Supplementary materials and methods

Plant material, growth conditions and histological analyses

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was the wild-type for the generation of all plant material described in this study. General plant handling and transformation protocols as well as ß-glucuronidase (GUS) staining followed standard procedures (Weigel and Glazebrook, 2001). The *ces-1* line was identified in the SIGnAL database (SALK Institute) and corresponds to line SALK_082100. This line segregated kanamycin resistance 3:1 and was defined by the absence of CES transcript.

For hypocotyl elongation assays mother plants were cultivated in a controlled environment of 16 hr/ 8 hr light/dark cycle (100 µmol $m^{-2}s^{-1}$ white light) at a temperature of 21°C/ 17°C (±1). Seeds of all lines used in one experiment were harvested at the same time and plated on ATS medium (Lincoln *et al.*, 1990). Hypocotyl elongation assays were performed as described previously (Poppenberger *et al.*, 2005). For hypocotyl growth response assays 24-epiBL (OlChemIm Ltd, Olomouc, Czech Republic) and Brz2001 (Sekimata *et al.*, 2001) were dissolved in DMSO and added to ATS medium in the required concentrations. Seeds were incubated on vertical plates either in the dark (following a 4 hr light impulse) or in the light and hypocotyl elongation was measured at different time-points. For each experiment the 20 tallest seedlings originating from 40 seeds (to correct for late germination) were analyzed in 3 replicates and the standard error (SE) was calculated.

For quantitative PCR analysis 10-day-old seedlings were grown vertically on ATS plates and transferred to flasks containing liquid ATS medium and incubated for 48 hr on an orbital shaker at 20 rpm. 24-epiBL and Brz2001 were then added to the flasks in the indicated concentrations and after incubation seedlings were ground in liquid nitrogen.

Western blotting

For Western blot analysis 100 mg of plant material was ground to a fine powder and extracted with 300 µl loading buffer (66 mM TRIS/HCl pH=6.8, 133 mM DTT, 2.7% SDS, 13% glycerol and 0.01% bromophenol blue). 20 µl of this extract were separated by SDS-PAGE (10% gel) and blotted onto Immobilon P (Millipore Cooperation, Bedford, MA, USA). Membranes were probed with a rabbit anti-c-Myc antibody. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, CA) was used as secondary antibody and detected by enhanced chemiluminescence using CDP-Star reagent (Amersham Biosciences).

Protein interaction assays and kinase assays

For yeast two-hybrid assays, CES, BEE1 and BEE3 were PCR amplified from Col-0 cDNA using specific primers and cloned into GAL4 bait (pGADT7) and prey (pGBKT7) vectors, respectively (Clontech). The sequenced constructs were introduced into the yeast two-hybrid strain PJ69-4A (James *et al.*, 1996) and ß-galactosidase activity was assayed.

Kinase assays were performed as described previously (Rozhon *et al.*, 2010).

