SUPPLEMENTAL INFORMATION

Supplemental Figure Legends

Figure S1. Visual representation of the co-complex interaction network
A network formed by KPAP1 poly(A) polymerase (75 mM of KCl), KPAF1 and 2, and small ribosomal subunit (S17) and large ribosomal subunit (L3) proteins was obtained from MS data (Table S1). A cutoff of five unique peptides was set to establish a pairwise contact in Cytoscape environment (www.cytoscape.org), which is reflected by blue edges connecting two nodes. Unique targets (proteins co-purifying with only one tagged polypeptide) are grouped by each source (tagged polypeptides, yellow boxes). Targets shared by two or more sources (co-complex interactions) are indicated in green boxes. Protein identities and experimental peptide counts for each node can be found in the Cytoscape Database File S1.

Figure S2. Effects of KPAF1, 2 and Dual KPAF1-2 RNAi Knockdowns on Relative mRNA Abundance
(A) Real-time RT-PCR analysis of RNAi-targeted nuclear mRNAs, mitochondrial mRNAs and rRNAs in KPAF1 and 2 procyclic RNAi cell lines and KPAF1 in the bloodstream RNAi cells. The RNA levels were normalized to β-tubulin mRNA. P, pre-edited mRNA; E, edited mRNA. Error bars represent the standard deviation from at least three replicates. The thick line at ‘‘1’’ reflects no change in relative abundance upon RNAi induction; bars above or below represent an increase or decrease, respectively. KPAF2 knockdown in BF cells did no produce growth phenotype and was not analyzed further.
(B) Dual KPAF1-KPAF2 knockdown in procyclic parasites. RNAi was induced for indicated periods of time in PF cell line. Total RNA was separated on 5% polyacrylamide/ 8M urea gel, transferred onto the membrane and probed for fully edited and pre-edited RPS12 mRNAs, 9S and 12S mitochondrial rRNAs, and cytosolic 18S rRNA as loading control.

Figure S3. Additional Controls for the *in vitro* 3′ Adenylation-Uridylation Reaction
(A) Purified recombinant KPAP1 and RET1 were resolved on 8%-16% polyacrylamide-SDS gel and stained with Sypro Ruby.
(B) Proteins were added to the reaction mixture containing 5′-labeled 80-mer RNA, only UTP or only ATP. The reaction products were separated on 8% polyacrylamide/ 8M urea gels. Note similarity of extension profiles with Fig. 3A.
(C) To verify the A/U composition of long extension products (Fig. 3C), the following reactions were set up: 1) pure U-tails addition by RET1; 2) pure A-tails addition by yeast poly(A) polymerase (6 kU/ml, USB) and 3) A/U-addition by RET1, KPAP1 and KPAFs in the presence of [α-^{32}P]ATP and UTP). The NTP concentrations were reduced from 50 to 10 μM to decrease the processivity of all reactions. After incubation for 5, 15 and 45 minutes reactions were terminated by adding EDTA to 15 mM and aliquots were digested with RNase A (1 μg/ml) for 15 min at 37 ^{o}C. Undigested and digested products were separated on 12% polyacrylamide/ 8M urea gel alongside the 5′ labeled substrate RNA 80-mer. As expected, the substrate RNA, pure U-extensions and A/U-extension were efficiently degraded while the pure A-tails were refractory to RNase A digestion.

Figure S4. Rapid Affinity Purification of Ribosomal Subunits in the Presence of Puromycin
Purification was performed in a parallel with experiment shown in Fig. 5 except that puromycin was added to cell extract at 0.1 mg/ml. RNA was eluted from magnetic beads and separated on a 5% polyacrylamide/8M urea gel. The membrane was probed for fully edited and pre-edited RPS12 mRNAs, 12S and 9S mitochondrial rRNAs, and cytosolic 5.8S rRNA as contamination control. [dT], total RNA was treated with RNase H in the presence of 18-mer [dT] to remove poly(A) tails. Beads, IgG-coated magnetic beads were incubated with extract from parental cells. M, RNA length marker in nucleotides. In contrast to Fig. 5C, note nearly complete absence of 12S rRNA in purified SSU.

Figure S5. De novo Synthesis of Cytochrome *c* Oxidase Subunit 1 and Cytochrome *b* is Undetectable in Bloodstream Form Parasites
Analysis of translation products in "single marker" (Wirtz et al., 1999) strain of BF *T. brucei*. Cells were labeled with EasyTag labeling mix (PerkinElmer Life Sciences) in isotonic buffer supplemented with 0.1 mg/ml of cycloheximide. Cells were collected by centrifugation, dissolved in SDS gel loading buffer and fractionated by two-dimensional electrophoresis. Gels were stained with Coomassie Brilliant Blue R250 (inset panels) and exposed to X-ray film (large panels).

Supplemental Database File Legend

Cytoscape Database File S1.
Protein interaction network (Fig. S1) was constructed in Cytoscape program available for free download at http://www.cytoscape.org/.

Supplemental Table Legends

Table S1. Proteins Identified in TAP-purified Complexes
Number of unique peptides for each polypeptide is indicated. Probability of mitochondrial localization, as predicted by Mitoprot (http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html), and structural motifs identified by similarity searches are indicated. Proteins detected in the putative RNA binding complex 1 (Panigrahi et al., 2007) are designated as MRB1.

Table S2. Pentatricopeptide Repeat (PPR)- and Tetratricopeptide Repeat (TPR)-Containing Proteins in Trypanosoma brucei
KPAF: kinetoplast polyadenylation/uridylation factors; KRIPP: Kinetoplast Ribosomal PPR-repeat containing protein. PPR proteins predicted by data mining (Pusnik et al., 2007) are indicated as (+). KRIT: Kinetoplast Ribosomal TPR-repeat containing protein. PPR and TPR proteins from mitochondrial ribosomal preparations investigated by (Zikova et al., 2008) are indicated as (X).

