Supporting Information

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SI Materials and Methods

RNA Expression Analysis. For RT-PCR analysis, total RNA was isolated from 4-day-old seedlings using the RNeasy Mini Kit (Qiagen) and used as template for first-strand cDNA synthesis using the RevertAid First Strand cDNA Synthesis kit from Fermentas, according to manufacturer's instructions.

Quantitative RT-PCRs were performed using the LightCycler FastStart DNA Master SYBR Green I kit (Roche) on a Roche LightCycler apparatus, according to manufacturer's instructions. Cycling conditions were as follows: 95 °C for 10 min, 45 cycles at 95 °C for 5 s, 60 °C for 5 s, 72 °C for 15 s, and 1 cycle at 95 °C for 5 s, 63 °C for 15 s. Dissociation kinetic analysis of the amplification products confirmed that only the expected products were amplified. A negative control without cDNA template was always included for each primer combination. Data represent mean values of 3 technical replicates on 3 independent biological repeats, and were calculated as the quantification of specific PCR amplification products normalized to the constitutive control actin. The value of WT (i.e., nontreated WT seedling) was set at 1.

Histology and Microscopy. Preparations were imaged with the Leica video microscope DMI 6000B. For fluorescence microscopy, roots were stained with 33 μM FM4-64 and imaged under a Leica-SP2 confocal microscope (DD488/543, excitation/emission 543/650 nm for FM4-64 and excitation/emission 488/515 nm for GFP).

For histological sections on SAMs, 7-day-old seedlings were fixed in 10% formaldehyde, 5% acetic acid, and 50% ethanol for 20 min under vacuum, and then overnight at 4 °C. Samples were rinsed in water, dehydrated in an ethanol series, and infiltrated with resin using the Technovit 7100 kit (Histo-Technik), according to manufacturer's instructions using a series of graded resin/ethanol solutions. Tissue sections (4 μm) were cut and stained with 0.5% Toluidine Blue for 20 s.

For scanning electron microscopy (Hitachi S-3000), SAMs from 12 DAG were dissected under the binocular and mounted directly. Samples were slowly frozen at −18 °C on the Peltier stage, and then observed under a partial vacuum with the Environmental Secondary Electron Detector (ESED) mode (90 Pa, 12 kV).

