Supplementary Material

Kaempferol alleviates steatosis and inflammation during early non-alcoholic steatohepatitis association with LXRα-LPCAT3 signaling pathway

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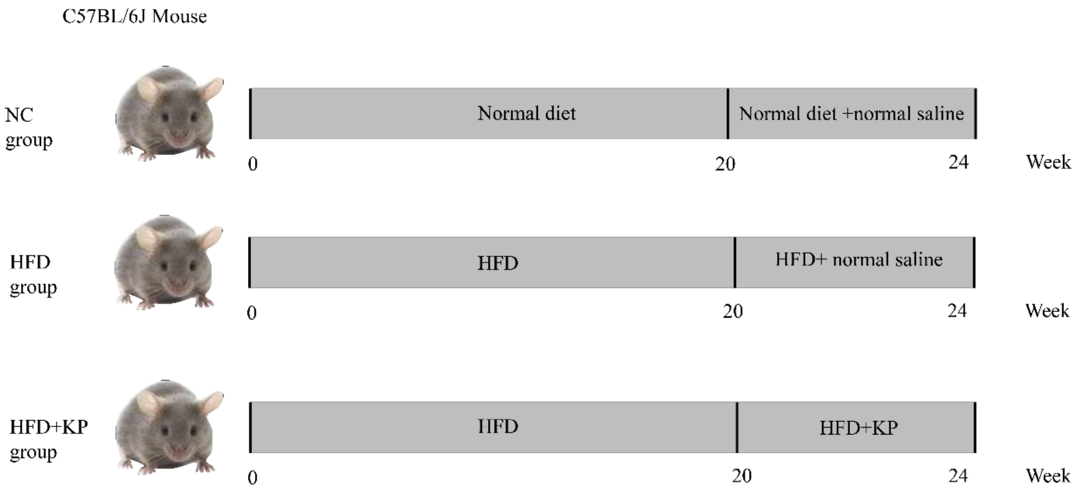
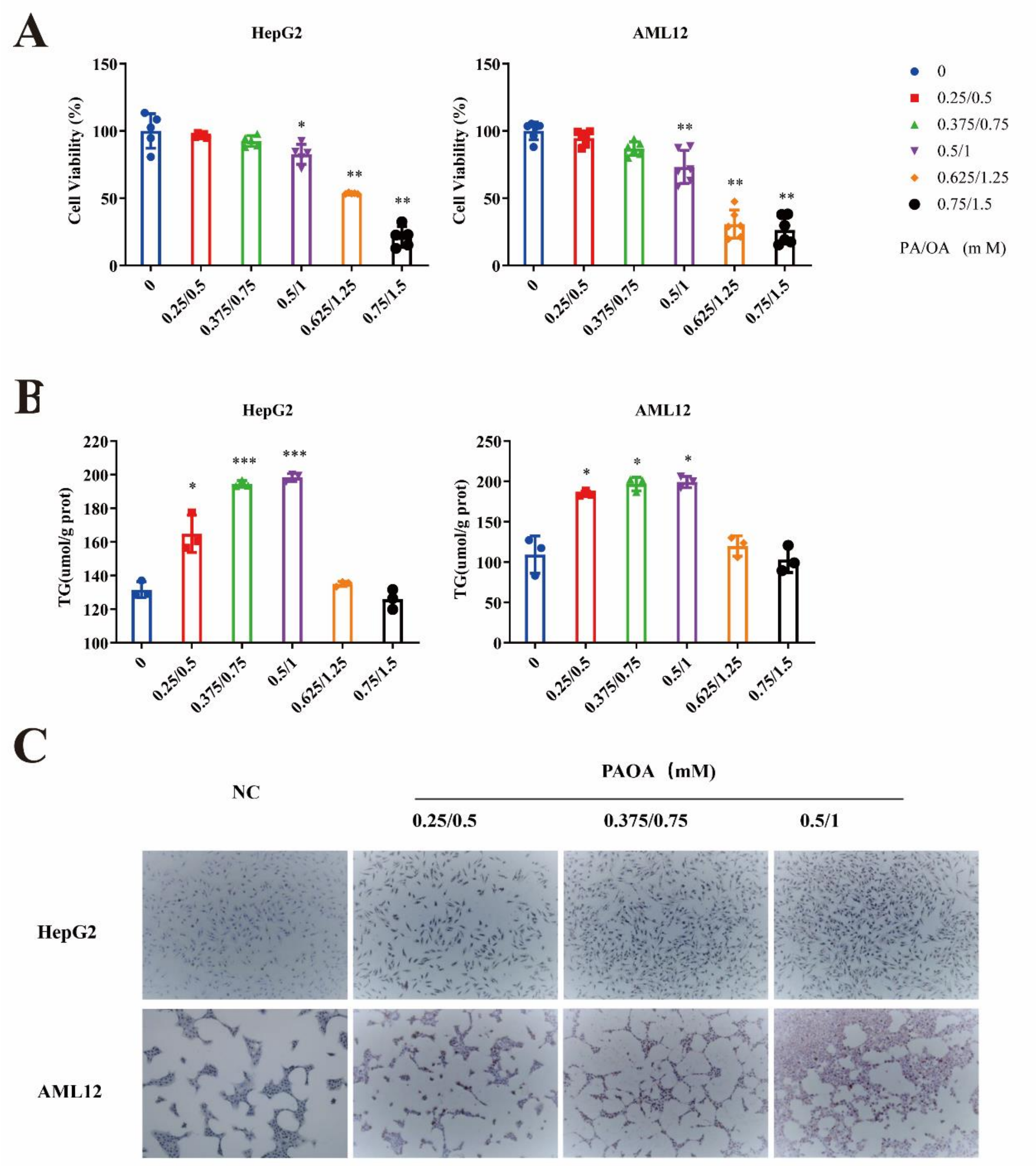


