Supplemental Table 1. TCRβ repertoire of naïve $D^bPA_{224}^+TRBV29^+CD8^+$ T cells in B6 mice.

CDR3β	M1	M2	M3	M4	M5	M6
SWGGEQ	2	-	1	-	1	-
SWGERL	2	-	-	-	1	-
SPDRGAL	2	-	-	-	-	-
SLGDEQ	1	-	-	-	1	1
AAGREQ	1	-	-	-	-	-
GGDWGAGDTQ	1	-	-	-	-	-
SDEDIQAP	1	-	-	-	-	-
SFGDTQ	1	-	-	-	-	-
SFGGEV	1	-	-	-	-	-
SGGGEQ	1	-	-	-	-	-
SLGEEQ	1	-	-	-	-	-
SPGQAP	1	-	-	-	-	-
SPTDWGRYEQ	1	-	-	-	-	-
SPYEQ	1	-	-	-	-	-
STGDEQ	1	-	-	-	-	-
SWGEEV	1	-	-	-	-	-
TEDRGRL	1	-	-	-	-	-
TRGETL	1	-	-	-	-	-
SLGAEQ	-	1	-	1	-	-
SLGTEV	-	1	-	1	-	-
SSGEAP	-	1	-	-	1	-
SLDRGEV	-	1	-	-	-	-
GDWTGGFNQDTQ	-	1	-	-	-	-
SDATQGYAEQ	-	1	-	-	-	-
SEGDTQ	-	1	-	-	-	-
SGTGEQ	-	1	-	-	-	-
SLGGDSDY	-	1	-	-	-	-
SLGGEV	-	1	-	-	-	-
SPDRGEV	-	1	-	-	-	-
SPGTG	-	1	-	-	-	-
SWDRGTL	-	1	-	-	-	-
SWGAEV	-	1	-	-	-	-
TWDRGEV	-	1	-	-	-	-
SGGDEQ	-	-	1	-	1	-
SYGDEQ	-	-	1	-	-	-
AGGAEQ	-	-	1	-	-	-
SDGTKFSNERL	-	-	1	-	-	-
SFGSEV	-	-	1	-	-	-
SFGVEQ	-	-	1	-	-	-
SFSGQL	-	-	1	-	-	-
SLGDTQ	-	-	1	-	-	-
SLSPERL	-	-	1	-	-	-
SRGTKV	-	-	1	-	-	-
SSPGQQ	-	-	1	-	-	-
SSPQEQ	-	-	1	-	-	-
TNTGQL	-	-	1	-	-	-
TPGAEQ	-	-	-	3	-	-
SLGGYEQ	-	-	-	2	-	-
TGGERL	-	-	-	1	-	1
ENYAEQ	-	-	-	1	-	-
SAYEQ	-	-	-	1	-	-
SDGGVP	-	-	-	1	-	-
SFGAEQ	-	-	-	1	-	-
SQGETL	-	-	-	1	-	-
SSGERL	-	-	-	1	-	-
SSPGQA	-	-	-	1	-	-
SSSGAK	-	-	-	1	-	-
TGGSDY	-	-	-	1	-	-
SWGDEQ	-	-	-	-	2	-
SSYEQ	-	-	-	-	1	-
EDRGFAEQ	-	-	-	-	1	-
SFGERL	-	-	-	-	1	-
SLGAEV	-	-	-	-	1	-
SLSGEQ	-	-	-	-	1	-
SPLGEGQNTL	-	-	-	-	1	-
SPSGQQ	-	-	-	-	1	-
SQGGAP	-	-	-	-	1	-
SSPSEQ	-	-	-	-	1	-
SSTGKQ	-	-	-	-	1	-
SWGVEV	-	-	-	-	1	-
SDQ	-	-	-	-	-	4
ESAPTEV	-	-	-	-	-	1
KGVEEQ	-	-	-	-	-	1
SFGTEV	-	-	-	-	-	1
SFNWGGAETL	-	-	-	-	-	1
SGGAEQ	-	-	-	-	-	1
SLGERL	-	-	-	-	-	1
SLSWGGGEQ	-	-	-	-	-	1
SQGQAP	-	-	-	-	-	1
SWTGEV	-	-	-	-	-	1
Total	**21**	**15**	**14**	**17**	**18**	**15**

mRNA from all individual $D^bPA_{224}^+TRBV29^+CD8^+$ cells isolated from naïve B6 mice was reverse transcribed followed by two rounds of nested TRBV29-specific PCR amplification. The PCR products were purified and sequenced using the internal TRBV29 oligonucleotide primer.

