SUPPLEMENTARY DATA

Activation of ERK1/2 ameliorates liver steatosis in leptin receptor deficient (*db/db*) mice via stimulating ATG7-dependent autophagy

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Running title: ERK1/2 regulates liver steatosis via autophagy

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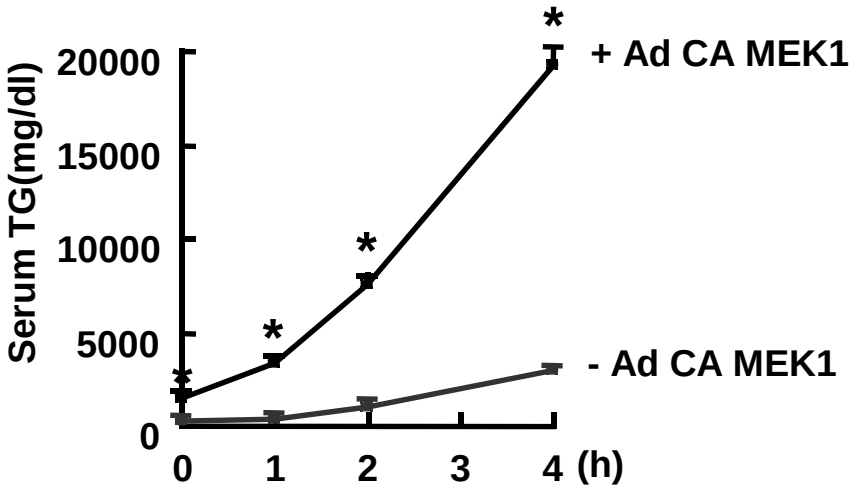
Supplementary Figure 1. VLDL secretion is enhanced by Ad-CA MEK1 in *db/db* mice. *db/db* mice were injected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) as indicated for five days then fasted for 4 h, followed by intraperitoneally injection of poloxamer 407 at 1 mg/g body weight, TG levels in serum of tail vein blood taken at different time point as indicated. Values are means ± SEMs (n=7) and analyzed by two-tailed student t-test. *: $p < 0.05$.