Table S3. Proteins Identified in Purified Ribosomal Particles
Number of unique peptides for each polypeptide is provided. Peptide counts obtained from ultra-rapid- and tandem affinity-purified samples are referred to as "beads" and "TAP", respectively. The "TAP" columns are reproduced from Table S1. Unique peptide counts obtained by (Zikova et al., 2008) for TAP-purified ribosomal subunits are provided as reference. (+): Proteins detected in density gradient-purified mitochondrial ribosomes from *L. tarentolae* (Maslov et al., 2006; Maslov et al., 2007).

Table S4. Common Contaminating Proteins
Based on inferred functions, structural motifs and network analysis, listed proteins have been excluded from consideration as potential components of mitochondrial RNA processing and translation complexes.

Table S5. Oligonucleotides used in this study

Name	Sequence, 5′-3′	Polarity	Application
Northern Blotting			
A304	TGAACAATCAATCATGGTAATAAGTAGACGATG		oligo probe 12S rRNA
A504	ACGGCTGGCATCCATTTC		oligo probe 9S rRNA

A 851	GGAAGCCAAGTCATCCATCGCGACACGTTGTGGGAGCC GTGG		oligo probe 5.8S
A872	GGGGACCATTCGGACTGCAGCCG		Oligo probe $tRNA^{Cys}$
A798	TAATTAAATCTTCTCATTGTCACTGTCTTATACTACGATT GAGTTTGTAT		Oligo probe CO3 gRNA [147]
A343	TGGTAAAGTTCCCCGTGTTGA		Oligo probe 18S rRNA
A359	CGACGGAGAGCTTCTTTTGAATA	sense	unedited RPS12 mRNA
A360	CCCCCCACCCAAATCTTT	anti	unedited RPS12 mRNA
A357	CGTATGTGATTTTTGTATGGTTGTTG	sense	edited RPS12 mRNA
A358	ACACGTCGGTTACCGGAACT	anti	edited RPS12 mRNA
A302	ACTAAGCAACCAAATCCTCCAATAAACATTC	sense	CO1 mRNA
A301	TGCCTATAACTATGGGTGGGTTTACAAAC	anti	CO1 mRNA
B108	ATAATTATCATATCACTGTCAAAATCTGATTCGTTATCG GAGTTATAGTATAT	sense	gA6[14]
A209	AAATATGTTTCGTTGTAGATTTTTATTATTT	sense	Cyb
A208	CCCATATATTCTATATAAACAACCTGACA	anti	Cyb
A213	CAAACCAACAAACAAATACAAATCAAAC	sense	A6 eidted
A212	GATTTATTTTGGTTGCGTTTGTTATTATG	anti	A6 edited
qRT-PCR			
A345	TGACGCCGGACACAACAG	anti	β-tubulin
A344	TTCCGCACCCTGAAACTGA	sense	β-tubulin
A343	TGGTAAAGTTCCCCGTGTTGA	anti	18S rRNA
A342	CGGAATGGCACCACAAGAC	sense	18S rRNA
A503	ATTAGATTGTTTTGTTAATGCTATTAGATG	sense	9S rRNA
A504	ACGGCTGGCATCCATTTC	anti	9S rRNA

A304	TGAACAATCAATCATGGTAATAAGTAGACGATG	anti	12S rRNA
A303	GGGCAAGTCCTACTCTCCTTTACAAAG	sense	12S rRNA
A302	ACTAAGCAACCAAATCCTCCAATAAACATTC	anti	CO1
A301	TGCCTATAACTATGGGTGGGTTTACAAAC	sense	CO1
A296	AGATAATTCAGTAACAAGGCCAGCAACAAG	anti	ND1
A295	GGACTGCTTCTTGATGGATTACGTTTACC	sense	ND1
A298	TGCTATAAATACTAAACCCAACACAATTACACTATC	anti	ND4
A297	CAATCTGACCATTCCATGTGTGACTACC	sense	ND4
A300	GCGTGTATTAATGCTGATACTGGGATAGG	anti	ND5
A299	TTTCTATATGTTTGTTAGTAGGATGTGCGTTC	sense	ND5
A294	AAAGCCAATACAAATACAAAGGTAACTTAG	anti	Murf1
A293	GTTTACTACTTGCATGTCTCTTTCTTTG	sense	Murf1
A312	ATTACAGTGTAACCATGTATTGACATT	sense	CO2 pre/ed
A313	TTCATTACACCTACCAGGTTCTCT	anti	CO2 pre
A314	ATTTCATTACACCTACCAGGTATACAA	anti	CO2 ed
A203	GAAACCAGATGAGATTGTTTGCA	sense	CO3pre
A204	TTCATTCCAACTAAACCCTTTCC	anti	CO3pre
A205	TTGTGTTTTATTACGTTGTATCCAGTATTG	sense	CO3ed
A206	CGAAAGCAAACTCACAACACAAA	anti	CO3ed
A352	AACAAATCTCTTTACCCCCTTCAG	anti	ND3pre
A351	GAATGGGAGATGGGTTTTGG	sense	ND3pre
A292	CGTTGTTGTTTGTGGTTT	sense	ND3ed
A293	ACAAATAATGGAATTTAACAATACA	anti	ND3ed
A353	GCATCCCGCAGCACATG	sense	ND7pre
A354	CTGTACCACGATGCAAATAACCTATAAT	anti	ND7pre
A355	GCGGGCGGAGCATTATT	sense	ND7ed
A356	GATCTACGGTCCCCTCTTTCCT	anti	ND7ed
B183	AAGCCCATTTTGAGCAGGAG	sense	ND8pre
B184	TTGGCAAAAATCTGTCGGGC	anti	ND8pre
B266	GTTGTATTGCTTGTCGTT	sense	ND8ed
B267	ACAATGAATGCGTAATGG	anti	ND8ed

