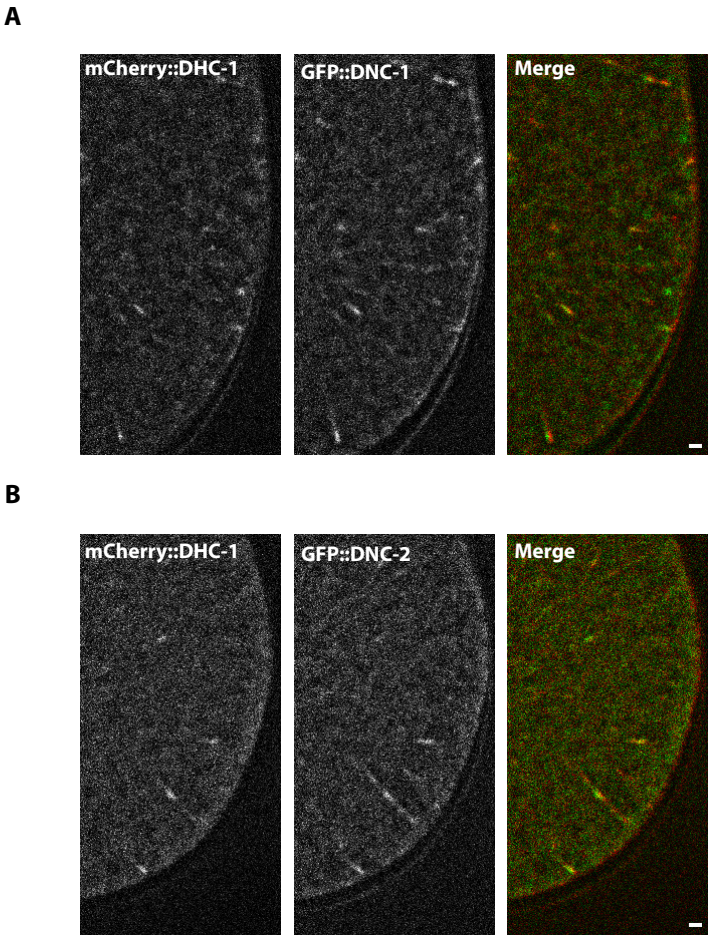
Supplementary Figures

Supplementary Figure 1. **Dynactin components track MT plus-ends together with DHC-1.**

(A, B) Representative simultaneous dual-color SDCLM images of mCherry::DHC-1 (red) and either GFP::DNC-1(A) or GFP::DNC-2 (B) localization during mitosis. Images are averages of 5 consecutive frames taken from 100 ms stream-lapse movies, after background subtraction by a gaussian blur filter. Scale bars, 1 μm.

