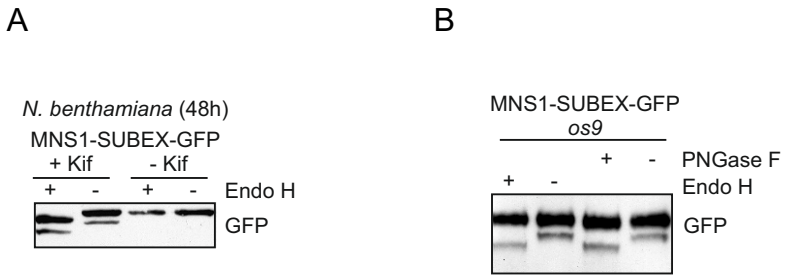

Figure S1. Endo H and PNGase F digestion of MNS1-SUBEX-GFP.
(A) MNS1-SUBEX-GFP was transiently expressed in *N. benthamiana* in the presence or absence of kifunensine (Kif) and subjected to Endo H digestion. (**B**) MNS1-SUBEX-GFP expressed in *Arabidopsis os9* was subjected to Endo H and PNGase F digestion.

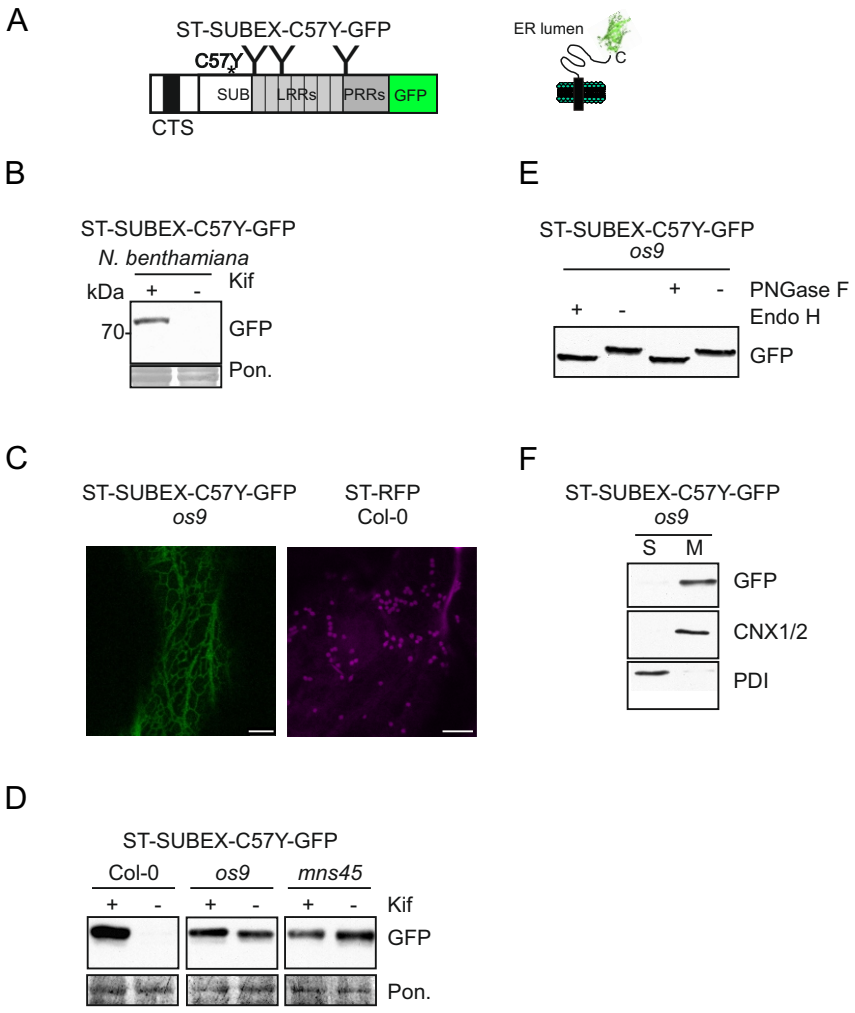

Figure S2. The ERAD pathway is dominant over Golgi targeting/retention signals from rat α2,6-sialyltransferase (ST).

