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:

Kalchhauser et al, Supplementary Fig.1

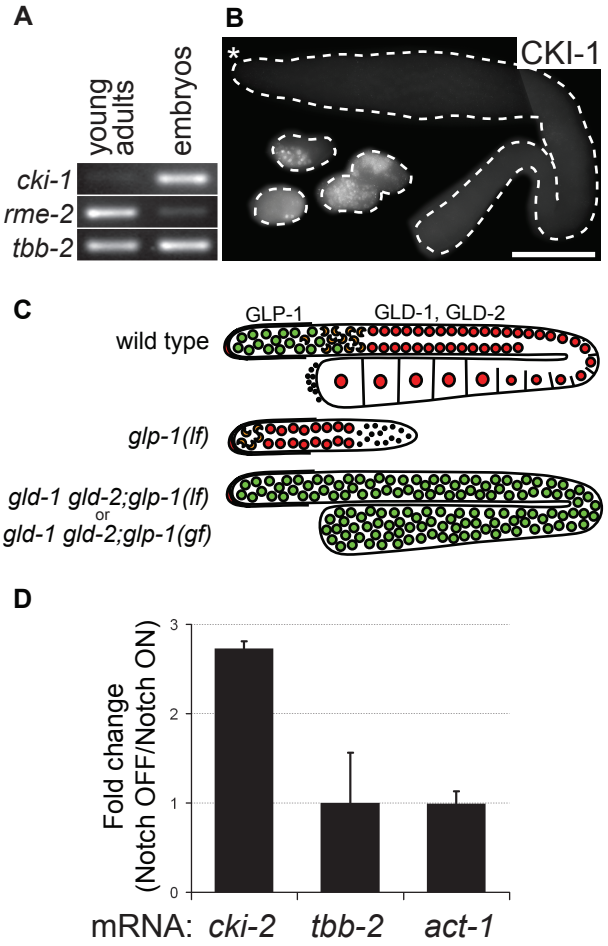


Figure S1

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: 50µm. 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

