Supplementary Figures for:

Similar evolutionary trajectories in an environmental Cryptococcus neoformans isolate after human and murine infection

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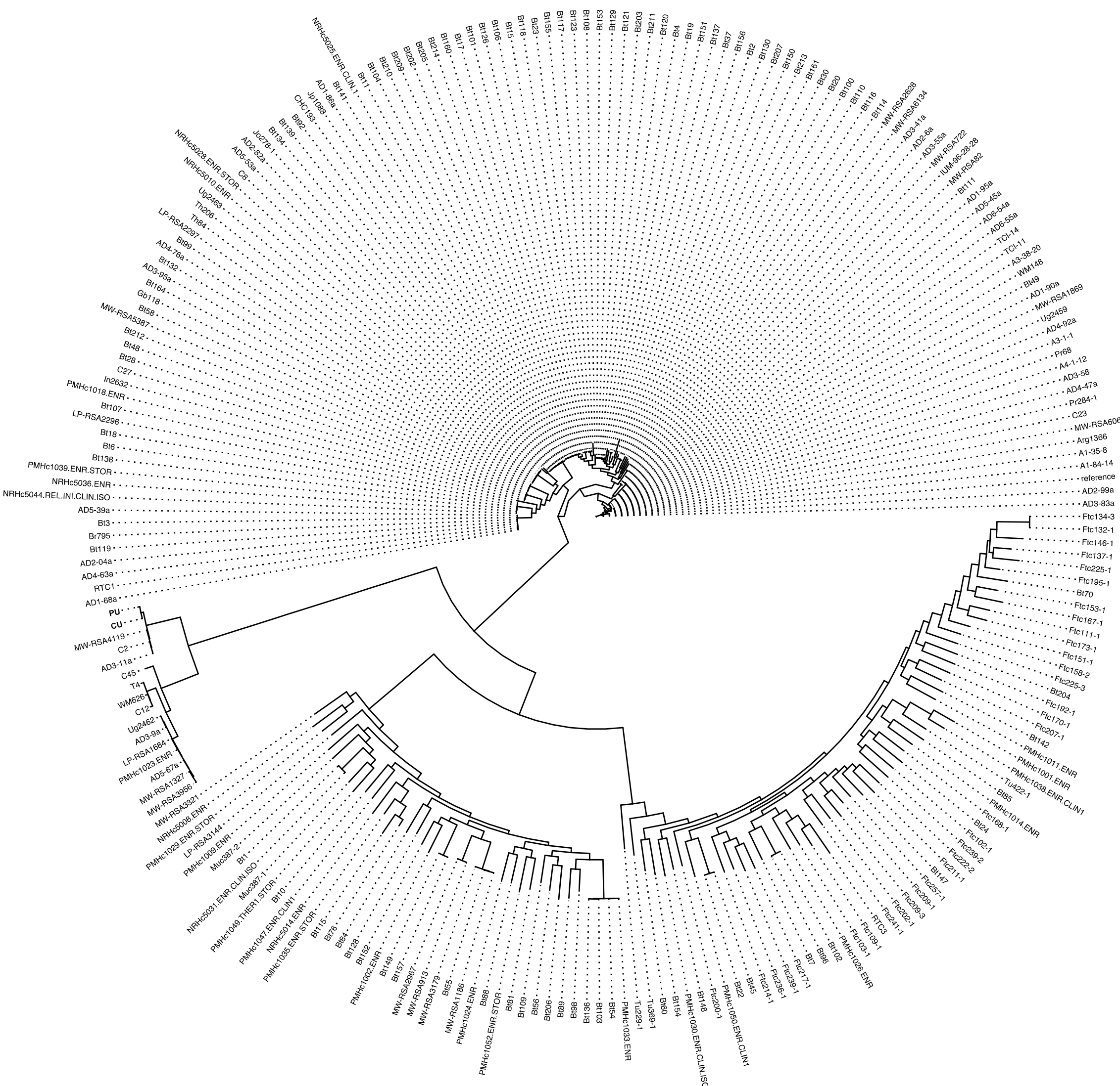
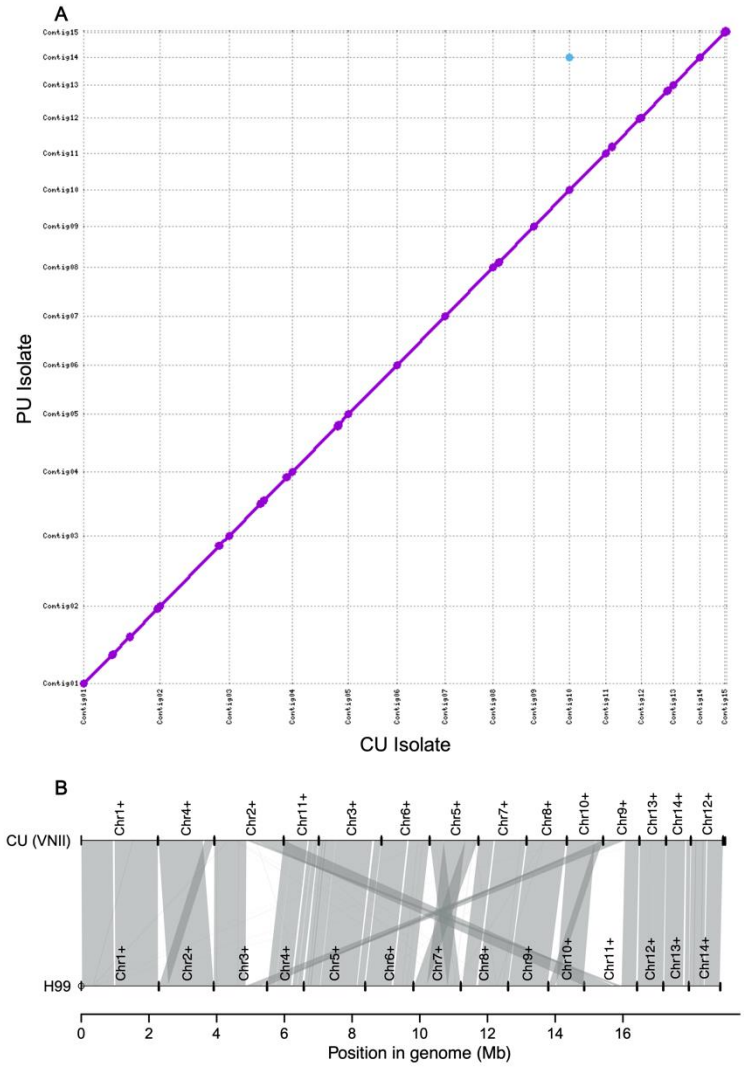
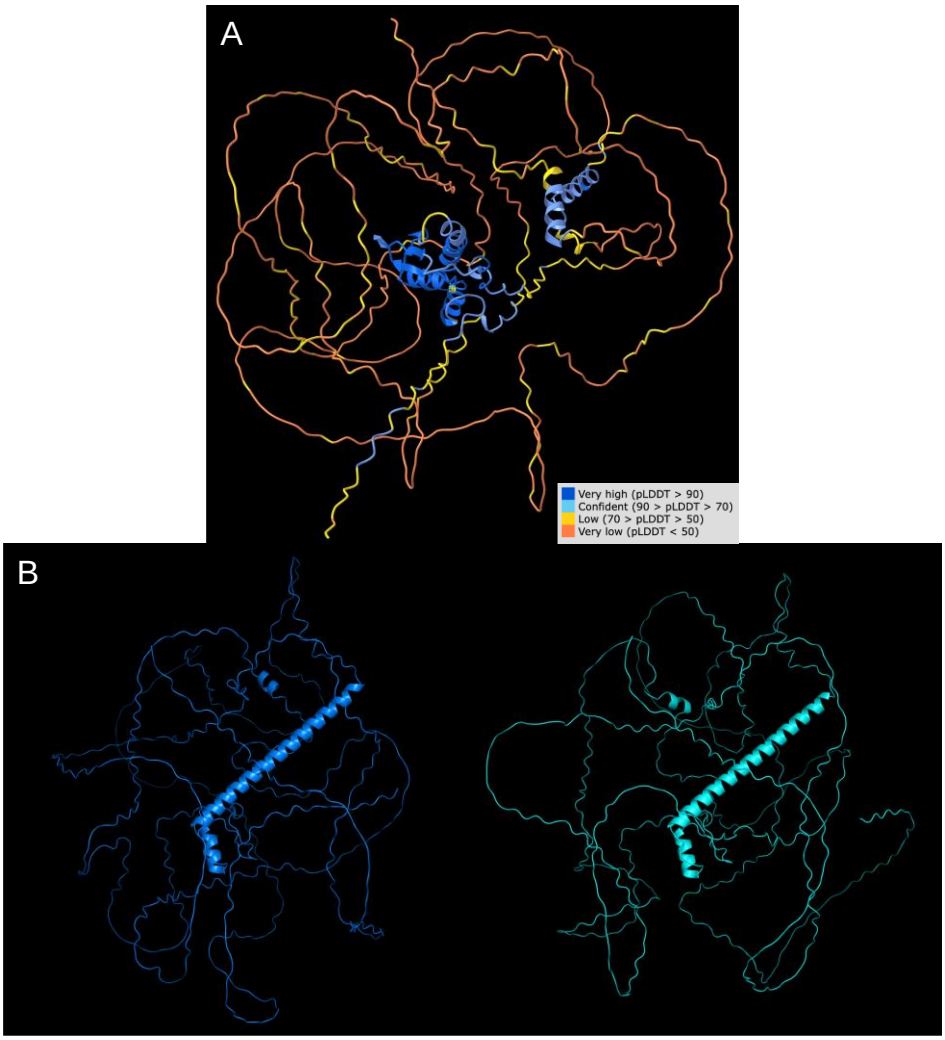


Supplementary Figure 1. Cockatoo and patient strain phylogeny. Maximum likelihood phylogeny of Desjardins et al. (13) isolates with the cockatoo (CU) and patient (PU) strains represented within the VNII lineage. All lineages show 100% bootstrap support.



Supplementary Figure 2. Synteny between genome assemblies. (A) CU and PU genome assembly alignments, with sequences aligning in the forward orientation in purple, and sequences aligning in the reverse orientation in blue. The single blue alignment is a small repetitive sequence in the telomeres of chromosomes 10 and 13. (B) Synteny between CU and *C. neoformans* H99 genome assemblies.



