Supplemental Figure Legends

Figure S1. *DHR38* mutant larvae have normal resistance to starvation and normal levels of glucose and triacylglycerol. (A) w^{1118} control, *DHR38*^{Y214}/*Df(2)Ketel* mutant (*DHR38*$^{-}$) and *dHNF4*$^{\Delta 33}$ mutant (*HNF4*$^{-}$) late second instar larvae were transferred to moist filter paper at 25°C and the fraction of surviving animals was scored after 24 and 48 hrs starvation. The survival of *DHR38* mutant larvae is similar to that of the controls, while ~50% of the *dHNF4* mutants, used as a positive control, had died after 24 hrs starvation (16). (B) Glucose levels are normal in *DHR38* mutant larvae. w^{1118} control and *DHR38*^{Y214}/*Df(2)Ketel* (*DHR38*$^{-}$) late second instar larvae were either collected or starved for 24 hrs, and homogenates were assayed for either protein and glucose. Glucose levels were normalized to the amount of protein and are presented relative to the level in fed controls. (C) Midguts dissected from fed or 24 hr starved w^{1118} control and *DHR38*^{Y214}/*Df(2)Ketel* (*DHR38*$^{-}$) mutant larvae were stained with Oil Red O. Similar depletion of lipid is seen upon starvation in both control and *DHR38* mutant larvae. Error bars represent SEM, n≥3 independent samples of 5-40 animals each.

Figure S2. *Amy* and *Pgm* expression are markedly reduced in *DHR38* mutants. RNA isolated from fed w^{1118}, *DHR38*^{Y214}/+, *DHR38*^{56}/+, *Df(2)Ketel*/+, *DHR38*^{Y214}/*Df(2)Ketel*, *DHR38*^{56}/*Df(2)Ketel* and *DHR38*^{Y214}/*DHR38*^{56} larvae was analyzed by northern blot hybridization to detect *Amy-p* and *Amy-d*, and *Pgm* expression. Blots were hybridized with *rp49* as a control for loading and transfer.

Figure S3. Disruption of either *Amy* or *Pgm* by RNAi has no effect on glycogen levels. (A) Control third instar larvae starved for 24 hrs that carry either the midgut-specific *mex-GAL4* driver or a *UAS-Amy(RNAi)* transgene (black bars) have the same level of glycogen as larvae in which both *Amy-d* and *Amy-p* have been inactivated by RNAi (grey bar). Due to the close identity between the *Amy-d* and *Amy-p* nucleotide sequences, the RNAi transgene targets both genes. RNA isolated from animals of the above genotypes were analyzed by northern blot hybridization to detect *Amy* transcripts, demonstrating efficient reduction in *Amy-d* and *Amy-p* expression by RNAi. (B) Control fed second instar larvae that carry either the widely-expressed *tub-GAL4* driver or a *UAS-Pgm(RNAi)* transgene (black bars) have the same level of glycogen as larvae in which *Pgm* has been inactivated by RNAi (grey bar). RNA isolated from animals of the above genotypes was analyzed by northern blot hybridization to detect *Pgm* transcripts, demonstrating efficient reduction in *Pgm* expression by RNAi. Error bars represent SEM, n≥3 independent samples of 5-40 animals each.

Figure S4. The *Amy-p* and *Amy-d* loci contain NBREs that have been conserved in related *Drosophila* species. Sequences of related *Drosophila* species were aligned using GenomeVISTA (http://genome.lbl.gov/cgi-bin/GenomeVista). The site at +2152 can be bound *in vitro* by DHR38 protein (not shown).

Figure S5. *DHR38* mutant pupae have normal levels of glycogen and ATP. w^{1118} control and *DHR38*^{Y214}/*Df(2)Ketel* mutant (*DHR38*$^{-}$) animals were grown to 72 hrs after puparium formation, when the adult cuticle starts to form and approximately one day before adult eclosion. Both glycogen (A) and ATP (B) levels are normal in mutant pupae. Metabolite levels were normalized to the amount of protein and are presented relative to the level in control animals. Error bars represent SEM, $n \geq 4$ independent samples of 3-5 animals each.

Figure S1.

