

Fig. S1. *At5g58100 (KNS3*) is the causative gene for the ER retention of NIP5;1.

(A) GFP-NIP5;1 in root epidermal cells of F1 plants between EMS mutants. Plants were grown on solid medium containing 30 μ M B for 7–10 days. Scale bar=10 μ m. (B) Summary of rough mapping using the F2 generation between ecotype *Ler* and line1–3, line10–6, line14–3, and line15–2. Recombination rates (%) at each marker position are indicated.



Fig. S2. GFP-NIP5;1 shows polar localization in mutants of *KNS3* and its homologs.

Polar localization of GFP-NIP5;1 in root epidermal cells of *nip5;1-1* (WT), a *KNS3* single mutant (*kns3-3*), and a triple mutant (*kns3-2 knsth1-1 knsth2-1*). Plant roots were stained with FM4-64 for 1 min and an optical longitudinal section of the root meristem zone was imaged by a confocal microscope. Plants were grown with 30 μ M B for 4–7 days. Scale bar=10 μ m.



Fig. S3. Growth of kns3 mutants under different B conditions.

(A) WT, *kns3-2*, *kns3-3*, and *nip5;1-1* were grown on solid medium contained 0.03, 0.3, 3, and 30 μ M B for seven days. Fresh weights of shoots and root lengths were measured from 8–13 plants. Data represent the mean ± SD. The asterisks indicate mutants that showed significant differences to the WT (Dunnett' s test, **P*<0.01). (B) WT, *kns3-3*, and *nip6;1-1* plants were grown hydroponically supplied with 0.1 μ M B under a short-day condition (8 h/16 h light/dark cycle) for 30 days. Arrows indicate young rosette leaves with reduced expansion. (C) WT, *kns3-3*, and *nip6;1-1* plants were grown hydroponically supplied with 1 and 100 μ M B under a long-day condition (16 h/8 h light/dark cycle) for 30 days. An arrow indicates the flowers of the main stem. (D) Boron concentrations in tissues of WT, *kns3-2*, and *kns3-3* plants grown hydroponically with 30 μ M B for 10–12 days and then with 1 or 100 μ M B for 15 days. Roots, rosette leaves, and shoot apices (1.5 cm from top) were harvested from three to four plants. Data represent the mean ± SD. No significant differences were observed (Mann-Whitney U-test, *P*>0.05). Scale bar=1 cm.



Fig. S4. Topologies of KNS3, KNSTH1, and KNSTH2.

Topologies of KNS3 (A), KNSTH1 (B), and KNSTH2 (C) as predicted by AlphaFold protein structure database.



Fig. S5. Pollen structure in kns3, knsth1, and knsth2 multiple mutants.

SEM images of pollens from WT, *kns3-2*, *kns3-2 knsth1-1*, *kns3-2 knsth2-1*, *knsth1-1 knsth2-1*, and *kns3-2 knsth1-1 knsth2-1* mutants. Plants were grown in pots with vermiculite supplied with 1/1000 diluted Hyponex solution (Hyponex Japan). Scale bar=10 µm.



Fig. S6. K941, K943, and I944 in the C-terminal tail of KNS3 are important for its trafficking from the Golgi to the ER.

(A, B) mCherry-KNS3 WT and variants were introduced with GFP-HDEL (A) or ST-YFP (B) in *N.benthamiana* leaf epidermal cells by agroinfiltration. Asterisks indicate the vacuole. Scale bar=10 μ m.



Fig. S7. Mutations in the C-terminal tail of KNSTH2 do not affect its ER localization.

(A, B) mCherry-KNSTH2 WT and variants were introduced with GFP-HDEL (A) or ST-YFP (B) in *N.benthamiana* leaf epidermal cells by agroinfiltration. Scale bar=10 μm.



Fig. S8. Localization of mCherry-KNS3 and KNSTH2 is unchanged in single mutants of their homologs.

(A-D) mCherry-KNS3 and GFP-HDEL (A) or Man1-GFP (B) in protoplasts from WT (Col-0), *knsth1-1*, and *knsth2-1* Arabidopsis leaf mesophyll cells. Pearson' s coefficients of mCherry-KNS3 with GFP-HDEL (C) or Man1-GFP (D). Data represent the mean \pm SD of 10 to 16 protoplasts. No significant differences were observed (Dunnett' s test, *P*>0.05). (E-H) mCherry-KNSTH2 and GFP-HDEL (E) or Man1-GFP (F) in protoplasts from WT (Col-0), *kns3-3*, and *knsth1-1* Arabidopsis leaf mesophyll cells. Pearson' s coefficients of mCherry-KNSTH2 with GFP-HDEL (G) or Man1-GFP (H). Data represent the mean \pm SD of 10 to 11 protoplasts. No significant differences were observed were observed (Dunnett' s test, *P*>0.05). Scale bar=10 µm.



