SUPPLEMENTARY MATERIAL

Phosphoinositides control the localization of HOPS subunit VPS41, which together with VPS33 mediates vacuole fusion in plants

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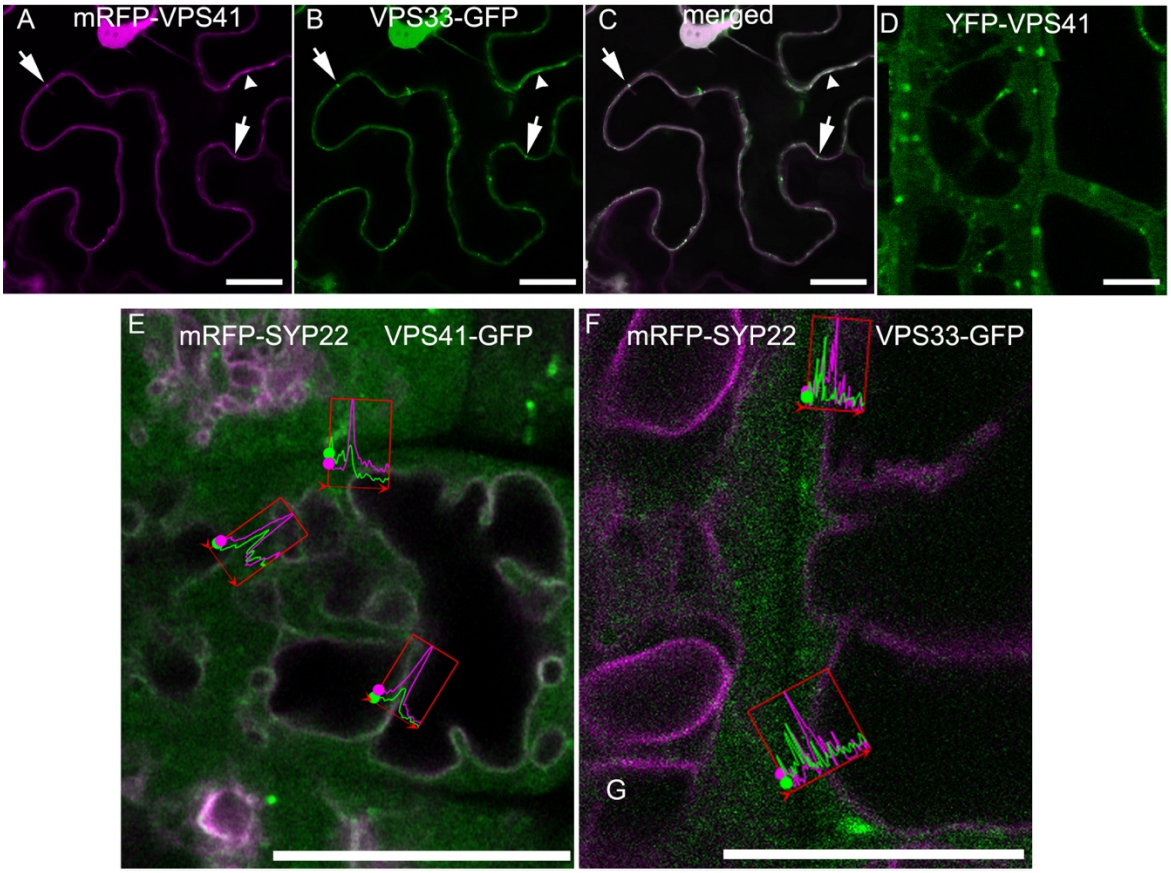

Figure S1. Subcellular localization of HOPS subunits VPS41 and VPS33.
(A-C) Co-localization analysis of RFP-VPS41 (A) with VPS33-GFP (B) in epidermis cells of *N. benthamina* leaves. The merged imaged is shown (C). Co-localization was detected in punctate structures (arrows), the nucleus and at the cell periphery (arrowheads).
(D) Localization analysis of YFP-VPS41 in Arabidopsis roots.
(E) Co-localization analysis of gVPS41-GFP with mRFP-SYP22 in Arabidopsis roots. Fluorescence signal intensity for gVPS41-GFP and mRFP-SYP22 overlapping across the tonoplast is shown.
(F) Co-localization analysis of gVPS33-GFP with mRFP-SYP22 in Arabidopsis roots. GFP fluorescence is not found at the tonoplast, which shows absence of VPS33 from the vacuole.
All scale bars: 20µm.

