Supplementary Materials for

Cell Non-Autonomous Activation of Flavin-containing Monooxygenase Promotes Longevity and Healthspan

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This PDF file includes:

Materials and Methods.

Growth conditions. Standard *C. elegans* procedures were used as previously described (*28-30*). Worms were maintained on solid Nematode Growth Medium (NGM) using UV-arrested *E. coli* OP50 throughout life except where RNAi was used. All experiments took place at 20°C except where otherwise noted in **Table S2**. All strains used in this study are listed in **Table S3**.

Lifespans. Lifespans were carried out as previously described (*28-30*). Gravid adults were placed on NGM plates seeded with UV-killed OP50 lawns for an 8-12 hour timed egg-lay. Subsequently, adults were removed from the plates and eggs were allowed to develop. At late L4/early adult stage worms were transferred to FUdR to prevent the development of progeny. Approximately 75 worms were placed on each NGM + FUdR plate seeded with 20x UV-killed OP50. A minimum of two plates per strain per condition were used. Worms were transferred periodically to prevent starvation and avoid contamination. Worms were scored as dead when they did not move in response to prodding under a dissection microscope. Worms that crawled off the plate were not considered.

RNAi Lifespans. RNAi experiments were carried out as described except all plates contained 1mM β-D-isothiogalactopyranoside (IPTG) and 25 μg/ml carbenicillin and the corresponding RNAi clone from the Vidal or Ahringer RNAi library. All RNAi experiments were performed on second generation worms whose mothers were placed on RNAi as eggs. All RNAi's were sequence verified before use.

sDR Lifespans. sDR lifespans were carried out as previously described (*11*). Worms for sDR experiments were treated like other lifespans, except at day 3 of adulthood they were transferred to plates containing either 10^11 (ad lib) or 10^9 (sDR) bacterial lawns. Worms were then transferred every other day until they stopped eating.

Q35 Paralysis Assay. Paralysis studies were carried out similarly to lifespan experiments, except instead of scoring for head movement, they were scored for the ability to move the posterior half of their bodies.

Stress Tests. For mortality tests, eggs derived from bleaching gravid adults were plated onto 10cm NGM plates and grown until they reached the L4 growth stage. Approximately 20 worms in triplicate were transferred into individual wells of 96-well plates containing S medium and OP50. Stressors were then added at equal volume to the wells and the plates were placed in orbital shakers at room temperature (~22°C). After 24 hours the worms were removed from the wells, rinsed and plated on solid NGM plates and allowed to recover an additional 24 hours before scoring. Heat experiments were performed similarly in liquid culture in a heated incubator and were repeated on solid NGM plates. In both cases worms were removed at the listed time points, after which they were given 24 hours to recover. Growth assays in tunicamycin were performed similarly, except ~20 eggs were placed in 96-well plates with tunicamycin and allowed to develop for 72-96 hours.

Brood Size. Worms from a timed egg-lay were plated individually on 35mm NGM plates, and once egg-laying began, mothers were transferred daily to new plates. Eggs were allowed to grow to L3 before counting.

Development. Development was measured by watching gravid adult mothers lay eggs under a microscope, followed by noting the date and time and moving the egg to a new plate. Each egg was then followed through development and watched hourly during young adulthood to score the time of first egg lay.

Pumping and thrashing. Worms were treated as for lifespans, except at the age-points indicated at least 10 worms per strain were assayed for their pumping rate under 100X total magnification. Because food availability affects pumping rate, only worms on the lawn were considered. For thrashing, worms were placed in a drop of M9 solution and individual body bends were counted at maximum rate. Both thrashing and pumping rates were examined individually for a minimum of 20 seconds at maximum rate to obtain the final rate shown.

Autofluorescence microscopy. For autofluorescence, worms were measured as previously described (*1*) using a using a Zeiss SteREO Lumar.V12 microscope. Worms paralyzed in 10mM azide were placed on glass 8-well slides and measured for DAPI fluorescence with an excitation filter of 365 nm and a 445 nm emission band-pass filter. Image J software (http://rsb.info.nih.gov/ij/java) was used to calculate the integrated density of the autofluorescence. Strains tested include the entire list of genes, both dependent and independent (as controls) of *hif-1*, reported by Bishop *et al.* (*31*) and Shen *et al.* (*12*) to be downstream of hypoxic signaling (both through *vhl-1* mutation and hypoxia).

GFP microscopy. For GFP fluorescence, we used a Nikon Eclipse E600 microscope under the FITC-HYQ filter set (excitation 450-495 nm and emission band-pass filter 510-550 nm) and quantified the images with Image J. Worms for these experiments were grown to L4, transferred to FUdR for 24 hours and then transferred to their final condition overnight (16-20 hours). The final conditions were standard fed/normoxia, 0.1% O_2, and fasted (no food on plate). Data shown in text are representative experiments, whereas supplemental figures show all data compiled.

Quantitative PCR. RNA was isolated from L4 or day 2 adult worms using a Direct-zol RNA miniprep kit (Zymo) and cDNA was prepared via the iScript cDNA Synthesis Kit (Biorad). *fmo-2* expression levels were measured using the SYBR® Green (Biorad) quantitative RT–PCR (qRT–PCR) system. mRNA levels were normalized using previously published controls, *act-1* and *ama-1* (*32*). Primers used for *fmo*-2 levels were 5'- GACGTGGCAGTTGAGCAATC -3' and 5'- ACACACGATGAGCTGGCTTT -3'. Gene expression was calculated using standard curves.

Worm sorting. The COPAS™ BIOSORT was used for high-throughput sorting and dispensing of *C. elegans* using size and fluorescence parameters. Worms were sorted

by size (time of flight and extinction), gated for L1 through adult worms, and results were graphed as scatterplots of individual worms.

Hypoxia. Hypoxic conditions were derived by flowing hydrated 0.5% or 0.1% oxygen through air-tight containers in 20°C incubators (*33*). Gas mixtures provided by Airgas and certified standard to 2% of target oxygen concentration. All hypoxic experiments were exposed to as little room air as possible from the point of hypoxia.

Strain Construction. Strains were constructed using the Mos1 single-copy insertion technique previously described (*7*). Briefly, using the Gateway modular system (Invitrogen), we created insertion plasmids with the listed promoter, the listed gene, and a 3' region (pCFJ326) containing the tbb-2 UTR operon linker followed by GFP. The plasmid cocktail was injected as suggested by the Jorgensen lab (https://sites.google.com/site/jorgensenmossci/Home) and rescued worms were heat-shocked and then PCR verified for location and sequence identity.

Statistical Analysis. P-values for lifespan and paralysis analyses were derived using the log-rank statistical test. Individual experiments and their statistics are provided in **table S2**. A two-tailed Student's t-test was used to derive p-values for other comparisons such as brood size, thrashing, pumping, fluorescence, and relative mRNA levels. For each experiment where there were more than two strains, t-tests were performed on each strain individually compared to the control strain. All error bars shown in Figures represent the standard error of the mean.

Supplementary References:

28. T. L. Kaeberlein *et al.*, Lifespan extension in Caenorhabditis elegans by complete removal of food. *Aging Cell* **5**, 487-494 (2006).
29. E. D. Smith *et al.*, Age- and calorie-independent life span extension from dietary restriction by bacterial deprivation in Caenorhabditis elegans. *BMC Dev Biol* **8**, 49 (2008).
30. G. L. Sutphin, M. Kaeberlein, Dietary restriction by bacterial deprivation increases life span in wild-derived nematodes. *Exp Gerontol* **43**, 130-135 (2008).
31. T. Bishop *et al.*, Genetic analysis of pathways regulated by the von Hippel-Lindau tumor suppressor in Caenorhabditis elegans. *PLoS biology* **2**, e289 (2004).
32. Y. Zhang, D. Chen, M. A. Smith, B. Zhang, X. Pan, Selection of reliable reference genes in Caenorhabditis elegans for analysis of nanotoxicity. *PLoS One* **7**, e31849 (2012).
33. E. M. Fawcett, J. W. Horsman, D. L. Miller, Creating defined gaseous environments to study the effects of hypoxia on C. elegans. *J Vis Exp*, e4088 (2012).

