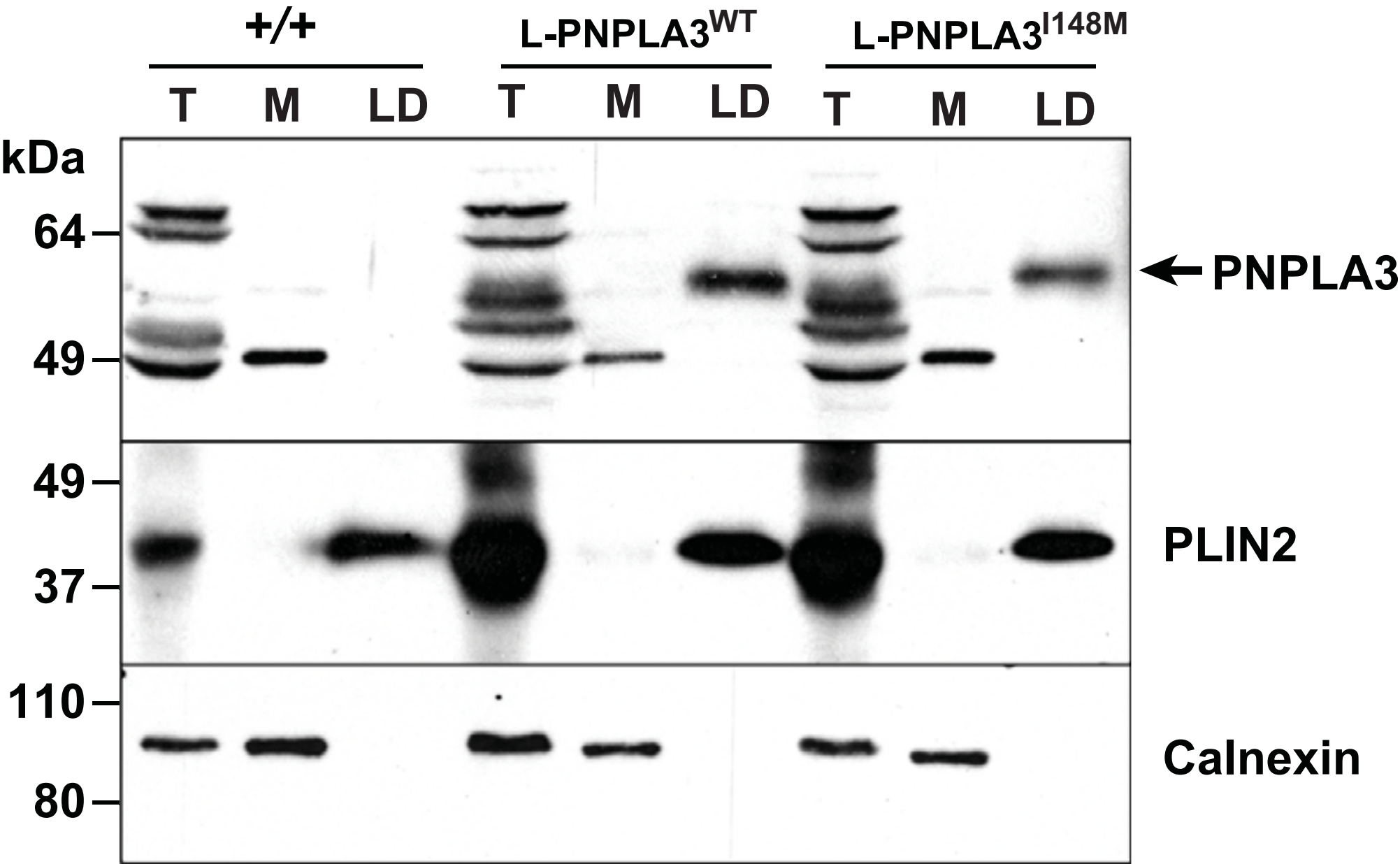
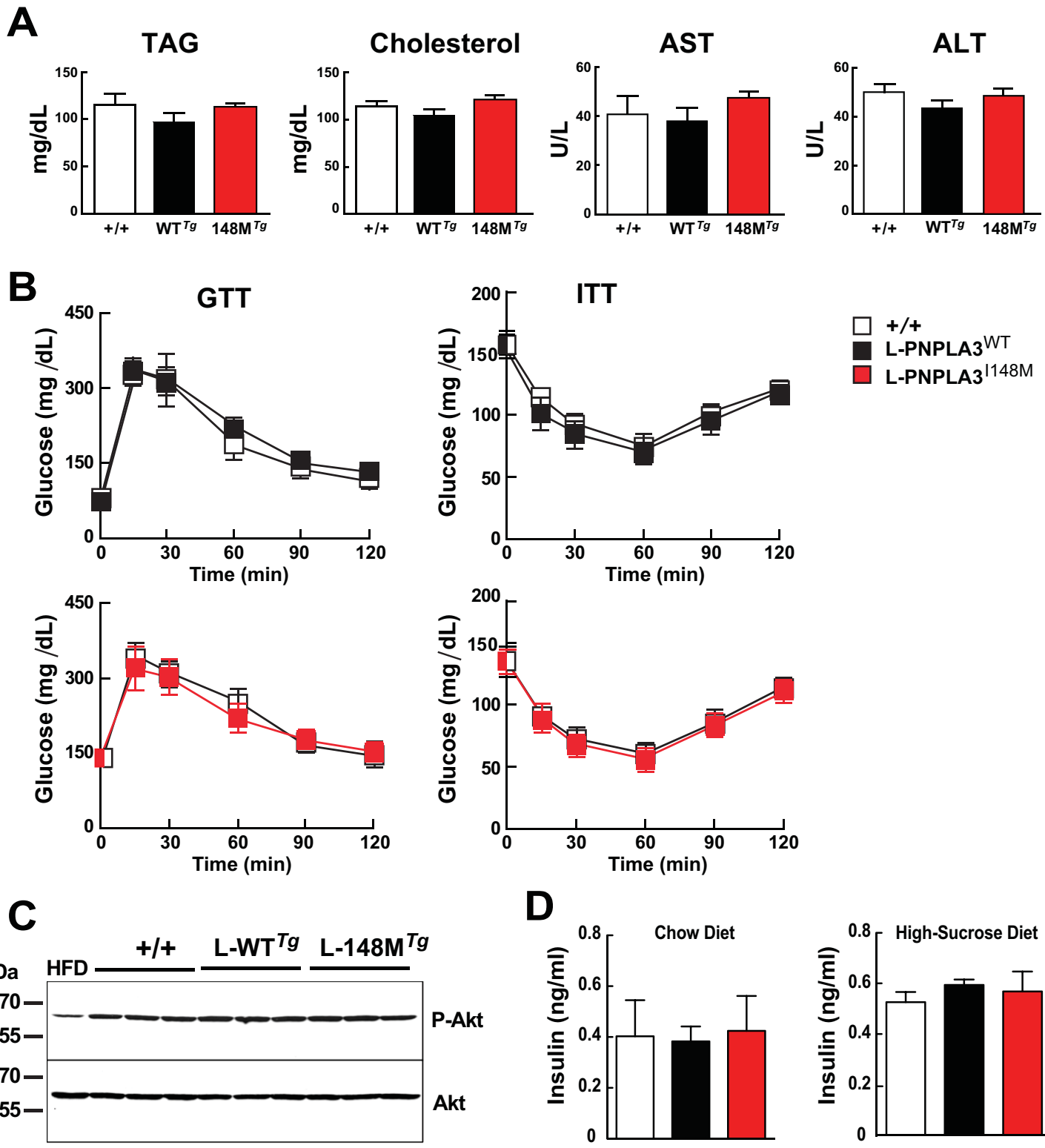
