

# Supplemental Materials

*Molecular Biology of the Cell*

Gibeaux et al.

## SUPPLEMENTAL FIGURES

### **SUPPLEMENTAL FIGURE S1: General characteristics of nuclei movements in hyphae.**

The main axis of the hyphae was calculated by a principal component analysis using the coordinates  $(x, y)$  of all nuclei and hyphae tip, derived from the tracking. We then used the coordinate  $x$  of the nuclei along this axis to calculate global characteristics of nuclei motions, pooling the data from 12 hyphae. **(A)** The average speed of nuclei movement is directed towards the hyphae tip, and is approximately equal to the speed of hyphae growth. For each hypha, each dot indicates the mean speed of motion of one nucleus, while the vertical line indicates the speed of tip growth. **(B)** The mean and the variance of  $x(t+\tau) - x(t)$  is plotted as a function of the time interval  $\tau$ , including all time-points  $t$  for which this can be calculated. Points indicate the experimental data, while the lines indicate linear fits constrained to cross the origin. The good agreement shows that the nuclei move at an average speed of  $\sim 0.008 \mu\text{m/s}$ , with a diffusive component of  $\sim 0.011 \mu\text{m}^2/\text{s}$ . **(C)** The speed of a nucleus depends on its distance to the hyphae tip (negative here), with more distant nuclei moving slower. The line indicates the best linear fit minimizing the sum of the squared residuals that crosses  $(0,1)$ . These results indicate that the cytoplasmic flow is decreasing away from the hyphae's tip. This effect was however not included in our simulation, since it would not be consistent with the periodic boundary conditions. **(D)** Distribution of the separation between nuclei in live recordings and in simulations. The difference in the  $x$ -coordinates (i.e. along the hypha axis) of the nuclei centers, was used. A 'zero' value corresponds to side-by-side nuclei at the same position of the hyphae, but not overlapping. Values for all experimental data were plotted in blue (top), for 6 simulations with 3 cMTs in grey (middle) and for 6 simulations with 6 cMTs in orange (bottom). The separation means were of  $4.05 \pm 1.96 \mu\text{m}$ ,  $4.96 \pm 4.20 \mu\text{m}$  and  $4.92 \pm 3.92 \mu\text{m}$ , respectively. The red lines represent the outcome of randomly distributing the 5 nuclei. It has a theoretical mean of  $5 \mu\text{m}$ .

### **SUPPLEMENTAL FIGURE S2: Importance of cMT dynamics in modulating nuclear dynamics.**

**(A)** Characteristics of nuclear movements in "wild-type" and "mutant" simulations with nuclei nucleating 6 cMTs. The average duration of forward movements (left), the average duration of backward movements (middle left), the frequency of bypassing events (middle right) and the frequency of tumbling events (right) are plotted. Colors as in Figure 4. Circles stand for individual simulations; the thick black line marks the average value and the transparent gray box the standard deviation. **(B)** Plots of the frequencies of forward (left) and backward (middle) movements and their ratio (right) as a function of cytoplasmic flow in "wild-type" and "mutant" simulations with nuclei nucleating 3

cMTs. Colors as in panel A. **(C)** Plots of the frequencies of forward (left) and backward (middle) movements and their ratio (right) as a function of cytoplasmic flow in “wild-type” and “mutant” simulations with nuclei nucleating 6 cMTs. Colors as in panel A.

**SUPPLEMENTAL FIGURE S3: Consequence of organelle crowding on nuclear dynamics in simulations with nuclei nucleating 6 cMTs.**

**(A)** Characteristics of nuclear movements in the simulated ‘WT’, ‘empty’ and ‘highly crowded’ simulations. The average duration of forward movements (top), the average duration of backward movements (middle top), the frequency of bypassing events (middle bottom) and the frequency of tumbling events (bottom) are plotted. The ‘empty’, ‘WT’ and ‘highly crowded’ data are plotted in blue, gray and orange, respectively. Circles stand for individual simulations; the thick black line marks the average value and the transparent gray box the standard deviation. **(B)** The frequencies of forward (top) and backward (middle) movements and their ratio (bottom) as a function of increasing cytoplasmic flow (from 0 to 0.02  $\mu\text{m/s}$ ). Colors as in panel A.