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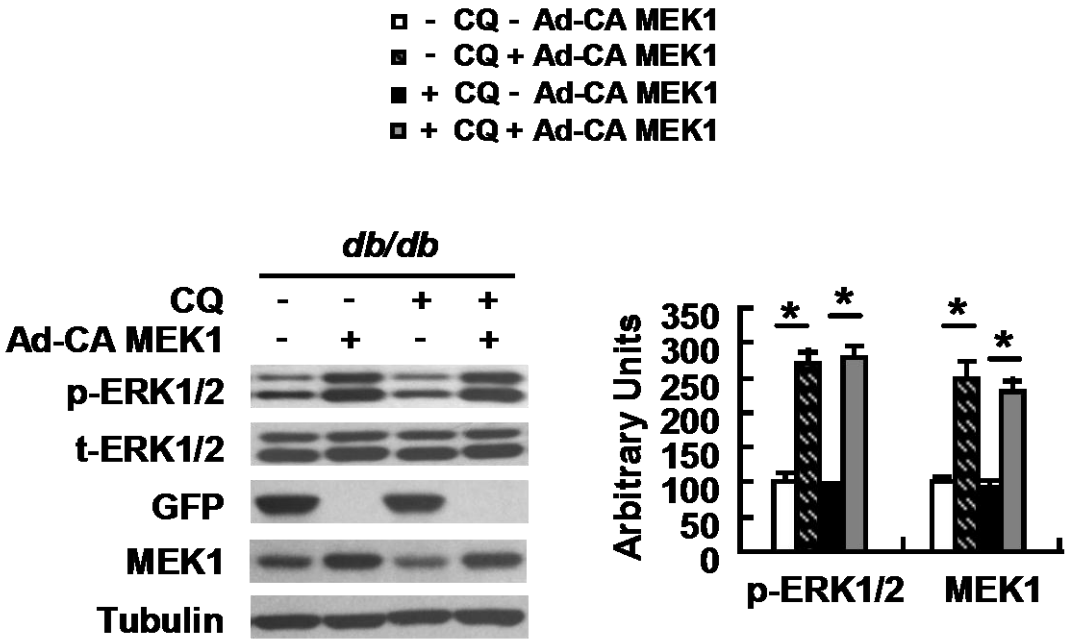
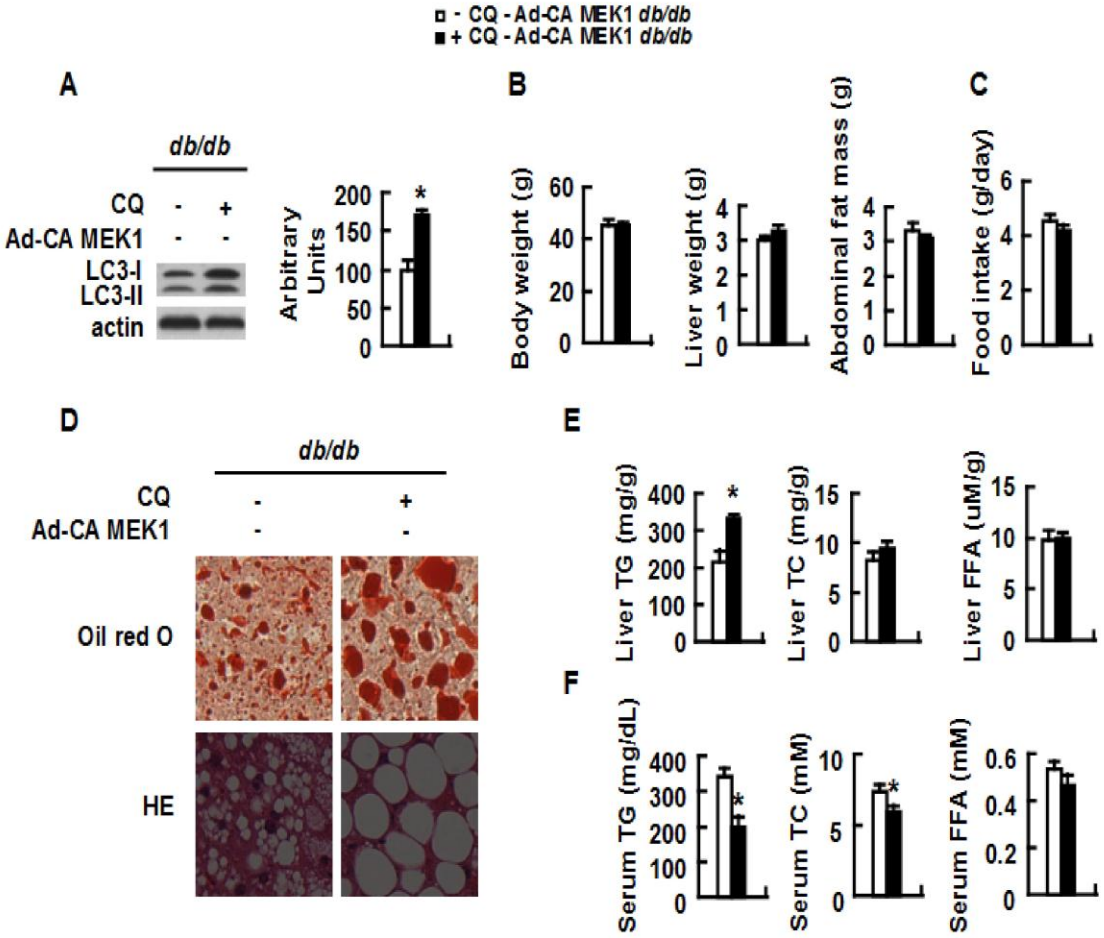
Supplementary Figure 2. The efficiency of adenoviral transfection in *db/db* mice treated with CQ (chloroquine). *db/db* mice were injected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) via tail vein injection, together with intraperitoneally injection of PBS or CQ for 14 days and livers were isolated, p-ERK1/2, t-ERK1/2, GFP and MEK1 proteins in the livers. Values are means ± SEMs (n=7) and analyzed by two-tailed student t-test. *: $p < 0.05$.



