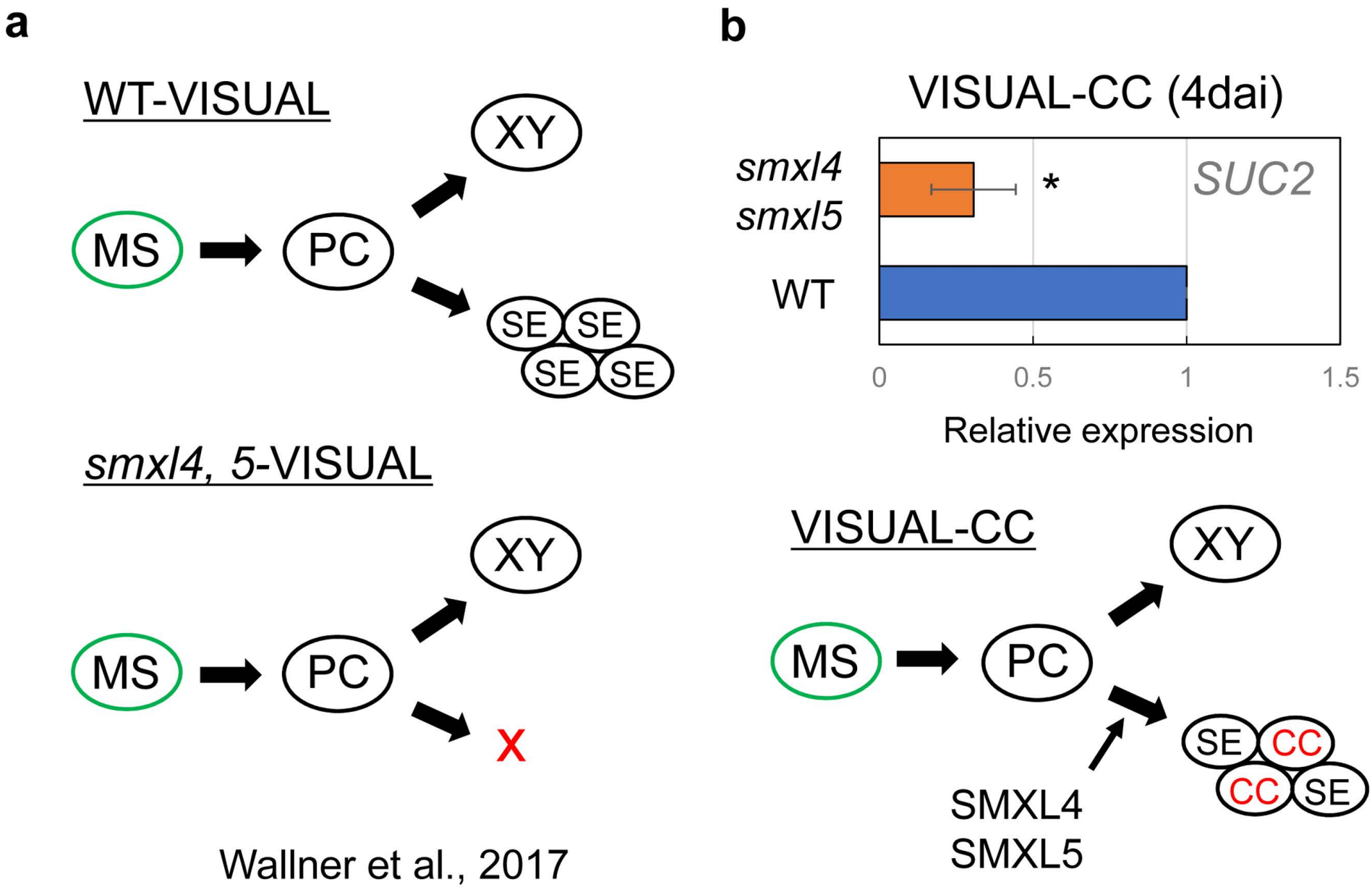
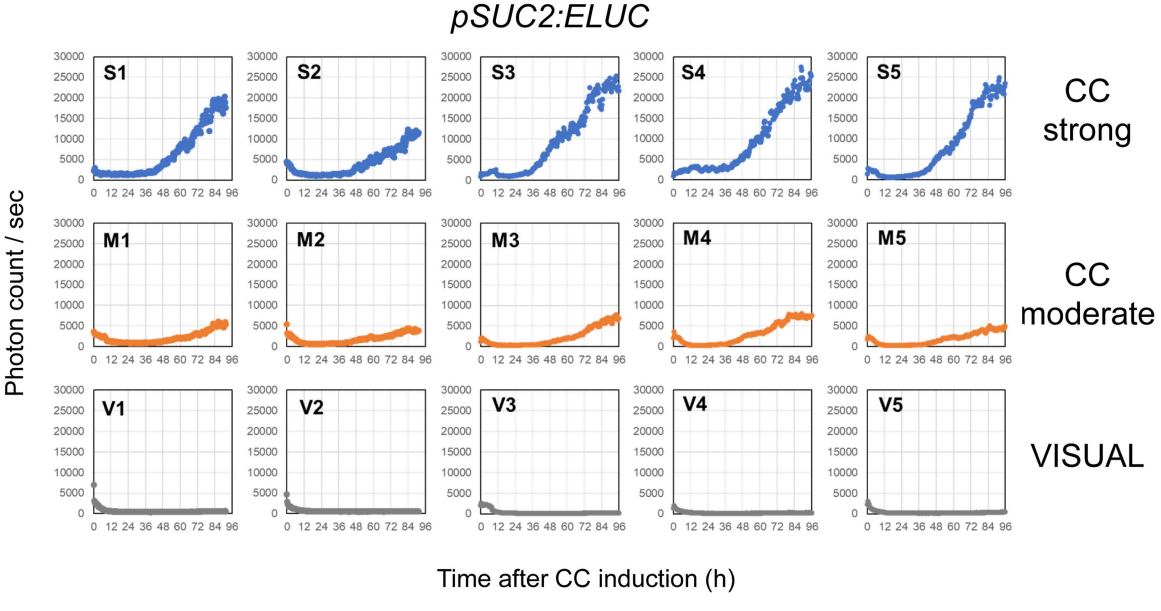
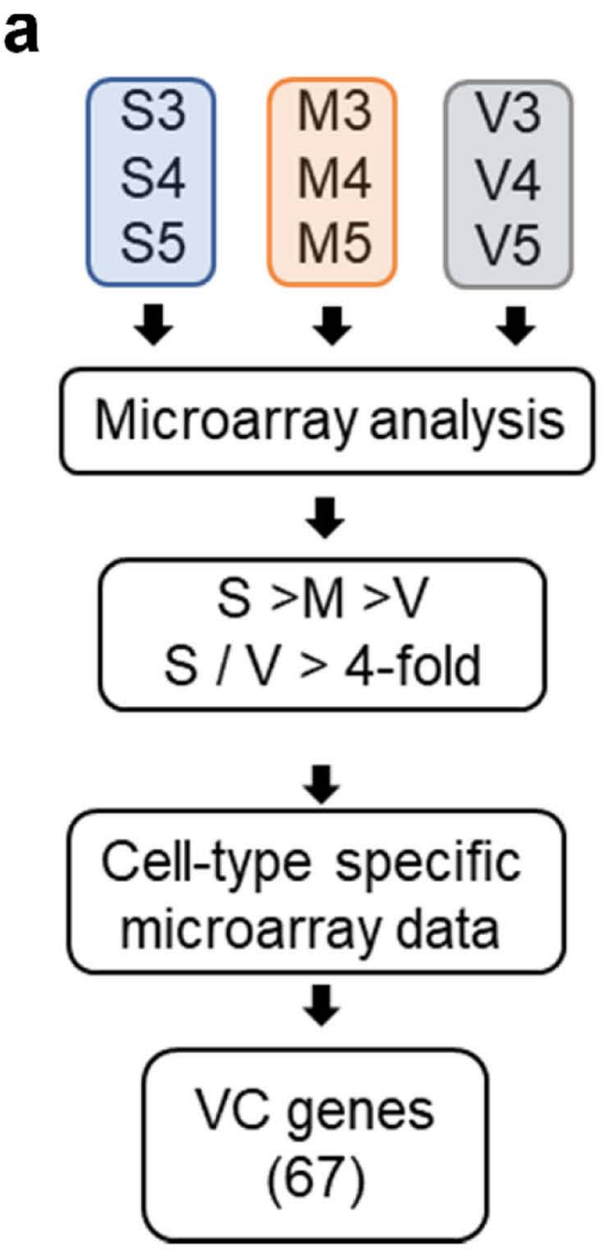
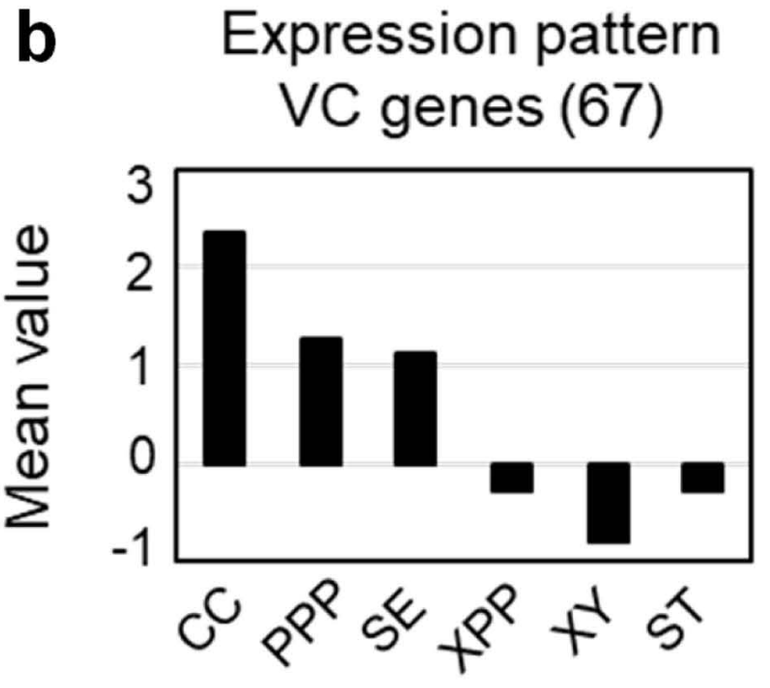


