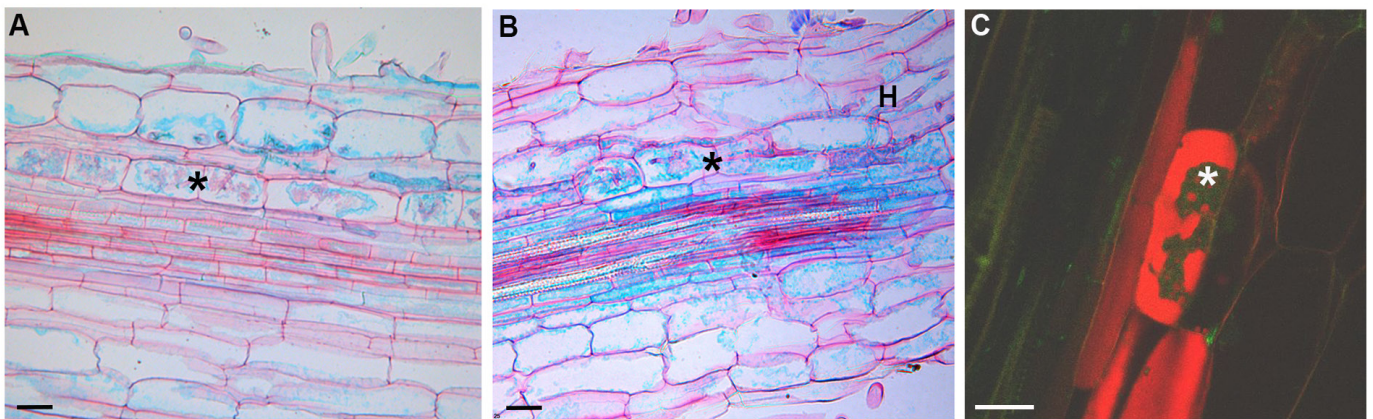
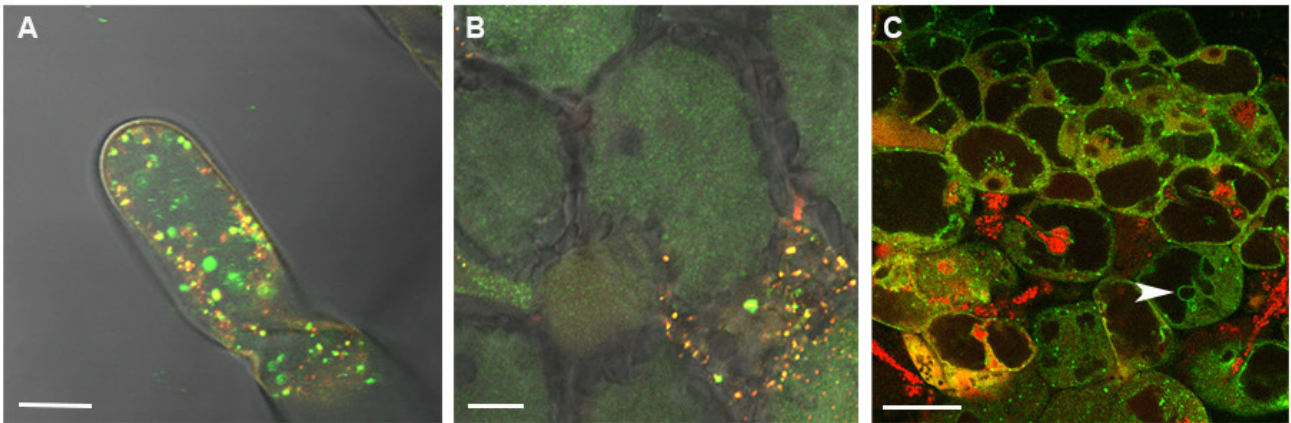


