

SUPPLEMENTARY DATA

**Supplementary Table 1. List of oligonucleotide primer pairs used in real time RT-PCR and RT-PCR analysis.**

primer	species	Forward	Reverse
FAM3A	Human	5'-GTGTCACATGGATCGTGGTC-3'	5'- TGCTCAATCAGCATCTTGTCC-3'
FAM3A	Mouse	5'-TCATGAGCAGCGTCAAAGAC-3'	5'-AGGGTACCTTCATGCAGTGG-3'
G6Pase	Mouse	5'-AGGAAGGATGGAGGAAGGAA-3'	5'-TGGAACCAGATGGAAAGAG-3'
PEPCK	Mouse	5'-ATCTTGGTGGCCGTAGACCT-3'	5'-CCGAAGTTGTAGCCGAAGAA-3'
PEPCK	Human	5'-ATGGCCCGCATTGTACCGCC-3'	5'-TCACATTGTGCACACGTCTCT-3'
FAS	Mouse	5'-CTGCCACAACCTTGAGGACA-3'	5'-CGGATCACCTCTTGAGAGC-3'
NFE2	Human	5'-TGGGACCATCTCCTTGTG-3'	5'-GGCAAGCCTCTTGAAACAC-3'
NFE2	Mouse	5'- TCTACTCCCCATGTCCCAGA-3'	5'-CAGCCTCCATACCCCTAGC-3'
OCT1	Human	5'-CAGAGCCAGTCAACACCAAAG-3'	5'-ATAGCGAGCCAACATCACC-3'
BACH1	Human	5'-TGTGCCAGAGGAAACAGTGAG-3'	5'-TAGGCTTCAAGACGCTGC-3'
GAPDH	Mouse	5'-AACTTGGCATTGTGGAAGG-3'	5'-ACACATTGGGGTAGGAACA-3'
β-actin	Mouse	5'-GGTGGGAATGGTCAGAAGG-3'	5'-GTACATGGCTGGGTGTTGA-3'
SREBP1	Mouse	5'-GGAGGCAGAGAGCAGAGATG-3'	5'-TTGCGATGTCTCCAGAAGTG-3'
ACC1	Mouse	5'-TGGAGCTAACCAACCAGCACTCC-3'	5'-GCCAAACCATCCTGTAAGCC-3'
CPT1 α	Mouse	5'-ATCGTGGTGGTGGGTGTGATAT-3'	5'-ACGCCACTCACGATGTTCTTC-3'
AOX	Mouse	5'-TGTCAATTCTACCAACTGTC-3'	5'-CCATCTTCTCAACTAACACTC-3'

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**Supplementary Table 2. siRNA sequence mixture against human NFE2 p45 subunit**

Dulex Name	SenseSeq	AntiSeq
NFE2-1(H)	GGAUUAUCCCUACAACUAUATT	UAUAGUUGAGGGAUAAUCCTT
NFE2-2(H)	GCGAAUAUGUAGAGAUGUATT	UACAUCUCUACAUAUUCGCTT
NFE2-3(H)	CGGACAAGAUUGUCAACUUTT	AAGUUGACAAUCUUGUCCGTT
NFE2-4(H)	GCCGGUAGAUGACUUUAUTT	AUUAAGUCAUCUACCGGCTT

H:human

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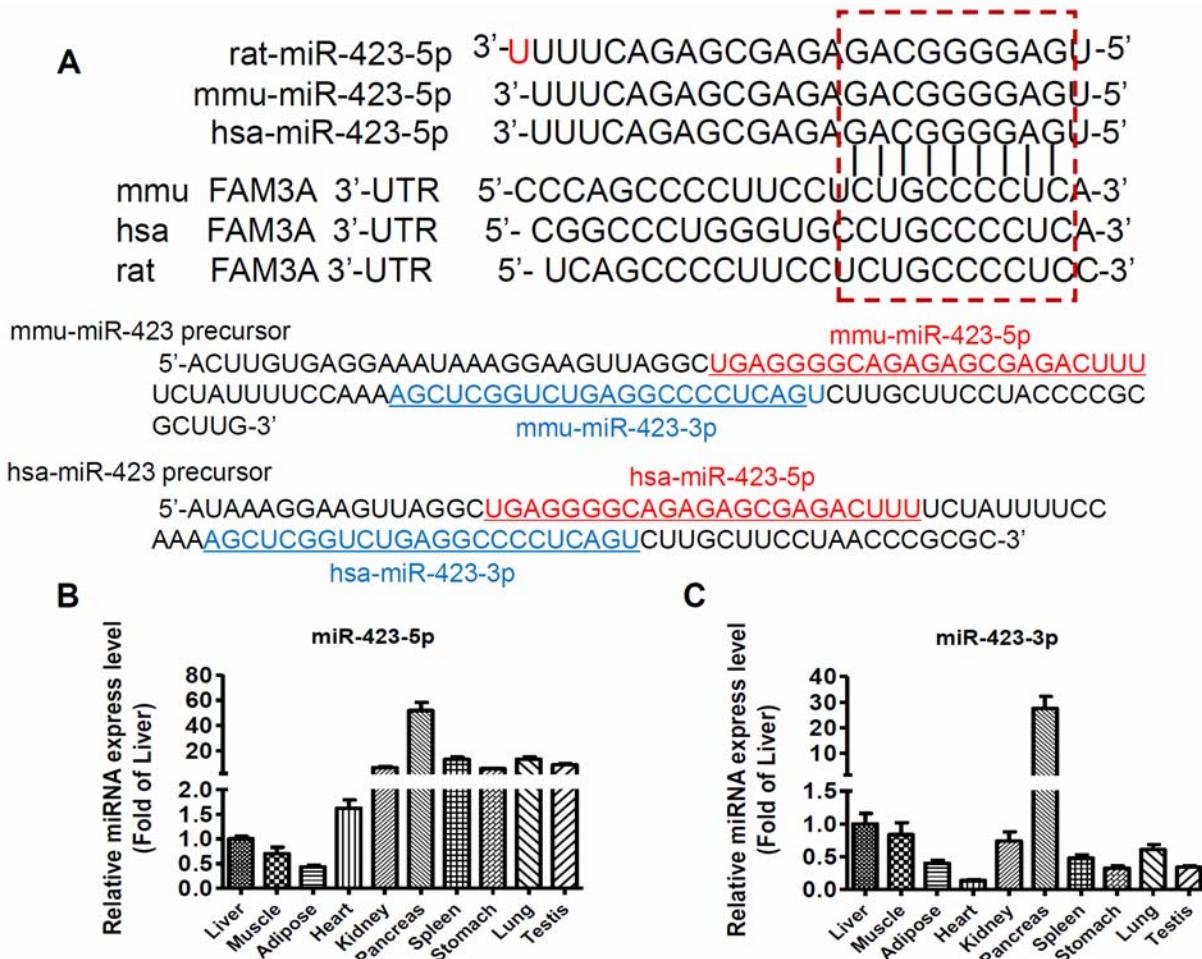
**Supplementary Table 3. Clinical and biochemical characteristics of healthy controls and patients with nonalcoholic fatty liver disease.**

