Supplemental Data

Oligomerization and catalytic parameters of human UDP-glucuronosyltransferase 1A10: Expression and characterization of the recombinant protein

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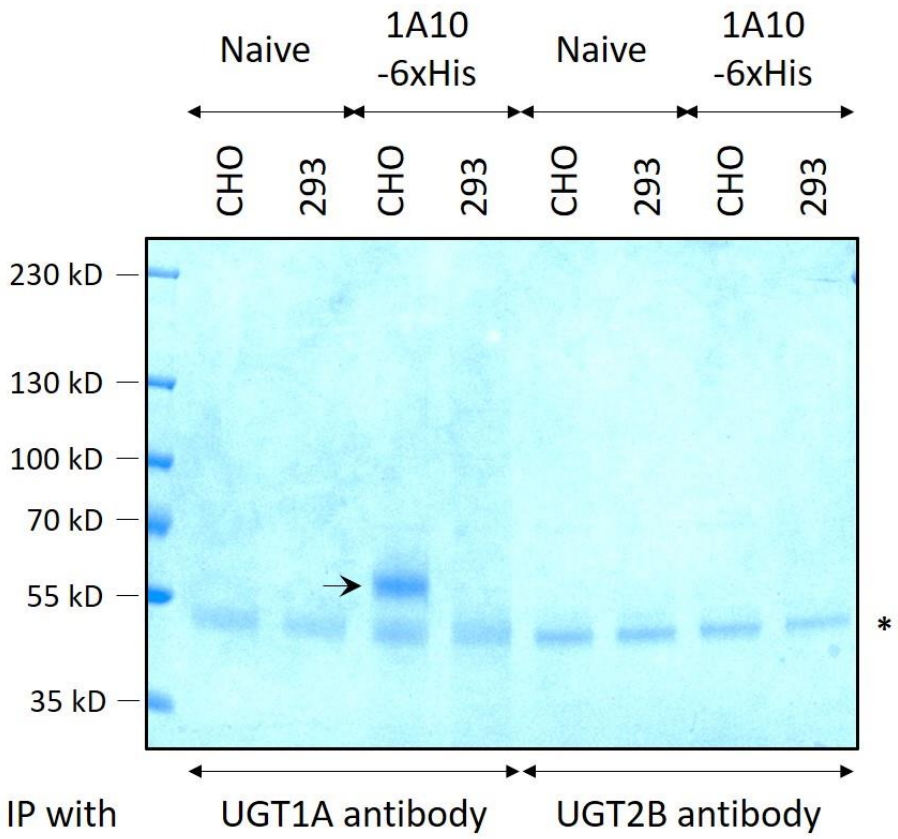
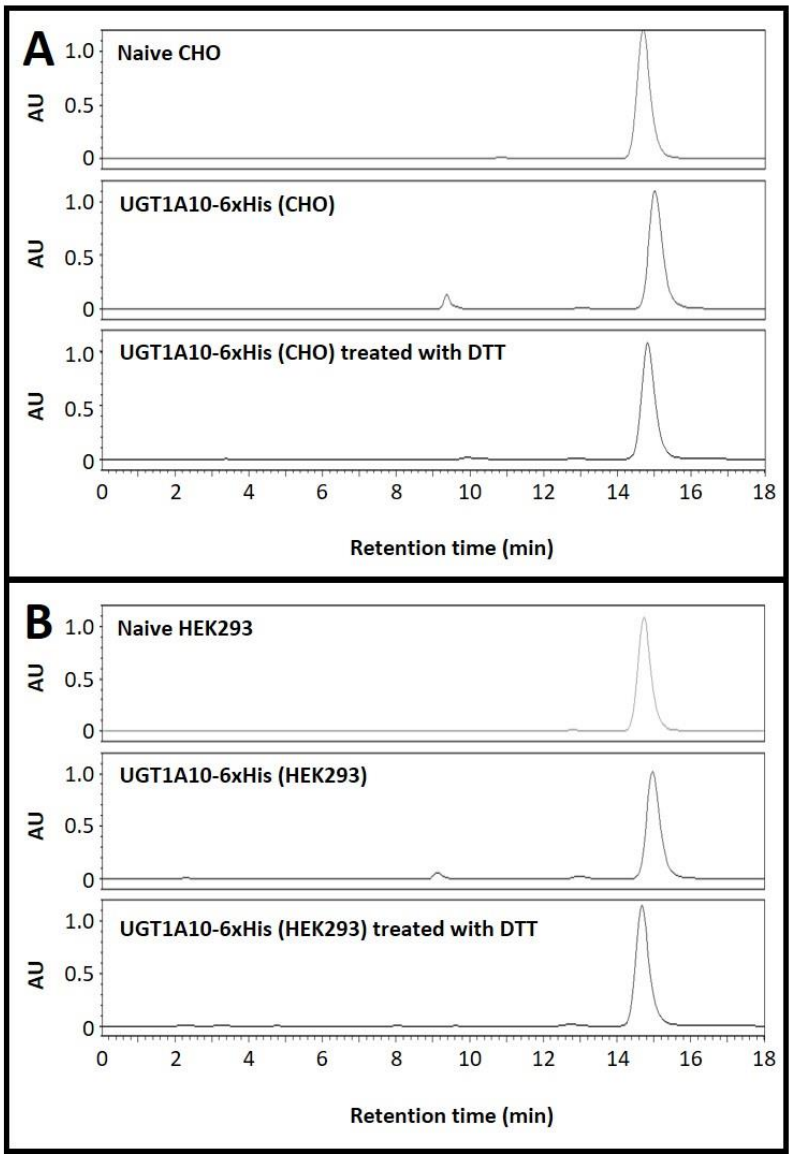
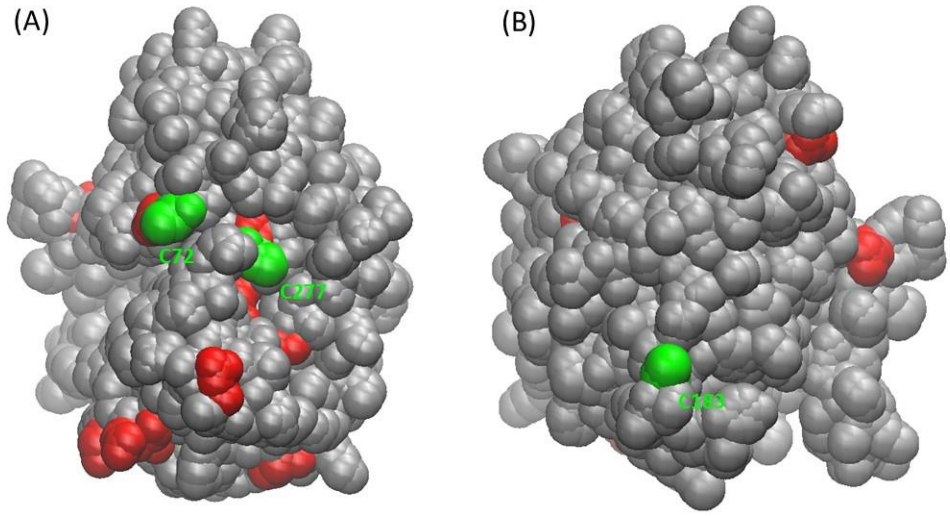


