Supplementary Materials for

Fmo induction as a tool to screen for pro-longevity drugs

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This PDF file includes:

Figs. S1 to S5

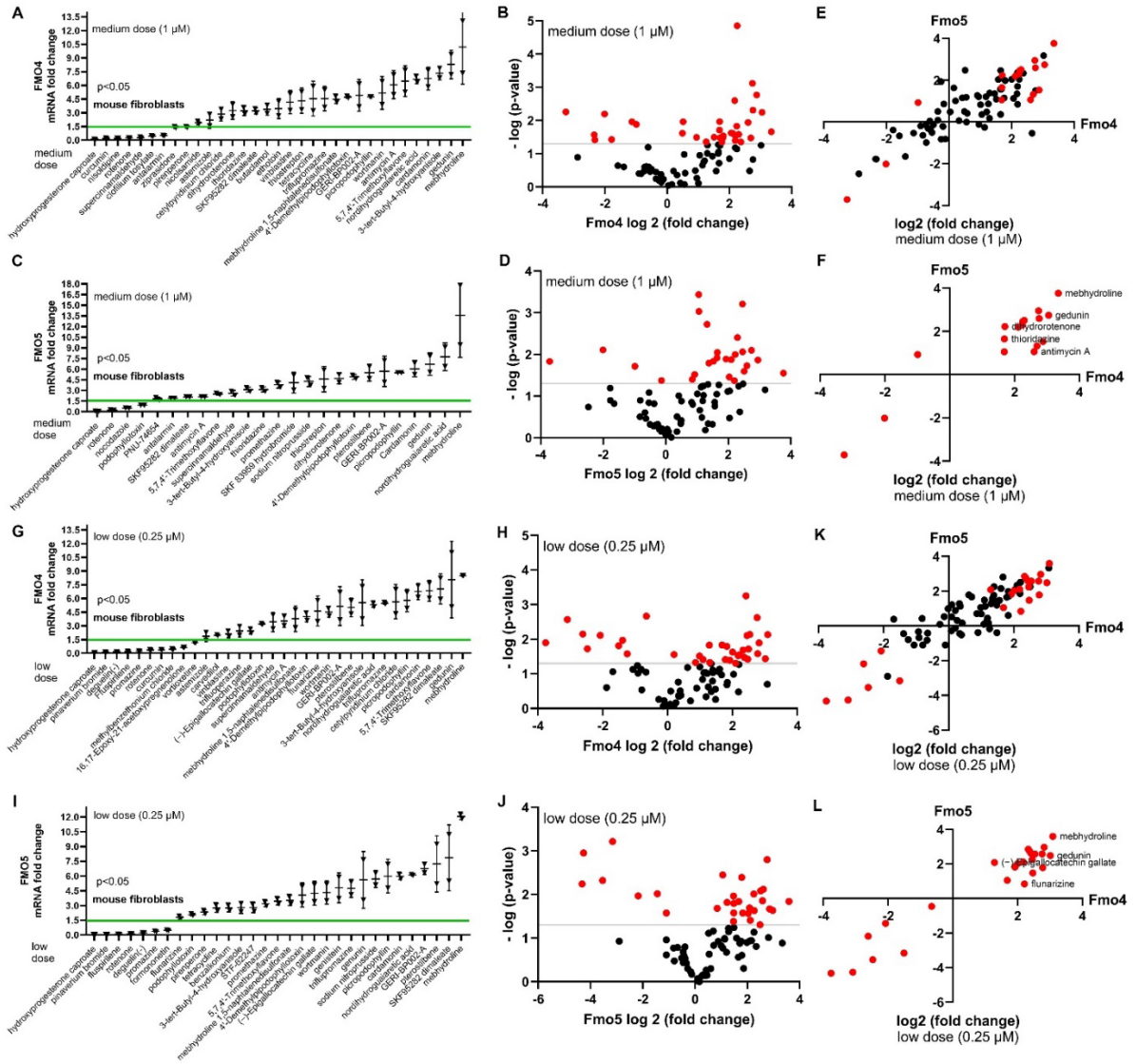

Figure S1. Drug hits that increase stress resistance in mouse fibroblasts also increase Fmo4 and Fmo5 levels under medium or low dose treatment. (**A**, **C**, **G** and **I**) Fmo4 and Fmo5 mRNA level fold changes after drugs treatment. Mouse fibroblasts from UM-Het3 mice were treated with indicated drugs on low dose of 0.25 μM or medium dose of 1 μM. Fmo4 or Fmo5 mRNA levels were then measured by qRT-PCR. All shown have $p < 0.05$ when compared to control (Welch Two Sample t-test, two-sided). Horizontal lines represent the mean, and error bars represent SD. The x axis values, shown on a Log2 scale in **B**, **D**, **H** and **J**, represent the change of mRNA levels. The y axis values, shown on a -log10 scale in **B, D**, **H** and **J**, represent the p-values. Drugs that affect the mRNA levels of Fmo4 or Fmo5 significantly are shown in red in **B, D**, **H** and **J**. (**E**, **F**, **K** and **L**) Drugs that co-regulate mRNA levels of Fmo4 and Fmo5 are shown by a scatter plot on a log2 scale fold change. Drugs that significantly increase both Fmo4 and Fmo5 mRNA levels are shown in red in the second quadrant.

