SUPPLEMENTARY INFORMATION

Glucosylceramide synthase (GlcT-1) in the fat body controls energy metabolism in *Drosophila*

Ayako Kohyama-Koganeya,[1,2] Takuji Nabetani,[1] Masayuki Miura,[2,3] Yoshio Hirabayashi[1,3*]

[1]Molecular Membrane Neuroscience, Brain Science Institute, RIKEN, Wako-shi, Saitama 351-0198, Japan

[2]Department of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan

[3]CREST, JST

*Correspondence

Supplementary Experimental Procedures

Primer list for real-time PCR analysis

rp49-F	cggatcgatatgctaagctgt
rp49-R	cgacgcactctgttgtcg
dGlcT-1-F	cacgtgctctgttggttcc
dGlcT-1-R	cgagccatgctggacaat
egh-F	caagctctttcacaagccgc
egh-R	tacatttcgccctcgatgaag
ACC-F	caacctgagcggactcaaag
ACC-R	cactgctcctttccaatgtattt
PEPCK-F	cccacttccctttatctctctc
PEPCK-R	acagaccgcaattgtcctg
FAS-F	tcgaggaatcgcctgattt
FAS-R	attgttgggcttgaaagcaa

Drosophila stocks

UAS-dMekk1, UAS-dATF2^{wt} GAL4, MS1096-GAL4 (kind gift from Dr. S. Ishii). MS1096-GAL4 driver was used to express transgenes specifically in the wing.

dGlcT-1 IR2 Construct. The inverted repeats (IR) were constructed in a head-to-head orientation by using a combination of tag sequences of PCR primers (AAG GCC TAC ATG GCC GGA CCG CCG CAA CGA TGT CAC ACC TAC and AAT CTA GAG GTA CCT CGG ACA TGT TCT GCA CCA TG). A ~500bp cDNA fragment of dGlcT-1 was amplified by PCR and inserted as an IR in a modified pUAST transformation vector, pUAST-R57, which possesses an IR formation site consisting of paired KpnI-CpoI and XbaI-SfiI restriction sites.

Supp Fig. S1

FB-Gal4 and Lsp2-Gal4 expression patterns were examined through the expression of GFP. FB-Gal4 induced transgene expression in the fat body during both larval and adult stages, whereas Lsp2-Gal4 induced transgene expression in the fat body only during

the larval stage, not the adult stage.

Supp Fig. S2

Quantitative RT-PCR analysis of dGlcT-1 mRNA of control, dGlcT-1-overexpressing (FB>dGlcT-1), and dGlcT-1 knockdown (FB>dGlcT-1 IR) flies. Lsp2-Gal4 (A) or FB-Gal4 (B) drivers were used to overexpress dGlcT-1 and dGlcT-1 IR specifically in the fat body, and then mRNAs were collected from the fat body. dGlcT-1 mRNA levels in dGlcT-1-overexpressing fat bodies were higher than that in controls. However, dGlcT-1 mRNA levels in dGlcT-1 IR-expressing fat bodies (dGlcT-1 knockdown) were reduced.

Supp Fig. S3

To confirm that dGlcT-1 knockdown line from VDRC Stock Center specifically downregulates dGlcT-1 expression, we generated another dGlcT-1 knockdown line (dGlcT-1 IR2) and measured whole-body TAG levels of 6~7day old male flies. n=5 for each genotype. TAG levels of both dGlcT-1 knockdown flies (dGlcT-1 IR and dGlcT-1IR2) were reduced than wild-type flies.

Supp Fig. S4

(A) Staining for the presence of lipid droplets that store TAG in the fat bodies of third (L3) instar larvae. Staining was strong in dGlcT-1-overexpressing fat bodies but weak in dGlcT-1 IR-expressing (dGlcT-1 knockdown) fat bodies.

(B) Histograms comparing the whole-body TAG levels of dGlcT-1-overexpressing and dGlcT-1 IR third (L3) instar larvae.

(C) Effects of altered dGlcT-1 expression in the fat body on larval hemolymph trehalose levels.

Supp Fig. S5

(A) dGlcT-1 was overexpressed or suppressed in the fat body of larvae using FB-Gal4, and proteins were collected from the fat bodies of these larvae. The level of dp38 phosphorylation was analyzed by Western blotting with phospho-p38 antibody.

