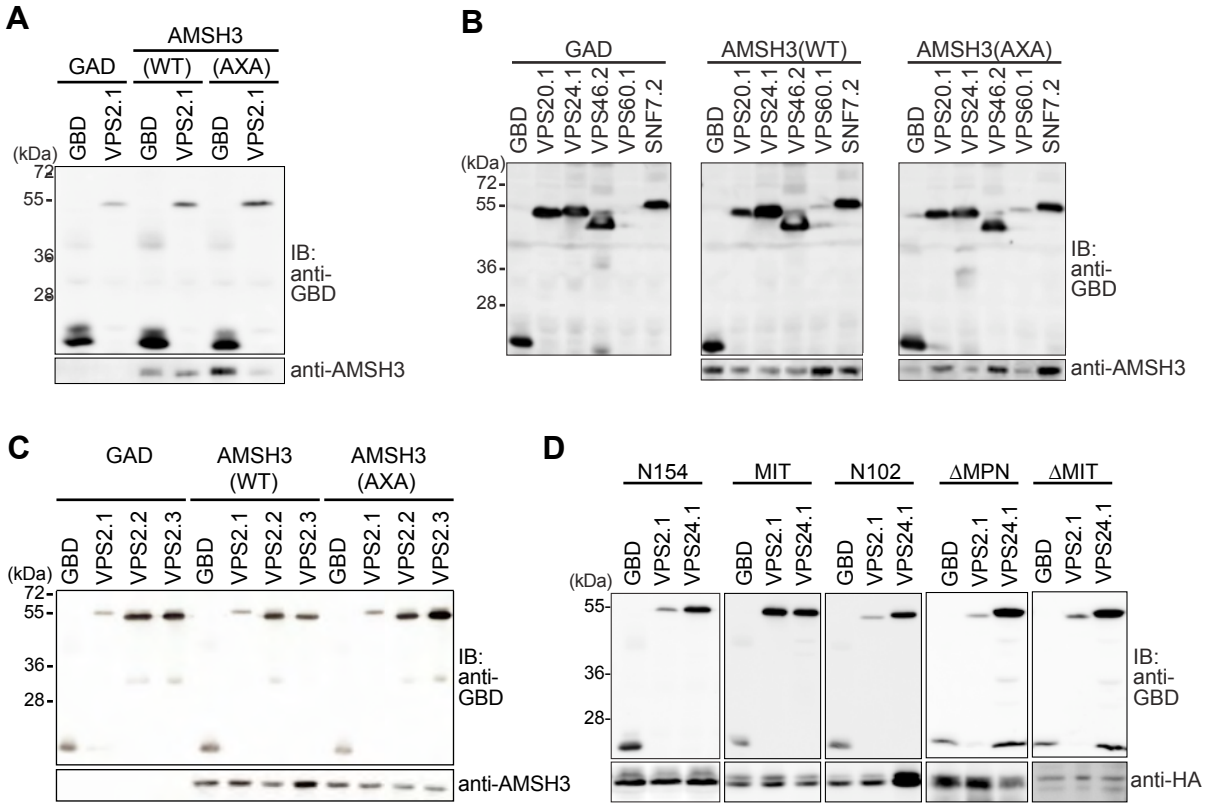
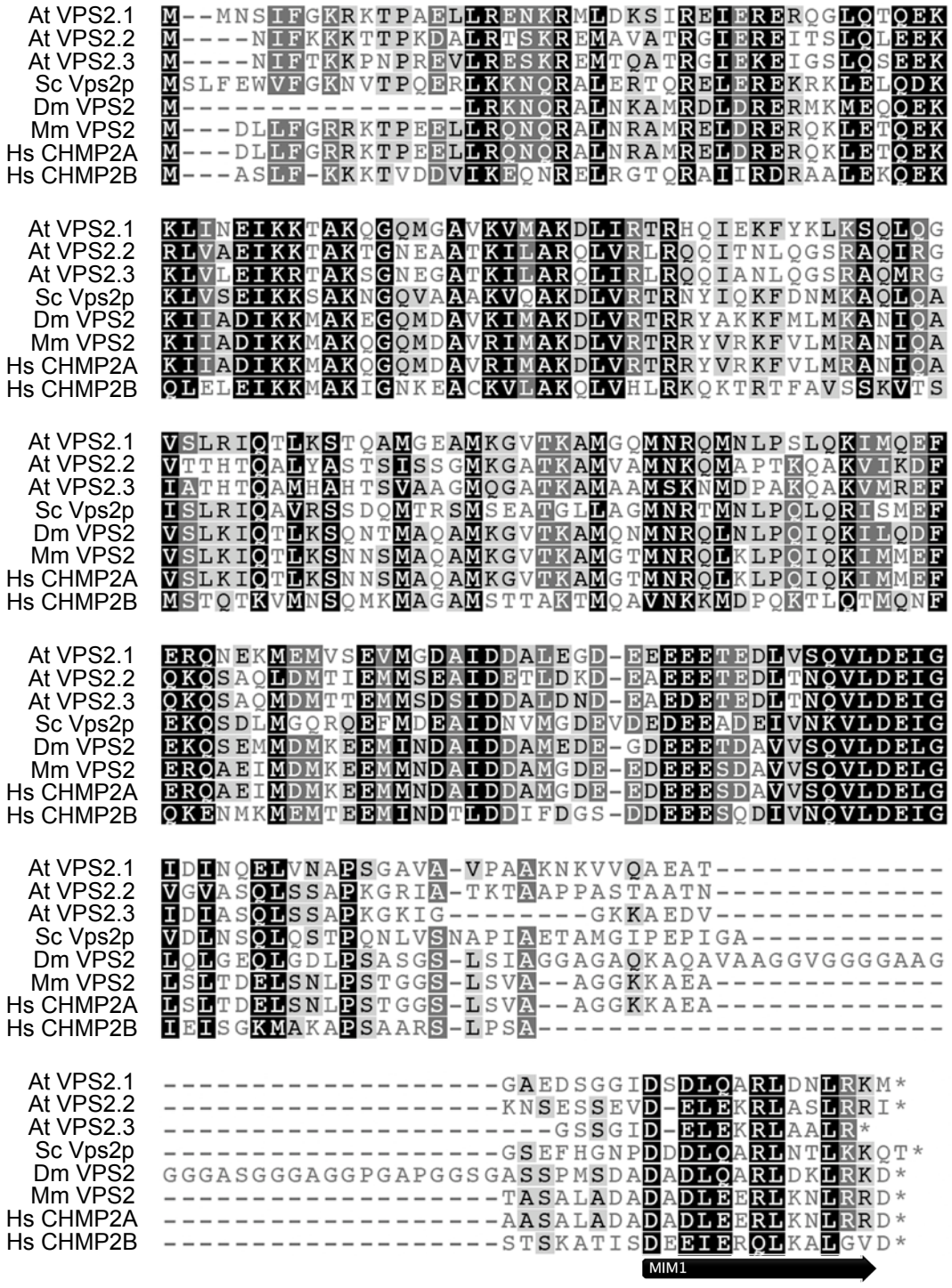