Supplementary Figure 1. Scheme figure with experimental approach of the establishment of high-fat diet induced NASH C57BL/6J mouse model. The mice in group NC were fed normal diet, and the mice in group HFD and HFD+KP were fed HFD for 24 weeks. At the beginning of the 21st week, KP (4mg/ml) was gavaged in the HFD + KP group at a dose of 0.5ml/100g on the basis of HFD feeding. At the same time, mice in NC and HFD groups received equal volume of normal saline.

NASH: non-alcoholic steatohepatitis; NC: normal control; HFD: high fat diet; KP: kaempferol.

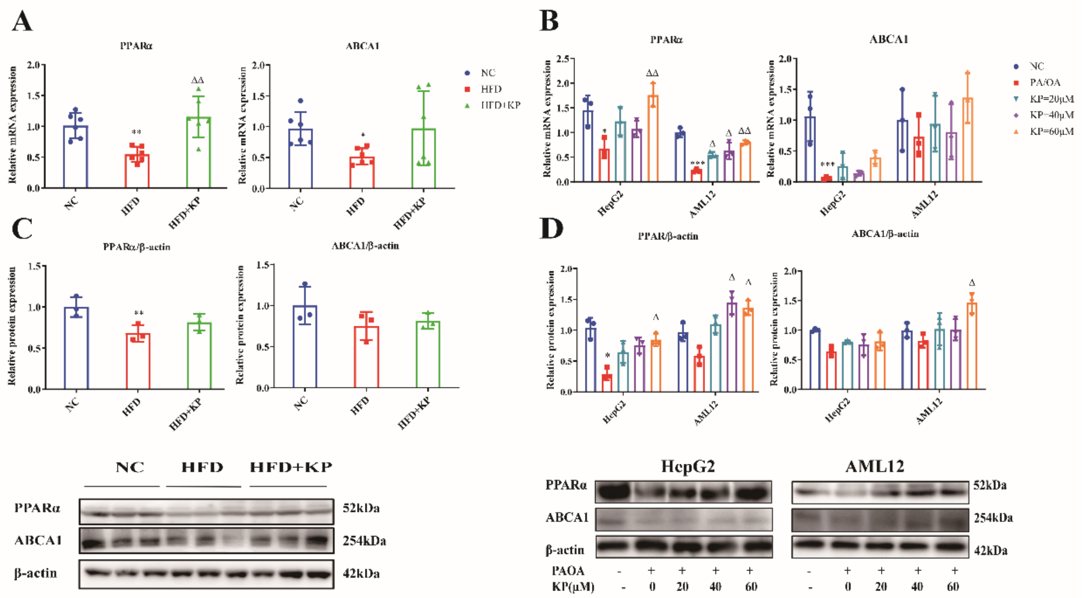


Supplementary Figure 2. The effect of different concentrations of PA/OA intervention on the cell viability, TG content and lipid droplet formation of HepG2 and AML12 cells. A: Changes in the survival rate; B: Changes of TG content; C: Oil red O staining in HepG2 and AML12 cells treated with different concentrations of PA/OA (x100).

