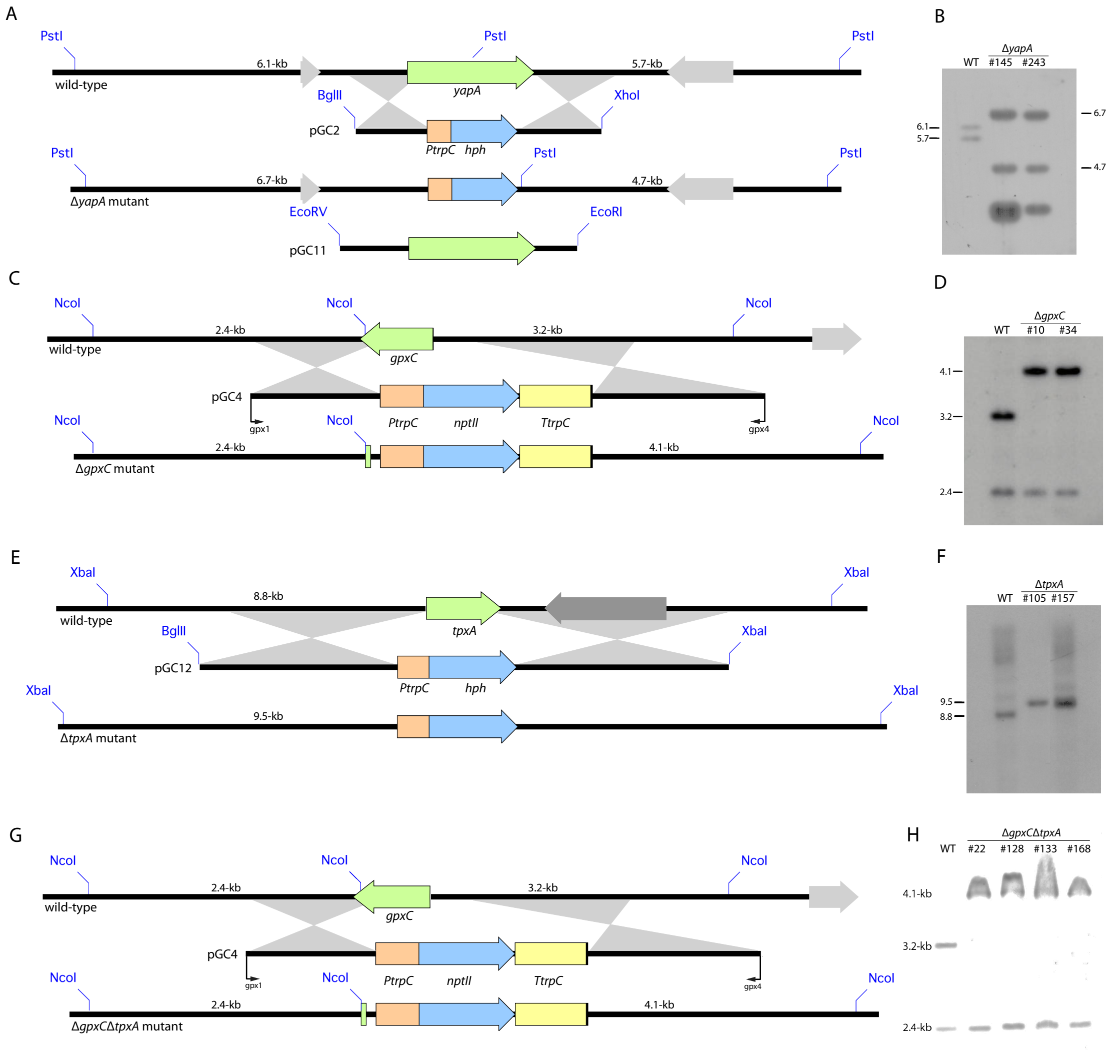
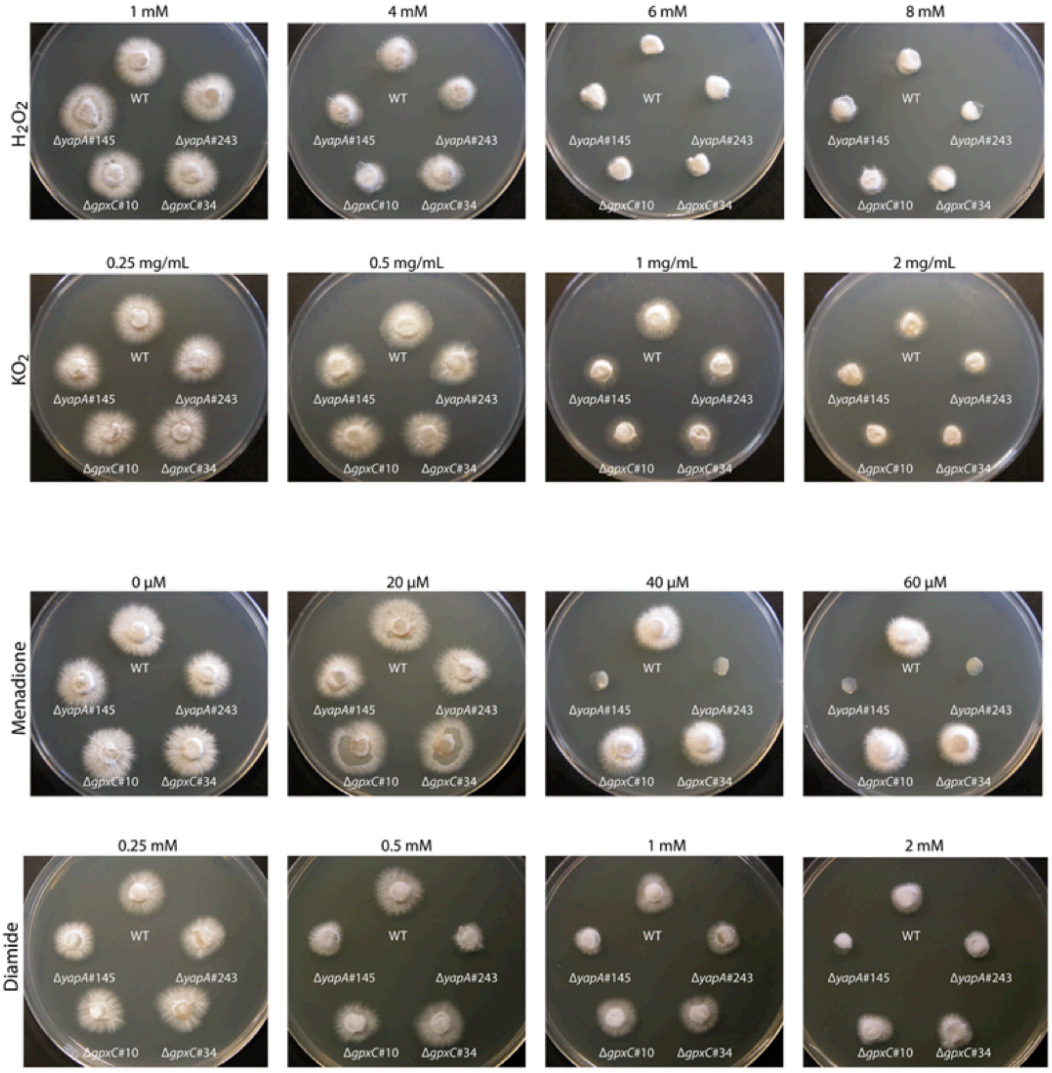