Fig. S1



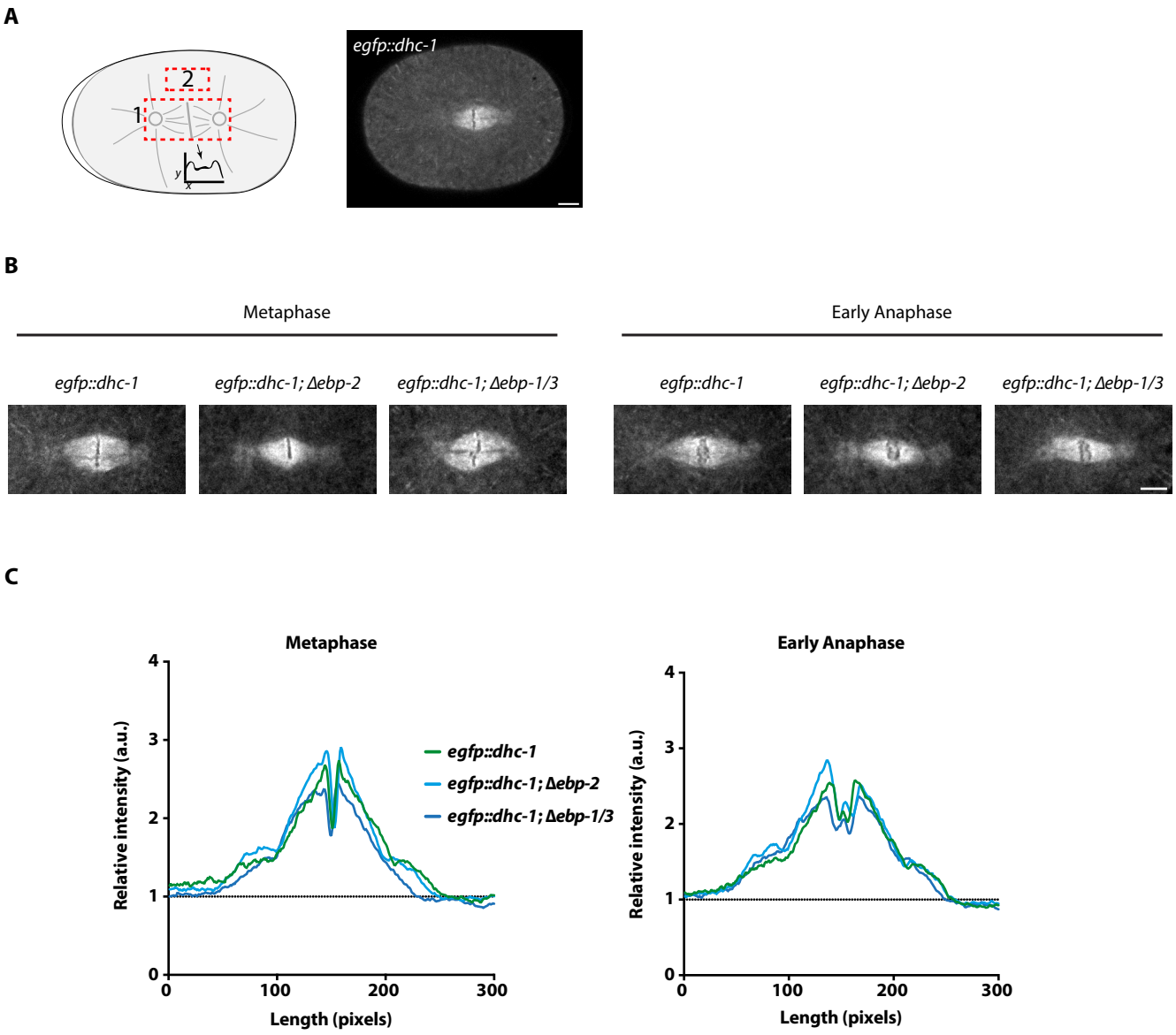
Supplementary Figure 2. **Dynein localization to the spindle midzone, kinetochores, kinetochore MTs and poles is not altered upon loss of EBP-1/2/3.**

(A) Schematic representation (left) of approach to measure eGFP::DHC-1 (right) intensity along the mitotic spindle. Scale bar, 5 µm.

(B) Representative SDCLM images of eGFP::DHC-1 localization in different wt or Δ*ebp* mutant backgrounds as indicated in each image, both during metaphase and early anaphase. Scale bar, 5 µm.

(C) Quantification of dynein distribution along the horizontal axis of the spindle (box 1 in (A)) in different genetic backgrounds as indicated in the graph, represented as average (N=4 spindles per condition) intensity normalized to average cytoplasmic values (measured in box 2 in (A)). Spindle length in pixels.

