Supplementary Infomation

Table 1. Primers used to PCR amplify the T-DNA insertion into *C. neoformans* genome (MAV30-MAV33) and to generate the fusion PCR to delete the *URA4* gene using the Geneticin-resistance gene as marker.

Name	Sequence	Reference
MAV30	5' GTAAAACGACGGCCAG 3'	Idnurm *et al.* (2004).
MAV31	5' CAGGAAACAGCTATGAC 3'	
MAV32	5' AACAGTTGCGCAGCCTGAATG 3'	
MAV33	5' AGAGGCGGTTTGCGTATTGG 3'	
MAV067	5' GTGATCAGTGCATTGCATGA 3'	deletion construct
MAV070	5’ CGGAGATGTTCGAGGAAGTGAGAAGAGATGTAGAAACTAGCTTCC 3’	
MAV072	5' TTATTGAGCAATTTCCCAACC 3'	
MAV097	5' ACAGGAAACAGCTATGACATGCGTGATATATCTGTGTTCG 3'	
MAV098	5' CGAACACAGATATATCACGCATGTCATAGCTGTTTCCTGT 3'	
MAV099	5' TGGCACTGGCCGTCGTTTTACACAGGATATCTATATTGATAG 3'	

Table 2. Real time PCR primers:

Gene	Name	Sequence	Reference
URA1	MAV203	5' TGCAAGCAGAGCTGTTCGGC 3'	current work
	MAV204	5' GGTTACCAGGCTGGGGTTCG 3'	
URA2	MAV234	5' GATTGTGTCTAGATCTGACG 3'	
	MAV202	5' GCCATTCGGACGAAAAGACC 3'	
URA3	MAV199	5' TATGGTCAAGACGCACTGCG 3'	
	MAV200	5' AACCGTGTTGCCGATATCGG 3'	
URA4	MAV197	5' TCAAAGGTGTCAAGTCCTAC 3'	
	MAV198	5' TTGAGATGTTCTTGTGAGCG 3'	
URA5	MAV195	5' AAGTCCGGCCGTCAATCCCC 3'	
	MAV196	5' CCTCACCATGGTCCTTCTTC 3'	
URA6	MAV243	5' CTCAAGGAGCTTGATGGTAAC 3'	
	MAV244	5' GCACCTTTCCGCTGGTGACC 3'	
URK1	MAV189	5' GTCCGGAAAGACTTCTGTAG 3'	
	MAV190	5' TAGACCGGGATCTCTGTAGC 3'	
URH1	MAV191	5' TGCGTACTTTCATGGTCCAG 3'	
	MAV192	5' CGATGTTTGTCAATGGACCG 3'	
CDD1	MAV193	5' CAGTAATCCAGCTTATGCCC 3	
	MAV194	5' GGGAATATCTGACTGCCTTC 3'	
GPDH1	MAV240	5' AGTATGACTCCACACATGGTCG 3'	Varma *et al* (1999)
	MAV241	5' AGACAAACATCGGAGCATCAGC 3'	

