Singh et al - Legends to Supplementary material

Supplementary Figure S1

Reproducibility of RNASeq results
Panels A-D show results as reads per million (RPM) for the unique gene set, with each spot representing one open reading frame. The datasets plotted are indicated above and on the axes.
The new wild-type results were highly reproducible (panel A) but deviated somewhat from the 3-year-old dataset (Manful et al., 2011) that we used as control for our previous analysis (Droll et al., 2013) (panel B). This is presumably due to changes in methodology and the increased coverage in our new data. Comparison of the *ZC3H11* RNAi results (panel C) with those for wild-type showed that most mRNAs are unaffected by ZC3H11 depletion (panel D). Panel E is regulation for each open reading frame in bloodstream forms (average RPM after ZC3H11 RNAi divided by average for wild-type) plotted against the effect of RNAi on the on the transcriptome of heat-shocked procyclics (data from Droll et al, 2013). Most mRNAs are not bound by ZC3H11, so for most mRNAs no effects are expected beyond those caused by growth inhibition.

Supplementary Figure S2

Alignment of selected Mkt1 homologues with the N-terminal PIN domains of human XPD and FEN1. Protein sequences were aligned in DNAStar using Clustal W, with manual adjustment. Amino acids identical to the consensus (at least two sequences identical) are highlighted in black and chemically similar residues are highlighted in grey. Similar groups were (DE), (HKR), (AGILV), (NQ), (FWY), (ST), (CM). Arrows indicate important amino acids for Mkt1p function of Fen1 or ATX-2 endonuclease function. Genes analysed were: Tb - *T. brucei* Tb927.6.4770; Dd- *Dictyostelium discoideum* Q54UD1_DICDI; Tg - *Toxoplasma gondii* locus TGME49_212250; Sc - *Saccharomyces cerevisiae* Mkt1p; Um - *Ustilago maydis* Q4P7L0_USTMA; XPD - *Homo sapiens* AAF89179.1; FEN1 - *Homo sapiens* AAX42675.1.

Supplementary Figure S3

Alignment of the Lsm domains of selected Pbp1 homologues. (A) Protein sequences were aligned in DNAStar using Clustal W, with manual adjustment. Amino acids identical to the consensus (at least two sequences identical) are highlighted in black and chemically similar residues are highlighted in grey. Similar groups were (DE), (HKR), (AGILV), (NQ), (FWY), (ST), (CM). Genes analysed were: Tb - *T. brucei* Tb927.8.4540; Sc - *Saccharomyces cerevisiae* Pbp1p; Af: *Aspergillus fumigatus* EDP56199.1; Sp: *Schizosaccharomyces pombe* NP_595684; Oryz: Dd- *Dictyostelium discoideum* XP_646184; Pf - *Plasmodium falciparum* PF14_0338; HsATX - *Homo sapiens* Ataxin-2, NP_002964.3. (B) Table showing percent identity of the sequences in (A).

Supplementary Figure S4

Alignment of selected Lsm12 homologues. Protein sequences were aligned in DNAStar using Clustal W. Amino acids identical to the consensus (at least two sequences identical) are highlighted in black and chemically similar residues are highlighted in grey. Similar groups were (DE), (HKR), (AGILV), (NQ), (FWY), (ST), (CM). Genes analysed were: Tb - *T. brucei* Sc - *Saccharomyces cerevisiae* Lsm12, Tg - *Toxoplasma gondii* XP_002368141; Dd

Dictyostelium discoideum XP_646772; Dm *Drosophila melanogaster* NP_572777; Tp *Thalassisosira pseudonana* XP_002294501; Hs *Homo sapiens* NP_689557

