

Expanded View Figures

Figure EV1. (relative to Fig 1). Characterization of *cest-2.2* mutant nematodes.

- A Representative survival curve of wt, *gas-1(fc21)* and *nuo-6(qm200)* mutant nematodes.
- B qRT-PCR of *cest-2.2* mRNA expression in dissected intestines that were isolated from wt, *nuo-6(qm200)*; *his-72(tm2066)*; *his-71(ok2289)* and *nuo-6(qm200)* mutants (one-way ANOVA with the Tukey's multiple comparisons test, $n = 3$ biological replicates).
- C CRISPR/Cas9 strategy for the generation of *cest-2.2(bon52)V*, with the resulting edited sequence below. In yellow: exon 1 fragment. In green: exon 11 fragment. In red: extra nucleotides inserted during CRISPR/Cas9 gene editing.
- D Representative survival curves of wt and *cest-2.2(lf)* nematodes fed with either control (solid lines) or *cco-1* (dashed lines) RNAi.
- E, F Representative lifespan assays of (E) *sod-2(ok1030)* and (F) *isp-1(qm150)* fed with control and *cest-2.2* RNAi.
- G qRT-PCR of *cest-2.2* mRNA expression in wt, *gas-1(fc21)*, *gas-1(fc21)*; *cest-2.2 O/E* and *cest-2.2(lf)*; *gas-1(fc21)*; *cest-2.2 O/E* ($n = 2$ biological replicates).
- H, I qRT-PCR of *cest-2.2* expression in (H) *gas-1(fc21)*; *cest-2.2 O/E* and (I) *gas-1(fc21)* animals fed with control and *cest-2.2* RNAi ($n = 2$ biological replicates).
- J Lifespan assay of *gas-1* mutants grown on control and *cest-2.2* RNAi bacteria.
- K, L Representative survival analyses of (K) *nuo-6(qm200)* versus *nuo-6(qm200)*; *cest-2.2 O/E* and (L) *mev-1(kn1)* versus *mev-1(kn1)*; *cest-2.2 O/E* nematodes.
- M Quantification of normalized body area of wt, *cest-2.2 O/E* and *cest-2.2(lf)* *C. elegans* (ordinary one-way ANOVA with the Dunnett's multiple comparison test, $n = 10-11$ animals).
- N Locomotory activity (arbitrary units) of wt, *cest-2.2 O/E* and *cest-2.2(lf)* nematodes (n of animals > 6).

Data information: In A, D, E, F, J, K, and L, the average median lifespan $\pm$ SEM across replicates is shown under the graphs, and additional information (e.g., n numbers) are reported in Appendix Table S1; in B, G, H, I, M, and N, bars represent average $\pm$ SEM). Across experiments, P -value summary is ns = not significant, $**P < 0.01$, $***P < 0.001$.

Source data are available online for this figure.

