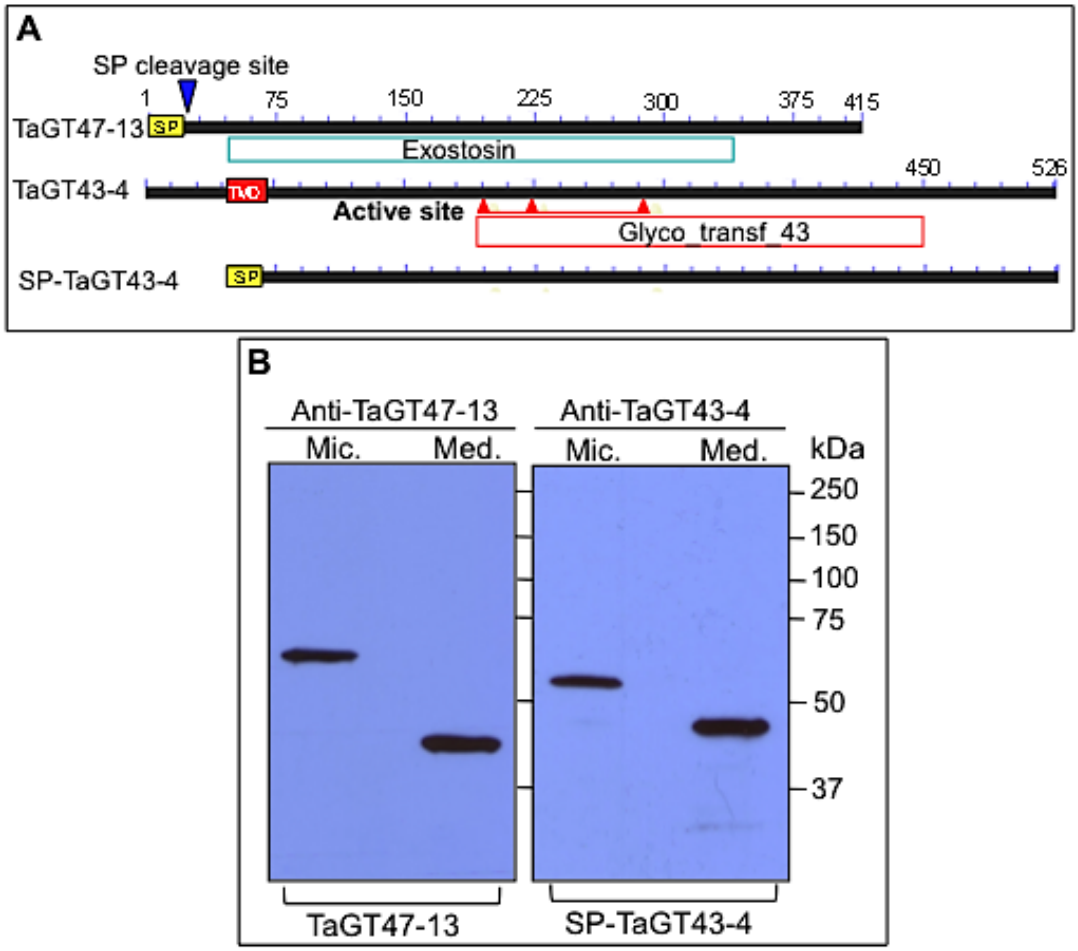
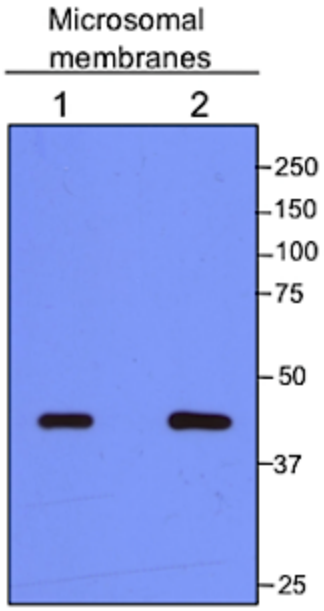
Supplemental Figure S1



