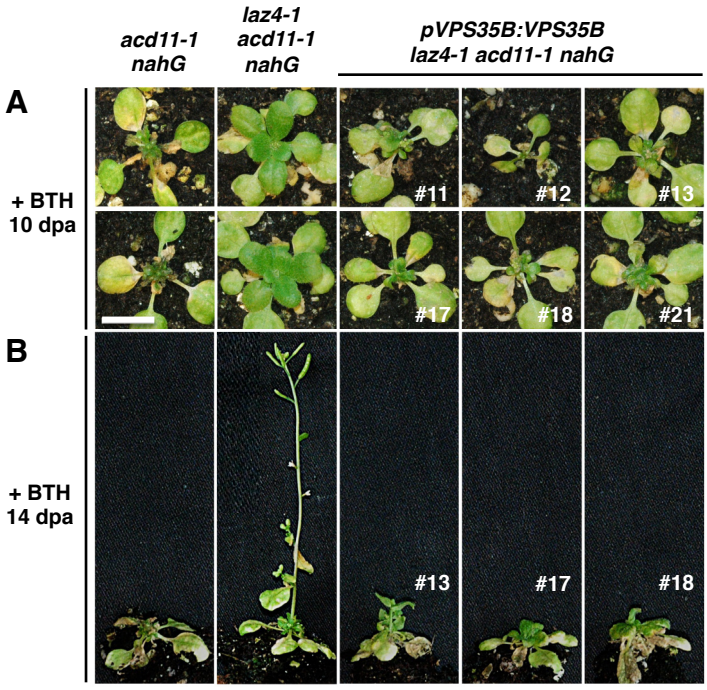
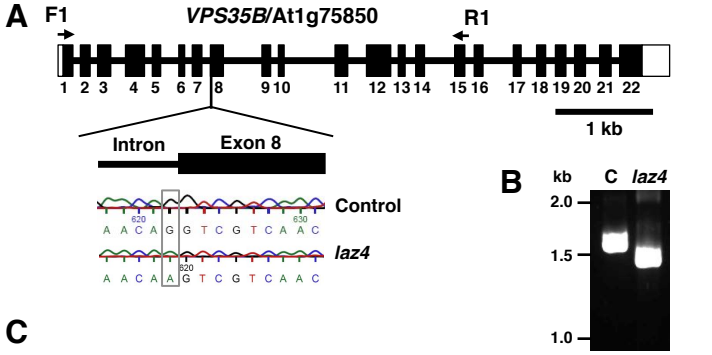


Supplemental Figure 1. Transgenic complementation of *laz4* mutant.

L*er laz4-1 acd11-1 nahG* plants were transformed with a 7.6 kb genomic fragment of the wild-type *LAZ4/ VPS35B* locus containing 1.5 kb of the predicted promoter, the transcribed region, and 300 bp of 3´ untranslated region. T3 progenies of several independent transgenic lines were selected on MS medium, transferred to soil and treated with 100 μM BTH (4 times every 2nd day).

(A) Rosette phenotypes of 34-day-old *acd11-1 nahG*, *laz4-1 acd11-1 nahG* (2 plants each), and six transgenic lines expressing *pVPS35B:VPS35B* in *laz4-1 acd11-1 nahG* backgrounds 10 days after BTH treatment. Bar indicates 1 cm.

(B) Reproductive development is restricted to *laz4 acd11-1 nahG* plants and inhibited by cell death activation in *acd11-1 nahG* and *pVPS35B:VPS35B* expressing *laz4-1 acd11-1 nahG* lines. Photos are of 38-day-old plants 14 days after BTH treatment. dpa, days post application.



C

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VPS35B  TGTGTCGAGGTGTTCAACATCCTATTCGTGGCCTCTTTTTGAGGAGTTATCTCGCTCAAGTGAGCAGGGATAAGTTACCT  480
laz4    TGTGTCGAGGTGTTCAACATCCTATTCGTGGCCTCTTTTTGAGGAGTTATCTCGCTCAAGTGAGCAGGGATAAGTTACCT  480

VPS35B  GAGATTGGCTCAGACTACGAAGGAGATGCAAATACTGTGATGGATGCTGTGGAGTTTGTGCTACAAAATTTCACAGAGAT  560
laz4    GAGATTGGCTCAGACTACGAAGGAGATGCAAATACTGTGATGGATGCTGTGGAGTTTGTGCTACAAAATTTCACAGAGAT  560

VPS35B  GAATAAGCTTTGGGTTCGAATACAGCATCAGGGACCAGGAACAGTTCGAGAAAAGCAGGAGAAAGAGAGGAACGAACTTC  640
laz4    GAATAAGCTTTGGGTTCGAATACAGCATCAGGGACCAGGAACAGTTCGAGAAAAGCAGGAGAAAGAGAGGAACGAACTTC  640