(B) Effects of altering dGlcT-1 expression on wing phenotype. We examined wing phenotype because dGlcT-1 expression affects the p38-ATF2 signaling pathway, and activation of the p38-ATF2 signaling pathway using the MS1096-GAL4 driver has been

shown to cause defects in wing formation (Sano *et al,* 2005). Overexpression or knockdown of dGlcT-1 did not result in obvious changes in wing phenotype (b and c). However, expression of dMEKK1 or dATF2 caused aberrant phenotypes to develop (d and g). Interestingly, the aberrant phenotypes were enhanced by dGlcT-1 overexpression (e and h), and suppressed by dGlcT-1 underexpression (f). Co-overexpression of both dMEKK1 and dGlcT-1 IR was lethal. (i) The MS1096-Gal4 driver used in this experiment is expressed throughout the wing.

Supp Fig. S6

(A) GSL core biosynthetic pathway of *Drosophila*. GlcCer is a common precursor of most complex GSLs. Egghead (egh) catalyzes the addition of a mannose residue to form a substrate for Brainiac (Brn), which catalyzes the addition of GlcNAc to the substrate, causing the GSL to elongate.

(B) Histograms comparing dGlcT-1 and egh mRNA expression levels in the fat body and whole body. Expression was examined by qRT-PCR.

(C) Histograms showing the effects of egh expression on stored TAG levels in the fat body.