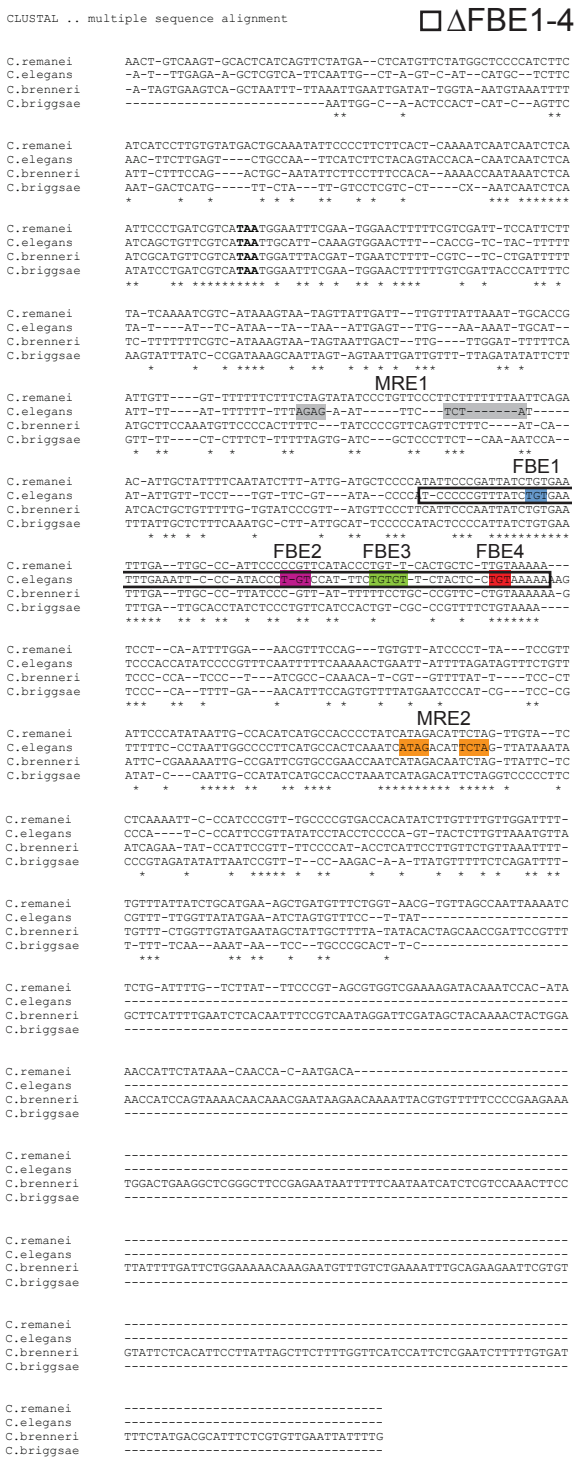


Figure S2

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

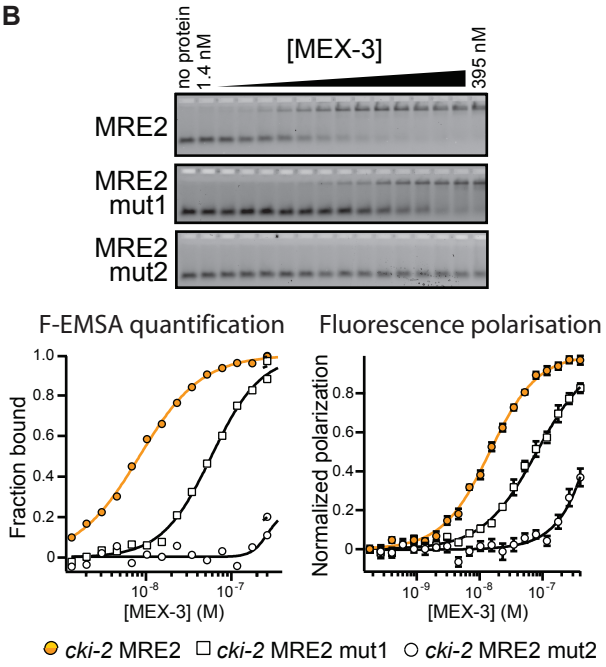
Alignment of *cki-2* 3'UTR sequences from *C.elegans, C.remanei, C.brenneri, and C.briggsae*, with the positions of both halfsites of MRE1 and MRE2 and the UGU trinucleotides of FBE1-4 indicated in colors. The region required for repression, ΔFBE1-4, is boxed. Bold: TAA, the stop codon of the *cki-2* S ORF. Nucleotides conserved between all four species are marked with an asterisk.

Kalchhauser et al, Supplementary Fig.3

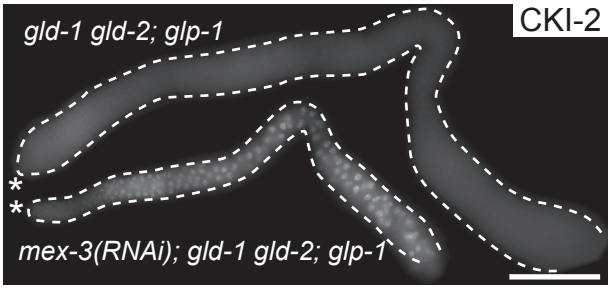
A

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

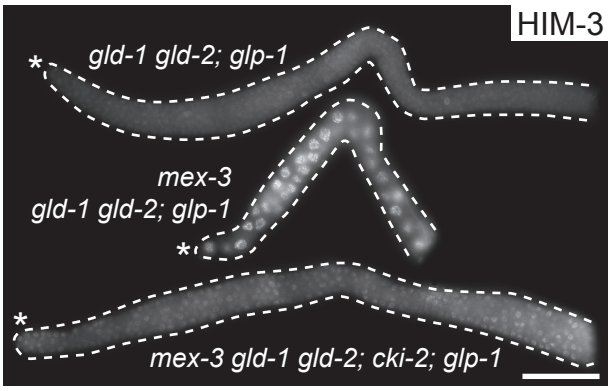


Figure S3

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. Kd,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: 50µm. Although MEX-3 is non-esssential in the wild-type gonad, these experiments demonstrate that, in the presence of additional mutations, MEX-3 prevents meiosis by repressing CKI-2 expression.

Kalchhauser et al, Supplementary Fig.4

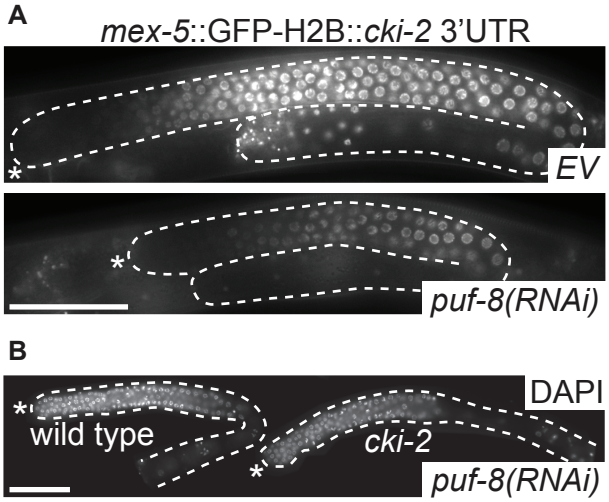


Figure S4

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	ACUCAAAUCAUAGACAUUCUAGUUAUAAAU
MRE2 mut1	ACUCAAAUCCCCCACAUCCCCGUUAUAAAU
MRE2 mut2	ACUCAAAUCCCCCACAUCCCCGUCCCCAAU
FBEa (*gld-1*)	AUAGAAUCAUGUGCCAUACAUCAUGUUG
FBE1 WT	UUUAUCUGUGAAUUUGAAAU
FBE1 mut	UUUAUCACAGAAUUUGAAAU
FBE2 WT	CAUACCCUGUCCAUUUCUGU
FBE2 mut	CAUACCCACACCAUUUCUGU
FBE3 WT	CAUUUCUGUGUUCUACUCCU
FBE3 mut	CAUUUCACAGUUCUACUCCU
FBE4 WT	CUACUCCUGUAAAAAAAGUC
FBE4 mut	CUACUCCACAAAAAAAAGUC

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.