Supplementary file 1

Methods:

1. LC-MS analysis for polyphenol profiling

Following the extraction, the honey extracts were concentrated 10-fold using a SpeedVac evaporator. The honey extracts and SPE-bound material were reconstituted in acetonitrile (5% v/v) containing 0.1% v/v formic acid. The resultant samples were analysed using the LCQ-DECA XP Plus system (LCMS-5TF-1343), which included a pump (Edwards 30 E2M30 vacuum pump), photo diode array detector (PDAD), ion trap (3D quadrupole), and mass spectrometer (50-4000 Da) from Thermo Scientific, USA. The gradient was composed of solvent A, ultra-pure water with 0.1% (v/v) formic acid, and solvent B, acetonitrile with 0.1% (v/v) formic acid. Then 20 µl of samples were eluted on gradients with t = 0–5 min and 2% solvent B, and t = 5–35 min and 2–40% solvent B. Finally, 3 different channels (280, 365 and 520 nm) were scanned by the PDAD using a C18 column (Synergi HydroC18 with polar end capping, 2 mm × 150 mm, Phenomenex Ltd, UK) at a flow rate of 200 µl/min.

2. HPLC analysis for sugar quantification

For this analysis, 1 ml of the standard solutions for the three sugars was added to a 10 ml volumetric flask to create the working sugar mixture solution. The remaining volume was then adjusted with distilled water. In order to prepare the sample, 2.5 g of the appropriate honey sample were dissolved in 25 ml of deionized water in a beaker. The resulting solution was placed in a 50 ml volumetric flask and filtered using a 0.45 µm nylon filter. After that, the right amount of solution was injected to a HPLC instrument, equipped with binary HPLC pump 1525 and PDA detector 2998 (Waters Corporation, Massachusetts, USA) and HPLC analysis was carried out. The 150 × 4.6 mm ZORBEX carbohydrate column (Agilent, USA; part no- 843300-908) was used for the separation process. The mobile phase for the chromatographic analysis was a 20:80 v/v mixture of distilled water and acetonitrile. Flow rate was maintained at 1.5 ml/min to inject the 10 µl sample. The column's temperature was kept constant at 27°C for the entire duration of the run.

3. Cytotoxicity assay

Briefly, $5x10^3$ HepG2 cells were seeded in 100 µl media per well in a 96-well plate and incubated for 24 h. Next, the cells were treated with different concentrations of LyH (10-100 mg/ml) in the presence or absence of palmitic acid (0.5 mM) for 24 h. After completion of required time of incubation, 20 µl of 5 mg/ml MTT was added and incubated for 4 h at 37 °C. Then, the media containing MTT was replaced by 150 µl of DMSO to allow the resultant formazan crystals to be dissolved and, with a microplate reader (Fluostar optima, BMG Labtech, Germany), the absorbance was measured at 570 nm. The % of cell survival was calculated from the resultant readings as-

$$\left[\frac{A570_{sample}}{A570_{control}}\right] * 100$$

4. Quantification of intracellular 2-NBDG by FACS

Briefly, after the termination of incubation of respective treatments and with or without insulin (100 nM) and 2-NBDG (100 µM), the cells were trypsinized and washed with cold PBS. After that the cells were resuspended into 300 µl of FACS buffer (1% BSA in PBS) and kept on ice. All the treatment groups were subjected to FACS (FACS lyric, BD Biosciences, San Diego, CA, USA) analysis for 2-NBDG (488/542 nm, blue laser) against the unstained control (cells with no 2-NBDG). All the instrument parameters were checked by performing QC test and the operating parameters for various treatment groups were maintained same throughout the experiments.

