SI Appendix

Protein diversity in discrete structures at the distal tip of the trypanosome flagellum

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Materials and Methods

Structure immunoprecipitation approach

Flagellar cytoskeleton preparation using 1 M NaCl

5 x 10^9 FCP1-YFP expressing procyclic cells were lysed with 1% Igepal CA-630 in PEME (100 mM PIPES pH 6.9, 1 mM MgSO4, 2 mM EGTA, 0.1 mM EDTA) supplemented with Protease Inhibitors (PIs: 50 µM Leupeptin, 7.5 µM Pepstatin A, 0.5 µM phenylmethylsulfonyl fluoride (Roche), 5 µM E-64-d (Enzo Life Sciences)). The lysate was spun for 5 min at 1800 g, and pellet (cytoskeletons) resuspended in cold 1 M NaCl in PEME supplemented with PIs, 200 µg/ml DNAse I and 50 µg/ml RNAse A. After a 30-min incubation on ice the material was spun for 20 min at 12000 g and pellet resuspended in PBS with PIs and 0.2% Igepal CA-630. Resulting flagellar cytoskeletons were sonicated for 8 x 10 s at 10 microns amplitude (Soniprep 150 ultrasonic disintegrator (MSE Sanyo)). The sonicated material (input) was incubated for 2.5 h with mouse monoclonal anti-GFP antibodies (Roche 11814460001) crosslinked to Dynabeads (Thermo Fisher Scientific) using dimethyl pimelimidate. The beads were pelleted and the supernatant (the unbound material) was retained for further analysis. After washes with PBS, 0.1% Igepal CA-630 the bound material was eluted from the beads by incubating them in 50 mM Tris pH 7.5, 0.3% SDS, 1 mM EDTA for 25 minutes with mild agitation. The bound and unbound material were fractionated by SDS-PAGE, proteins visualized by Sypro Ruby staining (Lonza), and each gel lane cut into horizontal slices. Proteins in the slices were digested by trypsin, resulting peptides separated using an Ultimate 3000 RSLCnano HPLC system (Dionex) and analyzed with an LTQ XLOrbitrap mass spectrometer (Thermo Scientific).

Peptides were identified by searching MS/MS spectra against a custom protein database based on *T. brucei* genome version 2.2 using the Central Proteomics Facility Pipeline version 1.2. (1). Proteins were identified at a 1% false discovery rate. Data from individual slices were pooled and protein abundance estimated using the SINQ tool (1). Protein species identified by at least two unique peptides and with probability = 1 in the bound material were considered. To calculate enrichment for proteins detected exclusively in the bound material, they were assigned an arbitrary abundance value in the unbound material, which was equivalent to half the SIN value of the least abundant protein of the unbound material.

Flagellar cytoskeleton preparation using 0.2 M NaCl

For the FC SIP experiment cytoskeletons were prepared from 1 x 10^{10} FCP1-YFP expressing procyclic trypanosome cells as in the 1 M NaCl procedure. For the control SIP of the tripartite attachment complex cytoskeletons were prepared from 1 x 10^{10} YFP-TAC102 (Tb927.7.2390) expressing cells (2). The cytoskeletons were resuspended in PEME, 0.2 M NaCl, PIs, sonicated for 3 x 10 seconds at 10 microns amplitude, followed by a 30 min incubation on ice. The material was spun for 30 min at 14000 g and pellet resuspended in 0.05% Igepal CA-630 in PBS, supplemented with PIs. Resulting flagellar cytoskeleton fragments were sonicated for 6 x 20 s at 10 microns amplitude and added to Dynabeads with anti-GFP antibodies. The subsequent immunoprecipitation step and sample analysis were performed as in the 1 M NaCl procedure, except for omitting the strong tubulin band from analysis, analyzing the rest with a Q Exactive mass spectrometer (Thermo Scientific), and searching MS/MS spectra against a database based on *T. brucei* genome version 9.0 using the Central Proteomics Facility Pipeline version 2.1. Protein species identified by at least two spectral counts in the bound material were considered.

Immunofluorescence staining

Procyclic cells were washed with PBS and settled on microscope slides. Cells were then either fixed for 30 min in -20^{o}C methanol, or, to extract cytoskeletons, incubated for 30 s in 1% Igepal CA-630 in PEME (100 mM PIPES pH 6.9, 1 mM MgSO4, 2 mM EGTA, 0.1 mM EDTA) and fixed in -20^{o}C methanol. Slides were transferred to PBS and stored at +4^{o}C from 30 min to several days.

The mouse monoclonal AB1 antibody and the rabbit polyclonal anti-FTZC and anti-ClpGM6 (for flagellum attachment zone length measurements) antibodies were used following published protocols (3-5). They were detected with anti-mouse IgM-specific antibodies conjugated with TRITC (Millipore) or with anti-rabbit antibodies conjugated with TRITC (Jackson). Samples were mounted into 90% glycerol supplemented with 1% 1,4-antioxidant diazabicyclo[2.2.2]octane and 100 ng/ml of 4',6-diamidino-2-phenylindole (DAPI).

Transmission electron microscopy

Cells were settled onto formvar coated nickel mesh grids (Agar Scientific) and incubated for 5 min with 1% Igepal CA-630 in PEME. After washes in PEME the grids were incubated for 30 min in 1% BSA in PBS, followed by a 45 min labelling with anti-GFP antibody (Invitrogen A11122) 500 x diluted in 1% BSA in PBS. The grids were washed with 1% BSA in PBS and labelled for 45 min with goat anti-rabbit antibody conjugated with 10nm gold (Sigma G7402) 50 x diluted in 1% BSA in PBS. Samples were fixed for 10 min in 2.5% glutaraldehyde in PEME, washed with PEME and H_2O and stained with 1 % aurothioglucose (UPS Reference Standard) in H_2O. The grids were allowed to dry before imaging them on a Tecnai12 TEM (FEI).

Figures

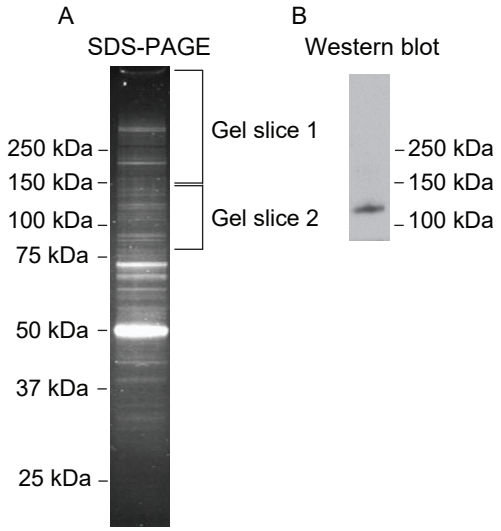

Figure S1

Proteomics indicates that the chimeric FCP1-YFP protein contains the C-terminal part of FCP1 and that the full-length FCP1 is also present.