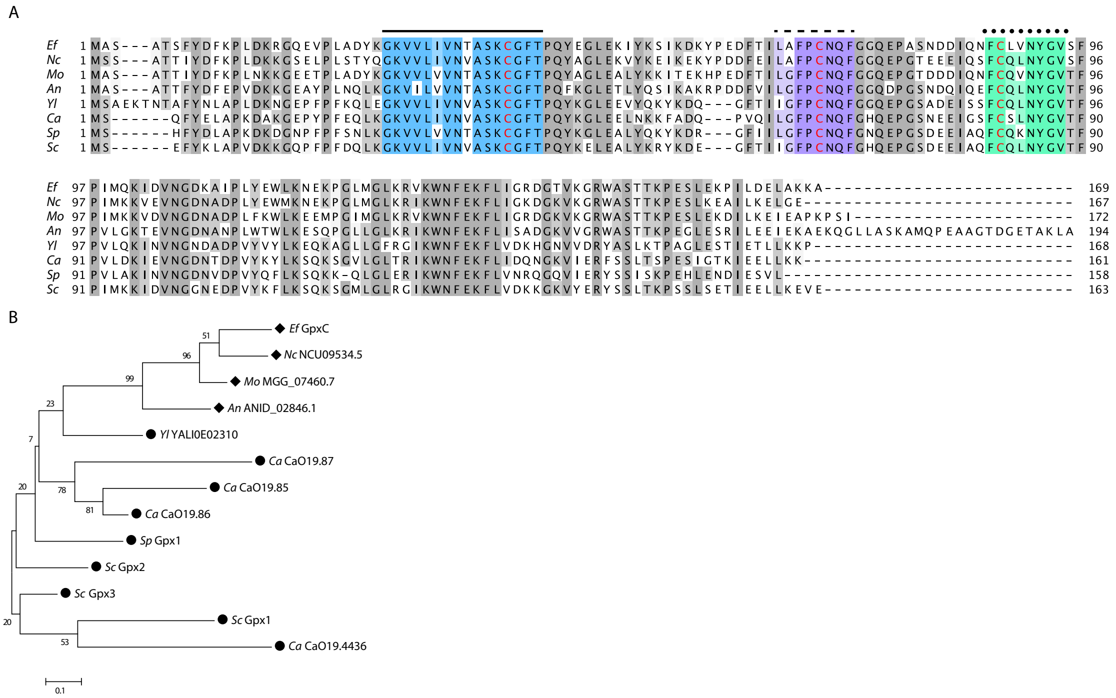


**SUPPL. FIG. 1.** Confirmation of *E. festucae yapA, gpxC and tpxA* single and *gpxC* and *tpxA* double deletions by Southern blotting (A) Schematic of the wild-type *yapA* locus, linearised replacement construct (pGC2), Δ*yapA* mutant genomic locus and complementation construct (pGC11). The regions of recombination between homologous sequences flanking the *yapA* locus and *hph* resistance cassette are indicated by grey shading. Restriction enzyme sites used to generate linear replacement (*Bgl*II, *Xho*I) and complementation fragments (*Eco*RV, *Eco*RI) and for Southern analysis (*Pst*I) are indicated. Grey arrows indicate genes flanking *yapA*. (B) Autoradiograph of Southern blot of *Pst*I digests (1 µg) of *E. festucae* WT/PN2278 (lane 1), Δ*yapA*#145/ PN2740 (lane 2) and Δ*yapA*#243/ PN2739 (lane 3) genomic DNA, probed with $^{32}$P-labelled pGC2. The expected fragment sizes are 6.1- and 5.7-kb for wild-type and 6.7- and 4.7-kb for the Δ*yapA* mutants. The additional 3.8-kb band corresponds to the size of the construct indicating it has integrated as a tandem repeat at the yapA locus. (C) Schematic of the wild-type *gpxC* locus, linearised replacement construct (pGC4) and Δ*gpxC* mutant genomic locus. The regions of recombination between homologous sequences flanking the *gpxC* locus and *npt*II resistance cassette are indicated by grey shading. Primer pair gpx1/gpx4 used to generate linear replacement fragment and restriction enzyme sites used for Southern analysis (*Nco*I) are indicated. Grey arrow indicates gene flanking *gpxC*. (D) Autoradiograph of Southern blot of *Nco*I digests (1 µg) of *E. festucae* Fl1/ PN2278 (lane 1), Δ*gpxC*#10/ PN2741 (lane 2) and Δ*gpxC*#34/PN2742 (lane 3) genomic DNA, probed with $^{32}$P-labelled pGC4. The expected fragment sizes are 3.2- and 2.4-kb for wild-type and 4.1- and 2.4-kb for the Δ*gpxC* mutants. (E) Schematic of the wild-type *tpxA* locus, linearised replacement construct (pGC12) and Δ*tpxA* mutant genomic locus. The regions of recombination between homologous sequences flanking the *tpxA* locus and *hph* resistance cassette are indicated by grey shading. Restriction enzyme sites used to generate the linear replacement fragment (*Bgl*II, *Xba*I) and for Southern analysis (*Xba*I) are indicated. Grey arrow indicates gene flanking *tpxA*. (F) Autoradiograph of Southern blot of *Xba*I digests (1 µg) of *E. festucae* Fl1/ PN2278 (lane 1), Δ*tpxA*#105/ PN2821 (lane 2) and Δ*tpxA*#157/ PN2822 (lane 3) genomic DNA, probed with $^{32}$P-labelled pGC12. The expected fragment sizes are 8.8-kb for wild-type and 9.5-kb for the Δ*tpxA* mutants. (G) Schematic of the wild-type *gpxC* locus, linearised replacement construct (pGC4) and Δ*gpxC* mutant genomic region for generation of the double mutant in a Δ*tpxA* background. The regions of recombination between homologous sequences flanking the *gpxC* locus and *npt*II resistance cassette are indicated by grey shading. The *gpxC* was deleted in the Δ*tpxA*#105 background. Primer pair gpx1/gpx4 used to generate linear replacement fragment and restriction enzyme sites used for Southern analysis (*Nco*I) are indicated. Grey arrow indicates gene flanking *gpxC*. (H) Autoradiograph of Southern blot of *Nco*I digests (1 µg) of *E. festucae* Fl1/ PN2278 (lane 1), Δ*gpxC*Δ*tpxA*#22/PN2831 (lane 2), Δ*gpxC*Δ*tpxA*#128/PN2828 (lane 3), Δ*gpxC*Δ*tpxA*#133/PN2829 (lane 4) and Δ*gpxC*Δ*tpxA*#168/PN2830 (lane 5) genomic DNA, probed with DIG-labeled gpx1/gpx4 PCR fragment from pGC4. The expected fragment sizes are 3.2- and 2.4-kb for wild-type and 4.1- and 2.4-kb for the Δ*gpxC*Δ*tpxA* mutants.



