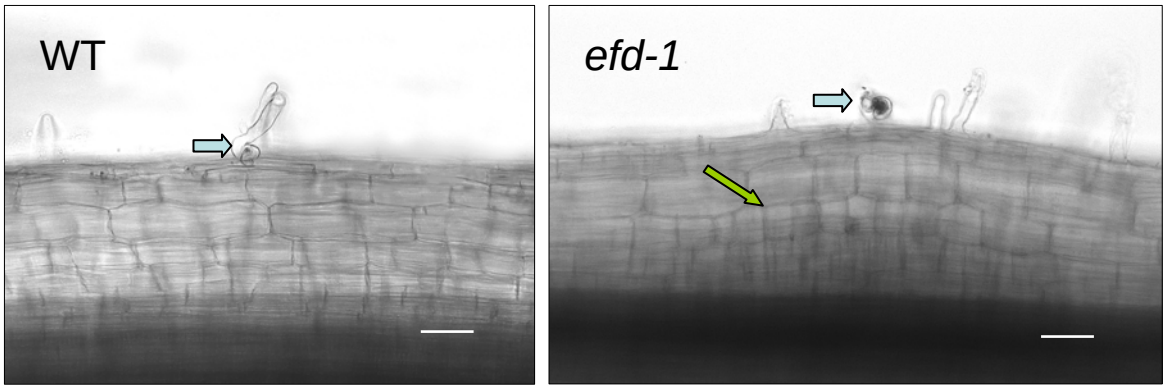
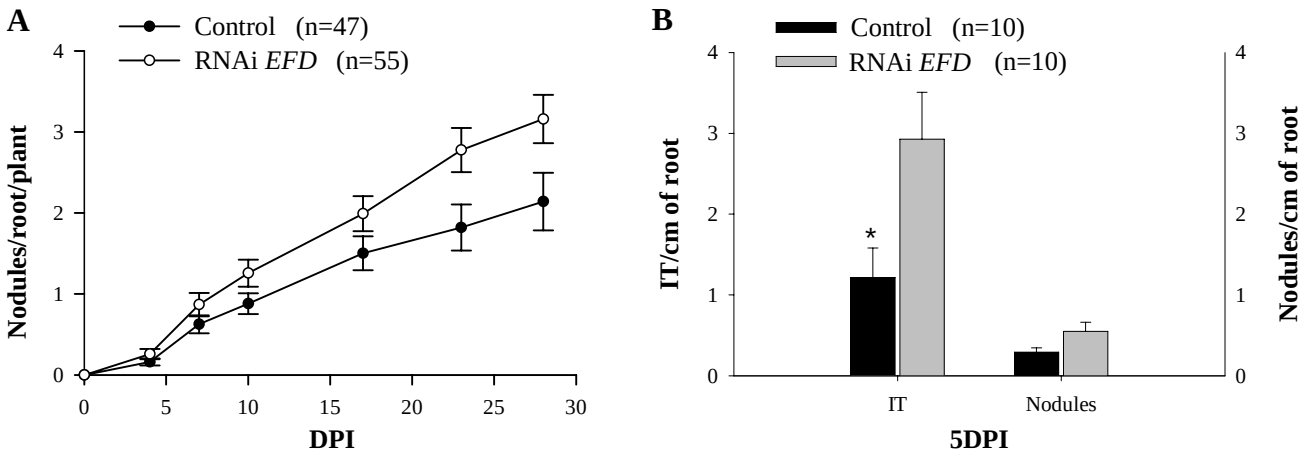
Supplemental Data. Vernié et al. (2008). EFD is an ERF transcription factor involved in the control of nodule number and differentiation in *Medicago truncatula*



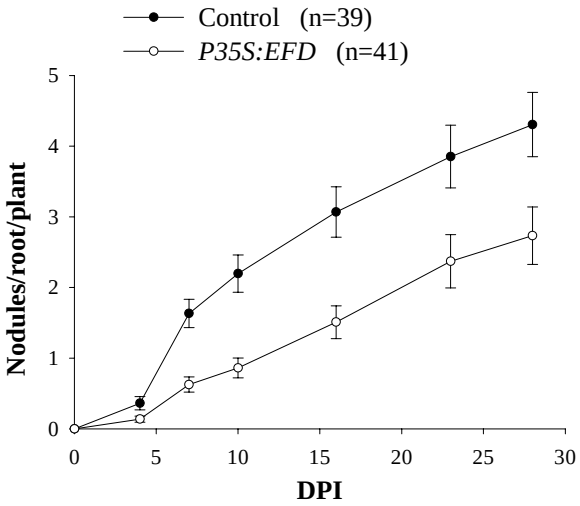
Supplemental Figure 1. Cortical cell divisions are frequently associated with root hair curls in the *efd-1* mutant but not in WT plants. Aeroponically grown plants, 21 days post inoculation with *S. meliloti hemA:lacZ.* The blue arrows show root hair curls and the green arrow indicates cortical cell divisions. *S. meliloti* bacteria are stained by a β-galactosidase assay. Bars= 50 µm.



