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Modulation of Longevity and Tissue Homeostasis by the *Drosophila* PGC-1 Homolog

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SUMMARY

In mammals, the PGC-1 transcriptional coactivators are key regulators of energy metabolism, including mitochondrial biogenesis and respiration, which have been implicated in numerous pathogenic conditions, including neurodegeneration and cardiomyopathy. Here, we show that overexpression of the *Drosophila* PGC-1 homolog (*dPGC-1/spargel*) is sufficient to increase mitochondrial activity. Moreover, tissue-specific overexpression of *dPGC-1* in stem and progenitor cells within the digestive tract extends life span. Long-lived flies overexpressing *dPGC-1* display a delay in the onset of aging-related changes in the intestine, leading to improved tissue homeostasis in old flies. Together, these results demonstrate that *dPGC-1* can slow aging both at the level of cellular changes in an individual tissue and also at the organismal level by extending life span. Our findings point to the possibility that alterations in PGC-1 activity in high-turnover tissues, such as the intestine, may be an important determinant of longevity in mammals.

INTRODUCTION

A progressive loss of mitochondrial energetic capacity is a common feature of multiple aspects of aging (Wallace, 2005). This may result from the age-related decline in the expression of genes important for mitochondrial electron transport chain (ETC) function observed in diverse organisms including humans (McCarroll et al., 2004; Zahn et al., 2006). A causal relationship is suggested by the fact that alterations in ETC activity are emerging as integrating phenomena in a number of life span-extending manipulations including dietary restriction (DR) (Guarente, 2008) and reduced insulin/TOR signaling (Bonawitz et al., 2007; Katic et al., 2007). More specifically, DR has been observed to result in an increase in mitochondrial biogenesis and/or respiratory activity in yeast, worms, flies, mice, and humans (Bishop and Guarente, 2007; Civitarese et al., 2007; Lin et al., 2002; López-Lluch et al., 2006; Nisoli et al., 2005; Zid et al., 2009). Furthermore, perturbation of mitochondrial ETC components has been shown to impair the ability of DR to promote longevity in yeast, worms, and flies (Bahadorani et al., 2010; Bishop and Guarente, 2007; Lin et al., 2002; Zid et al., 2009). These findings suggest that strategies to enhance mitochondrial biogenesis and/or energy metabolism may promote healthy aging.

In mammals, the PGC-1 family of transcriptional coactivators plays a central role in the regulation of mitochondrial biogenesis, respiration, and glucose homeostasis (Lin et al., 2005; Scarpulla, 2008b). Three members of this family have been identified based on sequence similarity to the founding member PGC-1α. PGC-1 family members promote mitochondrial biogenesis through coactivation of nuclear transcription factors, including nuclear respiratory factor-1 and -2 (NRF-1 and NRF-2) and estrogen-related receptor-α (ERRα) to induce the expression of genes encoding mitochondrial proteins (Puigserver and Spiegelman, 2003; Scarpulla, 2008a). Increased PGC-1 gene activity has been associated with health benefits in a number of pathogenic conditions, including various muscular (Handschin et al., 2007; Wenz et al., 2008) and neurodegenerative disorders (Cui et al., 2006; Zheng et al., 2010). Furthermore, increased expression of PGC-1α has been shown to protect against age-related sarcopenia (Wenz et al., 2009) and to improve respiration and gluconeogenesis under conditions of telomere dysfunction (Sahin et al., 2011). However, the role of PGC-1 coactivators in determining longevity remains poorly understood.

The fruit fly *Drosophila melanogaster* is an excellent model system to study the role of mitochondrial activity in the aging process (Cho et al., 2011). In addition, *Drosophila* has emerged as a premier model system to dissect the relationship between altered stem cell behavior, tissue homeostasis, and aging (Wang and Jones, 2011). Several discrete populations of adult stem cells have been reported in *Drosophila*, such as germline stem cells and intestinal stem cells (ISCs) in the midgut (reviewed in Wang and Jones, 2011). These stem cells reside in defined niches and play active roles in maintaining local tissue homeostasis, resembling the behavior of mammalian stem cells.

Here, we report that upregulation of the *Drosophila* PGC-1 homolog (*dPGC-1/spargel*) leads to an increase in multiple

markers of mitochondrial abundance and activity both during development and also in the adult stage. Furthermore, we find that targeted overexpression of *dPGC-1* in the digestive tract, including restricted expression in somatic stem cells (including ISCs) and immediate daughter cells, can extend adult life span. Upregulation of *dPGC-1* abrogates the precocious activation of ISC proliferation and delays the accumulation of misdifferentiated cells in the intestinal epithelium—two hallmarks of aging in this tissue. Furthermore, *dPGC-1* upregulation leads to improved intestinal integrity in old flies. Our findings demonstrate that *dPGC-1* gene activity is an important determinant of aging both at the tissue and organismal level.

RESULTS

Overexpression of *Drosophila* PGC-1 Leads to an Increase in Mitochondrial Activity

The *Drosophila* genome contains a single PGC-1 homolog, CG9809/*spargel*/*dPGC-1* (Gershman et al., 2007). A loss-of-function study reported that *dPGC-1* is required for the normal expression of multiple genes encoding mitochondrial proteins in the larval fat body (Tiefenböck et al., 2010); therefore, we predicted that increased *dPGC-1* might lead to an increase in mitochondrial abundance and/or activity. To investigate the physiologic and phenotypic consequences of overexpression of *dPGC-1*, we expressed *dPGC-1* using the GAL4/UAS system (Brand and Perrimon, 1993). We transformed flies with UAS-constructs containing the *dPGC-1* complementary DNA and performed 12 rounds of backcrossing into a w^{1118} background, which was used as a control strain in subsequent experiments. We confirmed that the *dPGC-1* transcript was upregulated in flies carrying both the *dPGC-1* transgene and a ubiquitous GAL4 driver line, *daughterless (da)*-GAL4 (Figures S1A and S1B available online). Increased expression of *dPGC-1* did not produce any gross changes in body size (Figure S1C) or obvious differences in size, morphology, or cell number of external structures, such as wings (Figure S1D).

To determine whether upregulation of *dPGC-1* can increase mitochondrial activity, we measured three independent mitochondrial markers: the amount of mitochondrial DNA (mtDNA), the enzymatic activity of citrate synthase, a key enzyme in the Krebs cycle and a widely used marker for mitochondrial density, and the abundance of HSP60, a mitochondrial matrix protein. First, we measured the amount of mtDNA, relative to the amounts of a nuclear DNA (nDNA) amplicon, in third-instar larvae and observed a 2.5-fold increase in response to *dPGC-1* upregulation (Figure 1A). This data are consistent with a *dPGC-1*-mediated increase in mitochondrial density per cell. Thoraxes, which consist primarily of flight muscle (a rich source of mitochondria), were used for the measurement of changes in mitochondrial abundance in adult flies: a 60% increase in mtDNA in response to *dPGC-1* upregulation was observed in adult thoraxes (Figure 1B). In accordance, there was a significant increase in citrate synthase activity in both larvae (Figure 1C) and adult thoraxes in *dPGC-1* overexpressing animals (Figure 1D). Similarly, western blots with antibodies against a mitochondrial matrix protein, HSP60, showed significant increases of HSP60 levels in both whole larvae and adult thoraxes of animals overexpressing *dPGC-1*, when normalized to loading control (Figures 1E and 1F).