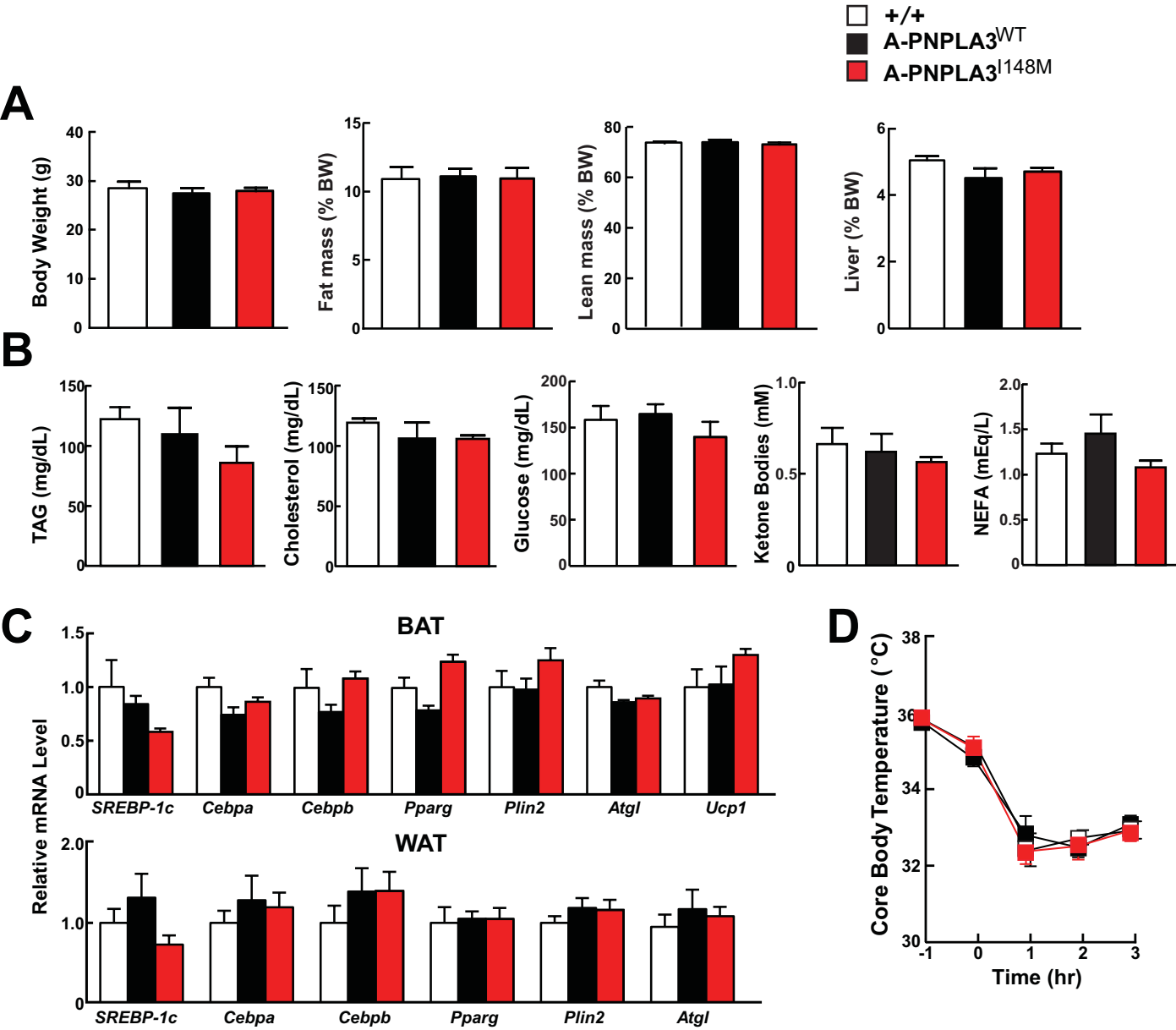
Supplemental Figure 1



