

Supplementary Information for

Posttranslational regulation of multiple clock-related transcription factors triggers cold-inducible gene expression in *Arabidopsis*

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Other supplementary materials for this manuscript include the following:

Datasets S1



Fig. S1. Genomic structure of the *DREB1* genes in five Brassicaceae species.

Α



Fig. S2. Alignment of promoter sequences of the *DREB1* genes in Brassicaceae species. Promoter sequences of *DREB1A* (A), *DREB1B* (B) and *DREB1C* (C) in five Brassicaceae species are aligned. Blue lines indicate the conserved regions of the three *DREB1* promoters in *Arabidopsis thaliana*, named Box I to VI (1).

В



Fig. S2. (continued)

С



Fig. S2. (continued)

			*	20		*		40			*	6	0	*		80		*		
CCA1	AL4G46300 AT2G46830 Carubv10022831m.g Bostr.25993s0083 Brara.E00100	 VIKTRKP VIKTRKP IIKTRKP VIKTRKP VVKTRKP	YTITKORI YTITKORI YKITKORI YTITKORI YTITKORI	RWTEE RWTEE RWTEE RWTEE RWTEE	EHNRFI EHNRFI EHNRFI EHNRFI EHNRFI	ALRL ALRL ALRL ALRL ALRL DALRL	YGRAW YGRAW YGRAW YGRAW YGRAW	QKIEI QKIEI QKIEI QKIEI	EHVA EHVA EHVA EYVA EHVA	ТКТА ТКТА ТКТА ТКТА ТКТА	VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH	IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF	SKVEK SKVEK SKVEK SKVEK SKVEK	EAESKGV EAEAKGV EAEAKGV EAEAKGV EAEAKGV	/A-MGC /A-MGC /A-IGC /A-LGE /P-VAC	QALDIA QALDIA QALDIA IALDIA QTLDIA	IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI	PKRKPSN PKRKPNN PKRKPSN PKRKPSN PKRKPNN	PYPRKT PYPRKT PYPRKT PYPRKT PYPRKT	 98 98 98 98 98
ГНΥ	Bostr.5325s0026 Carubv10008551m.g AT1G01060 AL1G11460 Brara.J00076 Brara.H03122	 LTKARKP LTKARKP LAKARKP LAKARKP LTKARKP LTKARKP	YTITKORI YTITKORI YTITKORI YTITKORI YTITKORI YTITKORI	RWTED RWTED RWTED RWTDD RWTED RWTED	EHERFLE EHERFLE EHERFLE EHERFLE EHDRFLE EHDRFLE	ALRL ALRL ALRL ALRL ALRL ALRL ALRL	YGRAW YGRAW YGRAW YGRAW YGRAW YGRAW	QRIEI QRIEI QRIEI QRIEI QRIEI QRIEI	EHIG EHIG EHIG EHIG EHIG EHIG	TKTA TKTA TKTA TKTA TKTA TKTA	VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH	IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF	TKLEK TKLEK TKLEK TKLEK TKLEK TKLEK	EAETKG EAETKG EAEVKG EAEAKG EAEAKG EAEAKG	P-VC(P-VC(P-VC(P-VC(P-VC(P-VC(ALDIE ALDIE ALDIE ALDIE ALDIE ALDIE ALDIE	IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI	PKRKPNI PKRKPNI PKRKPNI PKRKPNI PKRKPNI PKRKPNI	PYPRKP PYPRKP PYPRKP PYPRKH PYPRKP PYPRKP	 98 98 98 98 98 98 95