Supplemental Figure 2. A weak supernodulant phenotype is exhibited by *EFD* RNAi roots

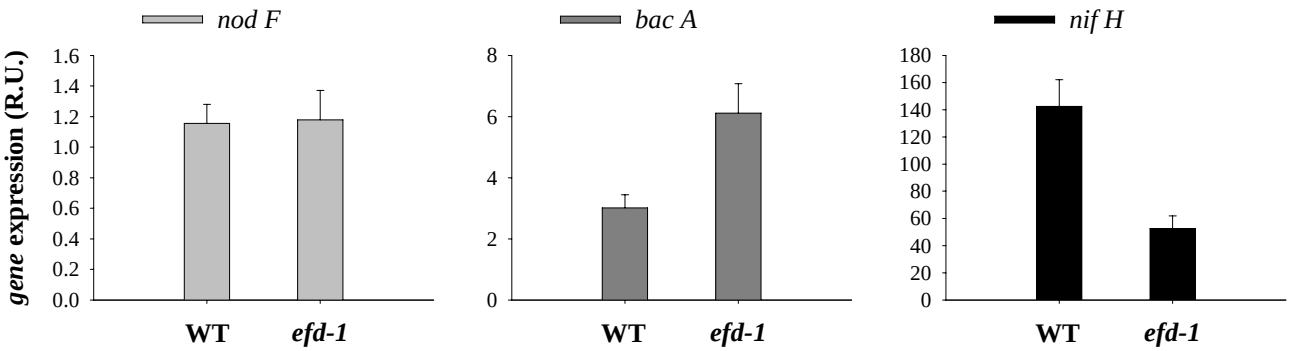
A: One-month-old transgenic roots transformed with *P35S*:RNAi *EFD* or with the empty cloning vector (control) were inoculated by wild type *S. meliloti*. Nodules were counted until 28 days post inoculation (DPI). The graph represents data from two biological repetitions. Error bars represent SE.

B: Number of infection threads (IT) and nodules per root at five days post inoculation counted on a third biological repetition of roots transformed with *P35S*:RNAi *EFD* or empty vector (control). Error bars represent SE. Star indicates statistically significant differences (Mann and Whitney test, $P<0.05$).



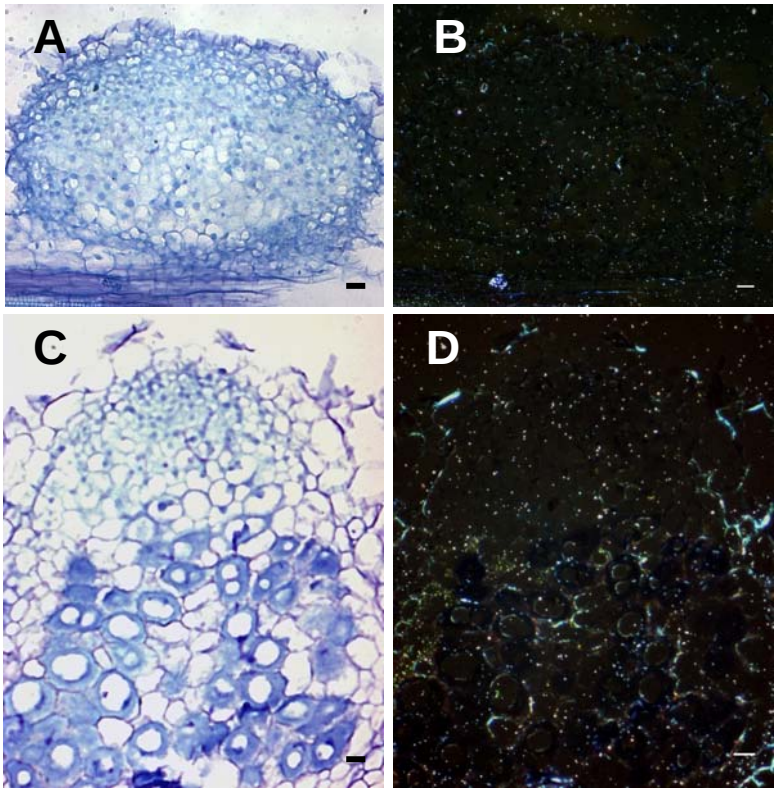
Supplemental Figure 3. A decrease in nodulation is exhibited by *EFD* overexpressing roots

One-month-old transgenic roots expressing *P35S:EFD* or empty vector (control) constructs were inoculated by wild type *S. meliloti*. Nodules were counted until 28 days post inoculation (DPI). Graph represents 2 biological repetitions. Error bars represent SE. Differences are statistically significant (Mann and Whitney test, $P<0.001$).

