Supplementary Information

MNK1 and MNK2 mediate adverse effects of high-fat feeding in distinct ways

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Table S1
Sequence of primers used in RT-qPCR

Gene	Forward	Reverse
Mknk 1 (MNK1)	GATTCCTCTGAGACTCCAAGTTAA	ACGCTTCTTCTTCCTCCTCTT
Mknk 2 (MNK2)	CCAGTGCCAGGGACATAGG	GCCACGCATCTTCTCAAACA
ACC1	GCAGTCTCCCAACTCCTACG	CCACCATCACTCAGCCGAT
***ATP citrate lyase* (*ACLY*)**	CAGACCTATGACTATGCCAAGAC	CAATGCTGCCTCCAATGATGA
GLUT4	AAAAGTGCCTGAAACCAGAG	TCACCTCCTGCTCTAAAAGG
CPT1a	AAACCTATTCGTCTTCTGGGATC	ATGTGCCTGCTGTCCTTGA
LXRα	ATCGCCTTGCTGAAGACCTCTG	GATGGGGTTGATGAACTCCACC
CCR2	ATTCTCCACACCCTGTTTCG	GATTCCTGGAAGGTGGTCAA
CCR5	CGAAAACACATGGTCAAACG	GTTCTCCTGTGGATCGGGTA
SREBP1c	GGAGCCATGGATTGCACATT	CCTGTCTCACCCCCAGCATA
HSL	CTC CAC ATG CCCCTC TAC AC	CAGAGCGCAAGCCACAAG
NRF2	TCCGCCAGCTACTCCAGGTTGG	TGGGCCCTGATGAGGGGCAGTG
C/EBPα	GAACAGCAACGAGTACCGGGTA	GCCATGGCCTTGACCAAGGAG
PPARγ	TCCGTGATGGAAGACCACTCGCAT	CAGCAACCATTGGGTCAGCTCTTG
Heme oxygenase 1 (HO-1)	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
CD36	TCATGCCAGTCGGAGACATGCTTA	AACTGTCTGTACACAGTGGTGCCT
IL-6	TCCATCCAGTTGCCTTCTTG	GGTCTGTTGGGAGTGGTATC
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
***TNF*-α**	TCTCAGCCTCTTCTCATTCCTGCT	AGAACTGATGAGAGGGAGGCCATT
MMP12	TTGGATTATTGGAATGCTGC	GCACATTTTGATGAGGCAGA
CD68	TTCTGCTGTGGAAATGCAAG	AGAGGGGCTGGTAGGTTGAT
CD206	CAGGTGTGGGCTCAGGTAGT	TGTGGTGAGCTGAAAGGTGA
STAT6	CTGGGGTGGTTTCCTCTTG	TGCCCGGTCTCACCTAACTA
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
MHCII	GACGCTCAACTTGTCCCAAAAC	GCAGCCGTGAACTTGTTGAAC
ADAM8	AGGATATTCAGCAGGTGTAGCAA	TGCTAAAGGTATAGCAGGAGTCG
B2M	CTGCTACGTAACACAGTTCCACCC	CATGATGCTTGATCACATGTCTCG

