Supplemental Figures and Legends

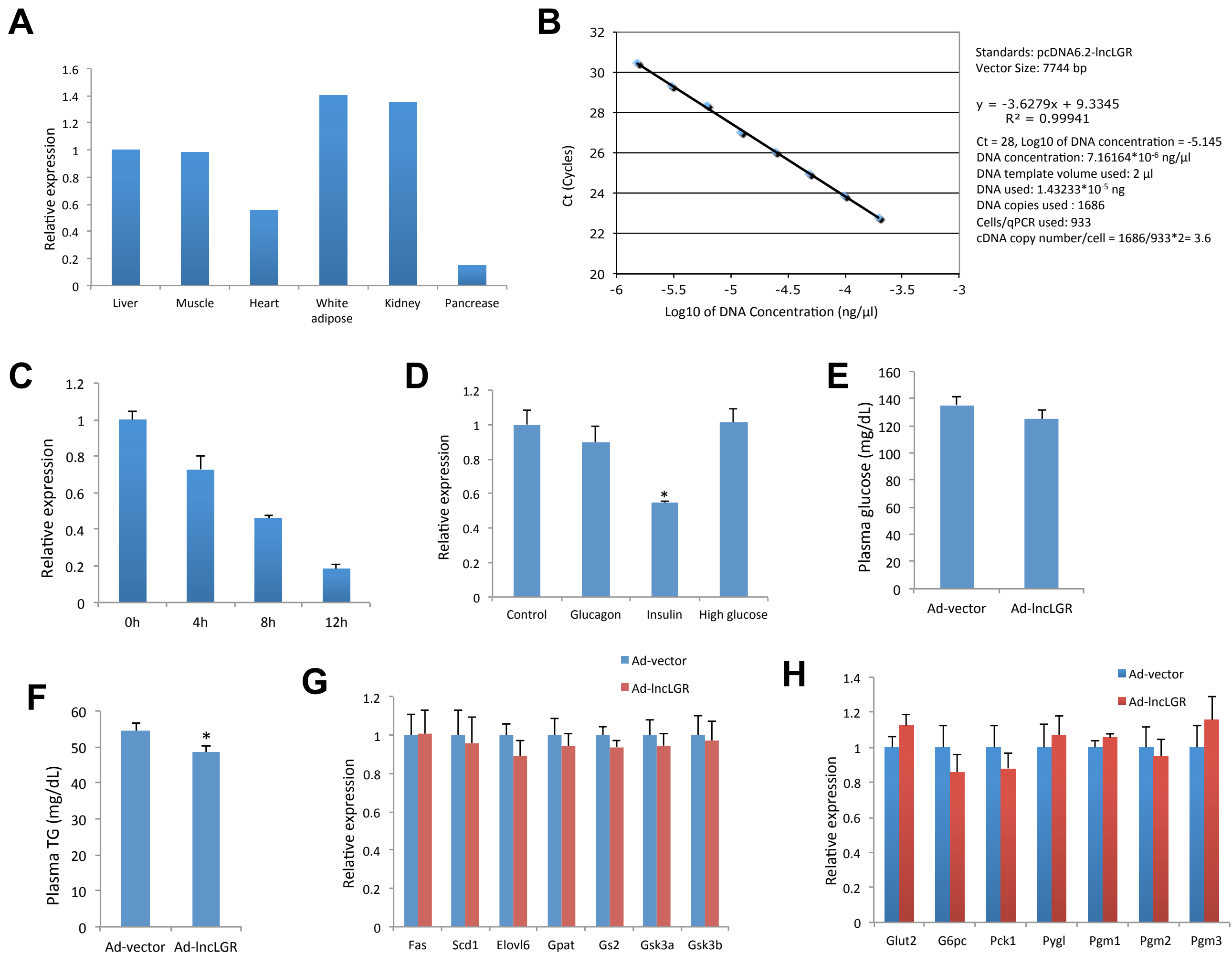

Figure S1, related to Figure 1. (A) Relative expression levels of lncLGR in different mouse tissues as compared to the liver expression levels (result of an independent experiment is also shown in figure S2D). (B) Quantification of lncLGR copy number in mouse primary hepatocytes. Total RNAs from $5*10^5$ cells were treated with Turbo DNase in a total volume of 30 µL, and an aliquot of 2.8 µL of treated RNAs were reverse transcribed in a final volume of 20 µL. RT products were diluted five-fold and 2.0 µL diluted RT products were subjected to qPCR. LncLGR cDNA concentration was quantified with a standard curve and it was calculated to be present in primary hepatocytes at ~3.6 copies per cell. (C) Expression of lncLGR in mouse primary hepatocytes treated with 0.5µg/ml actinomycin D for 0h, 4h, 8h and 12h (n=3 and the result is representative of two independent experiments). (D) Expression levels of lncLGR in mouse primary hepatocytes treated with control, 100nM glucagon, 100nM insulin, or 25mM glucose for 6 hours (n=3, and the result is representative of two independent experiments). (E) Blood glucose levels, (F) plasma TG levels and (G and H) liver gene expression levels in control and lncLGR overexpression mice (n=6) after a 4h food withdrawal. Error bars represent SEM, *$p<0.05$.

