Supplemental Figure S1

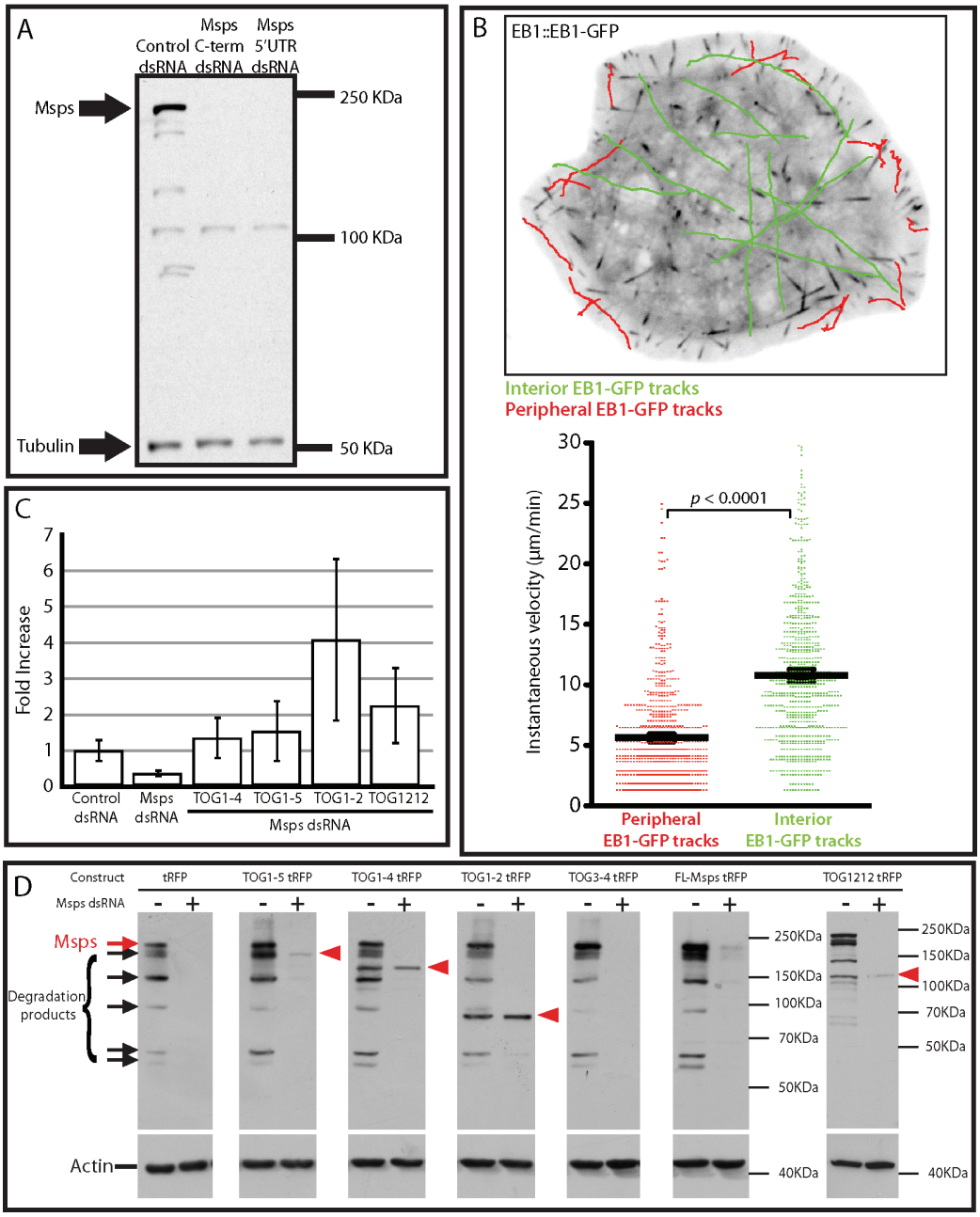

Figure S1. **Expression levels of endogenous and exogenous *Drosophila* Msps using a novel antibody raised against TOG domain 2.** (A) Rabbit polyclonal antibody raised against *Drosophila* Msps recognizes a 220KDa band in control treated S2 cells as well as a non-specific band at approximately 125KDa. Msps is depleted upon treatment with either C-terminally targeted dsRNA or dsRNA against the 5'UTR of Msps. Anti-α-tubulin is also present at 55KDa to represent equal protein load. (B) Representative EB1-GFP cell tracks from the cell interior (green) and EB1-GFP comets originating in the cell periphery (red), denoted as a 3µm region from the cell cortex that encompasses the actin-rich lamella (Iwasa and Mullins, 2007). Scatter plot of instantaneous velocities of EB1-GFP comets from the cell interior (green, right) or the cell periphery (red, left). Error bars represent 95% confidence intervals and center bar represents mean. N = 5 cells, approximately 7 EB1-GFP comets per cell, approximately 1000 velocity points per condition. (C) Relative fold increase in fluorescence of transiently transfected S2 cells expressing various Msps transgenes in the presence of either control dsRNA (left) or Msps

dsRNA (right) stained with Msps antibody. Error bars indicate standard deviation. (D) Western blots of cells from (C). Several degradation products are denoted (left) in control lysates and the Msps transgene where detectable is denote by red arrowhead. A separate blot from the same lysates is below to show equivalent protein load.

