Supplemental Information

Supplemental Figure Legends

Figure S1

Detecting slipping and buckling of MTs, related to Figure 1

To test our visual method to detect slipping versus captured MTs, and straight versus buckled MTs, we tracked a subset of MTs, quantified their behavior, and compared this to our visual analysis. (A) Cartoon that shows how we define *s*, the MT end displacement parallel to the barrier. We manually track the position of the end over time and record the component s of the displacement parallel to the barrier. (B) Cartoon that shows how the buckled/straight state of the MT is quantified. We manually track three points on the MT. The end of the MT, $\vec{x}_1$, the middle of the MT, $\vec{x}_2$, and the attachment of the MT at the centrosome $\vec{x}_3$. If the MT is buckled, $|\vec{x}_1 - \vec{x}_2| + |\vec{x}_2 - \vec{x}_3| > |\vec{x}_1 - \vec{x}_3|$ and the ratio *C* between these two quantities is larger than 1. (C) Sequence of images similar to Figure 1EF, showing a MT growing against a barrier coated with 10% dynein. Scale bar indicates 10 μm. (D) Example graph, corresponding to the MT shown in (C), in which the distance *s* of the MT end along the barrier is plotted against time. After reaching the barrier, this MT slips along the barrier until it is captured and stops moving. (E) Graph of the same MT as in (D) showing the ratio *C* over time. The MT remains straight. (F) Example graph, corresponding to the MT shown in Figure 1E, in which *s* is plotted against time. This MT samples the barrier until it is captured around 150-160 sec. (G) Graph of the same MT as in

(F) showing the ratio *C* over time. The MT is initially (slightly) buckled, until it is captured and straightens around 150-160 sec.

From these graphs we can determine whether MTs are slipping or captured, and buckled or straight. We consider MTs to be captured if their ends remain more than 5 frames (corresponding to 15 sec) at the same position (spatial resolution is limited by the pixel size). We consider MTs to be straight if $C<1.005$ (note however that strongly buckled MTs can have values of *C* around 1.2-1.3). We compared the tracking method with a visual analysis for 10 arbitrarily picked MTs at 100% dynein, without a multilayer of biotinylated-BSA and streptavidin (see Figure 1):

All times in (sec)	Total time buckled	Total time straight	Total time straight, captured	Total time slipping
Tracking method	1500	2454	1676	2159
Visual method	1688	2270	1688	2230

From this comparison we conclude that we do not find significant differences (given the error bars) between the two methods.

Figure S2

Effects of dynein on MT dynamics, related to Figure 2

(A,B) Barrier-attached dynein does not depolymerize GMPCPP-stabilized MTs. In (A), a time sequence of a GMPCPP-stabilized MT is shown that glides on a dynein-coated surface against a dynein-coated barrier (as in Figure 2). When the MT hits the barrier it buckles and eventually glides along the barrier. In (B), a kymograph of another GMPCPP-stabilized MT that glides against a barrier is shown (the edge of the barrier is indicated with a yellow line, to reveal the movement of the MT end). The first ~120 sec the MT glides towards the

barrier. As soon as it hits the barrier it stops gliding but does not depolymerize. After ~250 sec, the GMPCPP-stabilized MT end repositions on the barrier, and again stops moving. All GMPCPP-stabilized MTs we observed stopped moving against the barrier or started gliding along it. No depolymerization was observed. An upper estimate for the shrinkage velocity is given by ~0.2 nm/sec, which is much slower than the depolymerization velocity of ~65 nm/sec reported for the depolymerizing kinesin protein MCAK (Helenius et al., 2006). (C,D,E) Dynein and the slow moving dynein AAA3E/Q-GFP do not alter MT dynamics in solution. In (C), a time sequence of a shrinking MT, nucleated from an axoneme, decorated with the slow moving dynein AAA3E/Q-GFP is shown. Images were taken using spinning disk confocal microscopy. (D) Kymograph of a shrinking and growing MT in the presence of dynein AAA3E/Q-GFP. The end of the MT is indicated with a red dotted line. No rescues or slow shrinkage are observed (E) Parameters of MT dynamics, in the absence and presence of dynein in solution. (F) Kymographs of dynamic MTs nucleated from GMPCPP-stabilized MT seeds, gliding on surfaces coated specifically with dynein. The three examples show different shrinkage events for MTs gliding on surfaces incubated with 46 nM dynein. (G) Kymograph of a free dynamic MT nucleated from a surface-attached seed in the absence of dynein. (H) Time-weighted histograms of shrinkage velocities for 5 different gliding experiments after incubation with different concentrations of dynein (1 sample each), compared to shrinkage velocities of free MTs (2 samples) and MTs gliding against a dynein-coated barrier (see Figure 2). The dynein concentrations and average shrinkage velocities for these samples are indicated in the legend; all errors SD. The average MT gliding velocities in these samples were 11.2 ± 2.0 μm/min (23 nM

dynein), 11.4 ± 1.9 μm/min (46 nM dynein), 11.6 ± 1.3 μm/min (96 nM dynein), 7.1 ± 3.0 μm/min (128 nM dynein), and 7.4 ± 1.6 μm/min (160 nM dynein); all errors SD.

Figure S3

Dynamics of multiple MTs growing against barrier, related to Figure 3

Growth and shrinkage events of MTs, grown at a higher tubulin concentration (25 μM) and temperature (29 ^{o}C) than in Figure 3 (20 μM, 25 ^{o}C). Under these conditions, multiple MTs grow simultaneously. As MT bundles are more stable than individual MTs, higher trap stiffnesses than in Figure 3 were used for these experiments (0.08-0.17 pN/nm). The black curve shows growth in the presence of dynein (and ATP); the red and blue curves show examples of bundle growth in the absence of dynein that we previously studied under similar conditions (Laan et al., 2008). The arrows indicate collective catastrophes, when all MTs in the bundle have switched to a fast shrinking state. When multiple MTs are growing against the barrier, individual catastrophes are hard to observe since shrinkage of an individual MT is masked by the growth of the other MTs in the bundle. Fast shrinkage can only be observed following a collective catastrophe (Laan et al., 2008).