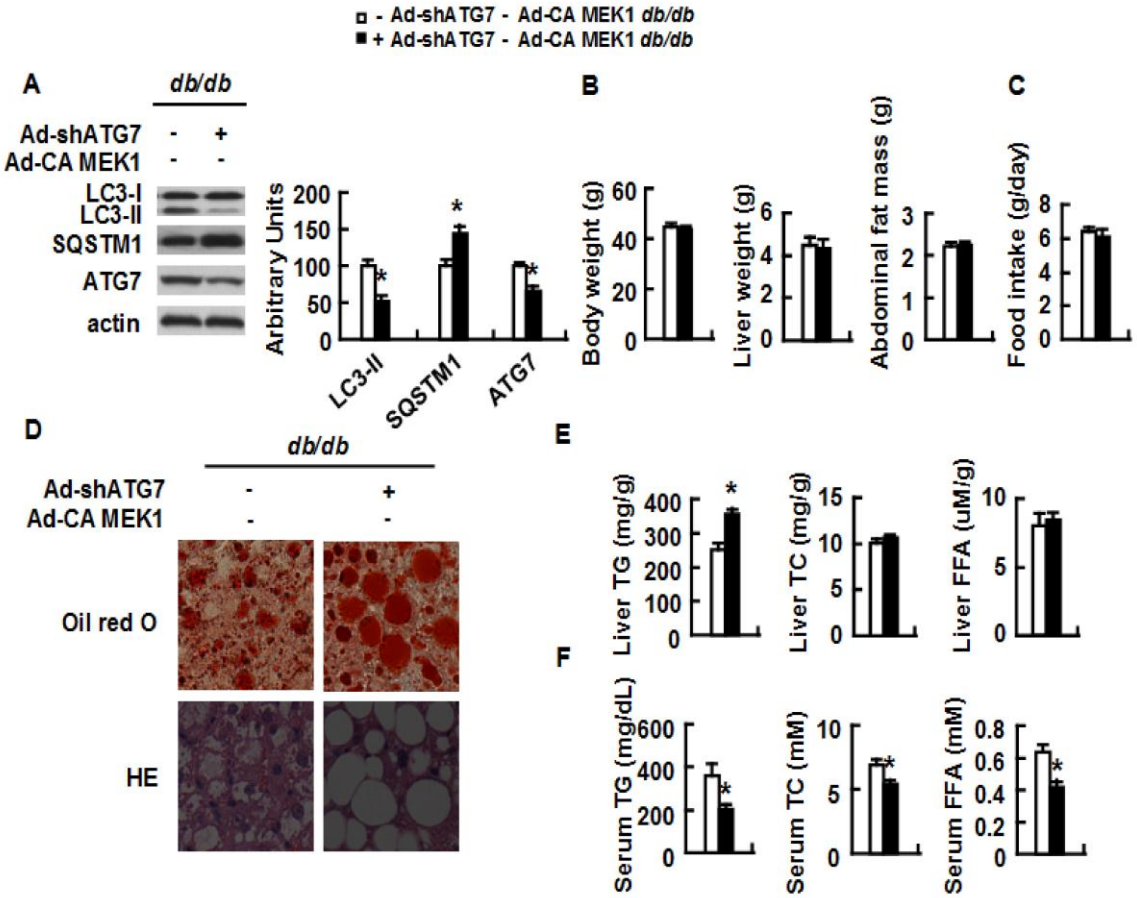
SUPPLEMENTARY DATA

Supplementary Figure 3. The effects of CQ treatment in *db/db* mice. *db/db* mice were injected with Ad-GFP (- Ad-CA MEK1) via tail vein injection together with intraperitoneally injection of PBS or CQ for 14 days and livers were isolated, LC3-II protein in the livers (A), body weight, liver weight, fat mass and food intake of mice under different treatment as indicated (B and C), Oil Red O and HE staining of representative liver sections (20×) (D), liver and serum TG, TC and FFAs (E-F). Values are means ± SEMs (n = 6-7/group) and analyzed by two-tailed student t-test. *: p <0.05.



