SUPPORTING MATERIALS AND METHODS

Quantitative real-time RT-PCR

Gene expression studies were performed with real-time RT-PCR (StepOne plus, Applied Biosystems) using the Power SyBR Green master mix (Applied Biosystems). Primers used are indicated in Supplemental Table S1. *Ubiquitin carboxy-terminal hydrolase* gene was used as internal standard for normalization of RNA levels. RNA purification and cDNA synthesis were carried out as previously described (Tejada-Jiménez et al., 2015)

Tejada-Jiménez M, Castro-Rodríguez R, Kryvoruchko I, Lucas MM, Udvardi M, Imperial J, González-Guerrero M (2015) *Medicago truncatula* Natural Resistance-Associated Macrophage Protein1 is required for iron uptake by rhizobia-infected nodule cells. Plant Physiol. **168:** 258-272

Table S1. Primers used in this study

Name	Sequence (5’ - 3’)	Use
5MtMOT1.2 +1689QF	GACCGAACTTTGCAGAAAGCA	Quantitative expression of *MtMOT1.2*
3MTMOT1.2 +1813QR	TGGGGATTTGGCTTGTCATCA	Quantitative expression of *MtMOT1.2*
5MtUBqF	GAACTTGTTGCATGGGTCTTGA	Quantitative expression of MtUbiquitin carboxyl-terminal hydrolase
3MtUBqR	CATTAAGTTTGACAAAGAGAAAGAGACAGA	Quantitative expression of MtUbiquitin carboxyl-terminal hydrolase
5MtMOT1.2-1446pGW	GGGACAAGTTTGTACAAAAAAGCAG GCTTTGTAACCTTTTGTATCCCTTTTG	*MtMOT1.2* promoter cloning into pGWB3 and pGWB13 vector
3MtMOT1.2pGW	GGGGACCACTTTGTACAAGAAAGCTG GGTAGGTCCAAGATTTTGTTTTTGGGAC	*MtMOT1.2* promoter cloning into pGWB3 vector
3MtMOT1.2 1361 GW	GGGGACCACTTTGTACAAGAAAGCTG GGTATGGTTTATCTTTTGTCAAATTCC	*MtMOT1.2* promoter cloning into pGWB13 and pGWB5 vector
5MtMOT1.2 GW	GGGGACAAGTTTGTACAAAAAAGCA GGCTATGGCAAACCAAAACTCTCATCC	*MtMOT1.2* promoter cloning into pGWB5 vector

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. *Medicago truncatula Molybdate Transporter 1.2* (*MtMOT1.2*) gene is expressed around the nodule vessels. Bleach-clarified image of section shown in Fig. 2C. Scale Bar = 25 μm.

Supplemental Figure S2. *Medicago truncatula* Molybdate Transporter 1.2 (MtMOT1.2) is located in the endodermis. Cross-section of a 28 days-post-inoculation (dpi) *M. truncatula* nodule expressing *MtMOT1.2-HA* and inoculated with a *Sinorhizobium meliloti* strain constitutively expressing the green fluorescent protein (GFP) (green). MtMOT1.2-HA was detected using an Alexa594-conjugated antibody (red). DNA was stained with DAPI (blue), and the Casparian strip shows autofluorescence in the blue channel. Top left panel, localization of DAPI-stained DNA (blue), *S.* meliloti (green) and the Casparian strip (indicated by arrow-heads). Top right panel MtMOT1.2-HA localization. Lower panel, overlay of the two previous panels. Scale bars= 25 μm.

Supplemental Figure S3. Autofluorescence control for the confocal immunolocalization of MtMOT1.2-HA. (A) Cross-section of a 28 days-post-inoculation (dpi) *M. truncatula* nodule expressing *MtMOT1.2-HA* and inoculated with a *Sinorhizobium meliloti* strain constitutively expressing the green fluorescent protein (GFP) (green). DNA was stained with DAPI (blue). Top left panel, transillumination image; top right panel, Alexa594 emission channel; lower left panel, *S. meliloti* GFP; lower right panel, DAPI-stained DNA. Scale bars= 0.2 mm. (B) Cross section of a 28 dpi *M. truncatula* nodules expressing *MtMOT1.2-HA*. Top left panel, transillumination image; top right panel, Alexa594 emission channel; lower panel, xylem autofluorescence. Scale bars= 0.1 mm.