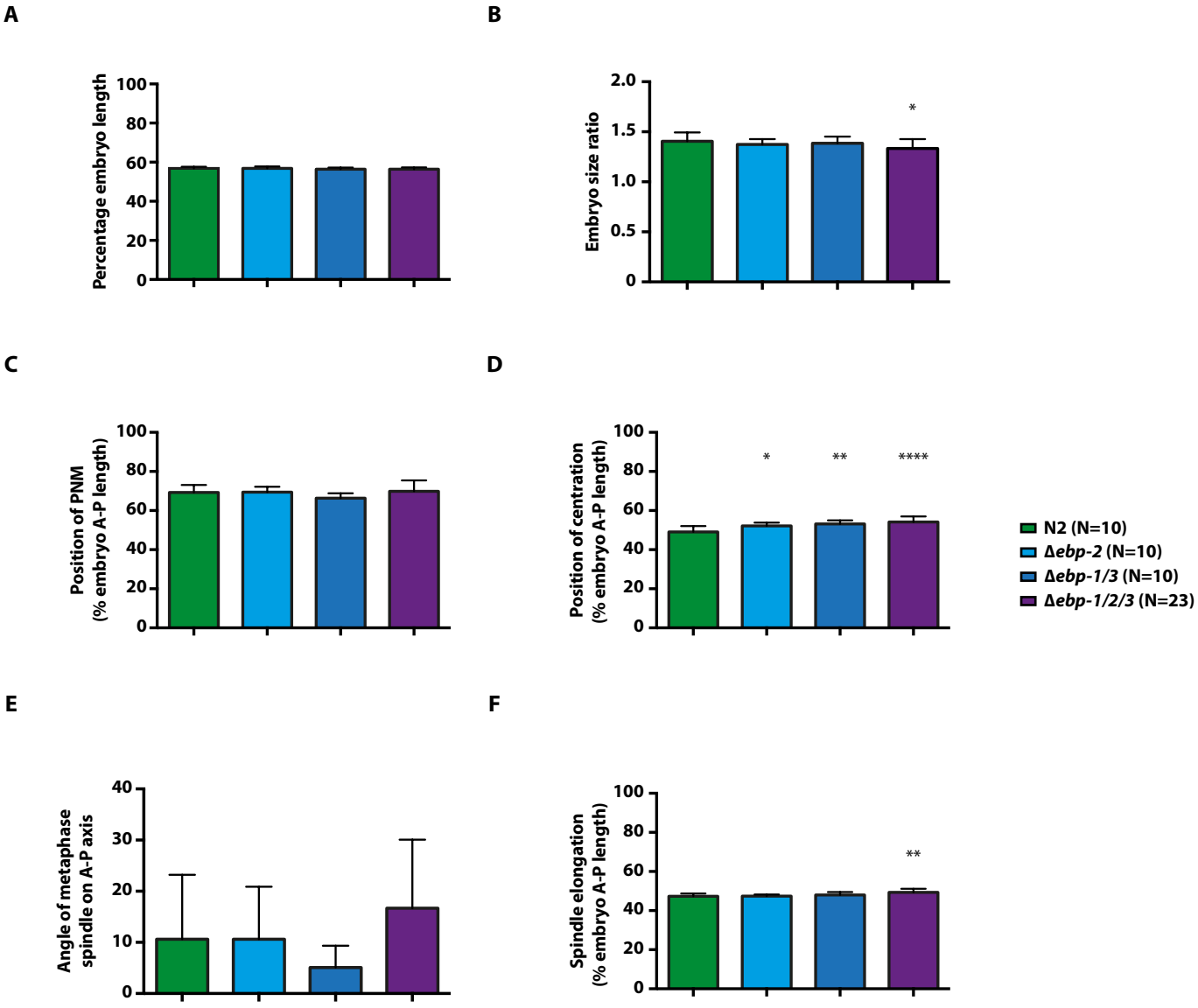
Fig. S2


A
2
1
y
x
egfp::dhc-1
B
Metaphase
Early Anaphase
egfp::dhc-1
egfp::dhc-1; Δebp-2
egfp::dhc-1; Δebp-1/3
egfp::dhc-1
egfp::dhc-1; Δebp-2
egfp::dhc-1; Δebp-1/3
C
Metaphase
Early Anaphase
Relative intensity (a.u.)
Length (pixels)
egfp::dhc-1
egfp::dhc-1; Δebp-2
egfp::dhc-1; Δebp-1/3
0
1
2
3
4
100
200
300

Supplementary Figure 3. **Characterization of key mitotic events in Δ*ebp* mutant embryos.**

Quantification of key mitotic events from DIC time-lapse movies of N2 (green), Δ*ebp-2* (light blue), Δ*ebp-1/3* (dark blue) and Δ*ebp-1/2/3* (purple) embryos during the first division. Asymmetry (A), position of pronuclear meeting (PNM, C) and centration (D) and spindle elongation (F) are plotted as a percentage of total embryo length, embryo size (B) as the ratio of embryo length over embryo height, and metaphase spindle angle as the angle relative to the embryo A-P axis. Bars represent average (N≥10) + SD, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$ compared to wt, no indication means no significant difference from wt. Unpaired Welch Student's *t*-test.

