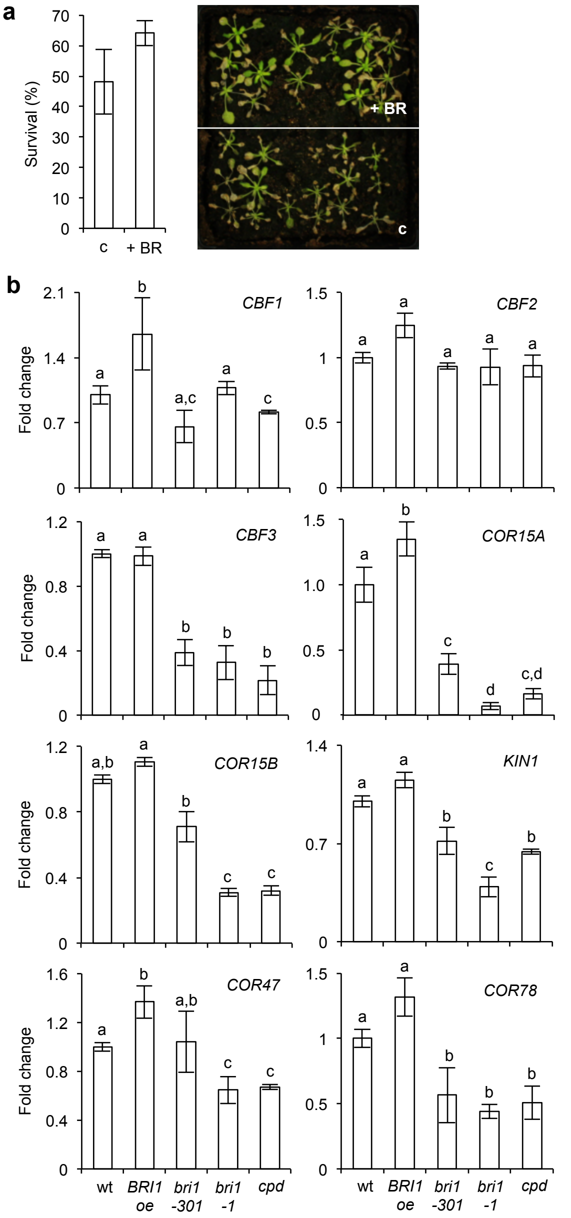

Figure S1. Basal freezing tolerance and basal levels of *COR* genes are altered by BRs. (a) Three-week-old, soil-grown arabidopsis plants were sprayed twice a day for 2 days either with 10 µM of epi-BL (BR) or with DMSO as a control (c), and a freezing tolerance assay was performed. Quantification (left) and representative plants (right) are shown. (**b**) Transcript levels of *CBFs* and downstream *COR* genes were determined by qPCR in 12-day-old seedlings of the wild type, *35S:BRI1-GFP* (*BRI1oe*), *bri1-301, bri1-1* and *cpd*. The error bars show SDs of at least 3 biological replicates. The letters indicate significant differences between genotypes ($P < 0.05$; ANOVA).

