Supporting Information

Wahl *et al.* 10.1073/pnas.0811271106

SI Text

Influenza PR8 Infection of Mice

Six-week-old male and female HLA-B*0702 transgenic H-2K^{b}D^b double-knockout C57BL/6 mice (obtained from the laboratory of Dr. Francois Lemonnier at the Institut Pasteur) were inoculated intranasally with 1000 EID50 mouse-adapted Influenza PR8 (kindly provided by the laboratory of Dr. David Woodland at the Trudeau Institute) or sterile endotoxin free saline (mock infection) at volume of 10 μl per nostril. Mice were monitored daily for weight loss. Splenocytes were harvested 12 days post infection, passed through a cell strainer and lymphocytes isolated by centrifuging cells over a lympholyte-M density gradient (Cedar Lane). Lymphocytes were stored at -180°C in 90% FBS 10% DMSO.

Lymphocytes were measured for peptide specific interferon gamma secretion via ELISPOT. Briefly, 10^5 lymphocytes were incubated with 10 μg/ml synthetic influenza peptide, 10 μg/ml synthetic HIV-NEF peptide RPMTYKAAL (negative control), and 4 μg/ml concanavalin A (positive control) in RPMI 1640 media for 24 hr at 37°C in triplicate wells of a 96-well PVDF membrane-bottomed plate coated with anti-IFNγ antibody (Cell Sciences). The assay was performed according to manufacturer's instructions. The number of IFNγ produced spots per well was enumerated with a Zeiss KS ELISPOT reader. Wells with greater than 2.6 standard deviations (SD) the number of spots produced in wells with only media and 10^5 lymphocytes were scored positive. Wells with greater than 2.6 SD SFU have a confidence level $\geq$ 99% ($\mu = x \pm (Z\sigma)/\sqrt{N}$). The data generated are illustrated as spot forming units (SFU) per 10^5 lymphocytes.
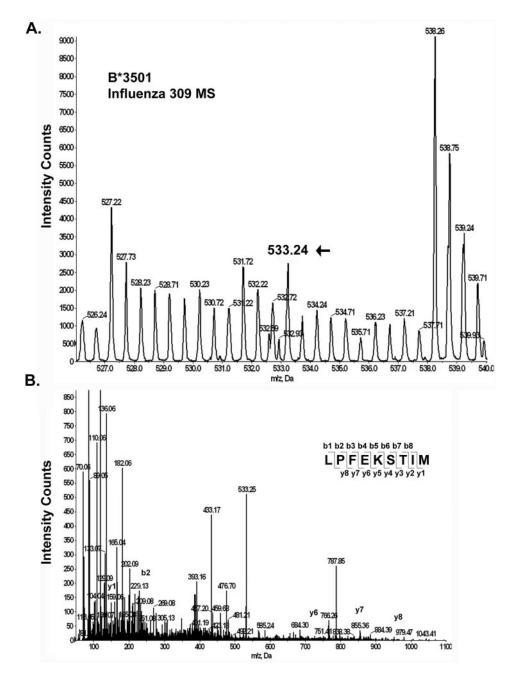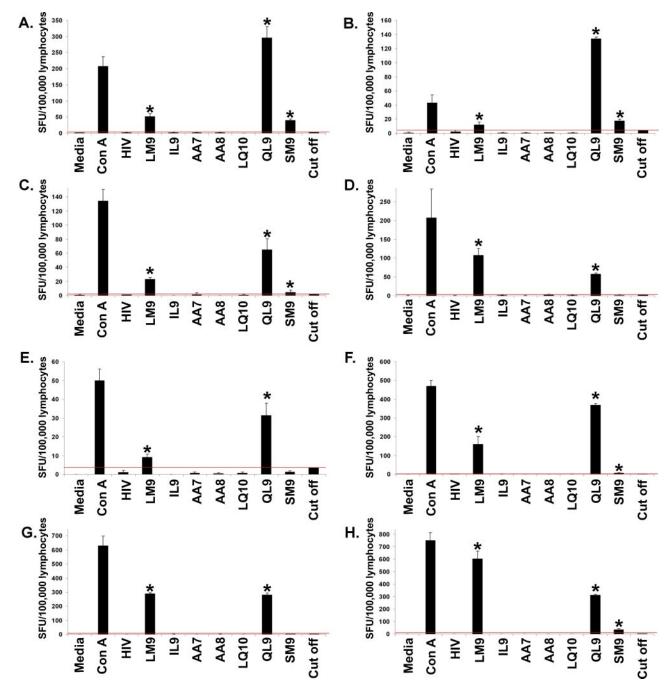
Fig. S1. Identification of NP 418–426 from 309 infected sHLA-B*3501 HeLa cells. (*A*) Peptides eluted from sHLA-B*3501 of 309 infected HeLa cells were separated by RP-HPLC and the fraction corresponding to the retention time of NP 418–426 was sprayed via nanospray into a Q-TOF mass spectrometer to create an MS ion map. (*B*) A peak at 533.2 *m/z* corresponding to the mass NP 418–426 was selected for MS/MS fragmentation. MS/MS fragmentation creates a series of b and y ions as the peptide is fragmented from the N (b ions) and C (y ions) terminus which are used to determine the peptide's amino acid sequence.

