A

```
RPL22α  ATGGTGCGTTTTTATTTTTTCTTTTTGATTTTCTCGTATCCCATGTAATCCTGATTGCAAA  60
RPL22a  ATGGTGCGTTTC---------------CTTTTCTCATATCCCATGTAACCCTCATTGCAAC  46
        *** *******           . ****** ************ *** ******* .

RPL22α  CGTTTGCAACATTGGGTTGACTGGCTCTTGTCCTAATCTTACCCATTTCCGCTAATATGA  120
RPL22a  TTCTTGCAATTTTGGGTTGT--GGATGCTGCGCTAAATTTAGCCATTTCCGCTAACGTGT  104
        ****** :*******: **.* ** ****: *** ************* .**:

RPL22α  CTCGTTTTCAAACAGCCGAAAGCTCCCTCCACTACCAAGAACGCAGCCGCTGGCAAGCCT  180
RPL22a  CTTGTGTTCAAACAGCCGAAAGCTCCCTCCGCCGCTAAGAACGCTGCCTCCGGCAAGCCT  164
        ** ** ********* **************  .* .* ********:***.* ********

RPL22α  CTCCACAAGTTCTTCGTTGACTGCTCCGTCCCCGTGAACGATTCTGTCTTCGACCTTGCC  240
RPL22a  CTTCACAAGTTCTACGTCGACTGCTCCGTTCCCGTGAACGATTCTGTCTTCGACCTCGCC  224
        ** ********* . * ** ********* ************************** ***

RPL22α  GCGTTTGAGAAGTTCCTCCACGACCGCATCAAGGTCGACGGCAAGCCTGGCCAGCTCGGC  300
RPL22a  GCTTTTGAGAAGTTTCTCCACGACCGCGTCAAGGTTGACGGCAAGCCCGGCCAGCTCGGC  284
        ** ********** *********** .*****  ** ************ *** ***********

RPL22α  GACGTTGTCGCTGTCCAGAAGGAGGGTGAGTGTGCCCAAAAAATCACTATTTGAATAATG  360
RPL22a  GATGTCGTCGCTGTCCAGAAGGAGGGTCAGTACGCCCGAAAAGTTAGTAGATGAGTTGTG  344
        ** ** ******************* ** ***. **** .**** .* * ** :***.*:. *

RPL22α  TTCTGATGGTCGTTGCACAGGTGCCAAGATCGTCCTCACTTCCCAAATCCCATTCTCCAA  420
RPL22a  TTCTGATGGTTGTTTCACAGGTGCCAAGATTGTCCTTACCTCTCAAATCCCCTTCTCCAA  404
        ********** *** .*************** ***** ** ** ******** .*******

RPL22α  GAGGTACCTCAAGTACCTTACGAAGAAGCACTTGAAGAAGAACTCTTTTGAAAACTTCCT  480
RPL22a  GAGGTATCTTAAGTACCTTACCAAGAAACACTTGAAAAAGAACTCTTTCGAGAACTTCCT  464
        ****** ** *********** *****.*******.*********** ** .********

RPL22α  CCGGTTAGTCATGTGTAACACCGCTGATATTATATATTAACATTTCTACAGCGTCGTTGC  540
RPL22a  CCGGTTAGTAACGTGTAGATACATTGATACTATATACTGATTGCCTCATAGTGTCGTTGC  524
        ********* .* *****..:.*. ***** ****** *.* : * ** ********

RPL22α  CACTTCCAAGGACACCTATTCCCTCAAGTACTTCAAGGTTGATCAGGATGAAGCTGAGGA  600
RPL22a  CACTTCCAAGGACACTTATTCCCTCAAATACTTCAAGGTTGACCAAGATGAAGCTGAGGA  584
        *************** *********** .************** **.**************

RPL22α  GGATGAACTCGCTTAA 616
RPL22a  GGATGAACTCGCCTAA 600
        ************ ***
```

B

```
        1        10        20        30        40        50        60
Rpl22α  MPKAPSTTKNAAAGKPLHKFFVDCSVPVNDSVFDLAAFEKFLHDRIKVDGKPGQLGDVVA
Rpl22a  MPKAPSAAKNAASGKPLHKFYVDCSVPVNDSVFDLAAFEKFLHDRVKVDGKPGQLGDVVA
        ******::****:.*******:***************************.**********
```

Figure S1. Alignment between *C. neoformans RPL22*α and *RPL22***a.** DNA sequence (A), and Rpl22α and Rpl22**a** proteins (B). In A introns are highlighted in red, and nucleotide changes that result in different amino acids between the Rpl22α and Rpl22**a** proteins are color coded with the corresponding amino acids boxed and highlighted in the same color in B. In B only the initial 60 amino acids are shown. Both in A and B, an asterisk (*) indicates positions which have a single, fully conserved residue, a colon (:) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix, and a period (.) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.
