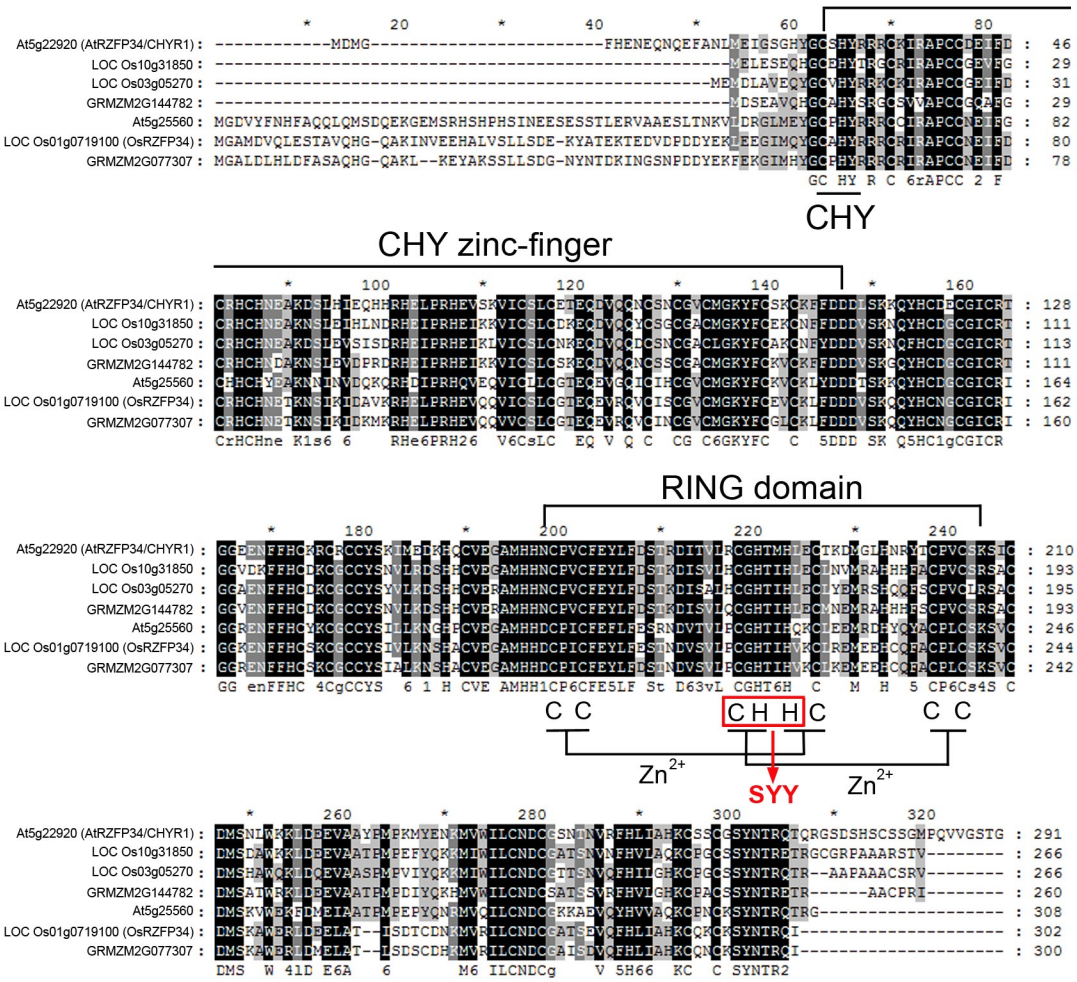
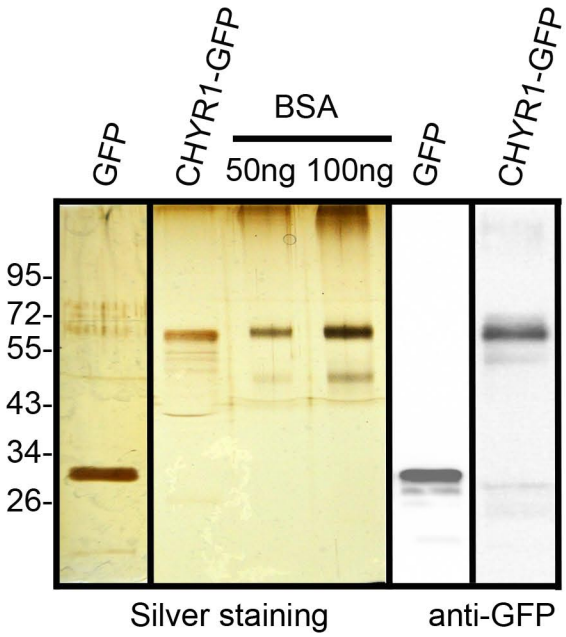


