Supplemental Information

Microtubule Dynamics Scale with Cell Size to Set Spindle Length and Assembly Timing

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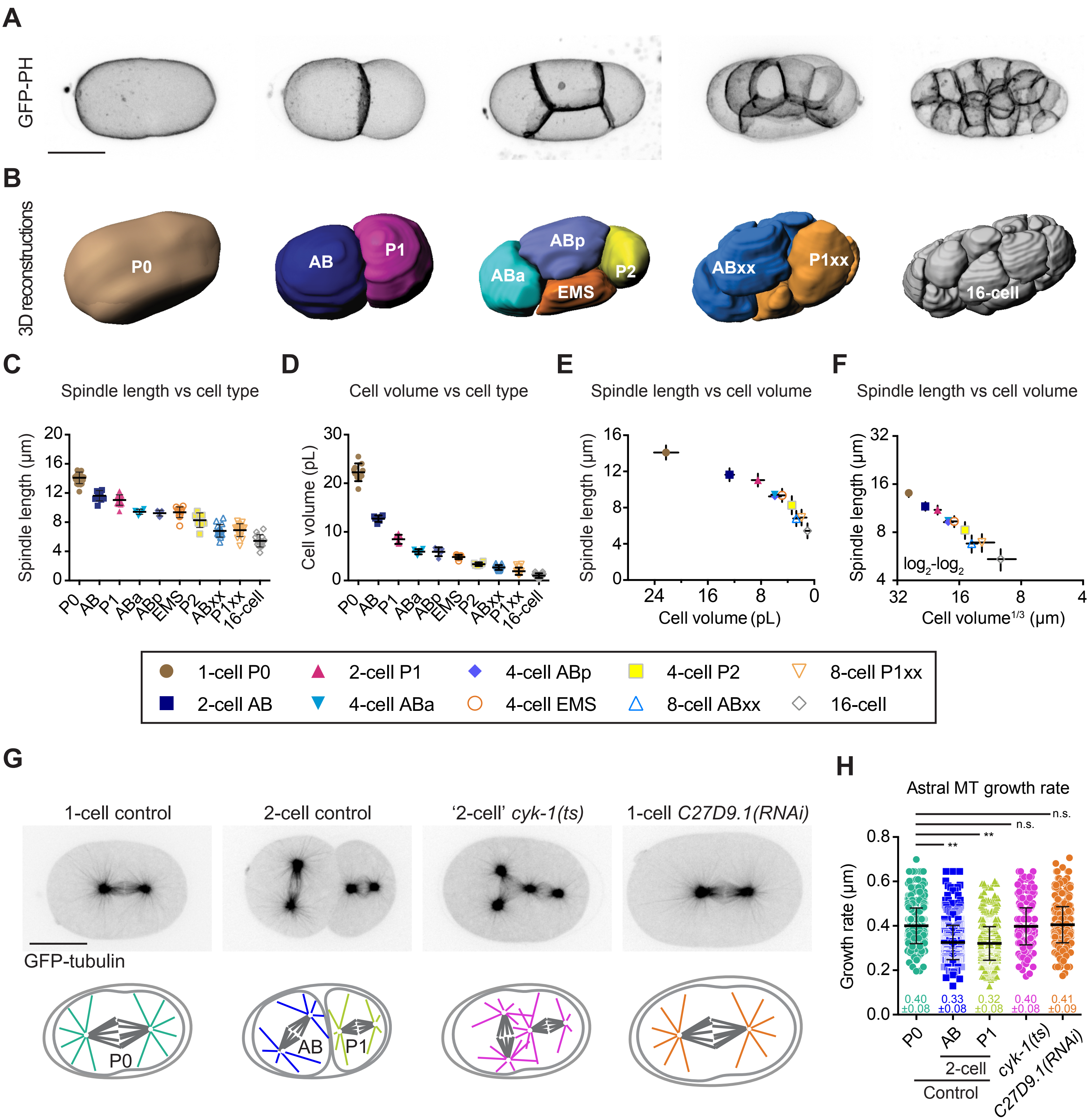
Figure S1



A
GFP-PH
B
3D reconstructions
P0
AB
P1
ABa
ABp
P2
EMS
ABxx
P1xx
16-cell
C
Spindle length vs cell type
Spindle length (μm)
D
Cell volume vs cell type
Cell volume (pL)
E
Spindle length vs cell volume
Cell volume (pL)
F
Spindle length vs cell volume
log2-log2
Cell volume1/3 (μm)
1-cell P0
2-cell AB
2-cell P1
4-cell ABa
4-cell ABp
4-cell EMS
4-cell P2
8-cell ABxx
8-cell P1xx
16-cell
G
1-cell control
2-cell control
'2-cell' cyk-1(ts)
1-cell C27D9.1(RNAi)
GFP-tubulin
H
Astral MT growth rate
Growth rate (μm)
n.s.
**
0.40 ±0.08
0.33 ±0.08
0.32 ±0.08
0.40 ±0.08
0.41 ±0.09
2-cell
Control
cyk-1(ts)
C27D9.1(RNAi)

Figure S1. Allometric Relationship Between Spindle Length and Cell Volume in the *C. elegans* Embryo. Related to Figure 1 and 2

(A) Still frames from live 2-photon imaging of *C. elegans* embryos expressing a GFP-tagged plasma membrane probe (Pleckstrin Homology (PH) domain) during the first five embryonic divisions (1- to 16-cell stage). Images correspond to maximal projections of z-stacks covering the entire thickness of the embryo. Scale bar, 20 µm.

(B) 3D surface reconstructions from live 2-photon imaging of *C. elegans* embryos expressing a GFP-tagged plasma membrane probe (PH domain) during the first five embryonic divisions (1- to 16-cell stage). Blastomere names are indicated except for the 16-cell stage. At the 8-cell stage, progeny of AB and P1 were grouped together as ABxx and P1xx respectively.

(C) Spindle length at anaphase onset in the different blastomeres during the first five embryonic divisions (1- to 16-cell stage). Each dot corresponds to an individual spindle. (Horizontal bars, mean; error bars, SD; n≥6 per type of blastomere).

