eLIFE
elifesciences.org

Figures and figure supplements

Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases

Martina Katharina Ried, et al.

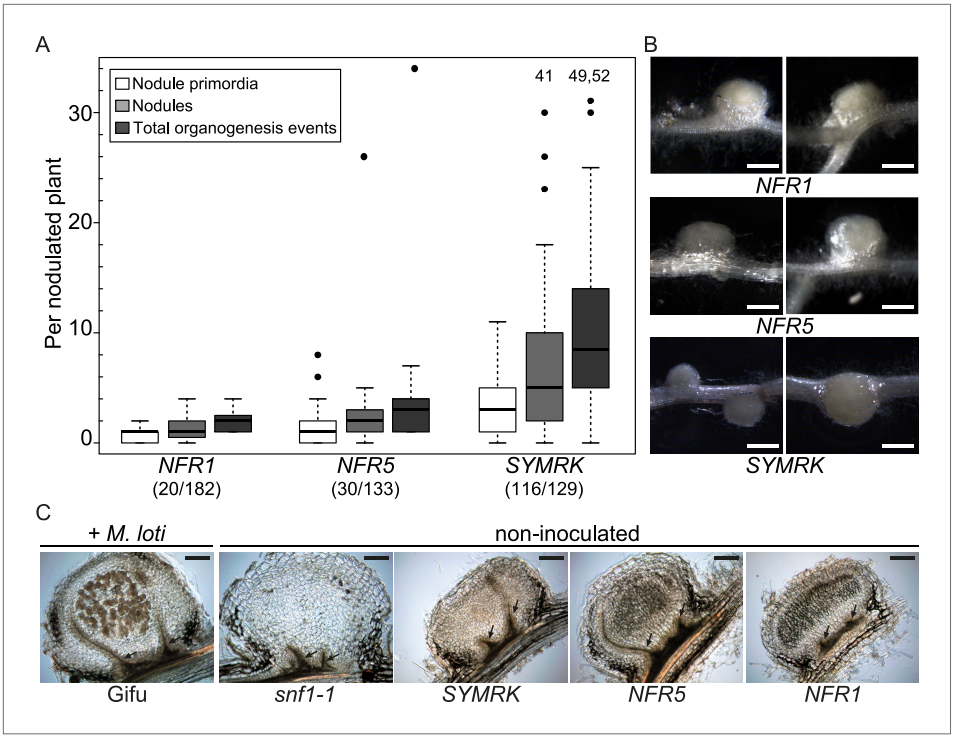


Figure 1. Symbiotic *RLKs* mediate spontaneous formation of root nodules. Hairy roots of *L. japonicus* Gifu wild-type transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. (**A**) Plot represents the numbers of nodule primordia (white), nodules (light grey) and total organogenesis events (dark grey; nodules and nodule primordia) per nodulated plant formed in the absence of rhizobia at 60 dpt. Number of nodulated plants per total plants is specified under each line label. Black dots, data points outside 1.5 interquartile range (IQR) of the upper quartile; numbers above upper whiskers indicate the values of individual data points outside of the plotting area. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. Plants transformed with the empty vector did not develop spontaneous nodules. (**B**) Pictures of spontaneous nodules on hairy roots expressing the indicated transgenes taken 60 dpt. Bars, 1 mm. (**C**) Micrographs of sections of spontaneous nodules on hairy roots expressing the indicated transgenes harvested at 60 dpt. Spontaneous nodules of 10-week-old *snf1-1* mutant plants were used as controls. Nodules of 10-week-old untransformed *L. japonicus* wild-type Gifu 6 weeks after inoculation with *M. loti* MAFF303099 *Ds*RED contained cortical cells filled with bacteria (brown colour) that are absent in spontaneous nodules. Arrows point to peripheral vascular bundles. Longitudinal 40 mm sections. Bars, 150 μm.