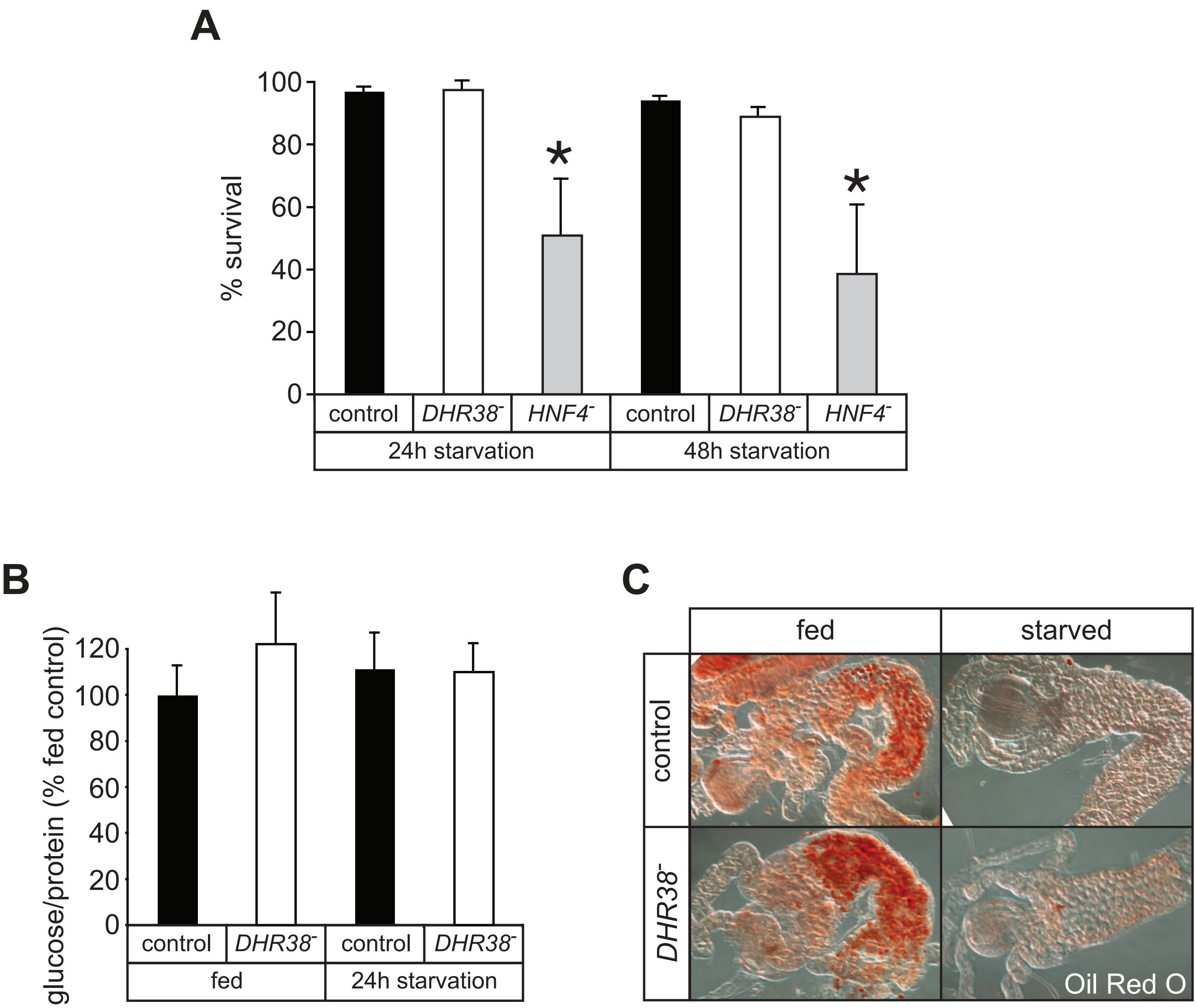
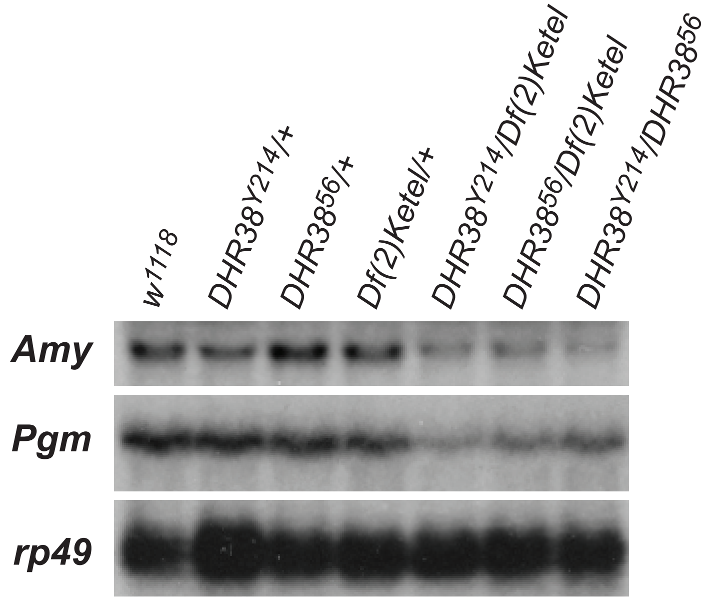

