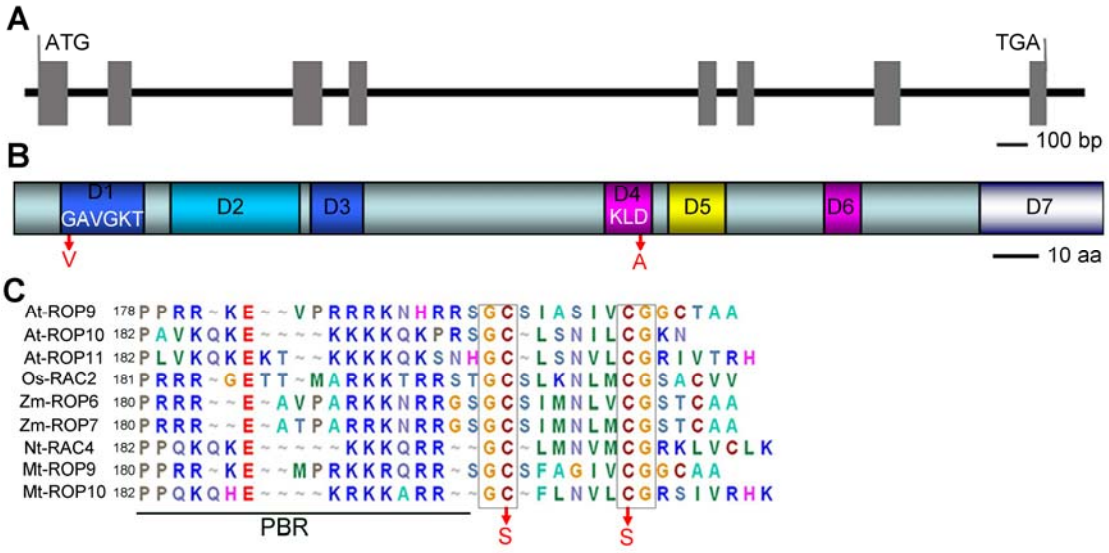
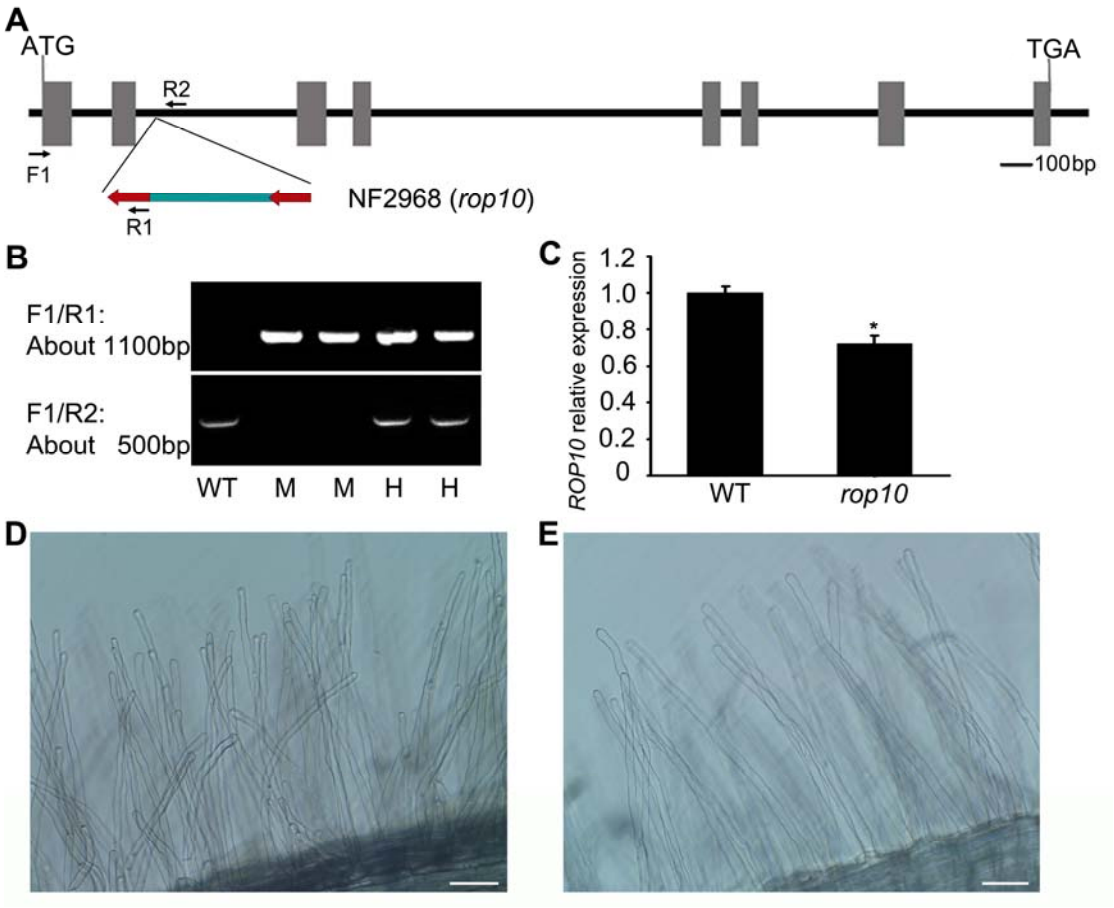


Supplemental Figure 1. Structure analysis of *M. truncatula ROP10.*
(A) *ROP10* gene structure. Translated regions of exons are indicated as gray boxes, introns and other non-coding regions as boldface lines.
(B) Schematic drawing of the ROP10 protein with GTPase domains (D1 and D3), GDP/GTP-binding domains (D4 and D6), effector/regulatory domain (D2), RHO insert region (D5), and membrane localization domain (D7). Constitutive active (CA) mutants of ROP10 are locked in the GTP-bound form by mutation of glycine (G) to valine (V) at position 17, whereas dominant negative (DN) mutants are locked in the GDP-bound form by mutation of aspartate (D) to alanine (A) at position 123.
(C) Alignment of membrane targeting/partitioning domains of type-II ROPs from different plant species. The conserved GC-CG motif is boxed in black and the polybasic region (PBR) is underlined. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Zm, *Zea mays*; Nt, *Nicotiana tabacum*; Mt, *M. truncatula*. The cysteine (C) residues of ROP10 at positions 203 and 197 were mutated to serine (S) individually or simultaneously.



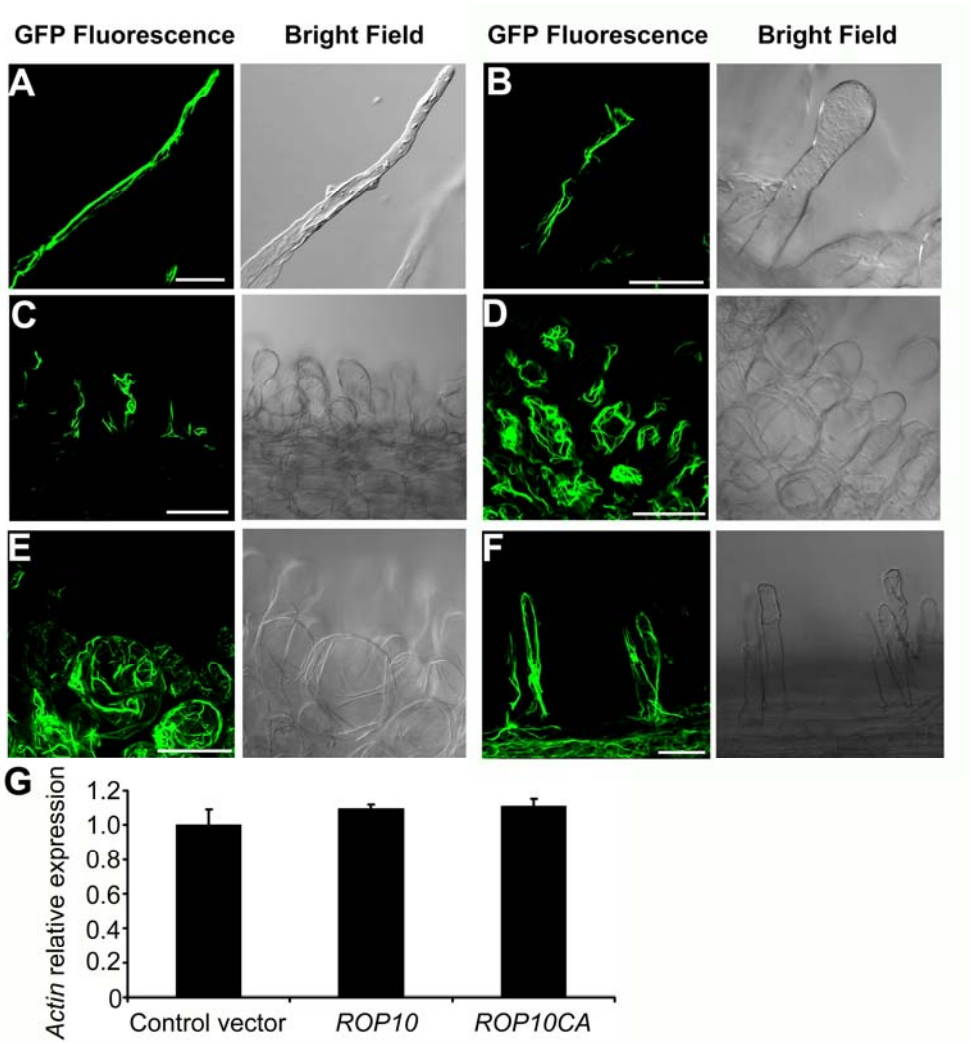
Supplemental Figure 2. Identification and phenotypic characterization of the *rop10* mutant.

