Supplemental material to:

Auxin response cell-autonomously controls ground tissue initiation in the early Arabidopsis embryo

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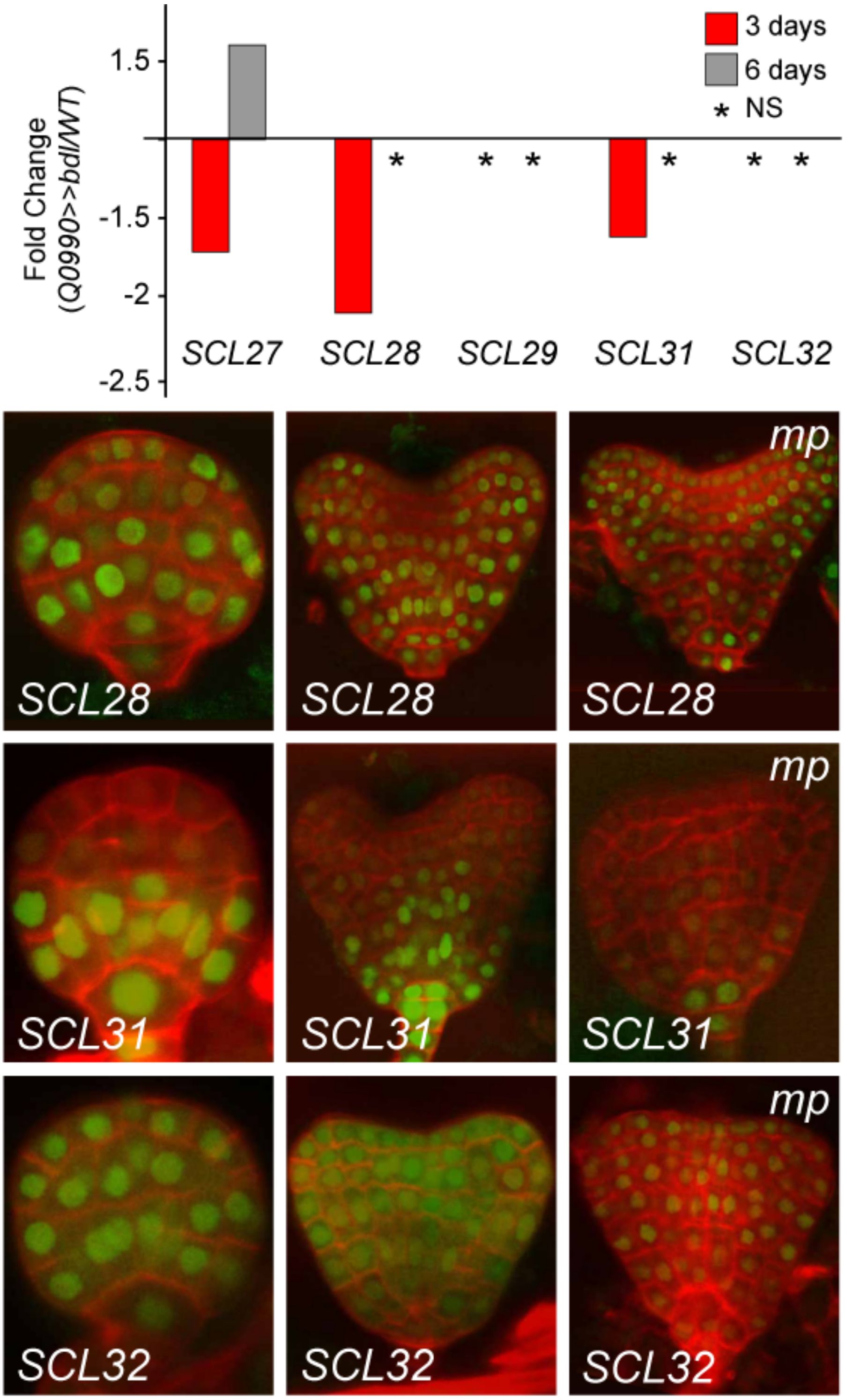
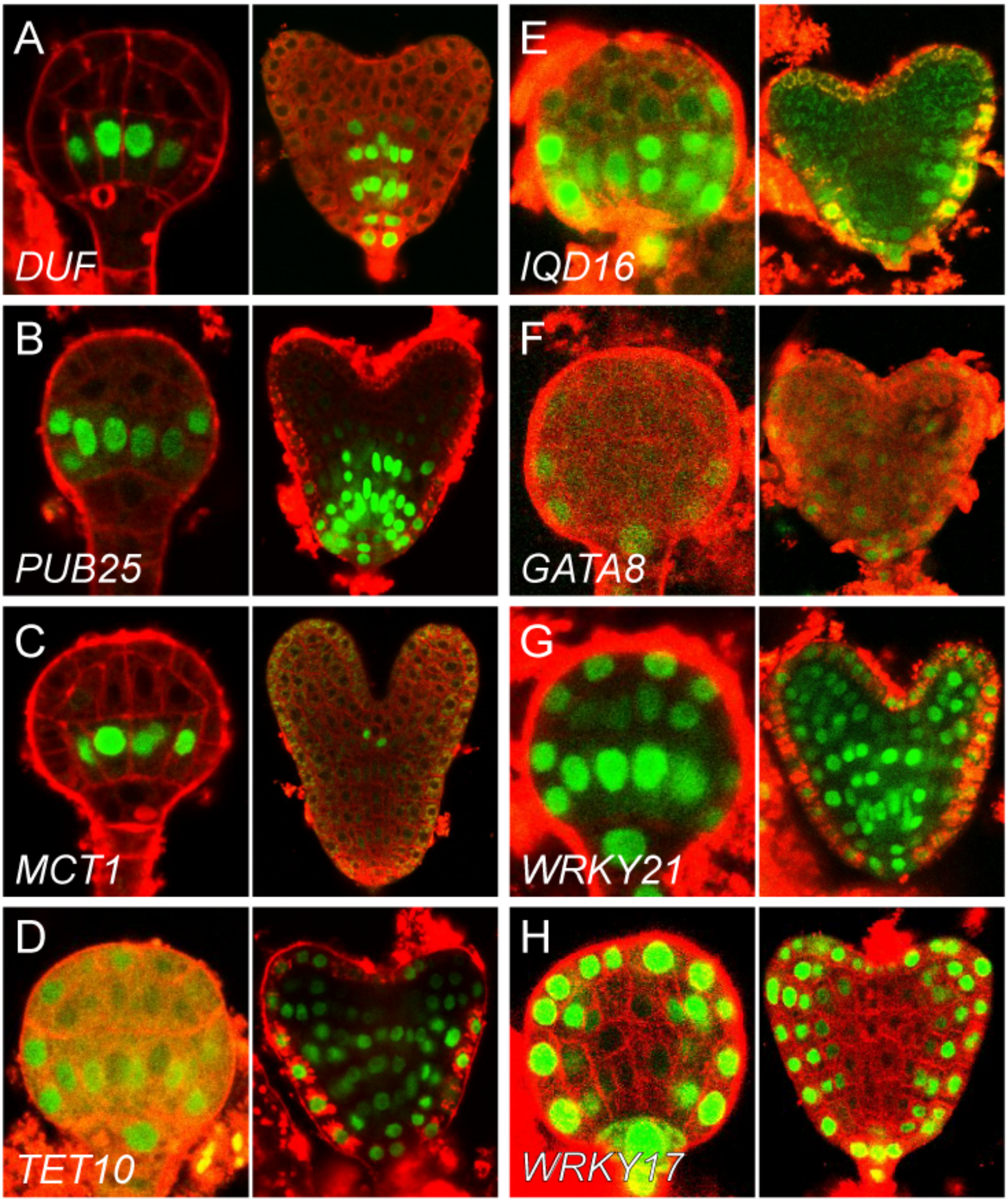