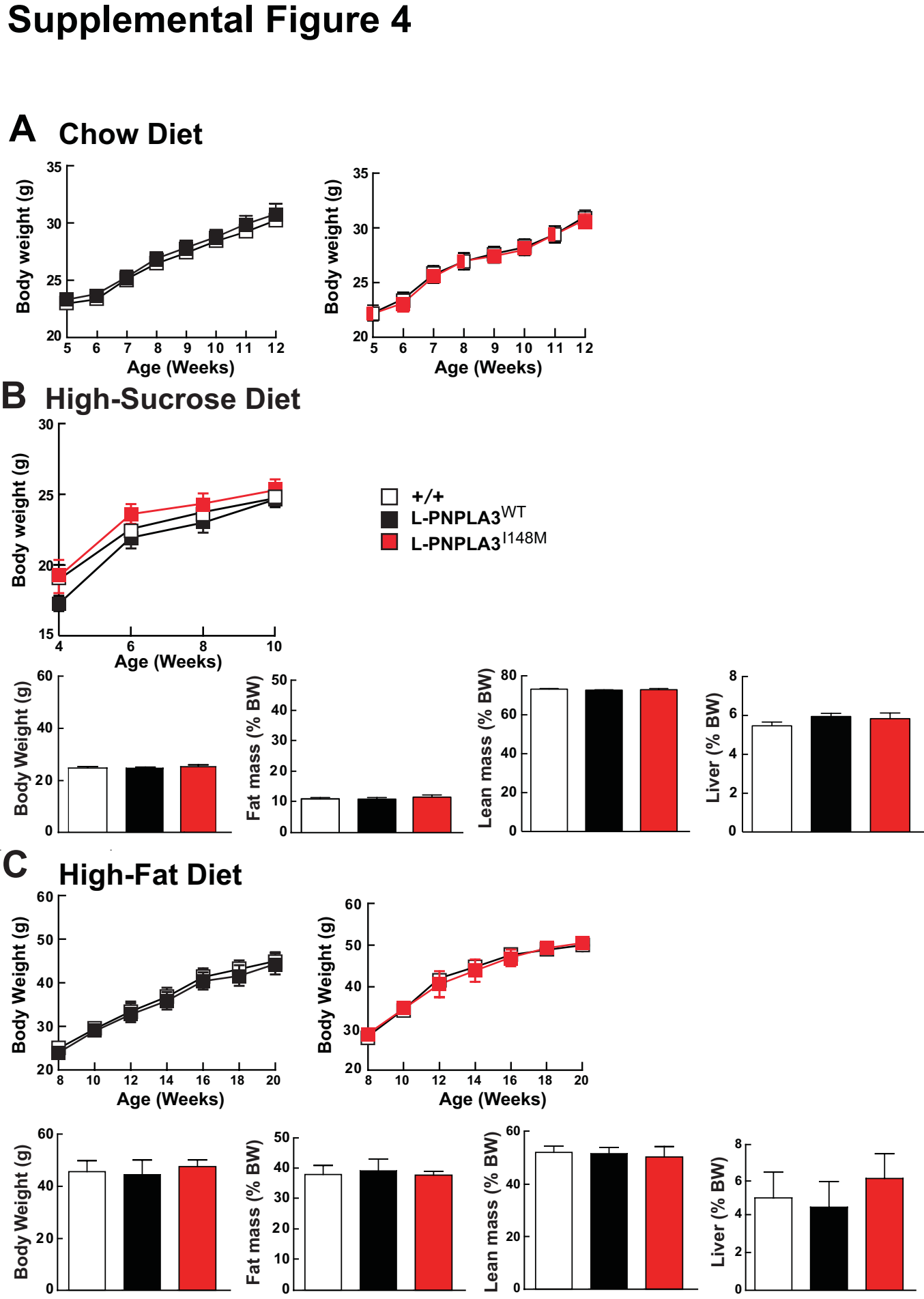
Supplemental Figure 2



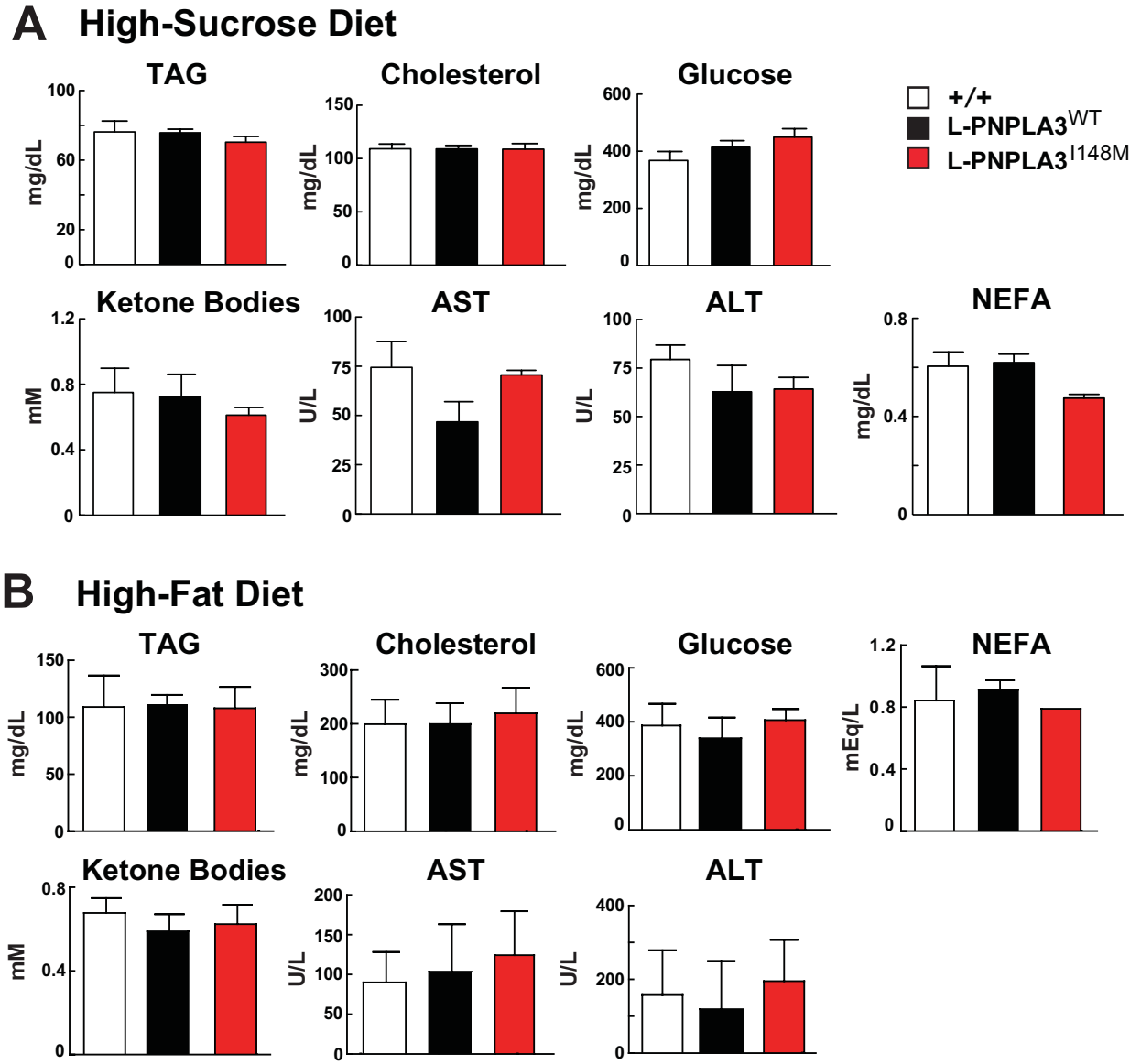
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