characteristic	control (n = 6)	NAFLD (n = 6)	P value
Gender (males/females)	3/3	3/3	—
Age (yr)	41.8 ±8.2	45.8±7.9	0.410860
BMI (kg/m <sup>2</sup> )	22.8±3.3	24.9±2.4	0.238565
Smoking	no	no	—
Waist circumference (cm)	79.2±9.6	87.7±6.4	0.102317
Diabetes Mellitus	no	no	—
Metabolic syndrome	no	no	—
Hypertension	no	no	—
Systolic blood pressure (mmHg)	110.0±6.3	116.0±9.2	0.217676
Diastolic blood pressure (mmHg)	73.3±8.2	78.7±5.8	0.220095
AST median (min-max, U/L)	16.8 (11-24)	19.2 (12-26)	0.410777
ALT median(min-max, U/L)	13.7 (8-22)	21.0 (11-31)	0.142687
Total cholesterol (mmol/L)	4.3±0.5	5.4±1.1	0.046149
HDL-cholesterol (mmol/L)	1.5±0.2	1.3±0.2	0.221911
LDL-cholesterol (mmol/L)	2.6±0.5	3.1±1.6	0.460173
Triglycerides (mmol/L)	0.8±0.3	2.9±2.0	0.025126

\*Notes: The human liver samples were among 11 pairs of human liver samples published in the previous study (Oncotarget. 2016 Jun 14;7(24):36207-36219).

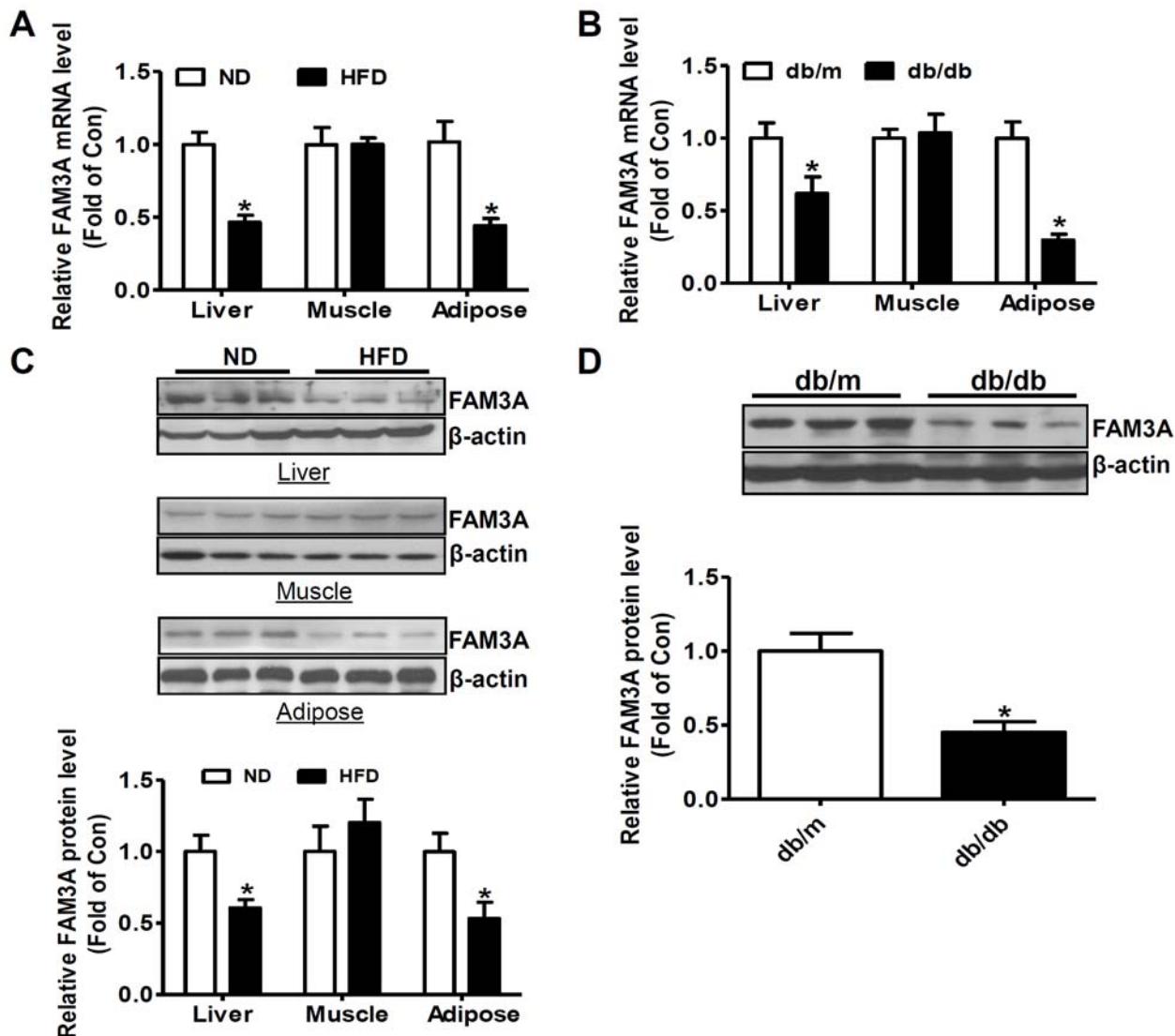
## SUPPLEMENTARY DATA

**Supplementary Figure 1. Prediction of human and mouse FAM3A mRNAs as a potential targets of miR-423-5p.** A) Bioinformatic analysis predicted a highly specific potential site for miR-423-5p in 3'-UTRs of human, rat and mouse FAM3A mRNAs . The location of miR-423-5p and miR-423-3p in mouse and human miR-423 precursor is also provided. B-C) Relative miR-423-5p (B) and miR-423-3p (C) expression in normal C57BL/6 mouse tissues. Adipose in the current study is epididymal adipose. The data are presented as mean ± SEM of 6-8 mouse tissues with normalization to liver tissues.



