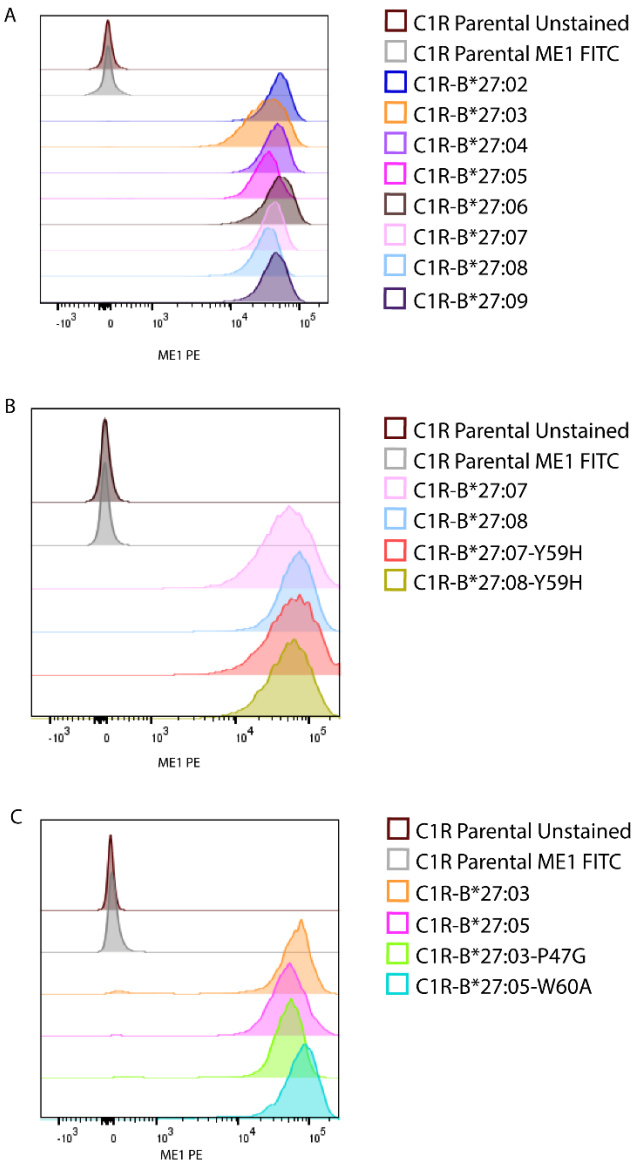