RVE1	AL6G28380 AT5G17300 Bostr.261850066 Carubv10001147m.g Brara.B00704 Brara.J01819 Brara.C00761	 APKVRKP APKVRKP APKVRKP APKVRKP APKVRKP APKVRKP APKVRKP	YTITKERI YTITKERI YTITKERI YTITKERI YTITKERI YTITKERI YTITKERI	RWTDE RWTDE RWTDE RWTDD RWTDE RWTDE RWTDE RWTDE	EHKKFVI EHKKFVI EHKKFVI EHSKFVI EHSKFVI EHNKFVI EHYKFVI	ALKL ALKL ALKL ALKL ALKL ALKL ALKL	YGRAW YGRAW YGRAW YGRAW YGRAW HGRAW HGREW	IRRIEI IRRIEI IRRIEI IRRIEI IRKIEI IRKIEI	BHVG BHVG BHVG BHVG BHVG BHVG GHVG	SKTA SKTA SKTA TKTA TKTA TKTA	VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH	IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF	SKVAR SKVAR SKVAR SKVAR SKVAR SKVAR SKVAR	EATGO EATGO EATGO EATGO ESSGO EASGO EATGO	GD-GSS GD-GSS GD-GSS GD-GSS GN-GSS GN-GSS GN-GSS	VEPIV VEPIV VEPIV SVEPIV SLEPIV SLEPIL SVEPIV	IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI	PKRKPAE PKRKPAE PKRKPAE PKRKPAE PKRRPME PKRRPME	PYPRKF PYPRKF PYPRKF PYPRKF PYPRKL PYPRKL PYPRKL	 96 96 96 96 96 96
RVE2	AL7G45670 AT5G37260 Bostr.9638s0043 Carubv10005376m.g Brara.E01373	 YLKTRKP YLKTRKP YLKSRKP YLKTRKP CL <mark>KT</mark> RKP	YTITKORI YTITKORI YTITKORI YTITKORI YTITKORI	KWTEA KWTEA KWTEA KWTEA	EHEKFVI EHEKFVI EHEKFVI EHEKFVI EHEKFVI	ALKL ALKL ALKL ALKL ALKL	YGRAW YGRAW YGRAW YGRAW YGRAW	IRRIE IRRIE IRRIE IRRIE IRRIE	EHVG EHVG EHVG EHVG EHVG	ТКТА ТКТА ТКТА ТКТА ТКТА	VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH	IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF	TKVAR TKVAR TKVAR TKVAR TKVAR	DFG DFG DFG DFG DFG	VSS VSS VSS VSS VSS	SE-SIE SE-SIE SE-TIE SE-TIE SEKSIE	IPPPRI IPPPRI IPPPRI IPPPRI IPPPRI	PKRKPME PKRKPME PKRKPME PKRKPME PKRKPME	PYP RKL PYP RKL PYP RKL PYP RKL PYP RKL	 92 92 92 92 93
