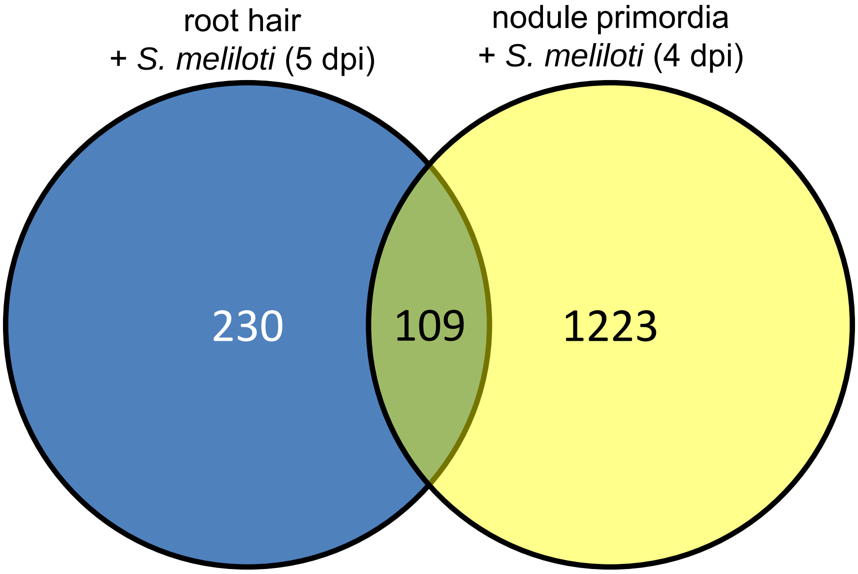
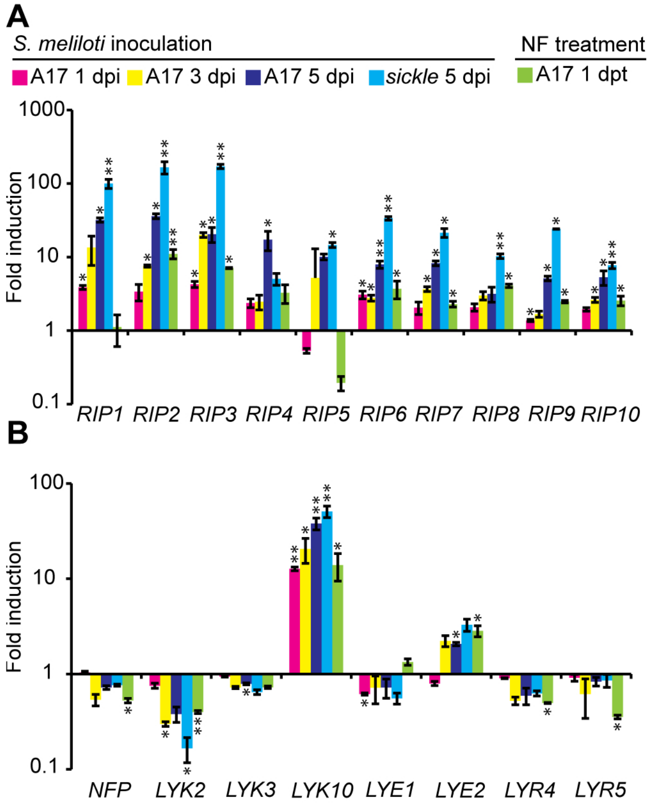
Supplemental Data. Breakspear et al. (2014). Plant Cell 10.1105/tcp.114.133496.



Supplemental Figure 1. Differentially Expressed Genes ($p<0.05$) in WT Plants Infected by Sm1021.

Left circle: numbers of genes significantly induced in root hairs harvested from WT plants 5 dpi with *S. meliloti*; right circle: genes induced in *S. meliloti* induced nodule primordia (MtGEA).

