

Figure S3

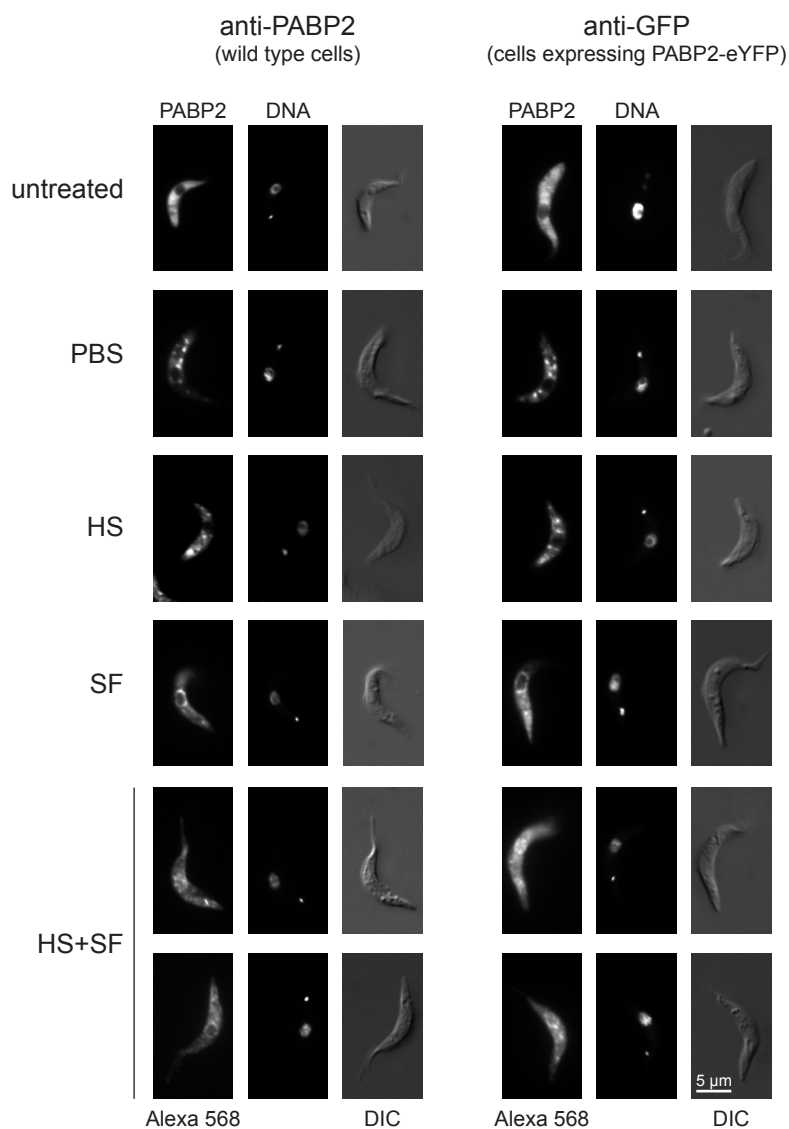


Figure S3: Detection of PABP2 in inducible RNP granules by immunofluorescence

2×10^7 cells, untreated or stressed by carbon source starvation (3 hours PBS), 2 h heat shock at 41°C (HS), sinefungin (SF) or 2 hours heat shock and sinefungin (HS+SF) were washed once in SDM79 without serum and heme, resuspended in 5 ml SDM79 without serum and heme and fixed for 15 minutes with 1 volume 8% paraformaldehyde in PBS at RT while rotating. Cells were washed once in PBS and allowed to settle on slides for 15 minutes. Slides were washed in 25 mM NH_4Cl for 10 minutes. Cells were permeabilized and blocked for one hour in blocking solution (taken from fluorescent antibody enhancer set for DIG detection, Roche) containing 0.5% saponin. Slides were washed, blocked for an additional 30 minutes without saponin, incubated with the first antibody (anti-LmPABP2 1:500 or anti-GFP, Invitrogen A11122, 1:100) for 60 minutes, washed 4 times in PBS, incubated with the secondary antibody (anti-mouse Alexa 568) and mounted in FluorSave (Calbiochem), containing Höchst33342 DNA stain at 5 $\mu\text{g}/\text{ml}$. Wild type cells stained with anti-GFP or cells stained with no antibody served as controls and showed significantly less signal (not shown).