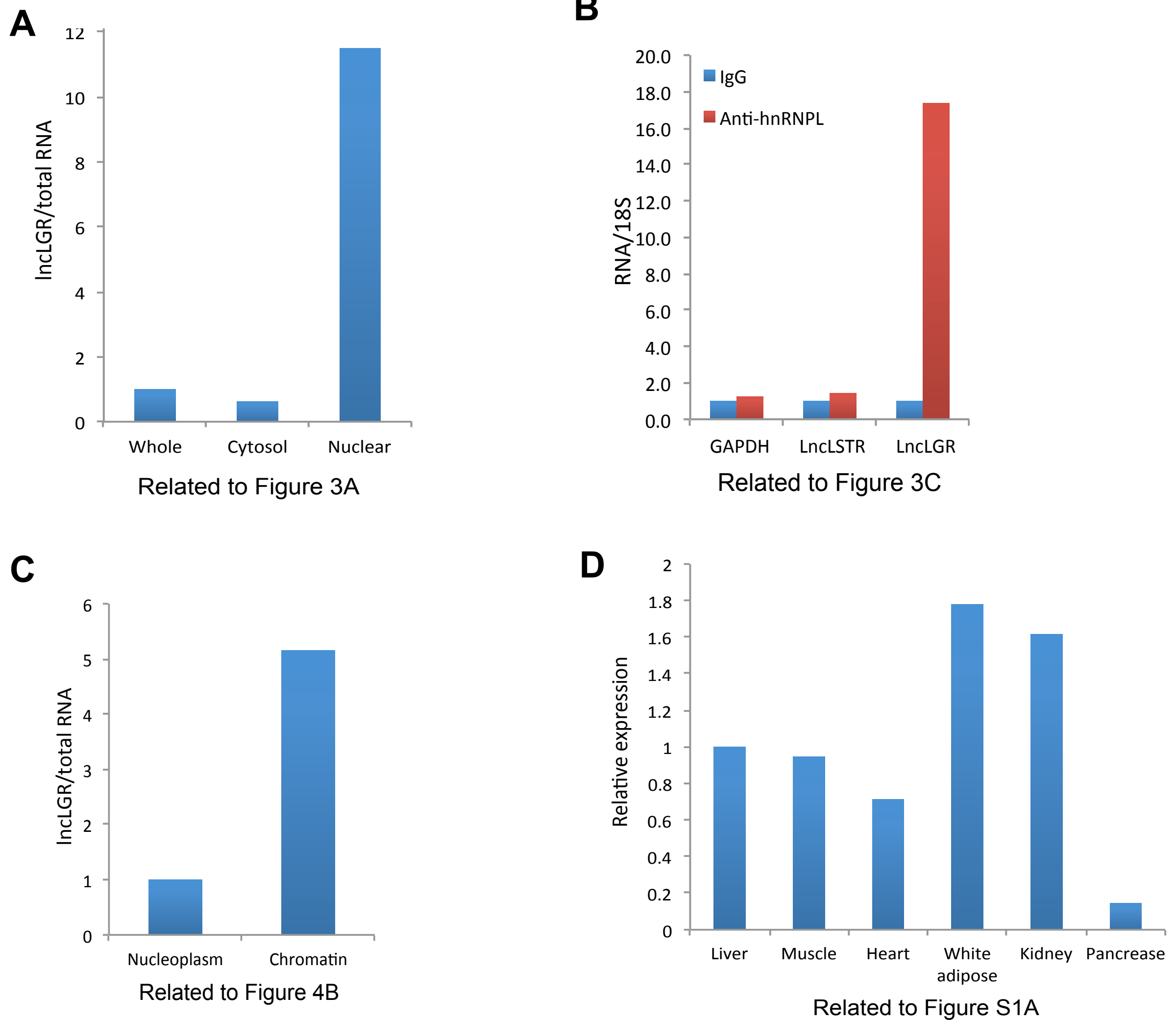

Figure S2, Independent experimental repeats. (A) Levels of lncLGR in whole cell, cytosolic, or nuclear fractions of liver tissue samples pooled from 4 mice. (B) Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), lncLSTR and lncLGR RNA levels in immunoprecipitates of liver tissue samples using an anti-hnRNPL antibody. (C) Levels of lncLGR in nucleoplasm and chromatin fractions of mice liver tissue samples. (D) Relative expression levels of lncLGR in different mouse tissues as compared to the liver expression levels.

Supplemental Table

Table S1, related to Experimental Procedures. A list of real-time PCR primers used.

Gene	Forward Primer	Reverse Primer
lncLGR	CGGCAGTTATCCACTTTCCT	GTGGCGTTGGGTTGGTAT
18s	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA
Gck	ATAGACAAGGGCATCCTGCT	CCACCACATCCATCTCAAAG
hnRNPL	CGTAAACAGCGTGCTTCTGT	CAAACTCCACCATAGCCTGA
Hk1	ATGCCAAGGAAATCTTGACC	CATACGTGCTGGACCGATAC
