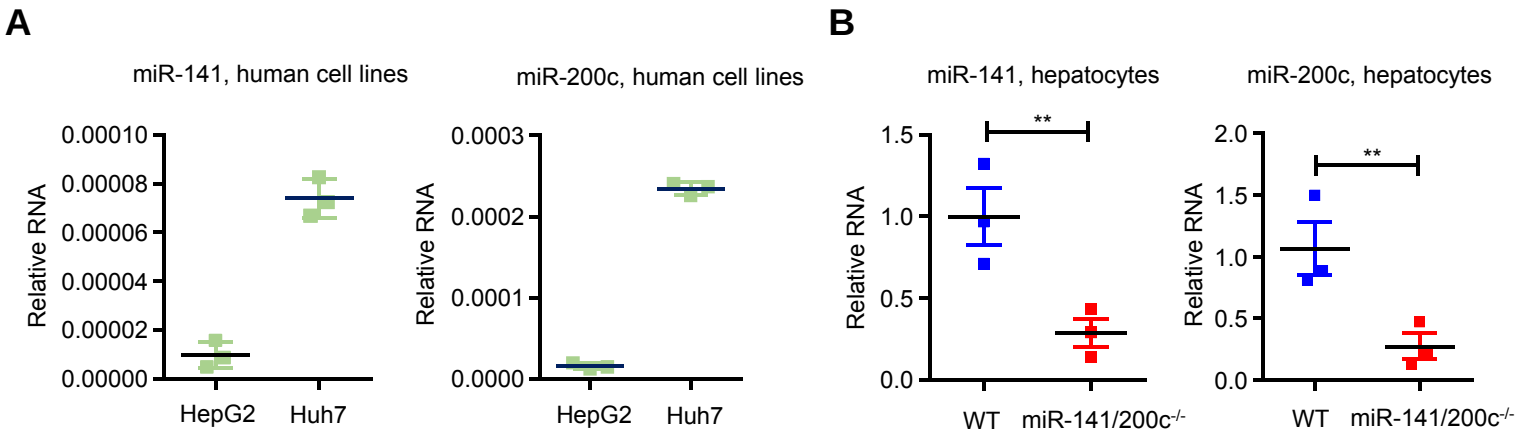
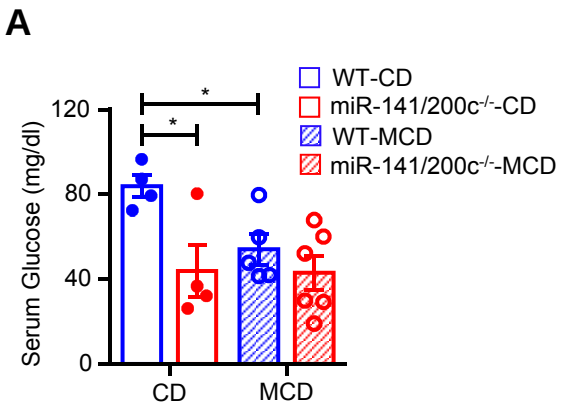
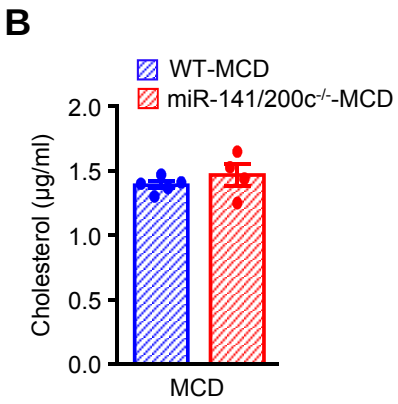
Supplementary Figure 1



Supplementary Fig. 1. qPCR analysis of miR-141 and miR-200c RNA expression levels (A) detected in two human hepatocellular carcinoma cell lines (HepG2 and Huh7) and (B) in primary mouse hepatocytes isolated from WT and miR-141/200c$^{-/-}$ mice (n=3 per group). Data are represented as mean ± SEM. A Students unpaired t-test was used to determine differences between WT and miR-141/200c$^{-/-}$ mice. * P<0.05 and ** P<0.01 indicate statistical significance.

