SUPPLEMENTARY INFORMATION

Selective nuclear export of mRNAs is promoted by DRBD18 in *Trypanosoma brucei*

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Running title: DRBD18 controls mRNA export in *Trypanosoma brucei*

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SUPPLEMENTAL FIGURE S1

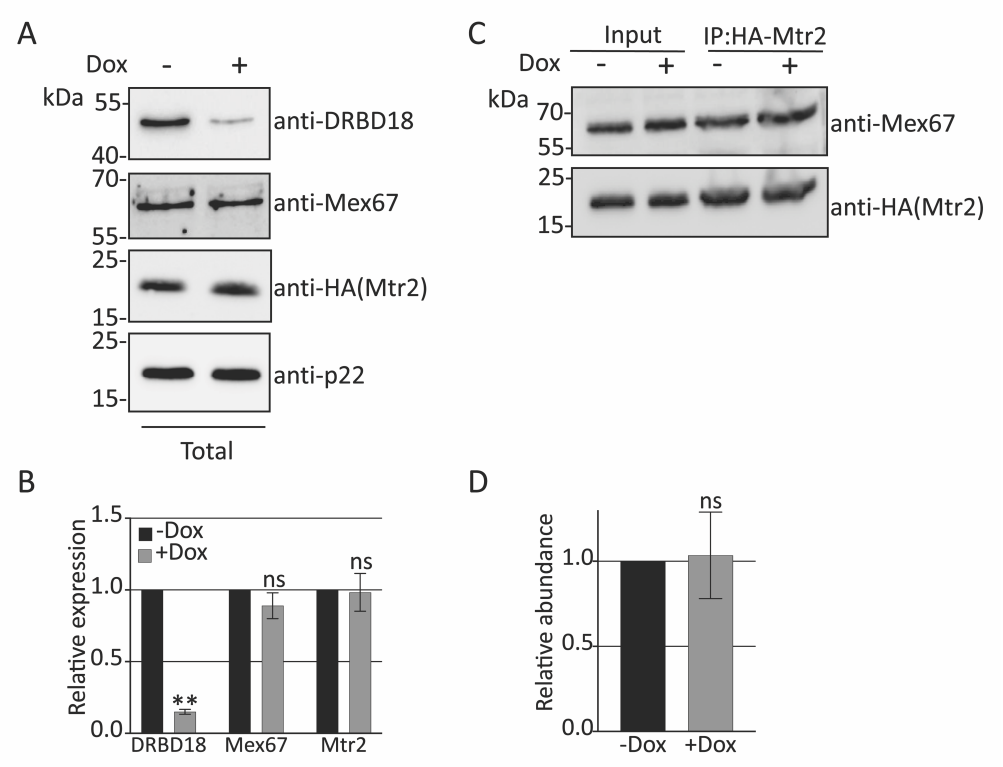

Figure S1. Effect of DRBD18 RNAi on the abundance and association of Mex67 and Mtr2. Cells harboring both 3XHA-tagged Mtr2 and the DRBD18 RNAi construct were grown for 23 h in the presence (+Dox) or absence of doxycycline (-Dox) to induce RNAi. **(A)** Steady state levels were determined by Western blot analysis of total cell lysates using anti-DRBD18, anti-Mex67 and anti-HA (Mtr2) antibodies. p22 served as a loading control. **(B)** Quantification of Western blots in (A). Protein levels were normalized to the expression of p22. The normalized protein expression from the +Dox was compared to that of the -Dox (which was set to 1) to calculate the relative expression in the DRBD18 knockdown. Bar graphs represent the average and standard deviation (SD) of four samples (two biological replicates, each with two technical replicates). **(C)** To assess the Mex67-Mtr2 association upon depletion of DRBD18, Mtr2 was immunoprecipitated using anti-HA antibodies. Bound proteins were eluted using 100 mM glycine and probed with anti-HA (Mtr2) and anti-Mex67 antibodies. **(D)** Quantification of Western blots in (C). The Mex67 signal was normalized to the anti-HA (Mtr2) signal. Two biological replicate experiments, each with two technical replicates, were performed. In all cases, significance was determined by unpaired t-test with Welch's correction. **P < 0.005 and ns = non-significant.