Next, we sought to address whether *dPGC-1* can stimulate mitochondrial oxidative metabolism. We employed blue native polyacrylamide gel electrophoresis (BN-PAGE) to examine the impact of upregulation of *dPGC-1* on the abundance of the respiratory chain enzyme complexes. A significant increase in the abundance of respiratory complexes I, III, IV, and V was observed in flies overexpressing *dPGC-1* (Figure 2A). To analyze the effect of elevated *dPGC-1* expression on respiratory chain activity, the rate of oxygen consumption was measured with a Clark-type oxygen electrode. The steps in respiration were compared in mitochondria isolated from *dPGC-1*-overexpressing flies and controls with substrates and inhibitors specific to individual respiratory complexes. In doing so, we observed that *dPGC-1* upregulation confers an increase in complex I-, II-, and IV-dependent respiration (Figure 2B and Figure S1E). Moreover, the respiratory control ratio (RCR), or state 3:state 4 respiration ratio, was significantly higher in mitochondria isolated from flies with increased *dPGC-1* expression (Figure 2C). Taken together, our data suggest that *dPGC-1* overexpression is sufficient to increase mitochondrial biogenesis and bioenergetic efficiency.

Drosophila PGC-1 Modulates Metabolic Stores and Free Glucose Levels

In mammals, PGC-1α regulates glucose homeostasis (Herzig et al., 2001; Yoon et al., 2001) and triglyceride (TAG) metabolism (Zhang et al., 2004). To determine whether *dPGC-1* also regulates fuel homeostasis, we examined metabolic stores and free glucose levels in *dPGC-1* overexpressing flies and controls. In *Drosophila*, metabolized nutrients are primarily stored as TAG and glycogen in the fat body, the insect equivalent of the mammalian liver and white adipose tissue. Using thin-layer chromatography, the most accurate method to measure stored TAG in *Drosophila* (Al-Anzi and Zinn, 2010), we observed ~20% reduction in TAG levels in *dPGC-1*-overexpressing flies (Figure 2D). In contrast, there was a significant increase in both the amount of stored glycogen (Figure 2E) and free glucose levels (Figure 2F) in *dPGC-1* overexpressing flies. Our data suggests that *dPGC-1* may play an important role in maintaining energy homeostasis in the fly-consistent with findings in mammals.

Effects of Tissue-Specific Overexpression of *Drosophila* PGC-1 on Longevity

As central regulators of energy homeostasis, PGC-1 coactivators provide an attractive target to modulate animal aging. Therefore, we examined the impact of targeted overexpression of *dPGC-1* in major tissues on *Drosophila* life span. First, we examined the effects of ubiquitous overexpression of *dPGC-1* mediated by *da-GAL4* and observed a moderate decrease in adult survival (Figures S2A and S2B). Similar effects were observed when *dPGC-1* was ubiquitously expressed using the mifepristone (RU486)-inducible driver Tubulin-Gene-Switch (Figure 3A and Figure S2C). To begin to examine tissue-specific effects of *dPGC-1*, we used a panel of Gene-Switch driver lines with recently characterized age-related expression patterns (Poirier et al., 2008). Life span was significantly increased when *dPGC-1* was induced with S_1106 (Figure 3B and Figure S2D), which is expressed in abdominal fat and the digestive tract

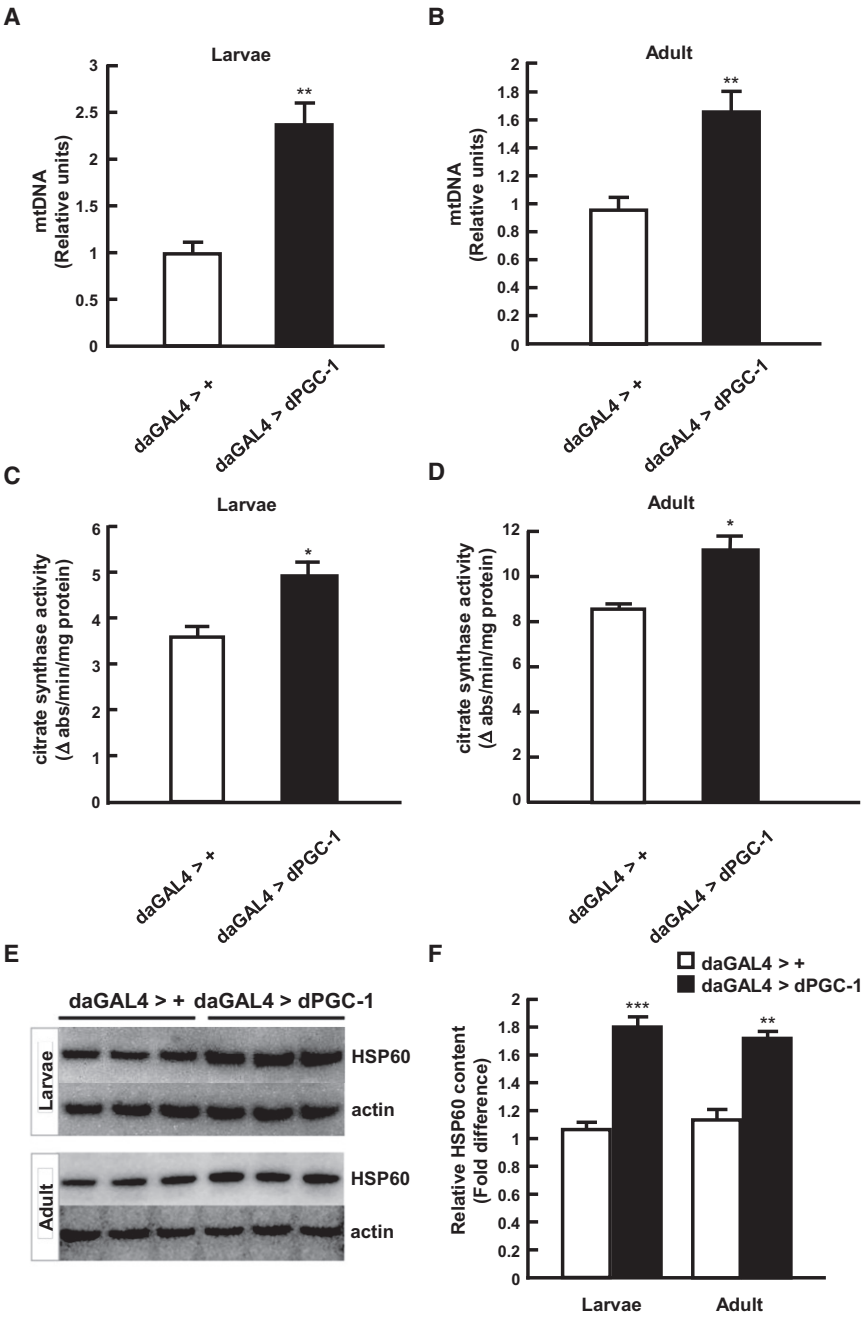

Figure 1. Overexpression of *dPGC-1* Increases Mitochondrial Markers

(A) Mitochondrial DNA (mtDNA) amount in larvae as determined by quantitative PCR (qPCR). Third-instar larvae that overexpress *dPGC-1* with *daughterless*-GAL4 driver (*UAS-dPGC-1*/*da*-GAL4) show an increase in the amount of a mtDNA amplicon (**p < 0.01, t test) when compared to controls (+/*da*-GAL4). Units are relative to the amounts of a nuclear DNA (nDNA) amplicon (n = 3, 5 larvae per replicate).

(B) mtDNA amount in adult female thoraxes as determined by qPCR. Thoraxes of female flies that overexpress *dPGC-1* show an increase in a mtDNA amplicon (**p < 0.01, t test) when compared to controls. Units are relative to the amounts of a nDNA amplicon (n = 3, 5 thoraxes per replicate).

(C) Citrate synthase (CS) activity in larvae. Third-instar larvae that overexpress *dPGC-1* show an increase in CS activity (*p < 0.05, t test) relative to controls (n = 3, 6 larvae per replicate).

(D) CS activity in adult female thoraxes. Thoraxes of female flies that overexpress *dPGC-1* show an increase in CS activity (*p < 0.05, t test) relative to controls (n = 3, 10 thoraxes per replicate).

(E) Western blot analysis of the mitochondrial matrix protein HSP60. Third-instar larvae and thoraxes of adult female flies that overexpress *dPGC-1* show increased ratios of mitochondrial HSP60 signal to actin signal (loading control), relative to controls.