5. Isolation of cytoplasmic and nuclear protein fractions

Briefly, after washed with cold PBS thrice, the cells were scraped with 200 µl of lysis buffer consisting of 10 mM HEPES-NaOH, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl and 0.5 mM β-mercaptoethanol and protease inhibitor cocktail and incubated for 30 min with occasional mixing. The mixture was incubated for further 2 min by adding 5 µl of 10% NP-40, followed by centrifugation at 16,000g for 15 min at 4 °C and the supernatant was collected as cytoplasmic fraction. The pellet was then washed with cold PBS and resuspended in 80 µl of nuclear lysis buffer (10 mM Tris-HCl, pH 7.6, 0.5% NP-40, 420 mM NaCl, 1 mM PMSF, 1 mM DTT, 2 mM $MgCl_2$ supplemented with protease inhibitor cocktail). After incubating in nuclear lysis buffer for 30 min, centrifugation was carried out at 16,000g for 15 min at 4 °C and the supernatant was collected as nuclear fraction, to which the NaCl concentration was adjusted to 150 mM by adding lower salt buffer (10 mM Tris-HCl, pH 7.6, 1 mM PMSF, 1 mM DTT, 2 mM $MgCl_2$, protease inhibitor cocktail) to perform the downstream SDS-PAGE analysis.

6. Immunoblot and immunofluorescence analysis

To perform the immunoblot analysis of targeted proteins, the respective protein fractions were quantified with Bradford assay and then 50 µg of each sample was resolved in the denaturing polyacrylamide gel electrophoresis. After that the proteins were transferred to PVDF membrane, blocked by 3% BSA and then subjected to immunoblot with respective primary and followed by secondary antibodies.

For immunofluorescence analysis, HepG2 cells were grown and cultured on poly-L-lysine coated coverslips and treated with respective test materials for required time period. After incubation, the cells were fixed with 4% paraformaldehyde for 20 min and permeabilized with 0.1% Triton X-100 followed by blocking with 1% BSA and then incubated with respective primary antibodies for overnight at 4°C. Anti-mouse and anti-rabbit secondary antibodies with FITC or TRITC conjugate were used for detection of proteins. The cells were stained with DAPI (1 µg/ml) for nuclear staining.

7. Staining of fatty acids

Briefly, the cells were fixed with 4% paraformaldehyde and washed with 60% isopropanol followed by incubation in Oil Red O solution for 15 min at room temperature. After the incubation, the excess Oil Red O was washed with 60% isopropanol and images were captured in PBS with a bright field microscope. For quantification, the bound Oil Red O was eluted by 100% isopropanol with gentle shaking and the OD was measured at 510 nm using 100% isopropanol as blank. For Nile Red staining, HepG2 cells were fixed as described earlier and then stained with Nile Red working solution (5 µg/ml) for 5 min at room temperature in dark. After that the cells were washed with PBS thrice and counterstained with DAPI (1 µg/ml) for 2 min to stain the nuclei. After washing of excess DAPI, the images were captured with a fluorescent microscope (EVOS FLoid, Invitrogen, Thermo Fisher, USA).

8. GC-MS analysis of cellular fatty acid contents

After the respective treatments, the cells were trypsinized and collected in cold PBS. Then 20 µl of methanolic 2N KOH solution was added to the pelleted cells and incubated for 10 min at 50°C in water bath with frequent shaking. After allowing to cool for 10 min, 100 µl of methanolic 5% HCl solution was added and further incubated for 10 min at 70°C in water bath with frequent shaking and then cooled it for 10 min. After adding 200 µl of petroleum ether it was incubated at 60°C for 2 min in shaking. The upper phase was then taken and transferred to the GC vials. A GCMS instrument of Agilent Technologies GC 7890B and MS 5977B system (Agilent Technologies, Santa Clara, USA) was used to analyse the samples. A DB-FastFame GC column (Agilent Technologies, part number- G3903-63011, 30 m X 0.25 mm X 0.25 µm) was used for separating the samples. Helium gas was used as carrier gas with column head pressure 13.8 psi. Total flow rate was 40 ml/min and injection volume were 1 µl. Inlet and detector temperature was 250°C and 260°C, respectively. The initial oven temperature was 50°C and held for 1 min and from which a temperature gradient was started with a linear increase to 175°C at 25°C/min and then a slower increase to 230°C at 4°C/min to separate closely eluted fatty acids and held there for 5 min.

