Supplementary Material

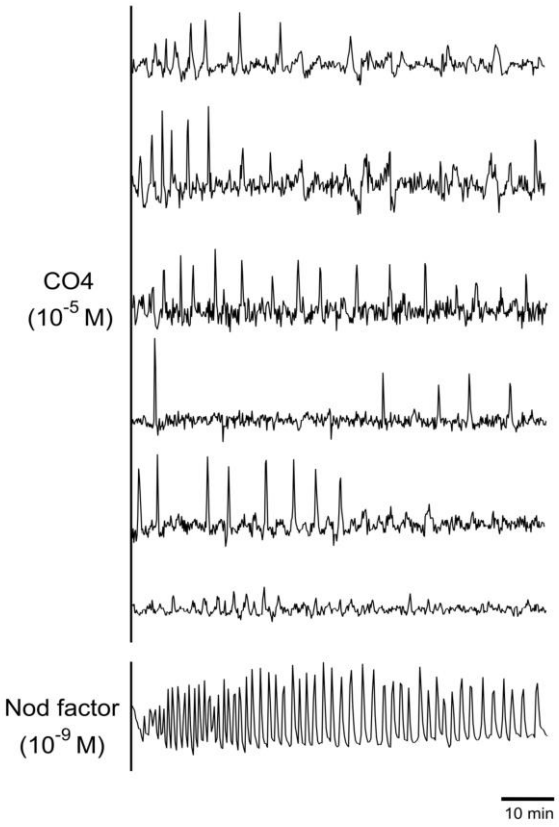

Figure S1: CO4 induced calcium responses in *M. truncatula* trichoblasts

Examples of calcium traces from root hairs of *M. truncatula* seedlings treated with 10^{-5} M CO4. Note the variation in responses. As a comparison a calcium trace from a root hair of *M. truncatula* treated with 10^{-9} M Nod factor is included. The y-axis is the ratio of YFP to CFP in arbitrary units.

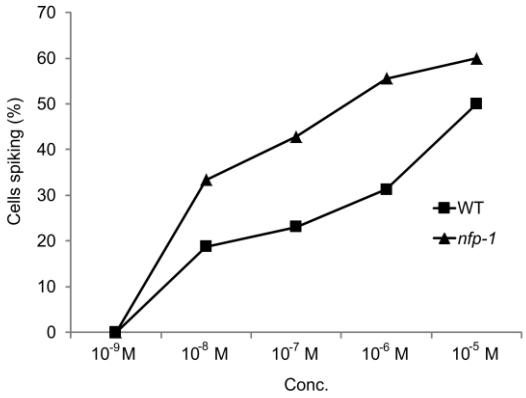

Figure S2: Dose response curves for CO4 in WT and *nfp-1*

The percentage of cells responding to different concentrations of CO4 in wild-type (WT) and *nfp-1* lateral roots. The dose response curve was performed on WT (75 cells on 6 plants) and *nfp-1* (59 cells on 5 plants) containing the calcium reporter cameleon.