Fig. S9. Phylogenetic tree of KNS3 and its homologs.

Protein sequences of KNS3 homologs (from the National Center for Biotechnological Information Database) in different plant species were used for alignment, and a maximum likelihood-based tree was generated using the MEGA X program. Bootstrap testing was conducted with 1000 replicates. Values are indicated at branch nodes.



Fig. S10. Expression pattern of KNS3.

Expression patterns of *KNS3* in whole plants and pollen. Data were obtained from the ePlant database (https://bar.utoronto.ca/eplant/).



Fig. S11. Expression pattern of *KNSTH1*.

Expression patterns of *KNSTH1* in whole plants and pollen. Data were obtained from the ePlant database (https://bar.utoronto.ca/eplant/).



Fig. S12. Expression pattern of *KNSTH2*.

Expression patterns of *KNSTH2* in whole plants and pollen. Data were obtained from the ePlant database (https://bar.utoronto.ca/eplant/).

	AT5G58100.1	Arabidopsis thaliana	-	-	-	-	R	Ρ	R	Α	Ρ	κ	Р	κ	Ι.	Ν	945
	LOC100779643	Glycine max	-	-	-	-	R	Р	R	R	Ρ	κ	Р	κ	L.	Ν	948
	LOC100800000	Glycine max	-	-	-	-	R	Р	R	R	Ρ	κ	Р	κ	L.	Ν	956
KNS3	LOC100255062	Vitis vinifera	-	-	-	-	R	Р	R	R	Ρ	κ	Р	κ	L.	Ν	938
clade	LOC4329337	Oryza sativa	-	-	-	-	R	Р	R	R	Ρ	κ	Р	κ	L.	Ν	951
	LOC103627145	Zea mays	-	-	-	-	R	Р	R	R	Ρ	κ	Р	κ	L.	Ν	953
	LOC9659220	Selaginella moellendorffii	-	-	-	-	-	Ρ	R	R	L	κ	Р	κ	L.	Ν	949
	LOC112291681	Physcomitrium patens	-	-	-	-	R	R	R	R	Ρ	κ	Р	κ	L.	Ν	953
1	AT3G28720.1	Arabidopsis thaliana	-	κ	R	D	R	L	F	R	Ν	κ	R	κ	Q	F	687
	LOC100818221	Glycine max	-	R	R	D	κ	L	F	R	Ν	κ	R	κ	Q	F	685
	JHK82_035474	Glycine max	-	R	R	D	κ	L	F	R	Ν	κ	R	κ	Q	F	686
	CK203_102423	Vitis vinifera	-	κ	R	D	κ	L	F	R	Ν	κ	R	κ	Q	F	673
clade	LOC4341393	Oryza sativa	-	κ	R	D	κ	L	F	R	s	κ	R	κ	Q	F	672
	LOC100279525	Zea mays	-	κ	R	D	κ	L	F	R	s	κ	R	κ	Q	F	671
	LOC9632901	Selaginella moellendorffii	-	R	R	E	Q	L	F	Α	s	κ	R	κ	R	F	678
	LOC112280368	Physcomitrium patens	Ν	κ	R	D	κ	F	L	v	Ν	κ	κ	κ	R	F	676
1	AT4G16180.2	Arabidopsis thaliana	-	-	-	-	-	-	-	-	-	s	Р	Ρ	s	R	820
	LOC100790294	Glycine max	-	-	-	-	-	-	-	-	-	-	s	Ρ	v	R	803
	LOC100812469	Glycine max	-	-	-	-	-	-	-	-	-	-	s	Ρ	v	R	803
	LOC100258488	Vitis vinifera	-	-	-	-	-	-	-	-	-	-	s	Ρ	v	R	809
KNSTH2	LOC4332629	Oryza sativa	-	-	-	-	-	-	-	-	-	-	s	Ρ	v	R	807
clade	LOC103634577	Zea mays	-	-	-	-	-	-	-	-	-	-	s	Ρ	v	R	804
	LOC9642494	Selaginella moellendorffii	-	-	-	-	-	-	-	-	-	s	G	D	κ	w	806
	SEMO_269607	Selaginella moellendorffii	-	-	-	-	-	-	-	-	-	s	G	D	κ	w	803
	LOC112293080	Physcomitrium patens	-	-	-	-	-	-	-	-	-	-	s	s	Е	R	820
	LOC112282869	Physcomitrium patens	-	-	-	-	-	-	-	-	-	-	s	s	Е	R	817

Fig. S13. Multiple alignments of the amino acid sequences of the C-terminal tail of KNS3 and its homologs.

