Supporting Information

The kynurenine pathway is essential for rhodoquinone biosynthesis in *Caenorhabditis elegans*

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List of Materials

TABLE S1. Statistical analysis of RQ_9 and Q_9 levels in mutant strains and RNAi knockdowns

Strain	**Avg pmol RQ_9/mg pellet**	**p value[a] (N = 3)**	**Avg pmol Q_9/mg pellet**	**p value (N = 3)**
N2	3.27 ± 0.15		17.03 ± 0.71	
afmd-1	1.51 ± 0.09	< 0.001	13.24 ± 1.80	0.0138
kynu-1	0	< 0.001	19.27 ± 1.76	0.0633
kmo-1	0.51 ± 0.07	< 0.001	13.49 ± 0.88	0.0027
haao-1	3.41 ± 0.61	0.360	14.41 ± 0.80	0.0065
EV	2.36 ± 0.20		9.48 ± 1.43	
kynu-1 (RNAi)	0.51 ± 0.05	< 0.001	9.95 ± 2.52	0.397
coq-3 (RNAi)	1.67 ± 0.12	0.003	7.01 ± 0.58	0.025
coq-5 (RNAi)	1.26 ± 0.17	< 0.001	5.21 ± 1.05	0.007
coq-6 (RNAi)	1.02 ± 0.04	< 0.001	5.10 ± 0.22	0.003
coq-7 (RNAi)	2.05 ± 0.20	0.068	6.29 ± 0.32	0.010
unc-22 (RNAi)	2.59 ± 0.60	0.283	9.48 ± 1.92	0.500

[a]The Student's T-test was used to analyze triplicate samples with significance noted at the $\alpha < 0.05$ level.
The standard deviation in each data set is represented by ± and shown as error bars in Figs. 2B, 2C and 4B.

TABLE S2. *C. elegans* strains used in this study.

Strain	Gene	Allele	Variation type	Nucleotide change	Genotype	Source
N2	*Bristol wild isolation*					CGC
NL2099	*rrf-3*	pk1426	deletion	3055 bp deletion	*rrf-3(pk1426) II*	CGC
Tm4924	*kynu-1*	tm4924	insertion/deletion	19 bp insertion 521 bp deletion	*kynu-1(tm4924) X*	NBPJ
Tm4529	*kmo-1*	tm4529	deletion	326 bp deletion	*kmo-1(tm4529) V*	NBPJ
Tm4547	*afmd-1*	tm4547	deletion	425 bp deletion	*afmd-1(tm4547)IV*	NBPJ
Tm4627	*haao-1*	tm4627	insertion/deletion	9 bp insertion 305 bp deletion	*haao-1(tm4627) V*	NBPJ
IH25					*kynu-1(tm4924)X; Ex[Pkynu-1::kynu-1::gfp, pRF4]*	This study

TABLE S3. Primers used for *kynu-1* reporter construction

Primer name	**Sequence**
kynu-1 FW pPD9577	acgctaacaacttggaaatgaaataccgaattagttttaatggac
kynu-1 RE pPD9577	ctttggccaatcccggggatccttcgctttcgacaatatgagcaac
pPD9577 RE	atttcatttccaagttgttagcgtatccatcg

TABLE S4. RNAi clones and TaqMan assays for RT-PCR

Strain	Gene	[a]Clone Number	Insert	Source	[b]TaqMan assay
E. coli HT115 (DE3)	*kynu-1*	DFCIp3320G0510040D	C15H9.7	Source Bioscience	Ce02495988_g1
	coq3	CUUkp3303J037Q	sjj_Y57G11C.11	Source Bioscience	Ce02467843_g1
	coq5	CUUkp3302K054Q	sjj_ZK652.9	Source Bioscience	Ce02449325_g1
	coq6	CUUKp3315A0214Q	sjj2_K07B1.2	Source Bioscience	Ce02479593_g1
	coq7/clk-1	CUUkp3302B242Q	sjj_ZC395.2	[c]gift	Ce02446729_g1
	unc-22	CUUkp3303K066Q	sjj_ZK617.1	[c]gift	Ce02465425_g1

[a]All clones were from Ahringer library, L4440 (pPD129.36) except for *kynu-1* was from Vidal library, pL4440_DEST
[b]Purchased from ThermoFisher Scientific (Rockford, IL, USA) with FAM-MGB, 20X
[c]Gift from Dr. Jennifer Watts, School of Molecular Sciences, Washington State University, Pullman, WA

TABLE S5. LC-MS parameters for each quinone

MS parameter	**Q_3**	**RQ_9**	**Q_9**
Dwell time (s)	0.1	0.1	0.1
Cone (V)	20	39	35
Collision (V)	20	30	30
Precursor mass $[M+H]^+$ (*m*/*z*)	387.2	780.6	795.6
Ion product mass $[M]^+$ (*m*/*z*)	197.2	182.2	197.2