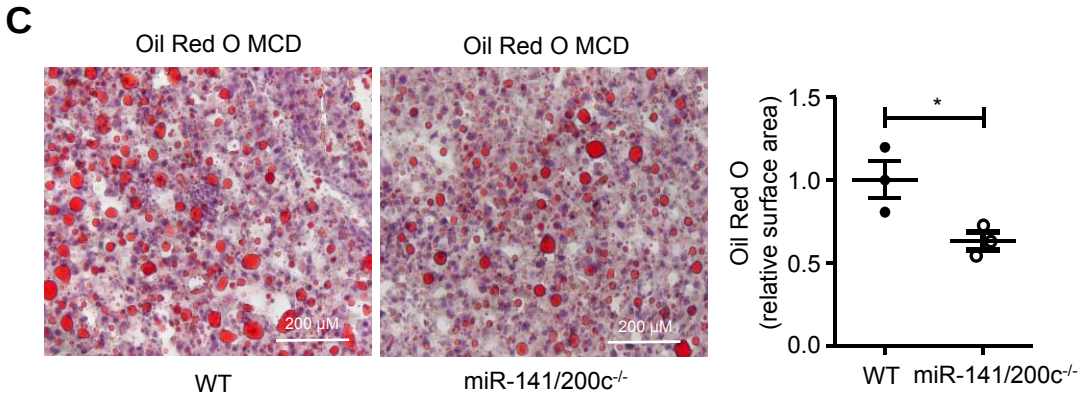
Supplementary Figure 2





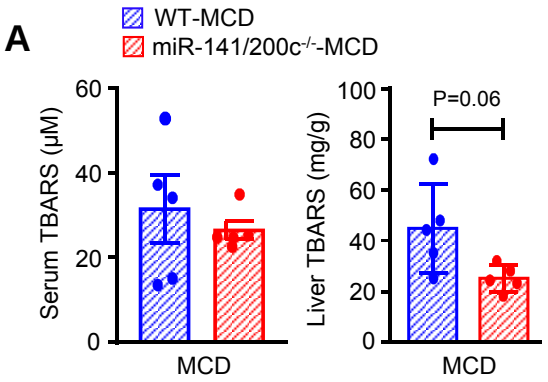
Supplementary Fig. 2A. Serum glucose levels in WT vs miR-141/200c$^{-/-}$ mice by calorimetric analysis (n=4-6 per group). Data are represented as mean ± SEM. * P<0.05 indicate statistical significance, one-way ANOVA with Newman-Keuls multiple comparisons test.

Supplementary Fig. 2B. Liver cholesterol levels in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers by GC-TOF mass spectrometry (n=5 per group). Data are represented as mean ± SEM.

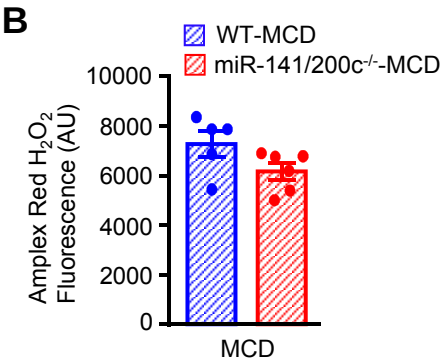


Supplementary Fig. 2C. Representative image of Oil red O staining showed reduced neutral lipid staining in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers. Images were quantified by digital image analysis using ImageJ software in 5 randomly chosen fields from 3 individual mice per group. Data are represented as mean ± SEM. Differences between WT and miR-141/200c$^{-/-}$ mice were compared using a Students unpaired t-test and * P<0.05 indicate statistical significance.

