Supplementary Figures

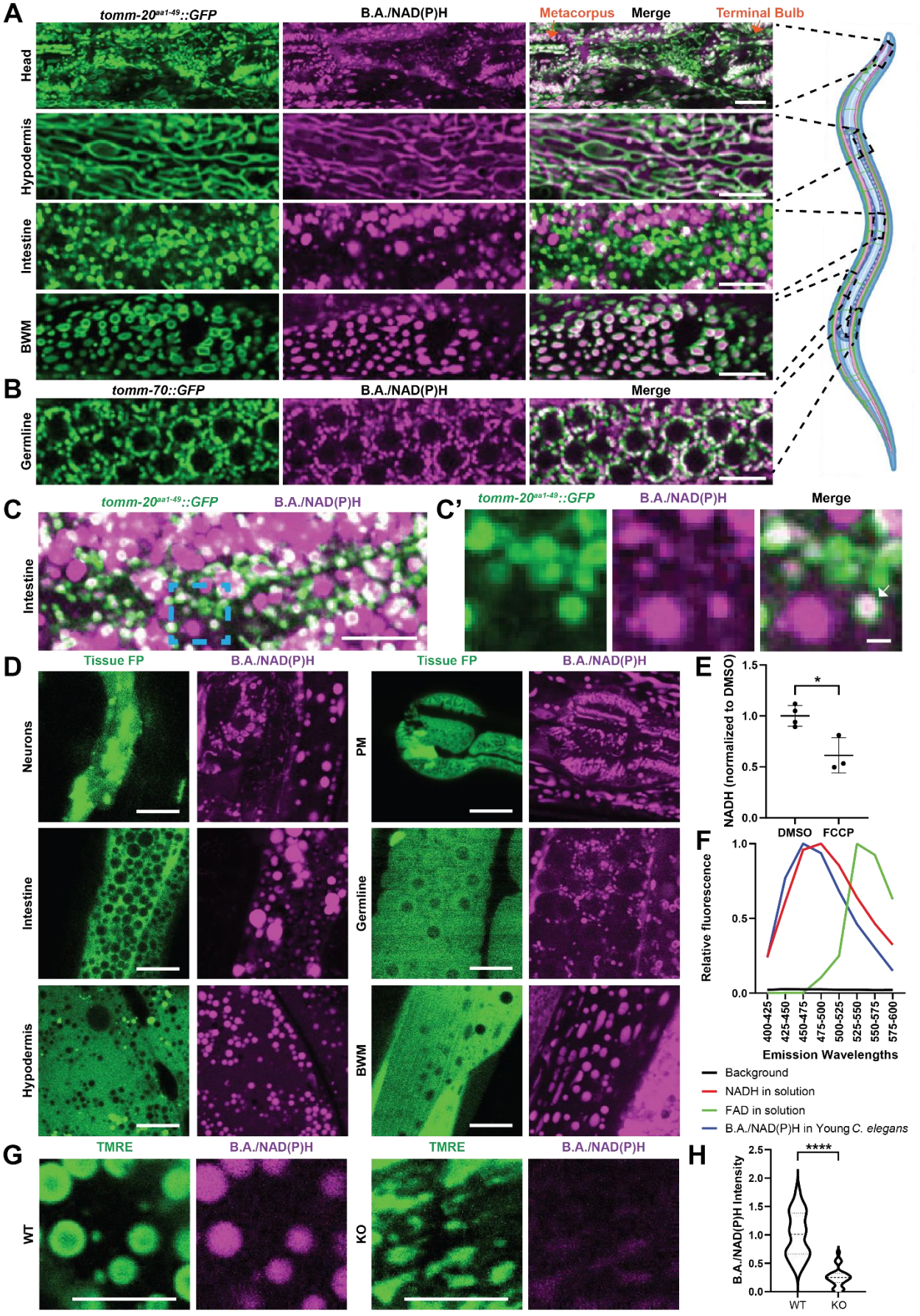

Figure S1 – Related to Figure 1. A-B) B.A./NAD(P)H (magenta) and TOMM-20^{aa1-49}::GFP (A; green) or *tomm-70::GFP* (B; green) images in head, hypodermis, intestine, BWM and germline cells of day 1 adult *C. elegans*. Orange arrows denote the metacorpus or terminal bulb of the PM. The *C. elegans* schematic to the right outlines the approximate location where each image was acquired throughout the *C. elegans* body. Created in BioRender. Morrow, C. (2024) https://BioRender.com/v72k420. C) A B.A./NAD(P)H (magenta) and TOMM-20^{aa1-49}::GFP (green) image of the intestine of day 1 adult *C. elegans*. Blue dashed square denotes inset location in C'. D) Images of B.A./NAD(P)H (magenta) in day 1 adult *C. elegans* harboring cassettes for tissue specific expression of a fluorescent protein localized to the cytosol of neuronal, intestinal, hypodermal, pharyngeal muscle, body wall muscle or germline cells (green). E) *C. elegans* were treated with either 1% DMSO or 10 mM FCCP for 15 minutes and then biochemically analyzed for NADH levels (N=3-4; Student's t test; mean ± SD). F) Emission intensity of NADH or FAD dissolved in solution or mitochondrial B.A./NAD(P)H in young (Day 1) *C. elegans* when excited by a 750 nm laser. G-H) WT or *T20D3.5/slc-25a51* deletion allele *ve652* (KO) *C. elegans* were treated with tetramethylrhodamine, ethyl ester (TMRE) to visualize mitochondria and then imaged and analyzed for B.A./NAD(P)H intensity in mitochondria in BWM cells. TMRE images were adjusted so that mitochondria could be seen in each condition and are not comparable across genotypes (n=22-28 *C. elegans*, Student's t test). Scale bars, 10 μm (A-D, G) 1 μm (C'). *$p < 0.05$.

