Supporting Information

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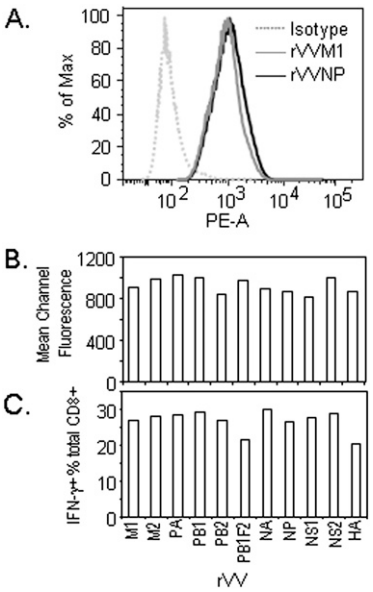

Fig. S1. Equivalent infection of B lymphoma cell lines (BLCLs) by influenza A virus (IAV) recombinant vaccinia viruses (rVVs). For screening antigenic IAV proteins, BLCL aliquots were infected individually with rVVs encoding each of the 11 IAV proteins at a multiplicity of infection (MOI) of 10 for 1 h at 37 °C in PBS containing 0.1% BSA (Sigma). The cells were then cultured in RF-10 for 4 h, washed, and further stained with mouse mAb TW2.3 (gift from Jonathan Yewdell and Jack Bennink, National Institutes of Health, Bethesda, MD). Fluorescence intensity of the rVV-M1– and rVV-nucleoprotein (NP) -infected BLCLs is shown as FACS plots for one experiment (*A*). Infected cells stained with only Phycoerythrin (PE)-conjugated goat anti-mouse Ig polyclonal Ab were included as controls (dotted line). The mean channel fluorescence values for 11 rVV-infected cells from two independent experiments were averaged and are shown in *B*. In *C*, the same rVV stocks used in *A* and *B* were used to infect the mouse L cells transfected with K^b (L-K^b; gift from James McCluskey, Melbourne University, Melbourne, Australia). Infected L-K^b cells (for 5 h) were coincubated (for another 4 h) with a murine $CD8^+$ T-cell line specific for an rVV epitope. As an indirect indication of the efficiency of infection, T-cell activation because of rVV antigen presentation was assessed by the IFN-γ intracellular cytokine staining (ICS) assay. This is representative of two independent experiments.

