Supplements to

Phytosulfokine Is Involved in Positive Regulation of Lotus japonicus Nodulation

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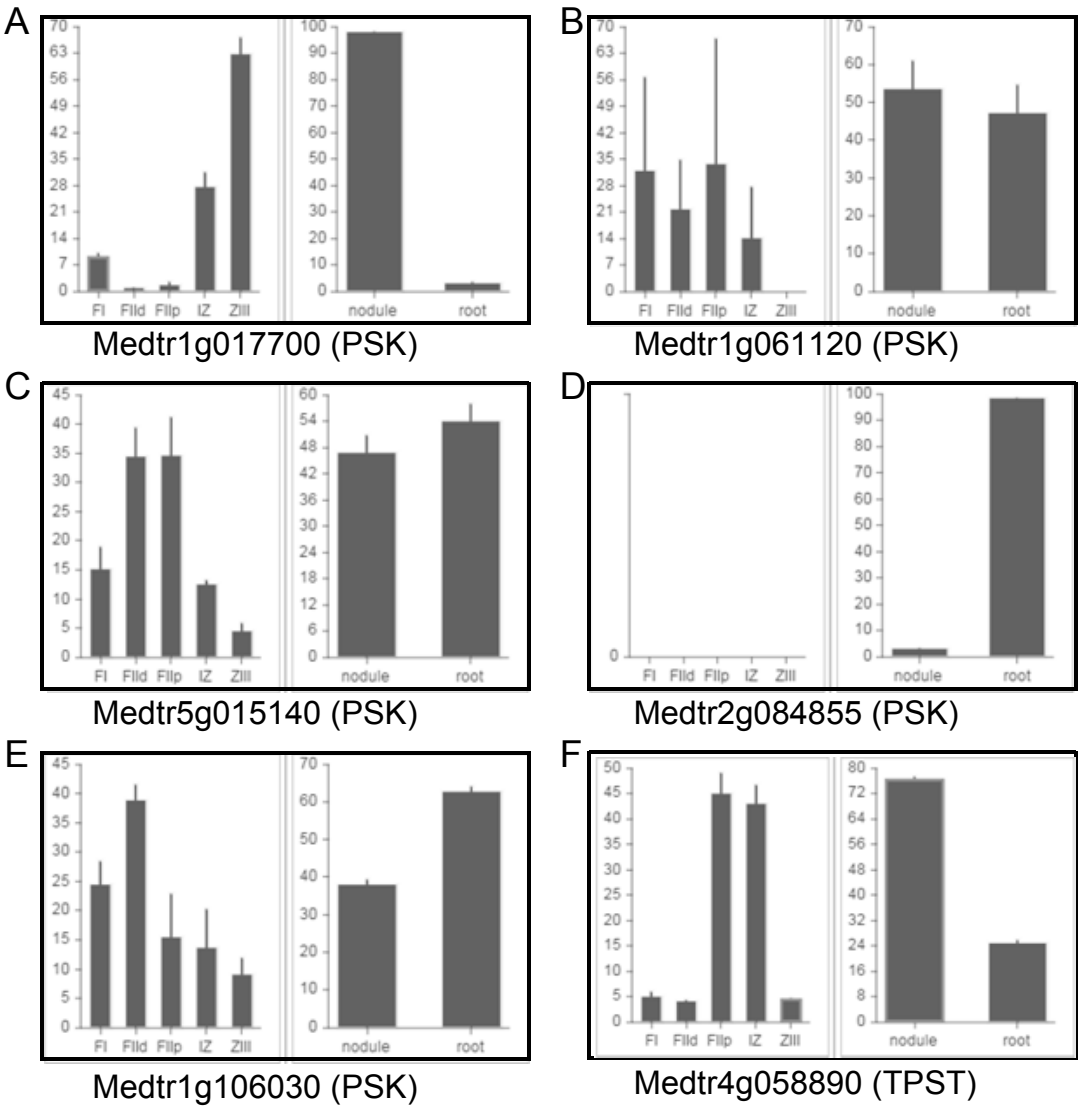
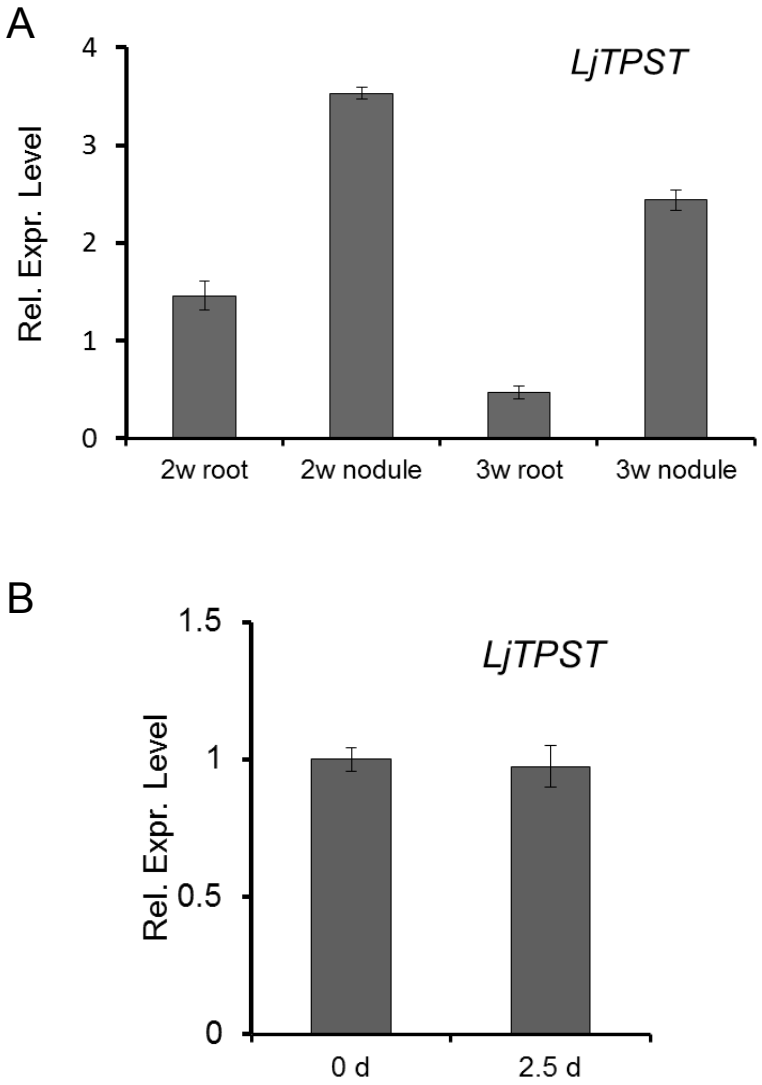
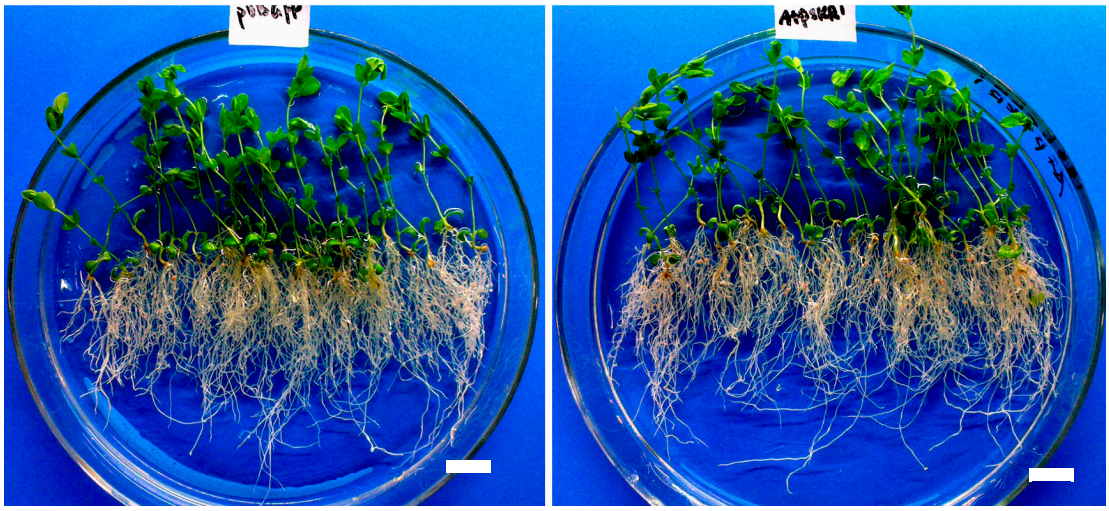