Supplementary Fig. 1| *smxl4 smxl5* mutants suppressed CC differentiation in VISUAL-CC
a, Schematic of the VISUAL differentiation process in the WT and *smxl4 smxl5*. The *smxl4 smxl5* double mutants were known to inhibit phloem differentiation in VISUAL. **b**, SUC2 expression at 4d after VISUAL-CC induction in the WT and *smxl4 smxl5*. Asterisks indicate significant differences using the Student's t-test (*$P < 0.05$, n = 3).



Supplementary Fig. 2| Raw data from time-course analysis of *pSUC2:ELUC* plants
An example of *pSUC2:ELUC* signals from individual samples is shown. Vertical axis indicates photon counts per second detected by the luminometer. Samples were classified based on LUC intensity.



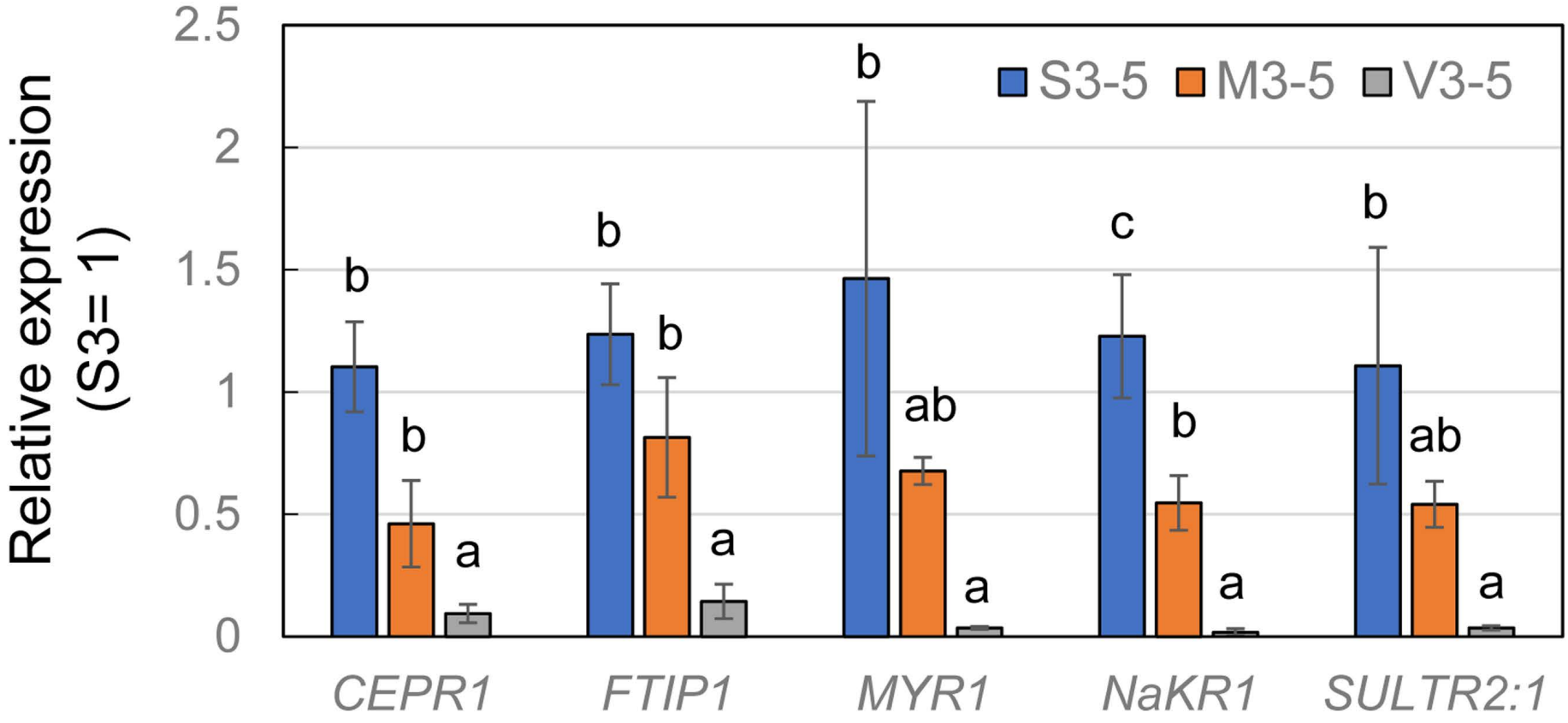


c

Gene functional classification

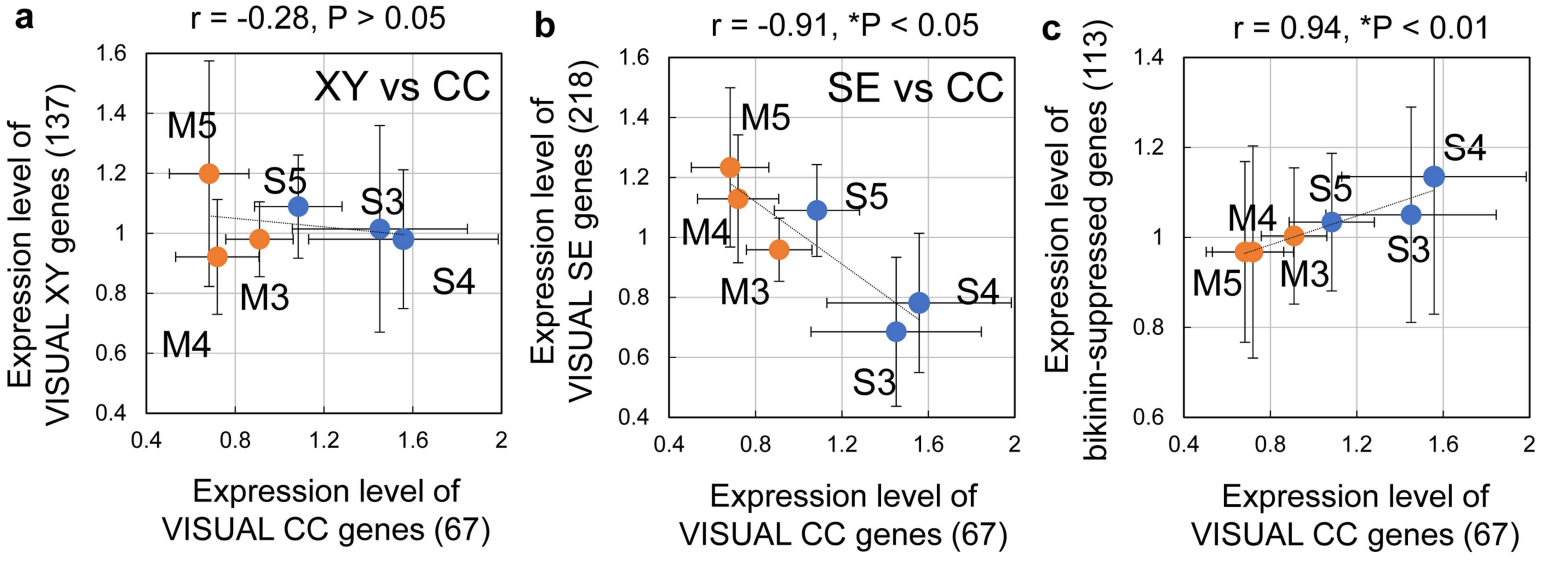
Gene functional clustering	Enrichment score
Peroxidase (4)	2.12
Transporter (10)	**0.82**
Transcription factor(8)	0.57

Supplementary Fig. 3| Characterization and molecular function of VC genes
