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Supplemental Information

Conversion of anterograde into retrograde trains is an intrinsic property of intraflagellar transport

Adrian Pascal Nievergelt, Ilia Zykov, Dennis Diener, Aditya Chhatre, Tim-Oliver Buchholz, Markus Delling, Stefan Diez, Florian Jug, Luděk Štěpánek, and Gaia Pigino

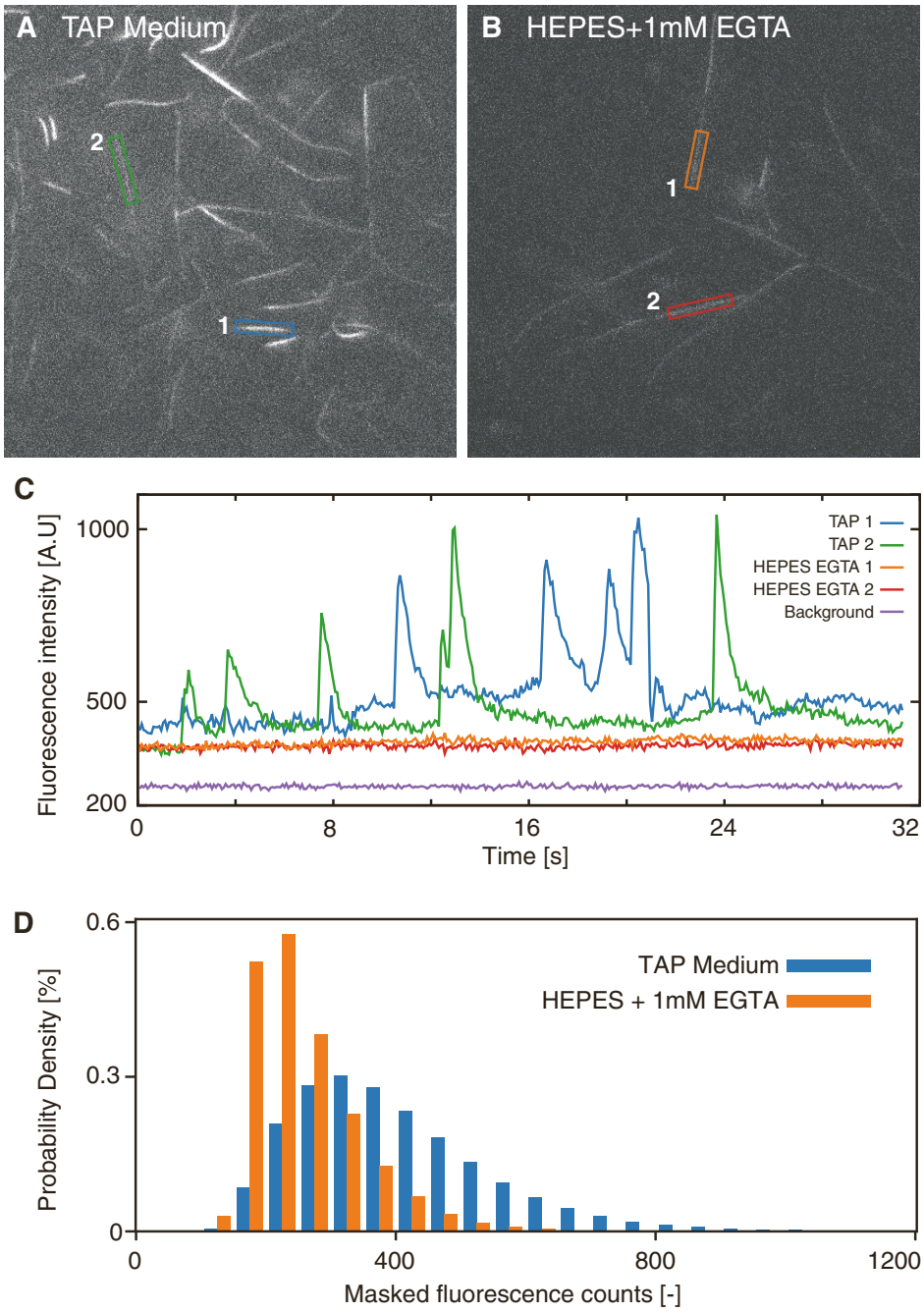

Figure S1: Quantification of the fluoresence of the *C.Reinhardtii* free-calcium-reporter (DRC4-GCaMP6) construct in calcium abundant and calcium depleted conditions, related to Figure 2. A) In calcium-abundant conditions (TAP Medium), cilia of DRC4-GCaMP6 cells show bright flashes of fluorescence usually correlated with touch or movement. **B)** In calcium depleted conditions (HEPES buffer with 1mM EGTA), cilia of DRC4-GCaMP6 show only faint background fluorescence. **C)** Time traces of fluorescent signal on select areas of cilia, showing characteristic decaying spikes of free calcium release in calcium abundant conditions and a constant background fluorescence without activity in calcium depletion. **D)** Density histogram of fluorescent intensity masked to places where cilia are visible in the respective movies for calcium rich and calcium depleted conditions showing higher overall fluorescence in calcium rich media.

