Cell Reports, Volume *30*

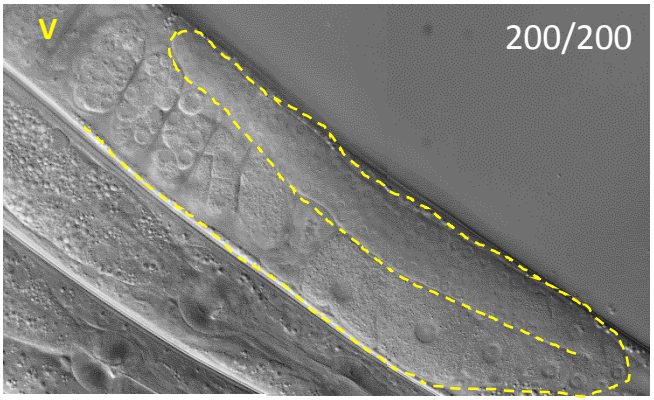
Supplemental Information

Regulation of Nucleotide Metabolism and Germline Proliferation in Response to Nucleotide Imbalance and Genotoxic Stresses by EndoU Nuclease

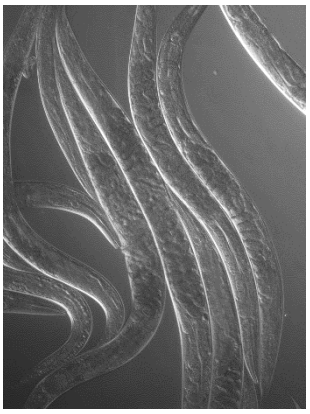
Fan Jia, Congwu Chi, and Min Han

Figure S1

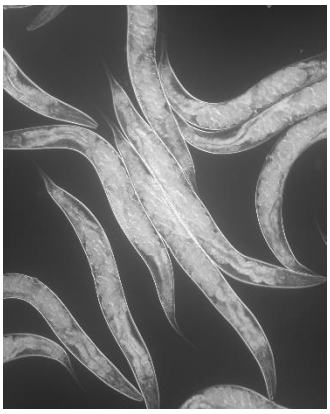
A



B



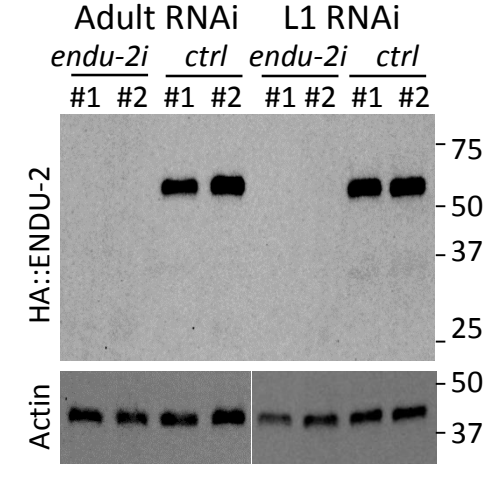
C



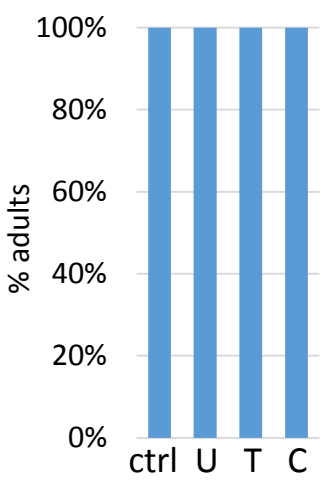
D

```
ENDU-2      NLFTTVDE-SIFTKKQYADLITT---YTQDLFTADVCKAEPAMGGFRKQYLQGVFNTFTA  403
XendoU      PLFQFVDEEKLKSRKTFATFISLLDNYEMDTGVAEVVTPEEI--AENNNFLDAI----LE  111
EndoU       PLFTYVNE-KLFSKPTYAAFINLLNNYQRATGHGEHFSAQEL--AEQDAFLREI----MK  236
             **  *:* .: ::  :* :*.    *      .:  . :    . .. :*  :

                                                    **        *
ENDU-2      TPMFASAFAYLQSINYKETSNLTNFKTKVLWPLWFGTYTRCKG-PLGSSGWEHVFSGEIK  462
XendoU      TKVMKMAHDYLVRKNQAKP--TRNDFKVQLYNIWFQLYSRAPGSRPDSCGFEHVFVGESK  169
EndoU       TAVMKELYSFLHHQNRY-G--SEQEFVDDLKNMWFGLYSRGNE-EGDSSGFEHVFSGEVK  292
            * ::   . :*   *        :    *  :**  *:*     .*.*:**** ** *

                470                                          *
ENDU-2      SNE-VDGQHDWVRYYTEQKADKMVYDGYYTHD-------ENLIGTFQYKWNGALKPKGGF  514
XendoU      RGQEMMGLHNWVQFYLQEKRKNIDYKGYVARQNKSRPDEDDQVLNLQFNWKEMVKPVGSS  229
EndoU       KGK-VTGFHNWIRFYLEEKEGLVDYYSHIYD---GPWDSYPDVLAMQFNWDGYYKEVGSA  348
             .: : * *:*:::* ::*   : * .:              :  :*::*.   *  *.

                                                          568
ENDU-2      FTGTSPAFDFSILSVCALAHGNG-GNCHFKVTNYPITVTSYLQDC--GDGSGTCLATAYP  571
XendoU      FIGVSPEFEFALYTIVFLASQEKMSREVVRLEEYELQIV--------VNRHGRYIGTAYP  281
EndoU       FIGSSPEFEFALYSLCFIARPGKVCQLS--LGGYPLAVRTYTWDKSTYGNGKKYIATAYI  406
            * * ** *:*:: ::  :*     .    :  * : :          .     :.***

ENDU-2      G---------- 572
XendoU      VLLSTNNPDLY 292
EndoU       VSST------- 410
```

E



F



G

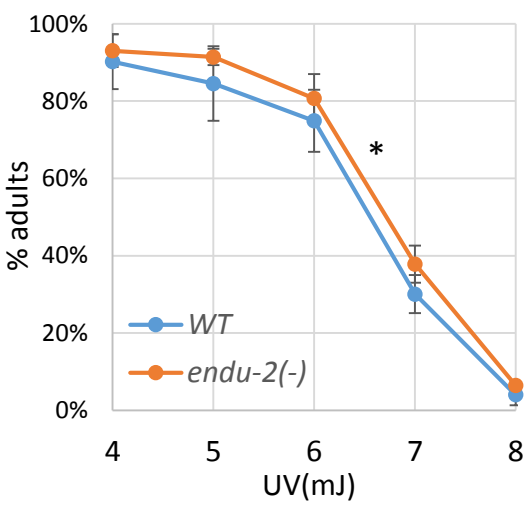


Figure S1, related to Figure 1. Additional phenotypes of *endu-2(-).*

(A) A picture showing a WT-looking germline in 3-day old *cddko; endu-2(ku544)* worms fed on low-pyrimidine food (*E. coli* OP50). The restored morphology was seen in 100% of the animals (n=200). v: vulva. Dashed line outlines the distal germline.

(B, C) Pictures of 4-day old *cddko* worms fed on pyrimidine-rich *cytR*$^{-}$ bacteria (B) and 4-day old *cddko; endu-2(ku544)* worms fed on low-pyrimidine food (*E. coli* OP50). Both are fertile but show severe egg-laying defects.

