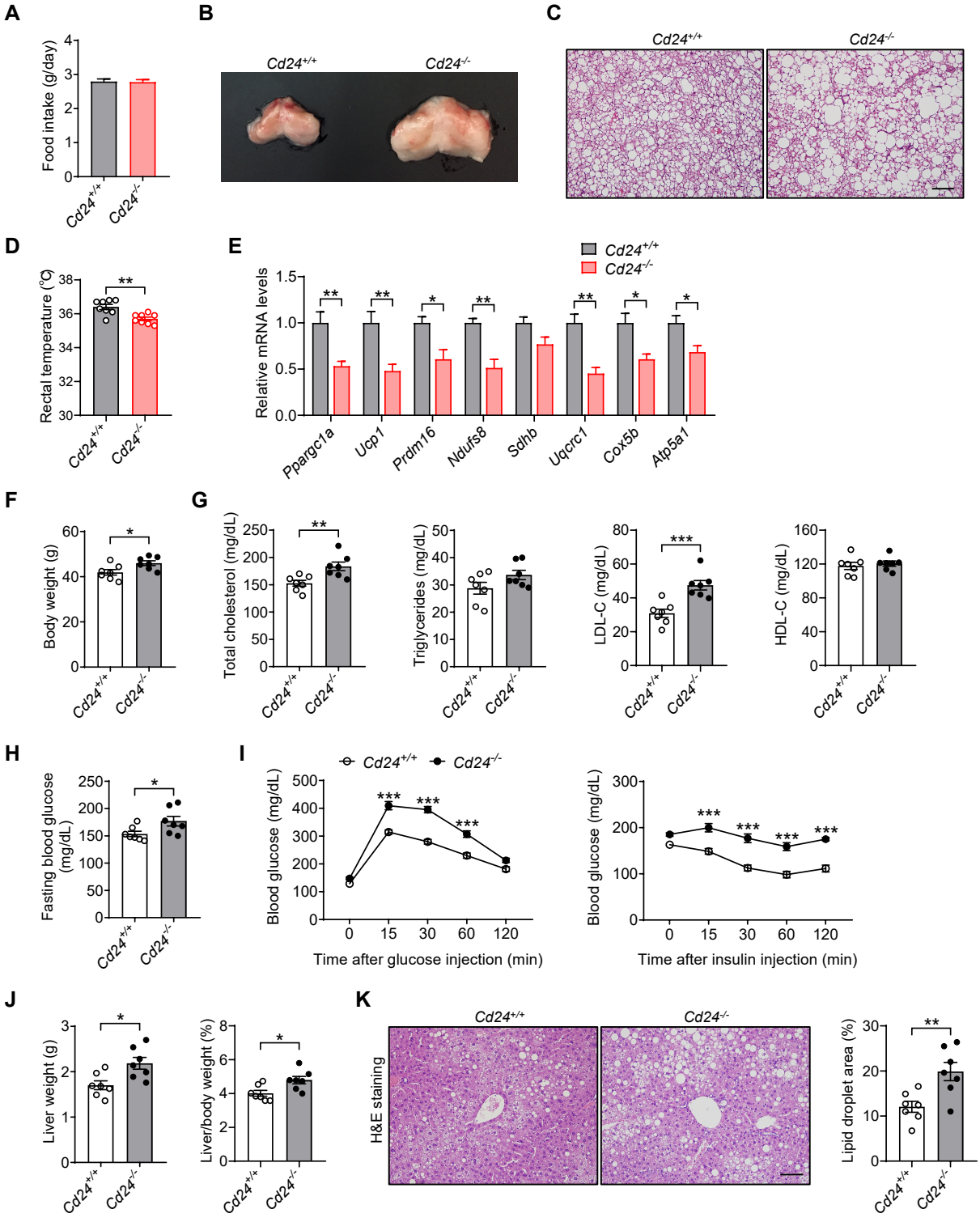
A
Food intake (g/day)
Cd24+/+
Cd24-/-
B
Cd24+/+
Cd24-/-
C
Cd24+/+
Cd24-/-
D
Rectal temperature (°C)
E
Cd24+/+
Cd24-/-
Relative mRNA levels
Ppargc1a
Ucp1
Prdm16
Ndufs8
Sdhb
Uqcrc1
Cox5b
Atp5a1
F
Body weight (g)
G
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
LDL-C (mg/dL)
HDL-C (mg/dL)
H
Fasting blood glucose (mg/dL)
I
Blood glucose (mg/dL)
Time after glucose injection (min)
Time after insulin injection (min)
J
Liver weight (g)
Liver/body weight (%)
K
H&E staining
Lipid droplet area (%)

Figure S1. Metabolic phenotypes of *Cd24*$^{-/-}$ mice, related to Figure 1.

(A-E) *Cd24*$^{-/-}$ mice and WT littermates were fed a HFD for 12 weeks.

(A) Food intake of mice in the indicated groups. n = 5 per group.

(B) Representative images of interscapular BAT of mice in the indicated groups.

(C) Representative images of H&E staining of BAT sections. Scale bar, 100μm.

(D) Rectal temperature of mice in the indicated groups. n = 8 per group.

(E) Relative mRNA levels of thermogenic and mitochondrial genes in BAT from mice in the indicated groups. n = 6 per group.

(F-K) *Cd24*$^{-/-}$ mice and WT littermates were maintained on a normal chow diet for 15 months.

(F) Body weight of mice in the indicated groups. n = 7 per group.

(G) TC, TG, LDL-C and HDL-C levels in the indicated groups. n = 7 per group.

(H) Fasting blood glucose levels of mice in the indicated groups. n = 7 per group.

(I) GTT and ITT results of mice in the indicated groups. n = 7 per group.

(J) Liver weight and liver/body weight ratio of mice in the indicated groups. n = 7 per group.