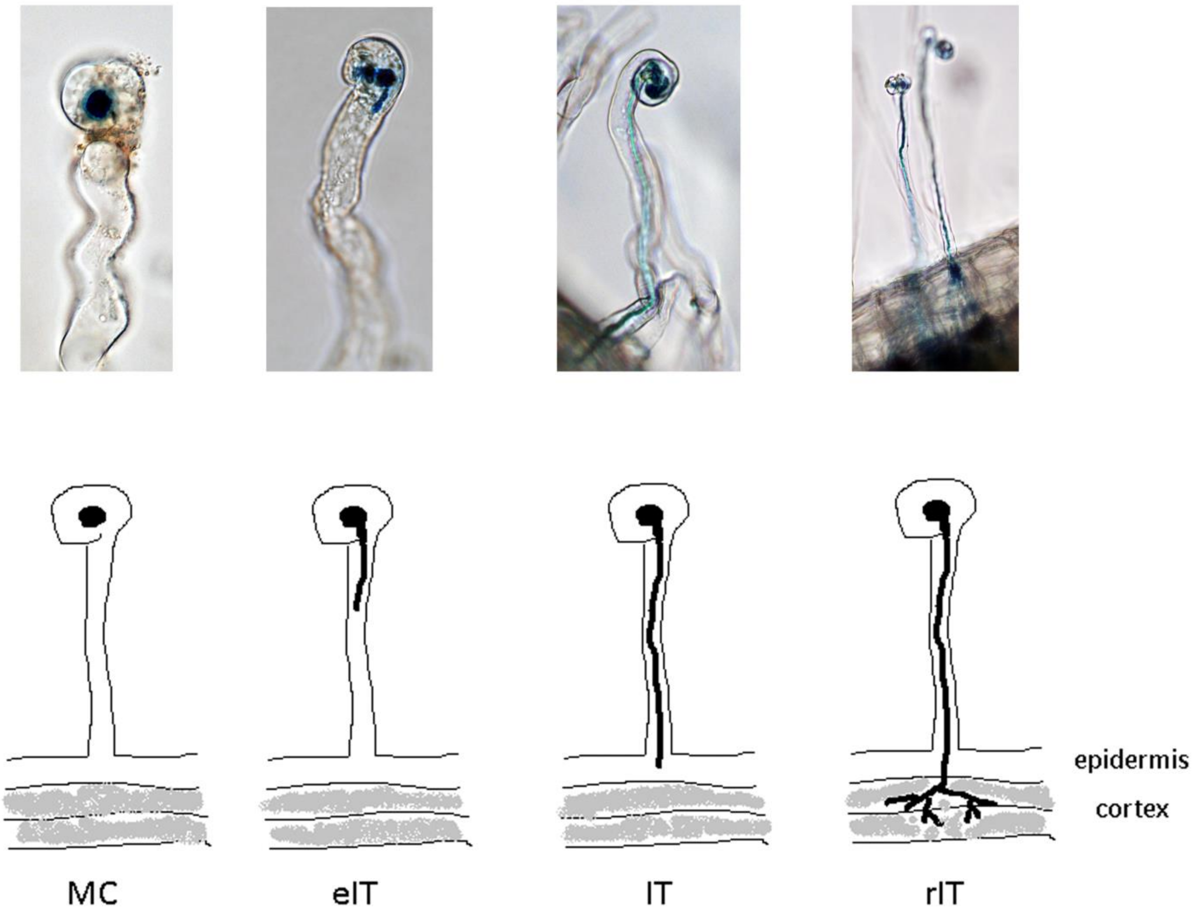


Supplemental Figure 2. Regulation of Genes Encoding Peroxidases and LYSM Proteins During Infection.

(A) Induction of Peroxidases (*RIP*s) in *M. truncatula* WT and *skl* mutant following inoculation with *S. meliloti* 1021 based on microarray data.

