Supplemental figure legends

Figure S1. General phenotypes of SH2B deficient flies and mice. (A) Genomic organization and P element insertion of the *dSH2B* gene (black boxes: coding exons; open boxes: UTRs; triangles: P elements). (B) Total RNA was extracted from adult flies (3 days) and reversely transcribed into cDNAs. dSH2B or β-actin cDNAs were amplified by PCR using dSH2B- or β-actin-specific primers. (C-D) Body weight of adult flies (3 days) (160 flies per group). Genotypes were: wild type (WT), $dSH2B^{D/+}$ (D/+), $dSH2B^{F/+}$ (F/+), $dSH2B^{D/D}$ (D/D), $dSH2B^{D/F}$ (D/F) and $dSH2B^{F/F}$ (F/F). (E) Pupation was recorded every 12 h (120 animals per group). (F) Left panel: WT or D/D males were mated with wild type females (10 mating pairs); right panel: WT or D/D females were mated with wild type males (10 mating pairs). Egg numbers were recorded daily. (G) Total TAG levels were measured in adult flies (3 days) under fed condition and normalized to total protein levels (32 flies per group). (H) Body weight and length (nose-anus) of male mice at 3 and 4 weeks of age (WT: n=10; KO: n=10), respectively. *$p<0.05$.

Figure S2. The *UAS-dSH2B* transgene alone does not alter fly body weight, lipid levels, or starvation resistance. (A) Body weight (120 flies per group) and total TAG levels (32 flies per group) of wild type (WT) and *UAS-dSH2B/+* adult flies. (B) Survival curves of WT and *UAS-dSH2B/+* flies (3 days) in response to starvation (120 per group).

Figure S3. dSH2B inhibits dFOXO. (A) S2 cells were cotransfected with expression vectors encoding dSH2B or V5-tagged dFOXO. Forty-eight h after transfection, cells were deprived of FBS for 6 h and treated with human insulin (0.1 μg/ml) for 15 min. Cells were immunostained with the indicated antibodies, and

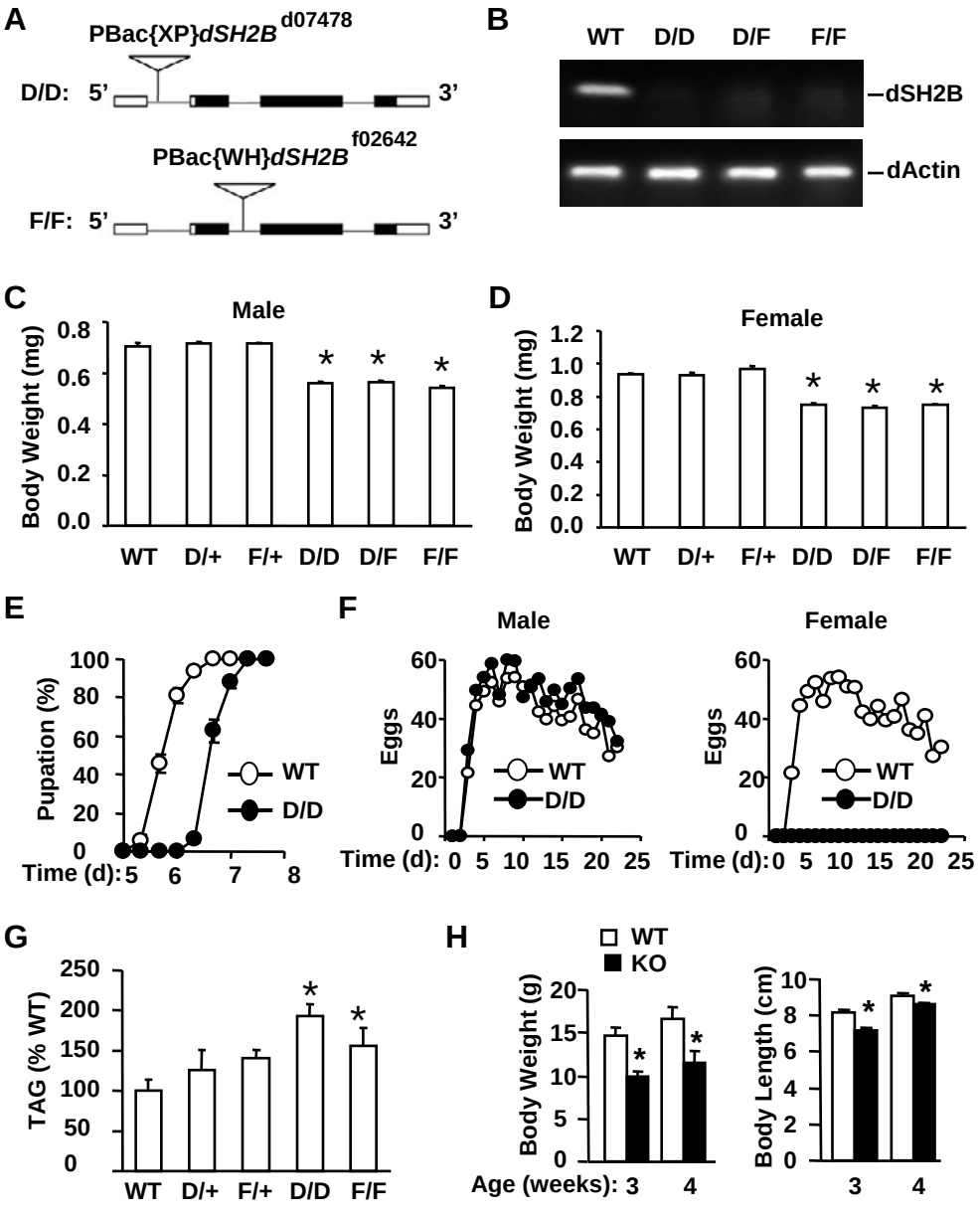
visualized using a confocal microscopy. Cells positive for cytoplasmic dFOXO were counted and normalized to total number of cells (four independent experiments). (B) Total RNA was prepared from the whole body of wild type (WT) and *dSH2B*$^{D/D}$ (D/D) adult flies under fed or starved (48 h) condition and used to measure *d4E-BP* and *dInR* mRNA abundance (normalized to RPL32 expression) by quantitative RT-PCR (32 flies per group). *p<0.05.