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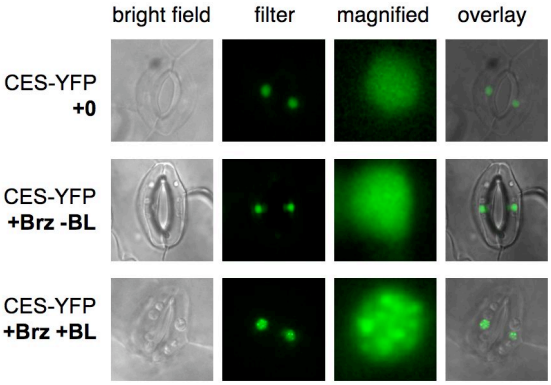
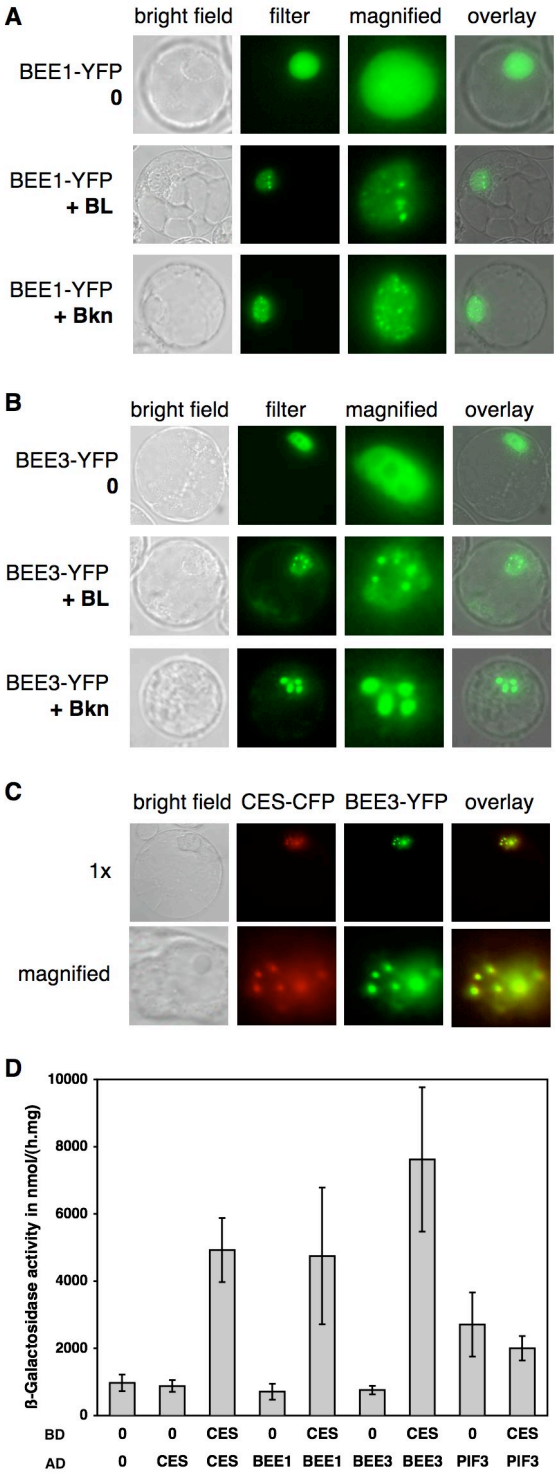
Supplementary figure legends

Figure S1 Analysis of CES-YFP subcellular localization in *Arabidopsis* plants stably transformed with $35S_{pro}$:CES-YFP. CES-YFP localizes diffusely to the nucleus in 14-day-old seedlings of $35S_{pro}$:CES-YFP. Nuclear compartmentalization was induced by application of 1 µM 24-epiBL for 2 h to plants pre-treated with 2.5 µM Brz2001 for 24 h. Application of Brz2001 on its own did not induce nuclear body formation.

Figure S2 Analysis of BEE1-YFP and BEE3-YFP subcellular localization and interactions of CES with BEE1 and BEE3 in yeast two-hybrid assays. (**A**) $35S_{pro}$:BEE1-YFP reporter expression in *Arabidopsis* protoplasts treated with 24-epiBL (1 µM) 2 hr as compared to untreated controls. (**B**) $35S_{pro}$:BEE3-YFP reporter expression in *Arabidopsis* protoplasts treated with 24-epiBL (1 µM) for 2 hr as compared to untreated controls. (**C**) Images of a representative $35S_{pro}$:CES-CFP and $35S_{pro}$:BEE3-YFP co-expressing protoplast after 2 hr of 24-epiBL treatment. (**D**) Quantitative yeast two-hybrid analysis of ß-galactosidase activity in cells transformed with combinations of CES and BEE1, BEE3 resp. as compared to control strains. Data points represent the average of three replicates. The standard deviation is shown.

Poppenberger *et al.* Supp Fig 1




A
bright field
filter
magnified
overlay
BEE1-YFP
0
BEE1-YFP
+ BL
BEE1-YFP
+ Bkn
B
bright field
filter
magnified
overlay
BEE3-YFP
0
BEE3-YFP
+ BL
BEE3-YFP
+ Bkn
C
bright field
CES-CFP
BEE3-YFP
overlay
1x
magnified
D
β-Galactosidase activity in nmol/(h.mg)
10000
8000
6000
4000
2000
0
BD
AD
0 0
0 CES
CES CES
0 BEE1
CES BEE1
0 BEE3
CES BEE3
0 PIF3
CES PIF3

Supplementary Table 3

BR-induced genes significantly up-regulated in *ces-D* and their expression in *35Sp:cMyc-CES-SRDX* plants.