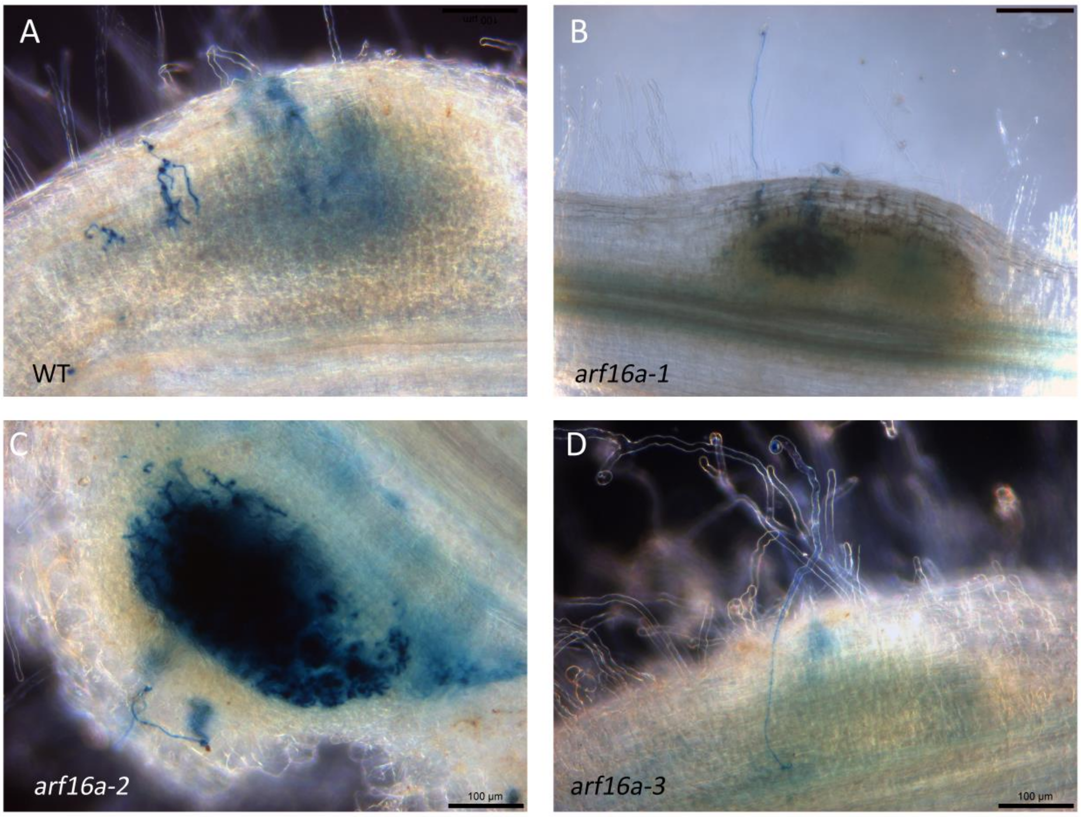
(B) Altered expression of LysM domain-containing proteins including: LYSM-receptor-like kinases (LYK), LYK-related (LYR) and LYE.

Significant inductions relative to control experiments are shown (* $P < 0.05$, ** $P < 0.01$). Error bars = SEM (n=3).



Supplemental Figure 3. Depiction of four categories of infection events which were used to characterize the infection phenotype of *mtarf16* mutants

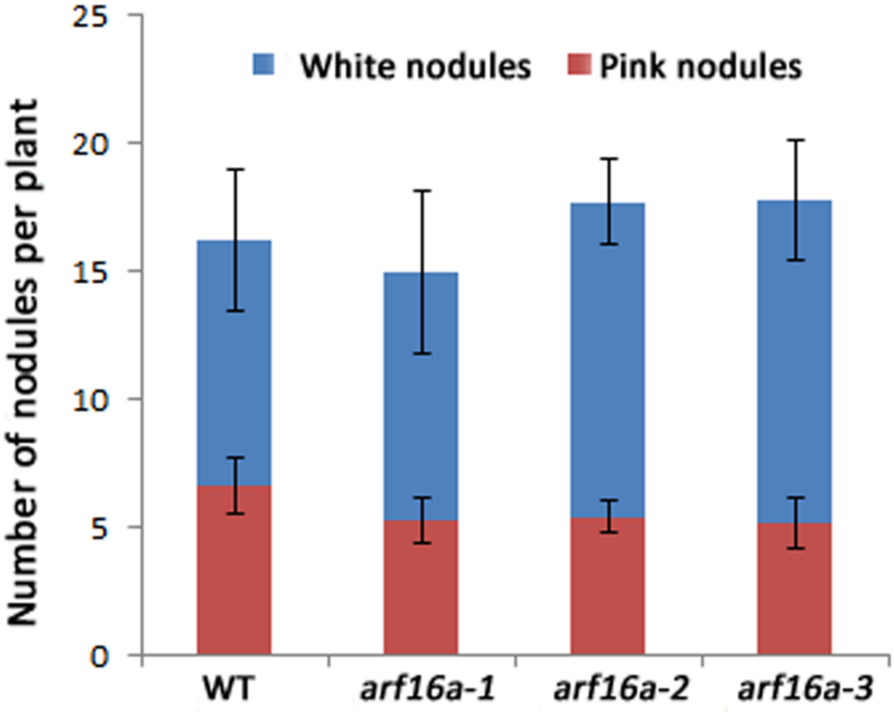
Upper panel: the four categories of infection events as shown by lacZ staining of *S. meliloti* (blue color). Bottom panel: Cartoon simplification of the four categories corresponding to the upper panels. Bold line indicates microcolony (MC) or infection threads (IT). Cortical cell layers were filled with grey. eIT, elongating infection thread. rIT, ramified infection thread.