RVE7	AT1G18330 AT3G10113 AL1G30700 Bostr.7128s0434 Carubv10009624m.g Brara.F01290	 VVKVRKP VVKVRKP IVKVRKP VVKVRKP VVKVRKP AAKVRKP	YTVTKORI YTVTKORI YTVTKORI YTVTKORI YTVTKORI YTVTKORI	KWSEE KWSEE KWSEE KWSEE KWSEE	EHDRFLI EHDRFLI EHDRFLI EHERFLI EHERFLI EHERFLI	AIKL AIKL AIKL AIKL AIKL AIKL	YGRGW YGRGW YGRGW YGRGW YGRGW YGRAW	IRQIQI IRQIQI IRQIQI IRQIEI IRQIEI	EHIG EHIG EHIG EHIG EHIG EHIG	TKTA TKTA TKTA TKTA TKTA TKTA	VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH	IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF IAQKFF	SKMAQ SKMAQ SKMAQ SKMTH SKMAQ SKVAR	EADSI EADSI EPDNI EADDI EADNI EADNI	RS-EGS RS-EGS RS-EGS RS-EGS RS-ERS RS-ERS RS-DGS	VKAIV VKAIV VKAVV NKAIV NKAIV NKAIV	IPPPRF IPPPRF IPPPRF IPPPRF IPPPRF IPPPRF	PKRKPAE PKRKPAE PKRKPAE PKRKPAE PKRKPAE	PYPRKS PYPRKS PYPRKS PYPRKS PYPRKS PYPRKS	 96 96 96 96 96
RVE3	Carubv10011679m.g AL1G10890 Bostr.5325s0070 Brara.J00041 AT1G01520	 MKKIRKP TKKIRKP TKKIRKP TKKIRKP TKKVRKP	YTITKSRI YTITKSRI YTITKSRI YTITKSRI YTITKSRI	NWTEQ NWTEQ NWTEQ NWTEQ NWTEQ	EHDKFLI EHDKFLI EHDKFLI EHDKFLI EHDKFLI	ALHL ALHL ALHL ALHL ALHL ALHL	F DRDW F DRDW F DRDW F DRDW F DRDW	KKIE KKIE KKIE KKIE KKIE	AFVG AFVG AFVG AFVG AFVG	SKTV SKTV SKTV SKTV SKTV	IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH	IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF	LKVOK LKVOK LKVOK LKVOK		b b b	IGTNEH IGTKEH IGTNEH IGTNEH IGTKEH	LPPPRE LPPPRE LPPPRE LPPPRE	KRK <mark>AKE</mark> KRKSNE KRKANE KRKANE	PYPQKA PYPQKA PYPQKA PYPQKA	 88 70 88 88 88
RVE5	AL6G52110 AT4G01280 Carubv10001399m.g Brara.l00054 Bostr.10064s0009	 TTKIRKP TTKIRKP ATKIRKP ATKIRKP MKKIRKP	YTIKKSRI YTIKKSRI YTIKKSRI YTIKKSRI YTIKKSRI	NWTDQI NWTDQI NWTDQI NWTEQI NWTEQI	EHDKFLI EHDKFLI EHDKFLI EHDKFLI EHDKFLI	ALHL ALHL ALHL ALHL ALHL ALHL	F DRDW F DRDW F DRDW F DRDW F DRDW	KKIEZ KKIEZ KKIEZ KKIEZ	AFVG AFVG AFVG AFVG AFVG	SKTV SKTV SKTV SKTV SKTV	VQIRSH VQIRSH VQIRSH VQIRSH VQIRSH	IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF	LKVOK LKVOK LKVOK LKVOK			GANEH GANEH GANEH GTNEH GTNEH	LPPPRE LPPPRE LPPPRE LPPPRE LPPPRE	YKRKASE YKRKASE YKRKAIE YKRKARE YKRKASE	PYPIKA PYPIKA PYPQKA PYPQKA PYPQKA	 88 88 88 88 88