DOI: 10.7554/eLife.03891.003

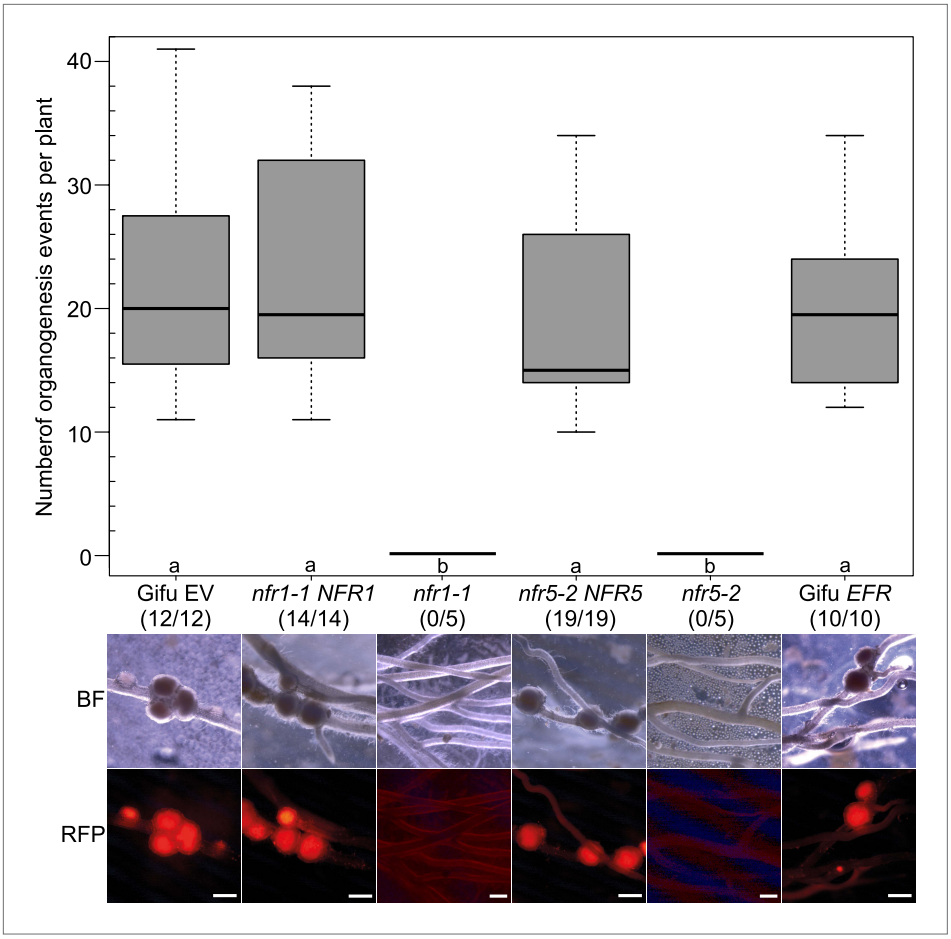


Figure 1—figure supplement 1. Expression of *NFR1* and *NFR5* from the *Ubiquitin* promoter restores nodulation in the *nfr1-1* and *nfr5-2* mutants, respectively. Hairy roots of *L. japonicus* Gifu wild-type transformed with the empty vector (EV) or with *pUB:EFR-mOrange* (*EFR*), the *nfr1-1* mutant transformed with *pUB:NFR1-mOrange* (*NFR1*) or the *nfr5-2* mutant transformed with *pUB:NFR5-mOrange* (*NFR5*) were generated. Untransformed *nfr1-1* and *nfr5-2* mutant plants served as control. Plot represents the number of organogenesis events (nodules and nodule primordia) per plant formed 15 days post inoculation with *M. loti Ds*RED. Numbers below each line label indicate the number of nodulated plants per total analysed plants. Representative pictures are shown. BF, bright field; RFP, RFP filter. Bars, 1 mm. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal–Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

DOI: 10.7554/eLife.03891.004

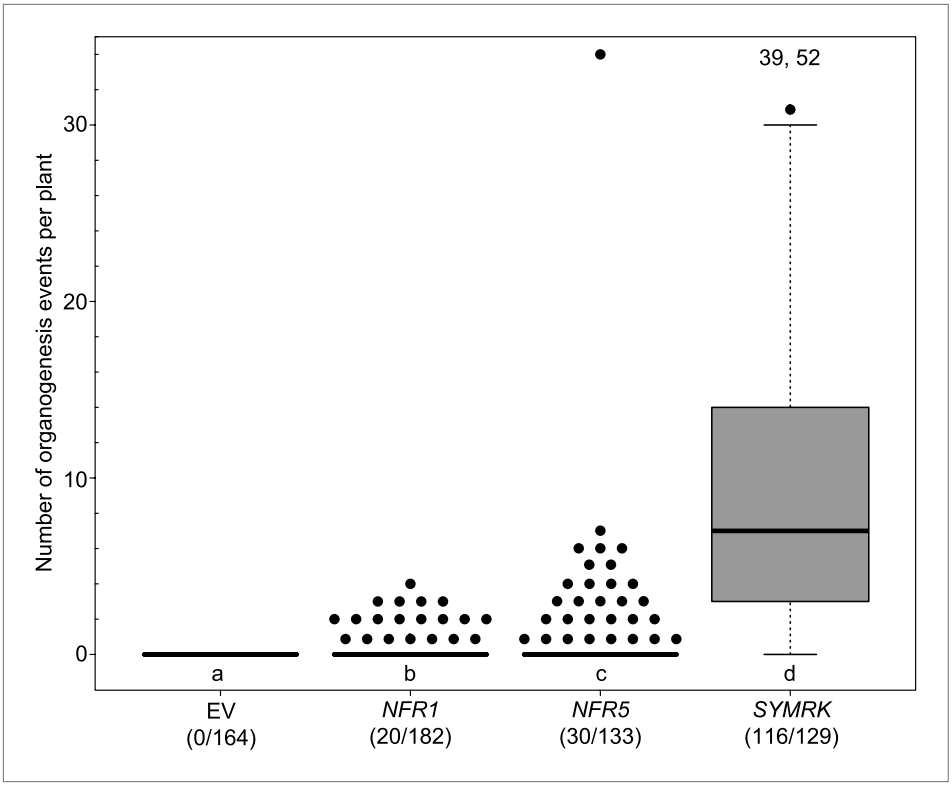


Figure 1—figure supplement 2. Statistical analysis of spontaneous root nodule formation. Hairy roots of *L japonicus* Gifu wild-type transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. Plot represents the numbers of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 60 dpt. Number of nodulated plants per total plants is specified under each line label. Black dots, data points outside 1.5 IQR of the upper quartile; numbers above upper whiskers indicate the values of individual data points outside of the plotting area. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal–Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

