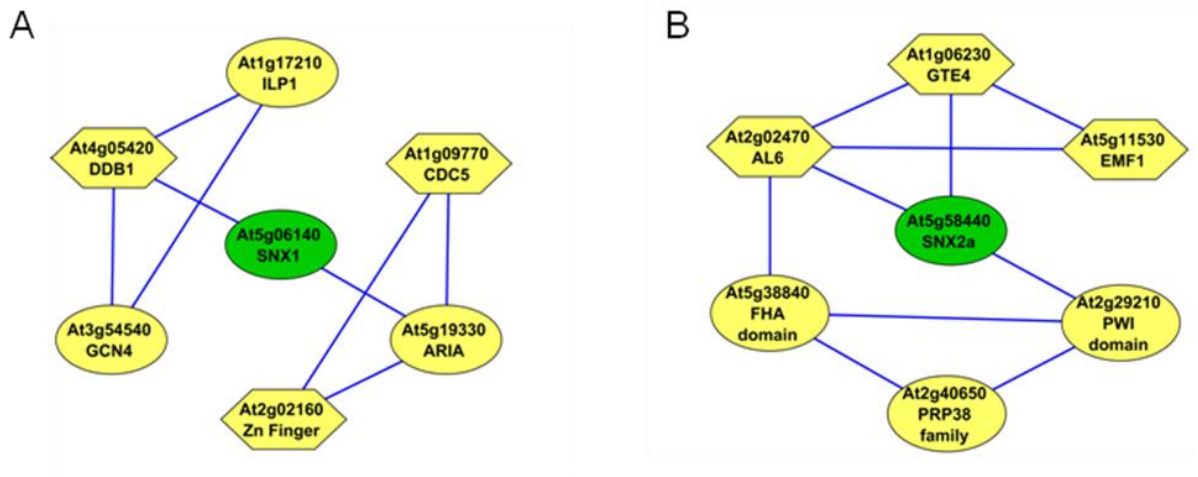
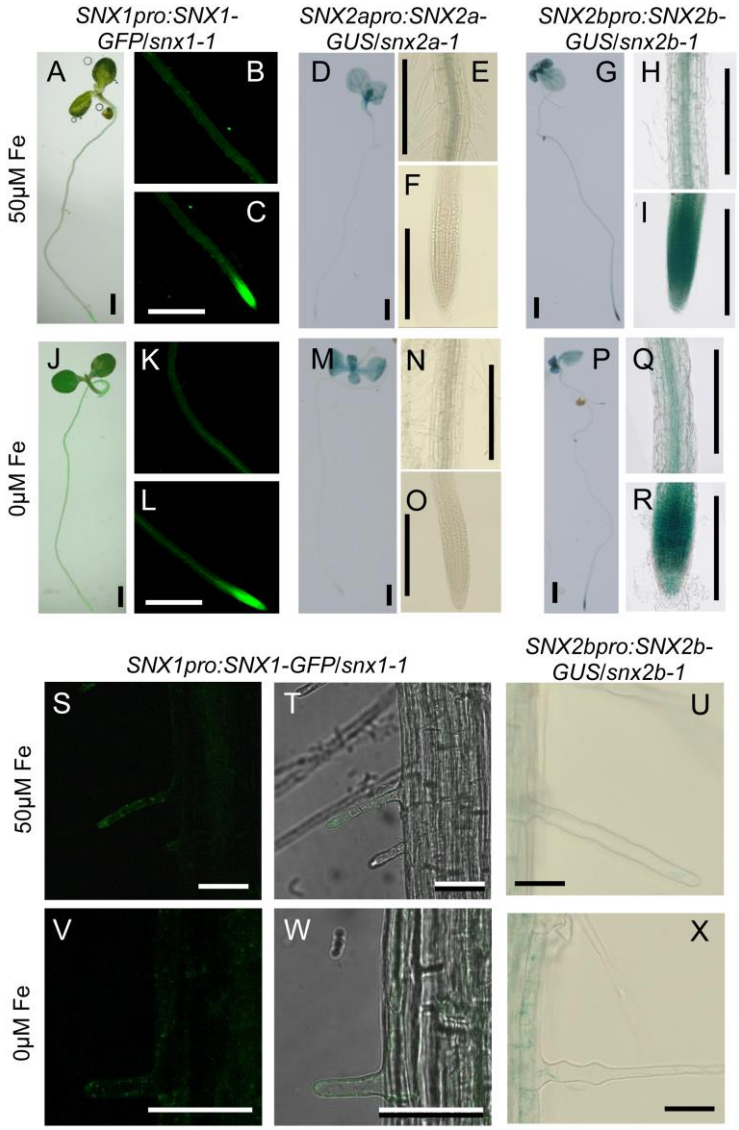



**Supplemental Figure 1. Gene coexpression networks for *SNX1* and *SNX2a***

(A) Gene coexpression network for *SNX1* gene. The list of genes can be seen in Supplemental Table 1.

