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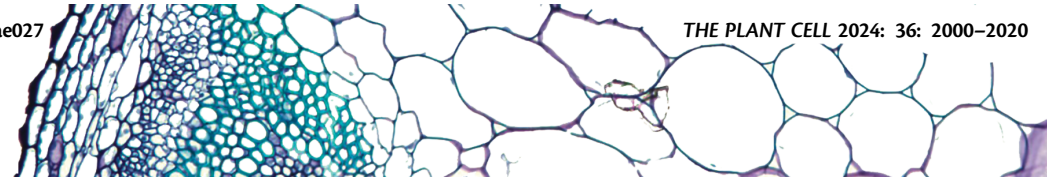

Research Article

Rice false smut virulence protein subverts host chitin perception and signaling at lemma and palea for floral infection

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Abstract

The flower-infecting fungus *Ustilaginoidea virens* causes rice false smut, which is a severe emerging disease threatening rice (*Oryza sativa*) production worldwide. False smut not only reduces yield, but more importantly produces toxins on grains, posing a great threat to food safety. *U. virens* invades spikelets via the gap between the 2 bracts (lemma and palea) enclosing the floret and specifically infects the stamen and pistil. Molecular mechanisms for the *U. virens*–rice interaction are largely unknown. Here, we demonstrate that rice flowers predominantly employ chitin-triggered immunity against *U. virens* in the lemma and palea, rather than in the stamen and pistil. We identify a crucial *U. virens* virulence factor, named UvGH18.1, which carries glycoside hydrolase activity. Mechanistically, UvGH18.1 functions by binding to and hydrolyzing immune elicitor chitin and interacting with the chitin receptor CHITIN ELICITOR BINDING PROTEIN (OsCEBiP) and co-receptor CHITIN ELICITOR RECEPTOR KINASE1 (OsCERK1) to impair their chitin-induced dimerization, suppressing host immunity exerted at the lemma and palea for gaining access to the stamen and pistil. Conversely, pretreatment on spikelets with chitin induces a defense response in the lemma and palea, promoting resistance against *U. virens*. Collectively, our data uncover a mechanism for a *U. virens* virulence factor and the critical location of the host–pathogen interaction in flowers and provide a potential strategy to control rice false smut disease.

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IN A NUTSHELL

Background: Rice false smut is an emerging fungal disease threatening rice production worldwide. This disease not only reduces grain yield but also introduces food toxins. The causative pathogen invades rice flowers via the gap between the 2 bracts (lemma and palea) enclosing the floret and specifically infects the stamens and pistils. The underlying mechanisms by which rice flowers defend against the pathogen and the pathogen counteracts this defense remain largely unknown.

Question: The optimal defense theory predicts that flowers should receive protection from constitutive defenses, rather than inducible defenses against herbivores. Do rice flowers deploy constitutive or induced defense against the false smut fungus? How does the pathogen overcome rice defense?

Findings: Chitin is a vital component of fungal cell walls and often elicits plant immunity. We show that rice flowers predominantly employ chitin-induced immunity against the false smut fungus in lemmas and paleas, rather than in stamens and pistils. We identify a secreted protein, named UvGH18.1, as a core virulence factor for the false smut pathogen. UvGH18.1 breaks down chitin to prevent host immune elicitation and further targets rice chitin sensors to impair chitin signaling, suppressing host immunity exerted at lemmas and paleas to gain access to stamens and pistils. Conversely, pretreating flowers with chitin promotes rice resistance to false smut, offering a potential strategy to control this disease.

Next steps: One next step is to identify plant receptors that recognize UvGH18.1 or other molecules derived from the false smut fungus. Another is to identify chemicals that inhibit UvGH18.1 function to block pathogen infection.

Introduction

Flower-infecting fungi cause many detrimental crop diseases, such as Fusarium head blight in wheat (*Triticum aestivum*; caused by *Fusarium graminearum*) (Xu and Nicholson 2009), Ergot disease in rye (*Secale cereale*; caused by *Claviceps purpurea*) (Tudzynski and Scheffer 2004), corn smut disease (caused by *Ustilago maydis*) (Brefort et al. 2009), and rice false smut disease (caused by *Ustilaginoidea virens*) (Sun et al. 2020). Floral pathogens can produce food toxins, such as deoxynivalenol (DON), ergot alkaloids, and diverse ustiloxins (Tudzynski and Scheffer 2004; Xu and Nicholson 2009; Zhou et al. 2012), imposing serious health hazards to humans and animals. To ensure food security, there is an urgent need to explore resistance genes and understand immune pathways that protect floral organs from these pathogens for crop breeding.

Rice false smut has become one of the most severe rice (*Oryza sativa*) diseases in south and east Asia (Zhou et al. 2012; Sun et al. 2020), and increasingly occurs in other rice-production areas worldwide, such as the Middle East and North America (Rush et al. 2000). This disease reduces rice yield and quality and introduces mycotoxins that inhibit cell division (Koiso et al. 1994). These mycotoxins are a great threat to human health. The causative pathogen *U. virens* specifically attacks developing rice spikelets at late booting stages. The pathogen spores germinate on the surface of the lemma and palea and the hyphae undergo epiphytical growth without penetrating host tissues. Instead, pathogen hyphae invade through the gap between the lemma and palea into the inner space of rice spikelets, and intercellularly infects stamen filaments, stigmas, styles, and lodicules (Ashizawa et al. 2012; Tang et al. 2013; Song et al. 2016). After successful colonization, *U. virens* forms massive mycelia to embrace all the inner floral organs, and ultimately develops into a fungal mass named false smut ball, which is the only visible symptom of rice false smut disease (Fan et al. 2016). However, the molecular basis employed by flowers to defend against *U. virens* and the counterstrategy employed by the pathogen remain largely unknown.

Chitin is a vital component of fungal cell walls. To fight against fungal pathogens, plants have evolved chitin-triggered immunity as a central defense pathway, which has been well characterized in plant leaf organs (see review (Gong et al. 2020)). For example, in rice leaves, CHITIN ELICITOR BINDING PROTEIN (OsCEBiP) functions as a major receptor with high affinity for chitin (Kaku et al. 2006; Hayafune et al. 2014). In addition, LYSIN MOTIF-CONTAINING PROTEIN4 (OsLYP4) and OsLYP6 serve as minor chitin receptors (Liu et al. 2012). As these chitin receptors lack a kinase domain, chitin signaling requires a co-receptor CHITIN ELICITOR RECEPTOR KINASE1 (OsCERK1) to activate downstream signaling components (Shimizu et al. 2010). In turn, downstream cytoplasmic kinases such as RECEPTOR-LIKE CYTOPLASMIC PROTEIN KINASE118 (OsRLCK118), OsRLCK176 and OsRLCK185 regulate chitin-induced Ca^{2+} influx, activation of MITOGEN-ACTIVATED PROTEIN KINASE (MAPK), and bursts of reactive oxygen species (ROS) (Wang et al. 2017a; Fan et al. 2018; Wang et al. 2019). However, it is unknown whether OsCEBiP/OsCERK1-mediated chitin-triggered immunity functions in rice flowers against *U. virens*.

Pathogens promote colonization by utilizing diverse effector proteins, which are promising molecular probes for identifying unknown but important aspects of host immunity and metabolism, because these effectors often target important host components to manipulate immunity and

metabolism (Win et al. 2012). *U. virens* is equipped with an arsenal of effectors, including 256 conventional effectors and 165 atypical effectors to facilitate infection (Zhang et al. 2021b). Dozens of effectors, such as SECRETED CYSTEINE-RICH EFFECTOR1 (SCRE1), UV_1261/SCRE2, and UV_5215, have been functionally investigated (Zhang et al. 2014, 2020; Fan et al. 2019; Fang et al. 2019), but only several effectors have been well characterized regarding their virulence mechanisms. The *U. virens* SCRE6 effector functions as a cysteine-rich phosphatase to dephosphorylate OsMPK6 in rice, resulting in accumulation of OsMPK6 which turns down rice immunity (Zheng et al. 2022). Effector UvSec117 targets HISTONE DEACETYLASE701 (OsHDA701), also a negative regulator of rice immunity, to transport OsHDA701 to the nucleus where OsHDA701 deacetylates histone H3K9 of defense-related genes (Chen et al. 2022a). In contrast, effectors SCRE1, SCRE4, and UvPr1a modulate positive immune regulators XA21-BINDING PROTEIN24 (XB24), AUXIN RESPONSE FACTOR17 (OsARF17), and SUPPRESSOR OF G2 ALLELE OF *skp1* (OsSGT1), respectively, to suppress rice immunity (Chen et al. 2022b; Qiu et al. 2022; Yang et al. 2022). We recently identified an *U. virens*-secreted protein CHITIN-BINDING PROTEIN1 (UvCBP1) that inhibits chitin-triggered immunity in rice (Li et al. 2022). Subsequent analysis predicted with high confidence that UvCBP1 possesses a conserved glycoside hydrolase family 18 (GH18) domain (Supplementary Fig. S1A; Wang and Kawano 2022). But it remains elusive whether UvCBP1 acts as a genuine glycoside hydrolase and whether this enzyme activity is required for the virulence function of UvCBP1.

In this study, we demonstrate that UvCBP1 indeed hydrolyzes chitin. The enzyme activity of UvCBP1 (hereafter renamed UvGH18.1) contributes to its ability to suppress host defense and to promote *U. virens* infection. Moreover, UvGH18.1 targets the chitin receptor OsCEBiP and co-receptor OsCERK1 independent of its chitinase activity, leading to impairment of chitin-induced dimerization of OsCEBiP and OsCERK1. We further found that OsCEBiP and OsCERK1 mediate the immune response to *U. virens* infection in lemma and palea, which form 2 protective bracts enclosing the floret and restrict the entry of *U. virens* into the inner floral organs.

Results

UvGH18.1 is a core virulence factor of *U. virens*

We previously failed to obtain knockout mutants for *UvGH18.1* (Li et al. 2022). Here, we used a CRISPR-Cas9-assisted gene replacement approach (Liang et al. 2018) and obtained multiple knockout mutants (Supplementary Fig. S1, B and C). We also constructed complemented strains by introducing a genomic fragment of *UvGH18.1* into a *uvgh18.1* mutant. The knockout mutant and complemented strains were further verified by immunoblot analysis with a UvGH18.1-specific antibody (Fig. 1A). The *uvgh18.1* mutant showed growth and colony morphology similar to the PJ52 wild-type and complemented strains on a culture medium, except for a slightly larger colony diameter (Fig. 1, B and C). Pathogenicity assays showed that *uvgh18.1* mutants grew poorly on rice and failed to develop any false smut balls on rice panicles, while the complemented strain restored the pathogenicity of the *uvgh18.1* mutant (Fig. 1, D and E; Supplementary Fig. S1D). Consistently, the *uvgh18.1* mutant triggered higher levels of H_2O_2 and defense gene expression in rice spikelets than PJ52 and the complemented strain (Fig. 1, F to H). These data suggest that *UvGH18.1* is indispensable for *U. virens* pathogenicity in rice, but not required for mycelial growth on culture medium.

U. virens infection process generally consists of 2 distinctive stages: the epiphytic growth stage on the surface of lemma and palea and the intercellular infection stage in inner floral parts, including stamen filaments, stigmas, styles, and lodicules (Fan et al. 2020). *U. virens* switches from the epiphytic growth stage to the intercellular infection stage at approximately 5 d postinoculation (dpi) (Fan et al. 2020). In consistence, the growth of *U. virens* increased rapidly from 5 to 7 dpi (Supplementary Fig. S2A), indicating that 5 to 7 dpi is a crucial stage for the successful *U. virens* colonization. Intriguingly, the *UvGH18.1* RNA level showed a sharp increase at 4 dpi and peaked at 6 dpi (Supplementary Fig. S2B), suggesting its virulence role in *U. virens* invasion from lemma/palea to stamen/pistil.

We next analyzed the polymorphism of *UvGH18.1* among over 50 *U. virens* field isolates that originated from different rice fields in China, to Japan and Nepal (Supplementary Data Set 1). We detected no polymorphisms in UvGH18.1 protein sequences from these tested *U. virens* isolates but found 1 InDel in an intron and 1 synonymous substitution in an exon which should not affect the protein (Supplementary Fig. S3). This finding indicates that *UvGH18.1* is highly conserved in the *U. virens* natural populations across wide areas.

UvGH18.1 acts as a chitinase effector to suppress host immunity

As the GH18 family contains chitinases and chitinase-like proteins (Kzhyshkowska et al. 2007), we tested whether UvGH18.1 has a chitinase activity. Chitinase activity was determined by recording fluorescence of 4-muthylumbelliferone released from the nonfluorescent substrate 4-methylumbelliferyl-β-D-N,N″,N″-triacetylchitotriose (4-MUF-$(GlcNAc)_3$, MUC3) as described previously (Thompson et al. 2001; Han et al. 2019). Recombinant MBP-UvGH18.1 protein, but not MBP protein, degraded MUC3 actively to release fluorescent 4-muthylumbelliferone, indicating a chitinase activity (Supplementary Fig. S4A). We next conducted a high-performance liquid chromatography (HPLC) analysis to assess whether UvGH18.1 could break down chitin oligomers into smaller molecules. We observed that MBP-UvGH18.1 but not MBP hydrolyzed chitin oligomer $(GlcNAc)_6$ into chitooligomers containing ≤4 monomer residues (Fig. 2A;