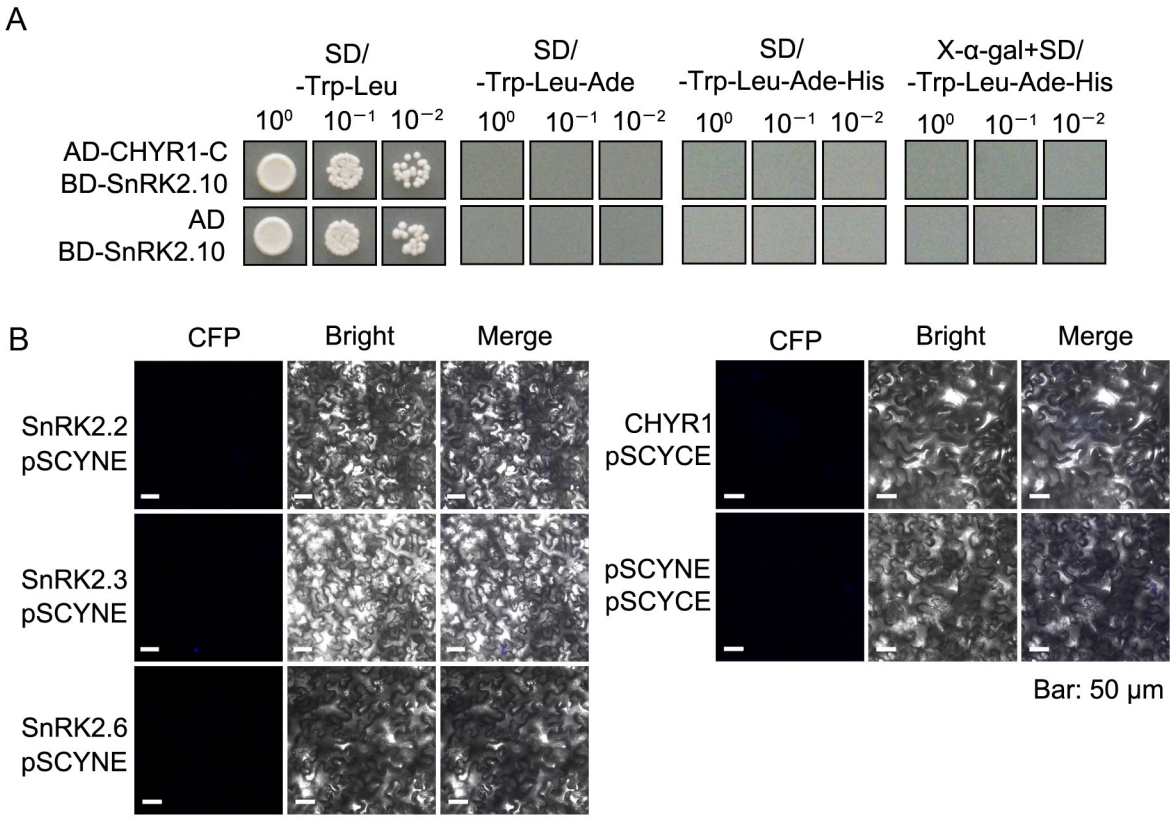
Supplemental Figure 1. Sequence Alignment of CHYR1 Homologs.

