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Supplementary Materials for

Adaptive advantages of restorative RNA editing in fungi for resolving survival-reproduction trade-offs

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

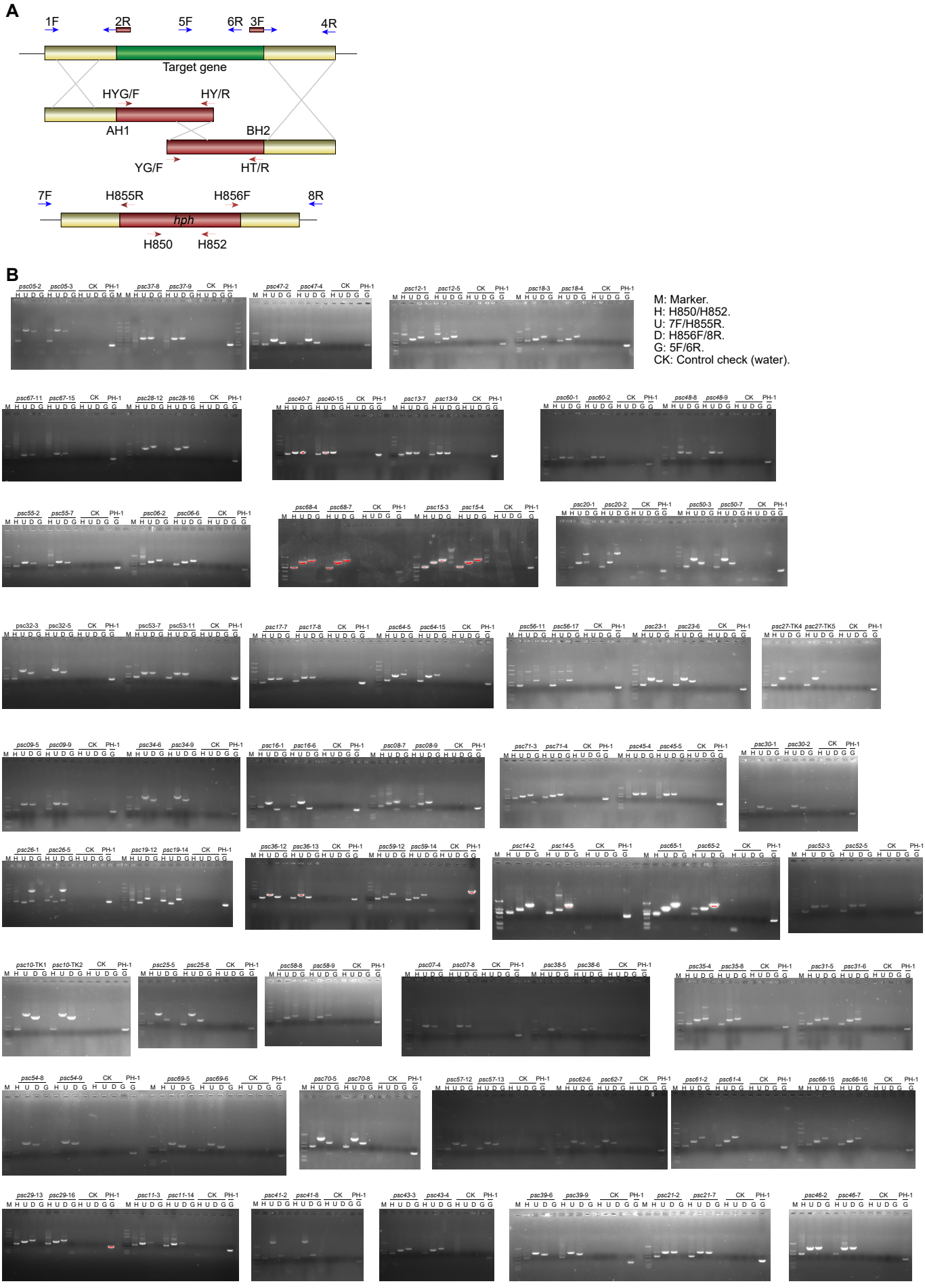

Fig. S1. The gene deletion strategy and PCR analysis of the deletion mutants of PSC pseudogenes. (**A**) Graphical representation of the split-marker approach used to replace the target gene with the hygromycin phosphotransferase (*hph*) cassette in this study. The location of PCR primers (F, forward and R, reverse) is indicated. (**B**) Confirming the deletion mutants of PSC pseudogenes by PCR analysis.

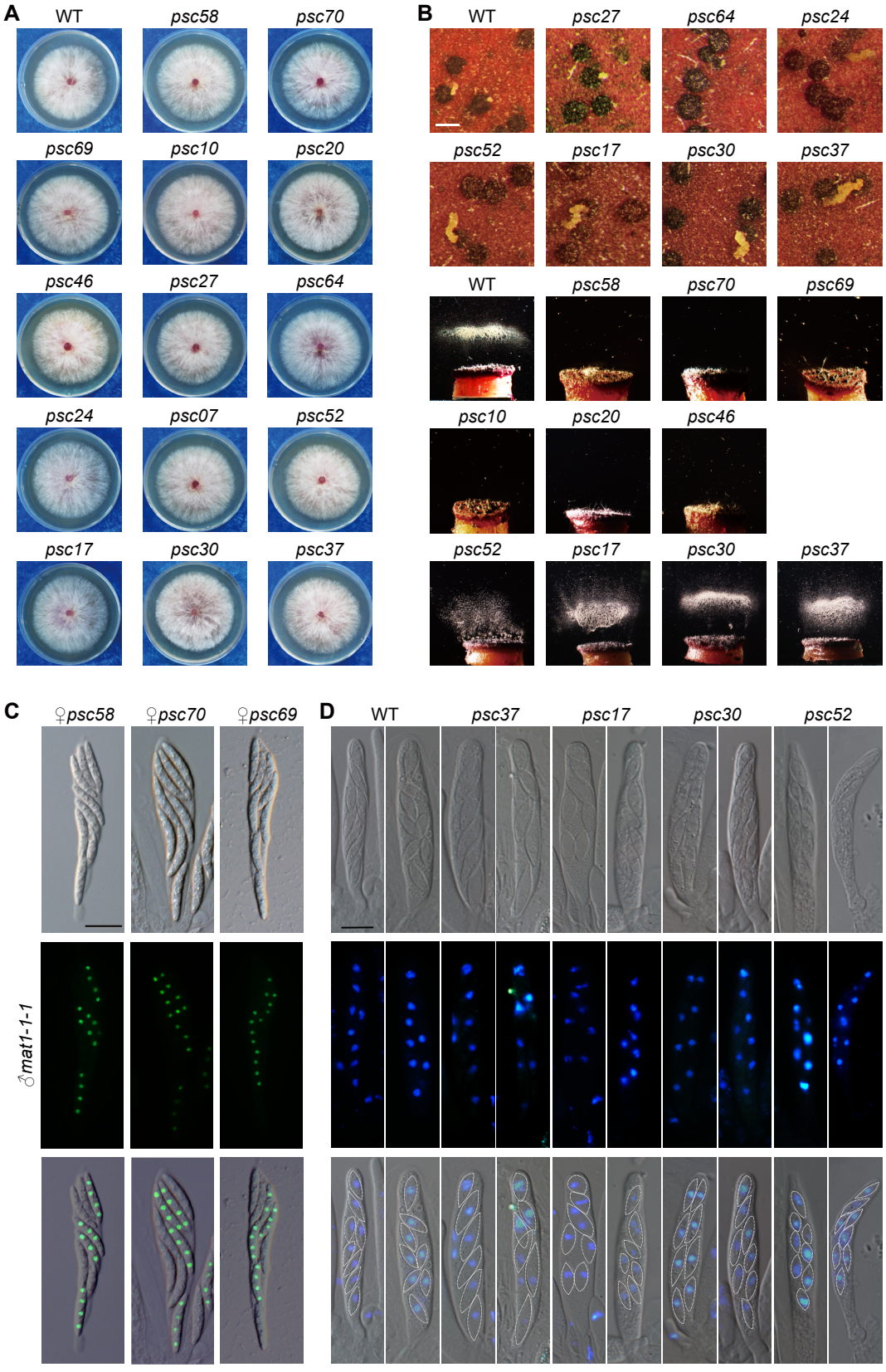

Fig. S2. Phenotypes of deletion mutants of PSC pseudogenes. (**A**) Three-day-old PDA cultures of PH-1 (WT) and the marked deletion mutants were examined for colony morphology. (**B**) The 7-dpf mating cultures of PH-1 (WT) and the marked deletion mutants were examined for perithecium formation (bar = 0.2 mm) and ascospore discharge. (**C**) Morphology of ascospores from crosses of the marked strains (♀) with the *mat1-1-1* H1-GFP (♂). Eight ascospores in each ascus showed 1:1 segregation for GFP signals. Bar = 20 µm. (**D**) Asci of the marked strains stained with DAPI and examined by epifluorescence microscopy at 6 dpf. The delimitation of ascospores is marked with white dashed lines. Bar = 20 µm.

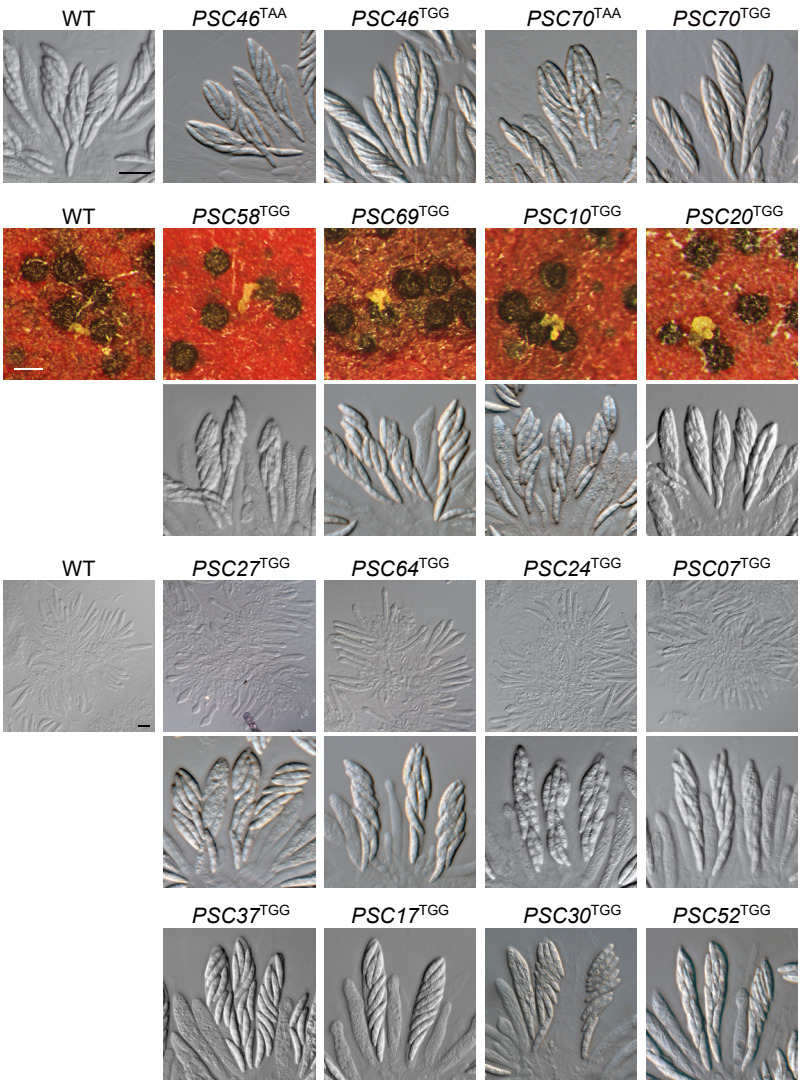

Fig. S3. Normal phenotypes of the edited (TGG) transformants of germ-needed PSC pseudogenes in sexual development. Mating cultures of the marked transformants were examined for perithecium formation (Bar = 0.2 mm) and/or asci and ascospores morphology (Bar = 20 μm) at 7 dpf.

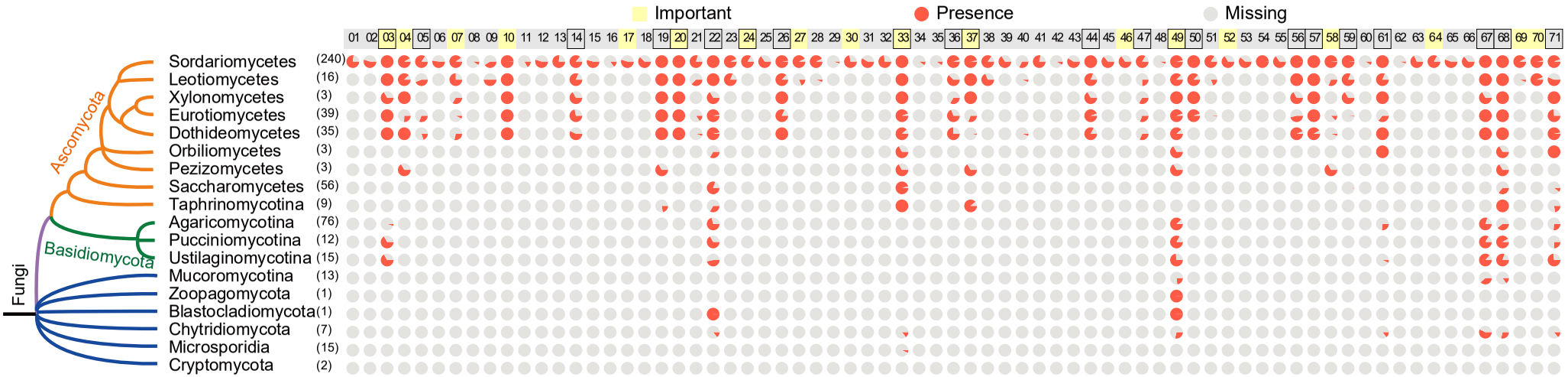

Fig. S4. Distribution of the orthologous genes of the 71 PSC pseudogenes in the fungal tree of life. The number of species in each taxon examined is indicated in the bracket. Each pie chart shows the proportion of fungal species with (presence) or without (missing) an ortholog. The names of the PSC pseudogenes with only detectable homologs in Pezizomycotina are boxed. The names of the 18 germ-needed PSC pseudogenes are highlighted by a yellow background.

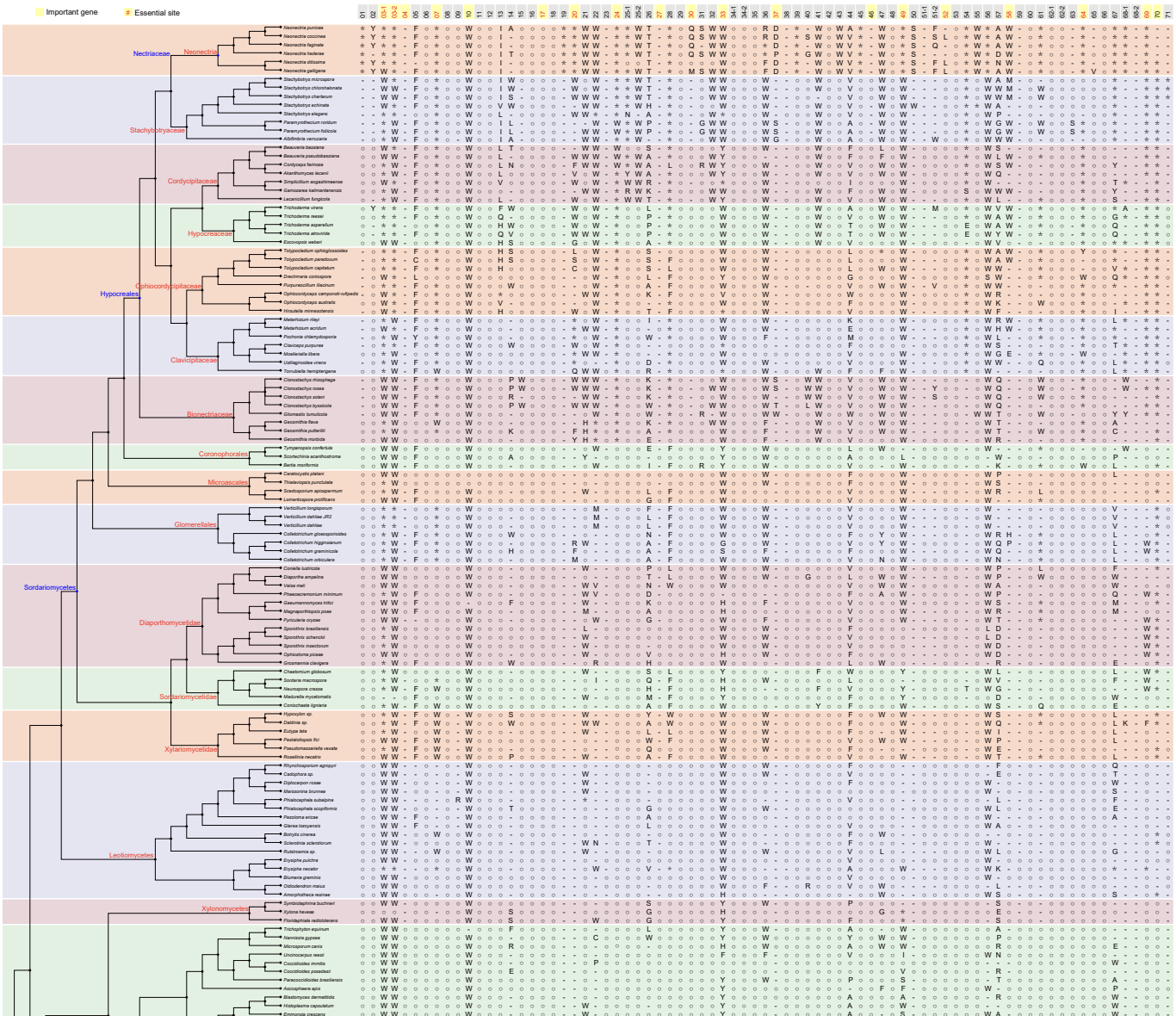

Fig. S5. Amino acid states at the PSC editing sites in the orthologous genes of the 71 PSC pseudogenes in 185 genomes of Ascomycota. The names of the 18 germ-needed PSC pseudogenes and the PSC editing sites essential for their functions are highlighted by a yellow background and red font, respectively. The one-letter symbol of amino acid residues at the PSC editing sites in each genome is shown. *, premature-stop codon TAG; -, missing the PSC editing site/region; ○, missing the orthologous gene. The taxon name is indicated on the phylogenetic tree.

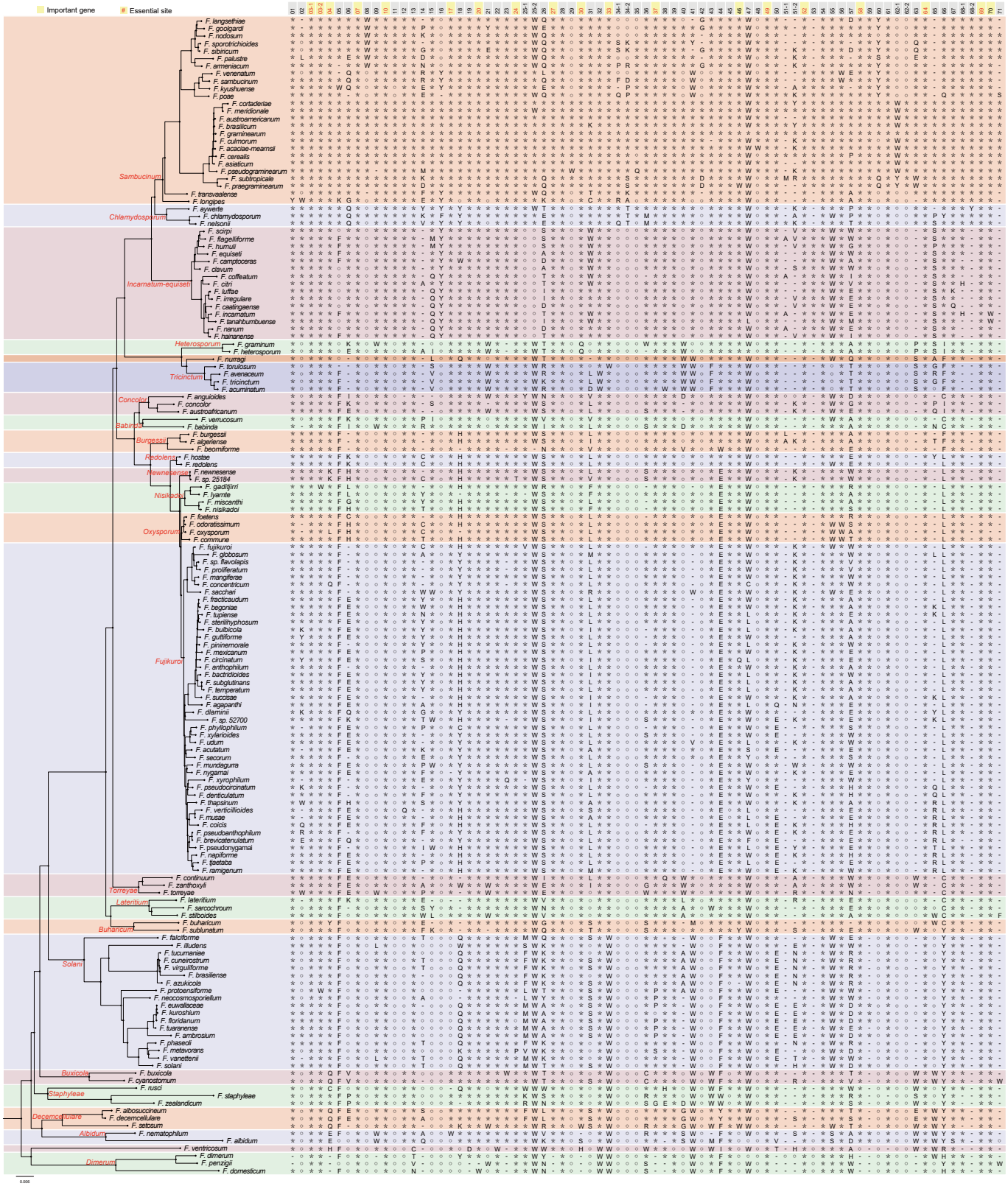

Fig. S6. Amino acid states at the PSC editing sites in the orthologous genes of the 71 PSC pseudogenes in 154 genomes of *Fusarium*. The names of the 18 germ-needed PSC pseudogenes and the PSC editing sites essential for their functions are highlighted by a yellow background and red font, respectively. The one-letter symbol of amino acid residues at the PSC editing sites in each genome is shown. *, premature-stop codon TAG; -, missing the PSC editing site/region; ○, missing the orthologous gene. The name of species complexes is indicated on the phylogenetic tree.

