

Hos2-mediated KatG1 decrotonylation orchestrates ROS scavenging in rice blast infection.

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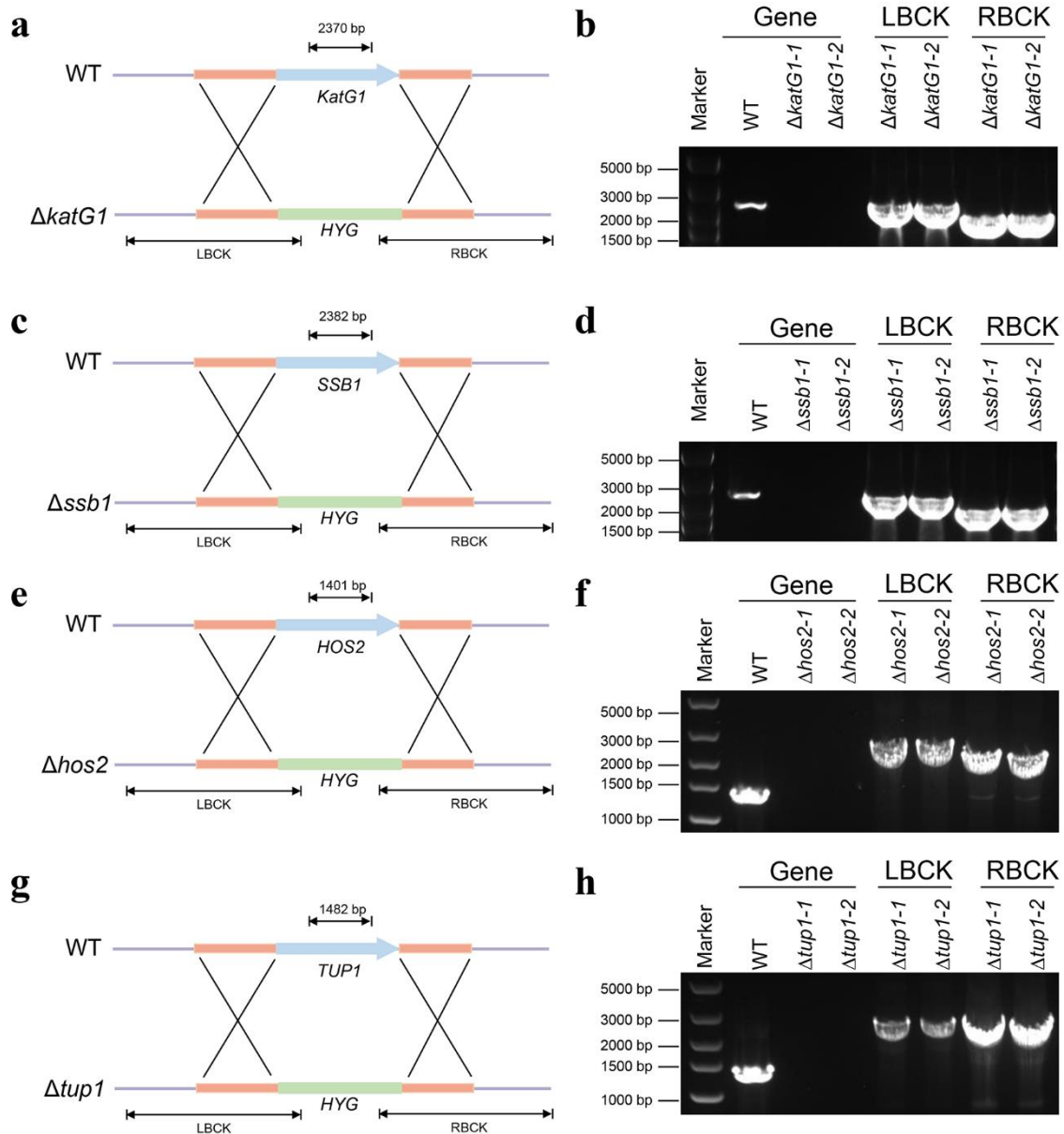