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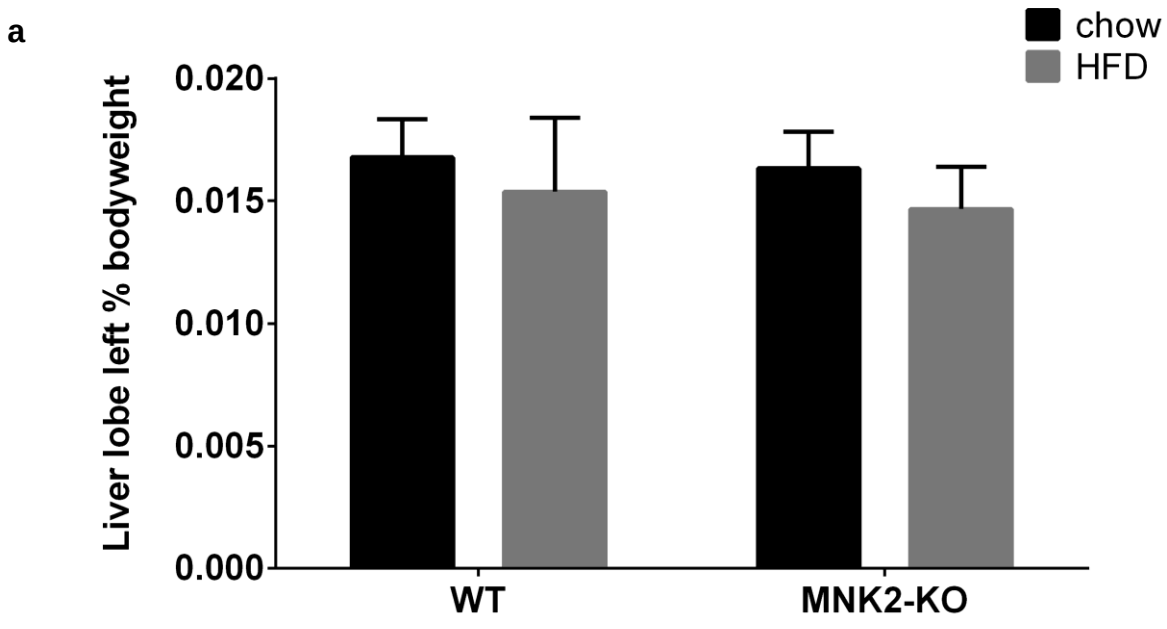
