SUPPLEMENTAL TABLES

Table S1. Origin and Nature of Lines

LINE	ORIGIN / NATURE
athb8-11	ABRC (CS6969); (Prigge et al., 2005); WT at the *ER* (AT2G26330) locus
athb8-27	ABRC (CS111153)
SHR::miR165a	Transcriptional fusion of *SHR* (AT4G37650; -2505 to -10; primers: "SHR HindIII F" and "SHR SalI R") to miR165a (AT1G01183; - 138 to +323 relative to the transcriptional start-site; primers: "SalI FWD – MiRNA 165" and "KpnI REV – MiRNA 165")
SHR::mATHB8	(Ohashi-Ito et al., 2013)
SHR::mATHB8:EAR	Translational fusion of SHR::mATHB8 (Ohashi-Ito et al., 2013) (primers: "SalI SHR Promoter FP" and "XhoI mATHB8 RP") to the sequence encoding the EAR portable repressor domain (Hiratsu et al., 2003) (primers: "EAR XhoI + KpnI Forward" and "EAR Reverse")
MP::ATHB8	Transcriptional fusion of *MP* (AT1G19850; -3281 to -1; primers: "MP BamHI Fwd" and "MP KpnI Rev") to the *ATHB8* (AT4G32880) cDNA (GeneBank accession: BT008798; ABRC: U24724;

	+1 to +2502; primers: “ATHB8 cDNA KpnI FWD” and “ATHB8 cDNA SmaI Rev”)
MP::mATHB8	Transcriptional fusion of *MP* (AT1G19850; -3281 to -1; primers: 63 “MP BamHI Fwd” and “MP KpnI Rev”) to the *ATHB8* (AT4G32880) cDNA (GeneBank accession: BT008798; ABRC: U24724; +1 to +2502; primers: “ATHB8 cDNA KpnI FWD” and “ATHB8 cDNA SmaI Rev”; “ATHB8mut165FWD” and “ATHB8mut165REV”)
ATHB8::nCFP	(Sawchuk et al., 2007)
MP::MP:YFP	Translational fusion of *MP* (AT1G19850; -3281 to +3815; primers: “MP Prom SalI Fwd” and “MP KpnI Rev-2”; “MP 3 kb SalI Fwd” and “MP 3 kb XhoI Rev”) to the sequence encoding EYFP (primers: “ECFP AflII F” and “ECFP AflII R”); rescues the root (240/240 seedlings), vein (Figure S1), and inflorescence (160/160 plants) defects of *mp-B4149*
mp-B4149	(Weijers et al., 2005)
RIBO::nCFP	ABRC (CS23898); (Gordon et al., 2007); WT at the *ER* (AT2G26330) locus
ATHB8::nYFP	(Sawchuk et al., 2007)
mp-U55	ABRC (CS8147); (Mayer et al., 1993; Donner et al., 2009)

mp-11	(Odat et al., 2014)
MP::MP	*MP* (AT1G19850; -3281 to +3830; primers: "MP Prom SalI Fwd" and "MP KpnI Rev-2"; "MP 3KB SalI Fwd" and "MP 3kb XhoI Rev"); rescues the root (169/176 seedlings), vein (Figure S1), and inflorescence (6/6 plants) defects of *mp-B4149*
bdl	(Hamann et al., 1999); introgressed into Col-0
MP::VP16:bdlΔI	Transcriptional fusion of *MP* (AT1G19850; -3281 to -1; primers: "MP BamHI Fwd" and "MP KpnI Rev-1") to a translational fusion of the sequence encoding the activation domain of the *Herpes simplex* virus protein 16 (VP16) (Sadowski et al., 1988) (primers: "VP16 NcoIF2" and "VP16 PstIR") to a 5'- terminally deleted *bdl* (Hamann et al., 2002) (+94 to +1229; primers: "BDL PstIF" and "BDL BamHIR"; "BDL mut F1", "BDL mut F2", "BDL mut F3", "BDL mut F4", "BDL PstIF", and "BDL MfeI mut R"; "BDLd1 PstI F" and "BDL BAMHI R")
iaa12-1	ABRC (CS25213); (Overvoorde et al., 2005)
tpl-1	ABRC (CS65909); (Long et al., 2002)
MP::MPΔPB1:GR	Translational fusion of *MP* (AT1G19850; -3427 to +2388; primers: "MP SalI Forward – Primer # 2" and "MP EcoRI Reverse") to the sequence encoding a fragment of the rat glucocorticoid receptor (GR) (Aoyama and Chua, 1997)

