Supplementary Data

SNAC1-targeted gene *OsSRO1c* modulates stomatal closure and oxidative stress tolerance by regulating hydrogen peroxide in rice

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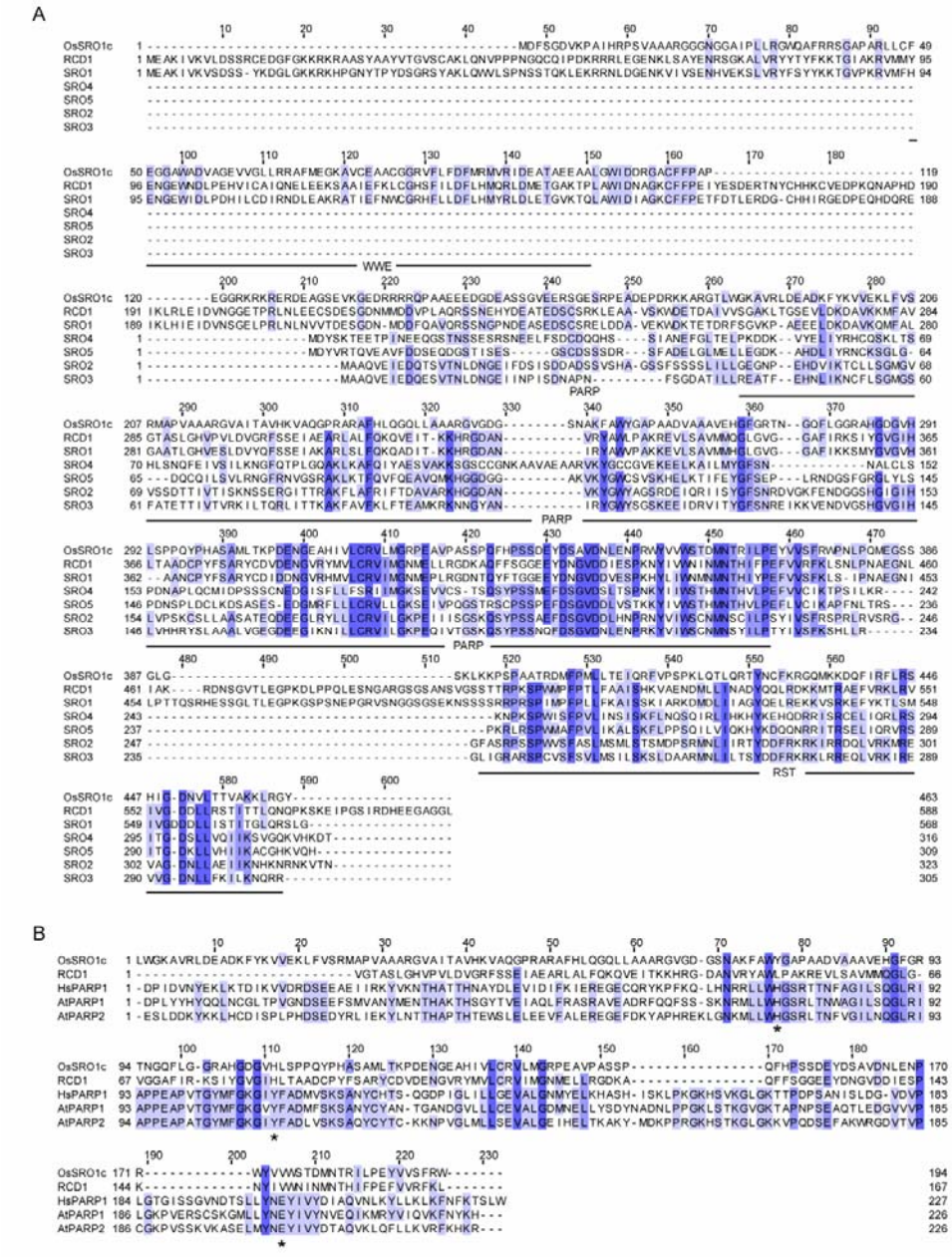
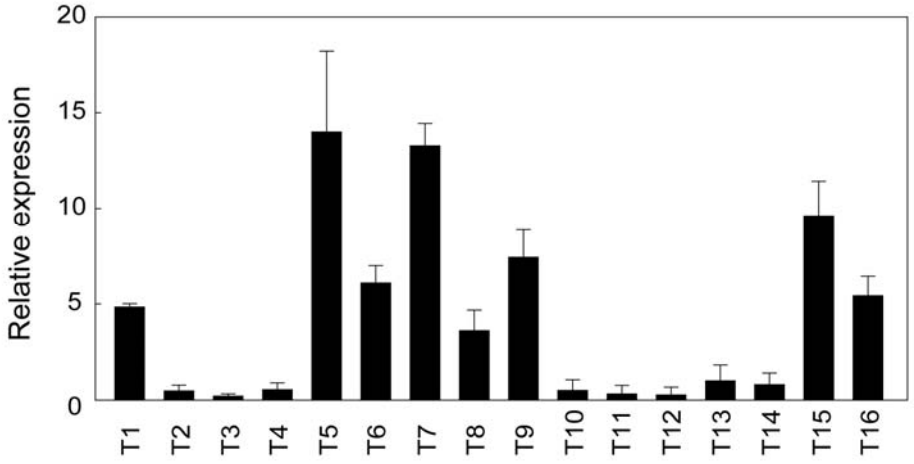
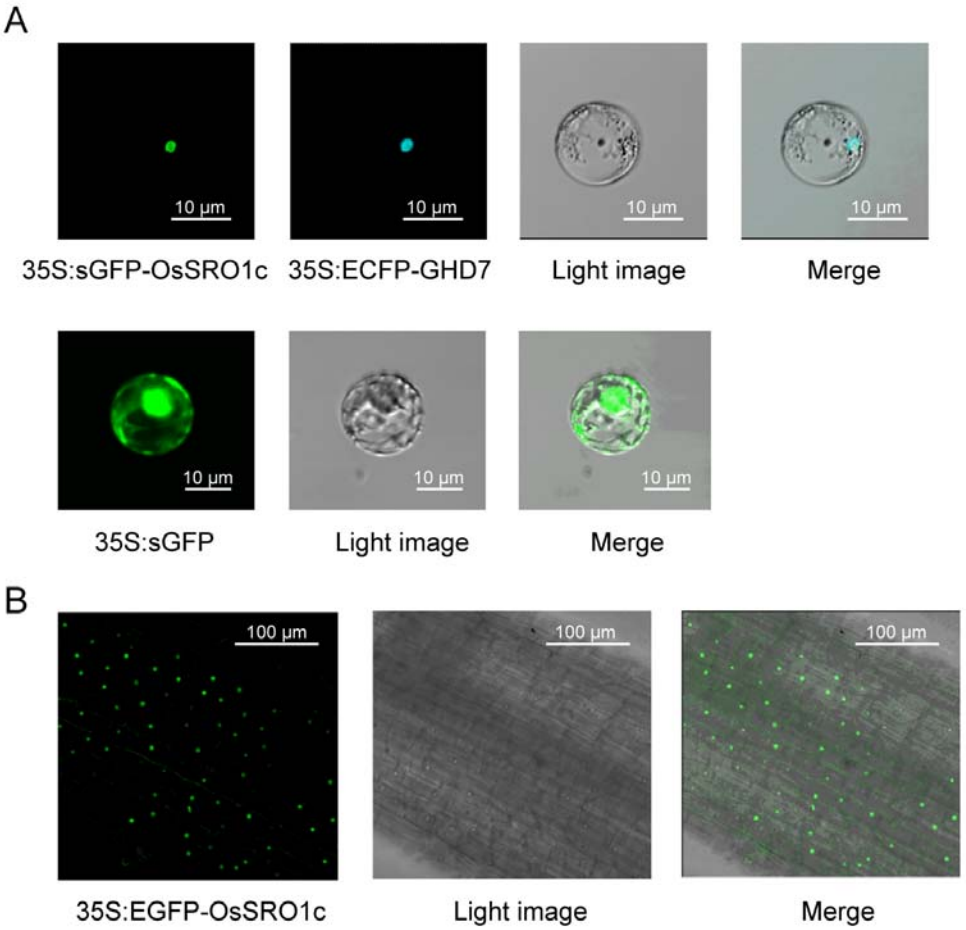
Supplementary Figures



