Supplementary Information

Materials and Methods

Plant growth conditions: Rice and maize seeds were surface sterilized as described [1,2] and allowed to pregerminate for 4 d at 30°C on moist filter paper, after which they were planted into pure quartz sand. For AM colonization assays, pregerminated rice and maize germlings were transferred to 50 ml cones and 750 ml pots, and inoculated with ~250 and 500 *R. irregularis* spores, respectively. For the mutant/wild type co-cultivation experiment, seeds of two tester plants were placed central to six donor seeds and inoculated upon planting as previously described [3]. The plants were grown in phytochambers with a 12h day/night cycle at 28/22°C and 60% humidity. Maize and rice plants were watered every other day and fertilized twice a week with full (maize) or one-half Hoagland (rice) solution containing 100 µM and 50 µM KH_2PO_4, respectively. In addition, rice was supplemented with 0.01% (w/v) Sequestrene Rapid (Syngenta Agro, Dielsdorf, Switzerland). For examining the effect of pharmacological complementation of *Osnope1* mutant inoculated with *R. irregularis* by exogenous application of GlcNAc, the above-described fertilizing solution was amended with either 1mM, 10mM or 100mM GlcNAc. Inoculation of rice roots with *Piriformospora indica* and *Magnaporthe oryzae* followed earlier reported protocols [1,4]. For rice GlcNAc transport assays, surface sterilized rice seeds were sown in one-half Murashige & Skoog medium (½ MS) and vertically grown in the dark for 5 days at 30°C before being transferred into hydroponic medium (see below). Arabidopsis seeds were stratified for 3 d at 4°C in the dark and then grown vertically on ½ MS medium containing 25 µg/ml basta for two weeks. Resistant plants were transferred to new ½ MS medium, and allowed to recover for one week under 21°C and 12h light/12h dark cycles and either used for subcellular localization of AtNope1

or grown in soil for additional 4-5 weeks under 8h light/16h dark regimes at 21°C for [^{3}H]GlcNAc uptake experiments with protoplasts.

Identification of maize and Arabidopsis *NOPE1*. *Dissociation* (*Ds*) mutagenesis of *ZmNope1* was performed following a strategy as previously described [9,10]. Briefly, individuals carrying either the selected donor *Ds* (*dDs*) element B.W06.0767C or B.S05.0412 (located at 264bp and 265kb, respectively, from *ZmNope1* with reference to the B73 v2 genome) and the stable transposase source *Ac-immobilized* (*Ac-im*) [11] in the color-converted W22 stock T43 [*r1-sc:m3*/*r1-sc:m3*; [12]] were used as males to pollinate multiple *r1-sc:m3*/*r1-sc:m3* females. A total of 4,320 test-cross progeny were germinated and screened for novel insertions of *Ds* in *ZmNope1* using a PCR-based strategy. Tissue was collected between 7 and 10 days after planting from pools of 10-18 seedlings using a 3.5 mm hole punch and DNA isolated following a modified Extract-n-amp ® (Sigma-Aldrich) extraction protocol, using 20% of the reagents in the recommended protocol (Add 20 µl Extraction solution to pooled leaf tissue, incubate at 95ºC 10', add 20 µl Dilution solution, vortex). One µl of Extract-n-amp ® template was used in a modified GoTaq Green ® (Promega) PCR reaction, in which 10% Betaine [5M] and 4% DMSO were added to a pair of gene-specific primers designed to span the *ZmNope1* gene-space and were combined with the 5' and 3' *Ds* end primers JSR01 and JGp3 to amplify flanking DNA adjacent to novel insertions (Supplement Table S1). From individuals of positive pools, tissue was recollected, DNA extracted (as above), and a second PCR reaction was performed to determine which individual of the pool carried the novel *Ds* transposition event. Furthermore, DNA was extracted and the PCR reaction was replicated using template from an additional seedling leaf in Ds-positive individuals to eliminate the possibility of recovering somatic events. Novel PCR products from individuals were gel purified according to the manufacturer's instructions (QIAquick ® Gel Extraction kit, Qiagen) and were sequenced to confirm each *Ds* insertion. Positive seedlings were

grown to maturity and self-pollinated to create a segregating homozygous *nope1::Ds* insertion stock. The *A. thaliana NOPE1* homologs were identified based on sequence similarities. The rice *OsNope1* cDNA and OsNope1 protein sequences were used as query for blastn and blastp searches in the Arabidopsis database (www.arabidopsis.org). Two *A. thaliana* genes, *A1g18000* and *At1g18010,* are identical in sequence and showed the highest similarities. Using the reciprocal blast strategy (RBS) confirmed rice *OsNope1* (*LOC_Os04g01520*) as the most similar rice sequence to *A1g18000* and *At1g18010*.

Phylogenetic analysis

Sequences for the viridiplantae-specific orthogroup corresponding to OsNope1 was downloaded from Phytozome V.11.1 (https://phytozome.jgi.doe.gov/pz/portal.html) as CDS sequences. Alignments were undertaken in the translation align function generated in Geneious version 7.1.8, and trees generated by FastTree, under default settings but under a GTR+I+gamma model of nucleotide substitution.

Mycorrhizal quantification: Performed by staining roots with Trypan Blue as described earlier [1]. For detailed inspection of fungal morphology, roots were stained with WGA-AlexaFluor488 conjugate (Invitrogen, Paisley, UK), counterstained with propidium iodide and imaged with a Leica SP5 confocal microscope (Leica Microsystems, Milton Keyes, UK).

Laser Scanning Confocal Microscopy: For the assessment of subcellular localization of *AtNope1*, roots of 7d old *A. thaliana* line *4731-y* were stained with propidium iodine and imaged with a Zeiss LSM 700 confocal microscope (Zeiss, Feldbach, Switzerland). Excitation/Emission windows used were: 488/500-550 nm for WGA; 488/590-660 nm for PI and 514/525-550 for YFP.

***Gigaspora rosea* germ tube branching bioassay**: Four *G. rosea* spores per plate were germinated and incubated at 2% [CO2] and 30°C in the dark in minimal medium supplemented with 10 µM quercetin and gelled with 0.6% W/V Phytagel (Sigma-Aldrich, Saint-Quentin Fallavier, FR). Seven days after inoculation, each spore produced a single germ tube growing upwards. Two small wells were produced into the gel on each side of the germ tube tip with a Pasteur pipette tip and 5 µl of the test solution (10^{-7} M GR24) in 0.1% acetonitrile (positive control), or 0.1% acetonitrile (negative control), or plant exudates (complemented with 0.1% final acetonitrile) were injected into each well. After 24 h, germ tube branching was recorded by counting newly formed hyphal tips. Three to five plates (9–15 spores) were used for each treatment. The mean numbers of branches for all conditions tested were compared by the Kruskal–Wallis test and, when significant, pair comparison was made by the student test. The experiment was three times independently repeated.

Nucleic acid extraction and manipulation. Rice genomic DNA was extracted by the urea extraction method [13], digested with *EcoR*I enzyme (Promega, Dübendorf, Switzerland) and treated with RNAse A (Sigma-Aldrich, Buchs, Switzerland). The digested DNA was separated by gel electrophoresis and transferred overnight to a nylon membrane (GE Healthcare, Glattbrugg, Switzerland). An 870 bp fragment of the phosphinothricin-acetyl-transferase resistance gene (bar) was amplified with primers MN047 and MN048 (Supplemental Table S4) from plasmid pTF101.1 [14] and used as probe for hybridization. For probe labelling and detection the non-radioactive DIG-system (Roche, Basel, Switzerland) was employed according to the manufacturer's guidance. Southern blot was performed according to standard protocols [15].

Plant RNA extraction, cDNA synthesis and quantitative RT-PCR were performed as described earlier [1]. Briefly, RNA was extracted from ~100mg of root (rice and maize) or cauline leaf tissue

(Arabidopsis) with Trizol prepared in the lab [16] and treated with DNaseI (ThermoFisher Scientific, Ecublens, Switzerland and Thermo Electron Basingstoke, UK) to remove contaminating gDNA. Absence of residual gDNA was confirmed by control PCR on DNAseI treated RNA before reverse transcription. First strand cDNA was synthesized using Superscript II reverse transcriptase (ThermoFisher Scientific, Ecublens, Switzerland and Thermo Electron Basingstoke, UK) combined with oligo(dT) primers (Promega, Dübendorf, Switzerland and Southhampton, UK). For detecting *NOPE1* transcripts from rice, maize or Arabidopsis lines, cDNA was synthesized using Superscript III reverse transcriptase (ThermoFisher Scientific, Ecublens, Switzerland and Thermo Electron Basingstoke, UK).

Fungal RNA was extracted from fungal mycelium produced from spores using RNeasy Plant Mini Kit (Qiagen, Hombrechtikon, Switzerland and Manchester, UK). Reverse transcription applied the same protocols as for plants described above. Gene specific primers for plant AM marker genes [1] and *AtNope1*, and fungal genes are described in Supplemental Table S2 and Supplemental Table S3, respectively. Real-time RT-PCR was performed as previously described [1]. The following constitutively expressed genes were used to normalize target gene transcript levels in each organisms: rice, *CYCLOPHILIN2* (*CP2*), *LOC_Os02g02890*; maize, *GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE* (*GAPDH*), *GRMZM2G046804*; Arabidopsis, *ISOPENTENYL-DIPHOSPHATE-DELTA-ISOMERASE II* (*IPP2*), *A13g02780*; *R. irregularis*, *ELONGATION FACTOR 1-α* (*RiEF*), fgenesh1_kg.21690_#_7.

