Supporting Information

The vault RNA of *Trypanosoma brucei* plays a role in the production of *trans*-spliced mRNA

Nikolay G. Kolev[1*], K. Shanmugha Rajan[2], Kazimierz T. Tycowski[3], Justin Y. Toh[1], Huafang Shi[1], Yuling Lei[1], Shulamit Michaeli[2], and Christian Tschudi[1]

From the [1]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, 295 Congress Avenue, New Haven, CT 06536, USA; [2]The Mina and Everard Goodman Faculty of Life Sciences and Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Ramat-Gan 52900, Israel; [3]Department of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06536, USA

Running title: *Trypanosome vtRNA affects mRNA metabolism*

*To whom correspondence should be addressed: Nikolay G. Kolev: Department of Epidemiology of Microbial Diseases, Yale School of Public Health, 295 Congress Avenue, New Haven, CT 06536, USA; nikolay.kolev@yale.edu; Tel.(203) 785-3556; Fax. (203) 785-7329.

Includes:

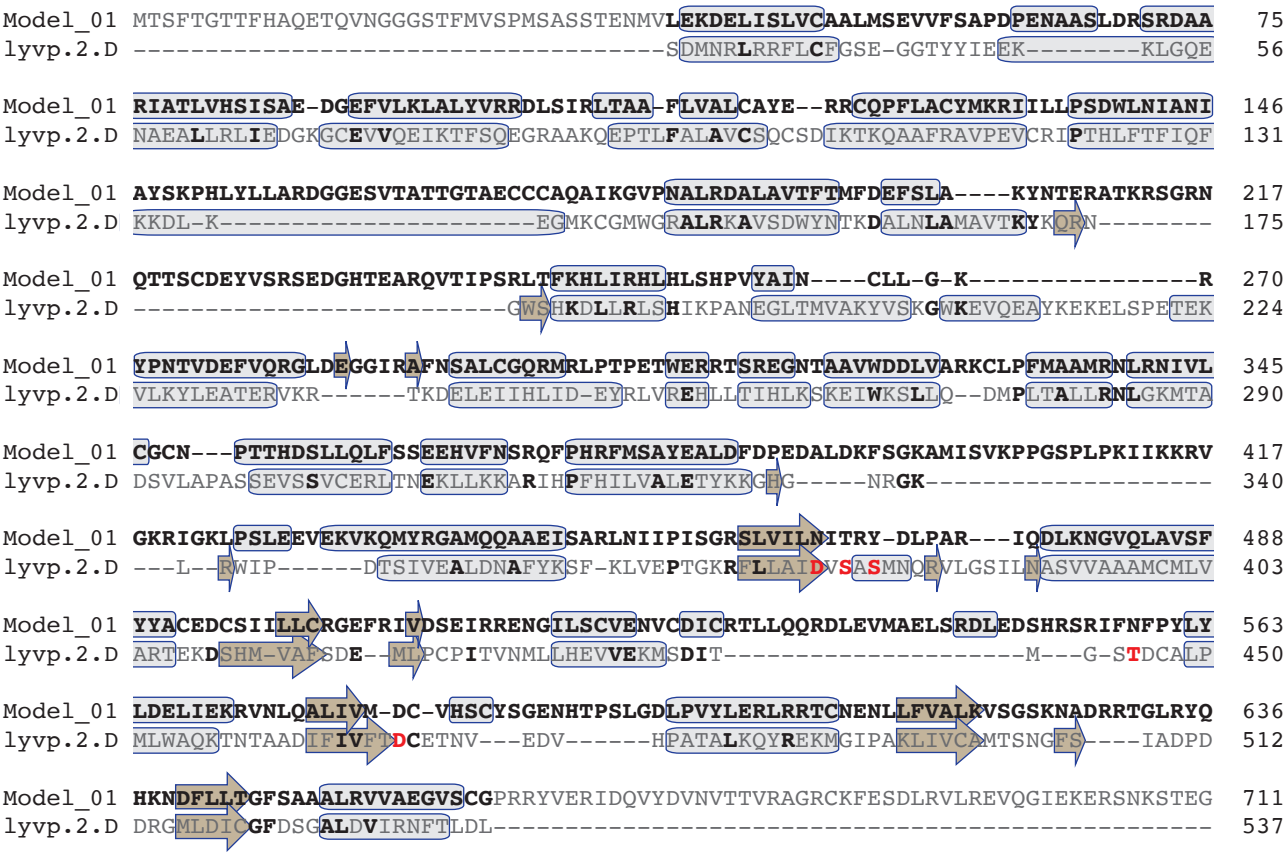

