Figure S1. No evidence for the localization of trypanosome ribosomal subunits to starvation stress granules

Co-expression of an N-terminal eYFP fusion of TbRPL18 and a C-terminal CerFP fusion of TbRPS30A from endogenous loci in procyclic trypanosomes. Fluorescence microscopy images (Z-stack projections) of untreated and starved (120 min PBS) cells are shown. An N-terminal fusion of the Arabidopsis RPL18 to a His$_6$-Flag tag integrates into polysomes (Zanetti et al., 2005) and a C-terminally GFP tagged RPS30 protein was used as a marker for the small ribosomal subunit in yeast, where it localizes to stress granules at robust heat shock (Grousl et al., 2009). Given that ribosomes are among the evolutionary most conserved structures, it is likely that the equivalent fusions of the trypanosome orthologues are functional.
Figure S2. The 463 proteins of the granule-enriched fraction (P4) identified by mass spectrometry were compared with proteins identified as regulators of mRNA stability in a genome wide tethering screen (Erben et al (2014)). Proteins that were identified in both studies are shown in red (overlap with proteins that cause an increase in the level of the reporter mRNA) or blue (overlap with proteins that cause a decrease in the level of reporter mRNA).

Erben, E.D., Fadda, A., Lueong, S., Hoheisel, J.D. and Clayton, C.E. (2014) A genome-wide tethering screen reveals novel potential post-transcriptional regulators in Trypanosoma brucei. PLoS Pathog, 10, e1004178.
| Gene Symbol | Genomic Position |
|-------------|------------------|
| CAF1        | Tb927.6.600      |
| NOT1        | Tb927.10.1510    |
| elf4G4      | Tb927.11.10560   |
| elf4G1      | Tb927.5.1490     |
| UPF1        | Tb927.5.2140     |
| Nui5        | Tb927.3.1920     |
| DRBD4       | Tb927.11.1400    |
| elf3-beta   | Tb927.11.9610    |

**Figure S3**

| untreated | DNA | merged | starvation | DNA | merged |
|-----------|-----|--------|------------|-----|--------|
| SCD6      |     |        | SCD6       |     |        |
| PABP2     |     |        | PABP2      |     |        |
| DHH1      |     |        | DHH1       |     |        |
| DHH1      |     |        | DHH1       |     |        |
| DHH1      |     |        | DHH1       |     |        |
| DHH1      |     |        | DHH1       |     |        |

Single plane

5 µm
Figure S4A

The posterior pole granule seems specific to starvation.

protein kinase, putative Tb927.7.3880

protein kinase, Tb927.9.6580

hypothetical, conserved Tb927.10.230
Figure S4B_page 1/2

| untreated       | DNA    | merged                 | starvation | DNA    | merged                 |
|-----------------|--------|------------------------|------------|--------|------------------------|
| hypothetical, conserved Tb927.4.3150 | DHH1   | DNA merged             | DHH1       | DNA merged             |
| hypothetical, conserved Tb927.4.4000 | DHH1   | DNA merged             |            |                    |
| kinesin Tb927.11.5300 | DHH1   | DNA merged             |            |                    |
| hypothetical, SAM dependent methyltransferase domain Tb927.9.2320 | DHH1 | DNA merged             |            |                    |
| serine/threonine protein phosphatase 2A regulatory subunit Tb927.1.1390 | DHH1 | DNA merged             |            |                    |
| protein kinase like domain protein Tb927.11.1340 | DHH1 | DNA merged             |            |                    |
| protein transport protein SEC13 Tb927.11.8120 | DHH1 | DNA merged             |            |                    |
Figure S4B_page 2/2

untreated

| DHH1 | DNA | merged |
|------|-----|--------|

starvation

| DHH1 | DNA | merged |
|------|-----|--------|

nucleoporin (Mlp-2)

Tb927.9.1340

hypothetical, conserved

Tb927.10.1150

5 µm

cell cycle dependency spindle pole body during anaphase (DeGrasse et al. 2009)
| Gene Description                      | Tbr Number | Tbr Start | Tbr End |
|--------------------------------------|------------|-----------|---------|
| polo-like protein kinase              | Tb927.6310 | 536       | 577     |
| UNC119                               | Tb927.4580 | 127       | 170     |
| dual specificity protein phosphatase  | Tb927.10.1620 | 84       | 124     |
| protein tyrosine kinase               | Tb927.8.5780 | 511      | 552     |
| protein phosphatase 1 regulatory subunit | Tb927.5.590 | 161      | 202     |
| serine/threonine protein kinase Nrk   | Tb927.8.6930 | 100      | 140     |
| protein kinase (Nek1-like)            | Tb927.10.5950 | 5668     | 6069    |

**Figure S4C page 1/3**

Untreated vs starved conditions are compared in the images. The images show the expression patterns of various proteins across untreated and starvation conditions, with magnified regions labeled as 'DHH1', 'DNA', and 'merged' to illustrate the protein localization and interaction. A scale bar indicating 5 µm is included to provide a sense of scale for the observed features.
untreated

DHH1 | DNA | merged

starvation

DHH1 | DNA | merged

ser/thr-protein kinase, putative Tb927.3.3190
adenosine kinase Tb927.6.2360
threonyl-tRNA synthetase Tb927.5.1090
cell division protein kinase 2 homolog (CRK1) Tb927.10.1070
mitogen activated protein kinase (CBPK1) Tb927.10.1478
protein kinase Tb927.9.12880
TPR-repeat-containing chaperone protein DNAJ Tb927.4.2220

Figure S4C page 2/3
Figure S4. Validation of the mass spectrometry data by testing the localization of fluorescent protein fusions at starvation: proteins not involved in mRNA metabolism that do not localize to starvation stress granules.

Fluorescence microscopy images of untreated and starved cells co-expressing the granule marker mChFP-DHH1 and the eYFP fusion of the granule candidate protein.
A) Proteins with a change in localization at starvation.
B) Proteins with a localization others than even cytoplasmic distribution.
C) Proteins with mainly cytoplasmic (and perhaps nuclear) distribution.
Figure S5. mRNA FISH of *RPL7a* and *DBP1* using the Stellaris system. More images of starved cells are shown as sum slices of a deconvolved Z-stack.
Figure S6. mRNA FISH of RPL7a and DBP1 using the Affymetrix system. More images of starved cells are shown.
Figure S7

Northern blots loaded with total RNA of untreated and starved cells were probed for DBP1 and rRNA (loading). The reduction in DBP1 mRNA upon starvation was quantified from three independent experiments, of which one gel is shown.