

FBF represses the Cip/Kip cell cycle inhibitor CKI-2 to
promote self-renewal of germline stem cells in *C. elegans*

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Supplemental Data

Inventory:

Supplemental Figures and Legends

Supplemental Methods

Supplemental References cited in Supplemental Methods

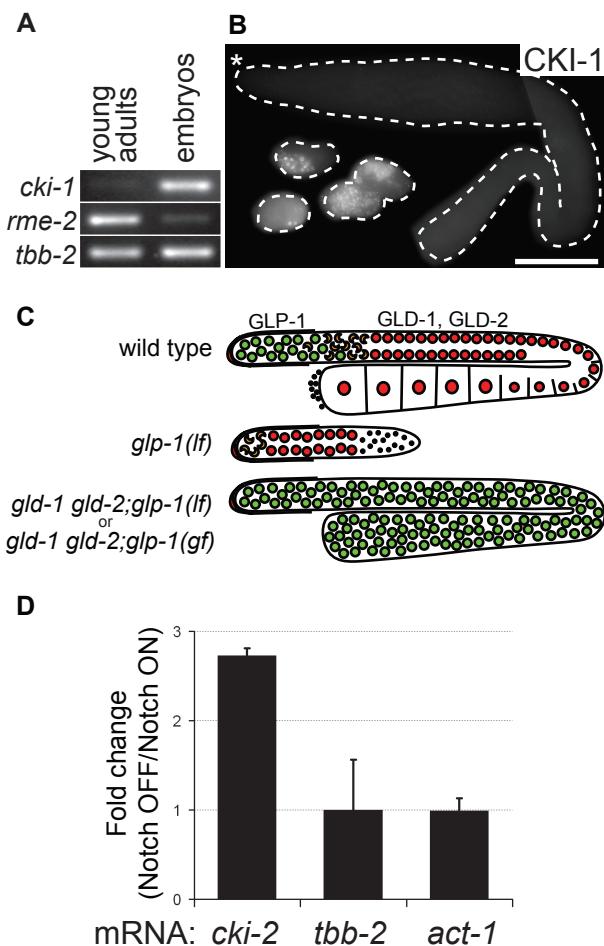


Figure S1

CKI-2, but not CKI-1, is predominantly expressed in the adult germ line.

(A) Semi-quantitative RT-PCR of indicated mRNAs. (B) Immuno-staining for CKI-1 protein on dissected gonads and embryos. Scale bar: 50 μ m. Both *cki-1* mRNA and CKI-1 protein can be detected in embryos, but are absent from non-gravid young adults. (C) A simplified view of GLP-1/Notch function in maintaining germline stem cells. In *glp-1(lf)* mutants, all germ cells enter meiosis, which depends on the pro-meiotic factors GLD-1 and GLD-2. In *gld-1 gld-2* mutants, germ cells proliferate independently of Notch signaling in either the presence of an additional loss-of-function mutation *glp-1(lf)* or a gain-of-function mutation *glp-1(gf)*. green, proliferating germline stem cells; yellow, nuclei entering meiosis; red, nuclei undergoing meiotic differentiation. (D) GLP-1/Notch signaling negatively regulates *cki-2* mRNA levels. The abundance of indicated mRNAs in gonads dissected from either *gld-1 gld-2; glp-1(lf)* (Notch OFF) or *gld-1 gld-2; glp-1(gf)* (Notch ON) gonads was measured by RT-qPCR. Control mRNAs: *tbb-2* (tubulin) and *act-1* (actin). Error bars = SEM, n = 3 (see Material and Methods).

Kalchhauser et al, Supplementary Fig.2

CLUSTAL .. multiple sequence alignment

□△FBE1-4

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C.remanei      AACT-GTCAAGT-GCACTCATCAGTTCTATGA--CTCATGTTCTATGGCTCCCATCTTC
C.elegans      -A-T-TTGAGA-A-GCTGCTCA-TTCAATTG--CT-A-GT-C-AT--CATGC--TCTC
C.brenneri     -A-TAGTGAACTCA-GCTAATTT-TTAATTGAAATTGATAT-TGTTA-AATGAAAATT
C.briggsae    ---AATTG-C-A-ACCTCACT-CAT-C-ACTTC
***          ***

