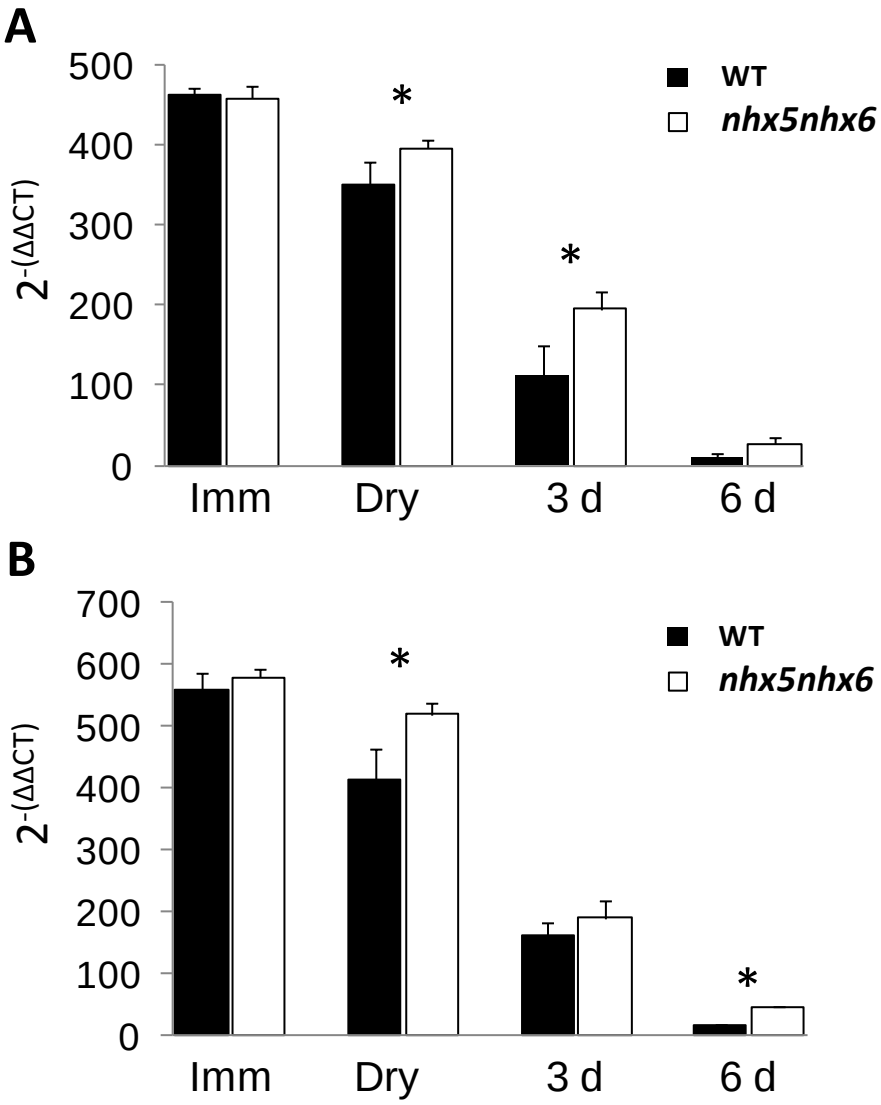
Supplemental Data. Reguera et al. (2015). Plant Cell 10.1105/tpc.114.135699



Supplemental Figure 1. Quantitative Real-Time RT-PCR of relative expression of two storage protein transcripts.

(A) Relative expression ($2^{-(\Delta\Delta CT)}$) of *12S CRA1 (*At5G44120); and **(B)** *2S (S1, S2, S3)* transcripts of samples taken at four different developmental stages, Immature seeds (Imm); Dry seeds (Dry); 3 days (3d) and 6 days (6d) after germination. Asterisk indicates $P \leq 0.05$. Bars represent the mean ± s.d. (n=6). The primers used amplify all 2S1, 2S2, and 2S3 transcripts (see Supplemental Table 3).