(B) Gene coexpression network for *SNX2a* gene. The list of genes can be seen in Supplemental Table 2.

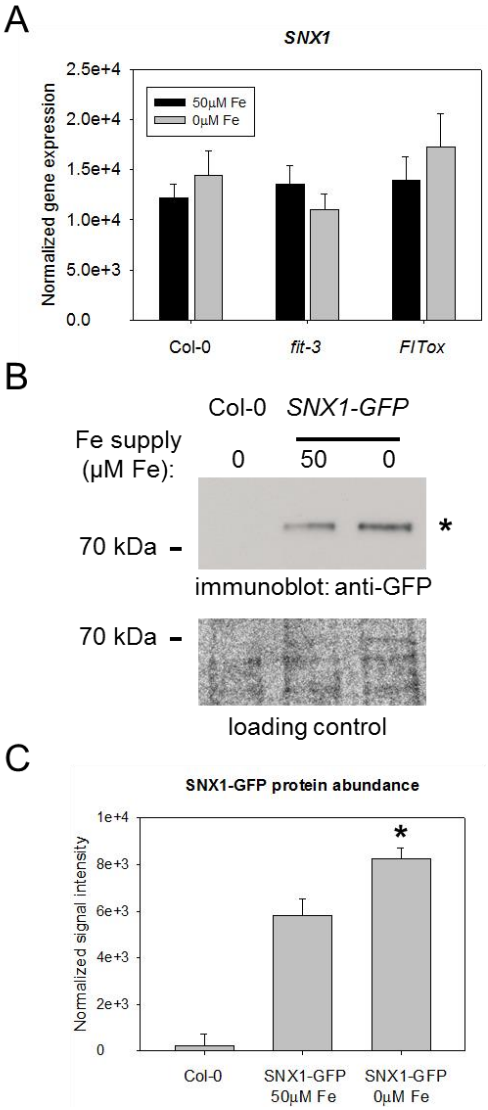



**Supplemental Figure 2. Expression pattern of *SNX* in the root**

(A) to (R) Analysis of reporter gene activity in *SNX1pro:SNX1-GFP*/*snx1-1* (A-C and J-L), *SNX2apro:SNX2a-GUS*/*snx2a-1* (D-F and M-O) and *SNX2bpro:SNX2b-GUS*/*snx2b-1* (G-I and P-R). Plants were grown for eight days under iron-sufficient (50µM Fe) or iron-deficient (0µM Fe) conditions and reporter gene activity was visualized either under fluorescence microscope (GFP), or using a brightfield microscopy after a chemical reaction (GUS). Bars: 2mm. Three independent experiments were made, showing similar results.

(S), (T), (V) and (W), *SNX1pro:SNX1-GFP*/*snx1-2* roots were investigated under confocal microscope. GFP signals can be seen in the root hair cells under both iron supply regimes. (S) and (V) GFP fluorescence, (T) and (W), merge of fluorescence and brightfield image. Bars: 50µm.

(U) and (X), higher magnification of *SNX2bpro:SNX2b-GUS*/*snx2b-1* roots. Blue reporter staining can be seen in the root hair cells under both iron supply regimes. Bars: 50µm.