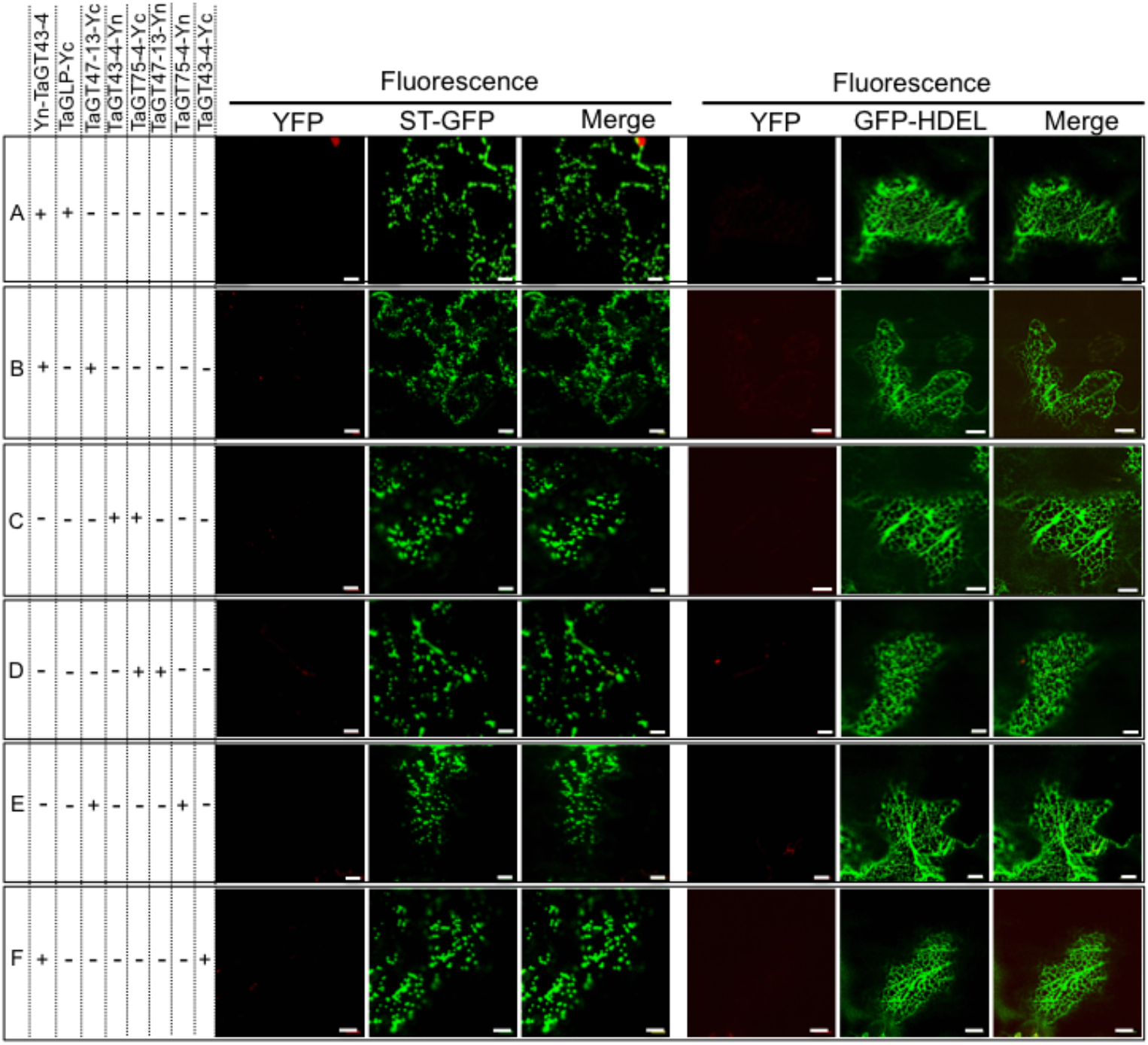
Supplemental Figure S1. NH_2-terminal secretion signal peptide (SP) of TaGT47-13 is functional. **Panel A**, schematic presentation of protein structures of TaGT47-13, TaGT43-4, and SP-TaGT43-4 (a chimeric protein in which the first 71 amino acids of TaGT43-4 were replaced with the first 31 amino acids of TaGT47-13). While TaGT43-4 is predicted as a type II membrane protein with a single transmembrane domain (TMD) located between amino acids 47 and 68 (predicted size of ~52kDa), TaGT47-13 is predicted to have a SP cleavage site located between amino acids 22 and 23 (predicted size of the processed protein is ~44kDa, according to amino acid sequence). **Panel B**, western blotting (~50 μg proteins per lane) using purified anti-TaGT47-13 and anti-TaGT43-4 to detect the presences of TaGT47-13 and SP-TaGT43-4 in microsomal membranes (Mic.) and culture medium (Med.) obtained from transgenic Pichia cells producing these two proteins individually. The data show that both TaGT47-13 and SP-TaGT43-4 were secreted in the culture medium (seen as bands of ~44kDa and ~48kDa, respectively), but some of unprocessed proteins (SP not cleaved) remained associated with microsomal membranes (seen as bands of ~56kDa and ~52kDa, respectively). Both proteins appeared to be secreted at similar rates. These results confirm that the first 31 amino acids of TaGT47-13 contain a cleavable SP, but the processing of this SP seems to be incomplete. Our current data and previous immunoblot data on the purified wheat XSC (Zeng et al., 2010) strongly suggest that the complex contains only unprocessed TaGT47-13.