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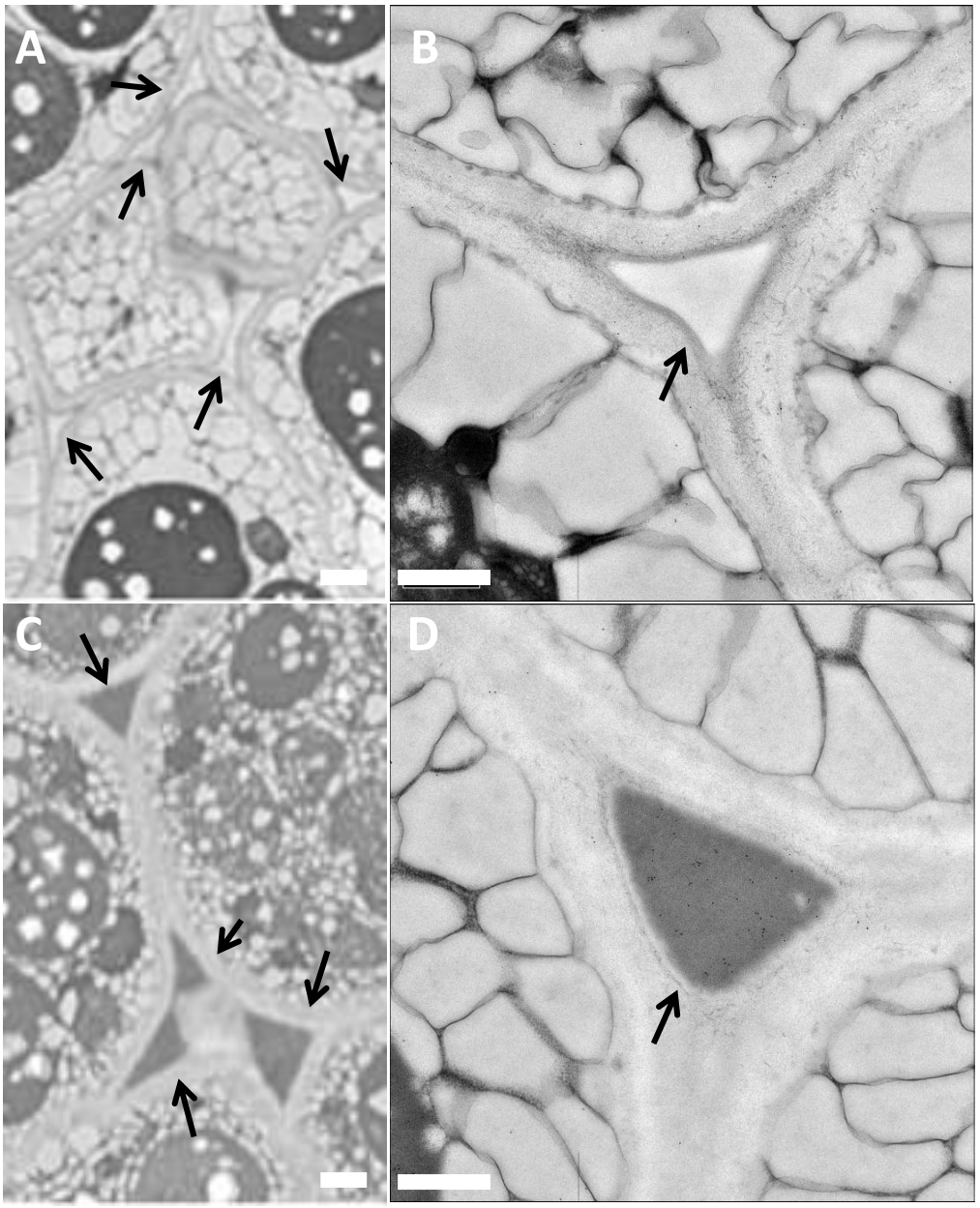

Supplemental Figure 2. Transmission electron micrographs of dry seed thin sections.

(A, B) Wild type

***(*C, D)** *nhx5 nhx6.* **(B), (D)** are an enlargement of **(A)**, and **(C)** respectively.

Note the enlargement of the apoplastic space in *nhx5 nhx6* and where an accumulation of electron dense material also exists (arrows). Bar is 0.025 μm in (A) and (C)and 0.5μm in (B)and (D).

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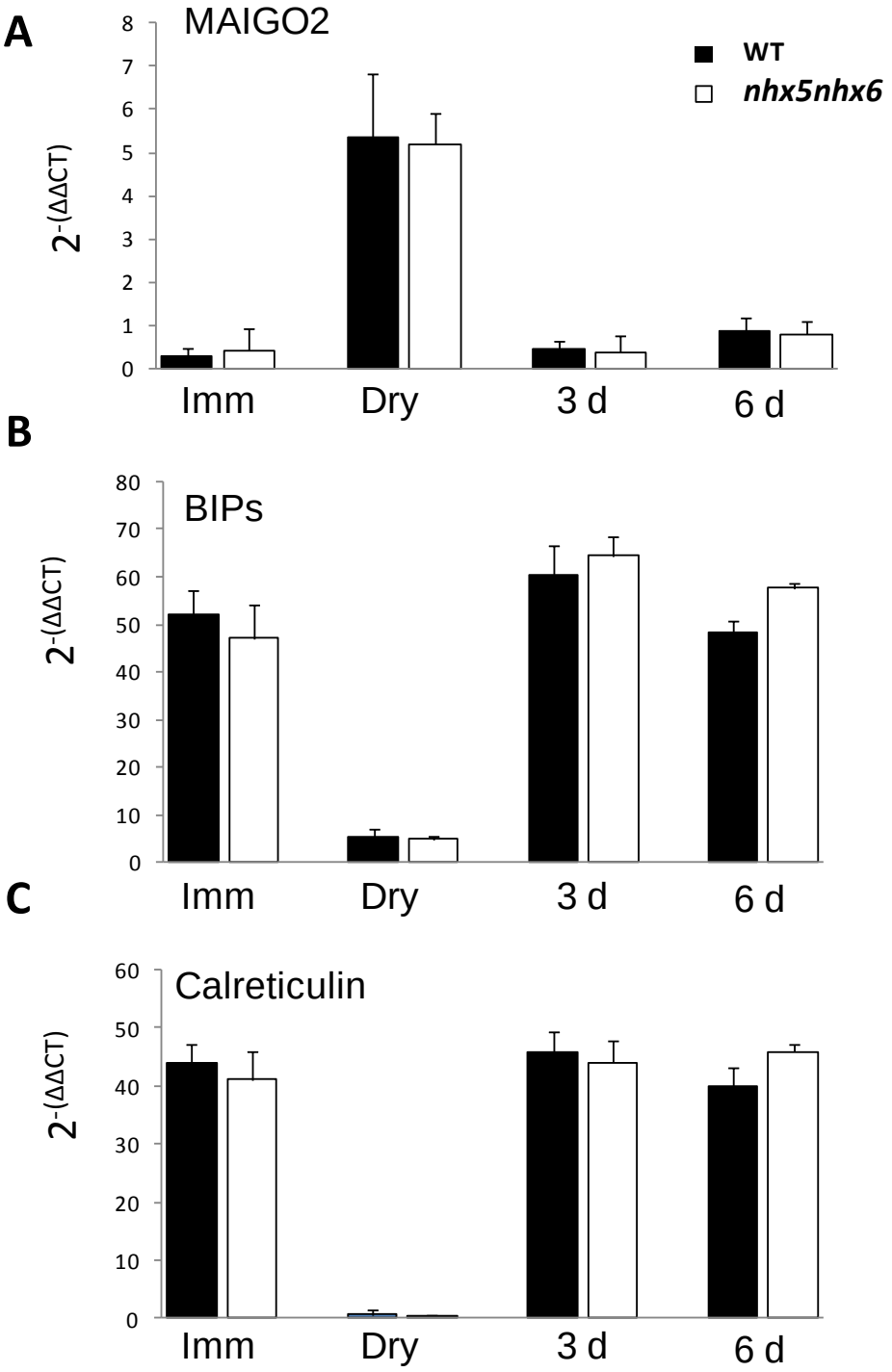

Supplementary Figure 3. Quantitative Real-Time RT-PCR of the relative expression of three genes related to the unfolded protein response (UPR-genes) in seeds and seedlings at different developmental stages.

