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Late Blight Effector Pi05910 Suppresses Plant Immunity by Promoting Destabilisation of Host Scaffold Protein RACK1

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ABSTRACT

Late blight, caused by oomycete pathogen *Phytophthora infestans*, is one of the most devastating diseases of potato and tomato. Plant resistance to pathogens relies on a sophisticated innate immune system, which is often targeted by pathogen-derived effectors. *P. infestans* secretes numerous RXLR effectors to suppress host immunity. In this study, we show that the *P. infestans* core effector Pi05910 functions as a virulence factor and targets the host scaffold protein Receptor for Activated C Kinase 1 (RACK1), which is identified to positively regulate potato resistance to late blight. Functional analyses showed that *StRACK1*-silencing in potato, and tobacco rattle virus (TRV)-based virus-induced *NbRACK1*-silencing in *Nicotiana benthamiana*, consistently increased host susceptibility to *P. infestans* colonisation, whereas transient expression of *NbRACK1* in *N. benthamiana* or stable overexpression of *StRACK1* in potato enhanced late blight resistance. Pi05910 physically interacts with RACK1 and promotes its destabilisation via the host 26S proteasome pathway. The destabilisation of RACK1 led to the inhibition of PAMP elicitor INF1-triggered plant immune responses, including reactive oxygen species burst and expression of defence-related genes such as *StWRKY7* and *StWRKY8*, and suppression of potato and *N. benthamiana* growth and global protein translation. Our findings reveal a strategy whereby a pathogen effector sabotages a key potato immune scaffold *StRACK1* to promote plant susceptibility to *P. infestans* infection, providing new insight into mechanisms of effector-triggered plant susceptibility.

1 | Introduction

A two-layer innate immune system was built up in plants during the long-term co-evolution with pathogens. Many pathogen-derived microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs) derived from pathogens activate pattern-triggered immunity (PTI) through cell surface pattern recognition receptors (PRRs), similar to innate immunity in animals, often by recognition of the same P/MAMPs (Jones et al. 2024; Zhou and Zhang 2020). Pathogens secrete virulence

molecules known as effectors to suppress PTI or interfere with plant physiological responses. Some effectors are either directly or indirectly recognised by intracellular nucleotide-binding leucine-rich repeat receptors (NLRs), triggering NLR-mediated immunity, commonly referred to as effector-triggered immunity (ETI) (Ngou et al. 2022; Zhang et al. 2020; Zhou and Zhang 2020). ETI enhances PTI to effectively activate immunity, indicating that PTI and ETI share similar signalling mechanisms, although they are mediated by different immune receptors (Ngou et al. 2022). In addition, the co-evolution of PTI

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and ETI receptors indicates that the functions of PTI and ETI are interdependent and synergistic, jointly providing complete plant immunity (Zhang et al. 2023).

Oomycete pathogens, particularly *Phytophthora*, encode numerous RXLR effectors that contain a conserved Arg-any amino acid-Leu-Arg (RXLR) motif and play key roles in the arms race between plants and pathogens (Wang, Pruitt, et al. 2022; Wang, McLellan, et al. 2023; Wawra et al. 2017). Multiple *Phytophthora* RXLR effectors have been reported to manipulate diverse host processes, including enzyme activity (Cao et al. 2024; Fan et al. 2018; Li et al. 2025), transcription (Qiu et al. 2023), translation (Lee et al. 2023), post-translational modifications (Wang, Li, et al. 2023), photosynthetic efficiency (Li, Liu, et al. 2024) and intracellular trafficking (Kim et al. 2024). Furthermore, interference with the stability of host target proteins is also an important strategy for effectors to exert virulence functions. The RXLR effector AVR8 of *P. infestans* suppresses plant immunity by targeting and destabilising a desumoylating isopeptidase DeSI2 (Jiang et al. 2023). *P. infestans* effectors Pi20303 and Pi20300 target and stabilise StMKK1, a potato MAPK cascade protein, leading to suppressed plant PTI response (Du et al. 2021). Avr3a-like effectors stabilise host negative immune regulators CAD7 proteins to suppress PTI immunity (Li et al. 2019). *P. infestans* effector Pi18609 promotes COP1-mediated degradation of StBBX27 to suppress potato immunity (Sun et al. 2025). *P. infestans* effector Pi06432 stabilises potato ubiquitin-like domain-containing protein StUDP to inhibit proteasome activity and host immunity (Wang, Li, et al. 2023).

RACK1 is an evolutionarily highly conserved scaffold protein from prokaryotes to eukaryotes, and orchestrates protein interactions in a way that allows it to regulate diverse cellular processes (Adams et al. 2011). In *Arabidopsis thaliana*, RACK1 proteins are composed of three members (RACK1A, RACK1B and RACK1C), with redundant functions to regulate multiple developmental processes including seed germination (Li, Zhang, et al. 2024), leaf development (Masood et al. 2023) and flowering (Li et al. 2021).

RACK1 is also reported to be involved in the regulation of plant immunity. In *Nicotiana benthamiana*, NbRACK1 is an important component of activation of the hypersensitive response (HR) triggered by co-expression of *P. infestans* effector PiAvr3a and its cognate resistance gene *R3a* (Hu et al. 2024). RACK1 functions as a scaffold that recruits immune-related proteins such as SGT1, HSP90, RbohB and CDPKiso2 to activate host immunity (Hyodo et al. 2019; Hu et al. 2024; Nakashima et al. 2008). In rice, overproduction of *OsRACK1A* confers rice floral resistance to false smut disease without yield penalty (Li et al. 2022). In *A. thaliana*, RACK1 plays prominent roles in regulating plant immunity via bridging the G β subunit and MEKK1, MKK4/5 and MAPK3/6 (Cheng et al. 2015). However, little is known about RACK1 proteins involved in regulating potato immunity to late blight.

A dual role of RACK1 in signalling and translational regulation has been recognised in eukaryotes. RACK1 and its orthologues are associated with the 40S small ribosomal subunit in human (Link et al. 1999), yeasts (Inada et al. 2002; Shor et al. 2003), *A. thaliana* (Chang et al. 2005) and algae (Manuell et al. 2005).

RACK1 is a vital component of the functional 80S ribosome and hence activates protein translation (Islas-Flores et al. 2015). RACK1 also activates the switch from canonical cap-dependent translation to R-motif-mediated translation through scaffolding MPK3/6 to phosphorylate eIF4G, eIFiso4G and PABP (Wang, Zhang, et al. 2022).

In this study, we found that stable overexpression of Pi05910, a candidate core RXLR effector of *P. infestans* (Yin et al. 2017), rendered potato more susceptible, suggesting its virulence function. We show that Pi05910 targets and destabilises host scaffold protein RACK1 to suppress potato resistance to late blight, PAMP elicitor INF1-triggered plant immune responses and translation via the 26S proteasome pathway. This research highlights a mechanism by which *P. infestans* promotes infection by suppressing scaffold protein RACK1-mediated host defence responses.

2 | Results

2.1 | Pi05910 Inhibits Potato Growth and Immunity

Our previous studies showed that the RXLR effector gene *Pi05910* of *P. infestans* is upregulated during early stages of plant infection (Yin et al. 2017). *Agrobacterium tumefaciens*-mediated transient expression of *Pi05910* in *N. benthamiana* also showed enhanced plant colonisation by *P. infestans* (Zhang et al. 2024). To further investigate Pi05910 function, we generated stable transgenic potato (cultivar Atlantic) lines overexpressing *GFP-Pi05910* (OE-Pi05910). The expression level of *Pi05910* in transgenic potato lines was determined by reverse transcription-quantitative PCR (RT-qPCR) (Figure S1a). Western blot analysis confirmed the expression and integrity of GFP-Pi05910 fusion protein (Figure S1b). Phenotypic assessment revealed that OE-Pi05910 potato plants exhibited reduced plant height compared to the control plants stably expressing *GFP* (Figure 1a). *P. infestans* inoculation assay showed that OE-Pi05910 potato plants were more susceptible to *P. infestans* (Figure 1b), with H₂O₂ accumulation significantly attenuated compared with that of OE-GFP potato transformants (Figure 1c). Consistently, biomass quantification showed a significant increase of *P. infestans* colonisation in OE-Pi05910 potato plants (Figure S2). Furthermore, the expression of defence-related genes *StWRKY7* and *StWRKY8* in transgenic potato lines were significantly attenuated compared with that of OE-GFP potato transgenic plants upon treatment with *P. infestans* PAMP elicitor INF1 (Figure 1d).

Taken together, these results demonstrate that *Pi05910* expression in potato inhibits both plant growth and disease resistance.