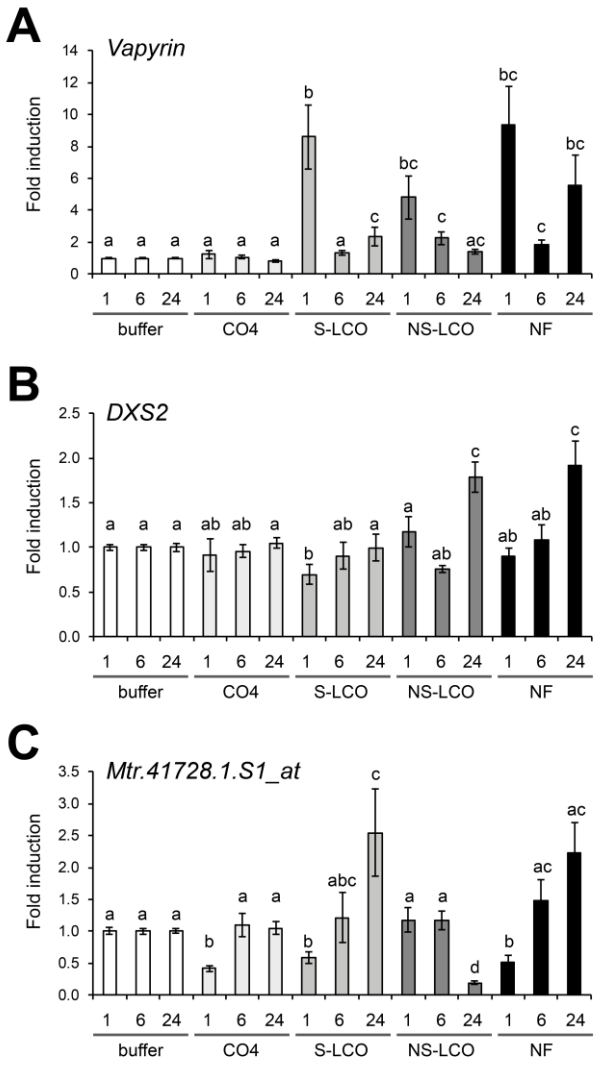

Figure S3. Specific gene induction in *M. truncatula* by the different LCOs

Induction of *Vapyrin* (A), *DXS2* (B) and *Mtr.41728.1.S1_at* (C) following 1, 6 and 24 h treatments with 10^{-10} M Nod factor (NF), 10^{-9} M S-LCO, 10^{-6} M NS-LCO and 10^{-5} M CO4. Values represent averages from three biological replicates and error bars are standard error. Letters denote significant induction in a two-tailed t-test ($p < 0.05$).

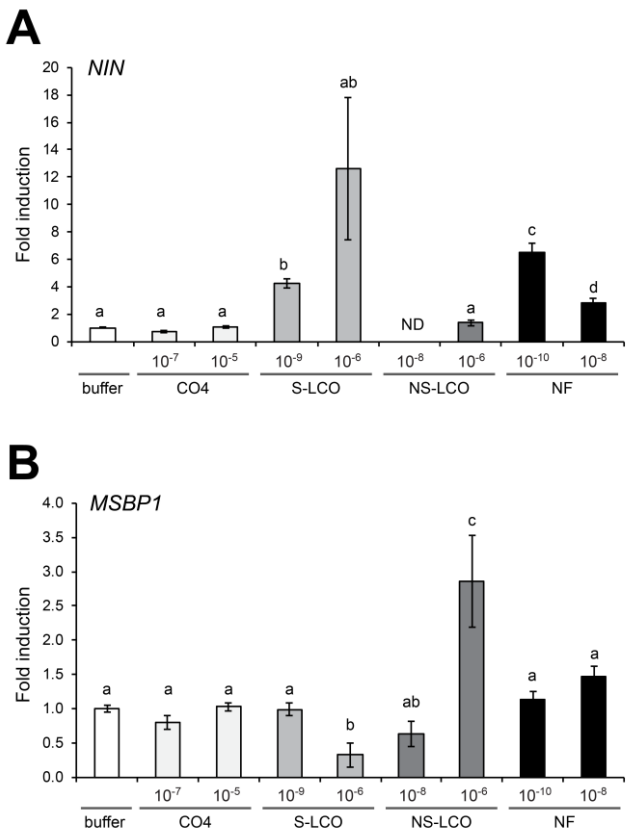

Figure S4. Gene induction in *M. truncatula* by the different LCOs is retained at higher concentrations

Induction of *NIN* (A) and *MSBP1* (B) following 24 h treatments with indicated concentrations of Nod factor (NF), S-LCO, NS-LCO and CO4. Values represent averages from at least two biological replicates and error bars are standard error. Letters denote significant induction in a two-tailed t-test ($p < 0.05$). ND: Not determined.

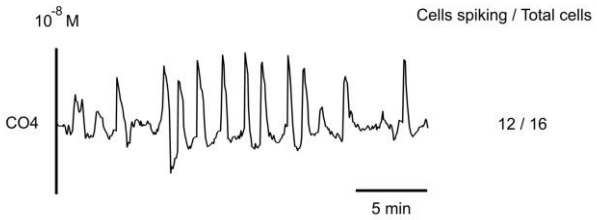

Figure S5: CO4-induced calcium responses in *M. truncatula* root organ cultures

A root organ culture of *M. truncatula* carrying nuclear-localised YC2.1 was assessed for responses to CO4. Treatments with 10^{-8} M CO4 were sufficient to activate calcium oscillations in root organ cultures. A representative calcium trace is shown and the number of responsive cells, relative to the total cells analysed is also shown.

