

Article

Glutamine Synthetase Contributes to the Regulation of Growth, Conidiation, Sclerotia Development, and Resistance to Oxidative Stress in the Fungus *Aspergillus flavus*

Sen Wang, Ranxun Lin, Elisabeth Tumukunde, Wanlin Zeng, Qian Bao, Shihua Wang and Yu Wang

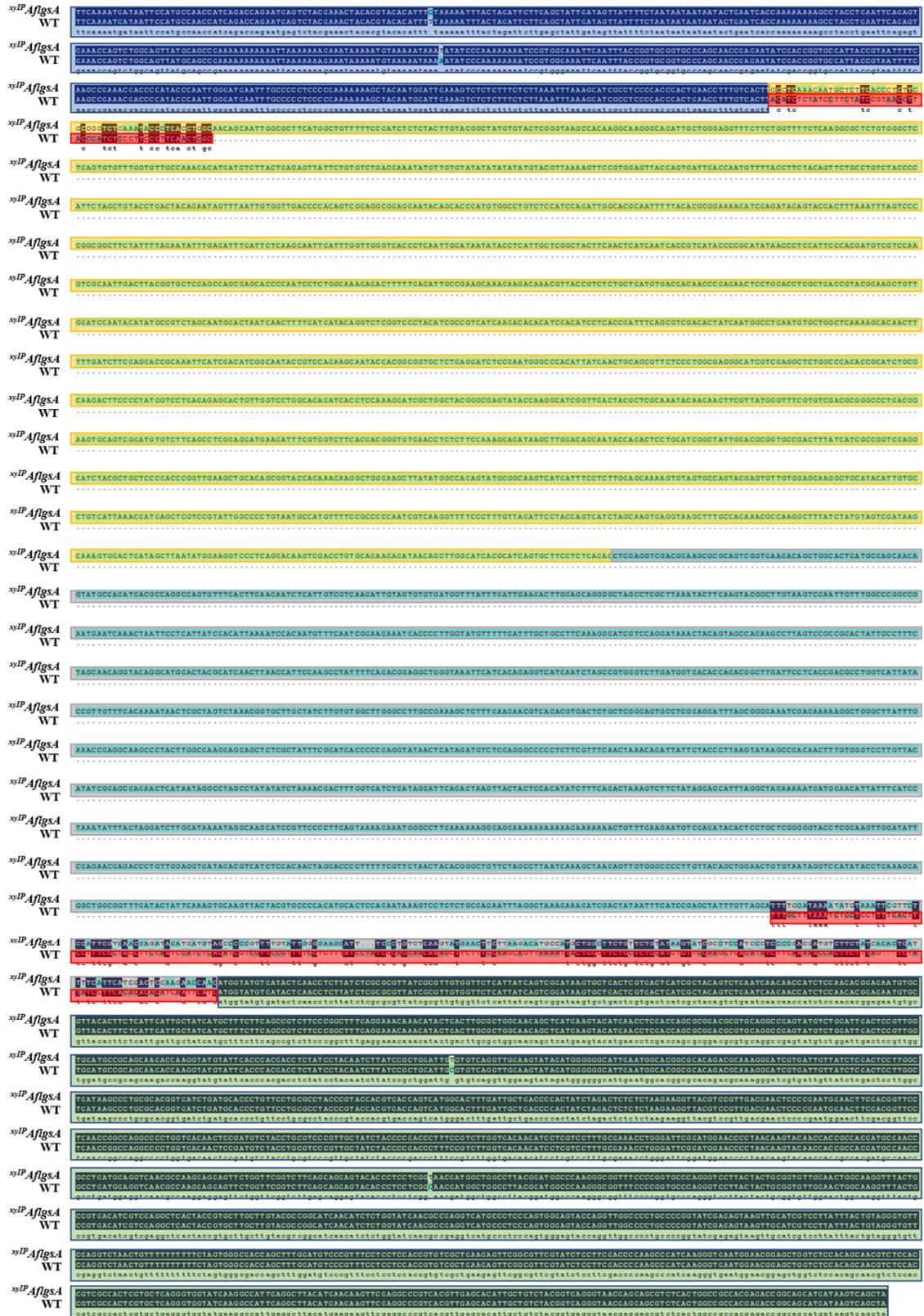


Figure S1. Genome sequencing validation of the *xyIP AflgsA* strain of *A. flavus*. *AflgsA* AP is the upstream non-coding region of the *AflgsA* gene. The mutations in the *AflgsA* CDS are synonymous mutations.