Supplementary Figure S5

Interactions of ZC3H11 with MKT1 and PBP1 and itself are RNA-independent.
These results are for procyclic forms.
A. Extracts from procyclic cells expressing ZC3H11-myc and either V5-PBP1 or V5-MKT1 (lanes 1-6), were subjected to immunoprecipitation with anti-myc in presence of RNaseA (lanes 1-3) or RNasin (lanes 4-6). Cells with no V5 tag served as negative controls (lanes 7-9). The precipitated proteins were analysed by Western blotting, using anti-myc, anti-V5, and, as control, anti-aldolase (aldo). In: input, U: unbound ($2x10^6$ cell-equivalents), E: eluate ($3.8x10^7$ cell-equivalents).
B. ZC3H11 interacts weakly with itself. Procyclic trypanosomes expressed V5-ZC3H11 (*in situ* tagged) and/or ZC3H11-myc (inducibly expressed, induced for 1 day with tetracycline). The parasites were incubated at 37°C, and MG132 was also added, to increase V5-ZC3H11 expression to detectable levels. Immunoprecipitation was with anti-V5 (lanes 1-6) or anti-myc (lanes 7-12). In each case, lanes 1-6 are from the same gel, as are lanes 7-12. The V5-ZC3H11 is probably less abundant than ZC3H11-myc. ZC3H11-myc reliably pulled down V5-ZC3H11, especially at 37°C (lane 9; negative control lane 12). The reciprocal precipitation of V5-ZC3H11 only pulled down an extremely small proportion of the (inducibly expressed) ZC3H11-myc (lane 3, negative control lane 6).
C. As in (B) but the parasites were incubated at 41°C for 1h.
D. Lysates were made from cells expressing V5-tagged MKT1 or V5-tagged ZC3H11 from the endogenous locus. The cells were grown at 27°C or overnight at 37°C. The "none" lane is the negative control (cells with no V5-tagged protein). At 27°C there is no difference with and without V5-ZC3H11. At this exposure there is a weak V5 signal at the height of V5-MKT1 even in the negative control, This could be flow-over from the V5-MKT1 lane.

Supplementary Figure S6

Yeast 2-hybrid assays. Photographs of alpha-galactosidase assay plates are shown. BD: DNA binding domain fusion (bait); AD: transcriptional activation domain fusion (prey). Blue colour indicates an interaction, white or pink no interaction. A: PBP interactions; B: interactions of trypanosome and yeast MKT1 and Mkt1; C: interactions of deleted versions of ZC3H11; D: interactions of N- and C-terminal fragments of MKT1; E: interactions of MKT1 with translation factors; F: interactions of CFB1D and its C-terminus with MKT1.

Supplementary Figure S7

Expression of lambda-N-myc proteins in cells expressing CAT-boxB reporters.
These are the proteins whose activities are listed in Table 3 and illustrated schematically in Figure 4.
Expression was induced for 1 day and proteins were detected by with anti-myc. In this experiment expression of full-length lambdaN-ZC3H11-myc was rather stronger than usual.

Supplementary Figure S8

MKT1 location and effects of RNAi against MKT1 or PBP1
A. RNAi was induced against either MKT1 or PBP1, in each case using two independent clones of procyclic or bloodstream-form trypanosomes. Cumulative growth curves are shown.

B. RNAi targeting MKT1 causes a decrease in protein synthesis, but this coincides with growth inhibition. RNAi was induced for one day in bloodstream forms (see panel A) then cells were pulsed with [^{35}S]-Methionine for 20 min. Proteins were separated by SDS-PAGE, then stained with Coomassie. The gel was dried and [^{35}S] detected by autoradiography. The two left hand panels include a Western blot to illustrate the decrease in *in situ* V5-tagged MKT1. The right-hand panel is a different experiment, in which we also analysed cells with RNAi targeting ZC3H11, which show a similar growth defect.
C. MKT1 mostly does not colocalise with SCD6 in starvation stress granules. Trypanosomes expressing MKT1-myc were starved for 3 h then stained for myc and SCD6. These are additional images similar to those shown in Figure 6.

Supplementary Figure S9

MKT1 depletion does not affect the procyclic-form heat shock response.
A. Cells with inducible RNAi were grown with tetracycline for the indicated times, then pulsed for 20min with ^{35}S-Methionine.
B. Expression of V5-MKT1 detected by Western blotting. The results were quantitated in Adobe Photoshop using the aldolase signal for normalisation.

Supplementary Figure S10

Role of the "HNPY" motif in interactions with MKT1.
Six open reading frames were chosen which had good coverage in the yeast 2-hybrid library before selection. A symbol is plotted at the start of each in-frame sequence read, with the number of reads at that position on the vertical axis. The position of the start of the HNPY-like motif is also shown, as indicated in the key on the Figure.