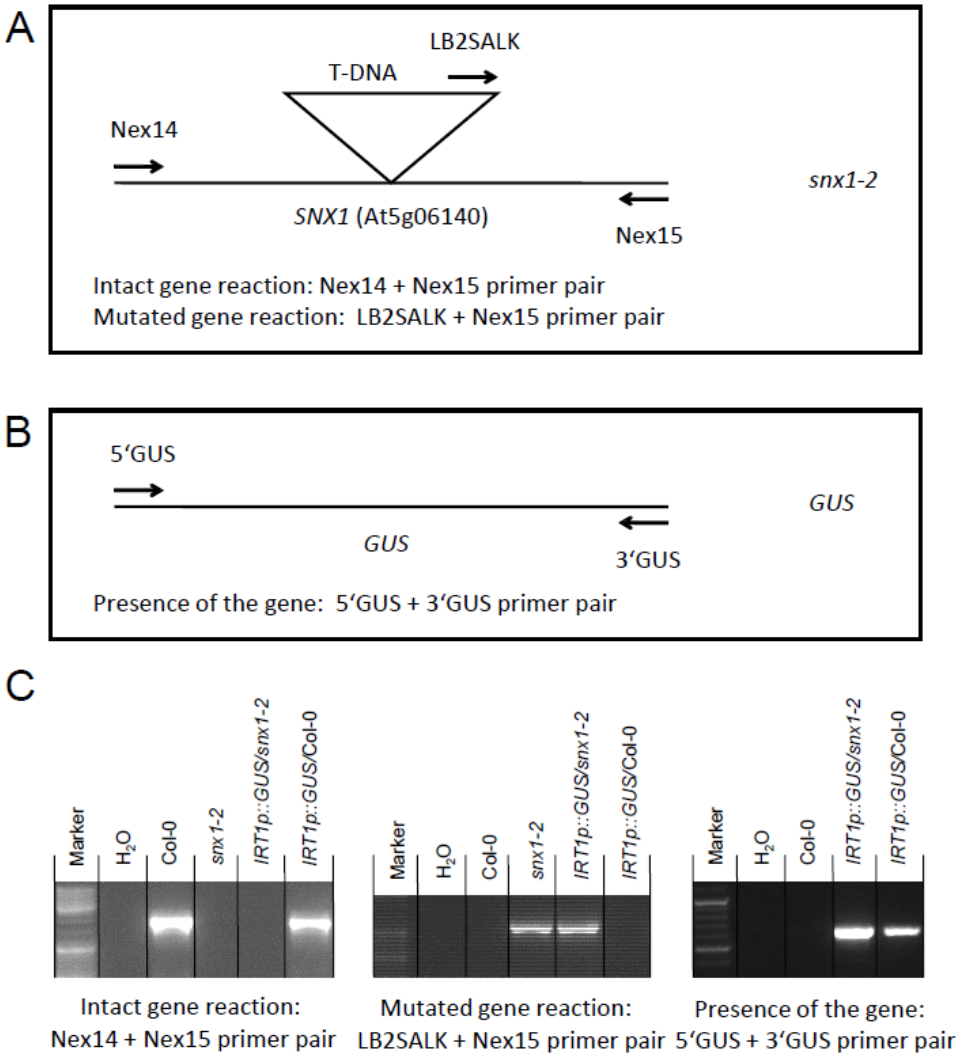



**Supplemental Figure 3. *SNX1* expression and protein abundance under iron deficiency**

(A) Analysis of *SNX1* gene expression in the roots of Col-0, *fit-3* and *FITox* plants grown under iron-sufficient (50µM Fe) or iron-deficient (0µM Fe) conditions by quantitative RT-PCR. The result of three biological repetitions is presented. Error bars represent standard deviation. No statistically significant differences were observed.

(B) Analysis of SNX1-GFP protein abundance in roots of *SNX1pro:SNX1-GFP/snx1-1* plants (labeled as *SNX1-GFP*) grown under iron-sufficient (50µM Fe) or iron-deficient (0µM Fe) conditions. A representative result is shown. Asterisk labels the position of the band corresponding to SNX1-GFP.

(C) Quantification of (B) based on three independent experiments. Error bars represent standard deviation. Asterisk indicates statistical significance ($p<0.005$).

