Supplemental Table S2. Oligonucleotide sequences used in this study.

Primer Description	Primer sequence	Reference if applicable
KREPB4 forward primer with *att*B1 site	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCAAAATGTTACGTGTTGAAAACTTG	This study
KREPB4 reverse primer (plus stop codon) with *att*B2 site	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATTTTTTCTTTGTGGCAAGG	This study
KREPB4 reverse primer (no stop codon) with *att*B2 site	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTTTTCTTTGTGGCAAGGC	This study
Forward primer floxed cassette amplification from SM06 and SM07 (Merritt and Stuart 2013)	GATAAGCTTATAACTTCGTATAGCATACA	(Merritt and Stuart 2013)
Reverse primer floxed cassette amplification from SM06 and SM07 (Merritt and Stuart 2013)	GACCCACTTAGGATCCATAACTTCGTA	(Merritt and Stuart 2013)
Forward primer blasticidin cassette amplification	ATGGCCAAGCCTTTGTCTCAA	(Merritt and Stuart 2013)
Reverse primer blasticidin cassette amplification	TTAGCCCTCCCACACATAACCA	(Merritt and Stuart 2013)
Forward primer hygromycin cassette amplification	ATGAAAAAGCCTGAACTCACC	(Merritt and Stuart 2013)
Reverse primer hygromycin cassette amplification	CTATTCCTTTGCCCTCGGAC	(Merritt and Stuart 2013)
KREPB4 PF and BF knockout outer 5’ fragment forward	CAGGATCAGGAACCACTTC	This study
KREPB4 PF and BF knockout outer 5’ fragment forward nested	AATGAAAAGTGTTACCTTGAG	This study
KREPB4 PF knockout outer 5’ fragment reverse	ATACGAAGTTATAAGCTTATCCAGAGTGAACCAAATTATAGC	This study
KREPB4 PF knockout inner 5’ fragment forward	TTGTACACAACGTTGCGGATG	This study

KREPB4 PF knockout inner 5' fragment reverse	ATACGAAGTTATAAGCTTATCGTTCAAAAACAGATACTGCCTC	This study
KREPB4 PF knockout outer 3' fragment forward	TTATGGATCCTAAGTGGGTCTCCGTTCCGTCGTCGCAGCA	This study
KREPB4 PF and BF knockout outer 3' fragment reverse	GAGTAGCGAATGCACGTCGT	This study
KREPB4 PF and BF knockout outer 3' fragment reverse nested	TGCAACCCCAGCCATACGCTTG	This study
KREPB4 PF knockout inner 3' fragment forward	TTATGGATCCTAAGTGGGTCGCGCCTGTGCGCGTGTG	This study
KREPB4 PF knockout inner 3' fragment reverse	AGAAGAACCACCAGCGATG	This study
KREPB4 BF knockout 5' fragment blasticidin reverse	TTGAGACAAAGGCTTGGCCATGTTCAAAAACAGATACTGCCTC	This study
KREPB4 BF knockout 5' fragment hygromycin reverse	GGTGAGTTCAGGCTTTTTCATGTTCAAAAACAGATACTGCCTC	This study
KREPB4 BF knockout 3' fragment blasticidin forward	TGGTTATGTGTGGGAGGGCTAAGCGCCTGTGCGCGTGTG	This study
KREPB4 BF knockout 3' fragment hygromycin forward	GTCCGAGGGCAAAGGAATAGGCGCCTGTGCGCGTGTG	This study
KREN1 in situ tagging pMOTag forward	AGATGCAACAGGCAAACACCTACTGCGAGCCAAATGGTGTCGTGAAAATCCCTT CTTTCCACGGGCGTCGCTACCTCTTTTAGGCATCTCGGTTGGTGCGGGTACCGG GCCCCCCCTCGAG	This study (Oberholzer et al. 2006)
KREN1 in situ tagging pMOTag reverse	GAATTCATTCATCACGAACTACGTTAGCAACCGAACAATGACGGATACTGCAGC GAAAAATAACACCCTCCCGTGTATTACCTCATCATCCAAATAATTATGGCGGCCG CTCTAGAACTAGTGGAT	This study (Oberholzer et al. 2006)
KREN2 in situ tagging pMOTag forward	ACAGCCCCGTTCTCGTTGCTCTGCAGAGGAGGCTGGACGATCAACTTCACGC CTGGGTATTCCTGAATTGAAGGACGCACTACTAGTGGCCGAATTAGTTGGTAC CGGGCCCCCCCTCGAG	This study (Oberholzer et al. 2006)
KREN2 in situ tagging pMOTag reverse	CTCCCGCACCACGGTATAAAGTTTGCTCAAACAAACAACATCTTATCTTCACCAC ACACATACGCAGACTTTGCCAGCCGGAAAGTGGGGAATGTAATCATGGCGGCC GCTCTAGAACTAGTGGAT	This study (Oberholzer et al. 2006)
KREN3 in situ tagging pMOTag forward	ACAAACCGGAAGGTTGCTGTTGACTTTTGAAGGGACTACTGAGCGTAGGGCGGC ACTCGCTACTAGCATGTGTTTTCCGTCGCTAGCACAATCGGCCGGCGGTACCGG GCCCCCCCTCGAG	This study (Oberholzer et al. 2006)
KREN3 in situ tagging pMOTag	CACTCCACAAAGAGGAAAAACCGAAGCCAAAAGCGCACCGGTGAAAGTATTTCGC AGAAAGAAGCCAAAGCGCAGGTATAAGCAAACCTCCAATGACGATTGGCGGCCGC	This study (Oberholzer