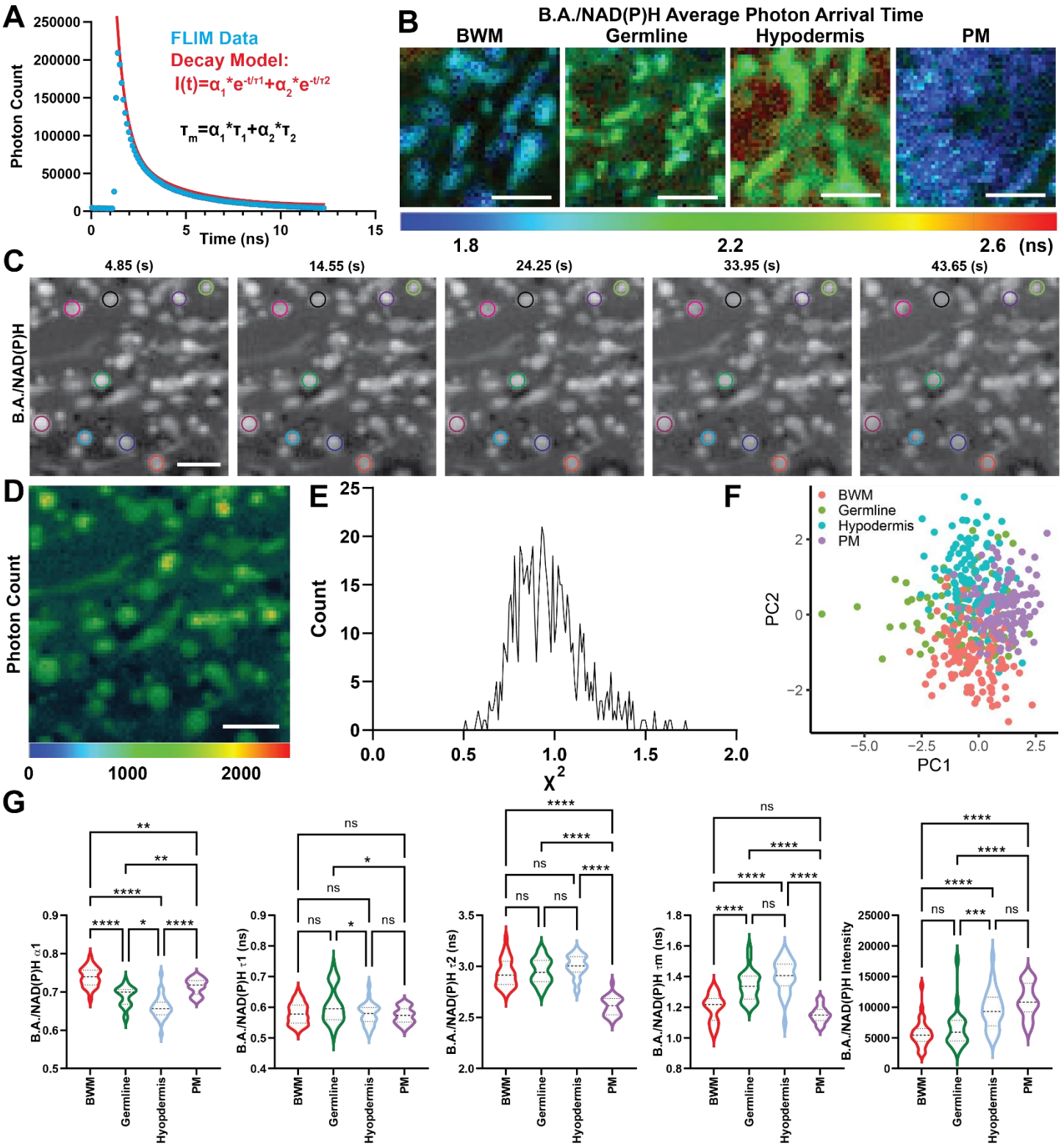

Figure S2 – Related to Figure 1. A) Representative FLIM data (blue) with a line of best fit (red) modeling the fluorescence decay and equations used to analyze B.A./NAD(P)H FLIM decay data. Tm represents the fluorescence lifetime. B) Representative FLIM images displaying the average B.A./NAD(P)H photon arrival time in germline, PM, BWM, and hypodermal cells in day 1 adult *C. elegans*. C) A time-lapse of B.A./NAD(P)H in the hypodermis across data collection for a FLIM image showing that mitochondria in *C. elegans* are lowly motile throughout ~1 minute during FLIM acquisition and can be analyzed on an individual level. D) Photon count image

for an example B.A./NAD(P)H FLIM image in the *C. elegans* hypodermis showing the number of photons collected in a representative FLIM image. E) Histogram of average χ2 values per mitochondria from day 1 adult *C. elegans* analyzed in this study showing the quality of fit by 2-component decay to B.A./NAD(P)H FLIM data. F-G) PCA (F) of non-redundant B.A./NAD(P)H FLIM endpoints (α1, T1, T2 and intensity) and plots (G) of B.A./NAD(P)H FLIM endpoints across germline, PM, BWM, and hypodermal cells of Day 1 adult WT *C. elegans* (n=22-30 *C. elegans* and 88-124 mitochondria per condition; Two-way ANOVA with post-hoc Tukey's test). Scale bars, 10 μm. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

