Supplemental Figures and Text.

PGC-1/*spargel* counteracts high fat diet-induced obesity and cardiac lipotoxicity downstream of TOR and Brummer ATGL lipase

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Figure S1

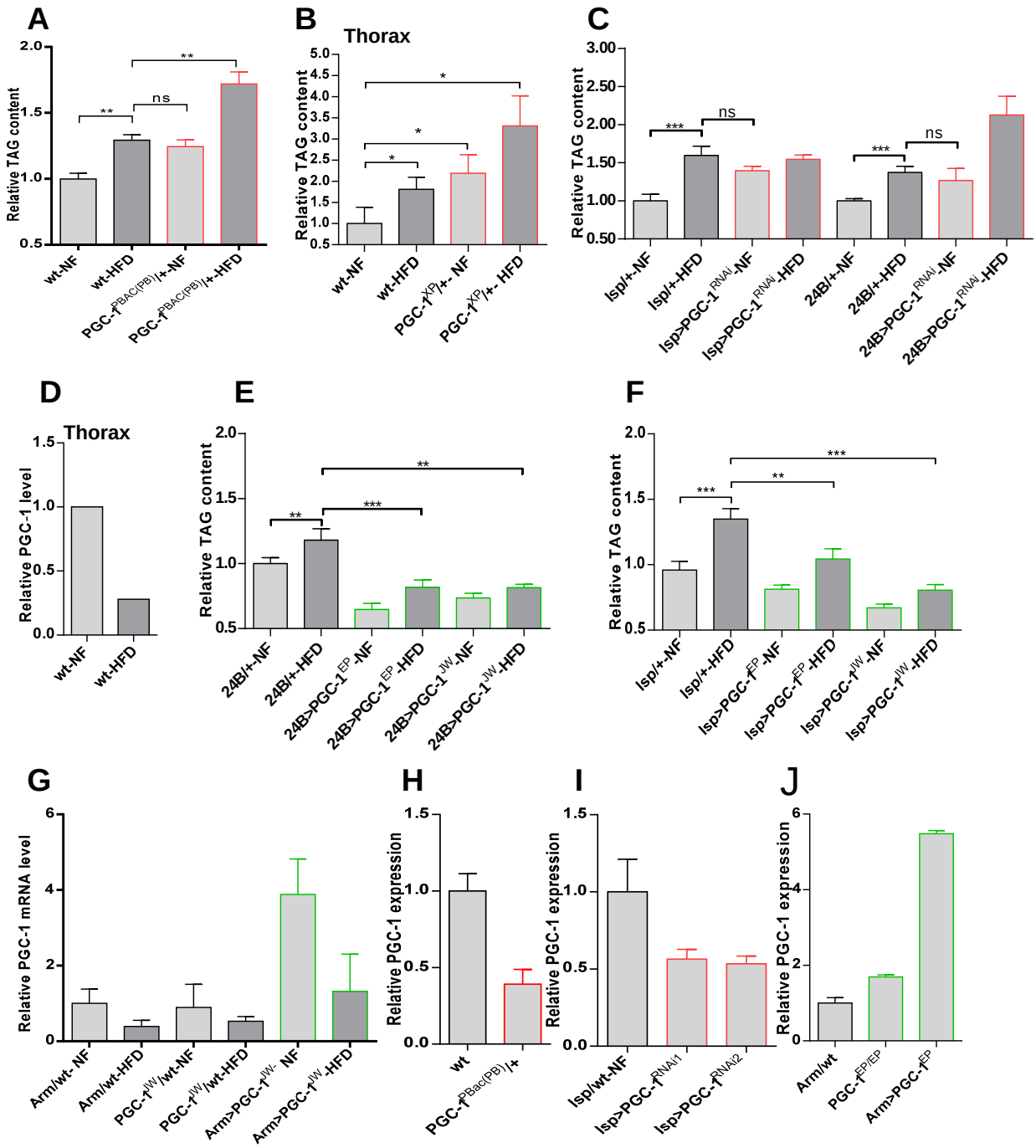
A
Relative TAG content
wt-NF
wt-HFD
PGC-1PBAC(PB)/+-NF
PGC-1PBAC(PB)/+-HFD
B
Thorax
Relative TAG content
wt-NF
wt-HFD
PGC-1XP/+- NF
PGC-1XP/+- HFD
C
Relative TAG content
Isp/+-NF
Isp/+-HFD
Isp>PGC-1RNAi-NF
Isp>PGC-1RNAi-HFD
24B/+-NF
24B/+-HFD
24B>PGC-1RNAi-NF
24B>PGC-1RNAi-HFD
D
Thorax
Relative PGC-1 level
wt-NF
wt-HFD
E
Relative TAG content
24B/+-NF
24B/+-HFD
24B>PGC-1EP-NF
24B>PGC-1EP-HFD
24B>PGC-1JW-NF
24B>PGC-1JW-HFD
F
Relative TAG content
Isp/+-NF
Isp/+-HFD
Isp>PGC-1EP-NF
Isp>PGC-1EP-HFD
Isp>PGC-1JW-NF
Isp>PGC-1JW-HFD
G
Relative PGC-1 mRNA level
Arm/wt- NF
Arm/wt-HFD
PGC-1JW/wt-NF
PGC-1JW/wt-HFD
Arm>PGC-1JW- NF
Arm>PGC-1JW-HFD
H
Relative PGC-1 expression
wt
PGC-1PBac(PB)/+
I
Relative PGC-1 expression
Isp/wt-NF
Isp>PGC-1RNAi1
Isp>PGC-1RNAi2
J
Relative PGC-1 expression
Arm/wt
PGC-1EP/EP
Arm>PGC-1EP

Figure S1. Reduced *PGC-1/srl* Function Mediates the Effect of HFD on Obesity, related to Figure 1.