VPS35B  GTGATCTTGTTGGAAAAAAATCTGCATGTTCTAGGACAGATAGAAGGTGTTGACCTTGAGATGTACAAAGAGACTGTTCTT  720
laz4    GTGATCTTGTTGGAAAAAATCTGCATGTTCTAGGACAGATAGAAGGTGTTGACCTTGAGATGTACAAAGAGACTGTTCTT  720

VPS35B  CCTAGGGTCTTAGAGCAGGTCGTCAACTGTAAAGATAAGTTGGCTCAGTATTATCTGATGGAGTGCATAATTCAAGTCTT  800
laz4    CCTAGGGTCTTAGAGCAG----------------------------------------------------------------  737

VPS35B  TCCTGATGAGTACCATTTGCAGACTCTTGAGACCTTATTGGCTGCTTGTACCCAACTGATGCCAACTGTTGACACGAAGA  880
laz4    ----------------------------------------------------------CCAACTGTTGACACGAAGA  757

VPS35B  TAGTGTTAACCCAACTAATGGACCGGTTATCAAACTATGCTGCCTCAAGTCCAGATGTGTTACACGAATTTTTGCAAGTG  960
laz4    TAGTGTTAACCCAACTAATGGACCGGTTATCAAACTATGCTGCCTCAAGTCCAGATGTGTTACACGAATTTTTGCAAGTG  837

VPS35B  GAAGCTTTTGCTAAATTGAGCAATGCAATTGGAAAGGTGATAGATACACAGCTTGAGATGCCTATTGTCGGAGCTATGAC 1040
laz4    GAAGCTTTTGCTAAATTGAGCAATGCAATTGGAAAGGTGATAGATACACAGCTTGAGATGCCTATTGTCGGAGCTATGAC  917

VPS35B  TCTGTTTGTCTCTCTTTTGACTTTTACTCTCCGAGTTCATCCTGATCGGCTTGACTATGTGGATCAAGTATTGGGTGCAT 1120
laz4    TCTGTTTGTCTCTCTTTTGACTTTTACTCTCCGAGTTCATCCTGATCGGCTTGACTATGTGGATCAAGTATTGGGTGCAT  997

VPS35B  GTGTAGTTAAGCTTTCCAGTGTGCCCAAGCTGGAGGACGCCCGTGCAATGAAACAAGTAGTGGCACTTTTGAGTGCTCCT 1200
laz4    GTGTAGTTAAGCTTTCCAGTGTGCCCAAGCTGGAGGACGCCCGTGCAATGAAACAAGTAGTGGCACTTTTGAGTGCTCCT 1077
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D

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laz4    MRTLAGVEDEDKWLAEGIAGIQHNAFFMHRALDANNLREVLKYSALMLSELRTSKLSPQKYYDLYMRAFDQLRQLEIFFK  80

VPS35B  DESRHGLPVVDLYELVQHAGNILPRMYLLCTVGSVYIKSKQAPSKDVLKDLVEMCRGVQHPIRGLFLRSYLAQVSRDKLP 160
laz4    DESRHGLPVVDLYELVQHAGNILPRMYLLCTVGSVYIKSKQAPSKDVLKDLVEMCRGVQHPIRGLFLRSYLAQVSRDKLP 160

VPS35B  EIGSDYEGDANTVMDAVEFVLQNFTEMNKLWVRIQHQGPGTVREKQEKERNELRDLVGKNLHVLGQIEGVDLEMYKETVL 240
laz4    EIGSDYEGDANTVMDAVEFVLQNFTEMNKLWVRIQHQGPGTVREKQEKERNELRDLVGKNLHVLGQIEGVDLEMYKETVL 240

VPS35B  PRVLEQVVNCKDKLAQYYLMECIIQVFPDEYHLQTLETLLAACTQLMPTVDTKIVLTQLMDRLSNYAASSPDVLHEFLQV 320
laz4    PRVLEQ----------------------------------------PTVDTKIVLTQLMDRLSNYAASSPDVLHEFLQV 279

VPS35B  EAFAKLSNAIGKVIDTQLEMPIVGAMTLFVSLLTFTLRVHPDRLDYVDQVLGACVVKLSSVPKLEDARAMKQVVALLSAP 400
laz4    EAFAKLSNAIGKVIDTQLEMPIVGAMTLFVSLLTFTLRVHPDRLDYVDQVLGACVVKLSSVPKLEDARAMKQVVALLSAP 359

VPS35B  LEKYSDIVTALTLSNYPRVMDHLDDGTNKVMAMLIIQSIMKTDSCISTADKVEVLFELIKGLIKDLDETNAEELDEEDFQ 480
laz4    LEKYSDIVTALTLSNYPRVMDHLDDGTNKVMAMLIIQSIMKTDSCISTADKVEVLFELIKGLIKDLDETNAEELDEEDFQ 439

