Supplemental Information

Homocysteine promotes hepatic steatosis by activating the adipocyte lipolysis in a HIF1α-ERO1α-dependent oxidative stress manner

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Supplementary Figures

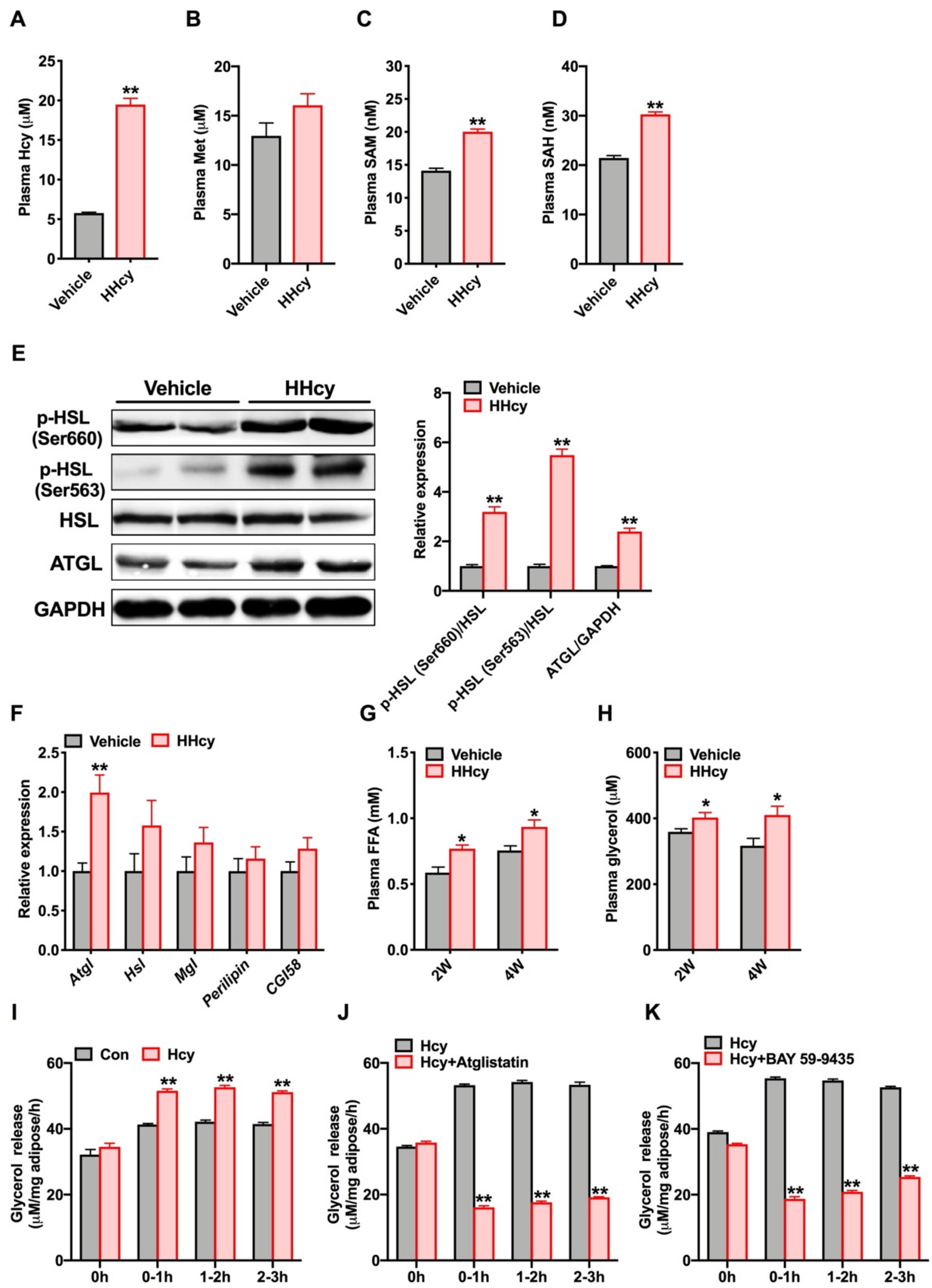

Figure S1. Hcy activates the lipolytic process in adipose tissue (Related to Figure 1)

(A-D) Levels of plasma Hcy, Met, SAM and SAH (n=6). Met, methionine; SAM, S-adenosylmethionine; SAH, S-adenosyl-L-homocysteine.