B187	AACATCGAGGAGTTTTGGGG	sense	ND9pre
B188	TTAAGGTTGCCCTGTTGTCG	anti	ND9pre
B268	TGTTGAAGTGTTATCCATT	sense	ND9ed
B269	TTAGAATTACAACGGTGAA	anti	ND9ed
A358	ACACGTCGGTTACCGGAACT	anti sense	RPS12ed
A357	CGTATGTGATTTTTGTATGGTTGTTG	sense	RPS12ed
A360	CCCCCCACCCAAATCTTT	anti sense	RPS12pre
A359	CGACGGAGAGCTTCTTTTGAATA	sense	RPS12pre
A207	ATATAAAAGCGGAGAAAAAAGAAAG	sense	Cybpre
A208	CCCATATATTCTATATAAACAACCTGACA	anti	Cybed/pre
B274	ATATAAATATGTTTCGTTGTAGATT	sense	Cybed
B275	CTAAACACACTCCACAAAT	anti	Cybed
B260	TTGCCGCCATATTACAGT	sense	A6ed
B261	TCTATAACTCCAATAACAAACCAAAT	anti	A6ed
B262	GAGAAGCAAGGAGGAGAA	sense	A6pre
B263	GCAAAGGCAATTCCCAAT	anti	A6pre
A348	GATTTTAATGTTTGGTTGTTTTAATTTAG	sense	Murf2pre
A347	AATATAAAATCTAGATCAAACCATCACA	anti sense	Murf2ed/pre
A346	GATTTTAAGATTGGCTTTGATTGA	sense	Murf2ed
B877	CTCTCAACACGATATGGCACATCTC	sense	KPAF1/PF
B878	AATCACTGCTGTCATTCTCCTTATACG	anti	KPAF1/PF
B298	GTGTTCCAAGCGGCGTTTAGGG	sense	KPAF1/BF
B299	GGCGAGCGTAGGCGGAGATTAG	anti	KPAF1/BF
A895	CCAGGGAGCGAGGGTAGATTTGTC	sense	KPAF2
A896	CCGTAGCGTGTCCATCAATCCATAAC	anti	KPAF2
A907	TGGCAGGTGGTGAGCGGAATG	sense	KRIPP1
A908	TCGGCGTACAAGATGCGGATTAGG	anti	KRIPP1
3′ End Analysis – cRT-PCR			
A394	CATTTTATGAGAAACACTTAAGCACAC	anti	CO1
CO1 lower	TTTTGACAAGCCTTCCTATTATTC		CO1

A398	ATAATAAAAAAAATAATAAAAATCTACAACGAA	sense	Cyb edited
Cyb lower	ATTATTATTTTTGTTGATAGTGTGTAGG	anti	Cyb edited
Plasmid construction			
A666	ATATCTAGAGTGTGAGTAACGCCTGGGATG	sense	KPAF1
A667	ATATCTAGAACCTTCCGTGTGAAAAGGTG	anti	KPAF1
A727	ACTAAGCTTCTTAGCAGACACCGCATTGAGGAGGT	sense	KPAF2
A728	AGCTCTAGACCATCACGTGGAACATTCAGTTTCTCC	anti	KPAF2
B294	GCGAAGCTTTGATTGTGCTGCAGGAGTTCC	sense	KPAF1
B295	CCGTCTAGATAAATCATCCGGCTCCAAACG	anti	KPAF1
A639	GACCTAGGATGTTCCAACGCCGGTTAG	sense	KPAF1
A781	CTCTCGAGAACATCCGCACCGGCAGCCATCTCC	anti	KPAF1
A784	GAAAGCTTATGTTTGCCTGTCGTCGGCTACTGGGACT	sense	KPAF2
A785	GACTCGAGCCACCGTCTTTTGCGTCGCGTAGCA	anti	KPAF2
A914	GAATTAATATGTTCCAACGCCGGTTAGTGCTGTGC	sense	KPAF1
A915	CTAGATCTCTAAACATCCGCACCGGCAGCC	anti	KPAF1
A780	CTAGATCTCTAAACATCCGCACCGGCAGCC	sense	KPAF1
B70	GCAGGATCCATGTTTGCCTGTCGTCGG	sense	KPAF2
B71	CGTAAGCTTTTACCACCGTCTTTTGCGTC	anti	KPAF2
B11	ACAAAGCTTATGCGCTCGCTCATAGC	anti	L3
B12	ACAGGATCCCTTCATACGTTTGTACTTCAG	sense	L3
B13	ACACCTAGGATGCTTCGGCGATCAC	anti	S17
B14	TGTCTCGAGAGGAGACTTCTTAAAGCTCTTG	sense	S17

Supplemental Experimental Procedures

Plasmid Construction

The RNAi plasmids were generated by amplifying the KRIPP1, KPAF1, KPAF2 gene fragments with A739/A740, A666/A667 and A727/728 primers, respectively, followed by cloning into p2T7-177 (Wickstead et al, 2002). For expression of TAP-tagged proteins: L3, S17, KRIPP1, KPAF1 and KPAF2 genes were PCR-amplified from genomic DNA using B11/B12, B13/B14, A639/A781, A784/A785 primer pairs, respectively, and inserted into MH-TAP vector (Jensen et al., 2007). For expression in E. coli, KPAF1 gene was amplified from genomic DNA with primers A914/A915 and cloned into pET15b vector (Novagen). To generate construct for co-expressing KPAF1 and KPAF2, corresponding genes using A780/A781 and B70/B71 pairs of primers, respectively, and cloned into pETDuet-1 vector (Novagen).