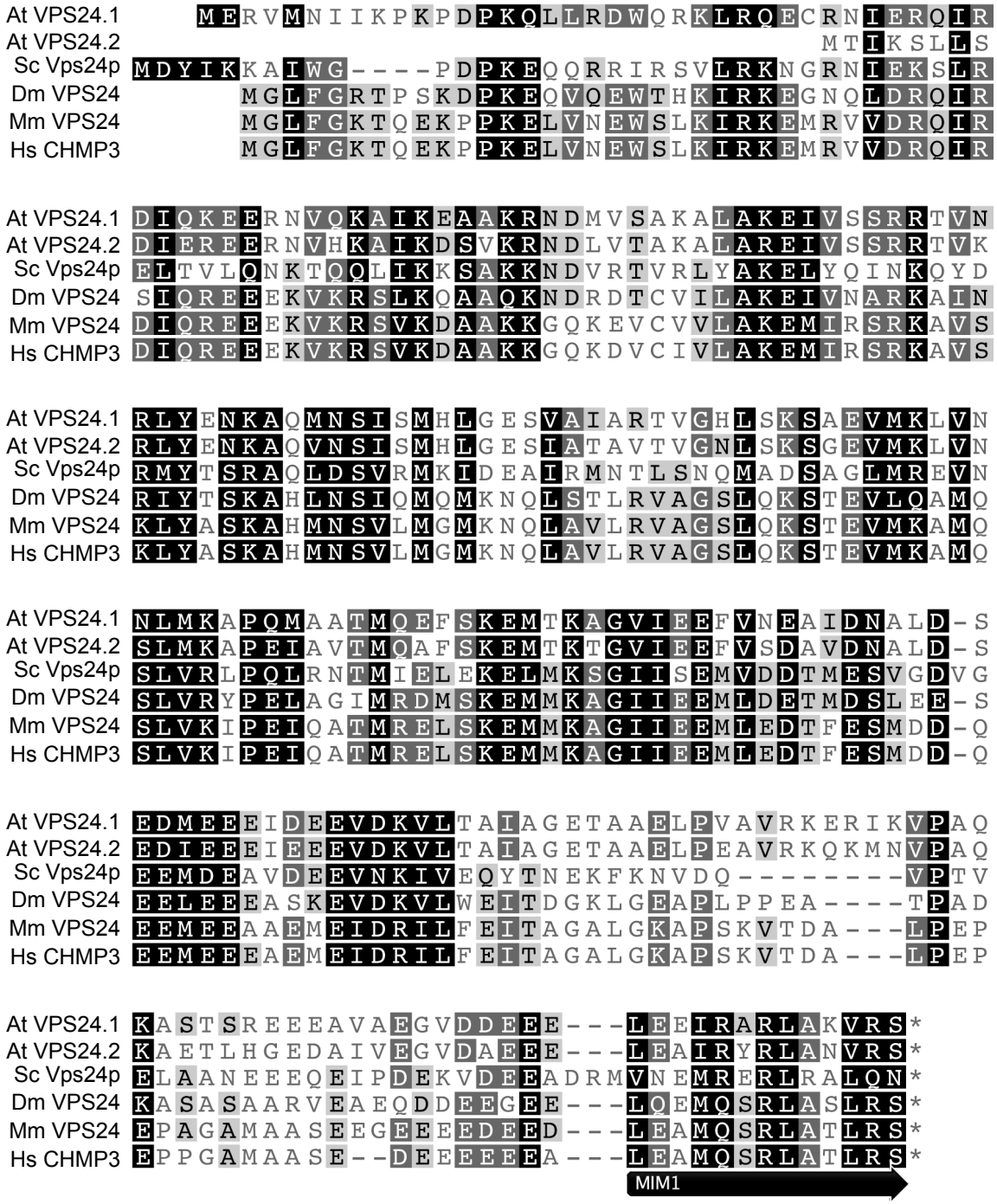
Supplemental Figure 1: Expression of Y2H constructs. (A), (B) and (C) Total proteins were extracted from yeast cells used in Figure 1A (A), 1B (B) and 2B (C) and subjected to immunoblotting using an anti-GAL4BD and an anti-AMSH3 antibody. (D) Expression of Y2H constructs shown in Figure 4B was verified by immunoblotting using an anti-GAL4BD and an anti-HA antibody.

