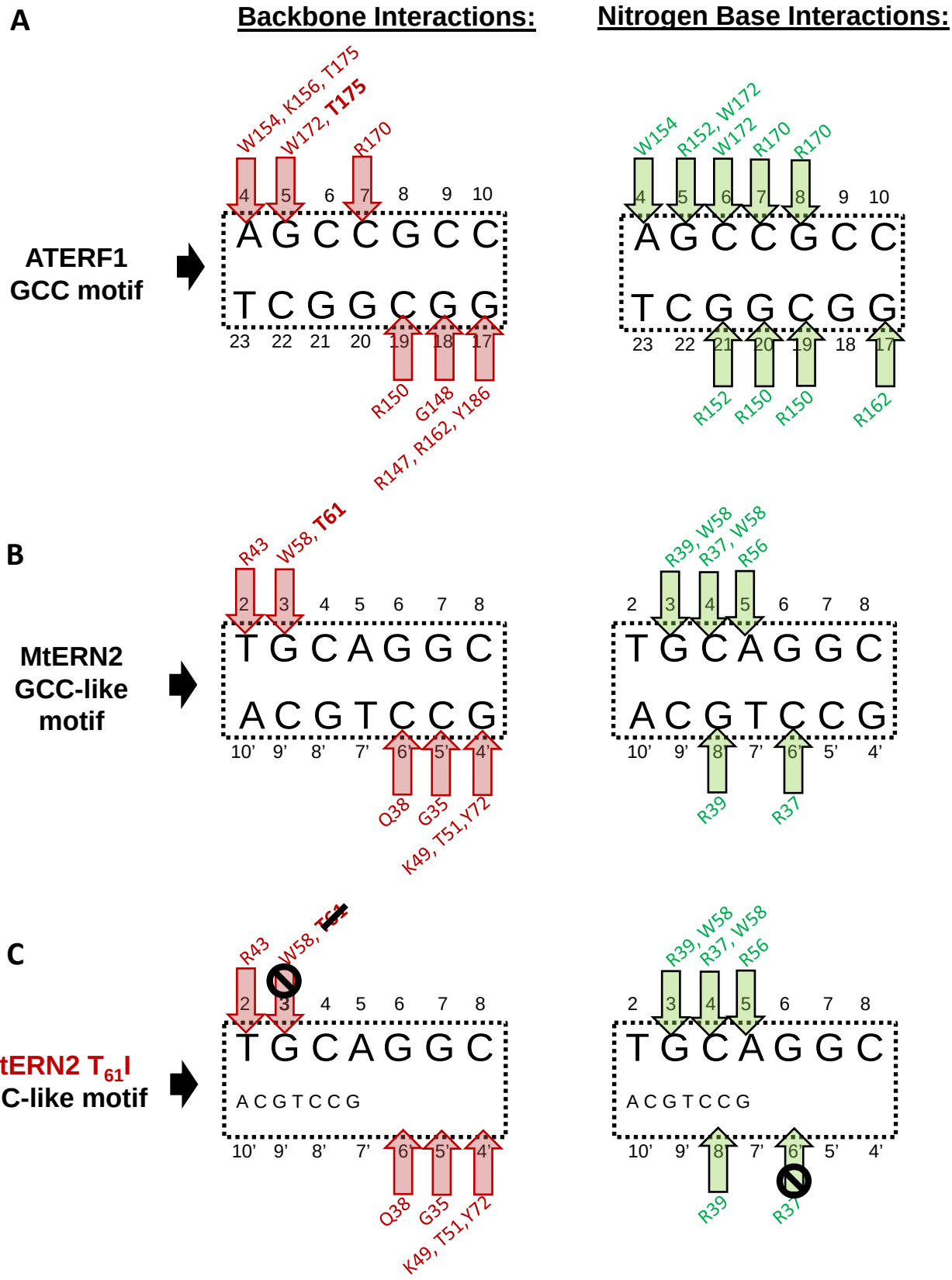
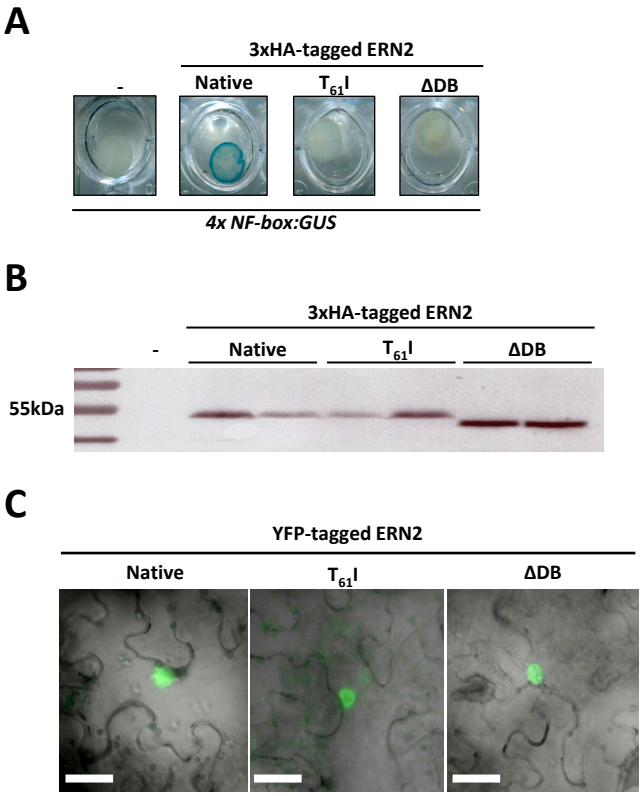


