

Fig. S1. Identification of *C. elegans* CDK-12. (A) Comparison of *C. elegans* cell-cycle class kinases with CDK-12 and CDK-9 proteins in other organisms. Rows indicate *C. elegans* kinases with the closest sequence similarity to the kinase input for BLAST (column head); columns show BLAST Expect (E) values of *C. elegans* kinases compared with known CDK12 and CDK9 proteins, as indicated. The following protein sequences were compared with the worm proteins using BLAST (Altschul et al., 1997): *S. cerevisiae* Ctk1 (Sc-Ctk1); *Drosophila melanogaster* CDK12 (Dm-CDK12); *Homo sapiens* CDK12 (Hs-CDK12); *S. cerevisiae* Bur1 (Sc-Bur1); *Drosophila melanogaster* CDK9 (Dm-CDK9); and *Homo sapiens* CDK9 (Hs-CDK9). Among the *C. elegans* kinases, the previously uncharacterized gene, B0285.1 (previously named *cdtl-7*; now named on wormbase.org and referred to in text as CDK-12) is the closest homolog to Ctk1 and other CDK12 proteins, whereas the *C. elegans* CDK-9 protein is the closest homolog to Bur1 and CDK9. These results support previous studies that reached a similar conclusion (Liu and Kipreos, 2000; Bartkowiak et al., 2010; Shaye and Greenwald, 2011). (B) Comparison of *C. elegans* cyclins with known cyclin K and cyclin T proteins. Comparisons presented as in A. *S. cerevisiae* Ctk2 (Sc-Ctk2), *Drosophila melanogaster* Cyclin K (Dm-CYCK), *Homo sapiens* cyclin K (Hs-CCNK), *S. cerevisiae* Bur2 (Sc-Bur2), *Drosophila melanogaster* Cyclin T (Dm-CYCT), *Homo sapiens* cyclin T (two genes: Hs-Hs-CCNT1 and Hs-CCNT2). The previously uncharacterized gene F43D2.1 (now identified in wormbase.org and referred to in text as *ccnk-1*) is the closest homolog to Ctk2 and cyclin K, whereas the *C. elegans* CIT-1.1 and CIT-1.2 proteins are the closest homologs to cyclin T. The longest annotated isoform was used for all BLAST searches. Red boxes indicate highest homology. ns, no significant homolog detected. (C) Predicted secondary structure comparisons for CDK12 kinases. Secondary structure predictions performed using (Cole et al., 2008) and the kinase domain of each was determined by the NCBI domain search (Marchler-Bauer et al., 2011). All CDK12 orthologs, including the *C. elegans* protein, contain largely unstructured N and C termini outside of the kinase domain that are not present on CDK9 kinases.

