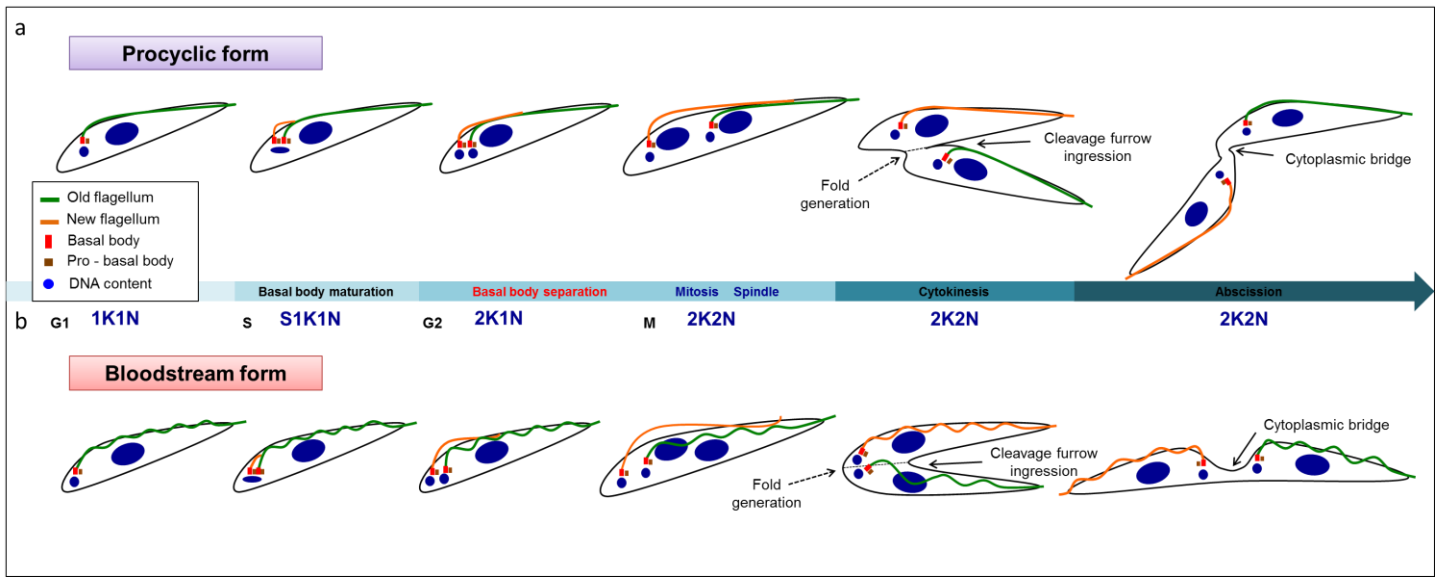


Fig. S1. *Trypanosoma brucei* shows differences in cell division according to the form Schematic representation of cell cycle in *Trypanosoma brucei*. a. The cell cycle in the insect stage procyclic form. b. The cell cycle in the mammalian bloodstream form.