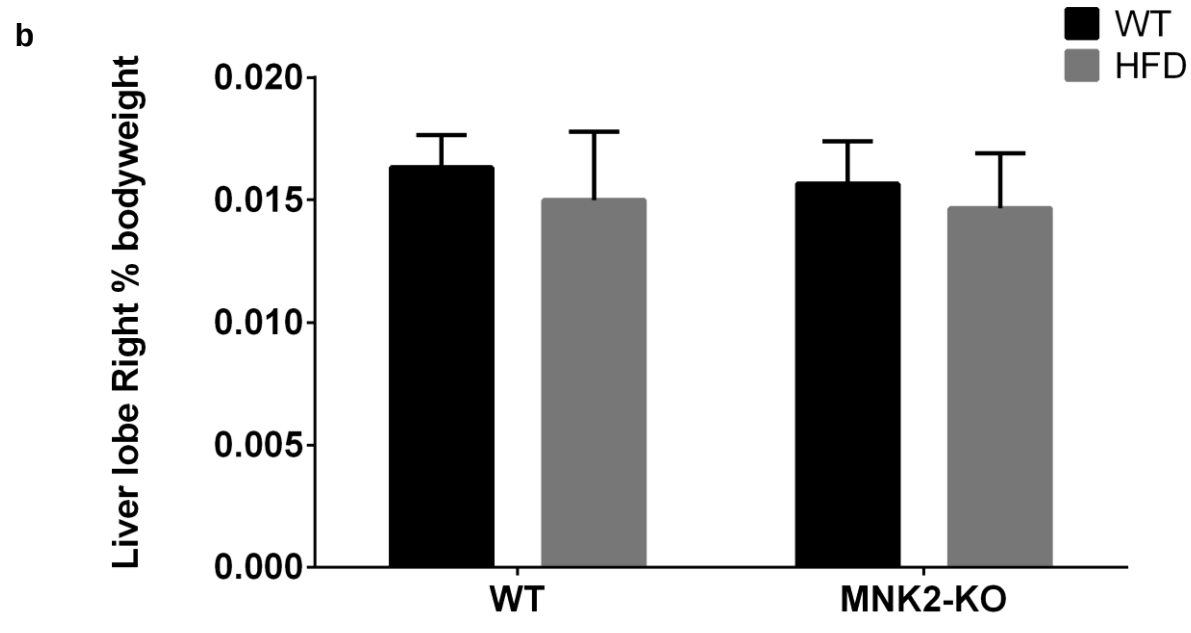
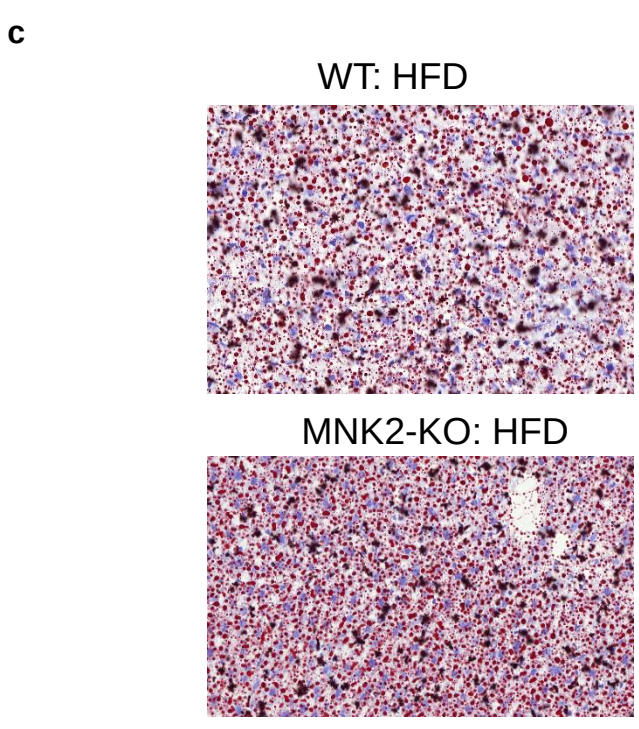
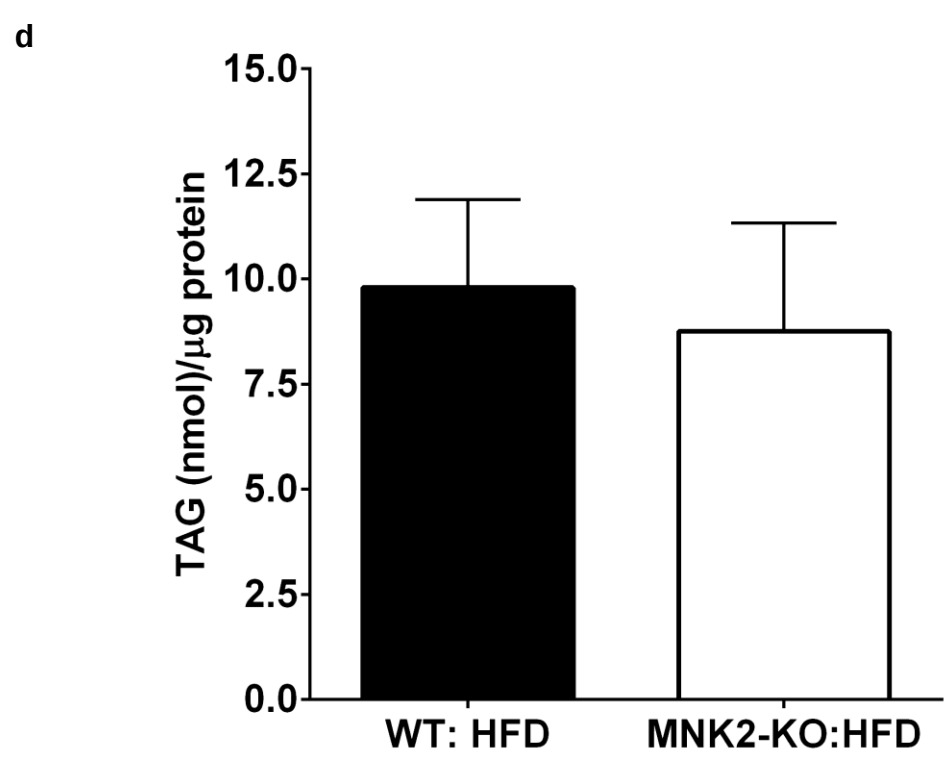