2.2 | Pi05910 Interacts With the Host Scaffold Protein RACK1

To dissect Pi05910-mediated plant susceptibility, we investigated its host target proteins using liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay. Three unique peptides of NbRACK1 (LC369496) were identified in

GFP-Pi05910-Immunoprecipitation (IP) samples but absent in the GFP-IP negative control. By performing a BLAST search in the Solanaceae Genomics Network (<https://solgenomics.net/>

[tools/blast](#)), we identified three RACK1 orthologs in the potato genome, designated as StRACK1A (PGSC0003DMP400027754), StRACK1B (PGSC0003DMP400050159) and StRACK1C

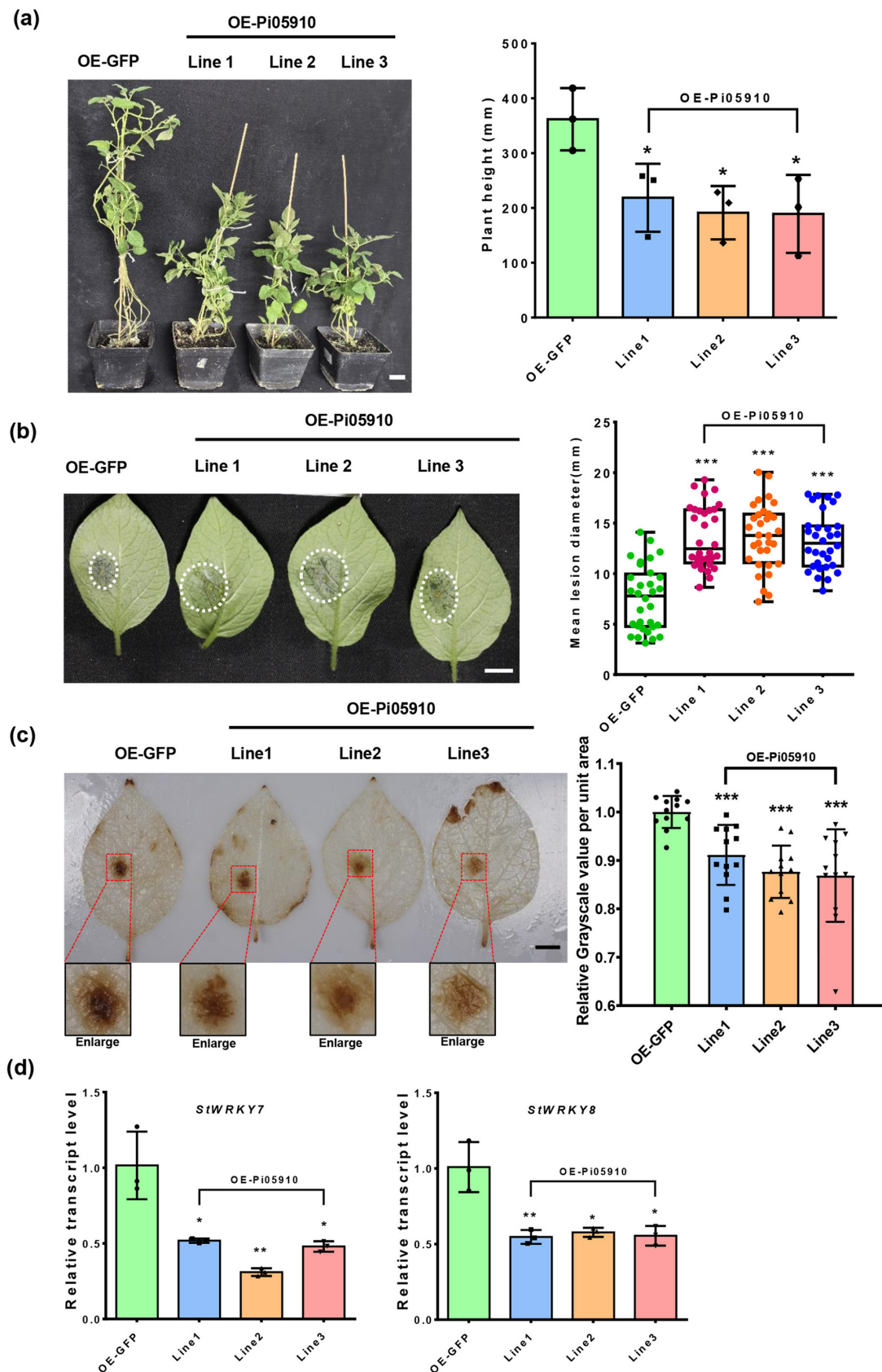


FIGURE 1 | Legend on next page.

FIGURE 1 | The RXLR effector Pi05910 of *Phytophthora infestans* inhibits potato growth and immunity. (a) Growth phenotype of three 40-day OE-Pi05910 transgenic Atlantic potato lines. Student's *t*-test was used for the statistical analysis ($*p < 0.05$, $n = 3$). Error bars indicate mean $\pm$ SE. The values were distributed in dots. Scale bar = 30 mm. (b) *P. infestans* infection assays on three OE-Pi05910 transgenic potato lines. The lesion size was measured at 4 days post-inoculation (dpi). Dotted white circles indicate lesion areas. Student's *t*-test was used for the statistical analysis ($***p < 0.001$). Scale bar = 10 mm. (c) 3,3'-diaminobenzidine (DAB) staining to indicate less reactive oxygen species (ROS) accumulation in three OE-Pi05910 transgenic potato lines at 3 dpi. Greyscale value plot shows the intensity of DAB staining in the image. The greyscale value of the OE-GFP control was set to 1. Student's *t*-test was used for the statistical analysis ($***p < 0.001$). The values were distributed in dots. Scale bar = 10 mm. (d) Reverse transcription-quantitative PCR (RT-qPCR) assay was performed at 3 h after treatment with 10 μ M INF1. *STEF-1 α* was used for normalisation in RT-qPCR assays. Student's *t*-test was used for the statistical analysis ($*p < 0.05$, $**p < 0.01$). Error bars represent mean values $\pm$ SD of 3 technical replicates ($n \geq 6$ biologically independent leaves).

(PGSC0003DMP400009959). StRACK1A is 91.2% identical to NbRACK1, while StRACK1B and StRACK1C share 91.8% and 93.3% identities with NbRACK1, respectively (Figure S3). Phylogenetically, RACK1 proteins are highly conserved across distant plant species (Figure S4a,b). RACK1 contains seven repeats of WD40 domains (Figure S4c).

Among the three StRACK1 proteins, StRACK1C shows the highest sequence similarity with NbRACK1. To confirm its interaction with Pi05910, a luciferase complementation imaging (LCI) assay was performed using nLuc-Pi05910 and StRACK1C-cLuc, nLuc-Pi17063 with NbRab-G3c-cLuc (Liu et al. 2025) combination as a positive control, and nLuc-Pi05910 with NbRab-G3c-cLuc or nLuc-Pi17063 with StRACK1C-cLuc combination as a negative control. The signal of luciferase catalytic activity restored by Pi05910 and StRACK1C proteins was strong, while no signals were detected in the negative control (Figure 2a). To further confirm their interaction, co-immunoprecipitation (co-IP) assay was performed using C-terminal FLAG-tagged Pi05910, co-expressed with C-terminal mCherry-tagged StRACK1C in *N. benthamiana*. Pi05910-FLAG was detected by the FLAG antibody when co-immunoprecipitated with StRACK1C-mCherry, but not with that of mCherry control (Figure 2b). Moreover, isothermal titration calorimetry (ITC) assay with purified recombinant proteins (Figure S5) showed that Pi05910 bound with StRACK1C in vitro (Figure 2c).

We further investigated the subcellular localisation of Pi05910 and the full-length StRACK1C. *A. tumefaciens* cells carrying Pi05910 and StRACK1C constructs were co-infiltrated in *N. benthamiana* leaves. Confocal microscopy showed their co-localisation in the nucleus and cytoplasm (Figure 2d). Bimolecular fluorescence complementation (BiFC) assay consistently showed their interaction in the host nucleus and cytoplasm (Figure S6a), western blot analysis confirmed the expression and integrity of nYFP-Pi05910 and cYFP-StRACK1C fusion proteins (Figure S6b). To determine whether Pi05910 also interacts with StRACK1A, StRACK1B and NbRACK1, LCI and co-IP assays were performed, with results showing stable interactions of Pi05910 with StRACK1A, StRACK1B and NbRACK1 (Figures 2b and S7a–d). Confocal microscopy showed that Pi05910 co-localised with StRACK1A or StRACK1B in the nucleus and cytoplasm (Figure S8).

Taken together, these results indicate that Pi05910 physically interacts with StRACK1A, StRACK1B, StRACK1C and NbRACK1.

2.3 | Pi05910 Promotes RACK1 Destabilisation via 26S Proteasome Pathway

Because StRACK1C shows the highest sequence similarity with NbRACK1, StRACK1C was selected for further research. In previous co-localisation analysis, we found that co-overexpression with Pi05910 decreased the fluorescence intensity of NbRACK1 and StRACK1C (Figure S9a–c), suggesting potential Pi05910-promoted destabilisation of RACK1 proteins. We investigated RACK1 protein abundance following co-overexpressing GFP-Pi05910 and StRACK1C-mCherry or NbRACK1-mCherry in *N. benthamiana*. The results showed that co-expression with Pi05910 led to a significant reduction in StRACK1C and NbRACK1 protein levels compared with co-expression with GFP (Figure S9d). In addition, we tested the influence of different agrobacterial concentrations ($OD_{600} = 0.1, 0.3, 0.5$) that carry GFP-Pi05910 or GFP on StRACK1C-mCherry stability. The results demonstrated that StRACK1C levels barely changed with the increasing concentrations of GFP-carrying agrobacteria, but decreased significantly with the increasing concentrations of Pi05910-carrying agrobacteria (Figure 3a), suggesting that Pi05910 promotes destabilisation of StRACK1C in a dose-dependent manner.

To determine mechanisms underlying Pi05910-promoted StRACK1C destabilisation, we first co-infiltrated agrobacteria carrying GFP-Pi05910 and StRACK1C-mCherry into *N. benthamiana* leaves and examined the mRNA abundance of StRACK1. The results showed that Pi05910 did not affect the transcript levels of StRACK1 (Figure 3b).

The 26S proteasome and autophagy are two major pathways that function in protein degradation in eukaryotic cells, and targets of pathogen effectors (Wang, Li, et al. 2023). To test by which manner StRACK1C destabilisation is promoted by Pi05910, GFP-Pi05910 was transiently co-expressed with StRACK1C-mCherry in *N. benthamiana* leaves and treated with the 26S proteasome inhibitor MG132 or the autophagy inhibitor 3-methyladenine (3-MA). Compared with infiltration with negative control dimethyl sulphoxide (DMSO), the destabilisation of StRACK1C was abolished in the presence of MG132 but not 3-MA (Figure 3c), indicating that Pi05910 promotes destabilization of StRACK1C through the 26S proteasome but not autophagy pathway. Consistently, NbRACK1 destabilisation is also promoted by Pi05910 through the 26S proteasome (Figure S10a,b).

