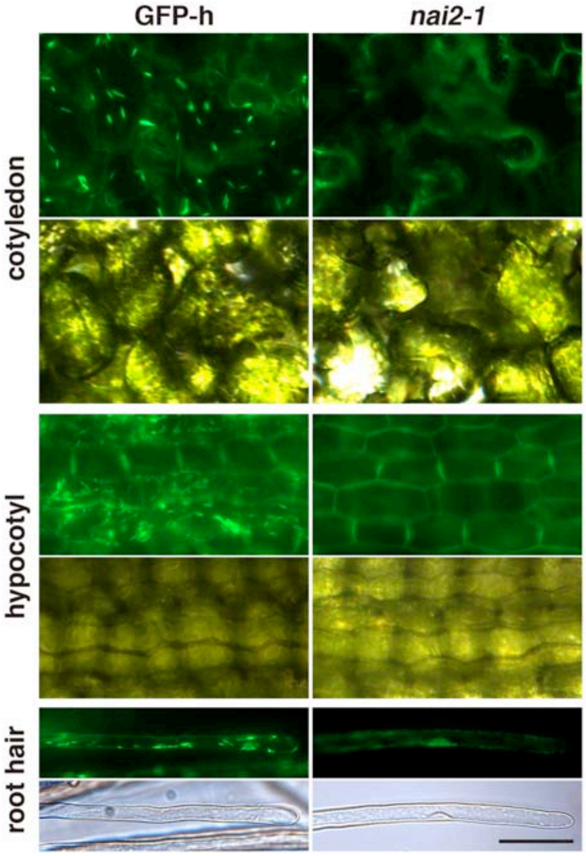
Supplemental Data. Yamada et al. (2008). NAI2 is an endoplasmic reticulum (ER) body component that enables ER body formation in *Arabidopsis thaliana*.



Supplemental Figure 1. GFP fluorescence and bright field images of the GFP-h plant and *nai2-1* mutant.

Cotyledons, hypocotyls and root hair cells of 7-day-old seedlings were inspected under a microscope. Upper panels are the GFP fluorescence images and lower panels are the blight field images. All panels are shown at the same resolution. Bar = 50 μm.

A

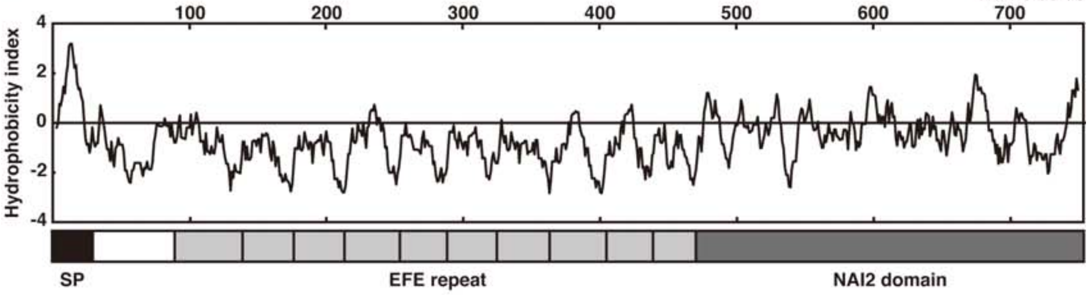
MEIYTMKTNFLVLALSLCILLSSFHEVSC 29 **Signal Peptide**

QDDGSGLSNLDLIERDYQDSVNALQGKDDEDQSAKIQSENQNNTTVTDKN 79
TISLSLSDESEV 91

GSVSDE**S**VGRS**S**L**L**DQ**I**KL**EFEA**HHNSIN**Q**AGSDGVKAESK 132 **EFE repeat**
DDD**EE**L**SA**HRQKM**LEEIE**H**EFEAA**SDS**L**K**QL**KTD**D**VNE 170
GND**EE**H**SAKR**Q**S**L**LEEIEREFEAA**TKE**L**E**QL**KVN**D**FTGD 209
KDD**EE**H**SAKR**K**S**M**LE**A**IEREFEAA**MEGIEA**L**KVS**D**STGS 248
GDD**EE**Q**SAKR**L**S**M**LEEIEREFE**GLEQLRASDSTA**D** 283
NNE**EE**HA**AK**GQ**S**L**LEEIEREFEAA**TES**L**K**QL**QVD**D**STE 321
DKEHFTA**AKR**Q**S**L**LEEIEREFEAA**TKD**L**K**QL**NDFTEG 358
SADD**E**Q**SAKR**NKM**LE**D**IEREFEAA**TIG**L**E**QL**KAN**D**FSEG 397
NNN**EE**Q**SAKR**K**S**M**LEEIEREFEAA**IGG**L**K**Q**IKVD**D**S 433
RNL**EE**E**SAKR**KII**LEE**M**EREFEEA**HSGINAKADKEE 469

