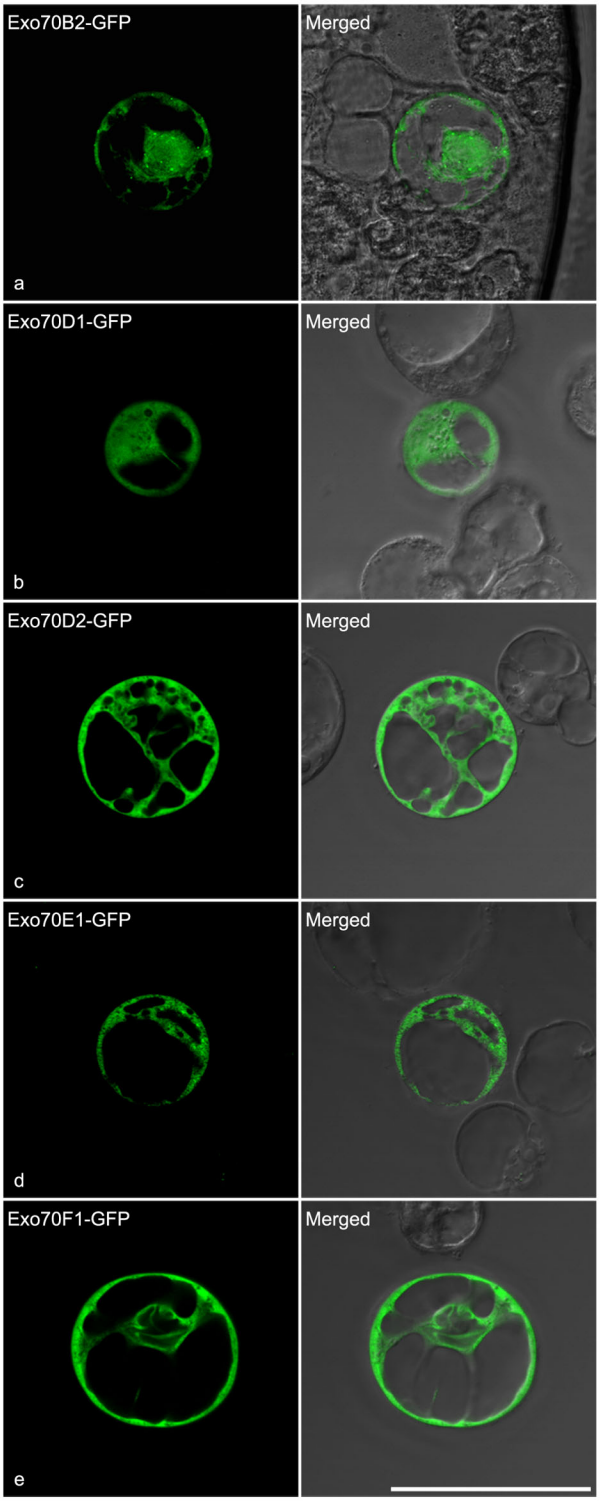
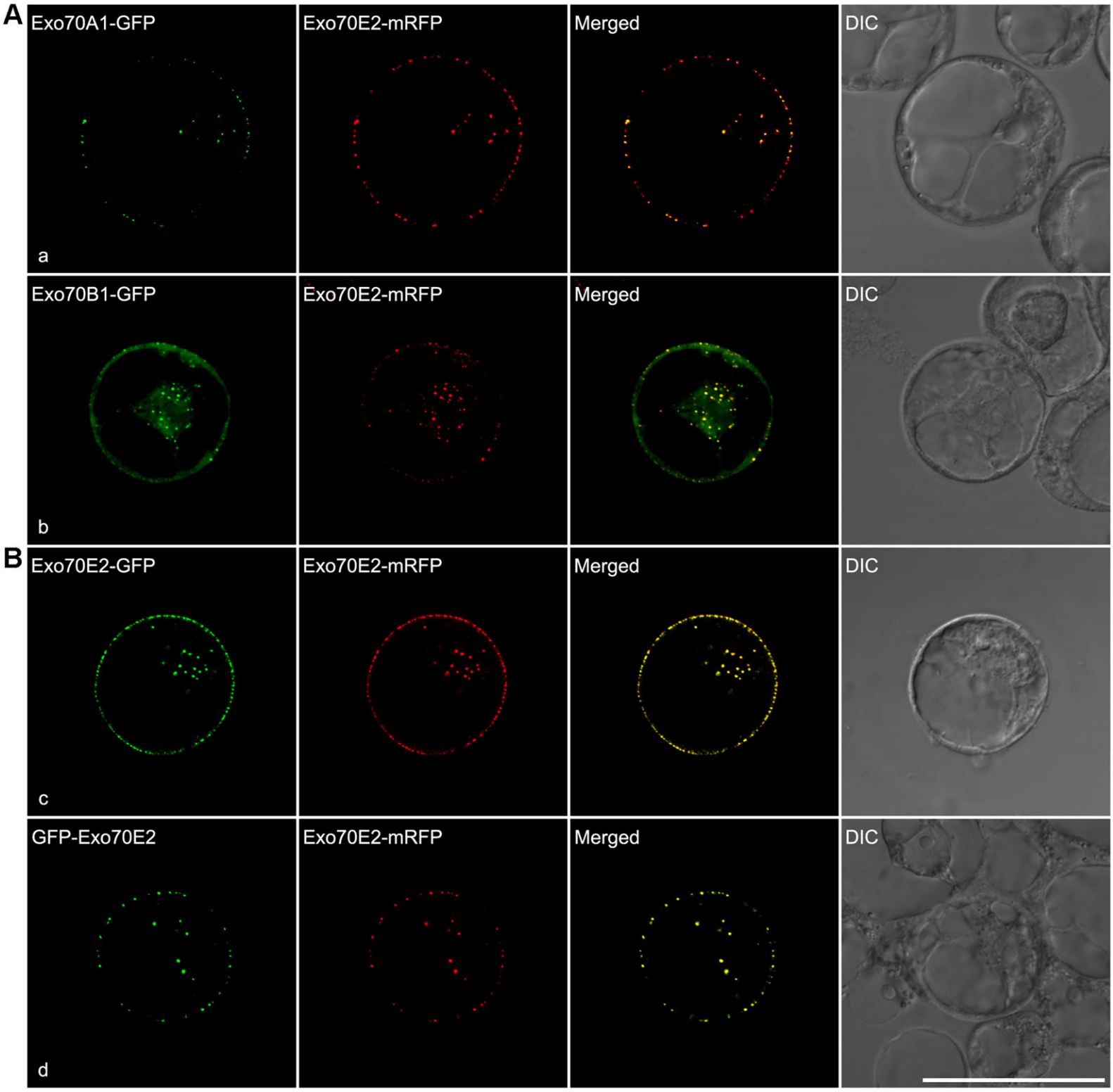


