**SUPPLEMENTARY MATERIAL**

**Figure S1. Regions of high expression on the mitochondrial chromosome identify the mitochondrial rRNA genes in *C. neoformans* H99 strain:** The depth of coverage plot of the whole H99 mitochondrial chromosome is shown. The *C. neoformans* large and small mitochondrial rRNA genes were clearly identified as the two regions of the mitochondrial genome with depth of coverage that is much higher than any other part of the chromosome.

**Figure S2. Scatterplot of all genes show that RNase H- and Ribo-Zero-treated libraries recapitulate the Unenriched libraries while Poly(A)-treated libraries underrepresent some genes:** In the RNase H- and Ribo-Zero-treated libraries, most genes have counts that are highly correlated with the Unenriched libraries, whereas Poly(A)-treated libraries have low counts for a number of expressed genes. For each biological replicate (subplot columns labeled “A”, “B”, and “C”), per-gene normalized read counts for each enrichment method are plotted as a function of the normalized read counts averaged across the Unenriched libraries. All annotated genes in the *C. neoformans* genome are plotted, excluding rRNA genes and genes containing coding-strand rRNA duplications. For libraries with technical replicates (RNase H and Ribo-Zero), only one of the replicates is shown. The same data are visualized on a linear scale (A) and a log scale (B).

**Figure S3. Scatterplot of protein-coding genes show that RNase H- and Ribo-Zero-treated libraries recapitulate the Unenriched libraries while Poly(A)-treated libraries underrepresent some protein-coding genes:** In the RNase H- and Ribo-Zero-treated libraries, most protein-coding genes have counts that are highly correlated with the Unenriched libraries, whereas Poly(A)-treated libraries have low counts for a number of protein-coding genes. This plot is the same as Figure S2, but only shows protein-coding genes, excluding genes containing coding-strand rRNA duplications. The same data are visualized on a linear scale (A) and a log scale (B).

**Figure S4. Scatterplot of annotated ncRNA genes show that the RNase H- and Ribo-Zero-treated libraries recapitulate the Unenriched libraries while Poly(A)-treated libraries underrepresent many ncRNA genes:** In the RNase H- and Ribo-Zero-treated libraries, most annotated ncRNA genes have counts that are well-correlated with the Unenriched libraries, whereas Poly(A)-treated libraries have low counts for almost all annotated ncRNA genes. This plot is the same as Figure S2, but only shows annotated ncRNA genes, excluding rRNA. The same data are visualized on a linear scale (A) and a log scale (B).

**Figure S5. The RNase H depletion method performs as well as the Ribo-Zero depletion method for annotated ncRNA genes when the outlier CNAG\_12993 is excluded:** When outlier ncRNA gene CNAG\_12993 is excluded, the RNase H depletion method has specificity for annotated ncRNA genes that is as good as the Ribo-Zero depletion method. Pearson correlations were calculated in the same way as Figure 4, except CNAG\_12993 was excluded from the analysis.

**Figure S6. Identification of genes underrepresented by the Poly(A) isolation method:** This plot is the same as Figure S2, with the genes that are significantly underrepresented in the Poly(A)-treated libraries colored red. The same data are visualized on a linear scale (A) and a log scale (B).

**Figure S7. Underrepresentation of nuclear protein-coding genes by the Poly(A) isolation method may be driven by mRNA degradation:** (A) The distribution of reads across nuclear protein-coding genes that are underrepresented by the Poly(A) isolation method suggests that these reads may be primarily mRNA degradation intermediates, and therefore that deadenylation may be driving this underrepresentation. (B) A comparison set of genes, randomly selected from protein-coding genes with a similar range of expression, display much more uniform expression. Read data is from Unenriched libraries.

**Figure S8. Visualization of reads across the predicted lncRNA genes supports the identifications of LncPipe:** Read depth across the 11 regions identified by LncPipe as encoding predicted lncRNA largely supports these predictions, while suggesting some need for optimization of LncPipe to adapt it to the characteristics of *C. neoformans* lncRNA.

**Table S1. Genes underrepresented by the Poly(A) isolation method:** Table details the genes that are substantially underrepresented in the Poly(A)-treated libraries. The gene name, chromosome, and gene type are included for each gene.

**File S1. RNase H depletion protocol:** The detailed protocol used to perform the RNase H depletion.

**File S2. Ensembl GTF with newly annotated mitochondrial rRNA:** A copy of the GTF genome annotation file for CNA3 of H99 *Cryptococcus neoformans* var. *grubii*, modified to include annotation for the mitochondrial rRNA genes.

**File S3. RNase H rRNA-targeting oligonucleotides:** DNA oligonucleotide sequences used in the RNase H depletion method to target H99 *C. neoformans* rRNA. This file was formatted to be directly pasted into the Eurofins order form.