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Supplemental Information

A PHABULOSA-Controlled Genetic Pathway Regulates Ground Tissue Patterning in the *Arabidopsis* Root

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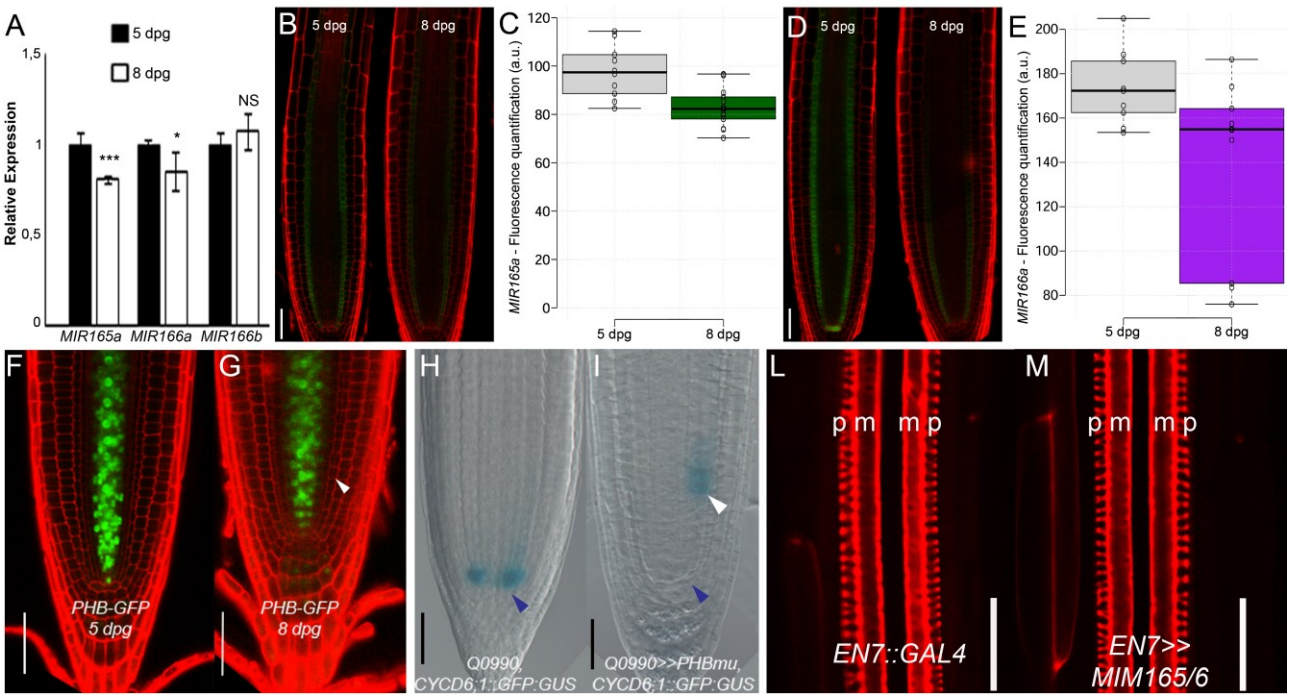
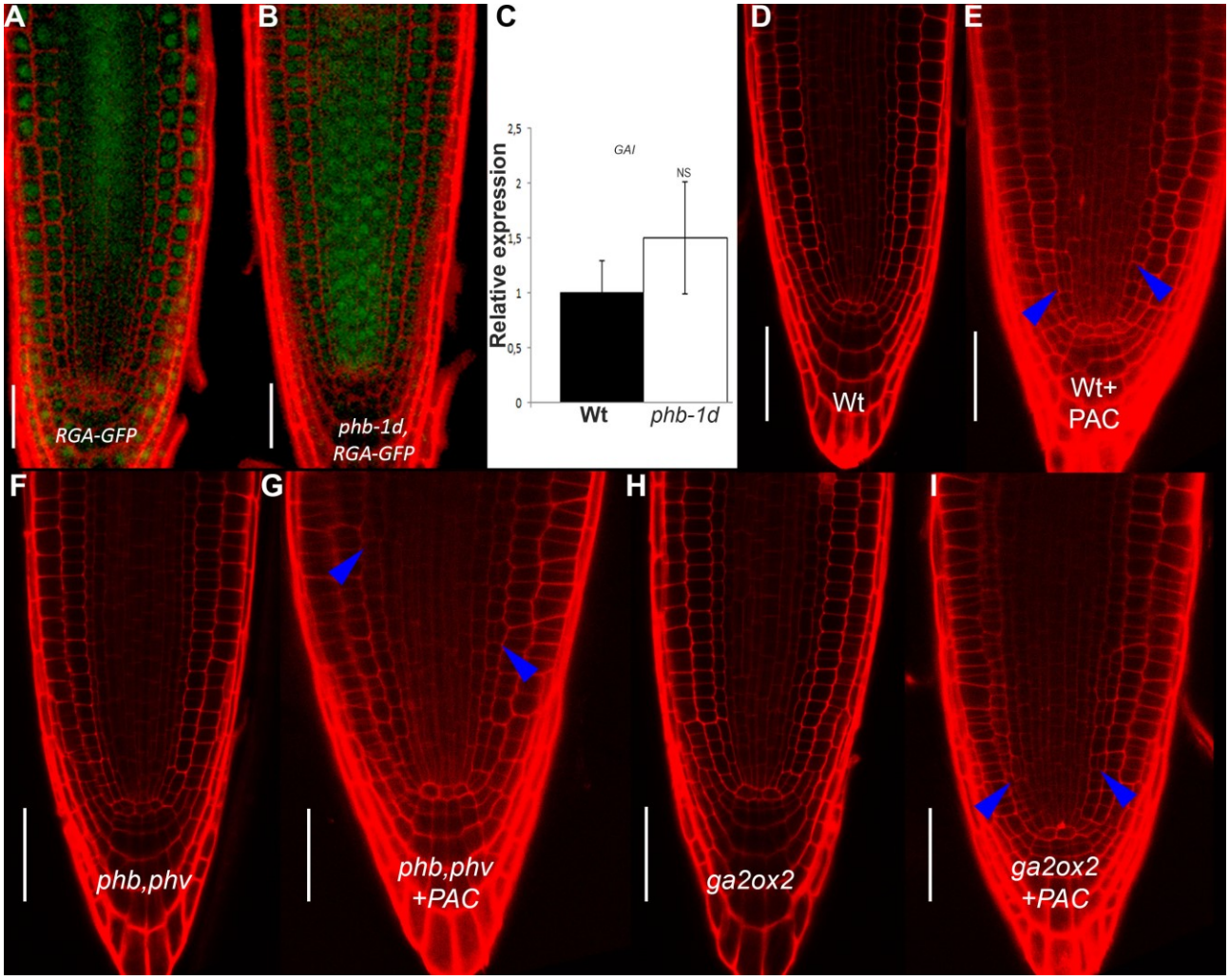
SUPPLEMENTAL DATA:

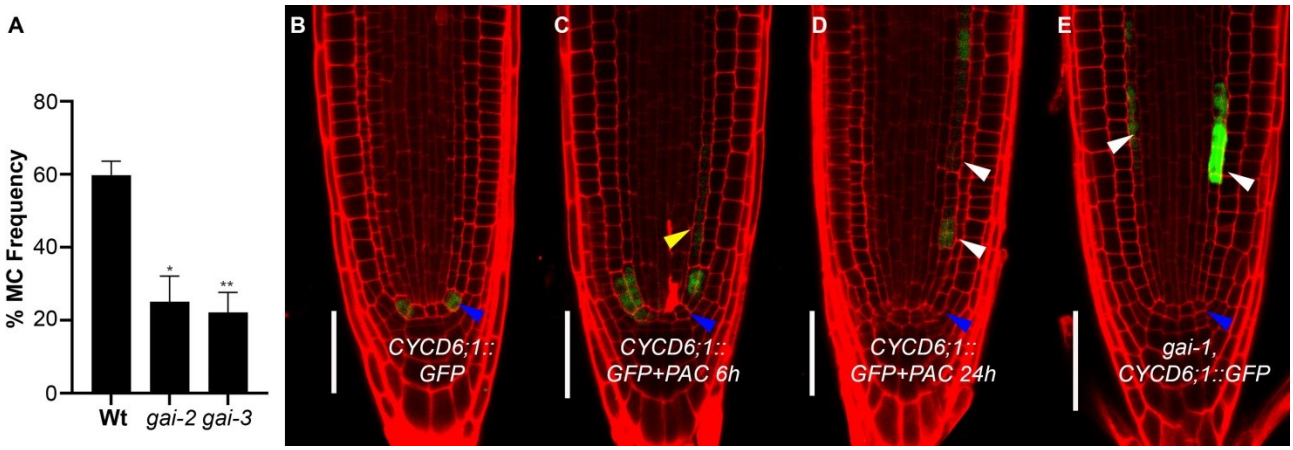


SD1: PHB directs *CYCD6;1* expression switch cell non autonomously. Related to Figure 1.
A) Relative expression of *pre-miR165a, pre-miR166a* and *pre-miR166b* in 5 and 8 dpg Wt plants. N=3, Student t test (*p<0,05, p **<0,01, p***<0,005, NS Not Significant). Error Bars: SD. B) Confocal images of *MIR165A::GFP* roots at 5 dpg and 8 dpg. Scale Bars: 50 µm. C) Fluorescence quantification of *MIR165A::GFP* line at 5 dpg (grey) and at 8 dpg (green) where center lines show the medians. 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; data points are plotted as open circles. p-value < 0.005, Student's t-test, n = 10, 14 sample points. D) Confocal images of *MIR165A::GFP* roots at 5 dpg and 8 dpg. Scale Bars: 50 µm. E) Fluorescence quantification of *MIR166A::GFP* line at 5 dpg (grey) and at 8 dpg (purple) where center lines show the medians. 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; data points are plotted as open circles. p-value < 0.05, Student's t-test, n = 9, 10 sample points. F,G) Confocal image of a root meristem of a *PHB-GFP* plant at 5 (F) and 8 dpg (G). H,I) Optical microscope images of a 5 dpg root meristem of *Q0990, CYCD6;1::GFP:GUS* (H) and *Q0990>>PHBmu:GFP, CYCD6;1::GFP:GUS* (I). Scale Bars: 50 µm, white arrowheads indicate MC formative asymmetric division, blue arrowheads CEI. L,M) Confocal images of 5 dpg basic fuchsin-clearsee stained *EN7::GAL4* (L) and *EN7>>MIM165/6* (M) roots. p: protoxylem, m:metaxylem. Scale Bars: 20 µm.