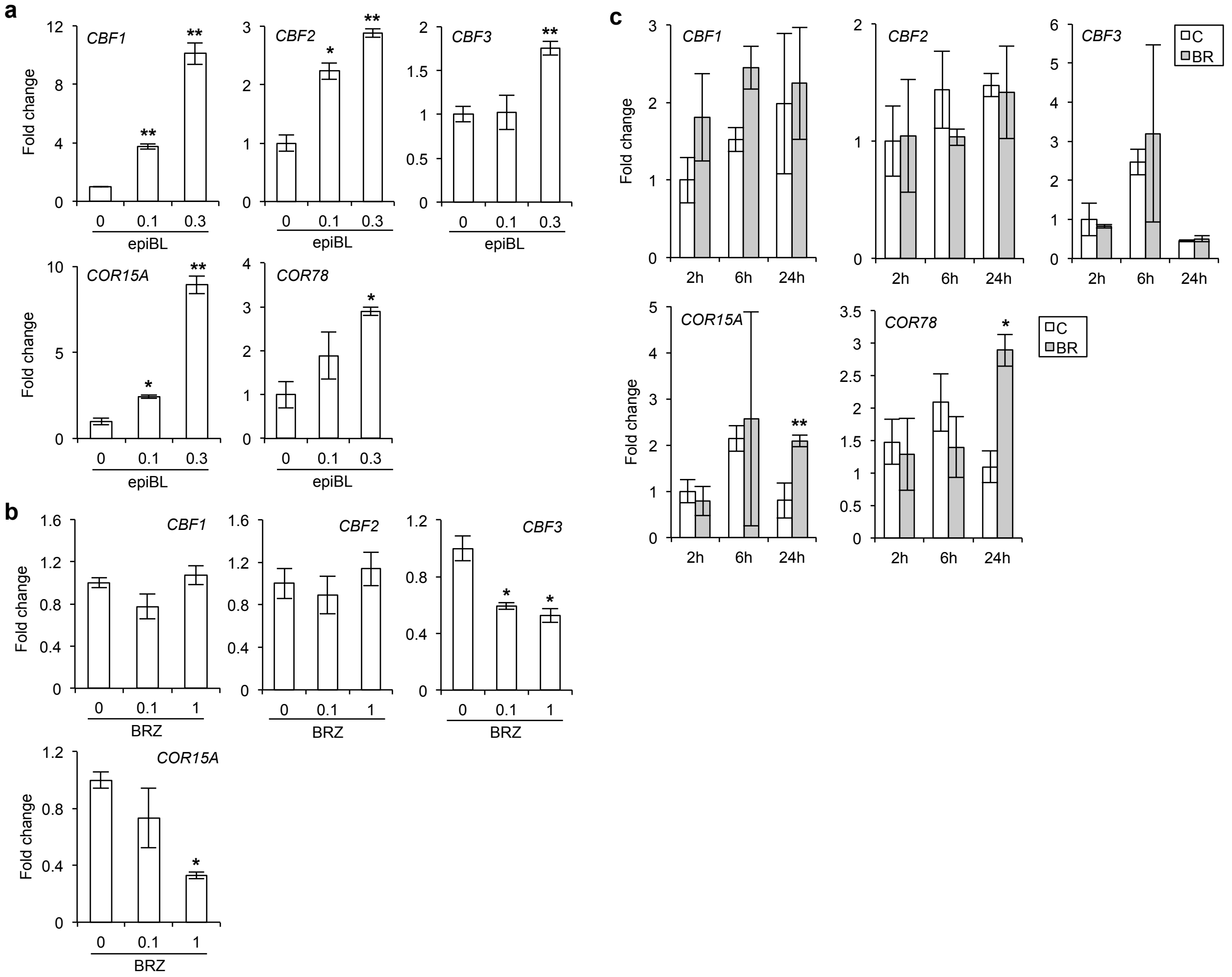

Figure S2. Application of BR promotes *COR* gene expression in arabidopsis. (a, b) Transcript levels of *CBFs* and *COR* genes were determined by qPCR in 10-day-old wild-type arabidopsis seedlings grown on ½ MS media supplemented with different concentrations (in µM) of epi-BL (b) or Brz (c). Error bars show SDs of 2 biological replicates. Asterisks indicate significant differences (* *P* < 0.05, ** *P* < 0.01; Student's t-test). (**c**) Effect of short-term epiBL treatments on the expression of *CBFs* and *COR* genes. Three-week-old, soil-grown wild-type plants were sprayed with either 10 µM of epiBL (BR) or DMSO as a control (c), and qPCRs were performed from plant material collected 2, 6 or 24 h after the treatment. Error bars show SDs of 2 biological replicates. Asterisks indicate significant differences (* *P* < 0.05, ** *P* < 0.01; Student's t-test).

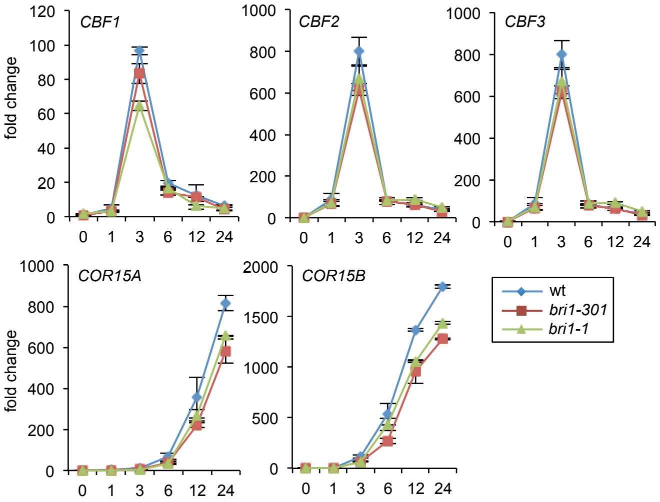

Figure S3. *CBF* and *COR* gene expression levels in BR signaling mutants following cold treatment. Transcript levels of *CBF* and *COR* genes in wild-type, *bri1-301* and *bri1-1* plants treated at 4 °C for the indicated periods of time (in hrs). Plants were grown on ½ MS plates in LDs at 21°C for 12 days and transferred to 4 °C. Samples were collected at the given time points, and mRNA levels were determined by qPCR. Error bars represent the SDs of typically 2 biological replicates.

