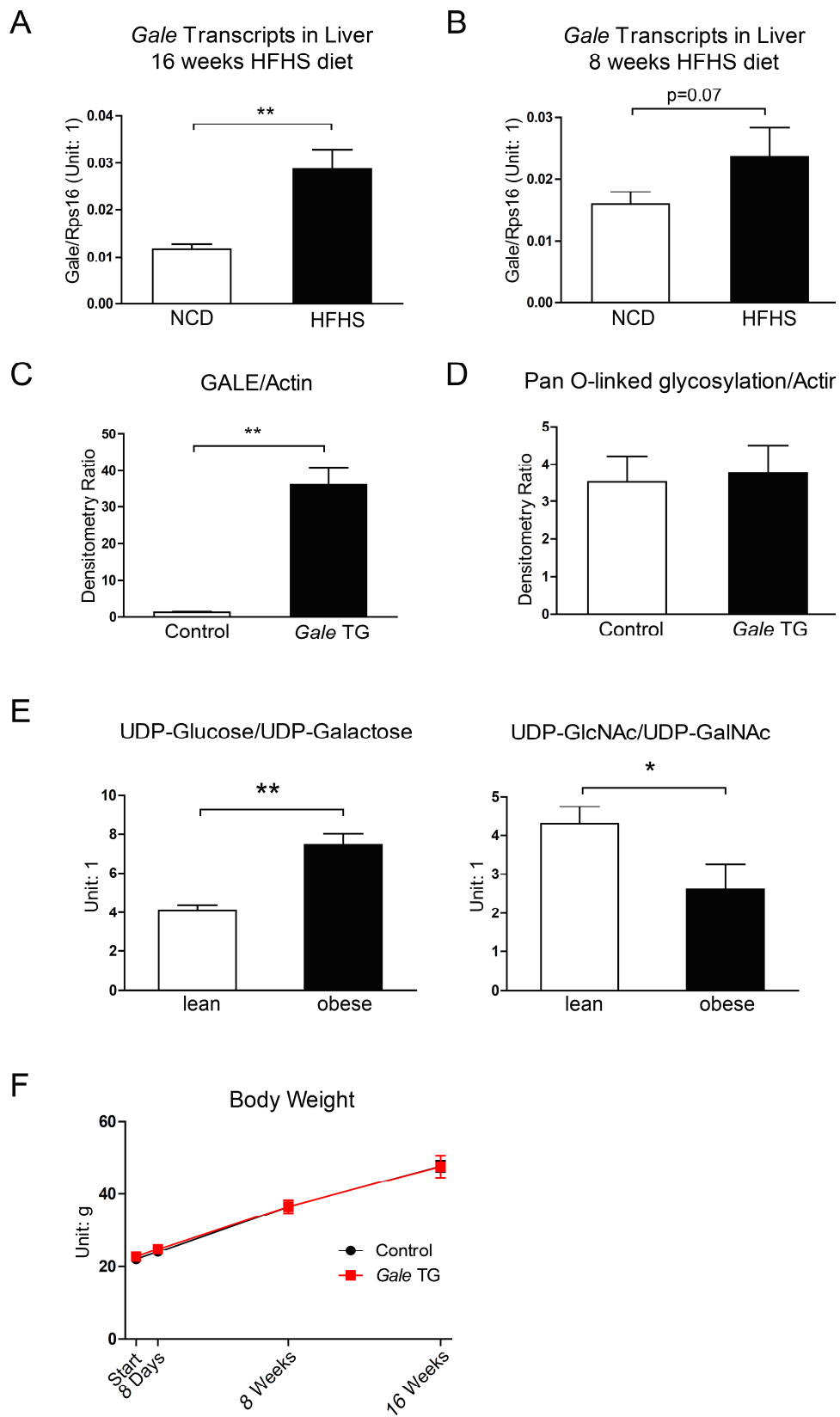SUPPLEMENTARY DATA

Supplementary Figure 1. Generation of hepatocyte-specific *Gale* TG mice. A. Hepatic *Gale* expression after 16 weeks HFHS diet (n = 10 for each group). B. Hepatic *Gale* expression after 8 weeks HFHS diet (n = 8 for each group). C. Quantification of GALE protein (n = 7 for control, n = 5 for *Gale* TG). D. Quantification of pan O-Linked glycosylation (n = 7 for control, n = 5 for *Gale* TG). E. UDP-Glucose/UDP-Galactose ratio and UDP-GlcNAc/UDP-GalNAc ratio in livers from lean and 22 weeks HFHS diet feeding induced obese mice (n = 9 and 4 for lean and obese mice). F. Body weight of control and *Gale* TG mice on HFHS diet. *p<0.05, **p < 0.01. Comparison by Student's T-test. All data are mean ± s.e.m.
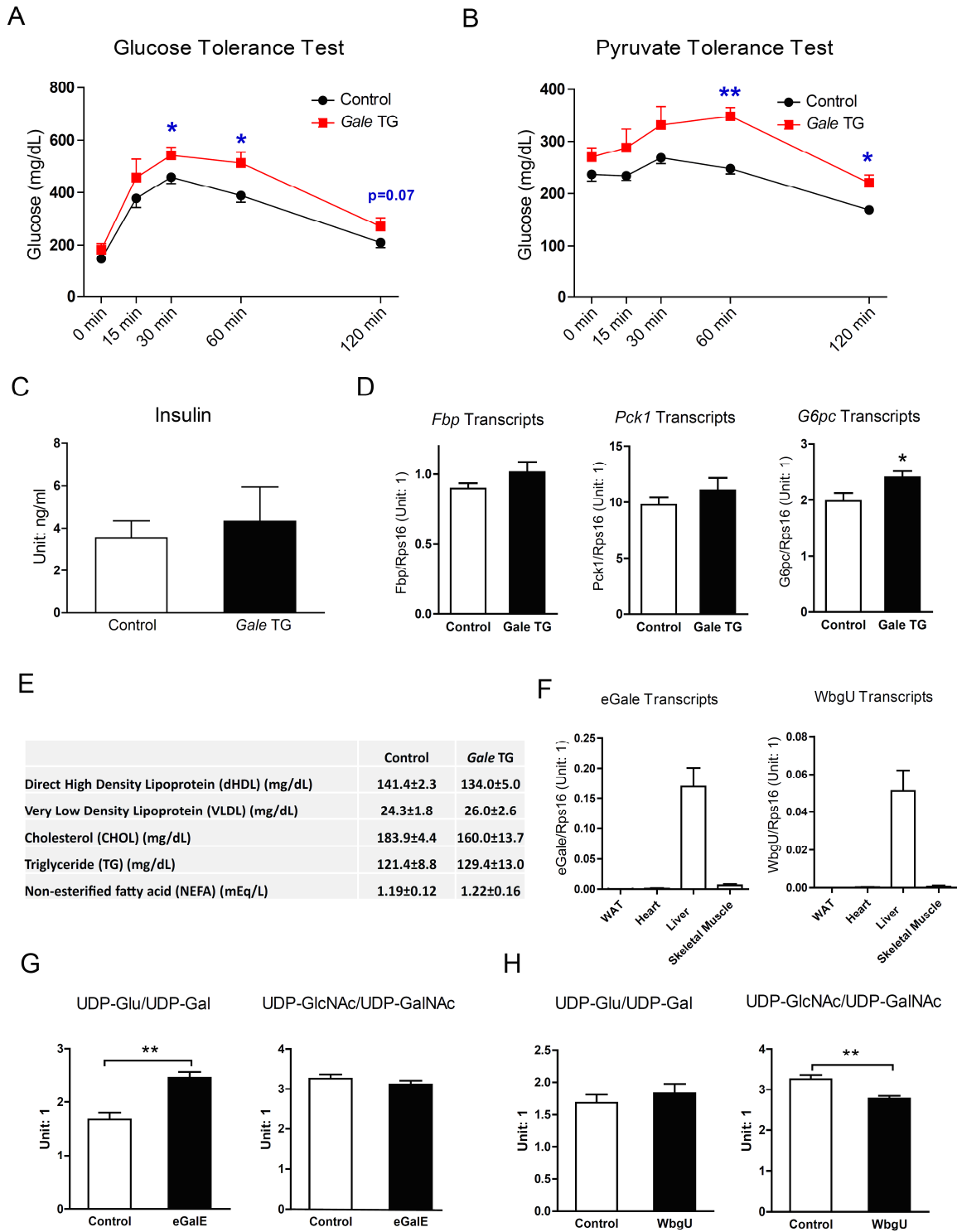
A