Supplemental Figure 1. Immunoprecipitation assay for UGT1A10-6xHis. Membrane-bound UGT1A10-6xHis protein was first extracted with 1.5% Triton X-100, followed by anti-UGT1A immunoprecipitation. Precipitated UGT1A10-6xHis was revealed by coomassie blue staining (see arrow). Asterisk marks the heavy chain of the immunoglobulin and this was used as an internal loading control.



Supplemental Figure 2. HPLC analysis of the formation of entacapone 3-*O*-glucuronide using microsomes prepared from UGT1A10-6xHis-overexpressing stable cell lines. Retention time was found to be about 9.4 min and 15 min for entacapone 3-*O*-glucuronide and entacapone. (A) Top, microsomal proteins prepared from naïve CHO cells; middle, membrane-bound UGT1A10-6xHis expressed in CHO cells; bottom, membrane-bound UGT1A10-6xHis expressed in CHO cells was incubated with 100 mM DTT for 1 h prior to glucuronidation activity assay. (B) Top, microsomal proteins prepared from naïve HEK293 cells; middle, membrane-bound UGT1A10-6xHis expressed in HEK293 cells; bottom, membrane-bound UGT1A10-6xHis expressed in HEK293 cells was incubated with 100 mM DTT for 1 h prior to glucuronidation activity assay.



Supplemental Figure 3. The modeled van der Waals surface of the UGT1A10 protein structure, showing three solvent-accessible cysteine residues (C72, C183, and C277 in green color). Panels A and B show two sides of the protein, with C72 and C277 on one side and C183 on the opposite side. Indicated in red color are solvent-accessible asparagine residues (potential glycosylation sites). C72 and C277 are close to the entrance of the enzyme active-site pocket. C72 or C277 of a UGT1A10 molecule may form a disulfide bond with C183 or C72 or C277 of another UGT1A10 molecule for crosslinking *via* intermolecular disulfide bonds. The crosslinking disulfide bonds involving C72 or C277 are expected to block the entrance of the enzyme active-site pocket and, thus, decrease the catalytic activity of the enzyme.