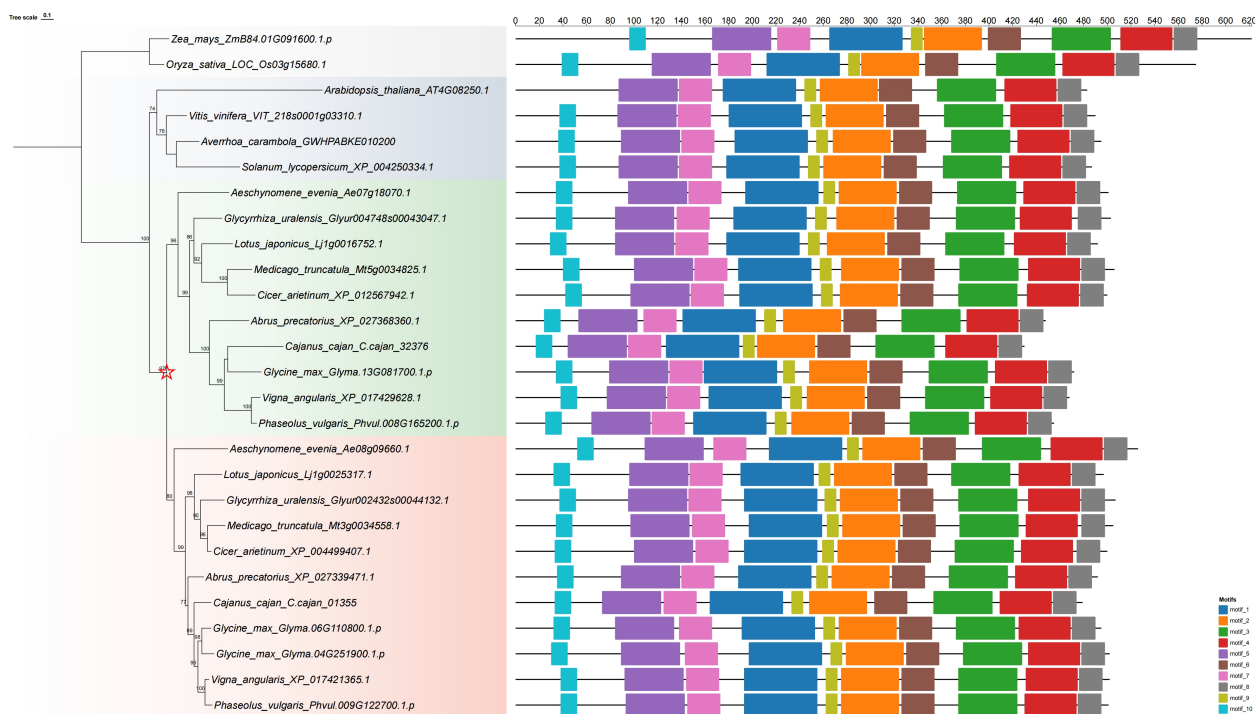
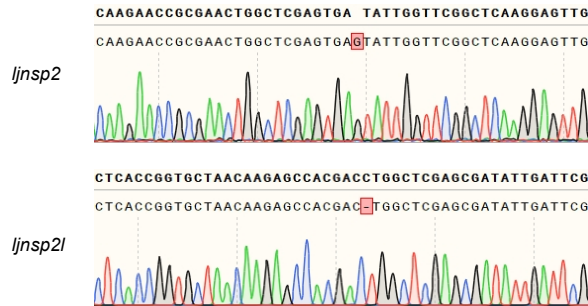
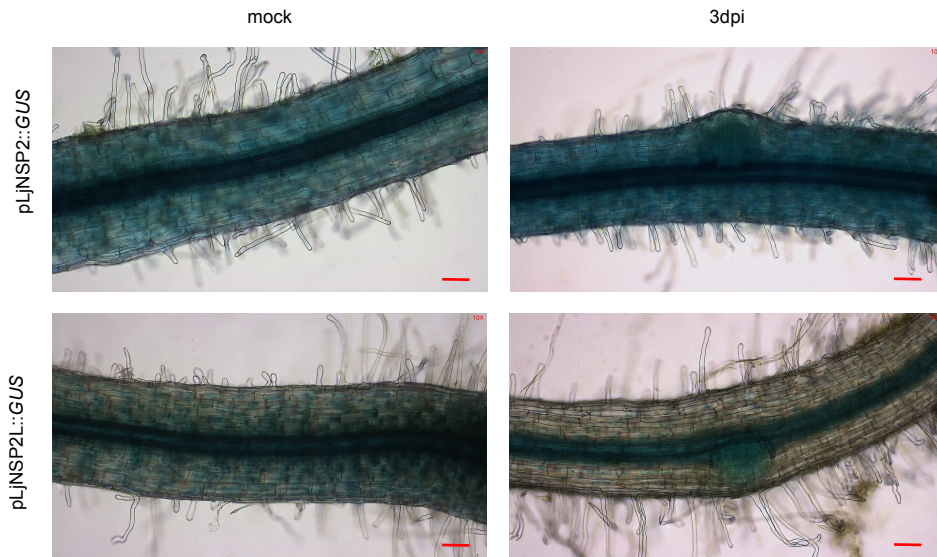




