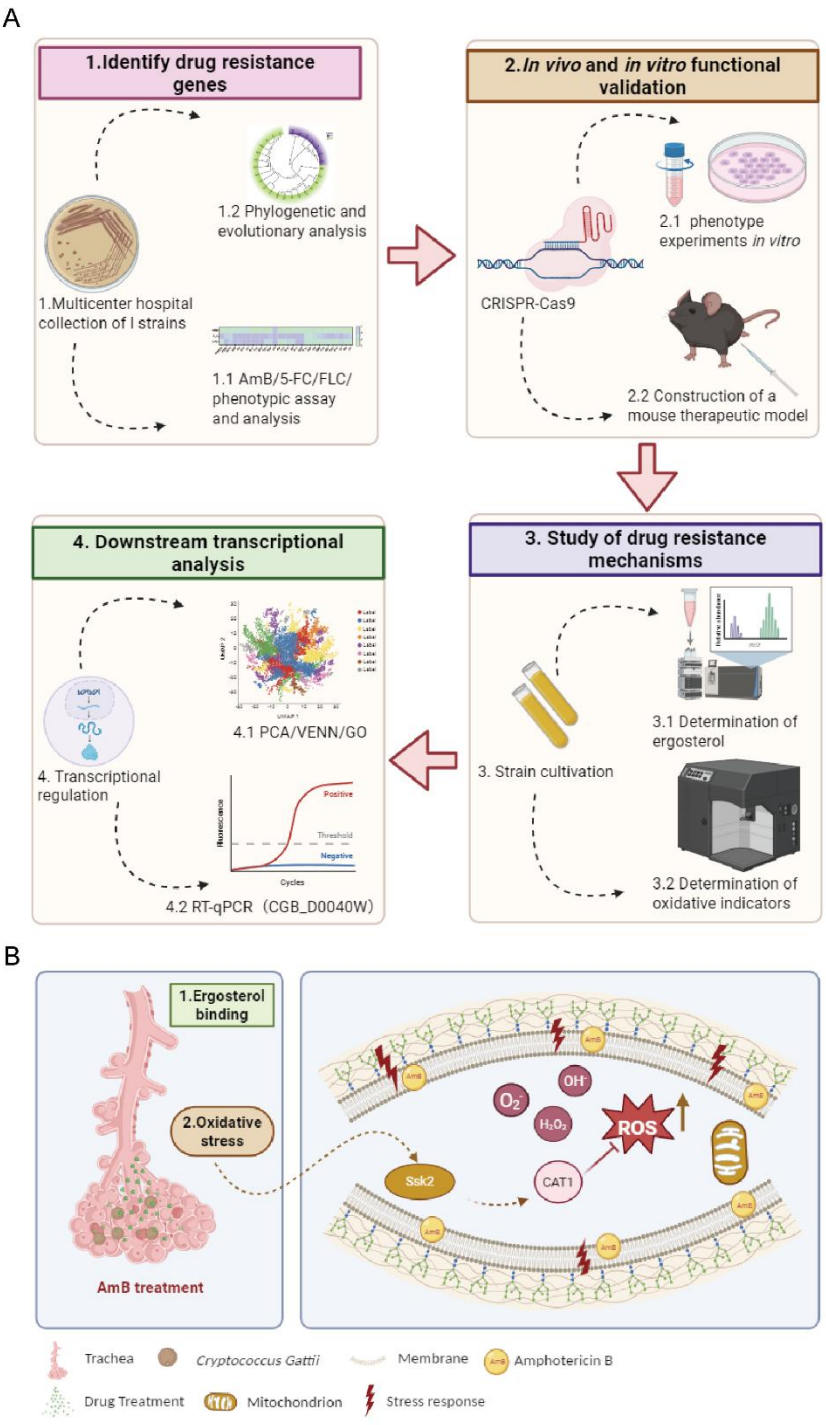
