

New *Phytologist* Supporting Information

Article title: The Auxin Response Factor MONOPTEROS controls meristem function and organogenesis in both the shoot and root through the direct regulation of *PIN* genes

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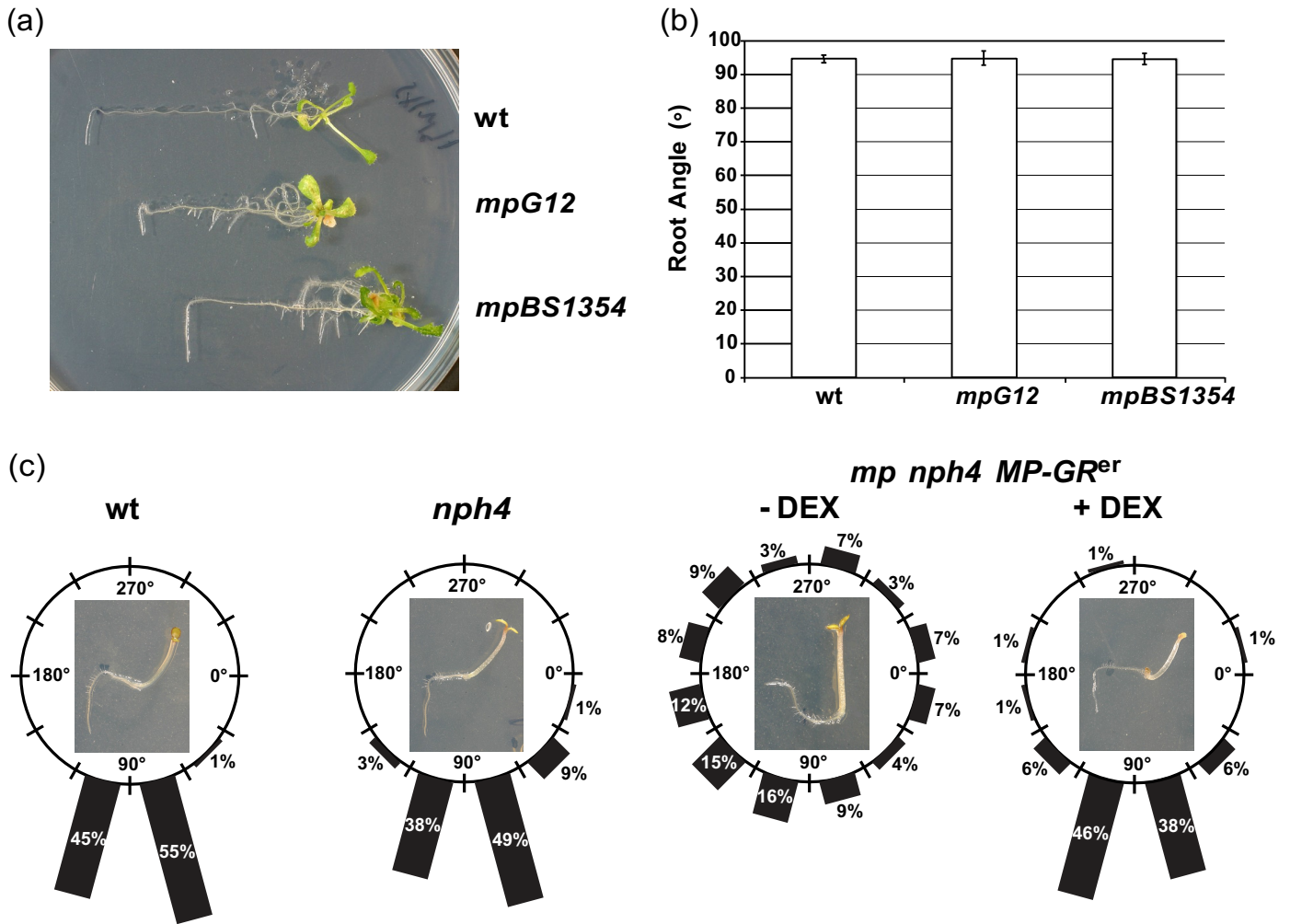


Fig. S1 *Arabidopsis* root gravitropic responses.

(a,b) *monopteros* (*mp*) seedlings were germinated and grown on 5 μ M α -naphthaleneacetic acid (NAA) for 16d. Individuals exhibiting adventitious rooting were transferred to fresh media lacking NAA and grown vertically for 5d. Vertically grown seedlings were then turned 90° clockwise and transferred into the dark for 24h. The position of each root relative to its original gravistimulated orientation (0°) was then measured. Control wild-type seedlings were treated as above except these were initially grown on 5 μ M NAA for 6d. (a) Representative seedlings of each genotype at the end of the assay with the gravity vector downward. (b) Quantitative assessment of root bending in response to gravitropic stimulus, showing that adventitious *mp* roots exhibit normal gravitropism. Columns represent mean values +/- s.e.m.