RVE6	Bostr.7305s0060 Brara.J00790 Carubv10026677m.g AT5G52660 AL8G27130 Brara.C01424	 SKKIRKP SKKIRKP SKKIRKP SKKIRKP SKKIRKP SKKIRKP	YTITKSRI YTISKSRI YTITKSRI YTITKSRI YTITKSRI YTITKSRI	SWTEP SWTEP SWTEP SWTEP SWTEP SWTEP	EHDKFL EHDKFL EHDKFL EHDKFL EHDKFL EHDKFL	ALQL ALQL ALQL ALQL ALQL ALQL	F DRDW F DRDW F DRDW F DRDW F DRDW F DRDW	KKIE KKIE KKIE KKIE KKIE KKIE	AFIG AFIG AFIG AFIG AFIG AFIG	SKTV SKTV SKTV SKTV SKTV SKTV	IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH	IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF	LKVOK LKVOK LKVOK LKVOK LKVOK LKVOK			GTAEH GTAEH GTGEH GTGEH GTGEH GTGEH	LPPPRI LPPPRI LPPPRI LPPPRI LPPPRI LPPPRI	PKRK <mark>AAF</mark> VKRKAAF VKRKAAF VKRKAAF VKRKAAF VKRKAAF	PYPQKA PYPQKA PYPQKA PYPQKA PYPQKA PYPQKA	 88 88 88 88 88 88
RVE4	AL6G12110 AT5G02840 Bostr.2128s0168 Carubv10001591m.g Brara.J02851 Brara.C00086	 EKKVRKA EKKVRKA EKKVRKA EKKVRKA EKKVRKA EKKVRKA	YTITKSRI YTITKSRI YTITKSRI YTITKSRI YTITKSRI YTISKSR(SWTEG SWTEG SWTEG SWTEG SWTEG SWTEG	EHDKFLE EHDKFLE EHDKFLE EHDKFLE EHDKFLE EHDKFLE	ALQL ALQL ALQL ALQL ALQL ALQL	F DRDW F DRDW F DRDW F DRDW F DRDW F DRDW	KKIEI KKIEI KKIEI KKIEI KKIEI	DFVG DFVG DFVG DFVG DFVG DFVG	SKTV SKTV SKTV SKTV SKTV SKTV	IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH	IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF	LKVQK LKVQK LKVQK LKVQK LKVQK LKVGK		b b b	IGTLAH IGTLAH IGTLAH IGTLAH IGTLAH IGTLAH	VPPPRI VPPPRI VPPPRI VPPPRI VPPPRI VPPPRI	PKRK <mark>AAF</mark> PKRKAAF PKRKAAF PKRKAAF PKRKAAF	PYPQKA PYPQKA PYPQKA PYPQKA PYPQKA PYPQKA	 88 88 88 88 88 88
RVE8	AT3G09600 Carubv10014328m.g Bostr.22252s0109 AL3G20820 Brara.E03078 Brara.A03523	 SKKVRKP SKKVRKP SKKVRKP SKKVRKP SKKVRKP SKKVRKP	YTITKSRI YTITKSRI YTITKSRI YTITKSRI YTITKSRI YTITKSRI YTITKSRI	SWTEE SWTEE SWTEE SWTEE SWTEE SWTEE	EHDKFLE EHDKFLE EHDKFLE EHDKFLE EHDKFLE EHDKFLE	ALQL ALQL ALQL ALQL ALQL ALQL	F DRDW F DRDW F DRDW F DRDW F DRDW F DRDW	KKIEI KKIEI KKIEI KKIEI KKIEI	DFVG DFVG DFVG DFVG DFVG DFVG	SKTV SKTV SKTV SKTV SKTV SKTV	IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH IQIRSH	IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF IAQKYF	LKVQK LKVQK LKVQK LKVQK LKVQK LKVQK		b	IGTLAH IGTLAH IGTLAH IGTLAH IGTLAH IGTLAH	VPPPRI VPPPRI VPPPRI VPPPRI VPPPRI VPPPRI	PKRK <mark>AAE</mark> PKRKAAE PKRKAAE PKRKAAE PKRKAAE	PYPQKA PYPQKA PYPQKA PYPQKA PYPQKA PYPQKA	 88 88 88 88 88 88

