

## Supplemental Data

**Supplemental Movie 1. LIC-GFP traffics from kinetochores to centrosomes.** A construct to express functional GFP-tagged LIC was made using *Dlic* cDNA and cloned into the pAc5.1 vector (Invitrogen). Further details on this construct will be published elsewhere (T. S. Hays, Y. He, and M.-G. Li, unpublished results). The movie presents time-lapse imaging of LIC-GFP during late prometaphase in wild type S2 cells. Small particles of LIC-GFP can be seen streaming from kinetochores toward the spindle poles. Images were collected at 1 fps and are displayed at 30 fps. The movie loops twice.

**Supplemental Figure 1. Distribution of LIC and HC in germline clones.** Fixed wild-type egg chambers show a similar enrichment of both proteins in the early oocyte (arrowheads). In *Dlic* mutant clones, neither HC nor LIC is enriched in a single cell, and instead both subunits are diminished and display a more homogeneous distribution pattern. Scale bar 5  $\mu$ m.

**Supplemental Figure 2. Tension, but not microtubule density, is reduced after LIC depletion. A and B)** Depletion of LIC decreases the distance between kinetochores, as measured using CID staining to mark kinetochore position. Scale bar = 5  $\mu$ m, n = 17 control cells (73 kinetochore pairs), 8 RNAi-treated cells (32 kinetochore pairs); error bars indicate SEM. **C)** Removal of LIC does not appear to significantly alter kinetochore microtubule density. S2 cells were fixed following cold treatment, which destabilizes non-kinetochore microtubules. Integrated tubulin fluorescence intensities were measured within a box of fixed dimension, slightly larger than the kinetochore and extending along the kinetochore fiber. Total integrated fluorescence was not significantly different between control and RNAi-treated cells. Error bars indicate SEM; n = 44 kinetochores from 11 control cells, and 70 kinetochores from 18 RNAi-treated cells.

**Supplemental Figure 3. GFP-LIC protein co-localizes with DHC at kinetochores in S2 cells.** Fixed cells were also stained with anti-CID as a marker for kinetochores. *Dlic*-RNAi decreases the GFP signal intensity to 14.2% ( $\pm$  3.6 SD) of the control signal. By comparison, significant levels of DHC remain at the kinetochore following depletion of GFP-LIC. Scale bar = 2  $\mu$ m.

**Supplemental Table 1.** Mad2 fluorescence at aligned kinetochores following dynein RNAi

RNAi	Mad2 fluorescence*	ratio to control	<i>n</i> <sup>§</sup>	<i>p</i>
<i>Dhc</i>	1286 ± 1101	1.69	28	<0.05
<i>Dic</i>	1557 ± 534	2.05	25	<0.001
<i>Dlic</i>	1525 ± 760	2.00	40	<0.001
<i>Tctex-1</i>	780 ± 288	1.02	17	0.878
Control	761 ± 445	--	24	--

\*mean ± SD

<sup>§</sup> *n* = number of kinetochores scored

**Supplemental Table 2.** Change in dynein heavy chain fluorescence at metaphase kinetochores following *Dlic* RNAi

	DHC fluorescence*	ratio to control	<i>n</i> <sup>§</sup>	<i>p</i>
<i>Dlic</i> RNAi	3186 ± 2241	3.8	18	<0.01
Control	841 ± 586	--	10	--

\*mean ± SD

<sup>§</sup> *n* = number of kinetochores scored

**Supplemental Table 3.** Kinetochores dynein fluorescence following RNAi and colchicine treatment

RNAi	DHC fluorescence*	ratio to control	<i>n</i> <sup>§</sup>	<i>p</i>
<i>Dlic</i>	2963 ± 1582	0.514	35	<0.001
<i>Dhc</i>	377 ± 415	0.0655	39	<0.0001
<i>Dic</i>	757 ± 756	0.131	32	<0.0001
<i>Tctex-1</i>	5210 ± 2173	0.905	39	0.499
Control	5759 ± 4421	--	31	--

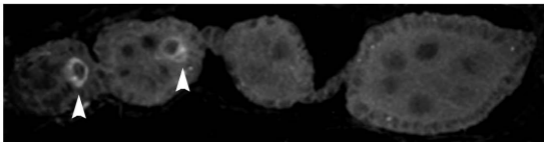
\*mean ± SD

<sup>§</sup> *n* = number of kinetochores scored

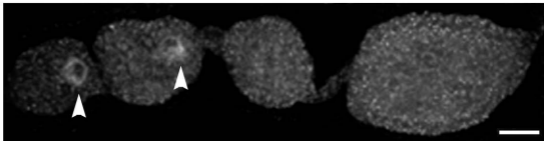
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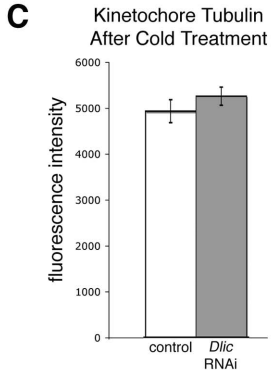
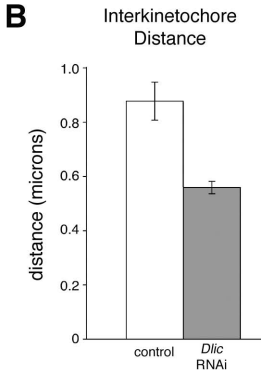
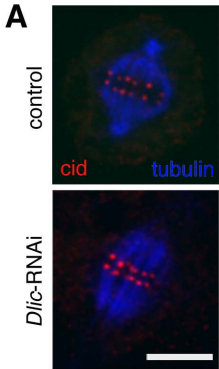
mutant

LIC



HC





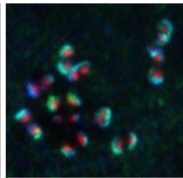
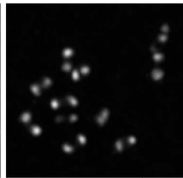
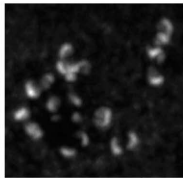
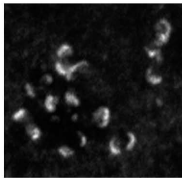
GFP-LIC

DHC

CID

merge

control

*DLIC* RNAi