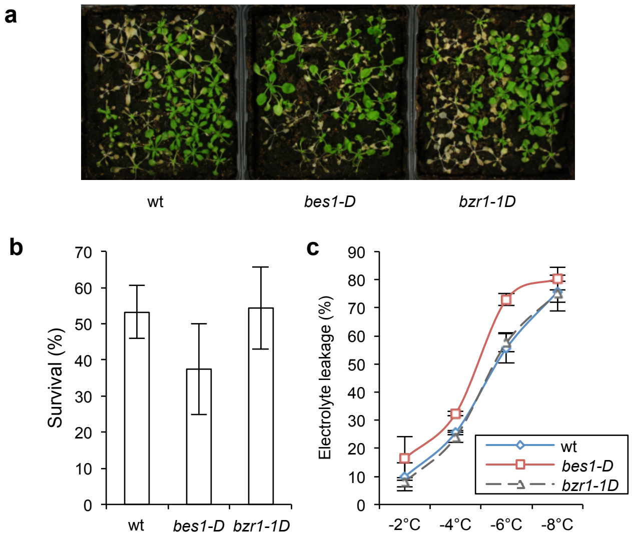

Figure S4. Freezing stress responses of *bzr1-1D* and *bes1-D.* (a,**b**) Freezing tolerance of non-acclimated *bzr1-1D* and *bes1-D* lines compared to that of the wild type. Plants were grown in soil under LD growth conditions at 21 °C. After 3 weeks, they were treated at −6 °C for 4 h. Survival was assessed after 2 weeks of recovery at 21 °C. (b) shows representative plants of each line, and (c) is the quantification of the results. Error bars show SDs of 3 biological replicates. (**c**) Electrolyte leakage in leaves of non-acclimated plants of wild-type, *bzr1-1D* and *bes1-D* plants grown under the same conditions as described in b and treated at the indicated temperatures. Error bars show SDs of 3 biological replicates.

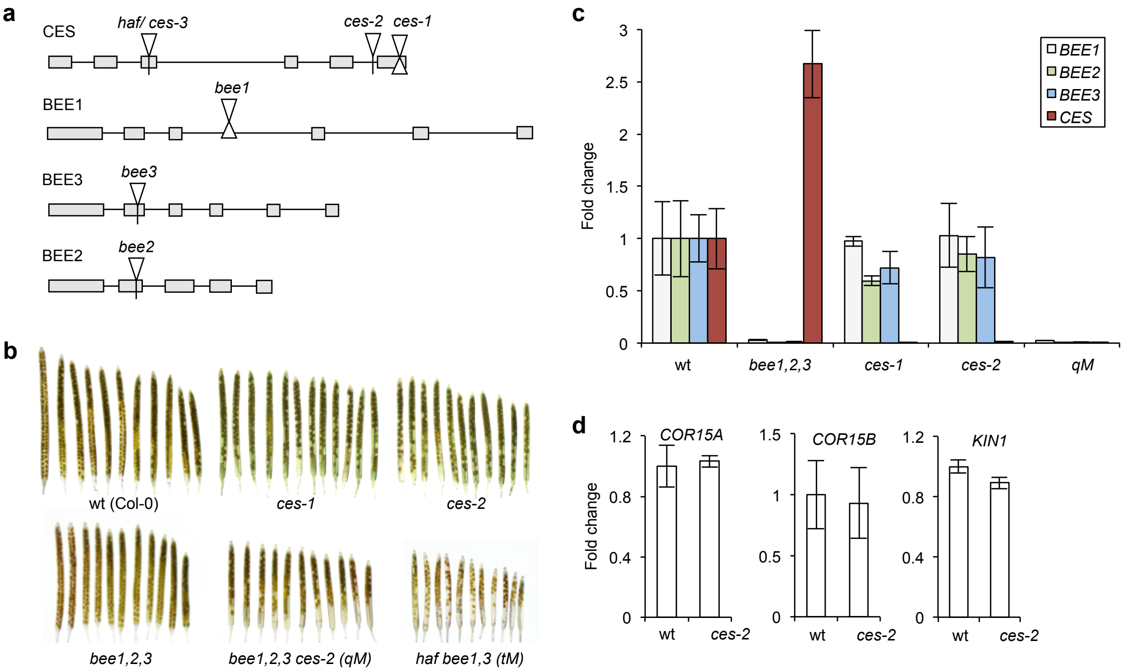

Figure S5. Generation and analysis of a *ces-2 bee1 bee2 bee3* quadruple mutant (*qM*). (**a**) Illustration of *CES*, *BEE1*, *BEE2* and *BEE3* gene organisation. Position of T-DNA insertions are indicated with triangles: *ces-1*: after bp 1475 in exon 6; *ces-2*: after bp 1380 in intron 5; *haf/ces-3*: in exon 3[22]; *bee1*: after bp 863 with a deletion of 105 bp (Δ864-967) in intron 3; *bee2*: after bp 519 with a duplication of 7 bp (AGGTGAA) in exon 2; and *bee3*: after bp 554 with a deletion of 4 bp (Δ555-558) in exon 2. (**b**) Silique-filling phenotype of siliques harvested from a single branch of 8-week-old plants. (**c**) Transcript levels of *BEE1*, *BEE2*, *BEE3* and *CES* in the different mutant lines. Floral tissues of 5-week-old plants were used for qPCR. SDs of 3 biological replicates are shown. (d) Transcript levels of *COR15A, COR15B* and *KIN1* in non-acclimated 12-day-old seedlings of *ces-2* mutant plants compared to those of the wild type. SDs of 3 biological replicates are shown.