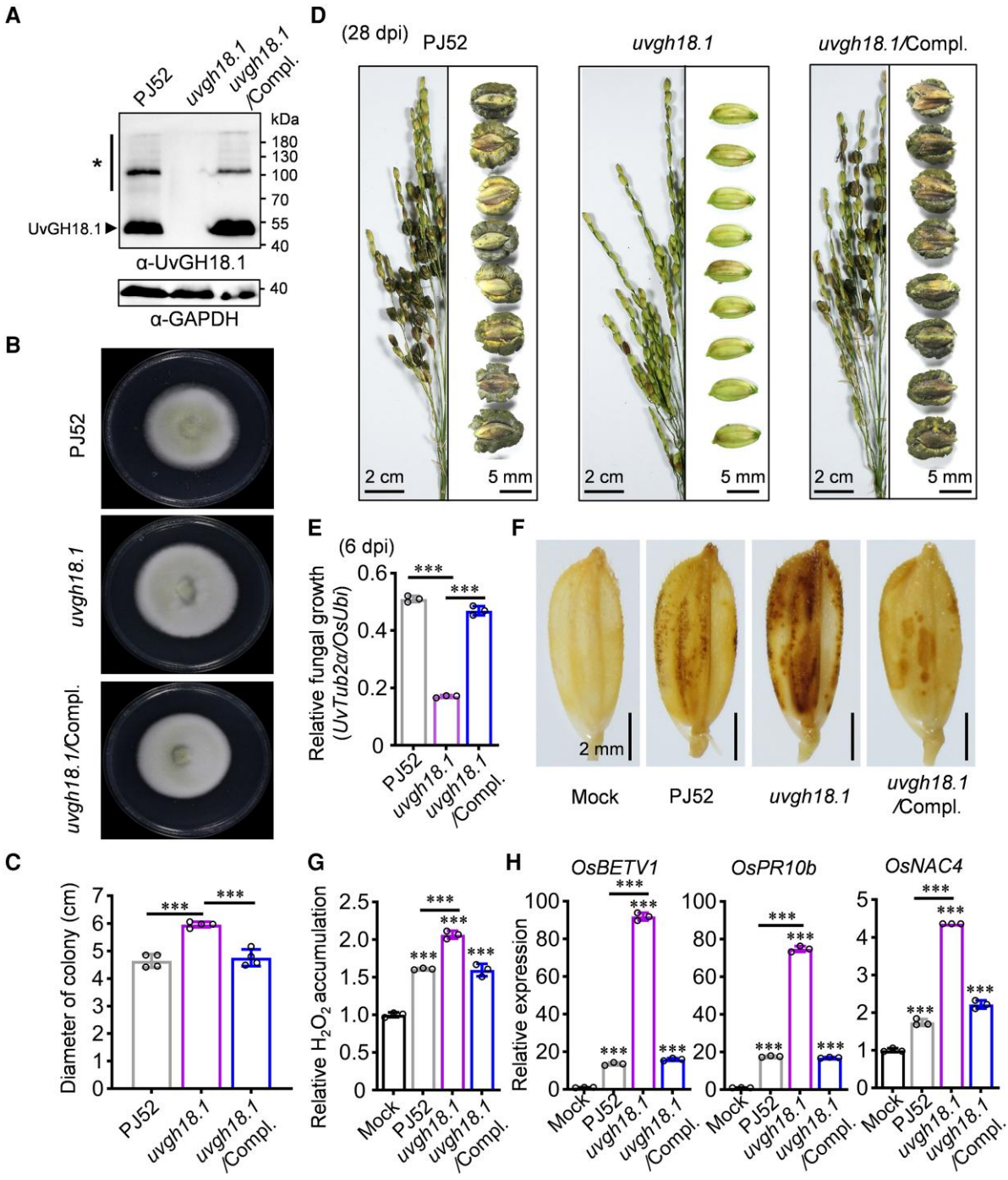

Figure 1. Loss-of-function of *UvGH18.1* disrupts false smut ball formation in rice flowers. **A)** Immunoblot validation of *UvGH18.1* knockout and complemented strains using UvGH18.1 antibody. Anti-GAPDH (Sangon Biotech, D110016) detection was used for loading control. *The higher molecular weight bands may represent modifications or protein complex of UvGH18.1. **B)** Top view of *UvGH18.1* knockout and complemented strains and wild-type PJ52 cultured in PSA media for 2 wk. **C)** Colony diameter of indicated strains cultured in PSA for 2 wk. Values are mean ± SD ($n = 4$ plates). **D)** Pathogenicity assay of *UvGH18.1* knockout, complemented strain, and PJ52. Inocula of indicated *U. virens* strains were injected into the panicles ($n \geq 30$ for each strain) of rice accession Q455 at the late booting stage. Disease phenotype was recorded at 28 dpi. **E)** Relative *U. virens* growth. Spikelet samples were collected at 6 dpi for qPCR. Relative *UvTub2α* levels were determined using *OsUbi* as the reference gene. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **F and G)** DAB staining **F)** and quantification of H_2O_2 **G)** in rice spikelets infected with indicated *U. virens* strains at 3 dpi. In **G)**, values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **H)** RT-qPCR analysis of defense-related genes in rice spikelets infected with indicated *U. virens* strains at 3 dpi. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). All *P* values were determined with 2-sided unpaired Student's *t*-test, compared to *uvgh18.1* **C, E)** or Mock **G, H)** unless otherwise indicated. ***$P < 0.001$.

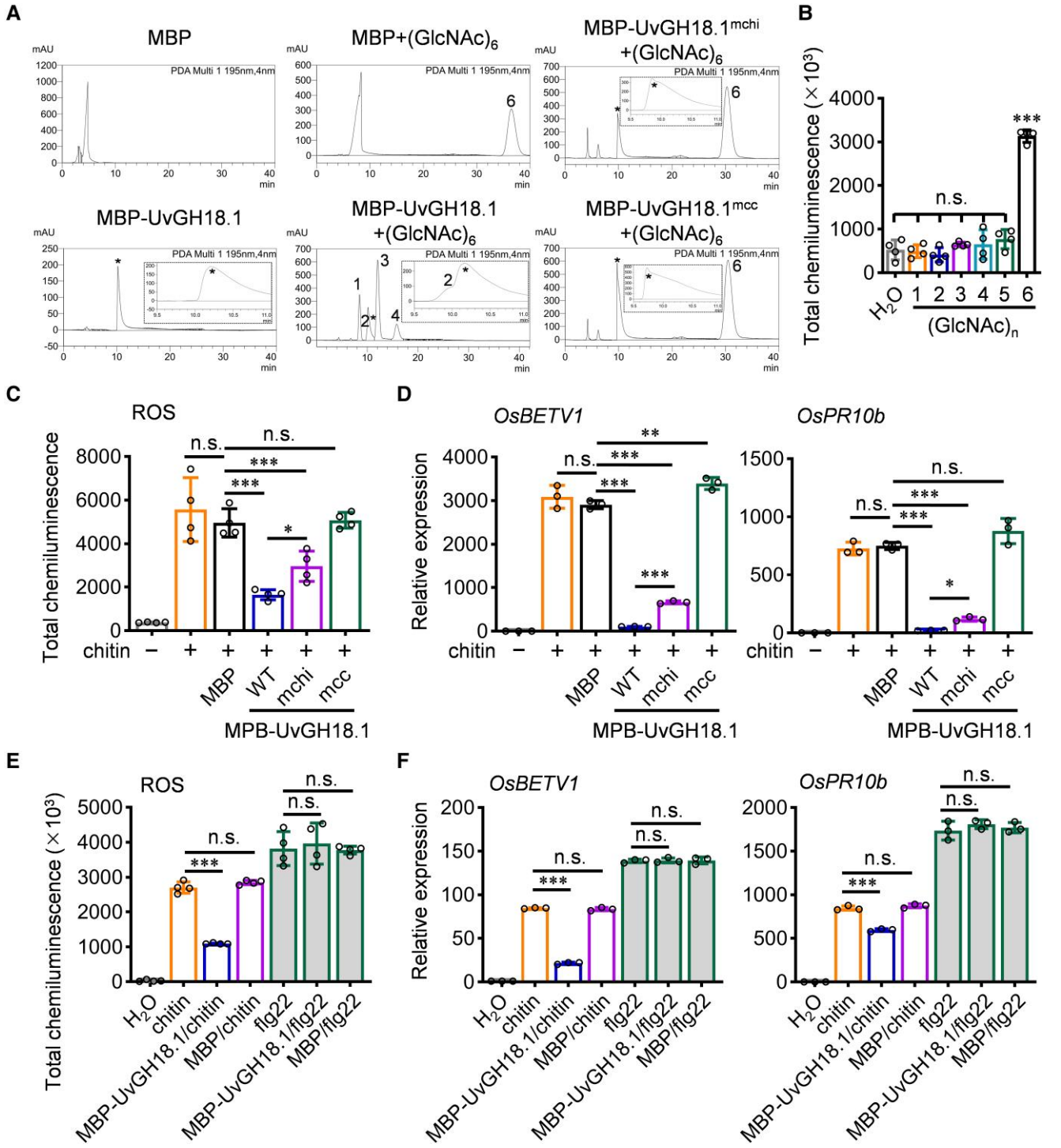

Figure 2. UvGH18.1 binds to and degrades chitin oligomers to prevent host chitin perception. **A)** HPLC analysis of the products derived from chitin oligomer $(GlcNAc)_6$ incubated with MBP-tagged UvGH18.1 and its mutant variants. MBP protein was used as a negative control. The peaks around 10 min are enlarged as inset graphs. The asterisks may indicate peaks of MBP-UvGH18.1 or its mutant variants. The numbers on peaks indicate the quantity of chitooligomer molecules. mchi, mutations (E185Q M251L) at the catalytic motifs of UvGH18.1. mcc, mutations (G179A Y186A L253F G259A) disrupting chitin-binding ability and chitinase activity of UvGH18.1. **B)** ROS production elicited by $(GlcNAc)_{1\text{-}6}$ in rice leaves. Values are mean ± SD ($n = 4$ leaf discs). **C)** ROS assay. The indicated recombinant proteins were incubated with chitin at 37 °C for 1 h, prior to elicitation of ROS bursts. Values are mean ± SD ($n = 4$ leaf discs). **D)** Expression analysis of defense-related genes *OsBETV1* and *OsPR10b* in rice spikelets treated with chitin incubated with the indicated recombinant proteins or buffer. H_2O-treated spikelets were used as mock controls. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **E and F)** ROS assay **E)** and expression analysis of defense-related genes **F)** in rice leaves treated with chitin/flg22 that were preincubated with the indicated recombinant proteins. Values are mean ± SD ($n = 4$ for **E**, 3 for **F**). All *P* values were determined with 2-sided unpaired Student's *t*-test, compared to H_2O treatment. $^*P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. n.s., not significant.

Supplementary Fig. S4B). In contrast to $(GlcNAc)_6$ which carries effective immune-eliciting activity, chitooligomers with ≤5 residues failed to triggered ROS bursts in rice leaves and induced much lower expression of defense-related genes in rice spikelets (Fig. 2B; Supplementary Fig. S4C). These results indicate that UvGH18.1 functions as a fungal chitinase that hydrolyzes immunogenic chitin oligomers into smaller molecules with minimal immunogenic activity.

Secreted fungal chitinase and chitinase-like effectors suppress chitin-triggered plant immunity (Fiorin et al. 2018; Martinez-Cruz et al. 2021). We first confirmed that UvGH18.1 was secreted from *U. virens* (Supplementary Fig. S4D), and then examined whether and how UvGH18.1 suppressed chitin-triggered immunity. We constructed 2 UvGH18.1 variants: UvGH18.1^{mchi}, carrying mutations at 2 conserved residues (E185Q M251L) in the catalytic pocket, that loses chitinase activity but retains chitin-binding ability; and UvGH18.1^{mcc}, carrying mutations at 4 conserved residues (G179A Y186A L253F G259A), that loses both chitinase activity and chitin-binding ability (Fig. 2A; Supplementary Fig. S4, A and E). We mixed chitin with/without purified MBP-UvGH18.1, MBP-UvGH18.1^{mchi}, MBP-UvGH18.1^{mcc}, or MBP control, and incubated them at 37 °C for 1 h. The resultant mixture was used to induce ROS production in *Nicotiana benthamiana* leaves and expression of defense-related genes in rice spikelets. The results showed that chitin pretreated with MBP-UvGH18.1 induced remarkedly less ROS than that pretreated with MBP or buffer. MBP-UvGH18.1^{mchi} was significantly less effective than MBP-UvGH18.1 in inhibiting chitin-induced ROS production, but still displayed significant inhibitory activity, presumably due to the remaining chitin-binding ability of MBP-UvGH18.1^{mchi} (Fig. 2C; Supplementary Fig. S4E). In contrast, MBP-UvGH18.1^{mcc} that lost chitinase activity and chitin-binding ability failed to prevent chitin from eliciting ROS (Fig. 2C). Expression of defense-related genes in rice spikelets showed similar trends: chitin-induced expression of *BETULA VERRUCOSE POLLEN ALLERGEN1* (*OsBETV1*) and *PATHOGENESIS-RELATED10b* (*OsPR10b*) was nearly completely blocked by MBP-UvGH18.1 and largely compromised by MBP-UvGH18.1^{mchi}, but not by MBP-UvGH18.1^{mcc} or MBP (Fig. 2D). These data indicate that chitinase enzyme activity and chitin-binding ability are both required for UvGH18.1 to suppress chitin-triggered plant immunity. By contrast, UvGH18.1 did not reduce flg22-triggered ROS production and defense-related gene expression in rice (Fig. 2, E and F), suggesting that the UvGH18.1 effector specifically inhibits chitin-triggered immunity.

UvGH18.1 interacts with OsCEBiP and OsCERK1

Two previous studies reported that *Magnaporthe oryzae* effector CHITINASE1 (MoChia1), a GH18 domain-containing protein, interacts with rice plasma membrane proteins MANNOSE-BINDING LECTIN1 (OsMBL1) and TETRATRICOPEPTIDE-REPEAT PROTEIN1 (OsTPR1) to modulate host immunity (Han et al. 2019; Yang et al. 2019). OsMBL1 is a jacalin-related mannose-binding lectin protein contributing to chitin perception and chitin-triggered rice immunity, which is suppressed by MoChia1 (Han et al. 2019). OsTPR1 is a tetratricopeptide-repeat family protein and functions as an immune receptor recognizing MoChia1 to regain chitin-triggered immunity in rice (Yang et al. 2019). We thus assessed whether UvGH18.1 might associate with OsMBL1 or OsTPR1. GST pull-down assays showed that, unlike MoChia1, UvGH18.1 did not interact with OsMBL1 or OsTPR1, although *OsMBL1* and *OsTPR1* were highly expressed in rice flowers (Supplementary Fig. S5, A and B).

As cell surface receptors OsCEBiP and OsCERK1 play important roles in antifungal immunity in rice (Gong et al. 2020), we asked whether UvGH18.1 could target OsCEBiP and OsCERK1 to modulate rice immunity. We first confirmed *OsCEBiP* and *OsCERK1* expression in rice floral organs (Fig. 3A) and then assessed their interactions with UvGH18.1. GST pull-down assays showed that MBP-OsCEBiP was pulled down by GST-UvGH18.1, and MBP-UvGH18.1 was pulled down by GST-OsCERK1, indicating direct interactions of UvGH18.1 with OsCEBiP and OsCERK1 (Fig. 3B). We next validated their interactions with a semi-in vivo co-immunoprecipitation (co-IP) assay. *OsCEBiP-3×FLAG* and *OsCERK1-3×FLAG* driven by their native promoters were separately expressed in rice protoplasts. Purified MBP-UvGH18.1 or MBP was incubated with the transfected protoplasts for 2 h prior to protein extraction and immunoprecipitation with anti-FLAG affinity beads. OsCEBiP-3×FLAG and OsCERK1-3×FLAG co-immunoprecipitated MBP-UvGH18.1, but not MBP (Fig. 3C). These data indicate that UvGH18.1 is physically associated with OsCEBiP and OsCERK1. Additional data showed that UvGH18.1 was not associated with OsLYP4 and OsLYP6 (Supplementary Fig. S5, C and D), 2 minor rice chitin receptors (Liu et al. 2012).

Mutations K137A and D178A in UvGH18.1 abolish its interactions with OsCERK1 and OsCEBiP, respectively

We defined the regions of OsCEBiP and OsCERK1 interacting with UvGH18.1 by generating a set of deletions and examining their interactions with UvGH18.1 in yeast two-hybrid (Y2H) assay. OsCEBiP-V1 (including the ectodomain of OsCEBiP lacking the signal peptide, the GPI-containing domain, and the putative transmembrane domain) failed to interact with UvGH18.1; however, deletion of the LysM1 domain on the basis of OsCEBiP-V1 restored the ability to interact with UvGH18.1 (Supplementary Fig. S6A). Because OsCEBiP-V4 (the ectodomain with deletion of LysM2) and OsCEBiP-V5 (LysM1 of OsCEBiP) caused auto-activation in our Y2H assays (Supplementary Fig. S6A), we could not further define the minimal interacting regions. For OsCERK1, its LysM1 domain, but not the kinase domain, interacted with UvGH18.1 (Supplementary Fig. S6A). We then conducted luciferase complementation imaging (LCI) experiments in *N. benthamiana*, which revealed the association of UvGH18.1 with the ectodomains of OsCEBiP and OsCERK1 (Supplementary Fig. S6B).

