

**Fig. S9. Measuring the plus-end directed velocity of the kinesin Kif4A.** (A) Expanding aster partially adhered to  $\kappa$ -casein coated coverslip with microtubules (Alexa647-tubulin; red) and Kif4A-GFP (green) visualized. TIRF image from a time-lapse sequence acquired using 2.1sec intervals for a total duration of 7.3min. (B) Kymographs of a microtubules (red) and Kif4A (green) along a microtubule that had a free plus end but was also part of an anti-parallel overlap (box in A). Scheme (*right*) shows the geometry of microtubules (red lines) before and after the time-lapse (top and bottom panels, respectively), and the inferred plus ends of the anti-parallel microtubules (yellow and blue solid lines); an antiparallel overlap is formed between the solid and dashed yellow lines. Apparent Kif4A velocity (measured from Kif4A kymographs) was corrected by velocity of microtubule sliding (measured from microtubule kymographs). (C) The velocity distribution of Kif4A moving on microtubules that are not part of AAIZs. Kif4A has a mean velocity of 26.4 ± 4.1 µm/min (mean±SD, N = 34 tracked Kif4A particles), an estimate for maximum velocity in our extract system.