Supplementary Fig. S1. Expression analysis of *MtPSK* genes in roots and nodules. The transcriptomic data were extracted from the public database *symbimics* (https://iant.toulouse.inra.fr/symbimics/). Nodules were divided into five regions, from the apical meristem (FI) to the nitrogen fixation zone (ZIII) (Roux et al. 2014). The region below apical meristem (FI) was collected as a distal fraction (FIId) and a proximal fraction (FIIp), corresponding to ZII cells undergoing differentiation and infection, respectively. IZ represents the interzone II–III, which separates ZII from the nitrogen-fixation zone ZIII.

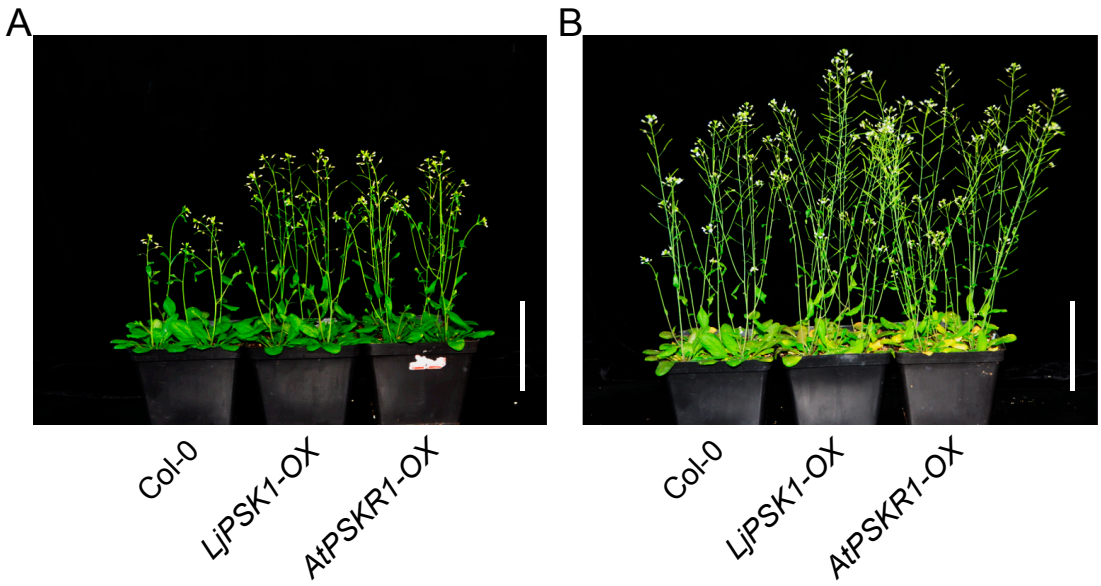


Supplementary Fig. S2. Expression analysis of *LjTPST* in roots and nodules. A. The *LjTPST* mRNA level was measured in 2-3 week-old roots and nodules, indicating constitutive expression of *LjTPST* in both tissues. The expression levels of *LjTPST* in nodules were higher than those in roots. B. The *LjTPST* mRNA level did not change significantly in the early stage of rhizobial inoculation. Error bars represents ± SE of two biological replicates.

