

A fungal pathogen suppresses host leaf senescence to increase infection

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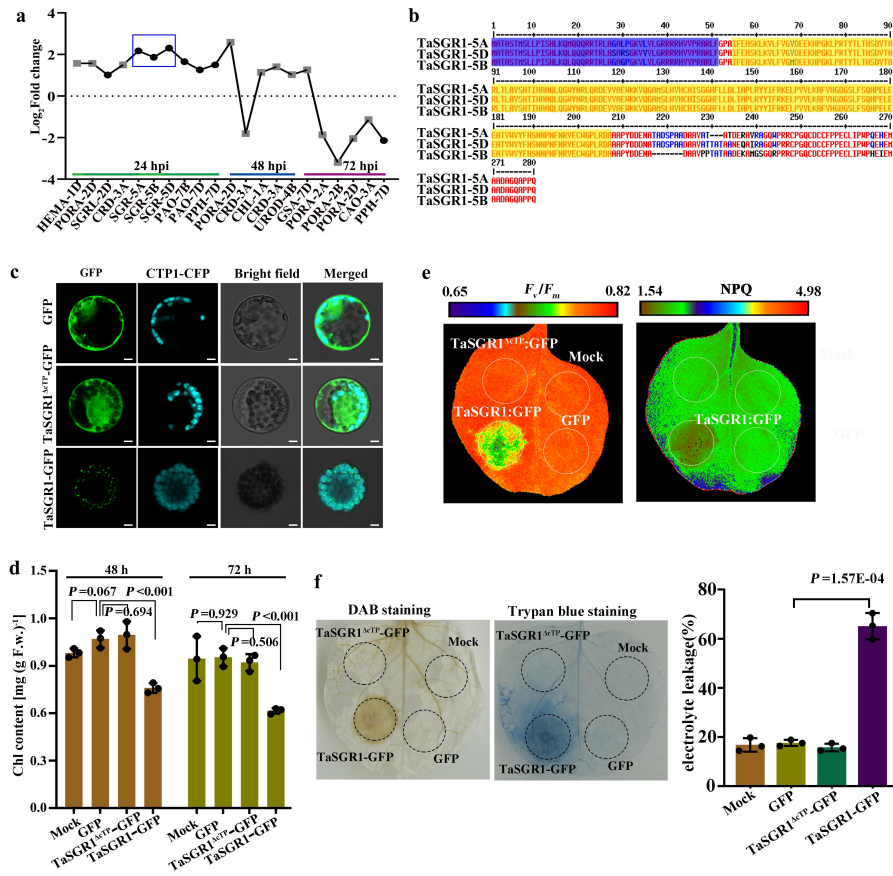
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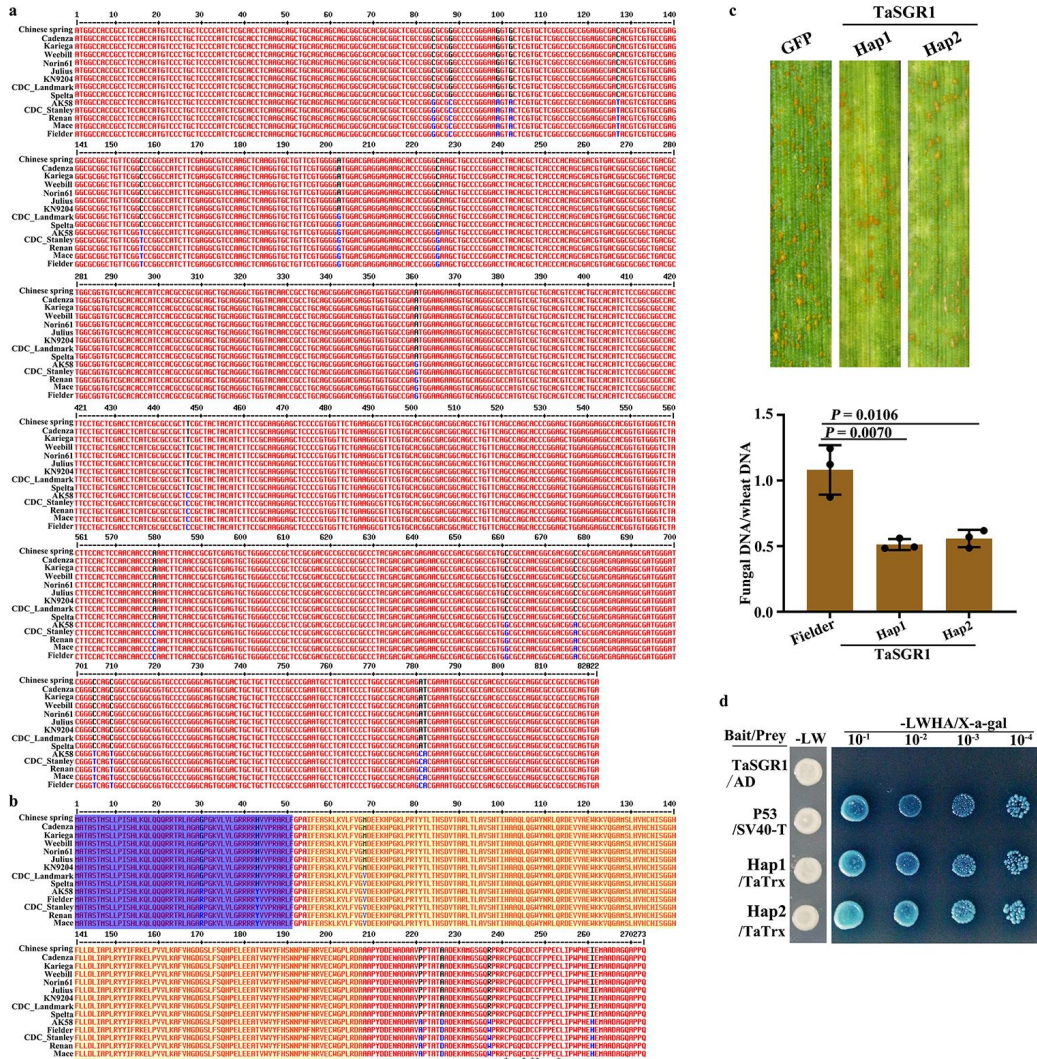
Supplementary Fig. 1



Supplementary Fig. 1 TaSGR1 expression contributes to leaf senescence and suppression of photosynthesis.