Figure S1. The von Willebrand factor A (vWFA) domain in *T. brucei* TEP1 lacks obvious metal ion-dependent adhesion site (MIDAS) motif. A SWISS-MODEL (Waterhouse *et al.*, 2018) sequence/structure alignment of the modeled *T. brucei* TEP1 TROVE and vWFA domains with the *X. laevis* Ro60 TROVE/vWFA domains. The highly conserved MIDAS motif in the *X. laevis* vWFA domain (D-x-S-x-S…T…D) is highlighted with red amino acids. Except the last aspartate in the motif, the identities of the amino acids in the corresponding positions in the *T. brucei* TEP1 vWFA are different.

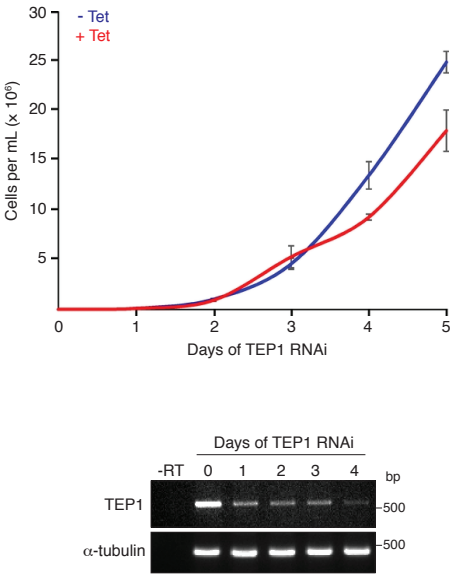

Figure S2. Effect of targetting TEP1 by RNAi on growth of procyclic cells. Uninduced cells carrying the TEP1 silencing construct (-Tet) were compared with cells induced for silencing (+Tet). Error bars, SEM; n=3. Semiquantitative RT-PCR assay is shown below for TEP1 and α-tubulin mRNAs. Reactions without reverse transcriptase (without RNAi induction) are shown in the first lanes (-RT).