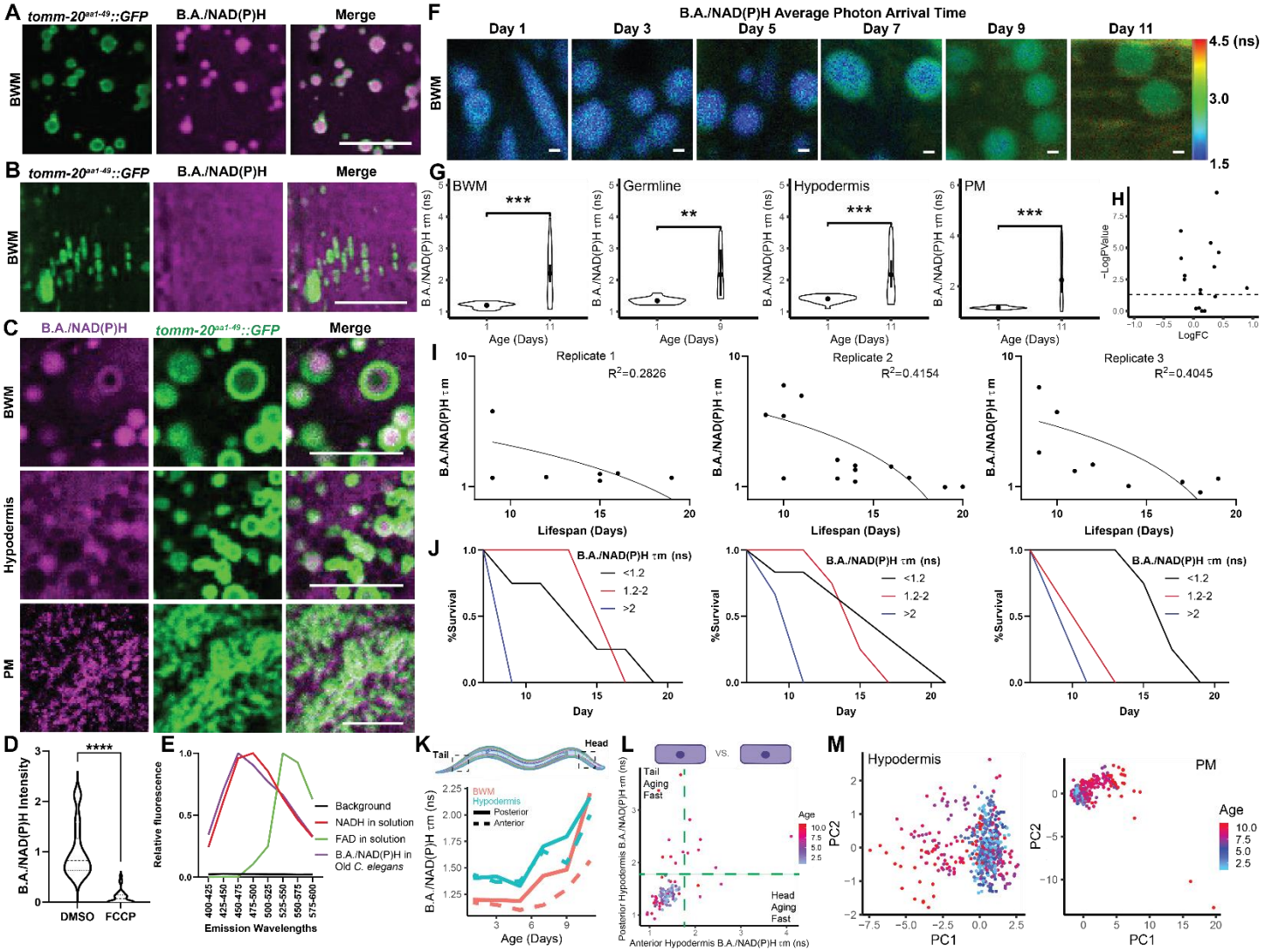

Figure S3 – Related to Figure 3. A-B) Two example images of B.A./NAD(P)H (magenta) and *tomm-20*$^{aa1-49}$*::GFP* (green) in BWM of day 11 adult *C. elegans*. A displays an example of a *C. elegans* at Day 11 where B.A./NAD(P)H is still enriched inside mitochondria and thus mitochondria can still be tracked, whereas B shows an example of a *C. elegans* at Day 11 that no longer has a clearly enriched mitochondrial B.A./NAD(P)H signal. C) Old (Day 11) *C. elegans* harboring a *tomm-20*$^{aa1-49}$*::GFP* expression cassette expressed ubiquitously (green) were imaged for B.A./NAD(P)H autofluorescence in the hypodermis, BWM and PM, showing that B.A./NAD(P)H puncta at Day 11 in each respective tissue still constitute mitochondria. D) Old (Day 11) *C. elegans* were treated with 1% DMSO or 10 mM FCCP and then imaged and analyzed for mitochondrial B.A./NAD(P)H fluorescence intensity (n=26-31 *C. elegans* per condition; Student's t test). E) Emission intensity of NADH or FAD dissolved in solution, or mitochondrial B.A./NAD(P)H in old (Day 11) *C. elegans* when excited by a 750 nm laser. F) Example images of B.A./NAD(P)H average photon arrival time in BWM mitochondria throughout aging (related to Fig. 1 analyses). G) B.A./NAD(P)H