Sciencexpress Reports

Cell nonautonomous activation of flavin-containing monooxygenase promotes longevity and health span

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Stabilization of the hypoxia-inducible factor 1 (HIF-1) increases life span and health span in nematodes through an unknown mechanism. We report that neuronal stabilization of HIF-1 mediates these effects in *C. elegans* through a cell nonautonomous signal to the intestine, which results in activation of the xenobiotic detoxification enzyme flavin-containing monooxygenase-2 (FMO-2). This prolongevity signal requires the serotonin biosynthetic enzyme TPH-1 in neurons and the serotonin receptor SER-7 in the intestine. Intestinal FMO-2 is also activated by dietary restriction (DR) and is necessary for DR-mediated life-span extension, which suggests that this enzyme represents a point of convergence for two distinct longevity pathways. FMOs are conserved in eukaryotes and induced by multiple life span–extending interventions in mice, which suggests that these enzymes may play a critical role in promoting health and longevity across phyla.

In nematodes, as in mammals, hypoxia-inducible factor (HIF) proteins have a central role in responding to changes in environmental oxygen (*1*). HIF proteins are transcription factors regulated by oxygen-dependent proteasomal degradation and are stabilized under low-oxygen conditions to modulate expression of hundreds of target genes to produce the hypoxic response (*2*). In mammals, constitutive stabilization of HIF through loss of the E3 ubiquitin ligase von Hippel-Lindau (VHL) protein leads to a disease characterized by angiomas and renal carcinomas (*3*), whereas in *Caenorhabditis elegans,* loss of the VHL homolog gene, *vhl-1*, improves proteostasis and increases life span (*4*, *5*). This difference likely reflects the fact that somatic cells of adult *C. elegans* are postmitotic, with little or no potential for tumor development, and raises the possibility that specific targets of HIF-1 that promote healthy aging in *C. elegans* may function similarly in mammals.

To understand how hypoxic signaling slows aging in worms, we identified genes downstream of HIF-1 that promote longevity and health span. We took advantage of the large reduction in age-associated autofluorescence observed in animals in which *vhl-1* is not expressed (*vhl-1* knockout) (*4*) to screen for known HIF-1–target genes required for this phenotype (fig. S1). Our screen identified 24 RNA interference (RNAi) clones that substantially increased autofluorescence in *vhl-1* animals, eight of which also reduced the long life span of *vhl-1* mutant animals (table S1 and fig. S2). Six of these RNAi clones had no effect on the life span of the wild-type reference strain (N2 Bristol), which indicated that they may function specifically to enhance longevity when HIF-1 is stabilized.

Having established a set of HIF-1–target genes necessary for the full longevity effect of activation of HIF-1, we tested whether any of these genes were sufficient to enhance longevity and health span. We used the Mos1 transposase–mediated single-copy insertion system (*6*) to overexpress a single copy of each of the six genes from the ubiquitous *eft-3* promoter (fig. S3). Depletion of the xenobiotic detoxification enzyme flavin-containing monooxygenase-2 (*fmo*-2) by RNAi showed it to be required for full life-span extension in *vhl-1* knockout animals (Fig. 1A). FMO-2 was also sufficient to extend life span on its own (Fig. 1B and fig. S3). Ubiquitous FMO-2 overexpression (FMO-2 OE) also improved multiple measures of health span, including enhanced maintenance of motility (measured by the ability to swim, or thrash, in liquid), pharyngeal pumping, and decreased age-associated autofluorescence (Fig. 1, C and D and fig. S4). FMO-2 OE animals did not show the decreased brood size or delay in development observed in animals lacking *vhl-1*; thus, these negative consequences of HIF-1 activation likely result from other HIF-1 targets and are separable from life-span and health-span extension (fig. S4).

Maintaining proteostasis is critical for healthy aging (*7*), and both dietary restriction (DR) and stabilization of HIF-1 enhance proteostasis in *C. elegans* (*4*, *8*). To determine whether FMO-2 enhances proteostasis, we examined the effect of FMO-2 OE on resistance to proteotoxic stress. The most notable effect of FMO-2 OE was resistance to proteotoxic stress within the endoplasmic reticulum (ER), as evidenced by reduced growth inhibition in response to

treatment of animals with tunicamycin (up to 10 μg/ml) and reduced mortality of animals treated with dithiothreitol (DTT, 7 mM) (Fig. 2, A and B). FMO-2 OE animals were also resistant to general proteotoxic stress induced by high temperature (Fig. 2C), reductive proteotoxic stress from 2-carboxyethyl phosphine hydrochloride treatment, and transgenic expression of an aggregation-prone polyglutamine peptide fused to yellow fluorescent protein (Q35::YFP) (*9*) (fig. S5).