Gale Transcripts in Liver
16 weeks HFHS diet

B

Gale Transcripts in Liver
8 weeks HFHS diet

C

GALE/Actin

D

Pan O-linked glycosylation/Actin

E

UDP-Glucose/UDP-Galactose

UDP-GlcNAc/UDP-GalNAc

F

Body Weight

Supplementary Figure 2. Metabolic phenotypes of *Gale*-overexpressing mice. A. GTT of mice after 8-week HFHS diet feeding followed by 8 days of transgene induction (n = 7 for both groups). 1.25 g kg-1 glucose via i.p. injection. B. PTT of mice after 8-week HFHS diet feeding followed by 8 days of transgene induction (n = 8 and 6 for control and *Gale* TG groups respectively). 1 g kg^{-1} pyruvate via i.p. injection. C. Fasting insulin for mice after 8-week HFHS diet feeding followed by 8 days of transgene induction (n = 7 and 5 for control and *Gale* TG groups respectively). D. Gluconeogenic genes in *Gale* liver 8 days post transgene induction (n = 6 for each group). E. Serum lipid profile and liver enzyme levels for mice after 8-week HFHS diet feeding followed by 3 weeks of transgene induction (n = 7 and 5 for control and *Gale* TG groups respectively). F. eGale and WbgU mRNA levels in white adipose tissue (WAT), heart, liver and skeletal muscle 4 weeks post AAV infection (n = 4 for each group in both experiments). G. UDPGluc/ UDP-Gal and UDP-GlcNAc/UDP-GalNAc ratios in eGale infected livers (n = 10 for each group). H. UDP-Gluc/UDP-Gal and UDP-GlcNAc/UDP-GalNAc ratios in WbgU infected livers (n = 10 for each group). *p < 0.05, **p < 0.01. Comparison by Student's T-test. All data are mean ± s.e.m.
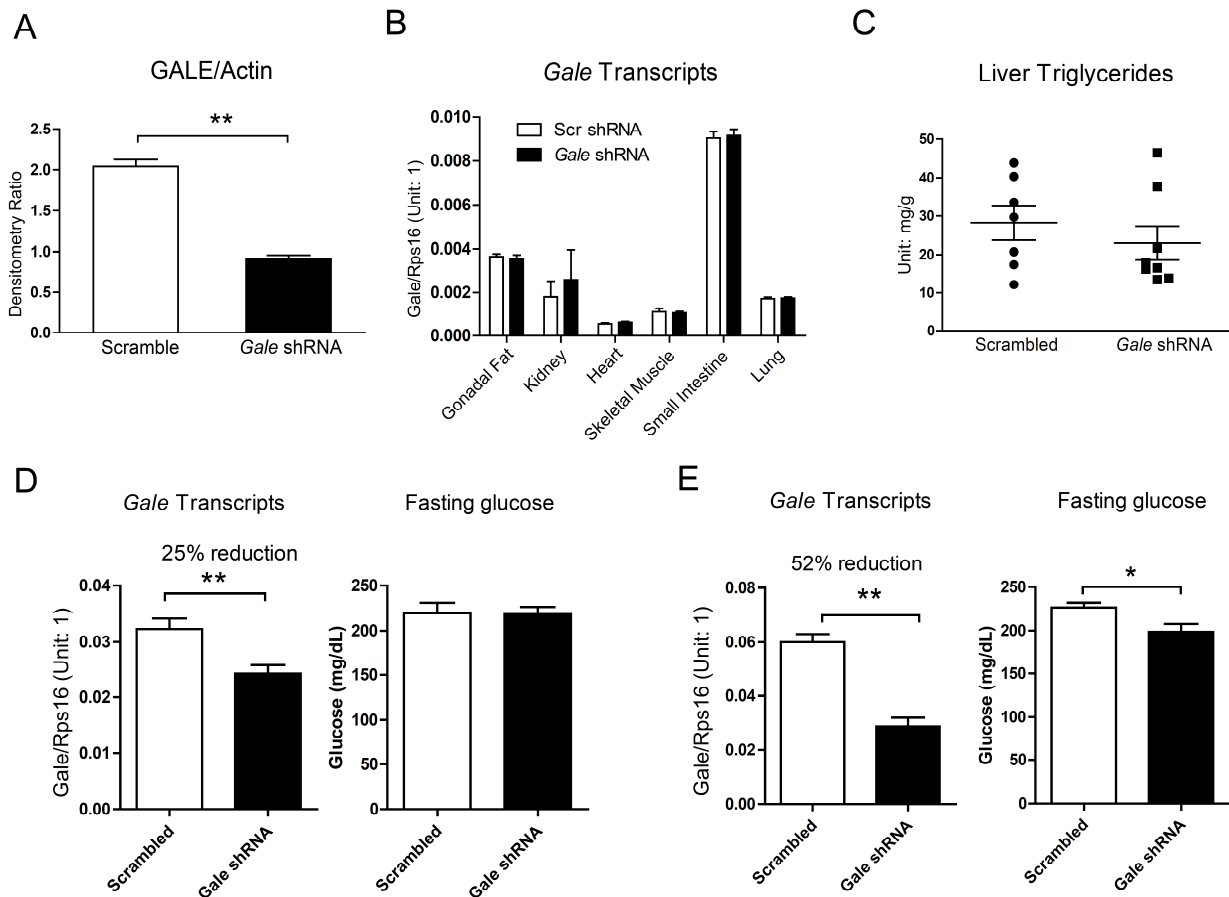
SUPPLEMENTARY DATA