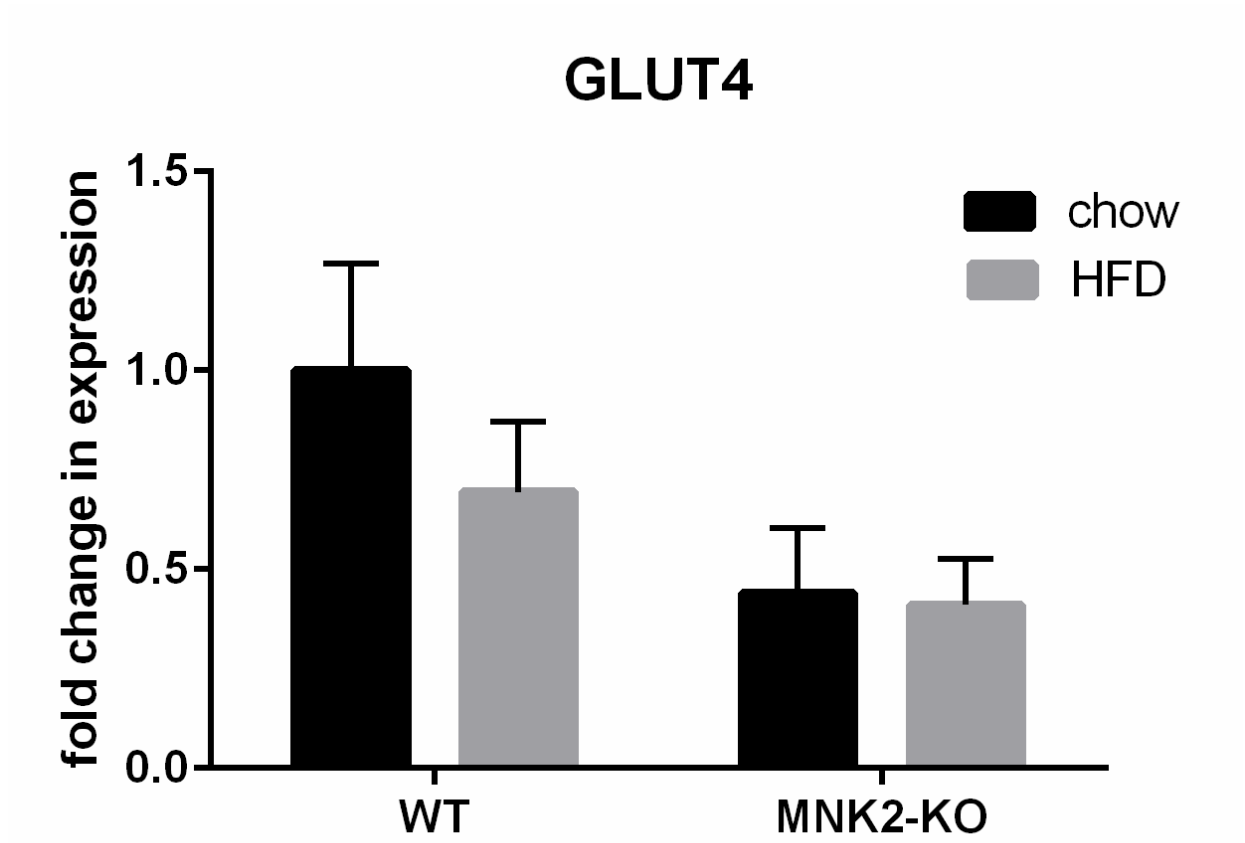






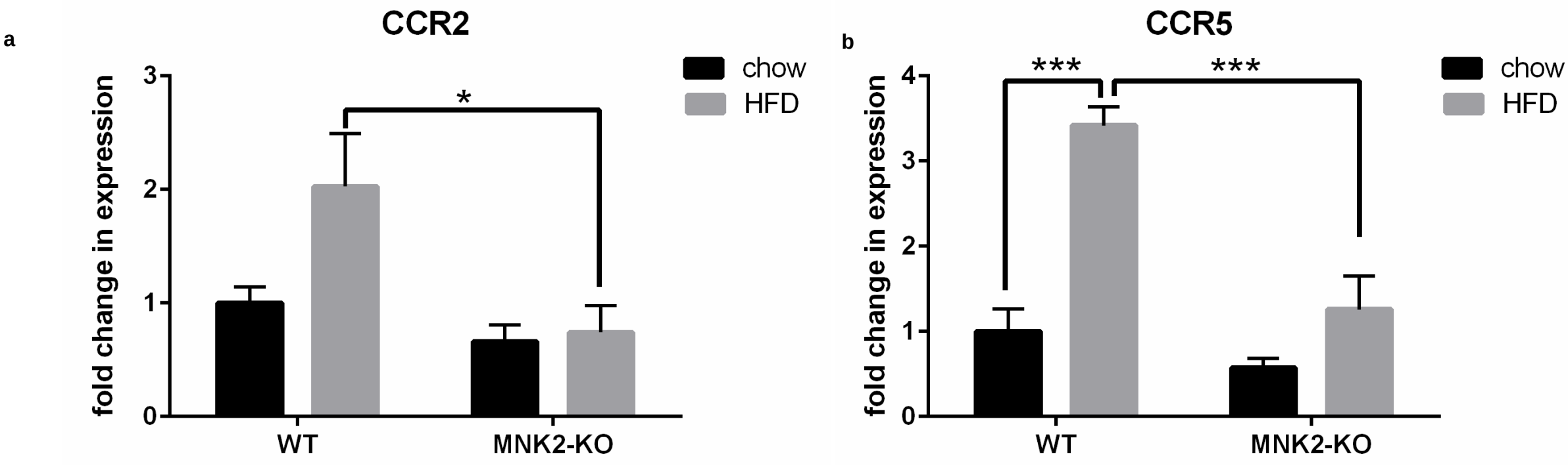
Supplemental Figure 1

Liver histology in WT and MNK2-KO mice (**A,B**) Liver weight of WT and MNK2-KO mice fed chow or the HFD. (n=8-9). Data are mean ± SEM. (**C**) H&E and Oil Red O staining of livers from high fat-fed WT and MNK2-KO mice, 20x magnification. (**D**) Liver total TAG content (n=4-5). Data are mean ± SEM.



