Supporting Online Material for

Checkpoint Proteins Control Survival of the Postmitotic Cells in *Caenorhabditis elegans*

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Materials and Methods

C. elegans strains and culturing

All nematodes were cultured using standard *C. elegans* methods as described by Brenner (*S1*). We thank Ronald Plasterk for the strain NL917 *mut-7*(*pk720*) and Cynthia Kenyon for the strain *daf-16(mu86).* N2, DA1113 *eat-2(ad1113)*, DR20 *daf-12(m20),* RB647 *cdc-25.3(ok358)*, MR142 *cdc-25.1(rr31) I; rrIs,* SJ4005 *zcIs4(hsp-4::GFP),* CL2070 *dvIs70(hsp-16.2::gfp; rol-6(su1006)*, CF1553 *muIs84((pAD76(sod-3::GFP))* and NL2099 *rrf-3(pk1426)* were obtained from the *Caenorhabditis* Genetics Center (CGC) in Minneapolis. The strains GL302 *cid-1(rf34::Tc4),* GL303 *cid-1(rf34::Tc4),* and GL304 *cid-1(rf34::Tc4)* were generated by backcrossing (2x) the mutant isolated from the mutator strain NL917 *mut-7*(*pk720*). GL305 *cid-1*(+/+) and GL305 *cid-1*(+/+) are control strains with wild-type *cid-1* generated by backcrossing (2x) the mutant isolated from the mutator strain NL917 *mut-7*(*pk720*). Worms were cultured at 20ºC or 25ºC as indicated. The strain GL310 was generated by crossing MR142 *cdc-25.1(rr31) I; rrIs* to wild-type N2 and selecting for progeny displaying no extra intestinal cells. We found no effect of the insertion on thermotolerance. GL311 and GL312 were generated by backcrossing RB647*cdc-25.3(ok358)* once. GL313 and GL314 were generated by backcrossing RB647*cdc-25.3(ok358)* twice. GL312 and GL313 are control strains with wild-type *cdc-25.3*.

Isolation and identification of the *cid-1* mutation

A number of previous publications have shown that selection for stress resistance yields mutants with extended life span (*S2*, *S3*). The transposon screen was performed by screening 1.5 million L1 larvae from the mutator strain NL917 *mut-7(pk720)* for their ability to survive a heat stress at 35ºC and exposure to lethal doses (40 mM) of hydrogen peroxide. Transposons were displayed using the Vectorette protocol. Lines established following two backcrosses were analyzed and the band co-segregating with the intrinsic thermotolerance (Itt) phenotype identified. The Itt phenotype assorted as a recessive trait. The position of the transposon was identified by sequencing of the flanking regions. Genetically stable lines were selected via allelic specific PCR of the *mut-7* gene. Sequencing of the flanking DNA followed by Blast analysis revealed the identity of the mutated gene.

Thermotolerance and life-span assays

Longitudinal thermotolerance assays were performed as described (*S4*). Briefly, synchronous 4- or 5-day-old adults were shifted from their normal growth temperature to 35°C and survival scored as indicated by means of touch provoked movement. Worms not responding to touch were scored as dead. In figures each point represents surviving fraction ± SEM. For each strain the results shown were obtained from two plates each with approximately 25 worms. In drug treatment experiments automated longitudinal thermotolerance assays were performed as described previously (*S5*). Life-span analysis assays were performed as described (*S4*). During the reproductive period worms were

transferred to fresh plates every day to maintain synchrony. After the fertile period worms were transferred to fresh plates every 3 to 5 days. Survival was scored by means of touch provoked movement and pumping of the pharynx. In the RNAi experiments the plates used were spotted with the feeding bacteria no more than 4 days prior to use. Controls were fed bacteria containing an empty vector. Survival data were analysed using the GraphPad Prism version 2, GraphPad Software Inc., San Diego Ca. Maximum life span was defined as the mean life span of the longest lived 20% of the cohort. A two-tailed t-test was used to determine significance between groups.

Generation of transgenic animals

A *cid-1::gfp* reporter construct was generated using a PCR fusion protocol (*S6*). A 2.5 kb region upstream of the K10D2.3 open reading frame was included. The cosmid K10D2 and the Fire vector pPD95.75 were used as templates for amplifying *cid-1* and GFP fragments, respectively. The following primers were used: Primer A 5'-TTGCCTCACTAAGACCTTGTTGAT-3', Primer A* 5'-CTTCTCTAGCAGCGGTCAATTCT-3', Primer B 5'-AGTCGACCTGCAGGCATGCAAGCTTTTGTTGTACGAGCGATGATAGTATGT-3', Primer C 5'-AGCTTGCATGCCTGCAGGTCGACT-3', Primer D 5'-AAGGGCCCGTACGGCCGACTAGTAGG-3', and Primer D* 5'-GGAAACAGTTATGTTTGGTATATTGGG-3'. Transgenic worms were generated by microinjecting wild-type N2 worms with the reporter construct.

