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Supplemental Information

An RNAi therapeutic targeting hepatic DGAT2 in a genetically obese mouse model of nonalcoholic steatohepatitis

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Figure S1

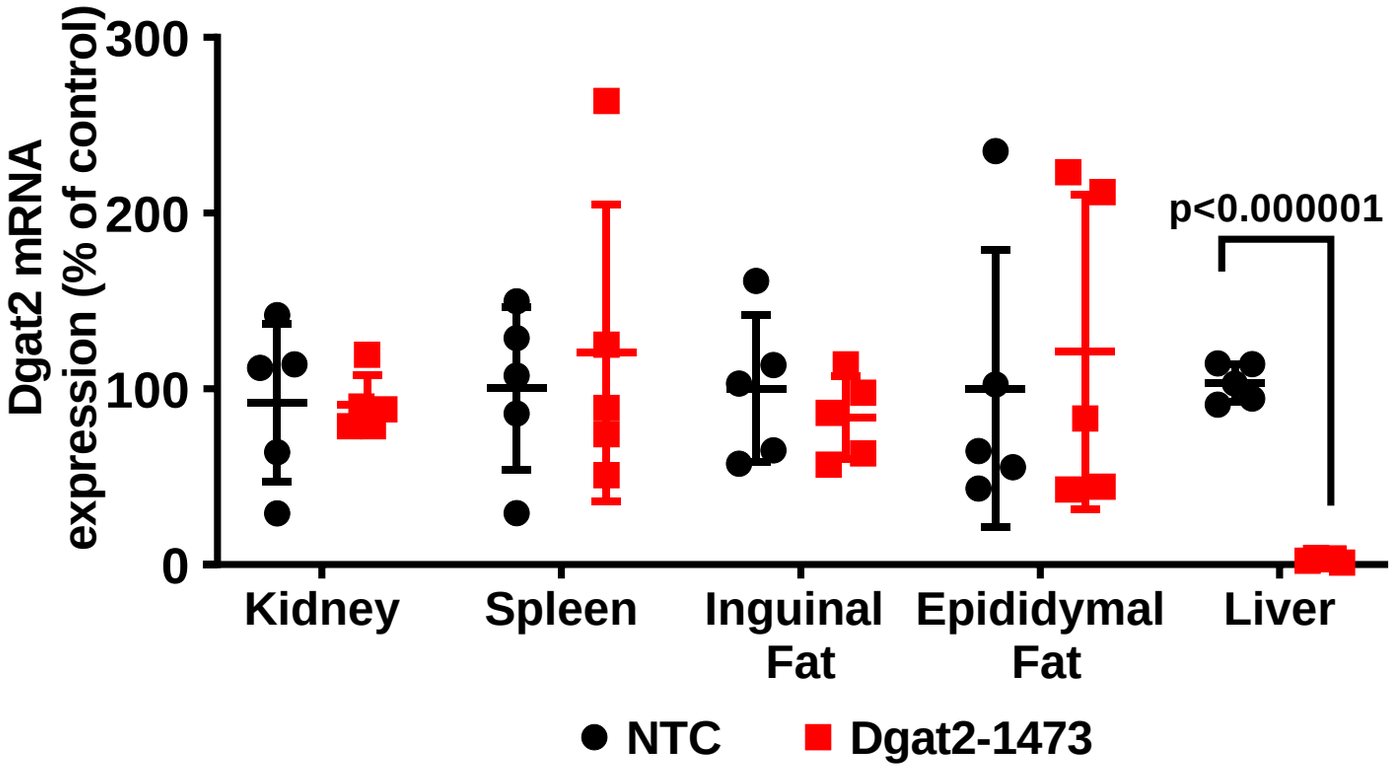
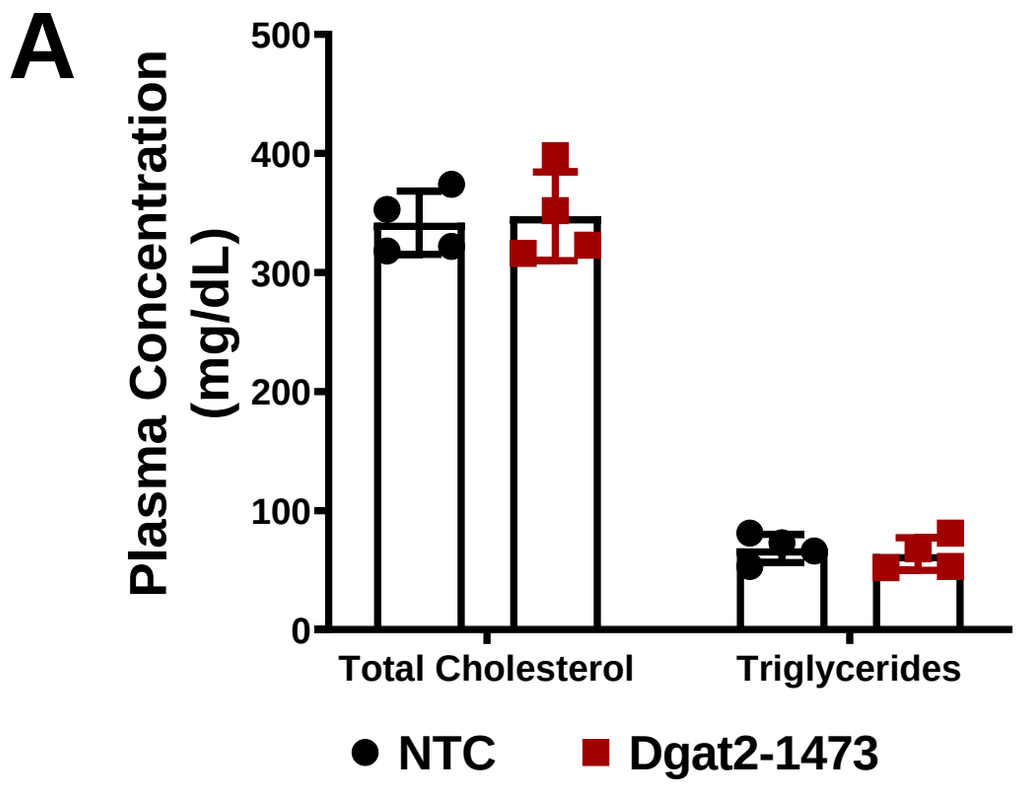


