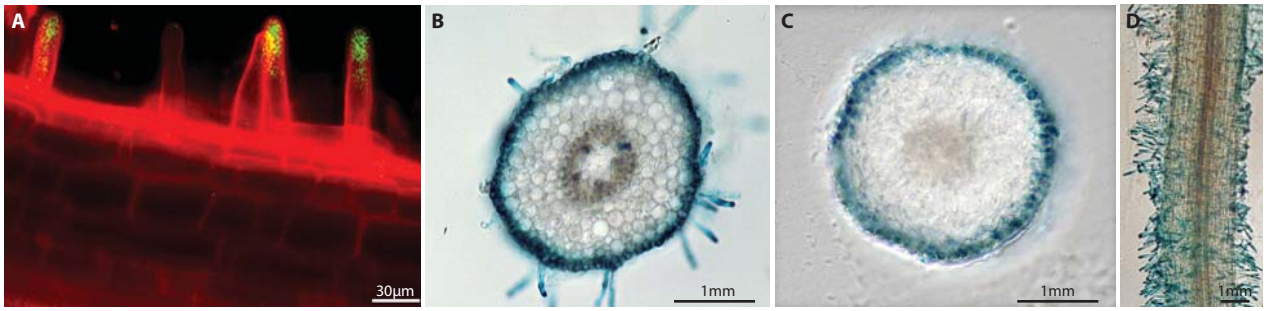
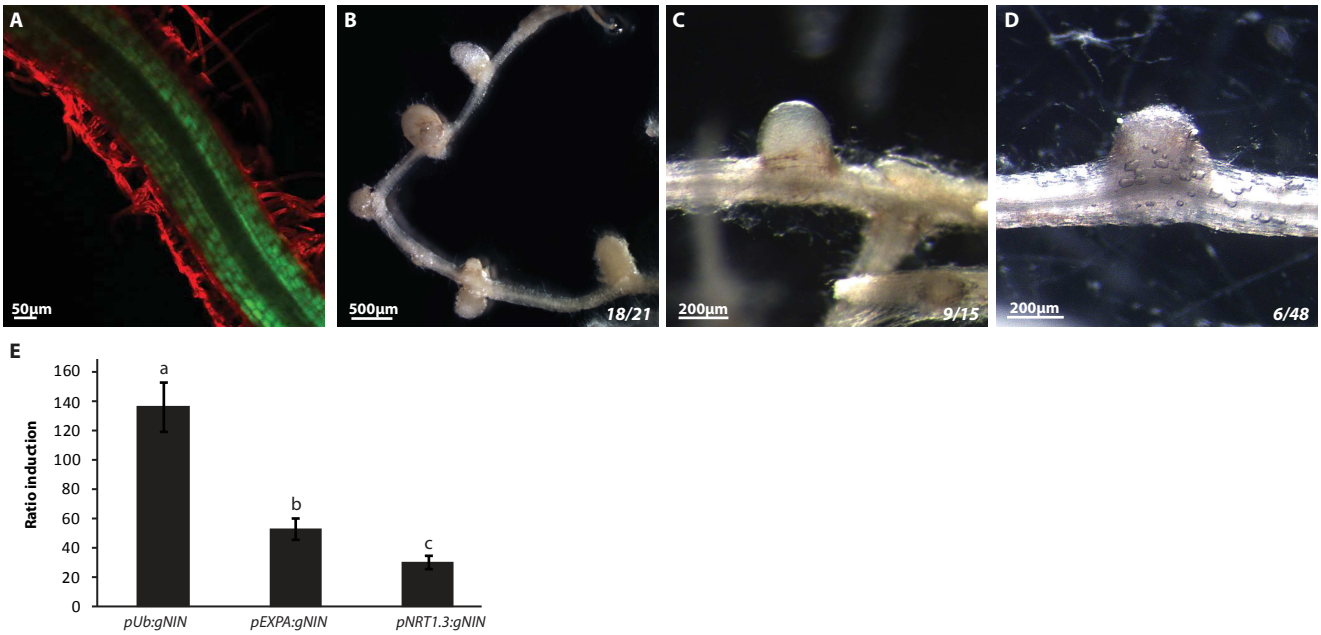


Supplemental Figure 1. *pEXPA* expression is restricted to epidermal cells of control or Nod factor treated *M. truncatula* roots
A. pEXPA:GFP-GUS was introduced into *M. truncatula* roots and the GFP signal (green color) was monitored after 2 hours of 10 nM Nod factor treatment. Propidium iodide was used to stain the membranes.
B-D. GUS reporter activity was detected in the epidermis of *M. truncatula* roots expressing the pEXPA:GFP-GUS fusion. Roots were treated with 10 nM Nod Factors for 48 hours (**B**) or buffer solution (**C-D**). **B** and **C** show transversal sections of roots.