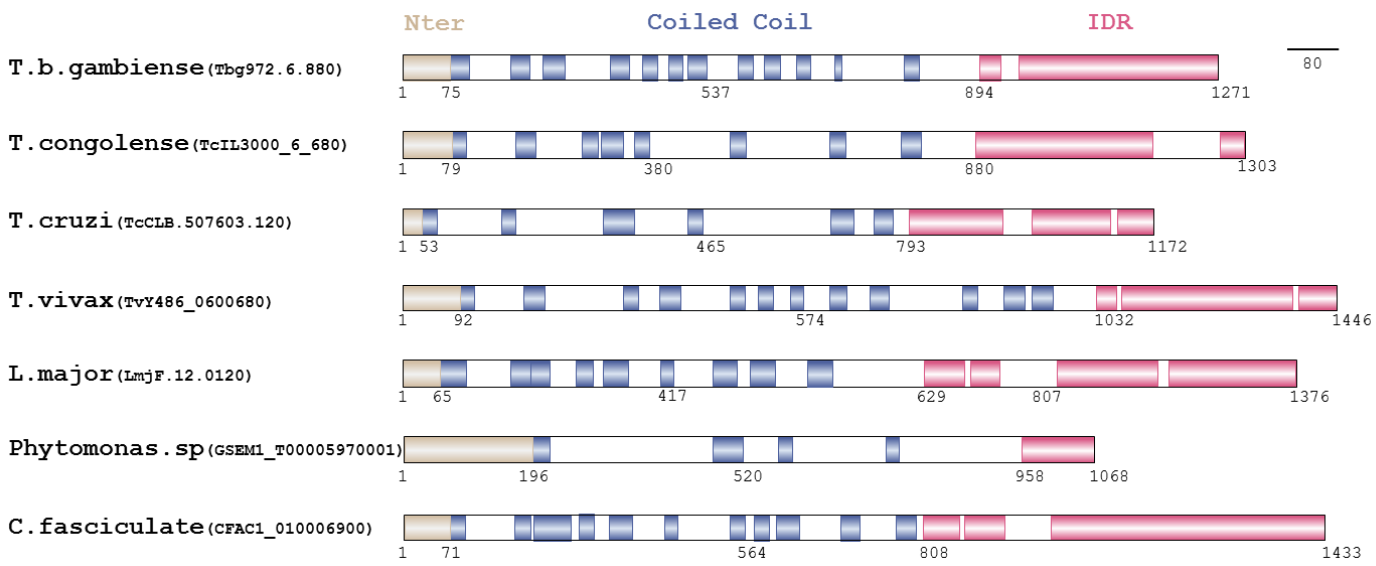


Fig. S2. TFK1 (Tb927.6.1180) is a coiled-coil kinetoplastid-specific protein

TFK1 protein orthologs of the class Kinetoplastida are aligned using ClustalW program (Madeira et al., 2019) and protein domains predicted by InterPro (Blum et al., 2021) are indicated with colored boxes: N-terminal region (Nter, brown boxes), coiled-coil domains (CC, blue boxes) and C-terminal intrinsically disordered region (IDR, pink boxes). The gene accession numbers encoding the putative proteins are indicated in parentheses.

