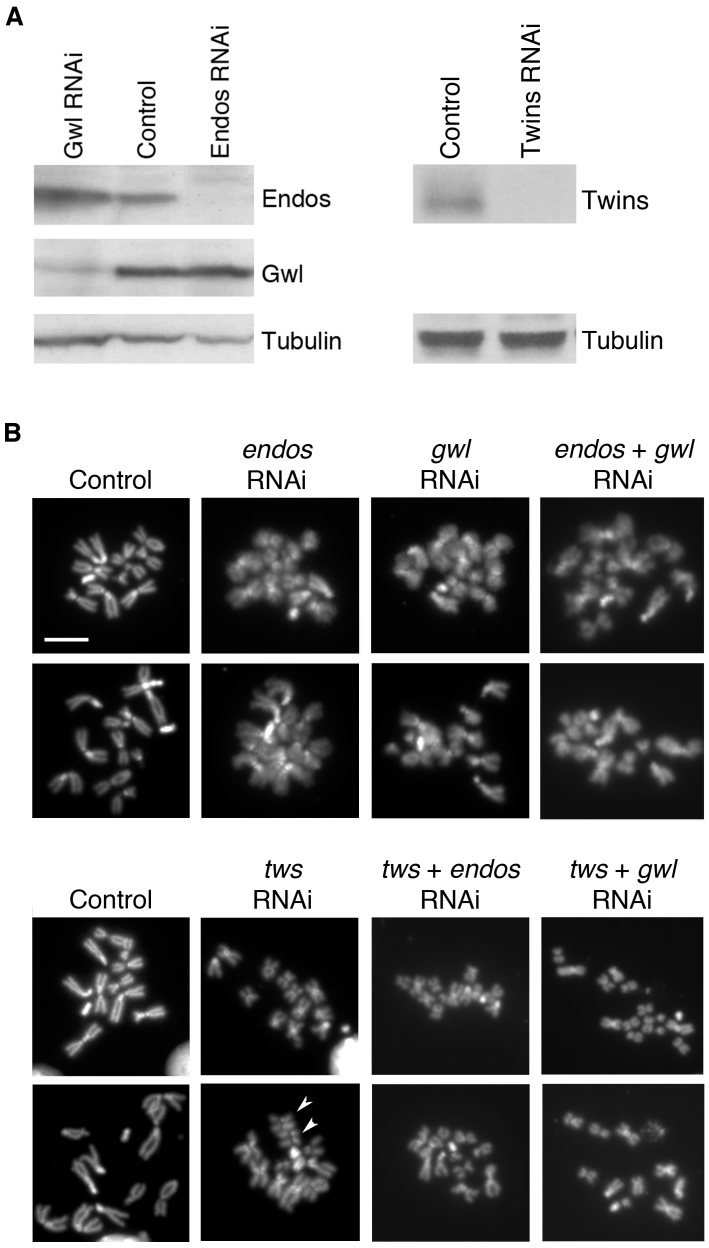


Figure S1 Condensation state of chromosomes from tissue culture cells subjected to *gwl-endos-PP2A/B55* pathway RNAi. **(A)** Depletion of Gwl, Endos, and Twins from S2 tissue culture cells by RNAi. S2 cells were treated with either control dsRNA or with dsRNAs for the indicated genes, and protein extracts were analyzed for the indicated component by Western blot. **(B)** Chromosome condensation phenotypes in S2 tissue culture cells depleted for components of the Gwl-Endos-PP2A/B55 module. S2 cells were treated with either control dsRNA or with dsRNAs for the indicated genes, and chromosome squashes were prepared as described in Materials and Methods. Chromosome undercondensation is obvious in *endos, gwl,* and *endos + gwl* RNAi cells. In contrast, RNAi for *twins* (the B55 regulatory subunit of PP2A) causes chromosome overcondensation: the chromosomes are shorter along their long axis. The *twins* RNAi phenotype is epistatic to that of *endos* or *gwl* RNAi.

