

***New Phytologist* Supporting Information**

Article title: MYB36 controls the boundaries of Arabidopsis lateral root primordia

Authors: Fernández-Marcos, M., Desvoves, B., Manzano, C., Liberman, L.M., Benfey, P.N., del Pozo, J.C., Gutierrez, C.

Article acceptance date:

The following Supporting Information is available for this article:

Fig. S1 Primary root phenotypes of MYB36^{oe} and *myb36* mutants.

Fig. S2 CASP1 and lignin production in *myb36* mutants.

Table S1 Primers used in this study.

Methods S1 ROS localization.

Methods S2 Lignin detection

Movie S1 Detection of MYB36-GFP in z-stack of a LRP (lateral view)

Movie S2 Detection of MYB36-GFP in z-stack of a LRP (front view)

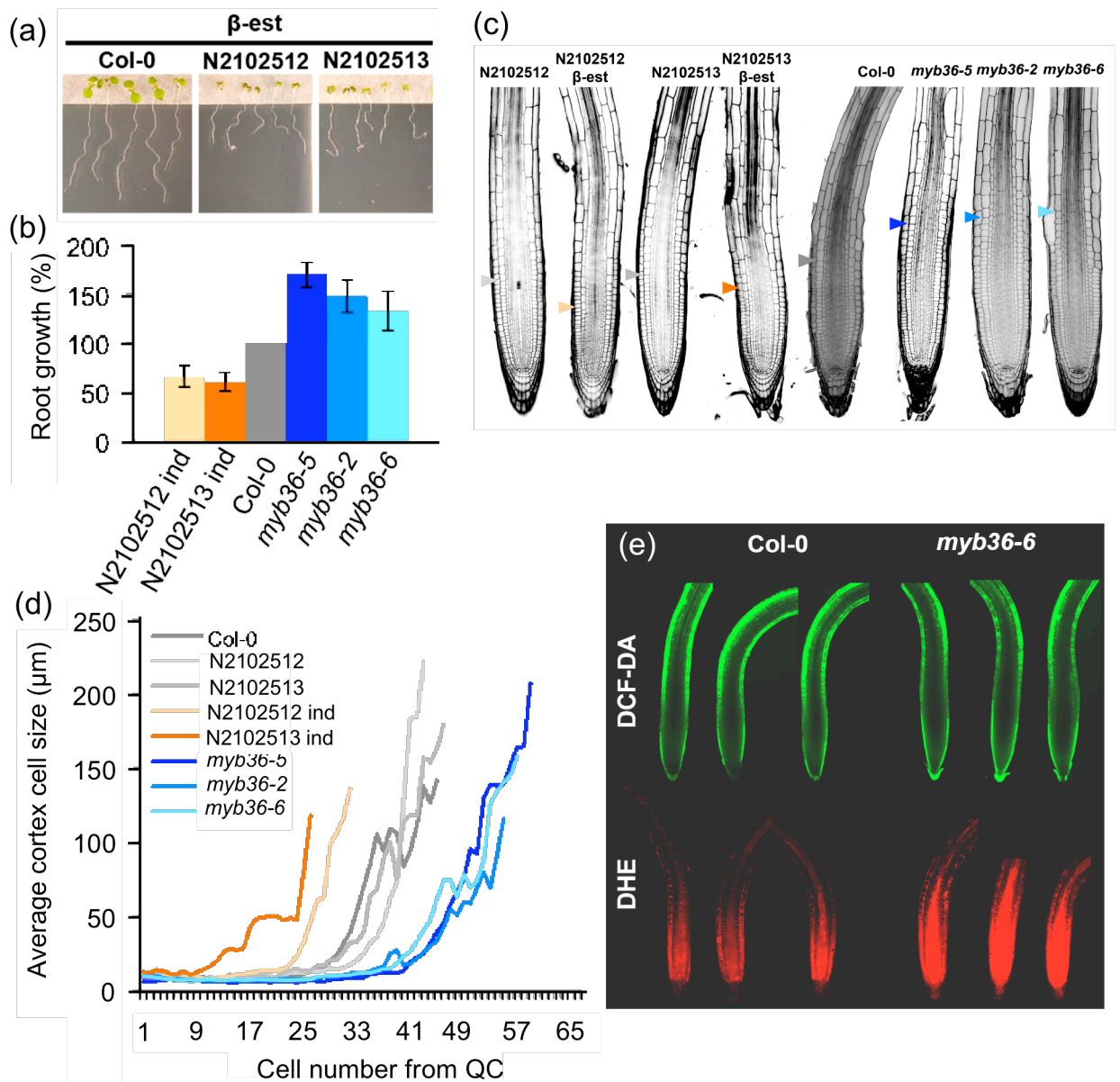


Fig. S1. Primary root phenotypes of MYB36oe and *myb36* mutants.