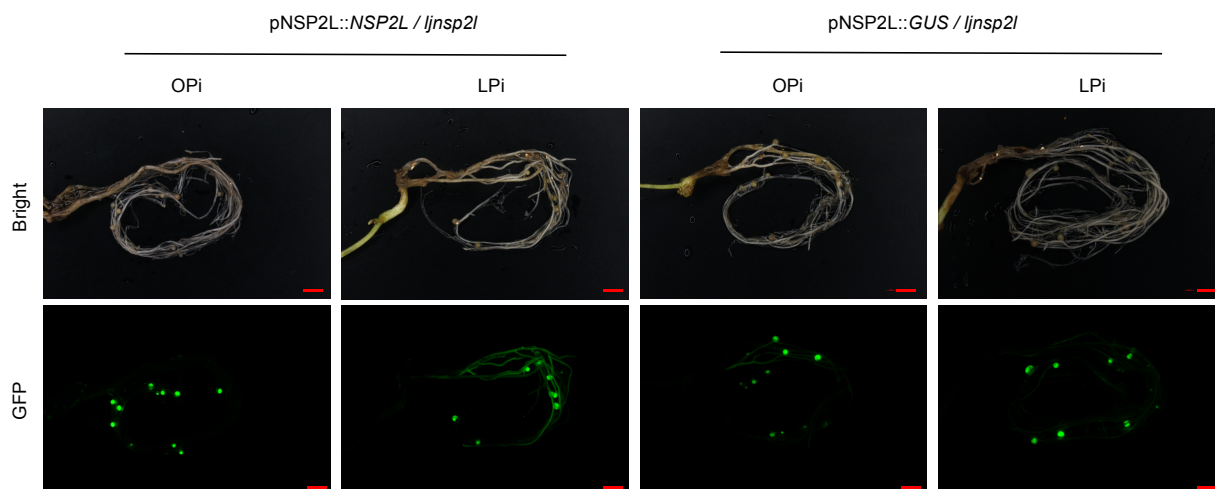
Supplemental Fig. S1. MEME-predicted conserved motifs of NSP2 homologs. The maximum likelihood phylogenetic tree of NSP2 homologs is shown together with the distribution of conserved motifs predicted using MEME. Full-length amino acid sequences were used for motif prediction, and ten conserved motifs were identified. Motifs 1–10 are displayed as colored boxes according to their positions in each protein sequence. Identical colors indicate the same MEME-predicted motif. Gray lines indicate protein length, and the scale at the top indicates amino acid positions. The NSP2 and NSP2L clades are indicated by shaded backgrounds.