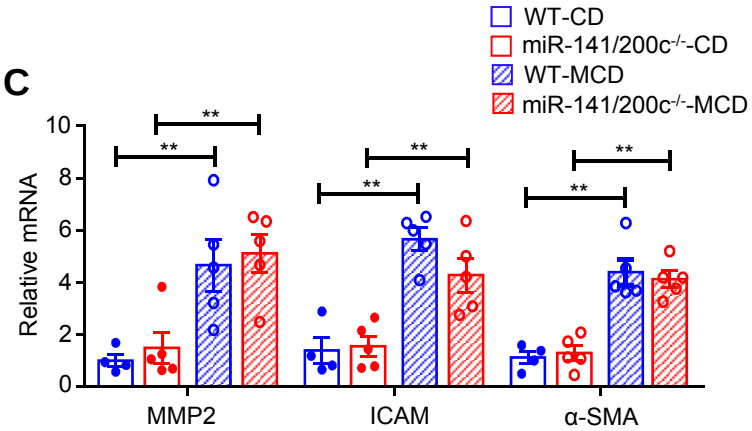
Supplementary Figure 3



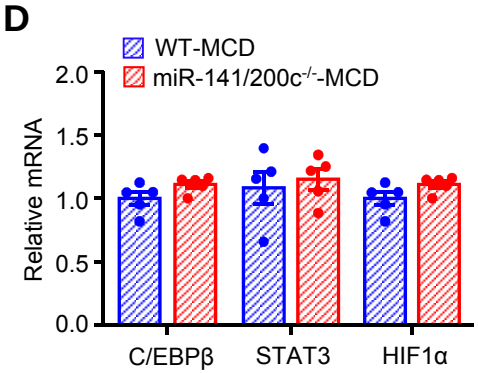
Supplementary Fig. 3A. TBARS expression in serum and livers of WT-MCD vs miR-141/200c$^{-/-}$-MCD (n=5 per group). Data are represented as mean ± SEM.



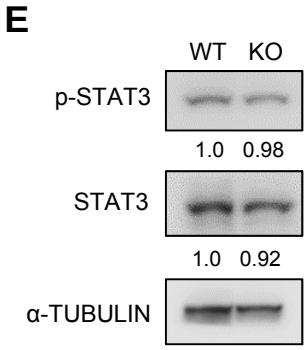
Supplementary Fig. 3B. Liver H_2O_2 levels in WT-MCD vs miR-141/200c$^{-/-}$-MCD (n=5-6 per group). Data are represented as mean ± SEM.



Supplementary Fig. 3C. qPCR of fibrosis-related gene expression in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers (n=4-5 per group). Data are represented as mean ± SEM. * P<0.05 and ** P<0.01 indicate statistical significance, one-way ANOVA with Newman-Keuls multiple comparisons test.

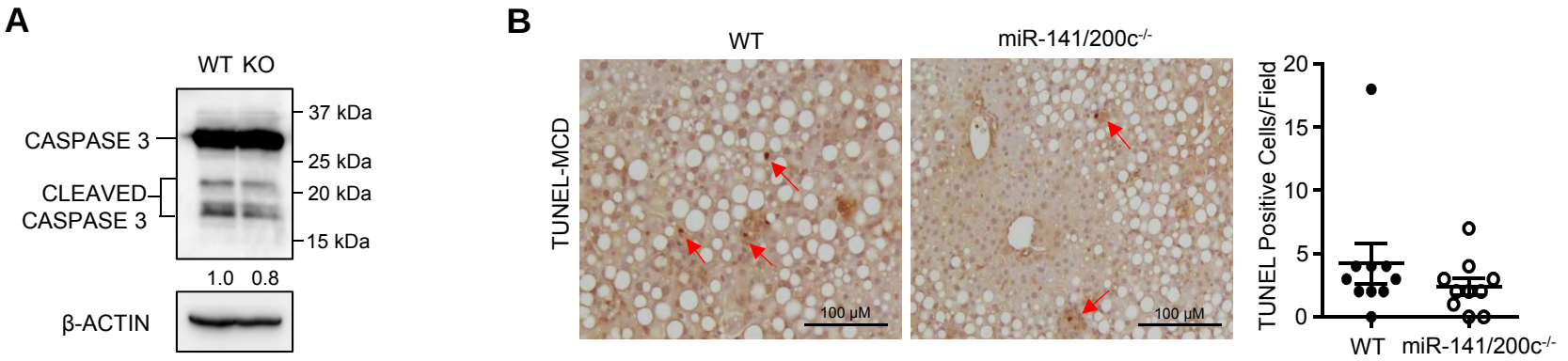


Supplementary Fig. 3D. qPCR of gene expression in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers (n=5 per group). Data are represented as mean ± SEM.



Supplementary Fig. 3E. Western blot analysis of p-STAT3 and STAT3 in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers. Densitometry of each blots relative to the loading control were quantified using ImageJ software. Samples were pooled from 5 individual mice per group.

