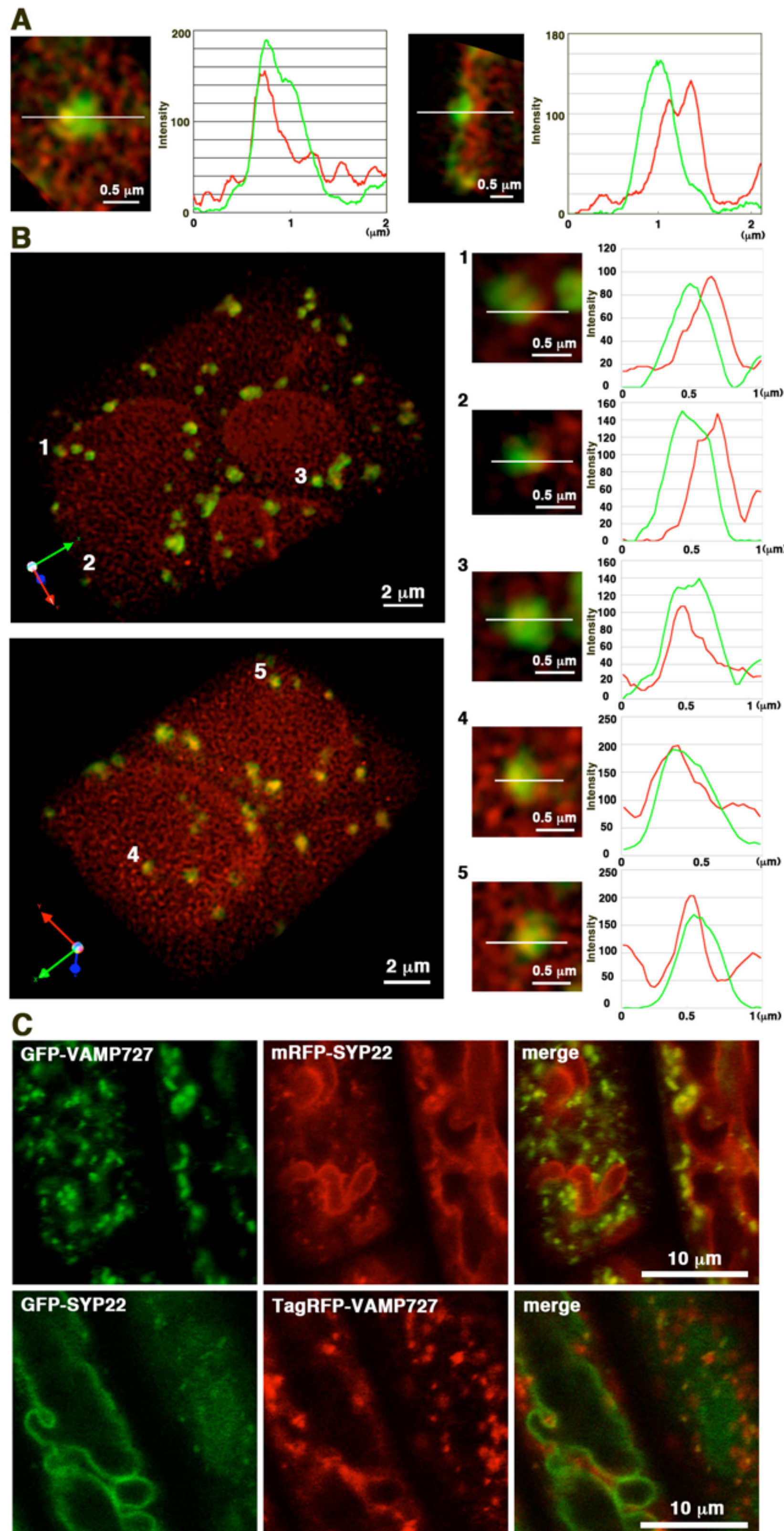
Supplemental Data. Ebine et al. (2008) A SNARE Complex Unique to Seed Plants Is Required for Protein Storage Vacuole Biogenesis and Seed Development of Arabidopsis thaliana.