DOI: 10.7554/eLife.03891.005

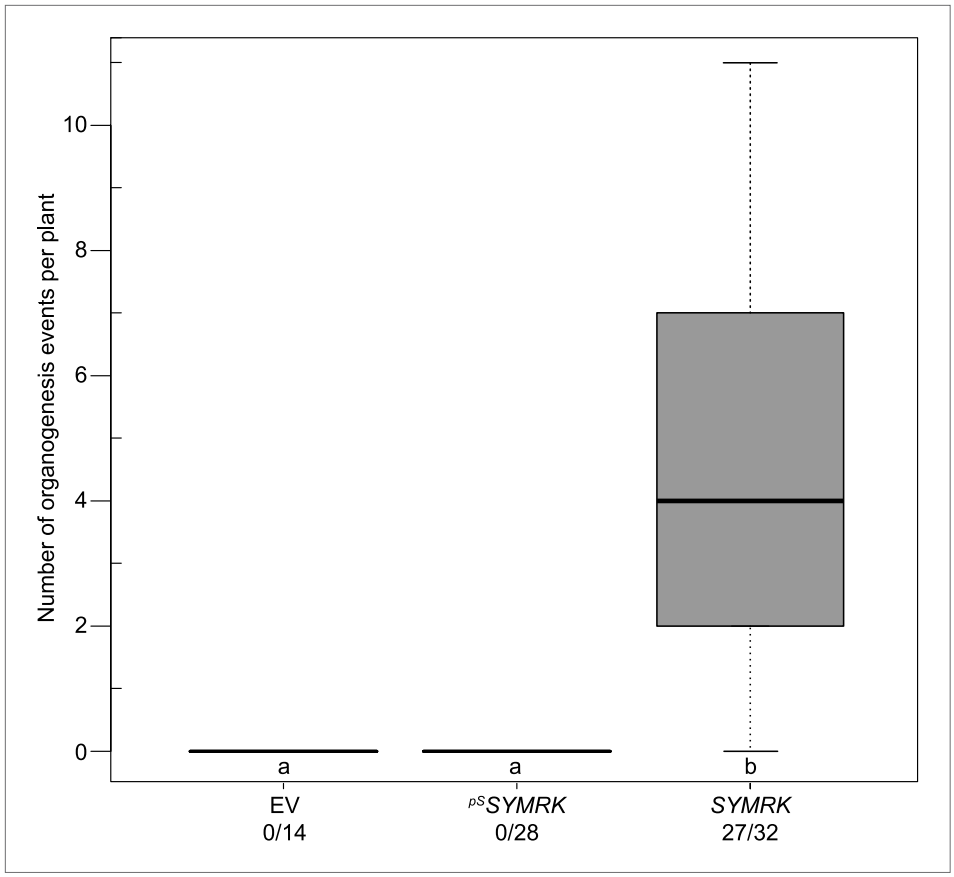


Figure 1—figure supplement 3. Expression of *SYMRK* from the native *SYMRK* promoter does not mediate spontaneous formation of root nodules. Hairy roots of *L. japonicus symrk-3* transformed with the empty vector (EV), *pUB:SYMRK-mOrange* (*SYMRK*), or *pSYMRK:SYMRK-RFP* (*^{pS}SYMRK*) were generated. Plot represents the number of total organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 21 dpt. Number of nodulated plants per total plants is specified under each line label. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal–Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.
DOI: 10.7554/eLife.03891.006

