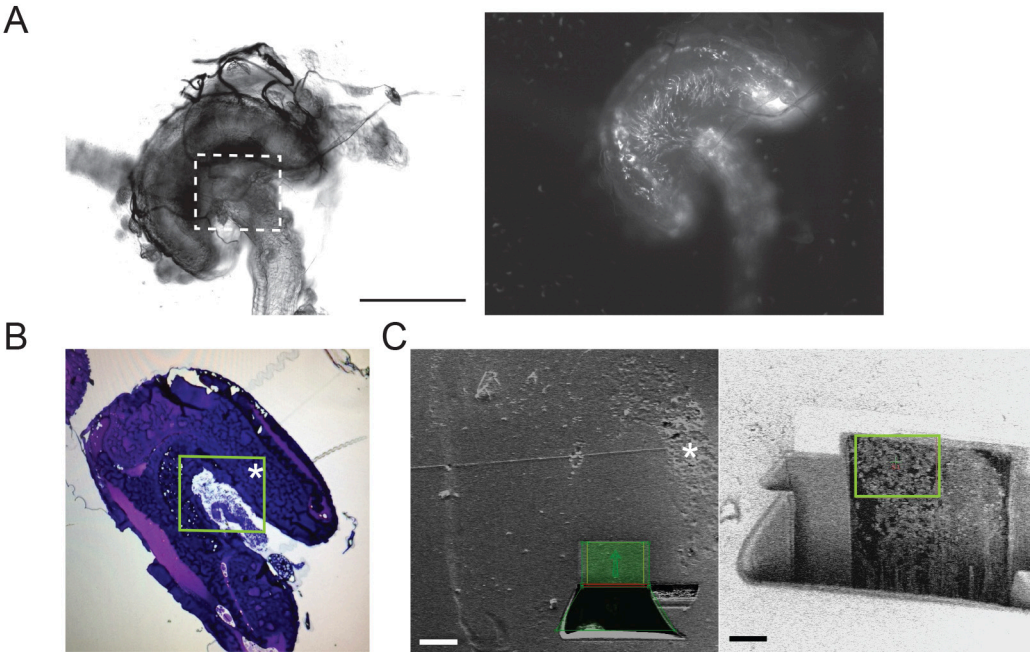


Fig. S1. Use of a correlative light and electron microscopy approach to detect trypanosomes in infected tsetse cardia. (A) Entire cardia isolated from a tsetse fly infected with trypanosomes expressing a TandemTomato fluorescent reporter (Calvo-Alvarez et al., 2018). Left panel: Brightfield image showing the general morphology of the cardia. The square indicates the region of interest for electron microscopy. Right panel: The same cardia viewed by fluorescence microscopy reveals the presence of trypanosomes in various positions within the organ. (B) A semi-thin section of the cardia stained with toluidine blue. Parasites are visible in the green square. The same region of interest was observed on the semi-thin section and on the block surface by scanning electron microscopy (white stars on B and C). (C) Different views of the bloc surface of the sample analysed by FIB-SEM after a cross-sectioning and a polishing by FIB. Parasites are visible on the side view (green squares). Scale bars: 20 µm (left) and 10 µm (right).

