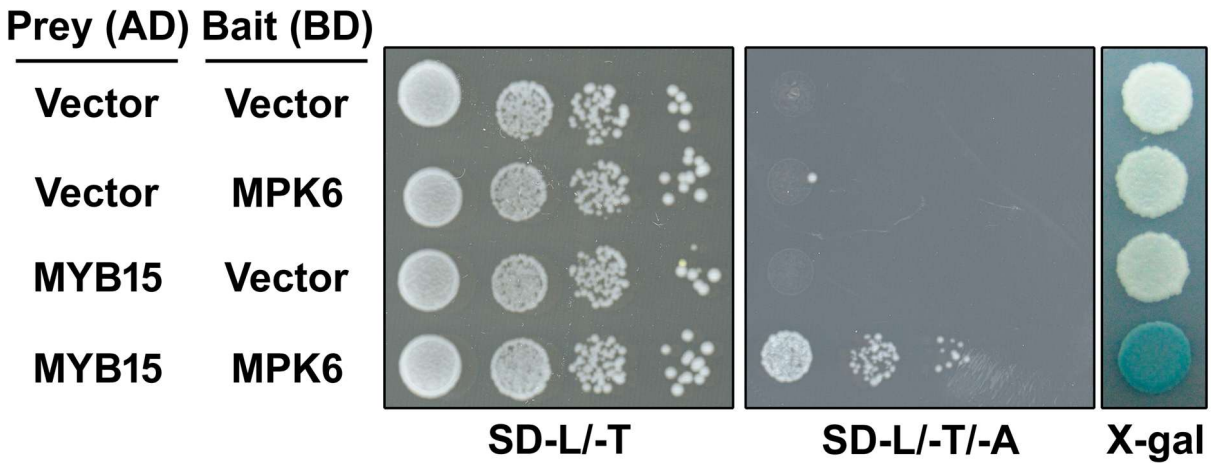

Figure S1. Yeast two-hybrid analysis showing the interaction of MYB15 with MPK6. The indicated prey and bait plasmids were co-transformed into yeast strain pJ69-4A. Shown are serial dilution growth tests of the transformants on selective SD medium lacking Leu and Trp (SD-L/-T) (control) and on SD medium lacking Leu, Trp, and Ade (SM-L/-T/-A) to demonstrate activation of the *ADE2* reporter gene. A filter lift assay for β-galactosidase activity (X-gal) is also shown. Blue color indicates high β-galactosidase activity caused by the activation of the *LacZ* reporter. BD indicates fusion to a plasmid containing the GAL4 DNA-binding domain, and AD indicates fusion to a GAL4 transcriptional activation domain.

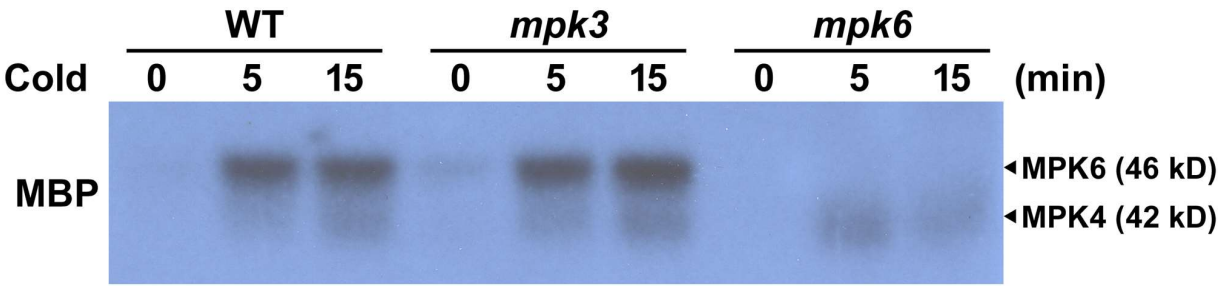

Figure S2. Cold-triggered activation of MPK4 and MPK6 *in planta*. Leaves of 3-week-old WT, *mpk3*, and *mpk6* plants were subjected to cold (4°C) treatment for the indicated times to induce MPK activity. Shown are autoradiographs of SDS-polyacrylamide gels containing embedded MBP depicting signals from an in-gel kinase assay on resolved proteins from extracts of treated leaves. The expected positions of MPK4 and MPK6 are indicated on the gels.