	(primers: “SpeI GR Forward” and “SacII + KpnI (Internal) GR Reverse”)
ATHB8::nQFP	Transcriptional fusion of *ATHB8* (AT4G32880; -2070 to -1; primers: “SalI 2KB ATHB8 Promoter Forward” and “ApaI 2KB ATHB8 Promoter Reverse”) to the sequence encoding 2xmTQ2-N7 (primers: “ApaI 2xmTurqoise Forward” and “KpnI 2xmTFP Reverse”)
R2D2	(Liao et al., 2015)
[TGTCTG]::nYFP	(Donner et al., 2009)
[TAGCTG]::nYFP	(Donner et al., 2009)
[TGTCAG]::nYFP	Transcriptional fusion of *ATHB8* (AT4G32880; -953 to -1; primers: “1NagARE” and “Athb8 R-5”) to the sequence encoding HTA6:EYFP (Zhang et al., 2005)
[TGTCTG]::nYFP	Transcriptional fusion of *ATHB8* (AT4G32880; -953 to -1; primers: “1NcARE” and “Athb8 R-5”) to the sequence encoding HTA6:EYFP (Zhang et al., 2005)

SUPPLEMENTAL REFERENCES

Aoyama T, Chua NH. 1997. A glucocorticoid-mediated transcriptional induction system in transgenic plants. *Plant J* **11**: 605–612.

Donner TJ, Sherr I, Scarpella E. 2009. Regulation of preprocambial cell state acquisition by auxin signaling in Arabidopsis leaves. *Development* **136**: 3235–3246.

Gordon SP, Heisler MG, Reddy GV, Ohno C, Das P, Meyerowitz EM. 2007. Pattern formation during de novo assembly of the Arabidopsis shoot meristem. *Development* **134**: 3539–3548.

Hamann T, Mayer U, Jurgens G. 1999. The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the Arabidopsis embryo. *Development* **126**: 1387–1395.

Hamann T, Benkova E, Baurle I, Kientz M, Jurgens G. 2002. The Arabidopsis BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes & Development* **16**: 1610–1615.

Hiratsu K, Matsui K, Koyama T, Ohme-Takagi M. 2003. Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in Arabidopsis. *Plant J* **34**: 733–739.

Liao CY, Smet W, Brunoud G, Yoshida S, Vernoux T, Weijers D. 2015. Reporters for sensitive and quantitative measurement of auxin response. *Nat Methods* **12**: 207–10, 2 p following 210.

Long JA, Woody S, Poethig S, Meyerowitz EM, Barton MK. 2002. Transformation of shoots into roots in Arabidopsis embryos mutant at the TOPLESS locus. *Development* **129**: 2797–2806.

Mayer U, Buttner G, Jurgens G. 1993. Apical-basal pattern formation in the Arabidopsis embryo: studies on the role of the gnom gene. *Development* **117**: 149–162.

Odat O, Gardiner J, Sawchuk MG, Verna C, Donner TJ, Scarpella E. 2014. Characterization of an allelic series in the MONOPTEROS gene of Arabidopsis. *Genesis* **52**: 127–133.

Ohashi-Ito K, Oguchi M, Kojima M, Sakakibara H, Fukuda H. 2013. Auxin-associated initiation of vascular cell differentiation by LONESOME HIGHWAY. *Development* **140**: 765–769.

