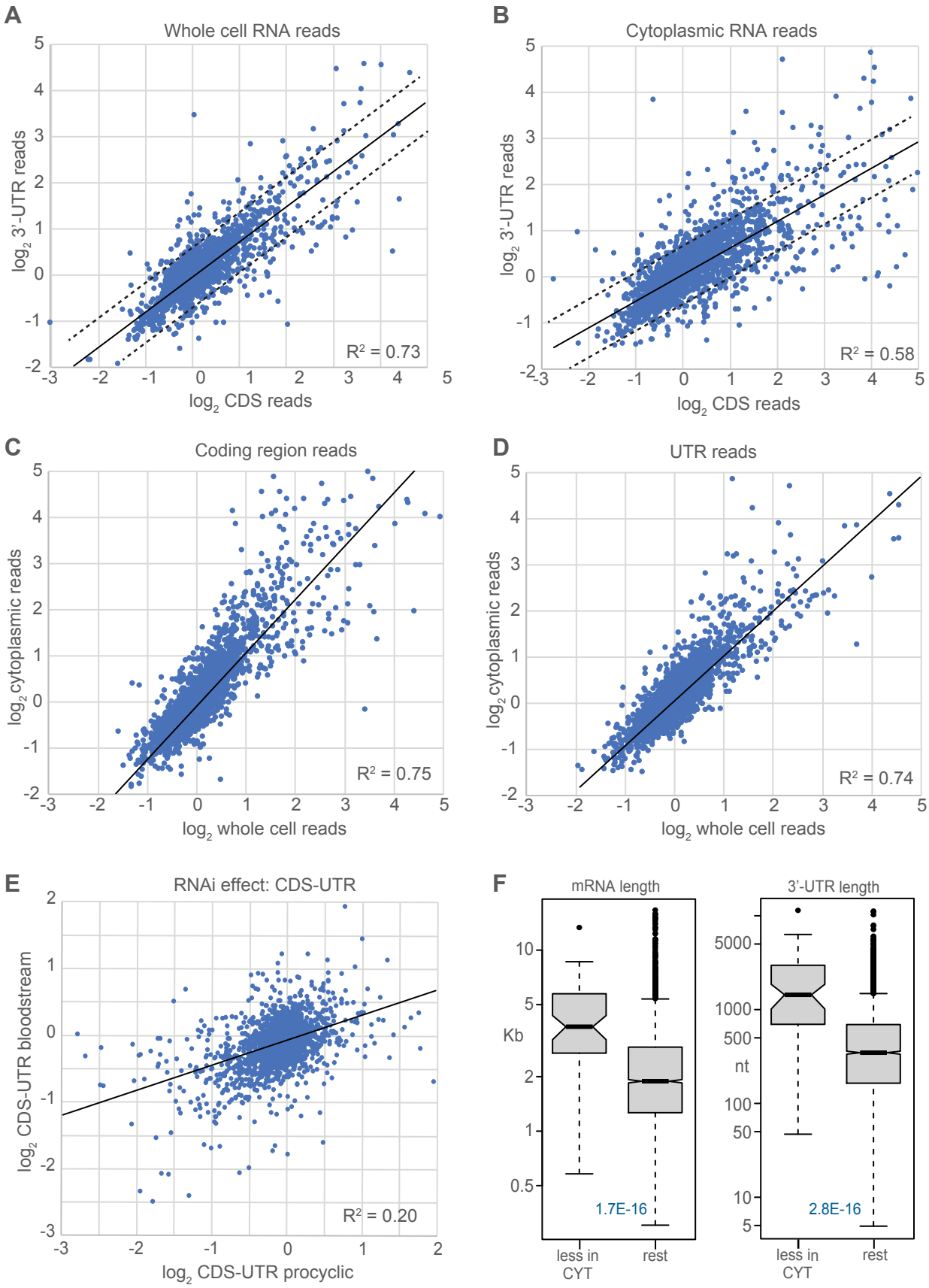

Supplementary Figure S4. Effects of DRBD18 depletion in procyclic forms on coding region and 3'-UTR read densities

This is a re-analysis of data from Mishra et al, 2021, showing poly(A)+ RNA after 19h RNAi.

A. Results for total poly(A)+ RNA are shown, with coding region (CDS) reads on the x-axis and 3'-UTR reads on the y-axis.

B. Results for cytoplasmic poly(A)+ RNA are shown, with coding region (CDS) reads on the x-axis and 3'-UTR reads on the y-axis.

C. Results for coding regions are shown, with whole cell poly(A)+ RNA reads on the x-axis and cytoplasmic reads on the y-axis.

D. Results for 3'-UTRs are shown, with whole cell poly(A)+ RNA reads on the x-axis and cytoplasmic reads on the y-axis.

E. Effects on the 3'-UTR relative to the CDS, for bloodstream forms (y-axis) and procyclic forms (x-axis. For each mRNA, the log2 fold change after RNA for the 3'-UTR was subtracted from the log2 fold change for the CDS. The magenta box encloses mRNAs for which the difference was less than 1.5-fold. A value of -1 means that relative to the coding region, 3'-UTR reads were half as abundant as before, suggesting relative accumulation of shortened mRNAs. A value of +1 indicates a doubling of 3'-UTR reads relative to the coding region.

F. Effects of RNAi on mRNA location in procyclic forms: mRNAs that showed a lower cytoplasmic RNA: whole cell RNA ratio after RNAi are significantly longer than other RNAs. Results are from Supplementary Table S4. First, we used DeSeq2 to calculated the ratios of cytoplasmic RNA to whole cell RNA for the -tet and +tet conditions. This yielded ratios for which we could see mRNAs that were present at significantly higher levels in whole cell RNA than in cyctoplasmic RNA (poorly exported or short half-life in the cytoplasm) or significantly higher in cytoplasmic RNA (very well exported or longer half-life in the cytoplasm). To find the effect of RNAi we simply, for coding regions, subtracted the log2 ratio for cells without RNAi from the log2 fold ratio for cells with RNAi. If the difference amounted to a decrease of at least 1.5-fold, the RNA was classified as having possible RNAi-induced retention in the nucleus (though this could also result from increased cytoplasmic instability). Student t-test results (in cyan) are for log-transformed values.