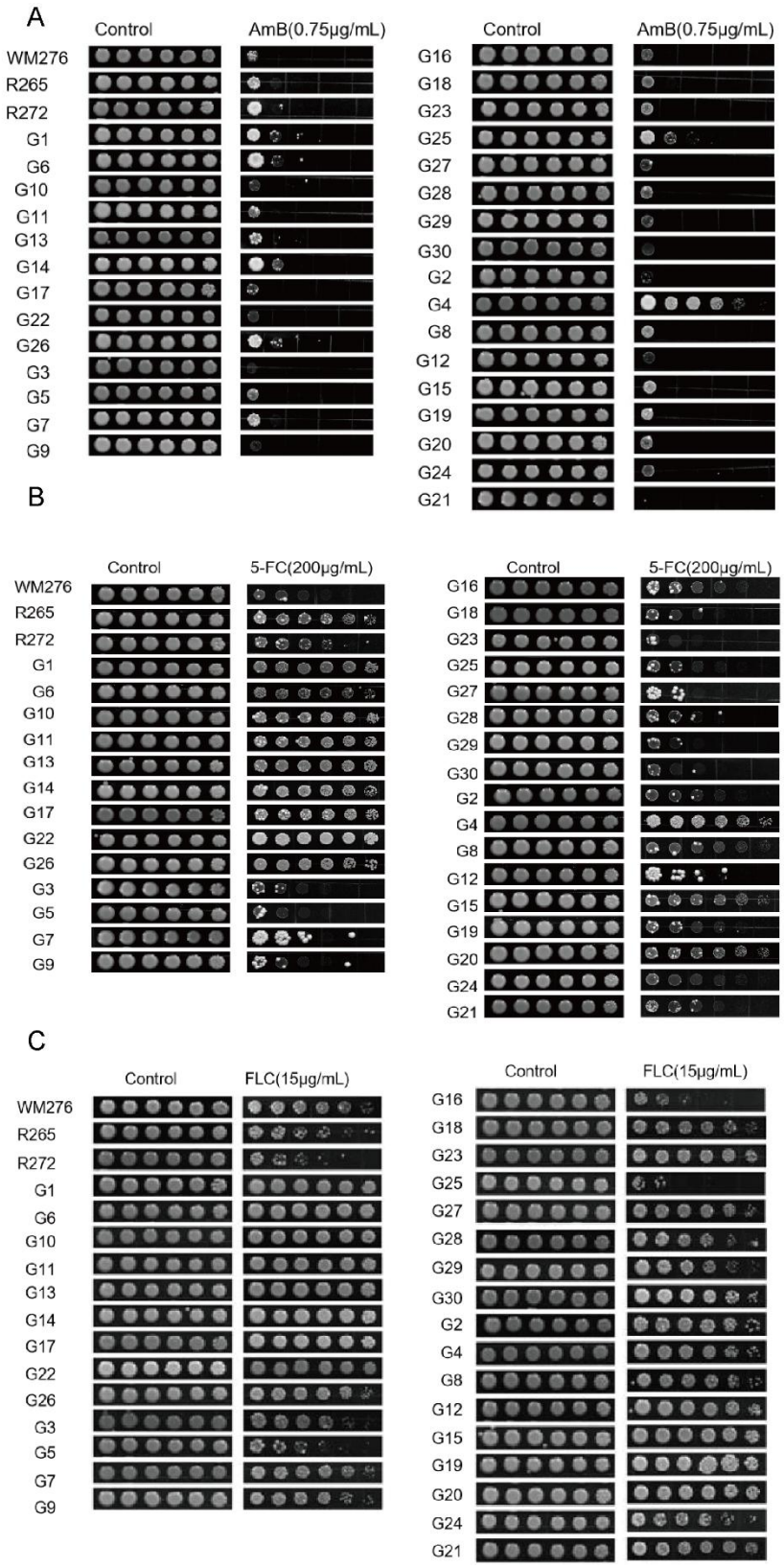
Supplements