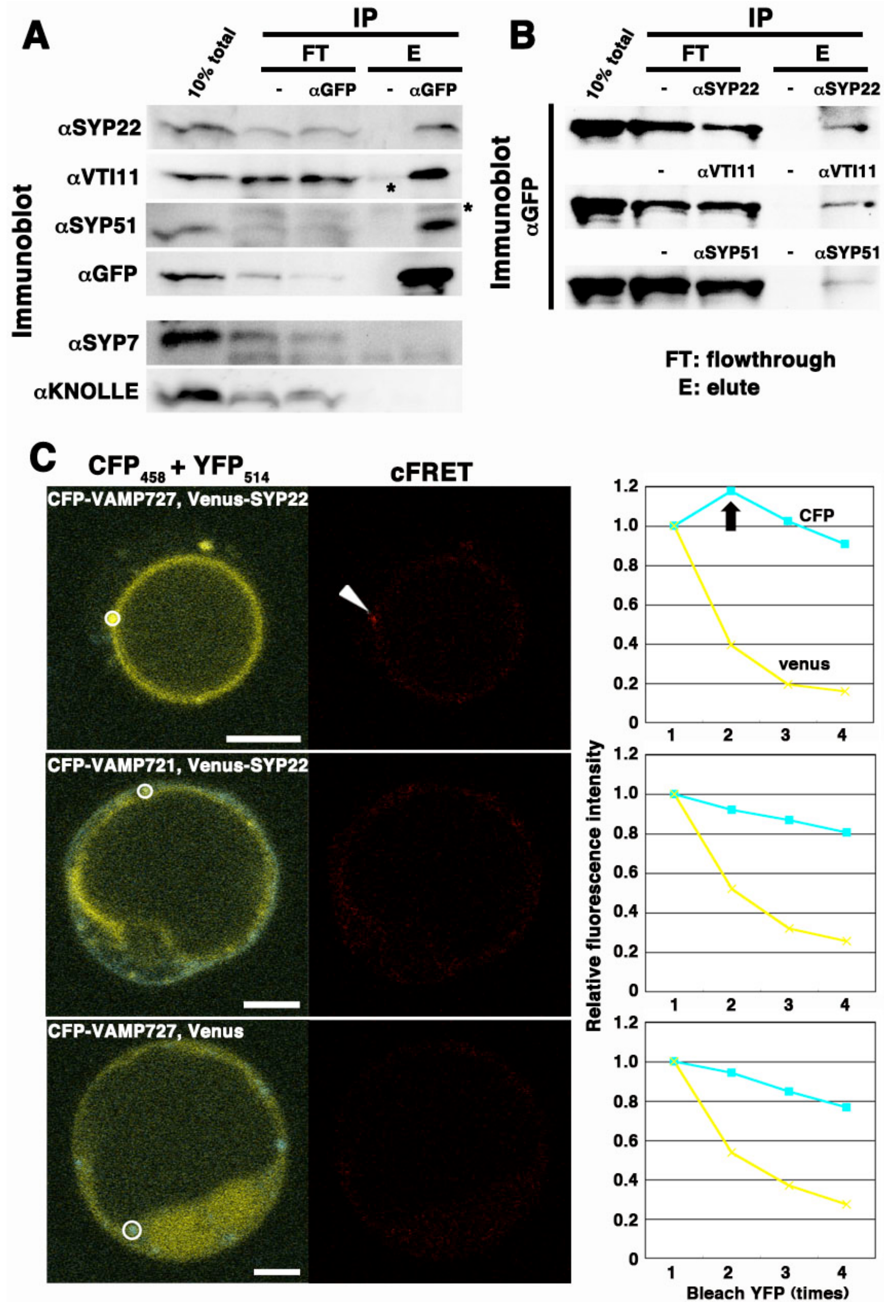


Supplemental Figure 1. Colocalization of VAMP727 and SYP22.

(**A**) Colocalization of GFP-VAMP727 and mRFP-SYP22 was quantified with a line-scan analysis application of ImageJ software. Intensity of signals in top-viewed (left) and side-viewed (right) images of the endosome presented in lower panel of Figure 5E was analyzed.

(**B**) Line-scan analysis of colocalization between GFP-VAMP727 and mRFP-SYP22 on endosomes. Fluorescence intensity of endosomes numbered in left panels, which show 3D-reconstructed images acquired with the high-performance microscope we developed, was quantified.

