Supplementary figures

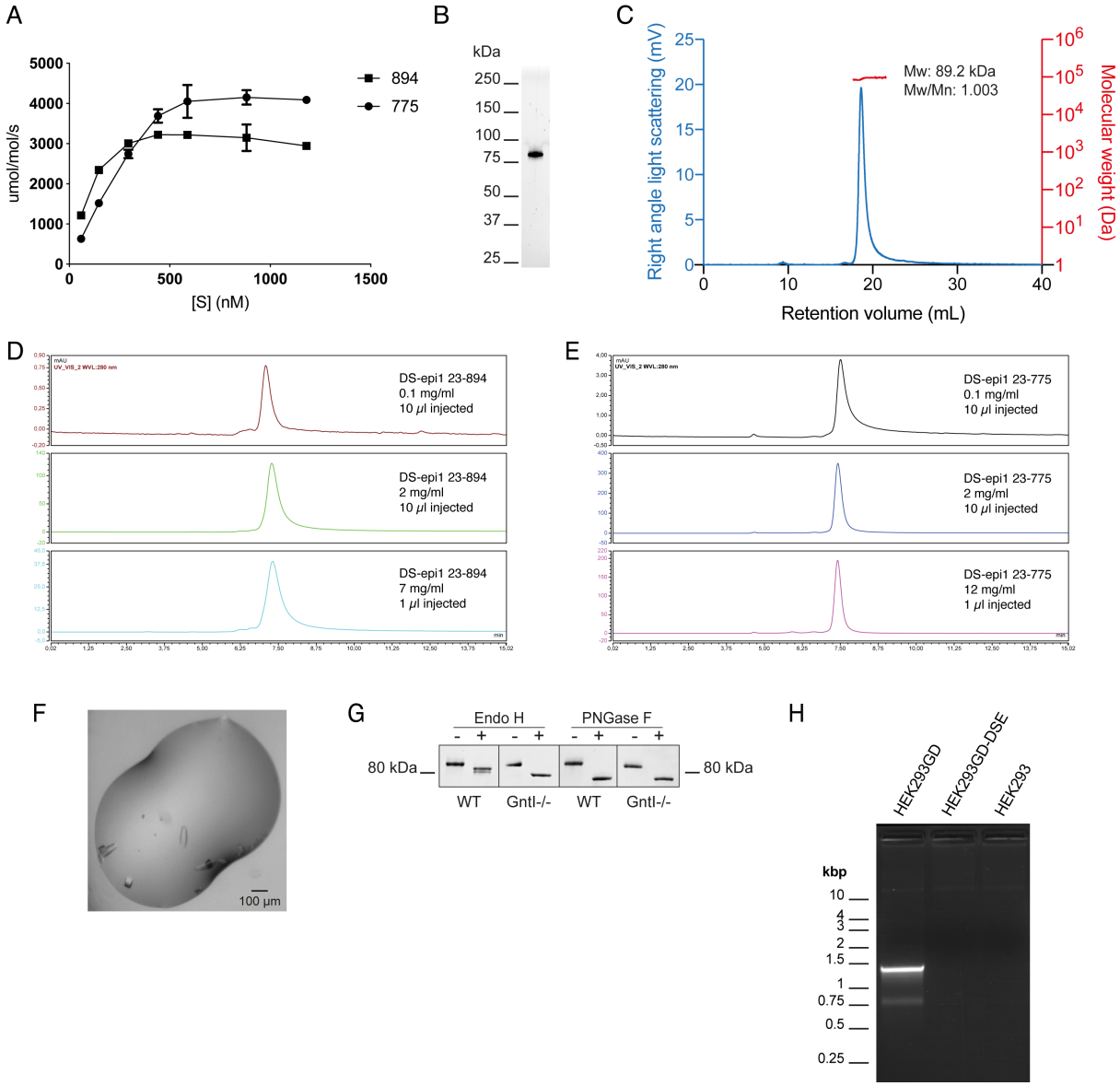

Fig. S1. Biochemical characterization of recombinant DS-epi1.
(A) Enzymatic activity of DS-epi1 23-894 and 23-775, using C5-tritiated chondroitin as substrate. Each (B) SDS-PAGE analysis (C) Multi-angle light scattering analysis, revealing a monomeric and monodisperse product of 89.2 kDa (theoretical Mw=87.2 kDa). (D) and (E) size-exclusion chromatography (SEC) analysis of DS-epi1 23-894 and 23-775 at different protein concentrations. Proteins were separated on an AdvanceBio SEC UHPLC column. (F) Crystals from a drop containing 200 mM $NH_4CH_3CO_2$, 100 mM MES pH 6.5, 30% v/v glycerol ethoxylate (15/4 EO/OH) and 6% xylitol (G) Glycosidase degradation and SDS-PAGE analysis (H) PCR analysis of the bacterial endoT gene using genomic DNA.

