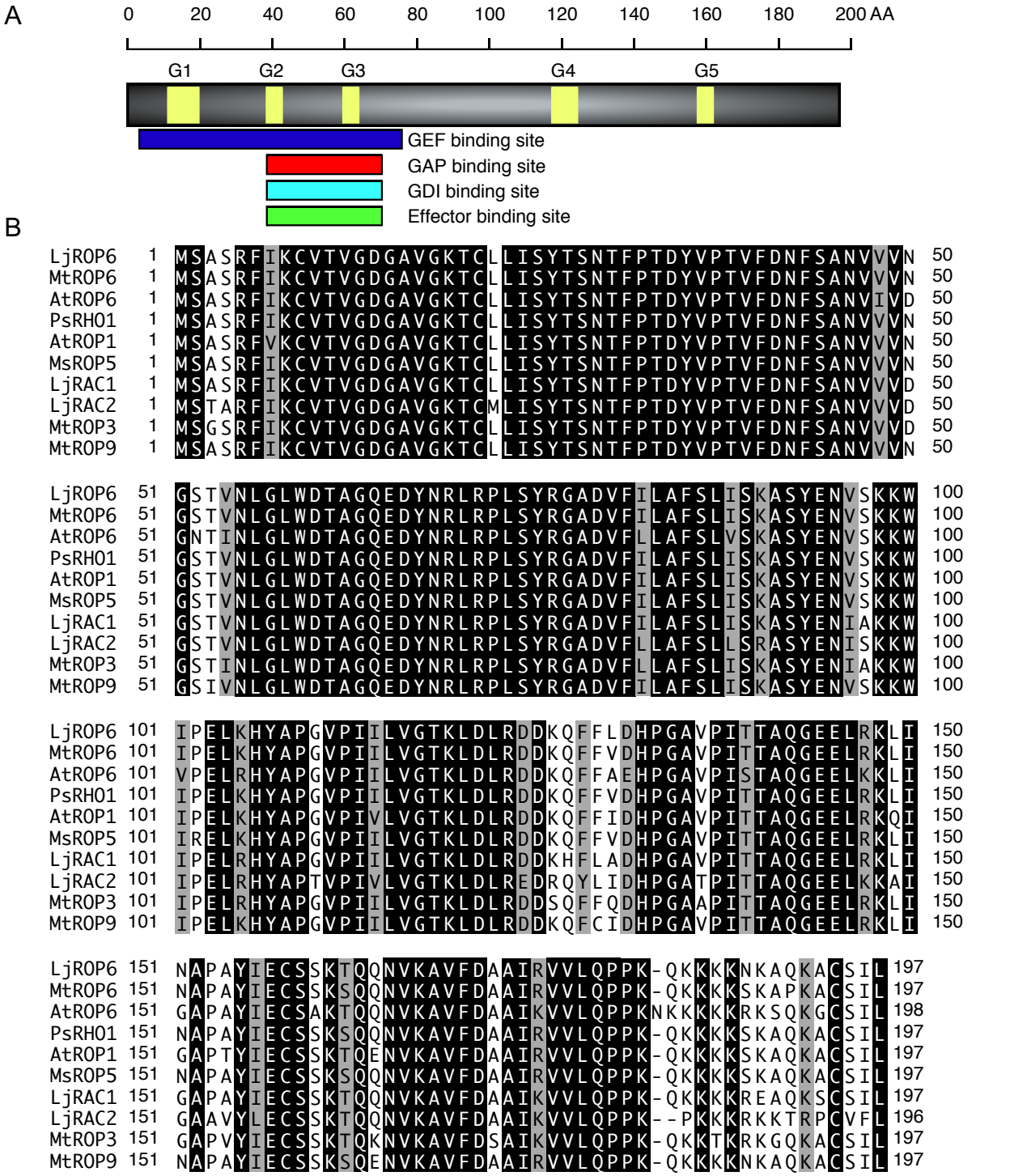
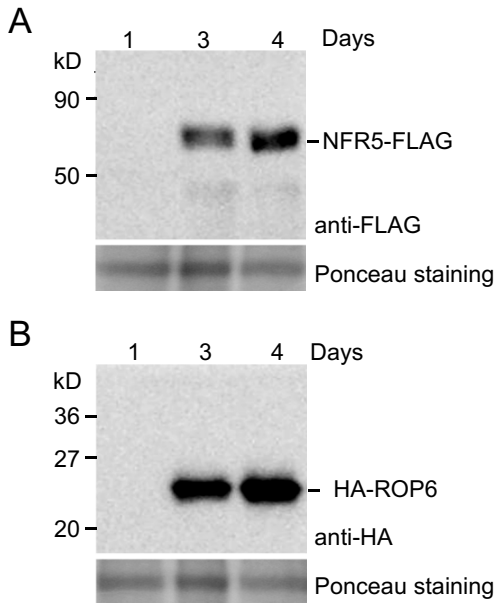


