Human umbilical cord mesenchymal stem cell-derived exosomes ameliorate liver steatosis by promoting fatty acid oxidation and reducing fatty acid synthesis

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Fig. S1

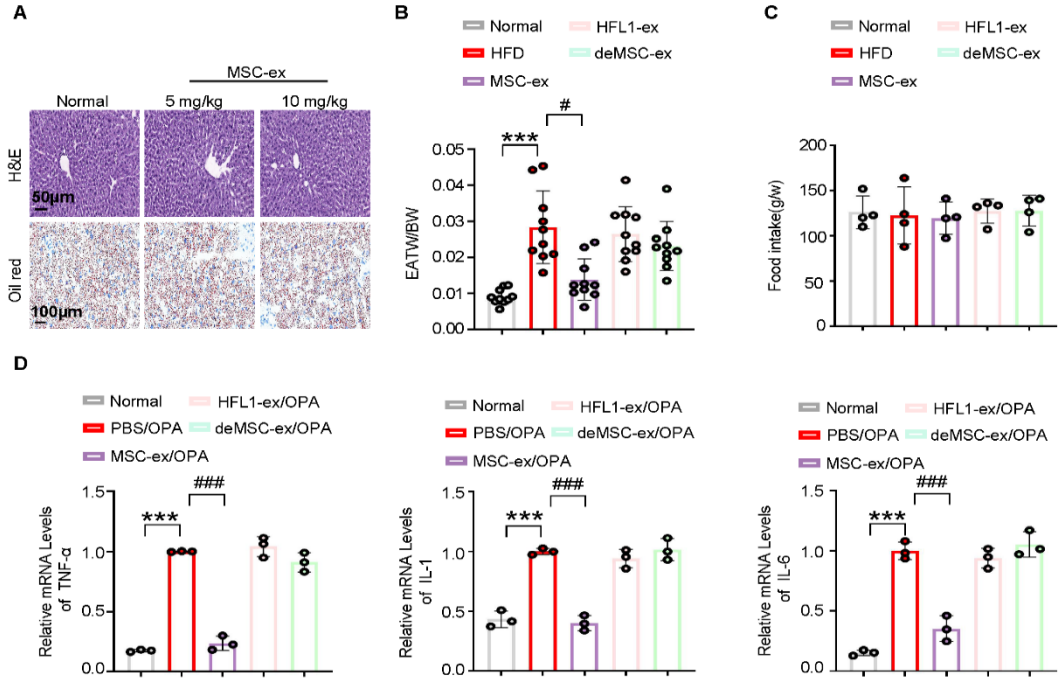

Fig. S1 MSC-ex inhibits weight gain of inguinal adipose tissue and inflammation in livers of HFD mice

(A) C57BL/6 mice were placed on high-fat diet (HFD, 40%) and administered 5 mg/kg and 10 mg/kg of MSC-ex *i.v.* From the 10th week to the 14th week of HFD feeding. Representative Images of Haematoxylin and eosin (H&E; upper; Scale bars, 50 μm) and oil red O (bottom; Scale bars, 20 μm) staining of liver sections. **(B)** Changes in the EATW/BW of mice (EATW/BW = relative weight of epididymal adipose tissue to body weight)**. (C)** The food consumption was measured by weighing the food used. **(D)** Inflammatory transcription factor expression at mrna level (n=3 in each group). Data are represented as the mean ± s.e.m. Statistical analyses was performed by a one-way ANOVA (panels **B**-**D**). *** P <0.001 versus normal chow diet group; # P <0.05, ### P <0.001 versus HFD group.