Supplemental Figure 4. Infection threads and nodule primordia from WT (R108) and *arf16a* mutants.

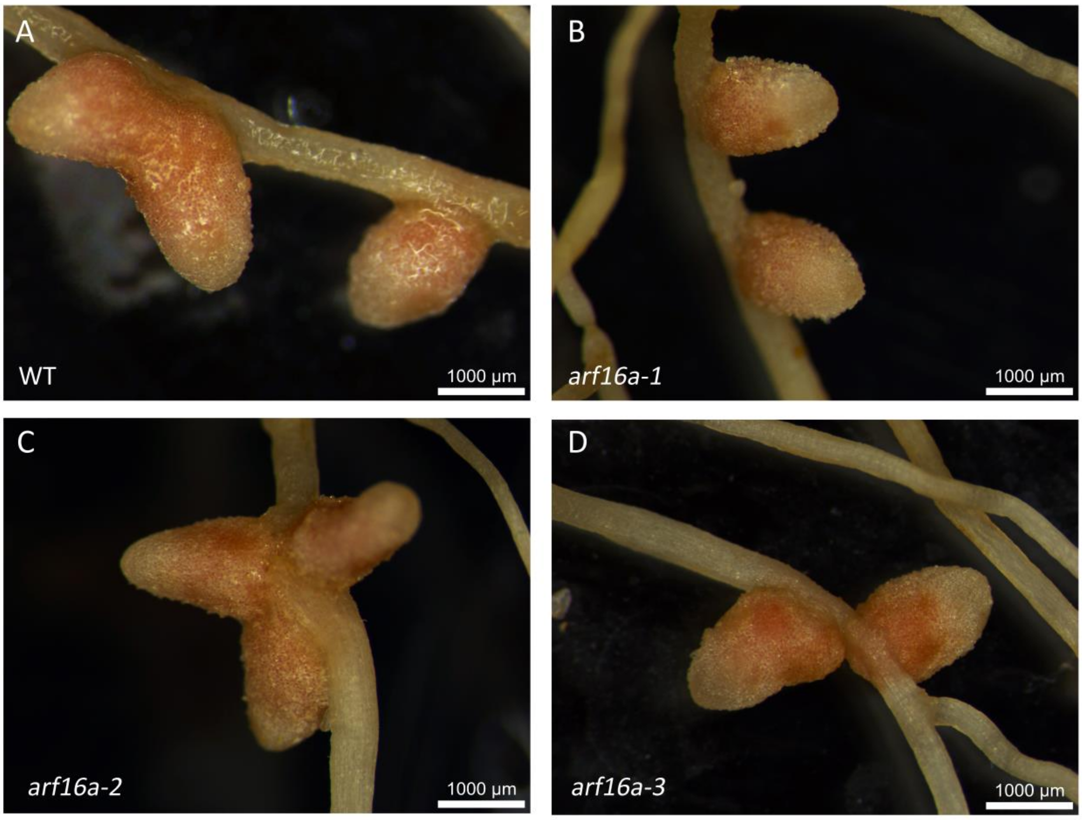
Some typical infection threads and/or nodule primordia from 7 dpi plants are shown.

