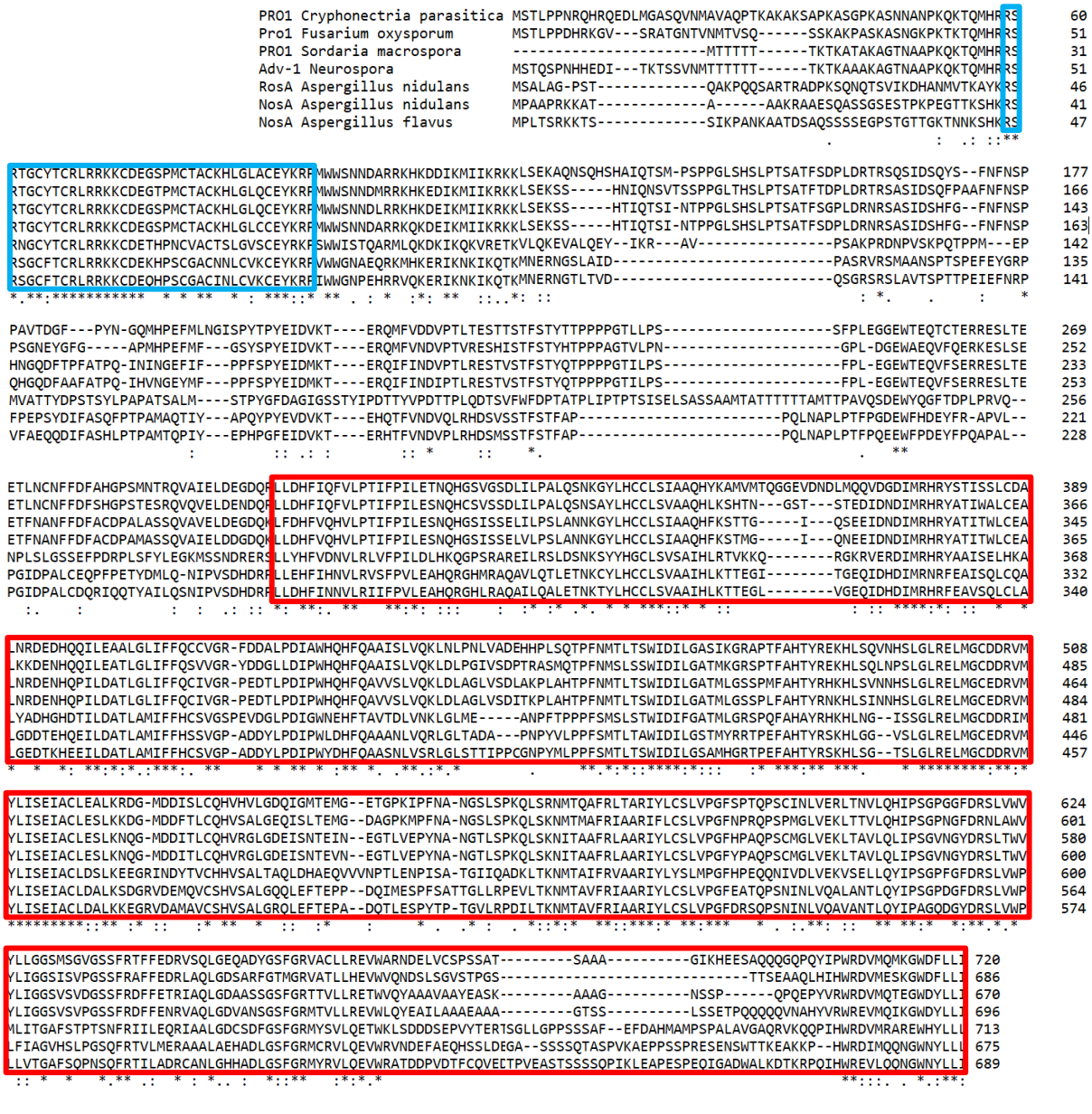
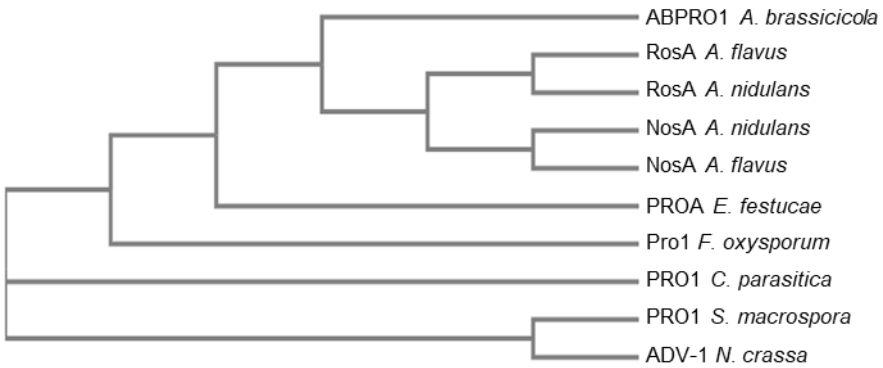
## Supplementary Figures



