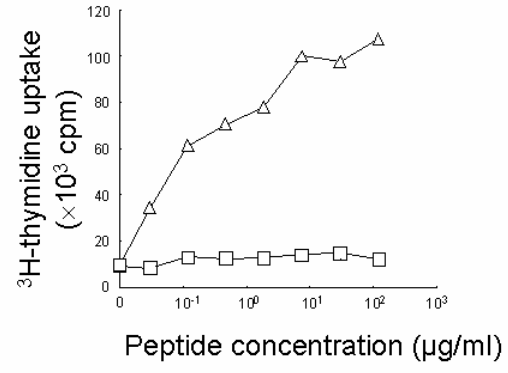
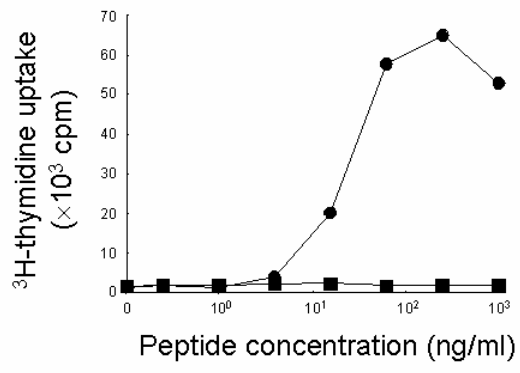


SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

Figure S1. Importance of the P5 peptide residue in T cell polyclonal response. Removal of the P5 side chain for both 2.5mi 9Q (left) and HEL₁₁₋₂₇ (right) peptides results in loss of reactivity. Mice were immunized with either 2.5mi 9Q or HEL₁₁₋₂₇ peptides (5 µg of peptide+CFA). Draining lymph nodes were harvested at day 8, and CD4⁺ T cells were purified by magnetic sorting and used for proliferation against increasing doses of the immunizing peptide or variants bearing Gly P5 in the presence of irradiated splenocytes. Identification: 2.5mi 9Q peptide (closed circles), 2.5mi 5G 9Q peptide (closed squares), HEL₁₁₋₂₇ peptide (open triangles), HEL₁₁₋₂₇ 5G peptide (open squares). Values are the mean of duplicates.

Figure S1.



SUPPLEMENTAL TABLES

Table S1. Amino acid sequences and N deletion/addition of CDR3 β segments of GAD₂₂₁₋₂₃₅ and GAD₂₂₁₋₂₃₅9E T cell hybridomas

CDR3 β sequences of GAD₂₂₁-reactive T hybridomas

Cell#	V	D	J	Amino acid sequence			Nucleic acid deletion/addition						comments
				Vb	nDbn	Jb	Vb del	add*	D5' del	D3' del	add**	Jb del	
cl.26	12-1	1	1-3	FCASS	DRA	GNTLYFGE	✓	-	✓	✓	-	✓	
cl.29	13-3	1	1-6	FCAS	RDRGD	SPLYFAA	✓	-	✓	✓	✓	✓	
cl.53	3	1	2-5	FCASS	PSGRG	DTQYFGP	✓	✓	✓	✓	-	✓	
cl.58	13-2	?	2-5	FCASG	ERY	QDTQYFGP	✓	✓	✓	✓	✓	✓	
cl.60	3	1	2-5	FCASS	PSGRG	DTQYFGP	✓	✓	✓	✓	-	✓	
cl.76	13-3	1	2-3	FCASS	EGN	SAETLYFGS	✓	-	✓	✓	✓	-	
cl.79	16	2	2-5	LCASS	PRDWG	QDTQYFGP	✓	✓	-	✓	✓	✓	
cl.82	3	2	2-1	FCASSL	DWG	YAEQFFGP	✓	✓	✓	✓	-	✓	
cl.86	16	1	1-3	LCASS	DQGAP	GNTLYFGE	✓	✓	✓	-	✓	✓	
cl.102	19	1	1-5	LCASSI	ST	NNQAPLFG	✓	✓	✓	✓	-	✓	
cl.103	13-2	?	2-5	FCASG	ERY	QDTQYFGP	✓	✓	✓	✓	✓	✓	
cl.105	19	1	1-5	LCASSI	ST	NNQAPLFG	✓	✓	✓	✓	-	✓	
cl.108	16	1	1-3	LCASS	DQGAP	GNTLYFGE	✓	✓	✓	-	✓	✓	

CDR3 β sequences of GAD₂₂₁9E-reactive T hybridomas

Cell#	V	D	J	Amino acid sequence			Nucleic acid deletion/addition						comments
				Vb	nDbn	Jb	Vb del	add*	D5' del	D3' del	add**	Jb del	
cl.34	13-2	1	1-6	FCAS	GTGG	YNSPLYFAA	✓	✓	✓	✓	-	✓	
cl.52	19	?	1-6	LCASS	RT	YNSPLYFAA	✓	✓	✓	✓	-	✓	
cl.60	2	1	2-7	FCASS	LRDRD	EQYFGP	✓	✓	-	✓	✓	✓	
cl.66	16	?	2-7	LCASSL	AGN	EQYFGP	✓	✓	✓	✓	✓	✓	
cl.92	16	1	2-7	LCASS	QGQGD	EQYFGP	✓	✓	✓	✓	✓	✓	
cl.110	3	1	2-5	FCASS	PSGRG	DTQYFGP	✓	✓	✓	✓	-	✓	
cl.119	19	2	2-5	LCASSI	EGGGG	QDTQYFGP	-	✓	✓	✓	✓	✓	
cl.142	16	?	2-7	LCASSL	AGN	EQYFGP	✓	✓	✓	✓	✓	✓	
cl.164	5	1	2-1	FCASSQ	GQA	NYAEQFFGP	✓	-	✓	✓	✓	-	
cl.183	13-3	1	2-3	FCASS	GHWGG	AETLYFGS	✓	✓	✓	✓	-	✓	
cl.187	13-3	1	2-3	FCASS	GHWGG	AETLYFGS	✓	✓	✓	✓	-	✓	

T cell hybridomas were isolated after immunization with GAD₂₂₁₋₂₃₅ or GAD₂₂₁₋₂₃₅9E peptides. Negatively charged amino acid residues in the first part of the CDR3 β segment are highlighted in bold and red. Vb del: deletion of nucleotide(s) on 3' part of BV germline, add*: addition of nucleotide(s) between BV and BD, D5' del: deletion of nucleotide(s) on 5' part of BD germline, D3' del: deletion of nucleotide(s) on 3' part of BD germline, add**: addition of nucleotide(s) between BD and BJ, Jb del: deletion of nucleotide(s) on 5' part of BJ germline ✓: deletion or addition of nucleotide(s), -: no deletion or no addition, ✓: deletion or addition of nucleotide(s) responsible for the coding of a negatively-charged residue in the first part of CDR3 β . The amino acid sequences after "CTC" in TRBV1, "LCG" in TRBV20, "CAW" in TRBV31, or "CAS" in all other TRBVs and before "YF", "FF" "TF" or "LF" in TRBJs were defined as CDR3 β . The N-terminal region of CDR3 β was defined as the first half of CDR3 β .