(D) Cell volume of the different blastomeres during the first five embryonic divisions (1- to 16-cell stage). Each dot corresponds an individual blastomere. (Horizontal bars, means; error bars, SD; n≥6 per type of blastomere).

(E) Average spindle length plotted over the average cell volume for the different blastomeres. (Error bars, SD).

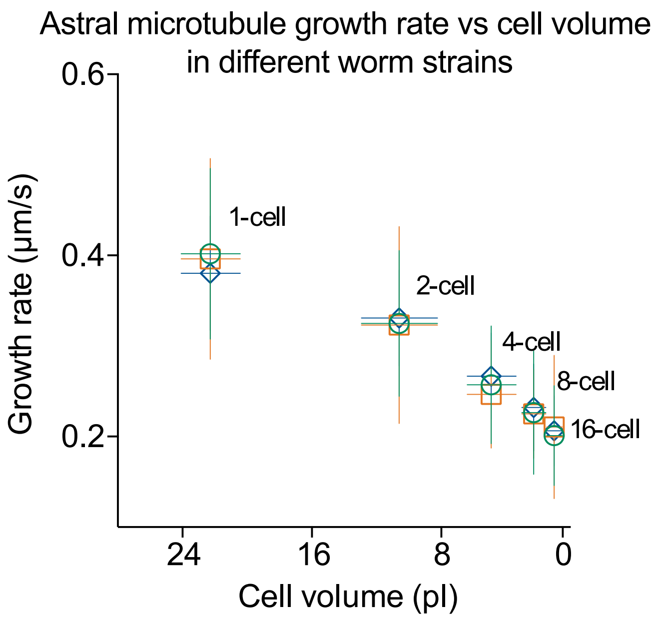
(F) Mean spindle length plotted over the cube root of the average cell volume on a $\log_2$-$\log_2$ scale for the different blastomeres indicated on the left. (Error bars, SD).

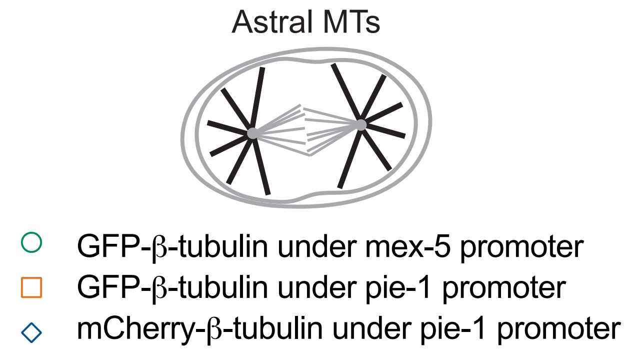
(G) From left to right, still images from confocal live imaging of a *C. elegans* control one-cell embryo, a control 2-cell embryo, a thermosensitive (ts) mutant embryo of the formin *cyk-1* at the '2-cell' stage after P0 cytokinesis failure, and an abnormally large *C27D9.1(RNAi)*-treated embryo. All express GFP-tagged β-tubulin. Corresponding schematics with color-coding for astral microtubules in different conditions shown at the bottom. Scale bar, 20 µm.

(H) Astral microtubule growth rates were measured at 25°C (restrictive temperature for the *cyk-1(ts)* mutant) for the indicated conditions. Color-coding for the different conditions corresponds to the schematics in (G). (Error bars, SD; one-way ANOVA with Dunnett's multiple comparisons test, **: $p \leq 0.01$, n.s.: $p > 0.05$).

Figure S2

A





B

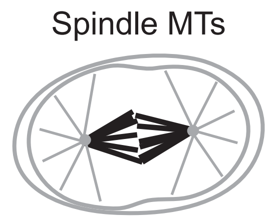
$$J = \frac{v_g f_r - v_s f_c}{f_c + f_r}$$

$$<L> = \frac{v_g v_s}{v_s f_c - v_g f_r}$$

J	:	Average growth velocity (µm.s^{-1})
$<L>$	:	Average MT length (µm)
v_g	:	Growth rate (µm.s^{-1})
v_s	:	Shrinkage rate (µm.s^{-1})
f_c	:	Catastrophe frequency (s^{-1})
f_r	:	Rescue frequency (s^{-1})

