Supplemental figure 1. **Purification of intact flagella from the *FLA1*^{RNAi} mutant**. *FLA1*^{RNAi} cells were grown without tetracycline (a) or induced to express *FLA1* dsRNA for 84h in the presence of 1µg of tetracycline per ml (b), leading to flagellum detachment. Vortex treatment allows separation of flagella from the cell body (c). After centrifugation on a 0.61 M sucrose cushion, fractions highly enriched in flagella were collected (d). (e-h) Flagella purified from *FLA1*^{RNAi} cells induced for 84h were fixed in glutaraldehyde and processed for scanning electron microscopy analysis. The sample is highly enriched in flagella but minor contaminating elements are occasionally detected (arrows).

Supplemental figure 2. **Purified flagella contain IFT proteins.** Flagella purified from *FLA1*^{RNAi} cells induced for 84h were fixed in PFA and permeabilised with detergent before double IFA with the axoneme marker MAb25 and a monoclonal antibody against IFT172 as indicated. Note the almost absence of DAPI signal.

Supplemental figure 3. **AK is found in the trypanosome flagellum membrane.** IFA on wild-type trypanosomes fixed in PFA (A) or in methanol (B) using the anti-*T. cruzi* AK antiserum reveals a typical membrane association (see enlarged portion in A). Detergent extraction leads to signal loss (C), in agreement with a membrane localisation. Left, IFA picture; right, phase contrast image merged with the DAPI signal (blue). Scale bar represents 5 µm.

Supplemental figure 4. **A 14-3-3 protein is present in the trypanosome flagellum.** IFA on wild-type trypanosomes fixed in methanol using the anti-*T. brucei* 14-3-3-I antiserum (green) and the axoneme marker MAb25 (red) reveals a flagellum signal on both whole cells (a) and detergent-extracted cytoskeletons (b).

Supplemental figure 5. **Primary structure of FLAM proteins.** The presence of unique protein domains (shown as boxes) was searched using the Pfam data base. The primary sequence of each FLAM protein is shown as line drawn to scale (bar is 50 kDa). NDK, nucleoside diphosphate kinase; PS, phytochelatin synthase; TPR, tetratricopeptide repeat; ADS, antimicrobial defensin ß signal; LRR, leucin-rich repeat; WD40, 40 amino acid motif terminating in a tryptophane-aspartate (or ß-transducin repeat); cNMP BD, cyclic nucleotide monophosphate binding domain; IQ, IQ calmodulin binding domain. No hits were found for FLAM8.

Supplemental figure 6. **FLAM proteins show different locations within the flagellum.** IFA on methanol-fixed whole cells using the anti-GFP (green) and the anti-axoneme marker Mab25 (red) on the cell lines expressing FLAM proteins fused to YFP as indicated. The panel on the right shows a 4-fold magnification of the white boxes. The white and yellow arrows indicate the most proximal part of the GFP signal corresponding to the exit of the flagellar pocket and to the base of the axoneme respectively. Note that the green signal is always slightly shifted towards the cell body in the first situation, which is expected for a PFR location. In contrast, FLAM6::YFP merges with the MAb25 signal. Scale bar is 5 µm.

Supplemental figure 7. **FLAM proteins are associated to the cytoskeleton.** IFA on PFA-fixed cytoskeletons using the anti-GFP on the cell lines expressing FLAM proteins fused to YFP as indicated. All FLAM proteins remain associated to the cytoskeleton. Scale bar is 5 µm.