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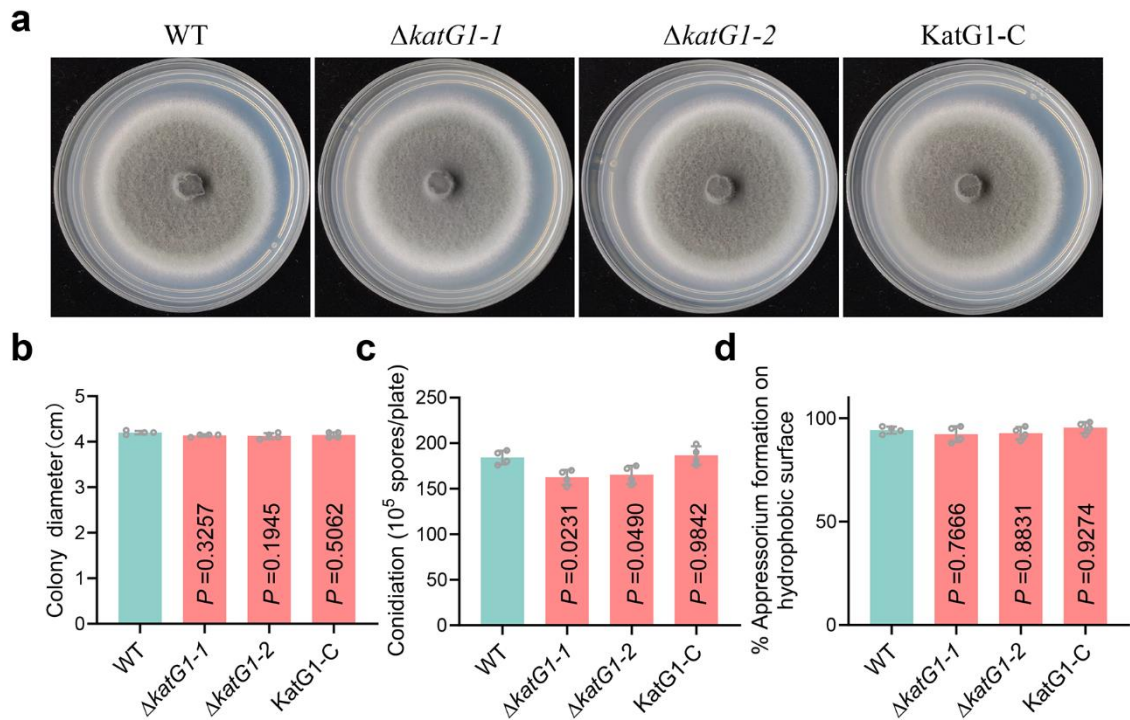
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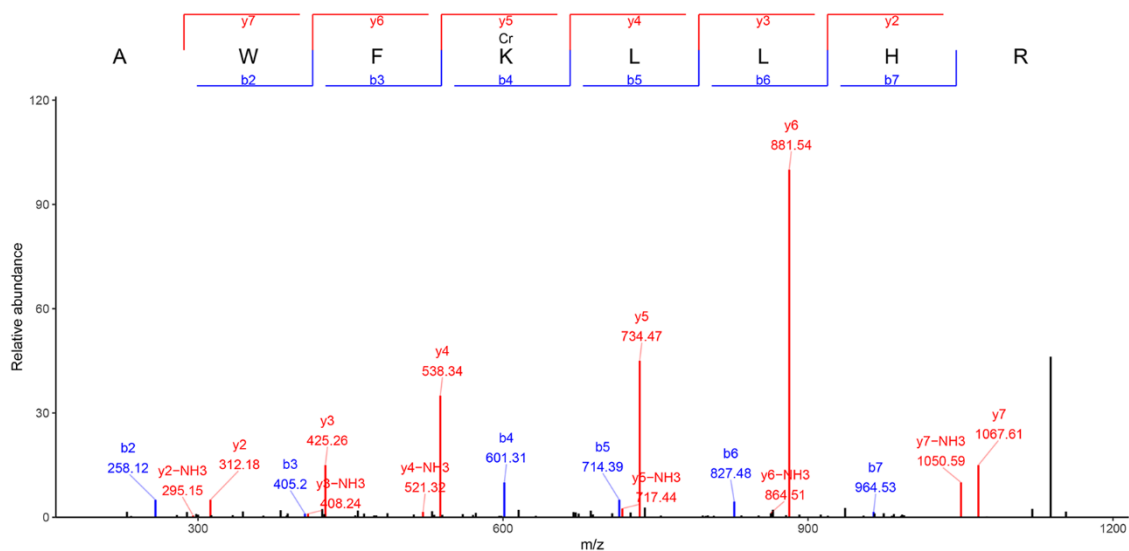