S1

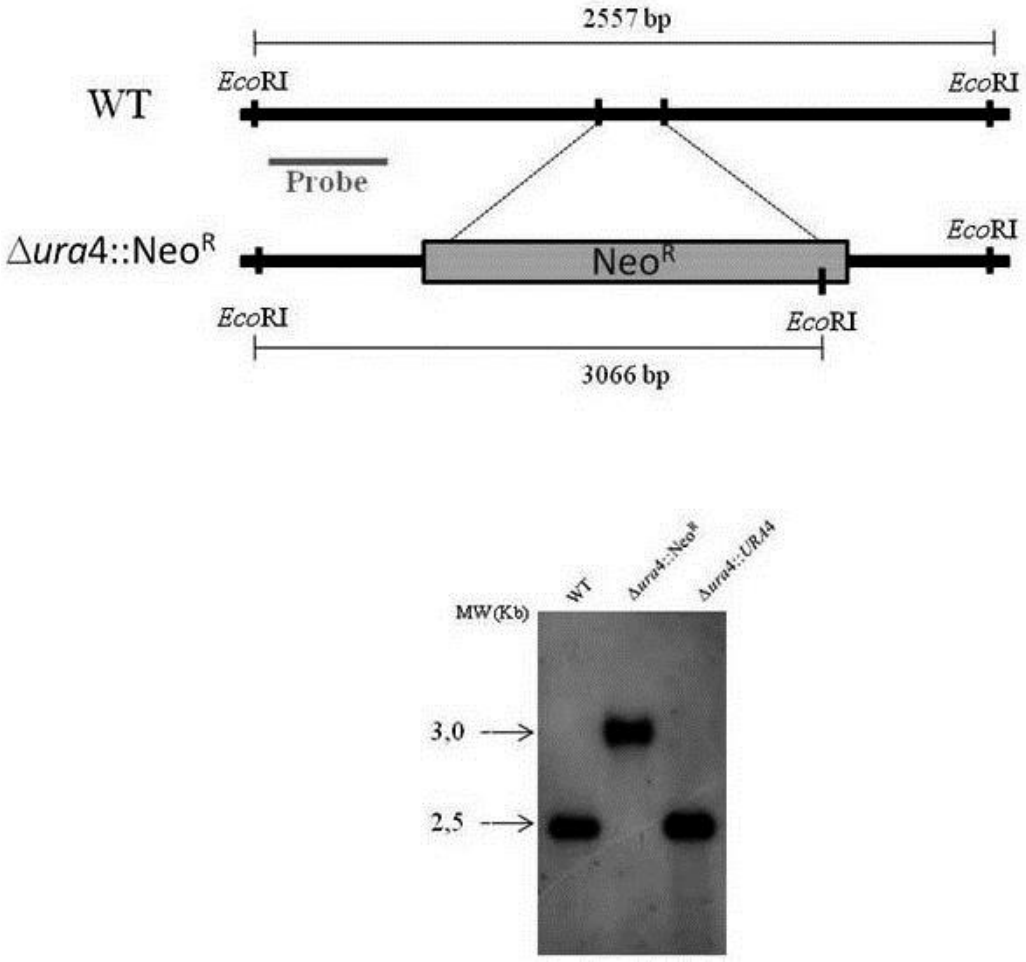

Figure S1: Deletion of *C. neoformans URA4* gene. (A) *Eco*RI restriction pattern of the wild type and *ura4 locus*. The orange box represents the G418 resistance gene (Neo^R) which replaced part of the ORF. The blue line represents the position of the probe used in the Southern blot. (B) Southern blot showing the bands which correlate to *Eco*RI restriction pattern: for wild type (KN99), *ura4*::Neo^R (FGC003) and reconstituted strain *ura4Δ*::*URA4* (FGC003R1). Numbers and arrow indicate the size and position of the bands. WT = wild type; MW = molecular weight in kilobases; bp= base pairs.

A

MV49 CHROMAT_ID=228877

194 aacatactaccccgtcgtaagggaatggaggaagaaggcatggtcgggaacatgcgcggagaagtccccag 118

117 tgacgctcacaaggtaaagaccactcaatttggagaatatgaagtacgtaaagatcatcagaagaacagaa 41

40 catctcaatcctcaatgccgaagttcacttcctcgaacatctccgtaactcg 1

B

atggaggaagaaggcatggtcgggaacatgcgcggagaagtccccagtgacgctcacaag

124 M E E E G M V G N M R G E V P S D A H K 143

aacatctcaatcctcaatgccgaagttcacttcctcgaacatctccgt

144 N I S I L N A E V H F L E H L R 159

Figure S2. Insertion mutagenesis locus. The neomycin resistance cassette interrupted the *C. neoformans URA4* gene neat the codon that encode the aminoacid argenine (R) at position 159. The sequenced region displayed represents the yielded sequence obtained from an inverted-PCR product from the locus where the cassette was inserted and it comprises an intron (shaded in gray) [A] that when spliced encoded the region represented in [B] from residue 124 to 159.