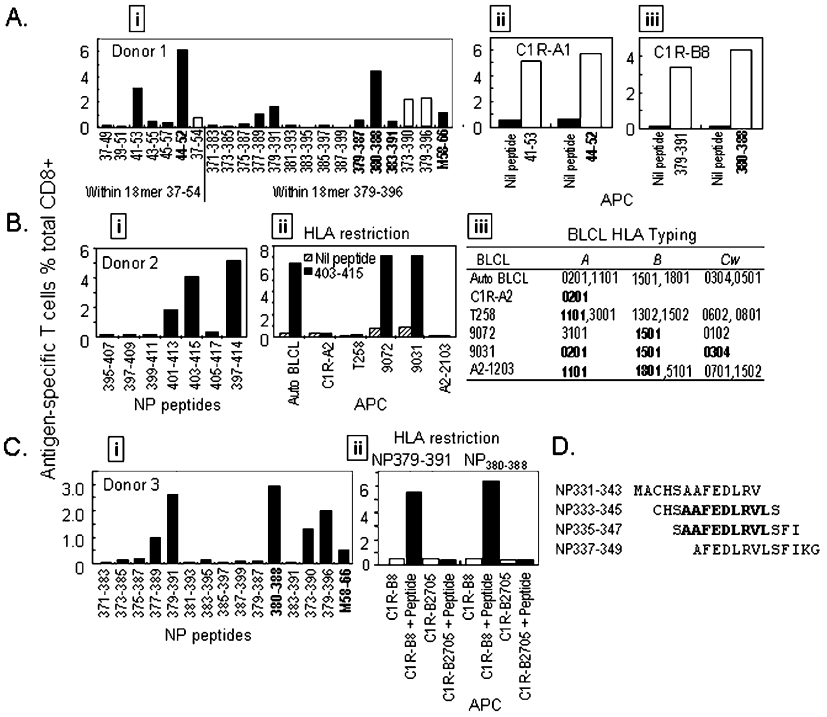

Fig. S2. Immunodominant T-cell epitopes in donors 1, 2, 3, and 5. Multispecificity cytotoxic T lymphocyte (CTL) lines were raised using IAV-infected peripheral blood mononuclear cells (PBMCs). (*A*) The 13mer peptides within 18mer NP37–54 and NP379–396 (identified from donor 1) were screened by ICS (*A*, i), with the control 18mer results being shown as white bars. The HLA restrictions for the 13mer NP41–53 and NP379–391 as well as the corresponding minimal peptides were confirmed with C1R-A1 and C1R-B8, respectively (*A*, ii and iii). (*B*) The line from donor 2 was used to screen 13mer peptides within the NP397–414 18mer (*B*, i). Then, NP403–415 was used to further pulse a panel of BLCL lines that shared one or more HLA alleles with donor 2 (*B*, ii and HLA typing info is shown in iii). The line from donor 3 was used to screen 13mer peptides within the NP379–396 18mer and the previously published HLA-B8–restricted $NP_{380–388}$ (*C*, i). The key sequence in NP379–391 was shown to be $NP_{380–388}$ and HLA-B8–restricted (*C*, ii). *D* illustrates the alignment of the four 13mers that showed stimulating capacity (at high peptide concentrations) for T-cell lines raised from donor 5's PBMCs with the NP331–348 18mer.

Table S1. Synthesized known NP peptides

IEDB reference ID	Name	Sequence	HLA restriction
1003540	NP44–52	CTELKLSDY	HLA-A1
1012379	NP174–184	RRSGAAGAAVK	HLA-B27
931	NP188–198	TMVMELVRMIK	HLA-A11
1003540	NP199–207	RGINDRNFW	HLA-B58
1013714	NP219–228	YERMCNILKG	HLA-B44
1003540	NP225–233	ILKGKFQTA	HLA-B8
1012379	NP265–273	ILRGSVAHK	HLA-A3
1000077	NP338–345	FEDLRVLS	HLA-B37
931	NP342–351	RVLSFIKGTK	HLA-A11
1007471	NP380–388	ELRSRYWAI	HLA-B8
1213	NP383–391	SRYWAIRTR	HLA-B27
315051	NP379–387	LELRSRYWA	HLA-B44

All of the above peptides share the PR8 NP sequences. IEDB, Immune Epitope Database.

Table S2. Conservancy of the peptide sequences within H1N1 and H3N2 viruses

Peptide	Peptide sequence	Frequency (%) of peptide variants			
		H3N2	Australian* H3N2	H1N1	Australian H1N1
NP140–150	HSNLNDATYQR†	33	64	18	—
NP140–150	HSNLNDTTYQR	67	31	82	61
NP140–150	HSNLNATTYQR	—	—	—	39
NP140–150	HSNLNDVTYQR	—	3	—	—
NP219–228	YERMCNILKG†	100	100	100	100
NP336–344	AAFEDLRLL†	92	100	92	100
NP336–344	AAFEDLRVL	8	—	—	—
NP336–344	VAFEDLRLL	—	—	8	—
NP404–412	GQISIQPTF†	23	65	75	17
NP404–412	GQISVQPAF	8	—	—	—
NP404–412	GQTSVQPTF	23	36	—	—
NP404–412	GQISTQPTF	—	—	16	48
NP404–412	GQISVQPTF	62	—	—	39
NP404–412	DQISVQPTF	—	—	8	—