Public ID		*ces-D*/Col-0			SRDX/Col-0			Gene	Description
		Ratio[a]	p-value[b]	q-value[c]	Ratio[d]	p-value[b]	q-value[c]	symbol	
At4g25810	1,3	4.92	0.0001	0.0046	2.42	0.0001	0.0129	XTR6	Xyloglucan-xyloglucosyl transferase
At4g16563	3	4.83	0.0000	0.0029	2.34	0.0000	0.0095	---	---
At1g35140	3	4.71	0.0016	0.0172	1.84	0.0137	0.0718	PHI-1	Phosphate-induced 1
At3g45970	3	4.68	0.0002	0.0073	1.16	0.3219	0.3537	AtEXLA1	Expansin-like
At1g62440	1	4.53	0.0185	0.0579	1.13	0.8141	0.5569	LRX2	Extensin; structural constituent of cell wall
At3g60140	2,3	3.99	0.0004	0.0097	0.22	0.0072	0.0522	DIN2	Dark-induced 2
At5g24030	3	3.75	0.0000	0.0040	1.55	0.0023	0.0324	SLAH3	SLAC1 homologue 3
At1g10550	1,3	3.66	0.0000	0.0026	1.82	0.0000	0.0083	XTH33	Xyloglucan-xyloglucosyl transferase
At2g35290	3	3.59	0.0006	0.0107	0.94	0.6106	0.4858	---	---
At2g19800	1,2,3	3.36	0.0003	0.0079	3.30	0.0020	0.0308	MIOX2	Myo-inositol oxygenase 2
At4g38400	1,2,3	3.25	0.0001	0.0060	1.17	0.3685	0.3790	AtEXLA2	Expansin-like
At5g57550	2	3.21	0.0173	0.0557	2.84	0.0025	0.0335	XTR3	Xyloglucan-xyloglucosyl transferase
At3g59350	3	3.12	0.0001	0.0048	1.36	0.0441	0.1269	---	---
At1g77640	3	3.12	0.0008	0.0126	0.86	0.3818	0.3859	---	---
At2g27920	2,3	3.07	0.0001	0.0056	0.73	0.0635	0.1530	SCPL51	Serine carboxypeptidase
At5g44680	3	2.97	0.0002	0.0064	0.80	0.0028	0.0353	---	---
At2g34510	1,2,3	2.95	0.0054	0.0307	1.46	0.0292	0.1029	---	Anchored to membrane
At4g08950	1,2,3	2.90	0.0019	0.0187	2.54	0.0003	0.0153	---	---
At2g47440	1,2,3	2.81	0.0003	0.0082	0.89	0.3103	0.3471	---	Heat shock protein folding
At2g42580	3	2.75	0.0001	0.0048	1.41	0.0106	0.0630	TTL3	Leaf vascular tissue pattern formation
At5g54380	3	2.71	0.0009	0.0131	1.18	0.2025	0.2784	THE1	Regulation of cell growth
At3g06070	3	2.56	0.0006	0.0108	0.46	0.0000	0.0074	---	---
At5g41080	3	2.54	0.0012	0.0151	1.96	0.0156	0.0757	---	---
At5g13220	3	2.50	0.0017	0.0179	1.83	0.0169	0.0786	JAS1	Jasmonate-associated 1
At1g23030	1,3	2.47	0.0006	0.0110	1.04	0.6621	0.5061	---	Ubiquitin-protein ligase activity
At5g57560	1,3,4	2.41	0.0013	0.0158	0.71	0.1628	0.2469	TCH4	Xyloglucan-xyloglucosyl transferase
At5g06720	1,2,3	2.41	0.0008	0.0123	1.59	0.0016	0.0272	---	Hydrogen peroxide catabolic process
At2g16060	4	2.38	0.0008	0.0123	1.64	0.0030	0.0361	AHB1	Oxygen transporter activity
