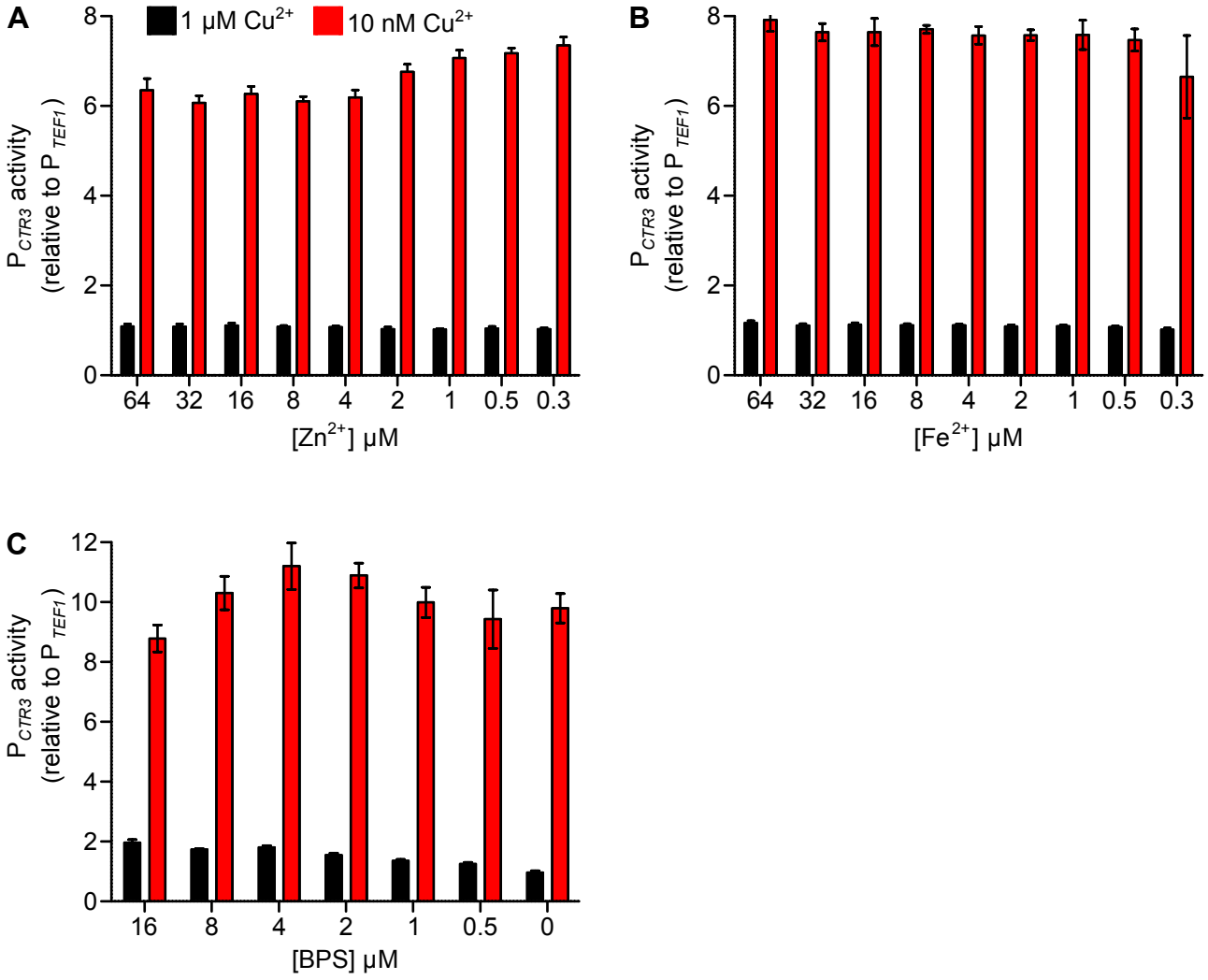
Supplemental Figure 4



S4 Fig. Zinc and iron do not regulate the *CTR3* promoter. *CTR3* promoter activity in 1 μM $CuSO_4$ (low *CTR3* promoter activity, black bars) or 10 nM $CuSO_4$ (high *CTR3* promoter activity, red bars) with high and low concentrations of zinc **(A)**, iron **(B)**, or the iron-specific chelator BPS **(C)**. **(A)** *H. capsulatum* yeasts were incubated in 3M medium (containing 4 μM $FeSO_4$ and 1 μM $CuSO_4$ or 10 nM $CuSO_4$) with different $ZnSO_4$ concentrations (0.3 μM to 64 μM). **(B)** *H. capsulatum* yeasts were incubated in 3M medium (containing 4 μM $ZnSO_4$ and 1 μM $CuSO_4$ or 10 nM $CuSO_4$) with different $FeSO_4$ concentrations (0.3 μM to 64 μM). **(C)** *H. capsulatum* yeasts were incubated in 3M medium (containing 4 μM $ZnSO_4$ and 1 μM $CuSO_4$ or 10 nM $CuSO_4$) with different BPS concentrations (0 μM to 16 μM). The *CTR3* promoter activity was assessed by fluorescence of wild-type yeasts with the *CTR3* promoter-*gfp* fusion (P_{CTR3}) after normalization to yeasts with the *TEF1* promoter-*gfp* fusion (P_{TEF1}) grown in identical conditions. After 72 hours incubation at 37°C, culture turbidity (optical density at 595nm) and GFP fluorescence (485 nm excitation, 528 emission) were measured. *TEF1* or *CTR3* promoter activity (GFP fluorescence) was normalized to the yeast density (OD_{595}) and the *CTR3* promoter activity then compared to that of the constitutively expressed *TEF1* promoter. Data represent the average relative *CTR3* promoter activity ± standard deviation among biological replicates (n = 3).