Supplemental Figure 1. Transient expression of various GFP fusions with selected members of Exo70.

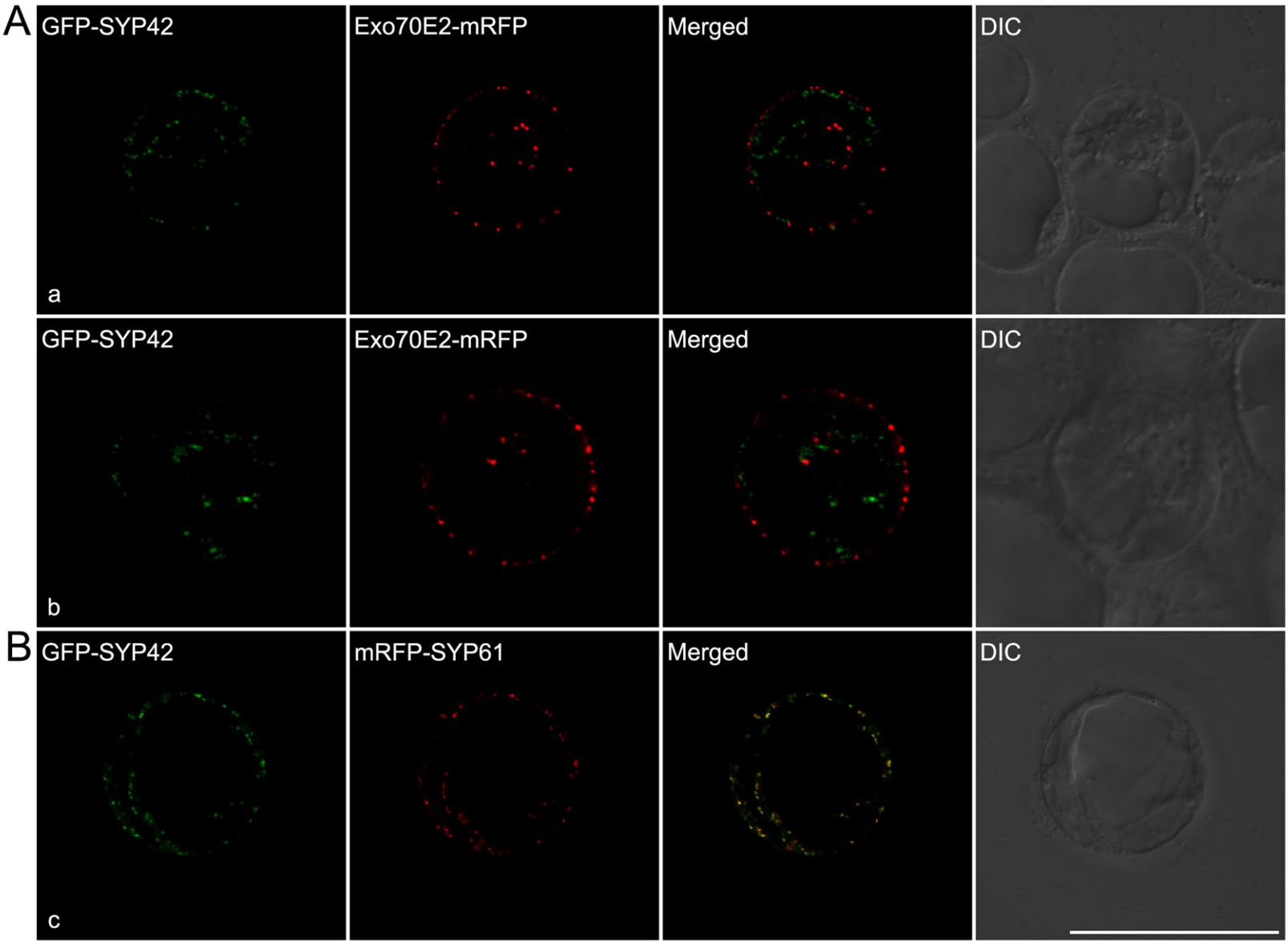
Various Exo70-GFP fusions were transiently expressed in *Arabidopsis* protoplasts, followed by confocal imaging. The corresponding cells were shown in the merged image. Scale bar = 50 μm.



Supplemental Figure 2. Colocalization of pairs of Exo70-XFP fusions.

A: Colocalization of Exo70E2-mRFP with Exo70A1-GFP or Exo70B1-GFP in *Arabidopsis* protoplasts.

