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Supplemental Information

A Grow-and-Lock Model for the Control of Flagellum Length in Trypanosomes

Eloïse Bertiaux, Benjamin Morga, Thierry Blisnick, Brice Rotureau, and Philippe Bastin

		Uniflagellated cells	Biflagellated cells	
			New F	Old F
Speed ($\mu m.sec^{-1}$)	**Anterograde**	1.65 ± 0.36 (329)	1.63 ± 0.40 (396)	1.90 ± 0.53 (419)
	Retrograde	5.09 ± 0.86 (297)	5.29 ± 0.60 (284)	5.38 ± 1.0 (261)
Frequency ($trains.sec^{-1}$)	**Anterograde**	1.13 ± 0.21	1.00 ± 0.22	1.04 ± 0.33
	Retrograde	1.59 ± 0.28	1.78 ± 0.84	1.7 ± 0.63

Table S1. IFT speed and frequency in growing and mature flagella of AnTat1E cells expressing TdT::IFT81, Related to Figure 1. The number of measured trains is given in parentheses. Since retrograde trains are more difficult to detect, only non-ambiguous ones were used for analysis, hence the reduced number. F, flagellum. There are no statistically significant differences for all parameters between uniflagellated and biflagellated cells with the exception of the speed of anterograde trains in the old flagellum of biflagellated cells compared to the new one (p=0.06).

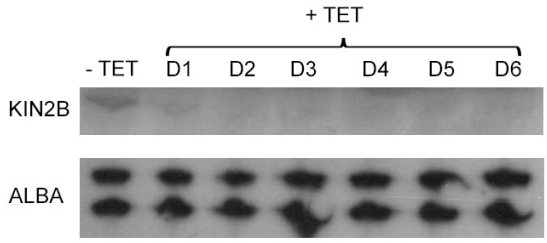

Figure S1. RNAi efficiently targets KIN2B at the protein level, Related to Figures 2 and 3.

Total protein samples of non-induced and induced *KIN2A2B*^{RNAi} cells were prepared after the indicated number of days. Proteins were separated by SDS-PAGE, transferred to a PVDF membrane that was incubated with the anti-KIN2B (top picture) or the anti-ALBA that detects ALBA3 and ALBA4 as loading control (bottom picture).

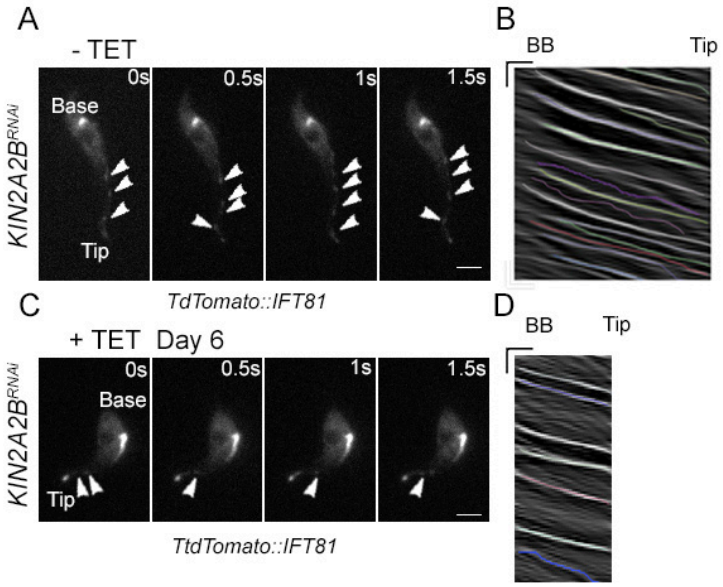

Figure S2. The frequency of IFT trains is reduced in *KIN2A2B*^{RNAi} cells, Related to Videos S5 and S6 and to Figures 2 and 3.

Live imaging of a non-induced (A) and a 6-day induced *KIN2A2B*^{RNAi} cell (B) expressing the TdT::IFT81 from its endogenous locus. Still images from Video S5 (A) and S6 (B) at the indicated time points showing the movement of IFT trains. White arrowheads indicate the successive position of anterograde IFT trains. Kymograph analyses from non-induced (C) and induced cells (D) show clear anterograde IFT traces that are highlighted in color. Note the difference in frequency between non-induced and induced cells. Horizontal scale bar is 2µm and vertical scale bar is 2s.