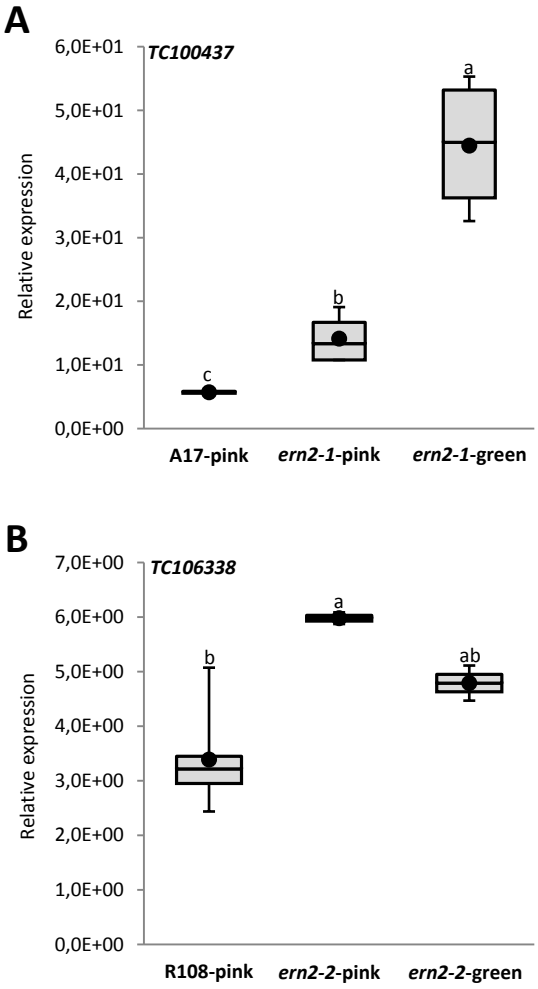
Supplemental Figure 1. Comparison of DNA Binding Interactions of AtERF1 and MtERN2 AP2/ERF Domains.

Interactions of key amino acids of AtERF1 (**A**) and MtERN2 (**B**) DNA binding domains with *GCC-box* and *GCC-like* sequences respectively. Arrows indicate conserved amino acid interactions with the sugar-phosphate backbone (red arrows) and/or with the bases (green arrows). When MtERN2 is mutated (**C**) the substitution of T_{61} by an isoleucine abolishes the H-bond interaction with the Guanine 3 and as a consequence negatively affects the interaction of the R_{37} residue with Cytosine 6'. These results were obtained via modeling and docking experiments using either the native or the mutated versions of MtERN2DB in the presence of the *GCC-like* motif (see Material and Methods).