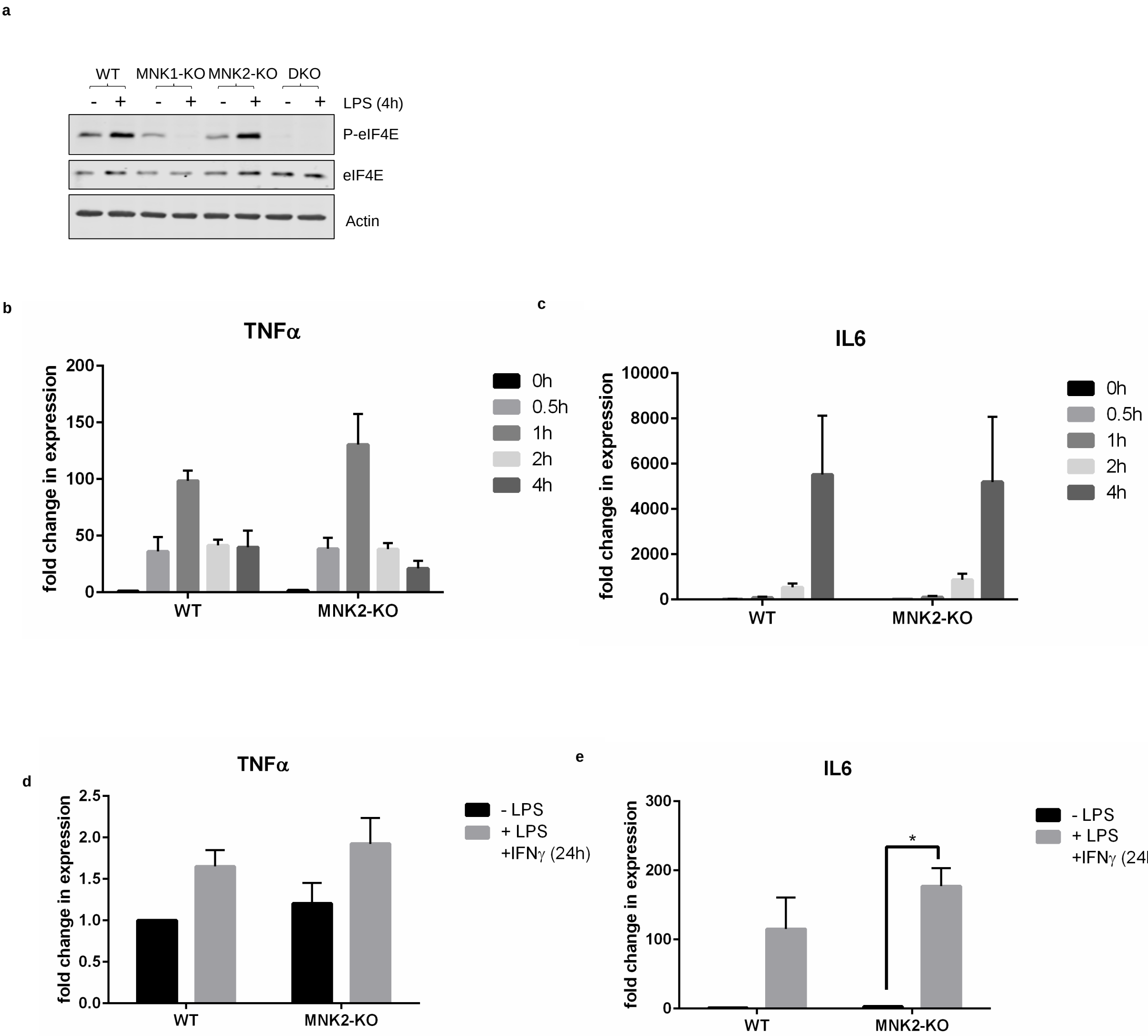
Supplemental Figure 2

Analysis of *GLUT4* mRNA levels in adipose tissue of WT and MNK-2-KO mice (A) Total RNA was isolated from gonadal adipose tissue from chow-fed and HFD-fed WT and MNK2-KO mice. The relative expression of mRNA for *GLUT4* was measured by qPCR (n=4). Data are mean ± SEM relative to WT chow.



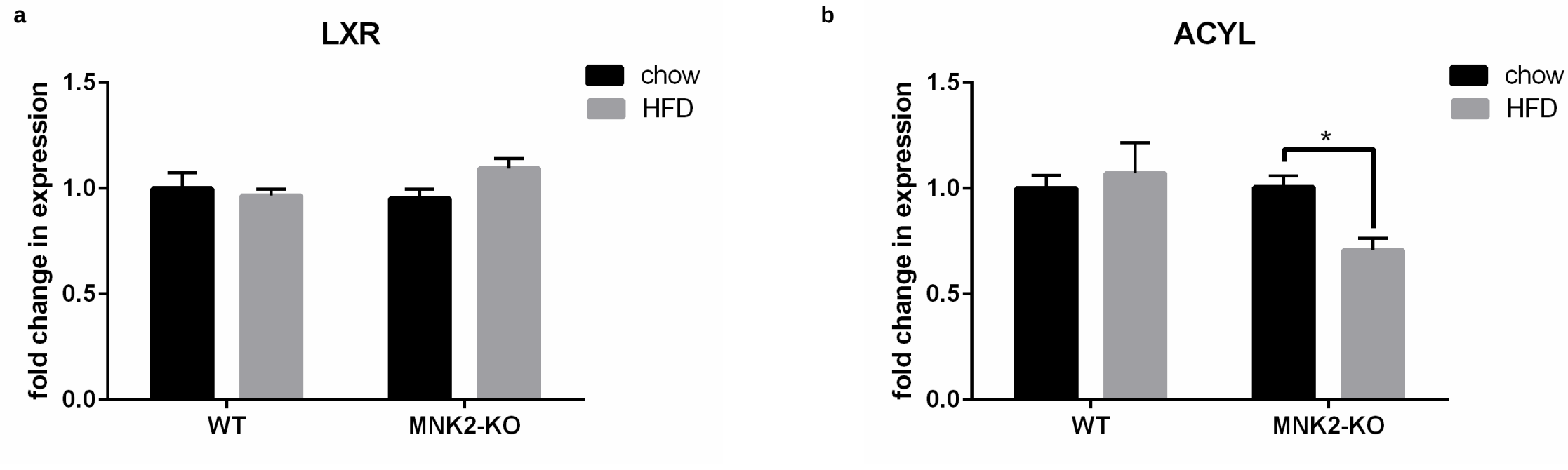
Supplemental Figure 3

Analysis of gene expression in fat tissue from WT and MNK2-KO mice. (**A-D**) Total RNA was isolated from gonadal adipose tissue from chow-fed and HFD-fed WT and MNK2-KO mice. The relative expression of mRNAs for macrophage markers (*CCR2* and *CCR5*) and tissue remodeling markers (*ADAM8 and MMP12*) were measured by qPCR, (n=4). Data are mean ± SEM relative to WT chow (two-way ANOVA followed by Tukey's post test) * P <0.05, ** P<0.01, *** P<0.001.