Relative expression ($2^{-(\Delta\Delta CT)}$) of **(A)** *MAIGO2 (*At3g47700), **(B)** *BIPs (BIP1 (*At5g20730) *, BIP2* (At5g42020) and *BIP3 (*At1g09080)) or **(C)** *calreticulin* (At1g56340) was not significantly different between wild type and *nhx5 nhx6* at any developmental stage. Error bars represent the mean ± s.d. n=4.

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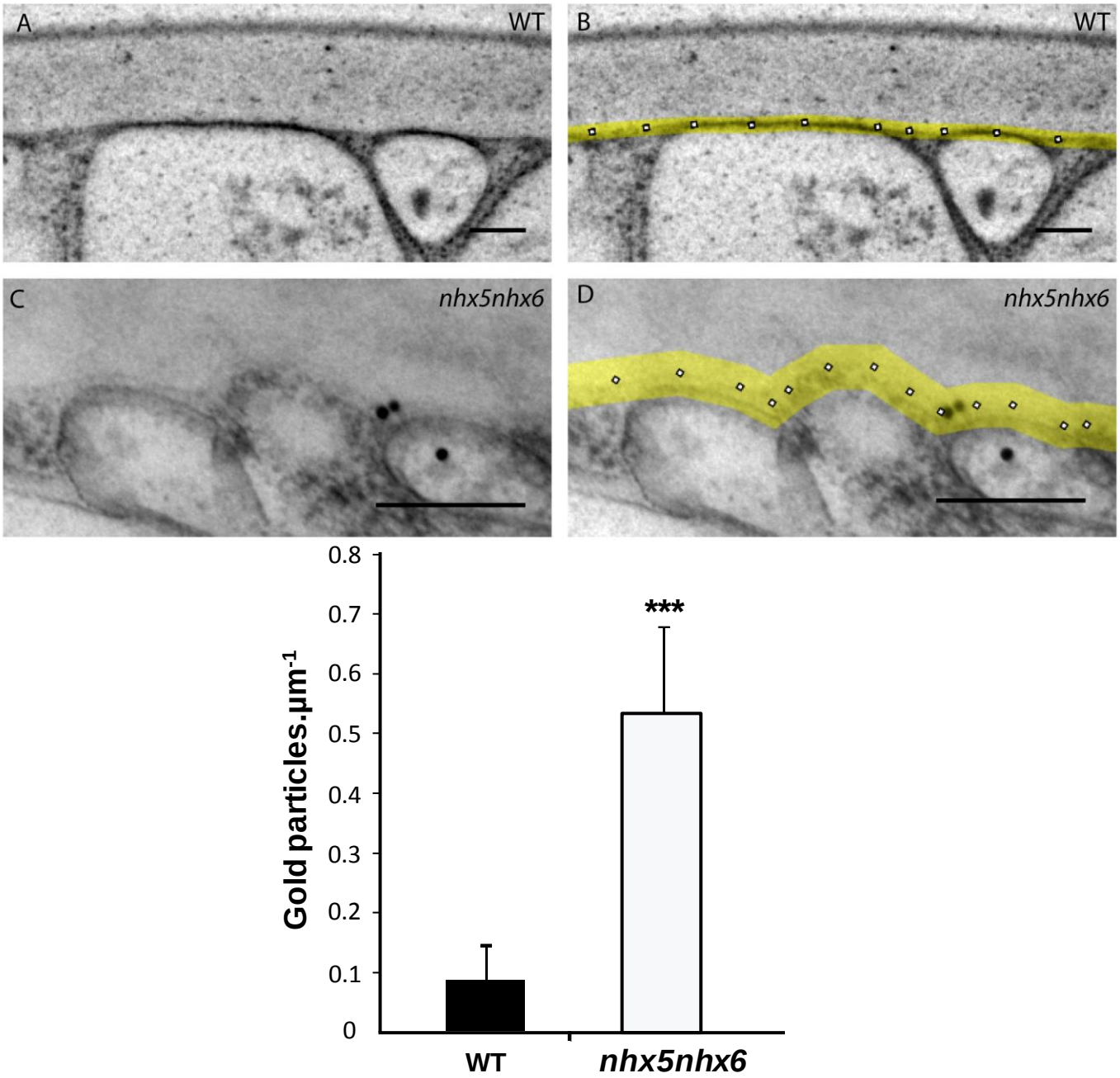

Supplemental Figure 4. Quantification of gold particles in high-pressure frozen-freeze substituted embryos labeled with anti-VSR antibody.
A region of interest spanning 30nm to each side of the plasma membrane (yellow ROI **B,D**) was delineated in order to quantify the number of gold particles contained within the ROI, in WT (**A,B)** (n=67.3µm of plasma membrane in 20 sections) and *nhx5 nhx6* **(C,D)** (n=25.3µm of plasma membrane in 9 sections). *** $P \leq 0.01$. Scale bar= 200nm.