A - The bound material fraction from the 1M NaCl SIP experiment (see Structure immunoprecipitation approach in Materials and Methods) was fractionated by SDS-PAGE. The gel lane was cut horizontally into slices, which were separately analyzed by mass spectrometry. Based on its predicted size of 237 kDa the full-length FCP1 is expected to be present in the slice 1 (top of the gel to 150 kDa).

B - A western blot of the bound material probed with an anti-YFP antibody. The chimeric FCP1-YFP protein was detected to migrate at around 120 kDa, and therefore was present in the slice 2 of the SDS-PAGE gel (150 kDa to 80 kDa).

C - Proteomics analysis of the gel slice 1 with respect to FCP1. Peptides identified by Mascot as stemming from this protein (red) are mapped on the FCP1 sequence. The coverage was 33% and the peptides were distributed along the sequence.

D - Mass spectrometry analysis of the gel slice 2 with respect to FCP1. Peptides identified as stemming from FCP1 (red) are concentrated in its C-terminal part. The coverage was 8%.

C Gel slice 1

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   1 MQMFLRRDST HCGTGTSHAS SLSPRYRHCC KVPFTFWASV LCIVSSTFLC
  51 SSGAASLVLR PNKPLVNVPF SVETTDQSIK ADIFVSRMST CDNTQIIGSK
 101 CKISKNAPCN FTVTSNDVGI DPWYDTKVRE VYICAGDAFP SLSLSVQLSL
 151 VEAIPRYLKR DVDNTIRFGD AVPVGTFITF HRNENCDDVS MIQGLPPAEL
 201 GSNREVRVTR SVQSVIYLCA KVPTSDGKTF VVPASVLLAV PRYDTNNTDA
 251 LRHTNETFRL EAVGNLVWAT FSQSPLCDPI LQDPVGGETL QVVNMEVSVP
 301 KGDYFLCNGW PYHKGGRLYS PSENKVTVRE YGVQPRTLYS GYGTRIKYTM
 351 DAATAAANDV KLVLDFYMYS NCTGAVRVDG PSGIQYRGPP VKKRRSRSDG
 401 GAVALPVSGT YYACLEGSTT AYPKARAAVV TVLPPPTVSF DEANVISGLD
 451 LTVLLSGHNA GRSGVITVGL SVTPECEELN SKGDVRVGAS SVTFRVPEDA
 501 LPNMTLCVAT PSSVDLQADE EPDEGYTYPV KNIATRRYKL KHRTLFVGVA
 551 ETIHLDANVT LKAGTTGYFS LDNCLTPVGE TYSMNATALY GVAFSTAGRH
 601 VLCVKTPGTE HPVNRPYSNV GNVMVYGPAE LSPASIVKGV STPVKVSAVP
 651 PEAPIVFSES ESCTPSISEV NATAEGEASV TVMRNTVGKV YVCVGYHDSD
 701 GGAWKVRVSG MLAVSDVEVF PRTVFVGVSN RVNFIVPDKT SLKGLKVLFK
 751 ETSDVNCGEI VDDGTAVAIR ITEVGPAYVV YTAVGMKKWK VCLWKGNGYE
 801 DVGLIQSQQQ LELVPDSPIG VVGLPLSMRF RGEALTALQP TRFLVSESIE
 851 SCQSAPGGGI RVYGEGWVDP ISGSAEPFVV QKAGELHVCV GWGDTNESYY
 901 LYGGKVSAED FAVDSLYAVR RSTNNFVARP LLKKASLFLV RCVNAASCKV
 951 PLNARICEEA TERYYTGPTT PLEDVLLGEY ALCQQDERRL GVAVGQKPLK
1001 VINPFTASFN VSLVRKHTPL RLTLDGGSLH IGASNLTVYI VPPELNCTEA
1051 NASYDSFPFA SGEHSKDVTI VKTPAVAKGA RVCVGISAYD KLPASTFNIF
1101 HYMTPATIIA GRSVTVESSG IQSGRVRIST LEACTDSILS EYDTVIANSM
1151 STLHVDKCKY RNRDLTDVYY CERGESGGYV MRGTMQLIHL DECPGGKQPA
1201 VRPVTALPAK QVTDYGIDKA ILTSPFLSTR SDCNGVLSQS RATMTPRYNR
1251 NLTFYVCTHT IRDPGYVFTT DGPTLSVENF RVVNPSIHGR VDSDSEVLGP
1301 ANLVMNYATR TPDTYLSDCA VCGEKIVAAP GLAETPVEEA VTLIGISGVK
1351 CVCVLGEQPT SPPIPVAEVL IITPPIVKKI DPAAVPTARF HAKLQTPVVS
1401 GAKPLYSLVP GEDAENPLAY LHNSDFRGVY LSENACATTL TGNSVGYVRP
1451 SGSVVLGPTF IPGRLPSVSL CVGTPAGNLS VVSEVEVSTD IIFPSSFVLG
1501 TEAEVYIPLS PNSAFRLRRD ASCGGEDVVP FFTTDEEARG NISFKNVNAA
1551 GLPSAGVWTL CQEVFDGSAT RPKIVKISAN GIAARVASAK QLLPIAQIET
1601 FDPTYYNIRG RDVLLGVPGV LYLMDDLYAE SLLPGFSTDR SCLRRNESHG
1651 SWALVGDDDA ASARRVSVTA QNGTDSIYFC ATTPVNRSVV SVPLSSSLRF
1701 IPPVSVFPSI VEPCKVTPLE SCRAPDSSHR QTVVRVIKGD CCNPTDKGVT
1751 VGEASEGSDG KCKLRMNHNK IRDYPAGTEF SVCAWDLTDN TYCVTLGNVK
1801 ASGDICGGSA WGPLGMGAVI AIIVAAVLLF LLLLLLAVCF LRRCCSKEKE
1851 ERQLVVADKM QLDNLDMSCI SGTSPDPEYL ESGNNPLLLG FYSGDGSPNT
1901 NTTARTMIDG MTSGVDDFAW GGDFPGRADW TSVEEQECDD RDQIALQEAR
1951 DRYNMALVFT DGIERIRVDA KELELQFDYS GEFSMYEGPT GRGVNVNPIP
2001 VTVPADHPDL LNAIYMRRAS QRRRLQSQTR EEIEVSVHDT MSEMSTDVHD
2051 TSRLTSYDMM SYTTTQHFYE ESTFMLESEA GRRMRLVNWE EEEWQAIVDA
2101 EFSDYVRLQQ AMRSIPLPLP QAVVLPFNST ATRGRDTINY SGHTHVDGEG
2151 VTVLPVYDTP PFHNPHAASV DEAQAYPNHD EESNVSLSCD SSHN
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D Gel slice 2