Seven CHYR1 homologs from different plant species including Arabidopsis (At5g25560), rice (Os10g31850, Os03g05270 and Os01g0719100) and maize (GRMZM2G144782 and GRMZM2G077307) are shown in the alignment. The red frame highlights key cysteine and histidine residues involved in Zn^{2+} ion chelation in the RING domain of CHYR1.



Supplemental Figure 2. Affinity Purification of CHYR1-GFP Interacting Protein for LC-MS/MS Analysis.

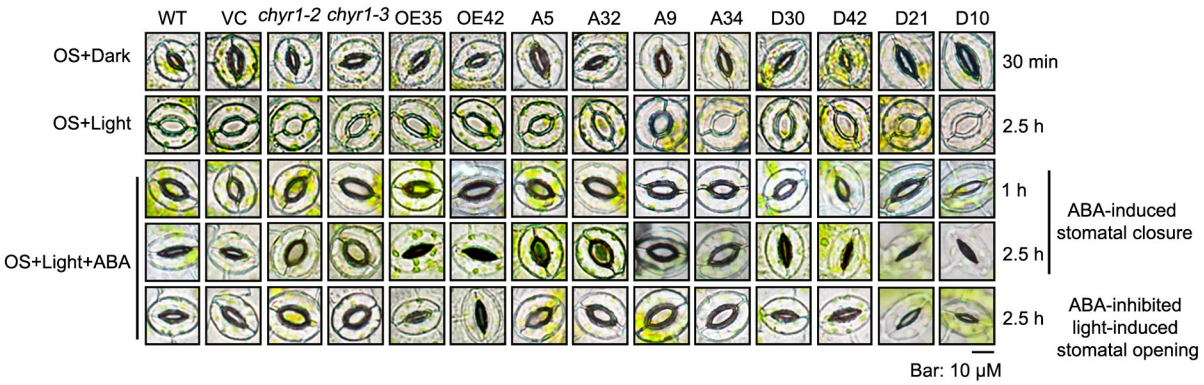
CHYR1-GFP and its interaction proteins purified with anti-GFP agarose from protein extracts of *35S:CHYR1-GFP* transgenic plants were detected by silver staining (left two panels) and immunoblotting with anti-GFP antibody (right two panels). GFP and its interacting proteins purified from protein extracts of *35S:GFP* transgenic plants were used as negative control in the following LC-MS/MS analysis. 50 ng and 100 ng bovine serum albumin (BSA) were loaded for protein quantification.



Supplemental Figure 3. Yeast Two-Hybrid Assay of CHYR1 and SnRK2.10 and Negative Controls in BiFC Assays of CHYR1 and SnRK2.2, SnRK2.3, and SnRK2.6.

