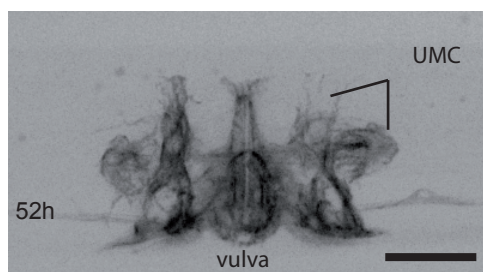
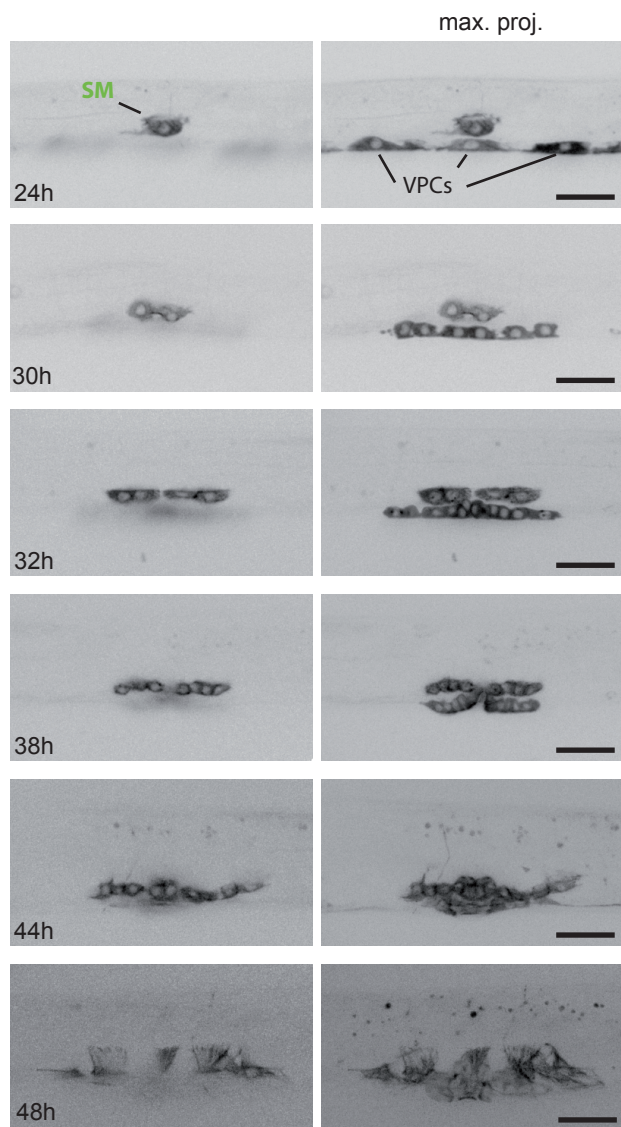


