

SUPPLEMENTAL DATA

Fig. S1. Colocalization of NPC1L1 variants with RFP-Rab11a. The EGFP-tagged wild-type or indicated variants of NPC1L1 were co-expressed with RFP-Rab11a in CRL-1601 cells, respectively. 24 hr after transfection, cells were fixed and examined by confocal microscopy. Scale bar=10 μ m.

Fig. S2. Colocalization of NPC1L1 variants with EHD1-DsRed. The EGFP-tagged wild-type or different variants of NPC1L1 were co-transfected with EHD1-DsRed into CRL-1601 cells, respectively. 24 hr after transfection, cells were fixed and examined by confocal microscopy. Scale bar=10 μ m.

Fig. S3. Colocalization of NPC1L1 variants with Calnexin. 24 hr after transfection of indicated NPC1L1 variants, cells were fixed, stained with anti-Calnexin antibody, and imaged with confocal microscopy. Scale bar=10 μ m.

Fig. S4. The different glycosylation forms of NPC1L1. Expression of EGFP-tagged wild-type NPC1L1 in CRL-1601 cell showed three different glycosylated forms: mature glycosylated form, core-glycosylated form and unglycosylated form. By glycosidase digestion with Endo H, the core-glycosylated form was converted to unglycosylated form (Endo H sensitive band), while the mature glycosylated protein remained unchanged (Endo H resistant band). Treatment with PNGase F would reduce all the glycosylated protein to unglycosylated form. The lower panel showed the glycosylation of Transferrin receptor under the same treatment which was used as a quality control of the experiment system. The Endo H resistant ratio of the protein was calculated by the indicated formula.

Fig. S5. The expression and degradation of NPC1L1 variants. (A) Quantification of the expression level and forms of NPC1L1 variants in Fig. 5A using densitometry and normalized against that of wild-type NPC1L1. (B-D) The amount of mature glycosylated and core-glycosylated form of NPC1L1 in Fig. 5A at each time point was quantified using densitometry, expressed as a percentage of the total NPC1L1 at time 0.

