

Table S2. Accession numbers for protein sequences retrieved from TriTryp database [1–3], GeneDB [4,5], NCBI database [6] or GenBank [7]

Gene	Organism	Accession number
KKT20	<i>Trypanosoma brucei</i>	Tb927.8.4760
	<i>Trypanosoma congolense</i>	TcIL3000_0_00100
	<i>Trypanosoma vivax</i>	TvY486_0804180
	<i>Trypanosoma cruzi</i>	TcCLB.419043.10
	<i>Angomonas deanei</i>	EPY28808
	<i>Crithidia fasciculata</i>	CFAC1_040021300
	<i>Leishmania mexicana</i>	LmxM.10.1227
	<i>Strigomonas culicis</i>	EPY28494
	<i>Bodo saltans</i>	BS19285
KKT3	<i>Trypanosoma rangeli</i>	ESL05960
	<i>Trypanosoma grayi</i>	XP_009309824
	<i>Crithidia fasciculata</i>	CFAC1_300091200
KKT2	<i>Crithidia fasciculata</i>	CFAC1_280060200
PLK	<i>Candida tenuis</i>	XP_006685766
	<i>Trichoplax adhaerens</i>	XP_002116022
	<i>Trypanosoma brucei</i>	Tb927.7.6310
	<i>Trypanosoma cruzi</i>	TcCLB.506513.160
	<i>Bodo saltans</i>	BS35140
	<i>Naegleria gruberi</i>	XP_002670439
	<i>Drosophila melanogaster</i> (Polo)	NP_524179
	<i>Homo sapiens</i> (Plk1)	NP_005021

KKT3 and KKT2 from other species were described previously [8].

Table S3. Trypanosome cell lines used in this study.

Strain	Description	Used in Figure
SmOxP9	Parental cell line that expresses TetR and T7 RNAP [9]	–
BAP391	TY-YFP-KKT20	1
BAP517	GFP-NLS-KKT2 DPB	4a, 4b
BAP296	GFP-NLS-KKT3 DPB	data not shown
BAP535	GFP-NLS-KKT2 DPB W1048A	4b
BAP538	GFP-NLS-KKT2 DPB Y1064A	4b
BAP327	GFP-NLS-KKT2	4c
BAP533	GFP-NLS-KKT2 W1048A	4c
BAP536	GFP-NLS-KKT2 Y1064A	4c
BAP308	TY-tdTomato-KKT2	–
BAP546	TY-tdTomato-KKT2, GFP-NLS-KKT20 DPB	4d
BAP550	TY-tdTomato-KKT2, GFP-NLS-KKT20 DPB W99A	4d
BAP551	TY-tdTomato-KKT2, GFP-NLS-KKT20 DPB Y112A	4d
BAP545	TY-tdTomato-KKT2, GFP-NLS-KKT20	4e
BAP547	TY-tdTomato-KKT2, GFP-NLS-KKT20 W99A	4e
BAP549	TY-tdTomato-KKT2, GFP-NLS-KKT20 Y112A	4e

Table S4. Plasmids used in this study.

Plasmid	Description
pEnT5-Y	TY-YFP tagging vector, Hygromycin [9]
pBA67	TY-YFP-KKT2 tagging vector, Hygromycin [8]
pBA148	TY-tdTomato tagging vector, Blasticidin [8]
pBA164	TY-tdTomato-KKT2 tagging vector, Blasticidin
pBA463	TY-YFP-KKT20 tagging at endogenous locus, Hygromycin
pDEX777	Inducible expression vector (GFP-TY fusion), integrate at 177 bp, Phleomycin [9]
pBA310	Inducible expression vector (GFP-NLS fusion), integrate at 177 bp, Phleomycin
pBA736	Inducible GFP-NLS-KKT2 DPB, integrate at 177 bp, Phleomycin
pBA366	Inducible GFP-NLS-KKT3 DPB, integrate at 177 bp, Phleomycin
pBA782	Inducible GFP-NLS-KKT2 DPB W1048A, integrate at 177 bp, Phleomycin
pBA785	Inducible GFP-NLS-KKT2 DPB Y1064A, integrate at 177 bp, Phleomycin
pBA425	Inducible GFP-NLS-KKT2, integrate at 177 bp, Phleomycin
pBA780	Inducible GFP-NLS-KKT2 W1048A, integrate at 177 bp, Phleomycin
pBA783	Inducible GFP-NLS-KKT2 Y1064A, integrate at 177 bp, Phleomycin
pBA748	Inducible GFP-NLS-KKT20 DPB, integrate at 177 bp, Phleomycin
pBA791	Inducible GFP-NLS-KKT20 DPB W99A, integrate at 177 bp, Phleomycin
pBA792	Inducible GFP-NLS-KKT20 DPB Y112A, integrate at 177 bp, Phleomycin
pBA747	Inducible GFP-NLS-KKT20, integrate at 177 bp, Phleomycin
pBA786	Inducible GFP-NLS-KKT20 W99A, integrate at 177 bp, Phleomycin
pBA790	Inducible GFP-NLS-KKT20 Y112A, integrate at 177 bp, Phleomycin

