

Appendix

Iron-deplete diet enhances *Caenorhabditis elegans* lifespan via oxidative stress response pathways

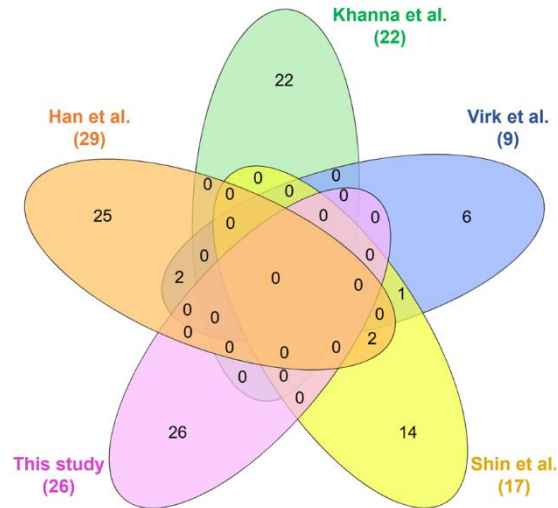
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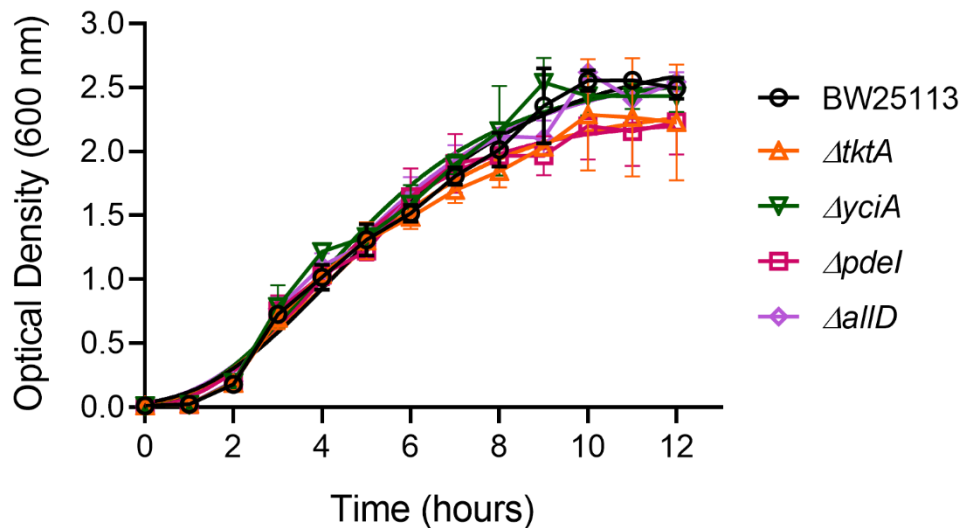
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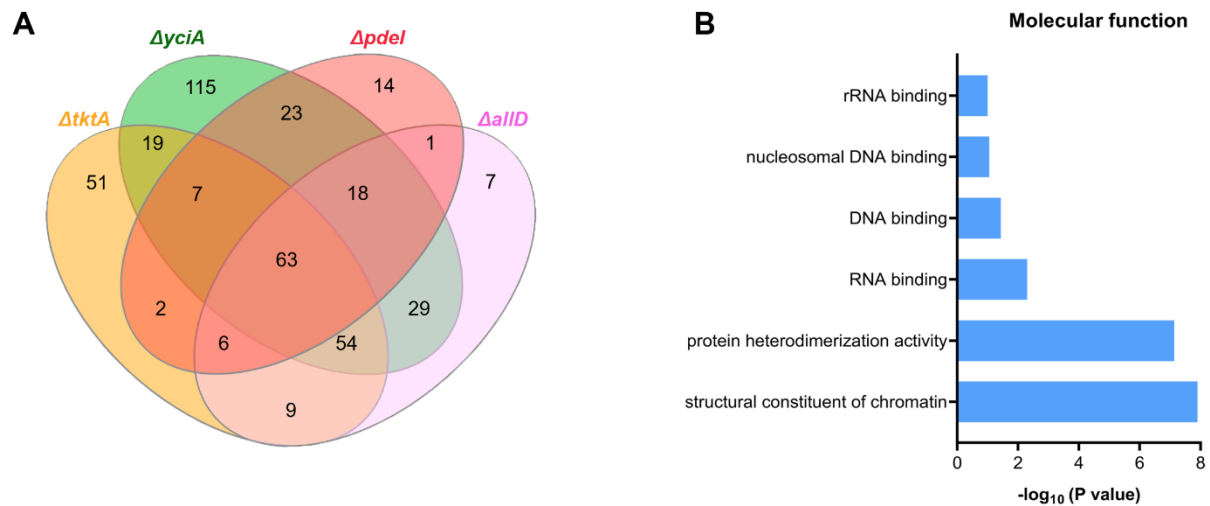
Appendix Figure S1. The pro-longevity *E. coli* mutants identified in this study differ from previously identified mutants