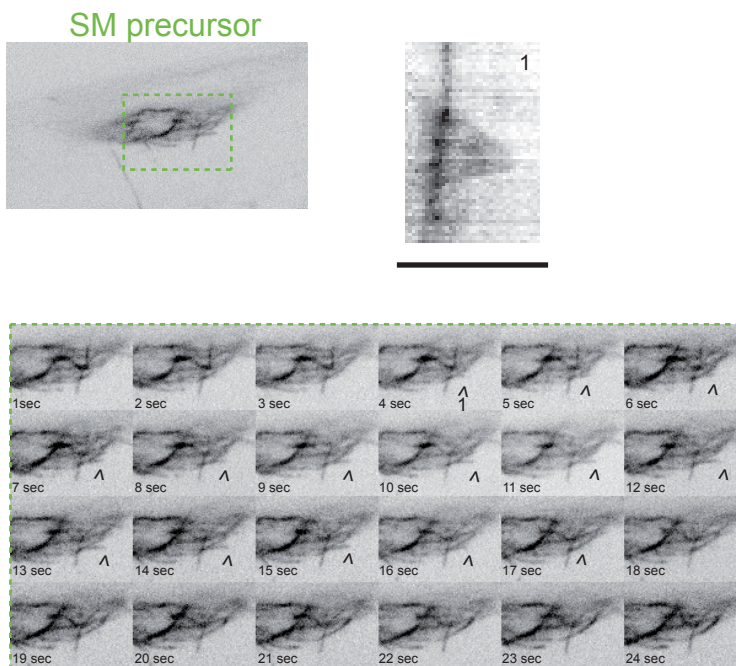
# Figure S1

**A**

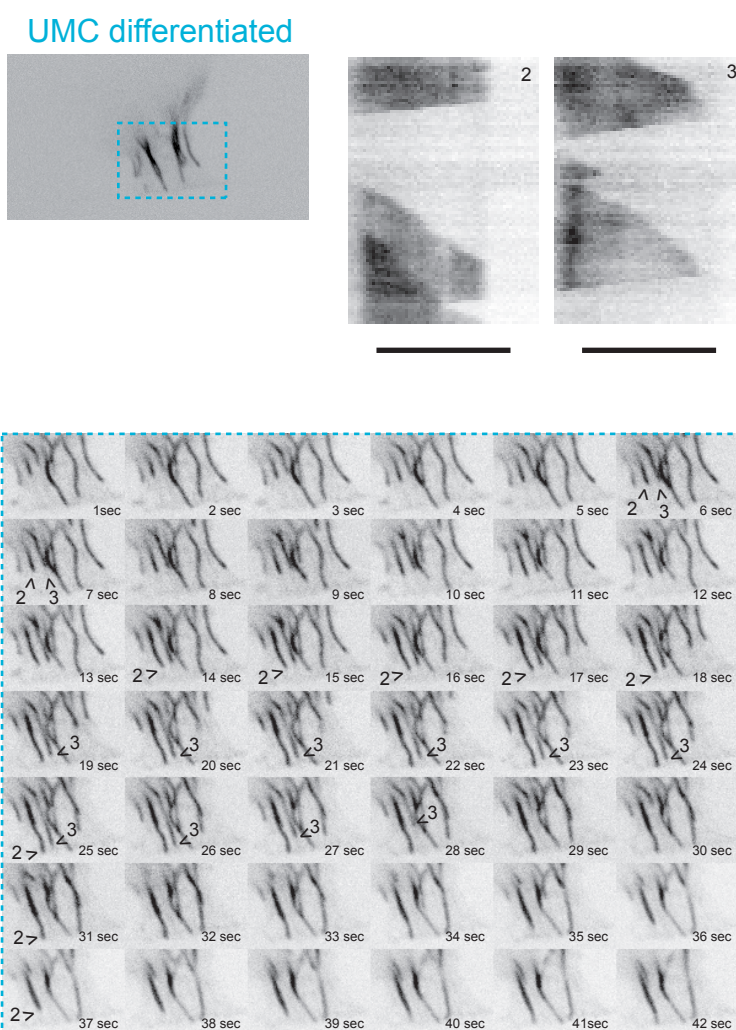


scale bars = 20 μm

**B**



**C**



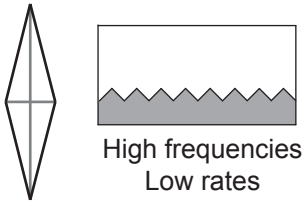
**Figure S1: Microtubule network visualized in SM lineage during post-embryonic development of *C. elegans*, related to Figure 1.**

(A) SM lineage described in Figure 1 followed during post-embryonic development in worms expressing GFP-tagged tubulin under the control of the *unc-62* promoter. Time is in hours from plating starved L1 worms on food. A z-stack through the entire thickness of the worm was acquired using a swept-field real-time confocal microscope. Images are oriented such that anterior is on left and ventral is down. Left: confocal sections containing the left SM. Right: maximal intensity projection of the z-series. Bottom: a maximal intensity projection of *unc-62p>GFP::tubulin* in an adult worm. SM: sex myoblast; VPC: vulval precursor cells; UMC: uterine muscle cells. Scale bars = 20  $\mu\text{m}$ . (B-C) Montages of a 1 sec interval time series of cropped area of a single confocal slice in SM (B) and UMC (C). These panels correspond to the dynamics data presented in Figure 1. Kymograph 1 corresponds to a MT tracked for 60 sec (#1) in an SM precursor (SM) extracted from Movie S1. Kymograph 2 corresponds to the MT bundle (#2) imaged in a UMC and kymograph 3 to a single MT in the same cell. Both kymographs represent 90 sec of tracking. Bundled MTs can be recognized in the kymograph by the increased grey value intensity where the two MTs are superimposed. Kymograph 2 and 3 were extracted from Movie S3. Scale bars = 5  $\mu\text{m}$ .