(F) Quantification of HSP60 western blot analysis. Densitometry measurements of (E) show increases in HSP60:actin signal ratios for both larvae (***p < 0.001, t test) and adult thoraxes of female flies (**p < 0.01, t test) that overexpress *dPGC-1*, relative to controls (n = 3, 5 larvae/thoraxes per replicate).

All assays in adults were carried out at 10 days of age. Data are represented as mean ± SEM. See also Figure S1.

(Poirier et al., 2008). To further narrow the tissue-specific requirements for *dPGC-1*-mediated longevity, we used the *TIGS-2* driver, which is expressed in the digestive tract but not fat body (Poirier et al., 2008). Induced expression of *dPGC-1* with this driver line resulted in a 33% increase in mean life span and a 37% increase in maximum life span in female flies (Figure 3C) and no major effect in male flies (Figure S2E). Notably, adult survival was not improved when *dPGC-1* was induced with the panneuronal driver ELAV-Gene-Switch (Figure 3D and Figure S2F). Similarly, muscle-specific expression of *dPGC-1* with a constitutive MHC-GAL4 driver did not promote life extension in male or female flies (data not shown). Two additional independent *dPGC-1* insertions were tested with *TIGS-2* and each resulted in enhanced longevity in female flies (Figures S2G and S2H). No major longevity effects were observed in male or female control flies exposed to RU486 (Figures S3A–S3H).

Together, the *$S_1$106* and *TIGS-2* life-span data support the idea that the digestive tract is an important target tissue in *dPGC-1*-mediated longevity.

Overexpression of *dPGC-1* in Intestinal Stem and Progenitor Cells Extends Life Span

As noted above, our tissue-specific longevity studies indicate that increased expression of *dPGC-1* in the digestive tract is sufficient to promote longevity. To validate and extend this finding, we set out to identify subsets of intestinal cells that are important in mediating this phenotype. Tissue homeostasis in the midgut is maintained by multipotent ISCs, which are distributed along

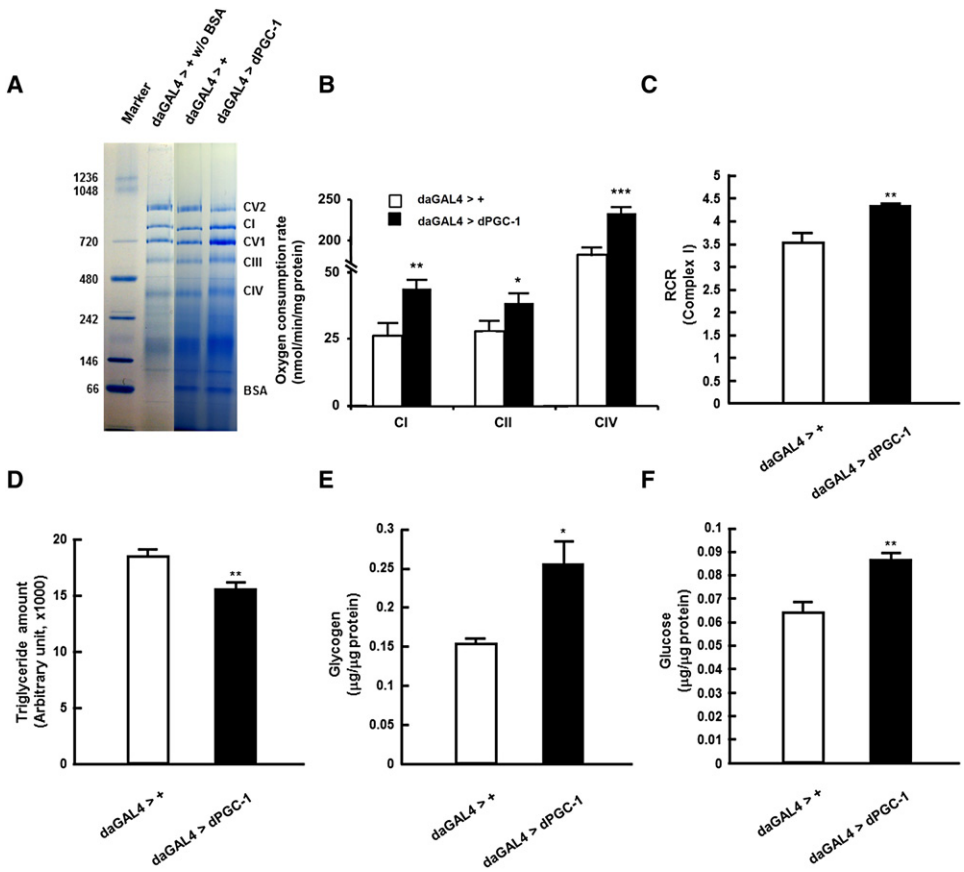

Figure 2. Regulation of Oxidative Metabolism, Metabolic Stores, and Glucose Levels by *dPGC-1*

(A) Blue-native polyacrylamide gel electrophoresis (BN-PAGE) analysis of respiratory complexes. Equal amounts of BSA (used as a loading control) were added to the total mitochondrial fraction isolated from thoraxes of female flies that overexpress *dPGC-1* (*UAS-dPGC-1/da*-GAL4) or controls (+/*da*-GAL4). Intensity of the bands corresponding to complexes I, III, IV, and V, relative to BSA, are increased in *dPGC-1* overexpressing flies compared to controls. CV2, complex V dimer; CI, complex I; CV1, complex V monomer; CIII, complex III; CIV, complex IV.

(B) Polarographic analysis of respiratory chain complex activities. Mitochondria isolated from female flies that overexpress *dPGC-1* show increased oxygen consumption by respiratory complexes I (**$p < 0.01$, t test), II (*$p < 0.05$, t test), and IV (***$p < 0.001$, t test) compared to controls (n = 6, 50 flies per replicate).

(C) ADP-coupled respiration status. Mitochondria isolated from female flies that overexpress *dPGC-1* display an increased complex I respiratory control ratio (RCR) (**$p < 0.01$, t test) relative to controls (n = 3, 50 flies per replicate).

(D) Triglyceride (TAG) content. Thin-layer chromatography and densitometry of female flies that overexpress *dPGC-1* shows a decrease in TAG content (**$p < 0.01$, t test) relative to controls (n = 3, 10 flies per replicate).

(E) Glycogen content. A colorimetric glycogen assay of female flies that overexpress *dPGC-1* shows an increase in glycogen content (*$p < 0.05$, t test) relative to controls (n = 3, 5 flies per replicate).

(F) Glucose content. A colorimetric glucose assay of female flies that overexpress *dPGC-1* shows an increase in glucose content (**$p < 0.01$, t test) relative to controls (n = 3, 5 flies per replicate).

All assays in adults were carried out at 10 days of age. Data are represented as mean ± SEM. See also Figure S1.

the basement membrane (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Division of an ISC gives rise to one daughter cell that retains stem cell fate and another daughter cell that becomes an enteroblast (EB), both expressing a transcription factor called Escargot (*esg*). Thus, expression of *esg* is often used as a surrogate marker for ISCs and EBs. After ISC division, the daughter EB does not divide again and differentiates into either a large, polyploid enterocyte (EC) or a small, diploid enteroendocrine (ee) cell that expresses Prospero (Pros) (Figure 6A). Cell-cycle arrest and differentiation of EBs are controlled by Delta-Notch signaling. While the ligand Delta (Dl) specifically accumulates in ISCs, it is quickly lost in newly formed EBs, which is accompanied by an activation of Notch signaling.