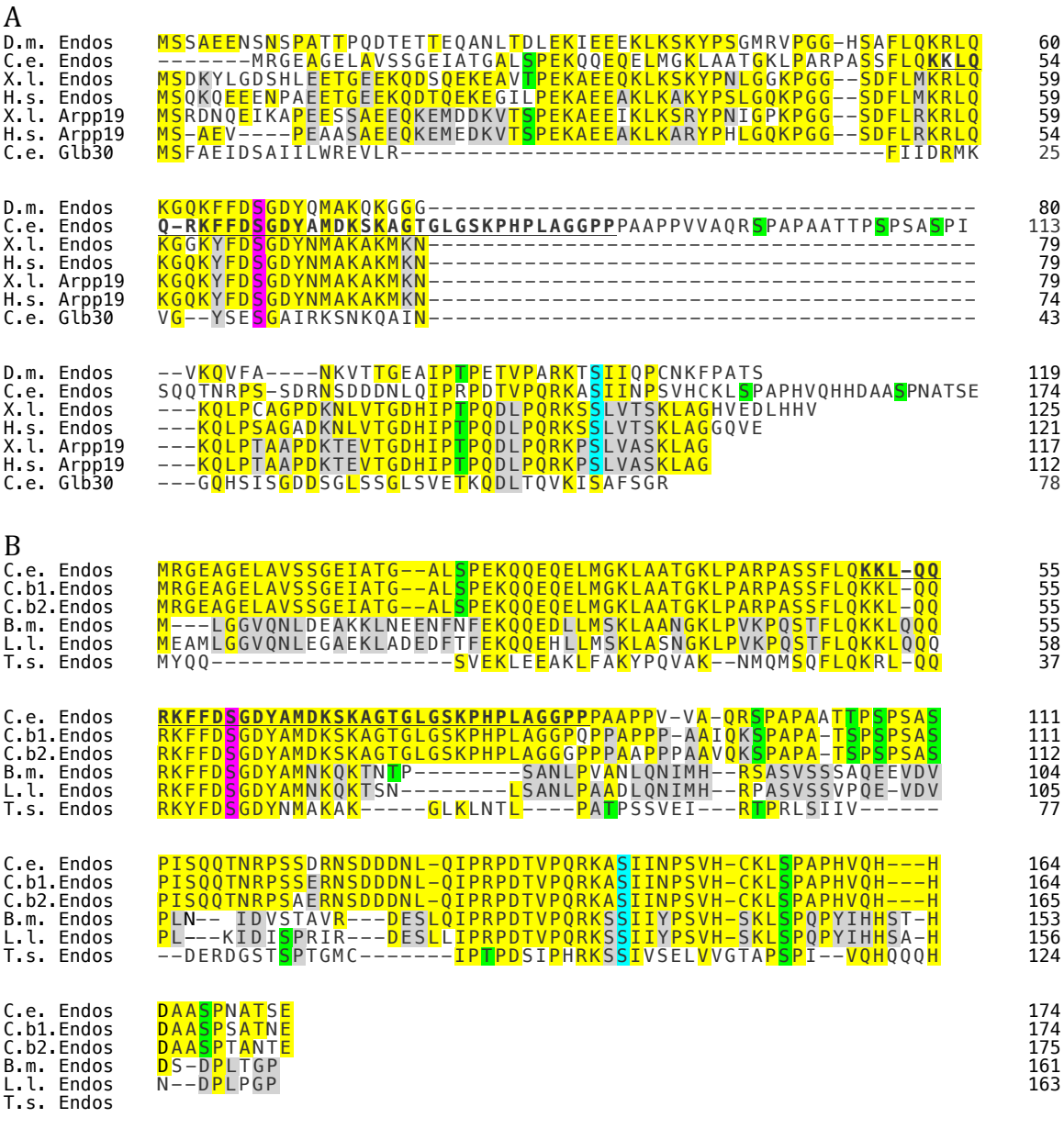


Figure S2 Endosulfine-family proteins. **(A)** Endosulfine in *Drosophila melanogaster* (D.m.) and *Caenorhabditis elegans* (C.e.) are compared with Endosulfine and its paralog Arpp19 in *Xenopus laevis* (X.l.) and humans (H.s.). Glb30 is a *C. elegans* globin family protein with limited homology to Endosulfine around the site targeted by Greatwall (*pink* shading). Amino acids that are identical between fly Endosulfine and other proteins are shaded in *yellow*, amino acids shared by other proteins but not fly Endosulfine are shaded in *grey*. *Green* shading indicates proline-directed S/T P sites potentially targeted by CDKs. The Ser illuminated by *aqua* shading is a site apparently phosphorylated by PKA and of unknown function. The underlined sequence in *C. elegans* Endosulfine is removed by the deletion allele *ensa-1(tm2810).* **(B)** Comparisons of nematode Endosulfine sequences. Species: *Caenorhabditis elegans* (C.e.), *Caenorhabditis briggsae* (C.b1.), *Caenorhabditis brenneri* (C.b2.), *Brugia malayi* (B.m.), *Loa loa* (L.l.), and *Trichinella spiralis* (T.s.). Colored shading as in part (A).