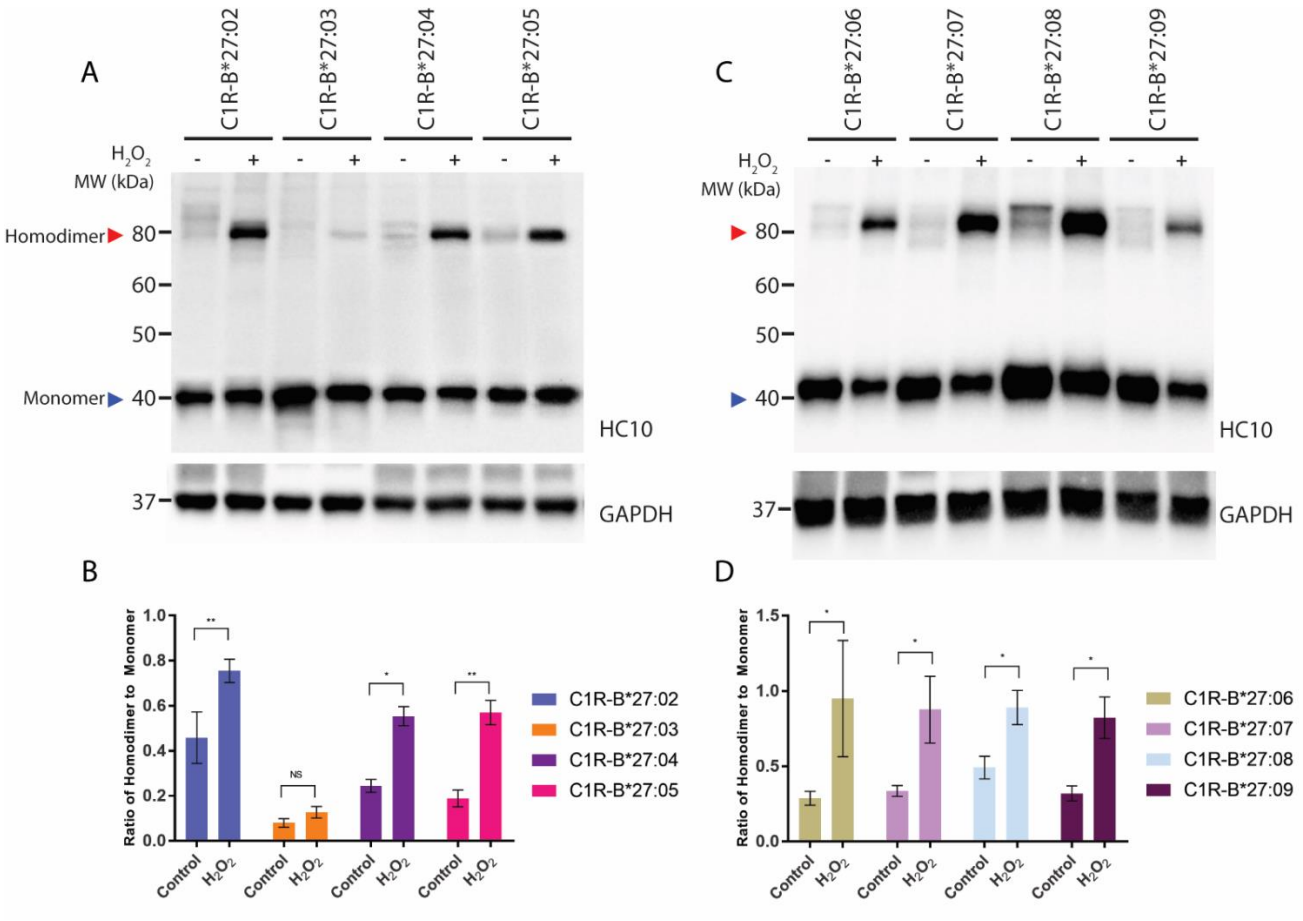