Supplemental Figure S2

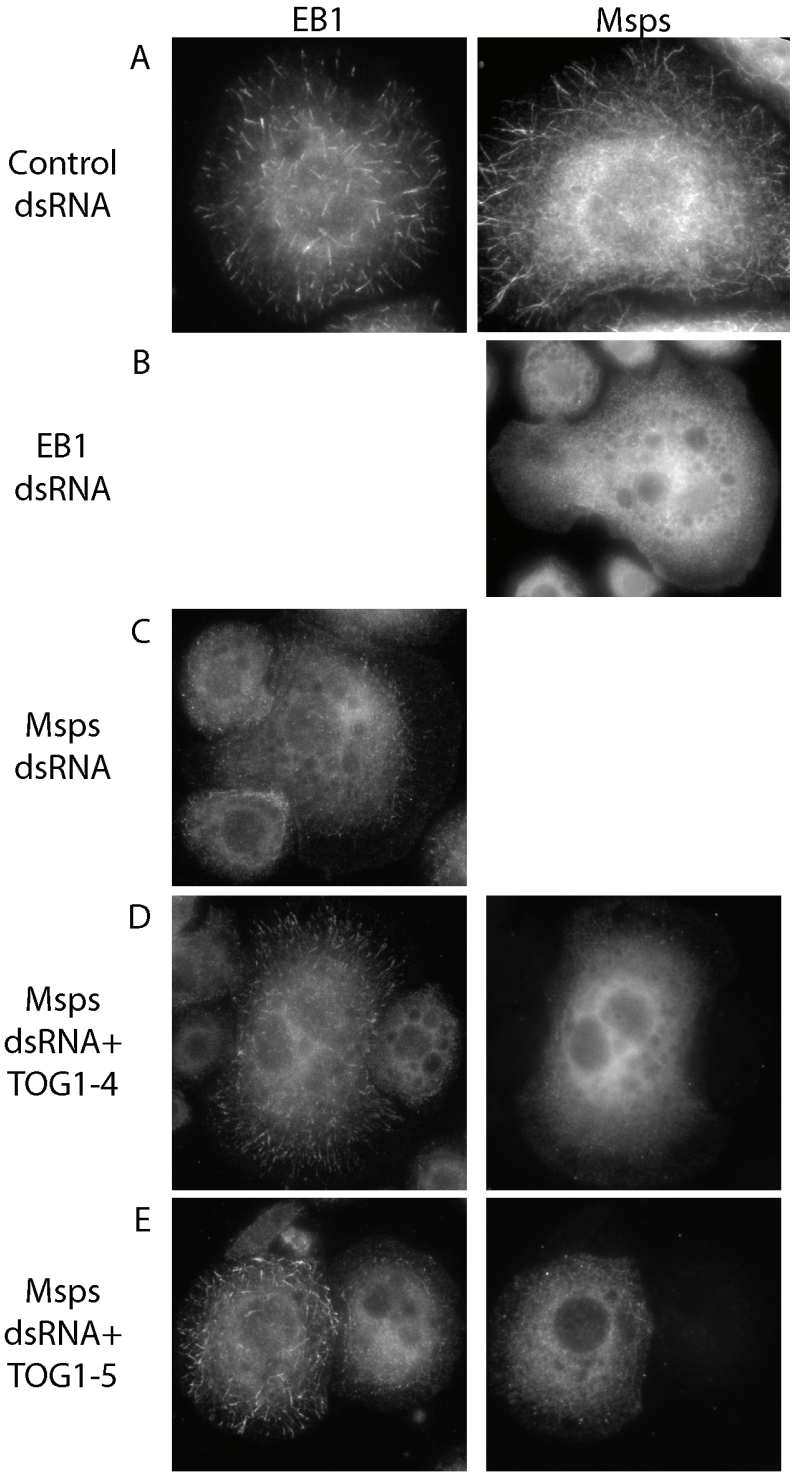

Figure S2. **Expression of Msps fragments TOG1-4 and TOG1-5 restore normal plus end localization to endogenous EB1.** S2 cells stained for endogenous EB1 (left) or Msps (right) treated with (A) control dsRNA, (B) EB1 dsRNA, (C) Msps dsRNA, (D) Msps dsRNA with TOG1-4, and (E) Msps dsRNA with TOG1-5.