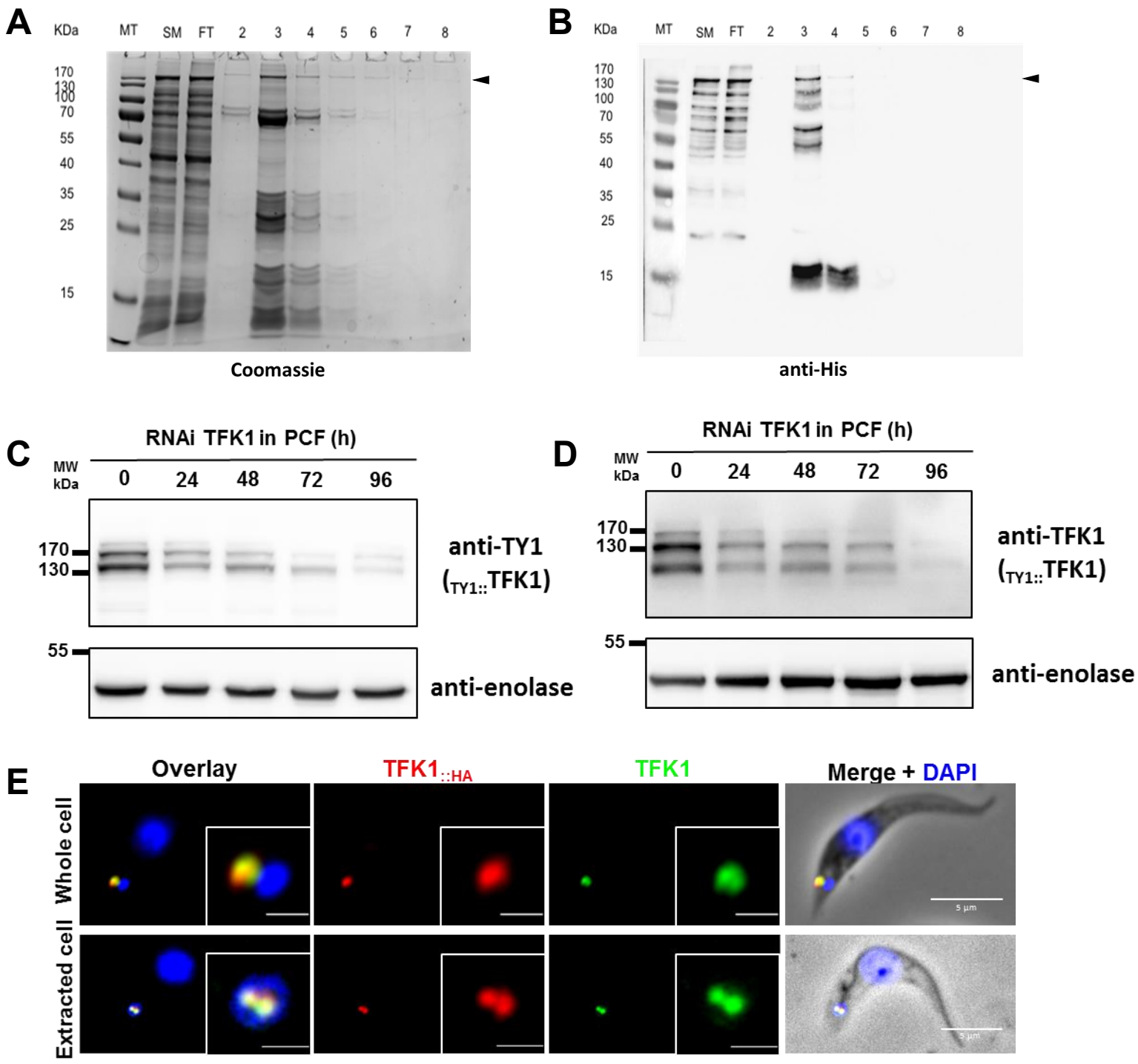
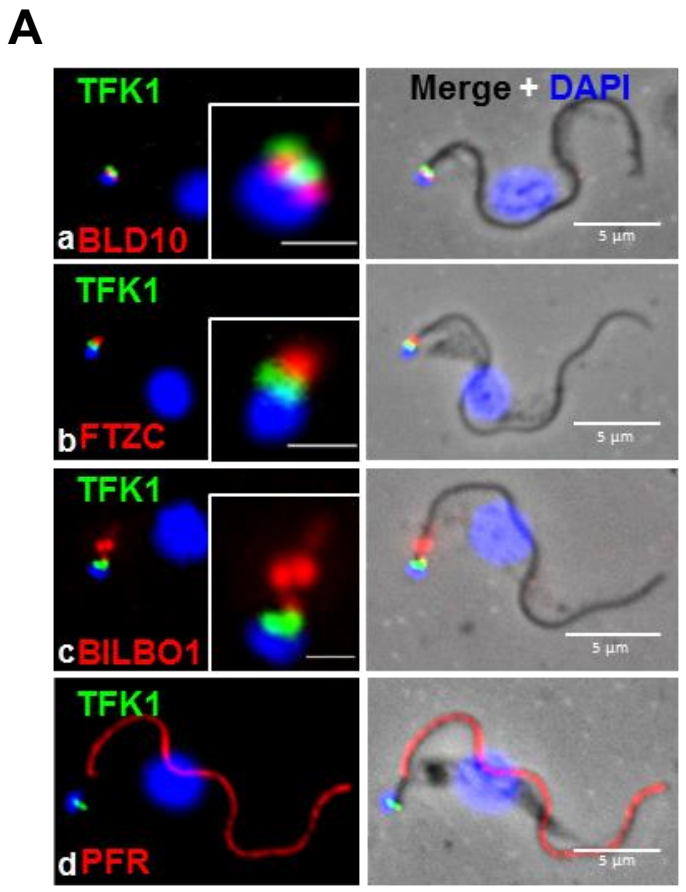


Fig. S3. Validation of the monoclonal antibody directed against TFK1 (anti-TFK1)

