Supplementary information 2 – Cloning procedure

HLA-DQ8 α chain construct

The HLA-DQ8 α chain construct was produced using overlapping PCR. The cDNA encoding the signal peptide and extracellular domain of the HLA-DQ8 α chain was amplified first using a forward primer that contained a *Xho*I restriction site in the non coding sequence (sequence 5-3' CCGCTCGAGATGATCCTAAACAAAGCTCTGATG), and a reverse primer that contained an 18 nucleotide base pair overlap with the N-terminus of the thrombin cleavage linker (sequence 5-3' GAACCAGGTCTGCTGACGACTCAGGCTCCCAGTGTTTC). The cDNA containing both the thrombin cleavage linker and the acidic leucine zipper was amplified using a forward primer (sequence 5'-3' TCGTCAGCAGACCTGGTTC) and reverse primer that contained a stop codon followed by a *Kpn*I restriction site in the non coding region (sequence 5'-3 CGGGGTACCTCACTGAGCCAGTTCCTTTTCC). The two PCR products were then joined together in an overlapping PCR reaction using the same forward primer that was used to amplify the HLA-DQ8 α chain cDNA (sequence 5-3'CCGCTCGAGATGATCCTAAACAAAGCTC TGATG) and the same reverse primer used to amplify the thrombin linker and leucine acidic zipper cDNA (sequence 5'-3 CGGGGTACCTCACTGAGCCAGTTCCTTTTCC). The resulting PCR product, containing the entire HLA-DQ8 α chain construct, was cloned into the baculovirus expression vector pFastBac™ Dual under the control of the p10 promotor using the *Xho*I and *Kpn*I restriction sites.

HLA-DQ8 β chain construct

The HLA-DQ8 β chain construct was produced by generating 4 separate fragments specifying the entire construct with overlapping regions that were then joined together in one overlapping PCR reaction. Fragment 1, containing the *Eco*RI restriction site, the signal peptide, the α-gliadin peptide (QQYPSGEGSFQPSQENPQ), Factor X linker and the first 18 nucleotide base pairs of the mature β chain, was generated synthetically using PCR with 4 overlapping primers that specified fragment 1 (sequences 5'-3 primer 1 forward CCGGAATTCATGTCTTGGAAGAAGGCTTTGCGAATCC CTGGAGGCCTTCGGGTAGCAACTGTGACCTTGATGC, primer 2 reverse CTGCTGACCGGTAGAGTC TCTGCCCTCAGCCACCGGGGTGCTCAGCATCGCCAGCATCAAGGTCACAG TTGC, primer 3 forward GACTCTACCGGTCAGCAGTACCCGTCCGGTGAGGGTTCCTTCCAGCCGTCC CAGGAGAATCCGCAGGGTGG, primer 4 reverse ATCCTCGGGAGAGTCTCTGGAACCACCACCGGAACCGCGACCCTC AA TTGAACCACCACCACCCTGCGGATTCTCC). Fragment 2, containing the mature extracellular domain of the β chain, was amplified from the full length wildtype cDNA using the forward primer (sequence 5'-3 CAGAGACTCTCCCGAGGAT) and reverse primer (sequence 5'-3 AGACTGAGCCCGCCACTC). Fragment 3, containing the last 18 nucleotides of the C-terminal β chain, and the thrombin cleavage linker and basic zipper, was amplified from the cDNA encoding the thrombin cleavage linker and leucine basic zipper using the forward primer containing the 18 nucleotides of the C-terminal β chain (sequence 5'-3 GAGTGGCGGGCTCAGTCTTCGTCAG

CAGACCTGGTTC) and reverse primer (sequence 5'-3 GCCTCAAAGATTCCTCCAAGCTGGGCGAGT TTCTTCTTG). Fragment 4, containing the last 19 nucleotides of the C-terminal basic leucine zipper, BirA biotinylation tag, hexahistidine tag, stop codon and a *Hind*III restriction site in the non coding sequence, was generated synthetically using PCR and two overlapping primers (sequence 5'-3 primer 1 forward CTTGGAGGAATCTTTGAGGCAATGAAGATGGAGCTGCGGGAC, primer 2 reverse CCCAAGCTTTCA ATGATGATGATGATGATGGTCCCGCAGCTCCATCTTC).

The four fragments were then joined together in an overlapping PCR reaction using the same forward primer that was used to generate fragment 1 (sequences 5'-3 CCGGAATTCATGTCTTGGAAGAAGGCTTTGCGAATCC) and the same reverse primer used to generate fragment 4 (sequences 5'-3 CCCAAGCTTTCAATGATGATGATGATGATGGTCCCGCAGCTCCATCTTC). The resulting PCR product, containing the entire HLA-DQ8 β chain construct, was cloned into the pFastBac™ Dual vector already containing the α chain construct, using the *Eco*RI and *Hind*III restriction sites and was under the control of the PH promotor.