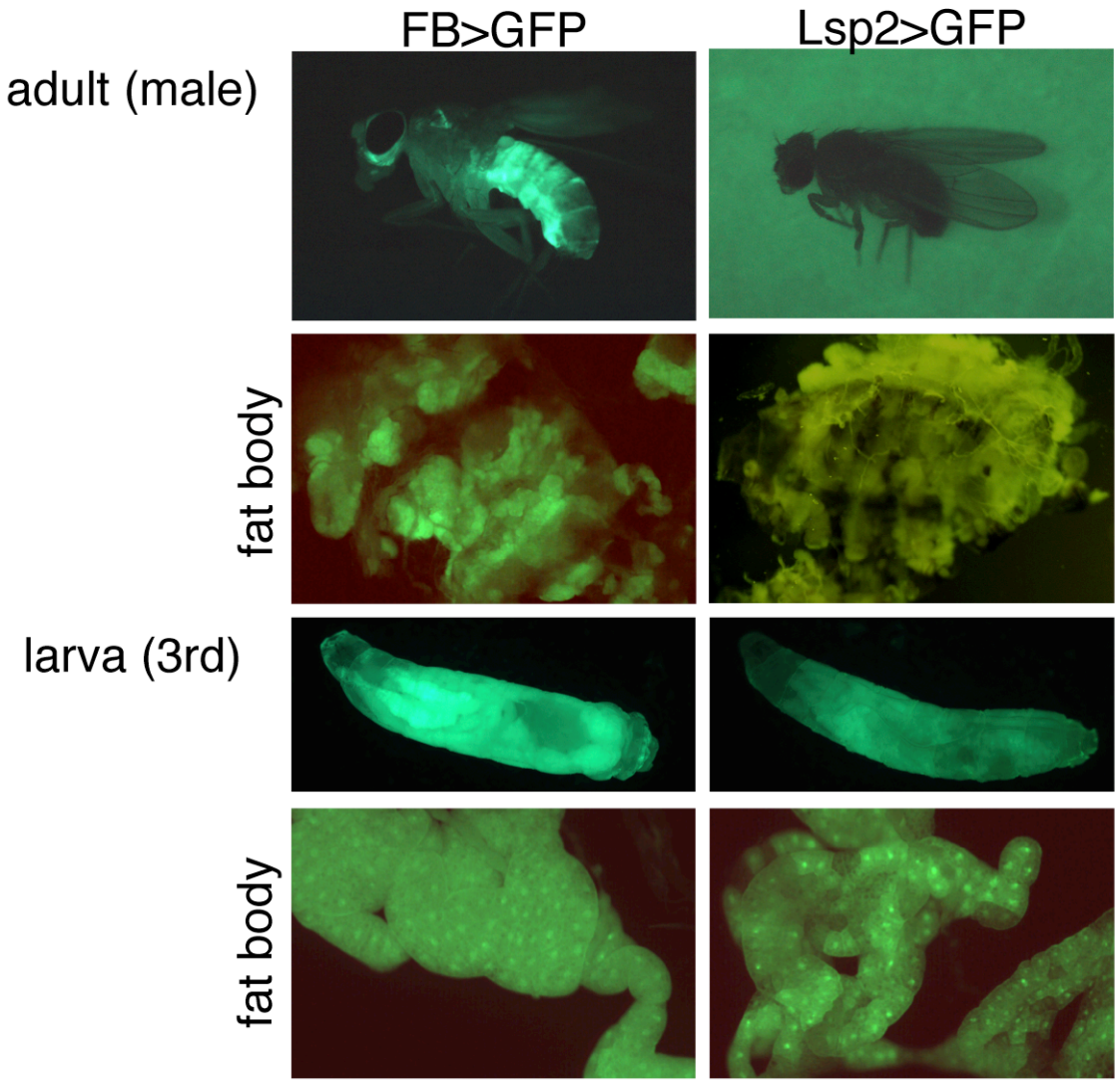
Supp Fig. S7

Model illustrating the role of dGlcT-1 on energy homeostasis in the fat body.

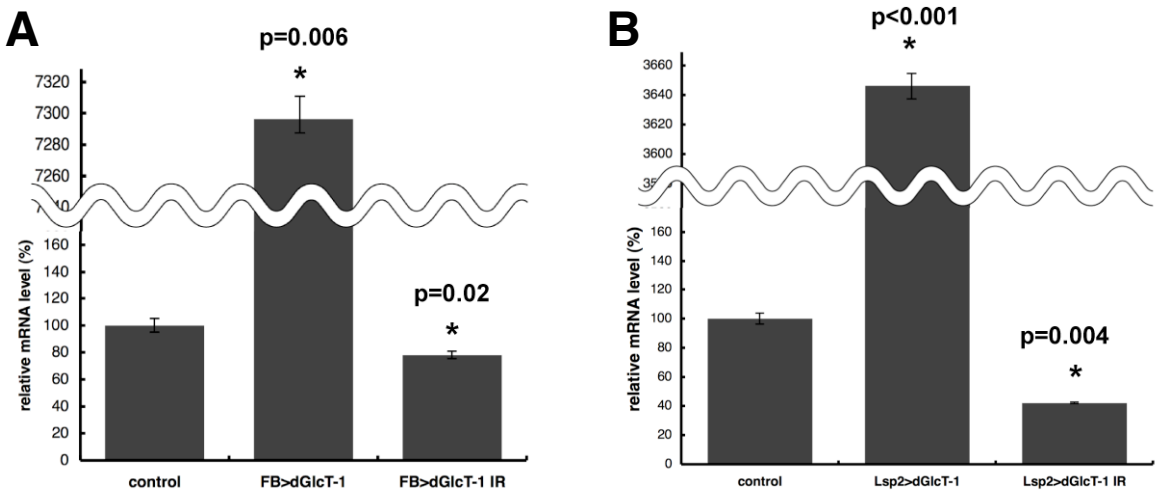
In the *Drosophila* fat body, dGlcT-1 generates GlcCer, which is a major component of lipid rafts. Lipid rafts may recruit proteins that activate the p38-ATF2 signaling pathway. As a result, PEPCK is activated, causing glyceroneogenesis to proceed. Furthermore, lipid rafts may