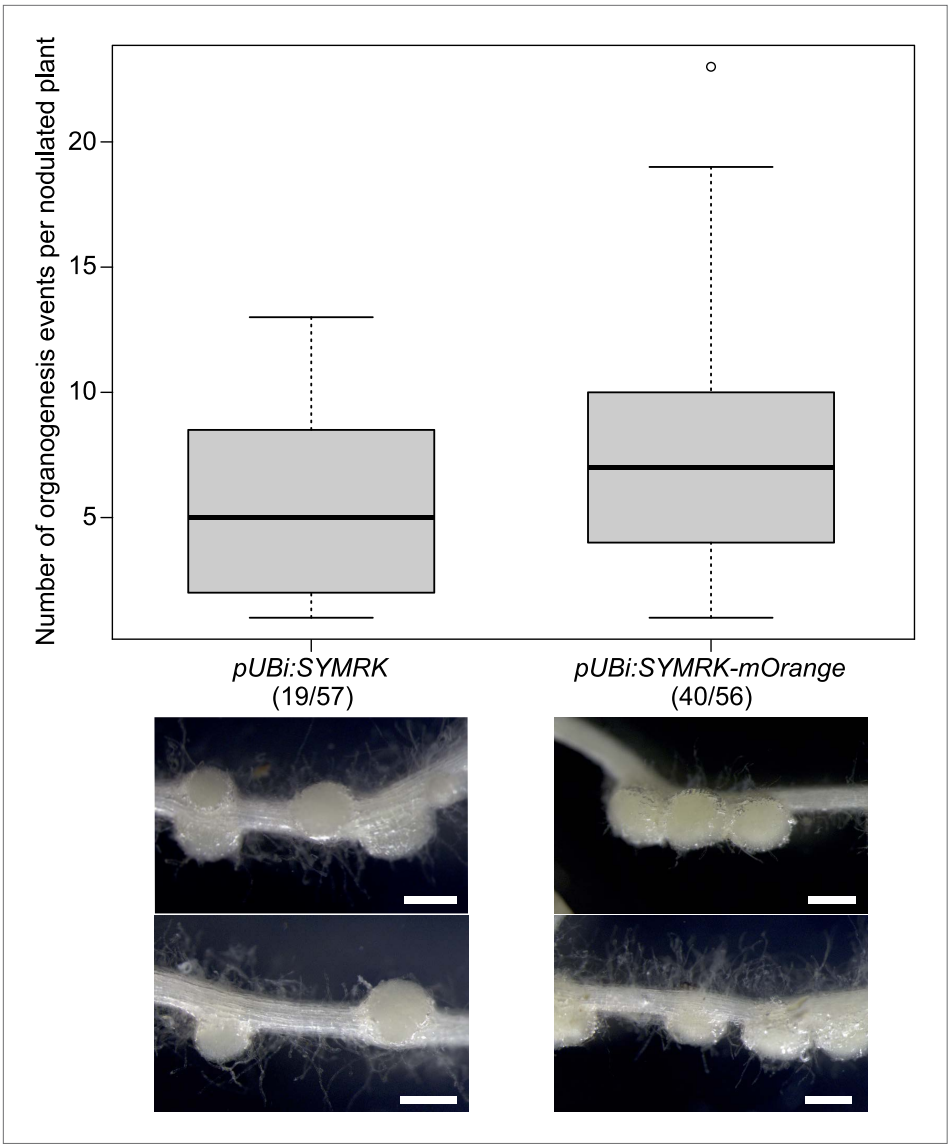


Figure 1—figure supplement 4. Expression of non-tagged *SYMRK* from the *Ubiquitin* promoter induces spontaneous formation of root nodules. Hairy roots of *L. japonicus symrk-3* transformed with *pUBi:SYMRK* (untagged) or *pUBi:SYMRK-mOrange* (C-terminally tagged) were generated. Plot represents the number of total organogenesis events (nodules and primordia) per nodulated plant formed in the absence of rhizobia at 42 dpt. Number of nodulated plants per total plants is specified under each line label. Dot, data point outside 1.5 interquartile range of the upper quartile. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. Plants non-transformed or transformed with the empty vector did not develop spontaneous nodules. A Kruskal–Wallis test followed by false discovery rate correction was performed for total organogenesis events per nodulated root system (p-value of 0.16) and for total organogenesis events per transformed root system (p-value of 1.2e-05). Numbers below each line label indicate the number of nodulated plants per total analysed plants. Representative pictures are shown. Bars, 0.5 mm.

DOI: 10.7554/eLife.03891.007

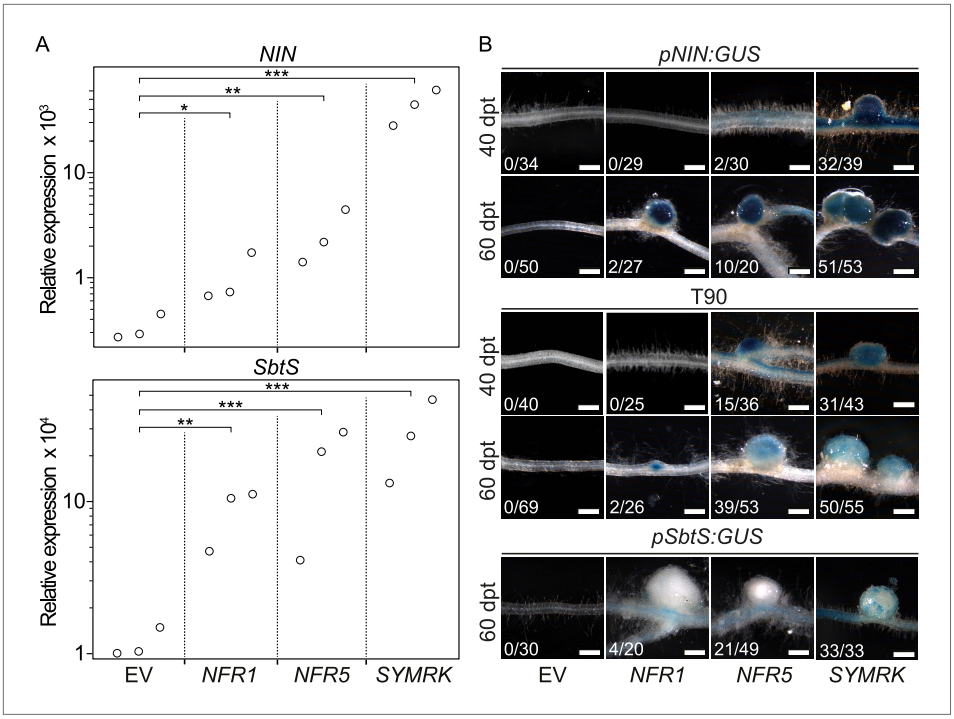


Figure 2. Symbiotic RLKs mediate spontaneous symbiosis-related signal transduction. Hairy roots of *L. japonicus* Gifu wild-type (**A**) or of three stable transgenic *L. japonicus* Gifu reporter lines (**B**)—carrying either the T90 reporter fusion, a *NIN* promoter:GUS fusion (*pNIN:GUS*), or a *SbtS* promoter:GUS fusion (*pSbtS:GUS*)—transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. (**A**) Relative expression of *NIN* or *SbtS* at 40 dpt was determined in three biological replicates for each treatment via qRT-PCR. Transcript levels in each replicate were determined through technical duplicates. Expression was normalized with the house keeping genes *EF1alpha* and *Ubiquitin*. Circles indicate expression relative to the *EF1alpha* gene. A Dunnett's test was performed comparing the transcript levels of *NIN* or *SbtS* detected for each treatment with those detected in the empty vector samples. Stars indicate significant differences from the EV control. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. (**B**) β-glucuronidase (GUS) activity was analysed by histochemical staining with 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) 40 and 60 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per number of total plants is indicated. Bars, 500 µm.