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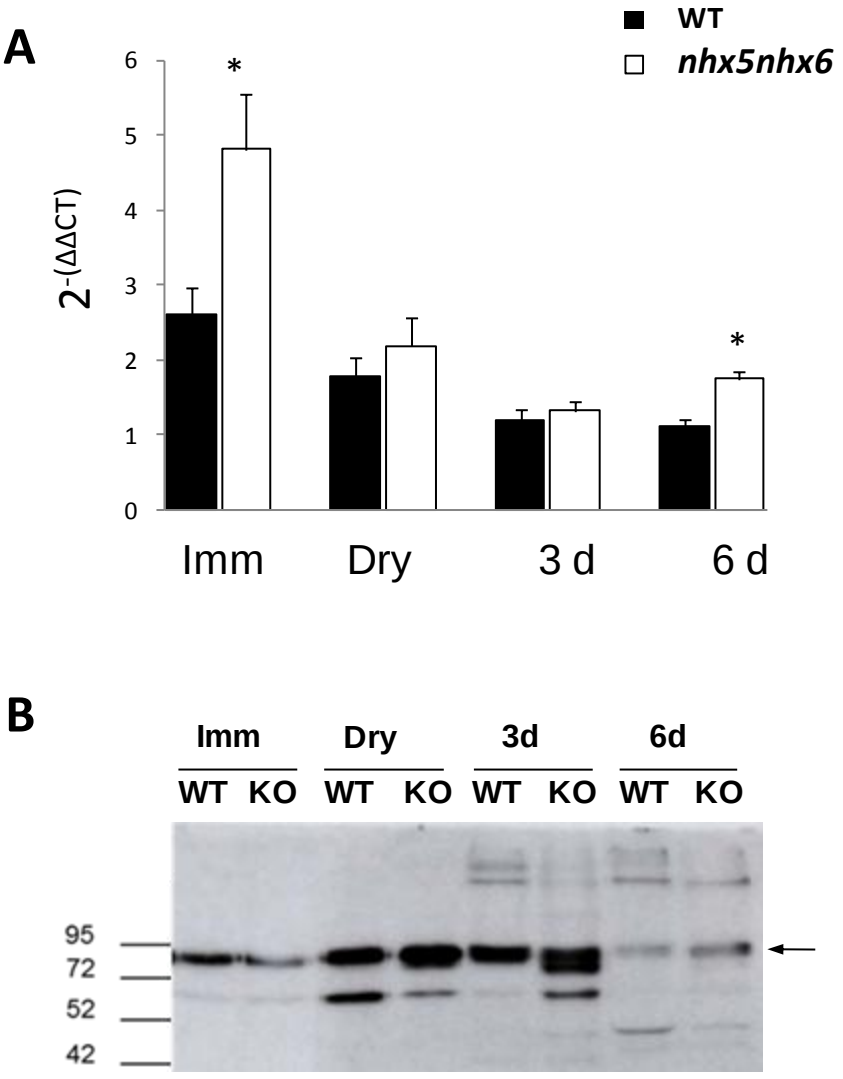

Supplemental Figure 5. Quantitative Real-Time RT-PCR of relative expression and immunoblot of protein abundance of VSR1 in developing seeds and germinating seedlings of *nhx5 nhx6*.

(A) Quantitative Real-Time RT-PCR showing relative expression ($2^{-(\Delta\Delta CT)}$) of *VSR1* transcripts; **(B)** and immunodetection of VSR1;1 at different stages of seed and seedling development in wild type (WT) and *nhx5 nhx6* (KO). Immature seeds (Imm); mature seeds (Dry); 3 days and 6 days after germination (3d) and (6d), respectively. Arrow points to predicted MW (~85kD) of VSR1. Bars represent the mean ± s.d. n=4. Asterisk indicates $P \leq 0.05$.

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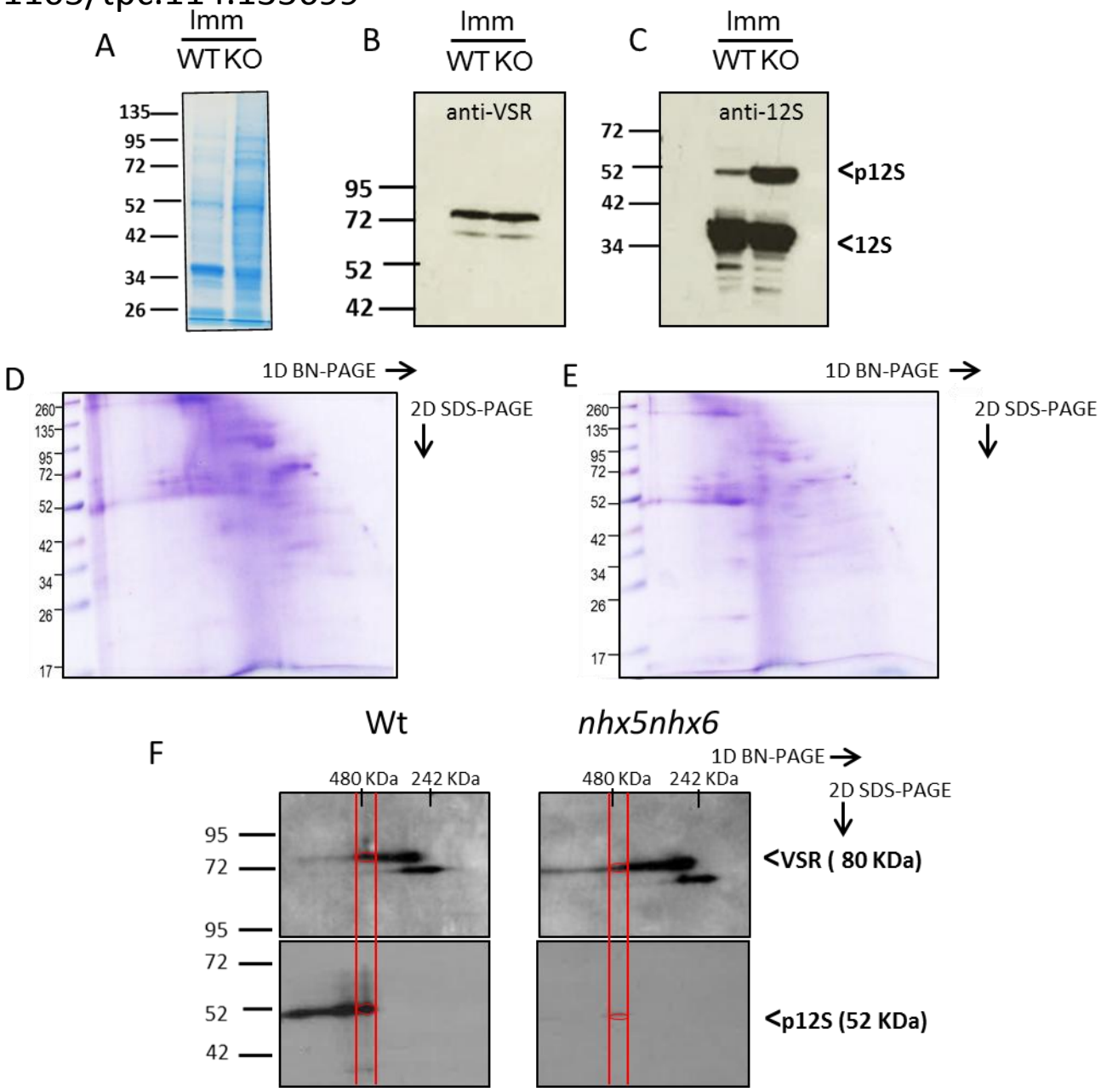