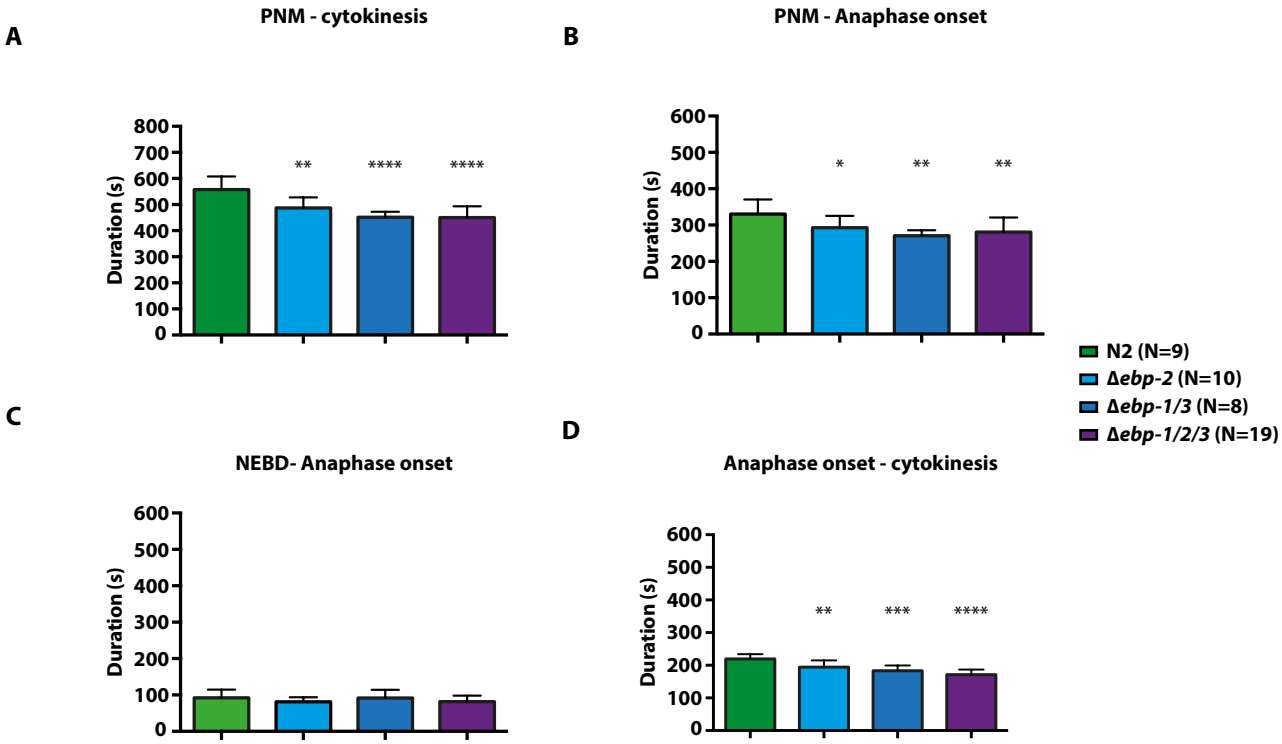
Fig. S3


A
Percentage embryo length
100
80
60
40
20
0
B
Embryo size ratio
2.0
1.5
1.0
0.5
0
*
C
Position of PNM
(% embryo A-P length)
100
80
60
40
20
0
D
Position of centration
(% embryo A-P length)
100
80
60
40
20
0
*
**
****
N2 (N=10)
Δebp-2 (N=10)
Δebp-1/3 (N=10)
Δebp-1/2/3 (N=23)
E
Angle of metaphase
spindle on A-P axis
40
30
20
10
0
F
Spindle elongation
(% embryo A-P length)
100
80
60
40
20
0
**

Supplementary Figure 4. **Quantification of timing of mitotic events in Δ*ebp* mutant one-cell embryos.**

Quantification of duration of mitotic events from DIC time-lapse movies of N2 (green), Δ*ebp-2* (light blue), Δ*ebp-1/3* (dark blue) and Δ*ebp-1/2/3* (purple) embryos during the first division. Duration in seconds. Bars represent average (N≥9) + SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared to wt, no indication means no significant difference from wt. Unpaired Welch Student's *t*-test.