Blue colour indicates lacZ staining of *S. meliloti* 1021. Bar = 100 µm (A, C and D) or 200 µm (B).



Supplemental Figure 5. Nodule numbers of *arf16a* mutants are not different from WT.

There were no significance differences in mean total nodule numbers between WT (R108) and *arf16a* alleles at 21dpi. (P>0.05, two-tailed Student's T-test). Error bar indicates standard error.



Supplemental Figure 6. Nodules of *arf16a* mutants have normal morphology.

Nodule phenotype of WT (R108) and *arf16a* mutants at 21dpi. Bar = 1000 μm.

Supplemental Table 1. Primers used in this study

Gene	**Model**	**Primer name**	**Primer sequence**
Genes of Interest			
SAUR1	Medtr8g094980	SAUR1-F	TGGTCCTCTCAAGCTTCCTT
		SAUR1-R	TGGTGATGATAGGGTGTGGA
GH3.1	Medtr5g016320	GH3.1-F	CTCAGAGTTTCTGACCAGTTCAG
		GH3.1-R	AGTAGGCTGTACAATAACTGACGAC
ARF16	Medtr1g094960	ARF16-F	GCGCCTTCTTCAGGTAGTGT
		ARF16-R	AGGTGTGAATGGTGAGAGATTG
IAA9	Medtr8g067530	IAA9-F	TGCTCATCGGTTGACAGTTC
		IAA9-R	CTTTCAGGCGACTGAGATCC
D27	Medtr1g471050	D27-F	TCAAGCAGCAACAGGAATCAG
		D27-R	AAATTTCTGTGAAGCCACGGTAG
CCD8	Medtr3g109610	CCD8-F	CTAGTGGAAAGATTGTGGCAAG
		CCD8-R	AGCAGTTACCCTCCCATCTTC
GDSL	Medtr5g083040	GDSL-F	TACCCAACCAAATACGGGCT
		GDSL-R	CAGGGCATATCATGTCAAGTTC
AMN3	Medtr8g022270	AMN3-F	GGTTATGAAACACAGGTTGGTG
		AMN3-R	GATTTCTTCAATATTGCCCTTGC
VRC2	Medtr7g074730	VRC2-F	TGGACCCTTTATTTGCCCTA
		VRC2-R	GAGAGGCTGCCAATGGATTA
Reference genes			
Actin	Medtr3g095530	Actin-F	TCAATGTGCCTGCCATGTATGT
		Actin-R	ACTCACACCGTCACCAGAATCC
PPA	Medtr6g084690	PP2A-F	CCGTTGTTGAGATCCTGCTT
		PP2A-R	TCCCACTTCCGGATAATGTT
Ubiquitin	Medtr1g066390	Ubiquitin-F	GCCGGAAAACAGCTAGAAGA
		Ubiquitin-R	GGAGACGGAGAACAAGGTGA
TIP41	Medtr1g107045	TIP41-F	GCTTTGCCACCTGTTGAAGT
		TIP41-R	AGCACCGCTTCCACAATAAG
Histone H3	Medtr4g097170	HH3-F	ccctggaactgttgctcttc
		HH3-R	cctgagcaatttcacgaacc
promoter cloning			
CCD8	Medtr3g109610	MtCCD8pro_attb1_F	ggggacaagtttgtacaaaaaagcaggctTCGCTCACCGGGACTAAAATTG
	Medtr3g109610	MtCCD8pro_attb1_R	ggggaccactttgtacaagaaagctgggtTTTGCTTTGAAATGGGGTTTC
VRC2	Medtr7g074730	MtVRC2pro_attb1_F	ggggacaagtttgtacaaaaaagcaggctTCTGGCATGCATGTGTGTTCAG
	Medtr7g074730	MtVRC2pro_attb1_R	ggggaccactttgtacaagaaagctgggtTAACCCTGTACCTCCAGTCAC
ARF16a	Medtr1g094960	MtARF16apro_attb1_F	ggggacaagtttgtacaaaaaagcaggctTCTGGTACCGTGAGATGAGGTG
	Medtr1g094960	MtARF16apro_attb1_R	ggggaccactttgtacaagaaagctgggtTGGTGTGGATGAGGAAAAAGG
GH3.1	Medtr5g016320	MtGH3.1pro_attb1_F	ggggacaagtttgtacaaaaaagcaggctTCGTTGGCATGATGTGATTTGG