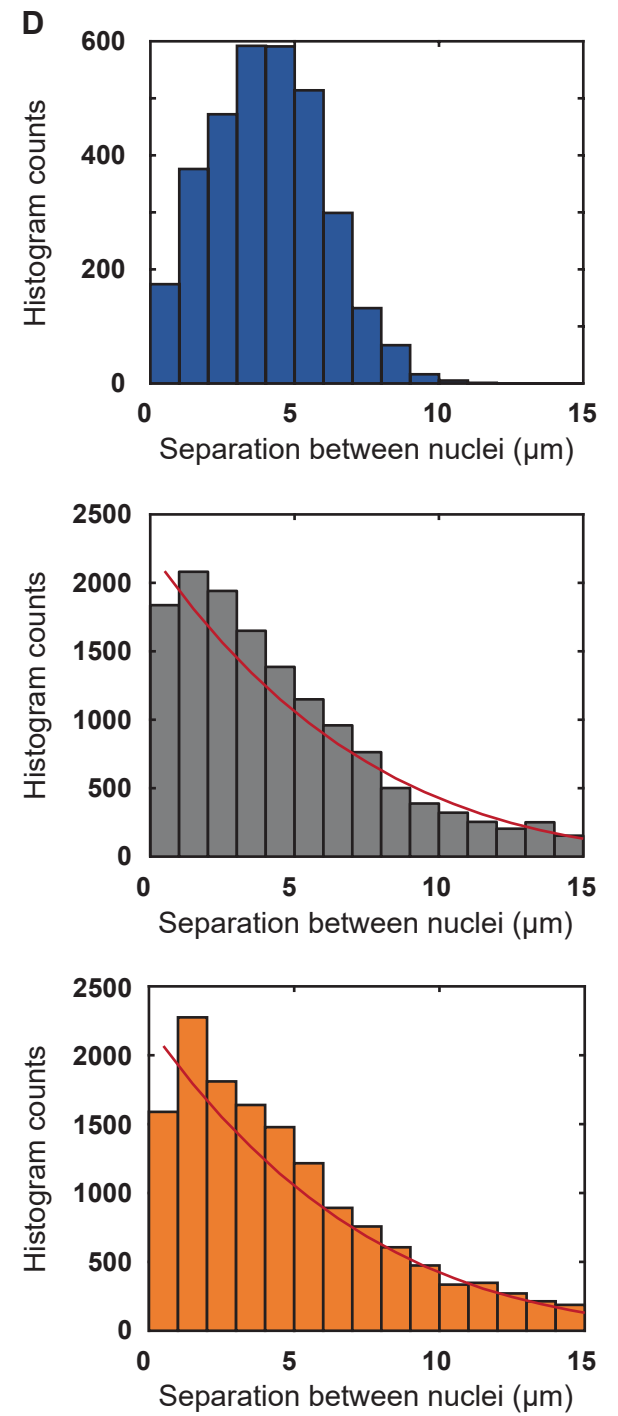
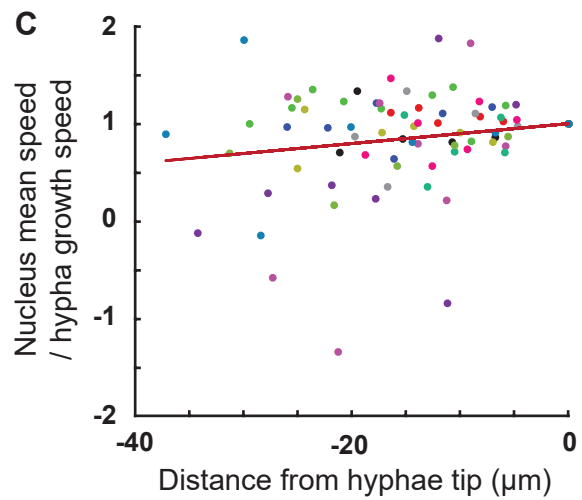