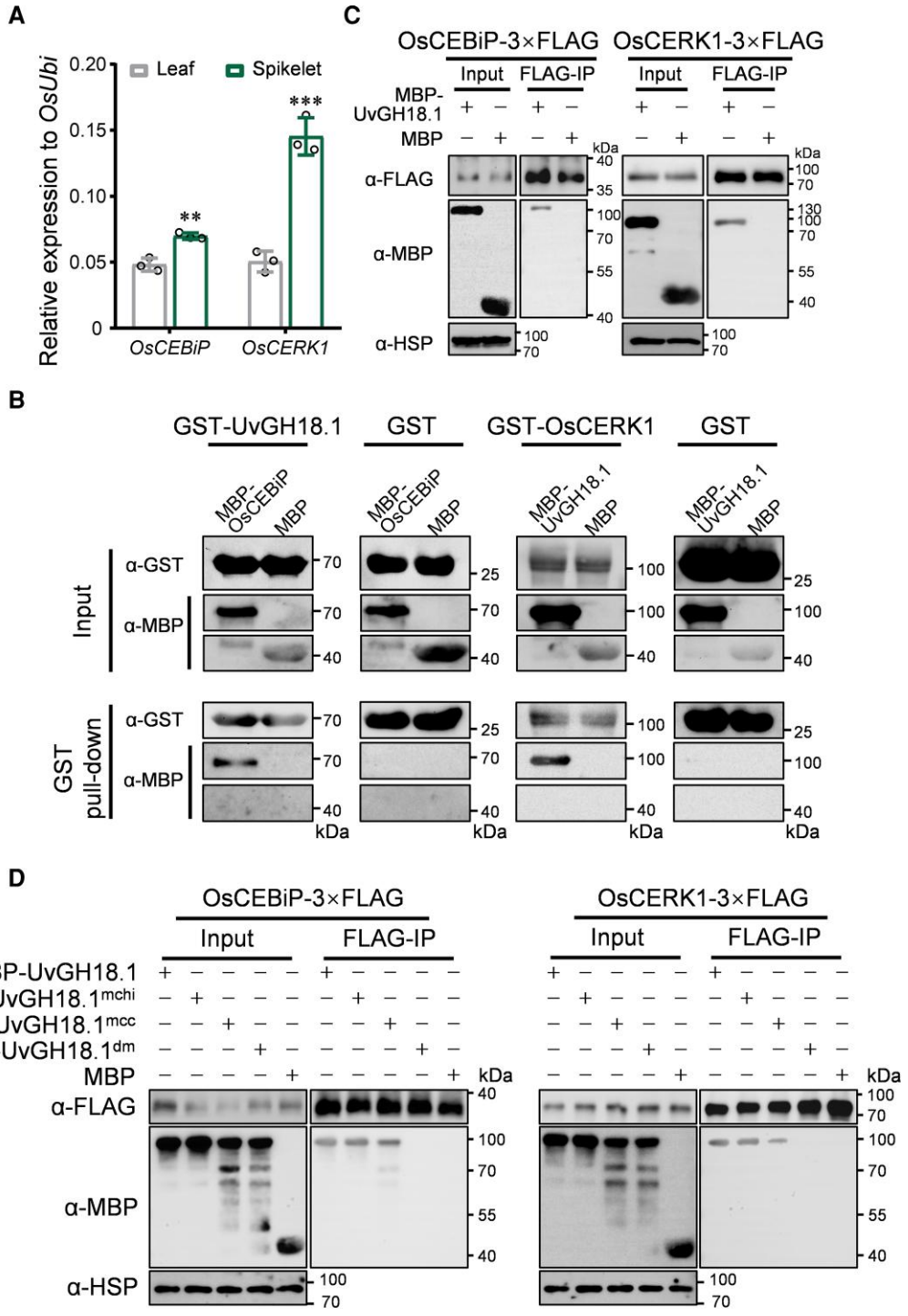

Figure 3. UvGH18.1 interacts with OsCEBiP and OsCERK1. **A)** RT-qPCR analysis of *OsCEBiP* and *OsCERK1*. Relative expression levels were calculated relative to that of *OsUbi*. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). *P* values were determined with 2-sided unpaired Student's *t*-test, compared to the leaf sample. $^{**}P < 0.01$. $^{***}P < 0.001$. **B)** In vitro GST pull-down assay. The recombinant proteins GST-UvGH18.1, GST-OsCERK1, MBP-UvGH18.1, and MBP-OsCEBiP were purified from *E. coli*. GST and MBP tag proteins were used as negative controls. **C)** Semi-in vivo co-immunoprecipitation (co-IP) assay. OsCEBiP-3×FLAG and OsCERK1-3×FLAG were expressed in rice protoplasts. Purified MBP-UvGH18.1 protein was incubated with the transfected protoplasts for 2 h. IP was conducted using anti-FLAG affinity gel and subjected to immunoblot analysis using FLAG and MBP antibodies. Anti-HSP detection was used for input control. **D)** Semi-in vivo co-IP assay for testing the interactions between OsCEBiP-3×FLAG or OsCERK1-3×FLAG and MBP-UvGH18.1 variants in rice protoplasts. IP was conducted using anti-FLAG affinity gel and subjected to immunoblot analysis using FLAG and MBP antibodies. Anti-HSP detection was used for input control. mchi, mutations of E185Q M251L causing loss of chitinase activity. mcc, mutations of G179A Y186A L253F G259A causing loss of chitin-binding ability and chitinase activity. dm, mutations of K137A D178A causing loss of interactions with OsCEBiP and OsCERK1.

To identify the key residues of UvGH18.1 responsible for interacting with OsCEBiP and OsCERK1, we generated a series of truncations and mutations for UvGH18.1. In LCI assay, we observed that the UvGH18.1$^{17-150}$ peptide interacted with the OsCERK1 ectodomain, while UvGH18.1$^{151-300}$ interacted with OsCEBiP ectodomain (Supplementary Fig. S7, A C, F, and H). To further define the interacting region of UvGH18.1, we made 48 NAAIRS constructs to replace amino acids across 17 to 304 of UvGH18.1, each replacing 6 amino acids with NAAIRS (Asn-Ala-Ala-Ile-Arg-Ser) (Supplementary Fig. S7, B and G). Replacement with NAAIRS is considered to have minimal effects on gross disruptions in protein secondary structures (Wilson et al. 1985; Wang et al. 2013). We found that replacement of aa 137 to 142 (m21 in Supplementary Fig. S7D) abolished interaction with OsCERK1, while replacement of aa 173 to 178 (m27 in Supplementary Fig. S7I) abolished interaction with OsCEBiP. We then conducted point mutation analysis on these 2 regions and found that mutations K137A and D178A in UvGH18.1 abolished its interactions with OsCERK1 and OsCEBiP, respectively (Supplementary Fig. S7, E and J). Simultaneous K137A and D178A mutations of UvGH18.1 (designated as UvGH18.1^{dm}) abolished interactions with both OsCERK1 and OsCEBiP in LCI and co-IP assays (Fig. 3D, Supplementary Fig. S8, A to D). UvGH18.1^{dm} also lost abilities to bind to and degrade chitin oligomers (Supplementary Fig. S8, E to G). Collectively, K137 and D178 are the key residues for UvGH18.1 to interact with OsCERK1 and OsCEBiP, respectively.

UvGH18.1 perturbs chitin-induced dimerization of OsCEBiP and OsCERK1 to attenuate host immunity

Dimerization of OsCEBiP and OsCERK1 induced by chitin binding is a prerequisite for intracellular immune signaling in Arabidopsis (*Arabidopsis thaliana*) and rice (Gong et al. 2020). We thus tested whether UvGH18.1 might affect the dimerization of OsCEBiP and OsCERK1 by conducting co-IP assays in rice protoplasts. We found that 10 min of chitin treatment induced interactions between OsCEBiP-3×FLAG and OsCEBiP-4×HA, OsCEBiP-4×HA and OsCERK1-3×FLAG, and OsCERK1-3×FLAG and OsCERK1-4×HA. When MBP-UvGH18.1 was incubated with the transfected protoplasts for 2 h prior to co-IP, the homo- and hetero-associations of OsCEBiP and OsCERK1 induced by chitin were almost completely abolished (Fig. 4A). On the contrary, MBP-UvGH18.1^{dm} that could no longer associate with OsCEBiP/OsCERK1 failed to disrupt the associations between OsCEBiP and OsCERK1 (Fig. 4, A and B). To exclude the possibility of chitin binding and degradation by MBP-UvGH18.1, we included MBP-UvGH18.1^{mchi} and MBP-UvGH18.1^{mcc} as controls. MBP-UvGH18.1^{mchi} and MBP-UvGH18.1^{mcc} were precipitated by both OsCEBiP and OsCERK1 and retained most of the ability to disrupt the OsCEBiP/OsCERK1 interactions (Figs. 3D and 4A). Blue native polyacrylamide gel electrophoresis (BN-PAGE) showed that OsCERK1-3×FLAG and OsCEBiP-4×HA formed dimers upon chitin treatment in the absence of MBP-UvGH18.1. MBP-UvGH18.1, but not mutant MBP-UvGH18.1^{dm}, clearly disrupted the dimerization of OsCERK1-3×FLAG and OsCEBiP-4×HA, significantly reducing the bands slightly larger than 132 kDa (Fig. 4B). The resulting smear bands above ~300 kDa appeared similar to the migration pattern of MBP-UvGH18.1, suggesting that OsCERK1-3×FLAG and OsCEBiP-4×HA were integrated into the complex of MBP-UvGH18.1 which oligomerized by itself (Fig. 4B). Altogether, these data indicate that UvGH18.1 disrupts chitin-induced dimerization of OsCEBiP and OsCERK1.

To determine whether the interference of dimerization of OsCEBiP and OsCERK1 by UvGH18.1 compromises immunity, we assessed the effects of UvGH18.1 on modulating rice defense responses. We added recombinant proteins (MBP-UvGH18.1, MBP-UvGH18.1^{dm}, MBP-UvGH18.1^{mchi}, MBP-UvGH18.1^{mcc}, and MBP) to rice leaf discs, incubated overnight to allow sufficient time for the recombinant proteins to interact with OsCEBiP and OsCERK1, and then treated leaf discs with chitin. The results showed that chitin-induced ROS production was inhibited by MBP-UvGH18.1, MBP-UvGH18.1^{mchi}, and MBP-UvGH18.1^{mcc}, but not by MBP-UvGH18.1^{dm} (no longer interacts with OsCEBiP/OsCERK1) and MBP (Fig. 4C). Similarly, chitin-induced expression of defense-related genes *OsBETV1* and *OsPR10b* was clearly suppressed by MBP-UvGH18.1, MBP-UvGH18.1^{mchi}, and MBP-UvGH18.1^{mcc}, but not by MBP-UvGH18.1^{dm} (Fig. 4D). Collectively, these data indicate that UvGH18.1 impairs dimerization of chitin receptor OsCEBiP and co-receptor OsCERK1, resulting in attenuation of chitin-elicited immune signaling in rice.

OsCEBiP and OsCERK1 are required for floral resistance to *U. virens*

We assessed the role of OsCEBiP/OsCERK1 in rice resistance to *U. virens* by generating *oscebip oscerk1* double mutants in a wild-type rice Q455 using the CRISPR-Cas9 technology (Supplementary Fig. S9). We inoculated their panicles with *U. virens* PJ52 and the *uvgh18.1* mutant at late booting stages of rice. The results showed that PJ52 developed more false smut balls on *oscebip oscerk1* panicles than on Q455 panicles (Fig. 5, A and B). Interestingly, the *uvgh18.1* mutant failed to form false smut balls on both Q455 and *oscebip oscerk1* plants (Fig. 5A). We further checked the infection process of the *uvgh18.1* mutant in rice panicles and found that *uvgh18.1* did infect spikelets of Q455 and *oscebip oscerk1* plants because mycelia have embraced the inner floral organs including stamens and pistils, even though no false smut balls were formed (Fig. 5A; Supplementary Fig. S10). This stage of mycelial growth has been reported to be a prerequisite for the symptom development of the false smut disease (Fan et al. 2020). Thus, we quantified the number of infected spikelets of *uvgh18.1*-inoculated panicles and found that the *uvgh18.1* infection rate was more than 3-fold higher in *oscebip oscerk1* than in Q455 (Fig. 5B).

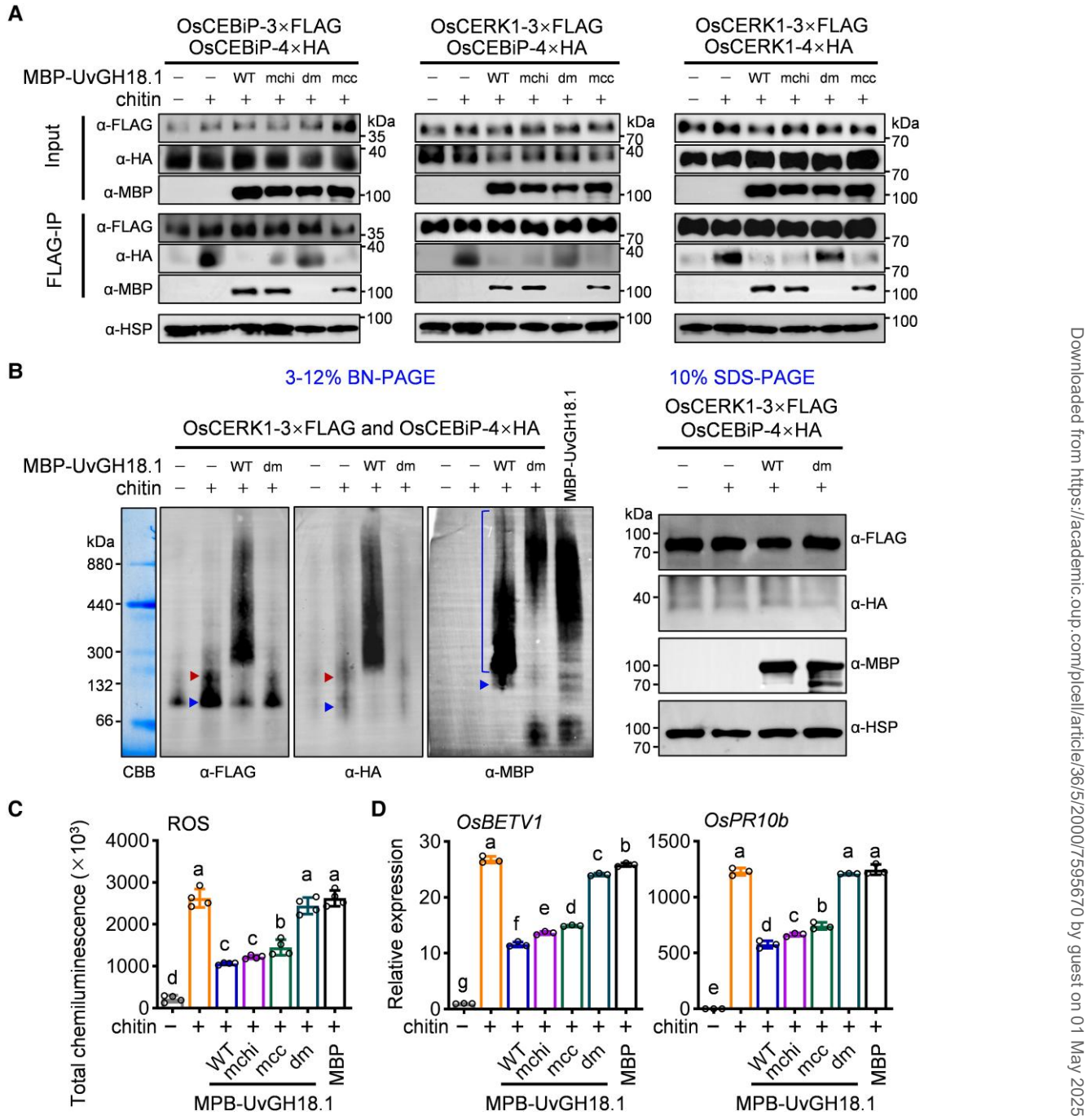

Figure 4. UvGH18.1 disrupts dimerization of OsCEBiP and OsCERK1 to compromise chitin-elicited immune signaling. **A)** Competitive co-IP assay. OsCEBiP and OsCERK1 tagged with FLAG or HA were co-expressed in rice protoplasts. Purified MBP-tagged UvGH18.1 and its mutant variants were incubated with the transfected protoplasts for 2 h, followed by chitin (10 μg ml^{-1}) treatment for 10 min. IP was conducted with anti-FLAG affinity gel and immunoblot analysis was performed with FLAG, HA, and MBP antibodies. mchi, mutations of E185Q M251L. mcc, mutations of G179A Y186A L253F G259A. dm, mutations of K137A D178A. **B)** BN-PAGE analysis. OsCEBiP-4×HA and OsCERK1-3×FLAG were co-expressed in rice protoplasts. Transfected protoplasts were incubated with MBP-UvGH18.1 or MBP-UvGH18.1^{dm} for 2 h, and then treated with chitin for 10 min to induce OsCEBiP and OsCERK1 dimerization. Total proteins were run in parallel on 3% to 12% blue native PAGE and 10% SDS-PAGE gels and subjected to immunoblot analysis using FLAG, HA, and MBP antibodies. Anti-HSP detection was used for input control. Upper triangles represent dimers, while lower triangles indicate monomers. The line indicates oligomers. CBB, Coomassie Brilliant Blue. **C** and **D)** ROS assay **C)** and expression analysis of defense-related genes **D)** in rice leaves. Prior to chitin elicitation, leaf discs were incubated with MBP-UvGH18.1 (WT) and its mutant variants overnight, allowing sufficient time for the recombinant proteins to interact with OsCEBiP and OsCERK1. Values are mean ± SD ($n = 4$ for **C**, 3 for **D**). Different letters indicate significant differences determined by 1-way ANOVA and Duncan's post hoc multiple comparison tests ($\alpha = 0.05$).

