SUPPLEMENTARY INFORMATION

This supplement contains three Supplementary Figures, one Table, and 6 time-lapse movies.

SUPPLEMENTARY FIGURE LEGEND

Figure S1: Methods for image analyses. (A) Spindle elongation rate analysis. To determine the elongation rate, time-lapse movies were used to analyze bipolar spindle-like structures. AurA-beads, which define spindle poles, were tracked using Metamorph and the distances between them determined (green and white lines show the tracks each beads had taken in the movie). The distance between AurA beads in each movie frame was imported into a Matlab program to identify 30-second over-lapping windows and to determine the AurA-bead separation rate in each of the windows. The elongation rate across all of the windows was then averaged to give the average elongation rate of the spindle. (B) To determine the extent of separation of AurA beads in MT structures, a nearest-neighbor analysis was performed. A Matlab image recognition program was used to identify AurA beads (marked with white dots) within MT structures. The distances between a bead and its nearest neighboring bead was identified (marked as white lines connecting the white dots), checked against the corresponding thresholded rhodamine-tubulin image, and measured. These numbers were then exported as Excel files to determine the average distances between AurA beads.

Figure S2: (A) Efficiency of LB3 depletion in *Xenopus* egg extracts. Western blotting analyses were performed using mock-depleted or LB3-depleted egg extracts. Immunodepletion using the antibody raised to the C-terminus of LB3 (LB3T, LB3 depletion) routinely removed at least 95% of LB3 in *Xenopus* egg extracts as compared to control depletion using non-immunized rabbit IgG (Mock depletion). Tubulin was used as a loading control. (B) Eg5 and LB3 do not interact with one another in *Xenopus* egg extracts. Eg5 was immunoprecipitated using two different polyclonal antibodies to the Eg5 tail region (Eg5-tail) and the first 545 amino acids of Eg5 (Eg5-N), while LB3 was immunoprecipitated using polyclonal antibodies raised against the C-terminus of the protein (amino acids 383-583, LB3T). These antibodies or control IgG from un-immunized rabbits were coupled to Affi-prep Protein-A beads. Both Eg5 and LB3 antibodies pulled down dynein as expected. However, Eg5 and LB3 did not immunoprecipitate each other. Tubulin is not present in any of the immunoprecipitate. (C) The effect of Monastrol on AurA bead-clustering as a function of time. Quantification of the bead-clustering index (clustered beads/separated beads) as a function of time with and without 100 μM Monastrol. The number of beads counted per condition per experiment was 400. Error bars, SEM. Graphs and error bars are based on averages from at least three experiments performed on different days. Quantifications were performed at the times indicated.

Figure S3. (A) Addition of an increasing concentration of Eg5 antibody caused a gradual decrease of the average distance between AurA beads. (B) The increase of the average distance between AurA-beads as a result of LB3 depletion could be reversed by inhibiting Eg5 activity using the Eg5 antibody. (C) Addition of an increasing concentration of Eg5 antibody caused a gradual increase of AurA-bead clustering. (D) The reduction of AurA-bead clustering caused by LB3 depletion can be reversed by inhibiting Eg5 activity using the Eg5 antibody. The number of beads counted for clustering per condition per experiment was 700. Error bars, SEM. Graphs and error bars are based on averages from at least three experiments performed on different days.

Figure S4. (A) Manipulating the egg extracts by immunodepletion increases AurA-bead clustering. Egg extracts that subjected to immunodepletion using control IgG coupled to

magnetic beads or Affi-prep beads exhibited a significant increase in clustering index as compared to the un-manipulated egg extracts. (B) LB3 does not increase the tendency of AurA-bead clustering but it hinders their separation during formation of spindle-like structures. Mock- or LB3-depleted egg extracts were used to assemble spindle-like structures from AurA beads. Bead clustering index were calculated at different time points. As expected, the decrease in bead clustering index was observed in both egg extracts as a function of reaction time. However, whereas there was no significant difference in bead clustering index at 3 min into the reaction, lower bead clustering was observed at later time points in LB3-depleted egg extracts than that of controls. * p-value < 0.05 (Student T test). The number of beads counted per condition per experiment was 400. Error bars, SEM. Graphs and error bars are based on averages from at least three experiments performed on different days.