Supplemental Figure 4

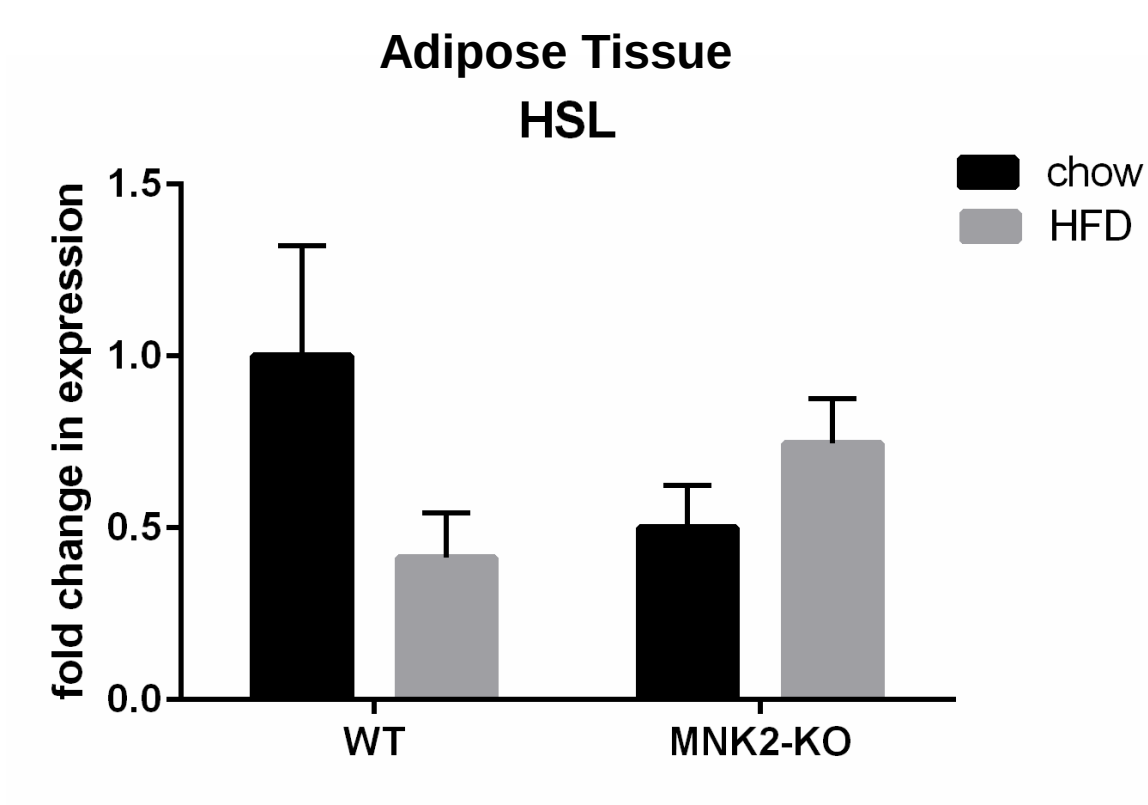
Analysis of gene expression in stimulated BMDMs from WT and MNK2-KO mice. (**A**) BMDMs isolated from WT, MNK1-KO, MNK2-KO and MNK1/2-DKO mice were cultured for 4 h in the presence or absence of LPS (250 ng/ml). Lysates were analysed by immunoblot using the indicated antibodies, representative of 3 independent experiments. (**B,C**) BMDMs isolated from WT and MNK2-KO mice were treated with LPS (250 ng/ml) for the times indicated. Total RNA was isolated and the relative expression of mRNAs for *TNFα* and *IL6* was measured by qPCR (n=3). Data are mean ± SEM relative to WT - LPS. (**D**,**E**) BMDMs isolated from WT or MNK2-KO mice were cultured for 24 h in the presence or absence of LPS (100 ng/ml) and IFNγ (20 ng/ml) to polarize the BMDMs towards an M1 phenotype. The mRNA expression levels of the following M1 markers *TNFα* and *IL-6* were measured by qPCR (n=3). Data are mean ± SEM relative to WT - LPS (2-tailed, unpaired Student's *t* test) * P <0.05.