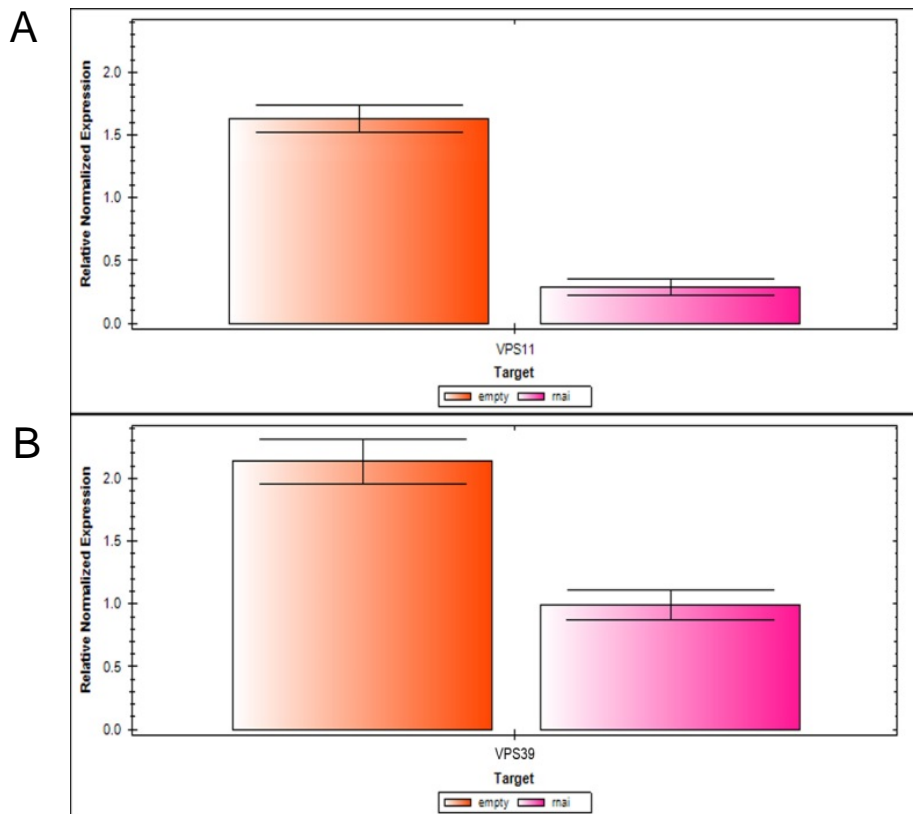
Supplemental Figure 1. Vacuoles in uninfected cells emit fluorescence corresponding to pH 5-5.5 whereas vacuoles in infected cells emit fluorescence corresponding to pH 7-7.5. Uninfected cells are distinguished from infected ones by their smaller size; the vacuoles of uninfected cells occupy near the whole area of the cell. Panel B shows the calibration of fluorescence of lysosensor Yellow/Blue at pH 3 to 8. IC, infected cell, (*) vacuole; UC, uninfected cell, bar:25 μ m. For analysis used a Leica DM 5500 Flu microscope with the filter cube A (DAPI). Bar:75 μ m



Supplemental Figure 2. The expression pattern of ProVPS11:GUS (A) and ProVPS39:GUS (B) in transgenic roots inoculated by *G. intraradices* shows that both VPS11 and VPS39 are expressed in the root cells which contain arbuscules. (C): Neutral Red staining to determine vacuolar pH indicates that the vacuole of the cell, containing the arbuscule (asterisk), retains acidic pH (C). H-hyphae, (*) – arbuscules. Bars:(A),(B):25 μ m, (C): 20 μ m.

