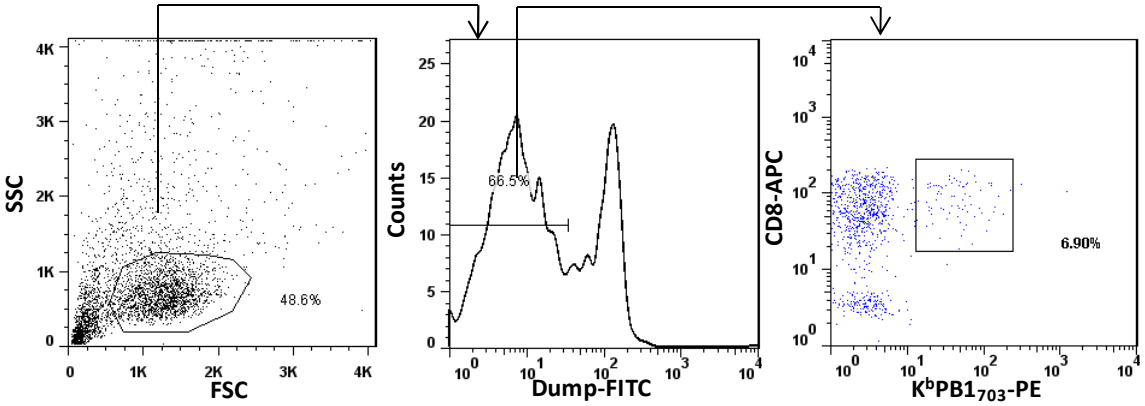
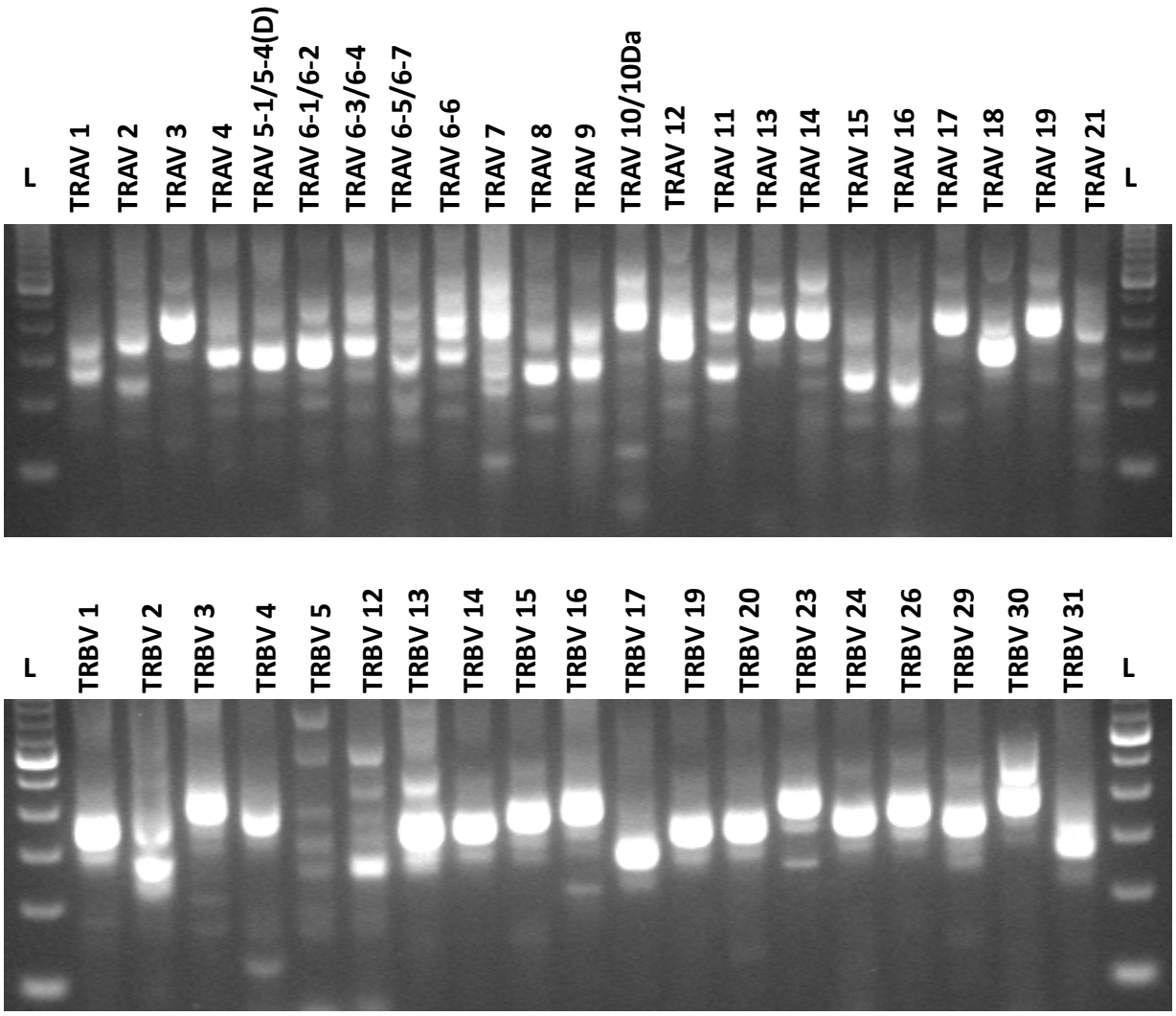


