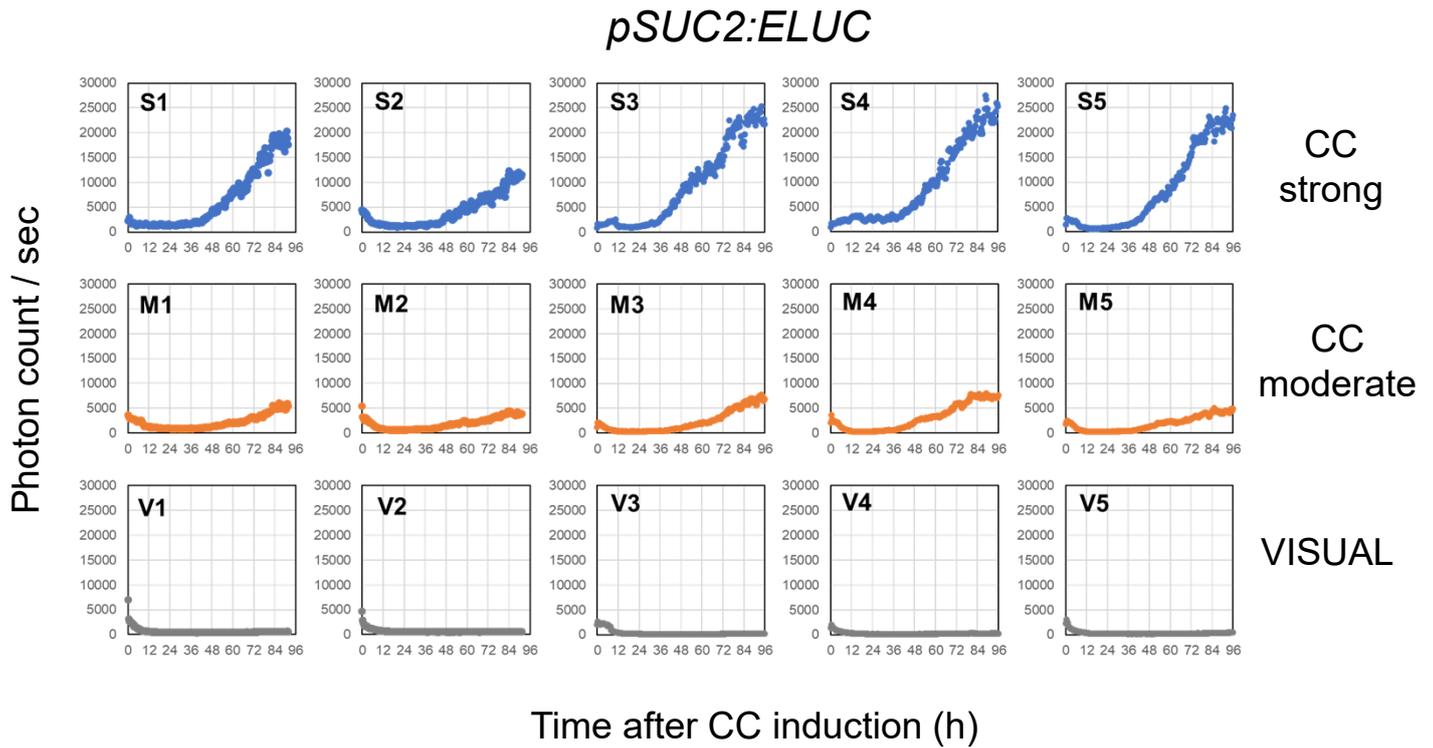


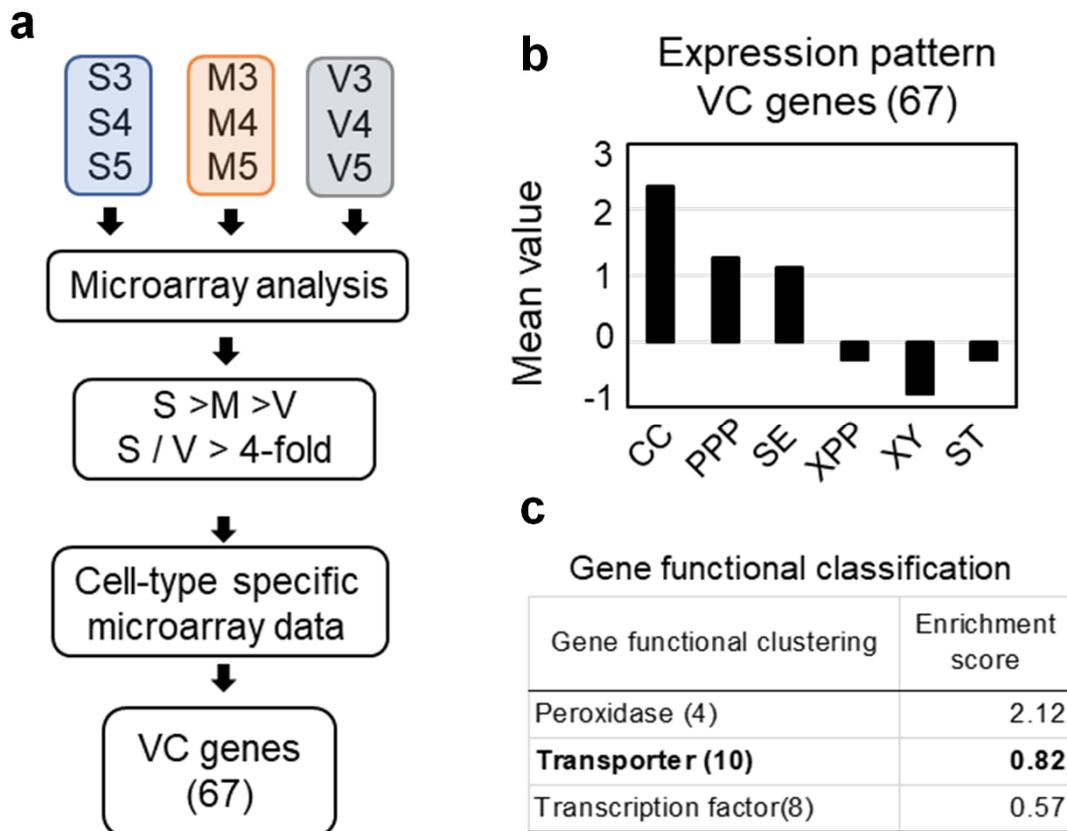
Supplementary Fig. 1| *smxl4 smxl5* mutants suppressed CC differentiation in VISUAL-CC

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 ($*P < 0.05$, $n = 3$).



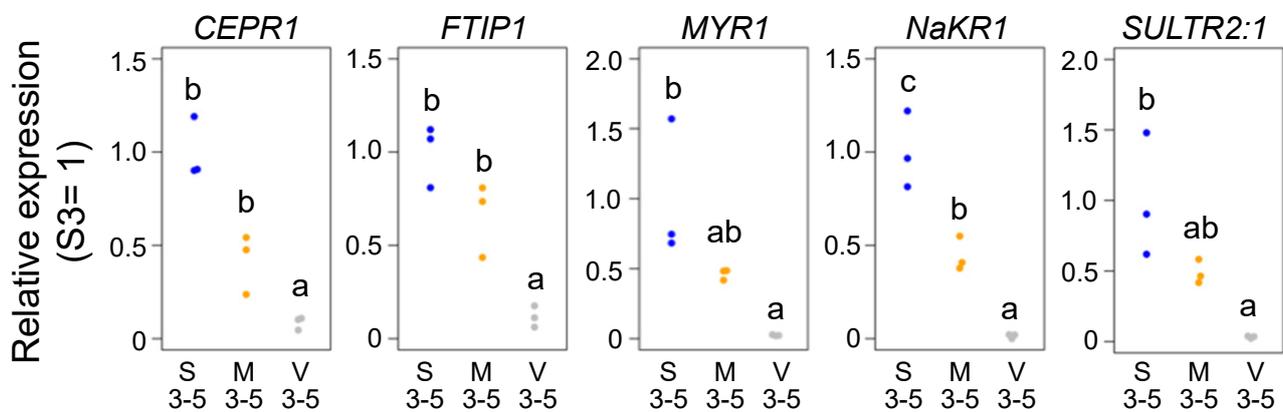
Supplementary Fig. 2 | Raw data from time-course analysis of *pSUC2:ELUC* plants

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.