Fig. S2. IFNγ ELISPOT responses of 8 HLA-B*0702 transgenic mice infected with 1,000 EID50 PR8. Splenocytes were isolated from 1,000 EID50 PR8 infected mice and incubated overnight with either medium alone, Con A (4 μg/mL), or synthetic peptide (10 μg/mL). IFN-γ ELIPSOT responses are illustrated for each mouse (*A–H*) in spot forming units (SFU) per 10[5] lymphocytes. The cut-off for a positive IFN-γ response (2.6 SD above wells with cells incubated with medium alone) shown in red for each mouse and positive responses are indicated with an asterisk. The peptide sequence of each influenza A ligand is abbreviated in the graphs above according to N- and C-terminal amino acids and length (i.e., LPFDRTTVM: LM9). Three influenza HLA-B*0702 peptides generated an IFN-γ response: NP 418–426 (LM9), NP 473–481 (SM9), and PB1 329–337 (QL9). All mice elicited a positive IFN-γ response to NP 418–426 (LM9) and PB1 329–337 (QL9) and 5/8 responded to NP 473–481 (SM9). The remaining directly discovered HLA-B*0702 ligands were not recognized in any of the 8 mice tested. Mock saline-infected mice tested negative for all influenza ligands (data not shown).

Table S1. Nucleoprotein 418–426 peptide sequences in human influenza A H1N1 and H3N2 strains

1	2	3	4	5	6	7	8	9	Influenza strain
L	P	F	D	R	T	T	V	M	H1N1
–	–	–	–	K	–	–	I	–	H1N1
–	–	–	–	–	P	–	I	–	H1N1
–	–	–	–	K	A	–	I	–	H1N1
–	–	–	E	K	–	–	I	–	H1N1
–	–	–	E	–	S	–	I	–	H1N1
–	–	–	–	K	I	–	I	–	H1N1
–	–	–	E	–	A	–	I	–	H1N1
–	–	–	G	K	–	–	I	–	H1N1
–	–	–	–	–	–	–	I	–	H1N1/H3N2
–	–	–	–	K	S	–	I	–	H1N1/H3N2
–	–	–	–	K	S	–	–	–	H1N1/H3N2
–	–	–	E	–	A	–	–	–	H1N1/H3N2
–	–	–	–	K	P	–	I	–	H3N2
–	–	–	E	–	S	–	–	–	H3N2
–	–	–	E	K	S	–	I	–	H3N2
–	–	–	E	K	S	–	–	–	H3N2
–	–	–	–	K	Q	–	I	–	H3N2
–	–	–	E	K	S	I	–	–	H3N2
–	–	–	E	–	A	–	I	I	H3N2
–	–	–	E	K	P	–	–	–	H3N2