Fig. S3. Alignment of the MYB binding domains of the CCA1, LHY, and RVE transcription factors in Brassicaceae species.



Fig. S4. Cold-inducible *DREB1A* expression in the *CCA1*, *LHY* and *RVEs* overexpression plants. (A-C) Expression levels of each transgene in transgenic *Arabidopsis* overexpressing the *GFP* fusion genes of *CCA1*, *LHY* and *RVEs*. (D-F) Cold-inducible *DREB1A* expression in the overexpression plants. The *GFP* fusion genes of *CCA1/LHY* (A, D), *RVE4/RVE8* (B, E) and *RVE1/RVE2* (C, F) were overexpressed using the CaMV 35S promoter in transgenic *Arabidopsis*. Two-week-old seedlings grown on agar plates were gradually chilled at 4°C for 3 h beginning 2 h (LL2), 6 h (LL6) and 14 h (LL14) after dawn. Gene expression before (N) and after cold stress (L) was measured. The asterisks indicate significant differences (***P*<0.01 as analyzed by one-way ANOVA followed by a Tukey's post hoc test) compared with the expression levels in the VC plants at each time point.



Fig. S5. Generation of multiple mutant plants of *CCA1*, *LHY* and *RVEs*. (A) Presence or absence of each transcript in each mutant plant was determined by derived cleaved amplified polymorphic sequences (dCAPS) assay for the *LHY* genes and RT-PCR for the *CCA1* and *RVE* genes. (B, C) Plant growth of the mutant plants of *RVE* genes used in Fig. 2A (B) and 2C (C). Sixteen-day-old seedlings grown on agar medium are shown. Black bars indicate 5 mm.



Fig. S6. Effects of the circadian clock on the expression of the *DREB1B* and *DREB1C* genes in response to the rapid temperature decrease. Two-week-old seedlings grown in soil pots under a 12-h light/12-h dark cycle were transferred to free-running conditions under continuous light from dawn. Cold treatments were initiated every 4 h from 2 h to 46 h after the beginning of the free-running conditions. At each time point, the seedlings were rapidly cooled at 4°C for 1 h. The transcript levels of each gene in the plants treated at 4°C were measured using the same samples as in Fig. 2G.



Fig. S7. Freezing tolerance of the multiple mutant plants of *CCA1*, *LHY* and *RVEs*. (A) Cold-inducible expression of genes downstream of DREB1 in the mutant plants of *CCA1*, *LHY* and *RVEs*. The asterisks indicate significant differences (*P<0.05, **P<0.01 as analyzed by one-way ANOVA followed by a Tukey's post hoc test) compared with the WT plants at each time point. (B, C) Freezing tolerance test. Non-acclimated seedlings were treated at -9° C for 0.5 h. Representative images (B) and survival rates (C) of the plants after recovery are shown. The symbols in the graph indicate the survival rate in four independent experiments. The asterisks indicate significant differences (*P<0.05, **P<0.01 as analyzed by one-way repeated measures ANOVA followed by a Tukey's post hoc test) compared with the WT plants at each time point.



Fig. S8. Expression of *CCA1*, *LHY* and *RVEs* under cold stress conditions. Twoweek-old seedlings grown in agar plates under a 12-h light/12-h dark cycle were incubated at 22°C or 4°C under continuous light from LL2. The bars refer to the means \pm standard deviation of three biological replicates. The asterisks indicate significant differences (***P* < 0.01 according to Student's *t*-test) compared with the expression in the WT plants at 22°C.



Fig. S9. Subcellular localization of RVE4 and RVE8 in transgenic *Arabidopsis* plants. (A) Subcellular localization of RVE4-sGFP driven by its own promoter in transgenic *Arabidopsis* plants. (B, C) Subcellular localization of RVE4-sGFP and RVE8-sGFP under cold stress conditions from 14 h (B, LL14) and 2 h (C, LL2) after dawn. (D) Subcellular localization of RVE4-sGFP and RVE8-sGFP under the recovery treatment from 4°C to 22°C. (E) Subcellular localization of RVE4-sGFP and RVE8-sGFP in response to various temperature decreases. Two-week-old seedlings overexpressing *RVE4-sGFP* and *RVE8-sGFP* driven by the CaMV *35S* promoter were used for GFP observation. Bars indicate 20 µm.



Fig. S10. Subcellular localization of RVE4-sGFP and RVE8-sGFP and expression of *DREB1A* under various stress treatments. (A) Subcellular localization of RVE4-sGFP and RVE8-sGFP under various stress treatments, such as heat stress (37°C), high light stress (1000 μ E), high salinity stress (250 mM NaCl) and ABA (50 μ M) treatment. Bars indicate 20 μ m. (B) Expression levels of *DREB1A* under various stress treatments. The bars refer to the means ± standard deviation of triplicates. Two-week-old seedlings overexpressing *RVE4-sGFP* and *RVE8-sGFP* driven by the CaMV 35S promoter were used for GFP observation. (C) Immunoblot analyses using transgenic *Arabidopsis* plants expressing *RVE4-sGFP* and *RVE8-sGFP* driven by their own promoters. Two-week-old seedlings grown on agar plates were warmed at 37°C up to 1 h from LL2.