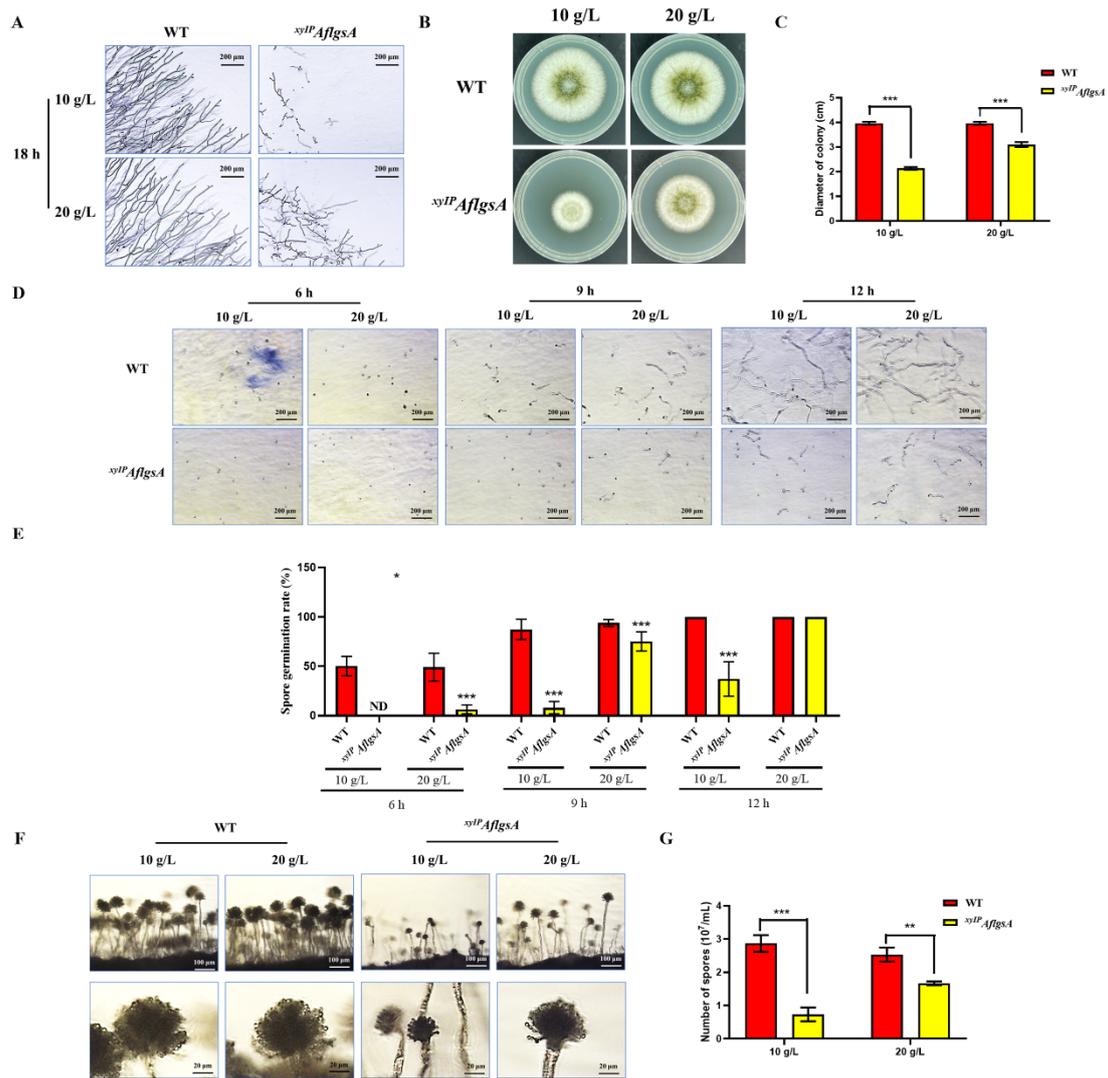


Figure S2. Analysis of the growth, mycelial morphology, conidial germination and conidiophore formation of WT and *xyIP AflgsA* strains. (A) The mycelial morphology of WT and *xyIP AflgsA* strains on the medium containing 10 g/L and 20 g/L xylose. (B) The colony morphology of WT and *xyIP AflgsA* strains. (C) Statistical analysis of the diameter from panel B. (D) The conidial germination of WT and *xyIP AflgsA* strains. (E) Statistical analysis of the conidial germination rate from panel D. (F) Microscopic view of the conidiophore formation of the above two *A. flavus* strains. (G) The number of conidia produced by the above two *A. flavus* strains. ND indicates no detection. ** indicates a significance level of $P < 0.01$, and *** indicates a significance level of $P < 0.001$ based on t tests with three replicates. The medium containing 10 g/L xylose: 5 g/L yeast extract, 10 g/L glucose, 10 g/L xylose, 1 mL/L trace elements, and 1.5% agar. The medium containing 20 g/L xylose (YXT medium): 5 g/L yeast extract, 20 g/L xylose, 1 mL/L trace elements, and 1.5% agar.

