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Figures and figure supplements

SIR-2.1 integrates metabolic homeostasis with the reproductive neuromuscular excitability in early aging male *Caenorhabditis elegans*

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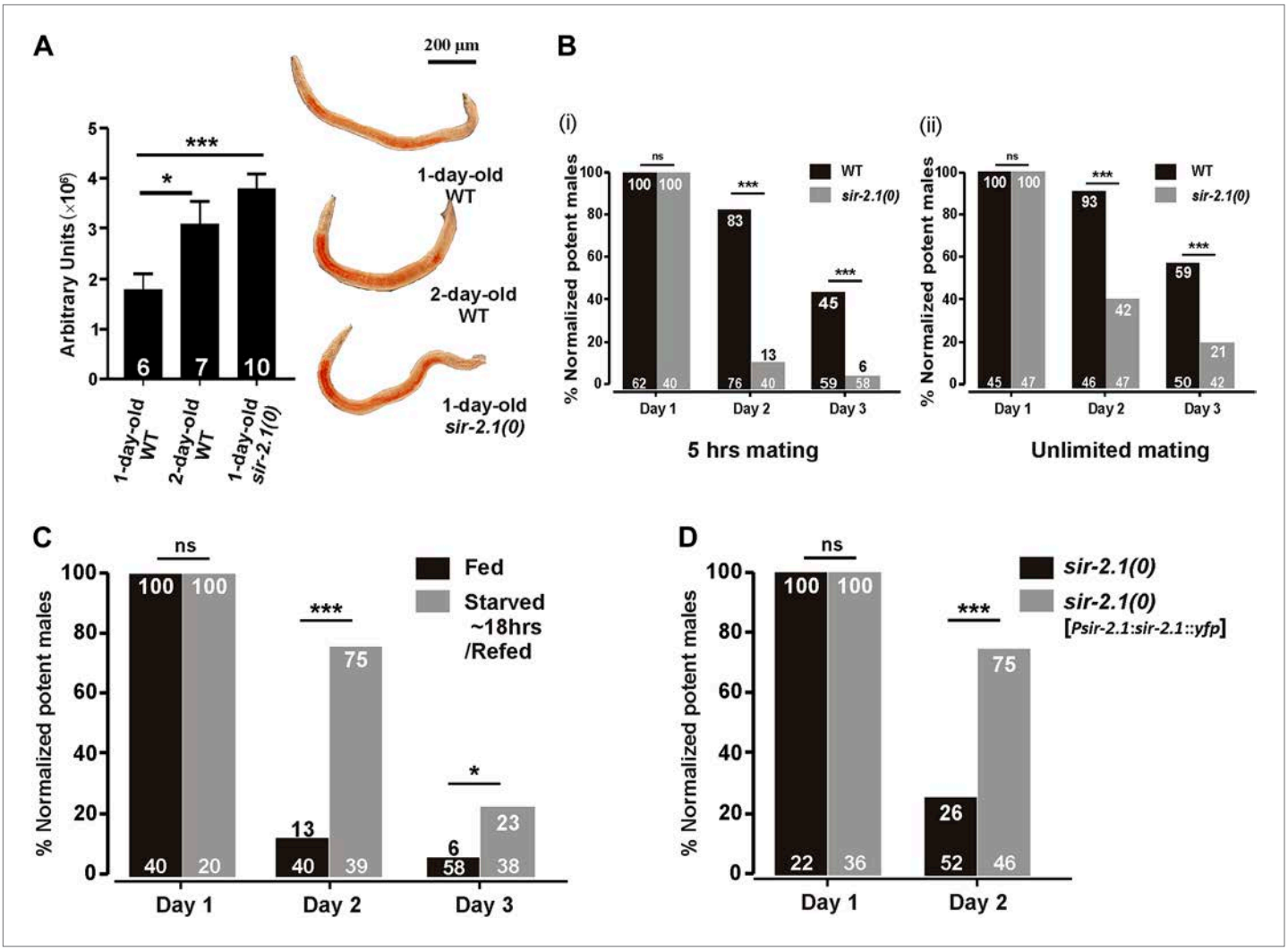

Figure 1. *sir-2.1(0)* males have altered lipid content and their mating ability deteriorates prematurely. (**A**) 1-day-old *sir-2.1(0)* and 2-day-old wild-type males have more lipid content than 1-day-old wild type. Left: quantification of fat staining, mean ± SEM, unpaired t-test. Right: representative images of fat staining. (**B**) Mating potency of wild-type and *sir-2.1(0)* males. Copulations were allowed to occur for 5 hr (i) or for an unlimited time (ii). The number of males in each assay is listed at the bottom of each bar. The numerical percentage of wild-type males that mated on day 1 was normalized to 100%. The normalization factor was then applied to the other experimental conditions. The normalized percentages for each day are listed on the top. Fisher's exact test was used to compare the mating potency prior to normalization. (**C**) Transient starvation reduces *sir-2.1(0)* mating deficiency. (**D**) Mating potency of *sir-2.1(0)* and rescued strain *sir-2.1(0); rgEX399 [Psir-2.1:sir-2.1::yfp]*. ns, not significant. Asterisks *, ** and *** indicate the $p<0.05$, 0.01, and 0.0001 in this paper, respectively.