(A) *ROP10* gene structure and the *Tnt1* insertion site. Exons are indicated by boxes and introns by boldface lines. The orientations of *Tnt1* are indicated.

(B) PCR identification of homozygous lines of the *Tnt1* insertion mutants. The wild type (WT, R108) plant has only a gene-specific band, whereas the homozygous lines (M) have only a *Tnt1*-DNA–gene specific band and the heterozygous lines (H) have two bands.

(C) qRT-PCR analyses of transcripts accumulation in *rop10* mutant and WT. *ACTINB* was used as a reference control gene. Statistical significance (*P < 0.05) was evaluated by Student's *t* test. Error bars indicate SE. Data presented are a representative of three independent experiments.

(D) and **(E)** The growth of root hairs of the WT **(D)** and the homozygous *rop10* mutant **(E)**. Bars=100 μm.



Supplemental Figure 3. Disorganized actin cytoskeleton in root hairs of *M. truncatula* transformed roots over-expressing *ROP10* or *ROP10* mutants.

(A) Root hairs transformed with the empty vector showing a characteristic arrangement of actin filaments with long strings running longitudinally.

(B) Root hairs over-expressing *ROP10* showing transversely aligned actin filaments in a swollen root hair tip.

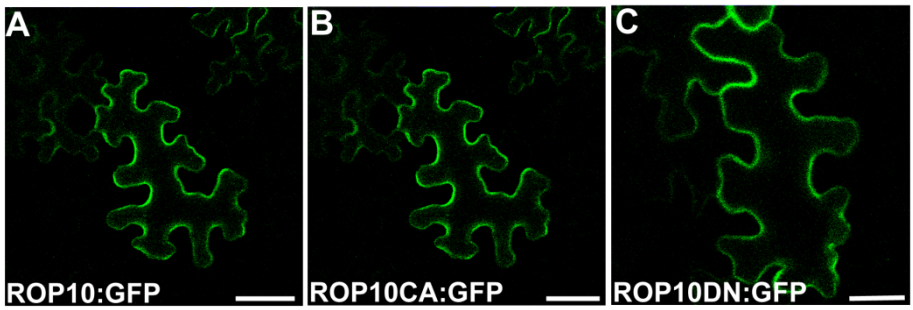
(C) Short swollen root hairs over-expressing *ROP10* showing distorted actin filaments.

(D) Ballooning root hairs over-expressing *ROP10* showing web-like aligned actin filaments.

(E) Ballooning root hairs over-expressing *ROP10CA* showing web-like aligned actin filaments.

(F) Root hairs over-expressing *ROP10DN* showing short actin filaments that are longitudinally aligned. Representative images of stained root hairs were showed. Bars=100 μm.

(G) qRT-PCR analyses of *ActinB* transcript levels in transformed roots over-expressing *ROP10* and *ROP10CA*. *EF1α* was used an internal reference. Error bars indicate SE. Data presented are representative of three independent experiments.



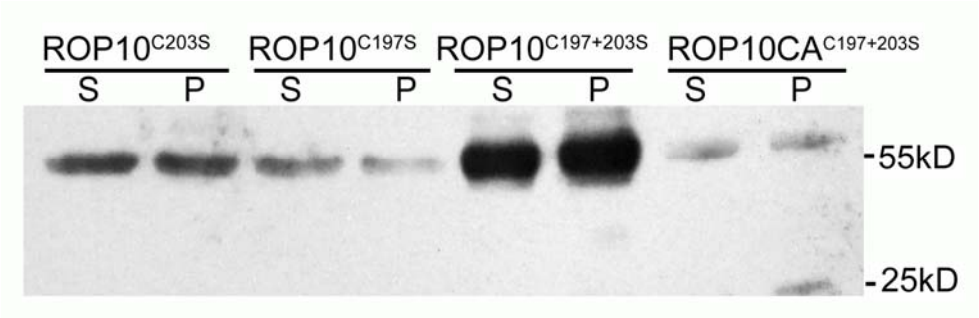
Supplemental Figure 4. Addition of 10 % (v/v) DMSO had no effect on PM localization of different GFP-fused ROP10 forms expressed in *N. benthamiana* leaf cells.

(A) Cells over-expressing ROP10:GFP.

(B) Cells expressing ROP10CA:GFP.

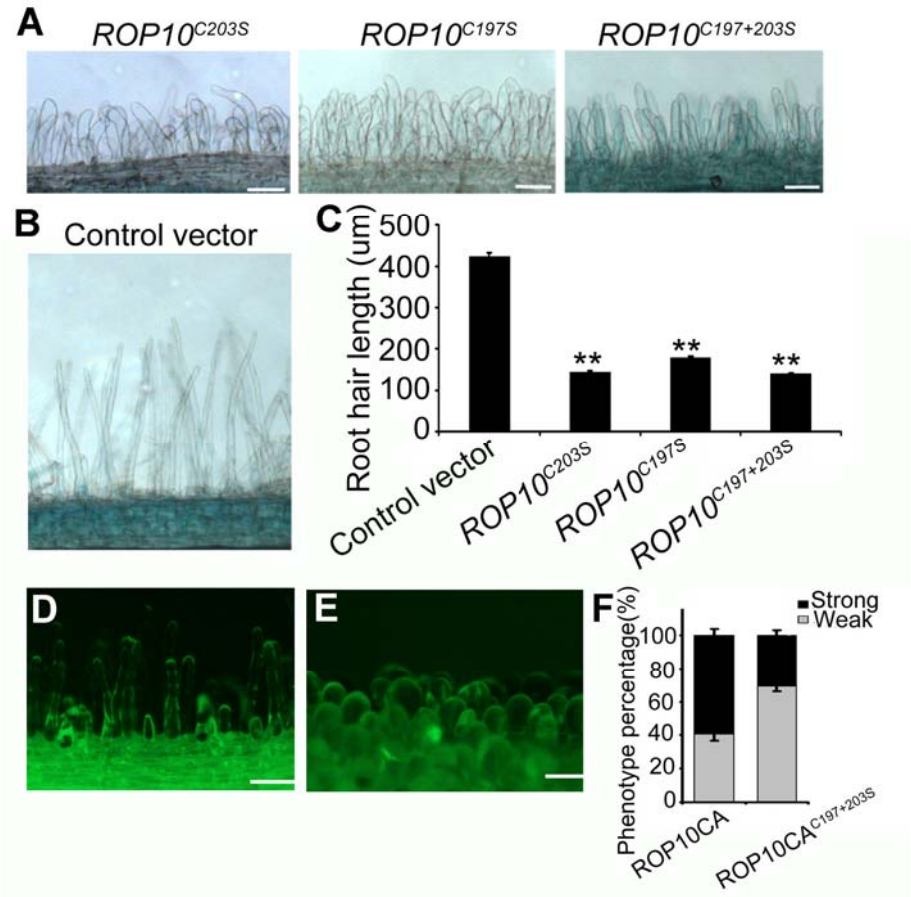
(C) Cells expressing ROP10DN:GFP.

