Identification of four unconventional kinetoplastid kinetochore proteins KKT22–25 in *Trypanosoma brucei*

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Summary

The kinetochore is a multi-protein complex that drives chromosome segregation in eukaryotes. It assembles onto centromere DNA and interacts with spindle microtubules during mitosis and meiosis. Although most eukaryotes have canonical kinetochore proteins, kinetochores of evolutionarily divergent kinetoplastid species consist of at least 20 unconventional kinetochore proteins (KKT1–20). In addition, twelve proteins (KKIP1–12) are known to localize at kinetochore regions during mitosis. It remains unclear whether KKIP proteins interact with KKT proteins. Here, we report the identification of four additional kinetochore proteins, KKT22–25, in *Trypanosoma brucei*. KKT22 and KKT23 constitutively localize at kinetochores, while KKT24 and KKT25 localize from S phase to anaphase. KKT23 has a Gcn5-related N-acetyltransferase (GNAT) domain, which is not found in any kinetochore protein known to date. We also show that KKIP1 co-purifies with KKT proteins, but not with KKIP proteins. Finally, our affinity purification of KKIP2/3/4/6 identifies a number of proteins as their potential interaction partners, many of which are implicated in RNA binding or processing. These findings further support the idea that kinetoplastid kinetochores are unconventional.

Keywords: Kinetochore, Kinetoplastid, *Trypanosoma brucei*, centromere, GNAT

1. Introduction

Kinetoplastids are a group of unicellular flagellated eukaryotes found in diverse environmental conditions [1]. It has been proposed that kinetoplastids may represent one of the earliest-branching eukaryotes based on a number of unique molecular features [2]. Understanding their biology could therefore provide insights into the extent of conservation or divergence among eukaryotes and lead to a deeper understanding of biological systems. Importantly, three neglected tropical diseases are caused by parasitic kinetoplastids: African trypanosomiasis, Chagas disease, and leishmaniasis, caused by *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* spp, respectively [3]. Although recent advances in public health and combination therapy have decreased the effect of these diseases, new drugs and druggable pathways are still very much needed against these diseases [4]. To this end, a more thorough understanding of the unique underlying biological mechanisms of kinetoplastids is critical.

One of such fundamental processes is the transmission of genetic material from mother to daughter cells, which is crucial for the survival of all organisms. Chromosome segregation in eukaryotes is driven by the kinetochore, a macromolecular protein complex that assembles onto centromeric DNA and captures spindle microtubules during mitosis [5]. Its structural core is typically composed of DNA-binding and microtubule-binding modules [6]. At least a fraction of core kinetochore proteins are present in nearly all sequenced eukaryotes, implying that most eukaryotes use a largely conserved mechanism of DNA and microtubule binding [7–9]. However, none of canonical kinetochore proteins have been identified in the genome of kinetoplastids [10,11]. To identify their kinetochore components, we previously carried out a YFP-tagging screen and identified a protein that forms kinetochore-like dots [12]. Affinity purification of this protein identified co-purifying proteins whose localizations were subsequently examined by microscopy. This process was repeated until saturation, leading to the identification of 20 proteins that localize at kinetochores in *T. brucei*. Chromatin immunoprecipitation followed by deep sequencing confirmed that they are indeed kinetochore proteins, and we therefore named them KKT1–20 (kinetoplastid kinetochore proteins). Although these proteins are highly conserved among kinetoplastids, their apparent orthologs are absent in other eukaryotes, suggesting that kinetoplastids have an unconventional type of kinetochore proteins [12,13].

Recently, KKT-interacting protein 1 (KKIP1) was identified as a protein distantly related to Ndc80/Nuf2 microtubule-binding proteins based on weak similarity in the coiled-coil regions [14]. However, KKIP1 apparently lacks the calponin homology (CH) domain, a critical feature of Ndc80/Nuf2 proteins. KKIP1 therefore does not appear to be a genuine Ndc80/Nuf2 ortholog. Nonetheless, KKIP1 localizes at kinetochores and its depletion causes severe chromosome segregation defects [14]. Immunoprecipitation of KKIP1 from chemically-crosslinked cells led to the identification of six additional proteins (KKIP2–7) that localize to kinetochore regions during mitosis [14]. Very recently, immunoprecipitation of KKIP2–7 from non-crosslinked cells identified a 9-subunit protein complex called the KOK (kinetoplastid outer kinetochore) complex that consists of KKIP2, 3, 4, 6, 8, 9, 10, 11, and 12 [15]. KKT proteins were not detected in the immunoprecipitates of KKIP2–12 or KKIP1 without chemical crosslinking [15]. It therefore remains unclear whether KKIP1–12 interact with KKT proteins in native conditions.

A hallmark of kinetochores in most eukaryotes is the presence of specialized nucleosomes containing the centromere-specific histone H3 variant CENP-A, which epigenetically specifies the position of kinetochore assembly, forms the primary anchorage point to DNA, and recruits other kinetochore proteins [16]. However, CENP-A is absent in kinetoplastids. It therefore remains unknown how their kinetochores assemble specifically at centromeres. *T. brucei* has 11 large chromosomes that have regional centromeres of 20–120 kb in size, as well as ~100 small chromosomes that lack centromeres [17–19]. Although kinetochore assembly sites on large chromosomes are apparently determined in a sequence-independent manner, the underlying mechanism remains a mystery.

To understand how unconventional kinetoplastid kinetochores perform conserved functions such as kinetochore specification, it is critical to have a complete constituent list. In this study, we report the identification of four additional kinetochore proteins in *T. brucei*.

2. Results

2.1 Identification of KKT22 and KKT23 in *Trypanosoma brucei*

Our previous immunoprecipitation of KKT3 that was N-terminally tagged with YFP (YFP-KKT3) did not result in co-purification of other kinetochore proteins [12]. To verify this result, we made a strain

that had a C-terminally YFP-tagged KKT3 (KKT3-YFP) as the sole copy of KKT3, and performed its immunoprecipitation using the same protocol. We detected a number of kinetochore proteins by mass spectrometry (Figure 1a and Table S1), suggesting that YFP-KKT3 was not fully functional. In addition to known kinetochore proteins, there were two uncharacterized proteins (ORF Tb927.9.6420 and Tb927.10.6600) that co-purified with KKT3-YFP in an apparently specific manner (Figure 1a). We tagged these proteins with an N-terminal YFP at the endogenous locus and found that they localized at kinetochores throughout the cell cycle (Figure 1b and 1c). Immunoprecipitation of these proteins showed that they specifically co-purified with other KKT proteins (Figure 1d and 1e). We therefore named them KKT22 and KKT23. These proteins were not detected in the immunoprecipitates of any other kinetochore protein [12], so it is likely that these proteins are closely associated with KKT3.

Homology search of KKT22 identified apparent orthologs in several kinetoplastids (Table 1), but not in *Bodo saltans* (a free-living kinetoplastid) or other eukaryotes. A profile-profile comparison using HHpred [20] did not reveal any obvious domain, except for a possible zinc hook motif of Rad50 (Figure S1). KKT23 has a Gcn5-related N-acetyltransferase (GNAT) domain [21–23], which is not found in any known kinetochore protein in other eukaryotes. In humans, TIP60 and KAT7/HBO1/MYST2 acetyltransferases (both are members of the MYST subfamily) are known to regulate kinetochore functions but are not part of core kinetochores [24,25]. We found that KKT23 co-purified with many KKT proteins (Figure 1e), implying that it is a core kinetochore protein in *T. brucei*. Interestingly, our sequence analysis failed to identify an obvious orthologous relationship with known GNAT subfamily members, suggesting that KKT23 forms a distinct subfamily. Our finding that an apparent ortholog of KKT23 is found even in divergent kinetoplastids (*Bodo saltans* and *Perkinsela*) (Table 1 and Figure S2) raises a possibility that it plays a fundamental role at the kinetoplastid kinetochore, which warrants further investigation.

2.2 Identification of KKT24

In our purification of YFP-KKT22, there was another kinetochore protein candidate (ORF Tb927.10.4200) (Figure 1d). We found that this protein in fact localized at kinetochores from S phase to anaphase (Figure 2a) and its immunoprecipitation confirmed specific co-purification with other

kinetochore proteins (Figure 2b). We therefore named it KKT24. Interestingly, KKT24 and KKIP1 share several similarities. Both proteins are predicted to consist mostly of coiled coils (Figure S3) [14], and their N-termini are located at the outer region of kinetochores, as judged by the formation of pairs of dots in metaphase (Figure 2b) [15,26]. However, our immunoprecipitation data do not support a possibility that KKT24 and KKIP1 form a stable complex (Figure 2b and see below). We also note that obvious orthologs for KKT24 and KKIP1 are not found in free-living *Bodo saltans*, an organism that has essentially all of KKT1–20 proteins (Table 1) [12,13].