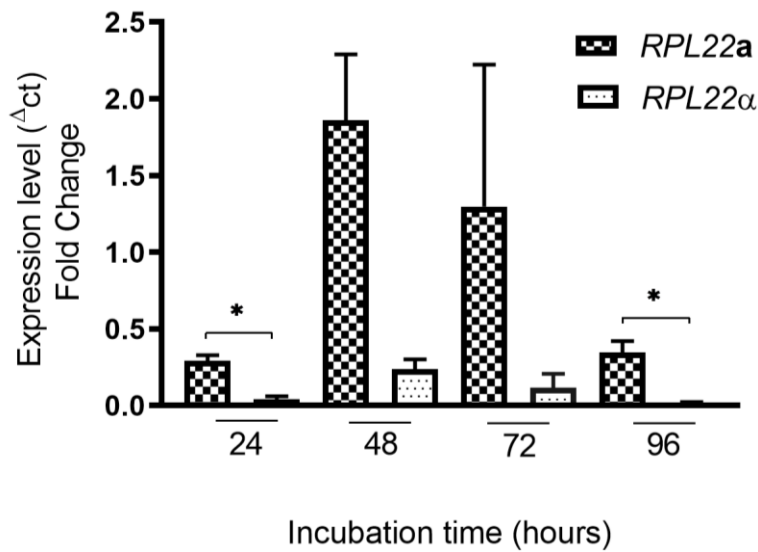
Figure S2 Expression of *RPL22*α and *RPL22***a** during vegetative growth of *C. neoformans.* WT strains H99α and KN99**a** were grown in YPD media for 24, 48, 72 and 96 h. Ct values were converted to expression level (fold change) through comparison with the endogenous reference *GDP1* ($^\Delta$ct analysis). The data presented in this supplemental figure were used as comparative condition to calculate the expression levels of *RPL22*α and *RPL22***a** during H99α x KN99**a** mating of Figure 4A. Asterisk indicates $p < 0.05$ for each *RPL22*α and *RPL22***a** comparison for the same day of incubation.
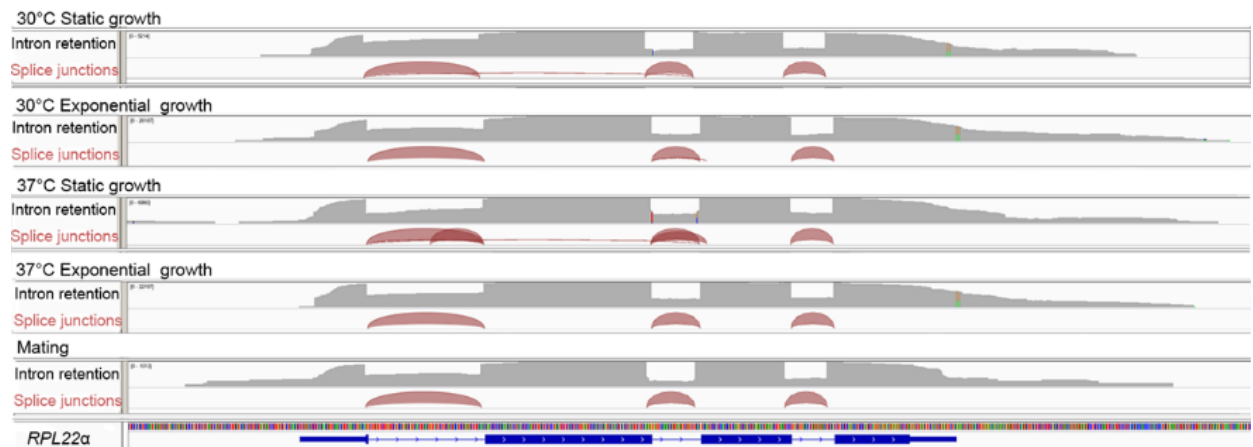