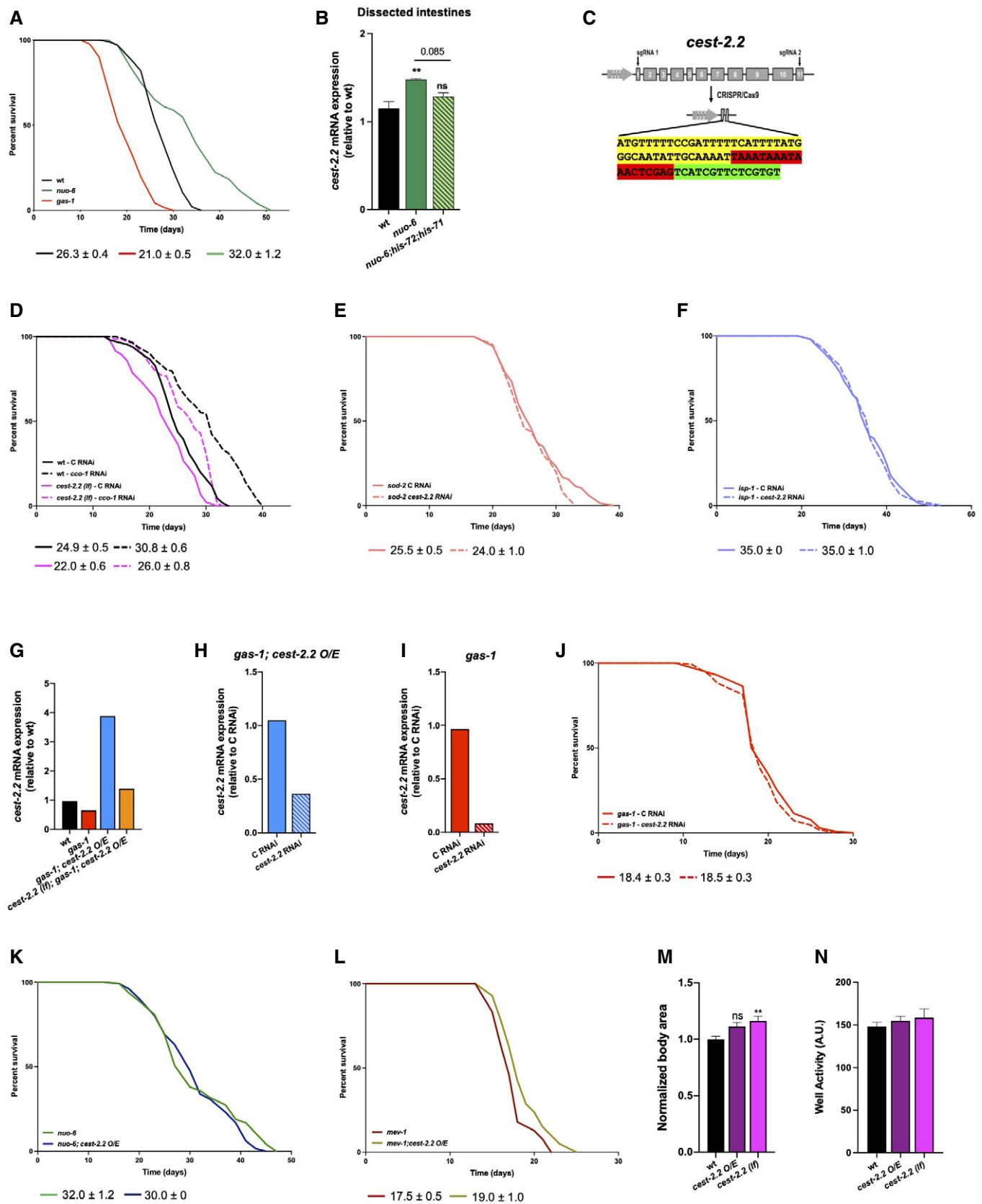


Figure EV1.

Figure EV2. (relative to Fig 2). Stress response in *cest-2.2* (lf) and *cest-2.2* O/E nematodes.

A–D qRT-PCR of *sod-3* mRNA expression with the indicated *C. elegans* strains and RNAi conditions (unpaired *t*-test, *n* = 3 biological replicates).

E–H qRT-PCR of *gst-4* mRNA expression with the indicated *C. elegans* strains and RNAi conditions (E and F unpaired *t*-test, G and H one-way ANOVA with the Dunnett's multiple comparison test, *n* = 3 biological replicates).

I Representative survival analysis of wt, *gas-1(fc21)* and *gas-1(fc21); cest-2.2* O/E mutant nematodes, in the presence or absence of 1mg/ml ascorbate (Vit C, dotted lines).

Data information: In A–H, bars represent mean $\pm$ SEM; in I, the average median lifespan $\pm$ SEM across replicates is shown under the graphs, and additional information (e.g., *n* numbers) are reported in Appendix Table S1. Across experiments, *P*-value summary is ns = not significant, **P* < 0.05.

Source data are available online for this figure.