(c) 2d after germination (DAG) light-grown seedlings were arranged vertically on agar media, turned 90° clockwise, and transferred into the dark for 24h. Root positions relative to the original gravistimulated orientation (0°) are depicted (gravity vector downward). *mp nph4 MP-GR^{er}* seedlings were grown in the absence of dexamethasone (-DEX), or were grown on 15 μ M DEX during the initial 2d light period then transferred to DEX-free media for 24h of gravistimulation (+DEX). Only *mp nph4 MP-GR^{er}* roots that exhibited significant growth were measured. Bars: 2mm.

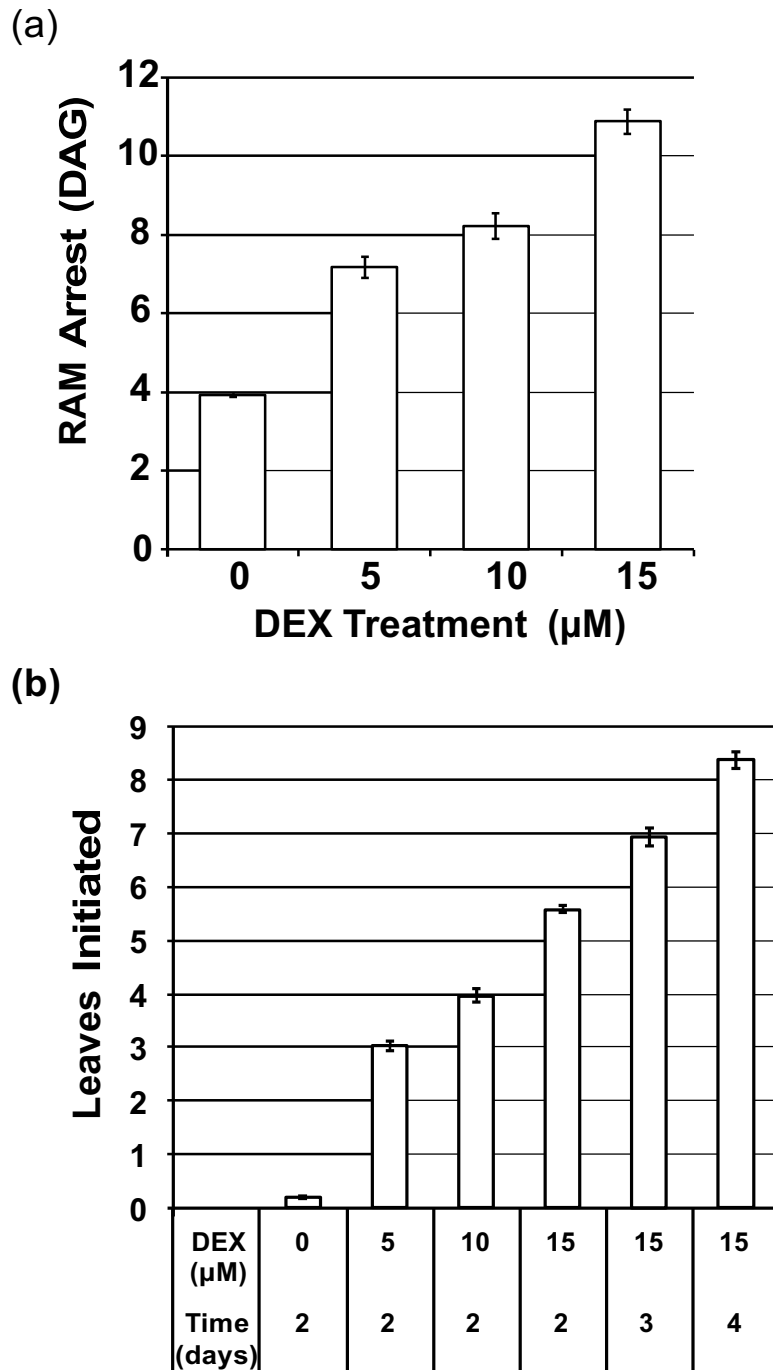


Fig. S2 *mp nph4 MP-GR^{er}* root and shoot apical meristem activity depends directly on dexamethasone (DEX) dosage in *Arabidopsis*.

(a) *mp nph4 MP-GR^{er}* seedlings were germinated and grown on varying concentrations of DEX for 2d. Individuals were subsequently transferred to DEX-free media, and the age of each seedling when root growth arrested was recorded. Columns represent mean values \pm s.e.m.