Figure S1

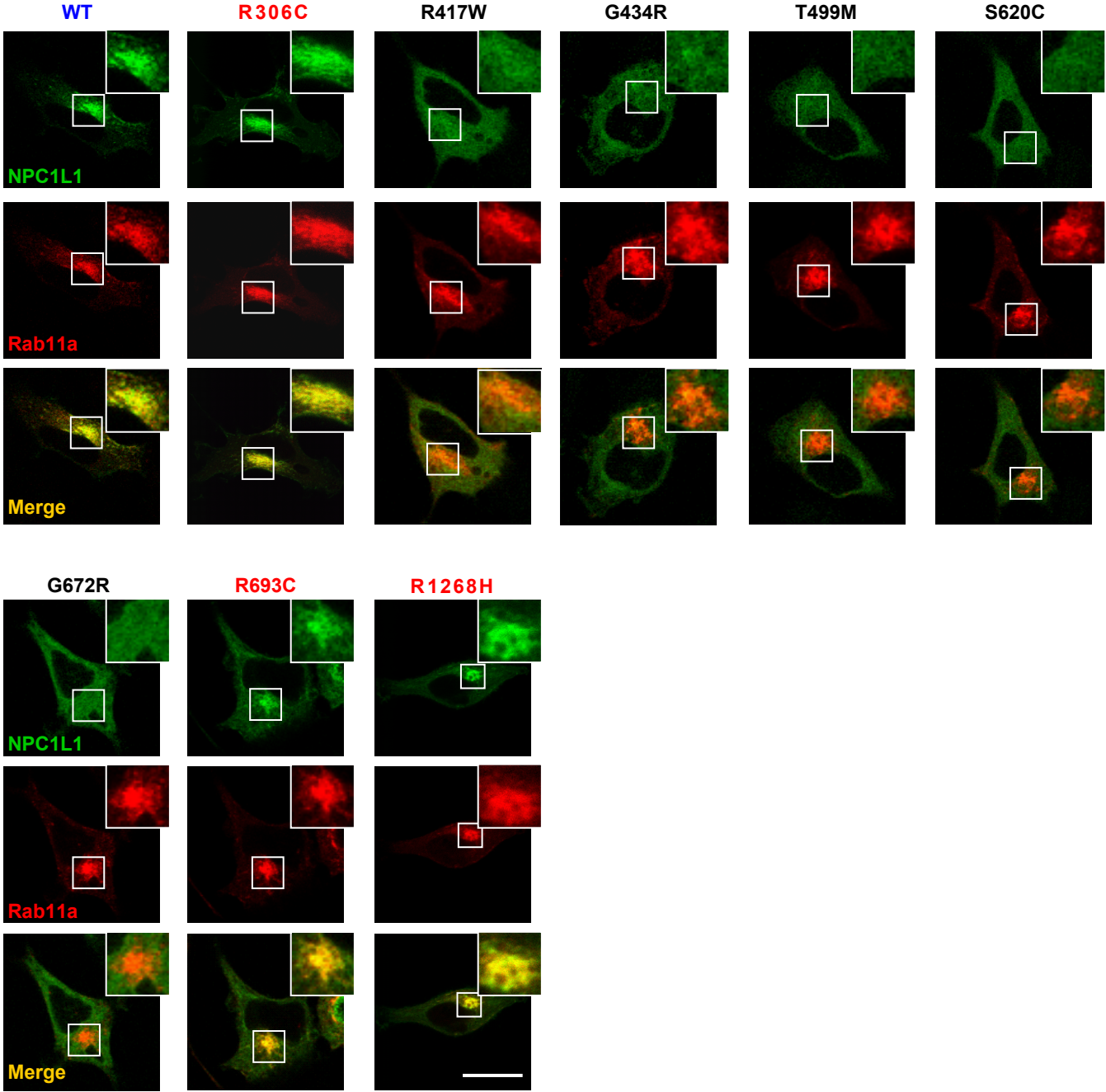


Figure S2

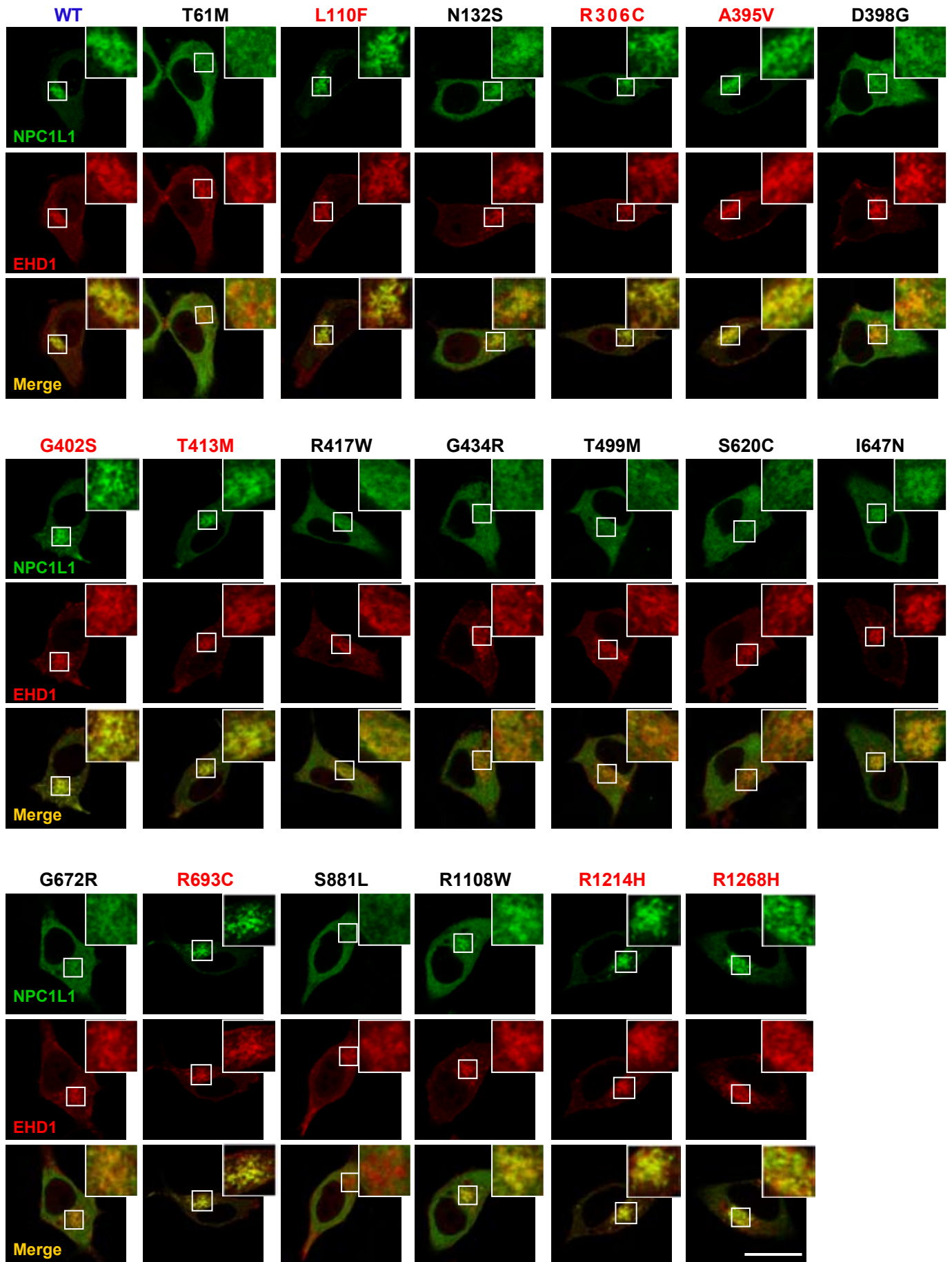