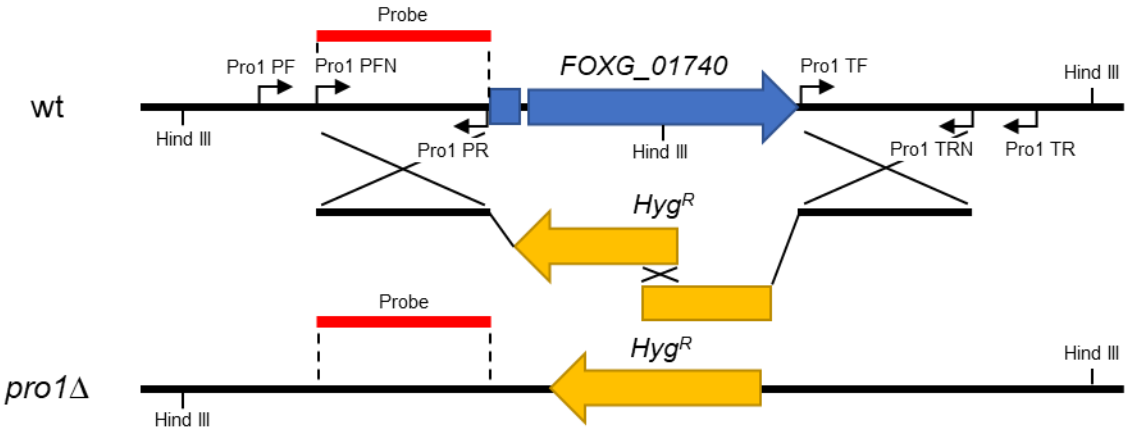

(A)



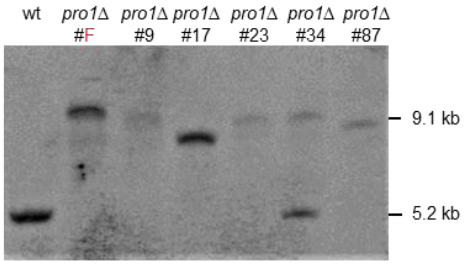

(B)

**Supplementary Figure S1.** Amino acid sequence alignment of the F. oxysporum Pro1 protein. (A) Sequence alignment of fungal Pro1 orthologues. The indicated full-length Pro1 protein sequences were obtained identified by BLASTP search in the

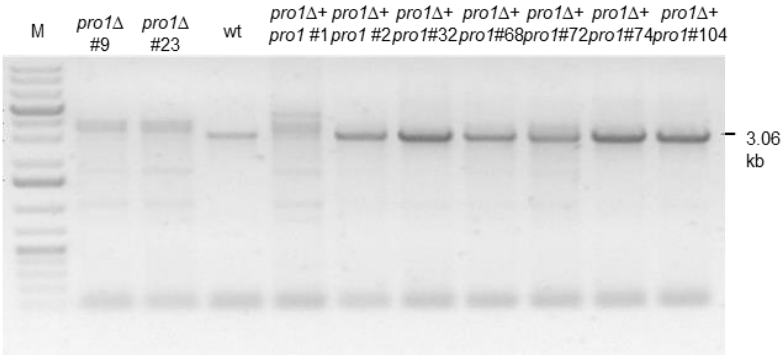
genome database of the National Center for Biotechnology Information, using the *Sordaria macrospora* k-hell PRO1 amino acid sequence (NCBI database: XP_003351793.1). Amino acid sequences of the different Pro1 proteins were aligned using Clustal W [38] and manually inspected. Asterisks, double dots and single dots indicate highly, less highly and moderately conserved residues. The GAL4-like $Zn_2Cys_6$ binuclear cluster DNA-binding domain is boxed in blue and the fungal specific transcription factor domain in red. (B) Phylogenetic tree of Pro1 orthologues based on sequence alignment of the complete predicted amino acid sequences was generated using the Clustal W Modelgenerator algorithm.