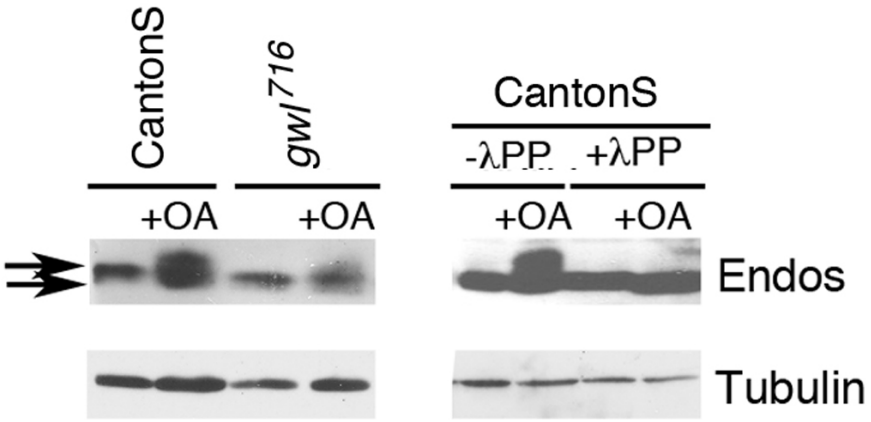


Figure S3 Phosphorylation of Endos by Gwl *in vivo*. Western blot developed with anti-Endos or anti-tubulin (loading control) antibodies to extracts from brains of control (Canton S) or *gwl*^{716} mutant third instar larvae as shown. In the lanes marked +OA, the brains were treated with okadaic acid for 2 hours to block the action of phosphatases. In the right panel, brain extracts were treated with lambda phosphatase to show that the slow-migrating forms of Endos are due to phosphorylation. The fraction of Endos in phosphorylated forms is significantly decreased in the *gwl*^{716} brains, but a small amount of phosphorylated Endos remains, presumably because Endos can be targeted by other kinases including PKA (DULUBOVA *et al.* 2001; MOCHIDA *et al.* 2010).