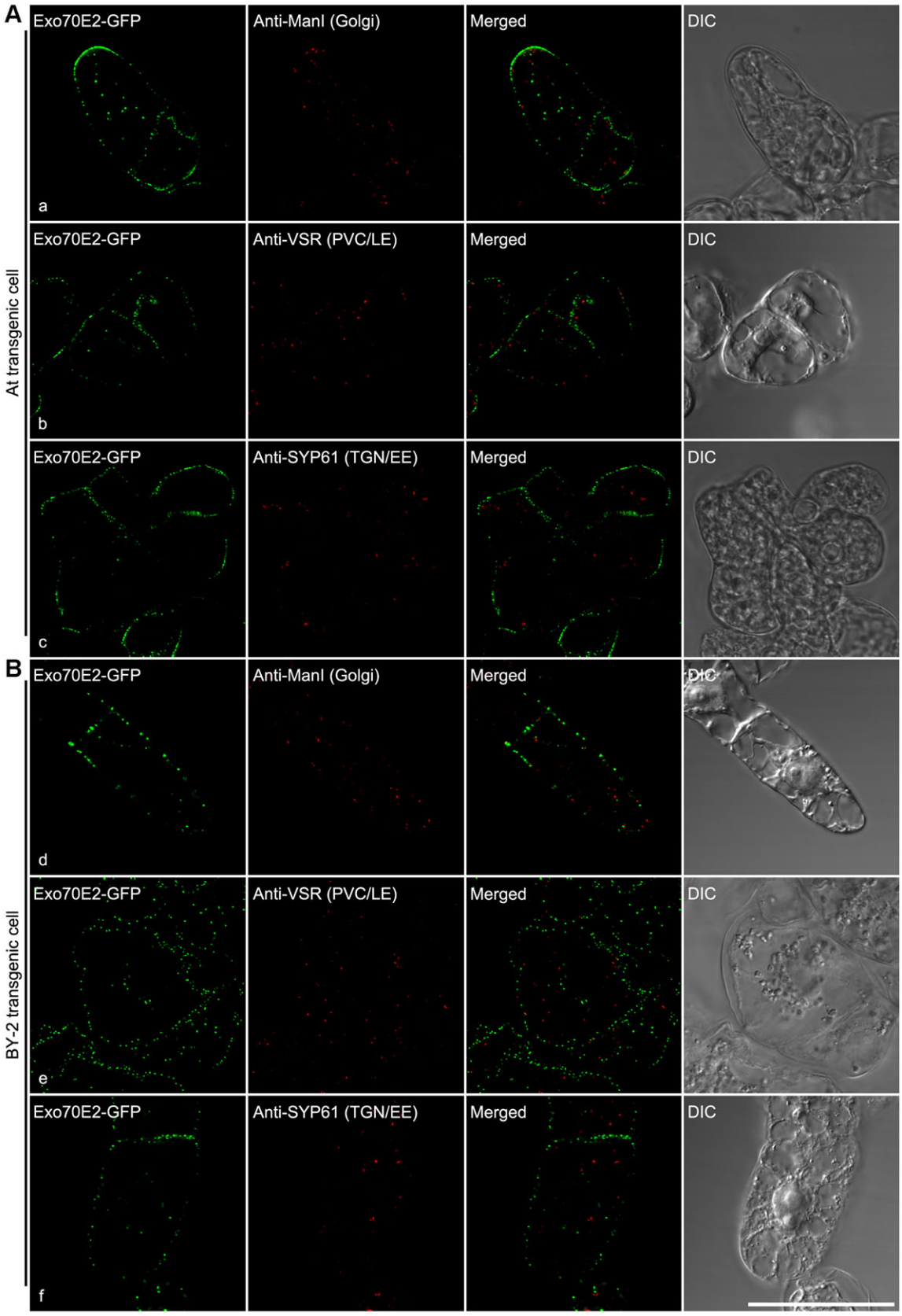
B: Colocalization of Exo70E2-mRFP with Exo70E2-GFP or GFP-Exo70E2 in *Arabidopsis* protoplasts. Scale bar = 50 μm.



Supplemental Figure 3. The TGN marker SYP42 is separated from Exo70E2-mRFP in *Arabidopsis* protoplasts.

A: Separation of GFP-SYP42 from Exo70E2-mRFP in *Arabidopsis* protoplasts (a and b).

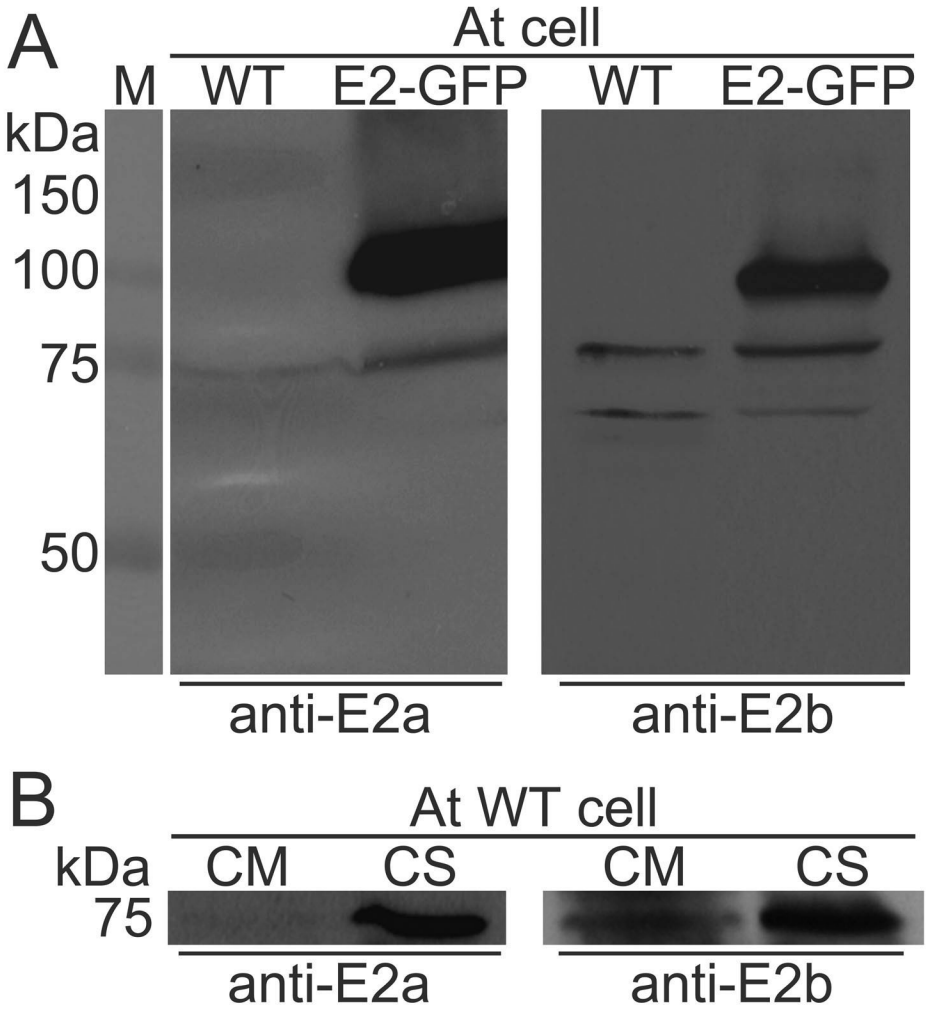
B: Colocalization of GFP-SYP42 with mRFP-SYP61 in *Arabidopsis* protoplasts (c). Scale bar = 50 μm.



