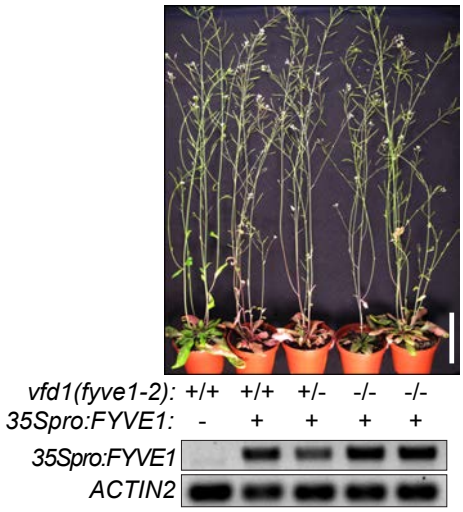
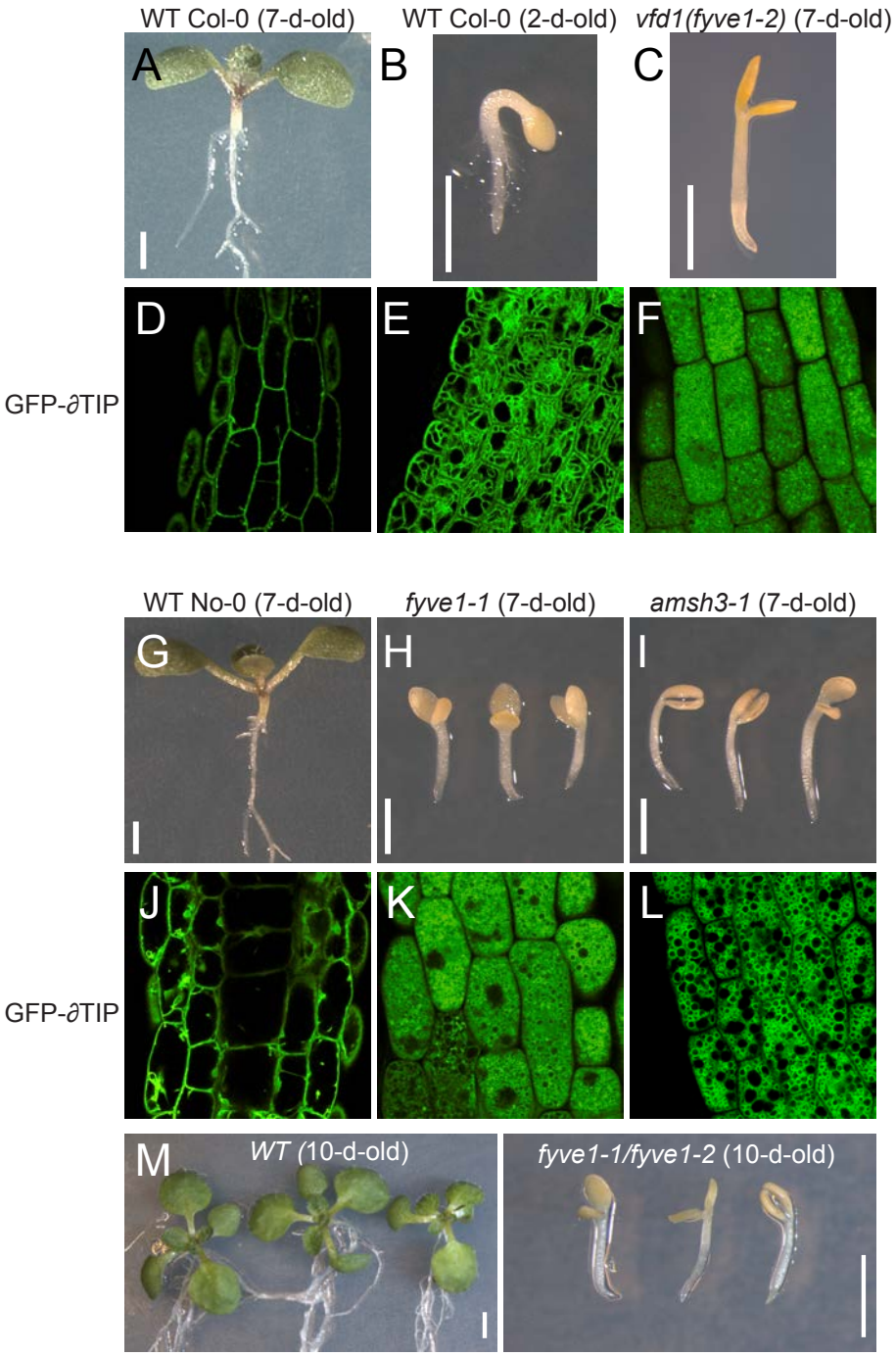
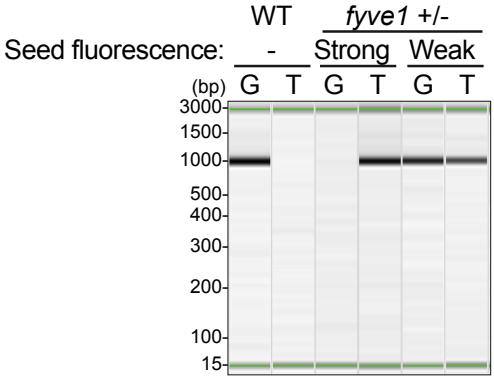