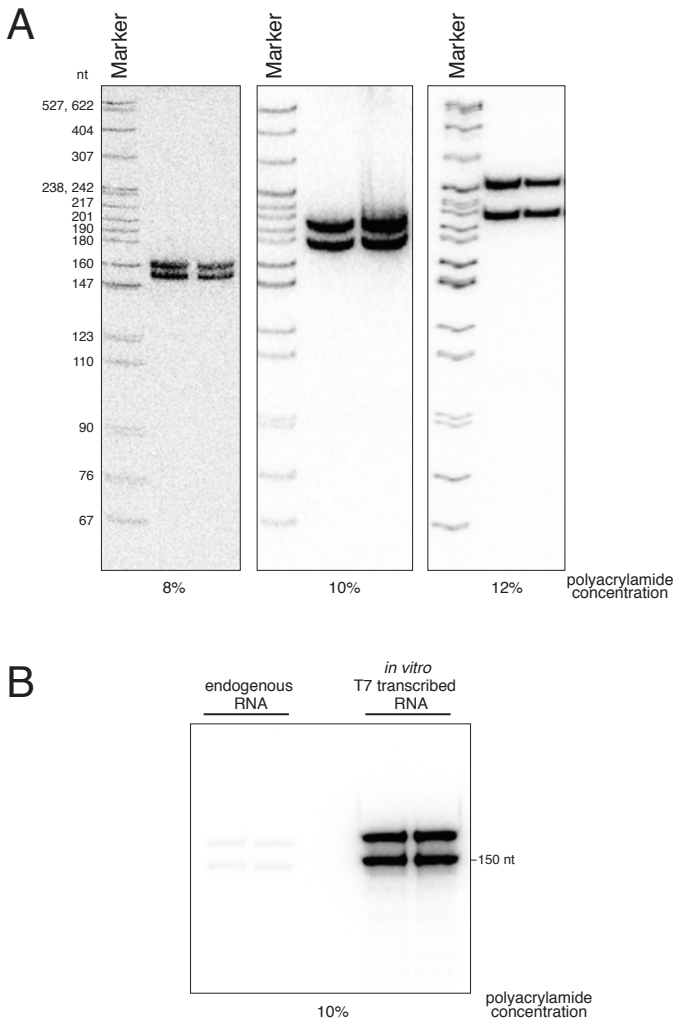

Figure S3. Aberrant migration of *T. brucei* vtRNA in denaturing polyacrylamide gels. *A*, Northern blots of vtRNA in *T. brucei* total RNA samples subjected to electrophoresis in polyacrylamide gels of the indicated percentages in the presence of 8M urea. Marker is a ^{32}P-labeled pBR322 DNA MspI digest. Portion of the middle panel is also shown in Fig. 1*A*. Note the difference in mobility in different percentage gels relative to the marker bands; *B*, comparison between the electrophoretic mobility of endogenous vtRNA (left) and vtRNA synthesized *in vitro* by T7 RNA polymerase run-off transcription (right).