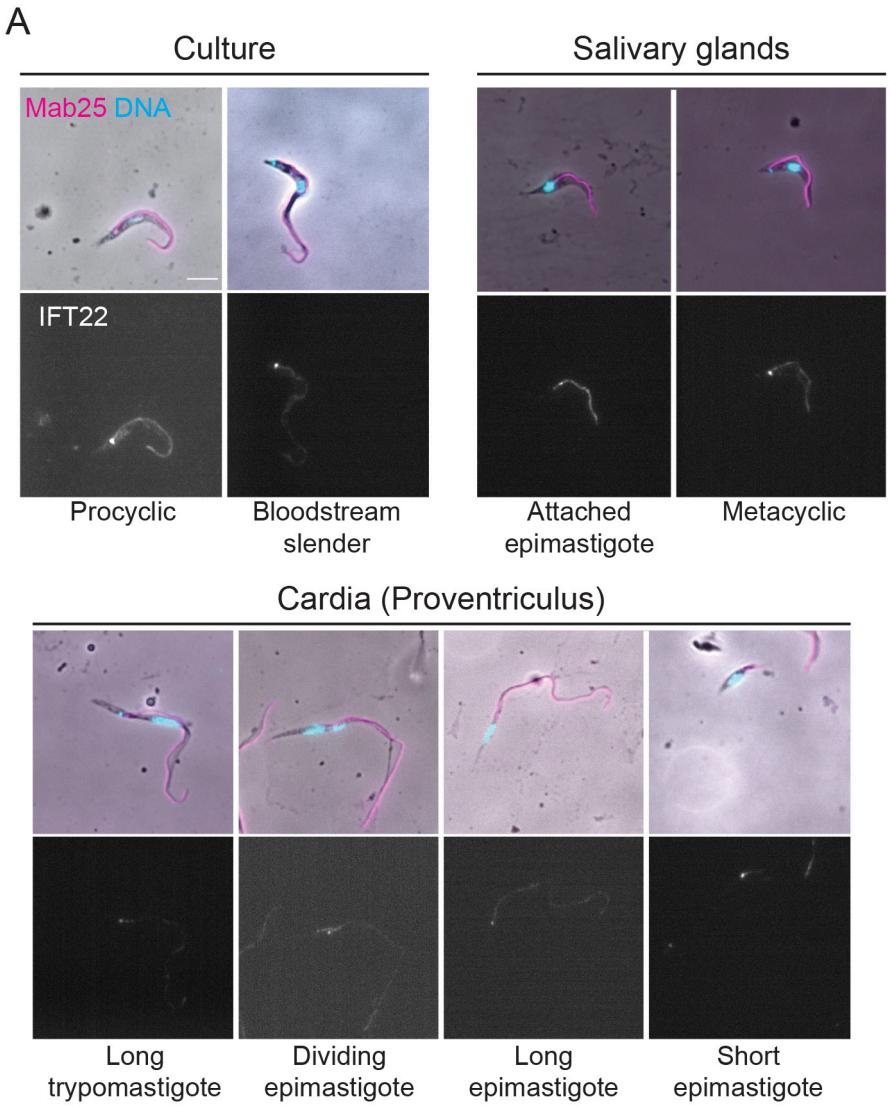
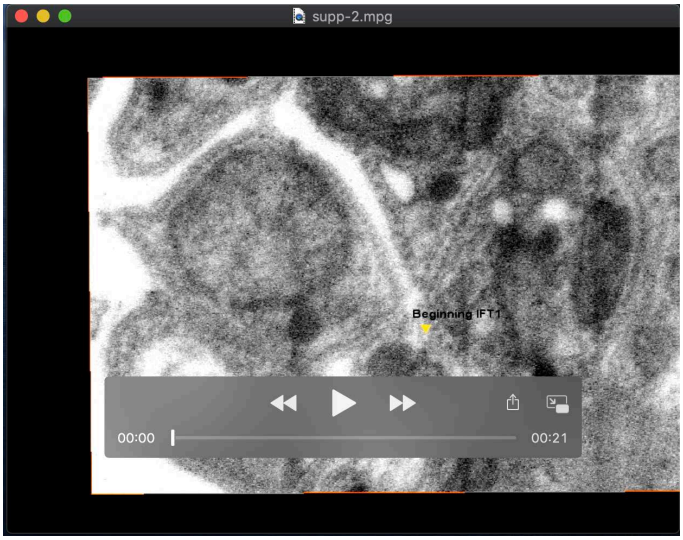
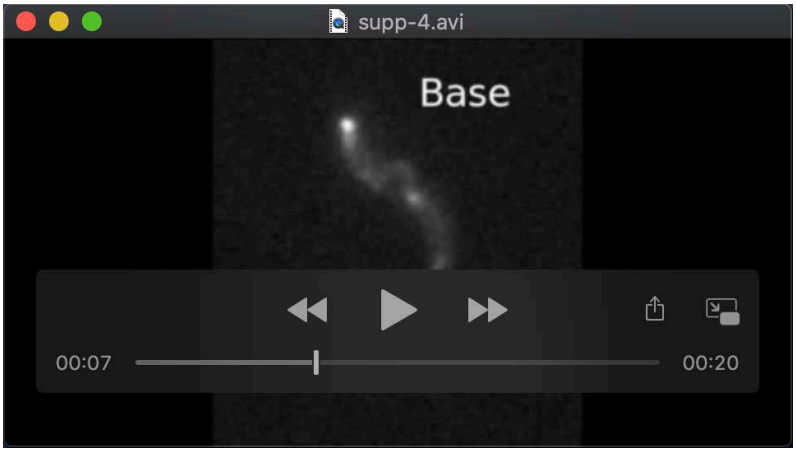