Figure S3. Intron retention and splice junction analysis for *RPL22*α following growth in several conditions. High retention of intron 1 at 30°C static growth. Detected alternative splicing at intron 1 and 2 at 37°C static growth.
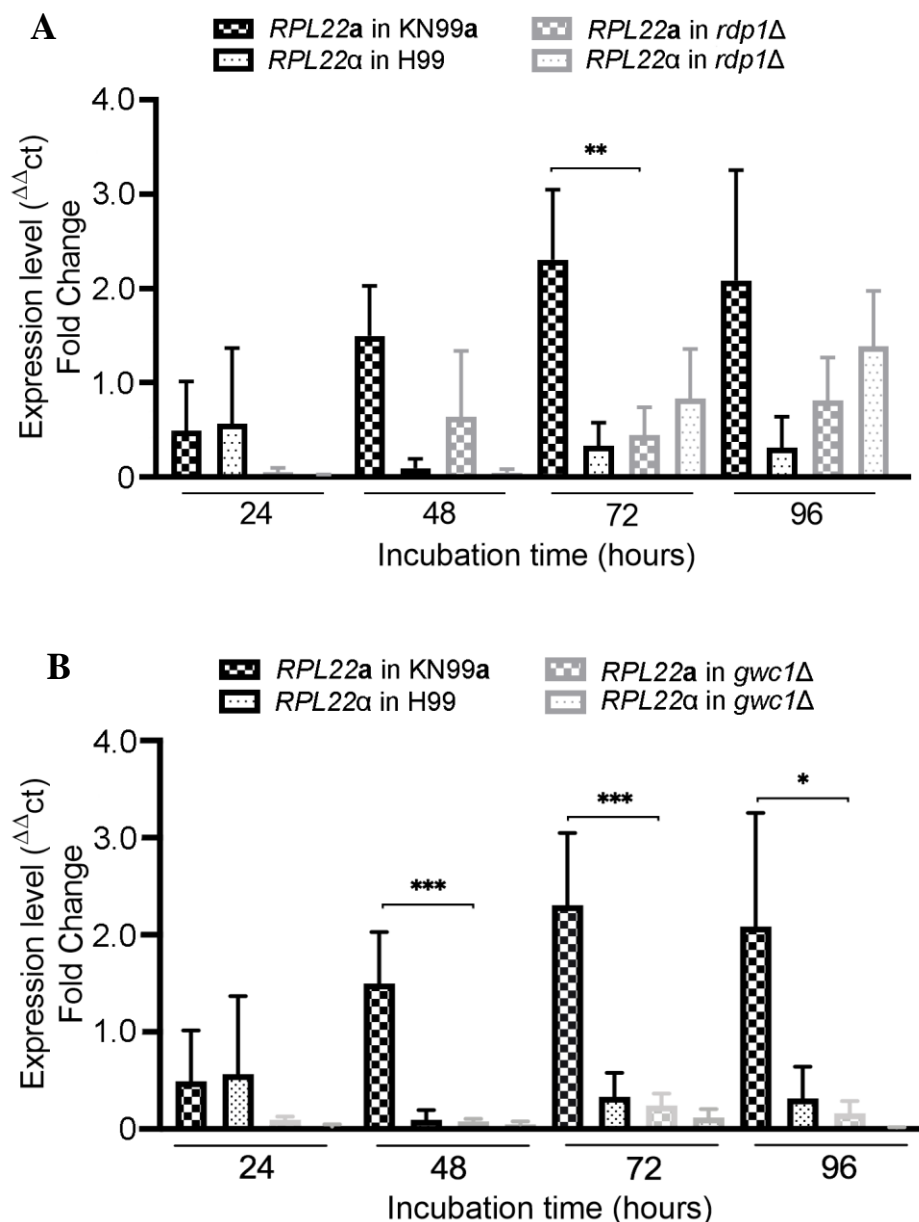
Figure S4. Comparison of the expression levels of *RPL22*α and *RPL22***a** in WT and RNAi mutants. RT-qPCR data from Figures 4A, 4C and 4D, were replotted to allow direct comparison of the *RPL22*α and *RPL22***a** expression levels during WT H99α x KN99**a** cross compared to *rdp1*Δ (A) and *gwc1*Δ (B) bilateral crosses for 24, 48, 72 and 96 h of incubation. Statistical analysis was carried out using one-way ANOVA with Tukey's multiple comparison test. Because on these comparisons we were interested in monitoring the changes in gene

