SUPPLEMENTARY DATA
FOR
"Functional characterization of two paralogs that are novel RNA binding proteins influencing mitochondrial transcripts of *Trypanosoma brucei*"

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Supplementary Table 1. Oligonucleotides designed in this study for cloning and quantitative Real-Time PCR

Cloning primers for generation of *T. brucei* cell lines

Name[1]	Forward Primer[3]	Forward Restriction Site	Reverse Primer[3]	Reverse Restriction Site	Amplicon Size (bp)	Amplicon coordinates[2]
MRB8170 RNAi sKD	CCGCTCGAGTTACGCACACTGCTCACA	XhoI	GCCGGATCCCTTCTTTTCGTCCCCACAAG	BamHI	257	128-384
MRB4160 RNAi sKD	GGACTCGAGTTCATAACTTCATTGAGCCCG	XhoI	CGCGGATCCATGACGGTGCTTCTTTTCGT	BamHI	263	64-326
MRB8170/4160 RNAi dKD	GGATCCCCTCCCGTTCTACTGCTGAG	BamHI	AAGCTTAGCAGTTGCATGAAGTGACG	HindIII	445	2111-2555
MRB8170 TAP	GACAAGCTTATGCGACGGCTAAGCCTTCT	HindIII	GACGGATCCACAAAATACCTTCGCTAACA	BamHI	2715	1-2715
MRB4160 TAP	CAGTCGACATGCGATGTCTTAGTCTTCT	XhoI	GCAGGATCCATAAAGCACCTTCGCTAACA	BamHI	2688	1-2688

Cloning primers for generation recombinant MRB8170 and MRB4160 proteins

Name[1]	Forward Primer[3]	Forward Restriction Site[3]	Reverse Primer[3]	Reverse Restriction Site
MRB8170-GST	TGTATCGCCGGAATTCATGCGACGGCTAAGCCTTCTT	EcoR1	TGCAGGTCGACATCGATCTAACAAAATACCTTCGCTAACAT	ClaI
MRB4160-GST	GGTTCCGCGTGGATCCGCAACAGCCGCAGGTTTTCAG	BamHI	GTCGACCCGGGAATTCCTAATAAAGCACCTTCGCTAACAT	EcoR1

Real-Time PCR Primers

Name[1]	Forward Primer	Reverse Primer	Amplicon size (bp)	Amplicon coordinates[2]
MRB8170	TACGCTCGATTCGCGACGCC	GGGTGTGTGAGCAGTGTGCGT	96	55-150
MRB4160	CGGAAAATTGGCGAGAAGTA	CCGGGGGAGAAGACTAAGAC	63	(-35)-27

Notes: 1 – MRB8170 = Tb927.8.8170; MRB4160= Tb927.4.4160; sKD= single knockdown; dKD= double knowckdown

2 – Coordinates within ORFs start with first adenosine in start codon.