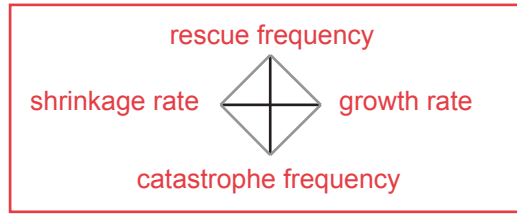
**Figure S2**

**A**

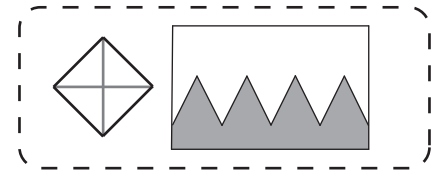
**Vertical elongation**



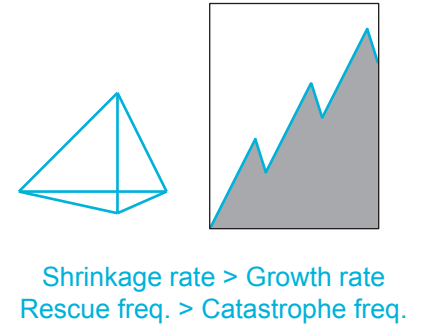
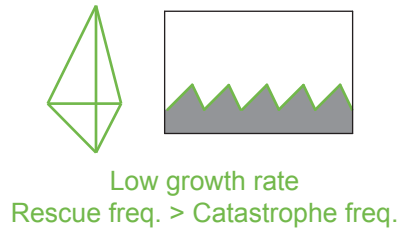
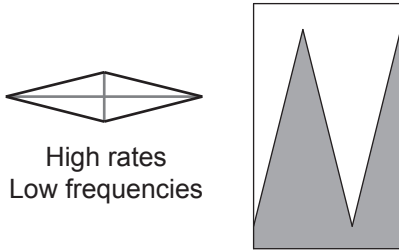
**Diamond Graphs**



Equal rates and frequencies



**Horizontal elongation**

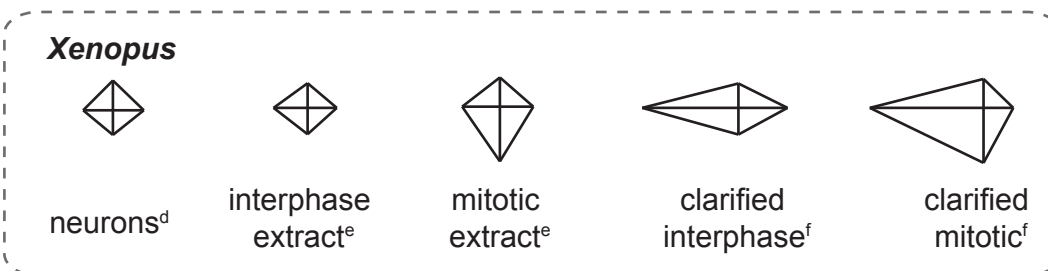
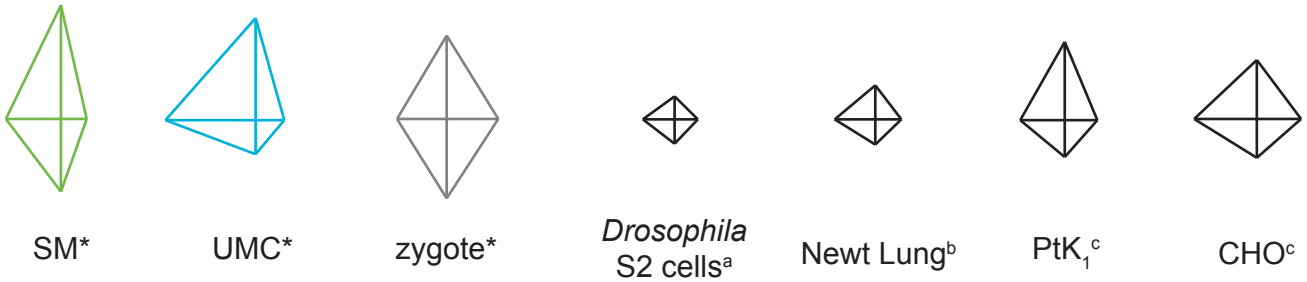