Fig. S11. GFP fluorescence of CCA1-sGFP and LHY-sGFP in response to cold stress. (A) GFP fluorescence of CCA1-sGFP and LHY-sGFP driven by their own promoters in transgenic *Arabidopsis*. Two-week-old seedlings overexpressing the *GFP* fusion genes of the genomic fragments, including the promoter regions of *CCA1* and *LHY*, were used for GFP observation. (B) GFP fluorescence of LHY-sGFP in transgenic *Arabidopsis* plants. Two-week-old seedlings overexpressing *LHY-sGFP* driven by the CaMV *35S* promoter in Fig. S4 were used for GFP observation. Bars indicate 20 µm.



Fig. S12. Regulation of CCA1 and LHY stability. (A) Effect of a 26S proteasome inhibitor on the cold response of LHY-sGFP. Two-week-old seedlings overexpressing *LHY-sGFP* driven by the CaMV *35S* promoter were used for GFP observation. White bars indicate 20 μ m. (B) Expression levels of the transgene in transgenic *Arabidopsis* overexpressing the s*GFP* fusion gene of *CCA1* Δ . (C) Plant growth of the transgenic plants overexpressing the s*GFP* fusion gene of *CCA1* Δ . Ten-day-old seedlings grown on agar medium are shown. The *CCA1-sGFP*-overexpressing plants are the same lines as shown in Fig. S4. Black bars indicate 5 mm.



Fig. S13. Subcellular localization of CCA1-sGFP and LHY-sGFP under various stress treatments, such as heat stress (37°C), high light (1000 μ E), high salinity stress (250 mM NaCl) and ABA (50 μ M) treatment. Two-week-old seedlings overexpressing *CCA1-sGFP* and *LHY-sGFP* driven by the CaMV 35S promoter were used for GFP observation. Bars indicate 20 μ m.



Fig. S14 ChIP assays of the CCA1, LHY and RVE proteins under the cold stress at subjective night. Enrichment of two regions around EEs in the *DREB1A* promoter (1 and 2; Fig. 6A) was measured using ChIP-qPCR analyses. The transgenic *Arabidopsis* plants used in Fig. 4A and Fig. 5A were analyzed. The cold stress treatment was started 14 h after dawn (LL14). The bars refer to the means \pm standard deviation in four replicates. The asterisks and "n.s." indicate significant (**P*<0.05, ***P*<0.01) and no differences (*P*>0.05), respectively, as analyzed by one-way ANOVA followed by a Tukey's post hoc test) between two samples.

Table S1. Number of the differential expression genes in the cold stress response and in the mutants.

# DEC	Cold (WT)				
# DEG	3 h	12 h			
UP	557	1503			
DOWN	463	3133			

# 050	cca1 lhy				rve48		quad			
# DEG	0 h	3 h	12 h	0 h	3 h	12 h	0 h	3 h	12 h	
UP	791	877	1005	92	282	480	429	760	765	
DOWN	1273	1206	1246	758	693	653	529	955	884	

 Table S2. Oligomers used in this study.