from Verde *et al.* 1992

C



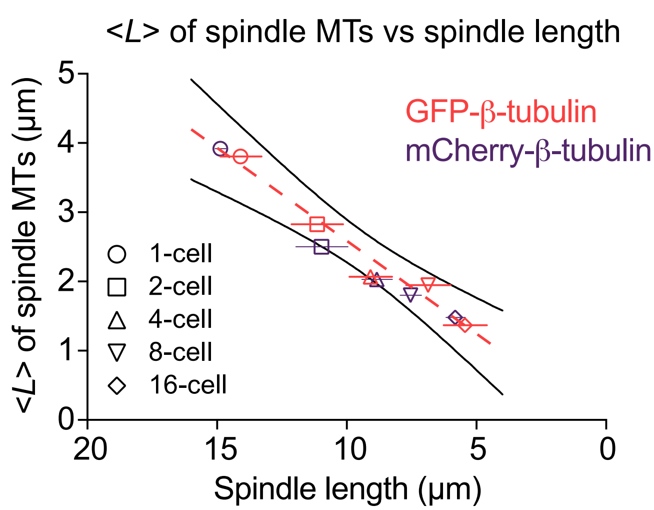


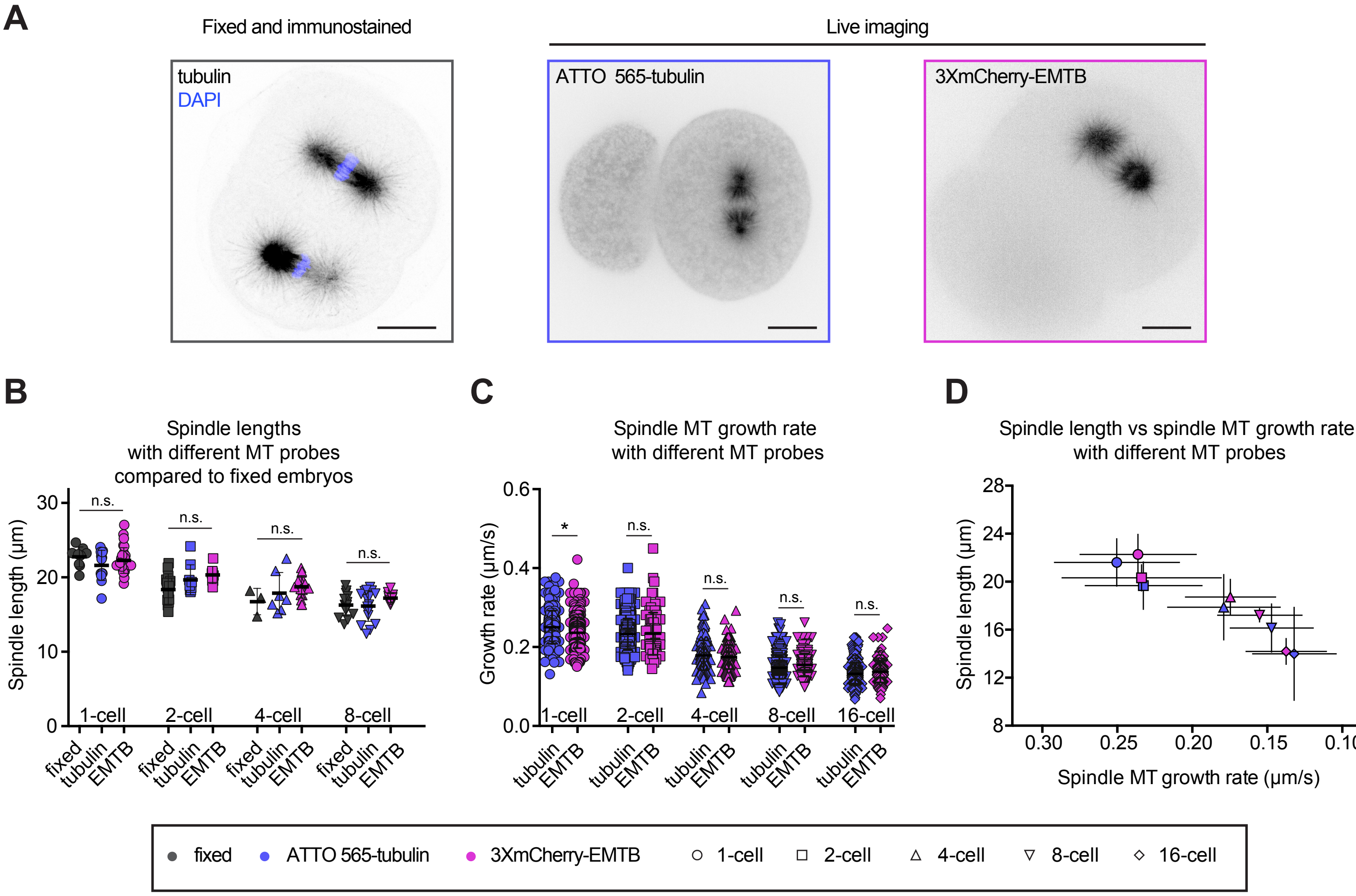
Figure S2. Fluorescent Protein Tags and/or Promoters Used to Drive β-tubulin Expression do not Affect Microtubule Dynamics in the *C. elegans* Embryo. Related to Figures 1 through 3

(A) Mean astral microtubule growth rate plotted over the average cell volume at each cleavage stage (1- to 16-cell stage) for the different worm strains indicated on the right. Astral microtubule growth rate was measured in three different worm strains during early embryonic development from the 1- to the 16-cell stage. Microtubule growth rate was tracked using GFP-tagged β-tubulin expressed under two different embryonic promoters (mex-5 or pie-1) or using mCherry-tagged β-tubulin (under the pie-1 promoter). ($n \geq 6$ per type of blastomere; error bars, SD).

(B) Formulas used for the calculation of *J* (estimated average growth velocity of a microtubule population) and *<L>* (estimated microtubule average length) from (Verde et al., 1992).

(C) Average theoretical microtubule length *<L>* calculated using the formula in (B) and plotted over the experimentally measured average spindle length in the indicated conditions (GFP-tagged β-tubulin under mex-5: red, mCherry-tagged β-tubulin under pie-1: purple). Since microtubules were in a bounded state ($J<0$) under all conditions, *<L>* could be calculated. Dotted red and black lines are the linear regression curve and 95% confidence interval respectively for the GFP-tagged β-tubulin under the mex-5 promoter (red) used in main Figures 1 to 3.

Figure S3



E

Spindle length (µm) in *P. lividus* embryos using different probes

	fixed embryos			ATTO 565-tubulin			3XmCherry-EMTB		
stage	mean (µm)	SD	N	mean (µm)	SD	N	mean (µm)	SD	N
1-cell	22.75	1.34	9	21.61	2.02	13	22.28	1.72	29
2-cell	18.36	2.11	20	19.68	2.02	9	20.33	1.14	8
4-cell	16.74	1.77	4	17.87	2.77	7	18.72	1.52	16
8-cell	16.27	1.45	16	16.14	2.05	17	17.21	0.6	14
16-cell	-	-	-	13.99	3.92	7	14.19	1.12	24

F

Spindle MT growth rate (µm/s) in *P. lividus* using different probes

	ATTO 565-tubulin			3XmCherry-EMTB		
stage	mean (µm/s)	SD	N	mean (µm/s)	SD	N
1-cell	0.25	0.04	276	0.24	0.04	381
2-cell	0.23	0.04	391	0.23	0.05	83
4-cell	0.18	0.04	187	0.17	0.03	173
8-cell	0.15	0.03	500	0.16	0.03	136
16-cell	0.13	0.03	341	0.14	0.03	182