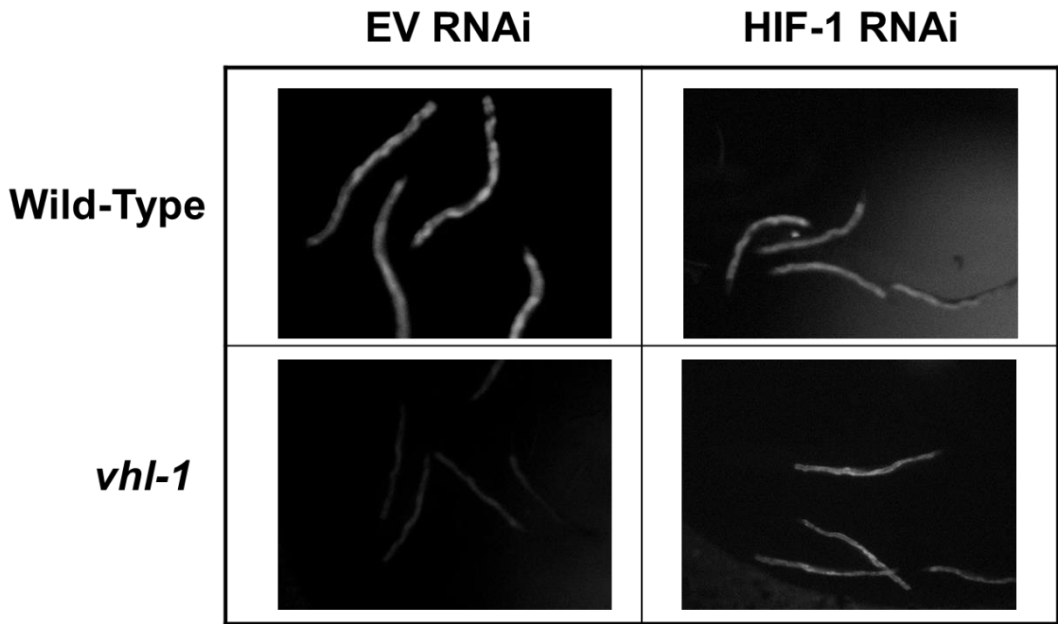


Fig. S1. A screen for age-associated autofluorescence in *vhl-1(ok161)* mutant worms. The basis of the screen is that *vhl-1(ok161)* knockout worms have a substantial decrease in autofluorescence that is abrogated by *hif-1* RNAi. Worms were imaged at 4-5 days of adulthood (day 5 shown in image) and again at 7-8 days of adulthood.

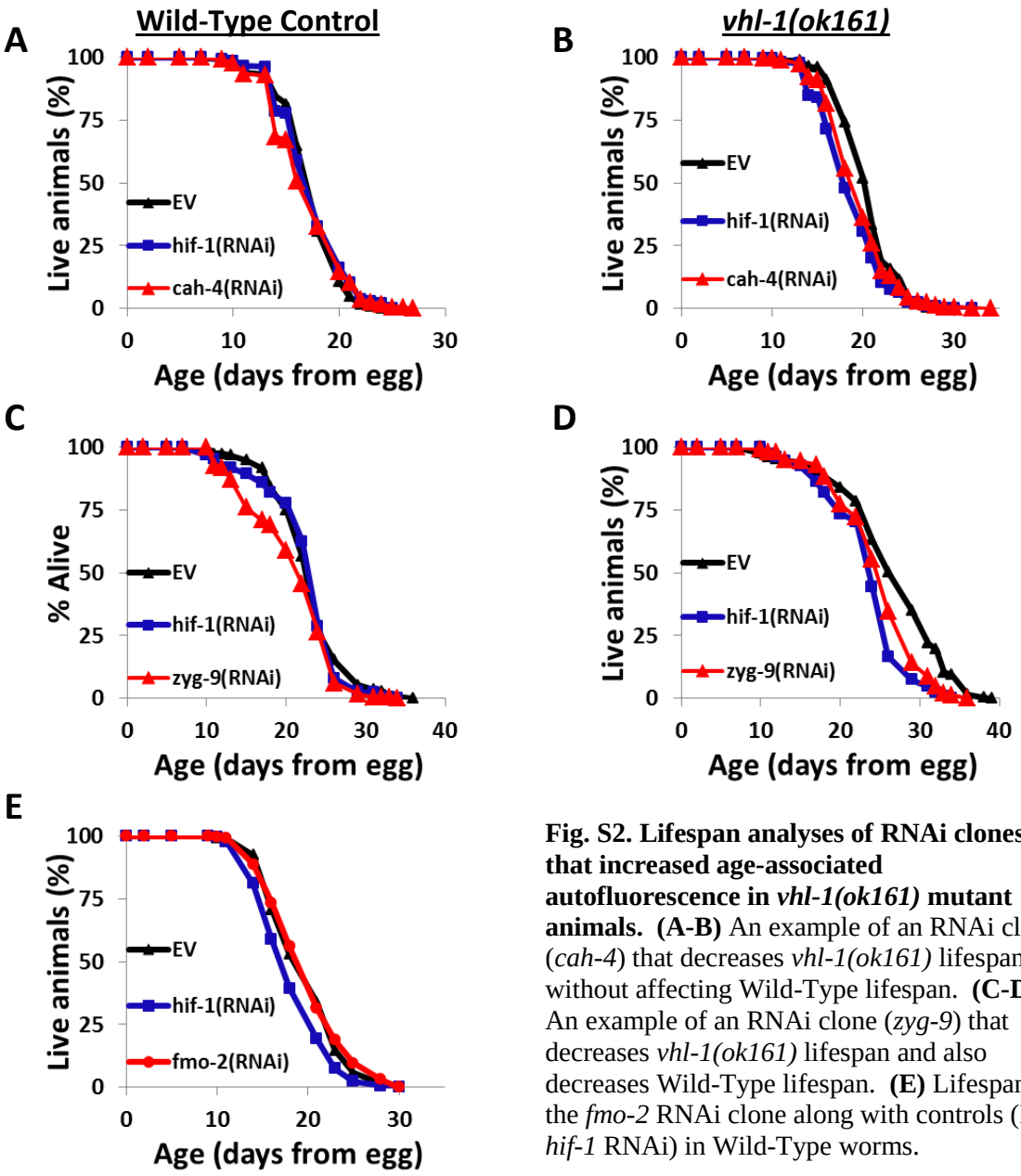


Fig. S2. Lifespan analyses of RNAi clones that increased age-associated autofluorescence in *vhl-1(ok161)* mutant animals. (A-B) An example of an RNAi clone (*cah-4*) that decreases *vhl-1(ok161)* lifespan without affecting Wild-Type lifespan. **(C-D)** An example of an RNAi clone (*zyg-9*) that decreases *vhl-1(ok161)* lifespan and also decreases Wild-Type lifespan. **(E)** Lifespan of the *fmo-2* RNAi clone along with controls (EV, *hif-1* RNAi) in Wild-Type worms.

A

B

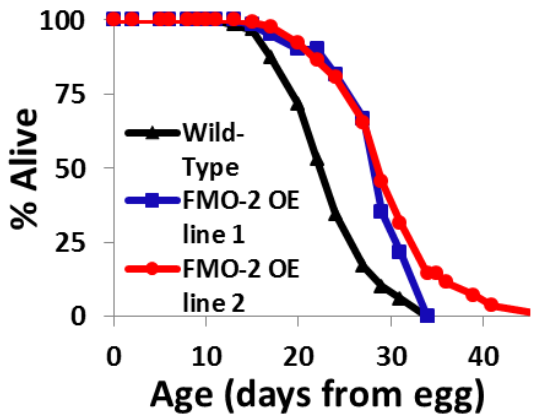


Fig. S3. (A) MosSCI vector arrangement for creating FMO-2 ubiquitous overexpression worms. **(B)** Lifespans of multiple independent lines of worms ubiquitously overexpressing (*peft-3*) FMO-2 in otherwise Wild-Type background.

