Supplemental Materials

Molecular Biology of the Cell

Boudreau et al.

Figure S1 | Partial *dhc-1*/*lis-1* and *zyg-12*/*sun-1* RNAi affect centrosome separation through separate mechanisms. (A)Representative maximum intensity projections of embryonic confocal stacks. All RNAi treatments were performed with L4 stage worms expressing H2B:GFP and γ-tubulin:GFP for 24 hours via feeding unless otherwise noted. Scale bar = 10 μm. (B)(C) Centrosome-centrosome distance and centrosome-male pronucleus distance were measured in 3D between respective centroids. Error bars are SEM and are represented as shaded areas around means. n of embryos analyzed per condition > 10. Statistics: *t* test where *P < 0.05. Time t = 0 s corresponds to centrosome separation.

Figure S2 | Cytoplasmic microtubule growth velocities prior to pronuclear migration. (A)(B)(C)(D)(E)(F)Frequency distributions of cytoplasmic microtubule growth velocities prior to pronuclear migration. The black dashed line represents a threshold over which microtubules are assumed to be accelerated. The grey dashed curves represent a gaussian distribution fit to control cytoplasmic microtubule growth velocities. n of microtubules analyzed per condition is > 50 across > 3 embryos. Frequency distributions between RNAi conditions were statistically non-significantly different ($p > 0.05$) using both Mann-Whitney and Kolmogorov-Smirnov tests. (G) Drawing illustrating the imaging plane, cytoplasmic microtubules in black with green plus ends representing EBP2, and representative single plane confocal image of embryos expressing EBP2:GFP. The yellow trajectory line represents a track for up to 10 frames (2.5 s) following the displayed frame using TrackMate. Scale bar = 10 μm.

Figure S3 | Quantification of endogenous DHC-1:mNeonGreen mean amounts on female and male pronuclear envelopes. (A)(B)Mean amounts of DHC-1:mNeonGreen (arbitrary units – a.u.) on the nuclear envelope in (A) 16 hour and 24 hour *dhc-1* RNAi treatments and (B) 24 hour *zyg-12* and *sur-6* RNAi treatments. Quantifications were performed using single slices of confocal stacks from embryos expressing mCherry:HIS-58 and endogenous DHC-1:mNeonGreen. n of embryos analyzed per condition is > 14. Time t = 0 s corresponds to NEBD.

Figure S4 | Pronuclear cross-sectional area measurements during pronuclear expansion. Pronuclear cross-sectional areas (μm^2) were measured using maximum intensity projections of mCherry:HIS-58 confocal stacks from embryos expressing mCherry:HIS-58 and endogenous DHC-1:mNeonGreen. Female and male pronuclei were analyzed in 24 hour *zyg-12* and *sur-6* RNAi treatments. n of embryos analyzed per condition is > 14. Time t = 0 s corresponds to NEBD.

Figure S5 | Cytosim-based computational simulations predict 24 hour *sur-6* RNAi effects on cortical Dynein motoring activity are not sufficient to affect centrosome separation. Predicted centrosome-centrosome distances in simulated control embryos and in embryos with a 50% reduction in cortical Dynein motoring velocity. Error bars are SEM and are represented as shaded areas around means of 10 simulation runs per condition. Time t = 0 s corresponds to centrosome separation.