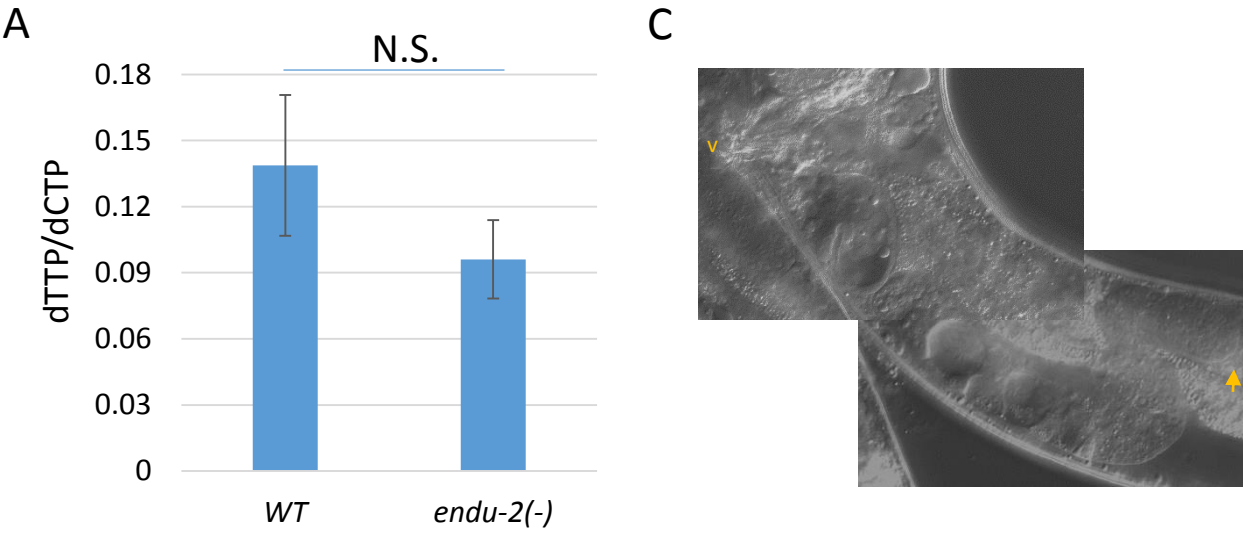
(D) Alignment of the homologous regions between *C. elegans* ENDU-2, Xenopus laevis XendoU (chain A, NP_001081040.1) and human EndoU (isoform1, NP_001165910.1) using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Stars indicate amino acids that are critical for the RNA binding or nuclease activities of XendoU and EndoU. T568 and H470 are noted.

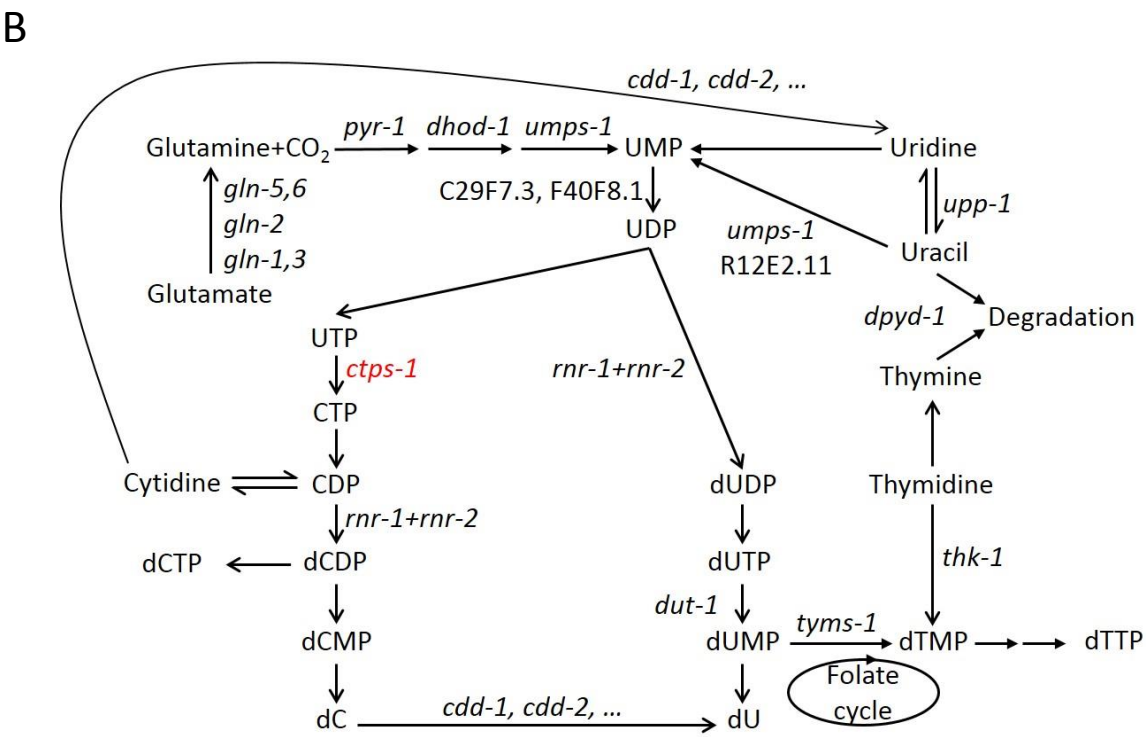
(E) Western blot showing successful knock-down of HA::ENDU-2 by both L1 RNAi and adult RNAi. The *endu-2(oe)* strain was used for the detection of HA::ENDU-2 fusion protein (see Methods).