H1N1 strains used for comparison with PR8-derived peptides included the full-length sequences of vaccine influenza strains available from National Center for Biotechnology Information (NCBI) influenza virus resource database: A/New Jersey/8/76, A/USSR/90/77, A/USSR/92/77, A/Brazil/11/78, A/Dunedin/27/83, A/Chile/1/83, A/Taiwan/1/86, A/Singapore/6/86, A/Texas/36/91, A/Bayern/7/95, A/Beijing/262/95, A/New Caledonia/20/99, A/Brisbane/59/2007, and A/Calfornia/7/2009 (pdm). H3N2 strains used for comparison with PR8-derived peptides included the full-length sequences of vaccine influenza strains A/Victoria/3/75, A/Texas/1/77, A/Bangkok/1/79, A/Philippines/2/82, A/Leningrad/360/86, A/Shanghai/11/87, A/Beijing/353/89, A/Beijing/32/92, A/Shangdong/9/93, A/Sydney/5/97, A/Moscow/10/99, A/Wellington/1/2004, A/California/7/2004, and A/Wisconsin/67/2005.

*Australian H1N1 (n = 24) and H3N2 (n = 40) sequences included the full-length sequences of viruses from 2000 to 2010 available from the NCBI influenza virus resource database accessed on December 1, 2010. Search criteria were Australia, NP, H3N2/H1N1, nonidentical sequences excluded, full length only, and from 2000 to 2010.

†These peptide sequences were identified and used in this study.

Table S3. Most published IAV HLA-A2 epitopes share the PR8 sequences

IEDB reference ID	Linear sequence	Position	Source molecule	Substitution in PR8
835	AIMDKNIIL	122–130	NS1	
1437	AIMDKNI*M*L	122–130	NS1	I
1012379	AIMDKNI*T*L	122–130	NS1	I
1012379	AIMDK*T*IIL	122–130	NS1	N
1012379	AIMDK*V*IIL	122–130	NS1	N
1008386	CVNGSCFTV	213–221	NA	
1003540	FLKDVMESM	166–174	PB1	
931	FMYSDFHFI	46–54	PA	
1013714	FSMELPSFGV	505–514	PB1	
1008386	GILGFVF*LT*	58–66	M1	TL
589	GILGFVFTL	58–66	M1	
315643	GILGFVFTLT	58–67	M1	
1000124	GKNTDLEVLMEWLKTRPILS	34–53	M1	
1008884	GLFGAIAGFI	344–353	HA precursor	
1013714	GLIYNRMGA	129–137	M1	
1012379	G*M*LGFVFTL	58–66	M1	L
931	ILGFVFTLTV	59–68	M1	
1013714	ILSPLTKGIL	51–60	M1	
1000120	KGILGFVFTLTV	57–68	M1	
1019199	*KL*YQN*PTTYI*	205–214	HA	NI . . . ENA.V
1000105	LTKGILGFVFTLTVPSERG	55–75	M1	
1013714	MMMGMFNML	407–415	PB1	
931	NMLSTVLGV	413–421	PB1	
1000124	RGLQRRRFVQNALNGNG	72–88	M1	
1013468	SGPLKAEIAQRLEDV	17–31	M1	
931	SLCPIRGWAI	75–84	NA	
931	SLENFRAYV	225–233	PA	
1213	SRYWAIRTR	383–391	NP	
1000124	TKGILGFVFTLTV	56–68	M1	
1013714	*T*TYQRTRAL	146–154	NP	A
1008884	VLLVSLGAI	541–549	HA precursor	
1013714	AL*Q*LLLEV	102–109	NS2	H
1013714	FNMLSTVLGV	412–421	PB1	
1013714	FVANFSMEL	501–509	PB1	
1013714	FVE*A*LARSI	45–53	PB1	T
1013714	ILGFVFTL	59–66	M1	
1013714	ITFMQAL*Q*LL	97–106	NS2	H
1013714	LLFLKVPA	7–14	PB1	
1013714	QI*AILVTTV*	25–33	NA	GNIISIW
1013714	QL*M*WALGENMA	365–375	PA	K
1013714	QLVWMACHSAA	327–337	NP	
1013714	RTMAWTVVNSI	84–94	PA	
1019592	DQAIMDKNIILKANFSV	120–136	NS1	
1019592	GMMMGMFNMLSTVLGVS	406–422	PB1	
1019592	I*S*I*A*IG*I*ISL*M*LQIGNI	13–29	NA	CLVV.L. . .I
1019592	LTKGILGFVFTLTVPSE	55–71	M1	
1019592	PPNFS*C*IENFRAYVDGF	220–236	PA	SL
1019199	*RL*YQN*PTTYI*	204–213	HA	QQNIYQNENA