FIGURE S1

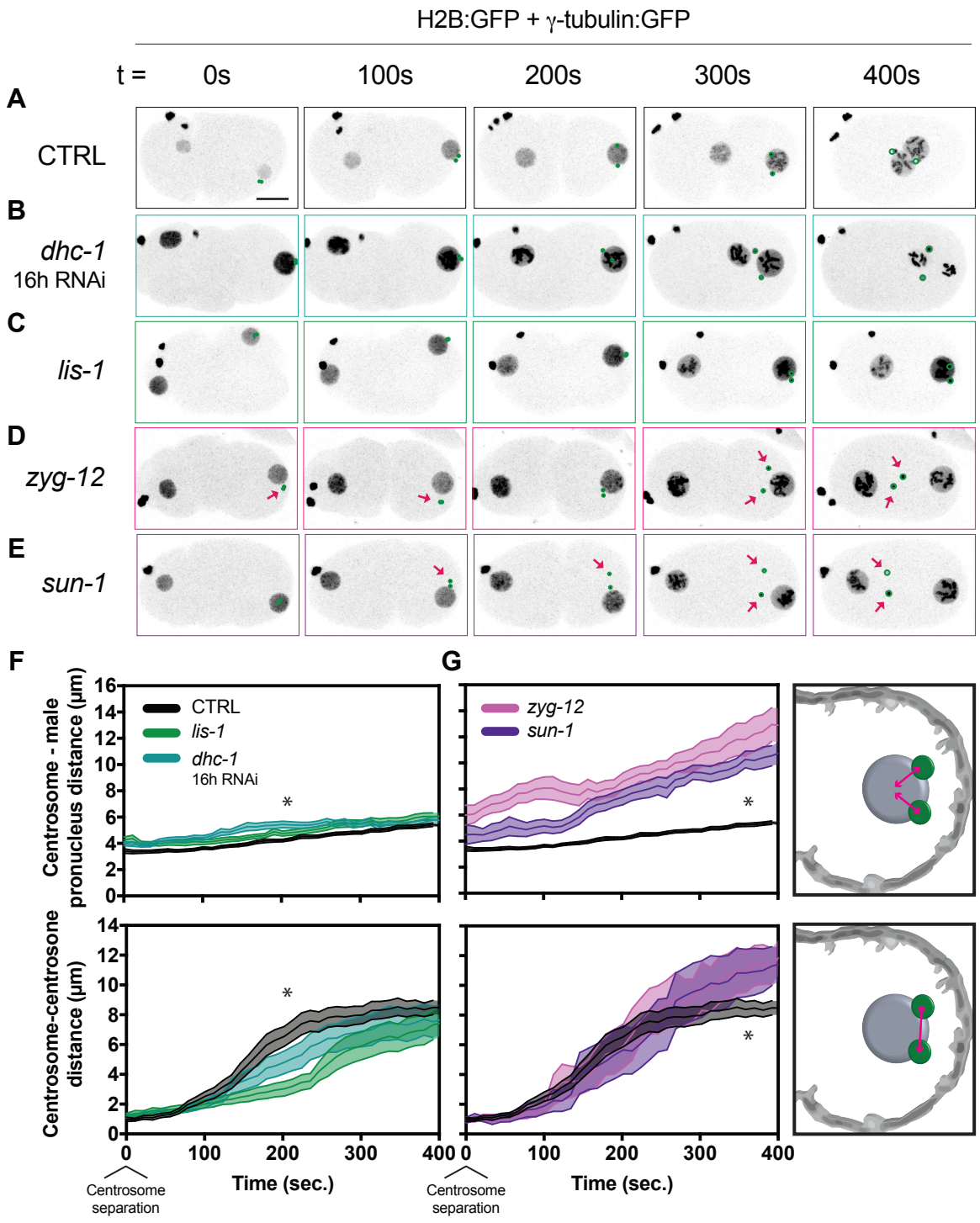

H2B:GFP + γ-tubulin:GFP
t =
0s
100s
200s
300s
400s
A
CTRL
B
dhc-1
16h RNAi
C
lis-1
D
zyg-12
E
sun-1
F
G
CTRL
lis-1
dhc-1
16h RNAi
zyg-12
sun-1
Centrosome - male pronucleus distance (µm)
Centrosome-centrosome distance (µm)
Time (sec.)
Centrosome separation
*