reverse	TCTAGAACTAGTGGAT	et al. 2006)
KREPB4 G163V site-directed mutagenesis	GAATTATATGTTCCTGGTCGAAAGCTTTGCGCGGC	This study
KREPB4 G163V site-directed mutagenesis	GCCGCGCAAAGCTTTCGACCAGGAACATATAATTC	This study
KREPB4 G163R site-directed mutagenesis	CCGCGCAAAGCTTTCGCGCAGGAACATATAATTCA	This study
KREPB4 G163R site-directed mutagenesis	TGAATTATATGTTCCTGCGCGAAAGCTTTGCGCGG	This study
KREPB4 E164A site-directed mutagenesis	TATGTTCCTGGGCGCAAGCTTTGCGCGGC	This study
KREPB4 E164A site-directed mutagenesis	GCCGCGCAAAGCTTGCGCCCAGGAACATA	This study
KREPB4 T280A site-directed mutagenesis	GTTCTCCATTACGTGTGCGCCAAGCACATTGAGGA	This study
KREPB4 T280A site-directed mutagenesis	TCCTCAATGTGCTTGGCGCACACGTAATGGAGAAC	This study
KREPB4 E284A site-directed mutagenesis	TGGCACACACGTAATGGCGAACATCATAGGCGAAC	This study
KREPB4 E284A site-directed mutagenesis	GTTCGCCTATGATGTTCGCCATTACGTGTGTGCCA	This study
KREPB4 H281A site-directed mutagenesis	GTCCTCAATGTGCTTGGCACAGCCGTAATGGAGAACATC	This study
KREPB4 H281A site-directed mutagenesis	GATGTTCTCCATTACGGCTGTGCCAAGCACATTGAGGAC	This study
KREPB4 PUF-triple (T280A/H281A/E284A) site-directed mutagenesis	GTCCTCAATGTGCTTGGCGCAGCCGTAATGGCGAACATCATAGGCGAACT	This study
KREPB4 PUF-triple (T280A/H281A/E284A) site-directed mutagenesis	AGTTCGCCTATGATGTTCGCCATTACGGCTGCGCCAAGCACATTGAGGAC	This study
KREPB4 S153A site-directed mutagenesis	AATTCACAAAGCTATCTGCCTCCACCGTAAGCGATAC	This study

