VLASTTAKAMEQMAGSSEQA	*identical to H3N2*	+	CD4	1	
	190-206	EQMAGSSEQAAEAMEIA	*identical to H3N2*	+	.	1	
	197-213	EQAAEAMEIASQARRMV	EQAA**EAMEIANQARQMV**	+	CD4	1	(6)
	204-219	EIASQARRMVQAMRAV	**EIANQARQMVQAMRTI**	+	CD4	3	(6)
	210-227	RRMVQAMRAVGTHPSSST	**RQMVQAMRTIGTHPNSSA**	+	CD4	3	(6)
	225-242	SSTGLRDDLLENLQTYQK	SSAGLRDNLLENLQAYQK	-	.	1	
	233-250	LLENLQTYQKRMGVQMQR	L**LENLQAYQKRMGVQMQR**	-	CD4	2	(8)
	241-252	**QKRMGVQMQRFK**	*identical to H3N2*	+	CD8	3	(8)
M2	7-21	**VETPIRNEW**GCRCND	**VETPTRNEW**ECRCSD	-	.	1	(9)
	13-29	NEWGCRCNDSSDPLVVA	NEWECRCSDSSDPIVVA	-	.	1	
	63-77	PSTEGVPESMREEYR	PATAGVPESMREEYR	+	.	1	
	69-84	PESMREEYRKEQQNAV	PESMREEYRQEQQSAV	+	CD8	1	
NP	1-19	MASQGTKRSYEQMETDGDR	MASQGTKRSYEQMETGGER	-	.	3	
	18-33	DRQNATEIRASVGKMI	ERQNATEIRASVGRMV	+	CD4	2	

24-41	EIRASVGKMIDGIGRFYI	EIRASVGRMVSGIGRFYI	-	CD4	1	
24-41	EIRASVGKMIDGIGRFYI	EIRASVGRMVSGIGRFYI	+	CD4	1	
32-49	MIDGIGRFYIQMCTELKL	MVSGIGRFYIQMCTELKL	+	CD8	1	
32-49	MIDGIGRFYIQMCTELKL	MVSGIGRFYIQMCTELKL	-	.	2	
40-57	YIQMCTELKLSDHEGRLI	YIQM**CTELKLSDY**EGRLI	+	CD8	3	(10)
56-71	LIQNSLTIEKMVLSAF	LIQNSITIERMVLSAF	+	.	1	
62-79	TIEKMVLSAFDERRNKYL	TIERMVLSAFDERRNRYL	-	.	1	
70-87	AFDERRNKYLEEHPSAGK	AFDERRNRYLEEHPSAGK	+	CD8	1	
102-119	GKWMRELVLYDKEEIRRI	GKWVRELILYDKEEIRRI	-	CD4	1	
102-119	GKWMRELVLYDKEEIRRI	GKW**VRELILYDKEEIRRI**	+	CD4	2	(6)
170-186	STLP**RRSGAAGAAVK**GI	STLP**RRSGAAGAAVK**GV	+	CD8	2	(9)
177-194	GAAGAAVKGIGTMVMELI	GAAGAAVKGVGTMVMELI	-	.	1	
192-208	E**LIRMVKRGINDRNFWR**	ELIRMIKRGINDRNFWR	+	CD4	1	(6)
192-208	ELIRMVKRGINDRNFWR	ELIRMIKRGINDRNFWR	-	.	1	
213-230	**RKTRSAYERMCNILKGK**F	RRTRIAYERMCNILKGKF	-	.	2	(11)
213-230	**RKTRSAYERMCNILKGK**F	RRTRIAYERMCNILKGKF	+	CD4	3	(11)
221-238	RMCNILKGKFQTAAQRAM	*identical to H3N2*	+	CD4	2	
229-246	KFQTAAQRAMVDQVRESR	KFQTAAQRAMMDQVRESR	-	CD4	1	
229-246	KFQTAAQRAMVDQVRESR	KFQTAAQRAMMDQVRESR	+	CD4	2	
237-253	AMVDQVRESRNPGNAEI	AMMDQVRESRNPGNAEI	-	.	1	
258-273	FLARSAL**ILRGSVAHK**	*identical to H3N2*	+	CD8	3	(10)
264-281	L**ILRGSVAHK**SCLPACAY	L**ILRGSVAHK**SCLPACVY	+	CD4	1	(6, 10)
307-324	LQNSQIYSLIRPNENPAH	LQNSQVFSLIRPNENPAH	-	.	2	
315-332	LIRPNE**NPAHKSQLVWMA**	*identical to H3N2*	+	.	1	(6)
329-346	VWMACHSAA**FEDLRLLSF**	VWMACHSAAFEDLRVSSF	+	CD8	1	(12)
329-346	VWMACHSAA**FEDLRLLSF**	VWMACHSAAFEDLRVSSF	-	CD8	1	(12)
337-353	A**FEDLRLLSF**IRGTKVS	AFEDLRVSSFIRGTRVV	-	CD8	1	(12)
356-373	G**KLSTRGVQIASNEN**MDN	GQLSTRGVQIASNENMEA	-	.	1	(13)
378-395	TLELRSGYWAIRTRSGGN	TL**ELRSRYWAI**RTRSGGN	-	.	1	(14)
386-403	WAIRTRSGGNTNQQRASA	*identical to H3N2*	+	CD4	1	
394-408	GNTNQQRASAGQTSV	GNTNQQRASAGQISV	+	.	1	
397-414	NQQRASAGQTSVQPTFSV	NQQRASAGQISVQPTFSV	+	.	1	
404-420	GQTSVQPTFSVQRNLPF	**GQISVQPTFSVQRNLPF**	+	CD4	2	(6)
411-428	TFSVQRNLPFEKSTIMAA	TFSVQRN**LPFERATIM**AA	-	.	2	(15)
419-436	PFEKSTIMAAFTGNTEGR	PFERATIMAAFTGNTEGR	-	.	1	