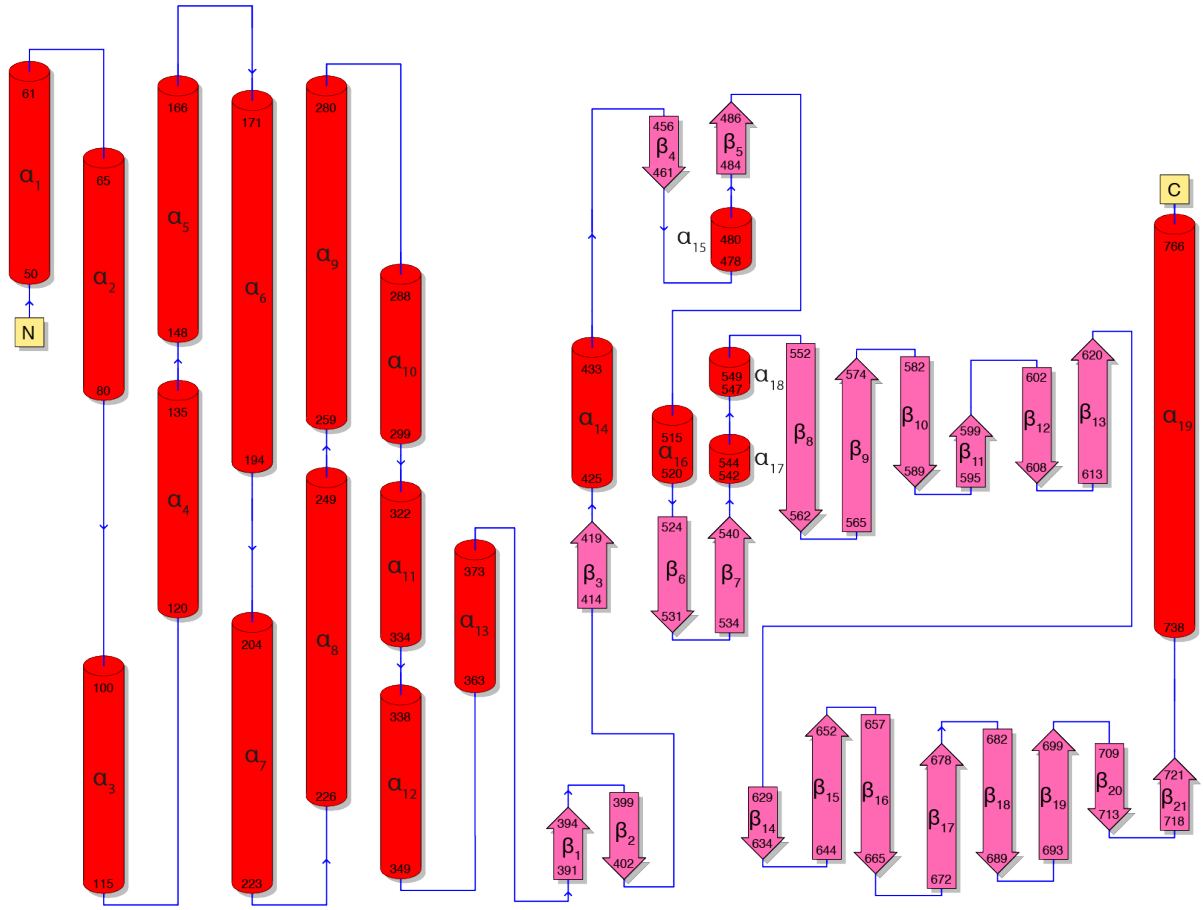

Fig. S2. Topology scheme of the crystal structure of DS-epi1. Alpha helices are shown as cylinders and labeled $\alpha_{1\text{-}20}$. Beta strands are shown as arrows and labeled $\beta_{1\text{-}21}$. Each corresponding starting and end amino acid is shown for each element and the N- and C-termini are marked with N and C. The scheme, based on HERA, was generated using PDBsum and further modified manually for clarity reasons.