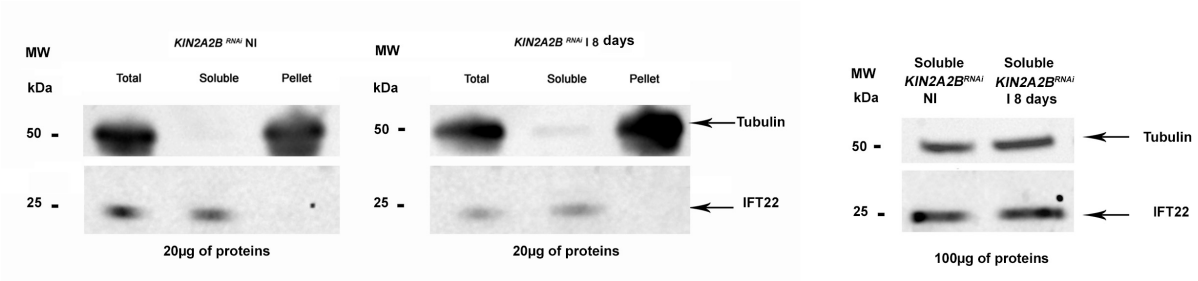

Figure S3. A soluble pool of tubulin is available, Related to Figures 2 and 3.
Samples from *KIN2A2B*^{RNAi} cells grown in non-induced or induced conditions for 8 days were run on gel, transferred to membranes and incubated with the indicated antibodies. Total protein samples, detergent-soluble and cytoskeletal fractions were analysed. Tubulin was detected with the TAT-1 monoclonal antibody whereas IFT22 (soluble marker) was detected with a mouse anti-IFT22 antiserum. Tubulin is detected in the soluble pool in all conditions. Since the cytoskeletal pool is comparatively more abundant [S1], a higher amount of protein was loaded to analyse the soluble pool alone (right panel). This confirmed the presence of a soluble pool of tubulin in both non-induced and induced conditions.

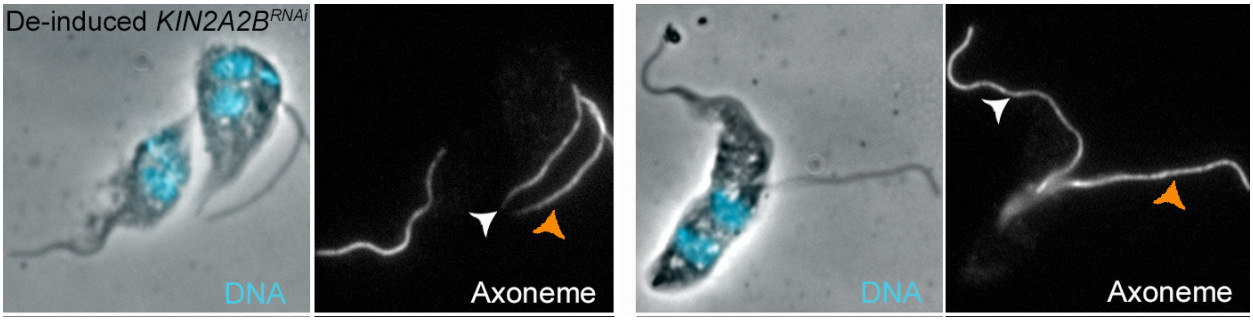

Figure S4. De-induction of RNAi in *KIN2A2B*^{RNAi} cells leads to the formation of longer new flagella that extend beyond the cell body, Related to Figure 4.

IFA pictures of a de-induced *KIN2A2B*^{RNAi} cell undergoing cytokinesis, stained with the Mab25 antibody targeting the axoneme. The first image shows phase contrast and DAPI staining (cyan) and the second one shows the axoneme staining (white). White and orange arrowheads indicate the old and the new flagellum, respectively.