Supplemental Figure S2



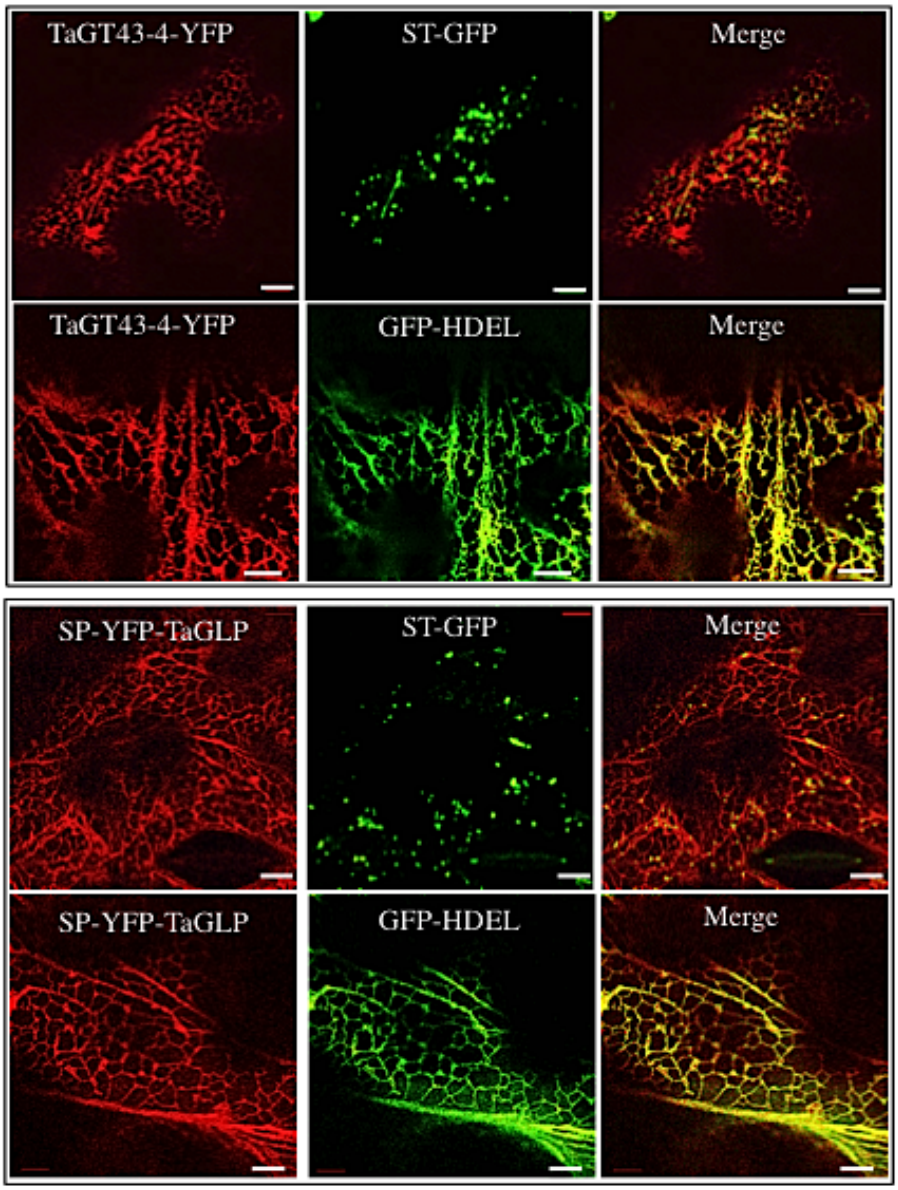
Supplemental Figure S2. Immunoblotting (~50µg proteins per lane) using anti-PsRGP1 to detect the presence of TaGT75-3 (lane 1) and TaGT75-4 (lane 2) in microsomal membranes obtained from transgenic Pichia cells expressing these proteins individually in combination with TaGT43-4 and TaGT47-13. The data show that TaGT75-3 and TaGT75-4 are recognized by anti-PsRGP1 and have similar estimated sizes (~40kDa).