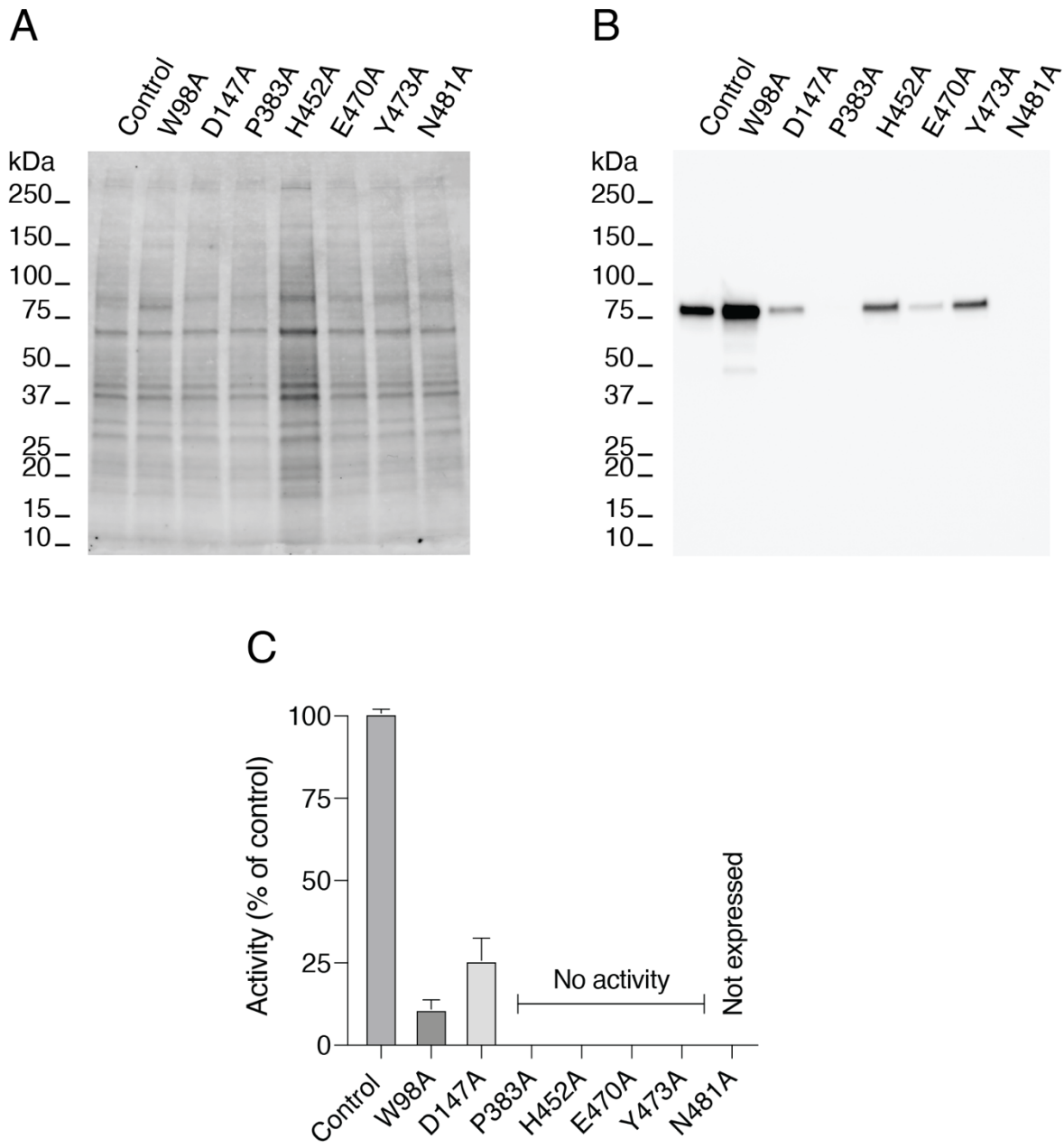

Fig. S3. Western blot and activity analysis of DS-epi1 23-775 point mutants. (A) Culture supernatants from HEK293 cells transfected with wild-type control plasmid or plasmids with point mutants were separated by SDS-PAGE and transferred to a PVDF membrane. Image shows total protein visualized using Bio-Rad stain-free imaging technology. (B) PVDF membrane probed with primary rabbit anti-DS-epi1 antibody and secondary anti-rabbit HRP conjugate, and developed using a chemiluminescent HRP substrate. (C) Activity analysis of DS-epi1 products using a C5-tritiated chondroitin substrate, followed by distillation and liquid scintillation counting. The activity of each product was normalized to the expression, as analyzed by western blot, and expressed as % of DS-epi1 23-775 wild-type control. The P383A product was barely visible after development of the western blot membrane, whereas the N481A product could not be detected. Each experiment was performed in duplicate and expressed as mean with standard deviation.