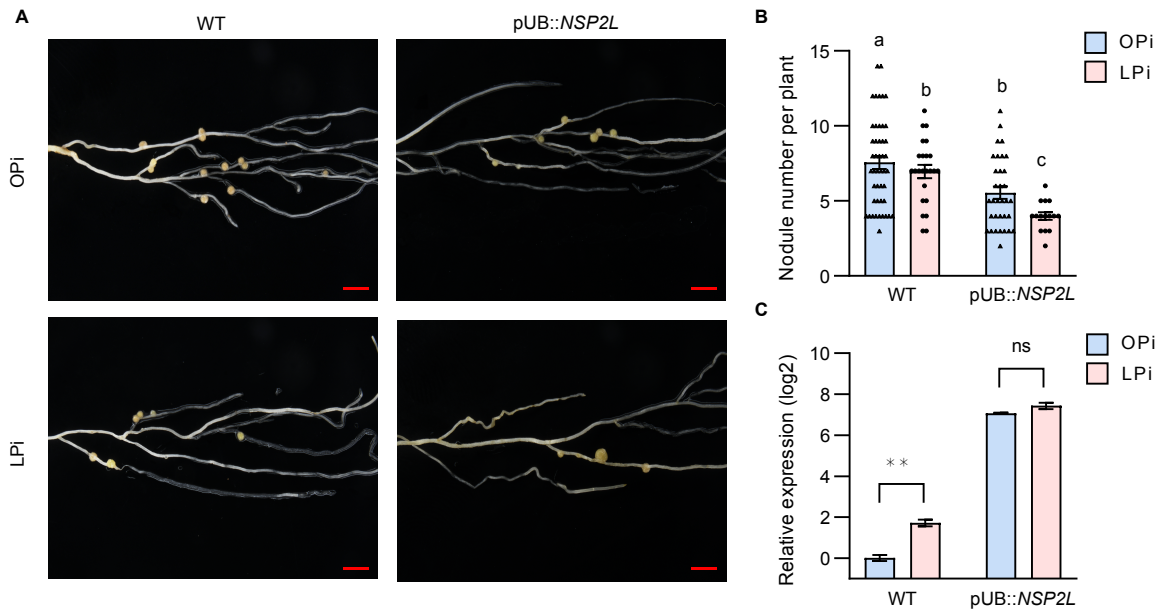
Supplemental Figure S2. CRISPR/Cas9-generated *ljnsp2* and *ljnsp2l* single mutants. Sanger sequencing chromatograms confirming the CRISPR/Cas9-induced mutations in *LjNSP2* and *LjNSP2L*. The *ljnsp2* single mutant carries a 1-bp G insertion around nucleotide position 415, which is predicted to introduce a premature stop codon at amino acid 177. The *ljnsp2l* single mutant carries a 1-bp C deletion at nucleotide position 360, which is predicted to introduce a premature stop codon at amino acid 125. Both mutations are predicted to cause frameshifts and premature termination of the encoded proteins.



Supplemental Figure S3. GUS staining of *LjNSP2* and *LjNSP2L* promoter activities in transgenic roots. Histochemical GUS staining of *Lotus japonicus* hairy roots carrying pLjNSP2::GUS or pLjNSP2L::GUS constructs. The 976-bp *LjNSP2* promoter fragment and the 1103-bp *LjNSP2L* promoter fragment upstream of the start codon were used to drive GUS expression. Transgenic roots were generated using *Agrobacterium rhizogenes* strain LBA1334 and collected under mock or rhizobial-inoculated conditions. Representative images are shown. Scale bars = 200 μ m.



Supplemental Figure S4. Hairy root transformation for native-promoter complementation of *ljnsp2l*. Bright-field and GFP fluorescence images of *ljnsp2l* hairy roots transformed with pLjNSP2L::LjNSP2L or pLjNSP2L::GUS. GFP fluorescence was used to identify transformed root systems, and non-transformed roots were removed before phosphate treatments and rhizobial inoculation. Nodules formed on transformed root systems were counted for complementation analysis. Scale bars = 2 mm.



Supplemental Figure S5. Constitutive expression of *LjNSP2L* affects nodulation. (A) Representative root phenotypes of WT and stable pUB::*LjNSP2L* transgenic plants grown under optimum phosphate (OPi) or low phosphate (LPi) conditions after inoculation with *Mesorhizobium loti*. (B) Nodule number of WT and pUB::*LjNSP2L* plants under OPi and LPi conditions at 3 weeks post inoculation with *M. loti*. (C) RT-qPCR analysis of *LjNSP2L* expression in WT and pUB::*LjNSP2L* roots grown under OPi or LPi conditions at 7dpi. Transcript levels were normalized to the endogenous ubiquitin gene. Relative expression values are shown on a log2 scale. Data are means $\pm$ SE. Different letters indicate statistically significant differences among groups in (B) ($P < 0.05$). Asterisks indicate statistically significant differences between indicated -comparisons in (C) (** $P < 0.01$); ns, not significant. Scale bars = 2 mm.