recruit the signaling pathway for FAS and ACC. Together with the p38-ATF2 signaling pathway, this pathway may stimulate TAG biosynthesis.

Supplementary Reference

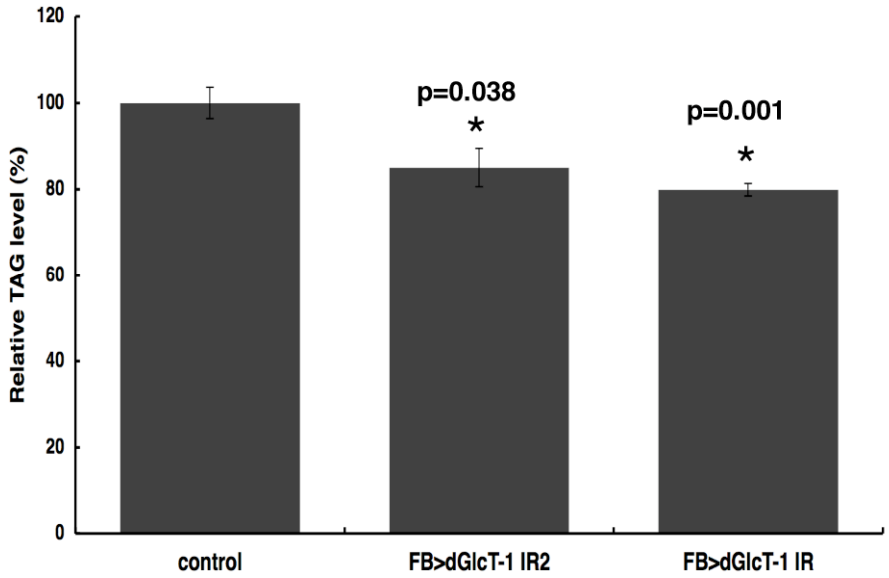
Sano, Y., Akimaru, H., Okamura, T., Nagao, T., Okada, M. and Ishii, S. (2005) Drosophila activating transcription factor-2 is involved in stress response via activation by p38, but not c-Jun NH(2)-terminal kinase. *Mol Biol Cell*, **16**, 2934-2946.