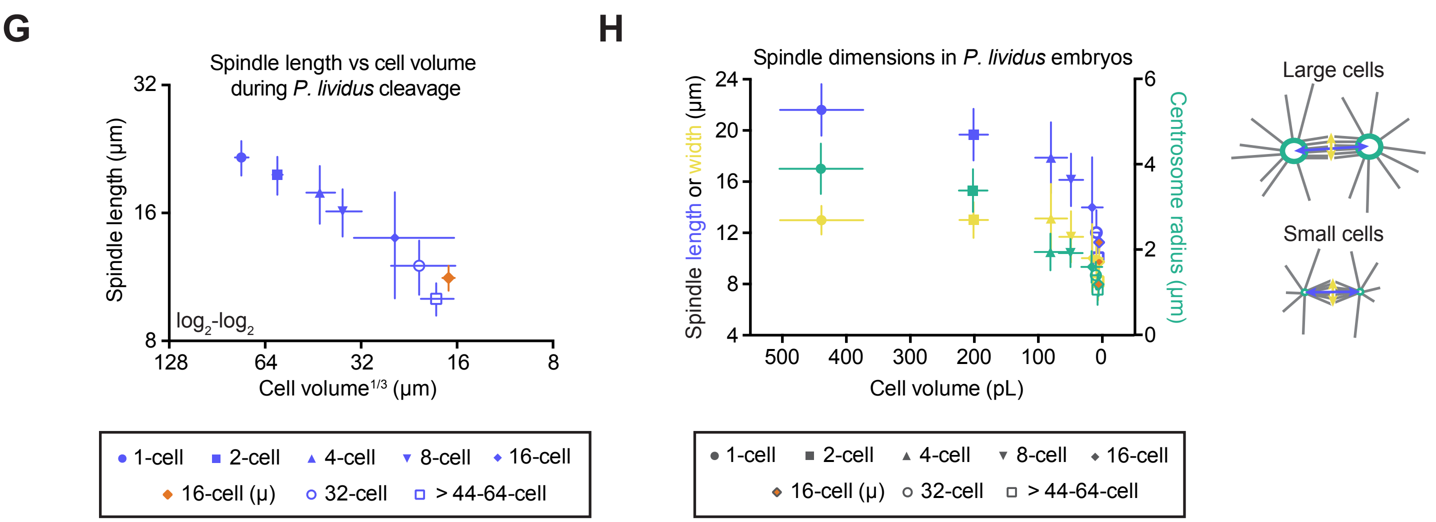


Figure S3. Probing the Allometric Relationship Between Microtubule Dynamics, Spindle Length/Width and Cell Volume in the *P. lividus* Embryo. Related to Figure 4

(A) Still images of 2-cell stage *Paracentrotus lividus* embryos with microtubules labeled using different methods. (Left) Fixed embryo with tubulin immunostaining and DAPI-labeled chromosomes. (Center and Right) Still images from live imaging of embryos injected with labeled pig brain tubulin (ATTO 565-tubulin) or the ensconsin microtubule-binding domain (3XmCherry-EMTB).

(B) Comparison of spindle lengths in immunostained embryos (grey), ATTO 565-tubulin injected live embryos (blue) or 3XmCherry-EMTB injected live embryos (magenta) at the indicated stages (Error bars, SD; one-way ANOVA comparison within each stage, n.s.: $p>0.05$). (n of analyzed spindle ≥ 4 per condition).

(C) Spindle microtubule growth rate measured with ATTO 565-tubulin (blue) or 3XmCherry-EMTB (magenta) at the indicated stages. (Error bars, SD; one-way ANOVA with Sidak's multiple comparisons test, **: $p\leq0.01$, n.s.: $p>0.05$). n(cells/stage)≥5, n(microtubules/stage)≥55.

(D) Average spindle length plotted over the corresponding mean spindle microtubule growth rate in the indicated cleavage stage and conditions (ATTO 565-tubulin in blue, 3XmCherry-EMTB in magenta). Key for the different cleavage stages shown in the bottom box. (Error bars, SD).

(E) Table corresponding to (B). Spindle length from the 1- to the 16-cell stage in *P. lividus* fixed embryos and in ATTO 565-tubulin or 3XmCherry-EMTB injected live embryos.

(F) Table corresponding to (C). Spindle microtubule growth from the 1- to the 16-cell stage in *P. lividus* in ATTO 565-tubulin or 3XmCherry-EMTB injected live embryos.

(G) Mean spindle length in *P. lividus* measured at anaphase onset and plotted over the cube root of the average cell volume for the different blastomeres on a $\log_2$-$\log_2$ scale. (Error bars, SD; n≥5 per stage).

(H) Mean spindle length (blue) and width (yellow) (left Y-axis) and centrosome radius (green) (right Y-axis) plotted over the corresponding average cell volume for the different stages. (Error bars, SD). A schematic representation of the spindle shape change observed between large and small cells in *P. lividus* embryos shown on the right.