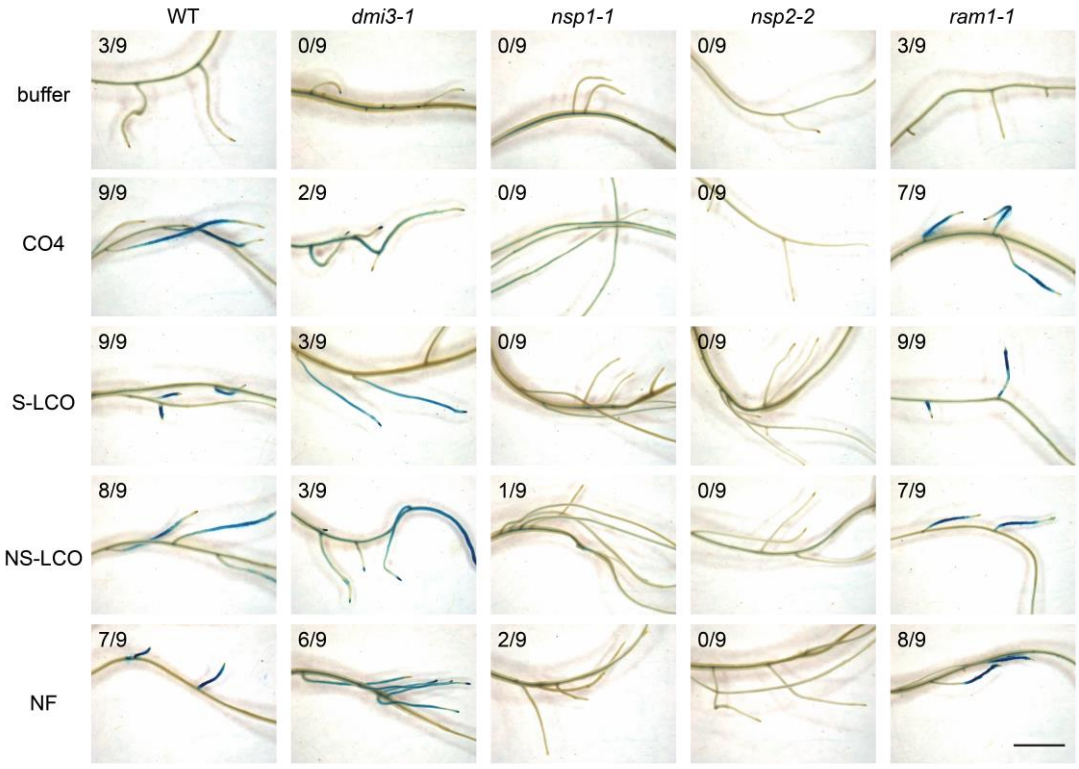

Figure S6. Induction of *pENOD11-GUS* in lateral roots

Induction of *pENOD11*-GUS in *M. truncatula* by 10^{-10} M Nod factor, 10^{-9} M S-LCO, 10^{-6} M NS-LCO and 10^{-5} M CO4 in lateral roots. The number of plants showing GUS staining, relative to the total number of plants analysed is indicated. The images are representative roots. The experiment was repeated three times with similar outcomes and all experiments are combined in the indicated numbers.

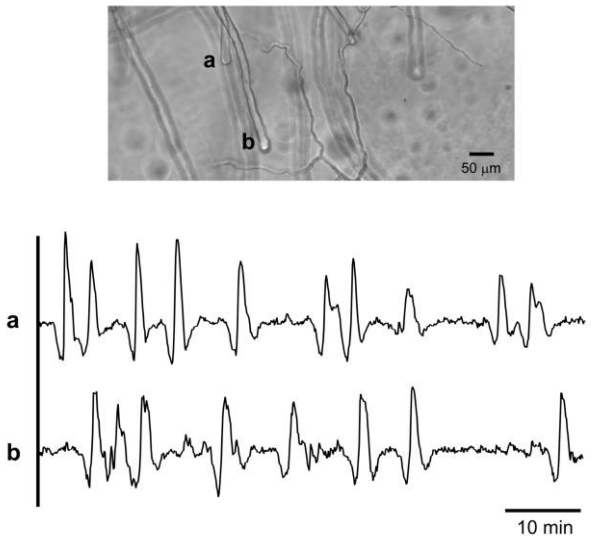

Figure S7: Calcium oscillations in *L. japonicus* trichoblasts induced by *R. irregularis*

L. japonicus plants were grown on plates containing *R. irregularis* in association with carrot root organ cultures. Trichoblasts in close proximity to *R. irregularis* (the branched hyphae in this image) were assessed for calcium responses. Two representative traces are shown that are calcium measurements of the two trichoblasts indicated with 'a' and 'b'.

