

Supplemental Table 1

Construct	Plasmid backbone	Restriction sites	PCR template	PCR Cloning strategy Primer pairs (F/R) and pairs of primers for chimeric constructs (N-terminal and C-terminal coding region) were given
HLA-DR α	pcDNA3.1 Zeo+	<i>HindIII</i> - <i>BamHI</i>	cDNA	Lapaque <i>et al.</i> (2009)
HLA-DR β	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>NotI</i>	cDNA	Lapaque <i>et al.</i> (2009)
CD8-DR α	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	HLA-DR α	Lapaque <i>et al.</i> (2009), designated CD8-DRA-extra in that study
CD8-DR β	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>NotI</i>	HLA-DR β	Lapaque <i>et al.</i> (2009)
CD8-DR β -K225R	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>NotI</i>	CD8-DR β	Lapaque <i>et al.</i> (2009)
CD8-DR β -K225C	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β - Δ 229	F: CTTCAGGAATCAGTGTGGACACTCTGGATGAGGATCCGAG, R: CAGAGTGTCCACACTGATTCCTGAAGTAGATGAACAG
CD8-DR β - Δ 233	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: TCGGATCCTCATGTTGGCTGCAGTCCAGAGT
CD8-DR β - Δ 229	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: AGGAATCAGAAAGGACACTCTGGATGAGGATCCGA
CD8-DR β - Δ 227	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCAGTGTCTTTCTGATTCCTGATTC
CD8-DR β - Δ 225	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATTTCTGATTCCTGAAGTAGATG
CD8-DR β -RNAK	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATTTAGCATTCTGAAGTAGATGAACAGC
CD8-DR β -RAQK	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATTTCTGAGCCCTGAAGTAGATGAACAGCC
CD8-DR β -ANQK	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATTTCTGATTAGCGAAGTAGATGAACAGCCCTG
CD8-DR β -RAAK	pcDNA3.1 Neo-	<i>EcoRI</i> - <i>BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATTTAGCAGCCCTGAAGTAGATGAACAGCC
CD8-DR β -HF 0	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>XhoI</i>	CD8-DR β	N-term: F: T7seq, R: CAGACCCAGTACAGCGCCCCGACTCCACTCAGTGCCTTGCTCTGTGCAGACCC C-term: F: GCTGTACTGGGTCTGCTCTTCGCTGGGACAGGGCTGTTTCATCGCATTTCAGGAATCAGAAAGG ACAC, R: Zeo2
CD8-DR β -HF 1	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>XhoI</i>	CD8-DR β	N-term: F: T7seq, R: CCCCTGTCCCTAGGAAGAGAGCGCCCAGAACAAAACCCCGCTCCACTCAGCATCTTGCTC C-term: F: TCTCTTCCTAGGGACAGGGGCTTTCATCTACTTCAGGAATCAGAA, R: Zeo2
CD8-DR β -HF 2	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>XhoI</i>	CD8-DR β	N-term: F: T7seq, R: GAAGAGCAGGCCAGAGCAAAGCCCCGACTCCACTAGCCATCTTGCTCTGTGCAGAC C-term: F: TCTGGCCCTGCTCTTCCTTGCTACAGGGCTGTTTCATCTACGCTAGGAATCAGAAAGGA CACTCTG, R: Zeo2
CD8-DR β -HF 3	pcDNA3.1 Zeo+	<i>EcoRI</i> - <i>XhoI</i>	CD8-DR β	N-term: F: T7seq, R: CCCAGTCCCAAGGAAAGCCAGGCCAGCACAAAACCAGCGACTCCACTCAGCATCTTGCT C-term: F: TTTCTTGGGACTGGGCTGGCTATCTACTTCAGGAATCAGAAAGG, R: Zeo2

