

Fig. S11. Partial co-localization of moving Kif4A and moving CPC clusters at AAIZ. (A) Stills from a time-lapse, two-color TIRF image sequence taken with a 2.73 sec time interval, and a time delay of  $\sim$ 1.36 sec between acquisition in the two channels. Kif4A-GFP was added at 3nM on top of endogenous Kif4A present in extract (left, green). Alexa647-anti-AurkB was added at  $0.1\mu g/mL$  to probe for endogenous CPC (middle, red). The right shows a merged image.

(B) 2x zoom-up of rectangle in (A) highlighting an antiparallel microtubule overlap and partial co-localization of the two probes. (C) Kymographs along the antiparallel MT overlap shown in (B). Note that the two probes move inwards at very similar rates (visualized as parallel lines in the kymographs). The fast-moving clusters localized at the outside of the AAIZ were moving inwards at  $\sim 25 \, \mu \text{m/min}$  in this example. We noted more co-localization between Kif4A-GFP and CPC clusters in static and slow moving clusters near the center. In fast moving clusters, the lines of Kif4A and CPC were staggered, presumably due to the time delay between acquisition in the green and red channels. Horizontal bars,  $10 \, \mu \text{m}$ . Vertical bars,  $1 \, \text{min}$ .