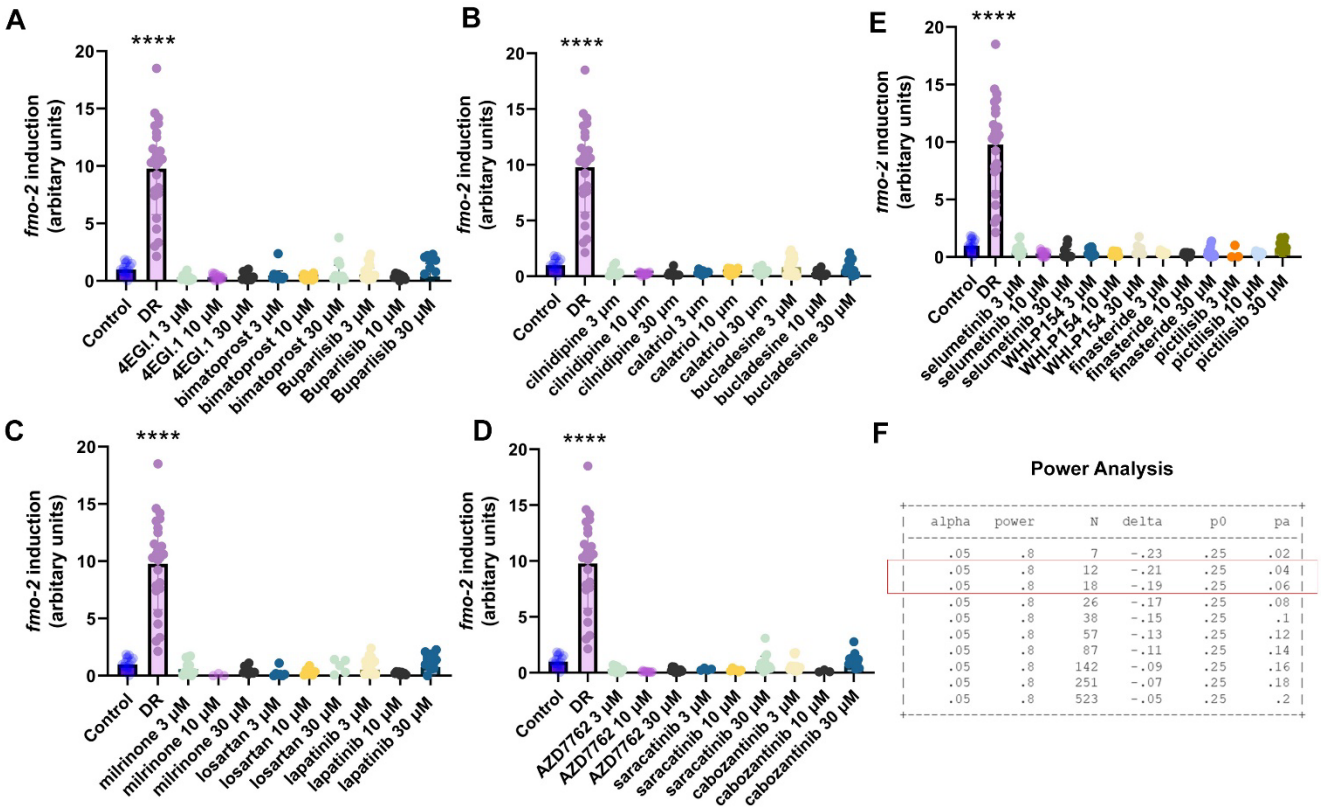

Figure S2. 16 drugs that do not increase stress resistance in mouse fibroblasts do not induce *C. elegans fmo-2*. (**A-E**) *fmo-2p::mCherry* transcriptional reporter strain was synchronized to L4 stage and treated with indicated drugs at indicated doses for 18 h. DR was shown as positive control that induces *fmo-2*. *fmo-2* levels were then measured by fluorescent microscopy. **** indicates P < 0.0001 when compared to control worms (Welch Two Sample t-test, two-sided). (**F**) power analysis. “pa” is the assumed percentage of random drugs induce *fmo-2*. “p0” is the percentage of enriched hits (~ 0.25 for 19 hits from 80 compounds). We assume no more than 5% (between 0.04 to 0.06 under “pa”) in random drugs induce *fmo-2*, to have 80% power (0.08 under “power”) to see the drug hits enriched