**B**

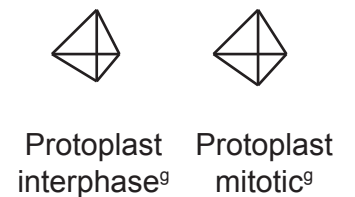
Species	<i>C.elegans</i>			<i>Drosophila</i>	Newton	Rat Kangaroo	Chinese hamster	<i>Xenopus</i>					Cowpea	
cell type	SM*	UMC*	1-4 cells zygote*	S2 cells <sup>a</sup>	lung epithelial cells <sup>b</sup>	PtK1 <sup>c</sup>	CHO <sup>c</sup>	neuron <sup>d</sup>	interphase extract <sup>e</sup>	mitotic extract <sup>e</sup>	i 218 <sup>f</sup>	m 218 <sup>f</sup>	Protoplast interphase <sup>g</sup>	Protoplast mitotic <sup>g</sup>
Growth rate (µm/sec)	0,1199	0,1534	0,4144	0,0883	0,12	0,1983	0,3283	0,1767	0,155	0,205	0,3833	0,1633	0,0833	0,1467
Shrinking rate (µm/sec)	0,4493	0,8495	0,3884	0,1883	0,2883	0,33	0,5367	0,1617	0,2133	0,255	0,9167	1,1167	0,3333	0,333
Catastrophe frequency (sec-1)	0,1774	0,0448	0,2011	0,011	0,014	0,054	0,061	0,012	0,018	0,116	0,018	0,116	0,02	0,034
Rescue frequency (sec-1)	0,3188	0,2782	0,2135	0,005	0,044	0,196	0,13	0,029	0,011	0,027	0,011	0,027	0,08	0,08

**C**

***Caenorhabditis elegans*\***



**Plants**



a, (Li *et al.*, 2011) ; b, (Cassimeris *et al.*, 1988) ; c, (Shelden and Wadsworth, 1993) ; d, (Tanaka and Kirschner, 1991) ; e, (Belmont *et al.*, 1990) ; f, (Parsons and Salmon, 1997) ; g, (Dhonukshe & Gadella, 2003)

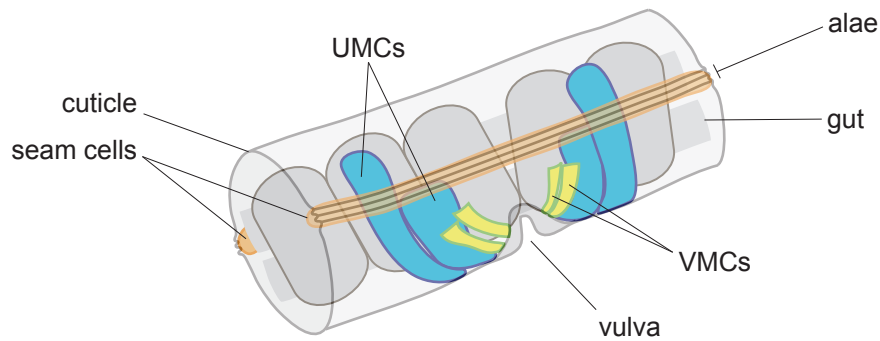
**Figure S2: Representation and interpretation of microtubule behavior using diamond graphs; related to Figure 1 and Figure 2.**

(A) Tutorial representation of MT behavior according to diamond graph shape. A square represents equal rates and equal frequencies, and a length change in any “strut” represents a change in that parameter. The simulated kymographs next to each diamond graph represent the corresponding trend in MT behavior. Green and blue simulated kymographs were created using the experimental data for SM and UMC, respectively (Figure 1 and 2). This non-stochastic simulation predicts that in the UMC, MTs increase in length and that eventually all tubulin is polymeric. This is distinct from the steady-state dynamics seen in the cell, likely because in cells the amount of available tubulin is regulated, and dynamic events are stochastic and controlled by multiple factors (*i.e.* subcellular regulation such as at the cell periphery) not accounted for in this simulation.