Table S2. Nucleotide sequences of GAD₂₂₁₋₂₃₅ and GAD₂₂₁₋₂₃₅^{9E} T cell hybridoma CDR3 β segments

CDR3 β sequences of GAD₂₂₁-reactive T hybridomas

Cell#	BV	BD	BJ	Nucleic acid sequence		
				Vb germline	nDbn	Jb germline
cl.26	12-1	1	1-3	ttc tgt gcc agc tct	gac agg g	ct gga aat acg ctc tat ttt gga gaa
cl.29	13-3	1	1-6	ttc tgt gcc agc ag	g gac agg ggg g	at tcg ccc ctc tac ttt gcg gca
cl.53	3	1	2-5	ttc tgt gcc agc agc	ccc tcg ggg agg ggg	gac acc cag tac ttt ggg cca
cl.58	13-2	?	2-5	ttc tgt gcc agc ggt ga	a cgg ta	c caa gac acc cag tac ttt ggg cca
cl.60	3	1	2-5	ttc tgt gcc agc agc	ccc tcg ggg agg ggg	gac acc cag tac ttt ggg cca
cl.76	13-3	1	2-3	ttc tgt gcc agc agt ga	a ggc aat	agt gca gaa acg ctg tat ttt ggc tca
cl.79	16	2	2-5	ctt tgt gca agc agc	ccc cgg gac tgg ggt	caa gac acc cag tac ttt ggg cca
cl.82	3	2	2-1	ttc tgt gcc agc agc tta g	at tgg gg	c tat gct gag cag ttc ttc gga cca
cl.86	16	1	1-3	ctt tgt gca agc agc	gac cag ggg gcg c	ct gga aat acg ctc tat ttt gga gaa
cl.102	19	1	1-5	ctc tgt gcc agc agt ata	tcc aca	aac aac cag gct cgg ctt ttt gga gag
cl.103	13-2	?	2-5	ttc tgt gcc agc ggt ga	a cgg ta	c caa gac acc cag tac ttt ggg cca
cl.105	19	1	1-5	ctc tgt gcc agc agt ata	tcc aca	aac aac cag gct cgg ctt ttt gga gag
cl.108	16	1	1-3	ctt tgt gca agc agc	gac cag ggg gcg c	ct gga aat acg ctc tat ttt gga gaa

CDR3 β sequences of GAD₂₂₁^{9E}-reactive T hybridomas

Cell#	BV	BD	BJ	Nucleic acid sequence		
				Vb germline	nDbn	Jb germline
cl.34	13-2	1	1-6	ttc tgt gcc agc gg	a aca ggg gg	c tat aat tcg ccc ctc tac ttt gcg gca
cl.52	19	?	1-6	ctc tgt gcc agc agt	cgg a	cc tat aat tcg ccc ctc tac ttt gcg gca
cl.60	2	1	2-7	ttc tgt gcc agc agc c	tc cgg gac agg gac	gaa cag tac ttc ggt ccc
cl.66	16	?	2-7	ctt tgt gca agc agc tta g	cg ggc a	at gaa cag tac ttc ggt ccc
cl.92	16	1	2-7	ctt tgt gca agc agc	caa gga cag ggc g	at gaa cag tac ttc ggt ccc
cl.110	3	1	2-5	ttc tgt gcc agc agc	ccc tcg ggg agg ggg	gac acc cag tac ttt ggg cca
cl.119	19	2	2-5	ctc tgt gcc agc agt ata g	aa ggt ggg ggg ggt	caa gac acc cag tac ttt ggg cca
cl.142	16	?	2-7	ctt tgt gca agc agc tta g	cg ggc a	at gaa cag tac ttc ggt ccc
cl.164	5	1	2-1	ttt tgt gcc agc agc caa g	ga cag gc	t aac tat gct gag cag ttc ttc gga cca
cl.183	13-3	1	2-3	ttc tgt gcc agc agt g	gt cac tgg ggg g	gt gca gaa acg ctg tat ttt ggc tca
cl.187	13-3	1	2-3	ttc tgt gcc agc agt g	gt cac tgg ggg g	gt gca gaa acg ctg tat ttt ggc tca

T cell hybridomas were isolated after immunization with GAD₂₂₁₋₂₃₅ or GAD₂₂₁₋₂₃₅^{9E} peptides.

Table S3. Amino acid sequences and N deletion/addition of CDR3 β segments of I-A⁹⁷BDC2.5 9G and 9D tetramer-reactive T cells

TCR CDR3 β sequences of I-A⁹⁷BDC2.5 9G/tet SP CD4 T cells

Cell#	V	D	J	Amino acid sequence			Nucleic acid deletion/addition						comments
				Vb	nDbn	Jb	Vb del	add*	D5' del	D3' del	add**	Jb del	
1	19	1	2-5	LCASS	LLGGR	DTQYFGP	✓	✓	✓	-	✓	✓	
	13-2	2	2-7	FCASSG	DWG	YEQYFGP	✓	-	✓	✓	-	✓	
3	29	1	2-1	FCASS	PTAGD	YAEQFFGP	✓	✓	✓	✓	✓	✓	
4	29	1	2-1	FCASS	PTAGD	YAEQFFGP	✓	✓	✓	✓	✓	✓	
9	3	1	2-7	FCASS	PDI	YEQYFGP	✓	✓	✓	✓	✓	-	
11	5	?	2-7	FCASSQ	DVQS	SYEQYFGP	-	✓	✓	✓	✓	✓	
13	13-2	1	1-1	FCASSG	EGGGG	TEVFFGK	✓	-	✓	-	✓	✓	
14	13-2	?	1-5	FCASSG	GE	NNQAPLFGE	-	-	-	✓	✓	✓	D:germline
	15	2	2-7	LCAS	SQTG	YEQYFGP	✓	✓	✓	✓	-	✓	
17	4	2	2-3	LCASS	YLGGH	AETLYFGS	✓	✓	✓	-	✓	✓	
19	5	1	2-7	FCASS	WDRG	YEQYFGP	✓	✓	-	✓	-	✓	
20	5	1	2-1	FCASSQ	GQA	NYAEQFFGP	✓	-	✓	✓	✓	-	
	15	1	2-4	LCASSL	DRGDG	QNTLYFGA	✓	-	✓	✓	✓	✓	
22	19	1	2-7	LCASSI	ELRQG	YEQYFGP	-	✓	✓	✓	-	✓	
24	5	?	2-2	FCASSQ	G	NTGQLYFGE	✓	-	✓	✓	-	✓	
25	19	1	2-5	LCASS	IRDR	DTQYFGP	✓	✓	✓	✓	✓	✓	
28	13-2	2	2-7	FCASSG	GTGGD	EQYFGP	✓	-	✓	✓	✓	✓	
29	13-2	2	2-3	FCASSG	GTGA	SAETLYFGS	✓	-	✓	✓	✓	-	
34	13-2	1	2-5	FCASSG	EGQ	NQDTQYFGP	✓	-	✓	✓	✓	-	
35	13-2	1	1-6	FCASSG	RG	NSPLYFAA	✓	-	✓	✓	✓	✓	D:germline
38	13-2	1	2-1	FCASSG	GTD	YAEQFFGP	✓	-	✓	✓	-	✓	
42	13-2	2	1-2	FCASSG	DA	NSDYTFGS	-	✓	✓	✓	-	-	D ⁺ :germline
43	31	1	2-1	LCAWS	LEGGA	YAEQFFGP	-	✓	✓	✓	✓	✓	
45	20	2	2-5	YLCGAR	TGGA	QDTQYFGP	✓	-	✓	✓	✓	✓	
52	4	2	2-3	LCASS	YLGGH	AETLYFGS	✓	✓	✓	✓	✓	✓	
53	13-2	?	2-5	FCASSG	GG	DTQYFGP	-	-	✓	✓	-	✓	D:germline
55	15	2	2-5	LCAS	SPTGGG	QDTQYFGP	✓	✓	✓	✓	✓	✓	
56	3	2	2-5	FCASS	FTGV	QDTQYFGP	✓	✓	✓	✓	✓	✓	
57	1	1	2-4	YCTCS	GQG	QNTLYFGA	✓	-	✓	✓	-	✓	
58	13-2	2	2-1	FCASSG	EDWG	NYAEQFFGP	✓	✓	✓	✓	-	✓	
63	19	1	1-2	LCASS	PTGEG	SDYTFGS	✓	✓	✓	✓	✓	✓	