at p = 0.05 (0.05 under “alpha”) for statistical test, 12 to 18 drugs (12 and 18 under “N”) need to be tested for *fmo-2* induction. We have tested 16 random compounds in A-E, and none of them induce *fmo-2*.

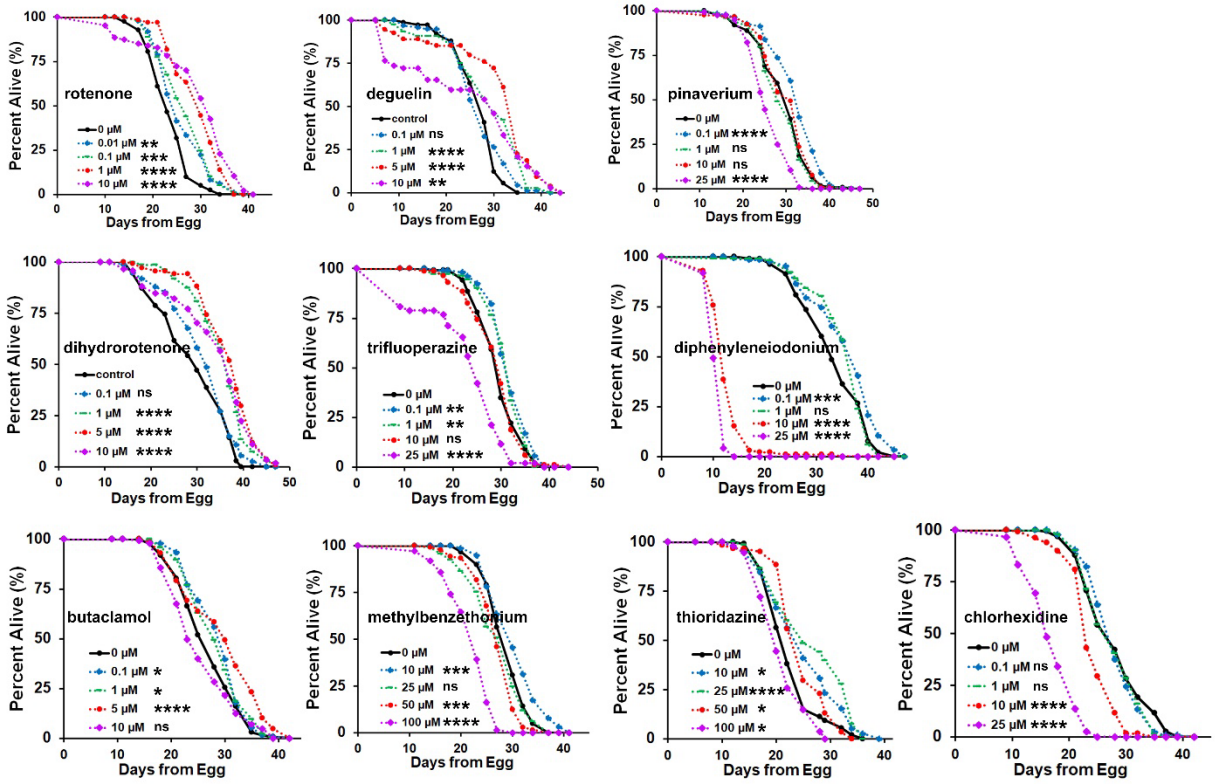

Figure S3. *fmo-2* inducers increase lifespan under different doses in *C. elegans*. Survival curves of wildtype (N2 Bristol) *C. elegans* after drug treatments. 9 out of the 10 *fmo-2* inducers extend lifespan at at least one dose. Wildtype *C. elegans* were synchronized to L4 stages, treated with indicated drugs, and then Lifespan was measured. **** indicates P < 0.0001; *** indicates P < 0.001; ** indicates P < 0.01; * indicates P < 0.05 when compared to control treated worms (log-rank test). p-values of lifespan curves comparisons were listed in Supplementary Table 3 by Log-rank test.