***9. In vivo* study**

The mice were housed in a proper condition, as per the guidelines of Institutional Animal Ethics Committee (IAEC) with *ad libitum* access of water and food and with a 12 h day-night cycle in conventional open-top cages with wheat straw used as bedding material. The temperature of the facility was maintained at 22-24°C. Normal chow was used as food pellet and for developing high fat induced diabetes, pork fat (lard) was used along with normal chow. The experiment was performed according to the approved protocol (BT/IAEC/2021/1/03) of the Institutional Animal Ethics Committee (IAEC) of Indian Institute of Technology Roorkee, Roorkee, India.

9.1 High fat diet and treatments

All the mice except the control group was given HFD (normal chow with lard) for 6 weeks, the control group was given normal chow. Body weight was monitored in every week

throughout the treatment period. After the end of sixth week, one single low dose of streptozotocin injection (35 mg/kg body weight, intraperitoneal) in 0.1 M citrate buffer (pH 4.5) was administered to the HFD-fed mice to develop insulin resistance, while the control group was given only 0.1 M citrate buffer. The development of insulin resistance was determined by checking the fasting blood glucose level (> 200 mg/dl). The doses of LyH (both low- 1.6 g/kg body weight/day and high- 3.2 g/kg body weight/day) were under the human RDI (recommended dietary intake) value range. Since, the major goal of this study was to establish honey as nutraceutical, all the treatments (metformin, honey, fructose and glucose, dissolved in water) were provided daily by oral gavage at the animal facility between 2:00-4:00 PM. Animals in the control group were given saline solution orally.

9.2 Tissue harvest and processing

Epididymal white adipose tissues (eWAT) were collected, dissected out and weighed. Blood was collected by cardiac puncture from each animal of every group to check various blood parameters. Liver, kidney and intestine were dissected out and preserved by fixing with 10% formaldehyde for histopathology and double immunohistochemistry. Some portions of liver tissues were immediately snap frozen by liquid nitrogen and preserved in -80°C freezer for protein extraction and western blot analysis (performed thrice for 3 individual animal samples). For double immunohistochemistry (IHC), paraffin embedded liver sections were incubated with mouse ChREBP and rabbit Glut4 primary antibodies, followed by anti-mouse FITC and anti-rabbit TRITC conjugated secondary antibodies, respectively (dilutions are given in supplementary table**, Table S2**). DAPI (1 µg/ml) was used to counterstain the nuclei. Finally, the sections were visualized under a fluorescent microscope (EVOS FLoid, Invitrogen, Thermo Fisher, USA).

9.3 Glucose tolerance test

On the termination of the respective treatments, fasting blood glucose were measured with a glucometer (BeatO, New Delhi, India). To perform glucose tolerance test, mice were fasted for 6 h and then glucose (1 gm/kg body weight) was administered intraperitoneally to each mouse. The respective treatments were administered 30 min before the glucose administration. Then blood glucose was monitored in different time points- 0, 15, 30, 60, 90 and 120 min.

9.4 Estimation of blood parameters

On completion of the treatment, animals were sacrificed, and blood was collected by cardiac puncture. Different blood parameters like cholesterol, triglycerides, creatinine, SGOT and SGPT were estimated by commercially available kits (Erba, Mannheim Germany) according to manufacturer's instruction.

9.5 ELISA

In order to check inflammation, two proinflammatory cytokines, IL-6 and TNF-α were checked by human IL-6 ELISA kit (Abbkine, catalogue # KET6017), human TNF-α ELISA kit (Abbkine, catalogue # KET6032), mouse IL-6 ELISA kit (Abbkine, catalogue #

KET7009) and mouse TNF-α ELISA kit (FineTest, catalogue # EM0183). The company suggested protocol was followed for all the analysis.