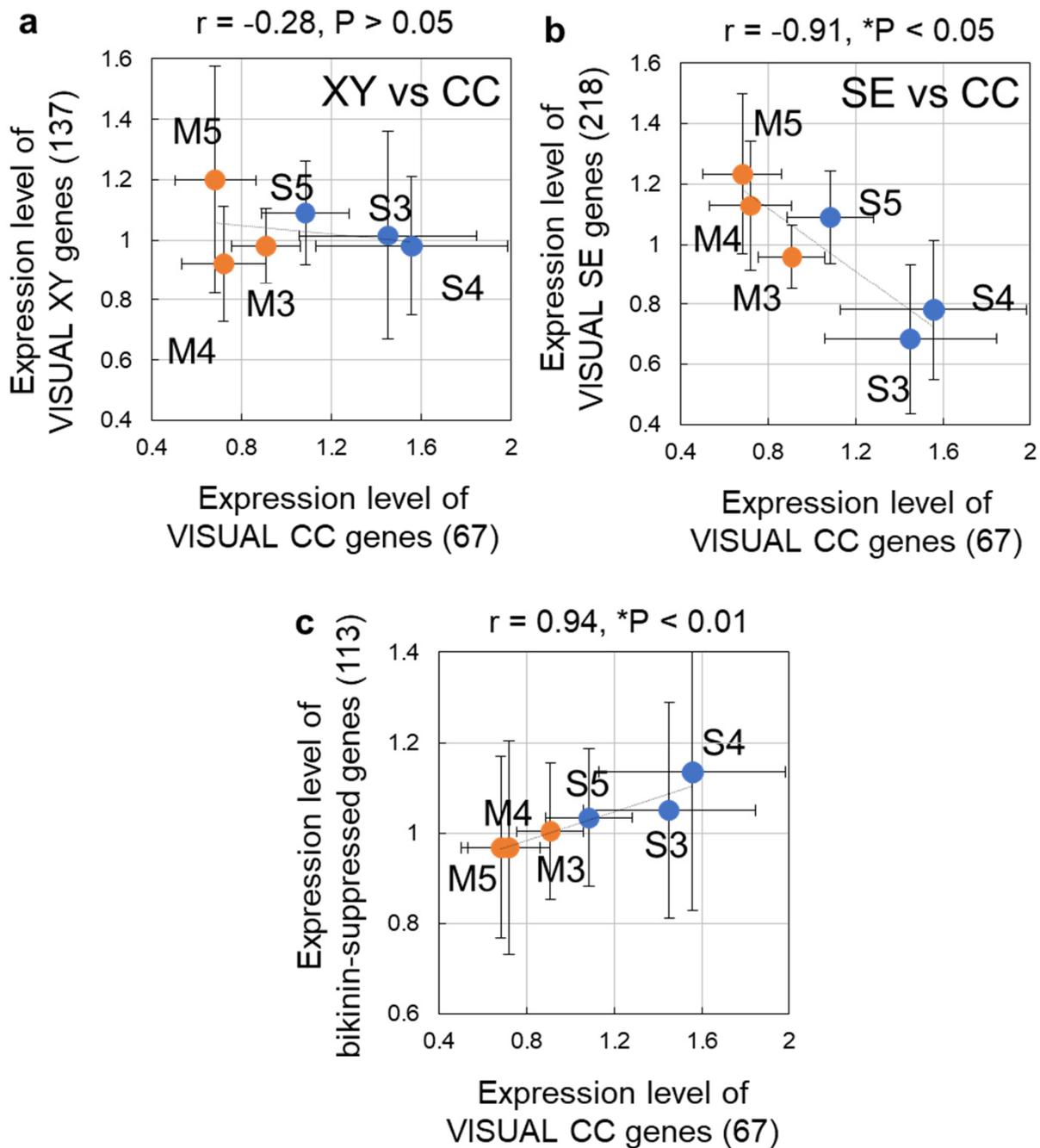
Supplementary Fig. 3| Characterization and molecular function of VC genes

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 dataset¹⁴. 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.