SUPPLEMENTARY TABLE LEGEND

Table S1: EC_{50} values for Monastrol as determined by different assays in this and previous studies. All numerical values are μM. Further information on the assays used can be found in the indicated publications.

SUPPLEMENTARY MOVIE LEGEND

Movie SM1. This movie shows two clustered AurA beads (green) initially nucleating one microtubule structure (red). The beads then separate from one another to form spindle poles, which gives rise to a bipolar spindle-like structure (red). Bead diameter, 2.8 μm. The movie shows the time period between 2 and 17 minutes of the spindle assembly reaction. Images were taken at a rate of 1 frame/5 seconds. Scale bar, 10 μm.

Movie SM2. This movie shows three clustered AurA beads (green) initially nucleating one microtubule structure (red). The separation of these beads from one another gives rise to a multi-polar spindle-like structure. Bead diameter, 2.8 μm. The movie shows the time period between 2 and 17 minutes of the spindle assembly reaction. Images were taken at a rate of 1 frame/5 seconds. Scale bar, 10 μm.

Movie SM3. This movie shows a field of AurA beads (green) engaging in microtubule (red) assembly. Well-isolated single AurA beads initially nucleate microtubule asters. When microtubules from different AurA beads come into contact, the distance between the beads are either maintained or increased as the reaction proceeded. The increase in distance is dependent on Eg5 activity (see the text). Bead diameter, 2.8 μm. The movie shows the time period between 2 and 17 minutes of the spindle assembly reaction. Images were taken at a rate of 1 frame/5 seconds. Scale bar, 50 μm.