SAKKQSGSAIPEVLGLGQSGGCSCSKQDEDSSIVIPTKYSIEDILSEESA 519 **NAI2 domain**
VQGTETSSLTASLTQLVENHRKEKESLLGHRVLTSPSIASSTSESSATSE 569
TVETLRAKLNELRGLTARELVTRKDFGQILITAASFEELSSAPISYISRL 619
AKYRNVIKEGLEASERVHIAQVRAKMLKEVATEKQTAVDTHFATAKKLAQ 669
EGDALFVKIFAIKKLLAKLEAEKESVDGKFKETVKELSHLLADASEAYEE 719
YHGAVRKAKDEQAAEEFAKEATQSAEIIWVKFLSSL 755

B



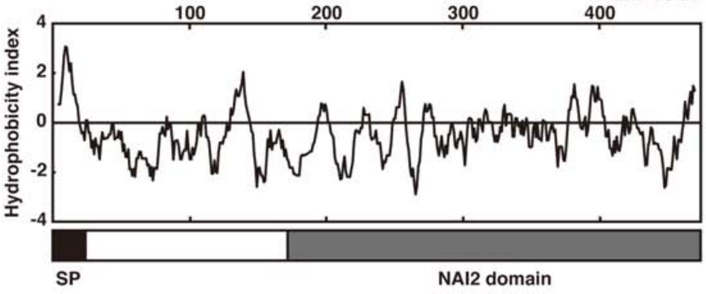
C

MGRKYVVLGLAVCLFLSSFNEVSC 24 **Signal Peptide**

QAEGGIESSNSEFQGSFLEEGEVEVTKIESESQSSSSEQNTKLSMSEENQ 74
EDSFDVDKFIESEAVSSREELGQSSSMDEVNRDLEAILDSLNKRDGSDDI 124
SKVGESMDAAGIAAAATNIVVEDGTSKRKSKVEETQEVVKFESESSSASS 174
E 175

SRRQSSSSYNSFNKGTGGSEMLGSLGISQSGSWRCYNQDKNGVSEEEDTS 225 **NAI2 domain**
IVIPKYDIDSIIKEESTSQGSSSKTSSLIASLTKIVEKHRKEKWSSGVSV 275
SVTKGASSTQTSETVEKLKVTLKKYRGLSARELVARSDFEEILATAARYE 325
ELSSASVSHISRLSMYRSVIKEGIKASQRVQLAYARTRLLKEMAVEKQKN 375
VDAELALVKALAERGDMLYVKIFAIKKLISKLEAEKYEVDMTFEKTVANL 425
SRVIEEASQAYEEYHVVVRKWKEEQASEEFSREAIERVEMVWVEFLSTL 474

D



Supplemental Figure 2. Structures of the Two NAI2 Homologues in *A. thaliana*.

(A) The deduced amino acid sequence of TSA1. The numbers show the amino acid residue positions. TSA1 contains a signal peptide, EFE repeats and a NAI2 domain. The bold characters indicate the conserved amino acids within the EFE repeats.

(B) Hydrophobicity index of TSA1. The structure of TSA1 is shown below.

(C) The deduced amino acid sequence of the At3g15960 protein. The numbers show the amino acid residue positions. The At3g15960 protein contains a signal peptide and a NAI2 domain but lacks EFE repeats.

(D) Hydrophobicity index of the At3g15960 protein. The structure of the At3g15960 protein is shown below.