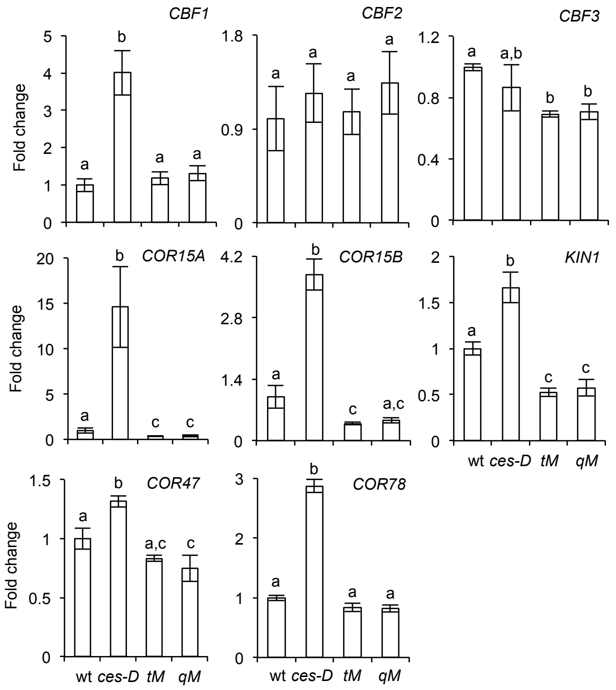

Figure S6. CES promotes *COR* gene expression. mRNA levels of *CBF* and *COR* genes were determined by qPCR in 12-day-old, non-acclimated *ces-D, tM* and *qM* plants grown on ½ MS medium. Error bars are SDs of 3 biological replicates. The letters indicate significant differences between genotypes ($P < 0.05$; ANOVA).

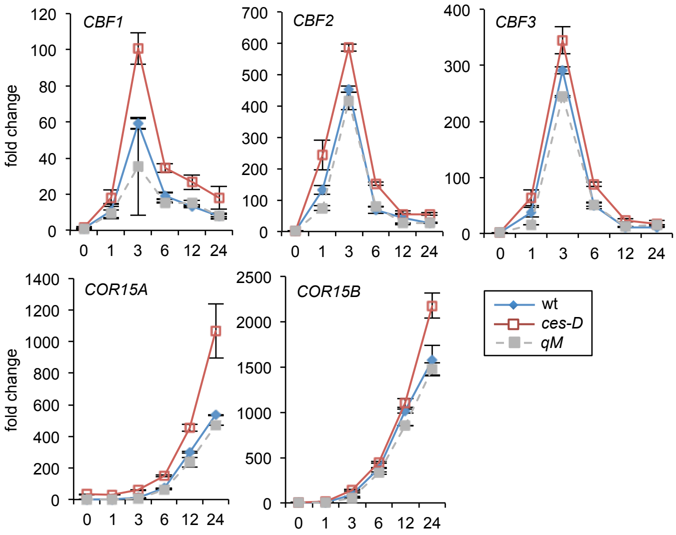

Figure S7. Transcript levels of *CBF* and *COR* genes in *ces* mutant plants following cold treatment. 12-day-old plants of wild-type, *ces-D* and *qM* plants treated at 4 °C for the indicated periods of time (in hrs). Error bars are SDs of typically 2 biological replicates.