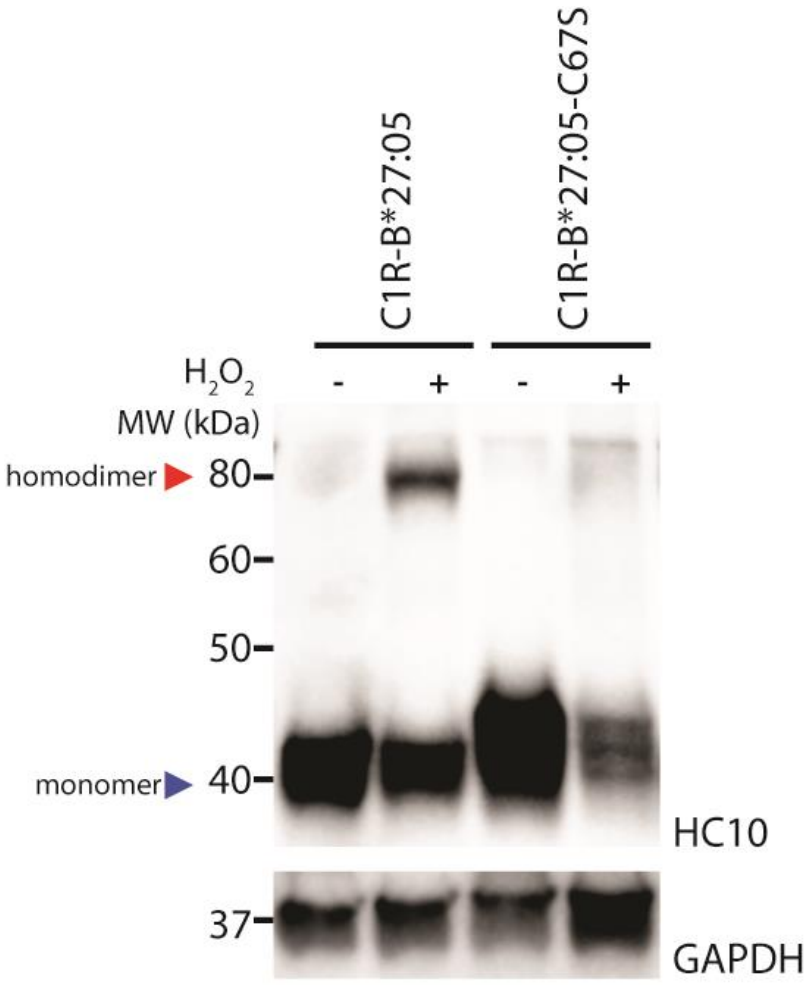

**Supplementary Figure 1. Expression levels of HLA-B27 allotypes**

HLA-B27 expression of **[A]** C1R-B*27:02 - C1R-B*27:09, **[B]** C1R-B*27:07-Y59H & C1R-B*27:08-Y59H and **[C]** C1R-B*27:03-P47G & C1R-B*27:05-P47G cells were tested by immunostaining with the mouse monoclonal antibody ME1 (HLA-B27 specific), followed by flow cytometry analysis. Anti-mouse FITC was used as secondary antibody. Unstained and ME1-stained parental C1R cells were used as negative controls.



