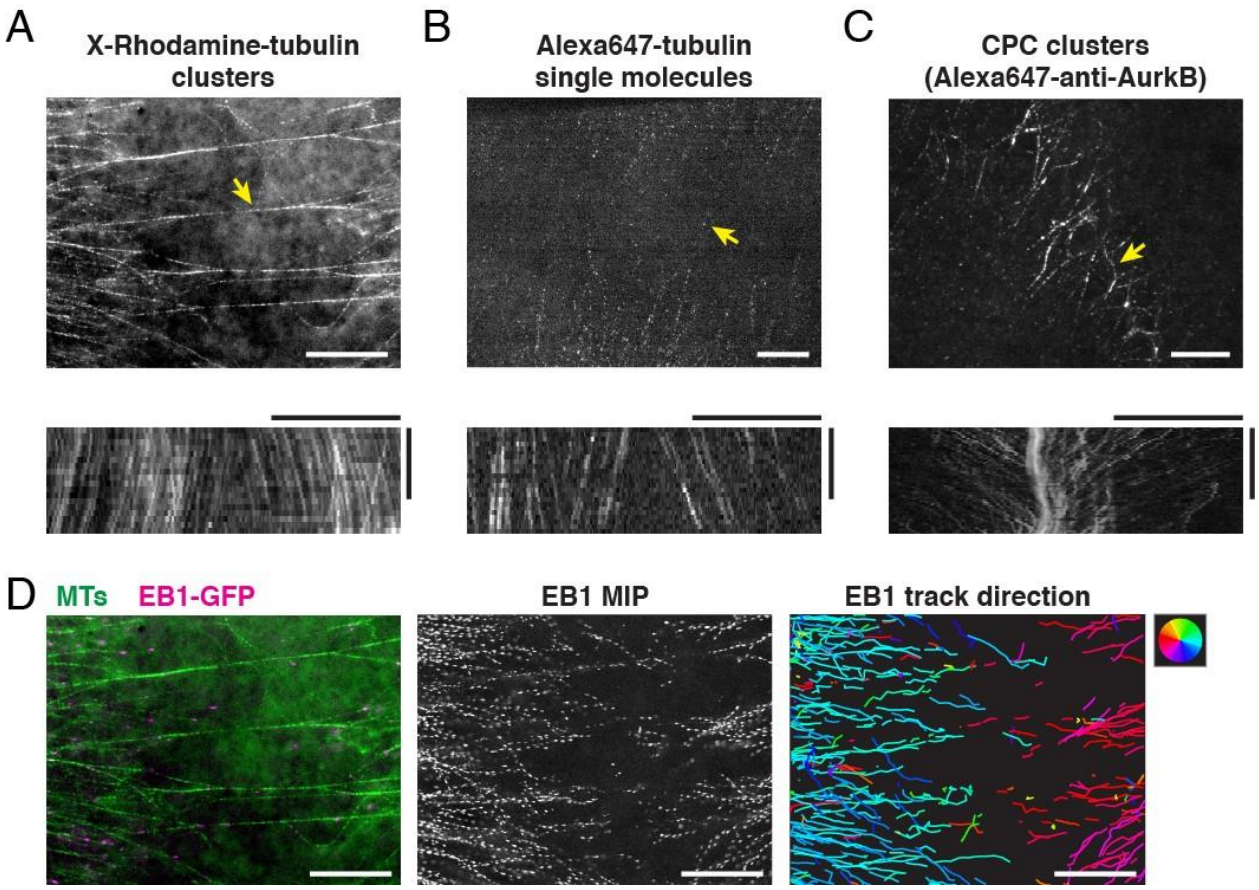

Fig. S8. Tubulin speckle imaging shows antiparallel microtubules sliding outward from overlap center. (A) Tubulin speckles were formed by uneven incorporation of the relatively hydrophobic X-rhodamine-tubulin into microtubules. The movement of these X-rhodamine tubulin clusters was imaged with time-lapse widefield microscopy at 5 sec intervals (movie S6). **(B)** Tubulin speckles were formed by single-molecule level incorporation of Alexa647-tubulin into microtubules. Time-lapse spinning disc confocal images were taken at 4 sec intervals (movie S8). **(C)** CPC clusters were visualized using Alexa647-IgG against AurkB and imaged with time- lapse spinning disc confocal microscopy at 1.3 sec intervals (same data as shown in fig. S7A, movie S4). **(A-C)** Still images from each time-lapse sequence *(top)*. Kymographs along the MT overlap bundles indicated by yellow arrow *(bottom)*. Horizontal bars, 20 µm. Vertical bars, 1
min. Kymographs show that while microtubules slide slowly outwards from the overlap centers at <5 µm/min, CPC clusters move inwards at 10-25 µm/min towards overlap center. This confirms that the fast inwards movement of the CPC is indeed motor-driven transport along microtubules. **(D)** Overlay of EB1-GFP image (magenta) on tubulin image (green) shown in (A); a still image from movie S7 *(left)*. Maximum intensity projection of EB1 images *(middle)*, and EB1 tracks colored by their mean direction *(right)* over the first half (2.5 min) of movie S7. Movie 7 illustrates the relationship between microtubule plus end growth (i.e. EB1 comet movement) and formation of antiparallel bundles. Horizontal bars, 20 µm.