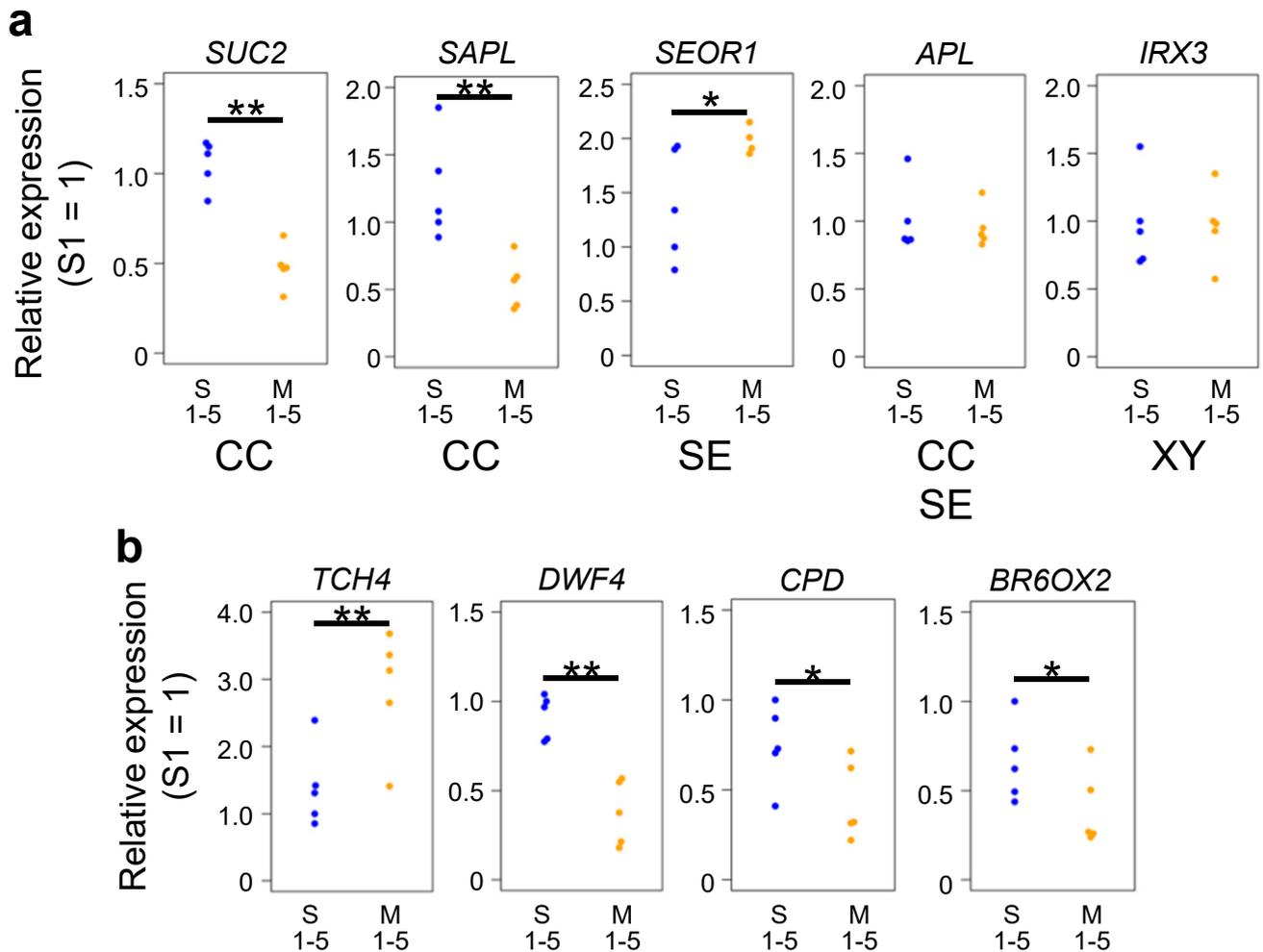
Supplementary Fig. 4 | Statistical differences in expression levels of CC-related genes among the S, M, and V samples

Expression levels of *CEPR*, *FTIP1*, *MYR1*, *NaKR1*, and *SULTR2:1* were confirmed 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).