Total RNA Isolation

Procyclic cells were grown in SDM 79 medium supplemented with 10% FBS and 0.01 mg/ml hemin chloride and necessary drugs to 4-6×10^6/ ml. Bloodstream cells were grown to ~10^6/ml in HMI-9 medium. Unless otherwise specified, 50 ml or 250 ml of respective cultures were centrifuged at 3000g for 10 min. Pellets were washed with ice-cold PBS, spun down under same conditions and stored at – 80^0C. Frozen cell pellets were immediately re-suspended in 4 ml of ice-cold Buffer D (4M guanidine isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5 % sarcosyl, 0.1 M β-mercaptoethanol). Cell lysate was mixed with 0.5 ml of 2M sodium acetate (pH 4.0) and 5 ml of water-saturated phenol. 1.5 ml chloroform/isoamyl alcohol 49:1 was added and mixture incubated for 15 min with gentle rocking at ambient temperature. After centrifugation at 10,000 g for 15 min, the supernatant was transferred into fresh tube and extracted with 5 ml of chloroform/isoamyl alcohol. RNA was precipitated with 5 ml of isopropanol at -20^0C for 1 hour, collected by centrifugation at 10,000 g for 15 min and washed with 80 % ethanol. The pellet was re-dissolved in 400 µl water and precipitated with ethanol by standard techniques. Expected yield is 600-800 µg of RNA with $OD_{260}/_{280}$ ratio of 1.6-1.8. For Northern blotting, RNA was treated with DNase I (Invitrogen) following the company's protocol. For real-time PCR and cRT-PCR, additional purification step with RNase Easy (Qiagen) columns was included.

Northern Blotting

The 20 cm-long, 8 mm-thick 1.8 % agarose formaldehyde gels were prepared in 1x MOPS buffer as described (Sambrook et al., 1989) and run for 3 hr at 100 V. The polyacrylamide/8M urea gels (5% for mRNA blotting and 10% for gRNA blotting) were prepared in 20x20x0.15 cm format, and run at 20 W for 2.5 hr. For agarose gels, 10 µg of RNA was resuspended in 20 µl of 1x loading buffer (Ambion). For acrylamide gels, 10 µg of RNA was resuspended in 95 % formamide, 10 mM EDTA, 0.05 % xylene cyanol, 0.05 % Bromphenol Blue buffer. Samples were heated at 65 ^{0}C for 5 min prior to loading. To visualize molecular mass markers on the membrane, 1 µg of the 0.1-2 kb RNA ladder (Invitrogen) was labeled with T4 RNA ligase and [α-^{32}P]pCp. RNA on agarose gel was transferred on to the BrightStar-Plus membrane (Ambion) in 10 × SSC, 10 mM NaOH buffer for 2 hours using vacuum blotter at 5 Psi (model 785, Bio-

Rad). RNA from the acrylamide gel was transferred to the same membrane in ½ TBE buffer for 2 hours at 90 V using a cooling blotter unit (TE series, Hoefer). The membranes were exposed twice to UV light at 120 mJ/cm^2 using HL 2000 HibriLinker (UVP) and stained with 0.04 % of Methylene Blue in 0.5 M Na Acetate (pH 5.0) to check transfer.

Following RNA transcripts were detected with single-stranded DNA probes produced by asymmetric PCR: COI, Cyb, A6 and RPS12 (edited and unedited). The template for asymmetric PCR was generated with oligonucleotides used for real-time RT-PCR analysis and gel-purified. The PCR reactions were set up with 10 pmol of 5′ labeled oligonucleotides and ~ 0.5 pmol of template. Membranes were pre-hybridized for 2 hours at 55 ^{0}C temperature using HL 2000 HibriLinker (UVP) in 20 ml of 6 × SSPE, 5 × Denhardt's solution, 0.5 % SDS, 0.2 mg/ml of denatured salmon sperm DNA. Hybridization was performed overnight in 4-5 ml of 6 × SSPE, 5 × Denhardt's solution, 0.5 % SDS, 20 µg/ml of yeast tRNA(Sigma), 3-4 × 10^6 cpm/ml of DNA probe. Membrane was washed 3 times with 2 × SSC, 0.1 % SDS solution at 55^{0}C and 1 time with 1 × SSC, 0.5 % SDS at 55^{0}C – 65^{0}C (temperature was increased if background appeared or near-melting point stringency was required, as in the case of 5′ pre-edited RPS12 mRNA). Membranes was stripped by boiling for 20 min in 0.1 % SDS and stored in plastic bag at -20^{0}C. Guide RNA and tRNA hybridizations with oligonucleotides were performed at 42^{0}C in ULTRAhyb -Oligo Hybridization solution as recommended by the manufacturer (Ambion).

Quantitative RT-PCR

cDNA was synthesized from 2 µg of total RNA with Applied Biosystems RT-PCR kit as recommended by manufacturer. qPCR reaction were performed SYBR® Green-Based Detection System from the same supplier. Samples were run in triplicate and the Ct values from each sample were averaged. Nuclearly encoded β-tubulin mRNA and 18s rRNA were used as internal normalization controls.

