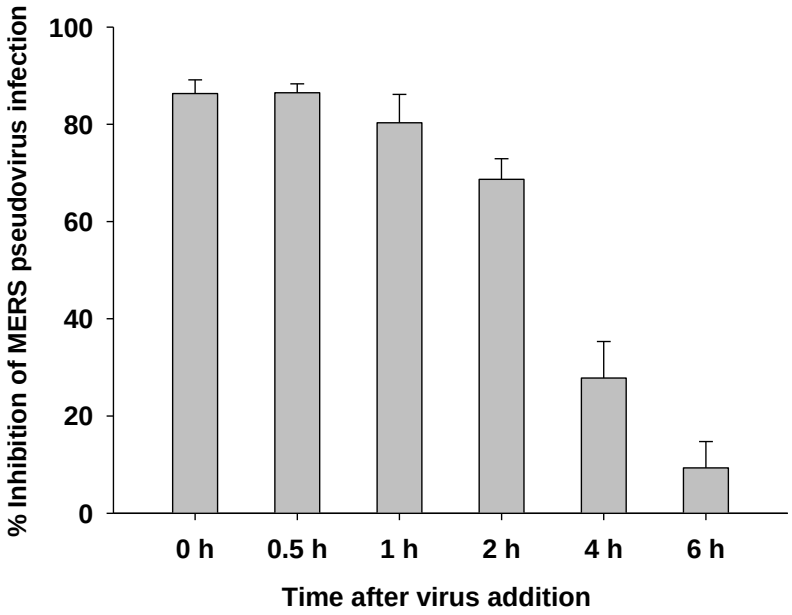
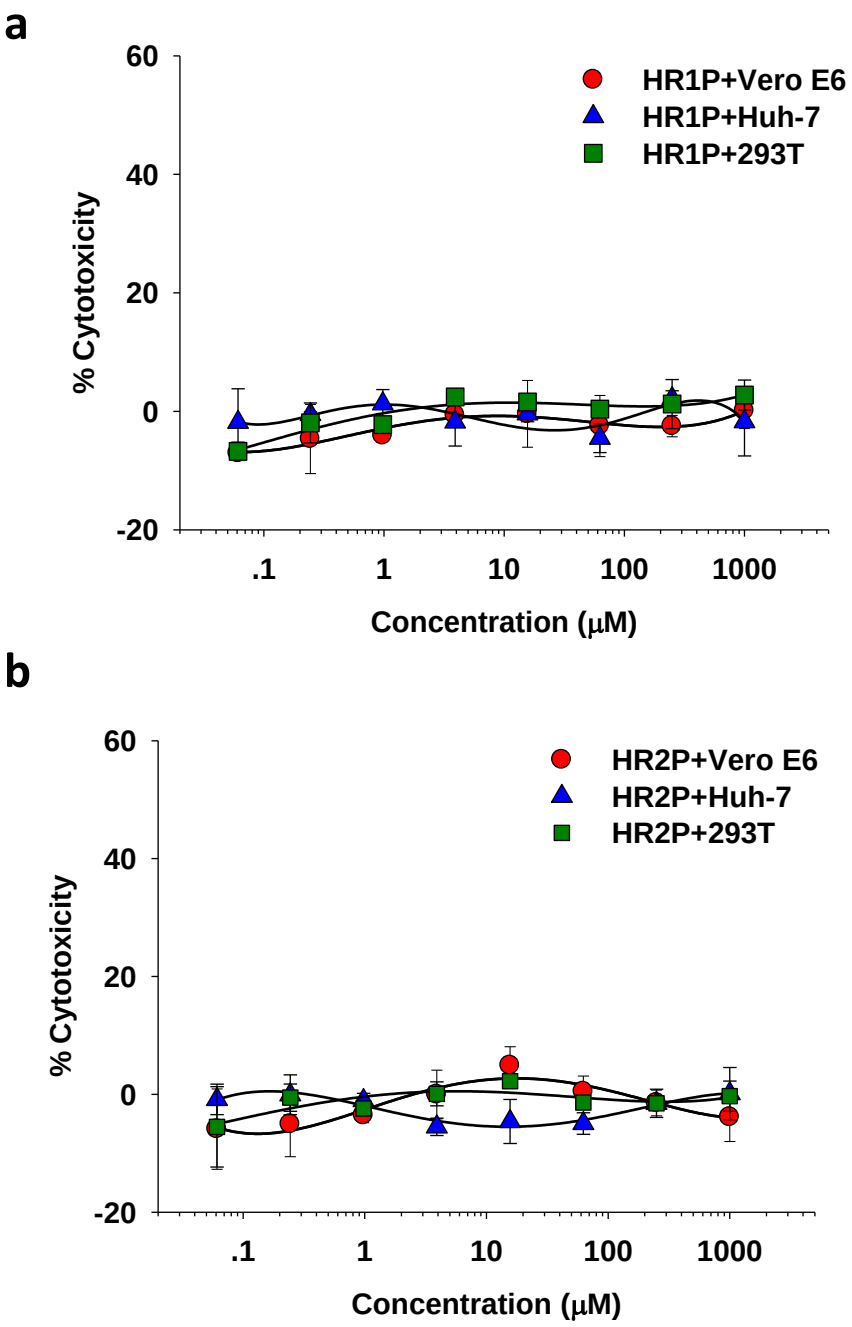
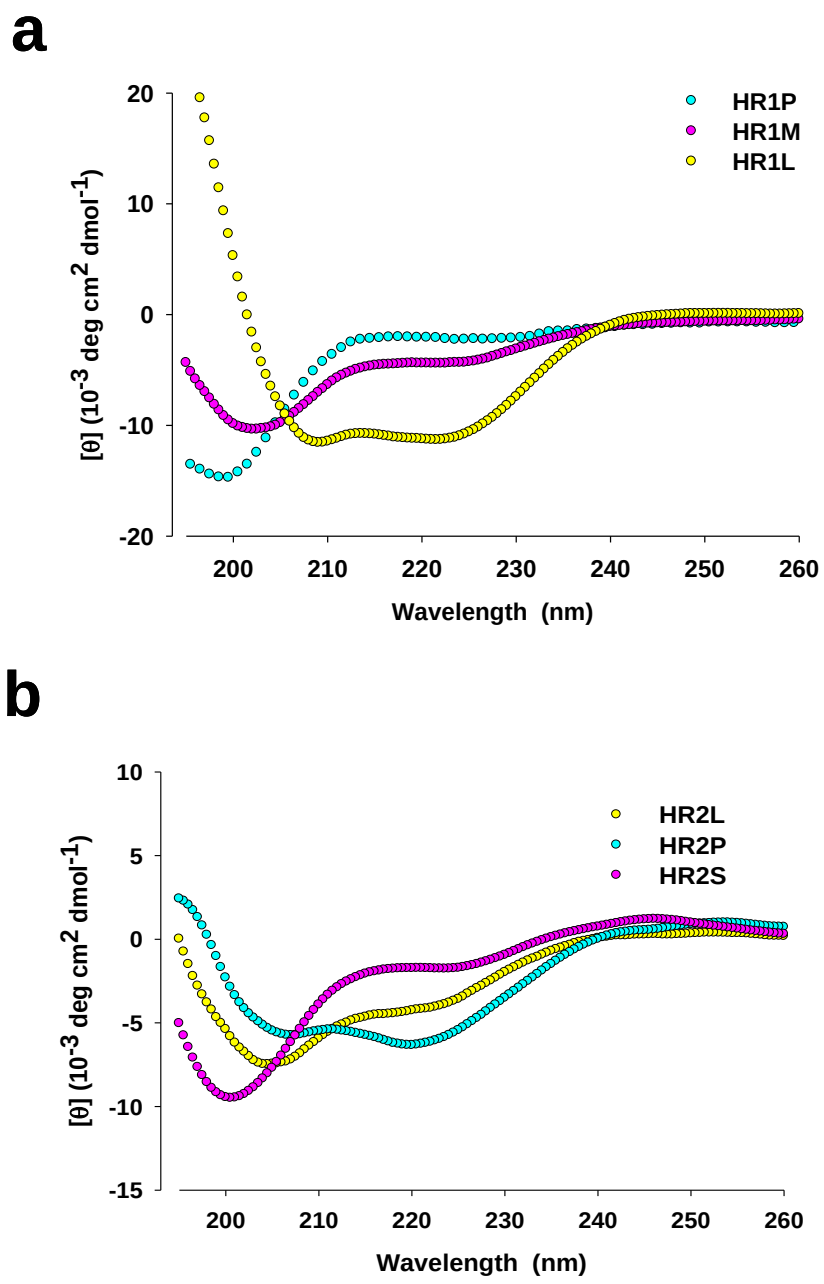


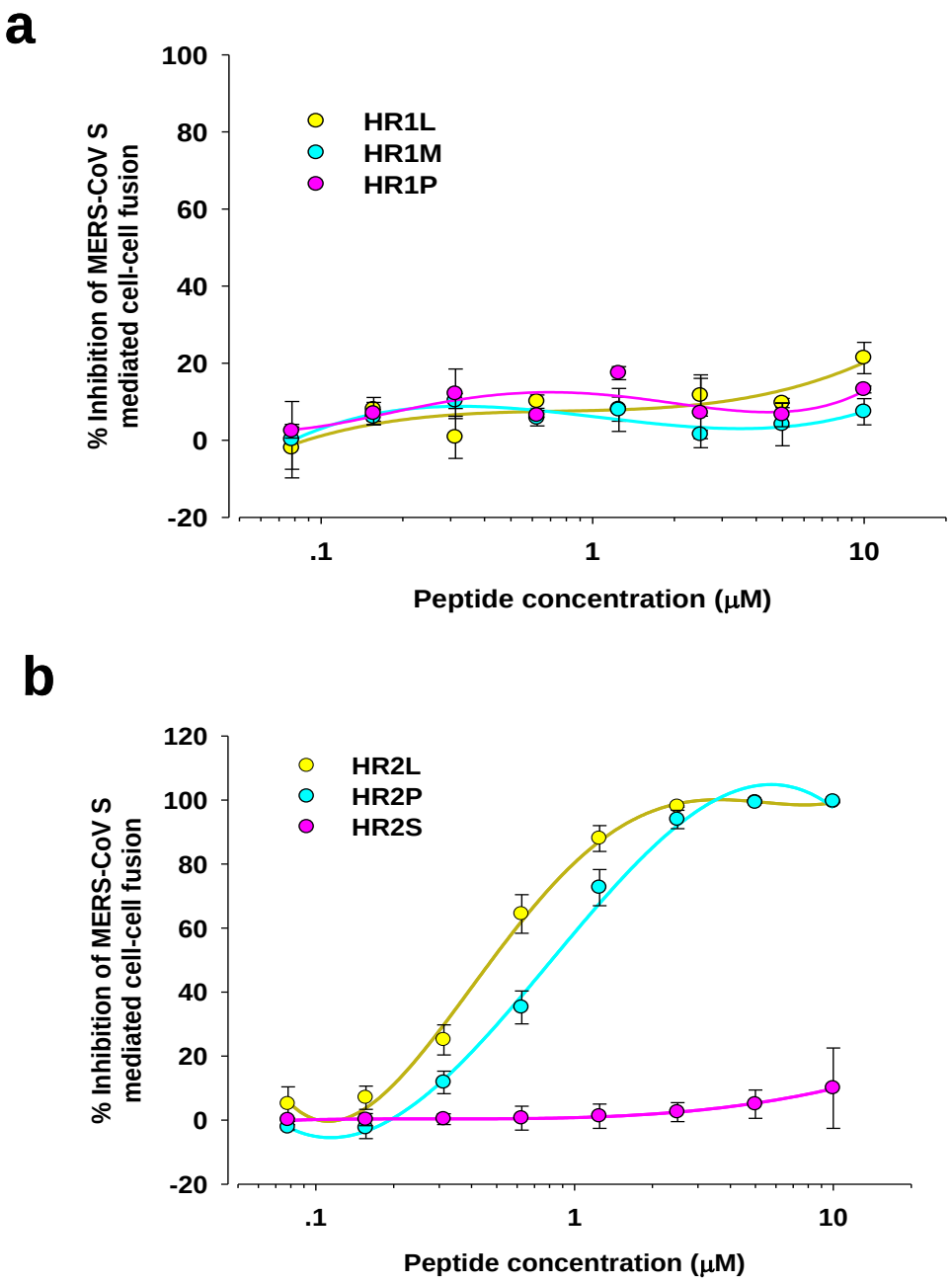
Supplementary Figure S1. Inhibition of HR2P on MERS pseudovirus infection at the different intervals postinfection. A time-of-addition experiment was performed to test the inhibitory activity of HR2P peptide on MERS pseudovirus infection in Huh-7 cells at different intervals between addition of virus and the peptide. The experiment was performed in triplicate and the data are presented as mean ± s.d. (error bar).