Supplementary figure 1. Flow cytometric staining profile and gating strategy for single cell sorting of K^bPB1_{703}-specific CD8 T cells. BAL from B6 mice infected intranasally with x31 influenza virus d10 post infection were stained with fluorochrome-conjugated tetrameric complexes of K^b-$PB1_{703}$ peptide, APC conjugated anti-CD8- antibody, FITC conjugated anti-CD4, anti-mouse pan macrophage marker, BM8 (F4/80), anti-CD11c and anti. The non-$CD8^+$ T cells were excluded using a dump gate based on CD4, F4/80, CD11c and CD11b staining and the $CD8^+$ $K^bPB1_{703–711}{}^+$ cells were single-cell sorted through the sort gate shown.



Supplementary figure 2. Agarose gel image showing validation of the individual TRAV and TRBV primers. Nested PCR products amplified by two rounds of PCR, using cDNA from total RNA isolated from resting CD8 T cells incorporating individual TRAV and TRAC specific primers (top panel) and TRBV- and TRBC-specific primers (bottom panel). L denotes the 100 bp ladder lane in the gel.

Supplementary Table 1: Paired co-expression analysis of TCRα and β on influenza K^bPB1_{703} -specific CD8 T cells by single cell multiplex RT-PCR–sequencing method. Cells with out-of-frame TCRα transcript are shown in bold. TCR V, D, and J nomenclature follows that of IMGT. Sequences with more than one family name call are shown in italics.