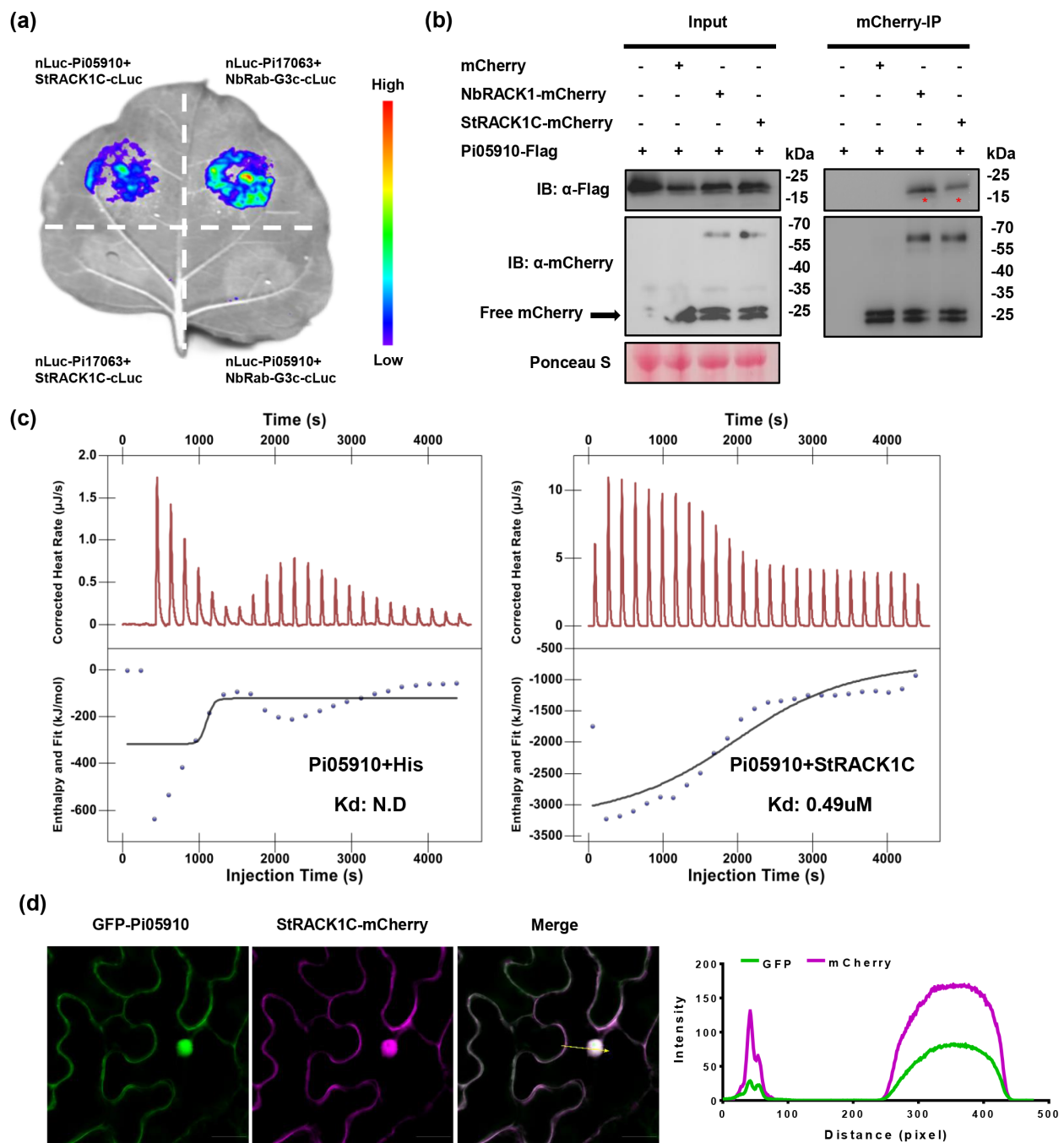


FIGURE 2 | Pi05910 interacts with host scaffold protein StRACK1C. (a) A luciferase complementation assay to indicate the interaction between Pi05910 and StRACK1C. nLuc-Pi17063 with NbRab-G3c-cLuc combination was used as a positive control, and nLuc-Pi05910 with NbRab-G3c-cLuc or nLuc-Pi17063 with StRACK1C-cLuc combination as a negative control. Leaves were photographed at 3 days post-infiltration (dpi). (b) Co-immunoprecipitation assay was performed with mCherry-trap beads. Total proteins were extracted from *Nicotiana benthamiana* leaves at 3 dpi. Co-precipitation was detected via western blot. Ponceau S indicates equal loading of protein samples. (c) In vitro interaction of Pi05910 with StRACK1C. Recombinant StRACK1C protein was titrated with identical concentrations of Pi05910 or His tag proteins in isothermal titration calorimetry assays. Kd, dissociation constant; N. D., not detectable. (d) Co-localisation was performed by co-expression of *StRACK1C-mCherry* and *GFP-Pi05910* in *N. benthamiana* leaves via agroinfiltration. Images were taken under a confocal microscope. Scale bars = 20 μ m. Fluorescence intensity was quantitated in the transect (yellow line) with ImageJ.

2.4 | Pi05910 Inhibits Protein Translation by Promoting Destabilisation of RACK1

RACK1 is a 40S ribosome-associated protein (Ceci et al. 2003; Sengupta et al. 2004), positively regulating

PABP/R-motif-mediated cap-independent translation reprogramming upon PTI induction in *Arabidopsis thaliana* (Wang, Zhang, et al. 2022). To investigate the role of NbRACK1 in protein translation, tobacco rattle virus (TRV)-induced gene silencing (VIGS) assay was employed to downregulate *NbRACK1*

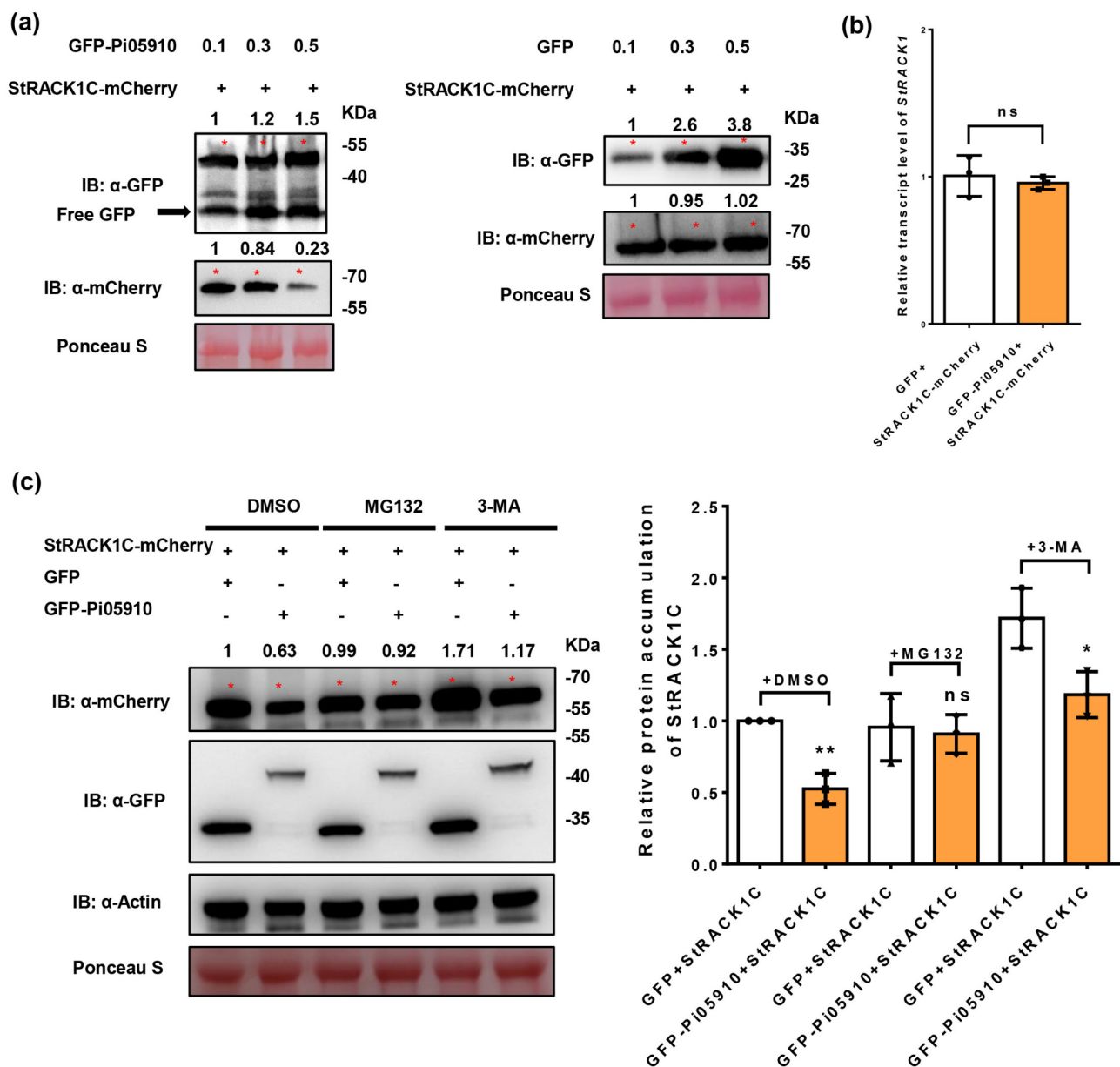


FIGURE 3 | Pi05910 promotes destabilisation of RACK1 via the 26S proteasome pathway. (a) Pi05910 destabilises StRACK1C in a dosage-dependent manner. The protein abundance of StRACK1C was detected by western blot and quantified using ImageJ. Different concentrations of agrobacterial suspensions carrying *GFP-Pi05910* or *GFP* constructs (OD_{600} values of 0.1, 0.3, 0.5) were co-infiltrated with *StRACK1C-mCherry* (OD_{600} value of 0.3) in *Nicotiana benthamiana*. (b) The relative transcript level of *StRACK1* was determined by reverse transcription-quantitative PCR with *NbActin* as an internal control. Student's *t*-test was used for the statistical analysis (ns = not significant). (c) Pi05910 destabilises StRACK1C in *N. benthamiana*. *StRACK1C-mCherry* was co-expressed with *GFP-Pi05910* or *GFP* control in *N. benthamiana* leaves, followed by treatment with MG132, 3-methyladenine (3-MA), or dimethyl sulphoxide (DMSO) at 36 h after agroinfiltration. Proteins were extracted at 12 h after treatments. Protein levels were quantified using ImageJ. Student's *t*-test was used for the statistical analysis (* $p < 0.05$, ** $p < 0.01$, ns = not significant). Error bars represent mean values $\pm$ SD of 3 technical replicates ($n \geq 6$ biologically independent leaves).