(A) Relative TAG content of wt and *PGC-1$^{/}$srl* mutant flies on NF or HFD. Results are expressed as the fold difference in whole fly TAG compared with wt-NF flies and are the mean ± SEM of at least 27 for all genotypes and conditions. *P < 0.05, one way ANOVA test. ns: not significant

(B) Relative TAG content in muscle rich thoracic tissue of *wt* and *PGC-1/srl* heterozygous flies. Results are expressed as the fold difference compared with wt-NF flies and are the mean ± SEM of 35 ≤ n ≥ 24. *P < 0.01, one way ANOVA test.

(C) Relative whole fly TAG content of control flies or flies with adipose-specific and muscle-specific knockdown of *PGC-1/srl*. Results are expressed as the fold difference compared with *lsp*/+-NF or *24B*/+-NF flies and are the mean ± SEM of 36 ≤ n ≥ 16. ***P < 0.001, one way ANOVA test.

(D) *PGC-1/srl* mRNA expression in the thorax of wt flies fed NF and HFD. Results are the mean of n = 20.

(E-F) Whole fly TAG content of flies with (E) muscle-specific or (F) adipose-specific *PGC-1/srl* overexpression. Results are expressed as the fold difference compared with lsp$^{/+}$-NF or 24B/+-NF flies and are the mean ± SEM of 24 ≤ n ≥ 21. **P < 0.01, ***P < 0.001, one way ANOVA test.

(G) Relative *PGC-1/srl* expression in controls (*Arm/wt*, *PGC-1^{JW}/wt*) and *PGC-1/srl* overexpression flies (*Arm/PGC-1^{JW}*) on NF or HFD. Results are expressed as the fold difference compared with *Arm/wt* on NF.

(H-J) Relative *PGC-1/srl* expression in *PGC-1^{PBAC}* (H), adipose-specific *PGC-1^{RNAi}* (I), and Arm>*PGC-1^{EP}* flies (J). Results are expressed as the fold difference compared with the indicated wt or NF-fed flies and are the mean ± SD of n = 20.

Figure S2

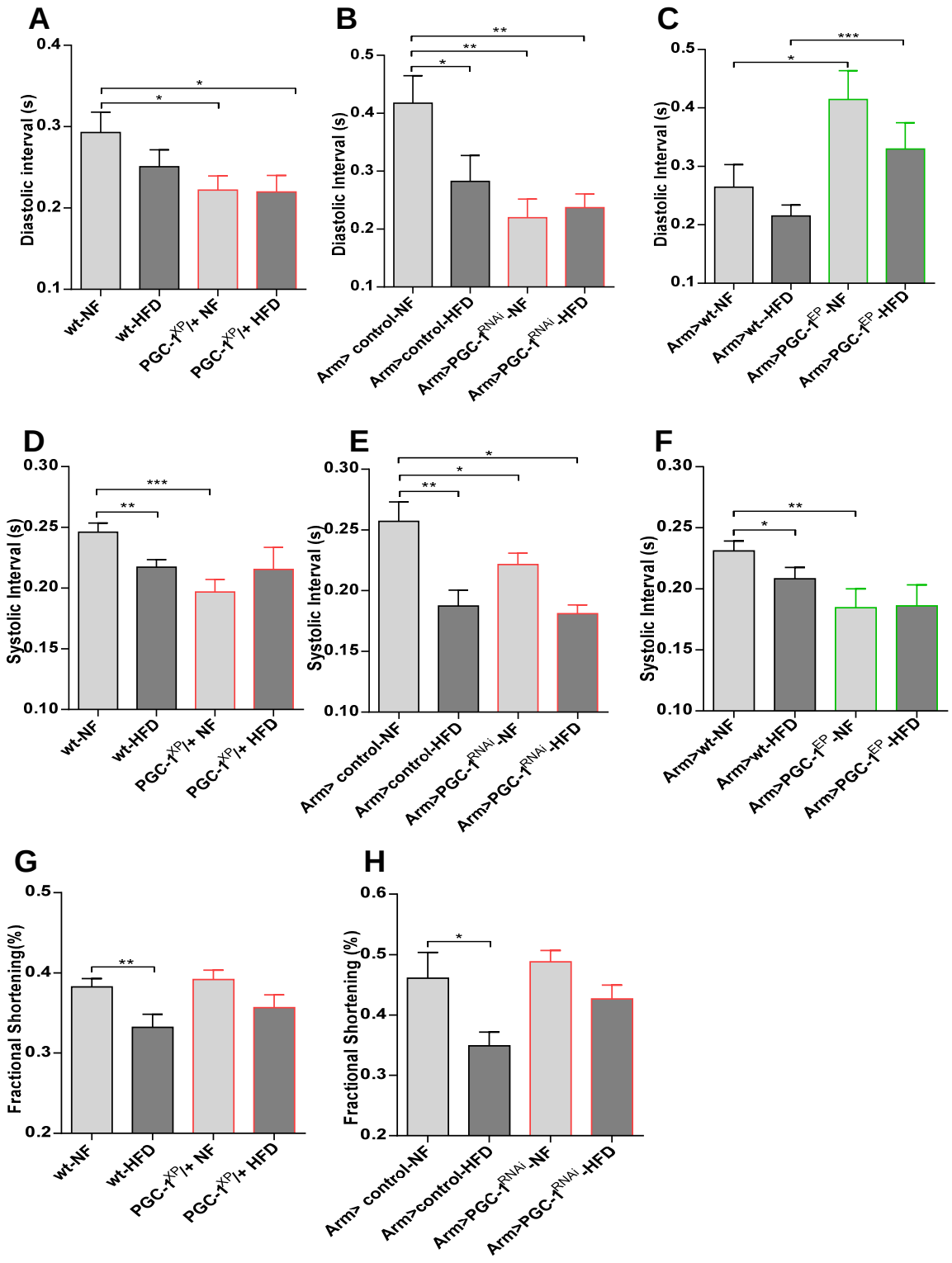
A
Diastolic interval (s)
0.4
0.3
0.2
0.1
*
*
wt-NF
wt-HFD
PGC-1XP/+ NF
PGC-1XP/+ HFD
B
Diastolic Interval (s)
0.5
0.4
0.3
0.2
0.1
**
**
*
Arm> control-NF
Arm>control-HFD
Arm>PGC-1RNAi -NF
Arm>PGC-1RNAi -HFD
C
Diastolic Interval (s)
0.5
0.4
0.3
0.2
0.1
***
*
Arm>wt-NF
Arm>wt--HFD
Arm>PGC-1EP -NF
Arm>PGC-1EP -HFD
D
Systolic Interval (s)
0.30
0.25
0.20
0.15
0.10
***
**
wt-NF
wt-HFD
PGC-1XP/+ NF
PGC-1XP/+ HFD
E
Systolic Interval (s)
0.30
0.25
0.20
0.15
0.10
*
*
**
Arm> control-NF
Arm>control-HFD
Arm>PGC-1RNAi -NF
Arm>PGC-1RNAi -HFD
F
Systolic Interval (s)
0.30
0.25
0.20
0.15
0.10
**
*
Arm>wt-NF
Arm>wt-HFD
Arm>PGC-1EP -NF
Arm>PGC-1EP -HFD
G
Fractional Shortening(%)
0.5
0.4
0.3
0.2
**
wt-NF
wt-HFD
PGC-1XP/+ NF
PGC-1XP/+ HFD
H
Fractional Shortening (%)
0.6
0.5
0.4
0.3
0.2
*
Arm> control-NF
Arm>control-HFD
Arm>PGC-1RNAi -NF
Arm>PGC-1RNAi -HFD