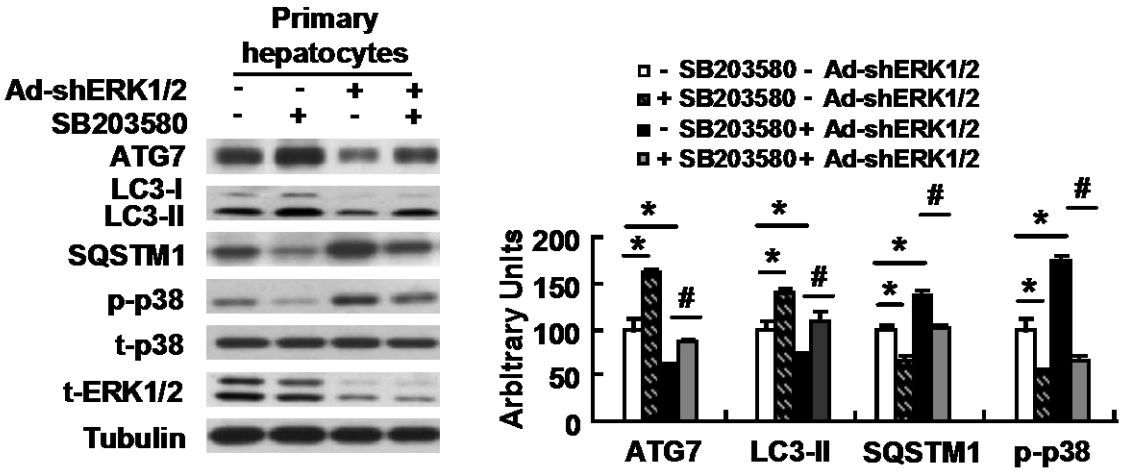
SUPPLEMENTARY DATA

Supplementary Figure 4. The effects of Ad-shATG7 in *db/db* mice. *db/db* mice were injected with Ad-GFP (- Ad-CA MEK1), Ad-scrambled (- Ad-shATG7) or Ad-shATG7 (+ Ad-shATG7), as indicated, via tail vein injection for 10 days and livers were isolated. LC3-II, SQSTM1 and ATG7 proteins in the livers (A), body weight, liver weight, fat mass and food intake of mice under different treatment as indicated (B and C), Oil Red O and HE staining of representative liver sections (20×) (D), liver and serum TG, TC and FFAs (E-F). Values are means ± SEMs (n = 6-7/group) and analyzed by two-tailed student t-test. *: p <0.05.



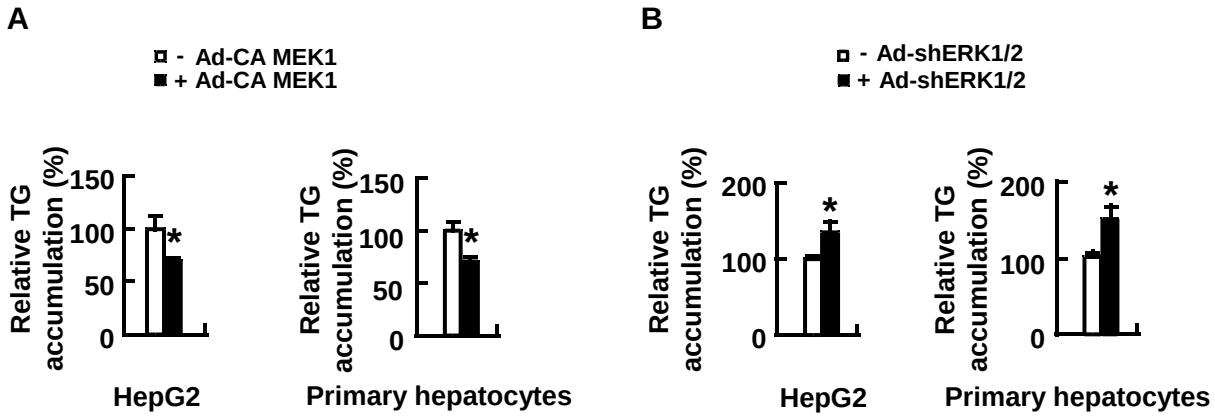
SUPPLEMENTARY DATA

Supplementary Figure 5. ERK1/2 regulates ATG7 and autophagy in a p38-dependent pathway in primary hepatocytes. ATG7, LC3-II, SQSTM1, p-p38, t-p38 and t-ERK1/2 proteins in primary hepatocytes infected with adenovirus as indicated for 48 h, followed by treatment with DMSO (- SB203580) or p38 inhibitor 15 uM SB203580 (+ SB203580) for 2 hours. Values are means ± SEMs for at least three independent in vitro experiments and analyzed by one-way ANOVA followed by the Student-Newman-Keuls (SNK) test. *: $p < 0.05$; #: $p < 0.05$.