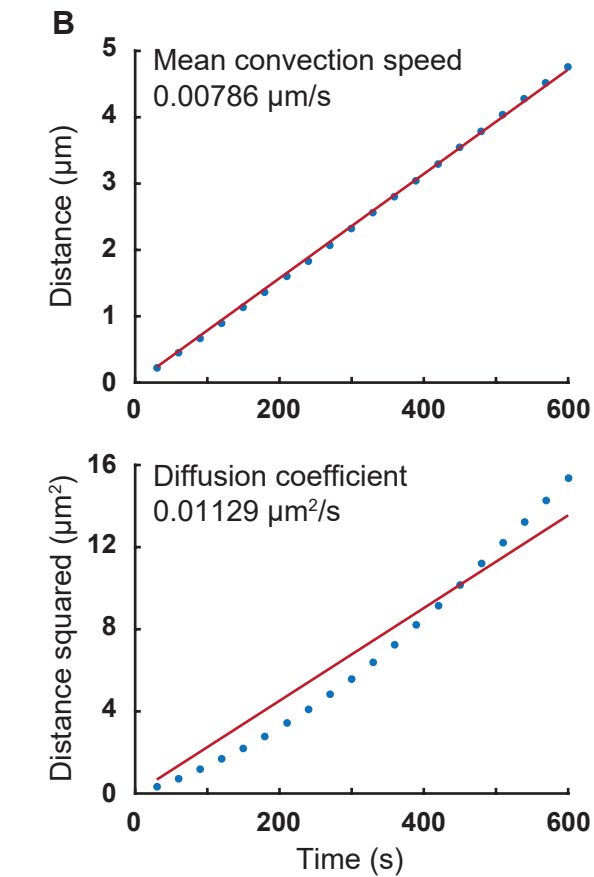
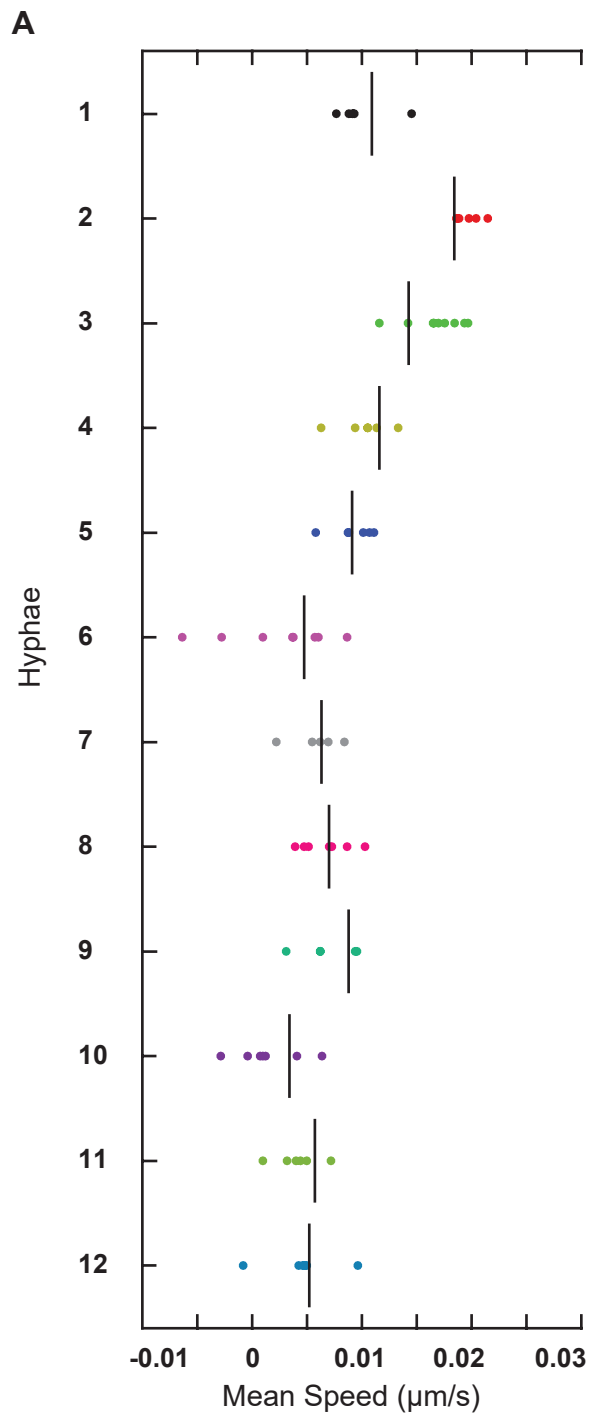