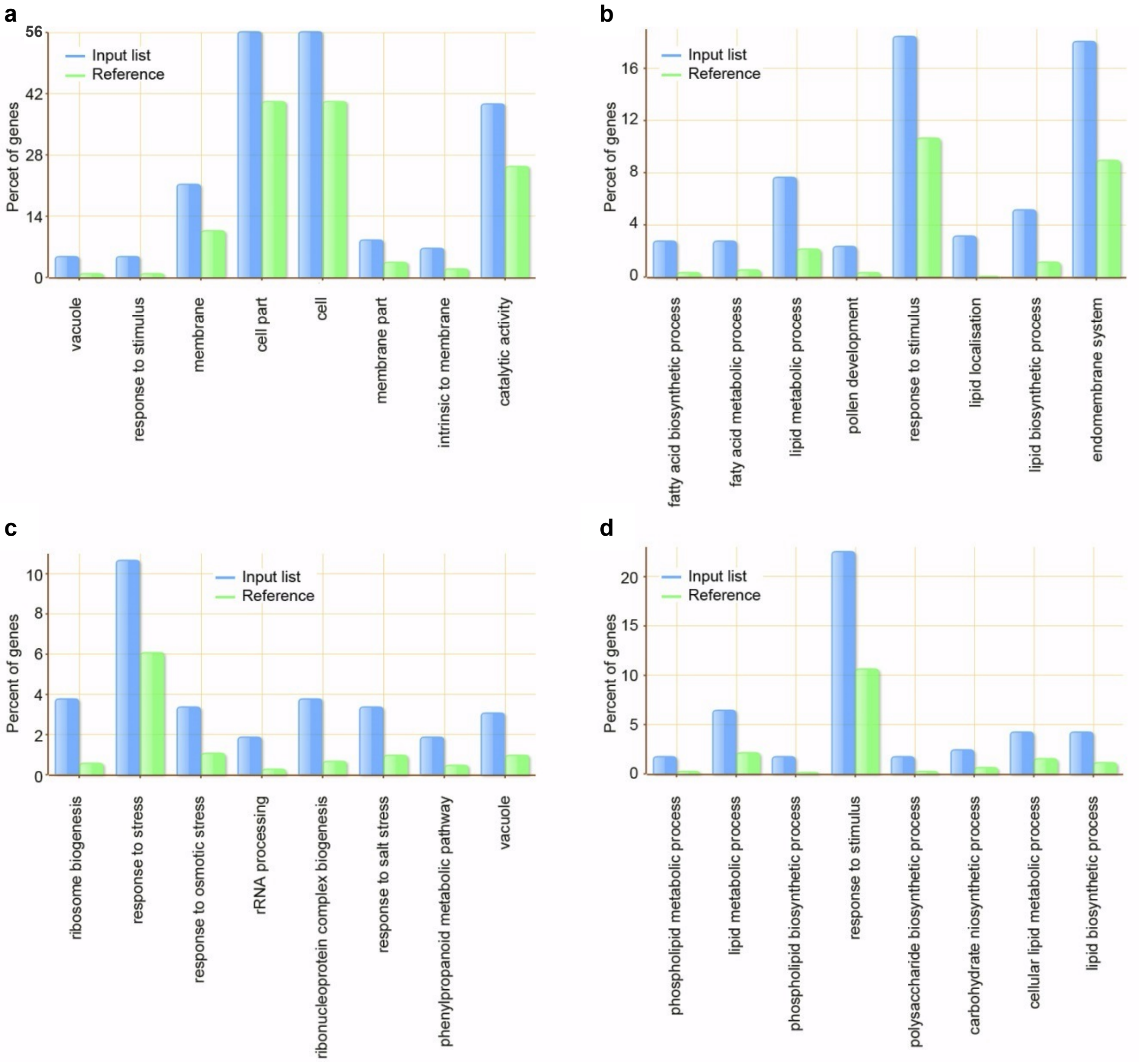

Figure S8. Enriched GO terms in *ces-D*. (**a,b**) GO terms enriched among CBF-independent, cold-induced genes up- (a) or down-regulated (b) in *ces-D* at 21 °C. (**c,d**) GO terms enriched among CBF-independent, cold-induced genes up- (c) or down-regulated (d) in *ces-D* at 4 °C. For full data sets see Tables S7 and S8.

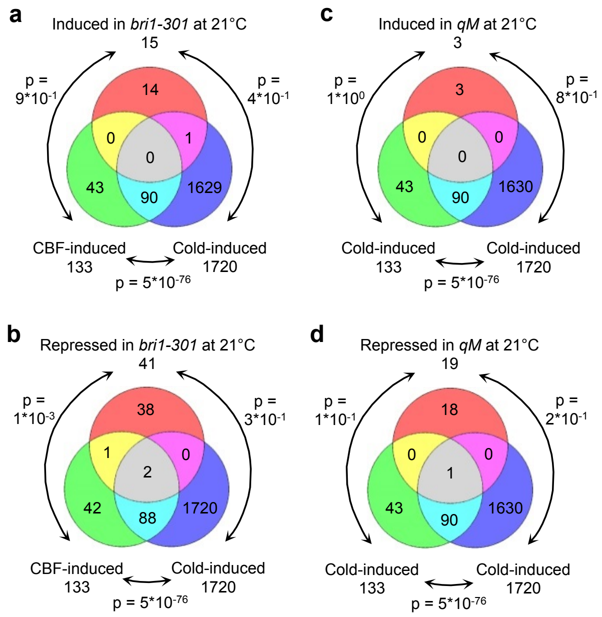

Figure S9. Whole transcriptome changes in *bri1-301* and *qM* as compared to wild type at 21 °C. (**a**) Overlap of genes induced in *bri1-301* as compared to wild type at 21°C, cold-induced and CBF-induced genes. (**b**) Same as (a) but for the *qM*. (**c,d**) Overlap of genes repressed in *bri1-301* (c) or *qM* (d) as compared to wild type at 21 °C, cold-induced and CBF-induced genes. p-values were calculated with the hypergeometric test.