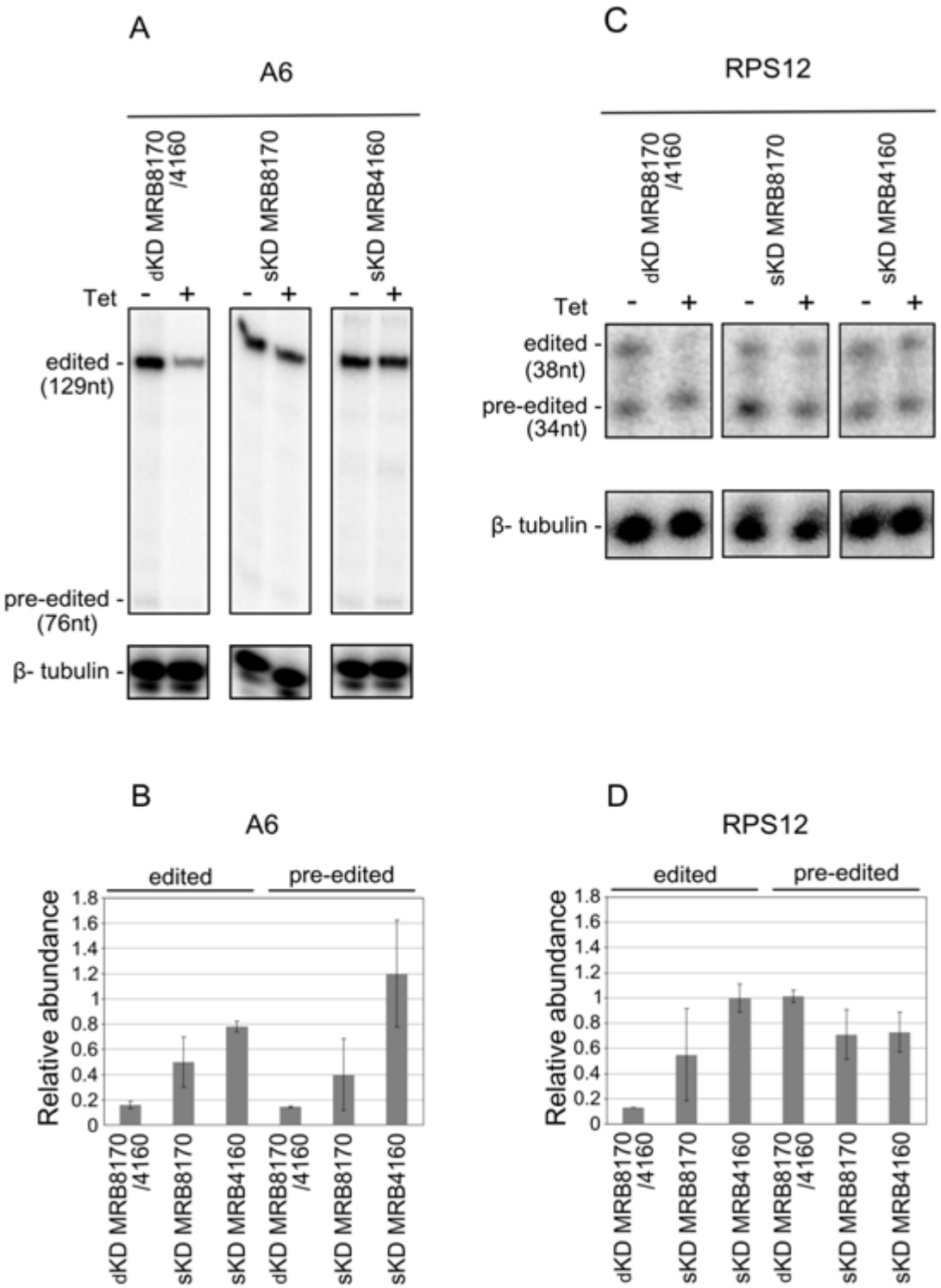
3 – Underlined sequence denotes restriction site of enzyme in right-hand column; Sequence given 5'→3'

SUPPLEMENTARY METHODS

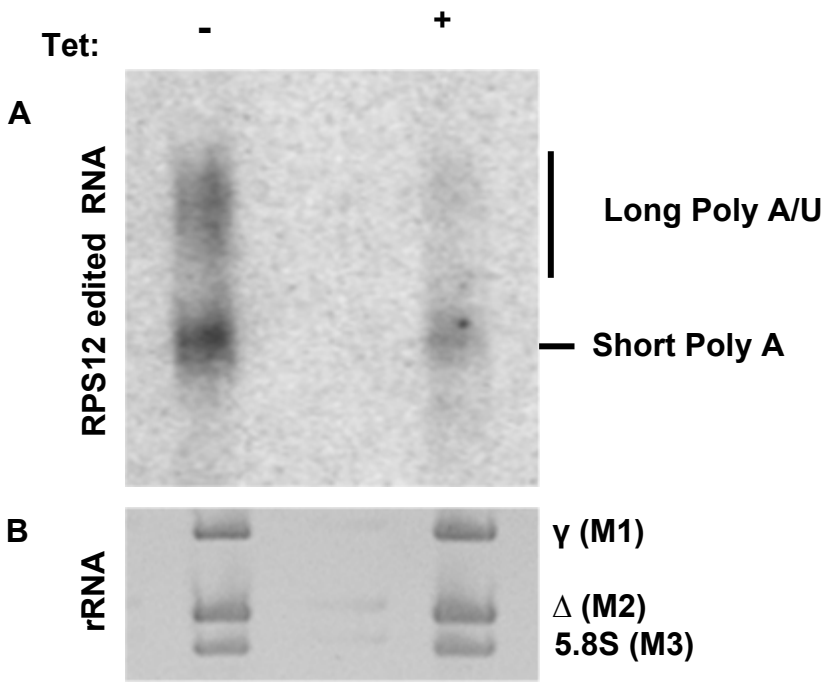
Spliced Leader PCR - A forward primer annealing to spliced leader sequence (SL1: 5'- AAC TAA CGC TAT TAT TAG AAC AGT TTC -3') was used in conjunction with a reverse primer annealing to known MRB4160 CDS sequence (5'-TCA ACA ACA CGT GTA ACT TC -3') in a spliced-leader (SL) mediated PCR to determine SL acceptor site of the transcript. Random hexamer primed cDNA using RNA harvested from the MRB8170 sKD was used as a template in the reaction that used the following program: 94ºC, 2 min; 30 cycles [94ºC, 20 sec; 52ºC 20 sec; 72ºC 45 sec]; 72ºC. The same reaction was also performed to determine the SL acceptor of MRB8170, but the data is not shown since it confirmed the annotated ORF.