RNA interference

Inactivation of gene function using RNAi was performed as described by Timmons *et al.* (*S7*). The primers used were cid-1f: 5'-TCCAAAAATGCCGGGTCA-3', cid-1r:5'- TGGGAACGCTTCTAGACGATC-3', chk-1f: 5'-CAGAAACGTGTGTCCGCTGT-3', chk-1r: 5'-TCGCAATGAGCACAATCCC-3', cdc-25f: 5'-GGAGAAACCAGTCATCGCGA-3' and cdc-25r: 5'-AGTCGAAACGGAGACCTGCA-3'. The fragments used to RNAi inactivate K10D2.3 (*cid-1*), Y39H10A.7 (*chk-1*) and K06A5.7 (*cdc-25.1*) were amplified using primer sets cid-1f/cid-r, chk-1f/chk-1r and cdc-25f/cdc-25r, respectively. The PCR amplified fragments were cloned into the TOPO pCR®2.1 vector and sequenced. The Not1/BamH1 fragments were sub-cloned into the pL4440 vector and plasmids were amplified in DH-5α cells. The RNAi bacteria feeding strain HT115 were transformed with Qiagen purified plasmids. Eggs were placed on agar plates (100 µg/mL ampicillin and 12.5 µg/mL tetracycline) spotted with the feeding bacteria. The phenotypes described were observed in the progeny generation. Control animals were fed bacteria carrying an empty L4440 construct. Identical phenotypes were observed using our RNAi constructs or when using the clones K10D2.3 and K06A5.7 from the RNAi library provided by Dr. Julie Ahringer and the MRC gene service following their protocol. Stronger phenotypes were often observed when using the RNAi sensitive strain *rrf-3(pk1426)* (*S8*).

Hydroxy urea resistance

Resistance to hydroxy urea (HU) was measured by letting worms develop on plates (3 cm) spotted with 60 µL HU (30.7 mg/mL) dissolved in M9 buffer. Eggs were placed on HU plates, and after 4 days, the developmental stage of the hatched worms was scored.

DNA staining

DNA was stained with 4'-6-diamidino-2-phenylindole (DAPI). Gonad dissections were performed after worms were paralyzed in 0.2 mM levamisole. The dissected gonads or whole animals were fixed with ice-cold methanol for 5 min before mounted on slides in mounting buffer containing DAPI.

UCN-01 treatment

The kinase inhibitor UCN-01 was dissolved in dimethylsulfoxide (DMSO) and spotted on top of the normal agar plates at the concentrations indicated. Control plates were spotted with DMSO only. Four-day-old adult worms were placed on the drug plates for 24 h before the effect of the drug treatment was assessed in longitudinal thermotolerance assays.

Fig. S1.