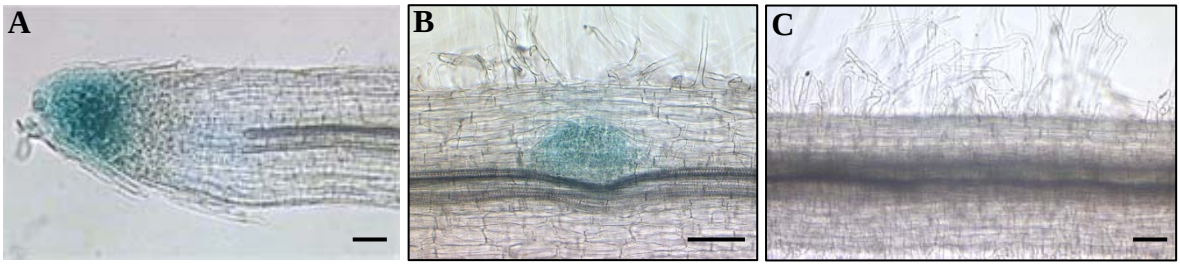


Supplemental Figure 4. Q-RT-PCR analyses of bacterial symbiotic genes expression in *efd-1* vs. wild type nodules.

nodF, *bacA* and *nifH* expression was measured on 10-day-old nodules of wild type (WT) *M. truncatula* or *efd-1* mutant**.** All data are from three biological repetitions and were normalized by *Pnp* (SMc00324) expression. Error bars represent SE.

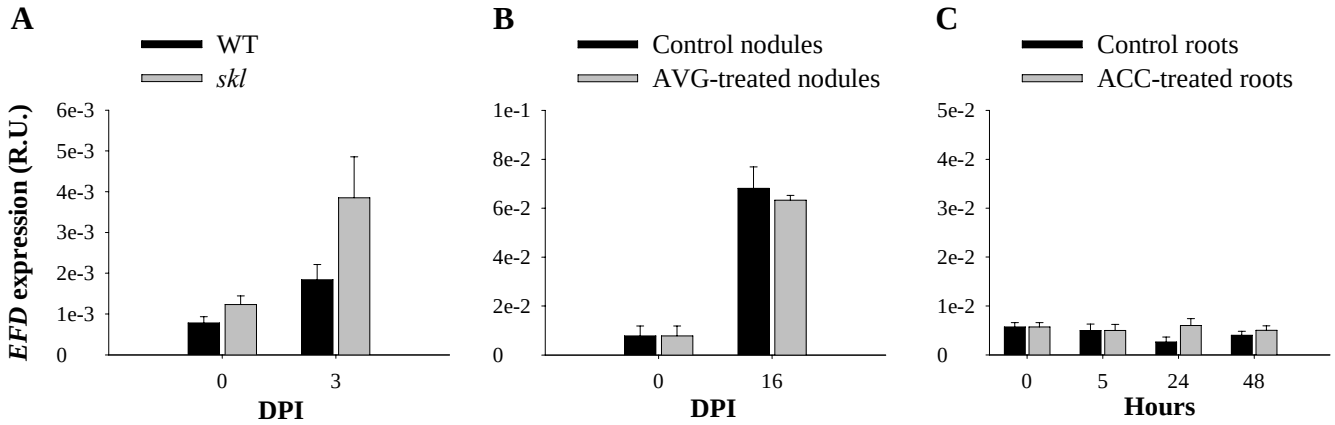


Supplemental Figure 5. *In situ* hybridization on nodule sections with a control ^{35}S-labelled *EFD* sense probe. **A, B:** 4-day-old nodules; **C, D:** 10-day-old nodules; **A, C:** bright field; B, D: dark field.
No hybridisation signals (white dots) are detected in B, D, as expected for a sense probe. Bars= 40 µm.



Supplemental Figure 6. *EFD* is expressed in root tips and root primordia and is not induced by purified Nod factors.

Localization of *EFD* mRNA, using a promoter:GUS fusion (blue color). The *PEFD:GUS* fusion is expressed in the root meristematic region (A) and in lateral root primordia (B) of non inoculated *M. truncatula* roots, but is not induced by a 48h incubation with 10^{-8}M Nod factors (C). Bars = 50 µm.