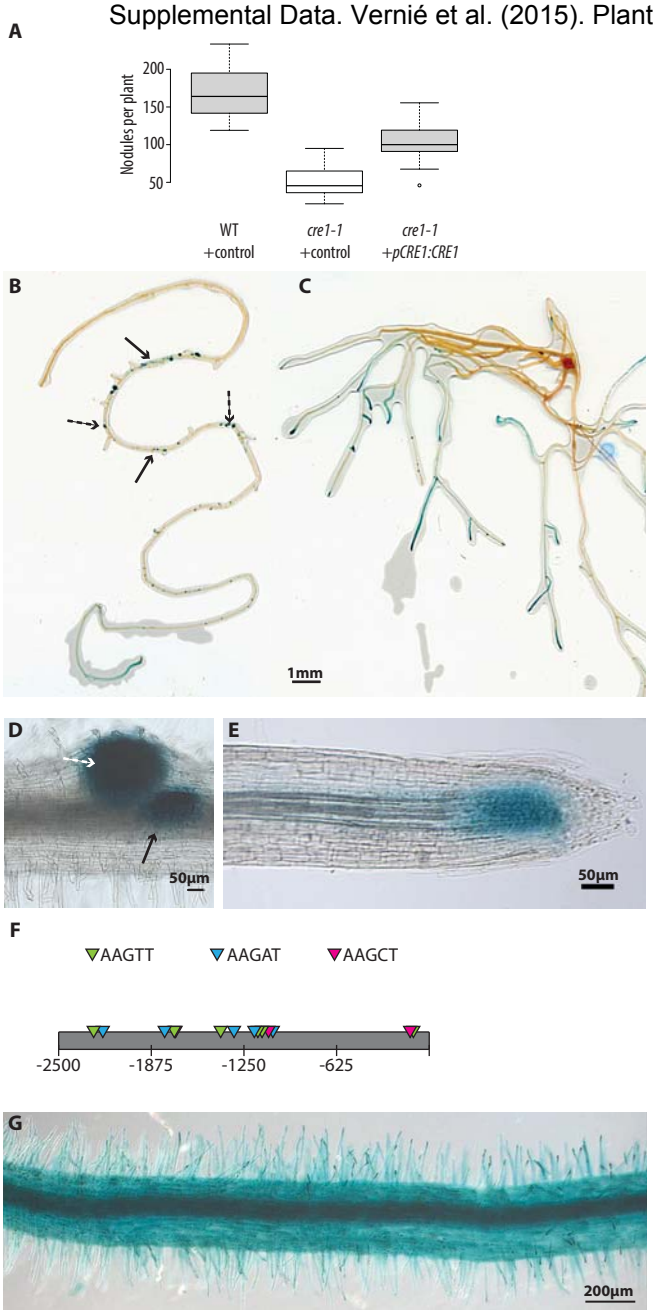


Supplemental Figure 2. Tissue specific expression of *NIN* in epidermal and cortical root tissues

A. *pNRT1.3:GFP* fusion expressed in wild type *M. truncatula* roots. The GFP signal is observed only in inner root tissues, associated with the root cortex and not in the root epidermis.

B-D. Spontaneous nodulation-like structures observed 15 weeks after transformation of wild type *M. truncatula* roots with *pUb:gNIN* (**B**), *pNRT1.3:gNIN* (**C**) and *pEXPA:gNIN* (**D**). Numbers indicate the number of transformed plants showing nodules out of the total number of transformed plants.

E. RT-qPCR analysis of *nin-1* roots transformed with the tissue specific constructs *pUb:gNIN*, *pEXPA:gNIN* and *pNRT1.3:gNIN*. The *NIN* expression was normalised with *EF1*. Bars represent the ratio relative to the control *nin-1* plants. Error bars represent SE (n=12). Different letters indicate statistical difference as determined by pairwise Wilcoxon rank sum test ($p<0.05$).



Supplemental Figure 3. *CRE1* expression is associated with cortical cell divisions in response to *S. meliloti*

A. Nodules numbers at 50 days post inoculation in wild type and in *cre1-1* roots transformed with a control vector or the pCRE1:CRE1 construct. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. n = 15 sample points.

B-E. *CRE1* expression at early stages of nodule organogenesis (6 DPI) measured using the *CRE1* promoter driving the expression of *GUS* in wild type (**B**, **D**) and *nin-1* (**C**) roots. *CRE1* symbiotic expression is localized in dividing cortical cells (black arrows) and young nodule primordia (dotted arrows).

