

Figure S1. *OsCTK1* positively regulates stomatal closure.

(A-B) Leaf blade width in cm (A) and morphological phenotype (B) of wild-type NIP, *Osctk1-2* and *Osctk1-3* plants before and after a 6°C treatment for 3 h and 9 h. The middle 2 cm section of the third leaf was photographed in (B).

(C) Histochemical GUS staining leaf tissues of the wild-type NIP and the *pOsCTK1::GUS* transgenic lines. Bars = 100 μm.

(D-I) Chilling response phenotypes of NIP, *Osctk1-2* and *OsCTK1* complementation transgenic lines in *Osctk1-2* (*OsCTK1CS#2*, *OsCTK1CS#4*, *OsCTK1CT#1* and *OsCTK1CT#2*) before and after a 6°C treatment, including leaf blade width (D), water loss (E), stomatal morphology (F), stomatal aperture (G), transpiration rate (mmol H₂O m⁻² s⁻¹) (H), and thermal imaging (I).

Dot plots in (G) are from 60 stomata per genotype time point. Bars in C represent 100 μm. Different letters in plots indicate significantly differences at $p < 0.05$ by Duncan's multiple range test among the means via ANOVA.

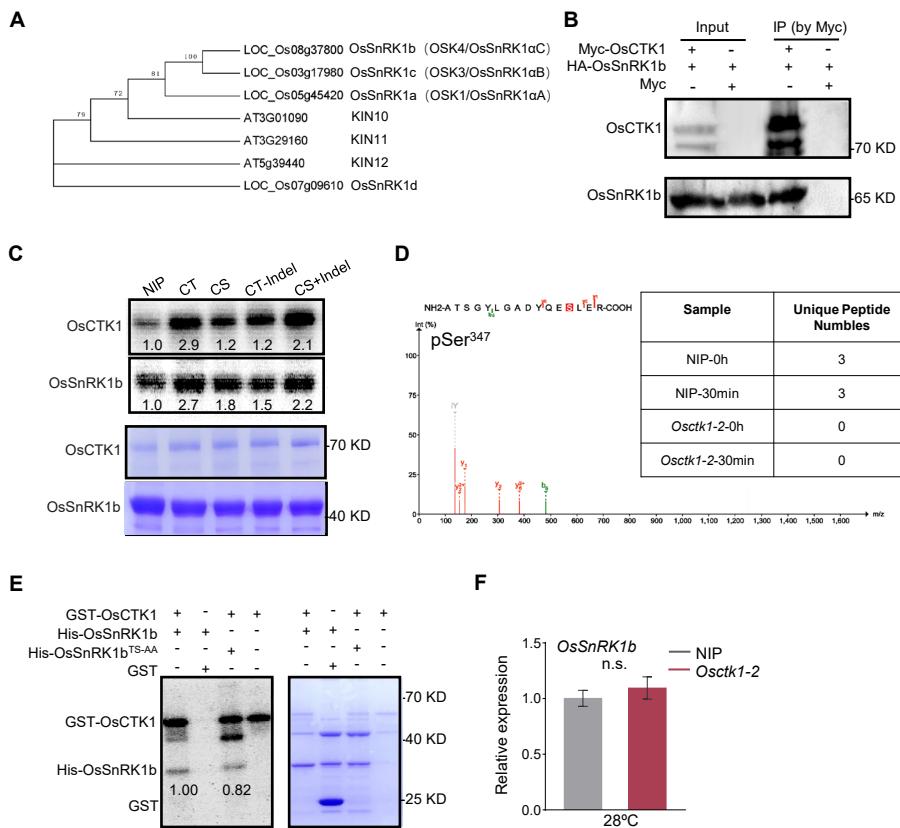


Figure S2. OsCTK1 phosphorylates and stabilizes OsSnRK1b.

(A) Phylogenetic tree of *SnRK1* orthologs in rice and *Arabidopsis* (NJ method). Numbers below branches indicate evolutionary distances (in substitutions per site), with values ≥ 70 shown. Shown in brackets are additional names used in literature.

(B) Immunoblots of total proteins (Input) and IPed proteins in the co-IP assay. OsCTK1-Myc was co-expressed with OsSnRK1b-HA in *N. benthamiana* via *Agroinfiltration*. Total proteins were IPed by anti-Myc antibody.