(F) *endu-2(-)* worms treated with 10mM HU do not show growth delay.

(G) *endu-2(-)* worms have reduced sensitivity to UV. L1 worms were radiated with UV light (UV Stratalinker 1800, Stratagene) at indicated dosage and the percentage of worms that reached adulthood was counted after 3 days. Error bars, standard deviation. Star indicates $p<0.01$ (Two-way ANOVA test).

Figure S2


A
N.S.
0.18
0.15
0.12
0.09
0.06
0.03
0
dTTP/dCTP
WT
endu-2(-)
C
v


B
cdd-1, cdd-2, …
pyr-1
dhod-1
umps-1
Glutamine+CO2
UMP
Uridine
gln-5,6
gln-2
gln-1,3
Glutamate
C29F7.3, F40F8.1
UDP
umps-1
R12E2.11
upp-1
Uracil
dpyd-1
Degradation
Thymine
UTP
ctps-1
rnr-1+rnr-2
CTP
Cytidine
CDP
dUDP
Thymidine
rnr-1+rnr-2
dCTP
dCDP
dUTP
thk-1
dut-1
dCMP
dUMP
tyms-1
dTMP
dTTP
Folate cycle
dC
cdd-1, cdd-2, …
dU

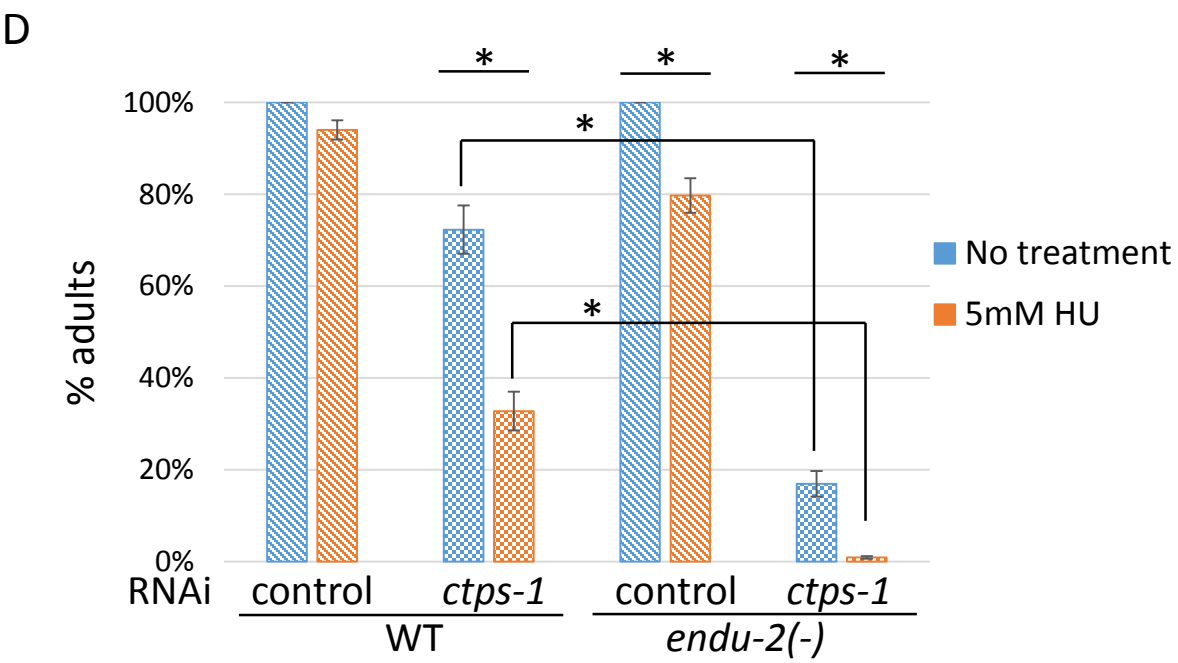

D
*
*
*
*
*
100%
80%
60%
40%
20%
0%
% adults
No treatment
5mM HU
RNAi
control
ctps-1
control
ctps-1
WT
endu-2(-)

Figure S2, related to Figure 2. A genetic screen identified that *ctps-1* positively interacts with *endu-2.*

(A) dTTP/dCTP ratio in WT and *endu-2(-)* single mutant. Error bars, standard deviation. *p=0.24* (Student's t-test, unpaired, two-tailed).

