Supplementary Information (Badjatia *et al.*)

Table S1 Accession numbers of relevant *T. brucei* genes

FIG S1 *CRK1* and *CRK3* expression silencing

FIG S2 *CRK9* silencing in bloodstream trypanosomes

FIG S3 *CRK7* silencing in procyclic trypanosomes

FIG S4 Anti-CRK9 chromatin immunoprecipitation

FIG S5 *CRK9* silencing does not affect SL RNA pseudouridylation

Table S2 List of oligonucleotides

Table S1 Accession numbers of relevant *T. brucei* genes

Gene	Accession number [1]
RPB1	Tb927.4.5020, Tb927.8.7400
CRK1	Tb927.10.1070
CRK3	Tb927.10.4990
CRK7	Tb927.7.1900
CRK9	Tb927.2.4510
MTR1	Tb927.10.7940
MTR2	Tb927.11.4890 (previously Tb11.02.2500)
MTR3	Tb927.9.12040 (previously Tb09.211.3130)
SNIP	Tb927.8.3710

[1] Accession numbers are from the GeneDB (www.genedb.org)/TritrypDB (www.tritrypdb.org) genome data bases.

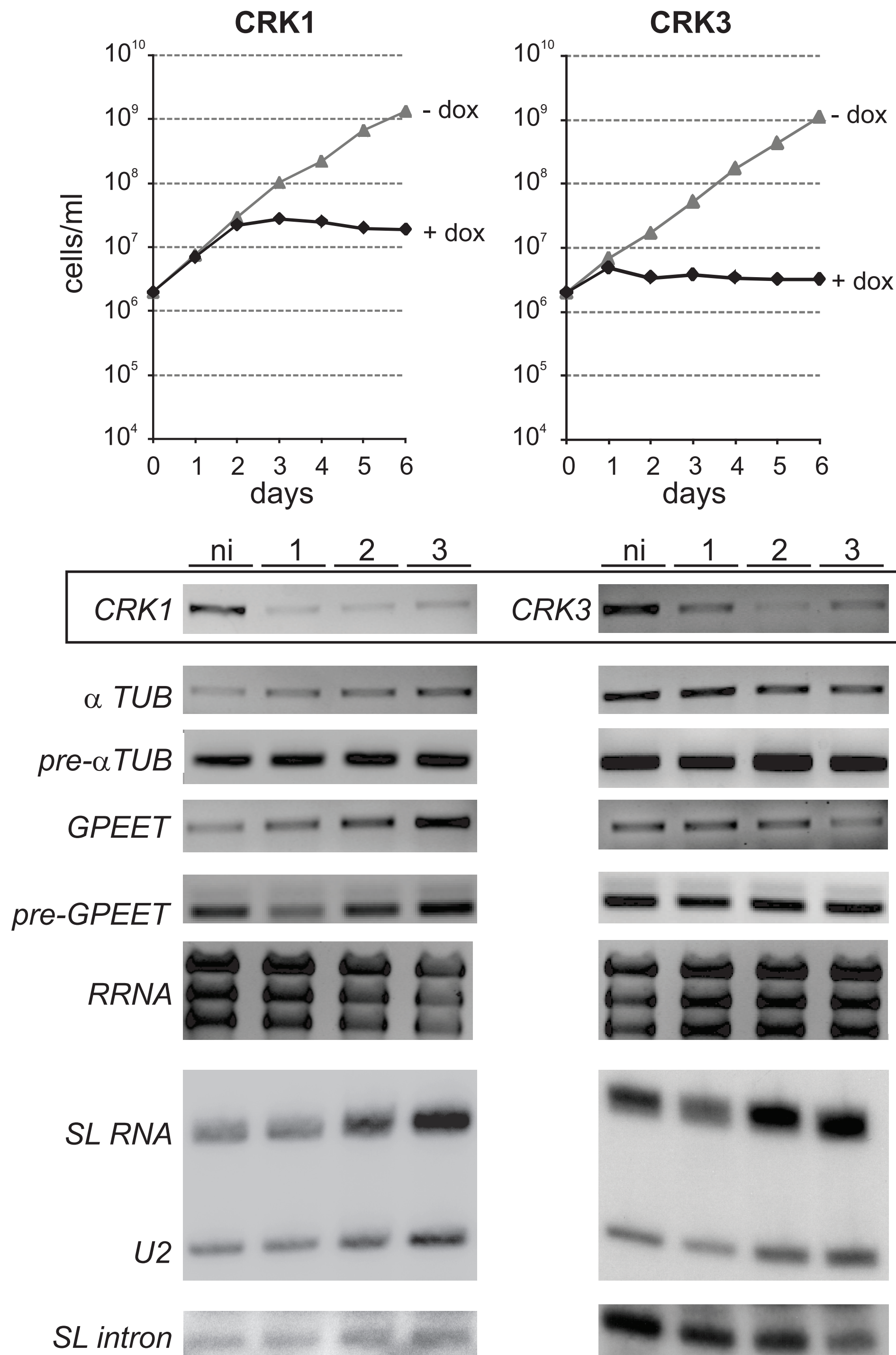

FIG S1 *CRK1* and *CRK3* expression silencing. For each knockdown, representative growth curves from one of three independently derived clonal cell lines were obtained in the absence and presence of the dsRNA synthesis-inducing reagent doxycycline (-/+ dox). *CRK* mRNA levels were determined by semi-quantitative RT-PCR of total RNA in non-induced (ni) cells and in cells that were silenced for 1 to 3 days (boxed panels). In the same RNA preparations, the relative abundances of α tubulin (α TUB) and of *GPEET* procyclin mature and pre-mRNAs were determined by semi-quantitative RT-PCR, those of the large ribosomal RNAs by ethidium bromide staining, and those of the SL RNA, the U2 snRNA, and the SL intron by primer extension assays.

CRK1 and *CRK3* silencing did not lead to a clear disturbance in gene expression patterns. In case of *CRK1* silencing, however, we did notice a reproducible, minor reduction in rRNA and compensatory increases in other RNAs suggesting a minor function of this kinase in the control of ribosome biogenesis.