Bars=50 μm.



Supplemental Figure 5. Immunoblot analyses of ROP10 and ROP10CA proteins with mutations of C residues in the GC-CG box.

Soluble (S) and insoluble pellet (P) membrane fractions of protein were fractionated on Suc density gradients from *N. benthamiana* leaf cells expressing ROP10^{C203S}:GFP, ROP10^{C197S}:GFP, ROP10$^{C197S+203S}$:GFP and ROP10CA$^{C197+203S}$:GFP. These mutant proteins were detected in the membrane fraction (P) and in the cytosol (S). Numbers on the right denote molecular mass in kD.



Supplemental Figure 6. Root hair phenotypes of *M. truncatula* roots over-expressing *ROP10* and *ROP10CA* mutants with mutations of C residues in the GC-CG box.

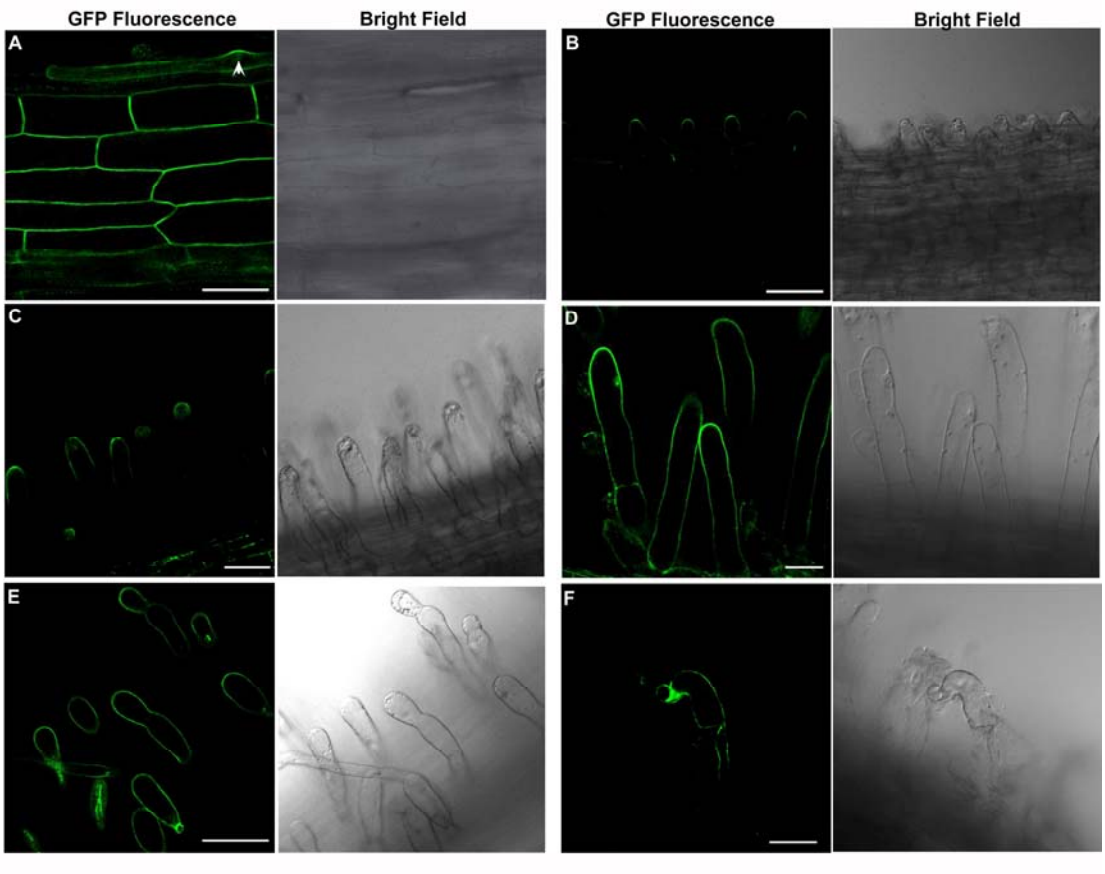
(A) Transformed roots expressing *ROP10*^{C203S}, *ROP10*^{C197S} and *ROP10*$^{C197+203S}$ generated short swollen root hairs to a lesser extent compared with over-expressing *ROP10* or *ROP10CA*.

(B) Roots transformed with the empty vector showed normal polarized tip growth of root hairs. Bars=100 μm.

(C) Length of root hairs in transformed roots expressing indicated proteins. Ten root cells were measured per transformed roots, 15 transformed roots were scored. Asterisks indicate a significant difference compared with the empty vector control by Student's *t* test (*P < 0.05; **P<0.01). Error bars are SE. This is a representative experiment that was repeated twice.

(D) and **(E)** Roots expressing *ROP10CA*$^{C197+203S}$ showed weakly swollen hairs **(D)** and strongly ballooning root hairs **(E)**. Bars=100 μm.

(F) The comparison of the rate of strong ballooning root hairs generated in transformed roots expressing *ROP10CA*$^{C197+203S}$ and *ROP10CA*.



Supplemental Figure 7. ROP10:GFP is localized at the apical PM of root hair tip and the PM of swelling tips and curling after NF treatment and rhizobial infection.

(A) ROP10:GFP is localized at the PM of root epidermal cells close to the root tip and the initial bulge (arrowhead), where root hair is formed.

(B) ROP10:GFP is localized at the PM in the apex of emerging root hairs.

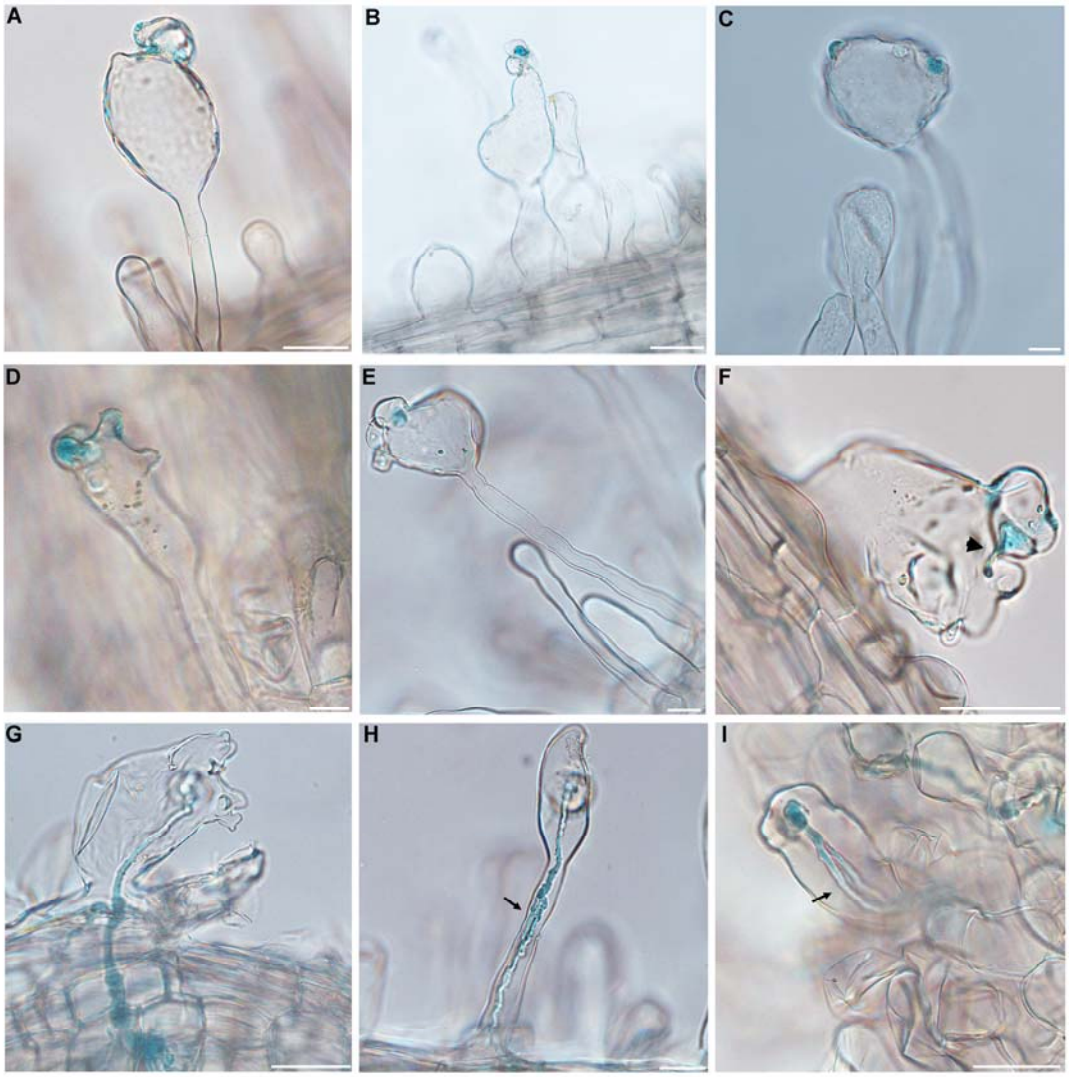
(C) ROP10:GFP is localized at the PM in the apical and subapical regions of elongating root hairs.