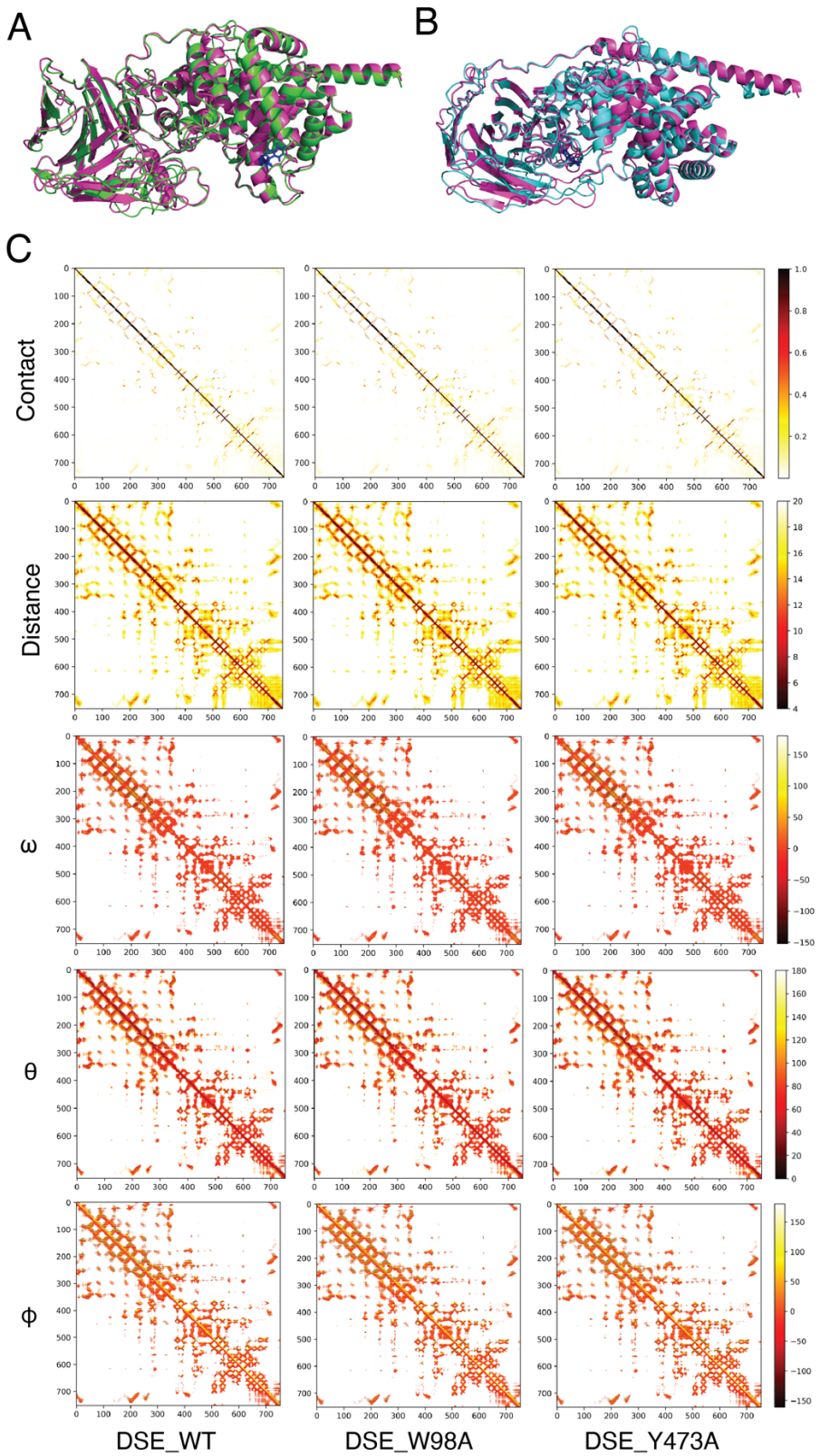

Fig. S4. DS-epi1 wild-type and mutant structure prediction using trRosetta (https://yanglab.nankai.edu.cn/trRosetta/). Structure predictions are based on a deep residual network for predicting inter-residue orientations and distances, followed by a Rosetta-constrained energy-minimization protocol to generate structure models. (A) Structural alignment between DS-epi1 WT (purple) and W98A mutant (green), with an RMSD of 0.43 Å (B) Structural alignment between DS-epi1 WT (purple) and Y473A mutant (turquoise), with an RMSD of 0.45 Å (C) Predicted inter-residue contacts/distances/orientations for DS-epi1 wild-type, W98A and Y473A. Residue number on x and y axis.