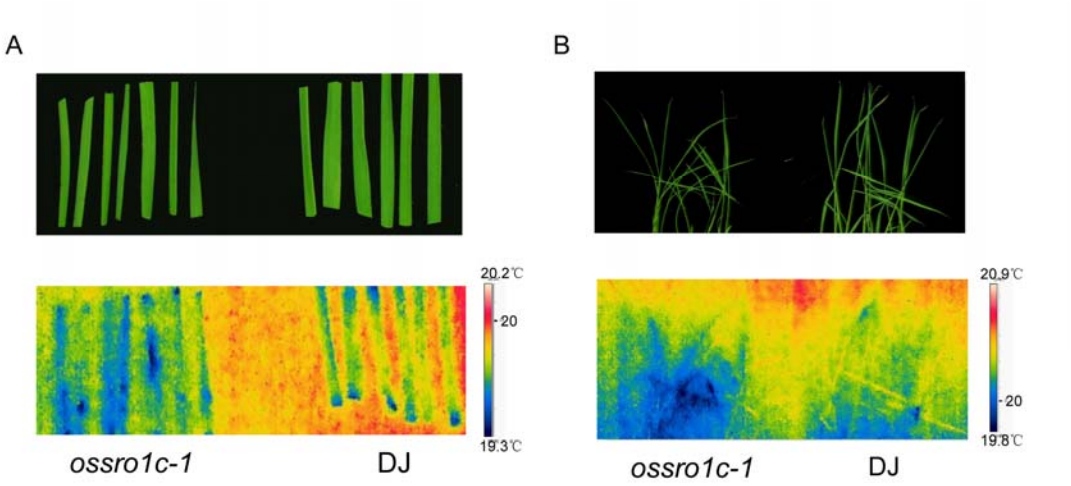
Supplementary Fig. S1. Multiple sequence alignment and sequence comparison. A, Multiple sequence alignment of OsSRO1c and *Arabidopsis* SRO proteins (RCD1, SRO1-5). B, Multiple sequence alignment of the PARP domain from human PARP1 (HsPARP1), *Arabidopsis* PARP-1 and -2 (AtPARP1 and AtPARP2), *Arabidopsis* RCD1, and OsSRO1c. Conserved ADP-ribosyl transferase catalytic triad, composed of three amino acids is indicated by an asterisk (*) below the alignment.



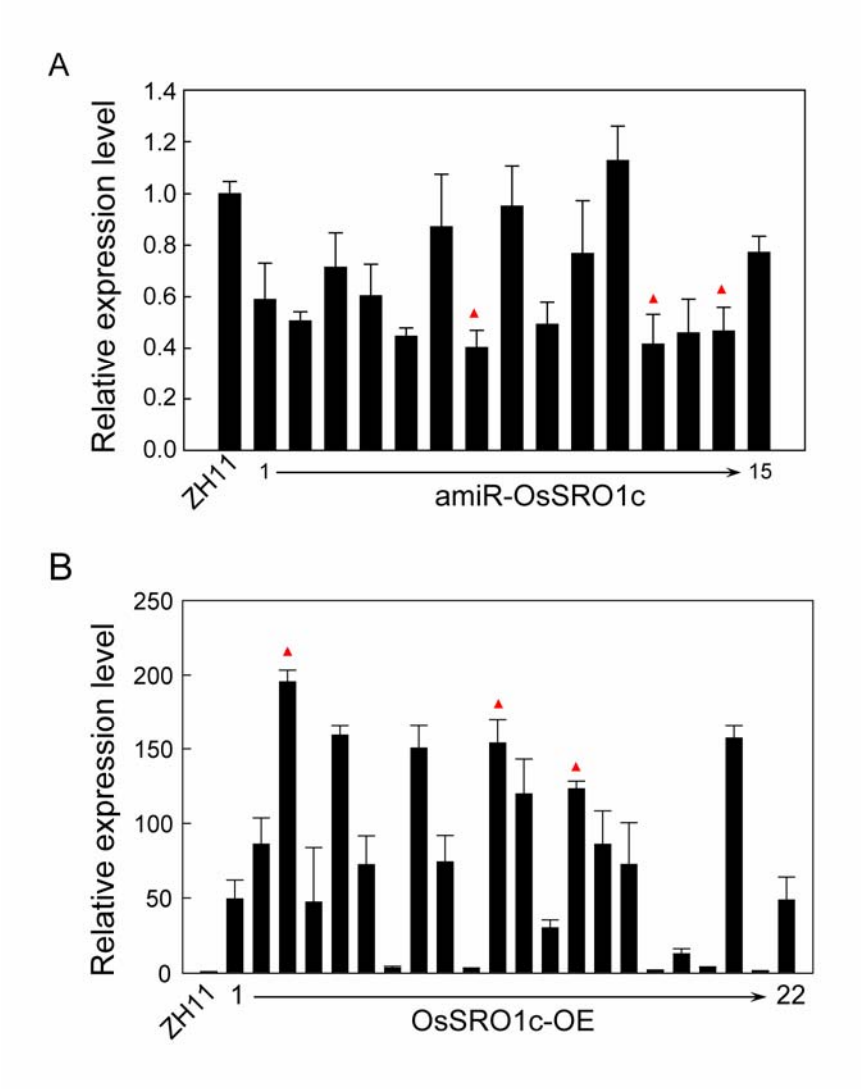
Supplementary Fig. S2. Tissue and organ expression pattern of *OsSRO1c*. The 16 organs/tissues are as follows: T1, calli; T2, seed; T3, radicle; T4, plumule; T5, nodes; T6, collar; T7, stem; T8, flag leaf; T9, sheath; T10, young panicle at stage 3; T11, young panicle at stage 4; T12, young panicle at stage 5; T13, young panicle at stage 7; T14, stamen; T15, secondary branches; and T16, hull. Error bars indicate SE based on three replicates.