VPS35B  EEQNSVARLIHMLDNEEPEEMLKIICVVRRHLMTGGPRRLPFTVPPLVFSAVRLVRQLESQGGDIAGEDSATPRKIFQIL 560
laz4    EEQNSVARLIHMLDNEEPEEMLKIICVVRRHLMTGGPRRLPFTVPPLVFSAVRLVRQLESQGGDIAGEDSATPRKIFQIL 519

VPS35B  NQTIEVLTSVPCPELALRLYLQCAEAASDCDLEPVAYEFFTQAFMLYEEEIADSKAQVTAIHLIVGTLQRINVFGVENRD 640
laz4    NQTIEVLTSVPCPELALRLYLQCAEAASDCDLEPVAYEFFTQAFMLYEEEIADSKAQVTAIHLIVGTLQRINVFGVENRD 599

VPS35B  TLTHKATGYSARLLKKPDQCRAVYACSHLFWVDDPDGIKDGERVLLCLRRALRIANAAQQMASATRGSSGPVTLFVEILN 720
laz4    TLTHKATGYSARLLKKPDQCRAVYACSHLFWVDDPDGIKDGERVLLCLRRALRIANAAQQMASATRGSSGPVTLFVEILN 679

VPS35B  KYIYFFEKGNPHITPSDIQSLIELINNEMQSDNGNTTIHSDPFFTSTLRYIKFIKQKGGLMGEKYDPIKL 790
laz4    KYIYFFEKGNPHITPSDIQSLIELINNEMQSDNGNTTIHSDPFFTSTLRYIKFIKQKGGLMGEKYDPIKL 749
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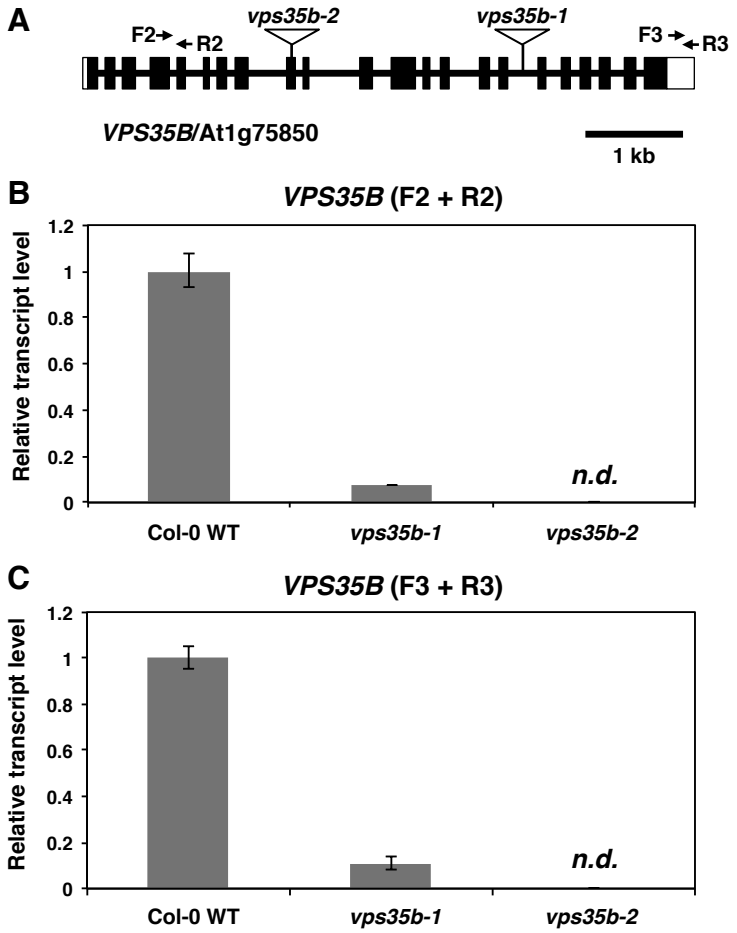
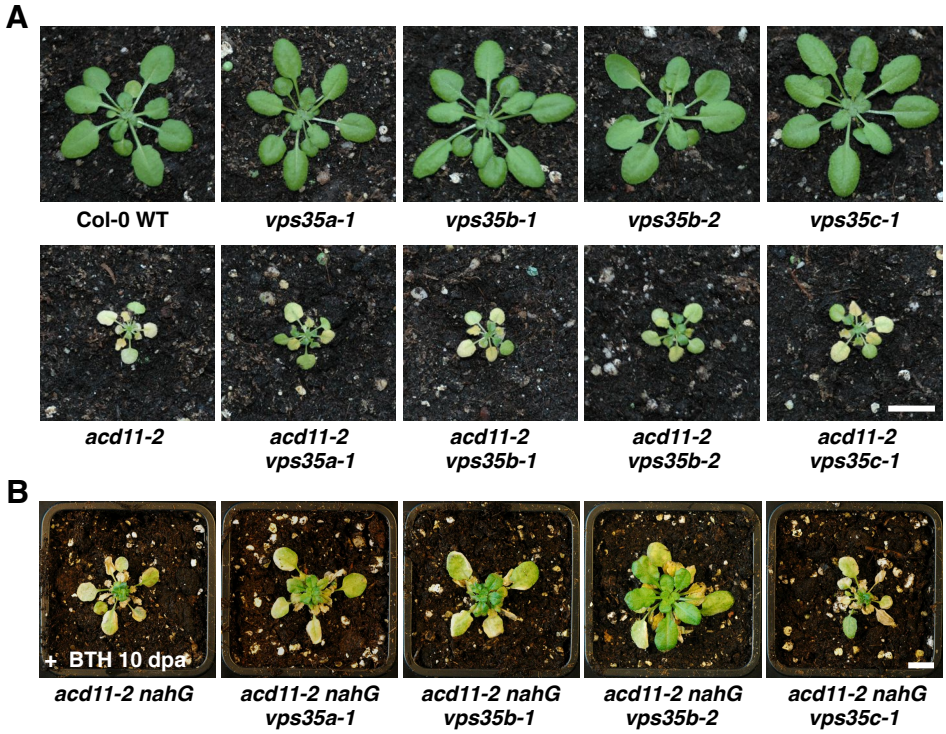
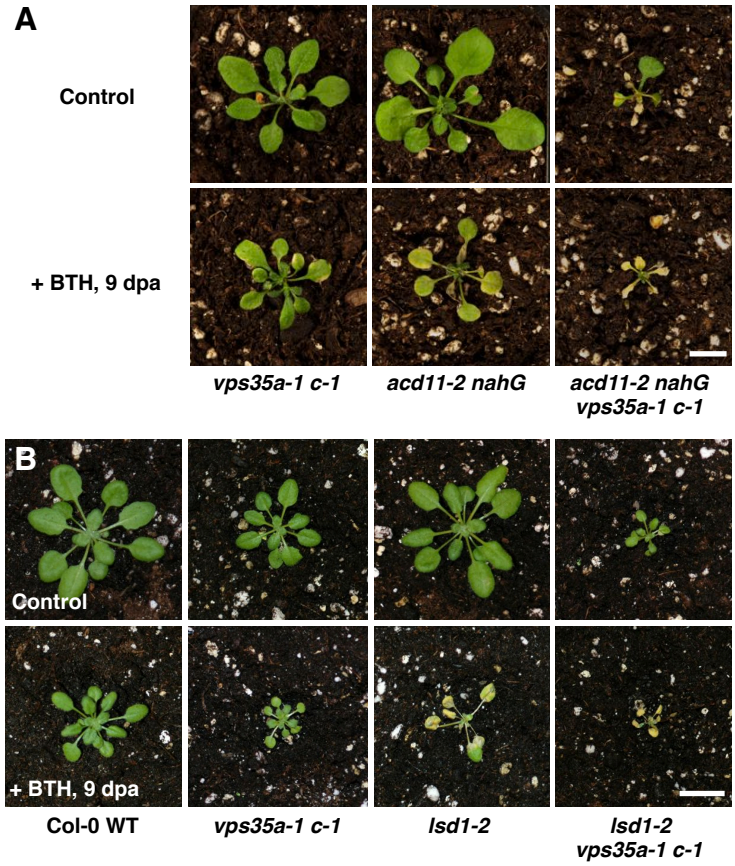
Supplemental Figure 2. EMS-induced intron splice mutation in *laz4* results in deletion of exon 8 from *VPS35B* transcripts.
(A) Exon-intron structure of *VPS35B* showing positions of EMS-induced G to A transition in *laz4* and exon-specific primers F1 and R1 used for PCR amplification. Filled boxes reflect exons of coding sequences, unfilled boxes are untranslated regions, and lines connecting exons represent introns.
(B) cDNA products amplified with F1 and R1 indicate reduced transcript size in *laz4* compared to the wild-type control (C).
(C) Sequencing of amplified cDNA products verified an in-frame 123 bp deletion (nucleotides 719-861) in *VPS35B* transcripts corresponding to the loss of entire exon 8. Alignment corresponds to nucleotides 400-1200 of wild-type *VPS35B* coding sequence.
(D) Sequence alignment of translated cDNAs reveals deletion of amino acids 247-287 from VPS35B protein in the *laz4* mutant.