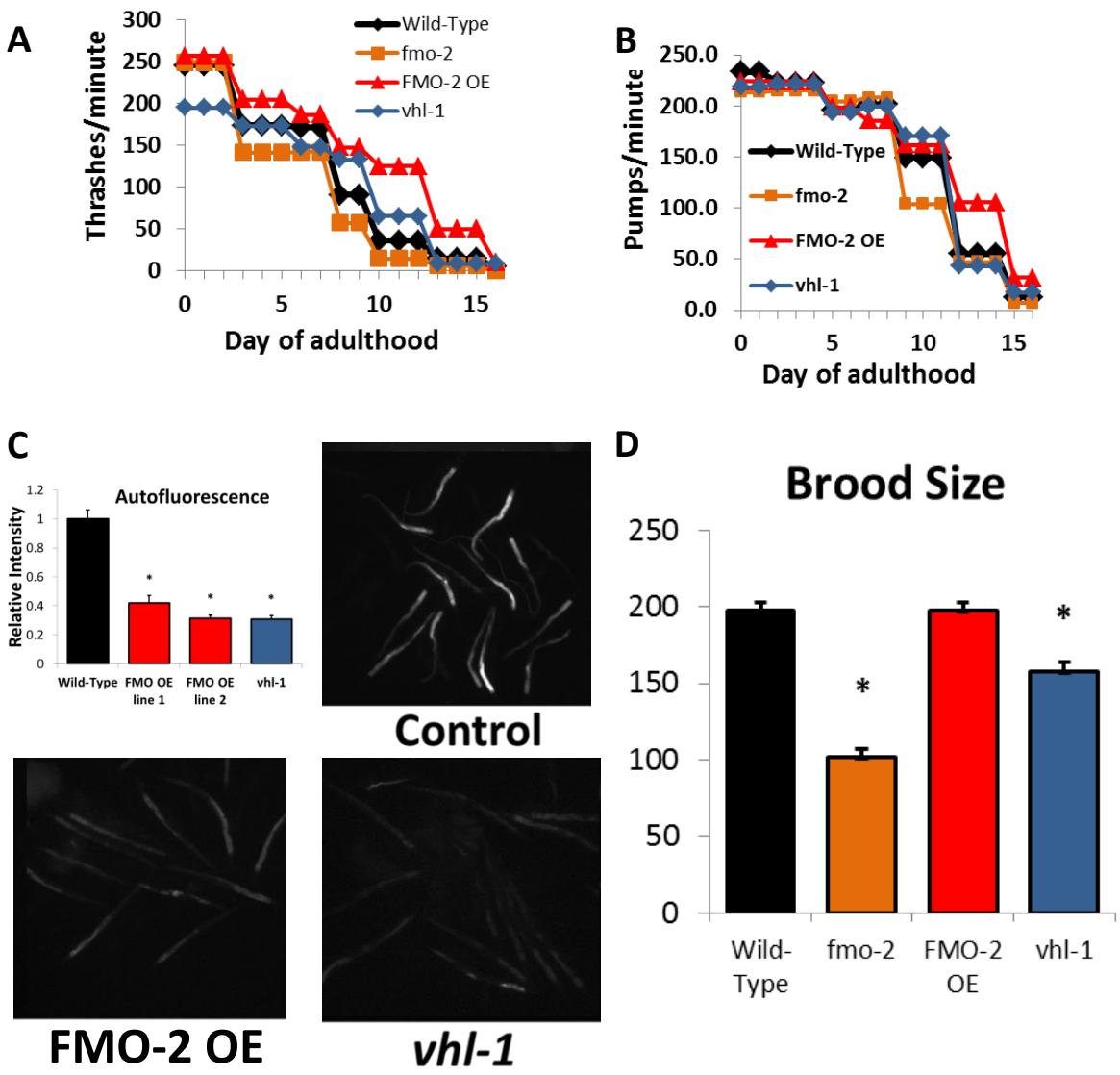


Fig. S4. Healthspan of FMO-2 OE worms is improved. (A-B) Time course of thrashing and pumping, respectively, for each strain (Wild-Type, *fmo-2(ok2147)*, FMO-2 OE, and *vhl-1(ok161)*) over time. **(C)** Bar graph and representative images of age-associated autofluorescence at day 5 of adulthood. **(D-E)** Average brood size and the rate of development (from egg to egg-laying adult) of each strain. (* denotes statistical difference from Wild-Type by individual t-tests, $p < 0.05$)

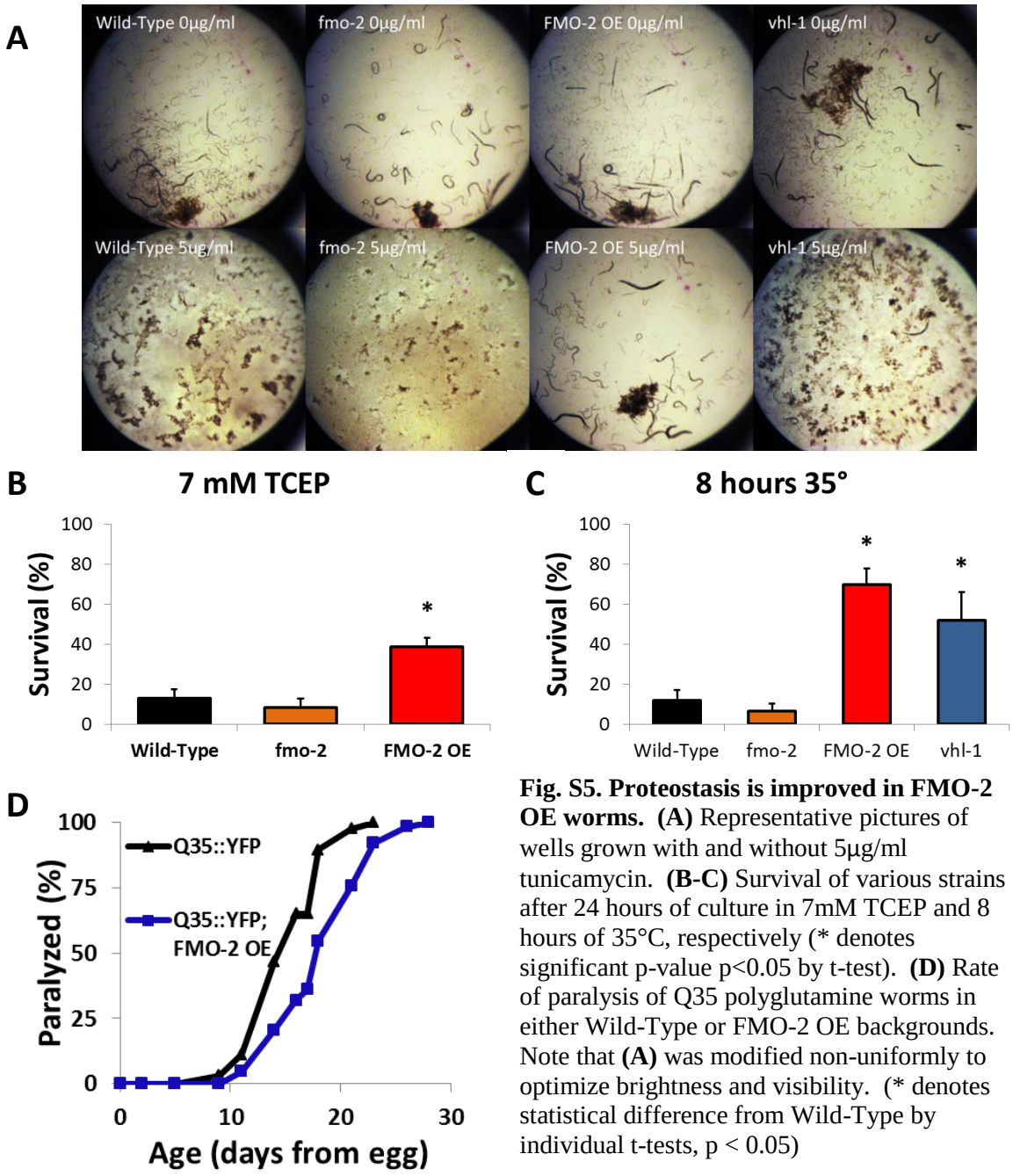


Fig. S5. Proteostasis is improved in FMO-2 OE worms. (A) Representative pictures of wells grown with and without 5µg/ml tunicamycin. **(B-C)** Survival of various strains after 24 hours of culture in 7mM TCEP and 8 hours of 35°C, respectively (* denotes significant p-value $p<0.05$ by t-test). **(D)** Rate of paralysis of Q35 polyglutamine worms in either Wild-Type or FMO-2 OE backgrounds. Note that **(A)** was modified non-uniformly to optimize brightness and visibility. (* denotes statistical difference from Wild-Type by individual t-tests, $p < 0.05$)