Supplemental Figure S4. Autofluorescence control for the *Nicotiana benthamiana* agroinfiltration assays. (A) Emission in the cyan fluorescent protein (CFP) channel (left panel), green fluorescent protein (GFP) channel (central panel); and overlay of the two with the transillumination image (right panel) of leaves infiltrated with the AtPIP2-CFP construction. Scale bars= 0.05 mm. (B) Emission in the CFP channel (left panel), GFP channel (central panel); and overlay of the two with the transillumination image (right panel) of leaves infiltrated with the MtMOT1.2-GFP construction. Scale bars= 0.1 mm.

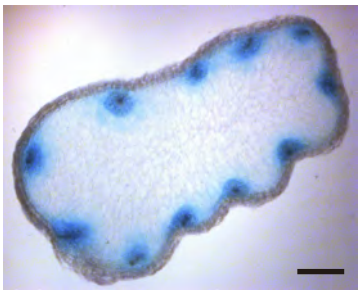
Supplemental Figure S5. Negative control for the immunodetection of MtMOT1.2-HA with gold-conjugated antibodies. Scale Bar = 0.5 μm.

Supplemental Figure S6. *Medicago truncatula* Molybdate Transporter 1.2 (MtMOT1.2) is not required for growth under non-symbiotic conditions under low-iron conditions. (A) Growth of representative WT and *mot1.2-1* plants watered with KNO_3. Scale bar= 1 cm. (B) Dry weight of shoots and roots. Data are the mean ± SD of at least 6 independently transformed plants. (C) Nitrate reductase activity. Nitrate reduction was measured in duplicate. Data are the mean ± SD.