(D) ROP10:GFP is localized at the PM in the apical and subapical regions, and flanks of swollen root hairs.

(E) Following treatment with 1 nM NodSm-IV(C16:2, S) for 24 h, ROP10:GFP was localized at the PM of swelling tips of swollen root hairs.

(F) After 7 d post-inoculation with *S. meliloti*, ROP10:GFP was localized at the PM of a curl of a deformed root hair.

Bars=100 μm.



Supplemental Figure 8. Root hairs of *M. truncatula* expressing *ROP10CA* showed aberrant rhizobial infection.

(A) A root hair showing two outgrowths, one of them surrounded by a patch of colonies.

(B) An infection site that is not initiated the infection thread.

(C) to **(E)** Root hairs with several outgrowths that are not initiated the infection thread.

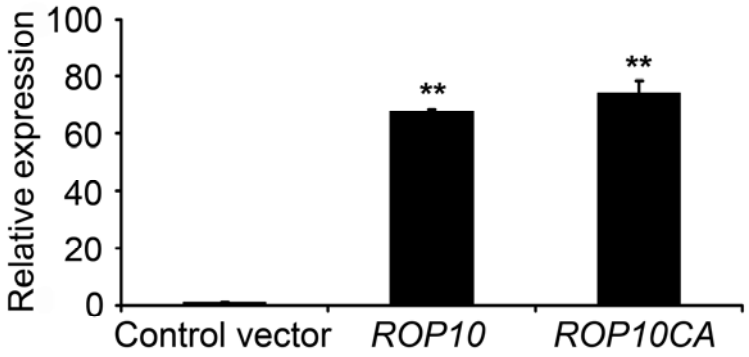
(F) An abnormal infection thread with a sac-like structure (arrowhead).

(G) Occasionally a few infection thread reached the root cortex.

(H) A new infection thread initiated from an abnormal infection thread with a sac-like structure (arrow). The infection thread reached the root cortex.

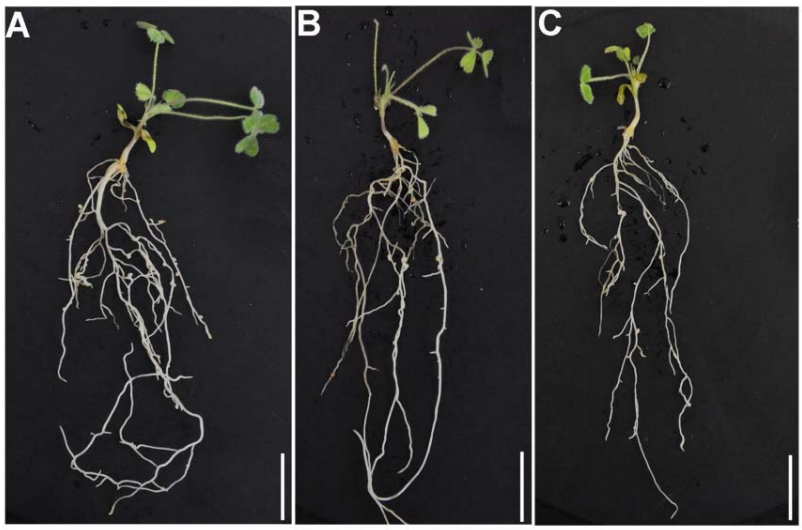
(I) A root hair with two infection threads (arrow).

Representative images of root hairs 7d post-inoculation with *S. meliloti* bacteria were shown. Bars=100 μm.



Supplemental Figure 9. qRT-PCR analysis of *ROP10* transcripts in *M. truncatula* roots over-expressing *ROP10* or *ROP10CA*.

Transformed roots over-expressing *ROP10* or *ROP10CA* were inoculated with *S. meliloti* harboring a constitutively-expressed *LacZ*. The transformed roots were collected at 7dpi for qRT-PCR analysis. The asterisks indicate a significant increase relative to the empty vector control ($P < 0.01$) as evaluated using Student's *t* test. Error bars indicate SE. Data presented are representative of three independent experiments.



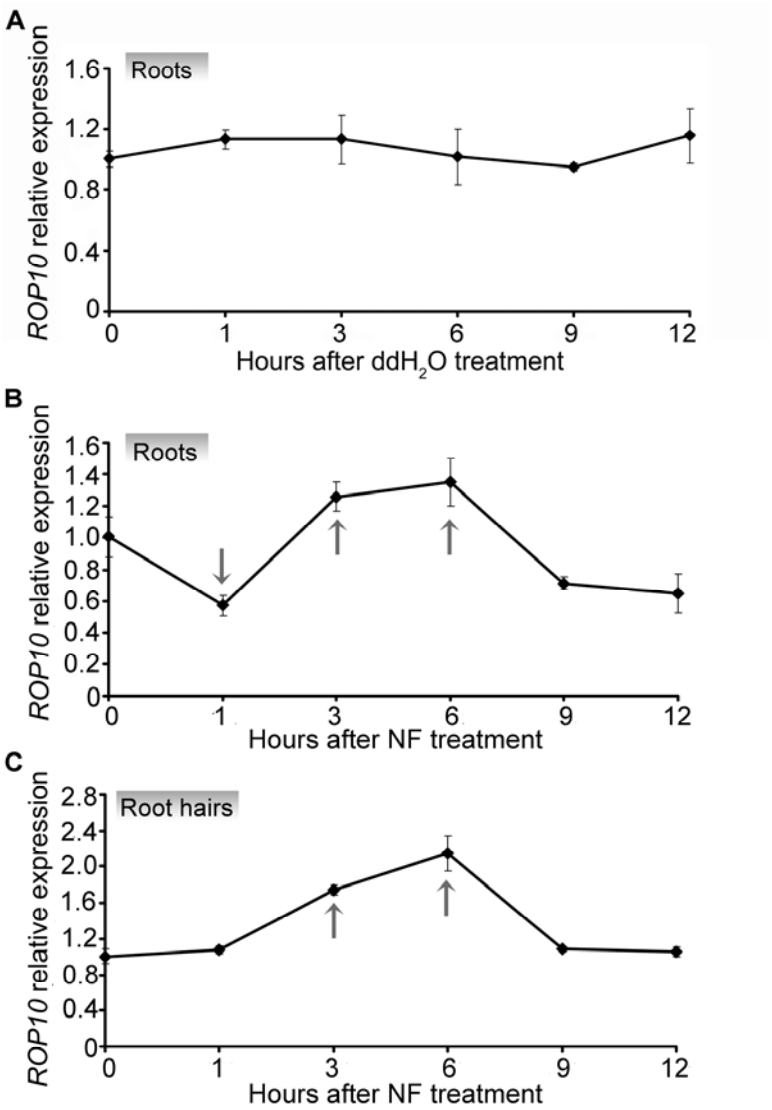
Supplemental Figure 10. Nodule formation on transformed *M. truncatula* roots over-expressing *ROP10* and *ROP10CA.*

(A) Roots transformed with the empty vector.

(B) Roots over-expressing *ROP10.*

(C) Roots over-expressing *ROP10CA*. Constructs were expressed in the transformed roots under the control of the *Ljubq1* promoter.

Pictures were taken 2 weeks post-inoculation with *S. meliloti*. Bars=1 cm.