Fig. S2

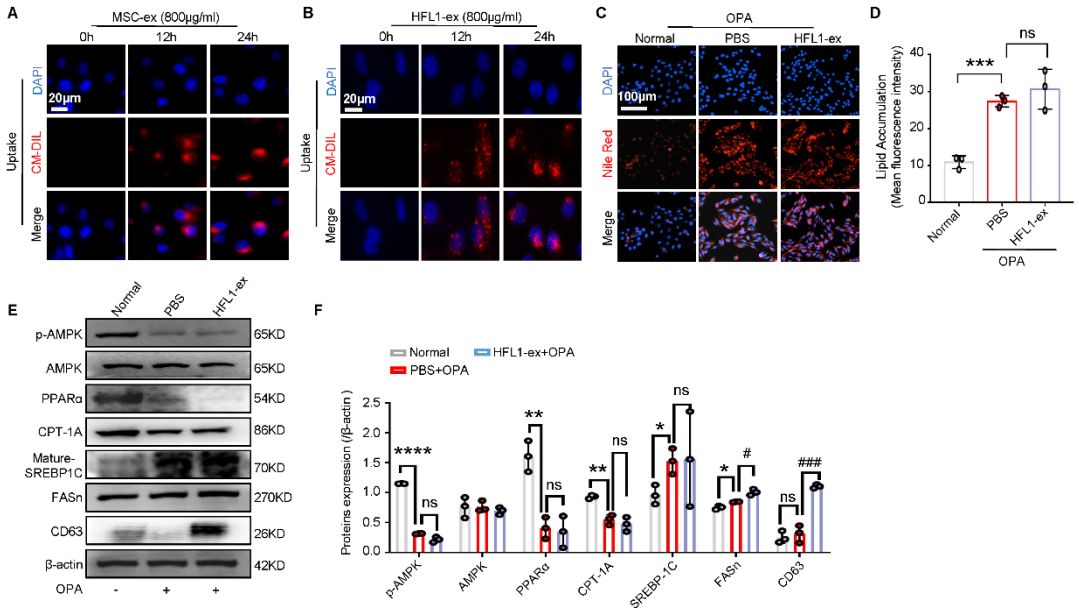

Fig. S2 HFL1-ex inactivates the AMPK signaling pathway and increases lipid accumulation (A-B) Representative fluorescent images of CM-DIL labeled MSC-ex (**A**) and HFL1-ex (**B**) in L02 cells at 0, 12, and 24 h. Scale bars, 100μm. (**C-D)** The intracellular lipid droplets in L02 cells subjected to oleate and palmitate (OPA) stimulation (2.0 mM, 2:1 ratio) in combination with HFL1-ex (800 μg/ml) or PBS treatment for 24 h were visualized by Nile red staining (**C**) and quantified by Image J for three random areas (**D**). Scale bars, 100 μm. (**E-F)** Immunoblotting of AMPK pathway proteins in L02 cells subjected to OPA stimulation (2.0 mM, 2:1 ratio) combined with HFL1-ex (800 μg/ml) or PBS treatment for 24 h (**E**) and quantification of the results (**F**). Data are represented as the mean ± s.e.m. Statistical analyses was performed by a one-way ANOVA (**D** and **F**). * *P* <0.05, ** *P* <0.01, *** *P* <0.001, **** *P* <0.0001 versus normal group; # *P* <0.05, ### *P* <0.001 versus PBS group.