To determine the full-length cDNA of rice *NOPE1* Gene-Race kit (ThermoFisher Scientific, Ecublens, Switzerland) was used to amplify 5' and 3' ends of rice *NOPE1* cDNA following manufacturer recommendations.

Generation of constructs and genetic transformation of *A. thaliana* and *C. albicans*: *A. thaliana* constructs were generated based on Gateway Cloning Technology (ThermoFisher

Scientific, Ecublens, Switzerland). Briefly, the silencing construct *pAtNope1* was generated by amplifying ~200 bp fragment complementary to the 5'end of the *At1g18000* coding region using primers MN042 and MN043, followed by cloning into pDONR207 (ThermoFisher Scientific, Ecublens, Switzerland) and transfer into the destination vector pB7GWIWG2-II [18]. To generate Ubq_{prom}::*YPF*::*AtNope1* for subcellular localization, *AtNope1* ORF was transferred from clone U14731 (http://signal.salk.edu/) into destination vector pNIGEL07 [19]. *A. thaliana* transgenic lines were generated by floral dipping as previously described [20]. Primers used to generate constructs for plant transformation are described in Supplemental Table S4.

The construct for genetic complementation of *C. albicans, pOsNope1,* was generated by synthesizing a codon optimized version of rice *NOPE1* cDNA for expression in *C. albicans* (http://www.idtdna.com/site). This *cDNA* version was then modified by amplification with primers carrying 50 bp of homology to the regions flanking the *CaNGT1* gene to replace the *CaNGT1* gene in plasmid pDDB57 by homologous recombination in *S. cerevisiae* [7]. The resulting *pOsNope1* plasmid and the corresponding parental *CaNGT1* plasmids were digested in the promoter region with *Bsa* BI and transformed into *C. albicans ngt1Δ* strain YJA2 [7] using a *URA3* gene for selection. Control *ngt1Δ* cells transformed at the same time received only the *URA3* gene. Resulting colonies were PCR-confirmed with primers shown in Supplemental Table S1 to contain the appropriate gene.

Library preparation and RNAseq sequencing. RNA extracted from treated *R. irregularis* spores was quantified using a Nanodrop fluorospectrometer (ThermoFisher Scientific Inc., Villebon sur Yvette, France) and quality was determined by electrophoresis on an Agilent Bioanalyzer (Agilent Technologies, Les Ulis Cedex, France). Samples with RNA Integrity Number (RIN) higher than eight were selected. RNAseq libraries were constructed as previously described [21]. The libraries were prepared according to Illumina's protocols using the Illumina

TruSeq RNA Sample Prep Kits v2 (Illumina Inc., San Diego, USA). Polyadenylated RNA was purified from at least 1µg of total RNA using beads containing oligo-dT. Subsequently, each mRNA sample was fragmented to generate double stranded cDNA for sequencing. Double-stranded cDNA was synthesized using SuperScript II Reverse Transcriptase (Life Technologies, Saint Aubin, France) and random primers for first strand cDNA synthesis followed by second strand synthesis using DNA Polymerase I and RNaseH (ThermoFisher Scientific Inc., Villebon sur Yvette, France) for removal of mRNA. Double-stranded cDNA was purified using Agencourt AMPure XP beads (Beckman Couter Inc., Villepinte, France) as recommended in the TruSeq RNA Sample Prep Guide. cDNAs were end-repaired by T4 DNA polymerase and Klenow DNA Polymerase, and phosphorylated by T4 polynucleotide kinase. The blunt ended cDNA was purified using Agencourt AMPure XP beads (Beckman Couter Inc., Villepinte, France). The cDNA products were incubated with Klenow DNA Polymerase (ThermoFisher Scientific Inc., Villebon sur Yvette, France) to add an 'A' base (Adenine) to the 3' end of the blunt phosphorylated DNA fragments and then purified using Agencourt AMPure XP beads. DNA fragments were ligated to Illumina adapters with a single 'T' base (Thymine) overhang at their 3'ends. Adapter-ligated products were purified using Agencourt AMPure XP beads and amplified in a Linker Mediated PCR reaction (LM-PCR) for 12 cycles using Phusion^{TM} DNA Polymerase and Illumina's PE genomic DNA primer set followed by purification using Agencourt AMPure XP beads. Size selection (ranging from 200-300 bp) was performed on E-gel (ThermoFisher Scientific, Villebon-sur-Yvette, France). Quality of finished libraries was assessed using an Agilent Bioanalyzer (Agilent Technologies, Les Ulis Cedex, France). Libraries were quantified by qPCR using the KAPA Library Quantification Kit (PN11 KK4824- KAPA Biosciences, Nanterre, France) to obtain an accurate quantification.

GlcNAc transport assays in *C. albicans* and *A. thaliana*: *C. albicans* GlcNAc uptake and

competition assay were performed as previously described [7]. Transport experiments using protoplast were performed by a slight modification of a previously described method [25]. Specifically, the abaxial epidermis of leaves from 6- to 8-week-old plants were abraded with P500 sandpaper, and immediately floated on medium A, containing 500 mM sorbitol, 1 mM CaCl2, and 10mM MES-KOH, pH 5.6, supplemented with 1 mg ml^{-1} BSA in petri dishes. Subsequently, leaves were incubated for 2 h at 30°C with their abaxial side on medium A containing 10 mg ml^{-1} cellulase R10 and 5 mg ml^{-1} macerozyme R10 (Serva Electrophoresis, Heidelberg, Germany). The suspensions with released protoplasts were collected into 50 ml Falcon tubes, each of which was underlied with 2 ml of Percoll solution, pH 6: 500 mM sorbitol, 1mM $CaCl_2$, and 20 mM MES in 100% Percoll (GE Healthcare, Glattbrugg, Switzerland). After centrifugation at 1500 g for 8 min at 4°C, the supernatant was aspired and the concentrated protoplasts were resuspended in the remaining solution. This solution was adjusted with medium A or Percoll solution to give a final Percoll concentration of about 35%. The protoplasts were overlaid with medium A containing 25% Percoll and medium A containing 2% Percoll. After centrifugation for 8 min at 1,200 g, the protoplasts were recovered from the upper interphase. The purified protoplasts were mixed with 2 parts of medium B: 500 mM betaine, 1 mM $CaCl_2$, and 10 mM MES-KOH, pH 5.6. For transport experiments, 50µl of medium A containing 50% Percoll was placed at the bottom of 400 µL polyethylene tubes and overlaid with 200µl of silicon oil AR200. Transport experiments were initiated by adding the substrate to protoplasts and terminated by overlaying 100 µL of the protoplast suspension on the preformed silicon oil gradients and a subsequent 20 s centrifugation at 10,000 g at the times indicated . The polyethylene tubes were frozen overnight. The bottoms of the frozen tubes were cut with a razor blade and the bottom of the tube was transferred to the scintillation liquid and counted directly. GlcNAc uptake values was estimated based on five biological replicates per genotype.

Statistical analysis: Biological replicates corresponded to individual plants of independently grown experiments. In experiments to evaluate the level of fungal, differences among genotypes were assessed per structure using the non-parametric Kruskal-Wallis test and *post hoc* Dunn test to assign means groups on the basis of pair wise comparisons (R statistics dunn.test::dunn.test; Dinno, 2016). To control for multiple testing, p-values were adjusted using the method of Benjamini and Hochberg (Benjamini and Hochberg, 1995) and significant differences called at $p < 0.05$. In GlcNAc and transcript accumulation experiments, differences among genotypes were assessed using ANOVA (R statistics; R Core Team, 2016) and *post hoc* means groups assigned using Tukey HSD (R statistics agricolae::HSD.test; de Mendiburu, 2016; $p < 0.05$).

Alexis Dinno (2016). dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package, version 1.3.2. https://CRAN.R-project.org/package=dunn.test; accessed June 2016. *de Mendiburu F. 2016.* agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-4. https://CRAN.R-project.org/package=agricolae; accessed June 2016. *R Core Team. 2016.* R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/; accessed June 2016.