These data provide additional evidence that barrier-attached dynein increases the catastrophe frequency. Note that the catastrophe frequency depends on the applied force (Janson et al., 2003), which is constantly increasing as the growing MTs push the bead away from the trap center. Nevertheless, we can compare the average time that MTs spent growing against the barrier before a catastrophe occurred and use this as an indication of differences in the catastrophe frequency. Under conditions where multiple MTs were simultaneously nucleated from the axoneme, we found long collective catastrophe times

against uncoated barriers (T_{cat} = 260 ± 60 sec, n=19, 8 exp). However, MTs growing against dynein-coated barriers, retained a much shorter catastrophe time (T_{cat} = 56 ± 6 sec, n=89, 3 exp) (p-value $6.0*10^{-41}$), indicating that frequent catastrophes prevented MT bundles from forming.

Note that for MTs growing against dynein-coated barriers at low tubulin concentration and temperature (Figure 3) the average catastrophe time was T_{cat} = 65 ± 17 sec (n=19 catastrophes, 6 exp). This was only slightly shorter than the time MTs spent growing against uncoated barriers, T_{cat} = 100 ± 20 sec (n=25, 4 exp) (p-value 0.047). However, it is possible that this relatively modest difference was due to the fact that under these conditions the time a MT needs to 'find' dynein molecules on the barrier is similar to the catastrophe time against an uncoated barrier.

Figure S4

Slipping-induced anisotropy of MTs in microfabricated chambers, related to Figure 4

Schematic picture of the analysis performed to measure anisotropy (A), and bar plot (B) of the results. The areas indicated in dark grey form the middle regions of the microfabricated chamber. The areas indicated in light grey form the corner regions of the microfabricated chamber. By comparing the fluorescence intensity in the corner regions with the middle regions, the MT anisotropy in the experiment was measured. Bar plot shows the intensity of the (corners/middles - 1) for different cases. The error is the SE. For all these cases a spinning disk fluorescence confocal microscopy image is shown. The cases are: (C) a MT aster in a microfabricated chamber without dynein, (D) a MT aster in a microfabricated chamber with high dynein amounts, (E) a MT aster on a surface, which is not confined in a

microfabricated chamber, and (F) an empty microfabricated chamber. Our theory predicts an anisotropic MT distribution with MTs mainly oriented towards the corners (Figure 6E). When pulling force generators are present, the orientation anisotropy is predicted to be less pronounced, since capturing of MTs by pulling force generators reduces the slipping distance (Figure 6D). This analysis indeed reveals that both with and without dynein more MTs are found in the corner regions of the microfabricated chambers than in the non-corner regions. As predicted, the effect is strongest if dynein is absent.

Figure S5

Example traces of aster position, related to Figure 5

150 s trajectories of singles centrosome for different conditions and regimes (similar to asters shown in Figure 4DEF). Statistics are shown in Figure 5. Squares represent the chamber boundaries. Note that here the chamber sizes are normalized. Not all chambers have the exact same size.

Figure S6

Theory of aster positioning, related to Figure 6

(A) Geometry for a MT aster with its organizing center at $\mathbf{r} = (x, y)$ in a square chamber. The MT orientation is described by the angle ϕ, the MT length is denoted by L. The angle between the MT orientation and the normal to the boundary is β. Numerical integration of Eq. (S1) is performed in separate intervals of ϕ which are indicated. Eight segments are distinguished, two of them are indicated (orange, yellow). Boundary conditions are imposed at $\phi = \bar{\phi}_k$ and integration is in the direction of the blue arrows. The corners at

$\phi = \phi_i$ are treated separately. The diameter of the geometry is 2*d*. (B) The effect of the binding rate (k_b) on the total pulling force ($\mathbf{F}^-$) on the centrosome. The centering stiffness, i.e. the linear response of the net pulling force to a small displacement from the center, $K^- = -dF_x^-/dx$ is shown as a function of the binding rate. At a very low binding rate ($2 \cdot 10^{-4}$ s^{-1}), MTs slip (left cartoon), but are rarely captured by dynein molecules before they undergo catastrophe. The pulling force is thus low. At an intermediate binding rate ($4 \cdot 10^{-3}$ s^{-1}), MTs slip (middle cartoon), but eventually get captured by dynein molecules such that pulling forces can be generated. At a high binding rate ($6 \cdot 10^{-2}$ s^{-1}) slipping is almost completely inhibited, which leads to more isotropic MT distribution (right cartoon). All MTs are being pulled upon, but the pulling forces balance each other resulting in a low total pulling force. (C) The effect of the boundary release rate of pushing MTs (k_{cat}) and the detachment rate of pulling MTs (k_{off}) on the total force, $\mathbf{F} = \mathbf{F}^+ + \mathbf{F}^-$, on the centrosome. The centering stiffness, i.e. the linear response of the net pulling force to a small displacement from the center, $K = -dF_x/dx$ is shown as a function of the rates k_{cat} and k_{off} with a black line and a magenta line, respectively. The data show that the magnitude of the centering force is similar for a broad range of values for k_{cat} and k_{off} (D) The effect of catastrophes of freely growing MTs on aster positioning. The centering stiffness is shown as a function of the catastrophe frequency of freely growing MTs, for the model with the length-dependent nucleation rate given by Eq. (S7). The centering and off-centering regions are denoted white and grey, respectively. The insets show the MT length distributions at a low, an intermediate, and a high catastrophe frequency (k_{cc} = 10^{-3} s^{-1}, 10^{-2} s^{-1} and 10^{-1} s^{-1}, respectively). Parameters are v_g = 2 μm/min and $k_{cat} = k_{cc}$. All remaining parameters are as in Figure 6D.

Supplemental Movie Legends

Movie S1, related to Figure 1C. MTs grow from a surface-attached centrosome against a gold barrier without dynein, 150x real time.

Movie S2, related to Figure 1D. MTs grow from a surface-attached centrosome against a gold barrier coated with dynein, 150x real time.