Supplemental Figure 4. Exo70-GFP-positive organelles are distinct from known secretory compartments.

Transgenic *Arabidopsis* (A) or tobacco BY-2 (B) cells expressing Exo70E2-GFP fusion proteins were chemically fixed and labeled with antibodies for known organelles as indicated, followed by confocal imaging.

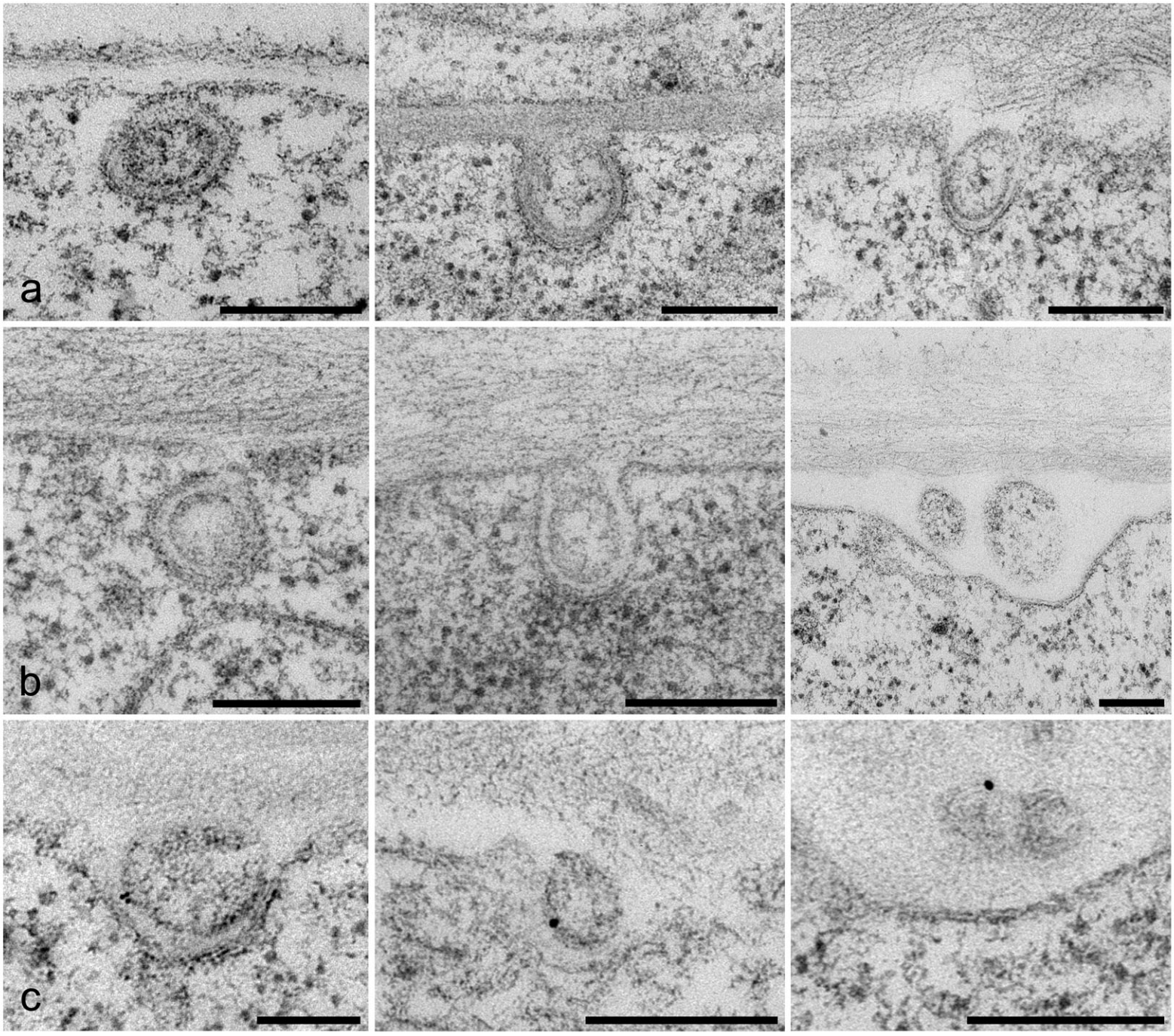
DIC, differential interference contrast; PVC, prevacuolar compartment; LE, late endosome; EE, early endosome; TGN, *trans*-Golgi network. Scale bar = 50 μm.



Supplemental Figure 5. Immunoblot characterization of endogenous Exo70E2 proteins in wild type *Arabidopsis* cells.