Supplementary Fig. 1. Replacement strategy and confirmation of *KatG1*, *HOS2* and *TUP1* deletion mutants. (a, c, e, g) The *KatG1*, *SSB1*, *HOS2* and *TUP1* genes were replaced by the split-marker approach method. Orange bars represent genomic regions upstream and downstream of the respective target gene coding sequence that was amplified and fused to segments of the hygromycin phosphotransferase (HYG) cassette. (b, d, f, h) PCR verification of the flanking sequences besides the replacement fragment by using primer pairs of LBCK/HCK-up and RBCK/HCK-down.

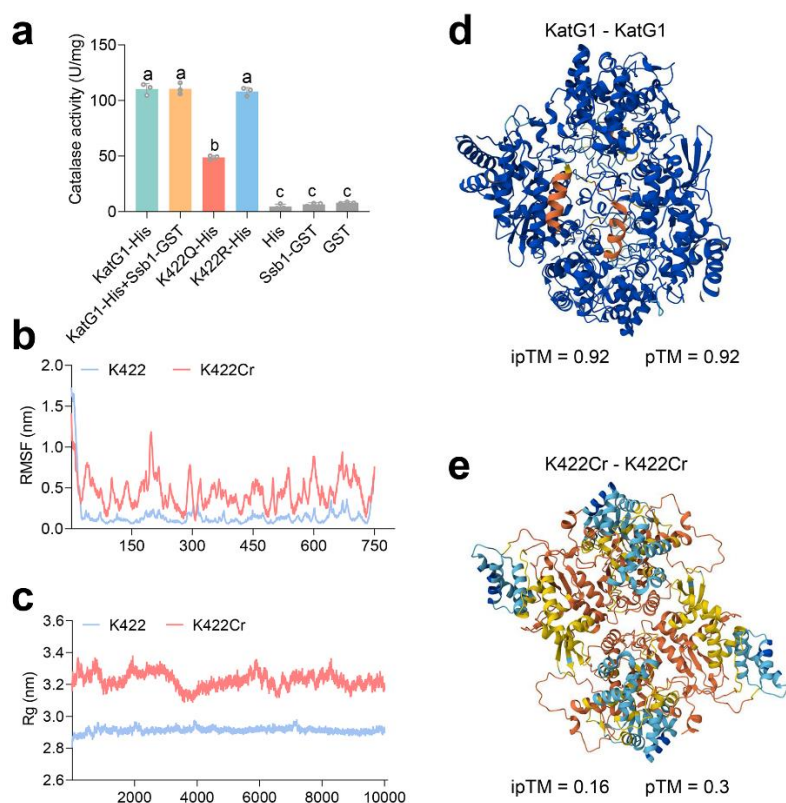


Supplementary Fig. 2 KatG1 is largely dispensable for normal vegetative growth, conidiation, and appressorium formation. (a) Colony morphology of the wild-type (WT, strain P131), two independent *katG1* deletion mutants ($\Delta katG1-1$ and $\Delta katG1-2$), and the complemented strain (KatG1-C). Strains were grown on complete medium (CM) plates at 28 °C for 5 days. (b-d) Statistical analysis of colony diameters (b), conidial production (c), and the percentage of appressorium formation on hydrophobic surfaces (d) for the indicated strains. Data are presented as mean $\pm$ SD ($n = 4$ independent biological replicates). Significant differences were determined via one-way ANOVA with Tukey's test ($P < 0.05$). Exact P values are provided in the Source Data.

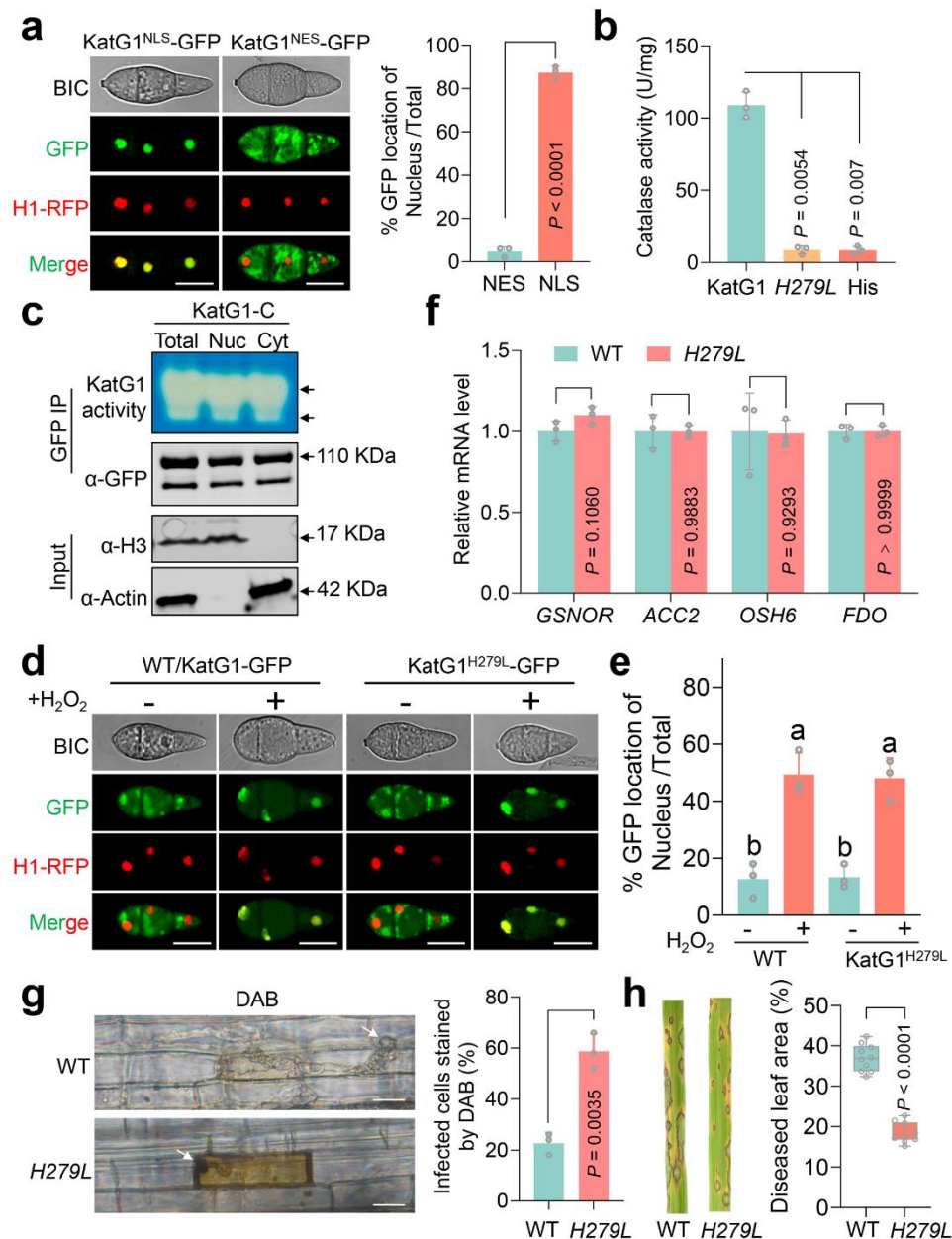
Experiment	Sequence	Length	Modified sequence	Charge	m/z	Mass error [ppm]	PEP	Score
KatG1 Rep1	AWFKLLHR	K4(Crotony)	AWFK(Crotony (K))LLHR	2	569.829643	-1.9886	0.00028849	104.76
KatG1 Rep2	AWFKLLHR	K4(Crotony)	AWFK(Crotony (K))LLHR	2	569.829643	-1.5314	0.00017152	113.1
KatG1 Rep3	AWFKLLHR	K4(Crotony)	AWFK(Crotony (K))LLHR	2	569.829643	-1.9749	0.000048706	140.12