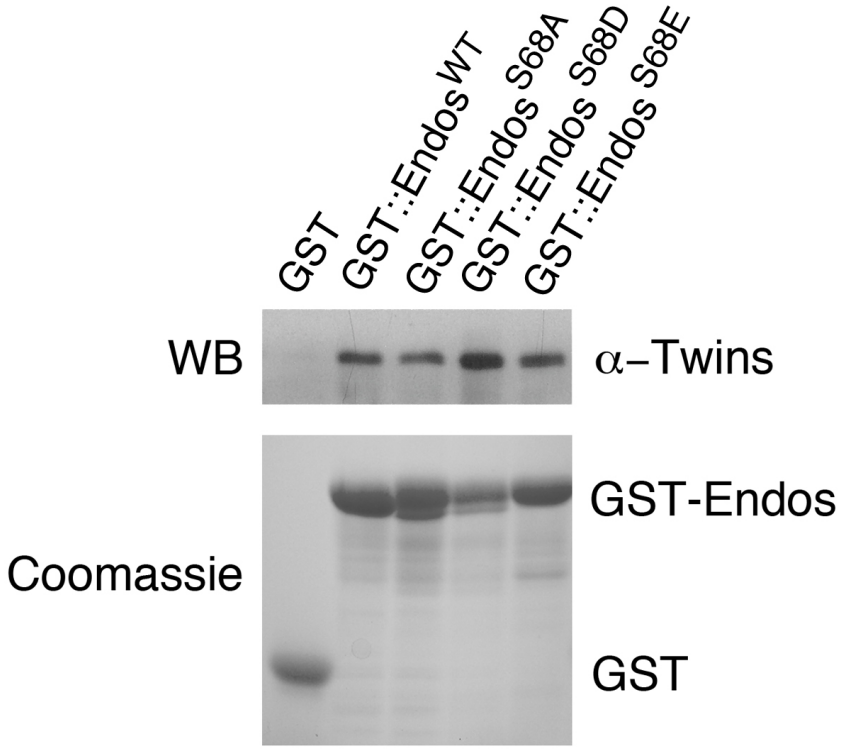


Figure S4 Physical interaction between Endos and B55/Twins *in vitro*. Pull-down experiment using glutathione-Sepharose beads bound with the same GST fusion proteins as in Figure 6, but incubated with recombinant, purified Twins protein instead of *in vitro* transcribed/translated ^{35}S-labeled Twins. (Top) Twins protein binding to beads, as detected on Western blots (WB). Depending on the sample, between 10% and 20% of the input Twins was retained on the beads (not shown). (Bottom) Coomassie blue-stained gel showing that the beads used for *in vitro* binding reactions had similar amounts of bound GST::Endos fusion proteins.