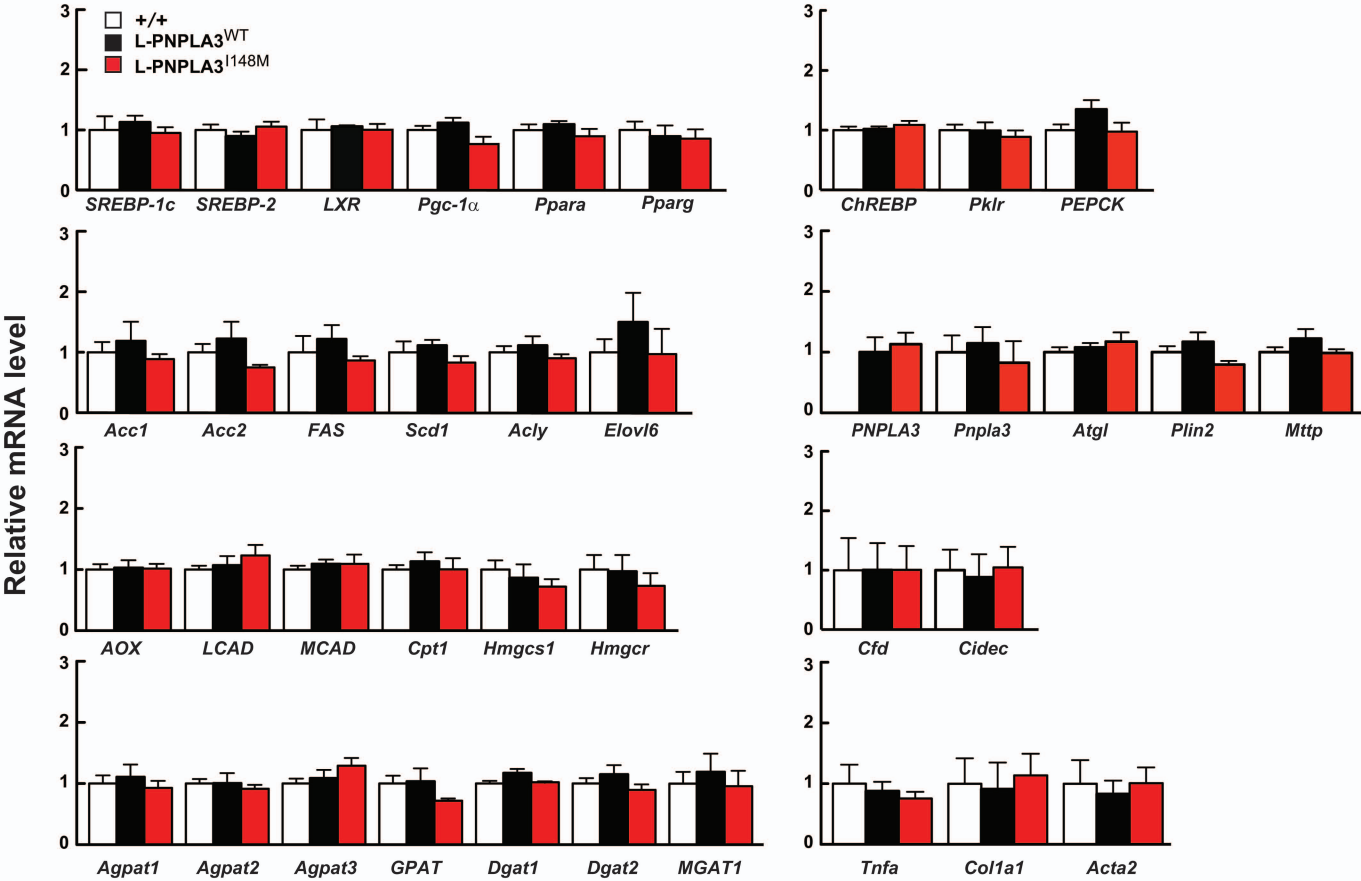


Figure 1B Perilipin 2

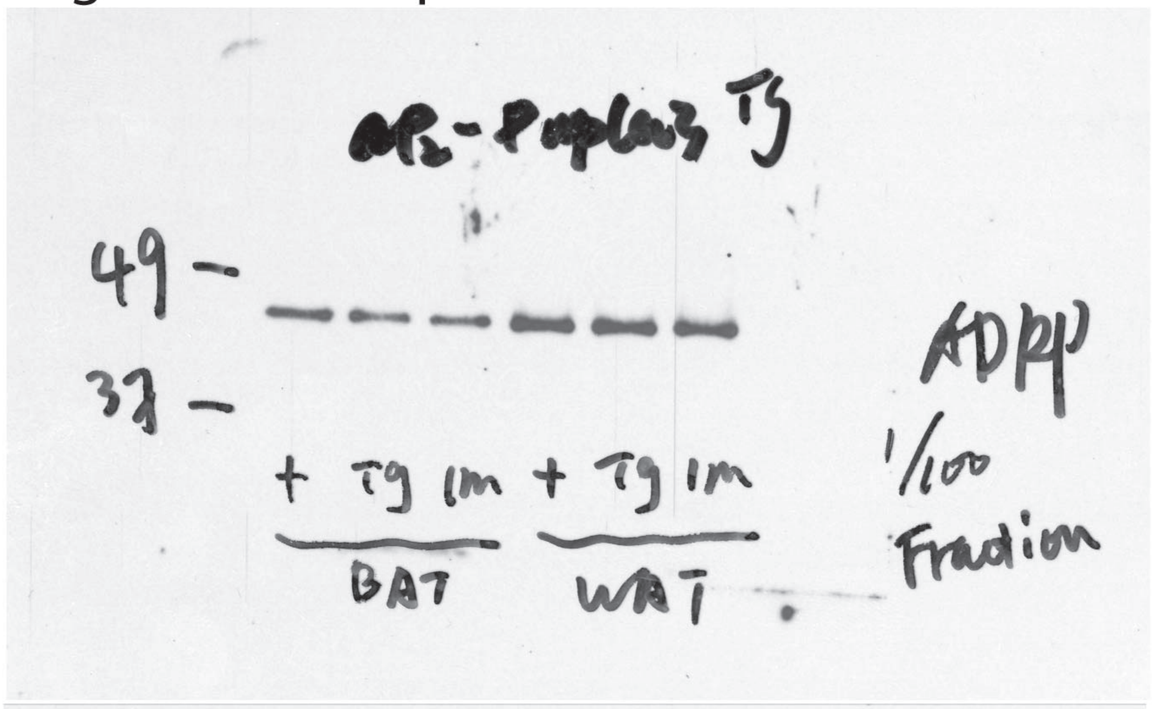
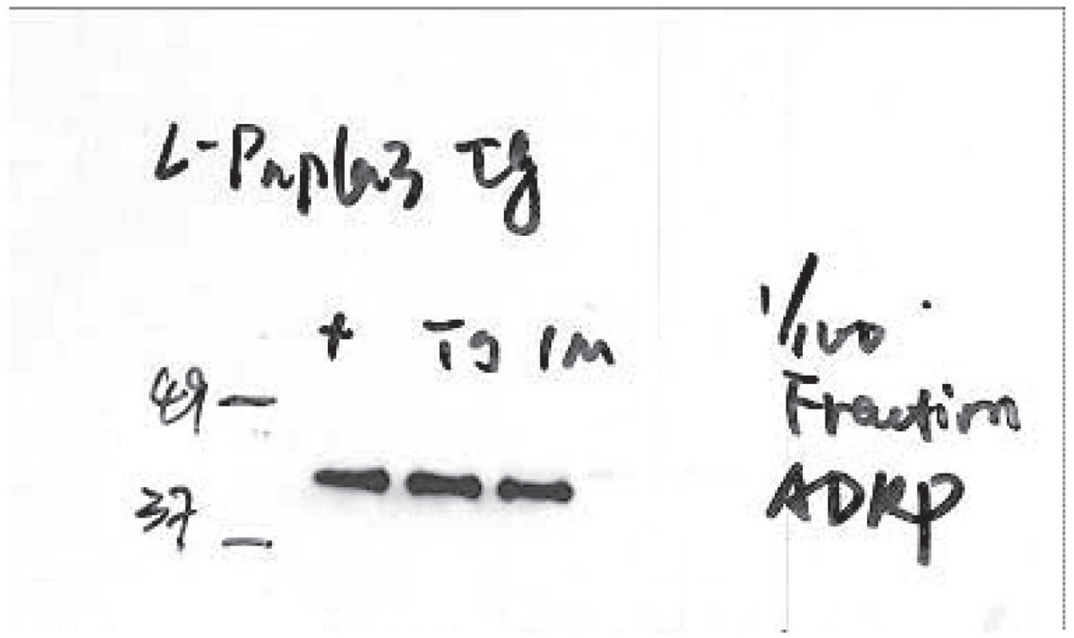