(a) Images showing the primary root length of Col-0, and lines N2102512 and N2102513 overexpressing MYB36 after β -estradiol treatment ($5 \mu\text{M}$).

(b) Effect of MYB36 overexpression (N2102512 and N2102513) or mutation (*myb36-5*, *myb36-2* and *myb36-6*) on primary root growth. Measurements were taken 3 days after treatment of 3 day-old seedlings with $5 \mu\text{M}$ β -estradiol and growth was followed for 7 days on MS agar plates. Values represent the mean \pm SD of at least 25 roots (except for the N2102512 and N2102513 lines where $n=5$).

(c) Confocal images of root meristem of N2102512 and N2102513 seedlings grown for 3 days and treated with $5 \mu\text{M}$ β -estradiol for 48h, and *myb36-5*, *myb36-2* and *myb36-6* mutants (7 dps) seedlings. Grey, orange and blue coloured arrowheads indicate the meristem boundary of each line.

(d) Average cell size in the cortical cell layer from QC of N2102512 and N2102513 seedlings treated or not with β -estradiol for 48h, and 7dps seedlings of Col-0, *myb36-5*, *myb36-2* and *myb36-6*.

(e) Hydrogen peroxide and superoxide detection in *myb36-6* and Col-0 root meristem using 2',7'-dichlorofluorescein diacetate (DCF-DA) and dihydroethidium (DHE), respectively.