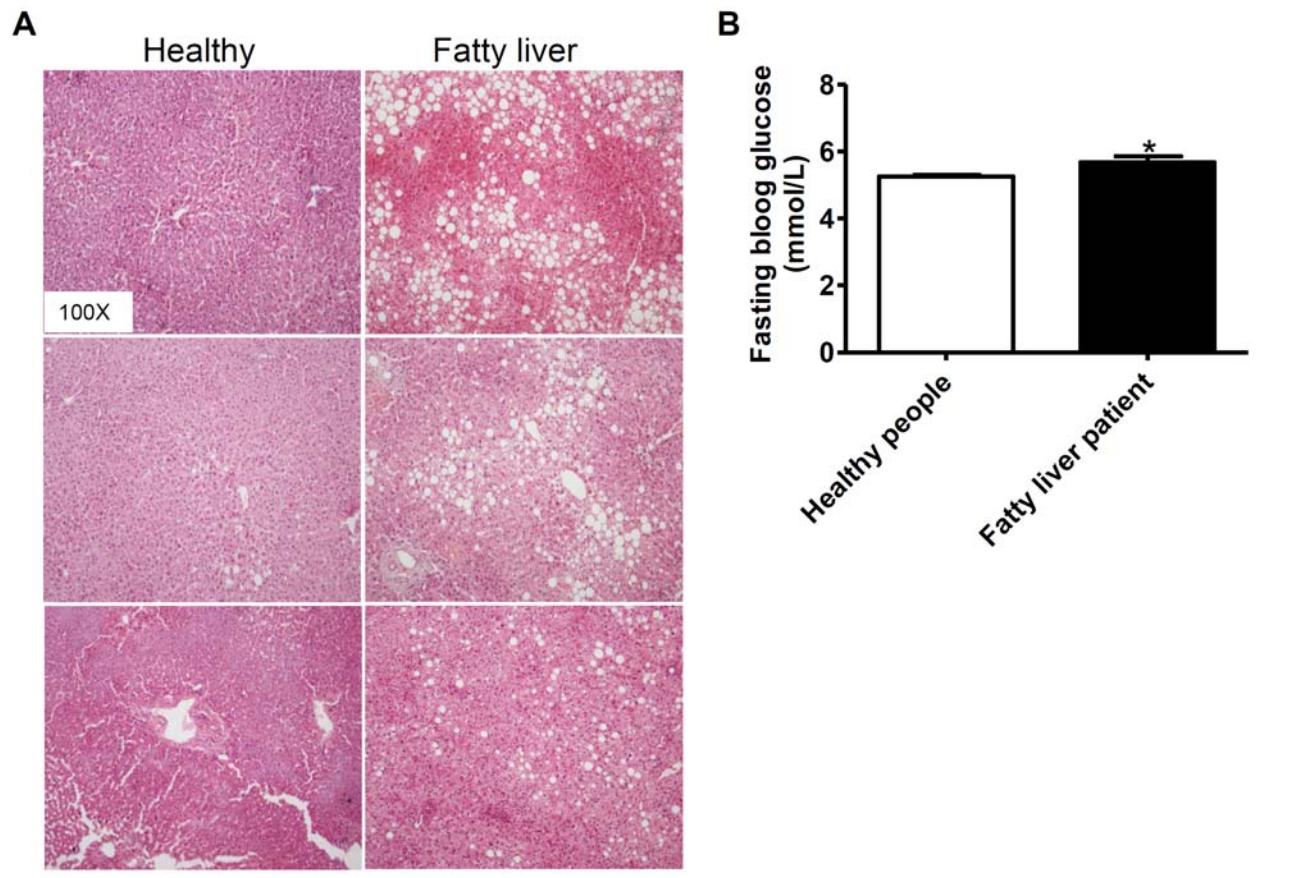
SUPPLEMENTARY DATA

**Supplementary Figure 2. FAM3A expression in tissues of HFD and db/db mice.** A-B) The FAM3A mRNA level in liver, muscle and adipose tissues of HFD (A) and db/db (B) mice. C) The FAM3A protein level in liver, muscle and adipose tissues of HFD mice. D) The FAM3A protein level in liver of db/db mice. N=6-8, \*P<0.05 versus ND or db/m mice. ND, normal diet; HFD, high fat diet.



## SUPPLEMENTARY DATA

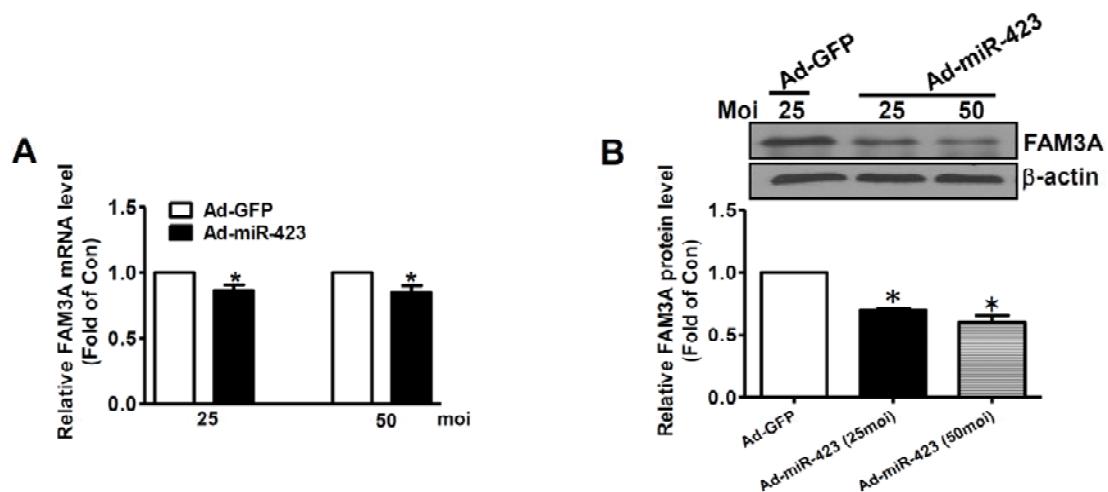
**Supplementary Figure 3. Metabolic characteristics of NAFLD patients.** A) Representative Hematoxylin and Eosin staining images of healthy and fatty liver of human. Significant lipid droplets were visualized in fatty livers, whereas no significant lipid droplets were detected in healthy livers. The magnifying power had been marked in the image. B) Fasting blood glucose was increased in NAFLD patients. N=6, \*P<0.05 versus healthy subjects.