Figure S2. As HFD, reduced *PGC-1/srl* function increases heart rate, **related to Figure 2.**

(A) Mean diastolic intervals of wt and *PGC-1* heterozygous flies on NF and HFD. Results are the mean ± SEM of 45 ≤ n ≥ 33. *P < 0.05, one way ANOVA test.

(B) Mean diastolic intervals of control and *PGC-1* knockdown flies. Results are the mean ± SEM of 20 ≤ n ≥ 22. *P < 0.05, **P < 0.01, one way ANOVA test.

(C) Mean diastolic intervals of control and *PGC-1*-overexpressing flies. Results are the mean ± SEM of 32 ≤ n ≥ 16. *P < 0.05, ***P < 0.001, one way ANOVA test.

(D–F) Mean systolic intervals of control and (D) *PGC-1* mutant flies, (E) *PGC-1* knockdown flies, and (F) *PGC-1*-overexpressing flies on NF and HFD. Results are the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, one way ANOVA test.

(G–H) Contractility changes (measured as % fractional shortening) in hearts from control and (G) *PGC-1* mutant flies, (H) *PGC-1* knockdown flies. Results are the mean ± SEM. Statistical significance for all experiments was determined using one-way ANOVA test, *P < 0.05, **P < 0.01, one way ANOVA test.

Figure S3

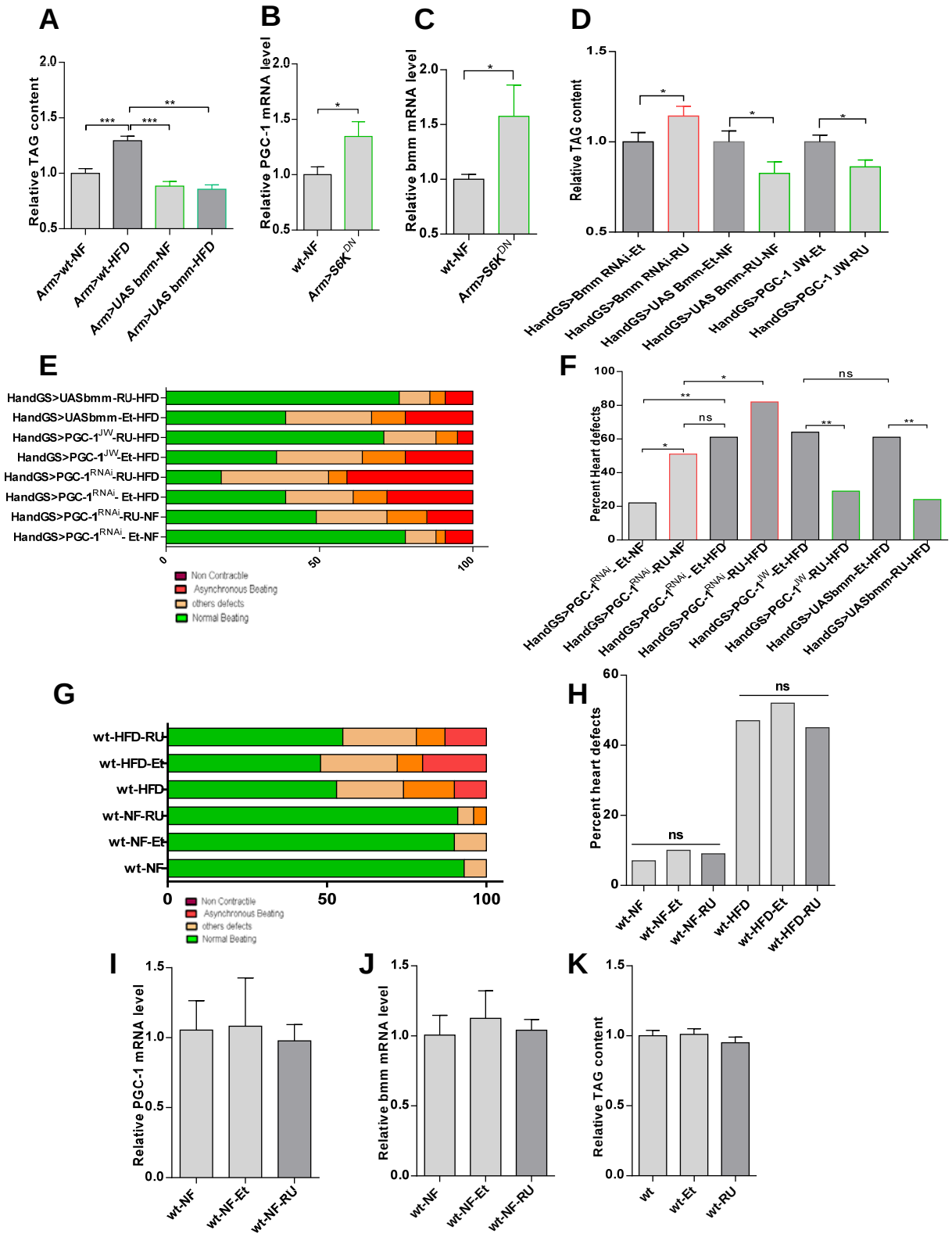
A
Relative TAG content
Arm>wt-NF
Arm>wt-HFD
Arm>UAS bmm-NF
Arm>UAS bmm-HFD
B
Relative PGC-1 mRNA level
wt-NF
Arm>S6K^DN
C
Relative bmm mRNA level
wt-NF
Arm>S6K^DN
D
Relative TAG content
HandGS>Bmm RNAi-Et
HandGS>Bmm RNAi-RU
HandGS>UAS Bmm-Et-NF
HandGS>UAS Bmm-RU-NF
HandGS>PGC-1 JW-Et
HandGS>PGC-1 JW-RU
E
HandGS>UASbmm-RU-HFD
HandGS>UASbmm-Et-HFD
HandGS>PGC-1^JW-RU-HFD
HandGS>PGC-1^JW-Et-HFD
HandGS>PGC-1^RNAi-RU-HFD
HandGS>PGC-1^RNAi- Et-HFD
HandGS>PGC-1^RNAi-RU-NF
HandGS>PGC-1^RNAi- Et-NF
Non Contractile
Asynchronous Beating
others defects
Normal Beating
F
Percent Heart defects
ns
HandGS>PGC-1^RNAi- Et-NF
HandGS>PGC-1^RNAi-RU-NF
HandGS>PGC-1^RNAi- Et-HFD
HandGS>PGC-1^RNAi-RU-HFD
HandGS>PGC-1^JW-Et-HFD
HandGS>PGC-1^JW-RU-HFD
HandGS>UASbmm-Et-HFD
HandGS>UASbmm-RU-HFD
G
wt-HFD-RU
wt-HFD-Et
wt-HFD
wt-NF-RU
wt-NF-Et
wt-NF
Non Contractile
Asynchronous Beating
others defects
Normal Beating
H
Percent heart defects
ns
wt-NF
wt-NF-Et
wt-NF-RU
wt-HFD
wt-HFD-Et
wt-HFD-RU
I
Relative PGC-1 mRNA level
wt-NF
wt-NF-Et
wt-NF-RU
J
Relative bmm mRNA level
wt-NF
wt-NF-Et
wt-NF-RU
K
Relative TAG content
wt
wt-Et
wt-RU