Multiple alignments of the C-terminal tails of KNS3 homologs were performed using Clustal Omega. The conserved amino acids are highlighted in gray.



Fig. S14. A working hypothesis of the functions of KNS3, KNSTH1, and KNSTH2 in an ER-Golgi cargo-receptor complex.

In our hypothetical model, KNS3, KNSTH1, and KNSTH2 form a cargo-receptor complex that interacts with boric acid channels and other cargoes in the ER, packaging them into COPII vesicles for transport to the Golgi. In the Golgi, the cargo-receptor complex separates from the cargo and returns to the ER via COPI vesicles. Boric acid channels and other PM cargoes are transported from the Golgi apparatus to the trans-Golgi network, and then to the PM via the secretion pathway.

	Na	Locati	Sequence			
	me	on	forward	reverse	uct	
		(Mbp)			size	
					Col-	
					0/Ler	
					(bp)	
Chromos	map	3	CGTGAACCCACTCGTTAC	TGCATTTCAACTTTAC	248/2	
ome 1	1-		ATT	CAACCA	04	
	3M					
	map	13.8	ATGTTGGATTCAAGCACT	AAGGTTCCGTCAGAC	200/1	
	1-		TCC	GTG	56	
	13.8					
	М					
	map	24.4	TACTCATGCGGATGCGGT	TCTCCCTCCCTTTTCT	187/1	
	1-		ТА	TGCT	32	
	24.4					
	М					
Chromos	map	3.5	ATCAACATCCGCAAAGTT	ACCTCCTTAGTCGCG	383/3	
ome 2	2-		CC	TGAAA	27	
	3.5					
	М					
	nga	16.3	GAGGACATGTATAGGAGC	TCGTCTACTGCACTG	150/1	
	168		CTCG	CCG	30	
Chromos	nga	4.6	CTCTGTCACTCTTTTCCTC	CATGCAATTTGCATCT	110/8	
ome 3	162		TGG	GAGG	5	
	map	18.2	TCGAGGACTTTTATTGAT	TGGATGAAAAGAAGG	265/1	
	3-		AGATTGAA	CAAGG	82	
	18M					
Chromos	NG	5.6	TGGCTTTCGTTTATAAAC	GAGGGCAAATCTTTA	154/1	
ome 4	A8		ATCC	TTTCGG	98	
Chromos	2.5	2.5	CCAAGACCAAAACCAAA	CATGCAATAGGCTTC	247/1	
ome 5	М		ACC	GGAGT	73	
	map	19.9	TGACAACTTTGGGCAATT	CGCATGATGCATAGC	246/2	
	5-		AAGA	AAAGT	09	
	19.9					
	М					

Table S1. SSLP markers for rough mapping.

Primer name	Sequence (5'>3')	Purpose		
KNS3 (1-34aa)	CTGATTAACACTCGAATGCGGAGATTCGGGGGCT	KNS3 (1-		
F		34aa) for		
KNS3 (1-34aa)	TCCTCCTCGCCCTTGCTCAATTGTGAAGCTCCATACGATA	In-Fusion		
R	GA	cloning		
mCherry F	TTGAGCAAGGGCGAGGAG	mCherry		
mCherry R	CTTGTACAGCTCGTCCATGCC	for In-		
		Fusion		
		cloning		
KNS3 (35-	GCATGGACGAGCTGTACAAGGGGGAACCGGAAGACGGCG	KNS3 (35-		
945aa) F	AA	945aa) for		
KNS3 (35-	AGTTGGATATCTCGATCAGTTGATCTTTGGCTTAG	In-Fusion		
945aa) R		cloning		
KNSTH2 (1-	CTGATTAACACTCGAGTCATGGAGTTGAGATCGG	KNSTH2		
36aa) F		(1 - 36aa)		
KNSTH2 (1-	TCCTCCTCGCCCTTGCTCAATTGCTGAGCCGAGTCGGTA	for In-		
36aa) R		Fusion		
		cloning		
KNSTH2 (37-	GCATGGACGAGCTGTACAAGCCTTTCCGTCGCGAGCCA	KNSTH2		
820aa) F		(37-820aa)		
KNSTH2 (37-	AGTTGGATATCTCGACTAGCGAGAAGGAGGAGA	for In-		
820aa) R		Fusion		
		cloning		
P937A F	TTAAGGGCTAGAGCTCCTAAGCCAAAG	Introductio		
P937A R	AGCTCTAGCCCTTAAGACAGCGTAAAG	n of		
		mCherry-		
		KNS3		
		P937A		
		mutation		
R938A F	AGGCCAGCTGCTCCTAAGCCAAAGATC	Introductio		
R938A R	AGGAGCAGCTGGCCTTAAGACAGCGTA	n of		
		mCherry-		
		KNS3		
		R938A		
		mutation		
P940A F	AGAGCTGCTAAGCCAAAGATCAACTGA	Introductio		

