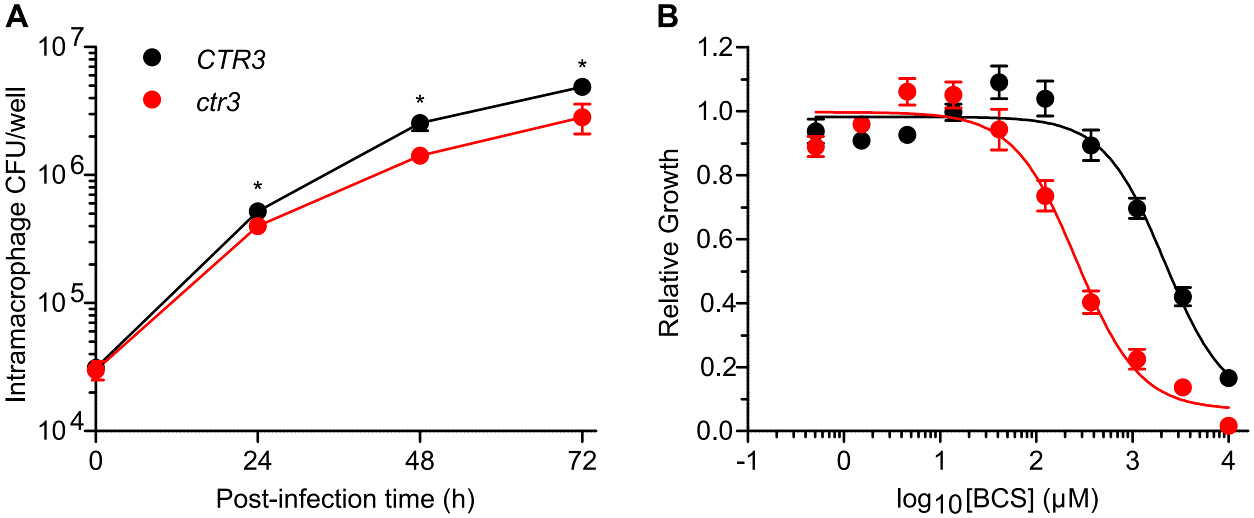
Supplemental Figure 2



S2 Fig. The Ctr3 requirement for yeast proliferation in macrophages and under copper limited conditions extends to a second phylogenetic species of *H. capsulatum* (G186A). Growth of the Ctr3-expressing parent strain (*CTR3*, black) and a strain in which the *ctr3* gene was deleted (*ctr3Δ*, red) of the G186A genetic background in macrophages **(A)** or in liquid media with limited copper **(B)**. **(A)** A strain in which the CTR3 locus was deleted was generated by allelic replacement (Sebghati et al., 2000) with a hygromycin expression cassette flanked by 2 kb upstream and downstream of the *CTR3* gene. Intracellular *CTR3* and *ctr3* mutant *H. capsulatum* yeasts were quantified over 72 hours following infection of P388D1 macrophages (MOI 1:1). Macrophages were lysed, the intracellular yeasts recovered, and the lysate plated on solid HMM medium to enumerate colony forming units (CFU). Data represent the average intramacrophage CFU ± standard deviation among infections with biological replicates (n = 3). Statistically significant differences between *CTR3* and *ctr3* proliferation at each day were determined by one-tailed Student's *t*-test and are indicated with asterisks (* $P < 0.05$). (B) Growth of Ctr3-expressing (*CTR3*, black) and the *ctr3* mutant (*ctr3*, red) strains grown in liquid HMM with the copper chelator BCS were determined by measurement of yeast metabolic conversion of resazurin to fluorescent resorufin (quantified by fluorescence: 530 nm excitation and 590 nm emission 90 minutes following addition of 1 mM resazurin) after 5 days of growth at 37°C. Relative growth was determined by normalization of yeast-dependent resazurin metabolism for each BCS concentration to that of yeasts grown in the absence of BCS. Dose-response curves were generated by non-linear regression and the IC_{50} for BCS treatment of *CTR3* and *ctr3* strains determined as 2,156 μM and 263 μM, respectively. Data represent average growth ± standard deviation among biological replicates (n = 3).