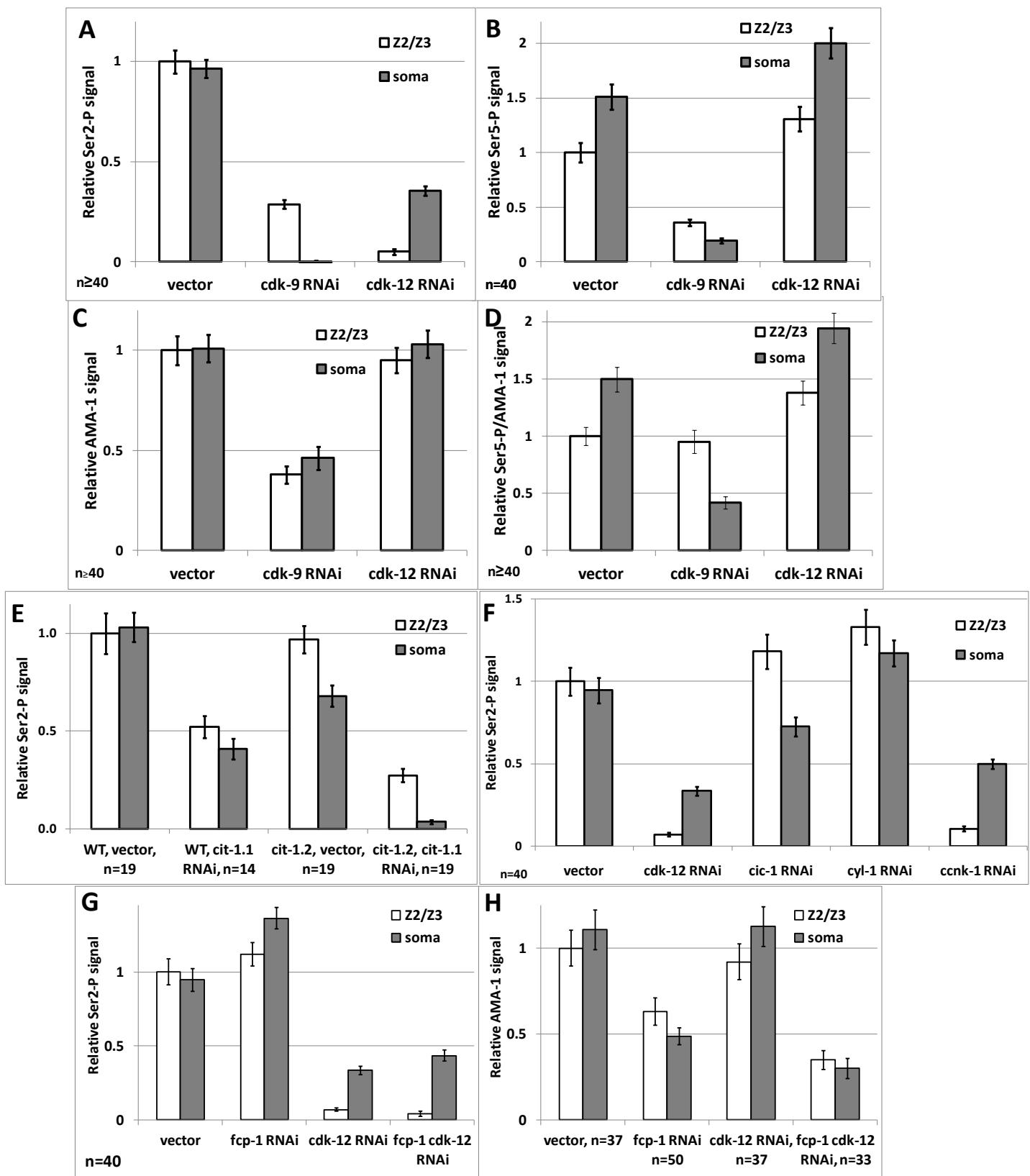


Fig. S2. Quantification of Pol II and CTD modifications in the embryo. This relates to Fig. 1. (A,G) Anti-Ser2-P immunofluorescence (E10 antibody). (B) Anti-Ser5-P (3E8) immunofluorescence. (C,H) Total anti-AMA-1 immunofluorescence. (D) Anti-Ser5-P signal normalized to total anti-AMA-1 immunofluorescence. (E) Anti-Ser2-P immunofluorescence of RNAi against *C. elegans* Cyclin T homolog, *cit-1.1* in WT or *cit-1.2(gk241)* mutants, showing that knockdown of both homologs phenocopies *cdk-9(RNAi)*. (F) RNAi targeting worm Cyclin K homologs. Knockdown of the closest sequence homolog, *ccnk-1*, phenocopies *cdk-12(RNAi)*. Signal intensities measured in Z2/Z3 or representative somatic nuclei (data are mean \pm s.e.m.) in the indicated RNAi conditions. Signal is normalized to Z2/Z3 immunofluorescence in vector-treated controls

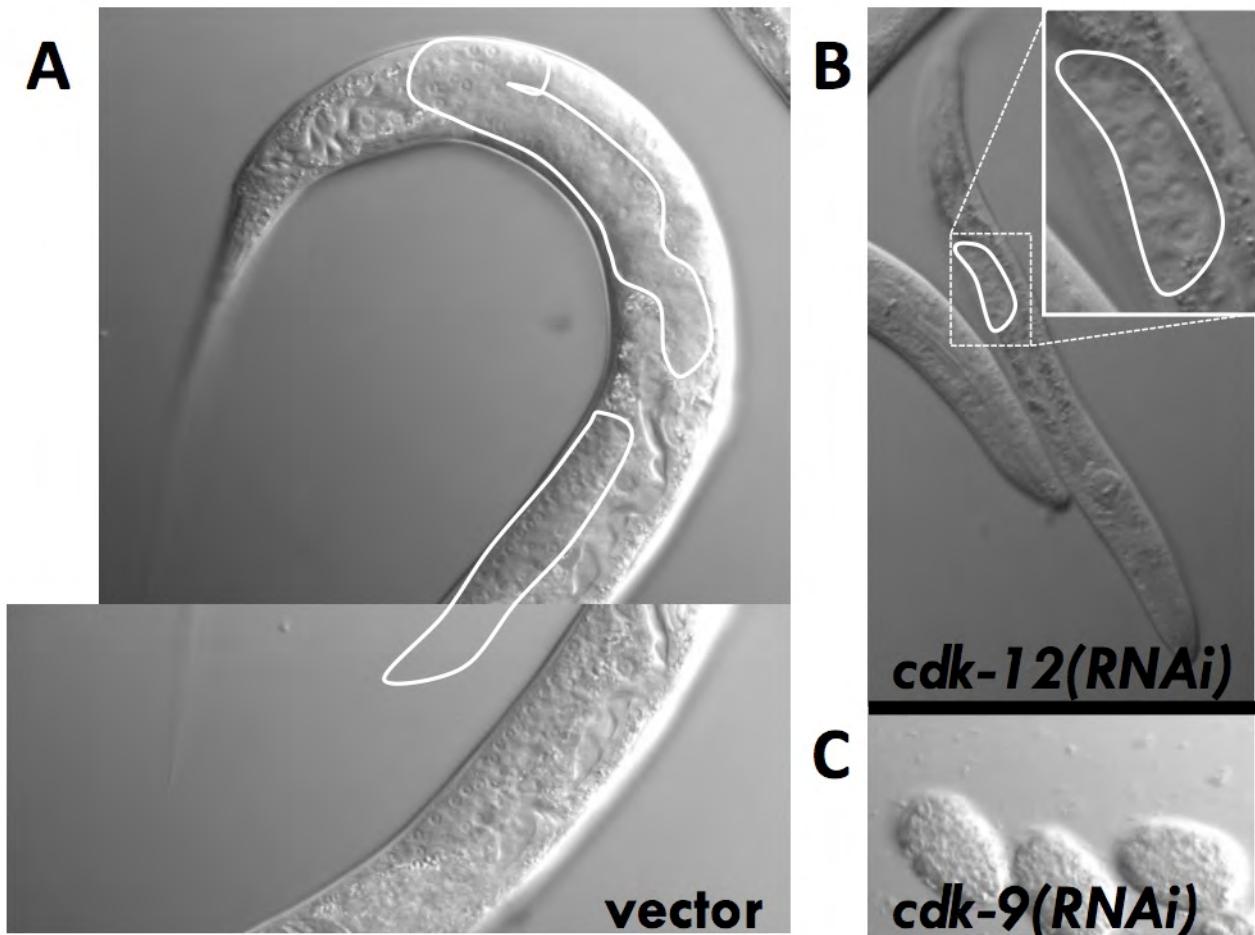


Fig. S3. Arrest phenotypes of CDK-9 and CDK-12 RNAi-treated animals. This relates to Fig. 1. DIC images of progeny ~2 days following indicated RNAi treatments. (A) Vector-treated animals develop normally. (B) Progeny of *cdk-12 (RNAi)* animals arrest after hatching. (C) Progeny of *cdk-9 (RNAi)* arrest as embryos (lower right). All images other than inset in B are at the same scale. Solid lines outline the germ cells and the inset in upper right shows a higher magnification of the larval gonad region, illustrating evidence of proliferation of the larval germ line.