(**A**) Schematic illustration of ST-SUBEX-C57Y-GFP. The signal peptide from SP-SUBEX-C57Y-GFP is replaced by the ST-CTS region. (**B**) Immunoblot of ST-SUBEX-C57Y-GFP transiently expressed in *N. benthamiana* leaves in the presence or absence of 20 μM kifunensine (Kif). Proteins were analysed 48 h after infiltration. (**C**) Confocal images of 12-day-old *Arabidopsis* seedlings expressing ST-SUBEX-C57Y-GFP. Scale bars = 7.5 μm. (**D**) Immunoblot analysis of protein extracts from 4-5-week-old *Arabidopsis* expressing ST-SUBEX-C57Y-GFP. (**E**) Endo H and PNGase F digestion of ST-SUBEX-C57Y-GFP from 4-5-week-old *Arabidopsis*. (**F**) Detection of ST-SUBEX-C57Y-GFP in soluble (S) and membrane fractions (M) of 12-day-old *os9* seedlings.

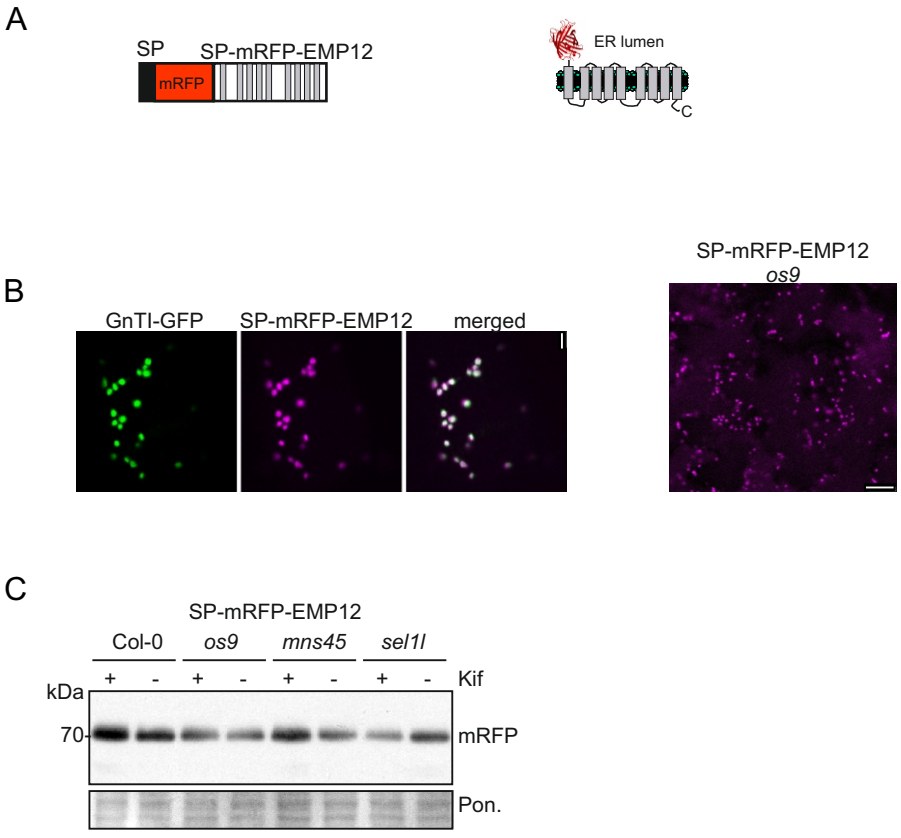

Figure S3. Golgi localization of SP-mRFP-EMP12.
(**A**) Schematic illustration of SP-mRFP-EMP12. The C-terminal part of *Arabidopsis* EMP12 carrying nine transmembrane domains is fused to SP-mRFP. (**B**) Confocal images of SP-mRFP-EMP12 (48 h after infiltration) transiently expressed in *N. benthamiana* leaves (scale bar = 25 µm) as well as of *Arabidopsis os9* leaves expressing SP-mRFP-EMP12 (scale bar = 10 µm). (**C**) Immunoblot analysis of protein extracts from 4-5-week-old *Arabidopsis* expressing SP-mRFP-EMP12.

