1 **Normal spindle positioning in the absence of EBPs and dynein plus-end**
2 **tracking in *C. elegans***

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13 **Short title: Two independent dynein populations at the cortex**

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1 **Abstract**

2

3 The position of the mitotic spindle is tightly controlled in animal cells, as it
4 determines the plane and orientation of cell division. Interactions between
5 cytoplasmic dynein at the cortex and astral microtubules generate pulling forces
6 that position the spindle. In yeast, dynein is actively delivered to the cortex
7 through microtubule plus-end tracking complexes. In animal cells, an
8 evolutionarily conserved Gα-GPR-1/2^{Pins/LGN}-LIN-5^{NuMA} cortical complex interacts
9 with dynein and is required to generate pulling forces, but the mechanism of
10 dynein recruitment to the cortex is unclear. Using CRISPR/Cas9-assisted
11 recombineering, we fluorescently labeled endogenous DHC-1 dynein in *C. elegans*.
12 We observed strong dynein plus-end tracking, which depended on the end-
13 binding protein EBP-2. Complete removal of the EBP family abolished dynein plus-
14 end tracking but not LIN-5-dependent cortical localization. The *ebp-1/2/3* deletion
15 mutant, which was viable and fertile, showed increased cortical microtubule
16 retention; however, pulling forces and spindle positioning were normal. These data
17 indicate that dynein recruited from the cytoplasm creates robust pulling forces.

18

1 The mitotic spindle determines the plane of cell cleavage through its position and
 2 interactions with the cell cortex (reviewed in refs. ^{1,2}). The position of the spindle
 3 thereby controls the relative size and location of daughter cells formed during cell
 4 division. In addition, dependent on the position of the mitotic spindle, polarized
 5 cells may divide either symmetrically or asymmetrically. Asymmetric cell divisions
 6 generate cell diversity and allow long-term retention of somatic stem cells during
 7 the development of organisms and maintenance of tissues. Thus, accurate
 8 positioning of the spindle is critical for a wide range of processes that include the
 9 formation of gametes, integrity of tissues, creation of different cell types, and
 10 coordination of stem cell proliferation and differentiation.

11 The *C. elegans* early embryo provides an important *in vivo* model for studies
 12 of regulated spindle positioning. The one-cell embryo divides asymmetrically
 13 based on an anterior-posterior (A-P) polarity axis established after fertilization³.
 14 Classical spindle severing experiments revealed that pulling forces acting from the
 15 cell cortex on astral microtubules (MTs) position the spindle, and that these forces
 16 are higher in the posterior than in the anterior⁴. This asymmetry in forces leads to
 17 displacement of the spindle towards the posterior, and allows cell cleavage to
 18 create two blastomeres of unequal size and developmental fate⁵.

19
 20 Genetic screens and biochemical experiments have revealed a variety of
 21 factors important for the generation and distribution of mitotic pulling forces.
 22 Among these are the evolutionarily conserved proteins G α , GPR-1/2^{PINS/LGN} and LIN-
 23 5^{Mud/NuMA6-10}. These proteins form a complex known as the force generator complex

(FGC), which is regulated downstream of A-P polarity, thus bridging cell polarity and mitotic force generation. Cortical pulling forces also depend on cytoplasmic dynein¹¹. This large multi-subunit protein complex is the major minus-end directed motor in the cell and is essential for a large variety of cellular processes, including bipolar spindle assembly, mitotic checkpoint regulation, centrosome positioning, and intracellular transport (reviewed in ref. ¹²). In the current model, the FGC recruits dynein to the cell cortex either directly or indirectly via an extended N-terminal region of LIN-5¹³. Dynein subsequently associates with astral MTs in an end-on configuration, inducing pulling forces via both MT depolymerization and minus-end directed movement. Both *in vivo* and *in vitro* studies support this model^{11,14–16}.

It remains unclear, however, how dynein is recruited to the cortex and how the dynamic instability of MTs is coupled to force generation and mitotic spindle positioning¹⁷. MT growth and shrinkage are spatiotemporally modulated by a large variety of MT-associated proteins (MAPs), a subset of which concentrates at the growing MT plus-end (reviewed in ref. ¹⁸). These MT plus-end-tracking proteins (+TIPs) form a highly interconnected and dynamic network, which is regulated in a cell cycle and position-dependent manner to fine-tune MT dynamics^{19–22}. Members of the end-binding (EB) protein family are seen as master regulators of the +TIP network, as they bind autonomously to the growing MT end and recruit multiple other +TIPs^{19,23–25} (reviewed in ref. ¹⁸).

Interestingly, dynein is known to behave as a +TIP in a variety of cellular contexts^{26–29}. Dynein plus-end tracking in mammals is classically regarded as a

1 'search-and-capture' mechanism, by which the complex finds its cargo molecules
 2 via the MT plus-end before initiating minus-end directed transport^{30,31}. In the
 3 budding yeast *S. cerevisiae*, dynein plus-end tracking appears coupled to off-
 4 loading at the cortex and association of dynein with its cortical anchor Num1³²⁻³⁴.
 5 Disruption of dynein plus-end recruitment is thus associated with spindle
 6 positioning defects in yeast. Interactions between EB1, CLIP-170, and the dynactin
 7 protein p150Glued also recruit the human dynein complex to the MT plus-end³⁵⁻³⁷.
 8 However, whether dynein plus-end tracking is generally involved in spindle
 9 positioning remains to be determined.

10 In this study, we explore the localization of dynein during mitotic pulling
 11 force generation in the one-cell *C. elegans* embryo. Using CRISPR/Cas9-mediated
 12 genome editing³⁸⁻⁴², we labeled the endogenous dynein complex and studied its
 13 dynamic localization during mitosis using a variety of techniques. We observed two
 14 populations of dynein at the cortex: an EB-protein dependent MT plus-end tracking
 15 dynein population and a LIN-5-dependent MT independent population. By
 16 generating single, double and triple EB protein family gene knock-out mutants, we
 17 show that spindle positioning and embryonic development can proceed as normal
 18 in the absence of dynein plus-end tracking, and despite alterations of MT
 19 dynamics.

20

1 Results

2

3 Visualization of the endogenous dynein complex

4 In order to visualize the dynamics of the dynein complex in the early *C. elegans*
 5 embryo we applied CRISPR/Cas9-mediated genome editing. We decided to target
 6 the dynein heavy chain as opposed to an accessory subunit in order to label all
 7 possible compositions of the dynein complex. In *C. elegans* the dynein heavy chain
 8 is encoded by *dhc-1*, which has been labeled with fluorescent proteins (FPs) at both
 9 its N- and C-terminus in different systems^{43–45}, resulting in targeting of the DHC-1
 10 tail and motor domain, respectively (Fig. 1A). To allow for functional comparison,
 11 we explored both strategies by inserting *mcherry* directly upstream of the *dhc-1*
 12 stop codon, and *mcherry* or *egfp* directly upstream of the *dhc-1* start codon. A linker
 13 region encoding 4 glycine residues was always inserted between FPs and *dhc-1* to
 14 preserve independent protein folding (Fig. 1A).

