

# Supplementary Materials: Chitin Deacetylase Homologous Gene *cda* Contributes to Development and Aflatoxin Synthesis in *Aspergillus flavus*

Table S1. Information of *cda* homologous gene in *A. flavus*

Gene name	Gene ID	Accession ID	Amino acid number
<i>cda1</i>	G4B84_000989	XP_041140747.1	609
<i>cda2</i>	G4B84_007455	XP_041147027.1	306
<i>cda3</i>	G4B84_009400	XP_041148937.1	431
<i>cda4</i>	G4B84_005217	XP_041144885.1	247
<i>cda5</i>	G4B84_003875	XP_041143589.1	241
<i>cda6</i>	G4B84_004417	XP_041144085.1	463

Table S2. The strains used in this study

strains	genotype	source
CA14 PTS	$\Delta ku70, \Delta pyrG$	[52]
Wild-type (WT)	$\Delta ku70, \Delta pyrG::pyrG$	[Prepared in our lab]
$\Delta cda1$	$\Delta ku70, \Delta cda1::pyrG$	this study
$\Delta cda2$	$\Delta ku70, \Delta cda2::pyrG$	this study
$\Delta cda3$	$\Delta ku70, \Delta cda3::pyrG$	this study
$\Delta cda4$	$\Delta ku70, \Delta cda4::pyrG$	this study
$\Delta cda5$	$\Delta ku70, \Delta cda5::pyrG$	this study
$\Delta cda6$	$\Delta ku70, \Delta cda6::pyrG$	this study
$cda6^C$	$\Delta ku70, \Delta cda6::pyrG, cda6::pyrG$	this study
$cda6$ -mCherry	$\Delta ku70, \Delta cda6::pyrG::cda6::mCherry$	this study
$cda6^{\Delta SP}$	$\Delta ku70, \Delta cda6::pyrG, cda6^{\Delta SP}$	this study
$cda6^{\Delta NodB}$	$\Delta ku70, \Delta cda6::pyrG, cda6^{\Delta NodB}$	this study
$cda6^{\Delta GPI}$	$\Delta ku70, \Delta cda6::pyrG, cda6^{\Delta GPI}$	this study
$cda6^{\Delta CBD1}$	$\Delta ku70, \Delta cda6::pyrG, cda6^{\Delta CBD1}$	this study
$cda6^{\Delta CBD2}$	$\Delta ku70, \Delta cda6::pyrG, cda6^{\Delta CBD2}$	this study

Table S3. Primers used for qPCR in this study

primer	sequence (5'-3')	remarks
<i>actin</i> -F	ACGGTGTCTGTCACAAACTGG	For $\beta$ - <i>actin</i>
<i>actin</i> -R	CGGTTGGACTTAGGGTTGATAG	
<i>cda6</i> -QF	TGCCAAAGGACCCATAGATACAC	For checking whether <i>cda6</i> is knocked out
<i>cda6</i> -QR	GGGCGACCTCATTCCACAGA	
<i>brlA</i> -F	GCCTCCAGCGTCAACCTTC	For spore formation related genes
<i>brlA</i> -R	TCTCTCAAATGCTCTTGCCCTC	
<i>abaA</i> -F	TCTTCGGTTGATGGATGATTTC	
<i>abaA</i> -R	CCGTTGGGAGGCTGGGT	For sclerotium formation related gene
<i>nsdC</i> -F	GCCAGACTTGCCAATCAC	
<i>nsdC</i> -R	CATCCACCTTGCCCTTTA	
<i>nsdD</i> -F	GGACTTGCGGGTCGTGCTA	
<i>nsdD</i> -R	AGAACGCTGGGTCTGGTGC	For toxin synthesis related genes
<i>aflO</i> -F	GTCGCATATGCCCCGGTCGG	
<i>aflO</i> -R	GGCAACCAGTCGGGTTCGG	
<i>aflS</i> -F	AAGCTAAGGCCGAGTCTGG	
<i>aflS</i> -R	CAGGTTGTGTTGCTGTTGATAG	
<i>aflR</i> -F	AAAGCACCTGTCTTCCCTAAC	
<i>aflR</i> -R	GAAGAGGTGGGTCAGTGTTGTAG	