Supplemental Figure S3

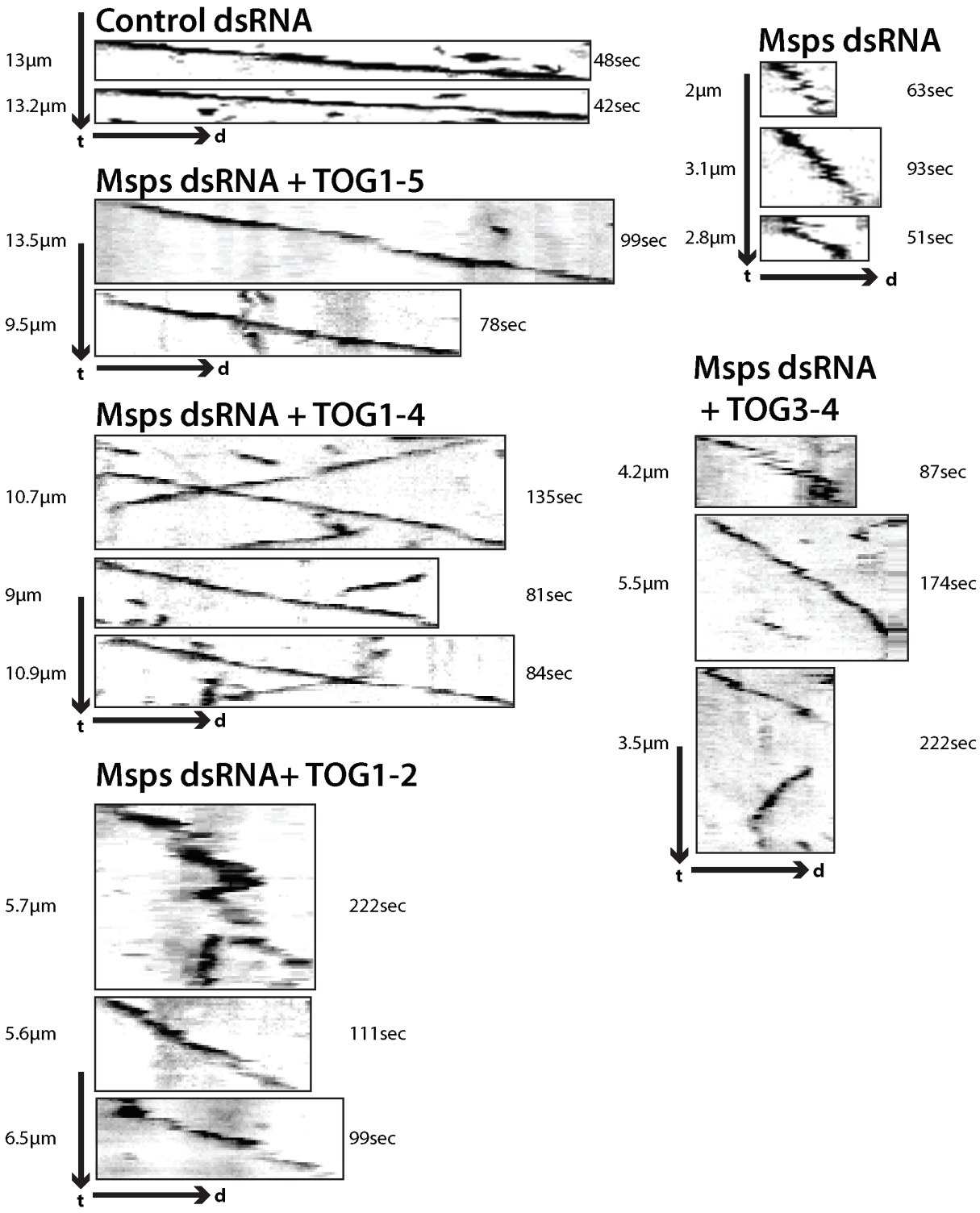

Figure S3. **Representative EB1::EB1-GFP kymographs of S2 cells treated with control or Msps dsRNA and transfected with Msps fragments.** Distance in micrometers is indicated to the left of each kymograph and the y axis of time is indicated to the right. Each kymograph represents the full life span of one EB1 comet for each condition.