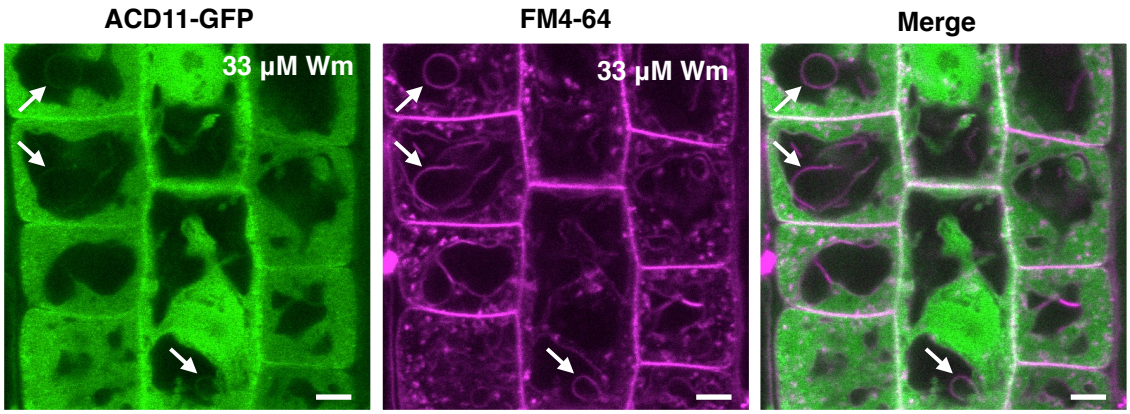
Supplemental Figure 3. Analysis of *VPS35B* transcript levels in T-DNA mutant alleles *vps35b-1* (SALK_014345) and *vps35b-2* (GABI_784C05).
(A) Structure of the *VPS35B* gene showing positions of T-DNA insertions (open triangles) and primers (not to scale) used for real-time quantitative RT-PCR.
(B)-**(C)** *VPS35B* transcript levels in Col-WT, *vps35b-1,* and *vps35b-2* using gene specific primer pairs positioned either **(B)** on exon 4 and 5 (F2, R2) or **(C)** in the 3´-UTR (F3, R3).



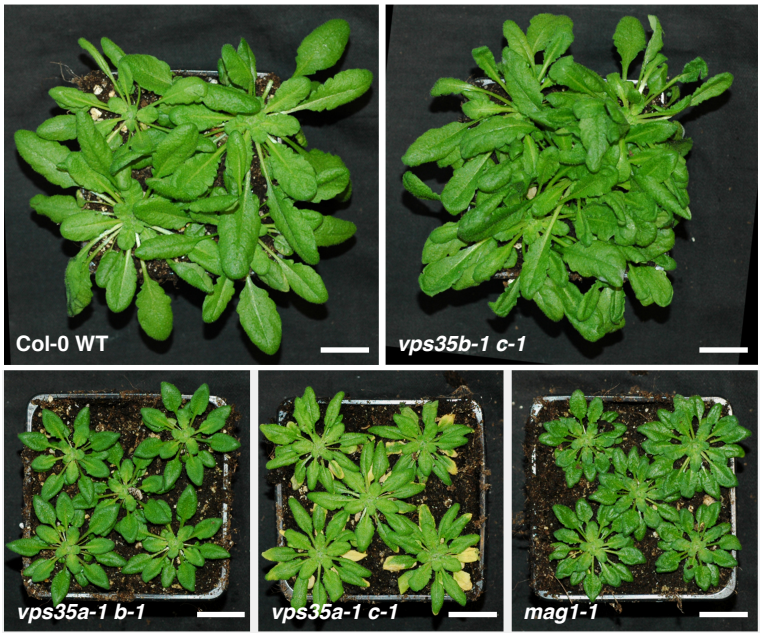
Supplemental Figure 4. Effect of single loss-of-function alleles in VPS35 genes on PCD in *acd11-2* and BTH-treated *acd11-2 nahG.*
(A) Col-0 wild-type (WT), *vps35a-1* (SALK_039689), *vps35b-1* (SALK_014345), *vps35b-2* (GABI_784C05), *vps35c-1* (SALK_099735) (upper panel) as well as *acd11-2, acd11-2 vps35a-1, acd11-2 vps35b-1, acd11-2 vps35b-2,* and *acd11-2 vps35c-1* (lower panel) were grown for 33 days under short-day conditions at 21°C. Bar = 1 cm.
(B) Cell death phenotype of *acd11-2 nahG* harboring *vps35a-1*, *vps35b-1*, *vps35b-2*, and *vps35c-1* in comparison to the *acd11-2 nahG* control. Photos were taken 10 days after BTH treatment of 4-week-old plants.
Bars indicate 1 cm. dpa: days post application.