Figure S4

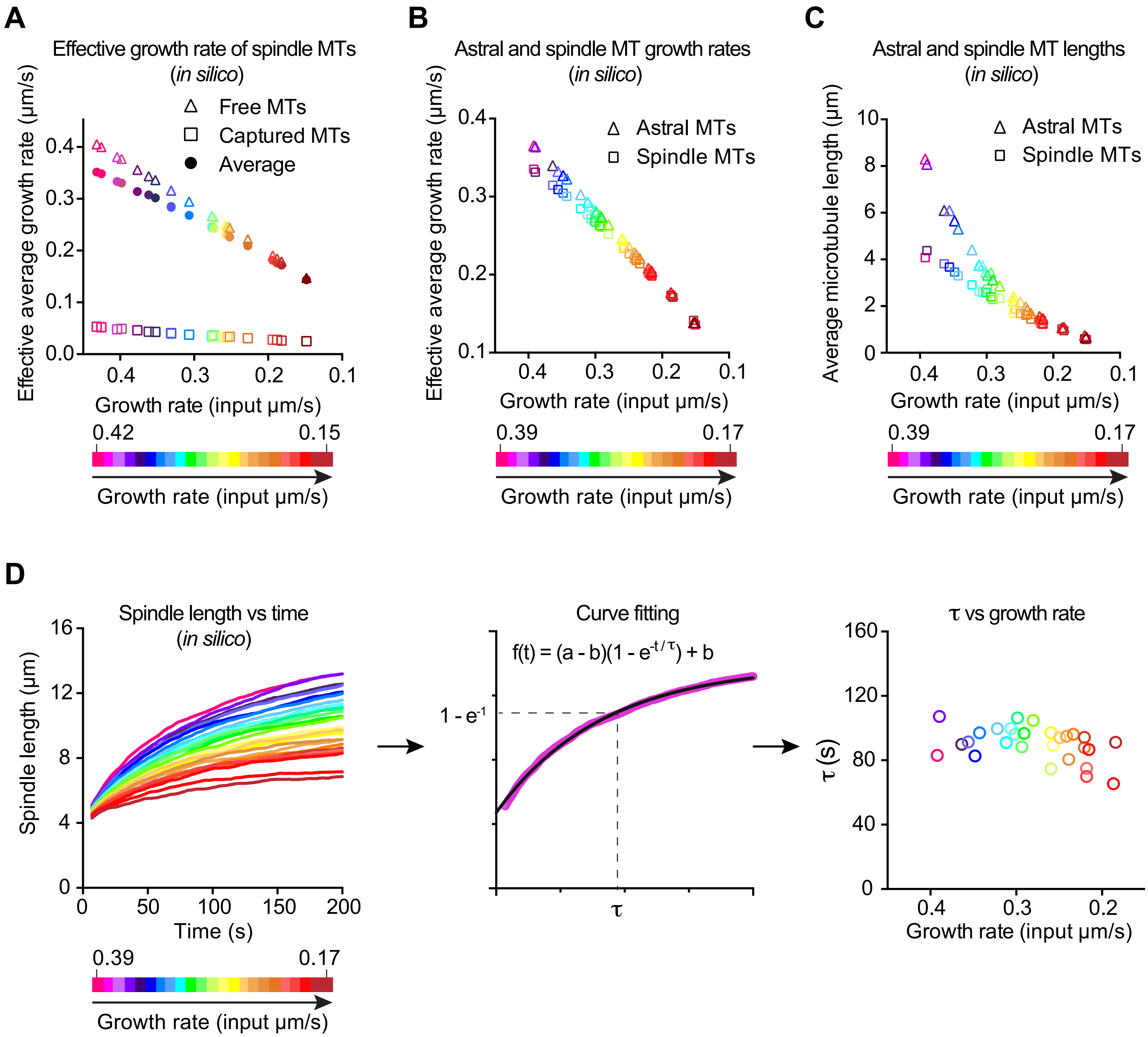

A
Effective growth rate of spindle MTs
(*in silico*)
Free MTs
Captured MTs
Average
Effective average growth rate (μm/s)
0.0
0.1
0.2
0.3
0.4
0.4
0.3
0.2
0.1
Growth rate (input μm/s)
0.42
0.15
Growth rate (input μm/s)
B
Astral and spindle MT growth rates
(*in silico*)
Astral MTs
Spindle MTs
Effective average growth rate (μm/s)
0.1
0.2
0.3
0.4
0.4
0.3
0.2
0.1
Growth rate (input μm/s)
0.39
0.17
Growth rate (input μm/s)
C
Astral and spindle MT lengths
(*in silico*)
Astral MTs
Spindle MTs
Average microtubule length (μm)
0
2
4
6
8
10
0.4
0.3
0.2
0.1
Growth rate (input μm/s)
0.39
0.17
Growth rate (input μm/s)
D
Spindle length vs time
(*in silico*)
Spindle length (μm)
0
4
8
12
16
0
50
100
150
200
Time (s)
0.39
0.17
Growth rate (input μm/s)
Curve fitting
f(t) = (a - b)(1 - e^{-t/τ}) + b
1 - e^{-1}
τ
τ vs growth rate
τ (s)
0
40
80
120
160
0.4
0.3
0.2
Growth rate (input μm/s)

Figure S4. Microtubule Dynamics and Spindle Assembly Timing in the Computational 3D Simulations. Related to Figure 5

(A) Simulated effective growth rate of spindle microtubules growing freely in the cytoplasm (triangles), captured at metaphase plate (squares), or the average of both populations (plain circles) plotted over the input growth rate. Color-coding of the input growth rate indicated at the bottom, from magenta (0.42 μm/s) to dark red (0.15 μm/s). See Table S3 and Methods for more details.

(B) Simulated effective growth rate of astral (triangles) and spindle (squares) microtubules plotted over the input growth rate. Color-coding of the input growth rate indicated at the bottom, from magenta (0.39 μm/s) to dark red (0.17 μm/s).

(C) Simulated average astral (triangles) and spindle (squares) microtubule lengths plotted over the input growth rate. Color-coding is the same as in (B).

(D) Spindle assembly timing is independent of the input microtubule growth rate in the computational 3D simulations. (Left) Simulated spindle length plotted over time at various microtubule growth rates for the simulation shown in Figure 5A. Color-coding of the growth rate indicated at the bottom, from magenta (0.39 μm/s) to red (0.17 μm/s). (Center) Example of curve fitting for one input growth rate (0.39 μm/s). *In silico* data fitted to single exponential functions $f(t) = (a - b)(1 - e^{-\frac{t}{\tau}}) + b$, where 'a' corresponds to the plateau, 'b' to the initial spindle length and 'τ' to the time constant required to reach ~63% of the maximal amplitude ($1 - e^{-1}$ when t = τ). (Right) The values of τ extracted for each input growth rate and plotted over the input growth rate.

Figure S5

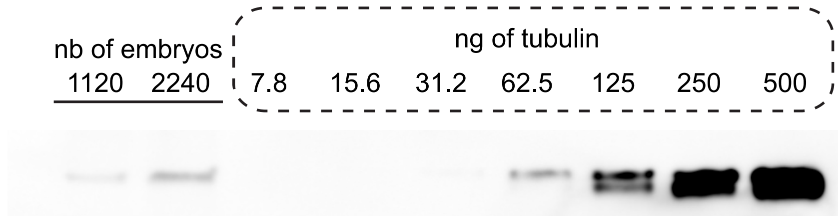

nb of embryos
1120 2240
ng of tubulin
7.8 15.6 31.2 62.5 125 250 500

Figure S5. Quantitative Western Blot for the Estimation of the Tubulin Concentration in Early *C. elegans* Embryos. Related to Figure 8

Quantitative western blot using an anti-α-tubulin antibody (DM1α) on 1- to ~64-cell stage embryo extracts and on increasing amounts of purified pig brain tubulin. Estimations of tubulin quantity and concentration are described in the Methods section.