Supplemental Figure S4

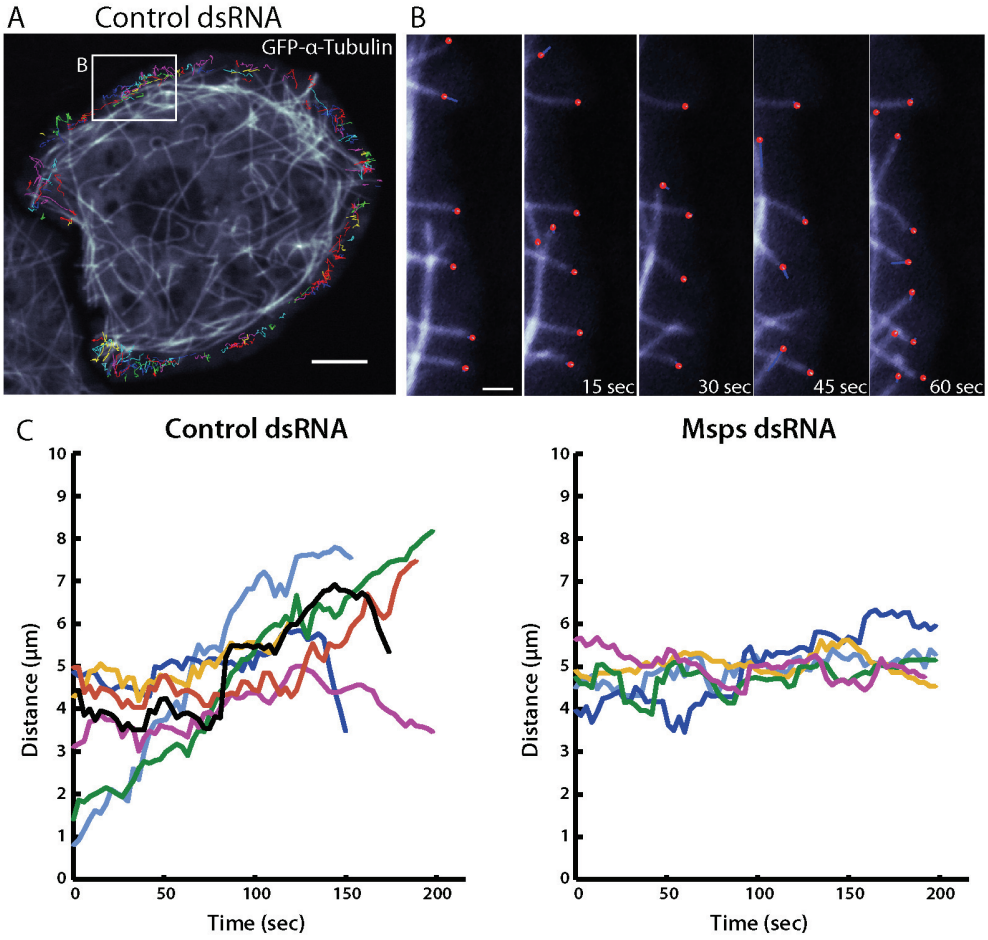

Figure S4. **Automated microtubule tracking algorithm.** (A) Representative control cell with trajectories of at least ten frames overlaid. Scale bar is 5 microns. (B) Zoom of inset from (A) showing 15 second intervals over 1 minute. Scale bar is 1.5 microns. (C) Microtubule lifetime plots of representative microtubules shown for Control dsRNA and Msps dsRNA treatments. Plots are arranged on the distance axis to indicate a mean distance of 5 microns.