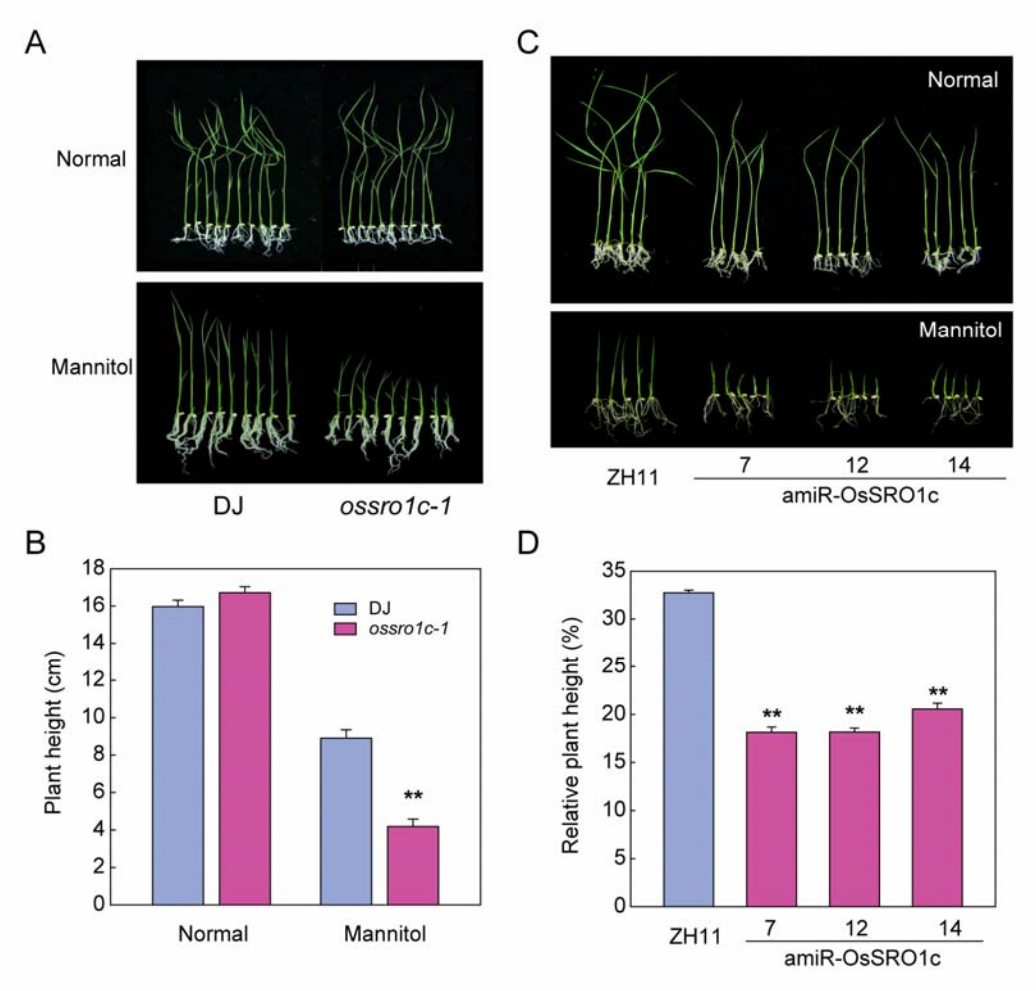
Supplementary Fig. S3. Subcellular localization of OsSRO1c. A, Subcellular localization of OsSRO1c in rice protoplast (ZH11 background). 35S:sGFP-OsSRO1c and 35:sCFP-GHD7 were co-transformed into rice etiolated shoot protoplasts. 35S:sGFP was transformed as control. B, Subcellular localization of OsSRO1c in transgenic rice (ZH11 background). OsSRO1c cDNA was fused to GFP and the construct was expressed in transgenic rice under the control of the cauliflower mosaic virus 35S promoter. Confocal image of the root was shown.



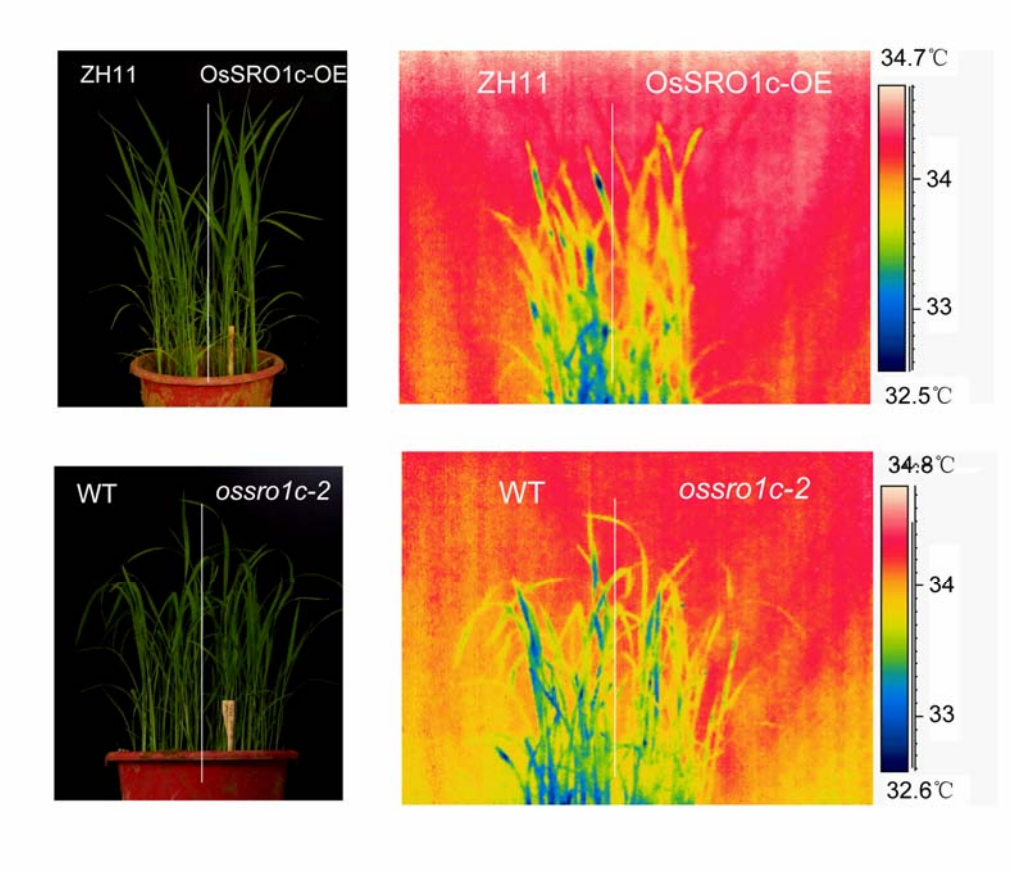
Supplementary Fig. S4. Thermal images of detached leaves (A) and drought-stressed seedlings (B) of DJ and *ossro1c-1* mutants. Visible image is on the top.