Supplementary Fig. 3 Identification of the K422 crotonylation site in KatG1 by LC-MS/MS. The top table lists the identification parameters of the crotonylated peptide (AWFK(Cr)LLHR) from three independent biological replicates, indicating high-confidence detection. The bottom panel shows a representative tandem mass (MS/MS) spectrum of this peptide. The annotated b-ion and y-ion series definitively map the crotonylation modification to the K422 residue of KatG1.

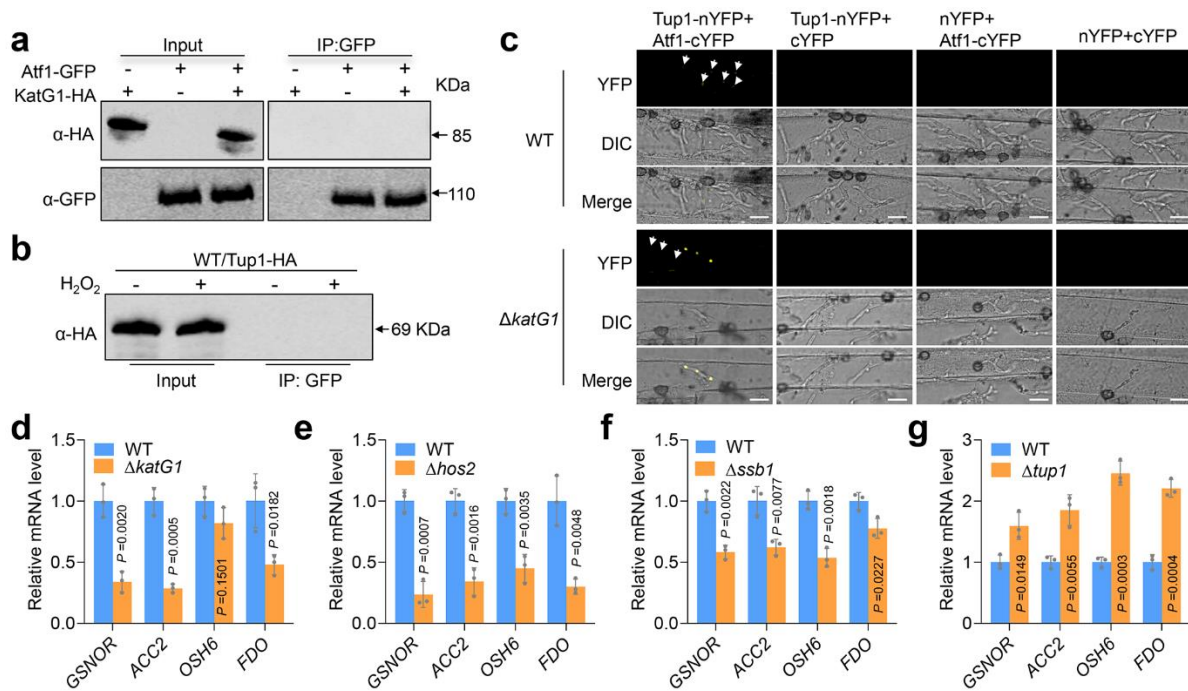


Supplementary Fig. 4 K422 crotonylation impairs KatG1 catalase activity and destabilizes the homodimer interface. (a) Catalase activity of purified KatG1 and its mutant proteins. The crotonylation-mimic mutant KatG1^{K422Q} exhibits significantly reduced activity, whereas the decrotonylation-mimic KatG1^{K422R} retains wild-type levels. Co-incubation of KatG1-His with Ssb1-GST shows that Ssb1 does not directly affect KatG1 enzymatic activity *in vitro*. Data in (a) are presented as bar graphs (mean $\pm$ SD) with individual data points from $n = 3$ independent experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons test. Different lowercase letters indicate significant differences ($P < 0.05$). Exact P values are provided in the Source Data file. (b, c) Root-mean-square fluctuation (RMSF) (b) and radius of gyration (Rg) (c) of wild-type KatG1 (K422, blue) and crotonylated KatG1 (K422Cr, red) during molecular dynamics simulations. The increased RMSF and Rg values indicate higher structural flexibility and instability in the crotonylated mutant. (d, e) AlphaFold3 predicted structural models of the wild-type KatG1-KatG1 dimer (d) and the crotonylated K422Cr-K422Cr dimer (e). Interface confidence scores (ipTM) and overall confidence scores (pTM) are indicated. The plummeting of ipTM from 0.92 (d) to 0.16 (e) demonstrates that K422 crotonylation drastically destabilizes the dimer interface.

