LFASS supplementary methods

Death fluorescence (DF) curve manual analysis. Fluorescence time-lapse recording data for each well were normalised. The maximum was chosen where a significant peak of fluorescence was observed. Fluorescence values for the first 15-20 time points were often inaccurate, yielding local maxima and minima, due to worm thrashing in the wells, and were therefore omitted for the determination of the fluorescence minimum and maximum. After normalization, the time of half-maximum fluorescence was determined.

DF curve automated analysis. Matlab 2014b and 2015a versions were used to write and execute the LFASS software package. **SupFig3_LFASS_scheme** describes the approach. Detailed documentation (description of functions and variables) is provided together with the package within the Readme_LFASS.txt file. Briefly, the program proceeds as follow.

(1) Matlab separates text and number matrices so that tags and values are stored in separate matrices. In .xlsx plate-reader files each row represents the fluorescence of a single well over time. The last column is the well identity/tag (attributes of the sample: age, genotype, drug treatment, bacterial type).

(2) For the fit to perform optimally and return median time of death in minutes, several assay parameters have to be informed by the user (see supplemental online methods). The time interval between two measurements of the same well allows for the results to be expressed in minutes. The noise threshold allows for discarding empty wells, and wells in which no fluorescence peak can be detected. The noise fluorescence threshold should be chosen above the fluorescence values measured in empty wells, and below the peak blue fluorescence value sample-containing wells. This threshold also depends on the number of worms per well, and all data treated in the same bulk analysis should have roughly the same number of worms per well. Max and min have to be identified for the normalisation of the data. Early fluorescence values can greatly fluctuate due to worm thrashing/swimming (in the absence of anaesthetic) associated with high or low fluorescence values that can exceed the relevant maximum and minimum. For this reason, the user must indicate in which time intervals min and max are to be expected. These intervals usually exclude the first 10-20 time points and have to include all the times of minimum or maximum fluorescence of all the data sets included in one analysis. Once the data are normalised, the sigmoid fit needs to be constrained within initial and final plateaux that match the min and the max values. Because of inaccuracies in measurements, to chose the best plateaux, they need to be fitted over several time points. To achieve this, a tolerance threshold is given for the min/max (i.e., 0.5/0.95). The fit function will then take into account time points around the min/max that are found within these tolerance thresholds (between 0 and 0.5, and between 0.95 and 1, respectively) and stop looking for additional points beyond. This will effectively define a fit region that encompasses the death-associated blue fluorescence burst, ignoring all other parts of the curve (see green dotted lines in supplemental figure 3 (3b) and (b) lower panels).

(3) To speed up computing, the program first excludes rows that do not need to be fitted such as parameter rows (date, time points, temperature with time, etc.) by keeping only rows with at least 6 consecutives numbers (non-data rows contain letters). It then uses the noise threshold defined earlier to exclude data rows that do not have values above this threshold, which eliminates most of the empty wells (3a). Inaccuracies in measurements are associated with noise spikes that can complicate

fitting. To limit their influence, the data are smoothened twice using the “smooth” function (4 other smoothing options were compared and this performed best). Min and max are found, data are normalized, and a fit interval is found. In the region to fit, curves are typically sigmoidal in shape. Because we are only interested in extracting the time of half-maximum (corresponding to the median time of death, Fig. S1), the critical region to fit is around this time point, and the sigmoid fit performs optimally.

(4) As the analysis progresses, a 4-column wide .txt result table is filled. Column 1 contains all the tags, column 2 reports the half-maximum time inferred from unfitted normalised raw data, column 3 reports half-maximum times obtained with the bulk fitting analysis, and column 4 reports the updated values obtained from bulk fitting and user-guided analyses. Dataset names and parameters (temperature, duration of assay) are filled in the first rows of column 1. “0” fill empty cells from non-data rows. “1” fill cells in data rows that did not pass the user-defined noise threshold. “NaN” fills cells in data rows that could not be fitted.

(5) When the fit does not converge for a given row, the user can re-analyse it giving attribute values that differ form the bulk analysis and that are better suited for this specific row. Typically, this allows for recovery of the 5% exploitable low quality data that are excluded by the bulk analysis. The result table is updated after each re-analysis until the user stops.

(6) Post-processing is performed in Microsoft Excel for data sorting and basic row/columns operations. Then statistical analysis and graphical representations are processed in GraphPad Prism.