a, Schematic of the selection process used to identify VISUAL-CC inducible genes. Expression levels of vascular-specific genes were determined using VISUAL-CC microarray data. **b**, Expression patterns of VC genes in the root stele obtained from a transcriptome dataset[14]. Mean values from Fig. 2A are shown. **c**, Functional classification of VC genes and VPP genes. Enrichment scores were calculated using David (https://david.ncifcrf.gov/). Transporter-related genes are over-represented in this category.

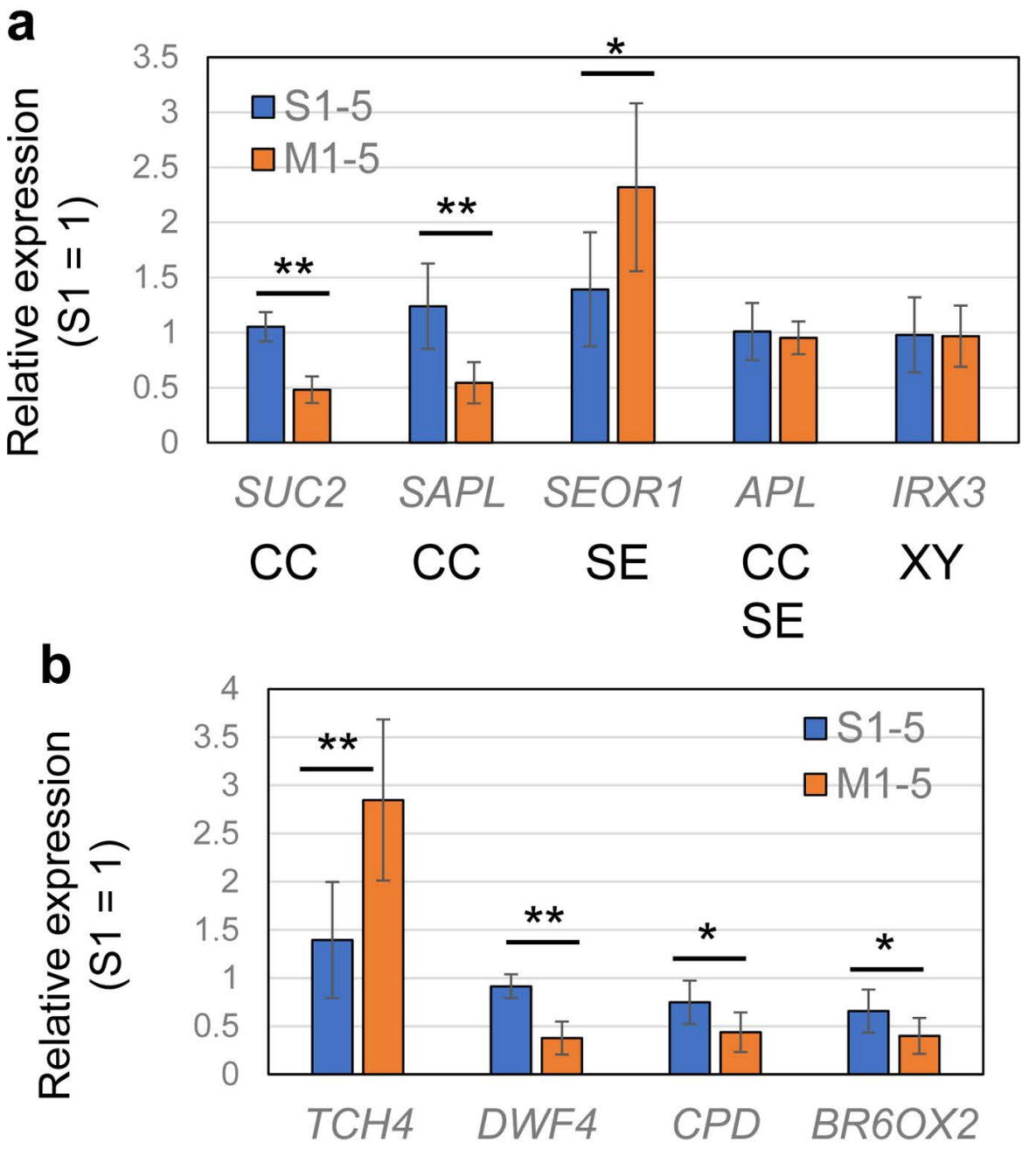


Supplementary Fig. 4 | Statistical differences in expression levels of CC-related genes among the S, M, and V samples

Expression levels of *CEPR, FTIP1, MYR1, NaKR1,* and *SULTR2:1* were quantified using qRT-PCR and compared statistically among the S3-5, M3-5, and V3-5 samples. Relative expression levels were calculated when the expression in S3 was set to 1. Statistical differences between samples are indicated by different letters (ANOVA, Tukey-Kramer method; n = 3; error bars indicate SD).