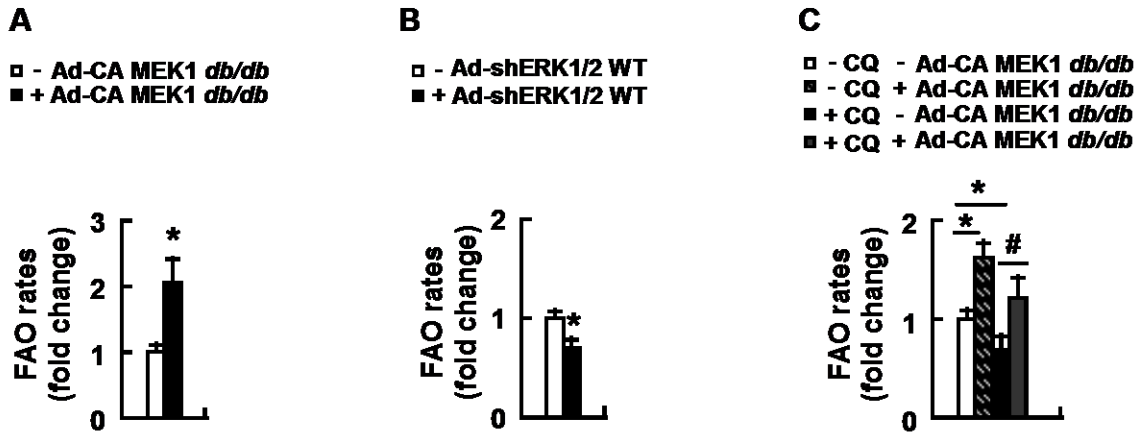
SUPPLEMENTARY DATA

Supplementary Figure 6. The effects of ERK1/2 on lipid accumulation in hepatocytes in vitro. The TG accumulation in HepG2 cells and primary hepatocytes infected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) (A), or infected with Ad-scrambled (- Ad-shERK1/2) or Ad-shERK1/2 (+ Ad-shERK1/2) (B). Values are means ± SEMs for at least three independent in vitro experiments and analyzed by two-tailed student t-test. *: $p < 0.05$.



SUPPLEMENTARY DATA

Supplementary Figure 7. The effects of ERK1/2 and autophagy on fatty acid oxidation (FAO) rates. FAO rates in primary hepatocytes of *db/db* mice infected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) (A), or WT mice infected with Ad-scrambled (- Ad-shERK1/2) or Ad-shERK1/2 (+ Ad-shERK1/2) (B), or *db/db* mice infected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) and treated with CQ (10 umol/L) for 8 hours (C). Values are means ± SEMs for at least three independent in vitro experiments and analyzed by two-tailed student t-test. *****: $p < 0.05$ in A-B, or one-way ANOVA followed by the Student-Newman-Keuls (SNK) test. *****: $p < 0.05$; $^{\#}$: $p < 0.05$ in C.