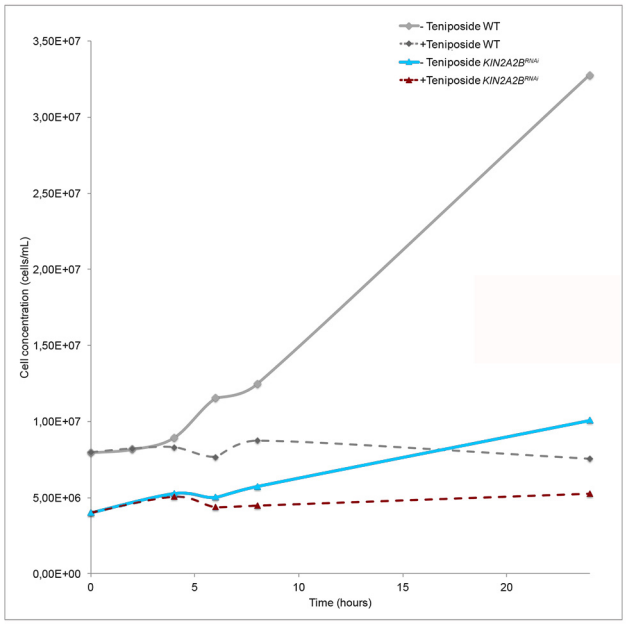

Figure S5. Teniposide blocks cell proliferation, Related to Figure 5.
Growth curve of wild-type cells untreated (grey continuous line) or treated with teniposide for 24h (grey dotted line) and 6 day-induced *KIN2A2B*^{RNAi} cells untreated (cyan continuous line) or treated with teniposide (purple dotted line).

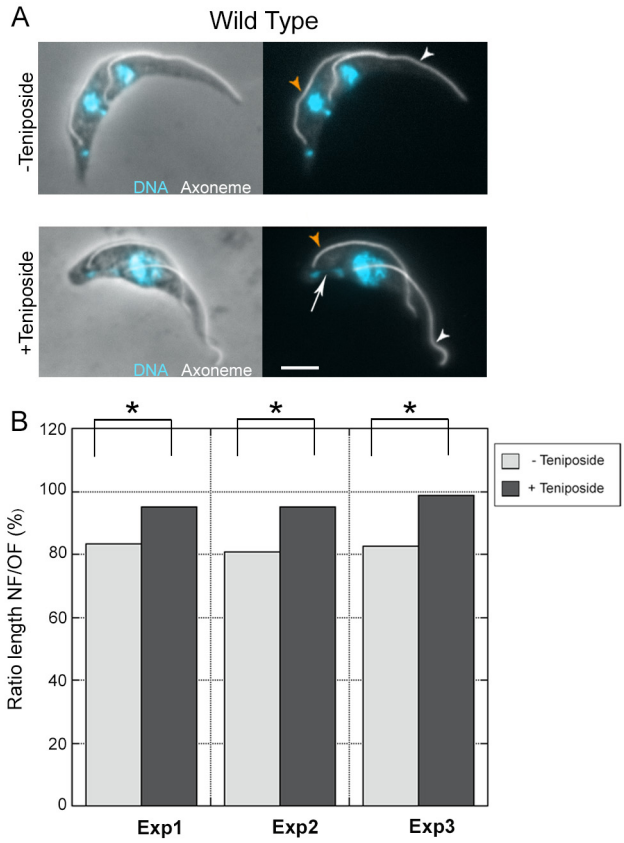

Figure S6. Inhibition of cell division and flagellum growth in wild-type cells, Related to Figure 5.

(A) IFA pictures of or wild-type cells that were left untreated (top panels) or treated for 24 hours with teniposide (bottom panels), stained with the Mab25 antibody targeting the axoneme (white) and DAPI labeling DNA (cyan). The left panels show the phase-contrast image merged with DAPI (cyan) and Mab25 signal (white). The right panels show the Mab25 signal (white) and DAPI (cyan). Orange and white arrowheads show the new and the old flagellum, respectively. The white arrows show the bridge linking the kinetoplasts after treatment with teniposide. Scale bar: 5µm. (B) Ratios between the length of the new flagellum and the old flagellum for wild-type cells treated (dark bars, n=150 cells) or not (white bars, n=180 cells) with teniposide during 8 hours. The results are shown for three independent experiments. Statistically significant differences are indicated with two stars ($p<0.0001$).

Supplemental reference

S1. Schneider, A., Sherwin, T., Sasse, R., Russell, D.G., Gull, K., and Seebeck, T. (1987). Subpellicular and flagellar microtubules of *Trypanosoma brucei brucei* contain the same alpha-tubulin isoforms. J Cell Biol *104*, 431-438.