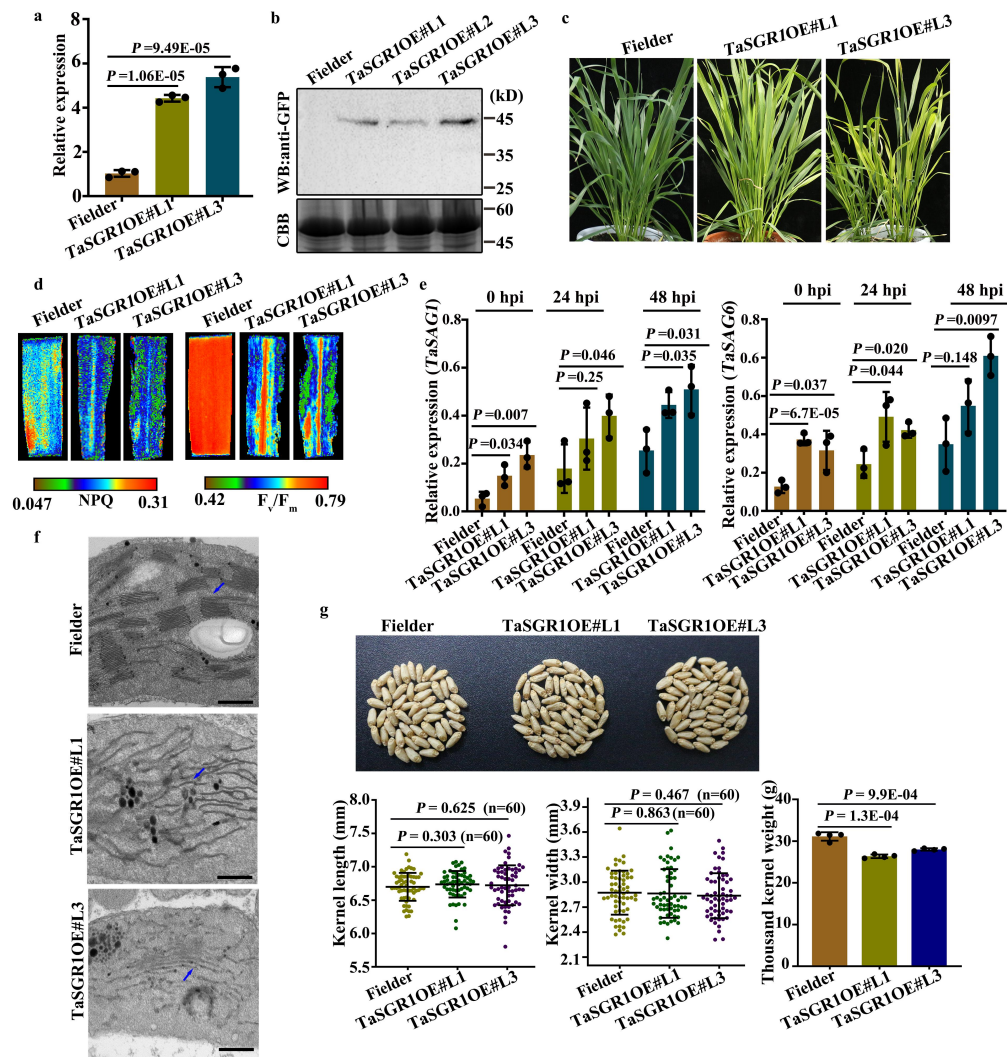
a The expression levels of Chl metabolism-related genes in wheat leaves infected with *Pst* from public transcriptome data (<http://www.wheat-expression.com/>). Genes related to Chl metabolism and Chl biosynthesis are indicated by closed circles and squares, respectively. The blue box indicates *TaSGR1* genes. **b** Three *TaSGR1* homeologs, located on chromosomes 5A, 5B, and 5D, exhibited high similarity. Their sequences were obtained from the Ensembl plant database (<http://plants.ensembl.org/index.html>), and sequence alignment was performed using the online platform at <http://multalin.toulouse.inra.fr/multalin/multalin.html>. The chloroplast-targeting peptides (cTP) and STAYGREEN domain are highlighted in blue and yellow, respectively. Red font indicates high consensus (> 90% similarity), whereas blue font indicates low consensus (90% < value < 50% similarity). **c** Subcellular localization of TaSGR1 in wheat protoplasts. Wheat protoplasts transfected with the marked vectors using PEG-mediated transfection were examined using confocal microscopy. CTP1-CFP was used as a marker protein to localize into chloroplasts. Bar = 5 μ m. Similar results are obtained from three independent biological experiments. **d** *TaSGR1* expression accelerated Chl degradation in *N. benthamiana*. TaSGR1 was transiently expressed in *N. benthamiana*, and Chl contents were determined using a multiscan spectrum. **e** The photosynthetic parameters (Fv/Fm and NPQ), measured at 48 hpi using a Chl fluorescence imager, were significantly affected by TaSGR1 overexpression in *N. benthamiana*. **f** H₂O₂ accumulation and necrotic cell death in plants overexpressing *TaSGR1*. H₂O₂ accumulation and necrotic cell death were detected via DAB staining (shown in brown) and trypan blue staining, respectively. Relative electrolyte leakage was measured in *N. benthamiana* transiently expressing TaSGR1 ^{Δ cTP}:GFP, TaSGR1:GFP, or GFP clone. In Supplementary Fig. 1d and 1f, data are expressed as means $\pm$ SD based on three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. Source data are provided as a Source Data file.

Supplementary Fig. 2



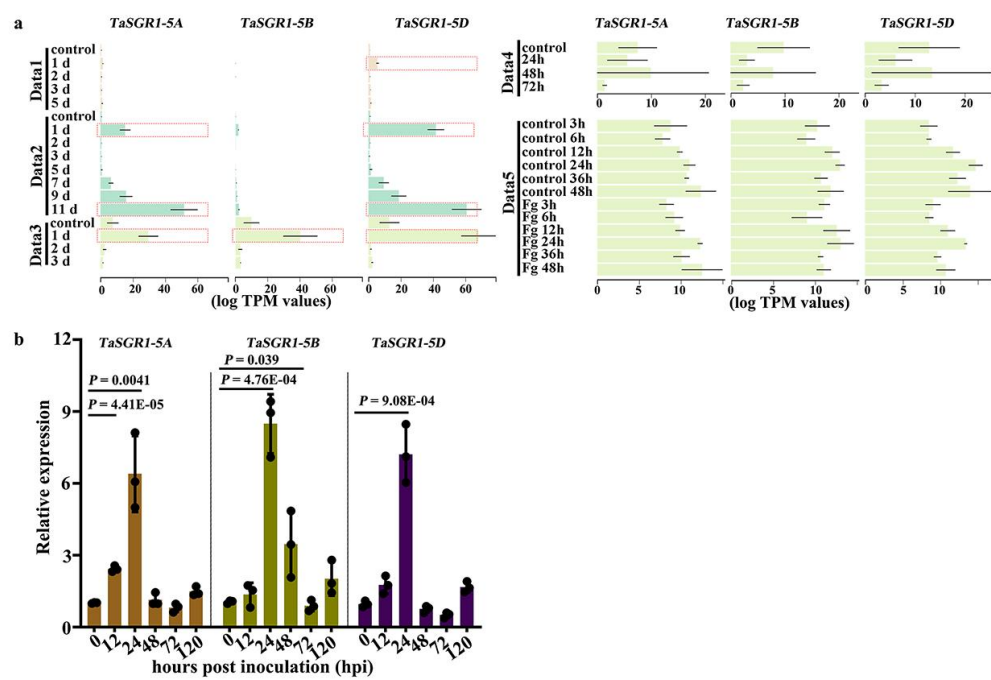
Supplementary Fig. 2 Detection of the conservation of TaSGR1-5B in wheat cultivars and the function of two haplotypes. **a** Nucleotide sequence alignments and **b** amino acid sequence alignments. The sequences of *TaSGR1-5B* genes were analyzed among 14 wheat cultivars from the WheatOmics database (<http://202.194.139.32/>). Sequence alignment was generated at <http://multalin.toulouse.inra.fr/multalin/multalin.html>. The cTP and STAYGREEN domain are highlighted in blue and yellow, respectively. Red font indicates high consensus (> 90% similarity). Variations in several bases or amino acids are marked in blue. Asterisks mark the conserved cysteine residues in the C-terminal region of *TaSGR1-5B*. **c** Two haplotypes of *TaSGR1* expressed in Fielder plants using BSMV promote plant resistance to *Pst*. Hap1 from Chinese spring and Hap2 from Fielder plant were transiently expressed in the leaves using BSMV, respectively, followed by inoculation with *Pst* CYR34, and disease phenotypes were observed at 14 dpi. The *Pst*/wheat biomass ratio in infected leaves at 120 hpi was measured using qPCR. **d** Two haplotypes of TaSGR1 interacted with TaTrx in yeast cells. P53/SV40-T and TaSGR1/AD were used as the positive and negative controls, respectively. Hap1 and Hap2 of *TaSGR1* were from Chinese spring and Fielder plant, respectively. Similar results are obtained from three independent biological experiments.

