Legends to Supplementary Information

Supplementary Figure 1

The CDC-48_UFD-1_NPL-4 complex is required for CMG helicase disassembly in *C. elegans*. (**a**) *cdc-48* RNAi leads to persistence of GINS and CDC-45 on chromatin during prophase and throughout mitosis (examples indicated by arrows). (**b**) Adaptors of CDC-48 in *C. elegans*. (**c**) *ufd-1* RNAi leads to persistence of GINS and CDC-45 on chromatin during prophase and throughout mitosis (examples indicated by arrows). (**d**) Equivalent experiment to that in Figure 1d, illustrating the effect of *npl-4* RNAi on embryos expressing GFP-MCM-3. To help visualise the small proportion of GFP-MCM-3 on chromatin in early metaphase (marked by an arrow), the experiment also included RNAi to the 3'UTR of endogenous MCM3 (this 3' UTR is not present in the GFP-MCM-3 transgene), to increase the incorporation of GFP-MCM3 into replication complexes. (**e**) *cdc-48* RNAi experiment, analogous to that in Figure 1d. (**f**) Homozygous *GFP-psf-1* worms were exposed to the indicated RNAi. Embryos were then isolated and processed as in Figure 1e-f. The middle panels show that the amount of CMG isolated from RNR-1 depleted extract was reduced compared to control (compare levels of MCM-7, MCM-2 and CDC-45), due to the inhibition of DNA replication in each embryonic cell cycle. In the right panels, loading of the GFP-PSF-1 IP samples was adjusted to obtain a similar level of CMG (compare MCM-2 and CDC-45). (**g**-**h**) Photobleaching experiments for GFP-SLD5 and GFP-MCM3, equivalent to the experiment in Figure 1h. The scale bars correspond to 5µm. Unprocessed scans of key immunoblots are shown in Supplementary Figure 8.

Supplementary Figure 2

CUL-2^{LRR-1} is required for removal of GINS from chromatin during S-phase in *C. elegans*. (**a**) *C. elegans* contain six families of cullin complexes, each with a specific cullin and a unique set of substrate adaptors. (**b**) Embryos from *GFP-sld-5 mCherry-H2B* worms were exposed to RNAi against the indicated cullins and processed as in Figure 2. Timelapse images are shown from S-phase to mid-prophase. (**c**) Six forms of the CUL-2 ligase in *C. elegans*, each with a unique substrate adaptor. (**d**) Embryos from *GFP-sld-5 mCherry-H2B* worms were exposed to RNAi against the indicated substrate adaptors of CUL-2 and processed as in Figure 2. Timelapse images are shown from S-phase to mid-prophase. RNAi for *zyg-11* produces meiotic defects and leads to abnormal nuclear morphology in the first embryonic cell cycle. Arrows in this figure indicate the persistent association of GFP-SLD-5 with mitotic chromatin in embryos treated with *npl-4* RNAi. Scale bars correspond to 5µm.

Supplementary Figure 3

A new pathway for CMG helicase disassembly acts during mitosis. (**a**) Embryos from *GFP-psf-1 mCherry-H2B* worms were exposed to the indicated RNAi and processed as in Figure 3. Timelapse images of the first embryonic cell cycle are shown from S-phase to metaphase. GFP-PSF1 initially persists on prophase chromatin following RNAi to components of CUL-2^{LRR-1} (the arrows denote examples), before being released in late prophase (indicated by asterisks). (**b**) Extended timecourses for the GFP-SLD-5 data presented in

Figure 2a-b. (**c**) Data from the first cell cycle, for the experiment in Figure 3b. (**d**) Embryos from *GFP-cdc-45 mCherry-H2B* worms were exposed to the indicated RNAi and processed as above. (**e**) Illustration of CMG disassembly defects produced either by depletion of CDC-48 / UFD-1 / NPL-4, or by depletion of components of CUL-2^{LRR-1}. Scale bars correspond to 5µm.

