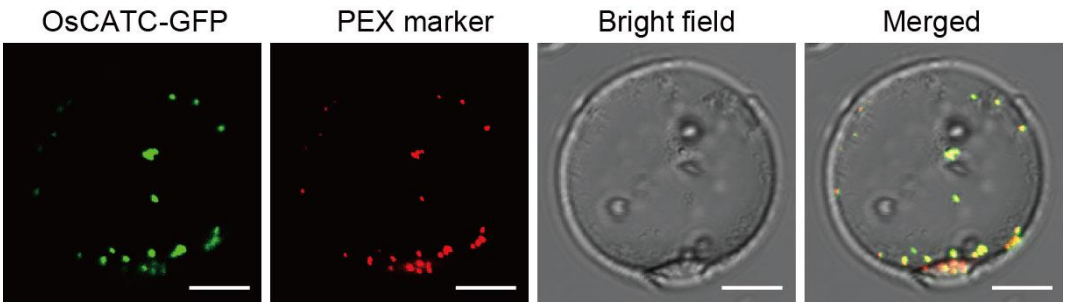
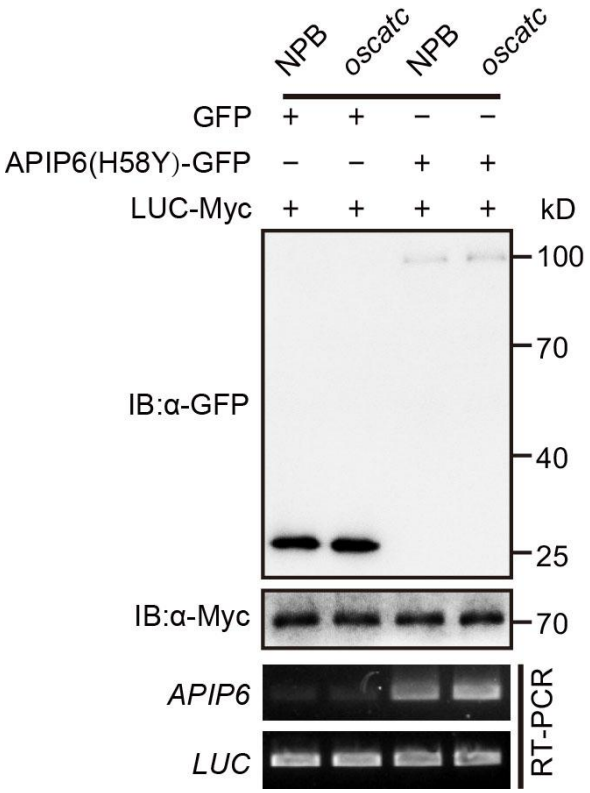


Supplemental Figure S1 Subcellular localization of OsCATC-GFP in rice protoplasts.

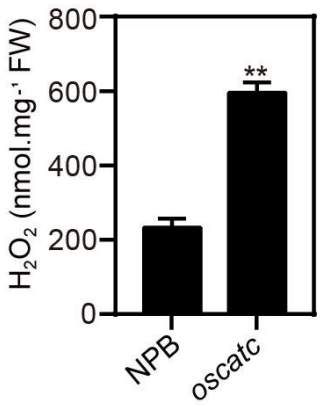
NPB protoplasts were transfected with *OsCATC-GFP* plasmid DNA and images were obtained using a laser-scanning confocal microscope (Zeiss LSM880) 16 h after transfection. OsPEX14-mCherry was used as a peroxisome marker. Bars: 10 μm. PEX, peroxisomes.



Supplemental Figure 2 The expression level of APIP6 in the protoplasts of *oscatc* mutants.

