Supplemental Information

Supplemental Figures

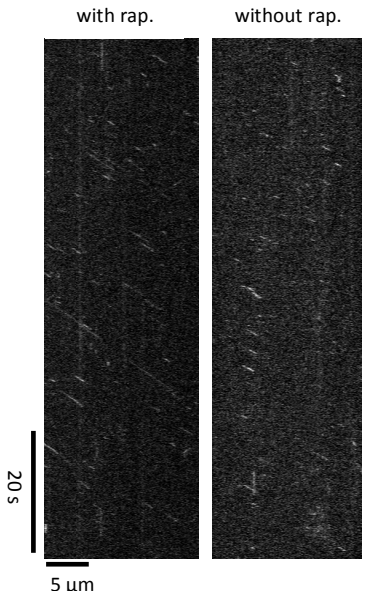

Figure S1: Kymograph of single molecule run length measurements for results displayed in Figure 2D. 1 nM Kin2GFPFRB was mixed with 20 nM EB1FKBP in the absence or presence of 100 nM rapamycin.

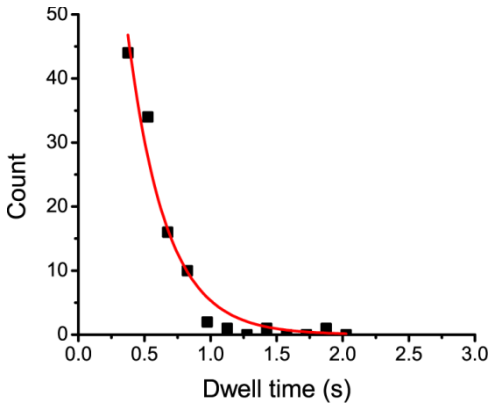

Figure S2: Duration of $EB1_{GFP}$ binding events on GTPγS microtubules in assay buffer without added KCl and KAc,. $EB1_{GFP}$ concentration was 1 nM, and buffer was 80 mM K-PIPES, 1 mM EGTP, 4 mM $MgCl_2$, pH 6.8. Data were fit to an exponential (red line) giving an average dwell time of 0.288 ± 0.28 s (mean ± SE of fit, N = 109). These data can be compared to dwell times of quantum dot-functionalized EB1 off-rates in normal assay buffer, shown in Figure 3C.

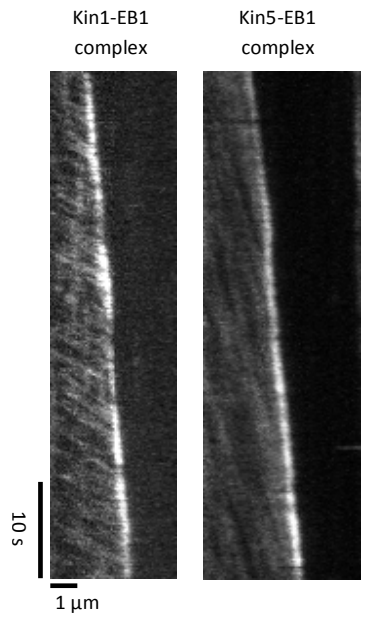

Figure S3: Kymograph of kin1-EB1 and kin5-EB1 tracks on dynamic microtubules. Experimental conditions were same as in Figure 4. Contrasting motor velocities can be seen by the different slopes of the single-motor tracks.

Supplemental Movies

Movie S1: Microtubule steering by $EB1_{FKBP}$-kin2GFP_{FRB} complex, related to Figure 3A. EB1: rapamycin: kinesin were used at a ratio of 10:10:1 with a kin2GFP_{FRB} concentration of 250 nM. Video was acquired using TIRF microscopy at 5 fps. The $EB1_{FKBP}$-kin2GFP_{FRB} complex highlights the growing microtubule plus-ends and during an encounter the plus-end of the growing microtubule is steered towards the plus-end of the immobilized microtubule.

Movie S2: Microtubule steering by $EB1_{FKBP}$-kin2GFP_{FRB} complex, related to Figure 3B. The video is from an independent experiment using conditions identical to Movie S1.

Movie S3: Microtubule steering by $EB1_{FKBP}$-kin1GFP_{FRB} complex, related to Figure 4B. Experimental conditions are the same as described in Movie S1 except 200 nM of kin1GFP_{FRB} was used. Similar steering of growing microtubule plus-ends was observed, confirming that kin1 also has the ability to steer growing microtubules when complexed to EB1.

Movie S4: Microtubule steering by $EB1_{FKBP}$-kin5GFP_{FRB} complex, related to Figure 4C. Experimental conditions are the same as described in Movie S1, except 25 nM of kin5GFP_{FRB} was used. In this movie the growing microtubule plus-end changes direction as it hits another microtubule laterally.