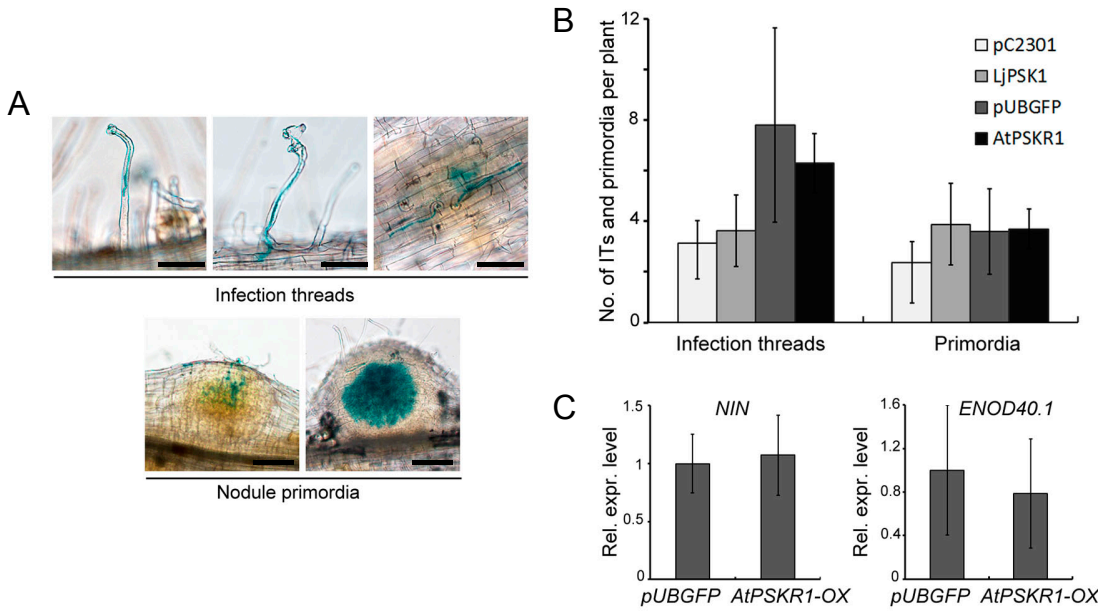


pUBGFP pUBGFP-*AtPSKR1*

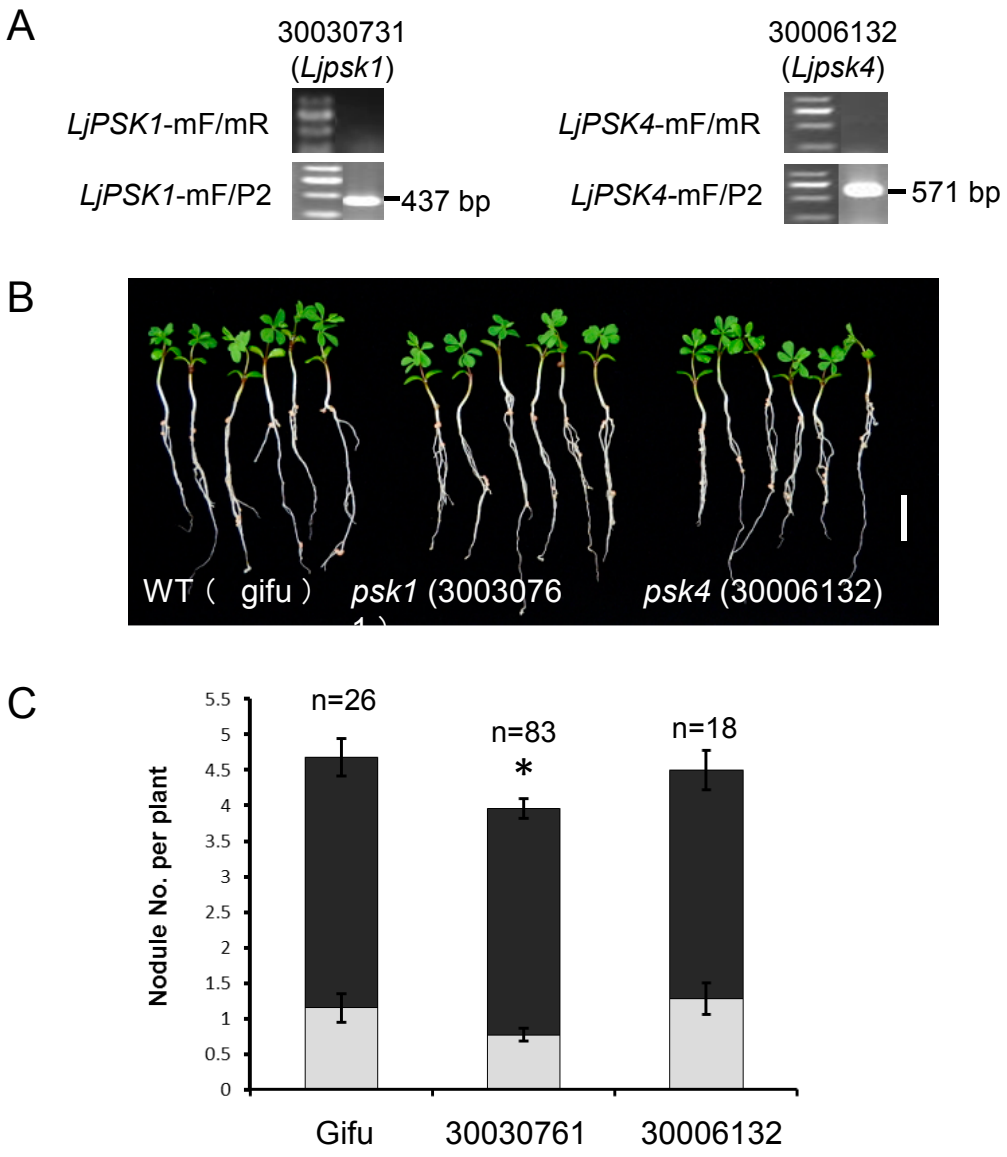
Supplementary Fig. S3. Phenotype of transgenic hairy roots expressing *AtPSKR1*. Transgenic hairy roots of *L. japonicus* were identified (see Method). The chimeric plants were transferred to growth cabinets, and supplied with half-strength B&D nitrogen-free medium for 6 days. Plants were then inoculated with *M. loti*. The roots and nodules were observed 12 days after inoculation. There was no significant difference in root growth between the control expressing the empty vector and the *AtPSKR1*-expressing line. Promoted nodulation was observed on the hairy roots expressing *AtSPKR1*. Bars, 1cm.