2.3 Identification of KKT25

Our purification of KKT24 led to the identification of another kinetochore protein candidate (ORF Tb927.8.2830) (Figure 2b), which indeed localized at kinetochores from S phase to anaphase (Figure 3a). We confirmed that this protein co-purified with various kinetochore proteins (Figure 3b) and therefore named it KKT25. Like KKT22 and KKT24, it is conserved in many kinetoplastids, but not in *Bodo saltans* or other eukaryotes (Table 1 and Figure S4). We failed to identify any obvious domain or predicted coiled coils in KKT25.

2.4 KKIP1 co-purifies with KKT proteins, not with KKIP proteins

A previous study by D'Archivio and Wickstead identified a putative kinetochore protein KKIP1 that localized to kinetochores [14]. Its immunoprecipitation from chemically-crosslinked trypanosome cells led to co-purification of many nuclear proteins including KKT proteins and KKIP2–7 [14]. However, KKT proteins were not detected in the KKIP1 immunoprecipitate without crosslinking [15], so the relationship between KKIP1 and KKT proteins remained unclear. While re-searching our previous mass spectrometry data [12] against the latest *T. brucei* proteome database, we found that KKIP1 was actually present in the immunoprecipitate of KKT2 (Figure 4a). We had therefore named it KKT21 but switched to use the name KKIP1 following the publication of the D'Archivio and Wickstead paper. Immunoprecipitation of KKIP1 using our protocol revealed co-purification with a number of KKT proteins (Figure 4b), showing that KKIP1 is a genuine kinetochore protein. It is important to mention that we did not detect KKIP2–7 in our KKIP1 immunoprecipitation sample. This raises a possibility

that previous identification of KKIP2–7 in the immunoprecipitate of KKIP1 from crosslinked cells was due to the artificial chemical crosslinking, which is consistent with the identification of many nuclear proteins in the same sample [14].

2.5 KOK subunits co-purify with a number of proteins with RNA-related functions

Immunoprecipitation of KKIP2–7 from non-crosslinked cells identified a complex called the KOK (kinetoplastid outer kinetochore) complex that consists of 9 KKIP proteins (KKIP2, 3, 4, 6, 8, 9, 10, 11, 12) and localizes at the outer region of kinetochores during mitosis [15]. It has been proposed that KKIP1 provides a linkage between inner kinetochores and the KOK complex, despite the fact that KKIP1 was not detected in the immunoprecipitates of any KOK components [15]. To reveal the relationship between the KOK complex and KKT proteins, we performed immunoprecipitation of KKIP2–7 using our purification protocol. Immunoprecipitation of KKIP7 did not reveal any specific interacting proteins (Table S1), while that of KKIP5 was unsuccessful despite multiple attempts (Table S1 and data not shown). In contrast, immunoprecipitation of KKIP2, KKIP3, KKIP4, and KKIP6 revealed a number of co-purifying proteins, including the KOK components (Figure 5a-d). Besides Tb927.3.3740 and Tb927.2.3160/Gar1 which were detected in the previous report [15], ten additional proteins were identified as apparent interactors of KKIP2/3/4/6, many of which have putative domains implicated in RNA binding, transcription, or splicing (Figure 5e). Interestingly, our HHpred analysis also revealed a similarity to CTD kinase subunit in KKIP6 as well as a putative RNA recognition motif (RRM) in KKIP4, KKIP9, and KKIP10 (Figure 5e). The functional significance of these factors for kinetochore functions, if any, remains to be determined. Although we did not detect significant amounts of KOK components in our immunoprecipitates of KKT proteins or KKIP1, several kinetochore proteins were detected in the immunoprecipitates of KOK components, especially KKIP3 (Figure 5b). Because KKIP1 did not co-purify with any KOK components using the same purification protocol (Figure 4b), kinetochore localization of the KOK complex may be mediated by KKIP3, rather than KKIP1.

3. Discussion

In this study, we identified four additional kinetochore components in *T. brucei* (KKT22–25). We also confirmed that KKIP1 is a genuine kinetochore protein. It is possible that KKIP5 is also a kinetochore protein based on its presence in the immunoprecipitates of KKT24 and KKT25 (Figure 2b and 3b) as well as observed chromosome segregation defects upon depletion of KKIP5 [27]. Our original definition of genuine kinetochore proteins in *T. brucei* was that any such protein should co-purify "only" with other kinetochore proteins (except for KKT4 and KKT20 that also co-purify with APC/C subunits) [12,13]. According to this definition, components of the KOK complex are not genuine kinetochore proteins because they co-purify with a number of factors that are implicated in RNA binding or processing. However, it has been clearly shown that KOK components localize at outer kinetochore regions at least during metaphase [14,15]. More importantly, our immunoprecipitation of KKIP3 revealed co-purification with several KKT proteins, suggesting that the KOK complex indeed localizes at kinetochores. Defects in chromosome segregation have not been reported after knock-down of KOK components or its interaction partners [15]. We speculate that the KOK complex might be involved in the segregation of small chromosomes, rather than large chromosomes, in *T. brucei*.

Identification of a kinetochore protein that has a GNAT domain reinforces the idea that kinetoplastid kinetochores are unconventional. It will be important to test whether the GNAT domain of KKT23 is important for kinetochore functions. Acetylation of unknown substrates (possibly histones or kinetochore proteins) at centromeres might mark the position of kinetochore assembly sites in kinetoplastids that lack CENP-A. It is noteworthy that the genome-wide tagging project in *T. brucei* has come to an end, which did not identify any additional kinetochore components [28,29]. It is possible that we now have a complete list of kinetochore components, which include KKT1–20, KKIP1, KKIP5, and KKT22–25. Characterization of their functions and structures is not only important for our better understanding of eukaryotic chromosome segregation machinery but also for the development of new drugs against kinetoplastid diseases.

4. Materials and methods

4.1. Cells

All cell lines, plasmids, and primers/synthetic DNA used in this study are listed in Table S2, S3, and S4, respectively. All cell lines used in this study were derived from *Trypanosoma brucei* SmOxP927 procyclic form cells (TREU 927/4 expressing T7 RNA polymerase and the tetracycline repressor to allow inducible expression) [30]. Cells were grown at 28 ˚C in SDM-79 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum [31]. The cell line carrying KKT3-YFP as the sole copy of KKT3 was made by deleting one allele of KKT3 by a fusion PCR method [32] using a neomycin gene cassette from pBA183, followed by tagging of the remaining allele with a C-terminal YFP using a PCR-based method with a blasticidin selection marker [33]. N-terminally YFP-tagged KKT22 was made by a PCR-based method using pPOTv7 (eYFP, blasticidin) [33]. Endogenous YFP tagging for KKT23–25 and KKIP1–7 was performed using the pEnT5-Y vector [34] with PCR products or synthesized DNA fragments using XbaI/BamHI sites. Endogenous tdTomato tagging of KKT2 was performed using pBA164 that has a blasticidin selection marker [13] or pBA809 that has a neomycin marker. pBA809 was made by subcloning of the KKT2 targeting fragment from pBA67 [12] into pEnT6-tdTomato [34] using XbaI/BamHI sites. All constructs were sequence verified. Plasmids linearized by NotI were transfected to trypanosomes by electroporation into an endogenous locus. Transfected cells were selected by addition of 25 µg/ml hygromycin (pEnT5-Y derivatives), 10 µg/ml blasticidin (pBA164 or pPOTv7-based PCR products), or 30 µg/ml G418 (pBA809 or pBA183-based PCR products).

4.2. Fluorescence microscopy

Cells were fixed with 4% paraformaldehyde for 5 min and images were captured at room temperature on a DeltaVision fluorescence microscope (Applied Precision) installed with softWoRx version 5.5 housed in the Micron Oxford essentially as described [13]. Images were processed in ImageJ [35]. Figures were made using Inkscape (The Inkscape Team) and converted to EPS or PDF format.

4.3. Immunoprecipitation and mass spectrometry

Immunoprecipitation was performed as previously described using mouse monoclonal anti-GFP antibodies (Roche, 11814460001) that had been pre-conjugated with Protein-G magnetic beads (Thermo Fisher Scientific, 10004D) with dimethyl pimelimidate (Sigma, D8388) [12]. Mass spectrometry was also performed essentially as previously described using a Q Exactive (Thermo Scientific) at the Advanced Proteomics Facility, University of Oxford [12]. Peptides were identified by Mascot (Matrix Science) using a custom *T. brucei* proteome database. Proteins identified with at least two peptides were considered as significant and shown in Table S1.

4.4. Bioinformatics

Search for homologous proteins were done using BLAST in the TriTryp database [36,37], Jackhmmer on the UniProtKB proteome database using a default setting (HmmerWeb version 2.39 [38]), or hmmsearch on select proteomes using manually prepared hmm profiles (HMMER version 3.0 [39]. A protein with the best score in a given species was considered as a putative ortholog if a reciprocal search using BLAST or Jackhmmer identified the starting query protein as the best hit in the original species. HHpred was carried out using pfamA_v32.0 and PDB_mmCIF70 databases [20]. Multiple sequence alignment was performed with MAFFT (L-INS-i method, version 7) [40] and visualized with the Clustalx coloring scheme in Jalview (version 2.10) [41]. Coiled coils were predicted using COILS [42]. Accession numbers for protein sequences were retrieved from TriTryp database [11,37,43–47] or UniProt [48].