SUPPLEMENTAL FIGURE S2

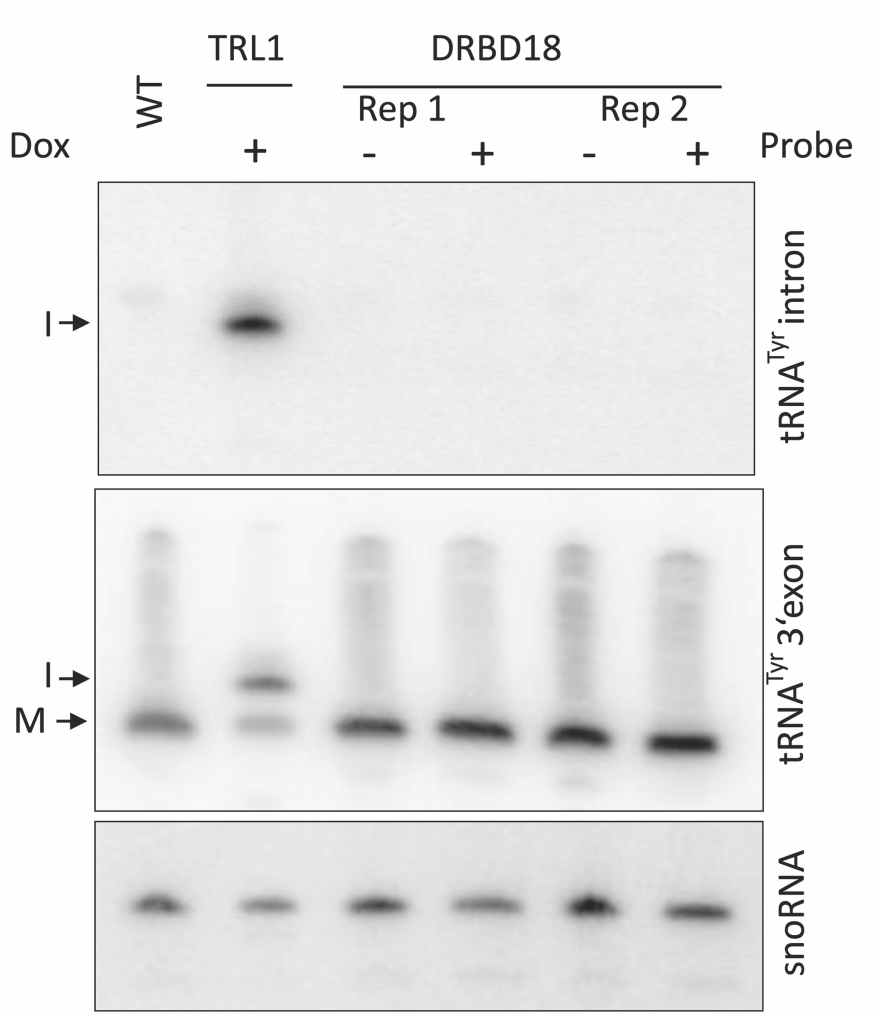

Figure S2. Downregulation of DRBD18 does not disrupt the export of intron-containing tRNA^{Tyr}.

Total RNA extracted from DRBD18 RNAi-induced (+Dox) and uninduced (–Dox) cells was separated on a urea gel, followed by Northern blotting. WT indicates total RNA extracted from strain 29-13 cells as a negative control, and TRL1 indicates RNA extracted from a tRNA ligase knockdown as a positive control for intron-containing tRNA^{Tyr} (Hegedűsová *et al.*, 2019). Probes for tRNA^{Tyr} 3' exon and intron were used to detect the accumulation of intron-containing tRNA (I) and mature tRNA (M), respectively. A snoRNA probe was used as a loading control.