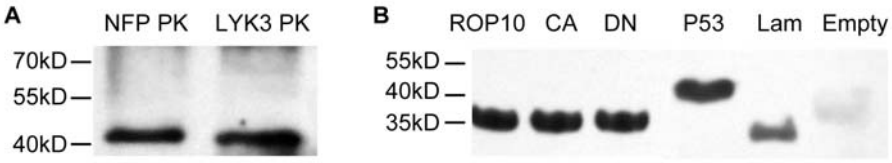


Supplemental Figure 11. *ROP10* is transiently up-regulated in response to NF treatment.

(A) and **(B)** qRT-PCR analyses of *ROP10* expression in untreated roots **(A)** and NF-treated roots **(B)** of five-day-old *M. truncatula* seedlings at different timepoints after treatment with 1 nM NodSm-IV(C16:2, S).

(C) qRT-PCR detection of *ROP10* expression in NF-treated root hairs. *ROP10* expression was normalized against *ACTINB* which was used as an internal reference.

Error bars indicate SE. Data presented are representative of at least three independent experiments.



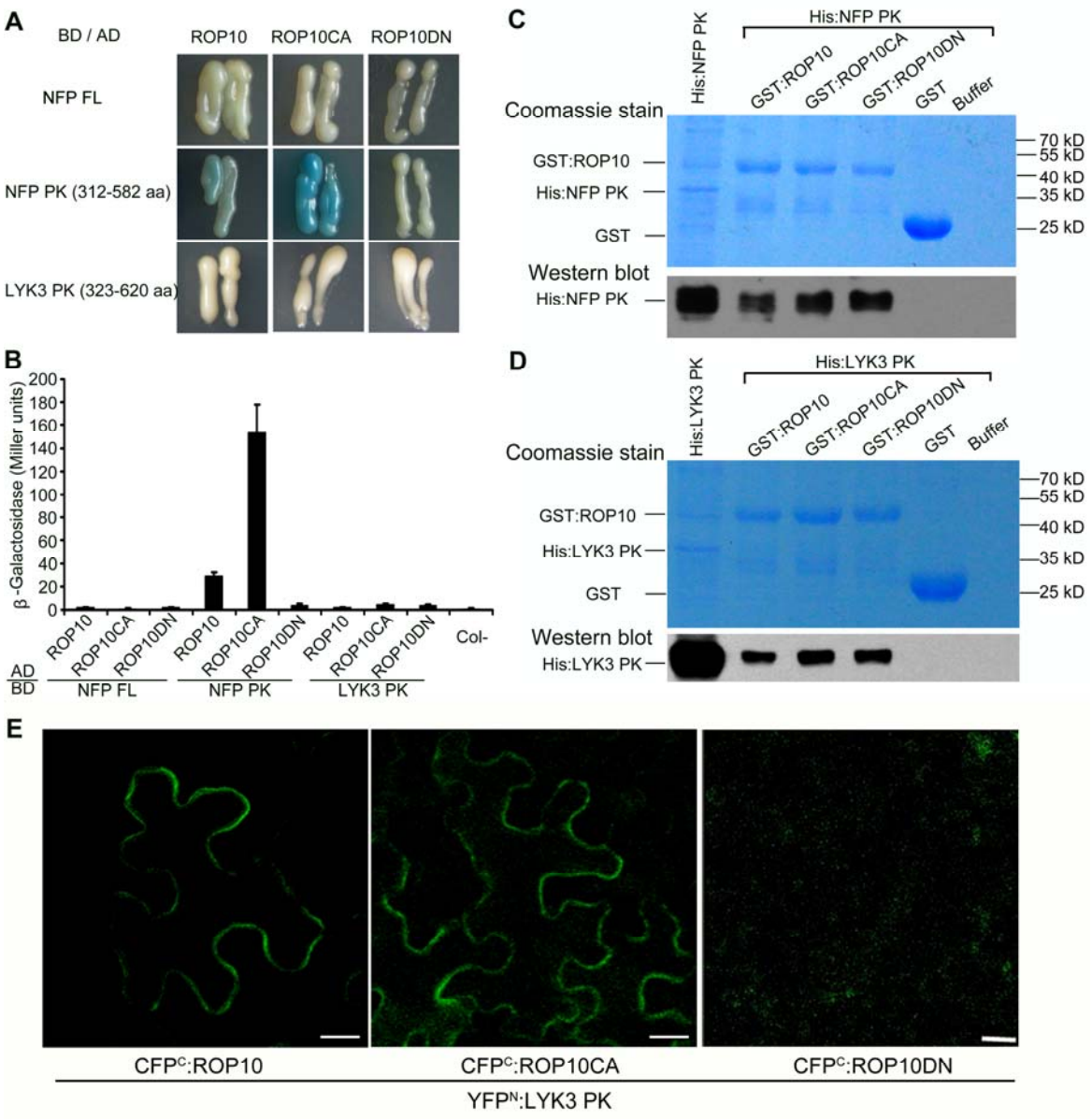
Supplemental Figure 12. Immunoblot analysis of protein expression in yeast cells.

A Gal4 DNA-binding domain (BD) fusion construct in pGBKT7 and a Gal4 activation domain (AD) fusion construct pGADT7 were co-transformed into the yeast strain AH109 using a LiAc-mediated yeast transformation protocol. Yeast cells co-transformed with bait and prey constructs were selected on SD media lacking Leu and Trp, and cultured in liquid SD selection medium broth lacking Leu and Trp. Soluble protein extracts were prepared from pelleted yeast cells for detection of protein expression.

(A) Recombinant proteins expressed from pGADT7 contained a hemagglutinin (HA) epitope and were detected using anti-HA monoclonal antibody.

(B) Recombinant proteins expressed from pGBKT7 contained a Gal4-BD epitope and were identified using anti-Gal4-BD monoclonal antibody.

Yeast cells containing no plasmid were cultured in YPDA broth used as a negative control.



Supplemental Figure 13. Interactions between ROP10 and NF receptors NFP and LYK3 of *M. truncatula*.

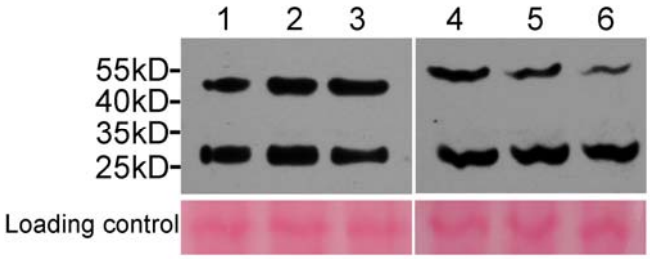
(A) Yeast two-hybrid assays. Full-length NFP (FL), NFP kinase domain (PK) and LYK3 PK were fused with LexA DNA-binding domain (BD) as baits; ROP10 and mutant forms (ROP10CA and ROP10DN) were fused with the B42 activation domain (AD) as preys. Yeast cells co-transformed with bait and prey constructs were grown on SD/-His-Trp-Ura medium containing X-Gal. The results indicate an interaction between NFP PK and ROP10 or ROP10CA.

(B) β-galactosidase activity of the yeast two-hybrid assay. Activities from yeast cells with indicated protein-protein combinations were quantified with CPRG as substrate. Col- indicates a negative control (no interaction for the combination pLexA and pB42AD. Error bars indicate SE. Data presented are a representative of at least three independent experiments.