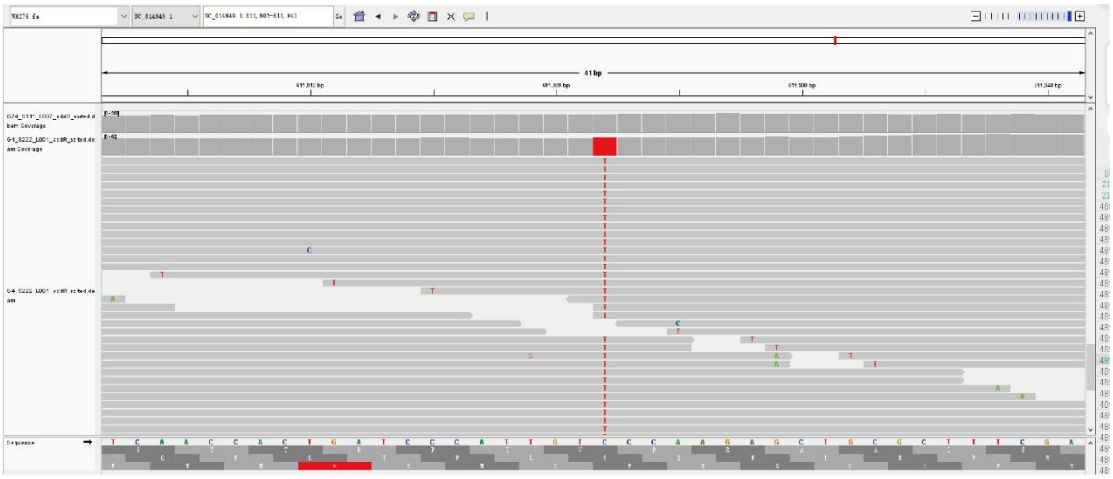
Graphical abstract: A: Experimental procedure. B: *SSK2* regulates the oxidative stress pathway by modulating the expression of *CAT1*, thereby mediating susceptibility of *Cryptococcus gattii* to AmB. AmB, amphotericin B; GO, Gene Ontology; LC-MS, liquid chromatography–mass spectrometry; ROS, reactive oxygen species.