Supplementary Fig. 3



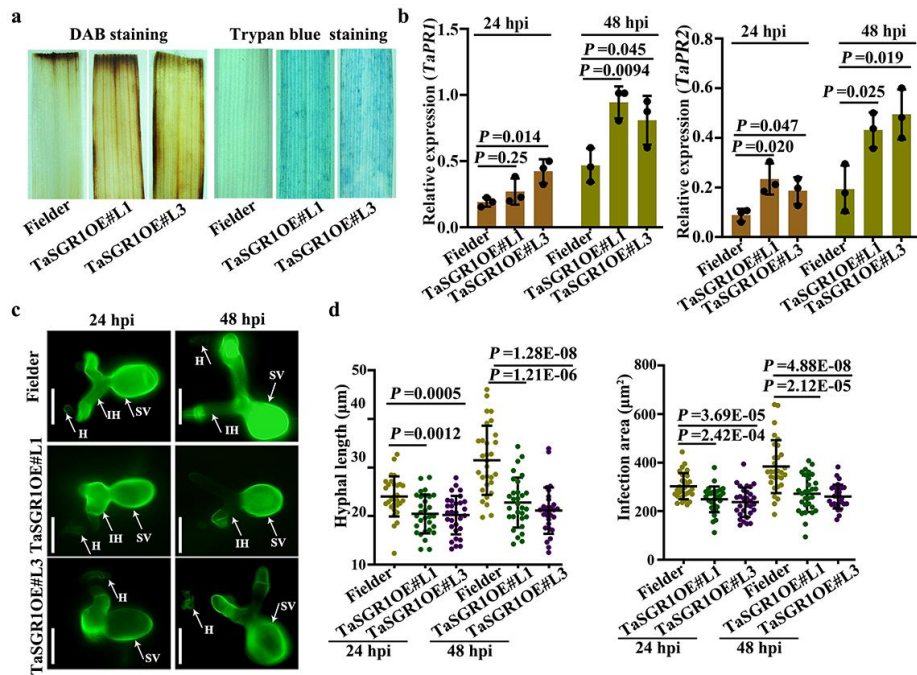
Supplementary Fig. 3 *TaSGR1*OE plants exhibited leaf senescence and impacted on wheat yield under greenhouse conditions. **a** Relative expression of *TaSGR1-5B* in Fielder and *TaSGR1*OE plants was detected via qPCR using *TaEF1a* as an internal control. The control for qPCR were Fielder plants. Data are expressed as mean $\pm$ SD from three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test by compared with Fielder plants. Source data are provided as a Source Data file. **b** Detection of TaSGR1 protein levels in Fielder and *TaSGR1*OE plants via western blot using the anti-GFP antibody. Rubisco protein was stained with Coomassie Brilliant Blue (CBB) as a loading control. **c** Two-month-old, soil-grown *TaSGR1*OE plants exhibited a chlorotic phenotype, which was not observed in the control Fielder plants. **d** The photosynthetic parameters (Fv/Fm and NPQ) of 2-month-old *TaSGR1*OE plants were measured using a Chl fluorescence imager. **e** The senescence-associated genes *SAG1* and *SAG6* were upregulated in transgenic wheat lines overexpressing *TaSGR1*. The control for qPCR were Fielder plants. Data are expressed as mean $\pm$ SD from three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. **f** Morphological changes in the chloroplasts of mesophyll cells in Fielder and *TaSGR1*OE plants. Each genotype was examined in 2-month-old wheat plants using transmission electron microscopy. Blue arrows indicate the thylakoids. Bar = 500 nm. Similar results are obtained from two independent biological experiments. **g** Overexpression of *TaSGR1* impacted on wheat yield under greenhouse conditions. Performance of harvested grains from representative plants is shown in upper panel. Statistical analysis of kernel length (n=60), kernel width (n=60), and 1000-kernel weight (n=4) were shown in lower panel, performing with GraphPad Prism 8.0. The *P* value represents the significant difference between Fielder and two *TaSGR1*OE lines (two-tailed unpaired Student's t-test). Source data are provided as a Source Data file.

Supplementary Fig. 4



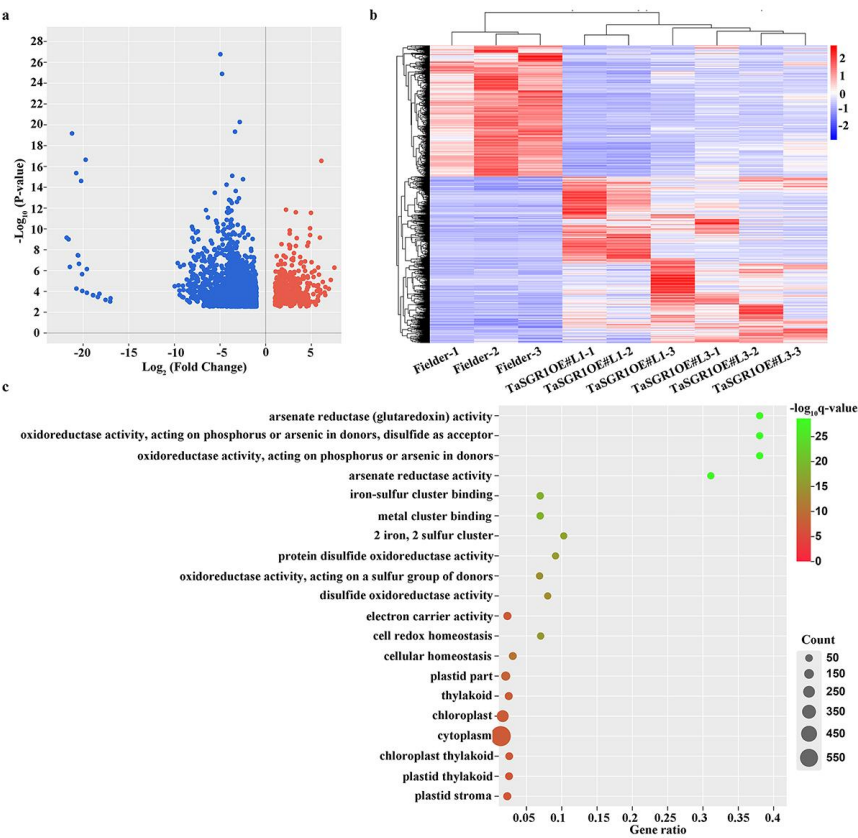
Supplementary Fig. 4 *TaSGR1* transcript levels were upregulated in *Pst*-infected wheat. **a** The three *TaSGR1* homeologs (*TaSGR1-5A*, *TaSGR1-5B*, and *TaSGR1-5D*) were upregulated early during infection with *Pst*. Data 1–5 represent the RNA-seq data from Avocet-Yr5+PST87/66, Vuka+PST87/66, N9134+*Pst* CYR31, N9134+*Blumeria graminis* f.sp.*tritici* isolate E09, and CM-82036+*Fusarium graminearum*, respectively, based on publicly available wheat RNA-seq data (<http://www.wheat-expression.com/>). Control refers to wheat leaves treated with ddH₂O. TPM, transcripts per million; h, hour; d, day. Error bars represent standard errors. **b** Relative expression of *TaSGR1* in *Pst* CYR23-infected Fielder plants. qPCR analysis was performed to assess the transcript levels of *TaSGR1*, with *TaEF1a* used as an internal control, employing the comparative threshold method ($2^{-\Delta\Delta C_t}$). Control refers to wheat leaves treated with ddH₂O. Data are expressed as means $\pm$ SD from three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test by comparing with Fielder plants treated with ddH₂O at different time points. Source data are provided as a Source Data file.