15

16 We obtained multiple homozygous viable knock-in strains using both strategies.
 17 However, C-terminal tagging of *dhc-1* resulted in both severe embryonic lethality
 18 ($72,5\% \pm 9,2$ vs. $0,2\% \pm 0,3$ in N2), and a strong reduction in brood size ($30,0 \pm 18,4$
 19 animals vs. $323,3 \pm 15,5$ in N2) (Fig. 1B). Observation of early embryos by
 20 differential interference contrast (DIC) microscopy revealed spindle positioning
 21 and cell division defects from the one-cell stage onward. These phenotypes are in
 22 accordance with perturbed dynein function⁴⁶, which we confirmed in two
 23 independent strains. This led us to conclude that C-terminal tagging of DHC-1

1 causes a partial loss of DHC-1 function, as a complete loss of function would not be
2 homozygous viable. We did not further study dynein dynamics using these strains.

3

4 In contrast, animals with homozygous N-terminally tagged DHC-1 were fully viable
5 ($0,1 \pm 0,2$ in *mcherry::dhc-1* vs. $0,5 \pm 0,4$ in *egfp::dhc-1* vs. $0,2\% \pm 0,3$ in N2), and
6 showed normal brood size ($325,0 \pm 38,8$ in *mcherry::dhc-1* vs. $318,8 \pm 34,2$ in
7 *egfp::dhc-1* vs. $323,3 \pm 15,5$ in N2) (Fig. 1B). Worms appeared healthy during all
8 stages of development, and we observed no obvious abnormalities in early
9 embryonic development for either of these strains by DIC microscopy. From these
10 data we conclude that N-terminal tagging of DHC-1 with either mCherry or eGFP
11 does not perturb dynein function.

12

13 Observation of adult worms by wide-field fluorescence microscopy revealed
14 expression of both mCherry::DHC-1 and eGFP::DHC-1 in all somatic tissues of the
15 worm. Notably, DHC-1 is enriched in the germ-line, which is known for the robust
16 silencing of transgenes (Fig. 1C, blow-up). Next we followed early embryos by time-
17 lapse spinning disc confocal laser microscopy (SDCLM). A diffuse cytoplasmic pool
18 of DHC-1 was present in the cytoplasm during all stages of the cell cycle; in
19 addition, DHC-1 was located at the nuclear envelope, centrosomes, kinetochores,
20 kinetochore MTs, central spindle, astral MTs and the cell cortex during mitosis (Fig.
21 1D). This localization pattern is in accordance with earlier results from
22 immunohistochemistry and transgene overexpression experiments^{11,43,46,47}. In
23 addition, we also noticed comet-like accumulations of dynein radiating from the

1 centrosomes to the cell periphery in a pattern that appeared to follow the astral MT
2 network during metaphase and anaphase.

3

4 **Dynein tracks MT plus-ends during mitosis**

5 To further explore this localization pattern, we co-expressed *Ppie-1*-driven *gfp::β-*
6 *tubulin*⁴⁸ with *mcherry::dhc-1* and imaged embryos by simultaneous dual-color
7 time-lapse SDCLM. We observed that dynein comets were MT-associated, most
8 likely concentrating at the growing plus-end (Fig. 2A, arrow, Supplementary Movie
9 1). Since dynein is a minus-end directed motor protein, we reasoned that cortex-
10 directed dynein comets most likely revealed a plus-end tracking population. To test
11 this possibility, we co-expressed *Ppie-1*-driven *ebp-2::gfp* as a germ-line MT plus-
12 end marker^{49,50} with *mcherry::dhc-1*. This revealed strong co-localization of dynein
13 and EBP-2::GFP comets (Fig. 2B, Supplementary Movie 2). Intensity profile analysis
14 of both mCherry::DHC-1 and EBP-2::GFP comets shows strong similarity between
15 the two, indicating that plus-end tracking of dynein is strongly similar to that of
16 EBP-2 (Fig. 2C). Next, we measured the cortex-directed velocity of mCherry::DHC-1
17 and EBP-2::GFP comets. Measurements indicated that comet velocities are almost
18 identical ($0,74 \pm 0,11 \mu\text{m/s}$ for EBP-2::GFP (N=116) and $0,71 \pm 0,13 \mu\text{m/s}$ for
19 mCherry::DHC-1 comets (N=57), $p=0,136$) (Fig. 2D). These results are in line with
20 earlier quantifications of MT growth speeds during metaphase ($0.72 \pm 0.02 \mu\text{m/s}$ ⁴⁹).
21 In order to confirm that DHC-1 plus-end tracking is not an artefact due to protein
22 tagging, and to find out whether other components of the dynein complex are
23 present at the MT plus-end, we also studied the localization of dynactin. This

1 protein complex is required for a broad range of dynein functions¹², one of which is
 2 spindle positioning in *C. elegans*⁵¹. Both GFP::DNC-1^{p150glued} and GFP::DNC-2^{p50/dynamitin}
 3 showed clear overlap with mCherry::DHC-1 comets (Supplementary Fig. 1). Thus,
 4 we conclude that the dynein comets we observe represent plus-end tracking of the
 5 endogenous dynein complex, with high similarity in localization to EB plus-end
 6 tracking.

7
 8 An important question is how MT plus-end tracking of the dynein complex relates
 9 to pulling force generation. Thus, we studied cortical dynein localization in mitosis
 10 by dual-color total internal reflection fluorescence (TIRF) microscopy. We observed
 11 the simultaneous appearance of dynein comets with end-on MT-cortex contacts at
 12 the cortex (Fig. 2E, Supplementary Movie 3). Interestingly, when MTs stopped
 13 growing (as judged by loss of EBP-2::GFP signal), we also observed loss of the
 14 concentrated mCherry::DHC-1 signal at the cortex (Fig. 2F, Supplementary Movie
 15 4). This indicates that most dynein is lost from the plus-end when the MT switches
 16 from a growing to a shrinking state.

17 To explore the functional relevance of dynein plus-end tracking to spindle
 18 positioning, we imaged dynein dynamics during cortical force generation. Single
 19 force generation events can be visualized by invaginations of the plasma
 20 membrane⁵². To visualize membrane invaginations, we used CRISPR/Cas9-
 21 mediated genome editing to generate a strain expressing PH::eGFP driven by *Peft-3*
 22 in all tissues including the germ-line. This transgene was integrated in the
 23 *CxTi10816* locus on chromosome IV previously used to achieve germ-line

1 expression of single-copy integrated transgenes⁵³. Interestingly, dual-color imaging
 2 of PH::eGFP and mCherry::DHC-1 reveals strong co-occurrence of dynein comets
 3 reaching the cortex and the formation of membrane invaginations in 13/25 events
 4 (Fig. 2G, Supplementary Movie 5). However, we could not detect enrichment of
 5 mCherry::DHC-1 after the initiation of membrane invaginations. This indicates that
 6 a significant part of the dynein population present in a comet at the MT plus-end
 7 dissipates upon membrane contact and is most likely not involved in the
 8 generation of cortical pulling forces.