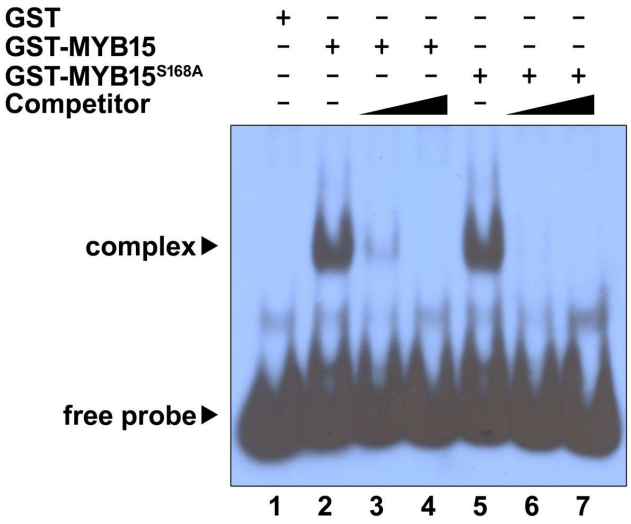

Figure S3. Analysis of the binding specificity of MYB15 or MYB15^{S168A} to *CBF3* promoter fragments. Shown are the results of an EMSA performed using a ^{32}P-labelled *CBF3* promoter fragment (-984 to -785 bp region) as a probe, except that GST-MYB15 and GST-MYB15^{S168A} proteins were incubated with a 100-fold (lanes 3 and 6) or 200-fold (lanes 4 and 7) molar excess of unlabeled probes (competitor) before adding labeled probes.

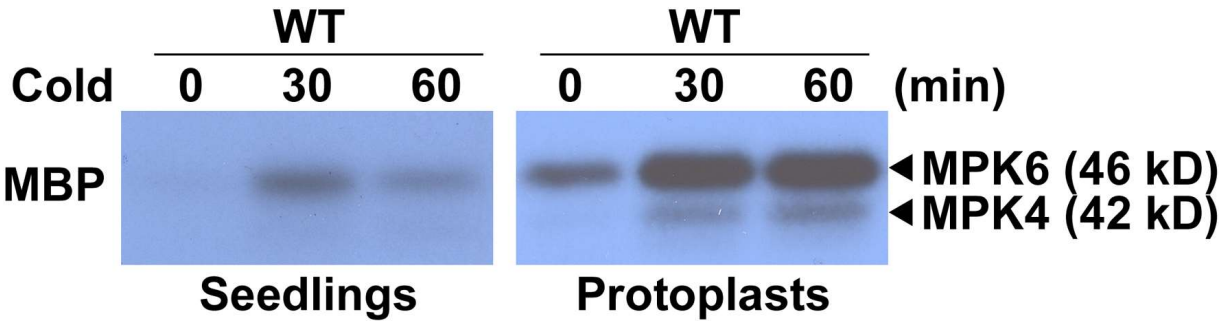

Figure S4. Comparison of the activities of cold-activated MPKs in wild-type (WT) seedlings and protoplasts. WT seedlings and protoplasts were subjected to cold (4°C) treatment for the indicated times to induce MPK activity. Shown are autoradiographs of SDS-polyacrylamide gels containing embedded myelin basic protein (MBP), depicting signals from an in-gel kinase assay on resolved proteins from extracted proteins. The expected positions of MPK4 and MPK6 are indicated on the gels.