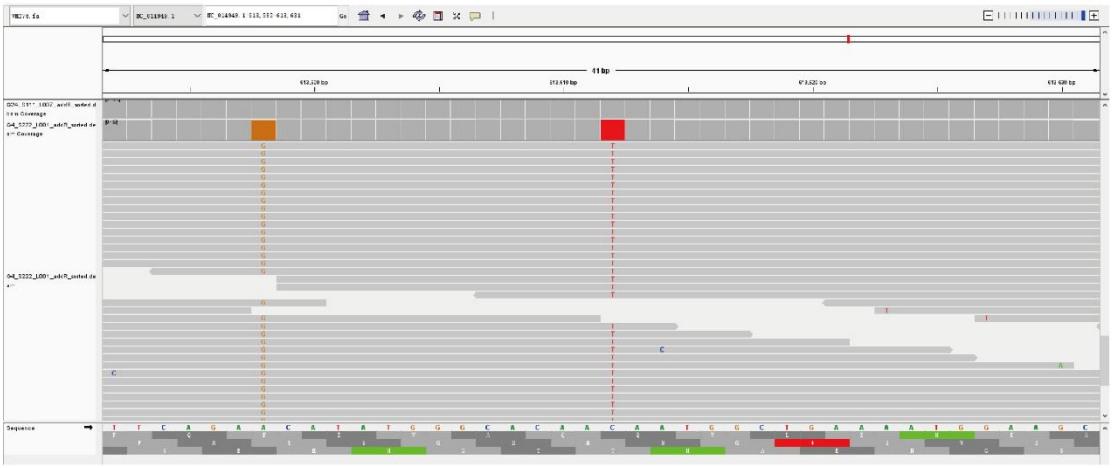
Supplementary Figure S1: Spot plate assay verifies the susceptibility of clinical *Cryptococcus gattii* strains to three therapeutic drugs. A: *C. gattii* susceptibility to AmB. B: *C. gattii* susceptibility

to 5-FC. C: *C. gattii* susceptibility to FLC. Representative results from one of three independent experiments are shown. 5-FC, 5-fluorocytosine; AmB, amphotericin B; FLC, fluconazole.