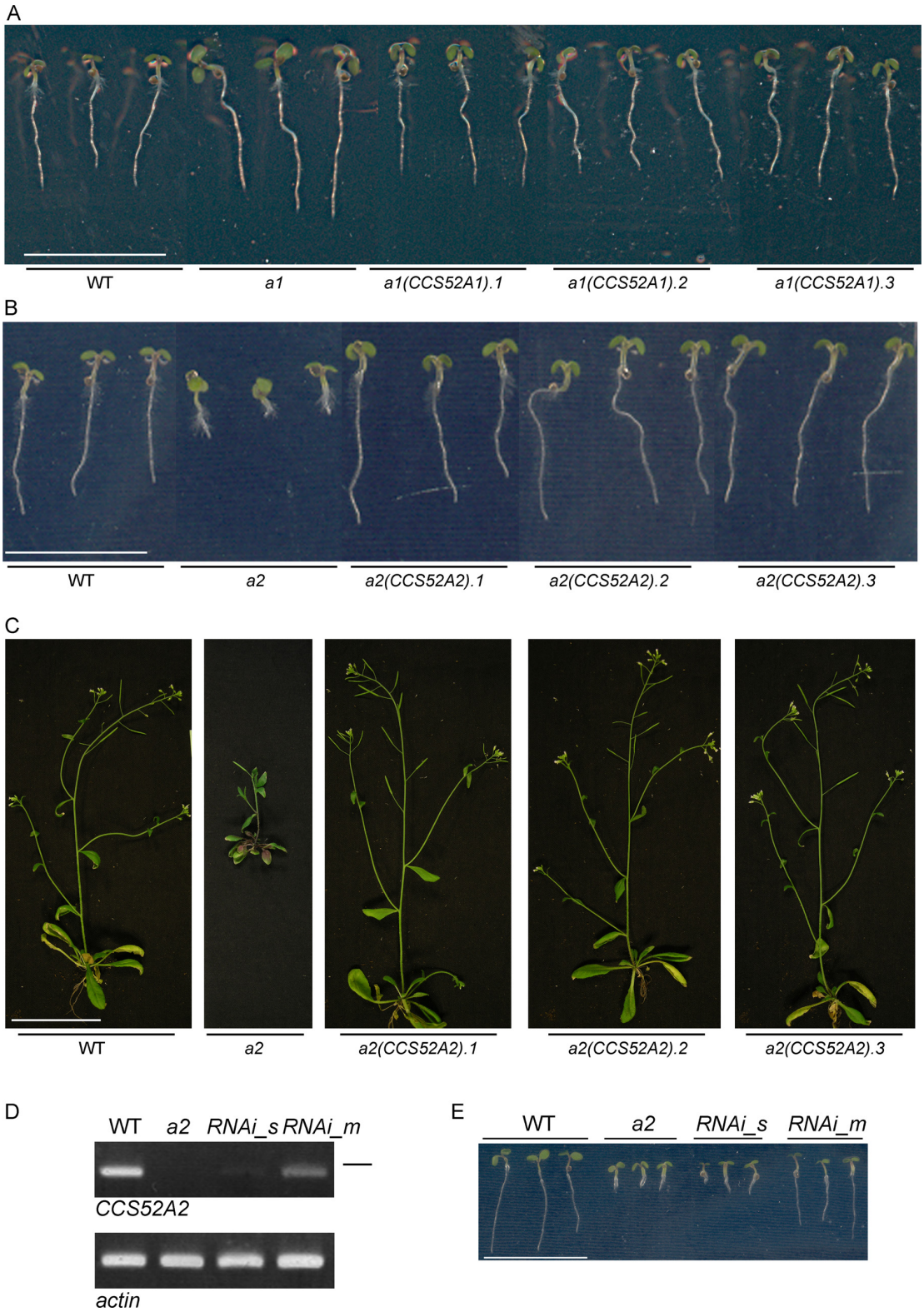

Fig. S1. Rescue of the *ccs52a* phenotype. (*A*–*C*) The *CCS52A1* (*A*) and *CCS52A2* (*B* and *C*) genomic region was introduced in the respective *ccs52a1* and *ccs52a2* mutant background. Three independent lines are shown in comparison with WT and *ccs52a1* (*a1*) and *ccs52a2* (*a2*) mutants at the seedling stage (*A* and *B*), and after 4 weeks of growth (*C*). (*D* and *E*) RT-PCR analysis (*D*) and phenotype (*E*) of *CCS52A2* RNAi lines after 4 days of growth. *CCS52A2* transcripts were strongly reduced to absent in the severely affected seedlings (RNAi_s) as in the *ccs52a2* KO lines (*a2*), and moderately reduced in the medium sized seedlings (RNAi_m). Amplification of the *ACT2* actin gene was used as reference. [Scale bars: 1 cm (*A*, *B*, and *E*); 5 cm (*C*); 0.5 kb (*D*).]