A

```
GGAAGCTTTAGAACACTGTGTCTTGTAATGGAAATATCCGCCATAAGAATGAATTTTCTC     60
                           M  E  I  S  A  I  R  M  N  F  L       11
GCGCTGGGTTTGTCTCTGTGTCTTGTTCTTTCGAGTTTCCATGGCGTTTCTTGTCAGGAT    120
A  L  G  L  S  L  C  L  V  L  S  S  F  H  G  V  S  C  Q  D       31
GATGGTGCTGCTAGTAGGTTGAGTCATCTGGATCTAATCGAACGTGAGTACCAAGATAGT    180
D  G  A  A  S  R  L  S  H  L  D  L  I  E  R  E  Y  Q  D  S       51
GTCAATGCTCTTCAAGGCAAGGAAGATCAGTCTGCAAGTATTCAGAGTGAGAACCAGAAG    240
V  N  A  L  Q  G  K  E  D  Q  S  A  S  I  Q  S  E  N  Q  K       71
AACACTACAGCGACTGATAAGAACACTATTTCTCTGTCTCTATCAGATGAATCTGAGGTT    300
N  T  T  A  T  D  K  N  T  I  S  L  S  L  S  D  E  S  E  V       91
GGAGGAGTGAGTGATGAAAGTGTTAAAAATTCGAGTCTGTTGGATGACATAGAACTTGAA    360
G  G  V  S  D  E  S  V  K  N  S  S  L  L  D  D  I  E  L  E      111
ATTGAAGCTCATCTCAATGGTCTTAACCAAGCTGGATCCGCTGATGTTAATGCTGAATCC    420
I  E  A  H  L  N  G  L  N  Q  A  G  S  A  D  V  N  A  E  S      131
AAATATGATGAAGTATTATCTGCACAGAGACAGAAGATGTTGGAAGACATCGAACGCGAT    480
K  Y  D  E  V  L  S  A  Q  R  Q  K  M  L  E  D  I  E  R  D      151
TTTGAAGCTGCTTCATCAGCAAGTTTGGAACAAATAAAGACTGATGAATTAAGTGAAGGA    540
F  E  A  A  S  S  A  S  L  E  Q  I  K  T  D  E  L  S  E  G      171
ATTAATGAGCAACAATCTGCAAAGAAACATAGTTTGCTGGAAGAGATCGAACGCGAATTT    600
I  N  E  Q  Q  S  A  K  K  H  S  L  L  E  E  I  E  R  E  F      191
GAAACTGCTACAAAAGATCTTGAACAACTAAAGGTTAATGACTTCACTGGGAACAAGCTT    660
E  T  A  T  K  D  L  E  Q  L  K  V  N  D  F  T  G  N  K  L      211
GACGAAGAACAATCTGCAAAGAGAAAAAGCATGCTTGAAGCTATTGAACGCGAGTTTGAA    720
D  E  E  Q  S  A  K  R  K  S  M  L  E  A  I  E  R  E  F  E      231
GCTGCTGTTGAAGGCCTTGAAGAACTAAAGGTTTCTGATTCTACCGGAAGCAAGGATGAT    780
A  A  V  E  G  L  E  E  L  K  V  S  D  S  T  G  S  K  D  D      251
GAAGAACAATCTGCAAAGAGACTAAGCGTGCTGGAAGAGATTGAACGGGAATTCGAAGCT    840
E  E  Q  S  A  K  R  L  S  V  L  E  E  I  E  R  E  F  E  A      271
GCTACAGAGAGCCTTAAGCAACTTCAAGTTGATGGTTCGAGTGAAGACACAGAACAAACT    900
A  T  E  S  L  K  Q  L  Q  V  D  G  S  S  E  D  T  E  Q  T      291
GGAAAGAGGCAAAGTATGCTTGACGAGATTGAACGTGAATTTGAAGCTGCTACACGTGAT    960
G  K  R  Q  S  M  L  D  E  I  E  R  E  F  E  A  A  T  R  D      311
CTTAAGCAGCTAAATGATTTCACTGAAGGAGCTGATGATGAACAATCTGCAAAGAGAAAC   1020
L  K  Q  L  N  D  F  T  E  G  A  D  D  E  Q  S  A  K  R  N      331
AGTGTAGTAGAAGAGATGGAACGTGAGTTTGAAGCTGCTACAAAAAAACTTAATTATTTA   1080
S  V  V  E  E  M  E  R  E  F  E  A  A  T  K  K  L  N  Y  L      351
GCTGAAGGCAGTGACAATGAAGAACAATCTGCAAAGAGGAAGACTATGCTTGAAGAGATG   1140
A  E  G  S  D  N  E  E  Q  S  A  K  R  K  T  M  L  E  E  M      371
GAACGTGAATTTGAAGCTGCCATTGGAGGTCTTAAACAGATCAAAGATGACGAATCTAAG   1200
E  R  E  F  E  A  A  I  G  G  L  K  Q  I  K  D  D  E  S  K      391
TACACTGAAGAACAAGCATCTAAGAGAAAGATAATGTTGGAAGAGATCGAACGCGAATTC   1260
Y  T  E  E  Q  A  S  K  R  K  I  M  L  E  E  I  E  R  E  F      411
GAAGAAGCTCGAAGTGGCTTTAGCACAAATGCTAATAAAGAAGGATCTGCAAAGAAACAT   1320
E  E  A  R  S  G  F  S  T  N  A  N  K  E  G  S  A  K  K  H      431
AGTATTACATTAGAGTCTCTTGGACTAGGACAGTCAGGTGTCTGTGGCTGTTTTAATCAA   1380
S  I  T  L  E  S  L  G  L  G  Q  S  G  V  C  G  C  F  N  Q      451
GACAAAGCTGGTTTAAAGCAAGACGAAGATGCTTCAATCGCTATATCAACAAAATATAGC   1440
D  K  A  G  L  K  Q  D  E  D  A  S  I  A  I  S  T  K  Y  S      471
ATAGAAGAGATCCTCACTGAAGAATCTTCACTCCAGGGCACAGATACTTCTTCTAGTCTC   1500
I  E  E  I  L  T  E  E  S  S  L  Q  G  T  D  T  S  S  S  L      491
ACTAAGTCTTTGACTCAACTAGTTGAGAATCACAGGAAAGAAAAGGAATCTCATAATGTG   1560
T  K  S  L  T  Q  L  V  E  N  H  R  K  E  K  E  S  H  N  V      511
CACACTTCATCCACAAGTGAATCAGCAGCCACATCAGAGACTGTAGAGAGCCTTAGAGCT   1620
H  T  S  S  T  S  E  S  A  A  T  S  E  T  V  E  S  L  R  A      531
AAACTGAGAGAGCTTCGTGGCTTAACCGCTCGTCAGCTCGTGACACGCCAAGATTTTGAG   1680
K  L  R  E  L  R  G  L  T  A  R  Q  L  V  T  R  Q  D  F  E      551
AGCATTATCCTTATGGCTGCAACTTTCGAAGAGCTAAGCTCAGCTCCCATCAGTTACATT   1740
S  I  I  L  M  A  A  T  F  E  E  L  S  S  A  P  I  S  Y  I      571
TCTAGGTTAGCTAAATACGGAAACGTCATAAAAGAAGGACTTGAAGCTTCTGAGAGAGTC   1800
S  R  L  A  K  Y  G  N  V  I  K  E  G  L  E  A  S  E  R  V      591
CACATGGCAAAGGCAAGAGCCACAATGCTCAAAGAGACTTCCAGGGAGAAGCAGATCTTC   1860
H  M  A  K  A  R  A  T  M  L  K  E  T  S  R  E  K  Q  I  F      611
GTGGACGCTAACTTCGAAGAGGCTAAGAAGCTTGCTCAAAGAGGAGACTCCTTGTACGTT   1920
V  D  A  N  F  E  E  A  K  K  L  A  Q  R  G  D  S  L  Y  V      631
AGAATCTTTGCGATCAAGAAACTGTTGAAGAAGCTTGAAACTGAGAGAGAATCTGTTGAT   1980
R  I  F  A  I  K  K  L  L  K  K  L  E  T  E  R  E  S  V  D      651
GTGATGTTTAAGGAGATTGTGAAAGGTCTTTCTCATCTTCTTGTTGATGCTTCTGAGGCC   2040
V  M  F  K  E  I  V  K  G  L  S  H  L  L  V  D  A  S  E  A      671
TATGAAGAGTATCATGGAGCTGTAAGGAAGGCTAAAGACGAGCAAGCAGCTGAGGAGTTT   2100
Y  E  E  Y  H  G  A  V  R  K  A  K  D  E  Q  A  A  E  E  F      691
GCGAGAGAGGCAACGCAGAGTGCAGAGATAATATGGGTTAAGTTTCTTAGTTCTCTTTAG   2160
A  R  E  A  T  Q  S  A  E  I  I  W  V  K  F  L  S  S  L  *      710
AGAACAGTTGAGATTCTTTGTTTGTCTTTAGAGCAAGTCTAGAGCTCTTGTTGGTTATTG   2220
TTTTCTACCTTGTGTTTATGTTCTGTTTCAGACTTTGTGTGTTGGTTATATCAGGAGAGA   2280
GAGAGATTTGGGAGATAGAGAGGGGAAAACAAATAATGTTTATTTTAGATTTCTAATAAA   2340
ATAAGCTTCCAATAATTCATAAAAAAAAAGGGTTGC                           2375
```

