Figures and figure supplements

SntB triggers the antioxidant pathways to regulate development and aflatoxin biosynthesis in *Aspergillus flavus*

Dandan Wu and Chi Yang *et al*.

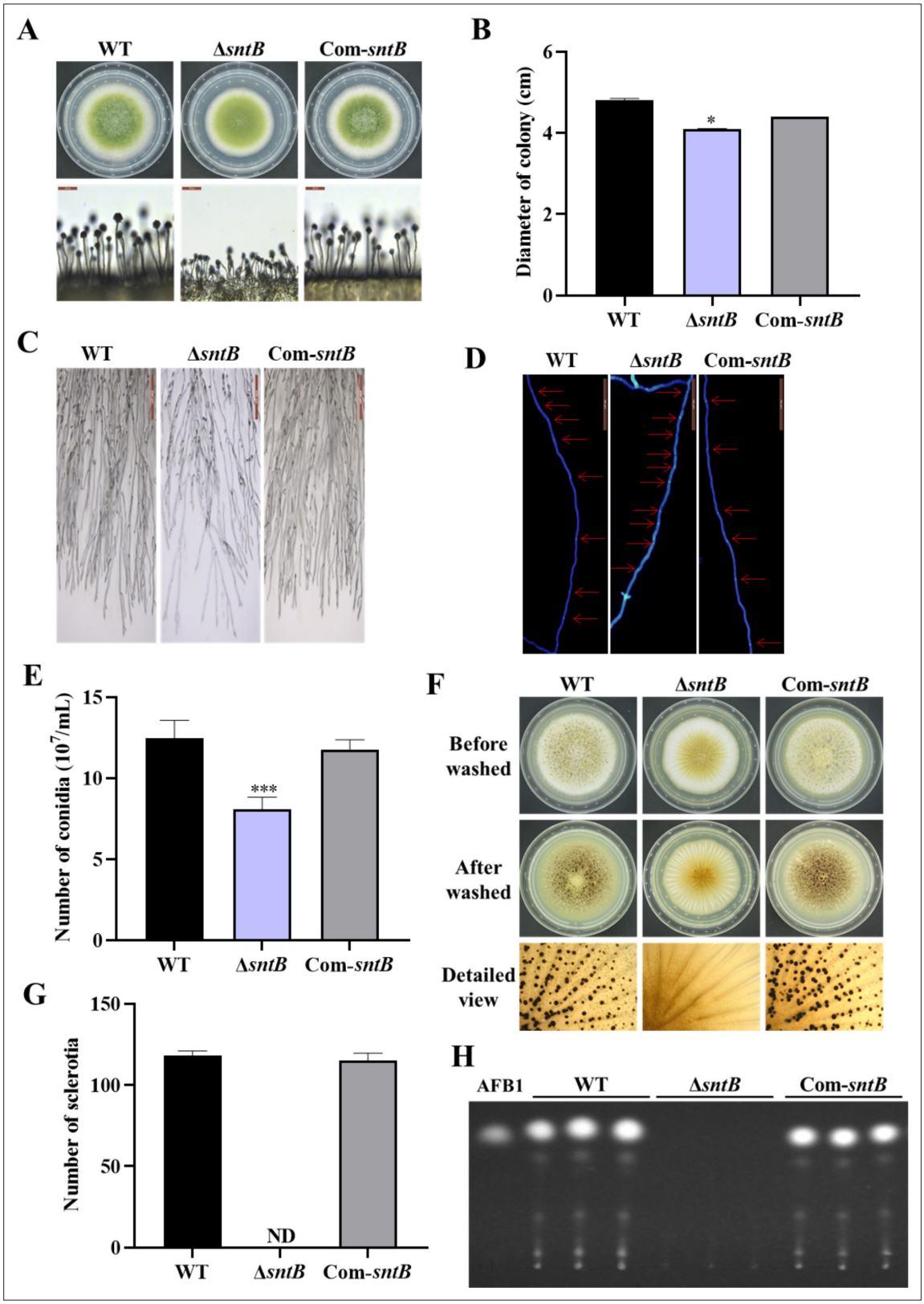

Figure 1. The functions of SntB in *A. flavus*. (**A**) The colonies of wild-type (WT), Δ*sntB*, and Com-*sntB* strains grown on potato dextrose agar (PDA) at 37°C in dark for 4 days. (**B**) The colony diameter statistics of the above fungal strains. (**C**) Microscopic examination revealed the difference in mycelia of each fungi strain at 37°C in dark, scale=200 μm. (**D**) Microscopic examination of the hyphal septum of each strain at 37°C in dark, scale=50 μm. (**E**) The spore production statistics. (**F**) All the above fungal strains were point-inoculated on CM medium and grown for 7 days at 37°C. (**G**) The number

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of sclerotia of the above fungal strains. ND=Not detectable. (**H**) AFB1 production of the above fungal strains was detected by TLC after the strains incubating at 29°C in PDB medium for 7 days.

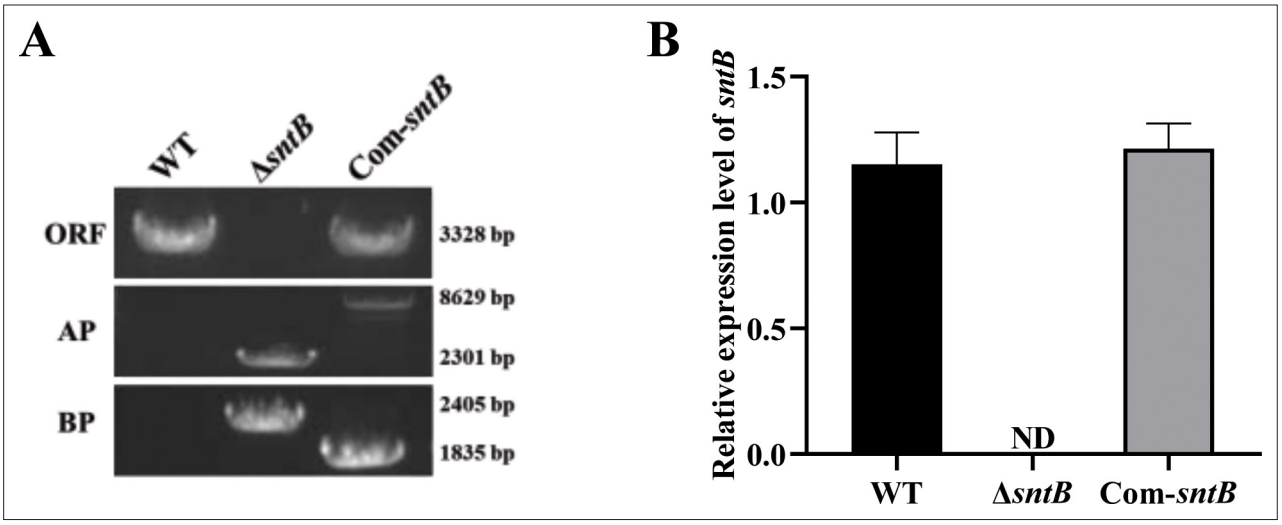

Figure 1—figure supplement 1. The construction of mutant strains. (**A**) PCR verification of gDNA in wild-type (WT), Δ*sntB*, and Com-*sntB* strains ('ORF' represents the *sntB* gene fragment, 'AP' represents the amplification of the fusion fragment upstream with primers *sntB*-p1 and P801, and 'BP' represents the downstream of the fusion fragment from primers P1020 and *sntB*-p4). (**B**) Quantitative RT-PCR (qRT-PCR) verification of the expression level of the *sntB* gene in WT and *sntB* gene mutant strains.