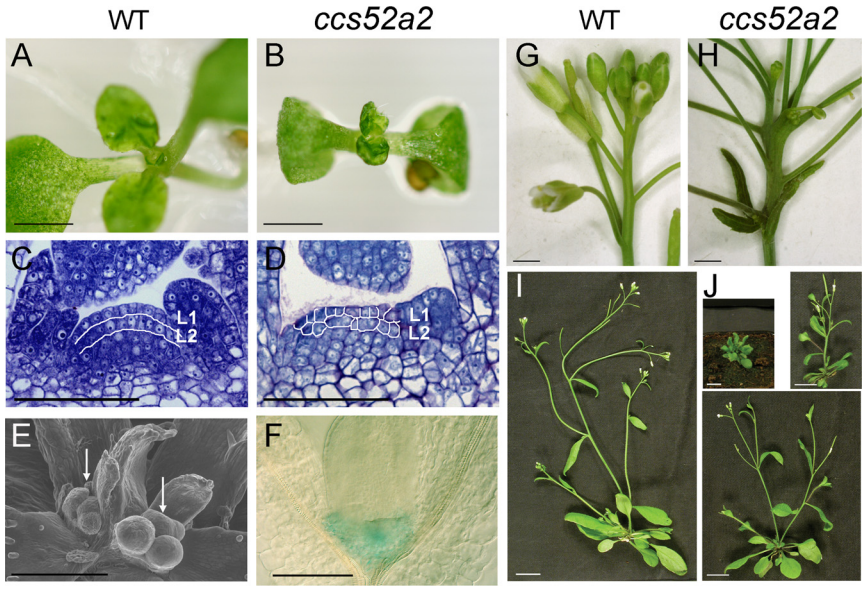

Fig. S2. The activity and structure of the SAM are disrupted in *ccs52a2* mutants. (*A* and *B*) Reduced meristematic activity as evidenced by retarded rosette leaf initiation was observed in *ccs52a2* mutants (*B*) compared with WT plants (*A*). (*C* and *D*) Toluidine blue-stained sections show disorganized cells in the SAM of *ccs52a2* (*D*) in comparison with the highly organized cells in the L1 and L2 layer of the SAM of WT seedlings (*C*). (*E*) SEM images of 12-day-old *ccs52a2* seedlings with fasciated shoot meristems (arrows). (*F*) GUS expression from the *CCS52A2* promoter in the SAM. (*G* and *H*) Fasciation of the primary stem in *ccs52a2* mutants (*H*) in comparison with WT stems (*G*). (*I* and *J*) Growth of WT (*I*) and *ccs52a2* (*J*) plants 4 weeks after germination. The *ccs52a2* phenotype varied from stunted bushy nonflowering plants to middle sized plants. [Scale bars: 1 mm (*A*, *B*, *G*, and *H*); 100 μm (*C*, *D*, and *F*); 200 μm (*E*); 1 cm (*I* and *J*).]