expression following genetic manipulation, we display only statistically significant differences (* for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$) of the expression levels of the same gene for the same day of incubation in separate mating reactions.
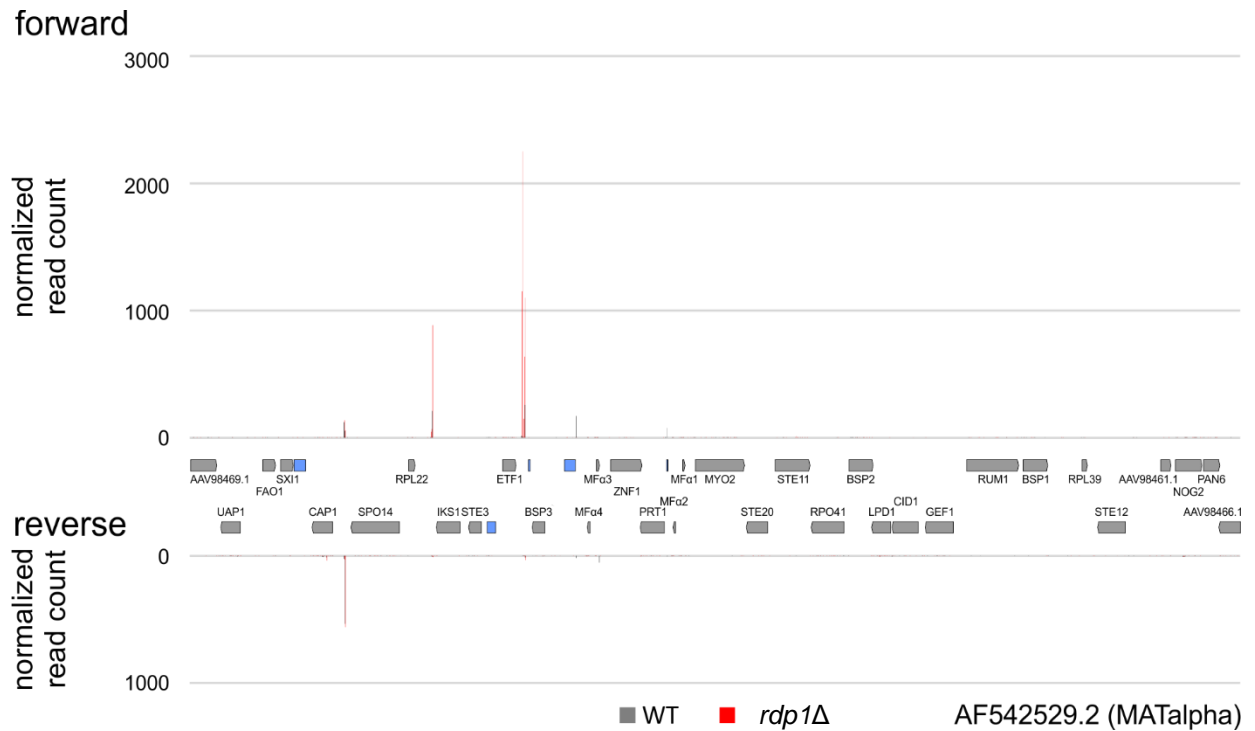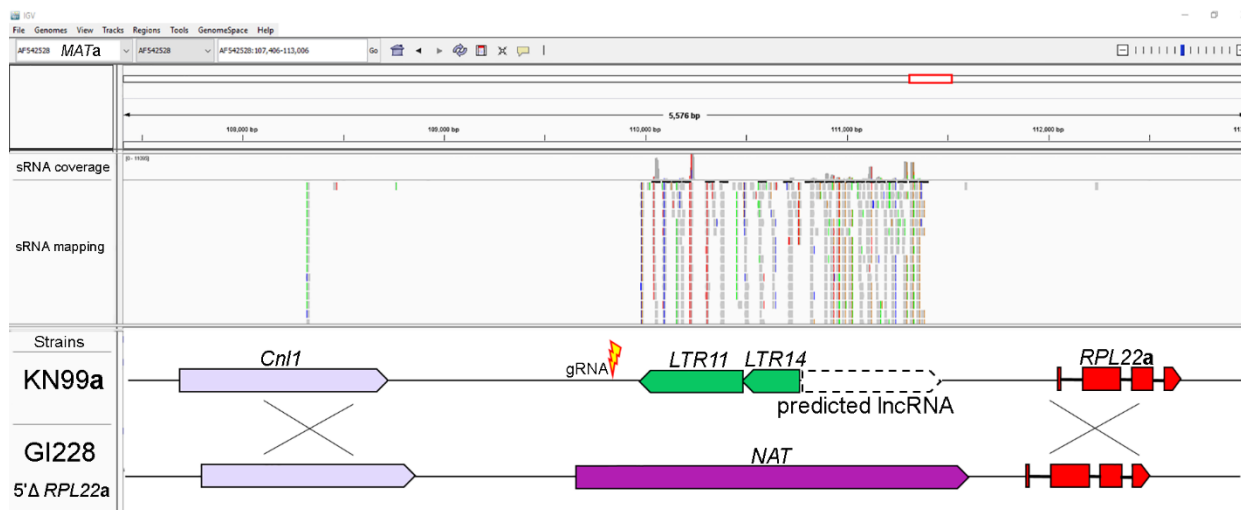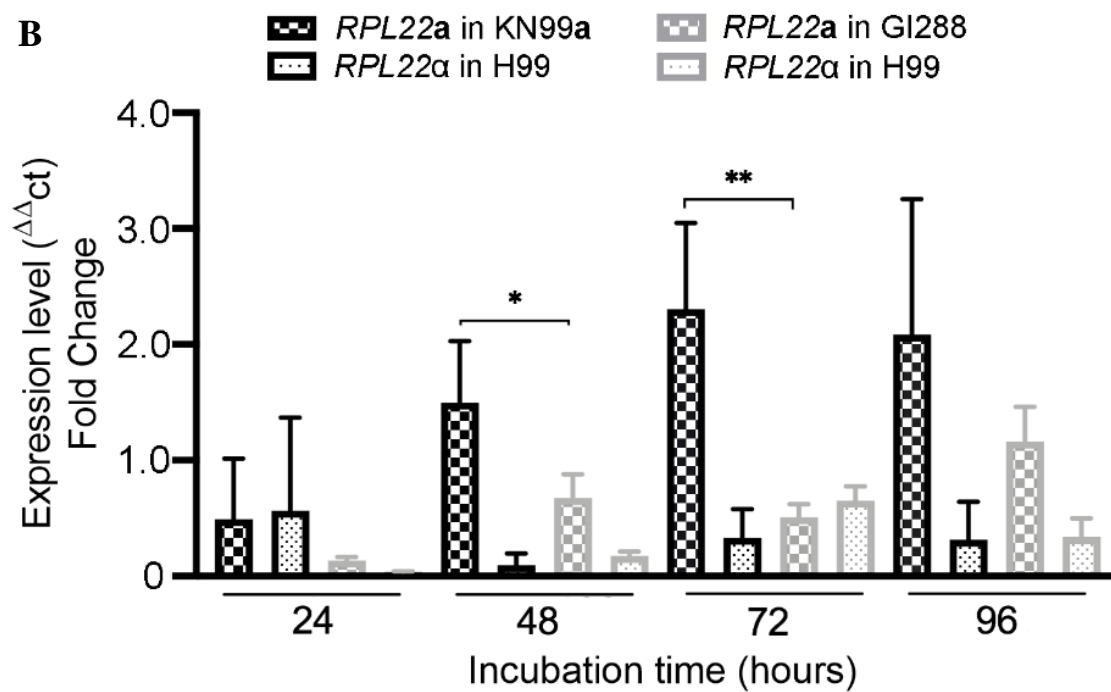
Figure S5. sRNA obtained during H99α x KN99**a** cross (black) and *rdp1Δ MAT*α x *rdp1Δ MAT***a** bilateral cross (red) were mapped to the reference *MAT*α locus of *C. neoformans* (accession number AF542529.2). Genes are represented in grey in the middle panel, and the *LTR* and transposable elements are in blue.