**SUPPL. FIG. 2.** Oxidative stress sensitivity of *E. festucae* deletion strains. Agar plugs 5 mm in diameter of strains indicated were inoculated onto PD medium containing a range of $H_2O_2$, $KO_2$, menadione and diamide concentrations as shown and cultured at 22ºC for 7 days.



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Ef  1 MAS---ATSFYDFKPLDKRGQEVPLADYKGKVVLIVNTASKCGFTPQYEGLEKIYKSIKDKYPEDFTIILAFPCNQFGGQEPASNDDIQNFCLVNYGVSF 96
Nc  1 MSS---ATTIYDFKPLDKKGSELPLSTYQGKVVLIVNVASKCGFTPQYAGLEKVYKEIKEKYPDDFEIILAFPCNQFGGQEPGTEEEIQSFCQLNYGVSF 96
Mo  1 MAS---ATTIYDFKPLNKKGEETPLADYKGKVVLVVNTASKCGFTPQYAGLEALYKKITEKHPEDFTIILGFPCNQFGGQEPGTDDDIQNFCQVNYGVTF 96
An  1 MAS---ATTFYDFEPVDKKGEAYPLNQLKGKVILVVNTASKCGFTPQFKGLETLYQSIKAKRPDDFVIILGFPCNQFGGQDPGSNDQIQEFCQLNYGVTF 96
Yl  1 MSAEKTNTAFYNLAPLDKNGEPFPFKQLEGKVVLIVNVASKCGFTPQYKGLEEVYQKYKDQ---GFTIIIGFPCNQFGGQEPGSADEISSFCQLNYGVTF 96
Ca  1 MS-------QFYELAPKDAKGEPYPFEQLKGKVVLIVNVASKCGFTPQYKGLEELNKKFADQ---PVQILGFPCNQFGHQEPGSNEEIGSFCSLNYGVTF 90
Sp  1 MS-------HFYDLAPKDKDGNPFPFSNLKGKVVLVVNTASKCGFTPQYKGLEALYQKYKDR---GFIILGFPCNQFGNQEPGSDEEIAQFCQKNYGVTF 90
Sc  1 MS-------EFYKLAPVDKKGQPFPFDQLKGKVVLIVNVASKCGFTPQYKELEALYKRYKDE---GFTIIGFPCNQFGHQEPGSDEEIAQFCQLNYGVTF 90

Ef 97 PIMQKIDVNGDKAIPLYEWLKNEKPGLMGLKRVKWNFEKFLIGRDGTVKGRWASTTKPESLEKPILDELAKKA-------------------------  169
Nc 97 PIMKKVEVNGDNADPLYEWMKNEKPGLMGLKRIKWNFEKFLIGKDGKVKGRWASTTKPESLKEAILKELGE---------------------------  167
Mo 97 PIMKKVDVNGDNADPLFKWLKEEMPGIMGLKRVKWNFEKFLIGRDGKVKGRWASTTKPESLEKDILKEIEAPKPSI----------------------  172
An 97 PVLGKTEVNGDNANPLWTWLKESQPGLLGLKRIKWNFEKFLISADGKVVGRWASTTKPEGLESRILEEIEKAEKQGLLASKAMQPEAAGTDGETAKLA  194
Yl 97 PVLQKINVNGNDADPVYVYLKEQKAGLLGFRGIKWNFEKFLVDKHGNVVDRYASLKTPAGLESTIETLLKKP--------------------------  168
Ca 91 PVLDKIEVNGDNTDPVYKYLKSQKSGVLGLTRIKWNFEKFLIDQNGKVIERFSSLTSPESIGTKIEELLKK---------------------------  161
Sp 91 PVLAKINVNGDNVDPVYQFLKSQKK-QLGLERIKWNFEKFLVNRQGQVIERYSSISKPEHLENDIESVL-----------------------------  158
Sc 91 PIMKKIDVNGGNEDPVYKFLKSQKSGMLGLRGIKWNFEKFLVDKKGKVYERYSSLTKPSSLSETIEELLKEVE-------------------------  163
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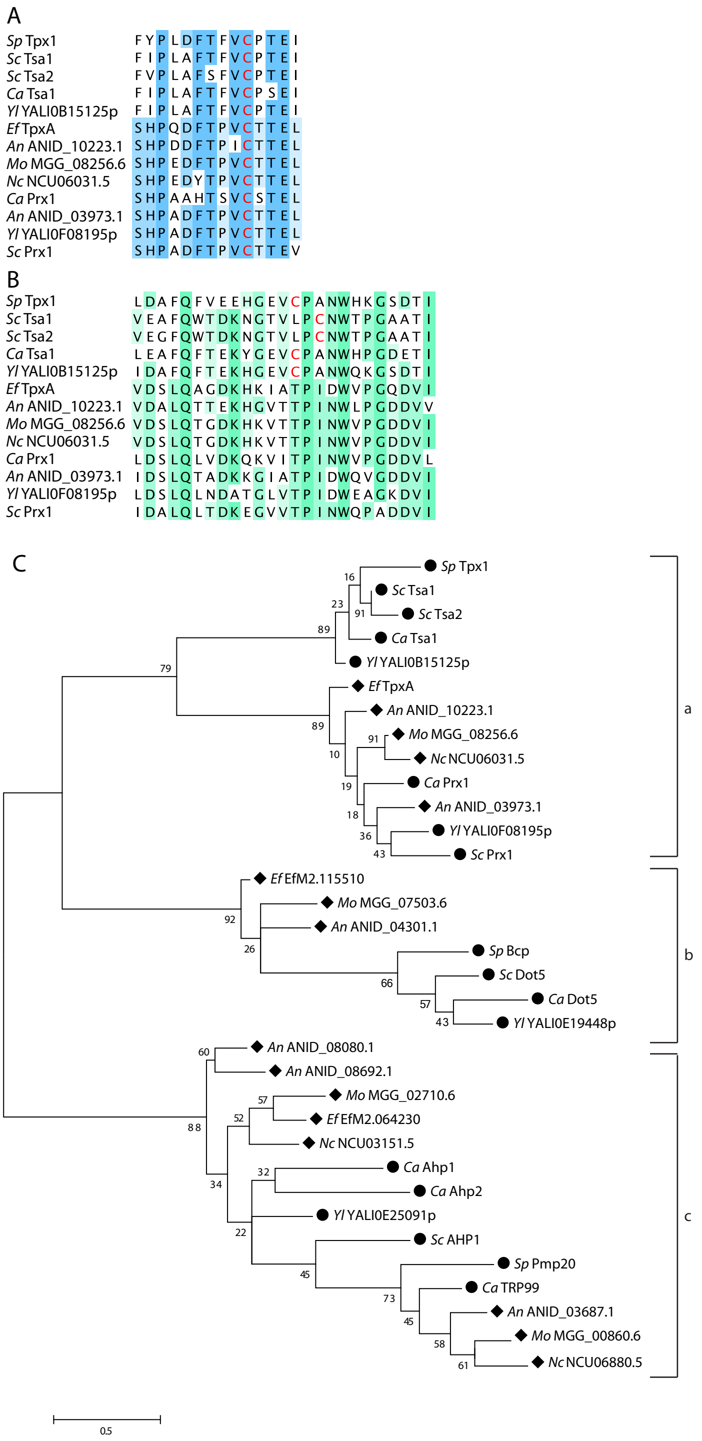
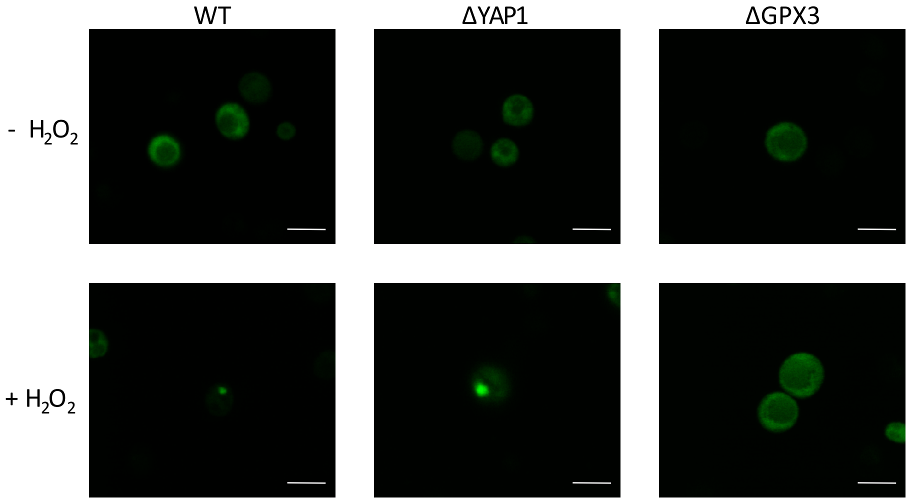
**SUPPL. FIG. 3.** Multiple sequence alignment and maximum-likelihood dendrogram of fungal GPx proteins. (A) Multiple sequence alignment of fungal GPx proteins. Solid line indicates active-site motif, dashed line indicates GPx family signature motif and dotted line indicates region containing the resolving cysteine. Conserved cysteine residues are highlighted in red. In species where multiple Gpx3-like proteins were present (*C. albicans*) the protein sharing the highest identity and similarity (CaO19.86, XP_714295.1) to *S. cerevisiae* Gpx3 was aligned. *Ef, Epichloë festucae*; *Nc, Neurospora crassa*; *Mo, Magnaporthe oryzae*; *An, Aspergillus nidulans*; *Yl, Yarrowia lipolytica*; *Ca, Candida albicans*; *Sp, Schizosaccharomyces pombe*; *Sc, Saccharomyces cerevisiae*.