Hk2	GGACGACGGTACACTCAATG	CCGCTGATCATCTTCTCAAA
Hk3	GGAGAATCAGGGTCTGAAGC	TGGCATCTCTTAGCAACTGG
Fas	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Scd1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
Elovl6	CGAACTGGTGCTTACATGCT	TGCTGAGCACAAATGCATAA
Gpat	CAACACCATCCCCGACATC	GTGACCTTCGATTATGCGATCA
Gs2	CGCTCCTTGTCGGTGACATC	CATCGGCTGTCGTTTTGGC
Gsk3a	GCGTTCCCAAGAAGTGGCTTA	GGTCCAGCTTACGCATAATCTG
Gsk3b	ATGGCAGCAAGGTAACCACAG	TCTCGGTTCTTAAATCGCTTGTC
GAPDH	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
lncLSTR	TGTAGGAGCCCGCAATGAA	CAACTTAAAGCTGCCCCATCA
Glut2	TCAGAAGACAAGATCACCGGA	GTCATAGCCGAACTGGAAGGA
G6pc	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
Pck1	CCCAAGGCAACTTAAGGGCTAT	CTGAGGTGCCAGGAGCAACT
Pygl	GAGAAGCGACGGCAGATCAG	CTTGACCAGAGTGAAGTGCAG
Pgm1	CAGAACCCTTTAACCTCTGAGTC	TCATTCATTCGAGAAATCCCTGC
Pgm2	CCGCTTCTACATGACCGAGG	GATGATGCAAGATACGGCAGG
Pgm3	TCAGTATGGGACTGCTGGATT	CCCAAAGGATCAACCAATTTCAC