Cell	CDR3α	CDR3β	TRAV	TRAJ	TRBV	TRBD	TRBJ
G1	SGNYAQGLTFGLGTRV	TGSSAETLYFGSGTRL	3-3	26	31	1	2-3
H1	SNNYAQGLTFGLGTRV	SGESAETLYFGSGTRL	3-3	26	14	2	2-3
A2	SANYAQGLTFGLGTRV		3-3	26			
B2	SGNYAQGLTFGLGTRV	TGSSAETLYFGSGTRL	3-3	26	31	1	2-3
C2	GNNYAQGLTFGLGTRV	SRDSAETLYFGSGTRL	3-3	26	4	2	2-3
F2	NSNYQLIWGSGTKLII	SWESAETLYFGSGTRL	3-3	33	4	2	2-3
G2	SGNYAQGLTFGLGTRV		3-3	26			
H2	**SMKFITRGSLSLDREP**	GETSAETLYFGSGTRL	3-3	23			
A3	GNNYAQGLTFGLGTRV	SRDSAETLYFGSGTRL	3-3	26	14	2	2-3
B3		SGESAETLYFGSGTRL			14	2	2-3
C3	SGNYAQGLTFGLGTRV	SGDSAETLYFGSGTRL	3-3	26	15	2	2-3
D3	SDNNRIFFGDGTQLVV		3-3	31			
E3		SWDSAETLYFGSGTRL			4	1	2-3
F3	SNNYAQGLTFGLGTRV	SGESAETLYFGSGTRL	3-3	26	14	2	2-3
G3		GGSSAETLYFGSGTRL			13-2	1	2-3
A4		GGDTSAETLYFGSGTR					
B4	**SGTMPRD*PSVLAPEY**	SQDSAETLYFGSGTRL	14-2	26	26	2	2-3
D4	**SDEGQMLTKSSLEKGH**	TPTDSAETLYFGSGTR	*6D-6 or 6D7-7*	30			
E4	SMDYANKMIFGLGTIL	GGDWGGSYEQYFGP	4D-3	47			
F4	SRNYAQGLTFGLGTRV	SGDSAETLYFGSGTRL	3-3	26	15	2	2-3
G4	SHNYAQGLTFGLGTRV	SGTSNERLFFGHGTKL	3-3	26	19	1	1-4
H4	SGNYAQGLTFGLGTRV	RDGWDSAETLYFGSG	3-3	26	20	2	2-3
A5	SNNYAQGLTFGLGTRV	SGESAETLYFGSGTRL	3-3	26	14	2	2-3
C5	SGNYAQGLTFGLGTRV	TGSSAETLYFG	3-3	26	31	1	1-3
A6	SNNYAQGLTFGLGTRV	SGESAETLYFGSGTRL	3-3	26	14	2	2-3
B6	SHNYAQGLTFGLGTRV		3-3	26			
C6	SSNYAQGLTFGLGTRV	GDSSAETLYFGSGTRL	3-3	26	1	1	2-3
D6	SNNYAQGLTFGLGTRV	SVDSAETLYFGSGTRL	3-3	26	26	2	2-3
E6	**SEHSLITTCFTSDLAP**	SGDSAETLYFGSGTRL	*7-4 or 7D4*	21	15	2	2-3
G6		GGSSAETLYFGSGTRL			13-2	1	2-3
H6		SGTSNERLFFGHGTKL			19	1	1-4
A7		SGESAETLYFGSGTRL			14	2	2-3
B7	SANSAGNKLTFGIGTR		3-3	17	13-1	2	2-3
C7	**V*LQRALLRIWHQTHC**	TGNSAETLYFGSGTRL			5	2	2-3
D7	SDNYAQGLTFGLGTRV		3-3	26			
E7	TGNYKYVFGAGTRLKV		3-3	40			
F7	SANNYAQGLTFGLGTR	RGTDSAETLYFGSGTR	3-3	26	19	1	2-3
G7		SFRDSAETLYFGSGTR			14	2	2-3
A8	SDNNRIFFGDGTQLVV	GRDSAETLYFGSGTRL	3-3	31	1	2	2-3
B8	SGNYAQGLTFGLGTRV		3-3	26			
C8	NSNYQLIWGSGTKLII	SWESAETLYFGSGTRL	3-3	33	4	2	2-3
D8	SANYAQGLTFGLGTRV	GGSSAETLYFGSGTRL	3-3	26			
E8	REGPGTGSNRLTFGKG	SLDSAETLYFGSGTRL	16D/ DV11	28	15	2	2-3
F8		SDARGEHERLFFGHGT			13-3	1	1-4
G8	SANNYAQGLTFGLGTR	RGTDSAETLYFGSGTR	3-3	26	19	1	2-3
H8	**SHNRQYRKTHLWTGD**	GDDSAETLYFGSGTRL	16D/ DV11	37	13-2	2	2-3
A9	SDNYAQGLTFGLGTRV	AGTSAETLYFGSGTRL	3-3	26	4	2	2-3
B9	SGNYAQGLTFGLGTRV	GEDSAETLYFGSGTRL	3-3	26	13-2	2	2-3
B10	SGNYQLIWGSGTKLII	SWDSNERLFFGHGTKL	3-3	33	4	1	1-4
C10		SRDSAETLYFGSGTRL			4	2	2-3
D10		SFRDSAETLYFGSGTR			14	2	2-3
E10	SANYAQGLTFGLGTRV	SGQDSAETLYFGSGTR	3-3	26			
G10	NSNYQLIWGSGTKLII	SWESAETLYFGSGTRL	3-3	33	4	2	2-3
H10	SGNYAQGLTFGLGTRV	SRDSAETLYFGSGTRL	3-3	26	15	2	2-3