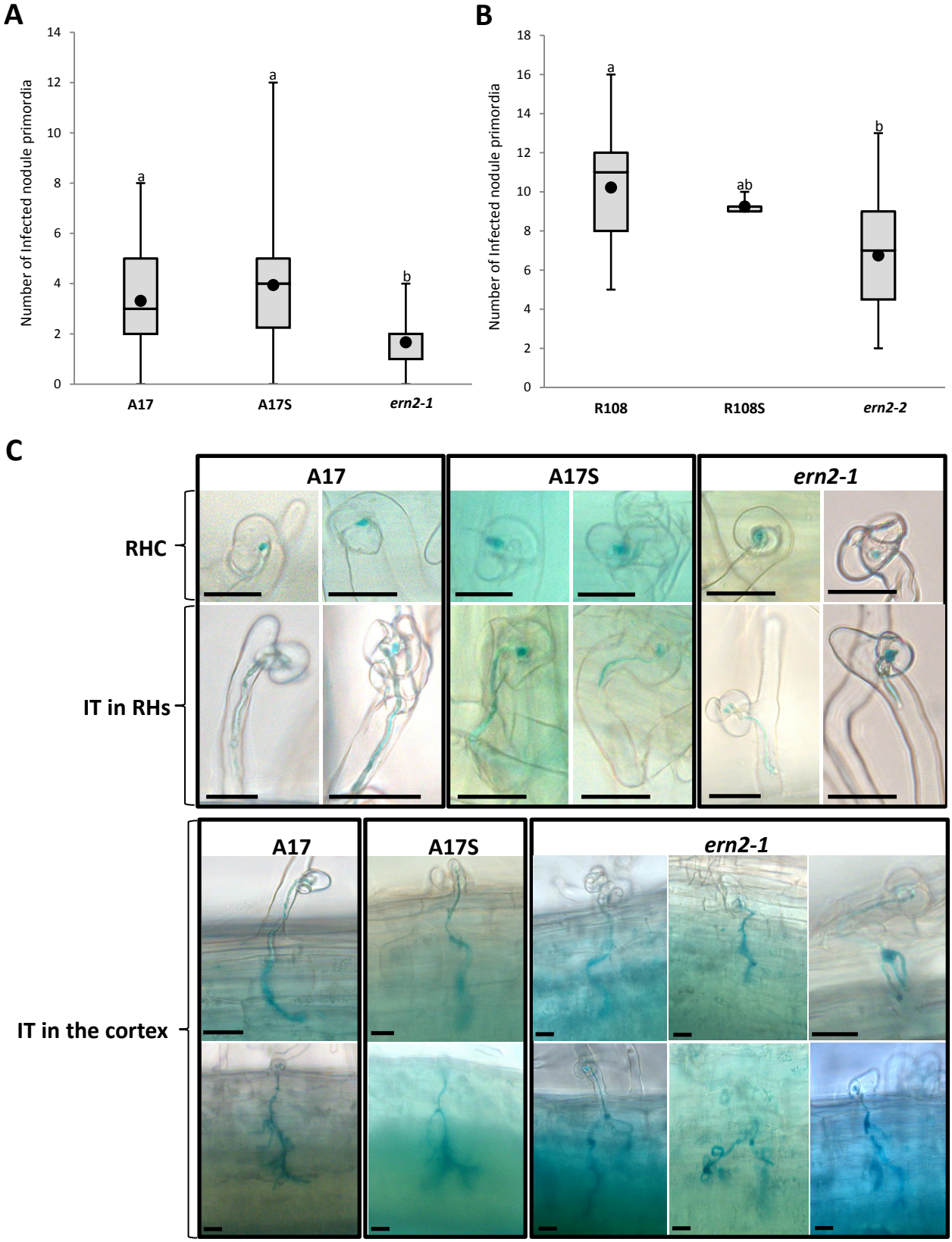


Supplemental Figure 2. The Mutated T_{61}I ERN2 Is Targeted to the Nuclear Compartment But Is No Longer Able to Activate Transcription.