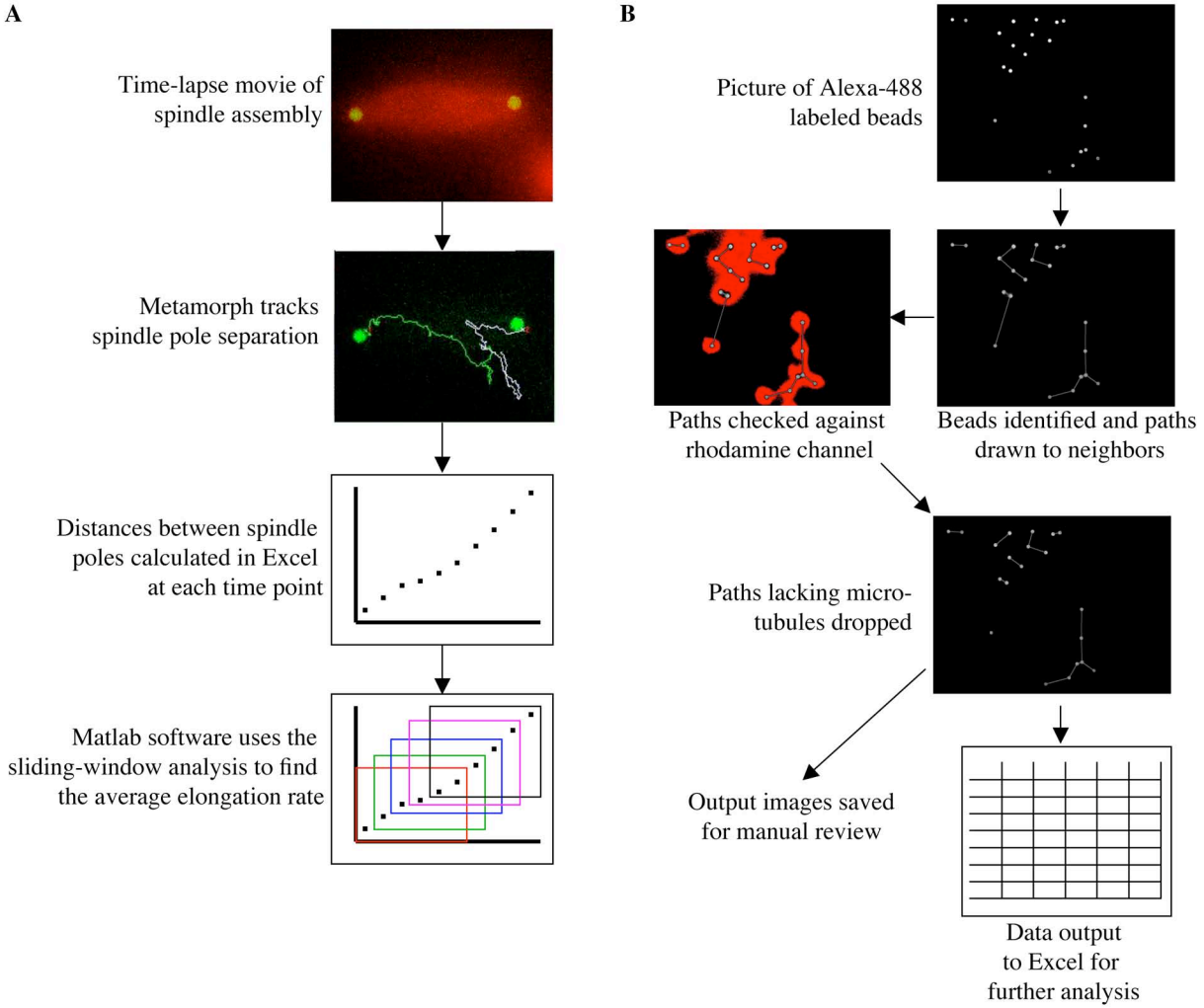
Movie SM4. This movie shows EB1-GFP tracking the plus ends of microtubules assembled from three AurA beads. Many microtubules grow from one bead toward the other bead indicating anti-parallel microtubule orientations in these spindle-like structures. Images were taken at a rate of 1 frame/second. Scale bar, 10 μm.

Movie SM5. This movie shows microtubule speckles in a bipolar spindle-like structure that is in the process of elongating. The beads are masked using the average intensity of the image background to allow better visualization of the speckles. The speckle analyses of this particular structure can be found in Fig. 1. Images were taken at a rate of 1 frame/3 seconds.