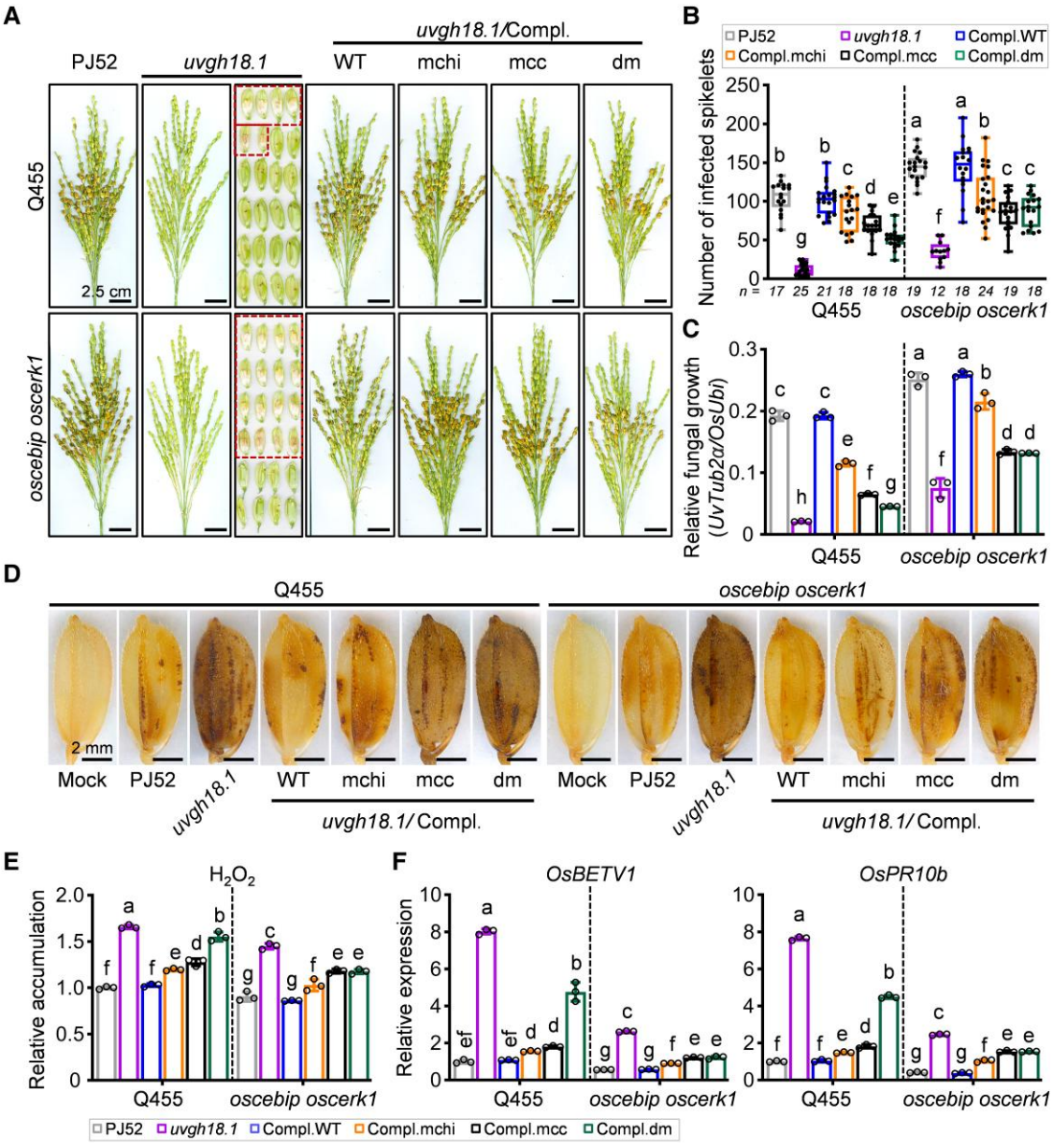

Figure 5. UvGH18.1 subverts chitin perception and OsCEBiP/OsCERK1-mediated immunity in rice flowers to promote *U. virens* infection. **A)** Disease phenotype of wild-type Q455 and *oscebip oscerk1* double mutant infected with *U. virens* PJ52, *uvgh18.1* knockout, and different complementation strains. False smut balls were formed on panicles infected with PJ52 and the complementation strains. Representative images of *uvgh18.1*-infected spikelets are indicated by red dotted rectangles. **B)** The number of diseased spikelets per panicle was box-plotted (sample size is indicated in the figure). Center line indicates the median value. Error bars indicate maximum and minimum values. Box limits indicate 25th and 75th percentiles. **C)** Relative *U. virens* growth at 6 dpi. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **D and E)** DAB staining and H_2O_2 quantification of rice spikelets collected at 3 dpi. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **F)** RT-qPCR of defense-related genes in rice spikelets of indicated rice lines infected with indicated *U. virens* strains. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). Different letters (in **B**, **C**, **E**, **F**) indicate significant differences determined by 1-way ANOVA and Duncan's post hoc multiple comparison tests ($\alpha = 0.05$).

PJ52 and *uvgh18.1* also grew more in *oscebip oscerk1* than in Q455 (Fig. 5C). In consistence, H_2O_2 accumulation and defense-related gene expression were significantly lower in *oscebip oscerk1* than in Q455 upon *U. virens* infection (Fig. 5, D to F). These data indicate that OsCEBiP and OsCERK1 are required for rice resistance to *U. virens*.

Targeting chitin and chitin receptors are both essential for UvGH18. 1 to promote *U. virens* infection

As the above data show that UvGH18.1 hydrolyzes chitin and targets host chitin receptors, we then tested how much UvGH18.1 contributes to *U. virens* pathogenicity by targeting chitin and OsCEBiP/OsCERK1. We complemented the

uvgh18.1 mutant with *UvGH18.1*, *UvGH18.1*^{dm}, *UvGH18.1*^{mchi}, and *UvGH18.1*^{mcc} separately (Supplementary Fig. S11) and inoculated them into the panicles of Q455 and *oscebip oscerk1* double mutant plants. In wild-type rice Q455, the *UvGH18.1*^{mchi}-, *UvGH18.1*^{mcc}-, and *UvGH18.1*^{dm}-complemented strains caused less diseased spikelets than the *UvGH18.1*-complemented strain and PJ52. The numbers of diseased spikelets per panicle were reduced by 18.1%, 32.5%, and 50.0% for the *UvGH18.1*^{mchi}-, *UvGH18.1*^{mcc}-, and *UvGH18.1*^{dm}-complemented strain, respectively, compared to the *UvGH18.1*-complemented strain (Fig. 5, A and B). In the *oscebip oscerk1* mutant rice, the numbers of diseased spikelets were decreased by 25.2% and 41.0% for the *UvGH18.1*^{mchi}- and *UvGH18.1*^{mcc}-complemented strains, respectively, compared to the *UvGH18.1*-complemented strain (Fig. 5, A and B). However, the diseased spikelet number for the *UvGH18.1*^{dm}-complemented strain in *oscebip oscerk1* was not significantly different from that of the *UvGH18.1*^{mcc}-complemented strain (Fig. 5B). Measurement of *U. virens* growth confirmed these results (Fig. 5C).

We further assessed the defense responses of rice spikelets infected with different complemented strains. The *UvGH18.1*-complemented strain elicited a comparable level of H_2O_2 in spikelets compared to PJ52, while the *UvGH18.1*^{mchi}- and *UvGH18.1*^{mcc}-complemented strain triggered more H_2O_2 accumulation in spikelets of both Q455 and *oscebip oscerk1*. In comparison with the *UvGH18.1*^{mcc}-complemented strain, the *UvGH18.1*^{dm}-complemented strain triggered more H_2O_2 accumulation in Q455 but not in *oscebip oscerk1* (Fig. 5, D and E). Similar trends were observed for expression of defense-related genes *OsBETV1* and *OsPR10b* (Fig. 5F). These data indicate that compared to wild-type UvGH18.1, mutants UvGH18.1^{mchi}, UvGH18.1^{mcc}, and UvGH18.1^{dm} carry a lower ability in sequentially decreasing order to suppress rice flower immunity. This is consistent with the data that UvGH18.1^{dm} lost both abilities to target chitin and OsCEBiP/OsCERK1, while UvGH18.1^{mcc} lost abilities to bind to and degrade chitin and UvGH18.1^{mchi} only lost the ability to break down chitin (Figs. 2A and 3D; Supplementary Figs. S4 and S8). Furthermore, the comparable levels of virulence displayed by *UvGH18.1*^{dm}- and *UvGH18.1*^{mcc}-complemented strains on *oscebip oscerk1* plants provides evidence that UvGH18.1 effectively inhibits OsCEBiP/OsCERK1-dependent immunity. Collectively, targeting chitin and chitin receptors are both required for UvGH18.1 to suppress rice immunity and promote *U. virens* virulence.

Chitin pretreatment enhances immunity against *U. virens* in flowers, significantly reducing formation of false smut balls

To verify chitin-inducible immunity in rice floral organs, we treated spikelets of *oscebip oscerk1* mutants and wild-type Q455 with pathogen elicitor chitin or flg22. We found that, in Q455 spikelets, chitin induced pattern-triggered immunity (PTI) responses, including phosphorylation of MAPKs, induction of defense-related gene expression, and accumulation of H_2O_2 (Fig. 6, A to C; Supplementary Fig. S12A), in consistence with a recent report (Song et al. 2021). However, in *oscebip oscerk1* mutant flowers, chitin induction of these PTI responses was almost completely blocked. By contrast, flg22 induced PTI responses in *oscebip oscerk1* to a similar level as in wild-type (Fig. 6, A to C; Supplementary Fig. S12A). These data indicate that rice flowers possess chitin-induced immunity mediated by OsCEBiP and OsCERK1.

Inducible floral defense responses drove us to test the efficiency of improving *U. virens* resistance by priming defense with elicitors. We pretreated panicles of wild-type Q455 and *oscebip oscerk1* double mutant plants with chitin solutions or water 24 h prior to *U. virens* inoculation. For wild-type plants, chitin pretreatment significantly reduced the number of false smut balls on panicles compared to water treatment; in contrast, chitin did not reduce the number of false smut balls on *oscebip oscerk1* panicles (Fig. 6, D and E). Consistently, chitin pretreatment reduced fungal growth by approximately 50% in wild-type panicles. Loss-of-function of OsCEBiP/OsCERK1 facilitated *U. virens* growth and could not be rescued by chitin pretreatment (Fig. 6F). This disease phenotype was negatively associated with H_2O_2 accumulation in spikelets (Supplementary Fig. S12, B and C). We also found that flg22 pretreatment improved host resistance to *U. virens* independent of *OsCEBiP*/*OsCERK1* (Supplementary Fig. S13). It remains to be examined whether OsFLS2, the rice homolog of receptor kinase FLAGELLIN SENSITIVE2 that recognizes bacterial flagellins (Wang et al. 2015), is involved in this flg22-triggered immunity against *U. virens*.

Lemma and palea are the main tissues where immune responses occur to protect flowers

As shown in Fig. 5A, the *uvgh18.1* knockout mutant was able to enter the inner space of Q455 spikelets, but the entry rate of *uvgh18.1* was significantly reduced compared to that of wild-type PJ52. This result prompted us to investigate how rice flowers restrict the entry of *U. virens*. Consequently, we examined the accumulation of H_2O_2 and the expression of defense-related genes in various tissues of spikelets after infection with *U. virens*. DAB staining results showed that high amounts of H_2O_2 were detected in the lemma and palea of Q455 spikelets in response to PJ52 and *uvgh18.1* infection, whereas no obvious H_2O_2 accumulation was observed in the stamen or pistil. In *U. virens*-challenged *oscebip oscerk1* spikelets, H_2O_2 accumulation was undetectable to barely detectable in lemma, palea, stamen, and pistil tissues (Fig. 7A). Similar trends were observed for the expression levels of defense-related genes: *OsBETV1* and *OsPR10b* were highly expressed in lemma and palea but not in stamen or pistil upon *U. virens* infection (Fig. 7B). These results indicate that the lemma and palea are the main tissues where OsCEBiP and OsCERK1 exert defense responses for preventing entry of *U. virens* into inner floral organs.