Supplementary Table 2. Comparative analysis of multiplex RT-PCR–sequencing method to that of MHC tetramer/Vβ antibody scanning. Dominant Vβs are shown in bold (n=9).

IMGT TRBV nomenclature[a]	Wilson, RK et al.[b]	No. of sequences	% of Total	Sequence data	Vβ Antibody data
TRBV1	Vβ2	16	3.70	3.70	2.362
TRBV2	Vβ4	4	0.93	0.93	0.696
TRBV3	Vβ16	5	1.16	1.16	
TRBV4	Vβ10	58	13.43	**13.43**	**16.3**
TRBV5	Vβ1	27	6.25	6.25	
TRBV12-1	Vβ5.2	19	4.40	6.02[c]	2.4[c]
TRBV12-2	Vβ5.1	7	1.62		
TRBV13-1	Vβ8.3	16	3.70	3.70	2.608
TRBV13-2	Vβ8.1	52	12.04	**16.2[d]**	**17.3[d]**
TRBV13-3	Vβ8.2	18	4.17		
TRBV14	Vβ13	51	11.81	**11.81**	**14.54**
TRBV15	Vβ12	30	6.94	6.94	0.32
TRBV16	Vβ11	17	3.94	3.94	5.19
TRBV17	Vβ9	7	1.62	1.62	1.64
TRBV19	Vβ6	52	12.04	**12.04**	**6.82**
TRBV20	Vβ15	2	0.46	0.46	
TRBV21	Vβ19	1	0.23	0.23	
TRBV23	Vβ20	1	0.23	0.23	
TRBV24	Vβ17	10	2.31	2.31	0.442
TRBV26	Vβ3.1	5	1.16	1.16	2.928
TRBV29	Vβ7	10	2.31	2.31	5.53
TRBV30	Vβ18	1	0.23	0.23	
TRBV31	Vβ14	23	5.32	**5.32**	**7.832**
		432	100	100	86.9

[a] and [b] Corresponding Vβ nomenclature (from http://www.imgt.org)

[c] Total percentage of Vβ 5.1 and Vβ 5.2 and corresponding sequence data

[d] Total percentage of Vβ 8.1 and Vβ 8.2 and corresponding sequence data

Supplementary Table 3. Forward and reverse TRAV specific primer sequences (both external and internal).