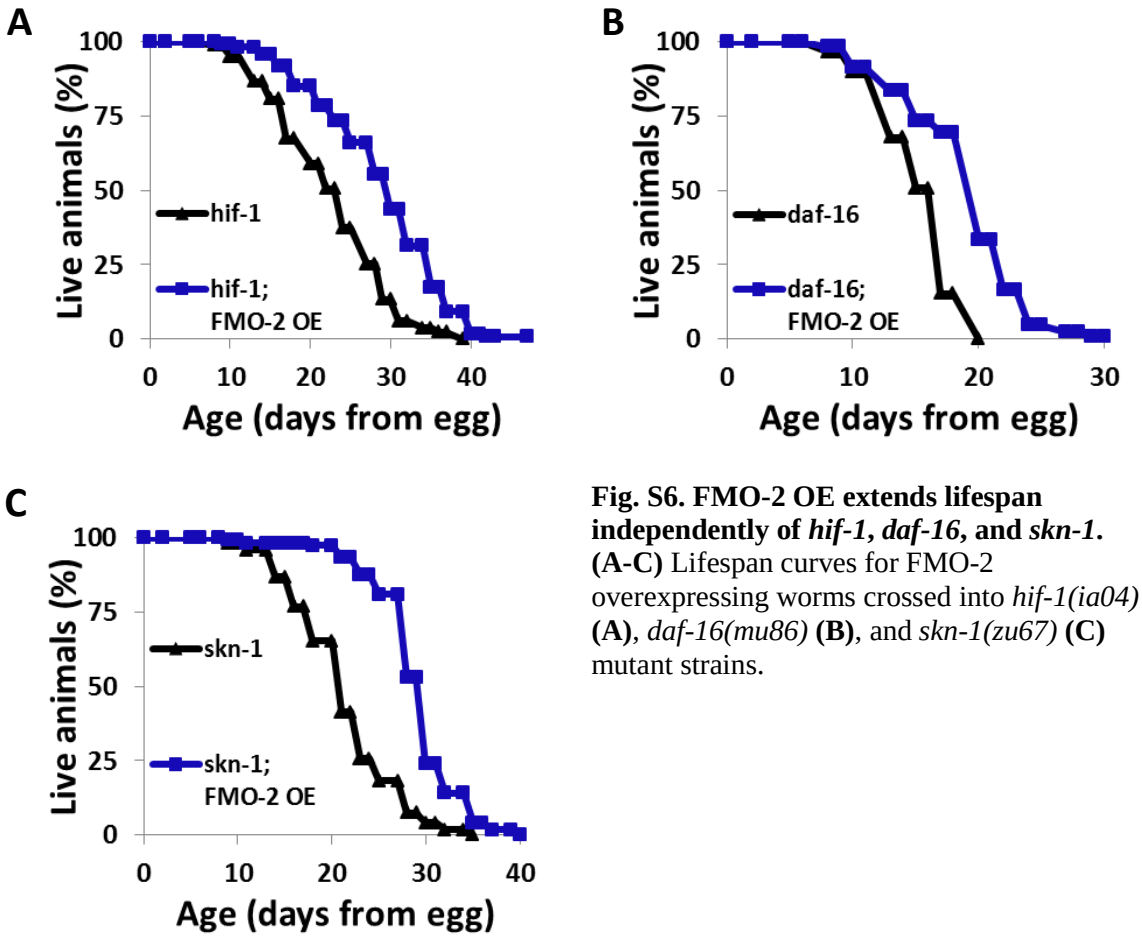
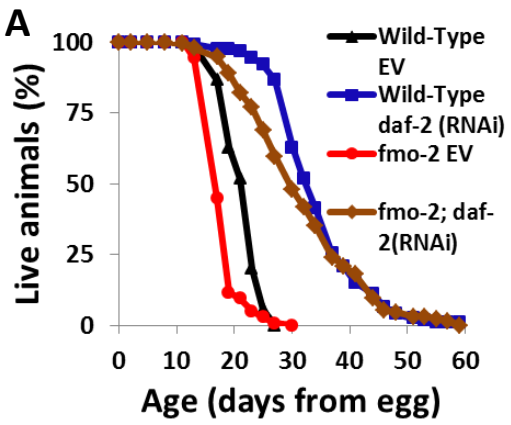
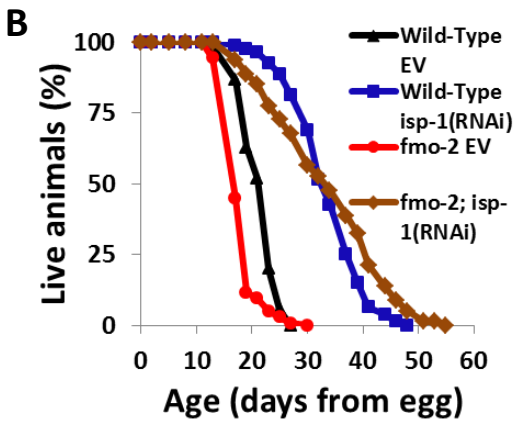


Fig. S6. FMO-2 OE extends lifespan independently of *hif-1*, *daf-16*, and *skn-1*. **(A-C)** Lifespan curves for FMO-2 overexpressing worms crossed into *hif-1(ia04)* **(A)**, *daf-16(mu86)* **(B)**, and *skn-1(zu67)* **(C)** mutant strains.





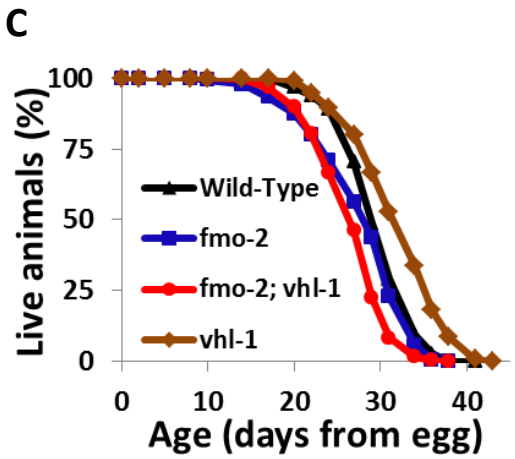


Fig. S7. *Fmo-2* is required for lifespan extension from mutation of *vhl-1* but not *daf-2* or *isp*-1. Epistasis of *fmo-2(ok2147)* knockout worms. Lifespans of Wild-Type and *fmo-2(ok2147)* worms on *daf-2* RNAi **(A)**, *isp-1* RNAi **(B)**, and crossed into *vhl-1(ok161)* knockout worms **(C)**.

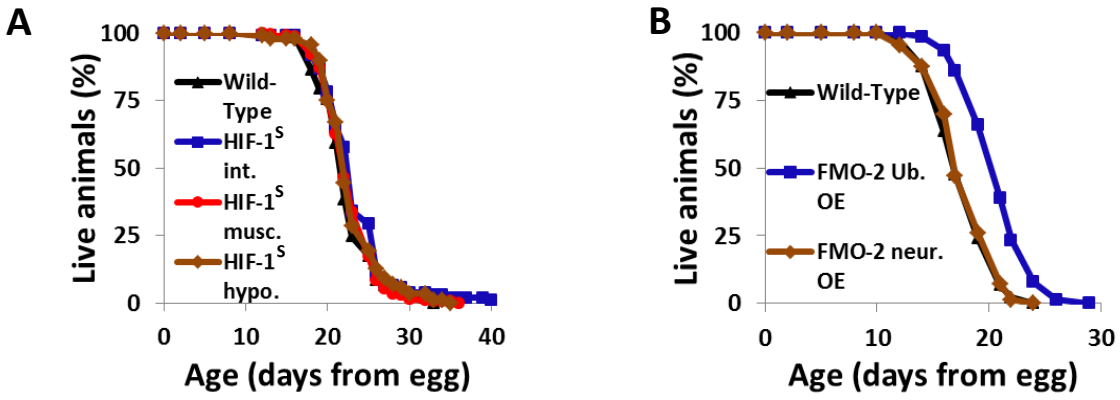


Fig. S8. Other tissue-specific overexpression of HIF-1 and FMO-2. (A) Lifespans of worms expressing non-degradable HIF-1 (HIF-1^{S}) under intestinal (pvha-6), muscle (punc-120), and hypodermal (pdpy-7) promoters. **(B)** Lifespans of worms overexpressing FMO-2 under the ubiquitous *eft-3* (Ub.) promoter and the neuronal *rab-3* (neur.) promoter.

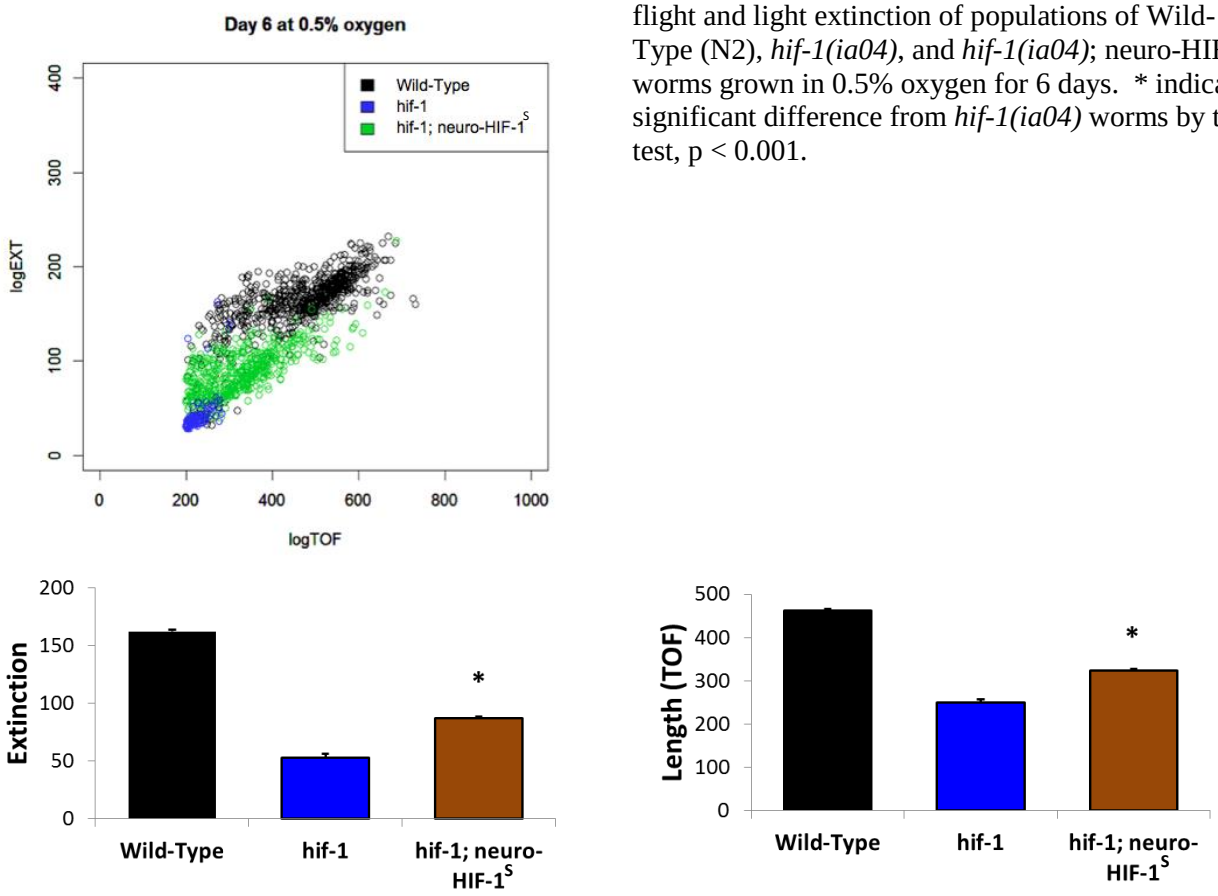


Fig. S9. Neuronal expression of HIF-1^S partially suppresses growth inhibition in hypoxia. Time of flight and light extinction of populations of Wild-Type (N2), *hif-1(ia04)*, and *hif-1(ia04)*; neuro-HIF-1^S worms grown in 0.5% oxygen for 6 days. * indicates significant difference from *hif-1(ia04)* worms by t-test, $p < 0.001$.