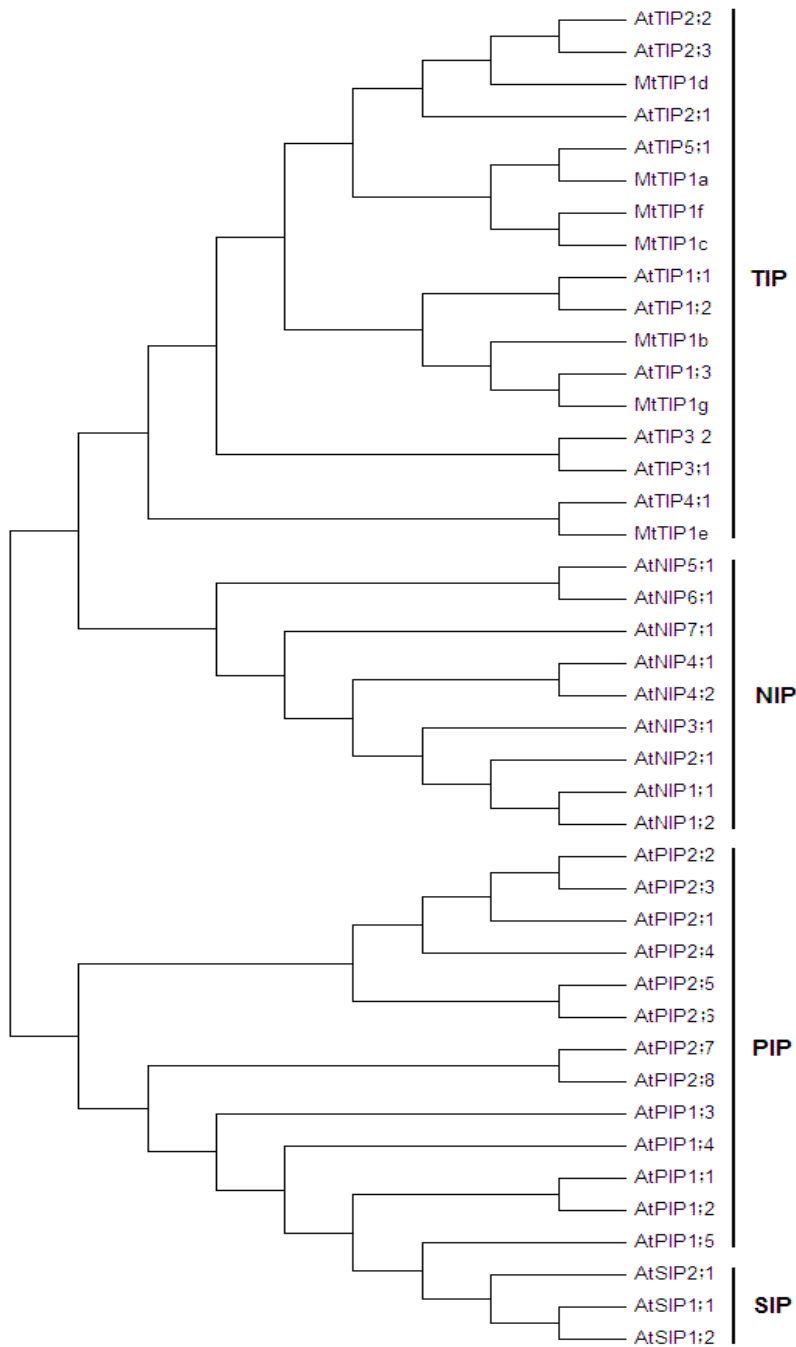


Supplemental Figure 3. Co-localization of endosome/vacuole molecular marker VTI11 with VPS proteins in ProVPS11:GFP-VPS11(A) and ProVPS39:GFP-VPS39 (B) transgenic cells. Co-localization shows that VPS proteins are located on the same structures- endosomes/young vacuoles- as a molecular marker VTI11. Tonoplast localization of GFP-VPS11 in ProUBQ3:GFP-VPS11 transgenic nodule (C). Arrow: tonoplast labelled by GFP. Bars: (A), (B): 10 μ m, (C): 20 μ m



Supplemental Figure 4. The level of gene silencing of VPS11 **(A)** and VPS39 **(B)** in 14 dpi nodules elicited on *ProE12:RNAi-VPS11* and *ProE12:RNAi-VPS39* transgenic roots ($P < 0.05$).

A



B

At-TIP1;2	MPTRNIAIGGVQEEVYHPNALRAALAEFISTLIFVFAGSGSGIAFNKITDNGATTPSGLV	60
Os-γTIP	MPIRNIAVG-SHQEVYHPGALKAALAEFISTLIFVFAGQGSGMAF SKLTGGGATTPAGLI	59
Mt-TIP1b	MPIRNIAVG-TPQEATHPDTLKAGLAEFISTFIFVFAGSGSGIAYNKLTNDGAATPAGLI	59
Mt-TIP1g	MPISRIAIG-NPSEFGKADALKAALAEFISMLIFVFAGEGSGMAYNKLTNNGAATPAGLV	59
Gh-γTIP1	MPISRIAIG-SPAEAGQADALKAALAEFISVLI FVFAGEGSGMAFNKLTDDGSSTPAGLV	59
Zm-TIP1-2	MPVSRIVG-APGELSHPDTAKAAVAEFISTLIFVFAGSGSGMAF SKLTDDGAATPAGLI	59
Mt-TIP1e	--MAKIALG-TTREATQPDCIQALIVEFIATFLFVFAGVGSAMTADKLSGD-----	48

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Mt-TIP1c      -MA-GIAFG-RLDDSFSGSIKAYIAEFISTLLFVVFAGVGSIAIAYGKLTSDAALDPAGLL 57
Mt-TIP1f      -MGRGIAFG-RFDDSFVSSIRAYVAEFISTLIFVVFAGVGSIAIAYAKLTSGAALDPAGLV 58
Mt-TIP1d      --MVKIGFG-TFDDSFSAASLKAYLSEFIATLIFVVFAGVGSIAIAYNDLTSDAALDPAGLV 57
Mt-TIP1a      -----MTG-----KLMPDASLNPTSLV 17
                AAA
At-TIP1;2     AAALAHAFGLFVAVSVGANISGGHVNPVTFGVLLGGNITLLRGILYWIAQLLGSVAACF 120
Os-γTIP       AAAVAHAFALFVAVSVGANISGGHVNPVTFGAFVGGNITLFRGLLYWIAQLLGSVACF 119
Mt-TIP1b      SASIAHAFALFVAVSVGANISGGHVNPVTFGAFVGGNITLLRGIVYIIAQLLGSIVASA 119
Mt-TIP1g      AASLSHAFALFVAVSVGANISGGHVNPVTFGAFVGGNITLIRGLLYWIAQLLGSVACL 119
Gh-γTIP1      AAAHALALFVAVSIGANISGGHVNPVTFGAFVGGNITLVRSLYWIAQLLGSVACF 119
Zm-TIP1-2     AASLAHALALFVAVSVGANISGGHVNPVTFGAFVGGNITLLKALVYVWVQQLLGSVACL 119
Mt-TIP1e      -----ALVGLFFVGHITIVRSILYWIDQLIASAAACY 80
Mt-TIP1c      AVAVCHGFALFVAVAVGANISGGHVNPVTFGLAVGGQITILTGFYWIAQLLGSIVACF 117
Mt-TIP1f      AVAVCHGFALFVAVSVGANISGGHVNPVTFGLAIGGQITILTGFYWIAQLLGSIVACF 118
Mt-TIP1d      AVAVAHAFALFVGVAAIANISGGHLNPAVTFGLAIGGNITILTGLFYWIAQLLGSIVASL 117
Mt-TIP1a      VGIASAFALSSVLYIAWDISGGHVNPVTFAMA VGGHISVPTALFYWVWVQQLIASVIACL 77