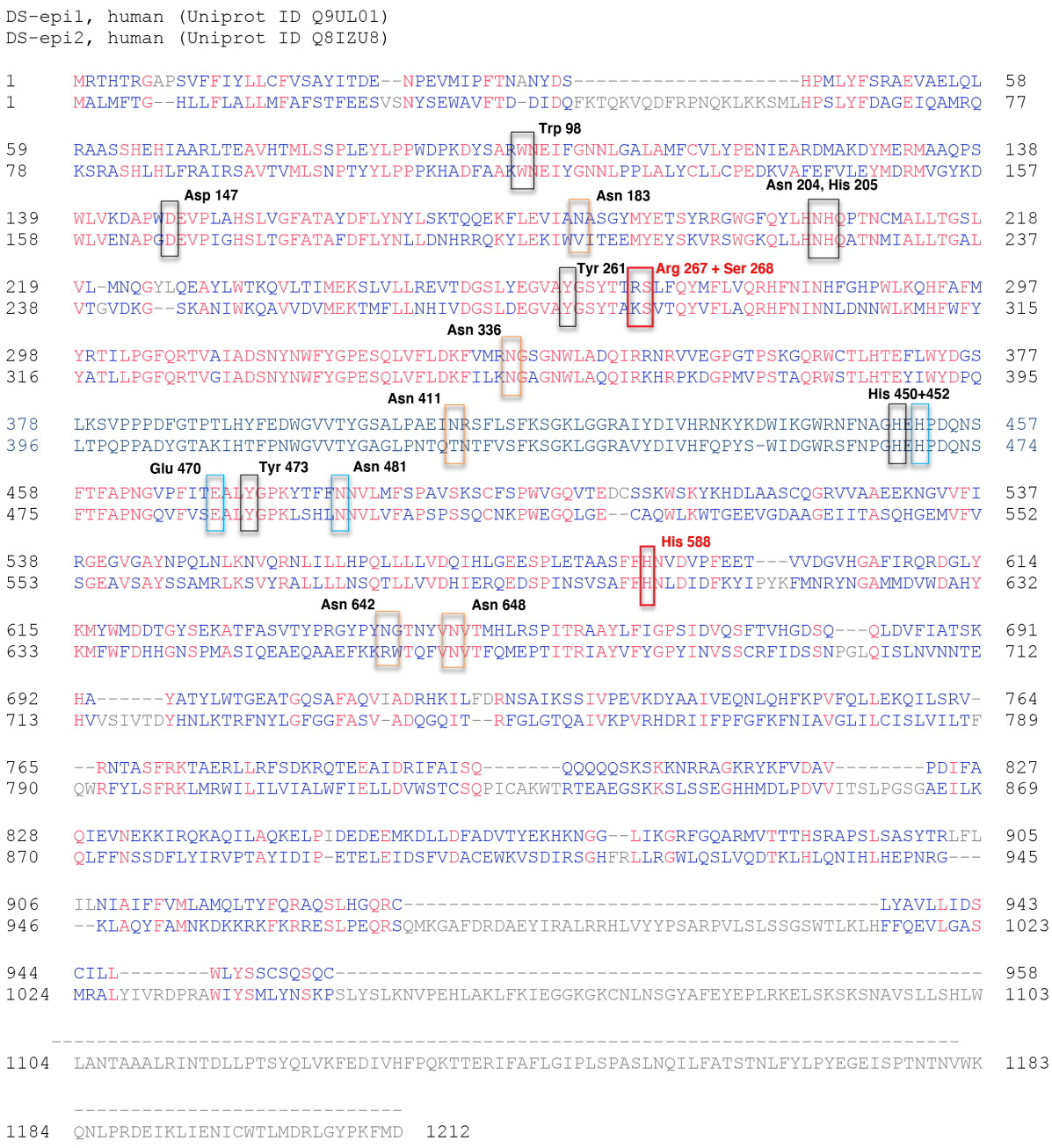

Fig. S5. Sequence alignment between human DS-epimerases. Sequences were aligned using COBALT Constraint-based Multiple Alignment Tool (https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi). Proposed active-site residues are marked with black rectangles, glycosylation sites are marked with brown rectangles, metal-coordinating sites are marked with blue rectangles and mutations linked to musculocontractural Ehlers-Danlos syndrome are marked with red rectangles.

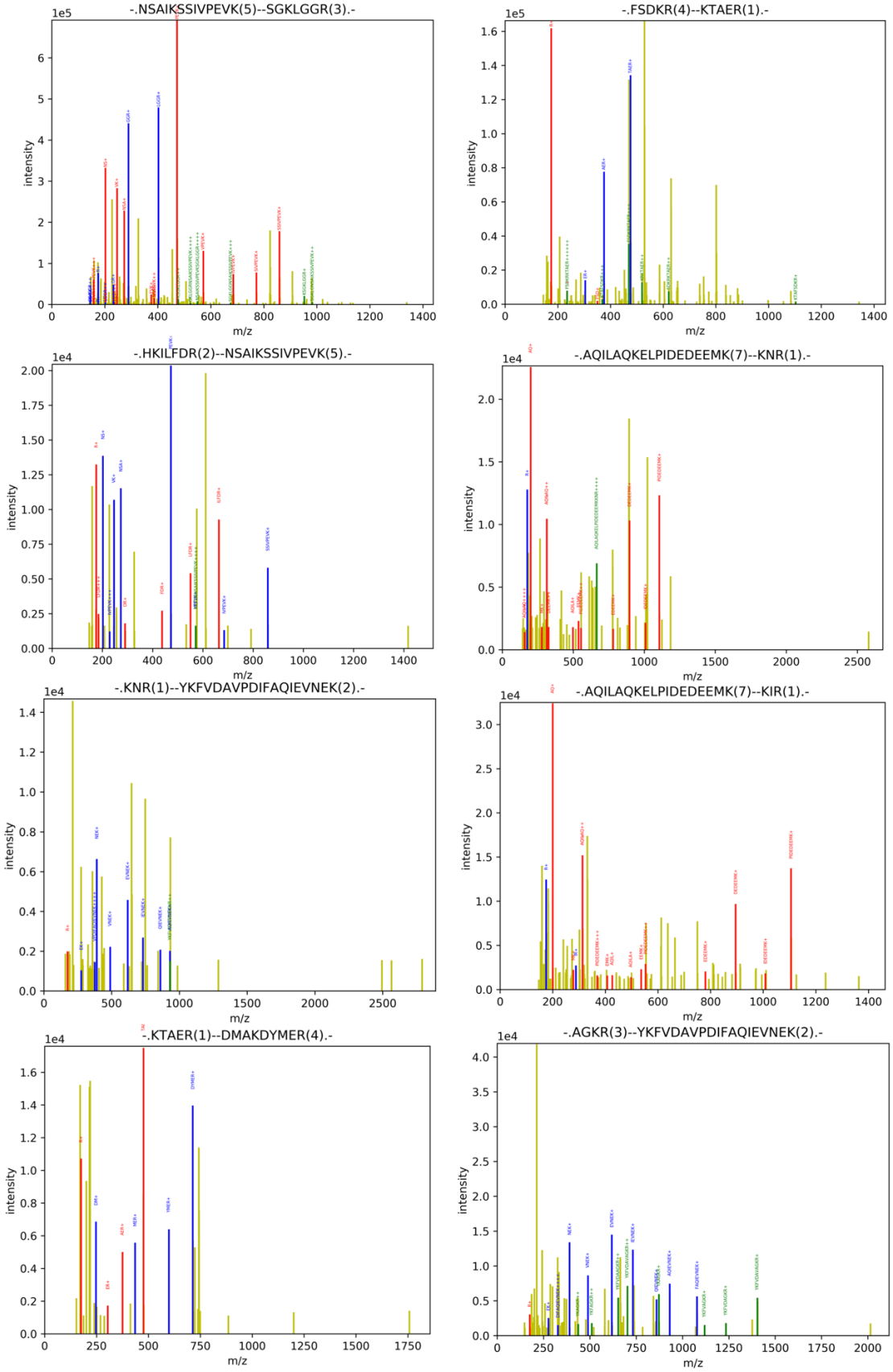

Fig. S6A. Supporting spectra for TX-MS table S2. In each spectrum figure, the title refers to the reference XL peptides. The yellow color shows the background spectrum which is found in the MS/MS sample. The red, blue, and green colors are corresponding to the first peptide fragments, second peptide fragments, and both peptides with cross-linking agent (DSS), respectively.