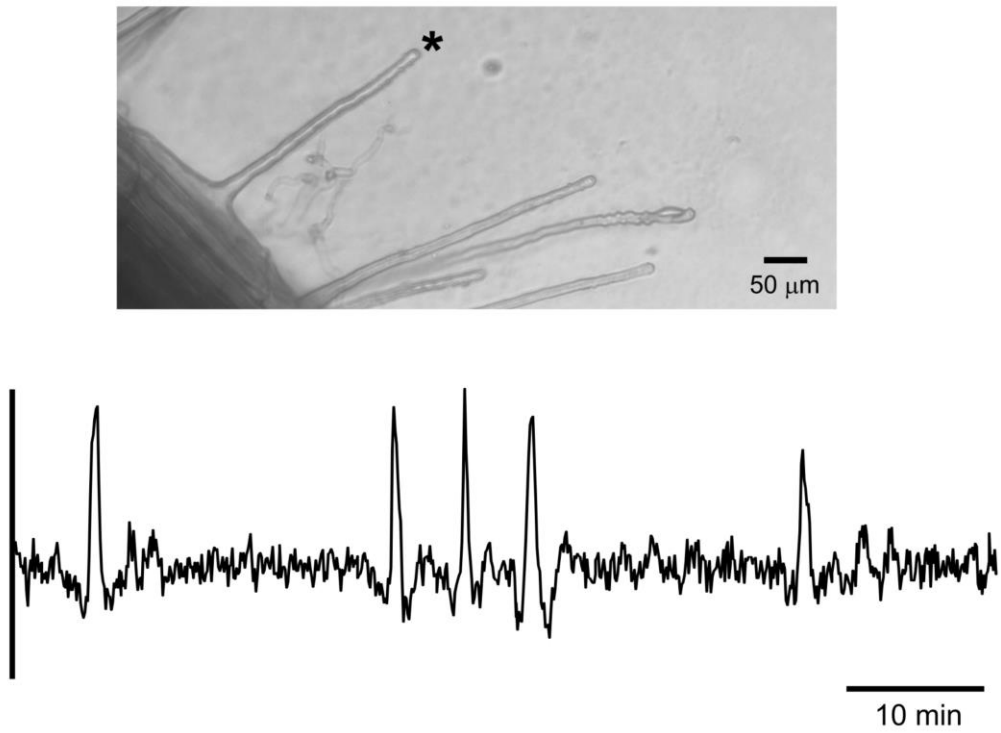

Figure S8: Calcium oscillations in rice trichoblasts induced by *R. irregularis*

Rice plants were grown on plates containing *R. irregularis* in association with carrot root organ cultures. Trichblasts in close proximity to *R. irregularis* (the highly branched hyphae in this image) were assessed for calcium responses. A representative trace is shown that is a calcium measurement of the trichoblast indicated with an asterisk.