Figure S3

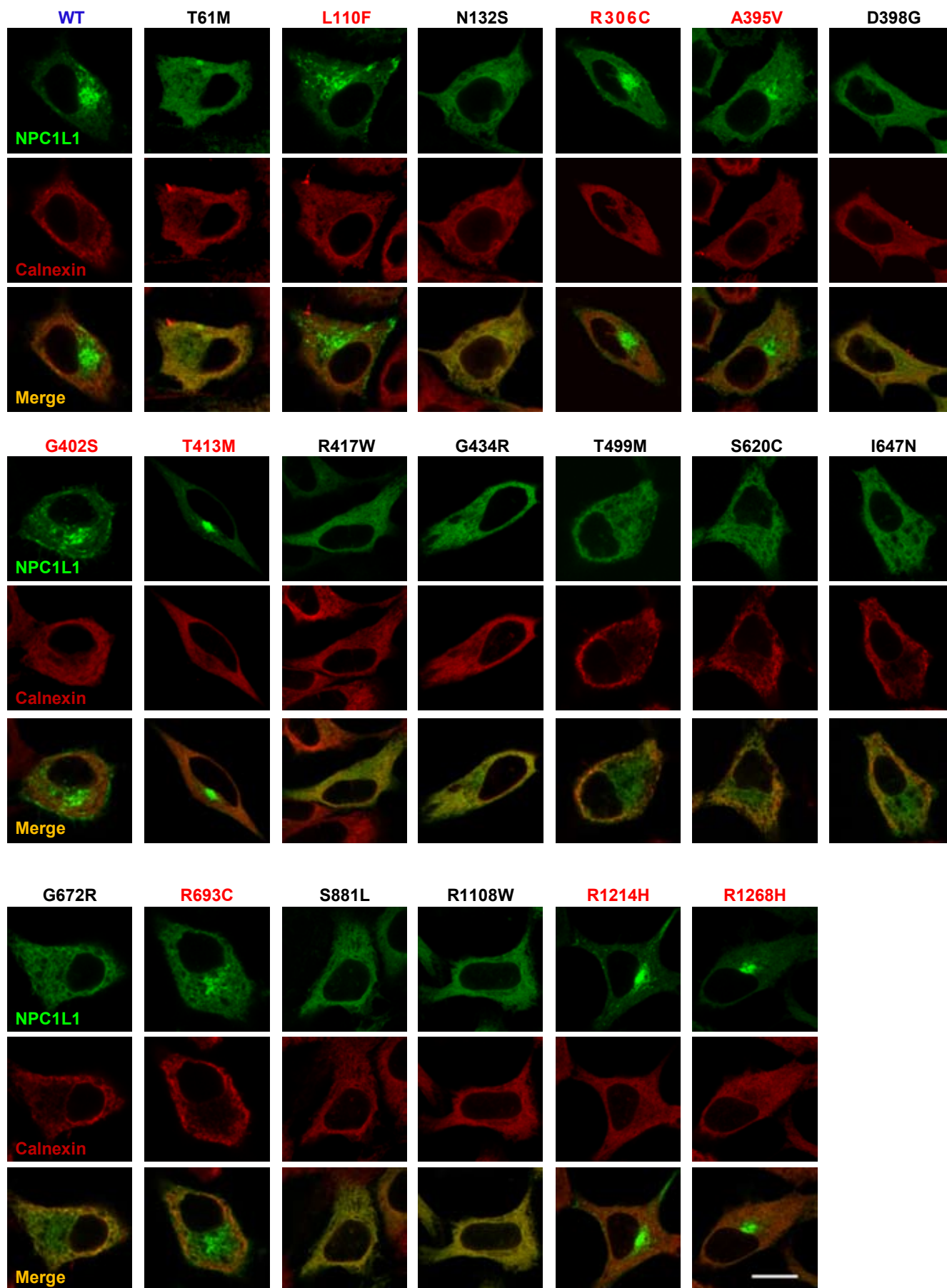
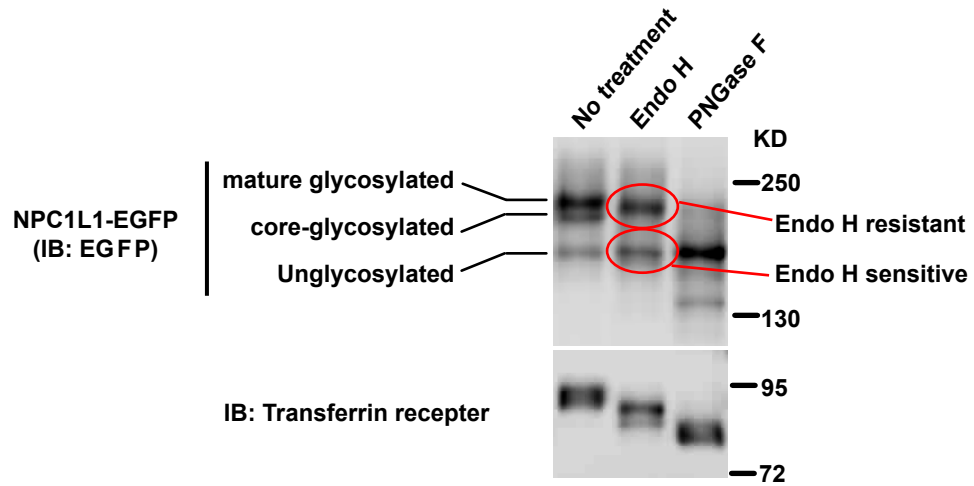