Movie S3, related to Figure 1E. MTs grow from a surface-attached centrosome against a gold barrier coated with 100% (no multilayer) dynein, 150x real time.

Movie S4, related to Figure 2B. Dynamic MT glides on a dynein-coated surface against a gold barrier coated with dynein, 150x real time.

Movie S5, related to Figure 4F. Aster moving in chamber with dynein at the barriers, 150x real time. Corresponding to Regime ii in Figure 4F.

Movie S6, related to Figure 4F. Aster centered in chamber with dynein at the barriers, 150x real time. Corresponding to Regime iii in Figure 4F.

Supplemental Tables

	#Exp	# Shrinkage events		V_{shrink} (F>0) [μm/min]	F_{pull} at release [pN]	V_{shrink} (F<0) [μm/min]
		MT-barrier connection lost at F<0	MT-barrier connection persists at F<0			
No Dynein	4	21	0	18 ± 2	--	--
Dynein plus ATP	6	5	10	5.1 ± 0.4	2.0 ± 1.4	1.1 ± 0.33
Dynein no ATP	3	7	0	0.15 ± 0.04	--	--

Table S1

Dynamics of shrinking MTs in the optical trap experiment, related to Figure 3

We analyzed the shrinkage events in which MT shrinkage allowed the bead to move all the way back to the zero-force position (excluding events where rescues occurred before). We counted the number of times the MT-barrier connection was lost after the zero-force position was reached and how many times the connection persisted, and thus a pulling force was generated. Only when dynein is present at the barrier and ATP is added, pulling forces are generated, with an average maximum force of 2 pN. In 3 of the 5 cases when no pulling forces were generated in the presence of dynein and ATP, the MT was shrinking with a velocity higher than 10 μm/min indicating that the MT did not interact with dynein. In the other 2 cases, it is possible that dynein detached before an observable force was reached. As observed in the gliding experiments, barrier-attached dynein slows down MT shrinkage (especially when a pulling force is generated). In the absence of ATP, this effect is dramatically enhanced, and no pulling forces are detected. The error on the shrinkage velocity is the standard error. The error on the force is the standard deviation.

	Regime i, MT Length<*d*		**Regime ii, MT Length ~*d***		**Regime iii, MT Length > *d***	
	<*x*/*d*> ± SD	**<*y*/*d*> ± SD**	**<*x*/*d*> ± SD**	**<y/*d*> ± SD**	**<*x*/*d*> ± SD**	**<*y*/*d*> ± SD**
No dynein	-0.10 ± 0.15	-0.32 ± 0.34	-0.10 ± 0.15	0.10 ± 0.20	-0.01 ± 0.23	-0.06 ± 0.23
Intermediate dynein	0.09 ± 0.29	0.30 ± 0.25	0.04 ± 0.18	-0.08 ± 0.12	-0.10 ± 0.17	0.03 ± 0.14
High dynein	0.19 ± 0.14	-0.47 ± 0.20	0.09 ± 0.33	-0.12 ± 0.32	-0.01 ± 0.10	0.05 ± 0.10

Table S2

Average positions of MT asters in microfabricated chambers, related to Figure 5

The average positions, normalized with the chamber half-width *d,* are calculated from all aster positions for every condition. They are tracked as described in the main text and as is shown in Figure 5. The standard deviation reveals the spread of the centrosome around the average position. As the chambers are symmetric, the average position should converge to the center position for a large number of data points.

Extended Experimental and Theoretical Procedures

Materials

Chemical reagents were obtained from Sigma, unless stated otherwise. MRB80 buffer consisted of 80 mM K-Pipes, 4 mM $MgCl_2$, 1 mM EGTA, pH = 6.8. Tubulin was purchased from Cytoskeleton Inc, Denver. GST-dynein331 and AAA3E/Q-GFP were purified according to (Cho et al., 2008; Reck-Peterson et al., 2006). The GST-dynein-331 was biotinylated via a halotag (Promega). It was tested in a gliding assay using a simple flow cell. The flow cell was sequentially incubated with 1.5 mg/ml biotinylated-BSA, 1.5 mg/ml streptavidin, 8 nM biotinylated dynein with 0.5 mg/ml BSA. AAA3E/Q-GFP was not biotinylated, and it was non-specifically attached to the glass surface. The flow cell was first incubated with 0.1 mg/ml κ-casein, and subsequently AAA3E/Q-GFP was flown in at 30 nM. After dynein incubation, the flow cell was flushed with MRB80, and taxol-stabilized MTs were added with 0.1 mg/ml κ-casein. The gliding speeds were 130 +/- 35 (SD; n=29) nm/sec for dynein, and 3.7 +/- 2.7 (SD; n=16) nm/sec for dynein AAA3E/Q-GFP, very similar to numbers reported before (Cho et al., 2008; Reck-Peterson et al., 2006). For dynein we also tested whether it binds in a rigor state to MTs in the absence of ATP. To make sure that no residual ATP coming from the dynein purification was present, the sample was washed with several flow cell volumes of buffer after it was incubated with dynein. After these washes, MTs did bind to the surface of the chamber, but no MT gliding was observed. To test that MTs were gliding on dynein and not on other motor proteins that can be present in the (commercial) tubulin stock (Cytoskeleton Inc.), we performed MT gliding experiments on dynein with brightly labeled seeds that grew dimly labeled MTs and

observed that the long dim end (the plus end) was always leading, indicating minus end directed motor activity. We furthermore tested that no MT gliding was observed when dynamic polarity marked MTs where added to a surface coated as in Figure 2 (i.e. with κ-casein and streptavidin), but without the dynein incubation step. In this test MTs remained floating in solution. In addition, we routinely grow MTs from centrosomes in our lab in the presence of the same tubulin stock using similar surface coatings. We never observe MTs being pulled from the centrosome such as we observe when the surface is incubated with dynein (Figure 2). The fact that we do not observe any non-dynein related motion in all these experiments is however not because motors are not present in the tubulin stock, but due to sufficient pre-coating of the surfaces. When we intentionally incubate the surface with high tubulin concentrations (50 μM) before coating the surface and adding dynamic MTs, we do observe fast (up to 50 μm/min), but erratic plus-end directed motion of polarity marked MTs, which is presumably due to the presence of residual kinesin in the tubulin stock.