Supplemental Figure 1. Complementation of the *vfd1(fyve1-2)* mutant. Photographs of *vfd1/fyve1-2* mutant lines that express a *35Spro:FYVE1* construct are shown in comparison to wild-type plant at the same age (upper panel). Expression of the transgene was verified by RT-PCR using transgene- and *ACTIN2*-specific primers (bottom panel). Scale bar: 6 cm.



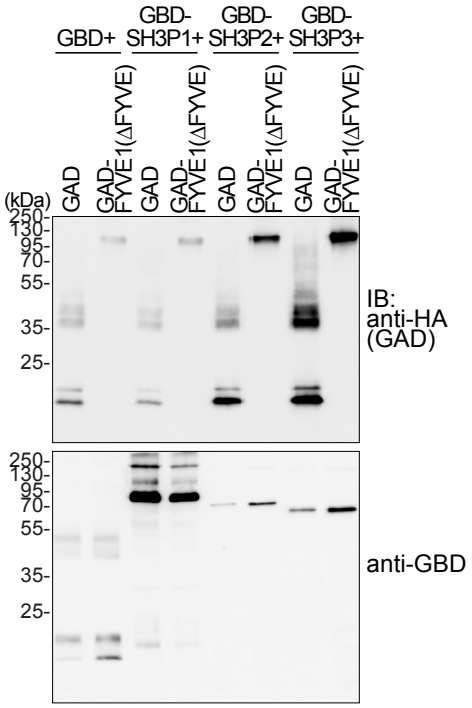
Supplemental Figure 2. Seedling- and vacuole phenotypes of *fyve1-1*, *fyve1-2* and *amsh3-1*.

(A to F) Photographs of the seedling and vacuole phenotypes of 7-day-old (A and D) and 2-day-old (B and E) wild type (Col-0) and 7-day-old *vfd1fyve1-2* mutant (C and F) are shown. Note that the *vfd1/fyve1-2* seedling is approximately the size of a 2-day-old wild-type seedling. Vacuolar membranes of the seedlings are visualized with the GFP=∂TIP mareker. (G to L) Photographs of the seedling and vacuole phenotypes of 7-day-old wild type (No-0) (G and J), 7-day-old *fyve1-1* (H and K) and 7-day-old *amsh3-1* (I and L) are shown. Scale bars: 1mm. (M) Transheterozygous *fyve1-1/fyve1-2* mutants show the same seedling lethal phenotype as the *fyve1-1-, fyve1-2* homozygous mutants. Scale bars: 2 mm.