We examined the interaction between *fmo-2* and other important longevity pathways. Life-span extension from stabilization of HIF-1 is genetically distinct from that regulated by both the insulin-like signaling pathway and DR (*4*, *5*, *10*). Life extension in FMO-2 OE animals appears not to require the rest of the hypoxic-response pathway, insulin-like signaling, or the phase II detoxification pathway, because it was not lost in *hif-1*, *daf-16*, or *skn-1* mutants, respectively (fig. S6). Thus, FMO-2 does not act through these transcription factors to promote longevity. Similarly, *fmo-2* appears not to be necessary for life-span extension produced by known aging-related pathways, because loss of *fmo-2* alone had only a modest effect on life span and did not prevent life-span extension in response to reduced insulin-like signaling caused by *daf-2* RNAi or inhibition of mitochondrial respiration caused by *isp-1* RNAi (fig. S7). However, *fmo-2* was required for life-span extension induced by DR, when the technique of periodic feeding and fasting, or sDR, is used (*11*) (Fig. 2D). To further explore the possibility that FMO-2 acts in both the hypoxic response and DR, we confirmed that *fmo-2* is transcriptionally induced by food deprivation by monitoring a reporter for *fmo-2* transcription fused with green fluorescent protein (*fmo-2p*::GFP) (Fig. 2, E and F). Unlike that caused by hypoxia (*12*), induction of *fmo-2* in response to fasting was not dependent upon HIF-1 (Fig. 2, E and F). This is consistent with our observation that life-span extension from DR does not require *hif-1* (*4*) and raises the possibility that DR and the hypoxic response converge on FMO-2 to promote longevity through distinct signal transduction pathways.

The simplest way HIF-1 might increase *fmo-2* expression is to bind the *fmo-2* promoter directly and promote transcription. Previous reports, and our results with transcriptional reporters, both indicate that FMO-2 is expressed predominantly in the intestine (*13*). In agreement with this, overexpression of FMO-2 under an intestinal promoter was sufficient to promote longevity (Fig. 3A). To test whether HIF-1 also acts in the intestine to promote longevity, we used transgenic nematodes with a nondegradable HIF-1 variant (*14*), referred to hereafter as HIF-1^S. Intestinal HIF-1^S had no effect on longevity (fig. S8), whereas neuronal HIF-1^S was sufficient to increase life span (Fig. 3B). Expressing HIF-1^S in neurons was also sufficient to rescue additional defects in *hif-1* knockout animals, including failure to develop in hypoxia (0.5% oxygen) (Fig. 3C and fig. S9), loss of vulval integrity during aging (fig. S10), and life-span extension from hypoxia during adulthood (fig. S11) (*15*, *16*). Neuronal HIF-1^S in animals lacking HIF-1 in other tissues was also sufficient to extend life span (Fig. 3D), which indicated that stabilization of HIF-1 in neurons alone is sufficient to extend life span in *C. elegans*, even without HIF-1 in other cell types.

Neuronal overexpression of *fmo-2* had no detectable effect on longevity (fig. S8). Thus, HIF-1 and FMO-2 appear to promote longevity and health span by acting in distinct tissues: HIF-1 in neurons and FMO-2 in intestine. Consistent with this model, transcription of *fmo-2* was significantly induced in the intestine by stabilization of HIF-1 in neurons in a background where *hif-1* is knocked out in all other tissues [*hif-1(ia04)*; neuro-HIF-1^S] as measured by both quantitative reverse transcription polymerase chain reaction (QRT-PCR) and by fluorescence in a reporter strain (Fig. 3E and fig. S12). In agreement with these results, depletion of *fmo-2* with RNAi prevented life-span extension in this strain (Fig. 3F), despite the inefficiency of RNAi in neurons (*17*), which indicated that neuronal HIF-1 signaling to intestinal FMO-2 is probably necessary for the longevity benefit.

