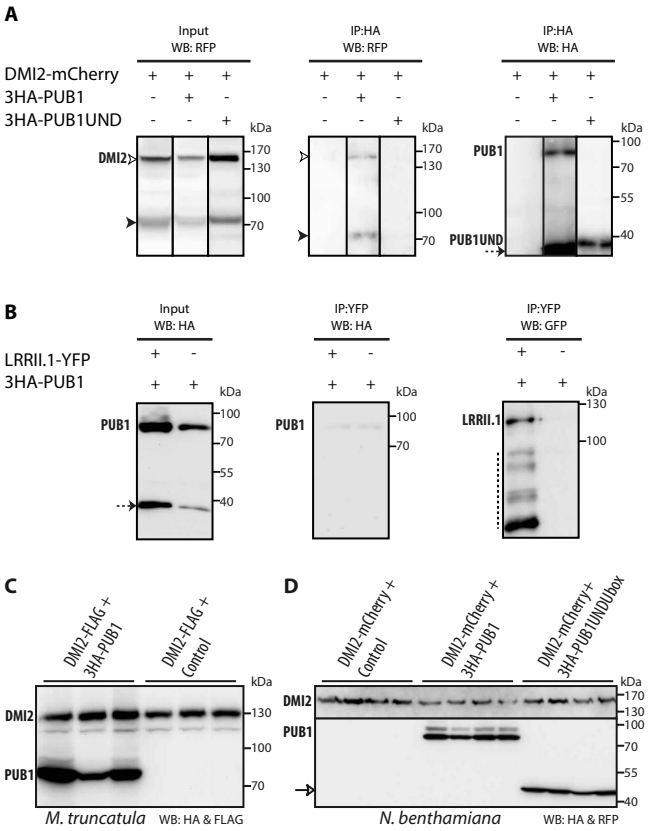


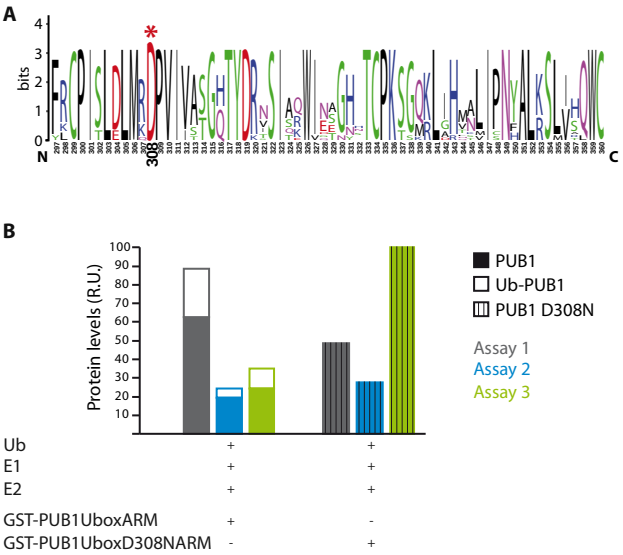

**Supplemental Figure 1. PUB1 interacts specifically with DMI2 but does not affect DMI2 stability *in planta***

**A.** DMI2 co-immunopurifies with PUB1 in *N. benthamiana*. DMI2-mCherry was co-expressed with 3HA-PUB1, 3HA-PUB1UND or a control strain and solubilized complexes were affinity purified with anti-HA beads. Solubilized proteins (input) and immunopurified proteins (IP) were analysed by Western blot (WB) using anti-HA or anti-RFP antibodies (mCherry-tagged proteins). DMI2 was co-purified by full length 3HA-PUB1 (~82 kDa) and not by 3HA-PUB1UND (~35 kDa). White and black arrowheads indicate DMI2-mCherry full length (>130kDa) and cleaved (~75 kDa), respectively. Dotted arrow indicates 3HA-PUB1 cleaved/degraded (~30 kDa).

**B.** PUB1 is not co-immunopurified with LRRII.1 in *N. benthamiana*. PUB1 was co-expressed with LRRII.1-YFP or a control strain and solubilized complexes were affinity purified with anti-GFP beads. Solubilized proteins (input) and immunopurified proteins (IP) were analysed by Western blot (WB) using anti-HA or anti-GFP antibodies (YFP-tagged proteins). 3HA-PUB1 (~82 kDa) was not co-purified by LRRII.1 (>95 kDa). Dotted arrow and dashed line indicate 3HA-PUB1 cleaved/degraded (~30 kDa) and LRRII.1-GFP cleaved products, respectively.