Overvoorde PJ, Okushima Y, Alonso JM, Chan A, Chang C, Ecker JR, Hughes B, Liu A, Onodera C, Quach H, Smith A, Yu G, Theologis A. 2005. Functional genomic analysis of the AUXIN/INDOLE-3-ACETIC ACID gene family members in Arabidopsis thaliana. *Plant Cell* **17**: 3282–3300.

Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE. 2005. Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development. *Plant Cell* **17**: 61–76.

Sadowski I, Ma J, Triezenberg S, Ptashne M. 1988. GAL4-VP16 is an unusually potent transcriptional activator. *Nature* **335**: 563–564.

Sawchuk MG, Head P, Donner TJ, Scarpella E. 2007. Time-lapse imaging of Arabidopsis leaf development shows dynamic patterns of procambium formation. *New Phytol* **176**: 560–571.

Weijers D, Benkova E, Jager KE, Schlereth A, Hamann T, Kientz M, Wilmoth JC, Reed JW, Jurgens G. 2005. Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators. *Embo J* **24**: 1874–1885.

Zhang C, Gong FC, Lambert GM, Galbraith DW. 2005. Cell type-specific characterization of nuclear DNA contents within complex tissues and organs. *Plant Methods* **1**: 7.

Table S2. Genotyping Strategies

LINE	STRATEGY
athb8-11	ATHB8: "Athb8 0.5" and "athb8attB2R"; *athb8-11*: "athb8 -5944" and "PD991- RB
athb8-27	ATHB8: "athb8-27 RP" and "athb8-27 LP"; *athb8-27*: "athb8-27 RP" and "Spm32"
mp-B4149	"MP 1498-s" and "MP2082-AS"; *Mse*I
mp-U55	"MP Seq 2061" and "U55 Geno Rev"; *Sml*I
mp-11	*MP*: "Sail_1265_F06LP" and "Sail_1265_F06RP"; *mp-11*: "LB3" and "Sail_1265_F06RP"
bdl	"bdl geno F" and "bdl geno R"; *Hae*III
iaa12-1	*IAA12*: "SALK_138684 LP" and "SALK_138684 RP"; *iaa12-1*: "LBb1.3" and "SALK_138684 RP"
tpl-1	"tpl Caps Genotyping Forward" and "tpl Caps Genotyping Reverse"; *Nco*I

Table S3. Oligonucleotide Sequences

NAME	SEQUENCE (5' TO 3')
SHR HindIII F	GAGAAGCTTGACAAAGAAGCAGAGCGTGG
SHR SalI R	TGGGTCGACTTAATGAATAAGAAAATGAATAGAAGAAAGGG
SalI FWD – MiRNA 165	ATTGTCGACCCACTCATCATTCCCTCATC
KpnI REV – MiRNA 165	AGCGGTACCCTTATAGAAAATACTTCGTTAGCTTG
SalI SHR Promoter FP	GGGGTCGACACATAAACCAGTAGACAT
XhoI mATHB8 RP	GGGCTCGAGTATAAAAGACCAGTTGAGG
EAR XhoI + KpnI Forward	TCGAGCTAGATCTGGATCTAGAACTCCGTTTGGGTTTCGCTTAAGGTAC
EAR Reverse	CTTAAGCGAAACCCAAACGGAGTTCTAGATCCAGATCATGC
MP BamHI Fwd	AAGGGATCCTCCGGGTTAATCAGTATTATTAC
MP KpnI Rev	ACAGGTACCACAGAGAGATTTTTCAATGTTCTG
ATHB8 cDNA KpnI FWD	GTCGGTACCATGGGAGGAGGAAGCAATAATAG
ATHB8 cDNA SmaI Rev	ATGCCCGGGATCATATAAAAGACCAGTTGAGG
ATHB8mut165FWD	ATAGGAATCGTTGCTATTTCTC