NC: normal control group; PA/OA: PA/OA group.

Blue: PA/OA=0mM; Red: PA/OA=0.25/0.5mM; Green: PA/OA=0.375/0.75mM; Purple: PA/OA=0.5/1mM; Orange: PA/OA=0.625/1.25mM; Black: PA/OA=0.75/1.5mM.

Compared with group PA/OA=0mM, *, P<0.05, **, P<0.01, ***, P<0.001.



Supplementary Figure 3. KP regulated lipid metabolism disorders both in vivo and in vitro. A: PPARα and ABCA1 mRNA levels in vivo; B: PPARα and ABCA1 mRNA levels in vitro; C: The protein expression of PPARα and ABCA1 in vivo; D: The protein expression of PPARα and ABCA1 in vitro. Quantification of mRNA and protein level expression was normalized to β-actin levels. PPARα：peroxisome proliferator-activated receptor α; ABCA1：ATP binding cassette transporter A1.

NC: normal control group; HFD: HFD group; HFD+KP: HFD+KP group; PA/OA: PA/OA group; KP: KP group; KP=20μM: KP low concentration group; KP=40μM: KP medium concentration group; KP=60μM: KP high concentration group. Compared with group NC, *, P<0.05, **, P<0.01. In Supplementary Figures 3A and C, compared with group HFD, △, P<0.05, △△, P<0.01. In Supplementary Figures 3B and D, compared with group PA/OA, △, P<0.05, △△, P<0.01.

Supplementary Table 1. Primers for real-time qPCR.

Genes	Sequence (5'to 3')	Base pair (bp)
mActin	mForwardPrimer 5'-GAGACCTTCAACACCCCAGC-3'	263
	mReverse Primer 5'-ATGTCACGCACGATTTCCC-3'	
mLXRα	mForwardPrimer 5'-CCAAAATGCTGGGGAACG-3'	125
	mReverse Primer 5'-GCGTGCTCCCTTGATGACA-3'	
mLPCAT3	mForwardPrimer 5'-CCCCACATCACAGACGACTATC-3'	192
	mReverse Primer 5'-TCTCACGGTCCCATTTTCATC-3'	
mPERK	mForwardPrimer 5'-GCTCAAAGACGAAAGCACAGAC-3'	153
	mReverse Primer 5'-CCCACCGAGAAAGACCGAC-3'	
meIF2α	mForwardPrimer 5'-ACCTGGATACGGTGCCTACG-3'	145
	mReverse Primer 5'-TCGAATTTTGACCGCTTGTG-3'	
mATF4	mForwardPrimer 5'-ATGGAGCAAAACAAGACAGCA-3'	180
	mReverse Primer 5'-TGCCTTACGGACCTCTTCTATC-3'	
mCHOP	mForwardPrimer 5'-AAACCTTCACTACTCTTGACCCTG-3'	184
	mReverse Primer 5'-GGGCACTGACCACTCTGTTTC-3'	
mATF6	mForwardPrimer 5'-AAAGTCCCAAGTCCAAAGCG-3'	116
	mReverse Primer 5'-CTAGGTTTCACTCTTCGGGATTC-3'	
mGRP78	mForwardPrimer 5'-TTGTCCCCTTACACTTGGTATTG-3'	170
	mReverse Primer 5'-TGTCTTTTGTTAGGGGTCGTTC-3'	
mIRE1α	mForwardPrimer 5'-TTTGTTTTCCGAGGACAGTTTG-3'	136
	mReverse Primer 5'-AGAAGTAGCGAAGCACGTTGG-3'	
mXBP1	mForwardPrimer 5'-CAAGTGGTGGATTTGGAAGAAG-3'	119
	mReverse Primer 5'-TCCATTCCCAAGCGTGTTC-3'	
mPPARα	mForwardPrimer 5'-CCTGAAAGATTCGGAAACTGC-3'	139
	mReverse Primer 5'-GACAAAAGGCGGGTTGTTG-3'	
mABCA1	mForwardPrimer 5'-CCTGTGGGGGCATCATCTAC-3'	178
	mReverse Primer 5'-TCCCATTGGACCCCGATAC-3'	
mMCP-1	mForwardPrimer 5'-GCTGACCCCAAGAAGGAATG-3'	184
	mReverse Primer 5'-TTGAGGTGGTTGTGGAAAAGG-3'	
mCCL5	mForwardPrimer 5'-ACCACTCCCTGCTGCTTTG-3'	129
	mReverse Primer 5'-CACTTGGCGGTTCCTTCG-3'	
mCXCL10	mForwardPrimer 5'- AAGTGCTGCCGTCATTTTCTG-3'	160
	mReverse Primer 5'- GGATAGGCTCGCAGGGATG-3'	
mTNF-α	mForwardPrimer 5'- CCCTCCAGAAAAGACACCATG-3'	183
	mReverse Primer 5'- CACCCCGAAGTTCAGTAGACAG-3'	
mIL6	mForwardPrimer 5'- AAATGATGGATGCTACCAAACTG-3'	137
	mReverse Primer 5'- CTCTGGCTTTGTCTTTCTTGTTATC-3'	
hActin	hForwardPrimer 5'- CTCCATCCTGGCCTCGCTGT-3'	268
	hReverse Primer 5'- GCTGTCACCTTCACCGTTCC-3'	
hLXRα	hForwardPrimer 5'-GAAACTGAAGCGGCAAGAGG-3'	152
	hReverse Primer 5'-AGCGCCGGTTACACTGTTG-3'	