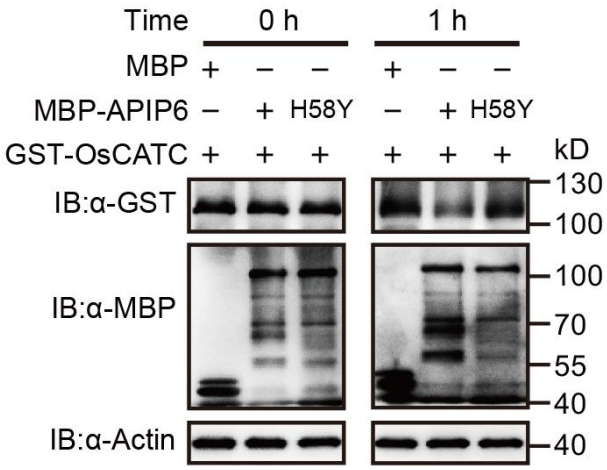
The expression level of APIP6 in the protoplasts of *oscatc* mutants. The *APIP6(H58Y)-GFP* plasmids were transiently expressed in the protoplasts of *oscatc* mutants. Samples were collected 24 h after transfection for protein and RNA extraction. The extracted protein and RNA were used for western blot and RT-PCR

analysis, respectively. LUC-Myc was used as an internal control. NPB, Nipponbare; IB, immunoblotting.



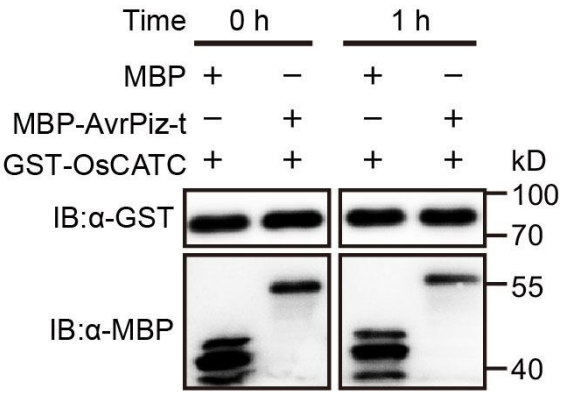
Supplemental Figure 3 H_2O_2 contents in 6-week-old NPB and *oscatc* mutant plants.

Leaves from 6-week-old plants were ground into fine powders in liquid nitrogen. Then the Hydrogen Peroxidase Assay Kit (Beyotime) was used to determine the H_2O_2 contents according to the manufacturer's instructions. FW, fresh weight. Values are means ± SE (n = 3, biological repeats). Asterisks represent statistically significant differences between samples (**$P < 0.01$ by Student's t-test). NPB, Nipponbare.



Supplemental Figure S4 OsCATC and APIP6 protein abundance in Figure 2E.

Recombinant purified OsCATC was used for catalase assays and mixed with MBP, MBP-APIP6 or MBP-APIP6(H58Y) for 1 h at 30 ℃ in the presence of rice extracts. The expressed proteins were immunoprecipitated with the anti-MBP or anti-MBP antibody. MBP was used as a negative control. MBP, maltose binding protein; IB, immunoblotting.



Supplemental Figure S5 OsCATC and AvrPiz-t protein abundance in Figure 2H. Purified OsCATC protein was mixed with MBP or MBP-AvrPiz-t for 1 h at 4 ℃ in 50 mM KH_2PO4 and 20 mM H_2O_2 for *in vitro* catalase assay. The expressed proteins were immunoprecipitated with the anti-MBP or anti-MBP antibody. MBP was used as a negative control. MBP, maltose binding protein; IB, immunoblotting.

Supplemental Materials and Methods

Plant materials and growth conditions

The *APIP6-RNAi* and *AvrPiz-t-OE* rice (*Oryza sativa* L.) lines in the NPB background were generated using *Agrobacterium*-mediated transformation of rice calli as previously described (Wang et al., 2016). The *oscatc* mutant in the NPB background was generated as described previously (Lin et al., 2012). Rice plants were grown in a growth chamber at 26℃/20℃ and 80%/60% relative humidity under a 12-h light/12-h dark cycle.

Inoculation of rice leaves with *Magnaporthe oryzae*

Rice leaves were inoculated by punch inoculation as previously described with slight modifications (Wang et al., 2021). Briefly, *Magnaporthe oryzae* (*M. oryzae)* isolate RO1-1 was cultivated on oatmeal agar for 1 week and placed under fluorescent lights

for 1 week at room temperature for spore induction. A spore suspension with a concentration of 5×10^5 spores/mL was used for punch inoculation of the last second leaves of 6-week-old plants. Disease symptoms on the leaves were evaluated 14 days after inoculation. Relative fungal biomass was calculated as described previously (Zhang et al., 2020).