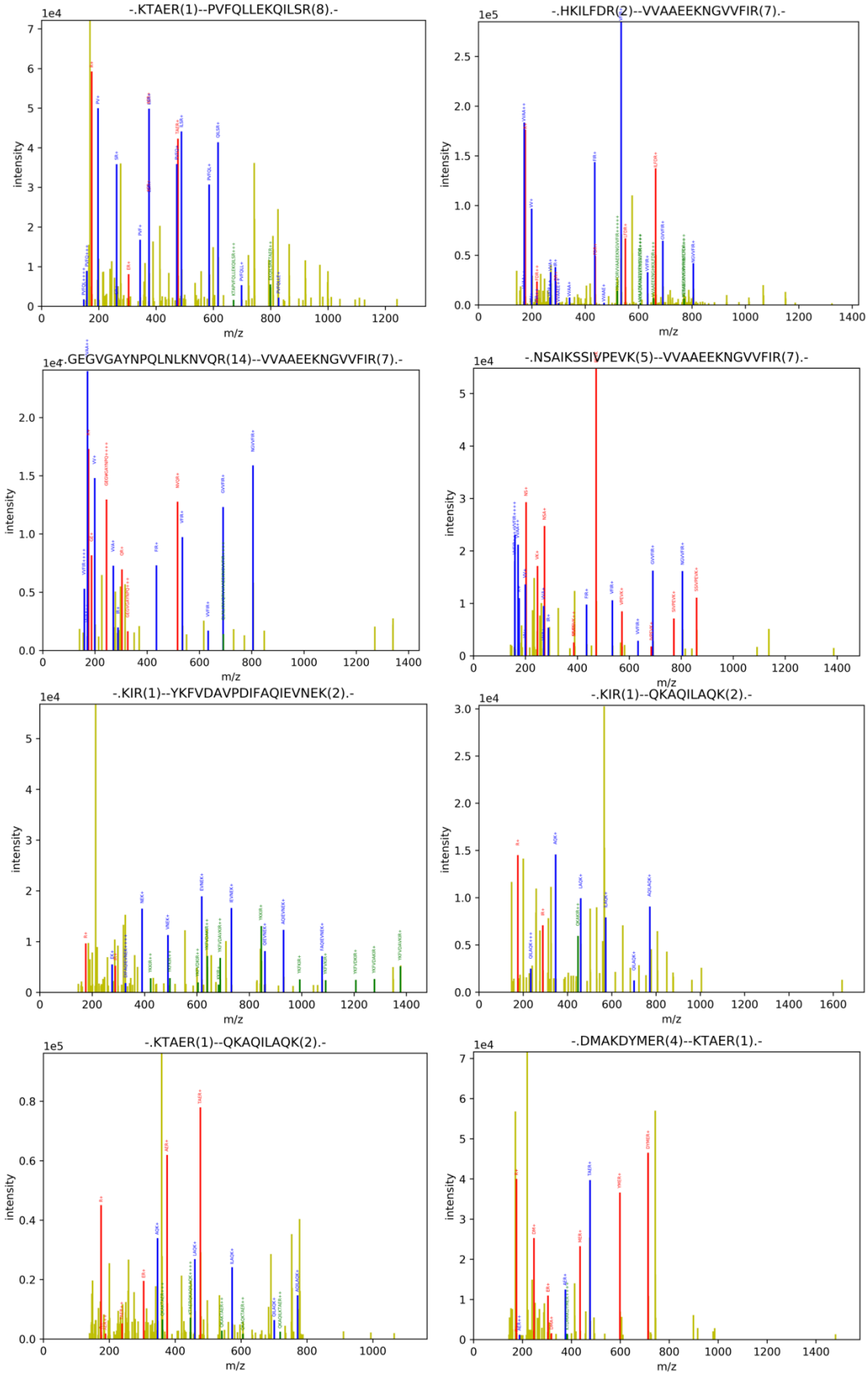

Fig. S6B. Supporting spectra for TX-MS table S2. In each spectrum figure, the title refers to the reference XL peptides. The yellow color shows the background spectrum which is found in the MS/MS sample. The red, blue, and green colors are corresponding to the first peptide fragments, second peptide fragments, and both peptides with cross-linking agent (DSS), respectively.