Venn diagram showing the overlap among *E. coli* mutants identified in previous genome-wide bacterial screens for mutants that extend *C. elegans* lifespan (Han *et al.*, 2017; Khanna *et al.*, 2016; Shin *et al.*, 2020; Virk *et al.*, 2016) and the mutants identified in this study.



Appendix Figure S2. *E. coli* mutants do not exhibit growth defects

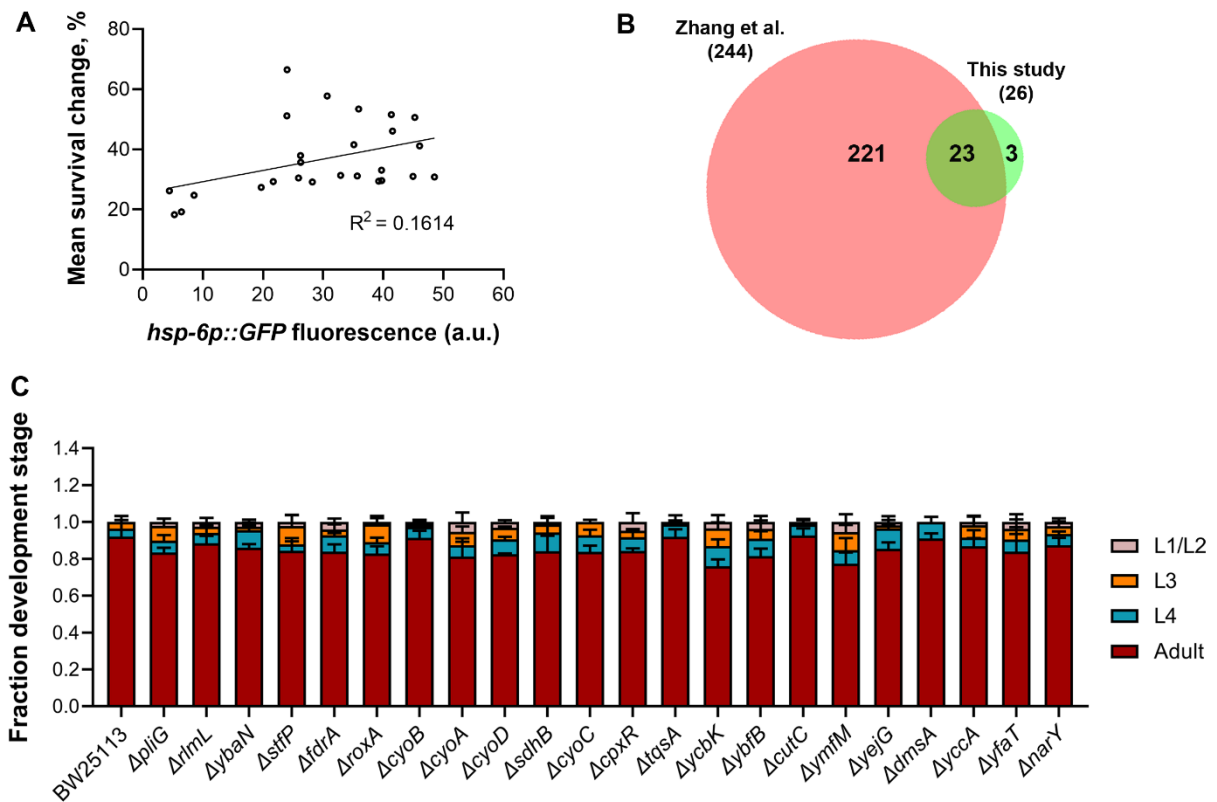
Growth curves of different *E. coli* strains in Luria-Bertani (LB) broth at 37°C. Data represent the mean and standard deviation from three independent experiments.