Table S1. Microtubule Dynamics Parameters in *C. elegans* Embryos. Related to Figures 1 to 3 and Figure S2

A

Astral microtubule dynamics in control and RNAi-treated embryos

Control		Growth rate (μm/s)			Shrinkage rate (μm/s)			Catastrophe freq. (s^{-1})			Rescue freq. (s^{-1})			Cell volume (pL)			Spindle length (μm)			*J*	<*L*>
stage	cell	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	(μm/s)	(μm)
1-cell	P0	0.4	0.09	2080	0.71	0.38	1129	0.19	0.07	2080	0.32	0.14	1129	22.27	1.83	10	14.09	0.79	24	-0.01	31.55
2-cell	All	0.32	0.08	1320	0.62	0.23	904	0.24	0.07	1320	0.39	0.14	904	10.6	2.35	12	11.14	0.99	23	-0.03	10.58
2-cell	AB	0.32	0.08	610	0.63	0.27	411	0.22	0.06	610	0.38	0.14	411	12.73	0.66	6	11.28	1.29	10	-0.02	10.94
2-cell	P1	0.32	0.08	791	0.61	0.23	545	0.24	0.07	791	0.41	0.14	545	8.48	0.93	6	11.04	0.72	13	-0.02	10.41
4-cell	All	0.26	0.07	2443	0.67	0.26	1630	0.24	0.08	2443	0.43	0.21	1630	4.9	1.52	23	9.08	0.82	33	-0.07	3.47
4-cell	Aba	0.26	0.06	330	0.64	0.22	178	0.25	0.08	330	0.49	0.18	178	5.93	0.54	6	9.42	0.36	6	-0.04	4.64
4-cell	Abp	0.25	0.07	385	0.67	0.24	227	0.23	0.07	385	0.44	0.23	227	5.97	1.04	5	9.25	0.39	7	-0.05	4.04
4-cell	EMS	0.26	0.07	975	0.7	0.28	634	0.25	0.1	975	0.51	0.2	634	4.84	0.44	7	9.35	0.46	12	-0.04	4.74
4-cell	P2	0.25	0.06	754	0.68	0.34	596	0.23	0.09	754	0.37	0.19	596	3.37	0.38	6	8.27	1.00	7	-0.07	2.63
8-cell	All	0.23	0.07	1647	0.57	0.22	1103	0.29	0.10	1647	0.47	0.18	1103	2.29	0.74	27	6.86	0.85	34	-0.07	2.31
8-cell	Abxx	0.23	0.07	1367	0.56	0.23	892	0.3	0.1	1367	0.48	0.19	892	2.69	0.5	13	6.81	0.85	16	-0.07	2.08
8-cell	P1xx	0.22	0.06	653	0.57	0.25	469	0.27	0.09	653	0.47	0.17	469	1.91	0.74	14	6.91	0.88	18	-0.06	2.4
16-cell	16-cell	0.2	0.05	639	0.51	0.21	413	0.32	0.11	639	0.45	0.18	413	1.02	0.48	18	5.44	0.84	15	-0.09	1.4
cls-2^{CLASP}(RNAi)		mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	(μm/s)	(μm)
1-cell	P0	0.3	0.06	321	0.6	0.18	272	0.23	0.06	321	0.35	0.14	272	-	-	-	8.97	0.94	8	-0.04	5.40
C27D9.1(RNAi)		mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	(μm/s)	(μm)
1-cell	P0	0.41	0.08	740	0.65	0.2	552	0.2	0.06	740	0.3	0.1	552	-	-	-	18.41	1.01	17	-0.008	39.55

Spindle microtubule dynamics in control and RNAi-treated embryos

Control		Growth rate (μm/s)			Shrinkage rate (μm/s)			Catastrophe freq. (s^{-1})			Rescue freq. (s^{-1})			Cell volume (pL)			Spindle length (μm)			*J*	<*L*>
stage	cell	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	(μm/s)	(μm)
1-cell	P0	0.3	0.07	1021	0.65	0.26	743	0.28	0.1	1021	0.44	0.18	743	22.27	1.83	10	14.09	0.79	24	-0.05	3.89
2-cell	All	0.26	0.06	1059	0.72	0.34	679	0.36	0.10	1059	0.70	0.29	679	10.6	2.35	12	11.14	0.99	23	-0.07	2.48
2-cell	AB	0.27	0.05	355	0.75	0.36	246	0.34	0.09	355	0.65	0.28	246	12.73	0.66	6	11.28	1.29	10	-0.07	2.63
2-cell	P1	0.26	0.06	704	0.71	0.33	433	0.37	0.1	704	0.72	0.3	433	8.48	0.93	6	11.04	0.72	13	-0.07	2.44
4-cell	All	0.21	0.05	625	0.66	0.30	405	0.34	0.09	625	0.72	0.29	405	4.9	1.52	23	9.08	0.82	33	-0.07	2.01
4-cell	Aba	0.22	0.04	47	0.54	0.17	43	0.31	0.07	47	0.62	0.18	43	5.93	0.54	6	9.42	0.36	6	-0.04	3.98
4-cell	Abp	0.22	0.05	62	0.51	0.13	53	0.32	0.07	62	0.6	0.16	53	5.97	1.04	5	9.25	0.39	7	-0.04	3.42
4-cell	EMS	0.22	0.05	359	0.69	0.31	226	0.34	0.1	359	0.78	0.33	226	4.84	0.44	7	9.35	0.46	12	-0.07	2.12
4-cell	P2	0.2	0.05	173	0.69	0.33	99	0.34	0.08	173	0.73	0.29	99	3.37	0.38	6	8.27	1.00	7	-0.09	1.57
8-cell	All	0.20	0.04	909	0.59	0.24	555	0.31	0.11	909	0.60	0.26	555	2.29	0.74	27	6.86	0.85	34	-0.07	1.78
8-cell	Abxx	0.2	0.04	247	0.54	0.21	173	0.28	0.09	247	0.52	0.19	173	2.69	0.5	13	6.81	0.85	16	-0.06	2
8-cell	P1xx	0.2	0.04	662	0.61	0.25	382	0.32	0.12	662	0.64	0.29	382	1.91	0.74	14	6.91	0.88	18	-0.07	1.74
16-cell	16-cell	0.17	0.05	422	0.65	0.31	288	0.33	0.1	422	0.77	0.29	288	1.02	0.48	18	5.44	0.84	15	-0.08	1.46
cls-2^{CLASP}(RNAi)		mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	(μm/s)	(μm)
1-cell	P0	0.25	0.06	556	0.63	0.20	458	0.33	0.12	556	0.52	0.22	458	-	-	-	8.97	0.94	8	-0.08	2.02
C27D9.1(RNAi)		mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	(μm/s)	(μm)
1-cell	P0	0.38	0.08	772	0.65	0.16	758	0.27	0.07	772	0.35	0.03	758	-	-	-	18.41	1.01	17	-0.07	5.78