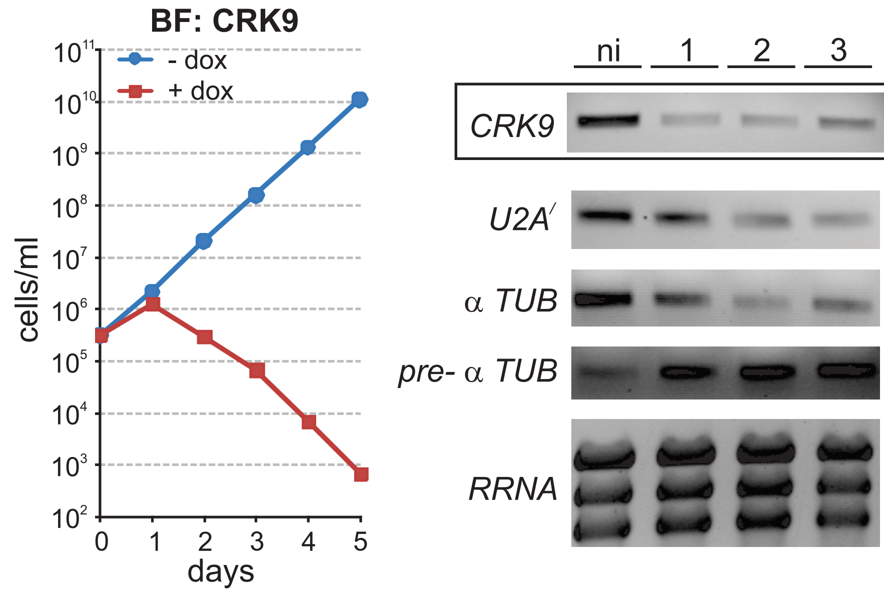

FIG S2 *CRK9* silencing in bloodstream trypanosomes. A representative growth curve in the absence and presence of the dsRNA synthesis-inducing reagent doxycycline (-/+ dox) of one of two independently derived clonal cell lines. Mature mRNAs of CRK9, the spliceosomal protein U2A′ and α tubulin (α TUB) as well as pre-mRNA of α tubulin (pre-α TUB) were analyzed by semi-quantitative reverse transcription (RT)-PCR in non-induced (ni) cells and cells that were silenced for 1, 2 or 3 days. The relative RNA abundances show the same trend as in procyclics: mRNA abundances decrease whereas the pre-mRNA level increases in *CRK9*-silenced bloodstream trypanosomes.

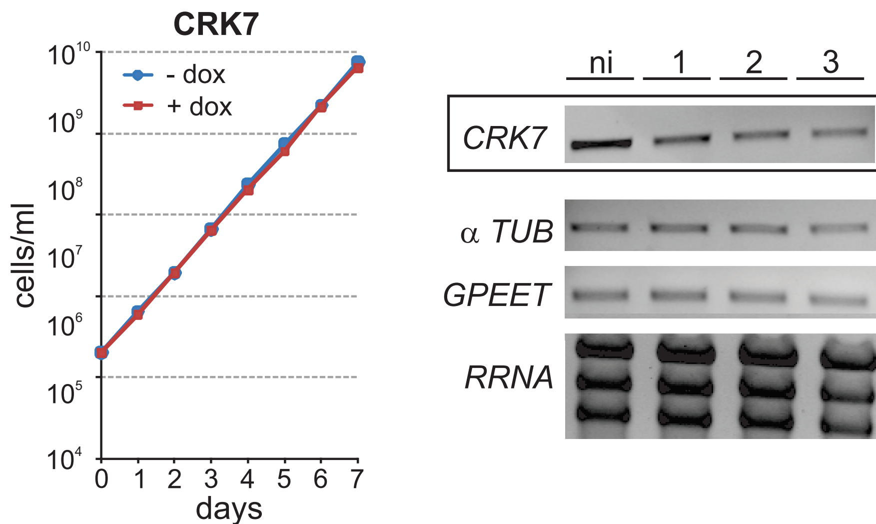

FIG S3 *CRK7* silencing in procyclic trypanosomes. A representative growth curve of one of three independently derived clonal cell lines obtained in the absence and presence of doxycycline (-/+ dox). Abundances of CRK7, α tubulin (α TUB) and of *GPEET* procyclin mRNA were analyzed in total RNA prepared from non-induced (ni) cells and from cells that were silenced for 1, 2 or 3 days by semi-quantitative RT-PCR. The large ribosomal RNAs were detected by ethidium bromide staining.