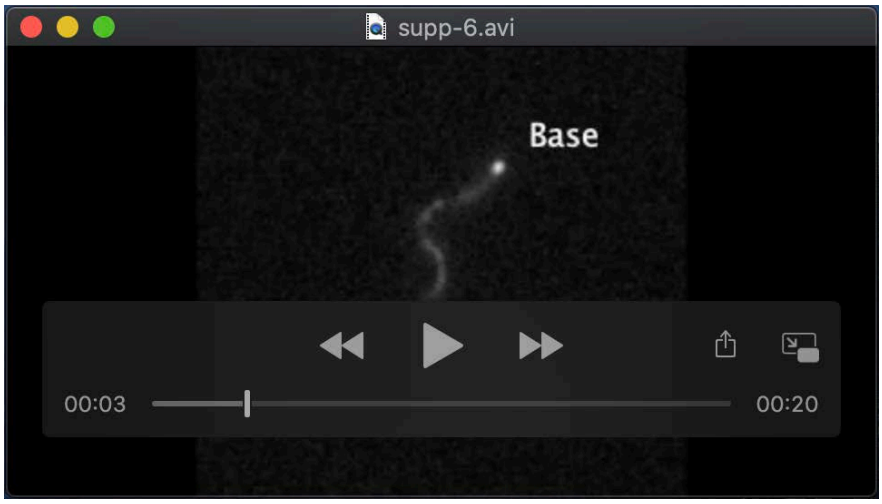
Fig. S2. IFT22 distribution during the *T. brucei* parasite cycle. (A) Parasites isolated from tsetse flies or grown in culture were fixed in cold methanol and stained with the Mab25 antibody to detect the axoneme (magenta) and the anti-IFT22 antibody (white). The top panels show the phase-contrast images merged with DAPI (cyan) and Mab25 signals (magenta). The bottom ones show the IFT22 fluorescent signal (white). Scale bar: 5µm. (B) Quantification of the total amount of IFT22 fluorescent signal in the flagellum normalized to flagellum length for each stage of the parasite cycle. The ROI was defined by the axonemal marker and used to measure the flagellum length. The fluorescence intensity directly reflects the total amount of IFT22 proteins present in the flagellum compartment. n=35 cells per stage. BSF: Slender bloodstream form, PC: Procyclic form, LT: Long trypomastigote, LDE: Long dividing epimastigote, SDE: Short dividing epimastigote, LE: Long epimastigote, SE: Short epimastigote, AE: Attached epimastigote, MT: Metacyclic trypomastigote.



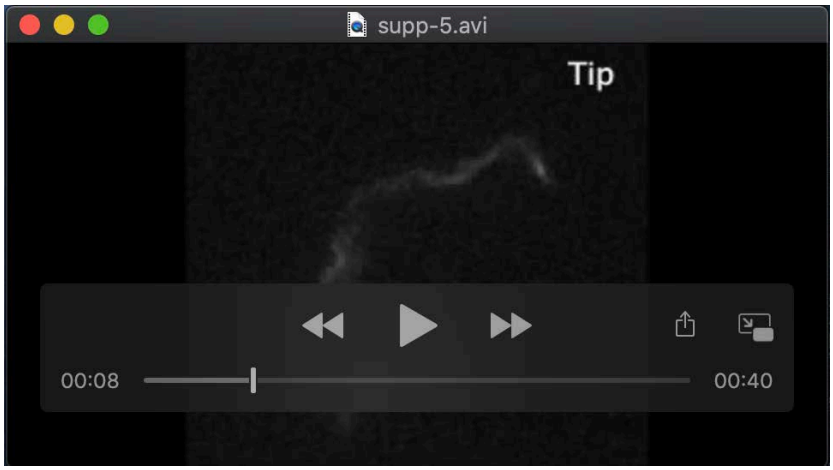
Movie 1. FIB-SEM analysis of parasites in the cardia. The region shown corresponds to that indicated by black squares at Figure 3. Two IFT particles are indicated by arrowhead: the first one (IFT1) is a long train found on doublet 8 and the second one is present on doublet 4 (IFT2). This video is related to Figure 3.