(C) and **(D)** *In vitro* pull-down assays for interactions between NFP PK or LYK3

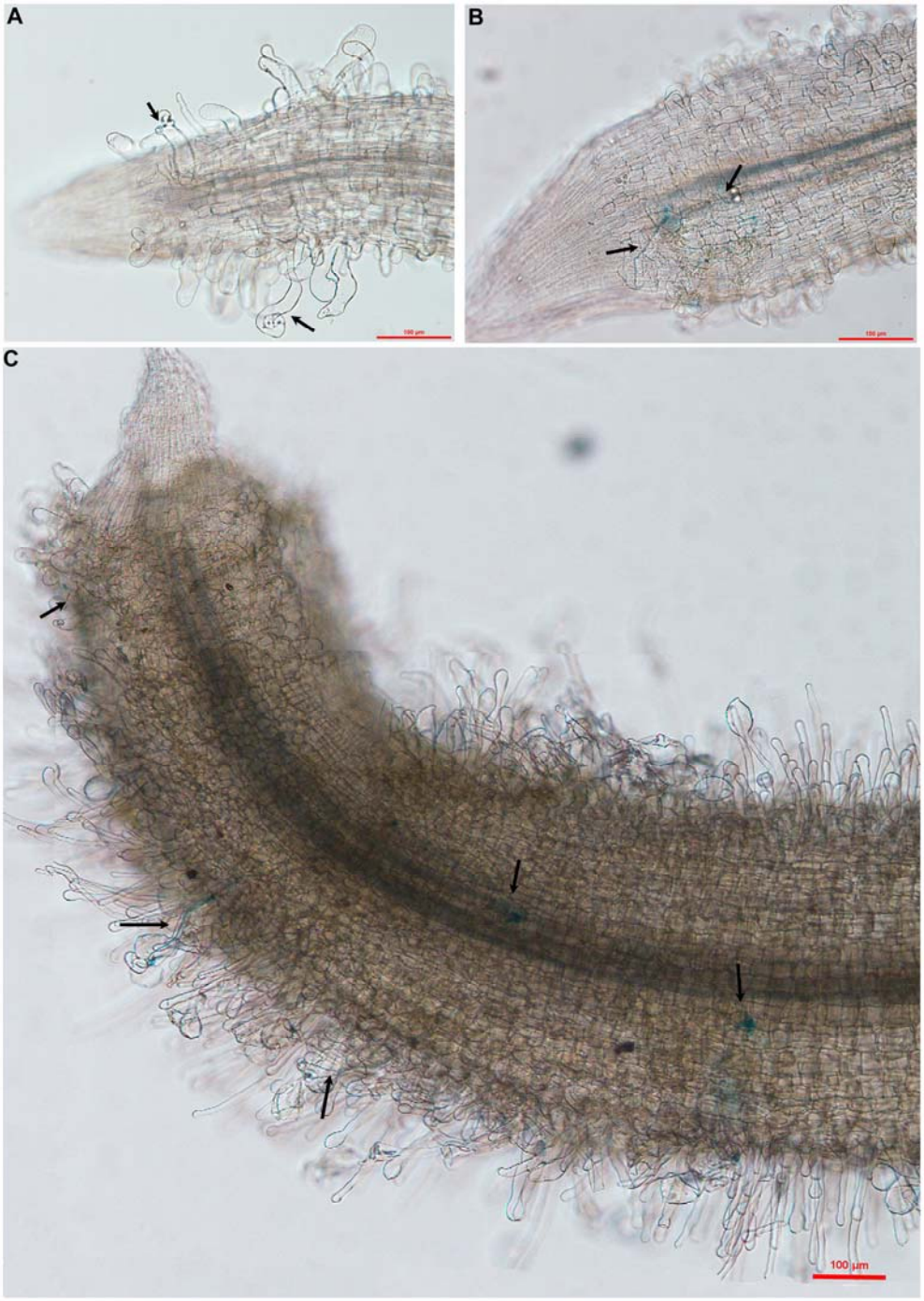
PK and different forms of ROP10. Purified GST:ROP10, GST:ROP10CA or GST:ROP10DN were immobilized on glutathione beads and mixed with His-tagged NFP PK or LYK3 PK. After washing, proteins pulled down by the glutathione beads were resolved by SDS-PAGE. The gels were stained with Coomassie brilliant blue G250 (upper panel) or used for Western blotting using anti-His antibodies (lower panel).

(E) BiFC analysis to test interactions between ROP10 or mutant forms and LYK3 PK. BiFC experiments were performed by co-expression of the indicated combinations in *N. benthamiana* leaf epidermal cells. Bars=50 μm.



Supplemental Figure 14. Immunoblot analysis of expressed fusion proteins of *N. benthaniana* for BiFC assays.

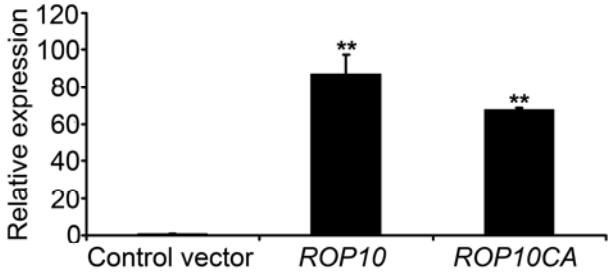
Protein expression was detected by immunoblotting with anti-His antibodies for CFP^C fusions and YFP^N fusions. Extracts from *N. benthaniana* epidermal cells co-transformed with constructs CFP^C:ROP10 and YFP^N:NFP PK (lane 1), CFP^C:ROP10CA and YFP^N:NFP PK (lane 2), CFP^C:ROP10DN and YFP^N:NFP PK (lane 3), CFP^C:ROP10 and YFP^N:LYK3 PK (lane 4), CFP^C:ROP10CA and YFP^N:LYK3 PK (lane 5), CFP^C:ROP10DN and YFP^N:LYK3 PK(lane 6).



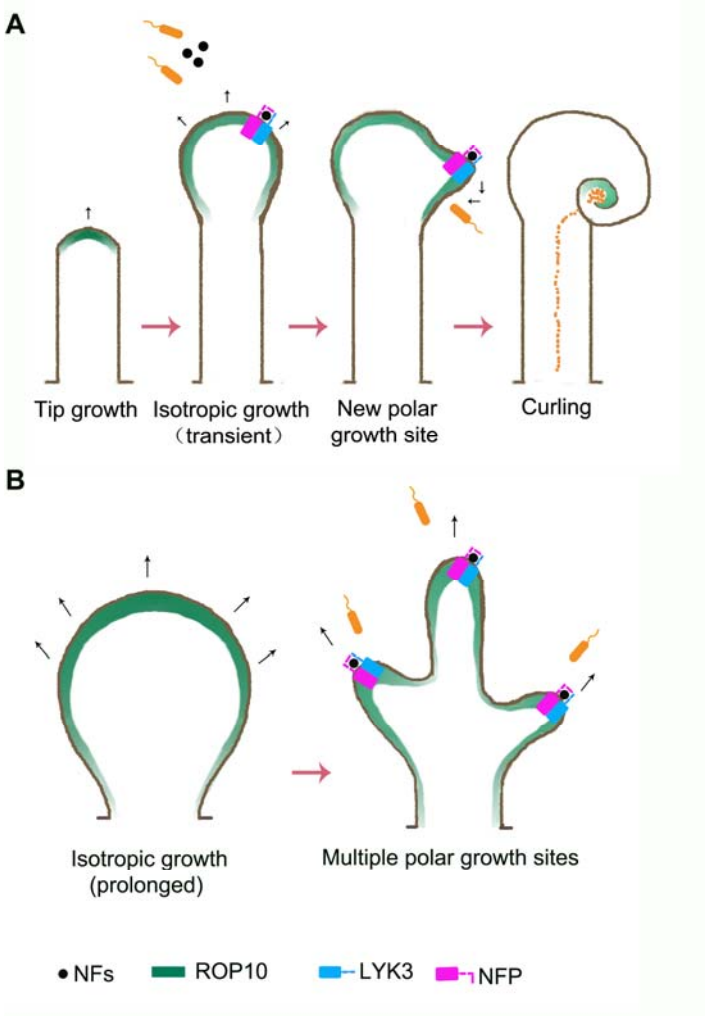
Supplemental Figure 15. Root hair deformation and rhizobial infection in *M. truncatula* roots over-expressing *ROP10* or *ROP10CA*.

(A) Root hair deformations close to root tips in transformed roots over-expressing *ROP10*.

(B) and **(C)** Rhizobial infections (marked by arrows) observed close to root tips in transformed roots over-expressing *ROP10* **(B)** or *ROP10CA* **(C)**. Transformed roots were inoculated with *S. meliloti* and photographed 7 d post-inoculation. Bacteria were detected by β-galactosidase activity using X-Gal (blue). Bars=100 µm.



Supplemental Figure 16. qRT-PCR analysis of *ROP10* transcripts in *M. truncatula* roots over-expressing *ROP10* or *ROP10CA* treated with NFs.
Transformed roots were incubated with 1 nM NodSm-IV(C16:2, S) and harvested after 12 h. Control roots were transformed with the empty vector. Asterisks indicate significant differences from the empty vector control ($P < 0.01$, Student's *t* test). Error bars indicate SE. Data presented are representative of three independent experiments.



Supplemental Figure 17. Proposed model for NFs temporal and spatial regulation of ROP10 PM-localization and activity to induce root hair deformation.