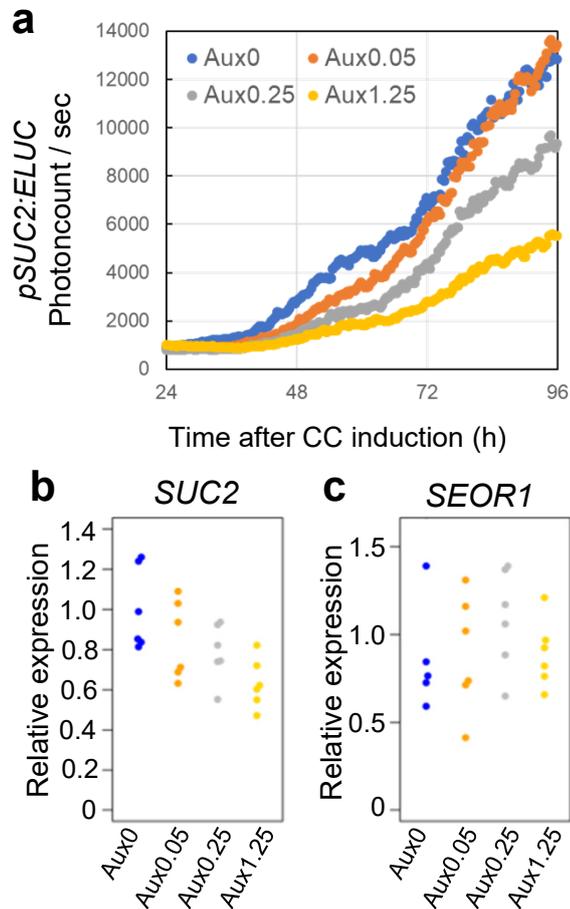
Supplementary Fig. 5 | Correlation analysis in microarray data between the S and M samples

a, VC genes (67) vs VX genes (137) **b**, VC genes (67) vs VS genes (218) **c**, VC genes (67) vs bikiniin-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

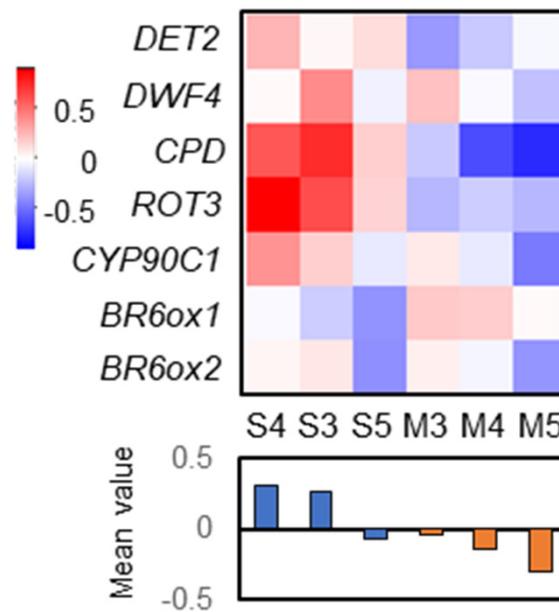
a, Expression levels of *SUC2* (as CC), *SAPL* (also as CC), *SEOR1* (as SE), *APL* (as CC+SE), and *IRX3* (XY) were quantified using qRT-PCR and compared statistically between the S and M samples. Asterisks indicate significant differences using the Student's t-test (** $P < 0.005$; * $P < 0.05$). **b**,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