Supplementary information

A supplementary Excel file has all the lists of identified proteins by mass spectrometry (Table S1). A supplementary PDF file has 4 figures, as well as details of cell lines (Table S2), plasmids (Table S3), and primers or synthetic DNA sequences (Table S4) used in this study.

Author's contributions. OON performed immunoprecipitation of KKT3 and KKIP1–7 and identified KKT22 and KKT23. BA performed immunoprecipitation of KKT22, KKT23, KKT25 and identified KKT24. PL performed immunoprecipitation of KKT24 and identified KKT25. All authors participated

in data analysis. BA wrote the manuscript. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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Figure legends

Figure 1. Identification of KKT22 and KKT23

(a) KKT3-YFP co-purifies with a number of KKT proteins, including two kinetochore protein candidates, KKT22 and KKT23. See Table S1 for all proteins identified by mass spectrometry. Cell line, BAP1123.

(b) YFP-KKT22 and (c) YFP-KKT23 localize at kinetochores throughout the cell cycle. K and N represent the kinetoplast (mitochondrial DNA) and nucleus, respectively. These organelles have distinct replication and segregation timings and serve as good cell-cycle markers. K* is an elongated kinetoplast and indicates that the nucleus is in S phase. Bars, 5 μm. BAP1454 and BAP1593.

(d) KKT22 co-purifies with KKT proteins and another kinetochore protein candidate, KKT24. BAP1490.

(e) KKT23 co-purifies with KKT proteins. BAP1549.

Figure 2. Identification of KKT24

(a) KKT24 localizes at kinetochores from S phase to anaphase. Note that YFP-KKT24 signals appear as pairs of dots (indicated by *). Bar, 5 μm. Cell line, BAP1819.

(b) KKT24 co-purifies with a number of KKT proteins, KKIP5, and another kinetochore protein candidate, KKT25. BAP1635.

Figure 3. Identification of KKT25

(a) YFP-KKT25 localizes at kinetochores S phase to anaphase. Bar, 5 μm. Cell line, BAP1820.

(b) KKT25 co-purifies with a number of KKT proteins and KKIP5. BAP1742.

Figure 4. KKIP1 co-purifies with KKT proteins, not with KKIP proteins

(a) Re-analysis of our previous mass spectrometry data [12] identifies KKIP1 in the KKT2 immunoprecipitate.

(b) YFP-KKIP1 co-purifies with a number of KKT proteins but none of other KKIP proteins. Cell line, BAP710.

Figure 5. KOK subunits co-purify with a number of proteins with RNA-related functions

(a-d) Mass spectrometry summary tables of KKIP2, KKIP3, KKIP4 and KKIP6 immunoprecipitates. Cell lines, BAP825, BAP826, BAP808, BAP828.

(e) Putative domains in the identified proteins and KOK subunits.

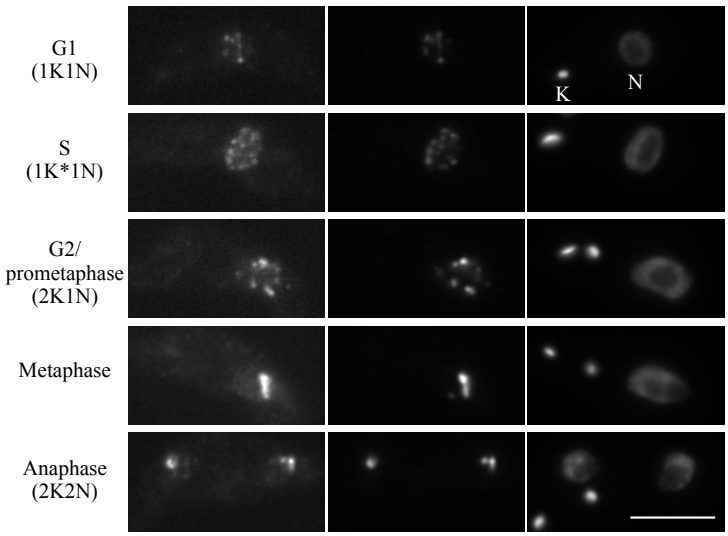
Table 1. Conservation of KKT22–25, KKIP1, and KKIP5 among kinetoplastids

	KKT22	KKT23	KKT24	KKT25	KKIP1	KKIP5
T. brucei	Tb927.9.6420	Tb927.10.6600	Tb927.10.4200	Tb927.8.2830	Tb927.5.4520	Tb927.7.6630
T. congolense		TcIL3000_10_5670	TcIL3000_10_3510	TcIL3000_0_41350	TcIL3000_5_5210	TcIL3000_0_33690
T. grayi	DQ04_05731010	DQ04_07341010	DQ04_12781000	DQ04_02041070	DQ04_01751000	DQ04_10231010
T. vivax		TvY486_1006570	TvY486_0019400	TvY486_0802340	TvY486_0503940	TvY486_0706450
T. cruzi	TcCLB.506509.60	TcCLB.510187.340	TcCLB.511467.50	TcCLB.504427.170	TcCLB.509539.40	TcCLB.510055.70
T. rangeli	TRSC58_06150		TraAM80_06991	TRSC58_01409	TRSC58_03083	TRSC58_00449
T. theileri	TM35_000182020	TM35_000022840	TM35_000421720	TM35_000132550	TM35_000061590	TM35_000202190
Blechomonas	Baya_113_0090	Baya_001_0150	Baya_039_0490	Baya_024_0050	Baya_086_0290	Baya_072_0080
Crithidia		CFAC1_250031900	CFAC1_240027400	CFAC1_150014500	CFAC1_020006200	CFAC1_090010600
Leptomonas		Lsey_0046_0080	Lsey_0122_0130	Lsey_0053_0040	Lsey_0120_0220	Lsey_0154_0070
Endotrypanum		EMOLV88_360026200	EMOLV88_350006300	EMOLV88_230010500	EMOLV88_050005000	EMOLV88_170008400
L. mexicana	LmxM.15.0825	LmxM.36.2100	LmxM.34.0180	LmxM.23.1610	LmxM.05.0010	LmxM.17.0430
Phytomonas	GSEM1_T00007051001	GSEM1_T00000676001	GSEM1_T00004838001	GSEM1_T00006110001	GSEM1_T00003924001	GSEM1_T00002551001
Paratrypanosoma	PCON_0021420	PCON_0016010	PCON_0019770	PCON_0076260	PCON_0068010	
Bodo saltans		BSAL_82255				
Perkinsela		XU18_2502				

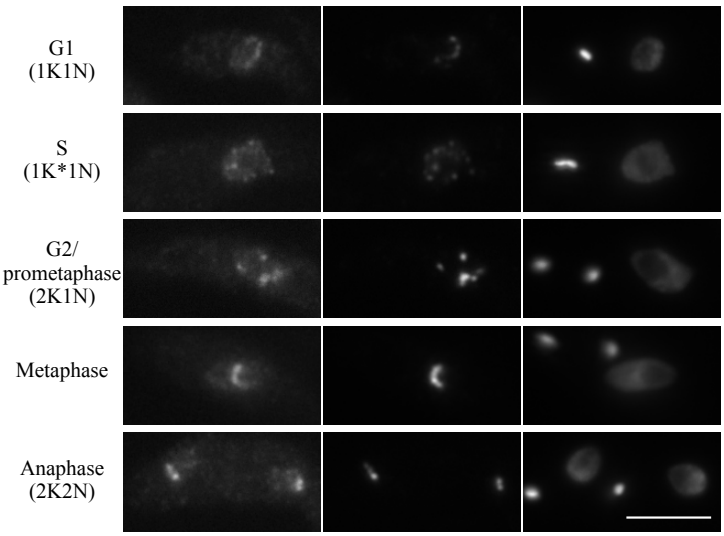
(*a*)

Proteins co-purified with KKT3-YFP	Mascot score	Number of peptides	Coverage
KKT3	4112	194	49.6%
KKT2	1096	39	35.9%
KKT7	961	43	52.0%
KKT1	788	32	19.9%
KKT8	395	12	37.3%
KKT9	208	8	24.3%
KKT10	188	7	18.9%
KKT6	175	13	33.7%
KKT4	133	6	21.2%
KKT19	132	6	20.5%
KKT14	127	4	14.2%
Tb927.9.6420 (KKT22)	119	6	33.9%
Tb927.10.6600 (KKT23)	105	7	33.3%
KKT11	101	4	21.9%

(*b*)



(*c*)



(*d*)