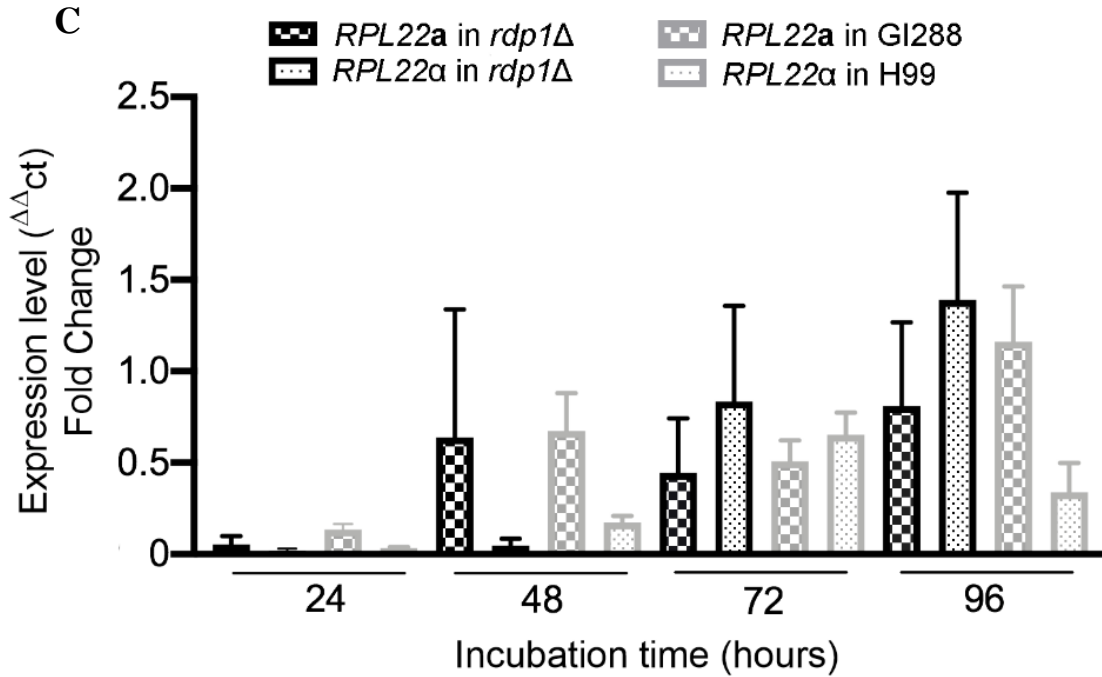
A

B

Figure S6. Analysis of 5′ control element of *RPL22***a** gene during sexual reproduction. (A) Schematic representation of the CRISPR/Cas9 strategy used to generate strain *C. neoformans* GI288 (5′ Δ *RPL22***a**) through deletion of the region targeted by sRNA upstream *RPL22***a** during H99α x KN99**a** cross. (B) RT-qPCR data from Figures 4A and 4D were replotted to allow direct comparison of the *RPL22***a** and *RPL22*α expression levels during WT H99α x KN99**a** cross compared to GI288**a** (5′ Δ *RPL22***a**) x H99α cross for 24, 48, 72, and 96 h of incubation. (C) RT-qPCR data from Figures 4C and 4D were replotted to allow direct comparison of the *RPL22***a** and *RPL22*α expression levels during *rdp1*Δ bilateral cross compared to GI288 x H99 cross for 24, 48, 72 and 96 h of incubation. For both B and C, statistical analysis was carried out using one-way ANOVA with Tukey's multiple comparison test. These comparisons were to monitor the changes in gene expression following genetic manipulation, and only statistically significant differences of the expression levels of the same gene in the same day of incubation in separate mating reactions are displayed (* for p<0.05, and ** for p<0.01).
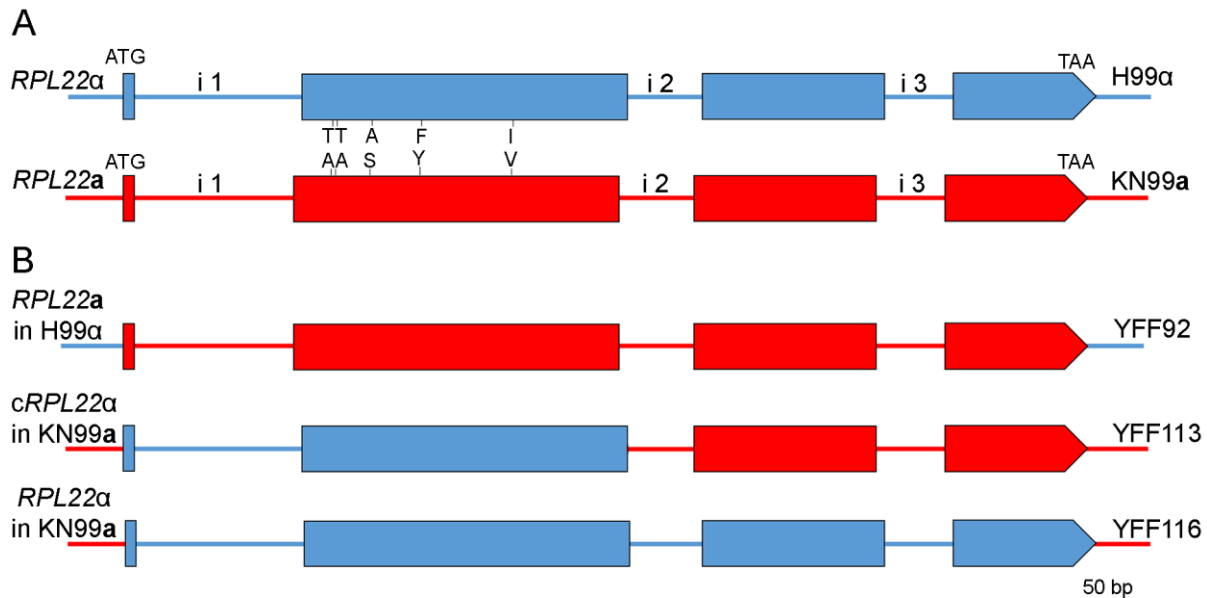
Figure S7. Features of the *RPL22α* and *RPL22***a** genes in *C. neoformans* WT H99α and KN99**a** (A), respectively, and in the *RPL22* exchange strains YFF92, YFF113, and YFF116 (B). Boxes represent exons. The start and stop codons, the different amino acids between the Rpl22α and Rpl22**a** proteins, and the introns (" i ") are indicated. The chimeric *RPL22α* is indicated as c*RPL22α* (c = chimera).