Supplemental Figure S4



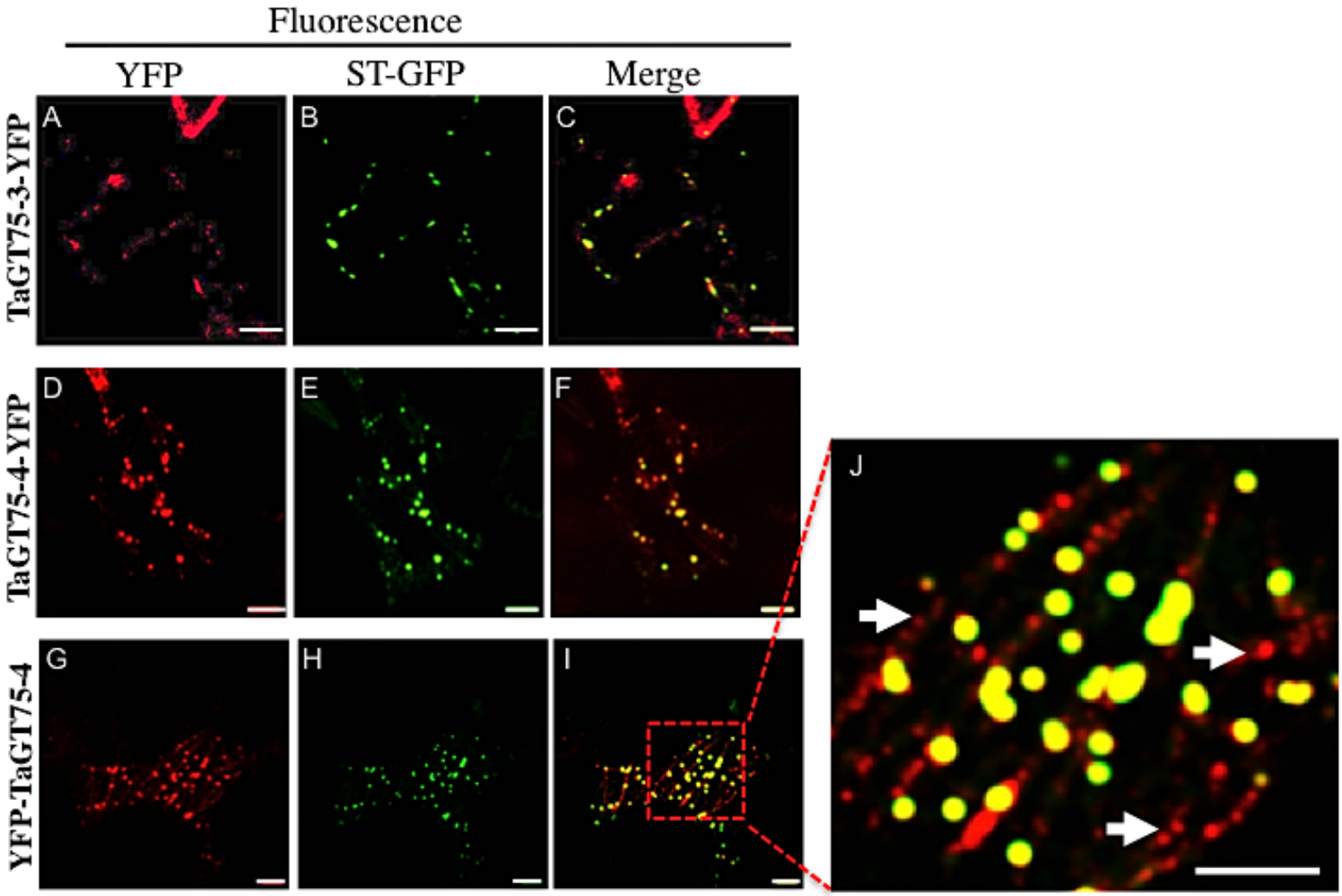
Supplemental Figure S4. Bimolecular fluorescence complementation (BiFC, split-YFP) assay of negative control combinations: [TaGT47-13-Yn and TaGT75-4-Yc], [TaGT47-13-Yc and TaGT75-4-Yn], [Yn-TaGT43-4 and TaGLP-Yc], [Yn-TaGT43-4 and TaGT47-13-Yc], [TaGT43-4-Yn and TaGT75-4-Yc], and [Yn-TaGT43-4 and TaGT43-4-Yc]. No YFP fluorescence was observed with any of these combinations, as Yn and Yc ends are likely located on opposite sides of the membrane

