Supplementary Materials for

KREH1 RNA helicase activity promotes utilization of initiator gRNAs across multiple mRNAs in trypanosome RNA editing

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The file includes: Table S1 and Figs. S1 to S7 with legends.

Other Supplementary Material for this manuscript includes the following: Tables S2-S6.

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Table S1. Primers used in this study

Primers	Sequence (5'-3')
KREH1_KO 5'UTR_For	CCGGGATCTCGAGCATTGGTGATGGCAAAACCTTCAGAG
KREH1 _KO 5'UTR _Rev	AATGGCAGGATCCGCTTATGTGCCGTATGCTAGCTAGA
KREH1 _KO 3'UTR_For	CCGAATGGTACCTTGCTGCGCAGTTTTGTTGCTGTCAGGACT
KREH1 _KO 3'UTR_Rev	CGGTCCTAAGCTTAGCTCGGATACGTCAATTATCCTCGCCA
KREH1_UTR_For	CTCTAGCTAGCATACGGCACATAAGCGGAT
KREH1_UTR_Rev	ACAAGTCCTGACAGCAACAAAACTGCGCAGCA
KREH1_ORF_For	ATAATAGGATCCCAAACTGCGAAAGTGTTCCATC
KREH1_ORF_Rev	ATAATAAAGCTTCCAACCACTGTCACGAGGTG
KREH1_WT_RECO_For	GGCAAGCTTATGCGCGCTCTGCGCTGCGTG
KREH1_WT_RECO_Rev	CCCCTCGAGTTACAGATCCTCTTCTG
KREH1_K168A_For	CCCACGGGTTCCGGTGCTACGGTGGCTTTCG
KREH1_K168A_Rev	AGCGAAAGCCACCGTAGCACCGGAACCCGTG
KREH1_E267Q_For	TTCCTGGTGTTCGACCAGGCTGACCGCCTGC
KREH1_E267Q_Rev	GCAGGCGGTCAGCCTGGTCGAACACCAGG
KREH1_RECO_RT_For	ATGCGCGCTCTGCGCTGCG
KREH1_RECO_RT_Rev	ACAGCAGGCGAGCGGAAGC
RESC13_10XTY_For	GGGAGCAGGCGATAGCGGAAGGAGATTGTAGCGCGCCCGCGCCTCCCGC CGAAGTGGCAGGTGATGCCAGTCAGAAGGTGGGTTCTGGTAGTGGTTCC
RESC13_10XTY_Rev	TTATTTACCCGCTTGACGCCAGTTTCCAGCGCTTGGCGGAACGGGGTGGG TATGCTACTAATATTCCCGTAAATAGGGTT CCAATTTGAGAGACCTGTGC
KREPB5-MHT_HindIII_For	CCCAAGCTTATGAGACGGGCTGTGGTACTC
KREPB5-MHT_BamHI_Rev	CCGGGATCCCCGCCCTCCCAGTGCCAG
KREH2_RT_For	GGGTGGTGATTGTTCCATTG
KREH2_RT_Rev	CCAATGCTTTCCAGTCGTTC
A6 gRNA-1_RT_For	ATACTATAACTCCAATGACGAAA
A6 gRNA-1_RT_Rev	AAAAATTATCATATCACTGTAAAACTG
A6 gRNA-2_RT_For	ATAAATACAACAATATAATAACTGTCG
A6 gRNA-2_RT_Rev	AAAAATTTAATCTCATATTCAACCT

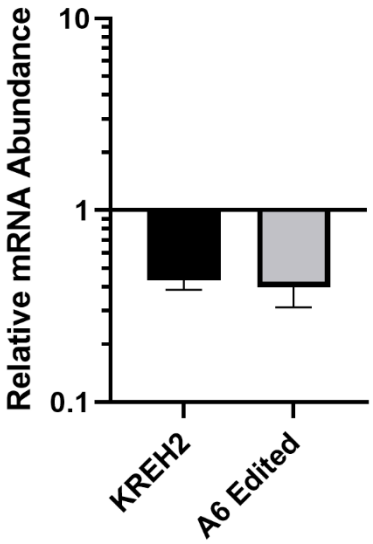

Figure S1. Relative RNA levels of A6 edited mRNA in KREH2 RNAi cell lines. RNA was isolated from uninduced and induced KREH2 cells after 2 days of induction and quantified by qRT-PCR using KREH2 ORF-specific primer sets recognize the levels of KREH2 mRNA and A6 edited mRNA. Relative RNA abundance represents RNA levels in induced cells compared to levels in uninduced cells. RNA levels were normalized to 18S rRNA, and numbers represent the mean and standard deviation of two biological replicates, each with three technical replicates.