9

10 Dynein plus-end tracking and *ebp-1/2/3* are not required for spindle **11 positioning and development**

12 Although disappearing during membrane invagination, it remained unclear
 13 whether the MT plus-end tracking population of dynein contributes to cortical
 14 pulling force generation. To investigate this possibility, we sought to specifically
 15 disrupt dynein plus-end accumulation by removing +TIPs. To date, MT plus-end
 16 tracking has not been thoroughly explored in *C. elegans*, though two homologs of
 17 the mammalian EBs, EBP-1 and EBP-2, have been shown to exhibit plus-end
 18 tracking activity when overexpressed in the embryo⁴⁹. An annotated third
 19 homolog, *ebp-3*, appears to have arisen by a duplication of the genomic region
 20 containing the *ebp-1* gene. This duplication likely occurred relatively recently, as
 21 judged by the almost complete sequence identity between *ebp-1* and *ebp-3*.
 22 However, alignment of the predicted protein sequences revealed that EBP-3 lacks
 23 the calponin homology (CH) domain, which is required for recognition of the MT

1 plus-end^{24,54}. Thus, we suspect that *ebp-3* is a pseudogene. Because of the high
2 sequence similarity between *ebp-1* and *ebp-3*, we refer to these genes collectively
3 as *ebp-1/3*.

4

5 We knocked down expression of *ebp-1/3* and *ebp-2* by RNAi, and imaged one-cell
6 *egfp::dhc-1* embryos during mitosis by time-lapse confocal microscopy. While loss
7 of *ebp-1/3* expression did not appear to affect eGFP::DHC-1 plus-end tracking, loss
8 of *ebp-2* expression abolished the appearance of dynein comets during mitosis
9 (Fig. 3A). Interestingly, this did not result in obvious spindle positioning or cell
10 division defects upon initial inspection.

11 To further explore the role of the EBPs and dynein plus-end tracking during
12 mitosis, and to circumvent the possibility of incomplete RNAi knockdown, we
13 generated knock-out alleles for both *ebp-2* and *ebp-1/3* (Fig. 3B). Making use of
14 CRISPR/Cas9, the neighboring *ebp-1* and *ebp-3* genes were removed together, by
15 excising a ~17 kb region (referred to as $\Delta ebp-1/3$). The *ebp-2* knock-out allele lacks
16 both the coding region as well as ~1 kb upstream of the start codon (referred to as
17 $\Delta ebp-2$). Both the $\Delta ebp-1/3$ and $\Delta ebp-2$ strains could be stably maintained as
18 homozygotes, indicating that neither *ebp-1/3* nor *ebp-2* are strictly required for
19 normal development and reproduction.

20 When crossed with *egfp::dhc-1*, we observed effects on dynein localization
21 identical to those of the RNAi experiments. Dynein still localized to the MT plus-end
22 in $\Delta ebp-1/3$; *egfp::dhc-1* embryos, while this localization was completely absent in
23 the $\Delta ebp-2$; *egfp::dhc-1* early embryos (Fig. 3C, Supplementary Movie 6). At the

1 same time, dynein localization to the nuclear envelope, spindle midzone and poles
2 did not appear to be affected in either case, which indicates that dynein depends
3 on EBP-2 specifically for recruitment to astral MT plus-ends (Supplementary Fig. 2).

4

5 While generally showing no defects in development, $\Delta ebp-1/3$ caused a slight but
6 statistically significant reduction in brood size ($312,50 \pm 42,53$ in $\Delta ebp-1/3$ vs.

7 $329,75 \pm 35,11$ in N2), while $\Delta ebp-2$ did not ($335,25 \pm 28,04$ in $\Delta ebp-2$ vs. $329,75 \pm$

8 $35,11$ in N2). In addition, $\Delta ebp-1/3$, but not $\Delta ebp-2$, displayed a significant but

9 biologically probably irrelevant increase in embryonic lethality ($0,47\% \pm 0,16$ in

10 $\Delta ebp-1/3$ and $0,00\% \pm 0,00$ in $\Delta ebp-2$ vs. $0,07\% \pm 0,14$ in N2) (Fig. 3D). In order to

11 assess whether development and reproduction could proceed in the absence of all

12 three EBPs, we crossed $\Delta ebp-1/3$ with $\Delta ebp-2$ to generate the triple knock-out

13 mutant $\Delta ebp-1/2/3$. Interestingly, this combination was viable and could be

14 maintained as triple homozygous animals. The triple mutant showed a reduction in

15 brood size ($240,38 \pm 62,45$ vs. $329,75 \pm 35,11$ in N2), which is stronger than the

16 observed reduction in $\Delta ebp-1/3$ animals and indicates some redundancy amongst

17 $ebp-1/3$ and $ebp-2$. Embryonic lethality remained low ($1,79\% \pm 1,96$ vs. $0,07\% \pm 0,14$

18 in N2), which is remarkable given the complete absence of EBPs and expected

19 profound disruption of the +TIP network. While $\Delta ebp-1/2/3$ worms generally show

20 no developmental defects, we did observe a partly penetrant pleiotropic

21 phenotype amongst adult worms. This included low frequencies of dumpy, sterile,

22 and/or uncoordinated animals, as well as lethal larvae that exploded through the

23 vulva. In addition, some triple mutant worms appeared to form small bulges in the

1 skin. Because of the low penetrance and unpredictable nature, we did not further
2 examine these abnormalities.

3

4 In order to assess whether spindle positioning was affected in the *Δebp* mutants,
5 we followed early embryos by DIC time-lapse microscopy and characterized key
6 mitotic events. Importantly, asymmetric positioning of the spindle and subsequent
7 asymmetric division of the one-cell embryo was not affected in *Δebp-1/3*, *Δebp-2* or
8 *Δebp-1/2/3* embryos (Supplementary Fig. 3A). *Δebp-1/2/3* embryos did exhibit a
9 slight change in geometry, as they attained a rounder shape compared to wild-
10 type (wt) embryos (Supplementary Fig. 3B). The position of pronuclear meeting
11 along the A-P axis of the embryo did not change significantly (Supplementary Fig.
12 3C), while centration of the nucleocentrosomal complex occurred slightly more
13 posterior in *Δebp-1/3*, *Δebp-2* and *Δebp-1/2/3* embryos. The angle at which the
14 metaphase spindle is set up did not change significantly (Supplementary Fig. 3E),
15 while elongation of the anaphase spindle was slightly increased only in the *Δebp-*
16 *1/2/3* mutant (Supplementary Fig. 3F). Regardless of these very small variations in
17 mitotic events, they did not appear to have any significant impact on the outcome
18 of mitosis. However, all *Δebp* strains did exhibit an accelerated progression through
19 mitosis compared to wt embryos (Fig. 3E and Supplementary Fig. 4A,
20 Supplementary Movie 7). This effect occurred in *Δebp-1/3* and *Δebp-2* mutants, but
21 was most dramatic in *Δebp-1/2/3*. Both the time to progress from pronuclear
22 meeting to nuclear envelope breakdown (NEBD) (Supplementary Fig. 4B), as well as
23 from the start of chromosome segregation until completion of furrow ingression

1 was significantly decreased (Supplementary Fig. 4D). We do not know the
2 mechanism behind the shortened M phase. However, there was no significant
3 reduction in the time between NEBD and anaphase onset (Supplementary Fig. 4C),
4 which indicates that the observed effect does not result from bypassing or
5 premature satisfaction of the spindle assembly checkpoint.