To examine the impact of targeted expression of *dPGC-1* in the ISCs and EBs, we used the constitutive *esgGAL4* driver line to overexpress *dPGC-1* and observed a significant lifespan extension in both male (Figure S4A) and female flies (Figure 4A) compared to isogenic control flies. Although *esgGal4* expression is restricted to ISCs and EBs in the intestine, it is also expressed in stem cells within malpighian tubules, germline and somatic stem cells in the testis, and in salivary glands (Biteau et al., 2010). Therefore, we made use of the recently described RU486-inducible *5961GS*, which recapitulates the *esgGal4* expression pattern in the digestive tract (ISCs/EBs and malpighian tubule stem cells) (Biteau et al., 2010; Mathur et al., 2010) but is not expressed in salivary glands (Biteau et al., 2010) or testis

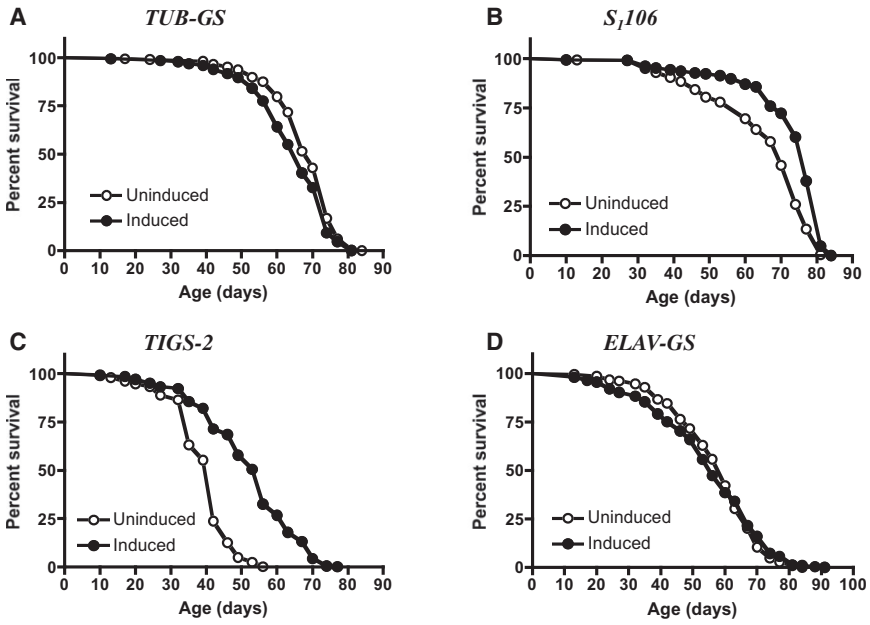

Figure 3. Effects of Tissue-Specific Overexpression of *dPGC-1* on Fly Longevity

UAS-dPGC-1 was crossed to Gene-Switch (GS) driver lines the ubiquitous *Tubulin* (*tub*)-*GS* driver (A), the abdominal fat and digestive tract driver *$S_1$106* (B), the digestive tract driver *TIGS-2* (C), and the panneuronal driver *ELAV-GS* (D), and life-span curves are shown as induced (5 μg ml^{-1} RU486 during development and 25 μg ml^{-1} RU486 from the onset of adulthood (black circles) added on top of the food or uninduced (−RU486, open circles).
(A) Life-span curves of *UAS-dPGC-1/tub-GS* females. A moderate decrease in survival was observed in response to RU486 ($p = 0.0029$).
(B) Life-span curves of *UAS-dPGC-1/ $S_1$106* females. An 11% increase in mean survival was observed in response to RU486 ($p < 0.0001$).
(C) Life-span curves of *UAS-dPGC-1/ TIGS-2* females. A 33% increase in mean survival was observed in response to RU486 ($p < 0.0001$).
(D) Life-span curves of *UAS-dPGC-1/ ELAV-GS* females. No impact on survival was observed in response to RU486 ($p = 0.8$). The significance of the difference between survival curves was analyzed with log-rank statistical test ($n > 195$ flies).

See also Figures S2 and S3. Survival data for male flies can be found in Figures S2C–S2F. Survival data for independent insertions of *dPGC-1* with *TIGS-2* can be found in Figures S2G and S2H. Survival data for control flies exposed to RU486 can be found in Figures S3A–S3H.

(C.K. and L.J., unpublished data). Consistent with a previous report (Biteau et al., 2010), we failed to detect *5961GS* expression in the brain, thorax, or abdomen. *5961GS*-mediated expression of *dPGC-1*, during both development and adulthood, resulted in a significant increase in life span (Figure 4B and Figure S4B). No major longevity effects were observed in control flies exposed to RU486 (Figures S4C and S4D). Next, we took advantage of the inducible nature the *5961GS* driver to determine whether adult-only induction of *dPGC-1* is sufficient to promote longevity. Indeed, *5961GS > dPGC-1* flies were significantly longer lived when exposed to RU486 exclusively in the adult stage (Figure 4C); again, no major effects on longevity were observed in control flies exposed to RU486 (Figure S4E). Taken together, our data with the constitutive *esgGAL4* and the inducible *5961GS* driver support a model whereby increased expression of *dPGC-1* in stem and progenitor cells within the digestive tract of adults is sufficient to extend life span.

dPGC-1 Extends Life Span Independently of Effects on Reproduction or Global Stress Resistance

To gain further insight into *dPGC-1*-mediated longevity, we examined a number of physiological and behavioral parameters in long-lived flies overexpressing *dPGC-1*. For these studies, we used *esgGAL4* because expression in the ISCs/EBs is stronger than with *5961GS* (Biteau et al., 2010), and the longevity effects are more pronounced with this driver. Importantly, there was no obvious difference in food consumption (Figure S5A) or body mass (Figure S5B) in long-lived flies compared to age-matched controls. As described earlier, we observed that ubiquitous upregulation of *dPGC-1* leads to alterations in metabolic stores and free glucose levels (Figures 2D–2F). Therefore, we examined these parameters in long-lived *esgGAL4 > dPGC-1* flies. Interestingly, *esgGAL4*-mediated expression of *dPGC-1* is sufficient to confer a decrease in triglyceride (TAG) levels in whole flies (Figure S5C). Although *esgGAL4 > dPGC-1* flies display normal glycogen stores (Figure S5D), restricted expression of *dPGC-1* is sufficient to produce a moderate increase in free glucose levels (Figure S5E).

Interventions that extend life span are often associated with a decline in reproductive output (Partridge et al., 2005). However, long-lived *esgGAL4 > dPGC-1* flies display normal fertility compared to age-matched isogenic controls (Figure S5F). Interestingly, we also failed to detect fertility defects in long-lived *dPGC-1* flies mediated by either *$S_1$106* or *TIGS-2* (S.B. and D.W., unpublished data). Another hallmark of extended longevity is an increase in the ability to withstand extrinsic stress (Lithgow and Walker, 2002). Therefore, we tested the ability of *esgGAL4 > dPGC-1* flies to survive under conditions of starvation and oxidative stress. However, we observed no difference in survival times when long-lived *dPGC-1* flies and controls were maintained on an agar-only diet to induce starvation (Figure S5G). In addition, long-lived *dPGC-1* flies displayed only a marginal increase in survival under hyperoxia (100% O_2; Figure S5H). Together, these data indicate that life extension mediated by overexpression of *dPGC-1* via *esgGAL4* acts independently of effects on reproduction or global stress resistance.

dPGC-1 Modulates Mitochondrial Activity and ROS Levels in the Aged Intestine

To better understand the relationship between *dPGC-1* gene activity and longevity, we examined *dPGC-1* messenger RNA (mRNA) levels in the intestine of young and aged flies. We observed that *dPGC-1* expression was dramatically decreased (~60%) in the intestine of aged control flies (Figure 5A). *esgGAL4 > dPGC-1* flies display an ~2-fold increase in *dPGC-1* mRNA levels in the intestine at both time points compared to