HLA-DQ2 α chain construct

The cloning procedure for the HLA-DQ2 α chain construct was essentially the same as that used for the HLA-DQ8 α chain. The cDNA encoding the signal peptide and extracellular domain of the HLA-DQ2 α chain was amplified first using a forward primer that contained an *Xho*I restriction site in the non coding sequence (sequence 5-3' CCGCTCGAGATGATCCTAAACAAAGCTCTG), and a reverse primer that contained a 20 nucleotide base pair overlap with the N-terminus of the thrombin cleavage linker (sequence 5-3' GGAACCAGGTCTGCTGACGACTCTGTGAGCTCTGACATAG). The cDNA containing both the thrombin cleavage linker and the acidic zipper peptide was amplified using the forward primer (sequence 5'-3' TCGTCAGCAGACCTGGTTC) and reverse primer that contained a stop codon followed by a *Kpn*I restriction site in the non coding region (sequence 5'-3 CGGGGTACCTCACTGAGCCAGTTCCTTTTCC). The two PCR products were then joined together in an overlapping PCR reaction using the forward primer (sequence 5-3' CCGCTCGAGATGATCCTAA) and reverse primer (sequence 5'-3 CGGGGTACCTCACTTATC). The resulting PCR product, containing the entire HLA-DQ2 α chain, was cloned into the baculovirus expression vector pFastBac™ Dual under the control of the p10 promotor using the *Xho*I and *Kpn*I restriction sites. Mutagenesis of a free cysteine at residue number 47 to a serine was then performed using the following primers, forward primer (sequence 5'-3 CCCTCCTCCCTTCTGCTGAGGAGAGTTATGAC) and reverse primer (sequence 5'-3 GTCATAACTCTCCTCAGCAGAAGGGAGGAGGG).

HLA-DQ2 β chain construct

The HLA-DQ2 β chain construct was produced using the HLA-DQ8 β chain construct that had been cloned into pGEM®-T Easy vector as a template, and by replacing the HLA-DQ8 peptide (QQYPSGEGSFQPSQENPQ) with the HLA-DQ2 peptide (QLQKFPQPELPYPQPQP), and the HLA-DQ8 β chain with the HLA-DQ2 β chain. Additional modifications were also made, an extra flexible glycine serine linker (GSGSGSGS) was added for the purposes of making tetramer, as well as extending the histidine tag from six residues to ten to increase protein purity post metal affinity purification.

First, the HLA-DQ2 peptide was produced synthetically with overlapping PCR using two primers that encoded the sequence from the start of the *Age*I site to the end of the *Mfe*I site (see HLA-DQ2 construct sequence), forward primer (sequence 5'-3 GACTCTACCGGTCAATTACAAAAATTTCCGCAACCGGAATTGCCGTACCC GCAAC) and reverse primer (sequence 5'-3 GACCCTCAATTGAACCACCACCACCCGGTTGCGGTTGCGGGTACGGCAAT TC). The resulting PCR product containing the HLA-DQ2 peptide, along with the HLA-DQ8 β chain construct in pGEM®-T Easy, was digested with *Age*I and *Mfe*I. The digested PCR product was then ligated into the digested HLA-DQ8 β chain construct in pGEM®-T Easy.

The extracellular domain of the HLA-DQ2 β chain was amplified using a forward primer that also encoded the *Mfe*I site (sequence 5-3' GTGGTTCAATTGAGGGTCGCGGTTCCGGTGGTGGTTCCAGAGACTCTCCC GAGGATT) and a reverse primer that also encoded the *Bam*HI site (sequence 5-3'

CGCCGCGGATCCGCGCGGAACCAGGTCTGCTGACGACTTGCTCTGGGCAG ATTCA) (see HLA-DQ2 construct sequence). The resulting PCR product containing the HLA-DQ2 β chain, along with the HLA-DQ8 β chain construct in pGEM®-T Easy, was digested with *Mfe*I and *Bam*HI. The digested HLA-DQ2 β chain was then ligated into the HLA-DQ8 β chain construct in pGEM®-T Easy which also contained the HLA-DQ2 peptide.

The extra flexible linker and the extended His Tag were then generated synthetically using overlapping PCR with a forward primer that started with the last two nucleotides of the basic leucine zipper (sequence 5'-3 AGGGTTCCGGTTCCGGTTCCGGTTCCCTTGGAGGAATCTTTGAGGCAATGA AGATGGAGCTGCG) and a reverse primer ending with the *Hind*III restriction site (sequence 5'-3 CCCAAGCTTTCAATGATGATGATGATGATGATGATGATGGTCCCGCA GCTCCATCTTCATTG) (see HLA-DQ2 construct sequence). The β chain construct was then amplified using the forward primer which began at *Eco*RI site (sequence 5'-3 CCGGAATTCATGTCTTGGAAG), and a reverse primer that ended with a 16 base overlap with the new flexible linker (sequence 5'-3 AACCGGAACCGGAACCCTGGGCGAGTTTCTTCTTG). These two PCR products were then joined together in one PCR reaction using the forward primer (sequence 5'-3 CCGGAATTCATGTCTTGGAAG) and reverse primer (sequence 5'-3 CCCAAGCTTTCAATGATGATG).