Supplemental Table 2. TCRβ repertoire of naïve $D^bPB1\text{-}F2_{62}{}^+TRBV19^+CD8^+$ T cells in B6 mice.

CDR3	M1	M2	M3	M4	M5
SPGTANTEV	3	-	-	-	-
SMGNTEV	2	2	-	-	-
SPGQNTEV	2	-	1	2	-
SPGTTNTEV	2	-	-	2	-
SMGANTEV	2	-	-	-	-
SPGTNTEV	2	-	-	-	-
SNWGTNTGQL	1	1	-	-	-
SPGTDTEV	1	-	1	2	-
SMGAQDTQ	1	-	1	-	-
SMGTEV	1	-	-	-	1
AWGVGSEQ	1	-	-	-	-
GQGLNSDY	1	-	-	-	-
GSGNTL	1	-	-	-	-
SAGAGTEV	1	-	-	-	-
SAGTANTEV	1	-	-	-	-
SAGTEV	1	-	-	-	-
SAGTGGAGQL	1	-	-	-	-
SIAILGVYEQ	1	-	-	-	-
SIAQYNSPL	1	-	-	-	-
SIGDISYEQ	1	-	-	-	-
SIGDPSNERL	1	-	-	-	-
SIGGARAEQ	1	-	-	-	-
SIGNANTEV	1	-	-	-	-
SLDWGGSAETL	1	-	-	-	-
SMGAGGYAE	1	-	-	-	-
SMGAGV	1	-	-	-	-
SMGDNSPL	1	-	-	-	-
SMGDTFSGNTL	1	-	-	-	-
SMGDTGQL	1	-	-	-	-
SMGESTEV	1	-	-	-	-
SMGGVDTEV	1	-	-	-	-
SMGLGGGQDTQ	1	-	-	-	-
SMGQFANTEV	1	-	-	-	-
SMGQGDSDY	1	-	-	-	-
SMGQGSNERL	1	-	-	-	-
SMGQGSTEV	1	-	-	-	-
SMGQQNTL	1	-	-	-	-
SMGSNQAP	1	-	-	-	-
SMGTGGPEV	1	-	-	-	-
SMGTGIEEQ	1	-	-	-	-
SMGTGNNSPL	1	-	-	-	-
SPGANTEV	1	-	-	-	-
SPGGNTEV	1	-	-	-	-
SPGLGAL	1	-	-	-	-
SPGQISNERL	1	-	-	-	-
SPGQYTEV	1	-	-	-	-
SPGSSYEQ	1	-	-	-	-
SPGTGGREQ	1	-	-	-	-
SPGTGGYEQ	1	-	-	-	-
SPGVANTGQL	1	-	-	-	-
SQGMGTGQL	1	-	-	-	-
SRGDYAEQ	1	-	-	-	-
STGAQDTQ	1	-	-	-	-
STGTGGAGTL	1	-	-	-	-
STGTGGL	1	-	-	-	-
STGTTNTEV	1	-	-	-	-
SWDISNTGQL	1	-	-	-	-
SWGANERL	1	-	-	-	-
SPGINTEV	-	1	1	-	-
GHTEV	-	1	-	-	-
RPGTAGTGQL	-	1	-	-	-
SAGDFYEQ	-	1	-	-	-
SAGTGGYEQ	-	1	-	-	-
SGGLSTGQL	-	1	-	-	-
SIAMGGQGTEV	-	1	-	-	-
SIGTGGFAEQ	-	1	-	-	-
SINWWEQ	-	1	-	-	-
SIWGGSAETL	-	1	-	-	-
SMGGWDTQ	-	1	-	-	-
SMGLTSAETL	-	1	-	-	-
SMGQGGYTEV	-	1	-	-	-
SMGTANTGQL	-	1	-	-	-
SMGWGDTQ	-	1	-	-	-
SPGASGNTL	-	1	-	-	-
SPGGAGTEV	-	1	-	-	-
SPGLGLDTQ	-	1	-	-	-
SPGLLEQ	-	1	-	-	-
SPGLSQNTL	-	1	-	-	-
SPGLVGDTQ	-	1	-	-	-
SPGQGGYAEQ	-	1	-	-	-
SPGSNSPL	-	1	-	-	-
SPGTGADTEV	-	1	-	-	-
SPGTGGFEQ	-	1	-	-	-
SPGTGSPEV	-	1	-	-	-
SPGTGTEV	-	1	-	-	-
SPGWGSQNTL	-	1	-	-	-
SSGGAGEQ	-	1	-	-	-
SSGTANTEV	-	1	-	-	-
STGTGQL	-	1	-	-	-
STGTHAEQ	-	1	-	-	-
SWGTNTEV	-	1	-	-	-
SQGFTEV	-	-	2	-	-
SRDISYNSPL	-	-	1	1	-
SAGTGDTEV	-	-	1	-	-
SDWGGYAEQ	-	-	1	-	-
SIGDFYEQ	-	-	1	-	-
SIGQGYYTEV	-	-	1	-	-