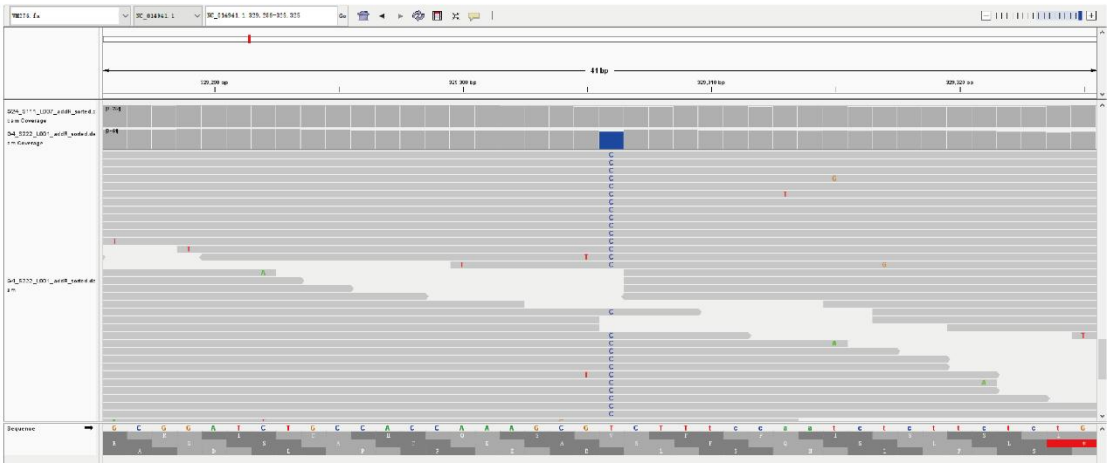
A



B



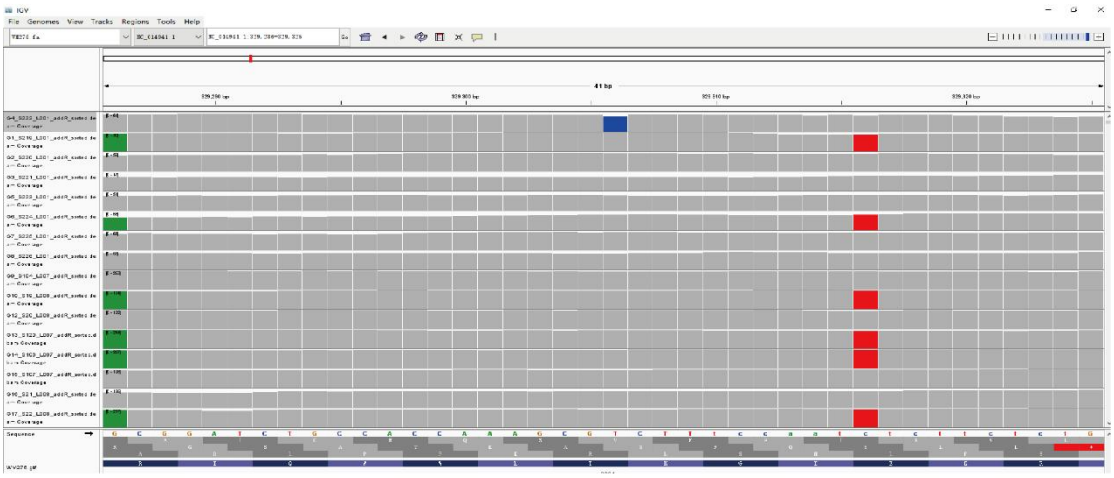
C



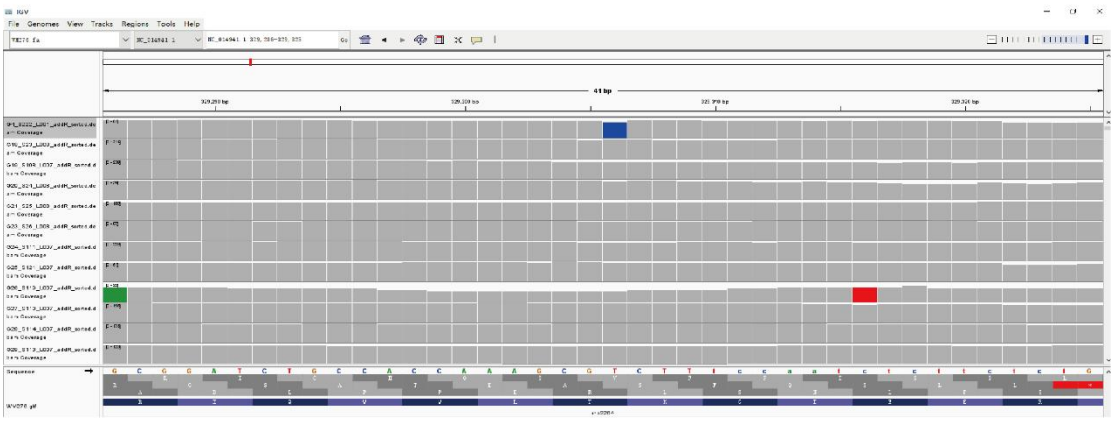
Supplementary Figure S2-1: Visualization of results from three point mutations. The read alignment results were visualized using IGV (version 2.14.0, Broad Institute, Cambridge, USA). The

sequencing reads of strains G4 and G24 were aligned to the reference genome. Distinct base variations at the target site between the two strains are displayed, confirming the genomic differences identified by variant calling, with different colors indicating mismatched bases relative to the reference. A: Blue indicates the c.793A>G point mutation in *SSK2*. B: Red indicates the c.3443G>A point mutation in *TCO2*. C: Red indicates the c.1753G>A point mutation in *TCO2*. IGV, Integrative Genomics Viewer.

