

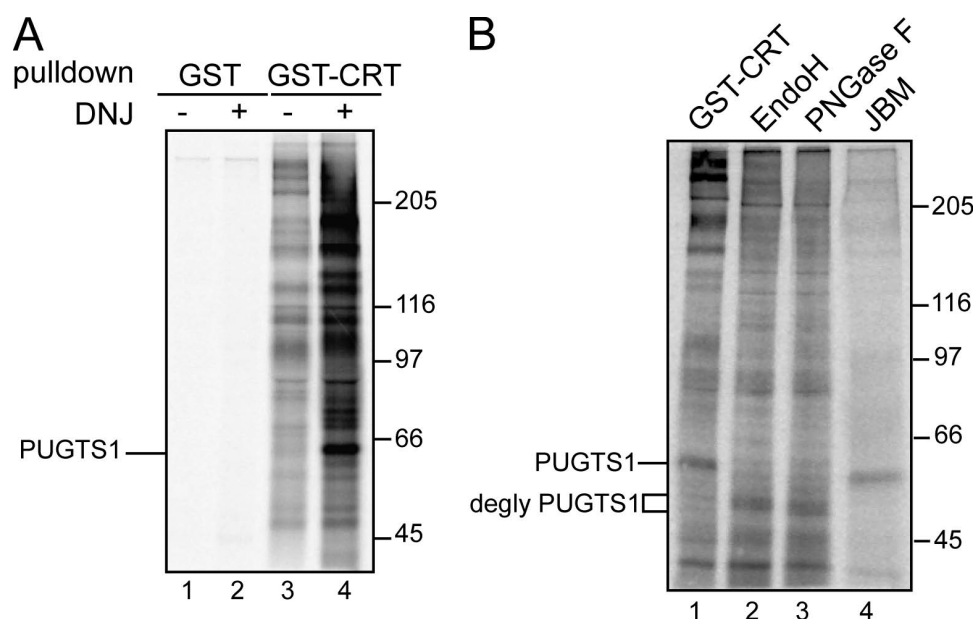
Pearse et al., <http://www.jcb.org/cgi/content/full/jcb.200912105/DC1>

Figure S1. **Identification of a putative UGT1 substrate.** (A) MI8-5 CHO cells were pulse labeled for 2 h in the presence or absence of DNJ. Samples were subjected to either GST or GST-calreticulin (CRT) pull-down. Putative UGT1 substrate 1 (PUGTS1), an ~60-kD protein, is indicated. Samples were resolved via 7.5% SDS-PAGE. (B) MI8-5 cells were pulse labeled for 30 min and chased for 30 min in the presence of DNJ. After GST-calreticulin pull-down, samples were treated with either endoglycosidase H (EndoH), peptide:N-glycanase F (PNGase F), or jack bean  $\alpha$ -mannosidase (JBM). (A and B) Molecular mass is indicated in kilodaltons.