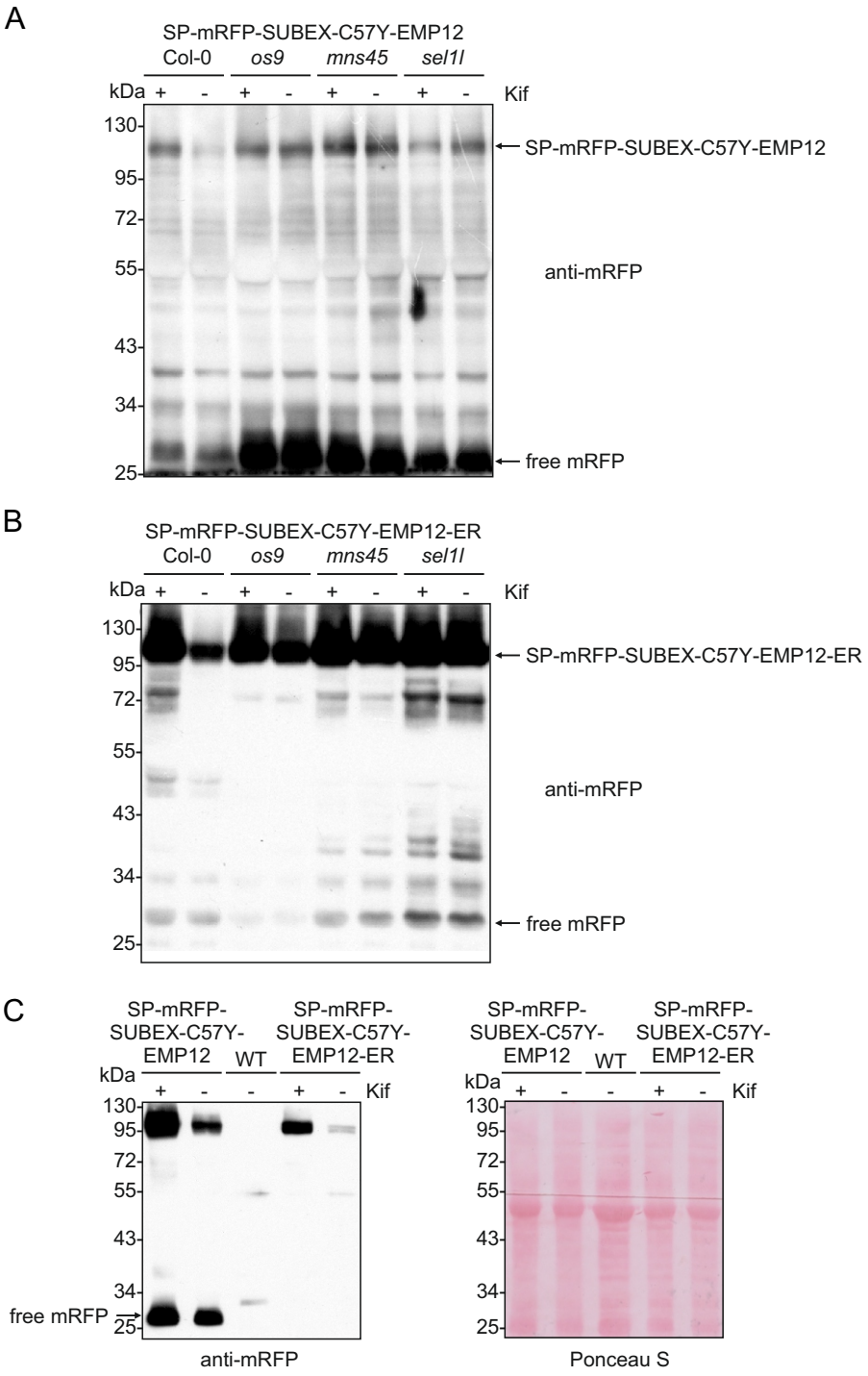

Figure S4. Detection of free mRFP on immunoblots.