Supplementary Figure S11

Interactions of three potential partners of MKT1. Extracts from bloodstream-form cells expressing V5-tagged proteins and/or MKT1-myc, were subjected to immunoprecipitation with anti-myc or anti-V5. The precipitated proteins were analysed by Western blotting, using anti-myc and anti-V5, and, as control, anti-aldolase. In: input, U: unbound ($2x10^6$ cell-equivalents), E: eluate ($3.8x10^7$ cell-equivalents).
A: Myc pull-down, V5-ZC3H38
B: Myc pull-down, V5-Tb927.5.1990
C. V5 pull-down, V5-Tb927.5.1990 or V5-ZC3H38
D. Myc pull-down, V5-Tb927.6.3090
E. V5 pull-down, V5-Tb927.6.3090

Supplementary Table S1

Plasmids and oligonucleotides made or used in this paper.

Supplementary Table S2

New RNASeq controls and effect of ZC3H11 RNAi in bloodstream forms.
These experiments were done because our previous analysis (Droll et al. 2013) used a single RNAi replicate and relied on a "wild-type" dataset made using older RNASeq technology.
The top sheet shows the unfiltered data. The second sheet (“Filter 10 reads..”) shows filtered results for the unique genes. All open reading frames represented by less than 10 reads, or 5

RPM in wild-type, or not represented in the published dataset, have been removed. The *ZC3H11* mRNA was reduced to below 10% of wild-type. The columns with shaded titles are new data. All of the other data are from Droll et al (2013) except for the results for poly(A)+ from Manful et al. (2011) which are indicated as "Manful". The results are illustrated in Supplementary Figure S1. For the comparisons between RNAi and wild type the colour code is: Dark blue: at least 2-fold decreased; light blue: at least 1.5-fold decreased; orange: at least 1.5-fold increased; red: at least 2-fold increased. For RNA binding, the colour code is red for RNAs that were at least 3-fold enriched in the ZC3H11 immunoprecipitation. The calculation of mRNAs/cell assumes that there are 20,000 mRNAs per parasite, without VSG.

Supplementary Table S3

All proteins associated with ZC3H11 after tandem affinity purification. The first sheet ("Possible ZC3H11 association) includes the confirmed complex components, and four proteins of unknown function that were strongly represented and have not been detected in other purifications. It also includes various proteins associated with RNA binding or translation which we have detected in some other purifications as well. The second sheet ("Possible contaminants") lists highly abundant proteins that we have seen in many other purifications (80), and proteins that are known to be in the nucleus, glycosome (86) or mitochondrion (87,88). Function: Function according to TritrypDB, with additional manual annotation. Category: classification of function (manual).

Supplementary Table S4

Results of high throughput yeast 2-hybrid screen with MKT1 as bait. Sheet 1 shows all in-frame gene locations which gave reads after selection, and the number of reads for each location. Sheet 2 is a list of unique genes giving at least one location with more than 20 reads. The sequence including the (H/N)(N/Q/E/D)PY motif, if present, is shown. "Locations per gene pGAD" is the total number of in-frame locations present in yeast containing the library and grown without any selection. "Peptides MKT1-TAP" is the number of peptides identified with at least 95% confidence in the MKT1 tandem affinity purification. Sheet 3, "Potential partners", is a list of genes from Sheet 2, with each gene listed only once. No clones: number of independent locations with at last 20 reads. Positive clone ratio: D divided by E (proportion of locations that were positive). Max reads: maximum number of reads obtained from a single location in the gene. Aa location: residue number of the most C-terminal location giving at least 20 reads. Motif start: amino acid residue at which the HNPY or poly(Q) motif starts, if present. Motif: motif found (see text). Manual Y2H: found (or not) in the manual two-hybrid screen. Other details as for Table 1.

Supplementary Table S5

All proteins associated with MKT1 after tandem affinity purification. Sheet 1 shows all proteins for which more than one peptide was identified, but with likely contaminants eliminated. Likely contaminants (Sheet 2) are proteins in other compartments, ribosomes and translation factors, proteins that have appeared in at least four other purifications, and metabolic enzymes. The third sheet includes all proteins identified with at least 2 peptides, and the fourth sheet is those for which only one peptide was identified. Previous MS: number of previous times identified. CNOT10: presence or absence (1 or 0) in CNOT10 tandem affinity purification. Polysomes: presence or absence in 4 different polysome purifications (C. Klein and C. Clayton, ZMBH, unpublished). MKT Y2H reads: maximum number of reads in the high-throughput MKT1 2-hybrid screen. CFB Y2H: maximum number of reads in the high-throughput CFB1 2-hybrid

screen. HNPY or Q6: The presence of a motif (HNPY-related or number of $(Q)_6$ repeats). Other annotation as for Table S1.