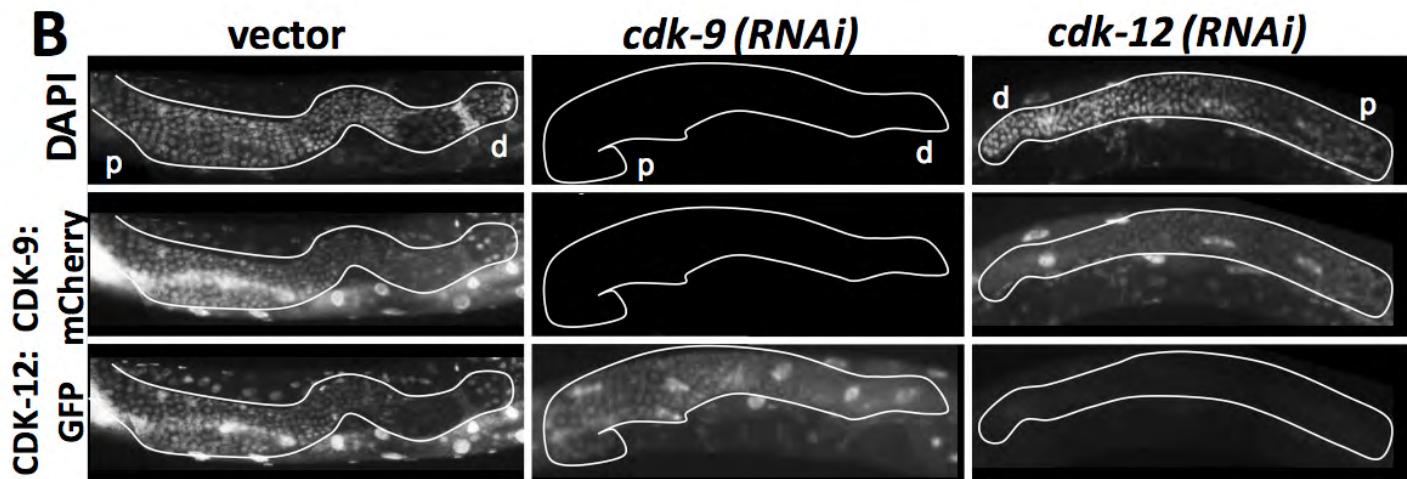
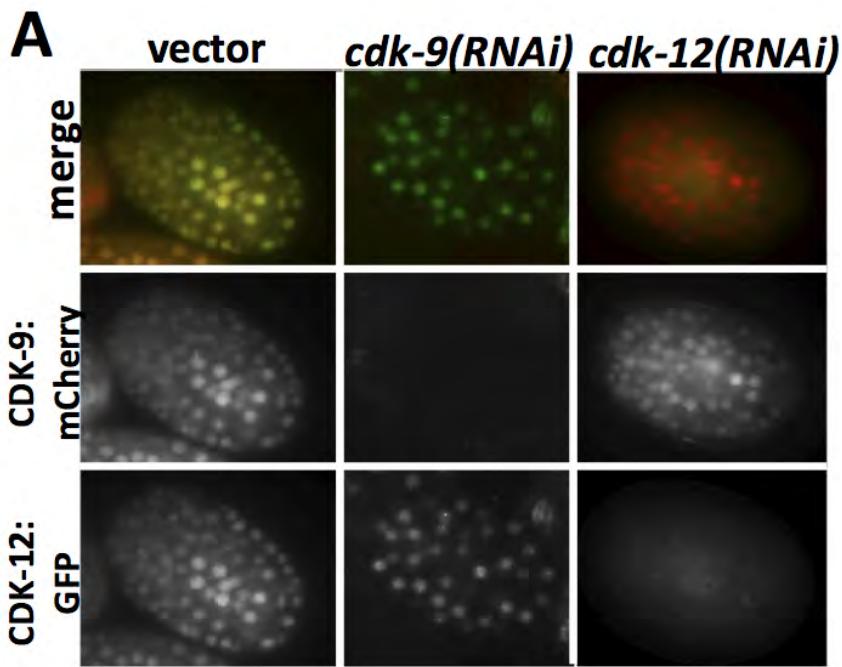


Fig. S4. Demonstration of *kinase(RNAi)* knockdown specificity and efficiency in CDK-9; CDK-12 double transgenic animals.
This relates to Figs 1 and 3. (A,B) CDK-9:mCherry and CDK-12:GFP transgenes targeted by RNAi against either show that the appropriate kinase was specifically and robustly knocked down in all embryonic nuclei (A) and germline (B, outlined regions) under the RNAi conditions used.