Supplemental Experimental Procedures

Animal experiments

All animal experiments were performed in accordance with and with approval from the NHLBI Animal Care and Use Committee. Male C57BL/6 (B6) mice were purchased from Jackson Laboratory at 8 weeks of age and housed 3-5 mice per cage with free access to water and normal chow diet (NIH-31), and animals were acclimatized to the housing condition for 10-14 days before experiments. Groups of co-housed mice were randomly assigned to experimental groups with age and weight matched between groups. For the fasting and refeeding study, mice were either allowed free access to food or were subjected to a twenty-four hour fast before being euthanized for tissue harvest. A third group was fasted for twenty-four hours and then allowed to feed ad libitum for another four hours before tissue harvest.

Measurement of liver TG, glycogen and G6P levels

Liver and plasma TG levels were measured using a kit from Sigma following the manufacturer's protocols. Liver glycogen and G6P were quantified with assay kits from Biovision.

Glucokinase Activity Assay

Hepatic glucokinase activity was measured using the method described before (Fujimoto et al., 2006). Briefly, liver samples were homogenized in buffer (20 µl buffer/mg tissue) containing 50 mmol/l HEPES (pH 7.4), 100 mmol/l KCl, 1 mmol/l EDTA, 5 mmol/l MgCl2, and 2.5 mmol/l dithioerythritol. Homogenates were centrifuged at 10000 g for 5 min and the supernatants were transferred to fresh tubes and further centrifuged at 100,000 g for 45 min to sediment the microsomal fraction. The postmicrosomal fraction (2 µl) was assayed in 150 µl medium containing 50 mmol/l HEPES, 100 mmol/l KCl, 7.5 mmol/l MgCl2, 5 mmol/l ATP, 2.5 mmol/l dithioerythritol, 10 mg/ml albumin, 0.5 or 100 mmol/l glucose, 0.5 mmol/l NAD^+, and 4 units/ml of G6P dehydrogenase (Leuconostoc mesenteroides, Sigma) at 37 °C. The reaction was initiated by adding ATP, and the rate of NAD^+ reduction was recorded at 340 nm for 40 min. Hexokinase activity was determined as the absorbance change in the presence of 0.5 mmol/l glucose. Glucokinase activity was determined as the absorbance change in the presence of 100 mmol/l glucose minus the absorbance change in the presence of 0.5 mmol/l glucose.

Nuclear and chromatin RNA fraction

To prepare mouse liver nuclear extract, frozen liver tissue samples were homogenized using a Dounce homogenizer with 15–20 strokes in nuclear isolation buffer (250mM sucrose, 10 mM Tris-HCl pH 7.5, 1mM EDTA with protease inhibitors). Nuclear pellets were collected by centrifugation at 1000g for ten minutes and resuspended in 1 ml RIP buffer (150 mM NaCl, 20 mM Tris pH 7.4, 1 mM EDTA, 0.5% Triton X-100 with protease inhibitors and RNaseOUT™). The lysates were mechanically sheared again using a Dounce homogenizer with 15–20 strokes. Nuclear membrane and other debris were pelleted by centrifugation at 12,000rpm for ten minutes. RNA isolation from chromatin and nucleoplasm was performed as previously described (Pandya-Jones and Black, 2009)

Isolation and culture of mouse primary hepatocytes

Primary hepatocytes were isolated from C57BL/6 mice fed with a normal chow diet. Mice were briefly anesthetized with Ketamine (100mg/kg) and Xylazine (10mg/kg), and the liver was perfused with Krebs Ringer buffer with glucose at a rate of 5ml/min for eight minutes, followed by continuous perfusion with the same buffer containing collagenase (Liberase TM Research Grade, Roche) for 10 minutes. Hepatocytes were harvested and purified with Percoll. The viability of hepatocytes was examined by trypan blue exclusion. Only cell isolates with viability over 90% were used. Hepatocytes were plated onto collagen-coated plates ($1x10^6$ cells/well in 6-well plates and $5x10^5$ cell per well in 12-well plates) in DMEM (no glucose) supplemented with 5.5mM glucose, 1xGlutaMAX™ and 10% Cosmic Calf Serum (CCS).