A

Glucose Tolerance Test

B

Pyruvate Tolerance Test

C

Insulin

D

Fbp Transcripts

Pck1 Transcripts

G6pc Transcripts

E

	Control	*Gale* TG
Direct High Density Lipoprotein (dHDL) (mg/dL)	141.4±2.3	134.0±5.0
Very Low Density Lipoprotein (VLDL) (mg/dL)	24.3±1.8	26.0±2.6
Cholesterol (CHOL) (mg/dL)	183.9±4.4	160.0±13.7
Triglyceride (TG) (mg/dL)	121.4±8.8	129.4±13.0
Non-esterified fatty acid (NEFA) (mEq/L)	1.19±0.12	1.22±0.16

F

eGale Transcripts

WbgU Transcripts

G

UDP-Glu/UDP-Gal

UDP-GlcNAc/UDP-GalNAc

H

UDP-Glu/UDP-Gal

UDP-GlcNAc/UDP-GalNAc

SUPPLEMENTARY DATA

Supplementary Figure 3. Liver weights of mice with chronic *Gale* overexpression. A. Wet liver weight normalized to body weight for mice initially fed with HFHS diet for 8 weeks before 16 weeks transgene induction with continued HFHS diet feeding (n = 5 for each group). B. Dry liver weight normalized to body weight for the same mice in Panel A (n = 5 for each group). Comparison by Student's T-test. All data are mean ± s.e.m.
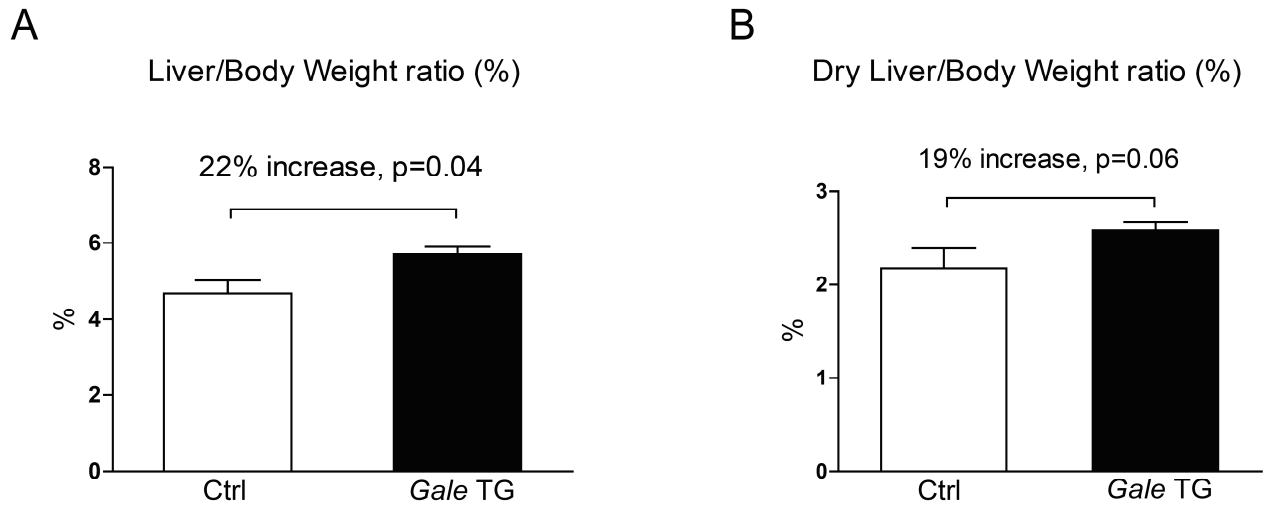
Supplementary Figure 4. Knocking down *Gale* improves glucose tolerance. A. Quantification of blots in Figure 4B. B. *Gale* mRNA levels in other tissues 4 weeks post scrambled or *Gale* shRNA AAV infection (n = 6 for each group). C. Liver triglyceride contents of mice 8 weeks post scrambled or *Gale* shRNA AAV infection (n = 7 and 8 for scrambled and *Gale* shRNA groups respectively). D. *Gale* transcripts (left) and fasting glucose (right) levels of mice 4 weeks post 5 E + 10 virus/mouse infection following 8 weeks HFHS diets pretreatment. E. *Gale* transcripts (left) and fasting glucose (right) levels of mice 4 weeks post 1 E + 11 virus/mouse infection following 8 weeks HFHS diets pretreatment. *p < 0.05, **p < 0.01. Comparison by Student's T-test. All data are mean ± s.e.m.