Having established a connection between neuronal HIF-1 signaling and intestinal FMO-2 activation, we explored potential signal transduction pathways by depleting signaling components and transcription factors chosen on the basis of previous reports and *in silico* promoter analysis (*18*, *19*). Although most of the factors examined had no effect (fig. S13), the serotonergic signaling pathway was both necessary and sufficient for the cell nonautonomous effect of HIF-1 signaling in neurons on expression of FMO-2 in intestine and subsequent longevity benefit of FMO-2. The 5-hydroxytryptamine$_7$ receptor *ser-7* and the rate-limiting enzyme in serotonin production, *tph-1,* were both required for the activation of FMO-2 in hypoxia and the longevity benefit from neuronal HIF-1^S or *vhl-1* mutation (Fig. 4, A to C, and fig. S14). In agreement with this, HIF-1^S expressed under the serotonergic *tph-1* promoter was sufficient to improve longevity to an extent comparable to that of pan-neuronal expression (Fig. 4D). A transcription factor with predicted binding to the *fmo-2* promoter, HLH-30, was necessary for either hypoxia or starvation to fully induce transcription of an FMO-2 reporter in the intestine (Fig. 4E). HLH-30 is necessary for life-span extension by DR (*20*), and our results indicate that it is also required to achieve maximal life-span extension from HIF-1 stabilization (Fig. 4F), although expression of HIF-1^S or deletion of *vhl-1* still partially increase life span in animals depleted of *hlh-30*.

Our results support a model in which the flavin-containing monooxygenase FMO-2 functions in the intestine to increase life span, improve health span, and enhance proteostasis in animals undergoing the hypoxic response or DR.

Further, intestinal *fmo-2* is regulated cell nonautonomously through serotonergic signaling originating in neurons, and subsequent activation of the transcription factor HLH-30 in the intestine (Fig. 4G). FMO-2 is thus an enzyme both necessary and sufficient for a majority of the beneficial effects of either of these longevity pathways. The FMO-2 substrates important for healthy aging in *C. elegans* remain unknown. It will also be of interest to directly assess whether FMOs may function in mammalian aging. There are five mammalian FMO proteins (FMO1 to 5) (*21*), similar to the five *C. elegans* FMOs (*13*), and all of these proteins came from a single ancestral FMO (*22*). In mammals, there is relatively limited information on the specific functions of each FMO beyond tissue-specific expression patterns and the role of FMO3 in a single human disease, fish-odor syndrome (*23*). Mammalian FMOs also have a major role in regulating cholesterol and fat metabolism (*24*, *25*). Abundance of FMO proteins is increased in the tissues, particularly liver, of several long-lived mouse models including Snell dwarf mice, Ames dwarf mice, growth-hormone receptor knockout mice, Little mice, dietary-restricted mice, and rapamycin-fed mice (*26*). Indeed, FMO3 mRNA is the most consistently induced mRNA under DR in mouse liver (*27*). Taken with the data presented here, these observations raise the possibility that activation of FMOs may be a conserved mechanism for enhancing protein homeostasis, improving health span, and extending life span and that appropriate activation of FMOs might promote healthy aging in mammals and people.

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ACKNOWLEDGMENTS

We thank C. Dolphin for the vector to make the *fmo-2p*::GFP reporter strains and A. Mendenhall for advice and help with injecting worms. Strains were provided by the *Caenorhabditis* Genetics Center. This work was supported by NIH grant R01AG038518 and an award from the Samsung Advanced Institute of Technology, Samsung Electronics Co., to M.K. and NIH grant K99AG045200 to S.F.L. S.F.L. and F.J.R. were supported by NIH Training Grant T32AG000057. S.F.L. was also supported by an American Federation for Aging Research Postdoctoral Fellowship. Additional support was provided by the University of Washington Healthy Aging and Longevity Research Institute, the University of Washington Nathan Shock Center of Excellence in the Basic Biology of Aging (NIH grant P30AG013280), and an award to M.K. from the M. J. Murdock Charitable Trust. D.L.M. is an Ellison Medical Foundation New Scholar in Aging and receives support from National Institute on Aging, NIH, R00AGA0033050. Additional data are available in the supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S14
Tables S1 to S3
References (*28*–*33*)