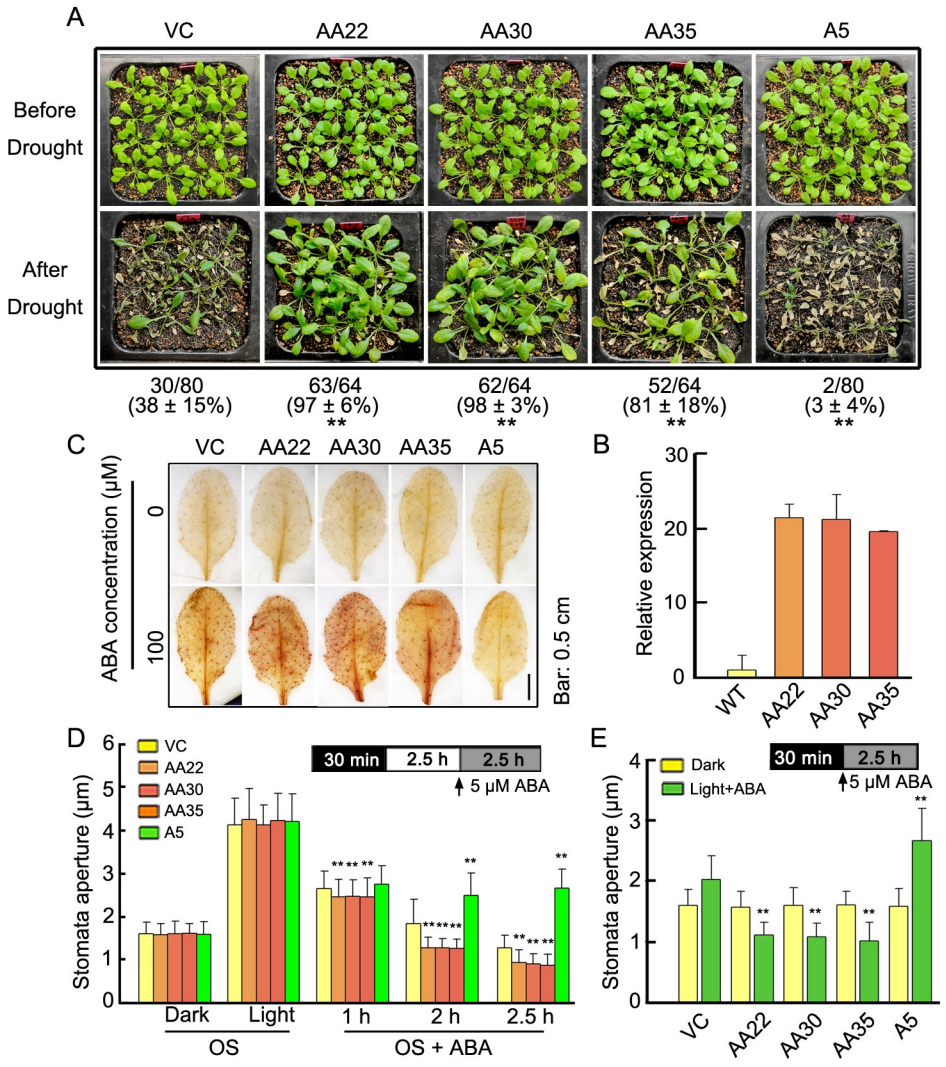
(A) Yeast two-hybrid assay of CHYR1 and SnRK2.10. Yeast cells expressing the indicated constructs were dropped on synthetic dropout (SD) medium without tryptophan and leucine (SD/-T-L), without tryptophan, leucine and adenine (SD/-T-L-A), without tryptophan, leucine, adenine and histidine (SD/-T-L-A-H), or without tryptophan, leucine, adenine and histidine with 20 mg/ml X-α-gal (X-α-gal+SD/-T-L-A-H).

(B) The construct pairs of pSCYNE-CHYR1 and pSCYCE-SnRK2.2, -SnRK2.3, -SnRK2.6 with empty vector were used as negative controls for the BiFC assays in **Figure 5B**. Left, middle, and right represent images of cyan fluorescence, brightfield, and merged, respectively.



Supplemental Figure 4. Representative Photos for Stomatal Aperture of WT, *chyr1* Mutants, *35S:CHYR1*, *35S:CHYR1*^{T178A} and *35S:CHYR1*^{T178D} Plants in ABA Treatment.

For ABA-induced stomatal closure, leaves from 4-week-old plants were incubated in OS in the dark for 30 min. After exposure to the light for 2.5 h, 5 μM ABA was added to the samples to observe the stomatal closure at 1 h and 2.5 h of ABA treatment. For ABA-inhibited light-induced stomatal opening, leaves from 4-week-old plants were incubated in OS for 30 min in dark, and then placed in the light in the presence of 5 μM ABA for 2.5 h. The representative photos showed stomatal apertures under different conditions. WT and empty vector control (VC) were used as controls.