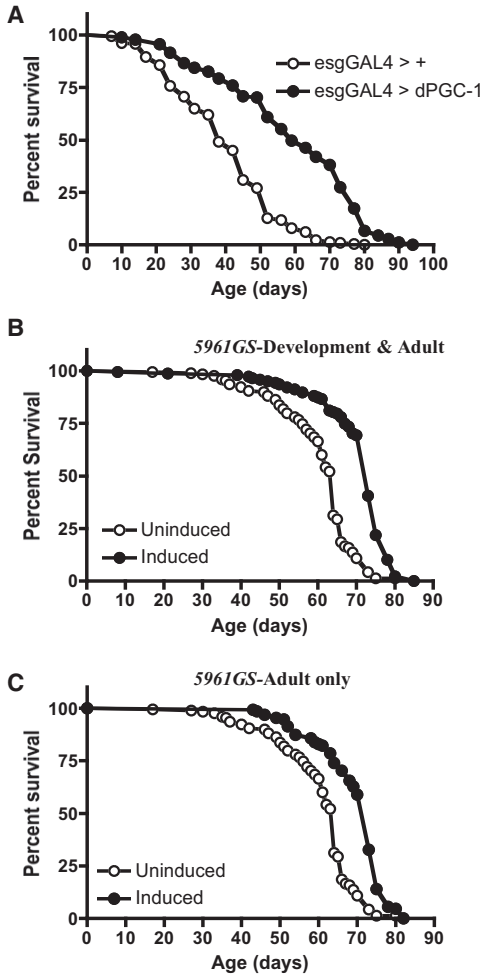

Figure 4. Overexpression of *dPGC-1* in Intestinal Stem and Progenitor Cells Extends Life Span

(A) Life-span curves of *esgGAL4* > *dPGC-1* females compared to isogenic controls. *UAS-dPGC-1* and the isogenic control strain (w^{1118}) were crossed to *esgGAL4*. A 49% increase in mean survival was observed in response to *dPGC-1* activation ($p < 0.0001$).

(B) Life-span curves of *UAS-dPGC-1/ 5961GS* females. *UAS-dPGC-1* was crossed to the *5961GS* driver and life-span curves are shown as induced (1 μg ml^{-1} RU486 during development and 5 μg ml^{-1} RU486 from the onset of adulthood (black circles) or uninduced (−RU486, open circles). A 19% increase in mean survival was observed in response to RU486 during both development and adulthood ($p < 0.0001$).

(C) Life-span curves of *UAS-dPGC-1/ 5961GS* females. *UAS-dPGC-1* was crossed to the the *5961GS* driver and life-span curves are shown as induced (5 μg ml^{-1} RU486 from the onset of adulthood (black circles) or uninduced (−RU486, open circles). A 17% increase in mean survival was observed in response to RU486 in the adult stage ($p < 0.0001$). The significance of the difference between survival curves was analyzed using log-rank statistical test ($n > 170$ flies).

See also Figures S4 and S5. Survival data for male flies can be found in Figures S4A and S4B. Survival data for control flies exposed to RU486 can be found in Figures S4C–S4E.

controls (Figure 5A). Next, we examined whether increased *dPGC-1* expression in the intestine impacts the activities of mitochondrial respiratory chain enzymes in the target tissue. Indeed, *esgGAL4* > *dPGC-1* flies display an increase in both mitochondrial complex I (Figure 5B) and complex II (Figure 5C) activities in the aged intestine compared to controls. Interestingly, complex IV activity was increased in the intestines of *esgGAL4* > *dPGC-1* flies, compared to controls, at 10 days but not 30 days (Figure 5D).

The cationic dye JC-1 can be used to measure mitochondrial membrane potential at a cellular level. In healthy cells, the negative charge established by the intact mitochondrial membrane potential allows this lipophilic dye to enter the mitochondrial matrix, where it accumulates and fluoresces red. When mitochondrial membrane potential is low, the dye remains monomeric in the cytoplasm and fluoresces green. Therefore, membrane potential can be determined by the presence of J-aggregates and measured by the ratio of green: red fluorescence. Using this approach, we examined mitochondrial activity in the midgut epithelium. In doing so, we discovered that aging results in a progressive loss of mitochondrial membrane potential in this region of the intestine; however, intestines of long-lived *esgGAL4* > *dPGC-1* flies showed significant maintenance of mitochondrial membrane potential, when compared to isogenic controls (Figures S6A–S6C).

PGC-1α is a potent regulator of reactive oxygen species (ROS) metabolism and is required for the induction of several ROS-detoxifying enzymes (St-Pierre et al., 2006). Therefore, we speculated that upregulation of *dPGC-1* may reduce ROS levels in the target tissue. To test this idea, we examined the endogenous levels of ROS in the intestines of control and *esgGAL4* > *dPGC-1* flies using dihydroethidium (DHE), a redox-sensitive dye that exhibits increased fluorescence intensity when oxidized (Owusu-Ansah and Banerjee, 2009; Owusu-Ansah et al., 2008). Targeted expression of *dPGC-1* in ISCs/EBs led to a reduction of DHE fluorescence in these cells and throughout the aged intestine (Figures 5E and 5F and Figures S6D and S6E). Therefore, upregulation of *dPGC-1* in stem cells and immediate daughter cells is sufficient to lower ROS levels throughout the intestinal epithelium in aged flies.

dPGC-1 Modulates Tissue Homeostasis in the Aged Intestine

Given the ability of *dPGC-1* expression in the intestine to maintain mitochondrial activity and lower ROS levels, we wanted to determine whether targeted expression of *dPGC-1* in ISCs/EBs was sufficient to delay the onset of previously characterized aging-related phenotypes in the intestine. In the *Drosophila* intestine, aging or stress results in a dramatic increase in ISC proliferation, which is accompanied by an accumulation of misdifferentiated daughter cells that express markers of both ISCs and terminally differentiated daughter cells (Biteau et al., 2008; Choi et al., 2008; Park et al., 2009). These cells retain expression of the stem cell markers, Esg and Dl, yet they are polyploid, suggesting that this population comprises EBs that that are blocked in the ability to terminally differentiate into functional enterocytes (Biteau et al., 2008; Choi et al., 2008; Park et al., 2009). The age-related increase in misdifferentiated cells disrupts epithelial integrity and tissue architecture, as revealed by staining for the membrane marker Armadillo (Arm), the *Drosophila* homolog of β-catenin. This leads to a loss of normal tissue homeostasis and severe deterioration of the midgut epithelium in aged flies (Biteau et al., 2008), which may impact gut function or integrity.