DOI: 10.7554/eLife.01730.003

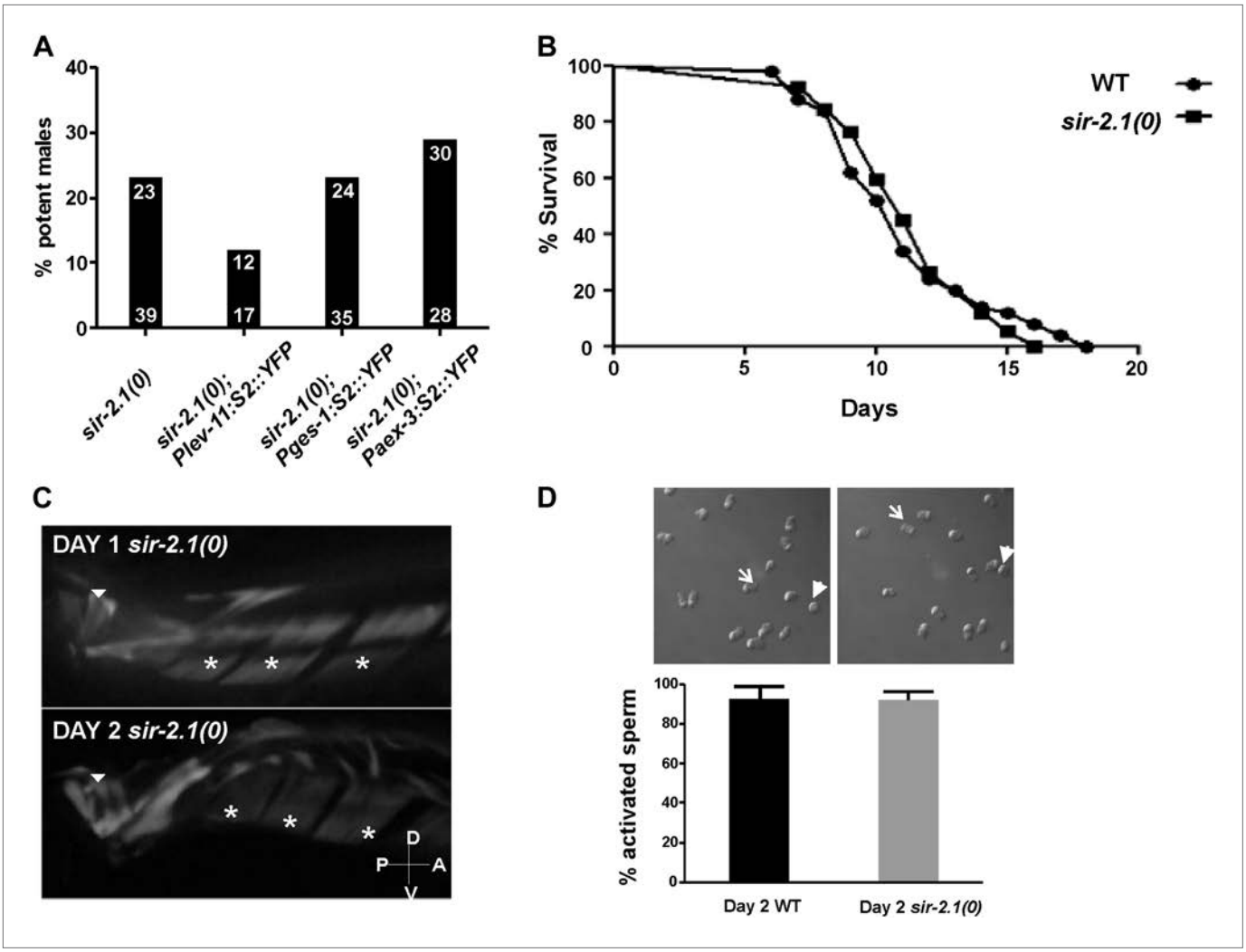

Figure 1—figure supplement 1. (**A**) Tissue specific expression of *sir-2.1* does not rescue the reduced mating potency of *sir-2.1(0)* males at day 2.
DOI: 10.7554/eLife.01730.004