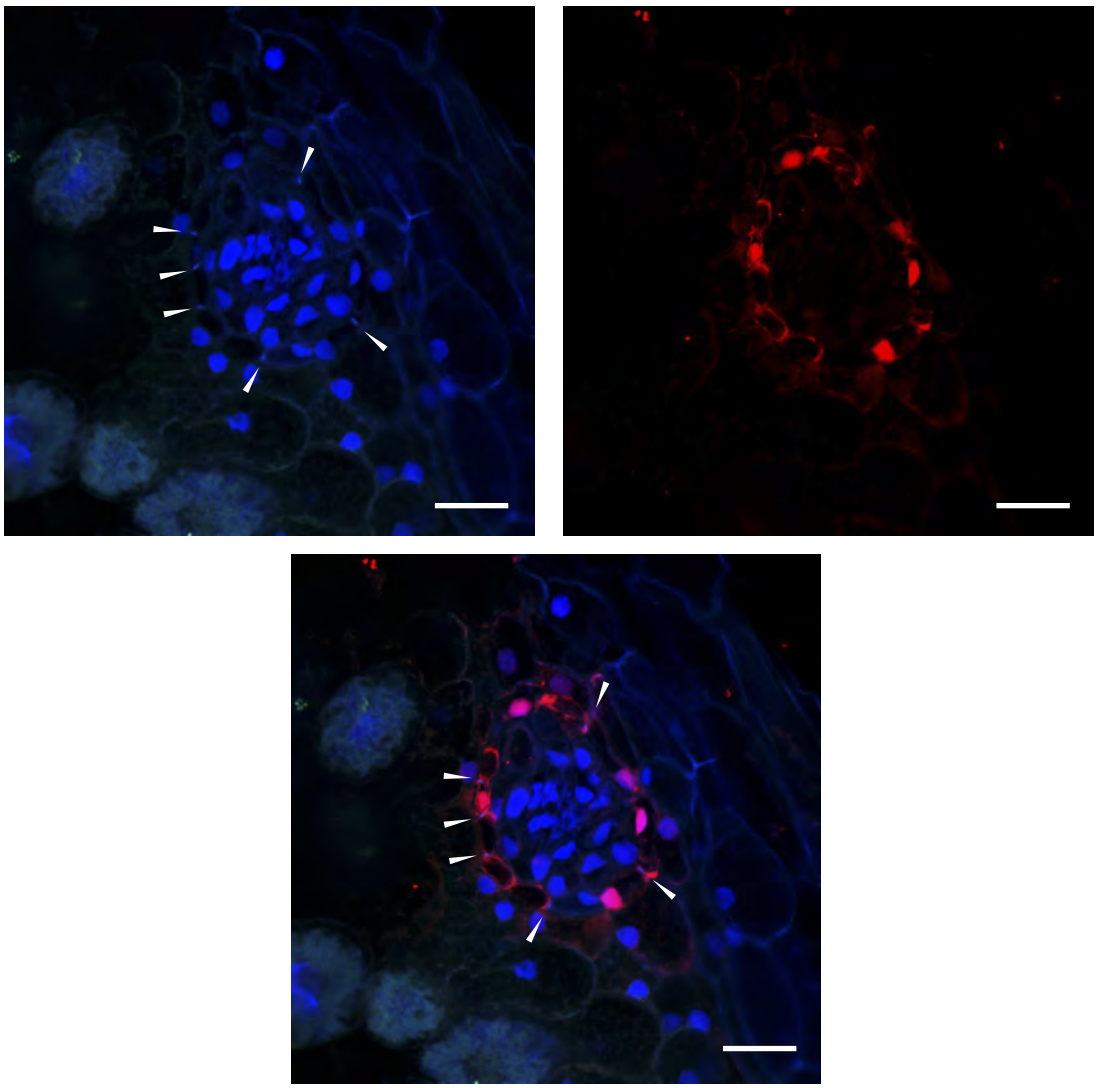
Supplemental Figure S7. Mutation of *Medicago truncatula Molybdate Transporter 1.2* (*MtMOT1.2*) does not affect nodule development. (A) Longitudinal section of 28 days-post-inoculation (dpi) nodules from wild type (WT) or mutant *mot1.2-1* plants stained with toluidine blue. Scale bars = 0.1 mm. (B) Kinetics of nodule appearance in WT and *mot1.2-1* plants. Data are the mean ± SD of 15 plants.

Supplemental Figure S8. Molybdate-dependence of the *mot1.2-1* phenotype. (A) Growth of representative 28 days-post-inoculation WT and *mot1.2-1* plants watered with standard 4 nM Mo nutrient solution or one fortified with 100 nM Mo. Scale bar= 1 cm. (B) Nodule fresh weight from 28 dpi WT and *mot1.2-1* plants watered with standard 4 nM Mo nutrient solution or one fortified with 100 nM Mo. Data are the mean ± SD of at least 12 plants. * indicates a statistically significant difference ($P<0.05$). (c) Nitrogenase activity of 28 dpi WT and *mot1.2-1* plants watered with standard 4 nM Mo nutrient solution or one fortified with 100 nM Mo. Data are the mean ± SD of at least 4 sets of 4 pooled plants. * indicates a statistically significant difference ($P<0.05$).

