MtARF4a	1	MEIDLNNEVIEVEKNALCHKECEKGFCFCVSCLSPSTCSSSSSTSPLVSSSYLELWHA
MtARF4b	1	MEIDLNDSIT <mark>EV</mark> KKNVCSNGK <mark>CEK</mark> SVCCVCTLS <mark>SSSSPTCSSSSSTS</mark> AIVSSSYLELWHA
MtARF4a	59	CAGPLTSLPKKGNVVVYFPQGHLEQ <mark>F</mark> ASFS <mark>P</mark> FKQLEIP <mark>N</mark> YDLQPQI <mark>F</mark> CRVVNVQLLANKE
MtARF4b	61	CAGPLTSLPKKGNVVVYFPQGHLEQ <mark>V</mark> AS <mark>LS</mark> LF <mark>SSLEIP</mark> TY <mark>G</mark> LQPQI <mark>LCRVVNVQLLANKE</mark>
MtARF4a	119	NDEVYTQVTLLPQAELAGMHMEGKEVEELEGDEEGDGGSPTKSTPHMFCKTLTVSDTSTH
MtARF4b	121	NDEVYTQV <mark>ALLPQAELAGMCLDDKEPEGLEADDEGNGR</mark> SPTKLASHMFCKTLT <mark>ASDTSTH</mark>
MtARF4a	179	GGFSVPRRAAEDCFPPL <mark>DYK</mark> LQRPSQELVAKDLHGV <mark>E</mark> WKFRHIYRGQ
MtARF4b	181	GGFSVPRRAAEDCFPPLVQHYEPVWICLFEDYK <mark>Q</mark> QRPSQELVAKDLHGV <mark>G</mark> WKFRHIYRGQ
MtARF4a	226	PRRHLLTTGWSIFV <mark>N</mark> QKNLVSGDAVLFLRG <mark>Q</mark> NGELRLGIRRA <mark>V</mark> RPRNGLPESIVGNQ <mark>N</mark> CY
MtARF4b	241	PRRHLLTTGWSIFV <mark>SQKNLVSGDAVLFLRG</mark> ENGELRLGIRRA <mark>ARPRNGLPESIIGNQ</mark> SCS
MtARF4a	286	PNFLSSVANAIS <mark>T</mark> KSMFHVFYSPRASHAEFVVPYQKY <mark>V</mark> KSIKNPMTIGTRFKMRIEMDES
MtARF4b	301	P <mark>S</mark> FLSSVANAIS <mark>A</mark> KSMFHVFYSPRASHADFVVPYQKY <mark>A</mark> KSIRNPVTIGTRFKMKF <mark>EMDES</mark>
MtARF4a	346	PERRCSSGMLIGINDLDPYRWPKSKWRCLMVRWDDDTETNHQDRVSPWEIDPSSPQPPLS
MtARF4b	361	PERRCSSGIVTGMSDLDPYKWPKSKWRCLMVRWDEDIGANHQDRVSPWEIDPSTSLPPLN
MtARF4a	406	IOSSPRLKKPRTGLLVASPNHLITGMNPNGISGMMGFEESVRSPKVLOGOENTGFMSLYY
MtARF4b	421	IQSSRRLKKLRTGLHVESPSHFITAGDSGFMDFDESIRSSKVLQGQEKTSFMSLYY
MtARF4a	466	GCDKWTNOPGFELSTSSHHONLASTGIGKWVTSSELMSVHPFSYAGEMESNNEPRVLOGO
MtARF4b	477	GCDTVTKQKEFDINSLR-HTNLASNGAR-KITSSEFTRIQPSSYADFTEMNRFPRVLQAQ
MtARF4a	526	ETCKIKSISGKVDENTGAWGTKPSEOSACEPYGDIDKS
MtARF4b	535	EIYPLRSLTGKVDLNLNSWGKTNVSYTKYNLHNATKLNFHSLGSEVLQNSSFPYGDIHKV
MtARF4a	564	NOA-SMFSSKHTSEMSDNVPENTPSIVAGDIRKEVGRSGSNLLPNEHKLODNVSASASLV
MtARF4b	595	G <mark>QGSSM</mark> LC <mark>SK</mark> PTNFQLG <mark>NV</mark> SFNTPSSQIGALRNEVGLSSFK-IRNEQKLQNDISAATS-L
MtARF4a	623	DTNRNAPNDNNVKGKANSCKLFGFPLSGEPSSONLONTAKRSCTKVHKOGSLVGRAID
MtARF4b	653	DANIRISNDENFKEMVNPCKLFGFSLSAAAETTSQNLQNSAKRSCTKVHKQGSLVGRAID
MtARF4a	681	LSRLS <mark>GYNDLLSELEKLFGMEGLLRD</mark> SDKGWRILYTDSENDIMVVGDDPWHEFCDVVSKI
MtARF4b	713	LSRLS <mark>SYNDLVSELERLFGMEGLLRD</mark> PDKGWRILYTDSENDIMVV <u>GDDPWHEFCDMVSKI</u>
MtARF4a	741	HIYTKEEVEKMTFGMMTNDDTHSCLDQAPVIIEPSKSSSVGQPDYSPTVVRV
MtARF4b	773	HIYTQEEVEKMTIGMMN-DDNQSCLEQTPLIMEASKSSSVGQPDSSTTVVRI