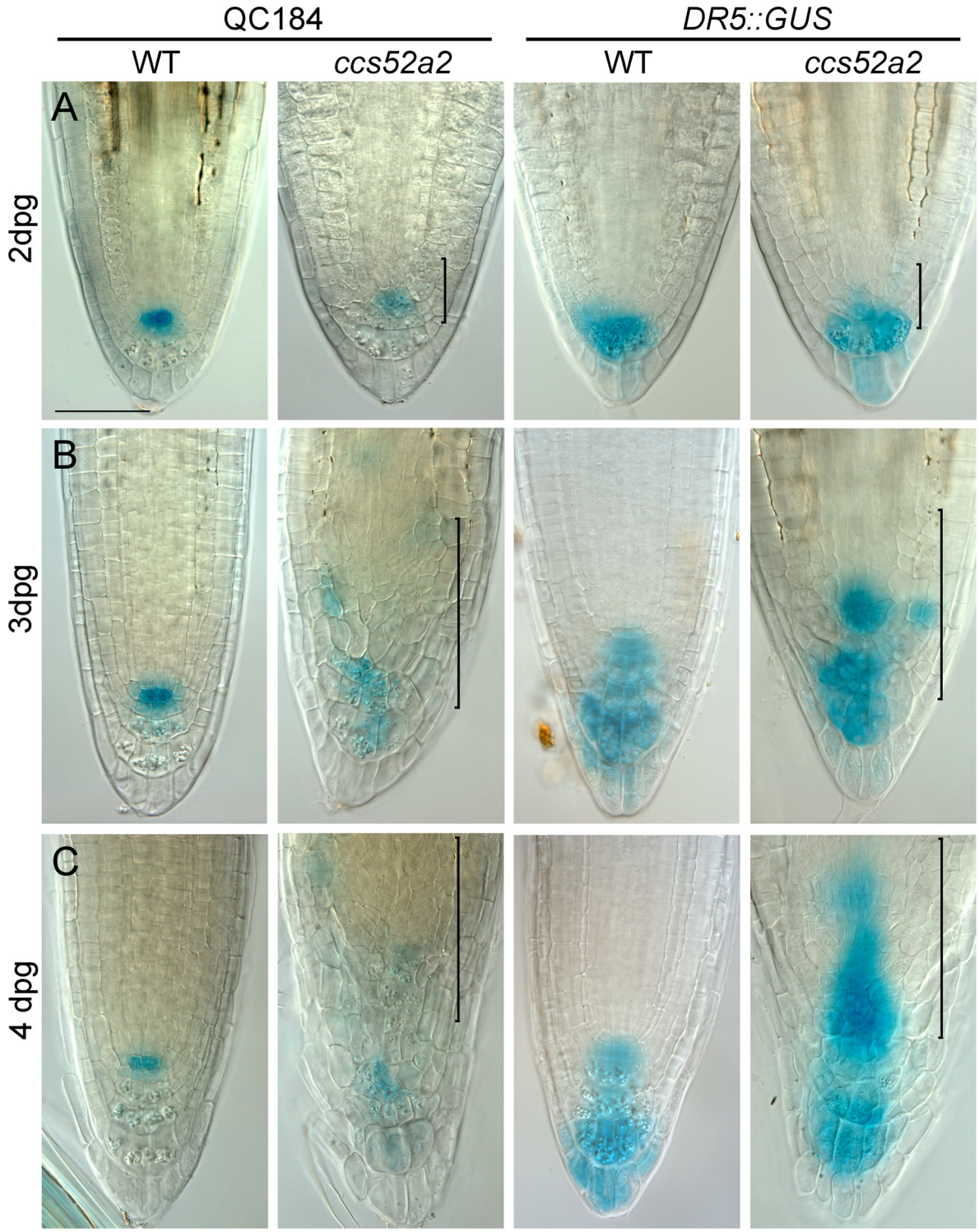

Fig. S3. Evolution of the *ccs52a2* root meristem (RM) phenotype after 2, 3, and 4 days of growth. Brackets indicate the region of cellular disorganization. (*A*) At 2 days postgermination (dpg), cellular disorganization in the *ccs52a2* RM is limited to the stem cell region. Expression in these roots from the QC184 marker becomes diffuse, whereas the auxin maximum as monitored by the *DR5*::*GUS* marker is still intact. (*B*) At 3 dpg, cellular organization in the whole distal part of the *ccs52a2* RM is disrupted. The QC184 marker is largely absent from the region just above the root cap in the mutant roots, and expression from the *DR5*::*GUS* marker is largely similar to WT plants, but has extended more proximal from the root tip. (*C*) At 4 dpg, the whole RM is disorganized in *ccs52a2.* Expression of the QC184 and *DR5*::*GUS* is similar as in *B*.

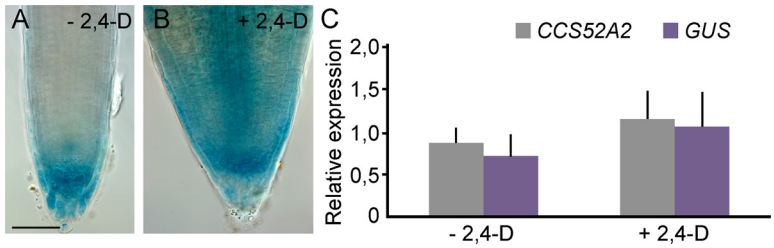

Fig. S4. Auxin does not stimulate the *CCS52A2* expression level. (*A* and *B*) Long-term auxin treatment with 5 μM 2,4-dichlorophenoxyacetic acid results in a spreading of the *CCS52A2*::*GUS* signal more proximally in the RM (*B*) in comparison with control treatments (*A*). (Scale bar: 50 μm.) (*C*) *GUS* and *CCS52A2* gene expression were measured by quantitative RT-PCR in the same experiment. Auxin did not stimulate *CCS52A2* or *CCS52A2* driven GUS expression. Histogram represents the quantification of specific PCR amplification products normalized to the constitutive *ACT2*.