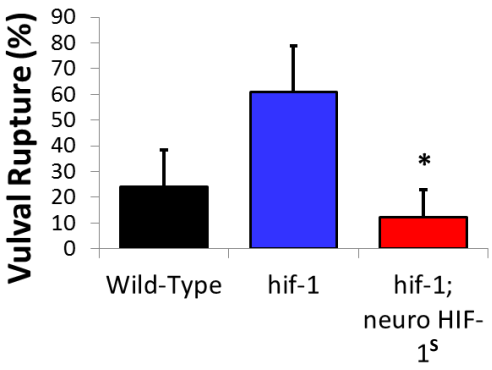


Fig. S10. Decreased rupture in *hif-1(ia04)* knockout worms with HIF-1 stabilized in the neurons. Levels of vulval rupture during aging (>day 10) in wild-type, *hif-1(ia04)*, and *hif-1(ia04)*; neuro-HIF-1^S stabilized worms. * indicates statistical difference ($p < 0.05$) from *hif-1(ia04)* by t-test.

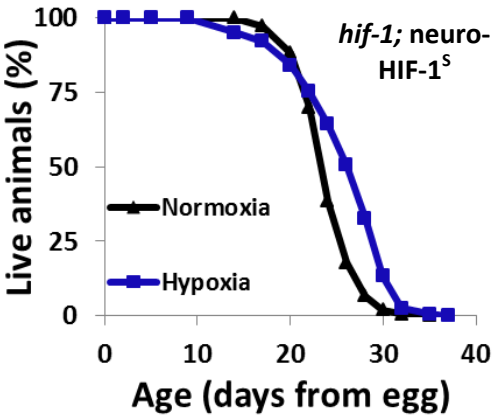


Fig. S11. HIF-1 in neurons is sufficient to allow lifespan extension from hypoxia during adulthood. Lifespans of *hif-1(ia04)* worms expressing stabilized HIF-1 only in the neurons (*hif-1(ia04); neuro*-HIF-1^{S}), where adults were kept in either normoxia (~21% oxygen) or hypoxia (0.5% oxygen).

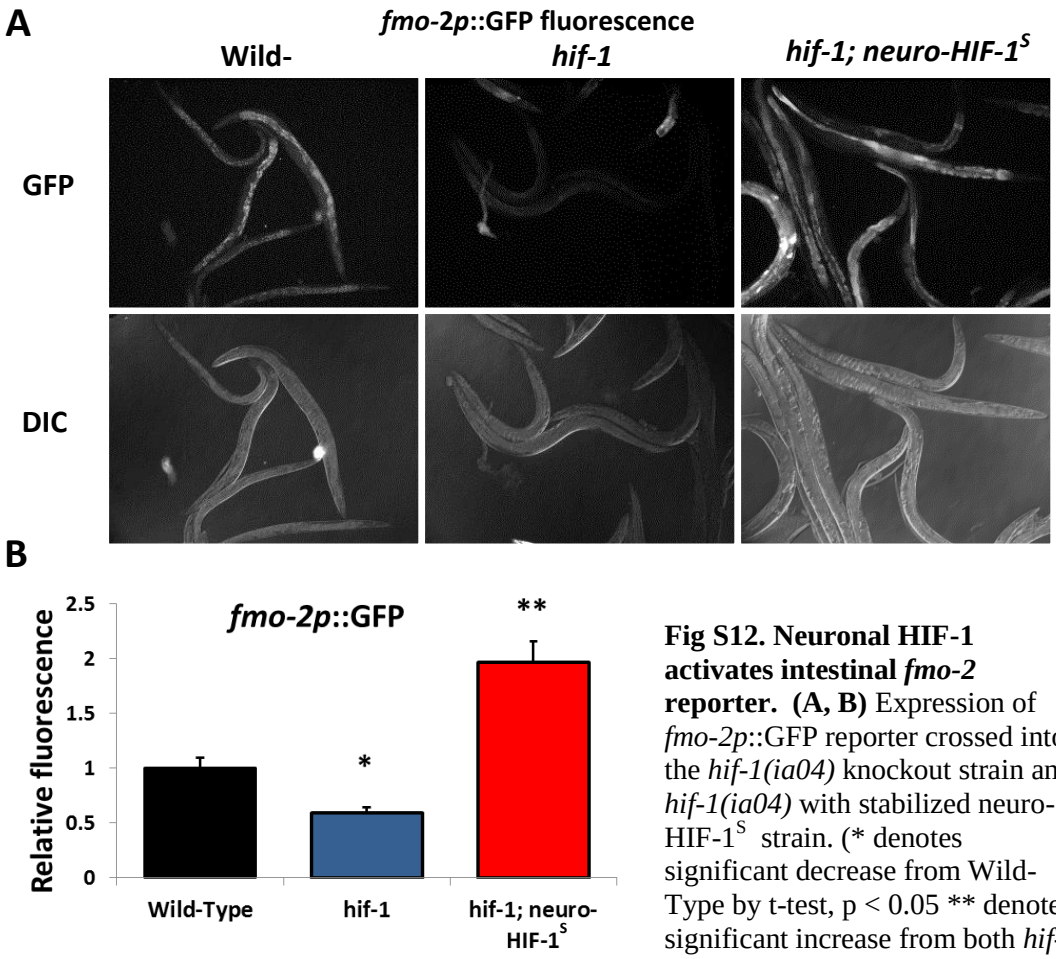


Fig S12. Neuronal HIF-1 activates intestinal *fmo-2* reporter. (A, B) Expression of *fmo-2p*::GFP reporter crossed into the *hif-1(ia04)* knockout strain and *hif-1(ia04)* with stabilized neuro-HIF-1^S strain. (* denotes significant decrease from Wild-Type by t-test, $p < 0.05$ ** denotes significant increase from both *hif-1* and Wild-Type by individual t-tests, $p < 0.05$.

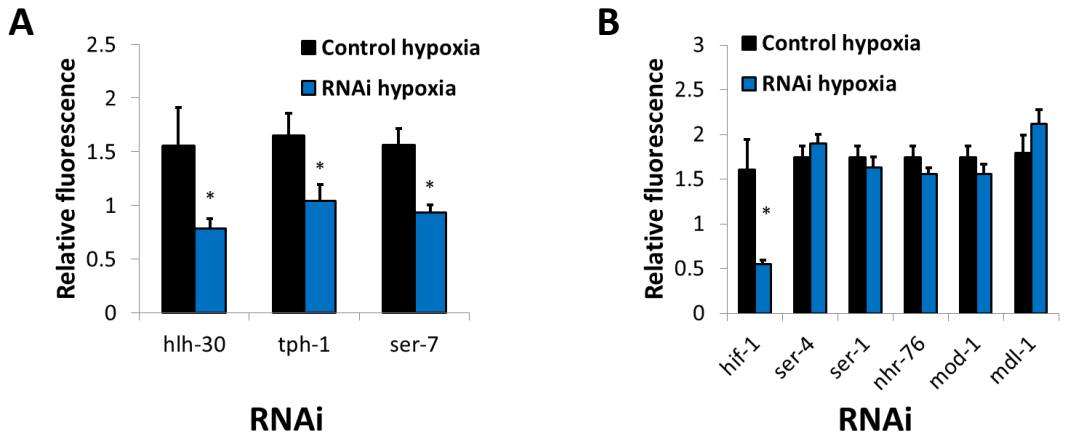


Fig S13. *Fmo-2* reporter fluorescence and lifespan analysis of hypoxic signaling. (A-B) Quantified *fmop*::GFP fluorescence of RNAi knockdown of transcription factors and signaling proteins in normoxia (~21% O_2) and hypoxia (0.1% O_2). (* denotes decrease from control hypoxia by t-test,
$p < 0.05$)

