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Supplemental Information

Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy

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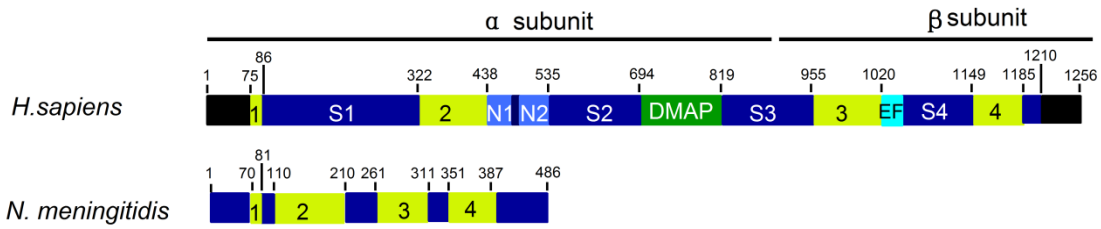
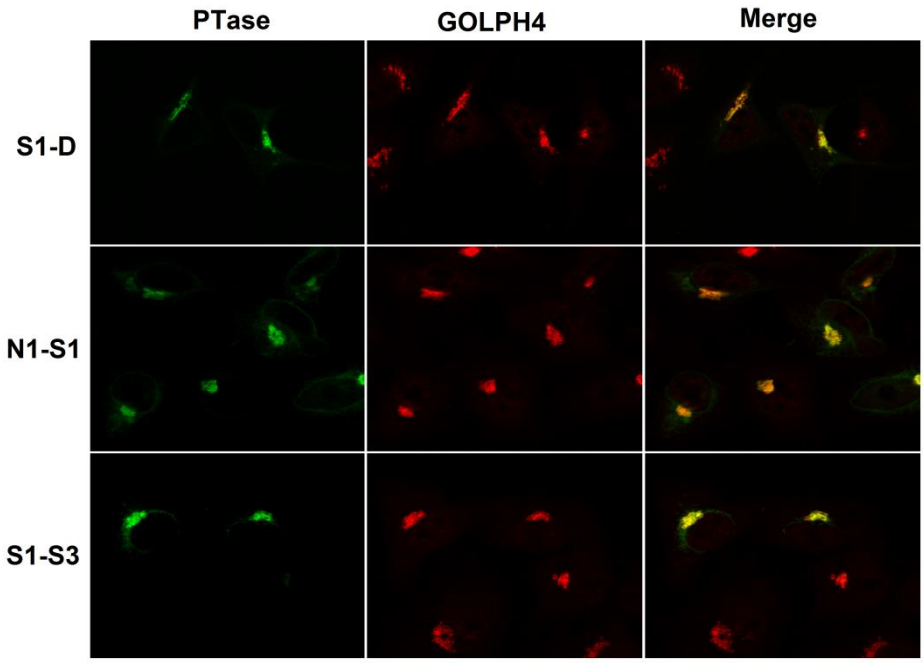
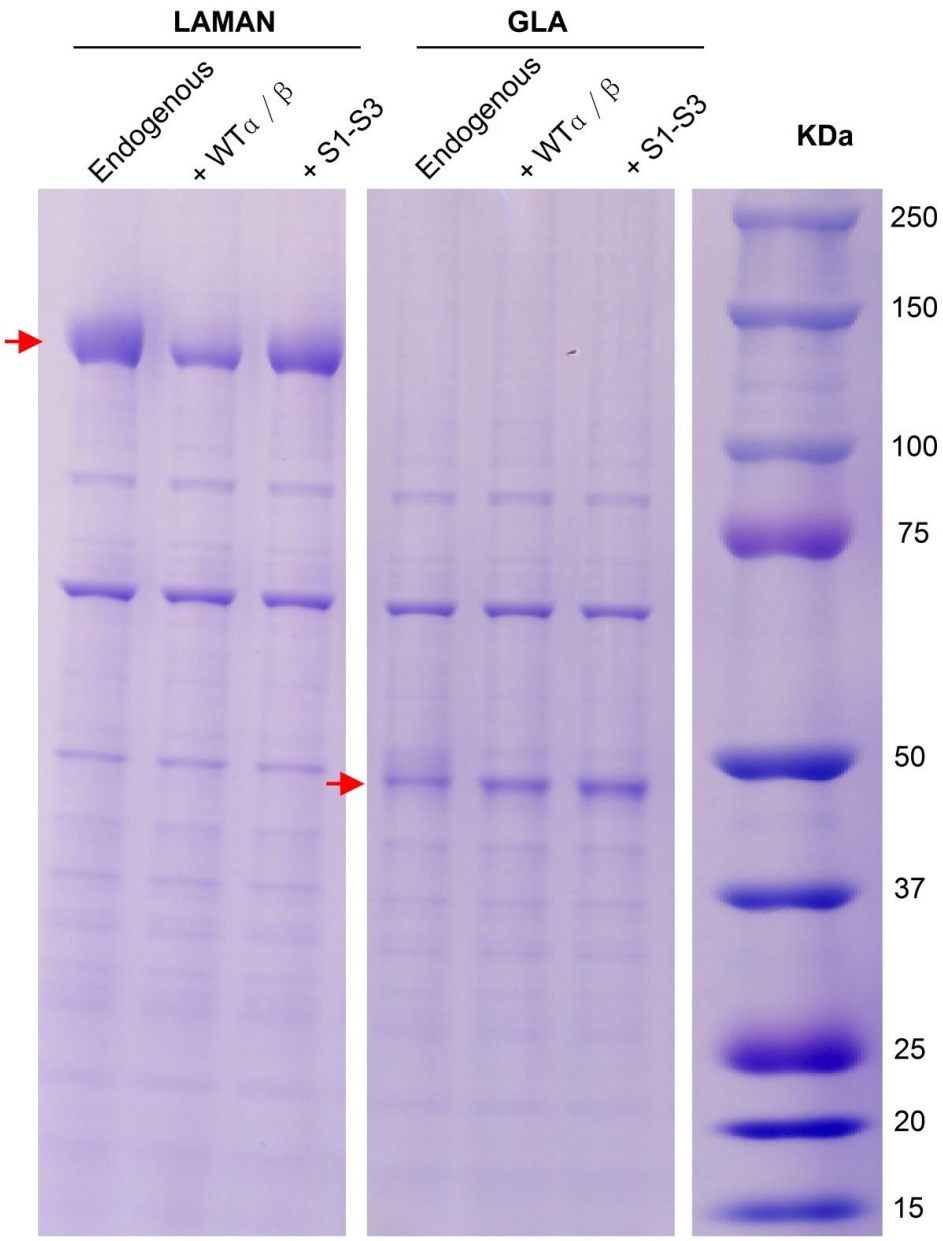


Supplementary Figure S1. **Organization of human GlcNAc-1-phosphotransferase domain structure.** Modular organization of the different domains of human GlcNAc-1-phosphotransferase α/β precursor and alignment with the *N. meningitidis* GlcNAc-1-phosphotransferase. See legend of Figure 1 for description of the various domains.



Supplementary Figure S2. **Golgi localization of α/β precursor mutants.** Confocal immunofluorescence images of GNPTAB$^{-/-}$ HeLa cells transfected with the indicated mutant cDNAs, and colocalized with the Golgi markers GOLPH4, respectively (see Materials & Methods).



Supplementary Figure S3. **Enzyme production by Expi293 cells.** Media was collected from Expi293 cells transfected with either LAMAN or GLA cDNA, or co-transfected with the cDNA for the lysosomal enzyme together with the cDNA for WT α/β precursor or the S1-S3 mutant. 10 ul media were resolved by SDS-PAGE and the gel stained with Coomassie Brilliant Blue R-250. Red arrows indicate the migration of the secreted lysosomal enzyme.