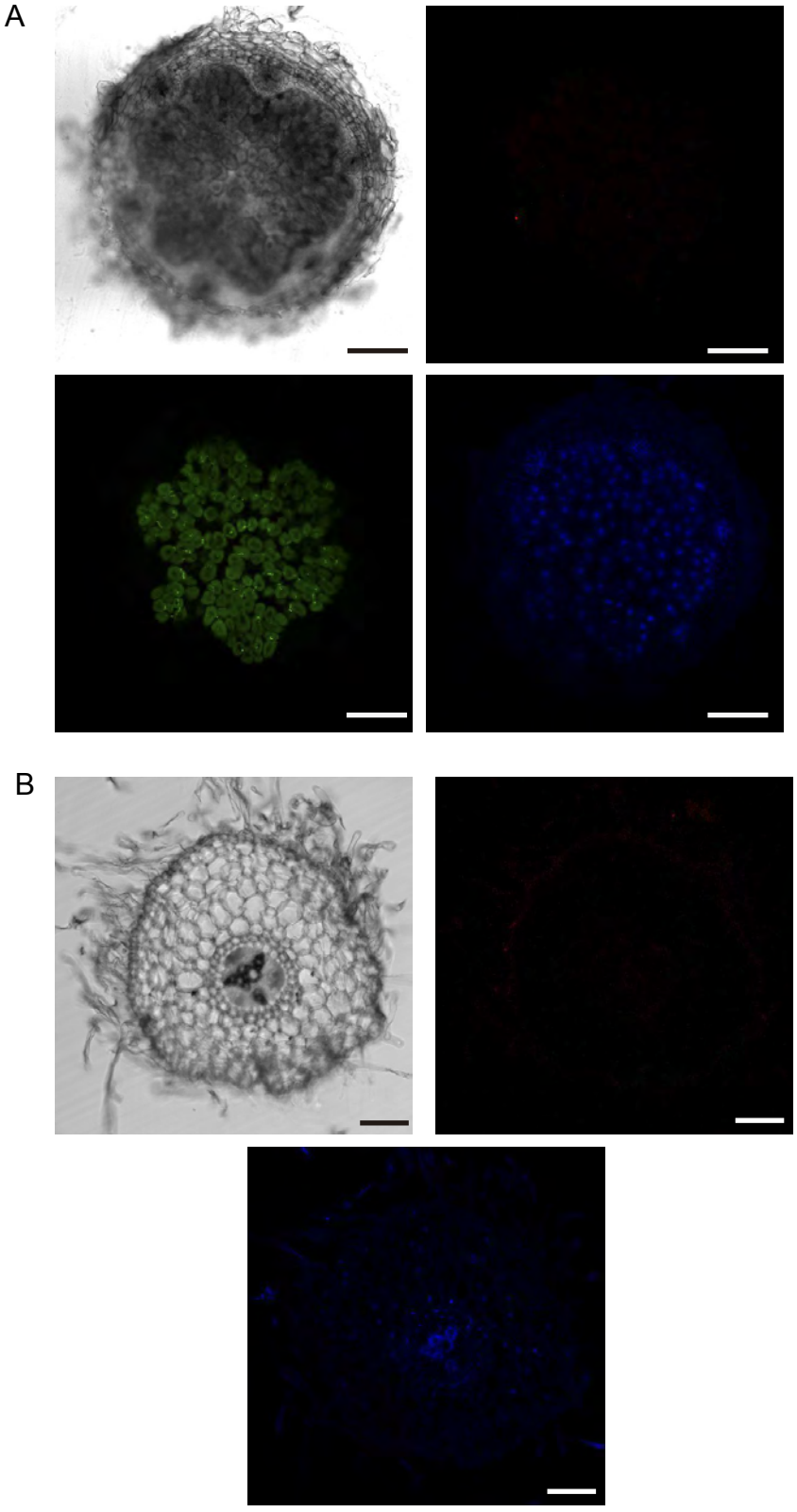
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