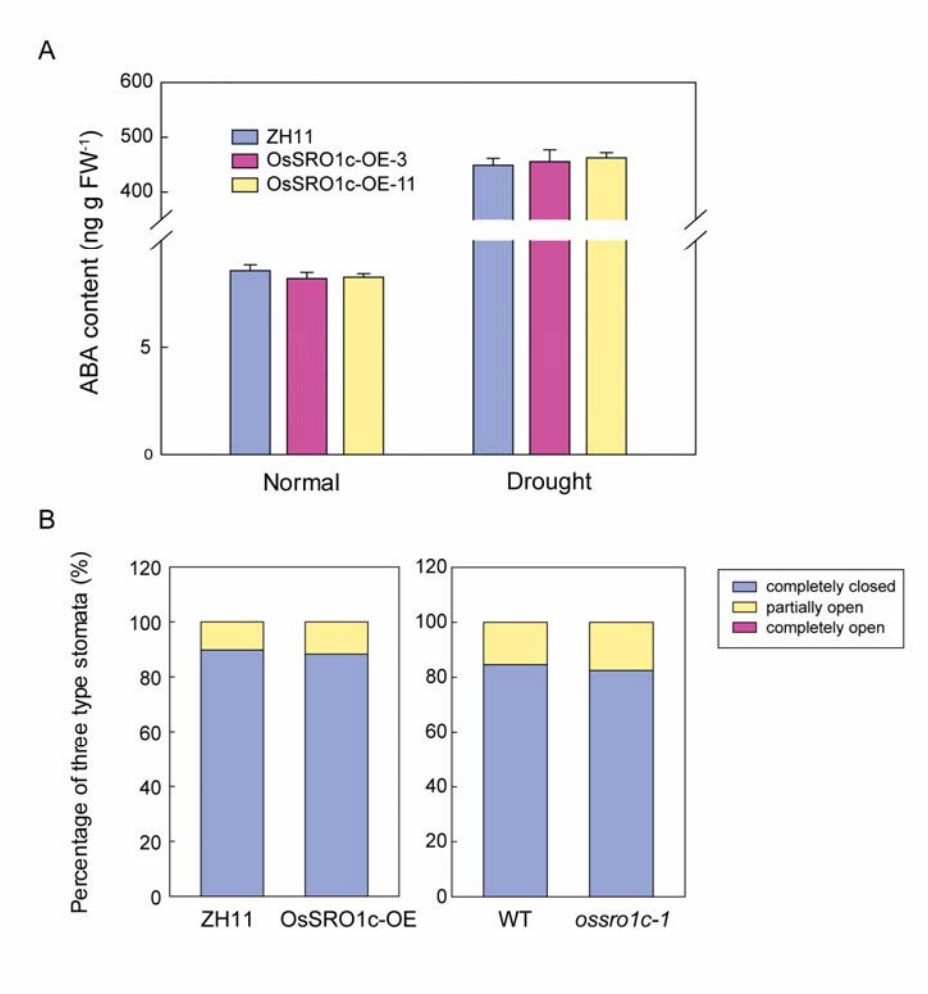
Supplementary Fig. S5. Transcript level of *OsSRO1c* in amiR-OsSRO1c (A) and *OsSRO1c*-overexpressing (B) plants. Error bars indicate SE based on three replicates. Red triangle indicates transgenic plants that were selected for further study.



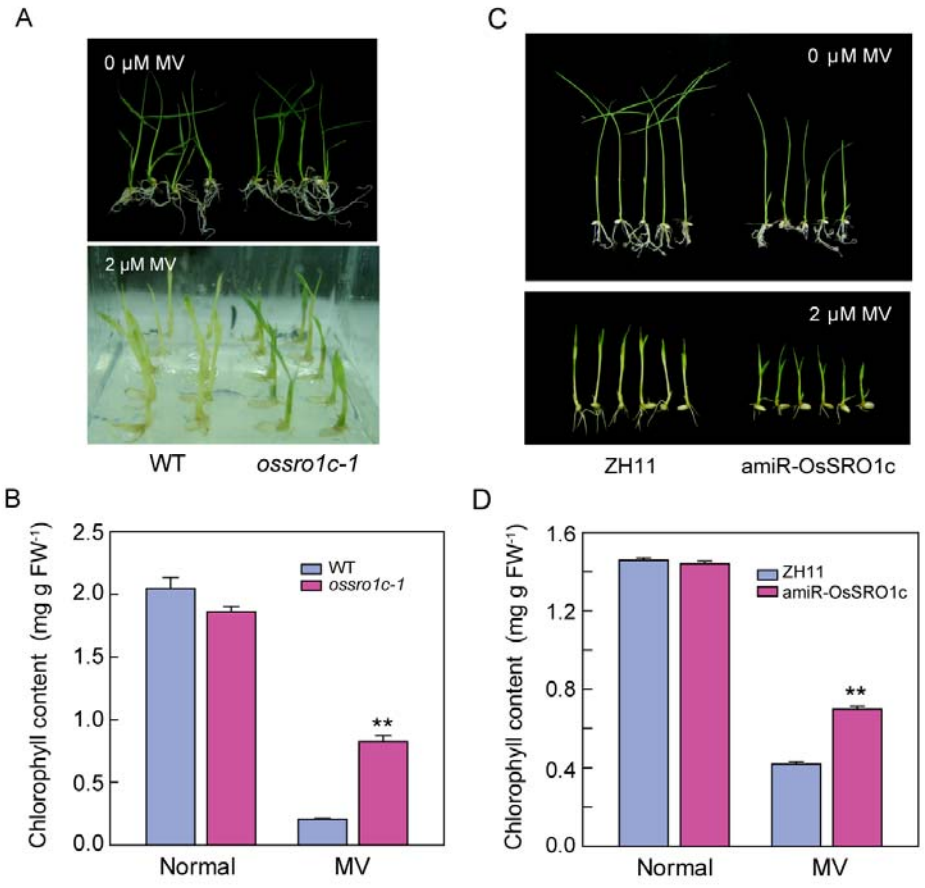
Supplementary Fig. S6. Suppression of *OsSRO1c* showed increased sensitivity to osmotic stress. A, Increased sensitivity of *ossro1-1* mutants to osmotic stress. B, Plant height of DJ and *ossro1c-1* mutant under normal and mannitol treatment (n = 10). C, Increased sensitivity of amiR-OsSRO1c plants to osmotic stress. D, Relative plant height of ZH11 and amiR-OsSRO1c plants under mannitol treatment (n = 3). Data represent mean ± SE. $^{**}P < 0.01$, t test.



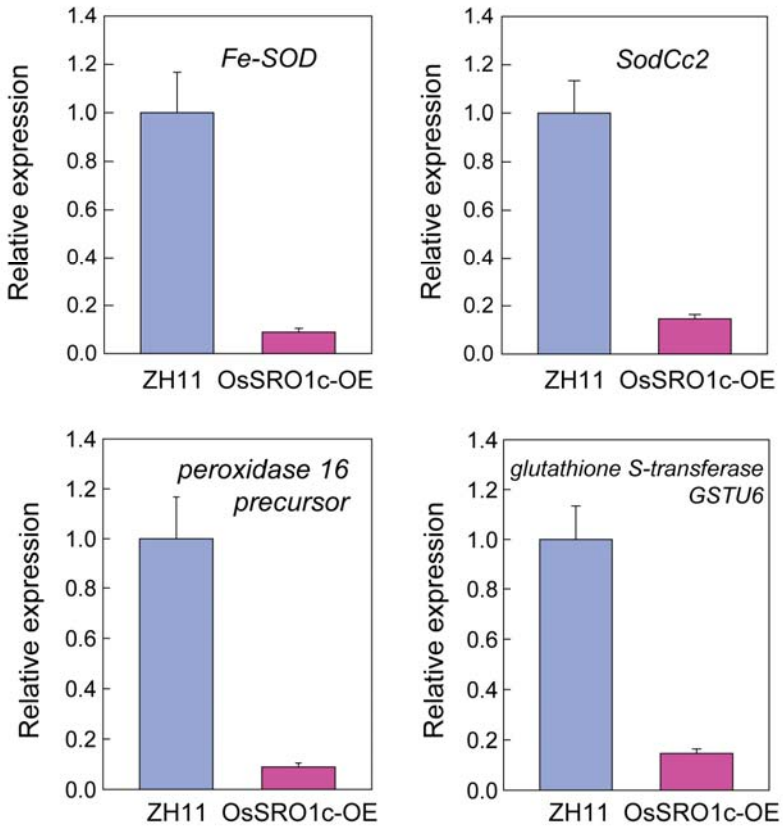
Supplementary Fig. S7. Thermal images of the *OsSRO1c*-overexpressing plants or *ossro1c-2* mutants. Visible image is on the left side.