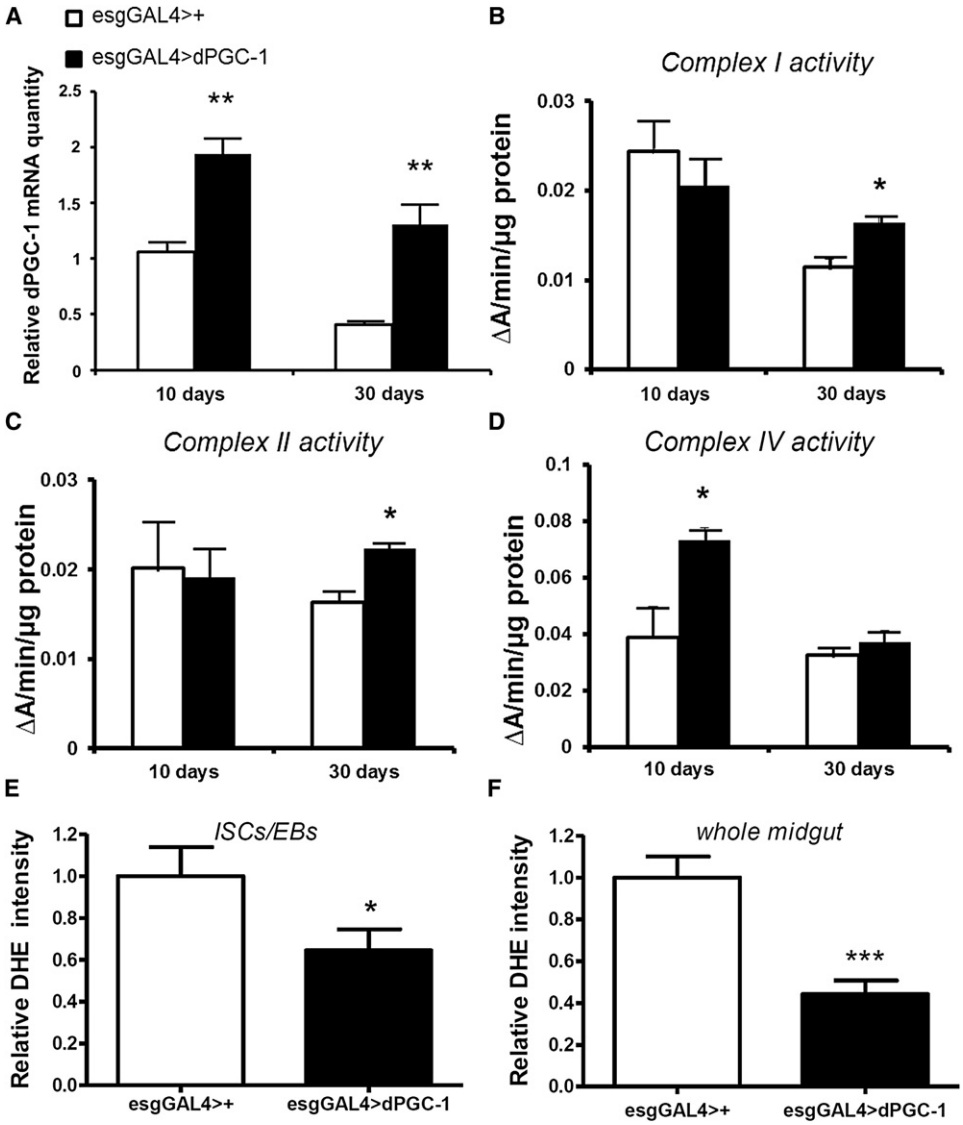

Figure 5. *dPGC-1* Modulates Mitochondrial Activity and ROS Levels in the Aged Intestine

(A) *dPGC-1* mRNA levels in the intestine at day 10 (young) and 30 (aged). *esgGAL4* > *dPGC-1* flies display increased expression of *dPGC-1* in the intestine at both ages (**$p < 0.01$, t test) compared to controls (n = 3, 5 guts per replicate).

(B) Complex I activity in the intestine at day 10 (young) and 30 (aged). *esgGAL4* > *dPGC-1* flies display increased complex I activity in the aged intestine (*$p < 0.05$, t test) compared to controls (n = 3, 15 guts per replicate).

(C) Complex II activity in the intestine at day 10 (young) and 30 (aged). *esgGAL4* > *dPGC-1* flies display increased complex II activity in the aged intestine (*$p < 0.05$, t test) compared to controls (n = 3, 15 guts per replicate).

(D) Complex IV activity in the intestine at day 10 (young) and 30 (aged). *esgGAL4* > *dPGC-1* flies display increased complex IV activity in the young intestine (*$p < 0.05$, t test) compared to controls (n = 3, 15 guts per replicate).

(E) ROS levels in ISCs/EBs. Dihydro-ethidium (DHE) fluorescence was assayed in ISCs/EBs from *esgGAL4* > *dPGC-1* flies and isogenic controls at day 30. DHE fluorescence was assayed throughout the entire Z stack and averaged to obtain a value representing the mean intensity of the entire Z stack per gut (n > 18 guts). *esgGAL4*-mediated activation of *dPGC-1* decreased DHE fluorescence in ISCs/EBs in the aged intestine (*$p < 0.05$, t test). Representative images can be found in Figure S6D.

(F) ROS levels in midguts. DHE fluorescence was assayed in midguts (200–500 μM anterior to the pylorus) from *esgGAL4* > *dPGC-1* flies and isogenic controls at day 30 (n > 10 guts). *esgGAL4*-mediated activation of *dPGC-1* decreased DHE fluorescence in the aged intestine (***$p < 0.001$, t test). Representative images can be found in Figure S6E.

Data are represented as mean ± SEM. See also Figure S6.

In order to determine whether increased expression of *dPGC-1* leads to a delay in the aging-related phenotypes and improved tissue homeostasis in the gut, we quantified *esg*-positive cells (GFP^+ as a consequence of a UAS-*gfp* reporter) in guts of flies overexpressing *dPGC-1* under control of the *esg* promoter. For quantification of ISCs/EBs, GFP^+ cells were

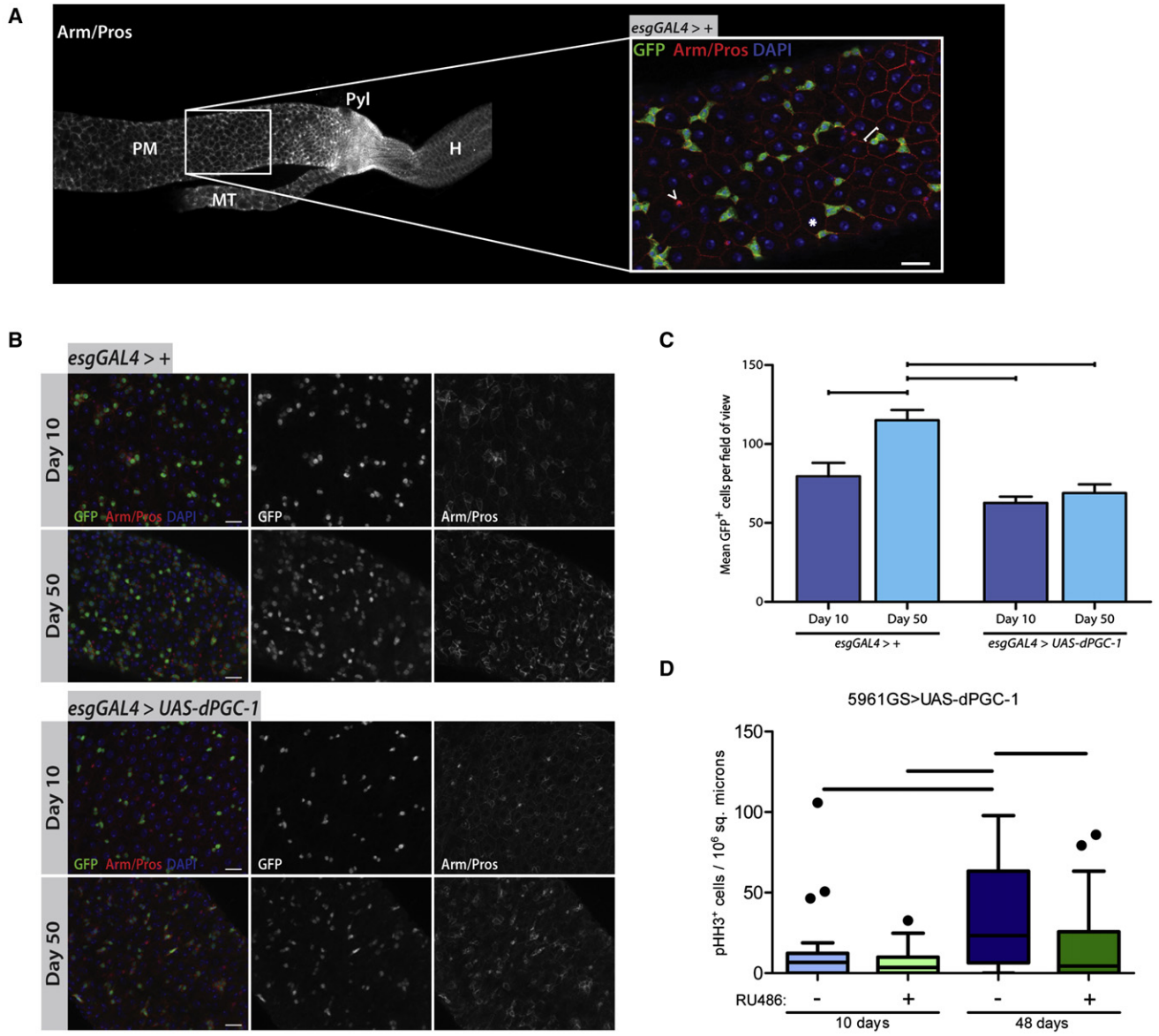

Figure 6. *dPGC-1* Modulates Tissue Homeostasis in the Aged Intestine

(A) Shown are adult *Drosophila* gut viewed with a 10× objective and stained with anti-Armadillo antibodies showing the posterior midgut (PM), malpighian tubules (MT), pylorus (Pyl), and hindgut (H) (left) and 40× view of the posterior midgut showing an ISC/EB nest (bracket), a polyploid enterocyte (*), and a Prospero$^+$ enteroendocrine cell (arrow head) (right).