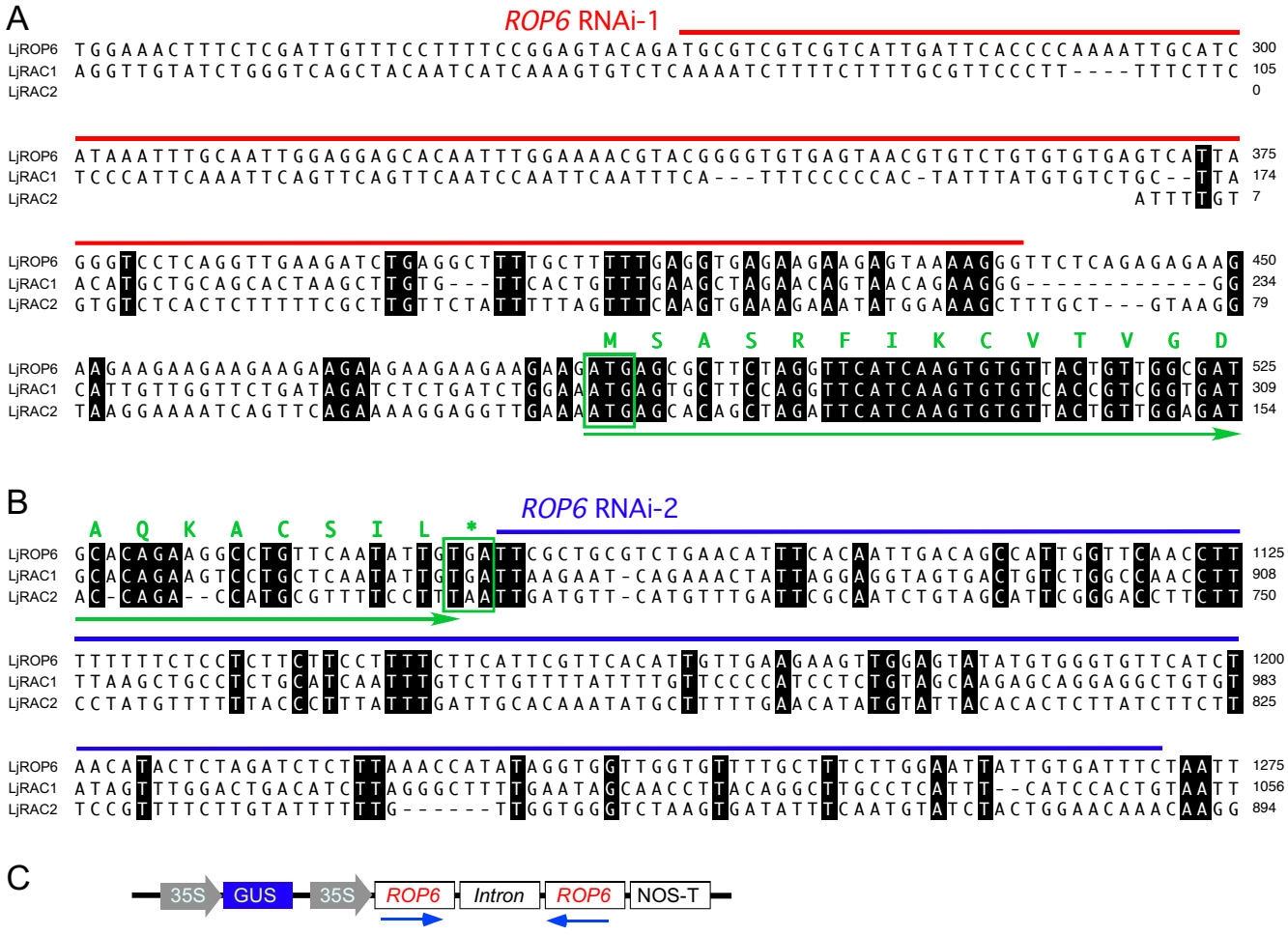
Supplemental Figure 1. Conserved Domains of LjROP6 Protein and its Homologs.

