Suppl. Fig. S1. Surface biotinylation of bloodstream-form *T. brucei*. Live, bloodstream-form *T. brucei* cells were surface-biotinylated, then subjected to immunofluorescence, with or without permeabilization, using antibodies to biotin and the intracellular marker BiP as indicated.

**Suppl. Fig. S2. Flagellum surface proteins are detergent-soluble.** Bloodstream trypanosomes expressing the indicated HA-tagged proteins from the flagellum surface dataset were lysed with non-ionic detergent. Detergent-insoluble proteins (P) were separated from detergent-soluble proteins (S) by centrifugation and subjected to immunoblot analysis using anti-HA antibodies. Antibodies against trypanin and BiP were used as markers for the insoluble and the soluble fraction, respectively. All flagellum surface proteins (FS) examined were detergent-soluble and four examples are shown.

**Suppl. Fig. S3. Immunofluorescence analysis of flagellar surface proteins.** Immunofluorescence analysis of bloodstream trypanosomes expressing the indicated HA-tagged flagellum surface proteins (FS105, FS29, FS163 or FS192). Cells were co-stained with anti-HA (green) and markers for the flagellar skeleton (Paraflagellar Rod, PFR, red) or flagellar pocket (Tomato Lectin, TL, red) as indicated. DNA was visualized with DAPI. FS105 localizes along the flagellum, but does not extend to the flagellum tip, as indicated by the extension of PFR staining (arrow) beyond FS105 staining (arrowhead), see inset for merged image. FS29 localizes to the flagellar pocket, as indicated by tomato
lectin co-staining (arrow, inset), and is also seen to extend to a position more anterior (arrowhead, inset), likely corresponding to endocytic/exocytic organelles that occupy the space between the flagellar pocket and nucleus [1]. FS163 is largely found anterior to the flagellar pocket, showing little or no overlap with tomato lectin staining (arrow, inset). FS192 is localized to the entire cell surface and may be enriched in the flagellar pocket region (arrow).

**Suppl. Fig. S4. FS179 is essential.** Growth curves of two independent, tetracycline-inducible FS179 RNAi lines grown in the absence (black lines) or presence (red lines) of tetracycline to induce RNAi. Within 24 hours post-induction, FS179 RNAi knockdown cells cease dividing.

**Suppl. Table ST1. Flagellum surface proteome (TbFSP): annotation and phylogenetic distribution of proteins identified in TbFSP.** A total of 201 proteins encompassing 158 protein groups (see text for detail) were identified in the *T. brucei* flagellum surface proteome (TbFSP). GeneDB annotation and NCBI GI reference number are provided for each protein. The table also lists the top BLAST hit in 12 reference organisms (*L. major, T. cruzi, P. falciparum, C. reinhardtii, M. brevicollis, C. elegans, D. melanogaster, H. sapiens, D. discoideum, A. thaliana, S. cerevisiae, C. merolae*). Proteins with a Expect value ≤1x10^{-10} are colored.

**Suppl. Table ST2. Summary of analysis of proteins in TbFSP.**
Suppl. Table ST3. Flagellum matrix proteome (TbFMP): annotation and phylogenetic distribution of proteins identified in TbFMP. A total of 687 proteins encompassing 666 protein groups were identified in the T. brucei flagellum matrix proteome (TbFMP). For details see legend for supplemental table ST1.

Suppl. Table ST4. Summary of analysis of proteins in TbFMP.

Suppl. Table ST5. Known T. brucei bloodstream-form flagellar membrane and flagellar pocket proteins. Table lists proteins previously demonstrated to be flagellar membrane and/or flagellar pocket proteins from bloodstream-form T. brucei used for Fig. 4.

Suppl. Table ST6. Proteins analyzed in this study. Table provides summary of immunolocalization results for proteins analyzed in this study.

Suppl. Table ST7. Homologs of flagellum surface (TbFSP) and flagellum matrix (TbFMP) proteins identified in proteomic analysis of the C. reinhardtii flagellum. Table lists proteins from TbFSP and TbFMP for which homologs were identified in flagellum proteome of C. reinhardtii [2] having BLAST Expect value of ≤1x10^{-10}.

Suppl. Table ST8. Intraflagellar transport (IFT) particle proteins identified in T. brucei flagellum proteomes.
Suppl. Table ST9. Components of the ubiquitin conjugating system identified in the flagellum matrix proteome.

Suppl. Table ST10. Details from mass spectrometric analysis of proteins in TbFSP and TbFMP.

Movie M1. Movement of an isolated flagellum. Movie shows motility of a single flagellum removed from the cell body by shearing. Recorded and played back at 30 frames per second.

REFERENCES FOR SUPPLEMENTAL FIGURES AND TABLES