Supplemental Figure S5

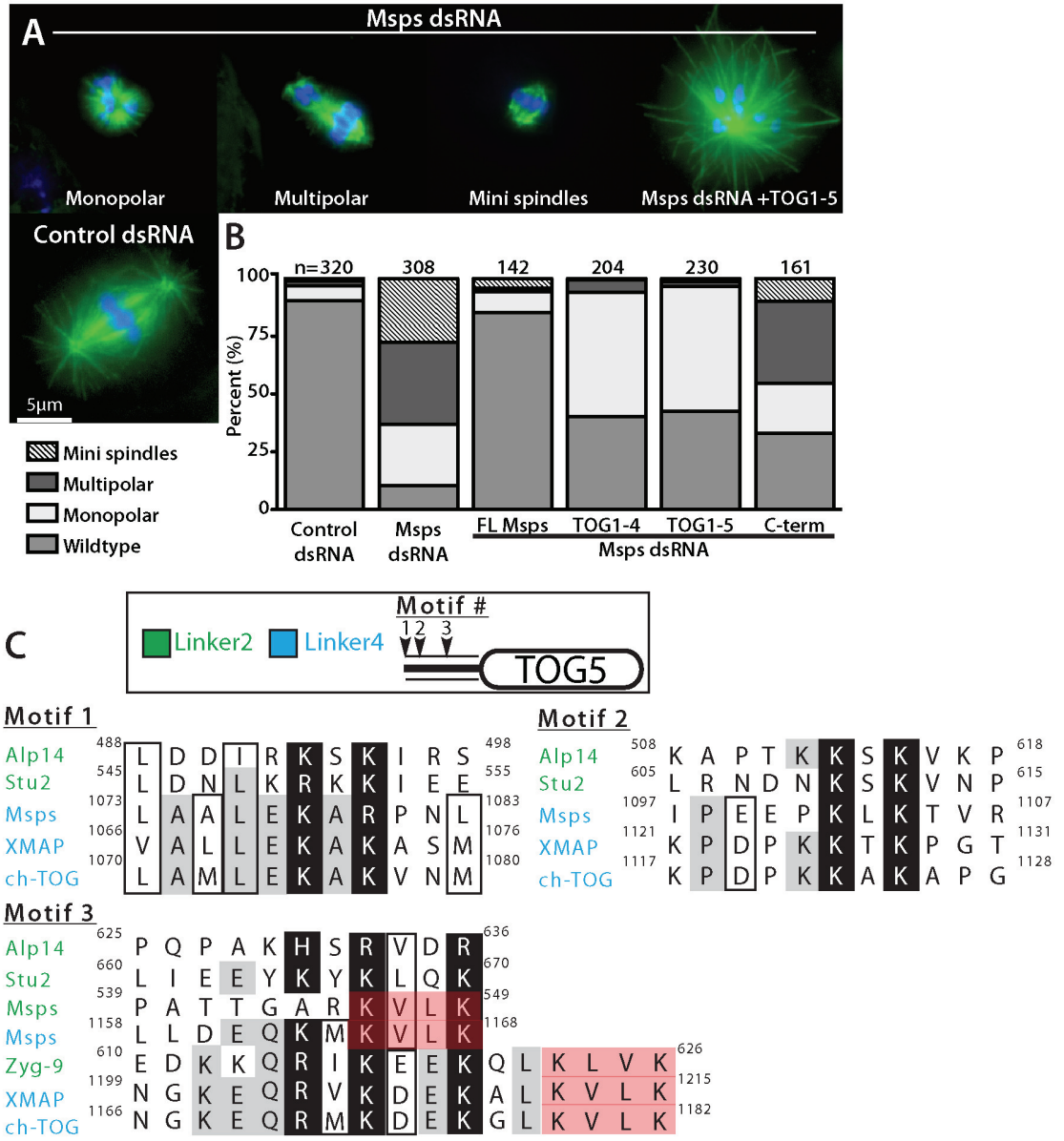

Figure S5 **Conserved motifs within the linker2 and linker4 of *Drosophila* Msps and other Dis1/XMAP215 members.** (A) Micrographs portraying representative spindle morphologies (microtubules in green and DAPi in blue) from Msps RNAi depletion (top) and control spindles (bottom, left). Msps RNAi-depleted cells rescued with TOG1-5 often displayed large monopolar spindles (top, far right). Scale bar for the control spindle applies to all micrographs. (B) Distribution of general spindle phenotypes between different RNAi conditions. All conditions were repeated three times with the addition of cdc27 RNAi at day 5 (see results) and the number of mitotic cells counted is indicated above each column. (C) Partially conserved motifs found in linkers of XMAP215/Dis1 members are labeled 1-3 based on their occurrence from NH_2-terminal to COOH-terminal within the linker. Green titled sequences indicate a position within the linker2 and blue within the linker4 where applicable. Outlined boxes are partial amino acid conservation, gray boxes absolute conservation in at least three members, black boxes indicate key positively-charged residues, and red boxes indicate the "KVLK" motif.

Online Supplemental Material

Movie 1. **The localization of Msps-GFP is spatially regulated in interphase *Drosophila* S2 cells.** S2 cell transfected with full length Msps-GFP (left) and mCherry-α-tubulin (right). Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 2. **Msps-GFP exhibits dynamic movements along the lattice of peripheral microtubules.** S2 cell transfected with Msps-GFP (left) and mCherry-Tubulin (right) showing the dynamic movement of discontinuous GFP-punctae on peripheral microtubules. Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 3. **Msps-GFP associates with growing and shrinking microtubules.