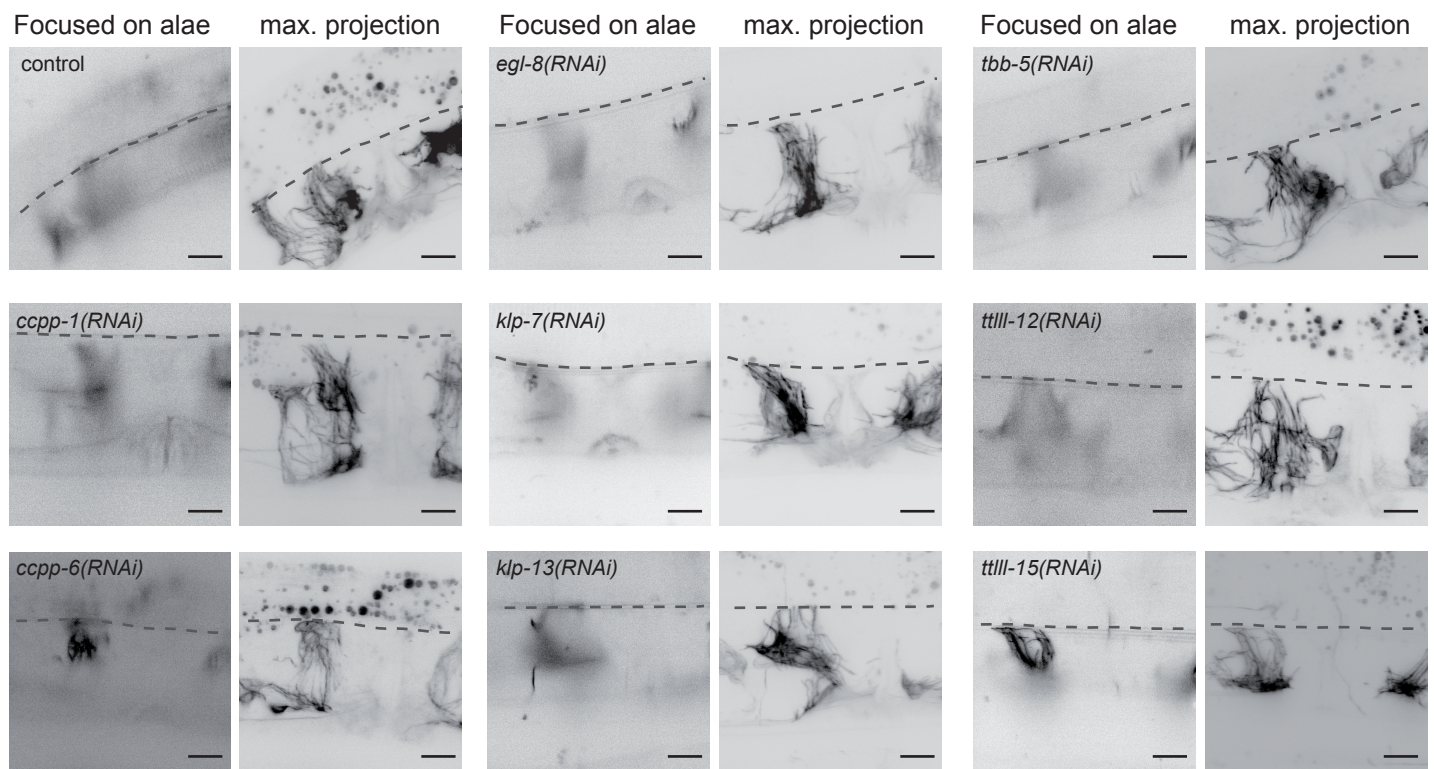
(B) Comparison of SM and UMC (Figure 1 and Table 1) with published data<sup>a-f</sup> of MT dynamics in different cell types. (C) Diamond graphs created with published data from (B) of MT dynamics of different cell types allow comparison with *C. elegans* SM, UMC and zygote (\*parameters measured in this study, with equal settings). For clarified *Xenopus* extract (218,000xg centrifugation supernatant)<sup>f</sup>, catastrophe and rescue frequencies were not available, we thus used these data from non-clarified extract<sup>e</sup> for the diamond graph.

## Figure S3

**A**



**B**



### Figure S3: UMC architecture and tissue interactions are preserved in Egl worms, related to Figure 5.

(A) Schematic volume view of the egg-laying apparatus in an adult worm. This view correspond to the central section corresponding to the central 1/5th of worm's length. UMCs: Uterine Muscle Cells, VMCs: Vulval Muscle Cells. (B) For every target inducing egg-laying defect (Figure 5), a single confocal plane displaying the location of seam cells (at the alae; dashed line) is represented next to a maximal intensity projection of the full z-stack of the same cell, in *unc-62p>GFP::tubulin* strain. Even though MT organization is affected by these protein depletions, the position of the UMCs within the tissue, and their cell-to cell contacts, are preserved. Scale bars = 20  $\mu\text{m}$ .

