

## SUPPLEMENTAL DATA

### **Supplemental FIGURE 1. DNA and RNA binding activity of AtCSP3.**

**A,** Purification of recombinant proteins expressed in *E.coli*. *E. coli* BL21 cells were transformed with pGEX-AtCSP3 and the recombinant protein was purified. Lane 1; soluble fraction from non-induced cells. Lane 2; soluble fraction from IPTG-induced cells. Lane 3; purified protein using GST-sepharose column chromatography and PreScission protease. **B,** Analysis of ssDNA and dsDNA binding activities of AtCSP3 by gel shift assay. Purified recombinant protein (from 0 to 500 pmol) was incubated with M13mp8 ssDNA or dsDNA and separated by agarose-gel electrophoresis. GST (500 pmol) was used as a negative control. **C,** RNA-binding activity of AtCSP3. Purified recombinant protein (from 0 to 250 pmol) was incubated with *in vitro*-transcribed luciferase mRNA and separated by agarose gel electrophoresis. The purified GST protein (B, 500 pmol; C, 250 pmol) was used as a negative control.

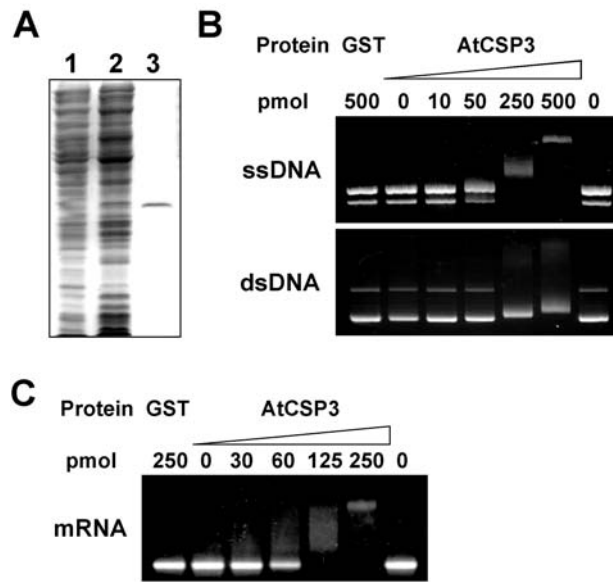
### **Supplemental FIGURE 2. Tissue- and developmental stage-specific expression and subcellular localization of AtCSP3.**

Bent cotyledon stage embryo (A), one-day-old germinating seed (B), two-day-old seedling (C), four-day-old seedling (D), root tip of four-day-old seedling (E), immature silique (F), flower (G and H), anther (I), pistil (J), three-week-old whole plant (I), and root (J). K, Localization of *pAtCSP3::AtCSP3::GFP* during cold treatment. Ten-day-old transgenic seedlings exposed to 4°C for 1 and 3 days (Scale bars, 20 μm).

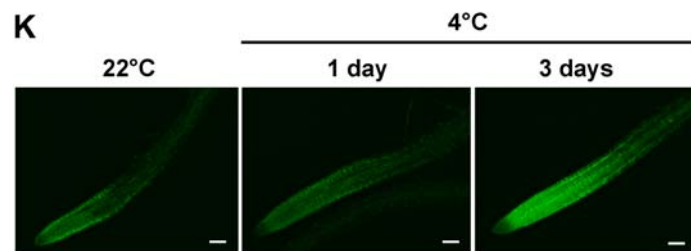
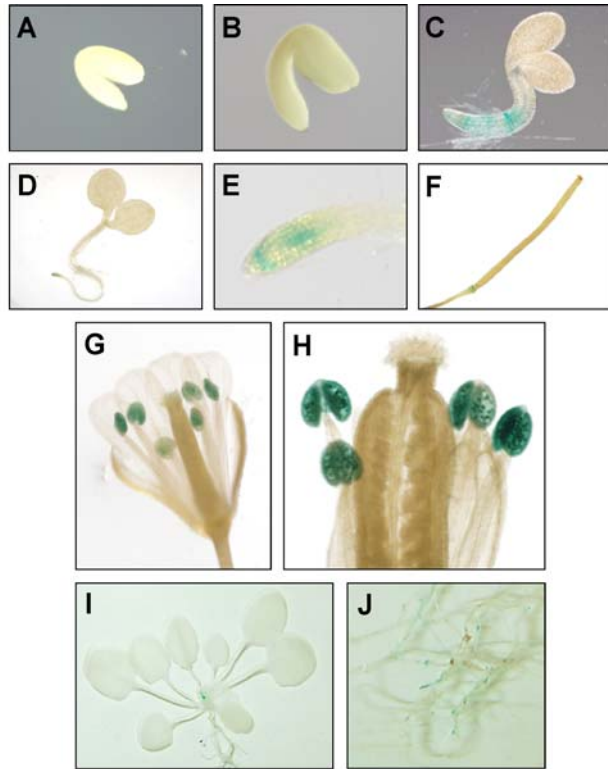
### **Supplemental FIGURE 3. Complementation of the freezing sensitive phenotype of *atcsp3-2* mutant.**