Axonemes (a generous gift from Matt Footer) were purified from sea urchins (Tselutin et al., 1999). Centrosomes were purified with the generous help of Claude Celati from human lymphoblastic KE37 cell lines (Moudjou, 1994).

Microfabrication and functionalization of gold barriers and chambers

Gold barriers (Figures 1 and 2) as well as chambers (Figure 4) were constructed and biotinylated as previously described (Laan and Dogterom, 2010; Romet-Lemonne et al., 2005). Afterwards the samples were checked in an FEI XL30 SFEG scanning electron microscope (Figure 4BC). The gold barriers (Figures 1 and 2) were 0.8-1.0 μm high. The

10 or 15 μm-wide square chambers (Figure 4) were ~2.6 μm deep, with either 100 or 700 nm thick gold layers sandwiched in between glass and silicon monoxide layers. The gold surfaces were specifically labeled with biotin via thiol chemistry and stored in ethanol (Laan and Dogterom, 2010; Romet-Lemonne et al., 2005). Microfabricated chambers were closed with a poly-dimethylsiloxane (PDMS) lid to achieve good sealing. The PDMS layer was fabricated on a 24x60 mm coverslip as previously described (Laan and Dogterom, 2010; Romet-Lemonne et al., 2005) to create a ~80 μm flat layer of PDMS on the coverslip. The PDMS layer together with the coverslip was thin enough to allow for microscopic observation with a high magnification oil immersion objective through the microfabricated chamber, looking from either side. The PDMS cover slips were stored before usage in a closed box for maximally 1 week.

Dynein molecules were specifically attached to biotinylated gold surfaces via biotin-streptavidin linkages and thorough blocking of the other sample surfaces. Glass surfaces were blocked with 0.2 mg/ml PLL-PEG (SurfaceSolutions, Switserland) for 5 minutes, rinsed with MRB80, blocked with 1 mg/ml κ-casein in MRB80 for 5 minutes, and rinsed. Subsequently a multilayer (to enhance the number of binding sites) of biotinylated-BSA and streptavidin was formed on the gold surfaces. A streptavidin mix (0.5 mg/ml streptavidin, 1 mg/ml κ-casein, 5 mg/ml BSA in MRB80) and a biotinylated-BSA-mix (1.5 mg/ml biotinylated-BSA, 1 mg/ml k-casein, 5 mg/ml BSA in MRB80) were sequentially introduced and incubated for 5 minutes to create a multilayer with 3 layers of streptavidin and 2 of biotinylated-BSA. Afterwards a solution with biotinylated dynein was introduced (20 nM biotinylated dynein, 1 mg/ml κ-casein, 5 mg/ml BSA in MRB80) for 5 minutes (in the case of control experiments without biotinylated dynein, this step was left out). In

experiments where the dynein density at the barrier was reduced (Figure 1), the streptavidin mix (0.5 mg/ml streptavidin, 1 mg/ml κ-casein, 5 mg/ml BSA in MRB80) was flown in only once after biotinylation of the gold, followed by a mixture of biotinylated dynein and biotinlylated BSA (20 nM total biotinylated protein in the ratios: 1% dynein, 10% dynein and 100% dynein, 1 mg/ml κ-casein, 5 mg/ml BSA in MRB80).

MTs growing from surface-attached centrosomes against dynein-coated barriers (Figure 1)

Microscope slides were cleaned in chromosulphuric acid. A 20 μl-flow cell was constructed by drawing two parallel lines of vacuum grease approximately 5 mm apart on a clean microscope slide, and mounting a coverslip containing biotinylated barriers on top. A solution of centrosomes in MRB80 was flown in and incubated for 5 minutes in order to let centrosomes adhere non-specifically to the glass surfaces. Centrosomes that did not stick were washed out by rinsing with two flow cell volumes of MRB80. Subsequently the glass surfaces were blocked and the barriers were functionalized with various densities of biotinylated dynein as described above. The flow cell was rinsed with MRB80 and the final tubulin mix was introduced (15 μM tubulin, 1 μM Rhodamine tubulin, 1 mM GTP, 1 mM ATP, 0.8 mg/ml κ-casein, 0.1% methyl cellulose (4000cP), and an oxygen scavenger system (20 mM glucose, 200 μg/ml glucose oxidase, 400 μg/ml catalase, 4 mM DTT) in MRB80). The flow cell was sealed and examined at 25^{o}C using spinning disk confocal microscopy. Movies were made with 561 nm laser light with a time lag of 3 seconds and 300 ms exposure, on a Leica microscope with a 100x 1.3 NA oil immersion objective equipped with a spinning disk confocal head from Yokogawa and a cooled EM-CCD

camera (C9100, Hamamatsu Photonics). We performed between 3 and 6 experiments for each condition (see table in Figure 1G). The data of the experiments without dynein were also used to obtain the dynamic parameters of MTs growing freely from centrosomes (no dynein, no barrier, see Table 1).

Dynamic MTs gliding on a dynein-coated surface against dynein-coated barriers (Figures 2 and S2AB)

This experiment was very similar to the one above, except that surface blocking was reduced by not blocking with PLL-PEG and by reducing the κ-casein concentration in the different steps to 0.1 mg/ml. Reduced blocking allowed non-specific binding of dynein to the glass surfaces. The presence of dynein was tested by adding stabilized MTs that were indeed observed to glide on the sample surface. Dynamic MTs were again nucleated by surface-attached centrosomes. At a certain MT length, the dynein on the surface pulled the MTs from the centrosome, which created dynamic MTs that were freely gliding with their plus-end leading. To create speckles on the MTs, the Rhodamine tubulin concentration was reduced to 0.5 μM. The dynamics of MTs that were gliding against barriers as well as MTs that were gliding freely were analysed from 7 different experiments (see below).