Figure S1: Dgat2-1473 targets *Dgat2* mRNA specifically in liver. Eight week old male C57BL6 were injected with either NTC (n=5) or Dgat2-1473 (n=5) subcutaneously. Four week after single injection, mice were sacrificed and *Dgat2* silencing was examined in kidney, spleen, inguinal fat, epididymal fat and liver via qPCR

Figure S2



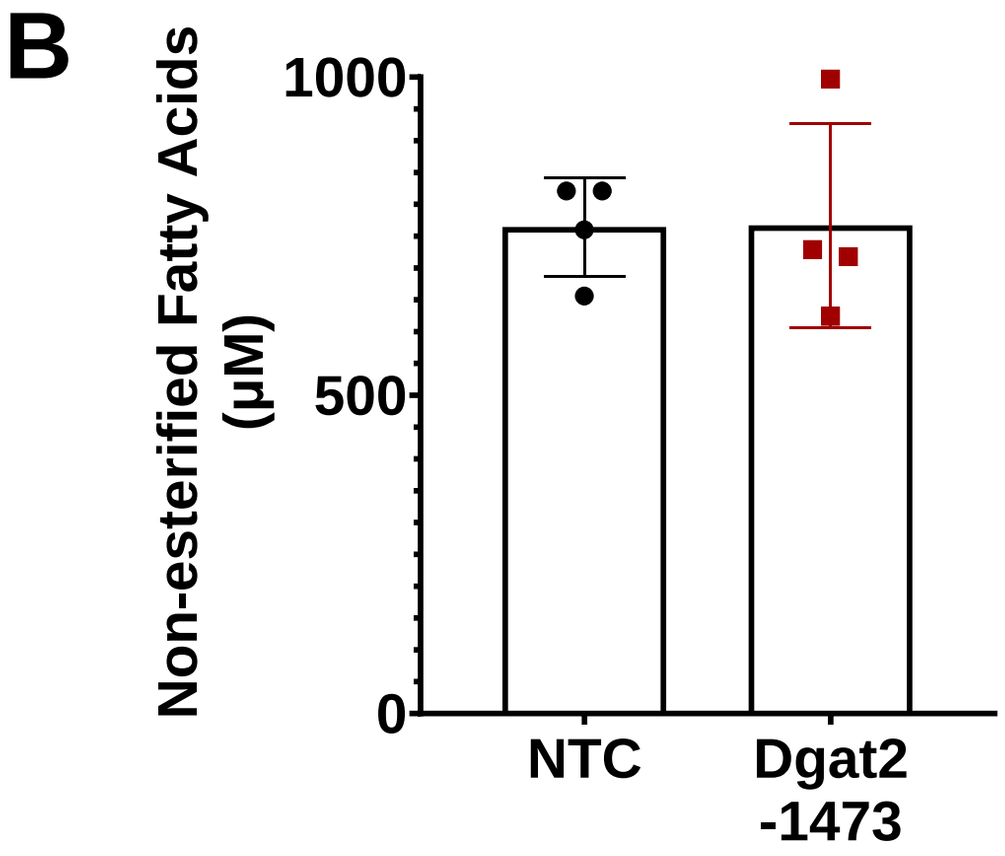


Figure S2: ***Dgat2*** **silencing did not alter (A) plasma total cholesterol, triglycerides or (B) non-esterified fatty acid levels in ob/ob NASH model.**