Two independent lines of *atcsp3-2* transformed with *pAtCSP3::AtCSP3* were analyzed for freezing tolerance. Survival rates in WT, *atcsp3-*

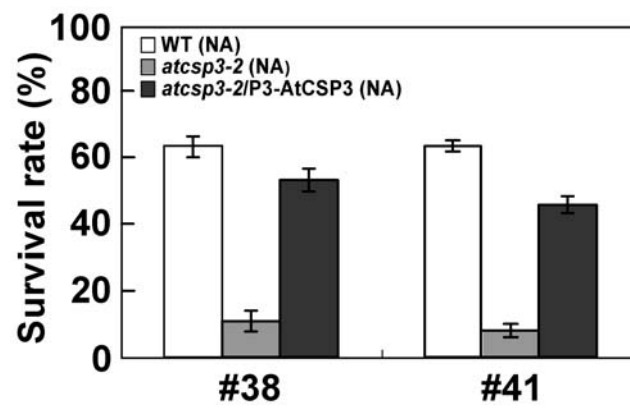
2 mutant, and *atcsp3-2/pAtCSP3::AtCSP3* plants (lines #38 and #41) were scored at 7 days after freezing treatment at -4°C (NA; non-acclimation).



Kim et al. Supplemental FIGURE 1.



Kim et al. Supplemental FIGURE 2.



Kim et al. Supplemental FIGURE 3.

Table S1. Genes down-regulated in *atcsp3-2* mutant.

AGI code	Gene description	Average fold	*Abiotic stresses
At2g17870	cold-shock DNA-binding family protein	12.2	-
At5g48430	unknown protein	7.71	S
At5g39580	peroxidase, putative (Aterox P62) (ATP24a)	5.47	C, S, O
At3g60140	glycosyl hydrolase family 1 protein	5.32	D, S, O
At1g08090	high affinity nitrate transporter 2.1 (ATNRT2:1, ACH1)	5.1	C
At1g02920	glutathione S-transferase 11(ATGSTF7)	5.02	C, D, S, O
At1g10070	branched-chain amino acid transaminase 2 (ATBCAT2)	4.75	S, O
At1g30730	FAD-binding domain-containing protein	4.63	C, D, O
At5g57220	cytochrome P450 (CYP81F2)	4.55	C, D, S, O
At1g02930	glutathione S-transferase, early responsive to dehydration 11 (ATGSTF6)	4.42	C, D, S, O
At5g40590	DC1 domain-containing protein	3.7	C, D, S
At3g47340	glutamine-dependent asparagine synthetase 1 (ASN1)	3.62	C, D, S, O
At4g21680	proton-dependent oligopeptide transport (POT) family protein	3.58	S, O
At5g64120	peroxidase, putative (Aterox P71) (ATP15a)	3.42	C, D, S, O
At5g57560	xyloglucan:xyloglucosyl transferase (TCH4)	3.04	C, D, S, O
At2g18690	expressed protein	2.81	C, D, S, O
At1g49570	peroxidase, putative (Aterox P10) (ATP5a)	2.8	D, O
At2g34930	disease resistance family protein	2.66	C, D, S, O
At4g31800	WRKY DNA-binding protein 18 (WRKY18)	2.64	C, D, S, O
At1g10550	xyloglucan:xyloglucosyl transferase 33 (XTH33)	2.63	-
At1g19020	expressed protein	2.57	C, D, S, O

Expression ratios (Col-0 plants/*atcsp3-2* mutant plants) > 2.5-fold. Values represent the mean of two replications.