Fig. S3

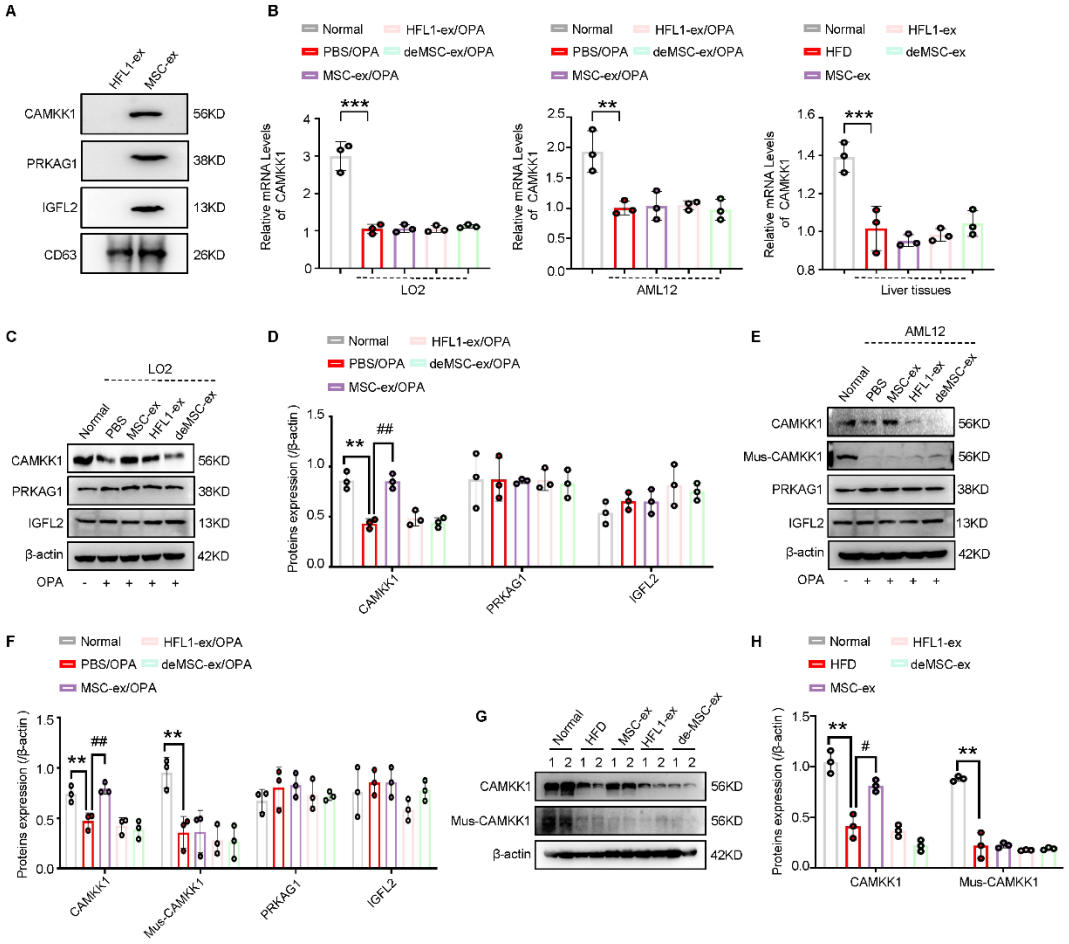

Fig. S3 Expression of CAMKK1 in HFL1-ex/MSC-ex, HFL1-ex/MSC-ex treated hepatocytes and livers

(**A)** CAMKK1, PRKAG1, and IGFL2 expression in MSC-ex and HFL1-ex were examined by immunoblotting. **(B)** QRT-PCR analyses of CAMKK1 mRNA expression (n=3 biological replicates per group). ** *P* <0.01, *** *P* <0.001 versus normal group. **(C-D)** The expression of CAMKK1, PRKAG1, and IGFL2 in L02 cells was detected by immunoblotting (**C**) and quantified (**D**). ** *P* <0.01 versus normal group; ## *P* <0.01 versus PBS group. **(E-F)** The expression of CAMKK1, PRKAG1, and IGFL2 in AML12 cells was detected by immunoblotting (**E**) and quantified (**F**). ** *P* <0.01 versus normal group; ## *P* <0.01 versus PBS group. (**G-H)** Immunoblotting analyses of CAMKK1 and

Mus-CAMKK1 proteins in mice placed on a HFD diet for 10 weeks followed by 10 mg/kg MSC-ex or 10 mg/kg HFL1-ex or MSC-ex-free conditional medium supernatant (deMSC-ex) and PBS treatment for 4 weeks (**G**) and quantification of the results (**H**). ** *P* <0.01 versus normal chow diet group; # *P* <0.05 versus HFD group. Data are represented as the mean ± s.e.m. Statistical analyses was performed by a one-way ANOVA (**B, D, F,** and **H**).