TCR V β sequences of I-A⁹⁷BDC2.5 9D/tet SP CD4 T cells

Cell#	V	D	J	Amino acid sequence			Nucleic acid deletion/addition						comments
				Vb	nDbn	Jb	Vb del	add*	D5' del	D3' del	add**	Jb del	
1	29	1	2-1	FCASS	PTAGD	YAEQFFGP	✓	✓	✓	✓	✓	✓	
5	19	1	2-1	LCASS	PTGGAD	YAEQFFGP	✓	✓	✓	-	✓	✓	
	29	1	2-1	FCASS	PTAGD	YAEQFFRP	✓	✓	✓	✓	✓	✓	
8	29	1	2-1	FCASS	PTAGD	YAEQFFGP	✓	✓	✓	✓	✓	✓	
9	15	1	1-1	LCASS	RQ	TEVFFGK	✓	✓	✓	✓	-	✓	
	3	2	2-7	FCASSL	TV	YEQYFGP	✓	-	✓	✓	✓	✓	
16	2	2	2-7	FCASSQ	DGLGG	EQYFGP	-	✓	✓	✓	-	✓	
18	29	1	2-1	FCASS	PTAGD	YAEQFFGP	✓	✓	✓	✓	-	✓	
26	13-3	1	1-3	FCASS	WDRA	GNTLYFGE	✓	✓	-	✓	-	✓	
27	13-3	1	1-3	FCASS	WDRA	GNTLYFGE	✓	✓	-	✓	-	✓	
32	2	2	2-3	FCASS	PNWGGT	ETLYFGS	✓	✓	✓	-	✓	✓	
33	31	1	2-3	LCAWS	LLGGR	AETLYFGS	-	-	✓	-	✓	✓	
35	1	2	2-4	YCTCSA	DWG	SQNTLYFGA	-	-	✓	✓	-	-	
36	13-3	1	2-5	FCAS	RDI	NQDTQYFGP	✓	-	✓	✓	✓	-	
42	13-3	1	2-7	FCAS	SGTGGG	YEQYFGP	✓	✓	-	-	✓	✓	
48	15	2	2-7	LCAS	SQTG	YEQYFGP	✓	✓	✓	✓	-	✓	
55	15	1	2-4	LCASSL	YK	NTRYFGA	✓	✓	✓	✓	-	✓	
61	4	1	2-7	LCASS	YGTD	SYEQYFGP	✓	✓	-	✓	✓	-	
75	1	1	1-1	YCTCSA	TTGT	NTEVFFGK	✓	✓	✓	✓	✓	-	
82	13-3	1	1-2	FCASS	GQP	NSDYTFGS	✓	✓	✓	✓	✓	-	

87	12-2	1	2-5	FCASSL	<u>GTGS</u>	DTQYFGP	-	-	-	✓	✓	✓	
95	29	1	2-1	FCASS	<u>PTAGD</u>	YAEQFFGP	✓	✓	✓	✓	✓	✓	
	4	1	2-7	LCASS	<u>YGTD</u>	SYEQYFGP	✓	✓	-	✓	✓	-	

Individual T cells sorted after tetramer labeling were used for RT-PCR and sequencing. Negatively charged amino acid residues in the first part of the CDR3 β segment are highlighted in bold and red. Vb del: deletion of nucleotide(s) on 3' part of BV germline, add*: addition of nucleotide(s) between BV and BD, D5' del: deletion of nucleotide(s) on 5' part of BD germline, D3' del: deletion of nucleotide(s) on 3' part of BD germline, add**: addition of nucleotide(s) between BD and BJ, Jb del: deletion of nucleotide(s) on 5' part of BJ germline ✓: deletion or addition of nucleotide(s), -: no deletion or no addition, ✓: deletion or addition of nucleotide(s) responsible for the coding of a negatively-charged residue in the first part of CDR3 β , +: first "D" in the Vb. The amino acid sequences after "CTC" in TRBV1, "LCG" in TRBV20, "CAW" in TRBV31, or "CAS" in all other TRBVs and before "YF", "FF" "TF" or "LF" in TRBJs were defined as CDR3 β . The N-terminal region of CDR3 β was defined as the first half of CDR3 β .

Table S4. Nucleotide sequences of CDR3 β segments of I-A⁹⁷BDC2.5 9G and 9D tetramer-reactive T cells