Supplemental Figure 1 (Isono)



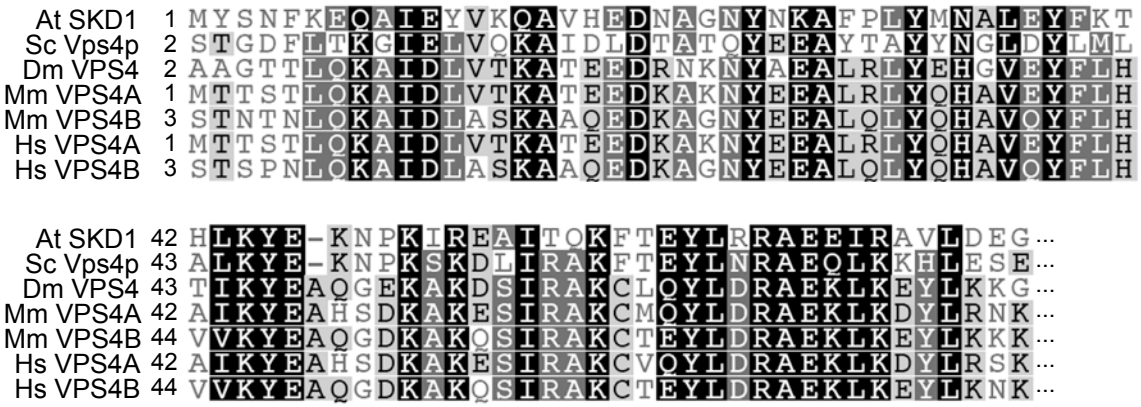
Supplemental Figure 2: Alignment of VPS2.1 (At VPS2.1), VPS2.2 (At VPS2.2) and VPS2.3 (At VPS2.3) with their counterparts from other organisms. Protein sequences of budding yeast Vps2p (Sc Vps2p; P36108-1), fruit fly VPS2 (Dm VPS2; Q8SXB1-1), mouse VPS2 (Mm VPS2; Q9DB34-1), human CHMP2A (Hs CHMP2A; O43633-1) and Hs CHMP2B (Q9UQN3-1) were aligned using the MUSCLE algorithm of Geneious software package. The previously described MIM1 (Obita et al., 2007) is indicated.

Supplemental Figure 2 (Isono)



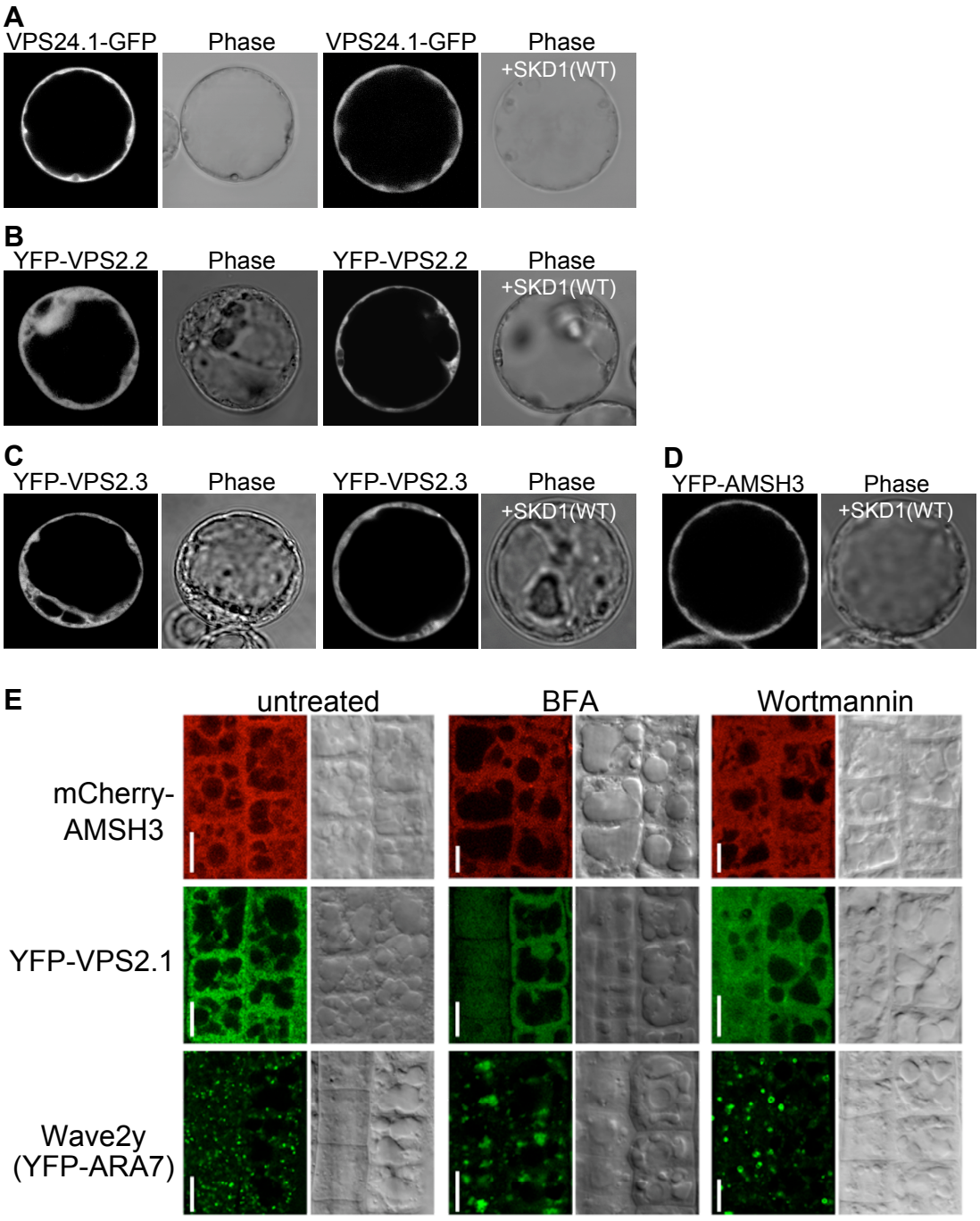
Supplemental Figure 3: Alignment of VPS24.1 (At VPS24.1) and VPS24.2 (At VPS24.2) with their counterparts from other organisms. Protein sequences of budding yeast Vps24p (Sc Vps24p; P36095), fruit fly VPS24 (Dm VPS24; Q9VN02), mouse VPS24 (Mm VPS2; Q9CQ10) and human CHMP3 (Hs CHMP3; Q9Y3E7) were aligned using the MUSCLE algorithm of Geneious software package. The previously described MIM1 (Obita et al., 2007) is indicated.