ATHB8mut165REV	GGAATCTGGTCCAGGCTTCATC
MP Prom SalI Fwd	CCCGTCGACGTATATATAAACAATACCACCTTATAAC
MP KpnI Rev-2	CATGGTACCTGCAGAATTAGCATACCACAC
MP 3 kb SalI Fwd	TCTGTCGACTCCGGGTTAATCAGTATTATTAC
MP 3 kb XhoI Rev	ATTCTCGAGTTAAGAGTTAAGACCACCTCC
ECFP AflII F	TTACTTAAGGTGAGCAAGGGCGACGAGC
ECFP AflII R	AGACTTAAGATTGTACAGCTCGTCCATGCC
VP16 NcoIF2	TTACCATGGCCCCCCCGACCGATGTC
VP16 PstIR	TTTCTGCAGCCCCACCGTACTCGTCAATTC
BDL PstIF	ATACTGCAGCTCGTGGTGTGTCAGAATTGGAC
BDL BamHIR	TACGGATCCACTAAACTGGGTTGTTTCTTTGTC
BDL mut F1	AATCTTCCGGCGGAGAGTGTTAGAGAATTGGG
BDL mut F2	GTGGGTAAAAGTAATCTTCCGGCGGAGAGTG
BDL mut F3	GTGTCAGAATTGGAGGTGGGTAAAAGTAATCTTCCG
BDL mut F4	CGTGGTGTGTCAGAATTGGAGGTGGGGAAGAGTAATC
BDL MfeI mut R	TAACAATTGGTGACCATCCTACCACTTGAC
BDLd1 PstI F	AAACTGCAGCGTGGAAAGAGCGTGGG

MP SalI Forward – Primer # 2	GGGGTCGACCGGATTCGTGATCTTCGTATCCCAT
MP EcoRI Reverse	ATTGAATTCGGTTCGGACGCGGGGTGTCGCAATT
SpeI GR Forward	GGGACTAGTGGAGAAGCTCGAAAAACAAAG
SacII + KpnI (Internal) GR Reverse	AATCCGCGGGGTACCTCATTTTTGATGAAACAGAAG
SalI 2KB ATHB8 Promoter Forward	CGCGTCGACCATTATAAATATCACGACTGTA
ApaI 2KB ATHB8 Promoter Reverse	ATTGGGCCCCTTTGATCCTCTCCGATCTCT
ApaI 2xmTurqoise Forward	ATTGGGCCCATGGTGAGCAAGGGCGAGGA
KpnI 2xmTFP Reverse	CGAGGTACCTCACTCTTCTTCTTGATCAGCTTCTG
1NagARE	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGTTGT CTCGTATTAAGGG
Athb8 R-5	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTGAT CCTCTCCGATCTCTC
1NcARE	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGTTAC CTGGTATTAAGGG
athb8-27 FP	TGTGAAGAATGGATCCACCTC
athb8-27 RP	AGTGGTCAACACCACTTGACC
Spm32	TACGAATAAGAGCGTCCATTTTAGAGTG

Athb8 0.5	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTTTG CTTCCAGAGACCAGCG
athb8attB2R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTGAT CCTCTCCGATCTCTC
athb8 -5944	GGTTTGGCATAAAAGTGCGG
PD991- RB	AAAACCTGGCGTTACCCAACT
MP 1498-s	CTCTCAGCGGATAGTATGCACATCGG
MP2082-AS	ATGGATGGAGCTGACGTTTGAGTTC
MP Seq 2061	CATAATGTTACTCTTCATGTACGCC
U55 Geno Rev	GTGCTGTTTGTTGGCGATTGG
Sail_1265_F06LP	GCTTCATCTCTTCAAGCAAGG
Sail_1265_F06RP	TCCCAAAGTCTCACCACTCAC
LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC
bdl geno F	GCTCAAATCTTGTGATGTGAGTG
bdl geno R	AGTCCACTAGCTTCTGAGGTTCCC
SALK_138684 LP	GTGGGGAAGAGTAATCTTCCG
SALK_138684 RP	CTTCTGCTCTTGACGTCTTGG
LBb1.3	ATTTTGCCGATTTCGGAAC