6

7 **LIN-5 recruits dynein to the cell cortex independently of its ability to track the** 8 **MT plus-end**

9 If MT plus-end tracking of the dynein complex is not required for spindle
10 positioning, then how does dynein reach the cortex? To find out, we assessed the
11 cortical localization of dynein in both wt and Δebp mutants. Previous studies have
12 attempted to visualize cortical dynein localization by immunohistochemistry^{11,13}.
13 While cortical dynein could be observed in two-cell embryos, immunostaining of
14 one-cell embryos did not show clear cortical localization. Time-lapse SDCLM of
15 *egfp::dhc-1* one-cell embryos revealed broad and transient regions of DHC-1
16 enrichment at the cortex during anaphase. These were most pronounced during
17 rocking of the spindle. Interestingly, this cortical enrichment of dynein was not
18 perturbed in $\Delta ebp-1/3$ or $\Delta ebp-2$ mutant embryos (Fig. 4A). Thus, we conclude that
19 EBP-1/3, and EBP-2-dependent plus-end tracking are not required for the cortical
20 localization of dynein. By contrast, knock-down of *lin-5* perturbed cortical dynein
21 localization but did not affect its localization to MT plus-ends (Fig. 4A).
22 Simultaneous dual-color imaging of embryos expressing endogenously-tagged
23 *egfp::lin-5* and *mcherry::dhc-1* showed that dynein comets do not co-localize with

1 LIN-5, whereas cortical dynein and LIN-5 co-localize at the cortex (Fig. 4B).
 2 Moreover, we previously observed cortical dynein localization in the absence of
 3 MTs, following treatment of *perm-1(RNAi)* embryos with 1 μ M nocodazole
 4 (Portegijs *et al.*, accepted manuscript). In the absence of astral MTs, as confirmed by
 5 tubulin and EBP-2 markers, dynein still localized to the cortex, and this localization
 6 strongly overlapped with and depended on LIN-5 (Fig. 4C). In addition, we
 7 visualized cortical dynein in the early embryo by time-lapse TIRF microscopy.
 8 Notably, this revealed two populations of dynein that could be genetically
 9 separated at the cortex: a population of bright large spots that represent dynein
 10 comets at the cell cortex (Fig. 4D, arrow), and a fraction of smaller, less dynamic
 11 spots that represent a LIN-5 dependent population (Fig. 4D, arrowhead). Most
 12 notably, the combination of Δ *ebp-2* with knock-down of *lin-5* resulted in a
 13 complete loss of cortical dynein (Fig. 4D, Supplementary Movie 8). Thus, two
 14 independent populations of dynein appear present at the cortex: an EBP-2-
 15 dependent plus-end tracking and a LIN-5-dependent cortical population. LIN-5 can
 16 most likely recruit dynein directly from the cytoplasm to the cortex, which would
 17 explain why plus-end tracking of dynein is not required for its cortical localization.

18

19 **Cortical pulling forces remain normal in the absence of end-binding proteins**
 20 **and dynein plus-end tracking.**

21 To assess the effect of the absence of EBPs and dynein plus-end tracking on
 22 anaphase pulling forces, we imaged embryos by DIC time-lapse microscopy during
 23 mitosis. Subsequently, we tracked anterior and posterior pole movements during

1 anaphase and calculated the average maximum amplitude, which is a read-out for
2 force generation during anaphase⁵⁵. Interestingly, rocking of both the anterior and
3 posterior centrosome was generally not affected by the removal of EBPs. We did
4 find a slight but significant increase in the maximum amplitude for the posterior
5 pole in the $\Delta ebp-1/3$ background, but this change was not present in the $\Delta ebp-$
6 $1/2/3$ background (Fig. 5A).

7 To quantify the forces generated during anaphase more directly, we
8 performed spindle severing assays with a focused UV laser beam⁴. Upon severing
9 of the central spindle during anaphase onset, the centrosomes move away from
10 each other with a velocity proportional to the pulling forces acting on their astral
11 MTs⁴ (Fig. 5B, Supplementary Movie 9). Remarkably, we did not find any significant
12 differences in average peak velocity between any of the Δebp mutants and wild-
13 type embryos for either pole (Fig. 5C). Thus, cortical pulling force generation is
14 robust and remains largely unaltered in the absence of EB proteins. We conclude
15 that dynein plus-end tracking is not required for cortical pulling force generation in
16 the one-cell *C. elegans* embryo.

17
18 The EB proteins not only determine dynein plus-end tracking but are also
19 thought to affect several aspects of MT dynamics. The direct effects of EBs on MT
20 dynamics are still unclear, because *in vitro* and *in vivo* studies have yielded
21 contradictory results^{25,56–58}. The prevailing view is that EBs increase the growth rate
22 as well as the catastrophe frequency of MTs *in vitro*, but have little effect on the
23 growth rate and suppress catastrophes in cells. However, the effect of complete

1 loss of EB family members on MT dynamics has not been reported in an established
2 *in vivo* system such as *C. elegans*. A hurdle for studying MT dynamics in the one-cell
3 embryo is the extremely dense MT network, with each centrosome of the spindle
4 concurrently nucleating about 300 MTs⁵⁰. This complicates the observation of
5 single MTs by GFP-tubulin labeling, and we could not use +TIP markers as an
6 alternative in this case. Therefore, we imaged the dynamics of labeled tubulin at
7 the cortex with the use of TIRF microscopy. By quantification of the end-on MT-
8 cortex contacts during early anaphase, we found a significant increase in residence
9 time in both $\Delta ebp-2$ and $\Delta ebp-1/2/3$, but not for $\Delta ebp-1/3$, mutants compared to
10 wild type (Fig. 5D, Supplementary Movie 10). This indicates that loss of EBP-2 either
11 reduces MT growth rate, catastrophe frequency or both, thereby possibly allowing
12 prolonged contact with the cell cortex. Interestingly, we also observed a reduced
13 MT density of the spindle midzone in $\Delta ebp-2$ and $\Delta ebp-1/2/3$ embryos
14 (Supplementary Fig. 5). This led to full or partial bisection of spindles, reminiscent
15 of *spd-1^{PRC1}* knock-down, which diminishes the mechanical strength required for
16 the midzone to counteract the forces acting on the centrosomes⁵⁹. We attribute
17 this effect to a potential reduction in MT polymerization. Collectively, these results
18 indicate that the +TIP network composition, dynein plus-end tracking, and MT
19 dynamics are altered by the loss of the EBPs. However, the net pulling forces are
20 not affected, possibly due to compensation by altered MT dynamics.