Supplementary Fig. 5



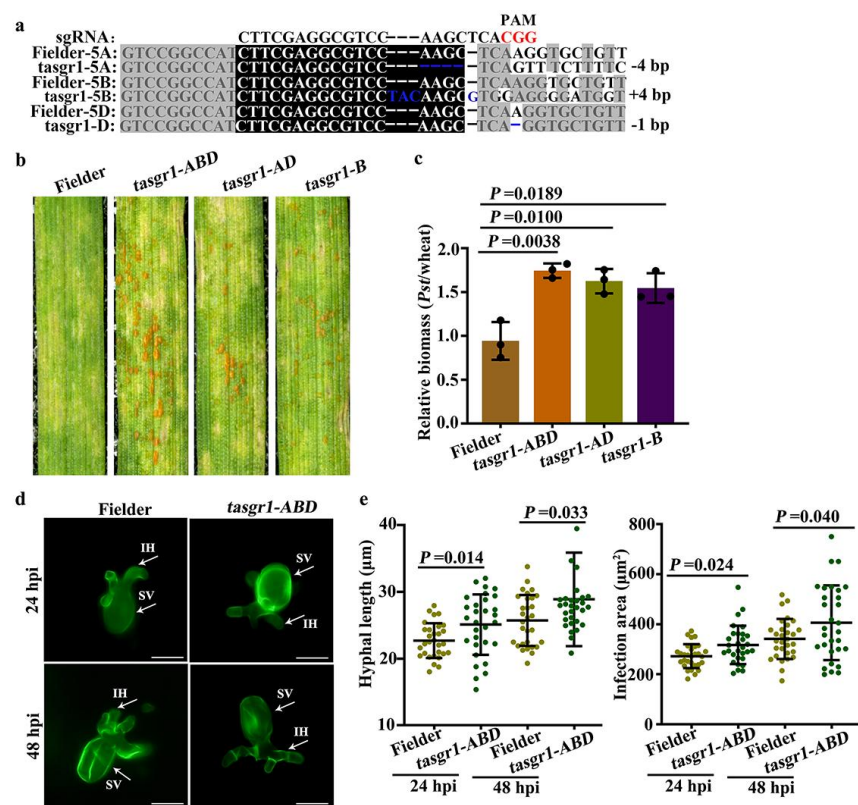
Supplementary Fig. 5 Overexpression of *TaSGR1* improved plant resistance to *Pst*. **a** H₂O₂ accumulation and necrotic cell death in *Pst* CYR31-infected *TaSGR1*OE plants at 120 hpi. These parameters were detected via DAB staining and trypan blue staining, respectively. Similar results are obtained from two independent biological experiments. **b** Relative expression of defense-related genes in *Pst* CYR31-infected *TaSGR1*OE plants at 24 and 48 hpi. qPCR analysis was performed to assess transcript levels of the corresponding genes, with *TaEF1a* as an internal control. Control refers to wheat leaves treated with *Pst*. Data are expressed as means $\pm$ SD from three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test by comparing the results with the control (Fielder plants). **c** Representative images of *Pst* hyphae from WGA-stained *TaSGR1*OE plants were obtained using an Olympus BX-51 microscope. SV, Substomatal vesicle; IH, infectious hyphae; H, haustorium; Bar = 20 μ m. **d** Hyphal length and infection area in *Pst* CYR31-infected *TaSGR1*OE plants were analyzed at 24 and 48 hpi using the CellSens Entry software. Means $\pm$ SD were determined from 30 infection sites across three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. Source data are provided as a Source Data file.

Supplementary Fig. 6



Supplementary Fig. 6 RNA sequencing analysis of differentially expressed genes in *Pst*-infected *TaSGR1OE#L1* and *TaSGR1OE#L3* plant. **a** Volcano plot of DEGs was performed to compare *Pst*-infected wheat leaves of Fielder, *TaSGR1OE#L1* and *TaSGR1OE#L3*. The red and blue points represent genes up- and down-regulated genes, respectively, in *TaSGR1OE#L1* and *TaSGR1OE#L3* plants compared with the Fielder plants. The threshold of significance (fold change > 2.0 and a q-value < 0.05) was selected for the differentially expressed genes. **b** Heatmap of DEGs in *Pst*-infected wheat leaves of Fielder, *TaSGR1OE#L1* and *TaSGR1OE#L3*. The transcript levels are shown with a red-blue colour scale, where red represents up-regulation and blue represents down-regulation. **c** GO enrichment analysis of DEGs from (a). GO categories within biological process, cellular component, and molecular function are separately grouped and arranged based on their rich factor ranks. The size of the circle represents the comprising gene number.

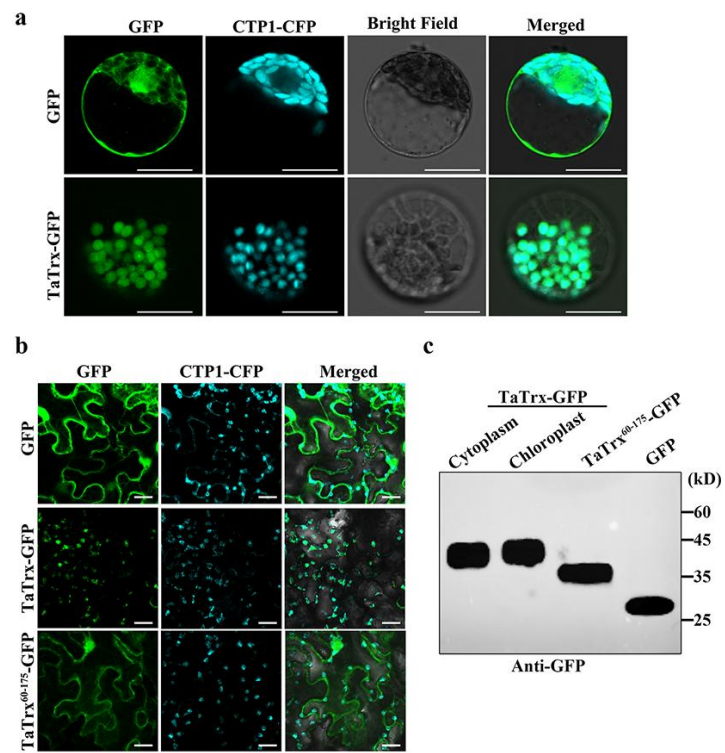
Supplementary Fig. 7



Supplementary Fig. 7 Knockout of *TaSGRI* impairs plant defense against *Pst*.

a Schematic representation of the *TaSGRI* structure and the gene sequences in Fielder and *tasgr1* plants at the sites targeted by sgRNAs. The *tasgr1* line contained frameshift mutations in *TaSGRI-5A* (4-bp deletion), *TaSGRI-5B* (4-bp insertion), and *TaSGRI-5D* (1-bp deletion). **b** *Pst* uredinia on the second leaves of *tasgr1-ABD*, *tasgr1-AD*, *tasgr1-B*, and Fielder plants were observed at 14 dpi with *Pst* CYR23. Similar results are obtained from three independent biological experiments. **c** The *Pst*/wheat biomass ratio was measured at 120 hpi via qPCR using DNA isolated from the same set of wheat plants shown in Supplementary Fig. 7b. Internal control genes *TaEF* and *PstEF* were used to normalize the DNA levels of wheat and *Pst*, respectively. Control refers to wheat leaves treated with *Pst*. Data are shown as the mean $\pm$ SD of three independent replicates from three leaf samples of different plants. **d** Representative images of *Pst* hyphae in WGA-stained *tasgr1* plants were captured using an Olympus BX-51 microscope. SV, Substomatal vesicle; IH, infectious hyphae; H, haustorium; Bar = 20 μ m. **e** Hyphal length and infection area in *Pst* CYR23-infected *tasgr1* plants were analyzed at 24 and 48 hpi using the CellSens Entry software. Means $\pm$ SD were calculated from 30 infection sites across three independent biological repeats from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. Source data are provided as a Source Data file.