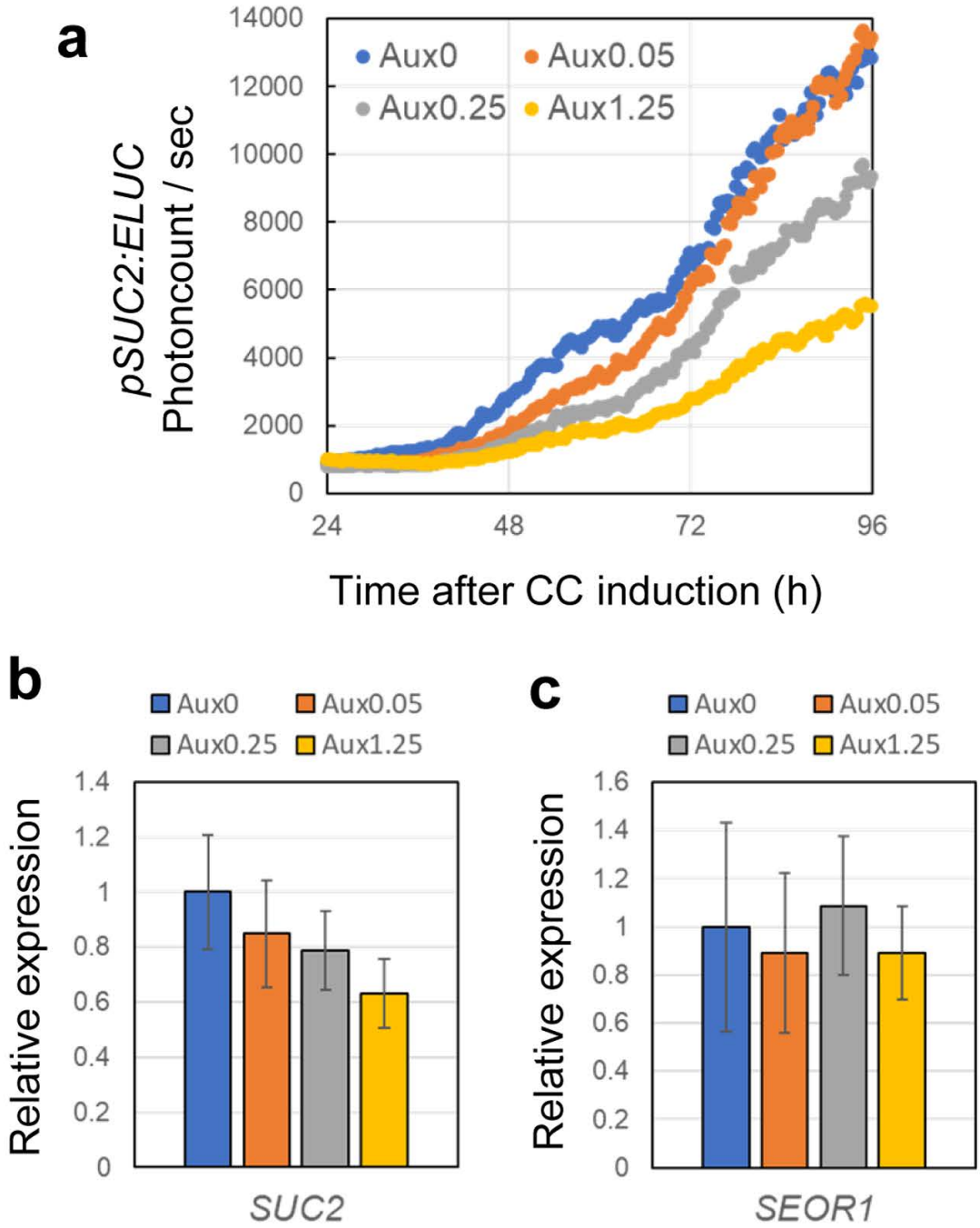


Supplementary Fig. 5 | Correlation analysis in microarray data between the S and M samples
a, VC genes (67) vs VX genes (137) **b**, VC genes (67) vs VS genes (218) **c**, VC genes (67) vs bikinin-suppressed genes (113). The Pearson correlation coefficient and *P*-value are marked above the chart. Error bars indicate SD.



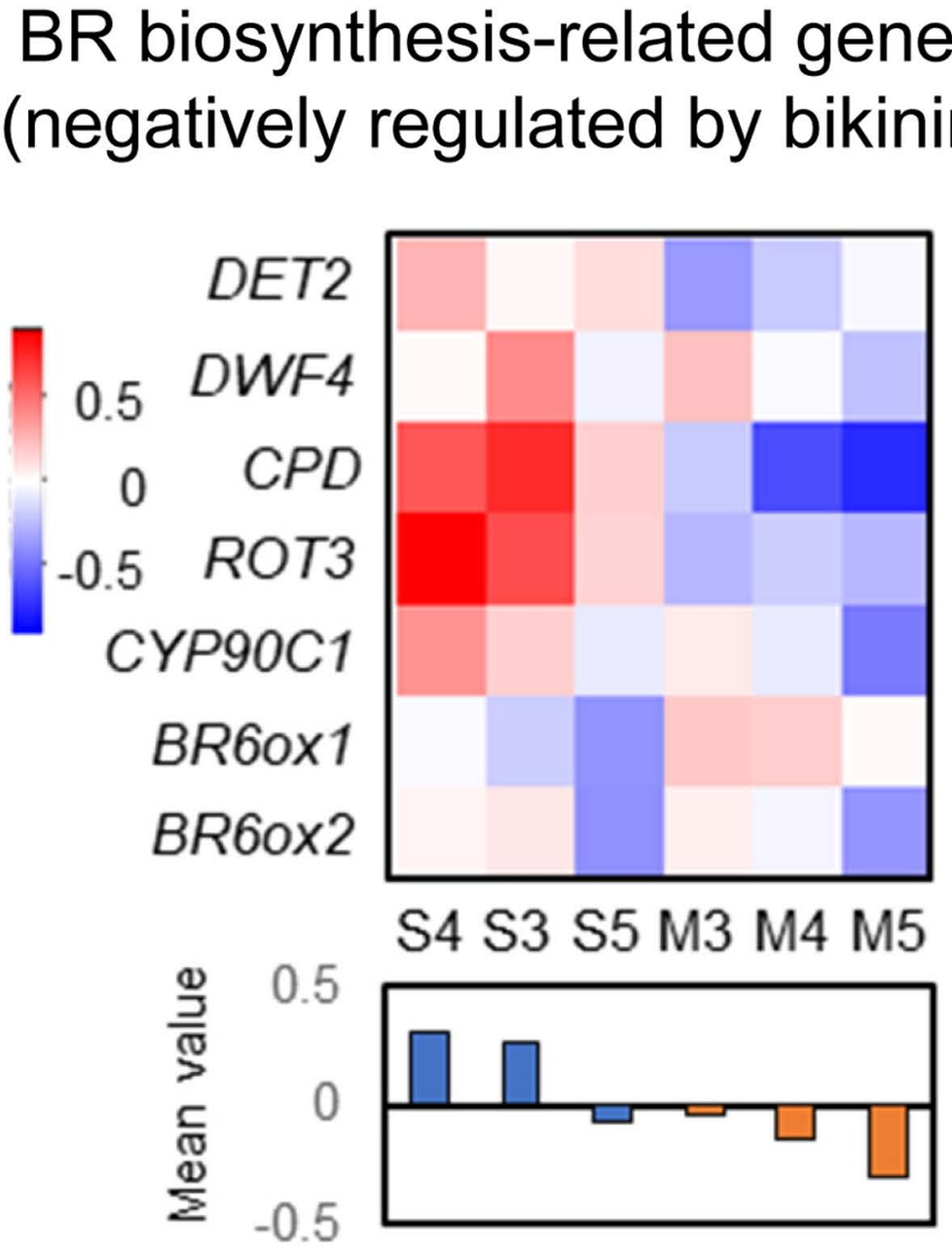
Supplementary Fig. 6| Statistical differences in expression levels between the S and M samples