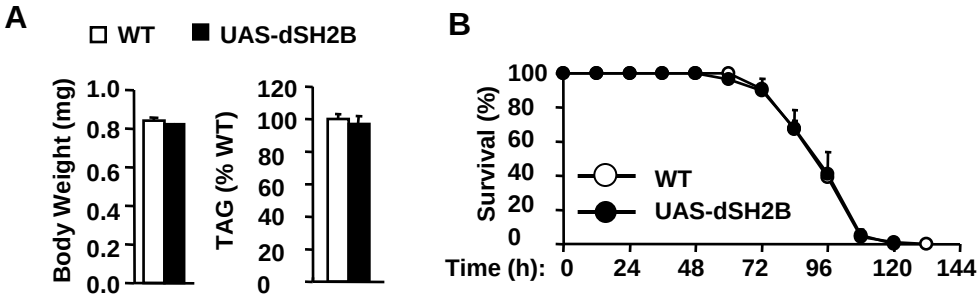
Figure S4. Fat body dSH2B regulates both lipid and glucose metabolism. (A) Tissue extracts were prepared from the heads or abdomens of adult flies (3 days) of the indicated genotypes, and immunoblotted with anti-dSH2B or anti-α-tubulin antibodies. (B) Body weight of *adh-GAL4/+;dSH2B*$^{D/D}$ (D/D-adh-Con) or *adh-GAL4/UAS-dSH2B;dSH2B*$^{D/D}$ (D/D-adh-dSH2B) flies (3 days) (100 flies per group). (C) Total TAG levels in *lsp2-GAL4/+* (lsp2-Con) and *UAS-dSH2B/+;lsp2-GAL4/+* (lsp2-Tg) third instar larvae (32 animals per group). (D) Hemolymph carbohydrates in lsp2-Con and lsp2-Tg third instar larvae (24 animals per group). (E) Total TAG levels in *ppl-GAL4/+* (ppl-Con) and *UAS-dSH2B/+;ppl-GAL4/+* (ppl-Tg) third instar larvae (32 animals per group). (F) Hemolymph carbohydrates in ppl-Con and ppl-Tg third instar larvae (24 animals per group). *p<0.05.