Supplementary Figure 4

The mitotic disassembly pathway for the CMG helicase requires UBXN-3 and is modulated by ULP-4. (**a**) Embryos from *GFP-sld-5 mCherry-H2B* worms were exposed to the indicated RNAi and processed as in Figure 4a. The arrows indicate persistent association of GFP-PSF1 with mitotic chromatin (throughout mitosis in the case of RNAi to *npl-4*, or after simultaneous RNAi to *lrr-1* + *ubxn-3*), whereas the asterisk denotes release of GFP-PSF-1 from chromatin in late prophase in embryos treated only with *lrr-1* RNAi. Scale bars correspond to 5µm. (**b**) Embryos from *GFP-cdc-45 mCherry-H2B* worms were processed as for Figure 4b. (**c**) Embryos from *GFP-psf-1 mCherry-H2B* worms were exposed to the indicated RNAi and processed as above. The data correspond to the AB cell in the second cell cycle and CMG components remained on chromatin until at or after nuclear envelope breakdown in 3/5 embryos treated with *lrr-1 ulp-4* double RNAi. The panel shows an example of an embryo where CMG persists on chromatin until nuclear envelope breakdown upon co-depletion of LRR-1 and ULP-4. (**d**) Data from a similar experiment, corresponding to the EMS cell in the third cell cycle. Note that in this case we also depleted the ATL-1 checkpoint kinase, to shorten the otherwise long cell cycle delay that is induced by the combination of *ulp-4 lrr-1*

double RNAi. CMG components remained on chromatin until late metaphase in 5/5 embryos treated with *lrr-1 ulp-4 atl-1* triple RNAi. CMG was extracted normally from chromatin during S-phase in embryos subjected to *ulp-4 atl-1* double RNAi (5/5 embryos tested), whereas *lrr-1 atl-1* double RNAi resembled *lrr-1* single RNAi treatment (CMG extracted before the end of prophase in 5/5 embryos).

Supplementary Figure 5

Additional supplementary material for experiments with *Xenopus* egg extracts. (**a**) In a similar experiment to that in Figure 6c, replisome disassembly was blocked during chromosome replication by addition of MLN4924 to *Xenopus* egg extracts. After isolation of chromatin and digestion of DNA, immunoprecipitation of LRR1 led to co-depletion of CUL2. (**b**) Analysis of ongoing DNA synthesis at the indicated timepoints for the experiment in Figure 6e-f, by addition of short pulses of α-dATP (see Methods). Data for repeats of this experiment are included in Supplementary Table 6. (**c**) Replication kinetics for the experiment in Figure 6h, measured by monitoring total incorporation of α-dATP into nascent DNA by the indicated timepoints (see Methods). Data for repeats of this experiment are included in Supplementary Table 6. Unprocessed scans of key immunoblots from this Figure are shown in Supplementary Figure 8.

Supplementary Figure 6

CUL2 is very highly conserved in vertebrates. Alignment of *Xenopus* CUL2 with the human and mouse orthologues, showing that the mammalian and frog proteins are almost identical. Moreover, previous work indicated that all key residues in CUL2 that contact EloB-C and substrate adaptors are 100% conserved between the human and frog orthologues[1].

Supplementary Figure 7

Validation of new antibodies generated in this study for *C. elegans* proteins. (**a**-**d**) In each case, RNAi was used to deplete the corresponding protein, before immunoblotting of embryonic extracts (upper panels). Ponceau S staining of the nitrocellulose membare (lower panels) provides a loading control in each case.

Supplementary Figure 8

Unprocessed scans of key immunoblots. (**a**) Raw immunoblot data for Figure 1e, with red boxes indicating the crops used to construct Figure 1e. (**b**) Equivalent data for Figure 1f. (**c**) Equivalent data for Figure 1g. (**d**) Equivalent data for Figure 6d. (**e**) Equivalent data for Figure 6h.

Supplementary Table 1

Factors targeted by RNAi in screen for components of the mitotic CMG disassembly pathway.

Supplementary Table 2

CUL-2^{LRR-1} associates with the isolated ‘post-termination’ replisome from *C. elegans*. Summary of mass spectrometry data for experiment shown in Figure 5b.

Supplementary Table 3

LRR-1 is required for CUL-2 to associate with the ‘post-termination’ worm replisome. Summary of mass spectrometry data for experiment shown in Supplementary Figure 5d.