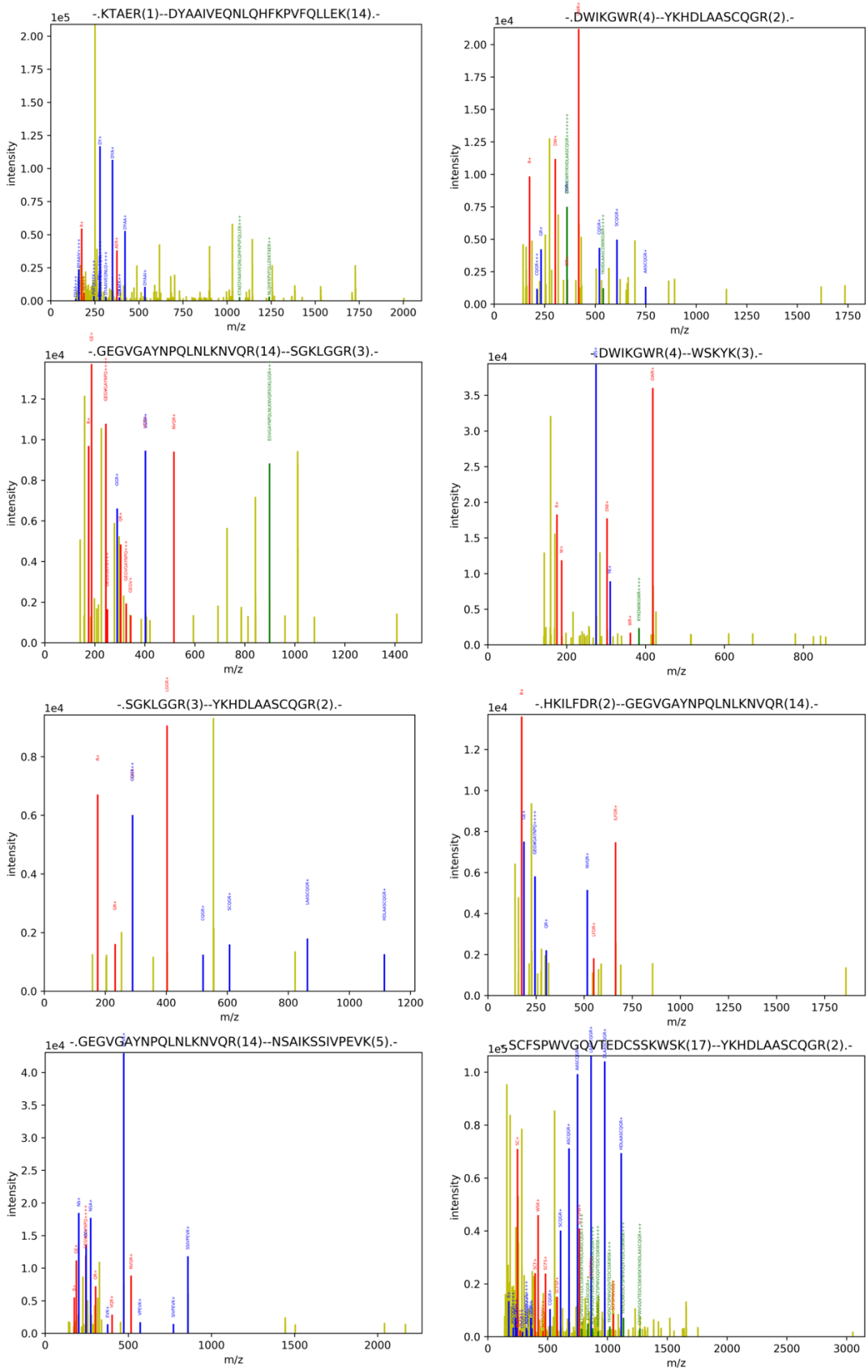

Fig. S6C. Supporting spectra for TX-MS table S2. In each spectrum figure, the title refers to the reference XL peptides. The yellow color shows the background spectrum which is found in the MS/MS sample. The red, blue, and green colors are corresponding to the first peptide fragments, second peptide fragments, and both peptides with cross-linking agent (DSS), respectively.