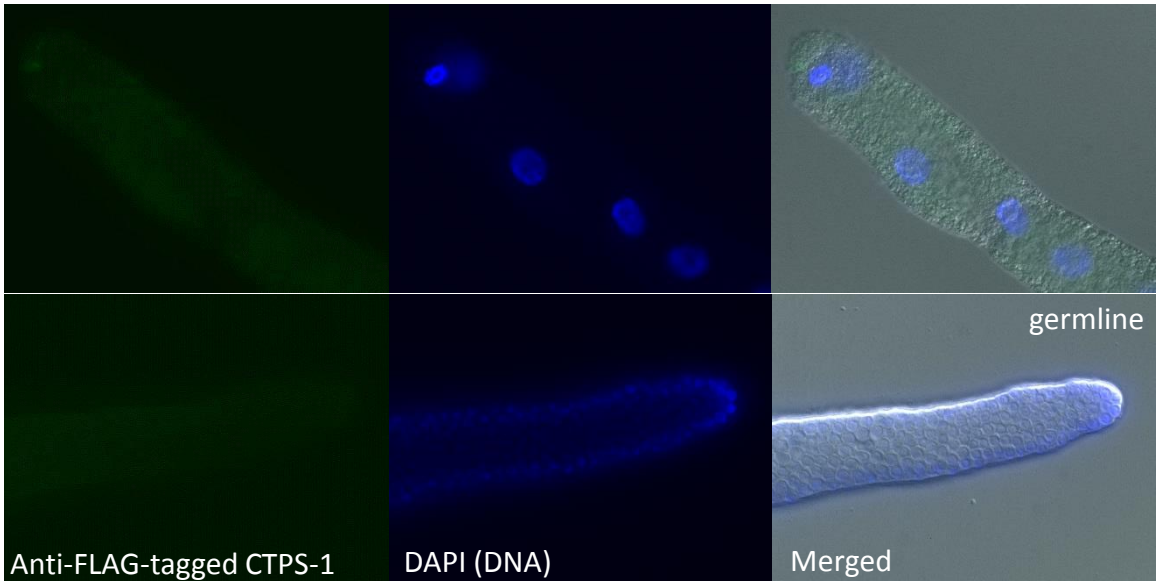
(B) A simplified diagram of the pyrimidine metabolism pathway and related genes in *C. elegans.*

(C) A picture of the disorganized germline of a *cddko* worm when fed diluted control RNAi food. This phenotype was suppressed when control RNAi was replaced with *endu-2* or *ctps-1* RNAi.

(D) Combined inactivation of *endu-2* and *ctps-1* further enhanced HU sensitivity. n≈200. Error bars, standard deviation. Stars indicate significant differences (Student's t-test, unpaired, two-tailed, with Bonferroni correction, $\alpha = 0.0018$).

Figure S3

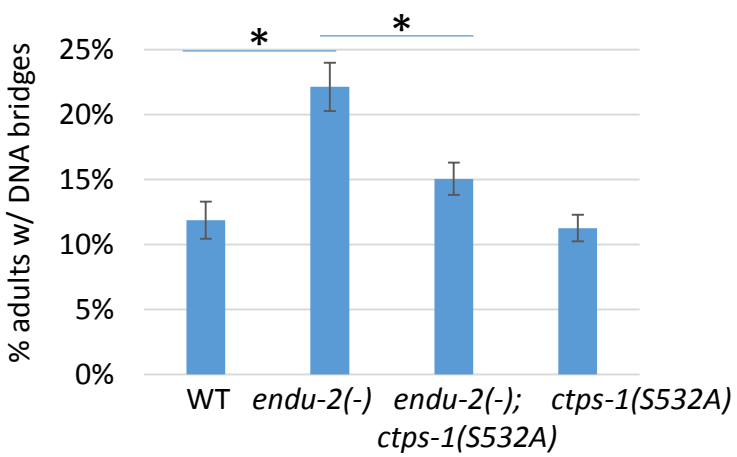
A



B

	phosphor/un-phosphor ratio		
#1	*WT*	*endu-2(oe)*	*endu-2(-), glp-1(RNAi)*
S532	0.35	0.27	1.03
S533	0	0.98	1.37
T530	0.25	0.21	0.63
#2	*WT*		*endu-2(-), glp-1(RNAi)*
S532	0.08		1.12
S533	0		0.41
T530	0.02		0