(A)

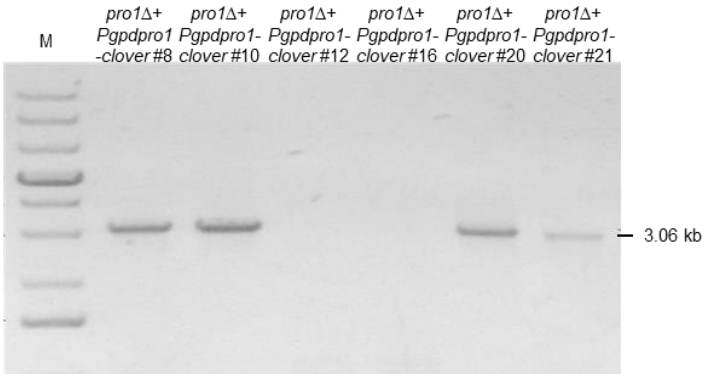



(B)




(C)

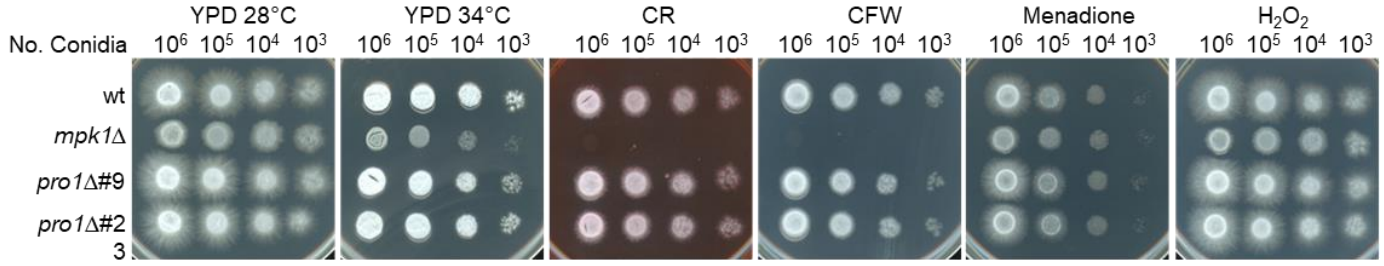

(D)