(A) Bacterial purified TFK1$_{::6His}$ (142 kDa indicated with black arrow heads) samples (SM: starting material, FT: flow through, 2-8: elutions) visualized by Coomasie blue staining and (B) by western blot using anti-His antibody. (C) and (D) western blots to monitor the protein level of TFK1 in endogenously tagged $_{TY1::}$TFK1 PCF (152.7 kDa) using both anti-TY1 and anti-TFK1 antibodies, respectively, before induction and 24 to 96 h post induction. (E) Immunofluorescence on detergent extracted (top panel) and whole cells (bottom panel) PCF showing the co-localization of TFK1 (green, anti-TFK1) and the endogenously tagged TFK1$_{::HA}$ (red, anti-HA antibody). Scale bar: 5 µm, inset 1 µm.



Ultrastructure expansion microscopy TFK1 diameter (nm)

Post-expansion		Expansion factor	Calculated		
Inner	Outer		Inner	Outer	
1597.3	2550.3	4.5	354.9	566.7	
1506.5	2470.4	4	376.6	617.6	
1570.2	2512.3	4.8	327.1	523.4	
1661	2661	4.8	346	554.3	
1699	2841.2	4.8	353.9	591.9	
1872.7	3036.2	4.5	416.1	674.7	
1593.4	2214.5	4.5	354.1	492.1	
1457.8	2253	4.5	323.9	500.6	
1497.7	2319.2	4.5	332.9	515.3	
1720.9	2471.5	4.5	382.8	549.2	
1617.6	2533		**356.8**	**558.6**	mean (nm)
124.6	257.8		28.3	56.8	s.d.

Fig. S4. TFK1 localizes to the distal region of basal bodes with a nine-dotted circular pattern

(A) Immunofluorescence on detergent-extracted BSF cells showing the co-localization of TFK1 (green, anti-TFK1) and BLD10 (red, BB marker), FTZC (red, transition zone marker), BILBO1 (red, flagellar pocket collar marker) and PFR (red, paraflagellar rod). Scale bars: 5 µm, inset 1 µm. (B) Top view of U-ExM experiments showing the labelling of TFK1 (green, anti-TFK1) composed of nine dots signal. The inner and outer diameters of the circular pattern indicated by the dashed circles were calculated from the measured distance (diameter)/expansion factor. The expansion factor is the ratio of the gel diameter after and before the expansion presented in the table (n=10). s.d.: standard deviation, K: kinetoplast, d: diameter, scale bars: 2 µm.