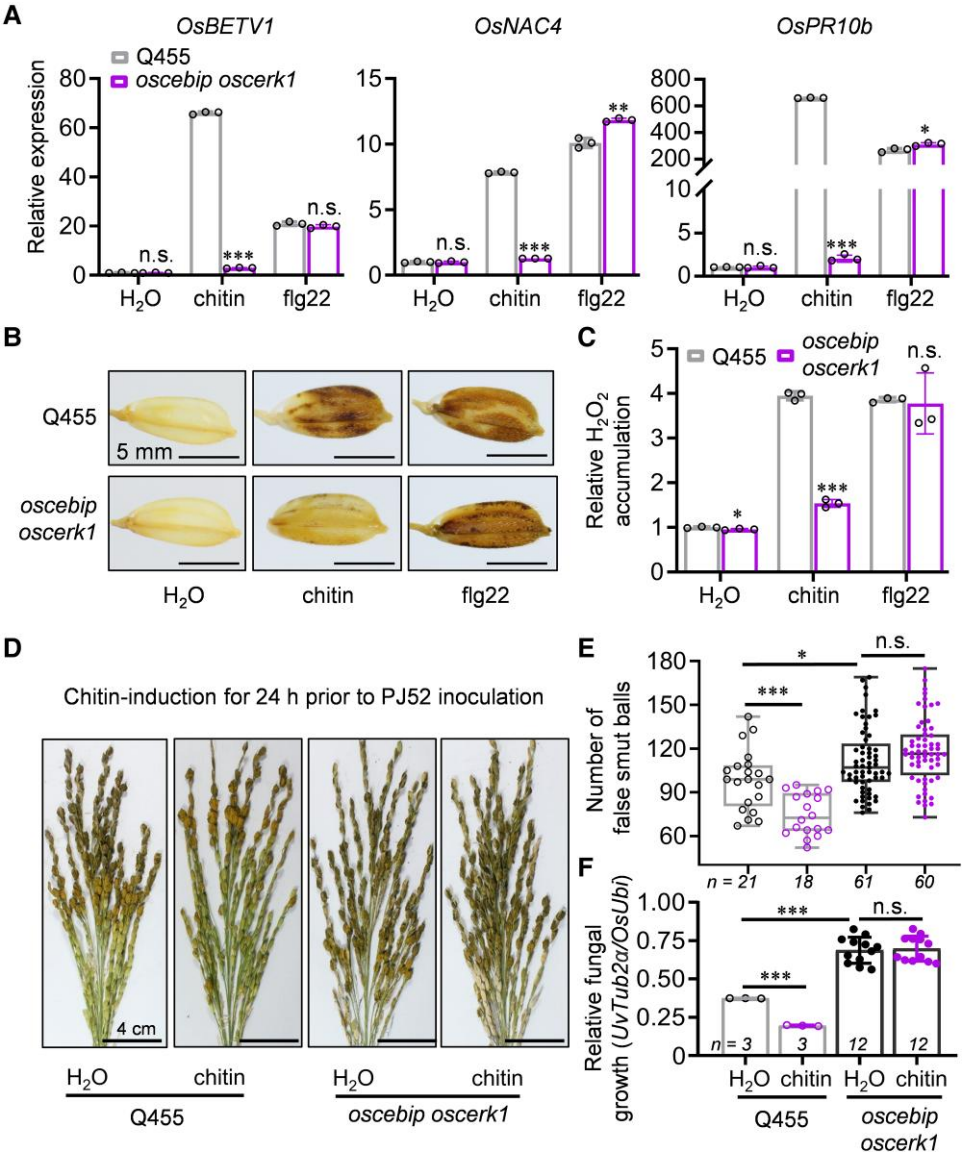

Figure 6. Pattern-triggered immunity functions in rice flowers and chitin pretreatment promotes rice resistance against *U. virens*. **A)** RT-qPCR analysis of defense-related genes in rice spikelets of *oscebip oscerk1* double mutant and wild-type Q455 rice treated with chitin (40 μg ml^{-1}) or flg22 (0.2 μM) for 10 h. Generation of *oscebip oscerk1* double mutant is presented in Supplementary Fig. S9. *OsUbi* was used as the reference gene. Water-treated sample of each rice line was used as the control and set at 1 for calculating relative gene expression. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **B** and **C)** DAB staining and quantification of H_2O_2 in rice spikelets of indicated rice lines treated with chitin or flg22 for 48 h. Water-treated Q455 sample was used as the control and set at 1 for calculating relative amount of H_2O_2. Values are mean ± SD ($n = 3$ replicates from 3 independent panicles). **D)** Disease phenotype of indicated rice lines infected with *U. virens* PJ52. Pathogen inoculation was performed 24 h post-chitin (40 μg ml^{-1}) treatment. Panicles with false smut balls were photographed around 4 wk postinoculation. **E)** The number of false smut balls per diseased panicle was box-plotted (sample size is indicated in the figure). Center line indicates median value. Error bars indicate maximum and minimum values. Box limits indicate 25th and 75th percentiles. **F)** Relative *U. virens* growth at 6 dpi. Values are mean ± SD (sample size is indicated in the figure). All *P* values were determined with 2-sided unpaired Student's *t*-test, compared to Q455 unless otherwise indicated. *$P < 0.05$. **$P < 0.01$. ***$P <$ 0.001. n.s., not significant.

To further determine the contribution of lemma and palea to immunity in flowers, we checked PTI responses in different floral parts of Q455 and *oscebip oscerk1* plants. DAB staining showed that both chitin and flg22 induced H_2O_2 production in lemma and palea of wild-type spikelets. By contrast, chitin failed to induce H_2O_2 accumulation in lemma and palea of *oscebip oscerk1* spikelets. Neither chitin or flg22 induced obvious H_2O_2 accumulation in stamens and pistils of wild-type or *oscebip oscerk1* spikelets (Fig. 7C). Consistently, chitin and flg22 induced *OsBETV1* and *OsPR10b* expression mainly in lemmas and paleas, much less in anthers, and weakly in stamen filaments, lodicules, and pistils. Chitin-induced

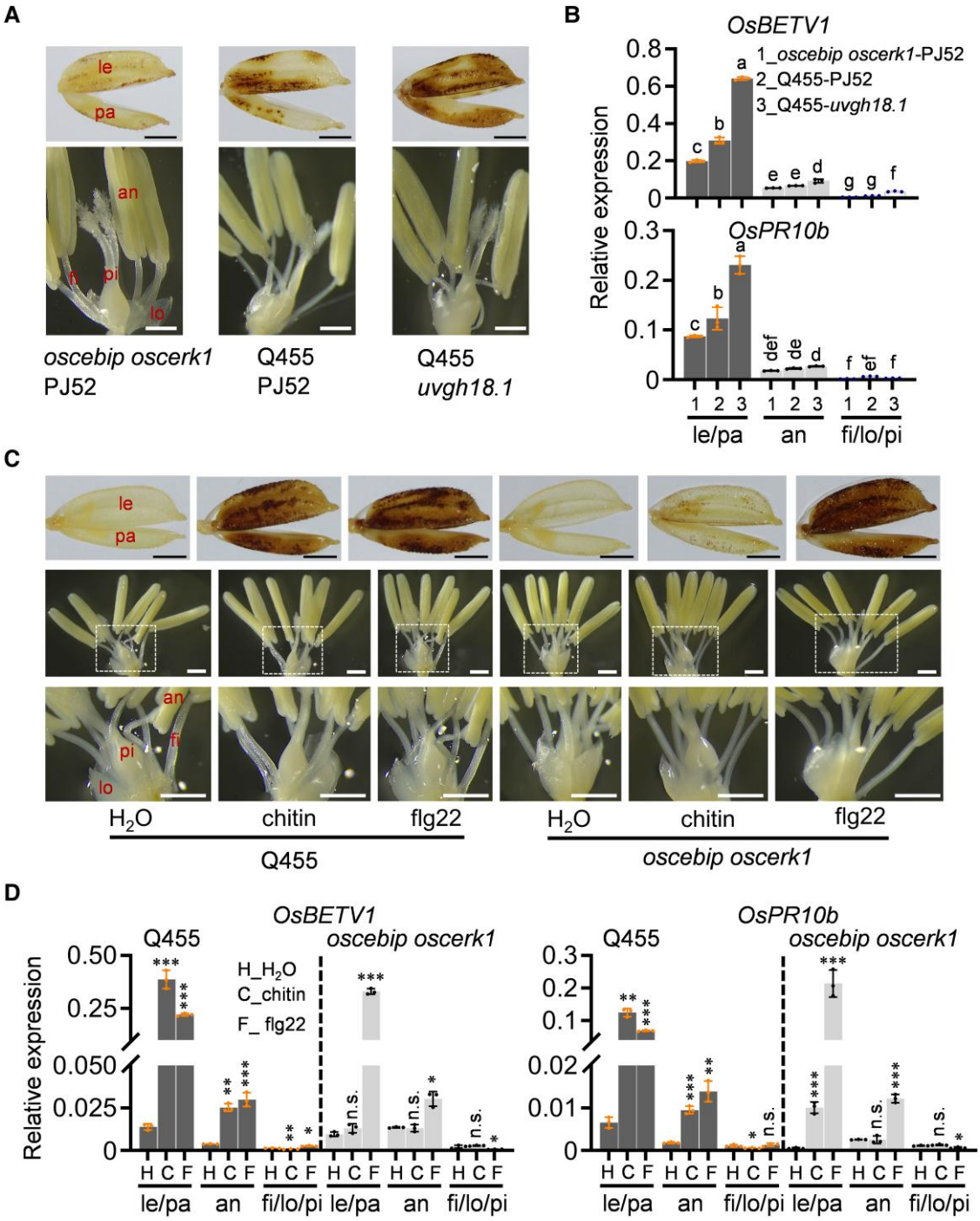

Figure 7. Rice flower immunity is predominantly deployed at lemma and palea. **A)** DAB staining. Rice spikelets challenged by *U. virens* PJ52 or the *uvgh18.1* mutant were collected for DAB staining at 3 dpi. Different floral organs were displayed. le, lemma; pa, palea; an, anther; lo, lodicule; pi, pistil. Scale bar, 2.0 mm (upper panel) or 0.5 mm (bottom panel). **B)** RT-qPCR analysis of defense-related genes in different floral organs from Q455 and *oscebip oscerk1* inoculated with PJ52 or *uvgh18.1* at 3 dpi. Expression levels were normalized to *OsUbi*. Values are mean ± SD ($n = 3$ replicates from pooled spikelet tissues). Different letters indicate significant differences determined by 1-way ANOVA and Duncan's post hoc multiple comparison tests ($\alpha = 0.05$). **C)** DAB staining of different floral organs from indicated rice lines treated with chitin (40 μg ml^{-1}) or flg22 (0.2 μM) for 48 h. White rectangles in the middle panel are enlarged in the bottom panel. Scale bar, 2.0 mm (upper panel) or 0.5 mm (middle and bottom panels). **D)** RT-qPCR analysis of defense-related genes in different floral organs from indicated rice lines treated with chitin or flg22 for 10 h. Expression levels were normalized to *OsUbi*. Values are mean ± SD ($n = 3$ replicates from pooled spikelet tissues). *P* values were determined with 2-sided unpaired Student's *t*-test, compared to H_2O. $^*P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. n.s., not significant.

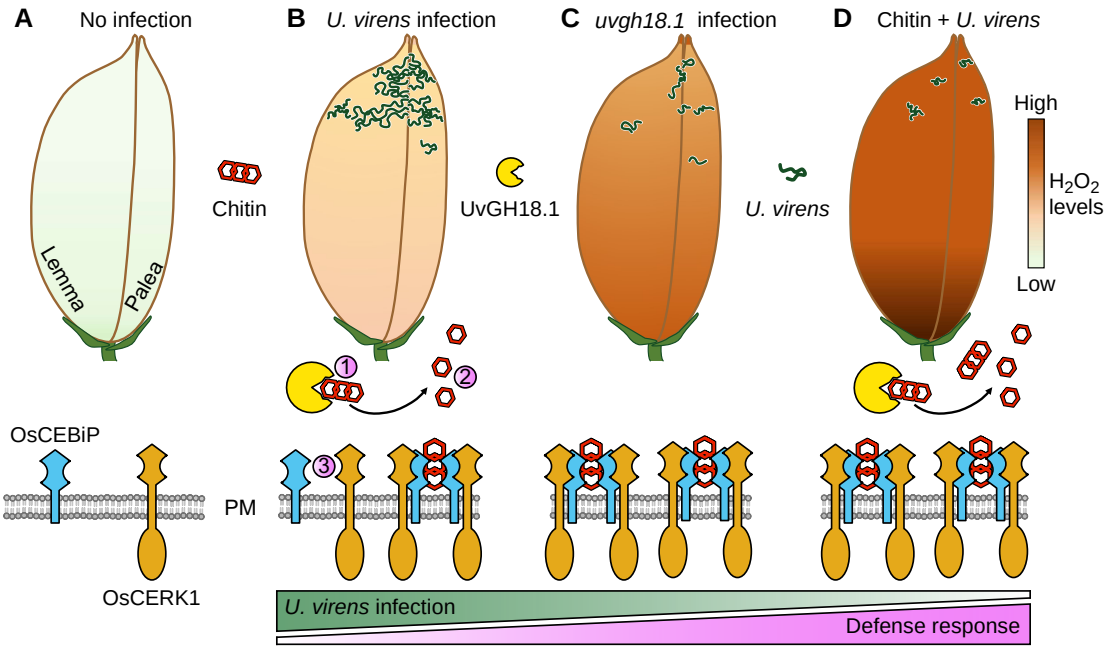

Figure 8. A model for the action of UvGH18.1 on chitin-triggered immunity at lemma/palea in a spikelet. **A)** Rice spikelet possesses OsCEBiP/OsCERK1-mediated chitin-trigged immunity mainly in the lemma and palea. **B)** *U. virens* suppresses chitin-triggered immunity in rice through UvGH18.1. On the one hand, the UvGH18.1 effector binds to (①) and degrades chitin (②) to prevent immune elicitation. On the other hand, it impairs chitin-induced dimerization of OsCEBiP and OsCERK1 (③) to attenuate immune signaling. **C)** Compared to the wild-type strain, the *U. virens* mutant lacking *UvGH18.1* (*uvgh18.1*) elicits a stronger rice defense response in the lemma/palea, which restricts pathogen growth. **D)** Pretreatment with chitin induces higher levels of H_2O_2 accumulation and expression of defense-related genes in the lemma/palea, limiting *U. virens* growth and potentially reducing its entry into inner floral organs. This approach proves effective in improving rice false smut resistance.

expression of *OsBETV1* and *OsPR10b* was largely compromised in lemmas and paleas of *oscebip oscerk1* spikelets, but not for flg22-elicited responses (Fig. 7D). These data indicate that inducible defense responses in rice flowers predominantly occur in lemmas and paleas.

Discussion

As summarized in Fig. 8, here we have identified *U. virens* UvGH18.1 as a core effector that binds to and degrades chitin oligomers to suppress chitin perception in rice flowers. We have also identified OsCEBiP and OsCERK1 as UvGH18.1 targets and revealed the mechanism by which UvGH18.1 disrupts host chitin signaling through binding to OsCEBiP and OsCERK1 and interfering with their dimerization. We have further identified the lemma and palea as the critical floral structure and location that exerts the immune response against *U. virens* to protect stamens and pistils. These findings carry several implications, including a strategy that exploits chitin- or flg22-elicited immunity in lemma/palea to improve rice false smut resistance.

The optimal defense theory predicts that flowers should receive protection of constitutive defense rather than inducible defense as they have high fitness value and are under high probability of attack (Zangerl and Rutledge 1996). This notion has been confirmed in studies with flower-feeding herbivores (Diezel et al. 2011; Li et al. 2017). However, it is rarely documented whether flowers deploy constitutive or induced defenses against pathogenic microbes. Compared to well-documented plant immunity in foliar organs, only limited information is available for immunity/resistance protein functioning in flowers. Here, through analysis on a core virulence factor of *U. virens*, we demonstrate that PTI plays a crucial role in rice flowers against *U. virens*, indicating that inducible defense acts in plant floral organs in response to pathogenic microbes.

We also reveal that rice flowers deploy inducible defense responses in specialized floral structures, i.e. the lemma and palea (Fig. 7). This is considered to be of high ecological value. Unlike individual leaves and roots that are often dispensable, the stamen and pistil are organs specialized for reproduction and indispensable. Thus, pathogen attack or activated immune responses can easily impair male–female gamete interaction and seed production. Therefore, the lemma and palea which enclose the pistil and stamen are ideal to provide protection against potential pathogens. Indeed, chitin and flg22 can elicit defense-related gene expression and H_2O_2 accumulation in the lemma and palea, which may restrict *U. virens* growth and reduce the chance of *U. virens* extending from the outer surface of rice spikelets to inner floral organs, to alleviate disease incidence (Figs. 5 to 7; Supplementary Fig. S10). Our discovery here validates the importance of the

lemma and palea in defending flowers against the false smut pathogen.