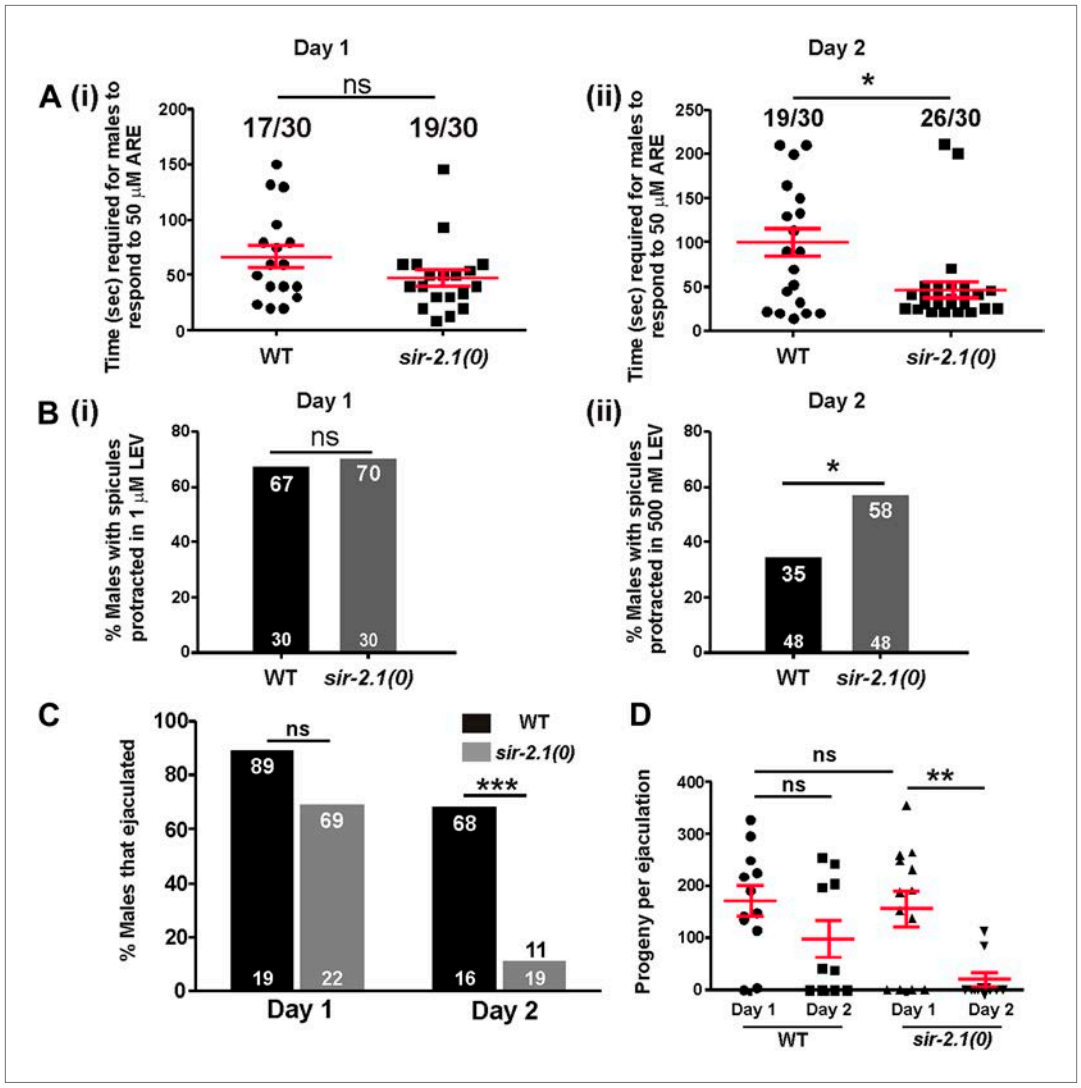

Figure 2. *sir-2.1(0)* males' sex circuitry becomes more excitable during aging, and those males display ejaculation defects. (**A**) 1-day-old wild-type and *sir-2.1(0)* males (n = 30) have similar response to the ACh agonist arecoline (ARE). The time required for those males to protrude their spicules out in 50 μM ARE solution are not significantly different (i) (unpaired t-test), whereas 2-day-old *sir-2.1(0)* males require significantly less time to respond to ARE (ii) (unpaired t-test). Mean and SEM are indicated. (**B**) 1-day-old wild-type and *sir-2.1(0)* males (n = 30) have similar response to the ACh agonist levamisole (LEV) (i). However, 2-day-old *sir-2.1(0)* males are more sensitive to LEV (ii). (Fisher's exact test). (**C**, **D**) 2-day-old *sir-2.1(0)* males have an ejaculation defect. (**C**) The percentages of 2-day-old wild-type and *sir-2.1(0)* males that ejaculated during copulation. (Fisher's exact test). (**D**) The numbers of cross progeny produced by individual 2-day-old wild-type and *sir-2.1(0)* with *unc-64(e240)* hermaphrodites. Mean and SEM are indicated (unpaired t-test).

DOI: 10.7554/eLife.01730.005

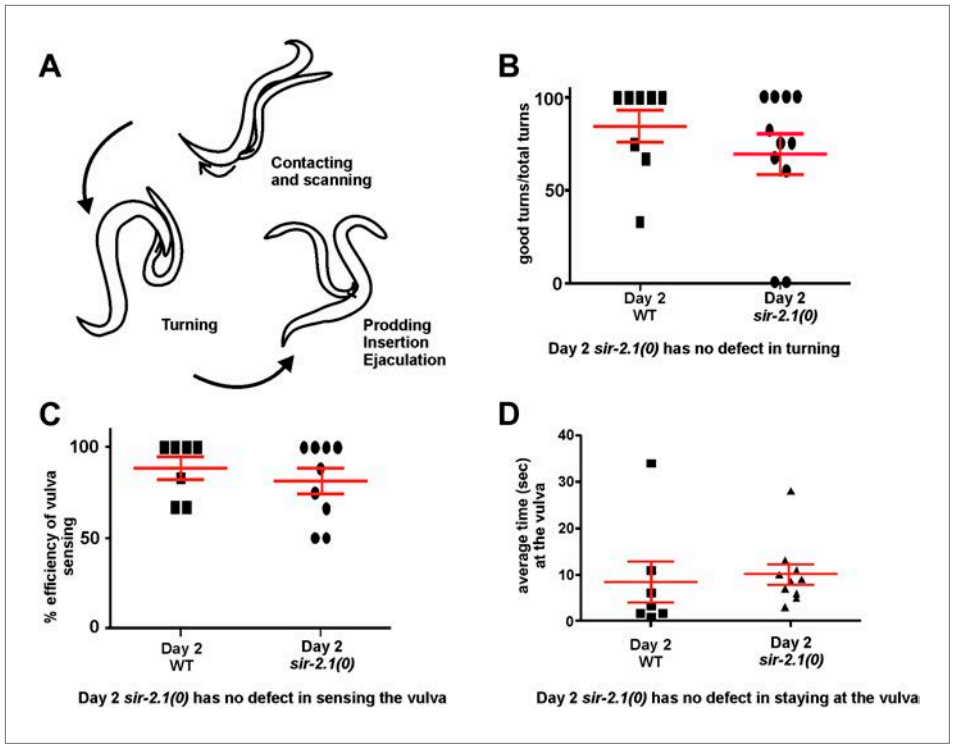

Figure 2—figure supplement 1. (A) A cartoon illustration of *C. elegans* male mating behavior.