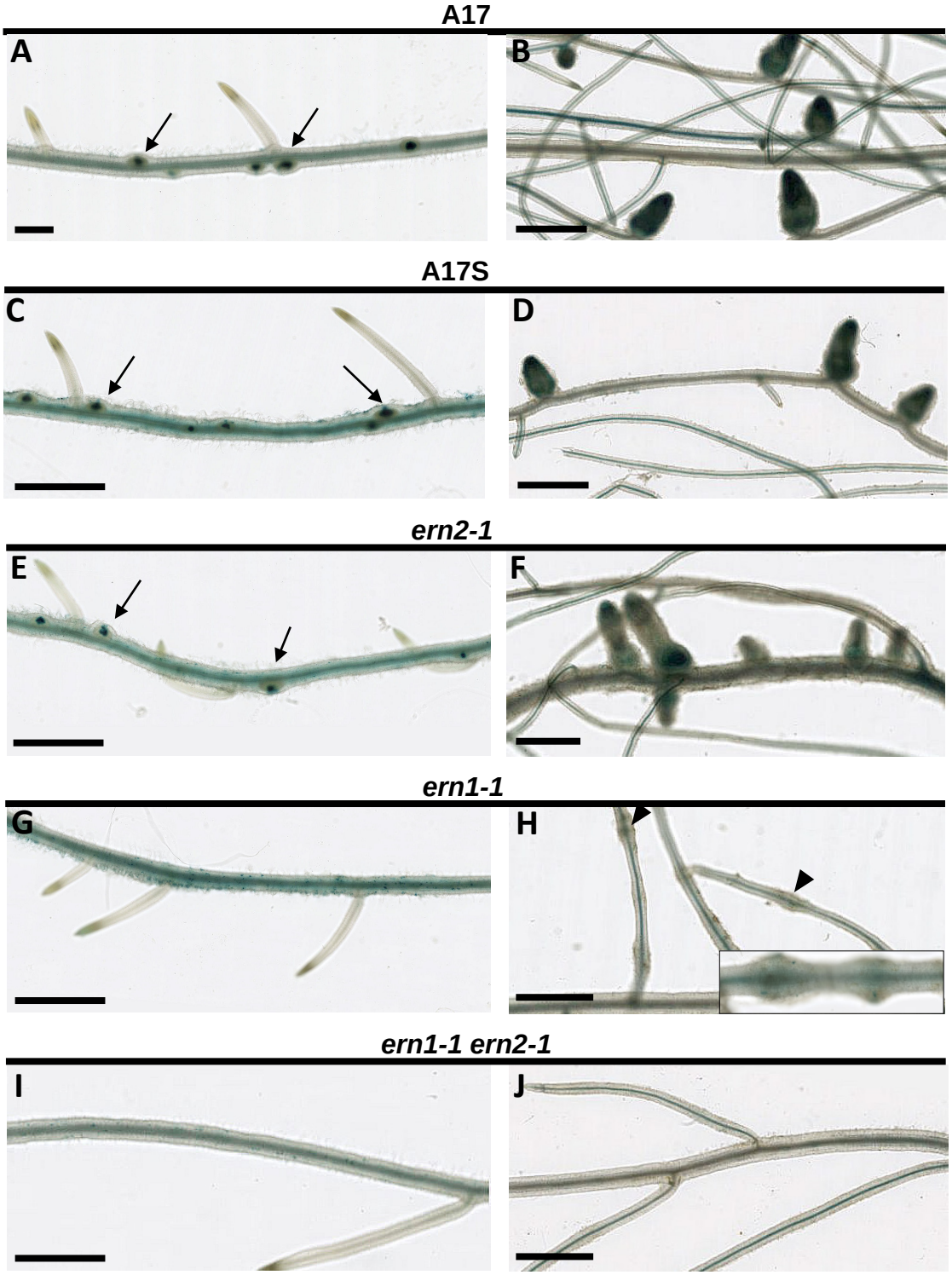
(**A**) The transcriptional activities of 3xHA-tagged ERN2 (native form), ΔDB (ERN2 deleted for the entire DNA binding domain) and T_{61}I (the critical point mutation in the ERN2 DNA binding domain) were evaluated by histochemical GUS assays in *N. benthamiana* leaves expressing the respective ERN2 proteins in the presence of the *4xNF-box:GUS* reporter. Leaf discs comprising the *4xNF-box:GUS* reporter alone were also included as a negative control (-). Only the native ERN2 version is capable of triggering *GUS* expression, as indicated by the blue staining. (**B**) Western blot analyses using anti-HA antibodies were performed with *N. benthamiana* leaf discs expressing the respective 3xHA-tagged ERN2 proteins as well as the reporter construct as negative control (-). This analysis shows that similar levels of ERN2 protein are present for both the ΔDB and T_{61}I variants. (**C**) YFP-tagged native, ΔDB and T_{61}I-ERN2 specifically accumulate in the nuclear compartment of *N. benthamiana* leaf cells. Scale bars=20 µm.