## SUPPLEMENTARY DATA

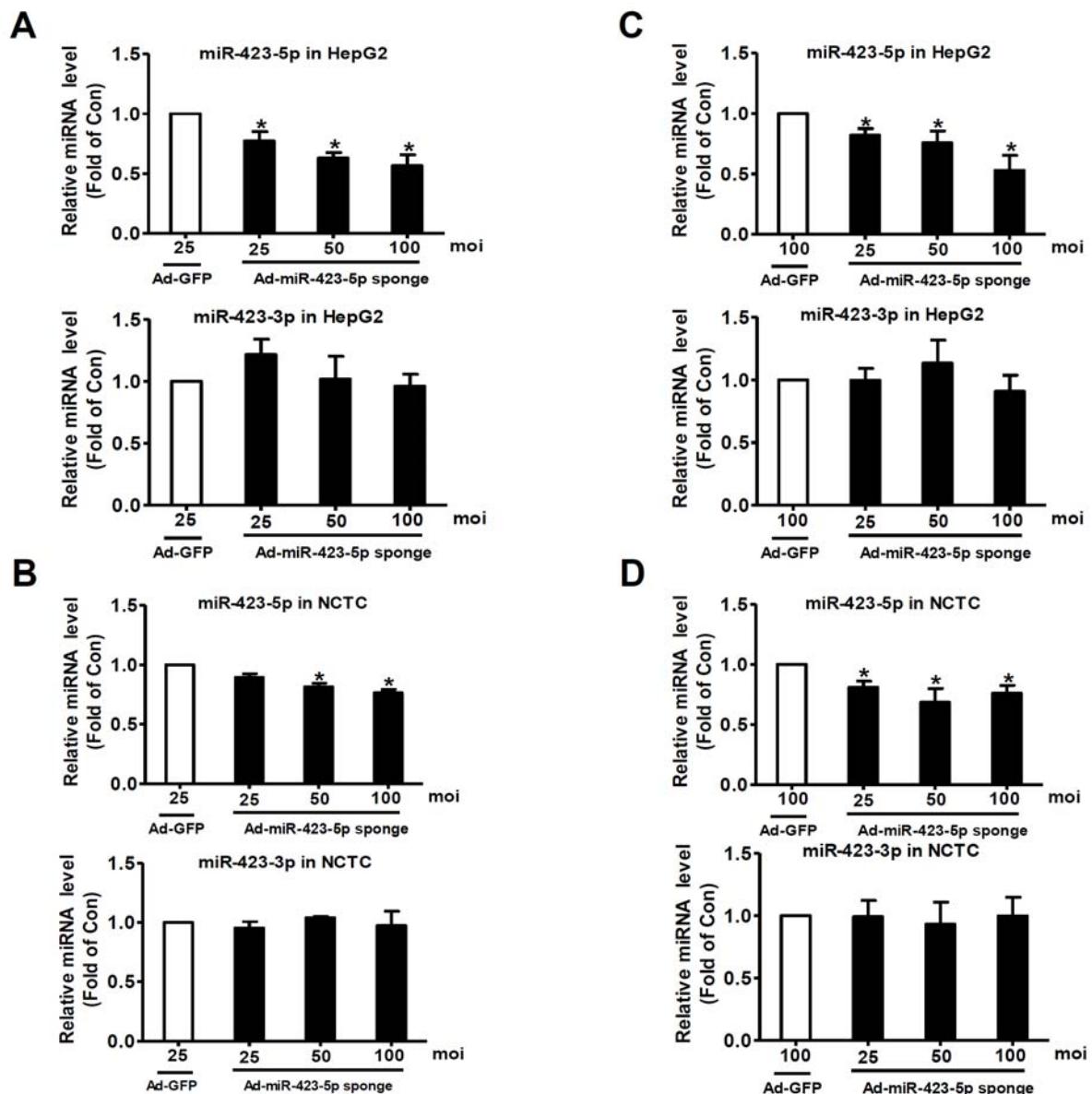
**Supplementary Figure 4. miR-423 overexpression repressed FAM3A expression in HepG2 cells.**

A-B) Adenoviral overexpression of miR-423 on FAM3A mRNA (A) and protein (B) levels in HepG2 cells. HepG2 cells were infected with Ad-miR-423 for 36 hours before analysis for FAM3A expression. N=3-5, \*P<0.05 versus control cells.



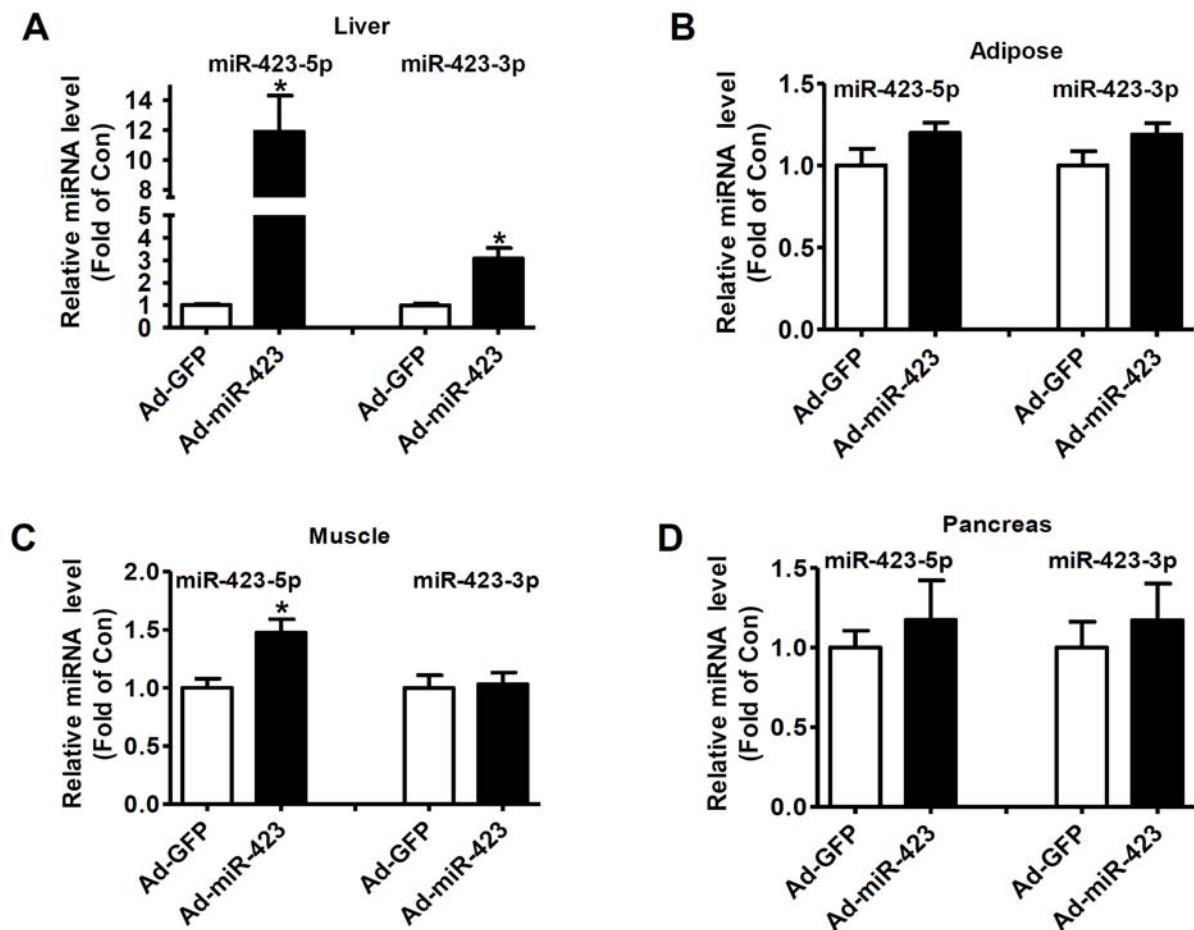
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**Supplementary Figure 5. Characterization of miR-423-5p sponge expressing adenoviruses.** The cells were infected with various dose of Ad-miR-423-5p sponge for 36 hours before analysis (25 or 100 MOI Ad-GFP as control, respectively). A-B) Viral infection reduced miR-423-5p expression without affecting miR-423-3p expression in HepG2 cells (A) and NCTC cells (B) (25 MOI Ad-GFP as control). N=3-5,\*P<0.05 versus control cells. C-D) Viral infection reduced miR-423-5p expression without affecting miR-423-3p expression in HepG2 cells (C) and NCTC cells (D) (100 MOI Ad-GFP as control). N=4,\*P<0.05 versus control cells.