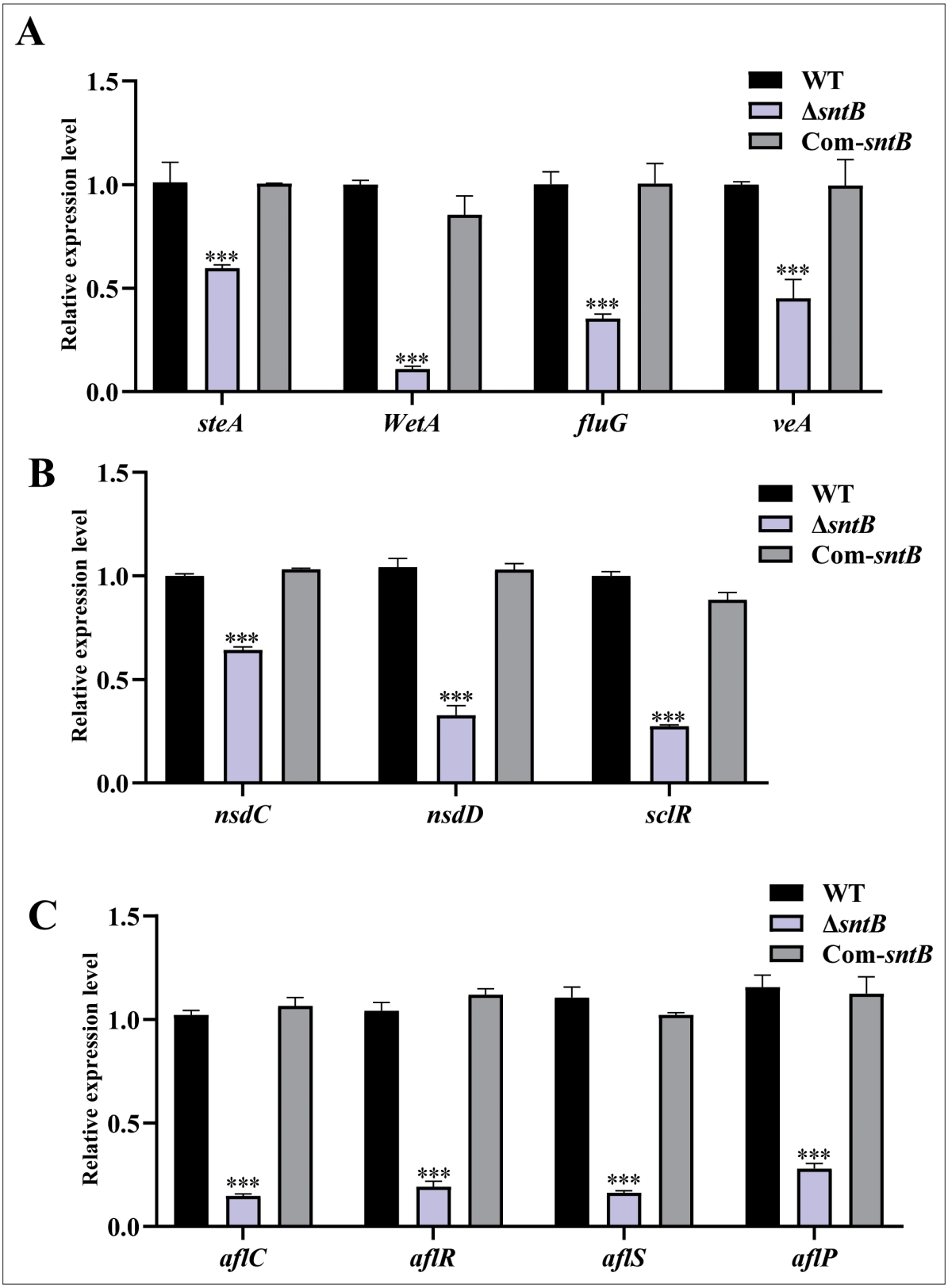

Figure 1—figure supplement 2. The expression of genes related to sporulation, sclerotia production, and aflatoxin synthesis. (**A**) The expression of sporulation-related genes *steA*, *WetA*, *fluG*, and *veA* in each strain at 48 hr. (**B**) The expression of sclerotia-associated genes *nsdC*, *nsdD*, and *sclR* in each strain at 48 hr. (**C**) The expression of aflatoxin-associated genes in each strain at 48 hr. The asterisk *** above the bars represents significantly different ($p<0.001$).