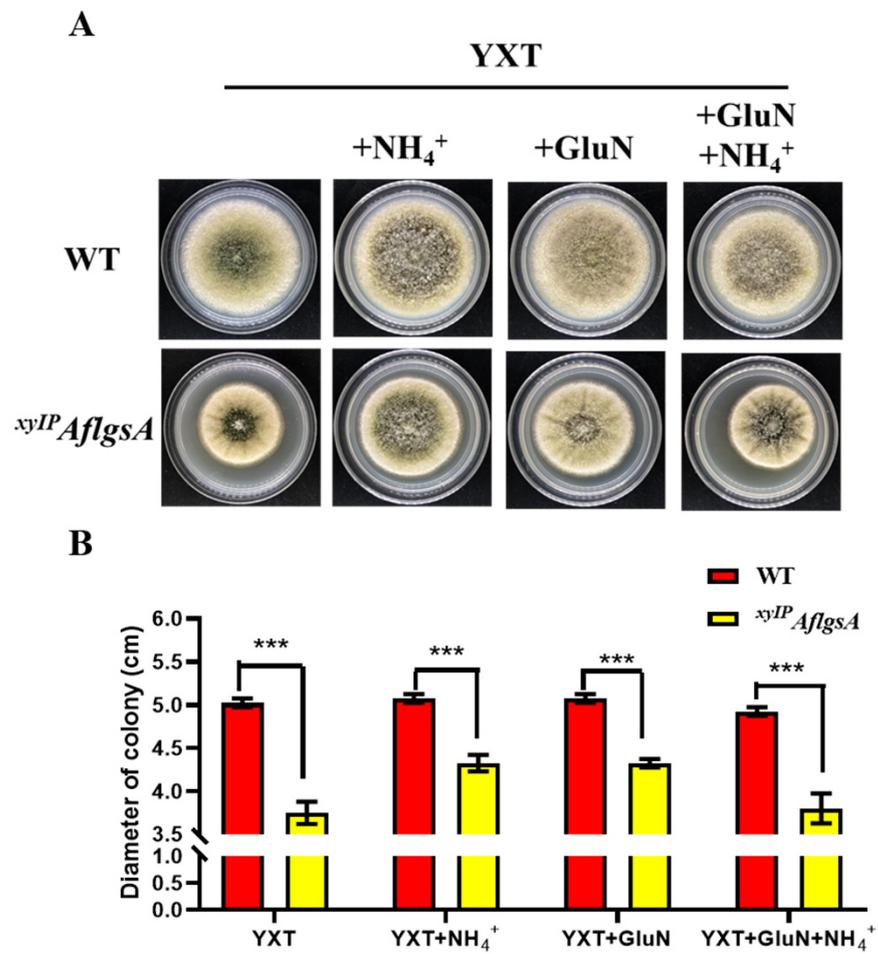


Figure S3. Analysis of the growth of WT and *xyIP AflgsA* strains. (A) The colony morphology of the WT and *xyIP AflgsA* strains on YXT medium containing 10 mM glutamine (GluN) or/and ammonium tartrate (NH₄⁺) as nitrogen sources. (B) Statistical analysis of the diameter from panel A. *** indicates a significance level of $P < 0.001$ based on t tests with three replicates.