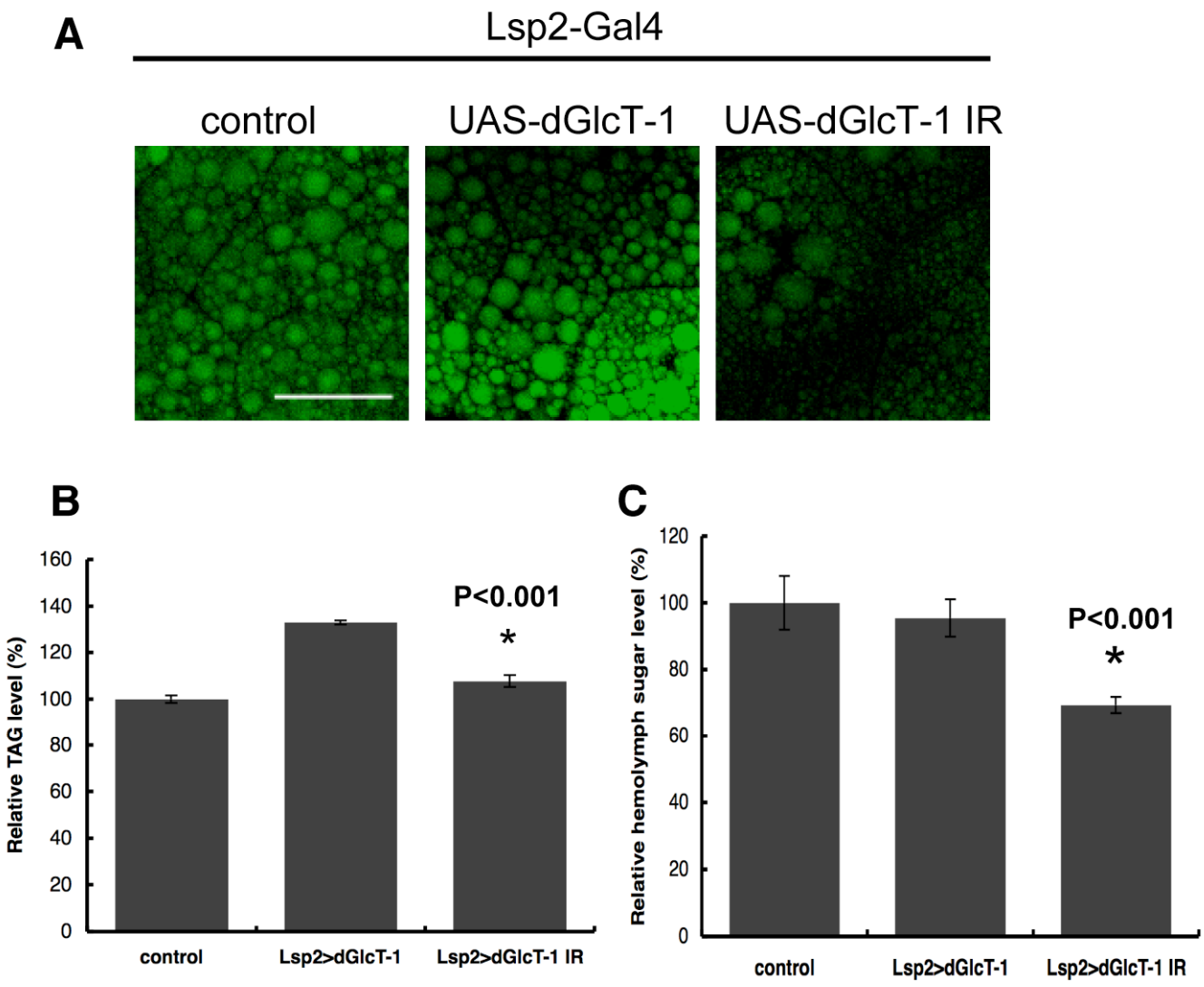
Supplemental Fig.S1



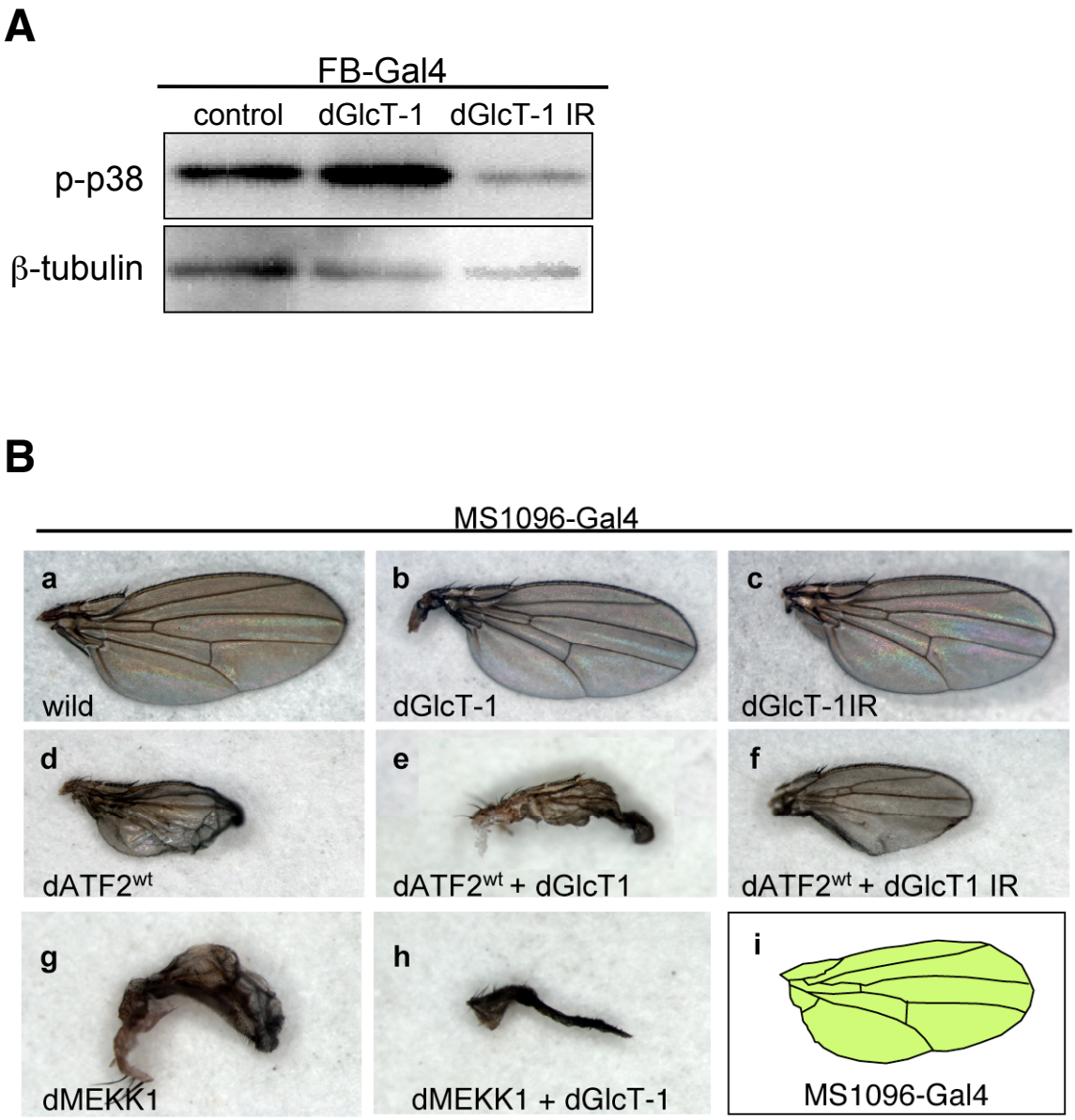
Supplemental Fig.S2