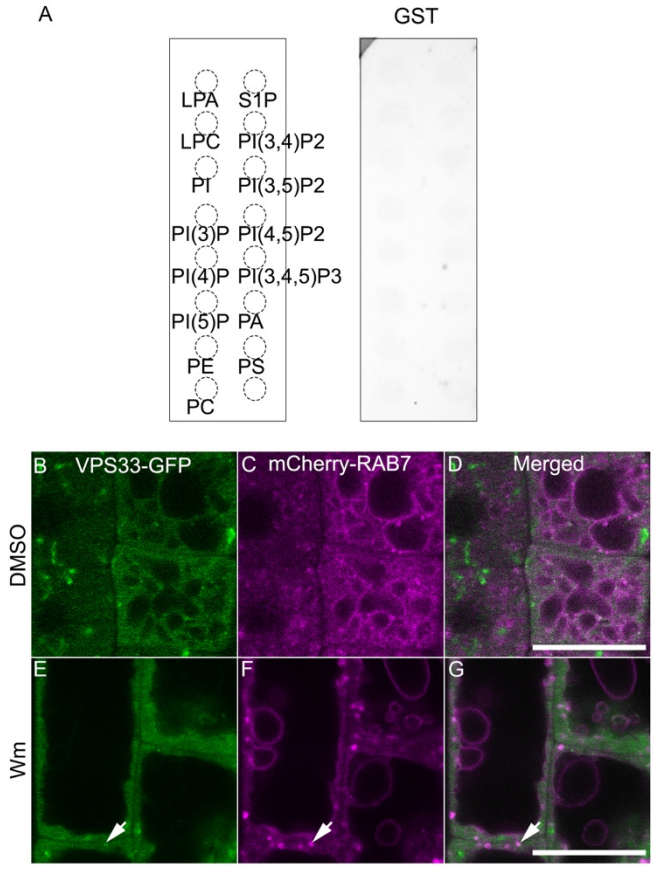

Figure S2. Wm treatment has no major effects on VPS33-GFP localization

(A) GST control for the lipid strip assay. GST alone does not bind to any of the lipids on the membrane.

(B-G) Wm treatment does not alter VPS33-GFP localization. Arabidopsis seedlings expressing VPS33-GFP (B and E) and mcherry-RAB7 (C and F) were treated with DMSO (B-E) or 33μM Wm (E-G) for 3h and observed by confocal microscopy. Merged images are shown (D and G). The arrow points to a Wm body also labeled by VPS33-GFP. Scale bar: 20μm.