Supplemental Table 1, continued

CD8-DRβ-HF 4	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: CAAGGAAGAGCAGGCCAGCCACAAAGCCCCGACTCCAGCCAGCATCTTGCTCTGTGC C-term: F: TGGCCTGCTCTTCCTTGGCGCTGGGCTGTTTCATCTACTTCAG, R: Zeo2
CD8-DRβ-HF 5	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: ACAGCCCAGTCCCAAGAGCGAGCAGTCCCAGTACAAAAGCCCCGACTCCACTCAGC C-term: F: TCTTGGGACTGGGCTGTTTCGCTTACTTCAGGAATCAGAAAGGACA, R: Zeo2
CD8-DRβ-HF 6	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: TCCCAAGGAAGAGAAGAGCCAGCACAAAACCCCCGACAGCACTCAGCATCTTGCTCTGTG C-term: F: TCTTCTCTTCCTTGGGACAGCTCTGTTTCATCTACTTCAGGAATCAG, R: Zeo2
CD8-DRβ- M ¹⁹⁹⁻²⁰⁴ G→A	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: GCCCAGCACGAAGCCAGCGGCAGCAGCAGCAGCCTTGCTCTGTGCAGACCC C-term: F: GGCTTCGTGCTGGGCGCTGCT, R: Zeo2
CD8-DRβ- G ²⁰⁵⁻²¹⁰ L→A	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: CCTGTCCAAGGAAGAGAGCAGCAGCAGCTGCAGCCCCGACTCCACTCAGC C-term: F: CTCTTCCTTGGGACAGG, R: Zeo2
CD8-DRβ- L ²¹¹⁻²¹⁶ G→A	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: CTGATTCCTGAAGTAGATGAACAGAGCTGCTGCTGCTGCTGCCAGGCCAGCACAAAG C-term: F: TCTGTTCATCTACTTCAGGAATCAG, R: Zeo2
CD8-DRβ- L ²¹⁷⁻²²¹ F→A	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: T7seq, R: CAGAGTGTCTTTCTGATTCCTAGCAGCAGCAGCGGCCCTGTCCCAAGGAAGAGC C-term: F: TAGGAATCAGAAAGGACACTCTG, R: Zeo2
CD8-DRβ- S ^{194-AAAAA}	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: 5'Prim, R: CCCCTCGTGTGCACTGC C-term: F: CAGTGCACACGAGGGGGGCCGCTGCTGCTATGCTGAGTGGAGTCGGG, R: Zeo2
CD8-DRβ- S ^{194-AAA}	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: 5'Prim, R: CCCCTCGTGTGCACTGC C-term: F: CAGTGCACACGAGGGGGGCCGCTGCTAGCAAGATGCTGAGTGGAGTCG, R: Zeo2
CD8-DRβ- S ^{197-AA}	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: 5'Prim, R: CCCCTCGTGTGCACTGC C-term: F: CAGTGCACACGAGGGGGTCTGCACAGGCTGCTATGCTGAGTGGAGTC, R: Zeo2
CD8-DRβ- S ^{194-AAAAE}	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: 5'Prim, R: CCCCTCGTGTGCACTGC C-term: F: CAGTGCACACGAGGGGGGCCGCTGCTGAGATGCTGAGTGGGA, R: Zeo2
CD8-DRβ- R ^{222-AAA}	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: 5'Prim, R: GAAGTAGATGAACAGCCCTGTC C-term: F: GACAGGGCTGTTTCATCTACTTCGAGCAGCGAAAGGACACTCTG, R: Zeo2
CD8-DRβ- R ^{222-EAA}	pcDNA3.1 Zeo+	<i>EcoRI-XhoI</i>	CD8-DRβ	N-term: F: 5'Prim, R: GAAGTAGATGAACAGCCCTGTC C-term: F: GACAGGGCTGTTTCATCTACTTCGAGGCAGCGAAAGGACACTCTG, R: Zeo2

Supplemental Table 1, continued

CD8-DR β -K ¹	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	F: 5'Prim, R: CGGGATCCTCATGCAGCAGCGGCAGCTGCAGCAGCTTTGTAGATGAACAGCCCTGTCCC
CD8-DR β -K ¹	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATGCAGCAGCGGCAGCTTTGAAGTAGATGAACAGCCCTGTC
CD8-DR β -K ²	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	F: 5'Prim, R: CGGGATCCTCATGCAGCAGCGGCAGCTGCAGCTTTAGCGAAGTAGATGAACAGCCC
CD8-DR β -K ³	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	F: 5'Prim, R: CGGGATCCTCATGCAGCGGCTTTAGCTGCAGCAGCGAAGTAGATGAACAG
CD8-DR β -K ⁴	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	N-term: F: T7seq, R: TCATGCAGCGGCAGCTTTTGCAGCAGCGAAGTAGATGAACAGCCCTGTC C-term: F: AGCTGCCGCTGCATGAGGATCCGAGCTCG, R : GATGCAATTTCTCATTATTATTAG
CD8-DR β -K ⁵	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	F: 5'Prim, R: CGGGATCCTCATGCAGCAGCGGCAGCTTTAGCAGCGAAGTAGATGAACAG
CD8-DR β -K ⁶	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β -K ⁵	F: 5'Prim, R: CGGGATCCTCATGCAGCTTTGGCAGCTGCAGCAGCGA
CD8-DR β -K ⁷	pcDNA3.1 Neo-	<i>EcoRI-BamHI</i>	CD8-DR β	F: T7seq, R: CGGGATCCTCATGCTTTAGCGGCAGCAGCGGCAGCGAAGTAGATGAACAGCCCTGTC
HLA-DR α -KK ^{215,219} RR	pcDNA3.1 Zeo+	<i>HindIII-BamHI</i>	HLA-DR α	F: 5'Prim, R: AATGGATCCTTACAGAGGCCCCCTGCGTTCTGCTGCATTGCTTCTGCGCACTCCCCTGATGATG
HLA-DR β	pcDNA3.1 Hygro+	<i>HindIII-BamHI</i>	cDNA , DRB1*0303	F: CCCCAAGCTTGGCCACCATGGTGTGTCTGAGGCTCCCTG R: AAGGATCCTAGCTCAGGAATCCTCTTGGCTG
HLA-DR β -wt	pcDNA3.1 Hygro+	<i>HindIII-BamHI</i>	CD8-DR β , HLA-DR β	N-term: F: 5'Prim, R: CTGTGCAGATTCAATCGATGCTCTCCATTCCACTG C-term: F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT , R: TATGGATCCTCAGCTCAAGAGTCTGTTGGC This construct was a chimera between the DR3 β extracellular domain and the perimembrane-transmembrane-cytoplasmic tail region of DR1 β (which was also used in the CD8-DR β reporters). A unique <u>Clal</u> site (underlined in primers) was introduced in the non-folded amino acid chain between the folded part of the Ig domain and the juxtamembrane region. This resulted in minimal amino acid substitutions (¹⁹¹ RS ¹⁹² SI) in that region and did not affect DR folding or antigen loading (the modified DR was bound by mAb 16.23 which only binds correctly folded, SDS-stable DR3 dimers; data not shown)