Table S5. Primers used in this study.

To make	Primers (all are listed 5' to 3')
pBA463	CDS targeting sequence with XbaI and NotI BA887: GATCGATC TCTAGA GGAGCAGGT AGGTGCGTATTATTTACT BA888: GATCGATC GCGGCCGC GAGTAAGCCCCATTTACCG 5'UTR targeting sequence with NotI and BamHI BA889: GATCGATC GCGGCCGC CTGTTTACCGCGCGTCTGAC BA890: GATCGATC GGATCC TCAGTGGTTAGTGGTGGCAG
pBA310	NLS with multiple cloning sites with synthetic XbaI and BamHI sites BA680: CTAGA ATCGATAC CGGT CGAGGACACAAGCGGTCACGTGAA CAATTGCCGCGGGATATCCATATGTTAATTAAGGCGCGCCTGATCAGTTT AAAC G BA681: GATCC GTTTAAACTGATCAGGCGCGCCTTAATTAACATATGGATATCCCGCGGC A ATTGTTACAGTGACCGCTTGTGTCCTCGACCGGTATCGAT T
pBA425	CDS targeting sequence with BamHI and AflII BA763: AGTCAC GGATCC TTCAATGTCTCACCAGCGAG BA768: AGCCAC CTTAAG TCATTTCCGGTAGACGTTGC
pBA736	CDS targeting sequence with BamHI and AflII BA1159: AGTCAC GGATCC ACGGCGTACCTGGCCCTTCC BA768: AGCCAC CTTAAG TCATTTCCGGTAGACGTTGC
pBA366	CDS targeting sequence with BamHI and AflII BA619: AGTCAC GGATCC CTAAGTGGTGAATGGAAACC BA620: AGCCAC CTTAAG TTACACGCCACCGTAGAGCAT
pBA747	CDS targeting sequence with PacI and AscI BA985: GATC TTAATTAA G AGGTGCGTATTATTTACT BA988: GATC GGCGCGCC CTACCTTTGTCCTCCTTTTAAAT
pBA748	CDS targeting sequence with PacI and AscI BA1157: AGTCAC TTAATTAA G ACTCCTTTCACTGCACTGGT BA988: GATC GGCGCGCC CTACCTTTGTCCTCCTTTTAAAT
KKT2 W1048A	BA1292: GATGACTTCCTAAGCGGGGCG <u>GCG</u> GTAAGGGTTTACTCTTTCATTG BA1293: CAATGAAAGAGTAAACCCTTACCGCCGCCCGCTTAGGAAGTCATC
KKT2 Y1064A	BA1294: CTGAAGTTGTTGTGATGTAT <u>GCT</u> TCTGTGCAACCCGGTAGGTAC BA1295: GTACCTACCGGGTTGCACAGAAGCATAACATCACAACAACCTTCAG
KKT20 W99A	BA1296: GTAATGAGGCCTACTGTCTAC <u>GCG</u> AGTGAGAGTGAGAGCTCTAG

BA1297:
CTAGAGCTCTCACTCTCACTCGCGTAGACAGTAGGCCTCATTAC
KKT20 BA1298:
Y112A CTAGAGCAATTGCACTTTGC GCC CAGATCCATCGAGACATTC
BA1299:
GAATGTCTCGATGGATCTGGGCGCAAAGTGCAATTGCTCTAG

Supplementary Reference

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