DOI: 10.7554/eLife.03891.008

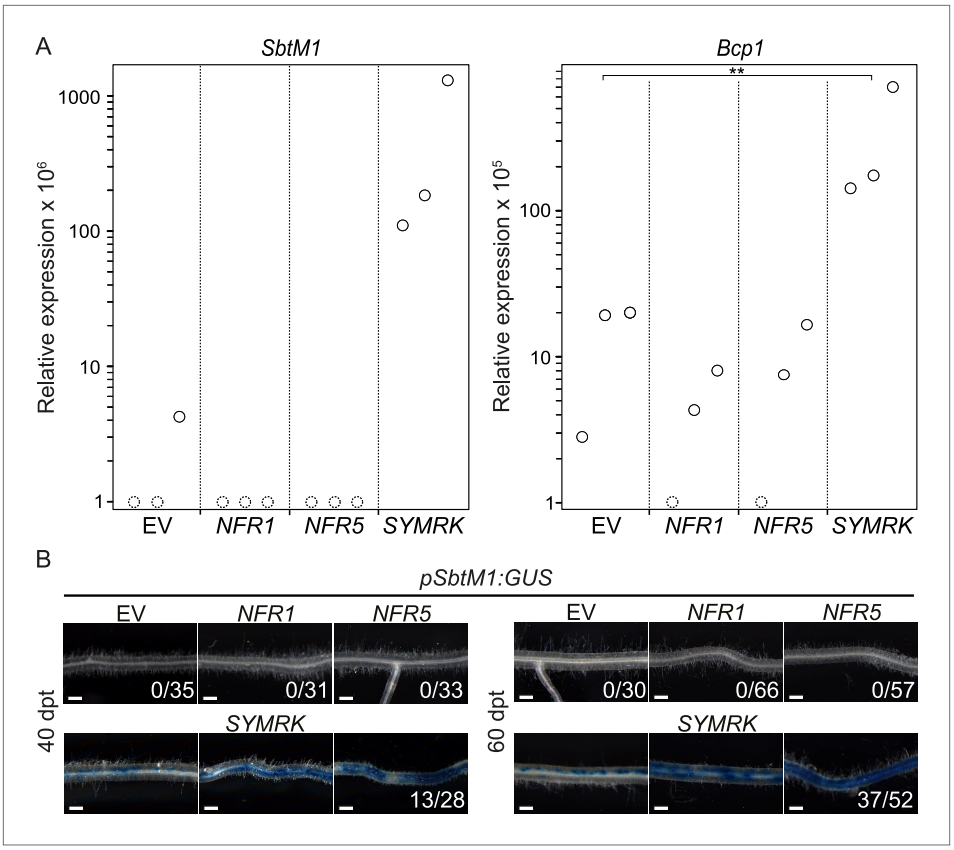


Figure 3. *SYMRK* mediates spontaneous AM-related signal transduction. Hairy roots of *L. japonicus* Gifu wild-type (**A**) or a stable transgenic L. *japonicus* MG20 reporter line carrying a *SbtM1* promoter:*GUS* fusion (*pSbtM1:GUS*) (**B**) transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. (**A**) Relative expression of *SbtM1* or *Bcp1* at 40 dpt was determined in three biological replicates for each treatment via qRT-PCR. Transcript levels in each replicate were determined through technical duplicates. Expression was normalized with the house keeping genes *EF1alpha* and *Ubiquitin*. Circles indicate expression relative to the *EF1alpha* gene. Dashed circles indicate that no transcripts could be detected for this sample. Samples in which the indicated transcript could not be detected were floored to 1. A Dunnett's test was performed comparing the transcript levels of *Bcp1* detected for each treatment with those detected in the empty vector samples. Stars indicate significant differences. **, $p < 0.01$. (**B**) GUS activity was analysed by histochemical staining with X-Gluc 40 and 60 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per total plants is indicated. Bars, 500 µm.

DOI: 10.7554/eLife.03891.009

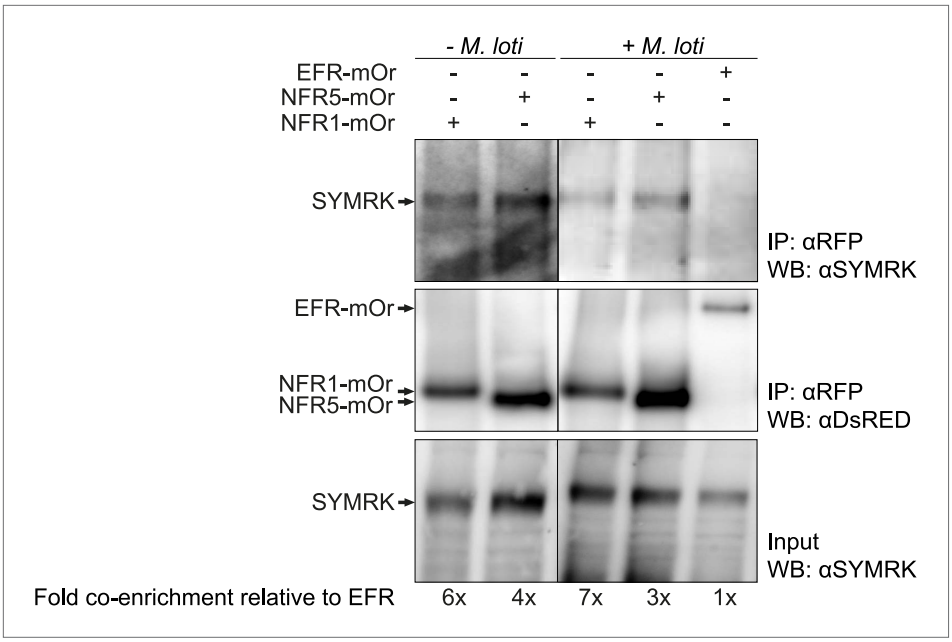


Figure 4. SYMRK associates with NFR1 and NFR5 in *Lotus japonicus* roots. Hairy roots of *L. japonicus* Gifu wild-type roots expressing *NFR1-mOrange* (NFR1-mOr), *NFR5-mOrange* (NFR5-mOr), or *EFR-mOrange* (EFR-mOr) under the control of the *Ubiquitin* promoter were extracted 10 days post inoculation with *M. loti Ds*RED or mock treatment. mOrange fusions were affinity bound with RFP magneto trap, and immuno-enrichment was monitored by immunoblot with and anti*Ds*RED antibody. Co-enrichment of endogenous SYMRK protein was monitored by immunoblot with an antiSYMRK antibody. Numbers below the western blot panels indicate the fold co-enrichment of SYMRK by NFR1 or NFR5 relative to the amount of SYMRK co-enriched with EFR. mOr, mOrange; IE, immuno-enrichment; WB, western blot.