Supplemental Table 1. **Proteins found in flagella purified from the *FLA1*^{RNAi} procyclic cell line.** The identified proteins are listed with Gene DB accession number, name where applicable,

and molecular weight. Proteins were ranked according to the total number of peptides (sum of unique peptides found in the pellet and the supernatant of the sample after shaving). The list of identified proteins was compared with that of the flagellum proteome obtained after detergent extraction and salt treatment (6). Proteins have been classified as present (yes, Y) or not (no, N) in this flagellar skeleton proteome. Known flagellar proteins are classified according to their location: MT, axoneme microtubules or associated; ODA, outer dynein arm; IDA, inner dynein arm; DRC; dynein regulatory complex; RSP, radial spoke protein; CP, central pair; AXO, other axonemal location (or sub-localisation not precisely determined); BB, basal body; PFR, paraflagellar rod; MM, membrane and/or matrix; IFT, intraflagellar transport. The columns "# of experiments" and "# of controls" indicates the number of samples where at least two peptides of a given protein could be identified. Ratio (exp/ctr) indicates the relative abundance of each protein in the flagellum fraction versus the cell debris fraction as calculated with Label Free Quantification (LFQ) values obtained from MaxQuant. In the case a protein was not encountered in the cell debris fraction, this ratio becomes infinite (∞).

Supplemental Table 2. **Recognised flagellar marker proteins and their presence or absence in flagella purified from the *FLA1*^{RNAi} cell line.** Proteins are classified according to their location: MT, axoneme microtubules or associated; ODA, outer dynein arm; IDA, inner dynein arm; DRC; dynein regulatory complex; RSP, radial spoke protein; CP, central pair; AXO, other axonemal location (or sub-localisation not precisely determined); BB, basal body; PFR, paraflagellar rod; MM, membrane and/or matrix; IFT, intraflagellar transport. They were selected because of experimental evidence in *T. brucei* or in a related species, or because of high conservation in flagellated species including trypanosomes. Proteins have been classified as present (yes, Y) or

not (no, N) in the flagellum proteome (FP) obtained after detergent extraction and salt treatment (6). The columns "# of experiments" and "# of controls" indicates the number of samples where at least two peptide of a given protein could be identified. Ratio (exp/ctr) indicates the relative abundance of each protein in the flagellum fraction versus the cell debris fraction as calculated with Label Free Quantification (LFQ) values obtained from MaxQuant. In the case a protein was not encountered in the cell debris fraction, this ratio becomes infinite (∞). In case a protein was not detected in the flagellar sample, the ratio could not be calculated (na, not applicable). References for the characterised proteins are found at the end of this file.

Supplemental Table 3. **Candidate novel proteins identified in flagella purified from the *FLA1*^{RNAi} cell line**. Proteins have been classified as present (yes, Y) or not (no, N) in other flagellum proteomes: FSP, flagellar surface proteome and FMP, flagellar matrix proteome from bloodstream *FLA1*^{RNAi} cells [study from Oberholzer et al. (45)], or purified flagella (stripped with detergent and salt) from wild-type procyclic trypanosomes [study from Zhou et al. (31)]. Proteins were ranked first by total peptide number and then by the ratio score between flagellar and cell debris fractions. Proteins that were investigated experimentally are indicated in red.

Supplemental Table 4. **List of all peptide sequences**. Compilation of all peptides identified by mass spectrometry, including any deviations from expected cleavage specificity, precursor charge and mass/charge (m/z) for each assignment and Peptide Identification Score(s).

Supplemental Table 5. **Label Free Quantification (LFQ) values.** LFQ values obtained from MaxQuant from all experiments.

Supplemental Table 6. **Plasmids for endogenous tagging of *FLAM* genes with *YFP*.** Fragments of the target gene at either the 5' or the 3' end (without stop codon) were synthesized containing flanking restriction enzyme recognition sequences for cloning and a naturally occurring unique restriction recognition site in-between (used for linearization). They were cloned into the respective plasmid backbone: either p3329 (allowing C-terminal eYFP tagging) or p2675 (allowing N-terminal tagging with eYFP). Before transfection in trypanosomes the plasmids were linearized in the target fragment with the indicated restriction enzymes and selection was applied with puromycin (PURO).

Supplemental Table 7. **Segments used to generate RNAi against *FLAM* genes.** Fragments of the target gene were selected using the RNAi algorithm and cloned in the pZJM vector.

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