SUPPLEMENTARY DATA

Supplementary Figure 5. Restoration of hepatic *Tff3* expression reuses glucose intolerance in *Gale* TG mice. A. *Tff3* mRNA levels in *Gale* overexpression and knocking down livers (n = 12, 12, 6, 6 for control, *Gale* TG, Scrambled shRNA and *Gale* shRNA groups respectively). B. Serum TFF3 levels in the same group of mice shown in Figure 5C (n = 7, 7, 8, 6 for Ctrl+*GFP*_AAV, *Gale*_TG+*GFP*_AAV, Ctrl+*Tff3*_AAV, *Gale*_TG+*Tff3*_AAV groups respectively). C. AST, ALT and Serum lipids levels from the same group of mice shown in Figure 5C. *p < 0.05. Comparison by Student's T-test. All data are mean ± s.e.m.
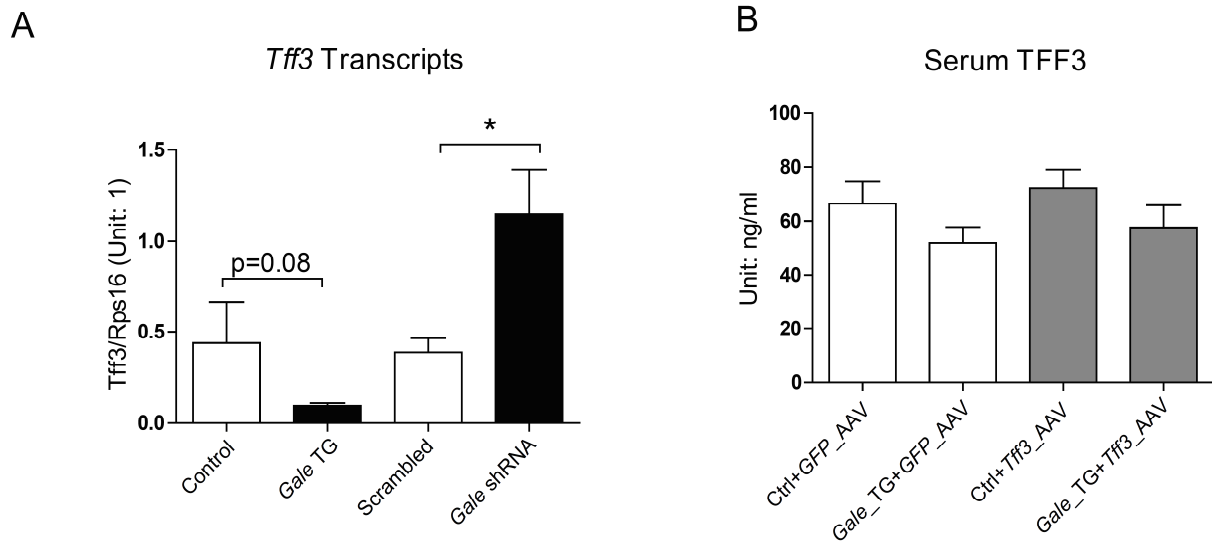
C

Serum Parameters	Ctrl+*GFP*_AAV	*Gale*_TG+*GFP*_AAV	Ctrl+*Tff3*_AAV	*Gale*_TG+*Tff3*_AAV
Triglyceride (TG) (mg/dL)	74±5.35	82.71±4.81	80±4.3	72.67±7.44
Non esterified fatty acids (NEFA) (mEq/L)	1±0.05	0.89±0.04	1.11±0.09	0.95±0.07
Aspartate transaminase (AST) (U/L)	185.67±7.76	155.71±15.13	163±17.25	155.5±53.82
Alanine transaminase (ALT) (U/L)	67±3.85	75.71±8.03	85.4±14.38	64.5±5.98

SUPPLEMENTARY DATA

Supplementary Figure 6. *Tff3* may be directly regulated by metabolites derived via GALE. A. Hepatic *Gale* mRNA levels in mice 1 hour post 1 g kg-1 body weight glucose or sucrose gavage (n = 8, 4, 6 for saline, glucose and sucrose groups respectively). B. Hepatic *Gale* mRNA levels in mice 1 hour post 15 μl g-1 body weight Intralipid gavage (n = 8 for each group). C. Hepatic *Gale*, *Galk*, *Galm* and *Galt* mRNA levels in NC and HFHS diet fed mice 1 hour post 1 g kg^{-1} body weight galactose gavage (n = 4 for each group). *p < 0.05, **p < 0.01. Comparison by Student's T-test. All data are mean ± s.e.m.