Table S1: Strains used in this study

Deletions	Resistance marker	Strain background	Strain designation	Source
cda1Δ	HYGR	KN99	LBCN369	Baker et al., 2007
cda1Δcda2Δ:A	HygR/NatR	*cda1Δ*	LBCN401	Baker et al., 2007
cda1Δcda2Δ:B	HygR/NatR	*cda1Δ*	LBCN635	Baker et al., 2007
cda1Δcda3Δ:A	HygR/PheR	F1 *cda1Δ* X *cda3Δ*	LBCN496	Baker et al., 2007
cda1Δcda3Δ:B	HygR/PheR	F1 *cda1Δ* X *cda3Δ*	LBCN525	Baker et al., 2007
cda2Δcda3Δ:A	NatR/PheR	*cda3Δ*	LBCN458	Baker et al., 2007
cda2Δcda3Δ:B	PheR/G418R	*cda3Δ*	NGCN927	Baker et al., 2007
cda1Δcda2Δ::*CDA2*	HygR/G418R	*cda1Δcda2Δ:*A	JLCN952	This study

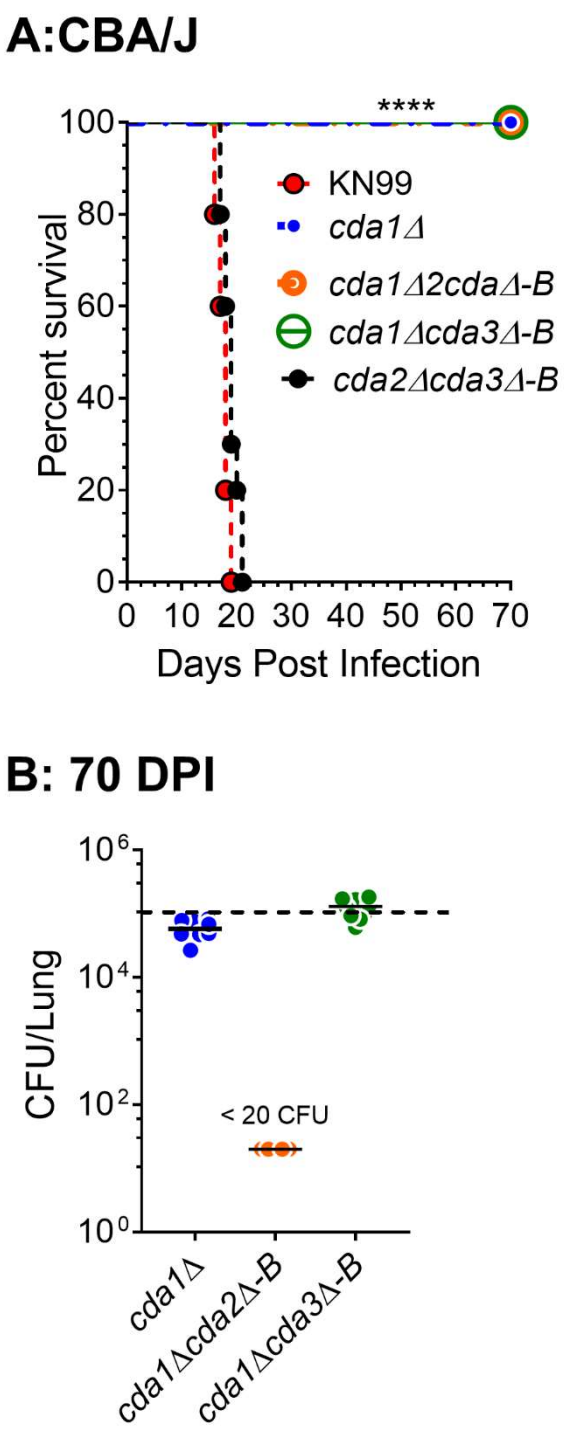

Fig. S1: **Deletion of *C. neoformans CDA2* or *CDA3* in conjunction with *CDA1* significantly attenuates fungal virulence in CBA/J mice**. **A**: Survival curves of mice infected intranasally with an inoculum equivalent to 10^5 CFU of each strain (isolate B). Ten mice (6 to 8 weeks old, female) were used for each group and the data is a representative of two independent experiments. ****, $P<0.0001$. Statistical analysis of survival rates was determined by log rank (Mantel-Cox) test. **B**: A solid line represents the median CFU per lung for each group. Each datum point represents one mouse's CFU ($n=10$). Lower limit of the detection is 20 CFU/lung. The dotted line represents CFU of the inoculum dose.