Fig. S4


A
PNM - cytokinesis
Duration (s)
800
700
600
500
400
300
200
100
0
**
****
****
B
PNM - Anaphase onset
Duration (s)
600
500
400
300
200
100
0
*
**
**
N2 (N=9)
Δebp-2 (N=10)
Δebp-1/3 (N=8)
Δebp-1/2/3 (N=19)
C
NEBD- Anaphase onset
Duration (s)
600
500
400
300
200
100
0
D
Anaphase onset - cytokinesis
Duration (s)
600
500
400
300
200
100
0
**
***
****

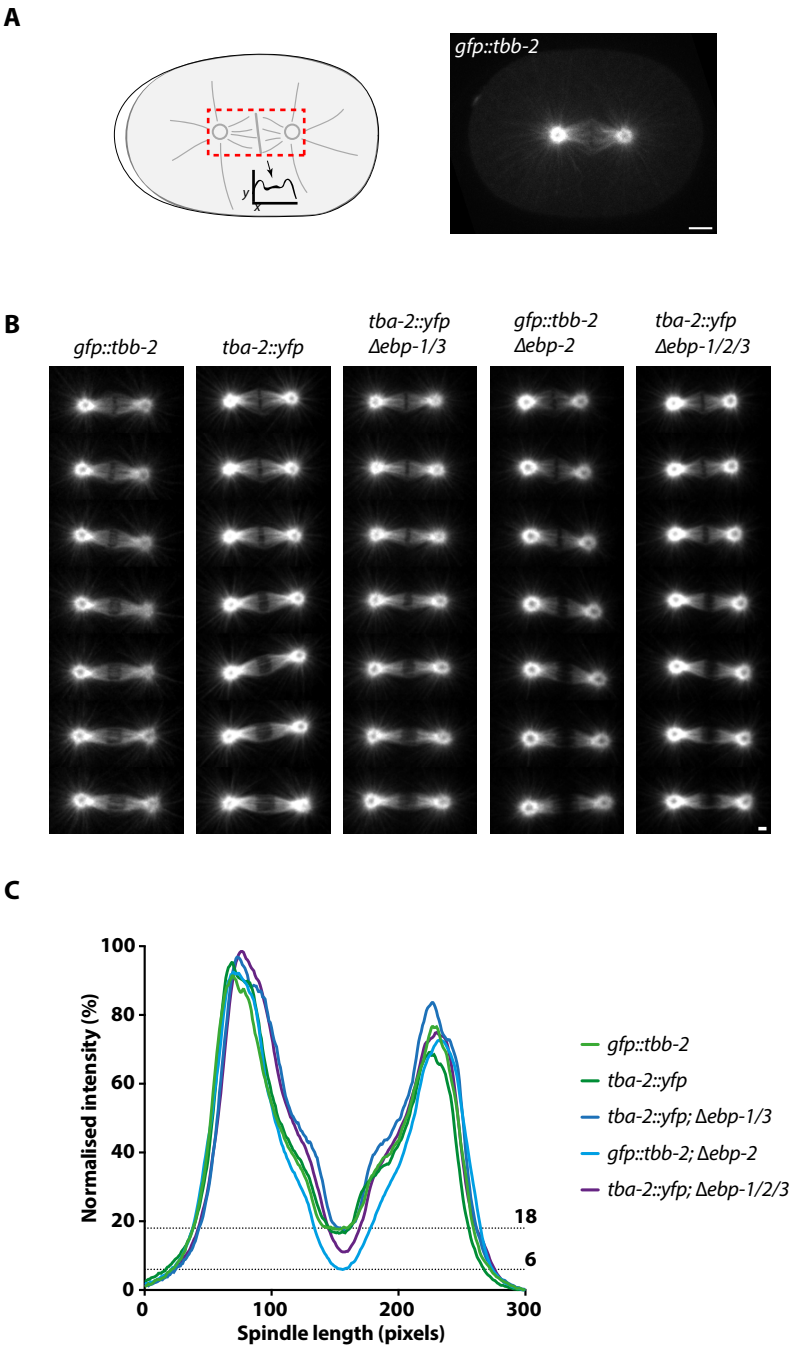
Supplementary Figure 5. **EBP-2 is required for proper spindle midzone establishment.**

(A) Schematic representation (left) of approach to measure MT (right) density along the mitotic spindle.