**C.** DMI2 stability is not affected by overexpression of *PUB1* in *M. truncatula* roots. A *ProDMI2-DMI2-FLAG* (~130kDa) line was transformed with *Pro35S-3HA-PUB1* or a control strain. Western blot (WB) analysis using anti-HA (PUB1) and anti-FLAG (DMI2) antibodies is shown with three biological replicates for each construct.

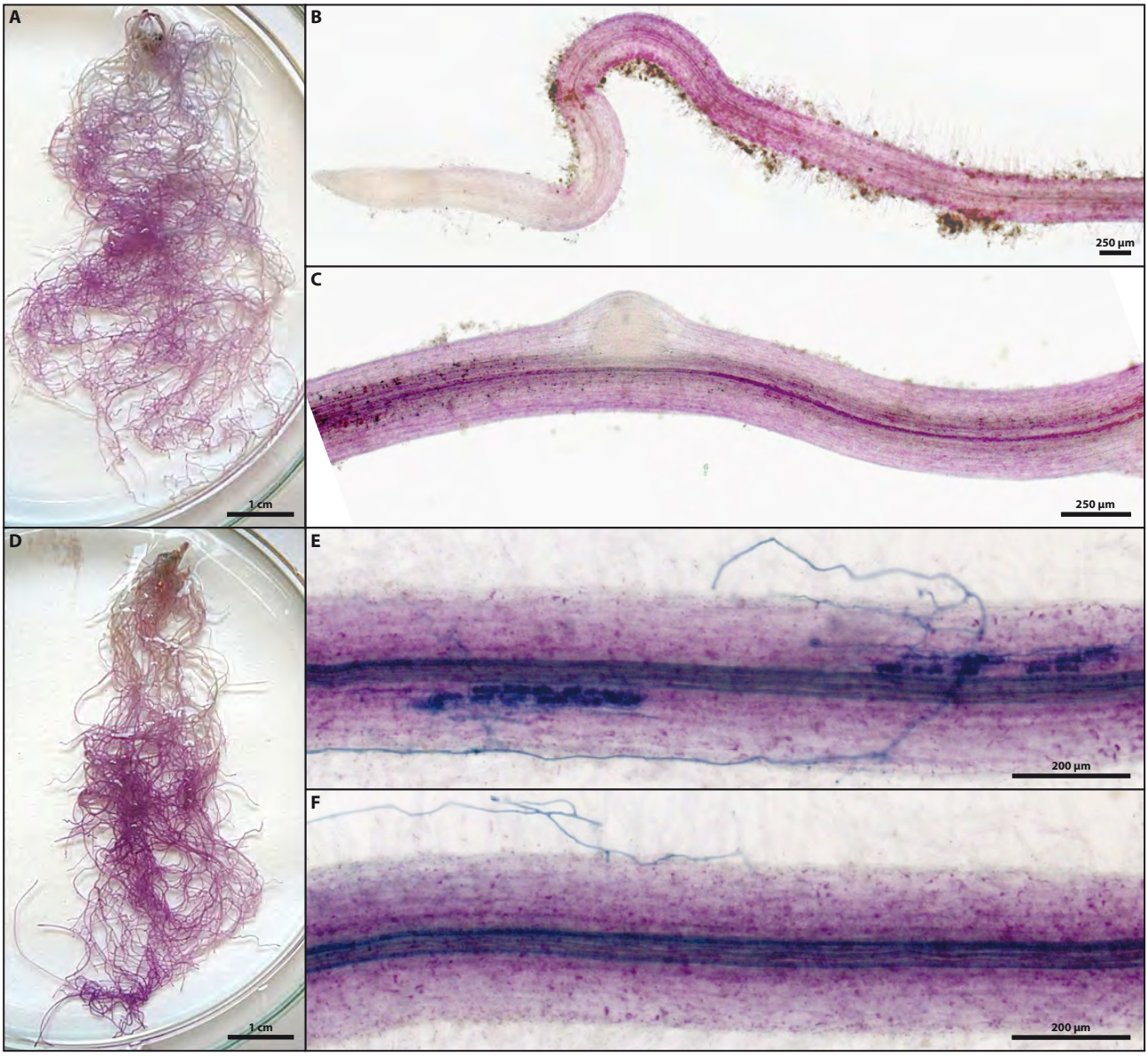
**D.** DMI2 stability is not affected by overexpression of *PUB1* in *N. benthamiana* leaves. Leaves were co-transformed with *Pro35S-DMI2-mCherry* (>130 kDa) and a control strain, *Pro35S-3HA-PUB1* (~82 kDa) or *Pro35S-HA-PUB1UNDUbox* (~49 kDa) constructs. WB analyses using anti-HA (different forms of PUB1) and anti-RFP (DMI2) antibodies are shown with four biological replicates for each construct. White arrow indicates 3HA-PUB1UNDUbox.