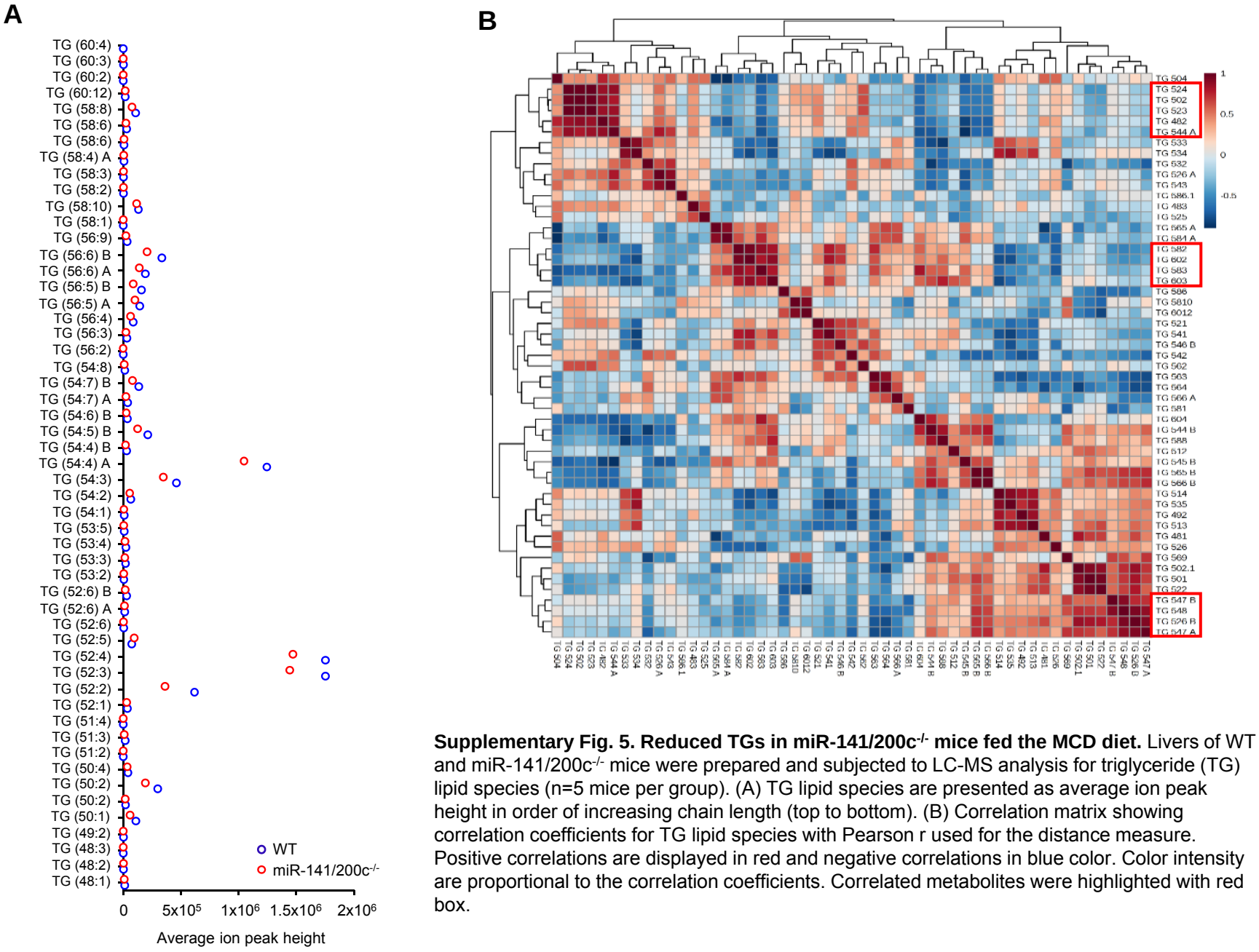
Supplementary Figure 4



Supplementary Fig. 4A. Western blot analysis for apoptosis marker, caspase-3, in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers Densitometry of the blot relative to the loading control was quantified using ImageJ software. Samples were pooled from 5 individual mice per group.