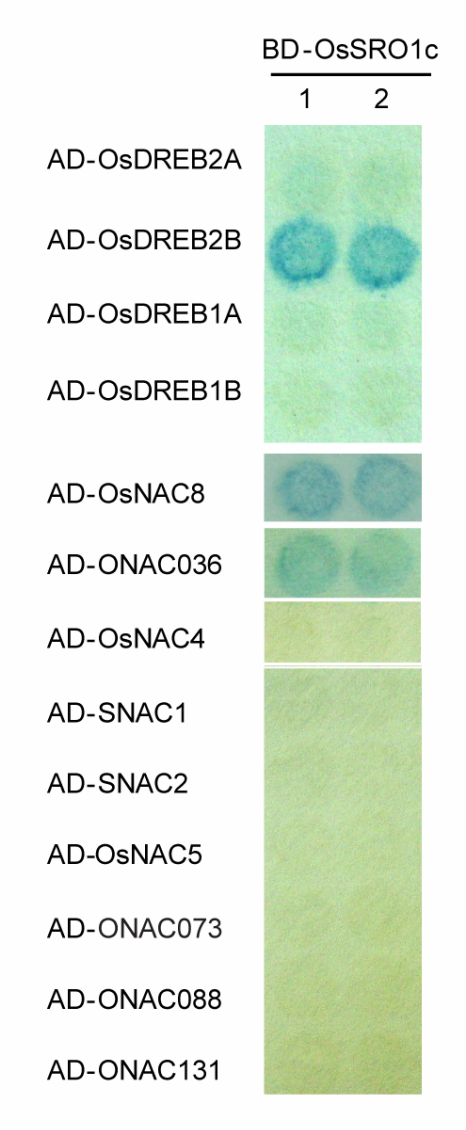
Supplementary Fig. S8. Endogenous ABA content and stomatal responses to ABA in *OsSRO1c*-overexpressing plants. A, The ABA content of ZH11 and *OsSRO1c*-overexpressing plants under normal and drought stress (n = 3). Data represent mean ± SE. B, The percentage of three levels of stomatal opening in wild-type (WT), *OsSRO1c*-overexpressing plants and *ossro1c-1* mutants under ABA treatment. Thirty five-day-old plants were treated with 50 μM ABA for 2h. 49 stomata of ZH11, 51 stomata of *OsSRO1c*-overexpressing plants, 65 stomata of WT and 63 stomata of *ossro1c-1* were randomly selected for analysis.



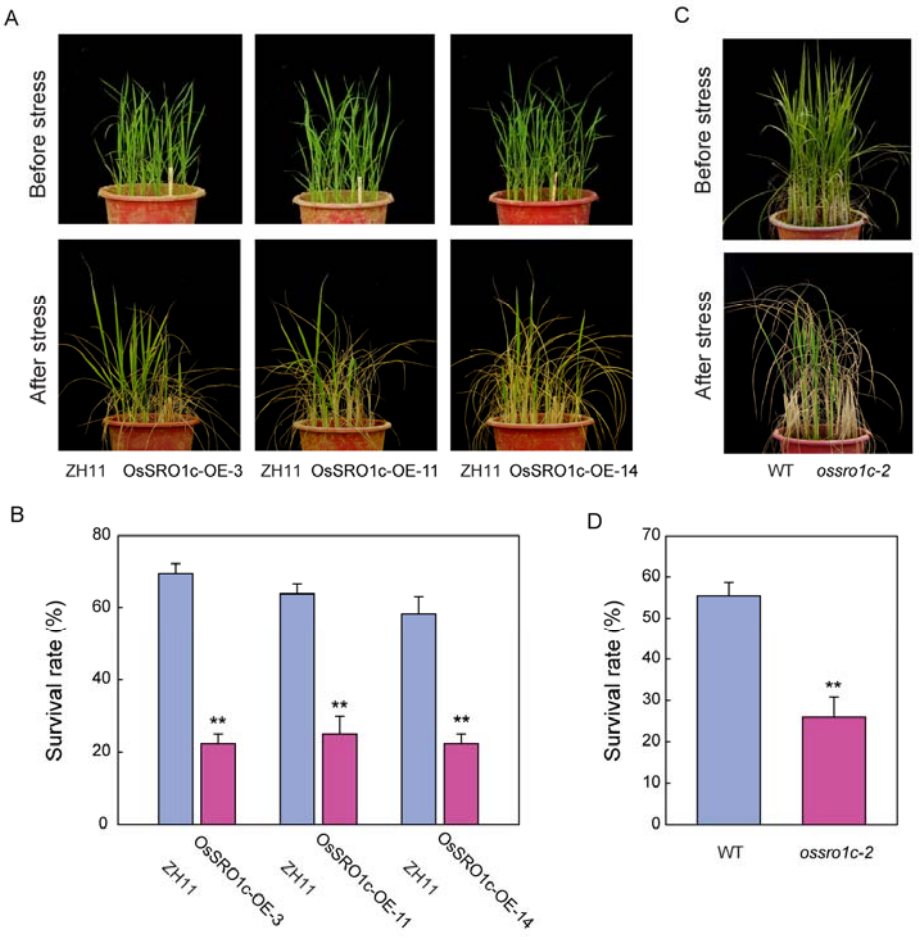
Supplementary Fig. S9. Loss of OsSRO1c function enhanced resistance to oxidative stress. A and C, *ossro1c-1* mutant (A) and amiR-OsSRO1c plants (C) enhanced resistance to oxidative stress. B and D, Total chlorophyll contents of wild-type (WT) and *ossro1c-1* mutants (B) or amiR-OsSRO1c plants (D) under normal and MV stress. Data represent mean ± SE ($n = 3$). $^{**}P < 0.01$, *t* test. FW, fresh weight.



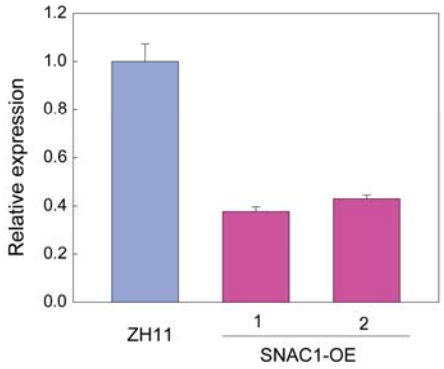
Supplementary Fig. S10. qPCR analysis expression of ROS-scavenging genes in *OsSRO1c*-overexpressing plants. *Fe-SOD* and *SodCc2* are two superoxide dismutase (SOD) genes. *Glutathione S-transferase GSTU6* and *peroxidase 16 precursor* were ROS-scavenging genes that up-regulated in *dst* mutant. Error bars indicate SE based on three replicates.