Figure S3

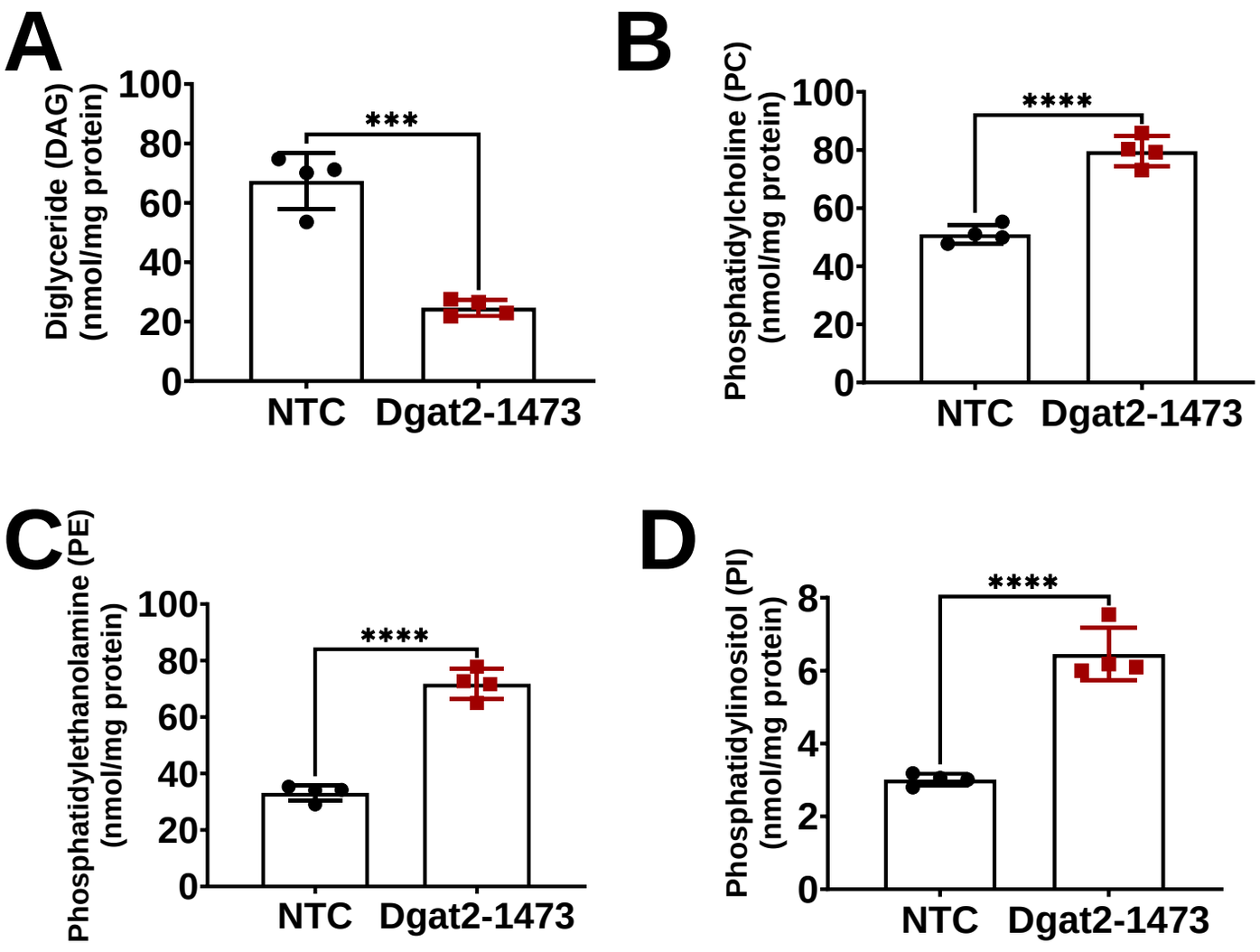
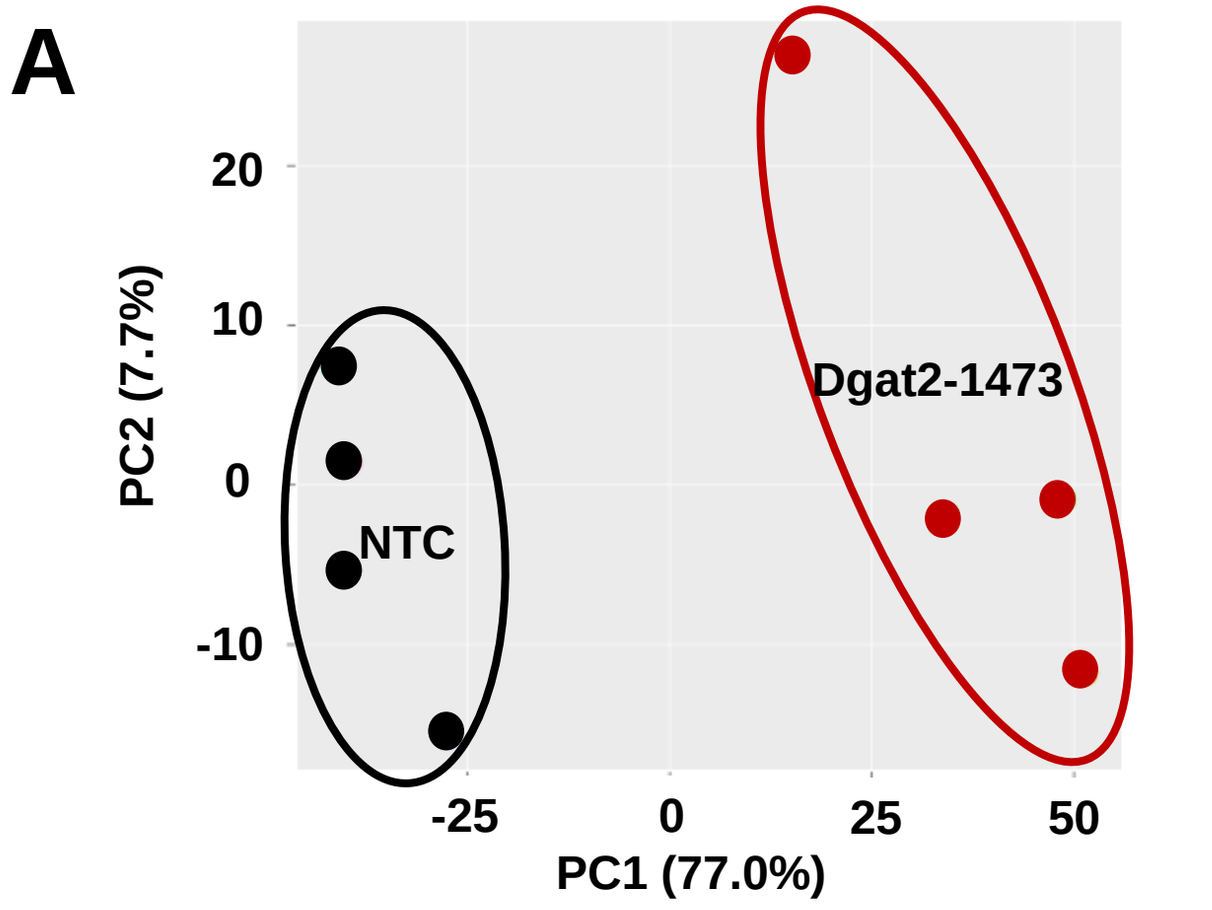