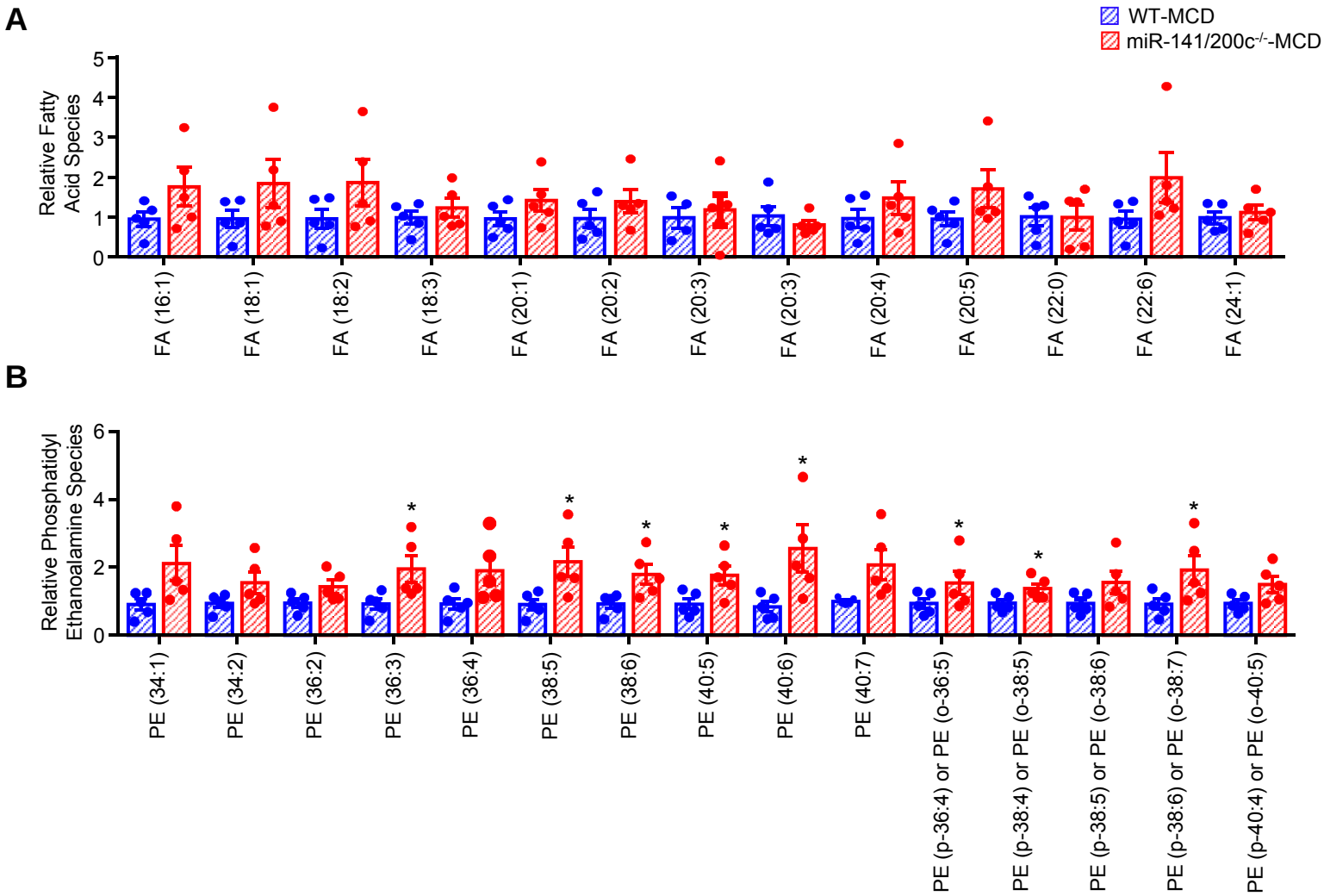
Supplementary Fig. 4B. Representative images of TUNEL assay for apoptosis in WT-MCD vs miR-141/200c$^{-/-}$-MCD livers. The total number of TUNEL positive cells were counted across 10 random high resolution fields and were expressed as average number of TUNEL positive cells per field (n=1 per group). Data are represented as mean ± SEM.