B

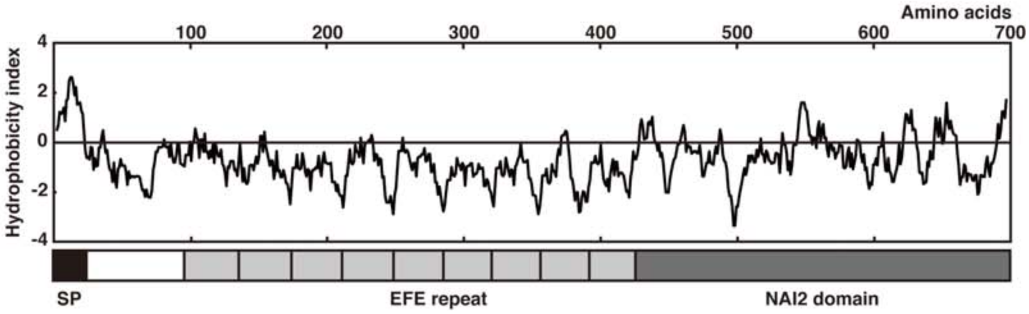
MEISAIRMNFLALGLSLCLVLSSFHGVSC 29 **Signal Peptide**

QDDGAASRLSHLDLIEREYQDSVNALQGKEDQSASIQSENQKNTTATDKN 79
TISLSLSDESEV 91

GGVSDE**S**V**K**NS**S**L**L**DD**IE**L**E**I**EA**HLNG**L**N**Q**AGSADVNAES 131 **EFE repeat**
KY**DE**VL**SA**Q**R**QK**MLE**D**IER**D**FEAA**SSASLEQIKTDELSE 170
GIN**E**Q**QSAK**KH**S**L**LEEIEREFE**T**A**TKD**L**E**QL**KVNDFTGN 209
KL**DEEQSAKR**K**SMLE**A**IEREFEAA**VEG**L**EE**L**KVSDSTGS 248
KD**DEEQSAKR**L**S**V**LEEIEREFEAA**TES**L**K**QL**QVDGS 284
SE**D**T**EQ**TG**KR**Q**SML**D**EIEREFEAA**TRD**L**K**QL**NDFTE 320
GA**D**D**EQSAKR**N**S**VV**EE**M**EREFEAA**TKK**L**NY**L**AEG 354
SDN**EEQSAKR**KT**MLEE**M**EREFEAA**IGG**L**K**Q**IKDDES 390
KYT**EEQ**AS**KR**KI**MLEEIEREFE**E**A**RSGFSTNANKEG 426