Figure S3. Adult-specific *PGC-1/srl* manipulation in the heart, related to Figure 3.

(A) TAG content of control and *bmm*-overexpressing (UAS-*bmm*) flies. Results are expressed as the fold difference in whole fly TAG compared with Arm>wt-NF flies and are the mean ± SEM of n = 36 for all genotypes. **P < 0.01, ***P < 0.001, one way ANOVA test.

(B-C) Relative *PGC-1/srl* (B) and *bmm* (C) mRNA levels in wt and dominant-negative *S6K* flies. Results are expressed as the fold difference compared with wt flies. *P < 0.05, student t-test.

(D) TAG content of control (ethanol vehicle) and RU treatment of *HandGS>bmm*^{RNAi}, *HandGS>UASbmm* and *HandGS>PGC-1*^{JW} flies. Results are expressed as the fold difference from wt in whole fly TAG. 36 ≤ n ≥ 18. *P < 0.05, ANOVA test.

(E) Graphical representation of the proportion of flies with adult cardiac specific manipulation of *PGC-1/srl* and *bmm* displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(F) Quantification of heart defects shown in D, 39 ≤ n ≥ 17. Statistics: Chi-square test. *Hand-GS>PGC-1*^{RNAi}-Et-NF vs. *Hand-GS>PGC-1*^{RNAi}-RU-NF, $\chi^2 = 6.44$; P < 0.05. *Hand-GS>PGC-1*^{RNAi}-RU-NF vs. *Hand-GS>PGC-1*^{RNAi}-RU-HFD, $\chi^2 = 4.79$; P < 0.05. *Hand-GS>PGC-1*^{RNAi}-Et-NF vs. *Hand-GS>PGC-1*^{RNAi}-Et-HFD, $\chi^2 = 7.69$; P < 0.01. *Hand-GS>PGC-1*^{RNAi}-RU-NF vs. *Hand-GS>PGC-1*^{RNAi}-Et-HFD, $\chi^2 = 0.47$; ns. *Hand-GS>PGC-1*^{JW}-Et-HFD vs. *Hand-GS>PGC-1*^{JW}-RU-HFD, $\chi^2 = 8.46$; P < 0.01. *Hand-GS>UAS-bmm*-Et-HFD vs. *Hand-GS>UAS-bmm*-RU-HFD, $\chi^2 = 5.57$; P < 0.05. *Hand-GS>PGC-1*^{JW}-Et-HFD vs. *Hand-GS>UAS-bmm*-Et-HFD, $\chi^2 = 0.03$; ns. *Hand-GS>PGC-1*^{JW}-RU-HFD vs. *Hand-GS>UAS-bmm*-RU-HFD, $\chi^2 = 0.20$; ns.

(G) Graphical representation of the proportion of flies displaying cardiac defects from ethanol or RU treatment of wt flies.

(H) Quantification of heart defects shown in G, 25 ≤ n ≥ 18.

(I-J) Relative expression of ATGL/*bmm* (E) or *PGC-1/srl* in the heart of wt flies treated with ethanol or RU, compared to non-treated wt.