Figure S2

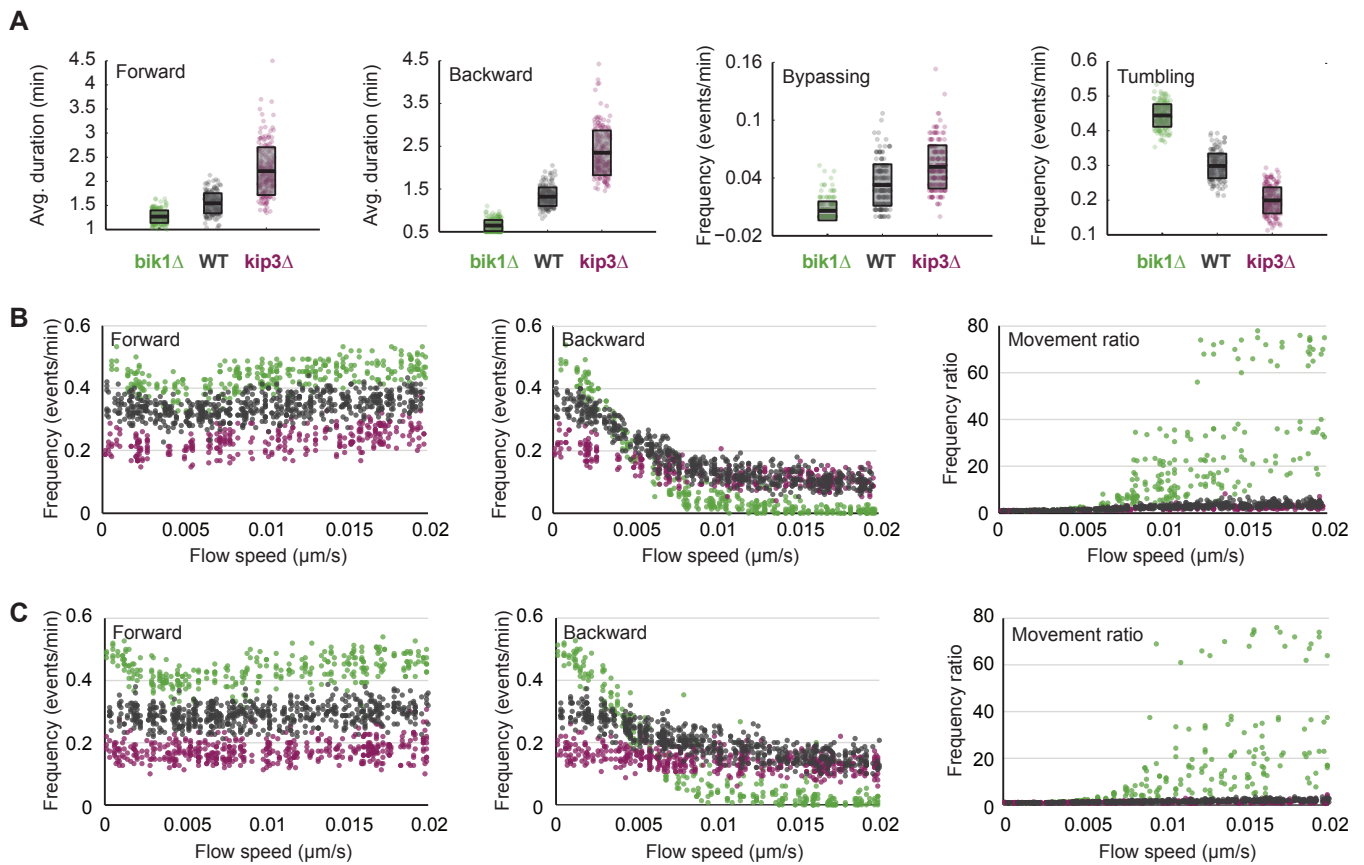
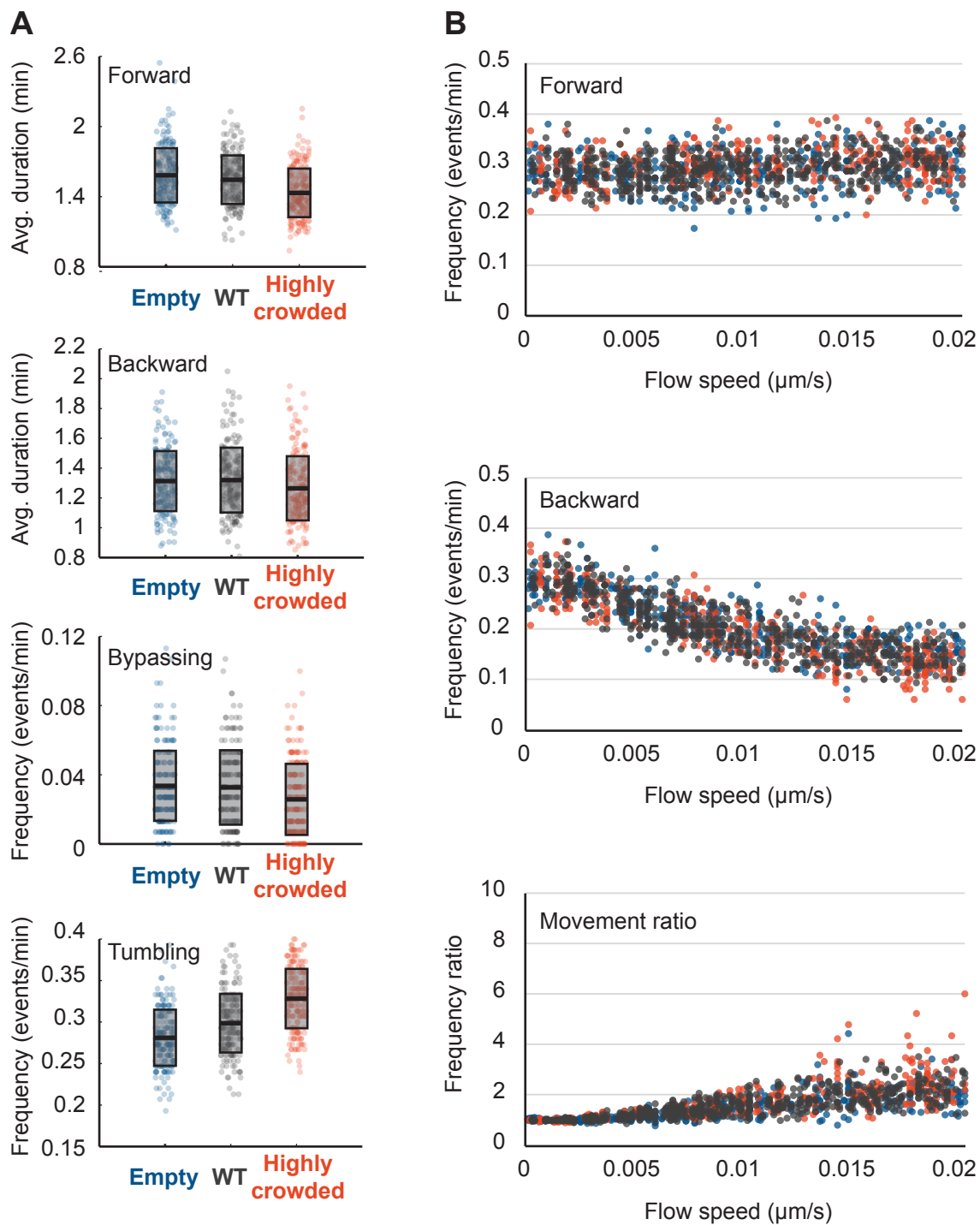