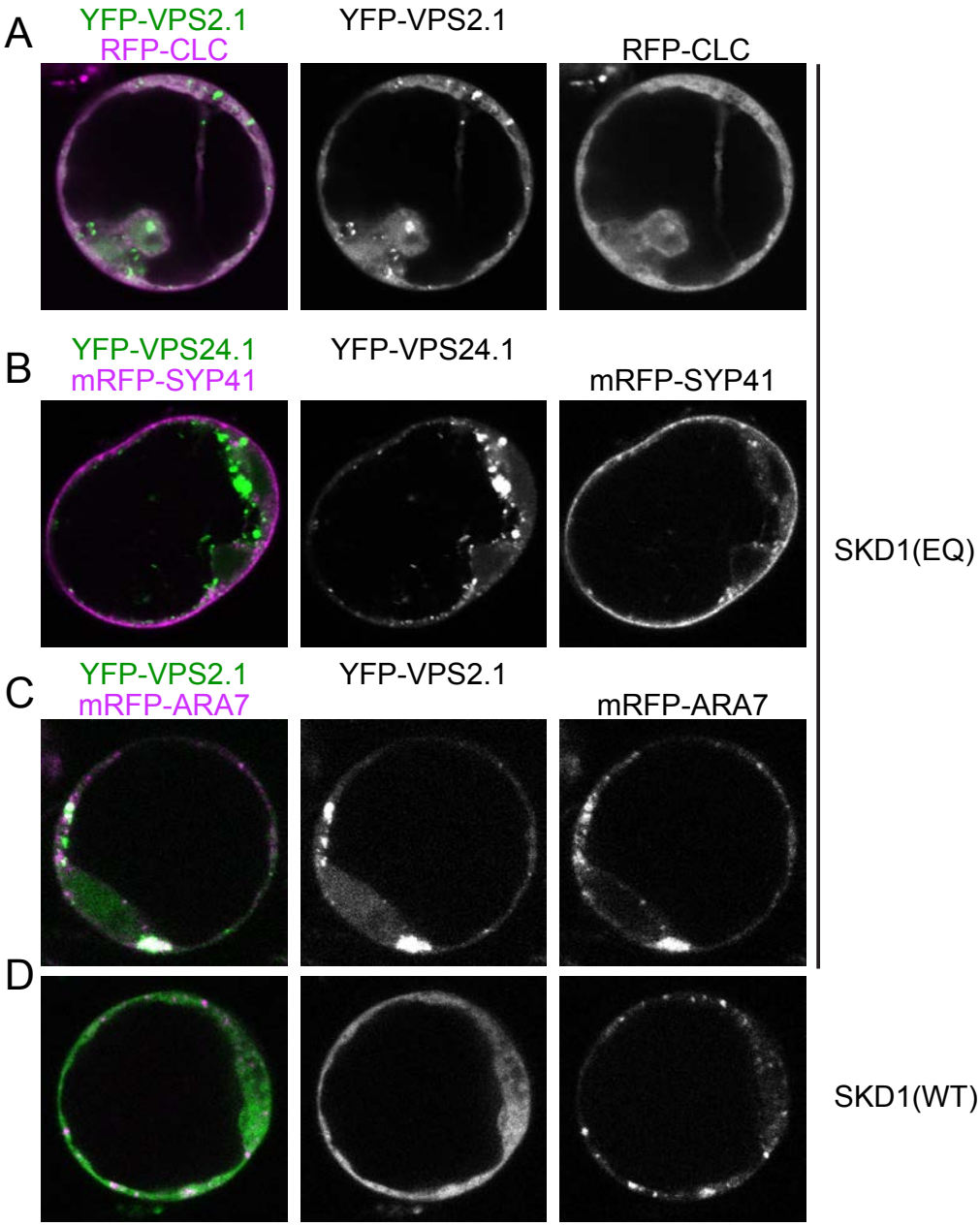
Supplemental Figure 3. Seeds with strong green fluorescence are homozygous for the *fyve1-1* transposon insertion.