Supplemental Figure S3



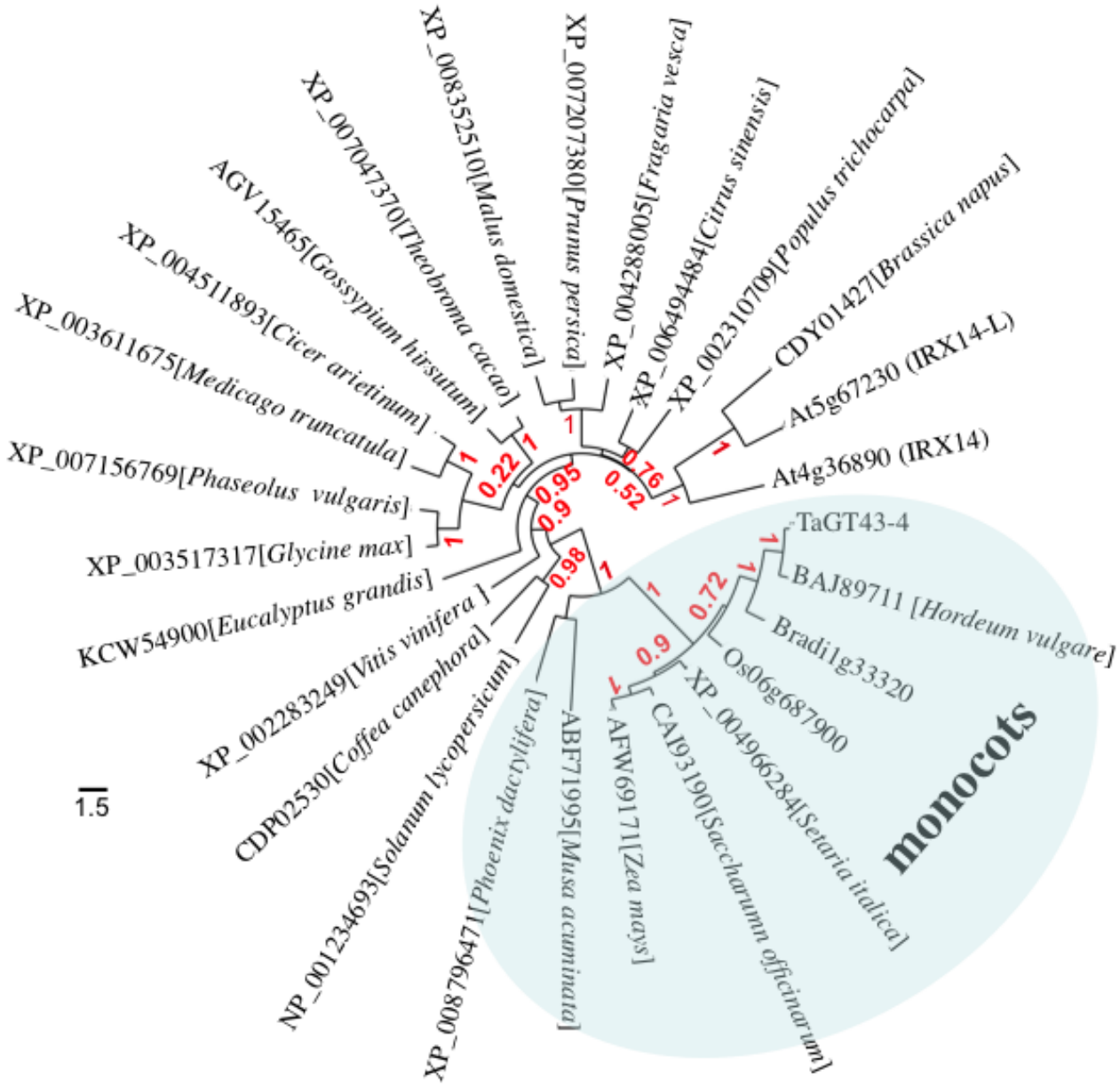
Supplemental Figure S3. Confocal images of epidermal cells of *N. tabaccum* leaves transiently expressing TaGT43-4-YFP or SP-YFP-TaGLP. ST-GFP and GFP-HDEL, *trans*-Golgi and ER markers, respectively, were used to show YFP localization. GFP and YFP fluorescence are shown in green and red, respectively, and their co-localization (merge) appears in yellow. Scale bars are 10 μm.