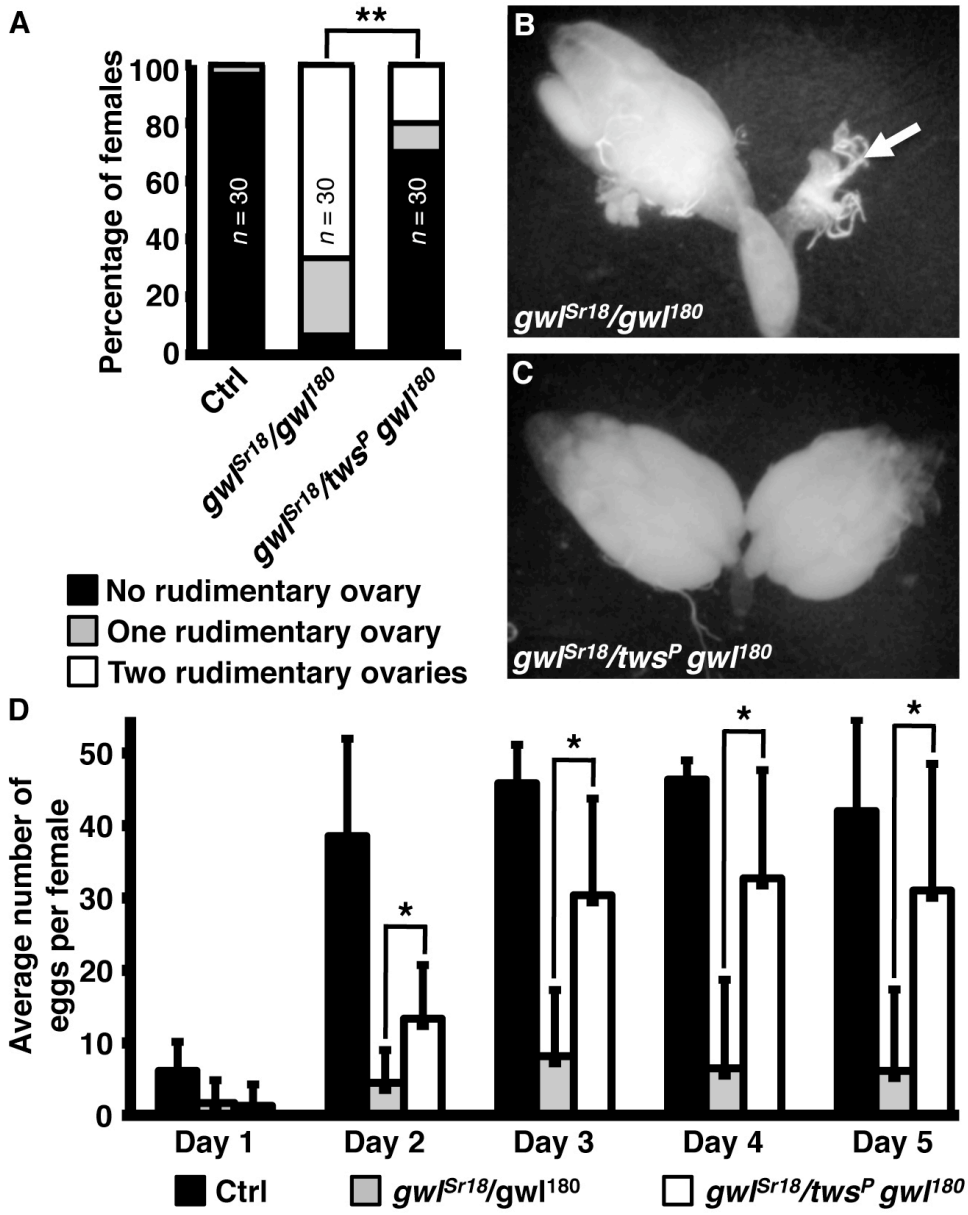


Figure S5 Heterozygosity for *twins* rescues the rudimentary ovary phenotye of *gwl* mutants. **(A)** Percentage of wildtype control (Ctrl), gwl^{Sr18}/gwl^{180}, or gwl^{Sr18}/tws^{P} gwl^{180} females carrying no, one, or two rudimentary ovaries. The number of analyzed females is shown inside the bars. Double asterisks indicate $p<0.001$. **(B)** Example of gwl^{Sr18}/gwl^{180} pair of ovaries, with a rudimentary ovary indicated by the arrow. **(C)** Example of a pair of ovaries in a gwl^{Sr18}/tws^{P} gwl^{180} female. **(D)** Average number of eggs laid per female per day. Error bars represent standard deviations. Asterisks indicate $p<0.05$.