FIGURE S2

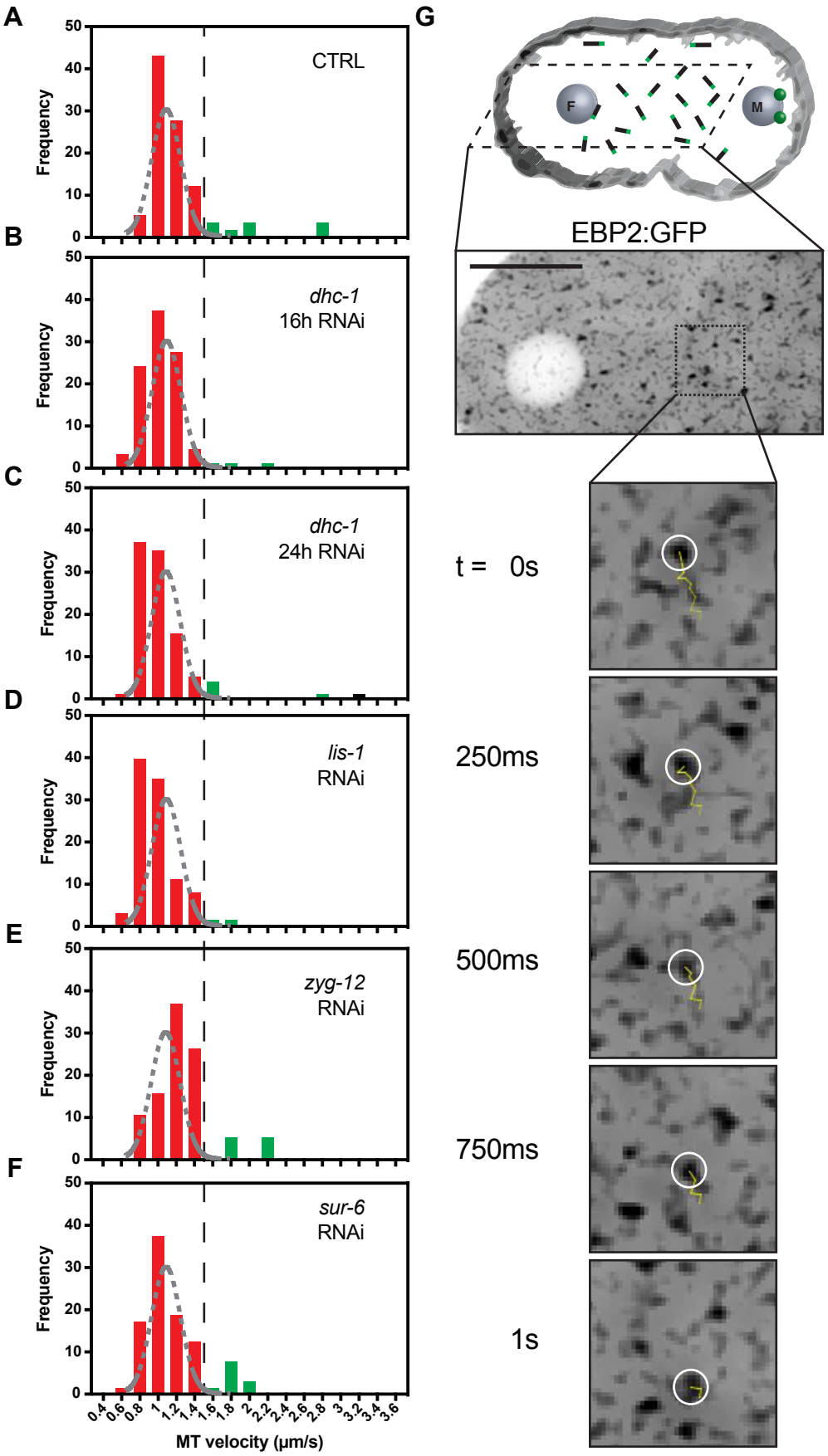

A
CTRL
B
dhc-1
16h RNAi
C
dhc-1
24h RNAi
D
lis-1
RNAi
E
zyg-12
RNAi
F
sur-6
RNAi
Frequency
MT velocity (µm/s)
G
F
M
EBP2:GFP
t = 0s
250ms
500ms
750ms
1s