Supplementary Fig. 8



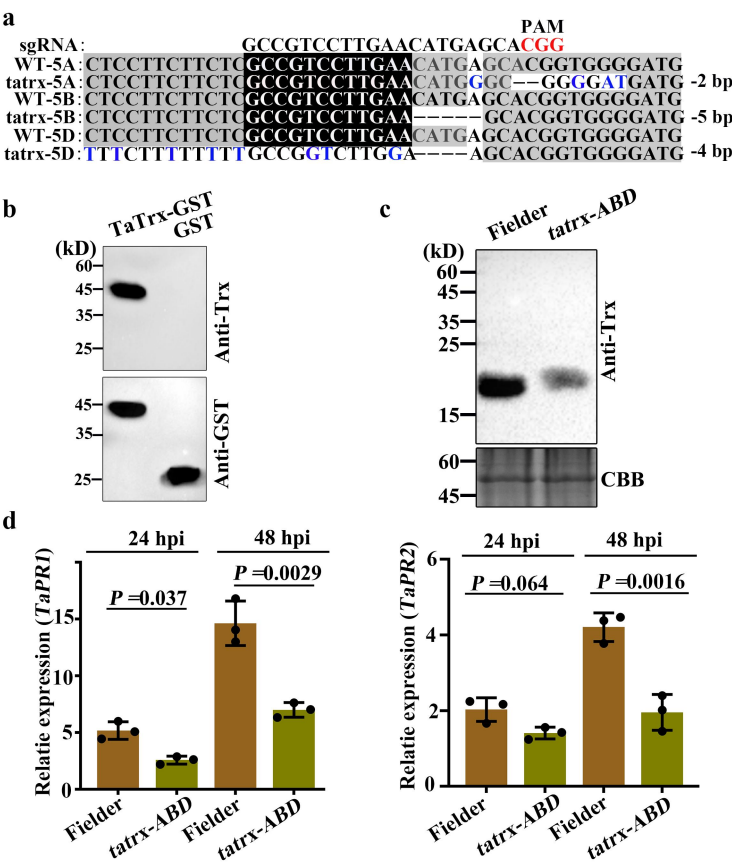
Supplementary Fig. 8 TaTrx-GFP was dependent on its N-terminal sequence for import into chloroplasts. a Subcellular localization of TaTrx in wheat protoplasts.

Wheat protoplasts were transformed with the marked vectors through PEG-mediated transformation and examined using confocal microscopy. CTP1-CFP was used as a marker protein to localize into chloroplasts. Bar = 20 μm .

b Subcellular localization of TaTrx in *N. benthamiana* leaves. The fluorescence signal from *N. benthamiana* leaves transiently expressing TaTrx-GFP was observed using confocal microscopy. CTP1-CFP was used as a marker protein to localize into chloroplasts. Bar=20 μm .

c Cytoplasmic proteins, and chloroplast proteins were isolated and detected via immunoblotting. The sizes of the corresponding proteins (TaTrx-GFP, TaTrx⁶⁰⁻¹⁷⁵-GFP) were detected via western blot analysis using an anti-GFP antibody. All experiments in this study were performed two to three times with similar results.

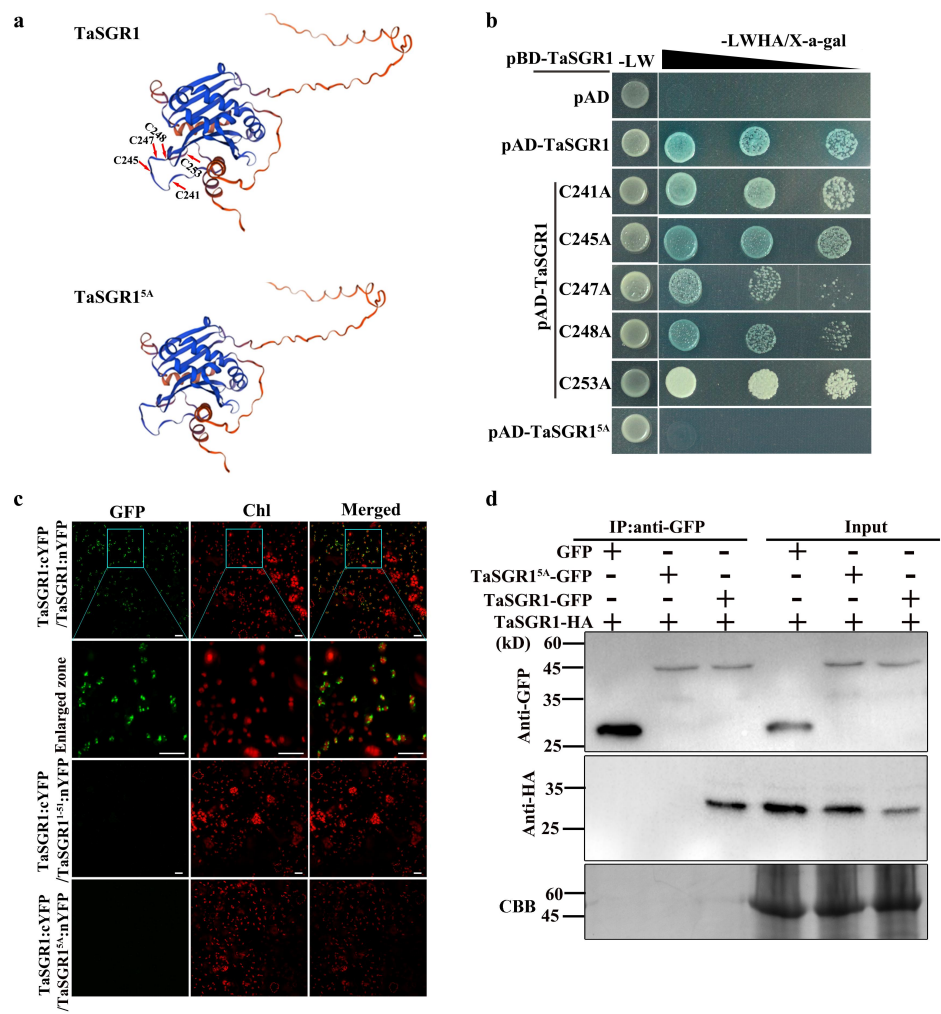
Supplementary Fig. 9



Supplementary Fig. 9 Knockout of *TaTrx* impairs plant defense against *Pst*. **a**