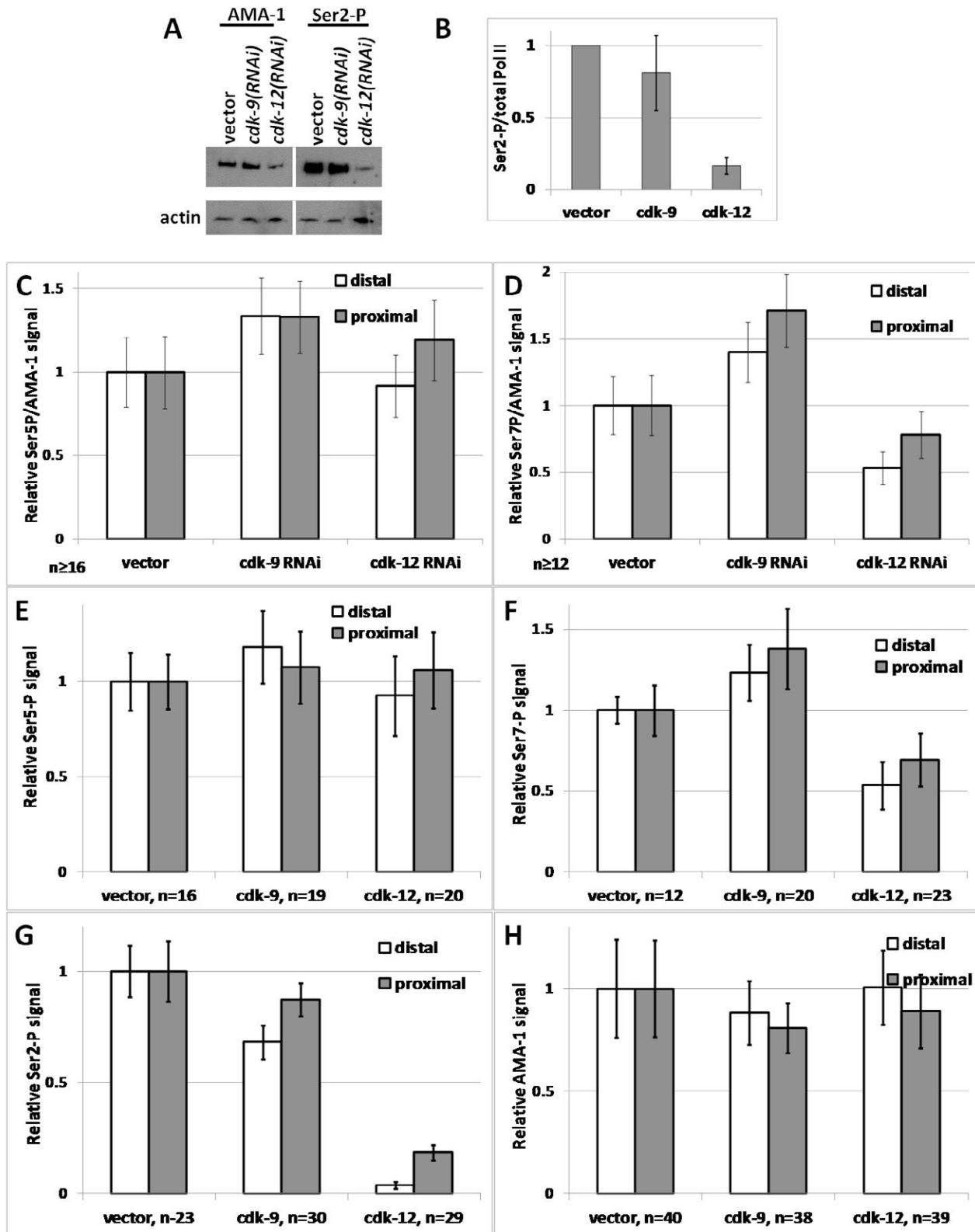


Fig. S5. Quantification of alterations in Pol II and CTD modifications following RNAi. This relates to Fig. 3. (A) Western blot of total protein extracted from adult worms following RNAi conditions. The same amount of protein was loaded in each lane. Total AMA-1 was detected using an antibody against the N terminus of AMA-1 and Ser2-P was detected using the H5 antibody. Decreases in total AMA-1 in *cdk-12(RNAi)* were not consistently observed. (B) Quantification of Ser2-P relative to total AMA-1 levels normalized to vector-treated sample. Error bars indicate s.e.m., $n=3$. (C-H) Quantification of immunofluorescence signals in the adult gonad. (C) Relative Ser5-P signal versus total AMA-1. (D) Relative Ser5-P signal versus total AMA-1. (E) Anti-Ser5-P (3E8) immunofluorescence. (F) Anti-Ser7-P (4E12) immunofluorescence. (G) Anti-Ser2-P (3E10) immunofluorescence. (H) Total anti-AMA-1 immunofluorescence. Signal intensities measured at the extreme distal or proximal end of the gonad. Error bars indicate s.e.m.. Signal is normalized to vector-treated signal.