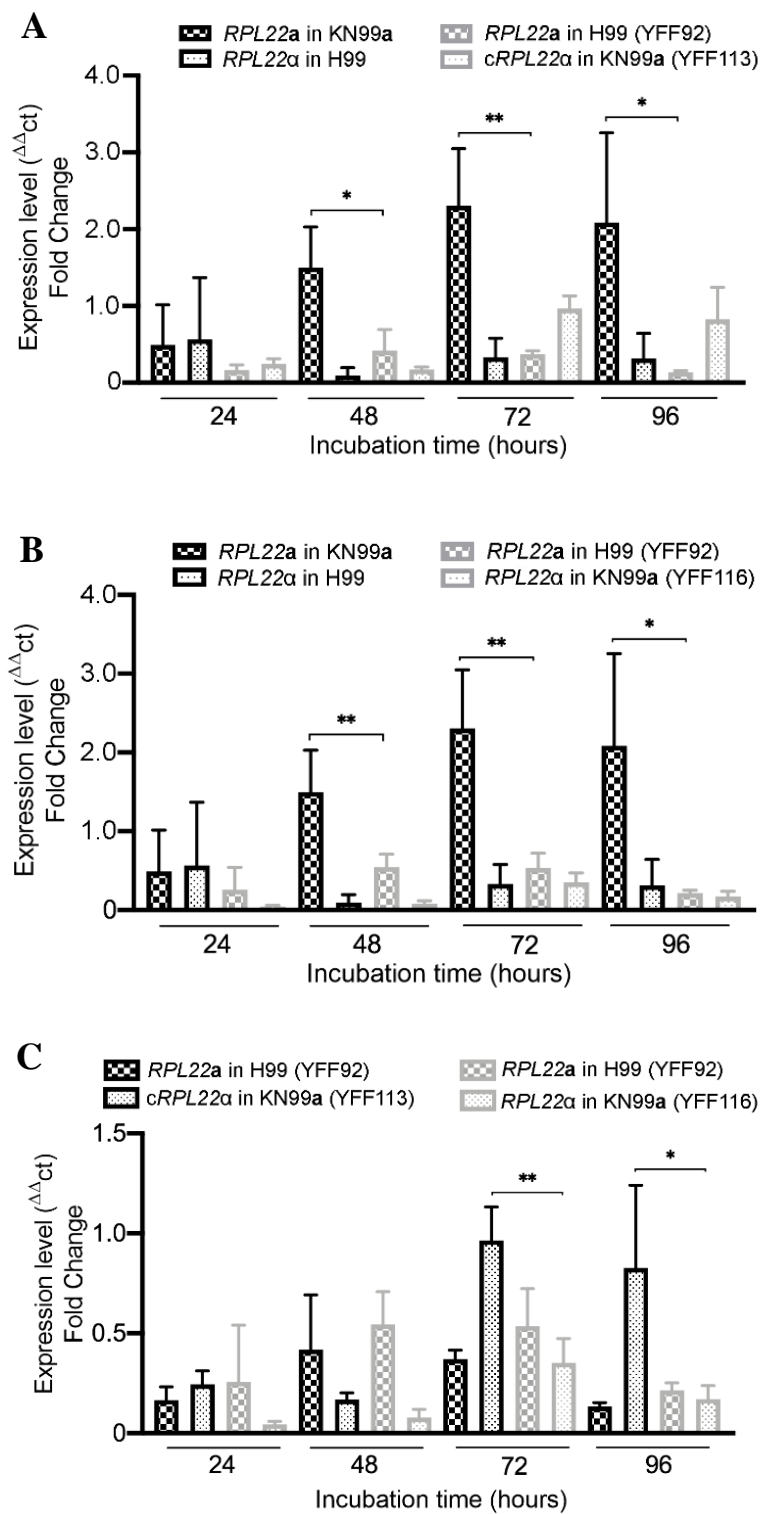
Figure S8. Comparison of the expression levels of *RPL22α*, c*RPL22α*, and *RPL22***a** during mating in the *RPL22* exchange strains. (A) RT-qPCR data from Figure 4A and Figure 5D were

replotted to allow direct comparison of the *RPL22*α and *RPL22***a** expression levels during WT H99α x KN99**a** cross compared to c*RPL22*α and *RPL22***a** expression levels during YFF92α x YFF113**a** cross for 24, 48, 72, and 96 h of incubation. (B) RT-qPCR data from Figure 4A and Figure 6B were replotted to allow direct comparison of the *RPL22*α and *RPL22***a** expression levels during WT H99α x KN99**a** cross compared to YFF92α x YFF116**a** cross for 24, 48, 72, and 96 h of incubation. (C) RT-qPCR data from Figure 5D and Figure 6B were replotted to allow direct comparison of the *RPL22*α and *RPL22***a** expression levels during YFF92α x YFF116**a** cross compared to c*RPL22*α and *RPL22***a** expression levels during YFF92α x YFF113**a** cross for 24, 48, 72, and 96 h of incubation. Statistical analysis was carried out using one-way ANOVA with Tukey's multiple comparison test. Because in these comparisons we were interested in monitoring the changes in gene expression following genetic manipulation, we display only statistically significant differences (* for p<0.05, and ** for p<0.01) of the expression levels of the same gene in the same day of incubation in separate mating reactions.