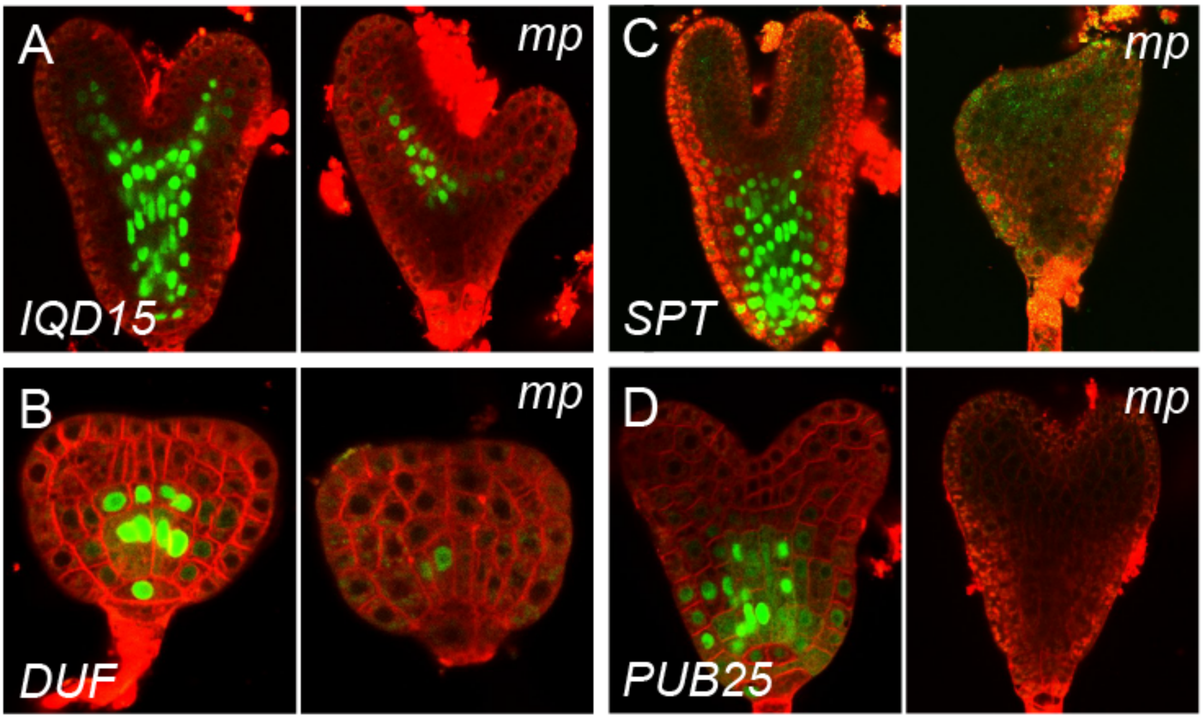
Supplemental Figure 1: Expression of GRAS family genes

(top) Differential gene expression of GRAS family genes downregulated in the *Q0990>>bdl* microarray (in fold change *Q0990>>bdl*/*Q0990>>WT*) in *Q0990>>bdl* embryos at 3 days (red columns) and 6 days (grey columns) after pollination. NS, not significant. (bottom) Expression of promoter-n3GFP (for *SCL28*) or nuclear tdTomato reporters (for *SCL31* and *SCL32*; all in green) in wild-type and *mp* mutant embryos. p*SCL27*-ntdTomato and p*SCL29*-ntdTomato were not expressed in embryos. Red counterstaining is Renaissance RS2200 signal.