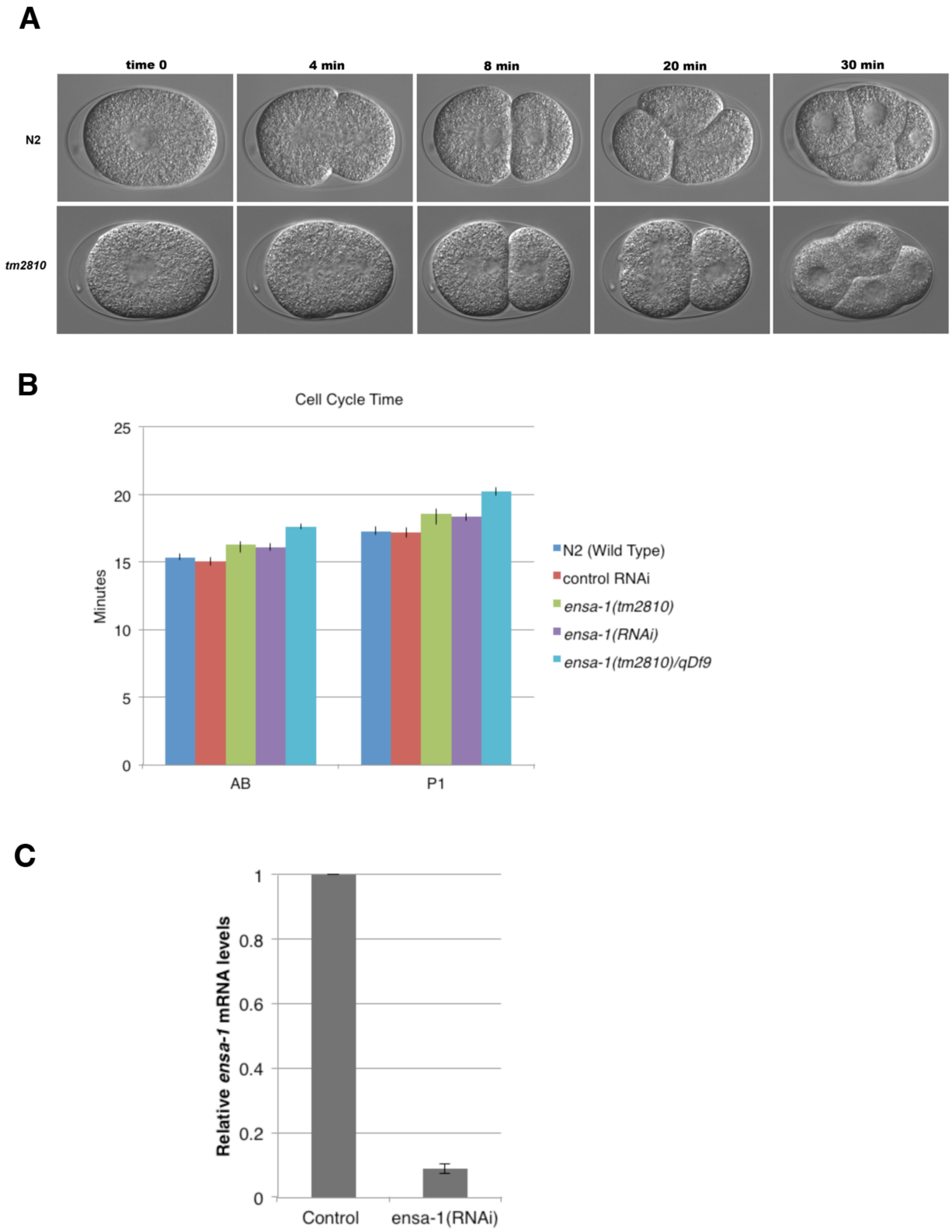
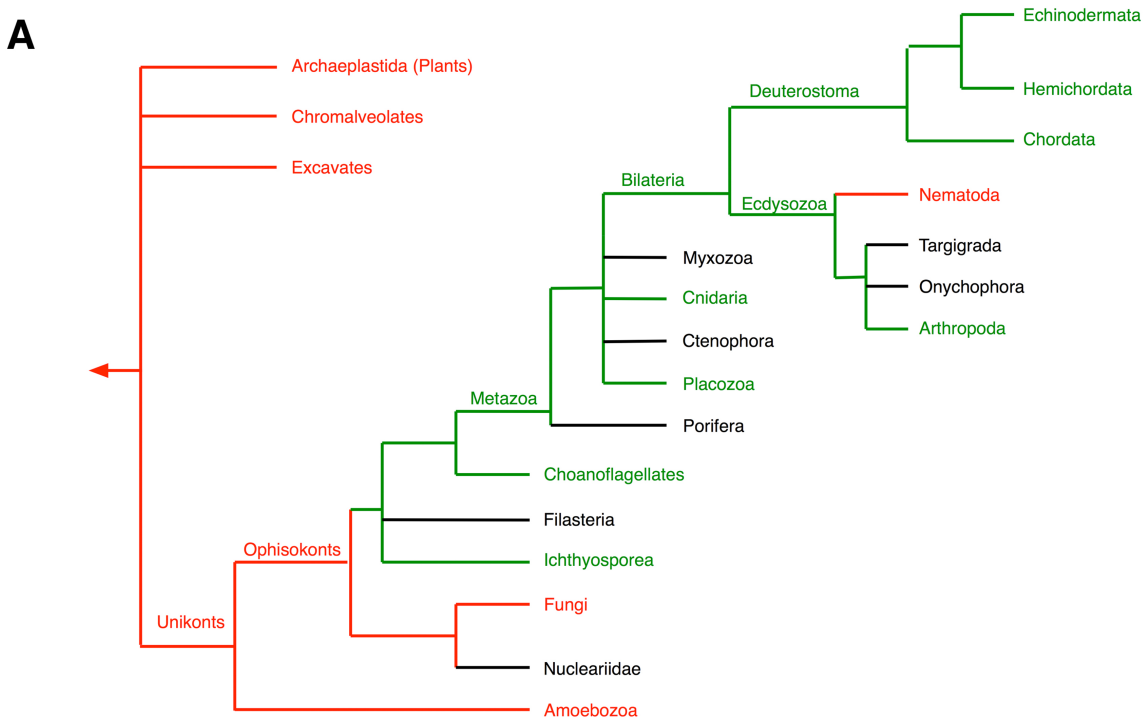


Figure S6 Ablation of *ensa-1* causes minor delays during the first two embryonic cell cycles in *C. elegans*. (A) Images are taken from a time-lapse movie at 20°C. Wildtype (top) and *ensa-1(tm2810)* embryos were mounted for viewing just prior to first cleavage. Mutant embryos initiate each of the first two divisions about 1 min later than wildtype. (B) Depiction of quantitative data from Table 5 for timing of the first two cell cycles of *ensa-1(tm2810)*, *ensa-1*(RNAi), and *ensa-1(tm2810)/qDf9* embryos. (C) Reduction of *ensa-1* mRNA by RNAi. qRT-PCR from control (L4440) and *ensa-1* RNAi-treated worms (see Materials and Methods).


