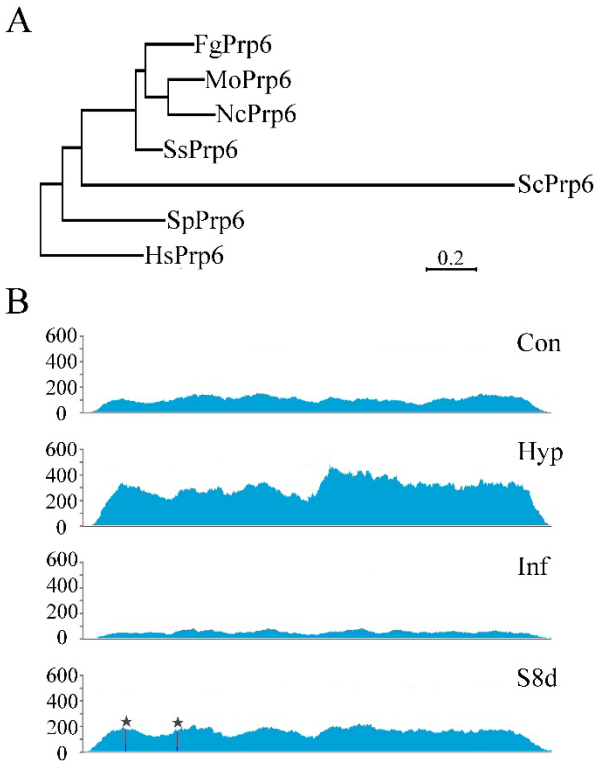
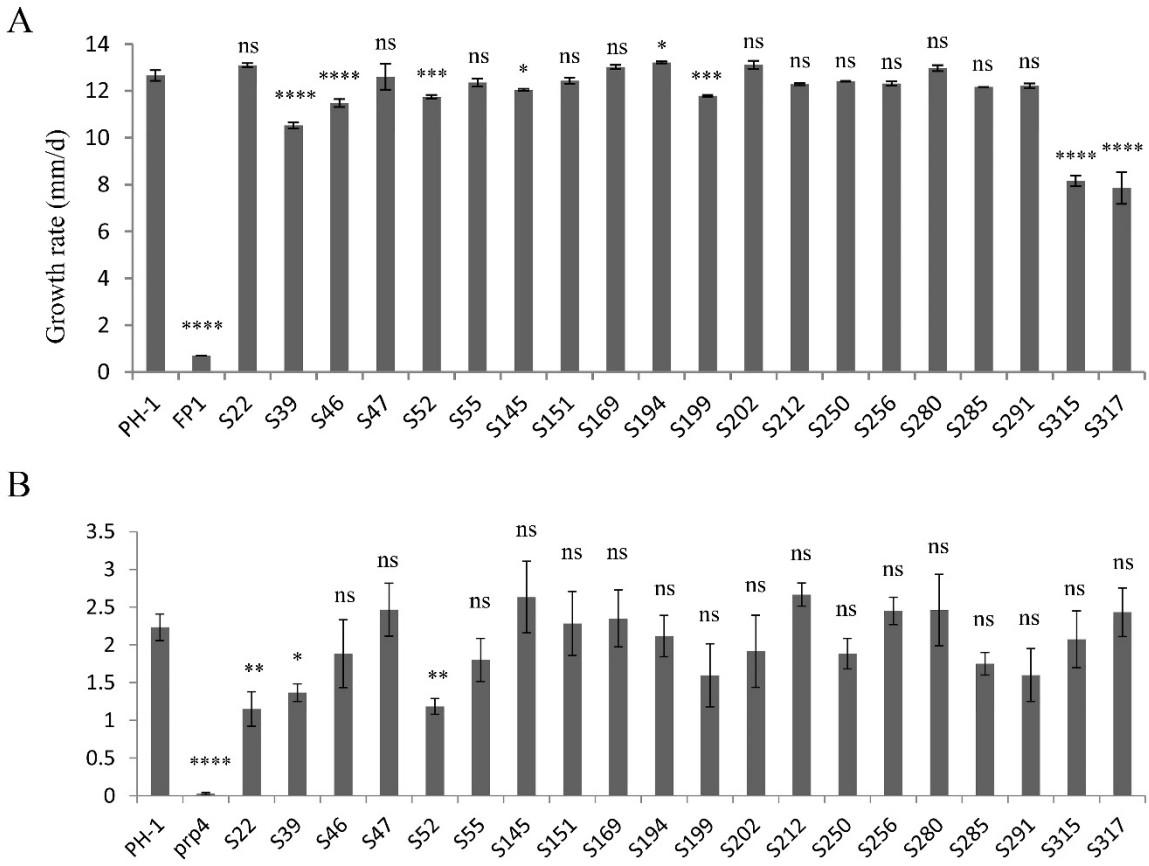
Appendix A PCR primers used in this study