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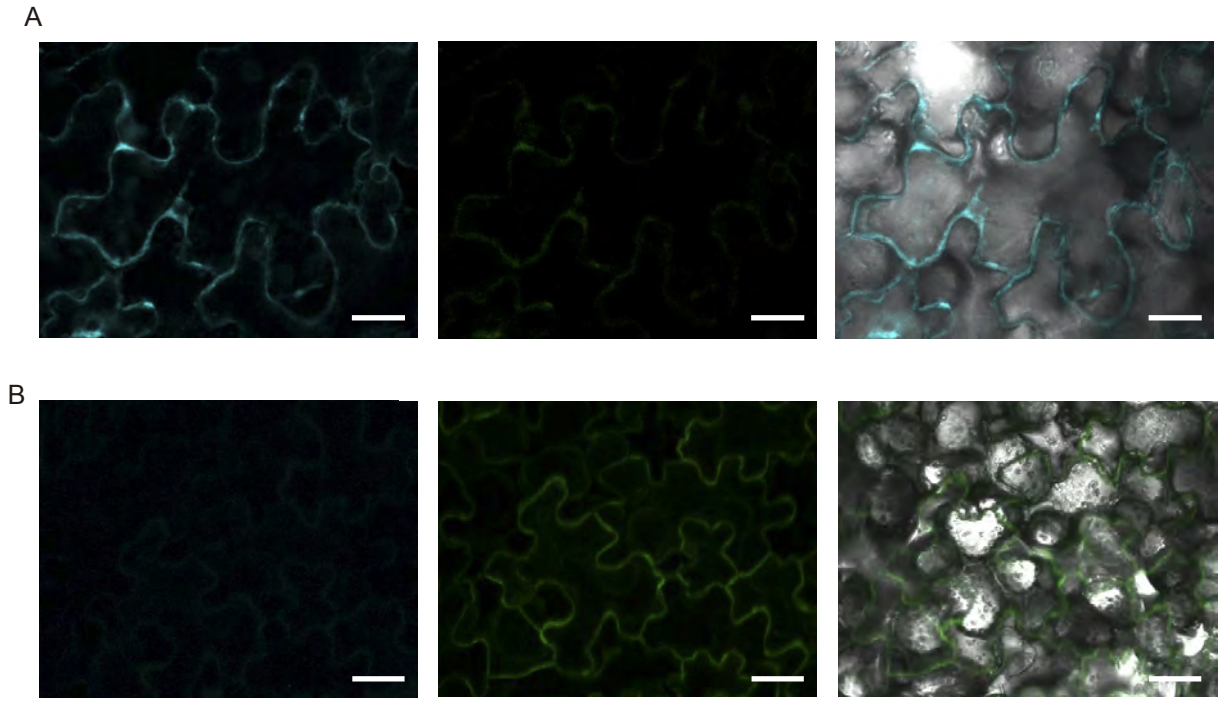

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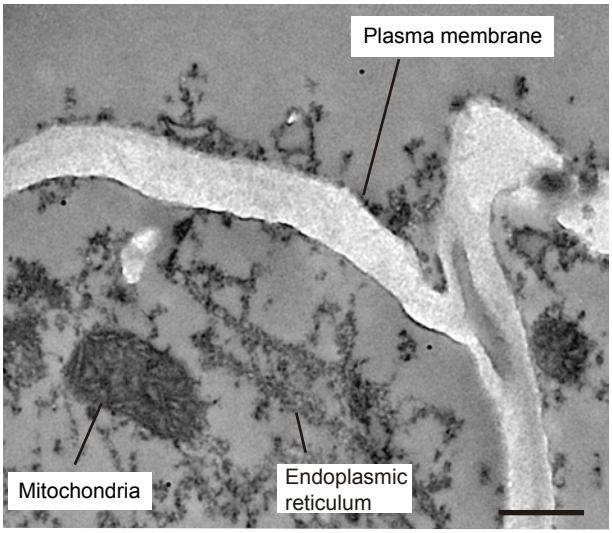