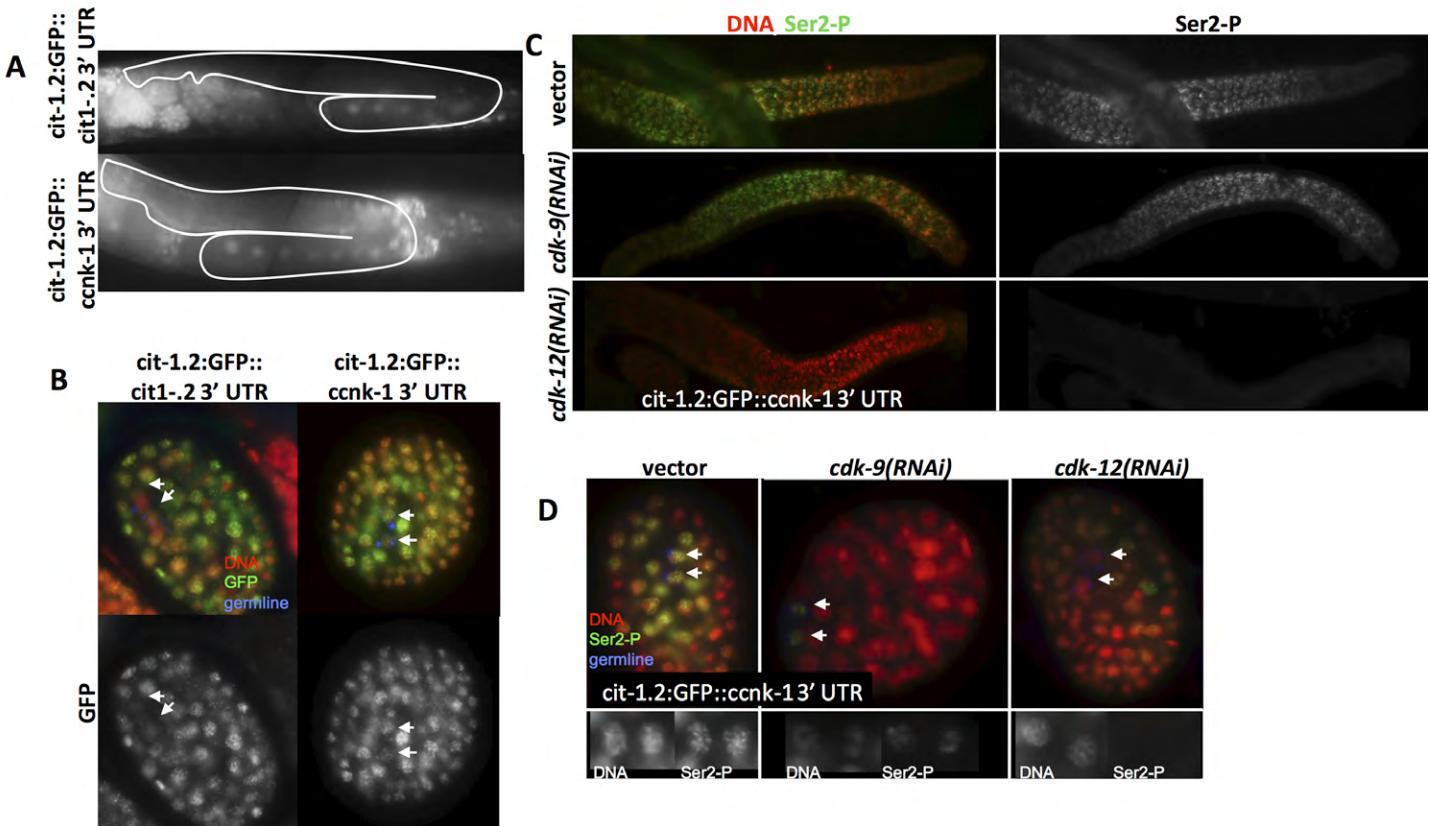


Fig. S6. Increased expression of cyclin T (CIT-1.2) does not change Ser2-P kinase requirements in germ cells. This figure relates to Fig. 4. We observed reduced CIT-1.2:GFP expression in germ cells, whereas we did not observe this with other transgenes tested. This suggests a possible correlation with the decreased requirement of CDK-9/cyclin T for Ser2-P in these cells. To test this, we increased cyclin T expression in the germline by replacing the *cit-1.2 3'UTR* with the *ccnk-1 3'UTR* in the CIT-1.2 construct. This resulted in substantially increased expression of CIT-1.2 in both embryonic and adult germ cells, but did not change the Ser2 kinase requirements. (A) GFP expression in an adult hermaphrodite transgenic animals expressing CIT-1.2:GFP with either its endogenous 3'UTR or *ccnk-1 3'UTR*. White outline is around gonad. (B) Anti-GFP immunofluorescence of MOS-SCI transgenic lines expressing CIT-1.2 with either its endogenous 3'UTR or *ccnk-1 K 3'UTR*. Blue, pgl staining and arrows indicate the primordial germ cells, Z2/Z3. (C) Anti-Ser2-P immunofluorescence images of dissected CIT-1.2:GFP:ccnk-1 3'UTR hermaphrodite gonads exposed to the indicated RNAi conditions. (D) Anti-Ser2-P immunofluorescence images of CIT-1.2:GFP:ccnk-1 3' UTR embryos exposed to the indicated RNAi conditions. Blue, pgl staining; arrows indicate the germ cells, Z2/Z3, expanded in inset below.

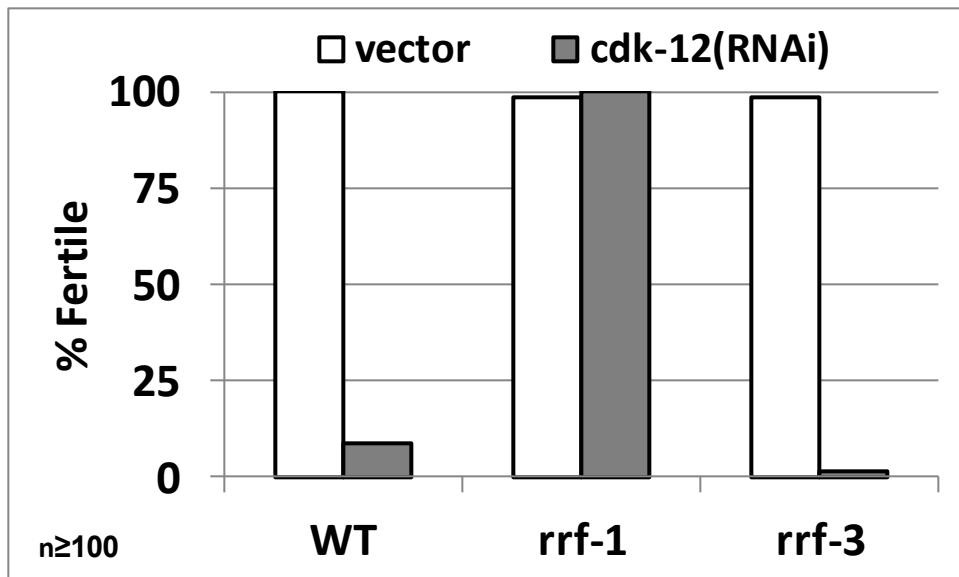


Fig. S7. Knockdown of CDK-12 specifically in the germline does not result in sterility. This figure relates to Fig. 5. Quantification of fertility in worms treated with RNAi from the L1 stage through adulthood. L1 worms hatched without food of the indicated genotype were placed on RNAi feeding plates for 72 hours. Plates were prepared by spreading ampicillin (final concentration 100 ng/ml) and IPTG (final concentration 1 mM) to NGM plates, dried for 12 hours and HT115 cells transformed with a cdk-12 RNAi construct was spread onto plate and dried for 24 hours before L1 worms were added. Fertility was scored by the presence of embryos within the ovary of the adult worm.