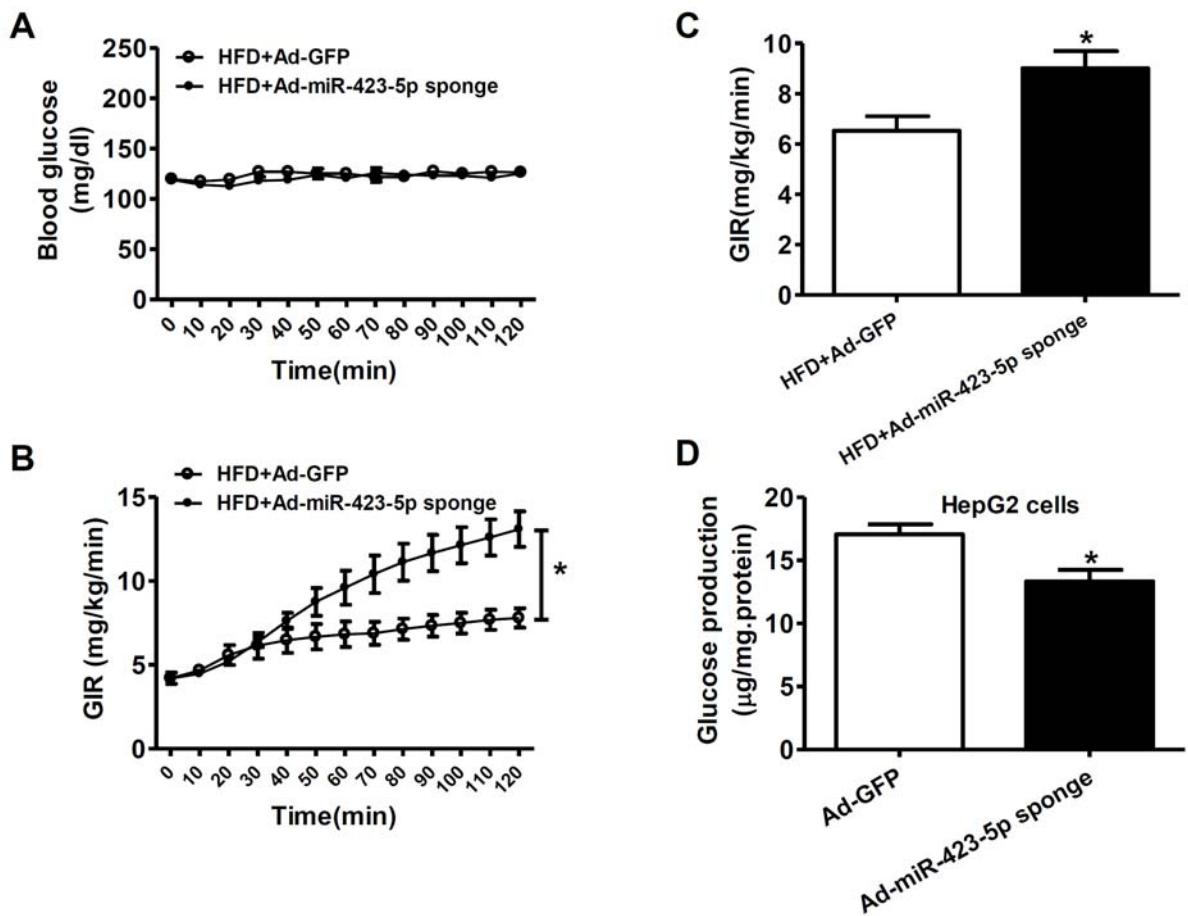
SUPPLEMENTARY DATA

**Supplementary Figure 6. Tail vein injection of Ad-miR-423 on miR-423-5p and miR-423-3p expression in C57BL/6J mouse tissues.** A-D) miR-423-5p and miR-423-3p expression in liver (A), adipose (B), muscle (C) and pancreas (D) tissues after viral injection. N=8-10, \*P<0.05 versus Ad-GFP-treated mice.