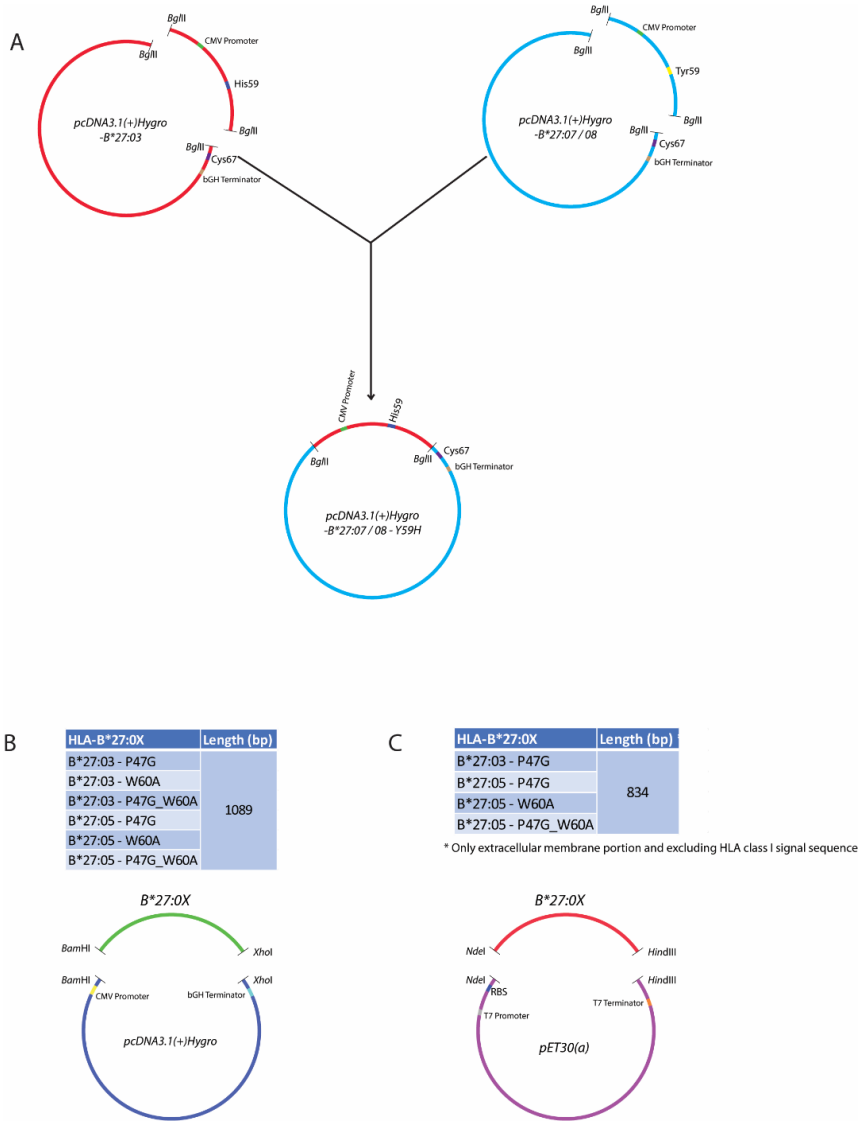

**Supplementary Figure 2. Effect of $H_2O_2$ treatment on the 8 most common HLA-B27 allotypes**

**[A]** Cell lysates from C1R-B*27:02 – C1R-B*27:09 cells were separated by a 12% SDS-PAGE gel and analyzed by immunoblotting using the HC10 antibody. The addition of $H_2O_2$ to the cells is indicated above the lanes. The red arrow indicates HLA-B27 homodimers and the blue arrow the monomeric forms. **[B]** Background-corrected densitometric analyses showing the ratio of HLA-B27 homodimer to monomer. n=3; mean ± SEM. P-value was calculated using the Welch's t test (*: $p < 0.05$, **: $p < 0.001$, NS: non-significant).