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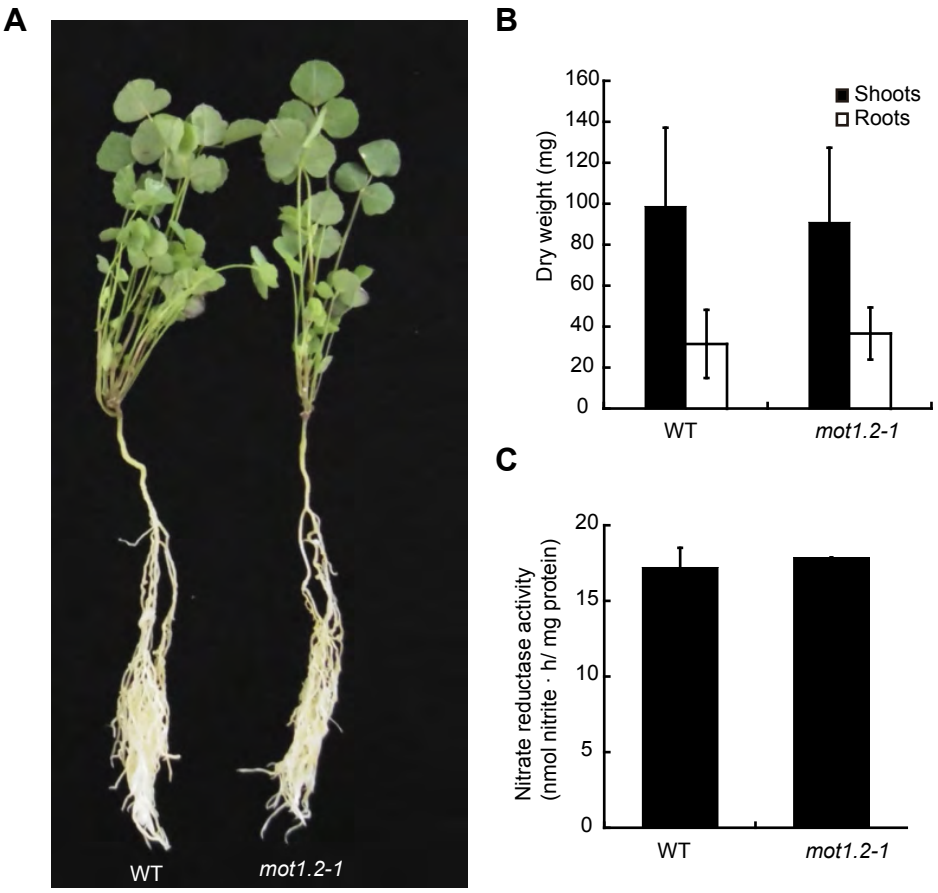
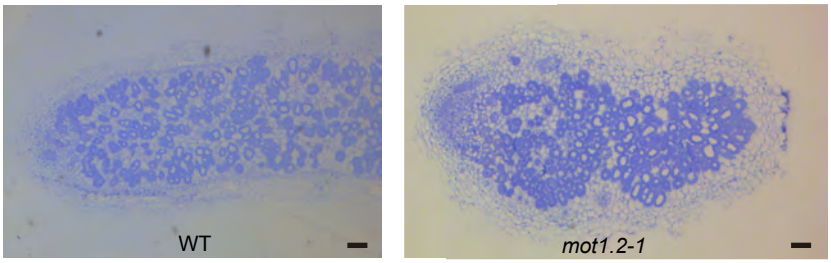
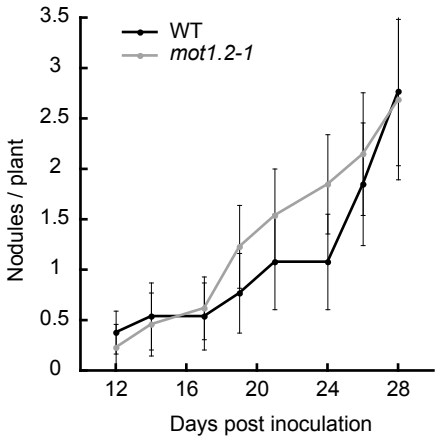


Figure S7

A


WT
mot1.2-1

B


WT
mot1.2-1
Nodules / plant
Days post inoculation
0
0.5
1
1.5
2
2.5
3
3.5
12
16
20
24
28

Supplemental Figure S8