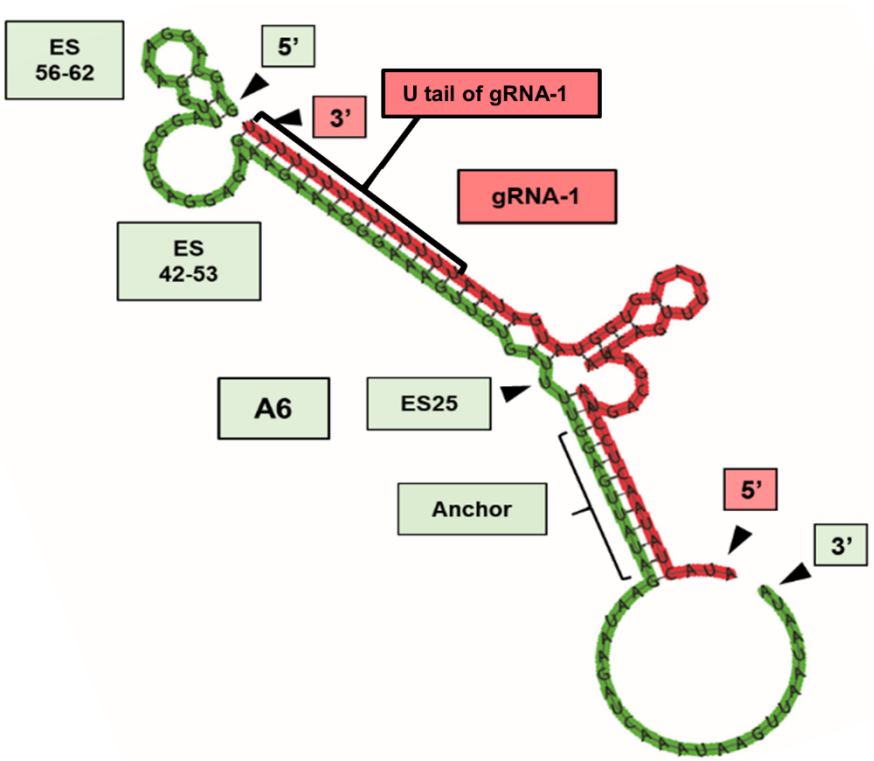

Figure S2. Structure prediction of 3' A6 pre-edited mRNA with A6 gRNA-1. This gRNA was denoted as alternate initiating gRNA (1) and previously reported to be utilized in our strain (2). Predictions were performed using ViennaRNA cofold function with a temperature setting of 27°C. mRNA, green; gRNA, red.

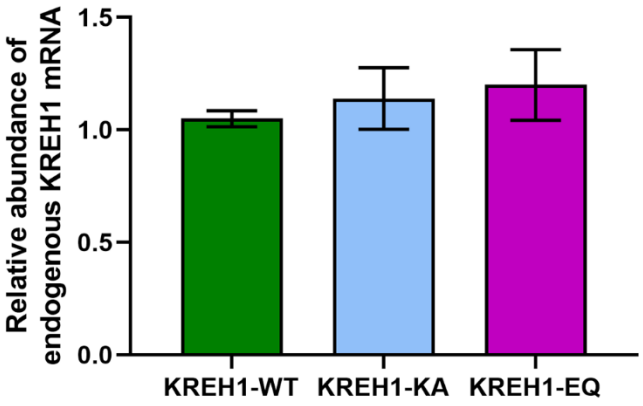

Figure S3. Relative endogenous KREH1 mRNA levels in cells overexpressing KREH1 variants. RNA was isolated from uninduced and induced KREH1-WT, KREH1-KA and KREH1-EQ cells after 3 days of induction and quantified by qRT-PCR using KREH1 ORF-specific primer sets recognize only the endogenous sequence. Relative RNA abundance represents RNA levels in induced cells compared to levels in uninduced cells. RNA levels were normalized to 18S rRNA, and numbers represent the mean and standard deviation of two biological replicates, each with three technical replicates.