KREPB4 S153A site-directed mutagenesis	GTATCGCTTACGGTGGAGGCAGATAGCTTTGTGAATT	This study
KREPB4 S153E site-directed mutagenesis	AACATATAATTCACAAAGCTATCCTCCTCCACCGTAAGCGATACCTCGG	This study
KREPB4 S153E site-directed mutagenesis	CCGAGGTATCGCTTACGGTGGAGGAGGATAGCTTTGTGAATTATATGTT	This study
KREPB4 S218A site-directed mutagenesis	CGACGCCTTATCGTCGGAAGCGTCACCATTTAAAACCATT	This study
KREPB4 S218A site-directed mutagenesis	AATGGTTTTAAATGGTGACGCTTCCGACGATAAGGCGTCG	This study
KREPB4 S219A site-directed mutagenesis	CGCCTTATCGTCGGCACTGTCACCATTTAAAACCATTTTACG	This study
KREPB4 S219A site-directed mutagenesis	CGTAAAATGGTTTTAAATGGTGACAGTGCCGACGATAAGGCG	This study
KREPB4 S218A S219A site-directed mutagenesis	CAGCCGACGCCTTATCGTCGGCAGCGTCACCATTTAAAACCATTTT	This study
KREPB4 S218A S219A site-directed mutagenesis	AAAATGGTTTTAAATGGTGACGCTGCCGACGATAAGGCGTCGGCTG	This study
KREPB4 S224A site-directed mutagenesis	CCACCATCAGCCGCCGCCTTATCGTCG	This study
KREPB4 S224A site-directed mutagenesis	CGACGATAAGGCGGCGGCTGATGGTGG	This study
KREPB4 S218E site-directed mutagenesis	CAGCCGACGCCTTATCGTCGGACTCGTCACCATTTAAAACCATTTTA	This study
KREPB4 S218E site-directed mutagenesis	TAAAATGGTTTTAAATGGTGACGAGTCCGACGATAAGGCGTCGGCTG	This study
KREPB4 S219E site-directed mutagenesis	ATCAGCCGACGCCTTATCGTCCTCACTGTCACCATTTAAAACCAT	This study
KREPB4 S219E site-directed mutagenesis	ATGGTTTTAAATGGTGACAGTGAGGACGATAAGGCGTCGGCTGAT	This study
KREPB4 S218E S219E site-directed mutagenesis	GCCACCATCAGCCGACGCCTTATCGTCCTCCTCGTCACCATTTAAAACCATTTTACGCAT	This study
KREPB4 S218E S219E site-	ATGCGTAAAATGGTTTTAAATGGTGACGAGGAGGACGATAAGGCGTCGGCTGATGGTGGC	This study

directed mutagenesis		
KREPB4 S224E site-directed mutagenesis	CGCCACCATCAGCCTCCGCCTTATCGTCGG	This study
KREPB4 S224E site-directed mutagenesis	CCGACGATAAGGCGGAGGCTGATGGTGGCG	This study
KREPB4 ORF BioMark forward	TCACCACAGTGTTCACGCCATACT	This study
KREPB4 ORF BioMark reverse	ATGTGCTTGGCACACACGTAATGG	This study
KREPB4 regulatable copy BioMark forward	CTAACGGTTCCGAATCCAGTG	This study
KREPB4 regulatable copy BioMark reverse (aldolase 3'UTR pLEW79)	GCTGTGCCATCAGATTACTCCG	(Wang et al. 2003)
V5 tag exclusive expressor BioMark forward	CCTCGGTCTCGATTCTACGC	(McDermott et al. 2015)
V5 tag exclusive expressor BioMark reverse	TGGGGATGGGCTTACCCAG	(McDermott et al. 2015)
COI BioMark forward	CCCGATATGGTATTTCCTCGTATAAA	(Carnes et al. 2008)
COI BioMark reverse	CCCCCATACCCTCTTCAGTCA	(Carnes et al. 2008)
ND4 BioMark forward	CAATCTGACCATTCCATGTGTGA	(Carnes et al. 2005)
ND4 BioMark reverse	TTTCAGCACAATACTTGCTAATAAAACA	(Carnes et al. 2005)
A6 BioMark pre-edited forward	TTGCCTTTGCCAAACTTTTAGAAG	(Carnes et al. 2005)
A6 BioMark pre-edited reverse	ATTCTATAACTCCAAAATCACAACTTTCC	(Carnes et al. 2005)
CYb BioMark pre-edited forward	ATATAAAAGCGGAGAAAAAAGAAAG	(Carnes et al. 2005)
CYb BioMark pre-edited reverse	CCCATATATTCTATATAAACAACCTGACA	(Carnes et al. 2005)
COII BioMark pre-edited forward	ATTACAGTGTAACCATGTATTGACATT	(Carnes et al. 2005)
COII BioMark pre-edited reverse	TTCATTACACCTACCAGGTTCTCT	(Carnes et al. 2005)
COIII BioMark pre-edited forward	GAAACCAGATGAGATTGTTTGCA	(Carnes et al. 2005)