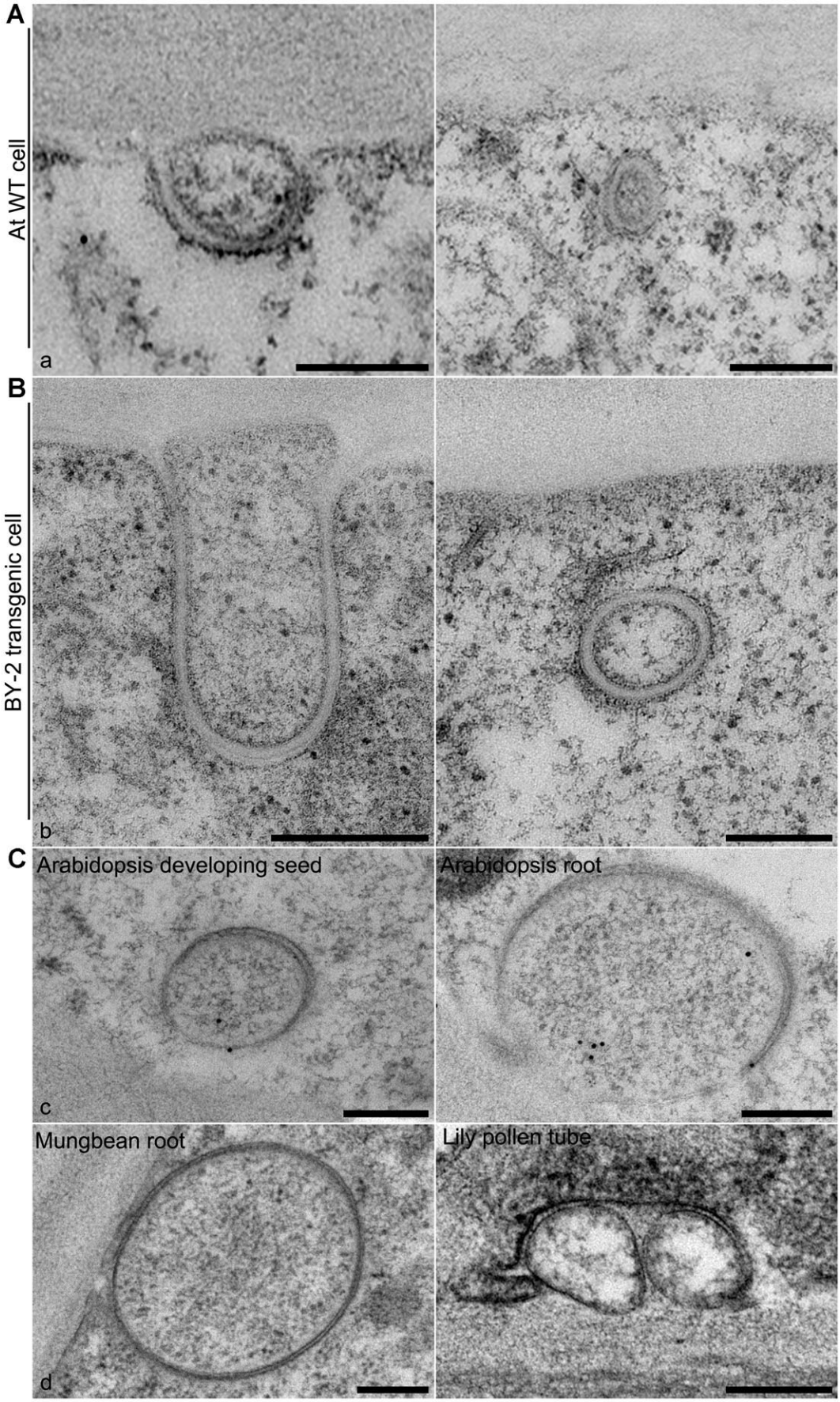
A. Endogenous Exo70E2 proteins are detected by either E2a or E2b antibodies in *Arabidopsis* WT cells and transgenic *Arabidopsis* E2-GFP cells.

B. Endogenous Exo70E2 proteins are detected by either E2a or E2b antibodies in *Arabidopsis* WT cells. CS = cell soluble proteins; CM = cell membrane proteins.