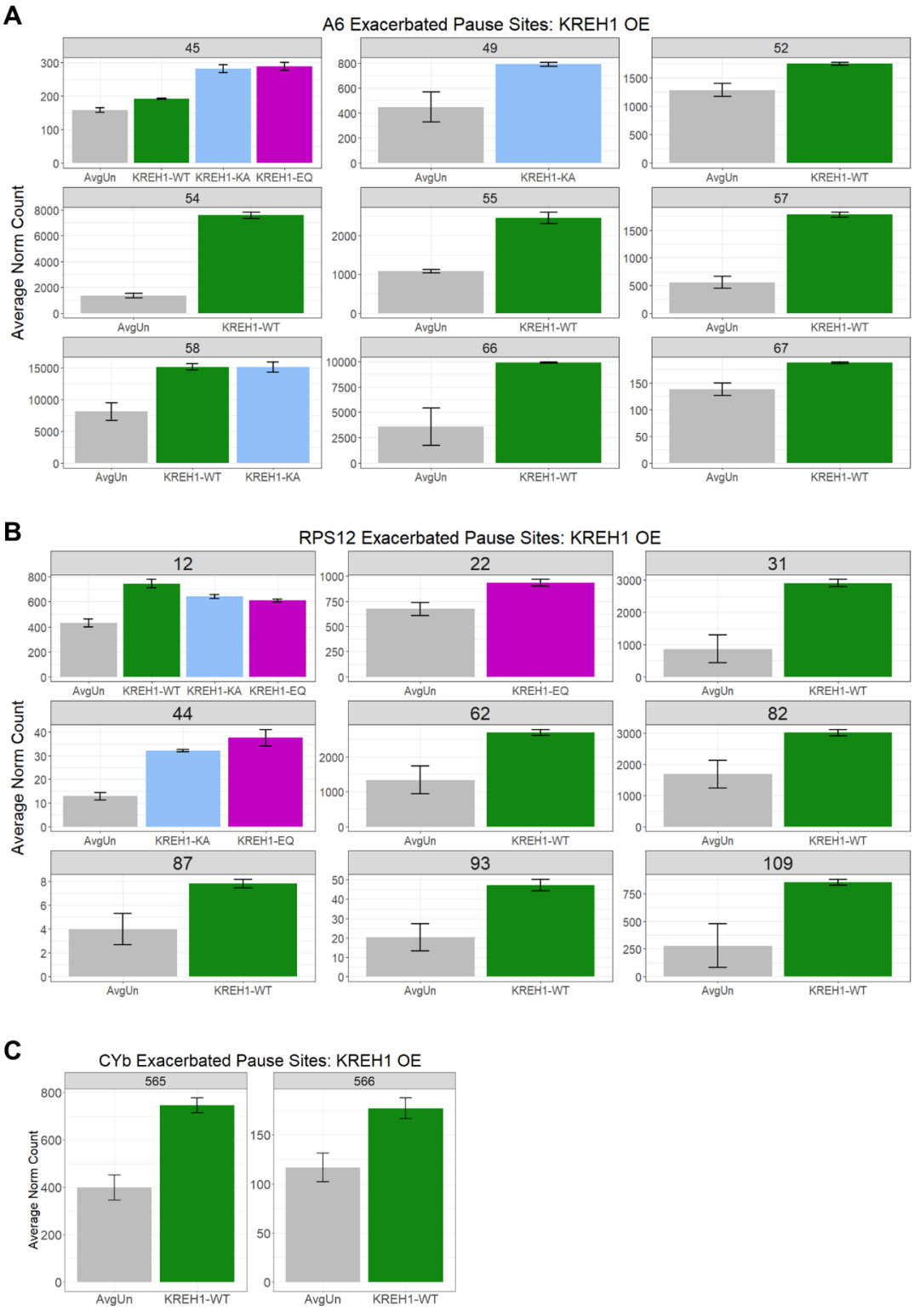

Figure S4. Quantification of EPS identified upon overexpression of KREH1 variants. Normalized counts at EPS in WT, KA mutants, and EQ mutants for A6 mRNA (A), RPS12 mRNA (B), and CYb mRNA (C) are shown.