SIGTANTGQL	-	-	1	-	-
SISAGGFAETL	-	-	1	-	-
SLGQGVTEV	-	-	1	-	-
SLGTGIYEQ	-	-	1	-	-
SMGDNYEQ	-	-	1	-	-
SMGEHTEV	-	-	1	-	-
SMGGFYAEQ	-	-	1	-	-
SMGHLNERL	-	-	1	-	-
SMGHYAEQ	-	-	1	-	-
SMGQAGTEV	-	-	1	-	-
SMGQGRGNTL	-	-	1	-	-
SMGQQDTQ	-	-	1	-	-
SMGQSYAEQ	-	-	1	-	-
SMGTDTEV	-	-	1	-	-
SMGTNQAP	-	-	1	-	-
SMGWGEEQ	-	-	1	-	-
SMSQGYSDY	-	-	1	-	-
SPEWGSAETL	-	-	1	-	-
SPGDSTEV	-	-	1	-	-
SPGLGASAETL	-	-	1	-	-
SPGQFNERL	-	-	1	-	-
SPGQGGWAP	-	-	1	-	-
SPGQINERL	-	-	1	-	-
SPGQTSGNTL	-	-	1	-	-
SPGSDTEV	-	-	1	-	-
SPGTGGAGTL	-	-	1	-	-
SPGTGVEQ	-	-	1	-	-
SPGTGVYEQ	-	-	1	-	-
SPGTNEQ	-	-	1	-	-
SPGTPNTGQL	-	-	1	-	-
SPGTVYEQ	-	-	1	-	-
SPLYEQ	-	-	1	-	-
SQGFAEQ	-	-	1	-	-
SQGNTEV	-	-	1	-	-
SSGTSSYEQ	-	-	1	-	-
STGDNSPL	-	-	1	-	-
STGLDTQ	-	-	1	-	-
STGTGRTEV	-	-	1	-	-
STVREV	-	-	1	-	-
SVSYEQ	-	-	1	-	-
SWGASTEV	-	-	1	-	-
SWGGNQAP	-	-	1	-	-
SWGSAETL	-	-	1	-	-
SWGSNTGQL	-	-	1	-	-
TPGTNTEV	-	-	1	-	-
VGGTNERL	-	-	1	-	-
SAGTNSDY	-	-	-	2	-
SPGLGGAETL	-	-	-	2	-
STGLGDTL	-	-	-	2	-
SPGQSNTGQL	-	-	-	1	2
GTGDYAEQ	-	-	-	1	-
SAGTGVVETL	-	-	-	1	-
SGGGNTEV	-	-	-	1	-
SGGVTEV	-	-	-	1	-
SMGGGAEQ	-	-	-	1	-
SMGGNTEV	-	-	-	1	-
SMGVQDTQ	-	-	-	1	-
SPGQYNSPL	-	-	-	1	-
SPGTGATGQL	-	-	-	1	-
SPGTGGQAP	-	-	-	1	-
SPGTGPSDY	-	-	-	1	-
SPGTGYAEQ	-	-	-	1	-
SPGTPNTEV	-	-	-	1	-
SPGTVEQ	-	-	-	1	-
SRGEYAEQ	-	-	-	1	-
SSGTGGAGQL	-	-	-	1	-
SSGTGVAETL	-	-	-	1	-
SSGTGVYEQ	-	-	-	1	-
SSGTTEV	-	-	-	1	-
STGAERL	-	-	-	1	-
STGDSYEQ	-	-	-	1	-
STGNGNTEV	-	-	-	1	-
SWGEYAEQ	-	-	-	1	-
SWGGGAEQ	-	-	-	1	-
SWGGGTEQ	-	-	-	1	-
TTGGYAEQ	-	-	-	1	-
SIGDWGGQNTL	-	-	-	-	2
SMGNANTEV	-	-	-	-	2
TSGTGDYAEQ	-	-	-	-	2
SAGLDTQ	-	-	-	-	1
SAGVTEV	-	-	-	-	1
SEGASYEQ	-	-	-	-	1
SGGDWNTEV	-	-	-	-	1
SGGNTEV	-	-	-	-	1
SIGGGGEQ	-	-	-	-	1
SIGWGGSYEQ	-	-	-	-	1
SIMLNQDTQ	-	-	-	-	1
SIWGDQDTQ	-	-	-	-	1
SIYSAETL	-	-	-	-	1
SMDWGAQNTL	-	-	-	-	1
SMGGAEV	-	-	-	-	1
SMGGTEV	-	-	-	-	1
SMGLQDTQ	-	-	-	-	1
SMGSSETL	-	-	-	-	1
SMGTADTQ	-	-	-	-	1
SMGTGGTEV	-	-	-	-	1
SMGTGVTEV	-	-	-	-	1
SMGTSEV	-	-	-	-	1
SPGDNSPL	-	-	-	-	1
SPGLGGADTQ	-	-	-	-	1
SPGLGSQNTL	-	-	-	-	1
SPGLGVYEQ	-	-	-	-	1