30 June 2015; accepted 03 November 2015
Published online 19 November 2015
10.1126/science.aac9257

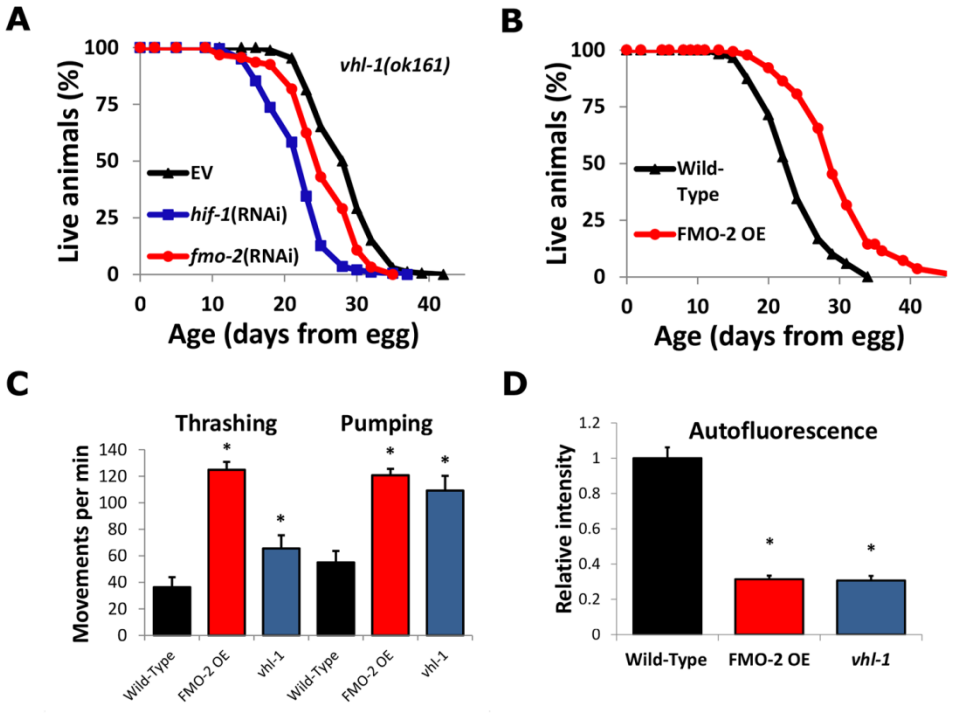

Fig. 1. A screen for age-associated autofluorescence identifies FMO-2 as a modulator of longevity and health span in the hypoxic response pathway. (**A**) Life spans of *vhl-1(ok161)* animals on empty vector (EV), *fmo-2* RNAi, or *hif-1* RNAi. (**B**) Life spans of wild-type worms and worms overexpressing FMO-2 ubiquitously (*eft-3* promoter, FMO-2 OE). (**C** and **D**) Thrashing, pumping, and autofluorescence measurements of wild-type, FMO-2 OE worms, and *vhl-1(ok161)* mutant worms during adulthood (day 10, 13, and 5, respectively). Statistically different (*$P < 0.05$) from wild type by individual *t* test for each strain. Error bars represent SEM; $N \geq 3$ for all experiments.