Supplementary Table 4

CUL2^{LRR1} associates with the ‘post-termination’ frog replisome. Summary of mass spectrometry data for experiment summarised in Figure 6a.

Supplementary Table 5

The ‘post-termination’ replisome associates with purified frog CUL2^{LRR1}. Summary of mass spectrometry data for an equivalent experiment to that shown in Figure 6c.

Supplementary Table 6

Statistics Source Data. Source data of all repeats of the experiments in Figure 6f, Figure 7d, Supplementary Figure 5b and Supplementary Figure 6g.

Supplementary Table 7

Antibody dilutions for immunoblots.

Supplementary Table 8

Sequence of oligonucleotide primers used to generate RNAi vectors for depletion of *C. elegans* proteins.

Supplementary Movie 1

The CMG helicase component PSF-1 does not associate with condensing chromatin during mitotic prophase or throughout mitosis. Video of a single optical section through an embryo expressing GFP-PSF-1 (left panel) and mCherry-Histone H2B (right panel) progressing throughout the first and second embryonic cell cycles. Images were acquired every 10 sec with a spinning disk confocal microscope and processed with ImageJ software. The female and male pronuclei are orientated respectively towards the left and right of the video.

Supplementary Movie 2

GFP-PSF-1 associates with condensing chromatin during prophase in embryos depleted for NPL-4 and remains on chromatin throughout mitosis. Images were acquired and analysed as for Supplementary Movie 1.

Supplementary Movie 3

FRAP analysis of GFP-CDC-45 after depletion of NPL-4. The movie was generated as above and shows an embryo expressing GFP-CDC-45 (left panel) and mCherry-Histone H2B (right panel). The female pronucleus (left side of the embryo) was photobleached during early S-phase (shown as a white disk in the video at 1'50") and the chromosomes from the female and

male pronuclei were then analysed during the following mitosis (see 19'30'' to 24'50''). No recovery of the GFP-CDC-45 signal was observed on the female chromatin, indicating that depletion of NPL-4 causes CDC-45 to persist on chromatin from S-phase until the end of mitosis.

Supplementary Movie 4

GFP-PSF-1 associates with condensing chromatin during prophase in embryos depleted for CUL-2, but is then released from chromatin during late prophase. Images were acquired and analysed as for Supplementary Movie 1. Note that depletion of CUL-2 leads to meiotic defects in the embryo and thus to abnormal nuclear morphology, reflecting the important role of CUL-2^{ZYG-11} during the second meiotic cell division [2, 3]. In addition, mitotic entry is delayed in the first embryonic cell cycle after depletion of CUL-2.

Supplementary Movie 5

GFP-PSF-1 associates with condensing chromatin during prophase in embryos depleted for LRR-1, but is then released from chromatin during late prophase. Images were acquired and analysed as for Supplementary Movie 1. The association of GFP-PSF-1 with prophase chromatin can be seen in the first embryonic cell cycle (P0 cell) from 3'20'' to 5'50'' and during the second cell cycle from 24'30'' to 26'10'' for the AB cell (left side of embryo) or from 27'30'' to 29'10'' for the P1 cell (right side).

Supplementary Movie 6

GFP-PSF-1 remains on chromatin throughout mitosis in embryos depleted for both UBXN-3 and LRR-1. Images were acquired and analysed as for Supplementary Movie 1.

Supplementary Movie 7

GFP-PSF-1 is released from chromatin before prophase in ubxn-3 RNAi embryos. Images were acquired and analysed as for Supplementary Movie 1.

References

1. Nguyen, H.C., Yang, H., Fribourgh, J.L., Wolfe, L.S. & Xiong, Y. Insights into Cullin-RING E3 ubiquitin ligase recruitment: structure of the VHL-EloBC-Cul2 complex. *Structure* **23**, 441-449 (2015).
2. Liu, J., Vasudevan, S. & Kipreos, E.T. CUL-2 and ZYG-11 promote meiotic anaphase II and the proper placement of the anterior-posterior axis in C. elegans. *Development* **131**, 3513-3525 (2004).
3. Sonneville, R. & Gonczy, P. Zyg-11 and cul-2 regulate progression through meiosis II and polarity establishment in C. elegans. *Development* **131**, 3527-3543 (2004).