Supporting experimental procedures

Methods S1

Plasmid construction

For promoter-*GUS* analysis and complementation tests, Gateway compatible destination vectors, *pEpi168::GW-p35S::GFP* and *pEpi308::GW-p35S::GFP*, were constructed as follows: Genomic sequences of the *LjEXPA7* promoter regions (168 and 308 bp) were amplified by PCR. Each fragment was digested with DraI and SpeI and then inserted into the HindIII (blunt)-SpeI site of *p35S::GW-p35S::GFP* (*P35S:GFP-gw*; Banba *et al.*, 2008). For the construction of the *pEpi400::GW-p35S::GFP* vector, the genomic sequence of the *LjEXPA8* promoter region (400 bp) was amplified by PCR. The fragment was digested with HindIII and SpeI and then ligated with *p35S::GW-p35S::GFP*. For co-transformation with CCaMK under the control of *pEpi308* and an inactive truncated CCaMK under the control of *p35S*, a Gateway compatible destination vector, *pEpi308::CCaMK-p35S::GW*, was constructed by introducing the reading frame cassette C.1 (RfC.1) of the Gateway vector conversion system (Invitrogen, Carlsbad, CA, USA) into the XhoI (blunt)-XhoI (blunt) site of *pEpi308::CCaMK-p35S::GFP*. All primer sets used for construction are listed in Table S2.

Information of entry clones of symbiotic genes and *GUSplus* (*GUS*$^{+}$) and conversion of these entry vectors into *pEpi* destination vectors are described below. cDNA sequences of *NFR5* and *NUP85* were amplified by PCR and cloned into pENTR/D-TOPO (Invitrogen, Carlsbad, CA, USA). cDNA sequence of *NUP133* was amplified by PCR and digested with *Sac*II and *Asc*I and then inserted into the

SacII-AscI site of pENTR/D-TOPO. The entry clones constructed in this study, and entry clones of *GUS*$^{+}$ (Yano *et al.*, 2008), *NFR1* (Nakagawa *et al.*, 2011), *CASTOR*, *POLLUX*, *CCaMK*, *CCaMK*^{T265D} (Banba *et al.*, 2008), an inactive truncated *CCaMK* (*CCaMK1-340*) (Shimoda *et al.*, 2012), *CYCLOPS* (Yano *et al.*, 2008), *NSP1* (Yokota *et al.*, 2010) and *NSP2* (Yokota *et al.*, 2010), were converted with the destination vector of *pEpi308::GW-p35S::GFP* by the LR reaction (Gateway LR clonase II Enzyme Mix; Invitrogen, Carlsbad, CA, USA).

Methods S2

RNA isolation from root hairs

Surface-sterilized seeds were germinated on sterilized filter paper on 1.5% agar containing half-strength B&D medium (Broughton and Dilworth 1971) in square plastic plates (Eiken Chemical Co., Tokyo, Japan, http://www.eiken.co.jp/). The plastic plates were incubated vertically for 2 days in a growth cabinet with a 16 h-day/8 h-night cycle at 24°C, and then the seedlings were inoculated with a suspension of DsRed-labeled *M. loti*. The plants were grown for an additional 2 days with the roots shielded from light. The roots were then collected and dropped immediately into a 100-ml stainless steel beaker filled with liquid nitrogen. The samples were stirred gently with a magnetic stirrer for 20 min. In this step, the root hairs were cut off from the roots. The liquid nitrogen containing root hairs was then filtered through a stainless testing sieve with a mesh aperture of 500 μm. Liquid nitrogen was evaporated in a 50-mL sample tube and root hairs were collected. The roots trapped by the testing sieve were also collected, as "stripped roots". Total RNAs were isolated by the CTAB method (Chang *et al.*, 1993) with some modifications. Briefly, the collected root hairs or stripped roots were ground

to a fine powder in liquid nitrogen, immediately dissolved in the extraction solution (2% CTAB, 100 mM Tris pH 9.5, 20 mM EDTA, 1.4 M NaCl, 1% 2-mercaptoethanol), and then incubated at 65°C for 10 min. After two successive extractions with chloroform, RNAs were precipitated using LiCl (final concentration 2.5 M) at -20°C overnight. The precipitated RNAs were collected by centrifugation, and were dissolved in distilled water. As necessary, the samples were further purified using an RNeasy Mini Kit (Qiagen, http://www.qiagen.com/) according to the manufacturer's instructions.

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