RNA extraction

Total RNA was extracted from the samples using a UNIQ-10 Column TRIzol Total RNA Isolation kit (Sangon Biotech). cDNA was generated from 1 μg total RNA in a 20 μL reverse-transcription reaction using a TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech).

Detection of ROS bursts

ROS detection was performed as previously described (Yuan et al., 2021; Zhang et al., 2022). Briefly, leaf disks were cut from 6-week-old plants and incubated in sterile distilled water for 12 h in the dark. Three disks were submerged in a 1.5 mL microcentrifuge tube (Axygen) containing 1 μL of horseradish peroxidase (Jackson Immuno Research), 100 μL of luminol (Bio-Rad Immun-Star horseradish peroxidase substrate 170-5040), and 1 μL 8 nM chitin (hexa-N-acetyl-chitohexaose), with ddH_2O for the control. The tube was immediately placed in a GloMax 20/20 luminometer (Promega) to measure luminescence at 1 min intervals for 30 min.

Subcellular localization of OsCATC-GFP

To observe the subcellular localization of OsCATC-GFP, the full-length coding sequences of *OsCATC* was fused to the N-terminus of GFP in the pAN580-GFP vector (Yu et al., 2018). The construct was introduced into rice protoplasts as previously described (Chen et al., 2006). All confocal images were captured under a laser-scanning confocal microscope (Zeiss LSM880).

Yeast two-hybrid assays

For the Y2H assays, the full-length coding sequences or specific domains of *OsCATC* and *APIP6* were amplified and cloned into the pGBKT7 or pGADT7 (Clontech) vector. The constructs were transformed into yeast strain AH109 following the manufacturer's instructions (Clontech Yeast Protocols Handbook). Primers are listed in Supplemental Table S1.

***In vitro* pull-down assays**

To generate GST- and MBP-tagged fusion proteins, the coding sequences of *OsCATC* and *APIP6* were amplified and inserted into pGEX4T-2 or pMAL-c2X. The pull-down assay was conducted as previously described (Cai et al., 2021). Immunoblot analysis was performed to detect the fusion proteins with anti-GST and anti-MBP antibodies. Primers are given in Supplemental Table S1.

Bimolecular fluorescence complementation assay

For the BiFC assay, the full-length coding sequences of *OsCATC* and *APIP6* were fused to the p2YC (cYFP) or p2YN (nYFP) vector. The recombinant plasmids were transformed into *Agrobacterium* EHA105, which were then co-infiltrated in *N. benthamiana* leaves as described previously (Waadt and Kudla, 2008). A laser-scanning confocal microscope (Zeiss LSM880) was used to detect the fluorescent signals between 48 h and 72 h after co-injection. All images captured with the following parameters (lasers: YFP: 488 nm, mCherry :561 nm; intensity: YFP: 723, mCherry: 780; collection bandwidth: 111 μm; gains: 1. All negative control were no fluorescence signals. Primers are given in Supplemental Table S1.

***In vivo* co-immunoprecipitation assays**

The full-length coding sequences of *OsCATC*, *APIP6(H58Y)*, and *AvrPiz-t* were amplified and cloned into the pYBA1143, pYBA1132 or pRTVcMyc vectors, respectively. The combinations were co-expressed in rice protoplasts. Total proteins were extracted from freshly harvested leaves in two volumes of NB1 buffer (50 mM Tris-MES, pH 8.0, 0.5 M sucrose, 1 mM $MgCl_2$, 5 mM DTT, 10 mM EDTA, and 1 ×

protease inhibitor cocktail) after 24 h culture and incubated at 4 ℃ for 30 min with rotation. The Co-IP assay was performed as previously described (Cai et al., 2021). Immunoblotting was performed using anti-GFP (598-7, MBL), anti-Flag (M185-7, MBL), and anti-HA (M180-7, MBL) antibodies. Primers are listed in Supplemental Table S1.

***In vivo* degradation assay**

For the *in vivo* OsCATC-APIP6 degradation assay, the OsCATC-HA plasmid was co-expressed with the APIP6-GFP and APIP6(H58Y)-GFP plasmids into NPB protoplasts, respectively. For the MG132 treatment in rice protoplasts, MG132 (20 μM) was added 12 h after co-expressed and incubated for 12 h before protein extraction. LUC-Myc vector was used as a negative control. Primers are given in Supplemental Table S1.