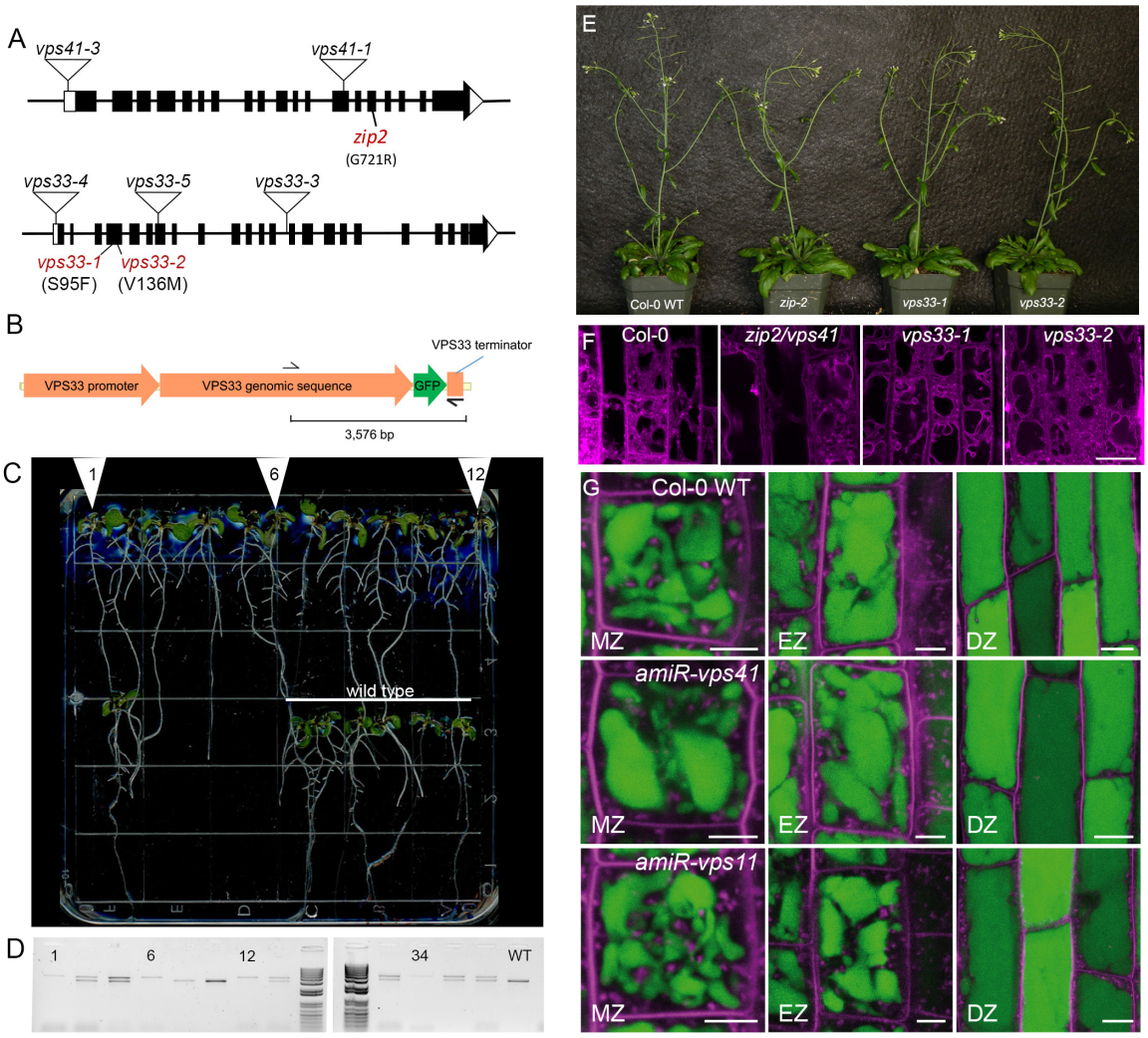

Figure S3. HOPS hypomorphic alleles have normal vacuole morphology.

(A) Schematic representation of the *VPS41* and *VPS33* coding sequences showing exons (black boxes), introns (lines), 5'UTR (white boxes) and 3' UTR (white arrows). T-DNA insertion alleles are shown with black triangles and hypomorphic alleles are shown in red.

(B) Genomic fusion construct for VPS33p::VPS33-GFP. Primers for genotyping complemented lines anneal to the genomic sequence and to the VPS33 terminator downstream of GFP. The fusion amplifies a 3,576 bp fragment. The wild type allele (without GFP) amplifies a 2,880 bp fragment.

(C-D) The genomic VPS33p::VS33-GFP construct complements *vps33-3* mutants. The progeny of transformed *vps33-3* /+ heterozygous plants were first screened for the presence of the original T-DNA insertion (*vps33-3*). Plants were then scored for the presence of the 3,576 amplicon and the absence of the wild type 2,880 bp fragment when using the primers indicated in (B). Plants labeled 1, 6 and 12 (C) are homozygous *vps33-3* and carry the VPS33p::VS33-GFP construct as they only amply the 3,576 fragment (D). The size of amplicon from the wild type control is shown.

(E) None of the mutants have major developmental defects in mature plants.