expression in *N. benthamiana*. A 306 bp fragment (466–771 bp) was selected to prepare *NbRACK1*-silenced plants (*TRV-NbRACK1*) (Figure S11a) and the relative transcript level of *NbRACK1* was confirmed by RT-qPCR (Figure S11c). Although *TRV-NbRACK1* plants developed narrow leaves and reduced heights, severe morphological defects such as chlorotic phenotypes were not observed (Figure S11b).

To examine the function of Pi05910 and RACK1 in the process of protein translation, agrobacteria cells carrying

mCherry and *Pi05910-mCherry* were infiltrated into *GFP*- or *TRV-NbRACK1* *N. benthamiana* leaves, followed by infiltration of leaves with $10 \mu\text{M}$ INF1, a PAMP elicitor of *P. infestans*. The surface sensing of translation (SUnSET) assay (Van Hoewyk 2016) was employed to examine de novo protein synthesis. The level of newly synthesised proteins from *N. benthamiana* following treatment with puromycin was measured using an anti-puromycin antibody. As in the in vivo translation system, de novo synthesised protein was barely detectable after treatment with cycloheximide (CHX),

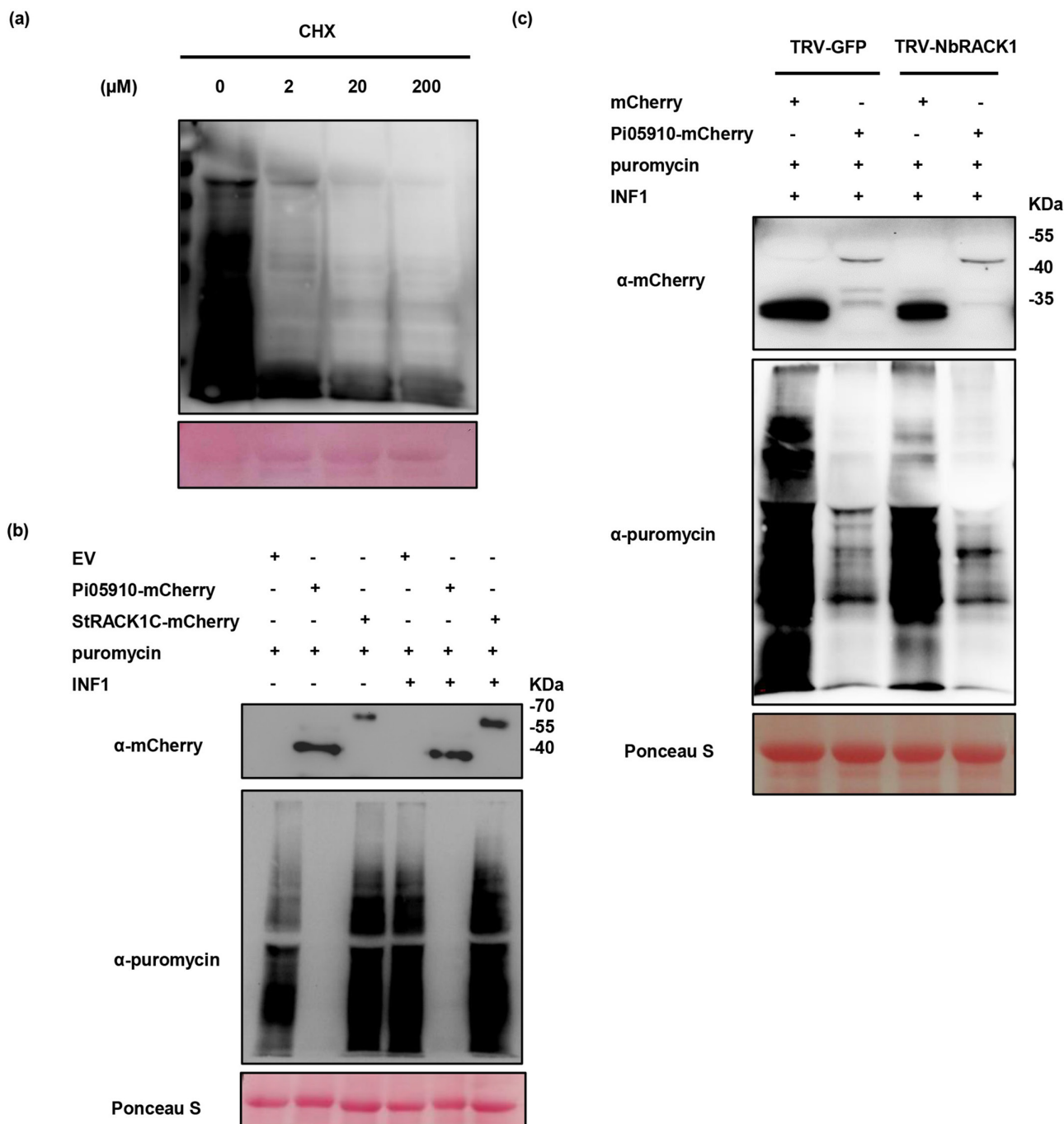


FIGURE 4 | Pi05910 inhibits protein translation by promoting destabilisation of RACK1. (a) De novo synthesised proteins were detected by western blot after treatment with cycloheximide (CHX). Ponceau S indicates equal loading of protein samples. (b, c) Detection of newly synthesised proteins in *Nicotiana benthamiana* leaves transiently expressing Pi05910- or StRACK1C-mCherry and TRV-GFP or TRV-NbRACK1. mCherry or empty vector (EV) was used as a negative control; the loading control was visualised by Ponceau S staining. The newly synthesised proteins were measured using an anti-puromycin antibody.

a translation inhibitor (Figure 4a). Both the overexpression of Pi05910 and silencing of NbRACK1 led to a significant reduction in de novo protein translation (Figure 4b,c). In addition, INF1 treatment or overexpression of StRACK1C increased protein translation compared with that of control (Figure 4b). Taken together, these results suggest that Pi05910 suppresses protein translation by promoting destabilisation of RACK1.

2.5 | RACK1 Is Required for Plant Resistance to *P. infestans*

To investigate the immune function of RACK1, we determined its expression pattern during *P. infestans* infection by RT-qPCR analysis. The transcript levels of NbRACK1 and StRACK1 were upregulated during *P. infestans* infection (Figures S12 and S13).

We then performed infection assay following its heterologous transient expression in *N. benthamiana* by agroinfiltration. Transient expression of *GFP-NbRACK1* followed by *P. infestans* infection produced significantly smaller lesions compared with the control *GFP-FLAG* (Figure S14a). Consistently, the *P. infestans* biomass was significantly lower in *GFP-NbRACK1*-expressing leaves compared to the control (Figure S14b). The expression and integrity of *GFP-NbRACK1* and *GFP-FLAG* fusion proteins were confirmed by western blotting (Figure S14c).

We further prepared *NbRACK1*-silencing *N. benthamiana* plants by agroinfiltration-mediated transient expression of *TRV-NbRACK1*, with *TRV-GFP* as control. The detached leaves were inoculated with *P. infestans* zoospores. At 6 days post-inoculation (dpi), the mean lesion size of *TRV-NbRACK1* leaves was significantly larger than that of the control *TRV-GFP* leaves (Figure 5a), indicating that silencing of *NbRACK1* promotes *P. infestans* colonisation. Consistently, the *P. infestans* biomass was significantly higher in *TRV-NbRACK1* leaves compared to that of *TRV-GFP* plants (Figure 5b).

To further examine the potential role of *RACK1* in the natural potato host of *P. infestans*, we generated *StRACK1*-RNAi (RNAi-1, -2 and -3) and *StRACK1C*-overexpression (OE-1, -2 and -3) transgenic lines using cultivar Atlantic. RT-qPCR analysis confirmed that the overexpression lines exhibited 12- to 19-fold higher *StRACK1* expression (Figure S15b), while the RNAi lines showed significantly reduced transcript levels (Figure S16b) compared with the control plants. No obvious growth phenotype differences were observed between OE-*StRACK1C* and OE-*GFP* transformants (Figure S15a), except that RNAi-*StRACK1* transformants showed reduced heights compared with wild-type (WT) after 7 weeks under the controlled condition (Figure S16a). Infection assays with *P. infestans* revealed decreased resistance in RNAi lines and increased resistance in OE lines, as indicated by lesion diameters and pathogen colonisation (Figures 5c, S15c,d and S16c).

Taken together, these results demonstrate that *RACK1* positively regulates plant resistance to *P. infestans* in both *N. benthamiana* and potato.