(C) In vitro kinase assay of OsSnRK1b by OsCTK1. Recombinant OsCTK1 (five variants) and OsSnRK1b proteins were separated by 12% SDS-PAGE after incubation in protein kinase buffer containing [γ -³²P] ATP. CS: chilling susceptible variant of OsCTK1, CT: chilling tolerant variant of OsCTK1 that has a 12-bp insertion. Top two panels are autoradiographs showing phosphorylation of proteins. Numbers are relative phosphorylation level of GST-OsCTK1 or His-OsSnRK1b normalized to that of NIP. Bottom two panels are Coomassie Brilliant Blue (CBB) staining of the proteins.

(D) Secondary mass spectra of peptides of OsSnRK1b identified by phospho-mass spectrometry.

(E) In vitro kinase assays of OsCTK1 on OsSnRK1b. Shown are autoradiograph (left panel) and CBB staining (right panel) of gels with proteins from the kinase reaction separated on 12% SDS-PAGE.

(F) Expression of *OsSnRK1b* in wild-type NIP and the *Osctk1-2* mutant at three-leaf seedling stage grown under at 28°C. Shown are relative expression analyzed by qRT-PCR with *OsActin* as the reference gene.

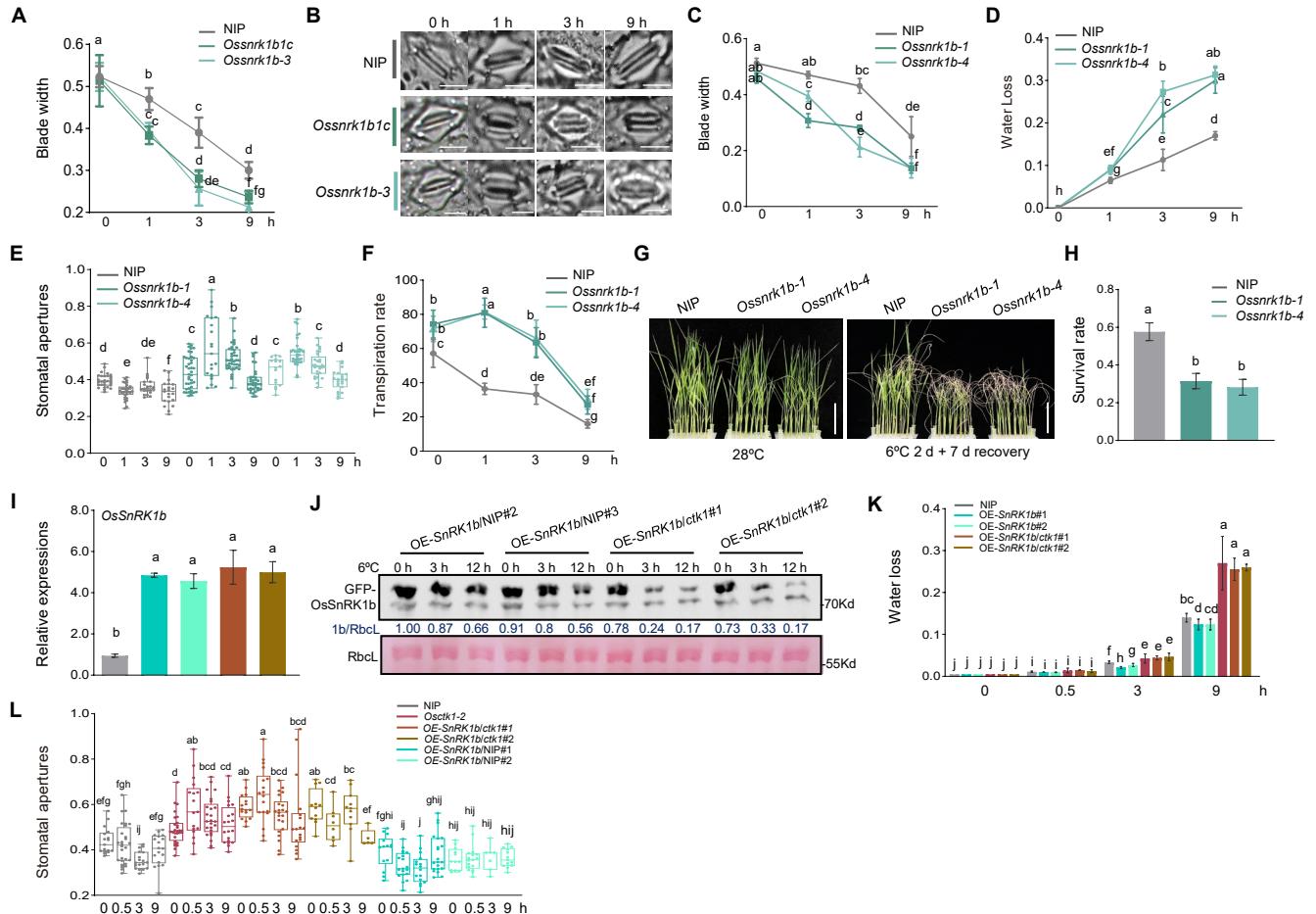


Figure S3. *OsSnRK1b* overexpression enhances stomatal closure and chilling tolerance.

