Supporting Information

Zhang et al. 10.1073/pnas.1400542111

SI Materials and Methods

Materials, Growth Conditions, and Treatments. All plants are in *Columbia* background. The *pMP::MP-GFP* transgenic line is a generous gift from Dolf Weijers (Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands) (1). For phenotype and some quantitative RT-PCR (qRT-PCR) analyses, seeds were grown on Murashige and Skoog (MS) agar plate (with 2% sucrose) (2) with ethanol or various concentrations of 2,4-D or 5 μM dexamethasone (DEX). To construct *35S::iaaM-HA*, the *iaaM* gene fused with 3× HA epitope was amplified into pHB vector (3). *35S::iaaL-HA* transgenic plants and the quadruple mutant *tir1 afb1 afb2 afb3* were as described (4). The *taa1 tar1 tar2* (CS16419) mutant was obtained from the Arabidopsis Biological Resource Center at Ohio State University. *MPΔIII/IV* (domain III/IV is deleted) (5) was amplified into pCambia1300 with 35S promoter driving the fusion with the hormone-binding domain of the rat glucocorticoid receptor (5). For *pXVE::bdl* construction, a gain-of-function form of *bdl* was generated and inserted into the pER10 vector (6). For β-estradiol treatment, *pXVE::bdl* seeds were grown on MS agar plate with 0.1% DMSO (as control) or 10 nM β-estradiol, respectively. The weak *mp* allele mp^{S319} (1) corresponds to SALK_021319. For time-course assay, the fusion gene *LUC-NLS-sGFP* was constructed to be driven by 511 bp upstream of *TMM* gene in pCambia1300 with nos terminator, and the resulting construct was introduced into *arf5-1*/+. The WT-like and *arf5-1*/− siblings segregated from *pTMM::LUC-NLS-sGFP;arf5-1*/+ were used for analysis. For transient expression analysis, 2-d-old seedlings were transiently treated with 150 nM 2,4-D for indicated time. To construct *pSTOMAGEN::LUC*, a 2-kb fragment directly upstream of the ATG of the *STOMAGEN* gene fused with *firefly* luciferase gene was cloned into pCambia1300, and the resulting construct was introduced into *arf5-1*/+ to generate *pSTOMAGEN::LUC;arf5-1*/+ transgenic lines. For constructing *pSTOMAGEN::GUS*, a 2-kb fragment directly upstream of the ATG of the *STOMAGEN* gene was amplified into pEarlyGate 164 (7), which carries a *β-glucuronidase* gene, and the resulting vector was introduced into *arf5-1*/+ to generate *pSTOMAGEN::GUS;arf5-1*/+ transgenic lines. The *pMP::MP-MYC-HA* fusion was generated by placing 6×MYC–3×HA epitope at the 3′ end of genomic *MP* fragment in pCambia1300 with a nos terminator. The construct was introduced into *arf5-1*/+ and complements the rootless mutant phenotype. For all analyses, seedlings were grown on MS medium with 2% sucrose under continuous white light of ∼90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 24 °C. For *STOMAGEN-RNAi* construction, miR319 backbone was used to construct artificial miRNA targeting *STOMAGEN* (8, 9) and the resulting construct was introduced into *arf5-1*/+. The WT-like and *arf5-1*/− siblings segregated from *STOMAGEN-RNAi;arf5-1*/+ transgenic lines were used for analysis. For *pXVE::MP-Myc* and *pXVE::MP* construction, PCR-amplified fragments encoding MP-Myc and MP were inserted into the pER10 vector, respectively (6). For β-estradiol treatment, transgenic *pXVE::MP-Myc* and *pXVE::MP* seedlings were treated with DMSO or 10 μM β-estradiol overnight, respectively.

Stomatal Phenotype Analyses. Cotyledons of 8-d-postgermination seedlings were transiently preserved in 5% (wt/vol) NaOH solution at 100 °C for ∼10 s, washed with distilled water, and placed in the clear solution (glycerol/chloral hydrate/water, 1:8:1, vol/wt/vol) overnight or for a few days. Two images at 200× magnification ($0.2\ mm^2$) were captured per cotyledon using Leica DM2500 microscope with Nomarski optics.