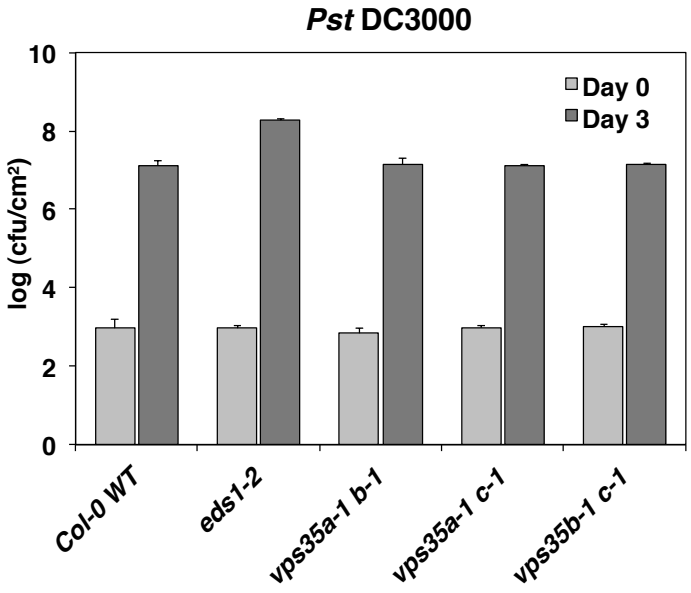
Supplemental Figure 5. Combined loss-of-function mutations in *VPS35A* and *VPS35C* do not suppress BTH-triggered autoimmune cell death in *acd11* and *lsd1*.
(**A**) *vps35a-1 c-1*, *acd11-2 nahG*, and *acd11-2 nahG vps35a-1 c-1* plants were grown for 7 days on MS plates and 21 days in soil under short-day conditions. After treatment with 100 μM BTH, plants were kept for additional 9 days under long-day conditions and photographs were taken in comparison to untreated controls (upper panel).
(**B**). Col-0 WT, *lsd1-2*, *vps35a-1 c-1,* and *lsd1-2 vps35a-1 c-1* plants were grown for 24 days under short day conditions and treated three times with 100 μM BTH. Photographs were taken 9 days after first treatment in comparison to untreated controls (upper panel).
Scale bars indicate 1 cm.



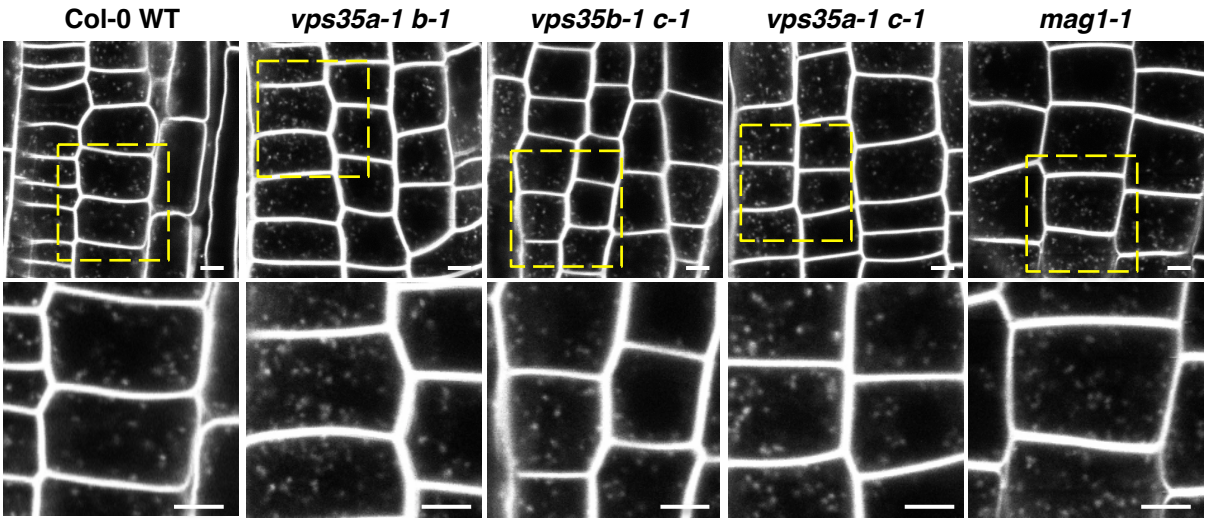
Supplemental Figure 6. ACD11 localizes to vacuole-like structures upon Wortmannin (Wm) treatment. Six-day-old ACD11-GFP expressing seedlings were stained for 5 min with FM4-64, followed by incubation in 33 µM Wortmannin for 90 min. ACD11-GFP colocalizes with FM4-64 on ring-like structures (arrows), suggesting that ACD11 trafficking lies in the late endosomal pathway. Bar = 5 µm



Supplemental Figure 7. Phenotypes of retromer deficient mutants. Different *vps35* double mutant combinations and *mag1-1* plants were soil-grown for 60 days under short-day conditions. *vps35a-1 c-1* plants show signs of early senescence which are absent from similarly dwarfed *vps35a-1 b-1* and *mag1-1* plants. Scale bars indicate 2 cm.