Fig. S4

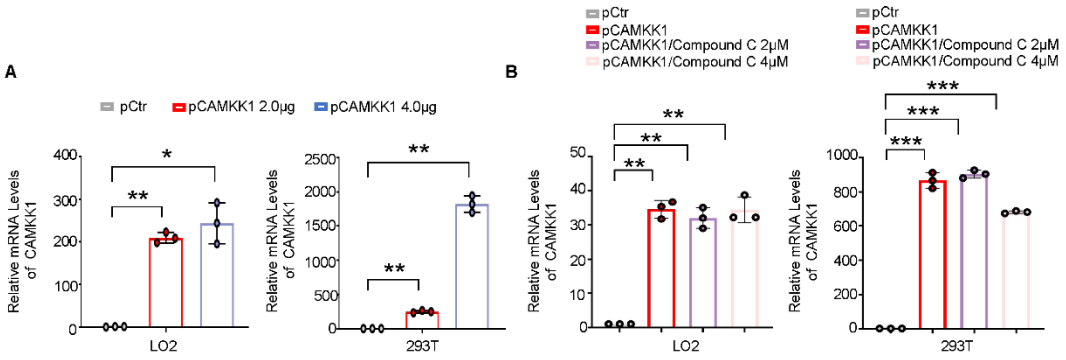

Fig. S4 CAMKK1 expression is not affected by Compound-C

(A) QRT-PCR analyses of CAMKK1 mRNA expression in L02 and 293T cells transfected with pCAMKK1 (2 μg and 4 μg) or empty plasmid (pCtr) and normalized to β-actin expression. n=3 biological replicates per group. (**B)** QRT-PCR analyses of CAMKK1 mRNA expression in L02 and 293T cells transfected with pCAMKK1 (4 μg) or pCtr with or without Compound-C (2 μM and 4 μM) treatment for 24 h. Expression was normalized to β-actin expression. n=3 biological replicates per group. Data are represented as the mean ± s.e.m. Statistical analyses by unpaired two-tailed student's t-test. * P <0.05, ** P <0.01, *** P <0.001 versus pCtr group.

Fig. S5

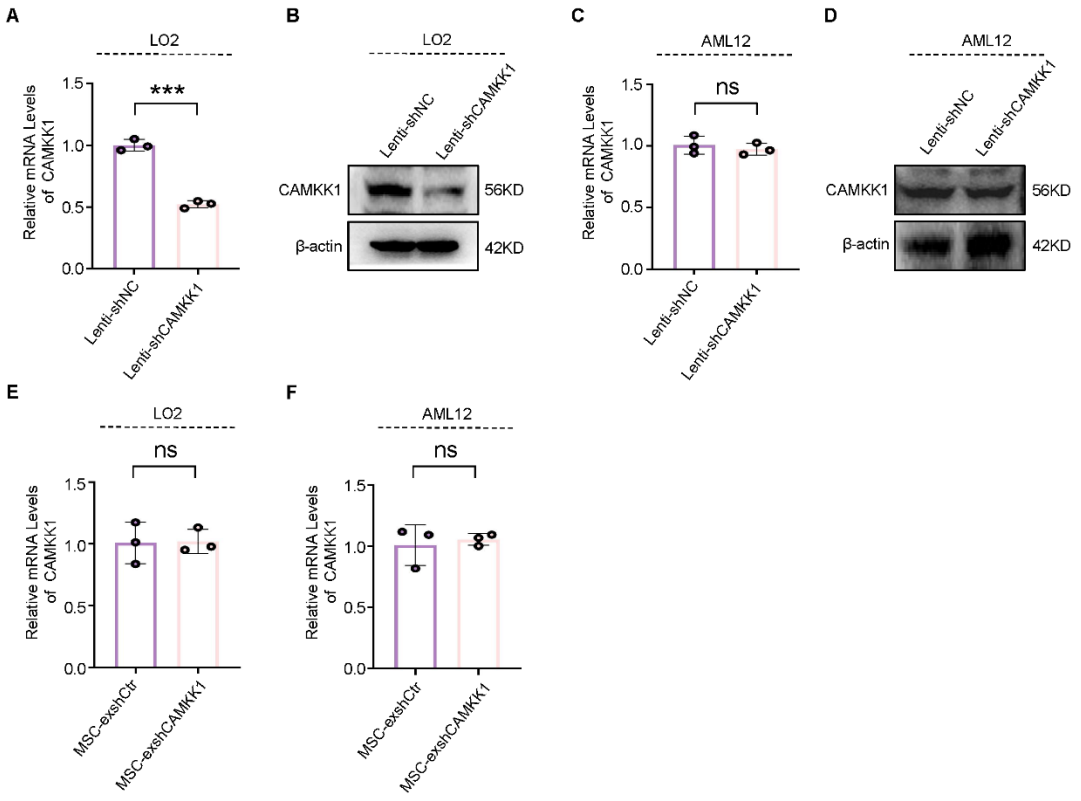

Fig. S5 Identification of CAMKK1 Knockdown in Lenti-shCAMKK1 transfected hepatocytes and MSC-ex^{shCAMKK1} treated hepatocytes (A) QRT-PCR analyses of CAMKK1 mRNA expression in L02 cells treated with recombinant lentivirus (pLKO) (pLKO.1-GFP-Puro-shCAMKK1 or pLKO.1-Puro-shRNA, 15MOI) for 72 h. n=3 in each group; *** *P* <0.001 versus Lenti-shNC group. **(B)** The expression of CAMKK1 in L02 cells was detected by immunoblotting. **(C)** QRT-PCR analyses of CAMKK1 mRNA expression in AML12 cells treated with recombinant lentivirus (pLKO) (pLKO.1-GFP-Puro-shCAMKK1 or pLKO.1-Puro-shRNA, 15MOI) for 72 h. n=3 in each group. **(D)** The expression of CAMKK1 in AML12 cells was detected by immunoblotting. **(E)** QRT-PCR analyses of CAMKK1 mRNA expression in L02 cells treated with MSC-ex^{shCtr} (800 μg/ml) or MSC-ex^{shCAMKK1} (800 μg/ml) for 24 h. n=3 in each group. **(F)** QRT-PCR