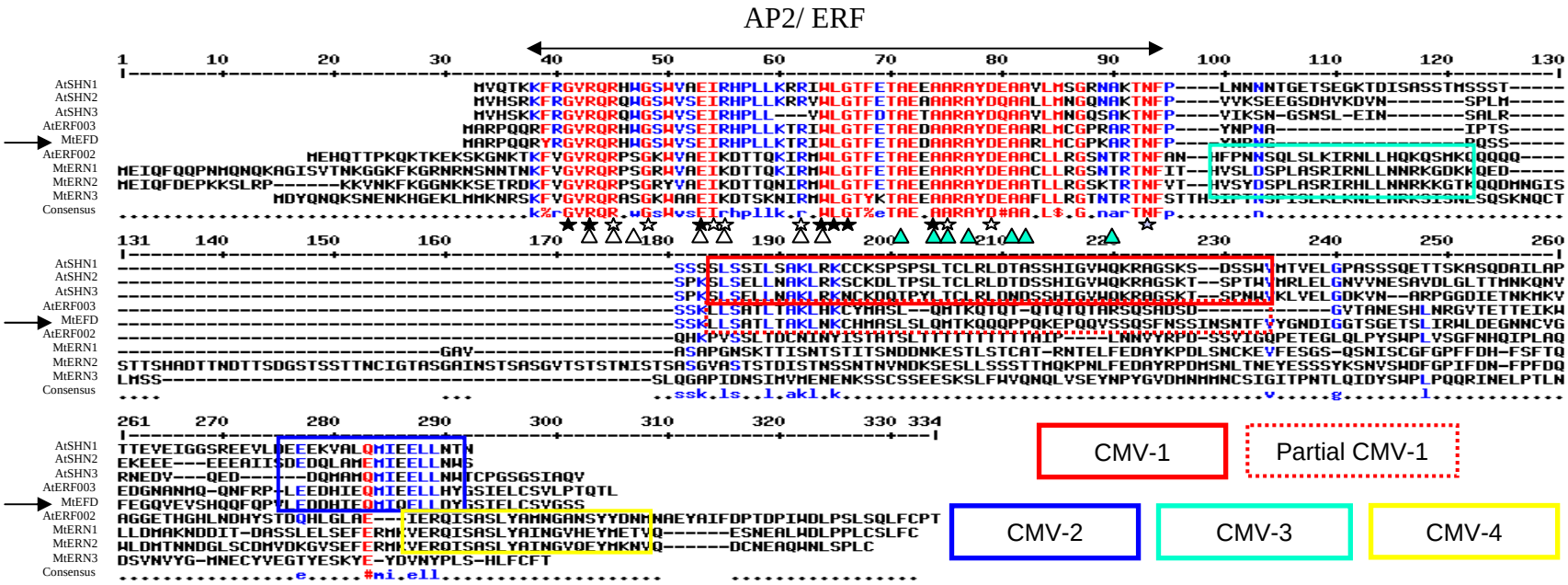
Supplemental Figure 7. *EFD* expression is not induced by ethylene

A: Q-RT-PCR analysis of *EFD* expression in wild type (WT) and *skl M. truncatula* roots at 0 and 3 days post inoculation (DPI) with wild type *S. meliloti.*

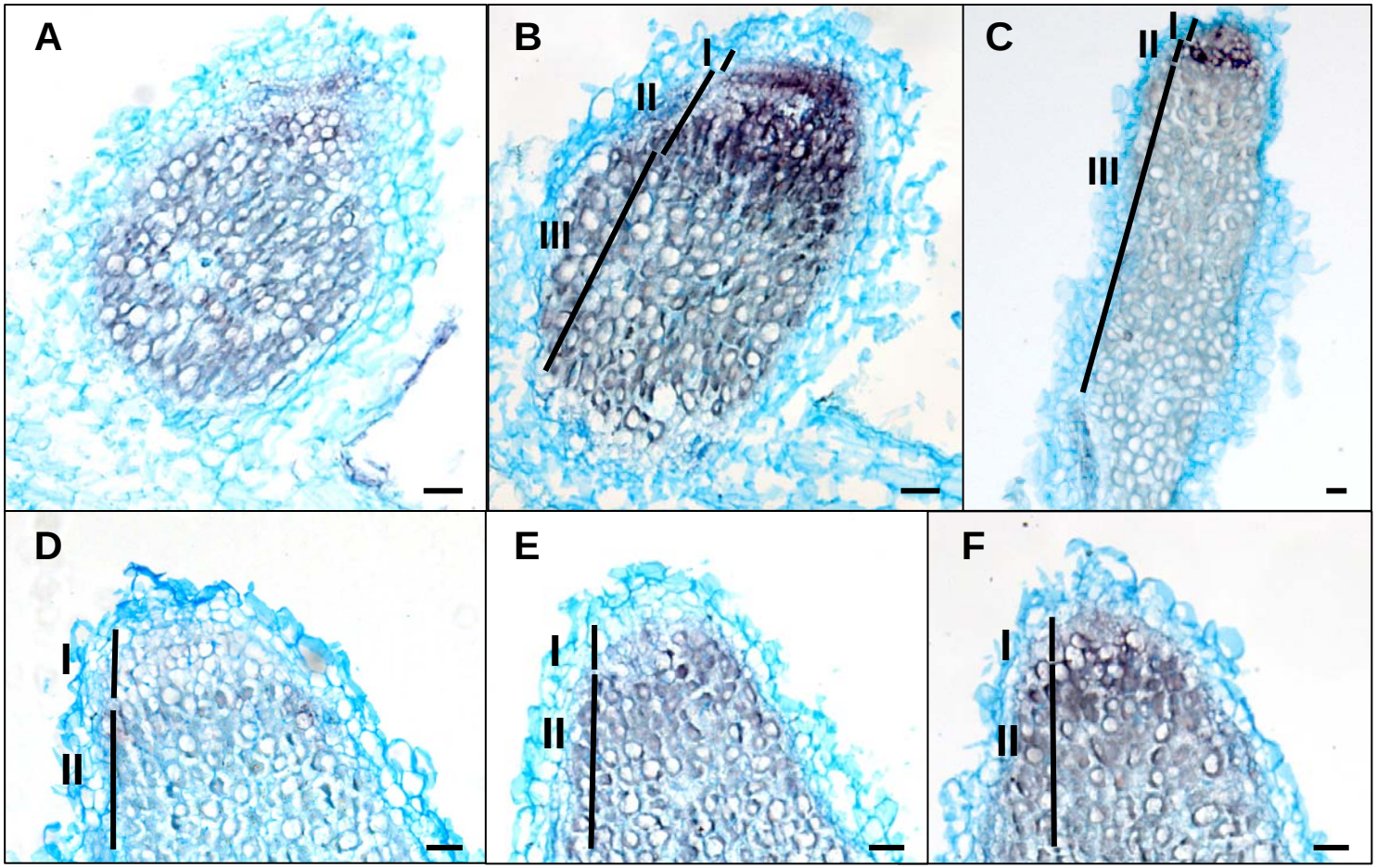
B: Q-RT-PCR analysis of *EFD* expression in 16-day-old nodules from wild type *M. truncatula* roots inoculated with wild type *S. meliloti*, grown on normal media (Control) or on media supplemented with 10µM AVG (Aminoethoxyvinyl-glycine).