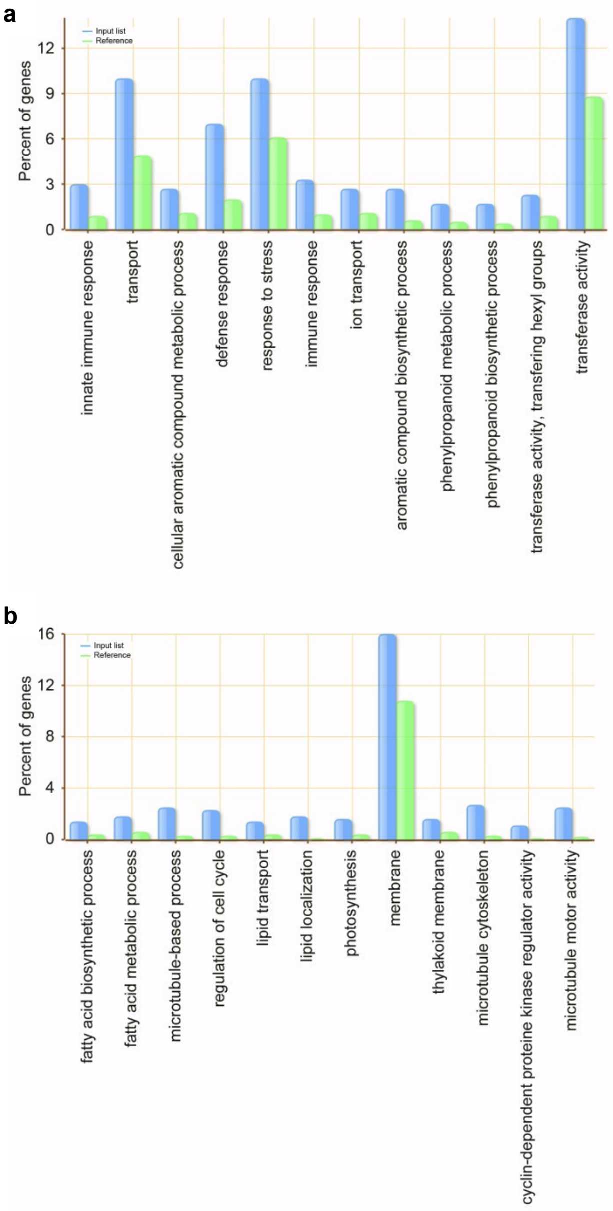

Figure S10. Enrichment of GO terms. (**a**) Enrichment of GO terms in the overlap of cold-induced genes that are not cold-induced in *bri1-301* at and in *qM* at 4 °C. (**b**) Enrichment of GO terms in the overlap of cold-repressed genes that are not cold-repressed in *bri1-301* and in *qM* at 4 °C. For complete data see Tables S13 and S14.

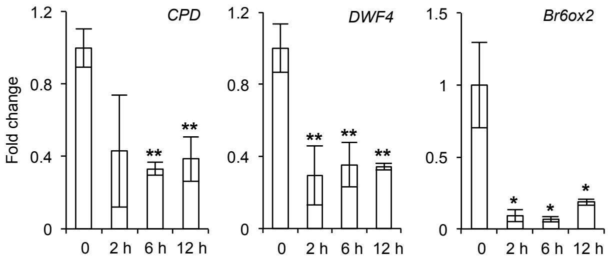

Figure S11. Impact of cold stress on the expression of BR biosynthetic genes. Transcript levels of *CPD, DWF4* and *Br6ox2* were determined in 3-week-old, soil-grown wild-type plants treated at 4°C for the indicated amounts of time by qPCR. Error bars show SDs of 3 biological replicates. Asterisks indicate significant differences (* $P < 0.05$, ** $P < 0.01$; Student's t-test).

Supplementary Table 1. List of genes present in the overlap of cold-induced genes and genes repressed in det2-1 shown in Figure 1a. CBF-induced and not CBF-induced genes are listed separately.

gene	symbol	full_name
CBF regulon		
AT5G15970	KIN2	
AT1G29395	COR413	COLD REGULATED 413
AT3G50970	LTI30	LOW TEMPERATURE-INDUCED 30
AT4G30650	NA	NA
AT1G20450	ERD10	EARLY RESPONSIVE TO DEHYDRATION 10
AT4G27560	NA	NA
AT5G23220	NIC3	nicotinamidase 3
AT1G62570	FMO GS-OX4	FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 4
AT5G24570	NA	NA
AT1G20440	COR47	COLD-REGULATED 47
AT3G22840	ELIP	EARLY LIGHT-INDUCABLE PROTEIN
AT3G09540	NA	NA
AT5G27930	NA	NA
AT2G02120	LCR70	LOW-MOLECULAR-WEIGHT CYSTEINE-RICH 70
AT5G48880	KAT5	3-KETO-ACYL-COENZYME A THIOLASE 5
AT2G17840	ERD7	EARLY-RESPONSIVE TO DEHYDRATION 7
AT4G19810	CHIC	CLASS V CHITINASE
AT3G12320	LNK3	NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 3
AT1G04240	IAA3	INDOLE-3-ACETIC ACID INDUCIBLE 3
AT5G55180	NA	NA
Not CBF regulon		
AT1G62710	BETA-VPE	beta vacuolar processing enzyme
AT1G22770	GI	GIGANTEA
AT4G10960	UGE5	UDP-D-glucose/UDP-D-galactose 4-epimerase 5
AT5G53970	TAT7	TYROSINE AMINOTRANSFERASE 7
AT5G62210	ATS3	Embryo-specific protein 3
AT4G22870	NA	NA
AT1G70900	NA	NA
AT2G21320	BBX18	B-box domain protein 18
AT3G51660	NA	NA
AT3G27210	NA	NA
AT1G19200	NA	NA
AT5G43150	NA	NA
AT3G49220	NA	NA
AT5G50100	NA	NA
AT4G25640	ATDTX35	DETOXIFYING EFFLUX CARRIER 35
AT5G19410	ABCG23	ATP-BINDING CASSETTE G23
AT2G43018	CPuORF17	CONSERVED PEPTIDE UPSTREAM OPEN READING FRAME 17
AT5G44670	GALS2	GALACTAN SYNTHASE 2
AT4G00080	UNE11	UNFERTILIZED EMBRYO SAC 11
AT1G17745	PGDH	3-PHOSPHOGLYCERATE DEHYDROGENASE
AT5G14640	ATSK13	SHAGGY-LIKE KINASE 13
AT5G50840	NA	NA
AT3G12700	NANA	NANA
AT5G53120	ATSPDS3	SPERMIDINE SYNTHASE 3
AT1G73390	NA	NA
AT1G12845	NA	NA
AT1G12370	PHR1	PHOTOLYASE 1
AT5G35970	NA	NA
AT1G65060	4CL3	4-COUMARATE:COA LIGASE 3
AT2G39130	NA	NA
AT3G44450	NA	NA
AT2G39450	ATMTP11	MANGANESE TRANSPORTER 11
AT1G78340	ATGSTU22	GLUTATHIONE S-TRANSFERASE TAU 22
AT5G40480	EMB3012	EMBRYO DEFECTIVE 3012
AT1G08650	ATPPCK1	PHOSPHOENOLPYRUVATE CARBOXYLASE KINASE 1
AT3G57780	NA	NA
AT3G05800	AIF1	ATBS1(ACTIVATION-TAGGED BRI1 SUPPRESSOR 1)-INTERACTING FACTOR 1
AT2G47000	ABCB4	ATP-BINDING CASSETTE B4