E. Non symbiotic expression of *CRE1* in root tips measured using the *pCRE1:GUS* construct.

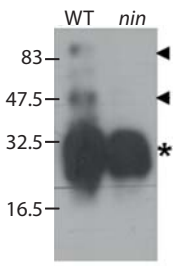
F. The *CRE1* promoter contains NIN-binding *cis* elements. Colored triangles indicate *cis* elements on the *CRE1* promoter (2500 bases).

G.*CRE1:GUS* expression observed in wild-type roots expressing *pEXPA:gNIN* (G). *GUS* expression (in blue) is occasionally localized in epidermis.

A

```
----------gcaAAGATgtgcaaacaaac
---actttgtcacaAAGATttag--------
-------ctcctccAAGATcttcttct----
tcttttcttttcttAAGCTt-----------
--tctctgccattaAAGCTaag---------
----------ataaAAGCTattttcaact-
----------tatAAGCTcatggttttctt
-----agtttgttcAAGATttagca------
--------tcatagAAGCTtttcattat---
-------tttacttAAGATtttaatgg----
---------tttctAAGATgtttatgctc--
---------tcaaaAAGCTattggtgttc--
----agtcctttgaAAGCTtgctt-------
---------ctgtcAAGATcaattgagta--
           AAG(A/C)T
```

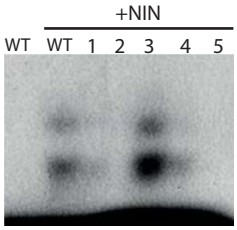
B



C

***ENOD11* promoter(-368 to -343)**

```
ggcctaAAGCTattattttaccagcc: WT
------c------------------: 1
-------c-----------------: 2
------AAGAT---------------: 3
--------t-----------------: 4
----------g---------------: 5
```

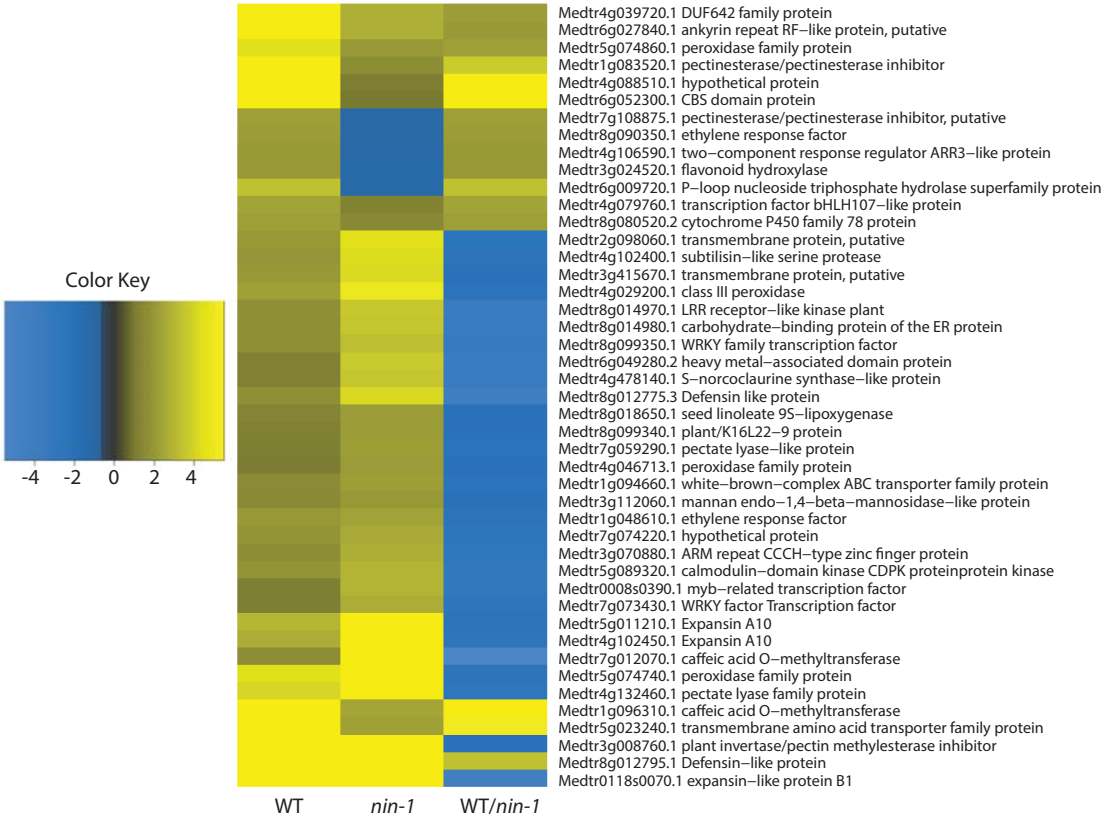


Supplemental Figure 4. NIN binds the NF-box

A. Identification of the NIN *cis* element by random binding site selection. The oligonucleotides that were bound by NIN are shown and all contained the AAGCT or AAGAT core sequences.