Supplementary Figure 5



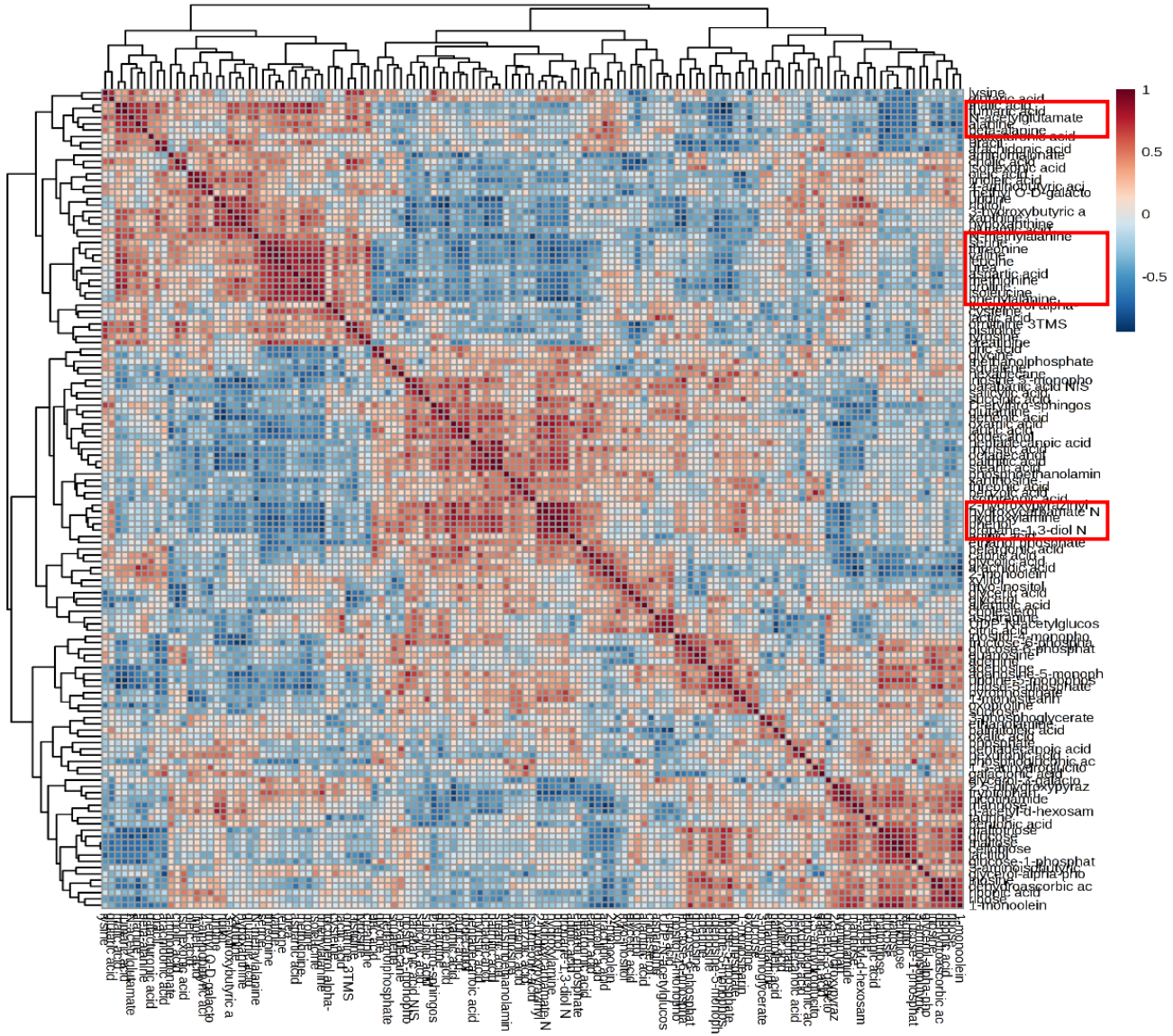
Supplementary Fig. 5. Reduced TGs in miR-141/200c$^{-/-}$ mice fed the MCD diet. Livers of WT and miR-141/200c$^{-/-}$ mice were prepared and subjected to LC-MS analysis for triglyceride (TG) lipid species (n=5 mice per group). (A) TG lipid species are presented as average ion peak height in order of increasing chain length (top to bottom). (B) Correlation matrix showing correlation coefficients for TG lipid species with Pearson r used for the distance measure. Positive correlations are displayed in red and negative correlations in blue color. Color intensity are proportional to the correlation coefficients. Correlated metabolites were highlighted with red box.