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C.remanei      ATCATCCTGTGTTATGACTGCCAATATTCCCCTTCCTACT-CAAATACTAACATCTCA
C.elegans      AAC-TTCTTGACT---CTGCCAA---TTCATCTTCTACAGTACACA-CATACTAACATCTCA
C.brenneri     ATT-CTTCCAG---ACTGC-AATATTCTTCCTTCCACA-AAAAAACTAACATCTCA
C.briggsae    AAT-GACTCATG---TT-CTA---TT-GTCCTCGTC-CT---CX-AATCAATCTCA
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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C.remanei      ATTCCCTGATCGTCATAATGGAAATTGCGA-TGGAACCTTTCTGTGATT-TCCATCTTC
C.elegans      ATCAGCTGTTGCTCATATAATGCAATTAAAGTGGAACTT---CACCG-TC-TAC-TTTT
C.brenneri     ATCCGATGTTGCTCATATAATGGATTACGAT-TGAACTTTCTGT---TC-CTGATTTT
C.briggsae    ATATCCTGATCGTCATAATGGAAATTGCGA-TGGAACCTTTCTGTGATACCCATTTT
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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C.remanei      TA-TCAAAATCGCT-ATRAACTAA-TAGTTATTGAT-TTGTGTTTAAATT-TCCACCG
C.elegans      TA-TAA-ATGCT-TC-ATAA-TA-TAA-ATTGACT-TTG---AA-AAAT-TCCAT-
C.brenneri     TC-TTTTTTCGTC-ATRAACTAA-TAGTAATTGACT-TTG---TTGGAT-TTTTCA
C.briggsae    AAGPATTTTC-CCGATAAAAGCAATTAGT-AGTAATTGATTGTTT-TTGGATATATCTT
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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MRE1

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C.remanei      ATTGTT---GT-TTTTTCTTCAGTATAATCCCGTTGTCCTCTTTTTAATTCAAGA
C.elegans      ATT-TT---AT-TTTTT-TTCTGAG-A-AT---TTC----TC---TCT------AG------
C.brenneri     ATGCTCCAAATGTTTCCCACTTTCG---TATCCCCGGTCACTGTTCTT---AP-CA-
C.briggsae    GTT-TT---CT-CTTCT-TTTTAGTG-ATC---GCTCCCTCT-CAA-AATCCA--*
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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FBE1

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C.remanei      AC-ATTGCTATTTCAAATCTT-ATTG-ATGCTCCCATATCCCGATTAACTCGTCAA
C.elegans      AT-ATTGTT-TCTC---TGT-PTC-GT---ATA-CCC-[CCCGGGTTTAACTCGTCAA
C.brenneri     ATCACTGCTGTTTIG-TGATATCCCGTT---ATGTCCTTCATCCCAAATTAATCGTCAA
C.briggsae    TTATATTGCTCTTCAAATGCT-ATTGCACT-TCcccATACTCCCAATTATCGTCAA
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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FBE2 FBE3 FBE4

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C.remanei      TTTGA---TTTC-CG-ATTCCCGGCTCATACCTGT---CAGTCGCTC-TGTTGAA---A
C.elegans      TTTGAAATT-C-CC-ATACCC-[CA]-TTCTGAG-T-CACTC-[CG]-----AG
C.brenneri     TTGA---TTGC-CC-TTATCCC-GTT-AT-TTTTCCTGC-CGGTCTGTTAAAAAA-G
C.briggsae    TTGA---TTGCACCTAATCTCCGTTGATCACAATGCT-G-CGCTTCTGTTAAAAAA-G
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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C.remanei      TCTCT---CA-ATTGGAA---AAGCTTTCAAG---TGTGTT-ATCCCTT-TA---TCGGT
C.elegans      TCCACACATATCCCGTTCAATTTTCAAACACTGAAATTTTAGTAGTTCTGTT
C.brenneri     TCCC---CA---TCC---T---ATGCC-C-AACAA-T-CGT---GTTTAT-T---TCC-CT
C.briggsae    TCCC---CA---TTT-GA---AAGATTCACTGTTTATGAATCCCAT-CG---TCC-CG
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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MRE2