Figure 1A Perilipin 2



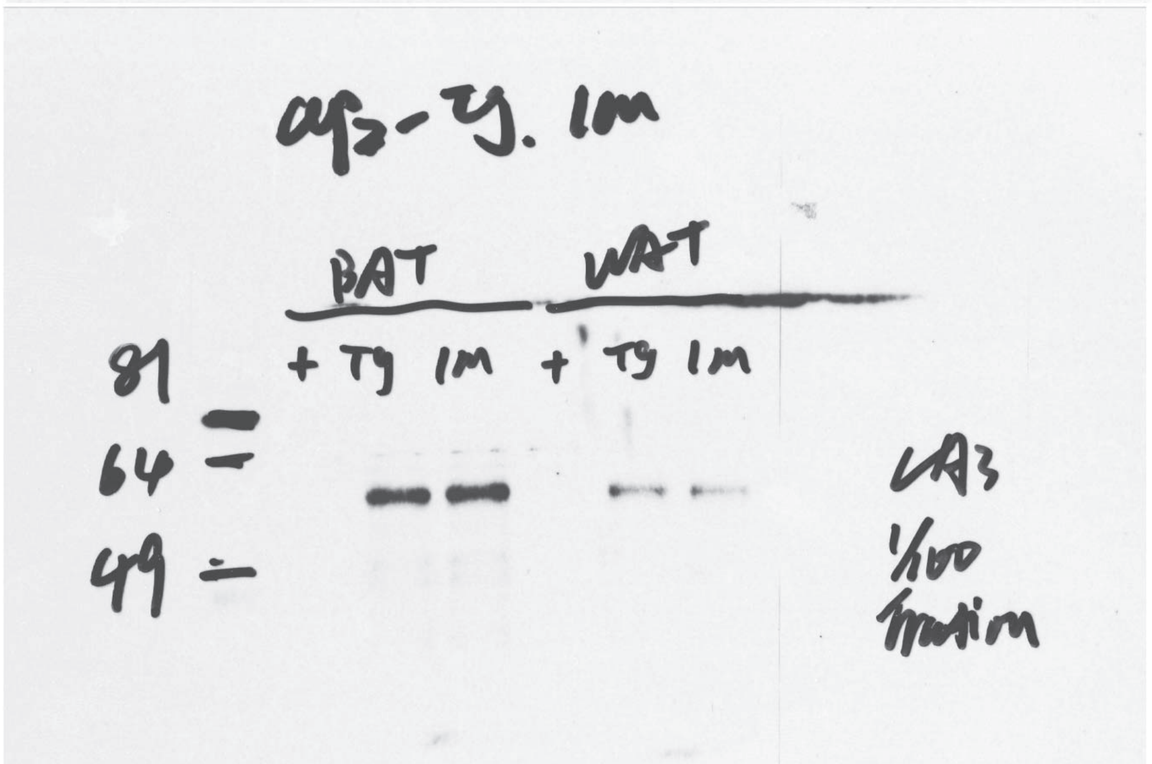


Figure 1B PNPLA3