1 Discussion

2

3 In this study, we investigated the recruitment of dynein in the generation of pulling
4 forces that position the mitotic spindle and determine the plane of cell division. We
5 took advantage of the well-established *C. elegans* one-cell embryo as an *in vivo*
6 model for spindle positioning, by combining targeted genome editing with high
7 spatial and temporal resolution microscopy, spindle severing assays, RNAi, and
8 drug treatment. Using CRISPR/Cas9-assisted recombineering, we tagged the
9 endogenous dynein complex and created knockout alleles for all three genes
10 encoding plus-end binding proteins of the EB1 family. The ability to study
11 endogenously expressed proteins allowed for reliable phenotypic characterizations
12 and observation of protein dynamics without overexpression.

13 Our first observation was that C-terminal tagging of the *dhc-1* dynein heavy
14 chain causes partial loss of function, as opposed to N-terminal tagging. It is
15 currently unclear whether this translates to dynein in other organisms. N-terminally
16 and C-terminally tagged yeast Dyn1 were reported to be functional⁶⁰. However,
17 Dyn1 lacks the C-terminal regulatory extension present in the cytoplasmic dynein
18 heavy chain of other organisms⁶¹ including *C. elegans*. A BAC transgene with a C-
19 terminally GFP-tagged mouse dynein heavy chain is commonly used in
20 mammalian studies⁶², normally in the presence of the endogenous DHC protein
21 and in cells in culture, which may depend less critically on dynein function. Tagging
22 the N-terminal DHC tail region instead of the C-terminal motor domain might be
23 the best option for future *in vivo* studies.

1 The next interesting observation was the concentration of the dynein
2 complex at the growing MT plus-ends in mitosis. This was previously described for
3 other systems, but to our knowledge not in *C. elegans*. The detection of dynein
4 plus-end tracking allowed us to quantify whether this transient dynein localization
5 forms part of the mechanism for cortical pulling force generation. Surprisingly,
6 pulling forces and spindle positioning remained unaltered in the absence of dynein
7 plus-end tracking and removal of the entire EB protein family. These observations
8 are in clear contrast with data from budding yeast, which indicate that plus-end
9 tracking provides an active system to deliver dynein to the daughter cell cortex.

10 *In vivo* and *in vitro* experiments with *S. cerevisiae* Dyn1 have implicated the
11 EB1-related protein Bim1^{EB1}, together with Bik1^{CLIP170}, Pac1^{LIS1}, and Kip2^{Kinesin} in
12 dynein plus-end recruitment^{63,64}. Yeast dynein is thought to be primed at the MT
13 plus-end for cortical anchorage by relieving its auto-inhibition^{32,33,65}. Offloading
14 from the MT plus-end to the cortex is dynactin-dependent, and allows cortical
15 anchorage by Num1 and correct spindle positioning^{32,34}. This two-step mechanism
16 was suggested to locally recruit dynein while at the same time minimizing the
17 need for modulation of motor activity.

18 In yeast, an estimated 30% of plus-end dynein appears delivered via
19 kinesin-mediated transport, while 70% is recruited from the cytoplasm³⁴. This,
20 together with the finding that Bik1^{CLIP170} can recruit dynein to the MT plus-end in
21 absence of Bim1^{EB1}⁶⁶, likely explains why depletion of Bim1^{EB1} from yeast cells does
22 not completely abolish dynein plus-end tracking⁶³. Isolated Dyn1 shows
23 constitutive minus-end-directed processive movement upon dimerization *in vitro*⁶⁰.

1 This indicates why kinesin plus-end directed transport is important for keeping
2 Dyn1 at the plus-end⁶⁴. While we did not explore the role of kinesin motors in the
3 context of dynein plus-end tracking, the effect of EBP-2 depletion suggests that
4 kinesin-mediated transport of dynein does not play a major role in *C. elegans*.
5 Possibly, *C. elegans* dynein regulation is more related to the human than to the
6 yeast dynein complex.

7 *In vitro* reconstitution studies have shown that the human dynein complex
8 is recruited to MT plus-ends by the large dynactin subunit p150Glued, which binds
9 to growing MT ends through EB1 and CLIP-170³⁷. As a major difference, yeast
10 dynein dimers are active on their own, while mammalian cytoplasmic dynein is in
11 an inactive conformation and requires adaptor-mediated dynactin binding for
12 processive movement^{67,68}. If both human and *C. elegans* dynein are kept inactive at
13 the plus-end, then the ‘tug-of-war’ with a potential kinesin should not be needed.
14 Kinesin and LIS1 dependent dynein transport has been observed, but its role may
15 be specific for certain cell types such as neurons⁶⁹. However, a recent study
16 indicates that LIS-1 is required for dynein plus-end tracking in *C. elegans* (Rodriguez
17 Garcia *et al.*, submitted). Interestingly, subcellular location-dependent adaptor
18 proteins bridge dynein and dynactin to activate dynein processivity, including the
19 coiled-coil proteins Bicaudal D at intracellular membranes, Rab11-FIB3 at Rab11-
20 endosomes and Spindly at the kinetochore. It is attractive to hypothesize that LIN-
21 5/NuMA acts as a dynein-activating adaptor at the cell cortex.

22

23 Could MT plus-end tracking of dynein play a role in asymmetric force generation?

1 In *S. cerevisiae*, dynein cortical recruitment is polarized by its ability to track the MT
2 plus-end, which directs dynein transport to the bud cortex³². However, our data
3 would suggest that dynein plus-end tracking is not in itself a polarizing mechanism
4 in *C. elegans*. We did not observe any obvious asymmetry in dynein localization to
5 the MT plus-end, and the spindle as well as the distribution of end-on MT contacts
6 were shown to be symmetric in the one-cell embryo⁵⁰. In addition, the presence of
7 cortical dynein and normal spindle positioning in the absence of EBPs indicates
8 that dynein plus-end tracking is not required for asymmetric spindle positioning.
9 This is further supported by data from nocodazole treatment of embryos, which
10 strongly suggests that LIN-5 can recruit dynein directly from the cytoplasm, as
11 opposed to MT-mediated delivery of dynein to the cortex. This is in accordance
12 with observations in nocodazole-treated cultured mammalian cells, which have
13 shown that cortical dynein recruitment does not require MT polymerization. At the
14 same time, the correct distribution of cortical dynein has been reported to depend
15 on a dynamic astral MT network⁷⁰. Thus, in *C. elegans* and mammalian cells, MT-
16 mediated delivery does not appear necessary for dynein localization to the cortex,
17 but dynamic MTs appear generally required for the correct distribution of dynein at
18 the cortex.

19
20 Based on our results, we propose that cortical recruitment of dynein in *C.*
21 *elegans* occurs by a mechanism different from the off-loading in yeast. We did not
22 observe off-loading or minus-end directed transport of dynein when MT plus-ends
23 reach the cortex. Although rapid diffusion appeared to prevent visualization, the

1 strong similarity and dependence in localization supports that dynein follows EBP-
 2 2 plus-end association and release. Intriguingly, we found that pulling force
 3 generation and spindle positioning are completely normal in the absence of EBPs.
 4 This was surprising, as we did observe effects on MT dynamics and +TIP network
 5 composition. It is worth noting that a minor dampening of centrosome rocking
 6 amplitudes was observed upon perturbation of *ebp-2* in a recent study
 7 complementary to ours (Rodriguez Garcia *et al.*, submitted). Our quantifications do
 8 not reveal such an effect in $\Delta ebp-2$ embryos, which might be explained by the use
 9 of different methods of quantification, reporter strains and mutant alleles.