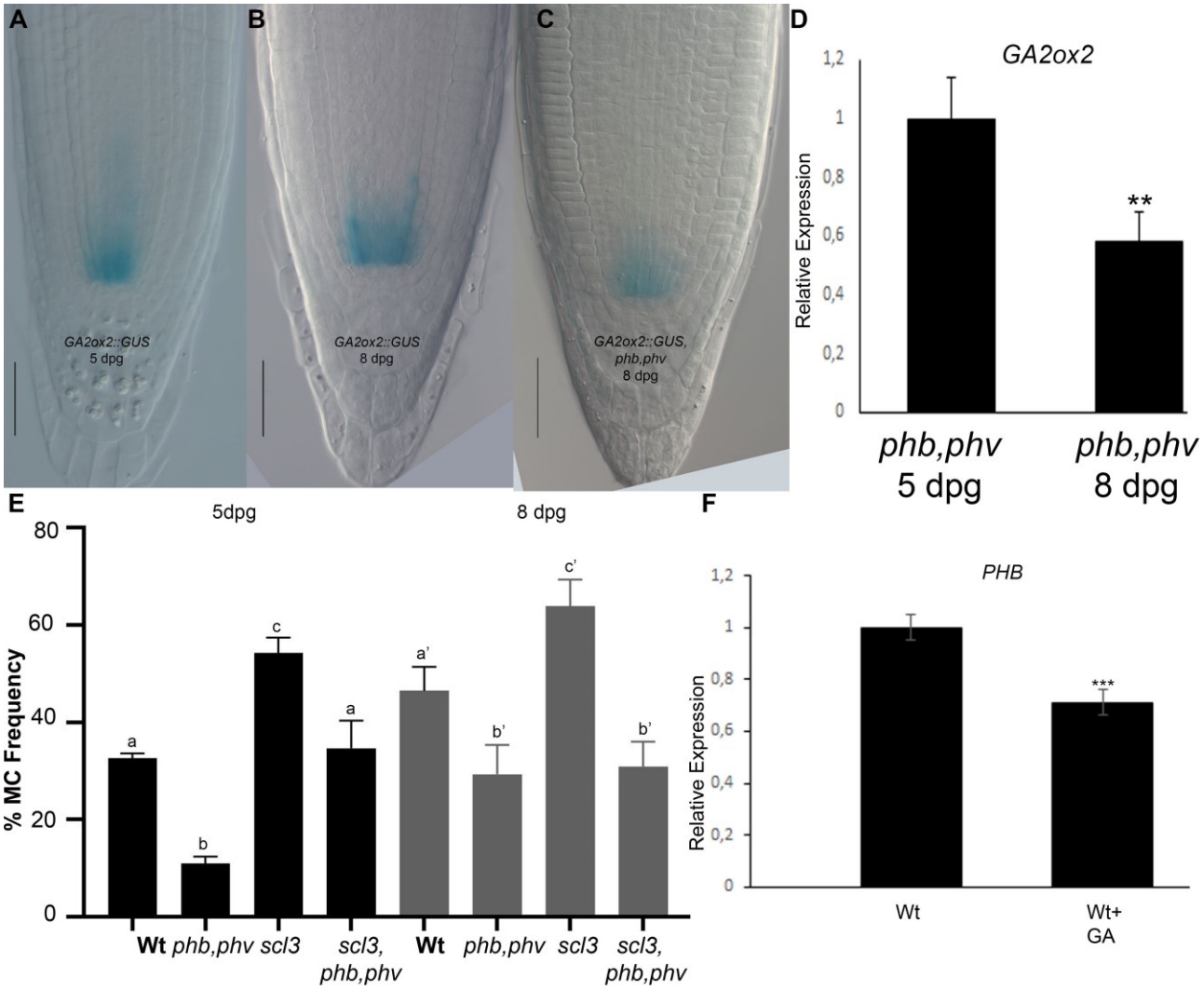
SD2: PHB regulates MC formation via GAs metabolism regulation. Related to Figure 2.

A-B) Confocal images of 5 dpg root meristems of *RGA-GFP* (A) and *phb-1d, RGA-GFP* (B). C) qRT-PCR of *GAI* in Wt and *phb-1d* mutants. N=3, Student t test (NS Not Significant). Error Bars: SD. D-I) Confocal images of 5 dpg root meristems of Wt (D), Wt+ PAC (50 μM 48h) (E), *phb, phv* (F), *phb, phv*+ PAC (50 μM 48h) (G), *ga2ox2* (H),*ga2ox2*+ PAC (50 μM 48h)(I). Scale Bars: 50 μm, blue arrowheads indicate the additional asymmetric division induced by PAC.



SD3: GAI directs *CYCD6;1* expression switch. Related to Figure 3

A) Histogram depicting the percentage of plants showing MC in Wt and *gai-2* and *gai-3* at 8 dpg N=3, Student t test (*p<0,05, p **<0,01). Error Bars: SD. ANOVA p<0,01. B-E) Confocal images 5 dpg the root meristem of *CYCD6;1::GFP:GUS* untreated Wt (B), treated (C-D) with PAC (50 μM) for 6 (C) and 24 (D) hours and *gai-1* mutant (E). Scale Bars: 50 μm, white arrowheads indicate MC formative asymmetric division, blue arrowheads CEI, yellow arrowhead shows the cells accumulating GFP signal prior to cell division.



SD4: PHB and PHV dependent GAs catabolism in the vasculature control MC formation via SCL3. Related to Figure 4.