RNA Isolation and Quantitative RT-PCR. Total RNAs were isolated using a RNAprep pure Plant kit (TIANGEN). cDNA was reverse-transcribed using iScript cDNA Synthesis kit (Bio-Rad). SYBR Premix Ex Taq II (TaKaRa) was used for qPCR on a CFX96 Real-Time System (Bio-Rad). *STOMAGEN*-specific signal was normalized relative to *ACTIN2* expression.

GUS Staining. GUS staining was performed as previously described (8) with minor modifications. Briefly, the plants were fixed with 90% acetone at 4 °C for at least 7 h, then washed with 64 mM Na_2HPO_4, 36 mM KH_2PO_4, and finally stained in wash solution with 10 mM EDTA, 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 0.1% (vol/vol) Triton-100, 10% methanol, and 1 $mg \cdot mL^{-1}$ X-Gluc (DMF) at 37 °C for 30 min to 1 h.

ChIP Assay. ChIP was performed with 2 g of 7-day postgermination (dpg) seedlings (dark-adapted overnight at 6 dpg) of *pMP::MP-MYC-HA* and WT according to the methods described previously (10) with minor modifications. Briefly, seedlings were harvested in dim green safe light and fixed in fixation buffer [0.4 M sucrose, 10 mM Tris·HCl (pH 8.0), 1% formaldehyde, 5 mM β-mercaptoethanol] by vacuum infiltration at 4 °C. Glycine was added to a final concentration of 0.125 M to stop the reaction under vacuum. Then seedlings were rinsed with water, ground into fine powder in liquid nitrogen, and homogenized in extraction buffer I [0.4 M sucrose, 10 mM Tris·HCl (pH 8.0)], filtered through four layers of Miracloth and centrifuged at 2,880 × *g* for 20 min at 4 °C. Pellet was then resuspended in extraction buffer II [0.25 M sucrose, 10 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 1% Triton-X 100, 5 mM β-ME, 1 mM Pefabloc (Roche Diagnostics), Complete Protease Inhibitor Cocktail (Roche Diagnostics)], centrifuged at 13,400 × *g* for 10 min, resuspended in nuclei lysis buffer [50 mM Tris·HCl (pH 8.0), 10 mM EDTA, 1% SDS, 1 mM Pefabloc, Complete Protease Inhibitor Cocktail]. Chromatin extracts were sonicated to ∼300- to 1,000-bp fragments with a sonicator (VCX 750; Sonics) and centrifuged at 12,000 rpm for 10 min. Soluble chromatin extracts were diluted in 10× ChIP dilution buffer [1.1% Triton-X 100, 1.2 mM EDTA, 16.7 mM Tris·HCl (pH 8.0), 167 mM NaCl, 1 mM Pefabloc, complete protease inhibitor cocktail], incubated with anti-HA antibody (1:2,000, sc-7392; Santa Cruz) for 3 h, then immunoprecipitated with Dynabeads Protein G (10 μL; Life Technologies) saturated previously with salmon sperm DNA and BSA. Immunocomplexes were then washed with four different buffers: low-salt buffer [150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl (pH 8.0)] once, high-salt buffer [500 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl (pH 8.0)] twice, LiCl wash buffer [0.25 M LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 2 mM EDTA, 20 mM Tris·HCl (pH 8.0)] twice, and TE [1 mM EDTA, 10 mM Tris·HCl (pH 8.0)] once on ice each for 10 min. The bound chromatin fragments were eluted with elution buffer (1% SDS, 0.1 M $NaHCO_3$) and reversed by incubating at 65 °C overnight. The mixture was treated with Proteinase-K to remove proteins. The genomic DNA was purified by phenol/chloroform/isoamyl alcohol (25:24:1) and ethanol precipitation. The resulting DNA was used for qPCR. IP/input was calculated by comparing the C_t values between immunoprecipitate and input. The NC1 fragment in the *STOMAGEN*

coding region and the NC2 fragment in the chromatin adjacent to 3′ UTR region of *STOMAGEN* gene were used as controls.