SAKKHSITLESLGLGQSGVCGCFNQDKAGLKQDEDASIAISTKYSIEEIL 476 **NAI2 domain**
TEESSLQGTDTSSSLTKSLTQLVENHRKEKESHNVHTSSTSESAATSETV 526
ESLRAKLRELRGLTARQLVTRQDFESIILMAATFEELSSAPISYISRLAK 576
YGNVIKEGLEASERVHMAKARATMLKETSREKQIFVDANFEEAKKLAQRG 626
DSLYVRIFAIKKLLKKLETERESVDVMFKEIVKGLSHLLVDASEAYEEYH 676
GAVRKAKDEQAAEEFAREATQSAEIIWVKFLSSL 700

C

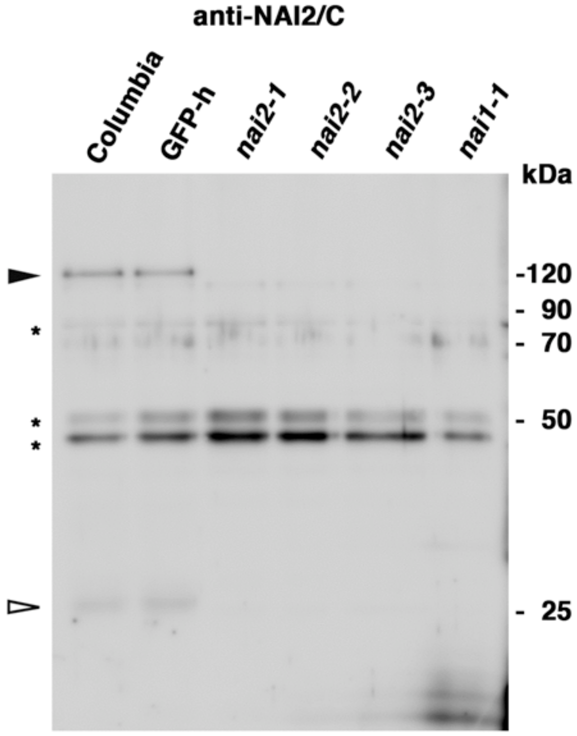


Supplemental Figure 3. Structure of the NAI2 Homologue in *B. napus*.

(A) Nucleotide and deduced amino acid sequences of TC74287. The numbers show the nucleotides or amino acid residue positions.

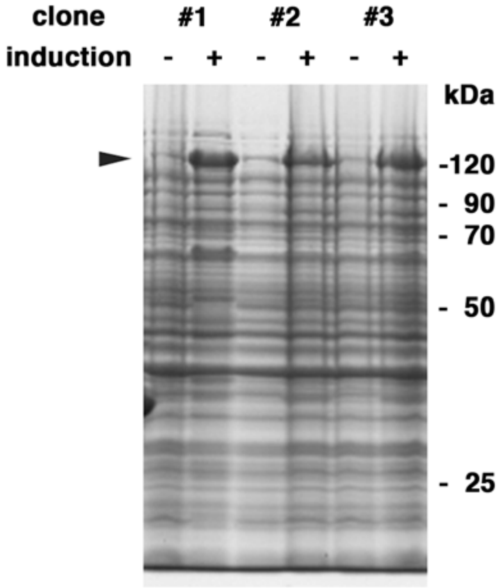
(B) The primary structure of the TC74287 protein. The numbers show the positions of amino acid residues. TC74287 consists of a signal peptide, nine EFE repeats, and a NAI2 domain. The bold characters show the conserved amino acids within the EFE repeats.

(C) Hydrophobicity index of the TC74287 protein. The structure of the TC74287 protein is shown below.



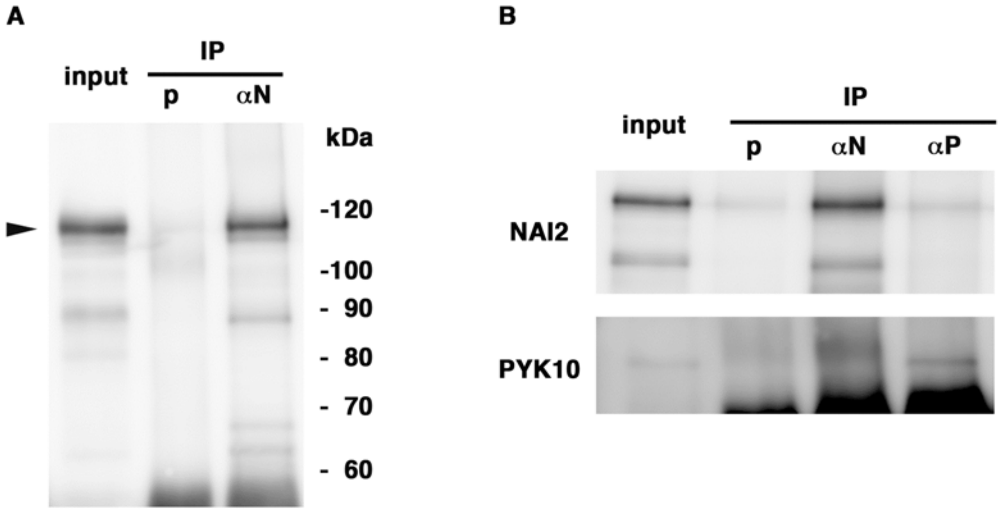
Supplemental Figure 4. Immunoblot analysis using antibody against NAI2/C.