At-TIP1;2     LLSFATGGEPFPAFGLSAGVGSNLNALVFEIVMTFGLVYTVYATAVDPKNGSLGTIAPIAI 180
Os-γTIP       LLRFSTGGLATGTFGLTG-VSVWEALVLEIVMTFGLVYTVYATAVDPKNGSLGTIAPIAI 178
Mt-TIP1b      LLVFTAS-SVPFGLSEGVGVPALVLEIVMTFGLVYTVYATAVDPKNGNIGI IAPIAI 178
Mt-TIP1g      LLKIATGGLETSAFSLSSGVGATNALVFEIVMTFGLVYTVYATAVDPKNGSLGTIAPIAI 179
Gh-γTIP1      LLKFSTGGMTTSAFSLSSGVGAMNAVVEIVMTFGLVYTVYATAVDPKNGNIGI IAPIAI 179
Zm-TIP1-2     LLKIATGGALGAFSLSAGVGMNAVVEIVMTFGLVYTVYATAVDPKNGDLGVIAPIAI 179
Mt-TIP1e      LLHFLSGGLTTPAHTLASGVVVEIVLTFLLFTYATMVDPKKAGLAGLPTLV 140
Mt-TIP1c      LLQFVTGGLETPIHSAEVEVPIGGVVTEI IITFGLVYTVYATAADPKKGSIGTIAPIAI 177
Mt-TIP1f      LLKYATGGTIPIHSAEVEVPIGGVVTEI IITFGLVYTVYATAADPKKGSIGTIAPIAI 178
Mt-TIP1d      LLNVVT-AKSVPTHGVAAGLNPIAGLVFEI IITFGLVYTVYATAADPKKGSIGTIAPIAI 176
Mt-TIP1a      FLKIVVGMHVPTYSAEEMTGFGASILEGILTFVLVYTYA-ARDTRRGQVSSSTGLTVI 136

                AAA
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Os-γTIP       GFIVGANILVGGAFDASMNPAVAVVSWTWNHVVYWGAPLIGGGLAGVIYEVLFVI 238
Mt-TIP1b      GFIVGANILVGGAFDASMNPAVAVVSWTWNHVVYWGAPLIGGGLAGVIYEVLFVI 238
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Gh-γTIP1      GFIVGANILAGGAFDASMNPAVAVVSWTWNHVVYWGAPLIGGGLAGVIYEVLFVI 239
Zm-TIP1-2     GFIVGANILAGGAFDASMNPAVAVVSWTWNHVVYWGAPLIGGGLAGVIYEVLFVI 239
Mt-TIP1e      GFVVGANILAGGAFSAASMNPARSFGPALVSGNWTDHVVYVWGAPLIGGGLAGVIYENFFI 200
Mt-TIP1c      GFIVGANILAGGAFSGGSMNPARSFGPAVVSNGFHDNWIYWGAPLIGGGLAGLIYGNVFM 237
Mt-TIP1f      GLIVGANILAGGAFSGGSMNPARSFGPAVLSGDYHNNWIYVWGAPLIGGGLAGVIYSYVFM 238
Mt-TIP1d      GFVVGANILAGGAFSGGSMNPARSFGPAVVSNGFADNWIYVWGAPLIGGGLAGLIYGDVFI 236
Mt-TIP1a      GLIAGASVLAAGPFSGGSINPACAFGSAS IAGTFRNQAVYVWGAPLIGAVVAGLLYDNVLF 196

At-TIP1;2     DENAHEQLP--TTDY--- 253
Os-γTIP       SH-THEQLP--TTDY--- 250
Mt-TIP1b      NS-THEQLP--TTDY--- 250
Mt-TIP1g      TPSSYEQLP--VTDY--- 252
Gh-γTIP1      APSTYEEVP--SADF--- 252
Zm-TIP1-2     GQRPHQQLPTTAADY--- 254
Mt-TIP1e      NR-DHVPLAVDEESY--- 214
Mt-TIP1c      HT-EHAPLS---SDF--- 248
Mt-TIP1f      PS-DHVPLA---SDF--- 249
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Mt-TIP1a      PSQNSDCIRGVSDGTVRV 214
    
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C

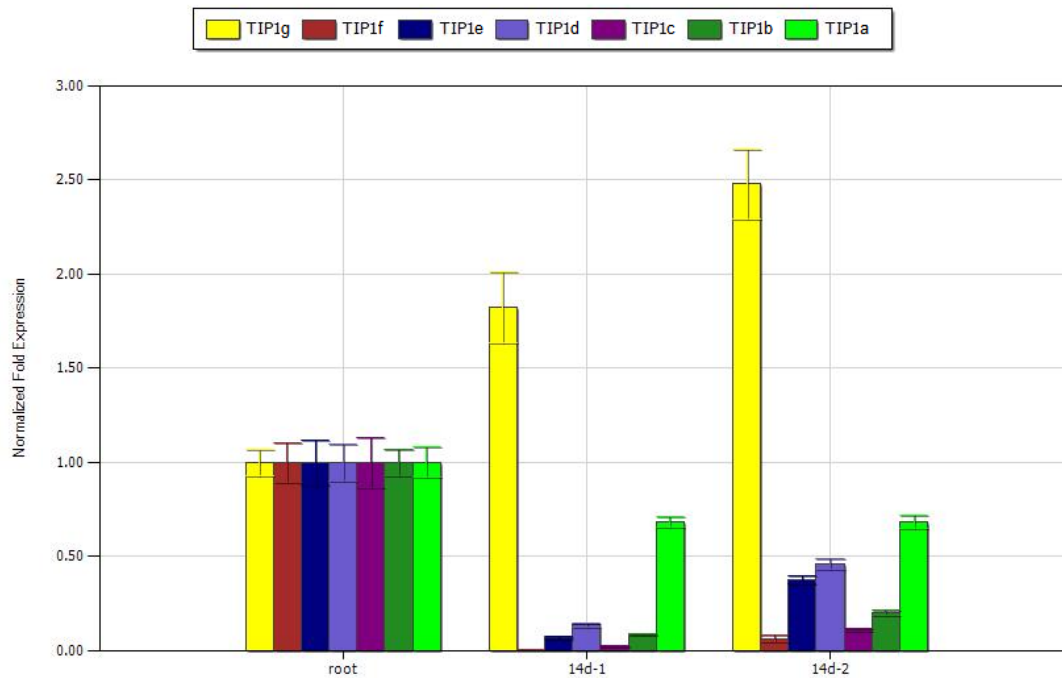
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Mt-TIP1d	Medtr5g012810
Mt-TIP1e	Medtr1g006490
Mt-TIP1f	Medtr2g101370
Mt-TIP1g	Medtr4g063090
At-TIP1;1	AT2G36830
At-TIP1;2	AT3G26520
At-TIP1;3	AT4G01470

At-TIP2;1	AT3G16240
At-TIP2;2	AT4G17340
At-TIP2;3	AT5G47450
At-TIP3;1	AT1G73190
At-TIP3;2	AT1G17810
At-TIP4;1	AT2G25810
At-TIP5;1	AT3G47440
At-PIP1;1	At3g61430
At-PIP1;2	At2g45960
At-PIP1;3	At1g01620
At-PIP1;4	At4g00430
At-PIP1;5	At4g23400
At-PIP2;1	At3g53420
At-PIP2;2	At2g37170
At-PIP2;3	At2g37180
At-PIP2;4	At5g60660
At-PIP2;5	At3g54820
At-PIP2;6	At2g39010
At-PIP2;7	At4g35100
At-PIP2;8	At2g16850
At-NIP1;1	At4g19030
At-NIP1;2	At4g18910
At-NIP2;1	At2g34390
At-NIP3;1	At1g31885
At-NIP4;1	At5g37810
At-NIP4;2	At5g37820
At-NIP5;1	At4g10380
At-NIP6;1	At1g80760
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At-SIP1;2	At5g18290
At-SIP2;1	At3g56950

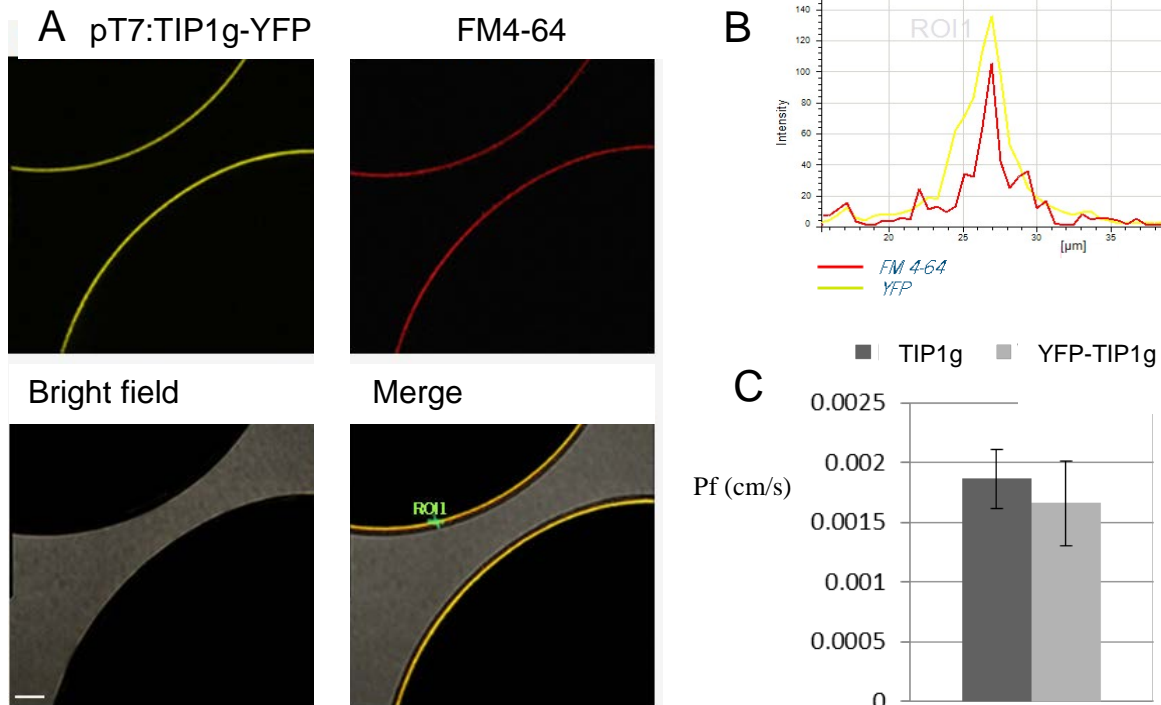
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Gene name	Corresponding gene locus	References
At-TIP1;2	AT3G26520	Bourguignon et al., 2007
Gh- γ TIP1	EF470294	Zhang et al., 2008
Os- γ TIP	RICYK333	Uchimiya et al., 1994
Zm-TIP1-2	AF326500	Jung et al., 2001

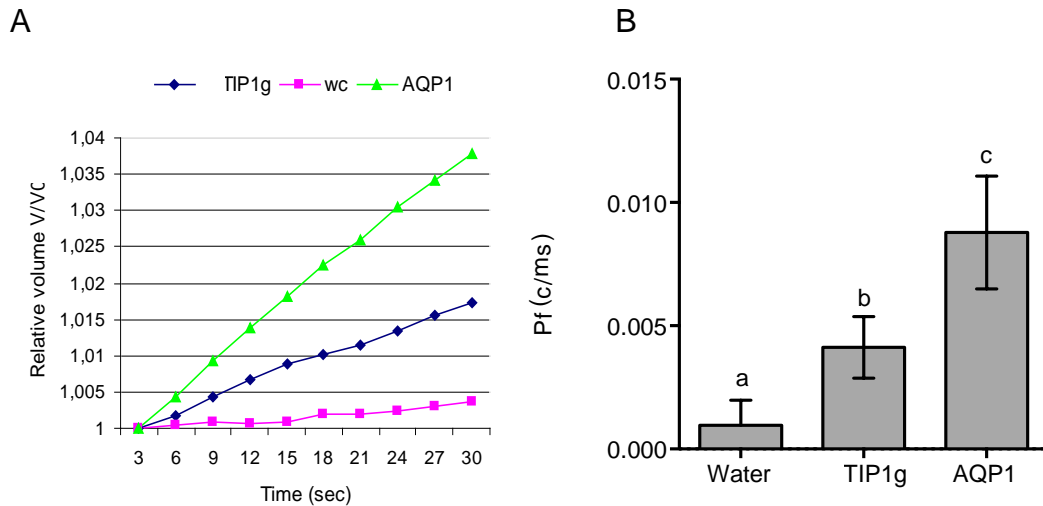
Supplemental Figure 5. (A) Phylogenetic comparison of transporters from *M. truncatula* and *A. thaliana*. Classification and naming of *A. thaliana* MIPs members as in Johanson et al. (2001). Phylogenetic analyses (bootstrap values of 500 replicates) were conducted using MEGA version 5 (Tamura et al., 2011). (B) ClastalW alignment of predicted amino acid sequence of *M. truncatula* aquaporins with another well studied water transporters. Transmembrane domains are shown with a line below the alignment; triangles indicate the NPA selectivity filter. (C) Accession numbers of gene sequences used in TIPs phylogenetic analysis. (D) Gene sequences used for multiple alignments of amino acid sequences



Supplemental Figure 6. Expression profile of *M. truncatula* TIP genes in roots and different zones of 14 day old nodules (14-1 – meristem and infection zone, 14-2 – fixation zone). Expression level was determined by qRT-PCR and normalized against transcription level of reference gene *M. truncatula* UBQ10.