(K) Relative TAG content of wt flies treated with ethanol or RU compared to non-treated wt. Results are expressed as the fold difference in whole fly TAG. 36 ≤ n ≥ 33.

Figure S4

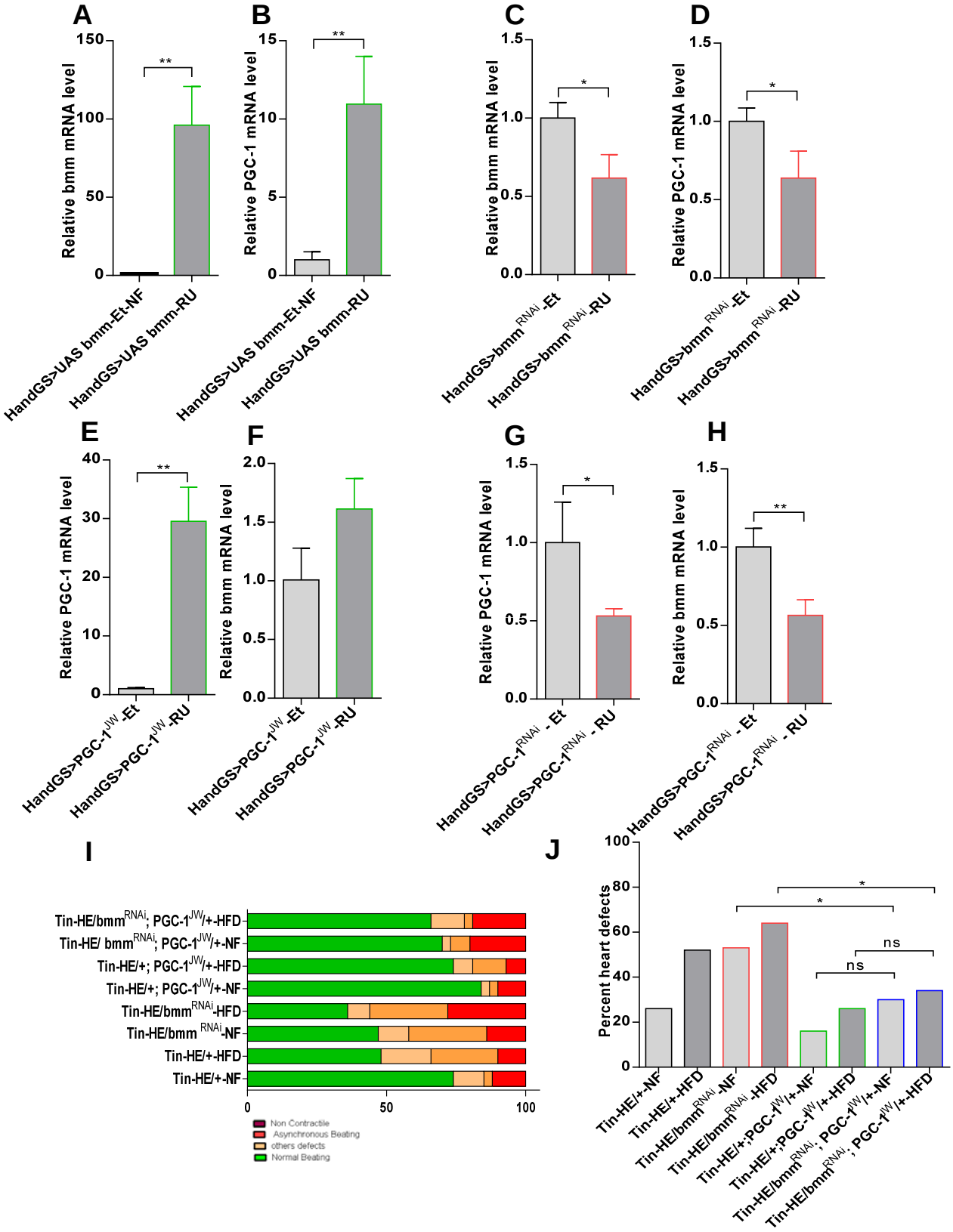
A
Relative bmm mRNA level
150
100
50
0
**
HandGS>UAS bmm-Et-NF
HandGS>UAS bmm-RU
B
Relative PGC-1 mRNA level
15
10
5
0
**
HandGS>UAS bmm-Et-NF
HandGS>UAS bmm-RU
C
Relative bmm mRNA level
1.5
1.0
0.5
0.0
*
HandGS>bmm RNAi -Et
HandGS>bmm RNAi -RU
D
Relative PGC-1 mRNA level
1.5
1.0
0.5
0.0
*
HandGS>bmm RNAi -Et
HandGS>bmm RNAi -RU
E
Relative PGC-1 mRNA level
40
30
20
10
0
**
HandGS>PGC-1 JW -Et
HandGS>PGC-1 JW -RU
F
Relative bmm mRNA level
2.0
1.5
1.0
0.5
0.0
HandGS>PGC-1 JW -Et
HandGS>PGC-1 JW -RU
G
Relative PGC-1 mRNA level
1.5
1.0
0.5
0.0
*
HandGS>PGC-1 RNAi - Et
HandGS>PGC-1 RNAi - RU
H
Relative bmm mRNA level
1.5
1.0
0.5
0.0
**
HandGS>PGC-1 RNAi - Et
HandGS>PGC-1 RNAi - RU
I
Tin-HE/bmm RNAi; PGC-1 JW/+-HFD
Tin-HE/ bmm RNAi; PGC-1 JW/+-NF
Tin-HE/+; PGC-1 JW/+-HFD
Tin-HE/+; PGC-1 JW/+-NF
Tin-HE/bmm RNAi -HFD
Tin-HE/bmm RNAi -NF
Tin-HE/+-HFD
Tin-HE/+-NF
0
50
100
Non Contractile
Asynchronous Beating
others defects
Normal Beating
J
Percent heart defects
100
80
60
40
20
0
*
*
ns
ns
Tin-HE/+-NF
Tin-HE/+-HFD
Tin-HE/bmm RNAi -NF
Tin-HE/bmm RNAi -HFD
Tin-HE/+;PGC-1 JW/+-NF
Tin-HE/+;PGC-1 JW/+-HFD
Tin-HE/bmm RNAi; PGC-1 JW/+-NF
Tin-HE/bmm RNAi; PGC-1 JW/+-HFD

Figure S4. Genetic interaction between *PGC-1/srl* and ATGL/*bmm,* related to Figure 4.