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 101 CKISKNAPCN FTVTSNDVGI DPWYDTKVRE VYICAGDAFP SLSLSVQLSL
 151 VEAIPRYLKR DVDNTIRFGD AVPVGTFITF HRNENCDDVS MIQGLPPAEL
 201 GSNREVRVTR SVQSVIYLCA KVPTSDGKTF VVPASVLLAV PRYDTNNTDA
 251 LRHTNETFRL EAVGNLVWAT FSQSPLCDPI LQDPVGGETL QVVNMEVSVP
 301 KGDYFLCNGW PYHKGGRLYS PSENKVTVRE YGVQPRTLYS GYGTRIKYTM
 351 DAATAAANDV KLVLDFYMYS NCTGAVRVDG PSGIQYRGPP VKKRRSRSDG
 401 GAVALPVSGT YYACLEGSTT AYPKARAAVV TVLPPPTVSF DEANVISGLD
 451 LTVLLSGHNA GRSGVITVGL SVTPECEELN SKGDVRVGAS SVTFRVPEDA
 501 LPNMTLCVAT PSSVDLQADE EPDEGYTYPV KNIATRRYKL KHRTLFVGVA
 551 ETIHLDANVT LKAGTTGYFS LDNCLTPVGE TYSMNATALY GVAFSTAGRH
 601 VLCVKTPGTE HPVNRPYSNV GNVMVYGPAE LSPASIVKGV STPVKVSAVP
 651 PEAPIVFSES ESCTPSISEV NATAEGEASV TVMRNTVGKV YVCVGYHDSD
 701 GGAWKVRVSG MLAVSDVEVF PRTVFVGVSN RVNFIVPDKT SLKGLKVLFK
 751 ETSDVNCGEI VDDGTAVAIR ITEVGPAYVV YTAVGMKKWK VCLWKGNGYE
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 851 SCQSAPGGGI RVYGEGWVDP ISGSAEPFVV QKAGELHVCV GWGDTNESYY
 901 LYGGKVSAED FAVDSLYAVR RSTNNFVARP LLKKASLFLV RCVNAASCKV
 951 PLNARICEEA TERYYTGPTT PLEDVLLGEY ALCQQDERRL GVAVGQKPLK
1001 VINPFTASFN VSLVRKHTPL RLTLDGGSLH IGASNLTVYI VPPELNCTEA
1051 NASYDSFPFA SGEHSKDVTI VKTPAVAKGA RVCVGISAYD KLPASTFNIF
1101 HYMTPATIIA GRSVTVESSG IQSGRVRIST LEACTDSILS EYDTVIANSM
1151 STLHVDKCKY RNRDLTDVYY CERGESGGYV MRGTMQLIHL DECPGGKQPA
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1251 NLTFYVCTHT IRDPGYVFTT DGPTLSVENF RVVNPSIHGR VDSDSEVLGP
1301 ANLVMNYATR TPDTYLSDCA VCGEKIVAAP GLAETPVEEA VTLIGISGVK
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2051 TSRLTSYDMM SYTTTQHFYE ESTFMLESEA GRRMRLVNWE EEEWQAIVDA
2101 EFSDYVRLQQ AMRSIPLPLP QAVVLPFNST ATRGRDTINY SGHTHVDGEG
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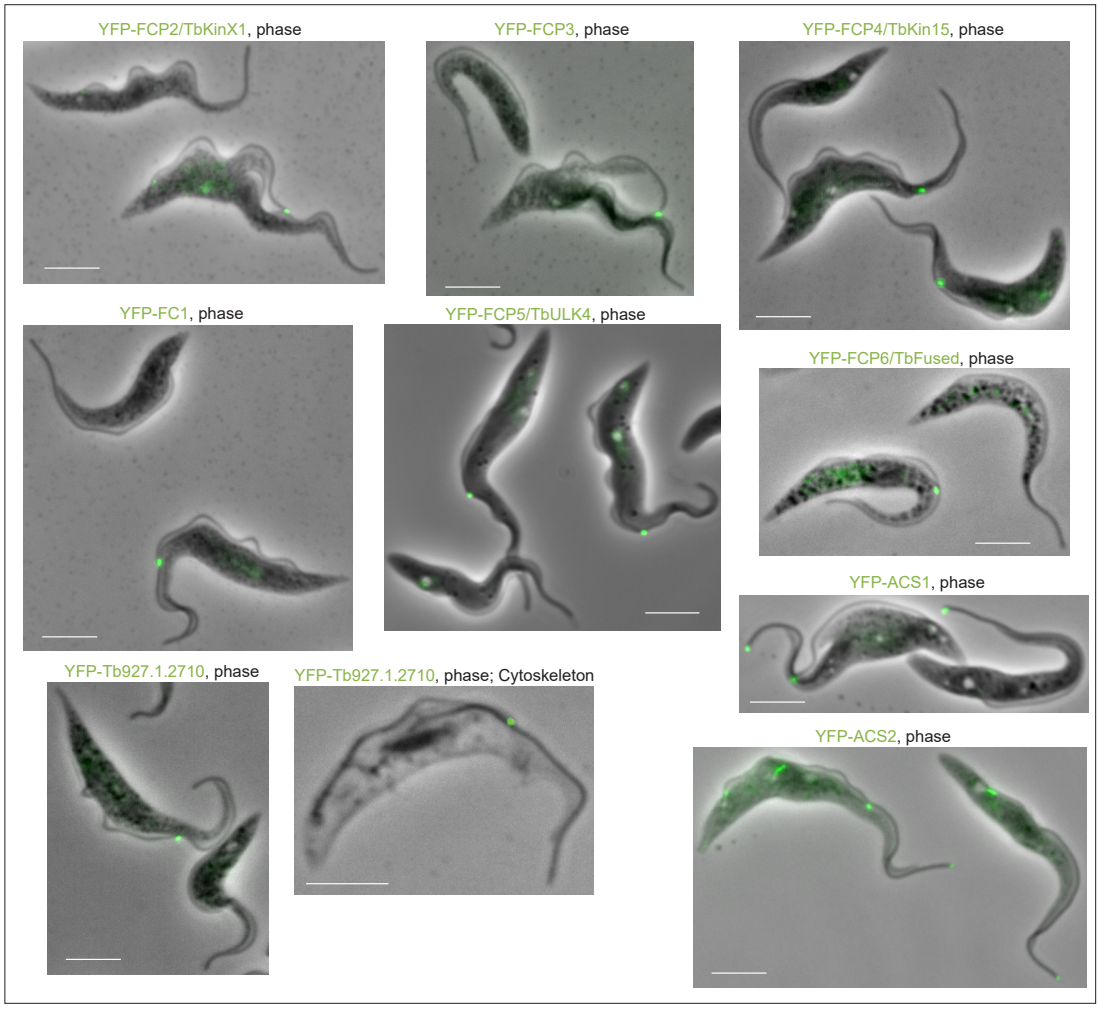

Figure S2

Fluorescence images of whole detergent untreated cells expressing YFP-tagged variants of the flagellum tip-localizing proteins (green) merged with phase contrast images. In the case of YFP-Tb927.1.2710 merged images of a cytoskeleton are also shown, demonstrating that the YFP signal is retained after detergent extraction. Scale bars denote 5 µm.