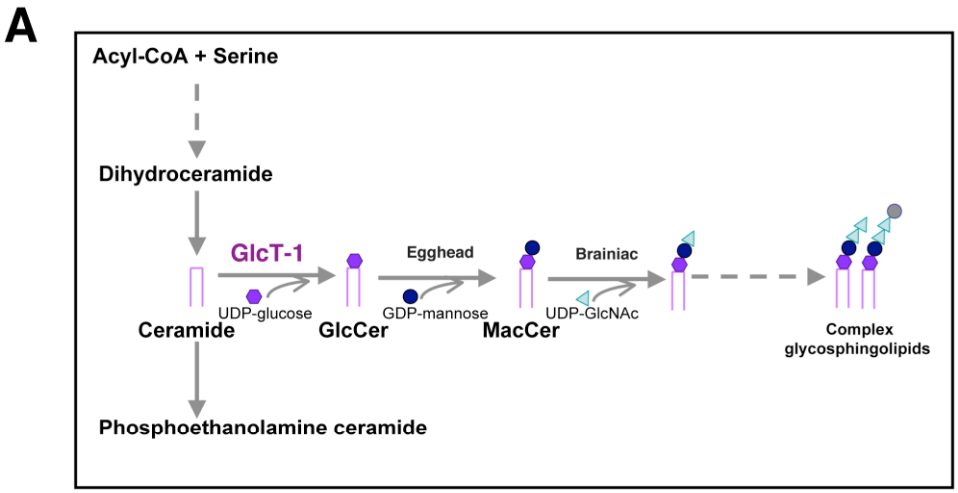
Supplemental Fig.S3

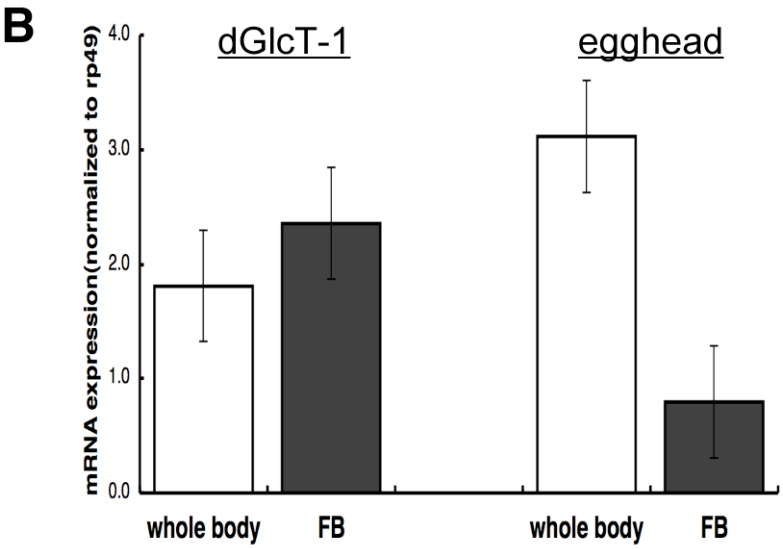


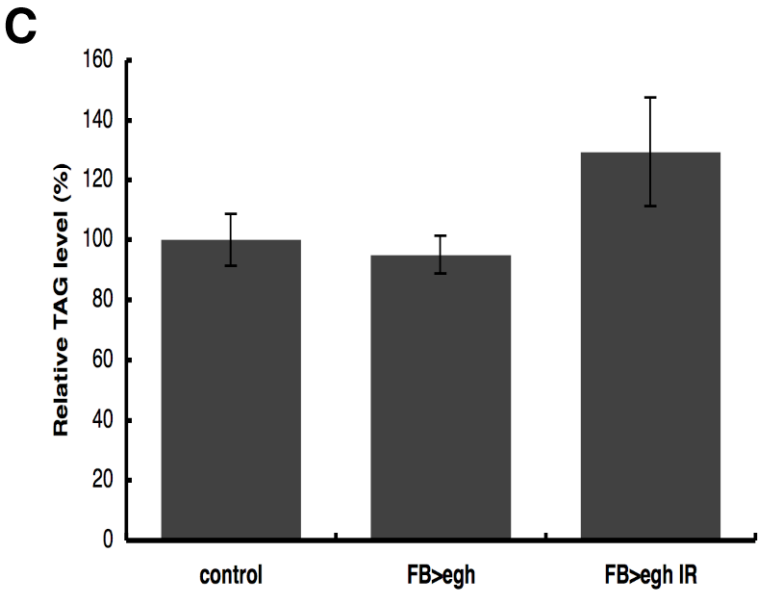