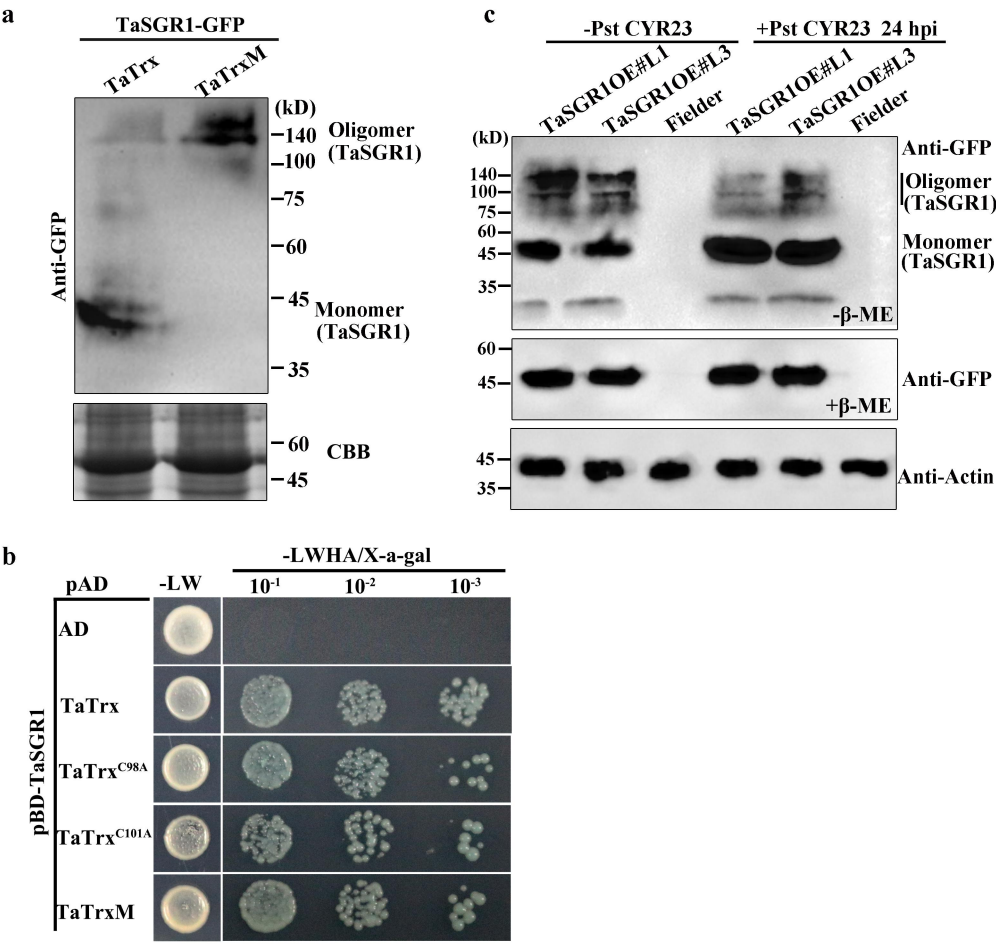
Schematic representation of the *TaTrx* structure and the gene sequences in Fielder and *tatr*x plants at the sites targeted by sgRNAs. The *tatr*x line contained frameshift mutations in *TaTrx-5A* (2-bp deletion), *TaTrx-5B* (5-bp deletion), and *TaTrx-5D* (4-bp deletion). **b** Immunoblotting tests showed that the Trx antibody recognized a TaTrx-GST expressed in *E. coli*. **c** Detection of TaTrx protein levels in *tatr*x plants and Fielder plants via western blotting with the commercial anti-Trx antibody. Rubisco protein was stained with CBB as a loading control. **d** Relative expression of the marked defense-related genes in *tatr*x plants at 0 and 24 hpi with *Pst* CYR23. Transcript levels were confirmed using qPCR and normalized to *TaEF1a*. Control refers to wheat leaves treated with *Pst*. Data are expressed as means $\pm$ SD from three independent biological repeats from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. All experiments in this study were performed two to three times with similar results. Source data are provided as a Source Data file.

Supplementary Fig. 10



Supplementary Fig. 10 TaSGR1 interacted itself dependent on its cysteine residues in the C-terminal region. **a** The overall structure of TaSGR1 and TaSGR1^{5A} with surface representations of their cysteine-rich sites, modeled using SWISS-MODEL (<https://swissmodel.expasy.org/>). **b** Detection of the interaction between TaSGR1 and its mutations. Five cysteines were individually changed to alanine in the following mutations: C241A, C245A, C247A, C248A, and C253A. TaSGR1^{5A} indicates that all cysteines at the C-terminal of TaSGR1 were replaced by alanine. **c** The interaction of TaSGR1 with itself was detected in the chloroplasts of *N. benthamiana* leaves transiently expressing the marked constructs via BiFC assays. TaSGR1¹⁻⁵¹ indicates the cTP of TaSGR1. The enlarged zone shows the localization of the TaSGR1–TaSGR1 interaction at higher magnification. Bar = 20 μm. **d** The TaSGR1–TaSGR1 interaction was confirmed via Co-IP assays. Western blots of total proteins extracted from *N. benthamiana* leaves and proteins eluted from magnetic beads were detected using anti-GFP and anti-HA antibodies, respectively. Rubisco protein was stained with CBB as a loading control. Similar results are obtained from three independent biological experiments. Source data are provided as a Source Data file.