(A-H) Relative expression of *bmm* (A, C, F, H) *and PGC-1/srl* (B, D, E, G) in the heart of ethanol vehicle or RU treated *Hand-GS>UASbmm*, *Hand-GS>bmm^{RNAi}*, Hand-GS>*PGC-1^{RNAi}*, HandGS>*PGC-1^{JW}* flies.

(I) Graphical representation of the proportion of flies with cardiac specific manipulation of *bmm* and/or *PGC-1/srl* displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(J) Quantification of heart defects shown in (A), 29 ≤ n ≥ 24. Statistics: Chi-square test. *Tin-HE/bmm^{RNAi}*-NF vs. *Tin-HE/bmm^{RNAi}*; *PGC-1^{JW}-NF*, χ^2 = 4; P < 0.05. *Tin-HE/bmm^{RNAi}*-HFD (n = 25) vs. *Tin-HE/bmm^{RNAi}; PGC-1^{JW}-HFD*, χ^2 = 5; P < 0.05. *Tin-HE/+; PGC-1^{JW}-NF* vs. *Tin-HE/bmm^{RNAi}; PGC-1^{JW}*-NF, χ^2 = 0.9; not significant (ns). *Tin-HE/+; PGC-1^{JW}*-HFD vs. *Tin-HE/bmm^{RNAi}; PGC-1^{JW}-HFD*, χ^2 = 0.2; ns.

Figure S5

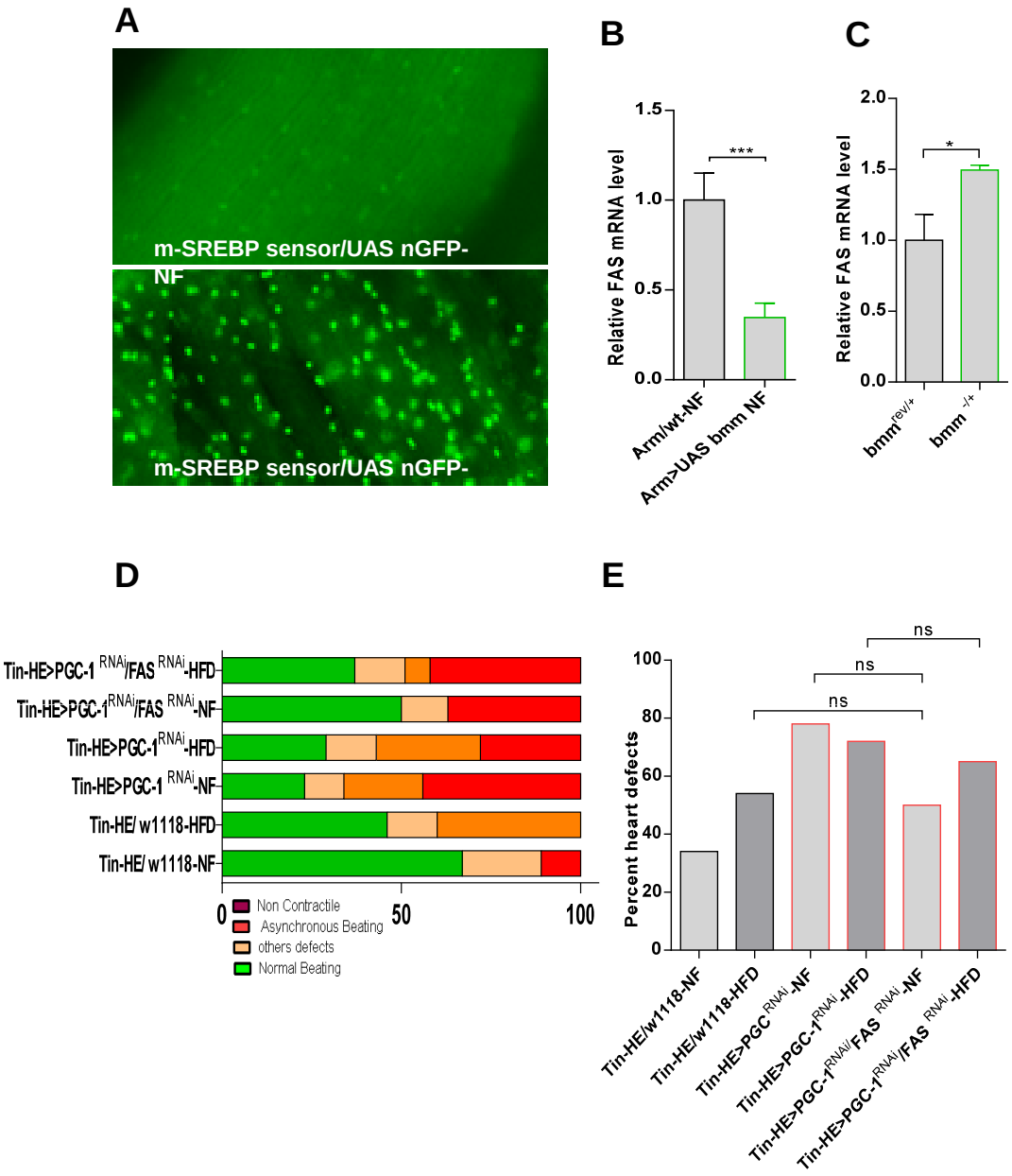
A
m-SREBP sensor/UAS nGFP-NF
m-SREBP sensor/UAS nGFP-
B
Relative FAS mRNA level
1.5
1.0
0.5
0.0
***
Arm/wt-NF
Arm>UAS bmm NF
C
Relative FAS mRNA level
2.0
1.5
1.0
0.5
0.0
*
bmm rev/+
bmm -/+
D
Tin-HE>PGC-1 RNAi/FAS RNAi-HFD
Tin-HE>PGC-1RNAi/FAS RNAi-NF
Tin-HE>PGC-1RNAi-HFD
Tin-HE>PGC-1 RNAi-NF
Tin-HE/ w1118-HFD
Tin-HE/ w1118-NF
0
50
100
Non Contractile
Asynchronous Beating
others defects
Normal Beating
E
Percent heart defects
100
80
60
40
20
0
ns
ns
ns
Tin-HE/w1118-NF
Tin-HE/w1118-HFD
Tin-HE>PGC RNAi-NF
Tin-HE>PGC-1RNAi-HFD
Tin-HE>PGC-1RNAi/FAS RNAi-NF
Tin-HE>PGC-1RNAi/FAS RNAi-HFD