Name	Sequence (5'-3')
RT-qPCR	
DREB1A_qRT_F	CGCTGACTCGGCTTGGA
DREB1A_qRT_R	GCATCACACATCTCATCCTGAAAC
DREB1B_qRT_F	AGTCAACATGCGCCAAGGAT
DREB1B_qRT_R	ATGTCCAGGCCATGATTCG
DREB1C_qRT_F	TGACGTGTCCTTATGGAGCTA
DREB1C_qRT_R	CTGCACTCAAAAACATTTGCA
RD29A_qRT_F	CTTGATGGTCAACGGAAGGT
RD29A_qRT_R	CAATCTCCGGTACTCCTCCA
COR15A_qRT_F	GTCAGAGTCGGCCAGAAAACTC
COR15A_qRT_R	AACAACGTAGTCTTTCGCTTTCTCA
CCA1_qRT_F	GGTGGACTGAGGAAGAAC
CCA1_qRT_R	GGAGAAAAATTTCTGAGCGTGAC
LHY_qRT_F	GAAGTCTCCGAAGAGGGTCG
LHY_qRT_R	TATTCACATTCTCTGCCACTTGAG
RVE4_qRT_F	GGCGGAAACTTCTACAGATGC
RVE4_qRT_R	AGCTTTCCTCACCTTCTTCTCC
RVE8_qRT_F	TCGTGGAGCAGAAGCTGATA
RVE8_qRT_R	TGGAGGCTGTTTAGCCTTTCTTAC
ELUC_qRT_F	AGGATCATCATACTGGACTCTGAA
ELUC_qRT_R	GAAGTTGTGCAGGCTCTCG
IPP2_qRT_F	GTATGAGTTGCTTCTCCAGCAAAG
IPP2_qRT_R	GAGGATGGCTGCAACAAGTGT
Construction	
CCA1pro_F_Apal	ATT <u>GGGCCC</u> ATGCATGGTTAGCTTAG
CCA1_F+1_EcoRV	GG <u>GATATC</u> CATGGAGACAAATTCG
CCA1_R-3_EcoRV	GG <u>GATATC</u> TGTGGAAGCTTGAGTTTC
CCA1_1014R_EcoRV	GG <u>GATATC</u> ACCACCTGAACTAAG
CCA1_1327_inverse_F	AGGCAAGAGGATGGCACCAATG
CCA1_1134_inverse_R	CTTTGATGCCTCGGAGTGTTC
LHYpro_F_Apal	ATT <u>GGGCCC</u> GGTTATTTCAATTAGATTC
LHY_F+1_Smal	TCC <u>CCCGGG</u> CATGGATACTAATACATC
LHY_R-3_Smal	TCC <u>CCCGGG</u> TGTAGAAGCTTCTCCTTC
RVE1_F+1_Smal	TCC <u>CCCGGG</u> CATGGCGTCGTCTCCG
RVE1_R-3_Smal	TCC <u>CCCGGG</u> TAAGTGGAGATGAATCTC
RVE2_F+1_Smal	TCC <u>CCCGGG</u> GATGGCTATGCAGGAAC
RVE2_R-3_Smal	TCC <u>CCCGGG</u> CCACAAAGGATATGA
RVE4pro_F_Apal	ATT <u>GGGCCC</u> TTTCAACACAACTCTTTAC
RVE4_F+1_Smal	TCC <u>CCCGGG</u> GATGACCTCAACCAA
RVE4_R-3_Smal	TCC <u>CCCGGG</u> AGAGCTTAAGTGTTC
RVE8pro_F_Apal	ATT <u>GGGCCC</u> TTGTTGTATTTGTTTCG

RVE8_F+1_Smal	TCC <u>CCCGGG</u> GATGAGCTCGTCGCCG
RVE8_R-3_Smal	TCC <u>CCCGGG</u> TATGATAAGAGGAC
SV40NLS_Xbal_S	CTAGAATGACTAGTCCTAAGAAGAAGAGGAAGGTTGGAACTAGTT
SV40NLS_Xbal_AS	CTAGAACTAGTTCCAACCTTCCTCTTCTTAGGACTAGTCATT
1AR_F_Xbal	GC <u>TCTAGA</u> GCTTCGCTGTGTATAG
1AR_R_Xbal	GC <u>TCTAGA</u> AATGGCGGAAGATATTTTAG
1AR_mCA/GA_inverse_F	TTTTTTAAACTCCGTCTTCGCCTTTTC
1AR_mCA/GA_inverse_R	AAAGTAATGCCACGTAAACTATACAC
1AR_mEE_R_Xbal	GC <u>TCTAGA</u> AATGGCGGAAAAAAAAAAAGAGG