Supplementary Figure 1. Alignment of MtARF4a and MtARF4b amino acid sequences. The multiple alignment was generated using the Clustal Omega algorithm available at EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/) and decorated with BOXSHADE (http://sourceforge.net/projects/boxshade/). Identical amino acids were shaded in black, whereas synonymous substitutions were shaded in gray. Locus IDs are MtrunA17_Chr4g0029671 (Medtr4g060460) and MtrunA17_Chr2g0326281 (Medtr2g093740) for ARF4a and ARF4b, respectively.



Supplementary Figure 2. Phylogenetic tree of amino acid sequences of Arabidopsis and *M. truncatula* members of the ARF family. All members of the Arabidopsis ARF family were retrieved from the TAIR database (https://www.arabidopsis.org/). *M. truncatula* ARF members linked to root development or nodulation were retrieved from were retrieved from the recently released version of the *M. truncatula* genome MtrunA17r5.0-ANR (https://medicago.toulouse.inra.fr/MtrunA17r5.0-ANR/, Pecrix et al. 2018). The tree was generated using MEGA X (Kumar et al, 2018) using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) is shown next to the branches (Felsentein et al, 1985). The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000). The ARF2 ARF3, and ARF4 clades were labelled with pink, green and blue circles, respectively.



Supplementary Figure 3. Heat map of *MtARF2*, *MtARF3*, *MtARF4a* and *MtARF4b* expression during lateral root and nodule formation. Log_2 Fold change of expression values at indicated time points upon induction of lateral root formation (Lat root) as compared with not induction of lateral roots or upon spot inoculation with droplets of *S. meliloti* suspension (Spotinoc) as compared with non inoculated roots are presented. Data were retrieved from Schiessl et al. (2019), where genes with Fold changes >1.5 were considered as differentially expressed. The asterisk indicates significant differences at each time point between induced lateral roots not induced roots or between spot inoculated roots and not inoculated roots with an adjusted p value ≤ 0.5 .