At3g47340	4	2.36	0.0005	0.0106	1.68	0.0524	0.1384	ASN1	Dark-induced; amino acid biosynthesis
At2g33570	3	2.35	0.0006	0.0110	1.11	0.1984	0.2753	---	---
At3g62720	3	2.33	0.0017	0.0176	1.28	0.0133	0.0705	ATXT1	Xylosyltransferase 1
At1g11260	2,3	2.31	0.0001	0.0048	1.32	0.0137	0.0717	STP1	Sugar transporter 1
At4g35320	2,3	2.28	0.0001	0.0059	0.75	0.0622	0.1521	---	---
At2g14900	2,3	2.27	0.0019	0.0188	1.09	0.2872	0.3336	---	Endomembrane system
At3g54030	3	2.25	0.0429	0.0950	3.15	0.0174	0.0796	---	---
At1g66160	3	2.25	0.0028	0.0222	1.20	0.0463	0.1301	---	---
At4g39830	2,3	2.25	0.0019	0.0186	0.88	0.0319	0.1075	---	L-ascorbate oxidase activity
At2g32150	2,3	2.21	0.0007	0.0117	0.88	0.0674	0.1568	---	Hydrolase activity
At3g50560	3	2.20	0.0005	0.0104	0.88	0.5540	0.4643	---	---
At1g44350	3	2.19	0.0069	0.0348	1.44	0.0221	0.0895	ILL6	IAA-leucine resistant (ILR)-like gene 6
At5g39580	3	2.17	0.0011	0.0145	2.66	0.0048	0.0438	---	Hydrogen peroxide catabolic process
At5g06870	3	2.16	0.0001	0.0059	1.01	0.8902	0.5800	PGIP2	Polygalacturonase inhibiting protein 2
At3g05900	3	2.16	0.0012	0.0148	1.26	0.0007	0.0192	---	---
At3g58620	3	2.16	0.0033	0.0244	1.10	0.7000	0.5189	TTL4	Tetratricopeptide-repeat thioredoxin-like 4
At2g28400	2,3	2.16	0.0003	0.0085	0.94	0.2643	0.3188	---	---
At4g31800	3	2.13	0.0006	0.0107	3.63	0.0001	0.0101	WRKY18	WRKY DNA-binding protein 18
At4g25260	2,3	2.11	0.0014	0.0162	1.02	0.7987	0.5518	---	Pectinesterase activity, shade avoidance
At3g06770	3	2.10	0.0002	0.0073	0.79	0.0146	0.0739	---	---
At3g30775	4	2.10	0.0002	0.0066	2.70	0.0036	0.0385	ERD5	Early responsive to dehydration 5
At2g34300	3	2.09	0.0056	0.0315	1.25	0.1425	0.2304	---	---
At5g58670	2,3	2.09	0.0067	0.0341	0.74	0.0096	0.0603	ATPLC1	Phospholipase C, lipid metabolic process
At4g31000	3	2.06	0.0035	0.0251	1.94	0.0417	0.1235	---	Calmodulin binding
At5g03120	3	2.03	0.0001	0.0047	1.00	0.9322	0.5920	---	---
At2g34930	1,2,3	2.02	0.0290	0.0757	3.63	0.0002	0.0134	---	Defence response
At5g01040	3	2.01	0.0016	0.0171	1.26	0.0773	0.1680	LAC8	Laccase 8
At1g76090	3	2.01	0.0001	0.0048	1.16	0.0832	0.1746	SMT3	Sterol methyltransferase 3
At1g03870	1,2,3	2.01	0.0017	0.0179	1.34	0.0700	0.1601	FLA9	Fasciclin-like arabinogalactan-protein 9