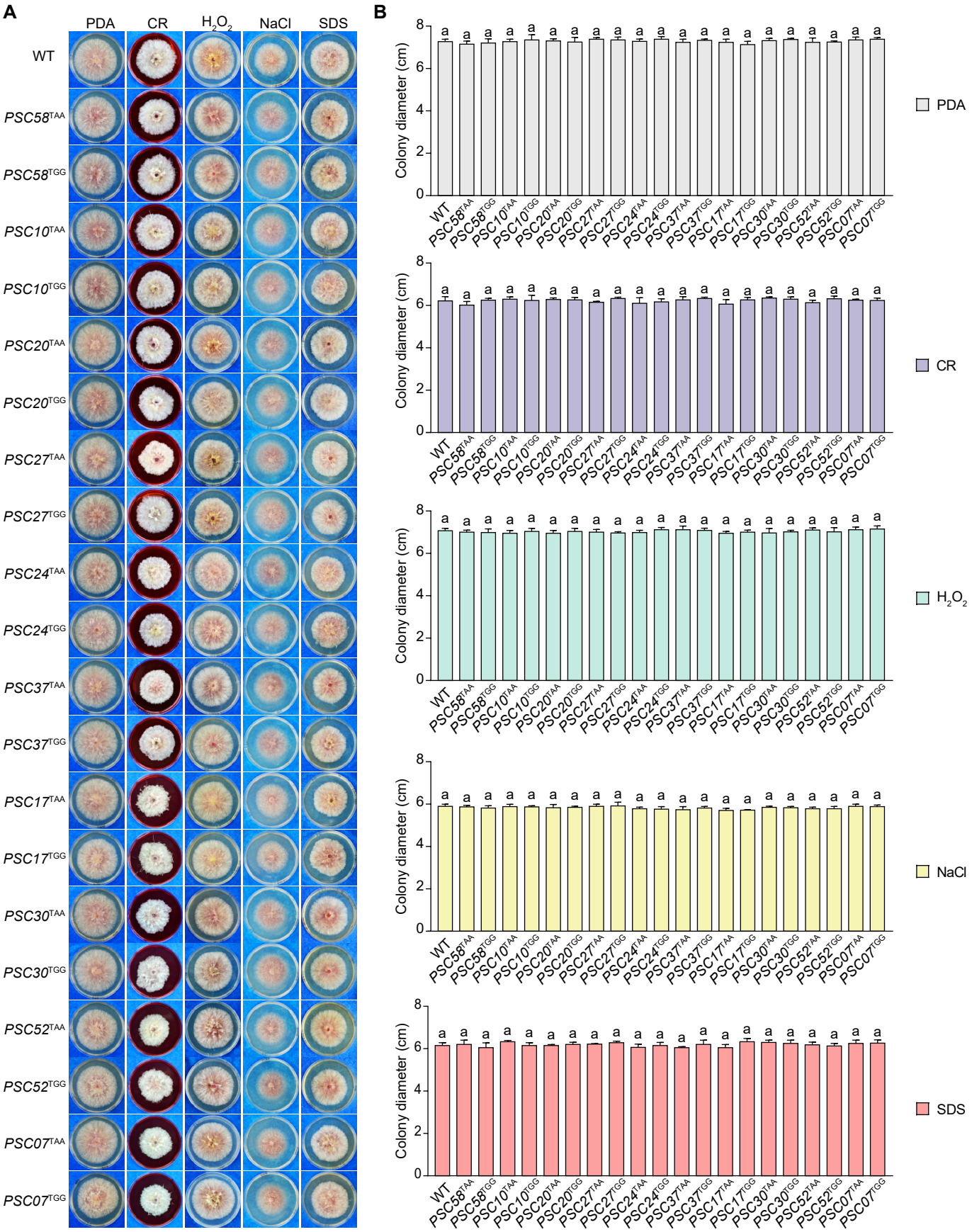

Fig. S7. Colonial morphology and growth rates of the uneditable (TAA) and edited (TGG) transformants under stressful conditions. (**A**) Colonies of PH-1 (WT) and the marked strains formed on PDA with or without 150 μg/ml Congo red, 0.05% H_2O_2, 0.7 M NaCl, and 0.01% SDS after incubation for 3 or 5 days. (**B**) Colony diameters of the marked strains. Mean and SD were calculated with data from three biological replicates (N = 3). The same letters indicate no significant differences based on one-way ANOVA followed by Turkey's multiple range test ($P > 0.05$).