Supplemental Figure 8. Growth of virulent strains of *Pst* DC3000 in Col-0 WT and *vps35a-1 b-1*, *vps35a-1 c-1*, *vps35b-1 c-1*, and *eds1-2* mutants 0 and 3 days after infection. Log-transformed values are means ± standard deviation (SD) with n = 3 in **(A)** and **(B)** or n = 4-6 in **(C)** and **(D)**.



Supplemental Figure 9. Early endocytosis is not affected in retromer mutants. Root tip epidermal cells were imaged after 5 min of FM4-64 staining. Early endosomes appear as scattered FM4-64 labeled punctate structures in the cytosol, and are readily visible in all genotypes (top panel), suggesting that FM4-64 uptake in retromer mutants is as efficient as in the wild-type control. Inset of the top panel is enlarged and shown in the bottom panel for better visualization of early endosomes. Bars = 5 µm.

ADDITIONAL SUPPLEMENTAL MATERIAL

Supplemental Table 1. Oligonucleotides.

Primer name	Sequence (5´-3´)
VPS35B(-1500)-F	CACCTCTCATCATGTGTTCACTGTATCAG
VPS35B-nostop-R	CAGCTTGATAGGGTCATATTTCTCACCCA
VPS35B-stop-R	CTTAGACAACGAAGAACATCTTGAGATAG
VPS29-TOPO-F	CAACATGGTGCTGGTATTGGCATTGG
VPS29-TOPO-R	CGGACCAGAGCTGGTAGTAGGGG
ACD11-5'UTR-F	CACCCTTGCAGCAAAGTAGATTCCGCG
ACD11-nostop-R	CCAATCAATACCGAGTTGCTT
VPS26A-TOPO-F	CACCATGAATTATCTTCTTGGAGCTTT
VPS26A-TOPO-R	TCAAGATGTCTCTTCCTTGAGCC
VPS26B-TOPO-F	CACCATGAATTATCTTCTTGGAGCCTTC
VPS26B-TOPO-R	TCAAGATGATGCGTCTTCCTTCACC
VPS35A-Y2H-F	ATATCCCGGGATGATCGCAGACGGATCAGAAGATGAA
VPS35A-Y2H-R	ATATGTCGACCTATACTTTGATCGCCTGGTATCTCTCACCAA
VPS35B-Y2H-F	ATATGAATTCATGAGAACGCTCGCCGGAGTAGAAG
VPS35B-Y2H-R	ATATGGATCCTCACAGCTTGATAGGGTCATATTTCTCA
VPS35C-Y2H-F	ATATGGATCCATGATCGCCGACGACGATGAGAAGT
VPS35C-Y2H-R	ATATCTGCAGTCATTCAAACCATTCCATTTTGATTTTCTC
VPS29-Y2H-F	ATATGAATTCATGAATTATCTTCTTGGAGCTTTCA
VPS29-Y2H-R	ATATGGATCCTCAAGATGTCTCTTCCTTGAGC
VPS26A-Y2H-F	ATATGAATTCATGAATTATCTTCTTGGAGCCTTC
VPS26A-Y2H-R	ATATGGATCCTCAAGATGATGCGTCTTCCTT
VPS26B-Y2H-F	ATATCCCGGGATGGAGTCTACTGCTAACGCACT
VPS26B-Y2H-R	ATATCTGCAGTCAAGCCTCCTTCTCCCC
VPS35B-F1	CACCATGAGAACGCTCGCCGGAGTAGAAG
VPS35B-R2	ACAAGTGGGGGAACAGTAAAAGGCAA
VPS35B-F2	GGTGTTCAACATCCTATTCGTG
VPS35B-R2	TTGCATCTCCTTCGTAGTCTGA
VPS35B-F3	ATATACTAGTCTCCTGTTGACCGTGCTTTT
VPS35B-R3	ATATGCGGCCGCCTAGCTAGGGCCAACGTTTTT
PP2A-F	TAACGTGGCCAAAATGATGC
PP2A-R	GTTCTCCACAACCGCTTGGT
RNA Helicase-F	CCATTCTACTTTTTGGCGGCT
RNA Helicase-R	TCAATGGTAACTGATCCACTCTGATG

Supplemental Table 2. Plasmids.