Figure S3



**Supplementary Table S1.** Quantitative description of nuclear dynamics in individual hyphae from live-imaging experimental data.

	Forward movements				Backward movements		Other movement features		Tumbling events		Bypassing events
	Weighted number of nuclei	Hypha growth speed ( $\mu\text{m/s}$ )	Frequency (events/min)	Average duration (min)	Frequency (events/min)	Average duration (min)	Frequency ratio	Average speed ( $\mu\text{m/min}$ )	Frequency (events/min)	Average duration (min)	Frequency (events/min)
<b>Movie 1</b>											
Source:	5	0.011	0.192	2.292	0.176	1.159	1.091	0.855	0.256	0.969	0.032
C. Lang, PhD Thesis, 2010 (see Video 1)	5	0.018	0.296	1.743	0.056	0.643	5.286	1.314	0.352	0.898	0.056
	7.76	0.014	0.258	1.970	0.124	0.875	2.083	1.147	0.304	0.907	0.015
	6	0.012	0.280	1.536	0.187	0.893	1.500	0.852	0.367	0.791	0.007
	5.8	0.010	0.310	1.444	0.200	0.983	1.552	0.784	0.310	0.856	0.007
<b>Movie 2</b>											
Source:	6.72	0.005	0.263	1.623	0.263	1.396	1.000	0.827	0.144	0.586	0.030
Gladfelter et al., 2006	5	0.006	0.280	1.810	0.220	1.121	1.273	0.858	0.213	0.609	0.027
	6.57	0.007	0.294	1.716	0.259	1.147	1.137	0.904	0.203	0.563	0.061
	5	0.009	0.272	1.735	0.224	1.107	1.214	0.947	0.272	0.735	0.064
<b>Movie 3</b>											
Source:	6.46	0.004	0.310	1.450	0.268	1.279	1.154	0.564	0.191	0.581	0.005
C. Alberti, PhD Thesis, 2001	5.61	0.006	0.309	1.317	0.268	1.111	1.156	0.615	0.250	0.702	0.006
	5.89	0.006	0.278	1.796	0.255	1.200	1.089	0.695	0.164	0.638	0.006