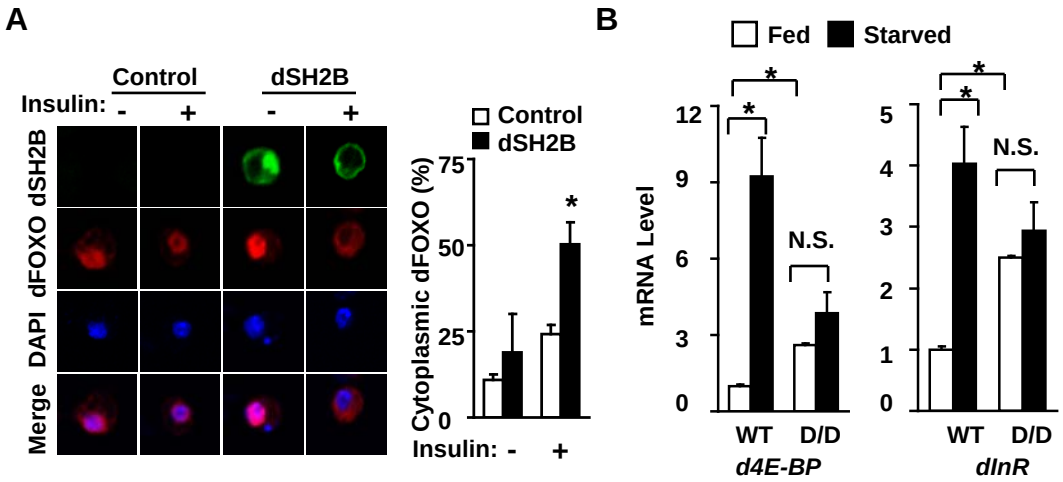
Figure S5. Neuron-specific overexpression of dSH2B does not alter metabolism and starvation resistance. (A) Tissue extracts were prepared from the heads or abdomens of adult flies (3 days) of the indicated genotypes, and immunoblotted with anti-dSH2B or anti-α-tubulin antibodies. (B) Body weight (100 flies per group) and total TAG levels (32 flies per group) of *elav-GAL4/+;dSH2B*$^{D/D}$ (D/D-elav-Con) or *elav-GAL4/UAS-dSH2B;dSH2B*$^{D/D}$ (D/D-elav-dSH2B) adult flies (3 days). (C) Survival curves

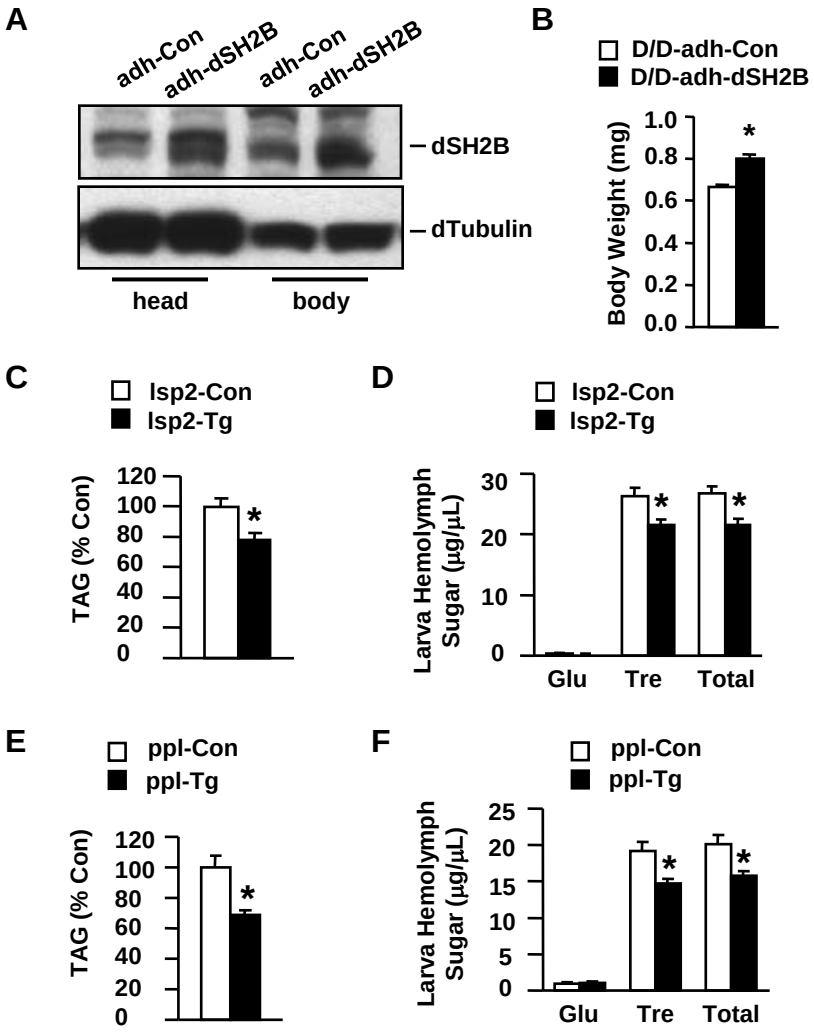
of D/D-elav-Con or D/D-elav-dSH2B adult flies (3 days) under starvation (100 flies per group).