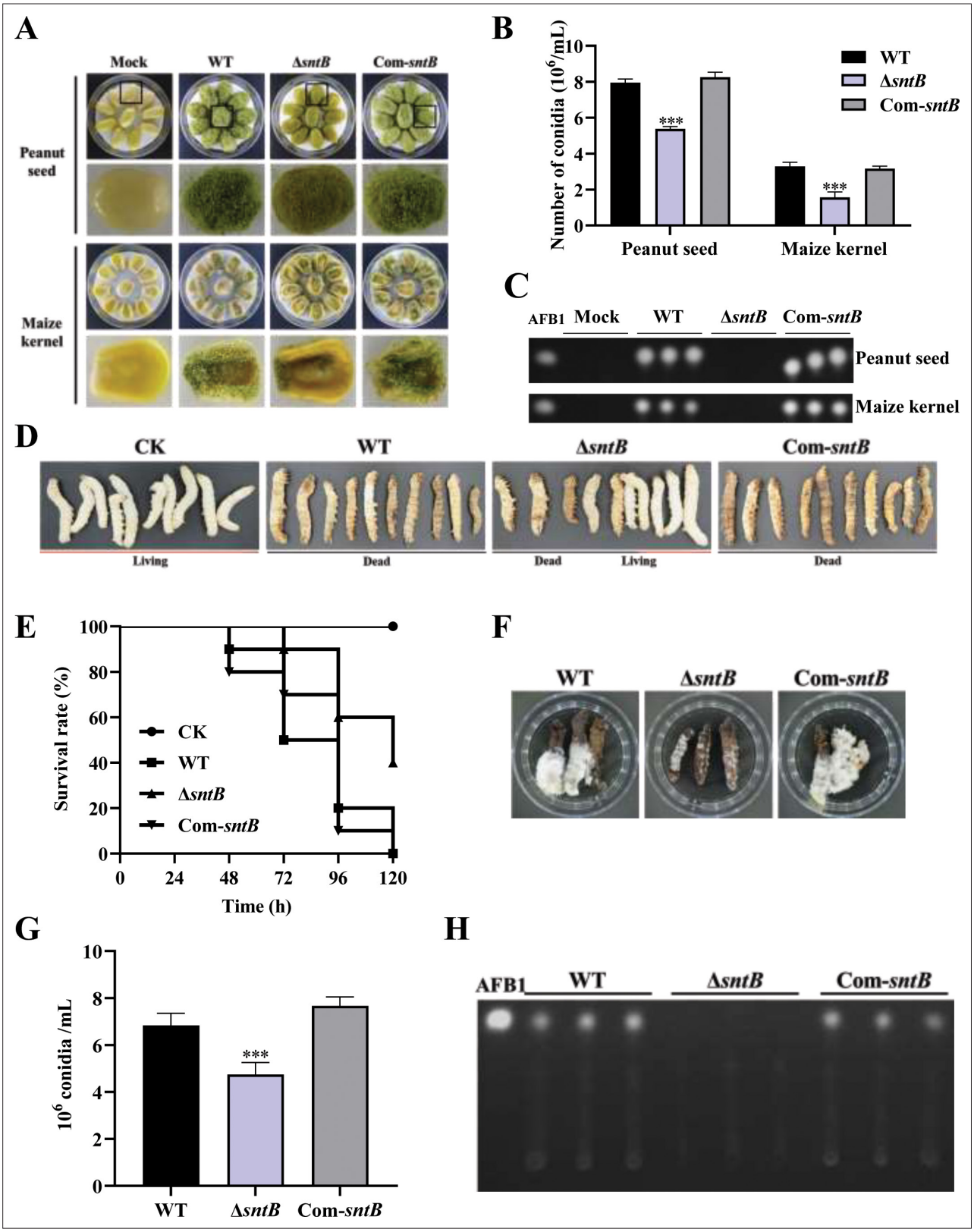

Figure 2. The role of SntB on the ability of *A. flavus* to colonize host. (**A**) Phenotype of peanut and maize kernels colonized by Δ*sntB*, Com-*sntB*, and wild-type (WT) strains at 29°C in dark for 7 days. (**B**) Statistical of the number of conidia on the surface of peanut and maize kernels. (**C**) TLC analysis to detect the yield of AFB1 in kernels infected by the above fungal strains after 7 days incubation. (**D**) Photographs of the silkworms infected by the above fungal strains. (**E**) The survival rate of silkworms in 5 days after injection of the above strains. (**F**) Photographs of the dead silkworms infected by *A. flavus*

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after 6 days incubation. (**G**) The spore production statistics of the above fungal strains on the dead silkworms shown in (**F**). (**H**) TLC analysis of AFB1 levels produced in infected dead silkworms in (**F**).

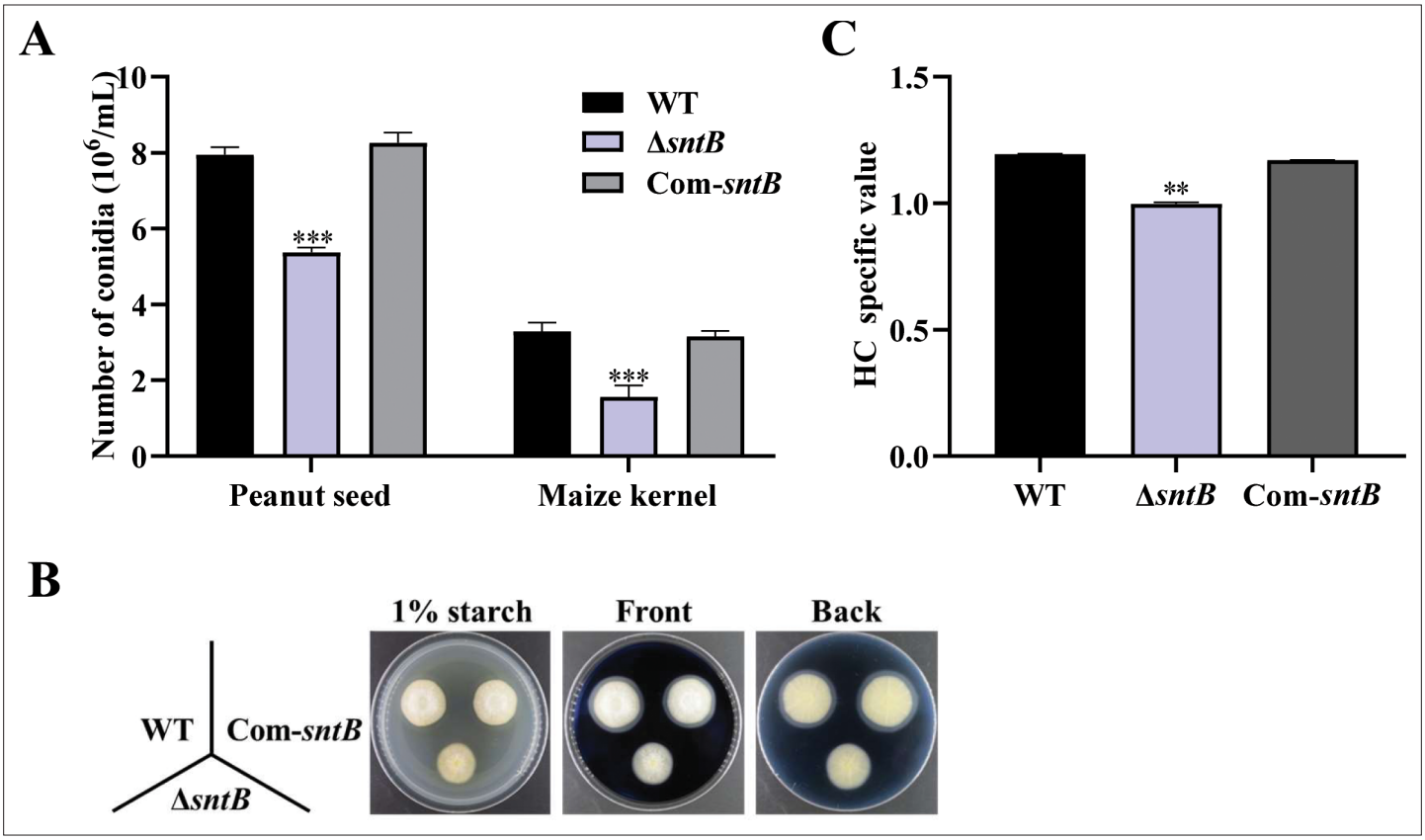

Figure 2—figure supplement 1. The changes of number of conidia, amylase, and lipase in wild-type (WT), Δ*sntB*, and Com-*sntB* strains. (**A**) Statistics of the number of conidia on the corn seed. (**B**) The phenotype of each strain on starch screening medium supplemented with 0.1% of soluble starch in darkness at 29°C for 3 days, followed by the addition of iodine solution. (**C**) HC value of the clear circle (outer diameter/inner diameter) of the salient analysis of the map. The asterisk ** above the bars represents significantly different ($p<0.01$).