SPGQGTPEV	-	-	-	-	1
SPGTKNTL	-	-	-	-	1
SPGVNSPL	-	-	-	-	1
STGDYAEQ	-	-	-	-	1
STGTGAYEQ	-	-	-	-	1
SVSGTGSYEQ	-	-	-	-	1
TGDISYNSPL	-	-	-	-	1
Total	**65**	**36**	**57**	**40**	**39**

mRNA from all individual $D^{b}PB1\text{-}F2_{62}{}^{+}TRBV19^{+}CD8^{+}$ cells isolated from naïve B6 mice was reverse transcribed followed by two rounds of nested TRBV19-specific PCR amplification. The PCR products were purified and sequenced using the internal TRBV19 oligonucleotide primer.

Supplemental Table 3. Public/repeated[A] CDR3β sequences in immune mice and their frequency in naive mice.

D^bNP_{366}	Freq	D^bPA_{224}	Freq	$D^bPB1\text{-}F2_{62}$	Freq
SGGGNTGQL	2/6	SFGAEQ	1/6	STGDYAEQ	1/5
SGGANTGQL	5/6	SFGGEV	1/6	SPGTNTEV	1/5
RGGANTGQL	5/6	SLDRGEV	1/6	SIGQYNSPL	0/5
SGGSNTGQL	3/6	SLGERL	1/6		
RGGSNTGQL	1/6	SSYEQ	1/6		
SARTANTEV	0/6	SFGGEQ	0/6		

mRNA from individual sorted cells isolated from pooled LN and spleen of naïve B6 mice was reverse transcribed followed by two rounds of nested TRBV-specific PCR amplification. The PCR products were purified and TCR CDR3 regions sequenced using the internal TRBV oligonucleotide primer.

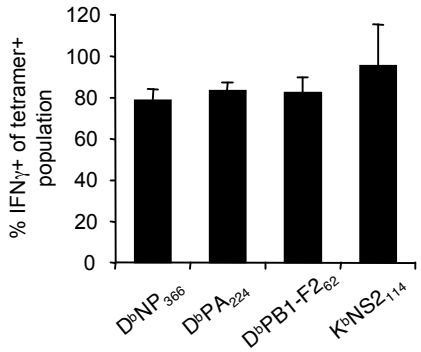
Data are from 6 (D^bNP_{366}, D^bPA_{224}) or 5 naïve mice ($D^bPB1\text{-}F2_{62}$), and 11 (D^bNP_{366}), 13 (D^bPA_{224}) or 19 immune mice ($D^bPB1\text{-}F2_{62}$), representing 759, 723, and 1507 CDR3β aa clonotypes, respectively.