** S2 cell transfected with Msps-GFP (left) and mCherry-Tubulin (right) shows growing and shrinking microtubule association. Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 4. **Msps localizes to plus ends and microtubule lattice, but not centrioles, in an EB1-dependent manner.** S2 cell transfected with Msps-GFP and treated with either control (left) or EB1 dsRNA (right). Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 5. **Msps TOG domains are sufficient to partially rescue microtubule polymerization and EB1 dynamics.** S2 cells transfect with EB1::EB1-GFP and a COOH-terminally tagged Msps construct (inset, bottom left), were treated with control or Msps dsRNA. Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 6. **Msps TOG domains 1-2 and 3-4 provide little rescue of microtubule polymerization and EB1 dynamics.** S2 cells transfect with EB1::EB1-GFP and a COOH-terminally tagged Msps construct (inset, bottom left), were treated with control or Msps dsRNA.

Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 7. **Double Mut Msps-GFP does not associate with the lattice of peripheral microtubules and results in gross alterations to the microtubule cytoskeleton.** S2 cells treated with Msps 5‘UTR dsRNA for 7 days and transfected with either wildtype Msps-GFP (top, left) or Double Mut Msps-GFP (bottom, left) and mCherry-Tubulin (right). Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).

Movie 8. **Mutation in the linker2 and linker4 motifs of full length Msps affect peripheral lattice association.** S2 cell transfected with either wildtype full-length Msps-GFP (left, green) or with Double Mutant full-length Msps-GFP (Double Mut Msps-GFP) and mCherry-Tubulin (right, red). Images were acquired at three second intervals using spinning-disc confocal microscopy (Yokogawa, Perkin Elmer).