A-C) Root meristems of *GA2ox2::GUS* plants at 5 (A) and 8 (B) dpg and 8 dpg *GA2ox2::GUS*,*phb,phv* plants (C). Scale Bars: 50 μm. D) qRT-PCR of *GA2ox2* in *phb,phv* mutants at 5 and 8 dpg. N=3, Student t test (p **<0,01). Error Bars: SD. E) Histogram depicting the percentage of plants showing MC in Wt and *phb,phv* and *scl3* and *scl3,phb,phv* at 5 and 8 dpg. N=3, Student t. Error Bars: SD. P<0,05 ANOVA. Letters indicate statistical significance. F) qRT-PCR of *PHB* in Wt plants untreated and treated with GA3 for 6 hours at 8 dpg. N=3, Student t test (p ***<0,05). Error Bars: SD.

Genotyping primers

Background	Forward	Reverse	Insertion
phb-13	ACCGTACCCTGGATTTAGCC	TTATCTAGATCCGGTGGATCCAAG	TCAAACGAACGACCAATTCACG
phv-11	CCCAATGGTCCACTTTCTTCA	GCCAGCAAATTTAGCAGAGGA	GCGTGGACCGCTTGCTGCAACT
gai-t6	CTAGATCCGACATTGAAGGA	AGCATCAAGATCAGCTAAAG	TCGGTACGGGATTTTCGCAT
gai-1	CTAGATCCGACATTGAAGGA	AGCATCAAGATCAGCTAAAG	AGCATCAAGATCAGCTAAAG
cycd6-1	AATTCGACGACCCATCTCTG	CTGCAATCACCGATGGTTTA	ATATTGACCATCATACTCATTGC
gai-2	TGTACCACTAGTTGCATGACAATC	AGCTTCGGCGAAGTAAGTAGC	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC
gai-3	TCGATAAGGTTCTTGGTGTGG	CAAAGGGTCACGAGTGAAGTC	GCGTGGACCGCTTGCTGCAACT
ga2ox2-1	GAAAACCCGAATCGTAAAAGC	GAGACGAGAAGAAATCGCATG	GCGTGGACCGCTTGCTGCAACT
scl3-1	AGCGCAGTTCTTTCTCATGAG	TTCCTCTGTTCTTTAACCCCC	GCGTGGACCGCTTGCTGCAACT

Table S1: Genotyping primers used in this study. Related to STAR Methods

qRT-PCR primers

Gene	Forward	Reverse
GADPH	TTGGTGACAACAGGTCAAGCA	AAACTTGTCGCTCAATGCA
OTC	TGAAGGGACAAAGGTTGTGTATGTT	CGCAGACAAAGTGGAAT GGA
PHB	GCTAACAACCCAGCAGGACTCCT	TAAGCTCGATCGTCCCACCGTT
GA2ox2	TCCGACCCGAACTCATGACT	CGGCCCGGTTTTTAAGAGAC
GAI	CTAGATCCGACATTGAAGGA	AGCATCAAGATCAGCTAAAG
PHV	GCTAATCTTCTCTCGATTGCGGAGGA	GCTCGATAGTACCACCATTTCCAGTG
MIR165A	GATCGATTATCATGAGGGTTAAGC	CTATAATATCCTCGATCCAGACAAC
MIR166A	GGGGCTTTCTCTTTTGAGG	CGAAAGAGATCCAACATGAATAG
MIR166B	GATTTTTCTTTTGAGGGGACTGTTG	CTGAATGTATTCAAATGAGATTGTATTAG

Table S2: qRT-PCR primers used in this study. Related to STAR Methods

ChIP-qPCR primers

Region	Forward	Reverse
A	CTGATCCATAGGCATCATGTA	TGCTTTCTGGTTGTAGGTTCTC
B	AGATCCAATCATTTTCCTCCAT	GTTTGGATAATTGGAAGAATTTTA
C	GACGGGCATTGGTGATTTATT	ATAGAAACATCCTTATCCTCAC

Table S3. ChIP-qPCR primers used in this study. Related to STAR Methods.