Supplementary Figures and Tables

Figure S1. Subcellular localization of YFP-NPSN11 and characterization of T-DNA insertion knockout allele *npsn11-2*

(A) Immunofluorescence localization of YFP-NPSN11 expressed from the *KNOLLE* promoter. YFP-NPSN11 (green) completely co-localizes with KNOLLE (red) at the cell plate (asterisk) and KNOLLE-positive endomembrane compartments during cytokinesis. In interphase cells, the YFP-NPSN11 signal is detected at the plasma membrane whereas KNOLLE has been degraded at the end of cytokinesis. DAPI-stained nuclei and chromatin are shown in blue in the merged image. Scale bar, 5 µm. **(B)** Schematic view of the *NPSN11* gene organization and the site of the T-DNA insertion (SALK_068094). CDS, yellow boxes; mRNA, green boxes (exons) and red arrows (5' UTR and 3' UTR). **(C)** Transcriptional analysis of *NPSN11*. No transcript was detected in *npsn11-2* homozygous mutants by RT-PCR. **(D)** Immunoblotting with anti-NPSN11 antiserum revealed no NPSN11 protein accumulation in *npsn11-2* mutants. Two nonspecific bands are detected by the anti-NPSN11 antiserum (arrowheads). *Col*, Columbia wild-type; *npsn11 -/-*, homozygous mutant; *npsn11 +/-*, heterozygous.

Figure S2. Cell plate localization of VAMP721 and VAMP722

(A-F) Immunofluorescence localization of VAMP721 (A,C,D,F) and VAMP722 (A,B,D,E) in root cells of wild-type (A,D), *vamp721* (B,E) and *vamp722* (C,F) seedlings. **(A-C)** VAMP721 and VAMP722 localize at the cell plate (asterisk) and endosomal structures. **(D-F)** VAMP721 and VAMP722 are localized in BFA compartments after BFA treatment for 1 h. DAPI-stained nuclei in (A-F) are shown in blue. Note the similar staining in (B) and (C) as well as (E) and (F), demonstrating that both VAMP721 and VAMP722 are localized at the cell plate and in BFA compartments. Scale bars, 5 µm.

Figure S3. *vamp721 vamp722* double mutant seedlings display cytokinesis defects

(A) 10-day-old *vamp721 vamp722* double mutant seedlings (right) and a wild-type (WT) seedling (left). The primary root fails to grow properly in double mutants and abnormal adventitious roots are formed (white arrowheads). **(B)** Cell-wall stubs (arrowheads) in cells of the primary root of a 10-day-old *vamp721 vamp722* mutant seedling seen on 3 µm sections stained with toluidine blue.

Figure S4. Cytokinesis-specific expression of VAMP721 or VAMP722 partially complements *vamp721 vamp722* double mutant

(A) Immunoblotting with anti-VAMP721/722 antiserum on *vamp721 vamp722 pKN::Myc-VAMP721* lines (lanes 1,2), *vamp721 vamp722 pKN::YFP-VAMP722* (lanes 3,5), *pKN::YFP-VAMP722* (lane 4), *vamp721 vamp722* (lane 6) and wild-type seedlings (lane C), demonstrated that there is no endogenous protein detectable in the complemented mutants and in the double mutant. Two *Myc-VAMP721* and three *YFP-VAMP722* transgenic lines were analyzed for expression. Ponceau staining was used as loading control. **(B**,**C)** The phenotype of *vamp721 vamp722* mutants (see Suppl. Figure S3) is partially complemented by the Myc-VAMP721 (B) or YFP-VAMP722 (C) protein expressed from the *KN* promoter. Note that there is an almost complete rescue of the primary root growth. **(D)** Complemented *vamp721 vamp722* double mutants (top) and *vamp721* +/- *vamp722* -/- *pKN::YFP-VAMP722* (bottom) plants 10 days after the transfer to soil. **(E)** Complemented double mutant (yellow arrow) shortly before bolting next to *vamp721* +/- *vamp722* -/- *pKN::YFP-VAMP722* plants.