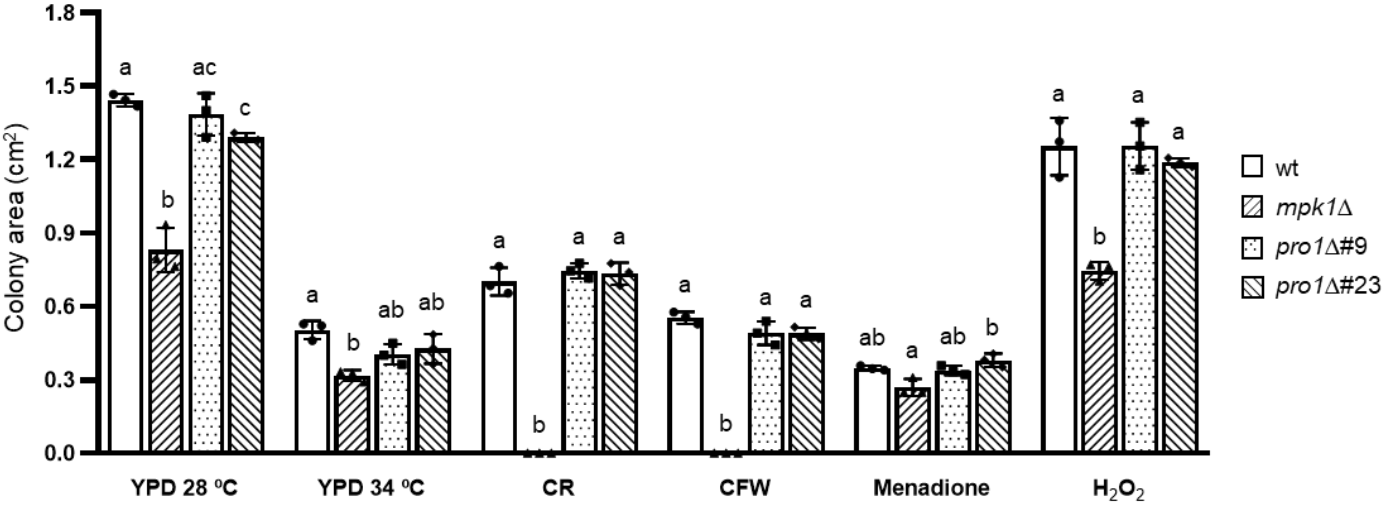

(E)

**Supplementary Figure S2.** Targeted replacement of the *pro1* gene in *F. oxysporum*. (A) Physical maps of the *F. oxysporum pro1* locus and of the split-marker gene replacement constructs obtained by fusion PCR. Relative positions of restriction sites, PCR primers and probe used in Southern blot are indicated. *Hyg*$^R$, *hygromycin* resistance gene. (B) Southern blot analysis of putative *pro1*Δ transformants. Genomic DNA of the wild type (wt) strain and six independent hygromycin resistant transformants was treated with

*Hind*III, separated on a 0.7% agarose gel, transferred to a nylon membrane and hybridized with a DNA probe corresponding to the 5′ flanking region of *pro1* indicated in (A). Molecular sizes of the hybridizing bands are indicated to the right. (C) Agarose gel electrophoresis of PCR products obtained using the primer pair CloverPro1 and Pro1compRnest with genomic DNA extracted from the indicated neomycin-resistant transformants. (D) Schematic representation of the *pro1*Δ *PgpdA-pro1-GFP* construct. (E) Agarose gel electrophoresis of PCR products obtained using the primer pair Gpda5 and Pro1Rev with genomic DNA extracted from the indicated neomycin resistant transformants. M, molecular size markers.




(A)




(B)

**Supplementary Figure S3.** Pro1 is required for the response to cell wall stress. (A) Colony phenotypes of the indicated strains grown on YPD (3% yeast extract, 1% peptone, 2% dextrose and 1.5% agar) medium at the indicated temperatures (28 or 34°C) or at 28 °C in the presence of the indicated compounds. Plates were spot inoculated with the indicated number of microconidia, incubated for 3 days (28°C) or 4 days (34 °C) and imaged. (B) Quantitative analysis of the colony area (cm²) was performed using the ImageJ software. The graph shows the values for the 104 colonies. Error bars represent standard deviations calculated from three replicate plates. Values with the same letter are not significantly different according to one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.05$). CFW, Calcofluor white; CR, Congo red.

## Supplementary Tables

**Supplementary Table S1.** Fungal Pro1 orthologues used for amino acid alignment.

| Organism | Protein | GenBank Accession Number |
|---|---|---|
| *Sordaria macrospora* | PRO1 | XP_003351793.1 |
| *Neurospora crassa* | ADV-1 | XP_958476 |
| *Cryphonectria parasitica* | PRO1 | ACI96308.1 |
| *Aspergillus nidulans* | RosA | CAD58393.1 |
| *Aspergillus nidulans* | NosA | CAJ76908.1 |
| *Aspergillus flavus* | NosA | RMZ40455.1 |
| *Epichloë festucae* | PROA | BAN17453.1 |
| *Fusarium oxysporum* f. sp. *lycopersici* | Pro1 | XP_018234693.1 |

**Supplementary Table S2.** Fungal strains used in this study.

| Strain | Background | Genotype | Reference |
|---|---|---|---|
| 4287 | 4287 | Wild type | [29] |
| *mpk1*Δ | 4287 | Knockout mutant for the *mpk1* locus | [10] |
| *fmk1*Δ | 4287 | Knockout mutant for the *fmk1* locus | [29] |
| *pro1*Δ#9 | 4287 | Knockout mutant for the *pro1* locus | This study |
| *pro1*Δ#23 | 4287 | Knockout mutant for the *pro1* locus | This study |
| *pro1*Δ+pro1 | pro1Δ#23 | Complemented knockout mutant | This study |
| *pro1*Δ+*PgpdA-pro1-GFP* | pro1Δ#23 | Constituve expression of *pro1* locus | This study |
| *fso1*Δ | 4287 | Knockout mutant for the *fso1* locus | [8] |
| *laeA*Δ | 4287 | Knockout mutant for the *laeA* locus | [39] |
| *veA*Δ | 4287 | Knockout mutant for the *veA* locus | [39] |
| *ste12*Δ | 4287 | Knockout mutant for the *ste12* locus | [7] |