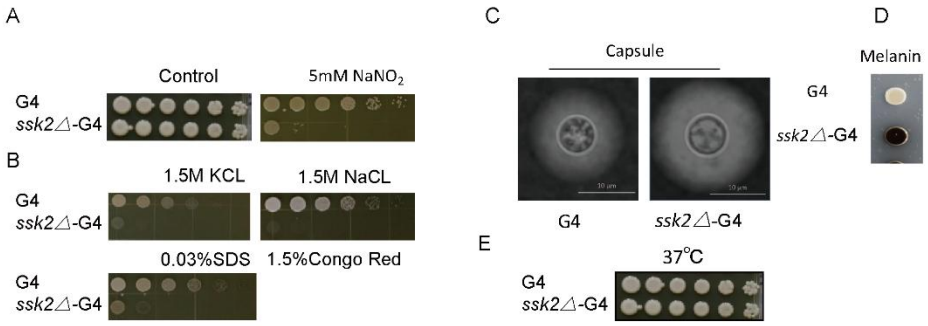
A



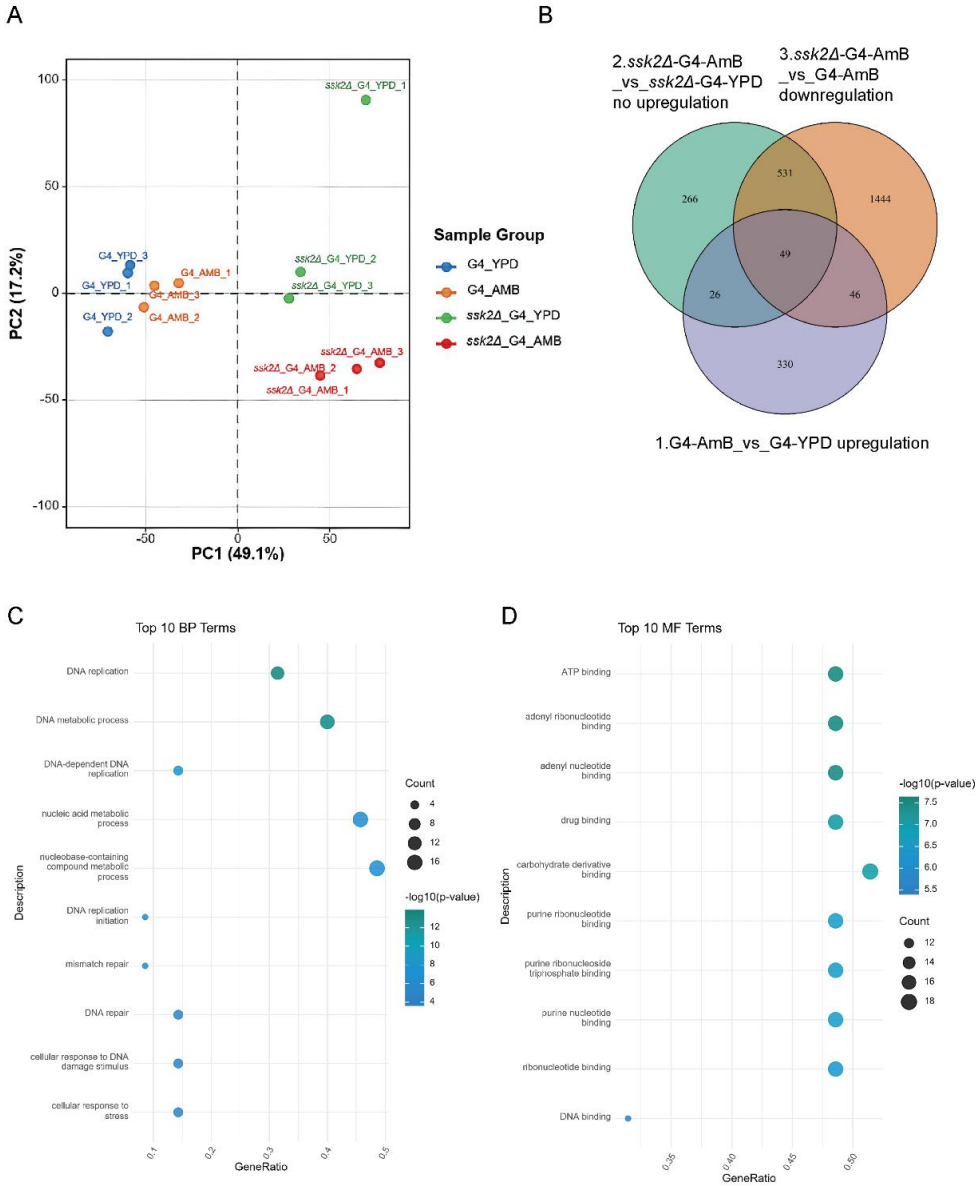
B



Supplementary Figure S2-2: The other 25 *Cryptococcus gattii* strains in IGV revealed no point mutations within the *SSK2* locus. With WM276 serving as the reference sequence, the blue boxes in the figure indicate the point mutations in *SSK2* identified in strain G4. No such mutations were observed at this locus in any of the other examined strains. IGV, Integrative Genomics Viewer.