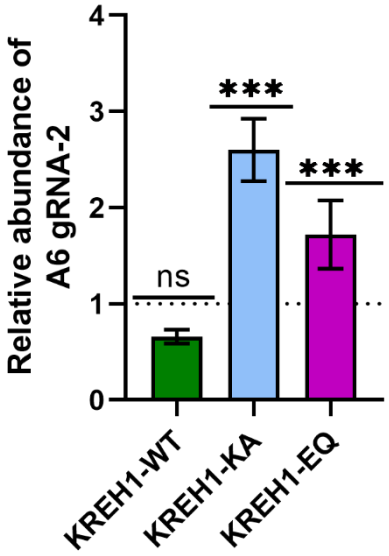

Figure S5. Abundance of A6 gRNA-2 was measured by qRT-PCR in the induced KREH1-WT, KREH1-KA and KREH1-EQ overexpressor lines (plotted relative to the level in the corresponding uninduced cells). Students t-tests were performed on n=6 replicates. ns = not significant; *** $p < 0.001$.

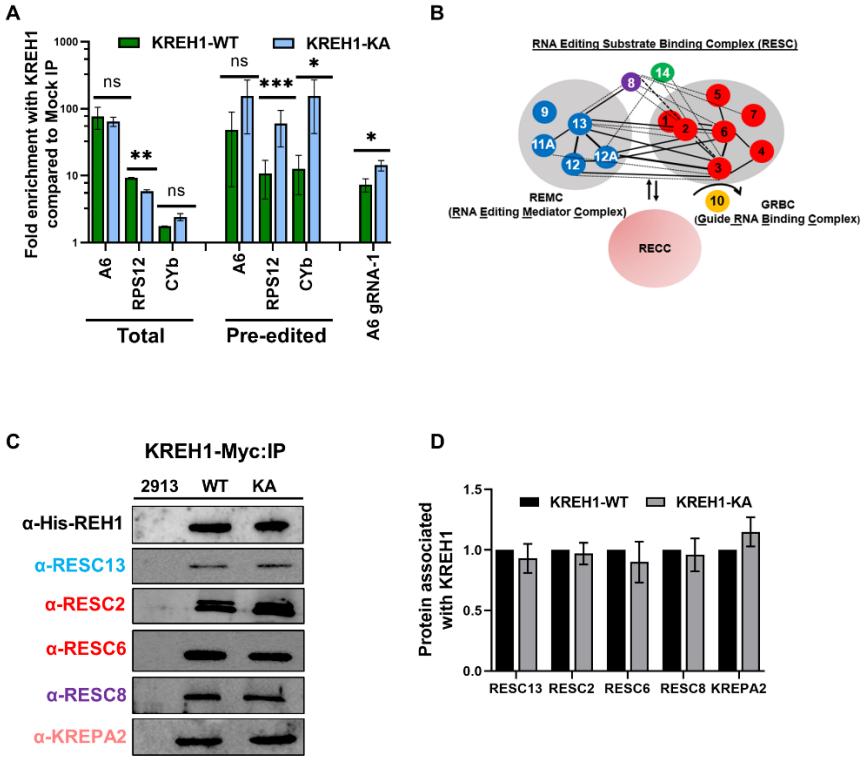

Figure S6: RNA immunoprecipitation and co-immunoprecipitation analysis of KREH1-WT and KREH1-KA. (A) Enrichment of total and pre-edited A6, RPS12, and CYb mRNAs and A6 gRNA-1 transcripts in KREH1-WT (green) and KREH1-KA (blue) immunoprecipitations relative to a negative antibody control was measured by qRT-PCR. 18S rRNA was used for the normalization. Values represent the mean of three biological replicates, each with three technical replicates. Students t-test was performed on n=3 replicates. ns, not significant; *p < 0.05; **p < .01; ***p < 0.001. **(B)** RESC and RECC organization. RESC proteins designated with their RESC numbers. REMC module, GRBC module, and organizer proteins are differentially colored. Black lines indicate direct interactions by yeast two-hybrid screen (3). Solid lines, strong interactions; dotted lines, weak interactions; thin lines, interaction in one direction; thick lines, interaction in both directions. Double arrows indicate that RECC transiently interacts with RESC. **(C)** Representative immunoprecipitation (IP) of KREH1-WT and KREH1-KA with α-myc antibodies from