Figure S5. Subcellular localization of GFP-SYP71, and gene expression in T-DNA insertion allele *syp71* and amiRNA knockdown transgenic lines

(A) Subcellular localization of GFP-SYP71 expressed from the *SYP71* promoter. In dividing cells, GFP-SYP71 labels the cell plate (asterisk). Scale bar, 5 µm. **(B)** Seedling phenotypes of T-DNA insertion allele *syp71* (GABI-KAT 367A08). Scale bar, 0.5 mm. **(C)** Schematic view of the *SYP71* gene organization and the site of the T-DNA insertion (GABI-KAT 367A08). The position of the amiRNA is also indicated. CDS, yellow boxes; mRNA, green boxes. **(D)** Transcriptional analysis of *syp71* homozygous GABI-KAT mutant plants by RT-PCR revealed knockout. **(E)** Effect of amiRNA(SYP71) on transcript accumulation of *SYP71* and its paralogs *SYP72* and *SYP73* in *syp71*^{amiR} seedlings of six independent lines. **(F)** Quantitative analysis of *syp71* (GABI-KAT line, sulfadiazine-resistant (Sul^{R})) and *syp71*^{amiR} seedlings (six independent lines). Phenotypic classification was validated by individual genotyping of at least ten seedlings for each phenotypic class and line.

Supplementary Tables

Table S1: Quantitative analysis of SNARE double mutants

(A) Embryos

	wild-type	**Mutant** [a]	**N**
syp71^{amiR} snap33	86%	14%	637
syp71^{amiR} npsn11	87%	13%	420

[a] Expected value is 25%. Only about half the mutant embryos are clearly phenotypically abnormal.

(B) Seedlings

	wild-type	***snap33***	***syp71^{amiR}***	***snap33 npsn11***	***syp71^{amiR} snap33***	**N**
snap33 npsn11	76%	18%		6% [a]		3467
syp71^{amiR} snap33	57%	19%	18%		6% [b]	498
syp71^{amiR} npsn11	75%		25% [c]			506

[a] Expected value for homozygous double mutants is 6.25%

[b] Expected value for homozygous double mutants is 6.25%

[c] Includes homozygous double mutants (exp. 6.25%) and *syp71^{amiR}* homozygous mutants that are heterozygous or homozygous wild-type for *NPSN11* (exp. 18.75%)

Embryos (A) and seedlings (B) analyzed were F1 progenies of the following plant genotypes:
snap33 npsn11 - parents: *snap33/SNAP33 npsn11/NPSN11* (self-pollinated)
syp71^{amiR} snap33 - parents: *RPS5A::GAL4/- snap33/SNAP33* crossed with *UAS::amiRNA(SYP71)/- snap33/SNAP33*
syp71^{amiR} npsn11 - parents: *RPS5A::GAL4/- npsn11/NPSN11* crossed with *UAS::amiRNA(SYP71)/- npsn11/NPSN11*
Whole-mount preparations of heart-stage embryos (A) were phenotypically classified as either mutant (i.e. abnormal) or wild-type by light microscopy. Phenotypic classification of

seedling progenies (B) was validated by genotyping individual seedlings (at least five) representative for each class.