CERK1 is a plasma membrane protein containing an extracellular LysM domain and an intracellular Ser/Thr kinase domain (Miya et al. 2007), and serves as a central hub for multiple pattern-recognition pathways. In PTI response, both Arabidopsis AtCERK1 and rice OsCERK1 have affinity for chitin oligomers and are indispensable for chitin signaling (Miya et al. 2007; Kouzai et al. 2014). However, AtCERK1 perceives fungal nonbranched β-1,3-glucan and bacterial peptidoglycan (PGN) (Willmann et al. 2011; Mélida et al. 2018), whilst OsCERK1 senses bacterial PGN and lipopolysaccharide (Liu et al. 2012; Desaki et al. 2018). In symbiotic signaling, OsCERK1 associates with the symbiotic receptor MYC FACTOR RECEPTOR1/LYSM DOMAIN RECEPTOR-LIKE KINASE2 (OsMYR1/OsLYK2) to mediate perception of arbuscular mycorrhizal fungi in rice (He et al. 2019), which is in competition with OsCERK1/OsCEBiP-mediated chitin signaling (Zhang et al. 2021a). Moreover, OsCERK1 can bind to rice cell wall-derived oligosaccharides to initiate damage-associated molecular pattern-triggered immune response (Yang et al. 2021). These findings pinpoint the vital roles of CERK1 in plant immune signaling. Here, we found that the *oscebip oscerk1* double mutant had almost completely abolished defense responses induced by chitin, suggesting that OsCERK1 and OsCEBiP together are the foundation for chitin-triggered immunity in rice flowers. Moreover, our findings suggest a conservative role for OsCEBiP/OsCERK1-mediated immunity in different plant organs.

Chitin-triggered immunity can be suppressed by phytopathogens through a diversity of strategies, such as by blocking chitin perception and by interrupting multiple steps of the chitin-induced immune signaling (see review (Gong et al. 2020)). To prevent chitin perception, pathogens may mask chitin with effector proteins, such as Ecp6, Slp1, MpChi, Mg3LysM, and MoAa91 (de Jonge et al. 2010; Marshall et al. 2011; Mentlak et al. 2012; Sanchez-Vallet et al. 2013; Fiorin et al. 2018; Li et al. 2020). Moreover, pathogens use effector VdPDA1 to deacetylate chitin (Gao et al. 2019) and chitinase effectors EWCAs and MoChia1 to degrade chitin oligomers (Han et al. 2019; Martinez-Cruz et al. 2021). Importantly, fungal pathogens can secrete effectors to directly target immune components in hosts. For example, *M. oryzae* deploys a chitinase effector MoChia1 to suppress plant immunity via interacting with rice OsMBL1, a jacalin-related mannose-binding lectin with a potential role in perceiving chitin (Han et al. 2019). AvrPiz-t from *M. oryzae* is able to interact with OsRac1 to suppress pathogen-associated molecular patterns (PAMPs)-triggered ROS bursts in rice (Bai et al. 2019). Multiple fungi can utilize a core effector NIS1 to promote infection by targeting conserved kinases BAK1 and BIK1 (Irieda et al. 2019). The bacterial AvrPtoB effector can ubiquitinate the cytoplasmic kinase domain of Arabidopsis CERK1 for degradation (Gimenez-Ibanez et al. 2009). Here, we demonstrate that UvGH18.1 not only possesses chitinase activity to hydrolyze chitin, but also the ability to interact with the ectodomains of rice chitin receptors OsCEBiP and OsCERK1 to block their chitin-induced dimerization (Figs. 2 to 4). The action of UvGH18.1 to disarm plant immunity in flowers by degrading the chitin signal and suppressing downstream immune signaling represents a strengthened counterstrategy employed by a fungal pathogen.

In the context that evidence for strong gene-for-gene type resistance to *U. virens* is lacking, rice may instead employ PTI to defend against *U. virens* by utilizing cell surface receptors to recognize *U. virens* factors like UvGH18.1, similar to OsTPR1 recognizing MoChia1 (Yang et al. 2019). It is also possible that PTI conferred by other yet-to-be-identified pattern recognition receptors may contribute to restricting the growth of *U. virens* in rice. For instance, a plasma membrane-localized protein OsMBL1 likely serves as a chitin sensor (Han et al. 2019). As OsMBL1 is not targeted by UvGH18.1 and the *OsMBL1* gene was highly expressed in rice flowers (Supplementary Fig. S5), chitin-OsMBL1 signaling may play a role against *U. virens*. Moreover, rice genome possesses a large number of genes encoding cell surface receptors, including >1,000 *RECEPTER-LIKE KINASES* (*RLKs*) and 90 *RECEPTER-LIKE PROTEINS* (*RLPs*) (Shiu et al. 2004; Fritz-Laylin et al. 2005). It is of interest to identify receptors that recognize PAMPs/effectors derived from *U. virens*, and to characterize their roles in rice resistance against *U. virens*.

Formation of false smut balls is critical to the development of rice false smut disease because these balls contain chlamydospores and sclerotia that serve as the primary inoculum sources in the disease cycle. More importantly, these balls produce mycotoxins that are poisonous to humans and animals (Fan et al. 2016; Sun et al. 2020). Disruption of *UvGH18.1* led to failure in false smut ball development on rice, and this defect could not be rescued by inoculating the *uvgh18.1* mutant into *oscebip oscerk1* panicles (Fig. 5A; Supplementary Fig. S10), indicating that UvGH18.1 may function more than by targeting chitin and host chitin receptors to promote false smut ball formation. Whether RECEPTOR FOR ACTIVATED C KINASE1A (OsRACK1A), another virulence target of UvGH18.1 (Li et al. 2022), could prevent the *uvgh18.1* mutant from developing false smut balls needs further investigation. Since UvGH18.1 is highly conserved in natural populations of *U. virens* (Supplementary Fig. S3), targeted inhibition of UvGH18.1 could potentially emerge as a prospective strategy for managing rice false smut disease.

Materials and methods

Fungal and plant materials, growth conditions, and disease assay

A virulent *U. virens* strain PJ52-2-5 (abbreviated as PJ52) was used in this work (Wang et al. 2016). It was isolated from a false smut ball naturally formed in rice (*O. sativa*) accession Pujiang 6 in Sichuan Province, China. PJ52 was stored as

mycelial clumps at −80 °C, and was reactivated on potato sucrose agar (PSA) at 28 °C before use. Rice accession Q455 was used in this study. For rice false smut disease assay, rice plants were grown in experimental fields under natural conditions and were maintained without spraying any fungicides at all growth stages of rice. *N. benthamiana* plants were grown in a growth room at 21 °C and 75% relative humidity under 10 h/14 h light (white fluorescent light intensity of 200 μmol m^{-2} s^{-1})/dark period.

Infection of rice with *U. virens* was performed as described previously with minor modifications (Fan et al. 2015). Briefly, the potato sucrose (PS)-cultured mixture of mycelia and conidia of *U. virens* strains were collected and blended for artificial injection with a syringe into rice panicles at late booting stages. Disease symptoms were photographed and disease severity recorded at around 4 wk postinoculation.

Constructs and transformation

To generate gene knockout for *UvGH18.1*, the 955-bp upstream and 1034-bp downstream sequences were amplified and subcloned into a gene replacement vector pRF-HU2 (Frandsen et al. 2012) to generate pRF-HU2-UvGH18.1. To improve homologous recombination efficiency in *U. virens* gene knockout experiments, the pCas9-tRp-gRNA-UvGH18.1 plasmid was constructed by introducing a *UvGH18.1*-specific gRNA spacer into the pCas9-tRp-gRNA vector following previous reports (Liang et al. 2018; Guo et al. 2019). The hygromycin resistance gene *Hph* in the pSK1044 vector (Yu et al. 2015) was replaced by a basta resistance gene *bar* amplified from the Pzp-Bar-Ex vector (Fan et al. 2019), resulting in vector SK1044-Bar. The *UvGH18.1* gene fragment (wild-type or mutated versions) including a 2.0-kb native promoter sequence and a 0.5-kb downstream sequence was amplified and ligated into the SK1044-Bar vector linearized by *Eco*RI-*Xho*I. The primers and gRNA spacer sequences are listed in Supplementary Data Set 2.

For purification of recombinant proteins, the coding sequences (lacking signal peptide-encoding fragments) of *UvGH18.1*, *OsCEBiP*, *OsCERK1*, *OsMBL1*, *OsTPR1*, *OsLYP4*^{ECD}, *OsLYP6*^{ECD}, and *MoChia1* were amplified with indicated primers (Supplementary Data Set 2) and ligated into the pMAL-c5x or pGEX-6p-1 vectors linearized by *Bam*HI-*Eco*RI.

To generate constructs for Y2H experiments, the coding sequence of *UvGH18.1* was amplified and cloned into the pGBKT7 vector to generate plasmids BD-UvGH18.1. The coding sequences of *OsCEBiP*, *OsCERK1*, and their truncations were amplified and cloned into the pGADT7 vectors to generate plasmids from AD-V1 to AD-V12 (Supplementary Fig. S6). The primer sequences and related information are listed in Supplementary Data Set 2.

To generate constructs for co-IP experiments in rice protoplasts, the coding sequences *OsCEBiP* and *OsCERK1* were amplified and individually cloned into the pRTVc-4×HA and pRTVc (tagging 3×FLAG during PCR amplification) vectors driven by their native promoters, to generate plasmids OsCEBiP-4×HA, OsCERK1-4×HA, OsCEBiP-3×FLAG, and OsCERK1-3×FLAG. The primer sequences and related information are listed in Supplementary Data Set 2.

To generate constructs for LCI experiments, *UvGH18.1* and its mutated/truncated versions were separately cloned into the cLUC vector. *OsCEBiP*^{ECD}, *OsCERK1*^{ECD}, *OsLYP4*^{ECD}, and *OsLYP6*^{ECD} were separately cloned into the nLUC vector. The primer sequences and related information are listed in Supplementary Data Set 2.

To make CRISPR-Cas9 constructs for simultaneous knockout of *OsCEBiP* and *OsCERK1*, the gene-specific guide RNAs targeting *OsCEBiP* and *OsCERK1* were designed with an online software toolkit CRISPR-GE (Xie et al. 2017) and subcloned into the pRGEB32 binary vector, resulting in the Cas9-OsCEBiP/OsCERK1 construct. Guide RNA sequences and primers are listed in Supplementary Data Set 2.

To knockout *UvGH18.1* in *U. virens*, gene replacement construct pRF-HU2-UvGH18.1 and pCas9-tRP-gRNA-UvGH18.1 were co-transformed into protoplasts of *U. virens* strain PJ52 as described by a previous study (Talbot et al. 1993). Knockout mutants were screened from hygromycin-resistant transformants by PCR. The positions of primers are indicated in Supplementary Fig. S1. For complementation assay, the SK1044-Bar-UvGH18.1/UvGH18.1^{mchi}/UvGH18.1^{mcc}/UvGH18.1^{dm} (basta resistance) constructs were introduced into *Agrobacterium* strain AGL1, and transformed into conidia of PJ52 following a previous report (Fan et al. 2019). Positive transformants were confirmed by PCR and immunoblotting with a UvGH18.1 antibody (Li et al. 2022).

To generate transgenic rice plants, the Cas9-OsCEBiP/OsCERK1 construct was introduced into rice accession Q455 via *Agrobacterium*-mediated transformation. Positive transgenic lines were confirmed by hygromycin resistance test and PCR.

Nucleic acid extraction, RT-qPCR, and PCR

U. virens mycelia were collected from PSA for extraction of genomic DNA using the CTAB method (Doyle and Doyle 1990) and total RNA with TRIzol reagent (Invitrogen). For quantification of *UvGH18.1* transcription level, *U. virens* RNA was reverse-transcribed using the ReverTra Ace PCR RT Kit (TOYOBO); the resultant cDNA was used for qPCR with SYBR Green mix (Qiagen) and gene-specific primers under a CFX Connect Real-Time System (Bio-RAD). *UvTub2α* was served as a reference gene. For determining relative fungal growth, the *UvTub2α* level was calculated relative to that of rice reference gene *OsUbi*. For polymorphism analysis of *UvGH18.1*, the full-length *UvGH18.1* gene was amplified from genomic DNA samples of tested *U. virens* isolates and directly sequenced at Sangon Biotech (Chengdu) Co., Ltd, China. Primers used in this study are listed in Supplementary Data Set 2.

To evaluate the effects of in vitro-purified proteins on chitin-induced expression of defense-related genes in rice spikelets, Chitin (40 μg ml^{-1}) was incubated with purified proteins (20 μg ml^{-1} each) at 37°C for 1 h, prior to injection

into rice panicles at the late booting stage. Spikelet samples were collected 24 h postinjection. RT-qPCR analysis was performed using *OsUbi* as a reference gene. Primers are listed in Supplementary Data Set 2.

ROS assays

To determine the bursts of ROS, leaf discs were floated on ddH_2O overnight and treated with ROS induction assay mixture (10 μg ml^{-1} chitin/0.2 μM flg22, 4 μM L-012, and 10 μg ml^{-1} horseradish peroxidase). To evaluate the effects of in vitro-purified proteins on chitin or flg22, their mixture (20 μg ml^{-1} for purified protein) was incubated at 37°C for 1 h, prior to elicitation of ROS. To evaluate the effects of in vitro-purified proteins on OsCEBiP/OsCERK1-mediated immune signaling, leaf discs were floated on ddH_2O for 6 h and then incubated with 20 μg ml^{-1} of recombinant protein overnight, prior to ROS elicitation. ROS was measured with a GloMax 96 microplate luminometer (Promega).

To examine H_2O_2 accumulation in rice spikelets, samples treated with chitin (40 μg ml^{-1}) or flg22 (0.2 μM) or inoculated with *U. virens* were collected at the late booting stage of rice. Samples were then stained with 3,3′-Diaminobenzidine tetrahydrochloride hydrate (DAB, 0.5 mg ml^{-1} pH 3.8) with vacuum for 1 h and decolorized with 75% v/v ethanol. The amounts of H_2O_2 were quantified with the Amplex Red Hydrogen Peroxide/Peroxidase Analysis Kit (Thermo Fisher Scientific) following the manufacturer's instructions. The absorbance at 560 nm was measured in a Spectral Scanning Multifunction Reader (Thermo Scientific Variskan Flash 4.00.53).

MAPK assay

At late booting stages of rice, fully-expanded spikelets were detached and floated on ddH_2O overnight, treated with chitin (40 μg ml^{-1}) or flg22 (0.2 μM), and then samples were collected at 0, 15, 30, and 45 min. Spikelet samples were collected for total protein extraction in Urea buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% v/v NP-40, 4 M Urea). Phosphorylation of MAPK proteins was detected by immunoblot with the phospho-p44/42 MAPK (T202/Y204) antibody (1:2,000 dilution, Cell Signaling, 4370T). Ponceau S staining was conducted for loading normalization.

Chitinase activity and chitin binding assays

Chitinase activity assay was conducted as previously described with minor modifications (Thompson et al. 2001; Han et al. 2019). Briefly, 20 μl of fluorescent substrate 4-methylumbelliferyl-β-D-N,N″,N″-triacetylchitotriose (1 mM in DMSO) was mixed with 150 μl of 200 mM sodium phosphate buffer (pH 6.7), and incubated for 20 min at 37 °C. The reaction was started by the addition of 30 μl of MBP-UvGH18.1, MBP-UvGH18.1^{mchi}, MBP-UvGH18.1^{mcc}, MBP-UvGH18.1^{dm}, or MBP (300 μg ml^{-1} each). After 2 h at 37 °C, the reaction was stopped by the addition of 50 μl of 3 M Na_2CO_3. The fluorescence of released by 4-methylumbelliferone was measured with an excitation wavelength of 390 nm and an emission wavelength of 448 nm in a Spectral Scanning Multifunctional Reader (Thermo Scientific Variskan Flash 4.00.53). Chitin binding assay was performed as previously described (Li et al. 2021).