Supplemental Figure 3. *ern2* Mutant Lines Exhibit a Nodule Senescence-Like Phenotype. Expression of senescence-associated marker genes coding for a cysteine protease *TC100437* (**A**) and for the vacuolar processing enzyme *TC106338* (**B**) were quantified in isolated 4 weeks-old pink nodules of *ern2* mutant lines (*ern2-1* in **A** and *ern2-2* in **B**) and compared with wild type nodules (A17 in **A** and R108 in **B**). Green senescent *ern2* nodules were also included for comparison. Data were obtained with 10-15 isolated nodules. One way Analysis of Variance followed by a Tukey HSD test of the values were performed (in **A**, p-value<0.001; in **B**, p-value<0.05). Classes sharing the same letter are not significantly different.

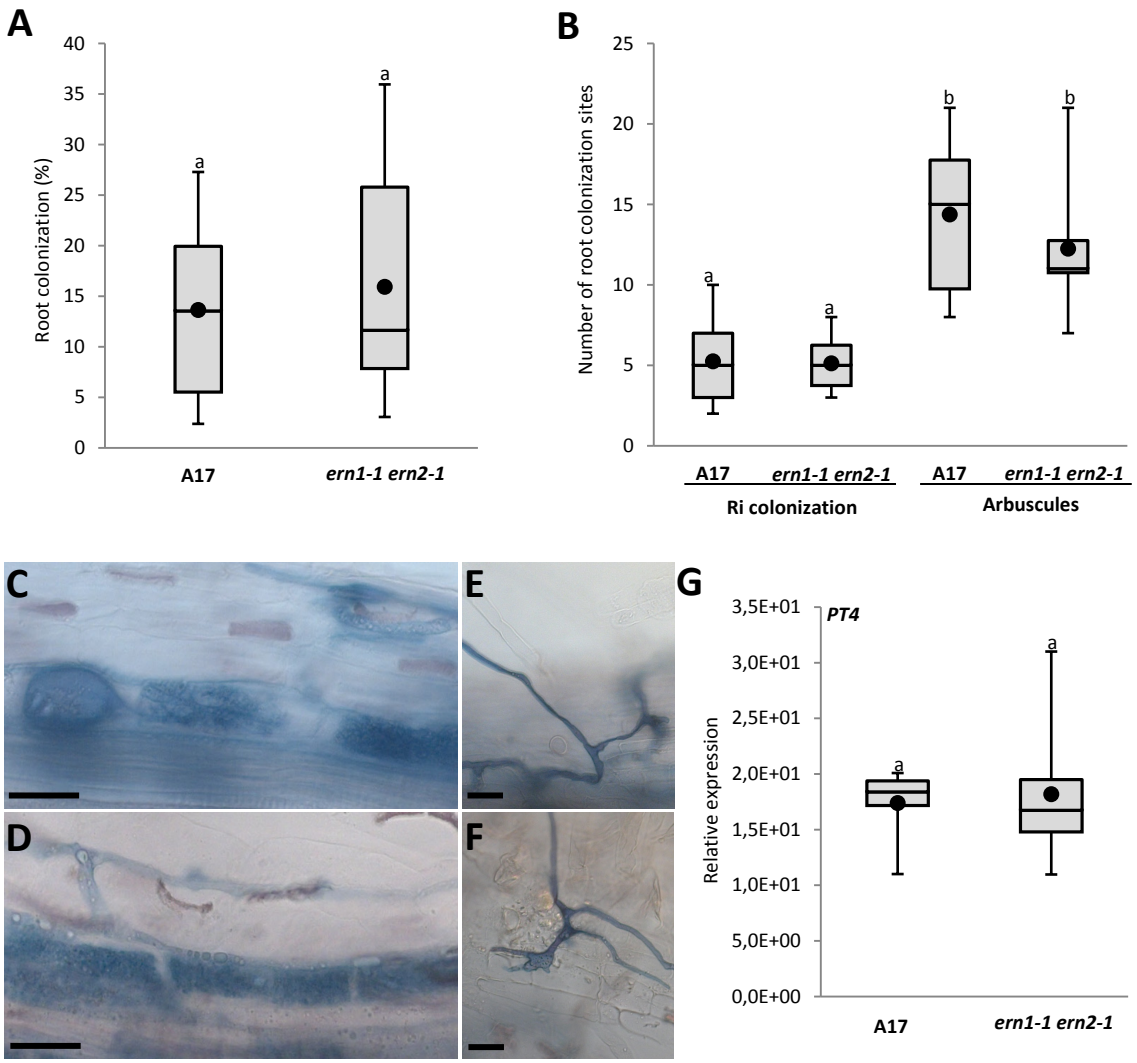


Supplemental Figure 4. *ern2* Mutants Display a Slightly Reduced Nodulation Phenotype.
M. truncatula plants cultivated in aeroponic growth conditions were analyzed 2-3 days post-inoculation with a *lacZ*-expressing *S. meliloti* strain, following histochemical staining for β-galactosidase activity of intact roots (blue in **C**). (**A**-**B**) Box plots showing the quantification of the number of infected nodule primordia observed in young seedlings of either wild-type (A17, A17S, R108, R1108S) or *ern2-1* and *ern2-2* inoculated roots. Box plots depict mean value (black circles), first and third quartile (horizontal box sides), minimum and maximum (outside whiskers). Results were obtained from 2 independent experiments with a total of 18-21 in **A** and 9-16 roots in **B**. One way Analysis of Variance (ANOVA) followed by a Tukey HSD test of the values were performed (in **A,** p-value<0.01; in **B** p-value<0.05). Classes sharing the same letter are not significantly different. (**C**) Different stages of rhizobial colonization were analyzed on roots of wild type (A17 and A17S) and mutant (*ern2-1*) plants: curled root hairs with the microcolony inside (RHC=Root Hair Curling), and infection thread (IT) progression within root hairs (RHs) or in the root cortex. Bars=20 µm.