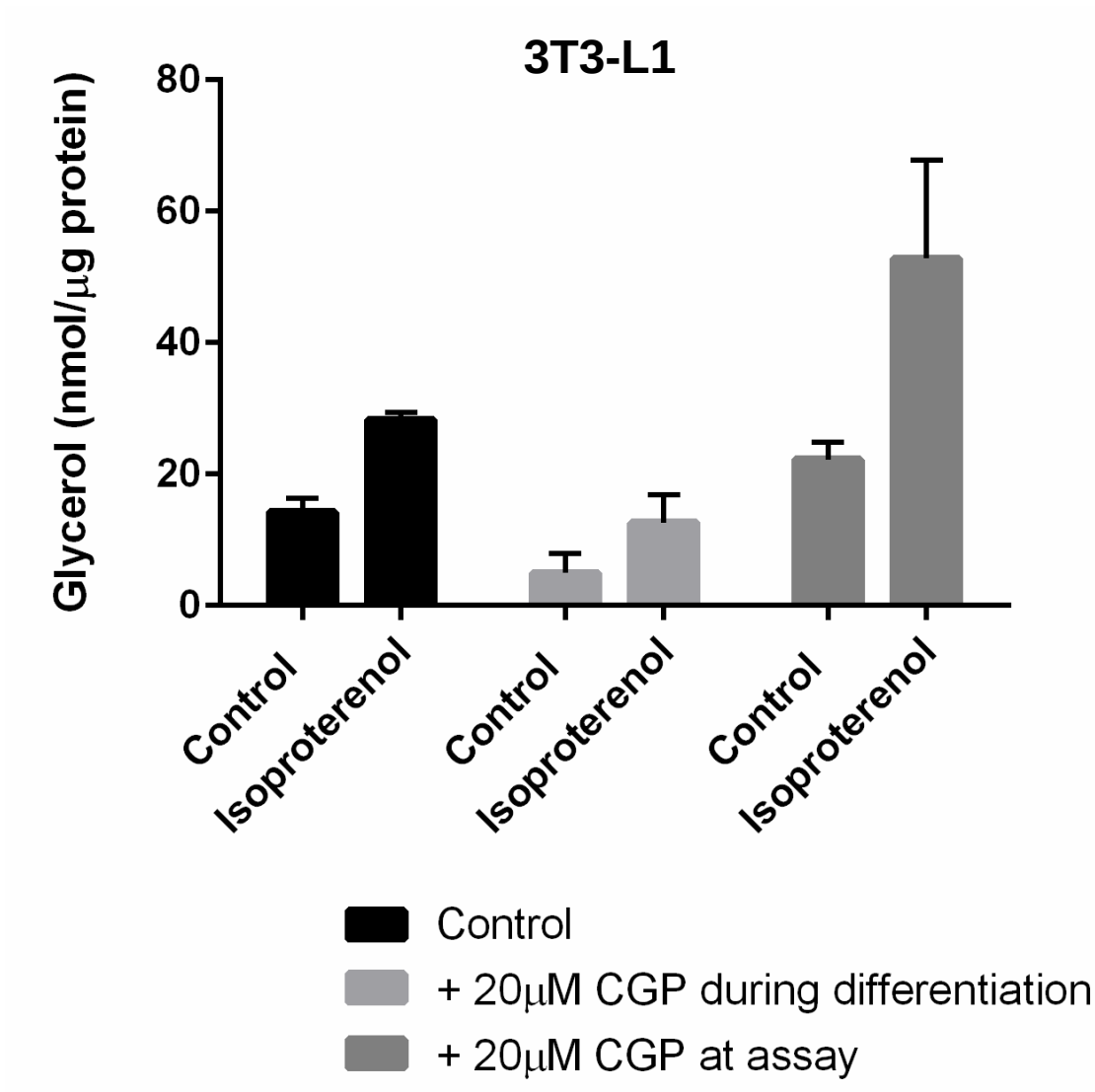
Supplemental Figure 5

Analysis of gene expression in liver from WT and MNK2-KO mice. (**A-C**) Total RNA was isolated from liver tissue from chow fed and HFD fed WT and MNK2-KO mice. The relative expression of *LXR*, A*CYL* and *PPARα* was measured by qPCR (n=4). Data are mean ± SEM relative to WT chow (2-tailed, unpaired Student's *t* test) * P <0.05.

a



b



Supplemental Figure 6

Effects of the MNKs on lipolysis. (**A**) Total RNA was isolated from gonadal adipose tissue from chow-fed and HFD-fed WT and MNK2-KO mice. The relative expression of *HSL* was measured by means of qPCR (n=4). Data are mean ± SEM relative to WT chow (**B**) Lipolysis assay: 3T3-L1 cells were differentiated for 9 days in the presence or absence of 20 µM CGP57380. Cells were treated with 100 nM isoproterenol for 3 h to induce glycerol production, in some cases 20 µM CGP57380 was added at the start of the isoproterenol treatment.