AT1G78050	PGM	PHOSPHOGLYCERATE/BISPHOSPHOGLYCERATE MUTASE
AT2G19580	TET2	TETRASPANIN2
AT1G56300	NA	NA
AT5G42720	NA	NA
AT2G29650	ANTR1	ANION TRANSPORTER 1
AT1G16300	GAPCP-2	GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE OF PLASTID 2
AT5G36220	CYP81D1	CYTOCHROME P450 81D1
AT2G22510	NA	NA
AT1G08430	ALMT1	ALUMINUM-ACTIVATED MALATE TRANSPORTER 1
AT3G17998	CPuORF30	CONSERVED PEPTIDE UPSTREAM OPEN READING FRAME 30
AT5G27990	TSR2	Pre-rRNA-processing protein TSR2
AT3G54140	ATPTR1	ARABIDOPSIS THALIANA PEPTIDE TRANSPORTER 1
AT3G53620	AtPPa4	PYROPHOSPHORYLASE 4
AT4G34980	SLP2	SUBTILISIN-LIKE SERINE PROTEASE 2
AT5G39710	EMB2745	EMBRYO DEFECTIVE 2745
AT1G44800	SIAR1	SILIQUES ARE RED 1
AT1G07180	ATNDI1	INTERNAL NON-PHOSPHORYLATING NAD (P) H DEHYDROGENASE
AT1G58270	ZW9	
AT3G53260	ATPAL2	PHENYLALANINE AMMONIA-LYASE 2
AT1G13930	NA	NA
AT1G43620	UGT80B1	UDP GLYCOSYLTRANSFERASE 80B1
AT4G02410	ATLPK1	L-TYPE LECTIN RECEPTOR KINASE IV.3

Supplementary Table 2. List of genes present in the overlap of cold-induced genes and genes induced in ces-D shown in Figure 3a. CBF-induced and not CBF-induced genes are listed separately.

gene	symbol	full_name
CBF regulon		
AT2G42540	COR15A	COLD REGULATED 15A
AT2G42530	COR15B	COLD REGULATED 15B
AT5G52310	COR78	COLD REGULATED 78
AT5G15960	KIN1	
AT1G29395	COR413-TM1	COLD REGULATED 413 THYLAKOID MEMBRANE 1
AT1G51090	NA	NA
AT4G12000	NA	NA
AT1G58360	AAP1	AMINO ACID PERMEASE 1
AT4G18270	ATTRANS11	ARABIDOPSIS THALIANA TRANSLOCASE 11
AT5G64260	EXL2	EXORDIUM like 2
Not CBF regulon		
AT2G28400	NA	NA
AT4G18280	NA	NA
AT2G32190	NA	NA
AT4G31800	ATWRKY18	ARABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 18
AT4G38400	ATEXLA2	EXPANSIN-LIKE A2
AT2G21590	APL4	
AT5G62360	NA	NA
AT4G23600	CORI3	CORONATINE INDUCED 1
AT3G11420	NA	NA
AT4G33550	NA	NA
AT3G11410	PP2CA	PROTEIN PHOSPHATASE 2C
AT5G58670	ATPLC	ARABIDOPSIS THALIANA PHOSPHOLIPASE C
AT2G33590	AtCRL1	
AT4G33950	ATOST1	OPEN STOMATA 1
AT5G18130	NA	NA
AT3G05400	NA	NA
AT1G18710	AtMYB47	MYB DOMAIN PROTEIN 47

Supplementary Table 15. Primers used in this study.