DOI: 10.7554/eLife.03891.010

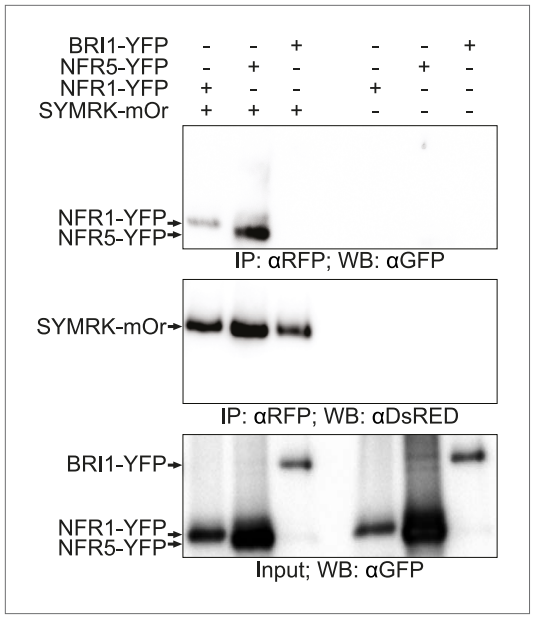
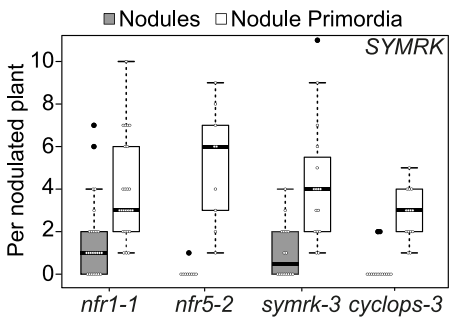


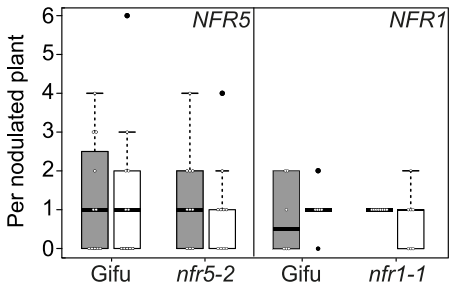
Figure 4—figure supplement 1. Full-length SYMRK associates with NFR1 and NFR5 in *Nicotiana benthamiana* leaves. *N. benthamiana* leaves were transiently co-transformed with constructs expressing NFR1-YFP, NFR5-YFP, or BRI1-YFP together with SYMRK-mOrange under the control of the CaMV 35S promoter. Leaf discs expressing the respective constructs were extracted 3 dpt. SYMRK-mOrange was immuno-enriched with RFP magnetotrap and monitored by immunoblot with an anti*Ds*RED antibody. Co-enrichment of NFR1-YFP, NFR5-YFP, or BRI1-YFP was monitored by immunoblot with an antiGFP antibody. mOr, mOrange; IE, immuno-enrichment; WB, western blot.

DOI: 10.7554/eLife.03891.011



Nodulated / total number of plants

Background	*SYMRK*	*^{pS}SYMRK*
nfr1-1	25/30	0/12
nfr5-2	9/12	0/13
symrk-3	16/21	0/8
nup133-1	0/14	-
castor-12	0/9	-
pollux-2	0/17	-
ccamk-13	0/9	-
cyclops-3	14/29	0/16



Background	*NFR5*	EV	*NFR1*	EV
Gifu	11/55	0/22	6/60	0/24
nfr1-1	0/42	0/12	7/56	0/23
nfr5-2	10/39	0/10	0/17	0/22
symrk-3	0/30	0/16	0/40	0/19
symrk-10	0/30	0/10	0/57	0/19
castor-12	0/25	0/14	-	-
ccamk-13	0/32	0/12	-	-
cyclops-3	0/22	0/15	-	-

Figure 5. Epistatic relationships between symbiotic *RLK* genes and common symbiosis genes. Hairy roots of *L. japonicus* Gifu wild-type and different symbiosis defective mutants transformed with *pUB:SYMRK-mOrange* (*SYMRK*) or *pSYMRK:SYMRK-RFP* (*^{pS}SYMRK*) (upper panel), or the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*) or *pUB:NFR5-mOrange* (*NFR5*) (lower panel) were generated. Plots represent the numbers of nodules (grey) and nodule primordia (white) per nodulated plant formed in the absence of rhizobia at 40 (*SYMRK*) and 60 (*NFR5* + *NFR1*) dpt. White circles indicate individual organogenesis events. Black dots, data points outside 1.5 IQR of the upper/lower quartile; bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. Table, fraction of nodulated per total number of plants. Plants transformed with *pSYMRK:SYMRK-RFP* or the empty *pUB* vector did not develop spontaneous nodules.