**Supplementary Table S2.** Nuclear dynamics quantification averages from microtubule dynamics simulations.

		Forward movements		Backward movements		Other movement features		Tumbling events		Bypassing events
		Frequency (events/min)	Average duration (min)	Frequency (events/min)	Average duration (min)	Frequency ratio	Average speed ( $\mu\text{m}/\text{min}$ )	Frequency (events/min)	Average duration (min)	Frequency (events/min)
<b>Flow = 0.009 <math>\mu\text{m}/\text{s}</math></b>										
<b>Simulation with 3 MTs per nucleus</b>										
bik1 $\Delta$	Average	<b>0.438</b>	<b>1.291</b>	<b>0.047</b>	<b>0.597</b>	<b>12.537</b>	<b>0.621</b>	<b>0.443</b>	<b>0.754</b>	<b>0.008</b>
	SD	0.032	0.113	0.023	0.114	8.439	0.014	0.031	0.055	0.016
kip3 $\Delta$	Average	<b>0.226</b>	<b>1.950</b>	<b>0.121</b>	<b>2.047</b>	<b>1.961</b>	<b>0.848</b>	<b>0.232</b>	<b>0.719</b>	<b>0.051</b>
	SD	0.035	0.408	0.028	0.526	0.537	0.047	0.040	0.066	0.020
<i>S. cerevisiae</i>	Average	<b>0.412</b>	<b>1.305</b>	<b>0.055</b>	<b>0.925</b>	<b>13.109</b>	<b>0.606</b>	<b>0.435</b>	<b>0.774</b>	<b>0.012</b>
	SD	0.040	0.130	0.035	0.362	13.302	0.025	0.032	0.063	0.016
<b>Simulation with 6 MTs per nucleus</b>										
bik1 $\Delta$	Average	<b>0.421</b>	<b>1.268</b>	<b>0.075</b>	<b>0.645</b>	<b>8.618</b>	<b>0.602</b>	<b>0.444</b>	<b>0.772</b>	<b>0.006</b>
	SD	0.039	0.130	0.046	0.130	7.919	0.030	0.033	0.059	0.010
kip3 $\Delta$	Average	<b>0.168</b>	<b>2.211</b>	<b>0.139</b>	<b>2.347</b>	<b>1.263</b>	<b>0.830</b>	<b>0.199</b>	<b>0.721</b>	<b>0.051</b>
	SD	0.029	0.496	0.029	0.523	0.367	0.056	0.038	0.085	0.022
<i>S. cerevisiae</i>	Average	<b>0.386</b>	<b>1.258</b>	<b>0.090</b>	<b>1.008</b>	<b>6.782</b>	<b>0.571</b>	<b>0.438</b>	<b>0.810</b>	<b>0.014</b>
	SD	0.054	0.132	0.054	0.310	5.665	0.041	0.034	0.065	0.016
<b>Flow = 0.013 <math>\mu\text{m}/\text{s}</math></b>										
<b>Simulation with 3 MTs per nucleus</b>										
bik1 $\Delta$	Average	<b>0.456</b>	<b>1.170</b>	<b>0.019</b>	<b>0.635</b>	<b>33.429</b>	<b>0.829</b>	<b>0.466</b>	<b>0.826</b>	<b>0.006</b>
	SD	0.029	0.103	0.015	0.187	21.843	0.017	0.029	0.058	0.014
kip3 $\Delta$	Average	<b>0.235</b>	<b>1.741</b>	<b>0.114</b>	<b>2.203</b>	<b>2.168</b>	<b>0.925</b>	<b>0.280</b>	<b>0.794</b>	<b>0.050</b>
	SD	0.034	0.378	0.025	0.569	0.607	0.043	0.039	0.079	0.022
<b>Simulation with 6 MTs per nucleus</b>										
bik1 $\Delta$	Average	<b>0.444</b>	<b>1.167</b>	<b>0.031</b>	<b>0.704</b>	<b>26.493</b>	<b>0.811</b>	<b>0.465</b>	<b>0.846</b>	<b>0.008</b>
	SD	0.039	0.101	0.033	0.219	23.849	0.032	0.030	0.060	0.015
kip3 $\Delta$	Average	<b>0.175</b>	<b>2.005</b>	<b>0.130</b>	<b>2.275</b>	<b>1.409</b>	<b>0.878</b>	<b>0.252</b>	<b>0.849</b>	<b>0.057</b>
	SD	0.029	0.393	0.027	0.601	0.397	0.050	0.035	0.093	0.023