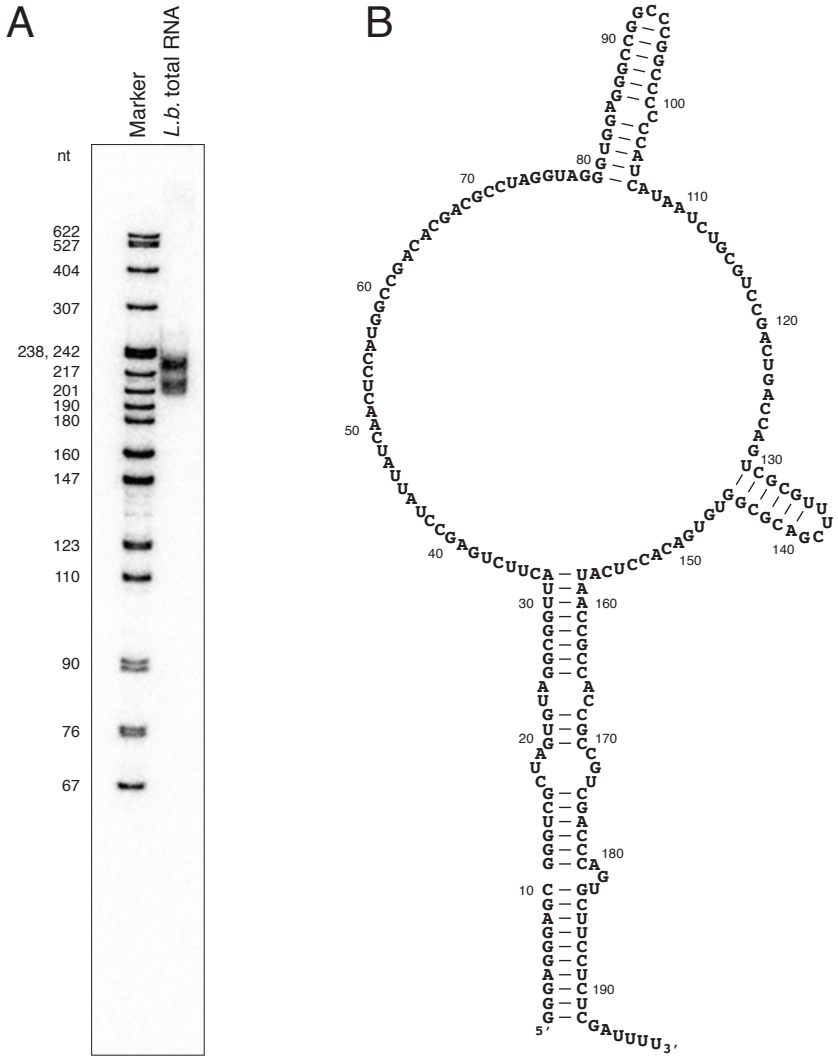

Figure S4. vtRNA of *Leishmania braziliensis*. *A*, Northern blot of total RNA from *L. braziliensis* with ^{32}P-labeled antisense oligodeoxyribonucleotide against the bioinformatically predicted *L. braziliensis* vtRNA. Marker is a ^{32}P-labeled pBR322 DNA MspI digest; *B*, predicted secondary structure model of vtRNA. Shown as double-stranded are only the terminal bulged helix and very stable hairpins emanating from the large internal loop.

***T. brucei* U2 snRNA gene**

CCAGCTGGTGAAACAACCATTCCTCAATCGGGGCGCGCTCCCTGCCATAGAGTGCTCAGTTCAATTTCACCGCAGTG
ACCGTCGCCGTAACCGCCGCCAGACTGCATCCACACGATAGAGGCACCAGGCTCCAACAGCCTGTTCAAAATTACCA
CCACAACCTGTAGTGGCGGTATCTTTCCAGGAAAGATTGGCCGCGTCTTGAGGCGCCGGGAGAAAAAAACATATGTA
CGGAGGTTCGAACCTGCGGCCCCCAAGACGTAAGCACGGAAGTGCAATTTACGACACCTGGTCCATTGCATCGACGG
AGCCCACCCGAATATATAATTACTTATTGAATTTATTCTTCGGTATTTCCTAGCTTGTTGCAGCCTACGGAACTTTT
GGATAAGGCGCTGCATATCTTCTCGGCTATTTAGCTAAGATCAAGTTATTAAACTGTTCTTATCAGAGTAACTCCTG
ATACGGGCCTTTGGCCCAAGGATCAAAACTGTTGGCTGTCCCGCGTTCTTCCGGGGTTCCACTTGTCCGGATGGAGC
GCGACGGTCGCAAACGTTTTCCGACAAAACCAACCACTGAACGTTGTAAGGCAGTTATTGGAACGACCCGCATTTCC
CATCACTTTATTCTCGCTTGGTGTATAC

***T. brucei* vtRNA gene**

CCTGGAACGCCGCATCCGAGGCCCCGCTACGATCGCGTGTTTTCAACGAGCGGAAGCGTGCCTCTCGAGATGAACGA
AGAAATGAGGAACACTATCCTCCAACAGTAGTGTCTATTGTGTTCCCGAGATTACCAAACTCACGAAACCAAATCAG
ATTCGGACAAACGGCGCACCCTCCGCAAATCCCGCCGGGCTTCCTTTCCACAACACAAAGCAGGTGCCGGAAGGTGA
ATAAAAAAGAGGGAAAAGTGGTAATCGAACCTTCAGCATCAATGGTAAAGCCACTCCACCATAACAACGGGACCGCG
ACGTAAATAAATAATCTAGCAATTAACCTTTTAATCAGCTAGAACCCCTTTGAATAGGAACCCGTGGAAAGCCATAC
GCCCGGGGCGGGCGCAAGGATTTCGGATACTTCTGCATAGCAAACAGCAAAGGGGCGTAGCTGTCGCCGAGGGAGGG
CACCGACGCCTTCCCCCCACCCCGTGGCCCTCGGCCGCACTGGACGCAAGTCCACCCCCTGTCCGAAGCCGCAATCG
CCCTTTTCGCACGACGACTCGCATCAACATTGCGTTTCAGTGTGCATTTATCTTTCCCCTTCATTCCTCAGCACACC
GCTTGAAGGGGATACCGCAGCTGGATTC