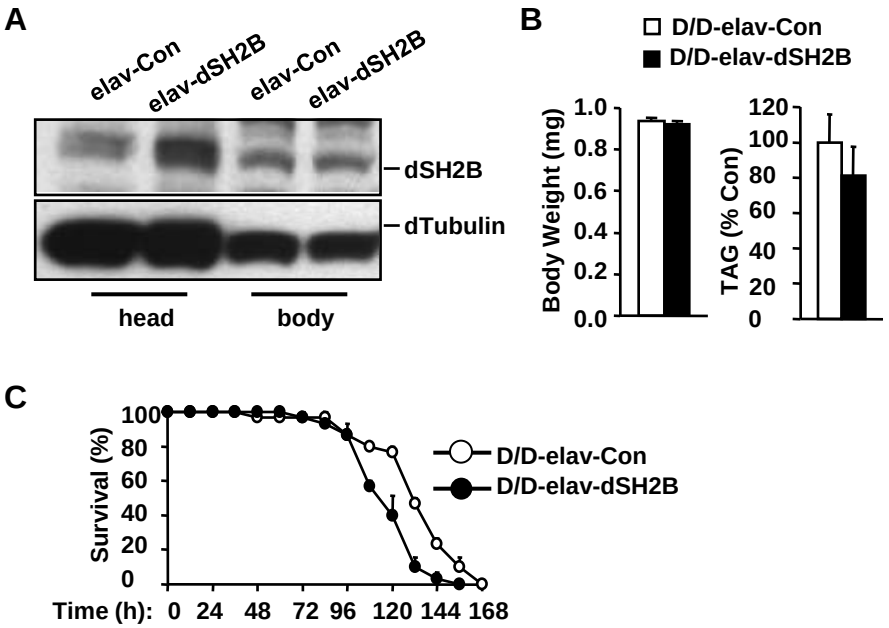
Figure S6. dSH2B regulation of lifespan. (A) Survival curves of WT or *dSH2B*$^{D/F}$ female flies backcrossed to w^{1118} for one time (140 per group). (B-C) Survival curves of WT and *UAS-SH2B* flies (100 flies per group). (D) Survival curves of *adh-GAL4/+* (adh-Con) or *adh-GAL4/UAS-dSH2B* (adh-dSH2B) flies (120 flies per group).