C



D

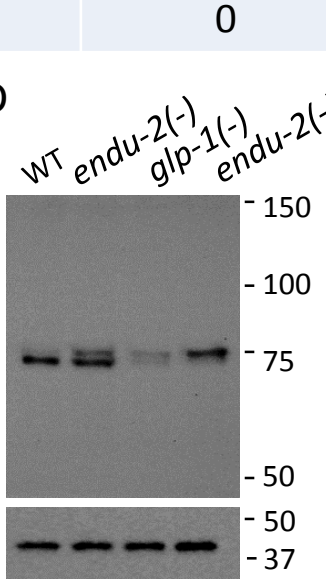


Figure S3, related to Figure 3. ENDU-2 regulates CTPS-1 phosphorylation.

(A) Negative controls for the immunostaining experiment in Figure 3D. WT worms that did not express the tagged FLAG::CTPS-1 protein were treated and imaged using the same conditions.

(B) Results of two independent mass-spec analyses. S532 was the only site that had a higher level of phosphorylation in *endu-2(-), glp-1* RNAi worms compared to WT or *endu-2(oe)* worms.

(C) *ctps-1(S532A)* partially suppressed the increased DNA bridge phenotype of *endu-2(-)*. Error bars, standard deviation. Stars indicate significant differences (Tukey's range test, FWER = 0.01).

(D) Western blot showing *glp-1(-)* mutation abolished the lower band in *endu-2(-); glp-1(-)* double mutant.

Figure S4

A

PhosphoMotif Finder - Results

Your query protein contains 21 Serine kinase / phosphatase motifs described in the literature and are underlined below.