(A-B) leaf blade width (A) and stomata images (B) of wild-type NIP, *Ossnrk1b-1c*, and *Ossnrk1b-3* before (0 h) and after treatment of 6°C for 1 h, 3 h and 9 h.

(C-F) Chilling responses of the *Ossnrk1b-1* and *Ossnrk1b-4* mutants compared to the wild-type NIP. Shown are leaf blade width (C), water loss (D), stomatal aperture (E), and transpiration rate (mmol H₂O m⁻² s⁻¹) (F) of seedlings before (0 h) and after 6°C treatment for 1 h, 3 h and 9 h. Bars = 100 μm.

(G-H) Chilling tolerance phenotypes of wild-type, *Ossnrk1b-1* and *Ossnrk1b-4* mutants at three-leaf stage: morphology (G) and survival rates (H). Three-leaf-stage seedlings were treated at 6°C for 2 days followed by 7 days of recovery at 28°C. Bar = 5 cm. Values are means of three replicates.

(I) Expression of *OsSnRK1b* in wild-type NIP, OE-*SnRK1b*/NIP and OE-*SnRK1b*/ctk1-2 plants at three-leaf seedling stage grown at 28°C. Shown are relative expression analyzed by qRT-PCR with *OsActin* as the reference gene.

(J) Immunoblot analysis of GFP-OsSnRK1b-GFP in OE-*SnRK1b*/NIP and OE-*SnRK1b*/ctk1-2 lines before (0 h) and after treatment of 6°C for 3 h and 12 h. Numbers are relative abundance of GFP-OsSnRK1b relative to the abundance in OE-*SnRK1b*/NIP#2 at 0 h.

(K-L) Water-loss (K) and stomatal aperture (L) of wild-type NIP, OE-*SnRK1b*/NIP, and OE-*SnRK1b*/ctk1-2 plants. Shown in (K) are means and standard deviations of 3 replica, shown in (L) are dot plots with 60 stomata per genotype and timepoint.

Different letters in all plots indicate significantly different levels as determined by ANOVA.

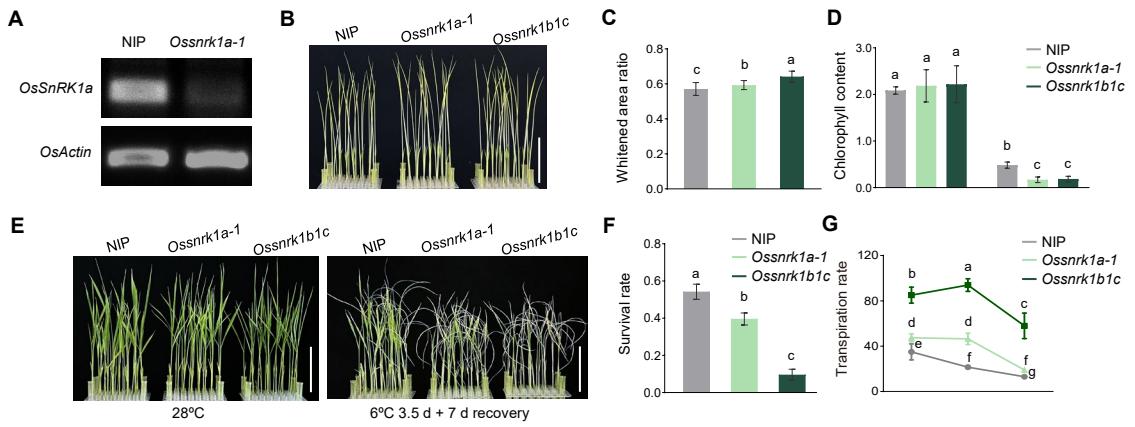


Figure S4. OsSnRK1b is required for starvation response.

(A) Expression of *OsSnRK1a* and *OsActin* analyzed by semi-quantitative RT-PCR in wild-type NIP and *Ossnrk1b*.