Supplementary Figure 6



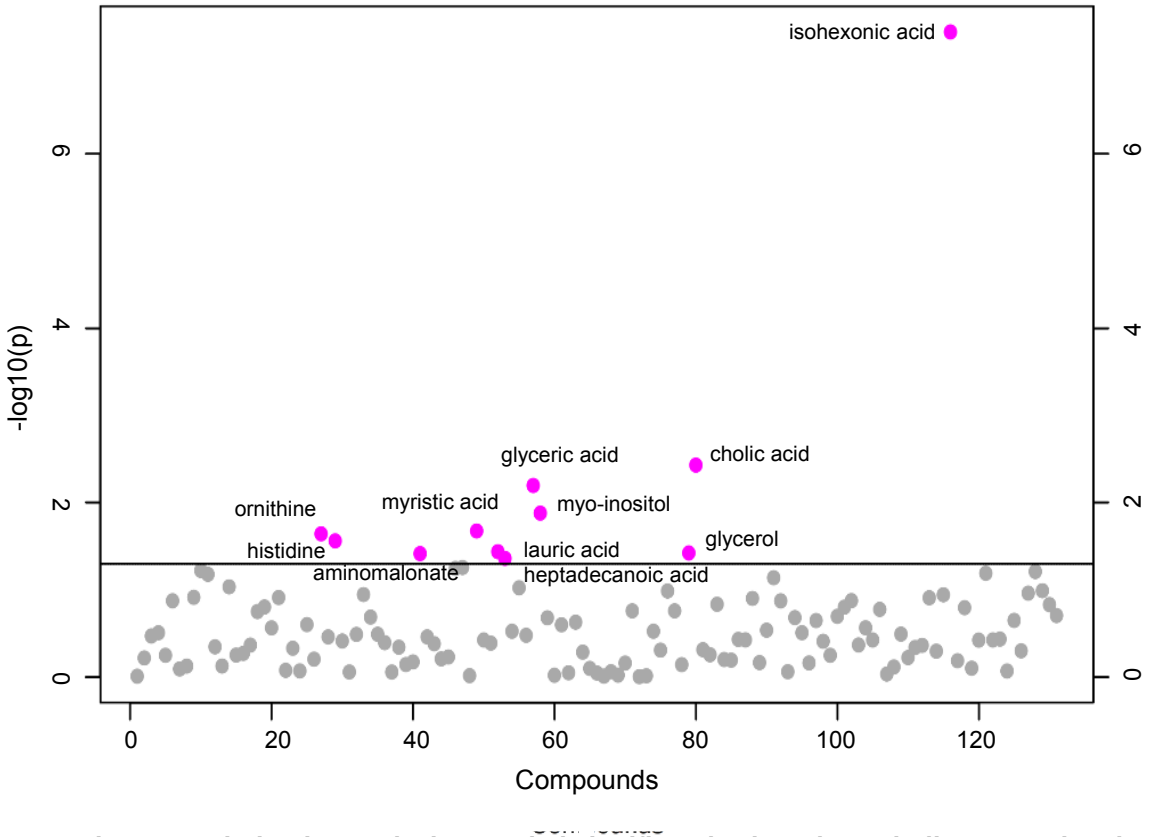
Supplementary Fig. 6. Lipidomics analysis revealed distinct changes in a number of lipid species associated with miR-141/200c. Livers of WT and miR-141/200c$^{-/-}$ mice were prepared and subjected to LC-MS for lipidomics analysis of various lipid classes (n=5 mice per group) including (A) fatty acids and (C) phosphatidylethanolamine (PE) lipid species and are expressed as fold change relative to WT mice. Data are represented as mean ± SEM. * $P < 0.05$ versus WT by Students unpaired t-test.

Supplementary Figure 7



Supplementary Fig. 7. Correlation matrix showing distinct changes in amino acids from metabolomics analysis. Livers of WT and miR-141/200c$^{-/-}$ mice were prepared and subjected to LC-MS for metabolomics analysis (n=5 mice per group). Correlation matrix showing correlation coefficients with Pearson r used for the distance measure. Positive correlations are displayed in red and negative correlations in blue color. Color intensity are proportional to the correlation coefficients. Correlated metabolites were highlighted with red box.

Supplementary Figure 8



Supplementary Fig. 8. Metabolomics analysis revealed significantly altered metabolites associated with miR-141/200c-deficiency. Livers of WT and miR-141/200c$^{-/-}$ mice were prepared and subjected to LC-MS for metabolomics analysis (n=5 mice per group). A Students t-test showing metabolites that are significantly altered between WT and miR-141/200c$^{-/-}$ mice. Each dot represents a metabolite plotted as a compound number (*x*-axis) and statistical significance (−log 10 (p-value), *y*-axis).