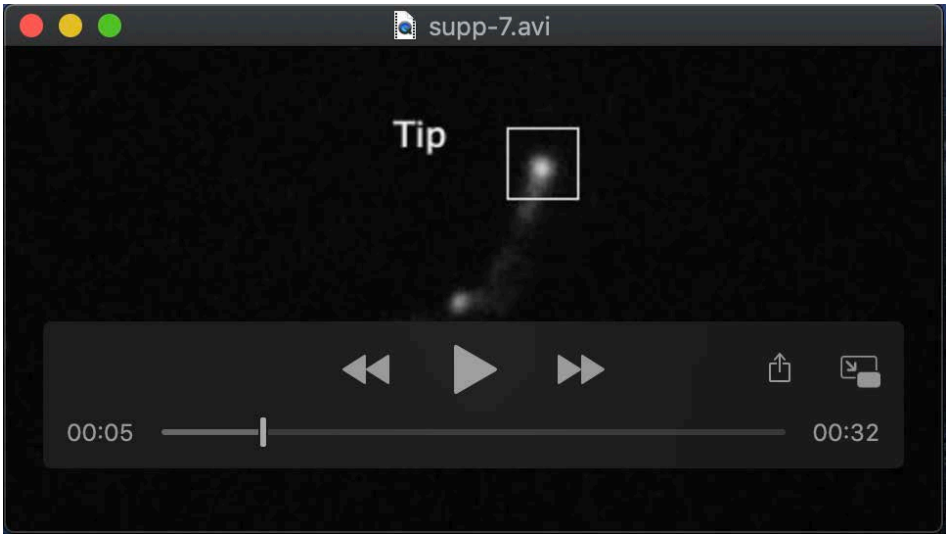
Movie 2. IFT trafficking in bloodstream (first in sequence) and procyclic cells grown in culture. Cells are expressing TdT::IFT81 following *in situ* tagging and display IFT trafficking. The bright signal at the base of the flagellum is clearly visible. The base and the tip of each flagellum are indicated. The video plays at real time. This video is related to Figure 5.



Movie 3. IFT trafficking in parasites isolated from the salivary glands showing first an attached epimastigote and then a metacyclic cell. Trypanosomes are expressing TdT::IFT81 following *in situ* tagging and display IFT trafficking. The bright signal at the base of the flagellum is clearly visible. The base and the tip of each flagellum are indicated. The video plays at real time. This video is related to Figure 5.



Movie 4. IFT trafficking in parasites isolated from the cardia of an infected tsetse fly. The sequence shows successively a long trypomastigote, a dividing epimastigote (trafficking visible in both old and new flagella), a long epimastigote (retrograde trains are nicely visible at the end of this sequence) and a short epimastigote with the typical accumulation of IFT proteins at the tip of the short flagellum. Cells are expressing TdT::IFT81 following *in situ* tagging and display IFT trafficking. The bright signal at the base of the flagellum is clearly visible. The base and the tip of each flagellum are indicated. The video plays at real time. This video is related to Figure 5.



Movie 5. Two examples of FRAP analysis of short epimastigote cells expressing TdT::IFT81. The distal portion of the flagellum was bleached with a brief laser pulse. Entry of new anterograde IFT trains in the flagellum can be monitored, they reach the distal tip where signal progressively comes backs before smaller, less bright, retrograde trains can be detected. The base and the tip of each flagellum are indicated. The square shows the portion of the flagellum that was photobleached. This video is related to Figure 6. The first cell goes briefly out of the plane of focus.