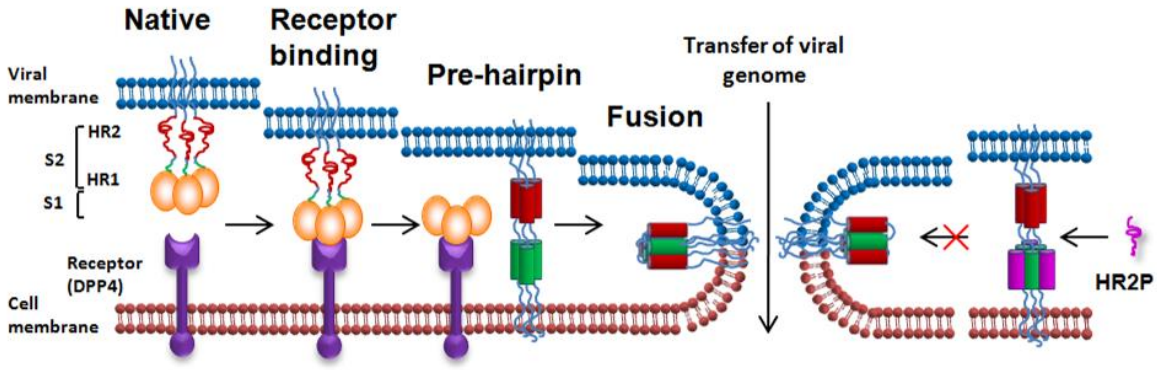
Supplementary Figure S2. Cytotoxicity of HR1P and HR2P peptides. The 293T, Huh-7 and Vero cells are used for testing the potential toxic effect of HR1P (a) and HR2P (b), determined by XTT assay. The experiment was performed in triplicate. The data are presented as mean ± s.d. (error bar) from a single representative experiment out of two repetitions.



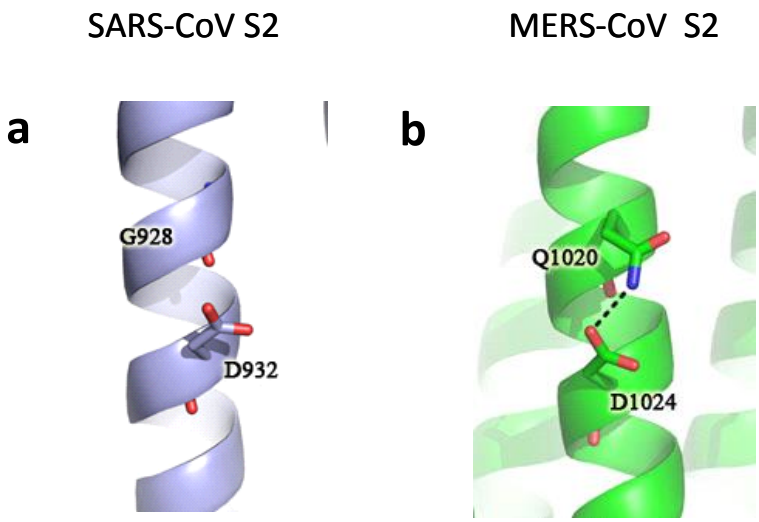
Supplementary Figure S3. The secondary structures of different lengths of HR1 and HR2 peptides. (**a)** HR1 peptides HR1L, HR1M, and HR1P; (**b**) HR2 peptides HR2L, HR2M, and HR2S.



