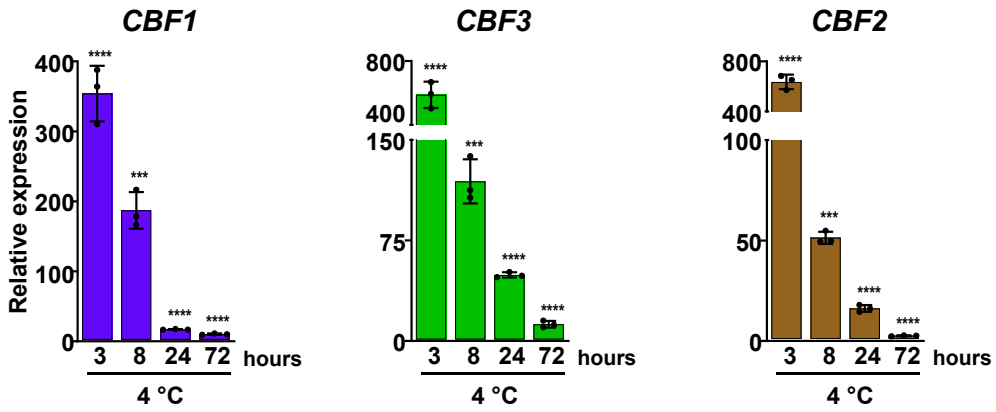
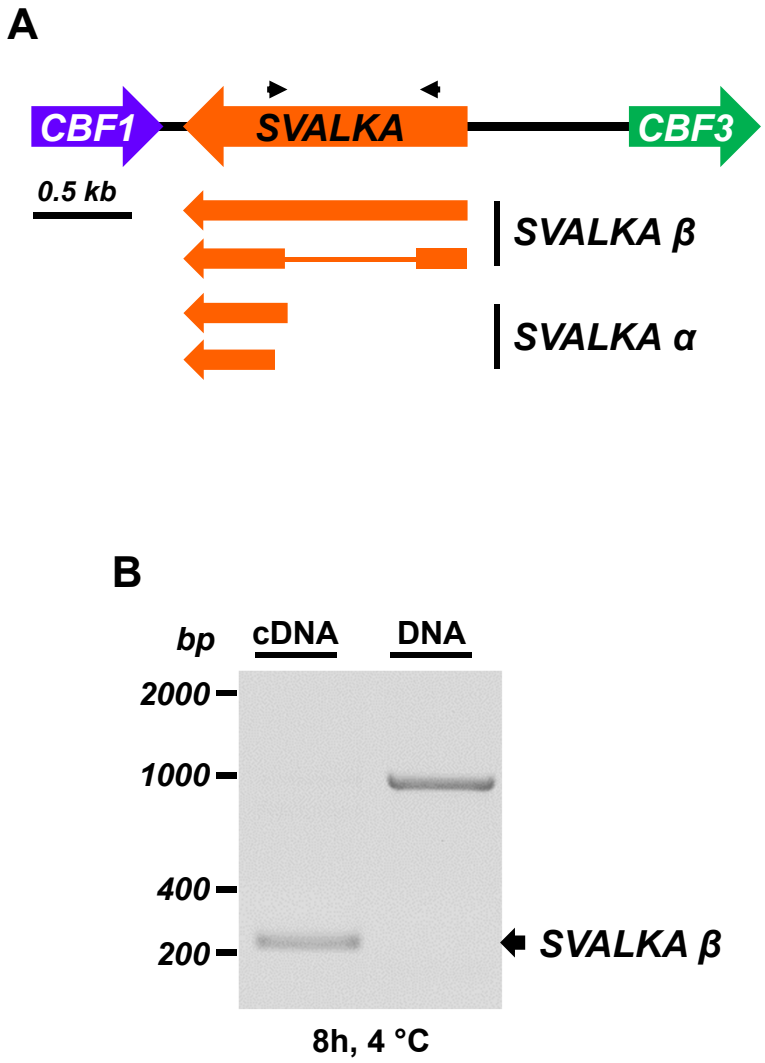
Supplemental Figure S1



