Supplemental information

Identifying *C. elegans* lifespan mutants

by screening for early-onset protein aggregation

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Supplementary Figures and Tables

Figure S1 Identification and cropping of anterior pharynx, Related to Figure 1. Pharyngeal image is split into anterior and posterior images. Binarization threshold applied to identify two largest objects, which are split at the midpoint of two centroids. Top of image is anterior, bottom is posterior.

Figure S2 Examples of factors that differentiate aggregate objects from non-aggregate objects, Related to Figure 1. Objects of certain size that are identified as aggregates exceed an intensity threshold relative to diffuse intensity. Objects of a certain intensity must be below a size threshold. Objects with high eccentricity are not identified as aggregates, as they are likely artifacts created by muscle fibers. Scale bar 10 µm.
Figure S3 Increased levels of protein aggregation observed with age and in \textit{hsf-1 (−)} mutants, quantified with the screening image processing algorithm, Related to Figure 3. (A) Increase in total aggregate intensity observed from Day 1 to Day 7 of adulthood. (B) Increase in total protein aggregation intensity observed in \textit{hsf-1 (sy411)} mutant relative to wild-type transgenic strain, **\textit{p}<0.01, ***\textit{p}<0.001, ****\textit{p}<1E-4.

Figure S4 Detection of aggregates in diverse image types, Related to Figure 2. (A) Sample images showing the low rate of aggregate identification in images with high diffuse intensity vs. low diffuse intensity. Ground Truth/Algorithm overlay ground truth binary (red) with algorithm binary (green). Overlap (yellow) is much higher in image with low diffuse intensity. (B) Predicted total intensity from algorithm plotted against actual total intensity from ground truth images. \(R^2=0.59\).
Figure S5 Dimensions of scaled-up microfluidic device for automated sorting. Related to Figure 4. The feature height on the device is about 60 µm. The imaging channel width was widened to 80 µm, and the gap between the valve and channel was widened to about 40 µm.
Figure S6 Characterization of isolated mutants that do not breed true to increased protein aggregation or that do not exhibit shortened lifespan. Related to Figure 5. A) Total area and (B) total intensity measurements for mutants that do not show a significant increase in aggregation. Numbers under box plots indicate the number of analyzed images. (C, D) Lifespan curves for identified aggregation mutants that did not display a reduction in mean population lifespan. Mutant 6 was also tested for lifespan given its p-value close to significance (as per Bonferroni correction, α=0.009).
Figure S7 Total area and total intensity measurements for short-lived mutants *kat-1* and *jkk-1* mutants, Related to Figure 6. Error bars are S.E.M. p-values using Mann Whitney U Test.
Figure S8 Pharynx morphology scoring. Related to Figure 6. A) A representative pharynx with a score of “1-least deteriorated”. B) A representative pharynx with a score of “2-somewhat deteriorated”, note the red arrowheads indicating the formation of vacuole-like structures. C) A representative pharynx with a score of “3-least deteriorated”, note the blue arrowhead indicating bending of the isthmus. Scale bar 10µM.

Figure S9 Lifespan for M3(x3), after three rounds of backcrossing with the parent strain DCD214. Related to Figure 6. Protein aggregation levels at Day 3 of adulthood were used to identify progeny populations that maintained the mutant phenotype. Mean lifespan is 19.3 days for wildtype and 14.6 for M3. Lifespan acquired in the presence of FUdR.
Table S1. Image features used in the Validation Algorithm, Related to Figure 2.

| Feature Number | Description                                      |
|---------------|--------------------------------------------------|
| 1             | Raw Pixel Intensity                              |
| 2             | Gradient Filter                                  |
| 3             | Difference of Gaussian Filter 1                  |
| 4             | Difference of Gaussian Filter 1                  |
| 5             | Standard Deviation                               |
| 6             | Laplacian of Gaussian Filter                     |
| 7             | Disk Filter                                      |
| 8             | Pixels Scaled to Maximum Image Intensity         |
| 9             | Background Subtracted Feature 1                  |
| 10            | Background Subtraction Feature 2                 |
| 11            | Smoothed Standard Deviation Feature 1            |
| 12            | Smoothed Standard Deviation Feature 2            |
| 13            | Range Filter 1                                   |
| 14            | Range Filter 2                                   |

Table S2 Initial aggregation measurements acquired for putative total area and total intensity mutants, ranked by z-score, Related to Figure 5.

| Total Aggregate Area Z-score | Total Aggregate Intensity Z-score |
|------------------------------|----------------------------------|
| 1 Mutant 1                    | 5.85                             |
| 2 Mutant 4                    | 5.64                             |
| 3 Mutant 3                    | 5.33                             |
| 4 Mutant 5                    | 4.59                             |
| 5 Mutant 6                    | 4.49                             |
| 6 Mutant 7                    | 4.35                             |
| 7 Mutant 8                    | 4.13                             |
| 8 Mutant 9                    | 4.03                             |
| 9 Mutant 10                   | 3.81                             |
| 10 Mutant 11                  | 3.65                             |
| 11 Mutant 2                   | 3.52                             |
| 12 Mutant 12                  | 3.06                             |
| 13 Mutant 13                  | 2.91                             |
| 14 Mutant 16                  | 2.75                             |
| 15 Mutant 14                  | 2.55                             |
| 16 Mutant 15                  | 2.2                              |
| 17 Mutant 17                  | 2.03                             |
| 18 Mutant 18                  | 1.55                             |
| 19 Mutant 19                  | 1.46                             |
| 20 Mutant 20                  | 1.27                             |
| 21 Mutant 27                  | 1.27                             |
| 22 Mutant 21                  | 0.86                             |
| 23 Mutant 22                  | 0.55                             |
| 24 Mutant 23                  | 0.47                             |
| 25 Mutant 24                  | -0.02                            |
| 26 Mutant 25                  | -0.76                            |
| 27 Mutant 26                  | -1.76                            |