Supplemental Movies

Movie S1. Laser wounding inhibits/induces mitoflashes, related to Figure 1.

Mito::cpYFP expressed in *C. elegans* epidermis (*juEx5344, col-19* promoter). Time lapse images were taken every 2 s on the spinning disk confocal, before and after femtosecond laser wounding at 5:02 (white arrow). A single mitoflash can be seen in the middle of the image at 01:16-01:22. Numerous mitoflashes can be seen around the wound site between 200-300 s post wounding. Single mitochondria can undergo repeated flashes (see Movie S2). Scale, 10 μm. Time: min.

Movie S2. Pro-oxidant PQ treatment induces mitoflashes before and after wounding in adult epidermis, related to Figure 2.

mito::cpYFP transgenic worms were transferred to agar pad in 2 μl 12 mM Levamisole with 0.1 mM PQ immediately before laser wounding. Time: min; scale, 10 μm.

Movie S3. mitoflashes after treatment with mitochondrial targeted antioxidant mitoTempo before and after wounding, related to Figure 2.

mito::cpYFP transgenic worms were incubated on 0.1 mM TPP or 0.1 mM mitoTempo plates for 1 h and then transferred to agar pad in 2 μl 12 mM Levamisole with TPP or mitoTempo for imaging. Time: min; scale, 10 μm.

Movie S4. Wounding triggers actin-based wound closure in the wild type and accelerated closure in the *sod-2 and isp-1* mutants, related to Figure 3.

GFP::moesin (juIs352) labels epidermal F-actin. The *sod-2 and isp-1* mutants show faster wound closure compared to WT. Scale, 10 μm; time: min.

Movie S5. Laser wounding triggers cytosolic Ca^{2+} elevation and mitochondrial Ca^{2+} uptake, related to Figure 4.

Cytosolic GCaMP3 (*juIs319, col-19* promoter, cGCaMP) and mito::GCaMP3 (*juEx4955, col-19* promoter, mGCaMP*)* fluorescence images were taken before and after laser wounding. Intensity code. Scale, 10 μm. Time: s. White arrow marks the wound site.

Movie S6. Mitochondrial Ca^{2+} uptake in WT and *mcu-1* mutant, related to Figure 5.

mito::GCaMP5(juSi103) fluorescence imaged on spinning disk confocal microscope before and after laser wounding. In the *mcu-1(ju1154)* mutant, the mito::GCaMP5 signal shows minimal change after wounding. Top: WT, Bottom: *mcu-1* mutant. Time: s; scale, 10 μm.

Movie S7. mitoflashes in WT and *mcu-1* mutant before and after wounding, related to Figure 5.

The mitochondrial Ca^{2+} uniporter MCU-1 is required for mitoflash induction after laser wounding. Top: WT, Bottom: *mcu-1* mutant. Time: min; scale, 10 μm.

Movie S8. Wounding induces local accumulation of eGFP:rGBD around wound site, related to Figure 6.

Laser wounding of P*col-19-eGFP::rGBD(juEx3025)* worms, intensity color code. Scale, 10 μm. Time: min; white arrow marks wound.

Supplemental Table

Table S1. Strains, Transgenes, and Plasmids, related to Experimental Procedures.

Strain	Transgene	Construct	Plasmid #
CZ17928	*juEx5344*	P*col-19*-mito::cpYFP	pCZ820
CZ16518	*juEx4796*	P*col-19*-mito::GFP	pCZGY2143
CZ17934	*juEx5350*	P*col-19*-mito::HyPer2	pCZ824
CZ16837	*juEx4955*	P*col-19*-mito::GCaMP3	pCZGY2162
CZ14748	*juIs352 I*	P*col-19*-GFP::moesin	pCZGY1576
CZ12680	*juEx3025*	P*col-19*-eGFP::rGBD	pCZGY2192
CZ18740	*juEx5614*	P*col-19*-mito::pHluorin	pCZ831
CZ13896	*juIs319*	P*col-19*-GCaMP3	pCZGY1435
CZ18058	*juSi103*	P*col-19*-mito::GCaMP5	pCZ829
CZ20761	*juEx6255*	P*col-19*-RHO-1	pCZ868
CZ20764	*juEx6258*	P*col-19*-RHO-1(G14V)	pCZ869
CZ20767	*juEx6261*	P*col-19*-RHO-1(C16A)	pCZ870
CZ20771	*juEx6265*	P*col-19*-RHO-1(G14V,C16A)	pCZ875
CZ20774	*juEx6268*	P*col-19*-RHO-1(C20A)	pCZ876
CZ20777	*juEx6271*	P*col-19*-RHO-1(C16A,C20A)	pCZ877
CZ20782	*juEx6276*	P*col-19*-SOD-2	pCZ879
QT47	*nzIs1*	P*hsp-16.2*-RHO-1(G14V)	QT#42

Notes: At least two transgenic lines per construct were tested; representative transgenes are listed. Constructs were injected at 10 ng/µl with the P*ttx-3*-RFP coinjection marker unless indicated. “mito” denotes the Cox8 mitochondrial matrix targeting sequence.

Supplemental Experimental Procedures

mitoSOX staining and imaging

MitoSOX Red (Molecular Probes, M36008) can react both with superoxide and with other mitochondrial oxidants (Zielonka et al., 2008); in *C. elegans* samples mitoSOX Red fluorescence is largely due to superoxide (Dingley et al., 2010). As the *C. elegans* cuticle is only minimally permeable to mitoSOX we performed needle wounding prior to mitoSOX staining. We transferred wounded worms to mitoSOX Red staining solution (5 μM in M9, prepared from a 5 mM stock in DMSO) immediately after wounding and stained in the dark with gentle shaking for 20 min at room temperature. Stained worms were washed three times with M9 before imaging using a 543 nm excitation laser.

Heat shock

Heat shock experiments were performed as previously reported (McMullan et al., 2006). Briefly, young adult worms (24 h after L4 stage) were first heat shocked for at 30 °C for 1 h, then incubated at 20 °C for 1 h. These worms were heat shocked again at 30 °C for 1 h and incubated at 20 °C for 1 h before proceeding to needle wounding.

Supplemental References

Dingley, S., Polyak, E., Lightfoot, R., Ostrovsky, J., Rao, M., Greco, T., Ischiropoulos, H., and Falk, M.J. (2010). Mitochondrial respiratory chain dysfunction variably increases oxidant stress in *Caenorhabditis elegans*. Mitochondrion *10*, 125-136.

McMullan, R., Hiley, E., Morrison, P., and Nurrish, S.J. (2006). Rho is a presynaptic activator of neurotransmitter release at pre-existing synapses in *C. elegans*. Genes Dev *20*, 65-76.

Zielonka, J., Vasquez-Vivar, J., and Kalyanaraman, B. (2008). Detection of 2-hydroxyethidium in cellular systems: a unique marker product of superoxide and hydroethidine. Nat Protoc *3*, 8-21.