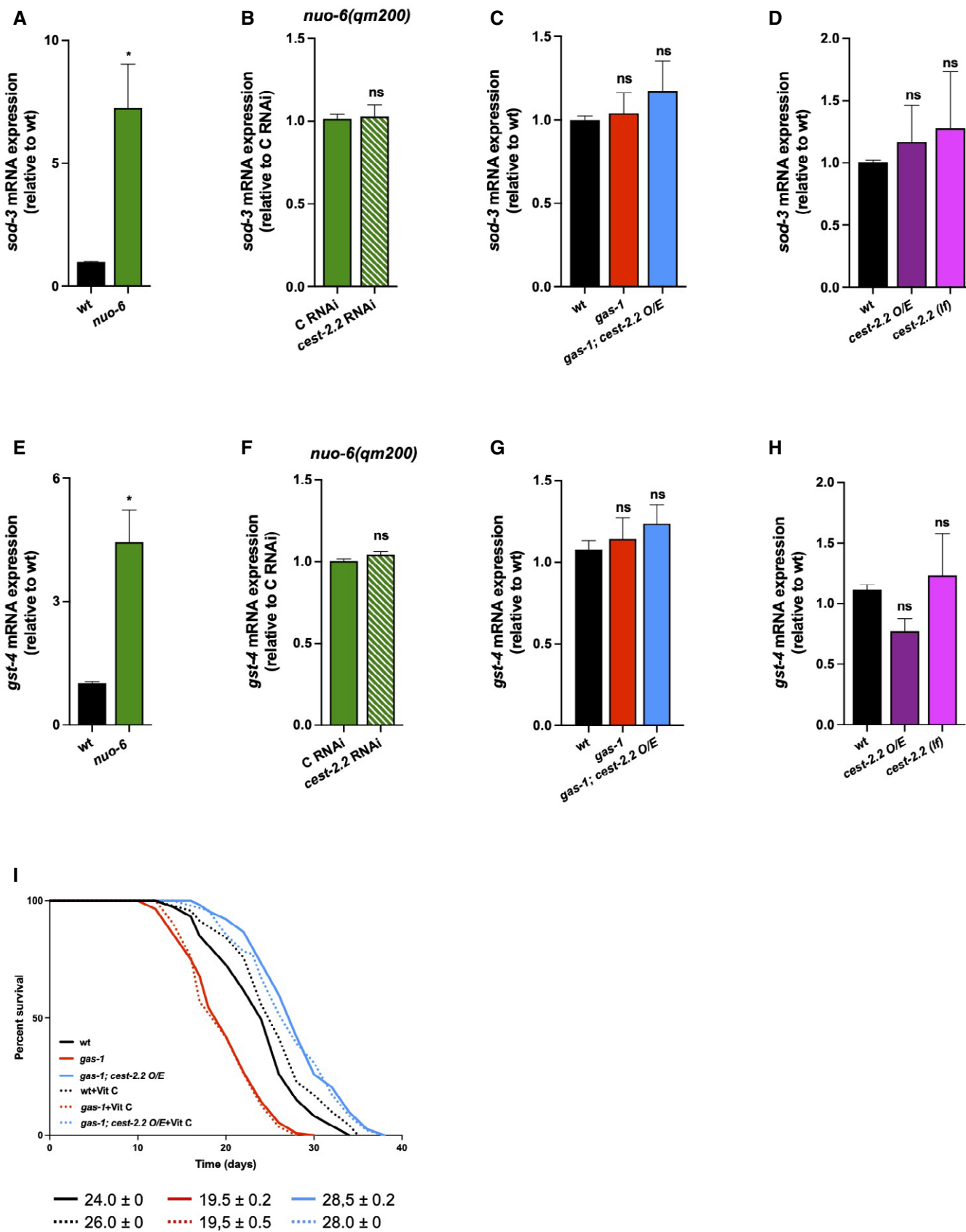


Figure EV2.