**Supplemental Figure 2. The D308N mutation in the U-box domain abolishes the ubiquitin ligase activity of the PUB1 protein**

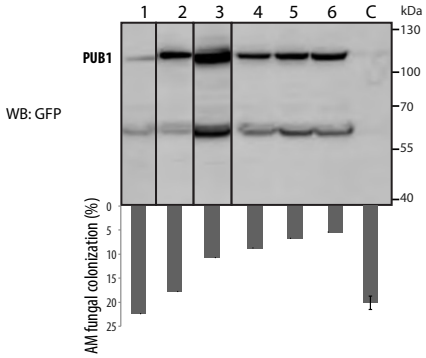
**A.** The D308N mutation is in a conserved amino acid of the U-box domain. According to Sift software, conserved PUB proteins were identified and used in WebLogo (http://weblogo.berkeley.edu/logo.cgi) to show the conservation of the 70 amino acids of the U-box domain. * indicates the D308N mutation.

**B.** GST-PUB1UboxD308NARM is not ubiquitinated *in vitro*. Graph shows protein quantification (PUB1 forms and higher bands named Ub-PUB1) in 3 independent assays performed as described and illustrated in Figure 1D.

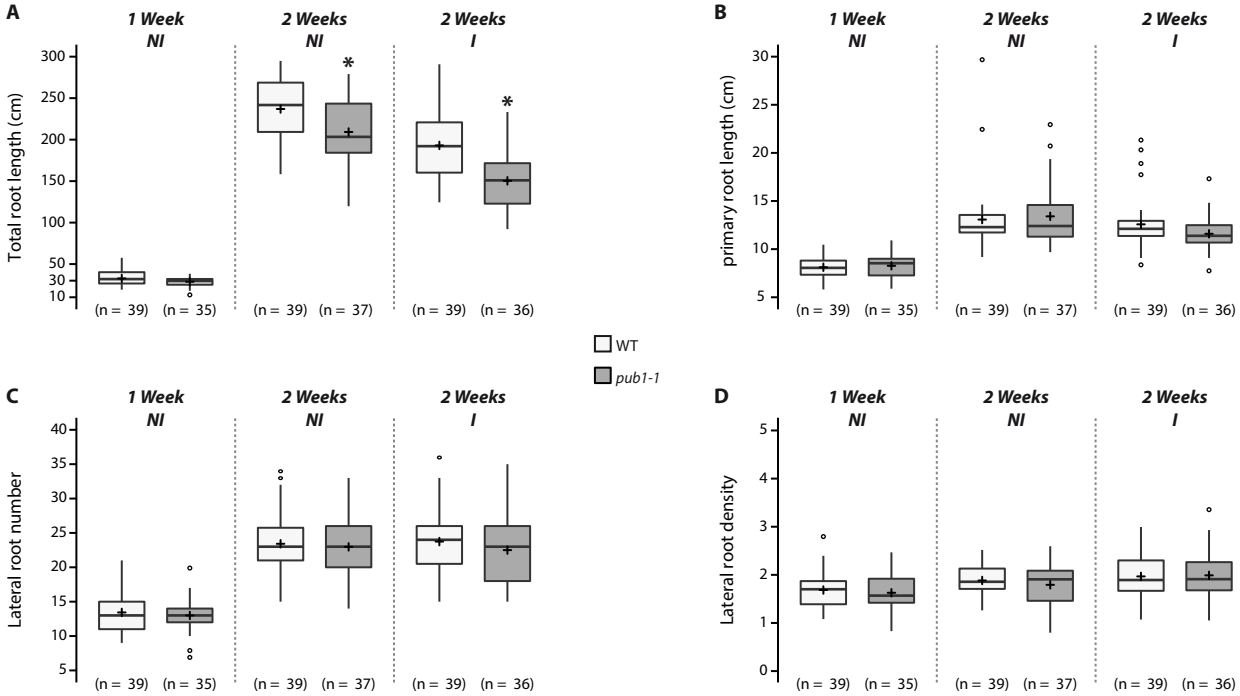



**Supplemental Figure 3. *DMI2* is expressed during AM symbiosis**

*A. rhizogenes* transformed roots of *M. truncatula* containing the *ProDMI2-GUS* construct were analyzed in uninoculated roots (**A**, **B**, **C**) or 10 days after *R. irregularis* inoculation (**D**, **E**, **F**). In uninoculated plants, GUS activity (in magenta) was observed in the whole root system (**A**) except in primary (**B**) and secondary (**C**) root meristems (as described in Bersoult et al. 2005). GUS staining of the whole colonized root system (**D**) looked like the uninoculated profile (**A**). Fungal structures were visualized by ink coloration (in blue) and the *DMI2* expression profile in mycorrhized zones (**E**) was similar to that observed in uncolonized regions (**F**). Blue staining of the vasculature system corresponds to an aspecific staining (**E**, **F**).