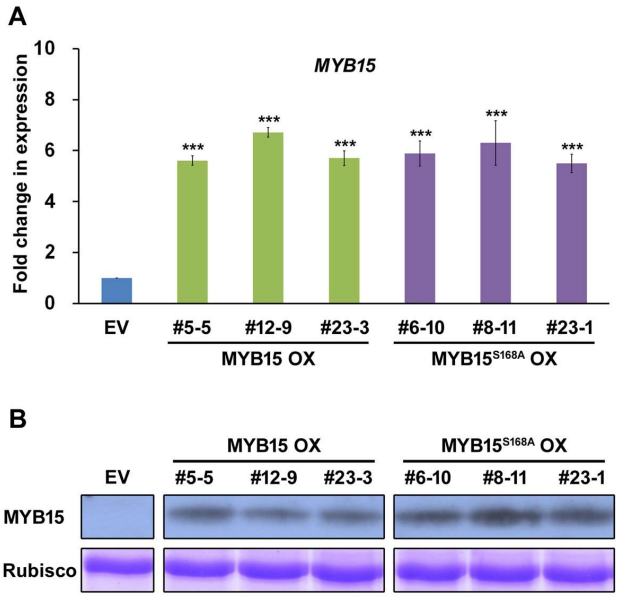

Figure S5. Characterization of MYB15 OX and MYB15^{S168A} OX transgenic plants. (A) Shown is an analysis of transcript levels of *MYB15* and *Tubulin* (loading control) by quantitative RT-PCR using total RNA prepared from 3-week-old Petri dish-grown seedlings harboring empty vector (EV) and T_3 homozygous progeny of three independent lines of *CaMV35S*:*3XFlag-MYB15* (MYB15 OX) and *CaMV35S*:*3XFlag-MYB15^{S168A}* (MYB15^{S168A} OX) transformants. Data are presented as the mean ± SD of three independent experiments. $P < 0.001$ (***) indicate statistically significant changes. (B) Shown is an immunoblot and Coomassie Brilliant Blue (CBB) staining of a polyacrylamide gel containing resolved total proteins isolated from the above seedlings. The gel blot was probed with anti-Flag antibody (anti-Flag antibody). The ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) band detected by CBB staining shows the amount of proteins loaded in each well.

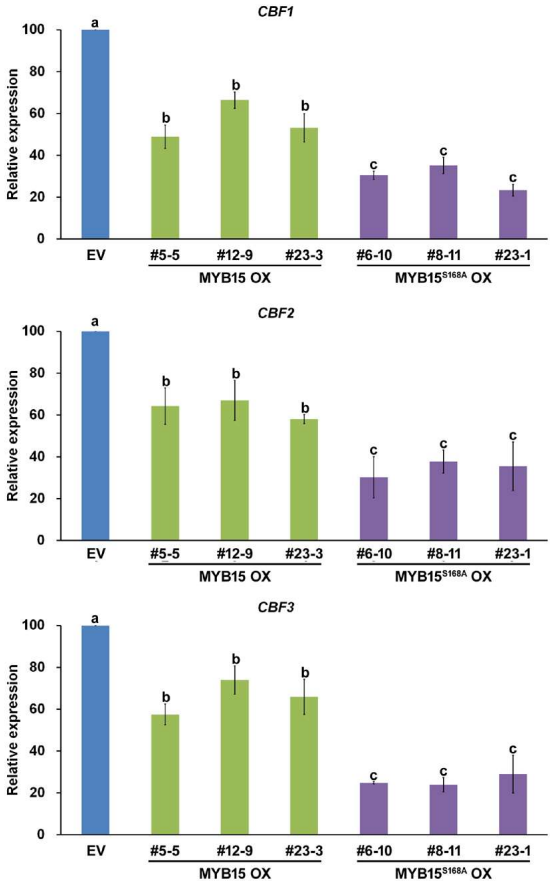

Figure S6. Comparison of transcripts of *CBF* genes from three, independent, cold-stressed MYB15 and MYB15^{S168A} lines. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis of *CBF* was performed using total RNA isolated from seedlings of the indicated lines subjected to cold (4°C) treatment for 3 h. cDNA was synthesized from 5 μg of total RNA and used as a template for RT-qPCR. Data are presented as the mean ± SD of three independent experiments. Different letters represent statistically significant differences between genotypes ($P < 0.001$).

Table S1. Primers used for construction and site-directed mutagenesis of *MYB15* plasmids.