Figure EV3. (relative to Fig 3). *cest-2.2* expression does not alter the expression of vitellogenin genes in *gas-1(fc21)*.

- A, B Representative images (A) and quantification (B) of adult *nuo-6* mutants fed with either empty vector (C RNAi) or RNAi against *cest-2.2* and stained with LipidTOX (scale bar = 100 μ m) (unpaired *t*-test, *n* = 8–11 animals).
- C ORO quantification (mean intensity $\pm$ SEM) in gonads and eggs of nematodes (one-way ANOVA with the Tukey's multiple comparisons test, *n* = 18–26 animals).
- D qRT-PCR of *vit-1*, *vit-2*, *vit-3,4&5*, and *vit-6* mRNA expression in the indicated mutant nematodes (one-way ANOVA with the Dunnett's multiple comparisons test, *n* = 4 biological replicates).
- E Lifespan assay of wt, *gas-1(fc21)* and *gas-1(fc21); cest-2.2 O/E* mutant nematodes grown on control (solid lines) and *vit-1* RNAi (dashed lines). Under the graph is the average median lifespan $\pm$ SEM across all replicates.
- F Quantification of individual fatty acids from total lipid content of 5-day-old nematodes (multiple *t*-tests with FDR correction, *n* = 5 biological replicates).
- G Sum of total fatty acids found in whole body extracts of 5-day-old mutant nematodes (*n* = 5 biological replicates). Data information: In B, C, D, F, and G, bars represent mean $\pm$ SEM; in E, the average median lifespan $\pm$ SEM across replicates is shown under the graph, and additional information (e.g., *n* numbers) are reported in Appendix Table S1. Across experiments, *P*-value summary is ns = not significant, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001.

Source data are available online for this figure.