DOI: 10.7554/eLife.03891.012

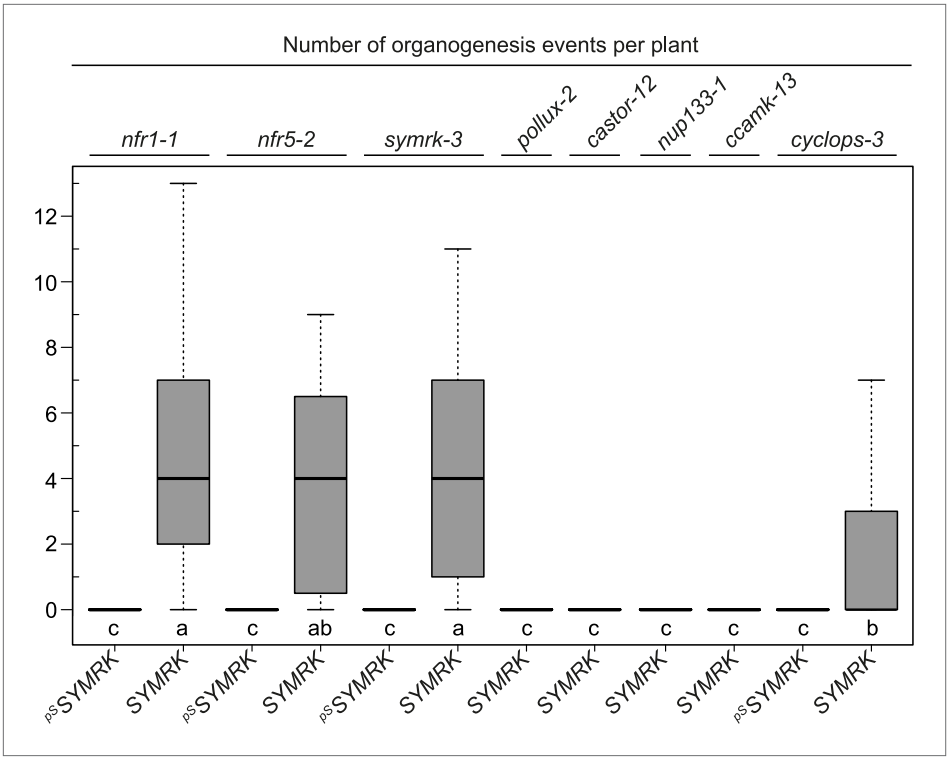


Figure 5—figure supplement 1. *SYMRK*-mediated spontaneous organogenesis events in *nfr1-1*, *nfr5-2*, and common symbiosis mutants. Hairy roots of different symbiosis defective mutants transformed with *pUB:SYMRK-mOrange* (*SYMRK*) or *pSYMRK:SYMRK-RFP* (^{pS}*SYMRK*) were generated. Plot represents the numbers of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 40 dpt. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal–Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

DOI: 10.7554/eLife.03891.013

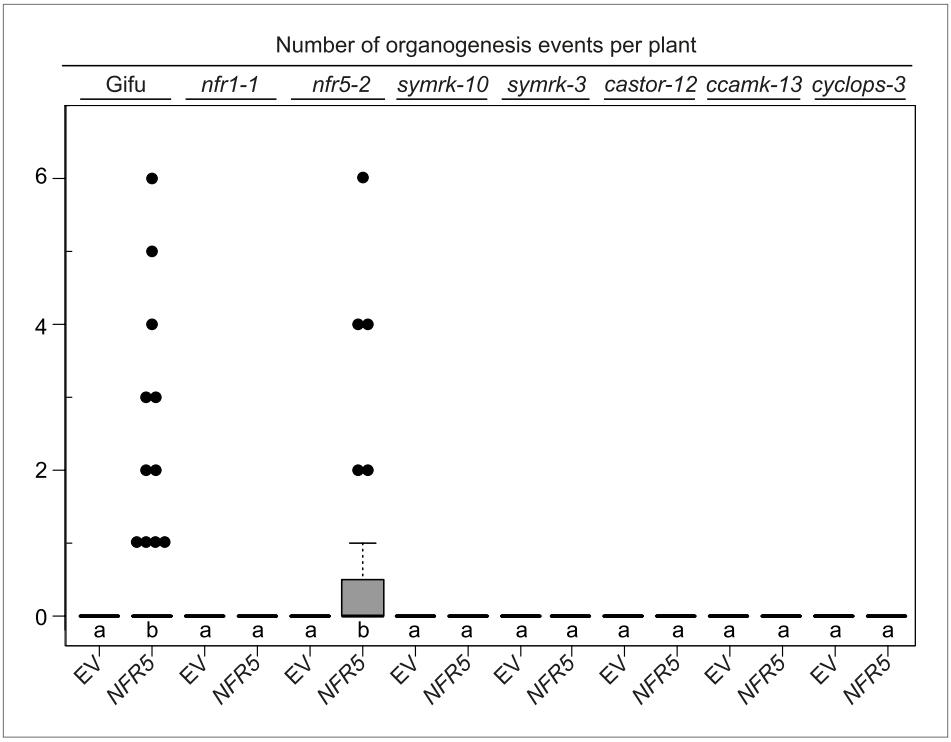


Figure 5—figure supplement 2. *NFR5*-mediated spontaneous organogenesis events in Gifu wild-type, *nfr1-1*, *nfr5-2*, and common symbiosis mutants. Hairy roots of *L. japonicus* Gifu wild-type and different symbiosis defective mutants transformed with the empty vector (EV) or *pUB:NFR5-mOrange* (*NFR5*) were generated. Plot represents the number of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 60 dpt. Black dots, data points outside 1.5 IQR of the upper quartile; bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal–Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.
DOI: 10.7554/eLife.03891.014