C57BL/6 mice (8 weeks old) were administered Hcy in the drinking water for 4 weeks. (E) Protein levels of ATGL and phosphorylation levels of HSL at the Ser660 and Ser563 residues (n=4). (F) The relative expression of genes involved in adipose tissue lipolysis (n=6).

C57BL/6 mice (8 weeks old) were administered Hcy in the drinking water for 2 or 4 weeks. (G, H) Plasma FFAs and glycerol levels after fasting for 24 h at the indicated time (n=6). (A) Mann-Whitney U test and (B-H) Two-tailed Student's t-test: *$P < 0.05$, **$P < 0.01$ compared to the vehicle group.

(I) Isolated mice eWAT were incubated with or without 500 μM Hcy for 8 h, the glycerol release per hour in freshly changed medium was examined (n=6). **$P < 0.01$ compared to the control group (J, K) After pretreatment with lipolysis inhibitor for 1 h, the eWAT were treated with Hcy (500 μM) for 8h. The glycerol release per hour in freshly changed medium was detected. Atglistatin, ATGL inhibitor, 10 μM; BAY 59-9435, HSL inhibitor, 5 μM. **$P < 0.01$ compared to the Hcy group. All of the data are presented as the means ± SEM.

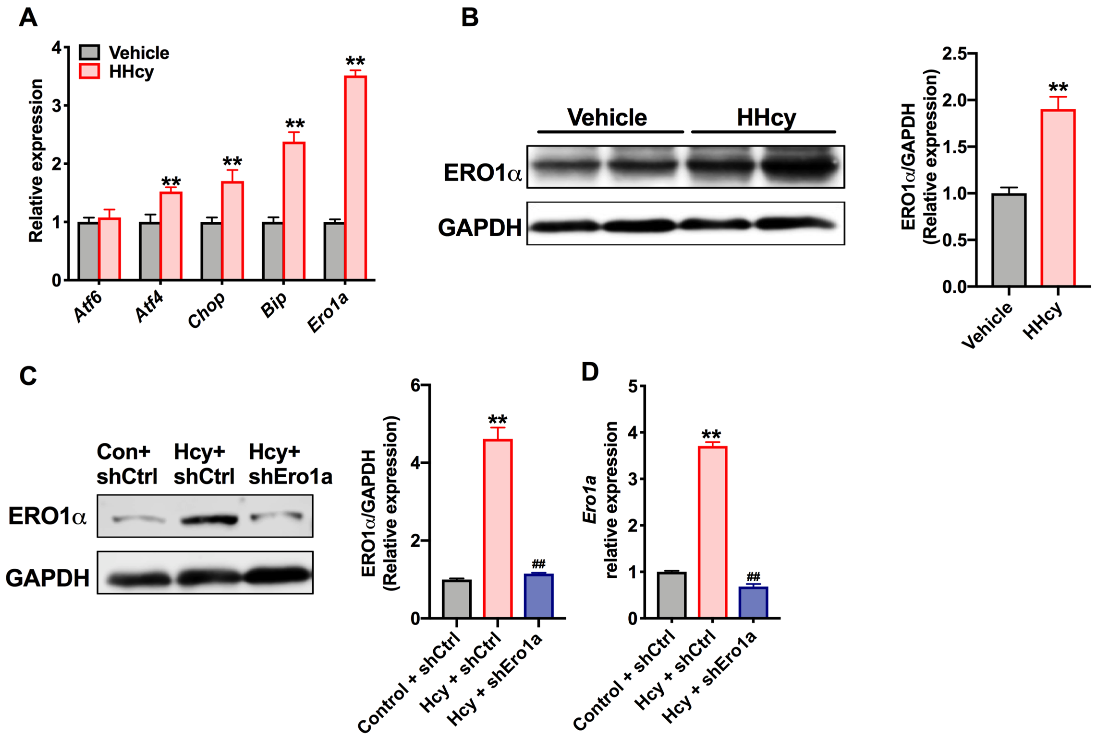

Figure S2. Hcy upregulates the expression of ERO1α (Related to Figure 4)

(A) The relative expression of genes related to ER stress markers in the vehicle or HHcy mice (n=6). (B) C57BL/6 mice (8 weeks old) were administered Hcy in the drinking water for 4 weeks. Protein levels of ERO1α (n=3). (C, D) 3T3-L1 cells transduced with lentiviral control shRNA (shCtrl) or shEro1a for more than 72 h were treated with 500 μM Hcy. The protein and mRNA levels of ERO1α (n=3 or 5).

All of the data are presented as the means ± SEM. (A, B) Two-tailed Student's t-test: $**P < 0.01$ compared to the vehicle group. (C, D) One-way ANOVA with Tukey's post-hoc test: $**P < 0.01$ compared to the control + shCtrl group. $^{##}P < 0.01$ compared to the Hcy + shCtrl group.

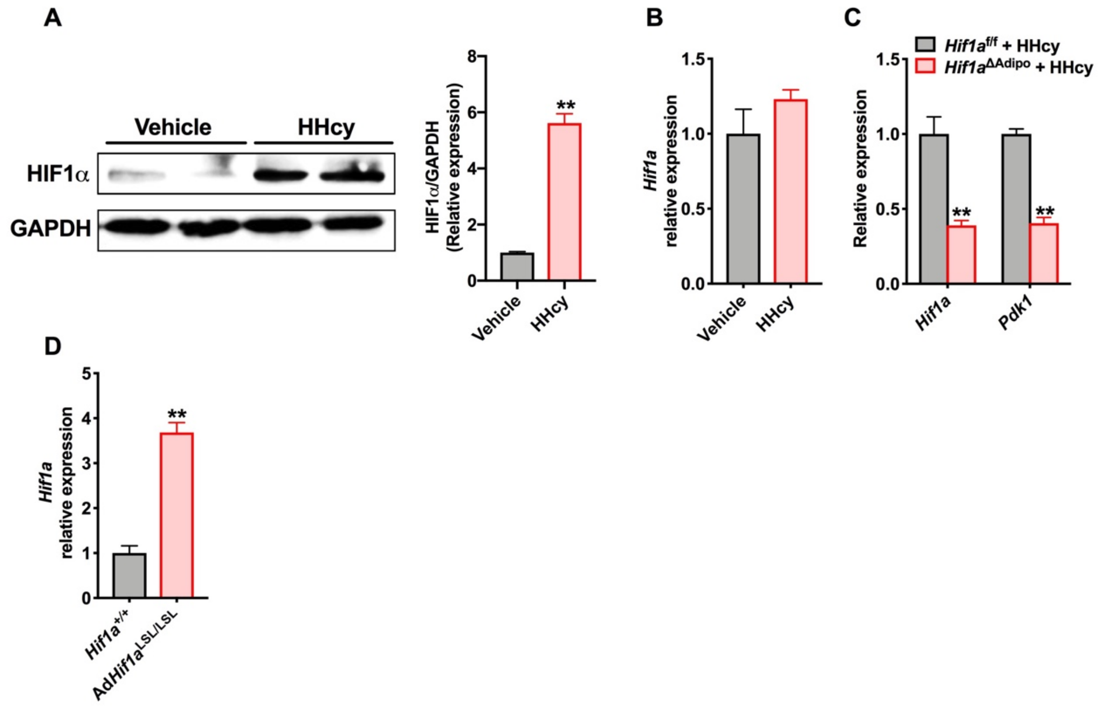

Figure S3. Activation of HIF1α mediates Hcy-induced ERO1α expression in adipocytes (Related to Figure 5)