(**C**) Colocalization of VAMP727 and SYP22 was also observed even when a different combination of fluorescent proteins was employed.



Supplemental Figure 2. VAMP727 Forms a Complex with SYP22, VTI11, and SYP51.

(**A**) and (**B**) Eluted samples (E) of immunoprecipitation were loaded with flow-through fractions (FT). Samples were prepared in the same way as samples used in Figure 6.

(**C**) Complex formation of VAMP727 and SYP22 was confirmed by fluorescence resonance energy transfer (FRET) analysis. CFP-VAMP727 and Venus-SYP22 under the regulation of 35S

promoter were subcloned in $pBSIIKS^+$ and pUC18, respectively, and introduced into protoplasts prepared from Arabidopsis suspension cells, as we previously described (Ueda et al., 2001). Protoplasts expressing fluorescent proteins were observed under the confocal laser scanning microscope, LSM510META (Zeiss). Fluorescence from CFP (477.9–520.7 nm) and Venus (531.4–563.5 nm), which were excited by the laser at 458 nm and 514 nm wavelengths, respectively (CFP_{458} and YFP_{514}), were measured. The effect of bleed through was estimated using images of cells expressing either of CFP-VAMP727 and Venus-SYP22. The corrected FRET (cFRET) was calculated according to Sorkin et al (2000). FRET was observed more efficiently between CFP-VAMP727 and Venus-SYP22 than between CFP-VAMP721 and Venus-SYP22 or CFP-VAMP727 and soluble Venus, which were confirmed by the acceptor bleach experiment (Miyawaki and Tsien., 2000). Fluorescence intensity in the circles was measured and plotted. The arrow indicates increase in fluorescence from the donor, CFP-VAMP727 upon bleaching the Venus-SYP22. The acceptor bleach experiment was repeated at least five times, which yielded similar results to those shown in the figure. It should be noted that the FRET was observed on the endosome (arrowhead), which was associated with a vacuole.

Supplemental Table 1. Primer sequences used for amplification of genomic fragments.

Primer name	Sequence
genomic VAMP727 for	CACCGAGGATGTCAAACTCAGACTGATC
genomic VAMP727 rev	CAAGTTTGATTGTCATCTGGAGATTTATC
genomic VAMP721 for	CACCATGCTCTGACTAAAAATGTT
genomic VAMP721 rev	AAGGATATGGCGGCTCCTCA
genomic VAMP713 for	CACCGTGAACTGTAATGTGTATTA
genomic VAMP713 rev	AGGAGGTTCTTGAAGCTACA
genomic SYP43 for	CACCCACCCTAACCGCACAAACTGGACATAC
genomic SYP43 rev	AGATGCACTGCGGACCTTAAGAGGTAA
genomic SYP22 for	CACCCAACACTTGCGTTAAGCTCCTTGTGTG
genomic SYP22 rev	CACTTCCTTAGACGACTCAGAGTTTCG
genomic SYP21 for	CACCGATGGTTGCTCAGCTTCAACCTTCTCT
genomic SYP21 rev	GTTTTCACCCAACACAGTTCAGTCAGC
genomic RHA1 for	CACCCTTTTGACCCTTGTGCAGTCGCC
genomic RHA1 rev	TCTGAAACTCACAGAGGCTTGATCTCAGAG

SUPPLEMENTAL REFERENCES

Miyawaki, A., and Tsien, R.Y. (2000). Monitoring protein conformations and interactions by fluorescence resonance energy transfer between mutants of green fluorescent protein. Methods Enzymol **327,** 472-500.

Sorkin, A., McClure, M., Huang, F., and Carter, R. (2000). Interactions of EGF receptor and Grb2 in living cells visualizedby fluorescence resonance energy transfer (FRET) microscopy. Curr. Biol. **10,** 1395–1398.

Ueda, T., Yamaguchi, M., Uchimiya, H., and Nakano, A. (2001). Ara6, a plant-unique novel type Rab GTPase, functions in the endocytic pathway of *Arabidopsis thaliana*. EMBO J. **20,** 4730-4741.