Reactions were carried out in the Eppendorf Realplex2S cycler under following conditions: 50°C for 2 min., 95°C for 10 min., followed by 45 cycles of 95°C for 15 sec. and 60°C for 1 min. Thermal dissociations were used to confirm generation of a single amplicon. PCR products were also analyzed by electrophoresis in 2.0% agarose gel. Oligonucleotide primers were designed with Beacon Designer 3 and purchased from Invitrogen. Relative changes in target amplicons were determined by using the Pfaffl method with PCR efficiencies calculated by linear regression using LinRegPCR (Ramakers et al., 2003).

Rapid Pulldown of TAP-tagged Proteins from Total Cell Lysate

Dynabeads were Conjugated with Rabbit IgG as described by (Oeffinger et al., 2007). PF cells were grown in 2 x 750 ml cultures to 10-15x10^6 cells/ml. Induction by adding tetracycline to 2 mg/L tet was initiated 1x10^6 cells/ml and cultivation continued for 60-72 hr. Cells were collected by centrifugation for 15 min at 5,000 g, washed twice with PBS+6 mM sucrose, frozen in N2 and kept at -80 ^{o}C. Expected yield is ~2 g (wet weight)**.**

Solutions

Lysis buffer: 50 mM Tris pH 7.8, 1% NP-40, 100 mM KCl, 10 mM $MgCl_2$, 1:50 dilution of Complete Protease inhibitor (Roche, stock is made by dissolving 1 tablet in 1 ml of water).

Wash buffer sans detergent: 20 mM Tris pH 7.8, 100 mM KCl, 10 mM $MgCl_2$
Wash buffer: 20 mM Tris pH 7.8, 100 mM KCl, 10 mM MgCl, 0.1% NP40.
TEV digestion buffer: 50 mM Tris-HCl, pH 8.0 (use 1M stock pH 7.5-7.6 at RT), 1mM EDTA, 100 mM KCl, 1 mM DTT, 0.1% NP40.

Cryo Powder Preparation

Pour N2 into grinder (Biospec Products, Cat. No. 206, Cryo-cup Grinder, use the smaller polypropylene pestle ball, 1 3/8'), poke the frozen pellet with spatula and transfer pieces into grinder cup with N2. Grind with nylon ball until cells turn into a very fine powder. This routinely takes 10-20 grinds, keep adding N2. Use N2 to transfer into N2-pre-cooled 50 ml tube. Powder can be stored at -80. The expected yield is ~1 g of powder.

Purification

All procedures are done at +4 ^{o}C. To 50 ml conical tube with frozen powder, add 3 ml of lysis buffer pre-warmed to 37 ^{o}C. Add 10 μl of TURBO DNase (Ambion) and incubate on Nutator at 4 C for 5 min. Add Wash buffer sans detergent to ~12 ml, incubate on Nutator for 5 min. Centrifuge extract for 10 min at 50,000 g. Immediately transfer the entire supernatant (including loose pellet) to 15 ml conical tube.

Pellet 20 mg of IgG-coated magnetic beads (Oeffinger et al., 2007) in 15 ml tube with a DynaMag-15 magnet (Invitrogen). Add extract and incubate on Nutator for 10 min. Pellet beads, remove supernatant with pipette. Rinse beads 1X with 12 ml of wash buffer with brief, mild vortexing, no incubation. Poor out washes while 15m-tube is held in the magnet, do not use pipette to remove wash buffer. Wash beads 5X with 12 ml of wash buffer for 5 min on Nutator. Transfer beads with wash buffer into 1.5 ml Eppendorf tube. Wash beads 2X with 1 ml of wash buffer.

For RNA isolation, resuspend beads in 100 μl of 1% SDS and incubate at 65C for 5 min with periodical mixing. Vortex, collect the beads, transfer the supernatant to a fresh tube. Wash the beads with 300 μl of 0.1 M NaoAc, pH 5.5. Vortex, collect beads, combine supernatants. Extract RNA with 400 μl of phenol/chloroform and with 400 μl of chloroform. Add 10 μg of glycogen and concentrate with sec-butanol to 100-150 μl. Precipitate RNA by adding 4 V of ethanol. For protein isolation, elute bound protein with 400 μl of TEV digestion buffer with 20U of TEV protease for 16 hr at 4 ^{o}C.

Analysis of Kinetoplast Translation in *T. brucei* Using Denaturing 2D Gels

Approximately 10^7 cells were collected in a 14 ml round-bottom sterile Falcon tube and washed twice with 2 ml of SoTE (0.6 M sorbitol, 20 mM Tris-HCl, pH 7.5, 2 mM EDTA) by centrifugation at 3000 g for 5 min at 20 ^{o}C in a swing bucket rotor. For bloodstream forms, the sorbitol buffer solution additionally contained 10 mM glucose (SoTEG). In the absence of glucose, bloodstream cells quickly loose the viability.

Pelleted cells were resuspended the in 90 μl SoTE (procyclics) or SoTEG (bloodstream parasites) supplemented with 1.5 μl 100 mM DTT and 1.5 μl of 10 mg/ml cycloheximide (freshly made with water) and incubated for 10 min at 27 ^{o}C with slow agitation (~100 rpm). Ten microliters of ^{35}S EasyTag Express Protein Labeling Mix (NEG772007MC) were added and incubation continued for additional 1 hour with slow agitation. Cells were spun down at 3000 g

for 3 min at 20 ^{o}C, resuspend in 100 µl of SoTE or SoTEG and transferred into a 1.5 ml Eppendorf tube.