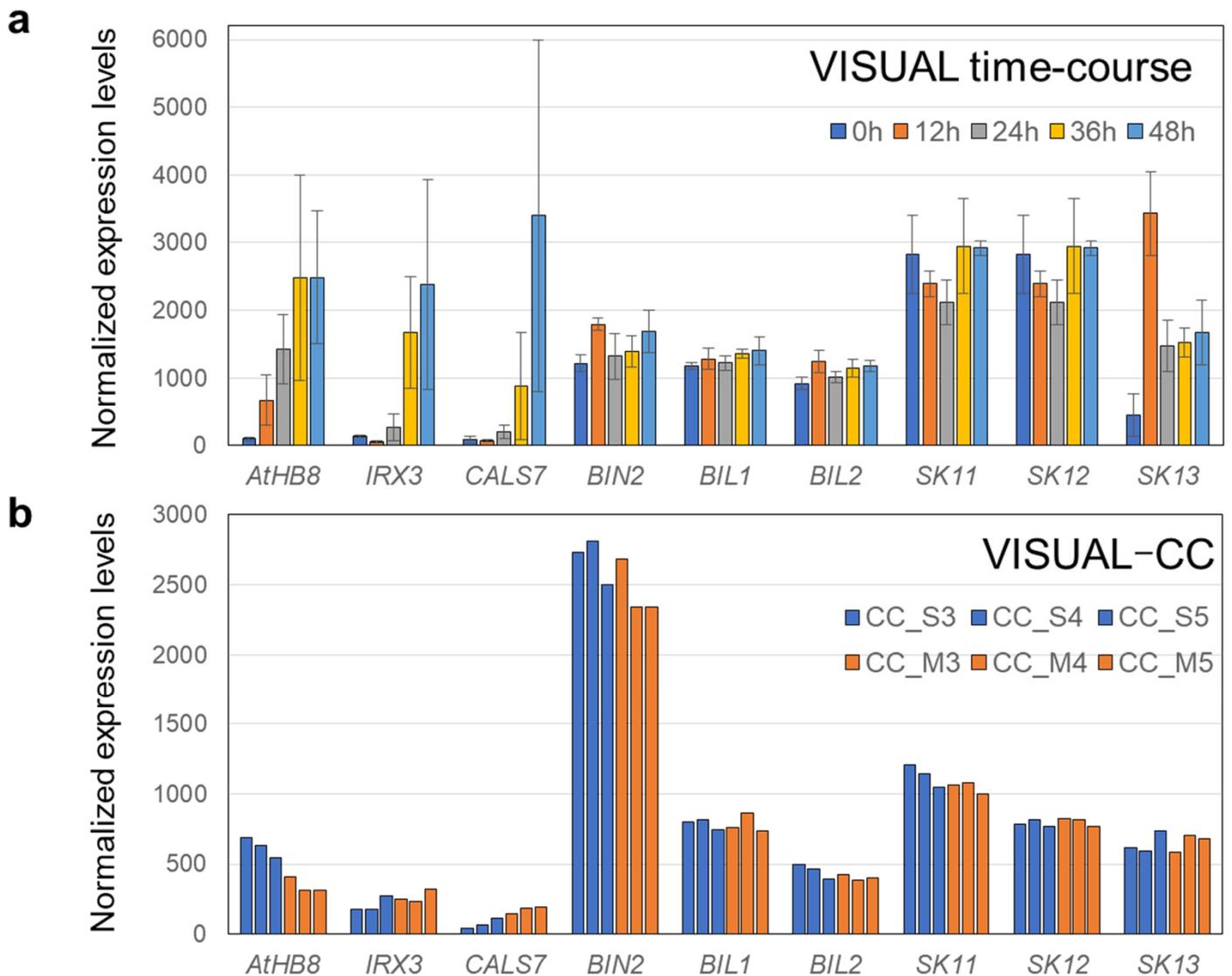
a, Time-course of $pSUC2:ELUC$ signal intensities in VISUAL-CC cultures containing different concentrations of auxin (mg/L). **b** and **c**, Expression levels of *SUC2* (**b**) and *SEOR1* (**c**) in VISUAL-CC samples from cultures containing different concentrations of auxin. There are no significant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

BR biosynthesis-related genes
(negatively regulated by bikinin)