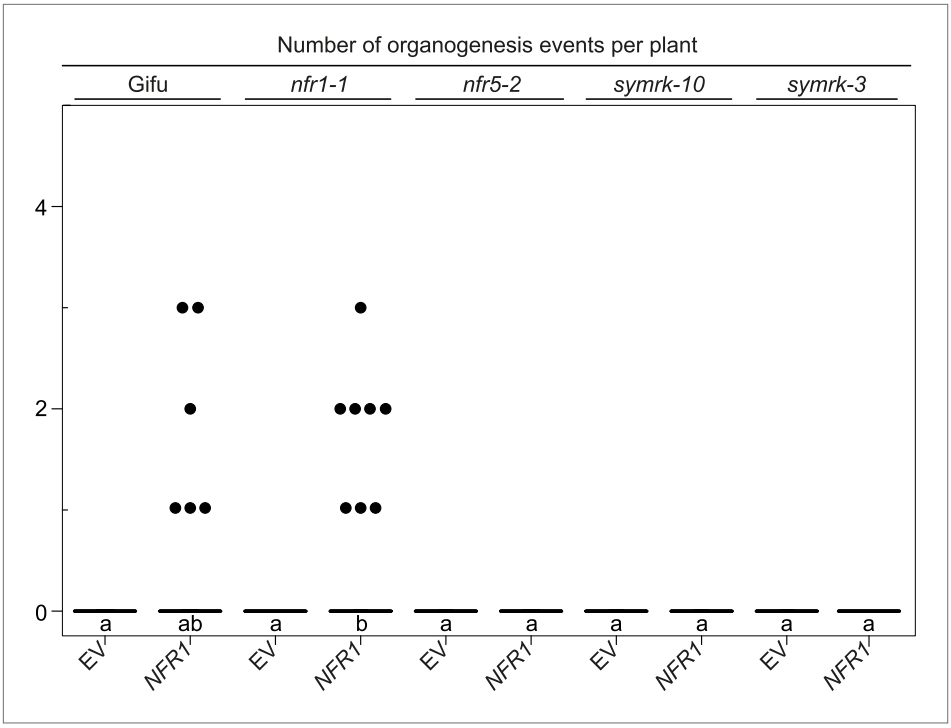


Figure 5—figure supplement 3. *NFR1*-mediated spontaneous organogenesis events in Gifu wild-type, *nfr1-1*, *nfr5-2*, *symrk-10*, and *symrk-3*. Hairy roots of *L. japonicus* Gifu wild-type and different symbiosis defective mutants transformed with the empty vector (EV) or *pUB:NFR1-mOrange* (*NFR1*) were generated. Plot represents the number of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 60 dpt. Black dots, data points outside 1.5 IQR of the upper quartile; bold black line, median. A Kruskal–Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

DOI: 10.7554/eLife.03891.015

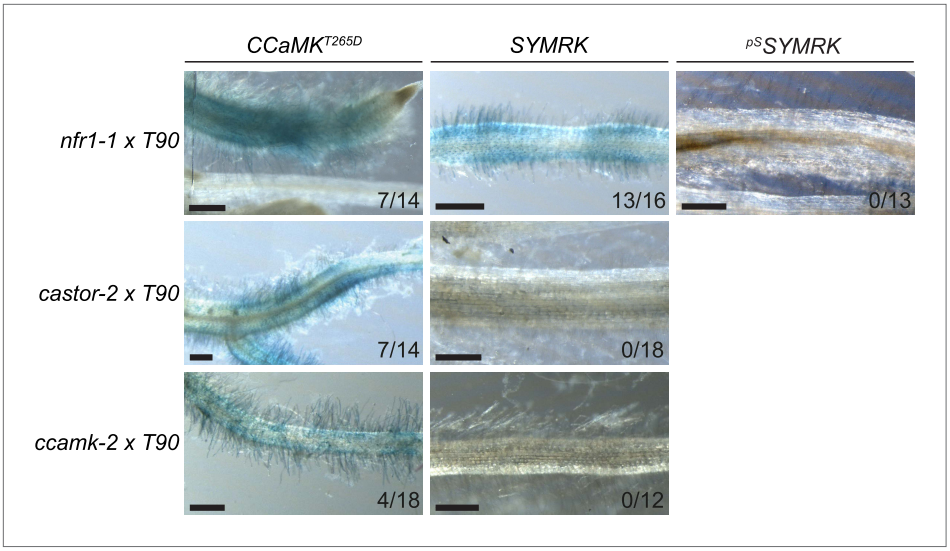


Figure 5—figure supplement 4. *SYMRK*-mediated activation of the symbiosis-specific T90 reporter in symbiosis-defective mutants. Hairy roots of three stable transgenic *L. japonicus* Gifu reporter lines homozygous for the T90 reporter fusion and the indicated mutant alleles transformed with *pUB:CCaMK*^{T265D} (*CCaMK*^{T265D}, a deregulated version of CCaMK), *pUB*:SYMRK-mOrange (*SYMRK*), or *pSYMRK*:SYMRK-RFP (^{pS}*SYMRK*) were generated and kept on agar plates for a total of 38 dpt (see 'Materials and methods'). The vast majority of transgenic root systems did not develop spontaneous nodules at this time point under these growth conditions. GUS activity was analysed by histochemical staining with X-Gluc at 38 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per total plants is indicated. Bars, 500 μm.

DOI: 10.7554/eLife.03891.016

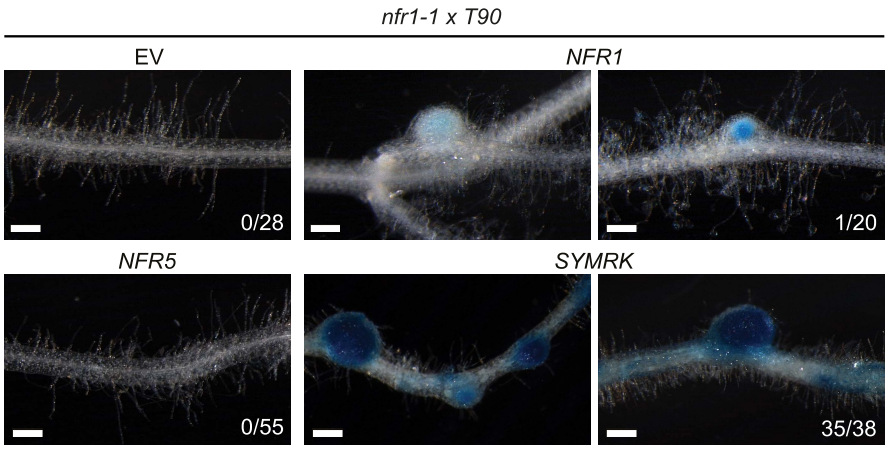


Figure 5—figure supplement 5. *NFR*-mediated activation of the symbiosis-specific T90 reporter in the *nfr1-1* mutant background. Hairy roots a stable transgenic *L. japonicus* Gifu reporter line homozygous for the T90 reporter fusion and the *nfr1-1* mutant allele transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. GUS activity was analysed by histochemical staining with X-Gluc at 60 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per total number of plants is indicated. Bars, 500 μm.

DOI: 10.7554/eLife.03891.017