(A) Before rhizobial inoculation, ROP10 is preferentially localized at the apical PM of elongating root hairs where it may help maintain polarized tip growth (arrow). Following rhizobial inoculation, NFs induce a transient up-regulation of *ROP10* in the growing root hairs. At the tip of these root hairs, GEF2 could rapidly activate ROP10, leading to ectopic accumulation of active ROP10 at the PM. These events culminate in isotropic growth of root hair tips (arrows). We also propose that active S-acylated GTP-bound ROP10 at the PM interacts with the NF receptors NFP and LYK3 in lipid rafts. This then results in initiation of a new growth site and root hair branching (arrow). Thus, formation of a curled root hair (arrow) allows entrapment of bacteria.

(B) In root hairs over-expressing *ROP10* or *ROP10CA*, activated ROP10 is recruited at the PM throughout the root hair stably and permanently. ROP10CA likely interacts with NF receptors at multiple sites, culminating in the initiation of multiple growth sites as well as numerous outgrowths. As a result, uncontrolled root hair deformation occurs, which is not beneficial for entrapment of bacteria.

Supplemental Table 1. Constructs used in various assays.

Constructs	Strategies	Applications
T-ROP10	KOD(Toyobo) PCR product of 1089 bp *ROP10* cDNA fragment amplified from A17 cDNA with primer pair 1 into pMD18-T via TA cloning (Takara)	T-cloning
T-ROP10CA	Site-directed mutagenesis(Takara MutanBEST Kit) on T-ROP10 with primer pair 2	
T-ROP10DN	Site-directed mutagenesis (Takara MutanBEST Kit) on T-ROP10 with primer pair 3	
T-ROP10^{C203S}	Site-directed mutagenesis (Agilent QuikChange Lightning Site-Directed Mutagenesis Kit) on T-ROP10 with primer pair 4	
T-ROP10^{C197S}	Site-directed mutagenesis(Takara MutanBEST Kit) on T-ROP10 with primer pair 5	
T-ROP10$^{C197+203S}$	Site-directed mutagenesis(Takara MutanBEST Kit) on T-ROP10 with primer pair 6	
T-ROP10CA$^{C197+203S}$	Site-directed mutagenesis(Takara MutanBEST Kit) on T-ROP10CA with primer pair 6	
pENTR/SD/D-ROP10/CA/DN/ CA$^{C197+203S}$	KOD(Toyobo) PCR product of 636 bp *ROP10* CDS amplified from T-ROP10/CA/DN/CA$^{C197+203S}$ with primer pair 7 into pENTR/SD/D via TOPO reaction (Invitrogen)	Entry cloning
pENTR/SD/D-ROP10/CA/DN/Δstop	KOD(Toyobo) PCR products of ROP10 CDS (Stop codon deleted) amplified from T-ROP10/CA/DN/CA$^{C197+203S}$ with primer pair 8 into pENTR/SD/D via TOPO reaction (Invitrogen)	
pENTR/SD/D-NFP PK	KOD(Toyobo) PCR product of 813 bp *NFP* PK fragment amplified from A17 cDNA with primer pair 9 into pENTR/SD/D via TOPO reaction (Invitrogen)	
pENTR/SD/D-LYK3 PK	KOD(Toyobo) PCR product of 897 bp *LYK3* PK fragment amplified from A17 cDNA with primer pair 10 into pENTR/SD/D via TOPO reaction (Invitrogen)	
BD-ROP10/CA/DN	KOD(Toyobo) PCR products of 636 bp *ROP10* CDS amplified from T-ROP10/CA/DN with primer 11 pair into *Eco*RI and *Xho*I sites of pGBKT7 (Clontech)	Yeast two-hybrid

AD-NFP FL	KOD(Toyobo) PCR product of 1830 bp *NFP* CDS amplified from A17 cDNA with primer pair 12 into *Eco*RI and *Xho*I sites of pGADT7 (Clontech)	
AD-NFP PK	KOD(Toyobo) PCR product of 813 bp *NFP* PK amplified from A17 cDNA with primer pair 13 into *Eco*RI and *Xho*I sites of pGADT7 (Clontech)	
AD-LYK3 FL	KOD(Toyobo) PCR product of 1863bp *LYK3* CDS amplified from A17 cDNA with primer pair 25 into *Eco*RI and *Xho*I sites of pGADT7 (Clontech)	
AD-LYK3 PK	KOD(Toyobo) PCR product of 897 bp LYK3 PK CDS amplified from A17 cDNA with primer pair 14 into *Eco*RI and *Xho*I sites of pGADT7 (Clontech)	
AD-ROP10/CA/DN	the *Eco*RI and *Xho*I fragments of BD-ROP10/CA/DN insert into pB42AD (Clontech)	
BD-NFP FL/ NFP PK/ LYK3 FL/LYK3 PK	the *Eco*RI and *Xho*I fragments of AD-NFP FL/ NFP PK/ LYK3 FL/ LYK3 PK insert into pLexA (Clontech)	
PUbi:ROP10/CA/DN/ $CA^{C197+203S}$	LR reaction (Invitrogen) of pENTR/SD/D-ROP10/CA/DN/ $CA^{C197+203S}$ and pUB-GW-GFP	Hairy Root Transformation
pCAMBIA2301-pRNAi	the *Kpn*I and *Sac*I fragment of pRNAi (Limpens et al., 2004) inserted into pCAMBIA2301	
P35S:$ROP10^{C203S}$/$ROP10^{C197S}$/$ROP10^{C197+203S}$	KOD(Toyobo) PCR products of 636 bp ROP10 CDS amplified from T-$ROP10^{C203S}$/$ROP10^{C197S}$/$ROP10^{C197+203S}$ with primer pair 15 into *Asc*I and *Spe*I sites of pCAMBIA2301- pRNAi	
pCAMBIA2300S	Modified based on pCAMBIA2300 and contains a double 35S promoter of Cauliflower mosaic virus and a terminator (*http://www.cambia.org/daisy/cambia/585.html)*	Subcellular Localization
2300S-GFP	the *Hin*dIII/*Eco*RI fragment of PA7-GFP inserted into pCAMBIA2300	
ROP10/CA/DN:GFP	KOD(Toyobo) PCR products of 633 bp *ROP10* (stop code deleted) amplified from T-ROP10/CA/DN with primer 16 into *Sal*I and *Spe*I sites of 2300S-GFP	

$ROP10^{C203S}$/$ROP10^{C197S}$ /$ROP10^{C197+203S}$/ $ROP10CA^{C197+203S}$:GFP	KOD(Toyobo) PCR products of 633 bp *ROP10* mutant(stop code deleted) amplified from T-$ROP10^{C203S}$/$ROP10^{C197S}$/$ROP10^{C197+203S}$/ $ROP10CA^{C197+203S}$ with primer pair 21 into *Sal*I and *Spe*I sites of 2300S-GFP	
NFP:mCherry/ LYK3:mCherry	KOD(Toyobo) PCR products of *NFP/LYK3* and *mCherry* amplified from BD-NFP FL/BD-LYK3 FL and pUB-mCherry-GFP with primer pairs 17 and 18, 19 and 20; amplicons used for construction of fusions protein by overlap extension PCR; construct cloned into the *Hin*dIII and *Eco*RI sites of 2300S-GFP	
CFP^{C}:ROP10/CA/DN	LR reaction (Invitrogen) of pENTR/SD/D-ROP10/CA/DNΔstop and pCCFP-X	BiFC Analysis
YFP^{N}:NFP PK/LYK3 PK	LR reaction (Invitrogen) of pENTR/SD/D-NFP PK/LYK3 PK and pNYFP-X	
GST:ROP10/CA/DN	KOD(Toyobo) PCR product of 636 bp *ROP10* CDS amplified from T-ROP10/CA/DN with primer pair 22 into *Eco*RI and *Xho*I sites of pGEX-6P-1	Pull-down Assay
His:NFP PK	KOD(Toyobo) PCR product of *NFP* PK fragments amplified from BD-NFP PK with primer pair 23 into *Eco*RI and *Xho*I sites of pET-28a (+)	
His:LYK3PK	KOD(Toyobo) PCR product of *LYK3* PK fragments amplified from BD-LYK3 PK with primer 24 into *Eco*RI and *Xho*I sites of pET-28a (+)	

Supplemental Table 2. Primers used for various constructs.