Appendix Figure S3. Different *E. coli* mutants induce similar transcriptomic changes in *C. elegans*

(A) Venn diagram showing the overlap among genes downregulated in N2 worms grown on *E. coli* mutants $\Delta tk t A$, $\Delta y c i A$, $\Delta p d e I$, and $\Delta a l l D$ compared to the BW25113 control.

(B) Gene ontology enrichment analysis of molecular function for the common 63 genes downregulated in N2 worms grown on *E. coli* mutants $\Delta tk t A$, $\Delta y c i A$, $\Delta p d e I$, and $\Delta a l l D$. The statistical analysis was performed using Fisher's exact test.

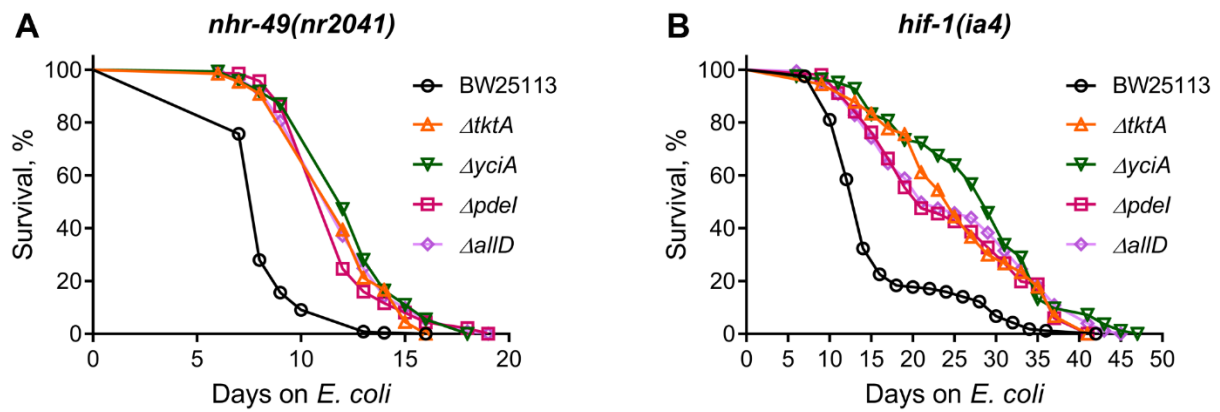


Appendix Figure S4. Iron supplementation rescues mutant diet phenotypes

(A) Correlation between *hsp-6p::GFP* fluorescence levels and the percent mean survival change in lifespan in wild-type N2 worms fed on different FAT-7-suppressing diets.

(B) Venn diagram showing the overlap between *E. coli* mutants identified by (Zhang *et al*, 2019) and those identified in this study. The Venn diagram was obtained using the web tool BioVenn (<https://www.biovenn.nl/>).

(C) Quantification of different developmental stages of N2 worms grown at 20°C for 60 hours after transferring synchronized L1 larvae onto *E. coli* BW25113 and mutant diets supplemented with 4 mM ferric chloride ($n = 3$ biological replicates; animals per condition per replicate >60). Data represent the mean and standard deviation from three independent experiments.



Appendix Figure S5. HIF-1 and NHR-49 are not required for lifespan extension on mutant diets

(A) Representative survival curves of *nhr-49(nr2041)* worms fed on $\Delta tktA$, $\Delta yciA$, $\Delta pdeI$, and $\Delta allD$ *E. coli* mutants along with the BW25113 control. $p < 0.001$ for all the mutant diets compared to the BW25113 control ($n = 3$ biological replicates; animals per condition per replicate > 60).

(B) Representative survival curves of *hif-1(ia4)* worms fed on $\Delta tktA$, $\Delta yciA$, $\Delta pdeI$, and $\Delta allD$ *E. coli* mutants along with the BW25113 control. $p < 0.001$ for all the mutant diets compared to the BW25113 control ($n = 3$ biological replicates; animals per condition per replicate > 80).

Appendix References:

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