In the experiments with stabilized GMPCPP MTs (Figure S2AB), no tubulin was added, so the MTs did not grow. Otherwise the assay was the same. When these MTs ran into barriers, no MT shrinkage could be detected within the observation time (by analysing the movement of speckled MTs). Therefore an upper estimate of the shrinkage velocity could be calculated by dividing the pixel size (110 nm) by the observation time (600 sec). This resulted in an upper estimate of the shrinkage velocity of 0.2 nm/sec.

Gliding assays with dynamic MTs nucleated by GMPCPP seeds (Figure S2FGH)

To verify if MT shrinkage was affected by gliding on increased dynein densities, we performed a set of conventional MT gliding experiments without barriers, in addition to the gliding experiments with barriers described above. Here we attached dynein specifically to the sample surfaces, and used stabilized GMPCPP-seeds as nucleation sites. Before incubation with dynein, sample surfaces were sequentially incubated with the following solutions to promote dynein binding: 0.2 mg/ml PLL-PEG-biotin, 1 mg/ml streptavidin, 0.5 mg/ml κ-casein, and 1% (w/v) Pluronic F-127 (all diluted in MRB80). Incubation with dynein was done at 8 nM, 16 nM, 34 nM, 45 nM, and 56 nM (all 1 sample). MTs were grown from gliding seeds using the following mix: 15 μM tubulin, 1 μM Rhodamine tubulin, 1mM GTP, 1mM ATP, 0.8mg/ml κ-casein, 0.1% (w/v) methyl-cellulose, and an oxygen scavenger system in MRB80. Note that for these experiments a different batch of tubulin was used than in all other experiments, which leads to slightly different numbers for the parameters of MT dynamics.

Dynamic MTs in the presence of dynein in solution (Figure S2CDE)

Cover slips and microscope slides were cleaned in chromosulphuric acid. A 20 μl-flow cell was constructed by drawing two parallel lines of vacuum grease approximately 5 mm apart on a clean microscope slide, and mounting a clean cover slip on top. A solution of axonemes in MRB80 was flown in and incubated for 5 minutes in order to let axonemes adhere to the glass surfaces. The concentration of the axonemes was tuned such that after preparation of the sample on average 5 axonemes could be seen in a field of view of the

microscope (35x25 μm^2). Axonemes that did not stick to the surface were washed out by washing with two flow cell volumes of MRB80. The flow cell was subsequently blocked with 0.2 mg/ml PLL-PEG in MRB80 for 5 minutes, rinsed with 10 flow cell volumes, blocked with 1 mg/ml κ-casein in MRB80 for 5 minutes and rinsed. Afterwards the tubulin mix (15 μM tubulin, 0.5 mg/ml κ-casein, 1 mM GTP, 1 mM ATP, and an oxygen scavenger system in MRB80), optionally with 8 nM dynein or 8 nM dynein AAA3 E/Q-GFP, was introduced and the sample was sealed. Samples were observed on an inverted microscope (DMIRB, Leica Microsystems, Rijswijk, The Netherlands) with a 100x 1.3 NA oil immersion objective by Video-Enhanced Differential Interference Contrast (VE-DIC) microscopy. The temperature in the sample was adjusted by a sleeve around the objective lens, which was controlled by thermoelectric coolers (Melcor). These experiments were performed at 20^{o}C. Images were recorded by a CCD camera (CF8/1, Kappa) and sent to an image processor (Argus 20, Hamamatsu). The resulting image stream was both recorded on a DVD and digitized online at a rate of 1 frame every 2 s (not shown). In the case of dynein AAA3 E/Q-GFP, the samples were also examined using spinning disk confocal microscopy (Figure S2CDE). Movies were made with a time lag of 3 seconds and 300 ms exposure, using 488 nm laser light. For the analysis of MT dynamics, DIC data were collected from three independent experiments for every condition. The plus-ends of the axonemes were identified by the presence of longer and faster growing MTs (Walker et al., 1988). Only plus-ends were analyzed.

Analysis of MT dynamics

To determine the percentage of buckling/straight MTs in contact with the barrier (Figure 1), we visually counted the number of buckling versus straight MTs in the movies. To determine the time MTs spend 'searching' along the barrier, we recorded the total time MT ends were (visually) observed to slip along the barrier and divided this by the total number of observed capture events. We found average search times of 220 sec for 100% dynein (10 events), 670 sec for 10% dynein (18 events), 890 sec for 1% dynein (22 events), and 2705 sec in the absence of dynein (4 events), indicating an increase in search time with decreasing dynein density (although the values for 1% and 10% are not significantly different; p-value 0.14, see definition below). For a subset of MTs, we compared this visual analysis to a more quantitative analysis as described in Figure S1. Here we quantified individual MT behavior over time to determine if they were buckled or straight, and to determine whether their ends were sliding or stationary along the barrier. We did not observe any significant differences in the results.

Catastrophe, barrier release, and rescue frequencies (Figures 1, 2, and S2) were determined by dividing the total number of observed catastrophe, release, or rescue events by the total time that the MTs were (visually) observed to grow (either free or gliding/buckling against a barrier), remain straight in stationary contact with the barrier, or shrink (only for free MTs). The error is the statistical error given by the frequency itself divided by the square root of the number of observed events. For $n<6$, no error bar is given as the number of events is too low. In this case, only an upper limit for the frequency is given corresponding to a 95% confidence interval. For large n, a 95% confidence interval corresponds to an error bar of roughly twice the statistical error. The p-value used to

determine whether two rates f_1 (from n_1 events in total time T_1) and f_2 (from n_2 events during total time T_2) are statistically different was calculated as follows: given that $f_1 < f_2$, the p-value of the null hypothes that the two rates are from the same Poisson process, is defined as the probability of observing n_2 or more events during time T_2, given frequency f_1: $P(n_2 + | f_1 T_2) = \sum_{i=n_2}^{\infty} \frac{f_1 T_2{}^i e^{-f_1 T_2}}{i!}$.