lifetimes (Tm) of young (Day 1) or old (Day 9 or 11) across germline, PM, BWM, and hypodermal cells (n=5-32 *C. elegans* per condition; moderated t test; mean ± SD). H) Volcano plot summarizing

statistical analyses of B.A./NAD(P)H FLIM endpoints comparing Day 1 and Day 11 *C. elegans* (moderated t test; related to Figure 3). I-J) Data in Fig. 2I-J separated by individual replicate. K) B.A./NAD(P)H FLIM lifetimes in anterior (dashed line) or posterior (solid line) hypodermal (blue) and BWM (red) cell mitochondria tracked across age (n=9-32 *C. elegans* per condition; median). L) Plot of anterior versus posterior hypodermal B.A./NAD(P)H lifetime colored by age. Each dot represents an individual *C. elegans* with each axis reporting the B.A./NAD(P)H lifetime (Tm) for each location in the hypodermis respectively within the same *C. elegans*. Green lines mark the edge of the young lifetime distribution (n=110 *C. elegans*). M) PCA plots of non-redundant B.A./NAD(P)H FLIM endpoints (α1, T1, T2 and intensity) colored by age from the hypodermis or PM. Each dot represents an individual mitochondrion (n=52-128 mitochondria across 15-32 *C. elegans* per condition). Scale bars, 10 μm (A,B,C) 1 μm (F). ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$.