SUPPLEMENTAL FIGURE S3

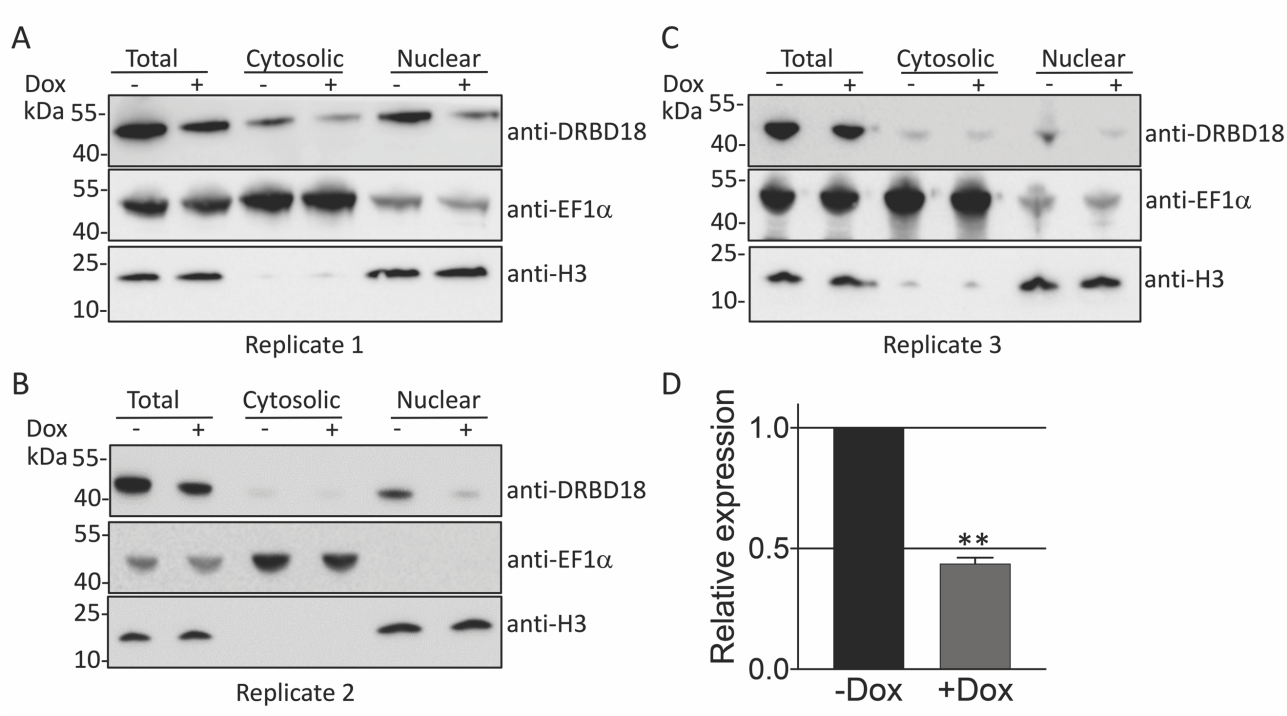

Figure S3. Confirmation of nuclear-cytosolic fractionation and DRBD18 knockdown.
Shown are the fractionations of the samples used for high throughput sequencing (A-C) and qRT-PCR (A and B). DRBD18 RNAi cells either uninduced (-Dox) or induced for 19 hours (+Dox) were subjected to nuclear-cytosolic fractionation. Total unfractionated extract (Total), cytosolic extract (Cytosolic), and nuclear extract (Nuclear) were subjected to Western blot analysis with anti-DRBD18, anti-EF1α, and anti-Histone H3 (H3) antibodies. (**D**) Bar graph represents the level DRBD18 in RNAi uninduced (-Dox) and induced cells (+Dox) in total extract from three biological replicates. Significance was determined by unpaired t-test with Welch's correction. **p <0.005.