C: Q-RT-PCR analysis of *EFD* expression in wild type *M. truncatula* roots treated by water (Control) or 50µM.ACC (1-aminocyclopropane-1-carboxilate).

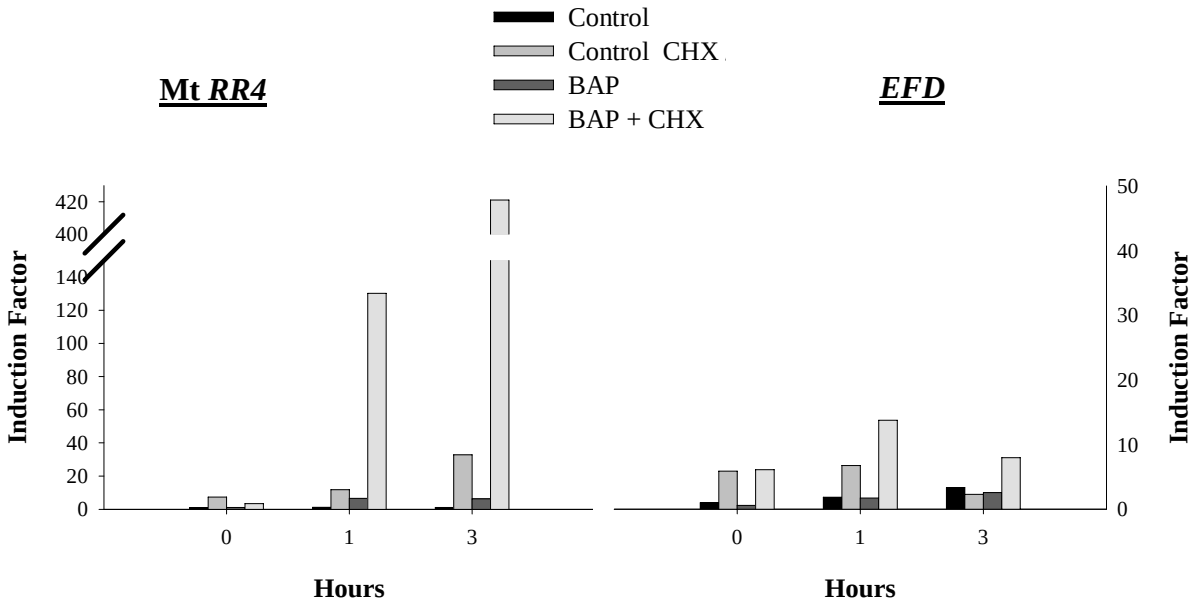
All data are from at least 2 biological repetitions and are normalized by Mt *EF1-α* expression. Error bars represent SE.



Supplemental Figure 8. Alignement analysis of group V ERF proteins from *Medicago truncatula* and *Arabidopsis thaliana*. Black stars indicate residues conserved in all ERF proteins, white stars those found in at least 95% of ERFs, white triangles residues predicted to make direct contacts with DNA and light blue triangles those implicated in the stabilisation of the ERF domain (Allen et al., 1998).