FIGURE S3

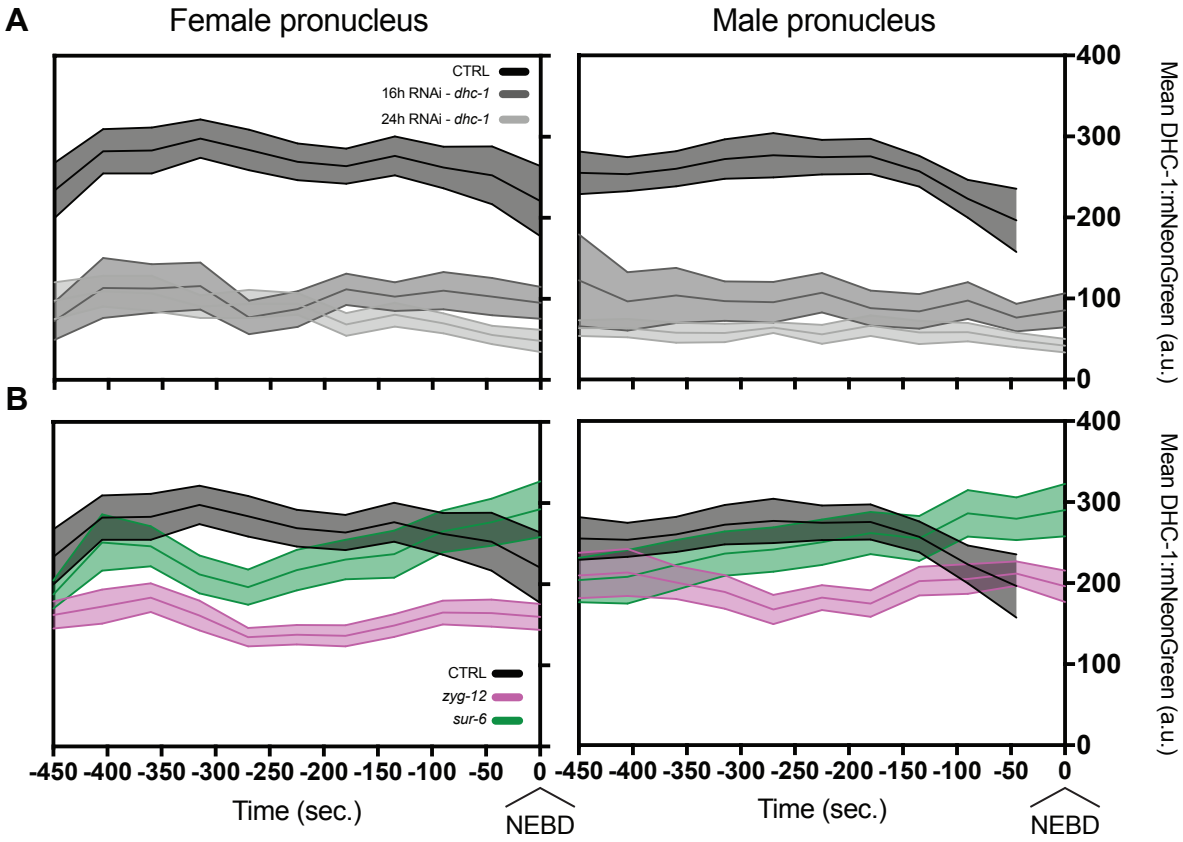

A
Female pronucleus
Male pronucleus
CTRL
16h RNAi - dhc-1
24h RNAi - dhc-1
B
CTRL
zyg-12
sur-6
Mean DHC-1:mNeonGreen (a.u.)
0
100
200
300
400
-450 -400 -350 -300 -250 -200 -150 -100 -50 0
Time (sec.)
NEBD