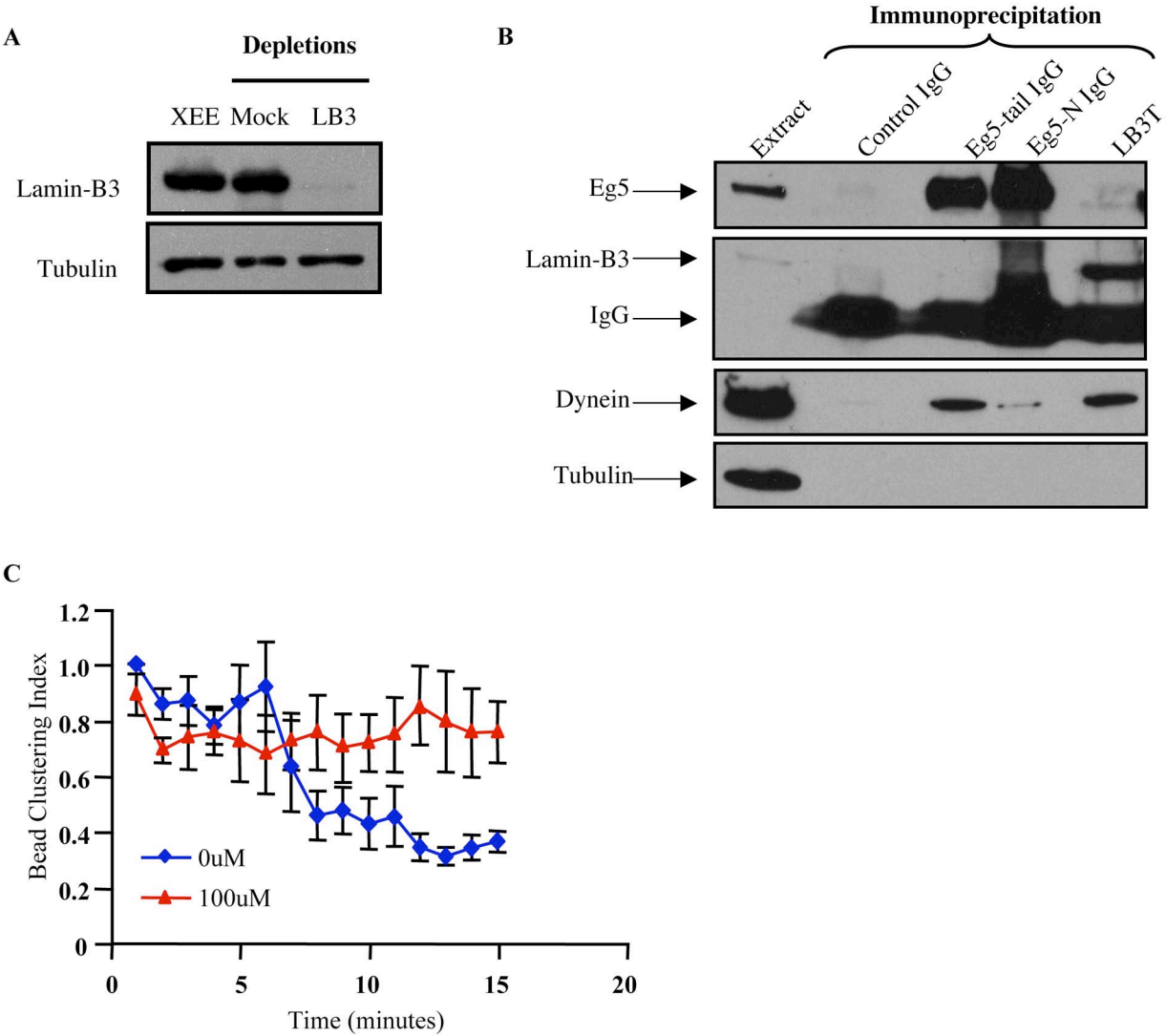
Movie SM6. This movie shows microtubule speckles in a sperm spindle. The speckle analyses of this particular spindle can be found in Fig. 4. Images were taken at a rate of 1 frame/3 seconds.

Note: All movies shown at 15 frames/second.