Recombinant Protein Purification and EMSA. For cloning MBP–MP and His-MP-DBD (residues 120–274), fragments encoding MP and MP-DBD were introduced into pMAL-c2X (New England BioLabs) and pET32a, respectively. MBP-MP and MBP were expressed in *Escherichia coli* (strain BL21), purified with amylose resin (NEB), and ultrafiltrated into TBS solution plus 10% glycerol. His-MP-DBD was expressed in *E. coli* (strain BL21), purified with Ni-NTA resin and ultrafiltrated into TBS solution plus 10% glycerol.

For the EMSA assay, a kit was used with 200 ng of MBP or MBP-MP and 20 fmol biotin-labeled probes, according to the manufacturer's instructions (Thermo Scientific).

DNA–Protein Pull-Down Assay. DNA–protein pull-down assay was performed as described previously (11, 12) with modifications. Briefly, 10 pmol biotin-labeled probes were first incubated with 10 μL streptavidin magnetic beads (Thermo Scientific) for 2 h in a buffer [10 mM Tris·HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 2.5% glycerol, 0.05% Nonidet P-40], then the free probes were washed out with the buffer. The probes-bound beads were incubated with 200 ng MBP or MBP-MP proteins unless otherwise stated in 100 μL of the same buffer supplemented with 5 μg fragmented salmon sperm DNA and 10 μg BSA for 1 h, then washed three times with 1 mL buffer for each time. The precipitates were eluted in SDS loading buffer and subjected to Western blot analysis with anti-MBP monoclonal antibody (NEB). For competition, 100 ng MBP or MBP-MP were preincubated with 100 pmol unlabeled probes in 100 μL of buffer supplemented with 5 μg fragmented salmon sperm DNA and 10 μg BSA for 1 h, then the mixture was incubated with 10 pmol biotin-labeled probes-bound streptavidin magnetic beads (10 μL) for 1 h. After three washes with 1 mL buffer for each time, the precipitates were eluted in SDS loading buffer and subjected to Western blot analysis with anti-MBP monoclonal antibody (NEB).

Luciferase and Dual-Luciferase Assays. For luciferase activity assay in *Arabidopsis*, seedlings were harvested and homogenized in 100 μL lysis buffer (Promega), centrifuged, and then 10 μL supernatant was incubated with 50 μL substrate solution for 10 s and the luciferase activity was recorded for 10 s by GloMax 20/20 Luminometer (Promega). Simultaneously, 3 μL supernatant was used for Bradford assay to determine protein amount. The ratio of luciferase activity to total protein amount was calculated and presented.

For dual-LUC assays, 2-kb or 1-kb fragments directly upstream of the ATG of the *STOMAGEN* or *ARR15* genes were cloned into pGreen0800-LUC, which carries both *renilla* (REN) driven by 35S promoter and *firefly luciferases* (*LUC*) genes (13). Deleted and mutated *STOMAGEN* promoter fragments were directly introduced into pGreen0800-LUC except that the fragment with deletion of the −500-bp region was first fused with minimal 35S (m35S), and mutation fragments were generated by site-directed mutagenesis in pBluescript SK+ vector. Effector constructs were generated by introducing CFP and MP-CFP into pKYL71, respectively. *Agrobacteria* (GV3101) harboring effector constructs (including *35S-CFP*, *35S-MP-CFP*) or reporter constructs were adjusted to OD_{600} = 0.6 in MS medium with 200 μM acetosyringone and 10 mM MES (pH 5.6), left at room temperature for 3 h, and a mixture of *Agrobacteria* harboring effector and reporter (MS medium/effector/reporter = 2:2:1, vol/vol/vol) was introduced into 3- to 4-wk-old tobacco (*Nicotiana benthamiana*) leaves by infiltration. After 40–48 h, leaves were collected for the dual-LUC assay.

Anti-MP Antibody Generation. The recombinant MP-specific fragment (residues 526–775) in middle region domain was used to immunize rabbit to generate anti-MP polyclonal antibody.