Figure S5. Genetic interaction between *PGC-1/srl* and *FAS,* related to Figure 5.

(A) Representative micrographs of sections of thoracic muscles of flies carrying a UAS-GFP/SREBP sensor and fed NF (top) or HFD (bottom). Sections were stained with anti-GFP antibody (green).

(B-C) Relative *FAS* mRNA levels in *bmm* overexpression (B) and mutant (C) flies. Results are expressed as the fold difference compared to Arm/wt (B) and bmm^{rev}(C). *P < 0.05, ***P < 0.001, student t-test.

(D) Graphical representation of the proportion of flies with cardiac specific manipulation of *PGC-1/srl* and/or *FAS* displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(E) Quantification of heart defects shown in (D), 20 ≤ n ≥ 12. Statistics: Chi-square test. *Tin-HE/+; PGC-1^{RNAi}-NF* vs. *Tin-HE/+; PGC-1^{RNAi}/FAS^{RNAi}*-NF, $\chi^2 = 0$; ns. *Tin-HE/+; PGC-1^{RNAi}-HFD (n = 12)* vs. *Tin-HE/+; PGC-1^{RNAi}/FAS^{RNAi}*-HFD, $\chi^2 = 0.34$; ns. *Tin-HE/wt*-HFD (n =20) vs. *Tin-HE/+; PGC-1^{RNAi}/FAS^{RNAi}*-HFD , $\chi^2 = 0.08$, ns. not significant (ns).

Supplemental movies

Movie S1: normal heart beating of wildtype flies fed NF, related to heart dysfunction graphs

Movie S2: HFD feeding induces increased heart rate, related to heart dysfunction graphs

Movie S3: Reduced *PGC-1/srl* function induces increased heart rate under normal conditions, related to heart dysfunction graphs

Movie S4: Reduced *PGC-1/srl* function induces increased heart rate under HFD conditions, related to heart dysfunction graphs.

Movie S5: HFD or reduced *PGC-1/srl* function causes non-contractile regions, related to heart dysfunction graphs.

Movie S6: HFD or reduced *PGC-1/srl* function causes heart asynchronous beating, related to heart dysfunction graphs.

Movie S7: HFD or reduced *PGC-1/srl* function causes dysfunctional ostia, related to heart dysfunction graphs.

Supplemental Experimental Procedures

Drosophila stocks

w^{1118} (wildtype controls), *PGC-1*^{XP} (CG9809^{d04518}), *PGC-1*^{PBAC} (CG9809^{c05624}), *PGC-1*^{EP} (CG9809^{EY0593}), and *Arm-Gal4* (ubiquitous driver) flies were obtained from the Bloomington *Drosophila* Stock Center. Flies containing a genomic rescue transgene (*PGC-1*^{GR}) were obtained from Christian Frei (Tiefenböck et al, 2010). UAS-*PGC-1*^{RNAi1} flies were obtained from the NIG Stock Center in Japan. UAS-*PGC-1*^{RNAi2} flies, GD controls lines and *bmm*^{RNAi} flies were obtained from the VDRC Stock Center in Vienna. *dPGC-1*^{JW} ("J" meaning Jones and "W" meaning Walker) was from L. Jones and D. Walker (Rera et al., 2011), *bmm*^{1} mutants, Bmm ^{Rev} and the UAS-*bmm* overexpression line were from R. Kuehnlein (Gronke el al., 2005), *TOR*$^{7/P}$ flies were as described in Luong et al. (2006), *S6K*^{DN} was from M. Stewart. Hand(GS)-Gal4 flies driving expression in myocardial as well as pericardial cells upon induction was generous gifts from L. Perrin; Monnier et al., 2013). The *lsp-Gal4* (fat body driver) was reported in Cherbas et al. (2003), and GMH5 and TinHE (myocardial-specific heart drivers) were described in Wessells et al. (2004). Hand4.2-Gal4 flies driving strong expression in myocardial as well as pericardial cells were from Z. Han (Han et al., 2006). pP{GAL4-dSREBPg} flies (SREBP cleavage sensor line) were from R. Rawson (Kunte et al., 2006). In this construct, the DNA-binding domain of SREBP (mature SREBP) is replaced with VP16-Gal4; thus, when SREBP is cleaved, VP16-Gal4 activates genes downstream of UAS sites (i.e., UAS-nGFP).

Triglyceride assay

For each genotype and gender, 36 flies were weighed and placed into a 96-well grinding plate (Brand Tech Scientific, 96 Deep Well plates Cat. # 701350). One metal ball (OPS Diagnostics, 5000 44C SS 5/32-inch, part # GBSS156-5000-01), three flies, and 600 µl of PBS containing 0.05% Triton X-100 were added to each well. The plate was transferred to a shaker (Talboys Cat. # 930145) and shaken for 3 min at the highest speed setting. The plate was centrifuged for 15 min at 4500 rpm and 4°C, and aliquots of 20 µl supernatant were transferred to a new plate containing 200 µl/well of lipid reagent (Thermo Electron, Cat. # TR22421/2780-250). The reaction mixture was incubated at 37°C for 10 min, and the absorbance at 550 nm was then measured (Spectramax M2e, Molecular Devices). Triglyceride (TAG) content was calculated from a standard curve constructed with triglyceride solutions of known concentrations (Thermo Electron, Cat. # TR222923) according to the manufacturer's instructions.