Supplemental Figure S1. The induction of *CBFs* strongly declines after 24 hours of cold exposure
Expression analysis of *CBF1*, *CBF3* and *CBF2* in 2-week-old WT plants exposed additionally to 4 °C for the indicated hours. In all cases, transcript levels, determined by RT-qPCR, are represented as relative to the values of the WT plants grown under control conditions. Primer sets 2, 5 and 7 in Figure 1A were used for expression analysis of *CBF1*, *CBF3* and *CBF2*, respectively. Asterisks indicate significant differences (***P<0.001, ****P<0.0001) between the transcript levels of each gene at the indicated time and the levels in WT plants grown under control conditions, as determined by one-sided *t* test. All data represent the mean of three independent experiments, and error bars show the standard deviation.

Supplemental Figure S2



Supplemental Figure S2. The β isoform is the *SVALKA* transcript analyzed in this study.
A, Schematic representation of *SVALKA* genomic region. Arrowheads indicate the position of the primers used in this work for *SVALKA* detection. The different long (β) and short (α) *SVALKA* transcripts generated in response to low temperature, including a long a spliced β isoform, are shown below. **B**, *SVALKA* transcripts, detected by RT-PCR with the primers indicated in **(A)**, in 2-week-old WT plants exposed 8 hours to 4 °C. Genomic DNA from the same plants was amplified by PCR with the same primers as a size reference.

Supplemental tables

Supplemental Table S1. Specific primers used in this study

Related figure	Name	Sequence (5'- 3')
Related to Figure 1	1-F (*CBF1*)	ATCTCTCCTCCGGCCAATAT
	1-R (*CBF1*)	TCAGGTAGATTCTTTTTCAAGTGTGT
	2-F (*CBF1*)	GTCAACATGCGCCAAGGATA
	2-R (*CBF1*)	TCGGCATCCCAAACATTGTC
	3-F (*CBF1*)	TTCGATAGTCGTTTCCATTTTTGT
	3-R (*CBF1*)	TTTTCCACTCGTTTCTACAACAAT
	4-F (*CBF3*)	GACACAAACTCCGTCTTCGC
	4-R (*CBF3*)	AGTTGGAGTGAGAGCATGCT
	5-F (*CBF3*)	CAACTTGCGCTAAGGACA
	5-R (*CBF3*)	TCTCAAACATCGCCTCAT
	6-F (*CBF3*)	TCAGTTTCAGTATAAGTGTGGGCT
	6-R (*CBF3*)	CTGAATCGGTTGTTTCGGTTT
	7-F (*CBF2*)	GAATCCCGGAATCAACCTGT
	7-R (*CBF2*)	CCCAACATCGCCTCTTCATC
	AT4G26410-F	GAGCTGAAGTGGCTTCCATGAC
	AT4G26410-R	GGTCCGACATACCCATGATCC
Related to Figure 2	KIN1-F	ATTCGGGTCAAATTTGGGAG
	KIN1-R	TGAATATAAGTTTGGCTCGTC
	XERO2-F	TTCCCGGTGGTCATCACTAG
	XERO2-R	GCGACTCAATGAAAGAAAGCCAC
	GOLS3-F	AGGGAAGACATCAAGATGCTT
	GOLS3-R	GCACATCAGCTTCAGACAAAT
	COR15B-F	CCAATGAAACTGCGACTGAG
	COR15B-R	ATGAGTGGTTGAATCAGGAC
Related to Figure 3	SVK-F	CTACCTCTTCCCCACCTT
	SVK-R	TCTTCTCGTTGTCGTCTTG

