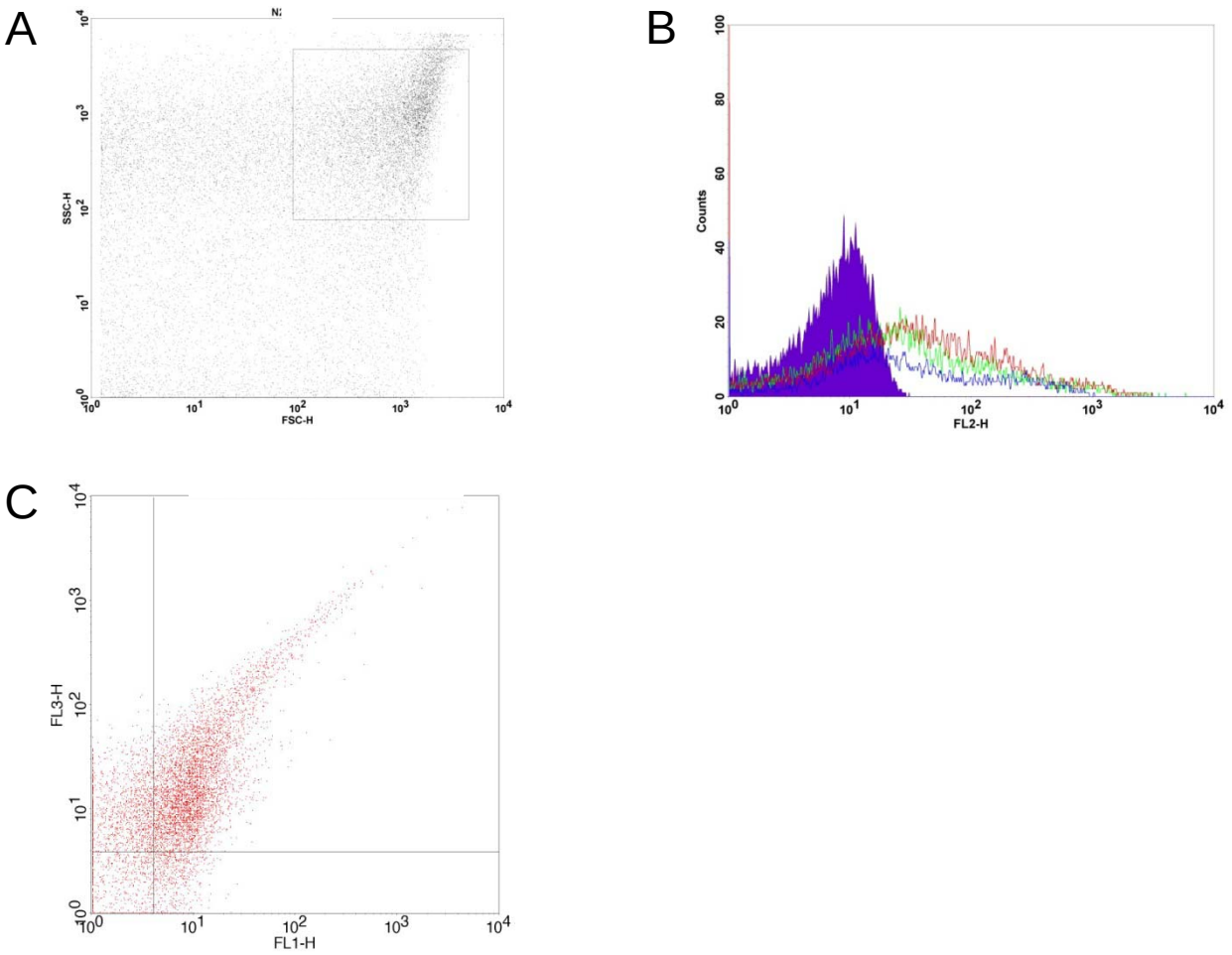

Selection of isolated mitochondria and ROS-sensitive dye analysis. Mitochondria were prepared as described in Material and Methods. A: A FACSCalibur flow cytometry cell sorter from Becton Dickinson equipped with a 488-nm Argon laser and a 635-nm red diode laser was used. Data from the experiments were analyzed using the CellQuest software (Becton Dickinson). To exclude debris, samples were gated based on light-scattering properties in the SSC (side scatter) and FSC (forward scatter) modes, and 20,000 events per sample within the region (gate) deliminated by a square in A. were collected, using the ”low” setting for sample flow rate. 99% of the particles in that region successfully stained with the mitochondria-specific dye Mitotracker Green. B. Isolated mitochondria were incubated with analysis buffer contain substrate (see Materials and Methods) and MitoSox (1μM) at room temperature for 1 hour and then sorted and the fluorescence of mitochondria in the gate measured. The purple area represents un-stained control. Paraquat (red line) was able to increase ROS generation over the untreated control (green line), while NAC (blue line) decreased the superoxide signal. Note that the X-axis shows a log scale. C. Isolated mitochondria (see A.) from wild-type worms were stained with both H_2DCF-DA (the signal plotted on FL1-H; 530 ± 15 nm channel) and Mitotracker Red (the signal plotted on FL3-H; ≥670nm channel). When particles were stained by both dyes (upper-right region), the signals were strongly correlated. Furthermore, 89.6 ± 2.4% (n=4) of the particles stained by H_2DCF-DA were also stained by Mitotracker Red.