Supporting Information

Heng Kang et al.

A MYB coiled-coil transcription factor interacts with NSP2 and is involved in nodulation in *Lotus japonicus*

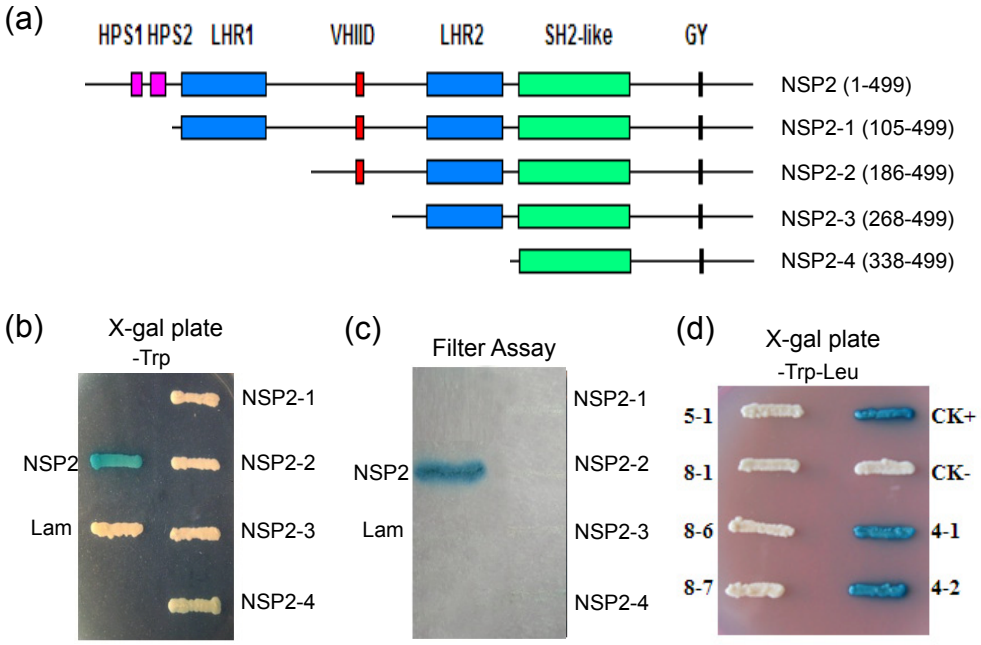
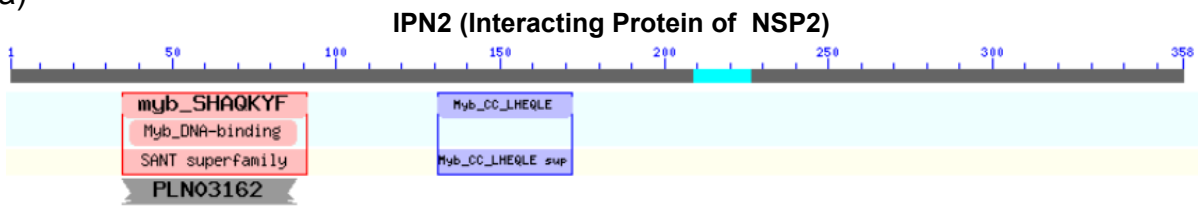


Fig. S1 Screens of NSP2-interaction proteins. (a) Schematic illustrations of NSP2 deletions. HPS, homopolymeric streches characteristic GRAS protein near to the N-terminus; LHR1 and LHR2, leucine heptad repeat; VHIID, putative DNA-binding sites; SH2, *Src*-homology 2. (b) Transcriptional activation assay of NSP2 was performed in the yeast strain Y187, which contains the *lacZ* reporter gene. Plasmids expressing either a fusion protein of the GAL4 DNA-binding domain with NSP2 or its deletion fragments were transformed into yeast strain Y187. The yeast cells were streaked on the SD/-Trp plate containing X-Gal (80µg/mL) for ß-galactosidase activity assay. When the N-terminal domain of NSP2 (1-105) is removed from NSP2 (105-499), no autoactivation was observed. The protein Lam (Clontech) was used as a negative control. (c) Colony-lift filter assay the same plate in (b). This method is more sensitive than in vivo plate assay using X-gal in the medium and can detect slight autoactivation. (d) Screening with the GRAS domain of NSP2 (105-499) revealed six clones on stringent selective medium (SD-Trp-Leu-His-Ade) and only two clones (4-1, 4-2) developed blue color on SD/-Trp-Leu plate containing X-Gal. The two clones showed identical nucleotide sequence with coding a gene named as IPN2. The combination of BD-53/AD-SV40 was used as CK+ and BD-Lam/AD-SV40 as CK- (Clontech).