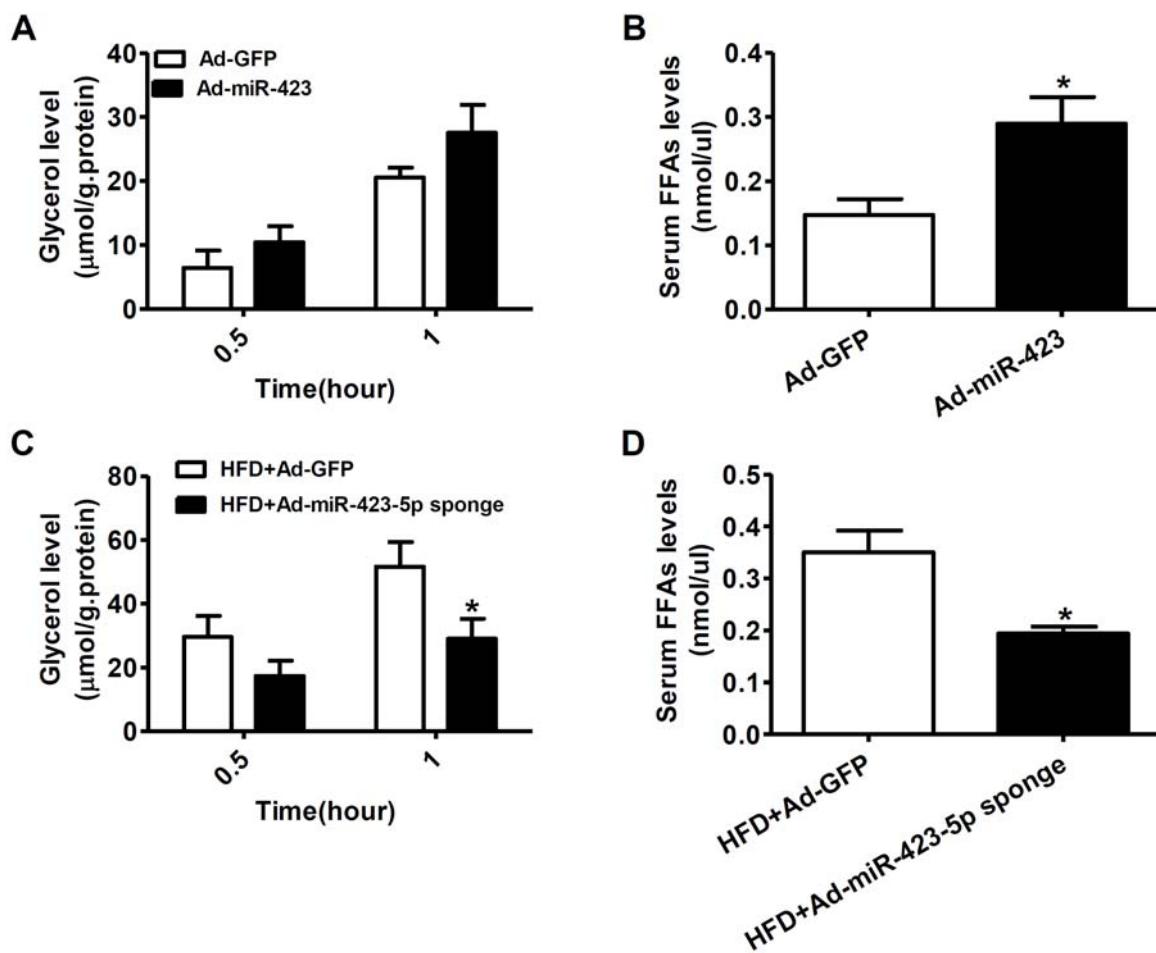
SUPPLEMENTARY DATA

**Supplementary Figure 7. Hepatic miR-423-5p inhibition enhanced global insulin sensitivity of HFD mice as evaluated by hyperinsulinemic-euglycemic clamp – A) Blood glucose level during hyperinsulinemic-euglycemic clamp. B) Glucose infusion rate (GIR) during hyperinsulinemic-euglycemic clamp. C) Average glucose infusion rate. N=4-5, \*P<0.05 versus Ad-GFP group of mice. D) Inhibition of miR-423-5p suppressed glucose production in HepG2 cells. N=5, \*P<0.05 versus Ad-GFP-treated cells.**



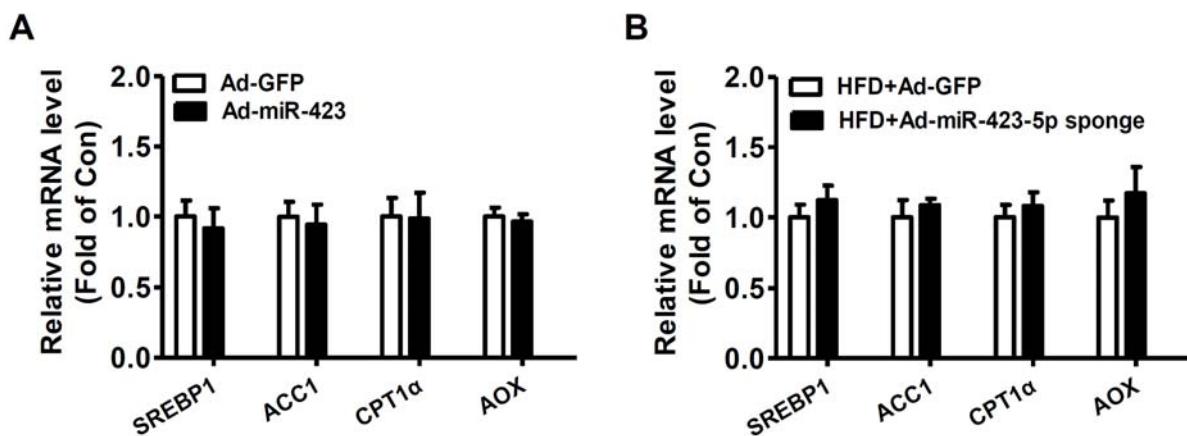
SUPPLEMENTARY DATA

**Supplementary Figure 8. Ad-miR-423-5p overexpression or knockdown on lipolysis of mouse epididymal adipose - A) Ad-miR-423 injection on lipolysis of epididymal adipose of normal mice. B) Ad-miR-423 injection increased serum FFAs levels in normal mice. Serum FFAs (C8 and longer) levels were determined using Free Fatty Acid Quantitation Kit (Sigma) according to the instruction protocol. FFAs, free fatty acids. C) Ad-miR-423-5p sponge injection repressed lipolysis of epididymal adipose of HFD mice. D) Ad-miR-423-5p sponge injection reduced serum FFAs levels in HFD mice. N=6-8, \*P<0.05 versus Ad-GFP group of mice.**



## SUPPLEMENTARY DATA

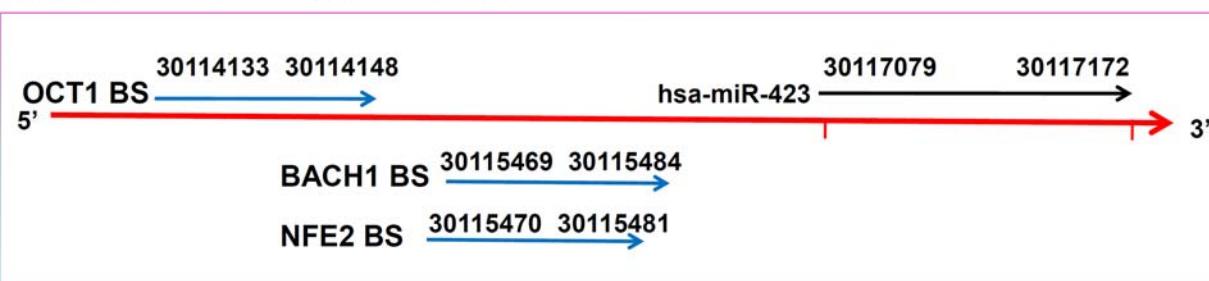
**Supplementary Figure 9. miR-423-5p overexpression or inhibition on lipid metabolic genes in normal or HFD mouse livers.** A) miR-423 overexpression on the mRNA levels of lipid metabolic genes in normal mouse livers. B) miR-423-5p inhibition on the mRNA levels of lipid metabolic genes in HFD mouse livers. N=8, there is no significant difference between two groups.