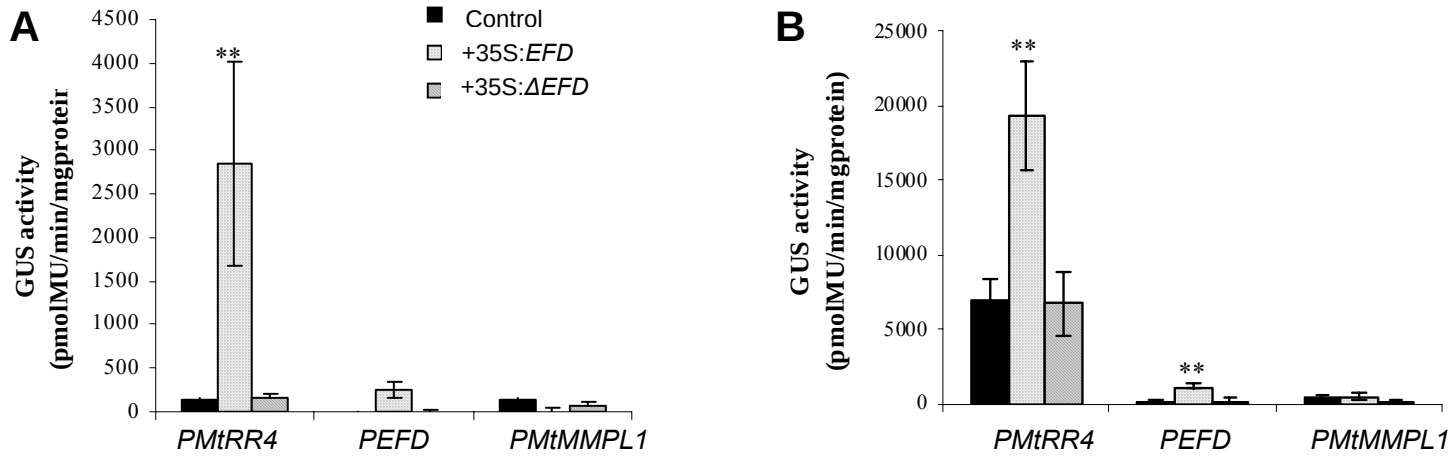


Supplemental Figure 9. Mt *RR4* and *EFD* are expressed in nodule zone II.
In situ hybridizations carried out on microtome sections of two-week-old (A, B) or four-week-old (C-F) nodules, using digoxigenin-labelled probes. D, E, F show a close up of results from hybridizations carried out on serial sections of the same nodule. **A:** sense control probe (note the homogenous signal in all internal nodule tissues); **B, E:** Mt *RR4* probe; **C, F:** probe for type-A response regulator gene family. **D:** *EFD* probe (note the absence of *EFD* hybridization signal in zone I; the weak *EFD* signal in zone II is validated by more sensitive *in situ* hybridizations carried out with radioactive probes, as shown in Figure 6E, F. Vertical or oblique bars indicate various nodule zones (I, II or III). Bars = 100µm.



Supplemental Figure 10. Mt *RR4* is a primary cytokinin response gene whereas *EFD* transcription is not regulated by cytokinins

Q-RT-PCR analysis of *RR4* and *EFD* induction in response to the cytokinin BAP (10^{-7}M) or control treatment at various incubation times (1 hour or 3 hours), with or without a cycloheximide (CHX) pretreatment (1 hour, 100 µM) to inhibit *de novo* protein synthesis. Induction factors were calculated in reference to non treated roots (control T0). Mt *RR4* induction by BAP alone was 6.5 after 1 hour and 6.3 after 3 hours, whereas no difference was observed for *EFD* between control and BAP-treated plants. Histograms represent the quantification of specific PCR amplification products for each gene normalized with the constitutive control *ACTIN11*. A representative example out of three biological experiments is shown.



Supplemental Figure 11. Trans-activation of *PRR4*, *PEFD* and *PMMPL1* in *Nicotiana benthamiana* by *P35S:EFD:HA*.

A. GUS activity at 24 h following co-transformation. **B.** GUS activity at 48 h following co-transformation. For both A. and B., leaves of *N. benthamiana* were co-transformed by *Agrobacterium tumefasciens* with *PRR4:GUS* , *PEFD:GUS* or *PMMPL1:GUS* plus either *P35S:EFD:HA* or *P35S:ΔEFD:HA* (ΔEFD is EFD deleted for its putative DNA binding domain). Controls correspond to leaves of *N. benthamiana* transformed with *PRR4:GUS*, *PEFD:GUS* or *PMMPL1:GUS* alone. GUS activity was measured using 10 µg of total protein extracts at 24 (**A**) and 48 (**B**) hours after transformation, using three biological repetitions. Error bars represent SE.

PRR4 was only significantly activated by EFD at 24 and 48 H and *PEFD* at 48H ($P<0.01$ for both, following Cumming et al. 2007). *PMMPL1* was not significantly activated.

	T0	1 DPI	3 DPI
WT	$7.8\ 10^{-4} \pm 1.4\ 10^{-5}$	$5.7\ 10^{-4} \pm 5.1\ 10^{-5}$	$1.8\ 10^{-3} \pm 3.7\ 10^{-4}$
efd-1	ND	ND	ND

Supplemental Table 1. Q-RT-PCR analysis of *EFD* expression in wild type (WT) and *efd-1* roots of *Medicago truncatula*, inoculated by wild type *Sinorhizobium meliloti*. Standard error came from three biological repetitions. DPI= days post inoculation. ND= non detectable.