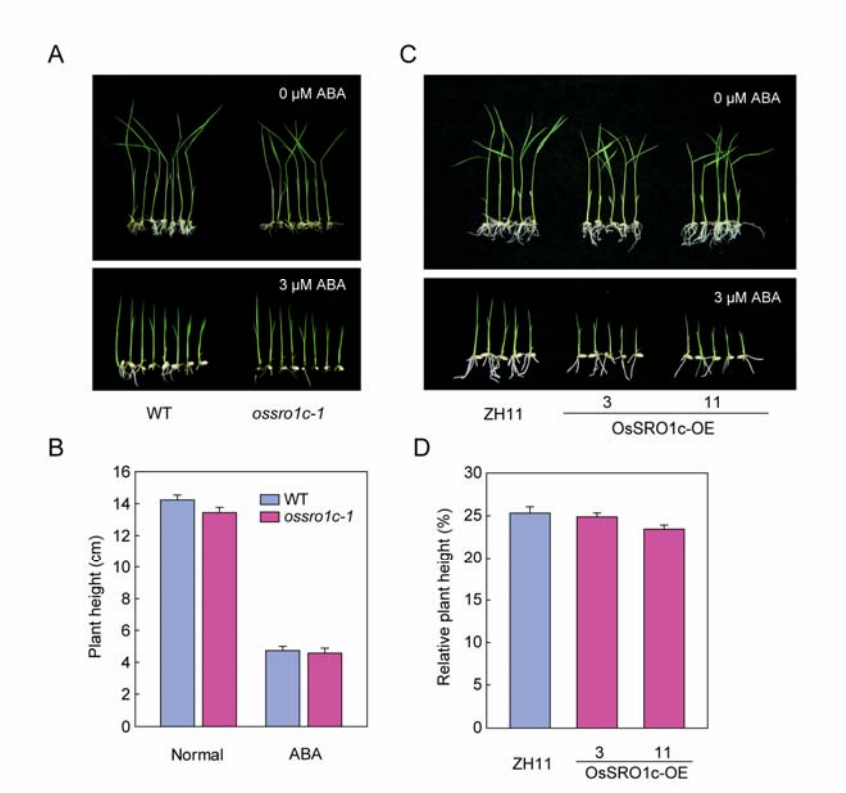
Supplementary Fig. S11. Pairwise interaction test of OsSRO1c with members of rice DREB and NAC family. Interaction was tested by the expression of *lacZ* reporter gene (blue on X-gal assay). 1 and 2 show two different colonies of each pairwise interaction test.



Supplementary Fig. S12. Overexpression of *OsSRO1c* caused increased sensitivity to drought stress. A and C, Increased drought sensitivity of *OsSRO1c*-overexpressing plants (A) and *ossro1c-2* mutants. Four-leaf stage plants were not watered for 10 d, followed by rewatering for 7 d. (C). B and D, Survival rate of wild-type and *OsSRO1c*-overexpressing plants (B) or *ossro1c-2* mutants (D) after drought stress. Data represent mean ± SE ($n = 3$). $^{**}P < 0.01$, *t* test.



Supplementary Fig. S13. Expression of *DST* in *SNAC1*-overexpressing plants. Error bars indicate SE based on three replicates.



Supplementary Fig. S14. Effect of ABA on seedling growth of *ossro1c-1* mutants (A and B) an *OsSRO1c*-overexpressing plants (C and D). 3-day-germinated seeds transplanted in either MS medium or MS medium supplemented with 3 μM ABA for 7 days. Data represent mean ± SE (n = 3).

Supplementary Table S1. Primer sequences used in this study.

Primer name	Primers sequence		Description
	sense primer (5'->3')	anti-sense primer (5'->3')	
(a) ChIP-PCR			
PA	TTCATATGTTTCTGCGGTTTCTTCT	TATTTTAGTACGGACGGAAGTTCGT	
PB	AAACTCCCGTAACCGTAGGATGATC	CCCAGGCGCCCAGCC	
PC	ATGCTGAATTTTGCTATTGTTAGGA	GTGTTCTGGGGCGGCG	
(b) T-DNA verification			
M1	TCGCCGATGTGGGA	GGTAAACTGGTACTTCTTGCT	*ossro1c-1* T-DNA verification
pGARP	TTGGGGTTTCTACAGGACGTAAC		Postech T-DNA-specific
M2	AAGTGGGAGCGGAAGACG	CGGCGATGGCAAAGGT	*ossro1c-2* T-DNA verification
T-DNA-3	TAATAACGCTGCGGACATCTA		SHIP T-DNA-specific
(c) construction for rice transformation			
SNAC1miRI	AGTATTTACACACGTTCCAGCATCAGGAGATTCAGTTTGA		P_{Ubi}:amiSNAC1
SNAC1miRII	TGATGCTGGAACGTGTGTAAATACTGCTGCTGCTACAGCC		
SNAC1miRIII	CTATGCTCGAAGGTGTGTAAATATTCCTGCTGCTAGGCTG		
SNAC1miRIV	AATATTTACACACCTTCGAGCATAGAGAGGCAAAAGTGAA		
SROmiRI	AGTACTGTTCGATTCTACGGACTCAGGAGATTCAGTTTGA		P_{Ubi}:amiOsSRO1c
SROmiRII	TGAGTCCGTAGAATCGAACAGTACTGCTGCTGCTACAGCC		
SROmiRIII	CTAGTCCCTAGTATCGAACAGTATTCCTGCTGCTAGGCTG		
SROmiRIV	AATACTGTTCGATACTAGGGACTAGAGAGGCAAAAGTGAA		

SROOE	CAGGGTACCGGGAGGGGTGATGGAC	CAGGGTACCACTATGACCGAACTCAAGAAT	P_{Ubi}:OsSRO1c (pU1301)
SROP	CAGGGATCCTAGCGAAATGATGATGCG	CAGAAGCTTGTGGGAGCGGAAGACG	$P_{OsSRO1c}$:GFP (DX2181)
SROGFP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGGCGACGTCAAG	GGGGACCACTTTGTACAAGAAAGCTGGGTAACTAAAGCAGCATCAGAAGA	P_{35S}:EGFP:OsSRO1c (pH7WGF2,0)

(d) construction for yeast transformation

SROHIS	ATAGAATTCAACTCGCCATTATACTATCC	ATAGAGCTCACCGACTTTGCCCTTTAC	pHIS2-$P_{OsSRO1c}$
SROY2H	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTCCGGCGACGTCAA	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTACACGACACGCACTG	pDEST32-OsSRO1c
SROWWW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGACTTCTCCGGCGACGTC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTACTCCTCCACGCCGGACGAC	
SROPARP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGGGGACGAGGCGTCGTC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATGGCAGGTTGGGCCACCTGA	
SRORST	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCAACCTGCCGCAGA	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTACACGACACGCACTGT	Cloning domains of OsSRO1c into GAL4 Y2H system (pDEST32)
SROWP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGGACTTCTCCGGCGACGTC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCGCCCGCCGAGAAACT	
SROPR1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCGCCCCGGGCAAGAG	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTACACGACACGCACTGT	
SROPR2	GGGGACAAGTTTGTACAAAAAAGCAGGCTCACACATCGTGCTGTGCC	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTACACGACACGCACTGT	

D1AADF	CAGGTCGACGATGTGCGGGATCAAGC	CAGGCGGCCGCGCATCGGAAGCCAGAA	
D1BADF	CAGGTCGACCATGGAGGTGGAGGAGG	CAGGCGGCCGCACACTTTTCAGTGCGAGATT	Cloning OsDREB1A, OsDREB1B, OsDREB2A and OsDREB2B into GAL4 Y2H system (pEXE-AD502)
D2AADF	CAGGTCGACGATGGAGCGGGGGG	CAGGCGGCCGCAGTAAGACGAAAACCGTAAATG	
D2BADF	CAGGTCGACGAAGGCAAGGAAGGCACC	CAGGCGGCCGCCCACCAACGAACCCAAT	
AD502F	TATAACGCGTTTGGAATCACT		pEXE-AD502 sequencing primer
AD502R	GTAAATTTCTGGCAAGGTAGA		pEXE-AD502 sequencing primer