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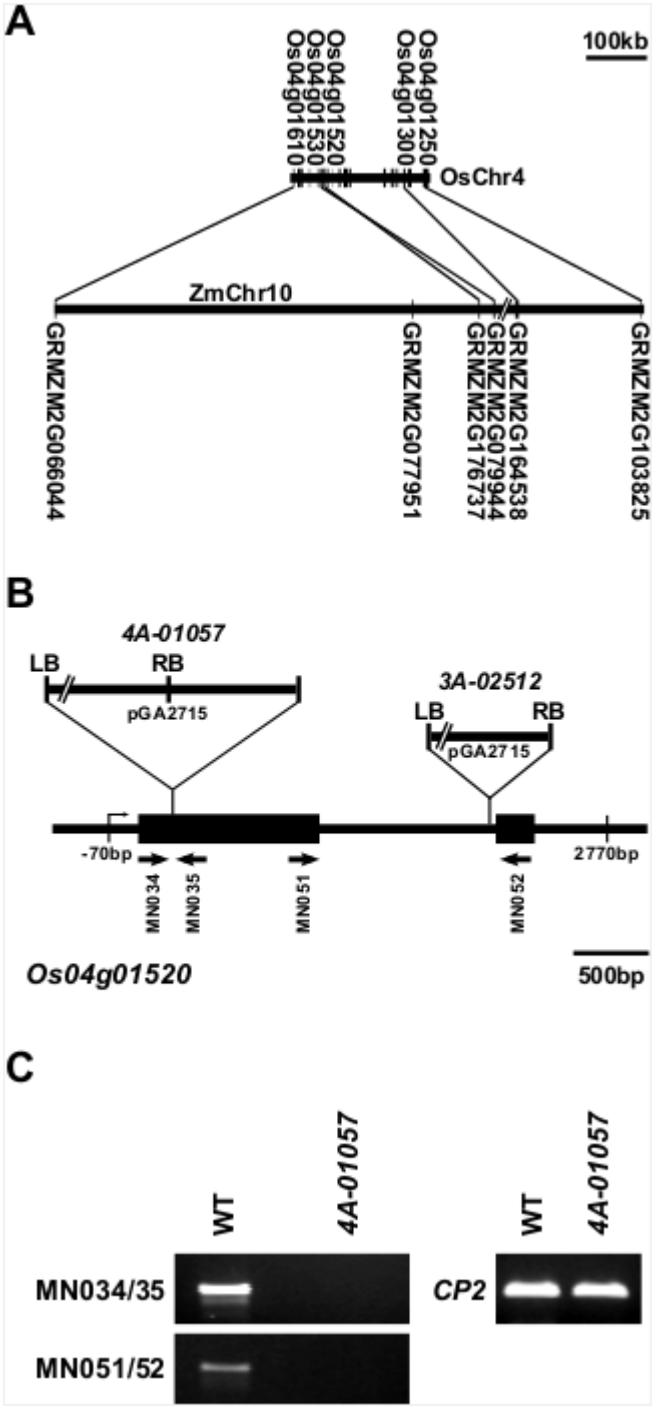
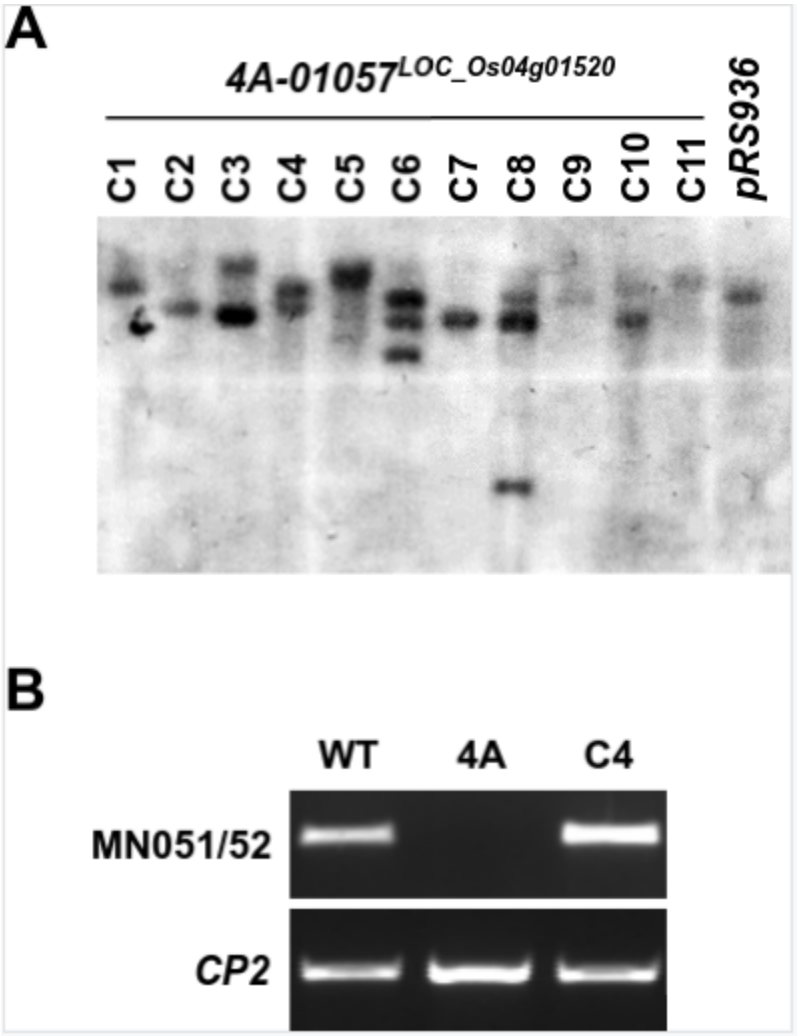
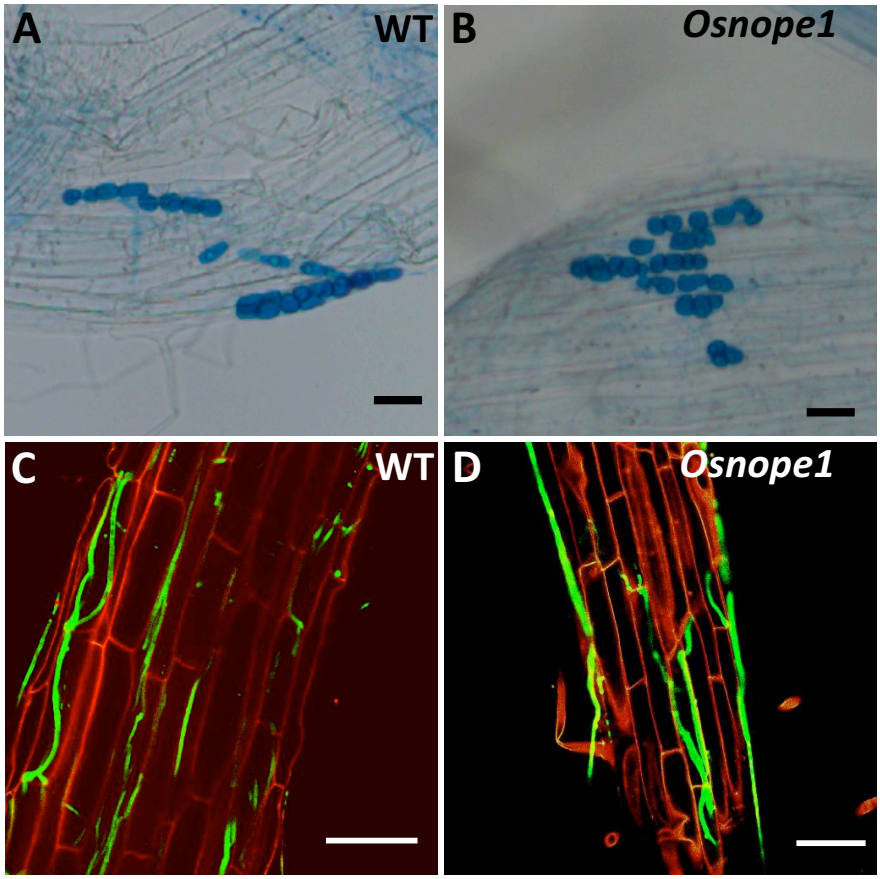
Supplemental Figure S1. **Identification of syntenic rice and maize *NOPE1* candidate genes. A**, Physical map of the *nope1* interval in syntenic regions of rice chromosome 4 (OsChr4) and maize chromosome 10 (ZmChr10). Maize and rice shown on a common scale with arrows indicating orientation. Double bar indicates a break in the representation of the maize chromosome. Annotated gene models are shown by bars and orthologous pairs connected with a dotted line. Only those rice genes assigned orthologs in the maize region are labeled by name. Maize and rice *NOPE1* homologs are indicated (underline). **B**, Structure of the rice gene *LOC_Os04g01520* and position of T-DNA insertions present in the lines in lines *4A-01057* and *3A-02512*. Sites of transcriptional initiation and termination are indicated relative to the start of translation, at -70bp and 2770bp, respectively. Arrows represent primers used in **C,** RT-PCR-based analysis of *LOC_Os04g01520* transcript levels in wild type (WT)and *4A-01057*. LB, left border; RB right border; *CP2*, *CYCLOPHILLIN (LOC_Os02g02890)*.