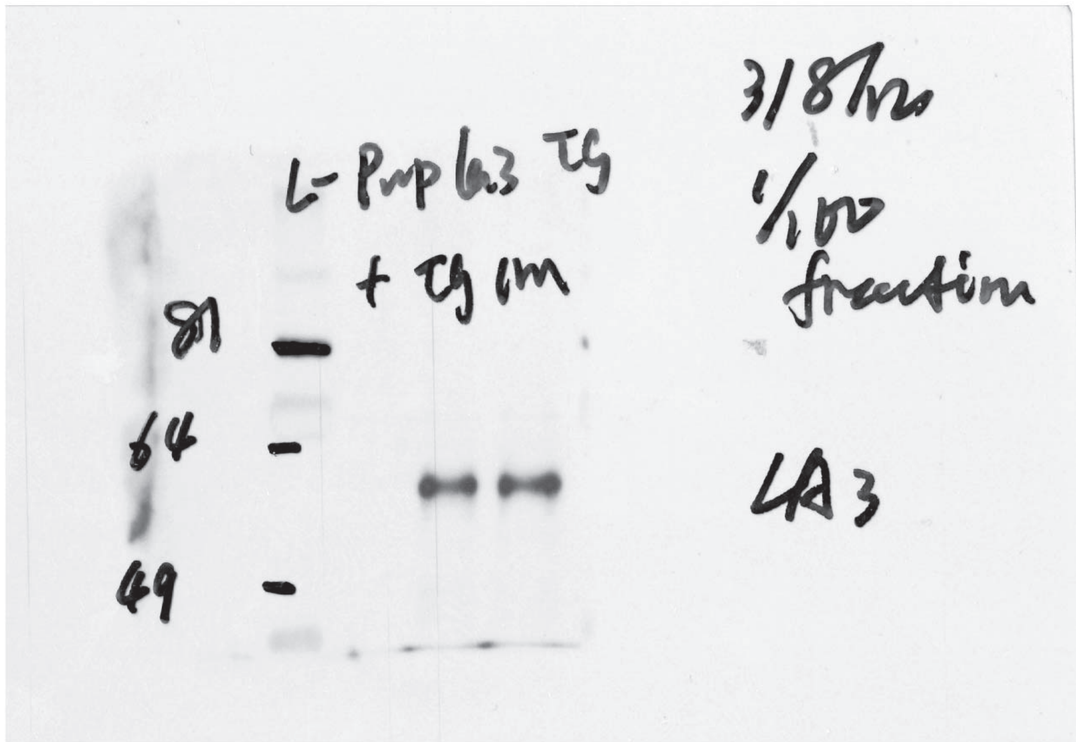
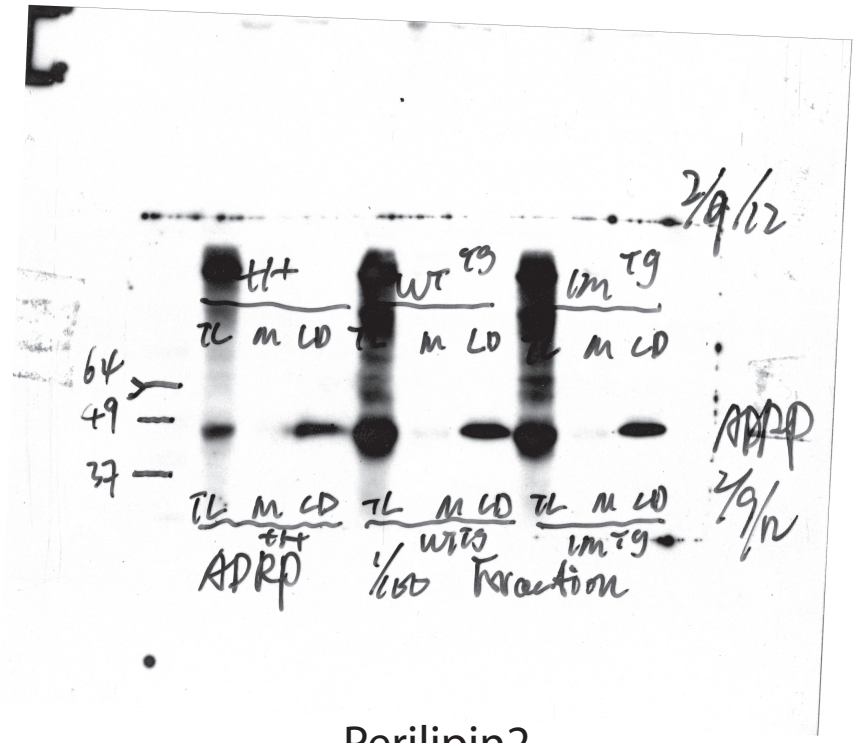
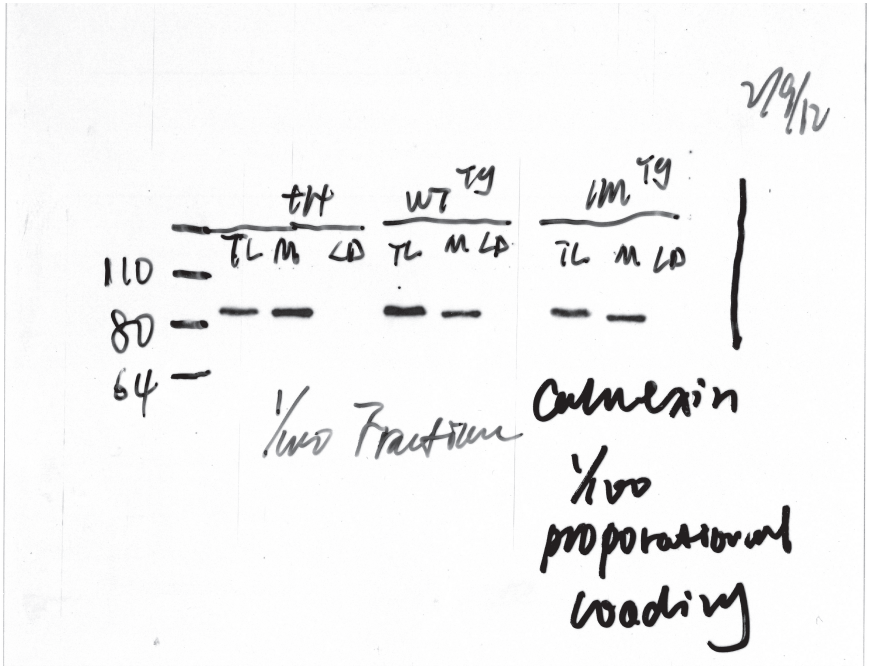