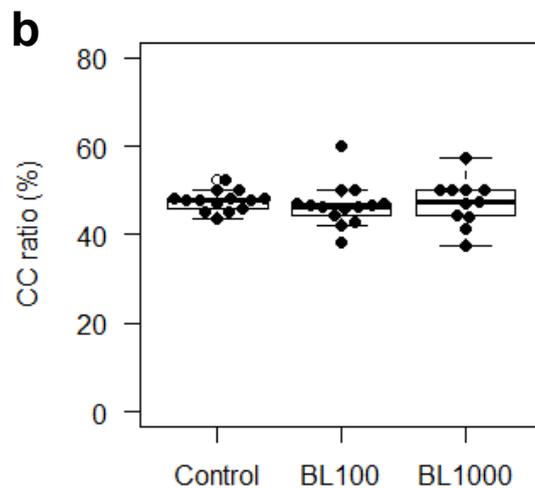
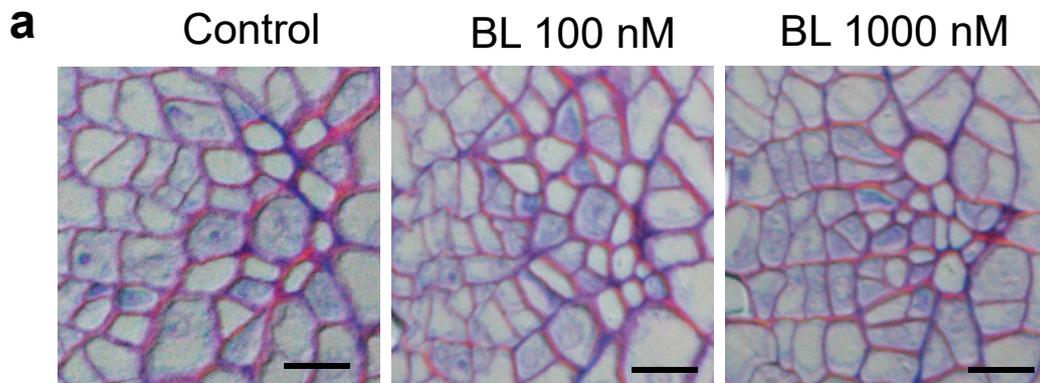
Supplementary Fig. 8| Heat map of expression levels of BR biosynthesis-related genes in S and M samples.

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.



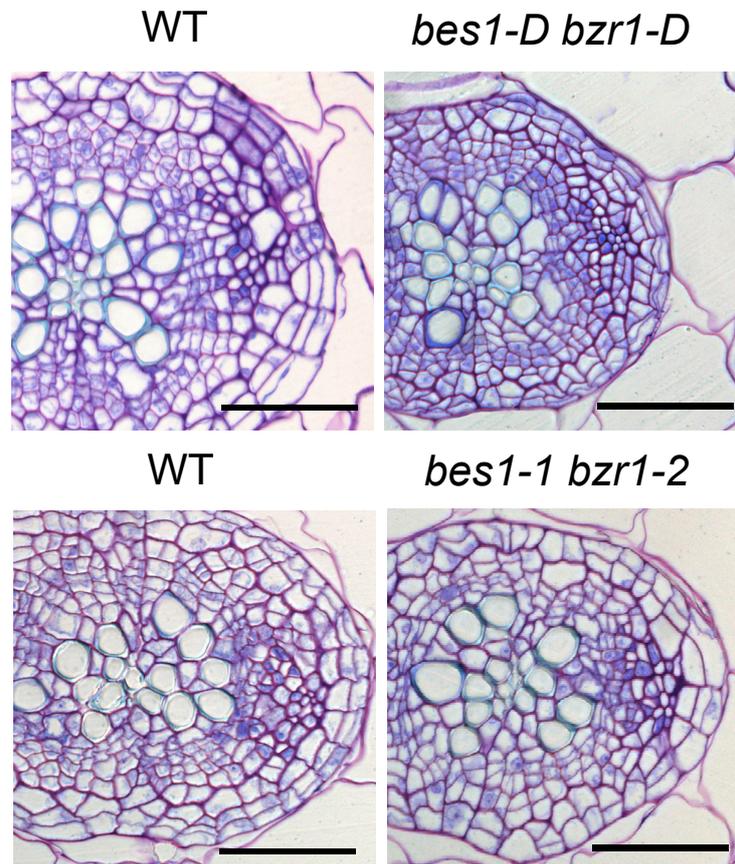
Supplementary Fig. 9| Expression pattern of GSK3s in VISUAL and VISUAL-CC transcriptome data

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. 10| 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. Scale bars: 10 μm .



Supplementary Fig. 11| Transverse sections of *bes1 b zr1* mutants

Toluidine blue-stained transverse sections for 11-day-old hypocotyls of WT, *bes1-D b zr1-D* (gain-of-function), and *bes1-1 b zr1-2* (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	GCAGTAATGGA ACTCCCTGAGA
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