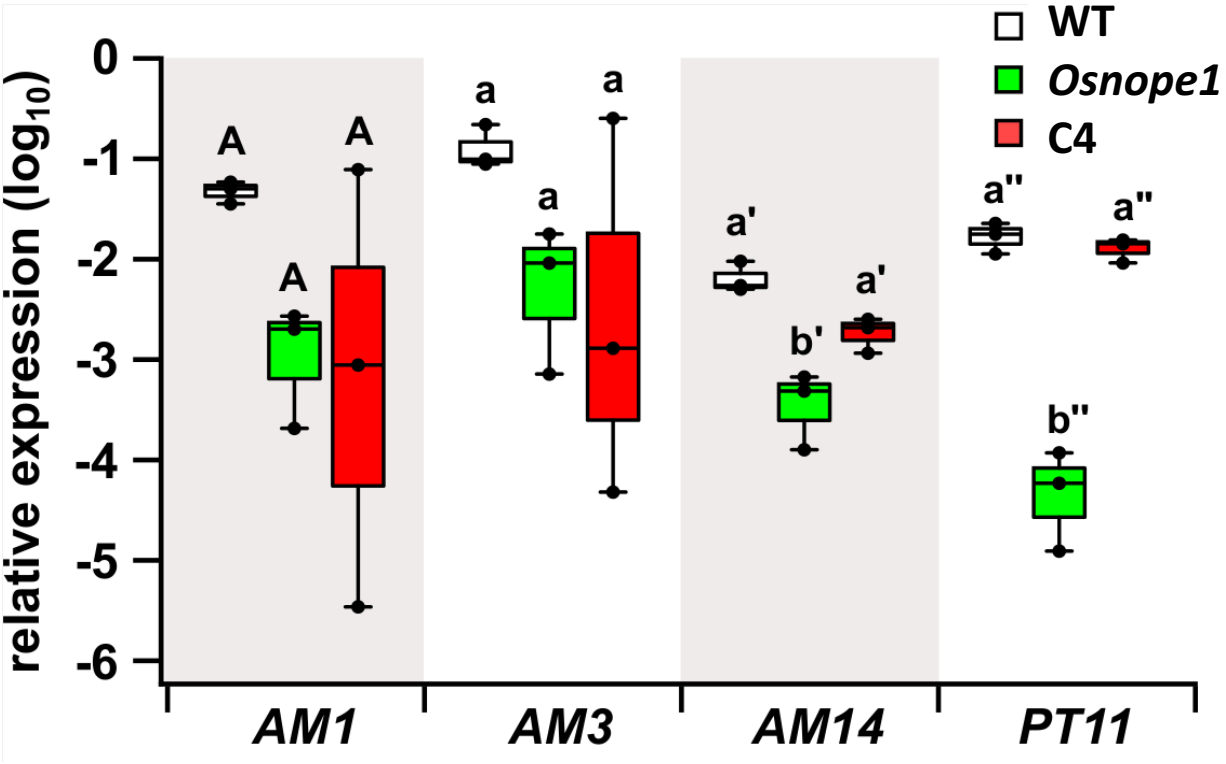
Supplemental Figure S2. **Genetic complementation of *4A-01057* with *LOC-Os04g01520*. A,** Southern blot analysis of independent *4A-01057*$^{LOC\_Os04g01520}$ transformants; the cDNA corresponding to the Basta resistance gene was used as a probe and plasmid pRS936 served as a positive control. **B**, Representative RT-PCR-based analysis of *LOC_Os04g01520* transcript levels in *4A-01057*$^{LOC\_Os04g01520}$ line C4 relative to wild type and *4A-01057* mutant. Primer location as indicated in Figure 1B. *CP2*, *CYCLOPHILLIN* (*LOC_Os02g02890*).



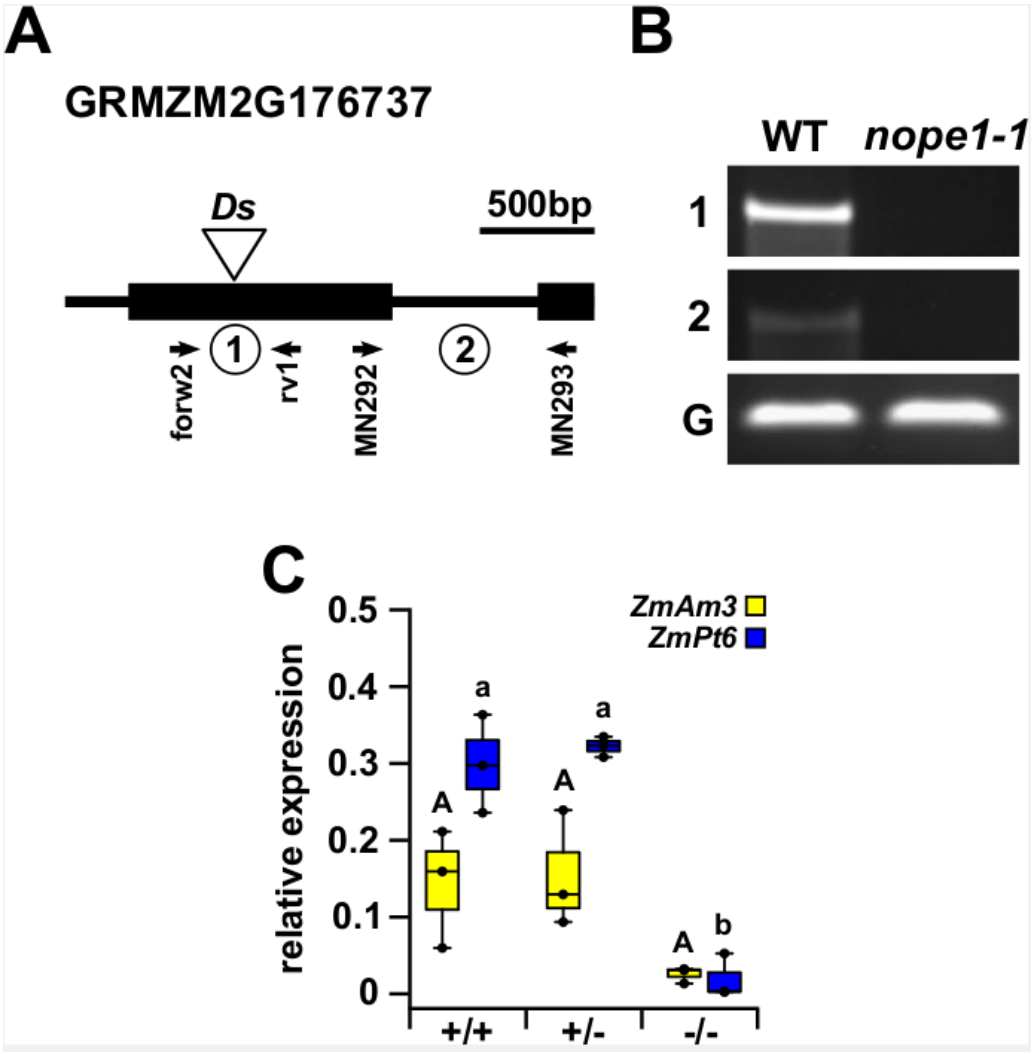
Supplemental Figure S3. Colonization of rice *nope1* roots by *Piriformospora indica* and *Magnaportha oryzae*. **A** and **B**, Trypan blue staining of wild type (**A**) and *nope1* (**B**) roots colonized by *P. indica* at 5wpi. **C** and **D**, Laser scanning confocal microscopy images of wild type (**C**) and *nope1* (**D**) roots infected with *M. oryzae* expressing GFP at 5 days post inoculation. Cell walls were counter-stained with propidium iodide (red) . Scale bars = 50μM.



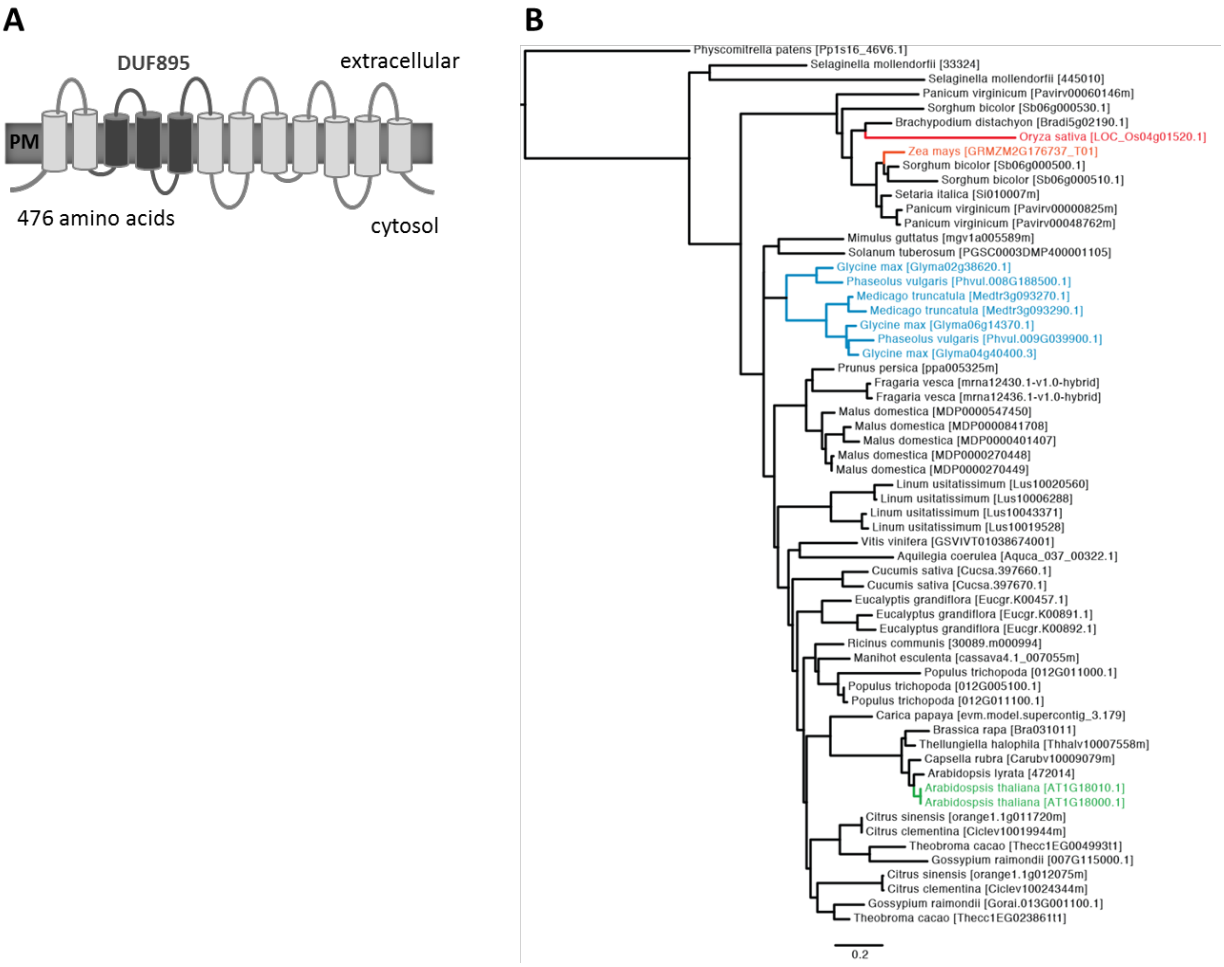
Supplemental Figure S4. qRT-PCR-based analysis of *AM1*, *AM3*, *AM14*, and *PT11* marker transcript accumulation in the roots of wild type, O*snope1-1*, and the complemented line C4, at 6 wpi with *R. irregularis*. Expression values were normalized against *CYCLOPHILLIN* (*LOC_Os02g02890*). Points represent individual plants. Means groups were calculated *post hoc* independently for each transcript and are indicated by letters ($p < 0.05$).