A

B

```
701                                                 750
elegans_cid-1  QEPVYEIVND KDHLKYMKEL KERLMIDGIL VNERKPIDYH TDDHRECVYG
   human_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~M GNEH..ISVH .PENSDCIQA
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               751                                                 800
elegans_cid-1  RNSLLRFK.. RVLKPGLNGK MAASMDGLSF VTKKGKKFIN .RWKKANVII
   human_cid1  DVNSDDYKGD KVYHPETGRK NEKEKVGF.. .TRKGKHLLT VDQKRGEHVV
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               801                                                 850
elegans_cid-1  LSEDSSEIDE KTKAADSNVS EIAEKLEETL KISSQKNIDV AVTSMPVSTE
   human_cid1  CGSTRNNESE STLDLEGFQN PTAKECEGLA TLDNKADLDG EFTSTEGTEE
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               851                                                 900
elegans_cid-1  IKP..EPIT. KLSNEAPVKI KNVAQPQCDL IEAPQES... LMN.....ES
   human_cid1  LEDSLNHFTH SVQGQTSEMI PSDEEEEDDE EEEEEEEPRL TINQREDEDG
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               901                                                 950
elegans_cid-1  MMESSFDETE SPMKTPIVQK PV.KQNATFG KST.ISTVIP DKQFCSEEFF
   human_cid1  MAFTNEDELD NTYTGSGDED ALSEEDDELG EAAKYEDVKE CGKHVERALL
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               951                                                1000
elegans_cid-1  IKLNS.NLKD .....IVSKS KSLKPSDFHY EFSTDLFCGD FEMEMKCTHC
   human_cid1  VELNKISLKE ENVFTCEEKN SPVDQSDFFY EFSKLIFTKG KSPTVVCSLC
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~MNISSAQF

               1001                                               1050
elegans_cid-1  D.GSHCVENC .P....MMEI PPIKKYQART PEDLKDIDDM IDKYYHE...
   human_cid1  KREGHLKKDC .PEDFKRIQL EPLPPF.TLT PKFLNILDQV CIQCYKDFSP
   yeast_cid1  IPGVHTVEEI EAEIHKNLHI SKSCSYQ.KV PNSHKEFTKF CYEVYNEIK.

               1051                                               1100
elegans_cid-1  NILDERRLKM LDHKIDELQS FLRKNYREDV TLTTFGSVMT GLSVNC..SD
   human_cid1  TIIED...QA REHIRQNLES FIRQDF.PGT KLSLFGSSKN GFTFGFKQSD
   yeast_cid1  ..ISDKEFKE KRAALDTLRL CLKR.ISPDA ELVAFGSLES GLAL..KNSD

               1101                                               1150
elegans_cid-1  IDICLRFGDG DVPPKDLTAK EVIQKTESVL RKCHLVKRVQ AIVTAKVPIV
   human_cid1  LDVCMTI.NG LETAEGLDCV RTIEELARVL RKHSGLRNIL PITTAKVPIV
   yeast_cid1  MDLCVLMDSR VQ..SDTIAL QFYEELIAEG FEGKFLQR.. ....ARIPII


              1151                                               1200
elegans_cid-1  K.FQ.VKLSN GAIIDVDISY YNILAIYNTA LLKEYSLWTP DKRFAKLALF