WDVKKRTDSSAELMQM

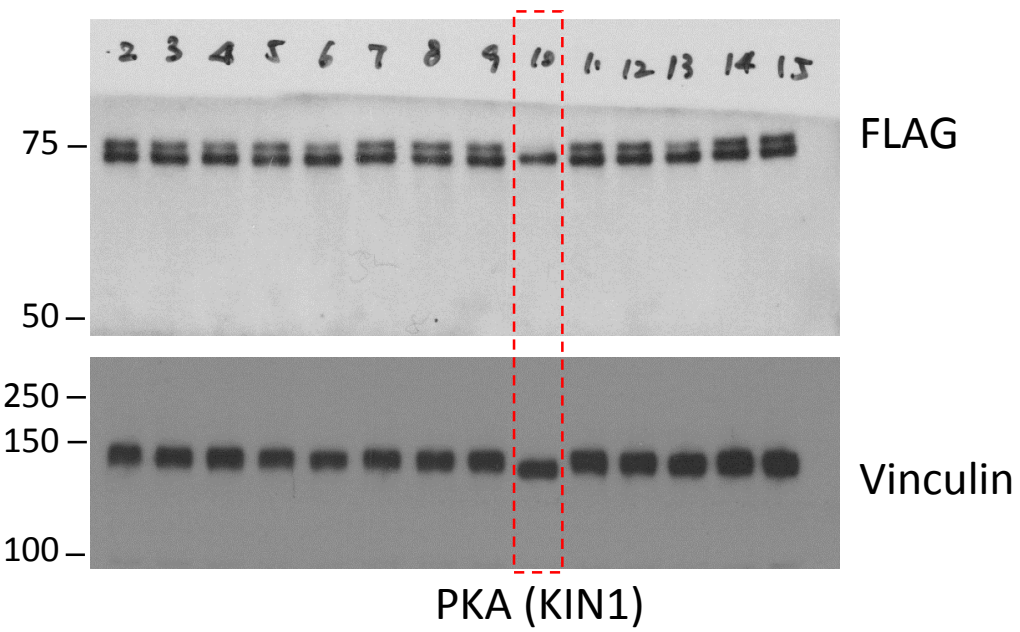
Sort by Position in query prot

PhosphoMotif

	Position in query protein	Sequence in query protein	Corresponding motif described in the literature (phosphorylated residues in red)	Features of motif described in the literature
1	4 - 7	KKRT	KXX[pS/pT]	PKA kinase substrate motif
2	4 - 7	KKRT	[R/K]XX[pS/pT]	PKC kinase substrate motif
3	4 - 7	KKRT	[R/K][R/K]X[pS/pT]	PKA kinase substrate motif
4	4 - 9	KKRTDS	[R/K]X[R/K][S/T]XpS	Akt kinase substrate motif
5	4 - 9	KKRTDS	[R/K]XRXXpS	MAPKAPK1 kinase substrate motif
6	5 - 7	KRT	[R/K]X[pS/pT]	PKA kinase substrate motif
7	5 - 7	KRT	[R/K]X[pS/pT]	PKC kinase substrate motif
8	5 - 9	KRTDS	KRXXpS	PKA kinase substrate motif
9	5 - 9	KRTDS	KXXX[pS/pT]	PKA kinase substrate motif
10	6 - 9	RTDS	RXXpS	Calmodulin-dependent protein kinase II substrate motif
11	6 - 9	RTDS	RXXpS	PKA kinase substrate motif
12	6 - 9	RTDS	RXX[pS/pT]	Calmodulin-dependent protein kinase II substrate motif
13	6 - 11	RTDSSA	X[pS/pT]XXX[A/P/S/T]	G protein-coupled receptor kinase 1 substrate motif