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C.remanei      ATTCACATATAATTG-CCACATCATGCCACCCTATCATGACATCTTAG-TTGTAA-TC
C.elegans      TTTTC-CCTAATTGCGCCCTCATGCCACTCAAAATC-[TG]ACATCTTAG-TTATAAATA
C.brenneri     ATTC-GAAAATTG-CGGATTCGGCCGAACCAATCATGACAACTTAG-TTATTC-TC
C.briggsae    ATAT-C---CAATTG-CCATATCATGCCACCTAAATCATGACATCTTAGGTCCCCCTC
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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C.remanei      CTCAAAATT-C-CCATCCGGT-TGCCCGGTGACACATATATCTGTGTTGTTGATTTT-
C.elegans      CCCA---T-C-CCATCCGGTATATCTTACCTCCCA-GT---FACTCTGTGTTAAATTCTA
C.brenneri     ATCAGAA-TAT-CCATCCGGT-TCCCCCAT-ACCTCATCTCTGTCTGTTAAATTCTT-
C.briggsae    CCCGTAGATATAATCCGGT-T-CG-AAGAC-A-A-TTATGTTTTCTGAGATTTT
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

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C.remanei      TGTTTATTATCTGATGAA-AGCTGATGTTCTGGT---AACG-TGTTAGCCAATTAAATC
C.elegans      CGTTT-TTGGTTATATGAA---ACTGATGTTTC-T-TAT
C.brenneri     TGTTT-CTGGTTATGAAATAGCTTATGTTT-TATACACTAGCAACCGATTCCCTT
C.briggsae    T-TT-TCAA---AAAT-AA---TCC---TGGCGCACT-T-C-G-
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

```

```

C.remanei      TCTG-ATTGG---TCTTAT-TTCCCGT-ACCGTGGCTGAAAGATAACAAATCCAC-ATA
C.elegans      GCTTCATTTGAATCTCACAATTCCGCAATAGGATTGATAGCTACAAAATCTGGA
C.brenneri     GCTTCATTTGAATCTCACAATTCCGCAATAGGATTGATAGCTACAAAATCTGGA
C.briggsae    GCTTCATTTGAATCTCACAATTCCGCAATAGGATTGATAGCTACAAAATCTGGA
***          * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

```

```

C.remanei      AACCATCTATAAA-CAACCA-C-AATGACA------
C.elegans      AACCATCTATAAA-CAACCA-C-AATGACA------
C.brenneri     AACCATCTATAAA-CAACCA-C-AATGACA------
C.briggsae    AACCATCTATAAA-CAACCA-C-AATGACA------

```

```

C.remanei      -----
C.elegans      -----
C.brenneri     TGGACTGAAGGCTCGGGCTTCCGAGATAATTTCATAATCTCGTCAAACCTCC
C.briggsae    TTATTTGATTCTGGAAAACAAAGATGTTGCTGAAAATTGAGAAGAATTCTGT

```

```

C.remanei      -----
C.elegans      -----
C.brenneri     GTATTCTCACATTCTTATTAGCTTCTGGTCTGATCCATTCTGAACTTTGTGAT
C.briggsae    GTATTCTCACATTCTTATTAGCTTCTGGTCTGATCCATTCTGAACTTTGTGAT

```