10. α-amylase inhibition assay

To study the alpha-amylase inhibition activity of LyH, different concentrations of LyH (1, 2, 5 and 10 mg/ml) dissolved in ddH_2O were taken and performed the alpha-amylase inhibition assay. Briefly, 1 ml of 0.5 units/ml α-amylase in 20 mM sodium phosphate buffer was mixed with 1 ml different concentrations (0.1, 0.2, 0.5 and 1 mg/ml) of LyH aqueous extract and incubated for 30 min at room temperature. Then equal volume of 0.5% starch solution was added and incubated for 3 min. Finally, 1 ml of colour reagent (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate and 2 M sodium hydroxide) was added and incubated at 85°C for 15 min to stop the reaction. The absorbance was measured @540 nm after cooling. Acarbose (2 mg/ml) was used as positive control. The % inhibition of activity was calculated as-

$$\left[\frac{X_A - X_B}{X_A}\right] * 100$$

Where, X_A= absorbance of control (100% enzyme activity), X_B= absorbance of samples.

11. α-glucosidase inhibition assay

Briefly, 100 µl of α-glucosidase enzyme (1 U/ml) was mixed with 50 µl of different concentrations of LyH aqueous extracts (0.1, 0.2, 0.5 and 1 mg/ml) and incubated for 10 min at 37°C. After that, 50 µl of 3 mM of pNPG (p-nitrophenyl-β-D-galactopyranoside; dissolved in phosphate buffer) was added and incubated further for 20 min at 37°C. Finally, 2 ml of 0.1 M Na_2CO_3 (dissolved in phosphate buffer) was added to stop the reaction. The absorbance was measured at 405 nm. Acarbose (2 mg/ml) was used as positive control. The % inhibition of activity was calculated as-

$$\left[\frac{X_A - X_B}{X_A}\right] * 100$$

Where, X_A= absorbance of control (100% enzyme activity), X_B= absorbance of samples.

12. Anti-glycation assay

Briefly, 500 µl of BSA (bovine serum albumin; 10 mg/ml) and 400 µl of glucose (50 mg/ml) were mixed properly. Then 100 µl of different concentrations of aqueous extract of LyH were added and incubated at 60°C for different time periods (24, 48, 72 and 96 h). After required time of incubation, 10 µl of trichloroacetic acid (TCA) was added and incubated for 10 min at 4°C. After that, the mixture was centrifuged at 13,000g for 5 min at 4°C and the supernatant was discarded. The pellet was then resuspended in 500 µl of PBS and the excitation and emission were measured at 370/400 nm. Quercetin (500 µg/ml) was used as positive control. The % inhibition of activity was calculated as-

$$\left[\frac{X_A - X_B}{X_A}\right] * 100$$

Where, X_A= fluorescence of control, X_B= fluorescence of samples.

Table S1: Details of primers used in this study

S. No.	Name of gene (Human)	Sequence (5' to 3')	T_m	Purpose
1.	*Glut4*	Forward- ccccttcctttcctctgcag	All the primers were designed to have similar T_m, that is 60°C.	qRT-PCR
		Reverse- gtcacacgaggggaatgagg		
2.	*ChREBP*	Forward- agctgcgggatgagattgag		qRT-PCR
		Reverse- ctggtcaaaacgctggtgtg		
3.	*SCD1*	Forward- ccacttgctgcaggacgata		qRT-PCR
		Reverse- ccaagtagaggggcatcgtc		
4.	*ELOVL6*	Forward- tgctcttcgaactggtgctt		qRT-PCR
		Reverse- tcctagttcgggtgctttgc		
5.	*NLRP3*	Forward- tctgtgtgtgggactgaagc		qRT-PCR
		Reverse- gagtctggtcagggaatggc		
6.	*NF-κB*	Forward- tgaagccctccaaaagcact		qRT-PCR
		Reverse- aagtttgagtttccccagctccc		
7.	*IL-1B*	Forward- gctggcagaaagggaacaga		qRT-PCR
		Reverse- ctgactgtcctggctgatgg		
8.	*β-actin*	Forward- gcatgggtcagaaggattccta		qRT-PCR (Internal control)
		Reverse- tgtagaaggtgtggtgccagat		
9.	*Glut4* promoter	Forward- gcagaatttggccgaggatc		ChIP-qPCR (-907 to -658 from TSS)
		Reverse- tcacaaccgaccacaccac		