   human_cid1  KFFF.THLRS G..LEVDISL YNTLALHNTR LLSAYS..AI DPRVKYLCYT
   yeast_cid1  KLTSDTKNGF GASFQCDIGF NNRLAIHNTL LLSSYT..KL DARLKPMVLL

               1201                                               1250
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elegans_cid-1  VKTWAKNCEI GDASRGSLS. .SYCHVIMLI SYL.QNCDPP VLPRLQEDFR
   human_cid1  MKVFTKMCDI GDASRGSLSF TSYAYTLMVL YFL.QQRNPP VIPVLQEIYK
   yeast_cid1  VKHWAKRKQI NSPYFGTLS. .SYGYVLMVL YYLIHVIKPP VFPNL...LL

               1251                                            1300
elegans_cid-1  SDNRERRLVD NWDTSFAQVE TSLLQRW... ..PKNKESCA QLLIGYFDYY
   human_cid1  GEKKPEIFVD GWNIYFFDQI DELPTYWSEC GFTKNTESVG QLWLGLLRFY
   yeast_cid1  SPLKQEKIVD GFDVGFDDKL EDIPP..... ..SQNYSSLG SLLHGFFRFY

               1301                                            1350
elegans_cid-1  S.RFDFRNFV VQCRR.EMIL SKMEKEW.P. .......... ...RP.LCVE
   human_cid1  TEEFDFKEHV ISIRR.KSLL TTFKKQWTS. .......... ...KY.IVIE
   yeast_cid1  AYKFEPREKV VTFRRPDGYL TKQEKGWTSA TEHTGSADQI IKDRYILAIE

               1351                                            1400
elegans_cid-1  DPFDLSHN.. ...LSSGVNK KMFVFIMKVF INSRAVFMSE KPPMNRDHTF
   human_cid1  DPFDLNHNF. ..TLGAGLSR KMTNFIMKAF INGRRVFGIP VKGFPKDYPS
   yeast_cid1  DPFEISHNVG RTVSSSGLYR IRGEFMAASR LLNSRSYPIP YDSLFEEAPI

               1401                                            1450
elegans_cid-1  LSSYQHQLLR KCNQGSAPTD R..QCHQCHR IGHFVESCPQ RALAKEARKR
   human_cid1  KMEYFFDPDV LTEGELAPND RCFTCRICGK IGHFMKDCPM R..RKVRRRR
   yeast_cid1  PPRRQKKTDE QSNKKLLNET DGDNSE~~~~ ~~~~~~~~~~ ~~~~~~~~~~

               1451                                       1493
elegans_cid-1  FGSNSTNSSY RSNDSGRGFK NGEEGGDRLT YHKRTYYHRS YNK
   human_cid1  DQEDALNQRY PE~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
   yeast_cid1  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~
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Fig. S1. The K10D2.3 gene encodes a protein homologous to caffeine induced death protein-1. (**A**) Gene model of *cid-1* (K10D2.3). The position of the 3.4 kb Tc4 transposon insertion *cid-1(rf34::Tc4)* is indicated (red box, not drawn to scale). (**B**) Pileup multiple alignment of *C. elegans* CID-1 (SWISS-PROT accession # Q09409), *S. Pombe* spCid1 (SWALL (SPTR) accession # 013833) and a human homolog (EMBL accession # AK055948) identified in the NEDO human cDNA sequencing project. ClustalW colour codes were used. Identical amino acids are marked in blue. Red indicates that more than half of the amino acids of a column are identical or belong to a group of amino acids with strong similarities. Orange indicates that more than half of the amino acids of a column belong to a group of amino acids with weak similarities. Numbers refer to the *C. elegans* CID-1. The PAP domains are found at amino acids 574 to 626 (not shown) and 1245 to 1296.