Supplemental Figure 3 (Isono)



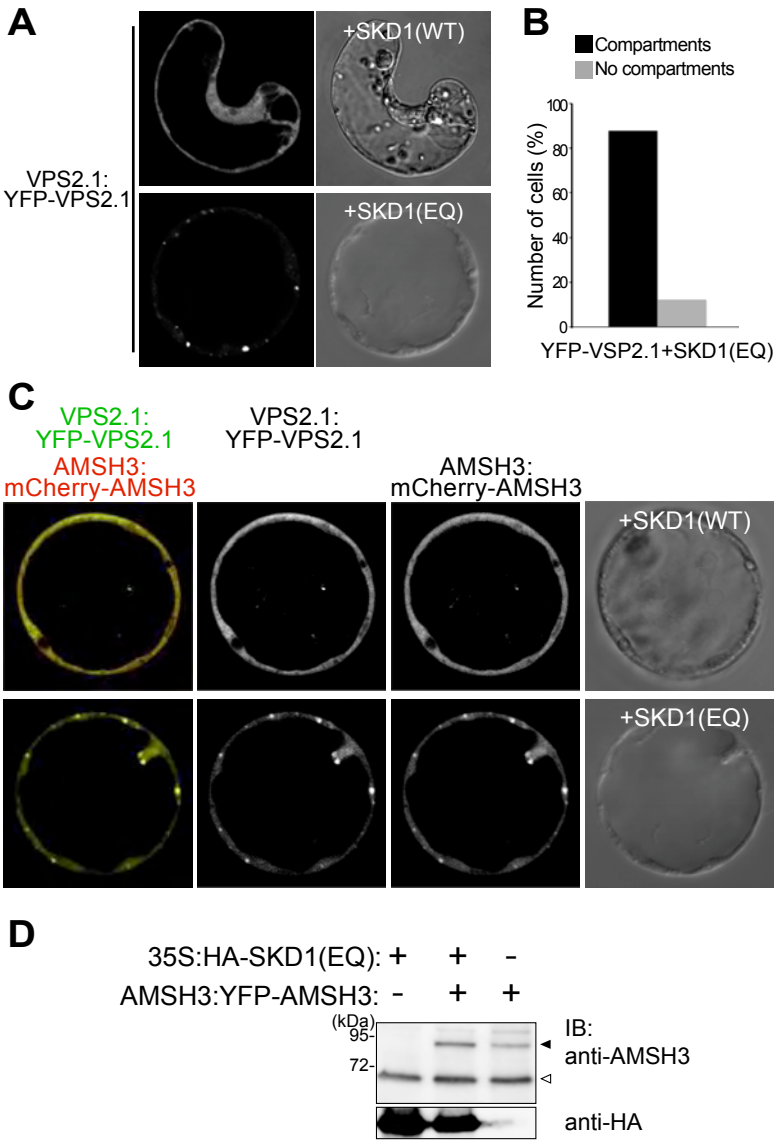
Supplemental Figure 4: Alignment of the MIT domain of Arabidopsis SKD1 (AtSKD1) and its counterparts from other organisms. Protein sequences of budding yeast Vps4p (Sc Vps4p; P52917), fruit fly VPS4 (Dm VPS4; Q9Y162), mouse VPS4A (Mm VPS4A; Q8VEJ9), mouse VPS4B (Mm VPS4B; P46467), human VPS4A (Hs VPS4A; Q9UN37) and human VPS4B (Hs VPS4B; O75351) were aligned using the MUSCLE algorithm of the Geneious software package.

Supplemental Figure 4 (Isono)