Figure 1A PNPLA3

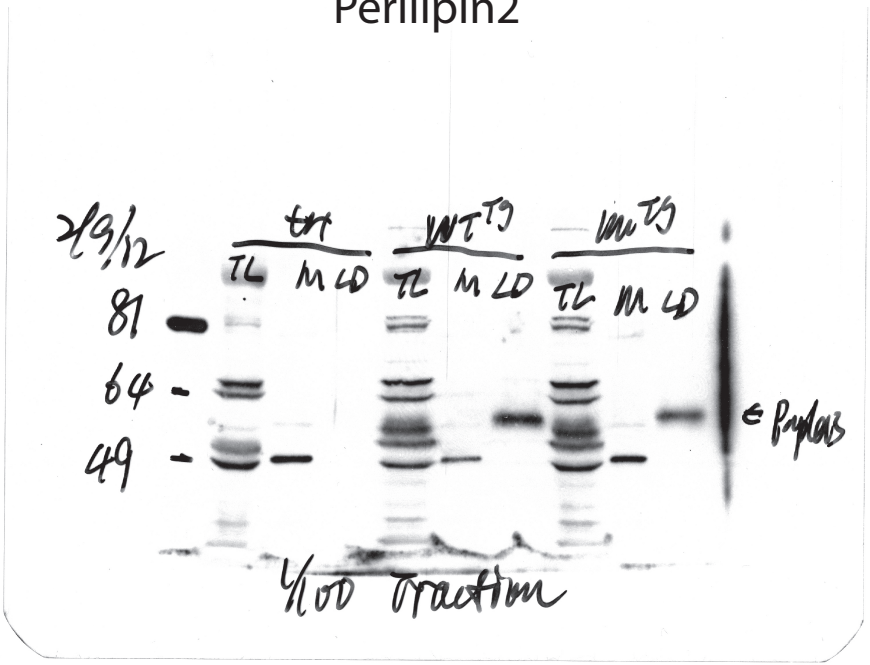
Full unedited gel for Figure 1



Perilipin2

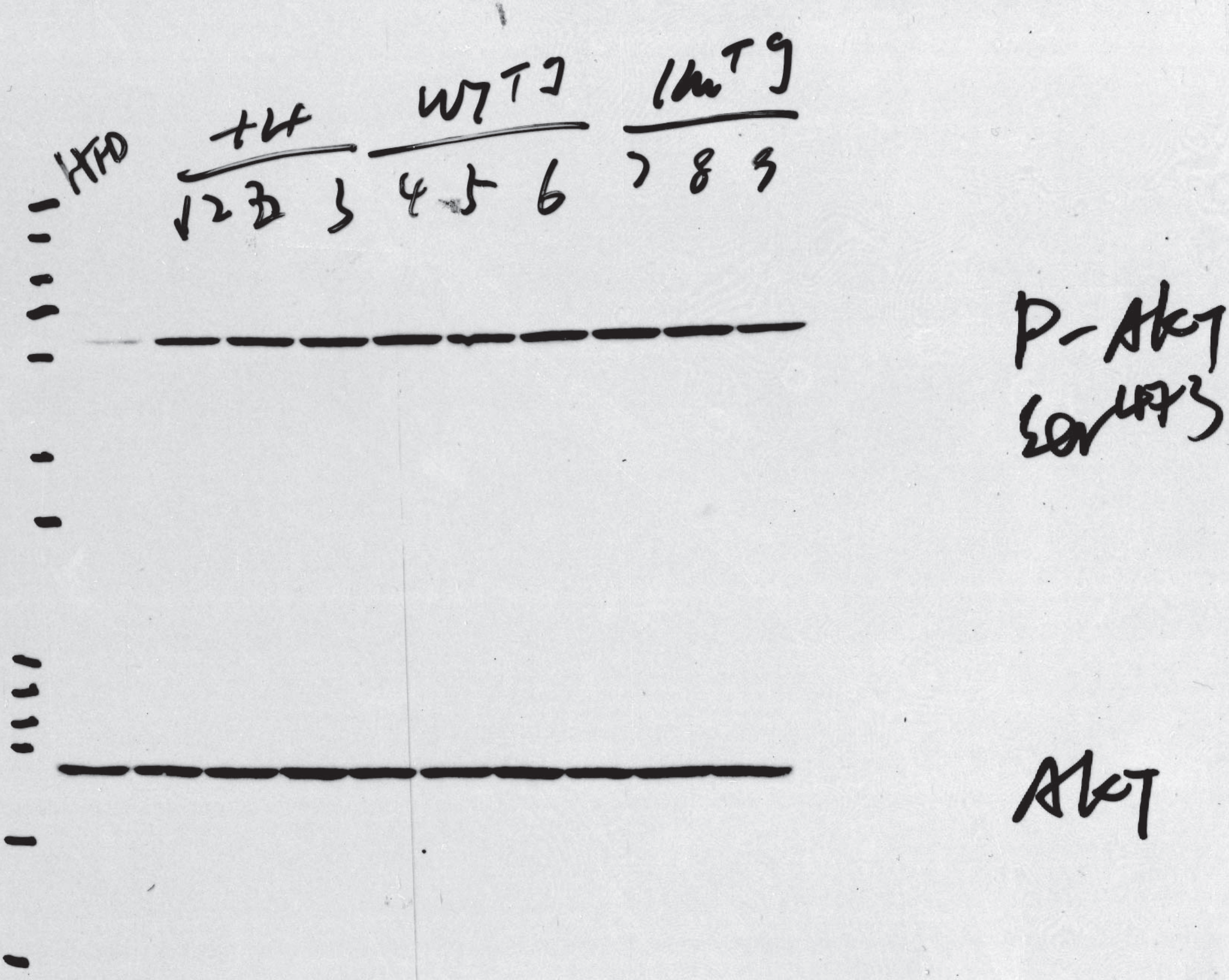


Calnexin



Human-PNPLA3

Full unedited gel for Supplemental Figure 1



Full unedited gel for Supplemental Figure 2

Supplemental Figures

Supplemental Figure 1

Immunoblot analysis of hPNPLA3 in total cell lysate (T), membranes (M) and lipid droplets (LD) from livers of wild-type (+/+), WT^{Tg} and $148M^{Tg}$ mice. Total cell lyates, membranes and lipid droplets were isolated from livers of the mice as described (18) and 1% of the total volume of each fraction was size-fractionated on an 8% SDS-PAGE gel. Immunoblotting was performed using a polyclonal anti-human PNPLA3 antibody (18). PLIN2 is a lipid droplet resident protein and calnexin is a membrane resident protein.