(B) Maximum-likelihood dendrogram of fungal GPx proteins. Values above branches indicate bootstrap values based on 2000 replicates. Gene IDs with associated GenBank accession numbers in parentheses are as follows: Ef, *E. festucae* GpxC EfM2.018640 (KC121578); Nc, *N. crassa* NCU09534.5 (XP_957919.1); Mo, *M. oryzae* MGG_07460.7 (XP_367549.2); An, *A. nidulans* ANID_02846.1 (XP_660450.1); Yl, *Y. lipolytica* YALI0E02310 (XP_503454.1); Ca, *C. albicans* CaO19.87 (XP_714296.1), CaO19.85 (XP_714294.1), CaO19.86 (XP_714295.1); Sp, *S. pombe* Gpx1 SPBC32F12.03c (NP_596146.1); Sc, *S. cerevisiae* Gpx2 YBR244W (NP_009803.1), Gpx3 YIR037W (NP_012303.1), Gpx1 YKL026C (NP_012899.1), Ca, *C. albicans* CaO19.4436 (XP_714081.1). ◆ = filamentous fungal species, ● = yeast species.



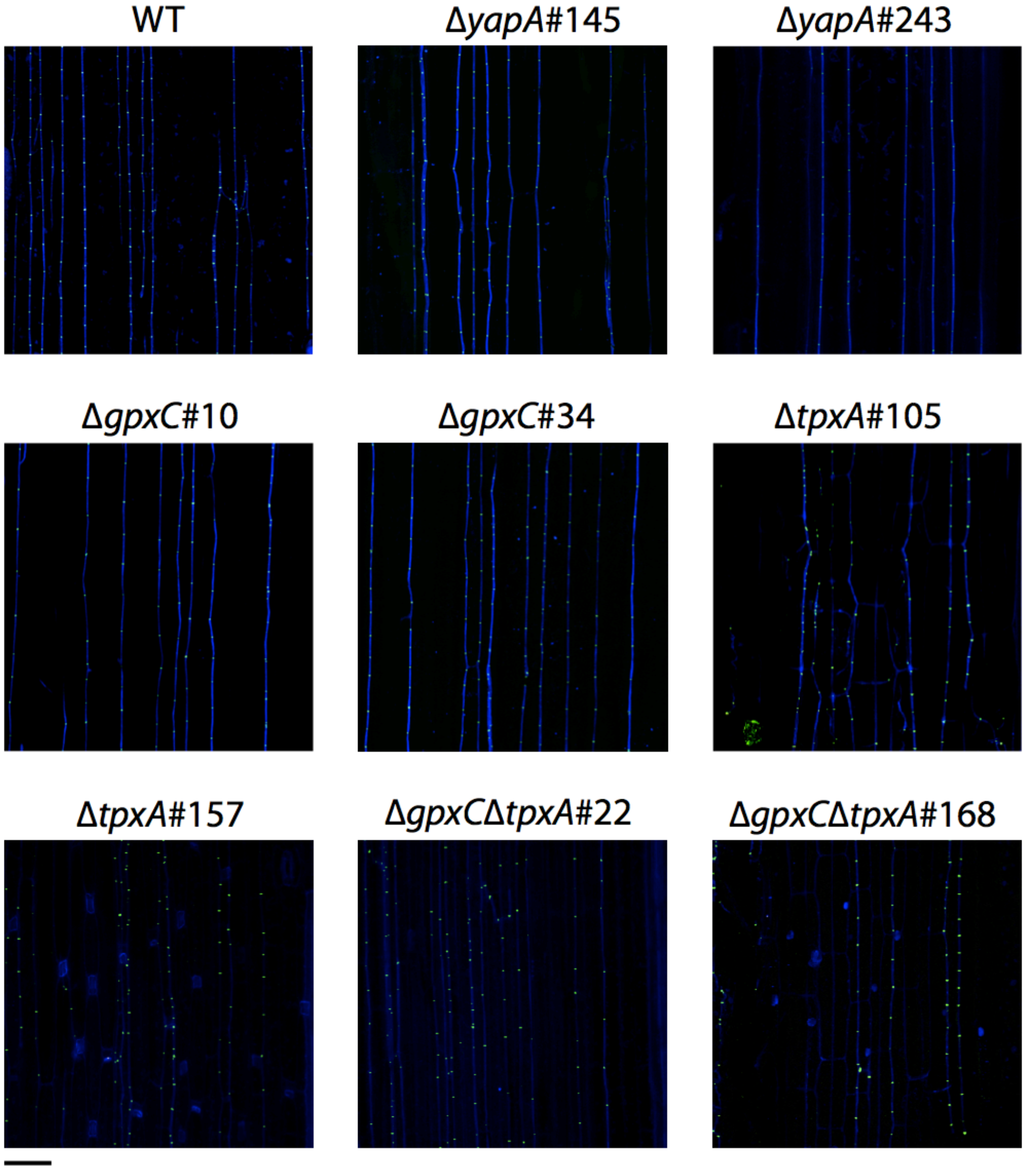
**SUPPL. FIG. 4.** Multiple sequence alignment and maximum-likelihood dendrogram of Prx signature motifs. (A) Multiple sequence alignment of Prx signature motifs. The first Prx signature motif from peroxiredoxin proteins from clade a in C. Conserved peroxidatic cysteine residues are highlighted in red. (B) The second Prx signature motif from peroxiredoxin proteins from clade a in (C). Conserved resolving cysteine residues, where present, are highlighted in red. *Sp, Schizosaccharomyces pombe*; *Sc, Saccharomyces cerevisiae*; *Ca, Candida albicans*; *Yl, Yarrowia lipolytica*; *Ef, Epichloë festucae*; *An, Aspergillus nidulans*; *Mo, Magnaporthe oryzae*; *Nc, Neurospora crassa*. (C) Maximum-likelihood dendrogram of fungal Prx proteins. Values above branches indicate bootstrap values based on 2000 replicates. Gene IDs with associated GenBank accession numbers in parentheses are as follows: Sp, *S. pombe* Tpx1 SPCC576.03c (NP_588430.1), Bcp SPBC1773.02c (NP_595117.1), Pmp20 SPCC330.06c (NP_587706.1); Sc, *S. cerevisiae* Tsa1 YML028W (NP_013684.1), Tsa2 YDR453C (NP_010741.1), Prx1 YBL064C (NP_009489.1), Dot5 YIL010W (NP_012255.1), Ahp1 YLR109W (NP_013210.1); Ca, *C. albicans* Tsa1

(XP_716082.1), Prx1 (XP_717002.1), Ahp1 (XP_720512.1), TRP99 (XP_715859.1), Ahp2 (XP_721312.1), Dot5 (XP_717789.1); Yl, *Y. lipolytica* YALI0B15125p (XP_500915.1), YALI0E25091p (XP_504381.1), YALI0F08195p (XP_505152.1) YALI0E19448p (XP_504146.1); Ef, *E. festucae* TpxA (EfM2.113210), Bcp-like (EfM2.115510), Pmp20-like (EfM2.064230); An, *A. nidulans* ANID_03973.1 (XP_661577.1), ANID_10223.1 (CBF85378.1), ANID_08080.1 (CBF73841.1), ANID_08692.1 (XP_681961.1), ANID_03687.1 (CBF75606.1), ANID_04301.1 (CBF77809.1); Mo, *M. oryzae* MGG_08256.6 (XP_362792.2), MGG_07503.6 (XP_367592.1), MGG_00860.6 (XP_368384.2), MGG_02710.6 (XP_366634.1); Nc, *N. crassa* NCU06031.5 (XP_959621.1), NCU06880.5 (XP_959227.1), NCU03151.5 (XP_964200.2). ◆ = filamentous fungal species, ● = yeast species.



**SUPPL. FIG. 5.** Subcellular localization of Yap1 in *S. cerevisiae***.** Confocal images of *S. cerevisiae* cells expressing *S. cerevisiae* Yap1-eGFP under the control of the *GAL1* promoter (pGC18) in WT (PN2871), Δ*YAP1* (PN2872) and Δ*GPX3* (PN2873) strains in the absence of $H_2O_2$ (- $H_2O_2$) and in cells treated with 0.4 mM $H_2O_2$ (+ $H_2O_2$) for 10 min. Bar = 5 µm

**SUPPL. FIG. 6.** Analysis of the *E. festucae*-*L. perenne* association. Photographs taken 8 weeks after inoculation with the following strains as indicated: *E. festucae* wild-type (WT), Δ*yapA*#145/ PN2740 and Δ*yapA*#243/PN2787, Δ*gpxC*#10/PN2741 and Δ*gpxC*#34/PN2742, Δ*tpxA*#105/PN2821 and Δ*tpxA*#157/PN2822 and Δ*gpxC*Δ*tpxA*#22/PN2831.



**SUPPL. FIG. 7.** Confocal analysis of the *E. festucae*-*L. perenne* association. Confocal depth series images of longitudinal sections through *L. perenne* pseudostem tissue, infected with *E. festucae* strains indicated as in Fig S6, stained with Alexafluor (WGA-AF488) and aniline blue. Images were generated by maximum intensity projection of 10 x 1 µm confocal z-stacks. Bar = 50 µm