A, LjROP6 protein contains G1-G5 subdomains of GTPase, a putative guanine nucleotide exchange factor (GEF)-binding site, a putative GTPase-activativing protein (GAP)-binding site, a putative guanine nucleotide dissociation inhibitior (GDI)-binding site, and an effector interaction site. B, Multiple sequence alignment of LjROP6 (GenBank acc. No. JF260911) and its homologs from *Lotus japonicus* (LjRAC1, Z73961; LjRAC2, Z73962), *Medicago truncatula* (MtROP3, AF498357.1; MtROP6, AF498358.1; MtROP9, AF498359.1); *Medicago sativa* (MsROP5, AJ966571.1); *Pisum sativum* (PsRHO1, L19093.1) and *Arabidopsis thalina* (AtROP1, NM_114989.3; AtROP6, AF031427.1). The alignment was performed with DNA Star software.



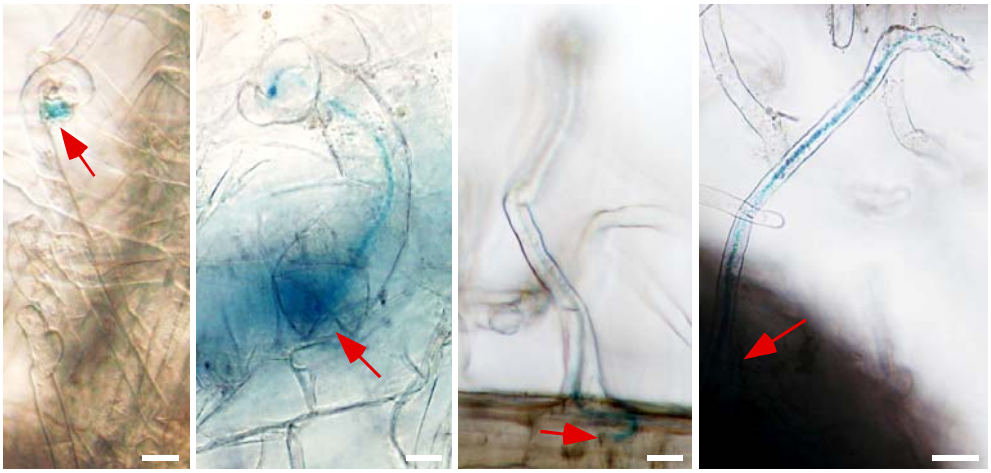
Supplemental Figure S2. Detection of Recombinant ROP6 and NFR5 Proteins Transfected Leaves of *Nicotiana benthamiana*.

Leaves of *Nicotiana benthamiana* were infiltrated with *Agrobacterium tumafaciens* cells carrying plasmids for transient protein expression of NFR5-FLAG **(A)** and HA-ROP6 **(B)**. Protein extracts were prepared from leaves at day 1, 3 and 4 after *Agrobacterium* infiltration. Proteins were resolved by 10% SDS-PAGE and transferred to nitrocellulose membrane. The membrane was stained with Ponceau dye for assessment of protein quantity and used as an internal loading control **(lower panel)**. Antibodies corresponding to the protein tags were used to detect the recombinant proteins **(upper panel)**. Molecular masses of marker proteins in kD are indicated.



Supplemental Figure S3. DNA Sequences *ROP6* RNAi Constructs.

The 5'- or 3'-untranslated regions (UTR) of *Lotus japonicus ROP6* (JF260911), *RAC1* (Z73961) and *RAC2* (Z73962) cDNA sequences were aligned with DNAMAN software (Lynnon Biosoft, Canada). Identical bases of the three sequences were shaded in black. The red line indicates the 5'-UTR of *ROP6* cDNA that was used for *ROP6* RNAi-1 construct **(A)**. The blue line indicates the 3'-UTR used for *ROP6* RNAi-2 construct **(B)**. The green lines indicate the coding region with the amino acid residues of ROP6. Boxed are the start codon ATG and stop codon TGA/TAA. The cDNA fragment was inserted in inverse orientations with an *Actin* intron between them and placed under the control of the CaMV35S promoter **(C)**. The two RNAi constructs were cloned in pCAMBIA1301-35S-int-T7 vector, which contained a GUS reporter construct. The two RNAi plasmids were used to generate transgenic hairy roots in *Lotus japonicus*. Note that the sequences used for RNAi constructs have very limited homology with the corresponding regions of *RAC1* and *RAC2,* the two *ROP6* homologs from *Lotus japonicus*. Expression of *ROP6* RNAi-1 and *ROP6* RNAi-2 did not knock down the expression of *RAC1* and *RAC2.*



Supplemental Figure S4. Classification of Infection Threads in Transgenic Hairy Roots.

Cellular distributions of infection threads. Transgenic hairy roots were inoculated with lacZ-labeled *M. loti* cells. Nine days post inoculation, hairy roots were stained with X-gal solution to reveal the locations of infection threads (ITs) and observed under microscope. Arrows indicate the cellular locations of infected thread tips. Bars = 50 μm.