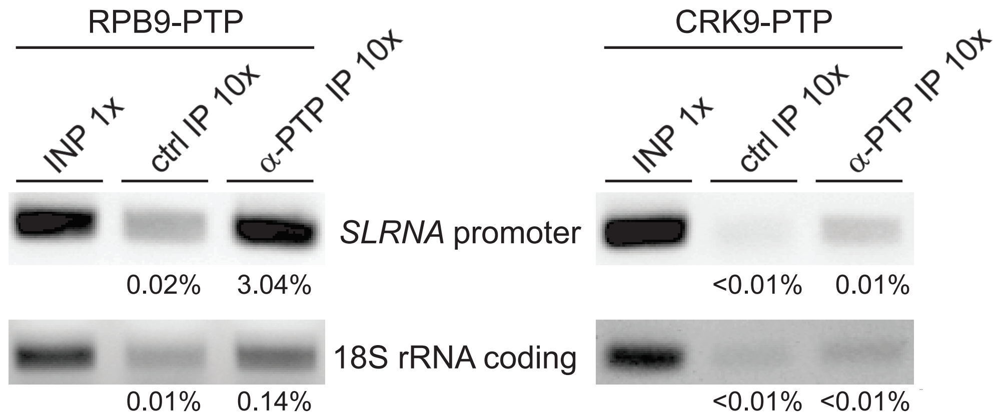

FIG S4 Anti-CRK9-PTP chromatin immunoprecipitation (ChIP). ChIP experiments were carried out with two cell lines expressing either the RNA pol II subunit RPB9 or CRK9 with a functional fusion of the composite PTP tag. Chromatin was precipitated with a ChIP-grade rabbit polyclonal anti-ProtA antibody (α-PTP IP). In negative control immunoprecipitations (ctrl IP) a comparable, rabbit-derived non-specific antibody was used. Precipitated DNA was analyzed by amplification of the *SLRNA* promoter region and, as a negative control, of the 18S rRNA coding region. For comparison, DNA of total chromatin (INP) was analyzed. The percent precipitation values specified under each lane were determined by qPCR.

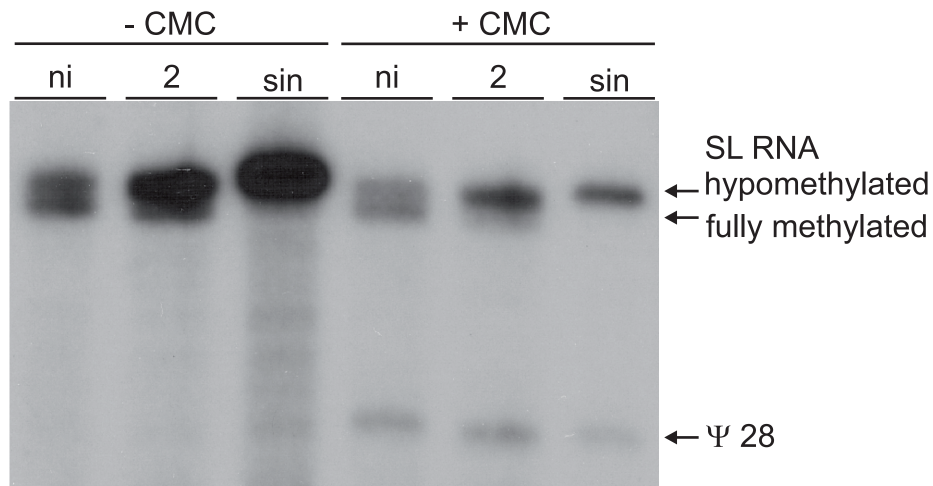

FIG S5 *CRK9* silencing does not affect SL RNA pseudouridylation. Total RNA, prepared from non-induced cells (ni), from cells in which *CRK9* was silenced for 2 days and from cells which were incubated with the general methyltransferase inhibitor sinefungin (sin) for six hours, was analyzed by primer extension with 5′ radiolabeled oligonucleotide SLf. RNA was either mock incubated or treated with *N*-cyclohexyl-*N*′-β-(4-methylmorpholinium)ethyl-carbodiimide *p*-tosylate (-/+ CMC) according to a published protocol (Bakin and Ofengand, 1993, Biochemistry 32, 9754-9762) to create an extension stop before the pseudoruridylated residue at position 28 (Ψ 28). To account for the SL RNA increase in *CRK9*-silenced and sinefungin-treated cells the amount of RNA in these samples was reduced by one half.