10

11 How could the embryo compensate for the observed effects on MT
 12 dynamics, and what is the relevance of dynein plus-end tracking in the context of
 13 spindle positioning? We propose that in a normal situation, dynein plus-end
 14 tracking could function as a local enrichment mechanism (Fig. 5E). By
 15 concentrating dynein at the plus-end, MTs could efficiently constitute complete
 16 FGCs with complexes of G α -GPR-1/2-LIN-5 waiting at the cortex (Fig. 5E, arrow 1).
 17 This local concentration of dynein could also increase the relative amount of
 18 complete FGCs present at the cortex. Thus, dynein plus-end tracking could be a
 19 back-up mechanism that ensures efficient force generation. In the absence of EBPs
 20 and thus dynein plus-end tracking, astral MTs would have to locate cortical
 21 complexes that anchor dynein directly from the cytoplasm (Fig. 5E, arrow 2). While
 22 this single mechanism was previously hypothesized to be less efficient⁷¹, we expect
 23 that the prolonged cortical residence of MTs might allow for successful probing of

1 the cortex. In addition, considering that the +TIP is a protein-dense network in
 2 which a limited amount of binding sites are available at any given time, cortical
 3 FGCs may associate with MTs more efficiently due to decreased crowding of MT
 4 tips in EB-depleted cells. Taken together, our work illustrates the complexity and
 5 robustness of molecular mechanisms controlling an essential cellular process such
 6 as spindle positioning.

7

1 **Materials and Methods**

2

3 ***C. elegans* strains**

4 A summary of the strains used in this study is included in Table 1. All strains were
5 maintained at 20 °C as described previously⁷², unless stated otherwise. Worms were
6 grown on plates containing nematode growth medium (NGM) seeded with OP50
7 *Escherichia coli* bacteria.

8

9 **Generation of CRISPR/Cas9 repair templates and gRNAs**

10 Homology arms of at least 1500 bp flanking the CRISPR/Cas9 cleavage site were
11 generated by PCR amplification from purified *C. elegans* genomic DNA using the
12 KOD polymerase (Novagen). PCR products were inserted into the pBSK backbone
13 by Gibson assembly (New England Biolabs). For the generation of *ph::egfp::lov* and
14 *egfp::dhc-1*, *egfp* was amplified from pMA-*egfp*, *ph* from *Pwrt-2::gfp::ph*⁷³ and *lov*
15 from *gfp::LOVpep::unc-54UTR*⁷⁴. For *mcherry::dhc-1* and *dhc-1::mcherry*, codon-
16 optimized *mcherry* was amplified from TH0563-PAZ-*mCherry* (a kind gift from A.
17 Hyman). Primers containing overlaps between PCR fragments, linker sequences
18 and mutated gRNA sites were synthesized (Integrated DNA technologies). For the
19 generation of gRNA vectors, oligonucleotides were annealed and inserted into
20 pJJR50 using T4 ligation (New England Biolabs). Vectors were used to transform
21 and purified from DH5alpha competent cells (Qiagen).

22

23

1 **CRISPR/Cas9-mediated genome editing**

2 Injection of adult *C. elegans* worms in the germ-line was carried out using an
3 inverted microscope micro-injection setup. Injection mixes contained a
4 combination of 30-50 ng/μl *Peft-3::cas9* (Addgene ID #46168⁴², 50-100 ng/μl
5 *u6::sgRNA* with sequences targeted against either *cxTi10816*, *dhc-1*, *ebp-1*, *ebp-2* or
6 *ebp-3*, 30-50 ng/μl of the repair template, 50 ng/μl PAGE-purified *pha-1* repair
7 oligonucleotide (Integrated DNA technologies), 60 ng/μl pJW1285 (Addgene ID
8 #61252⁷⁵), and 2.5 ng/μl *Pmyo-2::tdtomato* as a co-injection marker. Animals were
9 grown for 3-5 days at either 20 or 25 °C after injection, and transgenic progeny was
10 selected based on either expression of tdTomato in the pharynx or survival at the
11 non-permissive temperature (25 °C). Subsequent assessment of genome editing
12 events was carried out by either visual inspection using a wide-field fluorescence
13 microscope and/or PCR amplification using primers targeting the inserted FP and a
14 genomic region situated outside of the range of homology arms in case of *dhc-1*, or
15 sequences flanking the predicted cut sites as well as an internal control in case of
16 the *Δebp-1/2/3* knock-out mutants. The contexts of PCR-confirmed edited genomic
17 loci were further inspected by sequencing (Macrogen Europe).

18

19 **Quantification of embryonic lethality and total brood size**

20 In two separate experiments, N2, SV1598, SV1619 and SV1803, or N2, SV1868,
21 SV1872, SV1877 and SV1882 single L4-stage hermaphrodites were placed on OP50
22 feeding plates and kept at 20 °C. Animals were transferred to a new plate every day.

1 On each plate, embryonic lethality was scored after 24 hours, and brood size 48
2 hours after removal of the parent. Experiments were executed in quadruplicate.

3

4 **Microscopy**

5 For time- and stream-lapse imaging embryos were dissected from adult worms on
6 coverslips in 0.8x egg salts buffer 94 mM NaCl, 32 mM KCl, 2.7 mM CaCl₂, 2.7 mM
7 MgCl₂, 4 mM HEPES, pH 7.5⁷⁶, and subsequently mounted on 4% agarose pads.
8 Live-cell SDCLM imaging of one-cell embryos was performed on a Nikon Eclipse Ti
9 with Perfect Focus System, Yokogawa CSU-X1-A1 spinning disc confocal head, Plan
10 Apo VC 60x N.A. 1.40 oil and S Fluor 100x N.A. 0.5-1.3 (at 1.3, for photo-ablation)
11 objectives, Photometrics Evolve 512 EMCCD camera, DV2 two-channel beam-
12 splitter for simultaneous dual-color imaging, Cobolt Calypso 491 nm (100 mW),
13 Cobolt Jive 561 nm (100 mW) and Teem Photonics 355 nm Q-switched pulsed laser
14 controlled with the ILas system (Roper Scientific France/ PICT-IBiSA, Institut Curie,
15 used for photo-ablation), ET-GFP (49002), ET-mCherry (49008) and ET-GFPmCherry
16 (49022) filters, ASI motorized stage MS-2000-XYZ with Piezo Top Plate, and Sutter
17 LB10-3 filter wheel. The microscope was controlled with MetaMorph 7.7 software
18 and situated in a temperature-controlled room at 20 °C. For regular single- and
19 dual-channel imaging experiments, images were acquired in either stream-lapse
20 mode with 100 ms exposure, or time-lapse mode with 500 ms exposure and 5
21 second intervals, unless stated otherwise. Laser power was kept constant within
22 experiments. For spindle bisection assays, spindles were imaged after photo-
23 ablation in stream-lapse mode with 500 ms exposure time.