TCR CDR3 β sequences of I-A⁹⁷BDC2.5 9G/tet SP CD4 T cells

Cell#	BV	BD	BJ	Nucleic acid sequence		
				Vb germline	nDbn	Jb germline
1	19	1	2-5	ctc tgt gcc agc agt	cta ctg ggg ggg cgc	gac acc cag tac ttt ggg cca
	13-2	2	2-7	ttc tgt gcc agc ggt ga	c tgg gg	c tat gaa cag tac ttc ggt ccc
3	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca
4	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca
9	3	1	2-7	ttc tgt gcc agc agc	ccg gac at	c tat gaa cag tac ttc ggt ccc
11	5	?	2-7	ttt tgt gcc agc agc caa ga	t gtc caa ag	c tcc tat gaa cag tac ttc ggt ccc
13	13-2	1	1-1	ttc tgt gcc agc ggt ga	a ggg ggc ggg gg	c aca gaa gtc ttc ttt ggt aaa
14	13-2	?	1-5	ttc tgt gcc agc ggt gat g	gg gaa	aac aac cag gct cgg ctt ttt gga gag
	15	2	2-7	ctg tgt gcc agc ag	c cag act ggg	tat gaa cag tac ttc ggt ccc
17	4	1	2-3	ctc tgt gcc agc agc ta	c ctg ggg ggg ca	t gca gaa acg ctg tat ttt ggc tca
19	5	1	2-7	ttt tgt gcc agc agc	tgg gac agg ggg	tat gaa cag tac ttc ggt ccc
20	5	1	2-1	ttt tgt gcc agc agc caa g	ga cag gc	t aac tat gct gag cag ttc ttc gga cca
	15	1	2-4	ctg tgt gcc agc agt tta g	ac agg gga gac gg	t caa aac acc ttg tac ttt ggt gcg
22	19	1	2-7	ctc tgt gcc agc agt ata g	aa ttg aga cag gg	c tat gaa cag tac ttc ggt ccc ggc
24	5	?	2-2	ttt tgt gcc agc agc caa g	gg	aac acc ggg cag ctg tac ttt ggt gaa
25	19	1	2-5	ctc tgt gcc agc agt at	t agg gac cgg	gac acc cag tac ttt ggg cca
28	13-2	2	2-7	ttc tgt gcc agc ggt g	gg act ggg ggg ga	t gaa cag tac ttc ggt ccc
29	13-2	2	2-3	ttc tgt gcc agc ggt g	gg act ggg gct	agt gca gaa acg ctg tat ttt ggc tca
34	13-2	1	2-5	ttc tgt gcc agc ggt ga	a ggg cag	aac caa gac acc cag tac ttt ggg cca
35	13-2	1	1-6	ttc tgt gcc agc ggt gat	agg gga	aat tgg ccc ctg tac ttt ggc gca
38	13-2	1	2-1	ttc tgt gcc agc ggt g	gg aca g	ac tat gct gag cag ttc ttc gga cca
42	13-2	2	1-2	ttc tgt gcc agc ggt gat g	at g	ca aac tcc gac tac acc ttc ggc tca
43	31	1	2-1	ctc tgt gcc tgg agt ct	a gaa ggg ggc gc	c tat gct gag cag ttc ttc gga cca
45	20	2	2-5	tat ctg tgt ggt gct agg	act ggg ggg gcg	caa gac acc cag tac ttt ggg cca
52	4	2	2-3	ctc tgt gcc agc agc ta	c ctg ggg ggg ca	t gca gaa acg ctg tat ttt ggc tca
53	13-2	?	2-5	ttc tgt gcc agc ggt gat g	gg gg	a gac acc cag tac ttt ggg cca
55	15	2	2-5	ctg tgt gcc agc ag	c ccc act ggg ggg ggt	caa gac acc cag tac ttt ggg cca
56	3	2	2-5	ttc tgt gcc agc agc tt	t act ggg gta	caa gac acc cag tac ttt ggg cca
57	1	1	2-4	tac tgc acc tgc agt g	ga cag g	gt caa aac acc ttg tac ttt ggt gcg
58	13-2	2	2-1	ttc tgt gcc agc ggt ga	a gac tgg ggg	aac tat gct gag cag ttc ttc gga cca
63	19	1	1-2	ctc tgt gcc agc agt	ccg aca ggg gaa gg	c tcc gac tac acc ttc ggc tca

TCR V β sequences of I-A⁹⁷BDC2.5 9D/tet SP CD4 T cells

Cell#	BV	BD	BJ	Nucleic acid sequence		
				Vb germline	nDbn	Jb germline
1	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca
5	19	1	2-1	ctc tgt gcc agc agt	cca aca ggg ggc gct g	ac tat gct gag cag ttc ttc gga cca
	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca
8	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca
9	15	1	1-1	ctg tgt gcc agc agt	cga cag	aca gaa gtc ttc ttt ggt aaa
	3	2	2-7	ttc tgt gcc agc agc tta	act gt	c tat gaa cag tac ttc ggt ccc
16	2	2	2-7	ttc tgt gcc agc agc caa ga	t gga ctg ggg gg	t gaa cag tac ttc ggt ccc
18	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca
26	13-3	1	1-3	ttc tgt gcc agc agt	tgg gac agg g	ct gga aat acg ctg tat ttt gga gaa
27	13-3	1	1-3	ttc tgt gcc agc agt	tgg gac agg g	ct gga aat acg ctg tat ttt gga gaa
32	2	2	2-3	ttc tgt gcc agc agc c	ca aac tgg ggg ggc a	ca gaa acg ctg tat ttt ggc tca
33	31	1	2-3	ctc tgt gcc tgg agt ct	a ctg ggg ggg cgc	gca gaa acg ctg tat ttt ggc tca
35	1	2	2-4	tac tgc acc tgc agt gca ga	c tgg ggg	agt caa aac acc ttg tac ttt ggt gcg
36	13-3	1	2-5	ttc tgt gcc agc ag	g gac att	aac caa gac acc cag tac ttt ggg cca
42	13-3	1	2-7	ttc tgt gcc agc ag	c ggg aca ggg ggc ggt	tat gaa cag tac ttc ggt ccc
48	15	2	2-7	ctg tgt gcc agc ag	c cag act ggg	tat gaa cag tac ttc ggt ccc
55	15	1	2-4	ctg tgt gcc agc agt tta	tac a	aa aac acc ttg tac ttt ggt gcg
61	4	1	2-7	ctc tgt gcc agc agc ta	c ggg aca gaz	c tcc tat gaa cag tac ttc ggt ccc
75	1	1	1-1	tac tgc acc tgc agt gca	acg aca ggg a	ca aac aca gaa gtc ttc ttt ggt aaa
82	13-3	1	1-2	ttc tgt gcc agc agt g	gc caa c	ca aac tcc gac tac acc ttc ggc tca
87	12-2	1	2-5	ttc tgt gcc agc tct ctg	ggg aca ggg agt	gac acc cag tac ttt ggg cca
95	29	1	2-1	ttc tgt gct agc agt	cca acg gca gga g	ac tat gct gag cag ttc ttc gga cca

	4	1	2-7	ctc tgt gcc agc agc ta	c ggg aca ga	c tcc tat gaa cag tac ttc ggt ccc
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Individual T cells sorted after tetramer labeling were used for RT-PCR and sequencing.