Supplemental Figure 5. Phenotype Analysis of *35S:CHYR1^{S173AS208A}* Transgenic Plants.

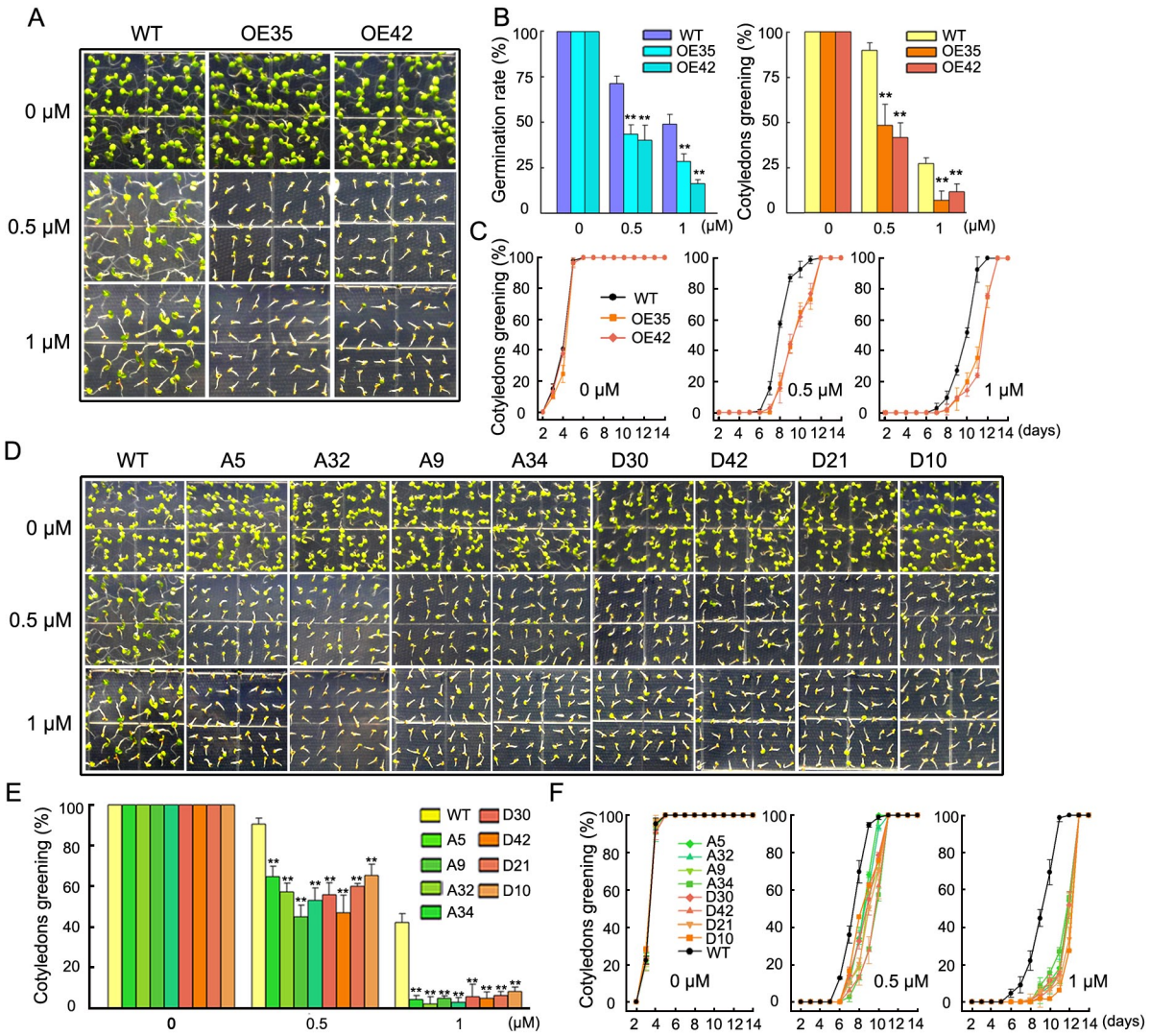
(A) Drought tolerance test of *35S:CHYR1^{S173AS208A}*. Water was withheld from 28-day-old plants for 14 days untill differences in wilting were observed. Photographs were taken after 3 days of rewatering. AA22, AA30 and AA35 denotes *35S:CHYR1^{S173AS208A}* transgenic lines 22, 30, and 35, respectively. *35S:CHYR1^{T178A}* transgenic plants (A5) were tested as a control. Values represent means ± SD from three biological replicates. Statistic significance between VC and the transgenics was determined by a *t*-test, *p < 0.05, **p < 0.01.