Immunoblot analysis of 7-day-old seedlings from the indicated strains using antibody against NAI2/C (amino acid residues 636-772). Arrowheads indicate the bands corresponding to the NAI2 polypeptide; closed, major bands; open, minor bands. Asterisks indicate the non-specific bands.



Supplemental Figure 5. SDS-PAGE of *E. coli* expressing NAI2.

The expression of recombinant NAI2 (His-tagged NAI2/ΔSP) was induced in *E. coli* (BL21-AI) by the addition of 0.2% L-arabinose to the growth medium. The total proteins were separated by SDS-PAGE and then subsequently stained with Coomassie brilliant blue. The arrowhead indicates the bands corresponding to His-tagged NAI2/ΔSP. The results of three independent clones are shown.



Supplemental Figure 6. Immunoprecipitation of NAI2 and PYK10

(A) The immunoprecipitation (IP) experiment was performed with preimmune serum (p) or anti-NAI2/C antibody (αN), and precipitated proteins were subjected to immunoblot analysis with anti-NAI2/ΔSP antibody. An arrowhead indicates NAI2 protein. Input lane shows one-twentieth of the amount used for immunoprecipitation.

(B) The immunoprecipitation (IP) experiment was performed with preimmune serum (p), anti-NAI2/ΔSP antibody (αN) or anti-PYK10 antibody (αP), and precipitated proteins were subjected to immunoblot analysis with anti-NAI2/ΔSP (upper) or anti-PYK10 antibody (lower). Input lane shows one-twentieth of the amount used for immunoprecipitation.

Supplemental Table 1. Genes that Are Coexpressed with the *NAI1* Gene According to ATTED-II.

Rank*	Correlation	Locus	Anotation
0	1.00	At2g22770	NAI1
1	0.73	At5g24290	integral membrane family protein
2	0.68	At3g15950	(NAI2)
3	0.67	At1g14120	2-oxoglutarate-dependent dioxygenase, putative
5	0.65	At3g20370	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
6	0.65	At3g16460	jacalin lectin family protein
7	0.63	At5g60890	ATR1 (ALTERED TRYPTOPHAN REGULATION)
8	0.63	At5g26260	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
9	0.62	At3g16450	jacalin lectin family protein
10	0.61	At3g16690	nodulin MtN3 family protein
11	0.60	At5g26280	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
12	0.60	At5g23830	MD-2-related lipid recognition domain-containing protein / ML domain-containing protein
13	0.60	At1g22550	proton-dependent oligopeptide transport (POT) family protein
14	0.60	At4g37410	CYP81F4 (cytochrome P450, family 81, subfamily F, polypeptide 4)
15	0.59	At3g09260	PYK10
16	0.57	At5g48000	CYP708A2 (cytochrome P450, family 708, subfamily A, polypeptide 2)
17	0.57	At5g23820	MD-2-related lipid recognition domain-containing protein / ML domain-containing protein
18	0.57	At1g64200	VHA-E3 (VACUOLAR H+-ATPASE SUBUNIT E ISOFORM 3)
19	0.56	At2g39310	jacalin lectin family protein
20	0.56	At2g38760	ANNAT3 (ANNEXIN 3, ANNEXIN ARABIDOPSIS 3)
21	0.55	At1g16370	transporter-related
22	0.55	At2g22330	CYP79B3 (cytochrome P450, family 79, subfamily B, polypeptide 3)
23	0.55	At3g58710	WRKY69 (WRKY DNA-binding protein 69)
24	0.55	At5g38030	MATE efflux family protein
25	0.54	At1g78660	ATGGH1
26	0.54	At5g20550	oxidoreductase, 2OG-Fe(II) oxygenase family protein
27	0.54	At5g47990	CYP705A5 (cytochrome P450, family 705, subfamily A, polypeptide 5)
28	0.54	At4g29270	acid phosphatase class B family protein
29	0.54	At4g29690	type I phosphodiesterase/nucleotide pyrophosphatase family protein
30	0.54	At5g48010	pentacyclic triterpene synthase, putative
31	0.53	At4g21600	bifunctional nuclease, putative
32	0.53	At5g52120	ATPP2-A14 (Phloem protein 2-A14)
33	0.53	At1g72140	proton-dependent oligopeptide transport (POT) family protein
34	0.53	At5g56760	AtSerat1;1 (SERINE ACETYLTRANSFERASE 52)
35	0.53	At3g46700	UDP-glycosyltransferase/ transferase, transferring glycosyl groups
36	0.53	At1g22440	alcohol dehydrogenase, putative
37	0.52	At5g63450	CYP94B1 (cytochrome P450, family 94, subfamily B, polypeptide 1)
38	0.52	At5g23840	MD-2-related lipid recognition domain-containing protein / ML domain-containing protein
39	0.52	At3g59340	
40	0.52	At4g13660	pinoresinol-lariciresinol reductase, putative

*, top 40 coexpressed genes.