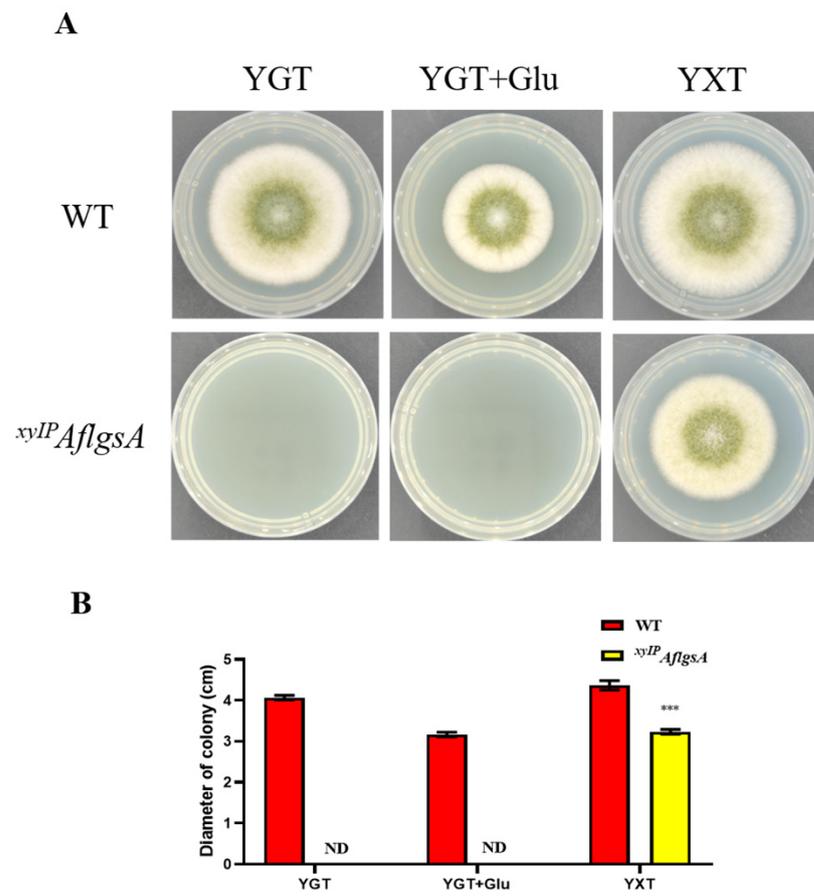


Figure S4. Analysis of the growth of WT and *xyIP AflgsA* strains. (A) The colony morphology of the WT and *xyIP AflgsA* strains on YGT medium containing 10 mM glutamate (Glu) or on YXT medium. (B) Statistical analysis of the diameter from panel A. ND indicates no detection. *** indicates a significance level of $P < 0.001$ based on t tests with three replicates.