Figure S3: ***Dgat2 silencing*** **in liver resulted in a remarkable decrease in diglycerides and increase in phospholipid levels in ob/ob mice with NASH (A)** Diglyceride levels **(B)** Phosphatidylcholine **(C)** Phosphatidylethanolamine **(D)** Phosphatidylinositol levels. (*: p<0.05, **:p<0.005, ***:p<0.0005, ****:p<0.00005)

Figure S4


A
20
10
0
-10
PC2 (7.7%)
NTC
Dgat2-1473
-25
0
25
50
PC1 (77.0%)

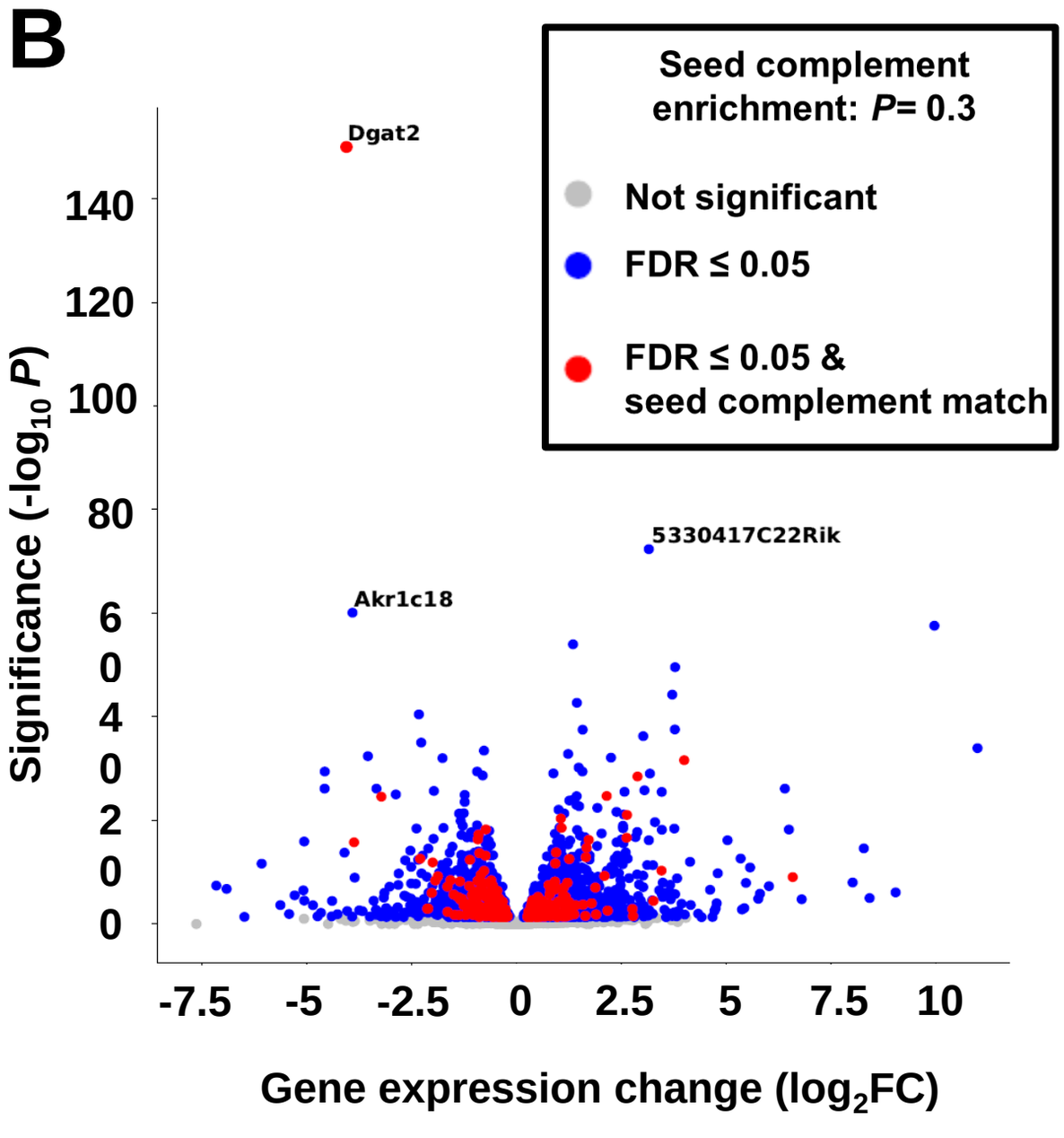

B
Seed complement enrichment: P= 0.3
Not significant
FDR ≤ 0.05
FDR ≤ 0.05 & seed complement match
Dgat2
5330417C22Rik
Akr1c18
140
120
100
80
60
40
20
0
Significance (-log10 P)
-7.5
-5
-2.5
0
2.5
5
7.5
10
Gene expression change (log2FC)

Figure S4: Dgat2-1473 did not produce significant detectable off-target silencing activity in liver *in vivo*. Total RNA samples were isolated from whole liver tissues from the study explained in Figure 4 and sent out for next-gen RNA sequencing. PolyA selection of mRNA species was used for the method of rRNA removal. The depth of the sequencing was 20-30 million reads/sample. The RNAseq pipeline in DolphinNext (Yukselen O, et.al.2020) was used to convert the fastq files into gene counts and the resulting estimates for gene expression were passed to DEBrowser (Kucukural A, et.al 2019) for **(A)** Principle Component Analysis (PCA) of the RNAseq database **(B)** Volcano plot of all differentially expressed genes and off-target silencing analysis. The seed enrichment p-value is calculated using a Fisher's exact test comparing the prevalence of the seed (guide 2-8) target in the 3' UTR of genes that are downregulated to the prevalence of the seed target in the 3' UTR of genes that are not downregulated.

Table S1

A

Antisense strands:

OLIGO ID	Modified Sequence
1464	P(mU)#(fA)#(mU)(mA)(mA)(fC)(mC)(mC)(mA)(mC)(mA)(mG)(mA)#(fC)#(mA)#(fC)#(mC)#(mC)#(mA)#(fU)
1473	P(mU)#(fU)#(mU)(mC)(mU)(fU)(mU)(mU)(mA)(mA)(mA)(mU)(mA)#(fA)#(mC)#(fC)#(mC)#(mA)#(mC)#(fA)
1476	P(mU)#(fA)#(mA)(mU)(mU)(fU)(mC)(mU)(mU)(mU)(mU)(mA)(mA)#(fA)#(mU)#(fA)#(mA)#(mC)#(mC)#(fC)
1093	P(mU)#(fG)#(mG)(mA)(mA)(fC)(mU)(mU)(mC)(mU)(mU)(mC)(mU)#(fG)#(mG)#(fA)#(mC)#(mC)#(mC)#(fA)
1094	P(mU)#(fU)#(mG)(mG)(mA)(fA)(mC)(mU)(mU)(mC)(mU)(mU)(mC)#(fU)#(mG)#(fG)#(mA)#(mC)#(mC)#(fC)

Sense strands:

OLIGO ID	Modified Sequence
1464	(mG)#(mG)#(mG)(mU)(mG)(mU)(mC)(fU)(fG)(fU)(mG)(fG)(mG)(mU)(mU)(mA)#(mU)#(mA)-TegChol
1473	(mU)#(mG)#(mG)(mG)(mU)(mU)(mA)(fU)(fU)(fU)(mA)(fA)(mA)(mA)(mG)(mA)#(mA)#(mA)- TegChol
1476	(mG)#(mU)#(mU)(mA)(mU)(mU)(mU)(fA)(fA)(fA)(mA)(fG)(mA)(mA)(mA)(mU)#(mU)#(mA)-TegChol
1093	(mG)#(mG)#(mU)(mC)(mC)(mA)(mG)(fA)(fA)(fG)(mA)(fA)(mG)(mU)(mU)(mC)#(mC)#(mA)-TegChol
1094	(mG)#(mU)#(mC)(mC)(mA)(mG)(mA)(fA)(fG)(fA)(mA)(fG)(mU)(mU)(mC)(mC)#(mA)#(mA)-TegChol