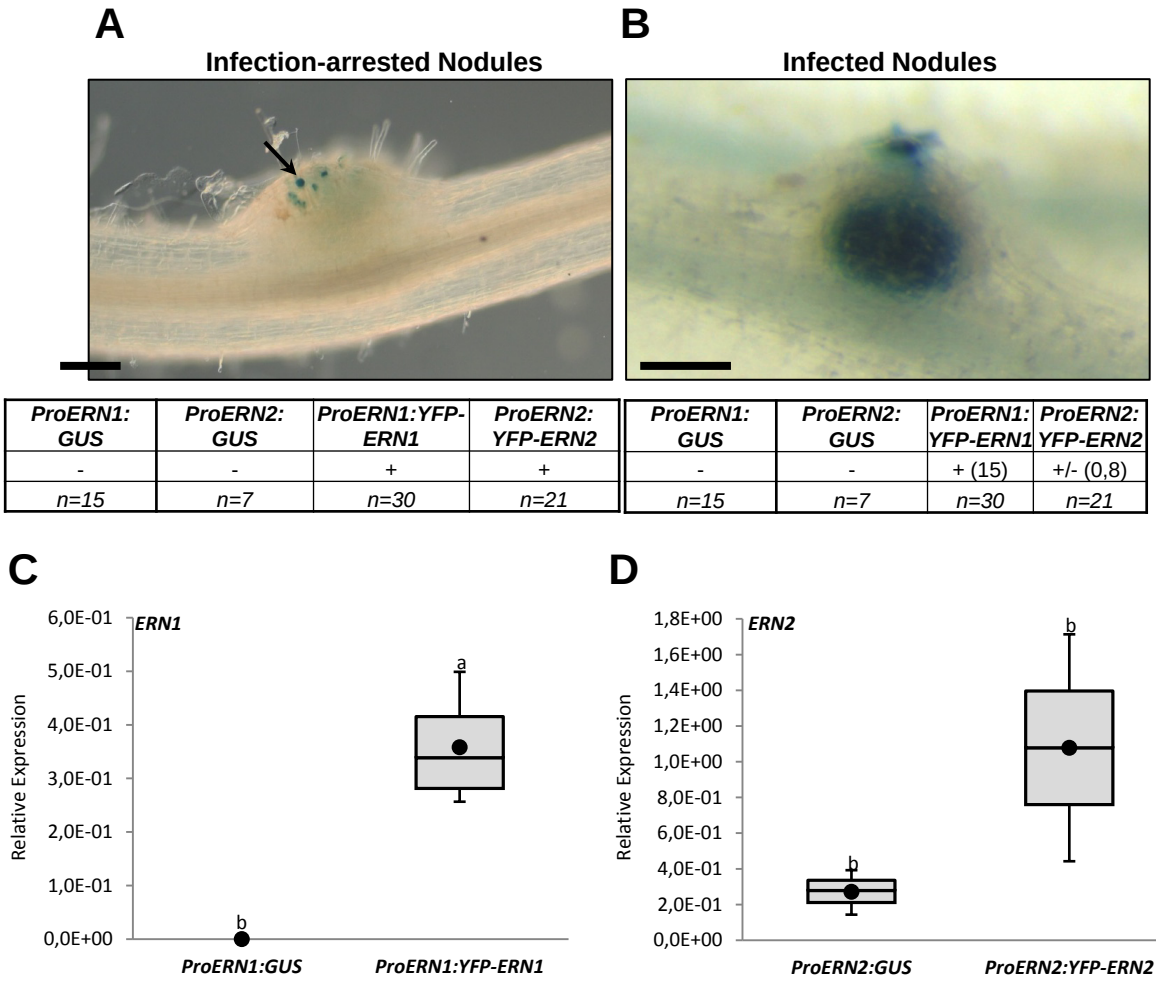


Supplemental Figure 5. The Double *ern1-1 ern2-2* Mutant Is Totally Defective for Root Nodulation.

S. meliloti-inoculated wild-type (A17), wild-type sibling (A17S), *ern2-1*, *ern1-1* and *ern1-1 ern2-1* plants were analyzed 3 days (**A, C, E, G** and **I**) and 4 weeks post-inoculation (**B, D, F, H** and **J**) with *lacZ*-expressing *S. meliloti*, following histochemical staining of β-galactosidase activity. Infected nodule primordia (arrows) and nodules are only observed in wild-type and *ern2-1* roots (**A-F**). Characteristic non-infected underdeveloped nodules with arrested ITs on top are only observed in *ern1-1* roots (arrowheads in **H** and detailed in enlargement) while no infection or underdeveloped nodules are observed in double *ern1-1 ern2-1* mutant roots (**I** and **J**). Data were obtained after analyses of approximately 40 independent plants from three-four biological experiments. Scale Bars=2mm.



Supplemental Figure 6. Colonization of A17 Wild-Type and *ern1-1 ern2-1* Mutant Roots by *R. irregularis*. The percentage of root colonization of A17 wild type and *ern1-1 ern2-1* roots was scored using the gridline intersect method after ink staining of *R. irregularis* inoculated roots harvested 3 weeks post-inoculation (3 wpi) (**A**). The number of root colonization sites was evaluated 2 weeks post-inoculation (2 wpi) by either scoring for early *R. irregularis* root colonization sites (Ri colonization, in **B**) with visible extraradical hypha but no detectable