Supplementary Materials

Materials and Methods

Caenorhabditis elegans *culture*

Maintenance of *C. elegans* was carried out according to standard procedures (116). *C. elegans* strains were cultured at 20°C on cholesterol-supplemented nutrient agar (OXOID) plates containing a lawn of freshly grown *Escherichia coli* OP50 cells.

Molecular biology

The *roGFP2-Orp1* gene was optimized for expression by changing the overall codon usage to 60% of most optimal codon usage in *C. elegans*, and by introducing five introns in the gene. This was carried out by using the software ''*C. elegans* Codon Adapter'' (http://worm-srv3.mpi-cbg.de/codons/cgi-bin/optimize.py). The *fbf-1* promoter and *tbb-2* 3′UTR region were selected to achieve expression in the entire germ line (83). Engineered transgenes were cloned into pCFJ150 and injected into strain EG6699 to create integrated single-copy *roGFP2-Orp1* transgenes using the MosSCI method (41).

Paraquat treatment

A paraquat stock solution was freshly made before each experiment and was added into NGM media the day before the plates were used. Worms were placed on plates with paraquat starting from the fourth larval stage.

Feeding RNAi

RNAi was applied by feeding bacteria expressing dsRNA to the worms. RNAi induction was performed as described in Ref. (123). Synchronized fourth larval stage worms were placed on NGM plates seeded with freshly induced RNAi bacteria.

Live imaging

One-day-old adult hermaphrodites were removed from the plate, anesthetized in a fresh mixture of 1 mg/ml Tricaine (ethyl 3-aminobenzoate methanesulfonate salt) and 0.1 mg/ml of tetramisole hydrochloride dissolved in M9 for 15–30 min before transferring them to an agarose pad under a coverslip for imaging. Gonads were imaged by collecting a 10×2 mm z-series after excitation at 405 and 488 nm. Microscopy was performed with a Nikon Eclipse TE2000-5 confocal microscope.

Image processing

Image processing was carried out as described in Ref. (87). In brief, images were saved as 16-bit tiff files and processed by ImageJ. Images were first converted to 32-bit tiff files, background was subtracted using an upper and lower threshold, and background values were set to ''not a number.'' Subsequently, 405-nm images were divided by 488-nm images pixel by pixel to create the ratio images, and displayed in false colors using the lookup table ''Fire.'' Relative H_2O_2 levels in these processed images were quantified by selecting eight equally sized rectangle areas in a single focal plane, followed by the calculation of the mean ratio in each of the selected areas.

Fluorimetry

In vivo H_2O_2 levels were quantified using *jrIs1[rpl-17p::HyPer], jrIs10[unc-119(+) rps-0p::roGFP2-Orp1]*, and the glutathion redox potential was determined with the strain *jrIs2[rpl-17p::Grx1-roGFP2]*(6,16). All strains were age synchronized through bleaching and scaled up on nutrient agar plates seeded with *E. coli* K12 bacteria at 20°C. Day 1 adults were washed twice with S-basal and the dense worm suspension was distributed as a linear dilution series (5–100%) over at least 10 wells of a black microtiterplate. H_2O_2 levels and the glutathione redox potential in live worms were determined through a fluorimetric assay as described in Ref. (6). Fluorescence was measured over a 30-min period at 25°C using a Wallac Victor^2 Multilabel Counter (PerkinElmer, Boston, MA) with 405- and 490-nm excitation filters and a 535-nm emission filter. For each sample, data were averaged over the 30-min measurement period. Data of at least three biological replicates were pooled per strain.