IEDB last accessed on January 16, 2011. Different sequences are italicized. M, matrix protein; NA, neuraminidase; NS, nonstructural protein; PA, acidic polymerase; PB1, basic polymerase 1; PB2, basic polymerase 2.

Table S4. Most published IAV NP epitopes share sequences with PR8 NP

IEDB reference ID	Object description	Position in NP	Substitution in PR8
315433	AAFEDLRVLSFI*R*G	335–349	K
1003542	AE*I*EDL*I*FL	251–259	F . . . T
1003542	AE*I*EDL*I*F*S*	251–259	F . . . T.L
1003540	CTELKLSDY	44–52	
1012379	CTELKL*T*D*Q*	44–52	S.Y
1012379	CTELKL*T*DY	44–52	S
1007471	ELRSRYWAI	380–388	
1000077	FEDLRVLS	338–345	
315051	FEDLRVLSF	338–346	
1003540	ILKGKFQTA	225–233	
1012379	ILRGS*I*AHK	265–273	V
1007471	ILRGSVAHK	265–273	
1401	KTGGPIY*K*R	91–99	R
1003542	KWMREL*V*LY	103–111	I
315051	LELRSRYWA	379–387	
315051	LELRSRYWAI	379–388	
1003691	LPFD*KP*TIM	418–426	
782	LPFD*KS*TIM	418–426	RT
1003691	LPFDRTTIM	418–426	
1012379	LPF*EKS*T*V*M	418–426	DRT
1012379	LPF*ER*ATIM	418–426	D.T
1012379	LPF*ERS*TIM	418–426	D.T
315048	LRSRYWAI	381–388	
315423	PKKTGGPIYRRVN	89–101	
1003540	RGINDRNFW	199–207	
1012379	RRSGAAGAA*I*K	174–184	V
1391	RRSGAAGAAVK	174–184	
931	RVLSFIKGTK	342–351	
315431	RYWAIRTR	384–391	
315021	SAAFEDLRVLSFIKG	335–349	
1000384	SAAFEDLRVLSFI*R*G	335–349	K
1002078	SRYWAIRTR	383–391	
931	TMVMELVRMIK	188–198	
315463	*T*TYQRTRAL	146–154	A
1013714	AE*I*EDL*I*FLA	251–260	F . . . T
1013714	FEDLRV*S*SF	338–346	L
1013714	QLVWMACHSAA	327–337	
1013714	RMVLSAFDER	65–74	
1013714	YERMCNILKG	219–228	

IEDB last accessed on January 16, 2011.

Table S5. Summary of the confirmed and discovered immunodominant epitopes

Epitope sequence	Position in NP (or M1)	HLA restriction	Donors	Binding motif
GILGFVFTL	58–66 (M1)	A*0201	6, 7, and 8	Yes
ELRSRYWAI	380–388	B*0801	1 and 3	Yes
CTELKLSDY	44–52	A*0101	1	Yes
HSNLNDATYQR	140–150[†]	B*1501	2 and 4	No
YERMCNILKG	219–228[†]	B*1801	2	No
AAFEDLRVL	336–344[†]	B*3503	5	No
GQISIQPTF	404–412[†]	B*1501	2	Yes

[†]Epitopes discovered in this study.