(F) No major defects in vacuole morphology were detected in the hypomorphic mutants. Col-0 wild type (control), *zip2/vps41*, *vps33-1* and *vps33-2* seedlings were stained with Lyso-Tracker Red and root tips were imaged by confocal microscopy. Scale bar: 20μm.
(G) Vacuole morphology of amiRNA lines without DEX induction. Seedlings from Col-0 WT (control), amiR-*vps11* and amiR-*vps41* were incubated for 72 h in media with DMSO (control) and stained with BCECF. Roots were imaged at the meristematic (MZ), elongation (EZ) and differentiation (DZ) zones.

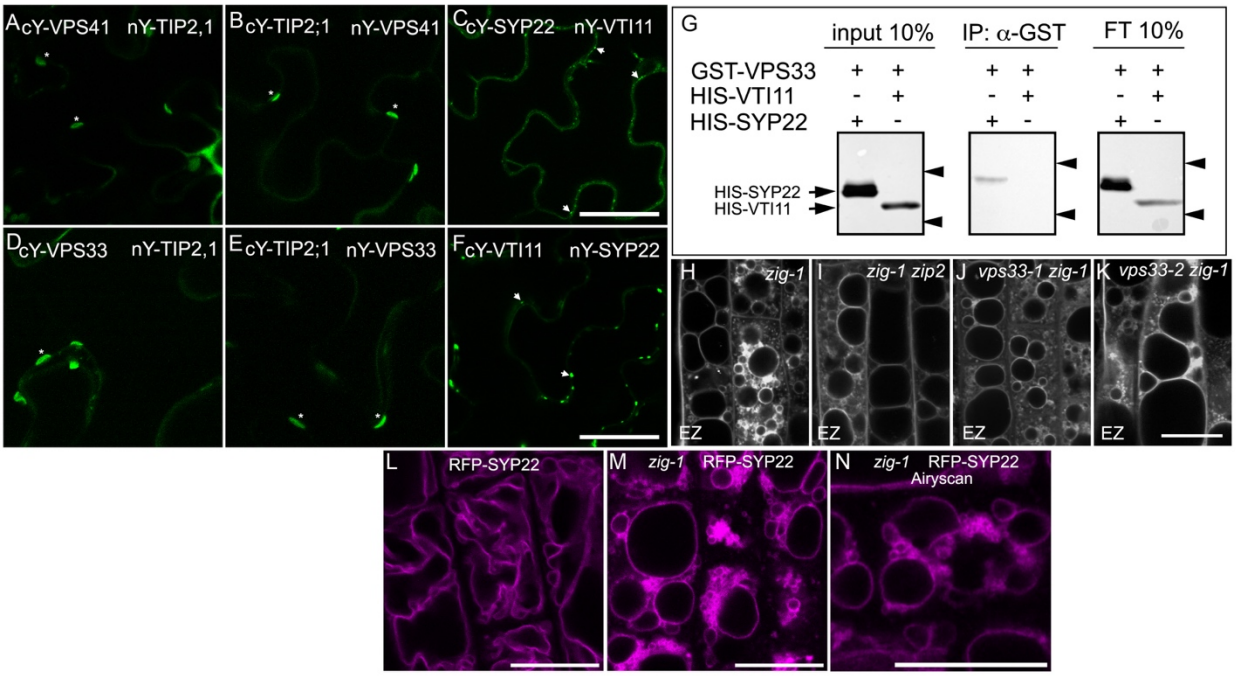

Figure S4. VPS41 and VPS33 interact and can bind the SNARE protein SYP22.
(A-F) *N. benthamiana* leaf cells were co-infiltrated with the BiFC combinations as indicated. N-terminal half (nY) or the C-terminal half (cY) of YFP were fused to VPS41, VPS33, SYP22, VTI11 or TIP2;1 (negative control). No fluorescence due to complementation was detected with either combination between the negative controls n/cYFP-TIP2,1 and c/nYFP-VPS41 (A, B) or c/nYFP-VPS33 (D, E). Large structures labeled with asterisks represent background noise. The two SNAREs, VTI11 and SYP22, interact with each other in punctate structures (arrows) regardless of the part of YFP that was used. Arrows point to a few punctate structures/endosomes where interaction is detected.
(G) VPS33 interaction with SYP22 SNARE *in vitro*. Recombinant GST-VPS41, GST-VPS33, HIS-SYP22 and HIS-VTI11 protein fusions were expressed in *E.coli* and purified by affinity chromatography. The purified proteins were mixed as indicated and immunoprecipitation was carried out with an α-GST antibody. Proteins from 10% input, the immunoprecipitate (IP, center) or 10% of the flow through (right, FT) were separated by SDS-PAGE and HIS-SYP22 or HIS-VTI11 were detected by western blot with an α-HIS antibody. Arrows indicate the expected sizes of HIS-SYP22 and HIS-VTI11. Arrowheads indicate the positions of 37 kDa and 25 kDa molecular weight markers.
(H-K) *zip2*/vps41 does not complement *zig1/vti11* in the root elongation zone. Seedlings were stained for 2h in Lysotraker Red. The fragmented vacuole phenotype of *zig-1/vti11* mutants was also observed in *zig-1/vti11 zip2/vps41* (I), *zig-1/vti11 vps33-1* (J), and *zig-1/vti11 vps33-2* (K) mutants when roots were imaged in the elongation zone. Scale bars: 20μm.
