

**Figure S1.** Growth curve of parental, Induced (+Dox) and uninduced(-Dox) for eYFP-RBP9 (*a*) and eYFP-RBP10 (*b*).



**Figure S2.** FACS analysis for the eYFP expression of the non-induced (C-), eYFP-RBP9 24 hours post-induction (t2) and eYFP-RBP10 10 hours postinduction (t2) cell lines



**Figure S3.** *Trypanosoma brucei* procyclic RNA-seq comparisons at t0, t1 and t2 post-induction of eYFP-RBP10 and eYFP-RBP9 proteins.



Log2 FC 24h pi (Wurst et al.2010 Microarray)

**Figure S4.** Scatter plot comparison of  $\geq$  2 FC Up-regulated and Down- regulated RNAs after RBP10 overexpression in PCFs. Log2 FC 10 h pi RNA- seq in the y-axis and Log2 FC 24 h pi microarray data from Wurst et al., 2010. n=99 genes (25 Up-regulated, 74 Down-regulated).



Figure S5. Distribution of BirA\*-RBP10 in sucrose density gradient fractions. Lysates of cell lines expressing the transgene for 10 h were fractionated by sucrose gradient centrifugation, and the absorption profiles at 254nm as well as western blotting using anti-myc antibodies were used to analyze the fractionation of BirA\*-RBP10. BiP protein was used as negative control of polysomal location and Ribosomal P0 as control of ribosomal protein presence in the different fractions.



**Figure S5.** Representation of the proteins present in each protein group in the  $\geq$ 10 Fold enriched fraction for the interactor and nascent peptide categories. (*a*). RBP9 biotinylated proteins present in the "Interactor" category at t2 are represented by Blue bars (n=167), light and dark grey bars represent the proteins falling within the "nascent peptide" category (n=41 and n=64, respectively). (*b*). RBP10 biotinylated proteins present in the "Interactor" category at t2 are represented by blue bars (n=171), light and dark grey bars represent the proteins falling within the "nascent peptide" category (n=13 and n=32, respectively). The categories were assigned based on GO terms or manually annotated and curated. Abbreviations: RHS: Retrotransposon Hot Spot Proteins; TCA: Tricarboxylic acid cycle; ETC: Electron transport chain



**Figure S6.** Normalized Spectral Index of DHH1 and SCD6 proteins after induction of BirA\*-RBP9, BirA\*-RBP10 or BirA\*-GFP.