C

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1   MAGSETSSPTDFAFEPPALPSGDAAFTCTPYKPEPLKPTFASPTGLSPPTGSFTPAANAA
61  PGHSYFPDSYNYGVSSASSFSSASASATGGSFSSVDNLPGISKNFVRPNLADTRRPATAG
121 GALQSRSPYSNFMMSSKNGMSGSMSQDAQKLGERGDMRRPEGTIEEGNEMLGLGGSTEED
181 GDEQGPQNVSPENENTVNPTLFPANRRSSAPQYNIPGSNWGQFPPSSSHDASNSSLGLPS
241 APAHMPMGYLQQQQAAHHNLNTQNRPPAFAGRPQTSDGLPSYTNYHGAVTLPSAQSIARQ
301 IPGMTDTTSFFHHSSGSAHLPFRDDRHFPSTNFPGDRAFTFDPLRSSLPPSFPGQIRASY
361 APPPPVPTAGSDSGGSSNNTNSTNDQMQFMSLSTGHGQKKRPRRRYEEIERLYPCGWNGC
421 EKSYGTLNHLNAHVMTQKHGEKRLPAEFKEMRKAWRKKKREHASAQASSQYMTNAAAWQQ
481 NYQRLSFASTSSAPDSDWDRRESSTSTVSVSTDGRPSFSYPTNYSWGVPPQGMPFQAGMM
541 SVDSRPSTSSSNVSSVSMDGTFISNQPTSASTSVMSMGPPTYLYLSGPPSLPGGVVSRRP
601 SAPQHVPMPPSNPVDGFRPADGDHPTPTMGNPFPTGPSGAGVVAGAGAGAGAAGQAGKAL
661 NFSTLTSPMKGSSGDFGSQFAFTR
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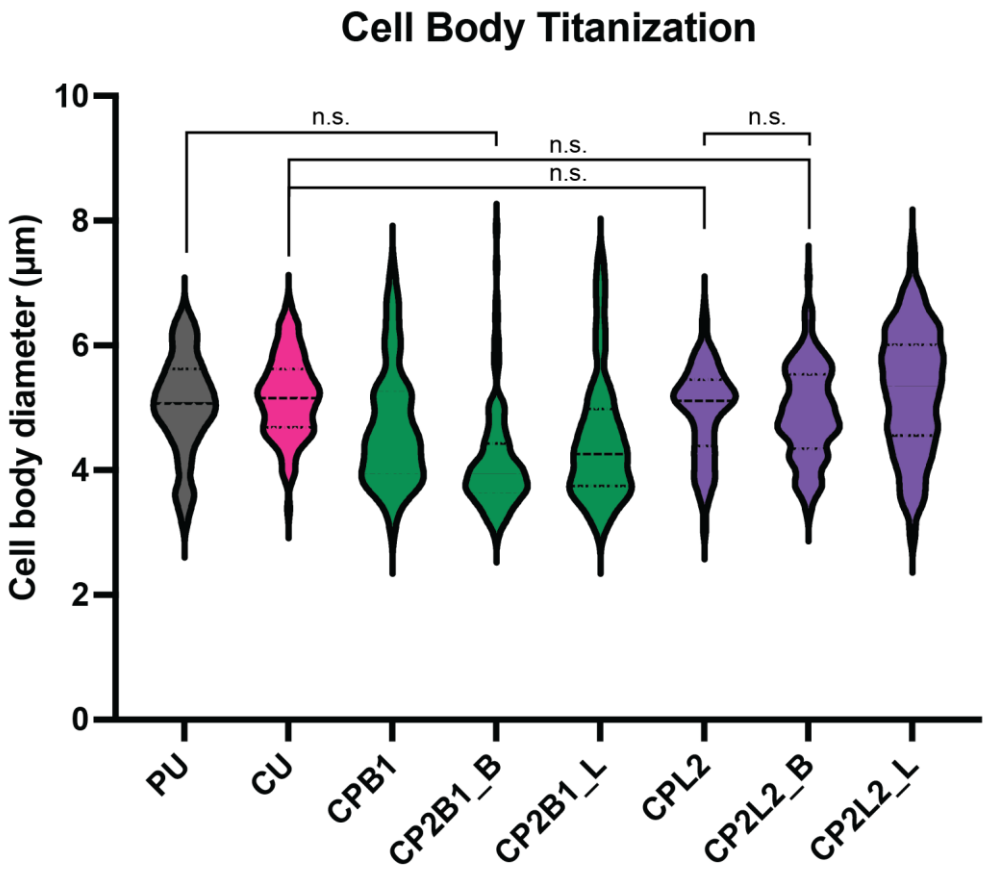
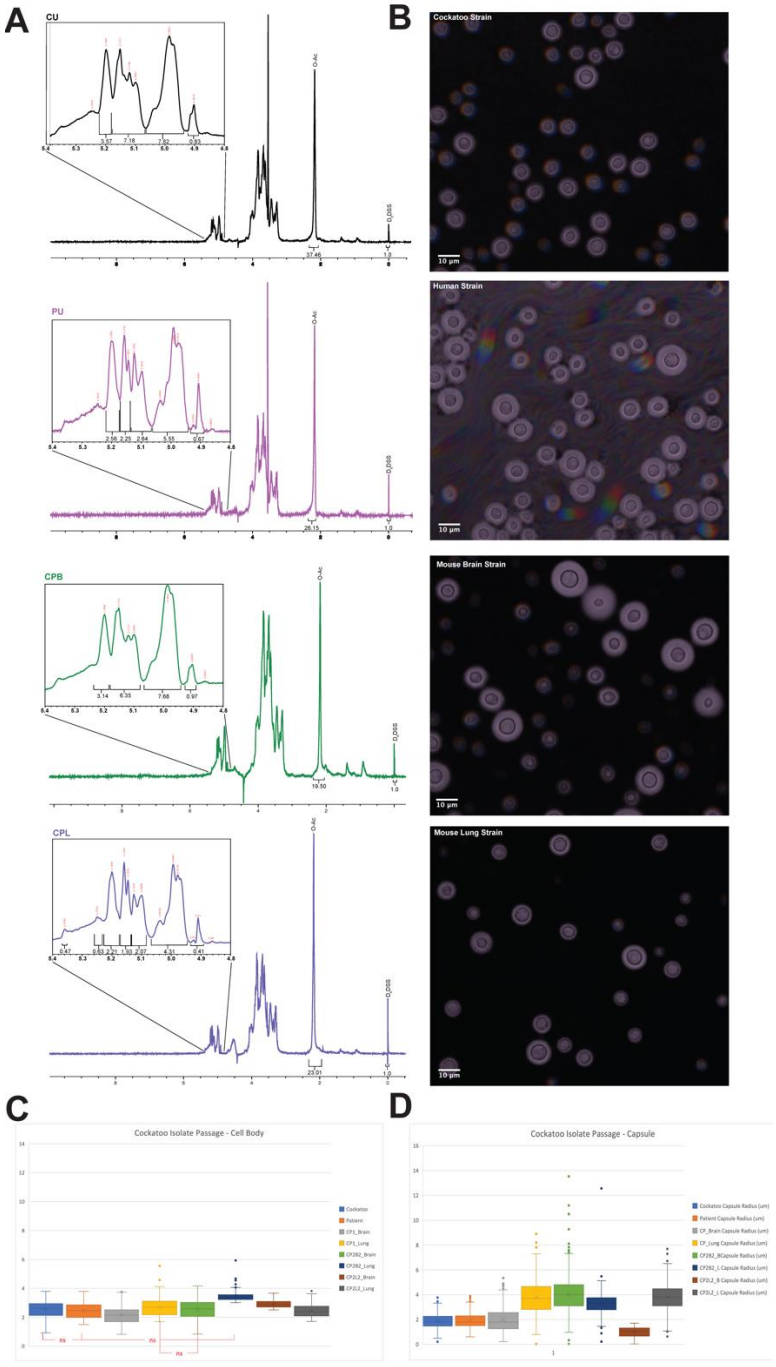
Supplementary Figure 3. Predicted structures for LQVO5_000317 and LQVO5_004463. (A) Structure of LQVO5_000317, as predicted