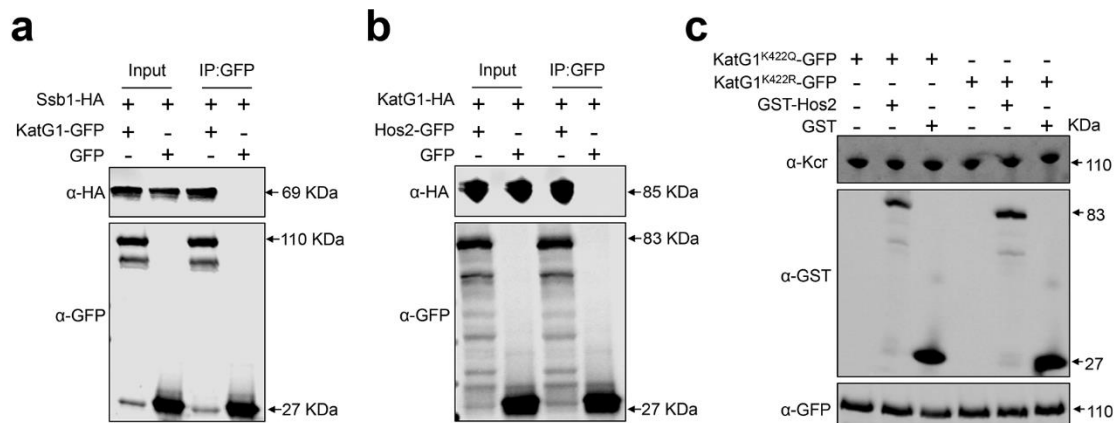


Supplementary Fig. 5 Subcellular localization controls and the decoupling of KatG1 enzymatic and signaling functions. (a) Confocal microscopy images validating the strictly cytoplasmic retention of KatG1^{NES}-GFP and the constitutive nuclear enrichment of KatG1^{NLS}-GFP under normal conditions. (b) *In vitro* catalase activity assay of recombinant wild-type KatG1, the H279L mutant, and a His-tag negative control purified from *Escherichia coli*. The H279L mutant exhibits a profound reduction in catalytic activity. (c) KatG1 exhibits inherent catalase activity in both the cytoplasm and the nucleus. Following GFP immunoprecipitation from total (Total), nuclear (Nuc), and cytoplasmic (Cyt) extracts of the KatG1-GFP strain, samples were subjected to an in-gel catalase activity assay (top) and immunoblotting (bottom). *M. oryzae* actin and histone H3 served as cytoplasmic and nuclear markers. Arrows indicate active KatG1 catalase bands. (d) Subcellular localization of KatG1^{H279L}-GFP

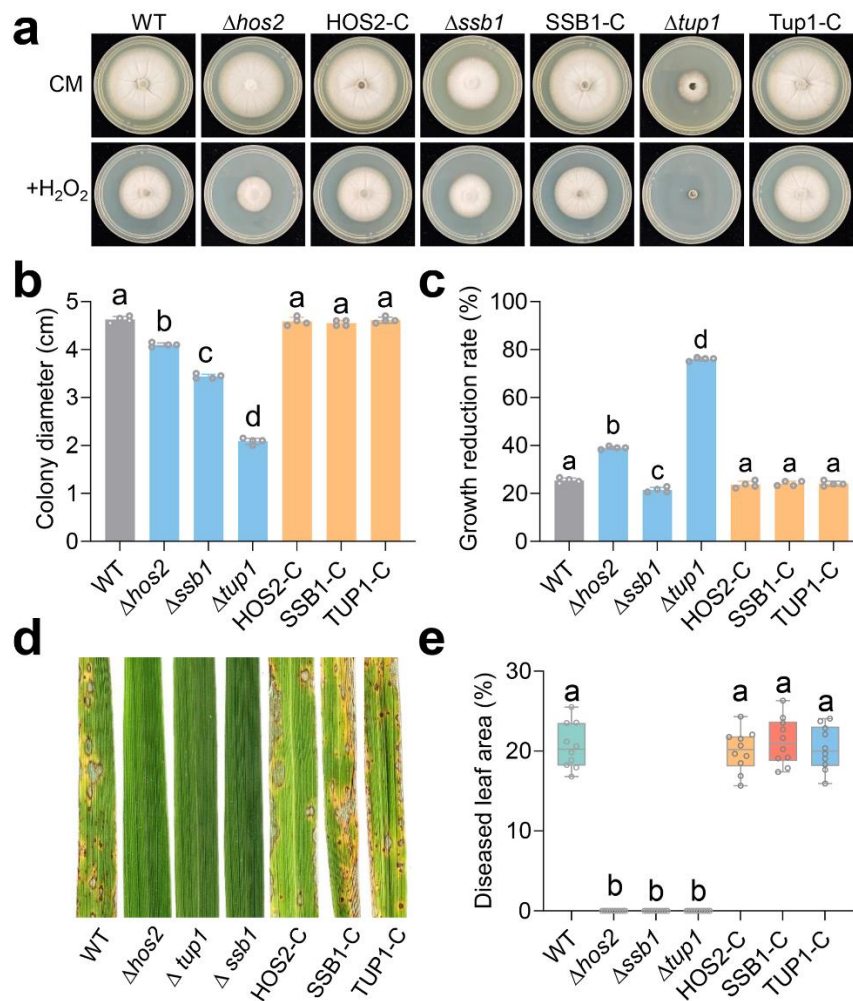
following 10 mM H₂O₂ treatment. H1-RFP marks the nucleus. H1-RFP serves as the nuclear marker. Scale bars, 10 µm. **(e)** Quantification of the nuclear GFP signal percentage (Nucleus/Total) for the strains shown in **(d)**. **(f)** The *H279L* mutant activates downstream target genes to levels comparable to wild-type. Relative mRNA levels of Atf1-targeted antioxidant genes (*GSNOR*, *ACC2*, *OSH6*, and *FDO*) under oxidative stress were quantified by RT-qPCR. **(g)** *In planta* DAB staining evaluating ROS accumulation in rice sheath cells infected by the WT and *H279L* mutant strains. Images and quantification show intense brown staining for the *H279L* mutant, indicating a failure to effectively scavenge host-derived ROS. White arrows indicate appressoria. Scale bars, 20 µm. **(h)** Pathogenicity assay on rice leaves. The bar chart shows the relative diseased leaf areas caused by the WT and the *H279L* mutant strains at 7 dpi. Significant differences were determined by a two-tailed Student's *t*-test for **(a, b, f, g, h)** and two-way ANOVA with Tukey's multiple comparisons test for **(e)**. Data are presented as mean ± SD of *n* = 3 independent biological replicates (for **h**, *n* = 10 leaves). Different lowercase letters indicate significant differences. Exact *P* values are provided in the Source Data.