Name	Sequence (5'-3')	Description
c10242/1f	cggagcaaggttagggatgga	Sequencing
c10242/2r	cccagagtttcacggataatgt	Sequencing
c10242/3f	caacgagggctcacccaatg	Sequencing
c10242/4r	tcaaggtcggagcctacggtta	Sequencing
prp6cxw1r	ctcctccagtcgtgccgct	Sequencing
prp6/1f	tgacgcattagggagcagcag	Knock out
prp6/2r	ttgacctccactagctccagccaagccaaaagctctctaggctccacac	Knock out
prp6/3f	gaatagagtagatgccgaccgcgggtttcgttgaccgtgatttcattgc	Knock out
prp6/4r	accaaacaaccctcctacacaa	Knock out
prp6/5f	aggacgatgaggaagccgac	Knock out
prp6/6r	ggtgcctggagcaaccgaat	Knock out
prp6/7f	ctacggtatctgggaggaagg	Knock out
prp6/8r	aataccccccaaccatcatctct	Knock out
prp4/1f	caacagcatggatcgtcggg	Knock out
prp4/2r	ttgacctccactagctccagccaagcccaggtcgtgatgtcgcaaactc	Knock out
prp4/3f	gaatagagtagatgccgaccgcgggtttgtcaccgccgttcgtatgt	Knock out
prp4/4r	cccgagtaatgcccttcacc	Knock out
prp4/7f	gcctttagaagtgtcgcagaa	Knock out
prp4/8r	ctttctgacttggtatccttgact	Knock out
prp4/5f	ccatcgcaacagaaccacagc	Knock out
prp4/6r	ccccagccaccatagtaagaatagt	Knock out
Prp6-phz65 /f	cgactcactatagggcgaattgggtactcaaattgccgtcaaagcacataacccta	BiFc
Prp6-phz65 /r	gctcaccatcgtggcgatggagcgttgttccagctcttcagcaac	BiFc
Prp6np/1f	cgactcactatagggcgaattgggtactcaaattggccgtcaaagcacataacccta	CO-IP
Prp6flag/4r	ctttataatcaccgtcatggtctttgtagtcttgttccagctcttcagcaac	CO-IP
$Prp6^{T199200A}$/2r	ccatgtcctcgtccataacagctgctcccatctgacccgcatc	Point mutation
$Prp6^{T199200A}$/3f	gatgcgggtcagatgggagcagctgttatggacgaggacatgg	Point mutation
$Prp6^{T199200D}$/2r	ccatgtcctcgtccataacatcgtctcccatctgacccgcatc	Point mutation
$Prp6^{T199200D}$/3f	gatgcgggtcagatgggagacgatgttatggacgaggacatgg	Point mutation
$Prp6^{T219211D}$/2r	ctccgatcttggcaaagttgtccatgtcgccatctgctgcatcgc	Point mutation
$Prp6^{T219211D}$/3f	gcgatgcagcagatggcgacatggacaactttgccaagatcggag	Point mutation
$Prp6^{T219211A}$/2r	ccgatcttggcaaagttggccatggcgccatctgctgcatcgc	Point mutation
$Prp6^{T219211A}$/3f	gcgatgcagcagatggcgccatggccaactttgccaagatcgg	Point mutation
$Prp6^{T252D}$/2r	gatagccttgtggatcgatactgtccgaggtgcctggagcaac	Point mutation
$Prp6^{T252D}$/3f	gttgctccaggcacctcggacagtatcgatccacaaggctatc	Point mutation
$Prp6^{T252A}$/2r	tagccttgtggatcgatactggccgaggtgcctggagcaac	Point mutation