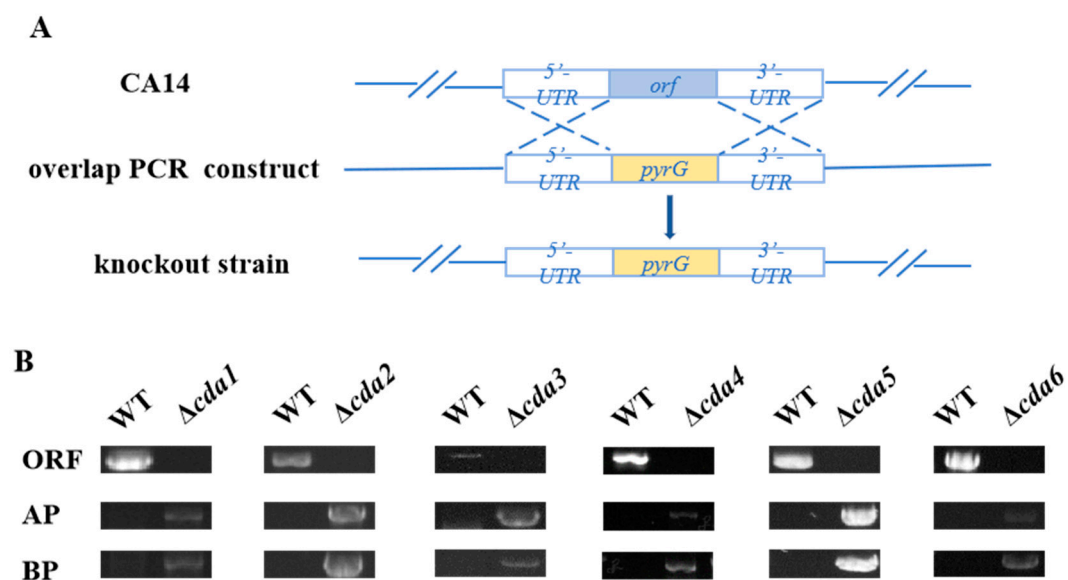


Figure S1. Construction of CDA mutants using homologous recombination. (A) Schematic showing the construction of CDA knockout mutants generated using homologous recombination. (B) Knockout mutations of *cda1-cda6* were validated by PCR in *A. flavus*.

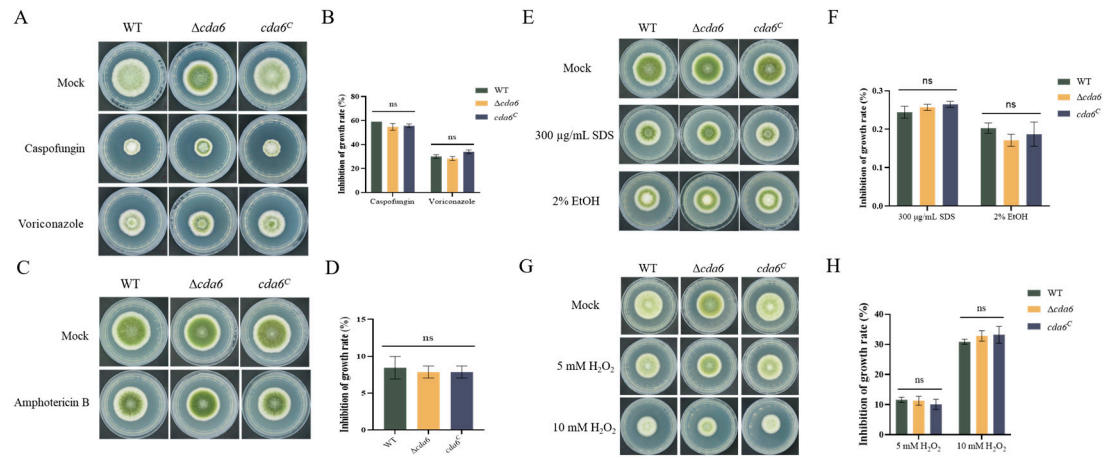


Figure S2 Role of *cda6* gene in response of *A. flavus* to stress. (A) Phenotypes of WT,  $\Delta cda6$ , and  $cda6^C$  strains in media without and with Caspofungin and Voriconazole, respectively. (B) Statistics of the inhibition rate of the above strains in media containing Caspofungin and Voriconazole. (C) Phenotypes of the above strains in media without and with Amphotericin B, respectively. (D) Statistics of inhibition rate of the above strains in media with Amphotericin B. (E) Phenotypes of WT,  $\Delta cda6$ , and  $cda6^C$  strains in YGT medium with 300  $\mu\text{g/mL}$  SDS and 2% EtOH, respectively. (F) Statistics of the inhibition rate of the above strains in media with SDS and EtOH. (G) Phenotypes of WT,  $\Delta cda6$ , and  $cda6^C$  strains in YGT medium with 5 mM and 10 mM  $\text{H}_2\text{O}_2$ , respectively. (H) Statistics of the inhibition rate of the above strains in media with 5 mM and 10 mM  $\text{H}_2\text{O}_2$ . ns indicates no significance ( $n = 3$ ).