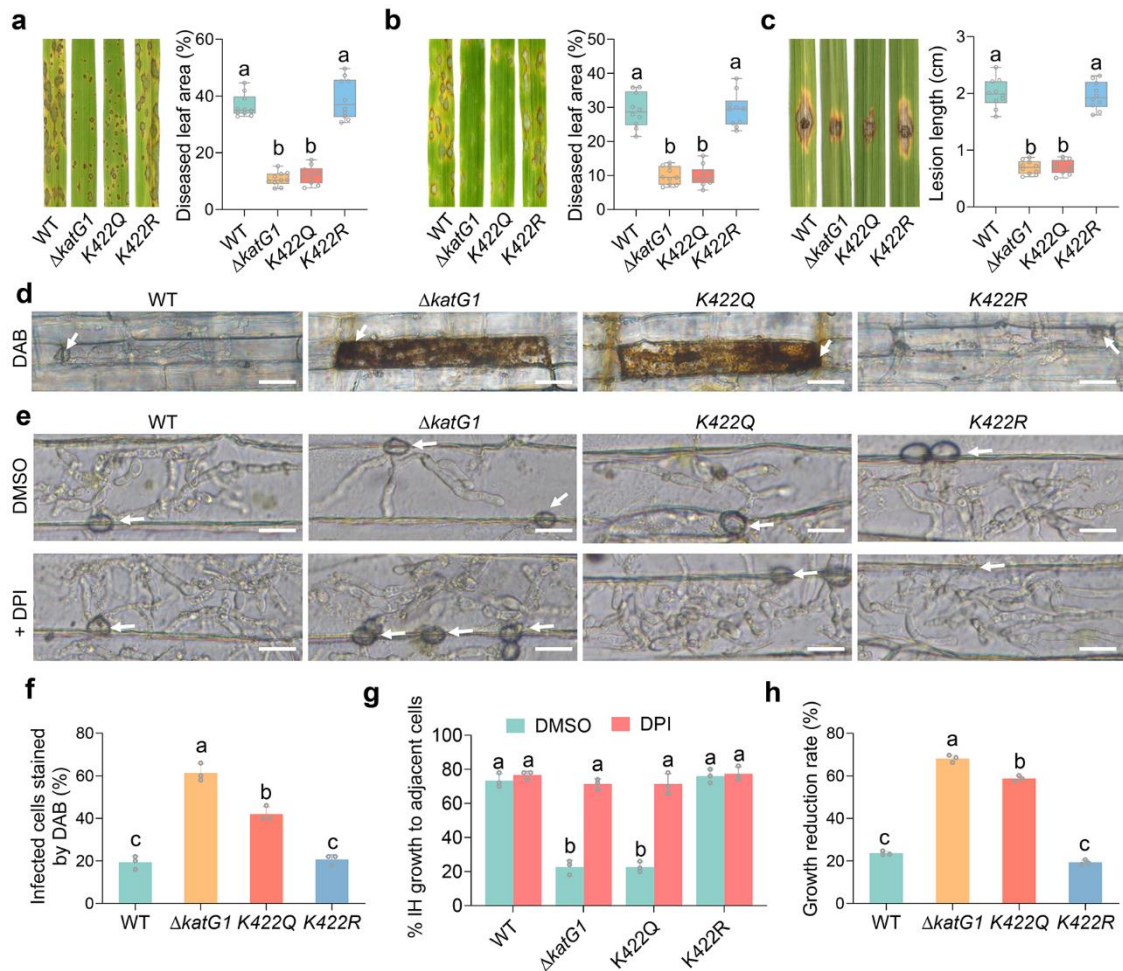
Supplementary Fig. 6 Transcriptional regulation of Atf1 targets by the Hos2-KatG1-Ssb1-Tup1 cascade. (a) Co-immunoprecipitation (Co-IP) assay showing no direct interaction between KatG1 and Atf1 *in vivo*. Total proteins from *M. oryzae* co-expressing KatG1-HA and Atf1-GFP were immunoprecipitated with anti-GFP beads. The absence of an α -HA band in the IP:GFP lane indicates a lack of physical association. (b) Negative control for the Co-IP assays. Total proteins extracted from the strain expressing Tup1-HA alone were immunoprecipitated using anti-GFP affinity beads. No non-specific binding was detected. (c) Bimolecular fluorescence complementation (BiFC) assay demonstrating the interaction between Tup1-nYFP and Atf1-cYFP in both wild-type (WT) and $\Delta katG1$ strains. White arrows indicate YFP fluorescent signals in the nuclei. Scale bars, 10 μ m. (d-g) Relative mRNA expression levels of Atf1-regulated target genes (*GSNOR*, *ACC2*, *OSH6*, and *FDO*) in the $\Delta katG1$ (d), $\Delta hos2$ (e), $\Delta ssb1$ (f), and $\Delta tup1$ (g) mutants compared to the WT strain. Data are presented as bar graphs (mean $\pm$ SD, $n = 3$ independent biological replicates) with individual data points. Statistical significance was determined using a two-tailed Student's t-test. Exact P values are visually indicated on the graphs and provided in the Source Data file.