Supplemental Fig.S4



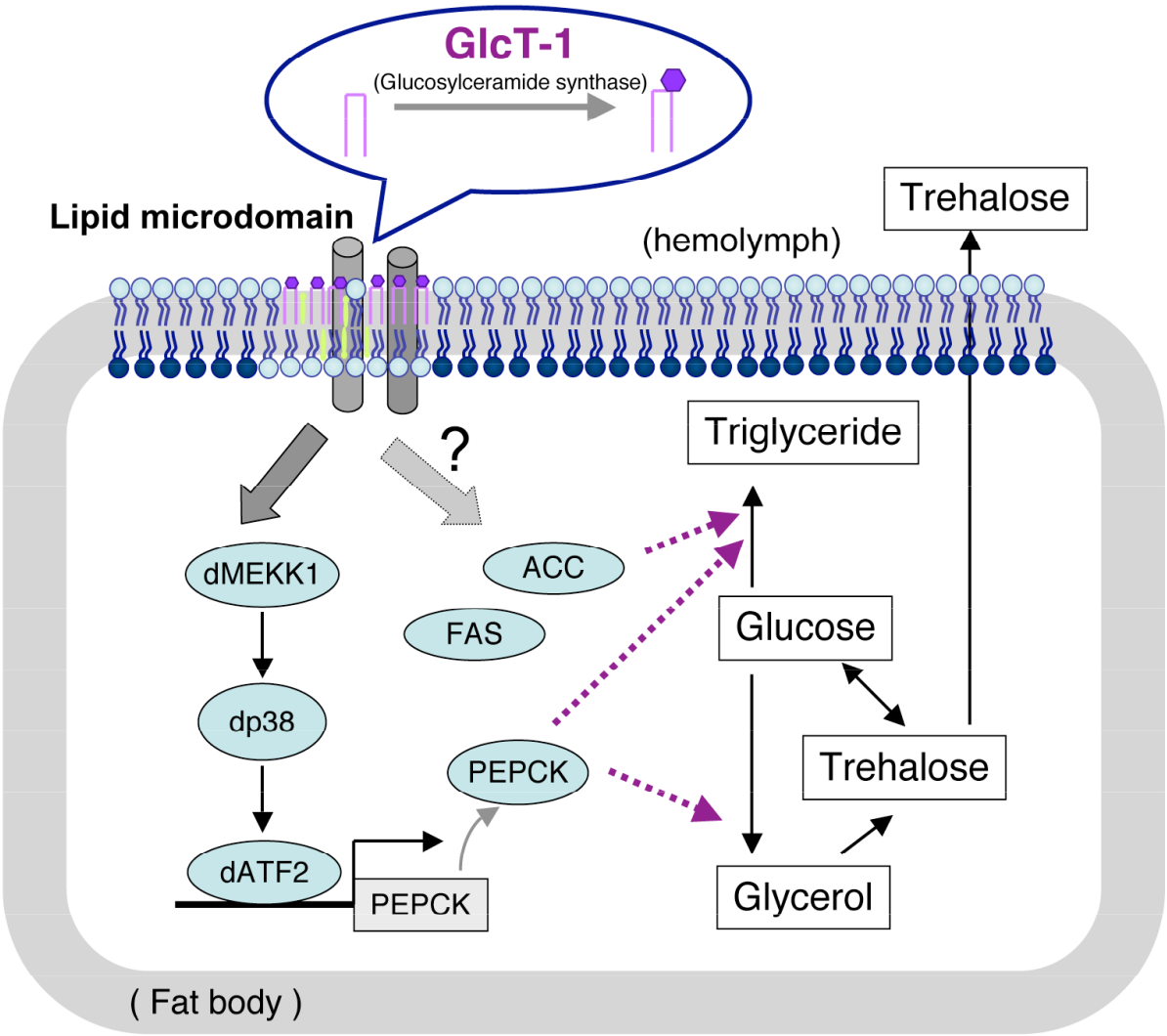
Supplemental Fig.S5







Supplemental Fig.S6



Supplemental Fig.S7