(K) Representative images of H&E staining of liver sections. Scale bar, 100μm. The lipid droplet area was quantified. n = 7 per group.

Data are mean ± SEM and representative of two or three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test (A, D-H, J, K) or two-way analysis of variance (ANOVA) (I).

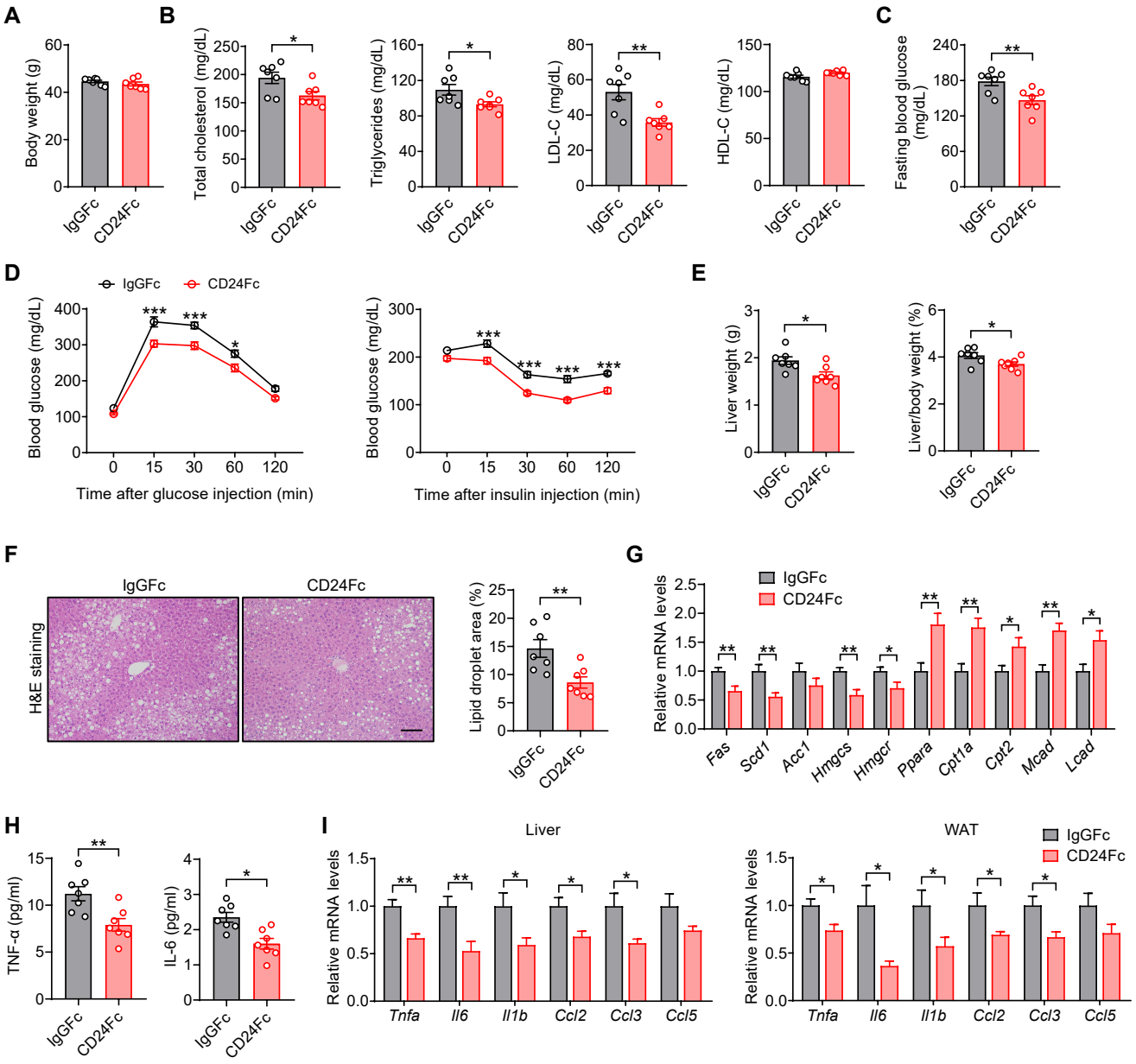
A
Body weight (g)
IgGFc
CD24Fc
B
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
LDL-C (mg/dL)
HDL-C (mg/dL)
C
Fasting blood glucose (mg/dL)
D
Blood glucose (mg/dL)
Time after glucose injection (min)
Time after insulin injection (min)
E
Liver weight (g)
Liver/body weight (%)
F
H&E staining
Lipid droplet area (%)
G
Relative mRNA levels
Fas
Scd1
Acc1
Hmgcs
Hmgcr
Ppara
Cpt1a
Cpt2
Mcad
Lcad
H
TNF-α (pg/ml)
IL-6 (pg/ml)
I
Liver
WAT
Tnfa
Il6
Il1b
Ccl2
Ccl3
Ccl5

Figure S2. CD24Fc therapy improves metabolic syndrome in DIO mice, related to Figure 2.

WT mice were fed a HFD for 8 weeks, then treated with CD24Fc or control IgGFc twice a week for 4 more weeks while continuing HFD.

(A) Body weight of mice in the indicated groups. n = 7 per group.

(B) TC, TG, LDL-C and HDL-C levels in the indicated groups. n = 7 per group.

(C) Fasting blood glucose levels of mice in the indicated groups. n = 7 per group.

(D) GTT and ITT results of mice in the indicated groups. n = 8 per group.

(E) Liver weight and liver/body weight ratio of mice in the indicated groups. n = 7 per group.

(F) Representative images of H&E staining of liver sections. Scale bar, 100 μm. Graph shows the quantitation of lipid droplet area. n = 7 per group.

(G) