Supporting Information

Floss et al. 10.1073/pnas.1308973110

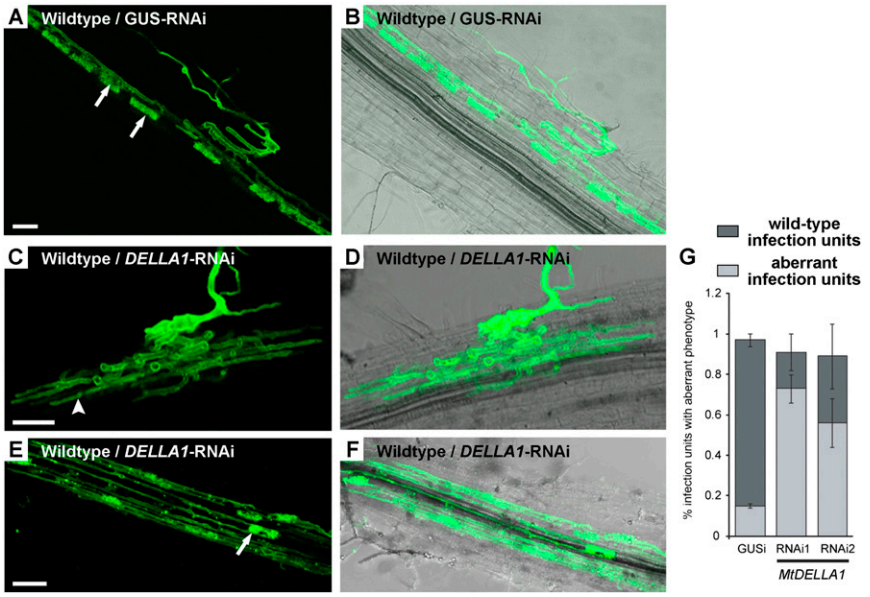

Fig. S1. Morphology of *Glomus versiforme* in *M. truncatula DELLA1*-RNAi roots. (*A* and *B*) Roots expressing a GUS-RNAi control construct colonized with *G. versiforme*. The fungal hyphae colonize the root cortex, and arbuscules are formed within inner cortical cells (arrows in *A*). (*C–G*) *DELLA1*-RNAi roots colonized with *G. versiforme* show an aberrant arbuscular mycorrhizal (AM) phenotype. (*C* and *D*) Infections may be blocked in the outer cortex or inner cortex (arrowhead in *C*). (*E* and *F*) Arbuscules are absent or present at very low levels (arrow in *E*). Two independent *DELLA1*-RNAi constructs were prepared and introduced into *M. truncatula* via *A. rhizogenes* transformation. The phenotypes observed in *C–F* were seen with both constructs. The *DELLA1*-RNAi-1 construct corresponds to a 746-nt region spanning the 5′ UTR and coding sequence (nucleotides −145 to +601 relative to the ATG). The *DELLA1*-RNAi-2 constructs targets a 251-nt region spanning the 5′ UTR and part of the coding sequence (−208 to +43 relative to the ATG nucleotide). The vector control is pHG8-GUS (1). (*G*) Quantification of the AM phenotype in *DELLA1*-RNAi roots: percentage of individual infection units showing the aberrant AM phenotypes (light gray bars) or wild-type infection units (dark gray bars) in roots expressing a GUS-RNAi control (GUSi) or the two independent *DELLA1*-RNAi constructs, *DELLA1*-RNAi1 and *DELLA1*-RNAi2. Thirty plants with *DELLA1* RNAi roots, generated from three independent experiments, were included in the quantitative analysis. $P < 0.0001$, Pearson χ^2 test. (Scale bars: 50 μm.)

1. Pumplin N, et al. (2010) Medicago truncatula Vapyrin is a novel protein required for arbuscular mycorrhizal symbiosis. *Plant J* 61(3):482–494.

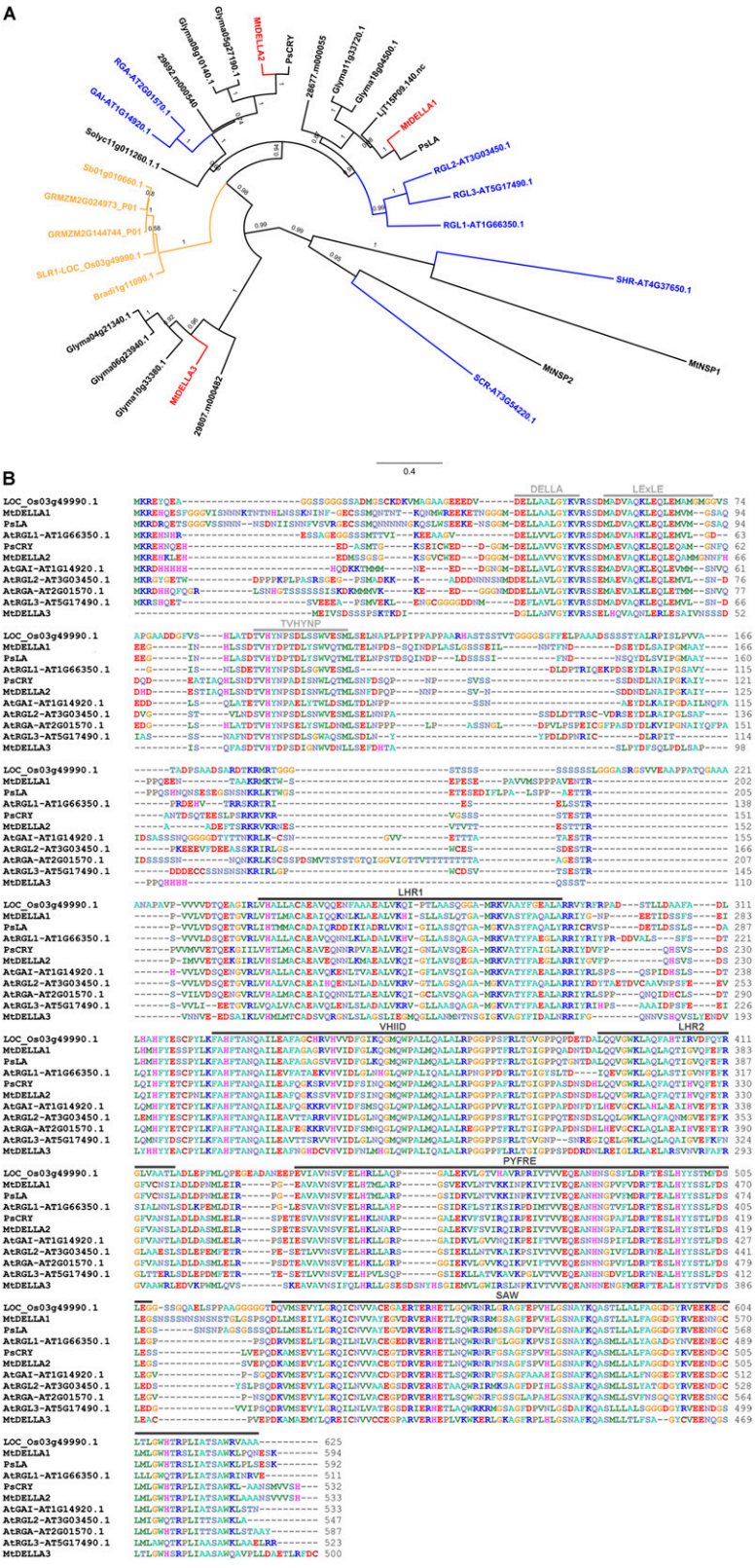

Fig. S2. (*A*) Phylogenetic unrooted tree of DELLA proteins. The phylogram was built using amino acid sequences from *Brachypodium distachyon*, rice, maize, sorghum, *Arabidopsis*, tomato, soy bean, castor bean, *Lotus japonicus*, pea, and *Medicago truncatula* DELLA proteins using the program FastTree (www.microbesonline.org/fasttree/). Numbers represent the posterior probabilities for each branch division using 100,000 generations. Sequences were obtained from the following databases: *B. distachyon*, rice, sorghum, soybean: Phytozome; maize, *L. japonicus*: PlantGDB; *Arabidopsis* (blue): TAIR; tomato: Sol Genomics. Monocot species are highlighted in yellow. PsLA and PsCRY accession numbers are reported in ref. 1. MtNSP1 and MtNSP2 are reported in ref. 2. In *M. truncatula*, three DELLA proteins (highlighted in red) could be identified: MtDELLA1 (contig_170694 and contig_69957), MtDELLA2 (contig_52215), and MtDELLA3 (contig_55897). (*B*) Amino acid sequence alignment of DELLA proteins from *M. truncatula*, rice, pea, and *Arabidopsis*. Gaps introduced to improve

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the sequence alignment are indicated by dashes. Amino acids are color-coded. Conserved N-terminal regulatory motifs (DELLA, LExLE, and TVHYNP) are indicated by light gray lines above the sequence. The C-terminal GRAS domain consists of the LHR1, VHIID, LHR2, PFYRE, and SAW subdomains, which are indicated by dark gray lines above the sequence.

1. Weston DE, et al. (2008) The Pea DELLA proteins LA and CRY are important regulators of gibberellin synthesis and root growth. *Plant Physiol* 147(1):199–205.
2. Hirsch S, *et al*. (2009) GRAS Proteins Form a DNA Binding Complex to Induce Gene Expression during Nodulation Signaling in *Medicago truncatula*. *Plant Cell* 21(2):545–557.

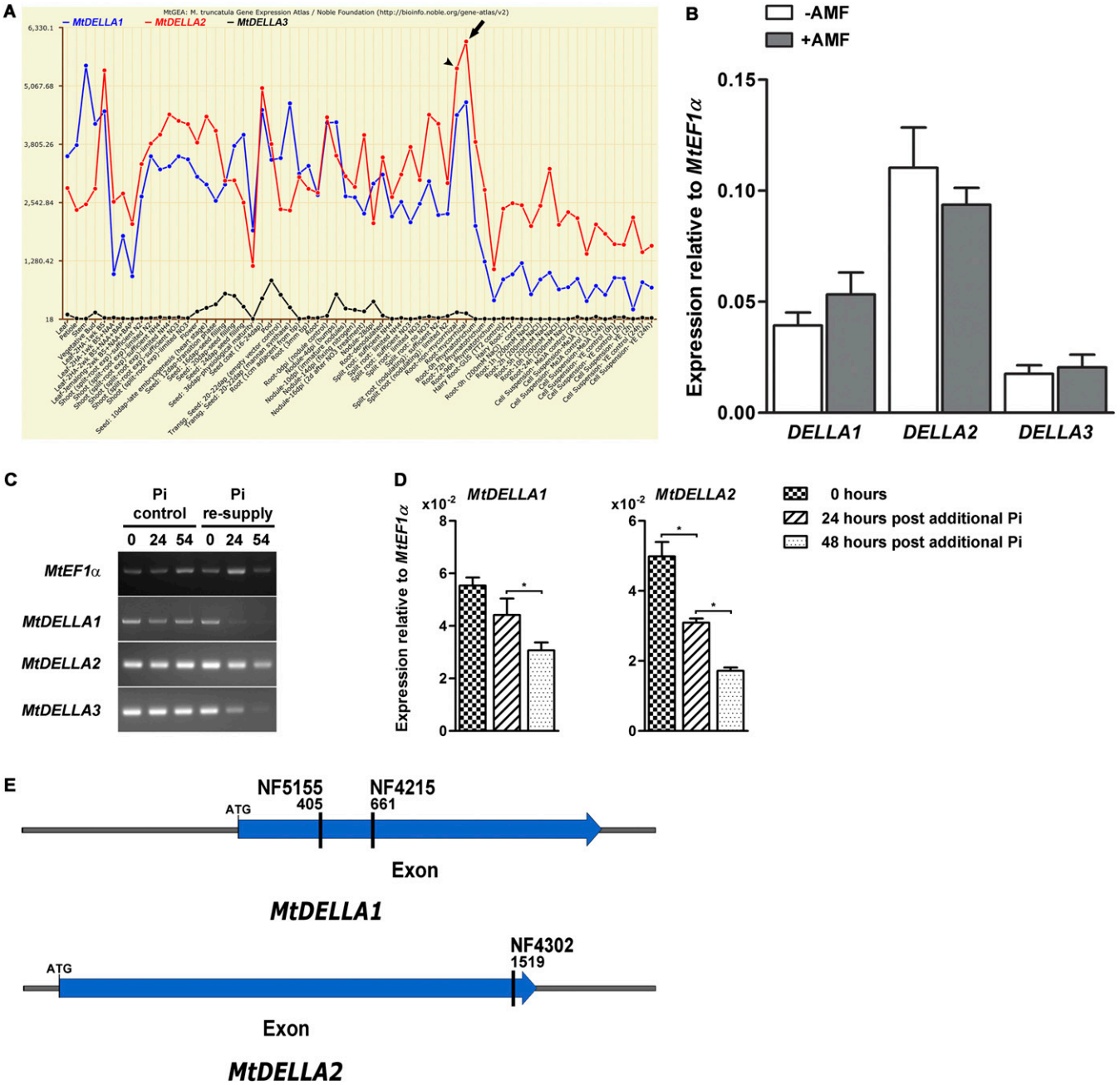

Fig. S3. *DELLA* gene expression and location of *Tnt1* insertions in *DELLA1* and *DELLA2*. Three DELLA genes are represented in the *M. truncatula* EST database: *MtDELLA1* (TC175700), *MtDELLA2* (TC182493), and *MtDELLA3* (TC191252 and TC191608). (*A*) Gene-expression profiles of *DELLA1*, *DELLA2*, and *DELLA3*. *DELLA1* (blue line) and *DELLA2* (red line) are coexpressed, and transcript levels are high in Pi-deprived roots (arrowhead) and during AM symbiosis (arrow). Expression profiles were extracted from the *Medicago truncatula* Gene Expression Atlas (MtGEAv2; http://mtgea.noble.org/v2/). (*B*) Expression of *DELLA1*, *DELLA2*, and *DELLA3* in noncolonized (white bars, −AMF) and colonized roots (gray bars, +AMF) of *M. truncatula* at 5 wk post planting (w.p.p.) with *G. versiforme* assayed by quantitative RT-PCR. Transcript levels of *MtDELLA1*, *MtDELLA2*, and *MtDELLA3* do not change significantly after colonization. Data are averages ± SEM ($n = 3$, where N denotes the number of independent root samples). (*C*) Transcript levels of *DELLA1, DELLA2*, and *DELLA3* in *M. truncatula* roots in response to phosphate assayed by semiquantitative RT-PCR. Plants were grown for 28 d in low-Pi conditions (1 μM KH_2PO_4) and were harvested at 0, 24, or 54 h following resupply with either 1 μM KH_2PO_4 or 1 mM KH_2PO_4 as described previously (1). (*D*) Expression of *DELLA1* and *DELLA2* in *M. truncatula* roots in response to phosphate assayed by quantitative RT-PCR. Plants were grown for 35 d in low-Pi conditions (20 μM KH_2PO_4) and were harvested 0, 24, or 48 h after the addition of 1 mM KH_2PO_4. Data are averages ± SEM ($n = 3$, where N denotes the number of independent root samples). *$P \leq 0.05$. (*E*) Location of the *Tnt1* insertion in *DELLA1* and *DELLA2*. The A of each ATG designates nucleotide 1. *Tnt1* insertions and positions (in nucleotides) are indicated. Two *della1* insertion lines were obtained (NF5155 and NF4215). Blue indicates the coding sequence. The coding sequences of *DELLA1* and *DELLA2* do not contain introns.

1. Liu JY, et al. (2008) Closely related members of the Medicago truncatula PHT1 phosphate transporter gene family encode phosphate transporters with distinct biochemical activities. *J Biol Chem* 283(36):24673–24681.

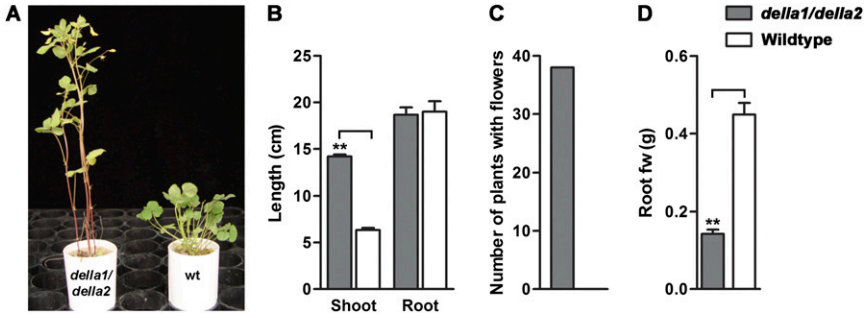

Fig. S4. *della1/della2* developmental phenotypes. (*A*) The *slender* shoot phenotype of *della1/della2*. (*B*) Shoot and root length of *della1/della2* mutant (gray bars) and wild-type plants (white bars). The shoot length of *della1/della2* mutants differs significantly from that of wild-type plants. Data are averages ± SEM ($n = 6$, where N denotes the number of independent samples). (*C*) Flowering time. At 35 d post planting (d.p.p.), flowers were observed on *della1/della2* plants but not on wild-type plants ($n = 42$). (*D*) Fresh weight (fw) of *della1/della2* and wild-type roots. Root weight of *della1/della2* plants differs significantly from that of wild-type plants. Data are averages ± SEM ($n = 6$, where N denotes the number of independent roots). $^{**}P \leq 0.01$.

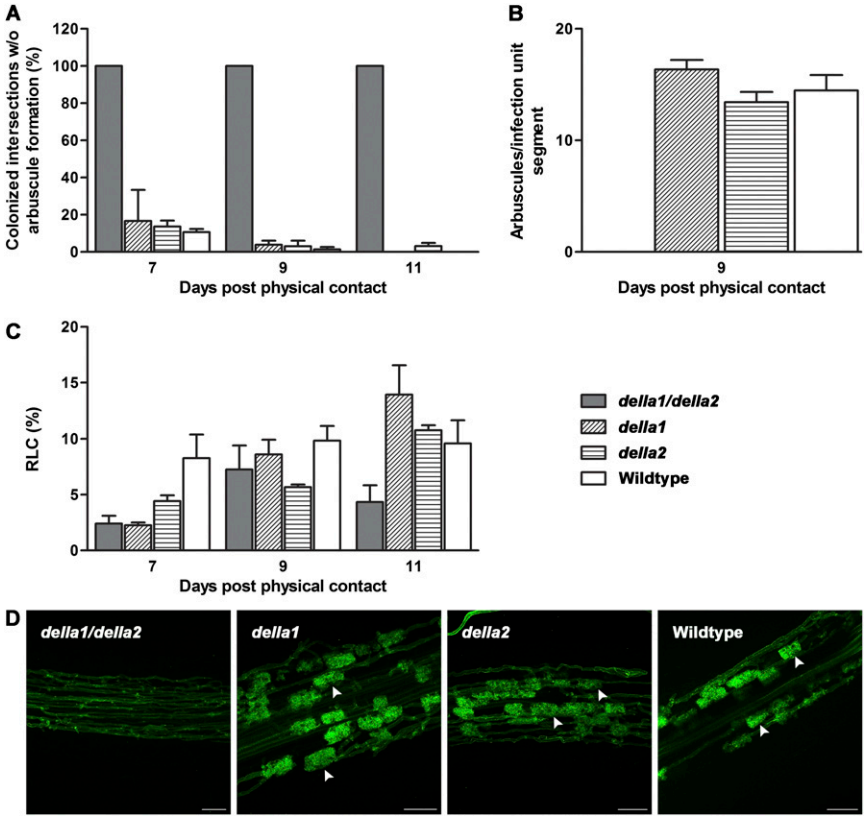

Fig. S5. AM phenotype of *della* mutants. (*A*) Arbuscule formation ($n = 3$, where N denotes the number of independent root samples), (*B*) arbuscule density ($n \geq 23$, where N denotes the number of segments), and (*C*) level of fungal colonization ($n = 3$, where N denotes the number of independent root samples) of *della1/della2*, *della1*, and *della2* mutants and wild-type plants grown in the double-cone system (1). Arbuscules are completely absent in *della1/della2* roots. The level of colonization of *della1/della2* roots does not differ significantly from that of wild-type roots. Data are averages ± SEM. RLC, root length colonized. (*D*) Laser-scanning confocal microscope images of *G. versiforme* in roots of *della1/della2*, *della1*, and *della2* mutants and wild-type plants. Arrowheads mark arbuscules. Arbuscules are absent in *della1/della2* roots. (Scale bars: 50 μm.)

1. Lopez-Meyer M, Harrison MJ (2006) An experimental system to synchronize the early events of development of the arbuscular mycorrhizal symbiosis. *Biology of Molecular Plant-Microbe Interactions*, eds Sánchez F, Quinto C, López-Lara IM, Geiger O (International Society for Molecular Plant-Microbe Interactions, St. Paul), Vol 5, pp 546–551

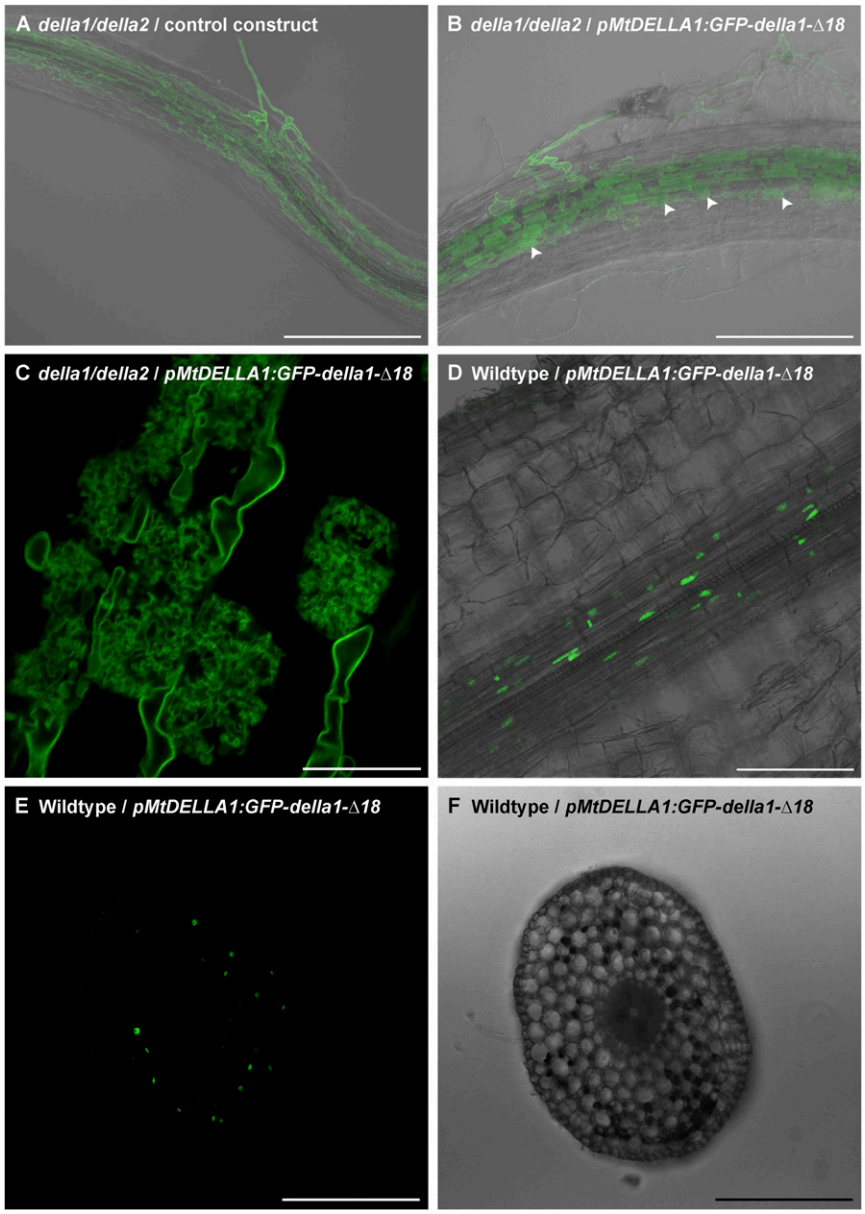

Fig. S6. AM phenotype of *della1/della2* expressing *pMtDELLA1:GFP-della1*-Δ18 and localization of MtDELLA1 in *M. truncatula* roots. (*A*–*C*) Laser-scanning confocal microscope images of *G. versiforme* in roots of *della1/della2* transformed with a control construct (*A*) or *pMtDELLA1:GFP-della1*-Δ18 (*B* and *C*). Arrowheads mark arbuscules. The expression of *pMtDELLA1:GFP-della1*-Δ18 complements the *della1/della2* mutant, and arbuscules show normal morphology. (*D*–*F*) Wild-type roots expressing *pMtDELLA1:GFP-della1*-Δ18. GFP signals are detected in the nuclei of cells of the vascular tissue and endodermis (*D*) and in some cortical cells (*E* and *F*). Note that the plane of the image in *D* is focused centrally through the vascular tissue, and the cortical cells that are in focus do not show any GFP signal. In *E* and *F*, the focal plane is set to reveal cortical cells in which the nuclei show GFP. (Scale bars: 250 μm in *A*, *B*, *E*, and *F*; 75 μm in *D*; 25 μm in *C*.)

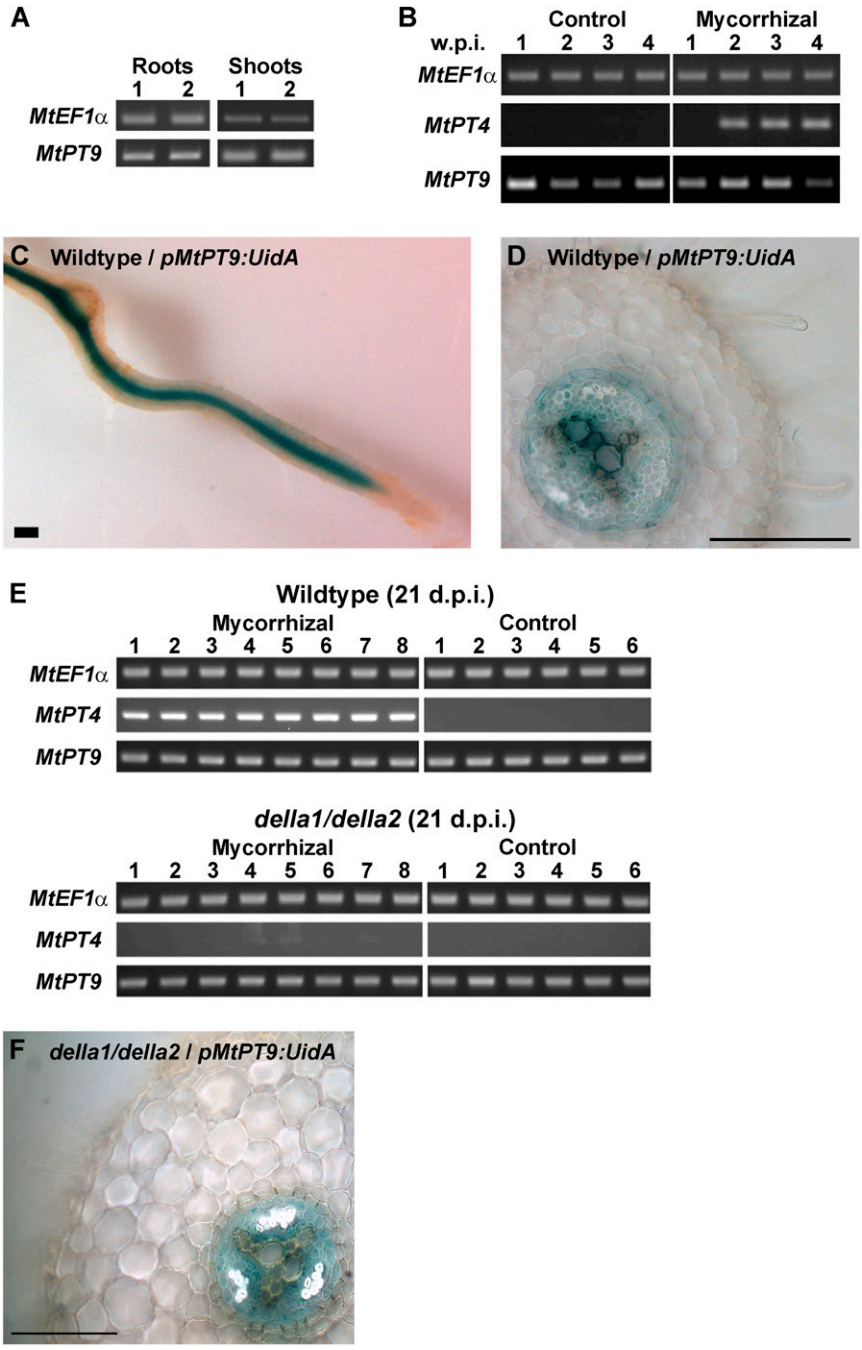

Fig. S7. Expression of *MtPT9* in wild-type plants and *della1/della2* mutants. (*A*) Expression of *MtPT9* in roots and shoots monitored by semiquantitative RT-PCR. Two independent root and shoot samples are shown. (*B*) Transcript levels of *MtPT9* and *MtPT4* in wild-type roots either mock-inoculated (control) or inoculated with *G. versiforme* (mycorrhizal) at 1, 2, 3, and 4 wk post inoculation (w.p.i.) assayed by semiquantitative RT-PCR. *MtPT4* expression is mycorrhiza-specific. *MtPT9* transcript levels do not change after inoculation with *G. versiforme*. (*C* and *D*) Histochemical staining for GUS activity in *M. truncatula* wild-type roots expressing *pMtPT9:UidA*. An *MtPT9* genomic fragment including 1,928 nt upstream of the ATG was amplified with primers adding 5′ XbaI and 3′ HindIII sites and was cloned into a modified version of the pCAMBIA2301 vector lacking the 35S promoter to create *pMtPT9:UidA*. The *MtPT9* promoter is active in vascular tissue and endodermis. (*E*) Expression of *MtPT9* in wild-type and *della1/della2* roots either mock-inoculated or inoculated with *G. versiforme* at 21 d post inoculation (d.p.i.) assayed by semiquantitative RT-PCR. Eight independent mycorrhizal root samples and six independent control root samples were analyzed. *MtPT9* transcript levels do not change in *della1/della2* mutants. (*F*) *della1/della2* roots expressing *pMtPT9:UidA*. The spatial expression pattern of the *MtPT9* promoter in *della1/della2* roots is the same as that seen in wild-type roots, namely in the vascular tissue and endodermis. (Scale bars: 100 μm.)

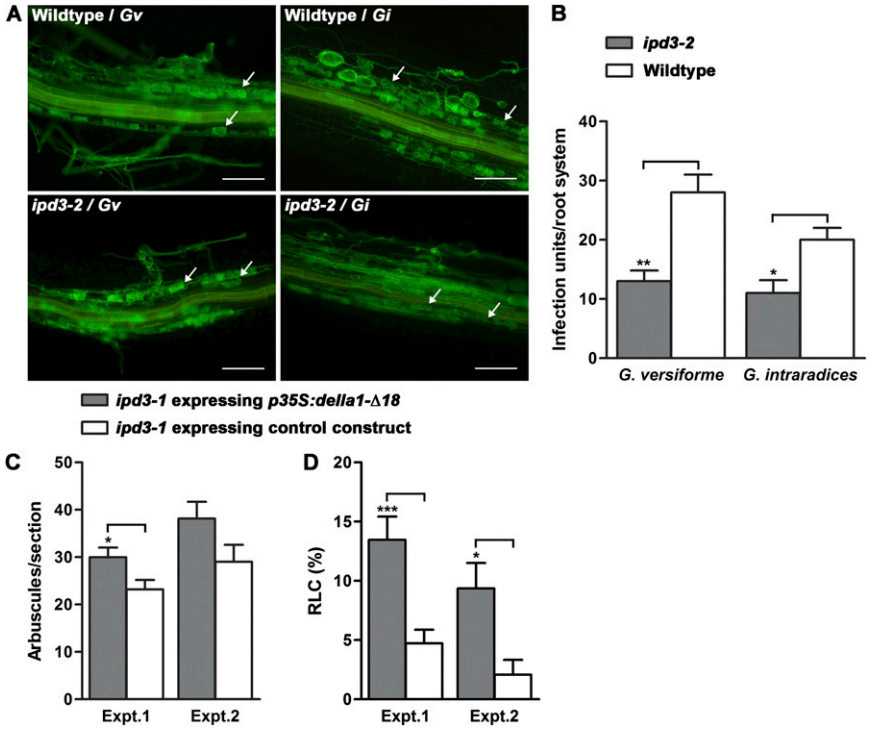

Fig. S8. AM phenotype of *M. truncatula ipd3* alleles. (*A*) Morphology of *G. versiforme* (*Gv*) or *Glomus intraradices* (*Gi*) in *M. truncatula* R108 (wild type) and *ipd3-2* roots at 6 w.p.i. Plants were inoculated with 600 surface-sterilized *G. intraradices* or *G. versiforme* spores. Arrows mark arbuscules. Arbuscules are formed in *ipd3-2* roots. (Scale bars: 100 μm.) (*B*) The numbers of infection units in *ipd3-2* and wild-type/R108 roots differ significantly. Data are averages ± SEM ($n \geq 6$, where *N* denotes the number of independent root samples). (*C* and *D*) Arbuscule density (*C*) and level of colonization (*D*) in *ipd3-1* roots expressing *p35S:della1*-Δ18 or a control construct at 29 d.p.i. with *G. versiforme*. Expression of *p35S:della1*-Δ18 results in an increase in arbuscule density and fungal colonization. Data are averages ± SEM from two independent experiments ($n \geq 5$, where *N* denotes the number of independent root systems). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

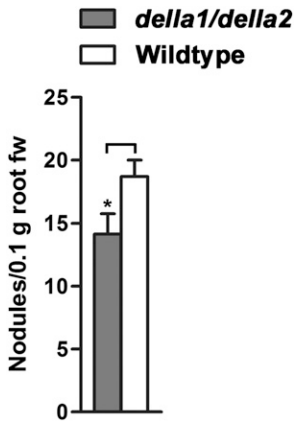

Fig. S9. Nodule numbers in *della1/della2* and wild-type root systems at 27 d.p.i. Fourteen-day-old seedlings were inoculated with *Sinorhizobium meliloti* strain 2011 ($OD_{600} = 0.2$). The plants were fertilized once a week with Fahraeus medium without nitrate. Nodules per root system were counted. The number of nodules is significantly lower in *della1/della2* mutants than in the wild-type segregant control. Data are averages ± SEM ($n \geq 16$, where *N* denotes the number of independent root systems). $^{*}P \leq 0.05$.

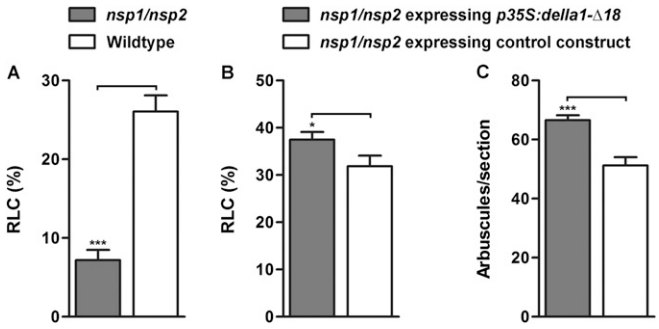

Fig. S10. AM phenotype in *M. truncatula nsp1/nsp2* roots colonized by *G. versiforme*. (*A*) Level of colonization of *nsp1/nsp2* and wild-type roots. Eighteen-day-old seedlings were transferred into pots partially filled with a sterile play sand/filter sand mixture (1:1 ratio). A 1-cm layer of play sand at the top of the mixture was inoculated with 200 surface-sterilized spores per plant and was covered by a sterile play sand/gravel mixture (1:1 ratio). Seedlings were fertilized every third day with modified half-strength Hoagland's solution containing full-strength nitrogen and 20 μM potassium phosphate. Plants were harvested 21 d.p.i. The colonization level in *nsp1/nsp2* roots is significantly lower than in wild-type roots. Data are averages ± SEM ($n = 8$, where N denotes the number of independent root samples). (*B* and *C*) The level of colonization by *G. versiforme* (*B*) and arbuscule density (*C*) in *nsp1/nsp2* in roots expressing *p35S:della1*-Δ18 and in a control construct at 23 d.p.i. Composite *nsp1/nsp2* plants were grown as described above. The expression of *p35S:della1*-Δ18 results in a significant increase in fungal colonization and arbuscule density. Data are averages ± SEM ($n \geq 11$, where N denotes the number of independent root samples). $^{*}P \leq 0.05$; $^{***}P \leq 0.001$. This *nsp1/nsp2* mutant was obtained from G. Oldroyd, John Innes Centre, Norwich, United Kingdom and was made by crossing *nsp1-1* with *nsp2-2*.

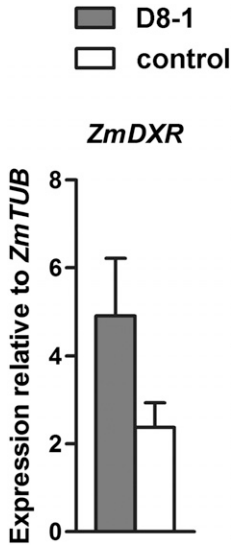

Fig. S11. Expression of *ZmDXR* in maize D8-1 and control roots assayed by quantitative RT-PCR at 10 wk post planting. Seed kernels of stock 121C, provided by the Maize Genetics COOP Stock Center, were planted into pots (one kernel per pot) partially filled with sterile Turface. A 1-cm layer of sterile play sand containing 1,600 surface-sterilized *G. intraradices* spores was spread equally onto the Turface layer and was covered by a 1-cm layer of Turface followed by a 2-cm layer of play sand. Plants were fertilized once a week with modified half-strength Hoagland's solution containing full-strength nitrogen and 20 μM potassium phosphate. Heterozygous D8-1 plants were selected based on their dwarf phenotype. The homozygous nondwarf plants were used as controls. Transcript levels of *ZmDXR* are higher in D8-1 plants ($P = 0.08$). Data are averages ± SEM ($n = 4$, where N denotes the number of independent root samples).

Table S1. Primers

Primer name	Sequence
DELLA1-RNAi-1-F	5′-GGGACAAGTTTGTACAAAAAAGCAGGCTTTGTCTCAACCCCTTTTCTC-3′
DELLA1-RNAi-1-R	5′-GGGACCACTTTGTACAAGAAAGCTGGGTTTCCACCGCCGGCGGTGGTG-3′
DELLA1-RNAi-2-F	5′-GGGACAAGTTTGTACAAAAAAGCAGGCTTGGTTTCCGTTCTTCCTT-3′
DELLA1-RNAi-2-R	5′-GGGACCACTTTGTACAAGAAAGCTGGGTAATCACACCCCCACCAAA-3′
Fusion PCR1-F	5′-GAGAGTCGACATGAAGAGAGAACACCAAGAAAG-3′
Fusion PCR1-R	5′-GAGCTACATCTCCACCACCGTTGGTTTCTTTTTC-3′
Fusion PCR2-F	5′-CGGTGGTGGAGATGTAGCTCAAAAGCTTGAACAGC-3′
Fusion PCR2-R	5′-GAGATGTACATCACTTGGACTCATTTTGTGGAAGC-3′
pMtDELLA1-A-F	5′-GTCGACGCGCTATCAGAAC-3′
pMtDELLA1-A-R	5′-AAGCTTTTTTTTTCTTTCTATAATTTGTTGA-3′
pMtDELLA1-B-F	5′-TATGTCGACGCGCTATCAGAACGCGC-3′
pMtDELLA1-B-R	5′-TATGTCGACTTTTTTTTTCTTTCTATAATTTGTTGATTAAATTGGTGAAG-3′
della1-Δ18-F	5′-TATTGTACAAGATGAAGAGAGAACACCAAGAAAGTTTTGGTGGGG-3′
della1-Δ18-R	5′-TATGCGGCCGCACGTGTCACTTGGACTCATTTTG-3′
pMtPT9-F	5′-TATGGATCCTTGCATCGCGCTCGTTG-3′
pMtPT9-R	5′-TATGTCGACTTTCTGATTTTGAAACTTGATATTGC-3′
Tnt1-F	5′-GCATTCAAACTAGAAGACAGTGCTACC-3′
gMtDELLA1-F	5′-ACCTTAACAGCAGCAAAAACATCA-3′
gMtDELLA1-R	5′-TCCCTTGCCTCACTCACTTT-3′
gMtDELLA2-F	5′-CATGATGAATCCACCATCGCT-3′
gMtDELLA2-R	5′-CGAGTTGAATCAGTGCGAAA-3′
MtEF-1α-F	5′-TGACAGGCGATCTGGTAAGG-3′
MtEF-1α-R	5′-TCAGCGAAGGTCTCAACCAC-3′
G. versiforme α-tubulin-F	5′-TGTCCAACCGGTTTTAAAGT-3′
G. versiforme α-tubulin-R	5′-AAAGCACGTTTGGCGTACAT-3′
MtPT4-F	5′-gacacgaggcgctttcatagcagc-3′
MtPT4-R	5′-gtcatcgcagctggaacagcaccg-3′
MtLEC5-F	5′-TCAAGTTGCTGAAACACATGAT-3′
MtLEC5-R	5′-GAGCAGAACCATTGCAACAA-3′
MtDELLA1-F	5′-AAGTCTAGGGTAAGTTGATGGGTTA-3′
MtDELLA1-R	5′-CTCATCCCTTGCCTCACTCAC-3′
MtDELLA2-F	5′-AGCCTGAGACAAGAGACCCA-3′
MtDELLA2-R	5′-TTACCACCAGCAGCAAGCTA-3′
MtDELLA3-F	5′-CAAGCATTGTAGCTCACTTAGCA-3′
MtDELLA3-R	5′-TGTGACAAGCAAACCCCTCAA-3′
MtKS-F	5′-AATTGGTGTTCCAACAATCTCC-3′
MtKS-R	5′-AAACAACTTTATCAAACAGAACTTTGG-3′
MtKO-F	5′-GCAGTTTAGTGAACATGCCAA-3′
MtKO-R	5′-GCCAACTCCTCCACGTAAAT-3′
GA20ox-F	5′-TTAGGAACTGGACCCCATTG-3′
GA20ox-R	5′-TGACCACAAAGGCATCTTCTT-3′
GA2ox-F	5′-TGATGTACTTTGCAGCACCA-3′
GA2ox-R	5′-AGACGTGAATCTCCCAATCG-3′
MtDMI3-F	5′-ACCGTGATGGAACAGTTGACA-3′
MtDMI3-R	5′-TCTTTGCTGATGCAGCCTGA-3′
MtIPD3-F	5′-GCGCTCAAGAAAAATGGCTGAAGC-3′
MtIPD3-R	5′-GCTTTAGTGATCGAACTTCCTTCTCAAGG-3′
MtNSP2-F	5′-GCTTCCAACAACAACGGTCC-3′
MtNSP2-R	5′-TAATCGCCTGCCGGTTTCTT-3′
MtNSP1-F	5′-GCGATTTCGCCACTGGATTC-3′
MtNSP1-R	5′-CAGCCTCGCCTTCCATCATT-3′
LjEF-1α-F	5′-GCAGGTCTTTGTGTCAAGTCTT-3′
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LjNSP1-F	5′-GAGGTCGAGCTTTGTTGAGG-3′
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LjNSP2-F	5′-CATCGACTCCATGATTGACG-3′
LjNSP2-R	5′-GGTTGTTGTTGTCGTGGTTG-3′
ADP-RF-F	5′-GCTCTCCAACAACATTGCCAAC-3′
ADP-RF-R	5′-GCTTCTGCCTGTCACATACGC-3′

Table S1. Cont.

Primer name	Sequence
TaPT10-F	5′-TGCATGACAAGTTGACAACACACG-3′
TaPT10-R	5′-AATCAATGCTCGTACATTCACACTG-3′
TaPT11-F	5′-TGCATGACACCACGCATCTTATC-3′
TaPT11-R	5′-GGTATCACACACAAGCAATCCTA-3′
ZmTUB-F	5′-AAGCGGACCTCGATGAGTTC-3′
ZmTUB-R	5′-GGGTGGGTACGCTAGCAGTAG-3′
ZmDXR	5′-GCTGGGGGCACCATGACAGG-3′
ZmDXR	5′-TGCAGGGACAGGGCTCAGGC-3′