Fig. S2.

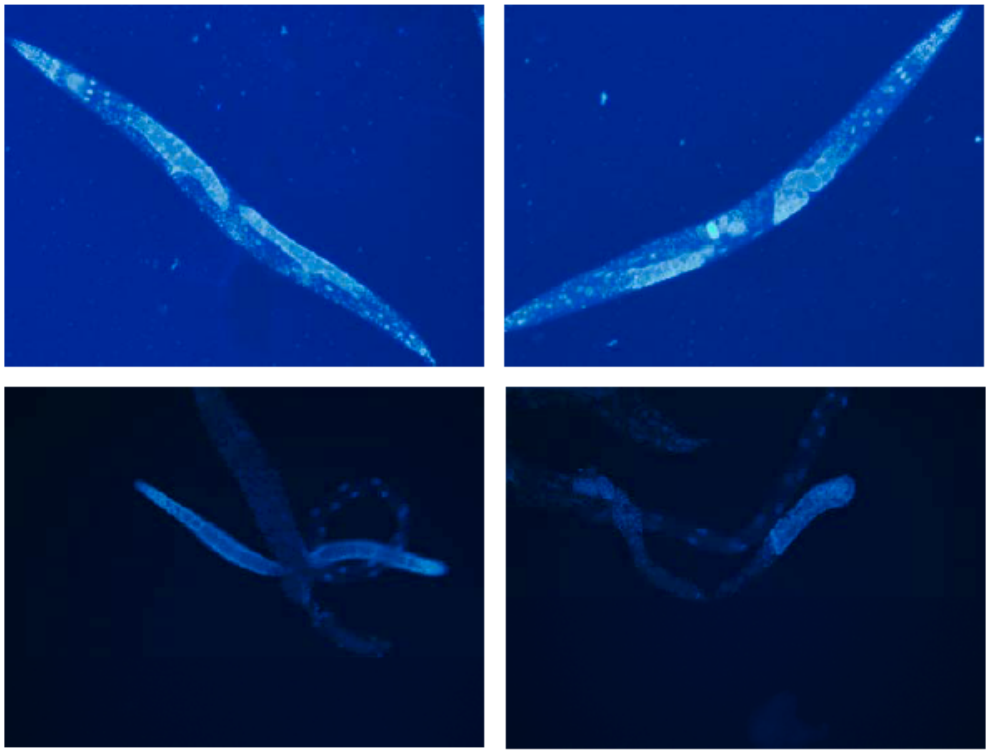

Fig. S2. *cid-1* mutants have abnormal gonads. DAPI staining of wild-type N2 (**left**) and *cid-1* mutants (**right**). Whole worms are shown in top panel. Bottom panels are stained dissected gonads.

Fig. S3.

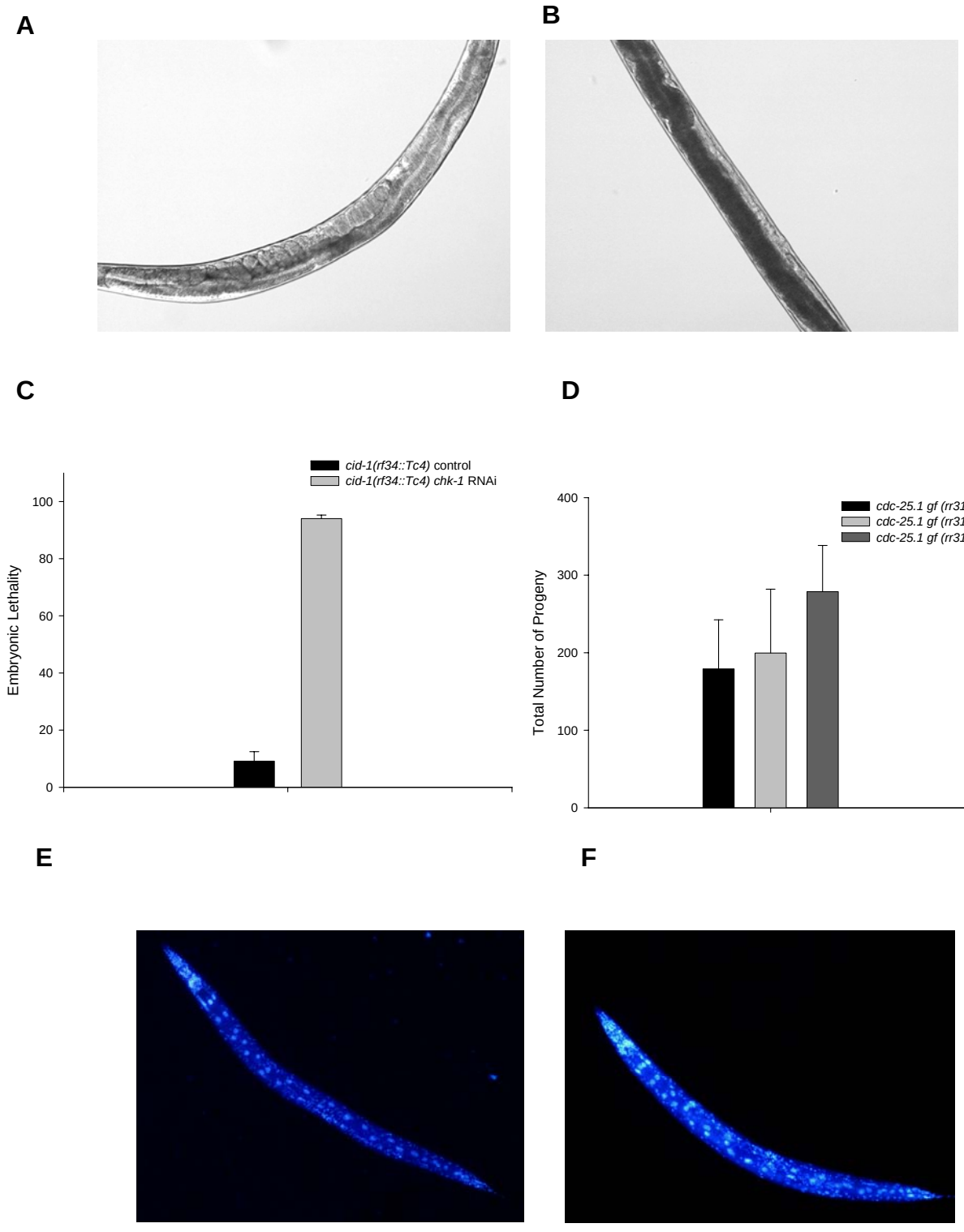
A
B
C
D
cid-1(rf34::Tc4) control
cid-1(rf34::Tc4) chk-1 RNAi
Embryonic Lethality
100
80
60
40
20
0
cdc-25.1 gf (rr31) Ctrl
cdc-25.1 gf (rr31) chk-1(RNAi)
cdc-25.1 gf (rr31) cid-1(RNAi)
Total Number of Progeny
400
300
200
100
0
E
F

Fig. S3. CID-1 and CHK-1 interact genetically in *C. elegans*. (**A**) Three-day-old wild-type N2 controls. (**B**) Three-day-old *chk-1(RNAi)* worms. The *chk-1(RNAi)* worms were sterile, they lacked oocytes and embryos in their uteri. (**C**) Percent embryonic lethality of *cid-1(rf34::Tc4)* and *cid-1(rf34::Tc4) chk-1(RNAi)* mutants. Results obtained from two independent experiments are shown. (**D**) Fertility of *cdc-25.1* gf mutant worms following inactivation of *cid-1*, *chk-1* and *cdc-25.1*. (**E**) DAPI staining of *chk-1(RNAi)* worm. (**F**) DAPI staining of *cdc-25.1(RNAi)* worm.

Fig. S4.

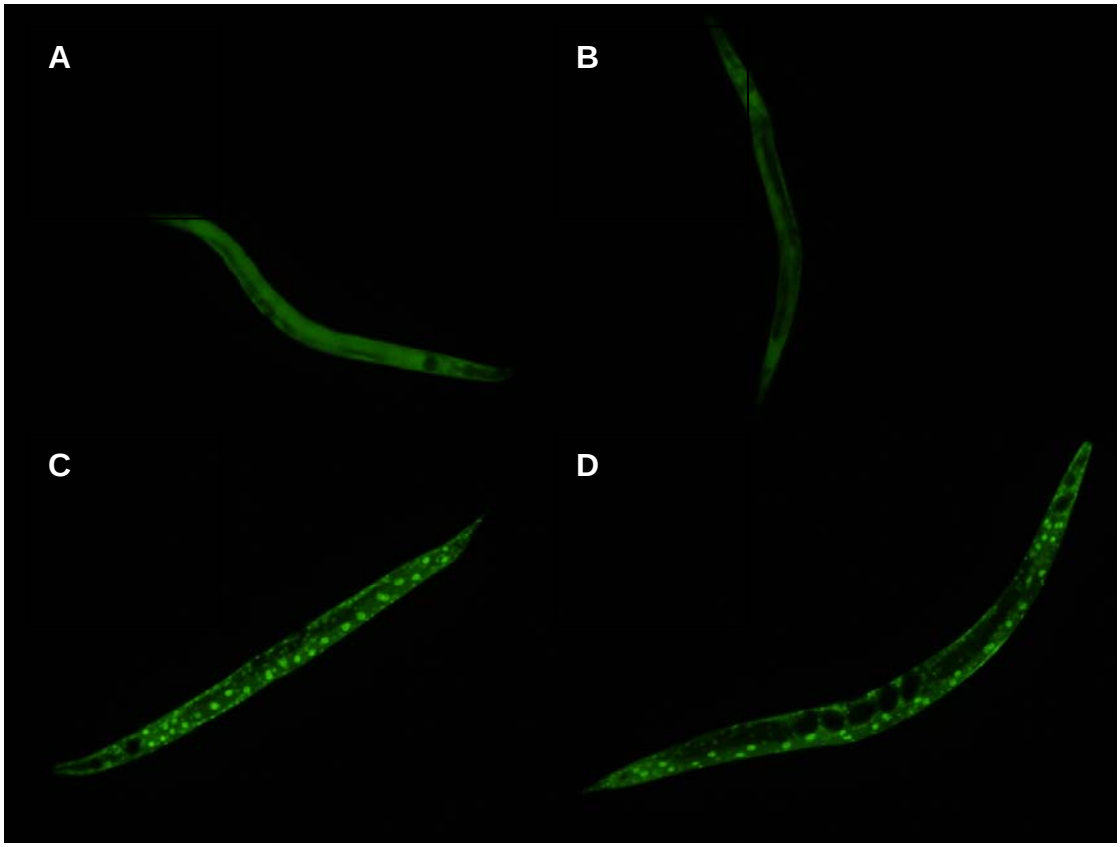

Fig. S4. Intracellular localization of DAF-16::GFP following inactivation of CHK-1. (**A**) TJ356 *chk-1(RNAi)*, no heat shock. (**B**) TJ356 control, no heat shock. (**C**) TJ356 *chk-1(RNAi)*, heat shocked. (**D**) TJ356 control, heat shocked.

Table S1. Summary of longitudinal thermotolerance assays. Log rank *P* values from comparisons between worms fed RNAi bacteria with and without the *chk-1* fragment inserted in the feeding vector are included. In each experiment the results shown were obtained from two plates each containing approximately 25 worms.

Strain		Survival (min) (mean ± SD)	*P* value Log Rank Test
Experiment I			
Wild-type N2		360 ± 33	
GL305 *cid-1(+/+)*		350 ± 26	
GL306 *cid-1(+/+)*		355 ± 29	
GL302 *cid-1(-/-)*	>	432	*P* < 0.0001
GL303 *cid-1(-/-)*	>	433	*P* < 0.0001
GL304 *cid-1(-/-)*	>	431	*P* < 0.0001
Experiment II			
Wild-type N2		408	
Wild-type N2 *chk-1(RNAi)*	>	442	*P* < 0.0001
daf-16(mu86)		366 ± 50	
daf-16(mu86) chk-1(RNAi)	>	435	*P* < 0.0001
Experiment III			
Wild-type N2		474 ± 42	
Wild-type N2 *chk-1(RNAi)*	>	571	*P* < 0.0001
daf-16(mu86)		433 ± 64	
daf-16(mu86) chk-1(RNAi)	>	500	*P* < 0.0001
Experiment IV			
Wild-type N2		442 ± 85	