```

C.remanei      -----
C.elegans      -----
C.brenneri     TTTCTATGACGGATTCTCGTGTGATATTG
C.briggsae    TTTCTATGACGGATTCTCGTGTGATATTG

```

Figure S2

Dissecting potential regulatory elements in the cki-2 3'UTR.

Alignment of *cki-2* 3'UTR sequences from *C.elegans*, *C.remanei*, *C.brenneri*, and *C.briggsae*, with the positions of both halfsites of MRE1 and MRE2 and the UGU trinucleotides of FBE1-4 indicated in colors.

The region required for repression, Δ FBE1-4, is boxed. Bold: TAA, the stop codon of the *cki-2* S ORF.

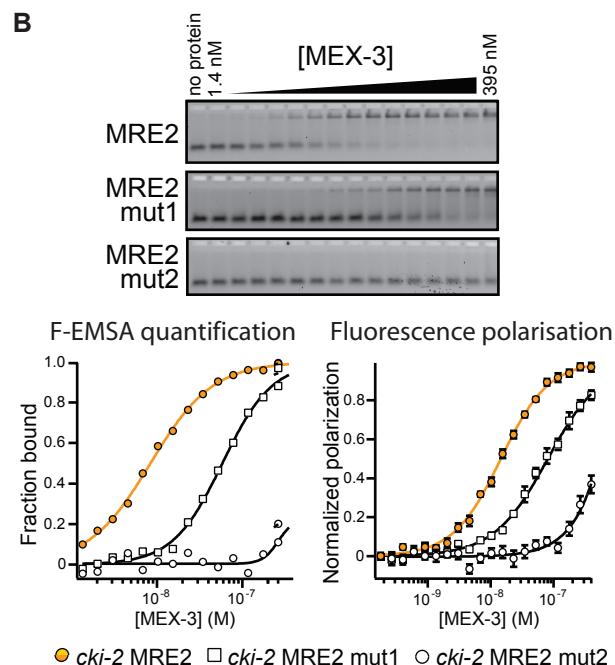
Nucleotides conserved between all four species are marked with an asterisk.

Kalchhauser et al, Supplementary Fig.3

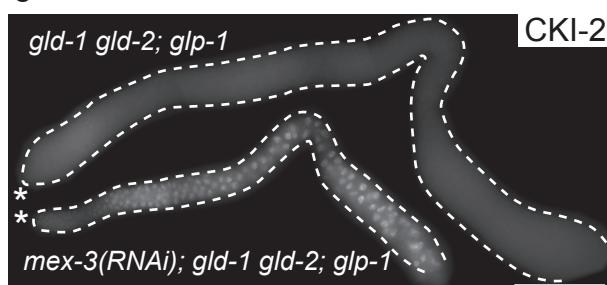
A

Oligo name	Kd, app (GS)	Kd, app (FP)
cki-2 MRE2	9 ± 1 nM	15 ± 2 nM
cki-2 MRE2 mut1	80 ± 20 nM	76 ± 4 nM
cki-2 MRE2 mut2	>500 nM	>500 nM

B



C



D

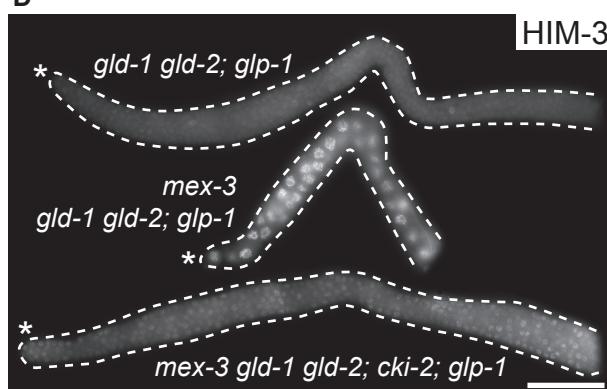


Figure S3

MEX-3 associates with MRE2 in the cki-2 3'UTR and regulates CKI-2 expression in certain genetic backgrounds.

(A) Affinities of MEX-3 to indicated RNA elements as determined by F-EMSA and FP assays. While MRE2 associates with MEX-3 tightly, MRE1 is predicted to be occluded by a stable hairpin (unpublished observation) and is not expected to associate with MEX-3. $K_{d,app}$, apparent equilibrium dissociation constant. For oligo sequences used see Table S1 in Supplemental Methods. (B) Top panel: F-EMSA gel pictures demonstrating association of MEX-3 to wild-type and mutated MRE2 from the *cki-2* 3'UTR. Bottom left: quantification of gel shifts shown above. The fraction of bound RNA was plotted as a function of protein concentration and fit to the Hill equation to determine the apparent equilibrium dissociation constant ($K_{d,app}$). Bottom right: affinity as determined by fluorescence polarization assays. Polarization is plotted as a function of protein concentration and fit to the Hill Equation to determine $K_{d,app}$. (C) CKI-2 immuno-staining on gonads (outlined) dissected from animals of the indicated genotype. MEX-3 prevents CKI-2 expression in *gld-1 gld-2; glp-1(lf)* gonads. (D) Immuno-staining for the synaptonemal complex component HIM-3 on dissected gonads (outlined) of indicated genotypes. Upregulation of CKI-2 in *mex-3 gld-1 gld-2; glp-1(lf)* gonads correlates with proliferation arrest and expression of the meiotic marker HIM-3. Removing *cki-2* from the *mex-3 gld-1 gld-2; glp-1(lf)* gonad rescues the proliferation defect and prevents accumulation of HIM-3. Scale bars: 50 μ m. Although MEX-3 is non-essential in the wild-type gonad, these experiments demonstrate that, in the presence of additional mutations, MEX-3 prevents meiosis by repressing CKI-2 expression.