B

Astral microtubule growth rates in different *C. elegans* strains

	mex-5p>GFP-β-tubulin			pie-1p>GFP-β-tubulin			pie-1p>mCherry-β-tubulin		
	mean	SD	n	mean	SD	n	mean	SD	n
1-cell	0.40	0.09	2080	0.40	0.11	295	0.38	0.06	321
2-cell	0.32	0.08	1320	0.32	0.11	188	0.33	0.05	333
4-cell	0.26	0.07	2443	0.25	0.06	234	0.27	0.05	442
8-cell	0.23	0.07	1647	0.23	0.05	197	0.23	0.05	378
16-cell	0.20	0.05	639	0.21	0.08	114	0.21	0.04	295

C

Spindle microtubule dynamics in different *C. elegans* strains

	Growth rate (μm/s)			Shrinkage rate (μm/s)			Catastrophe frequency (s^{-1})			Rescue frequency (s^{-1})			*J*	<*L*>
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	*μm/s*	*μm*
mex-5p>GFP-β-tubulin														
1 cell	0.30	0.07	1021	0.65	0.26	743	0.28	0.10	1021	0.44	0.18	743	-0.05	3.89
2 cells	0.26	0.06	1059	0.72	0.34	679	0.36	0.10	1021	0.70	0.29	679	-0.07	2.48
4 cells	0.21	0.05	625	0.66	0.30	405	0.34	0.09	625	0.72	0.29	405	-0.07	2.01
8 cells	0.20	0.04	909	0.59	0.24	555	0.31	0.11	909	0.60	0.26	555	-0.07	1.78
16 cells	0.17	0.05	422	0.65	0.31	288	0.33	0.10	422	0.77	0.29	288	-0.08	1.46
pie-1p>mCherry-β-tubulin														
1 cell	0.29	0.04	171	0.65	0.15	183	0.25	0.04	171	0.40	0.09	183	-0.05	3.78
2 cells	0.26	0.04	149	0.66	0.18	119	0.32	0.09	149	0.58	0.22	119	-0.06	2.80
4 cells	0.22	0.04	194	0.66	0.19	144	0.35	0.08	194	0.73	0.25	144	-0.07	2.04
8 cells	0.20	0.04	213	0.62	0.18	123	0.32	0.08	213	0.68	0.21	123	-0.07	1.92
16 cells	0.18	0.03	278	0.60	0.18	266	0.32	0.06	278	0.63	0.17	266	-0.09	1.35

Table S2. Microtubule Dynamics, Spindle and Cell Dimensions in *Caenorhabditis elegans* and *Paracentrotus lividus*. Related to Figures 4, 5, 8 and S4

A

C. elegans* and *P. lividus **spindle microtubule dynamics parameters, spindle lengths, and cell volume**

C. elegans	Growth rate (µm/s)			Shrinkage rate (µm/s)			Catastrophe freq. (s^{-1})			Rescue freq. (s^{-1})			Spindle length (µm)			Cell volume (pL)			*J*	<L>
stage	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	(µm/s)	(µm)
1-cell	0.30	0.07	1021	0.65	0.26	743	0.28	0.10	1021	0.44	0.18	743	14.09	0.79	24	22.27	1.83	10	-0.07	3.89
2-cell	0.26	0.06	1059	0.72	0.34	679	0.36	0.10	1059	0.70	0.29	679	11.14	0.99	23	10.6	2.35	12	-0.07	2.48
4-cell	0.21	0.05	625	0.66	0.30	405	0.34	0.09	625	0.72	0.29	405	9.08	0.82	33	4.9	1.52	23	-0.07	2.01
8-cell	0.20	0.04	909	0.59	0.24	555	0.31	0.11	909	0.60	0.26	555	6.86	0.85	34	2.29	0.74	27	-0.07	1.78
16-cell	0.17	0.05	422	0.65	0.31	288	0.33	0.10	422	0.77	0.29	288	5.44	0.84	15	1.02	0.48	18	-0.07	1.46

P. lividus	Growth rate (µm/s)			Shrinkage rate (µm/s)			Catastrophe freq. (s^{-1})			Rescue freq. (s^{-1})			Spindle length (µm)			Cell volume (pL)			*J*	<L>
stage	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	(µm/s)	(µm)
1-cell	0.25	0.04	276	0.47	0.19	104	0.14	0.04	276	0.23	0.07	104	21.61	2.02	13	438.81	65.0	13	-0.01	15.01
2-cell	0.23	0.04	391	0.42	0.18	189	0.14	0.04	391	0.22	0.08	189	19.68	2.02	9	201.19	22.38	9	-0.02	10.24
4-cell	0.18	0.04	187	0.37	0.09	103	0.15	0.04	187	0.24	0.07	103	17.87	2.77	7	80.1	25.05	7	-0.03	5.02
8-cell	0.15	0.03	500	0.36	0.11	276	0.13	0.03	500	0.23	0.06	276	16.14	2.05	17	48.77	18.73	17	-0.03	3.95
16-cell	0.13	0.03	341	0.37	0.12	189	0.13	0.03	341	0.24	0.08	189	13.99	3.92	7	15.74	16.39	7	-0.03	3.04
16-cell µ	0.11	0.01	92	0.35	0.07	72	0.12	0.03	92	0.26	0.06	72	11.26	0.76	5	4.91	0.66	5	-0.03	2.3
32-cell	0.11	0.02	150	0.38	0.15	55	0.12	0.03	150	0.26	0.09	55	12.03	1.75	9	9.28	6.38	9	-0.04	2.42
44-64-cell	0.11	0.02	97	0.35	0.11	58	0.15	0.04	97	0.28	0.09	58	10.05	0.88	5	6.43	2.29	5	-0.04	1.98