**Supplementary Table S3.** Nuclear dynamics quantification averages with cortical anchors amount decreased to 100 or 20 anchors per hypha

		<b>Forward movements</b>		<b>Backward movements</b>		<b>Other movement features</b>		<b>Tumbling events</b>		<b>Bypassing events</b>
		Frequency (events/min)	Average duration (min)	Frequency (events/min)	Average duration (min)	Frequency ratio	Average speed ( $\mu\text{m}/\text{min}$ )	Frequency (events/min)	Average duration (min)	Frequency (events/min)
<b>Flow = 0.009 <math>\mu\text{m}/\text{s}</math></b>										
<b>Simulation with 3 MTs per nucleus</b>										
100 anchors	Average	<b>0.367</b>	<b>1.444</b>	<b>0.110</b>	<b>1.040</b>	<b>3.537</b>	<b>0.674</b>	<b>0.369</b>	<b>0.749</b>	<b>0.021</b>
	SD	0.033	0.160	0.026	0.223	0.974	0.025	0.035	0.058	0.022
20 anchors	Average	<b>0.408</b>	<b>1.382</b>	<b>0.053</b>	<b>0.816</b>	<b>9.478</b>	<b>0.630</b>	<b>0.415</b>	<b>0.769</b>	<b>0.011</b>
	SD	0.032	0.143	0.021	0.282	5.708	0.014	0.033	0.059	0.017
<b>Simulation with 6 MTs per nucleus</b>										
100 anchors	Average	<b>0.329</b>	<b>1.516</b>	<b>0.144</b>	<b>1.170</b>	<b>2.407</b>	<b>0.682</b>	<b>0.339</b>	<b>0.732</b>	<b>0.022</b>
	SD	0.034	0.200	0.031	0.197	0.662	0.029	0.037	0.057	0.019
20 anchors	Average	<b>0.393</b>	<b>1.407</b>	<b>0.057</b>	<b>0.986</b>	<b>8.475</b>	<b>0.623</b>	<b>0.406</b>	<b>0.783</b>	<b>0.006</b>
	SD	0.033	0.156	0.021	0.319	5.592	0.016	0.032	0.054	0.014

**Supplementary Table S4.** Nuclear dynamics quantification averages with or without cytoplasmic organelle crowding.

		<b>Forward movements</b>		<b>Backward movements</b>		<b>Other movement features</b>		<b>Tumbling events</b>		<b>Bypassing events</b>
		Frequency (events/min)	Average duration (min)	Frequency (events/min)	Average duration (min)	Frequency ratio	Average speed ( $\mu\text{m}/\text{min}$ )	Frequency (events/min)	Average duration (min)	Frequency (events/min)
<b>Flow = 0.009 <math>\mu\text{m}/\text{s}</math></b>										
<b>Simulation with 3 MTs per nucleus</b>										
No organelles	Average	<b>0.323</b>	<b>1.610</b>	<b>0.160</b>	<b>1.138</b>	<b>2.084</b>	<b>0.774</b>	<b>0.301</b>	<b>0.692</b>	<b>0.028</b>
	SD	0.033	0.196	0.030	0.225	0.426	0.033	0.036	0.056	0.021
Large organelles	Average	<b>0.348</b>	<b>1.383</b>	<b>0.151</b>	<b>1.088</b>	<b>2.431</b>	<b>0.673</b>	<b>0.354</b>	<b>0.788</b>	<b>0.026</b>
	SD	0.030	0.158	0.031	0.202	0.631	0.029	0.030	0.061	0.022
<b>Simulation with 6 MTs per nucleus</b>										
No organelles	Average	<b>0.284</b>	<b>1.582</b>	<b>0.198</b>	<b>1.313</b>	<b>1.484</b>	<b>0.768</b>	<b>0.281</b>	<b>0.696</b>	<b>0.034</b>
	SD	0.033	0.196	0.030	0.225	0.426	0.033	0.036	0.056	0.021
Large organelles	Average	<b>0.296</b>	<b>1.432</b>	<b>0.191</b>	<b>1.264</b>	<b>1.587</b>	<b>0.670</b>	<b>0.328</b>	<b>0.770</b>	<b>0.026</b>
	SD	0.032	0.208	0.031	0.215	0.320	0.033	0.036	0.069	0.021