Supplementary Fig. S4. Vegetative growth of *Arabidopsis* plants overexpressing *LjPSK1-OX* or *AtPSKR1-OX*. Vegetative growth of transgenic *Arabidopsis* plants overexpressing *LjPSK1* or *AtPSKR1* were observed at 30th day (A) and 36th day (B) after transplanting to the growth cabinets. Wild type Col-0 plants served as a control. Results show promoted plant growth by overexpressing *LjPSK1* or *AtPSKR1*. Bars, 10 cm.



Supplementary Fig. S5. Rhizobial infection assay of transgenic hairy roots expressing *LjPSK1-OX* or *AtPSKR1-OX*. Transgenic hairy roots were inoculated with *M. loti* strain MAFF303099 that constitutively expresses a *lacZ* marker. Rhizobial infection and nodule primordium phenotypes were examined 7 days after inoculation from 10-15 transgenic roots. Infection threads and nodule primordia were photographed (A) and scored (B). Values represented the average (±SE) of total infection events or primordia per plant. Expression of early nodulation genes *NIN* (C) and *ENOD40* (D) in noninoculated hairy roots was not affected by overexpressing *AtPSKR1*. Values indicated average (±SD) of expression levels of 3 *AtPSKR1-OX* lines (n=3) and control hairy root lines (n=3).



Supplementary Fig. 6. Phenotypes of *Lotus japonicus psk1 and psk4* mutant plants. A. PCR assays of genomic DNA showing the identification of the *LORE1* insertion in homozygous mutant lines of *L. japonicus psk1 and psk4*. Primer pair F/P2 was used to amplify the insertion fragment, and F/R was used to amplify the uninserted genome. The PCR products correctly match the predicted sizes of DNA fragments as shown in agarose gels. The identity of these fragments were also confirmed by DNA sequencing. B. Normal root growth and nodulation in *L. japonicus psk1* and *psk4* mutant plants. Bar, 1 cm. C. Effect on nodule numbers per plant. Nodule numbers per plant were reduced slightly in *psk1* mutant. The effect was weak, yet

statistically significant (P = 0.0113). No significant change in nodule numbers per plant was observed in *psk4* mutant. Bar, 1cm.