Supplemental Figure 6. Protein loading controls for the identification of receptor-cargo complexes resolved using 2D blue native PAGE/SDS.

(A) Coomassie stained 1D SDS-PAGE (10% acrylamide) gel of wild type (WT) and *nhx5 nhx6* immature (Imm) seed protein extracts (15 μg of total protein were loaded per lane). Immunoblots using α-VSR **(B)** and α-12S **(C)**. 2D blue native PAGE/SDS gels stained with Coomassie of wild type **(D)** and *nhx5 nhx6* **(E)** extracts (45 μg of total protein were loaded). **(F)** Illustration of the method used to delineate the signals used for the quantification of VSR1;1 and 12S spots in complexes shown in Figure 5. Signals bound between two vertical red lines and highlighted within the red ovals were considered as the total VSR1;1 or 12S signal (area and intensity) as explained in Results and Methods. Apparent size of the denatured proteins are 80 kDa for VSR1;1 in (B) and 52kD and 34 kDa for p12S and 12S, respectively in (C).

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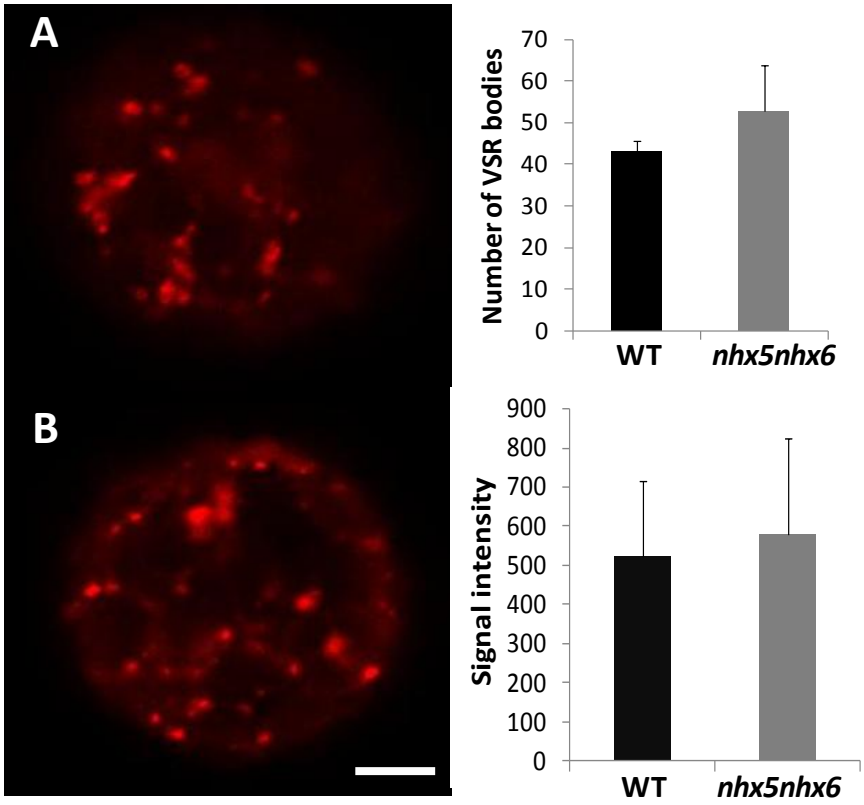

Supplemental Figure 7. **Expression of VSR1;1-mRFP in isolated protoplasts.**
Wild type **(A)** and *nhx5 nhx6* **(B)** protoplasts. Bars are ± S.D. and * designates $P \leq 0.05$. Scale bar is 5 μm.

