Lipid droplets purified from *Drosophila* embryos as an endogenous handle for precise motor transport measurements

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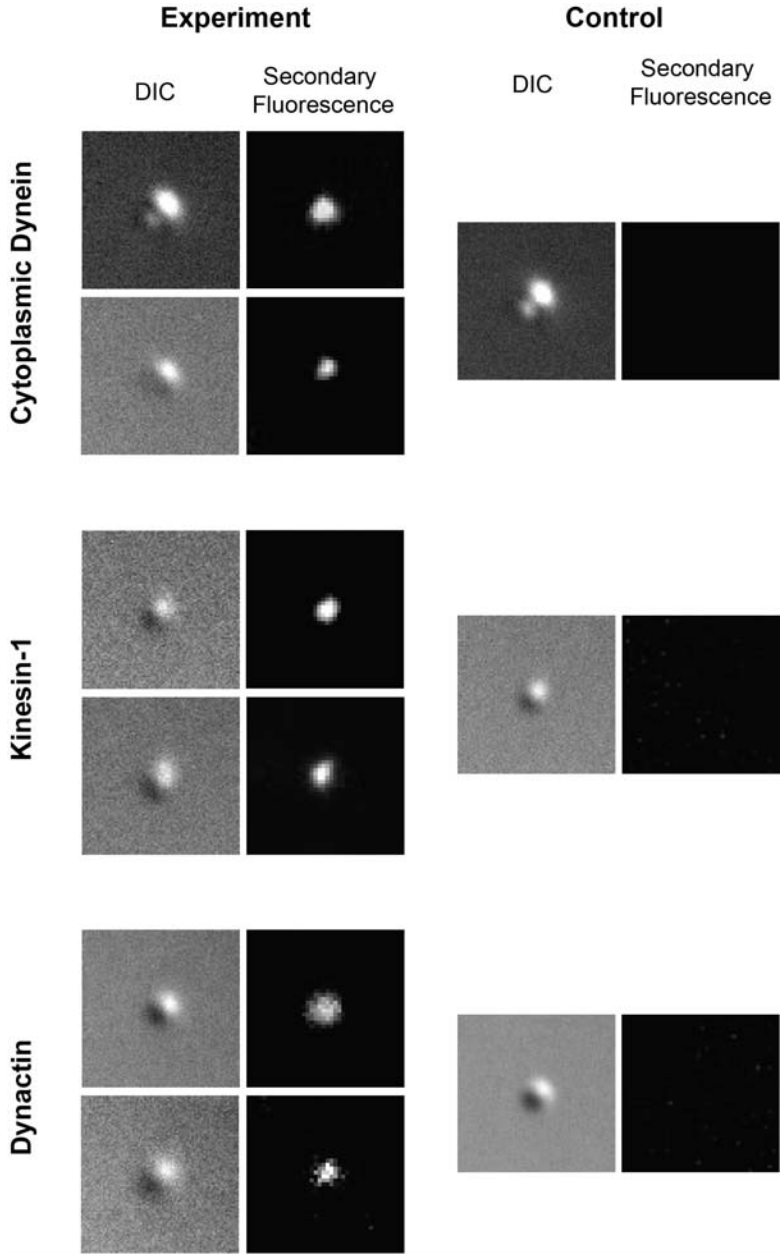
SUPPLEMENTARY FIGURES:



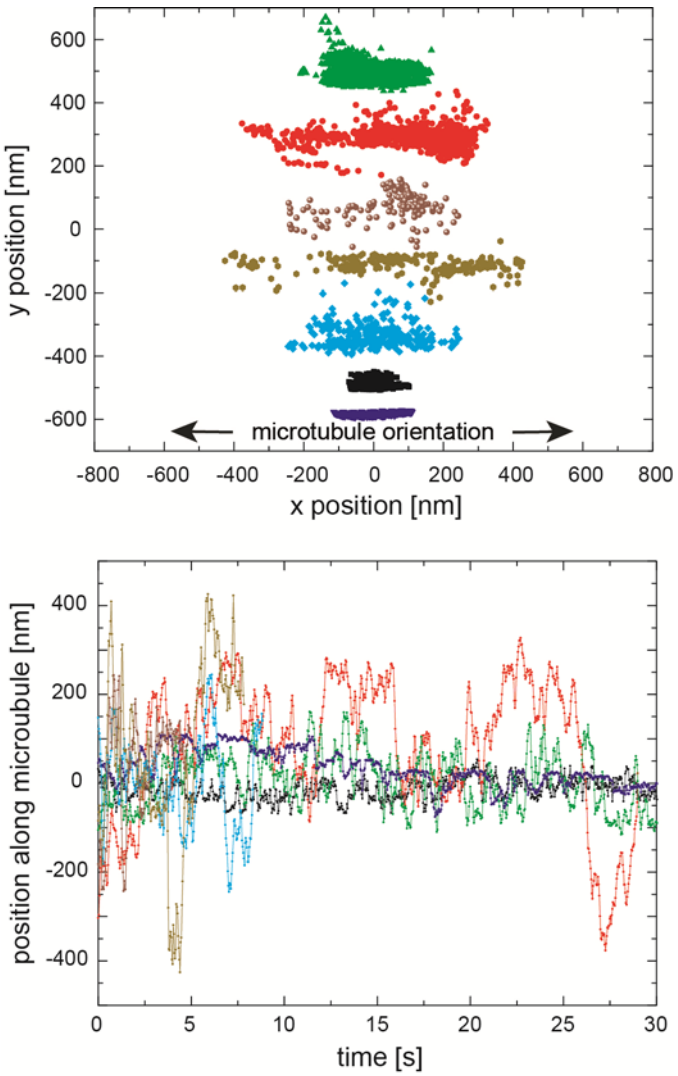
SUPPLEMENTARY FIGURE 1: **Purified lipid droplets retain cytoplasmic dynein, kinesin-1 and the dynactin complex.** Purified lipid droplets were incubated with antibodies against both motors and the $P150^{Glued}$ subunit of the dynactin complex and probed with fluorescently-labeled secondary antibodies. All panels show the Differential Interference Contrast (DIC) and the corresponding fluorescence image. Two examples for each motor protein are shown, but a bright fluorescence signal that coincides with the position of the lipid droplet was seen for all lipid droplets probed (n>12 for each motor). Dynactin was more variable with ~60% of the lipid droplets showing a bright fluorescence signal and the rest showing either dim or no fluorescence (n>20). No fluorescence was seen at the location of the lipid droplets in control experiments with the same secondary antibody but without the specific primary antibody for all three proteins. Lipid droplets were trapped from solution and both DIC and fluorescence images were recorded while the droplet was in the trap. The following conditions were used for fluorescence labeling:

Dynein: Purified lipid droplets were incubated overnight at 4°C with anti-DHC (DSHB 2C11-2, dilution 1:100). Cy3-labeled secondary antibody (Invitrogen A10521, dilution 1:100) was added and incubated for 6 hours on ice with an aluminum foil cover. For control experiments, purified lipid droplets were incubated with only secondary antibody.

Kinesin: Purified lipid droplets were incubated overnight at 4°C with anti-KHC (Cytoskeleton AKIN01, dilution 1:100). Cy3-labeled secondary antibody (Invitrogen A10520, dilution 1:100) was added and incubated for 4 hours on ice with an aluminum foil cover. For control experiments, purified lipid droplets were incubated with only secondary antibody.

Dynactin: Purified lipid droplets were incubated for 2 hours at 4°C with anti-DCTN1 (Thermo Scientific PA5-18095, dilution 1:100). Alexa Fluor 546-labeled secondary antibody (Invitrogen A21085, dilution 1:100) was added and incubated for 1 hour on ice with an aluminum foil cover. For control experiments, purified lipid droplets were incubated with only secondary antibody.

All images are of a $4\times4\ \mu m^2$ area.



SUPPLEMENTARY FIGURE 2: P**urified lipid droplets spend extended times in short-range back-and-forth motion** (A) scatter plots showing the positions of seven lipid droplets recorded by single particle tracking from video records at 30 frames/seconds. The scatter is clearly elongated along the direction of the microtubule while the droplets move back-and-forth as evident in the position versus time plot shown in (B). Excursions in either direction do not exceed a few hundred nanometers. Traces for the same lipid droplet have the same color in both panels.