HPLC analysis

HPLC analysis was conducted following a previous method with minor modifications (Martinez-Cruz et al. 2021). The chitinase activities of MBP-tagged UvGH18.1 and its mutant variants were separately assayed against the chitin oligomer $(GlcNAc)_6$ (Solarbio, 38854-46-5). The reactions were conducted in PBS buffer containing 80 μg ml^{-1} protein and 300 μg ml^{-1} chitin oligomers in a total volume of 200 μl and incubated at 37 °C for 1 h. After filtration, 30 μl of reaction products were directly analyzed using a SHIMADZU HPLC system equipped with a ShimNex HE 5 μm NH_2 column (4.6 × 250 mm). The mobile phase was 70% v/v acetonitrile with the flow rate of 0.7 ml min^{-1}. Chitin oligomers were detected by UV absorption at 195 nm. The MBP protein was used as a negative control.

GST pull-down assay

Recombinant proteins (MBP-OsCEBiP, GST-OsCERK1, MBP-UvGH18.1, MBP-UvGH18.1^{mchi}, MBP-UvGH18.1^{dm}, GST-UvGH18.1, GST-MoChia1, MBP-OsMBL1, MBP-OsTPR1, MBP-OsLYP4^{ECD}, MBP-OsLYP6^{ECD}, GST, and MBP) were purified from *E. coli* and used for GST pull-down assays as described previously (Wang et al. 2017b). Detection of GST- and MBP-fused proteins was performed with GST (1:2,000 dilution, Sangon Biotech, D110271) and MBP (1:2,000 dilution, NEB, E8032) antibodies, respectively.

Co-IP assay

For semi-in vivo co-IP assay, rice protoplast suspensions were prepared from etiolated rice seedlings grown on 1/2 MS medium for 6 to 7 d and used for transformation as described previously (Zhang et al. 2011). Protoplasts transformed with OsCEBiP/OsCERK1-related constructs were treated with 20 μl purified recombinant proteins MBP-UvGH18.1, MBP-UvGH18.1^{mchi}, MBP-UvGH18.1^{mcc}, or MBP-UvGH18.1^{dm} (40 μg ml^{-1} each) for 2 h, followed by IP with anti-FLAG affinity beads and immunoblot analysis with FLAG (1:2,000 dilution, Sigma-Aldrich, A8592), HA (1:2,000 dilution, Sigma-Aldrich, 12013819001), and MBP antibodies. Input proteins were immunoblotted with a HSP (1:2,000 dilution, Beijing Protein Innovation, AbM51099-31-PU) antibody. For competitive co-IP, chitin (10 μg ml^{-1}) was added to protoplast suspensions at 10 min before protein extraction.

BN-PAGE analysis

For BN-PAGE analysis, rice protoplasts transformed with OsCEBiP-/OsCERK1-related constructs were treated with 20 μl dialyzed recombinant proteins MBP-UvGH18.1 or MBP-UvGH18.1^{dm} (40 μg ml^{-1} each) for 2 h and then treated with chitin (10 μg ml^{-1}) for 30 min. BN-PAGE

experiments were conducted as described previously (Na Ayutthaya et al. 2020).

Y2H assay

The constructs of BD-UvGH18.1 and series of truncated versions of AD-OsCEBiP and AD-OsCERK1 were co-transformed into yeast strain AH109 (Clontech). Positive transformants were grown on SD/-Trp/-Leu or SD/-Trp/-Leu/-His/-Ade plates for 3 d at 30 °C, prior to photograph taking.

LCI assay

Agrobacteria carrying nLUC and cLUC constructs were infiltrated into the leaves of 5-wk-old *N. benthamiana* using a needleless syringe. Two days after infiltration, the leaves were soaked in 1 mM D-luciferin (Biovision) for 10 min in dark and examined under a plant molecular imaging system (FUSION-FX7.EDGE).

Sequence analysis

The genomic sequence of *UvGH18.1* was amplified from different *U. virens* isolates (Supplementary Data Set 1) with gene-specific primers (Supplementary Data Set 2) and identified by sequencing. Corresponding protein sequences were deduced by using online software (https://web.expasy.org/translate). Sequence alignment was conducted with the MultAlin software (Corpet 1988).

Statistical analysis

Significant differences were determined by 2-sided unpaired Student's *t*-test ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$) or by 1-way analysis of variance (ANOVA) and Duncan's post hoc multiple comparison tests ($\alpha = 0.05$). Data are presented as mean ± SD. The numbers of biological replicates are indicated in figures or figure legends. Statistical data are provided in Supplementary Data Set 3.

Accession numbers

Sequence data for rice and *U. virens* genes used in this article can be found in the GenBank database under the following accession numbers: *OsUBi* (LOC_Os03g13170); *OsTPR1* (LOC_Os10g34540); *OsMBL1* (LOC_Os01g24710); *OsBETV1* (LOC_Os12g36830); *OsPR10b* (LOC_Os12g36850); *OsNAC4* (LOC_Os01g60020); *OsCEBiP* (LOC_Os03g04110); *OsCERK1* (LOC_Os08g42580); *OsLYP4* (LOC_Os09g27890); *OsLYP6* (LOC_Os06g10660); *UvGH18.1* (UVI_02019410); and *UvTub2α* (UV8b_05680).

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Author contributions

J.F. and W.-M.W. conceived and designed the project. Y.L., Y.N., W.S., Fu Huang, and Z.-J.X. contributed to the planning of research. J.F., G.-B.L., J.L., J.-X.H., X.-H.H., G.-M.L., Y.-D.Z., X.-L.L., X.Z., J.-L.W., S.S., X.-X.L., Y.Z., Feng He, H.G., H.W., M.H., and J.-H.Z. performed the experiments and analyzed the data. Y.-Y.H., Z.-X.Z., J.-W.Z., S.-X.Z., Y.-P.J., M.P., X.C., J.W., W.L., and X.-J.W. analyzed the data. J.F., G.-B.L., and W.-M.W. wrote the manuscript with input from the other authors. G.-B.L., J.L., J.-X.H., and G.-M.L. contributed equally. All authors read and approved the final manuscript.

Supplementary data

The following materials are available in the online version of this article.

Supplementary Figure S1. Construction of *UvGH18.1* knockout mutants in *Ustilaginoidea virens*.

Supplementary Figure S2. *U. virens* growth and *UvGH18.1* expression during the infection process in rice flowers.

Supplementary Figure S3. UvGH18.1 is highly conserved in *U. virens* population.

Supplementary Figure S4. UvGH18.1 functions as a secreted chitinase.

Supplementary Figure S5. UvGH18.1 does not interact with OsMBL1, OsTPR1, OsLYP4, and OsLYP6.

Supplementary Figure S6. UvGH18.1 interacts with the ectodomains of OsCEBiP and OsCERK1.

Supplementary Figure S7. Key residues of UvGH18.1 required for interactions with OsCEBiP and OsCERK1.

Supplementary Figure S8. Simultaneous mutations of K137A and D178A in UvGH18.1 disrupt its interaction with OsCEBiP and OsCERK1.

Supplementary Figure S9. Generation of *oscebip oscerk1* double mutant.

Supplementary Figure S10. Infection of rice spikelets with the *uvgh18.1* mutant.

Supplementary Figure S11. Detection of UvGH18.1 in different *U. virens* strains.

Supplementary Figure S12. OsCEBiP and OsCERK1 contribute to chitin-triggered immunity against *U. virens* in flowers.

Supplementary Figure S13. Flg22 pretreatment promotes rice flower resistance against *U. virens*.

Supplementary Data Set 1. *Ustilaginoidea virens* isolates used for DNA polymorphism analysis.

Supplementary Data Set 2. Primers used in this study.

Supplementary Data Set 3. Results of statistical analyses in this study.

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Conflict of interest statement. None declared.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

Ashizawa T, Takahashi M, Arai M, Arie T. Rice false smut pathogen, *Ustilaginoidea virens*, invades through small gap at the apex of a rice spikelet before heading. J. Gen. Plant. Pathol. 2012:**78**(4):255–259. https://doi.org/10.1007/s10327-012-0389-3

Bai P, Park CH, Shirsekar G, Songkumarn P, Bellizzi M, Wang GL. Role of lysine residues of the *Magnaporthe oryzae* effector AvrPiz-t in effector- and PAMP-triggered immunity. Mol. Plant Pathol. 2019:**20**(4):599–608. https://doi.org/10.1111/mpp.12779

Brefort T, Doehlemann G, Mendoza-Mendoza A, Reissmann S, Djamei A, Kahmann R. *Ustilago maydis* as a pathogen. Annu Rev Phytopathol. 2009:**47**(1):423–445. https://doi.org/10.1146/annurev-phyto-080508-081923

Chen X, Duan Y, Qiao F, Liu H, Huang J, Luo C, Chen X, Li G, Xie K, Hsiang T, et al. A secreted fungal effector suppresses rice immunity through host histone hypoacetylation. New Phytol. 2022a:**235**(5):1977–1994. https://doi.org/10.1111/nph.18265

Chen XY, Li XB, Duan YH, Pei ZX, Liu H, Yin WX, Huang JB, Luo CX, Chen XL, Li GT, et al. A secreted fungal subtilase interferes with rice immunity via degradation of SUPPRESSOR OF G2 ALLELE OF *skp1*. Plant Physiol. 2022b:**190**(2):1474–1489. https://doi.org/10.1093/plphys/kiac334

Corpet F. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 1988:**16**(22):10881–10890. https://doi.org/10.1093/nar/16.22.10881

de Jonge R, van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, van der Krol S, Shibuya N, Joosten MH, Thomma BP. Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science. 2010:**329**(5994):953–955. https://doi.org/10.1126/science.1190859

Desaki Y, Kouzai Y, Ninomiya Y, Iwase R, Shimizu Y, Seko K, Molinaro A, Minami E, Shibuya N, Kaku H, et al. OsCERK1 plays a crucial role in the lipopolysaccharide-induced immune response of rice. New Phytol. 2018:**217**(3):1042–1049. https://doi.org/10.1111/nph.14941

Diezel C, Allmann S, Baldwin IT. Mechanisms of optimal defense patterns in *Nicotiana attenuata*: flowering attenuates herbivory-elicited ethylene and jasmonate signaling. J Integr. Plant Biol. 2011:**53**(12):971–983. https://doi.org/10.1111/j.1744-7909.2011.01086.x

Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990:**12**:13–15.

Fan J, Bai P, Ning Y, Wang J, Shi X, Xiong Y, Zhang K, He F, Zhang C, Wang R, et al. The monocot-specific receptor-like kinase SDS2 controls cell death and immunity in rice. Cell & Host Microbe. 2018:**23**(4):498–510.e5. https://doi.org/10.1016/j.chom.2018.03.003

Fan J, Du N, Li L, Li G, Wang Y, Zhou Y, Hu X, Liu J, Zhao J, Li Y, et al. A core effector UV_1261 promotes *Ustilaginoidea virens* infection via spatiotemporally suppressing plant defense. Phytopathol. Res. 2019:**1**(1):11. https://doi.org/10.1186/s42483-019-0019-5

Fan J, Guo XY, Li L, Huang F, Sun WX, Li Y, Huang YY, Xu YJ, Shi J, Lei Y, et al. Infection of *Ustilaginoidea virens* intercepts rice seed formation but activates grain-filling-related genes. J Integr. Plant Biol. 2015:**57**(6):577–590. https://doi.org/10.1111/jipb.12299

Fan J, Liu J, Gong Z-Y, Xu P-Z, Hu X-H, Wu J-L, Li GB, Yang J, Wang Y-Q, Zhou Y-F, et al. The false smut pathogen *Ustilaginoidea virens* requires rice stamens for false smut ball formation. Environ Microbiol. 2020:**22**(2):646–659. https://doi.org/10.1111/1462-2920.14881

Fan J, Yang J, Wang YQ, Li GB, Li Y, Huang F, Wang W-M. Current understanding on *Villosiclava virens*, a unique flower-infecting fungus causing rice false smut disease. Mol. Plant Pathol. 2016:**17**(9):1321–1330. https://doi.org/10.1111/mpp.12362

Fang AF, Gao H, Zhang N, Zheng XH, Qiu SS, Li YJ, Zhou S, Cui FH, Sun WX. A novel effector gene *SCRE2* contributes to full virulence of *Ustilaginoidea virens* to rice. Front Microbiol. 2019:**10**:845. https://doi.org/10.3389/fmicb.2019.00845

Fiorin GL, Sanchez-Vallet A, Thomazella DPD, do Prado PFV, do Nascimento LC, Figueira AVD, Thomma BPHJ, Pereira GAG, Teixeira PJPL. Suppression of plant immunity by fungal chitinase-like effectors. Curr Biol. 2018:**28**(18):3023–3030.e5. https://doi.org/10.1016/j.cub.2018.07.055

Frandsen RJ, Frandsen M, Giese H. Targeted gene replacement in fungal pathogens via *Agrobacterium tumefaciens*-mediated transformation. Methods in Molecular Biology. 2012:**835**:17–45. https://doi.org/10.1007/978-1-61779-501-5_2

Fritz-Laylin LK, Krishnamurthy N, Tor M, Sjolander KV, Jones JD. Phylogenomic analysis of the receptor-like proteins of rice and Arabidopsis. Plant Physiol. 2005:**138**(2):611–623. https://doi.org/10.1104/pp.104.054452

Gao F, Zhang BS, Zhao JH, Huang JF, Jia PS, Wang S, Zhang J, Zhou JM, Guo HS. Deacetylation of chitin oligomers increases virulence in soil-borne fungal pathogens. Nat Plants. 2019:**5**(11):1167–1176. https://doi.org/10.1038/s41477-019-0527-4

Gimenez-Ibanez S, Hann DR, Ntoukakis V, Petutschnig E, Lipka V, Rathjen JP. AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. Curr Biol. 2009:**19**(5):423–429. https://doi.org/10.1016/j.cub.2009.01.054

Gong B-Q, Wang F-Z, Li J-F. Hide-and-seek: chitin-triggered plant immunity and fungal counterstrategies. Trends Plant Sci. 2020:**25**(8):805–816. https://doi.org/10.1016/j.tplants.2020.03.006

Guo W, Gao Y, Yu Z, Xiao Y, Zhang Z, Zhang H. The adenylate cyclase UvAc1 and phosphodiesterase UvPdeH control the intracellular cAMP level, development, and pathogenicity of the rice false smut fungus *Ustilaginoidea virens*. Fungal Genet. Biol. 2019:**129**:65–73. https://doi.org/10.1016/j.fgb.2019.04.017

Han YJ, Song LL, Peng CL, Liu X, Liu LH, Zhang YH, Wang WZ, Zhou J, Wang SH, Ebbole D, et al. A *Magnaporthe* chitinase interacts with a rice jacalin-related lectin to promote host colonization. Plant Physiol. 2019:**179**(4):1416–1430. https://doi.org/10.1104/pp.18.01594

Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, Desaki Y, Arima S, Squeglia F, Ruggiero A, Tokuyasu K, et al. Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. PNAS. 2014:**111**(3):E404-413. https://doi.org/10.1073/pnas.1312099111

He J, Zhang C, Dai H, Liu H, Zhang X, Yang J, Chen X, Zhu Y, Wang D, Qi X, et al. A LysM receptor heteromer mediates perception of arbuscular mycorrhizal symbiotic signal in rice. Mol Plant. 2019:**12**(12):1561–1576. https://doi.org/10.1016/j.molp.2019.10.015

Irieda H, Inoue Y, Mori M, Yamada K, Oshikawa Y, Saitoh H, Uemura A, Terauchi R, Kitakura S, Kosaka A, et al. Conserved

fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. PNAS. 2019:**116**(2):496–505. https://doi.org/10.1073/pnas.1807297116

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. PNAS. 2006:**103**(29):11086–11091. https://doi.org/10.1073/pnas.0508882103

Koiso Y, Li Y, Iwasaki S, Hanaoka K, Kobayashi T, Sonoda R, Fujita Y, Yaegashi H, Sato Z. Ustiloxins, antimitotic cyclic peptides from false smut balls on rice panicles caused by *Ustilaginoidea virens*. J Antibiot. 1994:**47**(7):765–773. https://doi.org/10.7164/antibiotics.47.765