Table S2: Details of antibodies used in this study

S. No.	Antibody	Source	Catalogue No.	Host species	Dilutions
1.	Glut4	Elabscience	E-AB-30268	Rabbit	1:2000 (WB) 1:1000 (IF) 1:500 (IHC)
2.	ChREBP	Santa Cruz	sc-515922	Mouse	1:1500 (WB) 1:500 (IHC) 2 µg/reaction (ChIP)
3.	Glut1	Elabscience	E-AB-31556	Rabbit	1:2000 (WB)
4.	IL-6	GeneTex	GTX110527	Rabbit	1:500 (IHC)
5.	TNF-α	GeneTex	GTX110520	Rabbit	1:500 (IHC)
6.	Beta-actin	Santa Cruz	sc-47778	Mouse	1:2000 (WB) 2 µg/reaction (ChIP)
7.	Histone H3	GeneTex	GTX122148	Rabbit	1:2000 (WB)
8.	HRP-conjugated anti-mouse secondary antibody	Santa Cruz	sc-516102	Recombinant	1:5000 (WB)
9.	HRP-conjugated anti-rabbit secondary antibody	Santa Cruz	sc-2357	Mouse	1:5000 (WB)
10.	Goat anti-mouse IgG (H+L) secondary antibody (FITC-conjugated)	Invitrogen	62-6511	Goat	1:1000 (IHC)
11.	Goat anti-rabbit IgG (H+L) secondary antibody (TRITC-conjugated)	Santa Cruz	sc-2780	Goat	1:1500 (IF) 1:1000 (IHC)

Table S3: List of the ingredients of normal chow (NFD) and high fat diet (HFD)

Sr. No.	Compound Name	Retention time (min)	% of total energy	
			NFD	HFD
Amino Acids				
1	L-Leucine	6.355	0	0.3±0.002
2	L-Valine	7.244	0	4.2±0.008
3	L-Proline	8.373	0	2.3±0.01
4	Glycine	8.487	0	4.2±0.005
5	Serine	9.168	0.9±0.07	0.7±0.004
6	L-Threonine	9.467	1.0±0.004	2.8±0.001
7	L-Aspartic acid	9.684	1.2±0.03	4.4±0.01
8	beta-Alanine	10.042	0.3±0.06	1.3±0.04
9	L-5-Oxoproline	10.923	8.4±0.02	2.2±0.04
10	L-Glutamic acid	10.978	3.8±0.04	2.9±0.008
11	4-Aminobutanoic acid	11.336	0.9±0.005	3.9±0.003
12	L-Glutamic acid	12.414	0	8.4±0.02
13	Phenylalanine	12.493	0	1.4±0.008
14	Asparagine	13.027	0	3.4±0.05
15	L-Tryptophan	17.852	0	15.5±0.04
	Total amino acids		16.5±0.22	57.9±0.71***
Sugars				