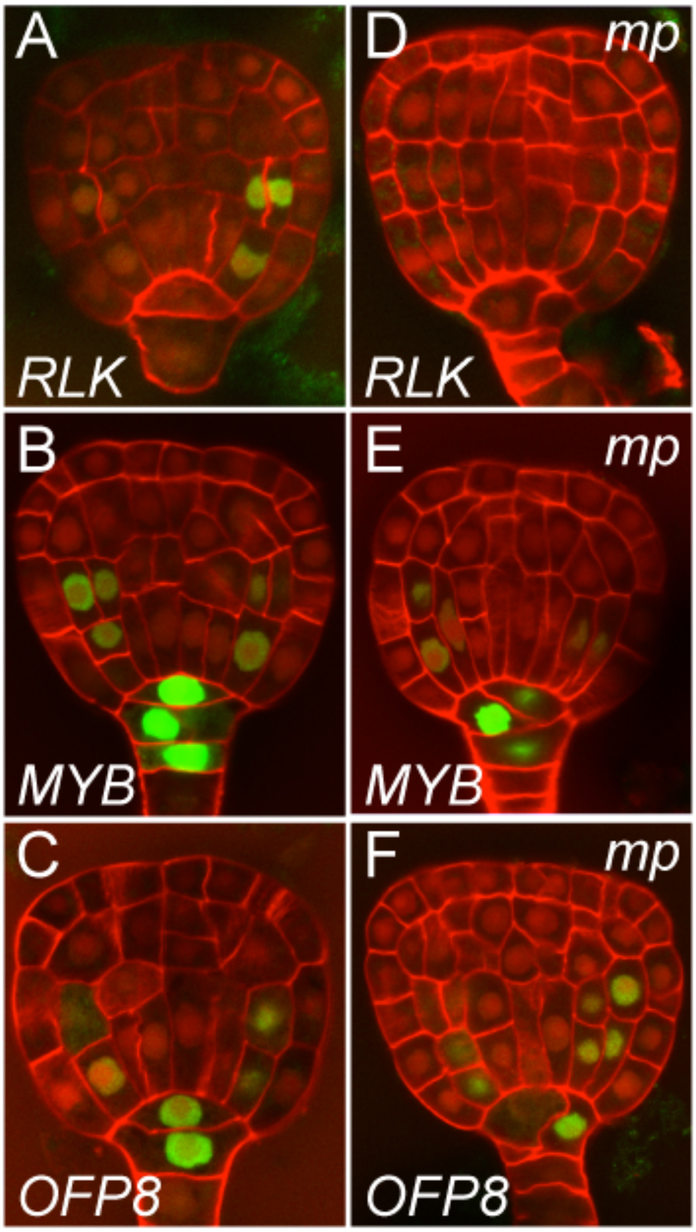
Supplemental Figure 2: Expression patterns of genes downregulated in globular stage Q0990>>bdl embryos

Expression patterns of transcriptional nuclear 3GFP reporter lines of *DUF/At1g05577* (A), *PUB25/At3g19380* (B), *MCT1/At1g37140* (C), *TET10/At1g63260* (D), *IQD16/At4g10640* (E), *GATA8/At3g54810* (F), *WRKY21/At2g30590* (G), *WRKY17/At2g24570* (H) genes in globular (left panel) and heart (right panel) stage embryos. Red counterstaining is FM4-64.



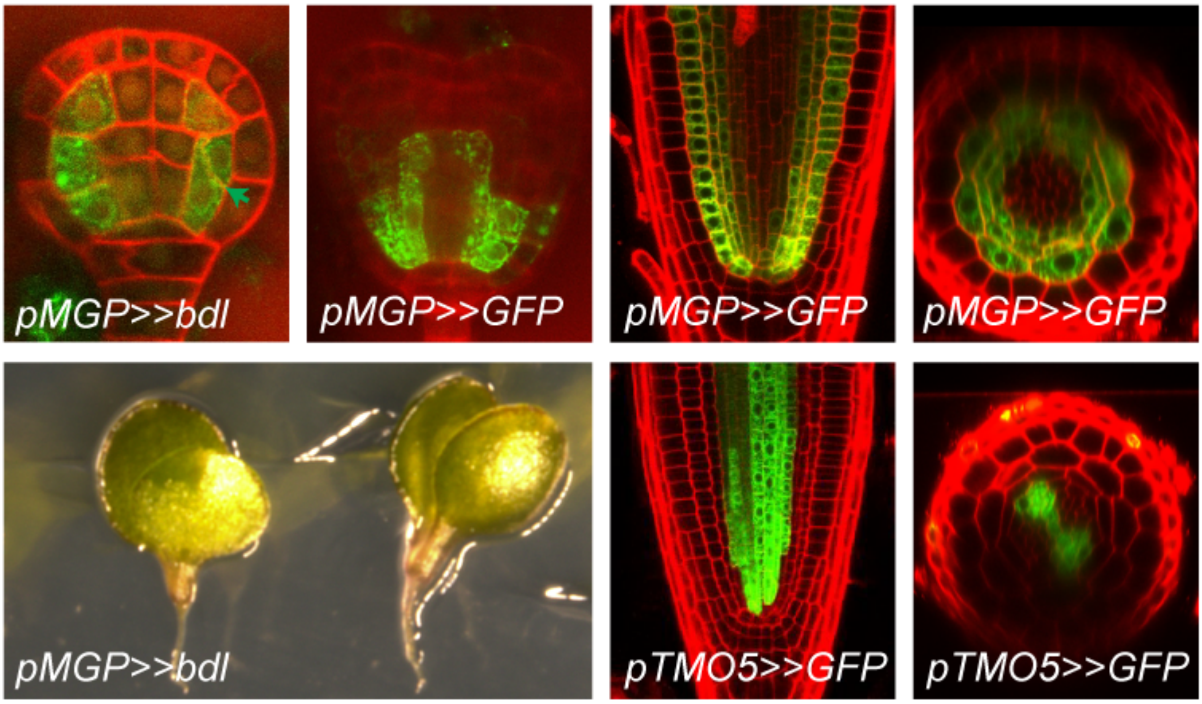
Supplemental Figure 3: Expression patterns of MP-dependent genes downregulated in globular stage Q0990>>bdl embryos

Expression patterns of transcriptional nuclear 3xGFP reporter lines of MP target genes in heart stage (A,C,D) or transition stage (B) wild-type and *mp* embryos. Images were taken at identical imaging settings. Red counterstaining is FM4-64.



Supplemental Figure 4: Expression patterns of ground tissue-expressed genes down-regulated in globular stage Q0990>>bdl embryos

Expression of promoter-n3GFP reporters for *RLK/At1g70250* (A,D), *MYB/At2g42660* (B,E) and *OFP8/At5g19650* (C,f) genes. Images show expression in globular stage wild-type (A,B,C) and *mp* mutant (D,E,F) embryos and were taken at identical imaging settings. Red counterstaining is Renaissance RS2200.