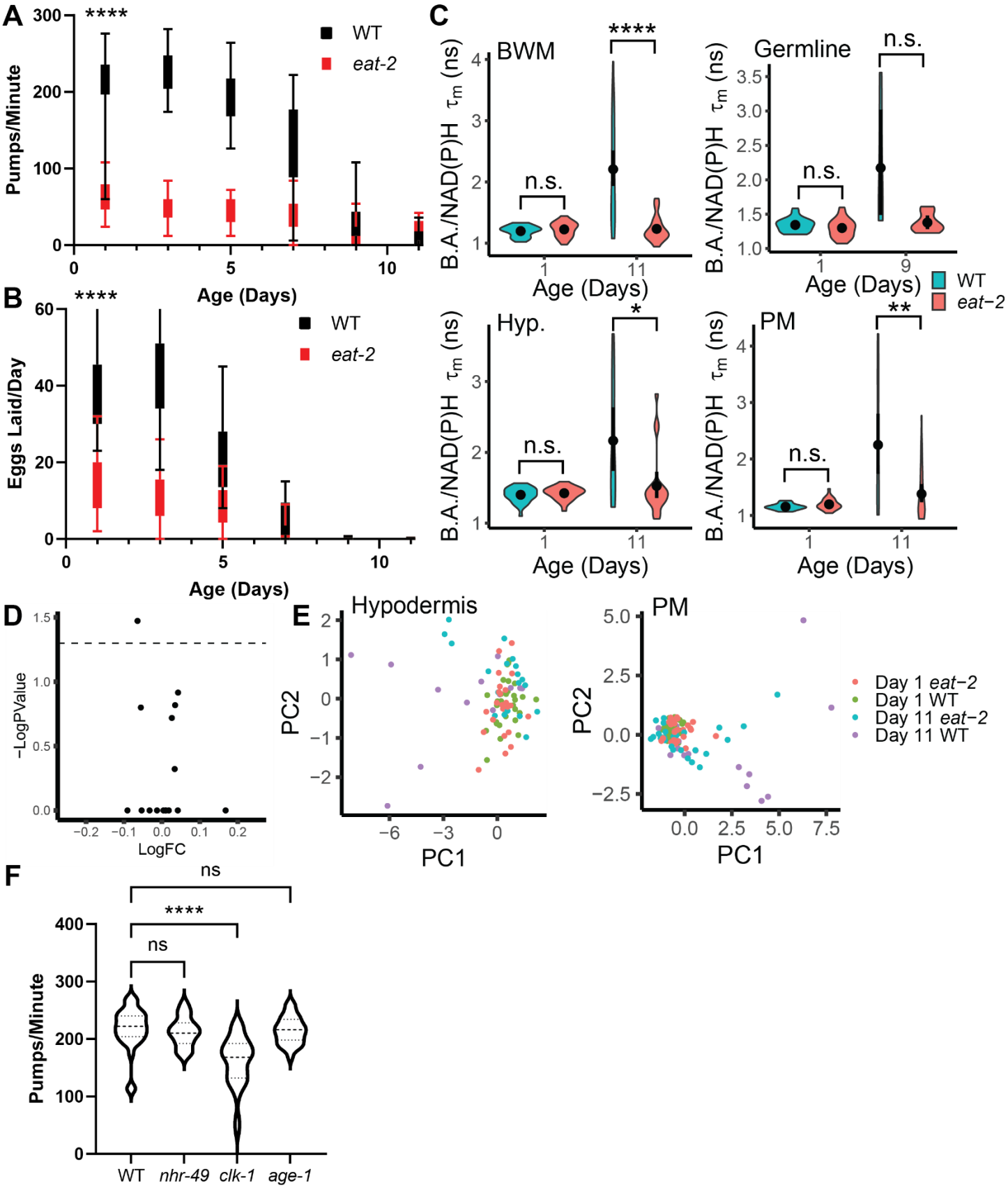

Figure S4 – Related to Figure 4. A-B) Plots of pharyngeal pumping rate (A) and egg laying rate (B) versus age in WT (black line) and *eat-2* (red line) *C. elegans* (n=29-30 *C. elegans* per condition, Two-way ANOVA with post-hoc Tukey's test; mean ± SD). C) Plots of B.A./NAD(P)H lifetimes in germline, PM, BWM, and hypodermal cell

mitochondria in young (Day 1) or old (Day 9 or 11) WT (blue) or *eat-2* (red) *C. elegans* (n=5-32 *C. elegans* per condition; moderated t test; mean ± SD). D) Volcano plot summarizing statistical analyses of B.A./NAD(P)H FLIM endpoints comparing Day 1 WT and *eat-2 C. elegans* to each other (moderated t test). E) PCA plots of non-redundant B.A./NAD(P)H FLIM endpoints (α1, T1, T2 and intensity) of Day 1 and Day 11 WT and *eat-2 C. elegans* PM and hypodermal mitochondrial B.A./NAD(P)H FLIM endpoints. Each dot is an individual *C. elegans* (n=17-30 *C. elegans* per condition). F) Day 2 adult WT, *nhr-49*, *clk-1* and *age-1* mutant *C. elegans* were analyzed for pharyngeal pumping rate (n=30 *C. elegans* per condition; Two-way ANOVA with post-hoc Tukey's test). ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$.