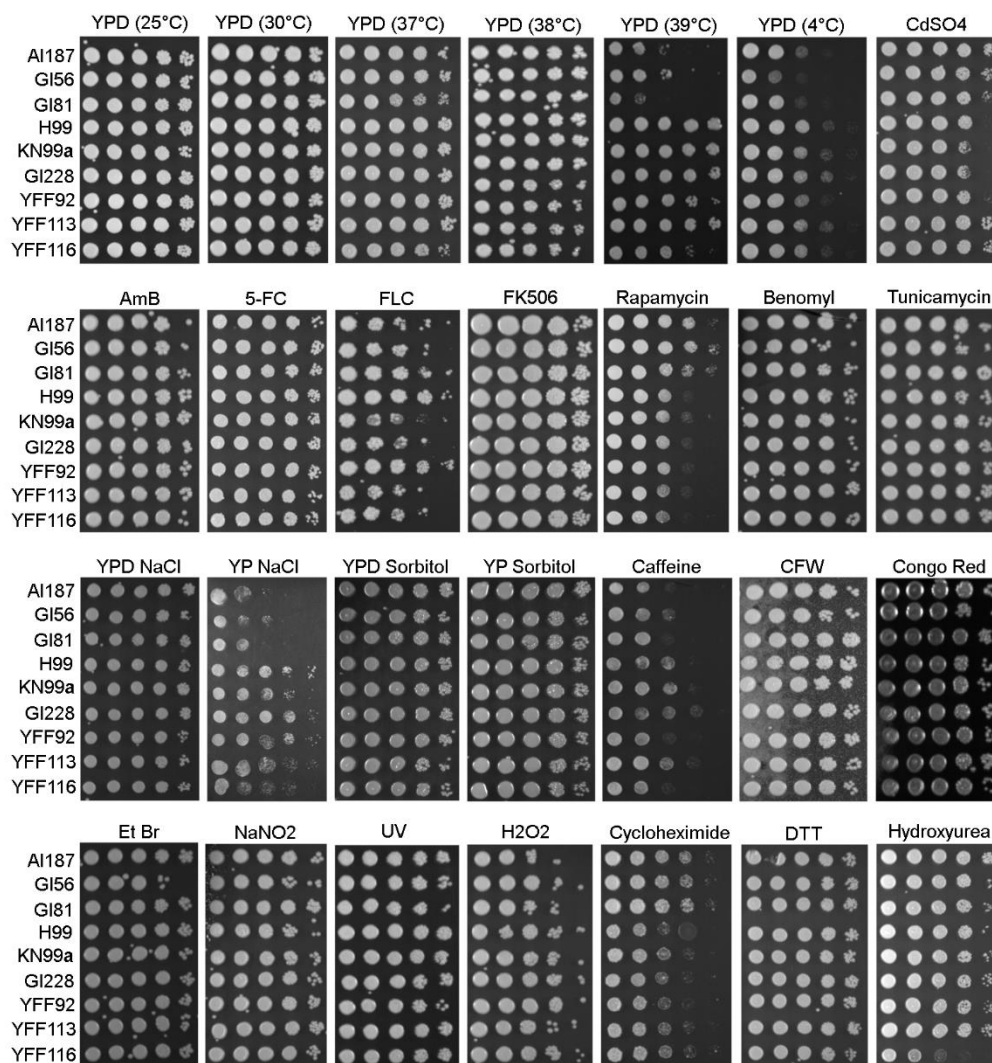
Figure S9. Phenotypic analysis of the *C. neoformans* heterozygous and mutant strains generated in this study (Table S1) on several stressors. For details see materials and methods.