(B) Immunofluorescence images evaluating intestinal homeostasis during aging. Control (upper panels: *esgGAL4, UAS-gfp*/+) and *dPGC-1* overexpressing (lower panels: *esgGAL4, UAS-gfp*/+ *; UAS-dPGC-1*/+) flies were aged 10 or 50 days and assayed for GFP$^+$ cells. Scale bars represent 20 μm.

(C) Quantification of total number of GFP$^+$ cells per field of analysis. Error bars represent SEMs with n > 24 midguts per treatment. The mean number of GFP$^+$ cells increased significantly from 10 to 50 days in control but not in *dPGC-1* overexpressing flies (one-way ANOVA with Tukey's HSD post hoc test; error bars represent $p < 0.001$).

(D) Quantification of total number of pHH3$^+$ cells in guts from induced (5 μg ml^{-1} RU486 from the onset of adulthood) or uninduced (–RU486) *5961GS* > *UAS-dPGC-1* flies. The box plot represents medians and quartiles, and whiskers indicate 1.5 times the interquartile range with n > 21 midguts per treatment. The median number of pHH3$^+$ cells increased significantly ($p < 0.05$) from 10 to 48 days in uninduced but not in *dPGC-1* overexpressing (induced) flies. Kruskal-Wallis test followed by a Dunn's multiple comparison test was used to assess differences between samples.

See also Figure S7.

counted in control flies (genotype: *esgGAL4, UAS-gfp*/+) or *dPGC-1* flies (genotype: *esgGAL4, UAS-gfp*/+ *; UAS-dPGC-1*/+), and at least 25 guts were examined for each time point (see the Experimental Procedures for details). Both the increase in misdifferentiated cells and the characteristic changes in tissue architecture were delayed in older animals expressing *dPGC-1* in ISCs/EBs (Figure 6B and Figure S7A). The average number of GFP$^+$ cells per FOV in 50-day old control females was

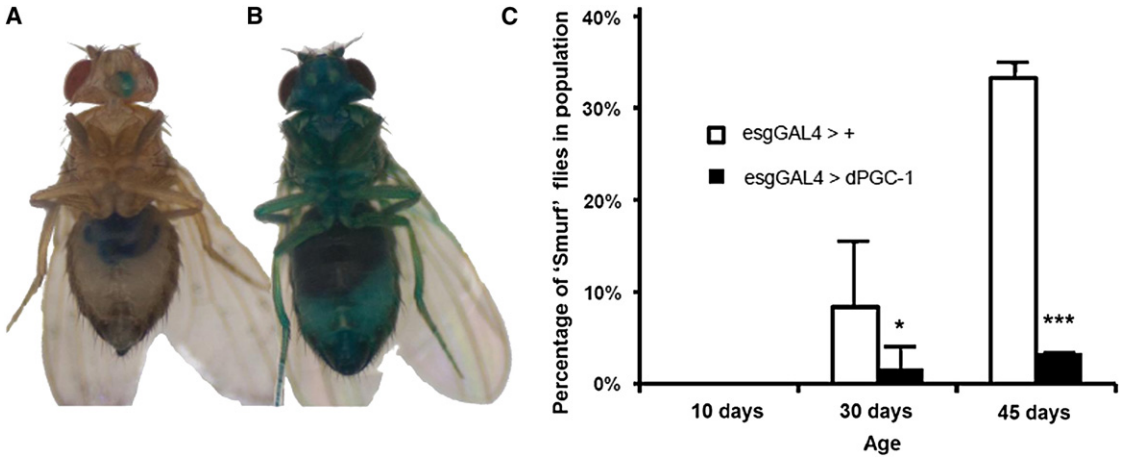

Figure 7. *dPGC-1* Modulates Intestinal Integrity in Old Flies

(A) A 10-day-old fly after consuming a nonabsorbed food dye (FD&C blue dye #1). The dye is restricted to the proboscis and digestive tract.

(B) A 45-day-old "Smurf" fly after consuming the same food dye. The blue dye is seen throughout the body due to loss of intestinal integrity.

(C) Analysis of intestinal integrity as a function of age. In control (*esgGAL4* > +) flies, the fraction of "Smurf" flies in the population increases with age. *esgGAL4*-mediated activation of *dPGC-1* improves intestinal integrity in aged flies. Binomial test *$p < 0.05$ at day 30 and ***$p < 0.001$ at day 45, n = 2 × 30 females for each genotype. Data are represented as mean ± SEM.

115.1 ± 6.4 (standard error of the mean [SEM]) (n = 26), whereas the average for 50-day old *dPGC-1* females was 69 ± 5.5 (n = 27) (Figure 6C). The same trend was observed in male flies (Figure S7B).

In addition to a decrease in the accumulation of misdifferentiated cells, we also observed a delay in the precocious activation of ISC proliferation, as measured by phosphorylation of histone H3 (pHH3), a marker of cell-cycle progression through mitosis. Forty-eight-day-old female flies expressing *dPGC-1* under the control of the inducible *5961GS* driver, induced from the onset of adulthood, contained significantly fewer $pHH3^+$ cells, when compared to uninduced age-matched sibling controls (Figure 6D). No difference in the number of $pHH3^+$ cells was observed in 10-day-old flies, indicating that the expression of *dPGC-1* delays the age-related increase in ISC proliferation. Furthermore, our data demonstrates that *dPGC-1* acts during the adult stage to abrogate the precocious activation of ISC proliferation, which occurs during aging.

Finally, we sought to determine whether *dPGC-1* expression in the digestive tract affects intestinal integrity as a function of age. To develop an assay of intestinal integrity, we examined flies of different ages that had consumed a nonabsorbable blue food dye (FD&C blue dye #1). As expected, we observed that in young flies (10 days) the dye is restricted to the proboscis and digestive tract after feeding (Figure 7A). However, in aged flies (>30 days) we observed a fraction of animals that displayed a strikingly different phenotype. In these animals, the blue dye was clearly visible throughout the body after feeding; subsequently, these flies were referred to as "Smurf" flies (Figure 7B). To exclude the possibility that this phenotype was due to a unique property of this dye, we fed aged flies a different non-absorbable red food dye (FD&C red dye #40) and observed a fraction of individuals that displayed red food dye throughout the body after feeding (M.R. and D.W., unpublished data). Therefore, we interpret the "Smurf" phenotype, i.e., the leakage of dye into the hemolymph and consequently all tissues, to reflect a defect(s) in intestinal integrity.

We next quantified the increase in "Smurf" flies as a function of age in control and long-lived *esgGAL4* > *dPGC-1* flies (Figure 7C). In control flies, the fraction of "Smurf" flies in the population increases dramatically with age; from 0% at 10 days to ~35% at 45 days of age. Strikingly, *esgGAL4*-mediated activation of *dPGC-1* retards the age-related onset of the "Smurf" phenotype. Therefore, an increase in *dPGC-1* expression within the digestive tract results in improved intestinal integrity in aged flies, which is consistent with the delay in disruption of apical-basal polarity in intestines from aging flies, as revealed by Arm staining (Figure 6B). Together, our analysis of proliferative homeostasis and tissue integrity strongly support a model whereby *dPGC-1* activity in somatic stem cell lineages within the digestive tract regulates tissue homeostasis in the aged intestine.