(e) construction for protoplast transformation

SROSL	CAGTCTAGAGGGAGGGGTGATGGAC	CAGTCTAGACCCTCGGAGTTTCTTGG	P_{35S}:OsSRO1c:sGFP (pM999-33)
SROBI	GGTACCTCCGGCGACGTCAAG	GAGCTCAACTAAAGCAGCATCAGAAGA	fusion of OsSRO1c to the N-terminal of YFP (pVYNER)
D2BBI	GGATCCGAAGGCAAGGAAGGCACC	GGTACCCAAGCCCTCAAAGAACTGAGA	fusion of OsDREB2B to the C-terminal of YFP (pVYCE)
UBIBI	TCTAGACCCGCACCCCCG	ACTAGTCTGGTCTTTCTTCTCCCTAGC	fusion of UBA to the C-terminal of YFP (pVYCE)
N8BI	TCTAGAAGTAGTCCACCCCAACGAC	CTCGAGGGTATGCTGCTGATCTCTCTG	fusion of NAC8 to the C-terminal of YFP (pVYCE)

(f) qPCR primers

				Primer efficiency
LOC_Os03g12820	TCCCTATGCTTCTGACGGAGAT	CAGTTGTACGTCCTCTGCAAAGTC	*OsSRO1c* primer set 1 for SA site in Fig.4	105.63%
LOC_Os03g12820	CTCCCACATCGGCGACA	ACCTTGCACTAGTACCCTCGGA	*OsSRO1c* primer set 2 for SB site in Fig.4	100.83%
LOC_Os03g60080	CATGGTCCCGTTCTGAGGTG	CACACGTTGCAGCATCGATC	*SNAC1*	104.23%
LOC_Os03g57240	ATCCAAGAAGGCAAGGTCAATC	CACACGAGGAGGAATTGGAAG	*DST*	101.40%
LOC_Os01g28030	GTCTCCAGGACCTCGTCGTC	AAAAGGTTGCAGTGCCCG	*peroxidase 24 precursor*	98.96%
LOC_Os10g38470	CGCCACCAACTGAAGTGACA	CTGTGAAACAGATGGAAACAATCC	*glutathione S-transferase GSTU6*	92.51%
LOC_Os06g48030	CCATGATGATCTTCGATTCTCACT	GCGGGAACAAATACAACAACTG	*peroxidase 16 precursor*	108.40%
LOC_Os06g05110	CGACGCCGAGGAATTTCTAG	AGGTGGTGTAAGTGTCTCTCATGC	*Fe-SOD*	108.24%
LOC_Os07g46990	ATTCCATGTGCACGCGC	GGATTGAAGTGTGGTCCAGTTG	*SodCc2*	104.78%
LOC_Os11g06390	TGGCATCTCTCAGCACATTCC	TGCACAATGGATGGGTCAGA	*Actin1*	100.60%

Supplementary methods S1. Supplementary methods for plasmid construction, rice transformation, stress treatments, physiological measurements, yeast assay, BiFC assay, and RT-PCR.

Stress treatments

To measure the transcript level of the *OsSRO1c* under stresses, ZH11 plants were grown in the greenhouse with a 14-h-light/10-h-dark cycle. Plants at the four-leaf stage were treated with various stresses and phytohormones treatment. For drought stress, the seedlings were grown for 7 d without water, and sampled at 0 d, 3 d, 5 d and 7 d, For salt stress, the seedlings were irrigated with 200 mM NaCl solution and sampled at 0 h, 3 h, 6 h and 12 h, For cold and heat shock stress, seedlings were transferred, respectively, to a growth chamber at 4°C and sampled at 0 h, 6 h, 12 h and 24 h and 42°C and sampled at 0 min, 10 min, 30 min and 2 h after treatment. For UV stress, seedlings were transferred to a tissue prepared room with UV lights (emission peak 254 nm; 1100 μW/cm2 at plant level; TUV30W, Philips, Nederland) and sampled at 0 h, 3 h, 6 h and 12 h. Rice leaves were wounded by cutting into pieces and floated on water at room temperature under continuous light for 1 h, 3 h and 6 h. For oxidative stress, seedlings were irrigated with 1% (v/v) H_2O_2 solution and sampled at 0 h, 2 h, 6 h and 12 h. For ABA treatment, 100 μM ABA were sprayed on leaves and sampled at 0 h, 2 h, 6 h and 12 h.

For stress testing of transgenic lines and mutant, *OsSRO1c*-overexpressing and amiRNA transgenic plants were selected by germinating seeds on MS medium containing 50 mg L^{-1} hygromycin. Wild-type and homozygous mutants were grown on MS medium or grown in soil. For drought stress at the seedling stage, mutant/transgenic plants and wild-type plants were growing in the same barrels filled with a mixture of soil and sand (1:1). The water supply was stopped to allow drought stress to develop at four-leaf stage. After all leaves completely rolled and recovery by re-water, surviving seedlings were photographed and analyzed. Drought stress testing at the later tillering stage was performed in a refined paddy field facilitated with a movable rain-off shelter. To evaluate mannitol stress tolerance, geminated seeds were transplanted in MS medium supplemented with 150 mM mannitol. After 7 d of growth, shoot length was measured. For the oxidative treatment, geminated seeds were transplanted in MS medium supplemented with 2 μM MV, After 7 d of growth, chlorophyll content was measured.

Physiological measurements

To measure the activity of ROS-scavenging enzymes, soluble proteins were extracted using 50 mM potassium phosphate buffer (pH 7.8). After centrifuged at 12,000 g for 15 min at 4ºC, the supernatant was used as the enzyme extract. Total SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method described previously (Giannopolitis and Ries, 1977). The 3 mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 10 μM EDTA and 100 μL enzyme extract. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. The activity of CAT and POX were measured using kit from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). One unit of CAT activity was defined as the amount of enzyme depleting 1 μmol H_2O_2 in 1 sec. One unit of POX activity was defined as the amount of enzyme for producing 1 μg substrate in 1 min. Quantitative measurement of H_2O_2 production was performed using the Amplex Red hydrogen peroxide/peroxidase assay kit (Molecular Probes) following the manufacturer's instructions. H_2O_2 was extracted from leaves according to the method described previously (Wu *et al.*, 2012)

Plasmid construction and rice transformation

A full-length cDNA of *OsSRO1c* isolated from *indica* rice Guang-lu-ai 4 was obtained from the Rice Indica cDNA Database (http://www.ncgr.ac.cn/ricd/) (Liu *et al.*, 2007). To generate the *OsSRO1c* overexpression constructs, the full-length cDNA of *OsSRO1c* was amplified from plasmid by PCR and the sequence-confirmed PCR fragment inserted into vector pCAMBIA1301U (pU1301) under the control of a maize ubiquitin promoter. The *OsSRO1c* artificial microRNAs (amiR-OsSRO1c) were constructed as described (Warthmann *et al.*, 2008). A 21-mer sequence targeting to the 3'UTR of *OsSRO1c* was used to replace the endogenous miRNA and miRNA* of osa-MIR528 were designed by Web MicroRNA Designer platform (WMD) (Ossowski *et al.*, 2008). Then the resulting artificial microRNAs were cloned into pU1301. The *SNAC1* artificial microRNAs (amiR-SNAC1) were constructed with the same process. To investigate the expression of *OsSRO1c*, the *OsSRO1c* promoter region (a fragment of 1.6 kb length upstream of the starting codon of the gene) was

amplified from the genomic DNA of Nipponbare and the sequence-confirmed PCR fragment cloned into pDX2181 to control GFP expression. For subcellular localization, the coding region of *OsSRO1c* cDNA was prepared by PCR, and cloned into the GATEWAY destination vector pH7WGF2,0 binary vector by recombination reaction (Karimi *et al.*, 2007). The primers are listed in Table S1. Both of the constructs were transformed into Zhonghua 11, a japonica rice that can be easily transformed, by the *Agrobacterium*-mediated transformation method (Hiei *et al.*, 1994; Lin and Zhang, 2005). The GFP fluorescence in different tissues of transgenic plants was observed by fluorescence stereomicroscope (MZ FLIII, Leica, Germany) or confocal laser-scanning microscopy (TCS SP2, Leica, Germany).