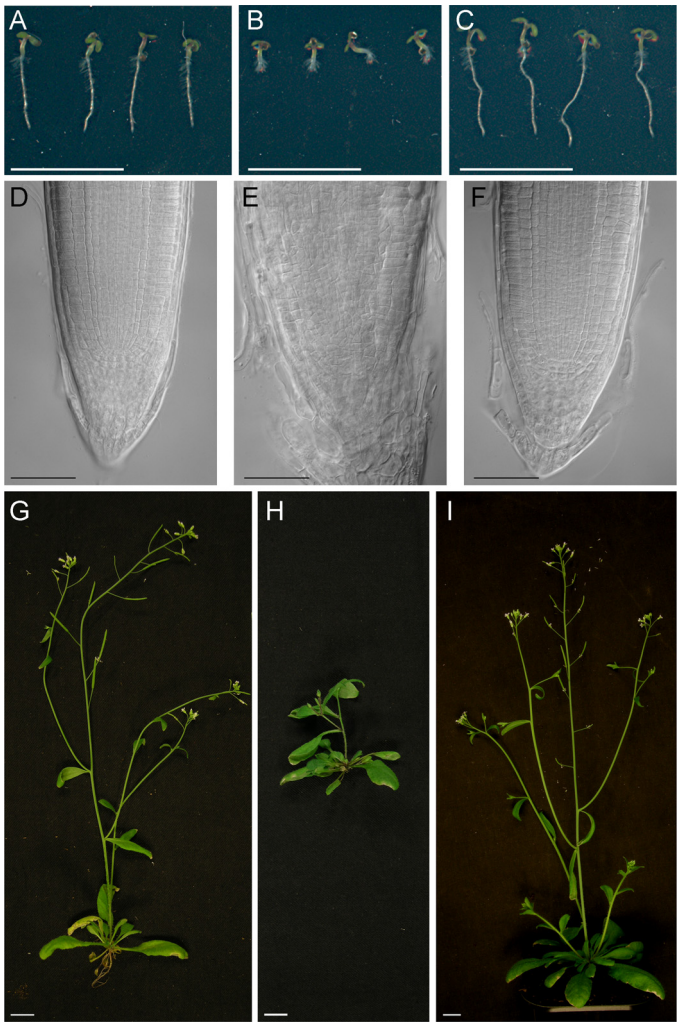

Fig. S5. CCS52A1 and CCS52A2 proteins are functional homologues. WT (*A*, *D*, and *G*), *ccs52a2* (*B*, *E*, and *H*), and *ccs52a2* mutants carrying the *CCS52A1* gene under control of the *CCS52A2* promoter (*C*, *F*, and *I*) are shown after 4 days (*A*–*F*) and 4 weeks (*G*–*I*) of growth. Expression of the *CCS52A1* gene from the *CCS52A2* promoter rescued the aerial (*I*) and root (*C*) phenotype of *ccs52a2*, including the organization of cells in the RM (*F*). [Scale bars: 1 cm (*A*–*C* and *G*–*I*); 50 μm (*D*–*F*).]

Table S1. Primer sequences used for cloning and RT-PCR

Fragment	Primer name	Primer sequence 5′→3′
CCS52A1 promoter construct	TATA-A1R Fw	GCGTCGAAAGAACTCGTCAGTCTTGTG
	PromANco1 Rv	CCATGGGTTTTTTTTTTTTGTTGACT
CCS52A2 promoter construct	PromA2L Fw	GCGTCGACCTGGCAAAACGGGAAAGG
	PromA2R Rv	CCATGTTTGATTTCAATCTCTTAC
CCS52A1 genomic construct for rescue	CCSA1-resc-pTopo-Fw	CACCCATTTTTTTTTAGTTTTTGGTTAAACGGCTAT
	CCSA1-resc-pTopo-Rv	GAAGAACAAGTAATATAAGAGAATGGTAAC
CCS52A2 genomic construct for rescue	CCSA2-resc-pTopo-Fw	CACCGATGACGAACAATGTTAACC
	CCSA2-resc-pTopo-Rv	GTCCCAAAAGCATTATGATC
CCS52A2 promoter construct for fusion with *CCS52A1* gene	CCSA2-resc-pTopo-Fw	CACCGATGACGAACAATGTTAACC
	PromA2-Rv-plus	GTAGGATCTTCTTCTTCCATGTTTGATTTCAATCTCTTAC
CCS52A1 gene construct for fusion with *CCS52A2* promoter	PromA1-Fw-plus	GTAAGAGATTGAAATCAAACATGGAAGAAGAAGATCCTAC
	CCSA1-resc-pTopo-Rv	GAAGAACAAGTAATATAAGAGAATGGTAAC
pro*CCS52A2*::*CCS52A1* gene construct	CCSA1-resc-pTopo-Fw	CACCCATTTTTTTTTAGTTTTTGGTTAAACGGCTAT
	CCSA2-resc-pTopo-Rv	GTCCCAAAAGCATTATGATC
*CCS52A1*construct for translational GFP fusion	CCSA1c-fus GFP Fw	CACCCATTTTTTTTTAGTTTTTGGTTAAACGGCT
	CCSA1c-fus GFP Rv	CCGAATTGTTGTTCTACCAAAGAAAGACGA
CCS52A2 construct for translational GFP fusion	CCSA2c-fus GFP Fw	CACCGATGACGAACAATGTTAACCGTGAAGGCTCTG
	CCSA2c-fus GFP Rv	CCGGATTGTTGTTCTACCAAAAGATAATGC
CCS52A2 RT-PCR	Atccs52A2-Fw	CTGTGAACACGCCGCAGCAGTG
	Atccs52A2-Rv	GTCTTGACATGATTCTCGAAATCAGG
ACTIN2 RT-PCR & QPCR	ACT2-Fw	TTGACTACGAGCAGGAGATGG
	ACT2-Rv	ACAAACGAGGGCTGGAACAAG
AUX/IAA1 QPCR	AUX/IAA1 QPCR Fw	GAATATGGAAGTCACCAATGGGC
	AUX/IAA1 QPCR Rv	GCAGGAGGAGGAGCAGATTCTT
AUX/IAA2 QPCR	AUX/IAA2 QPCR Fw	ATCAACCAGCTCACCAAGAACAA
	AUX/IAA2 QPCR Rv	CCGGGTAATCCAAGACATAGCTC
AUX/IAA5 QPCR	AUX/IAA5 QPCR Fw	CGTGAAAGTGAGTGTAGATGGAGC
	AUX/IAA5 QPCR Rv	ATCTCCAGCAAGCATCCAATCT
AUX/IAA19 QPCR	AUX/IAA19 QPCR Fw	GGTTAGGGTATGTGAAAGTGAGCA
	AUX/IAA19 QPCR Rv	CCAGTCTCCATCTTTGTCTTCGT