

**Figure S5. Experimental validation of** *T. brucei* genes that produce alternatively processed transcripts. Overlay of the number of reads (log<sub>2</sub>) from 5'-end- (blue) and 3'-end-enriched (red) libraries aligning to the shown regions of chromosome IV covering the transcripts produced for Tb927.4.4370 (*A*) and Tb927.4.4490 (*B*). Black arrows represent the annotated ORFs and purple arrows represent transcripts suggested by the internal tags (peaks in the pileups are indicated with red and blue downward arrows) and end-reads (not shown) alignment to the *T. brucei* genome. Note that Tb927.4.4370 is another example of a gene with a misanotated translation start codon. The positions of the regions hybridizing to specific probes are indicated by short black lines. (*C*) Northern blots of total RNA fractionated on denaturing agarose gels with the indicated (a – d) probes. Both probes a and b detect the full-length Tb927.4.4370 transcript. Probe a additionally detects a shorter transcript containing the Tb927.4.4370 ORF, while probe b additionally detects a shorter transcript that is a part of the 3' UTR of the full-length Tb927.4.4370 transcript. Probes c and d both detect the full-length Tb927.4.4490 ORF, while probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c additionally detects a shorter transcript containing the Tb927.4.4490 transcript. Probe c a