Supplementary Fig. 7 *In vivo* interaction of KatG1 with Ssb1 and Hos2, and *in vitro* site-specificity validation. **(a)** Co-immunoprecipitation (Co-IP) assay confirming the interaction between KatG1 and Ssb1. Total proteins were extracted from *M. oryzae* mycelia co-expressing KatG1-GFP and Ssb1-HA, and then immunoprecipitated with anti-GFP agarose beads. Immunoblots were probed with anti-HA and anti-GFP antibodies. The strain co-expressing Ssb1-HA and free GFP served as the negative control. **(b)** Co-IP assay confirming the interaction between Hos2 and KatG1. Total proteins from mycelia co-expressing Hos2-GFP and KatG1-HA were processed and analyzed similarly to (a). The strain co-expressing KatG1-HA and free GFP served as the negative control. **(c)** *In vitro* decrotonylation assay using natively immunoprecipitated KatG1 point mutants (K422Q and K422R) incubated with recombinant GST-Hos2. The crotonylation levels were detected by anti-Kcr immunoblotting. GST-Hos2 completely failed to reduce the crotonylation signals of the K422-substituted mutants, unequivocally confirming K422 as the specific target site.



Supplementary Fig. 8 Phenotypic analyses of the $\Delta hos2$, $\Delta ssb1$, and $\Delta tup1$ mutants. (a) Colony morphology of the wild-type (WT), deletion mutants ($\Delta hos2$, $\Delta ssb1$, $\Delta tup1$), and their corresponding complemented strains (HOS2-C, SSB1-C, TUP1-C) grown on complete medium (CM) with or without exogenous H₂O₂. (b) Statistical analysis of colony diameters on CM, showing significant vegetative growth defects in all three mutants. (c) Growth reduction rates (%) of the indicated strains under H₂O₂ stress. Notably, unlike the highly sensitive $\Delta hos2$ and $\Delta tup1$ mutants, the $\Delta ssb1$ mutant does not exhibit increased sensitivity to H₂O₂ *in vitro*. (d) Pathogenicity assays on rice leaves inoculated with the indicated strains. (e) Quantification of the diseased leaf area (%) corresponding to the infections in (d). The $\Delta hos2$, $\Delta ssb1$, and $\Delta tup1$ mutants almost completely lose pathogenicity. Data are presented as mean $\pm$ SD (b, c; $n = 3$ independent biological replicates) or box plots (center line, median; box limits, 25th–75th percentiles; whiskers, min to max; e; $n = 10$ biologically independent samples) with individual data points. Distinct lowercase letters denote significant differences determined via one-way ANOVA with Tukey's test ($P < 0.05$). Exact P values are provided in the Source Data.