A
Archaeplastida (Plants)
Chromalveolates
Excavates
Unikonts
Ophisokonts
Amoebozoa
Fungi
Nucleariidae
Metazoa
Choanoflagellates
Filasteria
Ichthyosporea
Bilateria
Porifera
Placozoa
Cnidaria
Ctenophora
Myxozoa
Ecdysozoa
Nematoda
Tardigrada
Onychophora
Arthropoda
Deuterostoma
Echinodermata
Hemichordata
Chordata

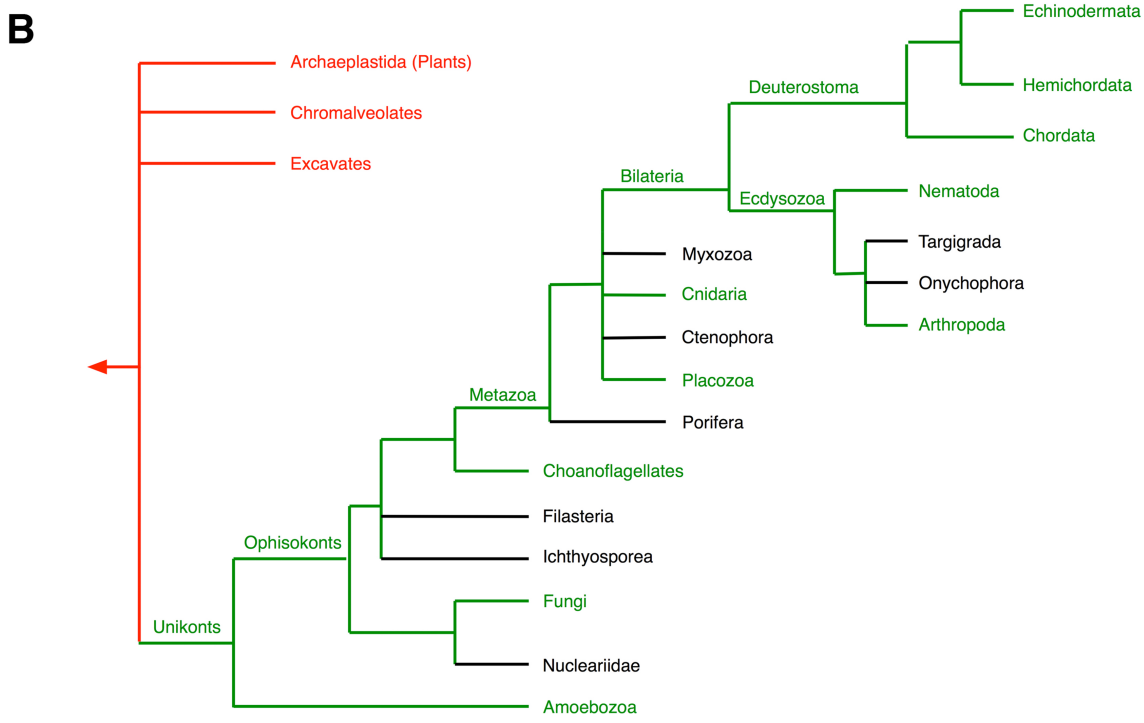

B
Archaeplastida (Plants)
Chromalveolates
Excavates
Unikonts
Ophisokonts
Amoebozoa
Fungi
Nucleariidae
Metazoa
Choanoflagellates
Filasteria
Ichthyosporea
Bilateria
Porifera
Placozoa
Cnidaria
Ctenophora
Myxozoa
Ecdysozoa
Nematoda
Tardigrada
Onychophora
Arthropoda
Deuterostoma
Echinodermata
Hemichordata
Chordata