(B-D) Starvation response phenotypes of wild-type NIP, *Ossnrk1a* and *Ossnrk1b1c* seedlings grown at 28°C for 4 days followed by dark for 7 days. Shown are the morphological phenotype (B), quantification of leaf yellowing area (C), and chlorophyll contents (ug/mg FW) (D) after starvation.

(E-G) Chilling tolerance phenotypes of wild-type NIP, *Ossnrk1a* and *Ossnrk1b1c* at three-leaf stage: morphological phenotypes (E), survival rates (F) and transpiration rate (G) after or during chilling.

Different letters indicate significantly different levels as determined by ANOVA in graphs. Bars equal 5 cm in (B) and (E).

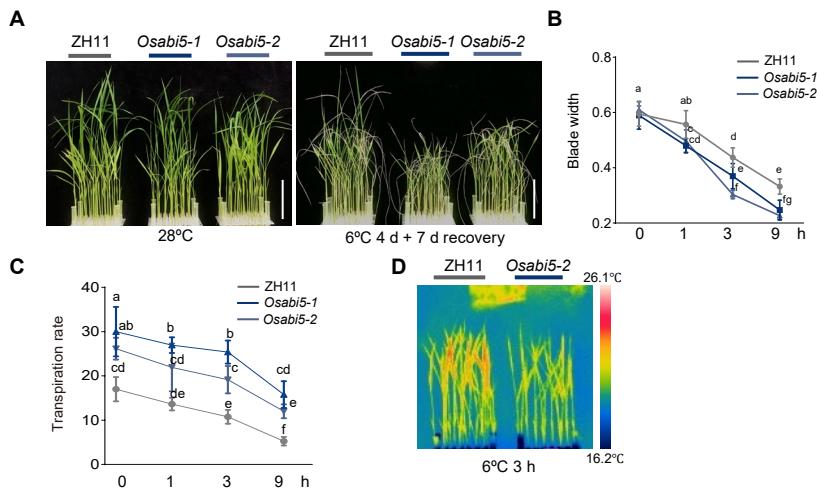


Figure S5. *OsABI5* regulates chilling tolerance.

(A) Chilling tolerance of wild-type (ZH11), *Osabi5-1* and *Osabi5-2*. Shown are three-leaf-stage seedlings treated at 6°C for 4 d followed by 7 d of recovery at 28°C. Bar=5 cm.

(B) Leaf blade width of wild-type NIP, *Osabi5-1* and *Osabi5-2* before (0 h) and after a 6°C treatment.

(C) Leaf transpiration of wild-type ZH11, *Osabi5-1* and *Osabi5-2* before (0 h) and after a 6°C treatment.

(D) Infrared thermal image of wild-type ZH11, *Osabi5-1* and *Osabi5-2* at 3 h of chilling treatment.

In (B) and (C), different letters indicate significant differences at $p < 0.05$ by Duncan's multiple range test among the means via ANOVA.

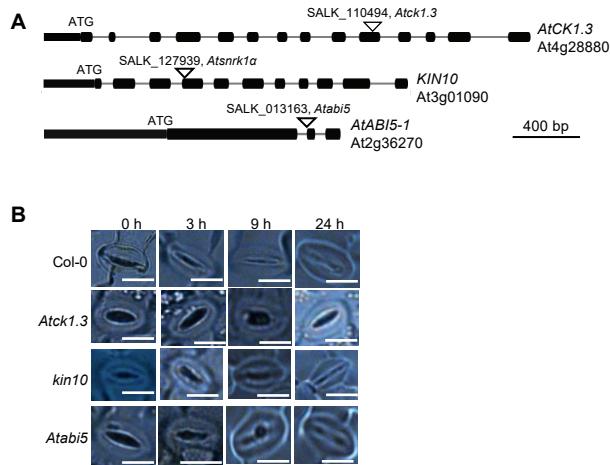


Figure S6. *AtCK1.3*, *KIN10* and *AtABI5* regulate stomatal movement under cold in *Arabidopsis*.

(A) Gene structures and T-DNA insertion sites of *AtCK1.3*, *KIN10* and *AtABI5* genes. Exons are indicated with filled black boxes. Lines between boxes represent introns. The insertion sites are shown with triangles, and the T-DNA orientations are indicated with arrows.

(B) Microscopic observation of stomata of wild-type *Col-0*, *Atck1.3*, *kin10* and *Atabi5-1* under cold treatment. Bar=150 μm.