[a] Ratio of the microarray intensity of the *ces-D* muatant and Col-0 controls.
[b] Student´s t-Test p-value.
[c] FDR q-value.
[d] Ratio of the microarray intensity of the dominat-negative over-expressor line CES-SRDX #203 and Col-0 controls.
References: 1, Goda *et al*. 2002; 2, Goda *et al*. 2004; 3, He *et al*., 2005; 4, Müssig *et al*., 2002

Supplementary Table 4

Genes involved in the regulation of transcription (GO 0006355) significantly up-regulated in *cesD*.

Public ID	*cesD*/Col-0			CES-SRDX/Col-0			Gene symbol	Description
	Ratio[a)]	p-value[b)]	q-value[c)]	Ratio[d)]	p-value[b)]	q-value[c)]		
At1g65330	26.51	0.0000	0.0028	0.91	0.9087	0.5854	PHE2	Agamous-like transcription factor
At1g18710	18.91	0.0087	0.0390	1.15	0.8588	0.5721	AtMYB47	Myb-type transcription factor, responsive to JA
At2g44910	14.61	0.0091	0.0400	1.67	0.2417	0.3046	---	Transcription factor activity, shade avoidance
At3g15170	13.57	0.0198	0.0603	0.69	0.6267	0.4925	CUC1	Shoot apical meristem formation
At2g28700	9.95	0.0157	0.0529	2.15	0.4733	0.4284	AGL46	Agamous-like, transcription factor activity
At5g53980	6.85	0.0028	0.0223	0.69	0.4032	0.3963	ATHB52	Homeodomain leucine zipper class I
At2g34600	5.58	0.0031	0.0236	2.43	0.0458	0.1294	JAZ7	Response to JA and chitin
At3g54340	5.00	0.0144	0.0504	9.35	0.0015	0.0261	AP3	Floral homeotic gene
At1g22130	3.28	0.0345	0.0835	1.09	0.9219	0.5891	AGL104	Agamous-like; pollen development, tube growth
At1g77640	3.12	0.0008	0.0126	0.86	0.3818	0.3859	---	Contains a AP2 domain
At1g01250	2.77	0.0265	0.0719	3.90	0.0008	0.0209	---	Putative transcription factor of unkown function
At4g28190	2.75	0.0009	0.0129	1.11	0.3676	0.3784	ULT1	Regulation of inflorescence meristem growth
At5g50570	2.75	0.0000	0.0040	0.91	0.3766	0.3832	---	Putative SBP-box binding transcription factor
At1g44830	2.70	0.0001	0.0061	1.74	0.0671	0.1564	---	ERF/AP2 transcription factor family
At3g58120	2.62	0.0004	0.0088	0.91	0.3981	0.3939	---	BZIP family transcription factors
At5g13220	2.50	0.0017	0.0179	1.83	0.0169	0.0786	JAS1	Overexpression enhances insensitivity to MeJa
At4g37850	2.50	0.0039	0.0265	1.54	0.3549	0.3718	---	Basic helix-loop-helix (bHLH) family protein
At1g21910	2.45	0.0016	0.0173	1.25	0.0819	0.1733	---	ERF/AP2 transcription factor
At3g16770	2.34	0.0001	0.0059	1.04	0.5265	0.4523	ATEBP	Suppressor of Bax-induced cell death
At4g36730	2.28	0.0004	0.0091	1.29	0.0670	0.1563	GBF1	bZIP transcription factor, G-box binding
At2g30590	2.25	0.0004	0.0088	1.48	0.0131	0.0702	WRKY21	WRKY DNA-binding protein, unknown function
At2g14210	2.20	0.0042	0.0272	0.90	0.8608	0.5726	ANR1	Agamous-like, response to nutrient
At2g42380	2.15	0.0255	0.0702	0.13	0.0000	0.0072	---	Heterodimerizes with AtbZIP61, binds G-boxes
At5g60850	2.14	0.0028	0.0225	0.52	0.0001	0.0116	OBP4	Zinc finger protein,
At4g31800	2.13	0.0006	0.0107	3.63	0.0001	0.0101	WRKY18	Pathogen-induced transcription factor
At5g10140	2.08	0.0006	0.0107	2.25	0.0000	0.0082	FLC	Agamous-like; negative regulation of flower development

[a)] Ratio of the microarray intensity of the *cesD* muatant and Col-0 controls.