RNA extraction and quantitative real-time PCR analysis

Total RNA was isolated from liver tissue samples or cells using Trizol reagent (Invitrogen). After Turbo DNA-free DNase treatment (Ambion), reverse transcription was carried out with the SuperScript® III First-Strand Synthesis system (Invitrogen) using 1 µg of RNA. Quantitative real-time RT-PCR was performed on a ViiA™ 7 Real-Time PCR System (Applied Biosystems Inc.). The PCR program was: 2 min 30 s at 95°C for enzyme activation, 40 cycles of 15 s at 95°C, and 1 min at 60°C. Melting curve analysis was performed to confirm the real-time PCR products. Primer sequences used are provided in Table S1. All q-PCR results are normalized to 18s.

Immunoblotting

For immunoblotting analyses, the cells and tissues were lysed in RIPA buffer (Cell Signaling Technology) containing phosphatase inhibitors (Sigma) and a protease inhibitor cocktail (Roche). The lysates were subjected to SDS–PAGE, transferred to polyvinylidene fluoride (PVDF) membranes, and incubated with the primary antibody followed by the fluorescence conjugated secondary antibody (LI-COR). The bound antibody was visualized using a quantitative fluorescence imaging system (LI-COR). The primary antibodies used were GCK antibody (sc-7908, Santa Cruz), Beta-actin antibody (8457, Cell Signaling), and hnRNPL antibody (ab6106, Abcam).

DNA and RNA binding assays with purified hnRNPL

Purification of flag tagged hnRNPL from transfected 293T cells was performed as previously described (Schwappacher et al., 2013). 1 µg biotin labeled RNAs were mixed with 1µg purified hnRNPL proteins in 300ul binding buffer (20mM Tris-HCl, PH 7.4, 150mM NaCl, 1mM EDTA, 5% Glycerol, 0.5% Triton X-100, 0.5mM DTT, 0.1 mg/ml BSA, 0.2mg/ml tRNA, 0.2mg/ml heparin with RNaseOUT™) and rotated at 4°C for 1 hour. Then 30µl washed Streptavidin-coupled Dynabeads (Invitrogen) were added and rotated for another 1 hour. The beads were collected and then washed with binding buffer for 5 times. Proteins bound to beads were detected by western blot. DNA pull-down with purified hnRNPL was performed as previously described (Li et al., 2015).

Chromatin immunoprecipitation (ChIP) analysis

ChIP assays of frozen liver tissue were performed using a Simple ChIP Enzymatic Chromatin IP kit (Cell Signaling Technology) according to the manufacturer's protocol. Immunoprecipitation was performed using an anti-hnRNPL antibody (ab6106, Abcam) or mouse IgG (ab81032, Abcam) as a negative control. Primers used for amplifying GCK and β-actin promoters were: Liver-Gck-chip-f, GTGATCAATCGTGTCAAGGG; Liver-Gck-chip-r, CCACTGGCAAGTATGACAGG; Neuroendocrine Gck promoter-f, TCTAC CAAGCTGGCAGTCAC; Neuroendocrine Gck promoter-r, TTGCCAAAGGCCTCTCT ACT; Actin-chip-f, GCTTCTTTGCAGCTCCTTCGTTG; Actin-chip-r, TTTGCACATG CCGGAGCCGTTGT. The PCR cycle parameters were 95°C for 5 minutes, then 27 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, followed by a final extension at 72°C for 5 minutes for both of the ChIP products and inputs. PCR products were resolved by electrophoresis in a 2% Agarose E-gel (Invitrogen).

REFERENCES

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Pandya-Jones, A., and Black, D.L. (2009). Co-transcriptional splicing of constitutive and alternative exons. Rna *15*, 1896-1908.