	Total number of ITs	ITs in epidermis		ITs in cortex		Nodules
Control (empty vector)	5.81 ± 1.01	5.12 ± 0.93	88.2%	0.69 ± 0.08	11.8%	0.71 ± 0.17
P35S :EFD :VP16	1.66 ± 0.96	1.52 ± 0.63	91.7 %	0.14 ± 0.06	8.3%	0.22 ± 0.09
Ratio Control / *P35S :EFD :VP16*	3.5	3.4		4.9		3.2

Supplemental Table 2. Infection thread formation is decreased in *EFD* overexpressing roots. Infection threads (ITs) were induced by *Sinorhizobium meliloti hemA:lacZ* and counted on ten independently transformed roots at 5 days post inoculation following β galactosidase revelation. Results are expressed per cm of root. Standard errors are indicated.

	nfp (C31)		*nsp1* (B85)		*hcl* (B56)	
	T_0	3dpi	T_0	3dpi	T_0	3dpi
EFD	$3.5\ 10^{-5} \pm 1.2\ 10^{-5}$	$6.9\ 10^{-5} \pm 3.5\ 10^{-5}$	$3.4\ 10^{-4} \pm 1.7\ 10^{-4}$	$2.1\ 10^{-4} \pm 5.0\ 10^{-5}$	$7.0\ 10^{-5} \pm 2.4\ 10^{-5}$	$5.3\ 10^{-5} \pm 1.2\ 10^{-5}$
Mt *ENOD11*	$6.7\ 10^{-5} \pm 5.7\ 10^{-6}$	$6.2\ 10^{-5} \pm 7.5\ 10^{-6}$	$7.9\ 10^{-}5 \pm 7.7\ 10^{-5}$	$7.0\ 10^{-}5 \pm 3.2\ 10^{-5}$	$5.1\ 10^{-}5 \pm 3.3\ 10^{-6}$	$1.1\ 10^{-3} \pm 7.8\ 10^{-4}$

Supplemental Table 3. Q-RT-PCR analysis of *EFD* and Mt *ENOD11* expression in roots of early symbiotic *Medicago truncatula nfp-1* (C31), *nsp1-1* (B85) and *hcl-1* (B56) mutants, inoculated by wild type *Sinorhizobium meliloti*. Standard error came from at least two biological repetitions.

Supplemental Tables 4, 5, and 6 are available in a separate .xls file online.

Supplemental Table 7. Primers used for *PEFD* sequencing

Name	Position	Sequence
MtC50408-369-5’	+ 1014	5’-CCAAACAACAACAACCACCA-3’
Tail Ap1	+ 932	5’-TTGTGGTCCATTTGGGTTGTATGG-3’
Tail Ap2	+ 913	5’-TATGGGAAATTGGTACGTGCTTTTG-3’
PMtERF1-714-PG310	+ 202	5’-AAAACACATGTGAAAGAGTTTTTCACA-3’
Tail Ap3	+ 69	5’-AATTTCAGAGACCCAAGAACCCCA-3’
PMtERF1-144-PG301	- 97	5’-GAAGTGAAGGTGAAGCAGCTTTGCACA-3’
PMtERF1-494-PG314	- 608	5’-GCCCATCGGATCGACGCAAGC-3’
PMtERF1-647-PG314	- 761	5’-ATGAGAAATTGAACCTCATCCCCG-3’
PMtERF1-725-PG339	- 1357	5’-GTTCTCACACACACCTATATAATGGTG-3’
PMtERF1-751-PG340	- 1533	5’-CCGCCATCGTTGCCTCCATCAA-3’
PMtERF1-499-PG358	- 1999	5’-TTTTATTGATCTCTACTTACCCA-3’
PMtERF1-649-PG358	- 2149	5’-CGGCAATTGGCATGTGTATG-3’