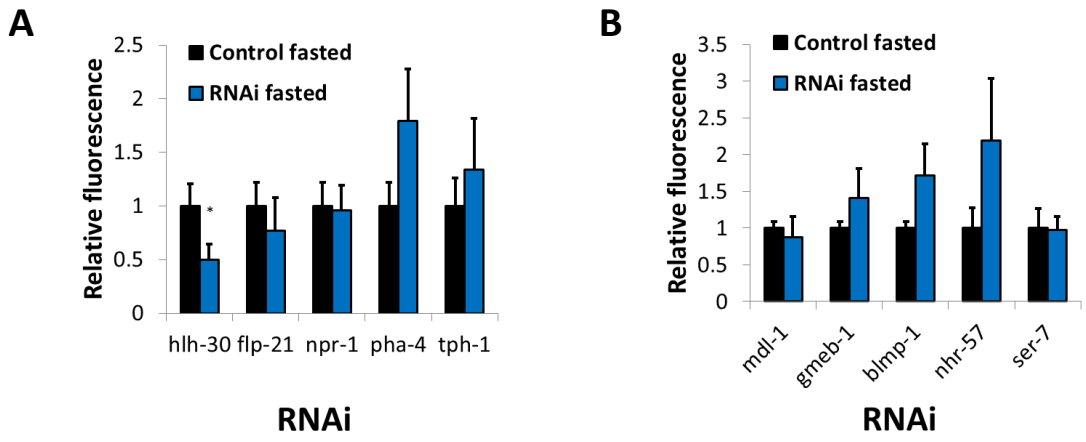


Fig S14. *Fmo-2* reporter fluorescence in fed and starved conditions. (A-B) Quantified *fmop*::GFP fluorescence of RNAi knockdown of transcription factors and signaling proteins in fed and fasted conditions, normalized to fasted control (EV) RNAi. * indicates statistical difference ($p < 0.05$) from Wild-Type in matching condition by t-test.

Table S1. RNAi clones that increase age-associated autofluorescence and their effects on lifespans of *vhl-1(ok161)* and Wild-Type worms.

	Gene/ORF	Decrease in *vhl-1* lifespan	p-value (vs *vhl-1*)	Lifespan Direction vs. N2	# of Exp.
1	inx-2/F08G12.10	No	>0.9	No	1
2	egl-9 (F22E12.4)	No	>0.9	No	3
3	Y59A8B.19	No	>0.9	No	2
4	dpf-6 (F44B9.1)	No	>0.9	No	2
5	F16G10.10	No	>0.9	No	1
6	Y44A6C.1	No	0.9	No	1
7	acdh-1 (C55B7.4)	No	0.8	No	2
8	dod-3 (C24B9.9)	No	0.85	No	1
9	clec-245 (C31G12.2)	No	0.284	No	3
10	hecd1 (C34D4.14)	No	0.77	No	1
11	Y19O10A.5	No	0.7	No	1
12	ncr-1 (F02E8.6)	No	0.36	No	1
13	F21D12.3	No	0.12	No	4
14	ZK896.4	No	0.18	No	1
15	F08G12.10	No	0.17	No	2
16	phy-2 (F35G2.4)	No	0.529	No	3
17	F59B10.4-unknown	Yes	0.07	Yes	2
18	*zyg-9* (F22B5.7)	Yes	8.4E-05	Yes	2
19	*cah-4* (R01E6.3)	Yes	9.5E-08	No	3
20	*fmo-2* (K08C7.5)	Yes	5.4E-12	No	3
21	K10H10.4- cysteine synthase	Yes	0.053	No	2
22	*rhy-1* (W07A12.7)	Yes	0.002	No	2
23	C01G6.9-cetriolin	Yes	2.6E-05	No	3
24	F28B4.3-unknown	Yes	0.005	No	5

Table S2. Lifespan Table.

Strain	Fig.	Experiment	Food	Temp.	n	median	mean	p- value
vhl-1 (ok161)		1	EV (RNAi)	20° C	183	31	28.1	N/A
vhl-1 (ok161)		1	*hif-1* (RNAi)	20° C	75	24	24.0	1.4E-07
vhl-1 (ok161)		1	*fmo-2* (RNAi)	20° C	127	26	26.6	0.00024
Wild-Type/Control		1	EV (RNAi)	20° C	189	24	24.4	N/A
Wild-Type/Control		1	*hif-1* (RNAi)	20° C	145	24	22.0	0.0006
Wild-Type/Control		1	*fmo-2* (RNAi)	20° C	184	24	22.3	0.00028
vhl-1 (ok161)	1A	2	EV (RNAi)	20° C	172	29	28.6	N/A
vhl-1 (ok161)	1A	2	*hif-1* (RNAi)	20° C	197	23	22.2	5.3E-30
vhl-1 (ok161)	1A	2	*fmo-2* (RNAi)	20° C	93	25	25.5	9.7E-06
Wild-Type/Control		2	EV (RNAi)	20° C	133	18	18.6	N/A
Wild-Type/Control		2	*hif-1* (RNAi)	20° C	203	16	17.3	0.00562
Wild-Type/Control		2	*fmo-2* (RNAi)	20° C	207	18	18.4	0.83742
vhl-1 (ok161)		3	EV (RNAi)	20° C	188	21	21.0	N/A
vhl-1 (ok161)		3	*hif-1* (RNAi)	20° C	176	21	19.9	0.00471
vhl-1 (ok161)		3	*fmo-2* (RNAi)	20° C	176	21	22.5	0.99853
Wild-Type/Control		3	EV (RNAi)	20° C	182	28	25.7	N/A
Wild-Type/Control		3	*hif-1* (RNAi)	20° C	238	23	22.8	7.2E-09
Wild-Type/Control		3	*fmo-2* (RNAi)	20° C	307	25	24.8	0.03649
Wild-Type/Control	1B	4	UV-treated OP50	20° C	119	24	23.8	N/A
FMO-2 OE ubiquitous-1	1B	4	UV-treated OP50	20° C	60	29	28.6	1.2E-09
FMO-2 OE ubiquitous-2	S3	4	UV-treated OP50	20° C	139	29	30.1	7.5E-16
Wild-Type/Control		5	UV-treated OP50	20° C	152	26	26.6	N/A
FMO-2 OE ubiquitous		5	UV-treated OP50	20° C	91	33	33.9	3E-26
fmo-2(ok2147)		5	UV-treated OP50	20° C	145	26	24.5	0.02399
Q35 (paralysis)	S5	6	EV(RNAi)	20° C	125	16	15.7	N/A
Q35; FMO-2 OE (paralysis)	S5	6	EV(RNAi)	20° C	194	18	18.7	5.8E-11
Q35 (paralysis)		7	UV-treated OP50	20° C	139	14	15.4	N/A
Q35; FMO-2 OE (paralysis)		7	UV-treated	20° C	145	21	20.3	2.1E-19

			OP50					
Q35 (paralysis)		8	UV-treated OP50	20° C	149	19	18.3	N/A
Q35: FMO-2 OE (paralysis)		8	UV-treated OP50	20° C	155	21	20.1	1.6E-5
Wild-Type/Control		9	UV-treated OP50	20° C	119	24	23.8	N/A
FMO-2 OE ubiquitous-1		9	UV-treated OP50	20° C	60	29	28.6	1.2E-09
FMO-2 OE ubiquitous-2		9	UV-treated OP50	20° C	139	29	30.1	7.5E-16
daf-16(mu86)	S6	9	UV-treated OP50	20° C	59	17	15.5	N/A
daf-16(mu86); FMO-2 OE	S6	9	UV-treated OP50	20° C	128	20	19.1	1.7E-08
hif-1(ia04)	S6	9	UV-treated OP50	20° C	83	24	22.6	N/A
hif-1(ia04) ::FMO-2 OE	S6	9	UV-treated OP50	20° C	121	30	28.9	1.1E-08
skn-1(zu67)	S6	9	UV-treated OP50	20° C	121	21	21.2	N/A
skn-1(zu67); FMO-2 OE	S6	9	UV-treated OP50	20° C	121	30	28.9	8.3E-23
Wild-Type/Control		10	UV-treated OP50	20° C	96	22	22.7	N/A
FMO-2 OE		10	UV-treated OP50	20° C	70	27	27.4	1.1E-05
daf-16(mu86)		10	UV-treated OP50	20° C	38	17	17.2	N/A
daf-16(mu86); FMO-2 OE		10	UV-treated OP50	20° C	78	20	20.2	0.0017
hif-1(ia04)		10	UV-treated OP50	20° C	70	23	23.6	N/A
hif-1(ia04); FMO-2 OE		10	UV-treated OP50	20° C	74	28	28.4	6.3E-05
skn-1(zu67)		10	UV-treated OP50	20° C	43	23	23.4	N/A
skn-1(zu67); FMO-2 OE		10	UV-treated OP50	20° C	78	26.5	26.6	0.00336
Wild-Type/Control		11	EV(RNAi)	20° C	73	23	21.0	N/A
Wild-Type/Control		11	*daf-2(RNAi)*	20° C	149	37	37.1	2.1E-33
Wild-Type/Control		11	*isp-1(RNAi)*	20° C	74	30	30.9	5.6E-20
fmo-2(ok2147)		11	EV(RNAi)	20° C	97	16	16.6	N/A
fmo-2(ok2147)		11	*daf-2(RNAi)*	20° C	131	30	31.5	1.9E-32
fmo-2(ok2147)		11	*isp-1(RNAi)*	20° C	63	34	33.3	2.9E-24