(B) DAB staining indicates different levels of ABA-induced H_2O_2 production in leaves of VC, *35S:CHYR1^{S173AS208A}* and *35S:CHYR1^{T178A}* lines. The red-brown staining indicates H_2O_2 generation. Representative photos are shown. Scale bar, 0.5 mm.

(C) Relative expression of *CHYR1* in the *35S:CHYR1^{S173AS208A}* lines analyzed by qRT-PCR. Expression of *CHYR1* in the WT was determined as 1.0. Values represent means ± SD (n = 3) from three technical replicates.

(D) ABA-induced stomatal closure in VC, the *35S:CHYR1^{S173AS208A}* and *35S:CHYR1^{T178A}* lines. Leaves from 4-week-old plants were incubated in OS in dark for 30 min. Then, after exposed to light for 2.5-h, 5 μM ABA was added to the samples to observe the stomatal closure at 1-h, 2-h and 2.5-h ABA treatment. Data were obtained from about 100 stomata for each sample. The error bars were obtained from different stomata. Values represent means ± SD (n > 100) from 3-5 biological replicates. Statistic significance between VC and the transgenics in each treatment was determined by a *t*-test, *p < 0.05, **p < 0.01.

(E) Comparison of ABA-inhibited light-induced stomatal opening among VC, *35S:CHYR1^{S173AS208A}* and *35S:CHYR1^{T178A}* plants. Leaves were incubated in OS for 30 min in dark, and then transferred to light in the presence of 5 μM ABA for 2.5-h. Data were obtained from about 100 stomata for each sample. The error bars were obtained from different stomata. Values represent means ± SD (n > 100) from 3-5 biological replicates. Statistic significance between VC and the transgenics in each treatment was determined by a *t*-test, *p < 0.05, **p < 0.01.



Supplemental Figure 6. ABA Sensitivity of *35S:CHYR1*, *35S:CHYR1*^{T178A} and *35S:CHYR1*^{T178D} Transgenic Plants During Seed Germination.

(A) Representative photo of seed germination of WT and *35S:CHYR1* lines in response to ABA.

(B) Statistical evaluation of the germination rate in terms of radicle emergence and cotyledon greening in response to the indicated ABA concentrations. Values represent means ± SD from three biological replicates. Statistic significance between WT and T3 generation of the transgenics in each ABA concentrations was determined by a *t*-test, *p < 0.05, **p < 0.01.

(C) Germination rate at different time points of WT and *35S:CHYR1* lines in terms of cotyledon greening. Values are presented as means ± SE (*n* = 3). *p < 0.05, **p < 0.01, by a *t*-test.

(D) Germination tests of WT, *35S:CHYR1*^{T178A} and *35S:CHYR1*^{T178D} transgenic lines in response to ABA.

(E) Statistical evaluation of the germination rate in terms of cotyledon greening in response to the indicated ABA concentrations. Values represent means ± SD from three biological replicates. Statistic significance between WT and T3 generation of the transgenics in each ABA concentrations was determined by a *t*-test, **p < 0.01.