## VIDEOS

**VIDEO 1:** Example of live-hyphae expressing GFP-tagged histone H4. DIC in gray and GFP signal in green.

### **VIDEO 2 to 9:**

These 14 movies represent various conditions, but the graphical representation is always identical, unless otherwise stated. The hypha (black) contains 5 nuclei (purple). One SPB (orange) on each nucleus may nucleate either 3 or 6 cMTs (white lines) that are elongating or shortening at the (distal) plus-end. MTs may be pulled by dynein motors that load on fixed anchors at the cortex. The anchor points are displayed gray, and then green if a dynein is engaged. Forces produced by dynein motors and transmitted along the MTs can move the nucleus. The simulation uses periodic boundary conditions, and objects leaving on the right side reappear on the left side, and vice-versa. The cytoplasm is also filled with various organelles. Unless otherwise noted, the simulation contains 550 spheres of diameter 0.4 $\mu$ m (mitochondria, orange) and 50 spheres of diameter 0.6 $\mu$ m (blue, large spherical organelles) and a uniform cytoplasmic flow is moving all objects from left to right at a speed of 0.009  $\mu$ m/s.

**VIDEO 2:** A “wild-type” simulation with 3 cMTs per nucleus.

**VIDEO 3.** A “wild-type” simulation with 6 cMTs per nucleus.

**VIDEO 4.** A “wild-type” simulation with 3 cMTs per nucleus. The same simulation as shown on Video 1 is displayed here, using a different style. The organelles are not displayed, and the nuclei are semi-transparent such that the SPB (orange) is always visible. The cortical anchors are drawn in dark gray when they are unbound/inactive, and green when they are loaded with bound/active dynein. The re-orientation of the SPB upon pulling is clearly visible, for example for the right-most nucleus during the interval 1400–1700s.

**VIDEO 5.** A simulation with *bik1 $\Delta$* -like MT dynamics. Everything is like the “wild-type” with 3 cMT/nucleus, except that MT growth and catastrophe rates were altered to represent the MT dynamics in *bik1 $\Delta$*  mutants (see Figure 4A-B).

**VIDEO 6.** A simulation with *kip3 $\Delta$* -like MT dynamics. Everything is like the “wild-type” with 3 cMT/nucleus, except that MT growth and catastrophe rates were altered to represent the MT dynamics in *kip3 $\Delta$*  mutants (see Figure 4A-B).

**VIDEO 7.** A simulation with *S. cerevisiae*-like MT dynamics. Everything is like the “wild-type” with 3 cMT/nucleus, except that MT growth and shrinkage as well as catastrophe rates were altered to represent the MT dynamics measured in *S. cerevisiae* (see Figure 4A-B).

**VIDEO 8.** A simulation with a reduced number of cortical anchors. Everything is like the “wild-type” with 3 cMT/nucleus, except that the number of anchors on the cortex has been reduced to 100 instead of 200.

**VIDEO 9.** A simulation with an even more reduced number of cortical anchors. Everything is like the “wild-type” with 3 cMT/nucleus, except that the number of cortical anchors has been reduced to 20 instead of 200.

**VIDEO 10.** A “wild-type” simulation with 3 cMTs per nucleus and no cytoplasmic flow (speed = 0  $\mu$ m/s.)

**VIDEO 11.** A “wild-type” simulation with 6 cMTs per nucleus and no cytoplasmic flow (speed = 0  $\mu$ m/s).

**VIDEO 12.** A “wild-type” simulation with 3 cMTs per nucleus at a high cytoplasmic flow (speed = 0.02  $\mu$ m/s).

**VIDEO 13.** A “wild-type” simulation with 6 cMTs per nucleus at a high cytoplasmic flow (speed = 0.02  $\mu$ m/s).

**VIDEO 14.** An “empty” simulation without organelles. The simulation does not contain the extra spheres that are used to represent mitochondria and large spherical organelles.

**VIDEO 15.** A simulation with “high” crowding. The simulation contains 550 spheres of 0.4  $\mu$ m in diameter (orange, simulating mitochondria) and 50 beads of 1.4  $\mu$ m in diameter (blue, simulating large spherical organelles in old hyphae).