Table S5. Amino acid sequences and N deletion/addition of CDR3 β segments of I-A⁹⁷HEL₁₁₋₂₇ 9G and 9E tetramer-reactive T cells

TCR V β sequences of I-A⁹⁷HEL₁₁₋₂₇/tet+ CD4 cells

Cell#	V	D	J	Amino acid sequence			Nucleic acid deletion/addition						comments
				Vb	nDbn	Jb	Vb del	add*	D5' del	D3' del	add**	Jb del	
1-2.2	13-3	1	1-1	FCASS	ERTGK	TEVFFGK	✓	✓	✓	✓	✓	✓	
1-2.3	13-3	2	2-5	FCAS	KTGG	NQDTQYFGP	✓	✓	✓	✓	-	-	
3-1.1.12	29	1	2-1	FCASSL	NRD	YAEQFFGP	✓	✓	✓	✓	-	✓	
3-1.1.35	13-3	1	1-3	FCASS	WDRA	GNTLYFGE	✓	✓	-	✓	-	✓	
3-1.1.39	15	1	1-6	LCASSL	EDRG	YNSPLYFAA	✓	✓	✓	✓	-	✓	
4-t2	29	1	2-7	FCASSL	SGTGGC	EQYFGP	-	✓	-	✓	✓	✓	
4-t7	4	1	1-2	LCAS	RPGTGA	SDYTFGS	✓	✓	-	✓	✓	✓	
4-1.1.3	13-3	1	1-3	FCASS	WDRA	GNTLYFGE	✓	✓	-	✓	-	✓	
4-1.1.11	19	2	2-7	LCASSI	GGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
4-1.1.13	19	1	2-5	LCAS	IPGH	QDTQYFGP	✓	✓	-	✓	-	✓	
4-1.1.14	5	2	2-7	FCASSQ	GGTGG	YEYQYFGP	✓	✓	-	✓	-	✓	
4-1.1.15	13-3	1	2-7	FCASS	ERQS	YEYQYFGP	✓	✓	✓	✓	✓	✓	
4-1.1.16	29	1	2-5	FCAS	RRDRE	DTQYFGP	✓	✓	-	✓	✓	✓	
4-1.1.23	13-3	1	1-3	FCASS	WDRA	GNTLYFGE	✓	✓	-	✓	-	✓	
4-1.1.34	3	1	1-2	FCASSL	DRVG	NSDYTFGS	✓	-	✓	✓	✓	✓	
4-1.1.53	13-2	2	2-7	FCASG	EGLGG	YEYQYFGP	✓	-	-	✓	-	✓	
4-1.1.54	5	1	2-7	FCASSQ	EGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
4-1.1.56	29	2	2-7	FCASSL	AGTGG	YEYQYFGP	✓	✓	-	✓	✓	✓	
4-1.1.58	13-3	1	2-7	FCASS	DRS	SYEQYFGP	✓	-	✓	✓	✓	-	
4-1.1.64	5	2	2-7	FCASSQ	EGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
4-1.1.65	2	1	1-2	FCASS	EGGTT	NSDYTFGS	✓	✓	-	✓	✓	-	
4-1.1.67	5	2	2-7	FCASSQ	DGTGG	YEYQYFGP	-	✓	-	✓	✓	✓	
4-1.1.73	29	1	2-1	FCASSL	SDPR	NYAEQFFGP	-	✓	✓	✓	✓	✓	
4-1.1.91	13-3	1	2-7	FCASS D	AGTGG	YEYQYFGP	-	✓	-	✓	-	✓	D:germline
4-1.1.96	13-3	1	1-3	FCASS	WDRA	GNTLYFGE	✓	✓	-	✓	-	✓	

TCR V β sequences of I-A⁹⁷HEL₁₁₋₂₇9E/tet+ CD4 cells

Cell#	V	D	J	Amino acid sequence			Nucleic acid deletion/addition						comments
				Vb	nDbn	Jb	Vb del	add*	D5' del	D3' del	add**	Jb del	
1-1-10	19	2	2-7	LCASSI	GGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
1-1-14	2	2	2-4	FCASSQ	DLV	SQNTLYFGA	-	✓	✓	✓	✓	-	
1-1-18	19	1	2-7	LCASSI	AGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
1-1-42	13-2	2?	2-4	FCASG	GGG	TLYFGA	✓	-	✓	✓	-	✓	
1-1-50	17	2	2-1	LCASS	ATGD	AEQFFGP	✓	✓	✓	✓	-	✓	
	20	1	1-6	LCGA	LRDR	NSPLYFAA	✓	✓	-	✓	✓	✓	
1-1-58	13-2	2?	2-4	FCASG	GGG	TLYFGA	✓	-	✓	✓	-	✓	
1-1-62	2	?	2-2	FCASSQ	DDA	NTGQLYFGE	✓	✓	✓	✓	✓	-	
1-1-63	13-3	2	2-7	FCASS	EGGTGG	YEYQYFGP	✓	✓	-	✓	✓	✓	
1-1-74	13-1	1	2-7	FCASS D	AGTGG	YEYQYEGP	-	✓	-	✓	-	✓	D:germline
1-1-78	5?	1	2-7	ASSQ	EGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
1-2-2	13-3	1	2-7	FCASS D	AGTGG	YEYQYFGP	-	✓	-	✓	-	✓	D:germline
1-2-10	1	2?	1-1	YCTCSA	AGGG	TEVFFGK	✓	✓	✓	-	✓	✓	
1-2-12	1	2?	1-1	YCTCSA	AGGG	TEVFFGK	✓	✓	✓	-	✓	✓	
	19	2	2-7	LCASSI	VGTGG	YEYQYFGP	-	✓	-	✓	✓	✓	
1-2-14	19	2?	2-7	LCASS	MGGR	YEYQYFGP	✓	-	✓	-	✓	✓	
1-2-34	29	2	2-7	FCAS	RDWID	EQYFGP	✓	-	✓	✓	✓	✓	
1-2-38	17	2	2-7	LCASSR	DLTGG	YEYQYFGP	-	✓	✓	✓	-	✓	
1-2-50	13-3	?	2-7	FCASS	ERS	SYEQYFGP	✓	✓	✓	✓	✓	-	
1-2-54	19	1	2-7	LCASSI	DRGE	QYFGP	-	-	✓	✓	✓	✓	
1-2-86	19	2	2-7	LCASSI	GGTGG	YEYQYFGP	-	✓	-	✓	-	✓	
1-2-89	19	2	2-7	LCASSI	SGTGG	YEYQYFGP	✓	✓	-	✓	✓	✓	

Individual T cells sorted after tetramer labeling were used for RT-PCR and sequencing. Negatively charged amino acid residues in the first part of the CDR3 β segment are highlighted in bold and red. Vb del: deletion of nucleotide(s)

on 3' part of BV germline, add*: addition of nucleotide(s) between BV and BD, D5' del: deletion of nucleotide(s) on 5' part of BD germline, D3' del: deletion of nucleotide(s) on 3' part of BD germline, add**: addition of nucleotide(s) between BD and BJ, Jb del: deletion of nucleotide(s) on 5' part of BJ germline ✓: deletion or addition of nucleotide(s), -: no deletion or no addition, ✓: deletion or addition of nucleotide(s) responsible for the coding of a negatively-charged residue in the first part of CDR3 β . The amino acid sequences after "CTC" in TRBV1, "LCG" in TRBV20, "CAW" in TRBV31, or "CAS" in all other TRBVs and before "YF", "FF" "TF" or "LF" in TRBJs were defined as CDR3 β . The N-terminal region of CDR3 β was defined as the first half of CDR3 β .