(F) Germination rate at different time points of WT, *35S:CHYR1*^{T178A} and *35S:CHYR1*^{T178D} transgenic lines. Values are presented as means ± SD (*n* = 3). **p < 0.01, by a *t*-test.

Supplemental Table 1. PCR Primer Sequences Used for This Research.

Name	DNA sequence (5' to 3')	Purpose
SALK_045606-LP	ACACATTGGTGTTTGTCCTCC	genotyping PCR
SALK_045606-RP	TGCGAATCTAATGGAGATTGG	genotyping PCR
SALK_117324-LP	AAACACCAATGTGTTGAAGGC	genotyping PCR
SALK_117324-RP	TCATGAAAATGTGCAAATTCG	genotyping PCR
SALK_LBb1.3	ATTTTGCCGATTTCGGAAC	genotyping PCR
CHYR1-RT-F1	ATGGATATGGGTTTCCATGAAA	RT-PCR
CHYR1-RT-R1	CTCAAAACAAACCGGGCA	RT-PCR
CHYR1-RT-R2	TTAACCGGTTGAACCAACAA	RT-PCR
18S rRNA-RT-For	AAACGGCTACCACATCCAAG	RT-RCR/qRT-PCR
18S rRNA-RT-Rev	CCTCCAATGGAATCCTCGTTA	RT-RCR/qRT-PCR
CHYR1-qRT-F	GCAGACATTGCCACAACGAA	qRT-PCR
CHYR1-qRT-R	TCGAGGAAGCTCATGTCTATGATG	qRT-PCR
RAB18-qRT-For	GGCTTGGGAGGAATGCTT	qRT-PCR
RAB18-qRT-Rev	TTGATCTTTTGTGTTATTCCCTTCT	qRT-PCR
RD29A-qRT-For	TGGACACGAATTCTCCATCA	qRT-PCR
RD29A-qRT-Rev	TTCCAGCTCAGCTCCTGATT	qRT-PCR
RD20-qRT-For	TTAGCTCCGGTCACCAGTCA	qRT-PCR
RD20-qRT-Rev	CATGTATGGTTTTGGTAATGTTTCC	qRT-PCR
LEA14-qRT-For	GATTTCTTCTGATCGACAAAACCTA	qRT-PCR
LEA14-qRT-Rev	AGCAAACCCAACTTATTACATTACG	qRT-PCR
DREB2A-qRT-For	GACCTAAATGGCGACGATGT	qRT-PCR
DREB2A-qRT-Rev	TCGAGCTGAAACGGAGGTAT	qRT-PCR
RbohF-qRT-For	CTGCGGTTTCGCCATTC	qRT-PCR
RbohF-qRT-Rev	TGTTTCGTCGGCTCTG	qRT-PCR
RbohD-qRT-For	ATTACAAGCACCAAACCAG	qRT-PCR
RbohD-qRT-Rev	TGCCAAGCCATAACATCA	qRT-PCR
ATGolS2-qRT-For	GACGAGTCTCTTGATTACAAGAATGTT	qRT-PCR
ATGolS2-qRT-Rev	AAACTGCTGAAGTGTCTGTTGC	qRT-PCR
Actin2-qRT-For	GCCATCCAAGCTGTTCTCTC	qRT-PCR
Actin2-qRT-Rev	GCTCGTAGTCAACAGCAACAA	qRT-PCR
CHYR1 CDS *Bam*HI For	atGGATCCATGGATATGGGTTTCCATGAAA	*35S:CHYR1/35S:CHYR1-cGFP*
CHYR1 CDS *Xho*I Rev	atCTCGAGACCGGTTGAACCAACAA	*35S:CHYR1/35S:CHYR1-cGFP*
CHYR1promoter *Hind*III For	aaGAATCCGATGATGACGAAGATATGCTTTT	GUS staining
CHYR1promoter *Eco*RI Rev	aaAAGCTTACTTTACCTAAAGTTTTGATCTTTC	GUS staining
CHYR1 RING CHH/SYY For	CTACGATCTGGTTACACTATGTATCTAGAA	CHYR1 RING mutagenesis
CHYR1 RING CHH/SYY Rev	TTCTAGATACATAGTGTAACCAGATCGTAG	CHYR1 RING mutagenesis
CHYR1 RING T178A For	AACAAGAGATATCGCTGTCCTACGAT	CHYR1 RING mutagenesis
CHYR1 RING T178A Rev	ATCGTAGGACAGCGATATCTCTTGTT	CHYR1 RING mutagenesis
CHYR1 RING T178D For	AACAAGAGATATCGATGTCCTACGAT	CHYR1 RING mutagenesis
CHYR1 RING T178D For	ATCGTAGGACATCGATATCTCTTGTT	CHYR1 RING mutagenesis
CHYR1 RING S173A For	GTTTGATGCAACAAGAGATATCACTGTCCT	CHYR1 RING mutagenesis
CHYR1 RING S173A Rev	AGGACAGTGATATCTCTTGTTGCATCAAAC	CHYR1 RING mutagenesis
CHYR1 RING S208A For	CTCCAAAGCCATATGTGACATGTCTA	CHYR1 RING mutagenesis
CHYR1 RING S208A Rev	TAGACATGTCACATATGGCTTTGGAG	CHYR1 RING mutagenesis
CHYR1 RING S173D For	GTTTGATGATACAAGAGATATCACTGTCCT	CHYR1 RING mutagenesis
CHYR1 RING S173D Rev	AGGACAGTGATATCTCTTGTATCATCAAAC	CHYR1 RING mutagenesis
CHYR1 RING S208D For	GCTCCAAAGATATATGTGACATGTCTAATC	CHYR1 RING mutagenesis
CHYR1 RING S208D Rev	GATTAGACATGTCACATATATCTTTGGAGC	CHYR1 RING mutagenesis
CHYR1 161th aa For	CACAATTGCCCGGTTT	In-gel kinases
CHYR1 216th aa Rev	CCACAGATTAGACATGTCACATAT	In-gel kinases
UBP CDS *Bam*HI For	ATGGATCCATGCAGATCTTTGTTAAGAC	Ubiquitin recombinant protein