***L. braziliensis* tRNA^{Ala} and U2 snRNA genes**

AAACTCCTTCTTTCCCGGACAAGCACCGCCCCCTCCCCTTACAATGACGAGCCGGGCCCTCTGGCCAGACCAAAAAA
AAAATGGAGAAGTTGGGTATCGATCCCAATACCTACCGCATGCTAAGCGGTCGCTCTACCATCTGAGCTACATCCCC
ACAACTGCTTCTCTTAAAAGGTCGATACGTTCAAAATAATGTGTTGGTGTAGTGGATCGAACCCGCGACCTAGTCGG
CAGTGCACTGCGGCGGCGCCCGTTGAACTACAAAACACGCTCGGCAGGAACGTCAGCTAACAGCGGCGCACCCCAGG
ACAAAACGGTGTAAGCCATGTGGTAGCCTCTCTGCTTTTGTGAACGCCTCGTGTAGCATATCTTCTCGGCTATTTAG
CTAAGATCATGTTTATAAACTGTTCTTATCAGAGTAACTCCTGATACTGCCTTCGGGCAAAGGAATAGAAATTATAA
GTCGAGGTTGTTTCCCTGGAGCTCCACCTTTCCAGGGGAGCAACTTCTTTTTTTTCCCGGGGCACAGCATCTTCAGC
AGAAGCTTGGCAAGCGTGCATTGCCCGCGGGCGGATGGTGGAACGAAGGCTGCTCCTGGGG

***L. braziliensis* vtRNA gene**

ACGACGAAAGAGATGTGCTATTTTTCGGCTCGGCTGGGTCTTTGTAAAAATTGACAGGAGCGGGGTTCGAACCCGCG
CCTACGAATAGAAGAGAACTTGAGTCTCCCGCCTTAGACCACTCGGCCATCCTGCCTTGTGGTTGTACGCTCTTTGC
TTCCTACCCACTGACGAAGTTAGCCGGAAAAATCGTCACCAGCAGGATTCGAACCTGCGCGCGAGATCGCAGTTGAT
TTCGAGTCAACCCCCGTAACCACTTGGGTATGGTGACCACGGTACGACGGGGATATGAATTGCCGTGTGGGTGAGGA
GGAAAAGGGCGATTGACGTTTGAGCTTGTGGGTCCTTGGCTTGTGACAGTGGGACGATAGGTGGTACCGTCCTGGCG
GTATCACCCTTTTTATTGGGAGGGAGCGGGTCGCTAGTGTAGGCGGTTACTTCTGAGCCTATTATCAACTCCATGGC
CGACACGACGCCTAGGTAGGGTGGAGGGCCGGCCCGGCCCCCCATCATAATCTGCGTCCGACTGACCAGTCGCGTTT
CGACGCGGTGTGACACCTCATAACCGCCACCGCCGTCGACCCAGTGCTTCCTCTCGATTTTTCTTGCCGCGGTGTCG
AGCGGGAAAGCTCTTCAGAGTAGTGCGTGCGTGCTAGAGCGTGCGAAGGGGGGGGGGTGAA

Figure S5. The organization of the vtRNA and U2 snRNA genes in *T. brucei* and *L. braziliensis*. The transcribed regions of the U2 snRNA, vtRNA and a tRNAAla genes are highlighted in grey. Black arrow indicates the start and direction of transcription for the tRNA and U2 snRNA genes and the direction and putative transcription start site for the vtRNA genes. Highlighed in blue and green are upstream box A and box B promoter elements, respectively, found in opposite orientation to the U2 snRNA and vtRNA transcribed sequences. Note that vtRNA genes also possess internal boxes A and B (Figs. 1 and 3). Nucleotides matching the consensuses for boxes A and B are in bold.

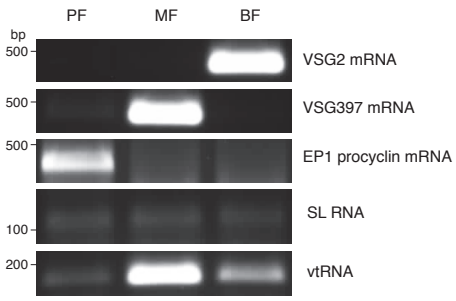

Figure S6. vtRNA has increased abundance in quiescent metacyclic *T. brucei* cells. Semi-quantitative RT-PCR reactions, using the same cell number equivalent of total RNA for procyclic, metacyclic and bloodstream-form trypanosomes. Ethidium bromide stained agarose gels are shown.