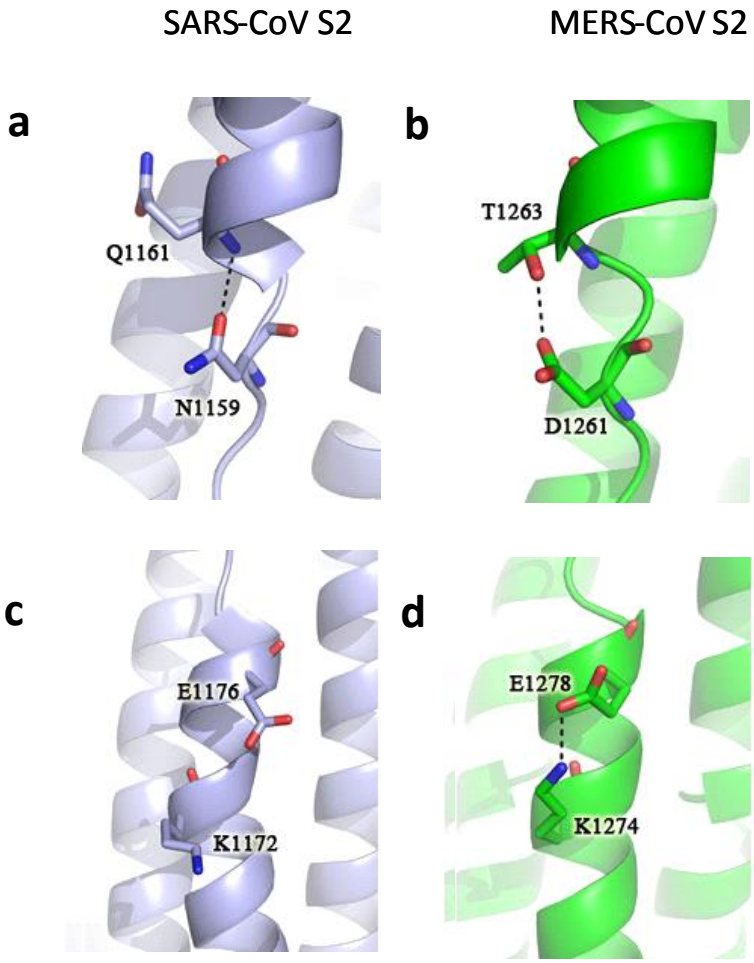
Supplementary Figure S4. Inhibitory activity of HR1 (a) and HR2 (b) peptides on MERS-CoV S mediated cell-cell fusion. Experiments were performed in triplicate and the data are expressed as means ± s.d. (error bar). The experiment was repeated twice and similar results were obtained.



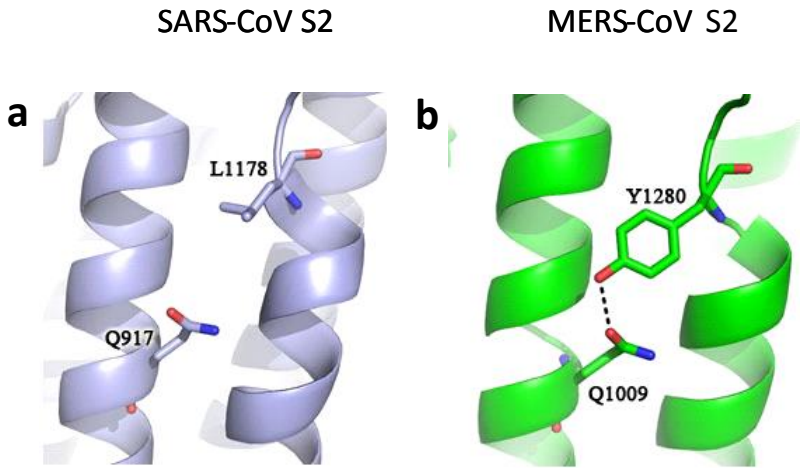
Supplementary Figure S5. Model of the MERS-CoV S protein S2 subunit-mediated membrane fusion and the mechanism of action of the fusion inhibitory peptide HR2P. In the native state, part of the HR1 domain in the S2 subunit, possibly in a random coil conformation, is shielded by the S1 subunit, and HR2 forms the stem of the spikes with partially helical structure. The entirety of S proteins on the viral surface may take an oligomeric form through self-association. In the receptor binding state, the S1 subunit binds with receptor DPP4 on the target cell surface. In the pre-hairpin state, the S1 subunit may be dissociated from the S2 subunit, which undergoes a series of conformational changes. The fusion peptide (FP) at the N-terminus of the S2 subunit inserts into the target cell membrane, resulting in exposure of the HR2-trimer and HR1-trimer. HR1-trimer at this stage serves as a target of the peptides derived from the HR2 domain. In the fusion state, the HR1 and HR2 helices associate with each other to form the 6-HB fusion core, drawing the viral and cellular membranes into close proximity for fusion and resulting in the formation of a fusion pore through which the viral genetic materials are released into the target cell. HR2P, the peptide derived from the HR2 domain, can bind to the HR1-trimer to form heterologous 6-HB and block viral fusion core formation, resulting in inhibition of MERS-CoV fusion with the target cell membrane. The 6-HB formation can occur on the cell plasma membrane or endosomal membrane when the virus enters into the cell through plasma membrane fusion or endocytosis pathway, respectively.