Supplemental Table S2. Buffers used in this study

Protocol	Name	Composition
Nuclear RNA immunoprecipitation	Honda buffer	20 mM Hepes KOH pH 7.4, 0.44 M sucrose, 1.25% Ficoll, 2.5% Dextran T-40, 10 mM $MgCl_2$, 0.5% Triton X-100, 5 mM DTT, 1 mM PMSF, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche) and 8 U/ml Ribolock RNase Inhibitor (Thermo-Scientific)
	Nuclei Wash buffer	20 mM Hepes KOH pH 7.4, 0.44 M sucrose, 10 mM $MgCl_2$, 5 mM DTT, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche), 1mM PMSF and 8 U/ml Ribolock RNase Inhibitor
	Lysis buffer	50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1 % SDS, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche), 1mM PMSF, 160 U/ml Ribolock RNase Inhibitor and 25 µM MG132 Proteasome Inhibitor
	Dilution buffer	16.7 mM Tris-HCl pH 8.0, 1.1% Triton X-100, 1.2 mM EDTA, 167 mM NaCl, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche), 1mM PMSF, 160 U/ml Ribolock RNase Inhibitor and 25 µM MG132 Proteasome Inhibitor
	Beads Wash buffer	20 mM Tris-HCl pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche), 1mM PMSF, 40 U/ml Ribolock RNase Inhibitor and 5 U/ml DNase I
	Elution buffer	50 mM Tris-HCl pH 8.0, 0.5% SDS and 40 U/ml RNase Inhibitor
Chromatin Immunoprecipitation	Extraction buffer	10 mM Tris-HCl pH 8.0, 0.25 M sucrose, 10 mM $MgCl_2$, 1% Triton X-100, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche) and 1mM PMSF
	Lysis buffer	50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche), 1mM PMSF and 1% SDS
	Dilution buffer	16.7 mM Tris-HCl pH 8.0, 1.1% Triton X-100, 1.2 mM EDTA, 167 mM NaCl, 1x cOmplete™ Protease Inhibitor Cocktail Mini, EDTA-free (Roche) and 1mM PMSF

Supplemental Table S3. Full names of the genes mentioned in this study

AGI	Gene Symbol	Brief description
AT4G25490	*CBF1*	Encodes a transcriptional activator that binds to the *DRE/CRT* regulatory element and induces cold-regulated (*COR*) gene expression increasing plant freezing tolerance.
AT4G25470	*CBF2*	Encodes a transcriptional activator that binds to the *DRE/CRT* regulatory element and induces cold-regulated (*COR*) gene expression increasing plant freezing tolerance.
AT4G25480	*CBF3*	Encodes a transcriptional activator that binds to the *DRE/CRT* regulatory element and induces cold-regulated (*COR*) gene expression increasing plant freezing tolerance.
AT4G07395	*SVALKA*	Encodes a long non-coding RNA involved in cold acclimation by limiting *CBF1* induction via RNAPII collision stemming from the *SVALKA-asCBF1* lncRNA cascade.
AT2G23380	*CLF*	Encodes a catalytic component of the PRC2 complex.
AT4G02020	*SWN*	Encodes a component of a large protein complex that can include VERNALIZATION 2 (VRN2), VERNALIZATION INSENSITIVE 3 (VIN3) and polycomb group of proteins FERTILIZATION INDEPENDENT ENDOSPERM (FIE) and CURLY LEAF (CLF).
	COLDAIR	Encodes a long noncoding RNA that is necessary for the repression of the floral repressor FLOWERING LOCUS C (FLC) during vernalization.
	COLDWRAP	Encodes an antisense long noncoding RNA
	AG-incRNA4	Encodes a long noncoding RNA that acts as a co-repressor with CURLY LEAF (CLF) to confer *AG* tissue-specific expression
AT5G15960	*KIN1*	Cold- and ABA- inducible gene. Possibly functions as an anti-freeze protein.
AT3G50970	*XERO2*	Encodes a dehydrin protein.
AT1G09350	*GOLS3*	Predicted to encode a galactinol synthase
AT2G42530	*COR15B*	Encodes COR15B, a protein that protects chloroplast membranes during freezing.
AT4G26410	RHIP1	Encodes a protein predicted to have a 3-stranded helical structure that interacts with both AtRGS1 and AtHXK1 in planta and is required for glucose-regulated gene expression.