Primer name	External Forward	Internal Forward
TRAV1	GGTTATCCTGGTACCAGCA	CTCCACATTCCTGAGCC
TRAV2	CATCTACTGGTACCGACAGG	ACTCTGAGCCTGCCCT
TRAV3	GGCGAGCAGGTGGAG	GCCCTCCTCACCTGAG
TRAV4	TCTGSTCTGAGATGCAATTTT	GGITIMAGGAACAAAGGAGAAT
TRAV5-1/5-4(D)	GGCTACTTCCCTTGGTATAAGCAAGA	ATYCGTTCAAATATGGAAAGAAA
TRAV6-1/6-2	CAGATGCAAGGTCAAGTGAC	GGAGAAGGTCCACAGCTC
TRAV6-3/6-4(D)	AAGGTCCACAGCTCCTTC	CAACTGCCAACAACAAGG
TRAV6-5/6-7(D)	GTTCTGGTATGTGCAGTATCC	TCCTTCCACTTGCAGAAAG
TRAV6-6	AGATTCCGTGACTCAAACAG	ACGGCTGGCCAGAAG
TRAV7	AGAAGGTRCAGCAGAGCCCAGAATC	CAKGRCYTCYYTCAACTGCAC
TRAV8	GAGCRTCCASGAGGGTG	AGAGCCACCCTTGACAC
TRAV9	CCAGTGGTTCAAGGAGTG	GCTTYGAGGCTGAGTTCAG
TRAV10/10a(D)	AGAGAAGGTCGAGCAACAC	CTACACTGAGTGTTCGAGAGG
TRAV11	AAGACCCAAGTGGAGCAG	AACAGGACACAGGCAAAG
TRAV12	TGACCCAGACAGAAGGC	GGTTCCACGCCACTC
TRAV13	TCCTTGGTTCTGCAGG	TGCAGGAGGGGGAGA
TRAV14	GCAGCAGGTGAGACAAAG	CTCTGACAGTCTGGGAAGG
TRAV15	CASCTTYTTAGTGGAGAGATGG	AYTCTGTAGTCTTCCAGAAATCAC
TRAV16	GTACAAGCAAACAGCAAGTG	ATTATTCTCTGAACTTTCAGAAGC
TRAV17	CAGTCCGTGGACCAGC	TATGAAGGAGCCTCCCTG
TRAV18	AACGGCTGGAGCAGAG	CAAGATTTCACCGCACG
TRAV19	GCAAGTTAAACAAAGCTCTCC	GCTGACTGTTCAAGAGGGA
TRAV21	GTGCACTTGCCTTGTAGC	AATAGTATGGCTTTCCTGGC
	External reverse	**Internal Reverse**
TRAC-Rev	GGCATCACAGGGAACG	GCACATTGATTTGGGAGTC

Supplementary Table 4. Forward and reverse TRBV specific primer sequences (both external and internal).

Primer name	External Forward	Internal Forward
TRBV1	TACCACGTGGTCAAGCTG	GTATCCCTGGATGAGCTG
TRBV2	CAGTATCTAGGCCACAATGC	GGACAATCAGACTGCCTC
TRBV3	CCCAAAGTCTTACAGATCCC	GATATGGGGCAGATGGTG
TRBV4	GACGGCTGTTTTCCAGAC	CAGGTGGGAAATGAAGTG
TRBV5	GGTATAAACAGAGCGCTGAG	GCCAGAGCTCATGTTTCTC
TRBV12	GGGGTTGTCCAGTCTCC	CCAGCAGATTCTCAGTCC
TRBV13	GCTGCAGTCACCCAAAG	GTACTGGTATCGGCAGGAC
TRBV14	GCAGTCCTACAGGAAGGG	GGTATCAGCAGCCCAGAG
TRBV15	GAGTTACCCAGACACCCAG	GTGTGAGCCAGTTTCAGG
TRBV16	CCTAGGCACAAGGTGACAG	GAAGCAACTCTGTGGTGTG
TRBV17	GAAGCCAAACCAAGCAC	GAACAGGGAAGCTGACAC
TRBV19	GATTGGTCAGGAAGGGC	GGTACCGACAGGATTCAG
TRBV20	GGATGGAGTGTCAAGCTG	GCTTGGTATCGTCAATCG
TRBV23	CTGCAGTTACACAGAAGCC	GCCAGGAAGCAGAGATG
TRBV24	CAGACTCCACGATACCTGG	GCACACTGCCTTTTACTGG
TRBV26	GGTGAAAGGGCAAGGAC	GAGGTGTATCCCTGAAAAGG
TRBV29	GCTGGAATGTGGACAGG	GTACTGGTATCGACAAGACCC
TRBV30	CCTCCTCTACCAAAAGCC	GGACATCTGTCAAAGTGGC
TRBV31	CTAACCTCTACTGGTACTGGCAG	CTGTTGGCCAGGTAGAGTC
	External reverse	**Internal Reverse**
TRBC-Rev	CCAGAAGGTAGCAGAGACCC	GGGTAGCCTTTTGTTTGTTTG