Supplemental Figure 7. YFP-TIP1g localizes on plasma membrane of injected oocytes. **(A)** Confocal image of expressing YFP-TIP1g oocytes. Plasma membrane is counterstained with FM4-64. **(B)** Signal overlapping in region of interest 1 (ROI1). **(C)** Water permeability coefficient values for oocytes injected with cRNA encoding TIP1g and YFP-TIP1g. Bar :0.5 μm

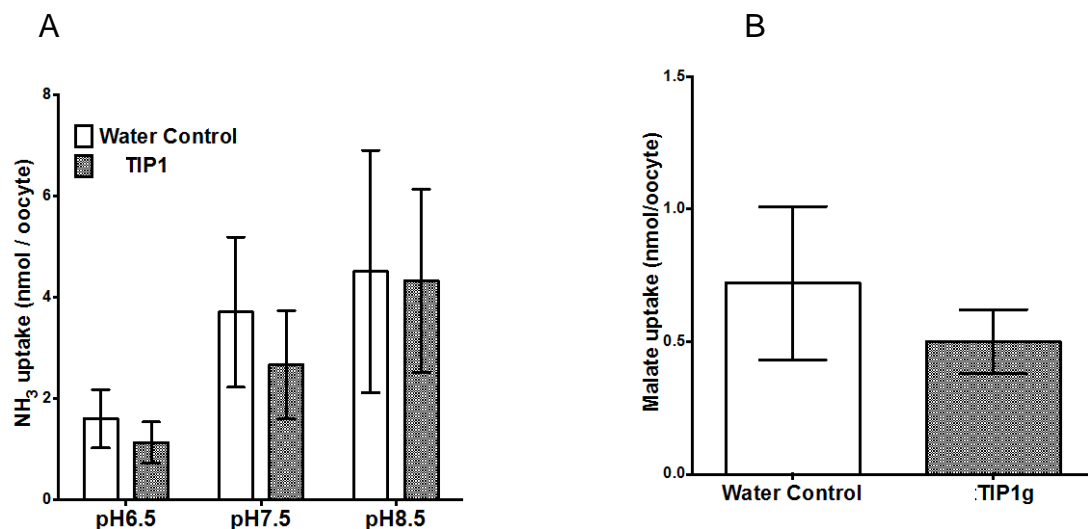


Supplemental Figure 8. Water-channel activity of TIP1g.

(A) The relative volume of oocytes injected with cRNA encoding TIP1g, human AQP1 or water following exposure to hypotonic media. The rate of oocytes swelling is plotted as V/V_0 versus time, where V is a volume at the certain time point and V_0 is the initial volume.

(B) Water permeability coefficient values for oocytes injected with cRNA encoding TIP1g, AQP1 or water. The difference was significant, that show that TIP1g is a functional water transporter.

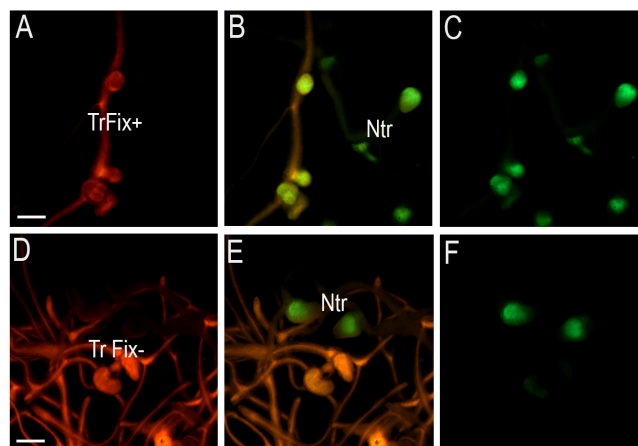
Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
TIP1g vs. Water	0.003166	0.001308 to 0.005025	Yes	***	0.0006
TIP1g vs. AQP1	-0.004654	-0.006185 to -0.003122	Yes	****	< 0.0001
Water vs. AQP1	-0.007820	-0.009742 to -0.005897	Yes	****	< 0.0001



Supplemental Figure 9. TIP1g does not transport ammonia and malate.

(A) NH₃ flux experiment. ¹⁵N content of TIP1g injected oocytes were same with water control in all pH tested. This result suggests that TIP1g is not a NH₃ transporter.

(B) Malate flux experiment. TIP1g injected oocytes in comparison with water control, show no significant differences, TIP1g does not transport malate.