(**A**) Immunoblot analysis of transgenic Col-0, *os9*, *mns45* and *sel1l* expressing SP-mRFP-SUBEX-C57Y-EMP12. The presence of cleaved free mRFP is indicated. The image corresponds to the one shown in Figure 4b and displays the molecular weight range from 25-130 kDa. (**B**) Immunoblot analysis of transgenic Col-0, *os9*, *mns45* and *sel1l* expressing SP-mRFP-SUBEX-C57Y-EMP12-ER. The presence of cleaved free mRFP is indicated. The image corresponds to the one shown in Figure 5e and displays the molecular weight range from 25-130 kDa. (**C**) Comparison of Col-0 expressing SP-mRFP-SUBEX-C57Y-EMP12 and Col-0 expressing SP-mRFP-SUBEX-C57Y-EMP12-ER on the same blot. A protein extract from a non-transformed Col-0 WT plant is included as a control.

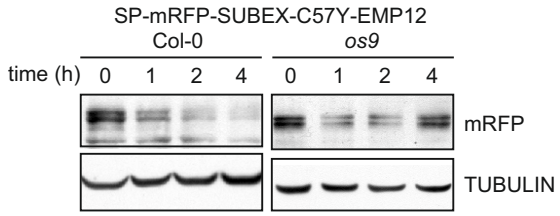

Figure S5. Degradation of SP-mRFP-SUBEX-C57Y-EMP12 in the presence of cycloheximide (CHX).

12-day-old *Arabidopsis* wild-type and os9 seedlings expressing SP-mRFP-SUBEX-C57Y-EMP12 were incubated for the indicated time with 100 µg/ml CHX. Proteins were extracted and subjected to SDS-PAGE followed by immunoblotting with antibodies against RFP and tubulin.

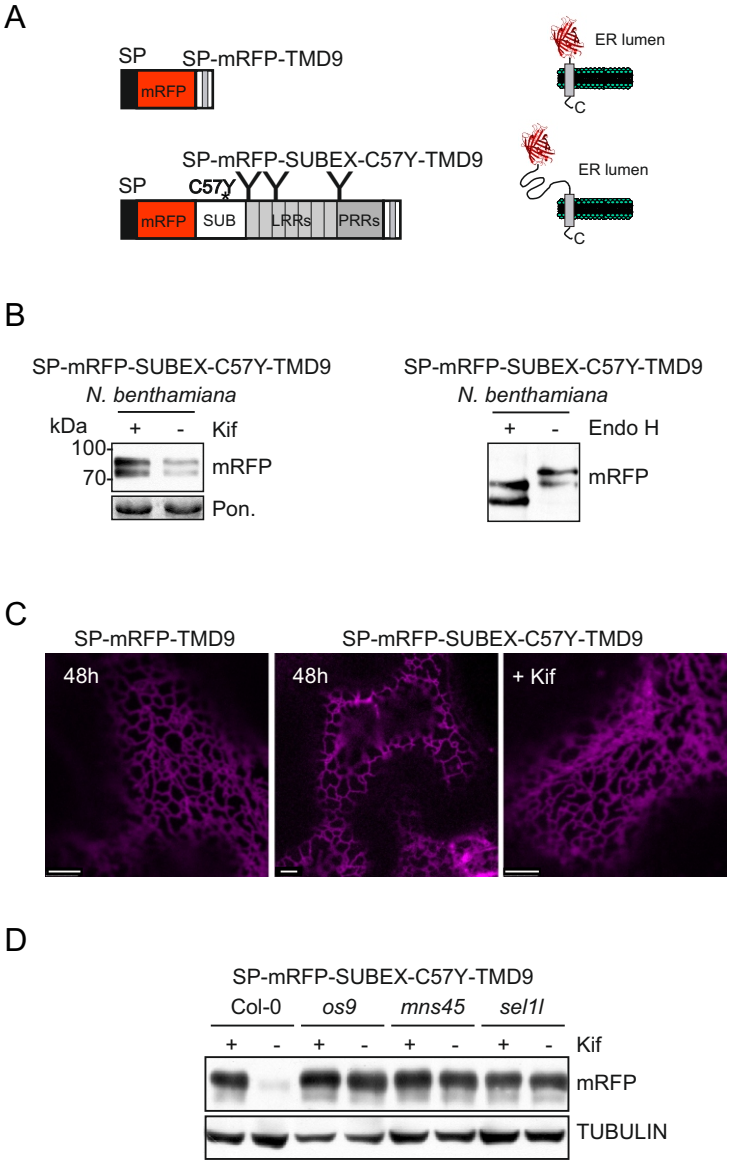

Figure S6. SP-mRFP-SUBEX-C57Y-TMD9 is retained in the ER and subjected to glycan-dependent ERAD.