Wild-type N2 *chk-1(RNAi)*	>	639	*P* < 0.0001
daf-16(mu86)		418 ± 54	
daf-16(mu86) chk-1(RNAi)	>	528	*P* < 0.0001
Experiment V			
daf-16(dr26)		350 ± 42	
daf-16(dr26) chk-1(RNAi)	>	406 ± 43	*P* < 0.0001
Experiment VI			
daf-12(m20)		490 ± 39	
daf-12(m20) chk-1(RNAi)	>	564	*P* < 0.0001
Experiment VII			
daf-12(m20)		436 ± 34	*P* < 0.0001
daf-12(m20) chk-1(RNAi)	>	467	
Experiment VIII			
Wild-type N2		412 ± 49	*P* < 0.0001
Wild-type N2 *cdc-25.1(RNAi)*		478 ± 65	
Experiment IX			
Wild-type N2		520 ± 66	*P* < 0.0001
Wild-type N2 *cdc-25.1(RNAi)*	>	560	
Experiment X			
Wild-type N2	>	438	*P* < 0.0001
cdc-25.1(rr31) I; rrIs1		423 ± 37	
Experiment XI			
Wild-type N2	>	477	*P* < 0.001
cdc-25.1(rr31) I; rrIs1		457 ± 39	

Experiment XII			
rrf-3(pk1426)		395 ± 37	*P* < 0.0001
rrf-3(pk1426) cid-1(RNAi)	>	431	
Experiment XIII			
rrf-3(pk1426)		582 ± 37	*P* < 0.0001
rrf-3(pk1426) cid-1(RNAi)	>	612	
Experiment XIV			
rrf-3(pk1426)		403 ± 42	
rrf-3(pk1426) F31C3.2 *(RNAi)*		411 ± 47	*P* = 0.3452
rrf-3(pk1426) F59A3.9 *(RNAi)*		412 ± 44	*P* = 0.4401
rrf-3(pk1426) K10D2.2 *(RNAi)*		420 ± 51	*P* = 0.0282
rrf-3(pk1426) K04F10.6 *(RNAi)*		431 ± 46	*P* = 0.0008
Experiment XV			
rrf-3(pk1426)		642 ± 43	
rrf-3(pk1426) F31C3.2 *(RNAi)*		655 ± 25	*P* = 0.2214
rrf-3(pk1426) F59A3.9 *(RNAi)*		634 ± 56	*P* = 0.0540
rrf-3(pk1426) K10D2.2 *(RNAi)*		636 ± 58	*P* = 0.3341
rrf-3(pk1426) K04F10.6 *(RNAi)*		618 ± 57	*P* < 0.0001
Experiment XVI			
Wild-type N2		410 ± 23	
Wild-type N2 F31C3.2 *(RNAi)*		411 ± 22	*P* = 0. 9757
Wild-type N2 F59A3.9 *(RNAi)*		424 ± 27	*P* = 0.0007
Wild-type N2 K10D2.2 *(RNAi)*		430± 19	*P* < 0.0001
Wild-type N2 K04F10.6 *(RNAi)*		407 ± 24	*P* = 0.4645
Experiment XVII			
eat-2(ad1113)		435 ± 33	
eat-2(ad11130) chk-1(RNAi)		548 ± 44	*P* < 0.0001
Experiment XVIII			
Wild-type N2		467 ± 43	
GL311 *cdc-25.3 (-/-)*	>	491	*P* < 0.0001
GL312 *cdc-25.3 (+/+)*		462 ± 44	*P* = 0.08
Experiment XIV			
Wild-type N2		375 ± 37	
GL313 *cdc-25.3 (-/-)*	>	521	*P* < 0.0001
GL314 *cdc-25.3 (+/+)*		416 ± 44	*P* < 0.0001
Experiment XX			
rrf-3(pk1426)		469 ± 33	
rrf-3(pk1426)cdc-25.3(RNAi)	>	498 ± 35	*P* < 0.0001
Experiment XXI			
rrf-3(pk1426)		478 ± 109	
rrf-3(pk1426)cdc-25.3(RNAi)	>	564 ± 100	*P* < 0.0001
Experiment XXII			
rrf-3(pk1426)		545 ± 132	
rrf-3(pk1426)cdc-25.2(RNAi)		648 ± 119	*P* = 0.0057
rrf-3(pk1426)cdc-25.4(RNAi)		569 ± 106	*P* = 0.5609

Table S2 Development time and fertility of *cid-1(rf34::Tc4)* mutant worms. Fertility is the total number of progeny (mean ± SD) through out the reproductive period. Student's t test *P* values from comparison to wild-type N2. (+/+) indicates wild-type *cid-1* gene. *n*, number of worms.