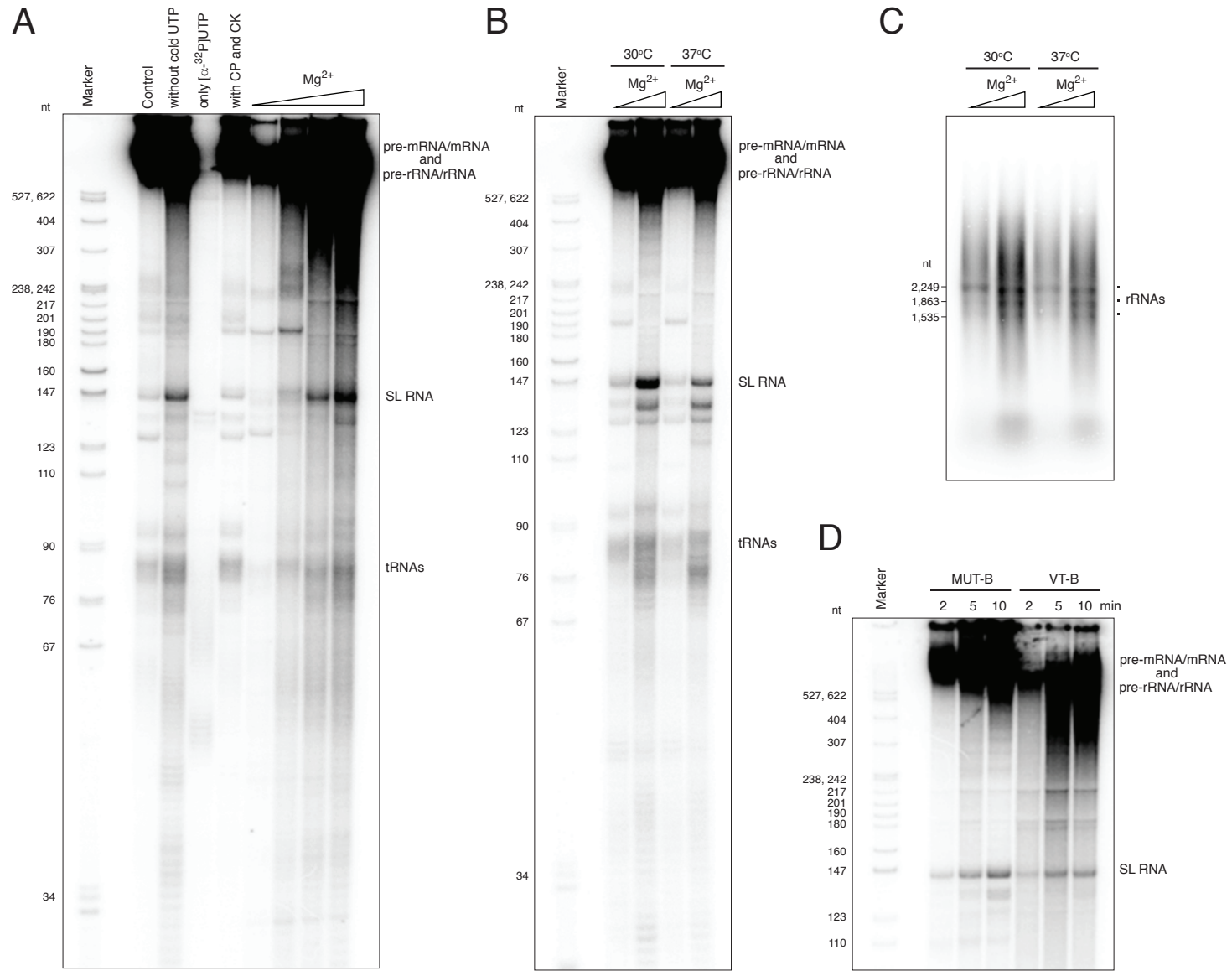

Figure S7. Optimization of the bloodstream-form permeable cell system. *A*, different reaction conditions for RNA synthesis after permeabilization. All reactions were incubated for 30 min at 30°C. Control – a reaction equivalent to the procyclic form permeabilized cell system, except sucrose in the buffer is substituted with glucose and ATP regeneration system is omitted; without cold UTP – a reaction containing ATP, GTP, CTP and [α-^{32}P]UTP, but no nonradioactive UTP; only [α-^{32}P]UTP – a reaction containing only [α-^{32}P]UTP, and no ATP, GTP, CTP or nonradioactive UTP; with CP and CK – a reaction with added creatine phosphate and creatine kinase; last 4 reactions – increasing concentration of Mg^{2+}, 1, 3, 6 and 10 mM. Marker is a ^{32}P-labeled pBR322 DNA MspI digest; *B*, reactions testing the combined effect on RNA synthesis of 3 or 10 mM Mg^{2+} and 30°C or 37°C. RNA is separated on denaturing 10% polyacrylamide gel. Marker is a ^{32}P-labeled pBR322 DNA MspI digest; *C*, reactions testing the combined effect on RNA