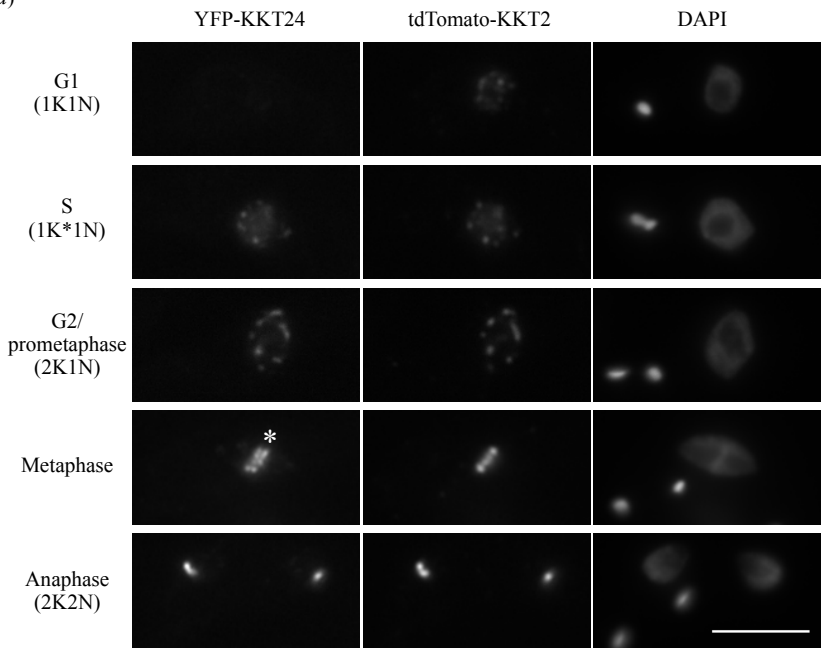
Proteins co-purified with YFP-KKT22	Mascot score	Number of peptides	Coverage
KKT3	9597	426	38.2%
KKT7	7351	273	49.1%
KKT1	4377	124	15.0%
KKT8	3940	103	34.8%
KKT2	3553	104	22.1%
KKT4	1595	40	19.8%
KKT6	1489	42	25.9%
KKT23	1045	38	7.8%
KKT10	999	46	16.8%
KKT14	815	39	16.2%
KKT5	640	23	15.4%
KKT9	579	35	32.2%
KKT19	533	26	22.1%
KKT12	394	11	15.0%
Tb927.10.4200 (KKT24)	178	11	6.0%
KKT22	85	10	15.8%

(*e*)

Proteins co-purified with YFP-KKT23	Mascot score	Number of peptides	Coverage
KKT3	5560	258	39.3%
KKT7	1775	62	37.9%
KKT22	1480	67	45.1%
KKT1	897	30	16.3%
KKT8	864	24	41.9%
KKT2	756	21	15.6%
KKT4	708	13	15.8%
KKT6	386	9	30.7%
KKT23	345	12	37.4%
KKT9	329	16	31.3%
KKT19	295	8	32.6%
KKT14	207	6	20.7%
KKT10	201	7	15.7%
KKT5	201	5	21.1%
KKT11	101	3	12.0%
KKT12	95	2	14.5%

Figure 1. Identification of KKT22 and KKT23

(*a*)

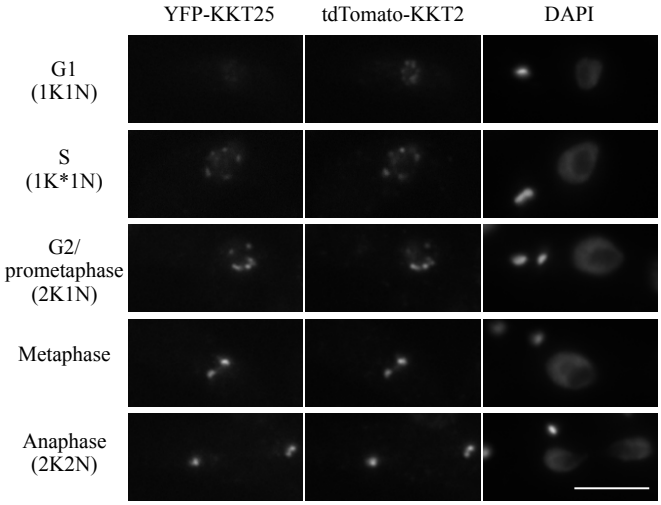


(*b*)

Proteins co-purified with YFP-KKT24	Mascot score	Number of peptides	Coverage
KKT7	4400	153	69.3%
KKT24	4191	126	60.2%
KKT1	3024	106	27.5%
KKT2	2735	88	38.7%
KKT9	2495	88	78.4%
KKT8	1913	52	73.2%
KKT4	1556	33	46.8%
KKT14	1098	29	31.5%
KKT10	908	40	37.2%
KKT19	724	34	36.0%
KKT11	688	24	49.0%
Tb927.8.2830 (KKT25)	684	32	42.2%
KKT6	565	20	45.4%
KKT5	481	19	34.2%
KKIP5	377	7	16.9%
KKT3	302	12	17.6%

Figure 2. Identification of KKT24

(*a*)



(*b*)

Proteins co-purified with YFP-KKT25	Mascot score	Number of peptides	Coverage
KKT24	8496	277	50.5%
KKT7	1123	48	32.8%
KKT25	881	28	49.4%
KKT8	531	16	29.1%
KKT2	420	14	13.0%
KKT1	404	19	20.6%
KKT4	387	8	13.0%
KKIP5	386	11	13.5%
KKT14	265	8	13.3%
KKT6	181	6	25.9%
KKT9	175	11	37.1%
KKT19	145	4	16.1%
KKT5	129	5	25.4%
KKT12	123	3	14.5%

Figure 3. Identification of KKT25

(*a*)

Proteins co-purified with YFP-KKT2	Mascot score	Number of peptides	Coverage
KKT2	1773	55	46.0%
KKT1	1023	31	23.5%
KKT7	701	22	45.5%
KKT8	538	17	59.6%
KKT11	302	8	32.3%
KKT10	295	13	27.7%
KKT16	291	4	43.3%
KKT9	287	9	32.2%
KKT6	238	6	28.3%
KKIP1	219	6	16.2%
KKT4	173	3	11.5%
KKT14	156	3	10.4%
KKT17	138	4	17.2%
KKT19	129	8	22.7%
KKT15	127	2	22.8%

(*b*)

Proteins co-purified with YFP-KKIP1	Mascot score	Number of peptides	Coverage
KKT1	4022	172	33.5%
KKT7	3824	176	78.9%
KKIP1	3607	182	61.3%
KKT9	2765	98	75.1%
KKT8	2216	81	80.7%
KKT2	1626	79	48.1%
KKT4	1529	58	54.9%
KKT14	710	28	46.3%
KKT6	641	24	42.4%
KKT11	614	32	62.9%
KKT10	448	25	33.5%
KKT19	440	23	35.7%
KKT5	404	23	40.4%
KKT15	301	14	33.4%
KKT3	203	18	17.9%

Figure 4. KKIP1 co-purifies with KKT proteins, not with KKIP proteins

(*a*)

Proteins co-purified with YFP-KKIP2	Mascot score	Number of peptides	Coverage
KKIP3	5752	270	85.8%
KKIP11	3596	164	64.3%
KKIP2	2157	71	30.0%
KKIP8	1625	72	40.5%
KKIP9	460	14	37.2%
KKIP10	258	12	37.3%
KKIP4	254	12	38.0%
KKIP6	204	4	8.2%
KKIP12	132	4	42.8%
Tb927.10.11600	90	3	12.4%
Tb927.3.3740	76	2	10.0%
KKT1	71	5	7.8%
KKT7	62	3	12.3%

(*b*)

Proteins co-purified with YFP-KKIP3	Mascot score	Number of peptides	Coverage
KKIP3	3357	157	78.5%
KKIP11	3222	142	66.5%
KKIP2	1571	44	26.8%
KKT1	880	27	23.3%
KKIP8	855	37	31.6%
KKT2	684	21	30.2%
KKT7	615	34	53.7%
KKT4	458	16	40.8%
KKIP9	456	16	34.5%
KKIP4	316	17	35.7%
KKIP10	312	12	32.4%
KKT14	257	10	20.0%
Tb927.10.11600	231	8	20.2%
KKIP6	164	4	15.1%
Tb927.3.3740	164	7	28.7%
KKT6	152	3	29.3%
Tb927.7.5590	136	4	11.6%
KKT24	118	5	13.2%
KKIP12	118	2	15.6%

(*c*)

Proteins co-purified with YFP-KKIP4	Mascot score	Number of peptides	Coverage
KKIP4	9534	367	72.3%
KKIP9	8129	291	71.7%
KKIP10	6115	292	68.2%
Tb927.3.3740	5233	231	85.0%
Tb927.10.11600	4158	184	58.4%
KKIP12	4141	143	80.4%
Tb927.7.5590	3609	164	35.3%
KKIP6	3536	145	64.0%
Tb927.11.7450	2078	96	55.4%
Tb927.11.2900	1596	45	77.8%
Tb927.2.3160	757	29	43.6%
Tb927.8.7400	592	30	20.0%
Tb927.10.170	591	28	47.3%
Tb927.5.3250	577	21	18.1%
Tb927.7.6320	522	15	20.8%
KKIP11	489	20	26.1%
KKIP8	489	13	20.9%
KKIP3	408	22	40.9%
Tb927.10.4740	330	17	87.3%