**Table S1. Organisms and plasmids**

| Strain | Relevant characteristic(s) | Source or reference |
|---|---|---|
| *S. cerevisiae* | | |
| PN2735 | MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 (BY4741; wild type) | Euroscarf, Frankfurt |
| PN2736 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YML007w::kanMX4 (Δ*yap1*) | Euroscarf, Frankfurt |
| PN2737 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YIR037w::kanMX4 (Δ*gpx3*) | Euroscarf, Frankfurt |
| PN2845 | BY4741; MATa; his3 Δ 1; leu2 Δ 0; met15 Δ 0; ura3Δ0; YML007w::kanMX4 pGC8 (Δ*yap1*/*ScYAP1*) | This study |
| PN2846 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YML007w::kanMX4 pGC6 (Δ*yap1*/*EfYapA*) | This study |
| PN2847 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YML007w::kanMX4 pYES2 (Δ*yap1*/pYES2) | This study |
| PN2848 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YIR037w::kanMX4 pGC7 (Δ*gpx3*/*ScGPX3*) | This study |
| PN2849 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YIR037w::kanMX4 pGC5 (Δ*gpx3*/*EfGpxC*) | This study |
| PN2850 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YIR037w::kanMX4 pYES2 (Δ*gpx3*/pYES2) | This study |
| PN2871 | MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 (WT/pGC18) | This study |
| PN2872 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YML007w::kanMX4 (Δ*yap1*/pGC18) | This study |
| PN2873 | BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YIR037w::kanMX4 (Δ*GPX3*/pGC18) | This study |
| *E. festucae* | | |
| PN2278 | Wild type (Fl1) | (Young *et al.*, 2005) |
| PN2739 | Fl1/Δ*yapA*::P*trpC-hph*; Hyg$^R$ (Δ*yapA*#243) | This study |
| PN2740 | Fl1/Δ*yapA*::P*trpC-hph*; Hyg$^R$ (Δ*yapA*#145) | This study |
| PN2787 | Δ*yapA/yapA;* PN2739/pGC11; Hyg$^R$; Gen$^R$ (Δ*yapA*#243/*yapA*) | This study |
| PN2788 | Δ*yapA/yapA;* PN2740/pGC11; Hyg$^R$; Gen$^R$ (Δ*yapA*#145/*yapA*) | This study |
| PN2741 | Fl1/Δ*gpxC*::P*trpC*-*npt*II-T*trpC*; Gen$^R$ (Δ*gpxC*#10) | This study |
| PN2742 | Fl1/Δ*gpxC*::P*trpC*-*npt*II-T*trpC*; Gen$^R$ (Δ*gpxC*#34) | This study |
| PN2821 | Fl1/Δ*tpxA*::P*trpC-hph*; Hyg$^R$ (Δ*tpxA*#105) | This study |
| PN2822 | Fl1/Δ*tpxA*::P*trpC-hph*; Hyg$^R$ (Δ*tpxA*#157) | This study |
| PN2828 | PN2821/Δ*gpxC*::P*trpC*-*npt*II-T*trpC*; Hyg$^R$; Gen$^R$ (Δ*gpxC*Δ*tpxA*#128) | This study |
| PN2829 | PN2821/Δ*gpxC*::P*trpC*-*npt*II-T*trpC*; Hyg$^R$; Gen$^R$ (Δ*gpxC*Δ*tpxA*#133) | This study |
| PN2830 | PN2821/Δ*gpxC*::P*trpC*-*npt*II-T*trpC*; Hyg$^R$; Gen$^R$ (Δ*gpxC*Δ*tpxA*#168) | This study |

| | | |
|---|---|---|
| PN2831 | PN2821/Δ*gpxC*::P*trpC*-*npt*II-T*trpC*; Hyg$^{R}$; Gen$^{R}$ (Δ*gpxC*Δ*tpxA*#22) | This study |
| PN2789 | PN2741/ pGC9/pJW19/pAN8-1; Hyg$^{R}$, Gen$^{R}$, Zeo$^{R}$ (Δ*gpxC:: eGFP-yapA, DsRed-stuA(NLS)*) | This study |
| PN2790 | PN2278/pGC9, pJH19; Hyg$^{R}$ (Fl1::*GFP-yapA, DsRed-stuA(NLS)*) | This study |
| PN2823 | PN2821/pGC10/ pJW19/pSF17.8; Hyg$^{R}$; Gen$^{R}$ (Δ*tpxA:: eGFP-yapA, DsRed-stuA(NLS)*) | This study |
| PN2824 | PN2278/pGC13; Gen$^{R}$ (Fl1::*PcatA-EGFP*) | This study |
| PN2836 | PN2739/pGC14; Hyg$^{R}$, Gen$^{R}$ (Δ*yapA#243::PcatA-eGFP-CL1*#1) | This study |
| PN2837 | PN2739/pGC14; Hyg$^{R}$, Gen$^{R}$ (Δ*yapA#243::PcatA-eGFP-CL1*#4) | This study |
| PN2838 | PN2278/pGC14; Gen$^{R}$ (Fl1::*PcatA-eGFP-Cl1*#8) | This study |
| PN2839 | PN2278/pGC14; Gen$^{R}$ (Fl1::*PcatA-eGFP-Cl1*#9) | This study |
| PN2840 | PN2278/pGC14; Gen$^{R}$ (Fl1::*PcatA-eGFP-Cl1*#10) | This study |
| PN2841 | PN2278/pGC14; Gen$^{R}$ (Fl1::*PcatA-eGFP-Cl1*#11) | This study |
| PN2842 | PN2740 Δ*yapA*#145/pGC14; Hyg$^{R}$, Gen$^{R}$ (Δ*yapA*#145::*PcatA-EGFP-CL1*#1) | This study |
| PN2843 | PN2740/pGC14; Hyg$^{R}$, Gen$^{R}$ (Δ*yapA#145::PcatA-EGFP-CL1#9*) | This study |
| PN2844 | PN2739 Δ*yapA*#243/pGC14; Hyg$^{R}$, Gen$^{R}$ (Δ*yapA*#243::*PcatA-EGFP-CL1*#10) | This study |
| PN2851 | PN2830/pGC9/pJW19/pAN8-1; Hyg$^{R}$, Gen$^{R}$, Zeo$^{R}$ (Δ*gpxC*Δ*tpxA::eGFP-yapA, DsRed-stuA(NLS)*) | This study |
| PN2874 | PN2278/pGC19/pJW19; Hyg$^{R}$ (Fl1::*YAP1-eGFP, DsRed-stuA(NLS)*) | This study |
| *E. coli* | | |
| PN4101 | One Shot® TOP10/pGC4; Amp$^{R}$ | This study |
| PN4103 | One Shot® TOP10/pGC2; Amp$^{R}$ | This study |
| PN4107 | One Shot® TOP10/pGC6; Amp$^{R}$ | This study |
| PN4108 | One Shot® TOP10/pGC5; Amp$^{R}$ | This study |
| PN4109 | One Shot® TOP10/pGC7; Amp$^{R}$ | This study |
| PN4110 | One Shot® TOP10/pGC8; Amp$^{R}$ | This study |
| PN4112 | One Shot® TOP10/pGC10; Amp$^{R}$ | This study |
| PN4113 | One Shot® TOP10/pGC9; Amp$^{R}$ | This study |
| PN4133 | One Shot® TOP10/pGC11; Amp$^{R}$ | This study |
| PN4135 | One Shot® TOP10/pGC12; Amp$^{R}$ | This study |
| PN4151 | One Shot® TOP10/pGC13; Amp$^{R}$ | This study |
| PN4174 | One Shot® TOP10/pGC14; Amp$^{R}$ | This study |
| PN4188 | One Shot® TOP10/pGC16; Amp$^{R}$ | This study |
| PN4193 | One Shot® TOP10/pGC18; Amp$^{R}$ | This study |
| PN4194 | One Shot® TOP10/pGC19; Amp$^{R}$ | This study |