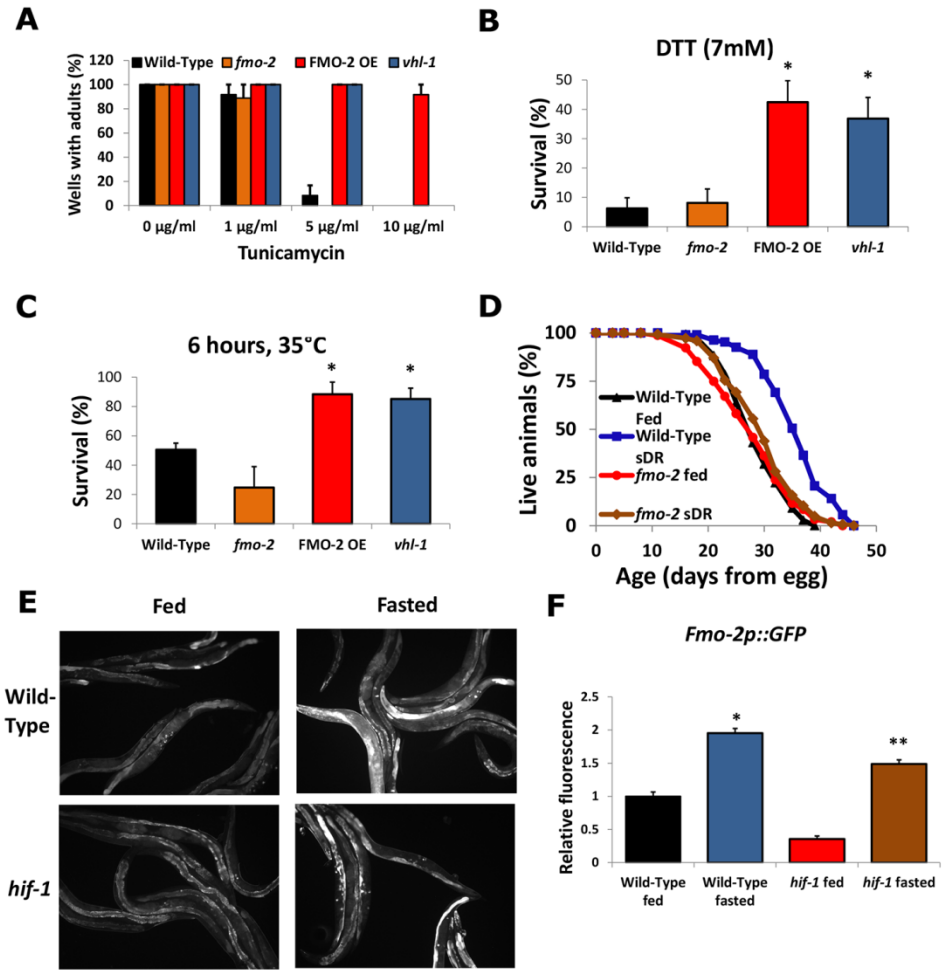

Fig. 2. FMO-2 modulates proteostasis and longevity downstream of HIF-1 and DR. (A to C) Control, *fmo-2(ok2147)*, *vhl-1(ok161)*, and FMO-2 OE resistance to tunicamycin (growth from egg), dithiothreitol [survival at the fourth larval stage (L4)], and heat (survival at L4). (D) Wild-type and *fmo-2(ok2147)* life spans on periodic DR (sDR). (E) *fmo-2p*::GFP reporter worms in fed and fasted conditions. (F) Quantitative measurements of *fmo-2* fluorescence shown in (E). Statistically different (*$P < 0.05$) from wild type (**$P < 0.05$) from *hif-1* by individual *t* test for each strain. Error bars represent SEM; $N \geq 3$ for all experiments.