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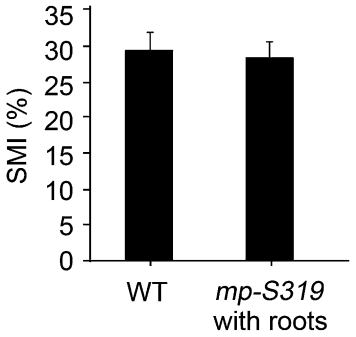

Fig. S1. There are no significant differences between the stomata and meristemoids indexes (SMIs) of WT and that of *mp-S319* homozygote with roots. The SMIs obtained from 8-d-old seedlings of WT and *mp-S319* homozygotes with roots segregated from *mp-S319* heterozygote. Data are means ± SDs (n = 10).

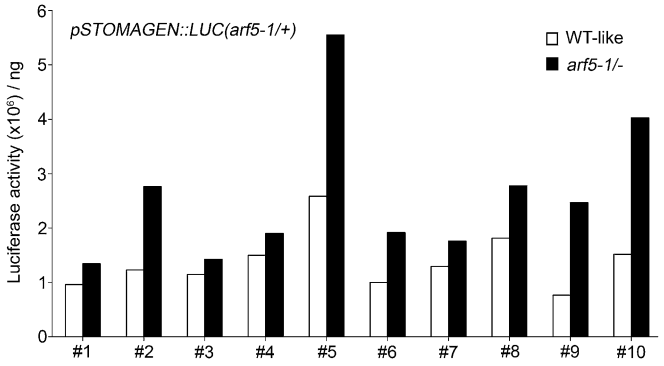

Fig. S2. Luciferase activities are higher in *arf5-1* than in WT-like progenies of *pSTOMAGEN::LUC;arf5-1*/+ transgenic seedlings. Ten independent *pSTOMAGEN::LUC;arf5-1*/+ lines were randomly selected. WT-like and *arf5-1*/– (rootless) seedlings were harvested from individual lines and subjected to analyses, respectively. Luciferase activities relative to total protein amount were calculated and presented.

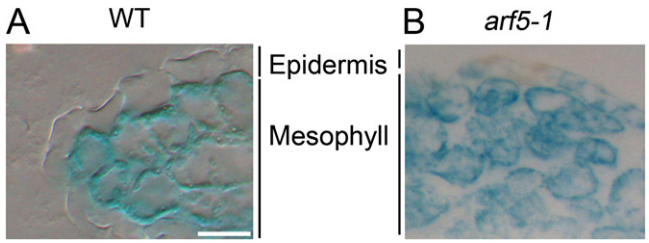

Fig. S3. GUS staining of *pSTOMAGEN::GUS* cotyledons. (*A* and *B*) Cross-section of the cotyledons of 7-dpg *pSTOMAGEN::GUS* seedlings show GUS activity in WT mesophyll (*A*) and in mesophyll and epidermis of *arf5-1*. (Scale bar: 20 μm.)