Plasmids	Description	Reference
pGWB501- pVPS35B:VPS35B	Binary, $Spec^R$ in bacteria, $Basta^R$ in plants	This study
pGWB504- pVPS35B:VPS35B-sGFP	Binary, $Spec^R$ in bacteria, Hyg^R in plants	This study
pNIGEL17- UBQ10:mCherry-SYP32	transgenic marker line	(Geldner et al., 2009)
pa1RTkan- pVHAa1:VHAa1-mRFP	transgenic marker line	(Dettmer et al., 2006)
pGreenII- pVPS29:VPS29-RFP	transgenic marker line	(Jaillais et al., 2007)
pNIGEL17- UBQ10:mCherry-RabG3F	transgenic marker line	(Geldner et al., 2009)
pSITE-3N1- 35S:VPS35B-YC	Binary, BiFC, $Spec^R$ in bacteria	This study
pSITI-3N1- 35S:VPS26B-YN	Binary, BiFC, $Spec^R$ in bacteria	This study
pGWB554- 35S:VPS29-mRFP	Binary, $Spec^R$ in bacteria, Hyg^R in plants	This study
pGWB554- 35S:mRFP	Binary, $Spec^R$ in bacteria, Hyg^R in plants	This study
pGBT9	Yeast two-hybrid BD vector, Kan^R	Clontech
pGAD424	Yeast two-hybrid AD vector, Amp^R	Clontech
pGBT9-VPS35B	BD fusion, Kan^R	This study
pGAD424-VPS29	AD fusion, Amp^R	This study
pGAD424-VPS26A	AD fusion, Amp^R	This study
pGAD424-VPS26B	AD fusion, Amp^R	This study
pUBN-Dest- UBQ10:GFP-LAZ5	Binary, $Spec^R$ in bacteria, $Basta^R$ in plants	This study
pUBC-Dest- UBQ10:ACD11-GFP	Binary, $Spec^R$ in bacteria, $Basta^R$ in plants	This study
pMDC32- 2x35S:GFP-ATG8A	Binary, Kan^R in bacteria, Hyg^R in plants	This study#
pENTR/D-TOPO	Entry vector for Gateway cloning, Kan^R in bacteria	Invitrogen
pENTR/D-TOPO-pVPS35B:VPS35B	Entry clone for pGWB504- pVPS35B:VPS35B-sGFP	This study
pENTR/D-TOPO-VPS35B	Entry clone for pSITE-3N1- 35S:VPS35B-YC	This study
pENTR/D-TOPO-VPS26B	Entry clone for pSITI-3N1- 35S:VPS26B-YN	This study
pENTR/D-TOPO-VPS29	Entry clone for pGWB554- 35S:VPS29-mRFP	This study
pENTR/D-TOPO-ACD11	Entry clone for pUBC-Dest- UBQ10:ACD11-GFP	This study
pENTR/D-TOPO-LAZ5	Entry clone pUBN-Dest- UBQ10:GFP-LAZ5	This study

#gift from Elena A. Minina (Uppsala, Sweden)

SUPPLEMENTAL METHODS

Transgenic complementation

For complementation analysis, a 7.6 kb fragment of *VPS35B* was amplified from genomic DNA (L*er acd11 nahG*) using the primer pair VPS35B(-1500)-F/VPS35B-stop-R (see Supplemental Table 1). After subcloning into pENTR/D-TOPO (Life Technologies, Carlsbad, CA, US), the fragment was recombined via the LR reaction into the gateway destination vector pGWB501 (Nakamura et al., 2009). The construct was verified by sequencing, electroporated into *Agrobacterium tumefaciens* strain GV3101 and transformed into *laz4-1 acd11-2 nahG*. Transgenic plants were selected on MS-plates with 20 µg/mL hygromycin B, and transferred to soil.

Quantitative RT-PCR

Total RNA was extracted from frozen samples using the RNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. Prior to cDNA synthesis, all RNA samples were DNase treated using the Turbo DNA-free Kit (Ambion, Austin, Texas, USA). Yield and integrity of the RNA were assessed using a NanoDrop micro photometer (NanoDrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis. First-strand cDNA was synthesized from 1 µg of total RNA by combined random hexamer and oligo dT priming using the First Strand cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD, USA). Oligonucleotide primers of *VPS35B* and two reference genes *PP2A* (At1g13320) and *RNA Helicase* (At1g58050) (Czechowski et al., 2005) were designed and their amplification efficiency optimized (Supplemental Table S1). Quantitative PCR reactions were performed using iCycler MyiQ2 (Bio-Rad, Hercules, CA, USA) and DyNAmo Flash SYBR Green qPCR Kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR amplifications were repeated at least three times on independent RNA samples. Calculations and statistical analyses were carried out as described in ABI PRISM 7700 Sequence Detection System User Bulletin #2 (Applied Biosystems, Austin, Texas, USA) (Avrova et al., 2003).

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