Table S2. Primer sequences

Primer	Sequence	Remark
V721 F	ATATCTAGAATGGCGCAACAATCGTTGATCT	cloning/genotyping
V721 R	CGTGAATTCTTAACACTTAAAC	cloning
V722 F	GATTCTAGAATGGCGCAACAATCGTTGAT	cloning
V722 R	CGAGAATTCTTATTTACCGCAGTTGAATC	cloning/genotyping
GABI-Kat T-DNA	CCCATTTGGACGTGAATGTAGACAC	genotyping
V721 geno AS	ACCGCAACAACACAATAGGCTGTACAACAG	genotyping
LBb Salk	GCGTGGACCGCTTGCTGAACT	genotyping
v722-3 sense	CTTATCAGCGATATATTGATGAATC	genotyping
NPSN 11 F	CATTCTAGAATGGATCCAATATCAGCG	cloning
NPSN 11 R	CATGAATTCTCAGTAATGGTTCCAGAG	cloning
N11 S SALK	GATGAATTCATGGATCCAATATCAGCGGTTAG	genotyping
N11 AS SALK	GATCTCGAGAATACATTTGTCAGTGGCAAC	genotyping
NPSN11 LP	GTCTTATTAGCTTCAAGTAAGTCTT	genotyping
NPSN11 RP	CTGAGCCTTGAGAGCTGCTGAAGTATC	genotyping
LBa1	TGGTTCACGTAGTGGGCCATC	genotyping
TAG3	CTGATACCAGACGTTGCCCGCATAA	genotyping
MH48 - SP2	GAACCGACTGGTTTTCAATACCACC	genotyping
GM S25 - FW4	GCTAGATCCTGGGCTTTCGATTTG	genotyping
KN start	CTTTCTAGAATGAACGACTTGATG	genotyping
KN stop	GTATCGAATTCTCAAGAAGAGCT	genotyping
X37-2 CIII	AAGGCTCTCTGGGACTCCGG	genotyping
X37-2 DIII	GGGATGGATATGGTGGTGC	genotyping
GABI-Kat LB	ATATTGACCATCATACTCATTGC	genotyping
SYP71 3'UTR as	GTAAGACACAAGCACACACTGGAT	genotyping
SYP71 Exon3 s	ACAACCGAAGAGCTTGCTGCG	genotyping/RT-PCR
SYP71 Exon8 as	ATAAGTATGCAGCGATACCCAGAAC	genotyping/RT-PCR
amiRNA-A	CTGCAAGGCGATTAAGTTGGGTAAC	cloning

amiRNA-B	GCGGATAACAATTTCACACAGGAAACAG	cloning
amiRNA71 I	GATTATCGATACAGAAGTTGCGGTCTCTCTTTTGTATTCC	cloning
amiRNA71 II	GACCGCAACTTCTGTATCGATAATCAAAGAGAATCAATGA	cloning
amiRNA71 III	GACCACAACTTCTGTTTCGATATTCACAGGTCGTGATATG	cloning
amiRNA71 IV	GAATATCGAAACAGAAGTTGTGGTCTACATATATATTCCT	cloning
Actin s	ATTCAGATGCCCAGAAGTCTT	RT-PCR
Actin as	TCTGTGAACGATTCCTGGACCTG	RT-PCR
SYP71 F	ATGACTGTGATCGATATTCTGACTAGAG	genotyping/RT-PCR
SYP71 R	CTGGTTCACGGTATCTTTAAGTCTA	genotyping/RT-PCR
SYP72 F	ATGCCGGTCATTGATATCATCTTCA	RT-PCR
SYP72 R	CTGCACAAGCTGTTTCTTGAGCCGA	RT-PCR
SYP73 F	ATGGGCGTAATTGATTTGATCACTAG	RT-PCR
SYP73 R	CTTTGTTACAGTATCCTTGAGCCTC	RT-PCR