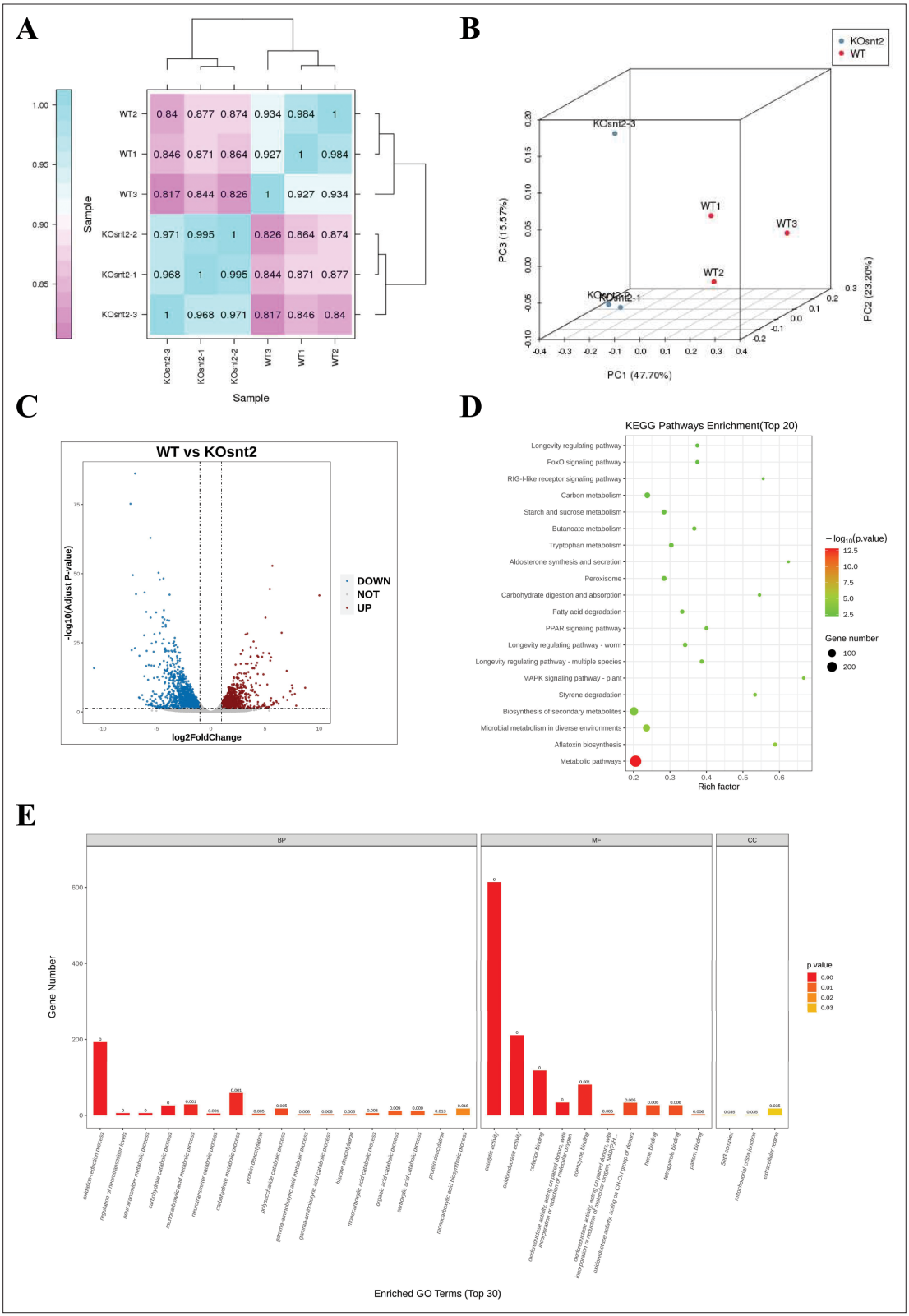

Figure 3. SntB chords global gene expression in *A. flavus*. (**A**) The Pearson correlation results shown by heatmap. (**B**) Principal component analysis (PCA) on six fungal samples, including three Δ*sntB* (KOsnt2) and three wild-type (WT) samples. (**C**) Volcano map reflecting the distribution of the differentially expressed genes. (**D**) Kyoto encyclopedia of genes and genomes (KEGG) analyses of the differentially expressed genes. (**E**) Gene ontology (GO) analyses of the differentially expressed genes.

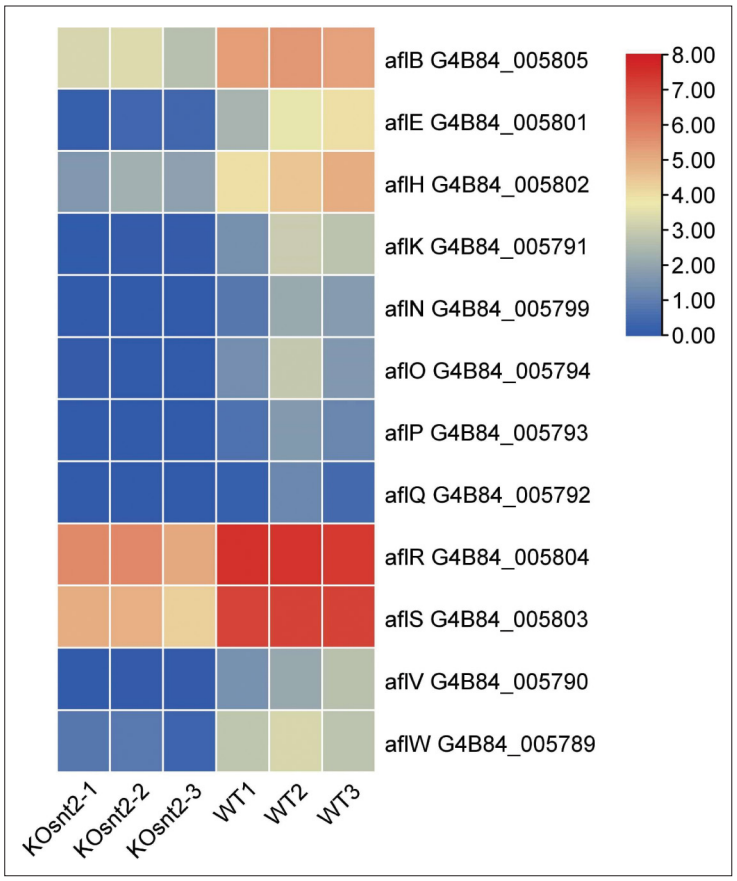

Figure 3—figure supplement 1. Heatmap of the differentially expressed genes (DEGs) related to oxidative response in transcriptome data drawn by TBtools.