DISCUSSION

Aging is associated with a decline of function at the organismal level that has origins in cellular deterioration and the loss of tissue homeostasis. Considerable attention has been focused separately on the roles of stem cells (Rando, 2006) and mitochondria (Guarente, 2008; Wallace, 2005) in the aging process, yet fundamental questions remain regarding the interplay between mitochondrial metabolism, stem cell behavior and life-span determination. In this study, we demonstrate that the *Drosophila* PGC-1 homolog is a potent inducer of mitochondrial activity and that overexpression in somatic stem cells within the digestive tract can slow aging at both the tissue and organismal level.

It is interesting to speculate upon the tissue-specific requirements for *dPGC-1*-mediated longevity. Although we failed to

observe life extension in response to ubiquitous, muscle or neuronal activation of *dPGC-1*, we cannot exclude the possibility that expression in subsets of muscle and/or neuronal cells or different levels of expression in these tissues could promote longevity. That said, we observed robust *dPGC-1*-mediated life extension with multiple driver lines that are expressed in the digestive tract. Consistent with our own findings, it was recently reported that intestinal homeostasis correlates with life span in a number of different genotypes including flies with altered Jun-N-terminal Kinase (JNK) or insulin/IGF signaling (IIS) activities (Biteau et al., 2010). One plausible explanation for such findings is that maintaining healthy intestinal function and/or integrity is an important determinant of fly life span. In our own study, using a nonabsorbed food dye, we demonstrate that there is a loss of intestinal integrity as a function of age. It is possible that this phenomenon could impact the survival of the animal by exposing the internal tissues and organs to toxins or pathogens. However, although the intestine appears to play an important role in modulating longevity, we cannot rule out the role of cell non-autonomous effects in *dPGC-1*-mediated longevity. In this regard, we observed that *esg-GAL4*-mediated expression of *dPGC-1* resulted in changes in lipid/carbohydrate metabolism in whole flies.

In this study, we report that *dPGC-1* expression declines in the aged intestine of control flies, whereas directed expression of *dPGC-1* in somatic stem cells within the digestive tract is sufficient to retard aging in this tissue. Importantly, our data indicate that manipulating *dPGC-1* in ISCs/EBs leads to maintenance of mitochondrial activity throughout the midgut, rather than exclusively in stem cells. This is not unexpected, as there is no transit amplifying population of daughter cells; the EBs differentiate directly into one of two lineages. Therefore, any manipulation of stem cell physiology could easily be passed onto directly differentiating daughter cells. The enterocytes are the predominant cell type in the intestine; therefore, changes in these cells likely account for most of the phenotypic differences that we observe. In the case of ROS levels, we observe that *dPGC-1* expression in ISCs/EBs leads to a reduction in ROS levels in the stem cells, as well as in the enterocytes (Figures 5E and 5F and Figures S6D and S6E).

Given the diverse roles that PGC-1 plays in metabolism (Lin et al., 2005), the relative contribution of each of these processes in modulating tissue homeostasis and longevity remains to be determined. However, our finding that *dPGC-1* modulates ROS levels in the aging intestine may provide mechanistic insight. ROS levels have been demonstrated to influence stem cell self-renewal and the onset of differentiation in multiple systems (Ito et al., 2004; Owusu-Ansah and Banerjee, 2009; Smith et al., 2000; Tothova and Gilliland, 2007). Indeed, it was recently reported that Nrf2, a master regulator of the cellular redox state, specifically controls the proliferative activity of ISCs, promoting intestinal homeostasis (Hochmuth et al., 2011). Our findings support a model whereby *dPGC-1*-mediated alterations in metabolism, including ROS metabolism, can retard aging of the intestine with significant consequences for animal life span.

It will be interesting to determine whether PGC-1 family members in other species also regulate tissue homeostasis in high-turnover tissues such as intestine.

EXPERIMENTAL PROCEDURES

Analysis of survivorship, fertility, stress resistance, food intake, lipid/carbohydrate levels, mtDNA amount, enzyme activity assays, oxygen consumption, qRT-PCR, BN-PAGE, and western blotting were conducted by standard methods and are available in the Supplemental Experimental Procedures.

Drosophila Strains

Tubulin-GS was provided by S. Pletcher. *Elav-GS* was provided by H. Keshishian. *$S_1$106* and *TIGS-2* were provided by L. Seroude. *5961GS* was provided by H. Jasper. *esgGAL4* was provided by A. Christiansen. All other stocks were provided by the Bloomington *Drosophila* Stock Center. We transformed flies with pUAST plasmids containing *dPGC-1* and performed 12 rounds of backcrossing into a w^{1118} background.

Dihydroethidium Staining

ROS levels were detected in live tissue based on previously described methods (Hochmuth et al., 2011; Owusu-Ansah and Banerjee, 2009; Owusu-Ansah et al., 2008). In brief, guts were dissected directly in Schneider's medium and were then incubated, protected from light, in 60 μM dihydroethidium (Invitrogen Molecular Probes) in Schneider's medium for 7 min (Dye freshly reconstituted each time in anhydrous DMSO). Three washes were performed for 5 min each in Schneider's medium at room temperature before mounting in ProlongGold antifade reagent containing DAPI (Invitrogen Molecular Probes). Midguts were imaged immediately after the staining procedure. For total ROS production from the midgut, Z stacks of regions 200–500 μM anterior to the pylorus were measured for mean signal intensity at 568 nM in Image J. For ROS output of ISCs/EBs, mean intensity of DHE signal was measured only in cell clusters identified by their esgGFP expression, their size, and their basal location within the intestinal epithelium. Pixel intensities of Z stacks, spanning from the basal to apical cell layers, for a minimum of 10 midguts were used for each of the quantifications. Statistical analysis was conducted on mean DHE intensities averaged from each midgut using a two-tailed, unpaired Student's t test.

Immunofluorescence, Quantification of ISCs/EBs, and pHH3$^+$ Cell Counts

Fixation of *Drosophila* intestines was carried out according to (Boyle et al., 2007), prepared according to standard procedures, and mounted in Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI) from Vector Laboratories. Primary antibodies used in this study included rabbit anti-GFP (1:5,000) from Molecular Probes, rabbit anti-phosphohistone H3 (1:200) from Millipore (06-570), and mouse anti-Armadillo (N2 7A1) (1:20) and mouse anti-Prospero (MR1A) (1:100), both obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. For the quantification of GFP$^+$ cells, images were acquired from sections imaged 250–500 μm anterior to the pyloric ring in the posterior midgut, and MetaMorph software (Molecular Devices, Downingtown, PA) was used to quantify GFP$^+$ cells. The average number of GFP$^+$ cells was obtained from at least 25 guts per treatment; means were compared with one-way ANOVA followed by Tukey's HSD posttest. Due to the smaller size of male posterior midgut, the field of view (FOV) was divided into nine equal sections, and the central region was used to quantify the average number of GFP$^+$ cells. To quantify pHH3$^+$ cells, images were acquired with a 20× objective one to two fields of view anterior to the pylorus. The numbers of pHH3$^+$ cells were normalized to gut area for each gut ($n > 21$ samples per treatment). Statistical significance was determined with a Kruskall-Wallis test followed by a Dunn's post hoc test.

Analysis of Intestinal Integrity

Quantification of intestinal integrity was based upon the distribution of a blue food dye (FD&C blue dye #1) post-feeding. In brief, two vials of female flies of each genotype were transferred onto fresh medium containing blue dye (2.5% w/v) at 9 a.m. for 150 min. Flies showing an extended blue coloration (not limited to the proboscis and crop) were considered "Smurf" flies.

Statistical Analysis

Unless indicated otherwise, significance was determined with a two-tailed, unpaired t test from at least three independent experiments and expressed as p values. Unless indicated otherwise, error bars represent the SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and seven figures and can be found with this article online at doi:10.1016/j.cmet.2011.09.013.

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