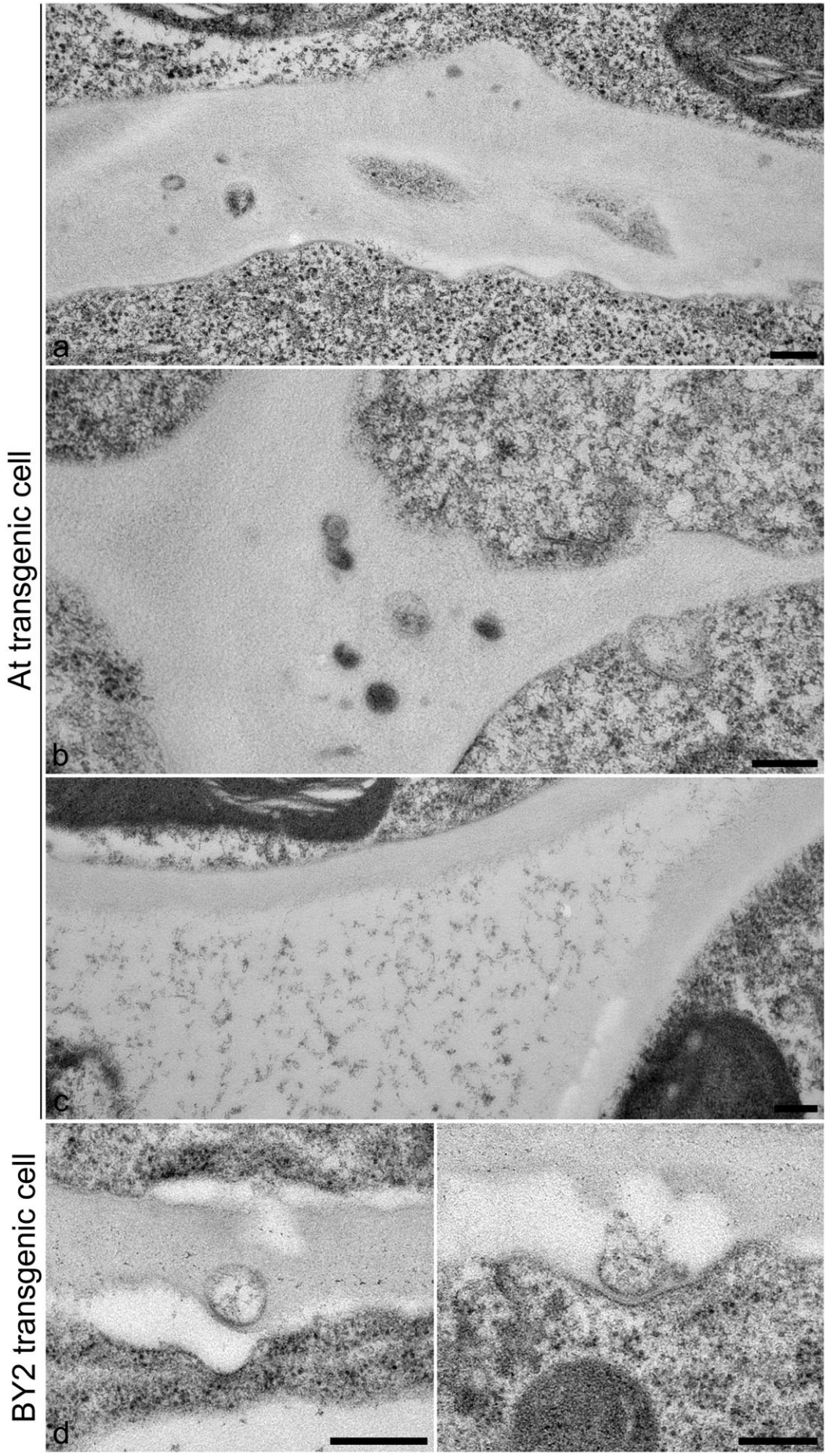
Supplemental Figure 6. Ultrastructure and fusion nature of EXPO in wild type *Arabidopsis* suspension culture cells.

Shown are examples of EXPO detected in ultra-thin sections prepared from high-pressure freezing/frozen substituted wild type *Arabidopsis* cells without antibody labeling (a, b) or with anti-E2a labeling (c). Scale bars = 200nm.



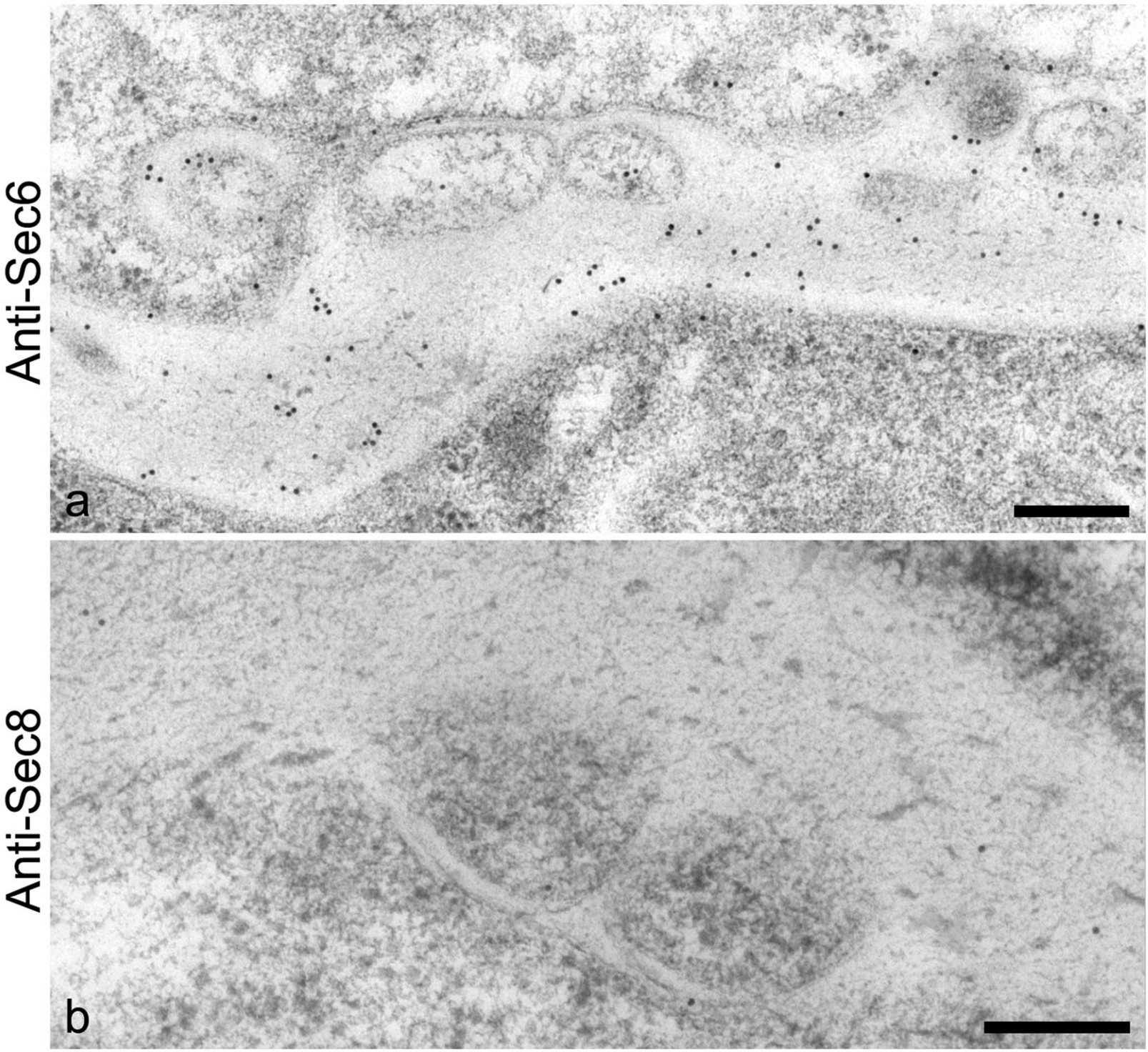
Supplemental Figure 7. Ultrastructure of EXPO in plant cells.