(*d*)

Proteins co-purified with YFP-KKIP6	Mascot score	Number of peptides	Coverage
KKIP9	6003	172	76.7%
KKIP4	5933	218	76.0%
KKIP10	5825	216	65.3%
KKIP12	3551	85	82.4%
Tb927.7.5590	2120	82	30.5%
Tb927.10.11600	1941	77	54.4%
Tb927.3.3740	1783	106	79.4%
KKIP6	1780	57	55.6%
Tb927.11.7450	588	26	30.1%
Tb927.5.3250	586	18	20.7%
Tb927.11.2900	584	18	60.8%
Tb927.4.5020	555	20	16.5%
Tb927.10.170	529	19	42.4%
KKIP3	497	23	40.7%
KKIP11	483	20	30.9%
Tb927.10.4740	310	14	87.3%

(*e*)

Name	Annotation and domains
Tb927.10.11600	**putative splicing factor, arginine/serine-rich domain**
Tb927.3.3740	**zinc-finger double-stranded RNA-binding**
Tb927.7.5590	domain of unknown function (DUF3883)
Tb927.11.7450	HIT zinc finger
Tb927.11.2900	HIT zinc finger
Tb927.2.3160	**H/ACA ribonucleoprotein complex subunit, Gar1/Naf1 RNA binding region**
Tb927.10.4740	**H/ACA ribonucleoprotein complex subunit Nop10**
Tb927.10.170	**H/ACA ribonucleoprotein complex subunit Cbf5, tRNA pseudouridine synthase**
Tb927.5.3250	**weak similarity to pre-mRNA-splicing factor 8**
Tb927.7.6320	BTB/POZ domain, regulator of chromosome condensation (RCC1) repeat
Tb927.8.7400	**RNA polymerase IIA largest subunit**
Tb927.4.5020	**DNA-directed RNA polymerase II subunit RPB1**
KKIP2	hypothetical protein
KKIP3	putative PDZ domain
KKIP4	**putative RNA recognition motif (RRM)**
KKIP6	**weak similarity to CTD kinase subunit gamma**
KKIP8	**poly(A) polymerase**
KKIP9	**putative RNA recognition motif (RRM)**
KKIP10	**putative RNA recognition motif (RRM)**
KKIP11	hypothetical protein
KKIP12	**RBP34, RNA-binding protein, putative RNA recognition motif (RRM)**

Figure 5. KOK subunits co-purify with a number of proteins with RNA-related functions

Supplemental Materials for

Identification of four unconventional kinetoplastid kinetochore proteins KKT22–25 in *Trypanosoma brucei*