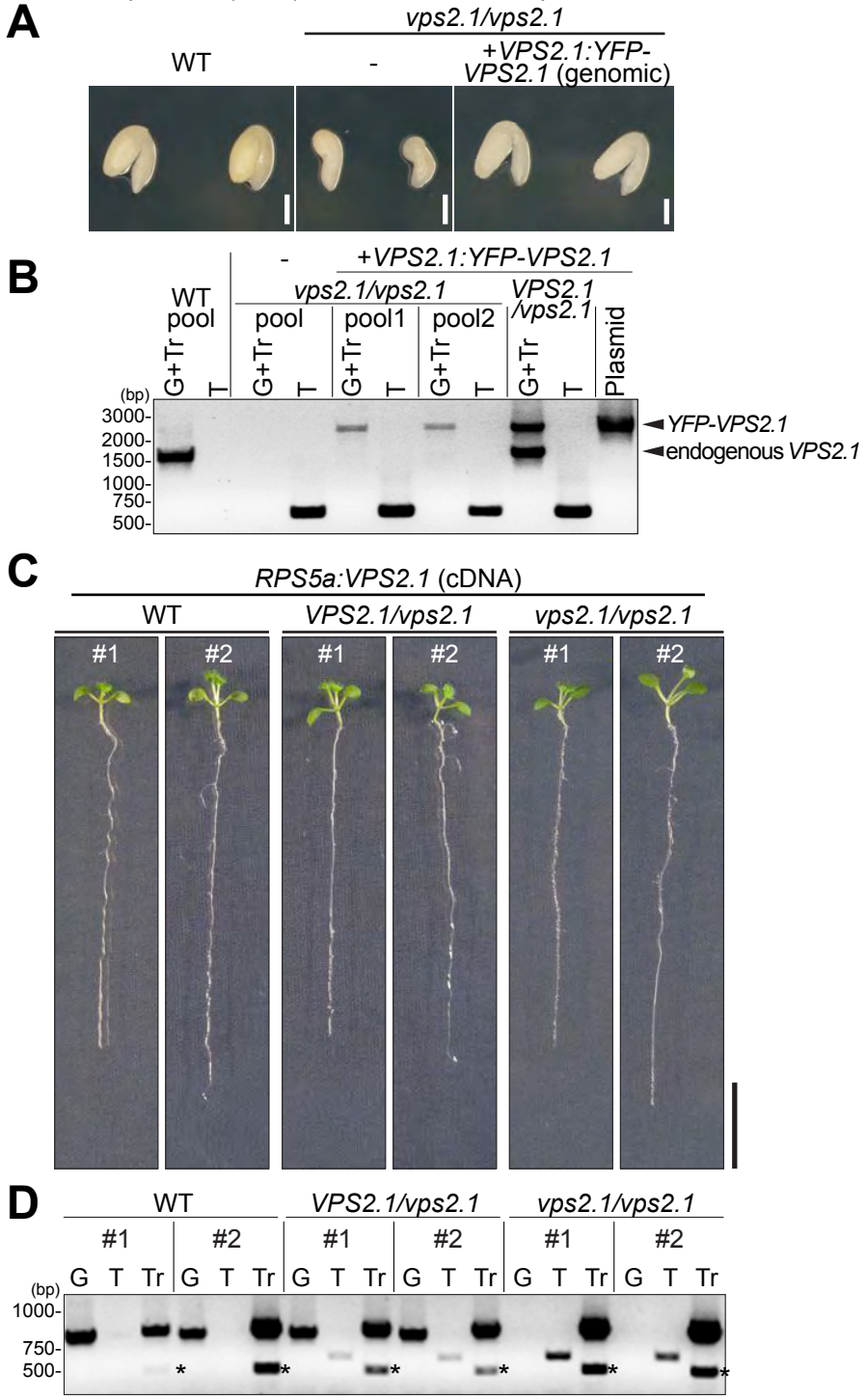
Supplemental Figure 5: VPS2 proteins, VPS24.1 and AMSH3 are localized to the cytosol. (A), (B) and (C) 35Spro:VPS24.1-GFP (A), UBQ10pro:YFP-VPS2.2 (B) and 35Spro:YFP-VPS2.3 (C) were transformed in Arabidopsis cell culture-derived protoplasts with or without 35Spro:HA-SKD1(WT) as indicated and observed under a confocal laser scanning microscope. (D) AMSH3pro:YFP-AMSH3 localization upon co-transformation with 35Spro:HA-SKD1 (WT) in cell culture derived protoplasts. (E) Localization of AMSH3 and VPS2.1 are both BFA and Wortmannin insensitive. Arabidopsis seedlings expressing one of UBQ10pro:mCherry-AMSH3, UBQ10pro:YFP-VPS2.1 or UBQ10pro:YFP-ARA7 (Wave2y) were treated with either 50 μM Brefeldin A (BFA) for 60 minutes or 33 μM Wortmannin for 90 minutes. Root epidermis cells were analyzed under a confocal laser scanning microscope. Note that while the localization of YFP-ARA7 is affected by the treatments, cytosolic localization of both AMSH3 and VPS2.1 remains largely unaffected. Scale bars: 10 μm.

Supplemental Figure 5 (Isono)