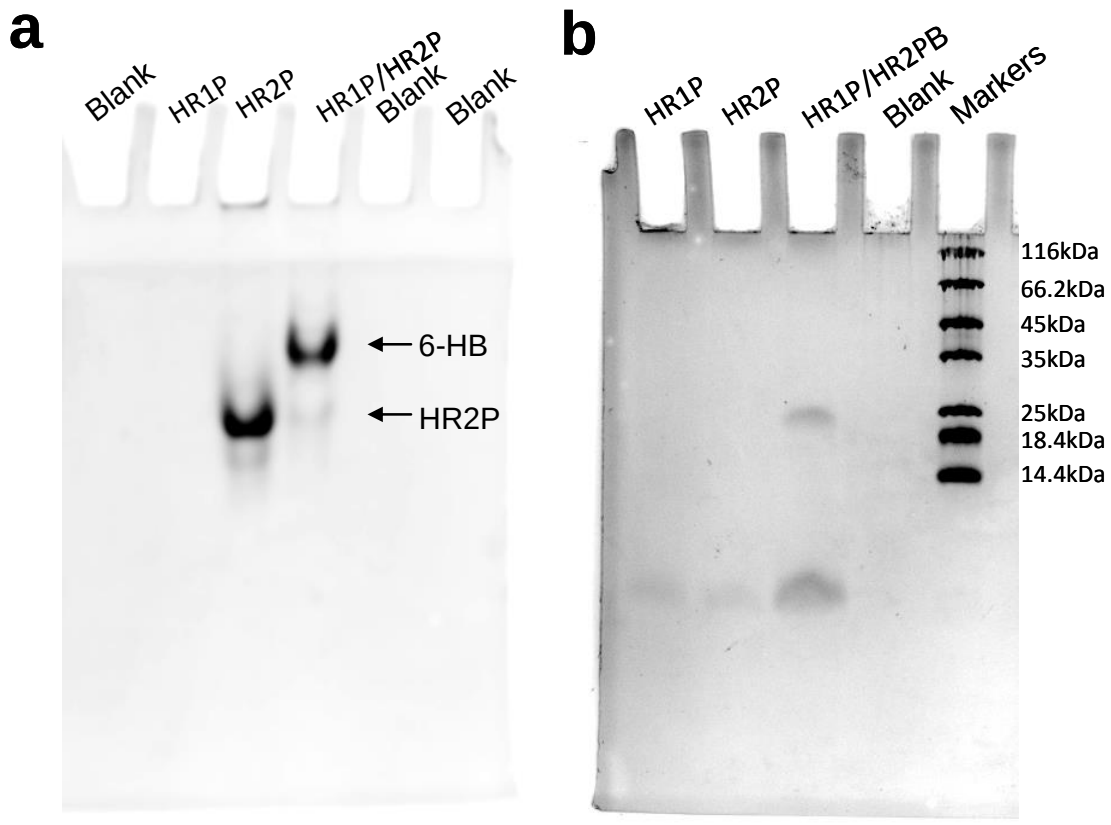
Supplementary Figure S6. The emerging hydrogen bond on HR1 in MERS-CoV. Residues G928 and D932 in HR1 domain in S2 subunit of SARS-CoV (**a**) correspond to Q1020 and D1024 in HR1 domain in S2 subunit of MERS-CoV (**b**), respectively. In the HR1 domain of MERS-CoV, the distance of the intramolecular hydrogen bond between Q1020 and D1024 is 2.55Å. The corresponding residues in the HR1 domain of SARS-CoV S2 could not form such hydrogen bond.



Supplementary Figure S7. Enhanced interactions inside the HR2 region in MERS-CoV. In the HR2 domain of SARS-CoV S2, N1159 binds to the main chain of Q1161 with a distance of 3.3Å (**a**). In the corresponding sites in the HR2 domain of MERS-CoV S2, D1261 binds to T1263 through their side chains with a distance of 2.76Å (**b**). K1172 and E1176 in the HR2 domain of SARS-CoV S2 show no interaction (**c**), while the corresponding residues (K1174 and E1178) in the HR2 domain of MERS-CoV S2 form a hydrogen bond with a distance of 2.60Å (**d**).



Supplementary Figure S8. Enhanced interaction between HR1 and HR2 in MERS-CoV. Q917 in HR1 and L1178 in HR2 of SARS-CoV S2 do not display an interaction bond (**a**), whereas the corresponding residues Q1009 in HR1 and Y1280 in HR2 of MERS-CoV S exhibit a hydrogen bond between HR1 and HR2 with a distance of 2.70Å (**b**).



Supplementary Figure S9. Images of the full gels presented in the main paper. (**a**) The image showing 6-HB formation between HR1P and HR2P by N-PAGE in Fig. 4a; (**b**) The image showing the molecular mass determined by gel electrophoresis in Fig. 4b.