Olga O. Nerusheva, Patryk Ludzia, and Bungo Akiyoshi

Supplemental Figures

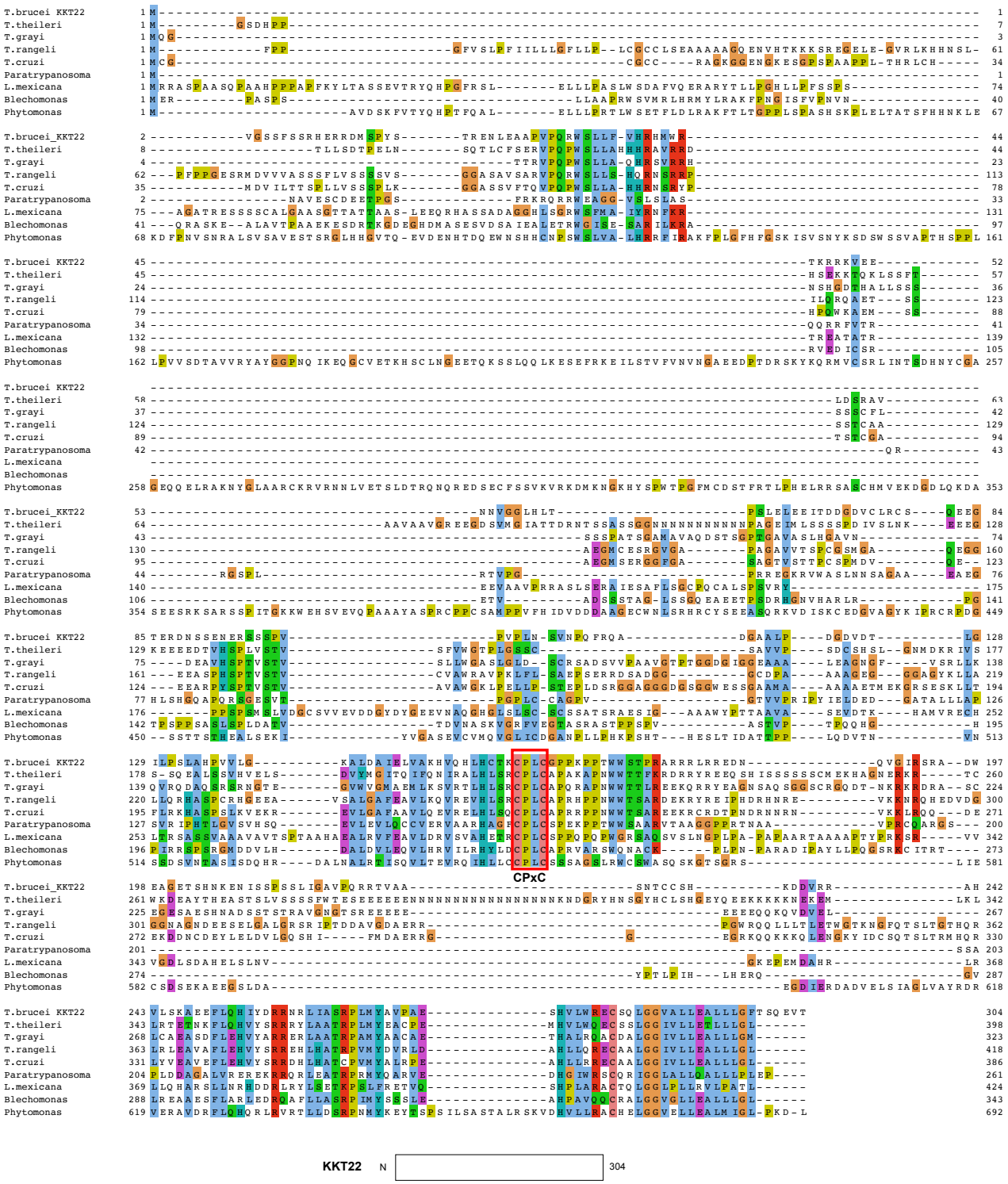


Figure S1. Multiple sequence alignment of KKT22

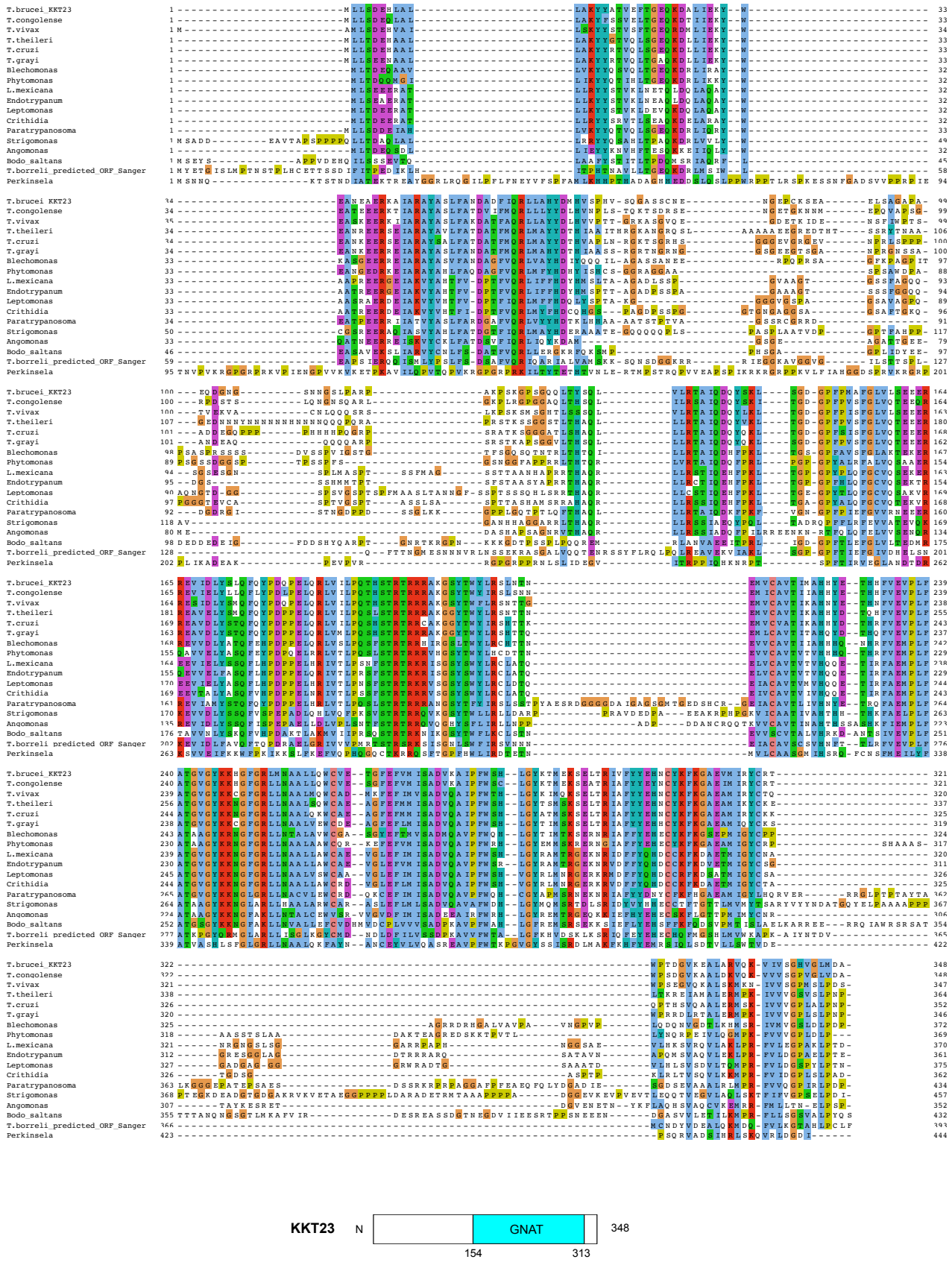


Figure S2. Multiple sequence alignment of KKT23

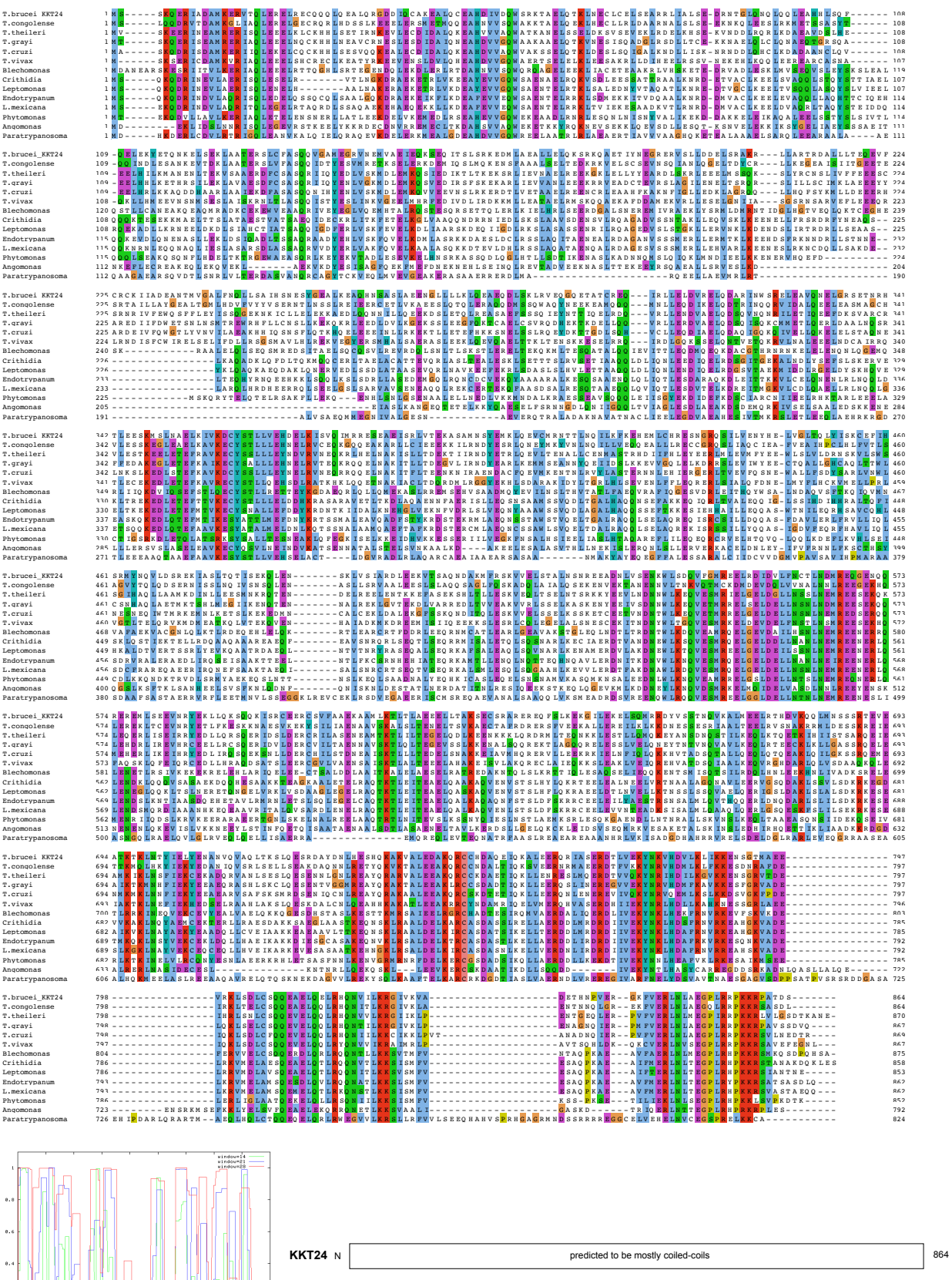


Figure S3. Multiple sequence alignment of KKT24 and its coiled-coil prediction

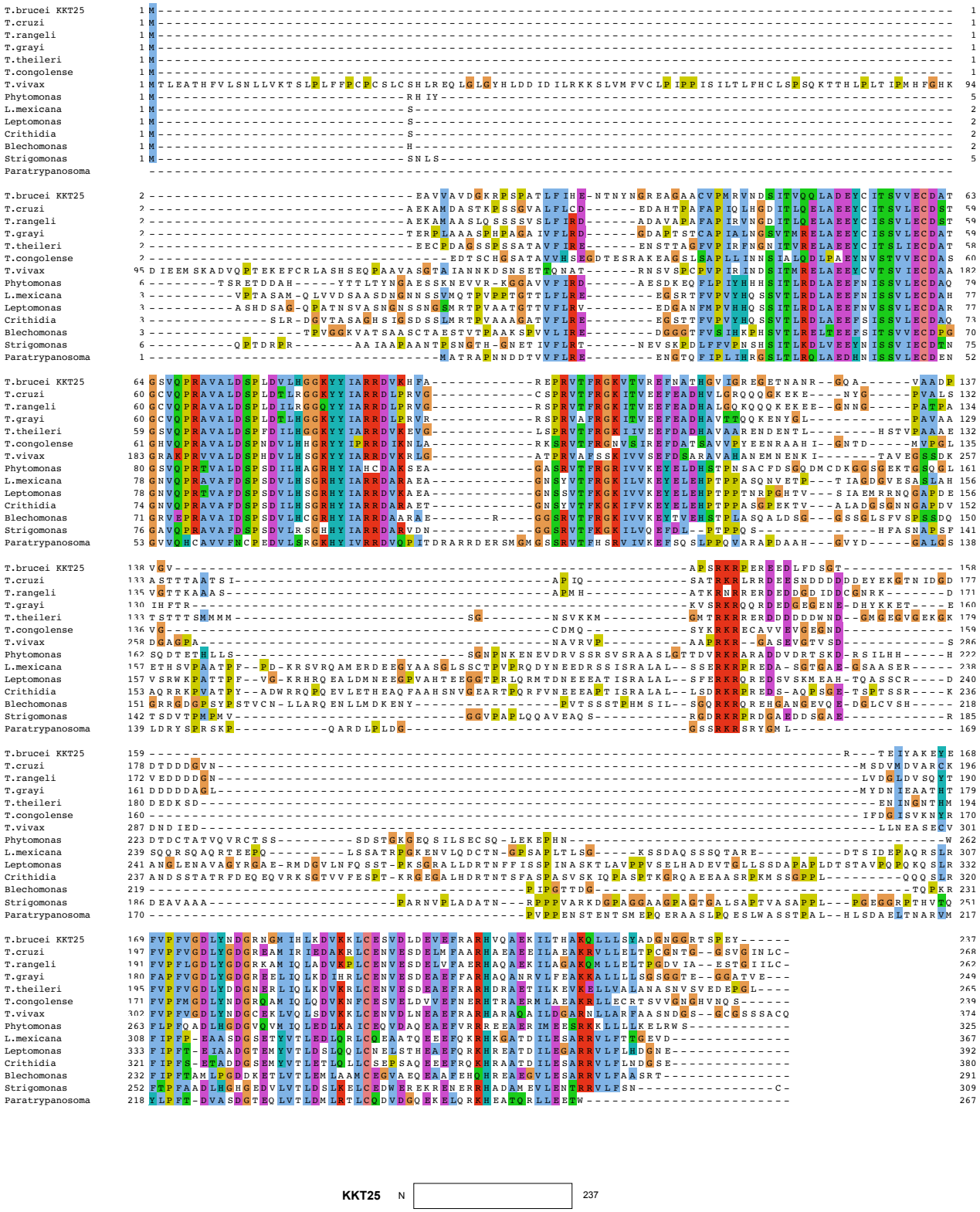


Figure S4. Multiple sequence alignment of KKT25

Supplemental Tables

Table S1. Lists of all proteins identified in the immunoprecipitates of KKT22–25, KKT2, and KKIP1–7 by mass spectrometry (Excel file).