B

GalNac-1473 antisense strand:

OLIGO ID	Modified Sequence
Dgat2-1473	vP(mU)#(fU)#(mU)(mC)(mU)(fU)(mU)(mU)(mA)(mA)(mA)(mU)(mA)#(fA)#(mC)#(fC)#(mC)#(mA)#(mC)#(fA)

GalNac-1473 sense strand:

OLIGO ID	Modified Sequence
Dgat2-1473	(mU)#(mG)#(mG)(mG)(mU)(mU)(mA)(fU)(fU)(fU)(mA)(fA)(mA)(mA)(mG)(mA)#(mA)#(mA)- GalNAc

Table S1: Chemically modified siRNA sequences. (A) Cholesterol conjugated chemically modified siRNA sequences for *in vitro* screening **(B)** Dgat2-1473 sequence for *in vivo* studies. (P: 5’ phosphate; vP: 5´-(E)-vinylphosphonate; (m):2’-*O*- methyl modification; (f): 2’-fluoro modification; #:phosphorothioate modification; Teg: triethyl glycerol; Chol: Cholesterol conjugate; GalNAc: trivalent GalNAc conjugate)

Table S2

GENE	Forward	Reverse
Dgat2 mouse	AGAATAAAGGATCTGCCCTGTC	TTCCACCTTAGATCTGTTGAGC
Dgat2 human	TCTCACGGAGGACCTGC	CACCAGCCAAGTGAAGTAGAG
18S	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCTGATT
SREBP-1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
SREBP-2	GCGTTCTGGAGACCATGGA	ACAAAGTTGCTCTGAAAACAAATCA
Fatty acid synthase	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Stearoyl-CoA desaturase-1	CCGGAGACCCCTTAGATCGA	TAGCCTGTAAAAGATTTCTGCAAACC
ChREBP-total	GCCTCCGCCAGACCTCACTG	AGTGCTGAGTTGGCGAAGGG
ChREBP-α	CGACACTCACCCACCTCTTC	TTGTTCAGCCGGATCTTGTC
ChREBP-β	TCTGCAGATCGCGTGGAG	CTTGTCCCGGCATAGCAAC
LXRα	GGATAGGGTTGGAGTCAGCA	GGAGCGCCTGTTACACTGTT

Table S2: Primer sequences used for qRT-PCR.