COIII BioMark pre-edited reverse	TTCATTCCAACTAAACCCTTTCC	(Carnes et al. 2005)
MURF2 BioMark pre-edited forward	GATTTTAAGATTGGCTTTGATTGA	(Carnes et al. 2005)
MURF2 BioMark pre-edited reverse	AATATAAAATCTAGATCAAACCATCACA	(Carnes et al. 2005)
RPS12 BioMark pre-edited forward	CGACGGAGAGCTTCTTTTGAATA	(Carnes et al. 2005)
RPS12 BioMark pre-edited reverse	CCCCCCACCCAAATCTTT	(Carnes et al. 2005)
ND3 BioMark pre-edited forward	GAATGGGAGATGGGTTTTGG	(Carnes et al. 2005)
ND3 BioMark pre-edited reverse	AACAAATCTCTTTACCCCCTTCAG	(Carnes et al. 2005)
ND7 BioMark pre-edited forward	GCGGGCGGAGCATTATT	(Carnes et al. 2005)
ND7 BioMark pre-edited reverse	GATCTACGGTCCCCTCTTTCCT	(Carnes et al. 2005)
A6 BioMark edited forward	GATTTATTTTGGTTGCGTTTGTTATTATG	(Carnes et al. 2005)
A6 BioMark edited reverse	CAAACCAACAAACAAATACAAATCAAAC	(Carnes et al. 2005)
CYb BioMark edited forward	AAATATGTTTCGTTGTAGATTTTTATTATTT	(Carnes et al. 2005)
CYb BioMark edited reverse	CCCATATATTCTATATAAACAACCTGACA	(Carnes et al. 2005)
COII BioMark edited forward	ATTACAGTGTAACCATGTATTGACATT	(Carnes et al. 2005)
COII BioMark edited reverse	ATTTCATTACACCTACCAGGTATACAA	(Carnes et al. 2005)
COIII BioMark edited forward	TTGTGTTTTATTACGTTGTATCCAGTATTG	(Carnes et al. 2005)
COIII BioMark edited reverse	CGAAAGCAAACTCACAACACAAA	(Carnes et al. 2005)
MURF2 BioMark edited forward	GATTTTAATGTTTGGTTGTTTTAATTTAG	(Carnes et al. 2005)
MURF2 BioMark edited reverse	AATATAAAATCTAGATCAAACCATCACA	(Carnes et al. 2005)
RPS12 BioMark edited forward	CGTATGTGATTTTTGTATGGTTGTTG	(Carnes et al. 2005)
RPS12 BioMark edited reverse	ACACGTCGGTTACCGGAACT	(Carnes et al. 2005)
ND3 BioMark edited forward	TGTTTTCGTTGTTGTTTGTGGTT	(Carnes et al. 2005)
ND3 BioMark edited reverse	CAATGTATAAAACACCAAACGTGAATT	(Carnes et al. 2005)

ND7 BioMark edited forward	GCATCCCGCAGCACATG	(Carnes et al. 2005)
ND7 BioMark edited reverse	CTGTACCACGATGCAAATAACCTATAAT	(Carnes et al. 2005)

Supplemental Table S3. Antibodies used in this study.