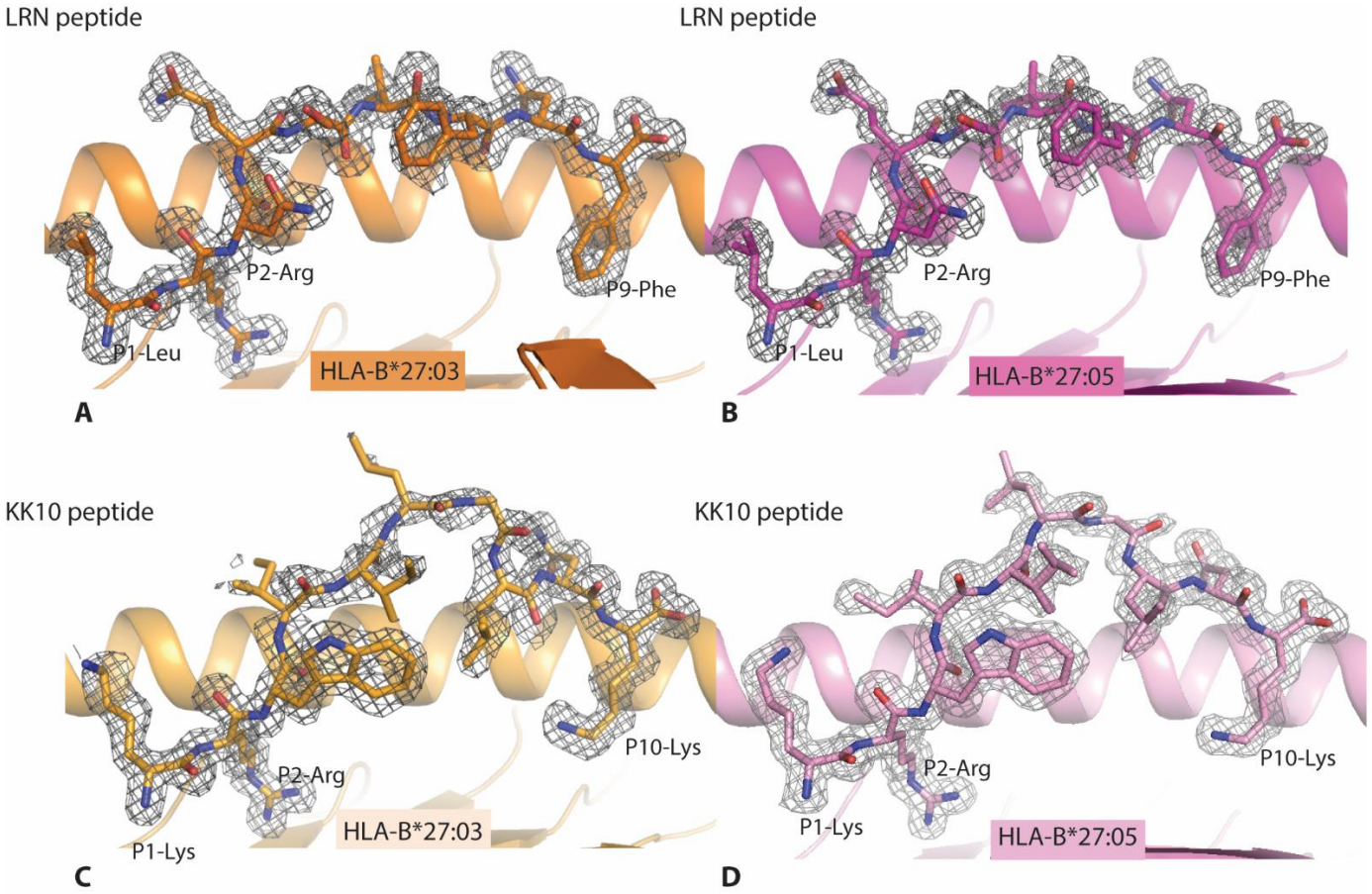

**Supplementary Figure 3. Effect of C67S on homodimer formation**

Cell lysates from C1R-B*27:05 and C1R-B*27:05-C67S cells were separated by 12% SDS-PAGE and analyzed by immunoblotting using the HC10 antibody and an antibody recognizing GAPDH as loading control. The addition of $H_2O_2$ to the cells is indicated above the lanes. The red arrow indicates HLA-B27 homodimers and the blue arrow the monomeric forms.