(L-M) RFP-SYP22 in *zig1* background is mis-localized to bright puncta, but it is still abundant at the tonoplast (M), compared to the Col-0 wilde type (L). Airyscan image is shown (N). Scale bar 20μm.

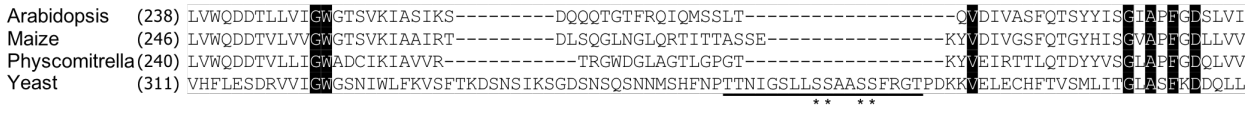

Figure S5. VPS41 sequence alignment.
Protein sequences were aligned using ClustalW using Vector NTI. Identical residues are shown with a black background. The ALPS motif from yeast VPS41 is underlined and important phosphorylation sites are marked with asterisks.

SUPPLEMENTARY TABLES

Table S1. Transmission efficiency for HOPS hypomorphic alleles.

Female	x	Male	HT:WT[a]	TE (%)[b]
vps41-1/+	x	WT	41:25	164[c]
WT	x	*vps41-1*/+	0:43	0[d]
vps33-3/+	x	WT	29:37	78.3[c]
WT	x	*vps33-3*/+	10:62	16.1[d]

[a] Number of heterozygous (HT) and wild type (WT) progeny from each cross.
[b] Transmission efficiency through either gamete calculated as TE (%) = (#hets/#WT) x 100 according to Howden et al. 1998.
[c] TE via the female gametes
[d] TE via the male gametes

Table S2. Summary of expression constructs.

Expression Construct	Entry vector (for Gateway)	Cloning or Gateway Destination vector	Experiment
pVPS33::gVPS33-GFP (VPS33-GFP)		pDGB_omega1	Protein localization in plants
pUBQ10::YFP-VPS41	pEntry-VPS41+	pUBQ10::GFP-GW	
pUBQ10::RFP-VPS41	pEntry-VPS41+	pUBQ10::RFP-GW	
pUBQ10::VPS33-GFP	pEntry-VPS33-	pUBQ10::GW-GFP	
T7::GST-VPS41	pEntry-VPS41+	pDEST15	Protein expression in *E. coli*
T7::GST-VPS33	pEntry-VPS33+	pDEST15	
T7::GST	scBTS	pDEST15	
T7::HIS-VTI11	pEntry-VTI11+	pDEST17	
T7::HIS-SYP22	pEntry-SYP22+	pDEST17	
35S::nYFP-VTI11	pEntry-VTI11+	pSITE-BIFC-nEYFP-C1	BiFC
35S::cYFP-VTI11	pEntry-VTI11+	pSITE-BiFC-cEYFP-C1	
35S::cYFP-VPS33	pEntry-VPS33+	pSITE-BiFC-cEYFP-C1	
35S::nYFP-VPS33	pEntry-VPS33+	pSITE-BIFC-nEYFP-C1	
35S::cYFP-TIP2,1	pEntry-TIP2;1+	pSITE-BiFC-cEYFP-C1	
35S:: nYFP-TIP2,1	pEntry-TIP2;1+	pSITE-BIFC-nEYFP-C1	
35S::cYFP-VPS41	pEntry-VPS41+	pSITE-BiFC-cEYFP-C1	
35S::nYFP-VPS41	pEntry-VPS41+	pSITE-BIFC-nEYFP-C1	
35S::nYFP- SYP22	pEntry-SYP22+	pSITE-BIFC-nEYFP-C1	
35S::cYFP-SYP22	pEntry-SYP22+	pSITE-BiFC-cEYFP-C1	
amiR-*vps11*		pRS300	Silencing by amiRNA
amiR-*vps41*		pRS300	