(b) *mp nph4 MP-GR^{er}* seedlings were germinated and grown on varying concentrations of DEX for 2 to 4d. Individuals with primary roots were subsequently transferred to DEX-free media and the number of leaves produced was recorded 21d later. Columns represent mean values \pm s.e.m.

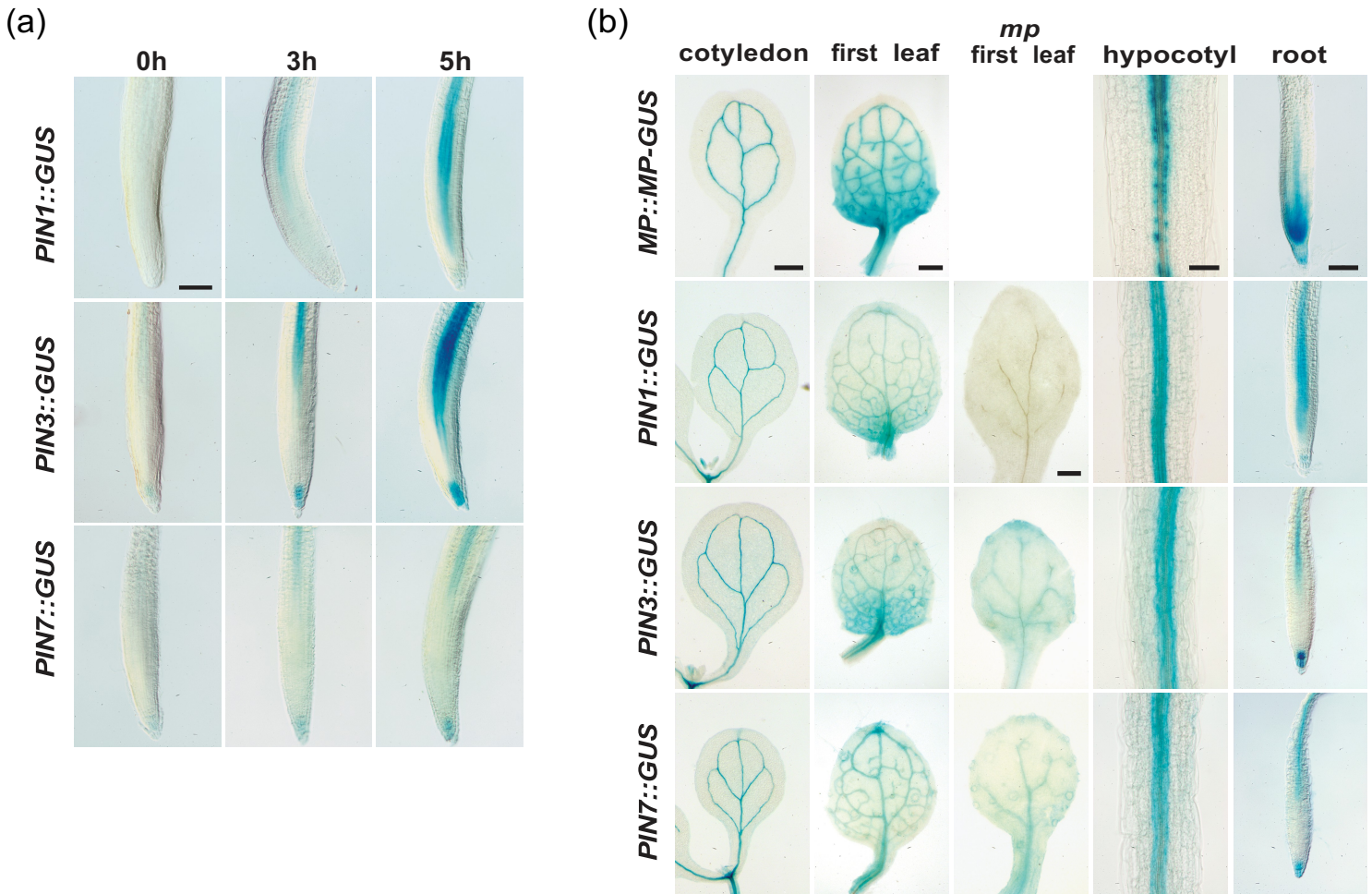


Fig. S3 *Arabidopsis* *PIN FORMED* (*PIN*:: β -glucuronidase (*GUS*)) expression patterns.

(a) Expression patterns of *PIN*::*GUS* transcriptional reporter genes in primary roots are shown. 5d after germination (DAG) seedlings were treated for 0, 3 or 5h with 1 μ M 2,4-dichlorophenoxyacetic acid (2,4-D). Each image shows a staining pattern that is representative of the majority of viewed samples. Bar: 0.1mm.