Figure S5

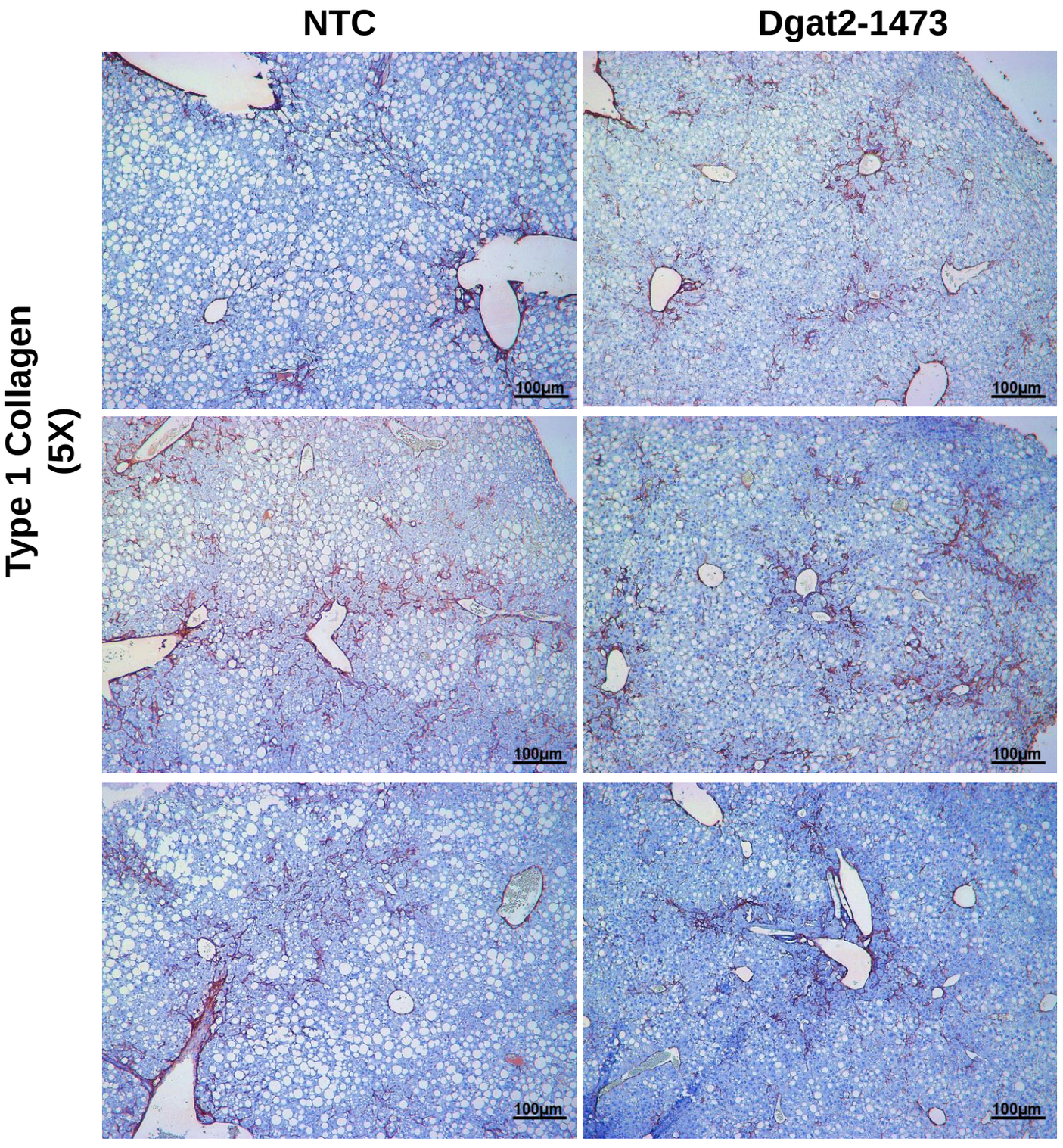


Figure S5: *Dgat2* silencing does not significantly alleviate the fibrosis in the liver of genetically obese NASH mice. Ten-week-old genetically obese ob/ob mice (n=4) were injected subcutaneously with either non targeting control NTC (10mg/kg) or Dgat2-1473 (10mg/kg) and provided a NASH-inducing diet (GAN diet) for 3 weeks. After 3 weeks mice were sacrificed. Histological examination of fibrosis via Type 1 collagen IHC.