Supplemental Figure 2

(**A**) Plasma levels of lipids and serum levels of aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) in 12-wk-old, chow-fed wild-type (+/+), WT^{Tg}, and $148M^{Tg}$ male mice (n=4/group) after a 4 h fast. (**B**) Glucose and insulin tolerance tests in 10-wk-old, chow-fed, male mice. After a 16 h fast, 1.5 g/kg of glucose was injected intraperitoneally (IP). Blood samples were collected from the tail vein at indicated times and glucose levels were measured using a glucometer. Insulin tolerance tests (ITT) were performed after a 4 h fast. A total of 0.75 U/kg body weight of human insulin was given IP and blood glucose levels were monitored. Values are means ± SEM (n=6/group). (**C**) Immunoblot analysis of total and phosphorylated hepatic Akt (P-Akt) in livers of 12-13 wk old male wild-type (+/+), WT^{Tg} and $148M^{Tg}$ mice on an ad lib chow diet (n=3/group). Total cell lysates (40 μg) were subjected to immunoblotting using Akt and phospho-Akt (Ser473) MAb (1:2,000 dilution) (Cell Signaling). Eight-week-old WT mice fed a high-fat diet (HFD) for 12 wks served as a positive control for the experiment. (**D**) Mean serum levels of insulin were measured after a 4 hour fast in plasma from 10-12 week old +/+, WT^{Tg} and $148M^{Tg}$ male mice (n=5/group) fed either a chow diet (left) or a high-sucrose diet (right) after a 4 hour fast.

Supplemental Figure 3

(**A**) Body composition of 12-wk-old, chow-fed wild-type (+/+), A-WT^{Tg} and A-148M^{Tg} male mice (n=4-5/group). Total lean mass and fat mass were measured by NMR and normalized to body weight as described in Methods. **(B)** Plasma levels of lipids, glucose, ketone bodies and nonesterified fatty acids (NEFA) (n=4-5/group). Values are means ± SEM. (**C**) Relative mRNA levels in BAT and WAT of 12-wk-old, chow-fed WT (+/+), A-WT^{Tg} and A-148M^{Tg} (n=4/group). Total hepatic RNA was subjected to Real-Time PCR for the indicated transcripts. Each value represents the amount of mRNA relative to the level in wildtype mice. Values are means ± SEM. *Cebpa*, CCAAT-enhancer-binding proteinα; *Cebpb*, CAAT-enhancer-binding proteinβ; *Plin2*, perilipin 2; *Atgl*, adipose triglyceride lipase; *Ucp1*, uncoupling protein 1. **(D)** Core body temperature after cold exposure in 11-13 wk-old, chow-fed mice. The core body temperature was monitored hourly, starting 1 hour prior to cold exposure (4^{o}C). Values are means ± SEM from 8 -12 mice in each group.

Supplemental Figure 4

(**A**) Body weight curves of male wild-type (+/+), WT^{Tg}, and 148M^{Tg} mice (n=5-10 mice/group) on chow (left panel), high-sucrose (middle panel) or high-fat (right panel) diets. Chow and high-sucrose diets were initiated at 4 wks of age and continued for 8 and 6 wks, respectively. The high-fat diet was initiated at 8 wks of age and continued for 12 wks. (**B**) Body composition of mice maintained on high-sucrose and high fat-diets. Lean and fat masses were measured and normalized as described in Methods.

Supplemental Figure 5

Plasma lipid levels and serum glucose, ketone bodies, NEFA, and liver enzyme (AST and ALT) levels in wild-type (+/+), WT^{Tg} and $148M^{Tg}$ mice on high-sucrose **(A)** or high-fat diet **(B).** Mice were fed the indicated diets as described in the legend to supplemental Figure 4. Values are means ± SEM (n=5-10 mice/group).

Supplemental Figure 6

Relative mRNA levels in livers of wild-type (+/+) and PNPLA3 transgenic mice (n=4/group) on a high-fat diet. The mice used in this experiment are described in the legend to Supplemental Figure 4. Total RNA was subjected to Real-Time PCR quantification and mRNA levels were expressed relative to levels in nontransgenic mice. Values are mean ± SEM. *Pgc-1α,* PPARγ co-activator 1α*; ChREBP*, carbohydrate responsive element-binding protein; *Pklr*, liver pyruvate kinase; *PEPCK*, phosphoenoylpyruvate carboxykinase; *Acc1*, acetyl-CoA carboxylase 1; *Acc2*, acetyl-CoA carboxylase β; FAS, fatty acid synthase; *Scd1*, stearoyl-CoA desaturase-1; *Acly*, ATP citrate lyase; *Elovl6*, ELOVL family member 6, AOX, acyl-CoA oxidase-1; LCAD, long chain acyl-CoA dehydrogenase; MCAD, medium chain acyl-CoA dehydrogenase; *Cpt*1, carnitine palmitoyltransferase 1; *Hmgcs1*, HMG-CoA synthase; *Hmgcr*, HMG-CoA reductase; *Agpat1*, 1-acylglycerol-3-phosphate-O-acyltransferase-1; *Agpat2*, 1-acylglycerol-3-phosphate-O-acyltransferase-2; *Agpat3*, 1-acylglycerol-3-phosphate-O-acyltransferase-3; *GPAT*, glycerol-3-phosphate acyltransferase; *Dgat1*, diglyceride acyltransferase-1; *Dgat2*, diglyceride acyltransferase-2; *MGAT1*, monoacylglycerol O-acyltransferase 1; *Atgl,* adipose triglyceride lipase; *Plin2*, perilipin 2; *Mttp*, microsomal TAG transfer protein; *Col1a1*, collagen, type 1, α1; *Acta2*, α–smooth muscle actin.