\*Abiotic stress response data were obtained from eFP Browser (>3-fold). C, cold; D, drought; S, salt; O, osmotic stress.

Table S2. Primers used for this study.

Gene name	Forward primer	Reverse primer
Cloning		
pGEX-AtCSP3	5'-TCTGTCGACATGAGCGGAGAC-3'	5'-TGCAGCGGCCGCTTAAGCAAC-3'
pINIII-AtCSP3	5'-TCTCATATGATGAGCGGAGAC-3'	5'-GCATCGGATCCTTAAGCAAC-3'
AtCSP3 promoter-GUS	5'-ATTCTACTGCA GTTGGGTAAAAGT-3'	5'-TCTTCCGGATCCATTAGATCAGAG-3'
AtCSP3 promoter::AtCSP3-GFP	5'-CACC CAACTAGGTATGCATGCATGGG-3'	5'-TTAAGCAACCGAAGTACATTCCCTC-3'
Overexpression of AtCSP3	5'-TCTAGACGAACAAAGTGCTTCG-3'	5'-GAGCTCTTAAGCAACCGAAGTA-3'
Probes		
AtCSP3 transgene	5'-TCTAGACGAACAAAGTGCTTCG-3'	5'-GAGCTCTTAAGCAACCGAAGTA-3'
AtCSP3 specific gene	5'-GTTGATGCCATAAGTTTGAGATCTT-3'	5'-AACAGCAATGCAGAGTATAAGTAAA-3'
Screening of <i>atcsp3-2</i> mutant		
T-DNA specific primer	5'-CGTCCGCAATGTGTTATTAAG-3'	5'-TACAATCCCTAGCGAAATGTC-3'
AtCSP3 gene specific primer	5'-TGTACGAACAAAGTGCTTCGA-3'	5'-TACAATCCCTAGCGAAATGTC-3'
RT-PCR		
AtCSP1 (At4g36020)	5'-ACGCCTAAGGATTTCCCA-3'	5'-ACACACAAAAGGACCAG-3'
AtCSP2 (At4g38680)	5'-CCAATCAAATGAGCGGAG-3'	5'-AGAACCGATCACTTGTGAG-3'
AtCSP3 (At2g17870)	5'-AAGTGCTTCGAGTTTTTGTTA-3'	5'-GCAGTGAACAGGTTGCAT-3'
AtCSP4 (At2g21060)	5'-AGAGAGATCTGATCGGTG-3'	5'-GAGAAGAGGGATCGATGA-3'
Peroxidase (At5g39580)	5'-ATGGGCTTGGTCCGATCATT-3'	5'-TTAATCACTGATTAATTAACCGC-3'
NRT2.1 (At1g08090)	5'-GACAAGACGGCCAAGTTC-3'	5'-CCGTAGAGAAGAACGAAGAT-3'
CYP81 (At5g57220)	5'-ATGGATTACGTTTTGATTGTTT-3'	5'-TTAAGCCAAGAGATTAGTCAT-3'
GSTF6 (At1g02930)	5'-CAAGAGCAATTACTCAATACA-3'	5'-GAGATTCACTTAAAGAACCTT-3'
DC1 (At5g40590)	5'-ATGTCTTACGACAATCGGT-3'	5'-TTAGATGCAATTGAGAGCTGC-3'
WRKY18 (At4g31800)	5'-GATAAAGGTTTCTTTTATGGAC-3'	5'-TCATGTTCTAGATTGCTCCAT-3'