by AlphaFold2, with prediction confidence indicated by ribbon color. (B) Structure of CNAG_05940 (left) and the extended LQVO5_004463 (right) protein, as predicted by AlphaFold2. (C) CNAG_05940 protein sequence with intrinsically disordered region's (>40 residues) underlined, Ser/Thr regions highlighted and colored by predicted glycosylation state

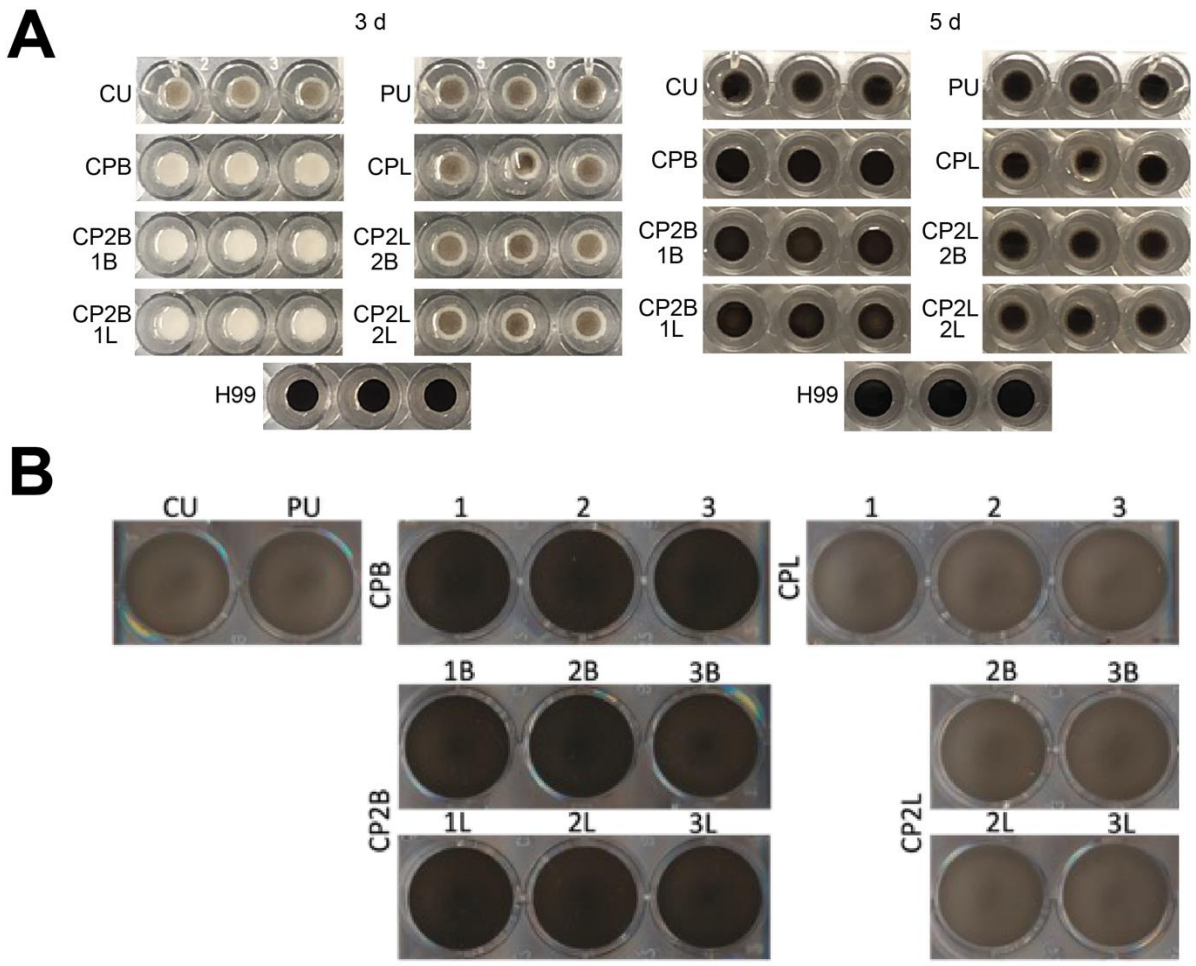
(red = glycosylated, green = un-glycosylated), and residues which differ in the extended LQVO5_004463 protein bolded (P>S and A>T).