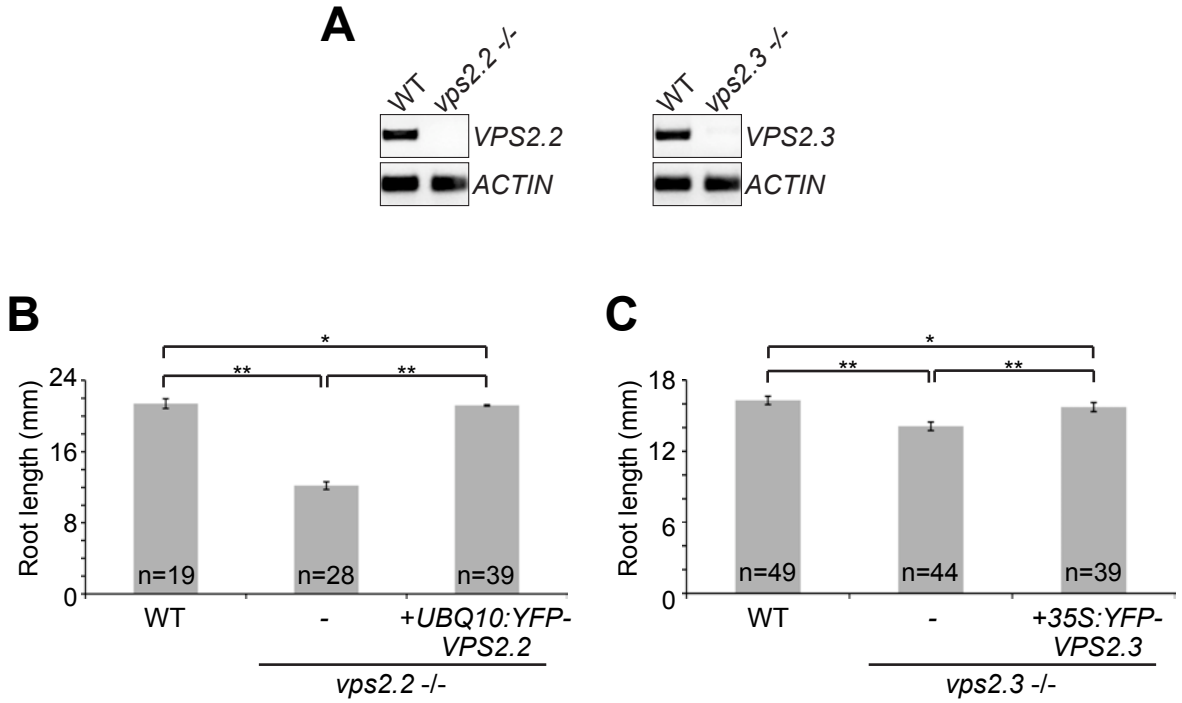


Supplemental Figure 6: Localization of VPS2.1 expressed under the native promoter. (A) VPS2.1pro:YFP-VPS2.1 co-expression with either 35Spro:HA-SKD1 (WT) or 35Spro:HA-SKD1(EQ) in Arabidopsis cell culture-derived protoplasts. (B) Quantification of the results of SKD1(EQ) co-expression in (A). Among the VPS2.1pro:YFP-VPS2.1 expressing cells, 87.8% show localization of the YFP fusion proteins in compartments (n=41). (C) VPS2.1pro:YFP-VPS2.1 and AMSH3pro:mCherry-AMSH3 were co-transformed with either 35Spro:HA-SKD1(WT) or 35Spro:HA-SKD1(EQ) in Arabidopsis cell culture-derived protoplasts. Both proteins localize to class-E compartments when expressed together with SKD1(EQ). (D) Verification of fusion protein expression in Arabidopsis cell culture-derived protoplasts. Total proteins were extracted from each sample and subjected to immunoblotting using an anti-AMSH3 and an anti-HA antibody. Open arrowhead: endogenous AMSH3; filled arrowhead: YFP-AMSH3.

Supplemental Figure 6 (Isono)

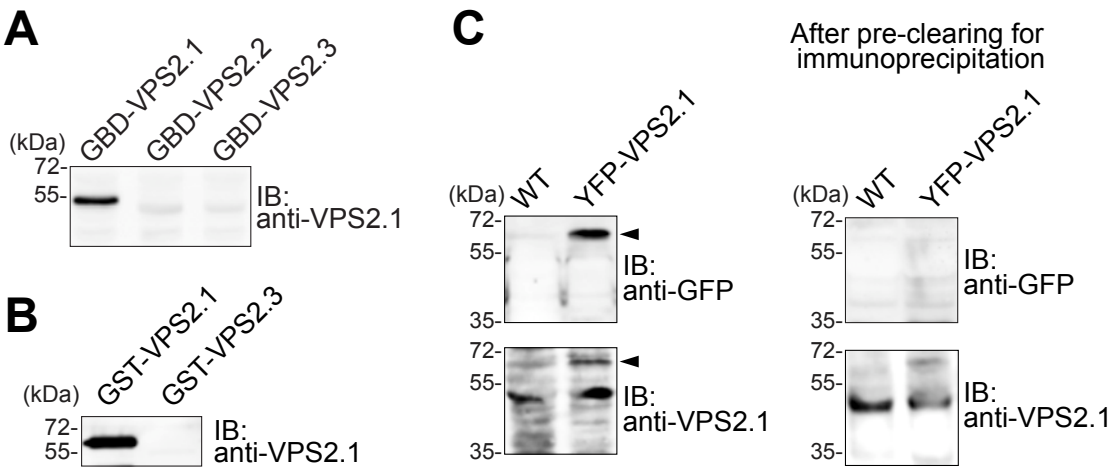


Supplemental Figure 7. Complementation of the *vps2.1* mutant. (A) Photographs of *vps2.1* embyos with or without the *VPS2.1pro:YFP-VPS2.1* (genomic) construct in comparison to wild type (WT) mature embryos. Scale bar: 0.2 mm. (B) The dissected embryos were genotyped in a pool of 10 embryos. Genotyping PCRs were performed for the endogenous gene and the transgene (lanes: G+Tr) and for the T-DNA (lanes: T). The sequences and combination of primers are shown in Supplemental Table 3 and Supplemental Methods, respectively. (C) Photographs of 13 days-old seedlings expressing *VPS2.1* (cDNA) under the *RPS5a* promoter in WT, *vps2.1* heterozygous or homozygous background. Scale bar: 1 cm. (D) Total DNA were extracted from seedlings photographed in C, and genotyping PCR was performed as in Figure 6C. Genotyping PCRs were performed for the endogenous gene (lanes: G), for the T-DNA (lanes: T) and for the transgene (lanes:Tr). The sequences and combination of primers are shown in Supplemental Table 3 and Supplemental Methods, respectively. * indicates an unspecific band.



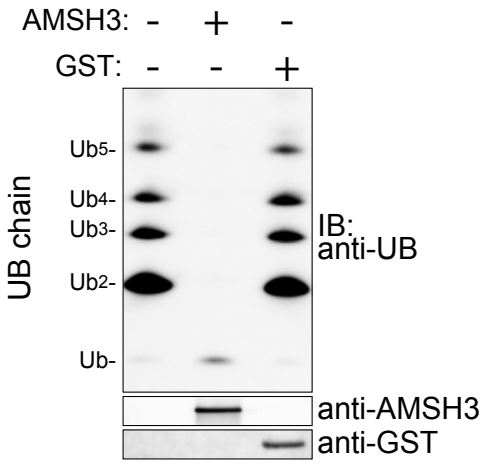
Supplemental Figure 8: Phenotype and complementation of *vps2.2* and *vps2.3* homozygous mutants. (A) RT-PCR of full length *VPS2.2* and *VPS2.3* in wild type (WT) and *vps2.2* (left panel) or *vps2.3* (right panel) homozygous mutants, respectively. *ACTIN* was used as a control. To verify the results, the RT-PCR was performed with two replicates and a representative result is shown. (B)(C) *UBQ10pro:YFP-VPS2.2* and *35Spro:YFP-VPS2.3* complement the growth defect phenotype of *vps2.2* and *vps2.3* mutants, respectively. Root length of 5 days-old wild type (WT) and *vps2.2* mutants with (+) or without (-) *UBQ10pro:YFP-VPS2.2* (B) or 4 days-old wild type (WT) and *vps2.3* mutants with (+) or without (-) *35Spro:YFP-VPS2.3* (C) are shown. Numbers of measured seedlings are indicated in the graph. * indicates no significant difference ($P>0.05$) and ** indicates significant difference ($P<0.01$). Error bars: SE.