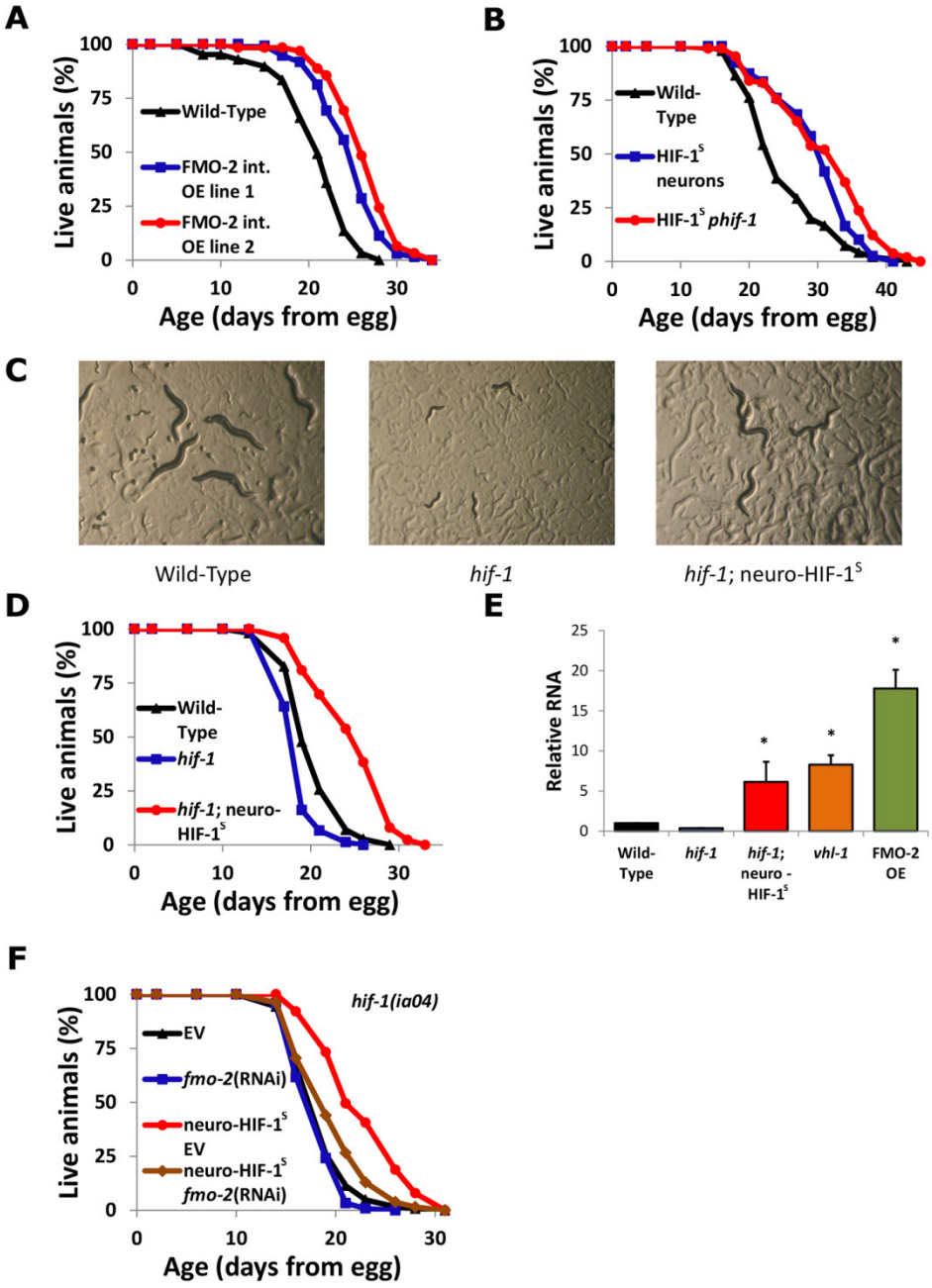

Fig. 3. Neuronal HIF-1 activates intestinal *fmo-2* to increase longevity. (**A**) Life spans of worms overexpressing FMO-2 under an intestinal *(vha-6p)* promoter. (**B**) Life spans of control worms and worms expressing HIF-1^S under neuronal (*unc-14p*) and ubiquitous (*hif-1p*) promoters. (**C**) Growth in hypoxia (0.5% oxygen) of wild-type, *hif-1(ia04)*, and *hif-1(ia04)*::neuro-HIF-1^S worms after 6 days from egg. (**D**) Life spans of control, *hif-1(ia04)*, and *hif-1(ia04)* worms with stabilized neuronal HIF-1. (**E**) QPCR measurement of *fmo-2* transcript in multiple strains. (**F**) Life spans of *hif-1(ia04)* and *hif-1(ia04)* worms with stabilized neuro-HIF-1^S in control (EV) and *fmo-2* RNAi. Statistically different (*$P < 0.05$) from wild type or (**$P < 0.05$) from *hif-1* by individual *t* test for each strain. Error bars represent SEM, $N \geq 3$ for all experiments.