Name		Sequence	
COR15A-pPCR-FW	5'	ACCTCAACGAGGCCACAAAGAAAG	3'
COR15A-pPCR-RV	5'	CGCTTTCTCACCATCTGCTAATGC	3'
COR15B-pPCR-FW	5'	CCTCAACGAAGCCACAAAGAA	3'
COR15B-pPCR-RV	5'	TTCCTCAGTCGCAGTTTCATT	3'
KIN-pPCR-FW	5'	AAGAATGCCTTCCAAGCCGGTCAG	3'
KIN-pPCR-RV	5'	TACACTCTTTCCCGCCTGTTGTGC	3'
COR47-pPCR-FW	5'	TCCCAGGACACCACGACAA	3'
COR47-pPCR-RV	5'	AATCCTCTGCTTTCTCGTCGT	3'
COR78-pPCR-FW	5'	GACGAAGTTACCTATCTCCG	3'
COR78-pPCR-RV	5'	CTTCCTTTGTCGTCGTTTCC	3'
CBF-pPCR-FW	5'	TTCGCTGACTCGGCTTGG	3'
CBF1-pPCR-RV	5'	CATGATTCGTGGTCGTCGT	3'
CBF2-pPCR-RV	5'	TCAAACATCGCCTCATCGTG	3'
CBF3-pPCR-RV	5'	GACACATCTCATCTTGAAAATT	3'
UBC-FW	5'	TCAAATGGACCGCTCTTATC	3'
UBC-RV	5'	CACAGACTGAAGCGTCCAAG	3'
CBF1-ChIP-FW	5'	ACACACGTCAGACAGCGAGT	3'
CBF1-ChIP-RV	5'	CGGAGGAGAGATAACGGATATG	3'
CBF2-ChIP-FW	5'	CAGTGATTGACAGCCTTGATA	3'
CBF2-ChIP-RV	5'	ATTTGGATATTTGTGGGGTCG	3'
CBF3-ChIP-FW	5'	CCATTGTCCATACCTTCTCT	3'
CBF3-ChIP-RV	5'	AAAGAGTGGGTATGGTCAAGA	3'
CBF1-luc-FW	5'	GATTGCTTCTCGAGGTACTATA	3'
CBF1-luc-RV	5'	TTGGCGTCTTCCATGGATCAGAGTACTCTG	3'
CBF1-luc-FW1	5'	TTGCTTCTCGAGGTACTATAAAAAAGTCATTCACA	3'
CES KO fwd	5'	GGCCATTGATTTCACTCTTCA	3'
CES KO rev	5'	ACTGACGTCACACACAAAAGA	3'
BEE1 KO fwd	5'	AACAATTACTTCACTTTCCCGG	3'
BEE1 KO rev	5'	TTGATTCTGTAGAGACTGGACATAAT	3'
BEE2 KO fwd	5'	GAAGATCAAAGCAGAGGATGAA	3'
BEE2 KO rev	5'	TTCACCTGTTTTGCGGATAA	3'
BEE3 KO fwd	5'	ACCTCTTCTGCTCAAGTTTCCA	3'
BEE3 KO rev	5'	CGAACTTGCTGCTGTAAGCTT	3'
LBb1	5'	GCGTGGACCGCTTGCTGCAACT	3'
CBF1 fwd comp	5'	TCGCTATGTACTATACACGTGTCATTCACAGAGACA	3'
CBF1 rev comp	5'	TGTCTCTGTGAATGACACGTGTATAGTACATAGCGA	3'
CBF1mt fwd comp	5'	TCGCTATGTACTATAAAAAAATCATTCACAGAGACA	3'
CBF1mt rev comp	5'	TGTCTCTGTGAATGATTTTTTTATAGTACATAGCGA	3'
CBF2 fwd comp	5'	TAGCTGTTTCTTATCCACGTGGCATTCACAGAGACA	3'
CBF2 rev comp	5'	TGTCTCTGTGAATGCCACGTGGATAAGAAACAGCTA	3'
CBF2mt fwd comp	5'	TAGCTGTTTCTTATCAAAAAAGCATTCACAGAGACA	3'
CBF2mt rev comp	5'	TGTCTCTGTGAATGCTTTTTTGATAAGAAACAGCTA	3'
CBF3 fwd comp	5'	TGTGGAGTCTCGTACCACGTGTCGCGTCACTTCACT	3'
CBF3 rev comp	5'	AGTGAAGTGACGCGACACGTGGTACGAGACTCCACA	3'
CBF3mt fwd comp	5'	TGTGGAGTCTCGTACAAAAAATCGCGTCACTTCACT	3'
CBF3mt rev comp	5'	AGTGAAGTGACGCGATTTTTTGTACGAGACTCCACA	3'
CPD-1	5'	CTTGCTCAACTCAAGGAAGAG	3'
CPD-2	5'	CTCGTAGCGTCTCATTAACCAC	3'
DWF4-3	5'	CCGTTGAAGAGCTTAGGGAAGAG	3'
DWF4-4	5'	CATTTCCCAATCGAAGAGTTTC	3'
BR6ox2-1	5'	AGCTTGTTGTGGGAACTCTATCGG	3'
BR6ox2-2	5'	CGATGTTGTTTCTTGCTTGGACTC	3'