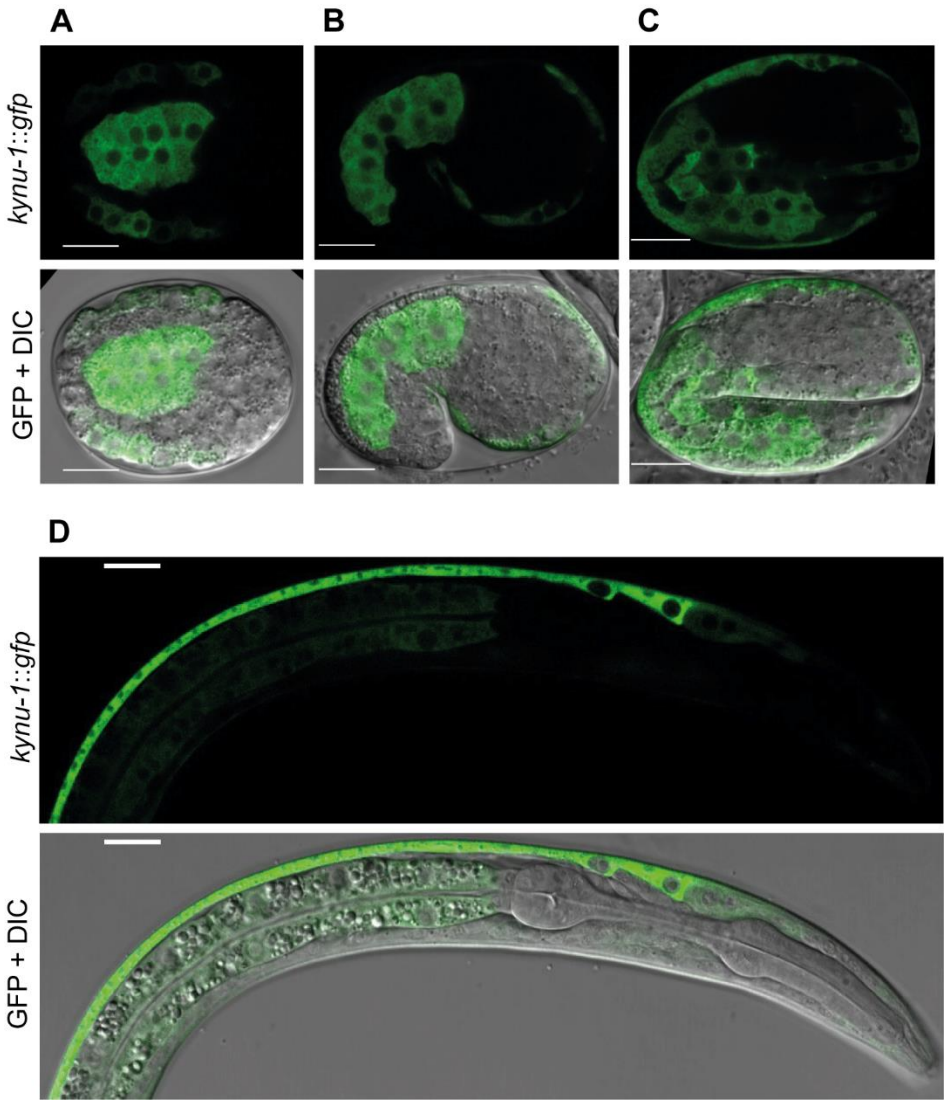

Figure S1. *kynu-1* is expressed during embryogenesis and the first larval stage in hypodermis and intestinal cells. Confocal images of selected planes show transgenic animals expressing the translational construct *Pkynu-1*::*kynu-1*::*gfp*. The stages shown are: (A) E16 dorsal view, (B) Comma lateral view, (C) 2-fold lateral view and (D) L1 lateral view. Scale bar 10 µm.

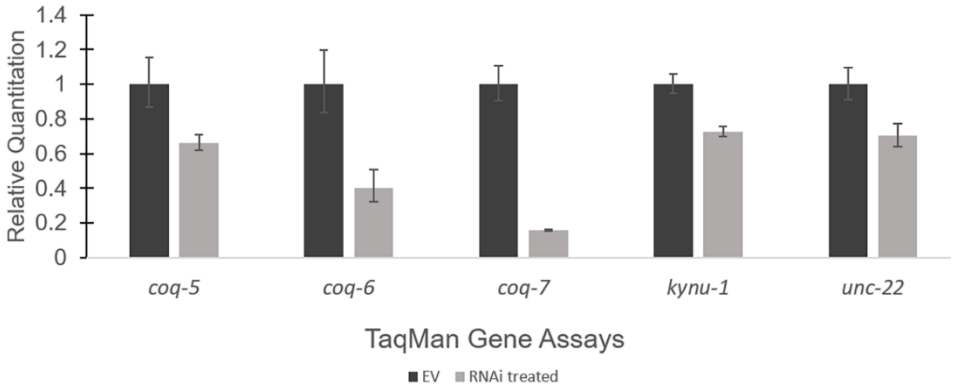

Figure S2. Quantitation of gene expression from *C. elegans* RNAi strains using RT-qPCR. A standard comparative C_T ($\Delta\Delta C_T$) experiment was performed with the TaqMan gene expression assays (Table S4) on a StepOnePlus™ Real-Time PCR system (Life Technologies, Waltham, MA). ROX™ dye was used as a passive reference and EV cDNA was used as an active reference in each experiment. Each cDNA sample and no RT control was tested in triplicate with each assay, and the average C_T values were generated for each biological sample with each gene target. ΔC_T and $\Delta\Delta C_T$ values were obtained in order to determine the range of fold-change values, comparing RNAi knockdowns to EV control. Relative quantitation (RQ) ranges were determined through standard propagation of error. TaqMan gene assays for *coq-5*, *coq-6*, *coq-7*, *kynu-1* and *unc-22* showed reduction of expression compared to the EV reference, using the *cdc-42* endogenous control. The relative quantitation values were significant for *coq-6* and *coq-7* (more than 2-fold smaller), and weakly significant for *coq-5*, *kynu-1*, and *unc-22*. The *coq-3* TaqMan assay did not allow for quantitation due to inconsistent amplification of *cdc-42* in the sample and reference.