Supplemental Table 8. Primers used in Q-RT-PCR

Gene	Forward	Reverse
EF1-α	CTTTGCTTGGTGCTGTTTAGATGG	ATTCCAAAGGCGGCTGCATA
Desmin	CAGCCTCAGTCCTCCAAATCACA	TAGGCCTGAGGTCACAGAGGT
Actin11	ACGAGCGTTTCAGATG	ACCTCCGATCCAGACA
EFD	CTCAAAGACACACTTCACAAACACG	CCACACATTAATCTTGCTGCTTCAT
Mt *RR4*	ATGCTTTTGTTCCGGGTTTA	CTGCACCTTCCTCCAAACAT
Mt *ENOD11*	CCACATGCAAAGATGGGACG	CAGCCTCCACCTAGCATCCA
Mt *MMPL1*	TTCAAAGGTCTGGGACTTGG	GCAAAACCAAGGGACAAAGA
nodF	CATTGGCATCATCAAGAACCG	TAAATCGCCGACTATTAACGCG
bacA	GGTCGGTCTTTGGTACGGTTTT	CGCTGATTGCGAAATTCCAG
nifH	CGGCTTTGCGATGCCTATT	GCATAGAGCGCCATCATCTCA
pnp	TGTCATCAGGCTTGCGGAA	ATCGCATTTTCGAGAGCGG
MtC50408	CAACGTTATAGAGGTGTACGCCAA	GGTGACGAATTTCAGAGACCCA
MtC00068	ATGGTGTGACCCTAGCATTCCTC	AACAGCGATGCTATACATCCCAAG
MtC90910	AGATTTCAGCAGATGTGGTAGCGT	CCCTTCAAACCACCCAAAGAG
MtC45510	CAAAAGGTGCTGCTGTCTGTGA	CCGGTTGCATACTAGAAGACCAAG
MtC00651	CACGACCACGAGGTGGTTTTT	ACCCTCTCCATACCAACCCCTT
TC106799	GGTCACAACCAATGGAAGCCT	GGACACATATCTGGTGGACATTCA
MtD19561	TGTCTTCACCCGACGTTCTTG	CAATCTAAAGAGTCATTCGGTGCC
MtC60355	TGACAAATCACGGCACCAAG	CAACATCAGCCTCACGAACATACT
MtC20017	TATTTGCAGCAGGTGACTGTCG	CCCTCTGAAATAGCCCATACCAC
MtD06543	CAAGAAGCGACGTTCAAGTCTCT	CCCTTGGTCTTTTGTGTTGGTT
MtC10245	TGTAAGAAAGGTGACGCGTGTG	CAGGATGAAGCCAACACTCGA
MtC60404	AGTGAAGGTTTGCTCCTGCCT	TATCCGCGTACCCGTATCGTA
MtC60095	CGGAACTGCAATTGATATGGCT	TTGAAGAGAGCTTGTGCCAGATG
MtD11051.3	ATCATTTTGGATACACGGCACA	TGCAGATGGAAAATTTGCCA
MtD01034.2	ATTACTGTGTGATGCTCCGCG	CCATCCTTCTGACTCCGAAACA
MtC93227	TCAACTGACAATGCTGCCACAT	GGAGTCCCATTCTTGACGTGATT
MtC61980	AGGAGACAGCTGCAAAGTTTCG	CGCTGAAACATCCCAAGAAGTC
MtD07324	CAAGAATTAGCCAAGGCGACA	AGCTCCAAATCCACCTTGACC
MtD02444	GATTCTGCTGCTTTCAGAATGAGG	CAAACACCAGCTTCCAAAGGAA
MtC61996	TGCCGAAGGCAGCATCTTTA	AGCATCAGATCCGATTCCTTCAC
MtD09485	TCCAACCCCAGAACAGTGAGA	ACTGAGCTACGAGAACCTCCCA
MtC00010.5	AGCATGGCACATTACGGTTTG	GCAGGCACGCAGATCTATCAA
MtD33991	CTTGCATTCCCCGAACTCTTT	ACATATCCCGCAGACCGAAA
MtD09577.1	ACAATCCCAACCTAGAGGACCAAT	CGGTAATCTTGGAACATGAGAACC
MtC00553	CTGCCACAAAATTTCAGTGTGG	CCCTAAATCACTAGTCGACGAACC
MtC10163	CAGTACCTGAAATGTGGTCACCG	CAACTGATCATAAGCTTCTCCCCA
MtC45082	AAATGCGGCTAGTGAACCTGAG	AAAAGCCCAGAAGCACGACA
MtC93014	TCTGCAACAGTCTTCATGGTGGT	CAACCCTCTCAACGTCCAACAA
MtC00043.1	TACACACTCTCCCTCCATTTTCCT	TGAGAAGCCAAAAGCTAAAGCAC
MtD34353	GCGTGCAATAATTCACCCAAG	GGCAGACTAGCTGAATCTGCAGTA
MtD05134	GAAAACGATCAAGTTCCCACTCC	GTGTTTGTTCTGGCACTTCTTCAG
MtD22297	CCTTGAACTACAACGGTTGGTCTT	ACTCACAAACCCCACGAAAATG
MtC40019	TGCTCGTGCACAAGGAAACA	TGCTCTCCGGAAGAAAACCAT
MtD00089	TACACAACGCAGGAAAACCACA	CTGGAAAAGACTGCTCAAGAAGGA
MtC20187	AATTCCATTGGTCCTCCCCA	GGTAGTCCCAACTTGAGCGATG
MtD00210	GCTTTGTATGGTGGTCTCGAGC	GCTTGATGCGACTTTCCAACA
MtC60677	CAAAGGCCCTGTTGTATCTTGTG	GGCTACAGAATCACGAGCTGCT
MtC10690	GGCCCAGGAACCATTAAGAAAT	TGTGTTGCTTCGTAGACCCAAA
MtC10582	TCGGATCTACTGTCCACTCTTTGG	TTGGCATGACGATACCGTGTC
MtC61034	GCTGGACTTGAATTTGACACCTCT	GGAATTGGTGTTGGTGGTGACT
MtC10265	GCCTGTTGATCCTTCACCACAT	GGTAAAGAGGCATGAGGCTCAGTA
MtC00344	TGAGTTATTTGCTCCCACACGAG	TGACGAACCCCTGAAGTAGCAG
MtC45620	GGCAGGCTTTTGAAGACAGTCTT	GCTGCAATGGCTGTTTGGATAT
MtS00134	AGGTGGAGGCTGGTTACCATAAG	GCCTACCACACAAACAACCATCA
MtD03185	CGTGTTTGGCTTGGAACCTTTA	GGAACTCGATAGCGGCATTGT
MtC60655.1	AACGCTTTGTATGGTGGTCTCG	ATGAGACTTTCCAGCAACCCG
MtC63234	ACAGCTTTACCGTCGATCACCTT	CCATCGATCATGTCTGCCATAG