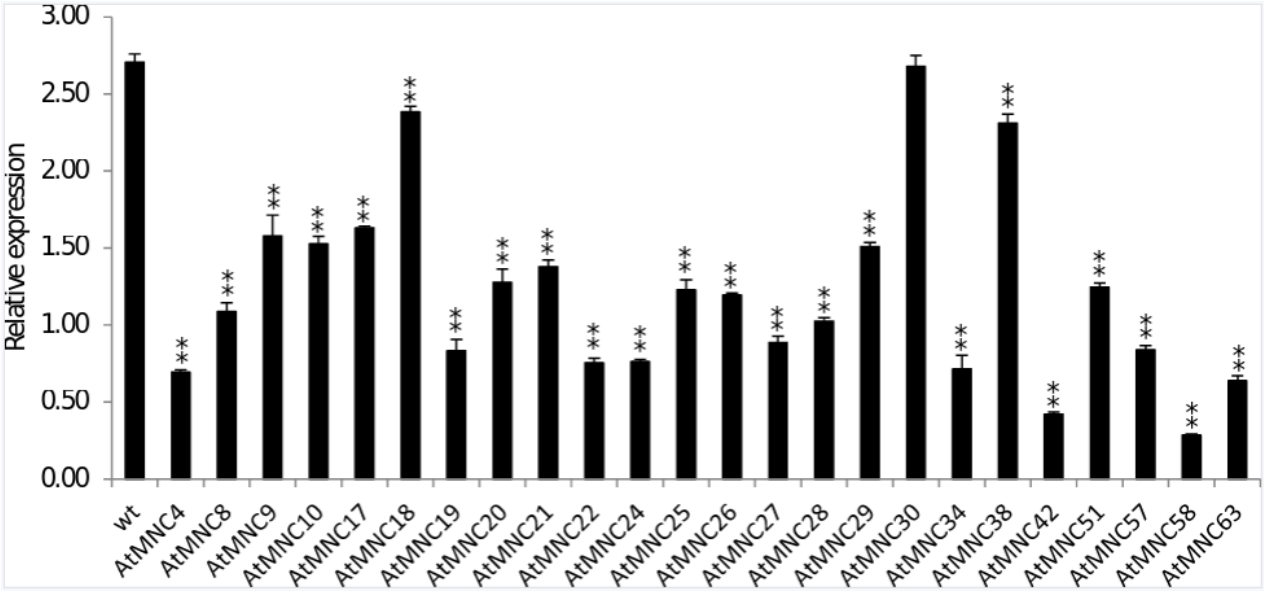
Supplemental Figure S5 A, *GRMZM2G176737* gene structure and position of *Ds* transposon insertion (triangle). Arrows indicate position of primers used in **B**; forw2, ZmLph2-forw2; rv1, ZmLph2-rv1 B, RT-PCR analysis of *GRMZM2G176737* transcript accumulation in the roots of plants homozygous for the *Ds* insertion *GRMZM2G176737::Ds* using primers flanking (1) and downstream (2) of the insertion site (reaction 1); *GAPDH, GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE* (*GRMZM2G046804*). **C**, qRT-PCR-based analysis of transcript accumualtion of *ZmAM3* (*GRMZM2G135244*) and *ZmPT6* (*GRMZM5G881088*) in maize plants segregating *GRMZM2G176737::Ds* at 6wpi with *R. irregularis*. Expression values were normalized against *GAPDH*. Points indicate individual plants. Means groups were calculated *post hoc* independently for the two transcripts and are indicated by letters ($p < 0.05$).



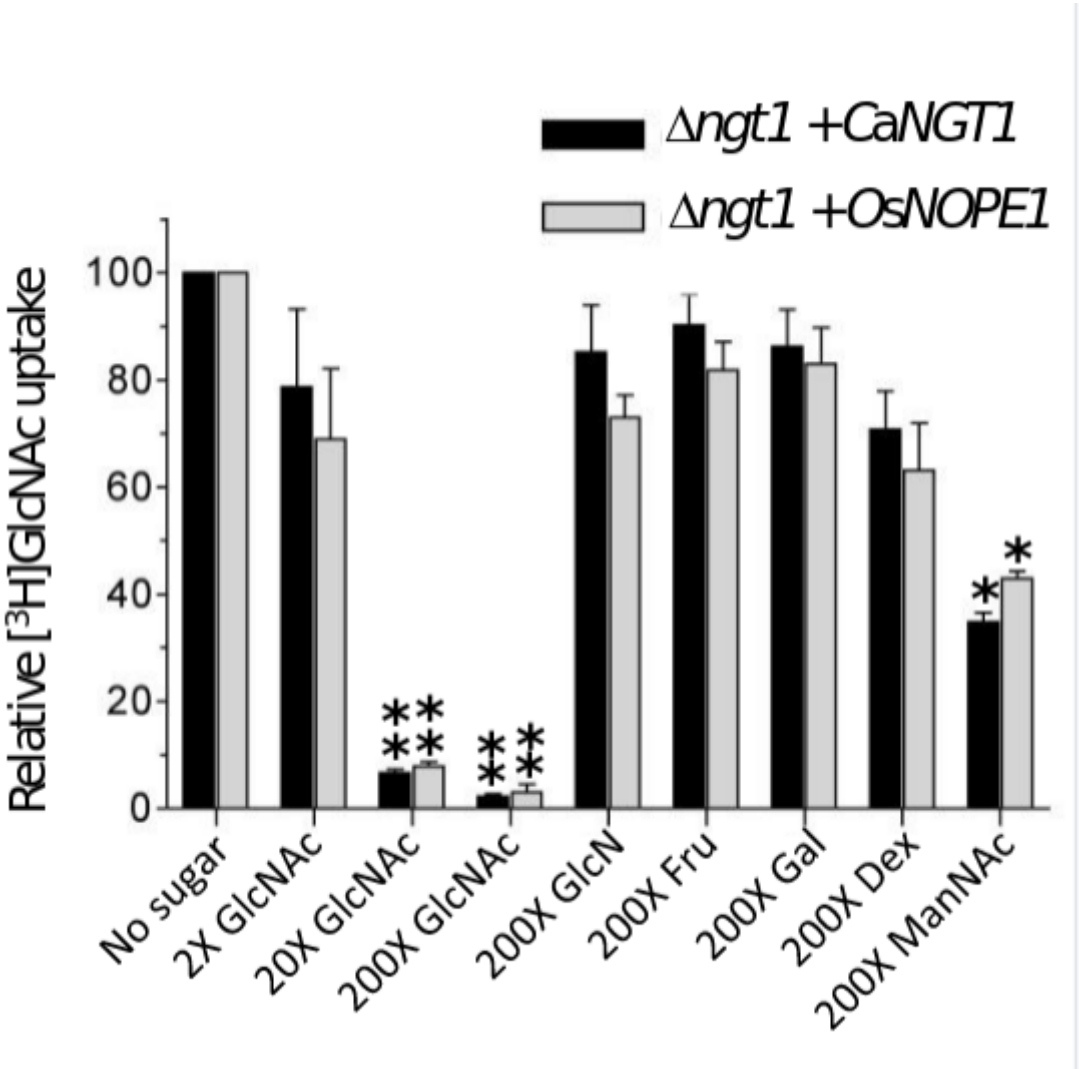
Supplemental Figure S6. NOPE1 belongs to the Major Facilitator Superfamily and orthologs occur in mycorrhizal and non-mycorrhizal plant species. **A**, Predicted OsNope1 topology with 12 transmembrane-spanning domains and DUF895 in N-terminal region; DUF895, Domain of Unkonwn Function895. **B**. Phylogenetic distribution of NOPE1 orthologs across the plant kingdom. Rice (red), maize (orange), legume (light blue) and Arabidopsis (green) orthologs are indicated.