1 Simultaneous dual-color TIRF imaging of embryos was performed on a Nikon
2 Eclipse Ti with Perfect Focus System, Nikon Apo TIRF 100x N.A. 1.49 oil objective,
3 Photometrics Evolve 512 EMCCD camera, Optosplit III beam-splitter for
4 simultaneous dual-color imaging, 488 nm (150 mW) and Cobolt Jive 561 nm (100
5 mW) lasers, ET-GFP (49002), ET-mCherry (49008) and ET-GFPmCherry (49022) filters,
6 ASI motorized stage MS-2000-XY System for Inversted Microscope Nikon Te/Ti
7 2000, and Sutter LB10-3 filter wheel. Acquisition was controlled with MetaMorph
8 7.7 software and the setup was situated in a temperature-controlled room at 20 °C.
9 For single- and dual-channel imaging experiments, images were acquired in
10 stream-lapse mode with 100 ms exposure time.

11 Single-color TIRF imaging of embryos was performed on either above-mentioned
12 TIRF setup, or on an identical TIRF setup in which lasers were controlled by the Ilas-
13 2 system (Roper Scientific France / PICT-IBiSA, Institut Curie), and image acquisition
14 was controlled with MetaMorph 7.8 software.

15 Live-cell wide-field time-lapse differential interference contrast (DIC) microscopy
16 imaging of embryos was performed on a Zeiss Axioplan upright microscope, with a
17 100x N.A. 1.4 PlanApochroma objective, controlled by AxioVision Rel 4.7 software,
18 at an acquisition rate of 1 image per 2 seconds with constant exposure time and
19 light intensity. Embryos were followed from pronuclear meeting until completion
20 of the first division. Images acquired by SDCLM and TIRF microscopy were
21 prepared for publication in ImageJ by adjusting brightness and contrast,
22 subtracting background and frame averaging as described in figure legends.

23

1 **RNA-mediated interference**

2 For RNAi experiments⁷⁷, either the gonads of young adults were injected with
3 double-stranded RNA targeting RNA molecules of interest (*perm-1* and *perm-1* +
4 *lin-5*) and grown for 20 hours at 15 °C, or L4 animals were grown on RNAi feeding
5 plates for 48 hours at 15 °C prior to imaging sessions (*lin-5*, *ebp-1/3*, *ebp-2*).

6

7 **UV laser spindle midzone severing**

8 Severing of the mitotic spindle was performed as described previously⁴. AZ224,
9 TH65, SV1874, SV1879 and SV1900 embryos were cultured and imaged at 20 °C,
10 and subjected to spindle bisection at anaphase onset, as judged by GFP::TBB-2 and
11 TBA-2::YFP tubulin localization. Peak centrosome velocities upon spindle severing
12 were subsequently tracked automatically using image intensity thresholding and
13 the FIJI TrackMate plugin.

14

15 **Data analysis**

16 All intensity profile measurements on SDCLM and TIRF data were generated using
17 ImageJ. Analysis of the timing of key mitotic events and position of the
18 nucleocentrosomal complex and centrosomes from DIC movies of N2, SV1868,
19 SV1872, SV1877 and SV1882 strains was performed by hand using ImageJ.
20 Quantifications of cortical MT residence time were performed by hand using the
21 FIJI plugin MtrackJ. Speeds of EBP-2::GFP and mCherry::DHC-1 comets during
22 metaphase were calculated based on angles of tracks in kymograph data
23 generated in ImageJ with the KymoResliceWide plugin. Numerical data processing

1 was performed using Excel 2011 (Microsoft). Line and bar graphs were generated

2 using Prism 6 (GraphPad software, inc.).

3

4

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2

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2

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13

1 **Author contributions**

2

3 AA, RS and SvdH designed the study, analyzed the data and wrote the paper. RS
4 performed the experiments.

5

6

7 **Competing financial interests statement**

8

9 The authors declare no competing financial interests.

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	ddl5 [[unc-119(+) + C47B2.3(genomic)::YFP]]
AZ224	Ruls57[Ppie-1::gfp::tbb-2]
SV1702	Ruls57[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	ojls5[pie-1::GFP::dnc-1 + unc-119(+)]; he250[mcherry::dhc-1]
SV1841	ojls57[Ppie-1::gfp::dnc-2 unc-119(+)]; he250[mcherry::dhc-1]
SV1857	abcls3[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]; Ruls57[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]; ddl5 [[unc-119(+) + C47B2.3(genomic)::YFP]]
SV1900	[unc-119(+) + C47B2.3(genomic)::YFP]; he278[Δebp-2]; he279[Δebp-1, ΔY59A8B.25, Δebp-3]

1 **Figures**

2

3 **Figure 1. Endogenous tagging of *dhc-1* using CRISPR/Cas9.**

4 (A) Schematic representation of *dhc-1* tagging strategies. Blue boxes represent the
 5 exons of the *dhc-1* locus on chromosome I. Red and green boxes represent *mcherry*
 6 and *egfp* sequences respectively. Orange boxes indicate linkers. Dynein cartoons
 7 (right) indicate the location of FPs in DHC-1 for C- (upper) and N-terminal (lower)
 8 strategies. (B) Quantification of embryonic lethality (left Y-axis) and brood size
 9 (right Y-axis) of *dhc-1* strains. Bars represent average (N=4 replicates) + SD, **** P <
 10 0.0001 compared to wt. No indication means no significant difference to wt.
 11 Unpaired Welch Student's *t*-test. (C) Wide-field fluorescence microscopy image of
 12 adult animal expressing mCherry::DHC-1. Blow-up indicates expression of
 13 mCherry::DHC-1 in the germ-line. Scale bar, 50 μ m. (D) Representative spinning
 14 disk confocal microscopy images of single eGFP::DHC-1 embryo in prophase (left),
 15 metaphase (middle) and anaphase (right). Scale bar, 5 μ m. All images were taken
 16 with the same exposure time and laser power, anterior to the left.

17

1 **Figure 2. Dynein tracks MT plus-ends during mitosis.**

2 (A, B) Representative simultaneous dual-color SDCLM images of one-cell embryos
3 during mitosis. mCherry::DHC-1 (red), GFP::TBB-2 (MTs, green, A), EBP-2::GFP (plus-
4 end, green, B). Arrow indicates dynein plus-end tracking. Yellow box indicates the
5 blow-up depicted in (C). (C) Intensity profile analysis of mCherry::DHC-1 (red) and
6 EBP-2::GFP (green) comets. Length in pixels, intensity in average (N=10 comets)
7 percentage of maximum. (D) Quantification of mCherry::DHC-1 (red, N=57 comets)
8 and EBP-2::GFP (green, N=116 comets) average comet velocity ($\mu\text{m/s}$) during
9 metaphase. Bars represent average + SD, ns not significant. (E, F) Representative
10 simultaneous dual-color TIRF images of mCherry::DHC-1 and GFP::TBB-2 (tubulin)
11 (E) or EBP-2::GFP (F) localization during early anaphase. Graphs indicate intensity
12 profile of mCherry::DHC-1 (red) and GFP::TBB-2 (E) or EBP-2::GFP (F) decoration of
13 the MT plus-end. Length in pixels, intensity in average (N=10 comets E, F)
14 percentage of maximum. (G) Representative simultaneous dual-color SDCLM
15 images of one-cell embryo expressing mCherry::DHC-1 (red) and PH::eGFP (green,
16 plasma membrane). Panels 4-6 are kymographs of the membrane invagination
17 indicated with the arrow in panel 3. Graph indicates single intensity profile analysis
18 of mCherry::DHC-1 (red) and PH::eGFP as measured along the box delineated in
19 kymograph, representative of N=25 events. Images are averages of 5 consecutive
20 frames taken from 100 ms stream-lapse movies, after background subtraction by a
21 gaussian blur filter. Scale bars, 1 μm .