Table S3. Summary of entry constructs generated for Gateway cloning.

Entry construct	Entry vector	Primers used	
pEntry-VPS41+	pENTR/D-TOPO	VPS41-Topo 1F	VPS41-Topo 1 NR
pEntry-VPS33+	pENTR4	VPS33-NotI-1F	VPS33+ NotI-1R
pEntry-VPS33-stop	pENTR4	VPS33-NotI-1F	VPS33- NotI-1R
pEntry-VTI11+	pENTR4	VTI11 NotI-1F	VTI11+ NotI-1R
pEntry-SYP22+	pENTR4	SYP22 Nco-1F	SYP22+ NotI-1R
pEntry-TIP2;1+	pENTR/D-TOPO	TIP2;1 TOPO 1F	TIP2;1 TOPO 1R
scBTS	pENTR/D-TOPO	Selote D. et al., 2015 Plant Physiology	

Table S4. List of primers.

Name	Sequence	Use
GBpVPS33-1F	GCGCCGTCTCGCTCGGGAGGCCTATTTCAGAGTTTATTTAACTAG	GoldenBraid cloning of VPS33 promoter
GBpVPS33-1R	5'GCGCCGTCTCGTTGTCTCTATGGTTTCTTGTTCAT	
GBpVPS33-2F	GCGCCGTCTCGACAAAAATATCCTAAGGAAGCACA	
GBpVPS33-2R	5'GCGCCGTCTCGCTCACATTGGACGATGATCAAGAGATGAT	
GBVPS33-1F	GCGCCGTCTCGCTCGAATGGCGCAGATCCCCAGCTT	
GBVPS33-1R	GCGCCGTCTCGCATCTCCCAACCCAAAAAAAAAAA	GoldenBraid cloning of VPS33 coding sequence (includes introns)
GBVPS33-2F	GCGCCGTCTCGGATGAAAGTTA GGAAATTTCGCG	
GBVPS33-2R	GCGCCGTCTCGGCGACGCTATGAATTTCATAAAG	
GBVPS33-3F	GCGCCGTCTCGTCGCACATTCAGAATGATATTGC	
GBVPS33-3R	GCGCCGTCTCGTAAGACCCGAAGACAA GATATG	
GBVPS33-4F	GCGCCGTCTCGCTTAACTTTCAGAATGGAACTACT	
GBVPS33-4R	GCGCCGTCTCGCTCACGAACCGCCTAGTTTTTCCATGA ATGTTTC	
GBVPS33ter-1F	5'GCGCCGTCTCGCTCGGCTTTCCGTAAACTGAGCGTTATGT	GoldenBraid cloning of VPS33 terminator sequences
GBVPS33ter-1R	GCGCCGTCTCGCTCAAGCGTAGCAGAAGTTGAAGTGAAAGG	
VPS33-14F	TGTACGTTTGTCGCTCTCCT	Genotyping VPS33-GFP transformants (Fig. S3B)
VPS33-8R	ACCATTCACTCTGCAACCACA	
VPS33-NotI-1F	GCGGCCGCATGGCGCAGATCCCCAGCTT	Gateway cloning of VPS33 CDS
VPS33+ NotI-1R	GCGGCCGCTCAGCCTAGTTTTTCCATGA	
VPS33- Not1-R	GCGGCCGCGAGCCTAGTTTTTCCATGAATG	
VPS41-Topo 1F	CACCATGGCTGCGGTTCCGCCT	Gateway cloning of VPS41 CDS
VPS41-Topo 1NR	CTACCGAGCGGACGCAGC	
VTI11 NotI-1F	GCGGCCGCATGAGTGACGTGTTTGATGG	Gateway cloning of VTI11 CDS
