Supplementary Information for

**Trypanosome RNA Editing Substrate Binding Complex integrity and function depends on the upstream action of RESC10**

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**Figure S1**

**Figure S1.** **Controls for RESC10 RIP experiments. *A,*** Schematic representation of the qRT-PCR primers used to detect the largest pool of mRNAs for a given pan-edited transcript in RIPs. Pre-edited mRNA, black; edited mRNA, gray; never edited regions (NE), white. RIP mRNA primers (black arrows) hybridize to the 5′ never-edited and 5′ most pre-edited region of the transcripts. For pan-edited mRNAs, RIP primers detect pre-edited and most partially edited RNAs, excluding RNAs that are fully edited. ***B,*** qRT-PCRanalysis of A6, COIII, RPS12, and CYb mRNAs in total RNA, comparing Relative RNA Abundance in uninduced and induced RESC10 RNAi cells. Numbers represent the mean and standard deviation of two biological replicates, each with three technical replicates (n=6).qRT-PCR was also used to validate the level of RESC10 knockdown in the biological replicate experiments (32%-38%). ***C,D,*** Comparison of RNA immunoprecipitated with RESC6-PTP (*C*) RESC13-HTM or (*D*) compared to a mock IP. RNA was detected using RIP mRNA primers described in *A* and primers designed to detect a subset of gRNAs using qRT-PCR. RNA levels were standardized against 18S rRNA, and numbers represent the mean and standard deviation of two biological replicates, each with three technical replicates.

**Figure S2**

 **Figure S2. HTS analysis of RPS12 transcripts from RESC10 replete and depleted cells using TREAT. *A,*** Average number of RPS12 transcripts for the 10 uninduced (Avg Un) and two RESC10 RNAi-induced samples at each EPS. ***B,*** Pre-edited and fully edited sequences for the region depicted below are shown with Editing Site numbers indicated. Below that is an analysis of the most abundant sequences that occur within the first and second gRNA-directed blocks (Editing Stop Sites; ESS 19–35) in two replicates of RESC10 depleted cells compared to ten uninduced controls. Average number (normalized counts) of sequences in the uninduced samples (UI) and the RESC10 knockdown (KD) are shown. ESS, editing stop site for that sequence; JL, number of editing sites that the junction sequence spans; Fold change, fold change in the number of sequences for each specific sequence in the RESC10 knockdown samples compared to the uninduced samples. Pre-edited sequences are displayed in black, edited RNA is indicated in red, and junctions are shown in blue. Lower case u, u inserted by editing; asterisk, encoded U deleted by editing.

**Figure S3**



**Figure S3.** **EPSs and most abundant sequences arising in CYb mRNA upon RESC10 depletion. *A,*** Average number of CYb transcripts for 10 uninduced (Avg Un) and two RESC10 RNAi-induced samples at each Exacerbated Pause Site (EPS). ***B,*** Pre-edited and fully edited CYb sequences for the region analyzed with EPS numbers indicated. Below are shown the most abundant sequences arising within the gCYb-1 and gCYb-2 directed blocks after depletion of RESC10. ESS, editing stop site for that sequence; JL, number of editing sites that the junction sequence spans; KD, number of sequences reads in RESC10 knockdown; UI, number of sequences reads in uninduced cells; Fold, fold change in the number of each specific sequence in the RESC10 RNAi samples compared to the uninduced samples. Pre-edited sequence, black; fully edited sequence, red. Lower case u, u inserted by editing; asterisk, encoded U deleted by editing.

**Figure S4**



**Figure S4.** **EPSs and most abundant sequences arising in MURF2 mRNA upon RESC10 depletion. *A,*** Average number of MURF2 transcripts for 10 uninduced (Avg Un) and two RESC10 RNAi-induced samples at each Exacerbated Pause Site (EPS) for the RESC10 knockdown. ***B,*** The most abundant sequences arising within the gMURF-2 directed block after depletion of RESC10. Pre-edited sequence, black; fully edited sequence, red; junction sequences, blue. Labels and other symbols as in Fig. S3.