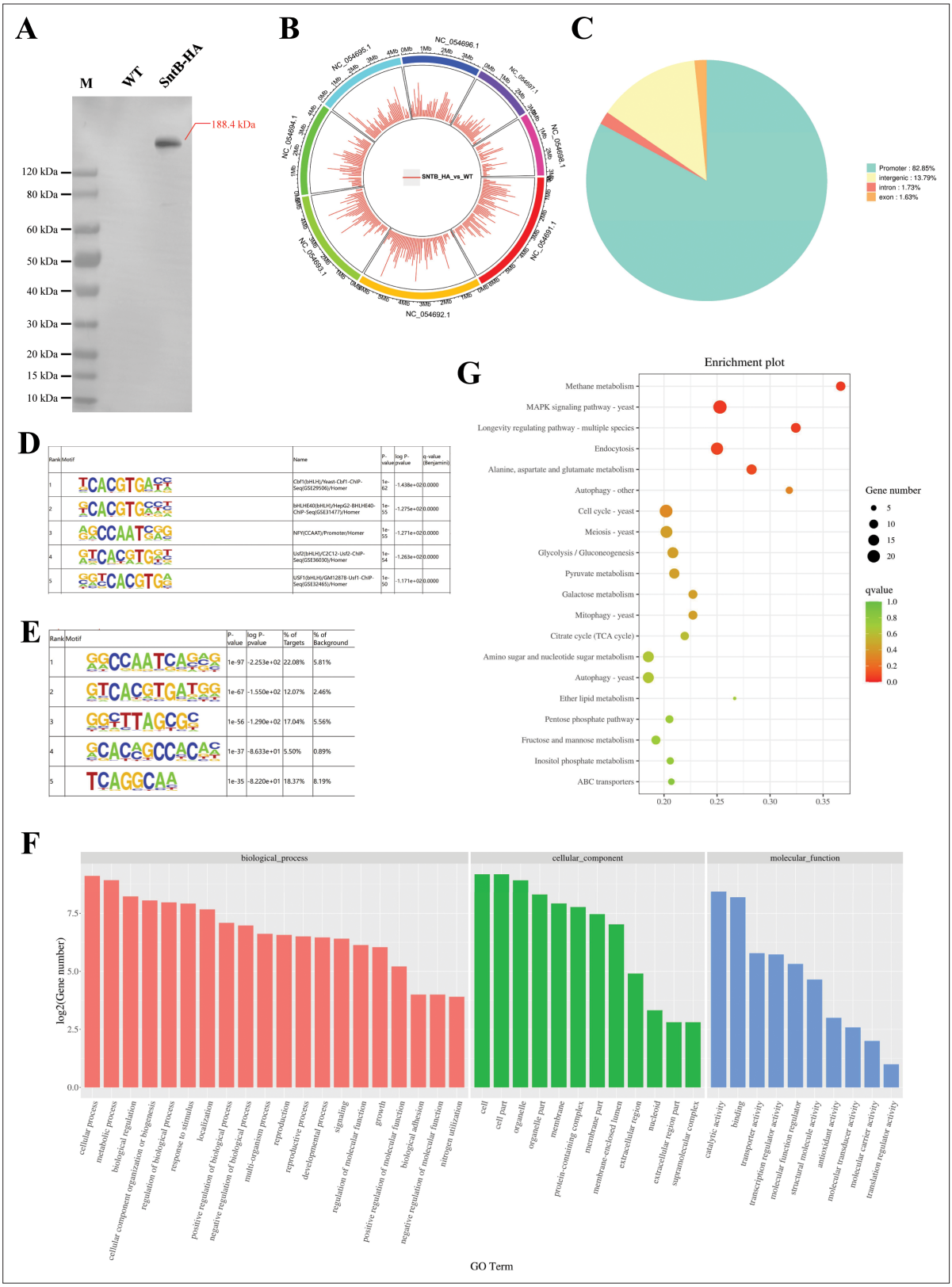

Figure 4. Characterization of the binding regions of SntB. (**A**) Verification of the construction of *sntB*-HA strain using western blot. M means the protein marker of PAGE-MASTER Protein Standard Plus (GenScript USA, MM1397). (**B**) The distribution of differently accumulated peaks on the genome. (**C**) Vennpie map of the differently accumulated peaks distribution on gene functional elements. (**D**) Enrichment of known motifs showing the top-ranked motif logos. (**E**) Enrichment of de novo motifs showing the top-ranked motif logos. (**F**) Gene ontology (GO) analyses of the differently accumulated peak-related genes. (**G**) Kyoto encyclopedia of genes and genomes (KEGG) analyses of the differently accumulated peak-related genes.