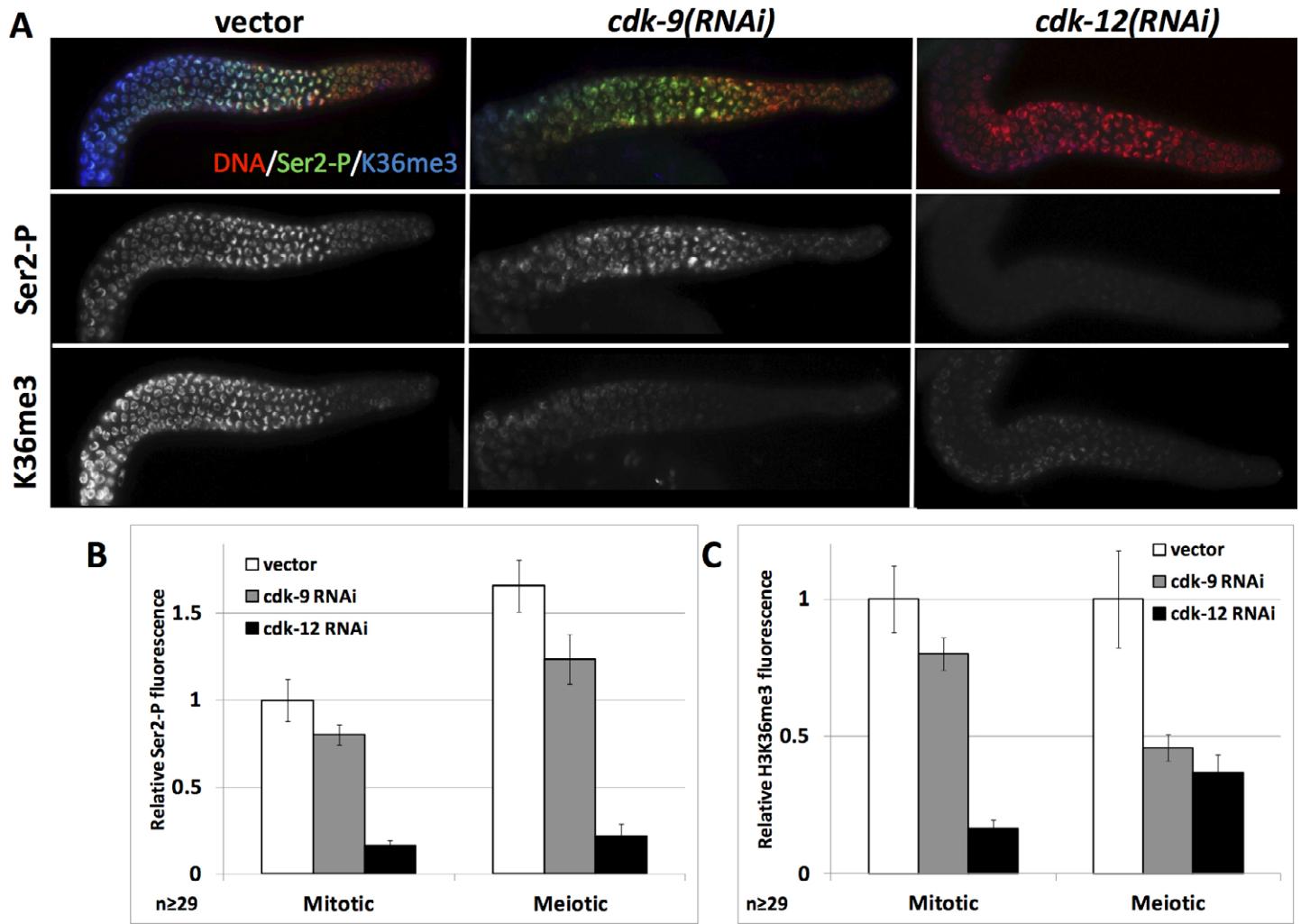


Fig. S8. CDK-9 and CDK-12 regulate transcription-dependent H3K36me3. This figure relates to Fig. 7. (A) Anti-Ser2-P and anti-H3K36me3 immunofluorescence images of dissected hermaphrodite gonads from *mes-4(bn85)* worms exposed to the indicated RNAi conditions. (B) Quantification of anti-Ser2-P immunofluorescence signal \pm s.e.m. (C) Quantification of anti-H3K36me3 immunofluorescence signal \pm s.e.m. Nuclei selected for analysis were from the most distal mitotic and meiotic regions of the gonad.

Table S1. Cyclin T RNAi embryonic lethality (n=17)

| RNAi treatment | cit-1.2:GFP | | cit-1.2(gk214) | | cit-1.2(gk214); cit-1.2:GFP | |
|--------------------|-------------|----------------------|----------------|----------------------|-----------------------------|----------------------|
| | vector | <i>cit-1.1(RNAi)</i> | vector | <i>cit-1.1(RNAi)</i> | vector | <i>cit-1.1(RNAi)</i> |
| Average # emb laid | 30±3 | 25±3 | 13±5 | 9±6 | 24±4 | 24±5 |
| Emb lethality | 0.19% | 0.23% | 30.34% | 100% | 0.24% | 0.00% |

Either *cdk-1.2:GFP* transgenic worms (KW2140), *cit-1.2(gk214)* mutant worms, or *cit-1.2(gk214)* mutants expressing the *cdk-1.2:GFP* transgene (KW2268) were exposed to vector only or *cit-1.1(RNAi)* treatment. Adult worms were transferred to individual plates RNAi. Worms laid embryos for 6 hours, the adult worm was killed and the number of embryos laid was counted. The number of embryos unhatched after 24 hours were counted and compared with the original number laid to calculate percent lethality.

Table S2. New transgenic lines

| Name | Description | Genotype | Plasmid |
|--------|----------------------------|---|---------|
| KW2112 | CDK-12:GFP | ckSi6 (unc-119, cdk-12:GFP) I; unc-119(ed3) III | pBAB66 |
| KW2157 | CDK-12 (D462N):GFP | ckSi9 (unc-119, cdk-12:D462N:GFP) I; unc-119(ed3) III | pBAB75 |
| KW2194 | CDL-12:GFP:mex-5 3' UTR | ckSi17 (unc-119, cdk-12:GFP:mex-5 3' UTR) I; unc-119(ed3) III | pBAB96 |
| KW2206 | CDK-12:GFP:pal-1 3' UTR | ckSi26 (unc-119, cdk-12:GFP:pal-1 3' UTR) I; unc-119(ed3) III | pBAB97 |
| KW2117 | CCNK-1:FLAG | ckSi10 (unc-119, ccnk-1:FLAG) II; unc-119(ed3) III | pBAB76 |
| KW2147 | CCNK-1:GFP | ckSi15(unc-119, ccnk-1:GFP)II; unc-119(ed3) III | pBAB88 |
| KW2115 | CDK-9:mCherry | ckSi4 (unc-119, cdk-9:mCherry) II; unc-119(ed3) III | pBAB61 |
| KW2167 | CDK-9:GFP | ckSi13 (unc-119, cdk-9:GFP) II; unc-119(ed3) III | pBAB86 |
| KW2159 | CDK-9(D235N):GFP | ckSi12 (unc-119, cdk-9D235N:mCherry) II; unc-119(ed3) III | pBAB85 |
| KW2195 | CDK-9:mCherry:mex-5 3' UTR | ckSi20 (unc-119, cdk-9:GFP:mex-5 3' UTR) II; unc-119(ed3) III | pBAB102 |
| KW2196 | CDK-9:mCherry:pal-1 3' UTR | ckSi21 (unc-119, cdk-9:GFP:pal-1 3' UTR) II; unc-119(ed3) III | pBAB103 |
| KW2096 | CIT-1.1:FLAG | ckSi2 (unc-119, cit-1.1:FLAG) II; unc-119(ed3) III | pBAB58 |
| KW2098 | CIT-1.2:FLAG | ckSi3 (unc-119, cit-1.2:FLAG) II; unc-119(ed3) III | pBAB59 |
| KW2140 | CIT-1.2:GFP | ckSi14(unc-119, cit-1.2:GFP)II; unc-119(ed3) III | pBAB87 |
| KW2104 | SPT-5:GFP | ckSi5 (unc-119, spt-5:GFP) II; unc-119(ed3) III | pBAB62 |
| KW2237 | CIT-1.2:FLAG:CCNK-1 3' UTR | ckSi25 (unc-119, cit-1.2:FLAG:ccnk-1 3' UTR) II; unc-119(ed3) III | pBAB110 |