Wild-Type/Control	S7	12	EV(RNAi)	20° C	126	23	21.5	N/A
Wild-Type/Control	S7	12	*daf-2(RNAi)*	20° C	126	34	34.6	1.7E-36
Wild-Type/Control	S7	12	*isp-1(RNAi)*	20° C	80	34	33.7	9.3E-28
fmo-2(ok2147)	S7	12	EV(RNAi)	20° C	139	17	18.3	1.4E-16
fmo-2(ok2147)	S7	12	*daf-2(RNAi)*	20° C	134	30	31.8	7.8E-34
fmo-2(ok2147)	S7	12	*isp-1(RNAi)*	20° C	80	34	33.7	3.3E-26
Wild-Type/Control	2D	13	Live OP50	20° C	144	28	28.6	N/A
Wild-Type/Control	2D	13	sDR	20° C	107	37	35.7	2.1E-17
fmo-2(ok2147)	2D	13	Live OP50	20° C	155	28	27.8	0.39864
fmo-2(ok2147)	2D	13	sDR	20° C	146	30	29.6	0.0323
FMO-2 OE		13	Live OP50	20° C	109	35	34.3	2.1E-17
FMO-2 OE		13	sDR	20° C	96	42	41.0	4.7E-10
Wild-Type/Control		14	Live OP50	20° C	72	28	28.2	N/A
Wild-Type/Control		14	sDR	20° C	79	35	34.1	2.5E-8
fmo-2(ok2147)		14	Live OP50	20° C	46	28	26.1	0.006
fmo-2(ok2147)		14	sDR	20° C	53	26	28.9	0.115
FMO-2 OE		14	Live OP50	20° C	74	31	31.5	0.001
FMO-2 OE		14	sDR	20° C	69	35	34.8	0.003
Wild-Type/Control		15	UV-treated OP50	20° C	124	29	27.8	N/A
fmo-2(ok2147)		15	UV-treated OP50	20° C	144	25	25.3	4.3E-5
vhl-1 (ok161); fmo-2(ok2147)		15	UV-treated OP50	20° C	82	25	24.5	
Wild-Type/Control	S7	16	UV-treated OP50	20° C	148	29	30.0	N/A
fmo-2(ok2147)	S7	16	UV-treated OP50	20° C	135	29	28.0	0.01
vhl-1 (ok161)	S7	16	UV-treated OP50	20° C	116	34	32.4	1.6E-12
vhl-1 (ok161); fmo-2(ok2147)	S7	16	UV-treated OP50	20° C	273	27	26.9	1.0E-13
Wild-Type/Control	S8	17	UV-treated OP50	25° C	137	17	17.9	N/A
FMO-2 OE Ub.-1	S8	17	UV-treated OP50	25° C	141	21	21.0	2E-16
FMO-2 OE Ub.-2		17	UV-treated OP50	25° C	143	21	20.7	1.2E-15
FMO-2 OE neurons	S8	17	UV-treated OP50	25° C	153	17	17.9	0.64447
Wild-Type/Control		19	UV-treated OP50	25° C	215	17	17.9	N/A

FMO-2 OE Ub.-1		19	UV-treated OP50	25° C	143	21	20.7	5.2E-17
FMO-2 OE Ub.-2		19	UV-treated OP50	25° C	138	21	19.9	1.2E-9
FMO-2 OE neurons		19	UV-treated OP50	25° C	153	17	17.9	0.599
Wild-Type/Control	3A	20	UV-treated OP50	25° C	126	21	20.9	N/A
FMO-2 OE intestine-1	3A	20	UV-treated OP50	25° C	133	26	24.9	3.6E-13
FMO-2 OE intestine-2	3A	20	UV-treated OP50	25° C	62	26	26.5	4.8E-15
Wild-Type/Control		21	UV-treated OP50	25° C	105	20	20.4	N/A
FMO-2 OE intestine-1		21	UV-treated OP50	25° C	42	25	24.4	1.9E-05
FMO-2 OE intestine-2		21	UV-treated OP50	25° C	58	26	24.8	9.5E-07
Wild-Type/Control	S8	22	UV-treated OP50	20° C	110	22	22.3	N/A
HIF-1^{S} hypodermis (pdpy-7)	S8	22	UV-treated OP50	20° C	88	22	22.8	0.24479
HIF-1^{S} muscle (punc-54)	S8	22	UV-treated OP50	20° C	203	22	22.7	0.25862
HIF-1^{S} intestine (pvha-6) line 1	S8	22	UV-treated OP50	20° C	106	23	23.3	0.04489
HIF-1^{S} serotonergic (ptph-1)		22	UV-treated OP50	20° C	174	27	27.4	5.9E-20
Wild-Type/Control		23	UV-treated OP50	20° C	96	22	22.7	N/A
HIF-1^{S} intestine (pvha-6) line 1		23	UV-treated OP50	20° C	44	22	22.8	0.94096
HIF-1^{S} intestine (pvha-6) line 2		23	UV-treated OP50	20° C	40	24	23.8	0.1788
Wild-Type/Control		24	UV-treated OP50	20° C	119	24	23.8	N/A
HIF-1^{S} intestine (pvha-6) line 1		24	UV-treated OP50	20° C	50	24	23.8	0.70606
HIF-1^{S} intestine (pvha-6) line 2		24	UV-treated OP50	20° C	84	23	23.2	0.48024
Wild-Type/Control		25	UV-treated OP50	20° C	188	22	21.4	N/A
HIF-1^{S} hypodermis (pdpy-7)		25	UV-treated OP50	20° C	124	22	22.6	0.05352

HIF-1^{S} muscle (punc-54)		25	UV-treated OP50	20° C	196	22	21.8	0.75311
Wild-Type/Control	3B	26	UV-treated OP50	20° C	96	24	25.2	N/A
HIF-1^{S} neurons (punc-14)	3B	26	UV-treated OP50	20° C	79	31	29.9	1.5E-06
HIF-1^{S} ubiquitous (phif-1)	3B	26	UV-treated OP50	20° C	106	34	31.1	3.1E-08
HIF-1^{S} serotonergic (ptph-1)		26	UV-treated OP50	20° C	74	29	28.0	0.00421
Wild-Type/Control		27	UV-treated OP50	20° C	186	20	19.1	N/A
HIF-1^{S} neurons (punc-14)		27	UV-treated OP50	20° C	74	23	21.9	5.6E-07
HIF-1^{S} ubiquitous (phif-1)		27	UV-treated OP50	20° C	71	22	21.4	0.00018
HIF-1^{S} hypodermis (pdpy-7)		27	UV-treated OP50	20° C	63	18	16.5	2.6E-05
HIF-1^{S} muscle (punc-54)		27	UV-treated OP50	20° C	53	20	18.9	0.70935
Wild-Type/Control	4D	28	UV-treated OP50	20° C	211	23	23.8	N/A
HIF-1^{S} neurons (punc-14)	4D	28	UV-treated OP50	20° C	121	26	24.6	0.03285
HIF-1^{S} serotonergic (ptph-1)	4D	28	UV-treated OP50	20° C	105	26	25.1	0.00014
hif-1(ia04); HIF-1^{S} neurons (punc-14) normoxia		29	UV-treated OP50	20° C	115	34	31.0	N/A
hif-1(ia04); HIF-1^{S} neurons (punc-14) hypoxia		29	UV-treated OP50	20° C	85	36	34.9	3.1E-06
hif-1(ia04); HIF-1^{S} neurons (punc-14) normoxia	S11	30	UV-treated OP50	20° C	214	24	24.4	N/A
hif-1(ia04); HIF-1^{S} neurons (punc-14) hypoxia	S11	30	UV-treated OP50	20° C	202	28	26.1	1.7E-07
Wild-Type/Control	3D	31	EV (RNAi)	20° C	258	19	20.5	N/A
hif-1(ia04)	3D	31	EV (RNAi)	20° C	242	19	18.8	
hif-1(ia04); HIF-1^{S} neurons (punc-14)	3D	31	EV (RNAi)	20° C	214	26	25.1	9.3E-27/ 6.5E-46
Wild-Type/Control		31	*fmo-2*(RNAi)	20° C	264	19	19.6	N/A
hif-1(ia04)		31	*fmo-2*(RNAi)	20° C	219	17	16.7	0.17677
hif-1(ia04); HIF-1^{S} neurons (punc-14)		31	*fmo-2*(RNAi)	20° C	238	17	17.5	5.2E-37