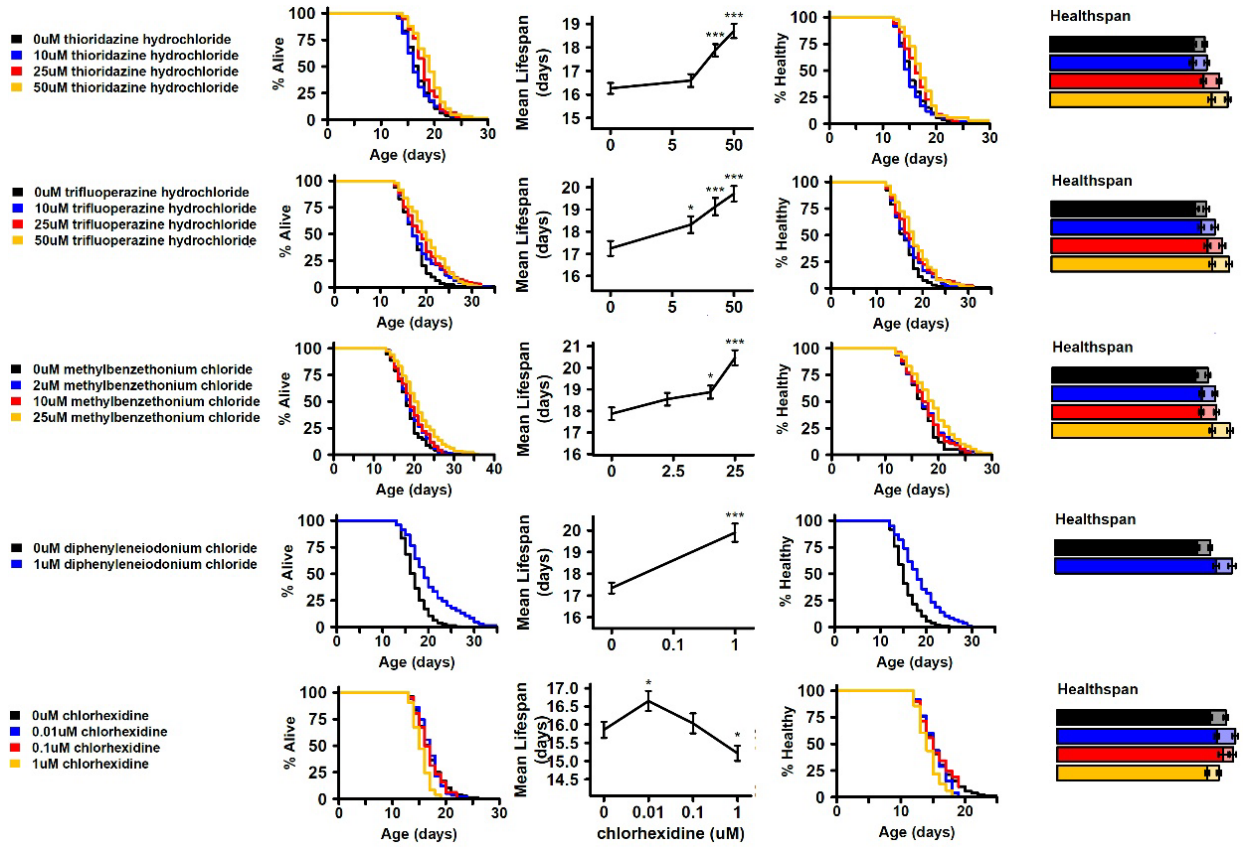

Figure S4. *fmo-2* inducers increase lifespan under different doses in *C. elegans* measured by lifespan machine. Survival curves, mean lifespan, and healthspan of wildtype (N2 Bristol) *C. elegans* after drug treatments. Thioridazine, trifluoperazine, methylbenzethonium, and diphenyleneiodonium extend lifespan more than 20% for at least one dose. Chlorhexidine extends lifespan marginally at 0.01 μM. Wildtype *C. elegans* were synchronized to L4 stage and treated with indicated drugs. Images were collected for worm every 8 hours using an autonomous robotic imaging platform and used for lifespan and healthspan quantification. Lifespans and healthspans were extended by these drugs under indicated concentration. Healthspan is defined here as the last day a worm can move a full body length. Full bars in right column indicate length of lifespan; solid portion indicates healthspan relative to lifespan for each animal. * P < 0.05, ***P < .001, when compared to control (Welch Two Sample t-test, two-sided).