Shown are examples of EXPO detected in ultra-thin sections prepared from high-pressure freezing/frozen substituted wild type *Arabidopsis* cells (A), transgenic tobacco BY-2 cells expressing Exo70E2-GFP (B), *Arabidopsis* developing seed and root (C, c), and mungbean root and lily pollen tube (C, d) as indicated. Scale bars = 200nm.



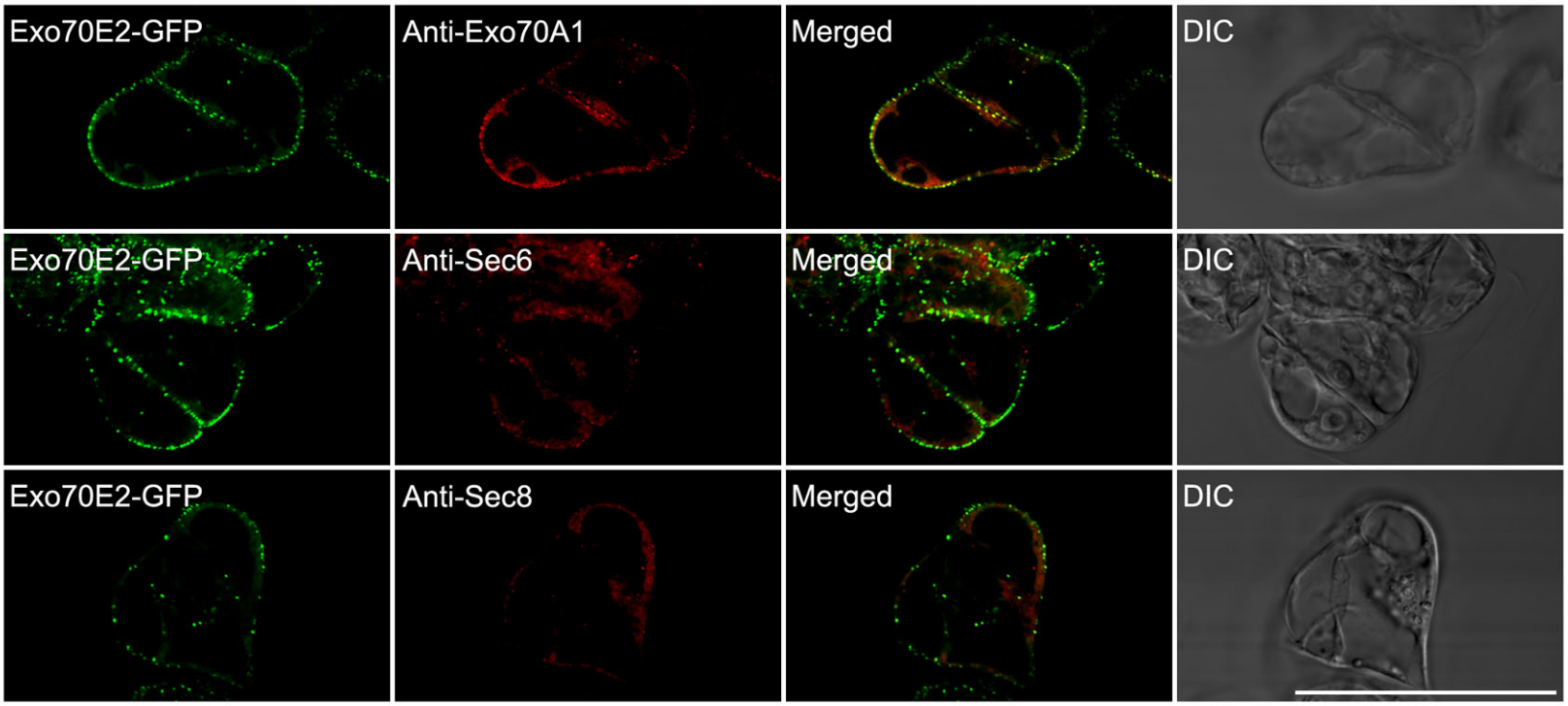
Supplemental Figure 8. ImmunoEM controls.

Immunogold labeling of ultra-thin sections cut from high-pressure freezing/frozen substituted samples of *Arabidopsis* transgenic cells and tobacco BY-2 transgenic cells using only gold particle-conjugated secondary antibodies. Scale bars = 200 nm.



Supplemental Figure 9. EXPOs are also labeled by Sec6 and Sec8 antibodies in *Arabidopsis* cells.