**Supplementary Table S3.** Oligonucleotides used in this study. Lowercase nucleotides were added to generate overlapping ends for fusion PCR.

| Primer | Annealing Temp. | Nucleotide Sequence 5′ – 3′ |
|---|---|---|
| Pro1 PF | 62°C | AGGTGTAGAGGAGCTTCGATT |
| Pro1 PR | 62°C | tttacccagaatgcacaggtacacttgtttaAGTGCAGCCATAGTCAGTTGT |
| Pro1 PFN | 62°C | GTTGGATGGGGGATTTTAGTG |
| Pro1 TF | 62°C | tggtcgttgtaggggctgtattaggtCTCGGAGAGGCACAACTGGGATAG |
| Pro1 TR | 62°C | GGGCATAGAGTGAAAGTGTTG |
| Pro1 TRN | 62°C | CTGGTTCTTTGTCCCTGCTG |
| Pro1 comp F | 62°C | ACAACTGACTATGGCTGCACT |
| Pro1 comp R | 62°C | ATGGTGTGATGATGCTGACCT |
| Pro1 comp R nest | 62°C | CCCAGTTGTGCCTCTCGGAG |
| Clover Pro1 | 62°C | acaaggacgatgacgataagggtggttctATGTCTACGCTGCCCCCCGA |
| Pro1 Rev | 62°C | AAAAGTCCCACCCCTTCGATT |
| GpdA15B | 62°C | GGATCCCGAGACCTAATACAGCCCCT |
| GpdA15 nest | 62°C | ACTAAATCGACTTCAGCAACA |
| TrpC8B | 62°C | GGATCCAAACAAGTGTACCTGTGCATTC |
| Sv40 rev nest | 62°C | CTAGATACCACATTTGTAGAGG |
| Gpda5 | 62°C | TCCAAGAACCTTTATTTCCCCT |
| Hyg-G | 62°C | ACGTTGCAAGACCTGCCTGAA |
| Hyg-Y | 62°C | GGATACCTCCGCTCGAAGTA |
| Act-q7 | 64°C | ATGTCACCACCTTCAACTCCA |

**Supplementary Table S4.** Plasmids used in this study.

| Plasmid | Origin/features | References |
|---|---|---|
| pAN7-1 | Derived from pUC18; *A.nidulans gpdA* promoter; hygromicin B phosphotransferase gene from *Streptomyces spp.* (*hph*); *A. nidulans trpC* terminator | [36] |
| *pUC57- 3xFomClover-3XFLAG* | Derived from pUC57; *A. nidulans gpdA* promoter; 3 copies in tandem of the *mClover3* gene codon-adapted to *F. oxysporum* (3x*FomClover*); 3 copies of FLAG. | [46] |
| *pUC57- 1xFomClover-3XFLAG* | Derived from *pUC57- 3xFomClover- 3xFLAG.* Two of the three copies of *FomClover3* were removed. | This study. |
| *NeoR* | Derived from pAc5-STABLE2-Neo and pAN7-1 plasmids. *A. nidulans gpdA* promoter; neomycin resistance gene (*neo*); *A. nidulans trpC* terminator. | [37] |

**Supplementary Table S5.** Statistical significance of tomato *Solanum lycopersicum* survival curves after inoculation with indicated *Fusarium oxysporum* strains, compared with the wild type. Data were analysed with the program GraphPad Prism version 8.0.1 and P values were calculated using the Log-rank (Mantel-Cox) test.

| Strain | P value |
|---|---|
| *fmk1*Δ | <0,0001 |
| *pro1*Δ#9 | 0,5131 |
| *pro1*Δ#23 | 0,6655 |
| *pro1*Δ#23+*pro1* | 0,9106 |
| *pro1*Δ#23+*PgpdA-pro1-GFP* | 0,5916 |
| H2O control | <0,0001 |