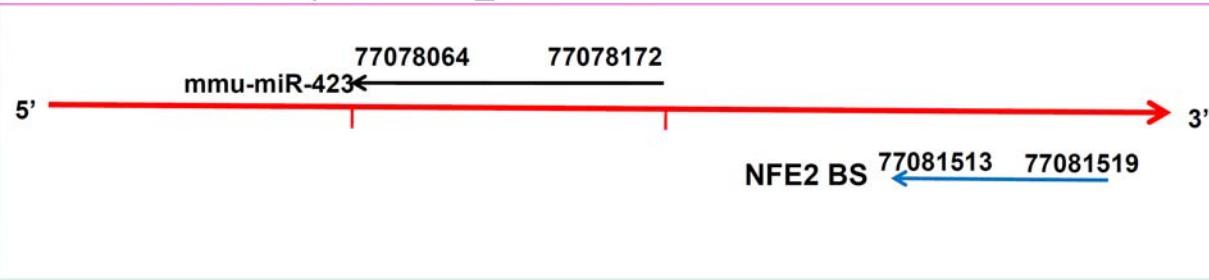
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**Supplementary Figure 10. Prediction and analysis of transcription binding sites in the promoters of human and mouse miR-423 precursor genes.** The binding sites in human and mouse *miR-423 precursor* promoters were analyzed using UCSC Genome Browser and TRANSFAC® 7.0 Public in the following website: <http://www.gene-regulation.com/pub/databases.html>. The potential sites with the highest scores were listed for human miR-423 precursor gene (NCBI Reference Sequence: NC\_000017.11); Homo sapiens chromosome 17, GRCh38.p2 Primary Assembly (Gene ID : 494335). chr17 30114133 30114148 V\$OCT1\_Q6 887 + 2.65; chr17 30115469 30115484 V\$BACH1\_01 823 + 2.18; chr17 30115470 30115481 V\$NFE2\_01 883 + 2.06. BS, binding site. A potential binding site of NFE2 is also predicted to be present in mouse miR-423 precursor gene promoter. Mus musculus strain C57BL/6J chromosome 11, GRCm38.p4 C57BL/6J. NCBI Reference Sequence: NC\_000077.6

Homo sapiens chromosome 17, GRCh38.p2 Primary Assembly  
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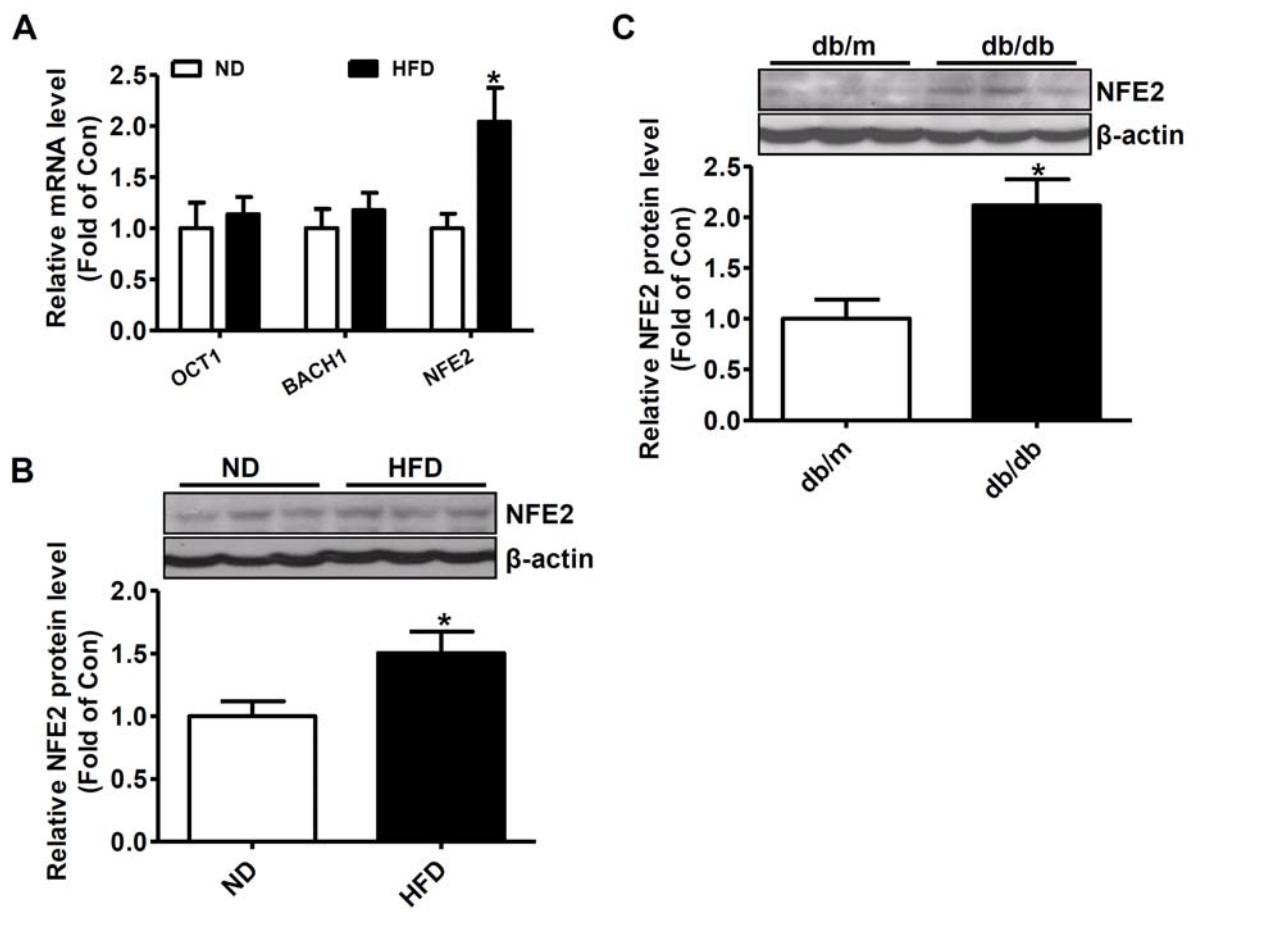


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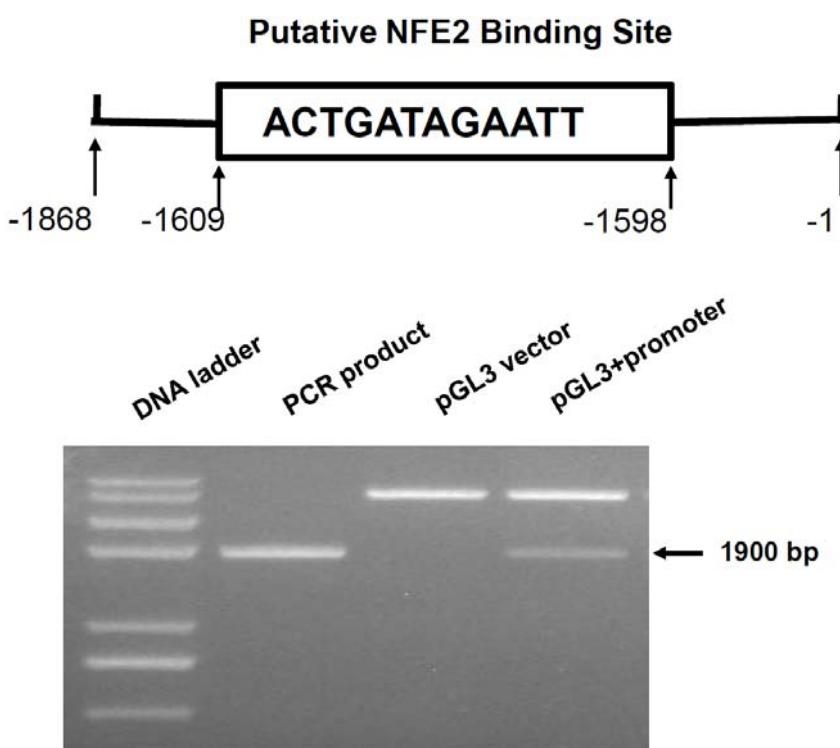
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**Supplementary Figure 11. NFE2 expression was increased in obese diabetic mouse livers.** A) NFE2 mRNA level was increased in the livers of HFD mice. B) NFE2 protein level was increased in the livers of HFD mice. C) NFE2 protein was increased in the livers of db/db mice. N=6, \*P<0.05 versus control mice.