Construct	Position	Sequence
GST-MYB15	F	5'-*ggatccATGGGAAGAGCTCCATGCTG*-3'
	R	5'-*gaattccccgggCTAGAGCCCGGCTAAGAGATCTTG*-3'
GST-MYB15 N (amino acids 1-172)	F	5'-*ggatccATGGGAAGAGCTCCATGCTG*-3'
	R	5'-*ctcgagCTAACTTGTCGAAGGCGATGTCG*-3'
GST-MYB15 C (amino acids 173-285)	F	5'-*ggatccGAGGTTTCTTCGATGACACT*-3'
	R	5'-*gaattccccgggCTAGAGCCCGGCTAAGAGATCTTG*-3'
MYB15 (yeast two-hybrid)	F	5'-*ggatccGAGGTTTCTTCGATGACACT*-3'
	R	5'-*gaattccccgggCTAGAGCCCGGCTAAGAGATCTTG*-3'
$MYB15^{T18A}$	F	5'-*GGACCATGGGCACCTGAAGAAGATCAAATC*-3'
	R	5'-*TTCTTCAGGTGCCCATGGTCCTCTCTTCAA*-3'
$MYB15^{S168A}$	F	5'-*TTTTCGACAGCGCCTTCGACAAGTGAGTT*-3'
	R	5'-*TGTCGAAGGCGCTGTCGAAAATAAGCTTTC*-3'

Table S2. Primers used to construct *MPK3*, *MPK4*, and *MPK6* plasmids.

Construct	Position	Sequence
His-MPK3	F	5'-*ggatccATGAACACCGGCGGTGGCCA*-3'
	R	5'-*gtcgacCTAACCGTATGTTGGATTGA*-3'
His-MPK4	F	5'-*ggatccATGTCGGCGGAGAGTTGTTT*-3'
	R	5'-*gtcgacCTACACTGAGTCTTGAGGAT*-3'
His-MPK6	F	5'-*ggatccATGGACGGTGGTTCAGGTCA*-3'
	R	5'-*gtcgacCTATTGCTGATATTCTGGAT*-3'
MPK3 (yeast two-hybrid)	F	5'-*ggatccATGAACACCGGCGGTGGCCA*-3'
	R	5'-*ctgcagCTAACCGTATGTTGGATTGA*-3'
MPK4 (yeast two-hybrid)	F	5'-*ggatccATGTCGGCGGAGAGTTGTTT*-3'
	R	5'-*ctgcagCTACACTGAGTCTTGAGGAT*-3'
MPK6 (yeast two-hybrid)	F	5'-*ggatccATGGACGGTGGTTCAGGTCA*-3'
	R	5'-*ctgcagCTATTGCTGATATTCTGGAT*-3'

Table S3. Primers used for quantitative RT-PCR.

Gene	Position	Sequence
MYB15	F	5'-*GGATTCGAGGTTTCTTCGATGACACT*-3'
	R	5'-*GGATTCCCCGGGCTAGAGCCCGGCTAAGAGATCTTG*-3'
CBF1	F	5'-*TGATACGACGACCACGAATC*-3'
	R	5'-*AGTAACTCCAAAGCGACACG*-3'
CBF2	F	5'-*TATGACGACGGATGCTCATG*-3'
	R	5'-*CTCCATAAGGACACGTCATC*-3'
CBF3	F	5'-*GATGACGACGTATCGTTATGG*-3'
	R	5'-*TACACTCGTTTCTCAGTTTTACAAAC*-3'
Tubulin	F	5'-*CCAACAACGTGAAATCGACAG*-3'
	R	5'-*TCTTGGTATTGCTGGTACTCT*-3'

Table S4. Mass spectrometry analysis of tryptic digests of unphosphorylated MYB15 and MPK6-phosphorylated MYB15.

Peptide sequence	Position of MYB15	No. of phosphate groups	(M+H)		Putative phosphorylation site
			Expected	Measured	
SESELADSSNPSGESLFSTS*PSTS	149-172	1 phospho	2482.9	2483.0	S168

*Amino acid residue that could potentially be phosphorylated by MPK6.