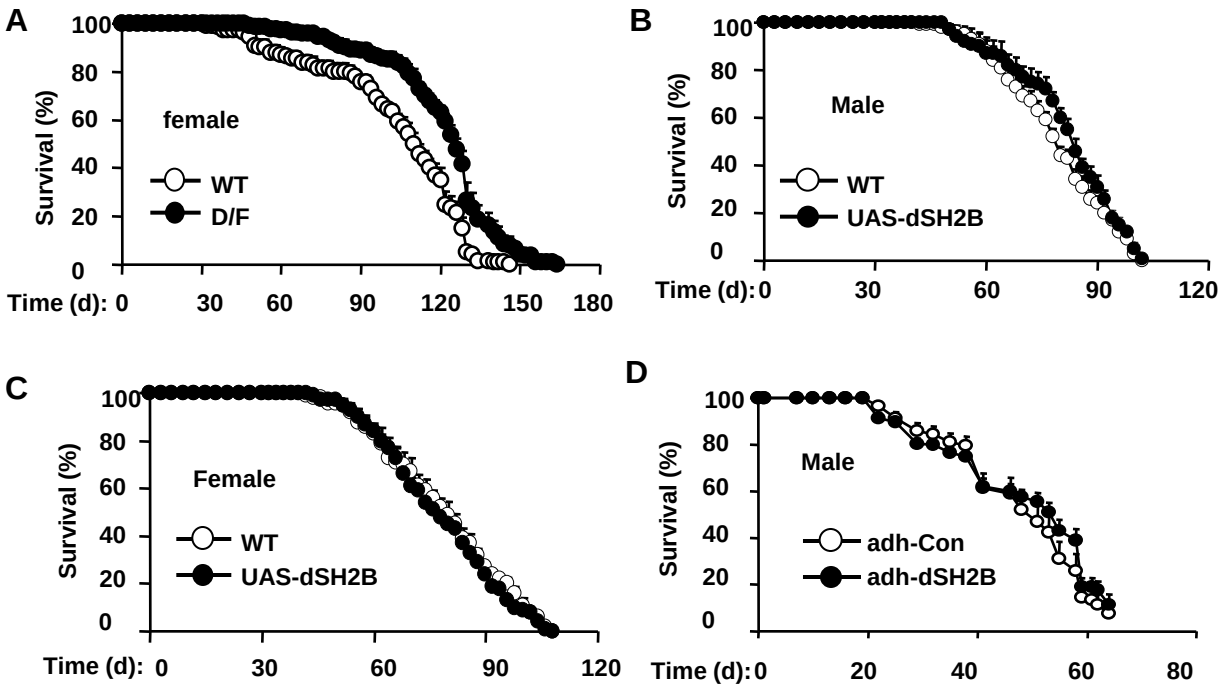

A
PBac{XP}dSH2B d07478
D/D: 5' 3'
PBac{WH}dSH2B f02642
F/F: 5' 3'
B
WT D/D D/F F/F
dSH2B
dActin
C
Male
Body Weight (mg)
0.0 0.2 0.4 0.6 0.8
WT D/+ F/+ D/D D/F F/F
D
Female
Body Weight (mg)
0.0 0.2 0.4 0.6 0.8 1.0 1.2
WT D/+ F/+ D/D D/F F/F
E
Pupation (%)
0 20 40 60 80 100
Time (d): 5 6 7 8
WT
D/D
F
Male
Eggs
0 20 40 60
Time (d): 0 5 10 15 20 25
WT
D/D
Female
Eggs
0 20 40 60
Time (d): 0 5 10 15 20 25
WT
D/D
G
TAG (% WT)
0 50 100 150 200 250
WT D/+ F/+ D/D F/F
H
WT
KO
Body Weight (g)
0 5 10 15 20
Age (weeks): 3 4
Body Length (cm)
0 2 4 6 8 10
3 4


A
WT
UAS-dSH2B
Body Weight (mg)
1.0
0.8
0.6
0.4
0.2
0.0
TAG (% WT)
120
100
80
60
40
20
0
B
Survival (%)
100
80
60
40
20
0
WT
UAS-dSH2B
Time (h):
0
24
48
72
96
120
144


A
Control
dSH2B
Insulin: - + - +
dSH2B
dFOXO
DAPI
Merge
Control
dSH2B
Cytoplasmic dFOXO (%)
75
50
25
0
*
Insulin: - +
B
Fed
Starved
mRNA Level
12
9
6
3
0
*
*
N.S.
WT
D/D
d4E-BP
5
4
3
2
1
0
*
*
N.S.
WT
D/D
dInR


A
adh-Con
adh-dSH2B
adh-Con
adh-dSH2B
dSH2B
dTubulin
head
body
B
D/D-adh-Con
D/D-adh-dSH2B
Body Weight (mg)
1.0
0.8
0.6
0.4
0.2
0.0
*
C
lsp2-Con
lsp2-Tg
TAG (% Con)
120
100
80
60
40
20
0
*
D
lsp2-Con
lsp2-Tg
Larva Hemolymph Sugar (μg/μL)
30
20
10
0
*
*
Glu
Tre
Total
E
ppl-Con
ppl-Tg
TAG (% Con)
120
100
80
60
40
20
0
*
F
ppl-Con
ppl-Tg
Larva Hemolymph Sugar (μg/μL)
25
20
15
10
5
0
*
*
Glu
Tre
Total


A
elav-Con
elav-dSH2B
elav-Con
elav-dSH2B
dSH2B
dTubulin
head
body
B
D/D-elav-Con
D/D-elav-dSH2B
Body Weight (mg)
1.0
0.8
0.6
0.4
0.2
0.0
TAG (% Con)
120
100
80
60
40
20
0
C
Survival (%)
100
80
60
40
20
0
Time (h): 0 24 48 72 96 120 144 168
D/D-elav-Con
D/D-elav-dSH2B


A
Survival (%)
100
80
60
40
20
0
female
WT
D/F
Time (d): 0
30
60
90
120
150
180
B
Survival (%)
Male
WT
UAS-dSH2B
Time (d): 0
30
60
90
120
C
Survival (%)
Female
WT
UAS-dSH2B
Time (d): 0
30
60
90
120
D
Survival (%)
Male
adh-Con
adh-dSH2B
Time (d): 0
20
40
60
80