Figure S6

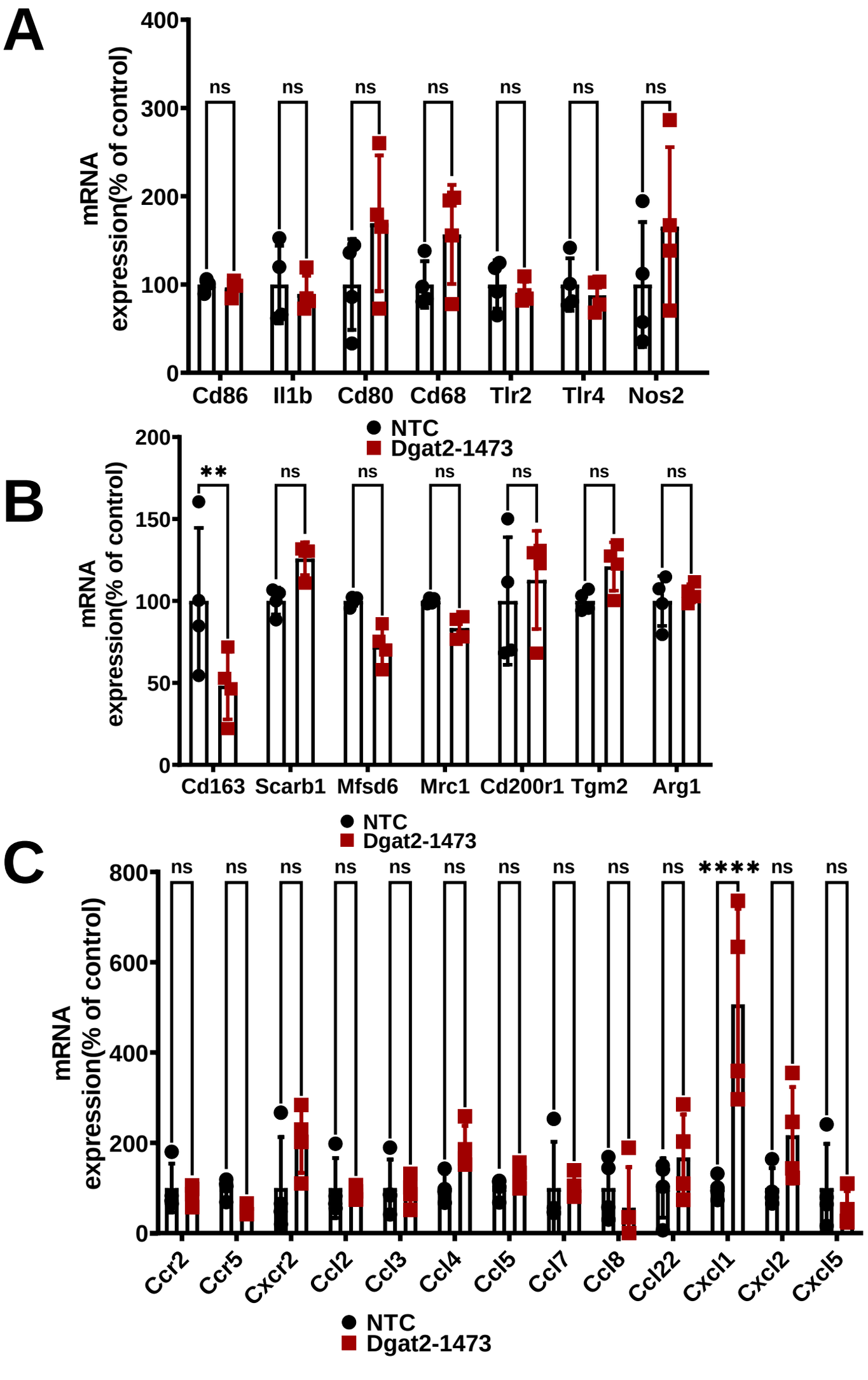


Figure S6: *Dgat2* silencing does not improve the inflammation in the liver of genetically obese NASH mice. mRNA expression levels of **(A)** M1 macrophage markers **(B)** M2 macrophage markers **(C)** Chemokines and their