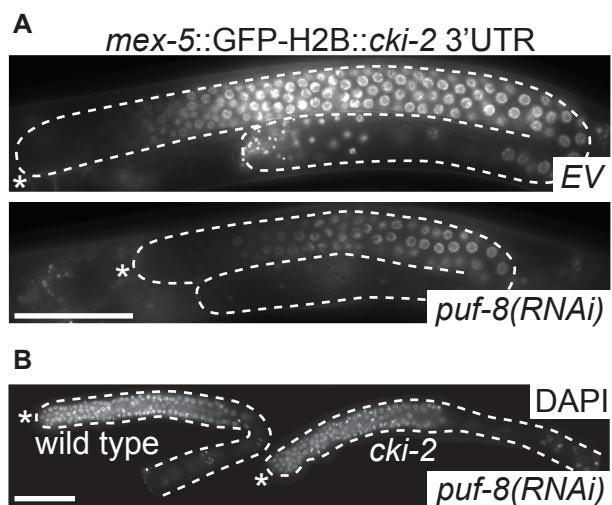


Figure S4

PUF-8 does not repress CKI-2.

(A) Live worms (gonads outlined) expressing the GFP::H2B reporter under the control of the wild type *cki-2* 3'UTR upon mock (EV: empty vector) or *puf-8* RNAi. Depletion of *puf-8* did not de-repress the reporter, though it did reduce germ line size as previously reported (Subramaniam and Seydoux 2003). (B) Dissected DAPI-stained gonads (outlined) of the indicated genotype upon RNAi of *puf-8*. *puf-8*(RNAi) caused germ line underproliferation in both wild-type and *cki-2* animals, indicating that this phenotype does not depend on CKI-2. Scale bars: 50μm

Supplementary Methods

Table S1

Oligonucleotide sequences used in *in-vitro* RNA binding assays.

MRE2 WT	ACUCAAAUCAUAGACAUUCUAGUUUAUAAA
MRE2 mut1	ACUCAAAUCCCCCACAUCGGGUUAUAAA
MRE2 mut2	ACUCAAAUCCCCCACAUCGGGUCCCCAAU
FBEa (<i>gld-1</i>)	AUAGAAUCAUGUGCCAUACAUGUUG
FBE1 WT	UUUAUCUGUGAAUUUGAAA
FBE1 mut	UUUAUCACAGAAUUUGAAA
FBE2 WT	CAUACCCUGGUCCAUUCUGU
FBE2 mut	CAUACCCACACCAUUUCUGU
FBE3 WT	CAUUCUGUGUUCUACUCCU
FBE3 mut	CAUUCACAGUUCUACUCCU
FBE4 WT	CUACUCCUGUAAAAAAAGUC
FBE4 mut	CUACUCCACAAAAAAAGUC

Table S2

Coordinates of regions deleted in the *cki-2* 3'UTR reporters

name	deleted nucleotides (of 520)
ΔM2	409-426
Δ1ΔM2	1-302, 409-426
Δ2ΔM2	106-235, 409-426
Δ3ΔM2	211-340, 409-426
Δ4	314-463
Δ5ΔM2	409-426, 409-426
ΔFBE1-4	246-313

Supplementary References

- Biedermann, B., J. Wright, et al. (2009). "Translational repression of cyclin E prevents precocious mitosis and embryonic gene activation during *C. elegans* meiosis." *Dev Cell* 17(3): 355-64.
- Ciosk, R., M. DePalma, et al. (2004). "ATX-2, the *C. elegans* ortholog of ataxin 2, functions in translational regulation in the germline." *Development* 131(19): 4831-41.
- Draper, B. W., C. C. Mello, et al. (1996). "MEX-3 is a KH domain protein that regulates blastomere identity in early *C. elegans* embryos." *Cell* 87(2): 205-16.
- Subramaniam, K. and G. Seydoux (2003). "Dedifferentiation of primary spermatocytes into germ cell tumors in *C. elegans* lacking the pumilio-like protein PUF-8." *Curr Biol* 13(2): 134-9.