1	Erythrose	10.195	0	0.5±0.09
2	2-Deoxy-D-ribose	11.116	0	7.7±0.07
3	d-Ribose	11.986	0.9±0.02	0
4	D-Arabinose	13.056	6.4±0.03	2.1±0.007
5	D-Fructose	15.381	28±0.27	41.6±0.04
6	d-Galactose	15.613	0	27.7±0.18
7	d-Glucose	15.665	7.8±0.05	29.4±0.08
8	D-(-)-Fructofuranose	17.648	0	1.5±0.03
9	D-(+)-Turanose	19.068	0	3.0±0.005
10	Lactulose	19.466	0	11.2±0.07
11	Sucrose	20.213	25±0.01	39.2±0.07
12	D-Lactose	20.646	1.6±0.03	10.9±0.54
13	Maltose	20.768	15.6±0.09	5.5±0.04
14	beta-Gentiobiose	21.272	4.2±0.57	6.9±0.25
15	D-(+)-Cellobiose	21.547	16.9±0.009	1.9±0.12
16	3-alpha-Mannobiose	22.027	0	4.5±0.024
17	Lactose	22.389	0	1.4±0.001
18	Melibiose	23.814	5.8±0.4	18.8±0.8
		Total sugars	112.2±0.47	213.8±0.078***
Fatty acids				
1	Palmitic Acid	16.64	4.4±0.025	11.9±0.9
2	Oleic Acid	17.801	0	8.5±0.22
3	Stearic acid	17.943	5.7±0.034	9.3±0.001
4	1-Monomyristin, 2TMS derivative	18.809	0	1.6±0.32
5	1-Monopalmitin	19.717	16.0±0.28	

				24.3±0.24
6	Glycerol monostearate	20.717	0	22.0±0.44
		Total fatty acids	26.1±0.27	77.6±0.47***

'*' was used to denote comparison between the NFD and the HFD. Depending on the p value i.e. 0.001, three * was used to indicate the statistical significance. NFD, non-fat diet (normal chow); HFD, high-fat diet.

F tests - ANOVA: Fixed effects, omnibus, one-way

Analysis:	A priori: Compute required sample size	
Input:	Effect size f	= 0.65
	α err prob	= 0.05
	Power (1-β err prob)	= 0.8
	Number of groups	= 7
Output:	Noncentrality parameter λ	= 17.7450000
	Critical F	= 2.3717812
	Numerator df	= 6
	Denominator df	= 35
	Total sample size	= 42
	Actual power	= 0.8398239

Figure S1: Sample size calculation for *in vivo* experiment by GPower software.