Growth and shrinkage velocities (Figures 2 and S2) were measured from manual fits to the growth/shrinkage parts of kymographs. The average velocity is the average over all events weighted with the time of the individual events. The error is the weighted standard deviation. For gliding MTs, the gliding velocity was determined by a manual fit to the movement of speckles on kymographs (see Figure 2). In these cases, the growth and shrinkage velocities were determined by subtracting the gliding velocity from the leading MT tip velocity. We determined the statistical significance of observed differences in growth or shrinkage velocities by using a standard student t-test. As datasets we used shrinkage or growth velocities weighted with the time of the individual events.

Optical trap experiments (Figures 3 and S3)

A clean cover slip with 7 µm-high SU-8 (photoresist) barriers, fabricated as previously described (Kerssemakers et al., 2006; Laan and Dogterom, 2010) was built into a flow system, which consisted of a channel cut in parafilm squeezed between a microscope slide and a cover slip. Here we did not use gold barriers, as gold barriers absorb the laser light that is used for trapping, thereby heating up the sample. In order to block the surface of the chambers, first a 0.2% agarose solution at 70^{o}C was flown in. The agarose was blow-dried by connecting a pump to the channel for a few minutes. Afterwards, a 0.1% Triton X100

solution was flown through to prevent bubble formation in the flow cell. A second blocking step was done by incubating the flow system for 10 min with a 0.1 mg/ml κ-casein solution in MRB80. Afterwards, in the case of an experiment with dynein, the flow cell was incubated with dynein (15 nM) for 20 minutes. The flow cell was rinsed with 100 μl of MRB80, to remove freely floating dynein and left-over ATP. A gliding assay showed that under these conditions dynein was indeed present on the surface (in this experiment, dynein attached non-specifically to all surfaces including the barriers).

The experiment started by flowing in axonemes and beads. First a bead was trapped in the point trap of our key-hole optical trap (Kerssemakers et al., 2006). Then an axoneme was caught in the line trap and non-specifically stuck to the bead. This construct was subsequently positioned in front of a barrier. Because the axoneme-bead construct is not infinitely stiff, the bead will be moved from the trap centre over a distance that is generally smaller than the motion of the axoneme tip away from the barrier. The conversion factor to relate bead displacement to displacement of the axoneme tip was measured by repeatedly pushing the barrier against the construct and plotting the subsequent bead displacement as a function of barrier displacement. After a first soft regime of approximately 50 nm, the conversion factor is constant over several hundreds of nm (Kerssemakers et al., 2006). Subsequently, the tip of the axoneme was positioned approximately 100 nm away from the barrier. Next, the chamber was rinsed to remove left over beads and axonemes and afterwards the tubulin mix (20 μM tubulin, 0.1 mg/ml κ-casein, 1 mM GTP, and optionally 1 mM ATP in MRB80) was added to trigger MT growth. The middle and bottom trace in Figure 3B are two experiments on the same axoneme-bead construct against the same dynein-coated barrier. Data were taken first in the absence (bottom trace) and then in the

presence (middle trace) of ATP. The temperature of the experiment was kept at 25^{o}C. In Figure S3, the tubulin concentration and temperature were raised to 25 μM and 29 ^{o}C. Under these conditions, multiple MTs were growing simultaneously (Laan et al., 2008). In some experiments with dynein present, the axoneme-bead construct was placed at the bottom surface of the chamber at the end of the experiment to let it glide on the dynein on the bottom surface. The gliding of the construct on the surface allowed us to confirm that we were looking at plus-end dynamics.

Axonemes and beads were imaged using VE-DIC. During the experiments, the position of the bead was tracked at 1 Hz online using a video-tracking routine. Afterwards, the DVD-recorded data were used to track the bead at 25 Hz. Before or after every experiment, the stiffness of the point trap was determined by analysis of the power-spectrum of the thermal fluctuations of a bead.

Data analysis of optical trap experiments

MT length was plotted against time by multiplying the bead displacement with the conversion factor. Shrinking events were identified by eye (Table S1; 3-6 independent experiments). Linear fits were made to a moving window of 4 frames (120 ms). The average shrinkage velocity and its standard error were determined by averaging over all fits over all events from all experiments for a given condition.. In the case of experiments with dynein on the barrier with ATP, shrinkage events above the zero position (center of the trap) and below zero were taken separately, because shrinkage events below zero corresponded to shrinking against a pulling force. For the experiments with dynein on the barrier without ATP, the whole event from the moment it started to shrink until the bead

reached the zero position was taken into account (including stretches of apparent MT length increase).

The catastrophe time was calculated as the average time MTs were observed to grow before a catastrophe occurred. For experiments with multiple MTs growing simultaneously (Figure S3), the catastrophe time reflects the time it takes for a MT bundle to undergo a collective catastrophe (Laan et al., 2008).

The force was plotted against time by multiplying the bead position with the trap stiffness. The average maximum force was calculated by averaging the maximum force of the shrinkage events that went below the zero bead position, with the error given by the standard deviation (Table S1).

Aster positioning experiments in microfabricated chambers (Figures 4 and S4)