14	7 - 10	TDSS	[pS/pT]XX[S/T]	Casein Kinase I substrate motif
15	7 - 10	TDSS	[pS/pT]XX[E/D/**pS*/pY***]	Casein Kinase II substrate motif
16	8 - 12	DSSAE	[E/D][pS/pT]XXX	b-Adrenergic Receptor kinase substrate motif
17	9 - 12	SSAE	pSXX[E/D]	Casein kinase II substrate motif
18	9 - 12	SSAE	pSXX[E/**pS*/pT***]	Casein Kinase II substrate motif
19	9 - 12	SSAE	[pS/pT]XX[E/D]	Casein Kinase II substrate motif
20	9 - 12	SSAE	[pS/pT]XX[E/D]	Casein Kinase II substrate motif
21	10 - 12	SAE	pSX[E/**pS*/pT***]	Casein Kinase II substrate motif

* **indicates the residue that has to phosphorylated already for the enzyme to recognize the motif**

B



C

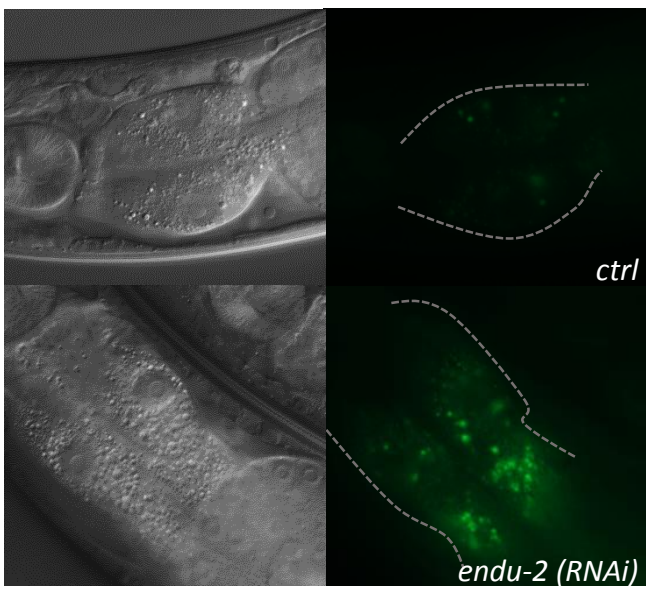


Figure S4, related to Figure 4. ENDU-2 regulates CTPS-1 phosphorylation through the PKA pathway.

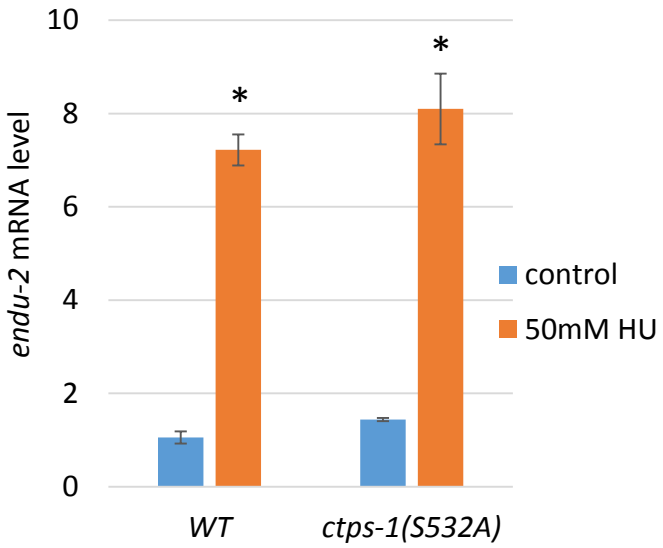
(A) Query results from a phospho-motif search using a small region of CTPS-1 protein sequence and the PhosphoMotif Finder (http://www.hprd.org/PhosphoMotif_finder).