	Medtr5g016320	MtGH3.1pro_attb1_R	ggggaccactttgtacaagaaagctgggtTGCCATGATGAAATGAAAGTTC
SAUR1	Medtr8g094980	MtSAUR1pro_attb1_F	ggggacaagtttgtacaaaaaagcaggctTCTGTCATTCATTGACAGCGTG
	Medtr8g094980	MtSAUR1pro_attb1_R	ggggaccactttgtacaagaaagctgggtTCTTTGTTGCTTTCCAAATTG
genotyping			
ARF16a	Medtr1g094960	ARF16a_geno_F2	gatgaggcagggataccaaaattc
	Medtr1g094960	ARF16a_geno_R2	ttgaatcaccagcaacaagc
Tnt1	NA	Tnt1R	CAGTGAACGAGCAGAACCTGTG
	NA	Tnt1F	TCCTTGTTGGATTGGTAGCC

Supplemental Table 2. Details for genes in Figure 13.

Gene name*	IMGAG gene model	Affymetrix Probeset	notes
PR4	Medtr1g080800	Mtr.8884.1.S1_at	Pathogen response protein
PR5	Medtr5g010635	Mtr.42989.1.S1_at	Pathogen response protein
NSP2	Medtr3g072710.1	Mtr.44789.1.S1_at	transcription factor
kinesin 3	Medtr2g082570.1	Mtr.38545.1.S1_s_at	kinesin
α-tubulin	Medtr1g106005.1	Mtr.28758.1.S1_s_at	α-tubulin
α-tubulin	Medtr1g106005.1	Mtr.8502.1.S1_s_at	α-tubulin
SAUR2	Medtr2g066830.1	Mtr.41652.1.S1_at	Auxin response gene
α-tubulin	Medtr1g106005.1	Mtr.37318.1.S1_at	α-tubulin
α-tubulin	no match	Mtr.34696.1.S1_s_at	α-tubulin
LYK3	Medtr5g086130.1	Mtr.142.1.S1_s_at	Nod factor receptor
RPG	Medtr1g062200.1	Mtr.1440.1.S1_at	unknown function
NSP1	Medtr8g020840.1	Mtr.6956.1.S1_at	transcription factor
HA1	Medtr8g006790.1	Mtr.43470.1.S1_at	H+ ATPase
NIN	Medtr5g099060.1	Mtr.28094.1.S1_at	transcription factor
NFYA1	Medtr1g056530.1	Mtr.43750.1.S1_at	transcription factor
NFYA2	Medtr3g106430.1	Mtr.1584.1.S1_at	transcription factor
ANN1	Medtr8g038210.1	Mtr.14183.1.S1_at	Annexin
VAPYRIN	Medtr8g056020.1	Mtr.42828.1.S1_at	unknown function
ERN1	Medtr7g085810.1	Mtr.7556.1.S1_at	transcription factor
ENOD11	Medtr3g082100.1	Mtr.13473.1.S1_at	unknown function
NPL	Medtr3g086320.1	Mtr.26489.1.S1_at	pectate lyase
FLOT4	Medtr3g106430.1	Mtr.42072.1.S1_at	Flotillin
PUB1	Medtr5g083030.1	Mtr.26500.1.S1_at	E3 ligase
RABA1	Medtr4g079350.1	Mtr.13500.1.S1_at	RAB GTPase
RABG1	Medtr6g039490.1	Mtr.43764.1.S1_at	RAB GTPase
RABGAP1	Medtr1g007590.2	Mtr.34702.1.S1_at	RAB-GTPase activating protein
RABGAP1	Medtr1g007590.1	Mtr.17571.1.S1_at	RAB-GTPase activating protein
ROPGEF14	Medtr5g025960.1	Mtr.10377.1.S1_at	ROP-Guanine nucleotide exchange factor

*Note that Cell Cycle, GA, SL, and Auxin genes are found in Supplemental Data Sets **4,5,6 and 8** respectively.