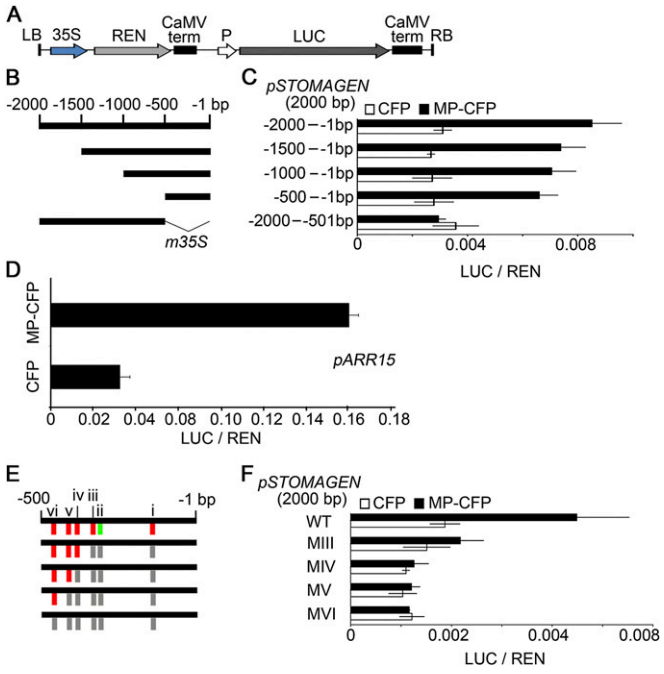

Fig. S4. Auxin response elements (AuxREs) within the −500-bp region mediate MP regulation of *STOMAGEN* expression in tobacco. (*A*) The construct for dual-LUC assay. P indicates the site for promoter insertion. LUC and REN represent *firefly* and *renilla* luciferase, respectively. (*B*, *C*, *E*, and *F*) Dual-LUC assay showing MP regulation of *STOMAGEN* promoter in tobacco leaves. *m35S*, minimal *35S* promoter. (*E*, *i–vi*) AuxREs at different positions. The gray boxes denote mutated AuxREs (GCCA or GAGCCA). WT and MIII-MVI (*F*) indicate wild-type promoter and promoters with 3–6 mutated AuxREs corresponding to *E*, respectively. (*D*) Dual-LUC assay showing MP regulation of *ARR15* promoter in tobacco leaves. Data are means ± SDs ($n = 4$, *C*) or ($n = 3$, *D* and *F*).

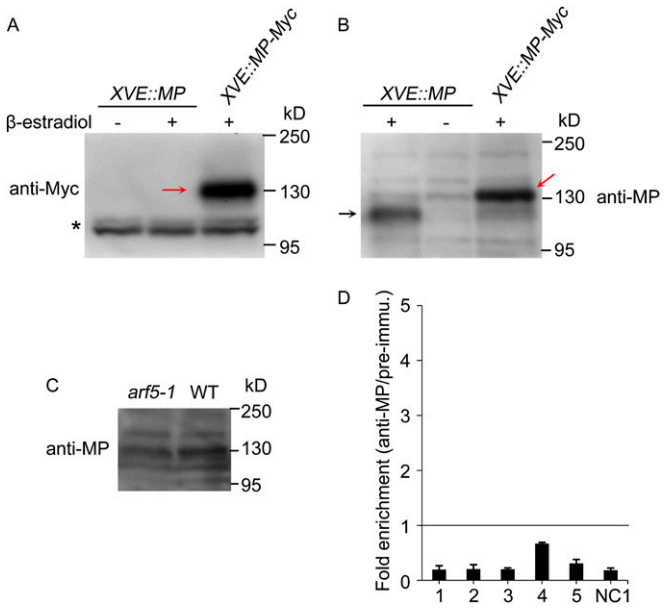

Fig. S5. Western blot and ChIP assays with anti-MP antibody in *Arabidopsis*. (*A*) Western blot with anti-Myc antibody. MP-Myc protein is expressed on β-estradiol induction in *pXVE::MP-Myc* seedlings. (*B* and *C*) Western blot with anti-MP antibody. Anti-MP antibody recognizes the induced MP and MP-Myc proteins but not the endogenous MP protein (*B*). No specific bands were detected by anti-MP antibody when WT and *arf5-1* samples were compared (*C*), indicating that anti-MP antibody cannot specifically recognize the endogenous MP protein. (*A* and *B*) Red arrows indicate the induced MP-Myc protein. Black arrow denotes the induced MP protein. Asterisk indicates nonspecific bands recognized by anti-Myc antibody. (*D*) Quantitative PCR of fragments (as in Fig. 4*A*) from ChIP of WT seedlings with anti-MP antibody (antiserum) and preimmune antiserum, respectively. Fold enrichment is expressed as the ratio of the enrichment by anti-MP antiserum to that by preimmune antiserum. Data are means ± SDs ($n = 3$). All ratios are not more than 1, indicating that this anti-MP antiserum does not specifically immunoprecipitate DNA fragments in the *STOMAGEN* promoter.