arbuscules, or the presence of cortical arbuscule structures (Arbuscules in **B**,). Values represent the percentage of root colonization after scoring about 600 to 800 root pieces of 1 cm length from 24 independent plants of each genotype (in **A**) or after scoring the entire root system of 20 independent plants of each genotype (in **B**). Box plots represent data from 2 independent experiments and depict mean value (black circles), first and third quartile (horizontal box sides), minimum and maximum (outside whiskers. (**C-F**) Arbuscule structures (**C** and **D**) and *Ri* colonization sites (**E** and **F**) observed respectively in A17 wild type (**C** and **E**) and in *ern1-1 ern2-1* (**D** and **F**) roots. Bars= 25 µm. (**G**) Quantitative RT-PCR analysis of relative expression levels of the *M. truncatula MtPT4* transcript in *R. irregularis*-inoculated roots (3 wpi). Data represent values of 6 (A17) and 7 (*ern1-1 ern2-1*) independent plants from two independent experiments. One way Analysis of Variance followed by a Tukey HSD test of the values were performed (in **A** and **G**, p-values>0.05; in **B**, p-value<0.001). Classes sharing the same letter are not significantly different.



ProERN1: GUS	ProERN2: GUS	ProERN1:YFP-ERN1	ProERN2: YFP-ERN2
-	-	+	+
n=15	n=7	n=30	n=21

ProERN1: GUS	ProERN2: GUS	ProERN1: YFP-ERN1	ProERN2: YFP-ERN2
-	-	+ (15)	+/- (0,8)
n=15	n=7	n=30	n=21

Supplemental Figure 7. Complementation of the Double *ern1-1 ern2-1* Mutant with either *ERN1* or *ERN2* Driven by their Respective Promoters.