DOI: 10.7554/eLife.01730.006

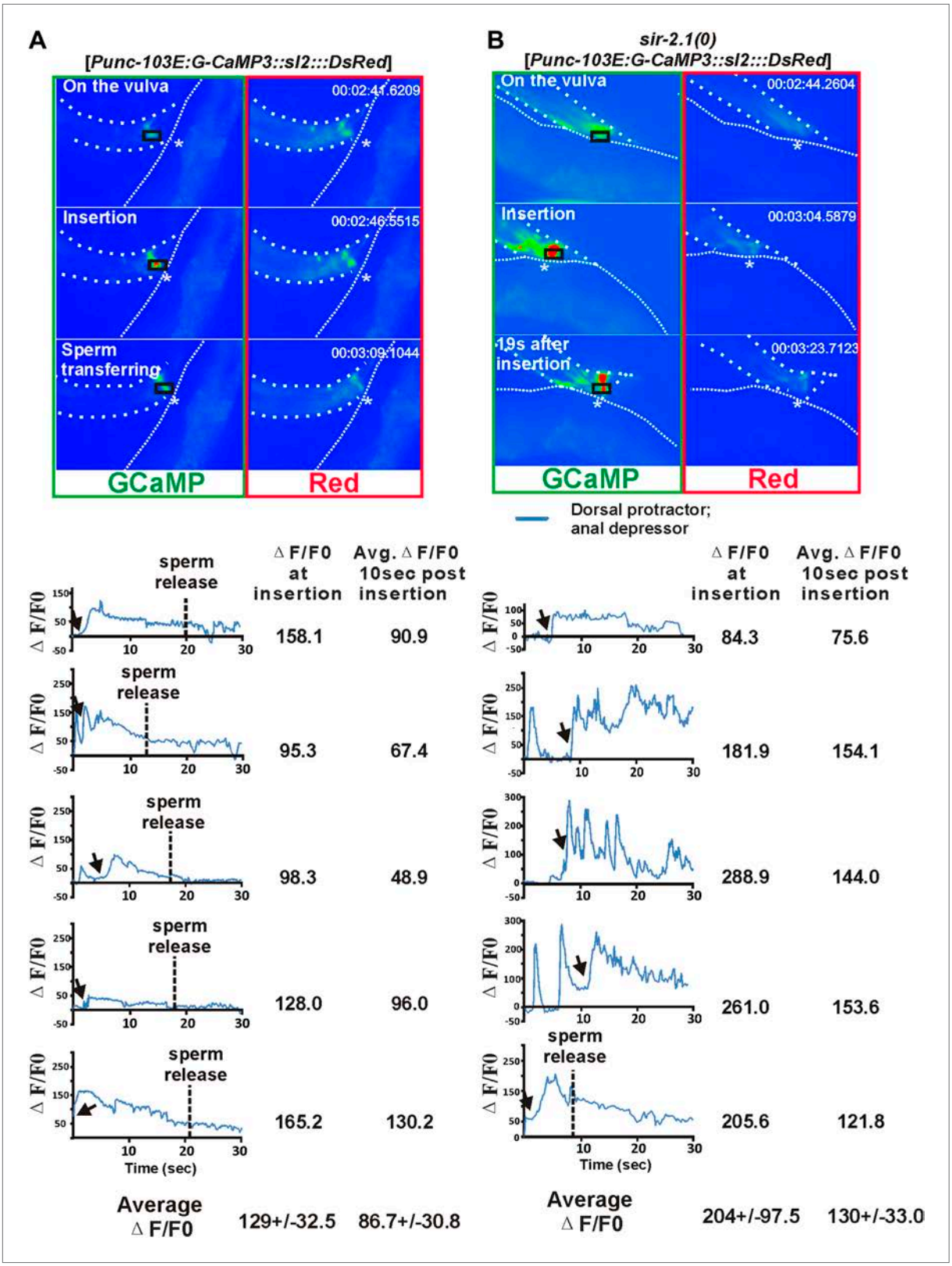

Figure 3. Ca^{2+} imaging of spicule-associated muscles in mating males. Pseudo-colored images of Ca^{2+} in the spicule muscles of 2-day-old wild-type and *sir-2.1(0)* males during mating (**A**) and (**B**) are representative frames to show the Ca^{2+} levels of the spicule-associated muscles during spicule insertion attempts, penetration and the start of sperm transfer (~10 s after insertion for wild type) or 19 s after insertion (for *sir-2.1(0)* males). The asterisks indicate

Figure 3. Continued on next page

Figure 3. Continued

the hermaphrodite vulva. Below the images, the Ca^{2+} transients in the protractor and anal depressor muscles (indicated by the black rectangle in **A** and **B**) are plotted for 5 individual wild-type (**A**) and *sir-2.1(0)* males (**B**), respectively.

DOI: 10.7554/eLife.01730.010

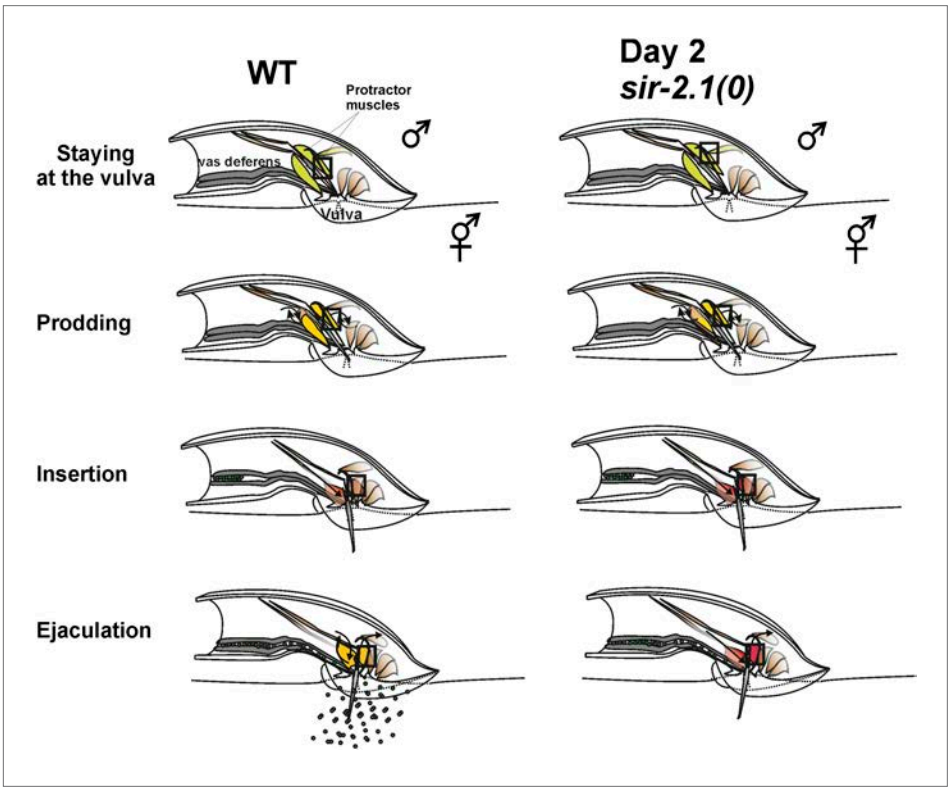

Figure 3—figure supplement 1. A cartoon illustrating the contractile changes of the spicule-associated muscles during intromission and ejaculation behaviors of a 2-day-old wild-type and *sir-2.1(0)* male, respectively.