Table S1. Primers used in this study

Purpose or construct name	Oligonucleotide	Sequence (5′ > 3′)
RT-qPCR	ACT2-S	AAGCTGGGGTTTTATGAATGG
	ACT2-AS	TTGTCACACACAAGTGCATCAT
	STOMAGEN-S	TAGGGTCGACAGCACCAACTTGTAC
	STOMAGEN-AS	TCATTTCCTTCGACTGGAACTTGCT
ChIP-qPCR	NC1-S	ATACGGTCTCCCTTCTCCCA
	NC1-AS	TAAATCCATTGTTTCCTTGC
	NC2-S	TTCGCCAATAGCGTTATCAAG
	NC2-AS	CCACCATCATTCACCATCATC
	1-S	TGTTCCTTGTCACCATACTTATTTG
	1-AS	AGATGTATACCAATCCTTCCGTTT
	2-S	CAATGTTTTGATGCTCTAGAAGGT
	2-AS	GCAAAAGAATTAAAGAGAGATAGG
	3-S	AAGTTCATACGAGTTCAAAGAGGAG
	3-AS	AACATGTCCAAACTGTTTTCTATAGACG
	4-S	ATTTAGTTGCCGACAAAAAGTAACT
	4-AS	TCCTACACTCTTGTCTCCCTATC
	5-S	GTGTGCACAAACCTCACCATTA
	5-AS	ACCAAATGTTGTGGAGAGAGATAAG
EMSA	P1-S	AAGGTTGTACCATGACATCCTGCTAGACACATGTCTCTCTCCCTATCTCTCTTTAATTCT
	P1-AS	AGAATTAAAGAGAGATAGGGAGAGAGACATGTGTCTAGCAGGATGTCATGGTACAACCTT
	P2-S	ATCATGTAGACAACACTCTAGCTAGAGAAGCTCTAGCATCGTCTATAGAAAACAGTTTGGACATGTTTTTGC
	P2-AS	GCAAAAACATGTCCAAACTGTTTTCTATAGACGATGCTAGAGCTTCTCTAGCTAGAGTGTTGTCTACATGAT
	P3-S	GCCGACAAAAAGTAACTAATAATTTGTAATTATTAGATGTAAGACAGGAGATGAGATAGGGAGACAAGAG
	P3-AS	CTCTTGTCTCCCTATCTCATCTCCTGTCTTACATCTAATAATTACAAATTATTAGTTACTTTTTGTCGGC
	P3-m-S	GCCGCCAAAAAGTAACTAATAATTTGTAATTATTAGATGTAAGCCAGGAGATGAGATAGGGAGCCAAGAG
	P3-m-AS	CTCTTGGCTCCCTATCTCATCTCCTGGCTTACATCTAATAATTACAAATTATTAGTTACTTTTTGGCGGC
	P4-S	CCATTAGATGATAAGTAGAAGACAAAACTTACAGCCAAACAGGCAAACTTATCTCTCTCC
	P4-AS	GGAGAGAGATAAGTTTGCCTGTTTGGCTGTAAGTTTTGTCTTCTACTTATCATCTAATGG
constructs		
pER10-bdl	BDL-1-S	CCCCTCGAGATGCGTGGTGTGTCAGAAT
	BDL-1-AS	GGACTAGTCTAAACAGGGTTGTTTCTTTGTCTATCC
	BDL-m-S	AAGTCAAGTGGTAGGATGGTCACCAATTGGGTTACACAG
	BDL-m-AS	CTGTGTAACCCAATTGGTGACCATCCTACCACTTGACTT
pCambia1300-nos	nos-S	GCCGGATCCGAATTCCATGGGCCCACTAGTTCGTTCAAACATTTGGCAATAAAG
	nos-AS	GGCCAATTGGCTGTCGAGGGGGGATCAATTCCC
pCambia1300-pTMM-LUC-NLS-sGFP	TMM-PRO-S	CCCAAAGCTTTGTTGCTCCATGGGCATGT
	TMM-PRO-AS	AAACTGCAGTTCTTAGTTGTTGTTGTTGTGTGAATG
	LUC-S	AAACTGCAGGGCGCGCCATGGAAGACGCCAAAAACATAAAG
	LUC-AS	GGGGTCACCTAGGCCATGGGGCCCCACGGCGATCTTTCCGCCCTTCTT
	NLS-sGFP-S	AGGGGGGCCCCCAAAGAAGAAGAGAAAGGTGGAAGATCCAATGAGTAAAG GAGAAGAACTTTTC
	sGFP-AS	GTATCCTAGGTTACAGCTCAGATCTTTTA
pCambia1300-pSTOMAGEN-LUC	STOMAGEN-PRO-1-S	GGGGTCGACTAGAAAAGATTTGATTCCTAAACA
	STOMAGEN-PRO-1-AS	GCCGGATCCTCTCTACTTCTTCTTCTTCTT
pEarlyGate 164-pSTOMAGEN-GUS	STOMAGEN-PRO-2-S	CACCTAGAAAAGATTTGCTTCCTAAACA
	STOMAGEN-PRO-2-AS	GGACTAGTTCTCTACTTCTTCTTCTTCTTGCCT
70S-MP-CFP	MP-1-S	TCCCCCCGGGATGATGGCTTCATTGTCT
	MP-1-AS	GGACTAGTTGAAACAGAAGTCTTAAGATCGTTAATG
pGreen0800-pSTOMAGEN-LUC	STOMAGEN-PRO-S	GGGGTCGACTAGAAAAGATTTGATTCCTAAACA
	STOMAGEN-PRO-AS	GGACTAGTTCTCTACTTCTTCTTCTTCTTGCCT
pGreen0800-pARR15-LUC	ARR15-PRO-S	GGGGTCGACTCGACGATTCTCACAAACC
	ARR15-PRO-AS	GGACTAGTTGTTTTCTCTCGGGAAAGTAAACA