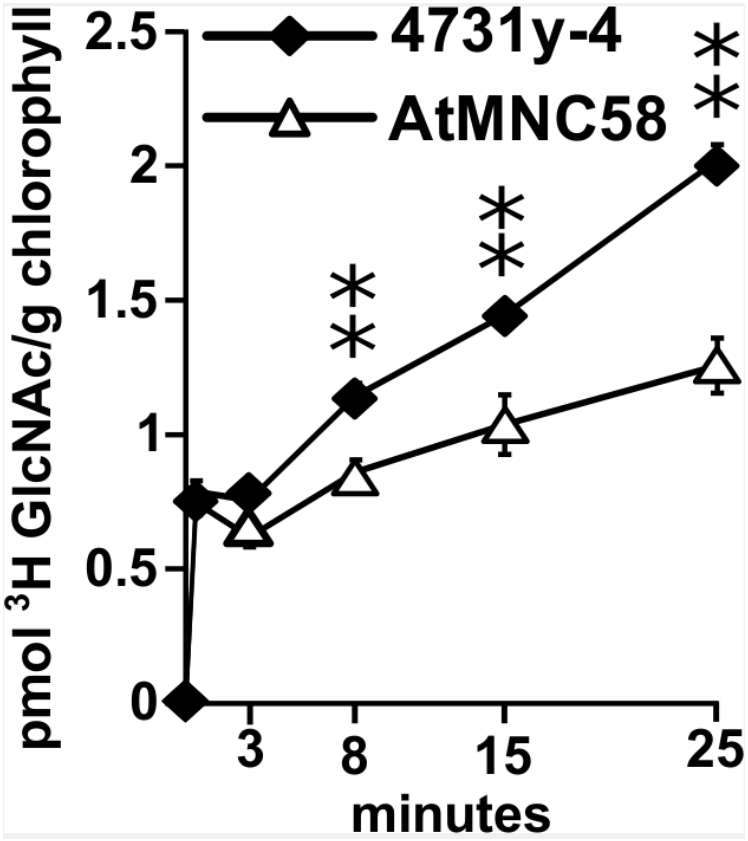
Supplemental Figure S7. Characterization of endogenous transcript levels of *AtNope1a* and *b* in *AtNope1*-RNAi lines. qRT-PCR-based analysis of endogenous mRNA levels in cauline leaves of *A. thaliana NOPE1* lines transformed with RNAi silencing construct pMN010. As both genes share identical sequences in the region complementary to the RNAi trigger, one primer pair allowed for the examination of endogenous transcript levels. Lines *AtMNC42* and *AtMNC58* showed the strongest *AtNope1* down-regulation and were selected for further experiments. Gene expression levels were normalized against the *IPP2, ISOPENTENYL-DIPHOSPHATE DELTA-ISOMERASE II* (*At3g02780*). Bars represent means of three technical replicates, error bars = s.e. Asterisks indicate lines with siginifcant *AtNope1*down-regulation (Tukey HSD, $^{**}P \leq 0.001$).



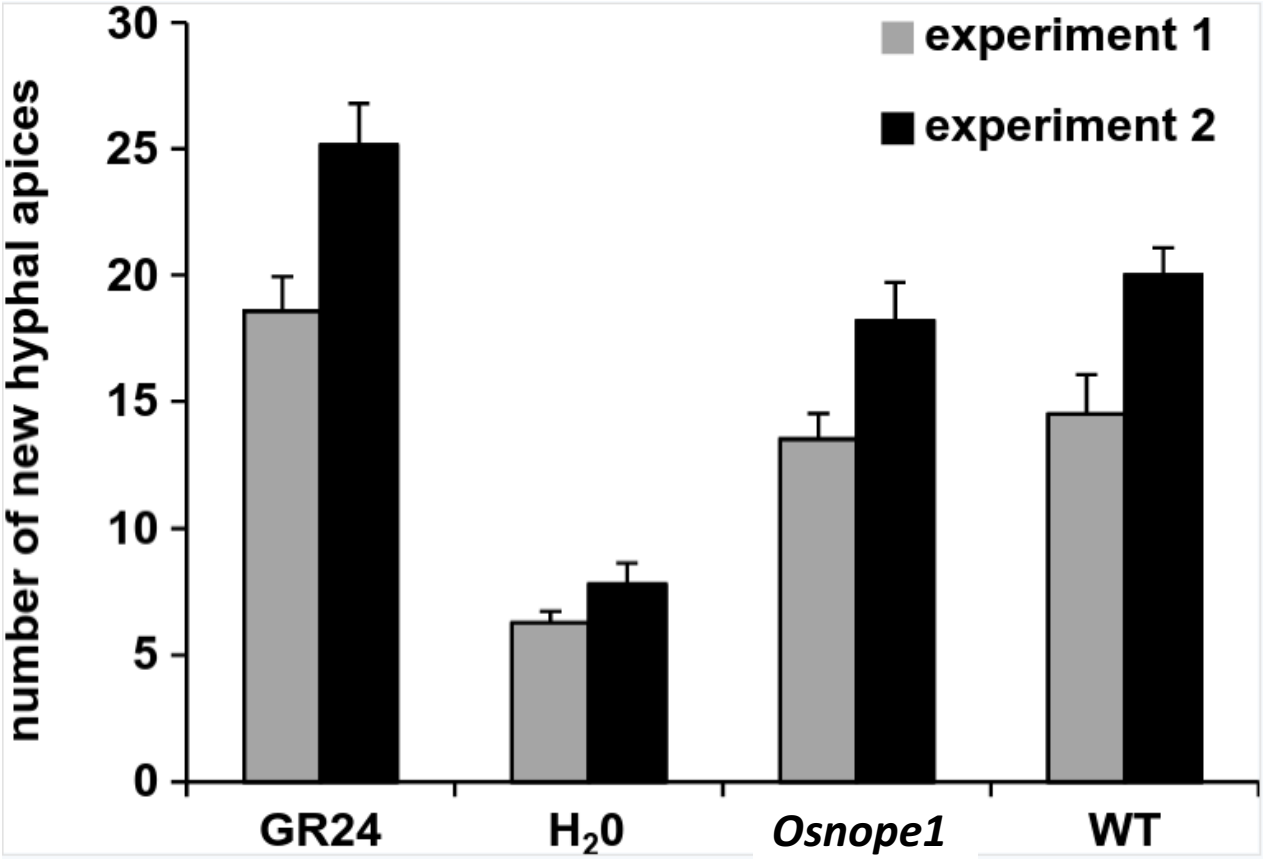
Supplemental Figure S8. OsNope1 exhibits specificity for GlcNAc in *C. albicans*. Uptake of [^{3}H]GlcNAc in *C. albicans* strains in the presence of 2 mM or 20 mM of indicated nonradioactive sugars. For each strain Kruskal-Wallis tests were performed to compare sugars with the no sugar control. Asterisks indicate statistical significance with respect to the no sugar treatment (Tukey HSD),**P* (20X and 200X GlcNAC) ≤ 0.001; ***P* (200X ManNAC) ≤ 0.05. GlcNAc, N-acetyl glucosamine; GlcN, glucosamine; Fru, fructose; Gal, galactose; Dex, dextrose; ManNAc, N-acetyl mannosamine. Means and SEs of at least four biological replicates.



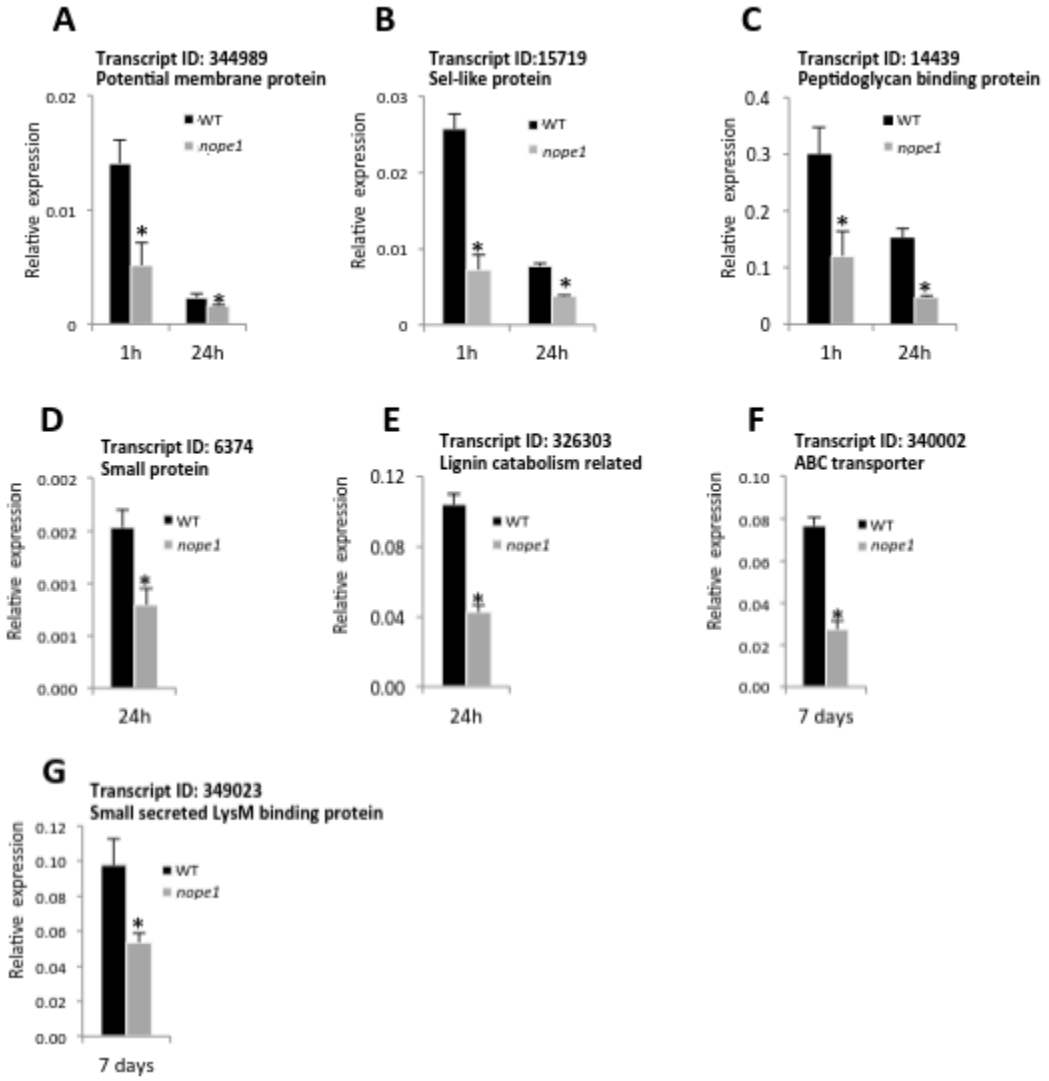
Supplemental Figure S9. Time course of [^{3}H]GlcNAc uptake in protoplasts of *A. thaliana AtNope1* overexpression (*4721y-4*) and down-regulated (*AtMNC58*) lines. Means and SEs of five biological replicates are shown. (**$P \leq 0.01$).