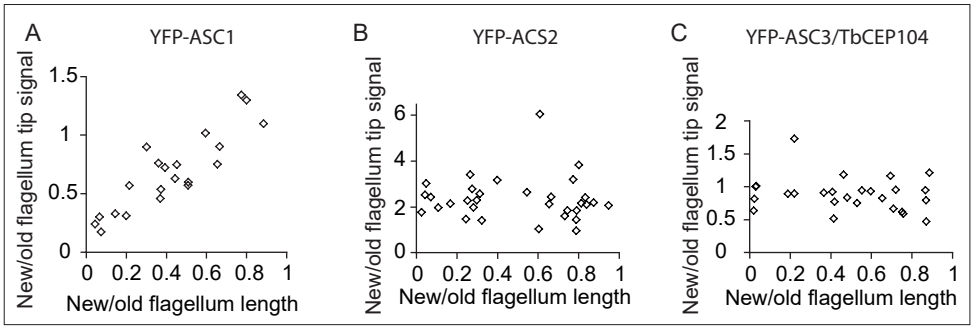

Figure S3

The ratio of YFP-protein signal intensities at the tip of the new versus the old flagellum plotted against the ratio of the new versus the old flagellum lengths.

A- YFP-ACS1

B- YFP-ACS2, the average new/old flagellum tip signal ratio was 2.2.

C- YFP-ACS3/TbCEP104, the average new/old flagellum tip signal ratio was 0.8.

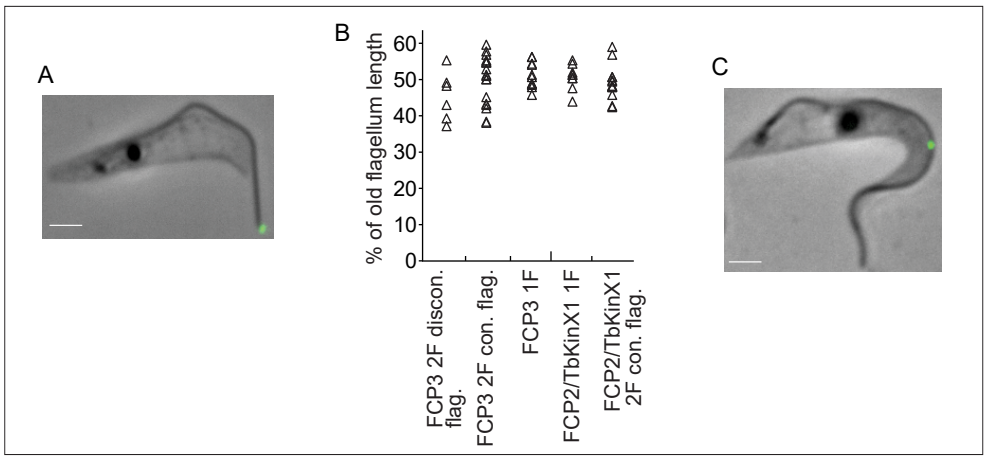

Figure S4

Certain FC constituents are retained in the flagella post cytokinesis.

A- An example of a 1F cell expressing YFP-FCP4/TbKin15 (green). In the population 10.4% of 1F cells possessed the flagellum-associated YFP signal, which was invariantly present at the flagellar tip. Scale bar denotes 2 μm.

B- The position of the YFP-FCP3 or YFP-FCP2/TbKinX1 signal along the flagellum (the old flagellum in the case of 2F cells in cytokinesis with connected or disconnected flagella) expressed as percentage of the length of the respective flagellum.

C- An example of a 1F cell expressing YFP-FCP3 (green). In the population 5.2% of 1F cells possessed the flagellum-associated YFP signal, which was present approximately half way along the flagellum. Scale bar denotes 2 μm.

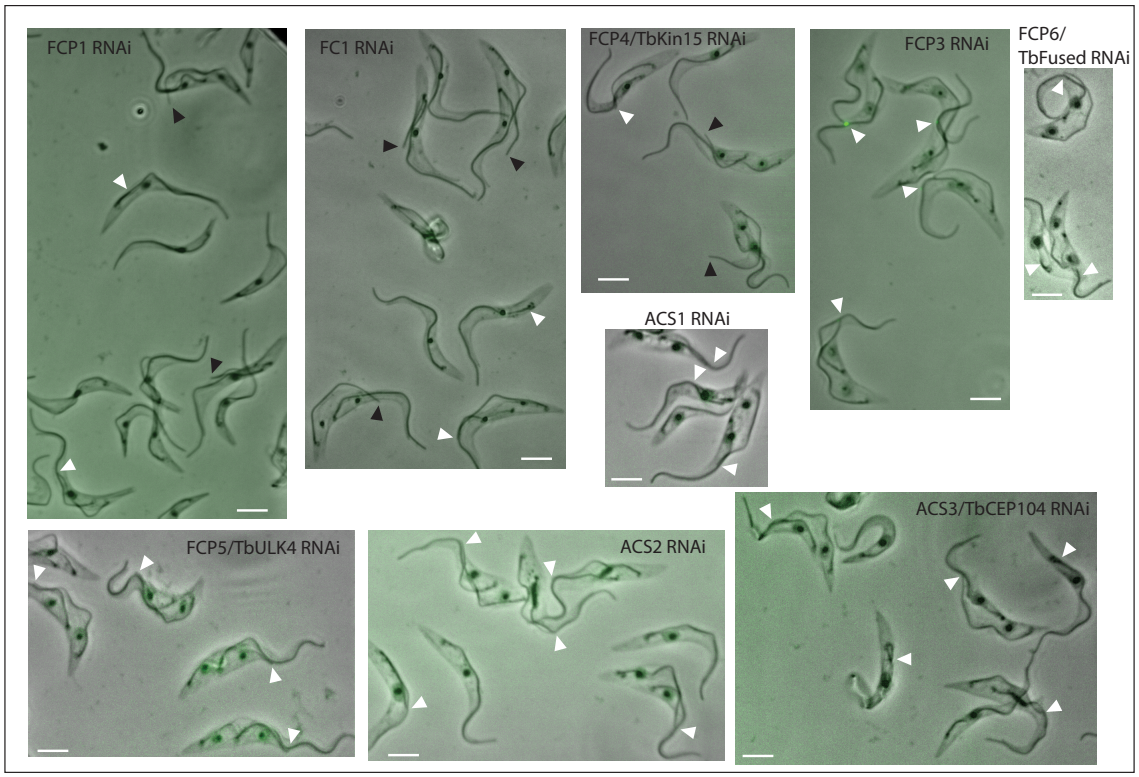

Figure S5

Phase contrast images of cytoskeletons prepared from cells depleted of individual FC or ACS constituents merged with the images of YFP fluorescence (green). The absence of detectable YFP signal in majority of cells indicates efficient protein depletion by RNAi. Black arrowheads denote tips of the new flagella not in contact with the old flagella, white arrowheads denote functional FCs. Scale bars denote 5 μm.