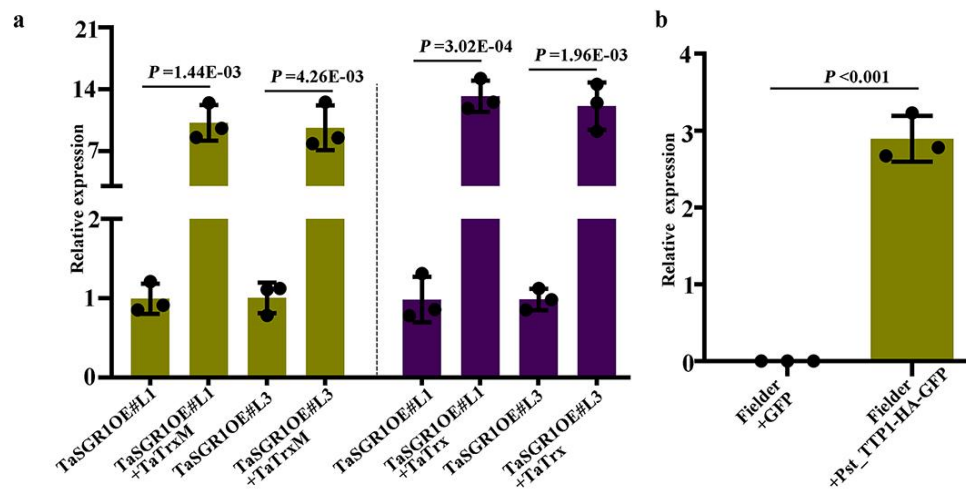
Supplementary Fig. 11



Supplementary Fig. 11 TaTrx reduced TaSGR1 from oligomer to monomer. a

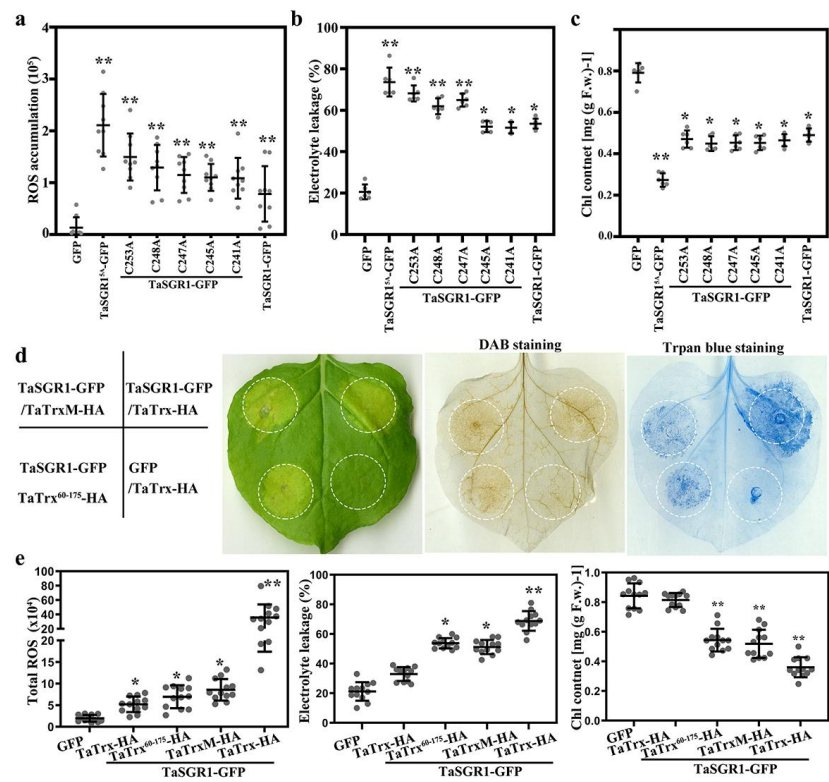
TaTrx, but not TaTrxM, reduced TaSGR1 from an oligomer to a monomer in *N. benthamiana*. TaTrx, and TaTrxM were transiently expressed in *N. benthamiana*, and total proteins were extracted and detected using the anti-GFP antibody, with Rubisco protein stained with CBB as a loading control. **b** Detection of the interaction between TaSGR1 and TaTrxM via Y2H assays. P53/SV40-T and TaSGR1/AD were used as positive and negative controls, respectively. Two conserved cysteines in the active site were changed to alanine in two mutations: TaTrx^{C98A} and TaTrx^{C101A} (each affecting one cysteine), whereas the TaTrxM mutation modified both cysteines. **c** The monomeric TaSGR1 were increased in *TaSGR1*OE plants inoculated with avirulent *Pst* CYR23. Total proteins extracted from the leaves of *TaSGR1*OE plants were detected using anti-GFP antibody. Actin proteins detected with anti-Actin antibody showed protein loading. Similar results are obtained from three independent biological experiments. Source data are provided as a Source Data file.

Supplementary Fig. 12



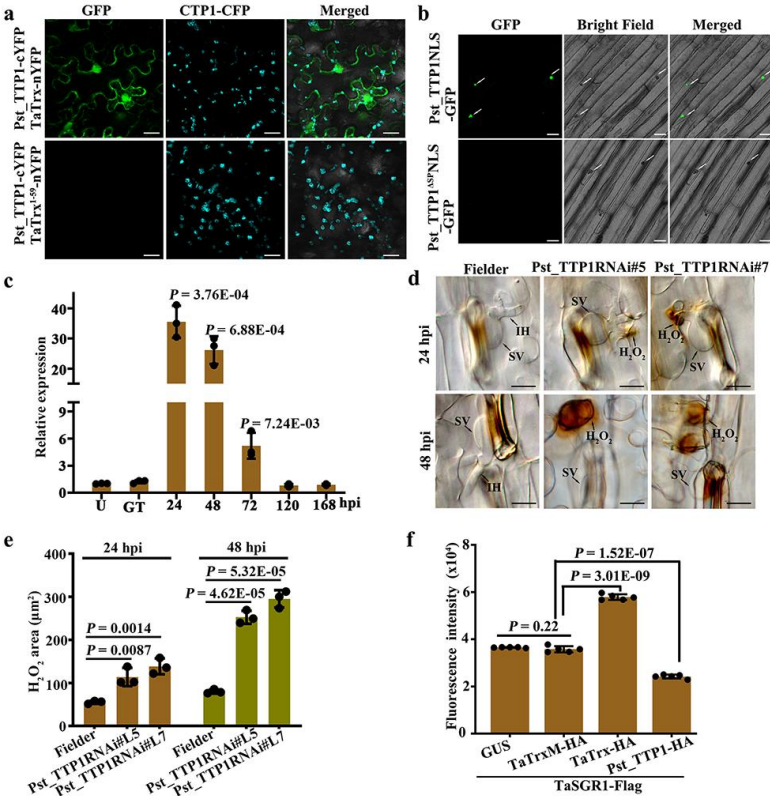
Supplementary Fig. 12 Expression analysis of the corresponding genes in wheat transiently expressing these constructs. **a** Relative expression of *Trx* genes from the same set of plants reported in Fig. 3d was detected using qPCR. The relative expression of *Trx* genes in *TaSGR1OE#L1* and *TaSGR1OE#L3* plants expressing *TaTrxM* and *TaTrx* was detected via qPCR using *TaEF1a* as an internal control. Control refers to Fielder plants. The yellowish-green and purple columns indicated the Fielder plant expressing *TaTrxM* and *TaTrx*, respectively. Data are expressed as mean $\pm$ SD (n = 3 biological replicates). The *P* value was determined using a two-tailed unpaired Student's t-test, comparing the results with those of control plants. **b** Relative expression of *Pst_TTP1* from the same set of plants reported in Fig. 6a was detected via qPCR. The relative expression of *Pst_TTP1* in Fielder plants expressing *Pst_TTP1* was analyzed via qPCR using *TaEF1a* as an internal control. The yellowish-green column indicated the Fielder plant expressing *Pst_TTP1*. Data are expressed as mean $\pm$ SD from three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test, comparing the results with those of control plants. Source data are provided as a Source Data file.

Supplementary Fig. 13



Supplementary Fig. 13 Monomeric TaSGR1 contributes to leaf yellowing and H₂O₂ accumulation. All TaSGR1 mutations, including C241A, C245A, C247A, C248A, C253A, TaSGR1^{5A}, and TaSGR1, were transiently expressed in *N. benthamiana*. Total Chl content and total ROS accumulation were determined using a multiscan spectrum over 60 min, and cell mortality was quantified via electrolyte leakage. **a** TaSGR1 mutations promote H₂O₂ accumulation in *N. benthamiana*. Data are presented as the mean $\pm$ SD (n = 10 leaves from three biological replicates), and significant differences were determined compared with the control (**, $P < 0.01$, unpaired two-tailed Student's t-test). **b** TaSGR1 mutations promote cell death in *N. benthamiana*. Data presented shown as the mean $\pm$ SD (n = 6 leaves from three biological replicates), and significant differences were determined compared with the control (**, $P < 0.01$, unpaired two-tailed Student's t-test). **c** TaSGR1 mutations accelerate Chl degradation in *N. benthamiana*. Data presented shown as the mean $\pm$ SD (n = 6 leaves from three biological replicates), and significant differences were determined compared with the control (**, $P < 0.01$, unpaired two-tailed Student's t-test). **d** TaTrx promoted TaSGR1-mediated H₂O₂ accumulation and cell death. The corresponding vectors were co-expressed in *N. benthamiana*. H₂O₂ accumulation and necrotic cell death were detected at 48 hpi via DAB staining (shown in brown) and trypan blue staining, respectively. **e** The H₂O₂ accumulation, cell death, and Chl content measurements reported in Supplementary Fig. 13d were obtained at 48 hpi. Data presented shown as the mean $\pm$ SD (n = 6 leaves from three biological replicates), and significant differences were determined compared with the control (**, $P < 0.01$, unpaired two-tailed Student's t-test). Source data are provided as a Source Data file.