DOI: 10.7554/eLife.01730.011

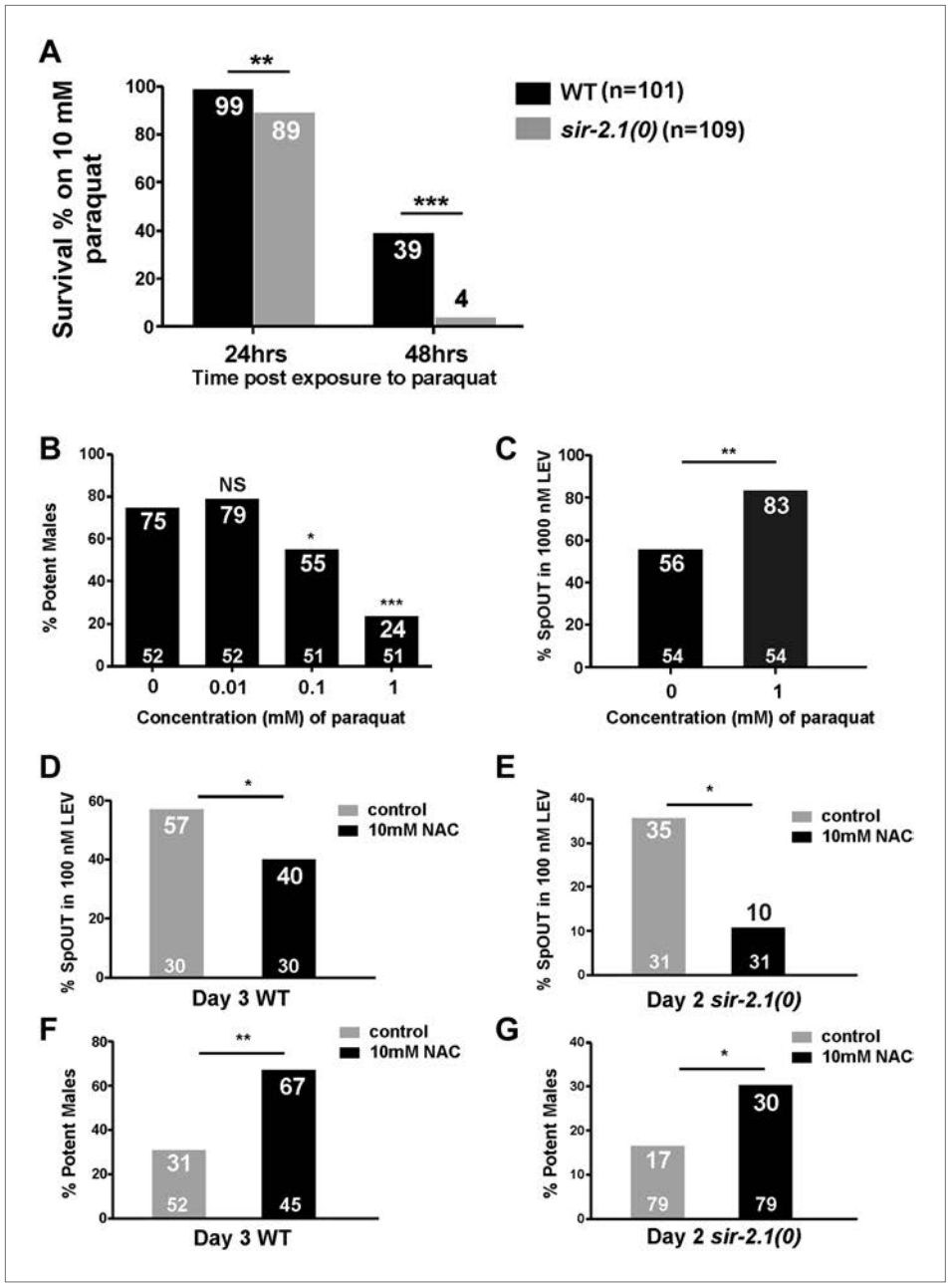

Figure 4. ROS contributes to the mating deterioration. (**A**) Survival rates of wild-type and *sir-2.1(0)* males on NGM containing 10 mM paraquat at 24 hr and 48 hr post paraquat exposure. (**B**) Mating potency of 1-day-old wild-type males exposed to 0.01, 0.1, and 1 mM paraquat. (**C**) The percentages of males with their spicules protruding out (SpOUT) in response to 1 μM levamisole (LEV) after treatment with 1 mM paraquat. (**D**–**G**) Exposing males to N-acetyl-cystine (NAC) improves mating. The percentages of 3-day-old wild-type (**D**) and 2-day-old *sir-2.1(0)* (**E**) males that protrude their spicules out in response to 100 nM LEV after NAC exposure. Mating potency of 3-day-old wild-type (**F**) and 2-day-old *sir-2.1(0)* (**G**) males after NAC exposure (Fisher's exact test).