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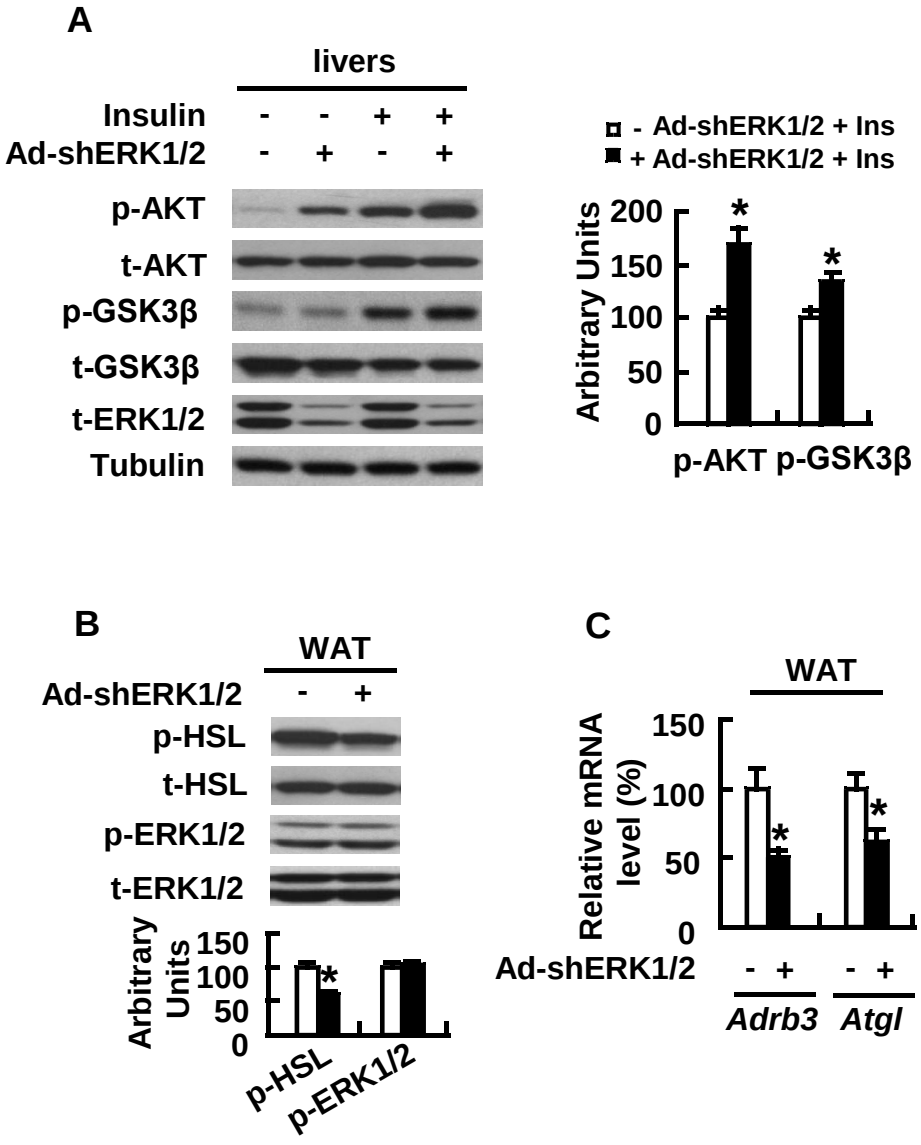
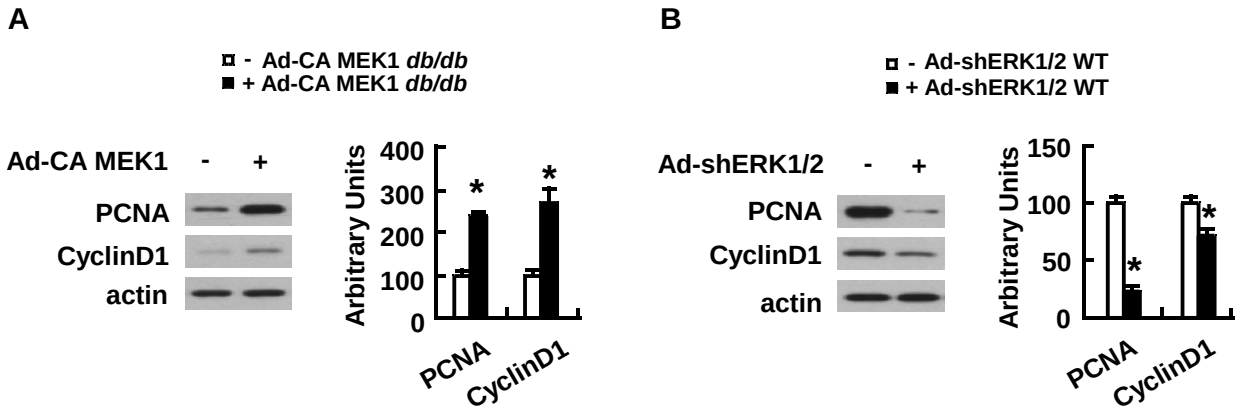
Supplementary Figure 8. The effects of ERK1/2 knockdown on Akt signaling in liver and expression of lipolysis-related regulators in white adipose tissue (WAT). WT mice were injected with Ad-scrambled (- Ad-shERK1/2) or Ad-shERK1/2 (+ Ad-shERK1/2) via tail vein injection for 10 days. Liver insulin signaling was evaluated before (- insulin) or after (+ insulin) 2U/kg insulin stimulation for 3 min. p-AKT, t-AKT, p-GSK3 β , t- GSK3 β and t-ERK1/2 proteins in the liver (A), p-HSL, t-HSL, p-ERK1/2 and t-ERK1/2 proteins, *Adrb3* and *Atgl* mRNAs in WAT (B and C), of WT mice injected with Ad-scrambled (- Ad-shERK1/2) or Ad-shERK1/2 (+ Ad-shERK1/2). Values are means ± SEMs (n = 6) and analyzed by two-tailed student t-test. *: p <0.05.