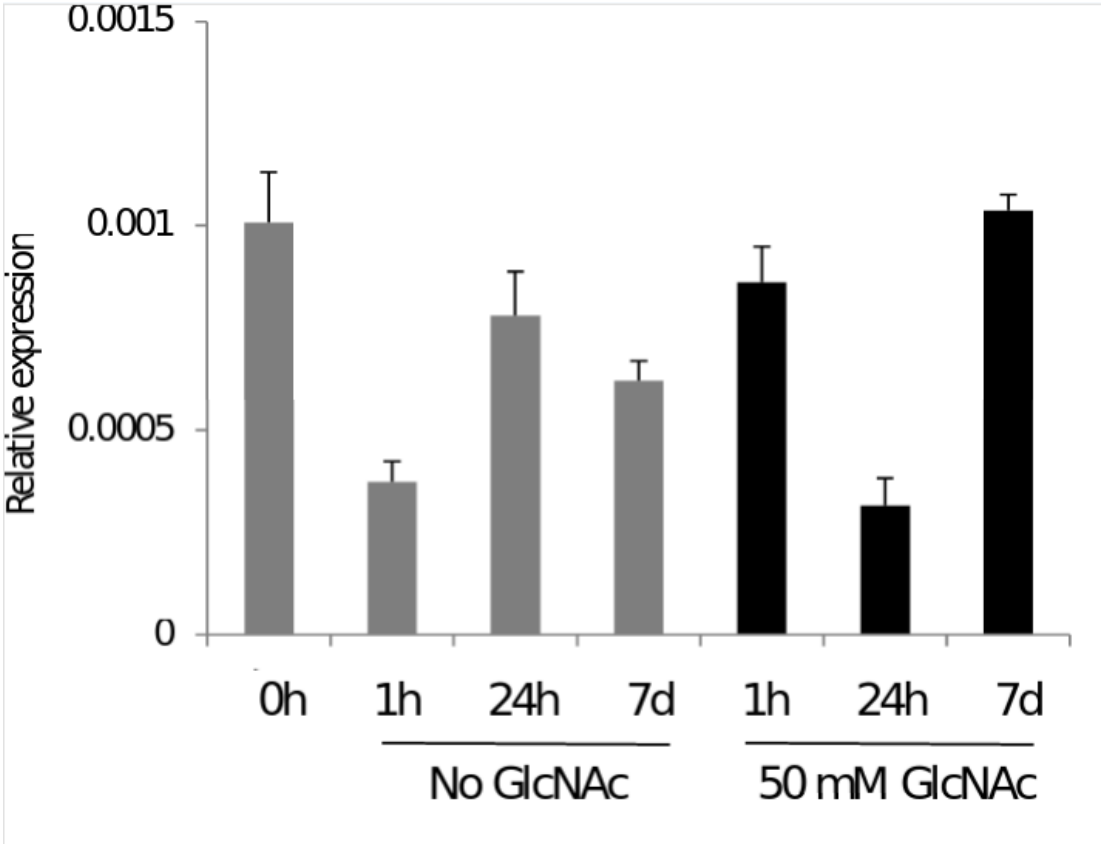
Supplemental Figure S10. Quantification of *G. rosea* hyphal apices in response to *Osnope1* and wild type root exudates. Number of new hyphal apices observed per germianted spore upon treatment with root exudates from *Osnope1-1* or wild type. Bars represent means of at least 9 spores, error bars = s.e. Two independent experiments were performed. For each one, no significant difference was observed between wt and *NOPE1* exudates treatments. Letters above bars indicate of number of new hyphae that were not significant in pair-wise comparison (Tukey (Kruskal–Wallis test, $P < 0.0001$). GR24, synthetic strigolactone.



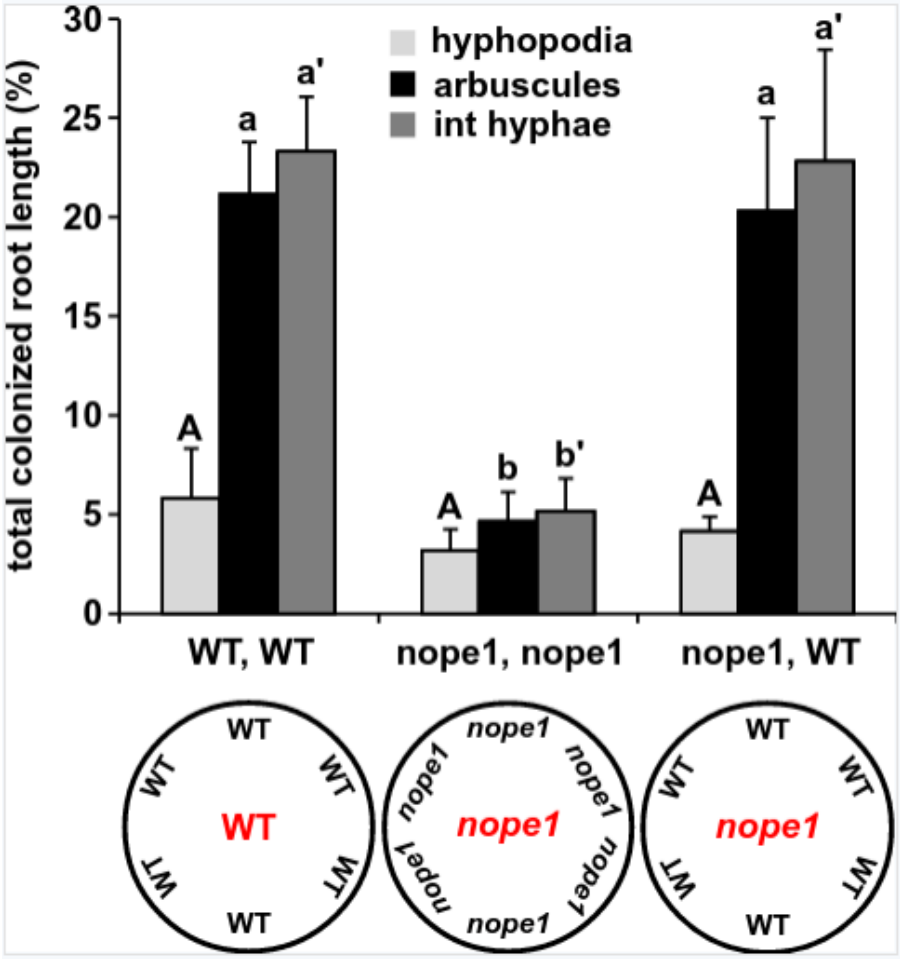
Supplemental Figure S11. qRT-PCR-based validation of representative genes identified by RNAseq analysis. Genes differentially expressed at 1h and 24h (**A-C**), at 24h (**D, E**) and at 7d (**F, G**). Gene expression levels were normalized to the *R. irregularis* housekeeping gene *ELONGATION FACTOR α, RiEF* (fgenesh1_kg.21690_#_7). Means and SEs of three biologically independent experiments are presented. (Tukey HSD *$P \leq 0.05$, one way ANOVA).