2.6 | Silencing of *RACK1* Attenuates Reactive Oxygen Species Production and INF1-Triggered Immune Responses

RACK1 is known as a scaffold protein that bridges MPK3/6 with the heterotrimeric G-protein complex during protease IV triggered PTI responses in *A. thaliana* (Cheng et al. 2015). To confirm the function of *NbRACK1* in INF1-triggered immunity, *TRV-NbRACK1* plants were treated with INF1. Transcript levels of the defence-related marker genes *NbWRKY7* and *NbWRKY8* showed substantially compromised levels in *NbRACK1*-silenced *N. benthamiana* leaves compared to the control following INF1 treatment (Figure S18). These results indicate that *NbRACK1* positively regulates INF1-triggered immune responses.

To detect whether *StRACK1* is involved in the PTI signalling pathway and reactive oxygen species (ROS) production, leaves of potato *StRACK1* RNAi transformants were inoculated with

P. infestans. DAB (3,3'-diaminobenzidine tetramine hydrochloride) staining showed decreased ROS accumulation in *P. infestans*-infected tissues in *StRACK1* RNAi lines (Figure 5d) but increased in OE-*StRACK1C* lines (Figure S17) compared to that of control. Furthermore, the expression of defence-related genes *StWRKY7* and *StWRKY8* (Figure 5e) in *StRACK1*-RNAi lines was significantly attenuated compared with that of the WT upon INF1 treatment. These results indicate that *StRACK1* positively regulates ROS production and INF1-triggered immune responses.

3 | Discussion

Plant pathogens secrete a large array of effectors to modulate host cell processes and facilitate a successful infection. Studies on the host targets of effectors provide molecular clues for understanding the outcome of plant-pathogen interactions. The core RXLR effector Pi05910 from *P. infestans* features a conserved protein sequence and is highly upregulated during early infection stages of *P. infestans* (Yin et al. 2017), has been shown to enhance host susceptibility to *P. infestans* (Zhang et al. 2024). In this study, we showed that the potato scaffold protein *StRACK1* is a virulence target of *P. infestans* effector Pi05910. We demonstrate that Pi05910 interacts with *StRACK1* and promotes its destabilisation via the 26S proteasome pathway, thereby disrupting host protein translation and INF1-triggered immune responses, leading to suppression of plant immunity and growth (Figure 6).

Plants have evolved intricate innate immune systems to defend against pathogen attacks. *RACK1* is a conserved scaffold protein and acts as a positive regulator of multiple immune-related processes (Cheng et al. 2015; Hu et al. 2024; Islas-Flores et al. 2015; Li et al. 2022). Previous studies indicated that *RACK1* in various plants regulates the resistance to multiple pathogens, such as maize to *Exserohilum turcicum* (Wang et al. 2014), *A. thaliana* to *Pseudomonas aeruginosa* (Cheng et al. 2015), rice to *Ustilaginoidea virens* (Li et al. 2022), and wheat to soil-borne viruses (Hu et al. 2024). We found that *RACK1* from both *N. benthamiana* and potato are responsive and upregulated during early stages of *P. infestans* infection and positively regulates INF1-triggered PTI responses and resistance to *P. infestans*, suggesting functional conservation of *RACK1* in different plant species.

Given the central role of *RACK1* in plant immunity, its targeting by pathogen effectors represents a common virulence strategy. For instance, the *U. virens* effector UvCBP1 disrupts the OsRACK1A-OsRBOHB interaction to suppress ROS bursts (Li et al. 2022). Here, using integrated approaches including IP-mass spectrometry, LCI, BiFC, ITC and co-IP, we provide evidence that Pi05910 targets *RACK1* and destabilises host scaffold protein *RACK1*, a central integrative hub in various signalling pathways via 26S proteasome pathway (Cheng et al. 2015; Masood et al. 2023; Wang, Zhang, et al. 2022). *RACK1* is indispensable for plant immunity and plant development, our results consistently showed that expression of *Pi05910* or silencing of *RACK1* led to morphological defects in plants. However, overexpression of *StRACK1C* did not induce obvious changes in growth phenotype. Plant metabolic network possesses a robust homeostatic mechanism: for proteins critical to plant growth

and development, the metabolic regulatory capacity provided by endogenous expression levels under normal conditions is already sufficient for their function, and additional expression

fails to further drive metabolic flux changes. However, when expression is insufficient, metabolic homeostasis is disrupted and the deficiency of key metabolites triggers pleiotropic

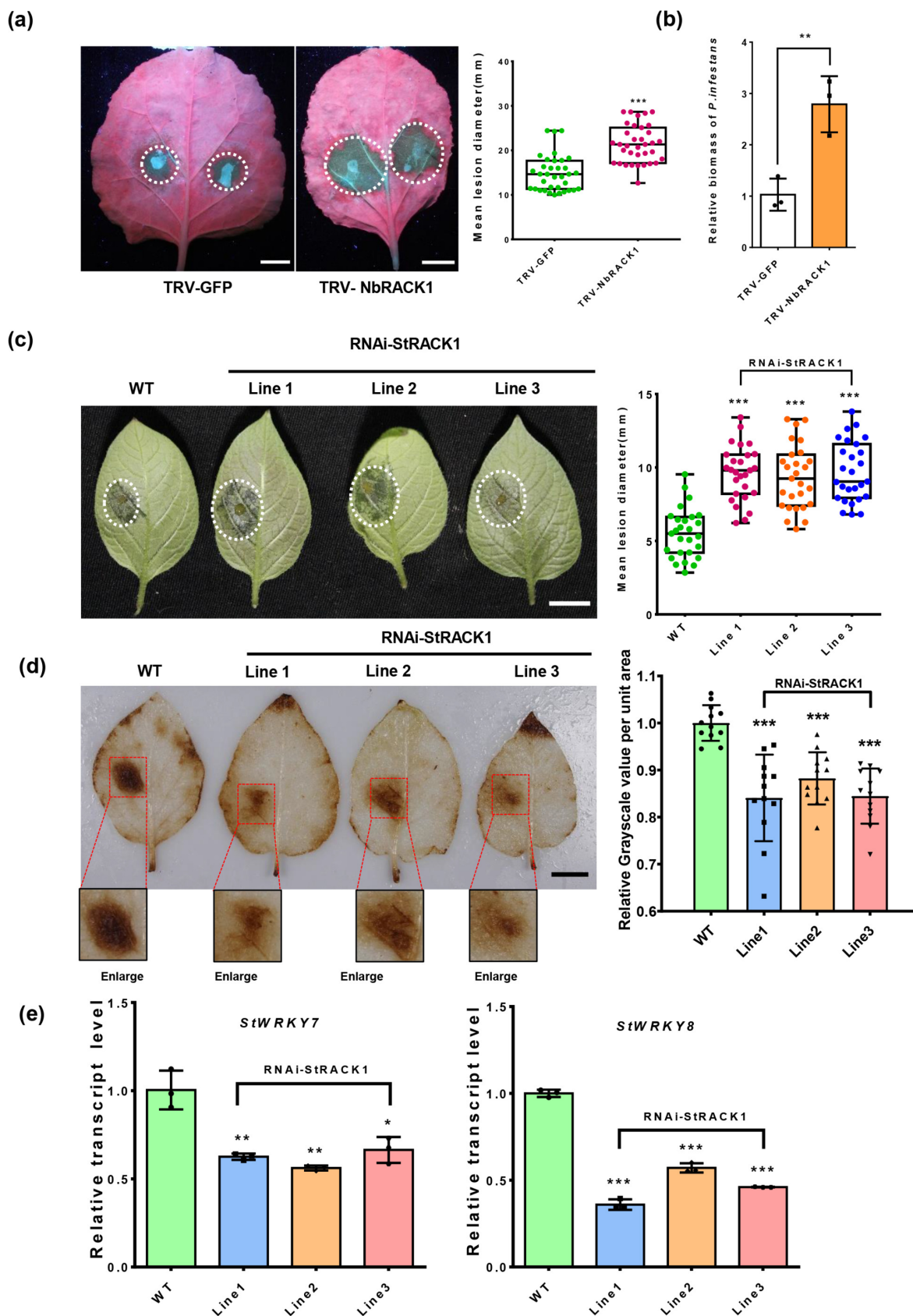


FIGURE 5 | Legend on next page.

FIGURE 5 | *RACK1* positively regulates late blight resistance and PAMP-triggered immunity (PTI) responses. (a) Infection of *NbRACK1*-silenced *Nicotiana benthamiana* leaves with *Phytophthora infestans* at 6 days post-inoculation (dpi). Dotted white circles indicate lesion areas. Student's *t*-test was used for the statistical analysis ($***p < 0.001$). Scale bar = 10 mm. (b) *P. infestans* biomass was quantified by quantitative PCR, with *NbActin* and *PiActin* served as internal standards for *N. benthamiana* and *P. infestans*, respectively. Student's *t*-test was used for the statistical analysis, ($**p < 0.01$). (c) Infection of three *StRACK1*-silencing potato transformants with *P. infestans* at 4 dpi. Dotted white circles indicate lesion areas. Student's *t*-test was used for the statistical analysis ($***p < 0.001$). WT, wild-type. Scale bar = 10 mm. (d) 3,3'-diaminobenzidine (DAB) staining to show less reactive oxygen species (ROS) accumulation in *StRACK1*-silenced potato transformants at 4 dpi. Greyscale value plot shows the intensity of DAB staining in the image. The greyscale value of WT control leaves was set to 1. Student's *t*-test was used for the statistical analysis ($***p < 0.001$). The values were distributed in dots. Scale bar = 10 mm. (e) Transcript levels of *StWRKY7/8* in three *StRACK1*-silenced potato transformants at 3 h after treatment with 10 μ M INF1, with *StEF-1 α* as an internal control. Student's *t*-test was used for the statistical analysis ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). Error bars represent mean values $\pm$ SD of 3 technical replicates ($n \geq 6$ biologically independent leaves).