Strain	Time to all individuals were egg laying (h)	Fertility	*P*	*n*
Wild-type N2 (+/+)	<73	296 ± 49		11
GL305 *cid-1* (+/+)	<73	311 ± 29	*P* = 0.35	13
GL302 *cid-1(rf34::Tc4)*	> 97	93 ± 15	*P* << 0.0001	10
rrf-3(pk1426)	<73	138 ± 25		8
rrf-3(pk1426) cid-1(*RNAi*)	<73	63 ± 23	*P* << 0.0001	10

Table S3. Summary of life-span assays. Log-rank *P* values from comparisons between worms fed RNAi bacteria with and without the indicated fragment inserted in the feeding vector. *n*, number of deaths recorded.

Strain	Survival (mean ± SD days)	n	Log-rank test *P* value	Temp
Experiment I				
Wild-type N2	15.3 ± 3.8	52		25 ºC
Wild-type N2 *chk-1(RNAi)*	17.1 ± 3.6	89	*P* = 0.0102	
Experiment II				
Wild-type N2	13.9 ± 3.7	66		25 ºC
Wild-type N2 *chk-1(RNAi)*	17.2 ± 3.3	118	*P* < 0.0001	
daf-16(mu86)	12.8 ± 2.6	115		
daf-16(mu86) chk-1(RNAi)	14.5 ± 2.4	110	P< 0.0001	
Experiment III				
Wild-type N2	14.6 ± 3.1	122		25 ºC
Wild-type N2 *chk-1(RNAi)*	18.5 ± 3.4	142	*P* < 0.0001	
daf-12(m20)	14.1 ± 2.8	131		
daf-12(m20) chk-1(RNAi)	16.2 ± 3.8	142	*P* < 0.0001	
Experiment IV				
daf-12(m20)	14.8 ± 4.1	145		25 ºC
daf-12(m20) chk-1(RNAi)	19.2 ± 5.6	177	*P* < 0.0001	
Experiment V				
daf-16(mu86)	12.3 ± 2.9	155		25 ºC
daf-16(mu86) chk-1(RNAi)	13.0 ± 2.4	177	*P* = 0.032	
Experiment VI				
Wild-type N2	19.7 ± 4.8	114		20 ºC
Wild-type N2 *chk-1(RNAi)*	22.4 ± 6.8	117	*P* < 0.0001	
daf-16(mu86)	16.1 ± 3.3	140		
daf-16(mu86) chk-1(RNAi)	16.1 ± 2.3	112	*P* = 0.280	
daf-12(m20)	18.7 ± 4.8	123		
daf-12(m20) chk-1(RNAi)	19.5 ± 5.3	168	*P* = 0.1250	
Experiment VII				
Wild-type N2	18.1 ± 5.6	151		20 ºC

Wild-type N2 *chk-1(RNAi)*	20.6 ± 5.4	99	*P* < 0.0001	
daf-16(mu86)	15.1 ± 3.1	181		
daf-16(mu86) chk-1(RNAi)	15.1 ± 2.1	96	*P* = 0.090	
daf-12(m20)	20.8 ± 4.8	178		
daf-12(m20) chk-1(RNAi)	20.8 ± 5.0	136	*P* = 0.3774	
Experiment VIII				
daf-2(e1368)	26.2 ± 7.0	70		
daf-2(e1368) chk-1(RNAi)	34.5 ± 9.8	112	*P* < 0.0001	20 ºC
Experiment IX				
Wild-type N2	14.9 ± 4.3	82		25 ºC
Wild-type N2 *cdc-25.1(RNAi)*	17.6 ± 6.1	116	*P* = 0.0022	
Experiment X				
Wild-type N2	18.6 ± 7.3	81	NS	20 ºC
Wild-type N2 *cdc-25.1(RNAi)*	18.2 ± 7.5	110		
Experiment XI				
Wild-type N2	21.9 ± 7.5	31	*P* = 0.08	20 ºC
Wild-type N2 *cdc-25.1(RNAi)*	24.9 ± 8.9	89		
daf-2(e1368)	31.2 ± 6.7 (40.0 ± 2.0)[b]	72	*P* = 0.39	
daf-2(e1368) cdc-25.1(RNAi)	28.4 ± 12.0 (46.5± 3.4)*	76		
Experiment XII				
Wild-type N2	23.7 ± 7.8	54	*P* = 0.0004	20 ºC
Wild-type N2 *cdc-25.1(RNAi)*	27.8 ± 10.8	166		
daf-2(e1368)	31.0 ± 7.7 (41.2 ± 3.4)*	89	*P* = 0.1123	
daf-2(e1368) cdc-25.1(RNAi)	32.1 ± 10.8 (48.1 ± 3.0)*	166		