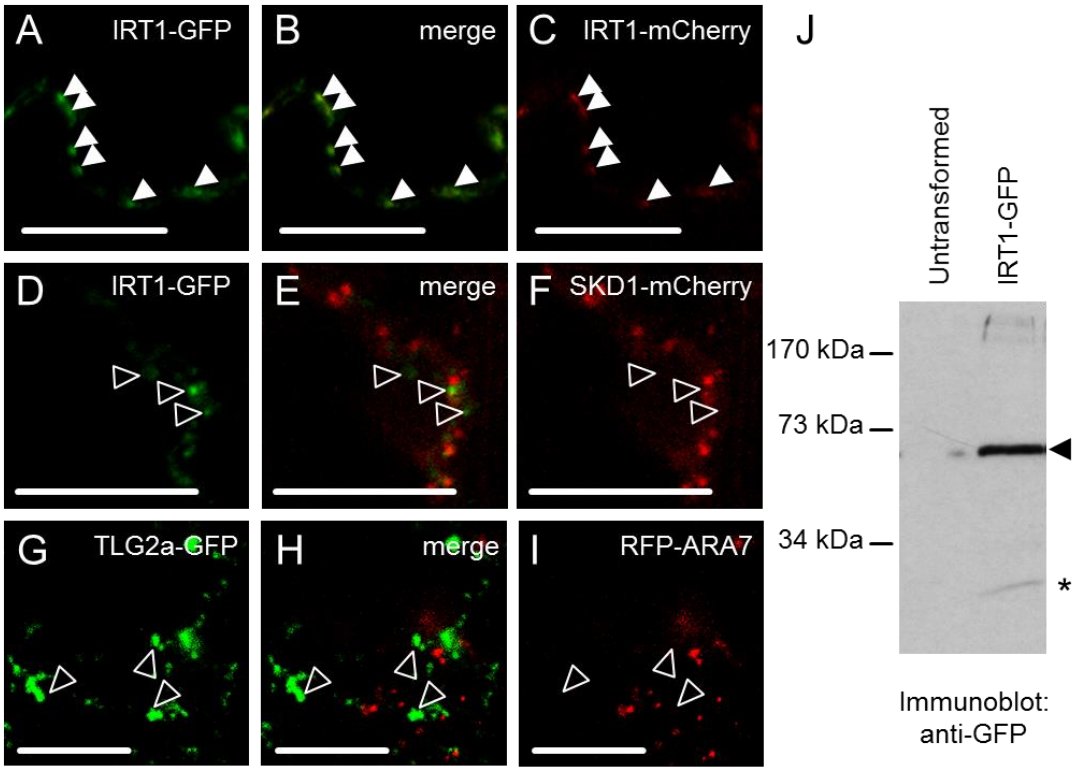



**Supplemental Figure 4. Verification of the *IRT1pro:GUS*/*snx1-2* line**

(A) Principal scheme for the verification of the presence of a T-DNA insertion in the *SNX1* gene. A positive PCR reaction with the Nex14 and Nex15 primer pair shows a wild type allele. A positive PCR reaction with the LB2SALK and Nex15 primer pair shows the presence of the T-DNA within the *SNX1* gene.

(B) Principal scheme for the verification of the presence of the *GUS* gene, revealed by the positive PCR reaction using the 5'GUS and 3'GUS primer pair.

(C) Examples of *IRT1pro:GUS*/*snx1-2* line verification by PCR. The line is homozygous for the T-DNA insertion within the *SNX1* gene (*snx1-2* allele) and contains the *GUS* gene, which was further verified to be homozygous by segregation analysis.




K

| GFP channel | mRFP channel | GFP/mRFP colocalization | mRFP/GFP colocalization |
|---|---|---|---|
| IRT1-GFP | mRFP-ARA7 | 1.94±7.52 | 4.17±6.21 |
| SNX1-GFP | mRFP-ARA7 | 71.23±5.62 | 84.31±9.45 |
| SNX1-GFP | IRT1-mCherry | 21.28±5.85 | 34.64±6.27 |
| SNX1-GFP | TLG2a-mCherry | 19.17±5.72 | 15.08±1.78 |
| IRT1-GFP | TLG2a-mCherry | 87.33±8.40 | 76.43±9.23 |
| IRT1-GFP | IRT1-mCherry | 94.19±4.13 | 95.31±8.15 |
| IRT1-GFP | SKD1-mCherry | 5.24±8.83 | 2.75±7.92 |
| TLG2a-GFP | mRFP-ARA7 | 1.56±5.52 | 1.38±2.95 |

**Supplemental Figure 5. IRT1 colocalization analysis**

(A-C) Coexpression of IRT1-GFP (A) and IRT1-mCherry (C). Filled arrowheads show examples of colocalization. Bars: 20µm.