PCR-based genotyping analysis was conducted using genomic DNA extracted from seeds from wild type or a *fyve1-1* heterozygous plant containing GFP-CT24. Among the seeds derived from a heterozygous mother plant, seeds with strong and weak green fluorescence observed. 10 seeds from each population shown in Figure 5A were subjected to genotyping analysis. Lanes G indicate gene-specific PCR products and Lanes T indicate transposon-specific PCR products. Note that the pool of strongly fluorescing seeds acorrespond to homozygous *fyve1-1*.



Supplemental Figure 4. Expression of the constructs used for YTH analysis.

Immunoblot of yeast total protein extracts from yeast cells used in Figure 8. Anti-HA and anti-GBD antibodies were used to detect GAD- and GBD-fused proteins, respectively. The GAD-fusion vector pGADT7 contains an HA-tag enabling the detection of the fusion protien with an anti-HA antibody.



Supplemental Figure 5. Coexpression of SKD1(EQ) specifically affect the late endosomal marker ARA7.
Localization of ESCRT-III subunits YFP-VPS2.1 or YFP-VPS24.1 together with RFP-CLC (A), the TGN/early endosome-marker mRFP-SYP43 (B) and the late endosome marker mRFP-ARA7 (C) upon co-expression with SKD1 (EQ). Localization of YFP-VPS2.1 and mRFP-ARA7 upon coexpression with SKD1(WT) is shown in (D). Note that only mRFP-ARA7 relocates to the SKD1(EQ)-induced class-E compartments together with the ESCRT-III subunit VPS2.1.

Supplemental Table 1: Primers used in this study

Primer	Sequence
CK40 FYVE1 GW fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGCAACA GGGAGATTAC
CK41 FYVE1 GW fw	GGGGACCACTTTGTACAAGAAAGCTGGGTTCAATGTG CGCTAACGAG
CK131 FYVE1 GW fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGCAACAG GGAGATTAC
CK140 ΔFYVE fw	GGCATTACTCAACCTATTAACAGGCTTTAT
CK141ΔFYVE rv	ATAAAGCCTGTTAATAGGTTGAGTAATGCC
EI511 Genotyping fw	GCGACATCACTAAACCC
EI512 Genotyping rv	AACCCACCAACATAAGAAC
EI528 FYVE fw Bam	AAGGGGATCCGCAACCGTAGCTGGTC
EI529 FYVE rv Sal	AAGGGTCGACAAAGCTAATGGATCGTCC
EI541 tFYVE1 qRT fw	GCAACAGGGAGATTACAATTCG
EI542 tFYVE1 qRT rv	TCGGAGTAGGATTTTGAAATTGA
MN53 SH3P2 GW fw	AAAAAGCAGGCTATGGATGCAATTAGAAAACA
MN54 SH3P2 GW rv	AGAAAGCTGGGTTGAAAACTTCGGACACTTTG
MN215 SH3P1 fw NcoI	AAGGCCATGGTGATCATCACAATCATC
MS8 SH3P1 rv XhoI	GGAACTCGAGTCACTGTTGCTTGGAGTT
MS9 SH3P3 fw EcoRI	GGAAGAATTCATGGATGCGTTTAGAAGAC
MS10 SH3P3 rv SalI	GGAAGTCGACTCAGTAAACTTCAGCAGCA
MS11 SH3P2 fw EcoRI	GGAAGAATTCAGTGATGCAATTAGAAAACA
MS12 SH3P2 rv SalI	GGAAGTCGACTCAGAAAACTTCGGACACT
SH3P2-up5 fw	CACCCACACCAATGGCGACATAAC
SH3P2-dw3 rv	CTATCAAATAAAAGAAGATCC
VC11 FYVE1(ΔFYVE) fw BamHI	AAGGGGATCCGTATGCAACAGGGAGATTACA
VC12 FYVE1(ΔFYVE) rv SalI	AAGGGTCGACTCAATGTGCGCTAACG
ACTIN fw	GGGCTGTTTTTCCCA
ACTIN rv	GGCCTTGGAGATCCA
Ds5-2a	TCCGTTCCGTTTTCGTTTTTTAC