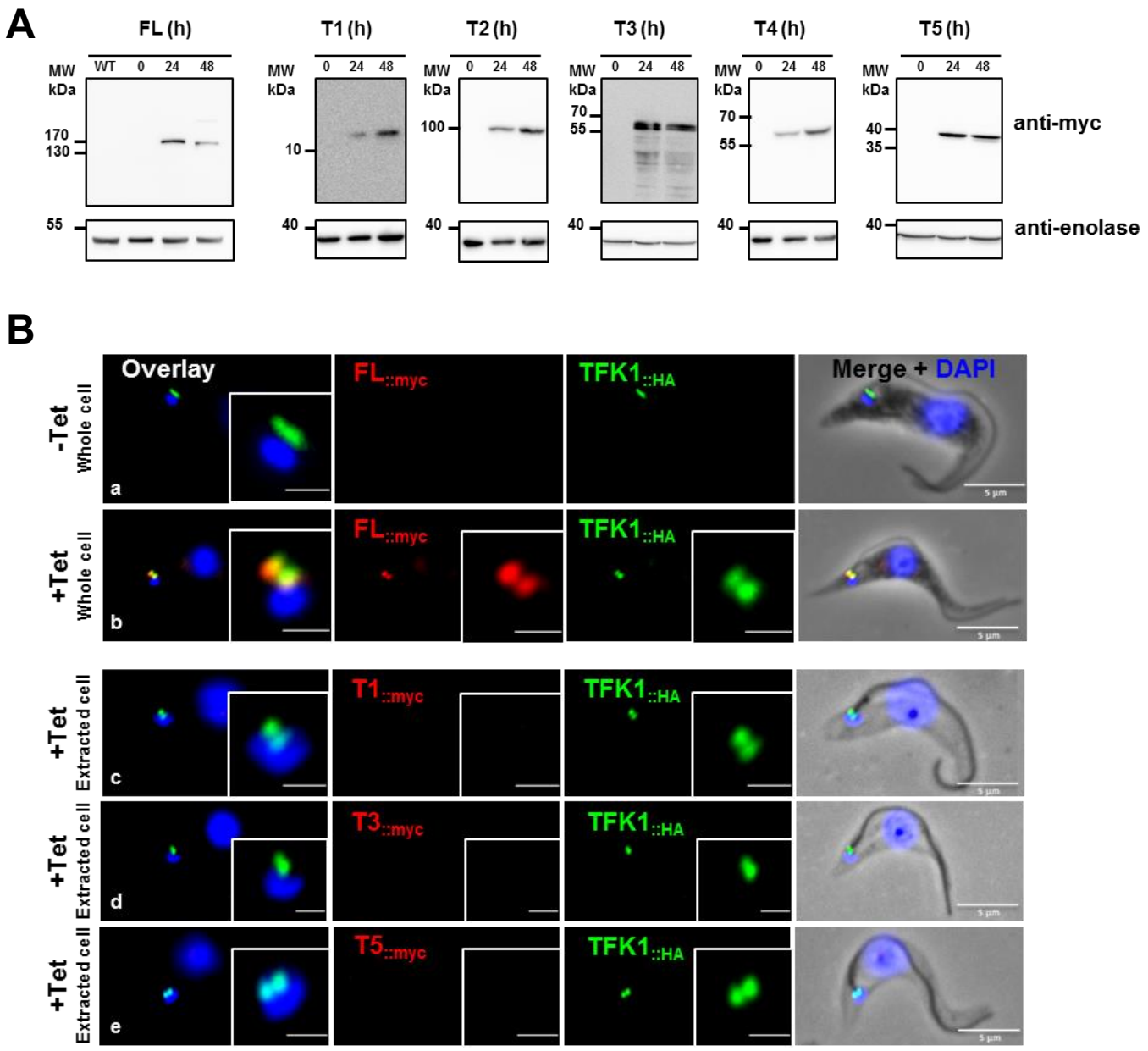


Fig. S5. Localization of ectopic expression and domains analysis of TFK1

(A) Western blot analysis after 24 h and 48 h induction of expression of FL TFK1$_{::myc}$ and truncations T1$_{::myc}$ (11.9 kDa), T2$_{::myc}$ (90.2 kDa), T3$_{::myc}$ (49 kDa), T4$_{::myc}$ (56.3 kDa) and T5$_{::myc}$ (38.4 kDa) in endogenously tagged TFK1$_{::HA}$ PCF cells, using anti-myc antibody. Anti-enolase was used as loading control. (B) Immunofluorescence on whole cells with labeling of TFK1::myc (red) in non-induced (a) and induced cells (b), and (c-e) immunofluorescence on detergent extracted induced cells of truncations (T1::myc, T3::myc, and T5::myc) (red) after 24 h of induction of expression in endogenously tagged TFK1::HA background (green) PCF cells Scale bars: 5 µm, insets 1 µm.