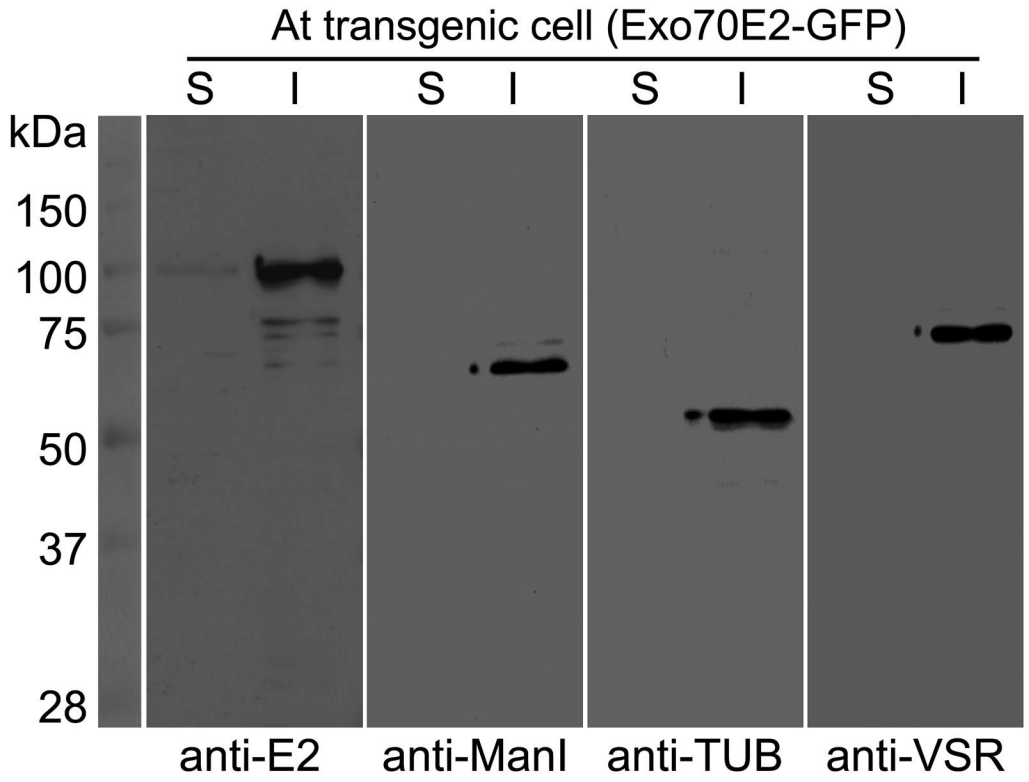
Immunogold labelling of sections cut from high pressure frozen/freeze-substituted samples of *Arabidopsis* cells using Sec6 and Sec8 antibodies as indicated. Scale bars = 200 nm.



Supplemental Figure 10. Immunofluorescence of Exo70E2-GFP *Arabidopsis* transgenic cells using different exocyst subunits antibodies.

Transgenic *Arabidopsis* cells expressing Exo70E2-GFP were chemically fixed and labeled with ExoA1 (a), Sec6 (b) and Sec8 (c) antibodies, followed by confocal imaging.

DIC, differential interference contrast. Scale bar = 50 μm.



Supplemental Figure 11. Immunoblot detection of endogenous *Arabidopsis* proteins.

S and I proteins were prepared from Day-7 transgenic *Arabidopsis* Exo70E2-GFP cells, followed by western blot detection using various antibodies as indicated. S, secretion medium proteins; I, intracellular proteins; TUB, tubulin.

Supplemental Table 1: Primers used in this study.

Exo70E2-GFP-Forward	5'- GGGACTAGTATGGCAGAGTTTGATTCCAAGG -3'
Exo70E2-GFP-Reverse	5'- GGGCTCGAGTCTCTTACGAGAGCTGCGCA -3'
GFP-Exo70E2-Forward	5'- GGGACTAGTATGGCAGAGTTTGATTCCAAGG -3'
GFP-Exo70E2-Reverse	5'- GGGCTCGAGTCATCTCTTACGAGAGCTGCGC -3'
Exo70E2 N-terminus-Forward	5'- GGGGAATTC ATGGCAGAGTTTGATTCCAAGGTTC -3'
Exo70E2 N-terminus-Reverse	5'- GGGCTCGAG TCAGTCTCCCAAAATCTGATCACAT-3'
Exo70E2 C-terminus-Forward	5'- GGGGAATTC TTTGAGTCAATTTCTACAGCTTGCTT-3'
Exo70E2 C-terminus-Reverse	5'- GGGCTCGAG TCATCTCTTACGAGAGCTGCGC-3'
Exo70A1-Forward	5'- GGGACTAGTATGGCTGTTGATAGCAGAATGGATC -3'
Exo70A1-Reverse	5'- GGGGGTACCCCGGCGTGGTTCATTCATAGACTTTC -3'
Exo70B1-Forward	5'- GGGCCCGGGATGGCGGAGAATGGTGAAGAGAAG -3'
Exo70B1-Reverse	5'- GGGACTAGTTTTTCTTCCCGTGGTAGTCCCTTTG -3'
Exo70B2-Forward	5'- GGGACTAGTATGGCTGAAGCCGGTGACGAGAATC -3'
Exo70B2-Reverse	5'- GGGGGTACCACTTGAGCTTTCCTTGAACAACCG -3'
Exo70D1-Forward	5'- GGGACTAGTATGGAACCACATGACCAAACTCAC -3'
Exo70D1-Reverse	5'- GGGGGTACCCTCGGATCGTCTTCTCAGATGCTG -3'
Exo70D2-Forward	5'- GGGACTAGTATGGCAACACCGGAGACTCGTGG -3'
Exo70D2-Reverse	5'- GGGGGTACCCTGAGACCGTCTCAAATGTGGGG
Exo70E1-Forward	5'- GGGTCTAGAATGGGAGAGTTTGAGGTAGATGG -3'
Exo70E1-Reverse	5'- GGGGGTACCTCTTCTATAAGAATTGTTCAAGG -3'
Exo70F1-Forward	5'- GGGTCTAGAATGGCCGCAACAACAACCACGGCGGCGAGTA -3'
Exo70F1-Reverse	5'- GGGGGTACCACTTTTCCTTCTCGGGTGGTGAAT -3'
SAMS2-Forward	5'- GGGTCTAGAATGGAGACTTTCCTATTCACATCTG -3'
SAMS2-Reverse	5'- GGGCTCGAGAGCTTGAGGTTTGTCCCACTT -3'