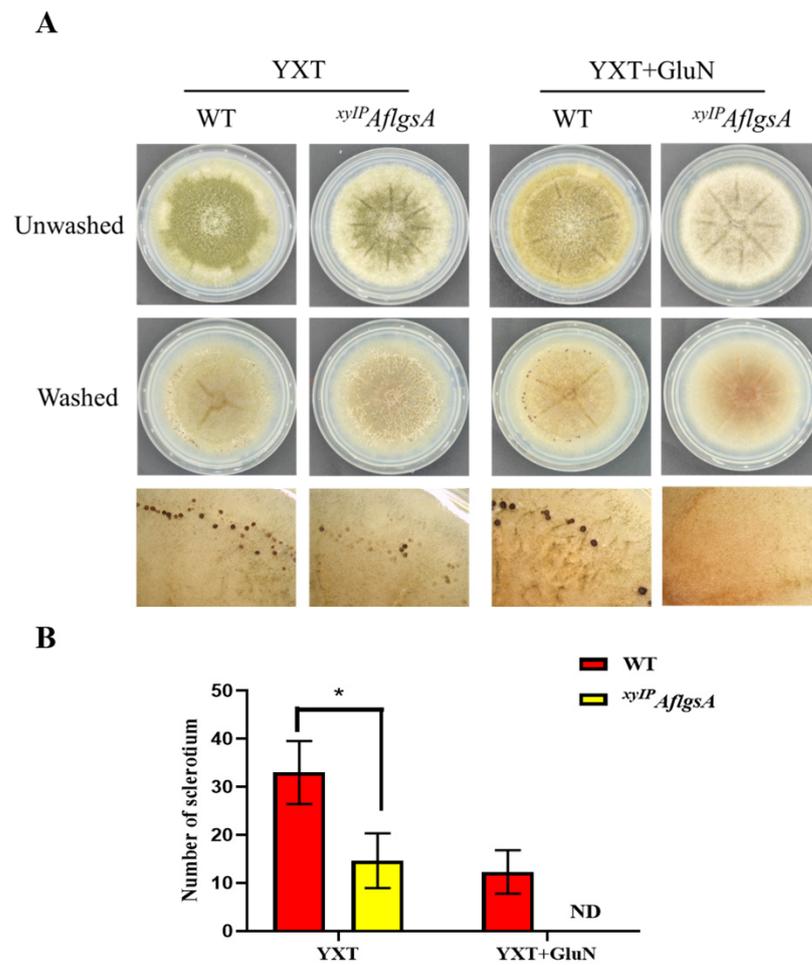


Figure S5. AflGsA regulates sclerotia formation in *A. flavus*. (A) Phenotypic observation of sclerotia formation in WT and *xyIP AflgsA* strains on YXT medium containing 10 mM glutamine (GluN). (B) The number of sclerotia produced by the above two *A. flavus* strains. ND indicates no detection. * indicates a significance level of $P < 0.05$ based on t tests with three replicates.