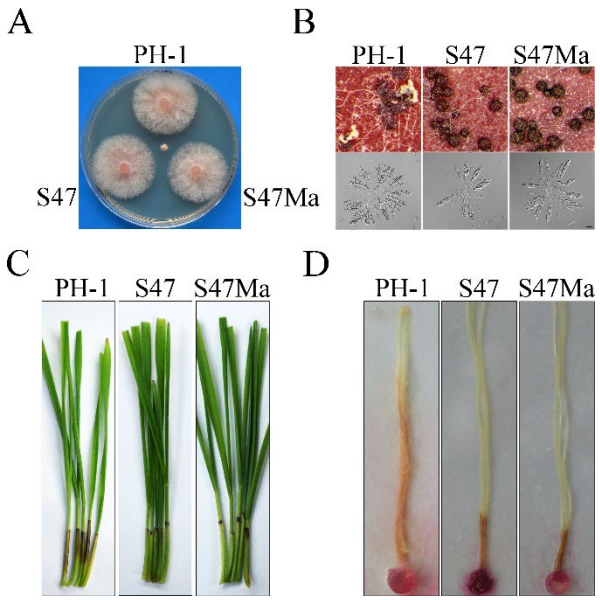
Prp6^{T252A}/3f	gttgctccaggcacctcggccagtatcgatccacaaggcta	Point mutation
Prp6^{T261D}/2r	gctgctttatttttatcgaggctgtccagatagccttgtggatcgatac	Point mutation
Prp6^{T261D}/3f	gtatcgatccacaaggctatctggacagcctcgataaaaataaagcagc	Point mutation
Prp6^{T261A}/2r	gctttatttttatcgaggctcgccagatagccttgtggatcgatac	Point mutation
Prp6^{T261A}/3f	gtatcgatccacaaggctatctggcgagcctcgataaaaataaagc	Point mutation



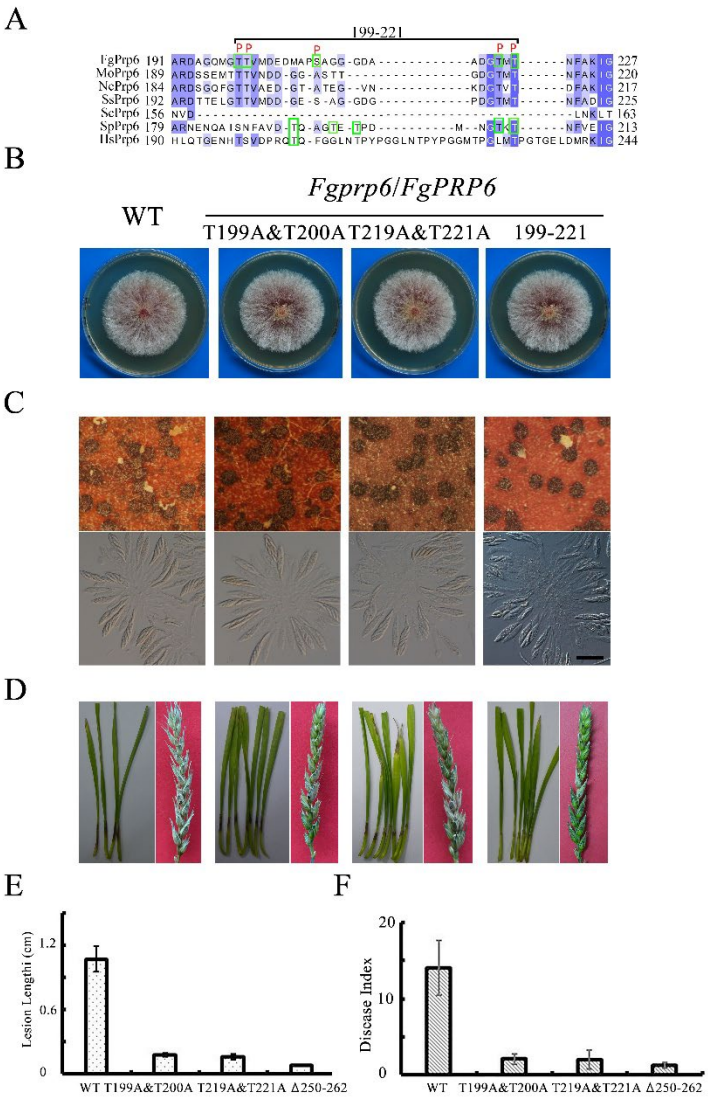
Appendix B Phylogenetic tree and RNAseq read coverages of *FgPRP6*. A. Neighbor-joining tree with 500 bootstrap replicates of phylogenetic relationships between Prp6 homologues in fungi. All of the Prp6 protein sequences were downloaded from the NCBI database and their accession numbers are as following: Fg (*Fusarium graminearum* XP_011319192.1), Nc (*Neurospora crassa* XP_011394664.1), Sp (*Schizosaccharomyces pombe* NP_596086.1), Sc (*Saccharomyces cerevisiae* NP_009611.1), An (*Aspergillus nidulans* XP_680716.1), Hs (*Homo sapiens* NP_036601.2). B. IGV Sashimi plots showing the read coverages of *FgPRP6* transcripts in RNAseq data of conidia (Con), hyphae (Hyp), infected wheat heads at 3 days post-inoculation (Inf), and perithecia at 8 days post-fertilization (S8d) . Editing sites were marked with asterisks.