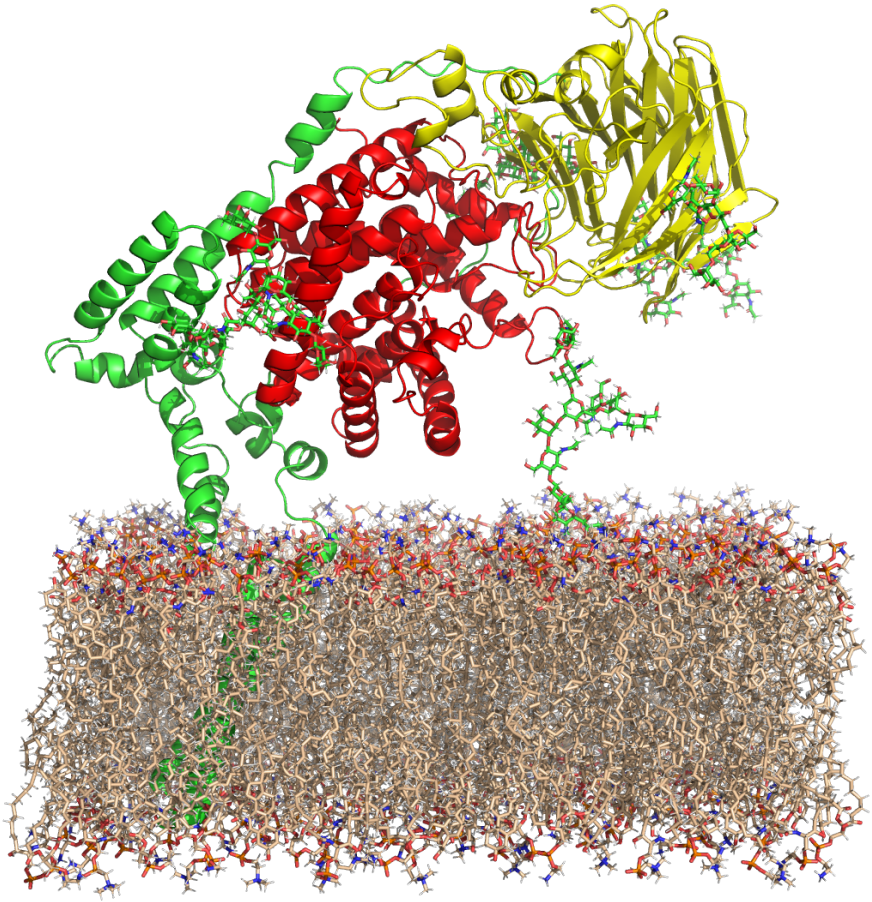

Figure S7. Model of full-length human DS-epi1. The model was constructed by glycan building, membrane construction/docking and energy minimization using the CHARMM web server (http://www.charmm-gui.org). The modeled glycans and C-terminal domain are shown in green.

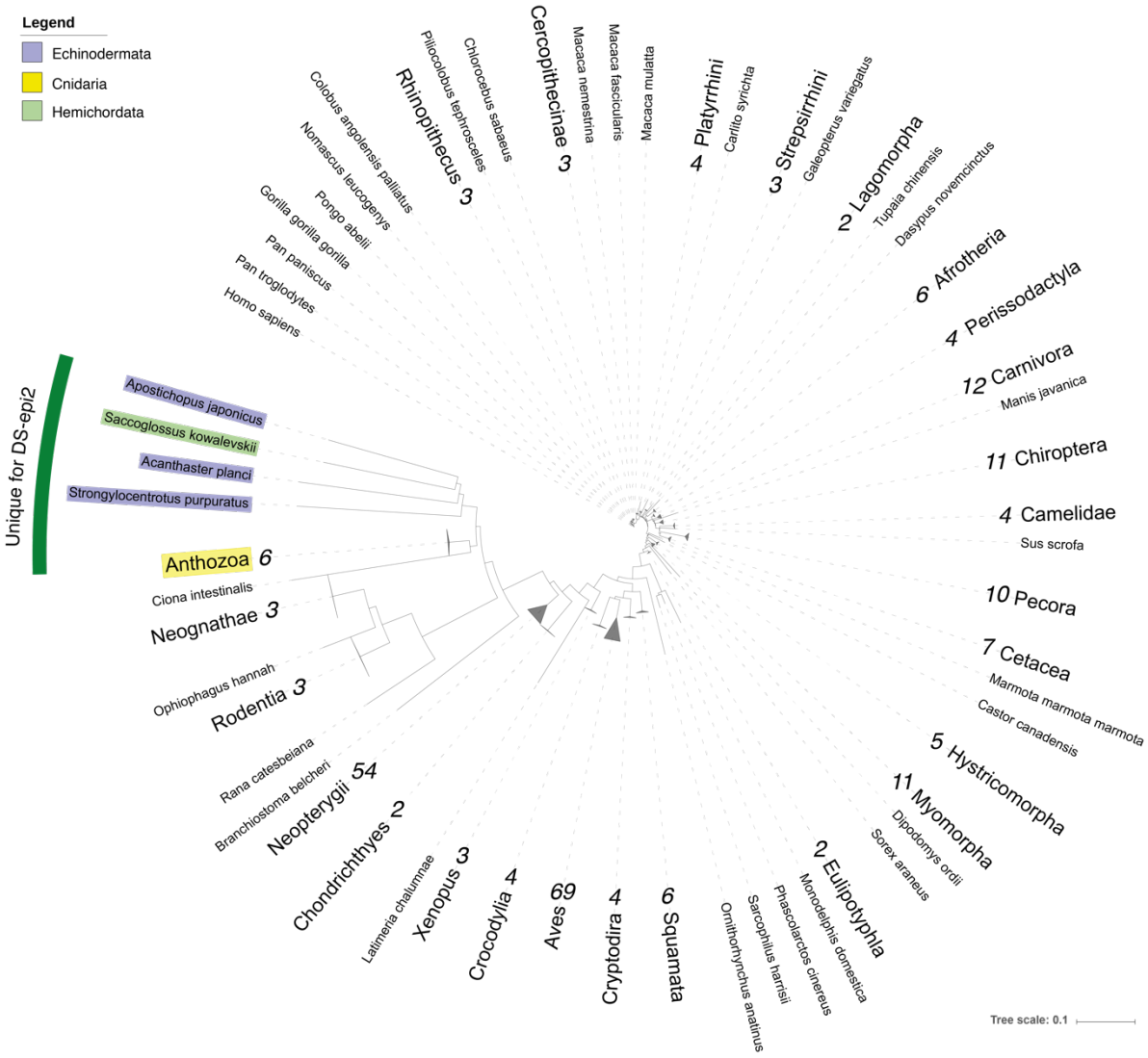

Fig. S8. Phylogenetic tree of species expressing DS-epi1 and/or DS-epi2, rooted in homo sapiens. All species identified were animals and the majority chordates. Non-chordates, all unique for DS-epi2, are colored according to the legend on the top left. Nodes with multiple species belonging to the same class, order or family were collapsed and the numbers adjacent to those nodes correspond to the number of species in each node. The isosceles triangle for each collapsed node is proportional to the number of species in the node.