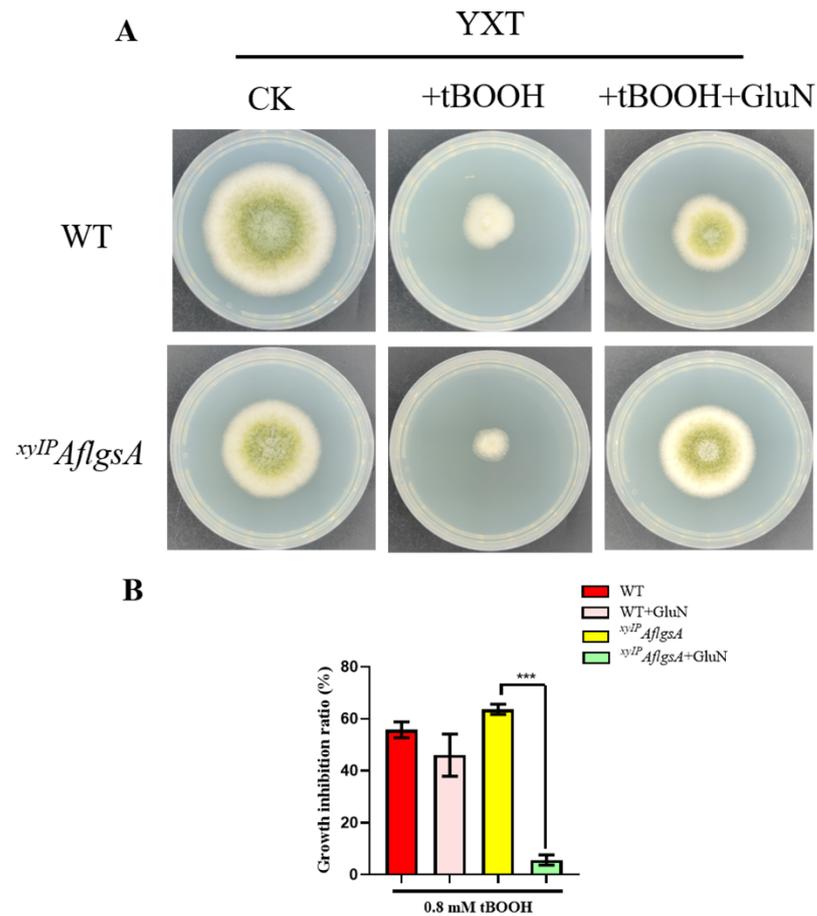


Figure S6. The role of AflGsA in resistance to oxidative stress in *A. flavus*. (A) Growth phenotype of the WT and *xyIP AflgsA* strains cultured in medium (with or without GluN) with 0.8 mM tBOOH oxidative stress. (C) The growth inhibition rate of different strains in media under oxidative stress (as in panel A). *** indicates a significance level of $P < 0.001$ based on one-way ANOVA with three replicates.