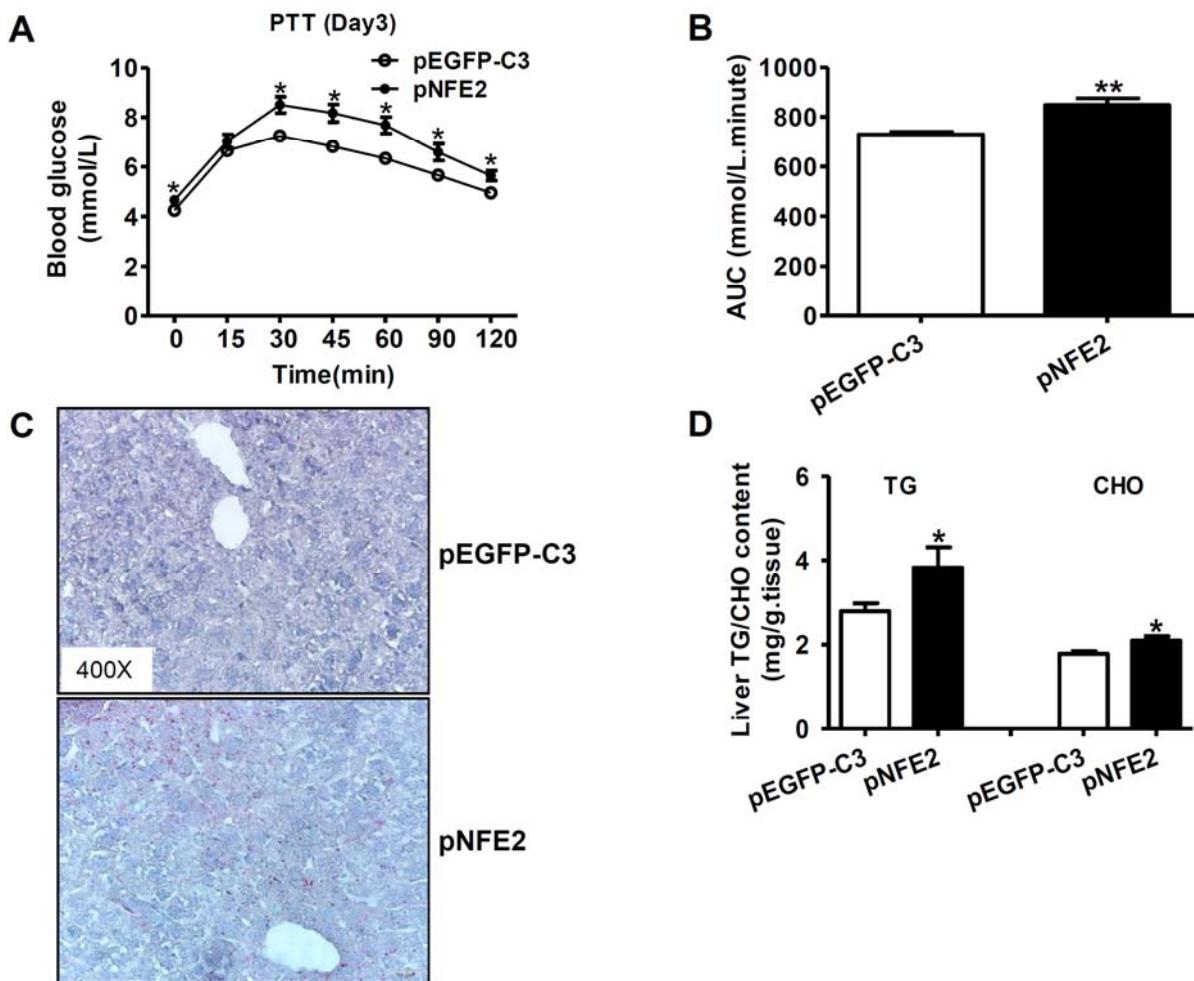
## SUPPLEMENTARY DATA

**Supplementary Figure 12. Cloning of human miR-423 precursor gene promoter.** A 1.9 kb miR-423 precursor gene promoter fragment was cloned from HepG2 cells. The start site of miR-423 precursor gene was referred as number 0. The promoter region of has-miR-423 containing the predicted NFE2 binding site was amplified using AmpliTaq gold DNA polymerase (Applied Biosystems) (Forward primer: 5'- CGACGGTGACCCAACTATTTCTGTCAACAAG-3'; Reverse primer: 5'- CCCAAGCTTTCCTCACAGTTCTCCCTCAAAC-3') using HepG2 cell genomic DNA as template. The PCR product was cloned into pGL3-basic vector using restriction enzyme MluI-HF and HindIII-HF, and confirmed by DNA sequencing.



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**Supplementary Figure 13. Hepatic NFE2 overexpression increased hepatic gluconeogenesis and lipid deposition in C57BL/6J mice.** At 72 hours post plasmid injection, PTT assays were performed as described in experimental procedure. A) NFE2 overexpression increased hepatic glucose production. B) AUC of the data presented in A. C-D) NFE2 overexpression increased lipid deposition in the liver. Representative images of liver samples stained with Oil Red O were shown in C, and quantitative data of TG and CHO content in liver were shown in D. N=8-10, \*P<0.05 versus control mice treated by pEGFP-C3.



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**Supplementary Figure 14. FFAs upregulated NFE2 and miR-423-5p expression in HepG2 cells.**  
Chronic exposure to high levels of FFAs upregulated NFE2 and miR-423-5p expression in HepG2 cells. Cells were exposed to indicated concentration of FFAs for 24 hours, and then NFE2 mRNA and miR-423-5p levels were analyzed. 0.2OA, 0.2mM oleate; 0.2PA, 0.2mM palmitate. N=5, \*P<0.05 versus control cells.