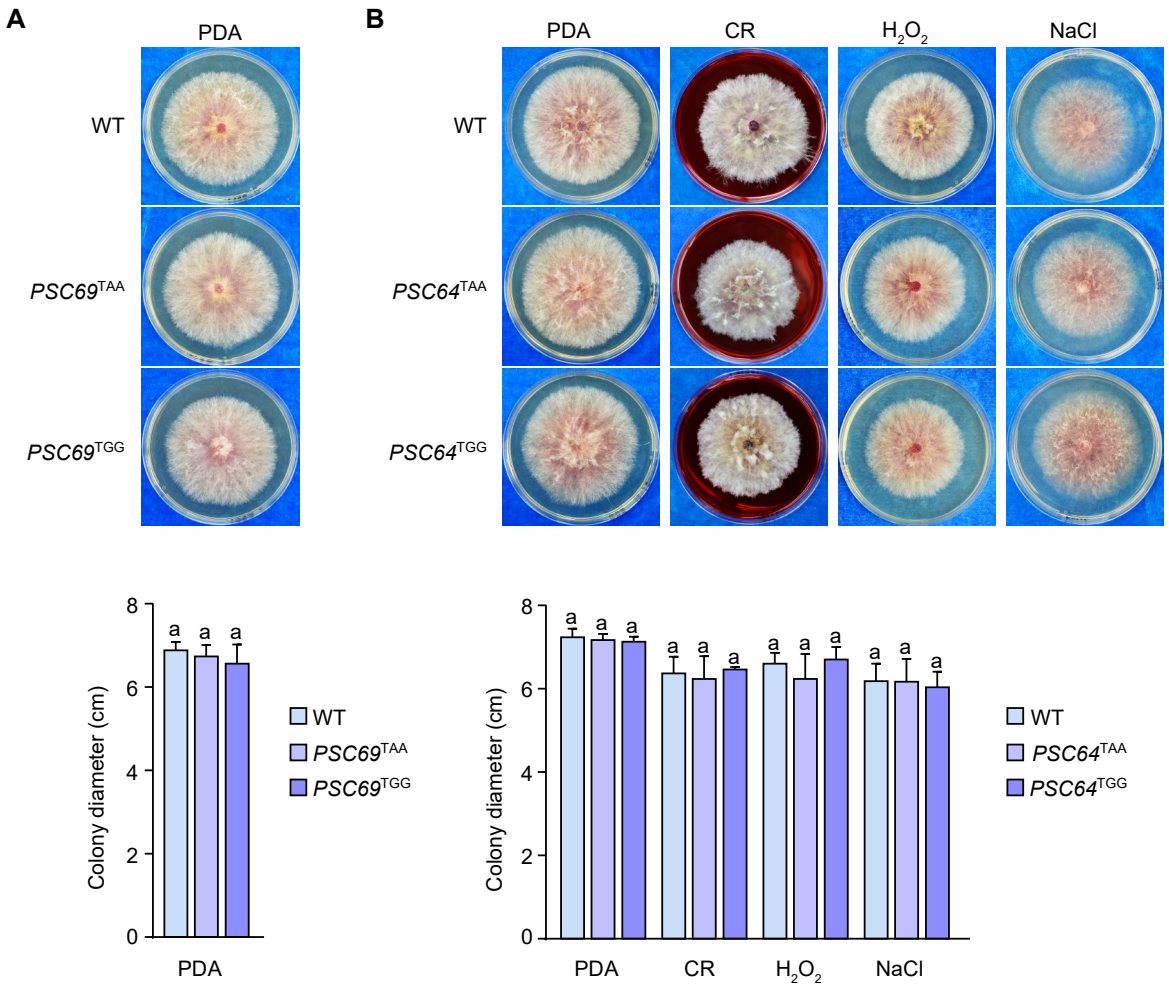

Fig. S8. Colonial morphology and growth rates of the uneditable (TAA) and edited (TGG) transformants of *PSC69* and *PSC64* under stressful conditions. (**A**) Colony morphology and colony diameters of PH-1 (WT) and the uneditable (TAA) and edited (TGG) transformants of *PSC69* formed on PDA plates after incubation for 3 days. (**B**) Colony morphology and colony diameters of PH-1 (WT) and the uneditable (TAA) and edited (TGG) transformants of *PSC64* formed on PDA with or without 150 μg/ml Congo red, 0.05% H_2O_2, and 0.7 M NaCl after incubation for 3 or 5 days. Mean and SD were calculated with data from three biological replicates (N = 3). Same letters indicate no significant differences based on one-way ANOVA followed by Turkey's multiple range test ($P > 0.05$).