In vivo binding assay of SNAC1

For ChIP assay, wild-type ZH11 were used for chromatin extraction and immunoprecipitation as described previously (Huang *et al.*, 2007). Briefly, 3-leaf-stage rice seedlings were treated with formaldehyde and the nuclei were isolated and sonicated using an Ultrasonic Crasher Noise Isolating Chamber (SCIENTZ, Ningbo Science Biotechnology Co.Ltd, China). The soluble chromatin fragments were isolated and reabsorbed with sheared salmon sperm DNA/protein A-agarose (Sigma-Aldrich, USA) to remove nonspecific binding. Immunoprecipitations with anti-SNAC1 rabbit polyclonal antibody (New-East Biosciences) or without any serum were performed as described. The precipitated DNA was analyzed by PCR using specific primer sets (see Table S1). Typically, 26 to 28 cycles of PCR were performed, and the products were analyzed by agarose gel electrophoresis.

Biochemical assay in yeast

Yeast one-hybrid assay was performed using the Matchmaker one-hybrid system (Clontech, Palo Alto, CA, USA). *OsSRO1c* promoter fragment was fused upstream to the HIS3 minimal promoter and served as reporter constructs. *SNAC1* was fused to the GAL4 activation domain in the vector pGADT7-Rec2 (Hu *et al.*, 2006) and cotransformed with the reporter vector (pHIS2-$P_{OsSRO1c}$) into yeast cell Y187 for determination of the DNA–protein interactions. Primers used for yeast constructs were listed in Table S1.

The yeast two-hybrid assay was performed using the ProQuest Two-Hybrid

System (Invitrogen, USA). To isolate interact protein of OsSRO1c, the coding region of *OsSRO1c* was amplified with primers SROY2H. The PCR product was cloned into the entry vector pDONR221 using the BP reaction and then into the vector pDEST32 using the LR reaction to generate bait vector with OsSRO1c fused to the GAL4 DNA binding domain. A prey stress mix (drought, high salt, cold, and ABA treated) cDNA library of rice was constructed by fusing cDNAs with the GAL4 activation domain in the pEXP-AD502 vector according to the manufacturer's instructions. The yeast strain Mav203 was transformed with the bait plasmid, and the cells containing the bait were transformed with the plasmid DNA of the prey cDNA library according to the method described previously (Gietz *et al.*, 1997). A total of 2.05×10^5 transformants were selected on synthetic complete selection medium containing 20 mM 3-AT (3-amino-1, 2, 4-Triazole) and lacking Leu, Trp, and His. Large yeast clones appearing within 7 days were picked out for testing of the *LacZ* reporter gene. Positive clones were isolated and co-transformed with pDEST32 to test their self-activation activities.

To test interaction of OsSRO1c and members of NAC family or DREB family，The full-length cDNA of OsDREB1A, OsDREB1B, OsDREB2A and OsDREB2B proteins were isolated and fusion to the transcriptional activation domain of GAL4. Nine members of stress responsive NAC transcription factors were also fused to the transcriptional activation domain of GAL4 for yeast two-hybrid assay. All these construction were co-transformed with OsSRO1c fused to the GAL4 DNA binding domain.

Subcellular localization and BiFC assays in rice protoplast

The rice protoplast isolation and transformation were based on the protocol for maize protoplasts and *Arabidopsis* protoplasts from Sheen's laboratory (Sheen, 1990; Yoo *et al.*, 2007) with minor modifications. Rice seeds were germinated on half-strength MS medium under light for 3 d, and then grown in the dark at 26 °C for 12 d. Etiolated young seedlings were cut into 0.5 mm pieces using sharp razors. Tissue was immediately incubated in enzyme solution (0.6 M mannitol, 10 mM MES (pH 5.7), 1.5% cellulase RS, 0.75% macerozyme, 0.1% BSA, 1 mM $CaC1_2$ and 50 μg mL^{-1} carbenicillin) for 4 h in the dark under gentle shaking (40 rpm). After incubation, protoplasts were passed through a 35 μm nylon mesh filter. One volume of W5 solution (154 mM NaCl, 125 mM $CaC1_2$, 5 mM KC1, 2 mM MES (pH 5.7)) was

added and the solution was centrifuged for 5 min at 100 g to pellet the protoplasts. After removing the solution, 5 mL cold W5 solution was added to re-suspend the protoplasts and the protoplasts were kept on ice for 30 min. Cells were re-suspended in MMG solution (0.6 M mannitol, 15 mM $MgC1_2$, 4 mM MES (pH 5.7)) for PEG-mediated transformation at 10^6 cells mL^{-1}. Cells were quantified using a hemocytometer. For transformation, 40% PEG (0.6 M mannitol, 100 mM $CaC1_2$, 40% v/v PEG 3350) and 10 µg plasmid were added to the protoplasts for 20 min. After incubation, 5 mL W5 solution was added then the protoplasts were incubated at 28°C in the dark overnight.

To investigate the subcellular localization of the OsSRO1c protein, the full open reading frame of OsSRO1c was cloned into pM999-33 vector, fused with the GFP reporter gene. To confirm the protein interactions by BiFC assays, full-length cDNAs of *OsSRO1c*, *OsDREB2B*, *OsNAC8*, and *UBA* were inserted into pVYNE(R) (fusion with the N-terminus of YFP) or pVYCE (fusion with the C-terminus of YFP) (Waadt *et al.*, 2008). Plasmids were purified using Plasmid midi kit (QIAGEN, Germany) according to the manufacturer's protocol. The plasmids were introduced into rice protoplasts according to the method described above. The expression of the fusion construct was monitored after 16 h of incubation in the dark, and images were captured with confocal laser-scanning microscope (TCS SP2, Leica, Germany).

RNA isolation and RT-PCR

The TRIZol reagent (Invitrogen, USA) was used according the manufacturer's instructions to extract total RNAs. Before reverse transcription, total RNA was treated with amplification-grade DNase I (Invitrogen, USA) for 15 min to degrade possibly contaminated residual genomic DNA. The cDNA templates were synthesized using Superscript II reverse transcriptase (Invitrogen, USA) according to the manufacturer's instructions. Real-time quantitative RT-PCR was performed on a 7500 real-time PCR system (Applied Biosystems, USA) using SYBR Premix Ex Taq (TaKaRa, China) according to the manufacturer's protocol. Rice *Actin1* gene (accession no. AK060893) was used as the endogenous control. The relative expression levels were determined as described previously (Livak and Schmittgen, 2001). The gene-specific primers are listed in Table S1.

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