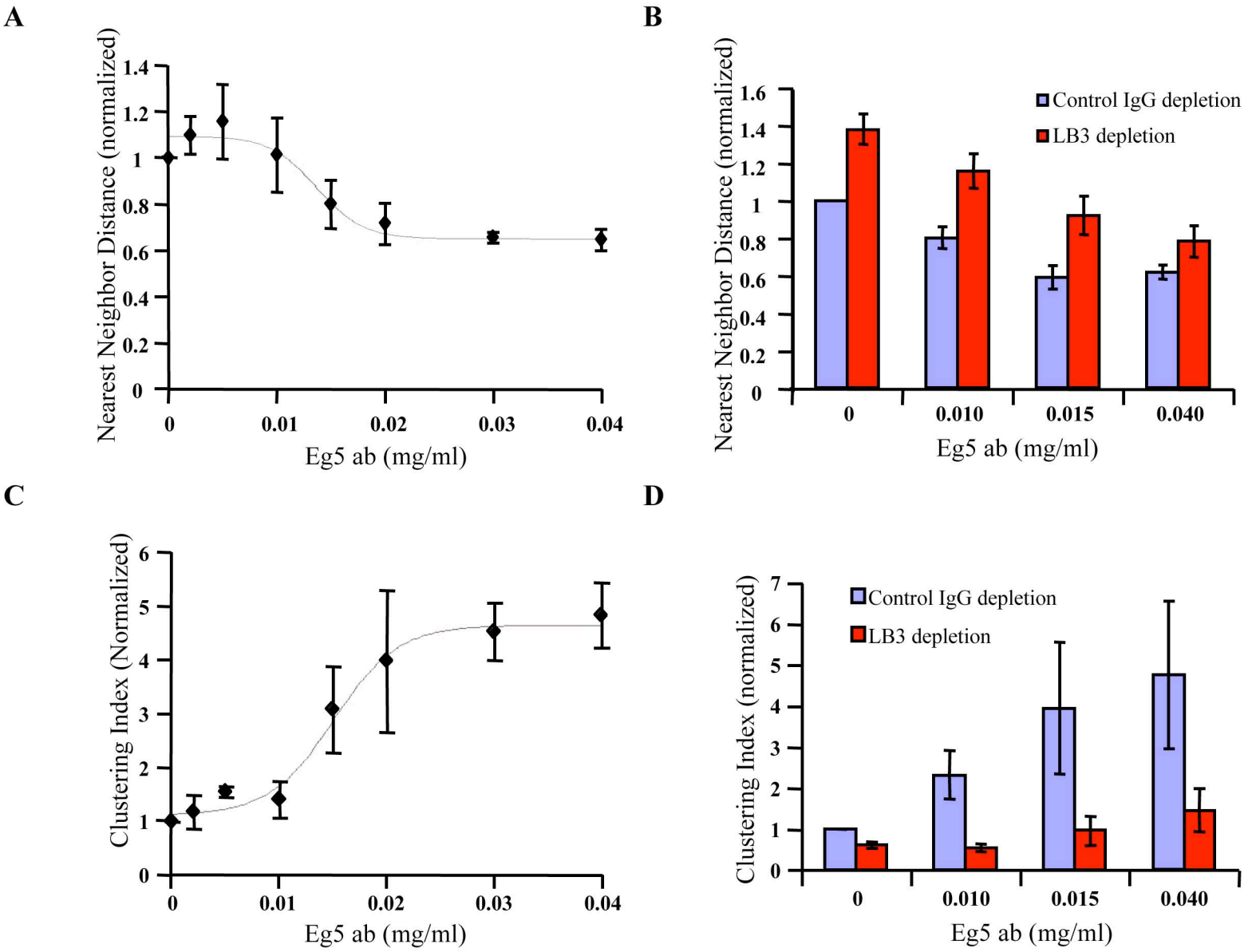
Supplementary Figure S1



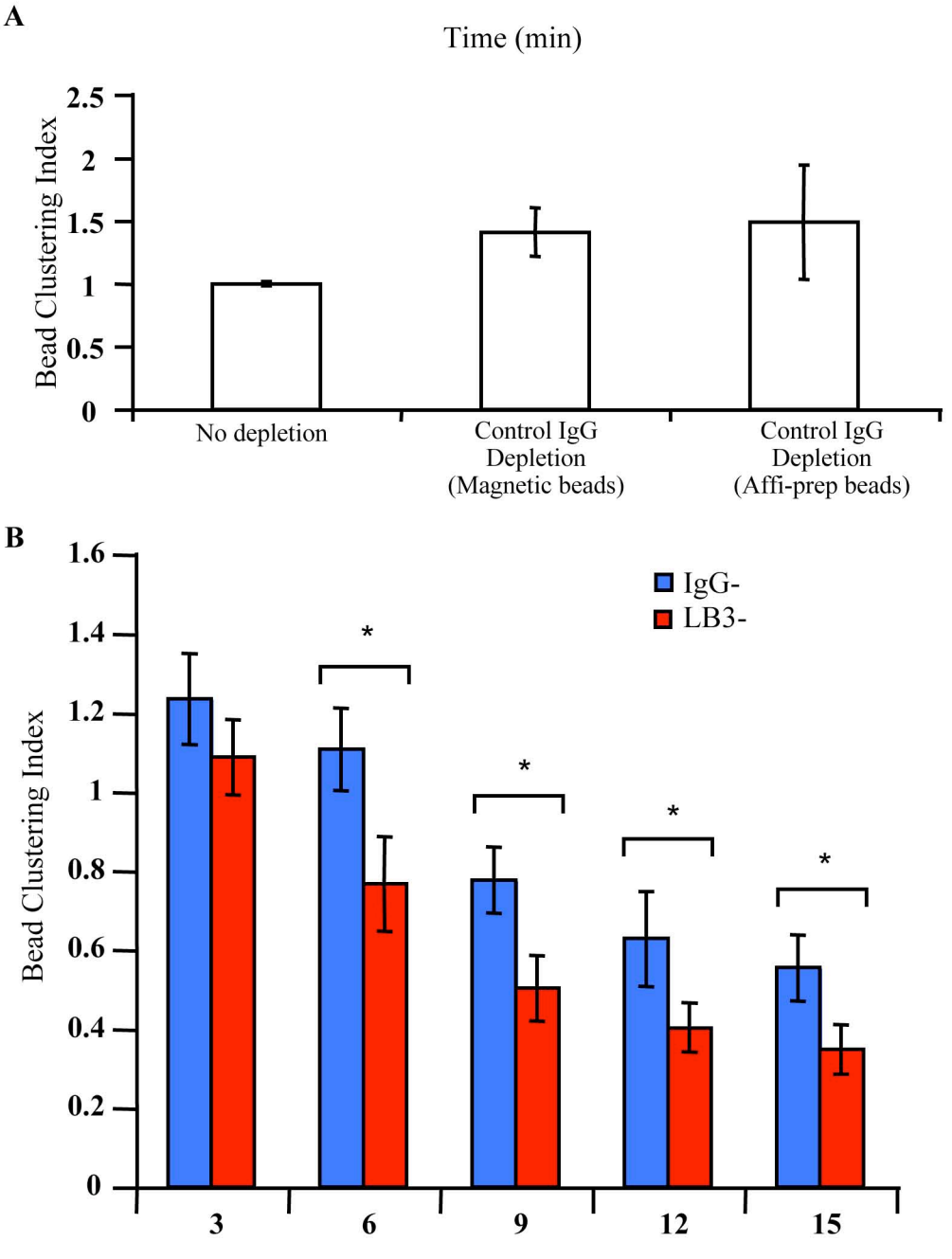
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Table S1

Assays used	Egg extract or pure proteins (μM)	Mock depletion (μM)	LB3 depletion (μM, this study)	References
Assay of initial separation of clustered AurA beads in *Xenopus* egg extracts (clustering index)	17	14	32	this study
Assay of nearest-neighbor distance between AurA beads in *Xenopus* egg extracts	21	11	16	this study
Assay of bipolar spindle assembly stimulated by sperm chromatin in *Xenopus* egg extract	20	NA	NA	Kapoor et al., 2000
Cytoblot screen used to discover Monastrol	22	NA	NA	Mayer et al.,1999
Eg5-driven microtubule motility assay	14	NA	NA	Mayer et al.,1999
ATP hydrolysis assay of the minimal motor domain of Eg5 in the absence of microtubules	6.3	NA	NA	Maliga et al., 2002
ATP hydrolysis assay of the minimal motor domain of Eg5 in the presence of microtubules	34	NA	NA	Maliga et al., 2002
FRET assay of ADP binding to the minimal motor domain of Eg5 in the absence of microtubules	5.2	NA	NA	Maliga et al., 2002