Table S6. Nucleotide sequences of CDR3 β segments of I-A⁹⁷HEL₁₁₋₂₇ 9G and 9E tetramer-reactive T cells

TCR V β sequences of I-A⁹⁷HEL/tet+ CD4 cells

Cell#	BV	BD	BJ	Nucleic acid sequence		
				Vb germline	nDbn	Jb germline
1-2.2	13-3	1	1-1	ttc tgt gcc agc agt ga	g cgg aca ggg aag	aca gaa gtc ttc ttt ggt aaa gga
1-2.3	13-3	2	2-5	ttc tgt gcc agc a	ag act ggg ggg	aac caa gac acc cag tac ttt ggg cca
3-1.1.12	29	1	2-1	ttc tgt gct agc agt tta	aac agg g	ac tat gct gag cag ttc ttc gga cca
3-1.1.35	13-3	1	1-3	ttc tgt gcc agc agt	tgg gac agg g	ct gga aat acg ctc tat ttt gga gaa
3-1.1.39	15	1	1-6	ctg tgt gcc agc agt tta g	ag gac agg ggg	tat aat tcg ccc ctc tac ttt cgg gca
4-t2	29	1	2-7	ttc tgt gct agc agt tta tc	t ggg aca ggg ggg tg	t gaa cag tac ttc ggt ccc
4-t7	4	1	1-2	ctc tgt gcc agc ag	a ccc ggg aca ggg gc	c tcc gac tac acc ttc ggc tca
4-1.1.3	13-3	1	1-3	ttc tgt gcc agc agt	tgg gac agg g	ct gga aat acg ctc tat ttt gga gaa
4-1.1.11	19	2	2-7	ctc tgt gcc agc agt ata g	gc ggg act ggg ggg	tat gaa cag tac ttc ggt ccc
4-1.1.13	19	1	2-5	ctc tgt gcc agc a	tt ccg gga ca	c caa gac acc cag tac ttt ggg cca
4-1.1.14	5	2	2-7	ttt tgt gcc agc agc caa g	ga ggg act ggg gg	c tat gaa cag tac ttc ggt ccc
4-1.1.15	13-3	1	2-7	ttc tgt gcc agc agt ga	g cga cag ag	c tat gaa cag tac ttc ggt ccc
4-1.1.16	29	1	2-5	ttc tgt gct agc a	gg agg gac agg gag	gac acc cag tac ttt ggg cca
4-1.1.23	13-3	1	1-3	ttc tgt gcc agc agt	tgg gac agg g	ct gga aat acg ctc tat ttt gga gaa
4-1.1.34	3	1	1-2	ttc tgt gcc agc agc tta g	ac agg gtg gg	a aac tcc gac tac acc ttc ggc tca
4-1.1.53	13-2	2	2-7	ttc tgt gcc agc ggt ga	g gga ctg ggg ggg	tat gaa cag tac ttc ggt ccc
4-1.1.54	5	1	2-7	ttt tgt gcc agc agc caa ga	a ggg aca ggg gg	c tat gaa cag tac ttc ggt ccc
4-1.1.56	29	2	2-7	ttc tgt gct agc agt tta	gcc ggg act ggg gga	tat gaa cag tac ttc ggt ccc
4-1.1.58	13-3	1	2-7	ttc tgt gcc agc agt ga	c aga ag	c tcc tat gaa cag tac ttc ggt ccc
4-1.1.64	5	2	2-7	ttt tgt gcc agc agc caa ga	g ggg act ggg ggg	tat gaa cag tac ttc ggt ccc
4-1.1.65	2	1	1-2	ttc tgt gcc agc agc	gaa ggg ggg aca a	ca aac tcc gac tac acc ttc ggc tca
4-1.1.67	5	2	2-7	ttt tgt gcc agc agc caa ga	t ggg act ggg ggt	tat gaa cag tac ttc ggt ccc
4-1.1.73	29	1	2-1	ttc tgt gct agc agt tta tc	c gat ccc aga	aac tat gct gag cag ttc ttc gga cca
4-1.1.91	13-3	1	2-7	ttc tgt gcc agc agt gat g	cc ggg aca ggg gg	c tat gaa cag tac ttc ggt ccc
4-1.1.96	13-3	1	1-3	ttc tgt gcc agc agt	tgg gac agg g	ct gga aat acg ctc tat ttt gga gaa

TCR V β sequences of I-A⁹⁷HEL_{9E}/tet+ CD4 cells

Cell#	BV	BD	BJ	Nucleic acid sequence		
				Vb germline	nDbn	Jb germline
1-1-10	19	2	2-7	ctc tgt gcc agc agt ata g	ga ggg act ggg ggg	tat gaa cag tac ttc ggt ccc
1-1-14	2	2	2-4	ttc tgt gcc agc agc caa ga	t ttg gtt	agt caa aac acc ttg tac ttt ggt cgg
1-1-18	19	1	2-7	ctc tgt gcc agc agt ata g	cc ggg aca ggg gg	c tat gaa cag tac ttc ggt ccc
1-1-42	13-2	2?	2-4	ttc tgt gcc agc ggt g	gg ggg gg	c acc ttg tac ttt ggt cgg
1-1-50	17	2	2-1	ctc tgt gct agc agt	gcg act ggg g	at gct gag cag ttc ttc gga cca
	20	1	1-6	ctc tgt ggt gct	ctc cgg gac aga	aat tcg ccc ctc tac ttt cgg gca
1-1-58	13-2	2?	2-4	ttc tgt gcc agc ggt g	gg ggg gg	c acc ttg tac ttt ggt cgg
1-1-62	2	?	2-2	ttc tgt gcc agc agc caa ga	t gat g	ca aac acc ggg cag ctc tac ttt ggt gaa
1-1-63	13-3	2	2-7	ttc tgt gcc agc agt ga	g ggg ggg act ggg ggt	tat gaa cag tac ttc ggt ccc
1-1-74	13-1	1	2-7	ttc tgt gcc agc agt gat g	ca ggg aca ggg gg	c tat gaa cag tac ttc ggt ccc
1-1-78	5?	1	2-7	gcc agc agc caa ga	a ggg aca ggg gg	c tat gaa cag tac ttc ggt ccc
1-2-2	13-3	1	2-7	ttc tgt gcc agc agt gat g	cg ggg aca ggg gg	c tat gaa cag tac ttc ggt ccc
1-2-10	1	2?	1-1	tac tgc acc tgc agt gca g	cg ggg ggc gg	c aca gaa gtc ttc ttt ggt aaa
1-2-12	1	2?	1-1	tac tgc acc tgc agt gca g	cg ggg ggc gg	c aca gaa gtc ttc ttt ggt aaa
	19	2	2-7	ctc tgt gcc agc agt ata g	ta ggg act ggg ggt	tat gaa cag tac ttc ggt ccc
1-2-14	19	2?	2-7	ctc tgt gcc agc agt at	g ggg ggg cg	c tat gaa cag tac ttc ggt ccc
1-2-34	29	2	2-7	ttc tgt gct agc ag	g gac tgg atc g	at gaa cag tac ttc ggt ccc
1-2-38	17	2	2-7	ctc tgt gct agc agt aga ga	t cta act ggg ggg	tat gaa cag tac ttc ggt ccc
1-2-50	13-3	?	2-7	ttc tgt gcc agc agt ga	a cgg ag	c tcc tat gaa cag tac ttc ggt ccc
1-2-54	19	1	2-7	ctc tgt gcc agc agt ata g	ac agg ggg ga	a cag tac ttc ggt ccc
1-2-86	19	2	2-7	ctc tgt gcc agc agt ata g	gg ggg act ggg ggg	tat gaa cag tac ttc ggt ccc
1-2-89	19	2	2-7	ctc tgt gcc agc agt ata	tcg ggg act ggg gga	tat gaa cag tac ttc ggt ccc