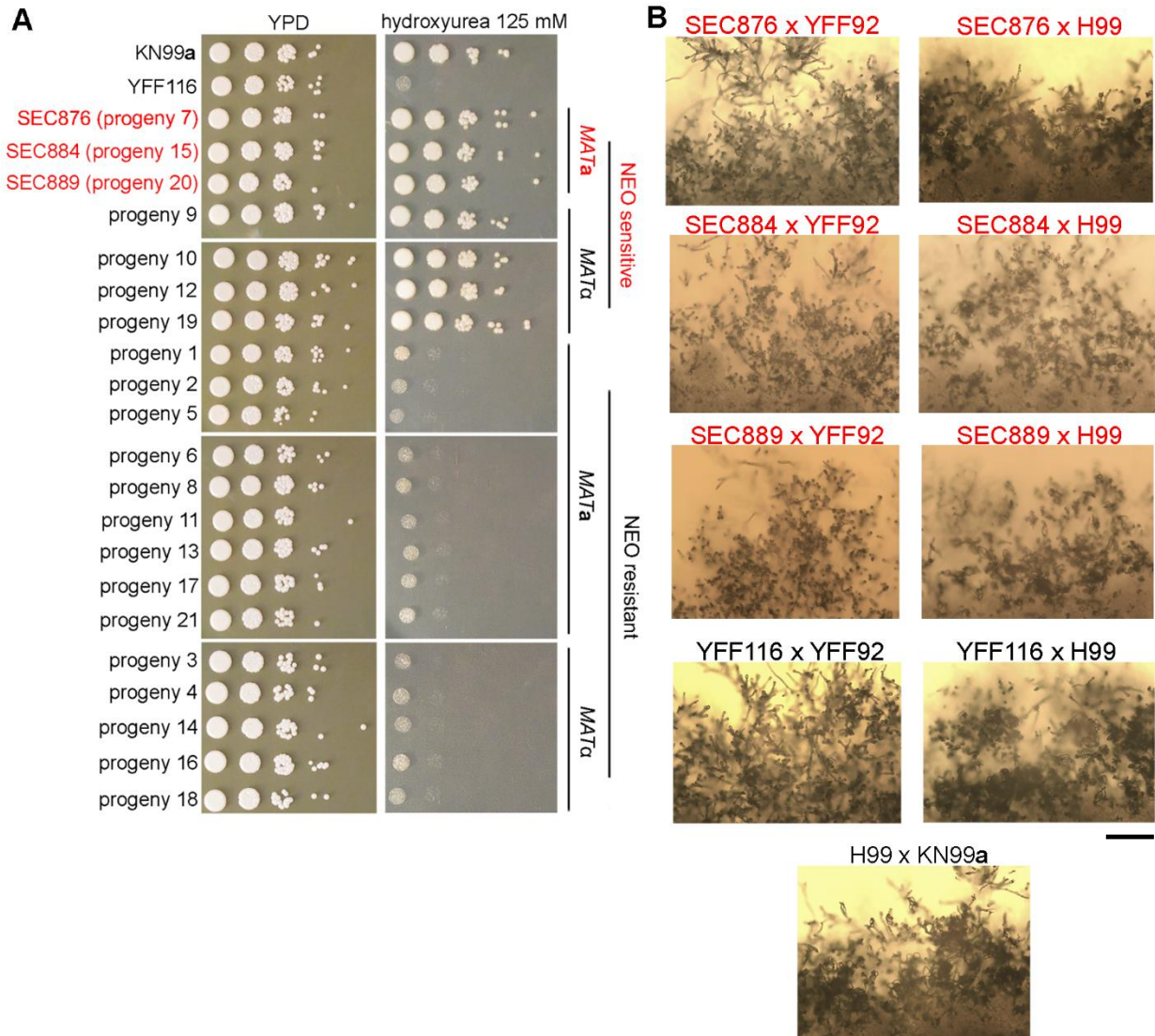
Figure S10. Genetic analysis of the markers segregating following the cross H99 x YFF116. 21 progeny that germinated after microdissection were 10-fold serially diluted and spotted on 125 mM hydroxyurea. NEO^S *MAT*a progeny 7 (SEC876), progeny 15 (SEC884), and progeny 20 (SEC889) were crossed with H99 and YFF92; control crosses were YFF116 x YFF92, YFF116 x H99α, and H99α x KN99**a**. Mating structures were photographed after 3 weeks on MS medium. The scale bar is 100 μm.
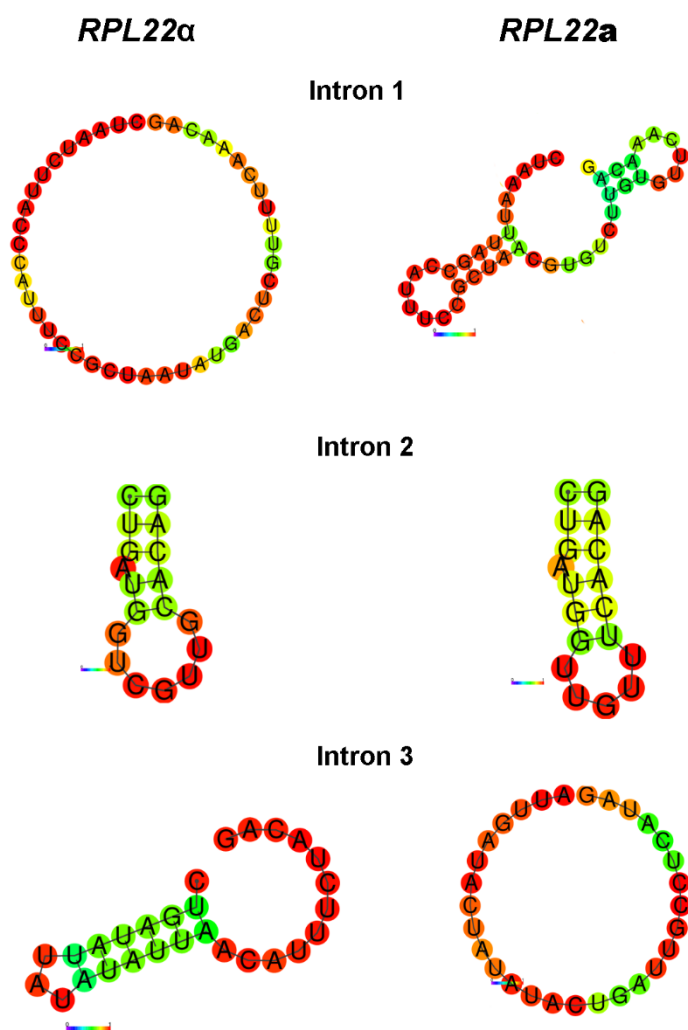
Figure S11. RNAfold secondary structure prediction of the regions including the branch sites and the acceptor sites of introns 1, 2, and 3 of the *RPL22*α and *RPL22***a** genes.