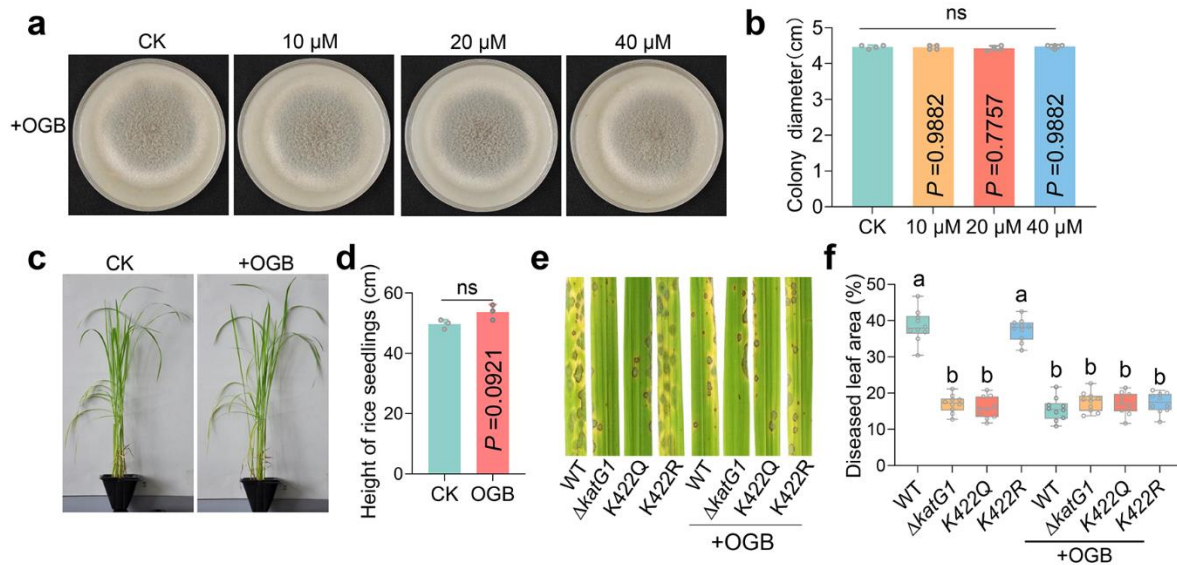


Supplementary Fig. 9 The K422 crotonylation site is essential for KatG1-mediated ROS scavenging and fungal virulence. (a, b) Pathogenicity spray assays on rice (a) and barley (b) leaves. Conidial suspensions of the wild-type (WT), $\Delta katG1$, and the crotonylation site mutants ($KatG1^{K422Q}$ and $KatG1^{K422R}$, indicated as $K422Q$ and $K422R$) were inoculated. Representative disease symptoms and quantified diseased leaf areas (%) at 5 days post-inoculation (dpi) are shown. (c) Wounded leaf assays. Mycelial agar plugs were inoculated onto wounded rice leaves. Representative images and quantification of lesion lengths (cm) at 5 dpi are shown. (d) DAB staining detecting ROS accumulation in rice leaf sheath cells infected by the indicated strains at 30 hpi. The $\Delta katG1$ and $KatG1$ -K422Q mutants trigger massive host ROS burst (dark brown). White arrows indicate appressoria. Scale bars, 20 μm . (e) Microscopic observation of invasive hyphae (IH) growth in rice leaf sheaths treated with DMSO (control) or 0.4 μM diphenyleneiodonium (DPI). White arrows indicate appressoria. Scale bars, 20 μm . (f) Percentage of DAB-stained infected cells corresponding to (d). Over 50 infected cells were evaluated per replicate. (g) Percentage of IH extending to adjacent cells corresponding to (e). DPI treatment partially restores the invasive growth defects of the $\Delta katG1$ and $KatG1^{K422Q}$ mutants. (h) Growth reduction rates (%) of the indicated strains under 12 mM H_2O_2 stress. Data are presented as box plots (center line, median; box limits, 25th–75th percentiles; whiskers, min to max; a–c, $n = 10$

biologically independent samples) or mean $\pm$ SD (**f–h**, $n = 3$ independent biological replicates; >50 infected cells were evaluated per replicate for **f**, **g**). Distinct lowercase letters denote significant differences ($P < 0.05$) determined via one-way (**a–c**, **f**, **h**) or two-way (**g**) ANOVA with Tukey's test. Exact P values are provided in the Source Data.

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Unlike the wild-type protein, the KatG1^{M6} mutant fails to translocate into the nucleus under oxidative stress. Scale bars, 10 μ m. **(e)** Quantification of the nuclear-localized GFP signals (%) corresponding to the observations in **(d)**. Data are presented as mean $\pm$ SD ($n = 3$ independent biological replicates). Significant differences were determined via a two-tailed Student's *t*-test **(b)** or two-way ANOVA with Tukey's test **(e)** ($P < 0.05$). Distinct lowercase letters indicate significant differences. Exact *P* values are provided in the Source Data.



Supplementary Fig. 11 Evaluation of OGB phytotoxicity and validation of KatG1 as its specific target. **(a)** Colony morphology of the wild-type strain grown on complete medium supplemented with various concentrations of OGB. **(b)** Statistical analysis of colony diameters corresponding to **(a)**. The results indicate that OGB does not inhibit the vegetative growth of *M. oryzae* *in vitro*. **(c, d)** Phytotoxicity evaluation of OGB on rice plants. Rice seedlings were sprayed with OGB or solvent control (CK). Representative photographs **(c)** and seedling heights (cm) **(d)** were recorded after 2 weeks. The data demonstrate that 10 mM OGB has no detrimental effect on rice growth. **(e)** Pathogenicity spray assays validating the specific on-target effect of OGB. Rice leaves were inoculated with the indicated strains (WT, Δ katG1, $KatG1^{K422Q}$, and $KatG1^{K422R}$) in the absence or presence of OGB. **(f)** Quantification of the diseased leaf area (%) corresponding to **(e)**. OGB treatment drastically suppresses the virulence of the WT and the highly active $KatG1^{K422Q}$ strains down to basal levels, but fails to further exacerbate the virulence defects of the low-activity $KatG1^{K422Q}$ or Δ katG1 mutants. Data are presented as mean $\pm$ SD **(b, n = 4; d, n = 3** independent biological replicates) or box plots (center line, median; box limits, 25th–75th percentiles; whiskers, min to max) with individual data points **(f; n = 10** leaves). Distinct lowercase letters indicate significant differences determined via one-way **(b)** or two-way **(f)** ANOVA with Tukey’s test ($P < 0.05$). “ns” denotes not significant via two-tailed Student’s *t*-test **(d)**. Exact *P* values are provided in the Source Data.