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## **Supporting Material**

### **Probing intracellular motor protein activity using an inducible cargo trafficking Assay**

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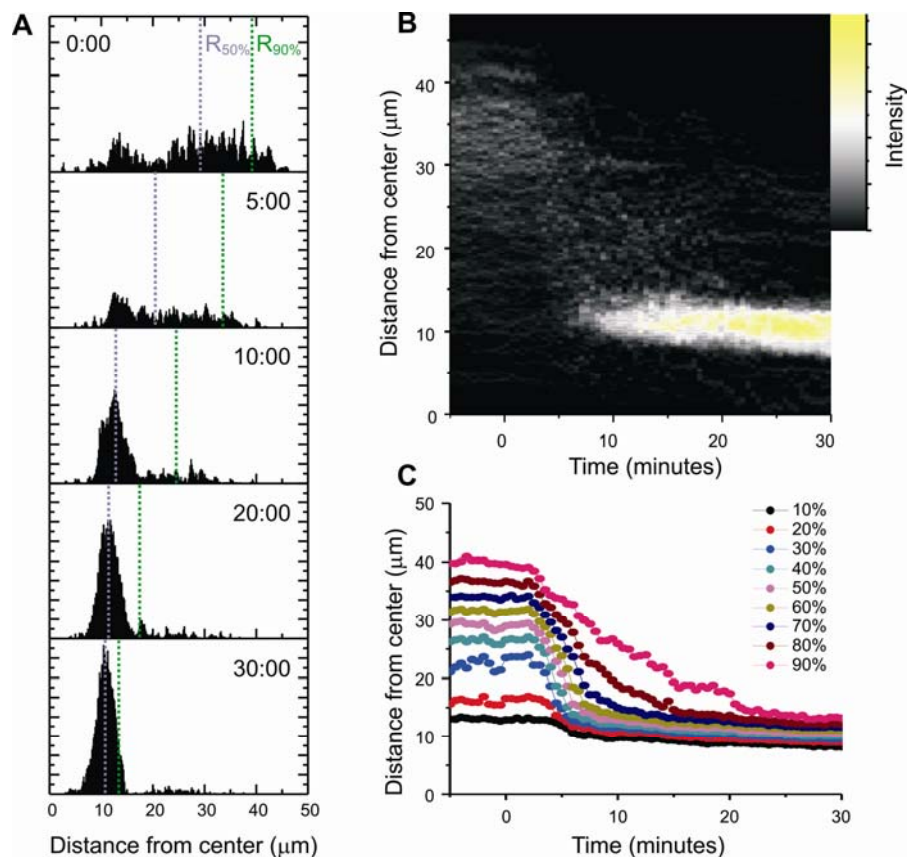
**Probing intracellular motor protein activity using an inducible cargo trafficking assay**

## Probing intracellular motor protein activity using an inducible cargo trafficking assay

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### SUPPLEMENTARY INFORMATION



### Supplemental Figure 1: Analysis of antagonistic motor action

(a) Histogram of intensity versus distance from center for the cell shown in Figure 3 (KIF17(1-547)-GFP-PEX26 + BICDN(1-594)-FRB) for the indicated time points. (b) The histograms of all frames of the time-lapse recording shown as a radial kymograph that directly visualizes all changes in peroxisome distribution upon motor protein recruitment. (c) Radii required to include a given fraction of peroxisomes (i.e. 10%, 20%, ..., 90%) versus time, calculated from the radial kymograph in b.

## **SUPPLEMENTARY VIDEO LEGENDS**

### **Supplemental video 1:**

This video corresponds to Fig. 2A,B and Fig. 3A,D,G. Peroxisome motility in a COS7 cell before and after recruitment of KIF1A(1-489)-FRB (Kinesin-3). The left part shows PEX3-RFP-FKBP, the right part Kif1A(1-489)-GFP-FRB. Addition of rapalog is indicated. Total time: 19:33 minutes. Acquired with 30 seconds between frames. 300x sped up. (AVI, 3.5 Mb)

### **Supplemental video 2:**

This video corresponds to Fig. 3B,E,H. Peroxisome motility in a COS7 cell before and after recruitment of FRB-DHC(1453-4644) (Dynein). Addition of rapalog is indicated. Total time: 60 minutes. Acquired with 30 seconds between frames. 600x sped up. (AVI, 2.4 Mb)

### **Supplemental video 3:**

This video corresponds to Fig. 3C,F,I. Peroxisome motility in a COS7 cell before and after recruitment of MyoVb(1-1090)-FRB (Myosin Vb). The left part shows PEX3-RFP, the right part Kif17-GFP-FRB. Addition of rapalog is indicated. Total time: 40 minutes. Acquired with 30 seconds between frames. 600x sped up. (AVI, 1.5 Mb)

### **Supplemental video 4:**

This video corresponds to Fig. 7B-E. Motility of peroxisomes (visualized by PEX3-RFP-FKBP) with attached KIF17 (Kinesin-2) in three COS7 cells before and after recruitment of BICN-FRB. Addition of rapalog is indicated. 2x2 pixels were binned to reduce video size. Total time: 37:30 minutes. Acquired with 30 seconds between frames. 600x sped up. (AVI, 4.7 Mb)

### **Supplemental video 5:**

This video corresponds to Fig. 7G,H. Bidirectional motility in COS7 cells of GFP-labeled peroxisomes with attached KIF17 (Kinesin-2) and BICDN(1-594)-FRB. Total time: 15 seconds. Acquired with 10 frames per second. Real time. (AVI, 0.8 Mb)