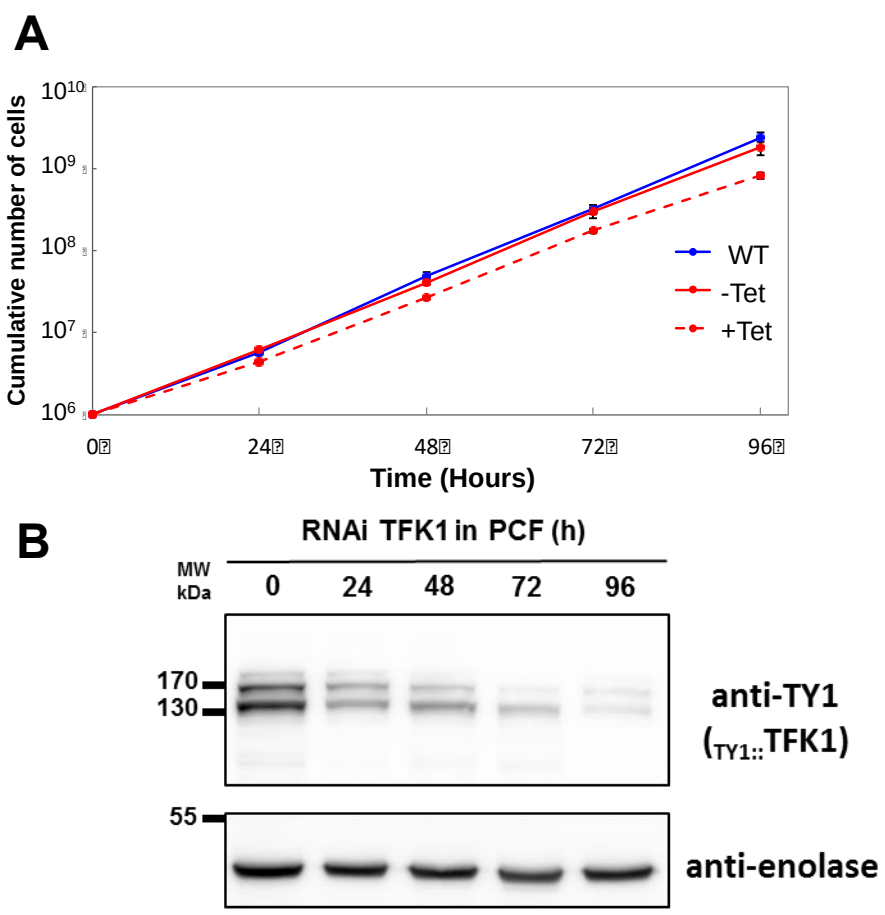


Fig. S6. TFK1 is not essential in PCF

(A) Growth curves of WT cells (blue line), non-induced cells (-Tet, red line) and tetracycline induced cells (+Tet, dashed red line) for TFK1 RNAi knockdown. (B) Western blot analysis of TFK1 protein level in endogenously tagged $_{TY1::}$TFK1 PCF using both anti-TY1 antibody before induction and 24, 48, 72 and 96 h post induction of TFK1 RNAi knockdown. Anti-enolase was used as loading control.