Supplemental Figure 8 (Isono)



Supplemental Figure 9: Analysis of the VPS2.1 specific antibody. (A-C) The VPS2.1 antibody is specific for VPS2.1. Immunoblotting with the anti-VPS2.1 antibody on total extracts from yeast carrying GBD-VPS2.1, GBD-VPS2.2 or GBD-VPS2.3 (A), on purified GST-VPS2.1 and GST-VPS2.3 (B) and on 50 µg of total extracts from transgenic plants carrying *UBQ10pro:YFP-VPS2.1* (C, left panels). Arrowheads indicate the position of YFP-VPS2.1. Note that though YFP-VPS2.1 can be detected in plant total extracts (C, left panels), the signal becomes almost undetectable after pre-clearing of the extract for immunoprecipitation (C, right panels).

Supplemental Figure 9 (Isono)



Supplemental Figure 10: DUB assay with only AMSH3 or GST. 250 µg of ubiquitin chains were incubated with 1 pmol of purified AMSH3 or GST as indicated. The reactions were stopped after 60 minutes, and samples were subjected to immunoblotting using anti-UB, anti-AMSH3 and anti-GST antibodies.

Supplemental Figure 10 (Isono)

Supplemental Table 1. Analysis of embryo development in wild type and *VPS2.1/vps2.1* mutants

Genotype	Transgene (segregating)	Walking stick stage	Torpedo stage or earlier	n
VPS2.1/VPS2.1	None	98%	2%	99
VPS2.1/vps2.1	None	85%	15%	225
VPS2.1/VPS2.1	*VPS2.1:YFP-VPS2.1*	98.7%	1.3%	233
VPS2.1/vps2.1	*VPS2.1:YFP-VPS2.1* (1)	93.6%	6.4%	202
VPS2.1/vps2.1	*VPS2.1:YFP-VPS2.1* (2)	95.8%	4.2%	240

Supplemental Table 2. Progeny analysis of wild type and *VPS2.1/vps2.1* heterozygous plants with *VPS2.1pro:YFP-VPS2.1* (genomic) or *RPS5apro:VPS2.1* (cDNA)

Genotype	Transgene (segregating)	Germinated	Ungerminated	Aborted	n
VPS2.1/VPS2.1	*VPS2.1:YFP-VPS2.1*	96.2%	3.8%	NA	213
VPS2.1/vps2.1	*VPS2.1:YFP-VPS2.1* (1)	83.1%	15.4%	1.5%	261
VPS2.1/vps2.1	*VPS2.1:YFP-VPS2.1* (2)	81.8%	16.8%	1.4%	143
VPS2.1/VPS2.1	*RPS5a: VPS2.1*	98.2%	1.8%	NA	156
VPS2.1/vps2.1	*RPS5a: VPS2.1* (1)	95.2%	3.4%	1.4%	145
VPS2.1/vps2.1	*RPS5a: VPS2.1* (2)	93.8%	4.9%	1.3%	239

Supplemental Table 3: List of primers used in this study.

Primer	Sequence (5' - 3')
ACT rv	GATGCACAGTTGAAGTGAACTTG
GAL4-AD	CTATTCGATGATGAAGATACCCCACCAAACC
GABI_8474	ATAACGCTGCGGACATCTACA
AtAMSH3RV	AAGGGTCGACGCTGCCCTCTTTTCCT
AK0 (AMSH3 BamHI fw)	TCGGGGATCCGTATGAAGATTGATCTGAAC
AK1 (VPS2.1 XhoI fw)	AAGGCTCGAGATGATGAATTCAATCTTCGG
AK2 (VPS2.1 SalI rv)	AAGGGTCGACTCACATTTTTCTAAGGTTAT
AK3 (VPS2.3 EcoRI fw)	AAGGGAATCCATGAACATCTTCACTAAG
AK4 (VPS2.3 SalI rv)	AAGGGTCGACCTATCTAAGCGCCGCCAA
AK21 (VPS2.1 SfiI fw)	CAGGCCGTCAAGGCCTATGATGAATTCAATC
AK22 (VPS2.1 NotI rv)	CAGCGGCCGCTCACATTTTTCTAAG
AK32 (VPS2.1 GW fw)	AAAAAGCAGGCTCCATGATGAATTCAATCTTCGG
AK33 (VPS2.1 GW rv)	AGAAAGCTGGGTATCAGAATCTTACGTGGCAGCT
AK38 (pVPS2.1 XhoI rv)	AAGGCTCGAGGATTCAATACCGAAAAAGGTG
AK41 (pVPS2.1 AscI fw)	AAGGGGAGCGCCACGTTGTTGTCGGGAAAATATG
AK44 (VPS2.1 D212N fw)	ACAGTGGAGGTATAAACAGTGACCTTCAAGC
AK45 (VPS2.1 D212N rv)	GCTTGAAGGTCACTGTTTATACCTCCACTGT
AK47 (VPS2.3 I199D fw)	TTGGCAGTTCTGGAGATGATGAACTGGAGAA
AK48 (VPS2.3 I199D rv)	TTCTCCAGTTCATCATCTCCAGAACTGCCAA