Supplemental Figure 5: Tools for tissue-specific bdl misexpression
pMGP>>bdl expression induces altered cell division orientation in the globular stage ground tissue precursors (green arrow). The *pMGP>>GFP* driver is expressed in a broader domain during transition stage and post-embryonically. *pMGP>>bdl* seedlings are rootless. The *pTMO5>>GFP* driver is specific to the xylem domain in the post-embryonic root tip.

Table S1: List of significantly downregulated genes in the *Q0990>>bdl* microarray on globular stage embryos. List of at least two-fold downregulated genes from a microarray on globular stage embryos in which MP activity was locally inhibited in the inner basal embryo cells that will acquire vascular and ground tissue identity, using the *Q0990>>bdl* two component system. The list shows genes that are significantly downregulated in all four replicates (q≤0,05). Below the bold line are genes that were less than two-fold downregulated or downregulated in less than four replicates. The same experiment was performed with heart stage embryos. For each gene, the fold change in early globular stage embryos (FC glob) and in heart stage embryos (FC heart) is shown. Gene functions were categorized as transcription factors (TF), cell wall remodelling genes (CW), genes involved in signaling (S), degradation (D), cell cycle (CC), auxin signalling (A), cytokinin activation (CK) and ethylene signalling (ETH). [a]Fold change is not significant (t-test, q>0,05). [b] Only three replicates. [c] Only two replicates, not significantly downregulated. [d] Only one replicate, not significantly downregulated.

AT number	Gene name	Gene function	FC glob	FC heart
AT1G74500	TMO7	TF	-8,80	-6,46
AT1G05577	Unknown (DUF)		-7,78	-2,18
AT2G30130	ASL5 (LBD12)	TF	-7,11	-5,98
AT5G34881	unknown, DUF784		-6,53	1,67[a]
AT3G11260	WOX5	TF	-5,23	1,32[a]
AT2G29350	SAG13, alcohol dehydrogenase		-4,98	-1,91
AT1G29270	unknown		-4,79	1,13[a]
AT5G59760	unknown, DUF1635		-4,67	-2,81
AT1G58270	ZW9		-4,29	1,32[a]
AT4G28720	YUC8	A	-4,09	1,41[a]
AT2G01420	PIN4	A	-4,08	1,00
AT2G03830	RGF8, secreted peptide	S	-3,99	1,43[a]
AT5G47440	PH/DUF828	S	-3,92	1,09[a]
AT3G48970	copper-binding family protein		-3,45	1,45[a]
AT5G37140	tRNA-splicing endonuclease		-3,33	1,38[a]
AT1G56010	NAC1	TF	-3,15	1,45[a]
AT1G80840	WRKY40, class IIa	TF	-3,12	1,01[a]
AT1G77145	unknown, DUF506		-3,09	-1,46[a]
AT1G37140	MCT1		-3,03	1,29[a]
AT3G04070	NAC47	TF	-3,03	-1,08[a]
AT5G57760	unknown protein		-3,01	1,36[a]
AT3G19380	PUB25	D	-2,96	-1,87
AT3G56770	bHLH106/TMO5-LIKE2	TF	-2,96	-1,16[a]
AT2G01430	ATHB17	TF	-2,94	-1,63
AT2G21050	LAX2	A	-2,93	1,07[a]
AT5G60810	RGF1, secreted peptide	S	-2,91	-1,19[a]
AT2G37210	LOG3, cytokinin-activating enzyme	CK	-2,84	-2,77
AT3G13960	AtGRF5	TF	-2,83	-1,11[a]
AT3G13857	unknown protein		-2,81	-7,09
AT3G26760	dehydrogenase		-2,81	-2,92
AT2G18500	OFP7	TF	-2,81	1,48[a]
AT2G42660	MYB-LIKE, SHAQKYF class	TF	-2,79	-3,00
AT5G06300	LOG7, cytokinin-activating enzyme	CK	-2,78	-1,56
AT3G52450	PUB22	D	-2,78	-1,20[a]
AT1G76420	CUC3	TF	-2,78	1,35[a]
AT4G25390	kinase	S	-2,78	1,17[a]
AT1G16220	PP2C	S	-2,76	-1,16[a]
AT5G05340	peroxidase	CW	-2,75	1,96[a]
AT1G05370	Sec14-like protein		-2,73	1,52
AT1G12610	DDF1 (AP2)	TF	-2,73	1,25[a]
AT3G17680	Kinase interacting protein-like		-2,73	-3,06
AT5G45480	unknown, DUF594		-2,71	1,14[a]
AT4G37650	SHR	TF	-2,68	-2,26
AT4G37810	unknown		-2,68	1,30[a]
AT5G15340	PPR containing protein		-2,68	1,46[a]
AT5G28720	unknown		-2,62	1,00[a]
AT3G45450	Protease chaperone-like	D	-2,59	1,21[a]
AT5G36960	unknown		-2,59	-1,05[a]
AT1G03220	Extrecellular peptidase	CW	-2,56	-1,33[a]
AT5G54020	DC1-domain Zn-finger	S	-2,56	-1,41[a]
AT2G35080	Aminoacyl tRNA synthetase		-2,54	-2,03
AT1G78860	Lectin	CW	-2,54	-3,59
AT4G17240	unknown		-2,53	1,13[a]
AT3G54920	PMR6, pectate lyase	CW	-2,53	1,19[a]
AT5G67390	unknown		-2,53	-1,58
AT5G19650	OFP8	TF	-2,52	-1,76
AT1G03840	MGP	TF	-2,50	-1,95
AT2G23050	NPY4	A	-2,45	-3,35
AT5G28640	AN3 (GIF)	TF	-2,44	1,04[a]
AT1G02810	pectinesterase	CW	-2,44	-3,00
At3g08030	unknown, DUF642	CW	-2,41	1,00
AT4G32800	TINY-LIKE (AP2)	TF	-2,39	1,50[a]
AT3G62100	IAA30	TF/A	-2,39	-1,25[a]

