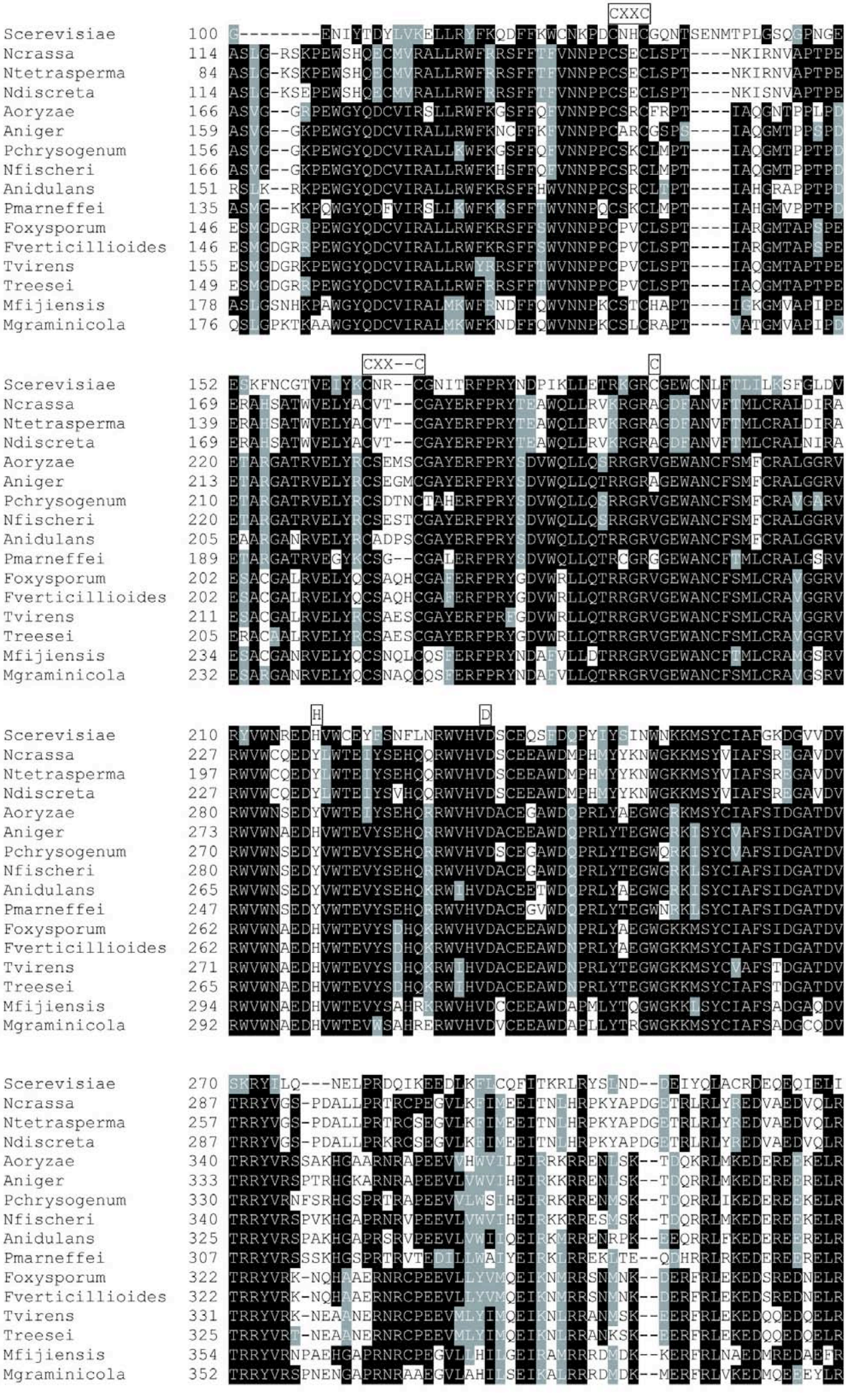
CXXC
CXX--C
C
H
D
Scerevisiae
Ncrassa
Ntetrasperma
Ndiscreta
Aoryzae
Aniger
Pchrysogenum
Nfischeri
Anidulans
Pmarneffei
Foxysporum
Fverticillioides
Tvirens
Treesei
Mfijiensis
Mgraminicola

Supplementary Fig. 1. Alignment of predicted fungal PNGase homologs and illustration of residues critical for activity. Amino acids corresponding to the CXXC motifs and the catalytic triad are indicated. *Aoryzae = Aspergillus oryzae; Aniger = Aspergillus niger; Pchrysogenum = Penicillium chrysogenum; Nfischeri = Neosartorya fischeri; Anidulans = Aspergillus nidulans; Pmarneffei = Penicillium marneffei; Mfijiensi = Mycosphaerella fijiensis; Mgraminicola = Mycosphaerella graminicola; Foxysporum = Fusarium oxysporum; Fverticillioides = Fusarium verticillioides; Tvirens = Trichoderma virens; Treesei = Trichoderma reesei; Ntetrasperma = Neurospora tetrasperma; Ndiscreta = Neurospora discreta.*

Supplementary Table 1. List of primers used for the generation of Png1 mutants; amino acids mutated are indicated in the left column.