Table S2. Trypanosome cell lines used in this study.

Name	Description
SmOxP9	Parental cell line that expresses TetR and T7 RNAP (Poon et al., 2012)
BAP412	heterozygous Δkkt3 (this study)
BAP1123	KKT3-YFP/Δkkt3 (this study)
BAP567	tdTomato-KKT2 (neomycin) (this study)
BAP1490	YFP-KKT22 (this study)
BAP1454	YFP-KKT22, tdTomato-KKT2 (neomycin) (this study)
BAP1549	YFP-KKT23 (this study)
BAP1593	YFP-KKT23, tdTomato-KKT2 (blasticidin) (this study)
BAP1635	YFP-KKT24 (this study)
BAP1819	YFP-KKT24, tdTomato-KKT2 (blasticidin) (this study)
BAP1742	YFP-KKT25 (this study)
BAP1820	YFP-KKT25, tdTomato-KKT2 (blasticidin) (this study)
BAP710	YFP-KKIP1 (Llauró et al., 2018)
BAP825	YFP-KKIP2 (this study)
BAP826	YFP-KKIP3 (this study)
BAP808	YFP-KKIP4 (this study)
BAP827	YFP-KKIP5 (this study)
BAP828	YFP-KKIP6 (this study)
BAP829	YFP-KKIP7 (this study)

Table S3. Plasmids used in this study.

Name	Description
pEnT5-Y	TY-YFP tagging vector, Hygromycin (Kelly et al., 2007)
pEnT6-tdTomato	TY-tdTomato tagging vector, Blasticidin (Kelly et al., 2007)
pPOTv7-eYFP	Vector for PCR only tagging (POT) of target genes, Blasticidin (Dean et al., 2015)
pBA183	Neomycin gene cassette used for gene disruption (p2705 derivative) (Kelly et al., 2007)
pBA67	TY-tdTomato-KKT2 tagging construct, Hygromycin (Akiyoshi and Gull, 2014)

pBA164	TY-tdTomato-KKT2 tagging construct, Blasticidin (Nerusheva and Akiyoshi, 2016)
pBA809	TY-tdTomato-KKT2 tagging construct, Neomycin (this study)
pBA1715	TY-YFP-KKT23 tagging construct, Hygromycin (this study)
pBA1803	TY-YFP-KKT24 tagging construct, Hygromycin (this study)
pBA2004	TY-YFP-KKT25 tagging construct, Hygromycin (this study)
pBA928	TY-YFP-KKIP1 tagging construct, Hygromycin (Llauró et al., 2018)
pBA1226	TY-YFP-KKIP2 tagging construct, Hygromycin (this study)
pBA1227	TY-YFP-KKIP3 tagging construct, Hygromycin (this study)
pBA1236	TY-YFP-KKIP4 tagging construct, Hygromycin (this study)
pBA1228	TY-YFP-KKIP5 tagging construct, Hygromycin (this study)
pBA1229	TY-YFP-KKIP6 tagging construct, Hygromycin (this study)
pBA1230	TY-YFP-KKIP7 tagging construct, Hygromycin (this study)

Table S4. Primers and synthetic DNA used in this study.

To make	Primer and synthetic DNA sequence (all are listed in the 5'-to-3' direction)
BAP412	SmOxP9 was transfected with a fusion PCR product consisting of: 1. Upstream targeting sequence proximal to KKT3, amplified from genomic DNA using primers BA909 and BA911, 2. Neomycin gene cassette amplified from pBA183 using primers BA903 and BA904, and 3. Downstream targeting sequence distal to KKT3, amplified from genomic DNA using primers BA914 and BA915. Clones were screened by PCR as follows BA941 and BA943: 1.4 kb in strains if carrying the deletion BA942 and BA943: 0.9 kb in strains if carrying the deletion BA909: GTGATGGTGTTGCATATATATAT BA911: TGGGAACTTAGTCAACCTCCTCGACTTTAGGGCGCTTTACTGGTAATATATAA BA903: CCTAAAGTCGAGGAGGTTGA BA904: CTCGATAAATAAATAGAAGTGC BA914: CAACAAAGCACTTCTATTTATTTATCGAGCATGCCTGTTTGTGCAGCTT BA915: GTACCCAAAGTGGAAAAAAG BA941: TGCTGAAGCATCCGCTGATA BA942: CCACCTATCTGCTGAAGTTG BA943: GTCGGTCTTGACAAAAAGAA
BAP1123	PCR-based C-terminal YFP tagging of KKT3 using pPOTv7 (eYFP, blasticidin) on BAP412 BA1821: GTAATGGAGTTTGTGAGGTGCTTGATGAGGAAAAATTCCCCCTTTCGGAGGAACTCAACCAGATGCTCTACGGTG GCGTGGGTTCTGGTAGTGGTTCC BA1822: GAAATGCGACAGCAGACGGAAACGGAAAAAAAAATAAAAAAAAAGAGAGGGCTATCTGTAATTCTTTACGTAC ATCACTTCCAATTTGAGAGACCTGTGC
BAP1454 and BAP1490	PCR-based N-terminal YFP tagging of KKT22 using pPOTv7 (eYFP, blasticidin) on BAP567 (for BAP1454) or SmOxP9 (for BAP1490) BA2129: TTGCCAATTCCACATTCGTGCATTTGGTGTTCCTCCTTTTTAACTAGACACATCACACCCCAGGCAACAGCCTAAA AAGAGTATAATGCAGACCTGCTGC BA2130: GCGGCCTCCAAATTTTCCCGTGTACTATAAGGGGACATATCTCGCCTTTCGTGCCGTGAGGAAAAGCTGGAGCCT ACCATACTACCCGATCCTGATCC
pBA1715	Synthetic DNA for the N-terminal tagging target sequence for KKT23 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTTTACTTAGTGATGAGCACCTGGCATTGCTGGCGAAGTACTACGCCAC
GGTAGAGTTCACAGGTGAACAGAAGGACGCGCTCATAGAAAAATACTGGGAGGCAAATGAAGCTGAGCGCAA
GGCCATCGCGAGGGCCTACGCATCGCTCTTTGCCAATGACGCCGACTTCATTCAGCGACTGCTTGCCCACTACG
ATATGCATGTTAGCCCGCATGTGAGTCAGGGTGCGAGCAGTTGCAATGAGAACGGCGGCCGCATCGACCACCC
ATCAATGCTTTGTACAAGAAGAGCATCTTTCCCCTTTCTTTTTTGTCCGACTCGGAAGGTGAAAGAAGATTGAAA
AGAGACAATATTGACAGCCATAGAGGGCTTGCAGCGTGGGATAGTGTGCGCACTGGGGGTGTACAAGTGGGTA
CGCAAAAAAATATTTCCCCCTCCCTCGAAATTTTGTTTCTGGCGTTTTCGTCACGCCTTTTTTTTTTTGTATTTGAG
GGTGCTGTGCATACGGATCCGATCGATCGATC

pBA1803 Synthetic DNA for the N-terminal tagging target sequence for KKT24 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTTCATCCAAGCAGGAGAGAATAGCCGATGCTATGAAGGAGCGCGTCAC
CCAACTAGAAAGGGAATTGAGGGAATGCCAGCAGCAGCTACAGGAGGCGTTGCAAAGAGGTGATGATATACAG
TGCGCCAAGGAAGCGTTACAGTGCGAAGCCCATGATATTGTGGATCAGTGGTCTAGGCGGCCGCCCGAATTGTTG
TTTTTCATCACTCACCTAATGTGGCATGGGAGTAATTCACATGATGTGACCCTACAAGGCGATCAAGATCTTTAC
AATAATAGCAATAATAATAATAGATTGTATGTGCCGTTATACTGTCATCCCTATCTCATTATCAACCGGTCTTGTG
TTGATGTATGGTACAACGAACCTCGGAAGTTTCCCTATAGTTTAACTACCACTTGCGATTGATATCCTCATCTTAT
TCTGCTTTAGTCGGATCCGATCGATCGATC