AT3G57010	strictosidine synthase		-2.38	-1.72
AT4G02290	AtGH9B13, glycosyl hydrolase	CW	-2.37	-1.63
AT5G48940	RCH1, LRR-kinase	S	-2.37	-4.91
AT1G63260	TET10		-2.36	-1.14[a]
AT5G48130	NPH3-like BTB-POZ	D/S	-2.35	-4.13
AT5G59790	unknown, DUF966		-2.35	-2.55
AT5G51450	RIN3, ubiquitin ligase	D	-2.34	1.14[a]
AT4G32540	YUC1	A	-2.33	1.15[a]
AT4G22860	unknown		-2.33	1.24[a]
AT5G40630	ubiquitin	D	-2.32	-2.04
AT3G25100	CDC45	CC	-2.31	1.33[a]
AT2G27240	unknown		-2.30	1.40[a]
AT1G70560	TAA1	A	-2.30	-1.19[a]
AT4G10640	IQD16	TF	-2.29	-1.82
AT2G45340	LRR RLK, LRR-IV	S	-2.29	-1.29[a]
AT1G01480	ACS2, ACC synthase	ETH	-2.28	1.06[a]
AT2G28550	TOE1 (AP2)	TF	-2.28	1.24[a]
AT2G44660	Glycosyltransferase	CW	-2.28	1.66[a]
AT3G62060	Pectinacetylesterase	CW	-2.27	1.25[a]
AT3G17840	ORK1, RLK, LRR-III	S	-2.27	1.01[a]
AT2G34700	Extensin	CW	-2.24	1.25[a]
AT3G59200	LRR-F-box gene	D	-2.23	-1.05[a]
AT2G45720	armadillo/beta-catenin repeat	S	-2.23	1.17[a]
AT1G33750	terpene synthase		-2.23	-2.38
AT2G18340	LEA domain		-2.23	-3.23
AT2G25980	jacalin lectin	CW	-2.21	-5.03
AT2G06850	EXGT-A1, hydrolase	CW	-2.20	-1.35[a]
AT4G25240	SKS1, oxidoreductase	CW	-2.20	1.16[a]
AT3G16490	IQD26	TF	-2.20	1.08[a]
AT4G29360	glycosyl hydrolase	CW	-2.19	1.14[a]
AT1G28400	unknown		-2.19	-1.87
AT1G18250	ATLP-1, thaumatin-like		-2.19	1.13[a]
AT3G16190	isochorismatase hydrolase		-2.18	1.21[a]
AT3G15170	CUC1	TF	-2.18	1.63
AT5G35770	SAP	TF	-2.18	-1.01[a]
AT5G22860	serine carboxypeptidase	D/S	-2.17	1.26[a]
AT1G53860	remorin		-2.17	-1.17[a]
AT4G37390	BRU6, GH3-2	A	-2.16	1.13[a]
AT3G24650	ABI3	TF	-2.16	1.08[a]
AT4G03292	nucleic acid binding	TF	-2.16	1.29[a]
AT2G18380	HANL1 (GATA20)	TF	-2.15	-1.13[a]
AT5G10520	RBK1, Rop-binding kinase	S	-2.15	1.41[a]
AT2G47930	AGP26	CW	-2.14	1.11[a]
AT3G17970	atToc64-III		-2.13	1.41[a]
AT4G31150	endonuclease		-2.13	1.44[a]
AT2G17860	pathogenesis-related thaumatin		-2.13	1.19[a]
AT3G15720	glycoside hydrolase	CW	-2.13	-2.28
AT1G23000	heavy-metal-associated domain		-2.12	1.21[a]
AT2G34650	PID	A	-2.11	-1.24[a]
AT4G10630	glutaredoxin		-2.11	1.10[a]
AT5G02070	kinase-related	S	-2.11	1.09[a]
AT5G48500	unknown protein		-2.10	-1.36[a]
AT4G19560	CYCT1	CC	-2.10	1.07[a]
AT1G62420	Unknown, DUF506		-2.09	-1.74
AT1G11130	SUB (RLK)	K/S	-2.09	1.03[a]
AT3G15540	IAA19	TF/A	-2.09	-2.78
AT3G17200	LTR Reverse Trancriptase-Like		-2.09	1.24[a]
AT2G45190	FIL/YAB1	TF	-2.09	1.44[a]
AT4G22010	SKS4, oxidoreductase	CW	-2.08	1.12[a]
AT2G32280	unknown		-2.08	1.26[a]
AT1G63100	SCL28	TF	-2.08	1.08[a]
AT5G28646	WVD2, Microtubule-binding	S	-2.08	-1.74
AT5G50890	lipase		-2.08	-1.02[a]
AT5G43810	ZLL	S	-2.07	-1.09[a]
AT1G14440	AtHB31	TF	-2.07	1.55
AT1G34030	40S ribosomal protein S18		-2.06	1.18[a]
AT2G20515	unknown		-2.06	-4.49
AT5G50915	bHLH137	TF	-2.06	1.42[a]
AT1G28110	SCPL45, carboxypeptidase	CW	-2.05	-2.32
AT4G07825	unknown		-2.05	1.12[a]
AT4G23950	Galactose-binding like	CW	-2.04	1.28[a]
AT4G00390	GeBP-like transc. regulator	TF	-2.04	-1.51[a]
AT4G28100	GPI-anchored protein	CW	-2.04	1.26[a]
AT5G62890	AtNAT6, ascorbate transporter		-2.04	-1.01[a]
AT2G28510	TMO6-LIKE2 (Dof2.1)	TF	-2.03	1.99
AT5G55480	SVL1, phosphoric diester hydrolase		-2.03	1.01[a]
AT5G01910	mannase	CW	-2.02	1.26[a]
AT2G28790	osmotin-like		-2.02	-1.18[a]
AT5G47230	ERF5 (AP2)	TF	-2.02	-1.65
AT1G04670	unknown		-2.01	-1.11[a]
AT1G70510	KNAT2	TF	-2.01	-3.80
AT3G29300	unknown		-2.00	1.04[a]