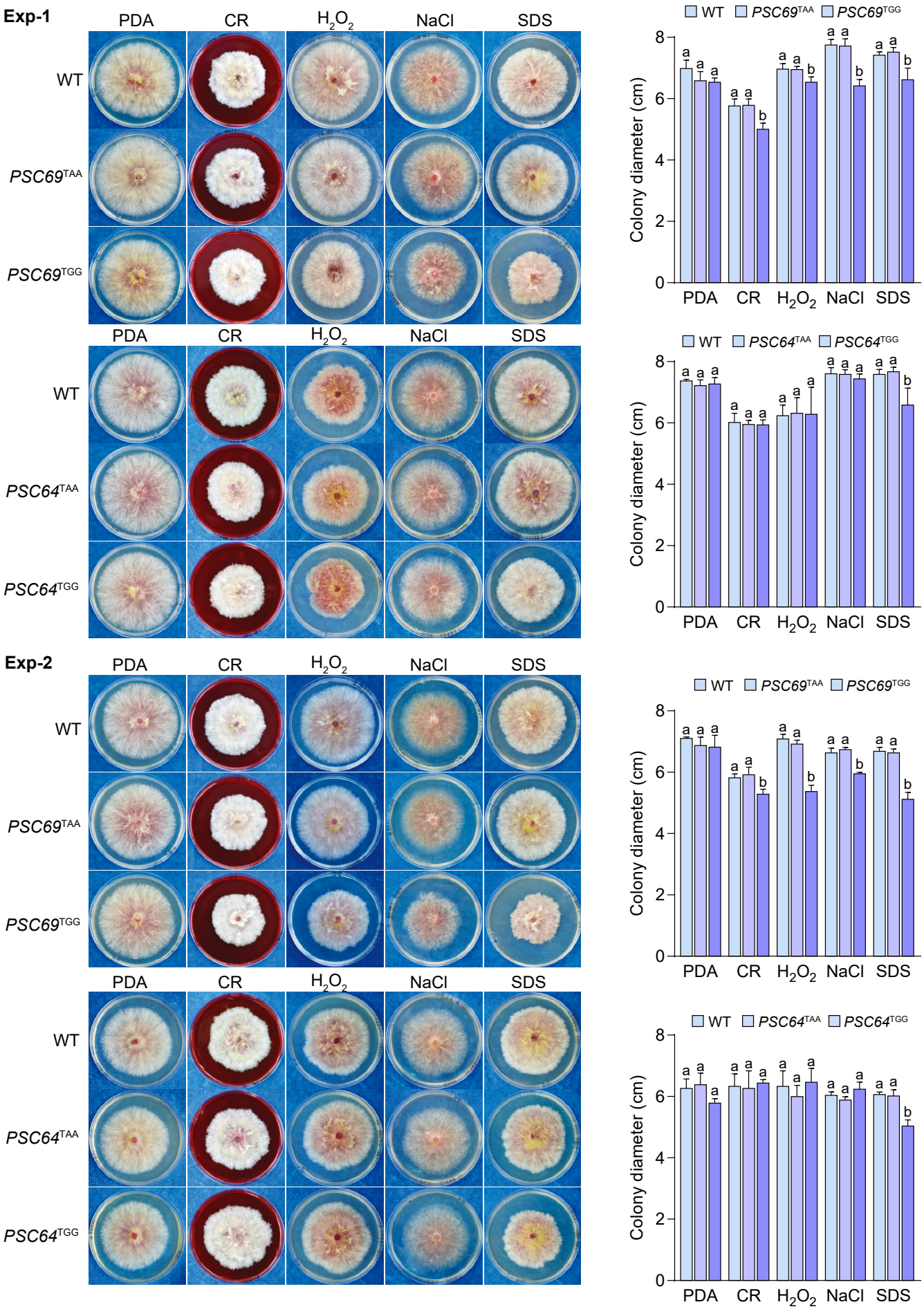

Fig. S9. Colonial morphology and growth rates of the uneditable (TAA) and edited (TGG) transformants of *PSC69* and *PSC64* under stressful conditions assayed in additional two independent experiments. Colonies of PH-1 (WT) and the marked strains formed on PDA with or without 150 μg/ml Congo red, 0.05% H_2O_2, 0.7 M NaCl, and 0.01% SDS after incubation for 3 or 5 days. Mean and SD were calculated with data from three biological replicates (N = 3). Different letters indicate significant differences based on one-way ANOVA followed by Turkey's multiple range test ($P < 0.05$).

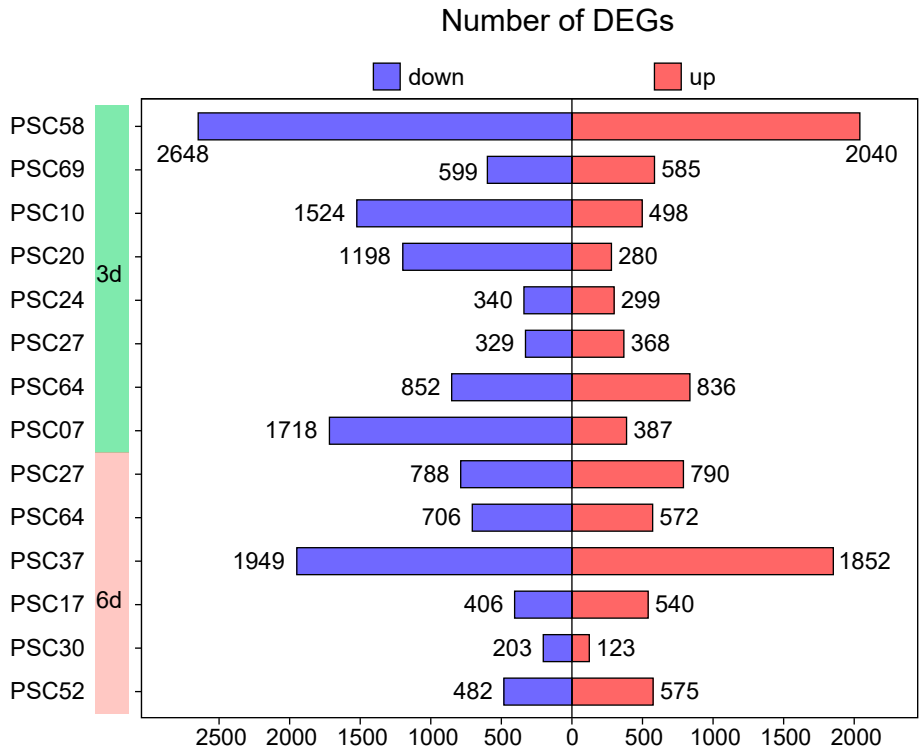

Fig. S10. Number of differentially expressed genes (DEGs) identified in deletion mutants of the marked PSC pseudogenes. 3d, 3-dpf perithecia; 6d, 6-dpf perithecia. down, down-regulated genes; up, up-regulated genes. Genes with FDR < 0.05 and |log2 (fold-change)| ≥ 1 were considered as DEGs. The number of down- and up-regulated genes in each sample is indicated.

Table S1-S6 (separate file)

Table S1. Information of the 71 PSC pseudogenes in *Fusarium graminearum*.

Table S2. Strains used in this study.

Table S3. Phenotypic description of deletion mutants of 71 PSC pseudogenes.

Table S4. The identified synonymous and nonsynonymous editing sites shared by *Fusarium graminearum* and *Fusarium neocosmosporiellum*.

Table S5. Primers used in this study.

Table S6. Information of Illumina DNA- and RNA-Seq data used in this study.

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