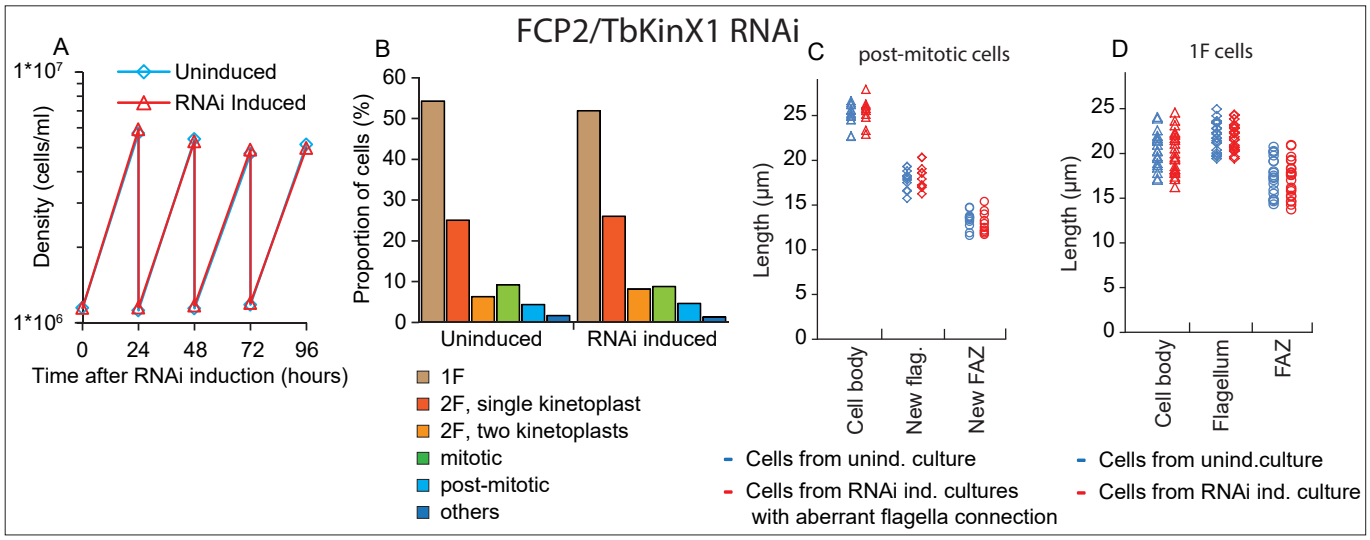

Figure S6

Depletion of FCP2/TbKinX1 has no major impact on cell cultures.

A- Growth curves of FCP2/TbKinX1 RNAi induced (red) and uninduced (blue) cell cultures. Cell densities were measured every 24 hours, and the cultures were subsequently diluted to approximately $1x10^6$ cells/ml.

B- Proportions of cells in various cell cycle stages in uninduced cell culture (left) and cell culture induced for 72 hours for FCP2/TbKinX1 RNAi (right). n > 500 cells for each condition.

C- Morphometric measurements of post-mitotic cells from uninduced culture, which had functional FCs (blue), and of post-mitotic cells with aberrant FCs due to FCP2/TbKinX1 RNAi (red).

D- Morphometric measurements of 1F cells from uninduced (blue) and FCP2/TbKinX1 RNAi induced cell cultures (red).

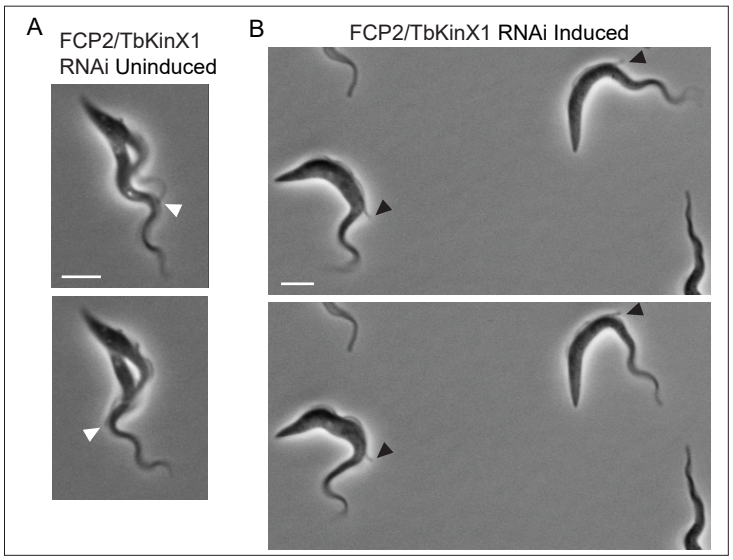

Figure S7

A- Two phase contrast images from a time-lapse movie of a live *T. brucei* cell with far progressed cytokinesis from an uninduced culture. Despite flagella beating the tip of the new flagellum is always connected to the old flagellum (white arrowheads), indicative of a functional FC. Scale bar denotes 5 µm.

B- Two phase contrast images from a time-lapse movie of live *T. brucei* cells from a FCP2/TbKinX1 RNAi induced cell culture. The cells are not in cytokinesis, as judged by the absence of a visible cleavage furrow, yet the tips of their new flagella are not in close contact with the old flagella (black arrowheads). Scale bar denotes 5 µm.

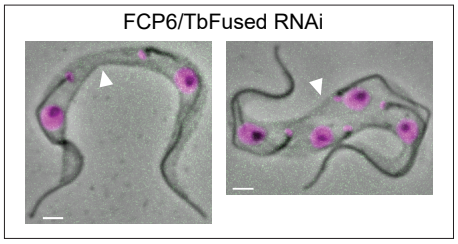

Figure S8

Examples of "double" cells, which are found in FCP6/TbFused RNAi induced cell cultures. Individual cells constituting these "double" cells are in a similar stage of the cell cycle (1F with a single kinetoplast on left, 2F post-mitotic on right), and their posterior ends are connected by a cytoskeletal bridge (arrowheads). Phase contrast images of cytoskeletons were overlaid with fluorescence images of DNA stain DAPI (magenta). Scale bars denote 2 µm.