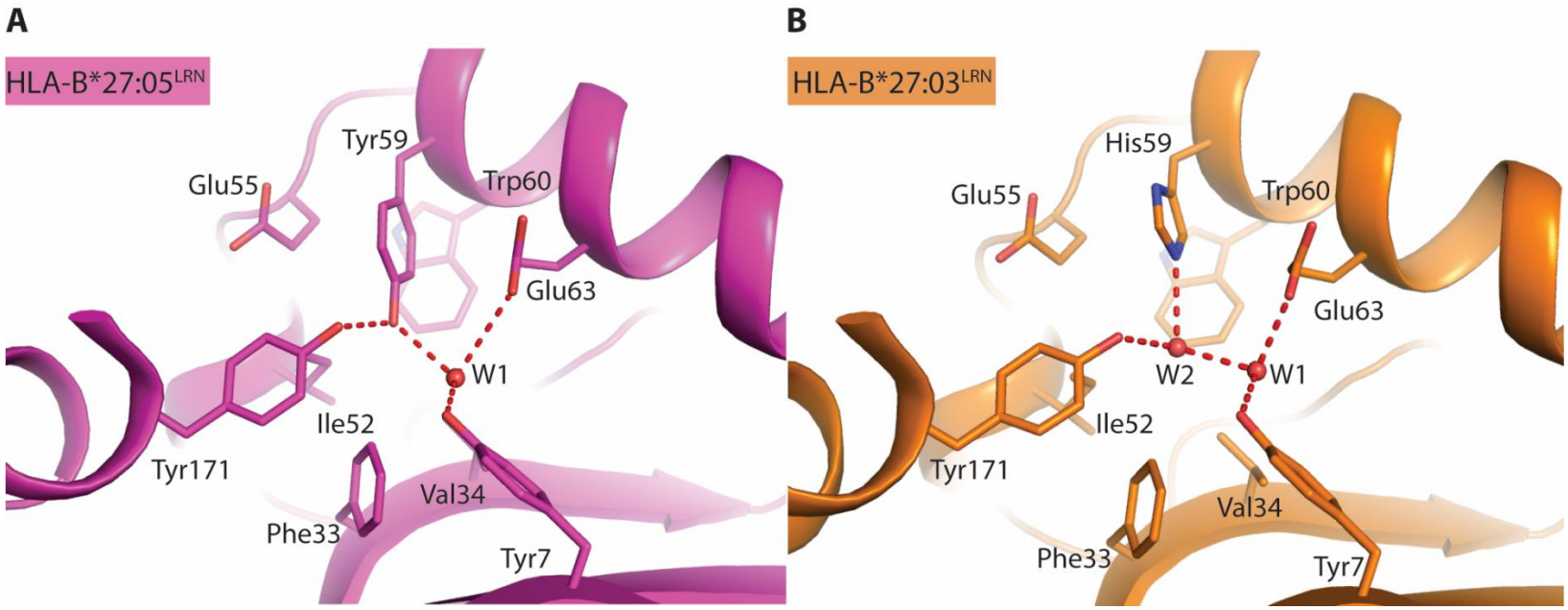

**Supplementary Figure 4. Cloning Method**

**[A]** Schematic illustration showing the creation of *pcDNA3.1(+)Hygro-B*27:07-Y59H* and *pcDNA3.1(+)Hygro-B*27:08-Y59H* by swapping the *Bgl*II region from *pcDNA3.1(+)Hygro-B*27:03* with the corresponding region in *pcDNA3.1(+)Hygro-B*27:07* and *pcDNA3.1(+)Hygro-B*27:08*. **[B,C]** Introduction of commercially synthesized *HLA*B*27:03* and *HLA-B*27:05* alleles containing the P47G and/or W60A polymorphisms into the *pcDNA3.1(+)Hygro* (B) or *pET30a* (C) vector for the expression in mammalian and bacterial cells, respectively.