DOI: 10.7554/eLife.01730.014

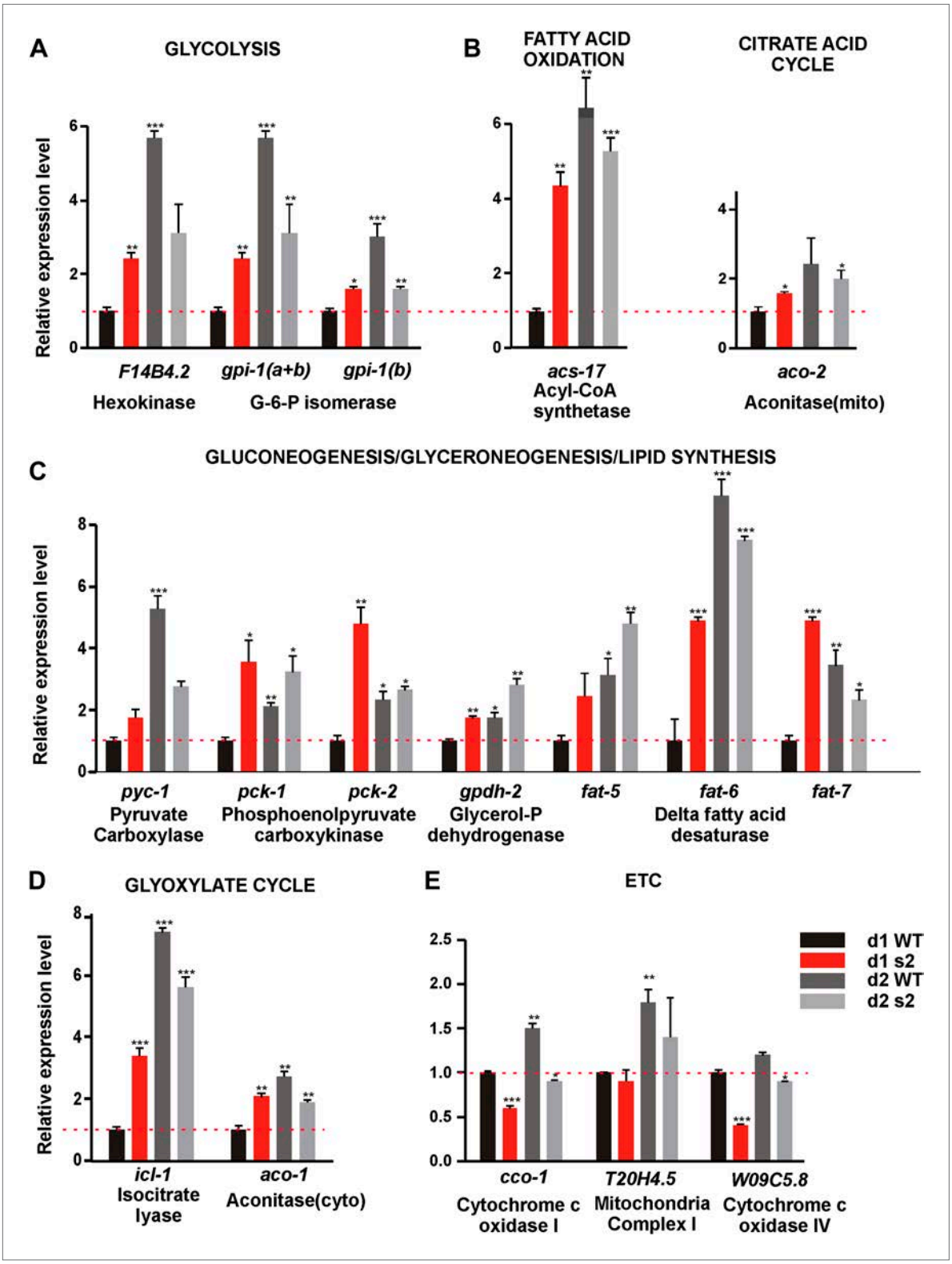

Figure 5. *sir-2.1(0)* males have altered expression of metabolic genes. Relative mRNA expression level of genes involved in metabolic processes such as glycolysis (**A**), TCA cycle (**B**), fatty acid oxidation(**C**), Gluconeogenesis/glyceroneogenesis/lipid synthesis (**D**), Glyoxylate cycle (**E**), and ETC (**F**) in 2-day-old wild type, 1-day-old, and 2-day-old sir-2.1(0) males relative to 1-day-old wild type. d1 WT refers to day1 wild type; d2 WT refers to day 2 wild type; d1 s2 refers to day1 *sir-2.1(0)*; d2 s2 refers to day 2 *sir-2.1(0)* (unpaired t-test compared to 1-day-old wild type).

