SUPP. MATERIALS AND METHODS

DNA constructs

Human *GNPTG*-FLAG (NM_032520.3) in pcDNA3.1(+) (Qian, et al., 2015) was modified by QuikChange site-directed mutagenesis to generate the variants p.G106S, p.G126S, p.C142Y and p.T286M. See Supp. Table S1 for the primer sequences. All mutations were confirmed by Sanger sequencing. Human *GNPTAB*-V5/His in pcDNA6 is described in Qian, et al., 2013.

Cell lines

GNPTG$^{-/-}$ HeLa cells were generated by CRISPR/Cas9-induced targeted double strand break as described (van Meel, et al., 2016). *GNPTG*$^{-/-}$ HeLa cells, control HeLa cells and HEK293 cells were maintained in DMEM (Mediatech) containing 110 mg/L sodium pyruvate and 4.5 g/L glucose, supplemented with 10% (vol/vol) FBS (Atlanta Biologicals), 100,000 U/L penicillin, 100 mg/L streptomycin (Life Technologies) and 2 mM L-glutamine (Mediatech).

Immunofluorescence microscopy

HeLa cells were transfected with WT or mutant human *GNPTG*-FLAG in pcDNA3.1(+) and human *GNPTAB*-V5/His in pcDNA6 in a 1 to 8 ratio using Lipofectamine® 3000 (Life Technologies), according to the manufacturer's protocol. The cells were fixed and stained for confocal immunofluorescence microscopy ~48h later (Qian, et al., 2013). The γ subunit was detected with antiserum generated to human γ GlcNAc-1-phosphotransferase (kind gift from Dr. T. Braulke), dilution 1:200; the αβ subunits with anti-V5 (Life Technologies/Novex), dilution 1:1000; mouse anti-GM130 (1:500) was from BD Transduction Laboratories and mouse anti-calnexin (1:150) from Millipore.

Analysis of N-linked glycans and Western blotting

HEK293 cells were transfected with the αβ and γ subunits of GlcNAc-1-phosphotransferase as described above. Approximately 48h after transfection the cells were lysed in 1% Triton X-100/PBS containing a protease inhibitor cocktail (Complete, Roche Diagnostics). To analyze the *N*-linked glycans, 20 μg of total protein in the cell lysates was treated for 3h at 37°C with neuraminidase, endoglycosidase H_f or N-glycosidase F as described (van Meel, et al., 2014). In all Western blots, 15-30 μg cell lysate in Laemmli sample buffer (with or without 0.1 M dithiothreitol (Sigma)) was subjected to SDS-PAGE and immunoblotting (van Meel, et al., 2014). The γ subunit was detected with mouse anti-FLAG M2 antibody (Stratagene), the αβ subunits with mouse anti-V5 (Life Technologies) and rabbit anti-α-tubulin (Abcam) antibody was used to check equal protein loading.

Rescue of *GNPTG*$^{-/-}$ HeLa cells

GNPTG$^{-/-}$ HeLa cells were transferred to 6-well plates and transfected with 100-500 ng DNA at ~60% confluency as described. The intracellular activities of a panel of lysosomal acid glycosidases were determined 3 days later using 1 mM 4-methylumbelliferyl-conjugated specific substrates (Sigma) in 50 mM sodium citrate buffer containing 0.5% Triton X-100 (pH 4.6) as described (Lee, et al., 2007). The enzyme activities were normalized to the total protein concentration of the cell lysates. In the case of the WT γ subunit, transfection with 100 ng DNA resulted in a maximum increase in intracellular lysosomal enzyme activities. The mutants were expressed at similar or higher levels as confirmed by Western blotting.

Supp. Table S1. Primer sequences used in QuikChange site-directed mutagenesis

Variant	Forward primer (5' → 3')	Reverse primer (5' → 3')
c.316G>A (p.G106S)	TAC AGT GGG ATC CTC AGC ATC TGG CAC GAG TGG G	CCC ACT CGT GCC AGA TGC TGA GGA TCC CAC TGT A
c.376G>A (p.G126S)	CCT TCA CGG GCA TGT GGA TGA GGG ACA GTG ACG CCT GCC GTT CCC GGA GCC GGC	GCC GGC TCC GGG AAC GGC AGG CGT CAC TGT CCC TCA TCC ACA TGC CCG TGA ACC
c.425G>A (p.C142Y)	GCA AGG TGG AGC TGG CGT ATG GAA AAA GCA ACC G	CGG TTG CTT TTT CCA TAC GCC AGC TCC ACC TTG C
c.857C>T (p.T286M)	CAC TTG GGC CAC GAG ATG CCC AGA GCC AAG TC	GAC TTG GCT CTG GGC ATC TCG TGG CCC AAG TG

Note that the nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.