P940A R	TGGCTTAGCAGCTCTTGGCCTTAAGAC	n of
		mCherry-
		KNS3
		P940A
		mutation
K941A F	GCTCCTGCTCCAAAGATCAACTGATCG	Introductio
K941A R	CTTTGGAGCAGGAGCTCTTGGCCTTAA	n of
		mCherry-
		KNS3
		K941A
		mutation
P942A F	CCTAAGGCTAAGATCAACTGATCGAGA	Introductio
P942A R	GATCTTAGCCTTAGGAGCTCTTGGCCT	n of
		mCherry-
		KNS3
		P942A
		mutation
K943A F	AAGCCAGCTATCAACTGATCGAGATAT	Introductio
K943A R	GTTGATAGCTGGCTTAGGAGCTCTTGG	n of
10/10/11		mCherry-
		KNS3
		K943A
		mutation
1944 A F		Introductio
		n of
1744 K		mCherry-
		KNS3
		1944 A
		mutation
N945A F		Introductio
NO45A P		n of
11773Λ Κ		mCherry_
		KNS3
		IN943A
C0164 F	TTOTOTOOTOOTOOTTOTOOOTAOTOO	Tutation
5810A F	TICICIGCICCICCITCICGCIAGICG	Introductio

S816A R	AGGAGGAGCAGAGAAGAAAATGACAAG	n of
		mCherry-
		KNSTH2
		S816A
		mutation
P817A F	TCTTCTGCTCCTTCTCGCTAGTCGAGA	Introductio
P817A R	AGAAGGAGCAGAAGAAGAAGAAAATGAC	n of
		mCherry-
		KNSTH2
		P817A
		mutation
P818A F	TCTCCTGCTTCTCGCTAGTCGAGATAT	Introductio
P818A R	GCGAGAAGCAGGAGAAGAAGAAAAAT	n of
		mCherry-
		KNSTH2
		P818A
		mutation
S819A F	CCTCCTGCTCGCTAGTCGAGATATCCA	Introductio
S819A R	CTAGCGAGCAGGAGGAGAAGAAGAAGAA	n of
		mCherry-
		KNSTH2
		S819A
		mutation
R820A F	CCTTCTGCTTAGTCGAGATATCCAACT	Introductio
R820A R	CGACTAAGCAGAAGGAGGAGAAGAGAA	n of
		mCherry-
		KNSTH2
		R820A
		mutation
LBb1.3	ATTTTGCCGATTTCGGAAC	Genotypin
		g for SALK
		T-DNA
		mutant
SALK_041228	TCAGTGCATACATATGCTGGC	Genotypin
LP		g for kns3-
SALK 041228	ACCATCAGGCTGTTGACATTC	2

RP		
SALK_061320	AAAGGAGCCAACCTTGAGAAG	Genotypin
LP		g for <i>kns3-</i>
SALK_061320	AAAGAAGCCTTTCCTTGATGC	3
RP		
SALK_027378	GTTCAAGAAAACGTCAGACGC	Genotypin
LP		g for
SALK_027378	CGTCAAGGTGGAGAGAGTGAG	knsth1-1
RP		
SALK_106609	CATCGGAGACTCTTTCCCTTC	Genotypin
LP		g for
SALK_106609	GCCCGTTGCAAGTATAATCAC	knsth1-2
RP		
LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC	Genotypin
		g for SAIL
		T-DNA
		mutant
SAIL_731_H0	AGGTGTAGGCTAGCGAGAAGG	Genotypin
3 LP		g for
SAIL_731_H0	ATGCTGTCCCATCACAGGTAC	knsth2-1
3 RP		
SAIL_670_H0	TAGCCGATGTAGATCCAATGC	Genotypin
1 LP		g for
SAIL_670_H0	ACCAGCCACAAGTATTCCTCC	knsth2-3
1 RP		