**Table S1: Microtubule dynamics data in SM and UMCs, related to Figure 1**

cell type	SM			UMCs					
population	total			total			on-track		
	mean	SEM	n	mean	SEM	n	mean	SEM	n
<b>Growth rate (<math>\mu\text{m}/\text{sec}</math>)</b>	0,12	0,003	121	0,15	0,003	169	0,18	0,004	51
<b>Growth length (<math>\mu\text{m}</math>)</b>	1,10	0,03	121	3,21	0,08	169	2,62	0,11	51
<b>Growth duration (sec)</b>	5,64	0,16	121	22,34	0,56	169	7,11	0,78	51
<b>Shrinking rate (<math>\mu\text{m}/\text{sec}</math>)</b>	0,45	0,03	121	0,85	0,02	169	0,91	0,04	51
<b>Shrinking length (<math>\mu\text{m}</math>)</b>	1,47	0,09	121	2,50	0,10	169	1,49	0,10	51
<b>Shrinking duration (sec)</b>	3,14	0,20	121	3,60	0,13	169	3,14	0,22	51
<b>Catastrophe (<math>\text{sec}^{-1}</math>)</b>	0,18	0,01	121	0,05	0,001	169	0,07	0,004	51
<b>Catastrophe (<math>\mu\text{m}^{-1}</math>)</b>	0,91	0,03	121	0,31	0,01	169	0,38	0,02	51
<b>Rescue (<math>\text{sec}^{-1}</math>)</b>	0,32	0,02	121	0,28	0,01	169	0,32	0,02	51
<b>Rescue (<math>\mu\text{m}^{-1}</math>)</b>	0,68	0,04	121	0,40	0,02	169	0,67	0,05	51

SEM: standard error of the mean. n = number of MTs. n(worms)  $\geq$  12



## Table S2 Legend

Alphabetical list of candidates tested for the reduction of brood size in L1 and L4 feeding (Figure 3). Closest known orthologs in *Drosophila*, mammals, *Xenopus* or yeasts are mentioned in the second column depending on the most commonly used name in the literature. Sequence names correspond to the annotation on Wormbase ([www.wormbase.org](http://www.wormbase.org)). Number of eggs laid per day and per worm correspond to an average of 3 to 5 worms isolated on a feeding plate after L1- or L4-feeding. Bold numbers represent a significant reduction of the brood size corresponding to less than 0.75 the number of eggs laid per control worms. This arbitrary threshold is voluntarily low and enables reducing false positive. Macroscopic phenotypes were scored using a dissecting scope as shown in Figure 3C and were notified in their respective column with the following abbreviations: Dev (developmental arrest); Pvl (protruded vulva); Ste (sterility); Egl (egg-laying defective). Lowercase and uppercase account for a penetrance of each phenotype being respectively lower than 50% or higher than 50%.



**Table S3: Dynamics parameters of UMC in egg-laying defective worms, related to Figure 5**

	CCPP-1			CCPP-6			TTL-12			KLP-13			TBB-5			KLP-7			EGL-8			TTL-15			CONTROL		
	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n
<b>Growth rate (<math>\mu\text{m}/\text{sec}</math>)</b>	0,20	0,004	303	0,14	0,005	259	0,20	0,005	299	0,15	0,01	169	0,15	0,01	180	0,15	0,004	211	0,22	0,01	315	0,18	0,01	215	0,18	0,003	353
<b>Shrinking rate (<math>\mu\text{m}/\text{sec}</math>)</b>	0,94	0,03	297	0,84	0,02	225	0,93	0,02	265	0,94	0,03	114	0,69	0,02	206	0,81	0,03	130	0,94	0,02	217	0,88	0,03	188	0,94	0,02	219
<b>Catastrophe frequency (<math>\text{sec}^{-1}</math>)</b>	0,08	0,003	303	0,05	0,002	259	0,06	0,002	299	0,05	0,003	169	0,07	0,004	180	0,06	0,003	211	0,06	0,002	315	0,06	0,003	215	0,07	0,002	353
<b>Rescue frequency (<math>\text{sec}^{-1}</math>)</b>	0,29	0,01	296	0,24	0,01	225	0,20	0,01	265	0,21	0,01	114	0,27	0,01	206	0,17	0,01	130	0,20	0,01	217	0,25	0,01	188	0,22	0,01	219