Experiment XIII				
Wild-type N2	22.2 ± 6.9	79	*P* = 0.084	20 ºC
cdc-25.1(rr31) I; rrIs1	19.1 ± 8.2	79		
Experiment XIV				
Wild-type N2	22.5 ± 6.1	32	*P* < 0.0003	20 ºC
cdc-25.1(rr31) I; rrIs1	18.2 ± 4.9	71		
Experiment XV				
Wild-type N2	22.0 ± 3.6	38	*P* < 0.0001	20 ºC
cdc-25.1(rr31) I; rrIs1	16.8 ± 4.1	30		
Experiment XVI				
Wild-type N2	19.2 ± 5.6	48	*P* < 0.0001	20 ºC
cdc-25.1(rr31) I; rrIs1	12.8 ± 2.5	55		
Experiment XVII				
Wild-type N2	13.2 ± 4.5	80	*P* = 0.05	25 ºC
cdc-25.1(rr31) I; rrIs1	12.3 ± 3.7	96		
Experiment XVIII				
Wild-type N2	14.8 ± 4.2	52	*P* < 0.0001	25 ºC
cdc-25.3(ok358)	20.3 ± 4.3	72		
Experiment XIX				

Wild-type N2	12.2 ± 4.3	46	*P* < 0.0001	25 ºC
cdc-25.3(ok358)	20.2 ± 6.2	33		
Experiment XX				
rrf-3(pk1426)	22.8 ± 5.0	67		
rrf-3(pk1426)cdc-25.3(RNAi)	24.0 ± 7.4	81	*P* = 0.0029	20 ºC
Experiment XXI				
rrf-3(pk1426)	19.2 ± 5.8	95	*P* < 0.0001	20 ºC
rrf-3(pk1426) cid-1(RNAi)	23.3 ± 5.8	110		
Experiment XXII				
rrf-3(pk1426)	21.4 ± 5.8	56	*P* < 0.0001	20 ºC
rrf-3(pk1426) cid-1(RNAi)	26.1 ± 5.6	39		
Experiment XXIII				
rrf-3(pk1426)	14.2 ± 3.5	77		25 ºC
rrf-3(pk1426) K104F10.6 (*RNAi*)	16.1 ± 4.5	88	*P* = 0.0033	
rrf-3(pk1426) F31C3.2 (*RNAi*)	14.2 ± 3.4	97	*P* = 0.9663	
rrf-3(pk1426) F59A3.9 (*RNAi*)	15.0 ± 4.1	105	*P* = 0.2435	
Experiment XXIV				
rrf-3(pk1426)	14.3 ± 4.0	115		25 ºC
rrf-3(pk1426) K104F10.6 (*RNAi*)	14.7 ± 4.4	127	*P* = 0.8510	
rrf-3(pk1426) F31C3.2 (*RNAi*)	15.6 ± 3.7	116	*P* = 0.2212	
rrf-3(pk1426) F59A3.9 (*RNAi*)	14.0 ± 3.8	120	*P* = 0.1390	
Experiment XXV				
Wild-type N2	21.9 ± 4.7	62		20 ºC
Wild-type N2 K104F10.6 (*RNAi*)	23.6 ± 5.1	71	*P* = 0.0311	
Wild-type N2 F31C3.2 (*RNAi*)	25.6 ± 6.0	85	*P* < 0.001	
Wild-type N2 F59A3.9 (*RNAi*)	21.3 ± 4.2	85	*P* = 0.3813	
Wild-type N2 K10D2.2 (*RNAi*)	23.1 ± 4.2	83	*P* = 0.03813	
Experiment XXVI				
Wild-type N2	18.9 ± 3.0	87		20 ºC
Wild-type N2 K104F10.6 (*RNAi*)	19.2 ± 3.0	87	*P* = 0.4708	
Wild-type N2 F31C3.2 (*RNAi*)	21.0 ± 3.4	113	*P* < 0.001	
Wild-type N2 F59A3.9 (*RNAi*)	19.8 ± 4.1	70	*P* = 0.0256	
Experiment XXVII				
Wild-type N2	16.4 ± 2.5	86		25 ºC
Wild-type N2 K104F10.6 (*RNAi*)	18.0 ± 1.9	82	*P* < 0.0001	
Wild-type N2 F31C3.2 (*RNAi*)	17.6 ± 2.1	62	*P* = 0.0012	
Wild-type N2 F59A3.9 (*RNAi*)	17.3 ± 2.4	92	*P* = 0.0022	
Wild-type N2 K10D2.2 (*RNAi*)	18.2 ± 1.7	71	*P* < 0.001	
Experiment XXVIII				
Wild-type N2	14.7 ± 3.2 (19 ± 1)*	90		25 ºC
Wild-type N2 *hsp-4(RNAi)* Eggs	11.5 ± 2.3 (14 ± 1)*	67	*P* < 0.0001	
Wild-type N2 *hsp-4(RNAi)* Adults	13.5 ± 2.9 (17 ± 0.4)*	116	*P* = 0.0027	

*Maximum life span.

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