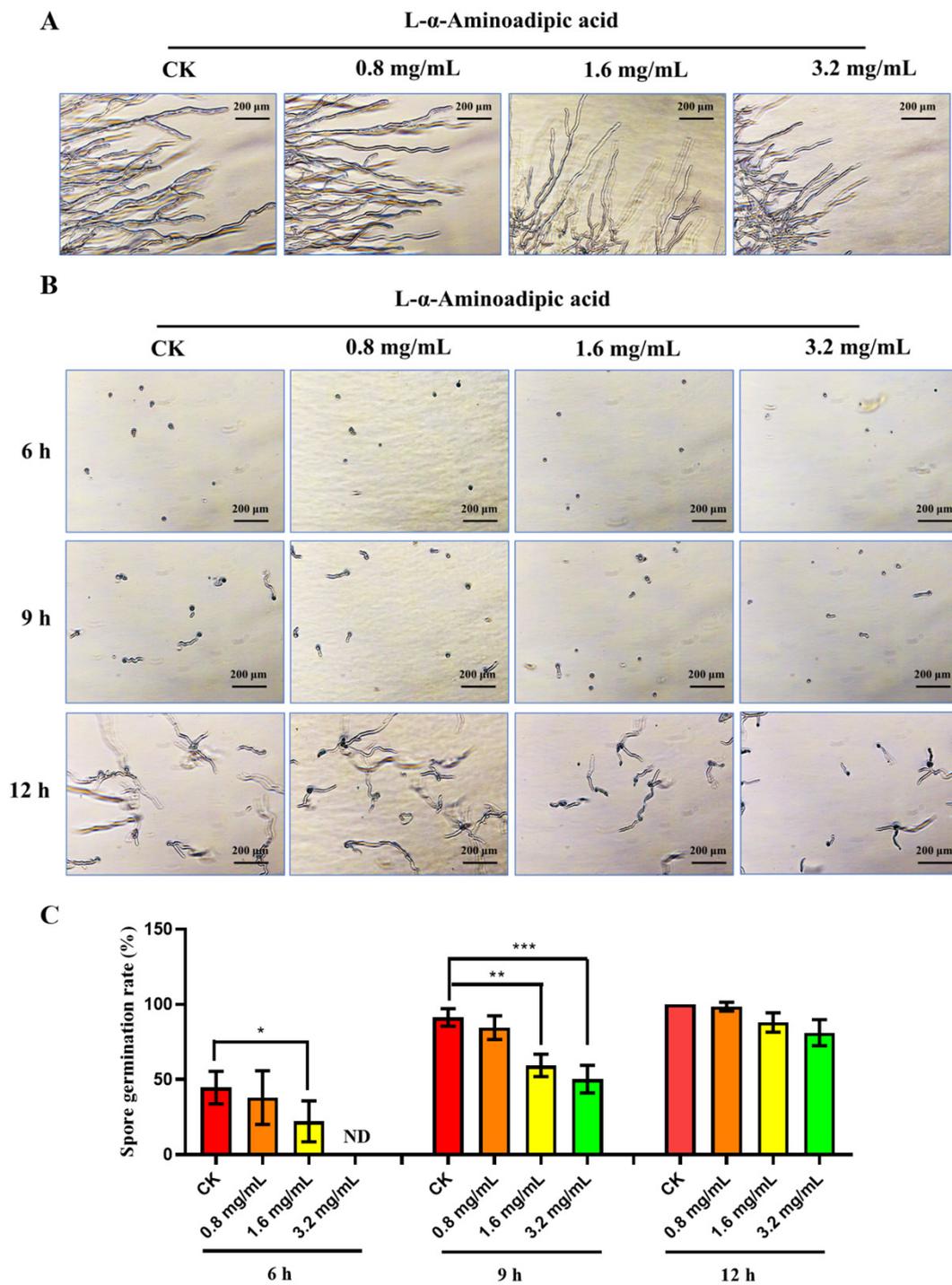


Figure S7. Effect of L- α -aminoadipic acid on mycelial morphology and conidial germination in *A. flavus*. (A) The mycelial morphology of the *A. flavus* WT strain was inhibited by L- α -aminoadipic acid (0–3.2 mg/mL). (B) The conidial germination of the *A. flavus* WT strain was inhibited by L- α -aminoadipic acid (0–3.2 mg/mL). (C) Statistical analysis of the conidial germination rate from panel B. ND indicates no detection. * indicates a significance level of $P < 0.05$, ** indicates a significance level of $P < 0.01$, and *** indicates a significance level of $P < 0.001$ based on one-way ANOVA with three replicates.

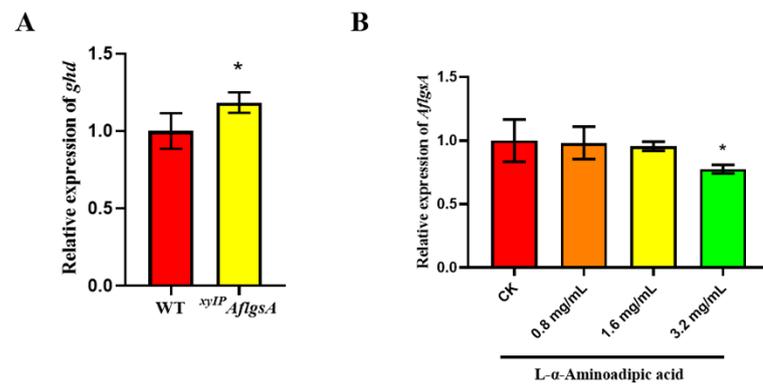


Figure S8. Relative expression of the genes in different strains. (A) Relative expression of the *ghd* gene in the WT and *xyIP AflgsA* strains. (B) Relative expression of the *AflgsA* gene of the WT strains treated with the inhibitor. * indicates a significance level of $p < 0.05$ based on t tests or one-way ANOVA with three replicates.

Table S1. Oligonucleotide primers used in this study.