MtARF2	1237	AACG <mark>TG</mark> GTGCCGT <mark>CATC</mark> CCCAGATTCTTCTTGTTCTTACCCGCGAAGCATCCTCAAA	
MtARF3	1225	GGATTCACTTCAGCAAAGCTA-GAATTTCCAGTTCCCAATGCGAT	
MtARF4a	1252	GGTCTACTGGTTGCCTCACCCAATCAC-CTCATCACTGGTATGAATCCTAATGGAATCAG	
MtARF4b	1297	GGTCTGCATGTTGAATCACCCAGTCAC-TTTATCACTGCTGGAGACAG	
		tasiARFs target site	
MtARF2	1293	AGTAAGCATGGACCCTTTGCCCACTAGTGGTTTCCAGAGGGTCTTGCAAGGTCAAGAATC	
MtARF3	1269	T <mark>GGAGCGTCAGACTTTGGGG</mark> AATC <mark>AT</mark> TAAGAT <mark>TC</mark> G <mark>AAAGGTCTTGCAAGGTCAAGAA</mark> T	
MtARF4a	1311	TGGAATGATGG <mark>GT</mark> TTTGAGGAGTC <mark>AG</mark> TAAGAT <mark>CA</mark> CCA <mark>AAGGTCTTGCAAGGTCAAGAA</mark> AA	
MtARF4b	1344	TGGCT <mark>T</mark> TATGGACTTTGA <mark>T</mark> GAGTCT <mark>ATAAGAT</mark> CAT <mark>CCAAGGTCTTGCAAGGTCAAGAA</mark> AA	
MtARF2	1353	ATCGACCTTGAGAGGAAATTTGGCTGAAAGCAACCACTCTTACACTGCAGAGAAGTCTGT	
MtARF3	1329	TATGGGCATGAATACTCCCTATGACACTATTAATGCTCAGAGTCCCCGGTTATA	
MtARF4a	1371	TACAGGTTTTTATGTCACTATACTATGGATGTGACAAAGTAACCAACCAGCCAGCTTT	
MtARF4b	1404	AACAAGTTTTATGTCACTATATTATGGATGTGACACAGTAACTAAGCAGAAAGAATT	
<i>MtARF2</i>	1413	TGCGTGGACTCCTGCAACTGATGAAGAAAAGATGCATGCTGTTTCTACTTCAAGAACGTA	
MtARF3	1383	TGAGCTAGGGAGGTGCTATCCTGGTTCAAACTGTTCCGGCGATTGCTGCAACAGGAAA	
MtARF4a	1428	TGAGTTGAG <mark>C</mark> ACTTCAAGTCAT <mark>C</mark> AAAAACCTTGCAT <mark>CAAC</mark> TGGAATAG <mark>G</mark> AAAAGT	
MtARF4b	1461	TGATATCAATTCTCTAAGACATACTAATCTTGCATCAAATGGAGCAAGAAA	
MtARF2	1473	TGGTTCAGAGAACTG <mark>CAT</mark> GCCAATGTCAAGGCAGGAACCA <mark>ACCT</mark> A <mark>TTC</mark> G <mark>GA</mark> CCTTCT <mark>G</mark> TC	
MtRF3	1440	<mark>A</mark> GAATGCACCCAGCGCCTTC	
MtARF4a	1485	<mark>ACTT</mark> AGTT	
MtARF4b	1512	ACTTCTGAGTTTACGAG	
		tasiARFs target sit	e
MtARF2	1533	GGGATTTGGTAGTACCCCTGAAGGCAAACATAACATGCTTACTCAATGGCCTGTA	
MtARF3	1464	TGATTT <u>TCCCTCTAAT</u> GGCAT <u>AGGCTTTG</u> GT <u>GAA</u> TCTTT <mark>CAG</mark> ATTCCAAAAGGTCTTGCA	
<i>MtARF4a</i>	1509	TGTTCATCCTTTCAGTTATGCAGGCTTTATGGAAAGTAACAACTTTCCAAGGGTCTTGCA	
MtARF4b	1536	GATTCAACCTTCTAGTTATGCAGACTTCACGGAAATGAACCGGTTTCCTACGGTCTTGCA	
MtARF2	1588	ATGCCACCTGGTCTGTCGCTAAATTTCTTGCACTCCAATATGAAAGG	
MtARF3	1524	AGGTCAAGAAATT <mark>CTTCTGTGCCCACCCTACG</mark> GACGAGCCTCGTTTGACGAGGCCCCG	
MtARF4a	1569	AGGTCAAGAAATATGT <mark>AAACTGAAATCC</mark> CTTTCAGGAAAAGTTGATTTCAACATTGGAGC	
MtARF4b	1596	AGCTCAAGAAATATATCCATTGAGATCCCTGACAGGAAACGTTGATCTAAACCTTAACTC	
MtARF2	1635	TTCTGCACAAGGCAGTGATAATGCAACTTACCAAGCTCAAGGGAATATGAGATA	
MtARF3	1581	TGGAAGTGGTTGCATTGGACGTTATGATGGTTATCCAATGCTCGG-CTCCAGAAATG	
MtARF4a	1629	TTGGGGAACCAAGTTTTCAATCTGCATGTTTTCCT	
MtARF4b	1656	TTGGGGTAAAACCAATGTTAGTTACACAAAATATAACCTGCATAATGC-AACCAAACTTA	

Supplementary Figure 4. Multiple alignment of nucleotide sequences of the region of *MtARF2*, MtARF3, MtARF4a and MtARF4b transcripts used for the design of ARF2/3/4 RNAi primers. The multiple alignment was generated the Clustal Omega algorithm available at EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/) and shaded with BOXSHADE (http://sourceforge.net/projects/boxshade/). Primers used for amplification of ARF2/3/4 RNAi fragments are colored in blue. Targets sites for tasiARFs were colored in green. Locus IDs are MtrunA17_Chr2g0282961 MtrunA17_Chr8g0385791 (Medtr8g100050), (Medtr2g014770), MtrunA17_Chr4g0029671 (Medtr4g060460) and MtrunA17_Chr2g0326281 (Medtr2g093740) MtARF2, MtARF3, MtARF4a and MtARF4b, respectively.



Supplementary Figure 5. Transcript levels of MtARF16a and MtARF19a in GUS RNAi and ARF2/3/4a/4b RNAi roots. Expression values were determined by RT-qPCR, normalized to MtHIS3L and expressed relative to the GUS RNAi sample, which was set at 1. Each bar represents the mean \pm SE of three biological replicates (whole root tissue from at least three composite plants were collected in each biological replicate) with three technical replicates each. The asterisk indicates statistically significant differences between GUS and ARF2/3/4a/4b RNAi roots in an unpaired two-tailed Student's t test with a p value ≤ 0.05 .



Supplementary Figure 6. Root and shoot phenotype of *arf4a* mutant plants at 15 days after germination (dag). Primary root length (A), lateral root length (B), lateral root density (C), shoot length (D) and number of true leaves (E) were measured in WT and *arf4a* mutant plants at 15 dag. Error bars represent mean ± SE of three biological replicates, each with at least 10 plants. Asterisks denote a statistically significant differences between WT and *arf4a* plants in an unpaired two-tailed Student's t test (**: $p \le 0.01$, ***: $p \le 0.001$, ****: $p \le 0.0001$). F. Illustrative pictures of shoots of WT and *arf4a* plants at 15 dag. Scale bar: 0.5 cm