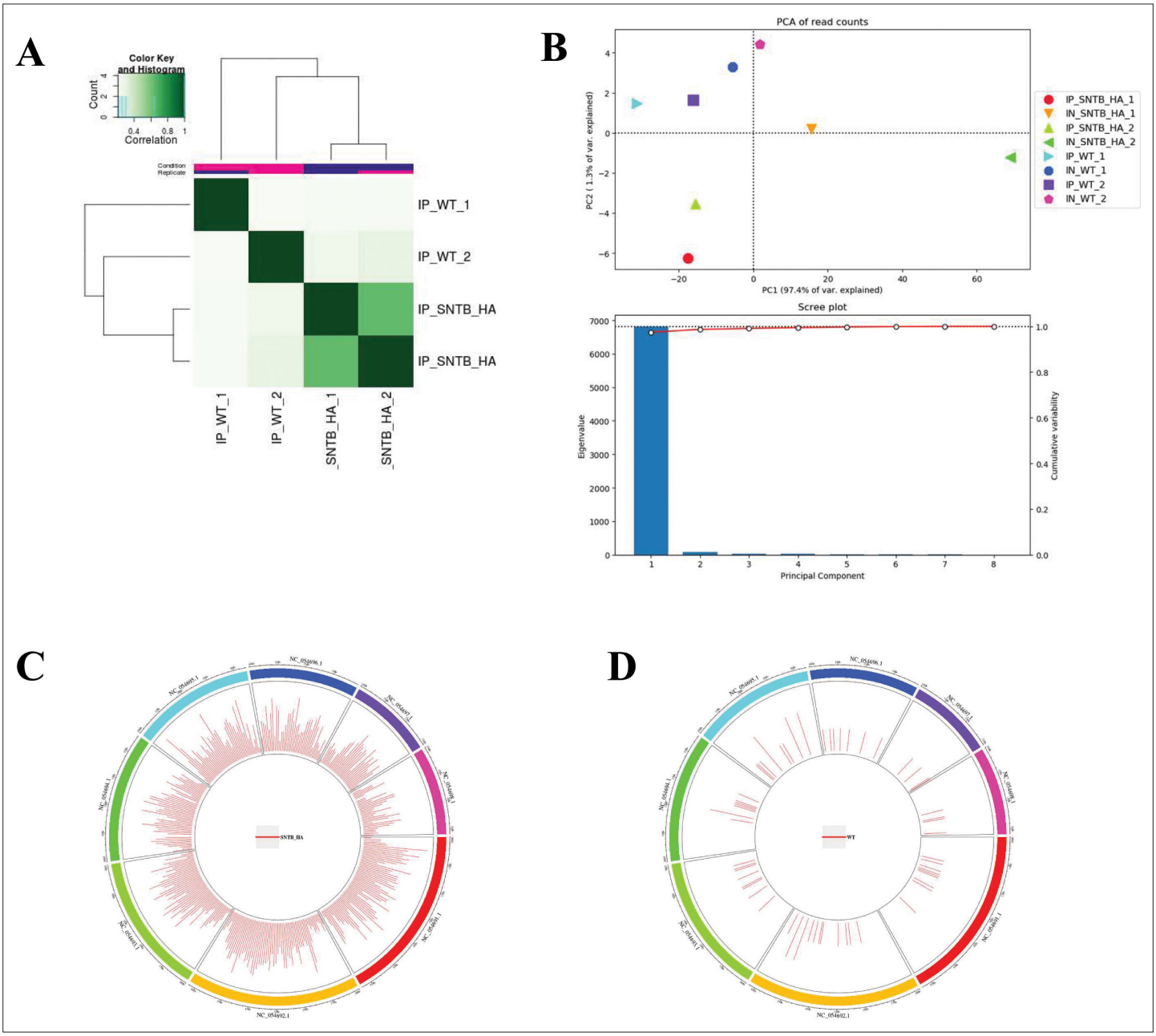

Figure 4—figure supplement 1. Sequence information of chromatin immunoprecipitation sequencing (ChIP-seq). (**A**) Heatmap. (**B**) Principal component analysis (PCA). (**C**) Peak distribution on the genome of SNTB-HA group. (**D**) Peak distribution on the genome of wild-type (WT) group.

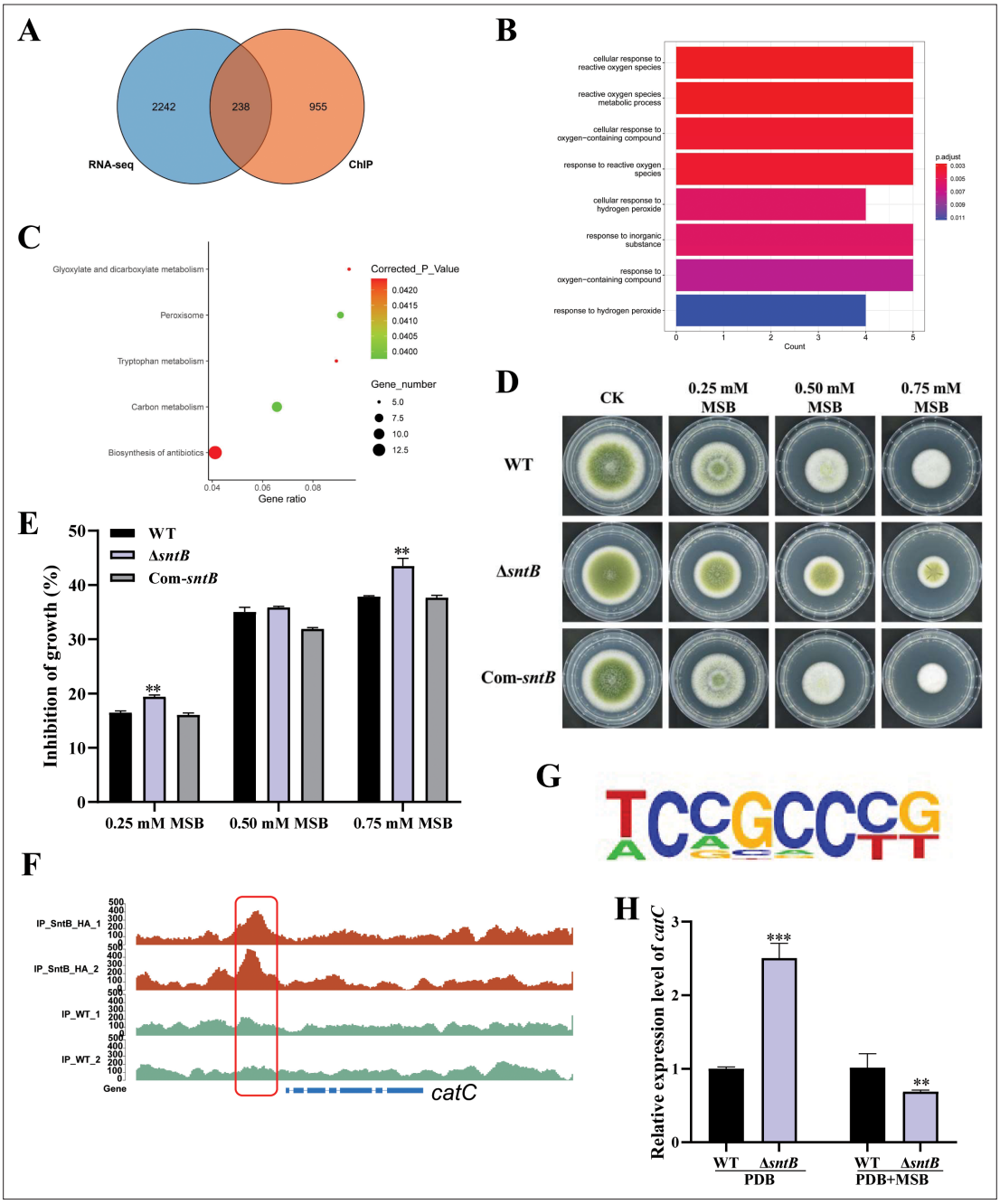

Figure 5. Integration of the results of chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) assays. (**A**) Venn diagrams of ChIP-seq and RNA-seq. (**B**) Gene ontology (GO) analyses of the common genes. (**C**) Kyoto encyclopedia of genes and genomes (KEGG) analyses of the common genes. (**D**) The phenotype of wild-type (WT), Δ*sntB*, and Com-*sntB* strains cultured in PDA containing a series concentration of menadione sodium bisulfite (MSB) for 3 days. (**E**) Statistical analysis of the growth inhibition rate of MSB to all the above fungal strains according to Panel D. (**F**) Comparison of the enrich levels of the SntB binding region of *catC* gene between WT and sntB-HA strains. (**G**) The motif logo in the SntB binding region of *catC* gene. (**H**) The relative expression level of *catC* in WT and Δ*sntB* strains with or without MSB treatment.