B

C. elegans **and** ***P. lividus*** **cell and spindle dimensions**

C. elegans	Spindle length (µm)			Spindle width (µm)			Metaphase plate thickness(µm)			Centrosome radius (µm)			Cell perimeter (µm)			Cell volume (pL)		
stage	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	error	N
1-cell	14.09	0.79	24	5.96	0.84	24	1.3	0.33	34	0.76	0.14	35	135.73	6.81	24	22.27	1.83	10
2-cell	11.14	0.99	23	5.35	0.47	23	1.09	0.24	56	0.59	0.13	63	94.62	9.2	27	10.6	2.35	12
4-cell	9.08	0.82	33	5.64	2.83	33	1.03	0.21	25	0.48	0.08	37	68.89	7.33	43	4.9	1.52	23
8-cell	6.86	0.85	34	3.99	0.47	34	1.19	0.36	51	0.44	0.08	47	48.69	5.7	39	2.29	0.74	27
16-cell	5.44	0.84	15	3.21	0.42	15	0.81	0.13	24	0.35	0.06	36	34.26	5.74	19	1.02	0.48	18

P. lividus	Spindle length (µm)			Spindle width (µm)			Metaphase plate thickness(µm)			Centrosome radius (µm)			Cell perimeter (µm)			Cell volume (pL)		
stage	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N	mean	error	N
1-cell	21.61	2.02	13	12.99	1.11	13	-	-	-	3.9	0.59	26	296.19	14.62	13	438.81	65.0	13
2-cell	19.68	2.02	9	13.01	1.4	9	-	-	-	3.39	0.5	18	228.39	8.47	9	201.19	22.38	9
4-cell	17.87	2.77	7	13.13	2.7	7	-	-	-	1.95	0.43	14	168.02	17.51	7	80.1	25.05	7
8-cell	16.14	2.05	17	11.69	2	17	-	-	-	1.93	0.33	34	142.41	18.23	17	48.77	18.73	17
16-cell	13.99	3.92	7	10.03	2.68	7	-	-	-	1.6	0.37	14	97.67	33.92	7	15.74	16.39	7
16-cell µ	11.26	0.76	5	9.74	1.62	5	-	-	-	1.19	0.27	12	66.26	2.96	5	4.91	0.66	5
32-cell	12.03	1.75	9	9.77	1.39	9	-	-	-	1.41	0.31	18	81.91	18.75	9	9.28	6.38	9
44-64-cell	10.05	0.88	5	8.19	1.45	5	-	-	-	1.07	0.36	10	72.49	8.59	5	6.43	2.29	5

Table S3. Parameters used in the simulations. Related to Figures 5 and S4

Parameter		Description/reference
Microtubules (MTs)		
Rigidity	30 pN.µm^2	Corresponds to a persistence length of 7 mm (Gittes et al., 1993)
Growth rate (input)	V_0 = 0.14 to 0.43 µm/s	Varied in each simulation between 0.14 and 0.43 µm/s (range of experimental growth rate observed in *C. elegans* in this study)
Effective growth rate	$V_g = V_0 * \exp(-F/F_{stall})$	Under antagonistic force (F) growth rate was reduced (Dogterom and Yurke 1997)
Stall force	F_{stall} = 5 pN	From Dogterom and Yurke, 1997
Shrinkage rate	0.65 µm/s	This study (average shrinkage rate of spindle MTs from 1- to 16-cell stage in *C. elegans*)
Rescue frequency	0.5 s^{-1}	This study (average rescue frequency of spindle MTs from 1- to 16-cell stage in *C. elegans*)
Catastrophe frequency (fc)	fc_0 = 0.3 ; fc_{stall} = 0.6 s^{-1}	fc_0 corresponds to the average catastrophe frequency of freely growing MTs (measured in this study). The catastrophe frequency of stalled MTs (fc_{stall}) was estimated to be twice that of free MTs ($2xfc_0$). In Cytosim catastrophe frequency can be increased with opposing force as described by Janson et al., 2003. Force-induced catastrophe was only considered in simulations using astral MTs confined in space (Figure 5A-C) Catastrophe frequency is expressed in Cytosim as: $fc = fc_{stall} / (1+ (fc_{stall}/fc_0 - 1)V_g/V_0)$
MT nucleation		MTs were only nucleated at centrosomes, the number of MTs per aster was constant
Centrosomes		
Viscosity	200 pN.s/µm^2	From Letort et al., 2016. In Cytosim the mobility of the beads used to mimic centrosomes is dependent on its effective viscosity (200 pN.s/µm^2). This does not influence MT mobility
Radius	0.5 µm	Average radius measured in this study
Fibers	50 or 500 MTs per aster	50 MTs when only spindle MTs were simulated. 500 MTs including 100 constrained spindle MTs when astral MTs were simulated
Spindle angle	0.6 rad	Constrains spindle MTs into a right circular cone with a semi-angle of ~30°. This angle was estimated from our measurements of spindle geometry (Table S2)
Metaphase plate		
Shape and dimensions	Solid cylinder	Cylinder dimensions varied in each simulation and were extrapolated from experimental data (Table S2). MTs were confined outside the plate
Couple		Couples are made of two singles called « hands » and link MTs to metaphase plate
Hand1	Hands binding to plate	Anchored to the metaphase plate. Avoid MT sliding away when attached to the plate
Hand2	Hands binding to MTs	Bind only free growing MTs with very high binding rate
Confinement space		
Cell space	unconfined or 3D ellipse	Dimensions of the ellipse were extrapolated from *C.elegans* embryo dimensions. In simulations with astral MTs, all objects were confined within the ellipse (Figure 5A-C).
Simulation parameters		
Time step	0.05 s	Nédélec et al., 2007
kT	0.0042 pN.µm	Corresponds to a temperature of 25°C
Viscosity	1 pN.s/µm^2	Cytoplasmic viscosity. Kole et al., 2005; Nedelec and Foethke, 2007; Letort et al., 2016