Individual T cells sorted after tetramer labeling were used for RT-PCR and sequencing.

Table S7. Data collection and refinement statistics for I-A⁹⁷GAD₂₂₁₋₂₃₅

Data processing	
Resolution range (Å)	38.45- 3.09 (3.15- 3.09)
Unique reflections	10,586 (509)
Completeness (%)	98.7 (99.4)
Redundancy	4.9
^a R _{sym} (%)	12.8 (68.2)
Average I/σ(I)	10.6 (1.8)
Refinement	
Reflections	10,023
Reflections(test)	563
^b R _{cryst} (%)	23.2 (28.7) ^c
^d R _{free} (%)	28.6 (31.2)
Residues/protein atoms	358/2924
^e Coordinate error (Å)	0.41
rmsd bonds/angles (Å/deg.)	0.003/0.638
Average B values(Å ²)	
I-A ⁹⁷	73.5
Peptide GAD ₂₂₁₋₂₃₅	67.4
^f Ramachandran statistics (%)	
Most favored	87.3
Additionally allowed	12.1
Generously allowed	0.3
Disallowed	0.3

Values in parentheses refer to the highest resolution shell.

^aR_{sym} = 100 Σ_h Σ_i | I_i(h) - <I(h)> | / Σ_h I(h) where I_i(h) is the *i*th measurement of the *h* reflection and < I(h) > is the average value of the reflection intensity.

^bR_{cryst} = Σ||F_o| - |F_c|| / Σ|F_o|, where F_o and F_c are the observed and calculated structure factor amplitudes within the set of reflections used for refinement.

^cShell resolution 3.4 - 3.1Å.

^d $R_{\text{free}} = \sum ||F_o| - |F_c|| / \sum |F_o|$ calculated for a randomly selected set of structure factors (~5%) throughout the resolution range and not used in refinement.

^eValues are based on maximum likelihood.

^fThe Ramachandran plot was generated with PROCHECK.

Table S8. Data collection and refinement statistics for TCR 21.30/I-A⁹⁷HEL₁₁₋₂₇

Data processing	
Resolution range (Å)	48.26 - 2.88 (2.98 - 2.88)
Unique reflections	43435 (1590)
Completeness (%)	68.9 (26.4)
Redundancy	1.99
^a R _{sym} (%)	11.2 (43.2)
Average I/σ(I)	13.0 (0.98)
Refinement	
Reflections	34208
Reflections (test)	727
^b R _{cryst} (%)	25.3 (37.3) ^c
^d R _{free} (%)	28.1 (42.1)
Residues/protein atoms	1614/12944
^e Coordinate error (Å)	0.54
rmsd bonds/angles (Å / deg.)	0.007/1.002
^f Average B values (Å ²)	
Monomer 1 (chains A,B,C,D,P)	
TCR 21.30	51
I-A ⁹⁷ HEL ₁₁₋₂₇	53
Monomer 2 (chains E,F,G,H,Q)	
TCR 21.30	95
I-A ⁹⁷ HEL ₁₁₋₂₇	89
^g Ramachandran statistics (%)	
Most favored	76.9
Additionally allowed	21.5
Generously allowed	1.4
Disallowed	0.1

Values in parentheses refer to the highest resolution shell.

^aR_{sym} = 100 Σ_h Σ_i | I_i(h) - <I(h)> | / Σ_h I(h) where I_i(h) is the *i*th measurement of the *h* reflection and < I(h) > is the average value of the reflection intensity.

^b $R_{\text{cryst}} = \sum ||F_o| - |F_c|| / \sum |F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes within the set of reflections used for refinement.

^cShell resolution 3.11 - 2.88Å.

^d $R_{\text{free}} = \sum ||F_o| - |F_c|| / \sum |F_o|$ calculated for a randomly selected set of structure factors (~2%) throughout the resolution range and not used in refinement.

^eValues are based on maximum likelihood.

^fAverage B values include TLS contributions. Significantly higher B values (>120 Å²) were obtained using the original data prior to anisotropic correction.

^gThe Ramachandran plot was generated with PROCHECK.

Table S9. Contacts between TCR 21.30 and I-A⁹⁷HEL₁₁₋₂₇**Contacts between TCR and HEL peptide**

CDR1 α :	C THR 27	-	P MET 12	3	VDW contacts	
	C THR 28	-	P HIS 15	3	VDW contacts	
	C LYS 37	-	P ASP 18	4	VDW contacts	+ 1 hydrogen bond
CDR3 α :	C GLY 110	-	P LYS 13	4	VDW contacts	
	C GLY 110	-	P HIS 15	2	VDW contacts	
	C SER 112	-	P LYS 13	3	VDW contacts	

Total contacts = 20

CDR1 β :	D ASN 28	-	P GLY 26	1	VDW contacts	
	D ASP 37	-	P ARG 21	7	VDW contacts	+ 1 hydrogen bond
	D TYR 38	-	P ARG 21	1	VDW contacts	
CDR3 β :	D TRP 108	-	P GLY 22	1	VDW contacts	
	D TRP 108	-	P SER 24	3	VDW contacts	
	D ASP 109	-	P TYR 20	2	VDW contacts	
	D ASP 109	-	P ARG 21	7	VDW contacts	+ 1 hydrogen bond
	D ARG 110	-	P ASP 18	8	VDW contacts	
	D ARG 110	-	P ASN 19	2	VDW contacts	+ 1 hydrogen bond