Movie S5: Negative control showing microtubule cross-over event in the absence of EB1. Related to Figure 3. Experiment was performed using conditions identical to Movie S3, but without $EB1_{FKBP}$. Microtubules are labeled weakly by moving $kin1GFP_{FRB}$ motors (200 nM), and no accumulation is observed at growing plus-ends. Note that encounters consist of microtubules crossing over one another without observable bending. Movie is 4x real time.

Supplemental Experimental Procedures

Cloning and protein expression

To make kin1, *Drosophila* conventional kinesin was truncated at position 559 and eGFP, FRB and a His_6 tag were added to the C-terminal sequentially. Kin2 was cloned by swapping the mouse KIF3A head and neck-linker into kin1 as previously described [1]. Kin5 was engineered by swapping head and neck-linker of XIKSP into kin1 and shortening the neck-linker to 14 aa as previously described [2]. Human EB1 was fused to FKBP and a His_6 tag at the C-terminal. All motors were expressed in bacteria and purified by Ni column chromatography as previously described [3], frozen in liquid N_2, and stored at -80^{0}C in storage buffer (50 mM K-phosphate, 300 mM NaCl, 2 mM $MgCl_2$, 100 µM ATP, 10 mM β-mercaptoethanol, 500 mM imidazole, pH = 7.2, with 10% sucrose added). EB1 was expressed and purified similarly, except that expression was induced with 0.5 mM IPTG and grown overnight at 23^{o}C. The cell pellet was resuspended in ice-cold buffer B (50 mM K-phosphate, 400 mM NaCl, 2 mM $MgCl_2$, 10 mM β-mercaptoethanol, pH 7.2) [4]. EB1 storage buffer consisted of 50 mM K-phosphate, 400 mM NaCl, 1 mM $MgCl_2$, 100 µM ATP, 5 mM DTT, pH = 7.0, with 10% sucrose added. At the highest concentrations used, EB1 and kinesin comprised 4% and 2.5% of the final volume, respectively; single-molecule investigations were carried out at concentrations 100-fold lower.

Microscopy assays and curve fitting

All experiments were carried out in assay buffer (80 mM K-Pipes, 85 mM KCl, 85 mM potassium-acetate, 1 mM EGTA, 4 mM $MgCl_2$, pH 6.8). Flow cells were assembled by attaching OTS-coated coverslips to glass slides with double-sided tape. The flow cell was first coated with 0.5 mg/ml neutravidin and blocked by 5% Pluronic F108 at room temperature. Then, the flow cell was incubated with Cy5- and biotin-labeled GMPCPP microtubules seeds at 35^{o}C. Microtubule polymerization was initiated by flowing in a buffer containing 20 µM free tubulin in assay buffer supplemented with 0.1% methyl cellulose, casein, 1 mM GTP, 1 mM $MgCl_2$, oxygen scavengers (glucose, glucose oxidase, catalase, β-mercaptoethanol) and proteins to be assayed. Flow cell temperature was maintained at 32^{o}C through an objective heater.

Run length and dwell time data were fit to single exponentials with no offset using Origin software.

To calculate tip/wall fluorescence ratio in Figure 4D, two line scans, were made perpendicular to the microtubules at the tip of microtubule and 1µm away from the tip, respectively. Peak intensities above the background were used to calculate the ratio.

Analytical gel filtration

A 300 µL sample of 5 µM kin2GFP$_{FRB}$ and 25 µM of EB1$_{FKBP}$ in assay buffer supplemented with 25 µM rapamycin was incubated on ice for 15 minutes before loading on to a Superdex 200 10/300 GL column (GE Healthcare). The fractions were eluted in assay buffer containing 1 µM rapamycin, 0.5 mL were fractions collected, and the absorbance monitored at 280 nm. SDS-PAGE gels were Coomassie stained, band intensities analyzed in ImageJ, and stoichiometries calculated by dividing each band intensity by its molecular weight.

Supplemental References

S1. Shastry, S., and Hancock, W.O. (2010). Neck linker length determines the degree of processivity in kinesin-1 and kinesin-2 motors. Curr Biol *20*, 939-943.

S2. Shastry, S., and Hancock, W.O. (2011). Interhead tension determines processivity across diverse N-terminal kinesins. Proc Natl Acad Sci U S A *108*, 16253-16258.

S3. Hancock, W.O., and Howard, J. (1998). Processivity of the motor protein kinesin requires two heads. J Cell Biol *140*, 1395-1405.

S4. Bieling, P., Kandels-Lewis, S., Telley, I.A., van Dijk, J., Janke, C., and Surrey, T. (2008). CLIP-170 tracks growing microtubule ends by dynamically recognizing composite EB1/tubulin-binding sites. J Cell Biol *183*, 1223-1233.