AT3G16130	ROP-GEF13		-10,4[c]	1,59[a]
AT1G25310	MEE8 (bHLH108)	TF	-9,67[d]	-1,02[a]
AT1G77730	pleckstrin homology domain		-8,13[c]	-1,58[a]
AT1G67520	lectin kinase		-6,17[c]	-2,33[a]
AT1G32770	NAC12	TF	-6,05[c]	-1,02[a]
AT2G24430	NAC38	TF	-4,36[d]	1,01[a]
AT1G70250	thaumatin-like RLK	S	-4,21[c]	2,36[a]
AT1G56030	Ubiquitin ligase	D	-3,80[c]	-2,40[a]
AT5G44460	CML43	S	-3,60[c]	3,98[a]
AT3G49380	IQD15	TF	-3,12[b]	-1,88
AT3G54810	BME3 (GATA8)	TF	-2,79[c]	1,44[a]
AT4G33880	bHLH85	TF	-2,75[c]	-1,50
AT2G31215	bHLH138	TF	-2,44[c]	1,20[a]
AT4G36930	SPT (bHLH24)	TF	-1,73	-1,19[a]
AT2G30590	WRKY21, class IId	TF	-1,72	-2,00
AT2G24570	WRKY17, class IId	TF	-1,69	-1,28[a]
AT4G39410	WRKY13, class IIc	TF	-1,68[a]	-1,22[a]
AT4G00210	LBD31	TF	-1,63	-1,25[a]

Table S2: Expression pattern analysis of putative MP target genes involved in root initiation. Schematic overview of embryo and root expression analysis of 37 putative MP target genes. * Expression in apical embryo region. [a] Expression in L1 layer SAM. [b] No expression in root meristem but expression in differentiation zone of the root. [c] Only lateral root cap and/or columella expression. [d] Weak expression in root meristem and stronger expression in differentiation zone. [e] Very strong expression in vasculature of the root meristem. N.a., not analyzed. Var, variable expression patterns between independently transformed lines of the same construct.

AT number	**Gene name**	**Observed expression pattern in the embryo**								**Expression observed in postembryonic root meristem?**
		Globular stage				**Heart stage**				
		hyp	**VSC**	**GSC**	**ep**	**qc**	**vas**	**gt**	**ep**	
A. Ubiquitous expression in globular stage embryo										
AT1G63260	*TET10*	X	X	X	X	X	X	X	X	n.a.
AT2G30590	*WRKY21*	X	X	X	X	X	X	X	X	X
B. Expression in VSC and GSC of globular stage embryo										
AT1G37140	*MCT1*		X	X						?
AT3G19380	*PUB25*	X	X	X	X	X	X	X	X	X
AT4G36930	*SPT (bHLH24)*		X	X	X	X	X	X	X	X
C. Expression in VSC of globular stage embryo										
AT1G05577	*DUF*		X			X	X			X
AT3G49380	*IQD15*		X			X	X			X
D. Expression in GSC of globular stage embryo										
AT2G42660	*MYB-LIKE*	X		X		X		X		X
AT5G19650	*OFP8*	X		X		X		X		X
AT1G70250	thaumatin-like *RLK*			X				X		X
E. Expression in hypophysis and epidermis										
AT3G54810	*GATA8*	X			X				X	X
AT4G10640	*IQD16*	X	X	X	X	X			X	n.a.
AT3G52450	*PUB22*	X			X	X			X	X
AT2G24570	*WRKY17*	X		X	X	X		X	X	X
F. Expression in apical embryo region										
AT1G14440	*ATHB31*				X*		X*		X*	n.a.
AT2G28510	*Dof2.1*						X*			X
AT1G70510	*KNAT2*								X*[a]	no
AT4G00210	*LBD31*								X*	X
G. Expression in differentiated columella cells of the torpedo stage embryo										
AT4G28720	*YUCCA8*									X[c]
H. Variable embryo expression among independently transformed plant lines										
AT4G33880	*bHLH85*	Variable expression pattern								X
AT5G50915	*bLH137*	Variable expression pattern								?
AT2G31215	*bHLH138*	Variable expression pattern								no
AT3G04070	*NAC47*	Variable expression pattern								X[d]
I. No embryo expression observed										
AT5G44460	*CML43*	No expression								X
AT1G12610	*DDF1*	No expression								no
AT5G47230	*ERF5*	No expression								var
AT2G30130	*LBD12*	No expression								X[e]
AT1G67520	Lectin kinase	No expression								X[c]
AT1G25310	*MEE8 (bHLH108)*	No expression								n.a.
AT1G32770	*NAC12*	No expression								no[b]
AT2G24430	*NAC38*	No expression								no
AT2G18500	*OFP7*	No expression								X[d]
AT1G77730	PH domain	No expression								var
AT3G16130	*ROP-GEF13*	n.a.								no
AT1G56030	Ubiquitin ligase	No expression								no[b]
AT4G39410	*WRKY13*	No expression								no[b]
AT1G80840	*WRKY40*	No expression								n.a.

Table S3: Primers used for cloning.