[A]Defined as being identified in ≥33% mice following primary i.n. influenza infection.

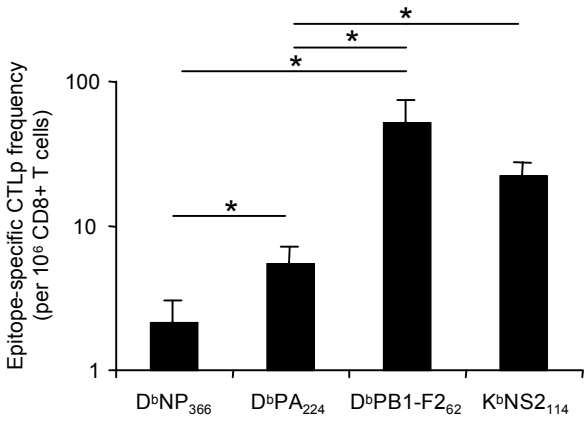
Supplemental Table 4. TCRβ repertoire of naïve and immune $K^bNS2_{114}^+$ $TRBV29^+$ $CD8^+$ T cells in B6 mice.

	IMMUNE		NAIVE			
CDR3β	**M1**	**M2**	**M1**	**M2**	**M3**	**M4**
SLRENTL	32	-	-	-	-	-
SYKNTEV	7	-	-	-	-	-
SLKNSDY	6	3	-	-	-	-
SLLDTEV	5	-	1	-	-	-
SPGLGGSYEQ	2	-	-	-	-	-
SRKNTEV	-	30	-	-	-	-
SLKENTL	-	7	-	-	-	-
SRVNTEV	-	6	-	-	-	-
SFKATEV	-	1	-	-	-	-
SLKENANCL	-	1	-	-	-	-
SSGKENTL	-	1	-	-	-	-
SLEGTQDTQ	-	-	2	-	-	-
RPHRDSDY	-	-	1	-	-	-
SGTPNTGQL	-	-	1	-	-	-
SLGQGNSP	-	-	1	-	-	-
SLNKNSDY	-	-	1	-	-	-
SLRQNTL	-	-	1	-	-	-
SLYGNSP	-	-	1	-	-	-
SPGQSQNTL	-	-	1	-	-	-
SPGTNAEQ	-	-	1	-	-	-
SPGTTSQNTL	-	-	1	-	-	-
SPTREGEQ	-	-	1	-	-	-
SRDNNQAP	-	-	1	-	-	-
SSGEPYEQ	-	-	1	-	-	-
SSGPERL	-	-	1	-	-	-
SSGTDQAP	-	-	1	-	-	-
SSPNERL	-	-	1	-	-	-
YSQNTL	-	-	1	-	-	-
RYAEQ	-	-	-	1	-	-
SLTDTEV	-	-	-	1	-	-
SPGNNNQAP	-	-	-	1	-	-
TRDNYAEQ	-	-	-	1	-	-
SLTNTEV	-	-	-	-	2	-
GGIKQNTL	-	-	-	-	1	-
SLGRLNQDTQ	-	-	-	-	1	-
SPGVENTL	-	-	-	-	1	-
SSGTENTL	-	-	-	-	1	-
SWGQNTEV	-	-	-	-	1	-
TDRALEQ	-	-	-	-	1	-
SLGNSQNTL	-	-	-	-	-	1
SLKNAEQ	-	-	-	-	-	1
SLSETEV	-	-	-	-	-	1
SRDNSYEQ	-	-	-	-	-	1
TOTAL	52	49	19	4	8	4