Antibody name	Raised in	Dilution	Source
V5 Epitope Tag Polyclonal Antibody	Rabbit	1 µg per IP with 1 × 10^8 cells 1/5000 WB	Rockland Immunochemicals 600-401-378
V5 Epitope Tag Monoclonal Antibody	Mouse	1 µg per IP with 1 × 10^8 cells 1/5000 WB	Life Technologies R960-25
c-Myc (9E10) Monoclonal Antibody	Mouse	1/200 WB	Santa Cruz sc-40
c-Myc (A-14) Polyclonal Antibody	Rabbit	1/200 WB	Santa Cruz sc-789
KREPA1 Monoclonal Antibody	Mouse	1/25 WB	(Panigrahi et al. 2001) P4D8-F6
KREPA2 Monoclonal Antibody	Mouse	1 mL per IP with 1 x 10^8 cells 1/12.5 WB	(Panigrahi et al. 2001) P1H3-D7
KREL1 Monoclonal Antibody	Mouse	1/50 WB	(Panigrahi et al. 2001) P3C1-G2
KREPA3 Monoclonal Antibody	Mouse	1/25 WB	(Panigrahi et al. 2001) P3C12-B6
KREPA6 Polyclonal Antibody	Rabbit	1/2000 WB	(Schnaufer et al. 2003)
GAP2 Monoclonal Antibody	Mouse	1/25 WB	(Allen et al. 1998) mAb53
mtHsp70 Monoclonal Antibody	Mouse	1/1000 WB	(Allen et al. 1998) mAb78

Supplemental References

Allen TE, Heidmann S, Reed R, Myler PJ, Goringer HU, Stuart KD. 1998. Association of guide RNA binding protein gBP21 with active RNA editing complexes in Trypanosoma brucei. *Molecular and cellular biology* **18**: 6014-6022.

Carnes J, Trotter JR, Ernst NL, Steinberg A, Stuart K. 2005. An essential RNase III insertion editing endonuclease in Trypanosoma brucei. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 16614-16619.

Carnes J, Trotter JR, Peltan A, Fleck M, Stuart K. 2008. RNA editing in Trypanosoma brucei requires three different editosomes. *Molecular and cellular biology* **28**: 122-130.

McDermott SM, Guo X, Carnes J, Stuart K. 2015. Differential Editosome Protein Function between Life Cycle Stages of Trypanosoma brucei. *The Journal of biological chemistry* **290**: 24914-24931.

Merritt C, Stuart K. 2013. Identification of essential and non-essential protein kinases by a fusion PCR method for efficient production of transgenic Trypanosoma brucei. *Molecular and biochemical parasitology* **190**: 44-49.

Oberholzer M, Morand S, Kunz S, Seebeck T. 2006. A vector series for rapid PCR-mediated C-terminal in situ tagging of Trypanosoma brucei genes. *Molecular and biochemical parasitology* **145**: 117-120.

Panigrahi AK, Gygi SP, Ernst NL, Igo RP, Jr., Palazzo SS, Schnaufer A, Weston DS, Carmean N, Salavati R, Aebersold R et al. 2001. Association of two novel proteins, TbMP52 and TbMP48, with the Trypanosoma brucei RNA editing complex. *Molecular and cellular biology* **21**: 380-389.

Schnaufer A, Ernst NL, Palazzo SS, O'Rear J, Salavati R, Stuart K. 2003. Separate insertion and deletion subcomplexes of the Trypanosoma brucei RNA editing complex. *Molecular cell* **12**: 307-319.

Wang B, Ernst NL, Palazzo SS, Panigrahi AK, Salavati R, Stuart K. 2003. TbMP44 is essential for RNA editing and structural integrity of the editosome in Trypanosoma brucei. *Eukaryotic cell* **2**: 578-587.