pBA2004 Synthetic DNA for the N-terminal tagging target sequence for KKT25 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTGAGGCTGTGGTCGCAGTGGATGGCAAACGTCCCAGCCCGGCGACTTT
GTTCATTCATGAAAACACTAACTATAATGGAAGGGAAGCGGGTGCGGCGTGCGTTCCCATGCGAGTCAACGATA
GCATTACTGTGCAACAACTCGCTGATGAGTACTGCATCACGAGTGTTGTGGAATGTGATGCCACGGGTAGTGTTC
AACCCCGAGCGGTGGCGCTAGATTCACCCCTTGATGTTCTCCACGGGGGAAAGTATTATATTGCCAGGCGTGATG
TGAAACATTTTGCGCGGGAACCCCGGGTAACATTCCGTGGAAAAGTTACCGTGAGGGAGTTTAATGCGACACAC
GGGGTAATTGGGCGGGAAGGGGAAACAAACGCGAACCGAGGACAAGCTGTCGCTGCGGATCCCGTAGGCGTAG
CCCCATCCCGCAAGCGGCCCGAACGGGAGGAAGACTTGTTCGATAGCGGCACACGCACTGAGATATATGCGAAG
GAGTACGAATTTGTGCCCTTCGTGGGGGATCTCTACAACGATGGAAGGAATGGCATGATTCATCTCAAAGATGTG
AAGAAACTCTGTGAAAGCGTAGATTTAGACGAAGTTGAGTTCAGAGCGCGACACGTACAAGCGGAGAAAATCCT
AACCCATGCAAAACAACTATTGCTTAGCTACGCGGACGGCAACGGCGGGAGGACCAGCCCCGAATACGCGGCCG
CGGCGTGGAGTGAAGTGCGTCATTGTGTGGTTGATCTTTGAGACGGAGTGTTACAGTTTCCTTTAAGGGGGGAAA
TGCTTGGTCTGCTTATTTATTATATCCACTCGTTTTAACGCTCCCGATAACCGCCAATGCGCCGCTTGGCGGGCAC
ACCGCCGCACACAAACAGGTGAGAAGACTCCCATATCATTTCTGATTCTCTTCTAGCTTTGGACTCAGTTACCTTA
AGGGAAGGAAGGGCAAGGGAACGTGGATCCGATCGATCGATC

pBA1226 Synthetic DNA for the N-terminal tagging target sequence for KKIP2 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTCCGAATTCGGCACCGATGAAAACAATACCGCCGAAGTCGCGTGTTCC
CCCCGATTGGATTCACCCGGCGCTGCATCGCCAGTGGCAGAGACGTGATAAATTGAGGACTCCACACGAATTGC
GACTTGAGGAATTAGATGTTCAACGAACGGAGATGGAGGAAGCGAGCCGCCGCATTATGAGCATTGTCTCAGAG
AAGAAGAGCGCACTTGAGAAGTTGGATCGTCAACGAGAGAAGGCAAAGGCAGGCGGCCGCATATATGTATATCT
ATATTTGTGTATACGTTGAGGGTGAACTCATCAATGTGTTACAATTTTTTCTTTTCTATTGCCCTCTTTTTTCTTTAT
TTGTGCGTTTCCACCCTACTAGTCGGTTCAATCCGAACCCGCACGCCTCTTTTTCCCCTTCTATCATTTATCATTAC
ATCACTGCTTCGCACTTTTATAACCGTCGTTGTTGGTTTGCTTATTTGCTGCTTGTGTCGCTGATCCGTTACGTGCT
TGAGGGATCCGATCGATCGATC

pBA1227 Synthetic DNA for the N-terminal tagging target sequence for KKIP3 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTGCTGGTGCGGAACAACGACAATTTCACAGCGTAGAGGAAGTTAGCGT
AGAGTTAGGTCGCGCATCACTGCATGCTTCTTGGGGTTTCAAGACATATGACGGTGTCTGCCCCCTTCGTGTACGT
GATGTAGCAGCGGAGATTCCTGAAGGTGTAAGGCGCGGTGATGAAATAATTGTAATTAACGGAATCCGACCAGG
GAGCTATGATGAGGGAATGTCATTGCTGCATCAGGCGCAGTCAACCGTTCGCGGCCGCACGTGGAGCCCGTACG
CCAAGGAGGTAAACCAAAAAGAGGGGGAAAATAACGAAGAGCAGGGACAGCAAGAACACGTTTTGGGATTTCG
AGGAATAAGTTCAATTTGGTGTCGGCTACGTTTGATCGTTTACGAAGGGTAGAAAAAAGTTCGTAGTAACGACCG
TACAACAAAAGGTAGCTGGGAAAAAATTGATTGAGTGACACGATTTTGTCATTGCGGCGGAAAACATTAATTCG
ATCGGCCACGAGGGATCCGATCGATCGATC

pBA1236 Following two PCR fragments were cloned into pEnT5-Y using XbaI and BamHI

- KKIP4 CDS targeting sequence with XbaI and NotI
BA1700: GATCGATCTCTAGAGGAGCAGGTTGGAACGCATTTAGCGG
BA1701: GATCGATCGCGGCCGCGCTCATCGATATTAGGCTGG
- KKIP4 5'UTR targeting sequence with NotI and BamHI
BA1702: GATCGATCGCGGCCGCATGAAGTGTCTCCTTGCTAC
BA1703: GATCGATCGGATCCCTTATCGCAGCGAAGAAAAG

pBA1228 Synthetic DNA for the N-terminal tagging target sequence for KKIP5 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTGACAGTGATACCATTTATTGTGATGAAAGCTCCGTAGCGAGTCTTTCA
CAGCCTCAAGGCCGTCCCGCGTTACGACTCGATTCCCTCCCCCCTACACCAATGGTCCTAACGCCATGTGACTCC
AACGTCACGGCATCCGCCAGGAAAAATCAACTAAAGCAGCAACGGCACCAGCACCGGTCATCATTTGCATGCAC
GGCGCTAACATCACCTTCACTTTCGCAGTCCGACTTCATACCTGAGACTCGCGGCCGCGGCACACCCGCGCATAC
ATCCCTCGGTTCTATACTCCTACTTGTGCATCGTCAAGTGAAGGAGGCACTTGTTTATAACATATATATATATATA

TATATATTATACGTTGCCGCCTCACTTCCATTTTGCGGTCATCGTCATCATTCATTCGCTTGAGCGAGCGGTTGTG
CTCACACAATAAAAATCAACAACAGTGTTGTACTGAGGAGAAACGAAGGGAAGGGGCATCGGTGGCAACAGCG
CATTGACAGGATCCGATCGATCGATC

pBA1229 Synthetic DNA for the N-terminal tagging target sequence for KKIP6 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTTCAACAGAGGAACTCGTGCAGCGGGTTGTACAAATGCAAATGACGAG
TCCTCATTTTGCGGAATATTGCGTTGCTCACGTCACAGATTCCAAACATAAGTTTTTATTCGATGCAACAGATAGT
GAAGAGAGGCGACTTTACTGCGCGCTTCTTCAGAGGATGAGGGGGGGATTTATTAGTGACGAGTCGAGGGAATG
TGCGGGAGCGACGGCGGCAGGAACGGAGCGTCAGCGGCAGCGGCATCGGCGCGGCCGCGCCAACATTTAAGGT
GAAGATTTTTGTTGTTTTTTTTTTGATTTGTTTACATTCAAATTATAATTAGTTTCACATTTATTCATTTAAAGTTGT
TGAATTAAATCGGGTGCGATAGGGTATTACACACGGGAGCAAACGCATTACGGTGAATTGCTTAACGTGTTTGAA
ACTTTTGCTCTGACGAGAGTTCGGATAAGAAGTTGTGTTGTGTGGATTAGGACGGACAGACAAGAAAAAAAAAA
AGAAAATTGGATCCGATCGATCGATC

pBA1230 Synthetic DNA for the N-terminal tagging target sequence for KKIP7 with XbaI and BamHI, cloned into pEnT5-Y

GATCGATCGATCTCTAGAGGAGCAGGTTCATCGTCAGAAGCGGTGAAGGCGCTTGCATCCCTGACGAAGGAAGA
GCTCATGCAGCGCGTGTTAGAGCTGCAAGGGAAGAATGCGGAGTTGTACGACGAGGTAGAACAGTTGCGGCAGC
GCCTCTCGCAAAACAGGATTCCGGACGTCAGCAACCCTCGTGTTTCGATTCGCCATTCGTGTGATGTCGGTTCGTG
TTCGCCCAGCAGGTTCAGTACGCTGGCAAGTAGCGTGGGGGGACAATACGGCGGCCGCGTCTTCTTTCGCCTGGT
GTGTGCTTTTTATTCTCTACCCCATTATTCTTCGCAAGCACTCTAGTTAACTATTTTTTATTTCTTTTTTTTACGTCT
CTGGGGACGGCAGGGACGTTTTGTGCGTTGCTGTCGTATTGAGTCTTAACACAGAGACCAAGAAAGAGGGACAA
TAGCCGCAATCTATACAGTATTTGTTGCCCAGATACGAGCGAGGGCCGGGAGAATATACATACTGGCATATAGG
CGGAGCGGGATCCGATCGATCGATC

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