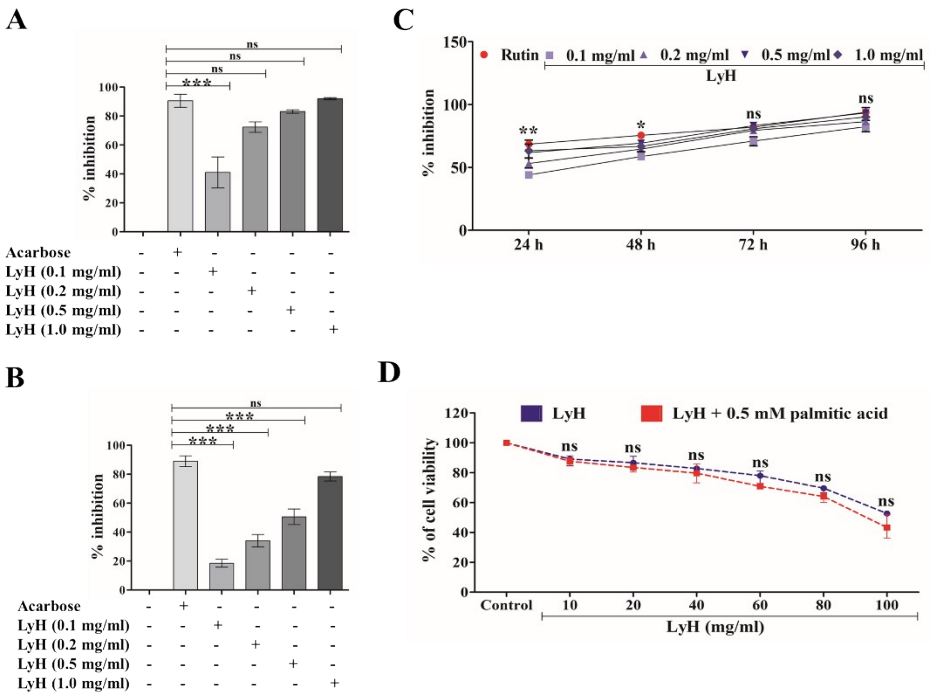

Figure S2: LyH possesses glucose lowering activity *in vitro*. HepG2 cells were treated with varying concentrations of LyH followed by estimation of its inhibition capacity of [A] α-amylase and [B] α-glucosidase using acarbose as the positive control having maximum inhibitory activity. [C] Anti-glycation activity of LyH compared to rutin used as positive control. [D] Cytotoxicity assay in HepG2 cells against LyH in the presence or absence of palmitic acid. Data represented (for three independent experiments) as mean±SEM. *, ** and *** were used to denote the statistical significances for $p < 0.05$, $p < 0.01$ and $p < 0.001$ probabilities, respectively.

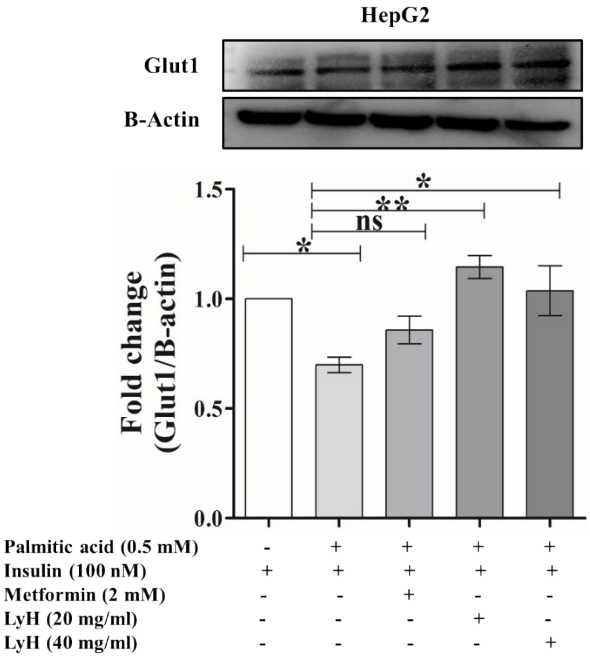

Figure S3: LyH regulates Glut1 expression in liver. Representative immunoblot showing the expression of Glut1 in HepG2 cells under various treatment conditions. The bar graph represents the quantitative densitometric scanning data of independent immunoblot experiments as shown above. Data represented (for three independent experiments) as mean±SEM. * and ** were used to denote the statistical significances for $p < 0.05$ and $p < 0.01$ probabilities, respectively. ns, non-significant.

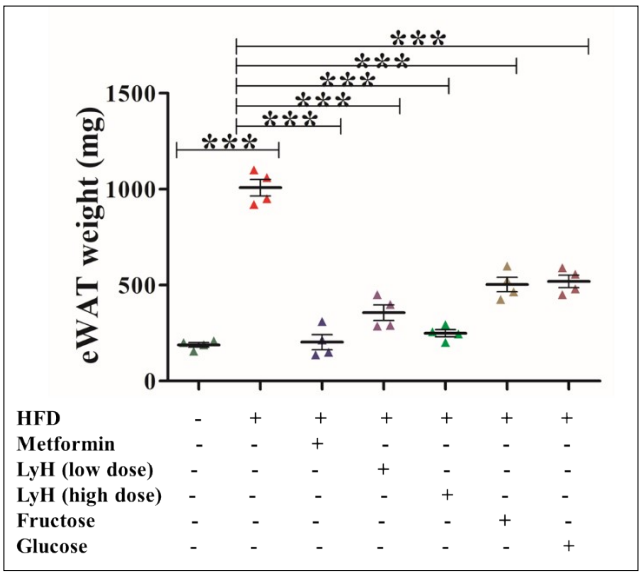

Figure S4: Weight of eWAT (epididymal white adipose tissue) of animals from different groups. Data represented as mean±SEM (n=4). *** was used to denote the statistical significances for $p < 0.001$ probability.