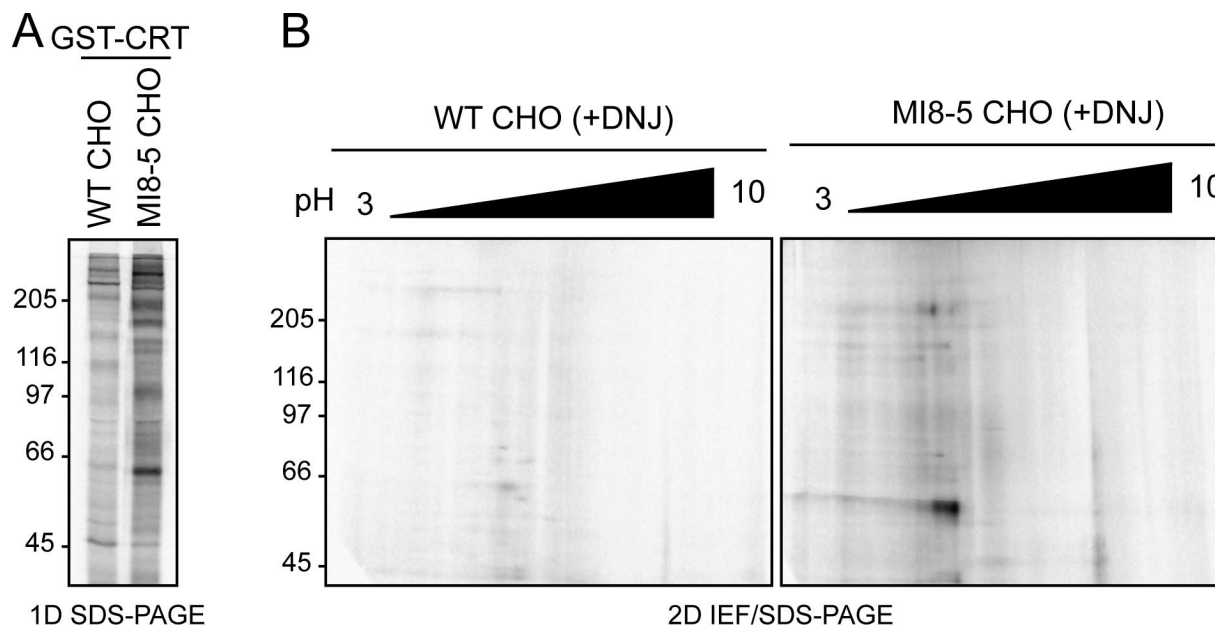


Figure S2. **Biochemical characterization of a putative UGT1 substrate.** (A) MI8-5 cells were pulse labeled for 60 min and chased for an additional 15 min in the presence of DNJ. After cell lysis, UGT1 substrates were isolated using the GST-calreticulin (CRT) pull-down assay and resolved by SDS-PAGE. (B) Samples were also resolved by isoelectric focusing (IEF) on pH 3–10 immobilized pH gradient strips. Immobilized pH gradient strips were then subjected to 10% SDS-PAGE. (A and B) Molecular mass is indicated in kilodaltons. WT, wild type.

