



Fig. S2. Microtubule plus tip tracking using EB1-GFP protein. (1) Asters were assembled between PEG-passivated coverslips, simultaneously for control and treatment samples (e.g. AurkB inhibition). Multiple AAIzs were imaged between 20 and 50 min of the assembly reaction, alternating between control and treatment conditions, using a spinning disk confocal microscope with a 40x oil objective. Time-lapse sequences were acquired of EB1-GFP with 1.5 sec intervals for a total duration of 2 min. Images of tubulin and AurkB were acquired at the beginning and end of each sequence. (2) Flow of cytoplasm was corrected by registering EB1 image sequences using the *StackReg* ImageJ plugin. (3) The *plusTipTracker* Matlab software was used to perform automated detection and frame-to-frame linking of EB1 to obtain growth tracks (see table S2 for parameters) (25). False tracks associated with bright centrosome beads, protein aggregates, and hot pixels in the camera were removed. Tracks were plotted and colored according to their mean direction. Top row images are zoomed up views of area marked by white square in EB1 image in **Step 1**. See Supplementary Methods section “Microtubule Plus Tip Tracking: Image Acquisition, Processing and Analysis” for more detailed description.