VTI11+ NotI-1R	GCGGCCGCTTACTTGGTGAGTTTGAAGT	
SYP22 Nco1-F	CCATGGGAATGAGTTTTCAAGATTTAGA	Gateway cloning of SYP22 CDS
SYP22+Not1-R	GCGGCCGCGATCAAGCTGCGAGTACTATAA	
TIP2;1 TOPO 1F	CACCATGGCTGGAGTTGCCTTT	Gateway cloning of TIP2;1 CDS
TIP2;1 TOPO 1R	TTAGAAATCAGCAGAAGCA	
VPS41-2F	GGGATGGTCTTGTGAGTAGT	Genotyping *vps41-1 and zip2*
VPS41-2R	GAGTAACAGCAATGAGGGGT	
VPS41-1F	GCACTCATAGAGATCTTCG	Genotyping *vps41-2*
VPS41-1R	ATTGCAGTAAACCCCTCCTC	
VPS41-4F	CCGTTCCTACGTAGCAGTCA	Genotyping *zip2*
VPS33-1F	TCAGTCTCATTGATCATCCCGACCTC	Genotyping tilling lines and *vps33-5*
VPS33-1R	CACTTCAGGCCTTCCCACCTGAAA	
VPS33-3F	CACGTTTGAGTGTTGACCTT	Genotyping *vps33-3*
VPS33-2R	TGCTGGGCAAGATGTATGTG	
VPS33-2F	AATGAGGCCCATTTCTAAGC	Genotyping *vps33-4*
VPS33-5R	GTCTGAACTGGTTCCGCAGTAAGATG	
LBb. 1.3	ATTTTGCCGATTTCGGAAC	Genotyping SALK lines
VTI11-4F	TGCTCGTGATGAGTTGCTAG	Genotyping *zig1*
VTI11-3R	TGGCGGCAATCAATGCGATG	

Primer A	AACAGGTCTCAAACACGAATTCCTGCAGCCCC	amiR* generation
Primer B	AACAGGTCTCTGCAGGGATCCCCCCATGGCGATGCC	
I miR-s (*vps11*)	5'GATAAAGTGCAAGCAGTACGCTTTCTCTCTTTTGTATTCC	
II miR-a (*vps11*)	GAAAGCGTACTGCTTGCACTTTATCAAAGAGAATCAATGA	
III miR*s (*vps11*)	GAAAACGTACTGCTTCCACTTTTTCACAGGTCGTGATATG	
IV miR*a (*vps11*)	GAAAAAGTGGAAGCAGTACGTTTTCTACATATATATTCCT	
I miR-s (*vps41*)	5-GATCTGTTTATAACAACAGGCCCTCTCTCTTTTGTATTCC	
II miR-a (*vps41*)	GAGGGCCTGTTGTTATAAACAGATCAAAGAGAATCAATGA	
III miR*s (*vps41*)	GAGGACCTGTTGTTAAAAACAGTTCACAGGTCGTGATATG	
IV miR*a (*vps41*)	GAACTGTTTTTAACAACAGGTCCTCTACATATATATTCCT	

Table S5. amiR* PCR Reactions

amiR-*vps* construct	**PCR reaction #**	**primer identifiers**
amiR-*vps11*	1	Primer A + IV miR*a (*vps11*)
	2	III miR*s (*vps11*) + II miR-a (*vps11*)
	3	Primer B + I miR-s (*vps11*)
	4	Primer A + Primer B
amiR-*vps41*	1	Primer A + IV miR*a (*vps41*)
	2	III miR*s (*vps41*) + II miR-a (*vps41*)
	3	Primer B + I miR-s (*vps41*)
	4	Primer A + Primer B