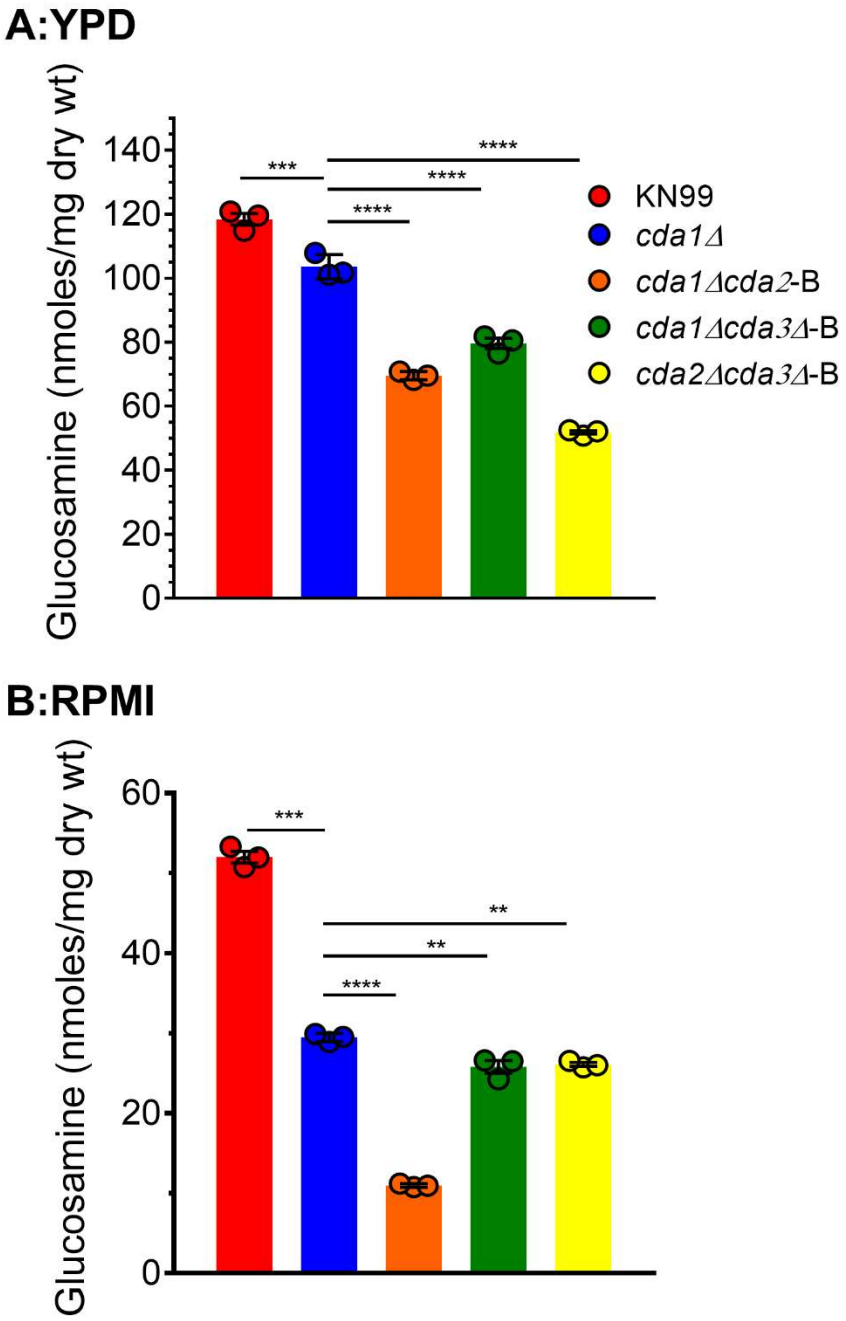

Fig. S2: **Deletion of *CDA2* in conjunction with *CDA1* further restricts chitosan production of *C. neoformans***. Quantitative determination of chitosan by the MBTH assay extracted from the cell walls of wild-type KN99 and various double CDA deletion strains. **A:** Cells were grown in YPD for 48 h collected, washed and used for the assay. **B**: Strains were initially grown in YPD for 36 h, collected, washed and inoculated at a cell density of 5×10^5 cells/mL into RPMI medium containing 10% FBS and incubated for 5 days at 37°C/5% CO_2. Cells were collected, washed and used for chitosan measurement. Data is the mean of three biological and two technical replicates (*n*=3). Significant differences between the groups were compared by one-way ANOVA followed by Bonferroni's multiple comparisons test. (****=p<0.0001 comparing chitosan amount of each strain to *cda1*Δ). Error bars represent standard errors of the mean.