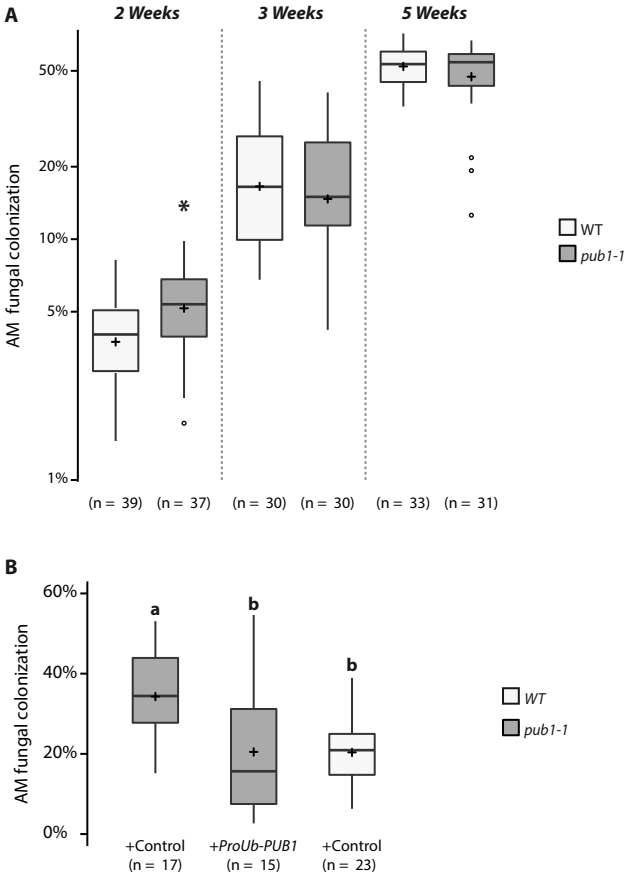

**Supplemental Figure 4. Overexpression of *PUB1* in *Medicago* roots**
A subset of *Medicago* roots transformed with *ProUb-GFP-PUB1* (number 1 to 6) or an empty vector (C). Transformed roots were selected with the selective marker Ds-Red and then GFP-PUB1 presence was checked by Western Blot using anti-GFP antibodies. Percentage of AM fungal colonization observed for these individuals are indicated at the bottom of the figure. For the empty vector (C), average of 23 individuals is indicated (see Figure 3). Error bar represent Standard Error.




**Supplemental Figure 5. *pub1-1* root architecture**

WT and *pub1-1* root systems were analysed for total root length (**A**), primary root length (**B**), number of lateral roots per primary root (**C**) and lateral root density (ratio of lateral root number/primary root length) (**D**). Measures were done on non inoculated (NI) roots of 1 and 2 weeks old and 2 wpi with *R. irregularis* (I). * indicate significant differences as determined by two sample-t-tests ($p \leq 0.05$).

Center lines show the medians, crosses the means and box limits indicate the 25th and 75th percentiles as determined by R software, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by circless. Numbers of samples are indicated in brackets (n).