(B) Western blot of *endu-2(-)* worms treated with RNAi of candidate kinases. Dotted red box highlights *kin-1* RNAi-treated samples which lost CTPS-1 phosphorylation. #2, #14 and #15 control, #3 *akt-1* RNAi, #4 *akt-2* RNAi, #5 *unc-43* RNAi, #6 *kin-3* RNAi, #7 *mak-1* RNAi, #8 *mak-2* RNAi, #9 *mnk-1* RNAi, #10 *kin-1* RNAi, #11 F47F2.1 RNAi, #12 *grk-2* RNAi, #13 *sgk-1* RNAi.

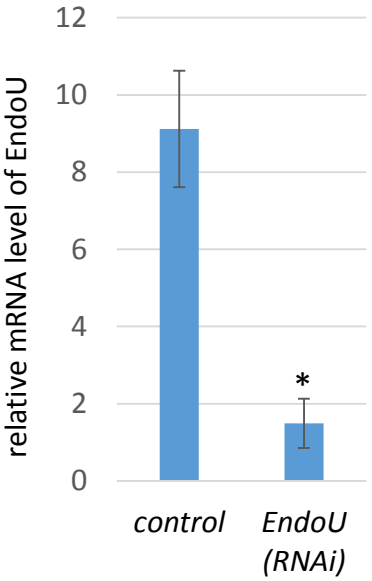
(C) High magnification (Under 63x Objective) images of the *atgl-1::gfp* strain. The first pair of intestinal cells had significantly higher GFP signal in *endu-2(RNAi)* worms compared to control.

Figure S5

A



B



C

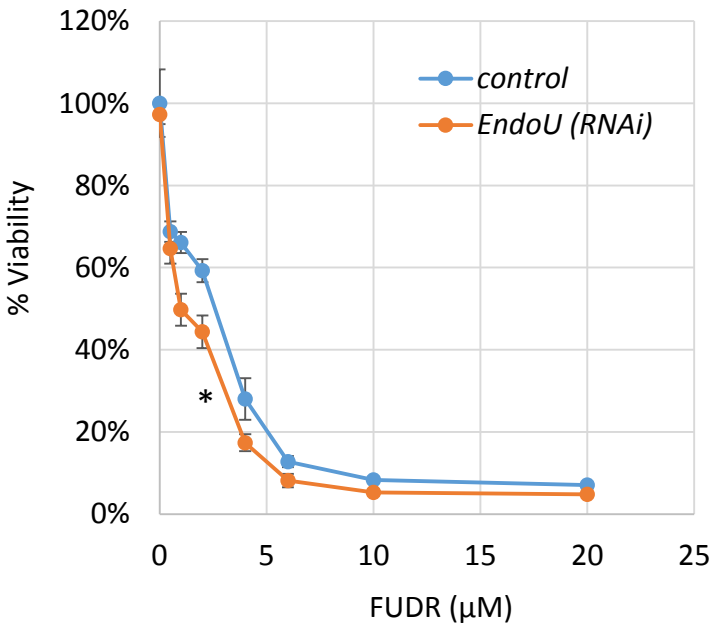


Figure S5, related to Figure 6. EndoU RNAi treated HeLa cells are hypersensitive to FUDR treatment.

(A) qPCR results show *ctps-1(S532A)* mutation did not affect the response of *endu-2* to HU treatment. Young adult WT or *ctps-1(S532A)* worms were treated with 50mM HU for 1 day. Star indicates significant differences (Student's t-test, unpaired, two-tailed, with Bonferroni correction, $\alpha = 0.008$).

(B) qPCR results for EndoU RNAi indicate efficient knockdown of the target. Star indicates $p<0.01$ (Student's t-test, unpaired, two-tailed).

(C) WST-8 cell proliferation assay results. EndoU RNAi-treated HeLa cells had reduced viability after treatment with FUDR. Star indicates $p<0.0001$ (Two-way ANOVA test). Error bars indicate standard deviation in all panels.