Table S2. Primers used in this research.

		localization						
		in Ara	bidopsis	protoplasts	in tobacco leaf cells			
	WT	ER, G	olgi	(Figs 6, 8)	ER, Golgi	(Fig. 6, Fig.		
						S6)		
	P937A	ER, G	olgi		ER, Golgi			
	R938A	ER, G	olgi		ER, Golgi			
	P940A	ER, G	olgi		ER, Golgi			
	K941A	ER, Go	olgi,		Golgi,			
KNS3		vacuo	ole		vacuole			
	P942A	ER, G	olgi	(Fig. 8)	ER, Golgi	(Fig. S6)		
	K943A	ER, Go	olgi,		Golgi,			
		vacuo	ole		vacuole	_		
	I944A	Golg	gi		Golgi,			
					vacuole			
	N945A	ER, G	olgi		ER, Golgi			
	WT	ER	_	(Figs 7, 9)	ER	(Fig. 7, Fig.		
						S7)		
	S816A	ER			ER	-		
KNSTH2	P817A	ER			ER	-		
	P818A	ER		(Fig. 9)	ER	(Fig. S7)		
	S819A	ER			ER	-		
	R820A	ER			ER			
			in Ara	bidopsis protopla	asts			
	WT			knsth1-1	knsth2-1			
KNS3 WT	ER, Gol	gi	I	ER, Golgi	ER, Golgi			
	(Figs 6, 8, F	ig. S8)		(Fig. S8)	(Fig	g. S8)		
VNCTU2	WT			kns3-3	kns	th1-1		
WT	ER			ER	I	ER		
VV I	(Figs 7, 9, F	ig. S8)		(Fig. S8)	(Fig	g. S8)		
KNIC2	WT		knstl	h1-1/knsth1-2	knsth2-	l/knsth2-3		
	Golgi		I	ER, Golgi	ER, Golgi			
1944A	(Figs 8, 10)			(Fig. 10)	(Fig. 10)			

Table S3. Summary of the localization of mCherry-KNS3 and mCherry-KNSTH2in Arabidopsis protoplasts and tobacco leaf cells.

Organism	Name	Protein length (aa)	NCBI protein number		
A 1'1 '	AT5G58100.1	945	NP_001318823.1		
Arabidopsis	AT4G16180.2	820	NP_001154242.2		
thanana	AT3G28720.1	687	NP_189514.1		
	LOC100779643	948	XP_003516388.1		
	LOC100800000	956	XP_003532318.1		
Glycine	LOC100818221	685	XP_003554877.2		
max	JHK82_035474	686	KAG5112205.1		
	LOC100790294	803	XP_003528615.1		
	LOC100812469	803	XP_003550564.1		
¥7'.'	LOC100255062	938	XP_010655027.1		
Vitis vinifera	CK203_102423	673	RVW26491.1		
	LOC100258488	809	XP_002273166.2		
_	LOC4329337	951	XP_015624632.1		
Oryza	LOC4341393	672	XP_015644546.1		
sativa	LOC4332629	807	XP_015632688.1		
7	LOC103627145	953	XP_008645675.1		
Zea	LOC100279525	671	XP_001145995.2		
mays	LOC103634577	804	XP_008655047.1		
	LOC9659220	949	XP_024530236.1		
Selaginella	LOC9632901	678	XP_002991685.1		
moellendorffii	LOC9642494	806	XP_024516482.1		
	SEMO_269607	803	EFJ08873.1		
	LOC112291681	953	XP_024395234.1		
Physcomitrium	LOC112280368	676	XP_024371568.1		
patens	LOC112293080	820	XP_024397913.1		
	LOC112282869	817	XP_024376759.1		
Chlamydomonas	CHLRE_16G680450	1079	XP_042916023.1		
reinhardtii	CHLRE_10G463300	1012	XP_042920595.1		

Table S4. List of	proteins collected	using BLAST in	the NCBI database.
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