Figure S1

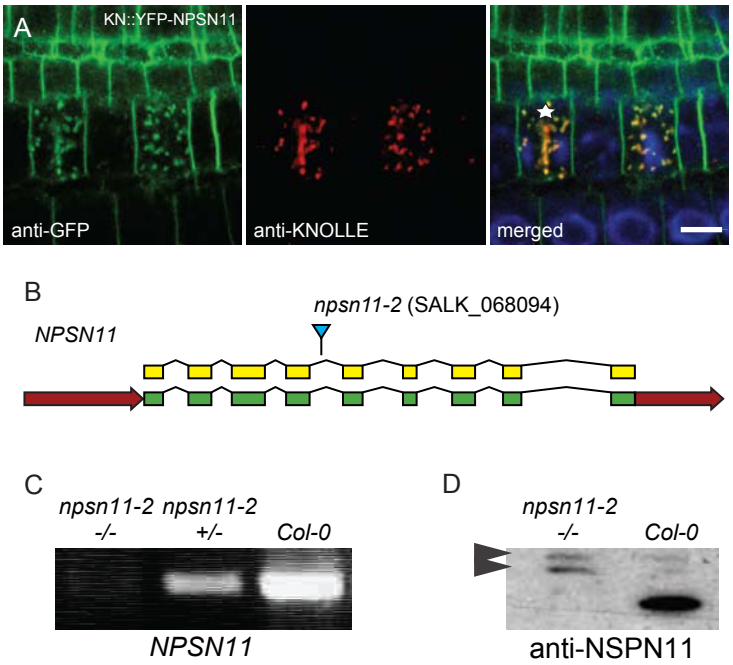

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Figure S2

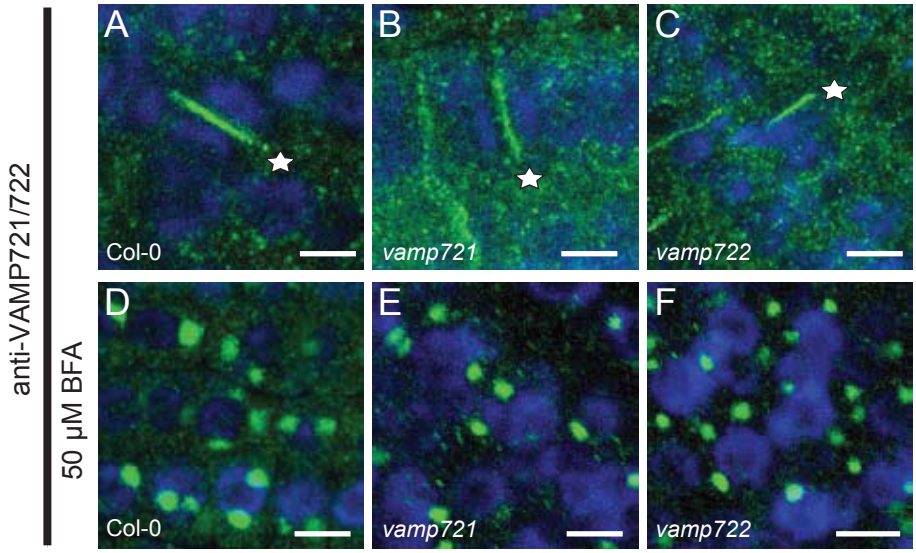

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Figure S3

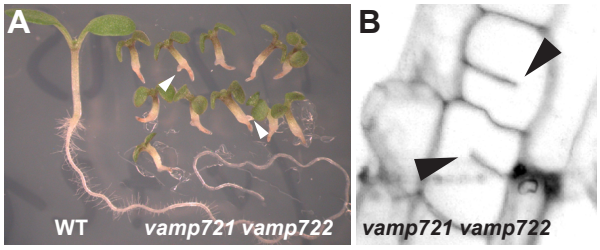

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Figure S4

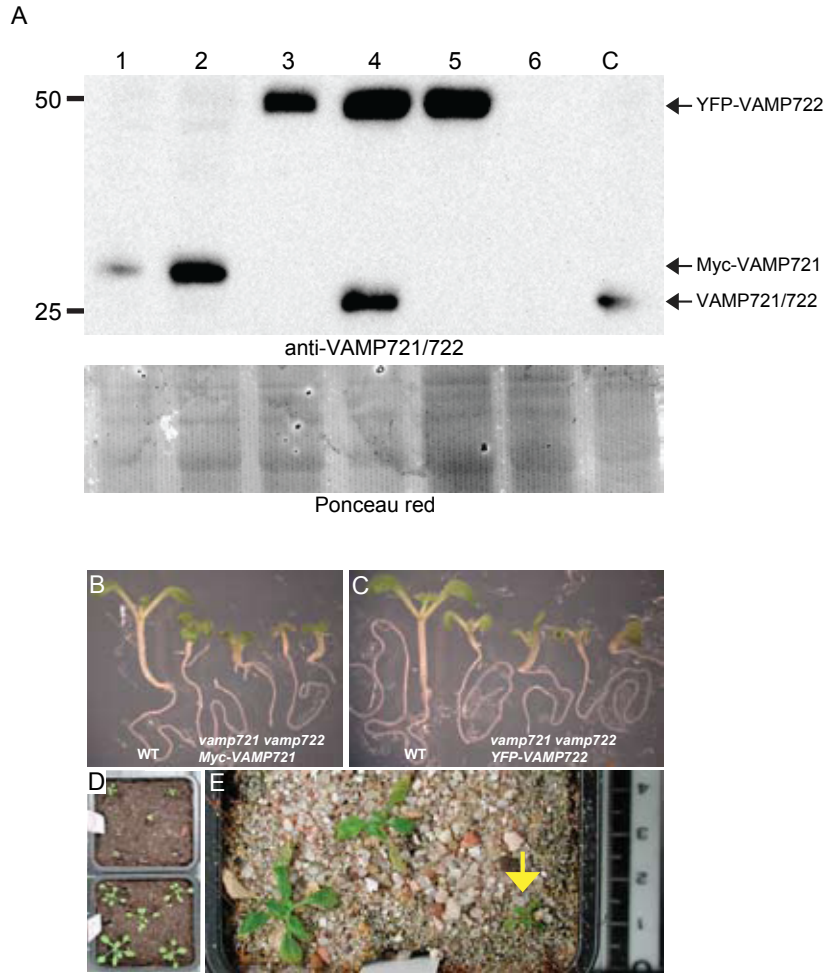

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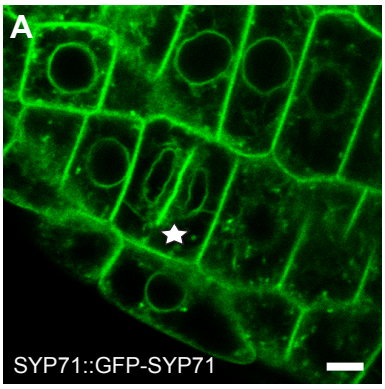
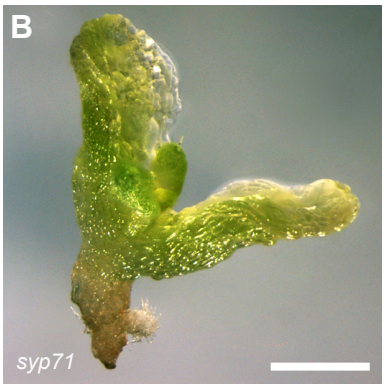