tpl Caps Genotyping Forward	GCCCTGAAAATGACATCGGT
MP PrimeTime Probe	/56-FAM/CAGACTCAC/ZEN/ AGGCCTTCTCTCGCCA/3IABKFQ/
MP PrimeTime Primer 2	TGTACCAGTGCCTCCAGAATTATC
MP PrimeTime Primer 1	TCCAGTCGCAGATCACATCAG
ACT2 PrimeTime Probe	/56-FAM/ACAGCACTT/ZEN/ GCCCAAGAGCATGA/3IABKFQ/
ACT2 PrimeTime Primer 2	TACTTCCTTTCAGGTGGTGCA
ACT2 PrimeTime Primer 1	GCTGACCGTATGAGCAAAGAAAT

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. MP::MP:YFP and MP::MP Functionalities in Vein Network Formation

Dark-field illumination of cleared first leaves 14 DAG. Top right: genotype. Scale bars: 0.5 mm.

Figure S2. ATHB8 *Expression Domains and MP and* RIBO *Expression Levels*

First leaves 4 DAG. Confocal laser scanning microscopy. Top right: reporter. Dashed green outline: second loop nuclei expressing ATHB8::nCFP (A,B) or ATHB8::nYFP (D,E). (B,E) Look-up table — ramp in C — visualizes expression levels. Scale bars (shown, for simplicity, only in A and D): 5 μm.

Figure S3. ATHB8 *Expression Domains and* RIBO *Expression Levels*

(A–F) First leaves 4 DAG. (A) Schematic of 4-DAG leaf — imaged in B–E — illustrating onset of *ATHB8* expression (red) — imaged in B — associated with second loop formation (Donner et al., 2009; Gardiner et al., 2011; Donner and Scarpella, 2013); increasingly darker gray: progressively older *ATHB8* expression domains. (B–E) Confocal laser scanning microscopy. (B) ATHB8::nYFP expression. (C) RIBO::nCFP expression. (D) Autofluorescence. (E) Overlay of images in B–D; red: ATHB8::nYFP expression; green: RIBO::nCFP expression; blue: autofluorescence. (F) RIBO::nCFP expression levels (mean ± SE) in nuclei at positions -2, -1, 1, and 2 — as defined in legend to Figure 3 — relative to RIBO::nCFP expression levels in nuclei at position 0 — as defined in legend to Figure 3 — during second loop formation. Difference between RIBO::nCFP expression levels in nuclei at position -2 or -1 and RIBO::nCFP expression levels in nuclei at position 0 was significant at $P<0.001$ (***) by One-Way ANOVA and Tukey's Pairwise test. Sample

population sizes: 27 leaves; position -2, 42 nuclei; position -1, 64 nuclei; position 0, 69 nuclei; position 1, 50 nuclei; position 2, 28 nuclei. Scale bars (shown, for simplicity, only in column 2): 5 μm.

Figure S4. mp-11 *and MP::MP Effects on* MP *Expression*

MP transcript levels in *mp-11* and MP::MP seedlings relative to *MP* transcript levels in WT (mean ± SE of three technical replicates for each of three biological replicates); seedlings 4 DAG; RT-qPCR. Difference between *mp-11* and WT, and between MP::MP and WT was significant at $P<0.001$ (***) by *F*-test and *t*-test with Bonferroni correction.

Figure S5. Summary and Interpretation.