Kouzai Y, Mochizuki S, Nakajima K, Desaki Y, Hayafune M, Miyazaki H, Yokotani N, Ozawa K, Minami E, Kaku H, et al. Targeted gene disruption of *OsCERK1* reveals its indispensable role in chitin perception and involvement in the peptidoglycan response and immunity in rice. Mol. Plant-Microbe Interact. 2014:**27**(9):975–982. https://doi.org/10.1094/MPMI-03-14-0068-R

Kzhyshkowska J, Gratchev A, Goerdt S. Human chitinases and chitinase-like proteins as indicators for inflammation and cancer. Biomark Insights. 2007:**2**:128–146. https://doi.org/10.1177/117727190700200023

Li GB, Fan J, Wu JL, He JX, Liu J, Shen S, Gishkori ZGN, Hu XH, Zhu Y, Zhou SX, et al. The flower-infecting fungus *Ustilaginoidea virens* subverts plant immunity by secreting a chitin-binding protein. Front Plant Sci. 2021:**12**:733245. https://doi.org/10.3389/fpls.2021.733245

Li GB, He JX, Wu JL, Wang H, Zhang X, Liu J, Hu XH, Zhu Y, Shen S, Bai YF, et al. Overproduction of OsRACK1A, an effector-targeted scaffold protein promoting OsRBOHB-mediated ROS production, confers rice floral resistance to false smut disease without yield penalty. Mol Plant. 2022:**15**(11):1790–1806. https://doi.org/10.1016/j.molp.2022.10.009

Li R, Wang M, Wang Y, Schuman MC, Weinhold A, Schafer M, Jimenez-Aleman GH, Barthel A, Baldwin IT. Flower-specific jasmonate signaling regulates constitutive floral defenses in wild tobacco. PNAS. 2017:**114**(34):E7205–E7214. https://doi.org/10.1073/pnas.1703463114

Li Y, Liu X, Liu M, Wang Y, Zou Y, You Y, Yang L, Hu J, Zhang H, Zheng X, et al. *Magnaporthe oryzae* auxiliary activity protein MoAa91 functions as chitin-binding protein to induce appressorium formation on artificial inductive surfaces and suppress plant immunity. mBio. 2020:**11**(2):e03304–e03319. https://doi.org/10.1128/mBio.03304-19

Liang YF, Han Y, Wang CF, Jiang C, Xu JR. Targeted deletion of the *USTA* and *UvSLT2* genes efficiently in *Ustilaginoidea virens* with the CRISPR-Cas9 system. Front Plant Sci. 2018:**9**:699. https://doi.org/10.3389/fpls.2018.00699

Liu B, Li JF, Ao Y, Qu J, Li Z, Su J, Zhang Y, Liu J, Feng D, Qi K, et al. Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. Plant Cell. 2012:**24**(8):3406–3419. https://doi.org/10.1105/tpc.112.102475

Mélida H, Sopeña-Torres S, Bacete L, Garrido-Arandia M, Jordá L, López G, Muñoz-Barrios A, Pacios LF, Molina A. Non-branched β-1,3-glucan oligosaccharides trigger immune responses in Arabidopsis. Plant Journal. 2018:**93**(1):34–49. https://doi.org/10.1111/tpj.13755

Marshall R, Kombrink A, Motteram J, Loza-Reyes E, Lucas J, Hammond-Kosack KE, Thomma BP, Rudd JJ. Analysis of two in planta expressed LysM effector homologs from the fungus *Mycosphaerella graminicola* reveals novel functional properties and varying contributions to virulence on wheat. Plant Physiol. 2011:**156**(2):756–769. https://doi.org/10.1104/pp.111.176347

Martinez-Cruz J, Romero D, Hierrezuelo J, Thon M, de Vicente A, Perez-Garcia A. Effectors with chitinase activity (EWCAs), a family of conserved, secreted fungal chitinases that suppress chitin-triggered immunity. Plant Cell. 2021:**33**(4):1319–1340. https://doi.org/10.1093/plcell/koab011

Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, Terauchi R, Nishizawa Y, Shibuya N, Thomma BPHJ, et al. Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease. Plant Cell. 2012:**24**(1):322–335. https://doi.org/10.1105/tpc.111.092957

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. PNAS. 2007:**104**(49):19613–19618. https://doi.org/10.1073/pnas.0705147104

Na Ayutthaya PP, Lundberg D, Weigel D, Li L. Blue native polyacrylamide gel electrophoresis (BN-PAGE) for the analysis of protein oligomers in plants. Current Protocols in Plant Biology. 2020:**5**(2):e20107. https://doi.org/10.1002/cppb.20107

Qiu SS, Fang AF, Zheng XH, Wang SZ, Wang JY, Fan J, Sun ZT, Gao H, Yang JY, Zeng QT, et al. *Ustilaginoidea virens* nuclear effector SCRE4 suppresses rice immunity via inhibiting expression of a positive immune regulator OsARF17. Int J Mol Sci. 2022:**23**(18):10527. https://doi.org/10.3390/ijms231810527

Rush MCA, Shahjahan KM, Jones JP. Outbreak of false smut of rice in Louisiana. Plant Dis. 2000:**84**(1):100. https://doi.org/10.1094/PDIS.2000.84.1.100D

Sanchez-Vallet A, Saleem-Batcha R, Kombrink A, Hansen G, Valkenburg DJ, Thomma BP, Mesters JR. Fungal effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. eLife. 2013:**2**:e00790. https://doi.org/10.7554/eLife.00790

Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, Minami E, Okada K, Yamane H, Kaku H, et al. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. Plant J. 2010:**64**(2):204–214. https://doi.org/10.1111/j.1365-313X.2010.04324.x

Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH. Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. Plant Cell. 2004:**16**(5):1220–1234. https://doi.org/10.1105/tpc.020834

Song JH, Wei W, Lv B, Lin Y, Yin WX, Peng YL, Schnabel G, Huang JB, Jiang DH, Luo CX. Rice false smut fungus hijacks the rice nutrients supply by blocking and mimicking the fertilization of rice ovary. Environ Microbiol. 2016:**18**(11):3840–3849. https://doi.org/10.1111/1462-2920.13343

Song T, Zhang Y, Zhang Q, Zhang X, Shen D, Yu J, Yu M, Pan X, Cao H, Yong M, et al. The N-terminus of an *Ustilaginoidea virens* Ser-Thr-rich glycosylphosphatidylinositol-anchored protein elicits plant immunity as a MAMP. Nat Commun. 2021:**12**(1):2451. https://doi.org/10.1038/s41467-021-22660-9

Sun W, Fan J, Fang A, Li Y, Tariqjaveed M, Li D, Hu D, Wang W-M. *Ustilaginoidea virens*: insights into an emerging rice pathogen. Annu Rev Phytopathol. 2020:**58**(1):363–385. https://doi.org/10.1146/annurev-phyto-010820-012908

Talbot NJ, Ebbole DJ, Hamer JE. Identification and characterization of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. Plant Cell. 1993:**5**(11):1575–1590. https://doi.org/10.1105/tpc.5.11.1575

Tang YX, Jin J, Hu DW, Yong ML, Xu Y, He LP. Elucidation of the infection process of *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) in rice spikelets. Plant Pathol. 2013:**62**(1):1–8. https://doi.org/10.1111/j.1365-3059.2012.02629.x

Thompson SE, Smith M, Wilkinson MC, Peek K. Identification and characterization of a chitinase antigen from *Pseudomonas aeruginosa* strain 385. Appl Environ Microbiol. 2001:**67**(9):4001–4008. https://doi.org/10.1128/AEM.67.9.4001-4008.2001

Tudzynski P, Scheffer J. *Claviceps purpurea*: molecular aspects of a unique pathogenic lifestyle. Mol. Plant Pathol. 2004:**5**(5):377–388. https://doi.org/10.1111/j.1364-3703.2004.00237.x

Wang C, Wang G, Zhang C, Zhu P, Dai H, Yu N, He Z, Xu L, Wang E. OsCERK1-mediated chitin perception and immune signaling requires

receptor-like cytoplasmic kinase 185 to activate an MAPK cascade in rice. Mol Plant. 2017a:**10**(4):619–633. https://doi.org/10.1016/j.molp.2017.01.006

Wang J, Liu X, Zhang A, Ren Y, Wu F, Wang G, Xu Y, Lei C, Zhu S, Pan T, et al. A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice. Cell Res. 2019:**29**(10):820–831. https://doi.org/10.1038/s41422-019-0219-7

Wang J, Yu H, Xiong GS, Lu ZF, Jiao YQ, Meng XB, Liu GF, Chen XW, Wang YH, Li JY. Tissue-specific ubiquitination by IPA1 INTERACTING PROTEIN1 modulates IPA1 protein levels to regulate plant architecture in rice. Plant Cell. 2017b:**29**(4):697–707. https://doi.org/10.1105/tpc.16.00879

Wang Q, Kawano Y. Improving disease resistance to rice false smut without yield penalty by manipulating the expression of effector target. Mol Plant. 2022:**15**(12):1834–1837. https://doi.org/10.1016/j.molp.2022.11.009

Wang S, Sun Z, Wang H, Liu L, Lu F, Yang J, Zhang M, Zhang S, Guo Z, Bent AF, et al. Rice OsFLS2-mediated perception of bacterial flagellins is evaded by *Xanthomonas oryzae* pvs. *oryzae* and *oryzicola*. Mol Plant. 2015:**8**(7):1024–1037. https://doi.org/10.1016/j.molp.2015.01.012

Wang W, Zhang Y, Wen Y, Berkey R, Ma X, Pan Z, Bendigeri D, King H, Zhang Q, Xiao S. A comprehensive mutational analysis of the *Arabidopsis* resistance protein RPW8.2 reveals key amino acids for defense activation and protein targeting. Plant Cell. 2013:**25**(10):4242–4261. https://doi.org/10.1105/tpc.113.117226

Wang YQ, Li GB, Gong ZY, Li Y, Huang F, Fan J, Wang WM. Stachyose is a preferential carbon source utilized by the rice false smut pathogen, *Villosiclava virens*. Physiol. Mol. Plant Pathol. 2016:**96**:69–76. https://doi.org/10.1016/j.pmpp.2016.09.003

Willmann R, Lajunen HM, Erbs G, Newman M-A, Kolb D, Tsuda K, Katagiri F, Fliegmann J, Bono J-J, Cullimore JV, et al. *Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. PNAS. 2011:**108**(49):19824–19829. https://doi.org/10.1073/pnas.1112862108

Wilson IA, Haft DH, Getzoff ED, Tainer JA, Lerner RA, Brenner S. Identical short peptide sequences in unrelated proteins can have different conformations: a testing ground for theories of immune recognition. PNAS. 1985:**82**(16):5255–5259. https://doi.org/10.1073/pnas.82.16.5255

Win J, Chaparro-Garcia A, Belhaj K, Saunders DGO, Yoshida K, Dong S, Schornack S, Zipfel C, Robatzek S, Hogenhout SA, et al. Effector biology of plant-associated organisms: concepts and perspectives. Cold Spring Harbor Symp. Quant. Biol. 2012:**77**(0):235–247. https://doi.org/10.1101/sqb.2012.77.015933

Xie XR, Ma XL, Zhu QL, Zeng DC, Li GS, Liu YG. CRISPR-GE: a convenient software toolkit for CRISPR-based genome editing. Mol Plant. 2017:**10**(9):1246–1249. https://doi.org/10.1016/j.molp.2017.06.004

Xu X, Nicholson P. Community ecology of gungal pathogens causing wheat head blight. Annu Rev Phytopathol. 2009:**47**(1):83–103. https://doi.org/10.1146/annurev-phyto-080508-081737

Yang C, Liu R, Pang J, Ren B, Zhou H, Wang G, Wang E, Liu J. Poaceae-specific cell wall-derived oligosaccharides activate plant immunity via OsCERK1 during *Magnaporthe oryzae* infection in rice. Nat Commun. 2021:**12**(1):2178. https://doi.org/10.1038/s41467-021-22456-x

Yang C, Yu YQ, Huang JK, Meng FW, Pang JH, Zhao QQ, Islam MA, Xu N, Tian Y, Liu J. Binding of the *Magnaporthe oryzae* chitinase MoChia1 by a rice tetratricopeptide repeat protein allows free chitin to trigger immune responses. Plant Cell. 2019:**31**(1):172–188. https://doi.org/10.1105/tpc.18.00382

Yang JY, Zhang N, Wang JY, Fang AF, Fan J, Li DY, Li YJ, Wang SZ, Cui FH, Yu JJ, et al. SnRK1A-mediated phosphorylation of a cytosolic ATPase positively regulates rice innate immunity and is inhibited by *Ustilaginoidea virens* effector SCRE1. New Phytol. 2022:**236**(4):1422–1440. https://doi.org/10.1111/nph.18460

Yu M, Yu J, Hu J, Huang L, Wang Y, Yin X, Nie Y, Meng X, Wang W, Liu Y. Identification of pathogenicity-related genes in the rice pathogen *Ustilaginoidea virens* through random insertional mutagenesis. Fungal Genet. Biol. 2015:**76**:10–19. https://doi.org/10.1016/j.fgb.2015.01.004

Zangerl AR, Rutledge CE. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. Am Nat. 1996:**147**(4):599–608. https://doi.org/10.1086/285868

Zhang C, He J, Dai H, Wang G, Zhang X, Wang C, Shi J, Chen X, Wang D, Wang E. Discriminating symbiosis and immunity signals by receptor competition in rice. PNAS. 2021a:**118**:e2023738118. https://doi.org/10.1073/pnas.2023738118

Zhang K, Zhao Z, Zhang Z, Li Y, Li S, Yao N, Hsiang T, Sun W. Insights into genomic evolution from the chromosomal and mitochondrial genomes of *Ustilaginoidea virens*. Phytopathol. Res. 2021b:**3**(1):9. https://doi.org/10.1186/s42483-021-00086-x

Zhang N, Yang J, Fang AF, Wang J, Li D, Li Y, Wang S, Cui F, Yu J, Liu Y, et al. The essential effector SCRE1 in *Ustilaginoidea virens* suppresses rice immunity via a small peptide region. Mol. Plant Pathol. 2020:**21**(4):445–459. https://doi.org/10.1111/mpp.12894

Zhang Y, Su J, Duan S, Ao Y, Dai J, Liu J, Wang P, Li Y, Liu B, Feng D, et al. A highly efficient rice green tissue protoplast system for transient gene expression and studying light/chloroplast-related processes. Plant Methods. 2011:**7**(1):30. https://doi.org/10.1186/1746-4811-7-30

Zhang Y, Zhang K, Fang A, Han Y, Yang J, Xue M, Bao J, Hu D, Zhou B, Sun X, et al. Specific adaptation of *Ustilaginoidea virens* in occupying host florets revealed by comparative and functional genomics. Nat Commun. 2014:**5**(1):3849. https://doi.org/10.1038/ncomms4849

Zheng X, Fang A, Qiu S, Zhao G, Wang J, Wang S, Wei J, Gao H, Yang J, Mou B, et al. *Ustilaginoidea virens* secretes a family of phosphatases that stabilize the negative immune regulator OsMPK6 and suppress plant immunity. Plant Cell. 2022:**34**(8):3088–3109. https://doi.org/10.1093/plcell/koac154

Zhou L, Lu S, Shan T, Wang P, Sun W, Chen Z, Wang S. Chemistry and biology of mycotoxins from rice false smut pathogen. In: **Melborn BJ, Greene JC**, editors. Mycotoxins: properties, applications and hazards. New York: Nova Science Publishers; 2012. p. 109–130.