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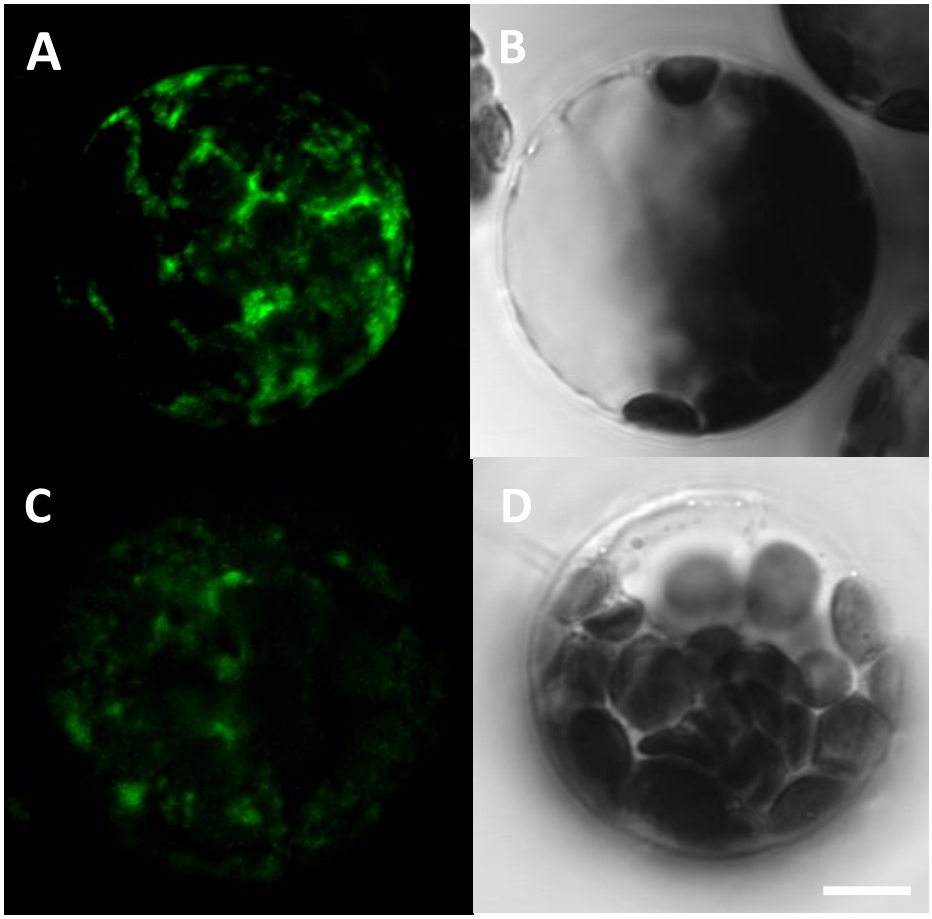
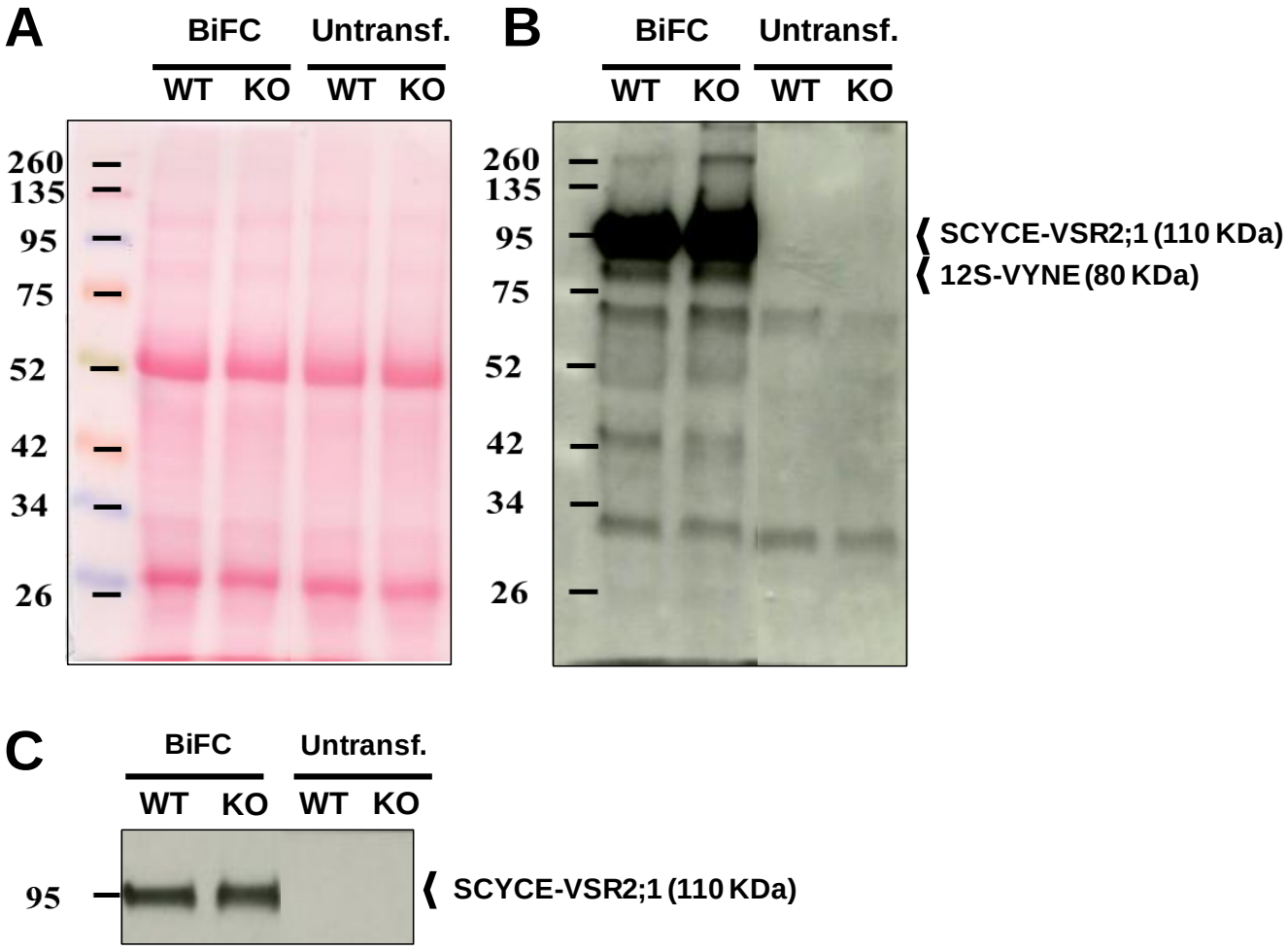