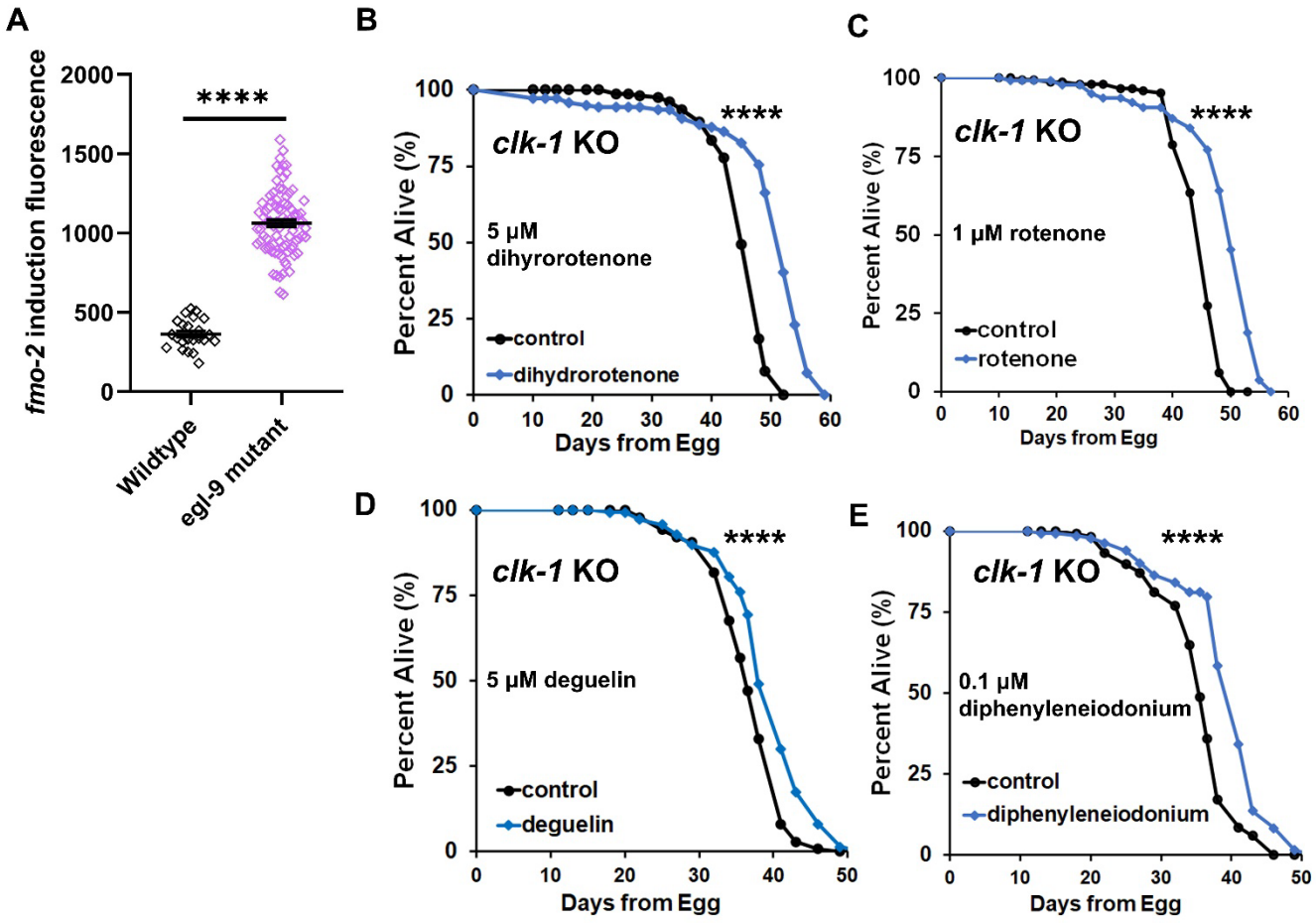

Figure S5. *fmo-2* inducers extend lifespan independent of mitochondrial respiration. (**A**) Quantifications of *fmo-2p::mCherry* in wildtype (N2 Bristol) and *egl-9* (sa307) mutant. *fmo-2* is induced significantly in *egl-9* mutant. (**B-E**) Survival curves of *clk-1* (qm30) mutant strain after drug treatments. These drugs further extend lifespan of *clk-1* mutant, suggesting extending lifespan through a mechanism independence of mitochondrial respiration pathway. *Clk-1* mutant strain was synchronized to L4, treated with indicated drugs, and lifespan was then measured. **** indicates $P < 0.0001$ when compared to control treated worms (log-rank test). p-values of lifespan curves comparisons were listed in Supplementary Table 3 by Log-rank test. ****P < .0001, when compared to control (Welch Two Sample t-test, two-sided).