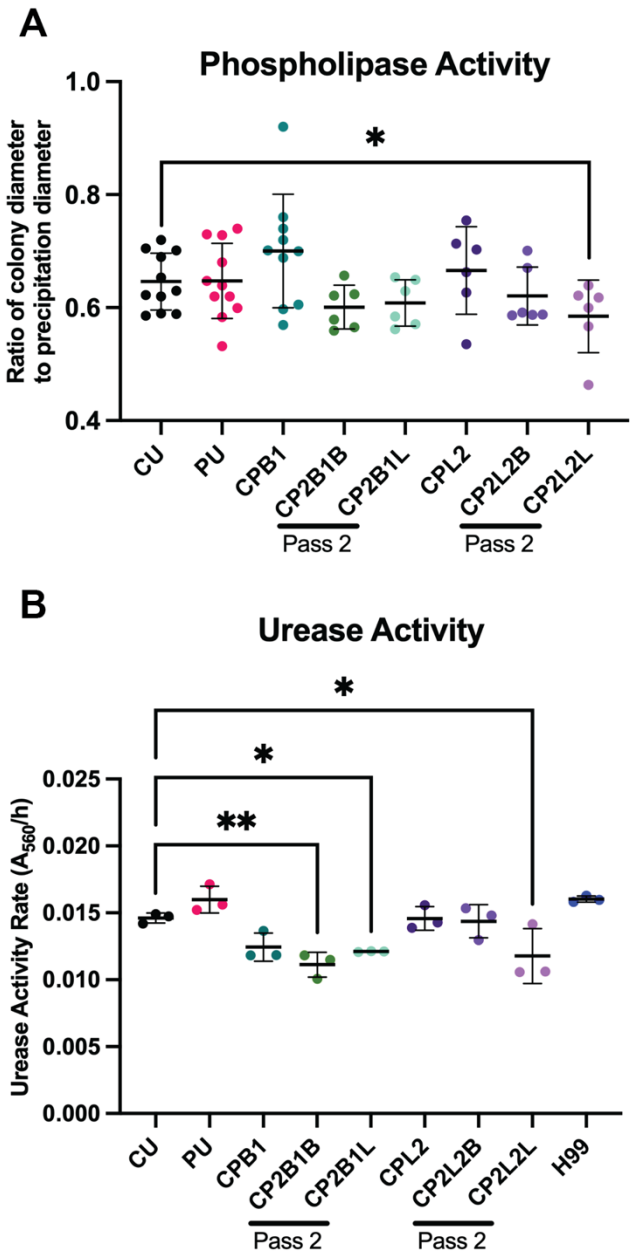
Supplementary Figure 4. Cell body measurements of strains and isolates after growth in titan cell inducing media. Quantitative capsule analysis (QCA) of CU, PU strains and mouse-passaged isolates for cell body diameter after growth in titan cell inducing media. Difference between cell body sizes is statistically significant (Tukey's multiple comparisons test, p-value < 0.05) unless indicated (ns).