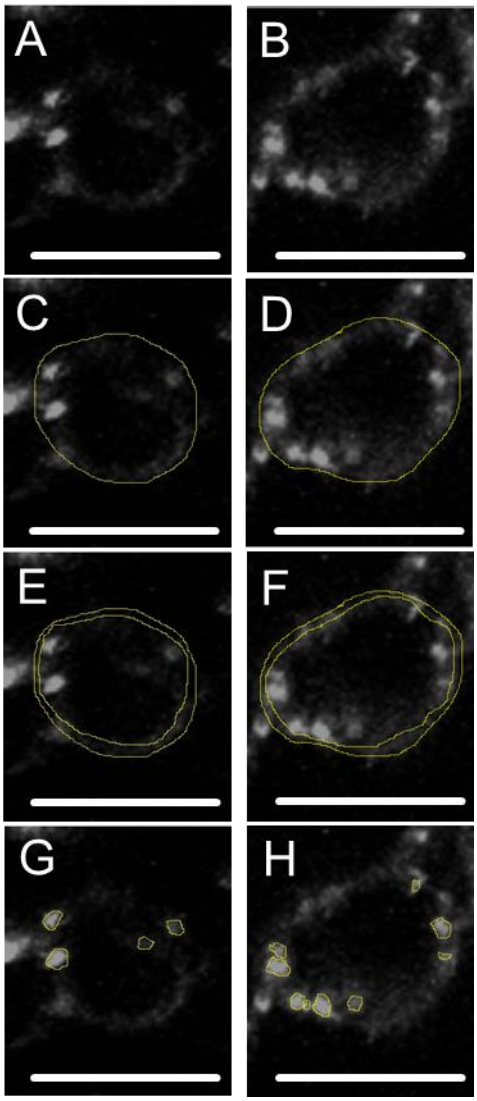
(D-F) Coexpression of IRT1-GFP (D) and SKD1-mCherry (F). Empty arrowheads show examples of signals in the green channel, which do not correspond to signals in the red channel. Bars: 20µm.

(continues on the next page)

(G-I) Coexpression of TLG2a-GFP (G) and RFP-ARA7 (I). Empty arrowheads show examples of signals in the green channel, which do not correspond to signals in the red channel. Bars: 20µm.

(J) Analysis of the integrity of the IRT1-GFP fusion expressed in tobacco. Tobacco leaf samples, either untransformed, or transformed with a *35Spro:IRT1-GFP*-containing expression construct, were tested. Arrowhead indicates the position of the full-length IRT1-GFP with an expected molecular weight of 62 kDa and the asterisk shows the position of free GFP at 27 kDa.

(K) Quantification of fluorescence signal colocalization in endosomal compartments. Results are given as percentage of signals detected in one channel colocalizing with signals in the second channel and vice versa. Error values represent standard deviations. For each couple, a minimum of 10 images were used for the analysis.




**Supplemental Figure 6. Quantification of IRT1 localization in root epidermis cells**

Original photos were converted to 8-bit greyscale (A, B). Outer boundaries (C, D), cell periphery (E, F) and cytoplasmic compartments (G, H) were sequentially selected and total signal intensity within the borders of the selected shapes were measured. An example is given with a cell from the *snx1-2* mutant (A, C, E, G) and Col-0 (B, D, F, H). Bars: 20µM

**Supplemental Table 1**. **Genes coexpressed together with *SNX1***

| AGI code | Name | Comment |
|---|---|---|
| At5g06140 | SNX1 | Sorting nexin |
| At2g02160 | CCCH-type | Zn finger |
| At1g09770 | CDC5 | MYB, essential for innate immunity |
| At1g17210 | ILP1 | Zn binding |
| At5g19330 | ARIA | ARM REPEAT PROTEIN INTERACTING WITH ABF2 |
| At3g54540 | GCN4 | Transporter, ATP binding |
| At4g05420 | DDB1A | DAMAGED DNA BINDING PROTEIN 1A |

**Supplemental Table 2. Genes coexpressed together with *SNX2a***

| AGI code | Name | Comment |
|---|---|---|
| At5g58440 | SNX2a | Sorting nexin |
| At1g06230 | GTE4 | GLOBAL TRANSCRIPTION FACTOR GROUP E4 |
| At5g38840 | FHA | Unknown FHA domain-containing protein |
| At2g40650 | PRP38 | RNA processing and binding |
| At5g11530 | EMF1 | Involved in regulating reproductive development |
| At2g02470 | AL6 | Alfin1-like family plant homeodomain-containing protein |
| At2g29210 | PWI | Splicing factor PWI domain-containing protein |