DOI: 10.7554/eLife.01730.015

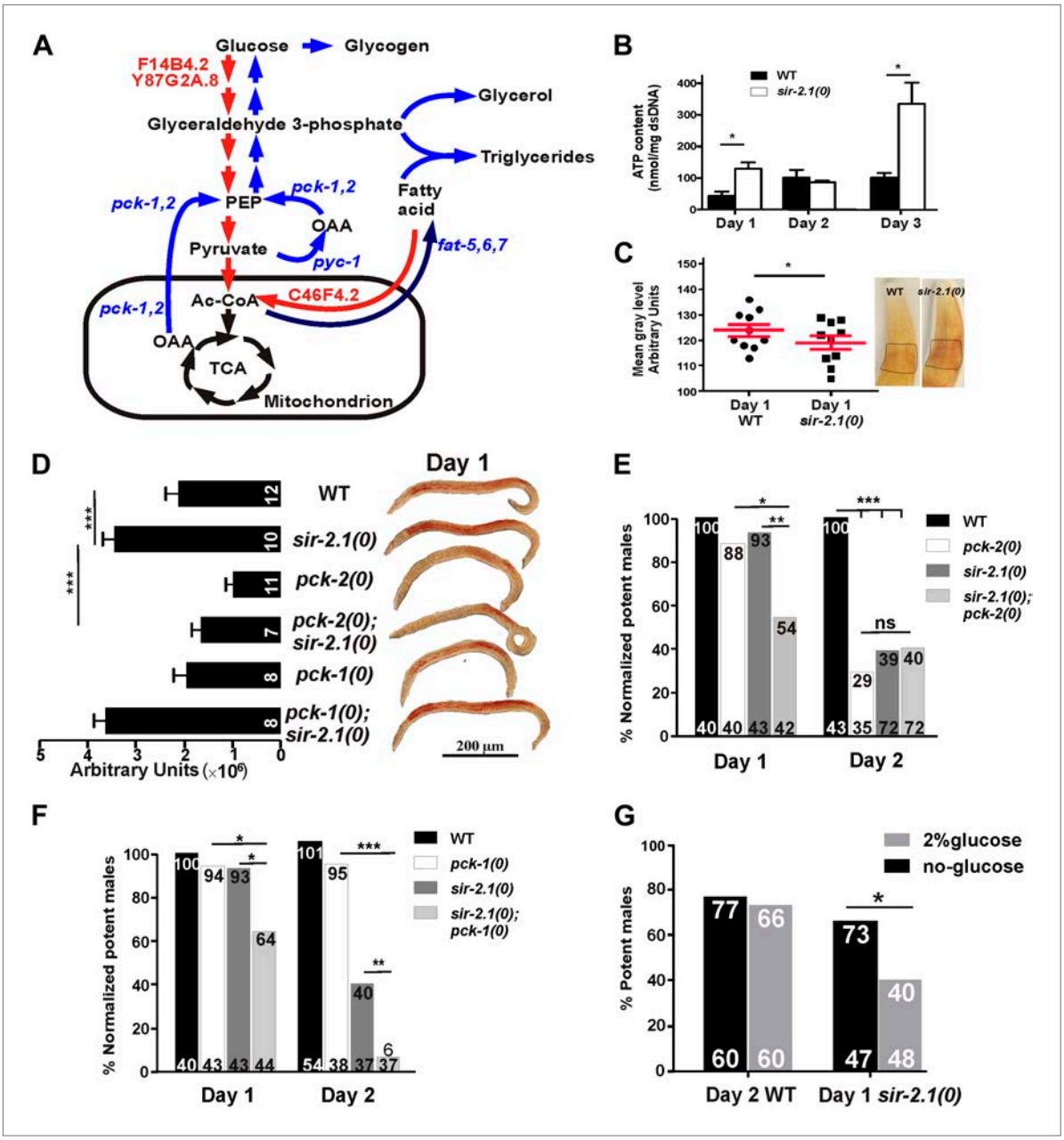

Figure 6. *sir-2.1(0)* males might have enhanced metabolism. (**A**) Schematic illustration of main metabolic enzymes which have altered expression in *sir-2.1(0)* males. Red arrows indicate catabolic pathways. Blue arrows indicate anabolic pathways. (**B**) ATP content measured in 1, 2 and 3-day-old wild-type and *sir-2.1(0)* males. (**C**) Glycogen staining in 1-day-old wild type and *sir-2.1(0)*. The glycogen staining level is quantified by measuring the mean gray level of the ROI indicated on the top right corner. The mean gray level is inversely correlated with the iodine stain. (**D**) Oil Red O staining of wild type and mutant *C. elegans* males. (**E**) *sir-2.1(+)* and *sir-2.1(0)* need *pck-2* to maintain their mating at day 2 and day 1 respectively. All percentages of mating potency are normalized to that of 1-day-old wild-type male. (**F**) *sir-2.1(0)* requires *pck-1* to maintain their mating at day 1 and day 2, while *sir-2.1(+)* males do not need *pck-2* to maintain their mating at either day 1 or day 2. (**G**) 2% glucose reduces 1-day-old *sir-2.1(0)* mating potency, but not 2-day-old wild-type males (Fisher's exact test).

DOI: 10.7554/eLife.01730.016

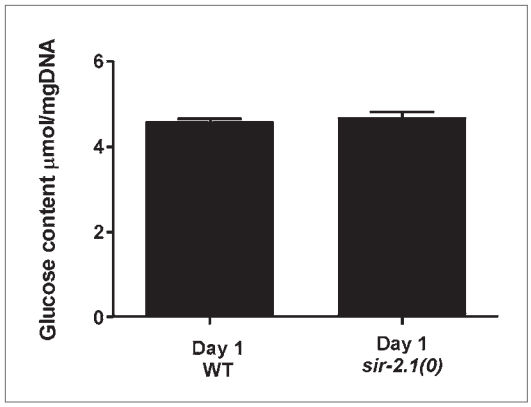

Figure 6—figure supplement 1. The level of glucose content is similar between 1-day-old *sir-2.1(0)* and wild-type males.

DOI: 10.7554/eLife.01730.017

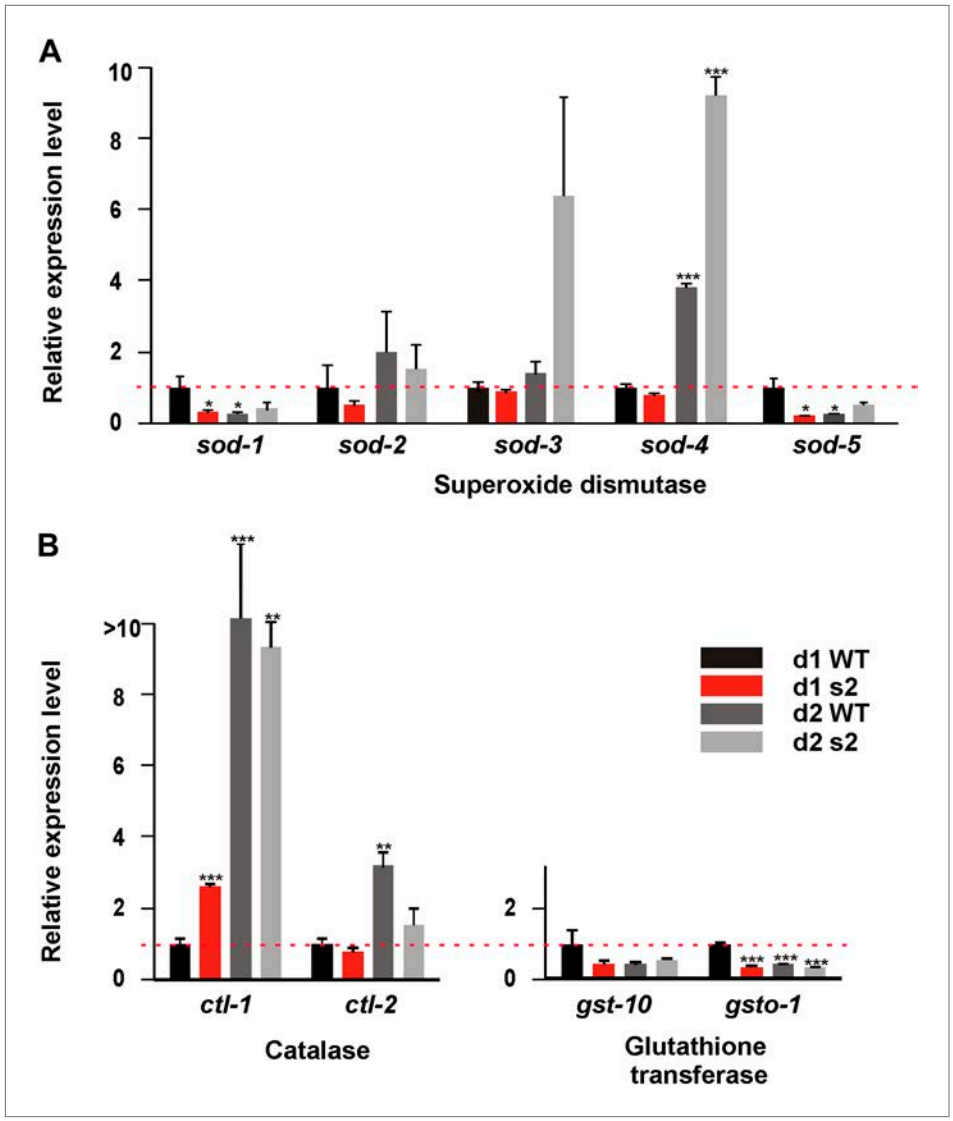

Figure 7. *sir-2.1(0)* males have compromised expression of anti-oxidant genes. Relative mRNA expression level of anti-oxidant genes superoxide dismutase (**A**), catalase (**B**), and glutathione transferase (**C**) in 1, 2-day-old wild type and *sir-2.1(0)* males (unpaired t-test).