a, Expression levels of *SUC2* (as CC), *SAPL* (also as CC), *SEOR1* (as SE), *APL* (as CC+SE), and *IRX3* (XY) were quantified using qRT-PCR and compared statistically between the S and M samples. Asterisks indicate significant differences using the Student's t-test (**P < 0.005; *P < 0.05). **b**, Expression levels of GSK3 activity-dependent genes were quantified using qRT-PCR and compared statistically in the S and M samples. Asterisks indicate significant differences determined using the Student's t-test (**P < 0.005; *P < 0.05).

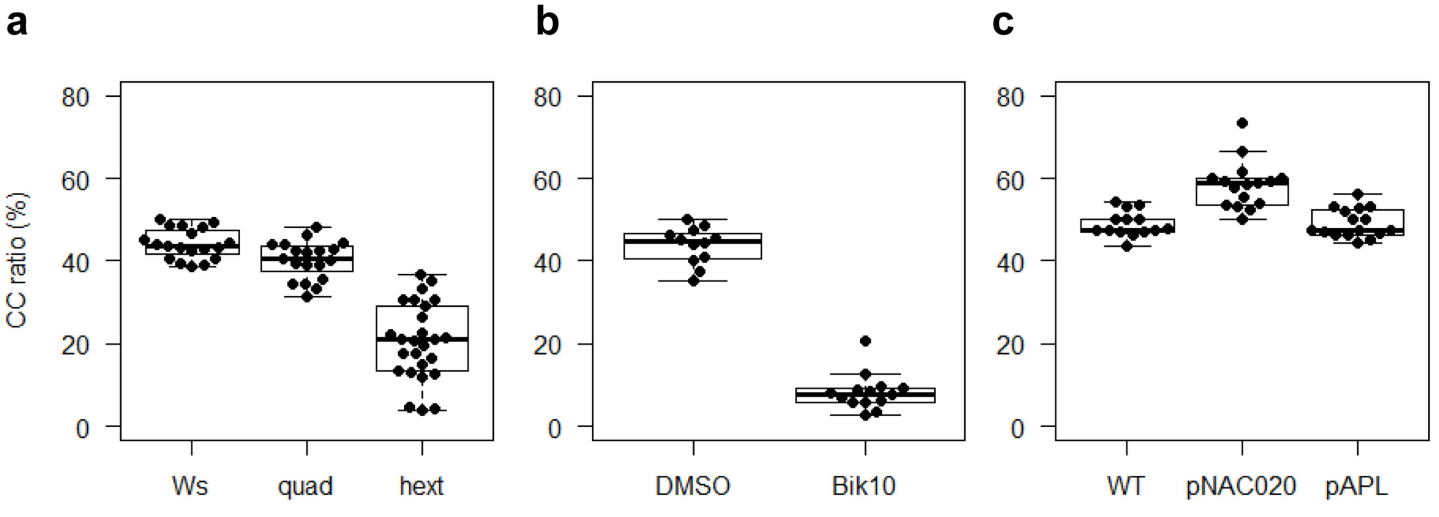


Supplementary Fig. 7| Auxin has only marginal effects on the formation of the SE-CC complex

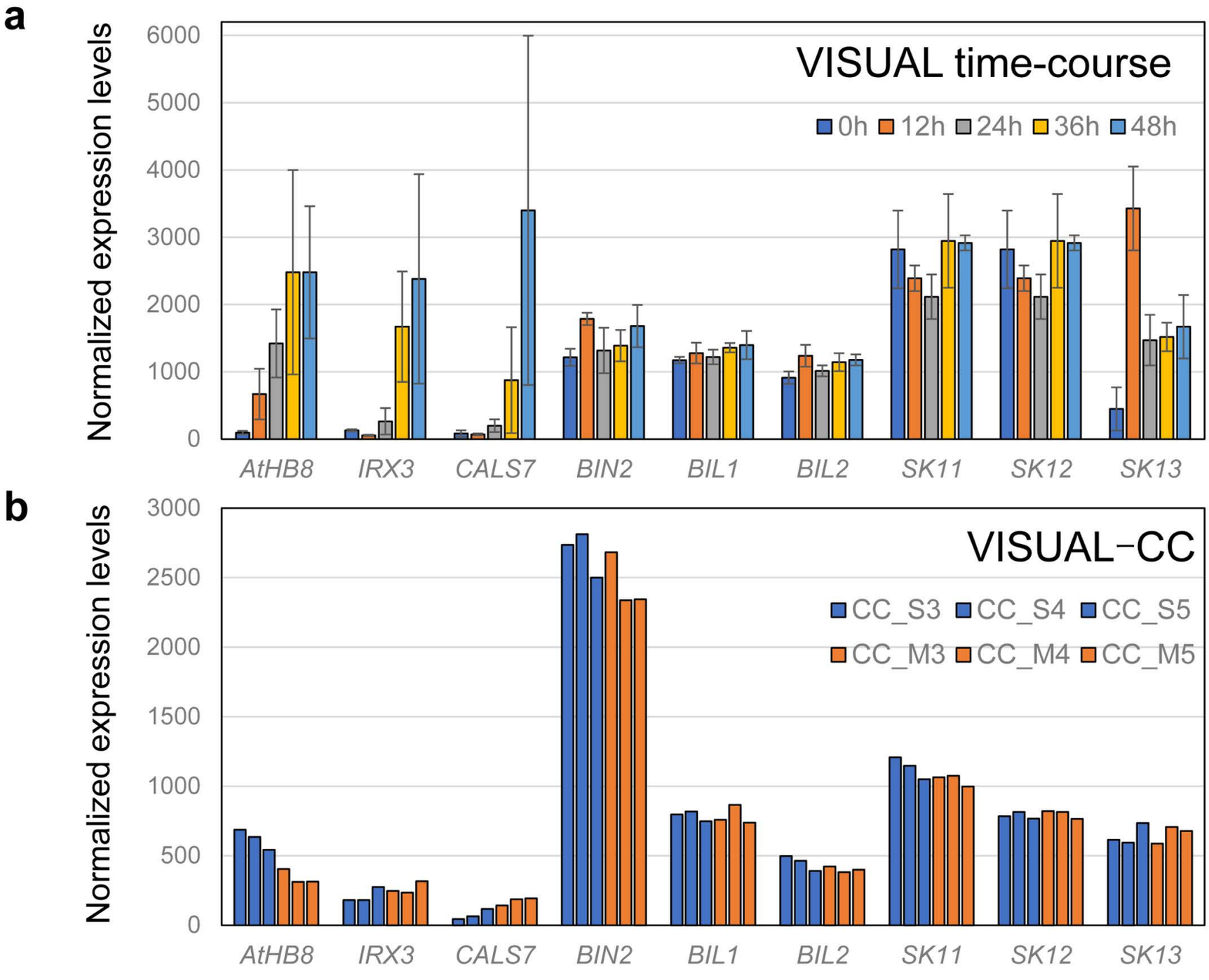
a, Time-course of *pSUC2:ELUC* signal intensities in VISUAL-CC cultures containing different concentrations of auxin (mg/L). **b** and **c**, Expression levels of *SUC2* (b) and *SEOR1* (c) in VISUAL-CC samples from cultures containing different concentrations of auxin. There are no significant differences (ANOVA, Tukey-Kramer method; n = 6; error bars indicate SD).