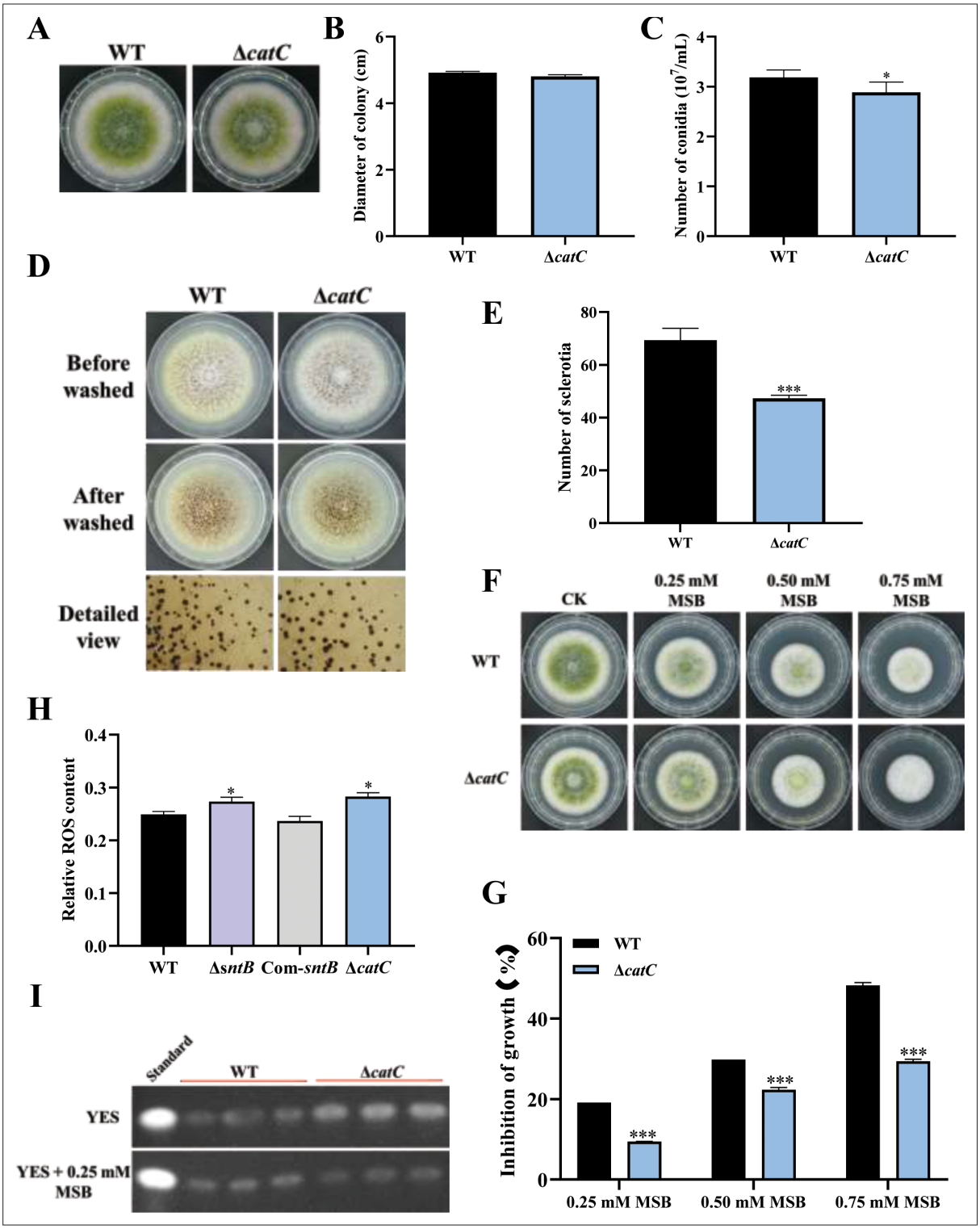

Figure 6. The functions of *catC* in *A. flavus*. (**A**) The colonies of wild-type (WT) and Δ*catC* strains grown on potato dextrose agar (PDA) at 37°C in dark for 4 days. (**B**) The colony diameter statistics of the above fungal strains. (**C**) The spore production statistics of the above fungal strains. (**D**) All above fungal strains were point-inoculated on complete medium (CM) and grown for 7 days at 37°C. (**E**) The number of sclerotia of the above fungal strains. (**F**) The phenotype of above strains cultured on PDA medium containing a series concentration of menadione sodium bisulfite (MSB) for 3 days. (**G**) Statistical analysis of the growth inhibition rate of MSB to all the above fungal strains according to (**F**). (**H**) Relative reactive oxygen species (ROS) levels in the WT, Δ*sntB*, Com-sntB, and Δ*catC* strains. (**I**) AFB1 production of the above fungal strains was detected by TLC after the strains incubating at 29°C in potato dextrose (PDB) medium for 7 days.

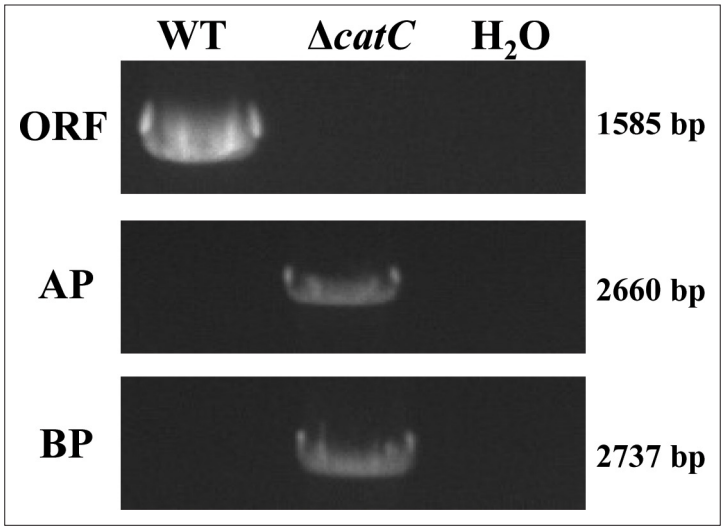

Figure 6—figure supplement 1. PCR verification of gDNA in wild-type (WT) and Δ*catC*.