Northern blotting - For Northern blot analysis of the edited RPS12 mRNA to visualize long and short forms of these molecules, 10 µg of RNA was resolved on 8%-acrylamide/7M-urea denaturing gel and transferred onto a Zeta-probe (Bio-Rad) membrane. A 5'-^{32}P-labelled primer designated RPS12edit reverse (Carnes et al., 2005) was subsequently used to probe the membrane over night at 45ºC in hybridization buffer (4 X SSC, 20 mM phosphate buffer pH 7.2, 7% SDS (w/v), 1X Denhardt's solution, 1 mg/ml salmon sperm DNA (Sigma)). The blot was subsequently washed once in wash buffer 1 (3 X SSC, 5% SDS (w/v), 25 mM NaH_2PO_4 pH 7.5) supplemented with 1 X Denhardt's solution at for 20 minutes followed by another 20 minute in wash buffer 2 (1 X SSC and 1% SDS), both at hybridization temperature. The radioactive signal was detected using the Storm PhosphorImager (Molecular Dynamics).

Supplementary Figure 1. *The MRB4160 ORF starts with an ATG internal to the one that was predicted for the gene.* An alignment of the first 63 nucleotides of the predicted *MRB4160* CDS with four sequenced clones from a SL-PCR of the 5′-end of the MRB4160 cDNA. The spliced leader sequence was omitted from the alignment, and its acceptor site is indicated (SL acceptor site). The actual start codon is boxed and labeled "Start".



Supplementary Figure 2. *The influence of simultaneous and individual MRB8170 and MRB4160 depletion on pan-edited RNAs.* Poisoned primer extension analysis was performed using primers and protocol as described in Ammerman et al. (2010) on total RNA from MRB8170 and MRB4160 double or single knockdown cell lines grown for 4 or 5 days, respectively, in the presence (+) or absence (-) of tetracycline. Radiolabeled primers annealing to pan-edited A6 (A) and RPS12 (C) mRNA (using abbreviations as defined in Figure 9) were used in the reverse transcription reaction to visualize edited and pre-edited forms of these transcripts (the sizes of which are indicated in parentheses). Radiolabeled oligonucleotide annealing to the cytosolic β-tubulin RNA was also introduced into the reaction and served for normalization of the obtained signal (bottom panel). The resulting bands from the reaction were quantified by densitometry and the average relative abundance of the A6 (B) and RPS12 (D) RNAs in the RNAi-silenced cells as compared to the non-induced controls from two replicates are given as bar graphs; standard deviation is indicated by whiskers.



Supplementary Figure 3. *The simultaneous downregulation of MRB8170 and MRB4160 results in depletion of edited mRNAs that bear both short polyA and long polyA/U 3'-tails.* Ten micrograms of total RNA from the dKD cells grown for 4 days in the presence or absence of the RNAi-inducing compound tetracycline (tet) was separated on a denaturing 8%-acrylamide gel, transferred onto a membrane and probed with a oligonucleotide that anneals fully edited RPS12 (A). In the tet- sample, two populations are visualized: a smear ranging in size from about 570 and 450 nts that represent the longA/U appended mRNAs and a smaller ~350 nts smear that contains the short A tail molecules. The levels of both populations are decreased in the tet+ lane. The ethidium bromide stained rRNA large subunits γ (M1; Tb927.3.3451; 214 nts), Δ (M2; Tb927.3.3453; 182 nts) and 5.8S (M3; Tb927.3.3448; 171 nts) are shown as a loading control (B).