DOI: 10.7554/eLife.01730.018

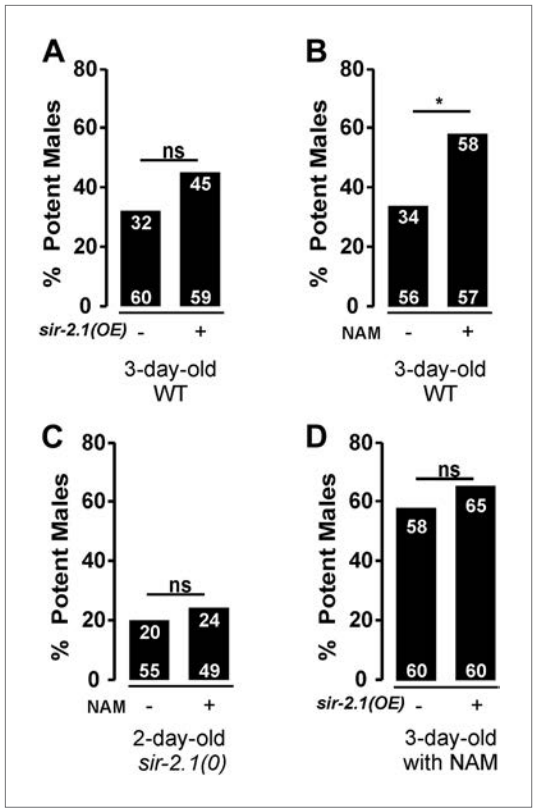

Figure 8. Exogenous nicotinamide improves mating during aging. *sir-2.1* overexpression cannot increase mating potency of 3-day-old wild type (**A**). However, feeding with a NAD^+ precursor nicotinamide (NAM) significantly improve the mating potency of 3-day-old wild type (**B**) but not 2-day-old *sir-2.1(0)* males (**C**). Overexpression of *sir-2.1* cannot further promote the effect of exogenous NAM (**D**).

DOI: 10.7554/eLife.01730.019

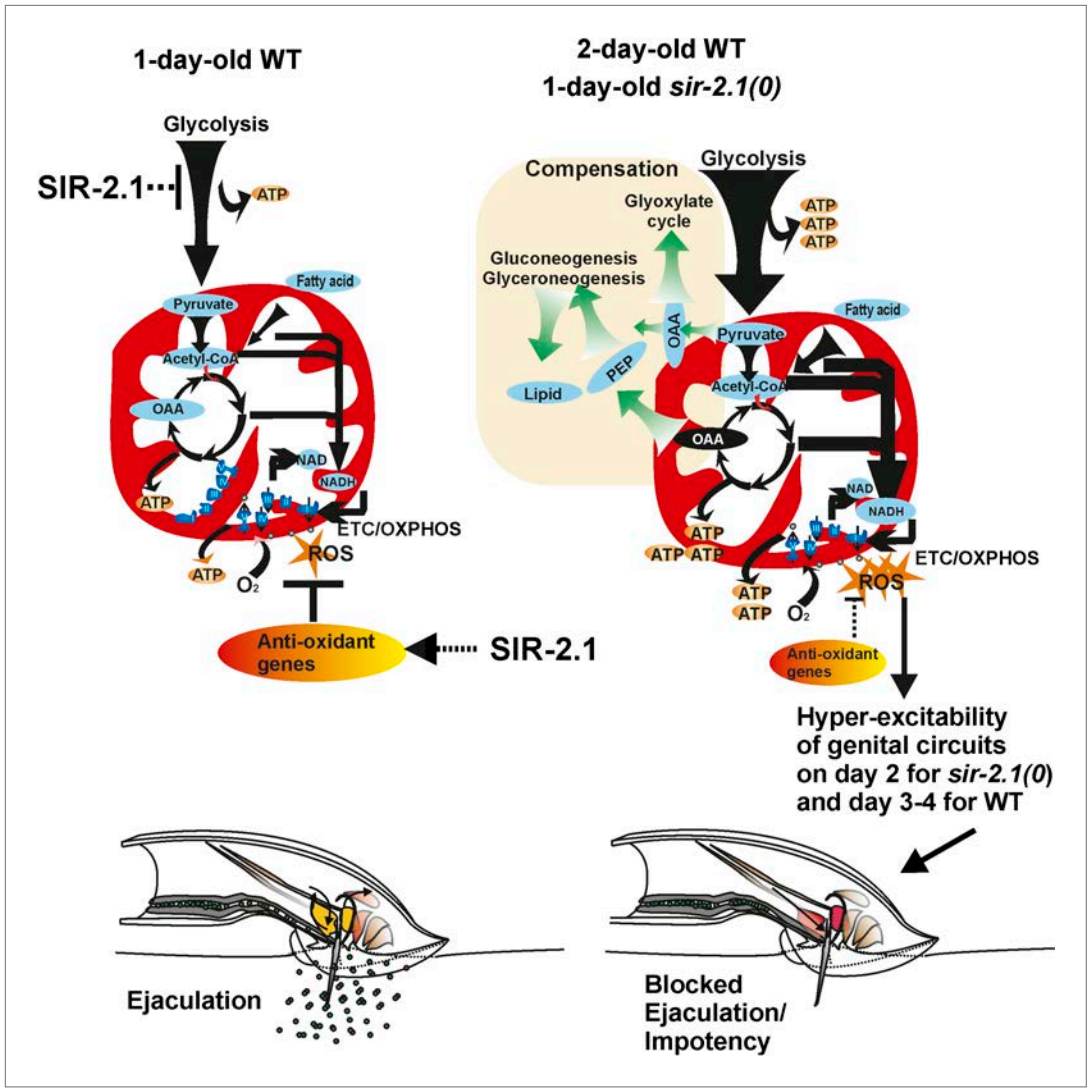

Figure 9. A cartoon of the metabolism and behavior that occurs in wild-type and *sir-2.1(0)* males during early aging. For successful reproductive behavior, SIR-2.1 is required to maintain proper carbon flow to meet the male's energy demands and balance the generation of ROS. In 1-day-old old *sir-2.1(0)* males, catabolism such as glycolysis and fatty acid oxidation is enhanced, and consequently, oxidative phosphorylation and generation of ROS are also increased. Without SIR-2.1, ROS accumulation by day 2 of adulthood can lead to hyperexcitability of the male's genital neuromuscular circuitry. This results in blocked ejaculation and impotency. It is possible that in 2- to 3-day-old wild-type males, the NAD^+-dependent SIR-2.1 activity declines due to a lower ratio of NAD to NADH; thus older wild-type males might have a similar physiology as 1-day-old *sir-2.1(0)* males.

DOI: 10.7554/eLife.01730.020