[b)] t-Test p-value.

[c)] FDR q-value.

[d)] Ratio of the microarray intensity of the dominat-negative over-expressor line CES-SRDX #203 and Col-0 controls.

Supplementary Table 5
Sequences of primers used in this study.

Name		Sequence	
Primers used for cloning and sequencing			
CESpGBKT7-fw	5'	AAGCATATGATGGCACGGTTTGAG CCATA	3'
CESpGBKT7-rv	5'	TGCGGATCCTCAAAAGGGTAATGTTGAACTG	3'
CESp2RT-fw	5'	GTACTCGAGCTAATGGCACGGTTTGAGCC	3'
CESp2RT-rv	5'	TGCGGATCCTCAAAAGGGTAATGTTGAACTG	3'
BEE1pGBKT7-fw	5'	TCTCAATTGAATTCATTATGGCAAATTTCGAGA	3'
BEE1pGBKT7-rv	5'	GAGGATCCAAAAGTCAAAGGGACCATG	3'
BEE3pGBKT7-fw	5'	CACATATGGCGAATCTCTCTTCTGA	3'
BEE3pGBKT7-rv	5'	CAAGGATCCAAAGTCAAAGGGTC	3'
CESfusions-fw-c	5'	CTATCAAGCTTGCCGATGGTGTTATA	3'
CEStranscGUS-rv-a	5'	CTCGGATCCTGCCATTAGAGACACTA	3'
pGWR8-BEE1-1	5'	GATATCACCATGGCAAATTTCGAGAATCT	3'
pGWR8-BEE1-2	5'	GCGGCCGCTAAGGGACCATGTTGATAAAT	3'
pGWR8-BEE3-1	5'	ACCATGGCGAATCTCTCTTCTGA	3'
pGWR8-BEE3-2	5'	GCGGCCGCTAAGGGTCCACGATGATGAATG	3'
pGWR8-CES-1	5'	ACCATGGCACGGTTTGAGCCATA	3'
PGWR8-CES-2	5'	GCGGCCGCTAAAGGGTAATGTTGAACTGAAATTAG	3'
SOER2	5'	GCAGGCATGCAAGCTTATCGATATCTAGA	3'
SOEL2	5'	TGATGTGATATCTAGATCCGAAACTATCA	3'
Primers used for RT-semiquantitative PCR			
At1g25320RT-fw	5'	GTATACTCATTCGGAGTGATCTTGCTA	3'
At1g25320RT-rv	5'	GATATGCTTCATCGGTGGACG	3'
At1g25340RT-fw	5'	GATTGTTCCACTTCCATGTCAGAA	3'
At1g25340RT-rv	5'	TGTCATCCATGTTCCACATTAGATC	3'
CESRT-fw	5'	CTCAGAAGCCAAAAGATGT	3'
CESRT-rv	5'	TCAAAAGGGTAATGTTGAA	3'
CPDRT-fw	5'	GGTGGAAAGTATTCTCATCGTT	3'
CPDRT-rv	5'	ATCACGGCGCTT CACG	3'
DWF4RT-fw	5'	GTCATCCTCAGGAAGTGGTAGT	3'
DWF4RT-rv	5'	TACAGAATACGAGAAACCCTAATAG	3'
ROT3-fw	5'	TATTAACGGGGTGTGGAGGA	3'
ROT3-rv	5'	CAAGTGAGATCGGAGAAGCA	3'
UBQ5-fw	5'	GTCCTTCTTTCTGGTAAACGT	3'
UBQ5-rv	5'	AACCCTTGAGGTTGAATCATC	3'
Primers used for RT-qPCR			
CPD-1	5'	CTTGCTCAACTCAAGGAAGAG	3'
CPD-2	5'	CTCGTAGCGTCTCATTAACCAC	3'
DWF4-3	5'	CCGTTGAAGAGCTTAGGGAAGAG	3'
DWF4-4	5'	CATTTCCCAATCGAAGAGTTTC	3'
ROT3-3	5'	CTTGTAACCCGGTACAGTTGC	3'
ROT3-4	5'	TCCGCTTCATCTTCACAGTC	3'
UBQ5-1	5'	ACCAAGCCGAAGAAGATCAA	3'
UBQ5-2	5'	ATGACTCGCCATGAAAGTCC	3'
Primers used for ChIP-semiquantitative PCR			
CPD-ChIP-1	5'	TTATCGAGCTACATATCAGCAATTC	3'
CPD-ChIP-2	5'	TTACTTAACACTTCCCAAAGTCTG	3'
CPD-ChIP-3	5'	CAGACTTTGGGAAGTGTTAAGTA	3'
CPD-ChIP-4	5'	GAAGAAGAAGATACTCCTAGTAGGAG	3'
CPD-ChIP-5	5'	CGTGATATATAAATATCAATCATCTACG	3'
CPD-ChIP-6	5'	GGAAACGAAAGAGATTGAGTTT	3'
CPD-ChIP-7	5'	CAATAGGAGACAAATAAGAGGTAGA	3'
CPD-ChIP-8	5'	GGACACCAAACGTTAAAACTAA	3'
CPD-ChIP-9	5'	AATACTCAAACAGTATATGGAAGAACCG	3'
CPD-ChIP-10	5'	GGCTGTGAGTATTTTGTAGGTTACAAA	3'
CPD-ChIP-13	5'	ATCCCAAGATTAGTATATATCTGCC	3'
CPD-ChIP-14	5'	CGTCTATGCGTACCTAAAGATC	3'
CPD-ChIP-15	5'	CTCTTACCCACTAACACAAGAGTATAGTATATTC	3'
CPD-ChIP-16	5'	CCAAGTGGCATACAAAGATGAAACAATT	3'
Primers used for ChIP-qPCR			
5S-F[a)]	5'	GGATGCGATCATACCAGCACT	3'
5S-R[a)]	5'	GAGGGATGCAACACGAGGACT	3'
CPD-qPCR-9	5'	ATCTGGGGATTTCACGTGTC	3'
CPD-qPCR-10	5'	GCTAAATCACATATCATAAAGCAAGC	3'
COR15a-ChIP-1	5'	ACAATTTCATGGCCGACCT	3'
COR15a-ChIP-2	5'	TTTCAGGCCACGTGTAATCA	3'