Supplemental Table 2. Genes that Are Coexpressed with the *NAI2* Gene According to ATTED-II.

Rank*	Correlation	Locus	Anotation
0	1.00	At3g15950	NAI2
1	0.91	At3g16460	jacalin lectin family protein
2	0.89	At3g09260	PYK10
3	0.86	At3g20370	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
4	0.84	At3g16450	jacalin lectin family protein
5	0.79	At2g39310	jacalin lectin family protein
6	0.79	At5g23820	MD-2-related lipid recognition domain-containing protein / ML domain-containing protein
7	0.77	At4g37410	CYP81F4 (cytochrome P450, family 81, subfamily F, polypeptide 4)
8	0.76	At5g26260	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
9	0.75	At5g26280	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
10	0.69	At1g14120	2-oxoglutarate-dependent dioxygenase, putative
11	0.69	At1g22550	proton-dependent oligopeptide transport (POT) family protein
12	0.69	At4g27860	integral membrane family protein
13	0.68	At4g29270	acid phosphatase class B family protein
14	0.68	At2g22770	NAI1
15	0.68	At4g13660	pinoresinol-lariciresinol reductase, putative
16	0.67	At1g73330	ATDR4 (Arabidopsis thaliana drought-repressed 4)
17	0.67	At5g23830	MD-2-related lipid recognition domain-containing protein / ML domain-containing protein
18	0.65	At1g70850	Bet v I allergen family protein
19	0.65	At3g43600	AAO2 (ALDEHYDE OXIDASE 2)
20	0.64	At1g15210	ATPDR7/PDR7 (PLEIOTROPIC DRUG RESISTANCE 7)
21	0.64	At3g21770	peroxidase 30 (PER30) (P30) (PRXR9)
22	0.64	At4g21600	bifunctional nuclease, putative
23	0.64	At1g31710	copper amine oxidase, putative
24	0.64	At5g24290	integral membrane family protein
25	0.63	At5g63600	flavonol synthase, putative
26	0.63	At2g21045	
27	0.63	At1g29280	WRKY65 (WRKY DNA-binding protein 65)
28	0.62	At5g64100	peroxidase, putative
29	0.62	At1g22440	alcohol dehydrogenase, putative
30	0.62	At4g11190	disease resistance-responsive family protein / dirigent family protein
31	0.62	At5g60890	ATR1 (ALTERED TRYPTOPHAN REGULATION)
32	0.62	At3g09270	ATGSTU8 (Arabidopsis thaliana Glutathione S-transferase (class tau) 8)
33	0.61	At4g14465	DNA-binding protein-related
34	0.60	At5g48000	CYP708A2 (cytochrome P450, family 708, subfamily A, polypeptide 2)
35	0.60	At3g16690	nodulin MtN3 family protein
36	0.60	At4g30170	peroxidase, putative
37	0.60	At3g44990	XTR8 (xyloglucan:xyloglucosyl transferase 8)
38	0.60	At4g17800	DNA-binding protein-related
39	0.60	At5g59090	subtilase family protein
40	0.60	At5g38030	MATE efflux family protein

*, top 40 coexpressed genes.

Supplemental Table 3. Proteins that Colocalize with NAI2 According to LOPIT Data.