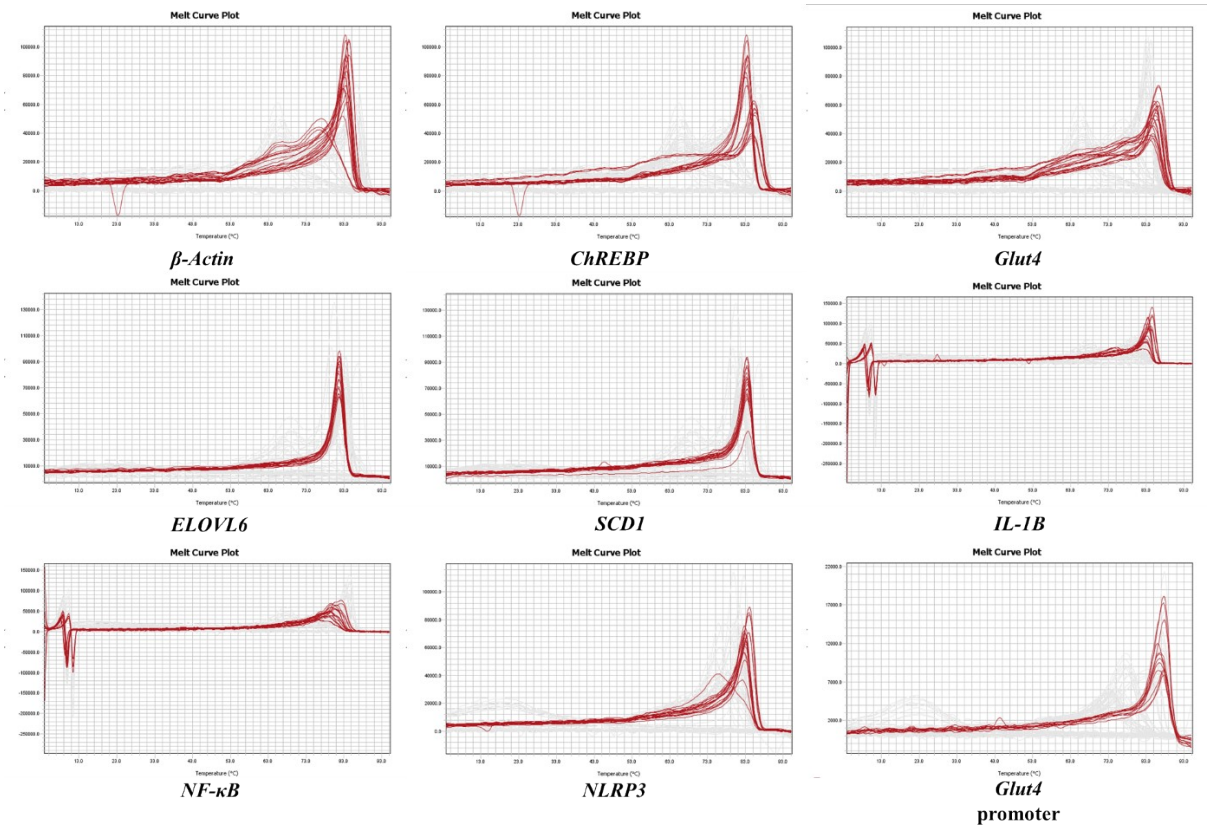

Figure S5: qRT-PCR Melt curve plots of primers used in this study.