Figure S7 Phylogenetic analysis of Gwl and Endos. Phylogenies are adapted from The Tree of Life Web Project (http://tolweb.org/tree/). For simplicity and also because of the lack of fully sequenced genomes, several clades among the Bilateria are not shown (including Ecdysozoa such as Priapulida, Loricefera, Kinorhyncha, and Nematomorpha; as well as Bilateria such as Rotifera and Platyhelminthes that are not categorized as Ecdysozoa). **(A)** Gwl. Green indicates lineages whose genomes include authentic Gwl [that is, proteins not only containing strong homologies with the Gwl kinase domain, but also containing the regions that ensure Gwl activation at M phase (particularly amino acids 180-222, 708-739, and 864-878 in the *H. sapiens* enzyme)]. Red indicates lineages without authentic Gwl genes, although some include genes for kinases closely related to Gwl such as Rim15 in *S. cerevisiae,* Cek1 in *S. pombe*, and IRE in the plant *Arabdipopsis thaliana.* Note particularly the absence of authentic Gwl in the Nematoda. Black indicates lineages for which the presence or absence of authentic Gwl genes cannot be determined based on available sequence information. Beyond the well-characterized authentic Gwl proteins in Chordata (e.g., human and frogs) and Arthropoda (e.g., flies), other presumed Gwl orthologs used to assemble this figure include: XP_001180570.1 in the Echinoderm *Strongylocentrotus purpuratus*, XP_002737566.1 in the Hemichordate *Saccoglossus kowalevskii*, XP_002167997.1 in the Cnidarian *Hydra magnipapillata*, XP_002108611.1 in the Placozoan *Trichoplax adhaerens*, XP_001745592.1 in the Choanoflagellate *Monosiga brevicollis*, and EFW39780.1 in the Ichthyosporean *Capsaspora owczarzaki.* **(B)** Endos. Green indicates lineages whose genomes encode at least one Endosulfine-like protein containing a motif homologous to that phosphorylated on human ENSA by Gwl or on yeast Igo1/2 by Rim15. Red indicates lineages in which fully sequenced genomes cannot be demonstrated to include Endosulfine-like genes. Black indicates lineages for which the presence or absence of Endosulfine-like genes cannot be determined based on available sequence information. The figure indicates the presence of an Endosulfine family gene precursor in a common ancestor to all the Unikonts. However, this conclusion is somewhat uncertain, as it is based on the results of tblastn searches of the genome of the Amoebazoan *D. discoideum* using the Igo1 amino acid sequence from *S. cerevisiae*. The hypothetical protein XM_639611.1 uncovered by this analysis has only low overall homology with Igo1, but the Dictyostelium protein includes the motif KYFSADWA, which is similar to the target site for Rim15/Gwl in Igo1/Endosulfine (KYFDSGDYA/N, with the phosphorylated site underlined; see Fig. S2).

Files S1-S3
Supporting Movies

Files S1-S3 are available for download at http://www.genetics.org/content/suppl/2012/05/25/genetics.112.140574.DC1.

File S1 Mitosis in a control larval neuroblast. A brain dissected from a late larva of genotype w^{1118}; *P{H2AvD-RFP}/+* expressing red fluorescent protein-labeled histone H2AvD was filmed as described in Materials and Methods. At t=0, the cell chosen for filming had already begun to condense its chromosomes and was thus in a prophase-like state. Note that the cell formed a clear-cut metaphase plate by t=10 min, and then entered anaphase by t=22 min. Still images from this sequence are shown at the top of Figure 3 in the text.

File S2 Mitosis in an *endos* null mutant larval neuroblast. A brain dissected from a late larva of genotype w^{1118}; *P{H2AvD-RFP}/+;* $endos^{215\text{-}4}$ /$endos^{215\text{-}4}$ expressing red fluorescent protein-labeled histone H2AvD was filmed as described in Materials and Methods. At t=0, the cell chosen for filming at the center of the field had already begun to condense its chromosomes and was thus in a prophase-like state. This cell was unable to progress further in the cell cycle even as late as t=90 min (compare with the control in File S1). Still images from this movie are shown at the bottom of Figure 3 in the text.

File S3 *ensa-1(tm2810) C. elegans* embryos develop at wildtype rates. Wildtype (top) and *ensa-1(tm2810)* embryos were mounted for viewing just prior to the first cleavage, and a time-lapse movie was made of their development at 20°C. Note that hatching of these two animals occurs in near synchrony, even though the mutant animal lacks the highly conserved site in Endos that can be phosphorylated by Gwl in other species. Still images from this movie are shown in Figure 9 of the text.

ADDITIONAL LITERATURE CITED IN SUPPORTING INFORMATION

DULUBOVA, I., A. HORIUCHI, G. L. SNYDER, J. A. GIRAULT, A. J. CZERNIK *et al.*, 2001 ARPP-16/ARPP-19: a highly conserved family of cAMP-regulated phosphoproteins. J Neurochem 77: 229-238.

MOCHIDA, S., S. L. MASLEN, M. SKEHEL and T. HUNT, 2010 Greatwall phosphorylates an inhibitor of protein phosphatase 2A that is essential for mitosis. Science 330: 1670-1673.