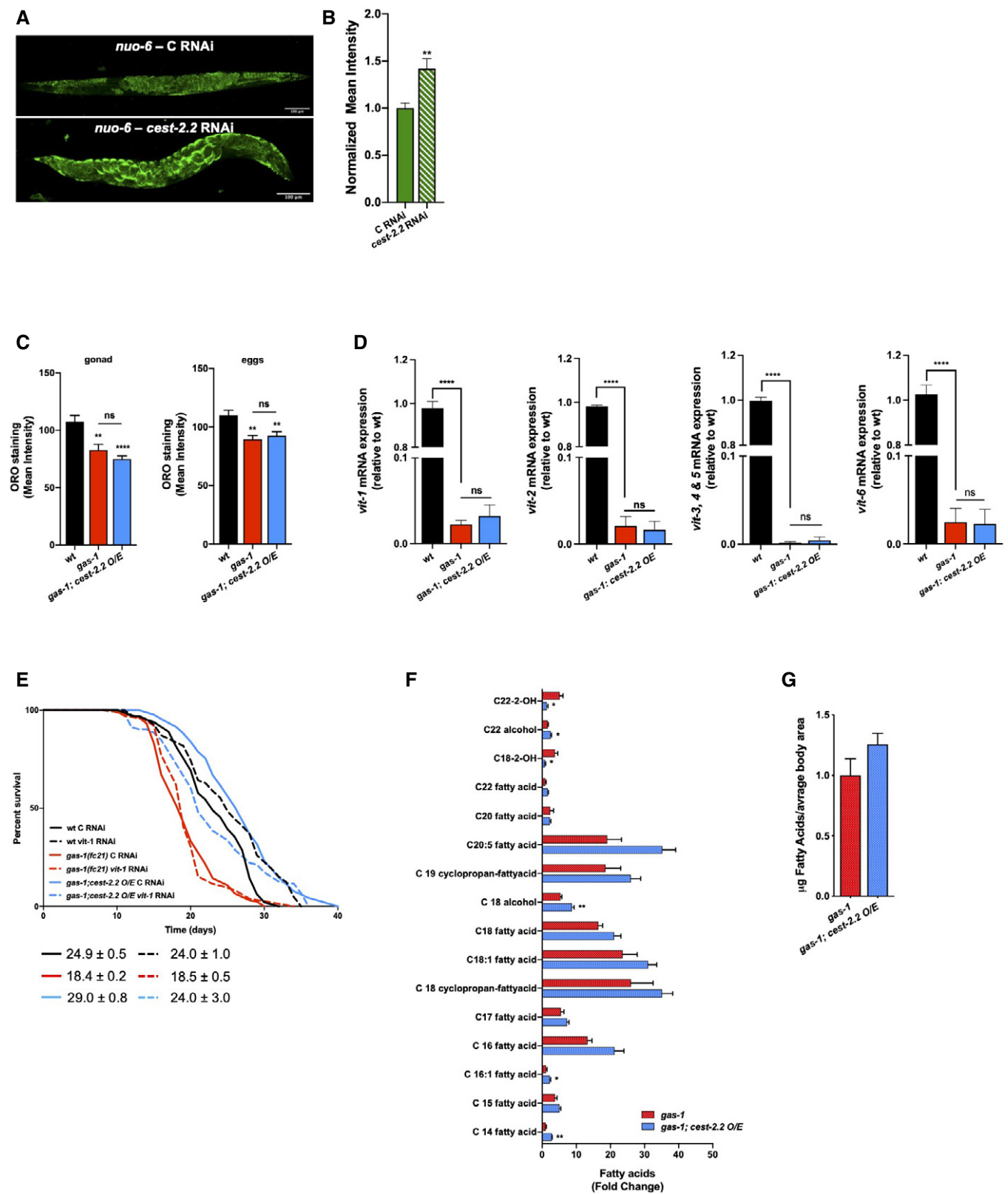


Figure EV3.

Figure EV4. (relative to Figs 4 and 5). CEST-2.2 connects lipids to oxaloacetate biosynthesis.

- A Representative images of LipidTOX-stained nematodes (scale bar = 100 μ m, inset scale bar = 50 μ m). Normalized mean intensity (relative to control RNAi) is reported on the right (Mann–Whitney test, *n*. of animals = 11).
- B qRT-PCR of *mdh-2* expression in wt nematodes grown on control and *mdh-2* RNAi expressing bacteria (unpaired *t*-test, *n* = 2 biological replicates).
- C Representative image of 5-day-old *gas-1(fc21)* (top panel) and *gas-1(fc21); cest-2.2 O/E* (bottom panel) animals fed with RNAi against *mdh-1* (scale bar = 100 μ m).
- D Representative Seahorse OCR profiling of control and oxaloacetate-treated wt nematodes. Exposure to oxaloacetate was from hatching and in 2 mM oxaloacetate-containing NGM plates (*n* = 9–10 biological replicates from three independent experiments).
- E Normalized maximal respiration of wt animals treated with oxaloacetate (unpaired *t*-test, *n* = 9 biological replicates from three independent experiments).
- F Representative OCR curve of 5-day-old *gas-1(fc21)* mutants supplemented with 1 mM nicotinamide mononucleotide (NMN), as measured by an XFe24 Seahorse Analyzer (*n* = 19–20 biological replicates from three independent experiments).
- G qRT-PCR of *pck-1* mRNA expression in wt nematodes grown on control and *pck-1* RNAi bacteria (*n* = 2 (*pck-1* RNAi)-3 (C RNAi) biological replicates).
- H, I Statistical analysis of OCR in (H) wt and (I) *gas-1(fc21)* nematodes fed with control and *mev-1* RNAi bacteria (one-way ANOVA with the Tukey's multiple comparisons test, *n* = 4–8 biological replicates from two independent experiments).

Data information: In A (right), B, E, G, H, and I, bars represent mean $\pm$ SEM; in D and F, data points represent mean $\pm$ SEM. Across experiments, *P*-value summary is ns = not statistical, **P* < 0.05, ****P* < 0.001, *****P* < 0.0001.

Source data are available online for this figure.