Table S1. Cont.

Purpose or construct name	Oligonucleotide	Sequence (5′ > 3′)
pSTOMAGEN mutation	M1-S	TTAGATGATAAGTAGAAGCCAAAACTTACAGCCAAAC
	M1-AS	GTTTGGCTGTAAGTTTTGGCTTCTACTTATCATCTAA
	M2/3-L-AS	TGGCTCCCTATCTCATCTCCTGGCTTACATCTAA
	M2/3-R-S	GCCAGGAGATGAGATAGGGAGCCAAGAGTGTA
	M4-S	TATTATATTTAGTTGCCGCCAAAAAGTAACTAATAAT
	M4-AS	ATTATTAGTTACTTTTTGGCGGCAACTAAATATAATA
	M5-S	CTATAGAAAACAGTTTGGCCATGTTTTTGCTTATAAT
	M5-AS	ATTATAAGCAAAAACATGGCCAAACTGTTTTCTATAG
	M6-S	GAGGAGCAAATCATGTAGCCAACACTCTAGCTAGAGA
	M6-AS	TCTCTAGCTAGAGTGTTGGCTACATGATTTGCTCCTC
pCambia1300-pMP-MP-MYC-HA	MP-G-S	CGACTCTAGAGGATCCGTGAATTACCAAGGCGTT
	MP-G-AS	AATCGATACCGGATCCGTGAAACAGAAGTCTTAAG
	MYC-HA-S	TAAGCTTGATATCGAATTCGGATCC
	MYC-HA-AS	CAGGGCCCGAGCTCGGTGACCTCAAG
pMAL-c2X-MP	MP-2-S	CGGAATTCATGATGGCTTCATTGTCTTGTGTT
	MP-2-AS	CCCCTCGAGTTATGAAACAGAAGTCTTAAGATC
genotyping		
mp-S319	LBb1.3	ATTTTGCCGATTTCGGAAC
	mp-S319-LP	CCTGGAAACTGATGAGCTGAC
	mp-S319-RP	CCTTCTTCACTCATCTGCTGG
arf5-1	LBb1.3	ATTTTGCCGATTTCGGAAC
	MP-1-LP	GAGAGGAAGTAAGCACCCGAC
	MP-1-RP	TCATTACATCCAGGCTCATCC