AT number	Gene name	Forward and reverse primer used for LIC cloning
AT1G14440	*ATHB31*	TAGTTGGAATGGGTTCGAAAACACATAACTTTCGATCCT TTATGGAGTTGGGTTCGAACTTTTTTAATTGGGTCTTCT
AT4G33880	*bHLH85*	TAGTTGGAATGGGTTCGAATTATTTTATCCATGGAACTTG TTATGGAGTTGGGTTCGAATTTTATATGTTTTGTTAACT
AT5G50915	*bLH137*	TAGTTGGAATGGGTTCGAATCTTAGAGTGAAGTAATGTA TTATGGAGTTGGGTTCGAAGAGAGAAGATTAAGGACTTG
AT2G31215	*bHLH138*	TAGTTGGAATGGGTTCGAATTGTTCAGAAGAAGAAGATC TTATGGAGTTGGGTTCGAAGAAGATGGTTCAATACTCTA
AT5G44460	*CML43*	TAGTTGGAATGGGTTCGAAAAACATGTAAATTTCATCGA TTATGGAGTTGGGTTCGAATGTTACTCTTCTTCTTAGTT
AT1G12610	*DDF1*	TAGTTGGAATGGGTTCGAAGTTTTTGAACAAAATAATCTTAAC TTATGGAGTTGGGTTCGAAAACTCAAAGTACCAAAAATTC
AT2G28510	*Dof2.1*	TAGTTGGAATGGGTTCGAAGCAAGCTCGCTCATTTCG TTATGGAGTTGGGTTCGAACCATGTAACAAAGGATCGAA
AT1G05577	*DUF966*	TAGTTGGAATGGGTTCGAACGTTCCGTGGTGAATCAATG TTATGGAGTTGGGTTCGAACTCTCTTTCTTTTTGTTTTGGTCT
AT5G47230	*ERF5*	TAGTTGGAATGGGTTCGAATCTCTGATTTGCTATATCAT TTATGGAGTTGGGTTCGAATGATAAAATTTTCAAAAAGC
AT3G54810	*GATA8*	TAGTTGGAATGGGTTCGAATTTACACTTTCTTAATTATC TTATGGAGTTGGGTTCGAAGTTTTACAATTATTGCAAGT
AT3G49380	*IQD15*	TAGTTGGAATGGGTTCGAAGTTTGCGATGGTAATTCTCT TTATGGAGTTGGGTTCGAACAAGATCGATCAACCTCGTC
AT4G10640	*IQD16*	TAGTTGGAATGGGTTCGAAGATCTACCAAGATCGAACAA TTATGGAGTTGGGTTCGAAATTGAATTAACGTTTTCTAAAAGC
AT1G70510	*KNAT2*	TAGTTGGAATGGGTTCGAATTTAGTATAGTAATCTCAAA TTATGGAGTTGGGTTCGAATAATGTGATCGTAGTGAGAT
AT2G30130	*LBD12*	TAGTTGGAATGGGTTCGAATTTACCATGGTTTTGTCTTA TTATGGAGTTGGGTTCGAACTTCTACTATGATCGTGATT
AT4G00210	*LBD31*	TAGTTGGAATGGGTTCGAATTTCACAAATATTTGAACTT TTATGGAGTTGGGTTCGAACTTTTATGTCAAAGACGTAA
AT1G67520	Lectin kinase	TAGTTGGAATGGGTTCGAAAACAAAGAACAGAACAGAAG TTATGGAGTTGGGTTCGAAAGTTTCAGAAAGATGGATGA
AT1G37140	*MCT1*	TAGTTGGAATGGGTTCGAAGTTTTATGTTTCATGATGAT TTATGGAGTTGGGTTCGAAGGAAGCTAAAGAAACTAGGA
AT1G25310	*MEE8 (bHLH108)*	TAGTTGGAATGGGTTCGAATCTATATGTATTTGATTAAA TTATGGAGTTGGGTTCGAATCTTTTGATAGTTTTAAGTG
AT2G42660	*MYB-LIKE*	TAGTTGGAATGGGTTCGAAAGGAATTGTGAATGAAGTGTC TTATGGAGTTGGGTTCGAAGTCTCTCCGGCGAGAGTGTCTTT
AT1G32770	*NAC12*	TAGTTGGAATGGGTTCGAATCTTCTACATAACGATTTCT TTATGGAGTTGGGTTCGAATAACGAAGATAGCAATATAT
AT2G24430	*NAC38*	TAGTTGGAATGGGTTCGAATGATTATATATTACGTTTAT TTATGGAGTTGGGTTCGAATTTATATGATCTTTAGTTTA
AT3G04070	*NAC47*	TAGTTGGAATGGGTTCGAAAAGAAAACGTTAACTAGATC TTATGGAGTTGGGTTCGAATGTTTAAAGGAATGATATTT
AT2G18500	*OFP7*	TAGTTGGAATGGGTTCGAATATTTTTAATGATTAAACGA TTATGGAGTTGGGTTCGAATTTTTTTTCTTTTCTTGTTT
AT5G19650	*OFP8*	TAGTTGGAATGGGTTCGAAAGATATTTAGTCTCTTGCTT TTATGGAGTTGGGTTCGAAGTTTAGAGAATATCGAGAGA
AT1G77730	PH domain	TAGTTGGAATGGGTTCGAAATTTCTTCAGATTGTACCAT TTATGGAGTTGGGTTCGAATGTTTGAAAACGTTCGAAGA
AT3G52450	*PUB22*	TAGTTGGAATGGGTTCGAACTTGGTCATGATTCTGGTCG TTATGGAGTTGGGTTCGAATTGACCCGCTCGAAAATATGAAAA
AT3G19380	*PUB25*	TAGTTGGAATGGGTTCGAATAAATAGAGACTATTTTCAT TTATGGAGTTGGGTTCGAATAAGAAACTTGAGAAACAGA
AT1G70250	thaumatin-like *RLK*	TAGTTGGAATGGGTTCGAATCTACCGTCTCTAGCCCTGA TTATGGAGTTGGGTTCGAATTTTCTTTCTTGGTAAATGGTAATG
AT3G16130	*ROP-GEF13*	TAGTTGGAATGGGTTCGAATCAATAATCCATTAGATATGG TTATGGAGTTGGGTTCGAATTCCATGATTTTCTTAAATC
AT4G36930	*SPT (bHLH24)*	TAGTTGGAATGGGTTCGAATGCATATGTATTACGGAAAC TTATGGAGTTGGGTTCGAATACACCAACAACAAAAAAAAAGC