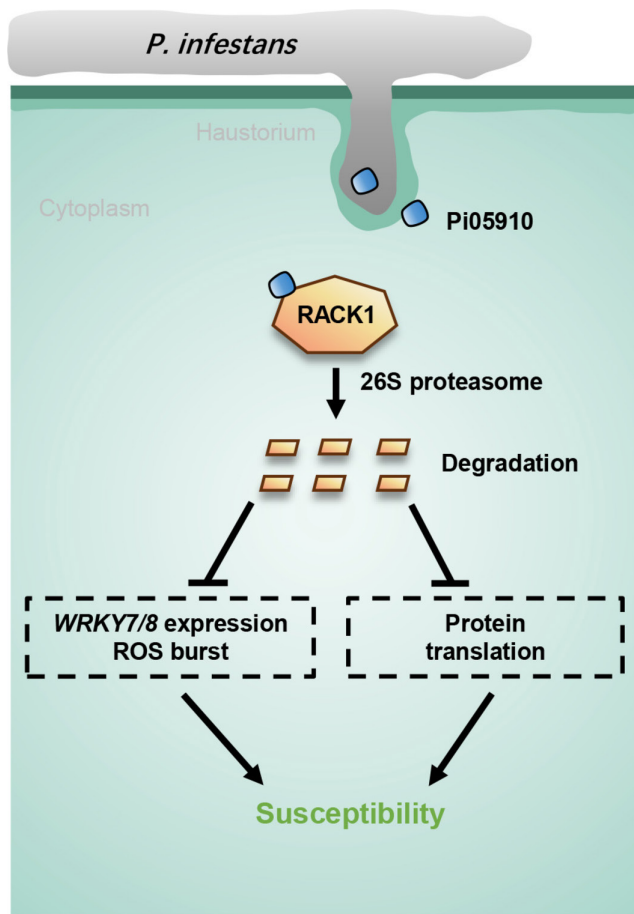


FIGURE 6 | A model for the role of Pi05910 in suppressing plant growth and immunity by targeting and promoting destabilisation of host scaffold protein RACK1. *Phytophthora infestans* effector Pi05910 targets and destabilises host scaffold protein RACK1, a positive plant immune regulator, via the 26S proteasome pathway, leading to inhibition of global protein translation and plant PAMP-triggered immunity (PTI) responses, including reactive oxygen species (ROS) burst and expression of defence-related genes.

developmental defects. For example, Li et al. (2018) generated *GmRACK1*-overexpressing soybean lines and found no obvious morphological differences between the overexpression lines and the wild type under normal conditions. In potato, when overexpressing *Snakin-1* (*SN1*), potato lines did not show remarkable morphological differences from the WT; however, *SN1*-silencing resulted in reduced height. The study further revealed that its

silencing led to significant changes in key metabolites such as tricarboxylic acid cycle (TCA) cycle intermediates, ascorbate and its precursors, whereas the overexpression lines were highly similar to the WT at the metabolic level (Nahirňak et al. 2012).

Interestingly, Pi05910 has been reported to promote destabilisation of glycolate oxidase (GOX) independent of the 26S proteasome pathway (Zhang et al. 2024), being different from its promoted degradation of RACK1 proteins. Our previous study (Zhang et al. 2024) demonstrated that GOX positively regulates ROS production in plants and Pi05910 suppresses both the enzymatic activity and protein stability of GOX. Therefore, the impact of Pi05910 on ROS (Figure 1c) may be due in part to its targeting of GOX. GOX mainly produces ROS in peroxisomes (Zhang et al. 2024), whereas RACK1 primarily regulates ROS generated by NADPH oxidases on the plasma membrane (Nakashima et al. 2008; Hyodo et al. 2019; Li et al. 2022). GOX and RACK1 represent two key nodes in the plant ROS regulatory network. They produce or modulate ROS via distinct subcellular pathways and jointly participate in plant immune responses. Further investigation into the cross-regulatory mechanism between these two proteins will facilitate improved understanding of the complex network of plant immunity.

Another key virulence strategy employed by pathogen effectors is the inhibition of host protein translation. For example, *P. infestans* effector Pi23226 inflates the size of the nucleolus, subverts 25S pre-rRNA processing by binding immature 25S rRNA and subsequently suppresses protein translation (Lee et al. 2023). We found that Pi05910 inhibits global protein translation by promoting destabilisation of RACK1 proteins. RACK1 is a ribosome protein that regulates cap-independent translation (Hyodo et al. 2019). RACK1 also activates the switch from canonical cap-dependent translation to R-motif-mediated translation through scaffolding MPK3/6 to phosphorylate eIF4G, eIFiso4G and PABP to promote plant immunity (Wang, Zhang, et al. 2022). Our data indicate that both *NbRACK1* and *StRACK1* are required for protein translation during INF1-triggered immune responses.

In summary, this study establishes that the *P. infestans* effector Pi05910 suppress plant immunity and protein translation by promoting destabilisation of the host RACK1 via 26S proteasome pathway. Our findings reveal a mechanism by which an oomycete effector disturbs host immunity through destabilising a conserved host scaffold protein, providing insights into effector-triggered plant susceptibility to oomycete pathogens.

4 | Experimental Procedures

4.1 | Materials and Plant Growth Conditions

Escherichia coli DH5 α and BL21 were used for plasmid extraction and expression and purification of recombinant proteins, respectively. *A. tumefaciens* GV3101 and AGL1 were used for transient expression and stable transformation, respectively. *P. infestans* 88069 was used for infection assay. *P. infestans* was cultured at 16°C on rye-sucrose agar (RSA) plates (60–80 g of rye was boiled in 750 mL ultrapure water, the filtrate was collected, 20 g sucrose added and the volume adjusted to 1 L with ultrapure water, and solidified with 8 g agar). *N. benthamiana* and potato (*Solanum tuberosum*) plants were grown in the growth chamber with 5000 lx (LED lamps, OUTRACE, OU016-U4B) and a 13/11 h light/dark cycle at 25°C.

4.2 | Plasmid Constructs

To perform transient gene overexpression assay, the full-length coding sequences of *RACK1s* were amplified by PCR and cloned into pART27 (Gleave 1992). The sequences of *Pi05910* and *RACK1s* were amplified by FastPfu DNA polymerase (Transgene), purified and cloned into the pART27-NGFP (Zhang et al. 2021) vector at EcoRI and XbaI sites (New England Biolabs) using MonClone Single Assembly Cloning Mix (Monad) or T4 DNA ligase (Thermo Scientific). *Pi05910* and *RACK1* gene sequences were inserted into pART27-CmCherry between the EcoRI and XhoI sites. To generate VIGS constructs, a 306 bp fragment of *NbRACK1* was amplified from *N. benthamiana* cDNA and cloned into the binary vector pTRV2, while *TRV2-GFP* was constructed as a control. A 261 bp DNA fragment of *StRACK1* was PCR-amplified, purified and the sense fragment was ligated into the pART27 vector at the XhoI and KpnI sites, and subsequently the antisense *StRACK1C* DNA fragment was cloned into the same vector at the XbaI and ClaI sites to generate the RNA interference (RNAi) construct *StRACK1-RNAi*. For BiFC assay, *Pi05910* was cloned into the StuI and KpnI sites of pDEST-VYNE(R) Gateway vector to form the *nYFP-Pi05910* plasmid. *StRACK1C* was cloned into the SpeI and XhoI sites of pDEST-VYCE(R) Gateway vector to form the *cYFP-StRACK1C* plasmid. For luciferase complementation assay, *Pi05910* was cloned into pCambia1300-nLUC while *StRACK1* family members were cloned into pCambia1300-cLUC. For expression and purification of recombinant proteins, all genes were inserted in the pET-32a vector. All constructs were confirmed by sequencing at Tsingke (Beijing, China). All primers used for plasmid constructions are listed in Table S1.

4.3 | Agrobacterium tumefaciens-Mediated Transient Gene Expression

Agrobacterium tumefaciens GV3101 or AGL1 carrying the target gene construct was centrifuged at 4000g for 5 min to collect agrobacterial cells. The precipitated cells were suspended in MES suspension with 100 μ M acetosyringone. The concentration of agrobacterial suspension was measured by spectrophotometer. Using a blunt syringe with the needle removed, agrobacterial suspensions at appropriate concentrations were infiltrated into leaves from the flat area between the veins on the back of the leaf (Elnahal et al. 2020; Meng et al. 2015).

4.4 | Confocal Microscopy Imaging

Confocal imaging was performed using an FV3000 confocal microscope (Olympus). Respective fusion constructs were co-expressed via agroinfiltration in *N. benthamiana* leaves 3 days before confocal observation. GFP and YFP were excited at 488 nm and detected at 500–540 nm. For mCherry observation, fluorescence was excited at 559 nm and detected at 580–630 nm.

4.5 | Infection Assays With P. infestans

To prepare zoospores, *P. infestans* was cultured on solid RSA at 16°C for 10–15 days, followed by flooding with 5 mL of precooled sterile distilled water. The plates were incubated for 1–2 h in a refrigerator at 4°C to fully release zoospores. To perform infection assays on *N. benthamiana* leaves, about 1000 *P. infestans* zoospores were drop-inoculated on detached leaves and placed in a dark incubator at 16°C. After 5–7 days, the lesion diameters were measured. To infect potato leaves, about 500 *P. infestans* zoospores were drop-inoculated on potato leaves and placed in a dark incubator at 16°C, and the lesion was scored as described above after 3–5 days. For measurement of the relative biomass of *P. infestans*, DNA was extracted from the lesion areas of at least 10 *N. benthamiana* or potato leaves and pooled. For quantitative PCR assays, genomic DNA was used employing primers specific to the *PiActin* and *NbActin* or *StEF-1 α* genes (Dong et al. 2025).