Supplementary Fig. 8| Heat map of expression levels of BR biosynthesis-related genes in S and M samples.
The upper panel shows a heat map of expression levels of 6 BR biosynthesis-related genes, which are downregulated by bikinin, in S and M samples. The lower panel indicates the mean value for each sample.

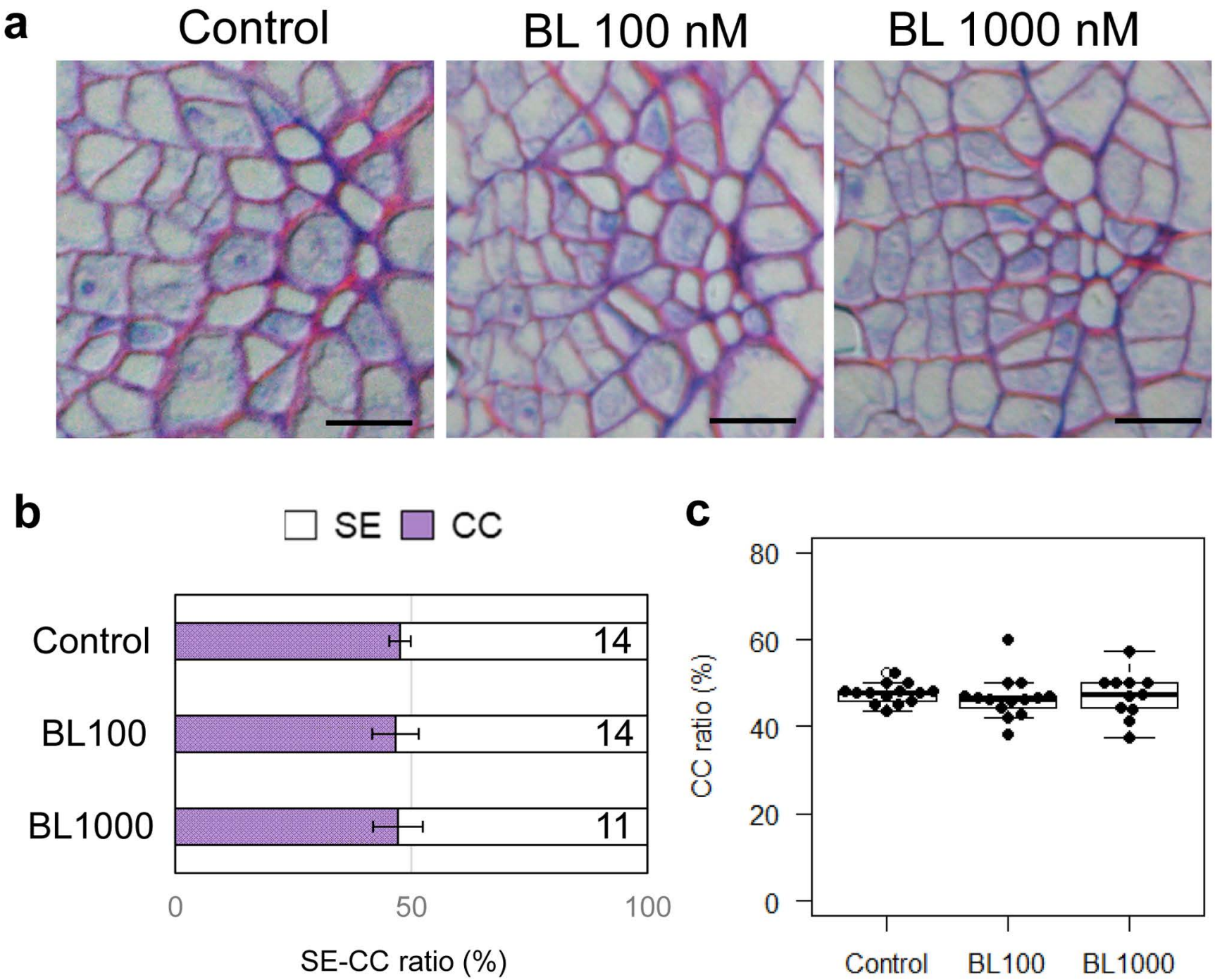


Supplementary Fig. 9| Distribution of the SE/CC ratio among samples
Box plots of Fig. 4e and Fig. 5c were shown. Median values were indicated by central lines. First (Q1) and third (Q3) quartile were shown as a box. Lines show the range of Q1+1.5x interquartile and Q3-1.5x interquartile. Dots indicated distributions of each plot.