## Supplemental Table 3. Primers used in this study

| Primer name | Primer sequence | Purpose | Origin |
|---|---|---|---|
| LB2SALK | ACCGAGCTCGAATTTCCCCG | *snx1* alleles genotyping | Jaillais et al., 2006 |
| Nex14 | GCATCCGTATCTGTCAGTCTCCGTC | *snx1* alleles genotyping | Jaillais et al., 2006 |
| Nex15 | AGTGCCTCGTTCTGCTATTGTTGCC | *snx1* alleles genotyping | Jaillais et al., 2006 |
| 5'GUS | CAGGAAGTGATGGAGCATCAG | *GUS* gene genotyping | Becker et al., 1994 |
| 3'GUS | TCGTGCACCATCAGCACGTTA | *GUS* gene genotyping | Becker et al., 1994 |
| FITrt1 | GGAGAAGGTGTTGCTCCATC | *FIT* RT-PCR | Wang et al., 2007 |
| FITrt2 | TCCGGAGAAGGAGAGCTTAG | *FIT* RT-PCR | Wang et al., 2007 |
| BHLH38rt1 | AGCAGCAACCAAAGGCG | *BHLH038* RT-PCR | Wang et al., 2007 |
| BHLH38rt2 | CCACTTGAAGATGCAAAGTGTAG | *BHLH038* RT-PCR | Wang et al., 2007 |
| BHLH39rt1 | GACGGTTTCTCGAAGCTTG | *BHLH039* RT-PCR | Wang et al., 2007 |
| BHLH39rt2 | GGTGGCTGCTTAACGTAACAT | *BHLH039* RT-PCR | Wang et al., 2007 |
| BHLH100rt1 | AAGTCAGAGGAAGGGGTTACA | *BHLH100* RT-PCR | Wang et al., 2007 |
| BHLH100rt2 | GATGCATAGAGTAAAAGAGTCGCT | *BHLH100* RT-PCR | Wang et al., 2007 |
| BHLH101rt1 | CAGCTGAGAAACAAAGCAATG | *BHLH101* RT-PCR | Wang et al., 2007 |
| BHLH101rt2 | CAGTCTCACTTTGCAATCTCC | *BHLH101* RT-PCR | Wang et al., 2007 |
| IRT1rt1 | AAGCTTTGATCACGGTTGG | *IRT1* RT-PCR | Wang et al., 2007 |
| IRT1rt2 | TTAGGTCCCATGAACTCCG | *IRT1* RT-PCR | Wang et al., 2007 |
| FRO2rt1 | CTTGGTCATCTCCGTGAGC | *FRO2* RT-PCR | Wang et al., 2007 |
| FRO2rt2 | AAGATGTTGGAGATGGACGG | *FRO2* RT-PCR | Wang et al., 2007 |
| EFc1 | ACTTGTACCAGTTGGTTATGGG | *EF1Balpha* RT-PCR | Wang et al., 2007 |
| EFc2 | CTGGATGTACTCGTTGTTAGGC | *EF1Balpha* RT-PCR | Wang et al., 2007 |
| EFg1 | TCCGAACAATACCAGAACTACG | *EF1Balpha* (genomic) RT-PCR | Wang et al., 2007 |
| EFg2 | CCGGGACATATGGAGGTAAG | *EF1Balpha* (genomic) RT-PCR | Wang et al., 2007 |
| SNX1F-RT | AAGTGAGGAAGCCACGAG | *SNX1* RT-PCR | this study |
| SNX1R-RT | GAGCTTGTCCTTTCGCAA | *SNX1* RT-PCR | this study |
| SNX1B1 | GGGGACAAGTTTGTACAAAAAAGCAGGCT ATGGAGAGCACGGAGCAGC | amplification of full-length SNX1 | this study |
| SNX1nsB2 | GGGGACCACTTTGTACAAGAAAGCTGGGT TTAGACAGAATAAGAAGCTT | amplification of full-length SNX1 | this study |
| I1B1 | GGGGACAAGTTTGTACAAAAAAGCAGGCT TTATGGCTTCAAATTCAGCACTT | amplification of full-length IRT1 | this study |
| FLI1B2 | GGGGACCACTTTGTACAAGAAAGCTGGGT TAGCCCATTTGGCGATAATCG | amplification of full-length IRT1 | this study |
| AtVPS4B1 | GGGGACAAGTTTGTACAAAAAAGCAGGCT ATGTACAGCAATTTCAAGGA | amplification of full-length SKD1 | this study |
| AtVPS4B2 | GGGGACCACTTTGTACAAGAAAGCTGGGT TACCTTCTTCTCCAAACTCCT | amplification of full-length SKD1 | this study |

## SUPPLEMENTAL REFERENCES

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