| | | |
|---|---|---|
| PN1862 | One Shot® TOP10/pSF15.15; Amp$^{R}$ | S. Foster |
| PN1866 | One Shot® TOP10/pSF17.8; Amp$^{R}$ | S. Foster |
| PN1687 | One Shot® TOP10/pII99; Amp$^{R}$ | (Namiki *et al.*, 2001) |
| PN4111 | One Shot® TOP10/pPN94; Amp$^{R}$ | (Takemoto *et al.*, 2006) |
| PN1390 | One Shot® TOP10/pAN8-1; Amp$^{R}$ | (Mattern *et al.*, 1988) |
| PN4134 | One Shot® TOP10/pJW19; Amp$^{R}$ | (Toews *et al.*, 2004) |
| PN4201 | One Shot® TOP10/pYES2; Amp$^{R}$ | Invitrogen |
| Plasmids | | |
| pGC2 | 1.1-kb *Bgl*II/*Kpn*I fragment 5′ of *yapA* amplified with yap1/yap2 and 1.2-kb *Hind*III/*Xho*I fragment 3′ of *yapA* amplified with yap3/yap4 in pSF15.15 | This study |
| pGC4 | 1.1-kb *Bgl*II/*Kpn*I 5′ of *gpxC* amplified with gpx1/gpx2 and 1.4-kb *Sal*I/*Sal*I fragment 3′ of *gpxC* amplified with gpx3/gpx4 in pSF17.8 | This study |
| pGC5 | 0.5-kb *Eco*RI/*Xba*I *gpxC* cDNA fragment amplified with gpx5/gpx6 in pYES2 | This study |
| pGC6 | 1.7-kb *Eco*RI/*Xba*I *yapA* cDNA fragment amplified with yap5/yap6 in pYES2 | This study |
| pGC7 | 0.5-kb *Hind*III/*Xba*I *gpx3* gDNA fragment amplified with gpx7/gpx8 in pYES2 | This study |
| pGC8 | 1.9-kb *Hind*III/*Xba*I *yap1* gDNA fragment amplified with yap7/yap8 in pYES2 | This study |
| pGC9 | 1.7-kb y *Eco*RI/*Cla*I *yapA* cDNA fragment amplified with yap27/yap28 and 0.8-kb *Cla*I/*NotI* EGFP fragment amplified with GCGFP1/GCGFP2 in *Eco*RI/*Not*I site of pPN94 (*yapA-EGFP*) | This study |
| pGC10 | 0.8-kb *Eco*RI/*Cla*I *EGFP* fragment amplified with EGFP1/EGFP2 and 1.7-kb *Cla*I/*Not*I *yapA* cDNA fragment amplified with yap29/yap30 in *Eco*RI/*Not*I site of pPN94 (*EGFP-yapA*) | This study |
| pGC11 | 3.7-kb *Eco*RV/*Eco*RI fragment from cosmid 28E7 in pSF17.8 | This study |
| pGC12 | 2.3-kb *Bgl*II/*Kpn*I fragment 5′ of *tpxA* amplified with tpx1/tpx2 and 2.5-kb *Bam*HI/*Xba*I fragment 3′ of *tpxA* amplified with tpx3/tpx4 in pSF15.15 | This study |
| pGC13 | 1-kb *Xba*I/*Eco*RI P*catA* fragment amplified with pcatA3/pcatA4, cloned into *Xba*I/*Eco*RI site of pGC10, 1.8-kb *Xba*I/*Xho*I P*catA-EGFP* fragment cloned into *Xba*I/*Xho*I fragment in pSF17.8 (P*catA-EGFP*) | This study |
| pGC14 | 1.2-kb *Sac*II/*Nde*I fragment containing a 48 bp insert 3′ of P*catA-EGFP* amplified in two steps with pCatAF1/CL1R1 and pCatAF1/CL1R2 and replacing the *Sac*II/*Nde*I fragment in pGC13 (P*catA-EGFP-CL1*) | This study |
| pGC16 | 1-kb P*tpxA* fragment amplified with tpx37/tpx41 and 0.7-kb EGFP-CL1 fragment amplified with tpx39/tpx40, recombined in yeast and *Xba*I/*Nde*I P*tpxA-EGFP-CL1* fragment cloned into *Xba*I/*Nde*I site of pSF17.8 (P*tpxA-EGFP-CL1*) | This study |
| pGC18 | 2.7-kb *Eco*RI/*Not*I *yap1-EGFP* fragment from pGC19 cloned into *Eco*RI/*Not*I site of pYES2 (P*GAL1-Yap1-EGFP*) | This study |

| | | |
|---|---|---|
| pGC19 | 2.0-kb *Eco*RI/*Not*I *yap1* fragment amplified with ScYap6/ScYap8 and 0.7-kb *EGFP* fragment amplified with GCGFP3/GCGFP4, recombined in yeast and *Eco*RI/*Not*I *YAP1-EGFP* fragment cloned into *Eco*RI/*Not*I site of pPN94 (P*TEF-YAP1-EGFP*) | This study |
| pSF15.15 | pSP72 containing 1.4-kb HindIII P*trpC-hph* from pCB1004 cloned into *Sma*I site Amp$^{R}$; Hyg$^{R}$ | S. Foster |
| pSF17.8 | Amp$^{R}$; Gen$^{R}$ | S. Foster |
| pII99 | P*trpC-npt*II-T*trpC*; Amp$^{R}$/Gen$^{R}$ | (Namiki *et al.*, 2001) |
| pPN94 | pSF14.14 containing 0.8-kb *Sal*I/*Xba*I *tef* promoter in *Xho*I/*Xba*I site and 0.6-kb *Eco*RI/*Bgl*II T*trpC* in *Eco*RI/*Bgl*II site | (Vanden Wymelenberg *et al.*, 1997) |
| pAN8-1 | P*gpd-sh ble*-T*trpC*; Amp$^{R}$/Zeo$^{R}$ | (Mattern *et al.*, 1988) |
| pJW19 | P*gpd-DsRed-stuA*(NLS), argB+ in pBluescript KS-; Amp$^{R}$ | (Toews *et al.*, 2004) |
| pYES2 | Amp$^{R}$; URA3 | Invitrogen |
| pCR4-TOPO® | Amp$^{R}$; LacZ$\alpha$-*ccdB* | Invitrogen |
| Cosmids | | |
| 28E7 | pMO-cosX clone Fl1 genomic DNA cosmid library containing the *yapA* gene and 5’UTR. | This study |

