**Supplemental Figure and Table Legends**

**Figure S1. Amino acid sequence alignment of the two PhyH domain containing *T. brucei* proteins.** *T. brucei* PhyH domain containing protiens, TriTrypDB IDs Tb927.3.3440 and Tb927.9.7260, were aligned with the *H. sapiens* PAHX and *M. musculus* PhyH proteins using Clustal Omega. An asterisk (\*) indicates positions with a single, fully conserved residue, a colon (:) indicates conservation between groups of strongly similar proteins, and a period (.) indicates conservation between groups of weakly similar properties. Amino acid residues essential for iron binding (yellow) and 2-oxogluterate binding (blue) in humans or mice are indicated. The table displays the percent identity matrix between the proteins as calculated using Clustal Omega.

**Figure S2. Amino acid sequence alignment between kinetoplastid MRB7260 homologues.** Protein sequences from *T. brucei,*  *Leishmania major, T. cruzi, T. vivax, T. brucei,* and *T. congolense* were aligned using Clustal Omega. The TriTryp Gene ID for each protein is listed in parentheses. An asterisk (\*) indicates positions which have a single, fully conserved residue, a colon (:) indicates conservation between groups of strongly similar proteins, and a period (.) indicates conservation between groups of weakly similar properties. The table displays the percent identity matrix between the proteins as calculated using Clustal Omega.

**Figure S3. Analysis of junction length at editing stop sites upon depletion of MRB7260.** **(A)** Percentage of the average junction length for all editing stop sites in RPS12 for the uninduced and MRB7260 RNAi induced samples. Junction lengths are divided into 4 categories based on the number of editing sites spanned: 0 (black), 1-10 (blue), 11-50 (yellow), and 50+ (green). **(B)** Average percentages of different categories of junction length: 0 (black), 1-10 (blue), 11-50 (yellow), and 50+ (green) at EPSs arising upon MRB7260 RNAi. **(C)** The sequences at all editing stop sites across RPS12 were grouped by junction length (as described above) and the proportion of sequences in each group was graphed from editing stop site 20 to 65 for the uninduced and induced samples. Black diamonds above the MRB7260 KD graph show the locations of EPSs for that protein knockdown. Open circles indicate editing stop sites in which junction length zero is significantly increased when MRB7260 is depleted in both replicates compared to the average of the uninduced samples.

**Figure S4.** ***In vitro* and *in vivo* assays to detectMRB7260 RNA-binding. (A)** UV cross-linking assays were performed using radiolabeled gA6[14] gRNA or an 79 nt fragment of pre-edited A6 mRNA with negative control proteins (p22 and MBP), a positive control protein (TbRGG2), and two MRB7260 variants (MBP-MRB7260-His and MRB7260-His). *In vitro* transcribed 32P-labeled RNA was first incubated with the indicated purified recombinant protein at RT. Reactions were subjected to UV cross-linking using the Stratalinker 2400 (Stratagene) for 10 minutes and then treated with RNase A. Proteins were then resolved on 10% (w/v) SDS-PAGE. Arrows indicate the size of full length proteins on both the Coomassie stained gel and the phosphorimage. **(B)** PF *T. brucei* cell lines (parental 29-13, TbRGG2-HTM, and MRB7260-MHT) were either untreated or exposed to UV treatment at 254 nm for either 200 or 400 mj/cm2. Denaturing precipitations via the His tags were then performed using protein lysates from these three cell lines. RNA was subjected to limited digestion, and remaining protein bound RNA was then labeled with [γ 32P]-ATP (3000Ci/mmol) using T4 polynucleotide kinase. Western blot using an anti-myc antibody was used to detect purified protein. The nitrocellulose membrane was then exposed to phosphor screen to detect RNA bound to the isolated protein.

**Figure S5. Circular dichroism spectrum of purified recombinant MRB7260.** Far UV spectra of MBP-MRB7260-His (585 nM) recorded in 1x PBS.

**Figure S6. MRB7260 knockdown variations at the level of RNA and protein.** Because specific anti-MRB7260 antibodies were not available until most of this study was complete, we were unable to evaluate protein levels for most experiments.  To estimate these protein levels, PF MRB7260 knockdown cells were grown for 1 day in the absence or presence of different concentrations of doxycycline (50-4000ng/ml) to vary the knockdown ofMRB7260. **(A)** Protein levels were analyzed via Western blot using anti-MRB7260 and anti-HSP70 (load control). **(B)** MRB7260 RNA levels were quantified by qRT-PCR. Relative RNA abundance represents RNA levels in doxycycline induced cells compared to levels in uninduced cells. RNA levels were normalized to β-tubulin and 18S rRNA levels and numbers represent the mean and standard error of 3 determinations.

**Table S1. Determination of Exacerbated Pause Sites (EPSs) in RPS12.** The number of sequences at each editing stop site (ESS) in RPS12 for replicates 1 and 2. The average uninduced sample includes the two uninduced MRB7260 samples from this study and 8 uninduced samples from a previous study (n=10) ([Simpson et al. 2017](#_ENREF_45)). The number of sequences at each editing stop site for each replicate of the induced samples are shown (norm. count) with the associated p and q values for that replicate. Sites are considered significantly increased if p < 0.05 and q < 0.05 and the norm. count is greater than the average of the uninduced. If an editing stop site is significantly increased in both replicates, this is an EPS and is shown in bold.

**Table S2. Oligonucleotide sequences used for this study.** List of the oligonucleotides used for cloning, qRT-PCR, and TREAT analysis.

**Table S3. Total fragments and unique sequences in the partially edited RPS12 sequence libraries. (A)** The breakdown of the number of fragments (decollapsed) in each sample (uninduced (-) and induced (+)) for the MRB7260 RNAi deep sequencing samples. Standard alignment (*i.e.* no non-T insertions, deletions or SNPs) and non-standard (*i.e.* with non-T errors) with a breakdown of the number of mismatches within the non-standard sequences (1, 2, >3) are shown. **(B)** The number of unique (collapsed) sequences represented in each category for the data set.