Labeled cells were recovered by centrifugation at 14,000 g for 5 min, dissolved by vortexing in 100 µl of 2X loading buffer (2% SDS, 27% glycerol, 0.25 M Tris-HCl (pH 6.8), 2% β-mercaptoethanol, 0.01% bromophenol blue) and incubated for 30 min at 37 °C. Samples were cleared by centrifugation at 14,000 g for 15 min and separated on 1 mm-thick 9.5% Tris-Glycine SDS gel (Laemmli, 1970) (stacking gel: ~3 cm; resolving gel: ~15 cm) at a constant current not exceeding 20 mA. The gel slice (~5 mm wide) was incubated for 30 min at 37 °C in 0.125 M Tris-HCl, pH 6.8, 1% β-mercaptoethanol, 1% SDS and transferred onto 1.5 mm-thick 14% gel (same dimensions as 9.5% gel) cast with a single broad-well comb. Sample buffer (2X, 500 µl) was loaded across the well and the gel was run at a constant current not exceeding 30 mA (usually at 6 mA overnight).

Gels were fixed for 40 min in 10% Acetic Acid, 50% MeOH, stained in Coomassie blue R250, destained in 10% acetic acid, 10% MeOH, and scanned. For radioautography, gels were washed three times in water for 20 min. incubated in 1M salicylate for 60 min, dried and exposed to X-ray film at -80 ^{o}C for five days.

Mass Spectrometric analysis by LC MS/MS

LC MS/MS was carried out by nanoflow reverse phase liquid chromatography (RPLC) (Eksigent, CA) coupled on-line to a Linear Ion Trap (LTQ)-Orbitrap mass spectrometer (Thermo-Electron Corp). The LC analysis was performed using a capillary column (100 µm ID x 150 mm long) packed with Polaris C18-A resin (Varian Inc., CA) and the peptides were eluted using a linear gradient of 2% to 35% B in 85 min at a flow of 300 nL/min (solvent A: 100% H2O/0.1% formic acid; solvent B: 100 % acetonitrile/0.1% formic acid). A cycle of one full FT scan mass spectrum (350-2000 m/z, resolution of 60,000 at *m*/*z* 400) followed by ten data-dependent MS/MS acquired in the linear ion trap with normalized collision energy (setting of 35%). Target ions already selected for MS/MS were dynamically excluded for 30 s.

Monoisotopic masses of parent ions and corresponding fragment ions, parent ion charge states and ion intensities from the tandem mass spectra (MS/MS) were obtained by using in-house software with Raw_Extract script from Xcalibur v2.4. Following automated data extraction, resultant peak lists for each LC MS/MS experiment were submitted to the development version of Protein Prospector (UCSF) for database searching similarly as described (Wang and Huang, 2008). The *T. brucei* database (www.genedb.org, v4) was used for database searching. Trypsin was set as the enzyme with a maximum of two missed cleavage sites. The mass tolerance for parent ion was set as ±20 ppm, whereas ±0.8 Da tolerance was chosen for the fragment ions. Chemical modifications such as protein N-terminal acetylation, methionine oxidation, N-terminal pyroglutamine, and deamidation of asparagine were selected as variable modifications during database search. The Search Compare program in Protein Prospector was used for summarization, validation and comparison of results. To determine the expectation value cutoff that corresponds to a percent false positive (% FP) rate, each project was searched against a normal database concatenated with the reversed form of the database. An algorithm in Search Compare automatically plots the expectation values versus % FP rate for each search result. Based on these results, we chose an expectation value cutoff for all peptides corresponding to ≤0.025% FP. At this false positive rate, false protein hits from decoy database was not observed. General protein identification is based on at least two peptides.

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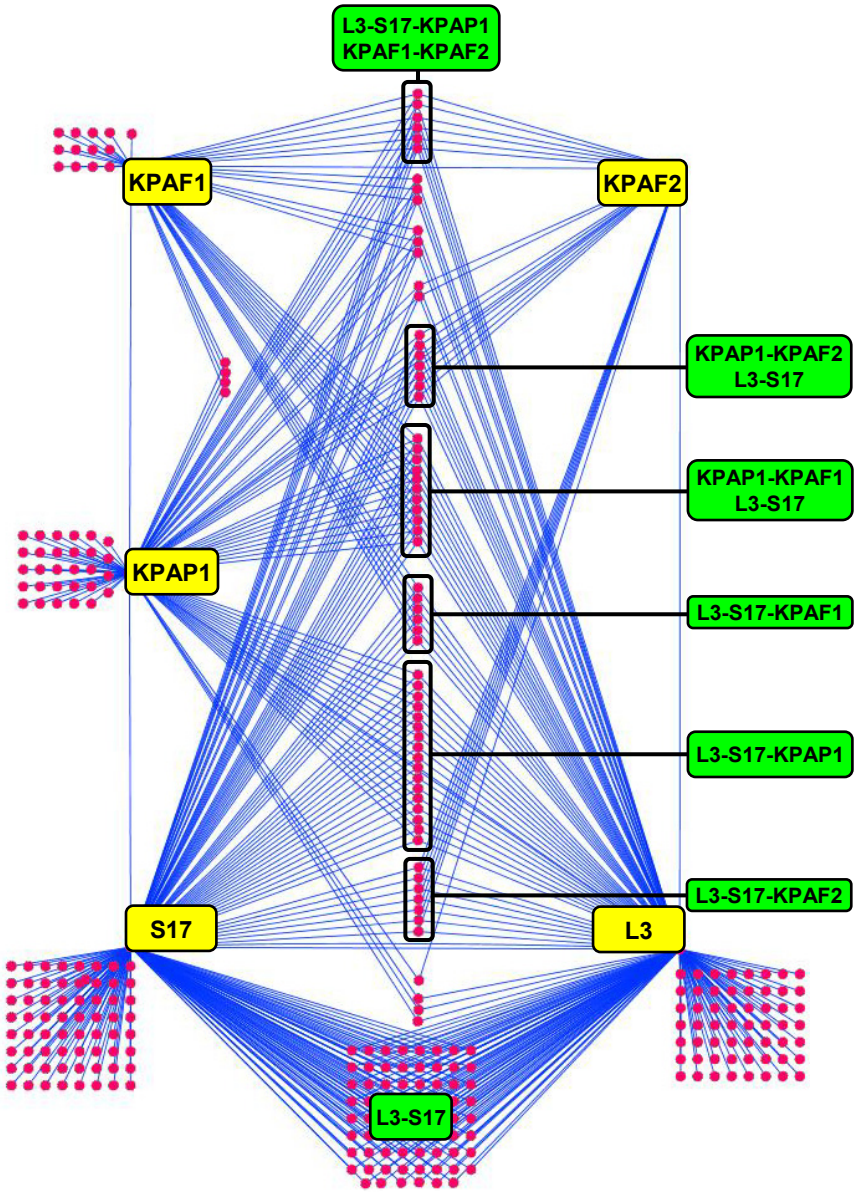