cell lysates of *T. brucei* overexpressing KREH1-WT or KREH1-KA proteins. Elution's of KREH1-WT and KREH1-KA were normalized, and RESC and RECC proteins were probed with the respective antibodies shown on the left. **(D)** Quantification of the experiment in B. The levels in the KREH1-WT samples were set to 1.0. Bar graphs represent the average and standard deviation of three biological replicates.

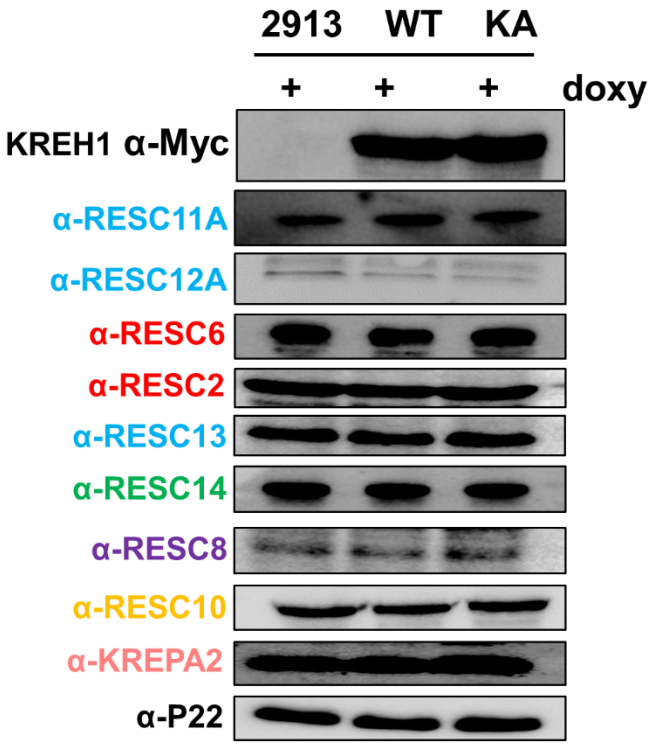

Figure S7. Effects of KREH1-WT and KREH1-KA overexpression on steady state levels of RESC and RECC proteins. KREH1-WT or KREH1-KA cells were induced with doxycycline (+ doxy) for three days, and the abundance of selected RESC proteins, and the KREPA2 RECC protein were measured by western blot. P22 is a loading control.

References:

1. Koslowsky, D., Sun, Y., Hindenach, J., Theisen, T., Lucas, J. (2014). The insect-phase gRNA transcriptome in *Trypanosoma brucei*. *Nucleic Acids Res.* **42**, 1873-1886.
2. Smith Jr, J.T., Doleželová, E., Tylec, B., Bard, J.E., Chen, R., Sun, Y., Zíková, A. and Read, L.K. (2020) Developmental regulation of edited CYb and COIII mitochondrial mRNAs is achieved by distinct mechanisms in *Trypanosoma brucei*. *Nucleic Acids Res.*, **48**, 8704-8723.
3. Ammerman, M.L., Downey, K., Hashimi, H., Fisk, J.C., Tomasello, D.L., Faktorová, D., Kafková, L., King, T., Lukes, J. and Read, L.K. (2012) Architecture of the trypanosome RNA editing accessory complex, MRB1. *Nucl. Acids Res.* **40**, 5637-5650.