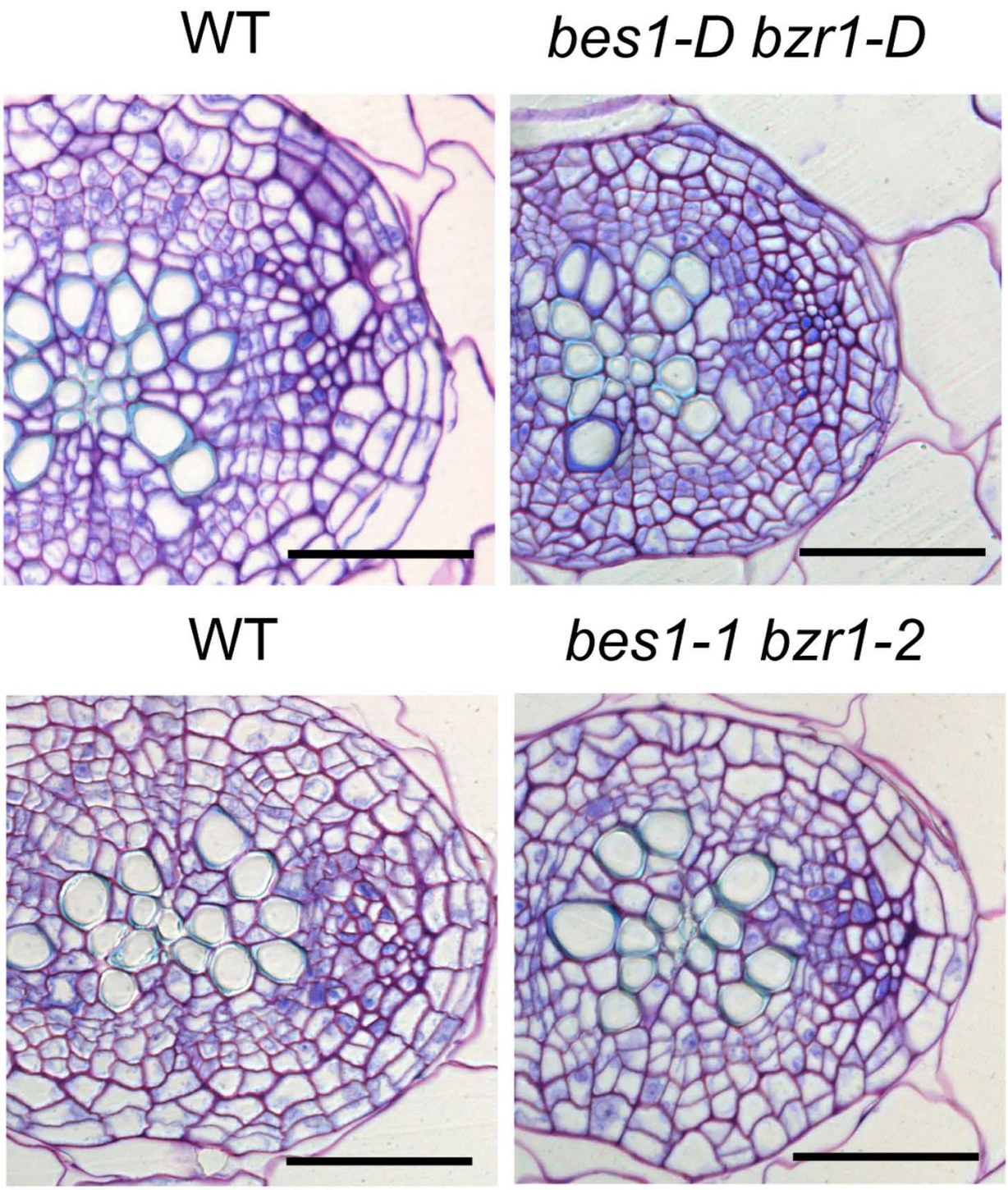
Supplementary Fig. 10| Expression pattern of GSK3s in VISUAL and VISUAL-CC transcriptome data

a, Normalized expression levels of procambium (*AtHB8*), xylem *(IRX3*), phloem SE (*CALS7*) and SKI/II GSK3 subgroup genes in VISUAL transcriptome data. Error bars indicate SD (n=3). **b,** Normalized expression levels of procambium (*AtHB8*), xylem *(IRX3*), phloem SE (*CALS7*) and SKI/II GSK3 subgroup genes in VISUAL-CC transcriptome data.



Supplementary Fig. 11 | Effect of brassinolide treatment on phloem development

a, Toluidine blue-stained transverse sections of mock-treated (DMSO) and bikinin-treated hypocotyls. SE: white empty cell; CC: dense purple cell. **b**, SE/CC ratios (%) in the WT treated with none (control), 100 nM BL, and 1000 nM BL were calculated from toluidine blue-stained sections (n = 11– 14). Numbers of individuals are marked. **c**, Box plot of (b) was shown. Scale bars: 10 μm.



Supplementary Fig. 12 | Transverse sections of *bes1 bzr1* mutants

Toluidine blue-stained transverse sections for 11-day-old hypocotyls of WT, *bes1-D bzr1-D* (gain-of-function), and *bes1-1 bzr1-2* (loss-of-function) mutant plants. Scale bars: 50 μm.

Supplementary Table 1. The list of VC genes (67).

AGI code	Description (based on TAIR)
At1g01470	Late embryogenesis abundant protein (LEA14)
At1g10380	Putative membrane lipoprotein
At1g12090	extensin-like protein (ELP)
At1g13380	Protein of unknown function (DUF1218) (DUF1218)
At1g13590	phytosulfokine 1 precursor (PSK1)
At1g22710	sucrose-proton symporter 2 (SUC2)
At1g49310	transmembrane protein
At1g49500	transcription initiation factor TFIID subunit 1b-like protein
At1g59740	NRT1/ PTR FAMILY 4.3
At1g59960	NAD(P)-linked oxidoreductase superfamily protein
At1g68740	PHO1;H1
At1g76130	alpha-amylase-like 2 (AMY2)
At1g77380	amino acid permease 3 (AAP3)
At2g02020	NRT1/ PTR FAMILY 8.4
At2g02130	low-molecular-weight cysteine-rich 68 (LCR68)
At2g04160	Subtilisin-like serine endopeptidase family protein (AIR3)
At2g19590	ACC oxidase 1 (ACO1)
At2g22860	phytosulfokine 2 precursor (PSK2)
At2g30070	potassium transporter 1 (KT1)
At2g37130	Peroxidase superfamily protein
At2g44380	Cysteine/Histidine-rich C1 domain family protein
At2g46690	SMALL AUXIN UPREGULATED RNA 32 (SAUR32)
At3g09260	BGLU23
At3g12730	SAPL
At3g12750	zinc transporter 1 precursor (ZIP1)
At3g14560	hypothetical protein
At3g14840	LYSM RLK1-INTERACTING KINASE 1 (LIK1)
At3g15950	DNA topoisomerase-related
At3g16450	JACALIN-RELATED LECTIN 33 (JAL33)
At3g16460	JACALIN-RELATED LECTIN 34 (JAL34)
At3g20370	TRAF-like family protein
At3g21770	Peroxidase superfamily protein
At3g23050	indole-3-acetic acid 7 (IAA7)
At3g60720	plasmodesmata-located protein 8 (PDLP8)
At3g63110	isopentenyltransferase 3 (IPT3)
At4g12470	azelaic acid induced 1 (AZI1)
At4g12550	Auxin-Induced in Root cultures 1 (AIR1)
At4g14465	AT-hook motif nuclear-localized protein 20 (AHL20)
At4g15660	GRXS8

At4g15690	GRXS5
At4g19840	phloem protein 2-A1 (PP2-A1)
At4g21960	Peroxidase superfamily protein
At4g27410	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein (RD26)
At4g32290	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
At4g32870	Polyketide cyclase/dehydrase and lipid transport superfamily protein
At4g35480	RING-H2 finger A3B (RHA3B)
At4g36410	ubiquitin-conjugating enzyme 17 (UBC17)
At4g37540	LOB domain-containing protein 39 (LBD39)
At5g01210	HXXXD-type acyl-transferase family protein
At5g01840	ovate family protein 1 (OFP1)
At5g02260	expansin A9 (EXPA9)
At5g02600	NaKR1
At5g07010	sulfotransferase 2A (ST2A)
At5g18240	myb-related protein 1 (MYR1)
At5g23820	MD2-RELATED LIPID RECOGNITION 3 (ML3)
At5g24800	BASIC LEUCINE ZIPPER 9 (BZIP9)
At5g26260	TRAF-like family protein
At5g26280	TRAF-like family protein
At5g28770	BASIC LEUCINE ZIPPER 63 (BZIP63)
At5g43380	type one serine/threonine protein phosphatase 6 (TOPP6)
At5g43580	UNUSUAL SERINE PROTEASE INHIBITOR (UPI)
At5g49660	CEPR1 / XIP1
At5g54130	Calcium-binding endonuclease/exonuclease/phosphatase family
At5g59080	hypothetical protein
At5g63710	Leucine-rich repeat protein kinase family protein
At5g64120	Peroxidase superfamily protein
At5g65970	Seven transmembrane MLO family protein (MLO10)