(A, B) Protein and mRNA levels of HIF1α in the eWAT of vehicle and HHcy mice (n=3 or 6). Two-tailed Student's t-test: $^{**}P < 0.01$ compared to the vehicle group. (C) The relative expression of the *Hif1a* and *Pdk1* genes in the eWAT of $Hif1a^{f/f}$ and $Hif1a^{\Delta Adipo}$ mice after 8 weeks on an HMD (n=6). Two-tailed Student's t-test: $^{**}P < 0.01$ compared to the $Hif1a^{f/f}$ + Hcy group. (D) The mRNA levels of HIF1α in the eWAT of $Hif1a^{+/+}$ and Ad$Hif1a^{LSL/LSL}$ mice (n=6). Two-tailed Student's t-test: $^{**}P < 0.01$ compared to the $Hif1a^{+/+}$ group. All of the data are presented as the means ± SEM.

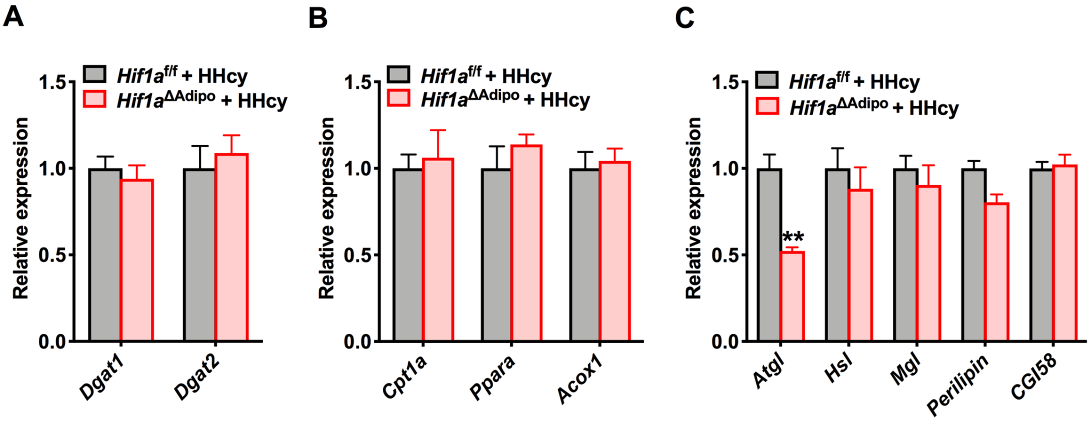

Figure S4. The lipolytic gene was downregulated in Hcy-treated *Hif1a*$^{\Delta Adipo}$ mice (Related to Figure 6)

(A-C) The relative expression of genes involved in TG synthesis, fatty acid oxidation and lipolysis (n=6). All of the data are presented as the means ± SEM. Two-tailed Student's t-test: $^{**}P < 0.01$ compared to the *Hif1a*$^{f/f}$ + HHcy group.

Supplementary Tables

Table S1

Characteristical data for nonhyperhomocysteinemia or hyperhomocysteinemia

Groups	Nonhyperhomocysteinemia	Hyperhomocysteinemia
Sex, M/F, n/n	13/39	43/15
Age, yr	43.5±1.7	47.1±2.3
Body mass index, kg/m^2	21.80±0.22	22.45±0.26
Homocysteine, μmol/l	9.72±0.31	29.96±2.15**
Triglycerides, mmol/l	0.75±0.02	1.64±0.10**
Total cholesterol, mmol/l	4.45±0.12	4.52±0.11
HDL cholesterol, mmol/l	1.51±0.05	1.17±0.03**
LDL cholesterol, mmol/l	2.63±0.09	2.85±0.10

Data are expressed by mean ± SEM. **$P < 0.01$ compared to the nonhyperhomocysteinemia group.