Primers	Sequence (5'-3')	Application
<i>gsA-A-F</i>	GGGGTGCATTATCTGGG	To amplify <i>AflgsA</i> upstream fragment
<i>gsA-A-R</i>	GGGTGAAGAGCATTGTTTGAGGCAAGTGACAAA GGTTCAGTGGG	
<i>pyrG-xyIP-F</i>	GCCTCAAACAATGCTCTTCACCC	To amplify of <i>pyrG</i> and <i>xyIP</i> fusion fragment
<i>pyrG-xyIP-R</i>	GTTGGTCTTCGAGTCGATGAATG	
<i>gsA-CDS-F</i>	CATTCATCGACTCGAAGAACCAACATGGTATGT GATACTCAACCTCT	To amplify <i>AflgsA</i> CDS fragment
<i>gsA-CDS-R</i>	TGAACTTATCAATGCTGCC	Nest-primers for fusion PCR
<i>gsA-xolap-F</i>	TCTATCGCATCGGACTGGC	
<i>gsA-xolap-R</i>	GAGACGCTCCTCGTTACCTT	
<i>gsA-A-F</i>	GGGGTGCATTATCTGGG	Identification of <i>xyIP AflgsA</i> transformants by PCR
<i>gsA-CDS-R</i>	TGAACTTATCAATGCTGCC	
<i>gsA-GS-F</i>	TGAAACACCGCAACAACG	Identification of <i>xyIP AflgsA</i> transformants by Genome sequencing
<i>gsA-GS-R</i>	GGAATACGAATGCTACCACCAC	
<i>AflgsA-F</i>	TGTCTACCTGCGTCCCCTTGC	qRT-PCR primers to detect the <i>AflgsA</i> gene transcript level
<i>AflgsA-R</i>	CGTGCGGTGGTTGTACTTGT	
<i>brlA-F</i>	GCCTCCAGCGTCAACCTTC	qRT-PCR primers to detect the <i>brlA</i> gene transcript level
<i>brlA-R</i>	TCTCTCAAATGCTCTTGCCTC	
<i>abaA-F</i>	CACGGAAATCGCCAAAGAC	qRT-PCR primers to detect the <i>abaA</i> gene transcript level
<i>abaA-R</i>	CATCACAATCCCTTACCTCCC	
<i>nsdD-F</i>	GGAAGTGGCGGTTCGTGCTA	qRT-PCR primers to detect the <i>nsdD</i> gene transcript level
<i>nsdD-R</i>	AGAACGCTGGGTCTGGTGC	
<i>nsdC-F</i>	GCCAGACTTGCCAATCAC	qRT-PCR primers to detect the <i>nsdC</i> gene transcript level
<i>nsdC-R</i>	CATCCACCTTGCCCTTA	
<i>ghd-F</i>	TCCGAGGCTTCCATCTACG	RT-PCR primers to detect the <i>ghd</i> gene transcript level
<i>ghd-R</i>	GAACAGTGAGCGGGCATT	
<i>cat-F</i>	TTTCTTCACGGCTCCTGGAC	RT-PCR primers to detect the <i>cat</i> gene transcript level
<i>cat-R</i>	CGAGCTCTTGACGTTGGAGT	
<i>sod-F</i>	CATGTCCACCAGTTCGGTGA	RT-PCR primers to detect the <i>sod</i> gene transcript level
<i>sod-R</i>	GCATCGGTCTTGAAGTTGCC	
<i>β-actin-F</i>	ACGGTGTGTCACAACTGG	qRT-PCR primers to detect the <i>β-actin</i> gene transcript level
<i>β-actin-R</i>	CGGTTGGACTTAGGGTTGATAG	
<i>β-tubulin-F</i>	CAACAACCTCCAGACCGCC	qRT-PCR primers to detect the <i>β-tubulin</i> gene transcript level
<i>β-tubulin-R</i>	CTCGTCCATACCCTCACC	