A
% survival
100
80
60
40
20
0
*
*
control
DHR38-
HNF4-
control
DHR38-
HNF4-
24h starvation
48h starvation
B
glucose/protein (% fed control)
120
100
80
60
40
20
0
control
DHR38-
control
DHR38-
fed
24h starvation
C
fed
starved
control
DHR38-
Oil Red O

Figure S2.



w1118
DHR38Y214/+
DHR3856/+
Df(2)Ketel/+
DHR38Y214/Df(2)Ketel
DHR3856/Df(2)Ketel
DHR38Y214/DHR3856
Amy
Pgm
rp49

Figure S3.

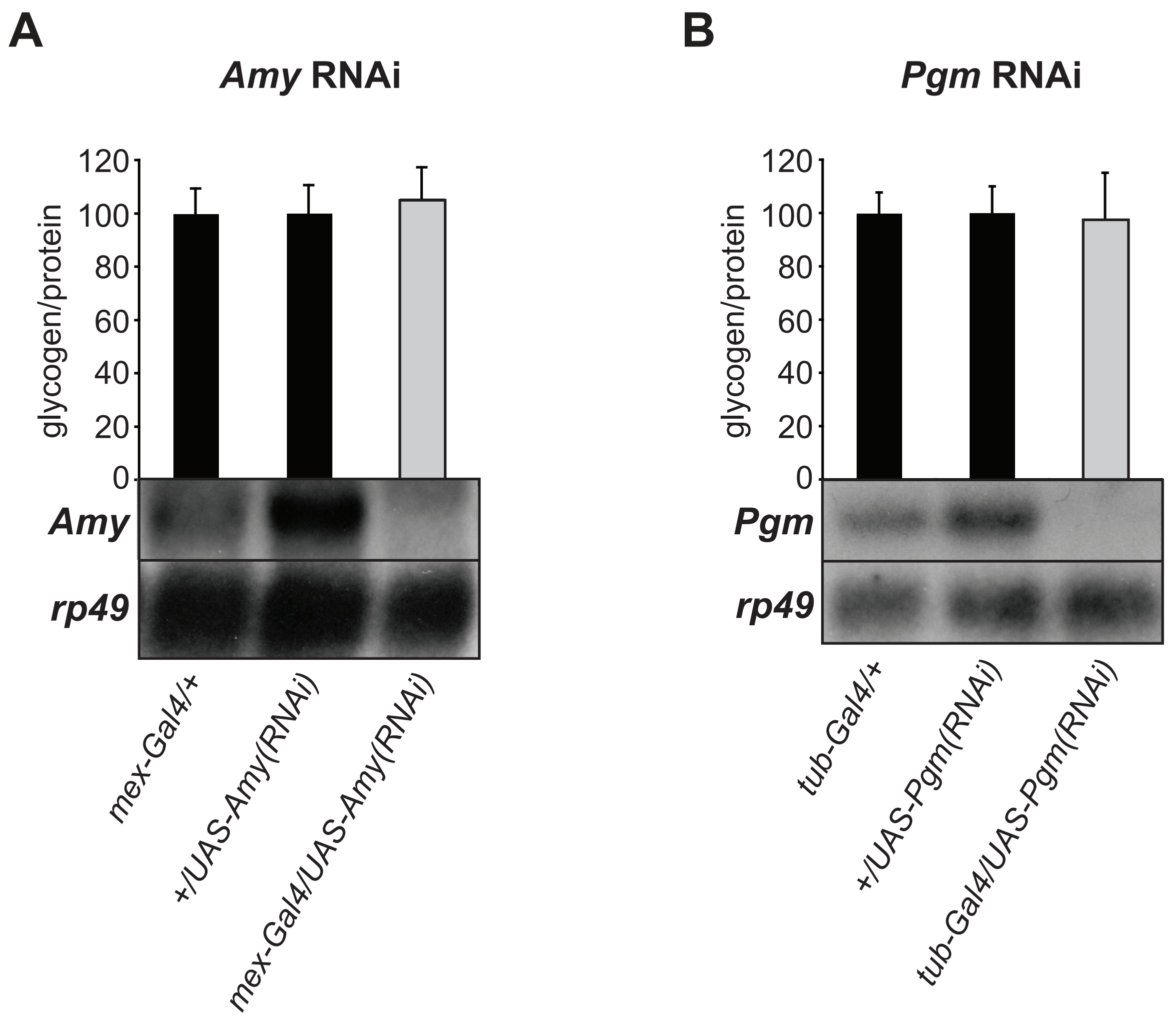

A
Amy RNAi
120
100
80
60
40
20
0
glycogen/protein
Amy
rp49
mex-Gal4/+
+/UAS-Amy(RNAi)
mex-Gal4/UAS-Amy(RNAi)
B
Pgm RNAi
120
100
80
60
40
20
0
glycogen/protein
Pgm
rp49
tub-Gal4/+
+/UAS-Pgm(RNAi)
tub-Gal4/UAS-Pgm(RNAi)

Figure S4.

Amy-p promoter -524

```
             NBRE      AAAGGTCA
      D. simulans CCAGTCAAGGCCAGAAGT