receptors. (ns: not significant, *: p<0.05, **:p<0.005, ***:p<0.0005, ****:p<0.00005)

Supplemental Figure 7

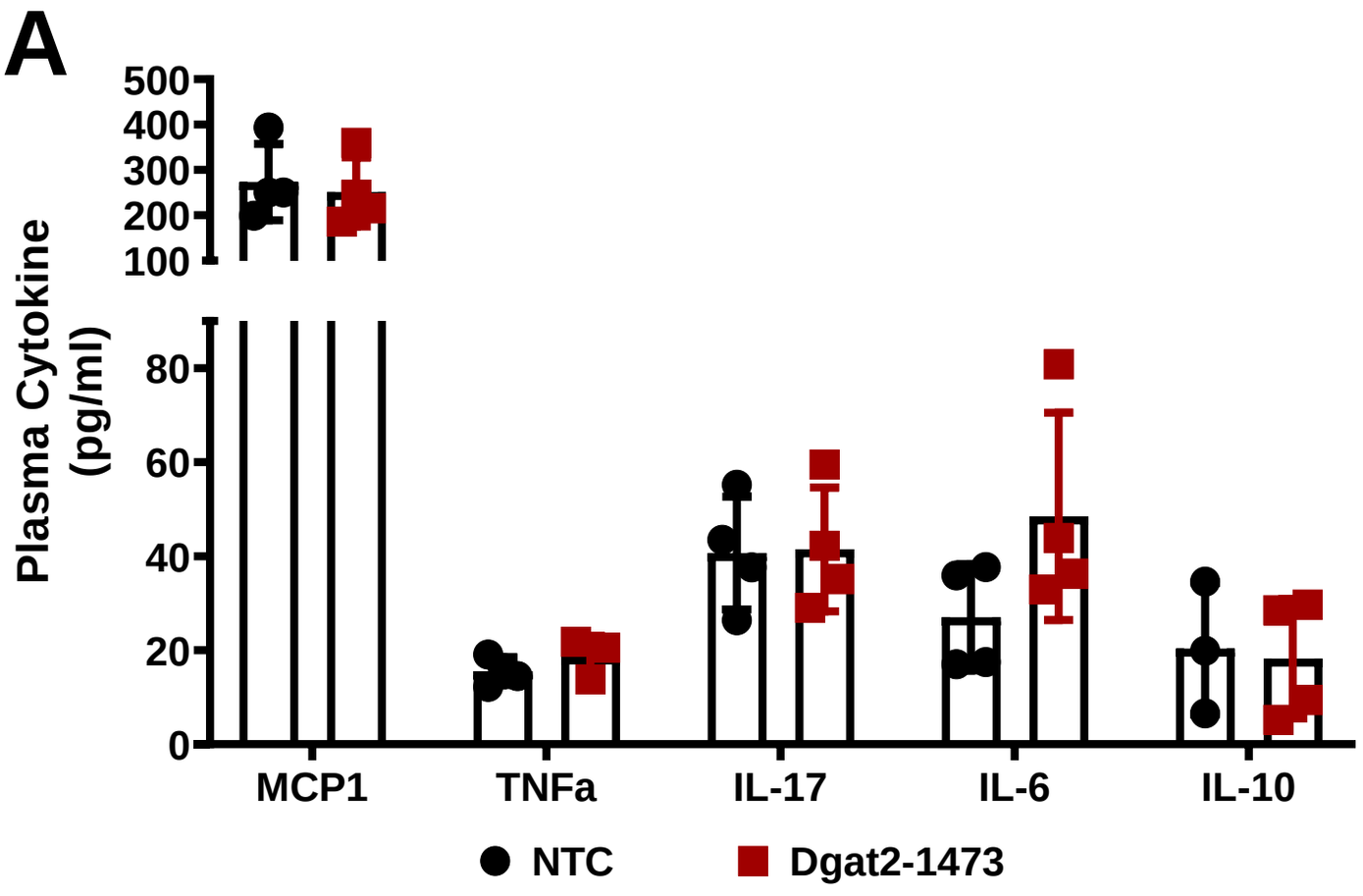


Figure S7: *Dgat2* silencing does not attenuate the plasma levels of inflammatory cytokines in genetically obese NASH mice.