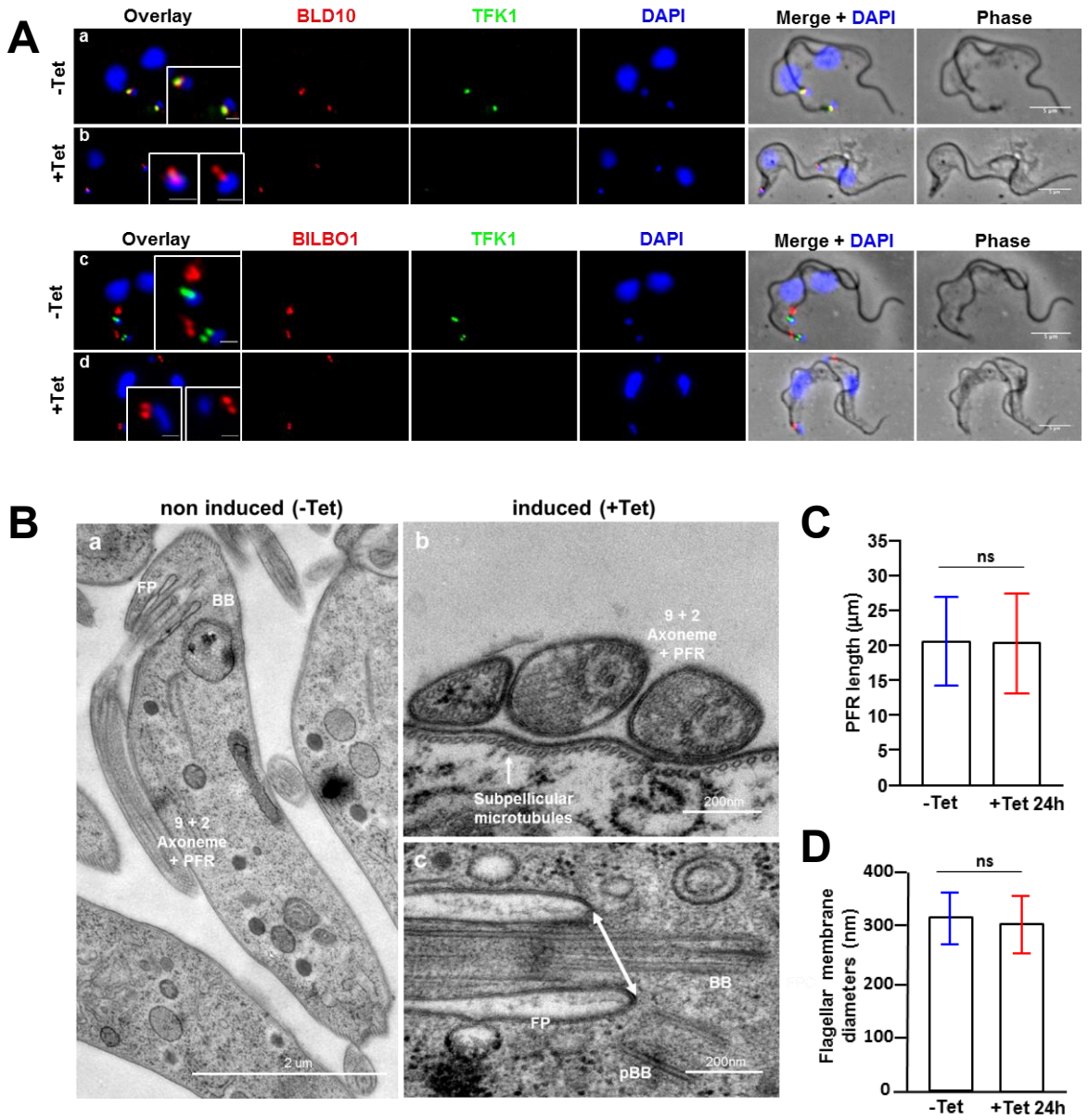


Fig. S7. Structural analysis of key organelles in TFK1 knockdown BSF cells

(A) Immunofluorescence on detergent-extracted BSF cells showing TFK1 (green) and BLD10 (BB marker, red) before (a, -Tet) and 24 h post induction of TFK1 RNAi knockdown (b, +Tet) and showing TFK1 (green) and BILBO1 (FPC marker, red) before (c, -Tet) and 24 h post induction (d, +Tet). Scale bar: 5 µm, inset 1 µm. (B) Transmission EM on thin sections of TFK1 RNAi non-induced (a) and induced 24 h (b, c) cells. PFR: paraflagellar rod, FP: flagellar pocket, FPC: flagellar pocket collar, BB: basal body, pBB: pro-BB. (C) Measurement of PFR length in BSF flagellar preparation labelled by anti-PFR antibody before (-Tet) and 24 h (+Tet) after TFK1 RNAi knockdown. (D) Measurement of the diameter of transition fibre structure from transmission EM images of longitudinal sections, by measuring the distance between the two sides of the flagellar pocket at the base of the flagellum (indicated by a white double-arrow on Bc) on non-induced and 24h-induced TFK1 RNAi knockdown BSF cells (n=19).