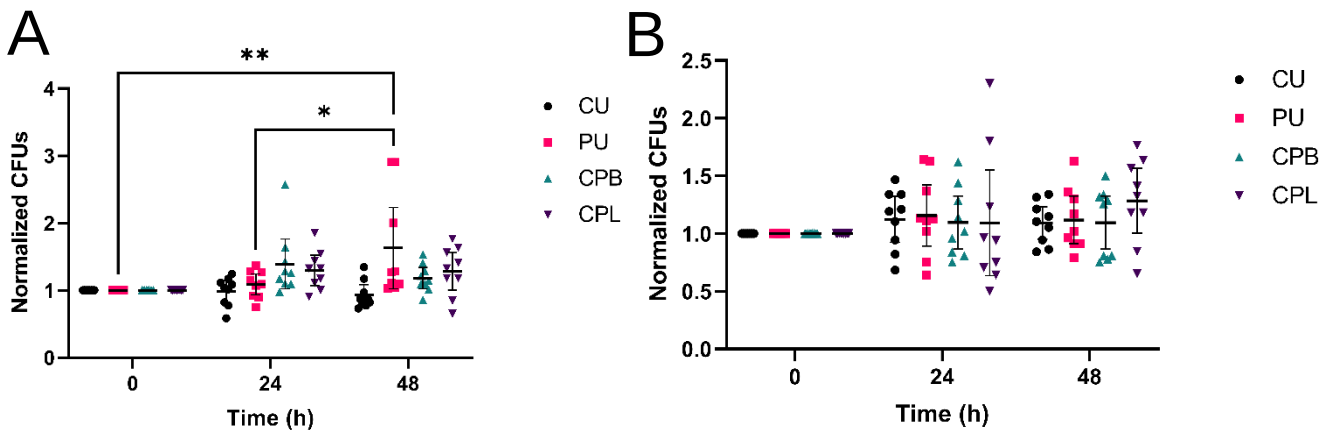
Supplementary Figure 5. Extended data for Capsular Characterization of Cockatoo, Human, and Mouse passage isolates. (A) 1D [^{1}H] NMR analysis of EPS isolated from Cockatoo and derivative strains. Integration of peaks and comparison to internal standard (d_6-DSS) shows variation in polysaccharide O-acetylation. (B) India Ink microscopy images of Cockatoo and derivative strains. (C, D) Quantitative capsule analysis (QCA) of cockatoo, patient, and mouse passage strains. Difference between sizes is statistically significant (Tukey's multiple comparisons test, p-value < 0.05) unless indicated (ns). Both cell body (C) and capsule radius (D) were compared.



Supplementary Figure 6. **Extended data for melanization.**
A) Melanization for each strain or isolate on solid agar. Each sample was run in triplicate, with the lab *C. neoformans* strain, H99, shown as reference. Melanization progress after 3 and 5 d are displayed on the left and right, respectively. B) Melanization in liquid media. Representative images of pigment production are displayed for cockatoo (CU), human (PU), first and second passage mouse isolates from brain (CPB, CP2B) and lung (CPL, CP2L). The mouse-derived brain isolates show different rates of pigment production compared to the original strain and other isolates, as melanization was faster in liquid media but slower on solid agar.



Supplementary Figure 7. Phospholipase and urease activity levels for passaged and unpassaged strains. (A) *C. neoformans* were inoculated onto egg yolk agar and incubated at 30˚C. After 72h of incubation phospholipase production was analyzed by measuring the ratio of colony diameter to precipitate + colony diameter on the plate, where a ratio value equal to 1.0 indicates a lack of phospholipase activity. (B) Urease activity for the indicated strains of *C. neoformans* grown at 30˚C in urea broth. Increased pH of culture media that results from the conversion of urea to ammonium was quantified by measuring the absorbance of cell culture media at 560 nm relative to a cell-free control.



Supplementary Figure 8. **Extended data for amoeba predation.** Normalized CFU counts of the four strains of *C. neoformans* following co-culture with (A) *A. castellanii* in A.c. buffer or (B) culture in A.c. buffer alone for 0, 24, and 48 hours. Data were normalized to the average CFU count at 0 hours for each of the three biological replicates for each strain. Bars represent the mean normalized CFU count with 95% confidence interval. n = 9 for all strains. *$p < 0.05$, **$p = 0.0039$.

Table S1. Fungal burden in mouse organs after each passage. Fungal burdens are reported as CFU/mg of organ weight.

Passage 1											
Brain						**Lung**					
CPB						*CPL*					
820						79					
Passage 2											
P1 Brain						**P1 Lungs**					
CP2B1_B	*CP2B1_L*	*CP2B2_B*	*CP2B2_L*	*CP2B3_B*	*CP2B3_L*	*CP2L1_B*	*CP2L1_L*	*CP2L2_B*	*CP2L2_L*	*CP2L3_B*	*CP2L3_L*
2191	11861	1786	2780	871	4762	-	-	0.3	1	2	10