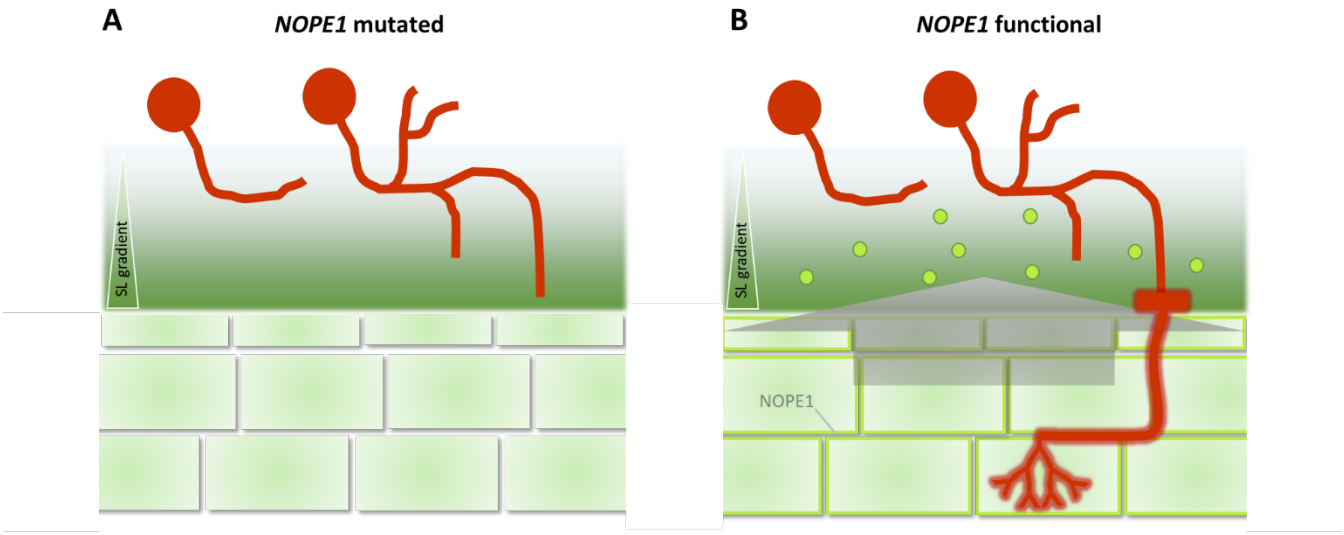
Supplemental Figure S12. *R. rhizophagus NGT1* mRNA levels in response to GlcNAc treatment. Time course qRT-PCR-based expression analysis of *RiNGT1* (MIX9501_16_76) in response to GlcNAc treatment (50mM). Gene expression levels normalized to *R. irregularis ELONGATION FACTOR α, RiEF* (fgenesh1_kg.21690_#_7). Means and SE of three biological replicates.



Supplemental Figure S13. Percentage of *R. irregularis* root length colonization at 7 wpi of 2 tester plants (central; red bold) that are surrounded by 6 donor plants (black bold). Means and SEs of five biological replicates are presented. Kruskal-Wallis tests with Benjamini-Hochberg adjustment for the post hoc tests were performed for individual fungal structures; *P* (hyphopodia) = 0.62, *P* (arbuscules, int hyphae) = 0.07. Post hoc pairwise comparisons of growth conditions that were not significantly different are indicated by letters on top of each bar. Comparisons correspond to bars with same letter case and apostrophes' number.



Supplemental Figure S14. Hypothetical model of NOPE1 function. Fungal perception of root-released strigolactones (SL) leads to increased hyphal branching and metabolic activity. The steep SL gradient adjacent to the root navigates the fungus towards the root. In the absence of functional NOPE1 (A) fungal responses to SL are intact, however efficient physical interaction is compromised. In the presence of functional NOPE1 (B) availability of a bioactive molecule in the rhizosphere conditions the fungus for symbiosis.



Supplemental Table S1: primers used for PCR analysis of mutant alleles

Primer name	Primer sequence	Used for
MN033	AGTCGGTATCGATTTGTTGGA	*OsNOPE1* genotyping (5' of T-DNA insertion)
MN034	GCCGTAATGAGTGACCGCATC	*OsNOPE1* genotyping (GUS RB)
MN035	TGGCGGGAGGGGTGGTGGTTGTAGTA	*OsNOPE1* genotyping (3' of T-DNA insertion)
MN051	AGGGCCTCAACAACGTCTTCTACT	*OsNOPE1* transcript analysis (RB exon1)
MN052	ACGGTAGTAAGCGCCCAATTCACA	*OsNOPE1* transcript analysis (LB exon2)
ZmLph2_forw2	CCTCATCTGCTTCTGCTG	*ZmNOPE1* genotyping (5' of Ds insertion)
JSR01	GTTCGAAATCGATCGGGATA	*ZmNOPE1* genotyping (Ds RB)
ZmLph2_rv1	ATCTTCCAGTTGGCGAAGAG	*ZmNOPE1* genotyping (3' of Ds insertion)
JGp3	ACCCGACCGGATCGTATCGG	*ZmNOPE1* genotyping
MN292	CCAACCAGCTCAGCTACAA	*ZmNOPE1* genotyping
MN293	GTCCTCCACCGACGAAAC	*ZmNOPE1* genotyping

Supplemental Table S2: primers used for qRT-PCR quantification of plant gene expression

Gene name	LOC_Os ID	Primer sequences
AM1	*LOC_Os04g04750*	**Fw:** ACCTCGCCAAAATATATGTATGCTATT **Rv:** TTTGCTTGCCACACGTTTTAA
AM3	*LOC_Os01g57400*	**Fw:** CTGTTGTTACATCTACGAATAAGGAGAAG **Rv:** CAACTCTGGCCGGCAAGT
AM14	*LOC_Os11g26140*	**Fw:** CCAACACCGTTGCAAGTACAATAC **Rv:** GCACTTTGAAATTGGACTGTAAGAAA
PT11	*LOC_Os01g46860*	**Fw**: GATGTTACGGATCTGGGTATATCGA **Rv**:CATATCCCAGATGAGCGTATCATG
NOPE1 (AM156)	*LOC_Os04g01520*	**Fw**: TCAGTGTGTTCCTTCCTTTGAAAG **Rv**: TTTCTATACCATGGCCCCTAAATC
CP2	*LOC_Os02g02890*	**Fw:** GTGGTGTTAGTCTTTTTATGAGTTCGT **Rv:** ACCAAACCATGGGCGATCT
ZmAM3	*GRMZM2G135244*	**Fw**: ATCTGTCGTTGCGTTCCTCT **Rv**: GCATCTATC ACTGCGGGAAT
ZmPT6	*GRMZM5G881088*	**Fw:** GATCCAGCTCATCGGTTTCT **Rv**: GAGCGTGGTGTGTTTGTTCT
GAPDH	*GRMZM2G046804*	**Fw:** CTTCGGCATTGTTGAGGGTTTG **Rv:** TCCTTGGCTGAGGGTCCGTC
AtNOPE1	*At1g1800/At1g1810*	**Fw:** TCGCCATTGGTTACGTGATGGACT **Rv:** ACACGAATGGTCCCGCAAACTCTA
IPP2	*A13g02780*	**Fw:** GTATGAGTTGCTTCTCCAGCAAAG **Rv:** GAGGATGGCTGCAACAAGTGT

Supplemental Table S3: primers used for qRT-PCR quantification of fungal gene expression

Gene product	Gene ID (JGI)	Primer sequences
RiNOPE1	MIX9501_16_76	**Fw:** GACGGGTCAGGGTGCTAT **Rv:** GCGATTGGAATCAAACTTCC
RiEF	fgenesh1_kg.21690_#_7	**Fw:** TGTTGCTTTCGTCCCAATATC **Rv:** GGTTTATCGGTAGGTCGAG
Protein ID: 344877	fgenesh1_kg.15196_#_1	**Fw:** GTTGAAACTCCACCACCACC **Rv:** TGTTGCACGACAGGTTCAAT
Protein ID: 15607	gm1.15607_g	**Fw**: CCTGAATGGGAGCATGTCGT **Rv**: AATGTTGGTACTTGCGCCTG
Protein ID: 14327	gm1.14327_g	**Fw**: AAGAGACTTGGTTTCCGGGT **Rv**: AAACCGCCAACTTCTACGAT
Protein ID: 6262	gm1.6262_g	**Fw:** TCCTACCCTTGGAGAAGCTAT **Rv:** ACCTCCAATAGTTGCCGCTA
Protein ID: 326191	MIX7972_36_14	**Fw**: CTCGTGTTATGCCTGACAGC **Rv**: ACTGGGTTGAACGAGGGATT
Protein ID: 339890	fgenesh1_kg.7458_#_1	**Fw:** ACTCGTGATGATCCAACTGG **Rv**: GTAGCTTCCCAACGACGTTC
Protein ID: 348911	fgenesh1_kg.22910_#_1	**Fw:** CGCGTGAATCAGGAGAATGG **Rv:** CCCGTAGCAGATAGGCCTTT

Supplemental Table S4: primers used for producing constructs for plant transformation.

Objective	Final vector	Sequence	Primer sequence
Genetic complementation	pTF101	*NOPE1*	**RS915:** AGATCTTGGAATCATGGAATGCCATTTG **RS916:** TCAGTGGAAGCTGGTTGGTGCAGA
		NOS terminator	**RS976:** CATATGCGTTCAAACATTTGGCAATAAAG **RS976:** GAGCTCCCCGATCTAGTAACATAGATGACAC
AtNOPE1 silencing	pB7GWIWG2-II	*AtNOPE1*	**MN042:** AAAAAGCAGGCTTATGAATGTGAGAGACGAGGGA **MN043:** AGAAAGCTGGGTTAGCAGCGGTTGGATCTACTT

Supplemental Table S5: *Candida albicans* strains used

Strain	Parent	Genotype
DIC185	BWP17	*ura3Δ::limm434/URA3 his1::hisG/ HIS1 arg4::hisG/ ARG4*
YJA2	BWP17	*ngt1Δ::ARG4/ngt1Δ::HIS1 ura3D::limm434/ ura3D::limm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*
SN956	YJA2	*ngt1Δ::ARG4/ngt1Δ::HIS1::CaNGT1-URA3 ura3D::limm434/ ura3D::limm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*
SN958	YJA2	*ngt1Δ::ARG4/ngt1Δ::HIS1::OsNGT1-URA3 ura3D::limm434/ ura3D::limm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*
SN960	YJA2	*ngt1Δ::ARG4/ngt1Δ::HIS1 ura3D::limm434/ ura3D::limm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG RPS10::P_{TDH3}-$ACT1_{term}$-URA3*