     D. sechellia CCAGTCAAGGCCAGAAGT
  D. melanogaster CCAGTCAAGGCCAGAAGT
        D. yakuba CCAGTCAAGGCCAGAAGT
        D. erecta CCAGTCAAGGCCAGAAGT
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Amy-d promoter -642

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             NBRE      AAAGGTCA
  D. melanogaster AGGCCAAAGTTCAGATTG
      D. simulans AGGCCAAAGTTCAGATTG
        D. yakuba AGGCCAAAGTTCAGATTG
        D. erecta AGGCCAAAGTTCGGATGG
     D. sechellia AGGCCAAAGTTCAGATTG
                  ************ *** *
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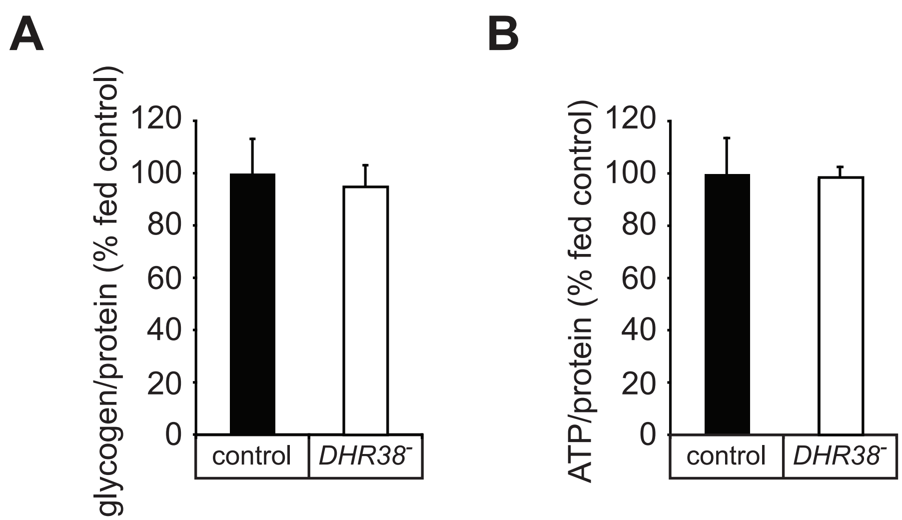
Figure S5.



A
B
glycogen/protein (% fed control)
ATP/protein (% fed control)
120
100
80
60
40
20
0
control
DHR38⁻