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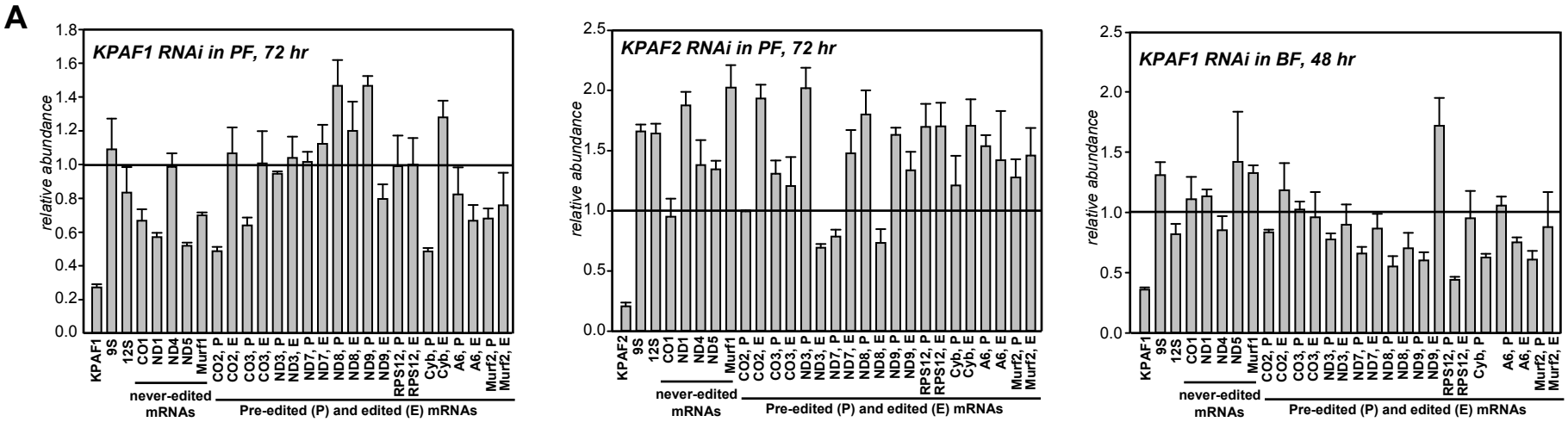