4.6 | LC-MS/MS Analysis

Total protein was extracted from *N. benthamiana* leaves using RIP A protein extraction buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 3 mM NaF, 1 mM sodium orthovanadate, 1 mM EDTA, 2% PVPP, 1 mM dithiothreitol and 1 mM PMSF) with a protease inhibitor cocktail (Sigma). GFP-trap agarose beads (Chromotek) were used for immunoprecipitation according to the manufacturer's protocol. Protein samples were digested into short peptides using trypsin hydrolysis then analysed by liquid chromatography–tandem mass spectrometry (Triple Quad 5500 + QTRAP Ready, AB SCIEX).

4.7 | LCI

To perform the LCI assay, *A. tumefaciens* GV3101 cells harbouring the designated plasmids (*nluc-Pi05910* and *cluc-NbRACK1*, *cluc-StRACK1A*, *cluc-StRACK1B* or *cluc-StRACK1C*) was adjusted to OD₆₀₀ = 0.3 and then were infiltrated into *N. benthamiana* leaves for transient expression. After 3 days, luciferase substrate (1 mM, beetle luciferin, potassium salt; Promega) was spread evenly over the agroinfiltration areas of leaves. After 5–10 min of dark treatment, in vivo imaging was performed using multispectral dynamic fluorescence microscopy (CCD).

4.8 | Co-Immunoprecipitation and Western Blot Analysis

Agroinfiltrated *N. benthamiana* leaves were harvested at 3 dpi and total proteins were extracted using RIP A protein extraction

buffer. Total protein extract was incubated with 25 μ L GFP-trap or mCherry-trap agarose beads (Chromotek) at 4°C for 4 h. The beads were then washed with IP buffer (10% glycerol, 25 mM Tris-HCl pH 7.5, 1 mM EDTA, 150 mM NaCl) for four to six times. The resuspended beads were boiled for 10 min at 100°C to dissociate the immunocomplexes. Western blot assays were as previously reported (Du et al. 2021). Primary antibodies included rabbit anti-GFP (ABclonal), mouse anti-FLAG (ABclonal) and mouse anti-mCherry (ABclonal), and were used at a 1:2000 dilution. Horseradish peroxidase-conjugated secondary antibodies were anti-rabbit (ABclonal) and anti-mouse (ABclonal) and were used at a 1:2000 dilution. Western blot bands were quantified with the ImageJ software. The images were first converted to 8-bit format and smooth continuous background removed with 50 pixels as parameter in ImageJ. The whole image was then inverted, and the integral greyscale value of each band was calculated. The greyscale value is a measure of the band intensity.

4.9 | In Vivo Translation Assays

De novo protein synthesis was examined in planta using a modified SUNSET assay (Lee et al. 2023). In brief, *N. benthamiana* leaf discs were incubated with 100 μ M puromycin for 2 h in 1/2 Murashige and Skoog (MS) medium (1/2 MS powder and sucrose, adjust pH to 5.8) under vacuum. Then, 30 μ g total protein extract was loaded onto a 4%–20% gradient SDS-PAGE gel, and the puromycin-polyptides were detected by western blotting with an anti-puromycin antibody (Assay Genie) at a dilution of 1:1000. For CHX treatment, leaf discs were incubated with CHX in 1/2 MS medium at 25°C for 4 h before puromycin treatment.

4.10 | Expression and Purification of Recombinant Fusion Proteins

To prepare recombinant proteins, *Pi05910* and *StRACK1C* constructs were created using plasmid pET32a and were transformed into *E. coli* BL21. *E. coli* cells were cultured at 37°C until an OD₆₀₀ value of 0.6–0.8. Protein expression was induced by adding 0.5 mM isopropyl- β -D-thiogalactopyranoside (IPTG). The culture was subsequently incubated at 18°C for 16 h to facilitate recombinant protein synthesis. The bacterial cells were precipitated by centrifugation at 6000g for 10 min at 4°C, washed twice with ice-cold Tris-HCl buffer, and resuspended in 20 mM Tris-HCl buffer (pH 8.0) containing 300 mM NaCl. Cell lysis was performed using an ultrasonic cell crusher (Ningbo Scientz Biotechnology). Cell debris was removed by centrifuging the lysate at 12,000g for 30 min at 4°C; the resulting supernatant, enriched with soluble proteins, was retained as the crude extract. Recombinant *Pi05910* and *StRACK1C* proteins were purified by immobilised metal affinity chromatography using a Ni NTA Beads 6FF agarose column (Smart-Life Sciences). Following equilibration with 5 mM imidazole, the column was washed and specifically bound proteins were eluted using a buffer supplemented with 250 mM imidazole. Eluted fractions were analysed by 10% SDS-PAGE and visualised by Coomassie brilliant blue staining.

4.11 | PAMP Treatment

The recombinant protein of *P. infestans* PAMP elicitor INF1 (Kamoun et al. 1998) was prepared, and its biological function was confirmed in our previous works (Liu et al. 2025). For PAMP treatment, 10 μ M INF1 or distilled water control treatment were directly infiltrated into leaves from OE-*Pi05910* and RNAi-*StRACK1* potato plants.

4.12 | Isothermal Titration Calorimetry

ITC-binding was measured by using a Nano-ITC instrument (Waters Corporation). The purified recombinant *StRACK1C* and *Pi05910* proteins were vacuum degassed before the test. *Pi05910* was added to the sample pool, titrated with *StRACK1C* at constant temperature and the heat change was recorded by Nano-ITC. Data were analysed by ITCRun software.

4.13 | VIGS Assay in *N. benthamiana*

The VIGS assay was performed following essentially procedure described by Li et al. (2020). In brief, *A. tumefaciens* GV3101 cells carrying *TRV1* and *TRV2-NbRACK1*, *TRV2-PDS*, or *TRV2-GFP* were adjusted to OD₆₀₀ of 0.2. *TRV2-PDS* was used to indicate silencing efficiency. At least 10 plants were used for silencing assays. VIGS was performed on 3-week-old *N. benthamiana* plants and *P. infestans* infection assays and in vivo translation assays were performed on plants 2 weeks post-agroinfiltration of TRV constructs.

4.14 | RNA Extraction and RT-qPCR Assay

Total RNA from *N. benthamiana* and potato leaves was extracted using an RNA extraction kit (Tiangen) following manufacturer instructions. Reverse transcription was performed according to the instructions of Primescript RT Master Mix Regent kit (TaKaRa). The real-time quantitative PCR assays were performed using Q7 (Life Technologies) (Gou et al. 2022).

4.15 | DAB Staining

DAB is specific to H₂O₂ and requires peroxidase activity to provide a rapid reaction rate, widely used to measure extracellular bursts of ROS (Yin et al. 2022). The potato leaves were treated with DAB solution 3–5 dpi with *P. infestans*, wrapped in tin foil and cultured in the dark for 6–8 h. After ethanol decolourisation, photographs were taken (Zhang et al. 2024). All DAB staining images are original photographs with no colour conversion or post-processing. The brownish-black chromogenic precipitate indicates moderately positive intrinsic DAB staining. To quantify the DAB staining, the images were first converted to 8-bit format and then inverted. Grey-scale value shows the intensity of DAB staining in the image. The integral grey-scale value of each band was calculated by ImageJ (Ai et al. 2021).

4.16 | Potato Transformation

To generate transgenic potato plants, stem segments of cultivar Atlantic were placed on R3B medium (MS-519, sucrose, agar, 1-naphthaleneacetic acid, 6-benzylaminopurine, pH 5.8), treated with PACM (MS-519, sucrose, casein hydrolysate, 2,4-D, kinetin, pH 6.5) for 24 h, followed by agroinfiltration with *A. tumefaciens* GV3101 suspension and transfer to the new R3B medium. After 48 h in the dark, the culture was transferred to ZCVK (MS-519, sucrose, agar, zeatin, cefotaxime, vancomycin, kanamycin, pH 5.8) medium until new buds grew. The buds were inserted into MS medium (MS-519, sucrose, agar, pH 5.8) containing kanamycin to select for potential transgenic potato lines (Wang, Li, et al. 2023).

4.17 | Statistical Analysis

Statistical analysis of gene expression and lesion diameter was based on Student's *t*-test. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = no significant difference). All data analysis experiments were repeated at least three times. GraphPad Prism software is used to process data. Statistical tests used for each panel are found in the corresponding legends.