AK51 (AMSH3-318 SalI rv)	AAGGGTCGACTAACAGCTTATCCACTAG
AK66 (VPS24.1 BamHI fw)	AAGGGGATCCATGGAGAGAGTGATGAAC
AK67 (VPS24.1 SalI rv)	TTGGGTCGACTTAGGATCTAACTTTAGC
AK68 (VPS60.1 BamHI fw)	AAGGGGATCCATGAGGAGAGTTTTCGG
AK69 (VPS60.1 SalI rv)	TTGGGTCGACTTAACCCCGGAGAGAAG
AK70 (AMSH3-48 BamHI fw)	AAGGGGATCCAGAATCGTATTCCTCTCCGT
AK71 (vps2_F2 fw)	ACTCGAAATCTACACAAGCGA
AK77 (VPS2.2 EcoRI fw)	AGGAATTCATATGAACATTTTCAAGAAG
AK78 (VPS2.2 SalI rv)	AGGTCGACTCAGATTCGTCGTAGCGA
AK88 (AMSH3-320 XhoI fw)	AAGGCTCGAGATGAGGATGAATCCCGTCAGG
AK90 (VPS24.1 GW fw)	AAAAAGCAGGCTCCATGGAGAGAGTGATGAACATC
AK92 (VPS24.1 GW rv)	AGAAAGCTGGGTAGGATCTAACTTTAGCGAGCC
AK97 (AMSH3-462 SalI rv)	AAGGGTCGACTTACGTCCAGGATGGTTGAG
AK100 (vps2_F2 fw-2)	TGGATACTTTCTCTCACAGGG
AK101 (VPS2.1 genomic rv)	TTGAAAAGTGATCAGCCTCAG
AK105 (pRPS5a fw)	CTCACGCTCTGTTTCTCTCACC
AK106 (pVPS2.1 fw)	TCACCTTTTTCGGTATTGAATC
EI14 (AMSH3 SalI rv)	AAGGGTCGACTTAGCGGAGATCGAGGACTT
EI141 (tAMSH3 KpnI)	AAGGGGTACAGCCAACGAGACGTGAGACG
EI180 (pAMSH3 NotI fw)	AAGGGCGGCCGCTGGTTTGGTAGCCTACTCAC
EI181 (pAMSH3 BamHI rv)	AAGGGGATCCCTCACCGTATCTGATTATAC

EI182 (ATG BamHI fw)	AAGGGGATCCATGAAGATTGATCTG
EI183 (YFP BamHI fw)	AAGGGGATCCATGGTGAGCAAGGGCGAGGA
EI184 (YFP BamHI rv)	AAGGGGATCCCTTGTACAGCTCGTCCATG
EI189 (SKD1 GW fw)	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGTACAGC AATTTCAAGGA
EI190 (SKD1 GW rv)	GGGGACCACTTTGTACAAGAAAGCTGGGTTCAACCTT CTTCTCCAAACT
EI 193 (SKD1 EQ fw)	ATTATTTTTGTTGATCAGATAGATTCTTTGTG
EI 194 (SKD1 EQ rv)	CACAAAGAATCTATCTGATCAACAAAAATAAT
VPS2.2 EcoRI fw	CAGAATTCATGAACATTTTCGAGAAGAAG
VPS2.2 BamHI rv	CAGGATCCTCAGATTCGTCGTAGCGA
VPS24.1 NdeI fw	CACATATGGAGAGAGTGATGAACATC
VPS24.1 BamHI rv	GAGGATCCTTAGGATCTAACTTTAGCGAG
VPS2.1 NdeI fw	GACATATGATGAATTCAATCTTCGGAA
VPS2.1 SmaI rv	GACCCGGGTCACATTTTTCTAAGGTTATCC
VPS2.3 NdeI fw	CACATATGAACATCTTCACTAAGAAAC
VPS2.3 EcoRI rv	TCGAATTCCTATCTAAGCGCCGCCAA
VPS46.1 EcoRI fw	TCGAATTCATGGGTAATACAGATAAGCTG
VPS46.1 BamHI rv	CTGGATCCTTAACCTCTGGCTTTAAGCT
VPS46.2 EcoRI fw	GAGAATTCATGGGTAACACAGATAAGC
VPS46.2 BamHI rv	TAGGATCCTTATCCTCTGGCTTTAAGCT

VPS60.1 EcoRI fw	TAGAATTCGCCCTTGAATTGATGAGGAGAGTTTTCGGC GCGA
VPS60.1 BamHI rv	CTGGATCCTTAACCCCGGAGAGAAGCT
VPS20.1 EcoRI fw	CAGAATTCATGGGGAATTTGTCGTGAAG
VPS20.1 BamHI rv	CTGGATCCTCAAGCCGGCAAACCTTC
SNF7.1 EcoRI fw	TAGAATTCATGATGAATCGGCTATTCGA
SNF7.1 BamHI rv	TAGGATCCTTAGAGGGCCATCTC
Actin FW	ATTCAGATGCCCAGAAGTCTTGTTC
Actin RV	GCAAGTGCTGTGATTTCTTTGCTCA

Supplemental Methods

Genotyping PCR: Genotyping PCR were performed with following primer combinations. For the *vps2.1* mutant primer paris AK2 and AK71 and GABI_8474 and AK2 were used. For *vps2.1* mutants with VPS2.1pro:YFP-VPS2.1 (genomic), primer pairs AK101 and AK106 and GABI_8474 and AK2 were used. For *vps2.1* mutants with RPS5apro:VPS2.1 (cDNA) primer pairs AK100 and AK101, GABI_8474 and AK2, AK2 and AK105 were used.

RT-PCR: Total RNA was extracted from 5-day-old Arabidopsis seedlings using the NucleoSpin RNA Plant (Macherey-Nagel) kit according to the manufacturer's instruction. One microgram of total RNA was subjected to reverse transcription by M-MuLV Reverse Transcriptase (Fermentas) to produce 20 µl of cDNA, and 1 µl cDNA was used for each PCR.

Root length measurement: Seeds were plated on MS growth medium supplemented with 1% sucrose grown vertically to continuous light. After 4 or 5 days of germination, plates were scanned and root length was measured with the IMAGE J software (http://rsb.info.nih.gov/ij).

Production of an anti-VPS2.1 antibody: For the production of an anti-VPS2.1 antibody, full-length VPS2.1 was purified from *E.coli* Rosetta (DE3) (Merck

Chemicals) strain using the GST-VPS2.1 clone. After the purification, the GST moiety was cleaved off with PreScission protease (GE Healthcare). The antibody was raised in rat (Eurogentec). The serum was purified with a HiTrap Protein G column (GE Healthcare) and subsequently with a VPS2.1 loaded NHS-activated column (GE Healthcare). The anti-VPS2.1 antibody was used in a dilution of 1:1000.