Rank*	Distance**	PC1	PC2	Locus	Annotation
0	0.000	-1.19	2.93	At3g15950	NAI2
1	0.105	-1.14	3.02	At1g56070	LOS1, elongation factor 2, putative / EF-2, putative
2	0.497	-0.93	2.51	At2g16060	non-symbiotic hemoglobin 1 (HB1) (GLB1)
3	0.518	-0.79	3.26	At3g16460	jacalin lectin family protein
4	0.525	-1.09	2.41	At5g26260	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
5	0.568	-1.76	2.93	At1g77120	alcohol dehydrogenase (ADH)
6	0.575	-1.72	3.15	At3g52930	fructose-bisphosphate aldolase, putative
7	0.592	-1.54	2.45	At5g24290	integral membrane family protein
8	0.649	-1.82	2.79	At5g02450	60S ribosomal protein L36 (RPL36C)
9	0.681	-0.58	2.62	At5g26280	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
10	0.692	-1.20	2.24	At4g04910	AAA-type ATPase family protein
11	0.693	-1.10	2.24	At1g69840	band 7 family protein
12	0.729	-1.40	2.23	At1g17290	alanine aminotransferase, putative
13	0.846	-0.36	3.11	At2g30860	glutathione S-transferase, putative
14	0.850	-1.63	3.65	At4g23850	long-chain-fatty-acid--CoA ligase / long-chain acyl-CoA synthetase
15	0.854	-2.04	2.89	At5g09810	actin 7 (ACT7) / actin 2
16	0.881	-1.78	2.28	At1g24510	T-complex protein 1 epsilon subunit, putative
17	0.928	-2.12	2.96	At4g17120	expressed protein
18	0.946	-2.07	2.57	At5g19550	aspartate aminotransferase, cytoplasmic isozyme 1 / transaminase A (ASP2)
19	1.027	-1.84	3.72	At2g31390	pfkB-type carbohydrate kinase family protein
20	1.054	-1.05	1.88	At3g01290	band 7 family protein
21	1.133	-1.01	1.81	At5g02870	60S ribosomal protein L4/L1 (RPL4D)
22	1.154	-2.31	3.21	At5g17920	5-methyltetrahydropteroyltriglutamate
23	1.163	-2.33	2.71	At4g13940	adenosylhomocysteinase / S-adenosyl-L-homocysteine hydrolase / AdoHcyase (SAHH)
24	1.208	-1.99	3.83	At4g17140	pleckstrin homology (PH) domain-containing protein
25	1.236	-2.33	2.44	At3g54840	Rab GTPase (ARA6)
26	1.249	-2.34	2.45	At3g07110	60S ribosomal protein L13A (RPL13aA)
27	1.274	-0.91	1.69	At5g63030	glutaredoxin, putative
28	1.279	-0.17	3.70	At5g27850	60S ribosomal protein L18 (RPL18C)
29	1.329	-1.40	1.62	At4g17170	Rab2-like GTP-binding protein (RAB2)
30	1.341	-1.97	1.84	At3g09630	60S ribosomal protein L4/L1 (RPL4A)

Principle component analysis was performed using LOPIT data to identify proteins that co-fractionated with NAI2 (Dunkley et al., 2006). On the 2-dimensional graph of principle component 1 (PC1) and principle component 2 (PC2), the co-fractionating proteins are located near each other (Dunkley et al., 2006). *, the top 30 of colocalizing proteins. **, distance from where NAI2 is located, as calculated by $\sqrt{(\mathrm{PC1}+1.19)^2+(\mathrm{PC2}-2.93)^2}$. The data for PYK10 and At4g27860 were not available.

Supplemental Table 4. Nucleotide Sequences of Oligonucleotide Primers Used in the Study.

Primer set name	Primer name	Sequence	Note
NAI2 genome	NAI2-gFW	5′-GGTTGAGTTTCTCAGCACTCTCTAGAAG-3′	Amplify a *NAI2* gDNA
	NAI2-gRV	5′-CAATCTAAGCTTGCGCCGTTCTCAGAG-3′	
NAI2/-SP	NAI2-expFW	5′-CAGGATGAAGGAACTGGAAG-3′	Amplify a *NAI2* cDNA (for *E. coli* expression)
	NAI2-RV	5′-TCAATTAAGTGAACTAAGAAACT-3′	
NAI2/C	NAI2-expCFW	5′-GCCAAATACAAAACCGTCAT-3′	Amplify a *NAI2* cDNA (for *E. coli* expression)
	NAI2-RV	5′-TCAATTAAGTGAACTAAGAAACT-3′	
PYK10	PYK10-FW	5'-CCATGGTTTTGCAAAAGCTTCCTC-3'	Amplify a *PYK10* cDNA
	PYK10-RV	5'-TTAAAGCTCATCCTTCTTGAGCGC-3'	
*Sal*I-PYK10	PYK10-NFW	5'-GACGGACCTGTTTGCCCGCC-3'	For a creation of *Sal*I site in PYK10
	PYK10-NRV	5'-GACATCTGCATTTGCTGGAGAG-3'	
GFP-*Sal*I	GFP-Sal/FW	5′-GTCGACATGGGCGGCATGGTGAGCAAGGGC-3′	Amplify a *GFP* cDNA (add *Sal*I site)
	GFP-Sal/RV	5′-GTCGACATCGTGGTGGTGGTGGTGGTGCCC-3′	

SUPPLEMENTAL METHOD

Immunoprecipitation

Total protein was extracted from 20 plants from 8-day-old *A. thaliana* seedlings with 350 μL of Tris-buffered saline (TBS, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl) and Protease Inhibitor Cocktail (1 tablet/50 mL, Boheringer Mannheim). The homogenate was centrifuged at 9,000 x g at 4°C for 3 min to remove cell debris. 100 μL of protein extract was mixed with 900 μL of TBS and 1 μL of antiserum over night at 4°C before being mixed with 20 μL of Protein G Sepharose (GE Healthcare Life Science) for 3 h. The Protein G Sepharose beads were recovered by centrifugation and washed several times with TBS. The bound proteins were eluted with 30 μL of 2x SDS-PAGE sample buffer (20 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, 40% (v/v) glycerol).