Appendix C Assays of growth rate and conidiation of the spontaneous suppressors. A. Growth rate of PH-1, *Fgprp4* mutant (FP1), and 20 spontaneous suppressors (S22, S39, ... and S317) was measured with 3-day-old PDA cultures. B. Conidiation of the same set of strains was measured with 5-day-old CMC cultures. Mean and standard errors were estimated with data from three independent measurements. The statistical significance for E and F was analysed by one way ANOVA followed by Dunnett's multiple comparisons test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

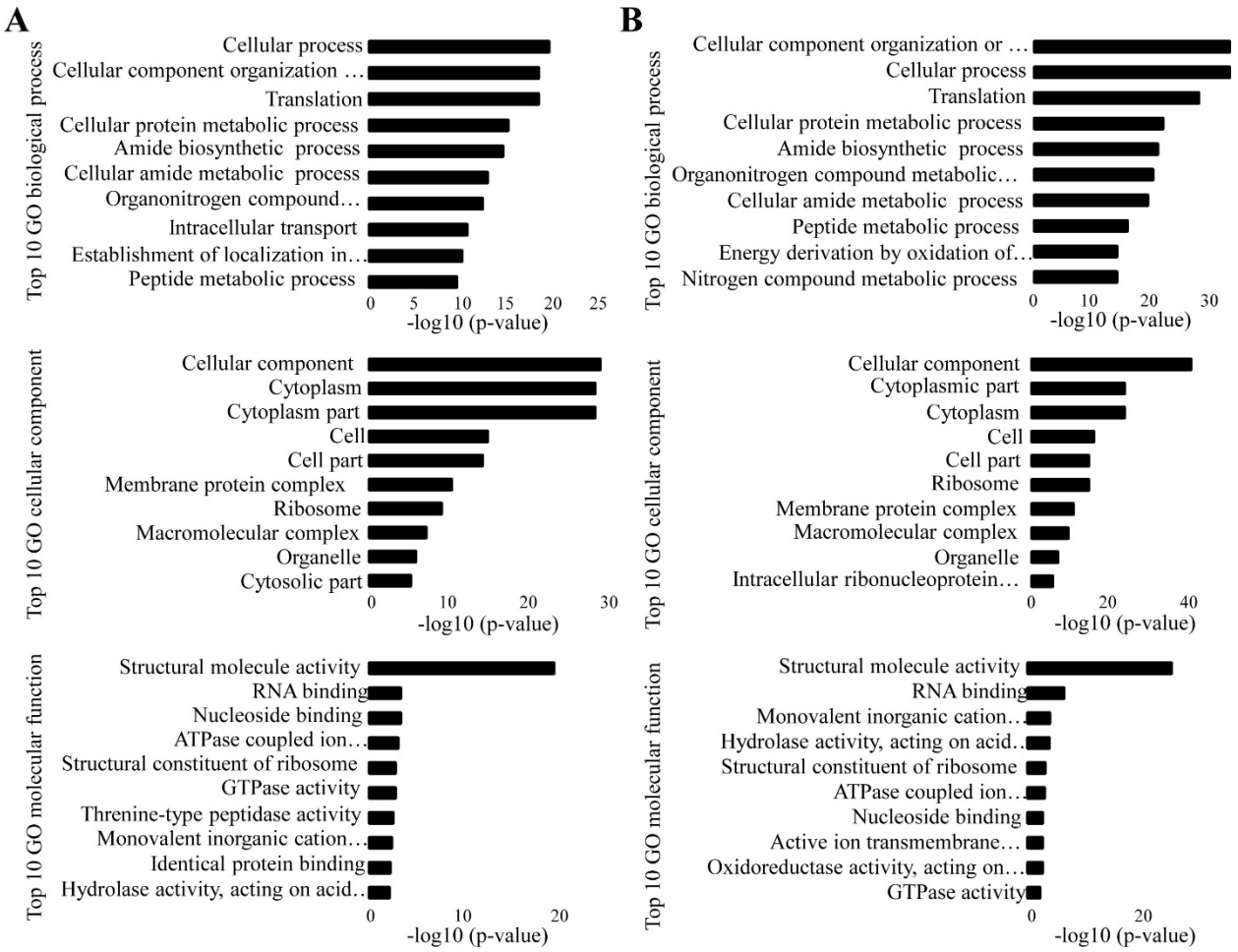


Appendix D Validation of suppressor mutation R230H. A. Two-day-old PDA cultures of the wide type (PH-1), *Fgprp4* mutant (inoculated in the center of the plates), S47 suppressor strain, and *Fgprp4/FgPRP6*^{R230H}-GFP transformant (S47Ma). B. Perithecia (upper row) and asci (lower row) produced by S47 and S47Ma. Bar = 20 μm. C. Wheat coleoptiles inoculated with PH-1, S47 and S47Ma were photographed at 7 dpi. D. Corn silks inoculated with culture blocks of PH-1, S47 and S47Ma were examined at 5 dpi.