The biotinylated microfabricated chambers and the PDMS lid were immersed in a mix of κ-casein (2 mg/ml) and BSA (5 mg/ml) in MRB80 for 15 minutes. Afterwards both surfaces were blow-dried using a N_2-flow. The PDMS coverslip and the microfabricated chamber coverslip were incorporated into a temporary flowcell, with Teflon-tape as a spacer and a metal block as a weight on top of the flowcell to keep the two coverslips tightly together. The flowcell was filled with a solution of κ-casein (2 mg/ml) and BSA (5 mg/ml) in MRB80 for 10 minutes, for extra blocking. Then biotinylated dynein was introduced as described above. The sample was rinsed with MRB80. Before the tubulin solution was introduced, the sample was placed on a metal block of 4^{o}C to prevent MT growth. The tubulin solution (centrosomes, 22 μM tubulin, 1.6 μM Rhodamine tubulin, 1 mM GTP, 1 mM ATP, an oxygen scavenger system, 0.5 mg/ml κ-casein, and 16% sucrose in MRB80)

was introduced in the flowcell, and left to mix by diffusion for 4 minutes. Afterwards the Teflon tape was carefully removed and the PDMS coverslip was firmly pressed on the microfabricated chamber coverslip for 2 minutes to create good sealing of the microfabricated chambers. The edges of the microfabricated chamber coverslip were sealed with hot candle wax. The sample was put at 28 ^{o}C for 2-10 min to initiate MT nucleation. Afterwards, the flowcell was examined at 25^{o}C using spinning disk confocal microscopy. The sample was imaged through the PDMS layer and therefore it could easily be checked whether the microfabricated chambers were well-sealed (Laan and Dogterom, 2010). Only asters located in well-sealed microfabricated chambers were considered for further analysis. Movies were made with 561 nm laser light with a time lag of 3 or 5 seconds and 300 ms exposure. Due to bleaching problems, individual asters could not be imaged longer than approximately 15 minutes. However, by sequentially imaging different asters, the positioning process could be monitored over ~3 hours. For every condition (no dynein, intermediate dynein, high dynein), centrosomes were imaged in 5 different samples.

Analysis of aster positions and movements in microfabricated chambers (Figures 5 and S5)

The position of the centrosome in the microfabricated chamber was tracked using the automatic ImageJ plugin, spot tracker, made by D. Sage, F.R. Neumann, F. Hediger, S.M. Gasser and M. Unser (Sage et al., 2005). In a home-written program in Matlab the edges of the chamber were manually tracked and compared to the centrosome position to calculate the normalized absolute x and y-position (|x/d| and |y/d|). If |x/d| and |y/d| < 0.2 we defined a centrosome to be centered. This means that the center position of the centrosome was

within the square center region of the chamber, which made up 4% of the total chamber area. The sides of this square center region were parallel to the chamber walls, and the center of mass of the square center region coincided with the center of mass of the chamber (see scheme in Figure 5A). A centrosome that moved could for example be centered for 38% of the time and not centered for 62% of the time. The percentage of time that the centrosome was centered was calculated by $\frac{\sum_{i=1}^{n} T_{i,+}}{\sum_{i=1}^{n} T_{i,+/-}} \times 100\%$, where *n* is the number of events, T_{+} is the time per event that the centrosome was centered, and $T_{i,+/-}$ is the total observation time per event. A centrosome was defined to move, if it moved more than 1 pixel (165 nm) per 150 s. Movement slower than this we attributed to noise, because by eye this movement appeared to be due to stage drift. Centrosome velocity was determined over 15 s time intervals (Figure 5).

Analysis of MT distributions in microfabricated chambers (Figure S4)

MT distributions were analyzed using a home-written program in Matlab. In this program only the immobile asters, of which the MTs did not buckle dramatically, were analyzed. In the analysis first the images of the microfabricated chamber were rotated such that the horizontal walls were parallel to the 0^{o} axis. The position of the corners of the microfabricated chambers was tracked manually. From the center of the centrosome a circle, with the maximum size that still fits in the microfabricated chamber, was defined (Figure S4A). Within this circle, a smaller circle, with the same geometrical center, but with a radius that is decreased with five pixels, was defined. The region between the two circles was divided into 8 equally sized regions, all 45^{o}, starting at -22.5^{o}, resulting in

"corner" (indicated in light grey) and "middle" (indicated in dark grey) regions. For every area the total fluorescence intensity was calculated. The total intensity in the corner regions was compared to the intensity in the middle regions. If the MTs slip, it is expected that there are more MTs in the corners than in the middles, which should result in a ratio of corners/middles larger than one (Figure S4B). This is indeed the case, indicating that there is slipping in the experiment. Note that the difference between the corners and the middles is at most on the order of 5-8%. This is due to the low S/N for MTs relative to the background fluorescence (S/N~1.25).

Theoretical description of aster mechanics in chambers (Figures 6 and S6ABC)

We describe the angular distributions of pushing and pulling MTs by $n^+(\phi,t)$ and $n^-(\phi,t)$, respectively, as a function of the angle ϕ between the MT direction and the *x*-axis and time *t*. They obey the kinetic equations (B1) and (B2). In steady state ($\partial_t n^+ = \partial_t n^- = 0$) the distributions obey:

$$\frac{dn^+}{d\phi} = -\frac{1}{\upsilon_\phi}\left[n^+ \frac{d\upsilon_\phi}{d\phi} + (k_{\text{cat}} + k_{\text{b}})n^+ - \frac{\nu}{2\pi} \right], \qquad \text{(S1)}$$

$$n^- = \frac{k_{\text{b}}}{k_{\text{off}}} n^+ . \qquad \text{(S2)}$$

Here $\upsilon_\phi(\mathbf{r},\phi) = \frac{1}{2}\xi^{-1} f^+(\mathbf{r},\phi) L(\mathbf{r},\phi)^{-1} \sin 2\beta(\mathbf{r},\phi)$, where $\mathbf{r}$=(x,y) is the position of the organizing center, $L(\mathbf{r},\phi)$ is the distance between this center and the MT contact with the boundary, and $\beta(\mathbf{r},\phi)$ is the angle between the MT orientation and the normal to the confining square geometry. In the minimal model, the pushing force $f^+ = \pi^2\kappa / L(\mathbf{r},\phi)^2$ is given by the buckling force. The friction coefficient associated with MT slipping at the

boundary is ξ. For the angle $\phi = \bar{\phi}$, at which the MT is perpendicular to the confining geometry $(\beta = 0)$, $\upsilon_\phi(\bar{\phi}) = 0$ and the angular density of pushing MTs is:

$$n^+(\bar{\phi}) = \tfrac{\nu}{2\pi}[1/(k_{\text{cat}} + k_{\text{b}} + \partial_\phi \upsilon_\phi)]. \qquad \text{(S3)}$$

Furthermore, because of symmetry, $\frac{dn^+}{d\phi}(\bar{\phi}) = 0$. This value $n^+(\bar{\phi})$ together with $\frac{dn^+}{d\phi}(\bar{\phi}) = 0$ is used as initial condition when equation (S1) is integrated numerically.