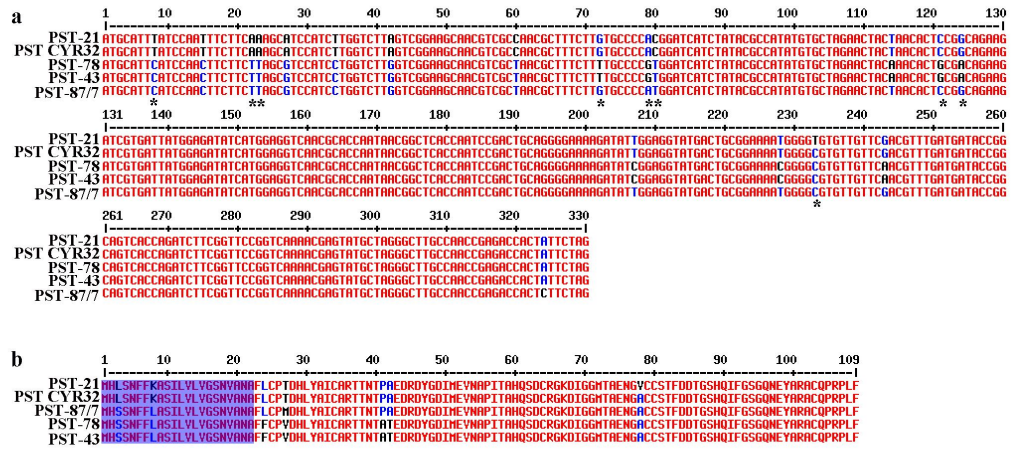
Supplementary Fig. 14



Supplementary Fig. 14 Pst_TTP1 is required for the full virulence of Pst. a

Pst_TTP1-TaTrx interaction was detected in *N. benthamiana* leaves via BiFC assays. CTP1-CFP was used as a marker protein to localize into chloroplasts. Bar = 20 μ m. **b** Secretion of Pst_TTP1 into wheat cells. Wheat coleoptiles were infected with *F. graminearum* PH-1 expressing Pst_TTP1NLS-GFP and Pst_TTP1 Δ SPNLS-GFP, respectively. Arrows indicate GFP signals in the nucleus. Bar = 20 μ m. **c** The pattern of Pst_TTP1 expression during Pst infection was determined via qPCR. The level of Pst_TTP1 expression was assayed using RNA isolated from urediniospores (U), germ tubes (GT), and leaves of Fielder plants inoculated with Pst CYR34, sampled at 24, 48, 72, 120, and 168 hpi. Means $\pm$ SD were determined based on three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. Source data are provided as a Source Data file. **d** Observation of H₂O₂ accumulation in DAB-stained leaves of Pst_TTP1RNAi wheat plants inoculated with Pst CYR31 at 24 and 48 hpi. Bar = 20 μ m. SV, substomatal vesicle; IH, infectious hyphae. **e** H₂O₂ accumulation in the leaves of Pst_TTP1RNAi plants was analyzed using the CellSens Entry software at 24 and 48 hpi. Means $\pm$ SD were determined based on three biological replicates from three leaf samples. The *P* value was determined using a two-tailed unpaired Student's t-test. **f** Fluorescence of ROS stained with H₂DCF-DA from the same set of *N. benthamiana* leaves expressing GUS-TaSGR1-Flag, TaTrxM-HA-TaSGR1-Flag, TaTrx-HA-TaSGR1-Flag, and Pst_TTP1-HA-TaSGR1-Flag shown in Fig. 6e. Means $\pm$ SD were determined based on three biological replicates from three leaf samples of different plants. The *P* value was determined using a two-tailed unpaired Student's t-test. Similar results are obtained from three independent biological experiments. Source data are provided as a Source Data file.

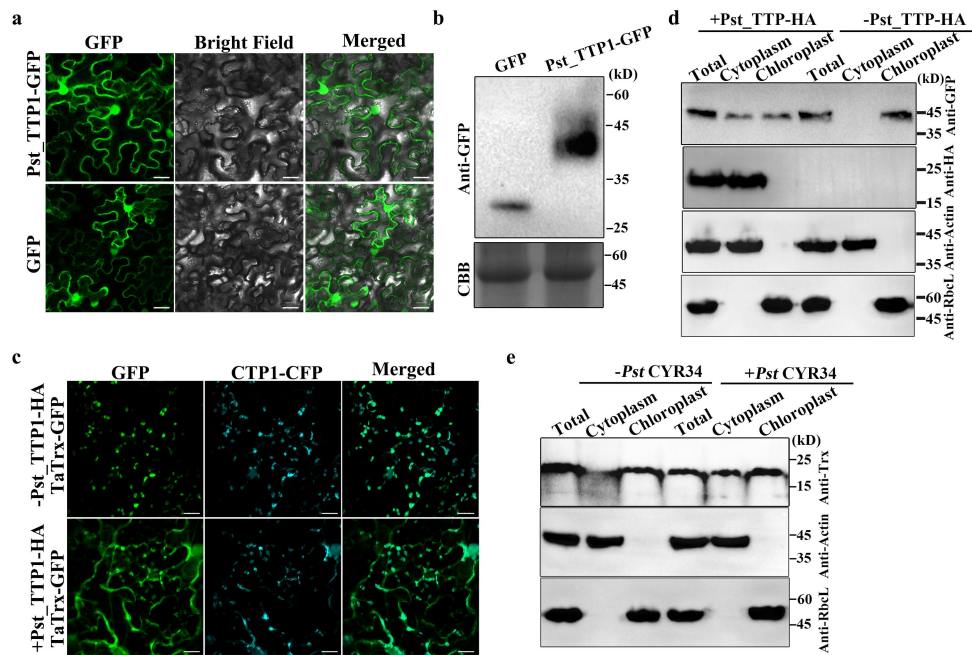
Supplementary Fig. 15



Supplementary Fig. 15 Detection of intraspecies polymorphism in Pst_TTP1. a

Nucleotide sequence alignments and **b** amino acid sequence alignments. The intraspecies polymorphism in *Pst_TTP1* was observed among five different *Pst* isolates, including one Chinese isolate (CYR32), three US isolates (PST-21, PST-43, and PST-78), and one UK isolate (PST-87/7). Their sequences were obtained from the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and sequence alignment was performed using the online platform at <http://multalin.toulouse.inra.fr/multalin/multalin.html>. The signal peptides are highlighted in blue. Asterisks indicate nonsynonymous nucleotide substitutions. Red font represents high consensus (> 90% similarity), whereas blue font represents low consensus (90% < value < 50% similarity).

Supplementary Fig. 16



Supplementary Fig. 16 Pst_TTP1 prevents TaTrx from entering chloroplasts. a

Subcellular localization of Pst_TTP1 in *N. benthamiana*. Pst_TTP1-GFP under the control of the 35S promoter was transiently expressed in *N. benthamiana* leaves. Bar = 20 μ m. **b** Western blots of proteins isolated from the same set of plants reported in Supplementary Fig. 16a were detected using the anti-GFP antibody. Rubisco protein was stained with CBB as a loading control. **c** Pst_TTP1 prevented TaTrx from entering chloroplasts in *N. benthamiana*. Leaf tissues transiently co-expressing TaTrx-GFP and Pst_TTP1-HA were examined using epifluorescence microscopy. CTP1-CFP was used as a marker protein to localize into chloroplasts. Bar = 20 μ m. **d** Pst_TTP1 promoted the accumulation of TaTrx in the cytoplasm in *N. benthamiana*. Western blots of proteins isolated from the same set of plants reported in Supplementary Fig. 16c were detected using the anti-GFP, anti-HA, anti-Actin and anti-RbcL antibodies. **e** Pst_TTP1 promotes the accumulation of TaTrx in the cytoplasm in *Pst* CYR34-infected Fielder plants. Total, cytoplasmic, and chloroplast proteins were isolated and detected via immunoblotting. Anti-Actin and anti-RbcL antibodies were used as markers for cytoplasmic proteins and chloroplast proteins, respectively. Similar results are obtained from three independent biological experiments.