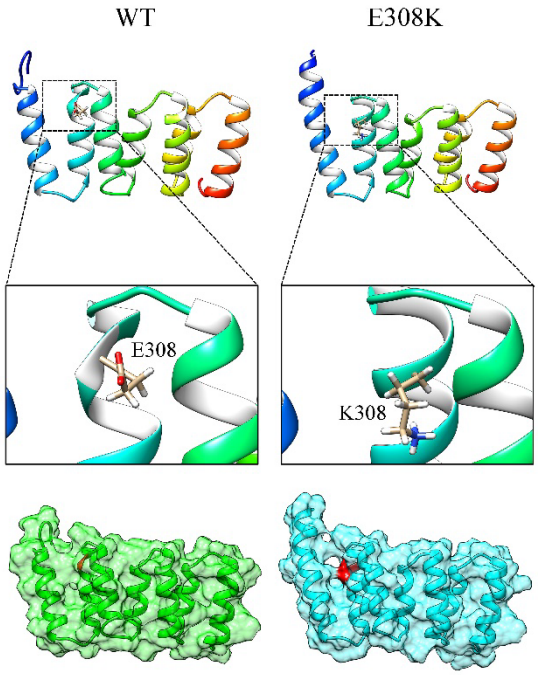


Appendix E Characterization of the conserved Prp4-phosphorylation sites of FgPrp6 with *S. pombe*. A. Alignment of the conserved Prp4-phosphorylation sites contained region in yeast Prp6 with sequences of *F. graminearum* and other model organisms. The conserved phosphorylation sites were boxed and labeled with letter P. B. Three-day-old PDA cultures of the wild type (PH-1), *Fgprp6*/*FgPRP6*$^{T199A\ \&\ T200A}$-3×FLAG, *Fgprp6*/*FgPRP6*$^{T219A\ \&\ T221A}$-3×FLAG and *Fgprp6/FgPRP6*$^{\Delta 199\text{-}221}$-GFP transformants. C. Perithecia (upper row) and asci (lower row) produced by the same set of strains as in B. Bar = 20 μm. D. Plant infection of the same set of strains on wheat coleoptiles (Left panel) and flowering wheat heads (Right panel). E. Average lesion length of wheat coleoptiles from three independent experiments. F. Mean disease index calculated from three independent experiments with more than five wheat heads/experiment. For E and F Bar=standard deviation, and the statistical significance was

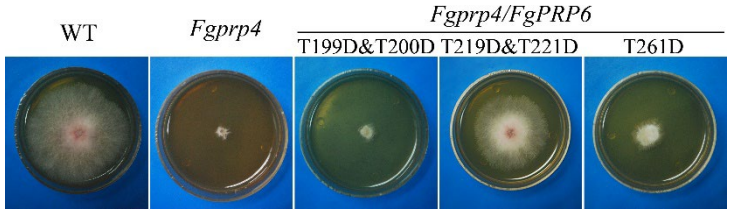
analysed by one way ANOVA followed by Dunnett's multiple comparisons test (****p < 0.0001).



Appendix F GO analysis of genes with intron retention rate ≥2-fold in *Fgprp6/FgPRP6*$^{\Delta 199\text{-}221}$-GFP (A) and *Fgprp6/FgPRP6*$^{\Delta 250\text{-}262}$-GFP (B).



Appendix G Three-dimensional modeling of FgPrp6 (271-393 aa). Tertiary structures of FgPrp6^{WT} (271-393 aa) and FgPrp6^{E308K} (271-393 aa) were predicted by I-TASSER server. Red color indicates the amino acid with suppressor mutation.



Appendix H Three-day PDA cultures of the wild type (PH-1), *Fgprp4* deletion mutant (*Fgprp4*), *Fgprp4*/*FgPRP6*$^{T199D\ \&\ T200D}$-3×FLAG, *Fgprp4*/*FgPRP6*$^{T219D\ \&\ T221D}$-3×FLAG and *Fgprp4*/*FgPRP6*^{T261D}-3xFLAG transformants.