Supplemental Figure 8. ***In vivo*** **interaction between 12S globulin and VSR2;1 is also compromised in** ***nhx5 nhx6.***

Interaction is visualized as bimolecular fluorescence complementation (BiFC) between p12S globulin fused at the C-terminus to the N-terminal half of Venus (12S-VYNE) and co-transformed with a construct expressing the C-terminal half of super cyan fluorescent protein (SCYCE) fused to the N-terminus of VSR2;1 (SCYCE-VSR2;1) in isolated mesophyll protoplasts. Images are 3D projections of a series of 7 z-stack images of **(A)** wild type and **(C)** *nhx5 nhx6*. **(B)** and **(D)** are the corresponding DIC light images of wild type and *nhx5 nhx6* respectively. Scale bar is 5 μm.

Supplemental Data. Reguera et al. (2015). Plant Cell 10.1105/tpc.114.135699



Supplemental Figure 9. **Abundance of VSR2;1 and 12S used in the bimolecular fluorescence complementation assay in isolated mesophyll protoplasts.**

SCYCE-VSR2;1 is the C-terminal half of super cyan fluorescent protein (SCYCE) fused to the N-terminus of VSR2;1. 12S-VYNE is the globulin 12S carrying the N-terminal half of Venus at its C-terminus. Both were cotransformed into protoplasts as described for Figure 8. Anti-GFP 1:1000 (Novus Biologicals, Littleton, CO) was used to immunodetect 12S-VYNE and SCYCE-VSR2;1 to determine their expression in WT and *nhx5nhx6* (KO) protoplasts.

(A) Ponceau S staining indicting the protein loading control of protoplast protein extracts transformed with 12S-VYNE and SCYCE-VSR2;1 (BiFC) and untransformed protoplasts (Untransf.). Wild type (WT) and *nhx5 nhx6* (KO) were loaded in alternate lanes. **(B)** Immunoblot of protein extracts (from [A]) probed using α-GFP to determine the protein abundance of both interaction partners in transformed mesophyll protoplasts. **(C)** Same as **[B]** but with reduced film exposure to indicate the unsaturated SCYCE-VSR2;1 signal. 20 µg of total protein were loaded per lane.

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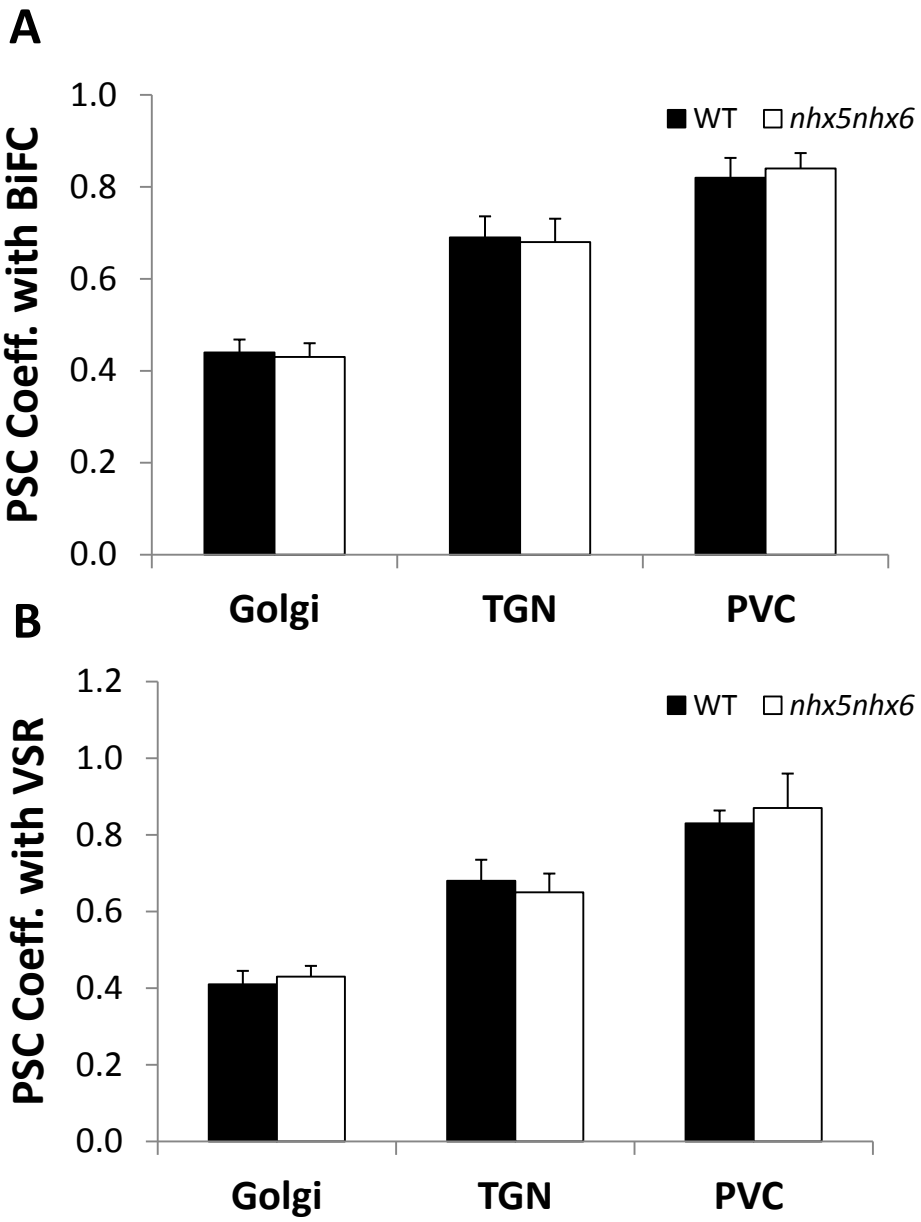

Supplemental Figure 10. Colocalization in bimolecular fluorescence complementation experiments.