Table S2. Primer sequences for qPCR

qPCR primers	Sequence (5' to 3')
m *Dgat1* u	TCCGTCCAGGGTGGTAGTG
m *Dgat1* l	TGAACAAAGAATCTTGCAGACGA
m *Dgat2* u	GCGCTACTTCCGAGACTACTT
m *Dgat2* l	GGGCCTTATGCCAGGAAACT
m *Atgl* u	CTGAGAATCACCATTCCCACATC
m *Atgl* l	CACAGCATGTAAGGGGGAGA
m *Hsl* u	CCAGCCTGAGGGCTTACTG
m *Hsl* l	CTCCATTGACTGTGACATCTCG
m *Mgl* u	AGGCGAACTCCACAGAATGTT

m *Mgl* l	ACAAAAGAGGTACTGTCCGTCT
m *Perilipin* u	GGGACCTGTGAGTGCTTCC
m *Perilipin* l	GTATTGAAGAGCCGGGATCTTTT
m *CGI58* u	TGGTGTCCCACATCTACATCA
m *CGI58* l	CAGCGTCCATATTCTGTTTCCA
m *Cd36* u	AGATGACGTGGCAAAGAACAG
m *Cd36* l	CCTTGGCTAGATAACGAACTCTG
m *Fabp1* u	ATGAACTTCTCCGGCAAGTACC
m *Fabp1* l	CTGACACCCCCTTGATGTCC
m *Fasn* u	AAGTTGCCCGAGTCAGAGAACC
m *Fasn* l	ATCCATAGAGCCCAGCCTTCCATC
m *Acc* u	GATGAACCATCTCCGTTGGC
m *Acc* l	GACCCAATTATGAATCGGGAGTG
m *Srebp1c* u	GGAGCCATGGATTGCACATT
m *Srebp1c* l	GCTTCCAGAGAGGAGGCCAG
m *Cpt1a* u	CTCCGCCTGAGCCATGAAG
m *Cpt1a* l	CACCAGTGATGATGCCATTCT
m *Ppara* u	AGAGCCCCATCTGTCCTCTC
m *Ppara* l	ACTGGTAGTCTGCAAAACCAAA
m *Acox1* u	GGGCACGGCTATTCTCACAG
m *Acox1* l	CATCAAGAACCTGGCCGTCT
m *ApoB* u	AAGCACCTCCGAAAGTACGTG
m *ApoB* l	CTCCAGCTCTACCTTACAGTTGA
m *Mttp* u	CTCTTGGCAGTGCTTTTTCTCT
m *Mttp* l	GAGCTTGTATAGCCGCTCATT
m *Ero1a u*	TTCTGCCAGGTTAGTGGTTACC
m *Ero1a l*	GTTTGACGGCACAGTCTCTTC
m *Hif1a u*	ATAGCTTCGCAGAATGCTCAGA

m *Hif1a l*	CAGTCACCTGGTTGCTGCAA
m *Atf6 u*	GACTCACCCATCCGAGTTGTG
m *Atf6 l*	CTCCCAGTCTTCATCTGGTCC
m *Atf4 u*	ATGGCGCTCTTCACGAAATC
m *Atf4 l*	ACTGGTCGAAGGGGTCATCAA
m *Chop u*	CTGGAAGCCTGGTATGAGGAT
m *Chop l*	CAGGGTCAAGAGTAGTGAAGGT
m *Bip u*	ACTTGGGGACCACCTATTCCT
m *Bip l*	ATCGCCAATCAGACGCTCC

Table S3. Primer sequences for ChIP

ChIP primers	Sequence (5’ to 3’)
Ero1a HRE1 u	GCCAGGTGTACCGGAGG
Ero1a HRE1 l	TGTCCACGCGAGCCT
Ero1a HRE2 u	TACCCCGACCCCGGAAG
Ero1a HRE2 l	CTCTGTCCACGCGAGCCT