(a)



(b)

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  1 MERMFPPKKPSTMNSHDRPMCVQGDSGLVLTTDPKPRLRWTVELHERFVDAVTQLGGPDK
 61 ATPKTIMRVMGVKGLTLYHLKSHLQKFRLGKQPHKEFNDHSIKDGMRASALELQRNTASS
121 SAMIGRNMNEMQIEVQRRLHEQLEVQKHLQLRIEAQGKYMQSILEKAYQTLAGENMASAA
181 TNLKGIGPQTIPDMGIMKEFGSPLGFSFQDLDLYGGGGGDQLELQQNMEKPPLDGFMPMN
241 HENLCLGKKRPNPYSGNNGKSPLMWSDDLRLQDLGSCLQDDPFKGDHHHQIQIAPPSLDR
301 GTEMDPMSEIYDSKPEEKKFDASMKLERPSPRRAPLGERMSPMITTGTMAQGRSSPFG
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Fig. S2 Structure features of IPN2 protein. (a) The National Center for Biotechnology Information (NCBI) conserved domain search of the IPN2 protein sequence identified an N-terminal region with homology to the SHAQKYR type myb_DNA binding domain belonging to the SANT (SWI3, ADA2, N-CoR and TFIIIB) superfamily and a coiled-coil domain with LHEQLE motif. (b) The deduced amino acid sequence of IPN2 contains 358 amino acid residues with a calculated molecular mass of approximately 40 kDa. The Myb-like domain and coiled-coil domain are highlighted in red and blue, respectively. A putative nuclear localization signal is underlined.