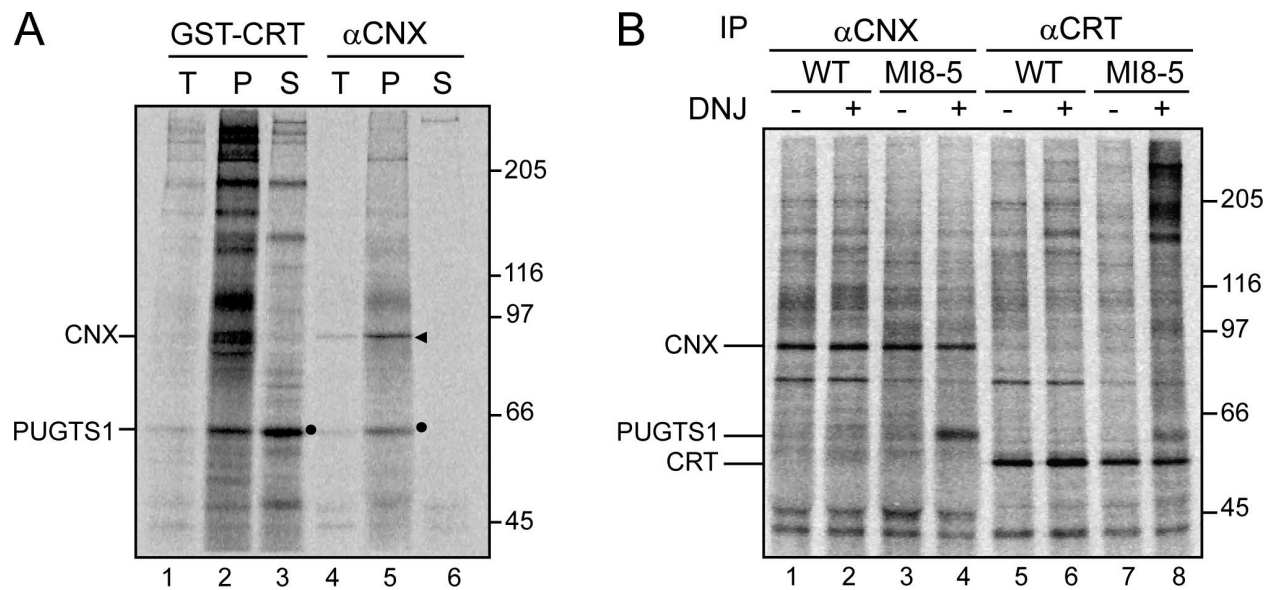


Figure S3. **Analysis of the solubility and chaperone binding for the UGT1 substrate.** (A) MI8-5 cells were pulse labeled for 30 min and chased for 30 min in the presence of DNJ. Cells were alkaline extracted and centrifuged to separate membrane (pellet [P]) and soluble (S) proteins. Samples were then subjected to the GST-calreticulin (CRT) pull-down assay or anti-calnexin (CNX) immunoprecipitation. The closed circles and the closed triangle represent the putative UGT1 substrate and calnexin, respectively. T, total fraction. (B) Wild-type (WT) and MI8-5 CHO cells were pulse labeled for 1 h in the presence or absence of DNJ. Endogenous radiolabeled substrates were isolated with calnexin and calreticulin antisera. PUGTS1, calnexin, and calreticulin are indicated. IP, immunoprecipitation. (A and B) Molecular mass is indicated in kilodaltons.

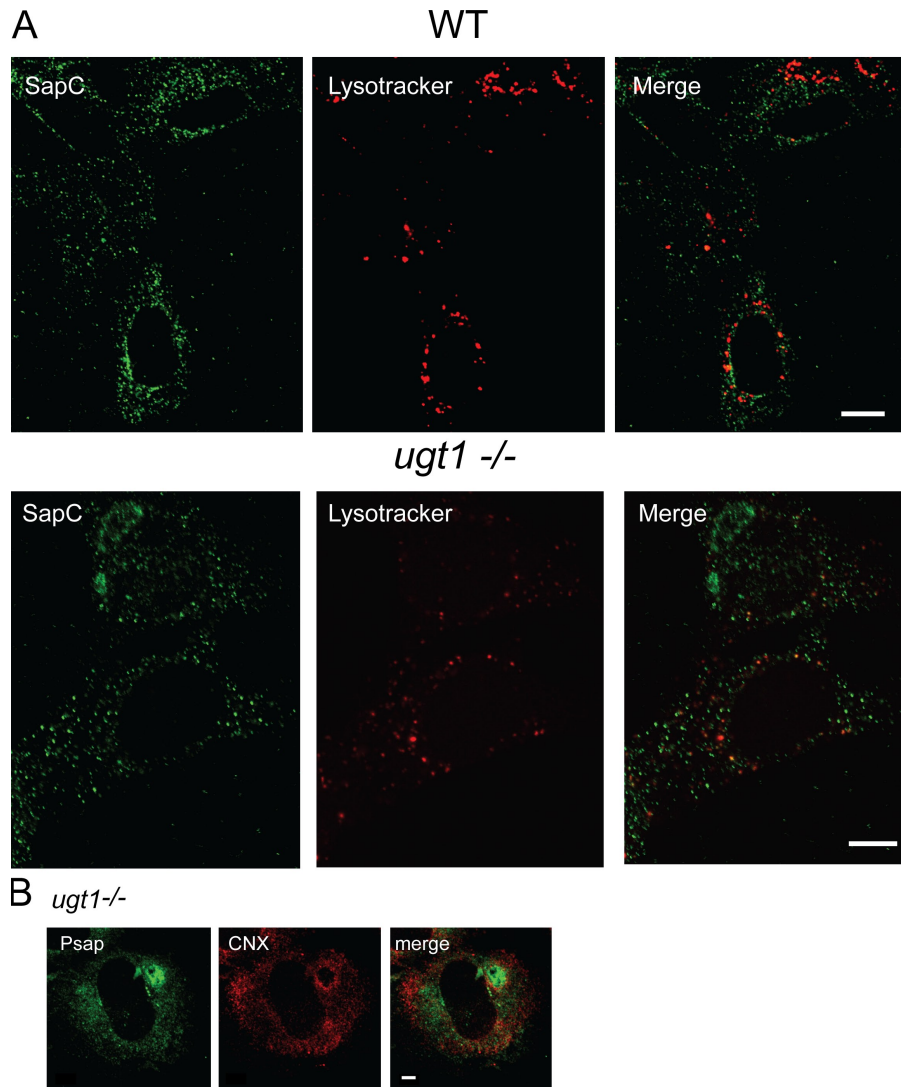


Figure S4. **Partial colocalization of prosaposin with lysosomes and the aggresome-like structures do not colocalize with calnexin.** (A) Wild-type (WT) and *ugt1*<sup>-/-</sup> cells were treated with 200 nM LysoTracker (Invitrogen) for 1 h at 37°C before fixation. Cells were immunostained with antiprosaposin antibodies as described in Materials and methods, and confocal images were obtained. SapC, saposin C. (B) Prosaposin (Psap) localization in *ugt1*<sup>-/-</sup> cells was determined by confocal immunofluorescence microscopy. Samples were double labeled with prosaposin and calnexin (CNX) antisera. Bars, 10 µm.