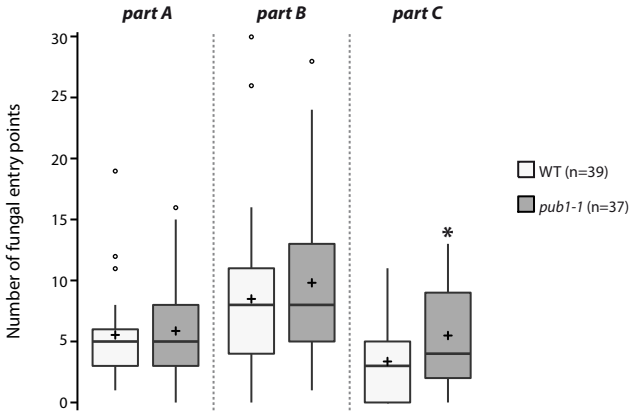



**Supplemental Figure 6. *ProUb-GFP-PUB1* construct complements the *pub1-1* AM root colonization phenotype**

**A.** Box plots show level of colonization per plant 2, 3 and 5 weeks post inoculation (wpi) with of *R. irregularis* (4000 sp $L^{-1}$) on wild type (WT) and *pub1-1* roots. Significant differences (* $p \leq 0.05$) were detected by Wilcoxon rank sum tests.

**B.** Wild type (WT) and *pub1-1* roots were transformed with an empty vector (control) or *ProUb-GFP-PUB1*. Transformed roots were inoculated with *R. irregularis* (1000 sp $L^{-1}$) and scored at 24 dpi. Different letters indicate significant differences as determined by non parametric multiple comparisons with Tukey correction test ($p \leq 0.05$).

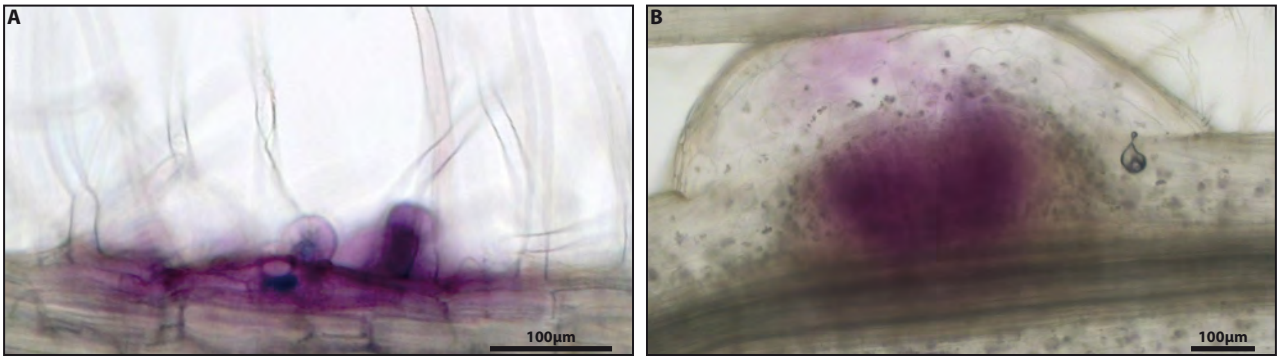
For details, see boxplot descriptions on Figure 3.




**Supplemental Figure 7. *PUB1* negatively regulates AM fungal entry and root responsive zone**

Box plots show number of fungal entry points counted per plant at 2 wpi with *R. irregularis* (4000 sp $L^{-1}$) on WT and *pub1-1* roots. Fungal entry points are shown for the entire root system (Total) and for each root part (A to C). Each root system was transversally divided in 3 equal parts: part A (oldest part), part B (middle part) and part C (youngest part).

Significant differences (* $p \leq 0.05$) were detected by Wilcoxon rank sum. For details, see boxplot descriptions on Figure 3.




**Supplemental Figure 8. The stable line *ProPUB1-GUS* shows *PUB1* induction during nodulation**
*GUS* expression (in magenta) in the stable line *ProPUB1-GUS* was observed 4 days post inoculation with *S. meliloti* containing a lac Z fusion (revealed in blue).
**A.** *ProPUB1-GUS* expression is associated with infected root hair cells.
**B.** *ProPUB1-GUS* expression is associated with the nodule primordium.