synthesis of 3 or 10 mM Mg^{2+} and 30°C or 37°C. RNA was separated on denaturing 1.2% agarose gel; *D*, testing the effect of incubation time after permeabilization in the presence of anti-vtRNA oligo (VT-B) or a mutant oligo control (MUT-B) on SL RNA levels. Marker is a ^{32}P-labeled pBR322 DNA MspI digest.

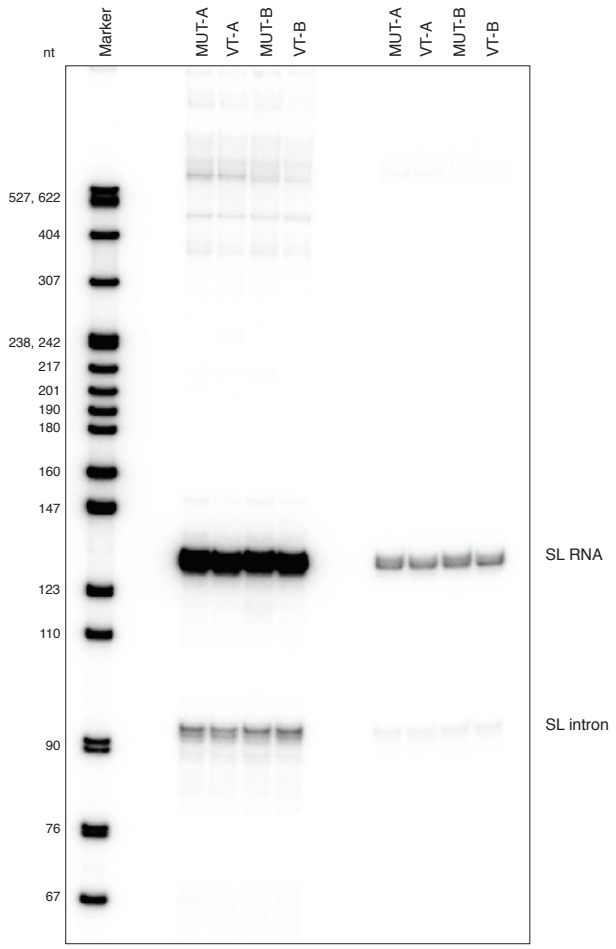

Figure S8. Lack of major defects in SL RNA capping and branched SL-intron formation in cells with reduced vtRNA levels. Cells were permeabilized in the presence of the indicated oligonucleotides and then incubated at 30ºC for 5 min with nonradioactive ribonucleoside triphosphates. Total RNA was prepared with Trizol reagent and used for primer extension reactions with ^{32}P-labeled primer annealing to positions 118 to 130 in the SL RNA. Since the *T. brucei* SL RNA cap is highly modified, primer extension gives distinct patterns of products for fully modified, partially modified and unmodified caps. Products were separated on denaturing 8% polyacrylamide gel. The two sets of lanes represent two different amounts of loaded samples.