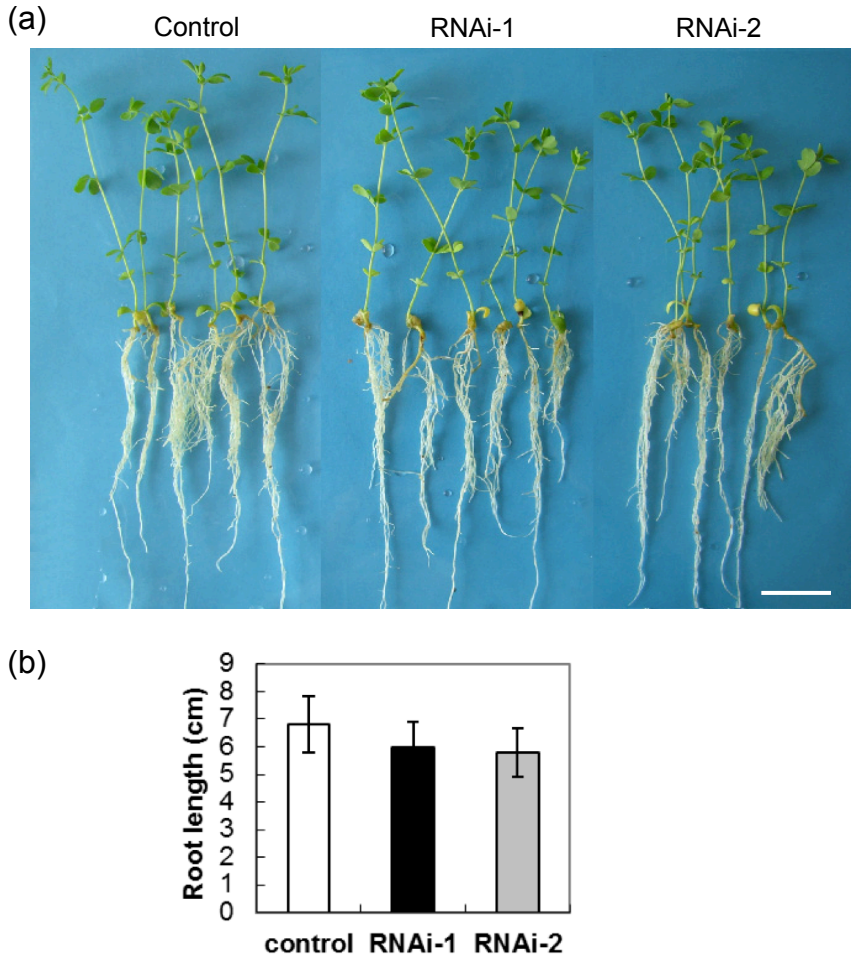

Fig. S3 Growth phenotypes of *IPN2*-RNAi roots under nonsymbiotic conditions. (a) Six representative images of plants transformed with *IPN2* RNAi-1 and RNAi-2 construct compared with plants that were transformed with an empty vector (control). The plants were grown under the condition same as the nodulation assay and the photographs were taken at 3 weeks after transferred to growth pots. Bar, 2 cm. (b) Total root length of vector control and two RNAi constructs transgenic roots were determined at 3 weeks. Histograms represent of 30 independent lines of each construct.

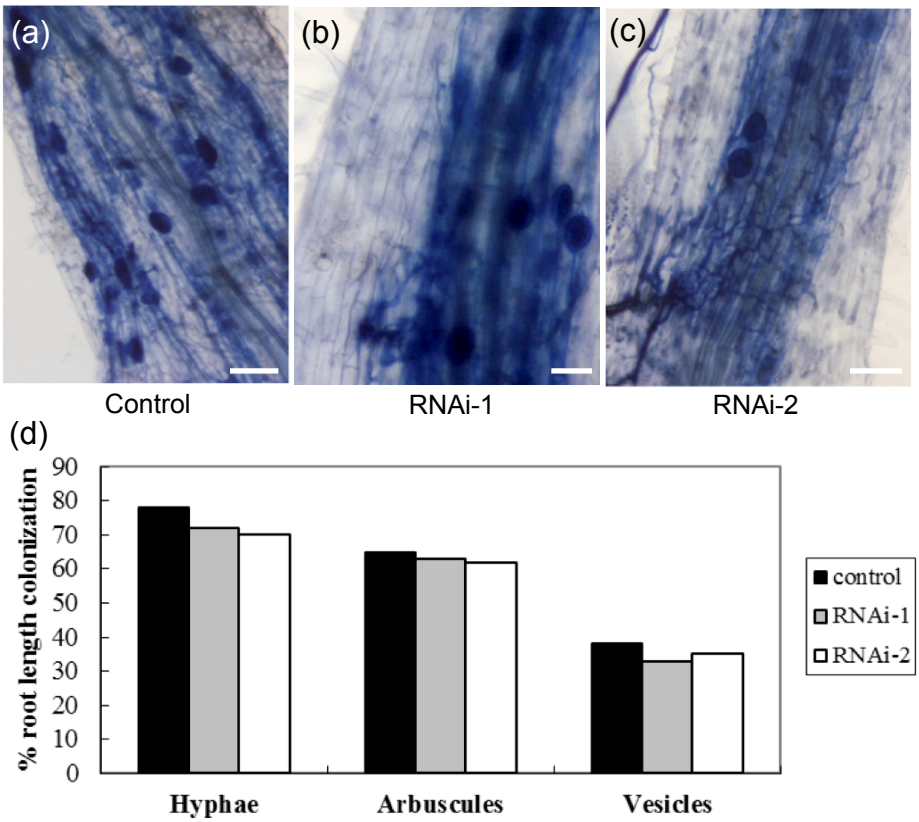

Fig. S4 Mycorrhization phenotypes of *IPN2* RNAi roots. (a) to (c) Light micrograph of trypan blue-stained roots 3 weeks after inoculation with *Glomus intraradices*. In the *IPN2* RNAi-1 (b) and RNAi-2 (c) roots, the AM fungus penetrated into the outer cell layers, colonized the root cortex, and formed arbuscules (ar) and vesicles (v), which did not differ from the vector control (a). (d) Mean hyphae colonization (Hyphae, %), arbuscular colonization (Arbuscules, %), and vesicular colonization (Vesicles, %) per root from vector control and two RNAi constructs transgenic roots after 3 weeks of cocultivated with *G. intraradices*. The data are presented as 15 individual transgenic plants each construct, and randomly scored in 4 roots per plant. Bars, 25 μm.