Genotyping

LBb1.3_SALK	ATTTTGCCGATTTCGGAAC
LB1_SAIL	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC
rve1_LP	AAGTGGAGATGAATCTCATGCTC
rve1_RP	CAAAGACCGCAGTTCAGATTC
rve2_LP	CAGATGCCTTTTACCTCAAGG
rve2_RP	ACGAACACTTCACCACAAAGG
rve3_LP	TGGCTTTCCACAACTTTCTTG
rve3_RP	TGTTAGTCCCACGCTCTGAAC
rve4_LP	AAATCAAACAGGCACAGGATG
rve4_RP	TTTTTACAACTTCCACACCGG
rve5_LP	TTTTGGCAGATTTGTCGATTC
rve5_RP	TCCACGCGGACATCTTTATAG
rve6_LP	GTGAAGAACGAAACAGGCAAG
rve6_RP	GGAGGGGAGTGAAGCTAATTG
rve8_LP	TTCAGCAAAATCAGGAACACC
rve8_RP	AGAGCTGGACAGAGGAAGAGC

RT-PCR

CCA1_RT_F	ATGGAGACAAATTCGTCTGGAG
CCA1_RT_R	CTTTGATGCCTCGGAGTGTTC
RVE1_RT_F	ATGGCGTCGTCTCCGTTGACTGC
RVE1_RT_R	TTATAAGTGGAGATGAATCTC
RVE2_RT_F	ATGGCTATGCAGGAACG
RVE2_RT_R	TCACCACAAAGGATATGATAA
RVE3_RT_F	ATGGTGACTGTAAACCCTAG
RVE3_RT_R	CTAACTGGCGTTGTAAGATG
RVE4_RT_F	ATGACCTCAACCAATCCG
RVE4_RT_R	CTAAGAGCTTAAGTGTTCATG
RVE5_RT_F	ATGGTGTCCGTAAACCCTAG
RVE5_RT_R	CTATTTCAAAGCTTTAGCGC
RVE6_RT_F	ATGGTCTCTAGAAATTCTG
RVE6_RT_R	TTAAGTAGAGATTTCAGGTGG
RVE8_RT_F	ATGAGCTCGTCGCCGTCAAG
RVE8_RT_R	TCATATGATAAGAGGACTTTC

dCAPs

lhy_dCAPs_F	CTCCAATCTTATTATGTCAACTCTC
lhy_dCAPs_R_BgIII	AGAATTCCCGACACTCGCATAGATC

EMSA

1AR-C_WT_S	CTAGACTTCGCCTTTTCTTTTGCCTCTAAAATATCTT
1AR-C_WT_AS	CTAGAAGATATTTTAGAGGCAAAAGAAAAGGCGAAGT
1AR-C_mEE_S	CTAGACTTCGCCTTTTCTTTTGCCTCTTTTTTTTTT
1AR-C_mEE_AS	CTAGAAAAAAAAAAAGAGGCAAAAGAAAAGGCGAAGT

ChIP-qPCR

DREB1A_ChIP1_F	TCTCGTTGTCGTCTTGCTTCTC
DREB1A_ChIP1_R	GTCTGCCTCGTTACCTACCTCTCA
DREB1A_ChIP2_F	CAAACTCCGTCTTCGCCTTTTC
DREB1A_ChIP2_R	GTTGGAGTGAGAGCATGCTGTTTTA
EIF4A1_ChIP_F	TGTTTTGCTTCGTTTCAAGGA
EIF4A1_ChIP_R	GCATTTTCCCGATTACAAC

Under bars indicate restriction enzyme sites.

Dataset S1 (separate file). List of the changes in gene expression in the cold stress response and mutants.

SI References

1. S. Kidokoro, K. Yoneda, H. Takahashi, F. Takahashi, K. Shinozaki, K. Yamaguchi-Shinozaki, Different cold-signalling pathways function in the responses to rapid and gradual decreases in temperature. *Plant Cell* **29**, 760–774 (2017).