SUPPLEMENTAL INFORMATION

Loss of miR-141/200c Ameliorates Hepatic Steatosis and Inflammation by Reprograming Multiple Signaling Pathways in NASH

Melanie Tran, Sang-Min Lee, Dong-Ju Shin, and Li Wang

Table of Contents

Supplementary Table 1. Sequences of primers used for quantitative-PCR

Gene	Forward Primer Sequence (5' – 3')	Reverse Primer Sequence (3' – 5')
miR-200c antisense	CDCTAATACTGCCGGGTAATG	
miR-141 antisense	GCCCGCTAACACTGTCTGGTAAAG	
miR-200c stem loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCATCA	
miR-141 stem loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCATCT	
Universal	GTGCAGGGTCCGAGGT	
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
HPRT	CACAGGACTAGAACACCTGC	GCTGGTGAAAAGGACCTCT
SREBP1c	TTGCTGGCTTGGTGATGCTATG	CTGGTGGAGGGCTGGAAGG
FAS	TCGGGTGTGGTGGGTTTGG	GCGTGAGATGTGTTGCTGAGG
MTTP	CATGCTTCTTCATCTGGTCCG	CACTTTGTCTTGCTGGGCCG
SCD1	AGTTCCGCCACTCGCCTAC	GATAGTCAGTTGCTCGCCTCAC
HMGCR	CCAAGCCCAATGAAGGGAAAGTC	CCACAGGAACAAGGCACACAG
LDLR	GTGAGGTTCCTGTCCATCTTCTTC	GTTCTTCAGCCGCCAGTTCC
CYP7A1	CACATCCTCCTGGCATTTACCC	TCCTCCTGTCTATGTCACACTACC
SAA1	TGATGGAAGAGAGGCCTTTCA	TCAGGCAGTCCAGGAGGTCT
IL-1β	CCTGTGTTTTCCTCCTTGCCT	GCCTAATGTCCCCTTGAATCAA
TNFα	GACCCTCACACTCAGATCATC	CCTCCACTTGGTGGTTTGCT
IL-6	TGATGCACTTGCAGAAAACA	ACCAGAGGAAATTTTCAATAGGC
IL-4	TGAACGAGGTCACAGGAGAA	CGAGCTCACTCTCTGTGGTG
IL-10	ATCGATTTCTCCCCTGTGAA	TGTCAAATTCATTCATGGCCT
F4/80	CCCCAGTGTCCTTACAGAGTG	GTGCCCAGAGTGGATGTCT
LYG6	GACTTCCTGCAACACAACTAC	ACAGCATTACCAGTGATCTCA

COL1A1	GCAGGGTTCCAACGATGTTG	GCAGCCATCGACTAGGACAGA
TGFβ	GGAGACCCCTGGATACCAAC	CAACCCAGGTCCTTCCTAAA
α-SMA	AGAGTTACGAGTTGCCTGATG	ATGAAGGATGGCTGGAACAG
iNOS	AATCTTGGAGCGAGTTGTGG	CAGGAAGTAGGTGAGGGCTTG
ARG1	TCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
C/EBPβ	CAACCTGGAGACGCAGCACAA	GGCAGCTGCTTGAACAAGTTC
HIF1α	GGGTACAAGAAACCACCCAT	GAGGCTGTGTCGACTGAGAA
STAT3	CAGAAAGTGTCCTACAAGGGCG	CGTTGTTAGACTCCTCCATGTTC
KLF4	GTGCCCCGACTAACCGTTG	GTCGTTGAACTCCTCGGTCT
IFR3	GGCTTGTGATGGTCAAGGTT	CATGTCCTCCACCAAGTCCT
ICAM	GTCTCGGAAGGGAGCCAAGTA	CGACGCCGCTCAGAAGAA
MMP2	ATGCGGAAGCCAAGATGTG	TTTCAGGGTCCAGGTCAGG
NFκβ	ATGATCCCTACGGAACTGGGCAAA	TGGGCCATCTGTTGACAGTGGTAT

Supplementary Table 2. Clinical and biochemical characteristics of patients with non-alcoholic steatohepatitis (NASH) non fatty or NASH fatty livers.

Characteristics	NASH non fatty (n=16)	NASH fatty (n=20)
Age (years)	57.2 ± 2.6	57.9 ± 1.8
Gender, male: female	7/9	6/14
MELD score	26.8 ± 2.1	30.2 ± 1.8
Total bilirubin (mg/L)	9.8 ± 3.2	13.8 ± 3.8
Creatinine (mg/L)	3.9 ± 0.7	2.1 ± 0.31*
Albumin (g/dL)	2.8 ± 0.14	3.0 ± 0.14
AST (U/L)	74.4 ± 14.9	66.4 ± 6.8
ALP (U/L)	225.5 ± 38.1	160.3 ± 20.9

Data are presented as means ± sem. MELD, model for end-stage liver disease; AST, aspartate transaminase, ALP; alkaline phosphatase. * $P<0.05$ *versus* NASH non-fatty by Students unpaired t-test.