	427-444	AAFTGNTEGRTSDMRAEI	AAFTGNTEGRTSDMRTEI	+	.	1	
	443-458	EIIRMMEGAKPEEVSF	**EIIRMMESARPEDVSF**	+	.	1	(6)
	449-466	EGAKPEEVSFRGRGVFEL	ESARPEDVSFQGRGVFEL	-	.	1	
	457-471	SFRGRGVFELSDEKA	**SFQGRGVFELSDEKA**	-	CD4	3	(11)
	470-487	KATNPIVPSFDMSNEGSY	KATNPIVPSFDMNNEGSY	+	.	1	
	470-487	KATNPIVPSFDMSNEGSY	KATNPIVPSFDMNNEGSY	-	.	1	
	478-493	**SFDMSNEGSYFFGDNA**	SFDMNNEGSYFFGDNA	-	CD4	1	(11)
NS1	6-23	VSSFQVDCFLWHIRKQVV	VSSFQVDCFLWHVRKRFA	-	.	2	
	14-30	FLWHIRKQVVDQELSDA	FLWHVRKRFADQELGDA	-	.	1	
	60-77	VGKQIVEKILKEESDEAL	AGKQIVERILEEESDKAL	+	.	2	
	68-85	ILKEESDEALKMTMVSTP	ILEEESDKALKMPASRYL	-	.	1	
	68-85	ILKEESDEALKMTMVSTP	ILEEESDKALKMPASRYL	+	CD4	1	
	76-92	STPASRYITDMTIEELSR	ALKMPASRYLTDMTLEEM	+	CD4	1	
	154-169	GAIVGEISPLPSFPGH	GAIV**GEISPLPSL**PGH	-	.	1	(12)
NS2	6-19	VSSFQDILLRMSKM	VSSFQDILVRMSKM	-	.	1	
	31-45	MITQFESLKIYRDSL	MITQFESLKLYRDSL	+	CD4	3	
PA	441-458	MRRNYFTAEVSHCRATEY	*identical to H3N2*	+	CD8	1	
PB1	21-38	TFPYTGDPPYSHGTGTGY	*identical to H3N2*	+	CD8	2	
	43-60	VNRTHQYSEKGKWTTNTE	*identical to H3N2*	+	.	1	
	57-73	TNTETGAPQLNPIDGPL	*identical to H3N2*	+	.	1	
	64-82	PQLNPIDGPLPEDNEPSGY	*identical to H3N2*	+	.	1	
	86-103	DCVLEAMAFLEESHPGIF	*identical to H3N2*	+	.	1	
	102-119	IFENSCLETMEVVQQTRV	IFENSCLETMEIVQQTRV	-	.	1	
	123-140	TQGRQTYDWTLNRNQPAA	*identical to H3N2*	+	.	1	
	270-287	GLPVGGNEKKAKLANVVR	*identical to H3N2*	+	.	1	
	316-333	MFLAMITYITKNQPEWF	RMFLAMITYITRNQPEWF	+	.	1	
	402-419	SLSPGMMMGMFNMLSTVL	*identical to H3N2*	+	CD4	1	
	410-426	GMF**NMLSTVLGV**SILNL	*identical to H3N2*	+	.	1	(2)
	417-433	TVLGVSILNLGQKKYTK	TVLGVSILNLGQKRYTK	-	.	1	
	417-433	TVLGVSILNLGQKKYTK	TVLGVSILNLGQKRYTK	+	CD4	1	
	432-449	TKTTYWWDGLQSSDDFAL	*identical to H3N2*	+	CD4	1	
	470-486	CKLVGINMSKKKSYINK	CKLVGINMSKKKSYINR	+	.	1	
	498-514	RYGFVANFSMELPSFGV	*identical to H3N2*	+	CD8	1	
	505-521	FSMELPSFGVSGINESA	*identical to H3N2*	+	.	1	
	562-579	HRGDTQIQTRRSFELKKL	*identical to H3N2*	+	.	1	