hif-1(ia04)	3F	32	EV (RNAi)	20° C	123	19	18.8	N/A
hif-1(ia04); HIF-1^{S} neurons (punc-14)	3F	32	EV (RNAi)	20° C	101	21	23.0	1.1E-13
hif-1(ia04)	3F	32	*fmo-2*(RNAi)	20° C	120	17	16.3	0.41089
hif-1(ia04); HIF-1^{S} neurons (punc-14)	3F	32	*fmo-2*(RNAi)	20° C	132	17	18.0	1E-07
hif-1(ia04)		33	EV (RNAi)	20° C	115	22	23.1	N/A
hif-1(ia04); HIF-1^{S} neurons (punc-14)		33	EV (RNAi)	20° C	122	28	27.1	1.5E-07
Wild-Type/Control		34	EV(RNAi)	20° C	258	17	18.5	N/A
Wild-Type/Control		34	*fmo-2*(RNAi)	20° C	264	19	19.6	N/A
hif-1(ia04)		34	EV(RNAi)	20° C	242	17	16.8	N/A
hif-1(ia04)		34	*fmo-2*(RNAi)	20° C	219	17	16.7	0.17677
hif-1(ia04); HIF-1^{S} neurons (punc-14)		34	EV(RNAi)	20° C	214	24	23.1	6.5E-46
hif-1(ia04); HIF-1^{S} neurons (punc-14)		34	*fmo-2*(RNAi)	20° C	238	17	17.5	5.2E-37
hif-1(ia04)		35	EV(RNAi)	20° C	123	17	16.8	N/A
hif-1(ia04)		35	*fmo-2*(RNAi)	20° C	120	17	16.3	0.41089
hif-1(ia04); HIF-1^{S} neurons (punc-14)		35	EV(RNAi)	20° C	101	19	21.0	1.1E-13
hif-1(ia04); HIF-1^{S} neurons (punc-14)		35	*fmo-2*(RNAi)	20° C	132	17	18.0	1E-07
Wild-Type/Control		36	EV(RNAi)	20° C	122	26	24.7	N/A
vhl-1 (ok161)		36	EV(RNAi)	20° C	195	28	27.9	3.48E-11
hif-1(ia04); HIF-1^{S} neurons (punc-14)		36	EV(RNAi)	20° C	142	27	26.9	3.28E-05
Wild-Type/Control		36	*ser-7*(RNAi)	20° C	87	21	21.1	4.02E-10
vhl-1 (ok161)		36	*ser-7*(RNAi)	20° C	100	20	19.1	2.69E-04
hif-1(ia04); HIF-1^{S} neurons (punc-14)		36	*ser-7*(RNAi)	20° C	123	21	21.2	0.525
Wild-Type/Control		36	*tph-1*(RNAi)	20° C	216	21	21.5	2.91E-12
vhl-1 (ok161)		36	*tph-1*(RNAi)	20° C	148	22.5	22.7	5.05E-05
hif-1(ia04); HIF-1^{S} neurons (punc-14)		36	*tph-1*(RNAi)	20° C	170	22.5	22.7	0.00264
Wild-Type/Control		36	*hlh-30*(RNAi)	20° C	81	20	18.4	8.93E-21
vhl-1 (ok161)		36	*hlh-30*(RNAi)	20° C	108	21	21.6	2.69E-12
hif-1(ia04); HIF-1^{S} neurons		36	*hlh-*	20° C	114	21	20.8	1.00E-06

(punc-14)			*30*(RNAi)					
Wild-Type/Control		37	EV(RNAi)	20° C	141	23	23.7	N/A
hif-1(ia04); HIF-1^{S} neurons (punc-14)		37	EV(RNAi)	20° C	72	27	27.2	1.96E-09
Wild-Type/Control		37	*ser-7*(RNAi)	20° C	145	23	22.8	0.165
vhl-1 (ok161)		37	*ser-7*(RNAi)	20° C	132	21	21.7	0.0127
hif-1(ia04); HIF-1^{S} neurons (punc-14)		37	*ser-7*(RNAi)	20° C	150	23	24.1	0.003
Wild-Type/Control		37	*tph-1*(RNAi)	20° C	138	21	21.7	1.12E-04
vhl-1 (ok161)		37	*tph-1*(RNAi)	20° C	110	23	23.7	4.45E-04
hif-1(ia04); HIF-1^{S} neurons (punc-14)		37	*tph-1*(RNAi)	20° C	122	23	23.9	2.22E-06
Wild-Type/Control		37	*hlh-30*(RNAi)	20° C	136	19	19.7	4.84E-16
vhl-1 (ok161)		37	*hlh-30*(RNAi)	20° C	53	23	23.2	3.75E-07
hif-1(ia04); HIF-1^{S} neurons (punc-14)		37	*hlh-30*(RNAi)	20° C	97	23	23.4	1.49E-13
Wild-Type/Control		38	EV(RNAi)	20° C	154	20	21.2	N/A
vhl-1 (ok161)		38	EV(RNAi)	20° C	150	25	26.2	1.79E-28
hif-1(ia04); HIF-1^{S} neurons (punc-14)		38	EV(RNAi)	20° C	155	32	30.4	1.51E-40
Wild-Type/Control		38	*ser-7*(RNAi)	20° C	153	25	23.4	3.05E-07
vhl-1 (ok161)		38	*ser-7*(RNAi)	20° C	163	20	21.0	8.06E-09
hif-1(ia04); HIF-1^{S} neurons (punc-14)		38	*ser-7*(RNAi)	20° C	152	22	23.1	0.313
Wild-Type/Control		38	*tph-1*(RNAi)	20° C	81	25	24.2	1.94E-09
vhl-1 (ok161)		38	*tph-1*(RNAi)	20° C	154	22	23.4	0.0437
hif-1(ia04); HIF-1^{S} neurons (punc-14)		38	*tph-1*(RNAi)	20° C	141	27	26.5	9.18E-06
Wild-Type/Control		38	*hlh-30*(RNAi)	20° C	68	20	19.2	2.58E-05
vhl-1 (ok161)		38	*hlh-30*(RNAi)	20° C	152	27	26.0	3.60E-23
hif-1(ia04); HIF-1^{S} neurons (punc-14)		38	*hlh-30*(RNAi)	20° C	138	22	22.4	1.39E-09

Table S3. List of strains.

Strain name	Genotype	Outcrossed	Source
N2 Wild-Type Control	**Wild-type Bristol isolate**	**N/A**	**J. Thomas**
ZG31	***hif-1 (ia4)***	**x 9**	**CGC**
CB5602	***vhl-1 (ok161)***	**x 5+**	**CGC**
CB1370	***daf-16 (mu86)***	**x 11**	**CGC**
VC1772	***skn-1 (ok2315)***	**X4**	**CGC**
OH7511	**otIs197[unc-14p::hif-1(P621A) + ttx-3::RFP]. *HIF-1^S* neurons**	**X4**	**CGC**
KAE13	***pvha-6::*HIF-1(P621A) + GFP (*HIF-1^S* intestine) line 1**	**X4**	**Injection**
KAE14	***pvha-6::*HIF-1(P621A) + GFP (*HIF-1^S* intestine) line 2**	**X4**	**Injection**
OH7251	**zdIs13 [tph-1p::GFP] IV. otEx3163[unc-120p::hif-1(p621A) + ttx-3::RFP]. (*HIF-1^S muscle)***	**X4**	**CGC**
OH7260	**zdIs13 [tph-1p::GFP] IV. otEx3168[hif-1p::hif-1(p621A) + ttx-3::RFP]. (*HIF-1^S phif-1)***	**X4**	**CGC**
OH7235	**zdIs13 [tph-1p::GFP] IV. otEx3154[dpy-7p::hif-1(p621A) + ttx-3::RFP] *(HIF-1^S hypodermis)***	**X4**	**CGC**
OH8014	**zdIs13 [tph-1p::GFP] IV. otEx3567[tph-1p::hif-1(p621A) + ttx-3::RFP] *(HIF-1^S serotonergic neurons)***	**X4**	**CGC**
KAE9	***p-eft-3::FMO-2 +GFP***	**X6**	**Injection**
KAE10	***p-eft-3::FMO-2 +GFP***	**X6**	**Injection**
VC1668	***fmo-2(ok2147)***	**X4**	**CGC**
	***hif-1(ia4);* otIs197[unc-14p::hif-1(P621A) + ttx-3::RFP]. *HIF-1^S* neurons**	**X6**	**Dana Miller**
AM140	***Q35* [unc54p::Q35::YFP] polyglutamine model**	**X5**	**CGC**
	***p-eft-3::FMO-2 +GFP; Q35* [unc54p::Q35::YFP]**	**N/A**	**Cross**
KAE8	***prab-3::FMO-2 OE neurons +GFP***	**X4**	**Injection**

KAE11	***pvha-6::FMO-2 OE intestine +GFP***	**X4**	**Injection**
KAE12	***pvha-6::FMO-2 OE intestine +GFP***	**X4**	**Injection**
	vhl-1 (ok161); fmo-2(ok2147)	**N/A**	**Cross**
	p-eft-3::FMO-2 +GFP; hif-1(ia4)	**N/A**	**Cross**
	p-eft-3::FMO-2 +GFP; daf-16(mu86)	**N/A**	**Cross**
	p-eft-3::FMO-2 +GFP; skn-1(zu67)	**N/A**	**Cross**