(B) Montages of representative SDCLM movies of one-cell embryos expressing either GFP::TBB-2 or TBA-2::YFP (MTs), in different wt or Δ*ebp* mutant backgrounds as indicated in each image. Montages are generated from seven consecutive frames from time-lapse movies acquired at a rate of 1 frame per 5 seconds, all acquired with the same laser power and exposure time of 500 ms. Scale bar, 1 µm.

(C) Quantification of MT abundance along the horizontal axis of the spindle in different genetic backgrounds during early anaphase as indicated in the graph, represented as average (N=7 spindles) intensity relative to the maximum value of all data sets. Spindle length in pixels.

Fig. S5


A
gfp::tbb-2
B
gfp::tbb-2
tba-2::yfp
tba-2::yfp
Δebp-1/3
gfp::tbb-2
Δebp-2
tba-2::yfp
Δebp-1/2/3
C
Normalised intensity (%)
100
80
60
40
20
0
0
100
200
300
Spindle length (pixels)
18
6
gfp::tbb-2
tba-2::yfp
tba-2::yfp; Δebp-1/3
gfp::tbb-2; Δebp-2
tba-2::yfp; Δebp-1/2/3

Online supplemental material

Supplementary Movie 1. **Co-localization of mCherry::DHC-1 comets with astral MTs.**
Simultaneous dual-color SDCLM movie of mCherry::DHC-1 (red) and GFP::TBB-2 (MTs, green) localization at the posterior pole during mitosis in a one-cell *C. elegans* embryo. Images were streamed with 100 ms exposure time, averaged for each 2 consecutive frames, and displayed at 15 frames per second. Movie corresponds to figure 2A.

Supplementary Movie 2. **Co-localization of mCherry::DHC-1 comets with EBP-2::GFP.**
Simultaneous dual-color SDCLM movie of mCherry::DHC-1 (red) and EBP-2::GFP (MT plus-end, green) localization at the posterior pole during mitosis in a one-cell *C. elegans* embryo. Images were streamed with 100 ms exposure time, averaged for each 2 consecutive frames, and displayed at 15 frames per second. Movie corresponds to figure 2B.

Supplementary Movie 3. **Cortical co-localization of mCherry::DHC-1 comets with astral MTs.**
Simultaneous dual-color TIRF movie of mCherry::DHC-1 (red) and GFP::TBB-2 (MTs, green) cortical localization during mitosis in a one-cell *C. elegans* embryo. Images were streamed with 100 ms exposure time, averaged for each 5 consecutive frames

after background subtraction by a gaussian blur filter, and displayed at 10 frames per second with the posterior to the right. Movie corresponds to figure 2E.

Supplementary Movie 4. **Cortical co-localization of mCherry::DHC-1 comets with EBP-2::GFP.**
Simultaneous dual-color TIRF movie of mCherry::DHC-1 (red) and EBP-2::GFP (MT plus-end, green) cortical localization during mitosis in a one-cell *C. elegans* embryo. Images were streamed with 100 ms exposure time, averaged for each 5 consecutive frames after background subtraction by a gaussian blur filter, and displayed at 10 frames per second with the posterior to the right. Movie corresponds to figure 2F.

Supplementary Movie 5. **Co-localization of mCherry::DHC-1 comets with PH::eGFP membrane invaginations.**
Simultaneous dual-color SDCLM movie of mCherry::DHC-1 (red) and PH::eGFP (plasma membrane, green) localization at the posterior pole during mitosis in a one-cell *C. elegans* embryo. Images were streamed with 100 ms exposure time, averaged for each 2 consecutive frames, and displayed at 10 frames per second. Movie corresponds to figure 2G.