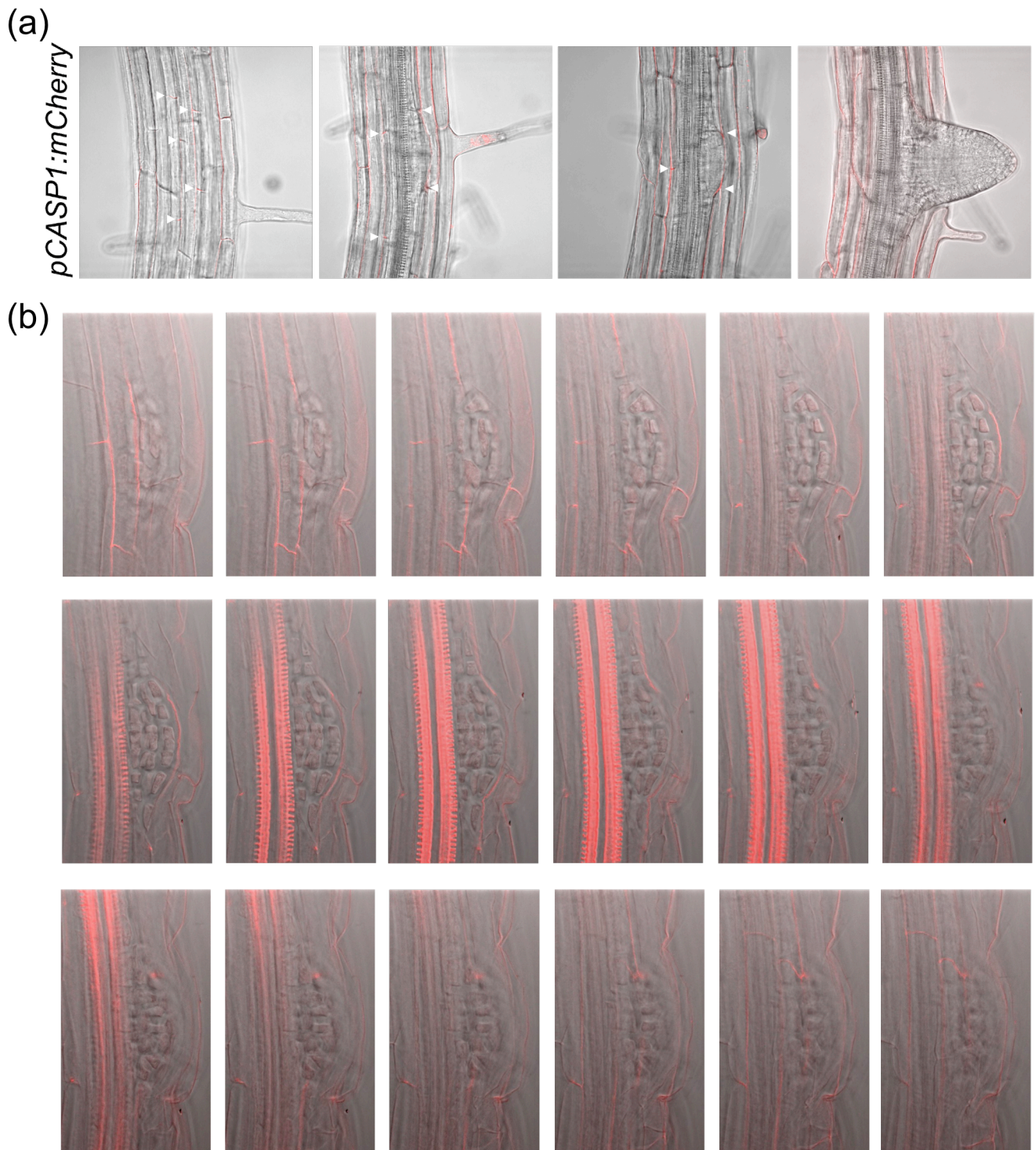


Fig. S2. CASP1 and lignin production in *myb36* mutants.

(a) CASP1 expression using pCASP1:mCherry reveals that CASP is not expressed in the LRP. White arrowheads point sites of CASP1 expression.

(b) Basic fuchsin staining of lignin in Col-0 seedlings reveals the absence of lignin deposition in the walls of LRPB cells.

Supplementary Methods

Methods S1. ROS localization

Hydrogen peroxide (H₂O₂) was visualized by incubating 7 dps Col-0 and *myb36* seedlings with 25 μM 2',7'-dichlorofluorescein diacetate (DCF-DA) in 10 mM Tris-HCl buffer, pH 7.4, for 30 min at 37°C in the dark, and washed for 30 min in 10 mM Tris-HCl buffer, pH 7.4.

Visualization was on an inverted Zeiss LSM 510 confocal microscope (excitation at 485 nm, emission at 530 nm). Superoxide (O₂^{•-}) was detected after incubation with 10 μM dihydroethidium (DHE) in the same buffer, for 1 hour at 37°C in dark. After washing, 30 min in the same buffer, seedlings were observed under the same conditions as for H₂O₂.

Potassium iodide (KI) treatment was performed by transferring Col-0 and *myb36* seedlings (5 dps) from MS media to MS containing KI 1mM for 3 days. The number of LRPs in stages IV and V was quantified in 10 Col-0 and *myb36-5* seedlings after treatment.

Methods S2. Lignin detection

Basic fuchsin staining was used to detect lignin accumulation inside LRPs. Col-0 seedlings (7 dps) were cleared as described {Malamy, 1997 #2557} and incubated in 0.01% (w/v; in ethanol) basic fuchsin for 5 min. Then roots were washed in 70% ethanol for 5 min and rehydrated (subsequent baths of 5 min in 40%, 20%, 10% and 5% ethanol). Samples were mounted in 50% glycerol and analyzed in an inverted Zeiss LSM 710 confocal microscope (excitation at 560 nm, emission at 645 nm).

Malamy JE, Benfey PN. 1997. Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* 124(1): 33-44.

Supplementary Table 1. Primers used in this study.

Genotyping of *myb36-2*

forward 5'-ACGCGGAGAGGACTTCATATAGTA-3';

reverse 5'-GAAGAAACATGGGCTTGCAT-3';

left border: 5'-CCGGACATGAAGCCATTTAC-3'.

ChIP experiments.

PER7 forward 5'-CGCCTTATCTTCCACGATTG-3';

PER7 reverse 5'-GTGTTTTGCTAGCCGGAGAT-3';

PER9 forward 5'-CTAGCCTTAATGGCGCAAAC-3';

PER9 reverse 5'-CGTATGTCCTCCTGATAGGGAA-3';

PER39 forward 5'-GCTTCGTTGCGGGTTGTGA-3';

PER39 reverse 5'-TTAGTTCGGAGGAGCAAGCT-3';

PER40 forward 5'-CCACGACTGTTTTGTCAATGGAT-3';

PER40 reverse 5'-TAGATTAGGAGGCGCCGTTT-3';

PER64 forward 5'-TTGTTGCTCTCTCTGGAGGTC-3';

PER64 reverse 5'-GGGGTTTAGTGTTGGGTCAA-3';

ACT2 forward 5'-CCGCTCTTTCTTTCCAAGC-3';

ACT2 reverse 5'-CCGGTACCATTGTCACACAC-3'