ScPng1	Forward primer	Reverse primer
N178T	GATTTCCTCGTTATaccGATCCAATTAAG	CTTAATTGGATCggtATAACGAGGAAATC
D179E, P180A	CCTCGTTATAACgaggcaATTAAGTTGCTGG	CCAGCAACTTAATtgcctcGTTATAACGAGG
I181W	TTATAACGATCCAtggAAGTTGCTGGAAAC	GTTTCCAGCAACTTccaTGGATCGTTATAAC
K182Q	AACGATCCAATTcagTTGCTGGAAACTAG	GTTTCCAGCAActgAATTGGATCGTTATAAC
I181W, K182Q	TTATAACGATCCAtggcagTTGCTGGAAACTAG	CTAGTTTCCAGCAActgccaTGGATCGTTATAAC
E185R	CCAATTAAGTTGCTGcgGACTAGAAAGGGAAGATGCG	GCATCTTCCCTTTCTAGTCcgCAGCAACTTAATTGGATCG
E185R, T186V	CAATTAAGTTGCTGcgagttAGAAAGGGAAGATGCG	CATCTTCCCTTTCTaactcgCAGCAACTTAATTGG
R187K, K188R	GTTGCTGGAAACTaaaaggGGAAGATGCGGTG	CACCGCATCTTCCcctttAGTTTCCAGCAAC
E193D	GGAAGATGCGGTgacTGGTGCAATTTATTTAC	AAATAAATTGCACCAgtcACCGCATCTTCCC
E193D, W194F	GGAAGATGCGGTgacttcTGCAATTTATTTAC	AAATAAATTGCAgaagtcACCGCATCTTCCC
W194F, C195A	AAGGGAAGATGCGGTGAAtttgccAATTTATTTACTTTGATTTTG	AAGTAAATAAATTggcaaaTTCACCGCATCTTCCCTTTC
L198V	GAATGGTGCAATgtaTTTACTTTGATTTTG	AATCAAAGTAAAtacATTGCACCATTCAC
L200M	GAATGGTGCAATTTATTTatgTTGATTTTGAAGTCGTTT	CGACTTCAAAATCAAcatAAATAAATTGCACCATTCACCG
L200M, I201L	GCAATTTATTTACTatgcttTTGAAGTCGTTTGGG	CAAACGACTTCAAaagcatAGTAAATAAATTGCACC
G206D, L207I	TTTTGAAGTCGTTTgacataGATGTTCGCTACG	GTAGCGAACATCtatgtcAAACGACTTCAAAATC
D208R, V209A	TCGTTTGGGTTAcgtgctCGCTACGTCTGG	CCAGACGTAGCGagcacgTAACCCAAACGACTTC
Y211W	GGGTTAGATGTTCGCtggGTCTGGAATAGAGAAG	CTCTATTCCAGACccaGCGAACATCTAACCCAAAC
N214C, R215Q	CGCTACGTCTGGtgtcaaGAAGATCATGTTTGG	AAACATGATCTTCttgacaCCAGACGTAGCGAAC
V219L	TAGAGAAGATCATcttTGGTGTGAATATTTTTC	AATATTCACACCAaagATGATCTTCTCTATTC
C221T	ATAGAGAAGATCATGTTTGGaccGAATATTTTTCAAATTTTT	ATTTGAAAAATATTCggtCCAAACATGATCTTCTCTATTCC
Y223I	GTTTGGTGTGAAattTTTTCAAATTTTTTG	CAAAAAATTTGAAAAaatTTCACACCAAACATG
F224Y	TTGGTGTGAAtacTTTTCAAATTTTTTGAATAGGTG	AAATTTGAAAAgtaTTCACACCAAACATGATCTTC
N226E, F227H	GAATATTTTTCAgagcatTTGAATAGGTGGG	CCACCTATTCAAatgctcTGAAAAATATTCAC
NcPNG1	Forward primer	Reverse primer
A208C	ACGAGTAAAGCGTGGAAGGtgTGGCGACTTTGCC	TGGCAAAGTCGCCAcaCCTTCCACGCTTTACTCG
Y235H	TGGGTGTGGTGCCAGGAAGACcACCTGTGGACCG	GTAAATTTCGGTCCACAGGTgGTCTTCCTGGCACCACAC