FIGURE S4

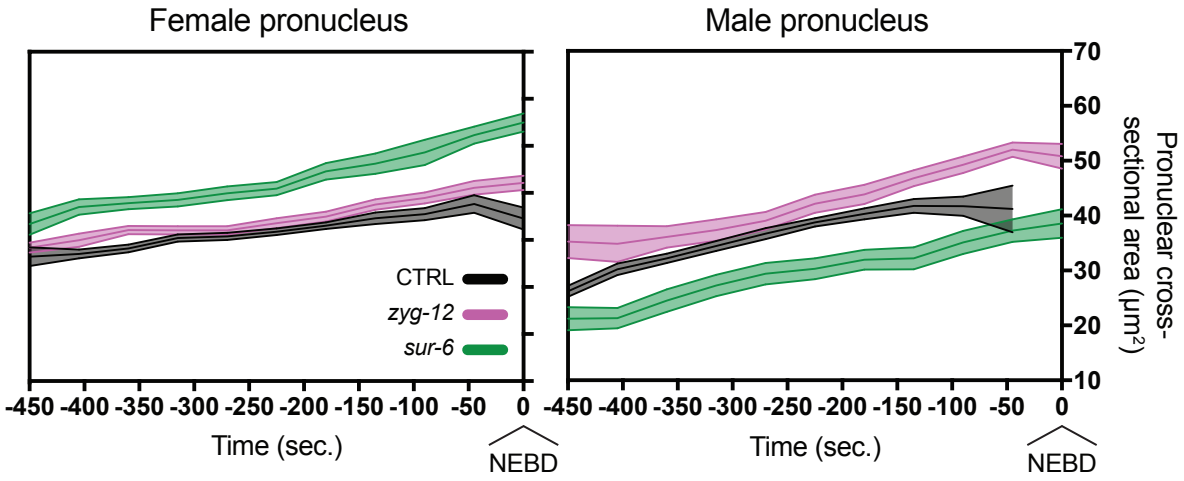
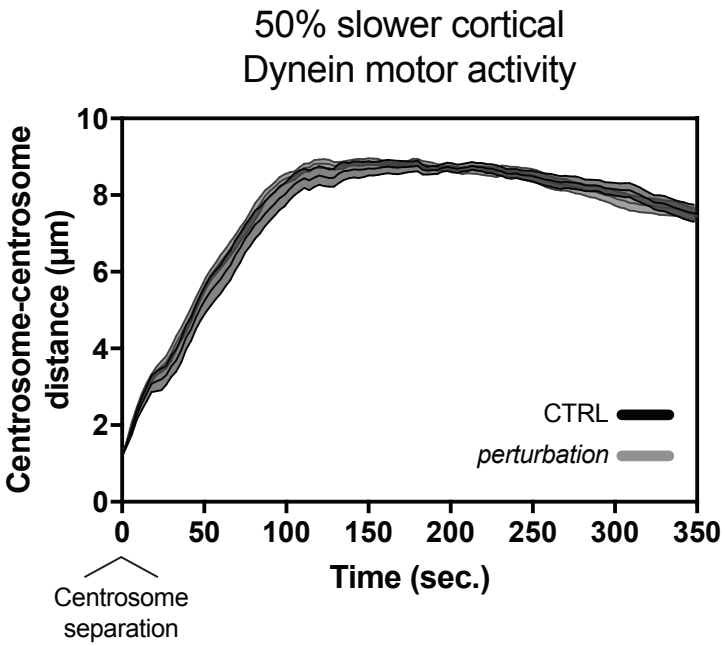

Female pronucleus
Male pronucleus
CTRL
zyg-12
sur-6
-450 -400 -350 -300 -250 -200 -150 -100 -50 0
Time (sec.)
NEBD
70
60
50
40
30
20
10
Pronuclear cross-sectional area (µm²)

FIGURE S5


50% slower cortical
Dynein motor activity
Centrosome-centrosome distance (µm)
10
8
6
4
2
0
0
50
100
150
200
250
300
350
Time (sec.)
CTRL
perturbation
Centrosome separation