Supplementary Table 5
(continued)

Name		Sequence	
COR15a-ChIP-3	5'	TGTTGGCCGACATACATTTG	3'
COR15a-ChIP-4	5'	TCGTTCTCATTTCCTTCACG	3'
COR15b-ChIP-1	5'	GATAATAGCAATGCGCAAAAA	3'
COR15b-ChIP-2	5'	TCTCGACCAATGAGAATCCA	3'
CYP718-ChIP-3	5'	ACATACAGCGAGGCCACTTG	3'
CYP718-ChIP-4	5'	TTGATGGGTCCTCTTCTACCTC	3'
CYP724a1-ChIP-1	5'	CATGAGGGTCCCAAATTACG	3'
CYP724a1-ChIP-2	5'	TTTCTCAAAAGAGGAATGTAAGAAA	3'
DIN11-ChIP-1	5'	TCAAGGGATTTGGATCACTCAC	3'
DIN11-ChIP-2	5'	TCATCACGTGTAAGTTAGTTTGGAG	3'
DWF4-ChIP-1	5'	CTCGTCTCGTCATGTCACTTTC	3'
DWF4-ChIP-2	5'	CAATGATTGCCGGAATGG	3'
JR2-ChIP-1	5'	ACAAGATTTAAGATCACCGAAGG	3'
JR2-ChIP-2	5'	GCCAATGGTTTTCACATTGTTC	3'
KIN1-ChIP-1	5'	CCGACATAAGGCAAAACTCGA	3'
KIN1-ChIP-2	5'	GAGTGTGGTGCCACGAGTAA	3'
PHE2-ChIP-1	5'	GAATTTGCGGTGGATGAGTT	3'
PHE2-ChIP-2	5'	TTTGGGAAACAATTTCAAGTTC	3'

[a)] Reference: Le *et al.* (2010)