22

Figure 3. **Analysis of dynein plus-end tracking and development in *Δebp* knock-outs.**

(A) Representative SDCLM images of one-cell embryos expressing eGFP::DHC-1 during metaphase, either untreated (wt, left) or following RNAi of indicated *ebp* gene(s) (middle, right). Blow-ups indicate the presence or absence of MT plus-end tracking, which was confirmed in N=5 embryos per condition. Images are averages of 5 consecutive frames taken from 100 ms stream-lapse movies, after background subtraction by a gaussian blur filter. (B) Schematic representation of *ebp-1/3* and *ebp-2* endogenous knock-out strategies. Blue and green boxes represent relevant genes mentioned in the text. Red boxes indicate the genetic duplication on chromosome V mentioned in the text. Grey bars represent the *Δebp-1/3* and *Δebp-2* deletions. (C) Representative SDCLM images of one-cell embryos expressing eGFP::DHC-1 during mitosis, in a wt (left), *Δebp-1/3* (middle) or *Δebp-2* (right) genetic background. Blow-ups indicate the presence or absence of MT plus-end tracking, which was confirmed in N=5 embryos per condition. Images are selected frames from 1000 ms exposure time-lapse movies, processed by background correction. (D) Quantification of embryonic lethality and brood size of *Δebp* mutants. Bars represent average (N=4 replicates) + SD, * P < 0.05, ** P < 0.01 compared to wt. Unpaired Welch Student's *t*-test. (E) Representative single images taken from time-lapse DIC movies of N2, *Δebp-2*, *Δebp-1/3* and *Δebp-1/2/3* one-cell embryos, aligned in time with PNM (first), centration (second), PSD (third) and cytokinesis completion (fourth column) in wild-type (N2). Times relative to pronuclear meeting are indicated in seconds for every frame. Scale bars, 5 μm.

1 Figure 4. **Cortical localization of dynein depends on LIN-5, but not on EBP-2-**
2 **mediated plus-end tracking.**

3 (A) Representative SDCLM images of eGFP::DHC-1 localization during anaphase,
4 under different RNAi conditions and in various genetic backgrounds as indicated
5 for each panel. Graphs indicate intensity profiles of eGFP::DHC-1 as measured in
6 boxes indicated in each superimposed panel. Length in pixels, intensity relative to
7 cytoplasmic values in average (N=24 measurements, 4 embryos per condition)
8 percentage of maximum. Images are selected frames from 1000 ms exposure time-
9 lapse movies, processed with background correction. Scale bar, 5 μ m. (B)
10 Representative simultaneous dual-color SDCLM images of mCherry::DHC-1 (red)
11 and eGFP::LIN-5 (green) localization during mitosis. Arrow indicates dynein plus-
12 end tracking, arrowhead indicates co-localization of dynein and LIN-5 at the cortex,
13 as confirmed in N=5 embryos. Images are averages of 10 consecutive frames taken
14 from 100 ms stream-lapse movie, after background subtraction by a gaussian blur
15 filter. Scale bar, 1 μ m. (C) Representative SDCLM images of either eGFP::DHC-1 or
16 mCherry::DHC-1; eGFP::LIN-5, in embryos treated with *perm-1* or *perm-1 + lin-5*
17 RNAi, and with 1 μ m nocodazole. Graphs indicate intensity profiles of eGFP::DHC-1
18 or mCherry::DHC-1 (red) and eGFP::LIN-5 (green) as measured in boxes delineated
19 in each superimposed panel. Length in pixels, intensity relative to cytoplasmic
20 values in average (N=10 measurements, 10 embryos per condition) percentage of
21 maximum. (D) Representative TIRF images of cortical eGFP::DHC-1 during early
22 anaphase, under different RNAi conditions and in various genetic backgrounds as
23 indicated for each panel. Arrow indicates large dot (plus-end dynein), arrowhead

- 1 indicates small dot (cortical dynein), as discussed in the text and observed for N=6
- 2 embryos per condition. Images are averages of 10 consecutive frames taken from
- 3 50 ms stream-lapse movie, after background subtraction by a gaussian blur filter.
- 4 Scale bar, 1 μm . Scale bar, 5 μm .
- 5

A

B

C

D

1 Figure 5. **Loss of EBP-1/2/3 affects MT dynamics but does not affect mitotic**
2 **pulling force generation.**

3 (A) Quantification of centrosome rocking during anaphase in wt and *Δebp*
4 embryos. The max. amplitude is presented as a % of embryo height for both the
5 anterior (green) and posterior (red) pole. Bars represent average + SD, * $P < 0.05$
6 compared to wt. Unpaired Welch Student's *t*-test. N-values are indicated below bar
7 graphs. (B) Example of spindle bisection using an ultraviolet laser (curved purple
8 line) in an embryo expressing GFP::TBB-2 (MTs). Five consecutive frames are shown,
9 each the average of 2 consecutive frames from a 500 ms exposure stream-lapse
10 movie. Arrows indicate the direction and arbitrary relative speed of displacement
11 upon spindle bisection. Anterior to the left. Scale bar, 1 μm . (C) Quantification of
12 average peak velocity ($\mu\text{m/s}$) upon spindle bisection for anterior (green) and
13 posterior (red) centrosomes. Bars represent average + SD, no indication means no
14 significant difference from wt. Unpaired Welch Student's *t*-test. N-values are
15 indicated below bar graphs. (D) Quantification of cortical residence time of end-on
16 MT contacts as visualized by TIRF microscopy of one-cell embryos expressing either
17 GFP::TBB-2 or TBA-2::YFP during early anaphase (schematically represented on the
18 left), in different *Δebp* mutant backgrounds. Representative image shown is an
19 average of 5 consecutive frames from a 250 ms stream-lapse movie, after
20 background subtraction by a gaussian blur filter. Scale bar, 1 μm . Average cortical
21 residence time in seconds. Bars represent average + SD, **** $P < 0.0001$ compared
22 to wt, ns not significant. Unpaired Welch Student's *t*-test. N=100 tracks for each

- 1 condition. (E) Model of force generation in wt (left) and $\Delta ebp-1/2/3$ (right) as
- 2 explained in the text.