analyses of CAMKK1 mRNA expression in AML12 cells treated with MSC-ex^{shCtr} (800 μg/ml) or MSC-ex^{shCAMKK1} (800 μg/ml) for 24 h. n=3 in each group. Data are represented as the mean ± s.e.m. Statistical analyses by unpaired two-tailed student's t-test.

Fig. S6

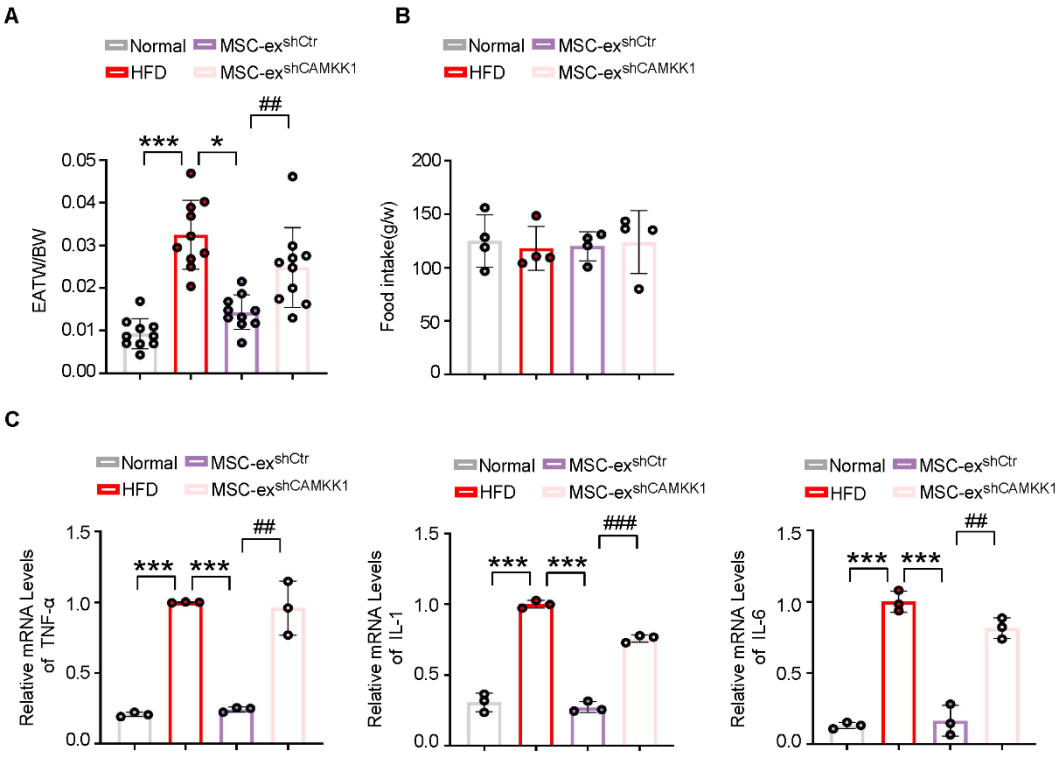

Fig. S6 MSC-ex^{shCAMKK1} reverses MSC-ex^{shCtr} decreased hepatic inflammation in the livers from HFD mice

(A) C57BL/6 mice were placed on a high-fat diet (HFD, 40%) and administered 10 mg/kg of MSC-ex^{shCtr} or MSC-ex^{shCAMKK1} *i.v.* from the 10th week to the 14th week of HFD feeding. As a control, the same volume of PBS was injected**.** Changes in the EATW/BW of mice (EATW/BW = relative weight of epididymal adipose tissue to body weight). **(B)** The food consumption was measured by weighing the food used**. (C)** Inflammatory transcription factor expression at mRNA level (n=3 in each group). Data are represented as the mean ± s.e.m. Statistical analyses was performed by a one-way ANOVA (panels **A**-**C**). * *P* <0.05, *** *P* <0.001 versus HFD group; ## *P* <0.01, ### *P* <0.001 versus MSC-ex^{shCtr} (10 mg/kg) group**.**