Supplementary Table 2

Primers used in this study for qRT-PCR

name	sequence (5'-3')
CPD-L	AACCCTTGGAGATGGCAGA
CPD-R	GTAACCGGGACATAGCCTTG
DWF4-L	TTCTCGTTATGACCAACCTAATCTC
DWF4-R	AGGATGACGCTCCGTTGTT
UBQ14-L	TCCGGATCAGCAGAGGTT
UBQ14-R	TCTGGATGTTGTAGTCAGCAAGA
APL-L	TGGATATTCAGCGCAACGTA
APL-R	TGCACTTCCATTTGCATCTC
SUC2-L	TAGCCATTGTCGTCCCTCA
SUC2-R	CCACCACCGAATAGTTCGTC
IRX3-L	TGACATGAATGGTGACGTAGC
IRX3-R	CATCAAATGCTCCTTATCACCTT
SEOR1-L	AAGACACCAACGCCTCCA
SEOR1-R	CGATAGCATAGGAGACACTATCAAGA
CALS7-L	GCAGTAATGGAACTCCCTGAGA
CALS7-R	GGCTGAATGGAATCTTGGTC
SAPL-L	AGAGCCATCTCCAGAAGTTCA
SAPL-R	CCTTCGAAGATCCAACATGG
TCH4-L	GCTCAACAAAGGATGAGATGG
TCH4-R	CCTCTTCGCATCCGTACAAT
BR6ox2-L	CCCATGGAGATGGATGGA
BR6ox2-R	CTTTCCAGGGCAAAGCCTA
SULTR2:1-L	AACGATCTCATGGCTGGTTTA
SULTR2:1-R	TTGCATAACCAATGCTCTGC
NaKR1-L	GCTCAGTTTTGGCCTGAGATT
NaKR1-R	GTGGTGAATCAGCCAGTCCT
CEPR1-L	TATGGCTGGCACCTATGGTT
CEPR1R	GATCGTTGCTTTGGACGAGT
FTIP1-L	GCGCAAGATGTTGAGCCTA
FTIP1-R	TTGTACTTTAACGAAAGCTTGAGG
MYR1-L	GAAGTAGACGAAAGTCACAGTGAGAG
MYR1-R	GGCATCACTTATGGGTAAGTTCA

Supplementary Method

VISUAL-CC

VISUAL-CC consists of two distinct steps; vascular stem cell formation and subsequent phloem differentiation. As the initial step, 6 or 7 day-old seedlings were cultured with the conventional VISUAL medium for 2 days in order to induce sufficient amount of (pro)cambial cells. After that samples are transferred into VISUAL-CC medium for SE-CC complex differentiation.

Materials

Growth of plant samples before VISUAL induction

1. MS growth medium: It contains 2.2 g/L MS Basal Medium (Sigma), 10 g/L sucrose and 0.5 g/L 2-morpholinoethanesulfonic acid monohydrate (MES) in Milli-Q water and the pH is adjusted to 5.7 with KOH. The solution is autoclaved at 120°C for 20 min and can be stored at room temperature up to several weeks.
2. Sterilizing solution: Sodium hypochlorite solution is diluted in Milli-Q water in the ratio 1:9 (v/v) and 0.1% of Triton-X100 is added. This solution is prepared immediately before the sterilizing procedure.
3. Sterilized 6-well plate (Sumilon)
4. Autoclaved Milli-Q water
5. Surgical tape
6. Continuous light chamber (22°C, 45–55 μmol/m^2/s)
7. Rotary shaker (Taitec)

VISUAL and VISUAL-CC

1. VISUAL base medium: It contains 2.2 g/L MS Basal Medium and 50 g/L D(+)-Glucose in Milli-Q water and the pH is adjusted to 5.7 with KOH The solution is autoclaved at 120°C for 20 min and can be stored at room temperature for several weeks.
2. VISUAL-CC base medium: It contains 2.2 g/L MS Basal Medium and 10 g/L D(+)-Glucose in Milli-Q water and the pH is adjusted to 5.7 with KOH The solution is autoclaved at 120°C for 20 min and can be stored at room temperature for several

weeks. Note that Glucose concentration is different from that of VISUAL base medium.

3. 2,4-D stock: 2.5 g/L 2,4-D stock dissolved in autoclaved Milli-Q water and sterilized through 0.22 μm filter units. Stored in small amounts in sampling tubes at -20°C.
4. Kinetin stock: 0.5 g/L Kinetin stock dissolved in 0.1 M KOH and sterilized through 0.22 μm filter units. Stored in small amounts in sampling tubes at -20°C.
5. Bikinin stock: 10 mM Bikinin stock dissolved in DMSO and sterilized through 0.22 μm filter units. Stored in small amounts in sampling tubes at -20°C.
6. Sterilized 12-well plate (Sumilon)
7. Surgical forceps
8. Continuous light chamber (22°C, 60–70 μmol/m^2/s)
9. Rotary shaker (Taitec)

Methods

Growth of plant samples before VISUAL induction

1. Sterilizing solution is added to the Arabidopsis seeds in 1.5 mL sampling tubes and gently mixed using a rotator for 5 min. The tubes are transferred inside a clean bench and allowed to stand for further 5 min. The sterilizing solution is then removed using a pipette and the seeds are washed with autoclaved Milli-Q water three times. The seeds are soaked in water at 4°C for 2 days to keep the germination timing constant.
2. 10 mL of the prepared MS growth medium is poured into each well of a 6-well plate. Seeds are sown at a density of 8-10 seeds/well containing the MS growth medium and the plate is sealed with surgical tape. The well plate is incubated for 6-7 days under continuous light (22°C, 45–55 μmol/m^2/s) with shaking at 110 rpm on a rotary shaker.

VISUAL

1. 2,4-D stock, kinetin stock and bikinin stock are defrosted at room temperature before use. The tubes are transferred inside a clean bench and added to the VISUAL base medium to obtain a final concentration of 1.25 mg/L 2,4-D, 0.25 mg/L kinetin and 10 μM bikinin. About 2.5 mL of the above medium is then added into each well of a

12-well plate.

2. A pair of sharp surgical forceps are used to cut the bottom half of Arabidopsis 6-7 day-old plants across the center of the hypocotyl and the roots are removed. About 4 of the Arabidopsis explants are then transferred carefully to each well containing the induction medium using forceps and the 12-well plate sealed with surgical tape. The explants are cultured for 2 days under continuous light (22°C, 60–70 µmol/m^2/s) with shaking at 110 rpm on a rotary shaker.

VISUAL-CC

1. 2,4-D stock, kinetin stock and bikinin stock are defrosted at room temperature before use. The tubes are transferred inside a clean bench and added to the VISUAL-CC base medium to obtain a final concentration of 0.25 mg/L 2,4-D, 0.25 mg/L kinetin and 1 µM bikinin. About 2.5 mL of the above medium is then added into each well of a 12-well plate. Note that auxin and bikinin concentration is decreased when compared to the VISUAL.
2. VISUAL-induced samples were transferred into the new CC medium and then cultured for 4 days under dark condition (22°C) with shaking at 110 rpm on a rotary shaker. Note that light severely affect CC differentiation ratio.