No.	Purposes	Primer sequences (5'-3')
1	ROP10	FW: ACAAAAACAAAACACTCATTTCATTA Rev: GAAATGCTTGAAAAATATTAGTCTG
2	ROP10CA	FW: ACGTAGCTGTAGGCAAAACCT Rev: CTCCAACTGTGACGCACTTG
3	ROP10DN	FW: CCAAATTGGCTCTCCGAG Rev: TTCCAACAAGTACAACAGGGAC
4	ROP10^{C203S}	FW: AGGCTGTTTCCTAAATGTCCTCTCTGGAAGGAGCAT Rev: ATGCTCCTTCCAGAGAGGACATTTAGGAAACAGCCT
5	ROP10^{C197S}	FW: GCTCTTTCCTAAATGTCCTCTGTG Rev: CTCGACGAGCTTTTTTCCTTT
6	ROP10$^{C197+203S}$	FW: GCTCTTTCCTAAATGTCCTCTCTG Rev: CTCGACGAGCTTTTTTCCTTT
7	ROP10-TOPO	FW: CACCATGGCTTCAACAGCTTCAAGATT Rev: TCATTTATGACGAACGATGCTCC
8	ROP10-TOPO2	FW: CACCATGGCTTCAACAGCTTCAAGATT Rev: TTTATGACGAACGATGCTCC
9	NFP PK-TOPO	FW: CACCATGGGTACAACGAATCTGAGTGAC Rev: ATCTAAACCAGATGTCAAGGA
10	LYK3 PK-TOPO	FW: CACC AGCTTGGATAATAAAATTGGTCA Rev: TCATCTAGTTGACAACAGATTTATG
11	BD-ROP10	FW: CGGAATTC ATGGCTTCAACAGCTTCAAGAT Rev: CCGCTCGAG TCATTTATGACGAACGATGCTCC
12	AD-NFP FL	FW: CGGAATTC CATAATTTGCATTTCCTCACA Rev: CCGCTCGAG CACTTGATTAACGAGCTATTACA
13	AD-NFP PK	FW: CGGAATTC GGTACAACGAATCTGAGTGAC Rev: CCGCTCGAG ATCTAAACCAGATGTCAAGGA
14	AD-LYK3 PK	FW: CGGAATTC AGCTTGGATAATAAAATTGGTCA Rev: CCGCTCGAG TCATCTAGTTGACAACAGATTTATG
15	ROP10mutant-OX	FW: AGGCGCGCCATGGCTTCAACAGCTTCAAGAT Rev: GACTAGTTCATTTATGACGAACGATGCTC
16	ROP10:GFP	FW: ACGCGTCGACATGGCTTCAACAGCTTCAAG Rev: GGACTAGTTTATGACGAACGATGCTCCTT
17	NFP:mCherry-1	FW: CCGCTCGAGATGTCTGCCTTCTTTCTTCCTTCTA Rev: GCCCTTGCTCACCATACGAGCTATTACAGAAGTA
18	NFP:mCherry-2	FW: TTCTGTAATAGCTCGTATGGTGAGCAAGGGCGAGGA Rev: TCCCCCGGGTTTTACTTGTACAGCTCGTCCATG
19	LYK3:mCherry-1	FW: CCGCTCGAG ATGAATCTCAAAAATGGATTA Rev: GCCCTTGCTCACCATTCTAGTTGACAACAGATTTAT
20	LYK3:mCherry-2	FW: ATCTGTTGTCAACTAGAATGGTGAGCAAGGGCGAGGA Rev: TCCCCCGGGTTTTACTTGTACAGCTCGTCCATG

21	ROP10mutant-GFP	FW: ACGCGTCGACATGGCTTCAACAGCTTCAAG Rev: GGACTAGTTTATGACGAACGATGCTCCTTCCAG
22	GST:ROP10	FW: CGGAATTCATGGCTTCAACAGCTTCAAG Rev: CCGCTCGAGTCATTTATGACGAACGATGCT
23	His:NFP PK	FW: CGGAATTCGGTACAACGAATCTGAGTGAC Rev: CCGCTCGAGTATCTAAACCAGATGTCAAGGA
24	His:LYK3 PK	FW: CGGAATTC AGCTTGGATAATAAAATTGGTCA Rev: CCGCTCGAGTTCTAGTTGACAACAGATTTATGAGA
25	AD-LYK3 FL	FW: CGGAATTC ATGAATCTCAAAAATGGATTACTAT Rev: CCGCTCGAG TCATCTAGTTGACAACAGATTTATG
26	*rop10* mutant identification	F1: ACAAAAACAAAACACTCATTTCATTA R1: TCCTTGTTGGATTGGTAGCCAACTTTGTTG R2: ATTGATTTCAGGGATGTAGACTTG

FW: forward primer; Rev: reverse primer. Nucleotides used for site directed mutagenesis were shown in red; restriction enzyme sites in primers were underlined.

Supplemental Table 3. Primers used for qRT-PCR

Genes	Primer sequences (5'-3')
ENOD11	FW: TAGGGCTTGCTGATAAATCTC Rev: TAATTGGAGGCTTGTAAGTAGG
ROP10	FW: GCAGATGTCTTTGTCTTGGCTTT Rev: GAGTTCCTCACCTTGCTCAGTAGTC
ActinB	FW: ACCGAATGAGCAAGGAAATCAC Rev: ATTGACCCTCCAATCCAGACAC
EF1α	FW: AAGCGTGTGATCGAGAGATTC Rev: TGTCCATCCTTAGAGATACCAG

FW: forward primer; Rev: reverse primer.

Supplemental Methods

Protein Extraction from Yeast Cells for Western Blot Analysis

Yeast proteins were extracted according to the Yeast Protocols Handbook (Clontech). Fresh diploid colonies on SD/-LW selection plates were inoculated into 5 mL liquid SD/-LW medium and grown at 28°C for 36h. The yeast cell were centrifuged at 4,000 rpm for 5min and then washed with cold sterile water. After centrifugation at 4,000g for 5 min, the pellets were frozen immediately in liquid nitrogen and stored at -70°C for subsequent analysis. The cell pellets were thawed by 200 µL of lysis buffer (8 M urea, 5% SDS, 40 mM Tris-HCl, pH 6.8, 0.1 mM EDTA, 1% β-mercaptoethanol, 0.4 mg/mL bromphenol blue, 1mM PMSF and protease inhibitor solution) at 70°C for 10 min. The suspensions were added with 40 µL of glass beads, vortexed vigorously for 2 min and boiled for 5 min. Cell debris and glass beads were discarded after centrifugation at 12,000g for 10 min. The supernatant was transferred to a fresh 1.5 mL tube and analyzed by SDS-PAGE for western blotting. BD and AD samples were analyzed by using GAL4 BD monoclonal antibody (Sigma) and HA monoclonal antibody (CW BIO) respectively.

Yeast Two-Hybrid Assay in the LexA-based system

AD and BD constructs were co-transformed into the yeast strain EGY48 [p8op-lacZ] using a LiAc-mediated yeast transformation protocol according to the manufacturer's instructions (Clontech). The co-transformed yeast cells were then plated on SD/–His/–Trp/–Ura to select for colonies containing both hybrid plasmids and the reporter plasmid. At least 10 single colonies were transferred to SD/Gal/Raf/X-gal/-His-Trp-Ura plates and incubated for 3 days at 30°C to test for β-galactosidase activity of the LacZ reporter protein. Quantitative β-galactosidase analysis was performed using a liquid culture assay with chlorophenol red-β-D-galactopyranoside (CPRG) as substrate as described in the Yeast Protocols Handbook (Clontech).

Fusion Protein Extract from *N. benthaniana* Leaves for BiFC Assay

About 300mg *N. benthamiana* leaves co-expressing the constructs CFP^C fusions and YFP^N fusions were ground in liquid nitrogen and homogenized in 500µL lysis buffer [50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10% glycerol, 1mM EDTA, 1% SDS, 1% Triton X-100, 1 mM phenylmethanesulfonyl flouride (PMSF) and protease inhibitor mix] on ice for 15min. Extracts were centrifuged at 12,000rpm for 5 min at 4°C to remove large pieces of cell debris. Then the supernatants were transferred to a new tube and centrifuged again at 12,000 rpm for 15 min at 4°C. The final supernatant samples were analyzed by SDS-PAGE and Western blots using a His monoclonal antibody (CWBIO). His antibody was used at 1:8000, and secondary antibody was used at 1:8000 (Goat Anti-Mouse IgG-HRP, Abmart).