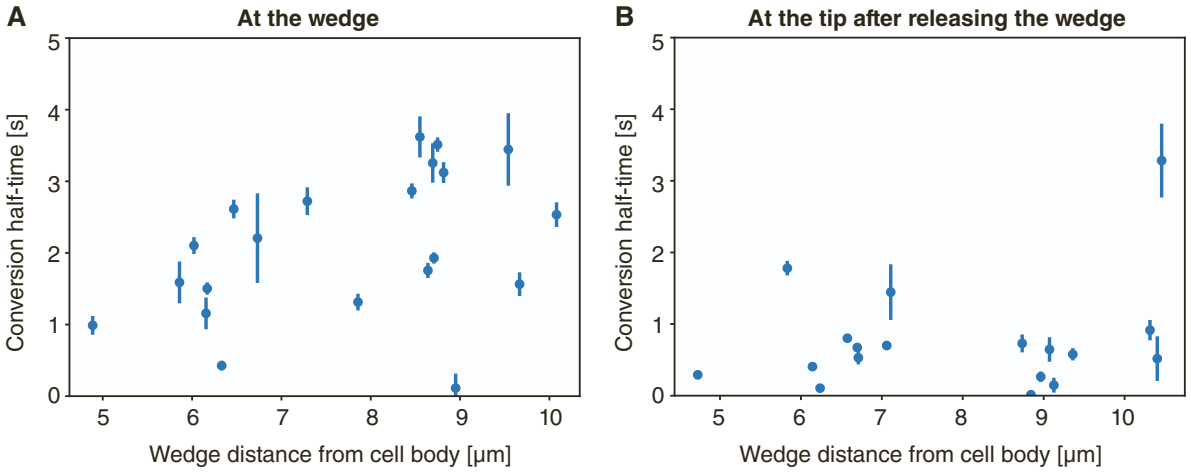

Figure S2: Conversion times for individual blocking experiments at different points along the cilium which show that IFT trains do not convert based on an internal timer, related to Figure 3. A) Conversion half-times at the basal side of the wedge with respect to the distance from the cell body where the wedge is applied on the cilium. **B)** Conversion half-times at the tip after the wedge has been released from a specific distance from the cell body after blocking. Data from 12 and 10 individual cells for wedge and tip respectively.

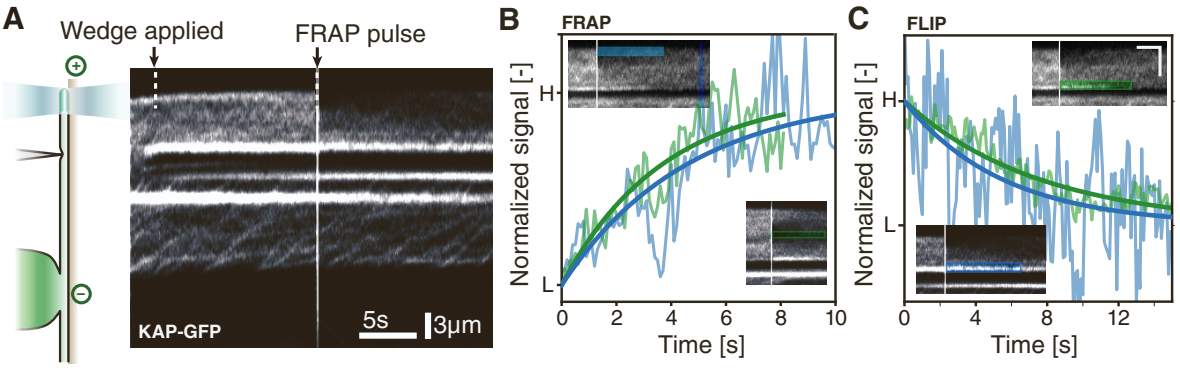

Figure S3: Photokinetics of the IFT motor kinesin-II in the isolated tip compartment showing the free diffusion of dissociated kinesin in the cilium, related to Figure 4. A) Fluorescence recovery after photobleaching (FRAP) experiment on free kinesin-II when creating a closed flagellar tip with the wedge, preventing both the exchange of KAP to the cell body and the arrival of new trains. **B)** Representative FRAP recovery rates and corresponding fits on two separate flagella. The blue curve was bleached the end of the tip while the green curve was bleached in the middle of the isolated tip compartment. Colored regions in insets mark the location of the fitted data. **C)** Fluorescence loss in photobleaching (FLIP) measurements on built up kinesin-II at the tip-side of the wedge. Colored regions in insets mark the location of the fitted data. Scale bar for all inserts: 4μm vertical, 4s horizontal.