(b) Expression patterns of a functional *MP*::*MP-GUS* translational reporter and *PIN*::*GUS* transcriptional reporter genes are shown. All expression patterns are depicted in wild type except for images in the “*mp* first leaf” column. Cotyledons and leaves are from 6DAG seedlings, while hypocotyls and roots are from 5DAG seedlings.

Bars: “cotyledon” 0.5mm; “first leaf” 0.2mm; “hypocotyl” and “root” 0.1mm.

Table S1 Reverse transcription-polymerase chain reaction (RT-PCR) primer sequences.

Gene	AGI number	Primer	Sequence (5'-3')
<i>PIN1</i>	At1g73590	forward	GATGTTTTCGCCCAACTGG
		reverse	TGTTACCGAAACTAACTCTTCCC
<i>PIN3</i>	At1g70940	forward	AAACCCGAAAGACGTTAATACG
		reverse	CTTCTTCTTTTCCGGCGAAAC
<i>PIN7</i>	At1g23080	forward	TGGTGGTACTAATATGACTCCTCGTC
		reverse	GGTACAGTTTCACCCGTTTCTATAGC
<i>ROC1</i>	At4g38740	forward	CAAACCTCTTCTTCAGTCTGATAGAGA
		reverse	GAGTGCTCATTCTTATTTCTGGTAG
<i>ACT7</i>	At5g09810	forward	GGTGAGGATATTCAGCCACTTGTCTG
		reverse	TGTGAGATCCCGACCCGCAAGATC

Table S2 Primer sequences used to create electrophoretic mobility shift assay (EMSA) probes.

Gene	AGI number	Primer	Sequence (5'-3')
<i>PIN1</i>	At1g73590	forward	AAATATGGTATTTTCTCAACGGAC
		reverse	ATCTTTTGTTTCGCCGAGAAGAG
<i>PIN3</i>	At1g70940	forward	CCAAGGAGTAATTGAGTGTTGCC
		reverse	AAGGAGACAAGATGGGCTGGAG
<i>PIN7</i>	At1g23080	forward	GTTTTCACAATCCGATTTTGTAC
		reverse	GTAAGAAATTAAGATGAAAAGAGAC

Table S3 Chromatin immunoprecipitation (ChIP) primer sequences.

Gene	AGI number	Primer	Sequence (5'-3')
<i>PIN1</i>	At1g73590	forward	GTGGTCTCTTCTCAACTCACT
		reverse	TTTTCCACCATTTGACAGAGCC
<i>PIN3</i>	At1g70940	A -forward	CCAAGGAGTAATTGAGTGTTGCC
		A -reverse	AATAAGGAGACAAGATGGGCTGG
<i>PIN3</i>	At1g70940	B -forward	TGAAAAGCAAAGATTAGGGGACAG
		B -reverse	TAAGAAGAAGGTCTACATGTGGCA
<i>PIN7</i>	At1g23080	A -forward	AAGTGGTGGAGAAGCTTCAAAGA
		A -reverse	TAAACGTTACTGAGAGATGGCCC
<i>PIN7</i>	At1g23080	B -forward	TATGTGTGACATGTAGACAGTCAGT
		B -reverse	AGCTTTCCCCTAATTTATGTACCAT
<i>PP2A</i>	At1g59830	forward	CAAACCAAAGACGAGCCAGAGC
		reverse	ACCGAATCGTTGTAAATCGAACAC
<i>ACT7</i>	At5g09810	forward	GGTGAGGATATTCAGCCACTTGTCTG
		reverse	ACCATGACACCAGTGTGCCT

Table S4a β -Glucuronidase (GUS)-staining conditions of auxin-induced *Arabidopsis* tissues.

Transgene	Tissue	[Fe salt] (mM)	Incubation time (h)
<i>PIN1::GUS</i>	root	1	2.75
<i>PIN3::GUS</i>	root	1	1
<i>PIN7::GUS</i>	root	1	3

Table S4b β -Glucuronidase (GUS)-staining conditions of *Arabidopsis* tissues not auxin treated.

Transgene	Tissue	[Fe salt] (mM)	Incubation time (h)
<i>MP::MP-GUS</i>	cotyledon	1	5.5
	leaf	5	23
	hypocotyl	1	23
	root	1	23
<i>PIN1::GUS</i>	cotyledon	5	5
	leaf	5	5
	hypocotyl	5	6
	root	0.5	1.75
<i>PIN3::GUS</i>	cotyledon	5	1.5
	leaf	5	1.5
	hypocotyl	5	2
	root	1	2
<i>PIN7::GUS</i>	cotyledon	5	2
	leaf	5	2
	hypocotyl	5	1
	root	1	3.25