SUPPLEMENTAL FIGURE S4

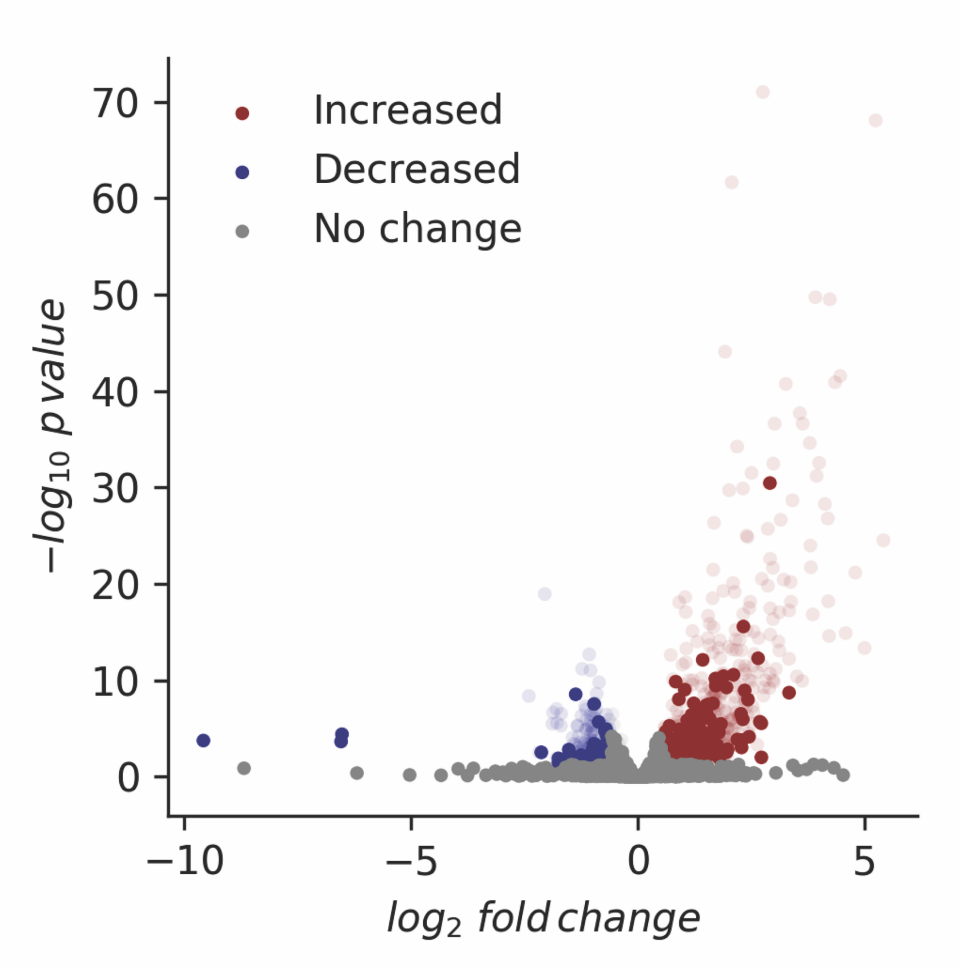

Figure S4. Volcano plot of transcript abundances in cytosolic fractions upon DRBD18 knockdown.

Transcripts decreased upon DRBD18 knockdown (blue) have corrected p-value < 0.05 and fold change < 1/1.5 (*i.e.*, log2 fold change < -0.58). Transcripts increased upon DRBD18 knockdown (red) have corrected p-value < 0.05 and fold change > 1.5 (*i.e.*, log2 fold change > 0.58). Grey indicates no significant change in transcript abundance upon DRBD18 knockdown. Those transcripts for which there was no change in whole cell RNA are opaque, while those with a significant change in whole cell RNA are transparent.

SUPPLEMENTAL FIGURE S5

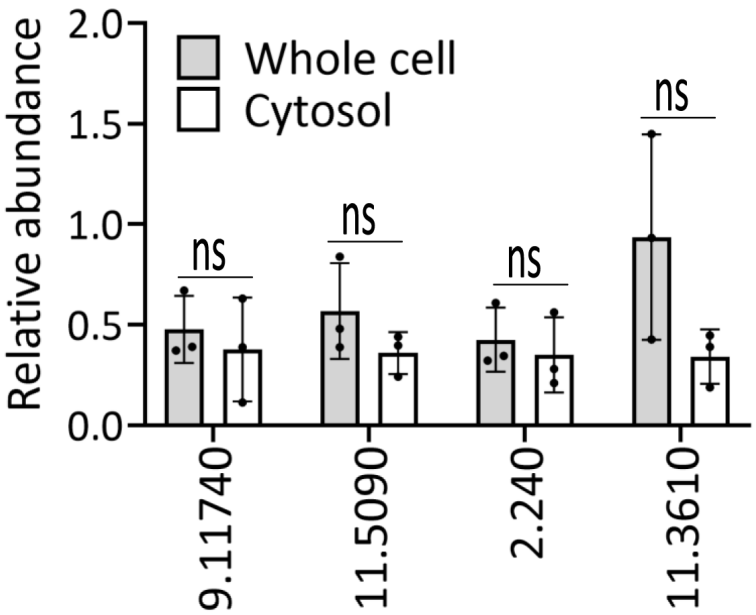

Figure S5. Relative abundance of selected transcripts in whole cell and cytosolic RNA in DRBD18 RNAi induced cells.

Whole cell or cytosolic RNA was isolated from PF *T. brucei* DRBD18 RNAi cells either uninduced or induced with doxycycline. Representative transcripts, identified by the last four to six digits of their TriTrypDB numbers, were quantified by qRT-PCR in whole cell and cytosolic fractions. Relative abundance represents RNA levels in induced (+Dox) cells compared to levels in uninduced (-Dox) cells. RNA levels were normalized to 18S rRNA. Values represent the mean of a three biological replicates, each with three technical replicates. Significance was determined by unpaired t-test. ns = non-significant.