SEM: standard error of the mean. n = number of MTs. n(worms)  $\geq 8$

**Table S4: Dynamics parameters of SM and UMC after CLS-1 and KLP-7 depletions, related to Figure 6**

<b>CONTROL RNAi (L4440)</b>									
cell type	SM			UMC					
population	total			total			on-track		
	mean	SEM	n	mean	SEM	n	mean	SEM	n
Growth rate ( $\mu\text{m}/\text{sec}$ )	0,13	0,004	72	0,17	0,004	167	0,19	0,01	87
Growth length ( $\mu\text{m}$ )	0,93	0,05	80	3,32	0,10	167	2,23	0,13	87
Growth duration (sec)	5,79	0,30	80	21,08	0,68	167	13,00	0,80	87
Shrinking rate ( $\mu\text{m}/\text{sec}$ )	0,67	0,06	76	0,93	0,02	269	0,84	0,05	88
Shrinking length ( $\mu\text{m}$ )	1,46	0,09	76	2,50	0,09	269	2,21	0,13	88
Shrinking duration (sec)	2,88	0,21	76	3,03	0,12	269	3,24	0,26	88
Catastrophe (sec-1)	0,15	0,01	80	0,05	0,002	167	0,08	0,01	87
Catastrophe ( $\mu\text{m}-1$ )	1,07	0,06	80	0,30	0,01	167	0,45	0,03	87
Rescue (sec-1)	0,35	0,03	76	0,33	0,01	269	0,31	0,03	88
Rescue ( $\mu\text{m}-1$ )	0,68	0,04	76	0,40	0,01	269	0,45	0,03	88
Time at cortex (sec)				3,8	0,2	159			
<b>CLS-1<sup>CLASP</sup> (RNAi)</b>									
cell type	SM			UMC					
population	total			total			on-track		
	mean	SEM	n	mean	SEM	n	mean	SEM	n
Growth rate ( $\mu\text{m}/\text{sec}$ )	0,14	0,01	229	0,15	0,004	242	0,21	0,01	72
Growth length ( $\mu\text{m}$ )	0,67	0,03	229	1,55	0,09	242	2,96	0,20	72
Growth duration (sec)	4,83	0,18	229	9,69	0,43	242	14,23	0,95	72
Shrinking rate ( $\mu\text{m}/\text{sec}$ )	0,81	0,03	194	0,94	0,05	144	1,26	0,09	59
Shrinking length ( $\mu\text{m}$ )	1,72	0,07	194	2,65	0,17	144	3,68	0,24	59
Shrinking duration (sec)	2,41	0,10	194	3,08	0,19	144	3,52	0,30	59
Catastrophe (sec-1)	0,21	0,01	229	0,10	0,01	242	0,07	0,01	72
Catastrophe ( $\mu\text{m}-1$ )	1,49	0,08	229	0,65	0,04	242	0,34	0,02	72
Rescue (sec-1)	0,42	0,02	194	0,33	0,02	144	0,29	0,02	59
Rescue ( $\mu\text{m}-1$ )	0,58	0,02	194	0,38	0,02	144	0,27	0,02	59
Time at cortex (sec)				3,2	0,2	101			
<b>KLP-7<sup>MCAK</sup> (RNAi)</b>									
cell type	SM			UMC					
population	total			total			on-track		
	mean	SEM	n	mean	SEM	n	mean	SEM	n
Growth rate ( $\mu\text{m}/\text{sec}$ )	0,18	0,01	308	0,10	0,003	149	0,11	0,01	65
Growth length ( $\mu\text{m}$ )	0,92	0,03	308	2,18	0,11	149	2,06	0,13	65
Growth duration (sec)	5,20	0,17	308	23,95	1,18	149	20,36	1,26	65
Shrinking rate ( $\mu\text{m}/\text{sec}$ )	0,57	0,02	293	0,63	0,02	156	0,62	0,03	66
Shrinking length ( $\mu\text{m}$ )	0,93	0,02	293	2,66	0,13	156	2,27	0,16	66
Shrinking duration (sec)	1,90	0,05	293	4,72	0,25	156	4,19	0,34	66
Catastrophe (sec-1)	0,19	0,01	308	0,04	0,002	149	0,05	0,003	65
Catastrophe ( $\mu\text{m}-1$ )	1,09	0,04	308	0,46	0,02	149	0,49	0,03	65
Rescue (sec-1)	0,53	0,01	293	0,21	0,01	156	0,24	0,02	66
Rescue ( $\mu\text{m}-1$ )	1,07	0,03	293	0,24	0,02	156	0,44	0,03	66
Time at cortex (sec)				10,0	1,1	96			

SEM: standard error of the mean. n = number of MTs. n(worms)  $\geq 10$

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### *C. elegans* genetics

MDX12 was generated by crossing JJ1753 and NK682. JJ1753: *unc-119(ed3)* III; *zuIs151* [pJN326: *nmy-2::mRFP*; *unc-119(+)*] was kindly provided by Jeremy Nance. NK682 contains an integrated transgene *qyIs119* generated by amplifying the sequence of GFP- $\beta$ -tubulin from pJH4.66 (*pie-1p::GFP:: $\beta$ -tubulin*) and placing it after the *unc-62* promoter region, which was amplified from the fosmid WRM0629aH06. This transgene allows expression of GFP::tubulin in vulval epithelium and SM lineage through post-embryonic development to the adult stage (Jiang et al., 2009). MT dynamics were measured in the early embryo using MDX20, which was generated by out-crossing XA3501 (*Ppie-1::GFP::H2B* ; *Ppie-1::GFP::tbb-2*) with N2 males in order to isolate the transgene (*pie-1::GFP::tbb-2*). The strain used for tissue specific RNAi was NK741: *rrf-3(pk1426)* II; *unc-119(ed4)* III; *rde-1(ne219)* V with an integrated transgene *qyIs138* [Punc-62::RDE-1; *unc-119(+)*; *Pmyo-2::YFP*].