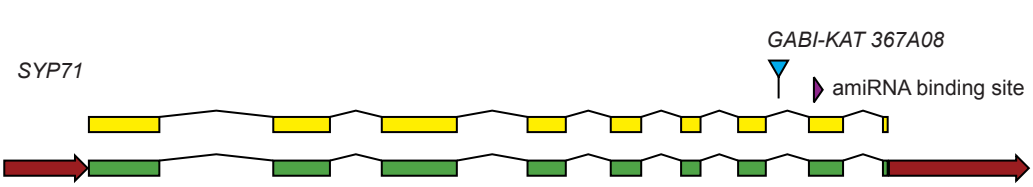
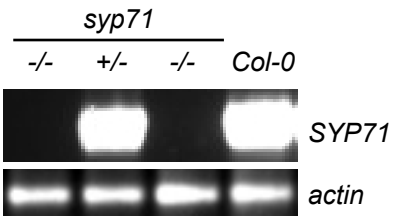
Figure S5





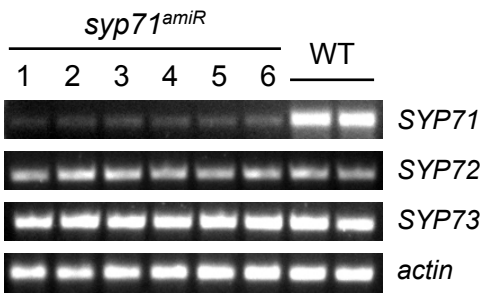




F

(1) GABI-Kat 367A08 (self-pollinated)

WT (Sul^R)	WT (Sul^S)	mutant	N
86%	11%	3%	2933



(2) *UAS::Gal4* +/+ crossed with *RPS5A::amiRNA(SYP71)* +/-

WT	mutant	N
50%	50%	455

Figure S5. Subcellular localization of GFP-SYP71, and gene expression in T-DNA insertion allele *syp71* and amiRNA knockdown transgenic lines

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