**Table S2. Primer sequences**

| Name | Sequence (5’ – 3’) | Used for |
|---|---|---|
| CL1 R1 | CAGGGAGGAGAACCAGTTCTTGCAGGCTAGAACTCGAGACTTGTACAGCTCGTC | P*catA-eGFP*-CL1 fusion construct |
| CL1 R2 | CATATGCAGGTGGATGACGAAGTGGGACAGGGAGGAGAACCAGTTCTTGCAG | P*catA-eGFP*-CL1 fusion construct |
| GCGFP1 | ATCGATGGTGCTGGTGCTGG | *yapA-eGFP* fusion construct |
| GCGFP2 | GCGGCCGCTTTACTTGTACAG | *yapA-eGFP* fusion construct |
| GCGFP3 | GCGGATAACAATTTCACACAGGAAACAGCGCGGCCGCTTTACTTGTACAG | P*TEF-YAP1-eGFP* fusion construct |
| GCGFP4 | GGTGCTGGTGCTGGTGCT | P*TEF-YAP1-eGFP* fusion construct |
| gpx1 | AGATCTAGCCTTTTAAGAAGCACAACG | 5’ *gpxC* replacement construct |
| gpx2 | GGTACCGAGTTTCACATGTGATCAGTA | 5’ *gpxC* replacement construct |
| gpx3 | GTCGACGGGATTTCATATGCTATG | 3’ *gpxC* replacement construct |
| gpx4 | GTCGACCTATGCATGTTAGCAACC | 3’ *gpxC* replacement construct |
| gpx5 | GAATTCATGGCCTCCGCCACGAGCTTCT | *S. cerevisiae* complementation |
| gpx6 | TCTAGATTAAGCCTTCTTCGCCAATTCGT | *S. cerevisiae* complementation |
| gpx7 | AAGCTTATGTCAGAATTCTATAAGCTAGCACC | *S. cerevisiae* complementation |
| gpx8 | TCTAGACTATTCCACCTCTTTCAAAAGTTC | *S. cerevisiae* complementation |
| gpx9 | ATGATGCCGCCACCTACGAAAT | screening *gpxC* for replacement |
| gpx10 | AGAACTCGTCAAGAAGGCGATAGA | screening *gpxC* for replacement |
| gpx11 | TATTCGGCTATGACTGGGCAC | screening *gpxC* for replacement |
| gpx12 | TACATACGGAGTAGTCCATAAACATA | screening *gpxC* for replacement |
| gpx15 | TCTTCCATACTGATCACATG | screening *gpxC* for replacement |
| gpx16 | ATCGATCTGTGAACACAGAC | screening *gpxC* for replacement |
| pcatA3 | TCTAGACGTTTTCATTGAGCAACA | P*catA-eGFP* fusion construct |
| pcatA4 | GAATTCTCTCGCGGTATTGGGG | P*catA-eGFP* fusion construct |
| pCatAF1 | CCGCGGATGCAGGTCAAAACACGCACTTTGCGAGTTTG | P*catA-eGFP*-CL1 fusion construct |
| ScYap6 | GTAACGCCAGGGTTTTCCCAGTCACGACGAATTCATGAGTGTGTCTACC | P*TEF-YAP1-eGFP* fusion construct |
| ScYap8 | CCCTTGCTCACAGCACCAGCACCAGCACCATCGATGTTCATATGCTTATT | P*TEF-YAP1-eGFP* fusion construct |

| | | |
|---|---|---|
| tpx1 | AGATCTAATAAGCTAAGGGTTCTC | 5’ *tpxA* replacement construct |
| tpx2 | GGTACCGAGTATATGTATGTATGATGC | 5’ *tpxA* replacement construct |
| tpx3 | GGATCCAATCAGGGAAGGCGAC | 3’ *tpxA* replacement construct |
| tpx4 | TCTAGACGTCCGTTATTGCTGCAA | 3’ *tpxA* replacement construct |
| tpx13 | CACACCAAGGCGTGAAACCCC | screening *tpxA* for replacement |
| tpx14 | CAGTCCATTCCCACGTCTTGGTC | screening *tpxA* for replacement |
| tpx15 | ACCCTCCTCCTTATTAGTAA | screening *tpxA* for replacement |
| tpx16 | TGCTCCTTCAATATCAGTTC | screening *tpxA* for replacement |
| tpx17 | AGCACTCGTCCGAGGGCAAA | screening *tpxA* for replacement |
| tpx18 | ACGCATCCATCTTTGCCGAAC | screening *tpxA* for replacement |
| yap1 | AGATCTCACGAGATGAAAAACGTTT | 5’ *yapA* replacement construct |
| yap2 | GGTACCGTGTTCTGTTGTTTGTTG | 5’ *yapA* replacement construct |
| yap3 | AAGCTTGACGGCGTTAAAGAACT | 3’ *yapA* replacement construct |
| yap4 | CTCGAGATGTGTACTCTGACGTTGT | 3’ *yapA* replacement construct |
| yap5 | GAATTCATGTCATCAAGTGGCAGCGG | *S. cerevisiae* complementation |
| yap6 | TCTAGACTAAGGCATGGTAGCGCCGT | *S. cerevisiae* complementation |
| yap7 | AAGCTTATGAGTGTGTCTACCGCCAAG | *S. cerevisiae* complementation |
| yap8 | TCTAGATTAGTTCATATGCTTATTCAAAGC | *S. cerevisiae* complementation |
| yap9 | ACGCACGAATGAATACAATAAACT | screening *yapA* for replacement |
| yap10 | GATTTGTGTACGCCAGACAGTCC | screening *yapA* for replacement |
| yap11 | CTGAACTCACCGCGACGTCTGT | screening *yapA* for replacement |
| yap21 | CCGACTTCACTCACAACATCAGC | screening *yapA* for replacement |
| yap22 | TCATCAAGTGGCAGCGGAGG | screening *yapA* for replacement |
| yap23 | ATTGACTGACGAGGGCCAG | screening *yapA* for replacement |
| yap27 | GAATTCATGTCATCAAGTGGC | *yapA-eGFP* fusion construct |
| yap28 | ATCGATAGGCATGGTAGCG | *yapA-eGFP* fusion construct |

## References

1. **Young CA, Bryant MK, Christensen MJ, Tapper BA, Bryan GT, Scott B**. 2005. Molecular cloning and genetic analysis of a symbiosis-expressed gene cluster for lolitrem biosynthesis from a mutualistic endophyte of perennial ryegrass. Mol. Gen. Genomics **274**:13-29.

2. **Namiki F, Matsunaga M, Okuda M, Inoue I, Nishi K, Fujita Y, Tsuge T**. 2001. Mutation of an arginine biosynthesis gene causes reduced pathogenicity in *Fusarium oxysporum* f. sp. *melonis*. Mol. Plant-Microbe Interact. **14**:580-584.

3. **Takemoto D, Tanaka A, Scott B**. 2006. A p67(Phox)-like regulator is recruited to control hyphal branching in a fungal-grass mutualistic symbiosis. Plant Cell **18**:2807-2821.

4. **Mattern IE, Punt PJ**. 1988. A vector of *Aspergillus* transformation conferring phleomycin resistance. Fungal Genetics Newsletter **35**:25.

5. **Toews MW, Warmbold J, Konzack S, Rischitor P, Veith D, Vienken K, Vinuesa C, Wei H, Fischer R**. 2004. Establishment of mRFP1 as a fluorescent marker in *Aspergillus nidulans* and construction of expression vectors for high-throughput protein tagging using recombination *in vitro* (GATEWAY). Curr. Genet. **45**:383-389.

6. **Vanden Wymelenberg AJ, Cullen D, Spear RN, Schoenike B, Andrews JH**. 1997. Expression of green fluorescent protein in *Aureobasidium pullulans* and quantification of the fungus on leaf surfaces. Biotechniques **23**:686-690.