A three-gene incoherent type-I feedforward loop (Mangan and Alon, 2003) activates *ATHB8* expression in narrow preprocambial stripes and leads to vein network formation. *MP* receives the auxin input and activates expression of intermediate-loop *AUX/IAA* genes like *BDL/IAA12*. Both *MP* and *AUX/IAA* genes jointly regulate expression of the stripe gene *ATHB8*, which converts the auxin input into vein-network formation output. Arrows indicate positive effects; blunt-ended lines indicate negative effects.

SUPPLEMENTAL FIGURES

Figure S1

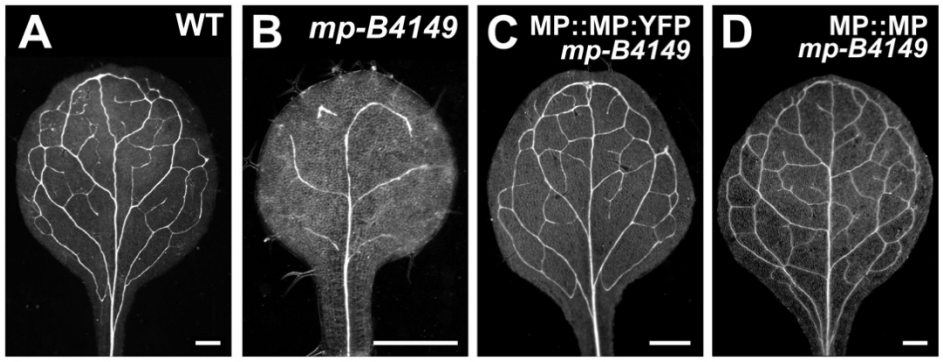

Figure S2

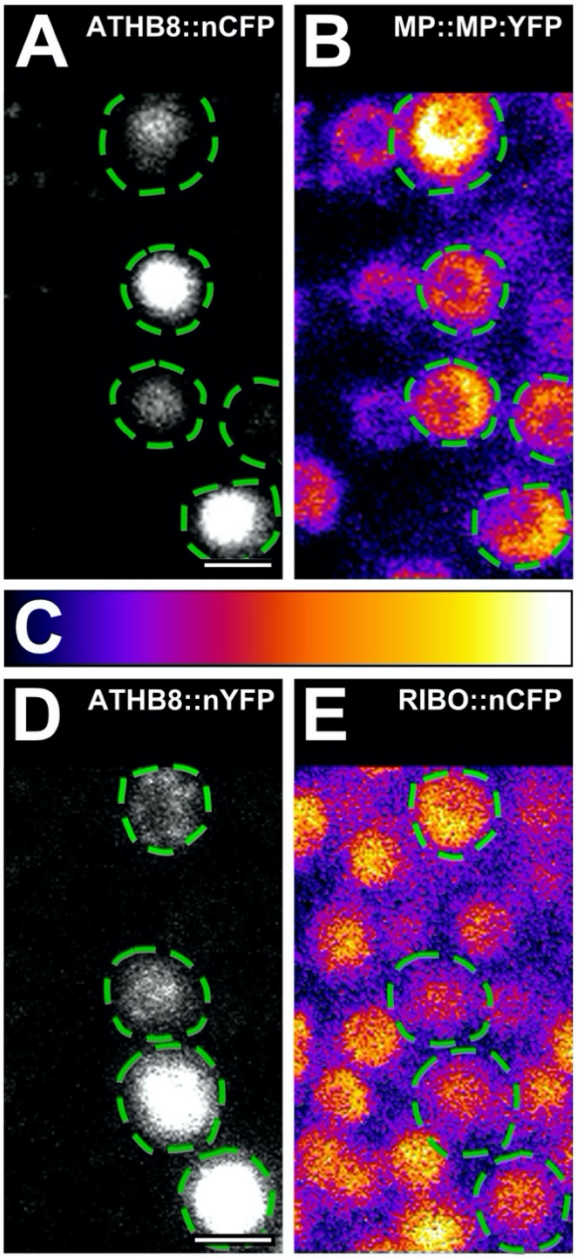

Figure S3

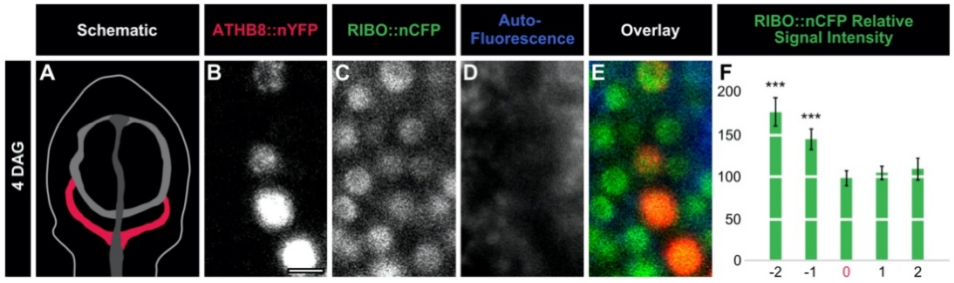
Schematic
ATHB8::nYFP
RIBO::nCFP
Auto-Fluorescence
Overlay
RIBO::nCFP Relative Signal Intensity
4 DAG
A
B
C
D
E
F
200
150
100
50
0
***
***
-2
-1
0
1
2

Figure S4

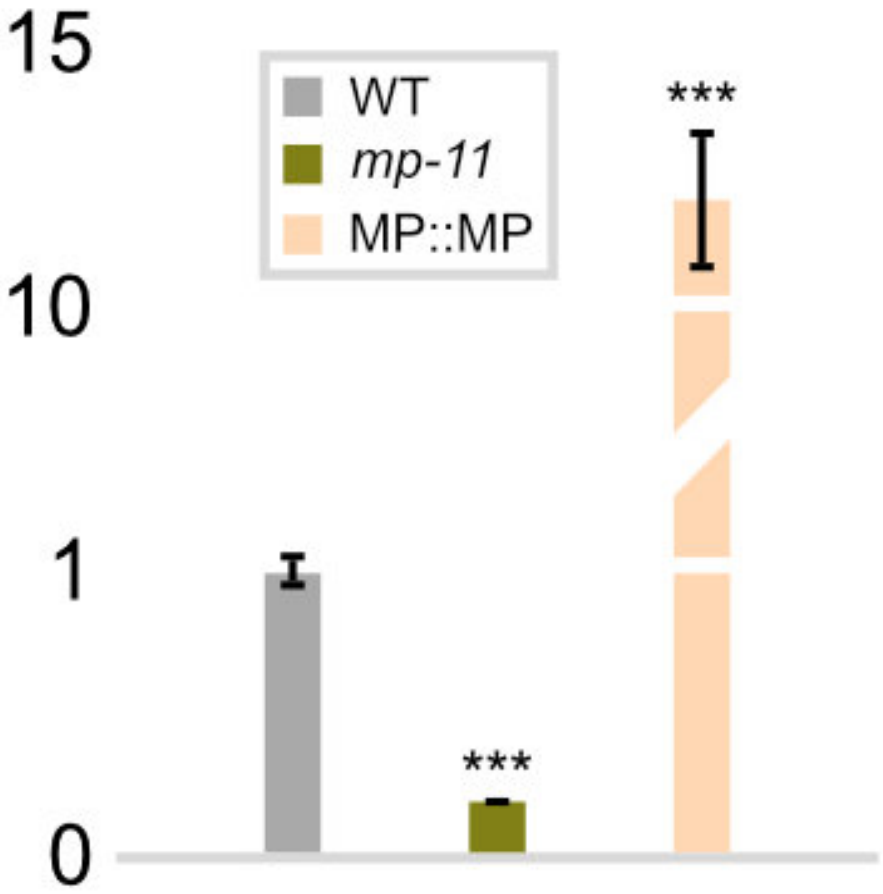

Figure S5