Supplemental Figure S5



Supplemengal Figure S5. Confocal images of epidermal cells of *N. tabaccum* leaves transiently expressing TaGT75-3-YFP (**Panels A-C**), TaGT75-4-YFP (Panels D-F), or YFP-TaGT75-4 (**Panel G-I**). ST-GFP, *trans*-Golgi marker, was used to show YFP localization. GFP and YFP fluorescence are shown in green and red, respectively, and their co-localization (merge) appears in yellow. Both TaGT75-3 and TaGT75-4 show similar subcellular localization, namely punctate pattern that localizes mostly with ST-GFP, but some of the fluorescence also localizes in certain areas at the surface of the ER (indicated by white arrows in **panel J**) that had no overlap with ST-GFP. Scale bars are 10 μm.

Supplemental Figure S6



Supplemental Figure S6. Maximum-likelihood phylogenetic analysis of TaGT43-4 and 26 homologous proteins from GT43 family. Sequences were obtained from NCBI, Phytozome 10.3, or CAZy databases. Phylogenetic tree was generated through the Phylogeny.fr platform using PhyML 3.0 program. Bootstrap values (based on 500 replicate) are indicated at the tree nodes. The scale measures evolutionary distance in substitution per amino acids. Alignment of protein sequeneces was generated through MUSCLE 3.7 program in the Phylogeny.fr platform. Accession numbers are indicated. The scale measures evolutionary distance in substitution per amino acids.

Supplemental Table S1. Primer sequences used to generate fluorescent-tagged constructs for confocal microscopy work (localization and protein-protein interactions). Underlined sequences correspond to restriction cutting sites (see Materials and Methods).