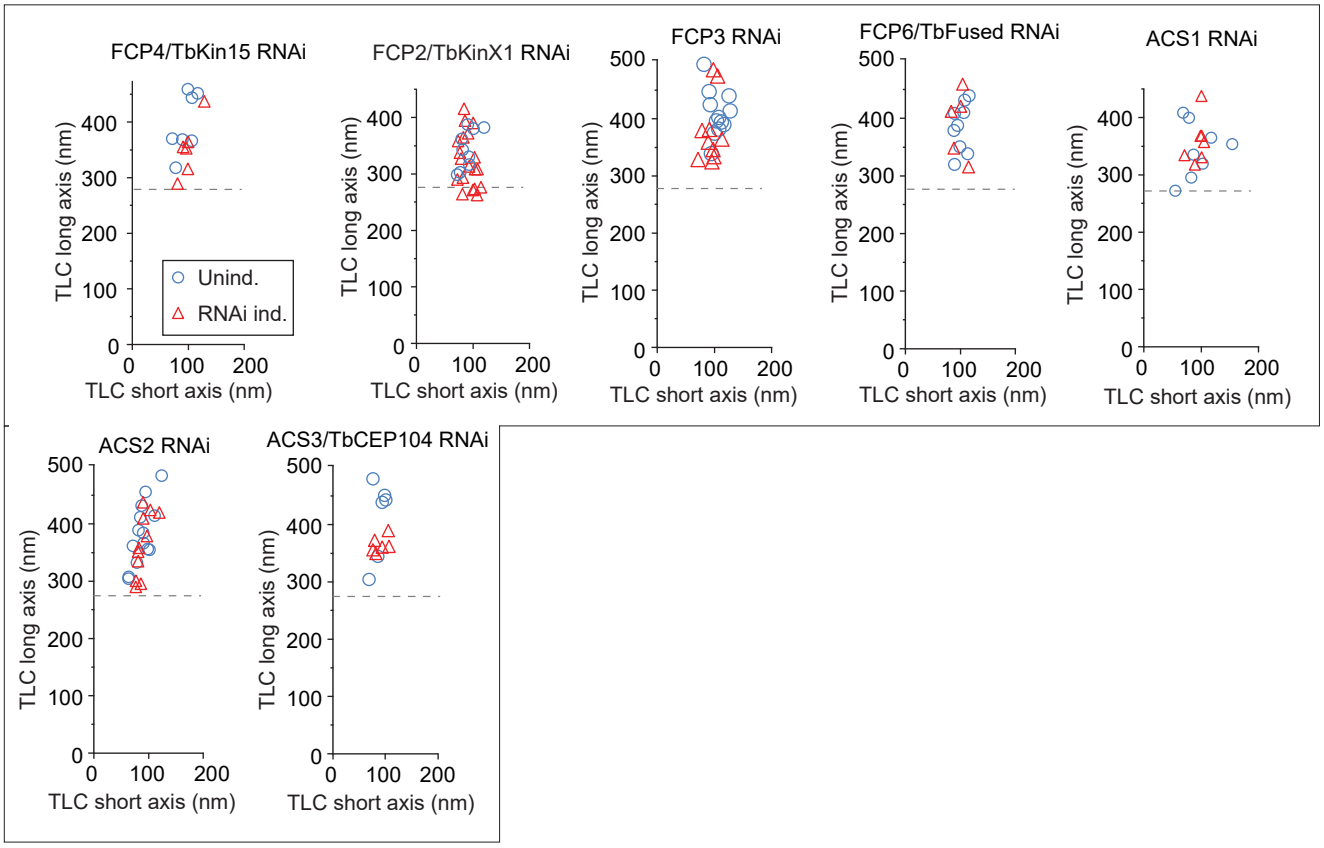

Figure S9

Plots of dimensions of the long axis of the TLC versus its short axis measured in cytoskeletons prepared from cells uninduced (blue circles) or induced (red triangles) for RNAi against individual FC or ACS constituents. Dotted lines indicate 280 nm, which is the smallest dimension of the long axis observed in uninduced cells. For none of the shown proteins is the TLC length difference between induced and uninduced cultures statistically significant at $P < 0.05$.

Figure S10
An alignment of kinesin motor domain sequences of FCP4/TbKin15, FCP2/TbKinX1 and rat Kinesin Heavy Chain 5C (RnKHC5C). Red boxes indicate four motifs involved in ATP binding and hydrolysis according to ref. 6.

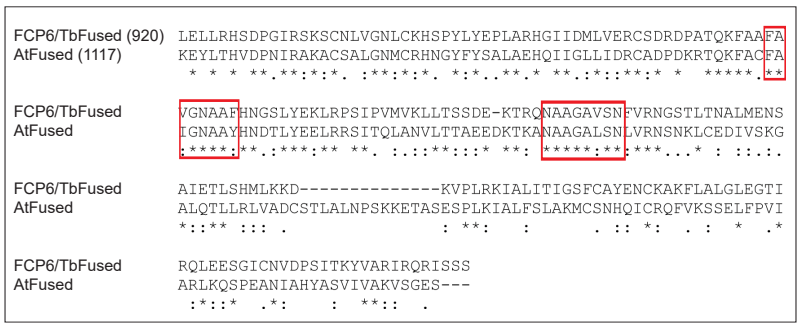

Figure S11
An alignment of C-terminal parts of FCP6/TbFused and of *Arabidopsis thaliana* Fused (Q2QAV0 in UniProtKB) proteins. Red boxes indicate motifs previously identified in *A. thaliana* Fused as required for interactions with *A. thaliana* kinesin-15s (7).

Tables

Table S1- Quantification of the YFP/AB1 signal localization measurements

YFP-tagged protein	YFP signal closer to the old flagellum	equal distance	YFP signal closer to the new flagellum
FCP4/TbKin15	2	2	14
ACS1	0	0	10
ACS2	0	2	8
ACS3/TbCEP104	0	0	10
FCP1	8	5	4
FC1	7	6	1
FCP2/TbKinX1	14	1	0
FCP3	17	1	2
FCP5/TbULK4	13	0	0
FCP6/TbFused	9	2	0

Table S2- Immunogold labeling quantification

			% of gold particles		
YFP-tagged protein	Number of FCs examined	Total gold particles	Old axoneme adjacent to TLC	TLC	Distal portion of new axoneme plus ACS
FCP1	12	325	9	**70**	21
FC1	12	162	12	**80**	7
FCP3	12	254	9	**76**	15
FCP4/TbKin15	5	337	1	18	**81**
ACS1	8	311	0	5	**95**
ACS2	14	129	4	9	**87**
ACS3/TbCEP104	6	27	7	15	**78**

Table S3- Bioinformatics analysis of the identified flagellum tip proteins

	Tritryp (Tritrypdb.org)							
Name	**Gene ID**	**Isoelectric Point**	**Molecular Weight**	**Protein Length**	**Trans-membrane domains** (Phobius.sbc.su.se)	**InterPro Domains** (Evalue below $1*10^{-10}$) (ebi.ac.uk/interpro/)	**Evolutionary conservation** (ebi.ac.uk/Tools/hmmer/)	**Publications regarding evolution, structure or function**
FCP1*	Tb927.8.940	5.25	237461	2194	2 (aa 34-59 and 1814-1841)	No	Trypanosoma cruzi, Leishmania, Leptomonas	ref. 8
FC1	Tb927.11.1340	8.44	127907	1156	0	0054833 Protein kinase-like (PK-like), aa 125-366	Leishmania, Leptomonas, Bodo, weaker homology to kinases from other eukaryotes	ref. 9
FCP2/TbKinX1	Tb927.3.4960	6.09	182491	1594	0	IPR001752 Kinesin Motor Domain, aa 54-409	well conserved accross eukaryotes	ref. 10 - classified as the kinetoplastid specific kinesin-X1 family member
FCP3	Tb927.8.7540	5.79	117856	1109	0	No	Trypanosoma cruzi, Leishmania, Leptomonas	
FCP4/TbKin15	Tb927.10.890	5.29	323347	2889	0	IPR001752 Kinesin Motor Domain, aa 4-391	well conserved accross eukaryotes	ref. 10 - classified as the ubiquitous kinesin-15 family member
FCP5/TbULK4	Tb927.11.8150	7.1	137803	1253	0	0054833 Protein kinase-like (PK-like), aa 1-272; 0054109 ARM repeat, aa 831-1228	well conserved accross eukaryotes	ref. 11
FCP6/TbFused	Tb927.11.4470	7.23	120659	1089	0	0054833 Protein kinase-like (PK-like), aa 1-255	well conserved accross eukaryotes	ref. 11
	Tb927.1.2710	8.69	75954	713	0	No	Trypanosoma cruzi, Leishmania, Leptomonas	
ACS1	Tb927.7.6180	4.5	29957	278	0	No	Trypanosoma cruzi	
ACS2	Tb927.11.450	6.85	90205	821	0	No	Trypanosoma cruzi, Leishmania, Leptomonas	
ACS3/TbCEP104	Tb927.10.14880	6.66	92733	850	0	No	well conserved in animals, Chlamydomonas, Giardia, Trichomonas, Leishmania	

