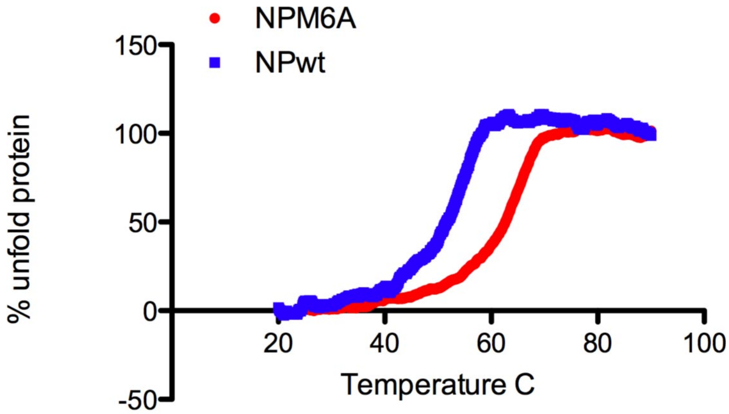
Supporting Information

Kedzierska *et al.* 10.1073/pnas.0810274105



H2Db-NP	51.8±0.7°C
H2Db-NP^{M6A}	63.3±0.7°C

Fig. S1. Thermostability of the H2D^{b}-NP$_{366}$ and H2D^{b}-NPM6A complexes. Thermostability measurements of recombinant class I complexes were performed using circular dichroism. The measurements for the thermal melting experiments were made at 218 and 220 nm for H2D^{b}-NP$_{366}$ and H2Db-NP^{M6A}, respectively, at intervals of 0.1 °C at a rate of 1 °C/min from 20 °C to 90 °C. The midpoint of thermal denaturation (T_m) for each protein was determined at the point at which 50% unfolding was achieved. The peptide–MHC complex with NP$_{366}$ WT epitope had a T_m of 51.8 ± 0.7 °C, irrespective of the concentration of the complex used for the thermostability assay. The H2D^{b} bound with the variant NPM6A epitope shows a higher thermostability with a T_m of 63.3 ± 0.7 °C, irrespective of the concentration of the complex used for the thermostability assay.

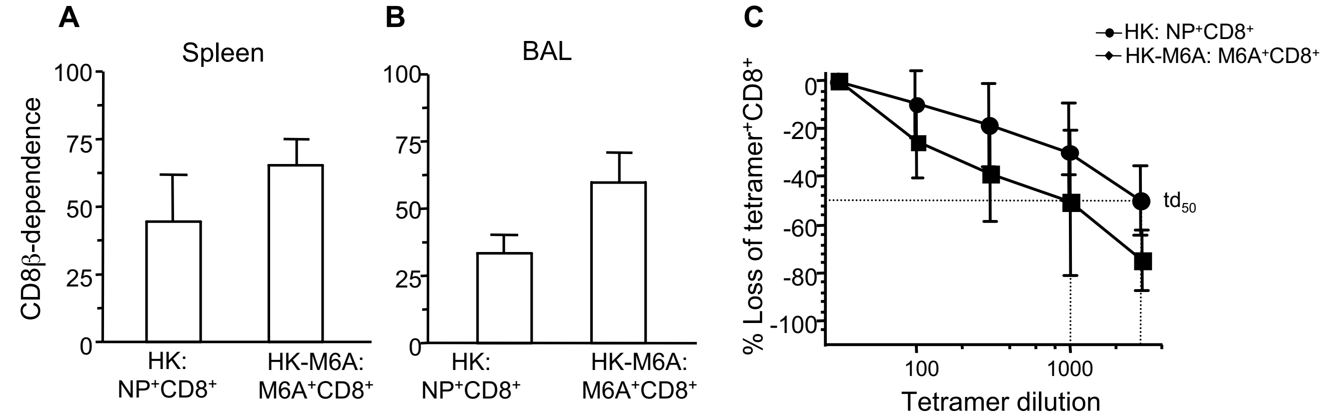


Fig. S2. TCR avidity for pMHC complex by CD8β dependence and tetramer dilution assay. pMHC-TCR avidity was assessed by two measures of the overall avidity: CD8β dependence (*A* and *B*) and tetramer dilution assays (*C*). Splenocytes (*A* and *C*) or BAL samples (*B*) were obtained from mice sampled on day 10 after infection with either the WT HK or mutant HK-NPM6A virus. (*A* and *B*) Lymphocytes were precultured in the presence or absence of anti-CD8β antibody (53.5–8) (10 μg/mL). Cells were then stimulated for 5 h with peptide, IL-2, and GolgiStop in the presence or absence of anti-CD8β antibody (5 μg/mL). After stimulation, cells were analyzed for CD8 and IFN-γ expression. Shown is the percentage of CD8$^+$ cells producing IFN-γ after stimulation in the presence of anti-CD8β blocking mAb. (*C*) Cells were stained with either the D^bNP_{336} or D^bNPM6A tetramers at 3-fold dilutions in the presence of NaAz, then washed and incubated with anti-CD8-FITC mAb. The progressive diminution in tetramer staining was measured. The Td50 value defines the time to 50% tetramer loss. Data represent mean ± SD for groups of 5 mice.