B. WT and *nin* roots (10-day-old) were treated with 10 nM Nod factor for 48 h. Total proteins (1.5 mg protein) were immunoprecipitated using the anti-NIN antibody. The immunoprecipitated proteins were detected by immunoblot analysis using the anti-NIN antibody. Arrows indicate NIN and asterisk indicates the immunoglobulin G. We could detect two products in wild type plants, both lacking in the *nin* mutant. The upper band is the predicted size of full length NIN. The lower band may represent a processed or partially degraded form of NIN.

C. A region of the NF-box in the *ENOD11* promoter spanning the AAGCT Cotif. The AAGCT/AAGAT motif and mutated motifs (1, 2, 4 and 5) were ^{32}P-radiolabeled and used in binding studies with NIN.



Supplemental Figure 5. A subset of Nod factor (100 pM) induced gene expression dependent on NIN
Heatmap of an Affymetrix-based expression analysis. Results for the probesets induced or repressed by at least two fold in *nin-1* vs WT are shown. One-week-old roots of wild type (WT) and *nin-1* plants were treated with Nod factors (100 pM) or BNM buffer for 24 hours. Three independent biological replicates were performed for each treatment. Fold change (Fc) calculations between buffer and Nod factor treatment conditions were evaluated statistically using an unpaired t-test algorithm for asymptotic p-value computation as implemented in Genespring GX11.5. All data presented have a p-value <0.05 in at least one of the genotype(s).

Supplemental Table 1: Nod factors induce *RR4* expression in wild type roots but not in the *nin-1* mutant roots.

Data were obtained in an Affymetrix-based expression analysis. Results for the probeset Mtr.9656.1.S1_at (RR4) are shown. One-week-old roots of wild type and *nin-1* plants were treated with Nod factors (100 pM) or BNM buffer for 24 hours. Three independent biological replicates were performed for each treatment. Fold change (Fc) calculations between buffer and Nod factor treatment conditions were evaluated statistically using an unpaired t-test algorithm for asymptotic p-value computation as implemented in Genespring GX11.5.

	p-value	Fc relative
WT	0.1	3.29
nin-1	0.5	1.26

Supplemental Table 2: Primers used in this work

Name	Forward	Reverse
pNIN	GGGGTACCCACTCAATGGTA	CATGCCATGGCCTTATAATTAAA GTTGTTTCTC
NIN (582-700)	GGGGACAAGTTTGTACAAAAAAG CAGGCTTCGTAGAAGCTGGTGAA GAATCTC	GGGGACCACTTTGTACAAGAAAG CTGGGTCTGCATTGCTCAACTCTG GGAAG
NIN (582-933)	GGGGACAAGTTTGTACAAAAAAG CAGGCTTCGTAGAAGCTGGTGAA GAATCT	GGGGACCACTTTGTACAAGAAAG CTGGGTCGGAGGATGGACTGCTG CTGCTGC
NIN Full Length and CDS	GGGGACAAGTTTGTACAAAAAAG CAGGCTTCATGGAATATGGTGGT GGGTTAGT	GGGGACCACTTTGTACAAGAAAG CTGGGTTCTAGGAGGATGGACTG CTGCTGCTG
ENOD11 (ChIP)	GTATCTGAGTAATGCAATCATAC G	CGTTTGTTTTATTGGCCATGG
CRE1 (ChIP-1)	CCTCAATCATTCATCATGCTTG	CTATGGAAGTCCCAAAAGATAC
CRE1 (ChIP-2)	CCAACAATGAATGTATGAGTAC	CACTATTTCTATGTGTTCCAACG
RR4 (ISH)	TTGTTCCGGGTTTAAAGGTG	ATCAAGAACGTCGGGTGAAG
pEXPA	GGGGACAAGTTTGTACAAAAAAG CAGGCTGCCTGGTCTGTGAAAGA ATTGTTA	GGGGACCACTTTGTACAAGAAAG CTGGGTGTGTTAAGATTCTGTATA AATTAATG
pNRT1.3	GGCACTATTTGTTACATGTG	GATATGCTTTGGCATTTAAC