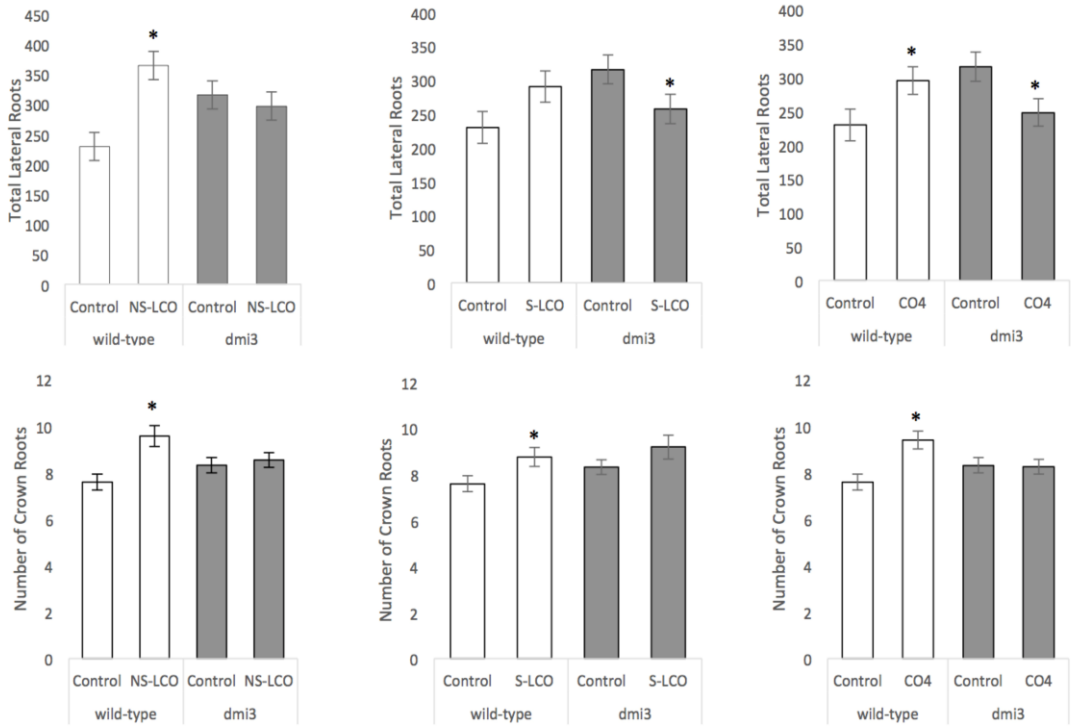

Figure S9. Promotion of root system development in rice by NS-LCO, S-LCO and CO4

The mean number of lateral roots and number of crown roots produced per rice plant is shown in response to treatments of 10^{-8} M NS-LCO, S-LCO or CO4 in both the wild-type (white) and *ccamk* (gray) rice lines. Controls indicate lines treated with the buffer. The results are the average of three experiments. The asterisk indicates statistical significance ($p < 0.05$) calculated using a t-test, assuming a normal distribution of the data, or a Wilcoxon signed-rank test when a normal distribution was not observed.

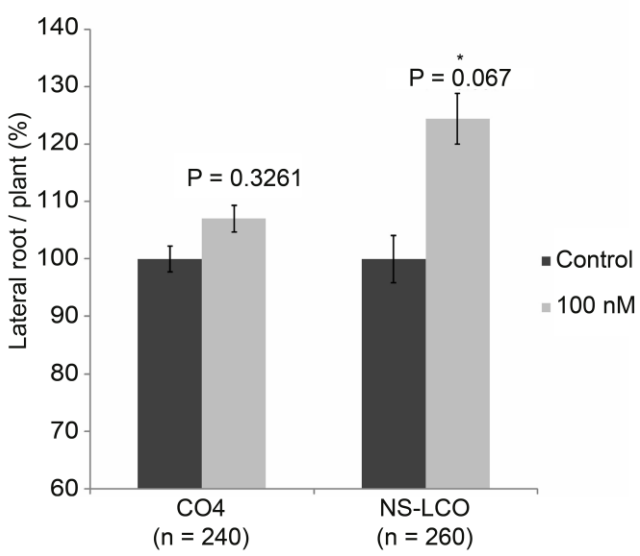

Figure S10: Promotion of lateral root emergence in *M. truncatula*

M. truncatula roots were treated with 10^{-7} M CO4 or 10^{-7} M NS-LCO and the effect on lateral root emergence was measured. The numbers in parentheses indicate the number of plants analysed. The significance of the difference to the control treated plants, as measured using a t-test, is indicated. The treatments are measured as fold induction relative to the control. Error bars are standard error.

Table S1: Cell and plant numbers assessed in Figure 8

	Medicago			*Lotus*			*Rice*		
	Trichoblast	Atrichoblast	No.	Trichoblast	Atrichoblast	No.	Trichoblast	Atrichoblast	No.
Nod factor									
10^{-9} M	8 / 10	7 / 9	2	9 / 10	11 / 12	2			
10^{-11} M	9 / 18	0 / 14	3	1 / 15	12 / 17	3			
NS-LCO									
10^{-5} M	9 / 11	11 / 18	3	0 / 10	10 / 11	2			
10^{-8} M	3 / 15	0 / 11	3	0 / 14	12 / 15	3			
CO4									
10^{-5} M							0 / 10	20 / 34	4
10^{-8} M							0 / 14	6 / 13	3
R. irregularis	6 / 14	ND	5	4 / 11	ND	4	4 / 10	ND	4

Table S2: Primers used for qRT-PCR in this study

Gene	Forward primer	Reverse Primer
EF1α	CTTTGCTTGGTGCTGTTTAGATGG	ATTCCAAAGGCGGCTGCATA
NIN	GCAATGTGGGGATTTAGAGATT	GGAAGATTGAGAGGGGAAGCTT
ERN1	GGAAGATGGTGCTGTTGCTT	TGTTGGATTGTGAACCTGACTC
MSBP1	CTGATAAGCCTCTTCTTATGG	TCCAACCTTAACGTACTTTCCC
MNR	TCAATGCCTATTGGCTCAACCTTGG	TCCCACTTCCACCGGCCAAC
Annexin 1	AACCTGCGGAGCGTGATGCTG	GCTGGCGAAGATGGCCGGAAG
Vapyrin	TCATCCTCCACAACAACAAGGT	TCAAGCACTTCTCTTATGTCATCCATTG
DXS2	TGGCCATCAGGCATATCCCCACA	TGCAATTGCCATACCAAGACCAGC
Mtr.41728.1.S1_at	AGCCTCAGTAACAACCGTGG	TGCAACAGCGACTACAAACC