Supplemental Table 1, continued

HLA-DRβ-K ¹	pcDNA3.1 Hygro+ HLA-DR3-DRβ-wt	<i>HindIII-XhoI</i>	CD8-DRβ-K ¹	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC Pair of universal primers that bound at the DRβ perimembrane region (F) and 3' terminal of the multiple cloning site on pcDNA3.1 Hygro+ (R). Insert was digested with <i>Clal</i> and <i>XhoI</i> (underlined) and inserted between the same sites in HLA-DR3-DRβ-wt. The reverse primer also contained a <i>M13F priming site</i> for screening for inserts by colony PCR (italics).
HLA-DRβ-K ¹	pcDNA3.1 Hygro+ HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ¹	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DRβ-K ²	pcDNA3.1 Hygro+ HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ²	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DRβ-K ³	HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ³	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DRβ-K ⁴	pcDNA3.1 Hygro+ HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ⁴	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DRβ-K ⁵	pcDNA3.1 Hygro+ HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ⁵	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DRβ-K ⁶	pcDNA3.1 Hygro+ HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ⁶	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DRβ-K ⁷	HLA-DR3-DRβ-wt	<i>Clal-XhoI</i>	CD8-DRβ-K ⁷	F: GAGCATCGATTGAATCTGCACAGAGCAAGATGCT, R: TATCTCGAGTGTA ^{AAACGACGGCCAGT} GATCAGCGGTTTAAACTTAAGC
HLA-DQβ	pCR2.1-TOPO	-	cDNA, HeLa.CIITA	F: ATAAGCTTCGCCACCATGCTCTTGAAGAAGGCTTTG, R: TACTCGAGTCTCAGGAGTCA ^{GTGCAGAAG} universal primer pair for DQB1, with <i>HindIII</i> and <i>XhoI</i> sites, <u>START</u> and <u>STOP</u> codons underlined
HLA-DRβ-DQβ ²¹⁵⁻²²⁹	pcDNA3.1 Zeo+	<i>HindIII-XhoI</i>	HLA-DRβ-wt, HLA-DQβ	C-term: F: 5'Prim, R: CCAAGCCCAAGGAAGAGCAGGCCCA N-term: F: TGCTCTTCCTTGGGCTTGGCCTTATCATCCGTCAA, R: TACTCGAGTCTCAGGAGTCA ^{GTGCAGAAG}

Supplemental Table 1, continued

HLA-DRβ ^{-DQβ215-221}	pcDNA3.1 Hygro+	<i>HindIII-BamHI</i>	HLA-DRβ ^{-DQβ215-229} , HLA-DRβ-wt	N-term.: F: 5'Prim, R: TCCTTTCTGATTCCTTTGACGGATGATAAGGCC C-term: F: CATCCGTCAAAGGAATCAGAAAGGACACTCTGG, R: Zeo2
EGFP-MARCH8	pEGFP C2	<i>EcoRI-KpnI</i>	pTracer/MARCH8	F: GCTTGGTACTAATACGACTCACTATAGGGA, R: <u>AGCGGTACCTAATGGTGATGGTGATGATG</u> MARCH8wt cloned from pTracer construct together with V5 and HIS tags, cut with <i>EcoRI</i> (on plasmid) and <u><i>KpnI</i></u> (underlined)
EGFP-MARCH8mut	pEGFP C2	<i>EcoRI-KpnI</i>	pTracer/MARCH8 mut	F: GCTTGGTACTAATACGACTCACTATAGGGA, R: <u>AGCGGTACCTAATGGTGATGGTGATGATG</u>

Further primer sequences:

T7seq: TAATACGACTCACTATAGGG
5'Prim: ACCCACTGCTTACTGGCTTATCG
Zeo2: CATGCCTGCTATTGTCTCCC

Supplemental Table 1.

List of primers used in the generation of constructs used in this study.