Table S2 List of oligonucleotides

Gene	Name	Usage	Sequence (5′ – 3′)
CRK1	CRK1 ApaI	RT-PCR of *CRK1* mRNA	TAGGGCCCGTTACGAGCGGCTTCAGAAGATTG
	CRK1 NotI	RT-PCR of *CRK1* mRNA	TACGGCCGGAACTCGACAGAAAAGTATGGGTG
CRK3	CRK3 ApaI	RT-PCR of *CRK3* mRNA	TAGGGCCCCGGAATCTGGAAAGCGCGAGC
	CRK3 NotI	RT-PCR of *CRK3* mRNA	GATCGCGGCCGCTACAATGCTTGGGGCGTTAACC
CRK7	CRK7 ApaI	RT-PCR of *CRK7* mRNA	TAGGGCCCGACCGATTTGTCGGCGGTGCTCTG
	CRK7 EagI	RT-PCR of *CRK7* mRNA	TACGGCCGGTACTCGTTGAAATATGTATGAC
CRK9	CRK9 RNAi-S	RT-PCR of *CRK9* mRNA	GTTGTTAAGAAAGAAAATTGAGG
	CRK9 3′ RT	RT-(q)PCR of *CRK9* mRNA	CGCCGCCGCTGCCAACCCCACTC
	CRK9 qPCR 5′	RT-qPCR of *CRK9* mRNA	GAGGAAGTGCAACAGCAGCG
	CRK9 UTR RNAi 5′	RT-PCR of *CRK9* mRNA	GATAAGCTTACGCGTAGGATGGAAGGGGTTGTGAC
	CRK9 UTR RNAi 3′	RT-PCR of *CRK9* mRNA	GATCTAGACTCGAGGCACTCGATACTTCAAAGAGC
	CRK9 Apa1	RT-PCR of *CRK9* mRNA	TAGGGCCCGCTCTGTGAATGAATATGTTCCCG
	HA antisense	RT-PCR of *CRK9* mRNA	TTAAGCGTAGTCAGGTACGTCGTAAGGGTA
α *tubulin*	α-Tub Fw q1	RT-(q)PCR of α tub. pre-mRNA	ACAGTTTCTGTACTATATTG
	α-Tub Rv q1	RT-(q)PCR of α tub. pre-mRNA	GTGGATGCAGATAGCC
	α-Tub Fw q3	RT-(q)PCR of α tub. mRNA	GTGCATTGAACGTGGATCTG
	α-Tub Rv q3	RT-(q)PCR of tub. mRNA	GAGAGTTGCTCGTGGTAGGC
GPEET	Pre-GPEET 5′	RT-(q)PCR of *GPEET* pre-mRNA	CATGTTCTCGTGATCGCTGC
	Pre-GPEET 3′	RT-(q)PCR of *GPEET* pre-mRNA	GCAGATAAAGGGAACGAGGTGC
	GPEET 5′	RT-(q)PCR of *GPEET* mRNA	ATGGCACCTCGTTCCCTTTATCTGC
	GPEET 3′	RT-PCR of *GPEET* mRNA	TTAGAATGCGGCAACGAGAGCAGC
	GPEET qPCR 3′	RT-qPCR of *GPEET* mRNA	CCTTTGCCTCCCTTCACGATAAC
18S rRNA	18SrRNAcoding5′	RT-(q)PCR of 18s rRNA	TCATCAAACTGTGCCGATTAC
	18SrRNAcoding3′	RT-(q)PCR of 18s rRNA	CTATTGAAGCAATATCGG
SLRNA	SL_PE	Primer extension of SL RNA & RT-(q)PCR of *SL RNA*	CGACCCCACCTTCCAGATTC
	SL sense	RT-(q)PCR of *SL RNA*	ACAGTTTCTGTACTATATT
U1 snRNA	U1_PE	Primer extension of U1 snRNA	CACTCAAAGTTTACTGCA
U2 snRNA	U2_PE	Primer extension of U2 snRNA & RT-(q)PCR of *U2 snRNA*	ACAGGCAACAGTTTTGATCC
	U2 5′	RT-(q)PCR of *U2 snRNA*	ATATCTTCTCGGCTATTTAGC
U5 snRNA	U5_PE	Primer extension of U5 snRNA	CCGCTCGAGGACACCCCAAAGTTT