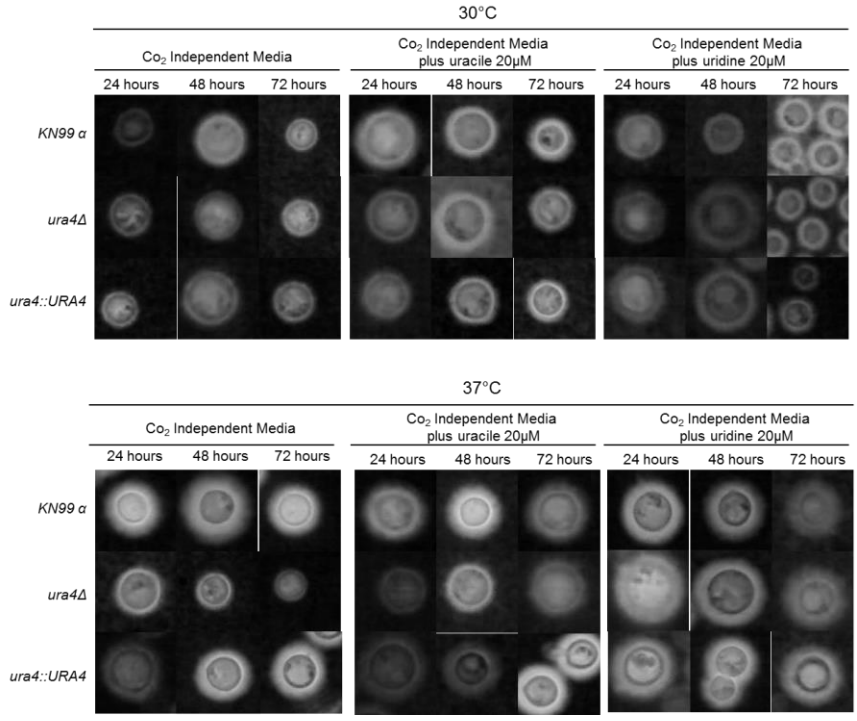

Figure S3 - Representative images for capsule production for *KN99α*, *ura4Δ* e *ura4+URA4* in CO2-indendent medium supplemented with uracil (20µM) or uridine (20 µM) at 30°C [A] and 37°C [B].

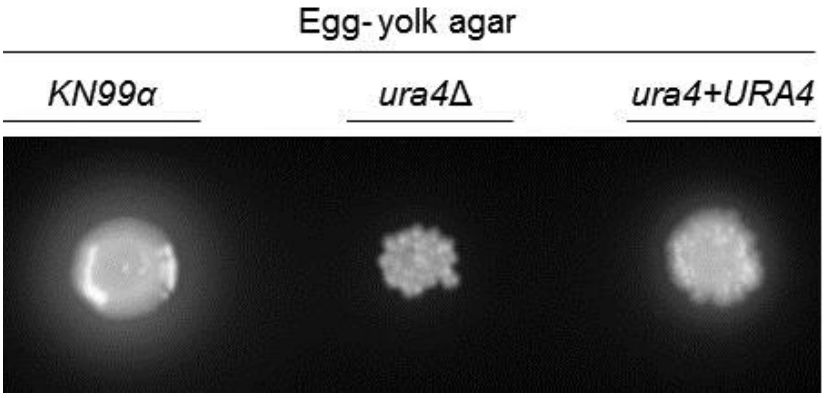

Figure S4. Extracellular phospholipase activity. Cultures were spotted on Egg-yolk agar plates and incubated at 30°C up to 72 hours. The halo observed around the colonies indicated the phospholipase activity and it was used to access the precipitation zone (Pz) on egg-yolk medium according to Price *et al.* (1982).