	570-587	TRRSFELKKLWDQTQSRA	TRRSFELKKLWEQTRSKA	-	.	1
	705-722	YRRPIGISSMVEAMVSRA	YRRPVGISSMVEAMVSRA	+	CD8	1
	705-722	YRRPIGISSMVEAMVSRA	YRRPVGISSMVEAMVSRA	-	CD8	1
PB2	61-78	KRITEMVPERNEQGQTLW	KRIIEMIPERNEQGQTLW	-	.	1
	205-221	YMLERELVRKTRFLPVA	*identical to H3N2*	+	CD8	1
	212-229	VRKTRFLPVAGGTSSIYI	VRKTRFLPVAGGTSSVYI	+	CD8	1
	212-229	VRKTRFLPVAGGTSSIYI	VRKTRFLPVAGGTSSVYI	-	.	1
	419-436	NFVNRANQRLNPMHQLLR	NFVNRANQRLNTMHQLLR	-	.	1
	544-561	SVLVNTYQWIIRNWEAVK	SVLVNTYQWIIRNWETVK	+	CD4	1
	590-607	SQYSGFVRTLFQQMRDVL	GQYSGFVRTLFQQMRDVL	-	.	1
	598-615	TLFQQMRDVLGTFDTTQI	TLFQQMRDVLGTFDTVQI	+	.	1
	683-699	TSGVESAVLRGFLIIGK	TAGVESAVLRGFLILGK	-	.	1

[A] Strains used: A/Vietnam/CL26/2004 (H5N1), A/New York 388/2005 (H3N2) (HA and NA), and A/New York 232/2004 (H3N2) (internal proteins).

H5N1 and H3N2 amino acid sequence variations are underlined.

[B] Recognition of H5N1 peptides.

[C] After depletion, most subdominant responses (< 40 SFU/million PBMCs) were detectable in neither CD4+ nor CD8+ T-cell depleted populations and therefore were excluded from the subsequent analysis of influenza-specific CD4+ and CD8+ T-cell distribution;

. , undetermined.

[D] Number of participants recognizing corresponding peptides.

[E] References for previously identified epitopes (sequences highlighted in bold).