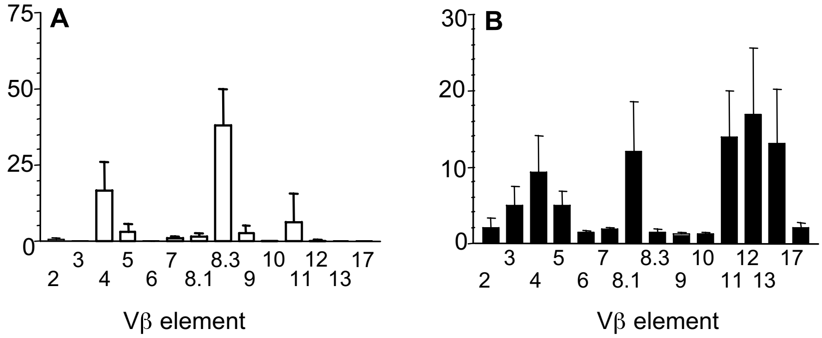


Fig. S3. TCRVβ usage in D^bNPM6A^+ $CD8^+$ T cell responses. WT D^bNP_{366} (*A*) or mutant NPM6A (*B*) $CD8^+$ T cell responses were generated by infection with the WT HK (*A*) or HK-NPM6A (*B*) viruses. Splenocytes were stained with the D^bNP_{366} (*A*) or D^bNPM6A (*B*) tetramers; anti-CD8 and anti-Vβ mAbs were conjugated with FITC, then the tetramer$^+$CD8$^+$ cells were analyzed for profiles of Vβ staining. Shown are mean $\pm$ SD values for groups of 4–5 secondarily-infected mice.

Table S1. TCR repertoire diversity of M6A$^+$CD8$^+$ T cells during primary (i.p.) and secondary (i.p.$\rightarrow$ i.n.) influenza-specific responses

Statistic	Primary M6A$^+$CD8$^+$	Secondary M6A$^+$CD8$^+$
Mice analyzed	4	6
TCRs sequenced	466	530
Predominant Jβ region	2S1, 2S4	2S4
Predominant CDR3β length, aa	8, 9	8, 9, 10
Different sequences	29	26
Repeated sequences	6	3
Vβ per mouse	2.8	2.2
Total sequences per Vβ	42.4	40.2
Clonotypes per Vβ	3.3	2.4
Clonotypes per mouse	9.0	5.0
Clonotypes with basic amino acid (R, K or H), %	79.3	96.2

Predominant CDR3β and Jβ is defined as $>$ 15%.

Table S2. Frequency of TCRβ amino acid sequences in M6A$^+$CD8$^+$ T cell responses

			Frequency					
CDR3β region	Jβ	Amino acid	M1	M2	M3	M4	M5	M6
Vβ4								
SQERGRGNTL	1S3	10			55			
SQKTVNQAP	1S5	9	22					
SQRQGYAEQ	2S1	9	12					
Vβ5.1/5.2								
SLDRKYEQ*	2S6	8					1	41
SLDRLYEQ	2S6	8						42
SLELKNTGQL	2S2	10					36	
SLGLKNTGQL	2S2	10					1	
SPHWGVYAEQ	2S1	10					3	
Vβ8.1/8.2								
GDGGRDTQ	2S5	8				43		
GPGGARSERL	1S4	10				2		
RDKNTEV	1S1	7			2			
SDAGRNQAP	1S5	9				5		
SDTGRRGAETL	2S3	11			37			
Vβ12								
RLGNYAEQ	2S1	8	11					
SLRGGASDY	1S2	9			53			
RGGNYAEQ	2S1	8	2					
RGGNHAEQ	2S1	8	1					
RQGNYAEQ	2S1	8		26				
RQRLGGTYEQ	2S6	10	5					
Vβ13								
SFRGRGTEV	1S1	9				1		
SFRGRQNTL*	2S4	9		32		33	31	
SLRGALAEQ	2S1	9		4				
SLRGLNQAP	1S5	9				8		
SLRGRQNTL	2S4	9					14	
SLWTTNTEV	1S1	9						38
RNRGRQNTL*	2S4	9		5			5	
Total sequences			53	67	147	92	91	121

M: individual mouse. Numbers of sequences for each clonotypes are shown. Please refer to ref. 1 for $D^bNP_{366}{}^+CD8^+$ TCR repertoire data. Secondary TCR repertoires are shown.

*Clonotypes correspond to repeated sequences.

1. Kedzierska K, Turner SJ, Doherty PC (2004) Conserved T cell receptor usage in primary and recall responses to an immunodominant influenza virus nucleoprotein epitope. *Proc Natl Acad Sci USA* 101:4942–4947.

Table S3. Data collection and refinement statistics for D^bNP-M6A

Data collection statistics	
Temperature	100 K
Space group	*P1*
Cell dimensions (a,b,c), Å	57.71, 69.69, 72.38, 99.94°, 111.04°, 110.48°
Resolution, Å	50–2.50
Total no. of observations	80,021 (8,859)
No. of unique observations	30,522 (3,335)
Multiplicity	2.6 (2.6)
Data completeness, %	94.9 (93.9)
I/σ_I	13.70 (5.04)
R_{merge}, %*	9.4 (27.3)
Refinement statistics	
Nonhydrogen atoms	
Protein	6,385
Water	35
Resolution, Å	2.50
R_{factor}, %†	22.0
R_{free}, %†	28.5
rmsd from ideality	
Bond lengths, Å	0.011
Bond angles, °	1.416
Ramachandran plot, %	
Most favored region	87.0
Allowed region	11.5
Generously allowed region	1.5
B factors, Å²	
Average *B* value	14.6
rmsd of bonded *B*	0.7

Values in parentheses are for the bin of highest resolution (approximate interval = 0.5 Å).

*$R_{merge} = \Sigma |I_{hkl} - [I_{hkl}]| / \Sigma I_{hkl}$.

†$R_{factor} = \Sigma_{hkl} || F_o | - | F_c || / \Sigma_{hkl} | F_o |$ for all data except ≈10%, which were used for R_{free} calculation.