(**A**) Schematic illustration of SP-mRFP-TMD9 and SP-mRFP-SUBEX-C57Y-TMD9. These chimeric proteins contain the C-terminal region of *Arabidopsis* EMP12 consisting of the last transmembrane domain and the C-terminal Golgi targeting and retention signal that faces the cytosol. (**B**) Immunoblot analysis of SP-mRFP-SUBEX-C57Y-TMD9 transiently expressed in *N. benthamiana* leaves in the presence or absence of 20 µM kifunensine (Kif) and after Endo H digestion. Proteins were analysed 48 h after infiltration. (**C**) Confocal images of SP-mRFP-TMD9 and SP-mRFP-SUBEX-C57Y-TMD9 (48 h after infiltration) transiently expressed in *N. benthamiana* leaves. Scale bars = 5 µm. (**D**) Immunoblot analysis of protein extracts from 4-5-week-old *Arabidopsis* expressing SP-mRFP-SUBEX-C57Y-TMD9.

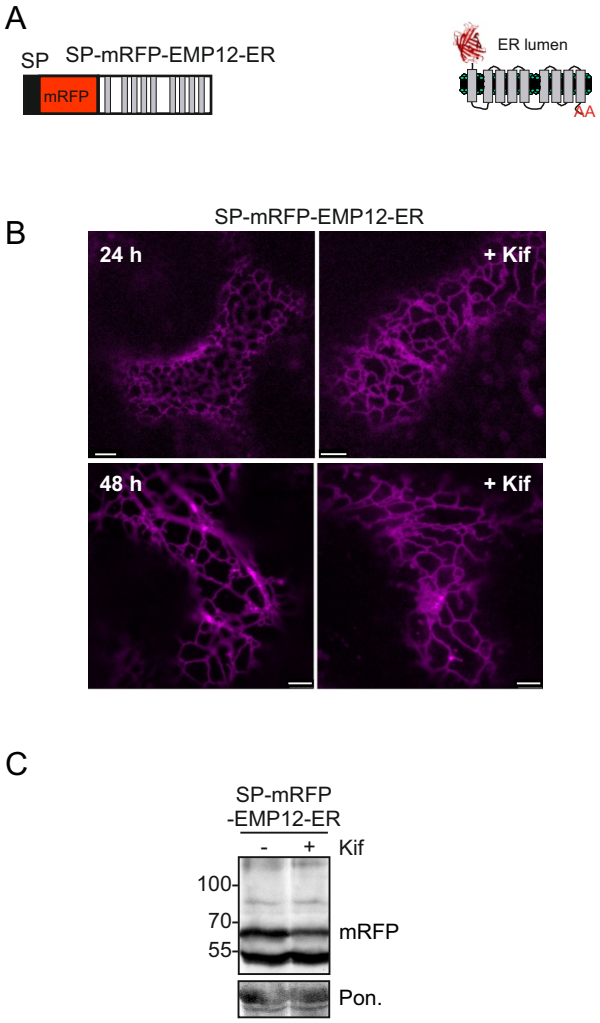

Figure S7. Subcellular localisation of SP-mRFP-EMP12-ER harbouring mutations in the ER export motif.

(**A**) Schematic illustration of SP-mRFP-EMP12-ER. The cytoplasmic C-terminal region carries two amino acid substitutions that inactivate the ER export signal of *Arabidopsis* EMP12. (**B**) Confocal images of SP-mRFP-EMP12-ER (24 and 48 h after infiltration) transiently expressed in *N. benthamiana* leaves. Scale bars = 5 µm. (**C**) Immunoblot analysis of SP-mRFP-EMP12-ER transiently expressed in *N. benthamiana* leaves in the presence or absence of 20 µM kifunensine (Kif) (48 h after infiltration).