hLPCAT3	hForwardPrimer 5’-GAAAAGGGCAAGGCAAAGTG-3’	114
	hReverse Primer 5’- CCAGGCGTTGGTGTTGATG-3’	
hPERK	hForwardPrimer 5’-CTCGGGAAAAGGTAATGCG-3’	119
	hReverse Primer 5’-ATCCATCTTTTCTTGCCACTTC-3’	
heIF2α	hForwardPrimer 5’-GATTGAGGAAAAGAGGGGTGTG-3’	154
	hReverse Primer 5’- TTTGGCTTCCATTTCTTCTGC-3’	
hATF4	hForwardPrimer 5’-CTCAGCACAGCCCCTCTACC-3’	141
	hReverse Primer 5’- CCAGTTTCTCACCCTTTACTTTTG-3’	
hCHOP	hForwardPrimer 5’-AACCAGGAAACGGAAACAGAG-3’	192
	hReverse Primer 5’- TTCACCATTCGGTCAATCAGA-3’	
hATF6	hForwardPrimer 5’-CTCAGCACAGCCCCTCTACC-3’	125
	hReverse Primer 5’-CCAGTTTCTCACCCTTTACTTTTG-3’	
hGRP78	hForwardPrimer 5’-GTCCTATGTCGCCTTCACTCC-3’	137
	hReverse Primer 5’-GCACAGACGGGTCATTCCAC-3’	
hIRE1α	hForwardPrimer 5’-GGAATTACTGGCTTCTGATAGGAC-3’	177
	hReverse Primer 5’-GTGCGTTTTCTGAAGTCTGGTC-3’	
hXBP1	hForwardPrimer 5’-ATGGATTCTGGCGGTATTGAC-3’	175
	hReverse Primer 5’-GAGAAAGGGAGGCTGGTAAGG-3’	
hPPARα	hForwardPrimer 5’- GCGAACGATTCGACTCAAGC-3’	119
	hReverse Primer 5’-ACATCCCGACAGAAAGGCAC-3’	
hABCA1	hForwardPrimer 5’-CTGCTAATTGCCAGACGGAG-3’	143
	hReverse Primer 5’-TACATCCAGGGCTGAAGTTCC-3’	
hMCP-1	hForwardPrimer 5’- CCTTCTGTGCCTGCTGCTC -3’	171
	hReverse Primer 5’- GCTTCTTTGGGACACTTGCTG -3’	
hCCL5	hForwardPrimer 5’- ACCACACCCTGCTGCTTTG -3’	168
	hReverse Primer 5’- GATGTACTCCCGAACCCATTTC -3’	
hCXCL10	hForwardPrimer 5’- TATTCCTGCAAGCCAATTTTG -3’	129
	hReverse Primer 5’- CTTTCCTTGCTAACTGCTTTCAG -3’	
hTNF-α	hForwardPrimer 5’- CACGCTCTTCTGCCTGCTG -3’	129
	hReverse Primer 5’- GGCTTGTCACTCGGGGTTC -3’	
mIL6	hForwardPrimer 5’- AAAGCAGCAAAGAGGCACTG -3’	137
	hReverse Primer 5’-TACCTCAAACTCCAAAAGACCAG -3’	