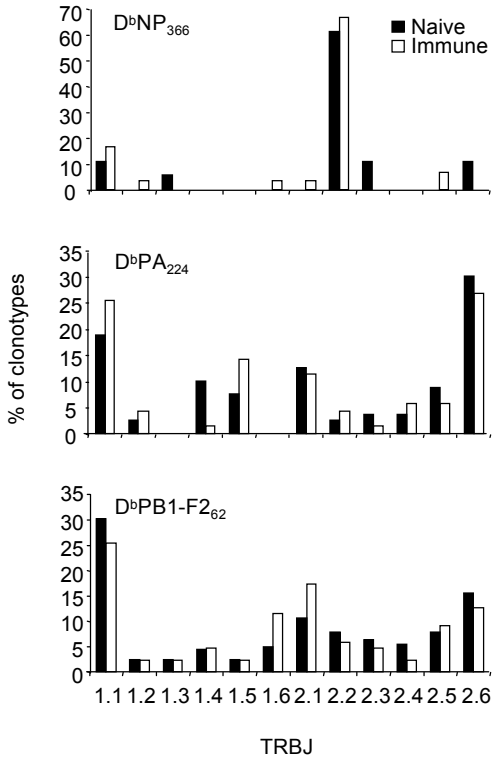
mRNA from individual sorted cells isolated from pooled LN and spleen of naïve or immune B6 mice was reverse transcribed followed by two rounds of nested TRBV29-specific PCR amplification. The PCR products were purified and TCR CDR3β regions sequenced using the internal TRBV29 oligonucleotide primer.



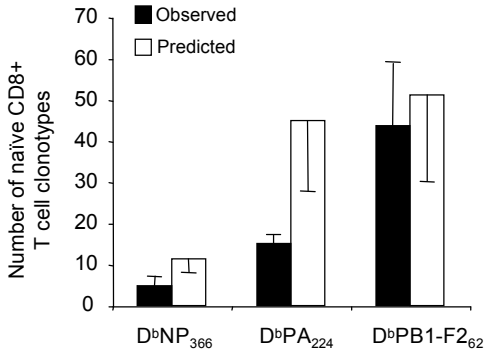
Supplental Figure 1. Equivalent binding of influenza pMHCI tetramers. Splenocytes harvested from mice 10d after primary i.n. influenza infection were either stained with either with the designated tetramers or restimulated in vitro with NP_{366}, PA_{224}, $PB1\text{-}F2_{62}$, or $NS2_{114}$ peptides followed by ICS for IFN-γ production. Shown are IFN-γ^+ cells expressed as a percentage of tetramer$^+$ cells (n=4 mice per group). Data are representative of 2 independent experiments.



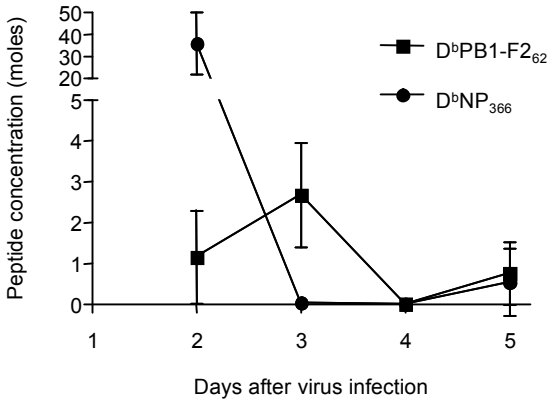
Supplementary Figure 2. Naive epitope-specific CTLp frequency relative to CD8+ T cell number. Epitope-specific CTLps were identified from the spleen and all major lymph nodes of naive B6 mice after enrichment with the indicated tetramers. CD8+ T cell number within the sample was determined from the total cell count and flow cytometric determination of % CD8+ cells, prior to magnetic enrichment. The frequency of naïve CTLp was then determined by dividing the number of naïve CTLps for each epitope specificity by the total number of CD8+ T cells and multiplying by 10^6.



