

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 NH₄CH₃CO₂, 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 α_{1-20} . Beta strands are shown as arrows and labeled β_{1-21} . 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.

DS-ep	i2, human (Uniprot ID Q8IZU8)	
1 1	MRTHTRGAPSVFFIYLLCFVSAYITDENPEVMIPFTNANYDSHPMLYFSRAEVAELQL MALMFTGHLLFLALLMFAFSTFEESVSNYSEWAVFTD-DIDQFKTQKVQDFRPNQKLKKSMLHPSLYFDAGEIQAMRQ	58 77
59 78	Trp 98 RAASSHEHIAARLTEAVHTMLSSPLEYLPPWDPKDYSARWNEIFGNNLGALAMFCVLYPENIEARDMAKDYMERMAAQPS KSRASHLHLFRAIRSAVTVMLSNPTYYLPPPKHADFAAKWNEIYGNNLPPLALYCLLCPEDKVAFEFVLEYMDRMVGYKD Asn 204, His 205	138 157
139 158	Asn 183 WLVKDAPW DEVPLAHSLVGFATAYDFLYNYLSKTQQEKFLEVIANASGYMYETSYRRGWGFQYLHNHOPTNCMALLTGSL WLVENAPG DEVPIGHSLTGFATAFDFLYNLLDNHRRQKYLEKIWVITEEMYEYSKVRSWGKQLLHNHOATNMIALLTGAL	218 237
219 238	Tyr 261 Arg 267 + Ser 268 VL-MNQGYLQEAYLWTKQVLTIMEKSLVLLREVTDGSLYEGVAYGSYTTRSLFQYMFLVQRHFNINHFGHPWLKQHFAFM VTGVDKGSKANIWKQAVVDVMEKTMFLLNHIVDGSLDEGVAYGSYTAKSVTQYVFLAQRHFNINNLDNNWLKMHFWFY	297 315
298 316	YRTILPGFQRTVAIADSNYNWFYGPESQLVFLDKFILKNGAGNWLADQIRRNRVVEGPGTPSKGQRWCTLHTEFLWYDGS YATLLPGFQRTVGIADSNYNWFYGPESQLVFLDKFILKNGAGNWLAQQIRKHRPKDGPMVPSTAQRWSTLHTEYIWYDPQ Asn 411 His 450+452	377 395
378 396	LKSVPPPDFGTPTLHYFEDWGVVTYGSALPAEINRSFLSFKSGKLGGRAIYDIVHRNKYKDWIKGWRNFNAGHEHPDQNS LTPQPPADYGTAKIHTFPNWGVVTYGAGLPNTOTNTFVSFKSGKLGGRAVYDIVHFQPYS-WIDGWRSFNPGHEHPDQNS Glu 470 Tvr 473 Asn 481	457 474
458 475	FTFAPNGVPFITEALYSPKYTFFNNVLMFSPAVSKSCFSPWVGQVTEDCSSKWSKYKHDLAASCQGRVVAAEEKNGVVFI FTFAPNGQVFVSEALYSPKLSHLNNVLVFAPSPSSQCNKPWEGQLGECAQWLKWTGEEVGDAAGEIITASQHGEMVFV	537 552
538 553	RGEGVGAYNPQLNLKNVQRNLILLHPQLLLLVDQIHLGEESPLETAASFFHNVDVPFEETVVDGVHGAFIRQRDGLY SGEAVSAYSSAMRLKSVYRALLLLNSQTLLVVDHIERQEDSPINSVSAFFHNLDIDFKYIPYKFMNRYNGAMMDVWDAHY Asn 642 Asn 648	614 632
615 633	KMYWMDDTGYSEKATFASVTYPRGYPYNGTNYVNYTMHLRSPITRAAYLFIGPSIDVQSFTVHGDSQQLDVFIATSK KMFWFDHHGNSPMASIQEAEQAAEFKKRWTQFYNYTFQMEPTITRIAYVFYGPYINVSSCRFIDSSNPGLQISLNVNNTE	691 712
692 713	HAYATYLWTGEATGQSAFAQVIADRHKILFDRNSAIKSSIVPEVKDYAAIVEQNLQHFKPVFQLLEKQILSRV- HVVSIVTDYHNLKTRFNYLGFGGFASV-ADQGQITRFGLGTQAIVKPVRHDRIIFPFGFKFNIAVGLILCISLVILTF	764 789
765 790	RNTASFRKTAERLLRFSDKRQTEEAIDRIFAISQQQQQQQSKSKKNRRAGKRYKFVDAVPDIFA QWRFYLSFRKLMRWILILVIALWFIELLDVWSTCSQPICAKWTRTEAEGSKKSLSSEGHHMDLPDVVITSLPGSGAEILK	827 869
828 870	QIEVNEKKIRQKAQILAQKELPIDEDEEMKDLLDFADVTYEKHKNGGLIKGRFGQARMVTTTHSRAPSLSASYTRLFL QLFFNSSDFLYIRVPTAYIDIP-ETELEIDSFVDACEWKVSDIRSGHFRLLRGWLQSLVQDTKLHLQNIHLHEPNRG	905 945
906 946	ILNIAIFFVMLAMQLTYFQRAQSLHGQRCLYAVLLIDS KLAQYFAMNKDKKRKFKRRESLPEQRSQMKGAFDRDAEYIRALRRHLVYYPSARPVLSLSSGSWTLKLHFFQEVLGAS	943 1023
944 1024	CILLWLYSSCSQSQC MRALYIVRDPRAWIYSMLYNSKPSLYSLKNVPEHLAKLFKIEGGKGKCNLNSGYAFEYEPLRKELSKSKSNAVSLLSHLW	958 1103
1104	LANTAAALRINTDLLPTSYQLVKFEDIVHFPQKTTERIFAFLGIPLSPASLNQILFATSTNLFYLPYEGEISPTNTNVWK	1183
1184	ONLPRDEIKLIENICWTLMDRLGYPKFMD 1212	

DS-epil, human (Uniprot ID Q9UL01)

Fig. S5. Sequence alignment between human DS-epimerases. Sequences were aligned using COBALT Constraint-based Multiple Alignment Tool (<u>https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi</u>). 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.