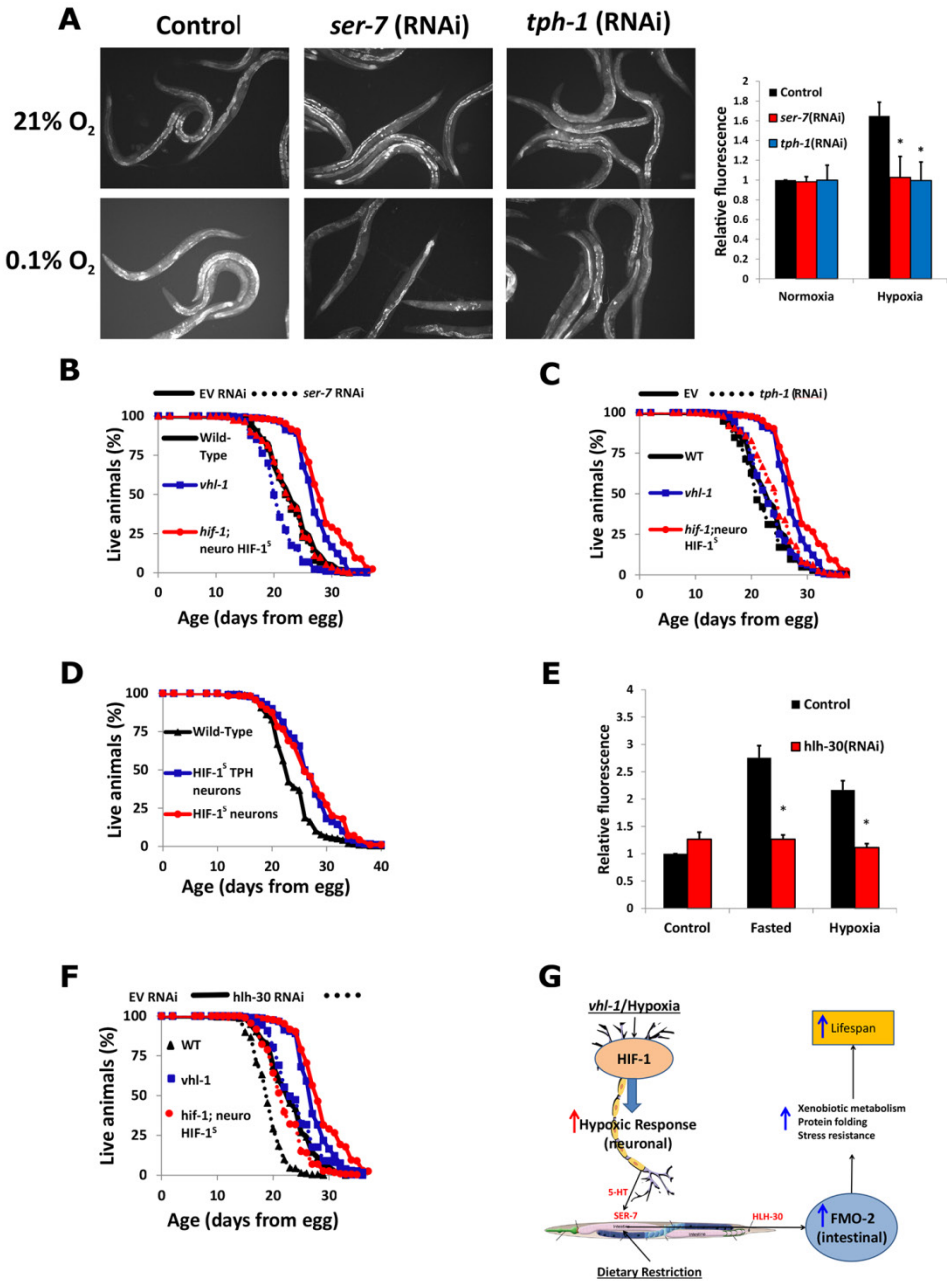

Fig. 4. The signaling pathway from neuronal HIF-1 to intestinal FMO-2 involves serotonin and HLH-30. (**A**) Fluorescence images and quantification of *fmo-2p*::GFP reporter worms in normoxia (~21% O_2) and hypoxia (0.1% O_2) on control, *ser-7*, and *tph-1*RNAi. (**B** and **C**) Life spans of wild-type, *vhl-1(ok161)* mutant, and *hif-1(ia04)* with stabilized neuro-HIF-1^S worms on control (EV, solid lines), *ser-7* RNAi (B, dashed lines) and *tph-1* RNAi (C, dashed lines). (**D**) Life spans of worms expressing HIF-1^S under the pan-neuronal (*unc-54p*) and serotonergic (*tph-1p*) promoters. (**E**) Expression of *fmo-2p*::GFP reporter in fed, fasted, and hypoxic conditions under control and *hlh-30* RNAi. (**F**) Life spans of wild-type, *vhl-1(ok161)* mutant, and *hif-1(ia04)* with stabilized neuro-HIF-1^S worms on control (EV, solid lines) and *hlh-30* RNAi (dashed lines) (**G**) Model of hypoxic response and DR converging on intestinal FMO-2. In graphs, statistical difference (*$P < 0.05$) from wild type by individual *t* test for each strain. Error bars represent SEM, $N \geq 3$ for all experiments.