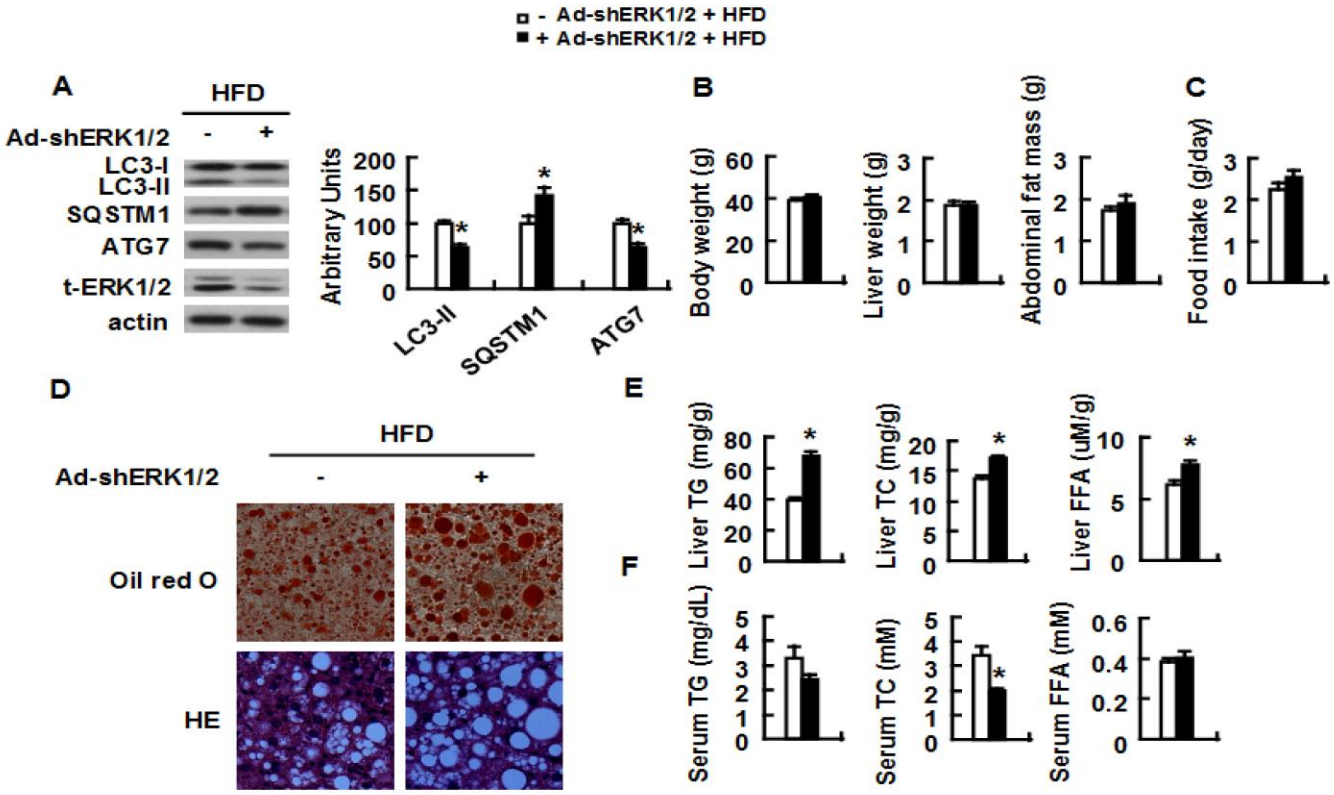
SUPPLEMENTARY DATA

Supplementary Figure 9. The effects of ERK1/2 on the expression of proliferation markers in liver. PCNA and CyclinD1 proteins in the livers of *db/db* mice injected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) (A), or WT mice injected with Ad-scrambled (- Ad-shERK1/2) or Ad-shERK1/2 (+ Ad-shERK1/2) (B). Values are means ± SEMs (n = 6-7/group) and analyzed by two-tailed student t-test. *: $p < 0.05$.