* - the *T. brucei* genome also contains a second gene coding for FCP1, Tb927.8.960 (ref. 8)

Table S4- Results of the SIP experiment on flagellar cytoskeletons prepared using 0.2 M NaCl. Data for proteins enriched over 15-fold in the FCP1-YFP SIP are shown. The majority of the proteins were present also in the bound material of the control SIP targeting YFP-TAC102 at the proximal end of the flagellum, and were not considered for further analysis. Half of the remaining proteins including two novel proteins (in red) localize to the new flagellum tip. The rest of the proteins have other localizations (in blue) as determined by the TrypTag project (tryptag.org, ref. 12).

Bound/ Unbound enrichment ranking	Protein ID	Description	Length aa	FCP1 Bound		FCP1 Unbound		Bound/Un-bound fold enrichment	TAC102 Bound	Note
				Spectral counts	MIC SIN (Protein abundance)	Spectral counts	MIC SIN (Protein abundance)		Spectral counts	
1	Tb927.11.6500	40S ribosomal protein S21, putative	196	10.00	6.55E-05		2.0065E-10	326538.7	2.00	
2	Tb927.11.2410	hypothetical protein, conserved	222	60.00	1.04E-05		2.0065E-10	51731.9	6.00	
3	Tb927.6.5120	60S acidic ribosomal protein P2, putative	107	5.00	2.48E-06		2.0065E-10	12359.8		likely ribosomal prot.
4	Tb927.10.2320	hypothetical protein, conserved	381	2.00	2.28E-06		2.0065E-10	11353.1		nucleus
5	Tb927.7.230	40S ribosomal protein S33, putative	103	8.00	2.10E-06		2.0065E-10	10456.0		likely ribosomal prot.
6	Tb927.10.3370	60S acidic ribosomal protein P2, putative	114	3.00	1.67E-06		2.0065E-10	8323.0	2.00	
7	Tb11.v5.1062	chaperone protein DNAj, putative	370	5.00	9.06E-07		2.0065E-10	4513.8	4.00	
8	Tb11.v5.0489	retrotransposon hot spot (RHS) protein, putative	573	4.00	6.09E-07		2.0065E-10	3037.1	1.00	
9	**Tb927.8.7540**	hypothetical protein	1109	11.00	5.56E-07		2.0065E-10	2771.0		**FCP3**
10	Tb927.5.1820	60S acidic ribosomal protein, putative	107	3.00	5.11E-07		2.0065E-10	2547.2	1.00	
11	Tb927.10.15350	histone H3 variant,H3V (h3vaR)	139	2.50	2.96E-07		2.0065E-10	1476.2	1.50	
12	**Tb927.11.1340**	hypothetical protein, conserved	1156	8.00	2.56E-07		2.0065E-10	1273.4		**FC1**
13	Tb927.9.5690	60S acidic ribosomal protein, putative	113	24.00	3.20E-05	2.00	2.67E-08	1199.9	8.00	
14	Tb927.9.9290	Polyadenylate-binding protein 1 (PABP1)	566	3.00	1.88E-07		2.0065E-10	936.5		cytoplasm
15	Tb927.2.470	retrotransposon hot spot protein 4 (RHS4), putative	860	3.50	1.12E-06	1.00	1.25E-09	899.0	1.00	
16	Tb927.4.4130	hypothetical protein, conserved	856	3.00	1.14E-07		2.0065E-10	570.1	3.00	
17	Tb927.2.5970	hypothetical protein, conserved	420	2.00	1.07E-07		2.0065E-10	535.3	3.00	
18	Tb927.11.11680	2-oxoglutarate dehydrogenase E2 component, putative	383	2.00	6.25E-08		2.0065E-10	311.7		basal body and cytopl.
19	Tb927.7.6360	histone H2A variant,H2Az (h2aZ)	179	12.00	2.31E-05	3.00	7.51E-08	307.7	5.00	
20	Tb927.10.6400	chaperonin HSP60, mitochondrial precursor (HSP60)	562	27.00	3.41E-06	2.00	1.22E-08	280.0	4.00	
21	Tb927.10.8430	40S ribosomal protein S12, putative	142	11.00	1.10E-04	4.00	4.29E-07	255.7	4.00	
22	Tb927.11.9720	40S ribosomal protein S27, putative	86	13.00	7.78E-06	1.00	3.17E-08	245.3	7.00	
23	**Tb927.11.8150**	protein kinase, putative	1253	3.00	3.87E-08		2.0065E-10	192.9		**FCP5/ULK4**
24	Tb927.1.2430	histone H3, putative	133	33.50	4.20E-04	7.00	2.31E-06	181.7	12.50	
25	Tb927.11.6140	40S ribosomal protein S15A, putative	130	24.00	1.21E-04	9.00	9.10E-07	132.6	8.00	
26	Tb927.9.15360	40S ribosomal protein S6, putative	250	23.50	1.06E-04	14.50	8.04E-07	131.7	16.00	
27	**Tb927.1.2710**	hypothetical protein, conserved	713	2.00	2.29E-08		2.0065E-10	114.3		**New flag. tip**
28	Tb927.10.7620	mitochondrial ATP-dependent zinc metallopeptidase, putative	657	2.00	3.16E-07	1.00	3.94E-09	80.2	1.00	
29	Tb927.6.3740	heat shock 70 kDa protein, mitochondrial precursor, putative	657	8.00	1.49E-06	3.00	2.15E-08	69.5	4.00	
30	Tb927.7.5340	hypothetical protein, conserved	498	3.00	4.63E-06	8.00	7.89E-08	58.7	2.00	
31	Tb927.5.4170	histone H4, putative	100	85.00	1.41E-04	10.00	2.59E-06	54.5	38.00	
32	**Tb927.8.940**	hypothetical protein, conserved	2194	85.00	7.27E-06	52.00	1.48E-07	49.0		**FCP1**
33	Tb927.11.11360	receptor for activated C kinase 1 (RACK1)	318	80.00	2.14E-05	8.00	4.40E-07	48.6	13.00	
34	Tb927.10.9650	hypothetical protein, conserved	1406	13.00	2.93E-06	18.00	6.14E-08	47.8	9.00	
35	Tb927.7.2300	Nucleoporin (TbNup132)	1203	10.00	3.46E-06	13.00	7.46E-08	46.4	7.00	
36	Tb927.10.7410	succinyl-CoA ligase [GDP-forming] beta-chain, putative	419	3.00	3.38E-07	1.00	7.29E-09	46.3	1.00	
37	Tb927.11.15990	nucleoporin Nup109 (TbNup109)	1001	11.00	6.32E-07	5.00	1.49E-08	42.5	7.00	
38	Tb927.11.9790	calmodulin, putative	163	4.00	9.90E-07	1.00	2.42E-08	40.9		paraflagellar rod
39	**Tb927.10.890**	kinesin, putative	2889	37.00	5.65E-07	9.00	1.44E-08	39.2	4.00	**FCP4/TbKin15**
40	Tb927.11.6300	40S ribosomal protein S5, putative	190	9.00	3.47E-06	2.00	1.06E-07	32.6	3.00	
41	Tb927.10.2240	hypothetical protein, conserved	588	4.00	5.27E-06	5.00	1.66E-07	31.9	2.00	
42	Tb927.4.3810	DNA-directed RNA polymerase II subunit 2, putative, (RPB2)	1190	8.00	5.48E-07	5.00	1.91E-08	28.8	2.00	
43	Tb927.9.5150	ribosomal protein S6, putative,NHP2/RS6-like protein (NHP2)	126	9.00	6.41E-06	3.00	2.55E-07	25.1	4.00	
44	**Tb927.3.4960**	kinesin, putative	1594	92.00	1.77E-06	24.00	7.33E-08	24.2		**FCP2/TbKinX1**
45	Tb927.10.190	40S ribosomal protein S6, putative	250	8.00	2.69E-06	3.00	1.16E-07	23.2	2.00	
46	Tb927.10.14180	protein transport protein SEC13, putative	374	6.00	7.91E-07	2.00	3.83E-08	20.6	2.00	
47	Tb927.8.6150	40S ribosomal protein S8, putative	220	42.00	1.87E-05	5.00	9.47E-07	19.7	8.00	
48	Tb927.1.1620	ATP-dependent DEAD/H RNA helicase, putative	739	4.00	7.65E-07	3.00	4.13E-08	18.5	1.00	
49	Tb927.11.2050	60S acidic ribosomal subunit protein, putative	324	68.00	9.35E-06	16.00	5.30E-07	17.7	15.00	
50	Tb927.4.470	snoRNP protein GAR1, putative	229	6.00	1.37E-06	2.00	7.98E-08	17.2	2.00	
51	Tb927.10.7680	GTPase activating protein, putative	530	9.00	1.62E-06	9.00	9.51E-08	17.0	13.00	
52	Tb927.10.15410	glycosomal malate dehydrogenase (gMDH)	323	14.00	6.59E-06	7.00	3.88E-07	17.0	6.00	
53	Tb927.11.6740	pumilio/PUF RNA binding protein 10, putative	683	19.00	9.88E-06	35.00	6.21E-07	15.9	22.00	
54	Tb927.10.14820	Mitochondrial ADP/ATP carrier protein 5c (MCP5c)	307	8.00	1.21E-05	12.00	7.94E-07	15.2	7.00	