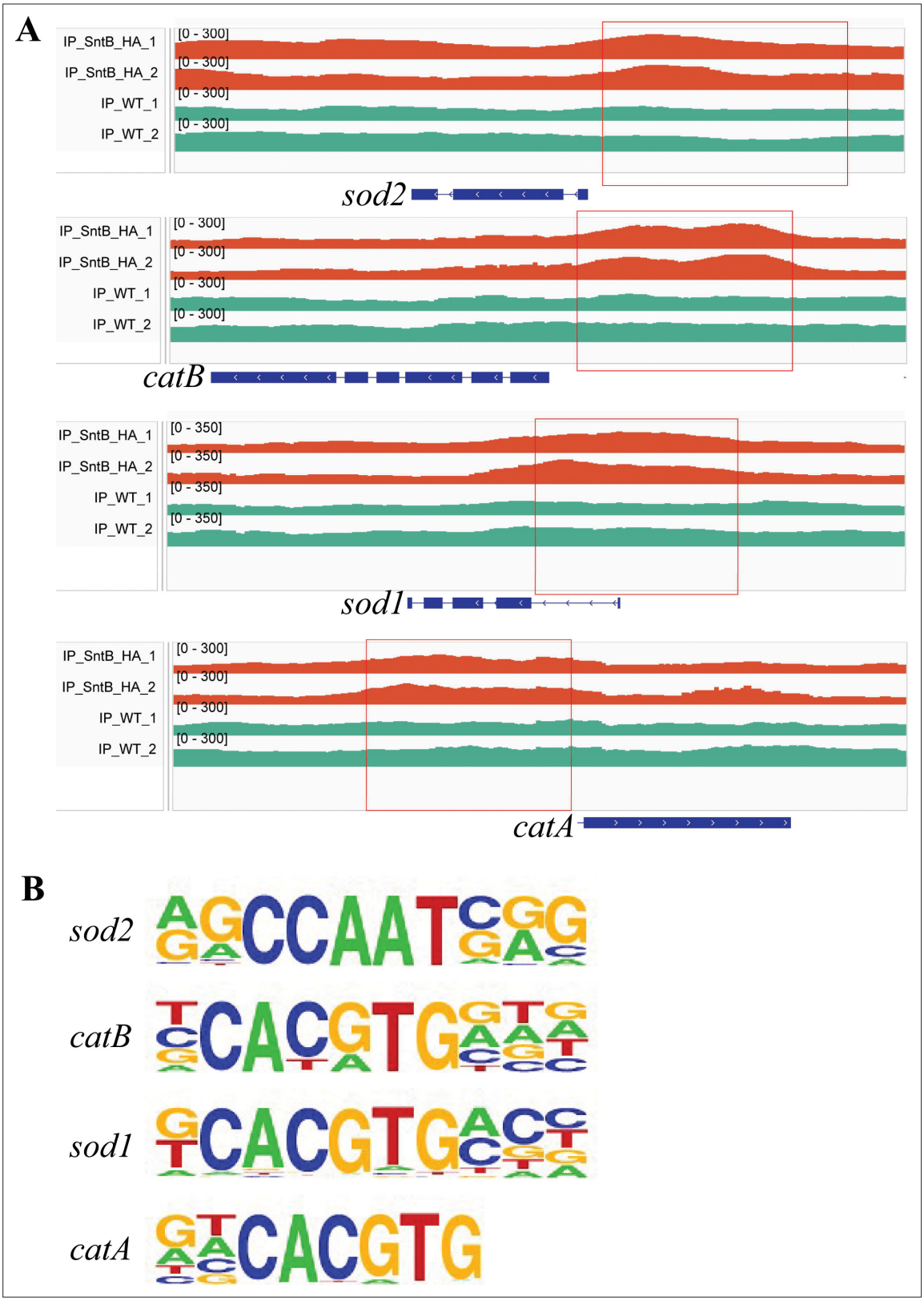

Figure 6—figure supplement 2. The binding region and motif of SntB on the *catA*, *catB*, *sod1*, and *sod2* genes. (**A**) Comparison of the enrich levels of the SntB binding region of *catA*, *catB*, *sod1*, and *sod2* genes between wild-type (WT) and sntB-HA strains. (**B**) The motif logo in the SntB binding region of *catA*, *catB*, *sod1*, and *sod2* genes.

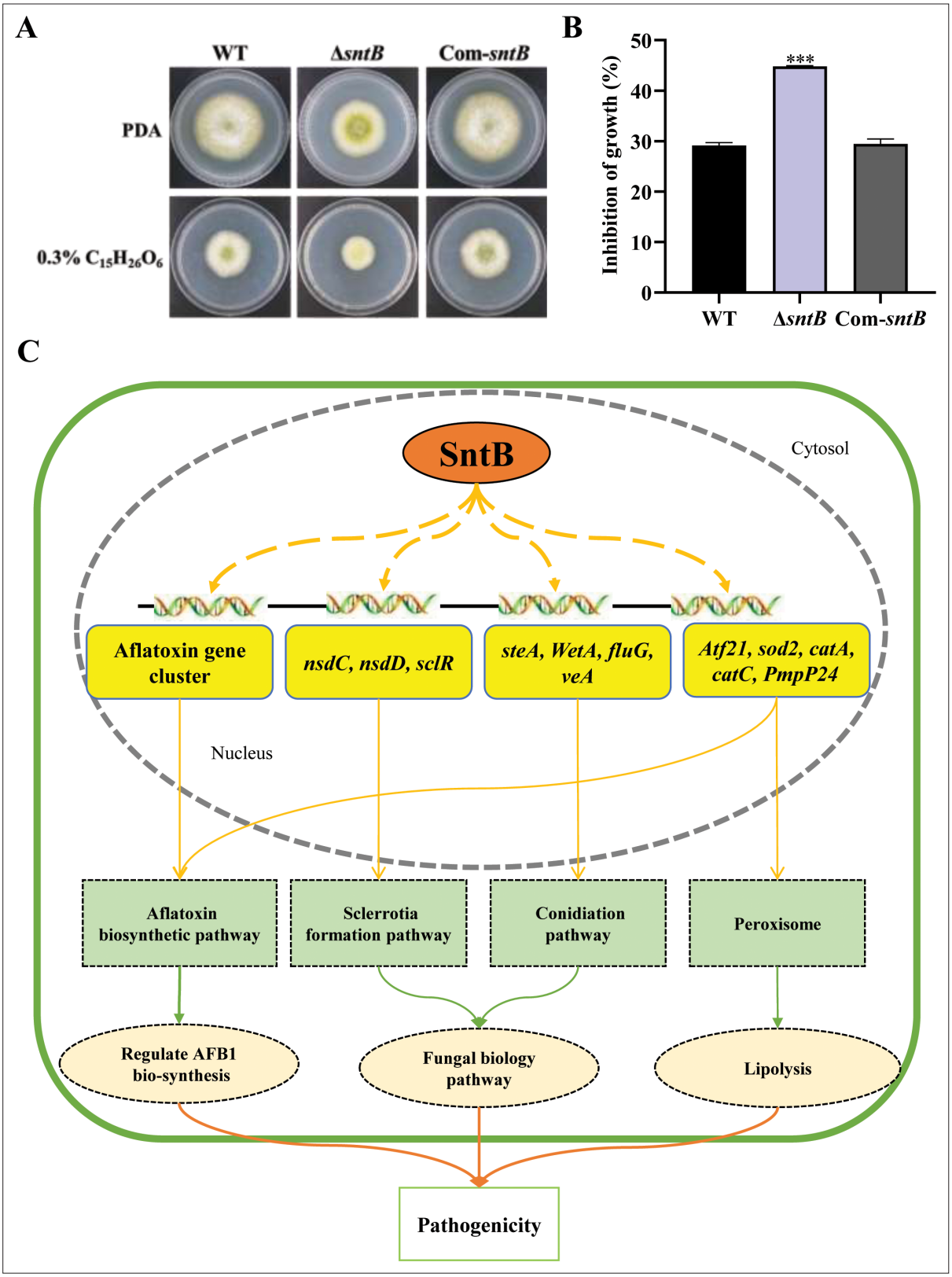

Figure 7. SntB regulate peroxisome biogenesis, fatty acid utilization, and fungal pathogenicity in *A. flavus*. (**A**) The phenotype of each strain on PDA medium containing 0.3% tributyrin. (**B**) Statistics of inhibition rates. The asterisk *** above the bars represents significantly different ($p<0.001$). (**C**) Mechanistic diagram of the bio-functions of SntB in *A. flavus*.