### Worm mounting and imaging conditions

Worms were anesthetized in 0.01% tetramisole in M9 buffer for 10 min before being transferred onto a 5% agarose pad. Worms were then covered with a poly-L-lysine coated coverslip. Coverslip was sealed with a mix of vaseline and paraffin wax and lanolin (1:1:1) and the imaging chamber was filled with M9 to prevent drying. To minimize out-of-focus light and maximize acquisition time, we used a real-time Swept Field Confocal (SFC, Nikon Canada, Mississauga, ON, Canada; and Prairie Technologies, Madison, WI, USA) using the 50  $\mu$ m slit mode without binning on a CoolSnap HQ2 camera (Photometrics, Tucson, AZ). For Z-series and volume view, 60X or 100X/1.4 NA Plan-Apochromat objectives were used to acquire confocal Z sections with 500  $\mu$ m steps. All SFC acquisitions and additional components including laser exposure setting were controlled by Elements software (Nikon). Acquisition of time-lapse for MT dynamics was performed with the 100X/1.4 NA Plan-Apochromat objective with the 1.5X optivar for 2 min with a time interval of 1 sec and less than 200 msec exposure time. A maximum of 5 movies (10 min of acquisition) were made on a single worm to avoid phototoxicity. At least 6 worms were imaged per condition.

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## SUPPLEMENTAL MOVIE LEGENDS

### **Movie S1: Microtubule dynamics in the SM of a *C. elegans* larva expressing GFP-tagged tubulin, related to Figure 1.**

Time-lapse imaging of a third larval stage (L3) *C. elegans* worm expressing GFP-tagged tubulin in the sex myoblast (SM) lineage and vulval precursor cells (not visible in this confocal plane). Images were acquired with 1 sec time interval at 150x magnification allowed visualization of MT dynamic instability in an intact developing nematode. Scale bar is 10  $\mu\text{m}$  and playback rate is 15 times real time (15 frames per second).

### **Movie S2: Microtubule dynamics imaged in a uterine muscle cell (UMC) of an adult *C. elegans* worm expressing GFP-tagged tubulin, related to Figure 1.**

Maximal projection of a 5  $\mu\text{m}$  z-stack (600 nm interval) imaged every 10 sec in an adult worm expressing GFP-tubulin in the SM lineage. The movie represents MT dynamics in a differentiated uterine muscle cell (UMC). Scale bar is 10  $\mu\text{m}$ . 1 frame (10 sec interval) is played every second: playback rate is 10 times real time.

### **Movie S3: Microtubule dynamics in a single confocal section in a uterine muscle cell (UMC), related to Figure 1.**

GFP-tubulin is imaged in a single confocal section of the dorsal extension of a UMC where the cell is attached to its neighboring seam cell. The time lapse is made with 1 sec time interval at 150x magnification. MTs abruptly depolymerize when they encounter the cell border. This movie is decomposed in a montage in Figure S2, showing single and bundled MT tracks. Scale bar is 10  $\mu\text{m}$  and playback rate is 15 times real time (15 frames per second).

### **Movie S4: Microtubule plus ends are oriented toward the cell periphery in the dorsal extension of the UMC, related to Figure 5.**

The dorsal extension of a UMC in an adult worm expressing GFP-tubulin has been imaged every 10 sec on a swept-field confocal microscope. The movie represents a volume view of a z-stack (600 nm interval). MTs are oriented toward the cell edge where UMCs are attached to the seam cells. Scale bar is 5  $\mu\text{m}$  and playback rate 10 times real time (1 frame per sec).

**Movie S5: Microtubule organization and dynamics are perturbed by KLP-7<sup>MCAK</sup> depletion, related to Figure 6.**

Time-lapse imaging of MT dynamics in a single confocal plane of a uterine muscle cell (UMC) in a *klp-7 RNAi* worm. Images were acquired every second at 150x magnification. The area of the cell imaged is the cell border of the UMC dorsal extension where it attaches to the neighboring seam cell. KLP-7<sup>MCAK</sup> depletion causes MT buckling. Scale bar is 10  $\mu\text{m}$  and playback rate is 15 times real time (15 frames per second).