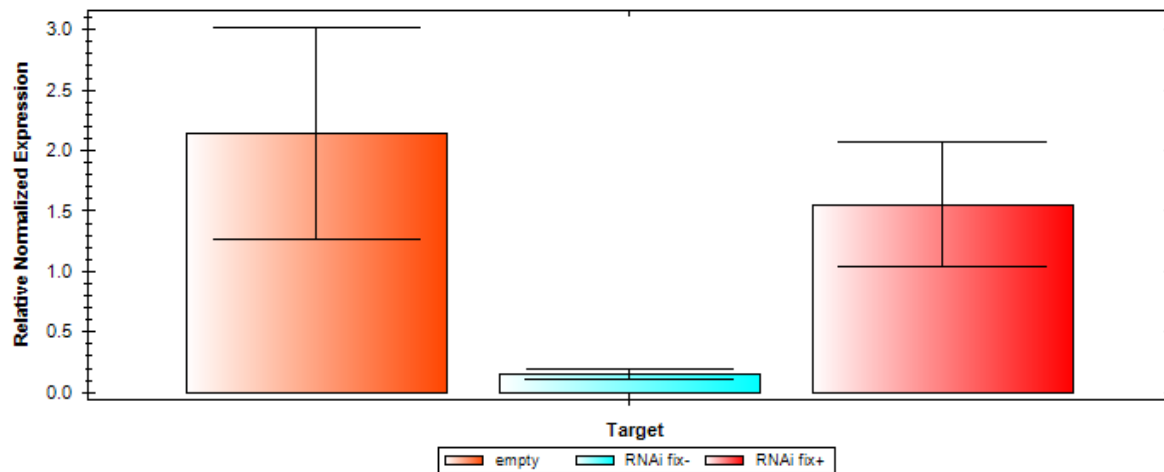


Supplemental Figure 10. The induction of the rhizobial *nifH* gene, detectable due to GFP fluorescence, permits discrimination between Fix+ and Fix- nodules on ProL-B:RNAi-TIP1g transgenic roots.

(A) to (C) Fix+ transgenic nodules (Tr Fix+) and Fix+ non-transgenic (Ntr) nodules.

(D) to (F) Fix- transgenic nodules (Tr Fix-) and Fix+ non-transgenic (Ntr) nodules.

All non-transgenic nodules are Fix+. The transgenic roots were selected according to DsRed emission (620/60) (A) and (D); rhizobial *nifH* induction was detected by GFP emission (525/50) (C) and (F) and the composite image were made by using YFP emission 560/40 (B) and (E). Bars:2 mm



Supplemental Figure 11. The level of *TIP1g* gene silencing in Fix^+ and Fix^- nodules harvested from *ProLB:RNAi-TIP1g* transgenic roots in comparison with an empty vector control. The difference between the control and Fix^- nodules is significant ($P < 0.05$).

Supplemental Table 1: primers used for cloning and PCR analysis of *VPS11*, *VPS39* and *TIP* aquaporins

Gene specific primers for cloning *VPS11* and *VPS39*:

VPS11-F TCCCCGGGATGTATCAATGGCGGAAGTT;
VPS11-R GGGGTACCTCAGAAG-CCACTGCTAGATGAT;
VPS39-F CGGGATCCATGGTGACAGTGCGTACG;
VPS39-R GGGGTACCTCATCGCTTCCTCAACTGA;

Promoter specific primers for *VPS11* and *VPS39* :

Pro VPS11- F CACCTATTCAAATTGAAAAACACAGAATATT;
Pro VPS11- R CGCCGCCGCGAT;
Pro VPS39- F CACCAAGGATTCAAACCCCGATCA;
Pro VPS39- R TTTGGTTACGAAATA-TTGAAGTTGA;

Primers for the analysis of the expression level by q-PCR for *VPS11* and *VPS39* :

VPS11- F TCAAGCAACGCAACTTCCTG;
VPS11-R TCAGGCACAAAGCTGATTGC;
Vps39-F AATCTACTCGCCGGAACAG;
Vps39-R TGACACAACAGGCTTCTTCG;

Gene specific primers for cloning *TIP1g*:

TIP1g-F CACCATGCCGATTTCTAGAATTGCA;
Tip1g-R TTAATAATCCGTGACAGGTAAGTCTG;

Promoter specific primers for *TIP1g*:

ProTIP1g- F CACCGCTTGTCTTGATTCATGGATTG;
ProTIP1g- R TGTTTTATATTTTTCTTTTCTCAAAGA;

Primers for the analysis of the expression level by q-PCR of *TIP* genes in roots and different zones of 14 day old nodules:

TIP1a-F ATTTACGCTGCAAGGGACAC;
TIP1a- R CACATGCAGGGTTGATTGAC;
TIP1b-F CGTTGACCCAAAGAAGGGTA;
TIP1b-R AAGTCCAGCAATTCCACCAC;
TIP1c-F GTCACCTTTGGATTGGCTGT;
TIP1c-R TCAGCTGCTGTGGCATAAAC;
TIP1d-F TTTTGTGTTGCTGGAGTTG;
TIP1d-R ACAGCTGGGTCAAATGTCC;
TIP1e-F CCCAAAGAAAGGAG-CACTTG;
TIP1e-R CCAACCCAATAAACCCAATG;
TIP1f-F GGTTCCATAG-TGGCATGCTT;
TIP1f-R AGCTGCTGTGGCATAACTG;
TIP1g-F AACACCAG-CAGGGTTGGTAG;
TIP1g-R CAAGCAACTGAGCAATCCAA.