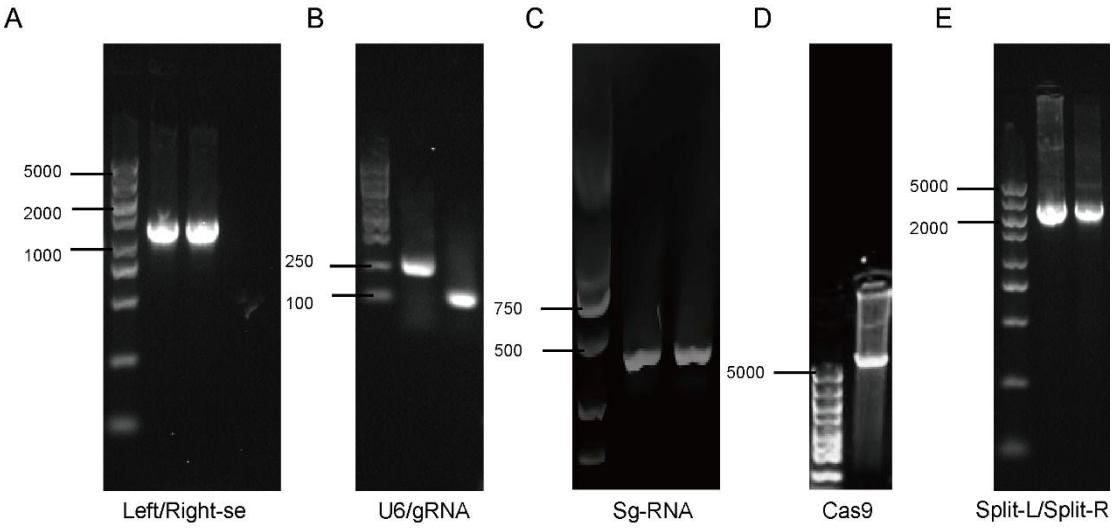


Supplementary Figure S3: Effect of *SSK2* deletion on *C. gattii* virulence factors. A: The antioxidant stress tolerance of *ssk2Δ*-G4 is reduced compared to G4. B: The resistance to cell wall osmotic pressure of *ssk2Δ*-G4 is reduced compared to G4. C: The amount of capsule produced by the *ssk2Δ*-G4 mutant strain was higher than that of G4. D: The G4 mutant strain exhibited increased melanin production compared to G4. E: Following *SSK2* deletion, the mutant strain showed no significant change in growth capacity at high temperatures compared to G4. Representative results from one of three independent experiments are shown.



Supplementary Figure S4: Effects of *SSK2* knockout on expression of downstream genes. A: Principal component analysis (PCA) of transcriptomes from G4 and *ssk2Δ*-G4 mutant strains under AmB treatment. PCA was performed based on the normalized counts of all differentially expressed genes. Projection of samples onto PC1 (49.1%) and PC2 (17.2%) revealed that, regardless of AmB treatment (distinguished by shape), the transcriptional profiles of G4 and *ssk2Δ*-G4 formed distinct clusters along the PC1 axis, while biological replicates (n = 3) within the same strain clustered closely together. B: The three groups are as follows: 1. (G4-AmB vs. G4-YPD), 2. (*ssk2Δ*-G4-AmB vs. *ssk2Δ*-G4-YPD), and 3. (G4-AmB vs. *ssk2Δ*-G4-AmB), comprising differentially expressed genes ($|\log_2 FC| > 2$, $Padj < 0.05$). The intersection of these three sets (49 genes) was identified as *SSK2*-dependent AmB stress core response genes for subsequent Gene Ontology enrichment analysis

(panels C and D). C: AmB stress core response genes essential for *SSK2* primarily affect the processes related to DNA replication and metabolism. D: The AmB stress core response genes, which *SSK2* depends on, primarily influence molecular functions such as drug and ATP binding. AmB, amphotericin B; log_2FC, log_2 fold change; *P*adj, adjusted *P* value; PC, principal component; YPD, yeast extract peptone dextrose medium.



Supplementary Figure S5: Gel electrophoresis of amplified fragments from the *TCO2* gene knockout process. A: Amplified homologous arms on both sides, approximately 1.2 kb. B: Amplified U6 and gRNA, approximately 200 bp and 100 bp, respectively. C: Amplified full-length sgRNA, approximately 400 bp. D: Amplified full-length Cas9, approximately 9 kb. E: Amplified Split-L and Split-R, approximately 3,000 bp. bp, base pairs; gRNA, guide RNA; kb, kilobase pairs; sgRNA, single-guide RNA.

Supplementary table S1

Supplementary Table S1 MIC of AmB against clinical *C. gattii* isolates

Strain	ID	Molecular Type	MIC （μg/mL）	Classification (WT/NWT)
C. gattii	WM276	VG Ⅰ	0.5	WT
	G3		0.25	NWT
	G5		0.25	NWT
	G7		0.25	NWT
	G9		0.5	NWT
	G16		0.25	NWT
	G18		0.5	NWT
	G23		0.5	NWT
	G25		0.5	NWT

	G27		0.5	NWT
	G28		0.5	NWT
	G29		0.5	NWT
	G2		0.25	NWT
	G4		1	NWT
	G8		0.5	NWT
	G12		0.25	NWT
	G15		0.5	NWT
	G19		0.5	NWT
	G20		0.5	NWT
	G24		0.5	NWT
	G21		0.5	NWT
	R272	VG Ⅱ	0.25	WT
	R265		0.5	WT
	G1		0.5	NWT
	G6		0.25	NWT
	G10		0.5	NWT
	G13		0.5	NWT
	G14		0.5	NWT
	G17		0.5	NWT
	G26		0.5	NWT

Note. *C. gattii*, *Cryptococcus gattii*; MIC, minimal inhibit concentration; WT, wild-type; NWT, Non-wild-type.