Measurement of H_2O_2 content

The measurement of H_2O_2 content was conducted by extracting from rice leaves according to a previously reported method (Liang et al., 2015) and quantified using the Hydrogen Peroxidase Assay Kit (Beyotime) according to the manufacturer's instructions.

Catalase Activity Assay

Half of a gram of rice leaf samples were ground into fine powders in liquid nitrogen and were extracted in 1 mL extraction buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.5% (v/v) Triton X-100, 10% (v/v) glycerol, and 1% (w/v) protease inhibitor cocktail). The supernatant was collected after centrifuged at 12,000 rpm for 10 min at 4 ℃ and protein concentration was measured using a BCA protein assay kit (CWBIO). The supernatant was used for catalase activity analysis with the Catalase Assay Kit (Beyotime) according to the manufacturer's instructions. The activity of purified OsCATC was measured at 240 nm in 50 mM KH_2PO_4 and 10 mM H_2O_2 as the absorbance decrease and was shown as units/mg (Weydert and Cullen, 2010). One

unit represents the amount of enzyme that catalyzes the decomposition of 1 mM H_2O_2 per minute at 30 ℃. To assess the effect of APIP6 on OsCATC activity, the purified MBP, MBP-APIP6, and MBP-APIP6(H58Y) proteins were mixed with OsCATC for 1 h at 30 ℃. For the effect of AvrPiz-t on OsCATC activity assay, the purified MBP and MBP-AvrPiz-t proteins were mixed with OsCATC for 1 h at 4 ℃. And the catalase activity was then determined.

Supplemental Table S1 Sequences of primers used in this study.

Primers Name	Primers sequence
AD-APIP6F	GGAGGCCAGTGAATTCATGGGTGCGAGGGAGGAGGT
AD-APIP6R	CGAGCTCGATGGATCCCTACATCCTTGGGGTGTGCATT
BD-OsCATCF	CATGGAGGCCGAATTCATGGATCCCTACAAGCACCGCC
BD-OsCATCR	GCAGGTCGACGGATCCTTACATGCTCGGCTTCGCGCTG
MBP-APIP6F	AAGGATTTCAGAATTCATGGGTGCGAGGGAGGAGGT
MBP-APIP6R	CGACTCTAGAGGATCCCTACATCCTTGGGGTGTGCATT
GST-OsCATCF	GGTTCCGCGTGGATCCATGGATCCCTACAAGCACCGCC
GST-OsCATCR	GTCGACCCGGGAATTCCTTACATGCTCGGCTTCGCGCTG
OsCATC-HAF	AGGAATTCGATATCAAGCTTATGGCGATGCGGCACCTCAT
OsCATC-HAR	TCGACGGTATCGATAAGCTTCAGGGGAAACTCTTTCT
APIP6-GFPF	CGGAATTCGATATCAAGCTTATGGGTGCGAGGGAGGAGGT
APIP6-GFPR	CGACGGTATCGATAAGCTTATCCTTGGGGTGTGCATT
OsCATC-GFPF	GGAATTCGATATCAAGCTTATGGATCCCTACAAGCACCGCC
OsCATC-GFPR	CGACGGTATCGATAAGCTTCATGCTCGGCTTCGCGCTG
nYFP-OsCATCF	CATTTACGAACGATAGTTAATTAAATGGATCCCTACAAGCAC
nYFP-OsCATCR	CACTGCCACCTCCTCCACTAGTCATGCTCGGCTTCGCGCTG
cYFP-APIP6F	CATTTACGAACGATAGTTAATTAAATGGGTGCGAGGGAGGAGGT
cYFP-APIP6R	CACTGCCACCTCCTCCACTAGTCATCCTTGGGGTGTGCATT
AvrPiz-t-MycF	CTGCAGGAATTCGATATCAAGCTTAGCTTCGTACAATGCAAC
AvrPiz-t-MycR	GAGGTCGACGGTATCGATAAGCTTTTGGCGCTGAGCCTGAG
q-MoPot2-F	ACGACCCGTCTTTACTTATTTGG
q-MoPot2-R	AAGTAGCGTTGGTTTTGTTGGAT
q-Actin1F	TGCTATGTACGTCGCCATCCAG
q-Actin1R	AATGAGTAACCACGCTCCGTCA

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