Name	DNA sequence (5' to 3')	Purpose
UBP CDS *Eco*RI Rev	ATGAATTCTAACCACCACGGAGCC	Ubiquitin recombinant protein
UBP-K48R-For	CTTATTTTCGCCGGAGAACAGCTAGAGGAT	Ubiquitin 48th Lys mutant to Arg
UBP-K48R-Rev	ATCCTCTAGCTGTTCTCCGGCGAAAATAAG	Ubiquitin 48th Lys mutant to Arg
UBP-K63R-For	TACAATATCCAGAGAGAATCCACCCTCCAC	Ubiquitin 63th Lys mutant to Arg
UBP-K63R-Rev	GTGGAGGGTGGATTCTCTCTGGATATTGTA	Ubiquitin 63th Lys mutant to Arg
SnRK2.2 CDS For	ATGGATCCGGCGACTAATT	Y2H & BiFC & Co-IP
SnRK2.2 CDS Rev	GAGAGCATAAACTATCTCTCCACTACTG	Y2H & BiFC & Co-IP
SnRK2.3 CDS For	ATGGATCGAGCTCCGGT	Y2H & BiFC & Co-IP
SnRK2.3 CDS Rev	GAGAGCGTAAACTATCTCTCCG	Y2H & BiFC & Co-IP
SnRK2.6 CDS For	ATGGATCGACCAGCAGTG	Y2H & BiFC & Co-IP
SnRK2.6 CDS Rev	CATTGCGTACACAATCTCTCC	Y2H & BiFC & Co-IP