Supplementary Movie 6. **Localization of eGFP::DHC-1 in Δ*ebp* mutant backgrounds.**

Single-color SDCLM movies of eGFP::DHC-1 localization during mitosis in one-cell *C. elegans* embryos of otherwise wt (left), Δ*ebp-1/3* (middle) and Δ*ebp-2* (right) genetic backgrounds. Images were acquired every 5 seconds with 1000 ms exposure time and equal laser power, background corrected and displayed at 5 frames per second with the posterior to the right. Movies correspond to figure 3A.

Supplementary Movie 7. **Time-lapse DIC movies of wt and Δ*ebp* embryos.**

Wide-field DIC movies of (from left to right) N2, Δ*ebp-2,* Δ*ebp-1/3* and Δ*ebp-1/2/3* one-cell *C. elegans* embryos during mitosis. Images were acquired every 2 seconds with 15 ms exposure time, equal transmitted light intensities and displayed at 20 frames per second with the posterior to the right. Movies correspond to figure 3E.

Supplementary Movie 8. **Two distinct cortical populations of eGFP::DHC-1.**

Single-color TIRF movies of eGFP::DHC-1 cortical localization in (from left to right) otherwise wt, Δ*ebp-1/3,* Δ*ebp-2, lin-5* RNAi and *Δebp-2 + lin-5* RNAi-treated one-cell *C. elegans* embryos. Images were streamed with 50 ms exposure time, averaged for each 10 consecutive frames after background subtraction by a gaussian blur filter, and displayed at 10 frames per second. Movies correspond to figure 4B.

Supplementary Movie 9. **UV-laser mediated spindle midzone severing.** Single-color SDCLM movie of GFP::TBB-2 (MTs) UV-laser-mediated spindle bisection at anaphase onset in a one-cell *C. elegans* embryo. Images were streamed with 500 ms exposure time and displayed at 15 frames per second with the posterior to the right. Movie corresponds to figure 5B.

Supplementary Movie 10. **Residence time of end-on MT-cortex contacts.** Single-color TIRF movie of GFP::TBB-2 (MTs) cortical localization in a one-cell *C. elegans* embryo. Images were streamed with 500 ms exposure time and displayed at 10 frames per second with the posterior to the right. Movie corresponds to figure 5D.

I. Table 1	
Strain	**Genotype**
N2	Wild type
SV1598	he248[dhc-1::mcherry]
SV1619	he250[mcherry::dhc-1]
SV1635	he244 [egfp::lin-5]; he250[mcherry::dhc-1]
TH65	ddIs15 [[unc-119(+) + C47B2.3(genomic)::YFP]]
AZ224	RuIs57[Ppie-1::gfp::tbb-2]
SV1702	RuIs57[Ppie-1::gfp::tbb-2]; he250[mcherry::dhc-1]
SV1703	he258[Peft-3::ph::egfp::lov::tbb-2-3'UTR]; he250[mcherry::dhc-1]
SV1803	he264[egfp::dhc-1]
SV1840	ojIs5[pie-1::GFP::dnc-1 + unc-119(+)]; he250[mcherry::dhc-1]
SV1841	ojIs57[Ppie-1::gfp::dnc-2 unc-119(+)]; he250[mcherry::dhc-1]
SV1857	abcIs3[Ppie-1::ebp-2::gfp; unc-119(+)]; he250[mcherry::dhc-1]
SV1868	he278[Δebp-2]
SV1872	he279[Δebp-1, ΔY59A8B.25, Δebp-3]
SV1873	he278[Δebp-2]; he263[egfp::dhc-1]
SV1874	he278[Δebp-2]; RuIs57[Ppie-1::gfp::tbb-2]
SV1877	he278[Δebp-2]; he279[Δebp-1, ΔY59A8B.25, Δebp-3]
SV1878	he279[Δebp-1, ΔY59A8B.25, Δebp-3]; he263[egfp::dhc-1]
SV1879	he279[Δebp-1, ΔY59A8B.25, Δebp-3]; ddIs15 [[unc-119(+) + C47B2.3(genomic)::YFP]]
SV1900	[unc-119(+) + C47B2.3(genomic)::YFP]; he278[Δebp-2]; he279[Δebp-1, ΔY59A8B.25, Δebp-3]