Author Contributions

Altyn Shayakhmetova: supervision. **Yisa Wang:** methodology, investigation, formal analysis. **Yuling Meng:** conceptualization, supervision, writing – original draft, writing – review and editing. **Liwen Ding:** conceptualization, supervision, project administration, funding acquisition, writing – original draft, writing – review and editing. **Song Liu:** methodology, investigation, writing – review and editing. **Xiran Li:** methodology, investigation. **Weixing Shan:** conceptualization, supervision, project administration, funding acquisition, writing – original draft, writing – review and editing. **Xiong Liu:** methodology, investigation, formal analysis, writing – review and editing, writing – original draft.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Expression and integration of GFP-Pi05910 fusion protein in transgenic potato. (a) Reverse transcriptase-PCR analysis shows that *GFP* and *Pi05910* are specifically expressed in transgenic potato lines. (b) Protein samples were separated by SDS-PAGE, and GFP-Pi05910 was detected by immunoblotting. Ponceau S staining indicates amounts of protein samples loaded. **Figure S2:** Quantification of *Phytophthora infestans* colonisation in inoculated transgenic potato lines overexpressing *Pi05910* (OE-Pi05910) using quantitative PCR. Genomic DNA was extracted from ten leaves at 4 dpi per treatment. *StEF-1α* and *PiActin* were used as reference genes for *Solanum tuberosum* and *P. infestans*, respectively. Student's *t* test was used for the statistical analysis (***p* < 0.01, ****p* < 0.001). Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 10 biologically independent leaves). **Figure S3:** Sequence alignment of RACK1 family proteins from *Nicotiana benthamiana* and *Solanum tuberosum*. The full-length amino acid sequences of RACK1 from *S. tuberosum* (St) and *N. benthamiana* (Nb) were aligned using MEGA7. Highly conserved sequences are highlighted in red. **Figure S4:** Phylogenetic and domain analysis of RACK1 proteins across plant species. (a) Phylogenetic tree constructed from full-length RACK1 amino acid sequences of *Nicotiana benthamiana* (Nb), *Arabidopsis thaliana* (At), *Oryza sativa* (Os) and *Zea mays* (Zm) using Phylogeny.fr (http://www.phylogeny.fr/simple_phylogeny.cgi). (b) Alignment of RACK1 sequences visualised using MEGA7. Highly conserved sequences are highlighted with a red mark. (c) Conserved domain prediction of RACK1 proteins from multiple species using the SMART database (<http://smart.emblheidelberg.de/smart/>), revealing seven conserved WD40 domains. **Figure S5:** Expression and purification of recombinant Pi05910 and StRACK1C proteins. Recombinant Pi05910 and StRACK1C proteins expressed in *Escherichia coli* and analysed by 10% SDS-PAGE gels followed by Coomassie brilliant blue staining. **Figure S6:** Bimolecular fluorescence complementation assay confirmed Pi05910 interacts with StRACK1C in the nucleus and cytoplasm. nYFP-Pi05910 was co-expressed with cYFP-StRACK1C in *Nicotiana benthamiana* leaves, and images were taken at 3 days post-inoculation. Scale bars = 60 μm. EV, empty vector. **Figure S7:** Interaction of Pi05910 with RACK1 family proteins. (a, b) Luciferase complementation imaging analysis showed the interaction between nLuc-Pi05910 and StRACK1A, StRACK1B, StRACK1C-cLuc and NbRACK1 in planta, with the combination of nLuc-Pi17063 with NbRab-G3c-cLuc used as positive control. Leaves were photographed at 3 dpi. (c) and (d) Co-IP assays performed with GFP-trap beads. Total proteins were extracted from *Nicotiana benthamiana* leaves at 3 days post-infiltration. Co-precipitation was detected via western blot. Ponceau S indicates protein samples loaded. **Figure S8:** Subcellular localisation of GFP-Pi05910 and StRACK1A/B-mCherry. StRACK1A/B-mCherry and GFP-Pi05910 were co-expressed in *Nicotiana benthamiana* leaves by agroinfiltration. Infiltrated leaves were observed under confocal microscopy at 3 days post-infiltration. Scale bars = 20 μm. Fluorescence intensity was quantitated in the transect (yellow line) with ImageJ. **Figure S9:** Pi05910 promotes destabilisation of RACK1. (a–c) Agroinfiltration of NbRACK1-mCherry, StRACK1C-mCherry and GFP-Pi05910, or GFP-NbRACK1 and Pi05910-mCherry into *Nicotiana benthamiana* leaves, with imaging at 3 days post-infiltration. Scale bar = 100 μm. (d) Detection of RACK1 stability by anti-mCherry antibodies using western blot assay. Protein loading is indicated by Ponceau stain. **Figure S10:** Pi05910 promotes NbRACK1 destabilisation via 26S proteasome pathway in *Nicotiana benthamiana*. (a) Confocal observation of NbRACK1-mCherry. NbRACK1-mCherry and GFP-Pi05910 or GFP were expressed in *N. benthamiana* leaves by agroinfiltration. Infiltrated leaves were observed at 3 days post-infiltration. Scale bar = 100 μm. (b) NbRACK1-mCherry was co-expressed with GFP-Pi05910 or GFP control in *N. benthamiana* leaves, and infiltrated with MG132 or dimethyl sulphoxide (DMSO) at 36 h post-agroinfiltration for 12 h. Protein levels were quantified using ImageJ. **Figure S11:** Morphology of NbRACK1-silenced *Nicotiana benthamiana* and the silencing efficiency. (a) Schematic graph of full length NbRACK1. The green region (466–711 bp) was used to construct TRV-NbRACK1. (b) The tobacco rattle virus vector harbouring a

partial fragment of NbRACK1 (TRV-NbRACK1) or GFP (TRV-GFP) was expressed in *N. benthamiana* by agroinfiltration. Images were taken 25 days post-infiltration. (c) The silencing efficiency of NbRACK1 was determined by reverse transcription-quantitative PCR. NbActin was used as the internal reference gene of *N. benthamiana*. Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 6 biologically independent leaves). Student's *t*-test was used for the statistical analysis (***p* < 0.001). **Figure S12:** The relative transcript levels of NbRACK1 in response to *Phytophthora infestans* infection. Expression of NbRACK1 in response to *P. infestans* infection were detected by reverse transcription-quantitative PCR. NbActin was set as the internal reference gene of *N. benthamiana*. Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 6 biologically independent leaves). Student's *t*-test was used for the statistical analysis (**p* < 0.05, ****p* < 0.001). **Figure S13:** Upregulation of StRACK1 during early stages of *Phytophthora infestans* infection. Expression of StRACK1 in potato to *P. infestans* infection were detected by reverse transcription-quantitative PCR. StEF-1α was set as the internal reference gene of potato. Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 6 biologically independent leaves). Student's *t*-test was used for the statistical analysis (***p* < 0.01, ****p* < 0.001). **Figure S14:** Overexpression of NbRACK1 attenuates *Phytophthora infestans* colonisation in *Nicotiana benthamiana*. (a) Representative photographs of *N. benthamiana* leaves of transiently overexpressing GFP-NbRACK1 plants infected with *P. infestans* at 7 days post-infiltration (dpi). Dotted white circles indicate lesion areas. Student's *t*-test was used for the statistical analysis (***p* < 0.001). Scale bar = 10 mm. (b) *P. infestans* biomass in inoculated leaves of OE-NbRACK1—*N. benthamiana* by quantitative PCR. Genomic DNA was extracted from samples at least 10 leaves at 7 dpi per treatment. NbActin and PiActin were served as internal standards for *N. benthamiana* and *P. infestans*, respectively. Student's *t*-test was used for the statistical analysis (**p* < 0.05). (c) The protein expression of FLAG-GFP and GFP-NbRACK1 in *N. benthamiana*. Protein expression was detected using anti-GFP antibodies via western blot. Ponceau S indicates amounts of protein samples loaded. Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 10 biologically independent leaves). **Figure S15:** Overexpression of StRACK1C reduces *Phytophthora infestans* colonisation in potato. (a) Representative photograph showing plants of three OE-StRACK1C transgenic Atlantic potato lines grown in a growth room for 40 days. Scale bars = 30 mm. (b) Expression levels of StRACK1 in OE-StRACK1C transgenic potato lines. Total RNA was extracted from transformant potato lines, with StEF-1α as internal standard. Student's *t* test was used for the statistical analysis (***p* < 0.001). (c) Representative photographs of the control and three OE-StRACK1C transgenic potato lines infected with *P. infestans* at 5 days post-inoculation (dpi). Dotted white circles indicate lesion areas. Student's *t* test was used for the statistical analysis (***p* < 0.001). The values were distributed in dots. (d) *P. infestans* biomass in inoculated leaves of OE-StRACK1C lines. The GFP and OE-StRACK1C lines infected with a *P. infestans* at 5 dpi. StEF-1α and PiActin served as internal standards for potato and *P. infestans*, respectively. Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 6 biologically independent leaves). Student's *t*-test was used for the statistical analysis (***p* < 0.001). **Figure S16:** Phenotype and *Phytophthora infestans*—colonisation of StRACK1-silencing potato lines. (a) Representative photograph showing plants of three RNAi-StRACK1 Atlantic potato lines grown in a growth room for 40 days. Scale bars = 30 mm. (b) Expression levels of StRACK1 in RNAi-StRACK1 potato lines. Total RNA was extracted from transformant potato lines, with StEF-1α as internal standard. Student's *t*-test was used for the statistical analysis (***p* < 0.01, ****p* < 0.001). (c) *P. infestans* biomass in inoculated leaves of RNAi-StRACK1 lines. The wild-type (WT) and RNAi-StRACK1 lines infected with *P. infestans* at 4 days post-inoculation. StEF-1α and PiActin served as internal standards for potato and *P. infestans*, respectively. Student's *t* test was used for the statistical analysis (***p* < 0.001). Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 6 biologically independent leaves). **Figure S17:** 3,3'-diaminobenzidine (DAB) staining to indicate increased reactive oxygen species (ROS) accumulation in three OE-StRACK1C transgenic potato lines at 3 days post-inoculation. Grey-scale value plot shows the intensity of DAB staining in the image. The grey-scale value of the

OE-GFP control was set to 1. Student's *t*-test was used for the statistical analysis (***p* < 0.01, ****p* < 0.001). Error bars represent mean values ± SD of three biological replicates (*n* ≥ 6 biologically independent leaves). The values were distributed in dots. Scale bar = 10 mm. **Figure S18:** Silencing of *NbRACK1* affects the expression of defence-related genes. Reverse transcription-quantitative PCR (RT-qPCR) assay was performed upon INF1 treatment. Leaves of *Nicotiana benthamiana* were treated with 10 μM INF1 3 h later and were collected. *NbActin* was used for normalisation in RT-qPCR assays. Student's *t*-test was used for the statistical analysis (***p* < 0.01). Error bars represent mean values ± SD of 3 technical replicates (*n* ≥ 6 biologically independent leaves). **Table S1:** Primers used in this study.