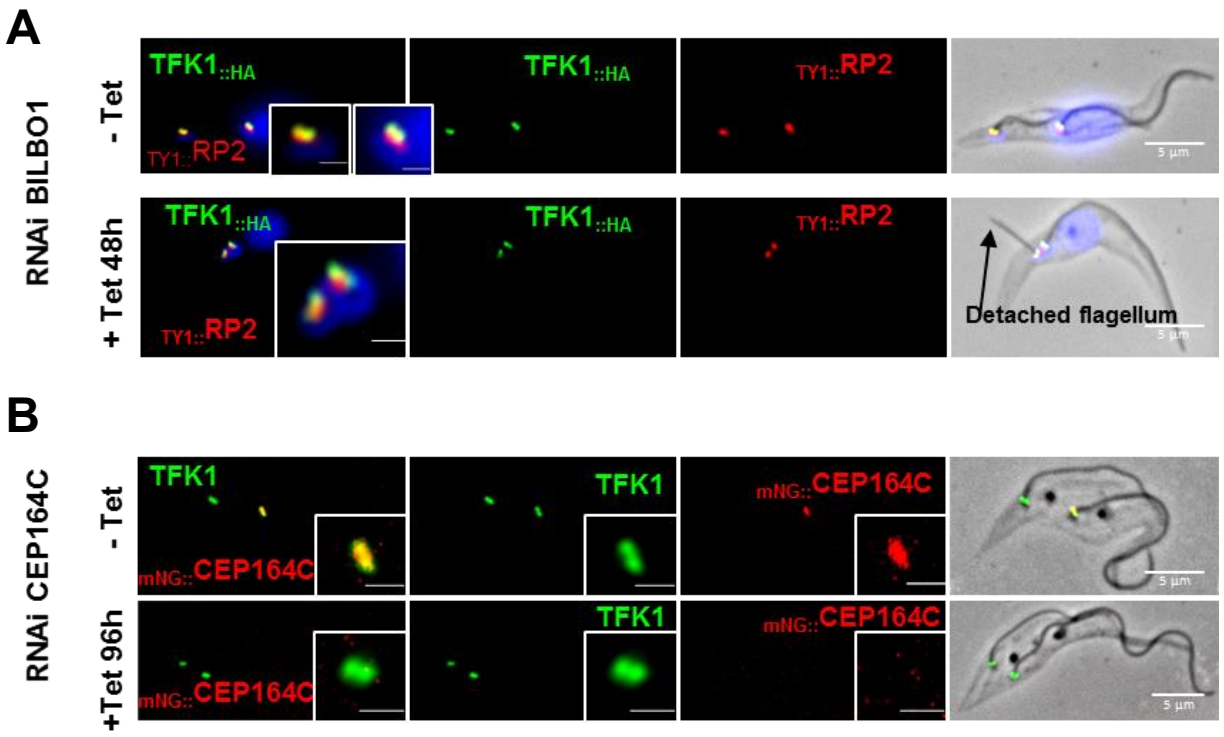


Fig. S8.TFK1 localization is not disturbed in BILBO1 RNAi or CEP164C RNAi knockdown cells and TFK1 and does not interact directly with TOEFAZ1 in Y2H assays (A) Immunofluorescence on detergent-extracted PCF cells showing the localization of TFK1$_{::HA}$ (green, anti-HA antibody) and $_{TY1::}$RP2 (red, anti-TY1 antibody) before (-Tet) and 48 h (+Tet 48h) after BILBO1 RNAi induction. (B) Immunofluorescence labelling of detergent-extracted PCF cells showing the localization of TFK1 (green, anti-TFK1) and $_{mNeonGreen::}$CEP164C (red, direct fluorescence) before (-Tet) and 96 h (+Tet 96h) after CEP164C RNAi induction. Scale bars: 5 µm, inset 1 µm. (C) Y2H interaction assay on – Histidine selective medium (-His) of TFK1 with TOEFAZ1/CIF1. Negative control: Lamin and T-antigen interaction, positive control: p53 and T-antigen interaction.

Table S1. Measurements of TFK1 immunogold labelling.

This table shows the measurements of the distance (indicated by the white double-arrow in Fig. 3C) from the center of the basal body to the gold labeling from transversal section iEM images (n=28). s.d: standard deviation.

Measurements of distance of TFK1 immunogold labelling on transversal section		
	Immunogold dot	Distance (nm)
micrograph n°21	1	160.8
	2	158.3
	3	172.8
	4	168.3
	5	160.6
micrograph n° 25	1	169.1
	2	163.4
	3	163.5
	4	178.5
	5	172.5
	6	166.2
	7	148.9
	8	163.4
micrograph n°6	1	221.7
	2	224.0
	3	180.5
	4	183.8
micrograph n°10	1	204.1
	2	204.2
	3	204.9
micrograph n°9	1	127.9
	2	141.3
	3	140.1
micrograph n°11	1	171.6
	2	187.1
	3	210.0
micrograph n°22	1	123.0
	2	154.0
	Mean (mn)	**172.3**
	s.d. (mn)	**25.9**