Table S5- Quantification of 2F dividing cells with aberrant flagella connection. Total numbers of counted cells are indicated in brackets.

	%			
Culture	**2F, single kinetoplast, single nucleus cells with aberrant connection**	**two kinetoplasts, single nucleus cells with aberrant connection**	**Mitotic cells with aberrant connection**	**Post-mitotic cells with aberrant connection**
Parental strain SMOX P9	0.0 (n=276)	0.0 (n=96)	0.0 (n=31)	2.6 (n=39)
FCP1 Uninduced	0.0 (n=131)	0.0 (n=37)	0.0 (n=26)	0.0 (n=24)
FCP1 RNAi Induced	1.4 (n=144)	7.5 (n=40)	27.7 (n=65)	45.6 (n=57)
FC1 Uninduced	0.0 (n=140)	0.0 (n=41)	5.7 (n=35)	7.4 (n=27)
FC1 RNAi Induced	2.6 (n=307)	11.3 (n=71)	19.1(n=68)	52.1 (n=71)
FCP4/TbKin15 Uninduced	0.0 (n=157)	0.0 (n=43)	0.0 (n=46)	0.0 (n=27)
FCP4/TbKin15 RNAi Induced	11.5 (n=174)	20.4 (n=49)	42.9 (n=49)	45.3 (n=64)
FCP2/TbKinX1 Uninduced	0.0 (n=348)	0.7 (n=147)	0.0 (n=104)	7.4 (n=88)
FCP2/TbKinX1 RNAi Induced	10.1 (n=503)	38.0 (n=205)	53.8 (n=156)	67.3 (n=98)
FCP2/TbKinX1 Knock out	5.8 (n=86)	33.3 (n=48)	45.5 (n=55)	66.7 (n=39)
FCP3 Uninduced	0.0 (n=154)	0.0 (n=67)	0.0 (n=30)	5.3 (n=19)
FCP3 RNAi Induced	0.0 (n=149)	0.0 (n=69)	0.0 (n=36)	0.0 (n=25)
FCP5/TbULK4 Uninduced	0.0 (n=121)	0.0 (n=27)	0.0 (n=25)	0.0 (n=25)
FCP5/TbULK4 RNAi Induced	0.0 (n=115)	0.0 (n=25)	0.0 (n=22)	0.0 (n=45)
FCP6/TbFused Uninduced	0.0 (n=136)	0.0 (n=52)	0.0 (n=34)	5.8 (n=86)
FCP6/TbFused RNAi Induced	0.0 (n=111)	0.0 (n=47)	0.0 (n=21)	10.4 (n=54)
ACS1 Uninduced	0.0 (n=147)	0.0 (n=31)	0.0 (n=48)	10.5 (n=38)
ACS1 RNAi Induced	0.0 (n=164)	0.0 (n=50)	0.0 (n=36)	5.9 (n=34)
ACS2 Uninduced	0.0 (n=100)	0.0 (n=50)	0.0 (n=59)	1.5 (n=66)
ACS2 RNAi Induced	0.0 (n=117)	0.0 (n=50)	0.0 (n=50)	9.8 (n=41)
ACS3/TbCEP104 Uninduced	0.0 (n=140)	0.0 (n=53)	0.0 (n=31)	0.0 (n=36)
ACS3/TbCEP104 RNAi Induced	0.0 (n=125)	3.8 (n=26)	0.0 (n=19)	6.5 (n=31)

Dataset

Part 1- Results of the SIP experiment on flagellar cytoskeletons prepared using 1 M NaCl

Part 2- Results of the SIP experiment on flagellar cytoskeletons prepared using 0.2 M NaCl

Part 3- Details on preparation of constructs for genetic modification of trypanosomes

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