Constructs	Primer sequences
Untagged TaGT43-4	Forward: 5'CAGGACGTCTAGAATGATGAAGCAGCTGCTGCCGCA3' Reverse: 5'CATGACCGTCGACtt**TCA**GTTGTGGACGTCGCCCTTCCG3'
TaGT43-4 -YFP	Forward: 5'CAGGACGTCTAGAATGATGAAGCAGCTGCTGCCGCA3' Reverse: 5'CATGACCGTCGACttGTTGTGGACGTCGCCCTTCCG3'
YFP-TaGT43-4	Forward: 5'GGTGCTGGATCCATGATGAAGCAGCTGCTGCCGCA3' Reverse: 5'GCGCAGGAGCTC**TCA**GTTGTGGACGTCGCCCTTCC3'
TaGT43-4 -Yn	Forward: 5'CAGGACGTCTAGAATGATGAAGCAGCTGCTGCCGCA3' Reverse: 5'CATGACCGTCGACttGTTGTGGACGTCGCCCTTCCG3'
TaGT43-4 -Yc	Forward: 5'CAGGACGTCTAGAATGATGAAGCAGCTGCTGCCGCA3' Reverse: 5'CATGACCGTCGACttGTTGTGGACGTCGCCCTTCCG3'
Yn-TaGT43-4	Forward: 5'GGTGCTGGATCCATGATGAAGCAGCTGCTGCCGCA3' Reverse: 5'GCGCAGGAGCTCTCAGTTGTGGACGTCGCCCTTCC3'
Untagged TaGT47-13	Forward: 5'CAGGACGTCTAGATGGAGAAGCCGCGGGCGATG3' Reverse: 5'CATGACCGTCGACtt**CTA**CCATGGCTTCAGGTCGCCCTTG3'
TaGT47-13-YFP	Forward: 5'CAGGACGTCTAGATGGAGAAGCCGCGGGCGATG3' Reverse: 5'CATGACCGTCGACttCCATGGCTTCAGGTCGCCCTTG3'
SP-YFP-TaGT47-13	Forward: 5'CAGGACGTCTAGATGGAGAAGCCGCGGGCGATG3' Reverse: 5'CATGACCGTCGACtt**CTA**CCATGGCTTCAGGTCGCCCTTG3'
TaGT47-13-Yc	Forward: 5'CAGGACGTCTAGATGGAGAAGCCGCGGGCGATG3' Reverse: 5'CATGACCGTCGACttCCATGGCTTCAGGTCGCCCTTG3'
TaGT47-13-Yn	Forward: 5'CAGGACGTCTAGATGGAGAAGCCGCGGGCGATG3' Reverse: 5'CATGACCGTCGACttCCATGGCTTCAGGTCGCCCTTG3'
Untagged TaGLP	Forward: 5'CAGGACGTCTAGATGGCATCACCCTCTTCCTTCC3' Reverse: 5'GCAGGTCGACtt**TTA**GTTGTGGTTGTTCTCCCAGAACTGA3'
TaGLP-YFP	Forward: 5'CAGGACGTCTAGATGGCATCACCCTCTTCCTTCC3' Reverse: 5'GCAGGTCGACttGTTGTGGTTGTTCTCCCAGAACTGA3'
SP-YFP-TaGLP	Forward: 5'CAGGACGTCTAGATGGCATCACCCTCTTCCTTCC3' Reverse: 5'GCAGGTCGACtt**TTA**GTTGTGGTTGTTCTCCCAGAACTGA3'
TaGLP-Yn	Forward: 5'CAGGACGTCTAGATGGCATCACCCTCTTCCTTCC3' Reverse: 5'GCAGGTCGACttGTTGTGGTTGTTCTCCCAGAACTGA3'
TaGLP-Yc	Forward: 5'CAGGACGTCTAGATGGCATCACCCTCTTCCTTCC3' Reverse: 5'GCAGGTCGACttGTTGTGGTTGTTCTCCCAGAACTGA3'
TaGT75-4-Yc	Forward: 5'CAGGACGTCTAGATGGCAGGGACGGTGACTGTG3' Reverse: 5'CATGACCGTCGACttCTTGGCTGCTGCCTTGCCG3'
TaGT75-4-YFP	Forward: 5'CAGGACGTCTAGATGGCAGGGACGGTGACTGTG3' Reverse: 5'CATGACCGTCGACttCTTGGCTGCTGCCTTGCCG3'
TaGT75-4-Yn	Forward: 5'CAGGACGTCTAGATGGCAGGGACGGTGACTGTG3' Reverse: 5'CATGACCGTCGACttCTTGGCTGCTGCCTTGCCG3'
TaGT75-3-Yc	Forward: 5'CAGGACGTCTAGATGGCCACCCCGACGACG3' Reverse: 5'CATGACCGTCGACttCTTGCTCTTCAGAGCAGGGCCG3'
TaGT75-3-YFP	Forward: 5'CAGGACGTCTAGATGGCCACCCCGACGACG3' Reverse: 5'CATGACCGTCGACttCTTGCTCTTCAGAGCAGGGCCG3'
TaGT75-3-Yn	Forward: 5'CAGGACGTCTAGATGGCCACCCCGACGACG3' Reverse: 5'CATGACCGTCGACttCTTGCTCTTCAGAGCAGGGCCG3'
YFP-TaVER2	Forward: 5'GGTGCTGGATCCATGGCCAAATTCCAGATTACACCGTTCCC3' Reverse: 5'GCGCAGGAGCTCTCAGACCGTGTAAACACCAAATGCGATG3'
TaVER2-Yn	Forward: 5'CAGGACGTCTAGATGGCCAAATTCCAGATTACACCGTTCCC3' Reverse: 5'CATGACCGTCGACttGACCGTGTAAACACCAAATGCGATG3'
TaVER2-Yc	Forward: 5'CAGGACGTCTAGATGGCCAAATTCCAGATTACACCGTTCCC3' Reverse: 5'CATGACCGTCGACttGACCGTGTAAACACCAAATGCGATG3'
IRX14-YFP	Forward: 5'GACGTCTAGATGAAGCTCTCTGCTTTACATCAGAG3' Reverse: 5'GACCGTCGACttGTTTCTTTCTTGATGCTTAGACGAAGATG3'
IRX14-M3-YFP	Forward: 5'GACGTCTAGATGATGAAGCAGCTGCTGC3' Reverse: 5'GACCGTCGACttGTTTCTTTCTTGATGCTTAGACGAAGATG3'