Table S3. Additional strains made

| Name | Description | Genotype |
|--------|--|--|
| KW2126 | CDK-12:GFP rescue of cdk-12 deletion | cdk-12(tm3846)III; ckSi6 (unc-119, cdk-12:GFP) I |
| KW2214 | CDK-12:GFP rescue of cdk-12 deletion | cdk-12(ok3664)III; ckSi6 (unc-119, cdk-12:GFP) I |
| KW2209 | CDK-12(D462N):GFP in balanced cdk-12 deletion | cdk-12(ok3664)/qC1 qIs26 (lag-2:GFP; rol-6) III; ckSi9 (unc-119, cdk-12 D462N:GFP) I; unc-119(ed3) III |
| KW2210 | CDK-12:GFP:mex-5 3' UTR in balanced cdk-12 deletion | cdk-12(ok3664)/qC1 qIs26 (lag-2:GFP; rol-6) III; ckSi17 (unc-119, cdk-12:GFP:mex-5 3' UTR) I |
| KW2211 | CDK-12:GFP:pal-1 3' UTR in balanced cdk-12 deletion | cdk-12(ok3664)/qC1 qIs26 (lag-2:GFP; rol-6) III; ckSi18 (unc-119, cdk-12:GFP:pal-1 3' UTR) I |
| KW2183 | CDK-9:mCherry rescue of cdk-9 deletion | cdk-9(tm2884) I; ckSi4 (unc-119, cdk-9:mCherry) II |
| KW2181 | CDK-9(D235N):mCherry in balanced cdk-9 deletion mutant | cdk-9(tm2884)/ht2 qIs48 (myo-2:GFP) I, III; ckSi12 (unc-119, cdk-9D235N:mCherry) II |
| KW2204 | CDK-9:mCherry:mex-5 3' UTR in balanced cdk-9 deletion | cdk-9(tm2884)/ht2 qIs48 (myo-2:GFP) I, III; ckSi20 (unc-119, cdk-9:mCherry:mex-5 3' UTR) II |
| KW2205 | CDK-9:mCherry:pal-1 3' UTR in balanced cdk-9 deletion | cdk-9(tm2884)/ht2 qIs48 (myo-2:GFP) I, III; ckSi21 (unc-119, cdk-9:mCherry:pal-1 3' UTR) II |
| KW2185 | CDK-12:GFP, CDK-9:mCherry | ckSi6 (unc-119, cdk-12:GFP) I; ckSi4 (unc-119, cdk-9:mCherry) II; unc-119(ed3) III |
| KW2268 | CIT-1.2:GFP rescue of cit-1.2 deletion | cit-1.2(gk241) III; ckSi14(unc-119, cit-1.2:GFP) II |