The *ern1-1 ern2-1* double mutant was transformed via *A. rhizogenes* with *ProERN1:YFP-ERN1*, *ProERN2:YFP-ERN2*, or with the negative control constructs *ProERN1:GUS* and *ProERN2:GUS*. Transformed root systems were analyzed 2 weeks post-inoculation (wpi) with *S. meliloti-lacZ* for the presence of non-infected arrested nodules (**A**) or infected nodules (**B**) after staining for *β-galactosidase*. Typical "*ern1*-like" non-infected underdeveloped nodules with arrested ITs on top (arrow in **A**) were observed with both *ProERN1:YFP-ERN1* and *ProERN2:YFP-ERN2* constructs while only *ProERN1:YFP-ERN1* could efficiently restore the formation of fully infected nodules (90% of composite plants with about 15 nodules/root system). Formation of infected nodules by *ProERN2:YFP-ERN2* complementation was a rare event (0.8 nodules/root system in 40% of composite plants). Quantitative RT-PCR analyses in total RNA samples from *ern1-1 ern2-1* roots (2wpi) transformed with the respective constructs confirmed the expression of *ERN1* (**C**) or *ERN2* (**D**) in complemented roots. Values represent average of 2-3 independent plants after normalization against reference transcript levels. One way Analysis of Variance (ANOVA) followed by a Tukey HSD test of the values were performed (in **C**, p-value<0.001; in **D**, p-value>0.05). Classes sharing the same letter are not significantly different. Scale bars=250 µm.

Supplemental Table 1. List of the Primers Used in this Study.

Name	Sequence 5'-3'
ERN2-219-Fw	CTAGCAGCAACATGAAAATTGAGG
ERN2-220-Rev	TAACTCGAAATATTAGTCTTTGCACGA
LTR6-Rev	GCTACCAACCAAACCAAGTCAA
ERN2-222-Rev	AGGTGAAAGATTCCATTGAGCTTC
ProENOD11-935-Fw	CGCATATCTGAATTCTTTTAAATCTAGACACG
GUS-1-Rev	TGCCCACAGGCCGTCGAG
ProERN1-654-Fw	TCTTTGGTGTTAATATGGGTGTG
ERN1-Rev1	TGTTGGATTGTGAACCTGACTC
ERN2-11-Fw	TCTCCTGTTCAATGGAAATTC
ERN2-1060-Rev	GTCTTTGCACGAGAATATACC
ERN2-Rev1	CACTGGCTGTGCCAATACAG
TC100437-Fw	CCTGCTGCTACTATTGCTGGATATG
TC100437-Rev	CACTCGCATCAATGGCTACGG
TC106338-Fw	AGTTCTGCCTGTTGTGGAATGTC
TC106338-Rev	GGTAGCTCCTGTCTGCCAATTAC
VPY-Fw	AAACCACCATCTGCACCTTC
VPY-Rev	ACCTCTTAGCGCACGAGTTC
RPG-Fw	AACCCTTCAGATGGCAAATG
RPG-Rev	TTCCCCCAGTTTAGATTCCAG
NIN-Fw	CGTCTTCTTCTTCGAGTGGGA
NIN-Rev	GTAATCCCATGCTGTCTGCA
ENOD12-Fw1	CCGGTGAATAAGCCACCACA
ENOD12-Rev1	GCTTGTGCACTGGTGATTCC
Ch-NFbox-Fw	AAATAATTGCAGGCCTAAAGCTAG
Ch-NFbox-Rev	CCTTATATAGAGGAAGGGTCTTGC
Pt4-Fw	CAAGAAAGATTAGACGCGCAA
Pt4-Rev	GTTTCCGTCACCAAGAACGTG
Mtr.3213.1.S1_at-Fw	ATCCCTCCGCAGTTTGTACC
Mtr.3213.1.S1_at-Rev	GCCAAAATTTGCAGAGCAAG
Mt0089_00067-842Fw	TCCTCGAACCTGTTGTTGGAA
Mt0089_00067-909R	GCGCAAGGAACTAAGGAAATCA
Mt0085_00020-4865F	GAAATCTGTCAGCTGCCAGCAT
Mt0085_00020-4928R	*CTAAACCGAGCCTCTTTCTTCCC*