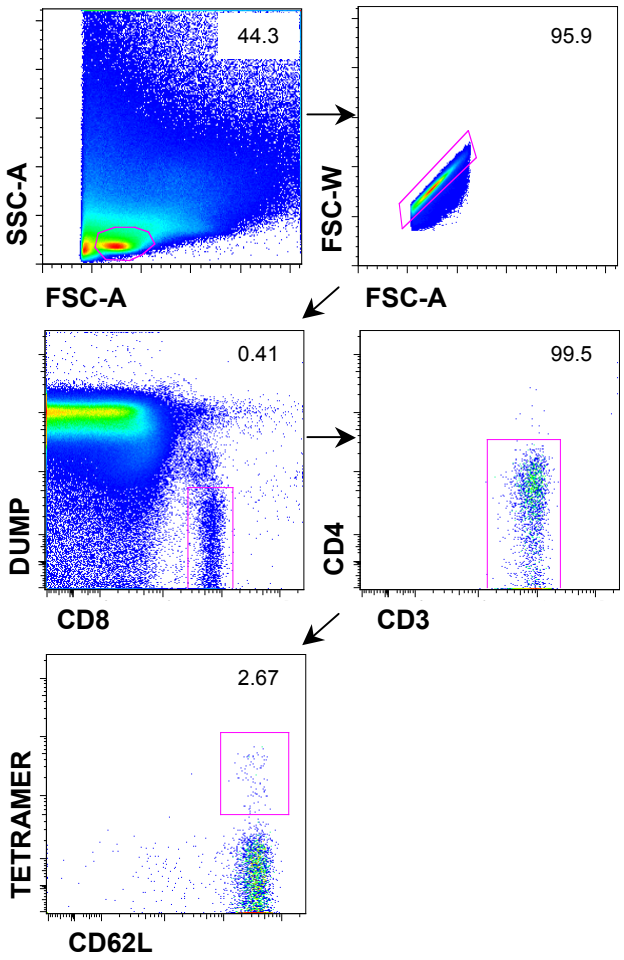
Supplemental Figure 3. TRBJ usage in naive and immune CTL populations. Individual naive or immune epitope-specific cells (identified as described in Methods) were individually sorted into wells of a 96 well plate, mRNA was reverse transcribed, followed by nested PCR with TRBV- and TRBC-specific oligonucleotide primers. PCR products were purified, sequenced and the TRBJ sequence identified according to IMGT nomenclature. Shown is the percentage of clonotypes (irrespective of abundance) from immune and naive repertoires utilizing specific TRBJ regions. Data was compiled from all of the naive sequences and sequences from at least 3 immune mice.



Supplemental Figure 4. Comparison of estimated and actual clonotype diversity. Using the non-parametric Chao1 method (38), the total number of naive clonotypes (richness) for each epitope was estimated and compared to the observed naive CTLp counts derived from the enrichment procedure. The Chao1 method utilizes the richness detected by sampling of the immune response (white column, approximately lower limit of error bar) and predicts the richness within the entire immune repertoire (column height), yielding an estimate of the lower limit of naive clonotype frequency. Error bars represent 95% confidence intervals. Calculations were performed using EstimateS (57) from all naive sequences and a database of 759, 723, and 1507 CDR3β aa clonotype sequences from 11 (D^bNP_{366}), 13 (D^bPA_{224}) or 19 immune mice ($D^bPB1\text{-}F2_{62}$), respectively (25, 30, 31). .



Supplementary Figure 5. Ex vivo presentation kinetics of D^bNP_{366} and $D^bPB1\text{-}F2_{62}$. MLN cells harvested from mice infected i.n. with x31 influenza virus at day 0, were co-cultured in 96 well plates with D^bNP_{366} or $D^bPB1\text{-}F2_{62}$-specific hybridoma cells (4-39, F2-54, respectively) for 24 hr. These hybridomas showed similar peptide sensitivity as determined by peptide titration experiments. Secreted IL-2 was measured using purified anti-IL-2 and biotin-anti-IL-2 (BD) antibodies in an established ELISA protocol. A standard curve was established using peptide pulsed, uninfected splenocytes, and epitope concentrations were calculated from the standard curve and the amount of IL-2 produced. Data is representative of 3 independent experiments.



Supplementary Figure 6. Gating strategy for identification of naïve epitope-specific cells following tetramer-based magnetic enrichment. Following staining with PE-conjugated tetramer (shown is a representative plot using D^bPA_{224} tetramer), cells are stained with anti-PE magnetic microbeads and passed twice over a magnetic column. Bound cells (comprising predominantly tetramer negative cells) are then eluted and single CD8+ dump (B220,F4/80, CD11b, CD11c)-, CD3+, CD4-, CD62Lhi, tetramer+ lymphocytes are identified using the gating strategy outlined, to discriminate between non-specific binders and true epitope-specific cells. Values represent the proportion of the parent population contained within the gate shown.