Table S4. Cloning methods

| Name | Parent vector | Description | Made from pBAB plasmid | Cloning method | Primers |
|--------|---------------|--|------------------------|---|--------------|
| pBAB30 | pCR blunt | cit-1.1 ORF | - | blunt ligation with PCR prod: cit-1.1 ORF | BB336, BB240 |
| pBAB31 | pCR blunt | cit-1.2 ORF | - | blunt ligation with PCR prod: cit-1.2 ORF | BB316, BB317 |
| pBAB33 | pCR blunt | cdk-12 ORF | - | blunt ligation with PCR prod: cdk-12 ORF | BB334, BB226 |
| pBAB34 | pCR blunt | cdk-9 5' ORF | - | blunt ligation with PCR prod: cdk-9 5' ORF | BB333, BB237 |
| pBAB35 | pCR blunt | spt-5 ORF | - | blunt ligation with PCR prod: spt-5 ORF | BB298, BB299 |
| pBAB36 | pCR blunt | Pcit-1.1 cit-1.1 ORF | pBAB30 | KpnI, NheI ligation with PCR prod: Pcit-1.1 | BB322, BB323 |
| pBAB37 | pCR blunt | Pcit-1.2 cit-1.2 ORF | pBAB31 | BamHI, NheI ligation with PCR prod: Pcit-1.2 | BB312, BB313 |
| pBAB39 | pCR blunt | Pcdk-12 cdk-12 ORF | pBAB33 | KpnI, NheI ligation with PCR prod: Pcdk-12 | BB320, BB321 |
| pBAB40 | pCR blunt | Pcdk-9 cdk-9 5' ORF | pBAB34 | KpnI, NheI ligation with PCR prod: Pcdk-9 | BB318, BB319 |
| pBAB41 | pCR blunt | Pcdk-9 cdk-9 whole ORF | pBAB40 | AvrII, SbfI ligation with PCR prod: Pcit-1.5 | BB238, BB332 |
| pBAB42 | pCR blunt | Pspt-5 spt-5 ORF | pBAB35 | KpnI, NheI ligation with PCR prod: Pspt-5 | BB300, BB301 |
| pBAB43 | pCR blunt | Pcit-1.1 cit-1.1 ORF FLAG | pBAB36 | SbfI, NotI ligation with PCR product: FLAG from pFS26 | BB330, BB331 |
| pBAB44 | pCR blunt | Pcit-1.2 cit-1.2 ORF FLAG | pBAB37 | SbfI, NotI ligation with PCR product: FLAG from pFS26 | BB330, BB331 |
| pBAB46 | pCR blunt | Pcdk-12 cdk-12 ORF GFP | pBAB39 | SbfI, NotI ligation with PCR product: GFP from pFS19 | BB326, BB327 |
| pBAB47 | pCR blunt | Pcdk-9 cdk-9 whole ORF mCherry | pBAB41 | SbfI, NotI ligation with PCR product: mCherry pFS26 | BB328, BB329 |
| pBAB48 | pCR blunt | Pspt-5 spt-5 ORF GFP | pBAB42 | SbfI, NotI ligation with PCR product: GFP from pFS19 | BB326, BB327 |
| pBAB49 | pCR blunt | Pcit-1.1 cit-1.1 ORF FLAG cit-1.1 3' UTR | pBAB43 | NotI, ApaI ligation with PCR prod: cit-1.1 3' UTR | BB310, BB311 |
| pBAB50 | pCR blunt | Pcit-1.2 cit-1.2 ORF FLAG cit-1.2 3' UTR | pBAB44 | NotI, ApaI ligation with PCR prod: cit-1.2 3' UTR | BB314, BB315 |
| pBAB51 | pCR blunt | Pccnk-1 ccnk-1 ORF FLAG ccnk-1 3' UTR | pBAB57+pBAB65 | KpnI, SbfI ligation | |
| pBAB52 | pCR blunt | Pcdk-12 cdk-12 ORF GFP cdk-12 3' UTR | pBAB46 | NotI, ApaI ligation with PCR prod: cdk-12 3' UTR | BB306, BB307 |
| pBAB53 | pCR blunt | Pcdk-9 cdk-9 ORF mCherry cdk-9 3' UTR | pBAB47 | NotI, ApaI ligation with PCR prod: cdk-9 3' UTR | BB304, BB392 |
| pBAB54 | pCR blunt | Pspt-5 spt-5 ORF GFP spt-5 3' UTR | pBAB48 | NotI, ApaI ligation with PCR prod: spt-5 3' UTR | BB302, BB303 |
| pBAB57 | pCR blunt | Pccnk-1 ccnk-1 ORF FLAG cit-1.1 3' UTR | pBAB49 | KpnI, SbfI ligation with PCR prod: Pccnk-1 ccnk-1 ORF | BB324, BB228 |
| pBAB58 | pCFJ151 | cit-1.1 FLAG | pBAB49 | blunt ligation after KpnI, ApaI digestion | |
| pBAB59 | pCFJ151 | cit-1.2 FLAG | pBAB50 | blunt ligation after BamHI, ApaI digestion | |
| pBAB60 | pCFJ151 | cdk-12 GFP | pBAB52 | blunt ligation after KpnI, ApaI digestion | |
| pBAB61 | pCFJ151 | cdk-9 mCherry | pBAB53 | blunt ligation after KpnI, ApaI digestion | |
| pBAB62 | pCFJ151 | spt-5 GFP | pBAB54 | blunt ligation after KpnI, ApaI digestion | |
| pBAB65 | pCR blunt | Pcit-1.2 cit-1.2 ORF FLAG ccnk-1 3' UTR | pBAB50 | NotI, ApaI ligation with PCR product: ccnk-1 3' UTR | BB308, BB309 |
| pBAB66 | pCFJ352 | cdk-12 GFP | pBAB52 | blunt ligation after KpnI, ApaI digestion | |
| pBAB68 | pCR blunt | cdk-9 5' ORF D235N | pBAB34 | Qiagen XL QC | BB385, BB386 |
| pBAB71 | pCR blunt | cdk-12 ORF D462N | pBAB33 | Qiagen XL QC | BB383, BB384 |
| pBAB75 | pCFJ352 | cdk-12 D462N GFP | pBAB66+pBAB71 | SphI, NheI ligation | |
| pBAB76 | pCFJ151 | ccnk-1 FLAG | pBAB51 | blunt ligation after KpnI, ApaI digestion | |
| pBAB79 | pCR blunt | Pspt-5 spt-5 ORF FLAG spt-5 3' UTR | pBAB54 | SbfI, NotI ligation with PCR product: FLAG from pFS26 | BB330, BB331 |
| pBAB80 | pCR blunt | Pcdk-12 cdk-12 ORF FLAG cdk-12 3' UTR | pBAB52 | SbfI, NotI ligation with PCR product: FLAG from pFS26 | BB330, BB331 |
| pBAB81 | pCR blunt | Pcit-1.1 cit-1.1 ORF GFP cit-1.1 3' UTR | pBAB49 | SbfI, NotI ligation with PCR product: GFP from pFS19 | BB326, BB327 |
| pBAB82 | pCR blunt | Pcit-1.2 cit-1.2 ORF GFP cit-1.2 3' UTR | pBAB50 | SbfI, NotI ligation with PCR product: GFP from pFS19 | BB326, BB327 |
| pBAB83 | pCR blunt | Pcdk-9 cdk-9 ORF GFP cdk-9 3' UTR | pBAB53 | SbfI, NotI ligation with PCR product: GFP from pFS19 | BB326, BB327 |
| pBAB84 | pCR blunt | Pccnk-1 ccnk-1 ORF GFP ccnk-1 3' UTR | pBAB51 | SbfI, NotI ligation with PCR product: GFP from pFS19 | BB326, BB327 |
| pBAB85 | pCFJ151 | cdk-9 D235N mCherry | pBAB61+pBAB68 | EcoNI, SphI ligation | |
| pBAB86 | pCFJ151 | cdk-9 GFP | pBAB83+pBAB61 | NotI, SphI ligation | |
| pBAB87 | pCFJ151 | cit-1.2 GFP | pBAB82+pBAB59 | NotI, NheI ligation | |

| | | | | | |
|---------|---------|----------------------------|---------------|--|--------------|
| pBAB88 | pCFJ151 | ccnk-1 GFP | pBAB84+pBAB76 | NotI, SphI ligation | |
| pBAB96 | pCFJ352 | cdk-12:GFP:mex-5 3' UTR | pBAB66 | NotI, SnaBI ligation with PCR product: mex-5 3' UTR as specified in (Merritt et al., 2008) | BB408, BB424 |
| pBAB97 | pCFJ352 | cdk-12:GFP:pal-1 3' UTR | pBAB66 | NotI, SnaBI ligation with PCR product: pal-1 3' UTR as specified in (Merritt et al., 2008) | BB410, BB425 |
| pBAB102 | pCFJ151 | cdk-9:mCherry:mex-5 3' UTR | pBAB61 | NotI, XhoI ligation with PCR product: mex-5 3' UTR as specified in (Merritt et al., 2008) | BB408, BB414 |
| pBAB103 | pCFJ151 | cdk-9:mCherry:pal-1 3' UTR | pBAB61 | NotI, Xhol ligation with PCR product: pal-1 3' UTR as specified in (Merritt et al., 2008) | BB410, BB415 |

Table S5. Primer sequences