The corners of the square separate four angular intervals. The corners correspond to the values $\phi = \phi_i(\mathbf{r})$; $\phi_1 = \arctan\frac{d-y}{d-x}$; $\phi_2 = \pi - \arctan\frac{d-y}{d+x}$; $\phi_3 = \pi + \arctan\frac{d+y}{d+x}$; $\phi_4 = 2\pi - \arctan\frac{d+y}{d-x}$. In addition, we introduce $\phi_0 = \phi_4 - 2\pi$. Since slipping MTs get trapped in the corners, we also consider the numbers $N_i^+(t)$ and $N_i^-(t)$ of pushing and pulling MTs at $\phi = \phi_i(\mathbf{r})$. The MT numbers in the corners satisfy:

$$\frac{dN_i^+}{dt} = J_i^- - J_i^+ - N_i^+ k_{\text{cat}} - N_i^+ k_{\text{b}} \qquad \text{(S4)}$$

$$\frac{dN_i^-}{dt} = N_i^+ k_{\text{b}} - N_i^- k_{\text{off}} \ . \qquad \text{(S5)}$$

Here $J_i^\pm = \lim_{\varepsilon \to 0} J_\phi(\phi_i \pm \varepsilon)$ with $J_\phi = n^+ \upsilon_\phi$ are MT currents entering/leaving the corners from both sides. The steady state solutions of $n^\pm(\phi)$ are obtained by integrating Eqs. (S1) and (S2) in the intervals $\phi_{i-1} < \phi < \phi_i$, starting from $\phi = \bar{\phi}_k$ ($\bar{\phi}_k = 0, \frac{\pi}{2}, \pi, \frac{3\pi}{2}$, see Figure S6A) towards the neighboring corners at $\phi = \phi_{i-1}$ and $\phi = \phi_i$, in negative and positive direction, respectively ($i = k$). In the intervals $\phi_{i-1} < \phi < \phi_i$ the functions L and β have values $L = \frac{d-x}{\cos\phi}$,

$\beta = \phi$ (i=1); $L = \frac{d-y}{\sin\phi}$, $\beta = \phi - \frac{\pi}{2}$ (i=2); $L = -\frac{d+x}{\cos\phi}$, $\beta = \phi - \pi$ (i=3); and $L = -\frac{d+y}{\sin\phi}$ $\beta = \phi - \frac{3}{2}\pi$ (i=4). For $\phi = \bar{\phi}_k$, $\upsilon_\phi(\bar{\phi}_k) = 0$. At these points $n^+(\bar{\phi})$ is given by Eq. (S3) and $dn^+/d\phi = 0$ because of local symmetry $\upsilon_\phi(\bar{\phi}_k + \delta\phi) = -\upsilon_\phi(\bar{\phi}_k - \delta\phi)$. As discussed above, these conditions provide an initial condition for the solution of (S1). From the distributions $n^+(\phi)$, the MT currents leaving the corner (J^+_{i-1}) at $\phi = \phi_{i-1} + \varepsilon$ and entering the corner (J^-_i) at $\phi = \phi_i - \varepsilon$ are calculated. The currents J^+_i and J^-_i are used to calculate $N^\pm_i$ in steady state ($dN^\pm/dt = 0$).

For a square geometry, the net pushing and pulling forces are thus

$$\mathbf{F}^\pm = \mp \int_0^{2\pi} d\phi\, n^\pm f^\pm \mathbf{m} \mp \sum_{i=1}^{4} N_i^\pm f^\pm \mathbf{m}. \qquad \text{(S6)}$$

The total number of MTs is $M = \int_0^{2\pi} d\phi (n^+ + n^-) + \sum_{i=1}^{4} (N_i^+ + N_i^-)$. Assuming that the number M of MTs is determined by the total number of nucleation sites in the steady state, the nucleation rate is given by $\nu = M\left(k_{cat} + k_b\right)/\left(1 + k_b / k_{off}\right)$.

Parameter values used in the model are: M = 50 MTs, k_{cat} = 10^{-4} s^{-1}, k_{off} = 10^{-3} s^{-1} (roughly estimated from our experimental data), f^- = 5 pN (Gennerich et al., 2007), κ = $3.3 \cdot 10^{-23}$ Nm^2 (Mickey and Howard, 1995). The parameter ξ=$5 \cdot 10^{-5}$ N·s/m was roughly estimated from slipping MTs observed in Figure 1, according to the equation: $\xi = \frac{\upsilon_s}{f_s}$, where υ_s, the velocity of slipping of the MT end parallel to the gold barrier, was measured and averaged during a slipping event. The component of the pushing force parallel to the barrier, f_s was calculated by $f_s = f^+ \sin\beta$ where β, the angle of the MT with the barrier

was averaged during slipping. The MT pushing force f^+ was estimated from the average MT length during slipping.

The effect of MT catastrophes on aster positioning (Figure S6D)

In our minimal model we assume that MTs can undergo catastrophes only when the MTs are in contact with the boundary. However, freely growing MTs can also undergo catastrophes, which reduces the number of MTs that reach the boundary. The reduced number of MTs in contact with the boundary is described by a length-dependent arrival rate ν of MTs at the boundary. For a square geometry and the centrosome close to the center we have

$$\nu = \frac{M e^{-k_{cc} L/v_g}}{\frac{1+I_0}{k_{cc}} + \frac{1+k_b/k_{off}}{k_{cat}+k_b} I_0} \qquad \text{(S7)}$$

Here *M* denotes the total number of MTs, including free MTs and the ones in contact with the boundary, k_{cc} denotes the free catastrophe frequency, v_g is the MT growth velocity, while $I_0 = \int_0^{2\pi} d\phi \frac{1}{2\pi} e^{-k_{cc} L(\phi)/v_g}$.

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