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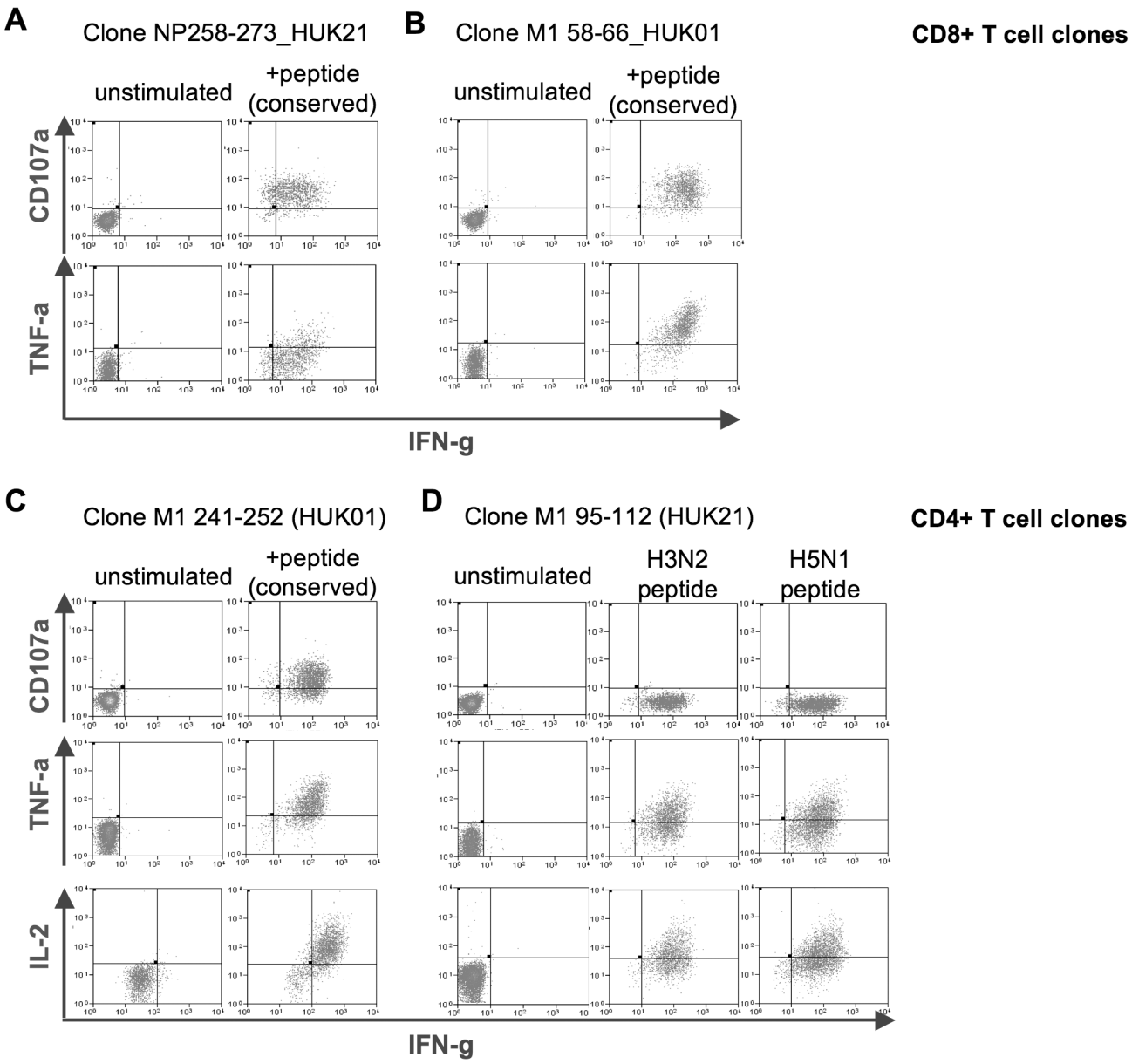

Supplementary Figure 2.

Polyfunctional capacities displayed by the H5N1 cross-reactive CD8+ (**A, B**) and CD4+ (**C, D**) T-cell clones derived from healthy individuals. Representative examples.