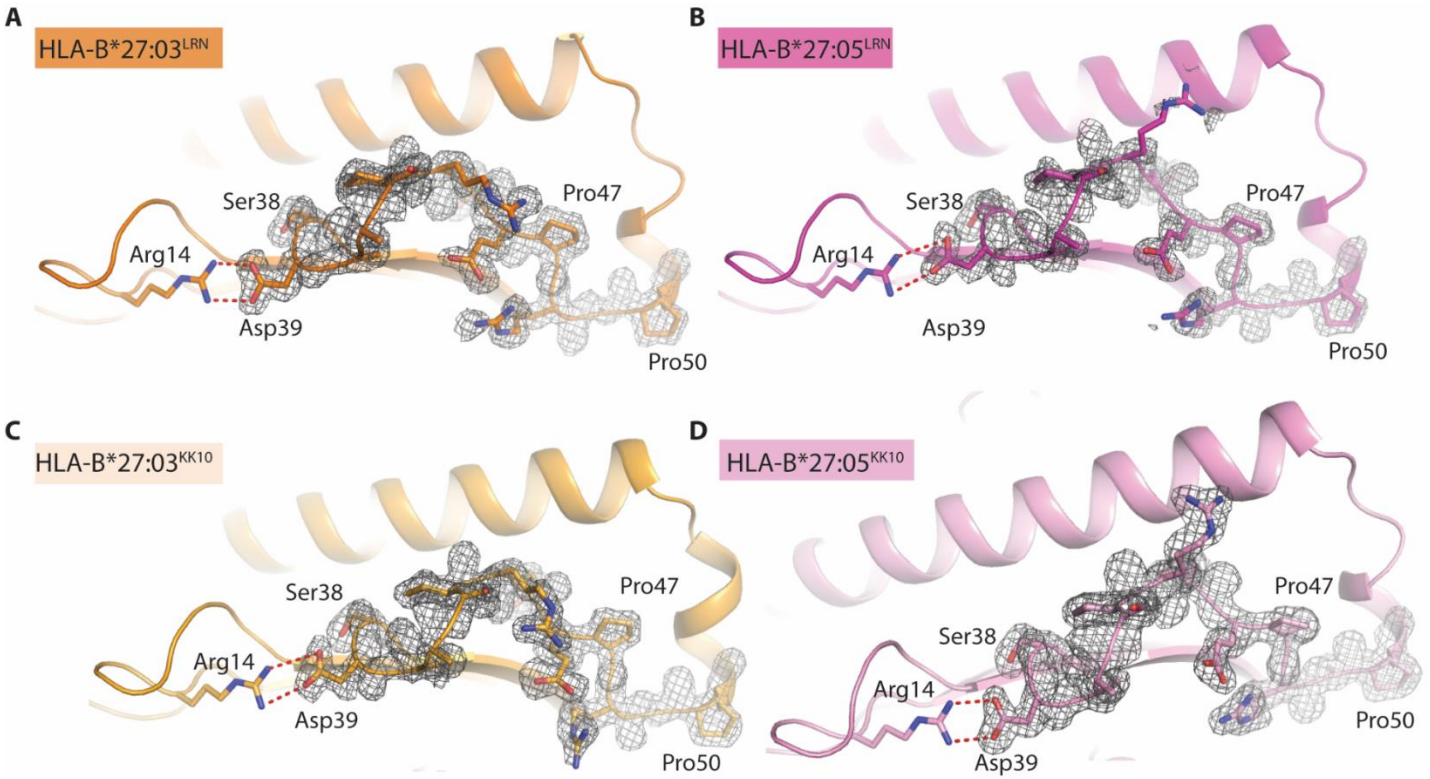

**Supplementary Figure 5. Density map of the peptide**

The four panels show the electron density map (2Fo-Fc at 1 σ in gray) for the LRN (top) or KK10 (bottom) peptides bound to HLA-B*27:03 (orange, A and C) or HLA-B*27:05 (pink, B and D). The peptide is represented in stick and colored accordingly to the HLA molecule, and the HLA molecule is represented as cartoon, with the α2-helix removed for clarity.




**Supplementary Figure 6. A Tyr59 in B2705 interaction, B His59 in B2703 interaction**

The panels show the interaction of the polymorphic residue at position 59 in HLA-B*27:05 (A, pink) and HLA-B*27:03 (B, orange). The red dashed lines represent hydrogen bond, the red sphere represent water molecules. In essence, the side chain of both Tyr59 and His59 are pointing down in the antigen-binding cleft. In HLA-B*27:05, the Tyr59 hydroxyl group is interacting directly or via a water mediated bond with the Tyr171 and Tyr7, respectively, as well as interacting with the hydrophobic residues Ile52, Phe33 and Val34. The aromatic ring of Tyr59 sits above the Trp60 and is sandwiched by two negatively charged Glu55 and Glu63. In HLA-B*27:03, the His59 has a similar environment than the Tyr59, with some localized movement. The lack of hydroxyl group allows for the presence of an additional water molecule bonding His59 and Tyr7 in HLA-B*27:03 structures.




**Supplementary Figure 7. Density map of the 3rd loop**

As per supplementary figure 5, the four panels show the electron density map (2Fo-Fc at 1 σ in gray) for the 3rd loop of the HLA-B*27:03 (orange, A and C) or HLA-B*27:05 (pink, B and D) molecules bound with the LRN (top) or KK10 (bottom) peptides. The 3rd loop, which ranges from residue 38 to residue 50, was involved in similar crystal packing interaction in all structures (with the α3 domain loop spanning from residues 195-198) and was stabilized by a salt bridge between the Asp14 and the Arg39 (red dashed lines). The electron density was clear for the 3rd loop in all structures permitting a detailed comparison of the impact of the polymorphic residue at position 59 onto the loop mobility