(A) Colocalization with bimolecular complementation bodies (BiFC) and with **(B)** VSR2;1 with Golgi, TGN and PVC markers in isolated mesophyll protoplasts. BiFC bodies resulted from the coexpression of Aleu-VYNE (aleurain fused at the C-terminus to the N-terminal half of Venus) with SCYCE-VSR2;1 (C-terminal half of Super Cyan fluorescent Protein fused to the N-terminus of VSR2;1) as described for Figure 8. For VSR, VSR2;1-GFP was used.

The subcellular markers were: (49aa) GmMan1 –RFP (Saint-Jore-Dupas et al., 2006) for the Golgi; Syp61-RFP (Foresti and Denecke, 2008) for the TGN and ARA7-mRFP for the PVC (Geldner et al. 2009). PSC is the Pearsons Correlation Coefficient obtained using the Coloc2 plugin of FiJi as described in Methods. Given the reduced BiFC in *nhx5 nhx6,* colocalization with each marker was assessed only where BiFC bodies existed. Error bars are s.d. ± 20-33 protoplasts. $P \leq 0.05$

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Supplemental Table 1. Constructs used for bimolecular fluorescence complementation experiments

Constructs	Primers	Template	Plasmid backbone	Cloning methods
pENTR-Aleu	B1-Aleu, B2-Aleu140aa	cDNA of Arabidopsis Col0	pDONR207 (Invitrogen)	BP reaction
Aleu-VYNE	N/A	N/A	pDEST-^{GW}VYNE (Gehl et al.,2009)	LR reaction with pENTR-Aleu
pENTR-12S	B1-12S, B2 12S	cDNA of Arabidopsis Col0	pDONR207 (Invitrogen)	BP reaction
12S-VYNE	N/A	N/A	pDEST–^{GW}SCYNE (Gehl et al., 2009)	pENTR-12S
SCYCE-VSR2;1	Xba-SP-SCYCEf, Spe-SCYCEr	pDEST-SCYCE(R)^{GW} (Gehl et al., 2009)	pH-VSR (Martinière et al., 2013)	XbaI/SpeI digestion and ligation
pENTR-Chimera	B1-GFP and VYNEr for VYNE fragment. SCYCEf and B2-GFP for SCYCE fragment.	pDEST-VYNE(R)^{GW} (Gehl et al., 2009) pDEST–^{GW}SCYNE (Gehl et al., 2009)	pDONR207 (Invitrogen)	BP reaction
VYNE-SCYCE chimera	N/A	N/A	pEarleyGate100 (Earley et al., 2007)	LR reaction with pENTR-chimera
Aleu-mRFP	N/A	N/A	pH7RWG2,0 (Karimi et al., 2007)	LR reaction with pENTR-Aleu

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Supplemental Table 2. List of primers used to generate constructs used for bimolecular fluorescence complementation experiments

Primer name	Sequences
B1-Aleu	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCTGCGAAAACAATC
B2-Aleu140aa	GGGGACCACTTTGTACAAGAAAGCTGGGTAAGCTGCTTCTGTGACCTT
Xba-SP-*VYNE*f	acTCTAGAATGAAGCAGCTTCTATGTTATCTTCCATGGCTTCTTCTTCTCTCTCTTGTGGTTTCCCC TTTTAACGAGGCTGCAGgaGTG*gtgagcaagggcgaggagctg*
Spe-*VYNE*r	ggACTAGTcc*aggcctgggcccaagatcctc*
Xba-SP-*SCYCE*f	acTCTAGAATGAAGCAGCTTCTATGTTATCTTCCATGGCTTCTTCTTCTCTCTCTTGTGGTTTCCCC TTTTAACGAGGCTGCAGgaGTG*gacaagcagaagaacggcatcaag*
Spe-*SCYCE*r	ggACTAGTcc*aggcctgggcccaagatcctc*
B1-GFP	GGGGACAAGTTTGTACAAAAAAGCAGGCTatggtgagcaagggcgaggagctgttc
B2-GFP	GGGGACCACTTTGTACAAGAAAGCTGGGTctacttgtacagctcgtccatgccgag
SCYCEf	atcaccgccgacaagcagaagaacggcatcaag
VYNEr	cttgatgccgttcttctgcttgtcggcggtgat
B1-12S	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGATGGCTCGAGTCTCTTCTCT
B2-12S	GGGGACCACTTTGTACAAGAAAGCTGGGTGAGCTGCAGCCACCCTTGGC

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Supplemental Table 3. List of primers used for quantitative real time PCR

Gene(s)	Identifier	Primer sequence
12S	At5G44120	F CCCCATCCTTCGCTTCATC R CCATTGCGTTTTGACGGATA
2S family	At4G27140 At4G27150 At4G27160	F CTCCTCACCAACGCTTCCAT R TGGCGTCATCTTCTTCGAACT
VSR1;1	At3G52850	F GTGGTCACCATATGGATAT R TACATGGAAACTTAAAGCTACT
BIPs	At5G28540 At5G42020 At1G09080	F CAAGGACGCTGTTGTCACTGTT R TCCTTGGTAGCTTGCCTTTGA
Calreticulin-1	At1G56340	F GAAACACACAGCTGGAAATTGG R AATCTGTAGTCCTCGCTAGTTTGGA
MAIGO 2	At3G47700	F TGATCTCTCTTCTTCTACCTCCGTTAC R GATCCTCTCCGGCTACATGCT
TIP4;1 like (reference gene)	At4G34270	F GTGAAAACTGTTGGAGAGAAGCAA R TCAACTGGATACCCTTTCGCA