Real-time quantitative PCR (qPCR) analysis

For whole fly samples, total RNA was extracted with Trizol reagent (Roche Diagnostics). Reverse transcription (RT) was performed on aliquots of 1 µg total RNA using a QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. qPCR amplification reactions were prepared with 5 µl of the RT reaction diluted in qPCR reaction mix (LightCycler FastStart DNA MasterPlus SYBR Green I, Roche) containing the PCR primers. Thermal cycling and fluorescence monitoring were performed in a LightCycler 2.0 sequence detector (Roche Diagnostics). Samples were analyzed in triplicate.

For heart-specific qPCR, a Cells-to-CT™ Kit (Ambion) was used. 20 hearts were dissected as described in the main text for semi-intact heart preparation, washed twice with PBS, and extracted and pool by group of 5 hearts in an Eppendorf tube containing 49.5 µl of Cells-to-CT lysis buffer and 0.5 µl DNase I. The lysis mixture was incubated for 5 min at 22°C, then 5 µl of Cells-to-CT stop solution was added and the mixture was incubated for 2 min at 20°C. The RT reaction was performed by mixing 20 µl of lysate with 30 µl of RT master mix (1× RT buffer, 1× primer, and 1× RT enzyme), and incubating in the thermal cycler. qPCR amplification was performed by mixing 5 µl of the RT reaction with 15 µl of Cells-to-CT qPCR master mix containing the appropriate PCR primers. Thermal cycling and fluorescence monitoring were performed in a LightCycler 2.0.

Western Blotting

Wildtype and *PGC-1/srl* mutant flies were aged for 5–10 days on NF and then placed on NF or HFD (NF containing 30% coconut oil) for a further 5 days. Twenty each of NF and HFD flies were homogenized in RIPA buffer (Thermo Scientific) containing phosphatase and protease inhibitors (Roche). Protein levels were quantified using Bradford Reagent (Bio-Rad, Cat. # 500-0201), and samples of 40 µg total protein were separated by SDS/PAGE using Novex NuPAGE® 4–12% Bis-Tris Gels (Invitrogen). Proteins were transferred to nitrocellulose membranes (Invitrogen) and the membranes were blocked with 1x TBS-T tween O/N. Blots were incubated for 1 h at room temperature with rabbit anti-phospho-Akt, rabbit anti-phospho-S6K (both Cell Signaling Technologies), rabbit anti-SREBP (BD Pharmingen), mouse anti-actin (Millipore), or mouse anti-GFP (Invitrogen) primary antibodies diluted 1:1000 in Tris-buffered saline containing 1xTBS-T tween and 5% BSA. After washing, blots were incubated for 1 h at room temperature with HRP-conjugated anti-rabbit or anti-mouse secondary antibodies (Amersham) diluted 1:5000 in 5% BSA in TBST. Blots were washed again and bands were visualized using the ECL plus enhanced chemiluminescence reagent (Amersham, Cat. # RPN2132).

Immunocytochemistry

Hearts were dissected as described above, incubated with 10 mM EGTA for 30 s to induce muscle relaxation, fixed with 4% paraformaldehyde in PBS for 20 min at room temperature, and then washed three times for 10 min with PBS containing 0.05% Triton X-100 (PBT). Hearts were transferred to a 96-well plate (10–15 per well) and incubated in blocking buffer (PBT containing 5% BSA) for 1 h. Mouse anti-GFP and anti-phalloidin primary antibodies were diluted 1:500 in PBT and added to the wells for 1 h at room temperature or overnight at 4ºC. Hearts were washed three times with PBT and incubated for 1 h at room temperature with the appropriate Alexa Fluor-conjugated secondary antibody diluted 1:1000 in PBT. Samples were washed three times with PBT, once with PBS, and then placed on a slide in a drop of Vectashield mounting medium. For staining of lipid droplets, a stock solution of 1 mg/ml Nile Red was prepared in acetone and stored protected from light at 4°C. Hearts were fixed with 4% formaldehyde for 20 min at room temperature, washed three times with PBS for 10 min each, and then incubated with 10 µg/ml Nile Red in PBS for 5 min. Stained hearts were washed with PBS and mounted in Vectashield for analysis. Images were acquired with a Zeiss ApoTome microscope using Zeiss imaging software.

GeneSwitch

GeneSwitch system uses a RU-dependent Gal4 that allow the spatiotemporal activation of specific UAS line in the fly (Monnier et al., 2012; Osterwalder et al., 2001; Roman et al., 2001). We established a stock solution of 25 mg/ml of RU486 (Mifepristone) dissolved in ethanol. For adult flies feeding, this stock solution was diluted to 100ug/ml directly into the fly food with or without HFD. Flies carrying Hand-GS-Gal4 Gene Switch (Hand-GS) drivers were crossed to PGC-1^{RNAi} for KD, UAS-*PGC-1*^{JW} and UAS-*bmm* flies for overexpression. The offspring Hand-GS>*PGC-1*^{RNAi}, Hand-GS>*PGC-1*^{JW} and Hand-GS>UAS-*bmm* flies aged 3-5days after eclosion were transferred into tubes (25 flies/tube) of normal fly food containing RU or vehicle (ethanol). After 5 days feeding, flies were transferred into new tubes containing NF or HFD with RU or vehicle for 5 more days. The fly hearts are then dissected for heart function analysis and qPCR experiments.

Statistical Analysis

Statistical analysis was done using Student's t-tests, ANOVA, chi-square or Kruskal-Wallis tests where appropriate. The analysis was performed using GraphPad Prism versions 5.00 and 6.00 for Windows.

Supplemental References

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