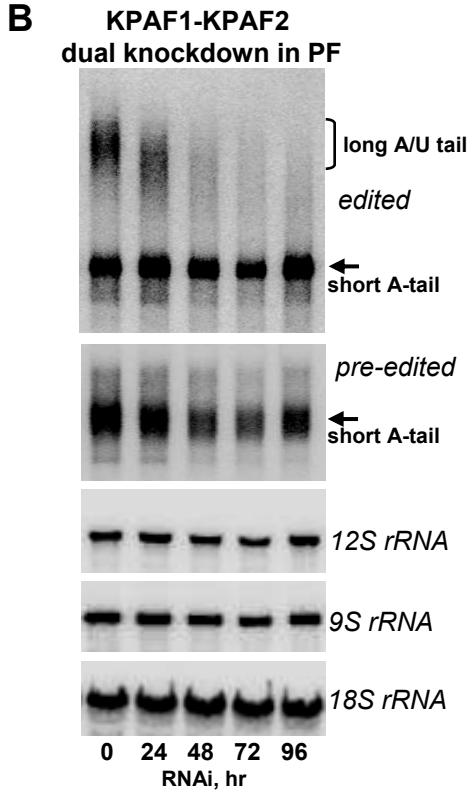

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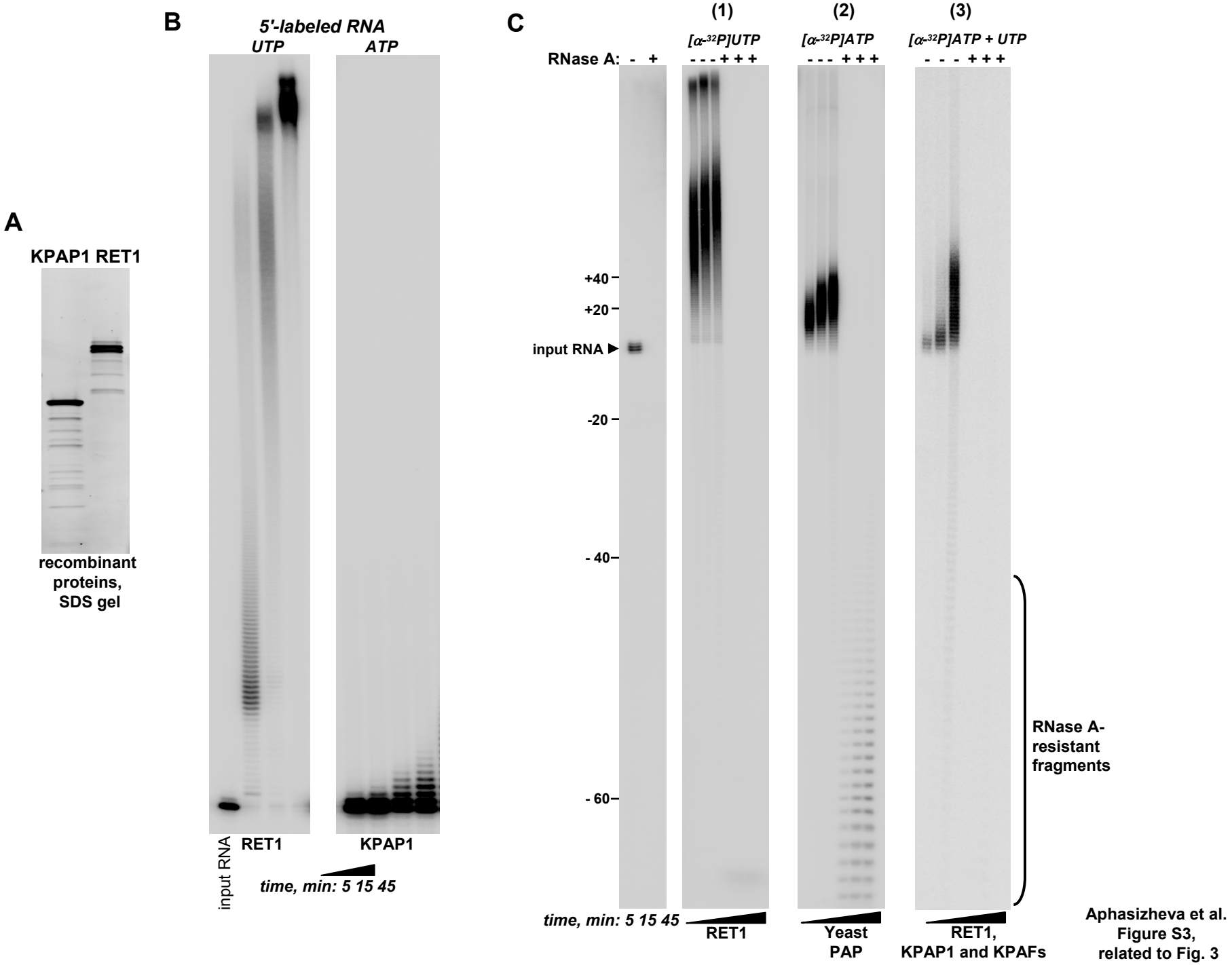

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Figure S3,
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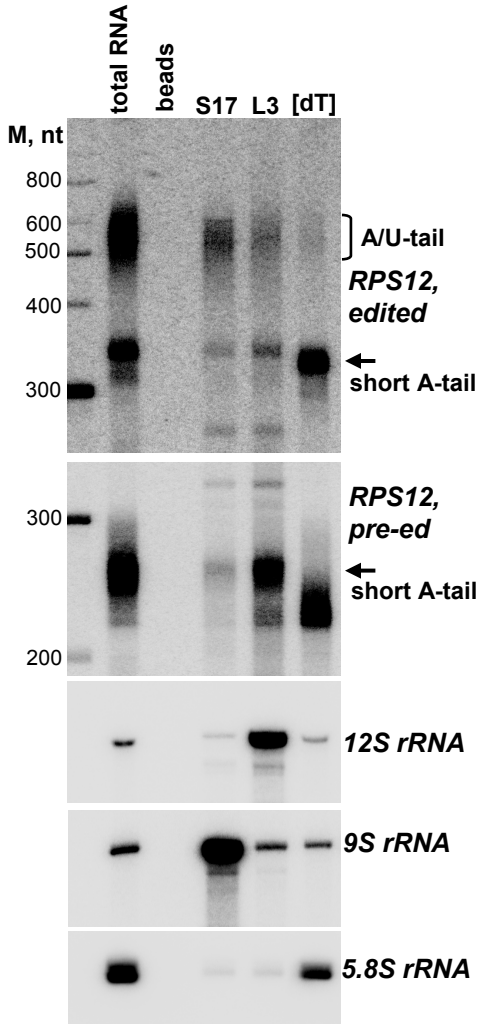
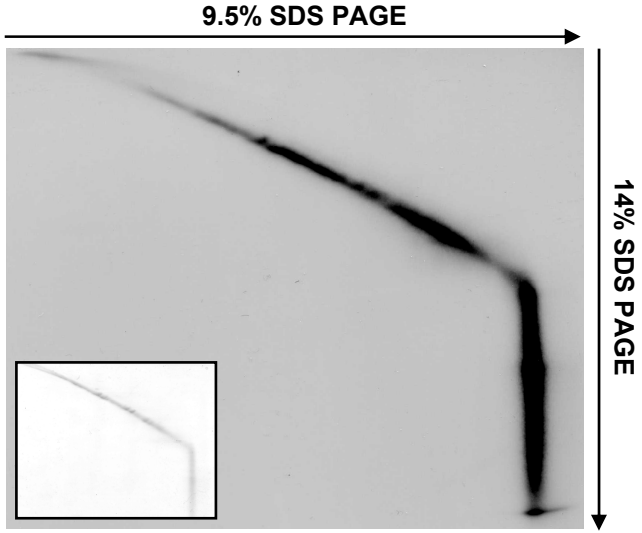


Aphasizheva et al. Figure S4, related to Fig. 5



Aphasizheva et al. Figure S5, related to Fig. 6