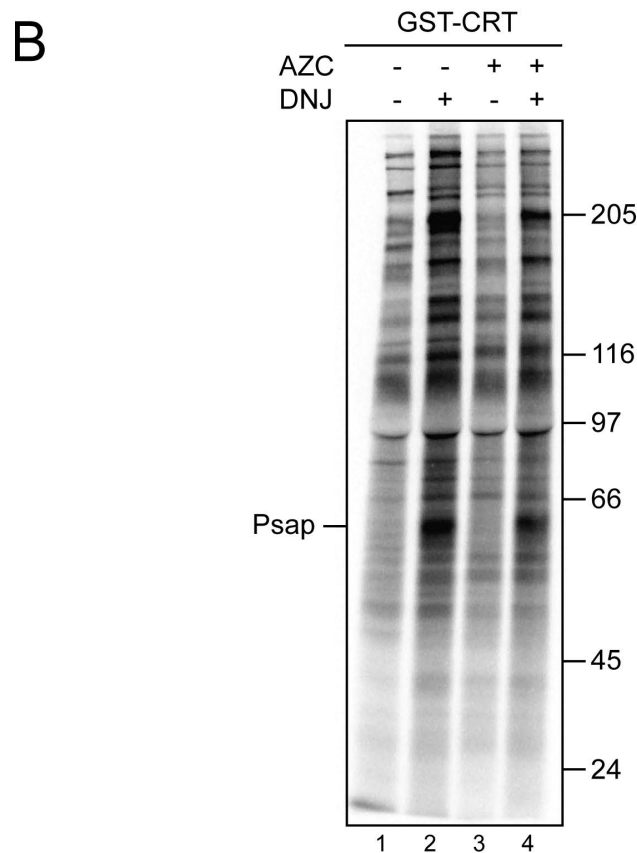
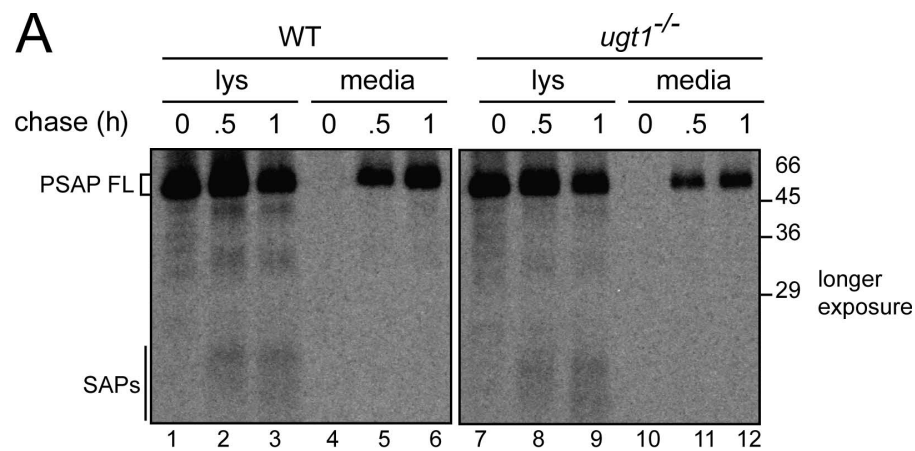


Figure S5. **Partial prosaposin processing to saposin and AZC does not increase the reglucosylation of glycoproteins.** (A) A longer exposure of the autorad for results presented in Fig. 3 A is depicted to allow for more definitive exposure of the smaller molecular mass saposin bands (SAPs). For details see Fig. 3 A. WT, wild type. (B) MI8-5 cells were starved for 1 h, followed by 2-h radiolabeling in [<sup>35</sup>S]Met/Cys. 20 mM AZC and 1 mM DNJ were included in the starvation and pulse media where indicated. Reglucosylated or monoglucosylated proteins were affinity purified with GST-calreticulin (CRT) and resolved by 7.5% reducing SDS-PAGE. (A and B) Molecular mass is indicated in kilodaltons. FL, full length; Psap, prosaposin.