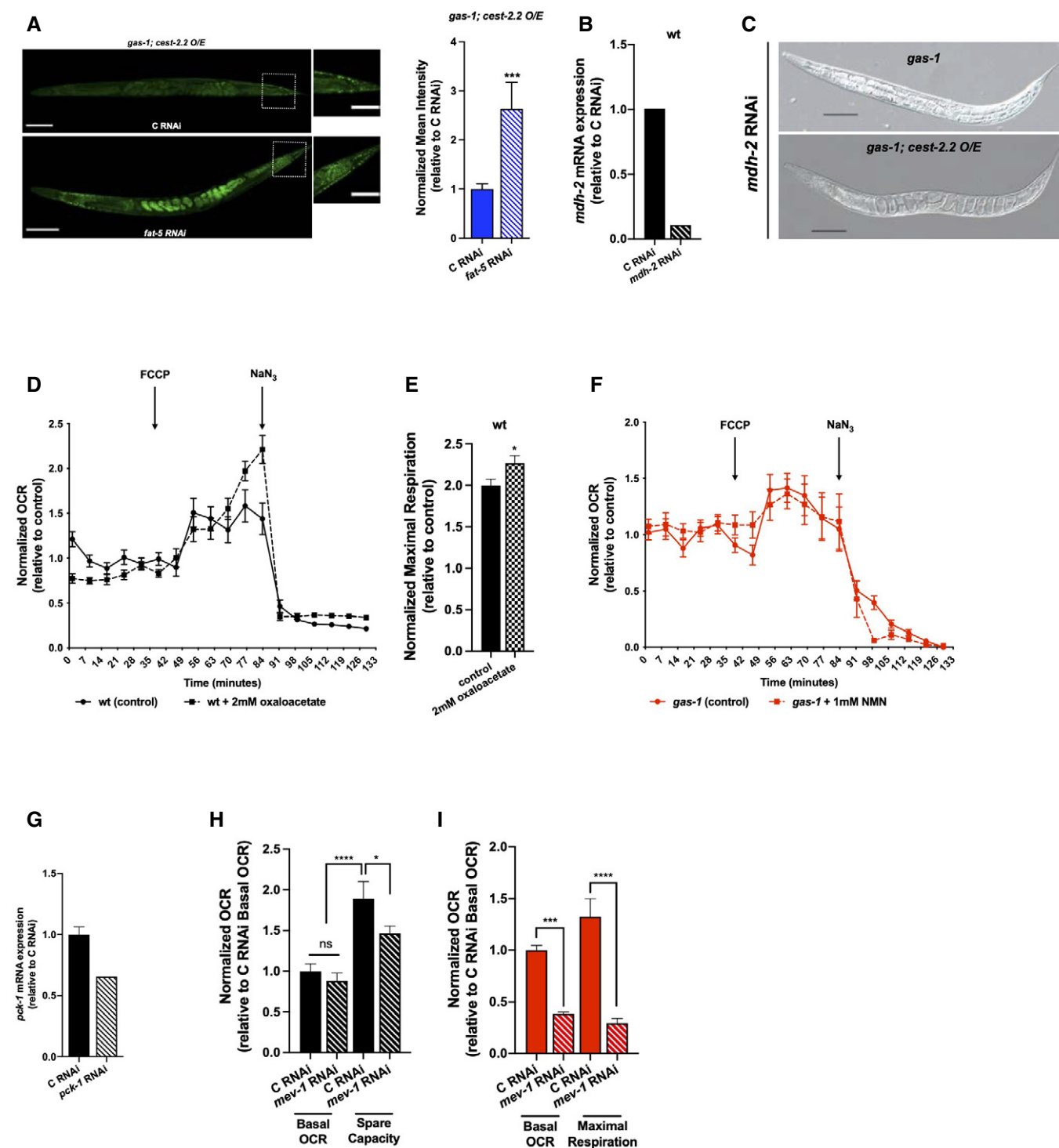


Figure EV4.