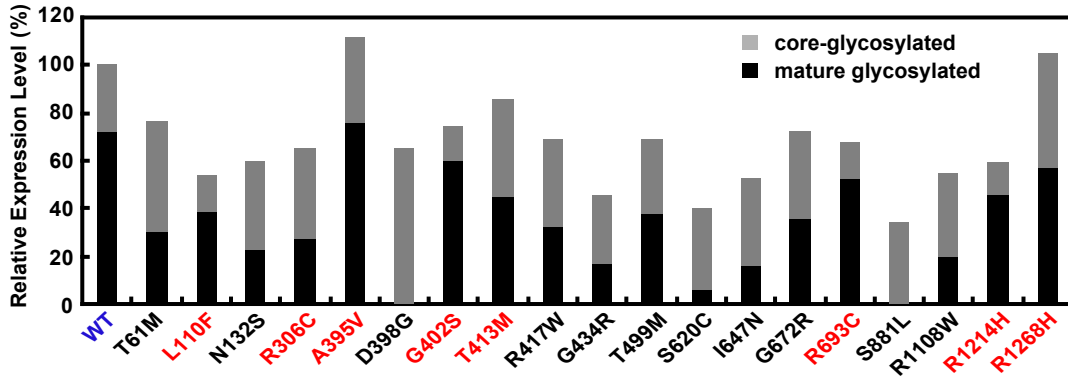
Figure S4



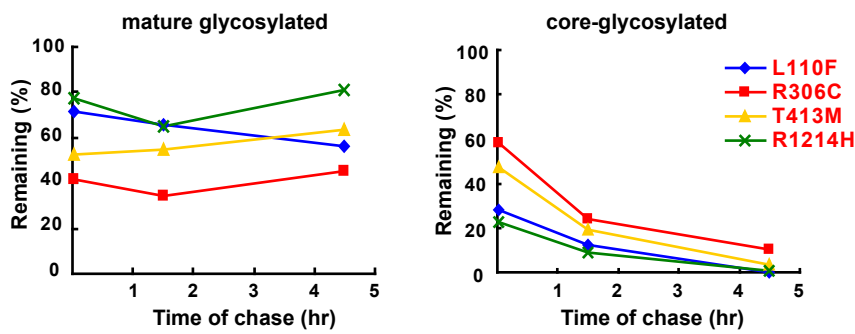
$$\text{Endo H resistant / Total (\%)} = \frac{\text{Endo H resistant}}{\text{Endo H resistant} + \text{Endo H sensitive}} \times 100\%$$

Figure S5

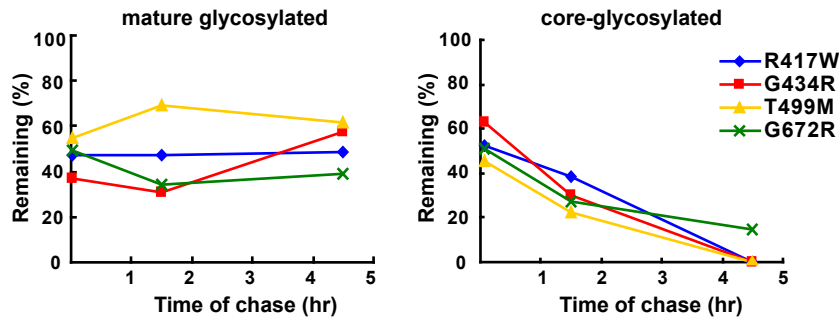
A



B



C



D