Total contacts = 35**Contacts between TCR and MHC**

C THR 27	-	B HIS 81	1	VDW contacts	
C THR 28	-	B THR 77	4	VDW contacts	
C THR 28	-	B HIS 81	3	VDW contacts	+ 1 hydrogen bond
C LYS 37	-	B ARG 70	1	VDW contacts	
C TYR 55	-	B ARG 70	1	VDW contacts	
C ALA 57	-	B GLU 69	6	VDW contacts	+ 1 hydrogen bond
C ALA 57	-	B ARG 70	3	VDW contacts	
C ALA 57	-	B ALA 73	1	VDW contacts	
C GLN 64	-	B ARG 72	1	VDW contacts	
C GLN 64	-	B ALA 73	4	VDW contacts	
C LYS 67	-	B GLU 69	5	VDW contacts	
C SER 84A	-	B THR 77	2	VDW contacts	
C ARG 85	-	B GLU 85	1	VDW contacts	
C GLU 107	-	B ARG 70	4	VDW contacts	+ 2 hydrogen bonds
C GLY 110	-	A GLU 55	4	VDW contacts	
C SER 112	-	A GLU 55	5	VDW contacts	
C GLY 113	-	A GLU 55	1	VDW contacts	
C GLY 113	-	A GLN 57	1	VDW contacts	
C ASN 114	-	A GLN 61	3	VDW contacts	+ 1 hydrogen bond

Total contacts = 56

D TYR 38	-	A GLN 61	6	VDW contacts	+ 1 hydrogen bond
D TYR 55	-	A GLN 57	0	VDW contacts	+ 1 hydrogen bond
D TYR 55	-	A GLN 61	1	VDW contacts	
D TYR 57	-	A GLN 57	5	VDW contacts	
D TYR 57	-	A LEU 60	2	VDW contacts	
D TYR 57	-	A GLN 61	5	VDW contacts	
D TYR 57	-	A ALA 64	2	VDW contacts	
D GLU 67	-	A GLN 57	3	VDW contacts	
D TRP 108	-	B TYR 60	2	VDW contacts	
D TRP 108	-	B TYR 66	1	VDW contacts	
D TRP 108	-	A HIS 68	2	VDW contacts	
D ASP 109	-	B TYR 66	2	VDW contacts	
D ARG 110	-	A ASN 62	5	VDW contacts	+ 1 hydrogen bond
D ARG 110	-	A GLN 61	3	VDW contacts	
D ARG 110	-	A ALA 65	1	VDW contacts	
D ARG 110	-	B ARG 70	1	VDW contacts	
D ALA 112	-	B ARG 70	0	VDW contacts	+ 1 hydrogen bond

Total contacts = 45

Chains A + B: I-A^{g7} alpha and beta chains, respectively. Chain P: HEL peptide

Chains C + D: TCR 21.30 alpha and beta chains, respectively.

Table S10. TCR 21.30 SPR measurements

Peptide	TCR 21.30	TCR 21.30 CDR3 β 97 E \rightarrow G
HEL ₁₁₋₂₇	K_{ass} : 18485 +/- 3997 M ⁻¹ s ⁻¹ K_{diss} : 0.0028 +/- 0.0005 s ⁻¹ K_D : 0.162 +/- 0.04 μ M	K_{ass} : 7990 +/- 12 M ⁻¹ s ⁻¹ K_{diss} : 0.030 +/- 0.0016 s ⁻¹ K_D : 3.76 +/- 0.2 μ M
HEL ₁₁₋₂₇ 9E	K_{ass} : 4013 +/- 735 M ⁻¹ s ⁻¹ K_{diss} : 0.019 +/- 0.0096 s ⁻¹ K_D : 5.02 +/- 2.8 μ M	K_{ass} : NM K_{diss} : NM K_D : NM

Affinity measurements of TCR 21.30 or mutant TCR 21.30 (CDR3 β 97 E \rightarrow G) with I-A^{g7}HEL₁₁₋₂₇ and I-A^{g7}HEL₁₁₋₂₇9E. Numbers are the average of 3 independent experiments. NM indicates a non-measurable reading. $K_D = K_{diss}/K_{ass}$.

Table S11. List of the TCR β specific primers used for isolating and sequencing the respective CDR3 β

Primer	Nucleotide sequence
TRBV1 external	CTA CAG ACC CCA CAG TGA C
TRBV2 external	AGT ATC TAG GCC ACA ATG C
TRBV3 external	GAA GCA GGA CAC ACA GGA C
TRBV4 external	AAT TGC TGA AGA TTA TGT TTA GC
TRBV5 external	GGA AAC AGC ACT CAT GAA CAC
TRBV12 external	CTC YTG GGA ACA AGT TCA GC
TRBV13 external	TGK GWR CAA AAC ACA TGG AGG C
TRBV14 external	TCT CCT TGA CAC AGT ACT GTC
TRBV15 external	GCA AGT CTC TTA TGG AAG ATG G
TRBV16 external	GAG AGC AGA ACC AAC AAA TGC
TRBV17 external	TGC AGC CAC TTT TGT GGA TAC
TRBV19 external	CAC ATG GTG ATG GTG GCA TC
TRBV20 external	TTC TGG GGC CTG GCT GTG
TRBV23 external	TTA CAC AGA AGC CAA GAT A
TRBV24 external	GCA GGC TCT TTT GTT GCT GG
TRBV26 external	CAA GAT ATC TGG TGA AAG GGC
TRBV29 external	GAA CAG GCC TTG TGG ACA TG
TRBV30 external	TGT CCT CCT CTA CCA AAA GC
TRBV31 external	GCT CAG ACT ATC CAT CAA TGG
TRBC external	CTA TAA TTG CTC TCC TTG TAG G

Primer**Nucleotide sequence**

TRBV1 internal	CTT GAA GAA TTC CCA GTA TCC C
TRBV2 internal	CTT ATG GAC AAT CAG ACT GCC
TRBV3 internal	GTG ACC CAG TTT CTA ATC ACC
TRBV4 internal	GTA AAC GAA ACA GTT CCA AGG C
TRBV5 internal	CTT TGG AAT GTG AGC AAC ATC
TRBV12 internal	GCA GAT TCT CAG TCC AAC AG
TRBV13 internal	ATG TAC TGG TAT CGG CAG GAC
TRBV14 internal	CTG TTT CCT TTT GGT GTG ACC
TRBV15 internal	CTA AAT TCA TCC TTC TCC ACT C
TRBV16 internal	CAA GAA GCA ACT CTG TGG TG
TRBV17 internal	GCA AGA GTT GGA AAA CCA GTG
TRBV19 internal	CAG GGA AAG GAT TGA GAC TG
TRBV20 internal	GAA GAA CCA TCT GTA AGA GTG G
TRBV23 internal	AAA ATG AAA GGC CAG GAA GC
TRBV24 internal	CCT GGT CAA AGA GAA AGG AC
TRBV26 internal	CTT CAG CAA ATA GAC ATG ACT G
TRBV29 internal	GAA TGT GGA CAG GAC ATG AG
TRBV30 internal	GCT GAC AGT CAA GTT GTT TCG
TRBV31 internal	CTA ACC TCT ACT GGT ACT GG
TRBC internal	AAG CCC CTG GCC AAG CAC AC