SUPPLEMENTARY DATA

Supplementary Figure 10. The effects of ERK1/2 knockdown on liver steatosis in mice fed a HFD for 3 months. HFD mice were injected with Ad-scrambled (- Ad-shERK1/2) or Ad-shERK1/ 2 (+ Ad-shERK1/2) via tail vein injection for 10 days and livers were isolated. t-ERK1/2, LC3-II, SQSTM1 and ATG7 in the livers (A), body weight, liver weight, fat mass and food intake (B-C) of HFD mice injected with Ad-scramble or Ad-shERK1/2, Oil Red O and HE staining of representative liver sections (20×) (D), liver and serum TG, TC and FFAs (E and F). Values are means ± SEMs (n = 6-7/group) and analyzed by two-tailed student t-test. *: p <0.05.



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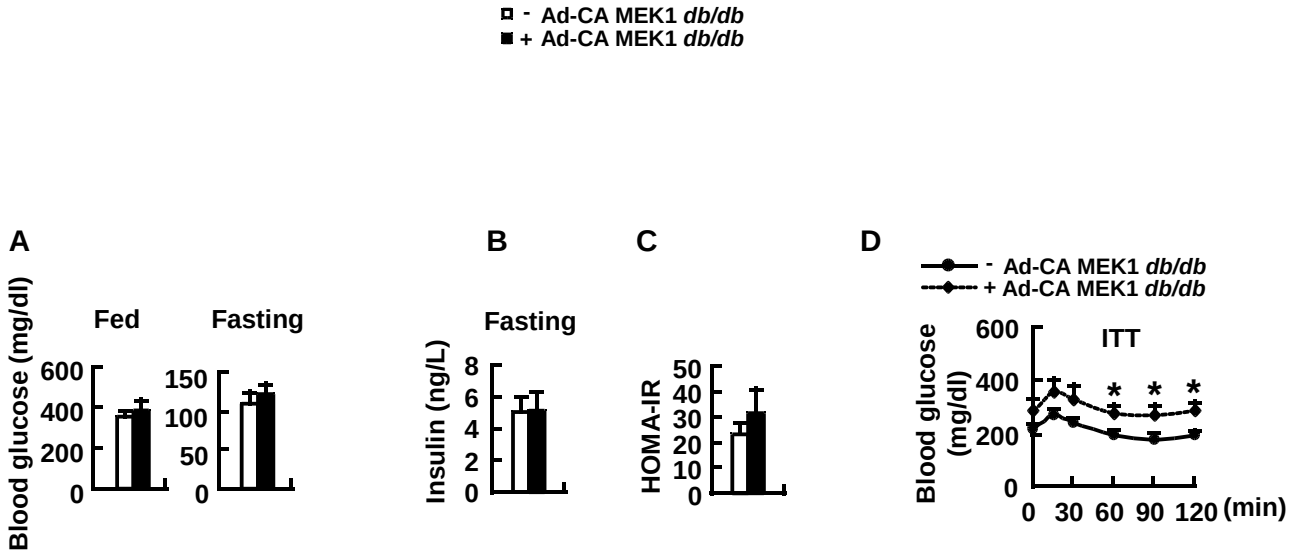
Supplementary Figure 11. The effects of ERK1/2 on insulin sensitivity in *db/db* mice. *db/db* mice were injected with Ad-GFP (- Ad-CA MEK1) or Ad-CA MEK1 (+ Ad-CA MEK1) via tail vein injection for 5 days. Fed and fasting blood glucose and fasting serum insulin levels (A and B), HOMA-IR index (C) and Insulin Tolerance Test (D). Values are means ± SEMs (n = 6-7/group) and analyzed by two-tailed student t-test. *: p <0.05.



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Supplementary Figure 12. The effects of ERK1/2 activation or knockdown on cell apoptosis. Primary hepatocytes were infected with Ad-GFP (-Ad-CA MEK1) or Ad-CA MEK1 (+Ad-CA MEK1) in a gradient for 48 hours or infected with Ad-scrambled (-Ad-shERK1/2) or Ad-shERK1/2 (+ Ad-shERK1/2) in a gradient for 72 hours. Cleaved PARP, cleaved caspas3, SQSTM1, LC3-II, p-ERK1/2, MEK1 and t-ERK1/2 proteins in primary hepatocytes infected with Ad-GFP or Ad-CA MEK1 (A). Cleaved PARP, cleaved caspas3, SQSTM1, LC3-II and t-ERK1/2 proteins in primary hepatocytes infected with Ad-scrambled or Ad-shERK1/2 (B). Values are means ± SEMs for at least three independent in vitro experiments and analyzed by two-tailed student t-test. *: $p < 0.05$.