Supplemental Figure legends

Figure S1. **Specificity of affinity-purified anti-MRB3010 polyserum.** Western blot of native MRB3010 at increasing concentrations of whole cell lysate (WCL) from *T. brucei* 29:13 cells. A Blast search of the peptide sequence used as antigen in rabbit produces a unique alignment to this protein in *T. brucei* (data not shown). A crossreacting band below MRB3010 represents some breakage of MRB3010 in the lysate (*).

Figure S2. **Native immunopurified subcomplexes of MRB1 that contain either REH2 (H2) or MRB3010 (3010).** Western analysis of H2 and 3010 in IP replicates with anti-REH2 and anti-MRB3010 antibodies from a whole cell lysate (WCL) from *T. brucei* 29:13 cells. H2 (~240 kDa) is often significantly fragmented in WCL, and 3010 (57.5 kDa) migrates slightly below IgG in the IPs, which is used as a loading control. The blot shown was split into two halves. Each half was treated separately with the indicated antibodies.

Figure S3. **Radiolabeled capping of gRNA in immunoprecipitations of REH2 and MRB3010 and titration of total RNA in mitochondrial extract.** IPs from ~2 mg of pre-cleared mitochondrial extract were performed as described in the methods section. Radiolabeled samples in direct IPs and total RNA dilutions were used as control to adjust identical uncapped samples and use comparable gRNA amounts in the Illumina libraries. Capped and uncapped samples were prepared in parallel and identical cuts of the latter were obtained. Eluted RNAs were ligated to 3' barcoded adaptors, and multiplexed prior to phosphatase/PNK treatment to add a single phosphate, 5' adaptor ligation, and RT-PCR. The asterisk indicates non-specific capping products usually detected in total RNA and mock IP samples but not in REH2 and MRB3010 IPs.

Figure S4. **RNAi-repression of REH2 has no major effect on gRNA steady-state at day 4 post-induction.** (A) Western blot of REH2 in whole-cell lysates upon REH2-RNAi repression at 0 and 4 days post-induction with tetracycline (Tet). The mitochondrial MP81 subunit of the RECC complex examined with specific antibodies is a control for sample loading and RNAi specificity. (B) [^{32}P]G-capping of gRNA 5' triphosphate with guanylyltransferase in whole-cell lysates upon REH2-RNAi repression at 0, 4 and 6 days post-induction. An asterisk denotes a non-specific labeled product typically seen in crude lysates and extracts that serves as loading control. The gRNA was resolved in 10% UREA-PAGE.