AT1G63260	*TET10*	TAGTTGGAATGGGTTCGAAATCTTCACAGTACCGTTTAT TTATGGAGTTGGGTTCGAATTTTTCAAGGTTGTTGCTTT
AT1G56030	Ubiquitin ligase	TAGTTGGAATAGGTTCATGGTGAAAAACTCCTATGTA AGTATGGAGTTGGGTTCTCAACGTCCCAACTCCTCAA
AT4G39410	*WRKY13*	TAGTTGGAATGGGTTCGAACTTCTAAATGGATAATGAAA TTATGGAGTTGGGTTCGAACTCGCAAAAGCTTGACGAAG
AT2G24570	*WRKY17*	TAGTTGGAATAGGTTCATGACCGTTGATATTATGCG AGTATGGAGTTGGGTTCTCAAGCCGAACCAAACACCA
AT2G30590	*WRKY21*	TAGTTGGAATGGGTTCGAACACCAATATCTAGTATTGGA TTATGGAGTTGGGTTCGAACTAAGAACCCTAATTTTTTC
AT1G80840	*WRKY40*	TAGTTGGAATGGGTTCGAAAATTCGAATATCAAATATAA TTATGGAGTTGGGTTCGAAGTAAATATATGTAGGATGAA
AT4G28720	*YUCCA8*	TAGTTGGAATGGGTTCGAATATAATATTTTCTCTAATAT TTATGGAGTTGGGTTCGAATCTTTTTTTATAAGTTTCTT
AT4G37650	*SHR**	TAGTTGGAATGGGTTCGAAGCATACAGGCATGCATAACAACC TTATGGAGTTGGGTTCGAAGACTGACTAGTCTAAAGAGAGTATCCGTTTT
AT2G45160	*SCL27*	TAGTTGGAATGGGTTCGAAGCGTGATCCATGTTATGTCATCGAG TTATGGAGTTGGGTTCGAACGCCTCCTCAACAACACAGAGTAAC
AT1G63100	*SCL28*	TAGTTGGAATGGGTTCGAATTTAGTCTAGTGATTAACTA TTATGGAGTTGGGTTCGAACCTCTACAAAATCTACCTAA
AT3G13840	*SCL29*	TAGTTGGAATGGGTTCGAAGTTCGAGTGCCTTGTCGGATTCTTC TTATGGAGTTGGGTTCGAATGAATAGATGATGAAAAAGGTATAATTTGTGAGTAGG
AT1G07520	*SCL31*	TAGTTGGAATGGGTTCGAACTGTTGATAGTCTCTCGCCAACACG TTATGGAGTTGGGTTCGAACCACACCTGAGTAATTCGATTCCTTCGTCC
AT3G49950	*SCL32*	TAGTTGGAATGGGTTCGAACGAACATGCCCTTATACGACAATTTGAGGCC TTATGGAGTTGGGTTCGAATTGAGTCTGGTTTTAGAGAGAAATGTACG
AT number	**Gene name**	**Forward and reverse primer used for cloning**
AT1G03840	*MGP*	AGGGCCCGGGGTAATATGT AAAGGATCCGAGATGGAGGATG
AT3G25710	*TMO5*	GTTGAACGTCGTGTGGGCTTC AACCGGATCCTTTTTGGTTTTT

Experimental Procedures

Genetic stocks

All plants were grown at 22°C under long day (16 hour) illumination. The *mpB4149* allele in Utrecht background and the *UAS::bdl* line were described in (1). The *mpS319* allele corresponds to SALK_021319. The *Q0990* enhancer trap line was generated by Jim Haseloff (University of Cambridge, UK) and obtained through the Arabidopsis Biological Resource Center (ABRC). The *shr-2* mutant was described in (2) and obtained from dr. Ikram Blilou. The *scr scz* mutant was described in (3) and obtained from dr. Liam Dolan. The pSHR:SHR-YFP and pSCR:YFP lines were described in (4) and obtained from dr. Ikram Blilou. *R2D2* and *DR5v2* reporters were described in (5).

Transgenic constructs

Transcriptional fusions were generated by PCR-amplifying 2 kb fragments upstream of the ATG from genomic DNA using Phusion Flash polymerase (Finnzymes), and introducing them into the pGreenIIKAN:LIC:SV40:3GFP:NOSt (pPLV04) or pGreenIIKAN:LIC:SV40:tdTomato:NOSt (pPLV11) vector using Ligation-Independent Cloning (6). Primers used for amplifying promoter fragments are listed in Table S3. All promoter fusion constructs were transformed into wild-type and *mpB4149* heterozygous plants by simplified floral dipping (6) using the *Agrobacterium* strain GV3101 (pSoup). For the *MGP* and *TMO5* promoter driver constructs, a 2.5 kb fragment upstream of the *MGP* gene and a 2.3 kb fragment upstream of the *TMO5* gene, respectively, was cloned in front of a GAL4VP16 transcriptional activator gene in the pGreen vector carrying a kanamycin-resistance cassette (7).

Microarray experiment

Q0990 plants were crossed to *UAS::bdl* plants to locally inhibit ARF activity in the embryo and to Columbia-0 wild-type plants to serve as a control. After three and six days, embryos from both crosses were separately isolated as described in (8) using a dissecting microscope and fine forceps (Dumont 55 forceps, catalog no. 11295-55, Fine Science Tools). Total RNA was extracted from pooled embryos (300-400 embryos for each biological replicate) following the protocol of RNAqueous-micro kit (Ambion, catalog no. 1927). Each biological replicate contained 300-400 isolated embryos.

The mRNA was subsequently amplified prior to labeling to increase the quantity as described in [S7]. Antisense RNA labeling was performed following the protocol of (9). The mRNA samples representing four biological replicates from experimental and control samples were labeled (two Cy3 and two Cy5) and hybridized to the microarray slides following the protocol described in http://ag.arizona.edu/microarray. The Arabidopsis 70-mer oligo array slides prepared by University of Arizona were used in all the microarray experiments (version ATV 3.7.2; http://ag. arizona.edu/microarray). Hybridized slides were scanned sequentially for Cy3- and Cy5-labeled mRNA targets with a ScanArray 4000 laser scanner at a resolution of 10 μm. The image analysis and signal quantification were performed using the QuantArray program (GSI Lumonics). Limma Software (10) was used to normalize and to determine the modulated genes from microarray data. Microarray data have been deposited to the NCBI Gene Expression Omnibus (Accession number GSE78695).

Microscopy

Differential interference contrast (DIC) microscopy was performed as described previously [S6], using a Leica DMR microscope. Basic Fuchsin staining was performed as described in (11). Microscopy was perfomed either on a Zeiss LSM510 confocal microscope or a Leica SP5/II equipped with HyD detectors. Membranes were counterstained using FM4-64 dye (Invitrogen). Alternatively, cell walls were stained with SCRI Renaissance 2200 (R2200; Renaissance Chemicals, UK). Both stains are visible as red signal in confocal images.

Supplemental References

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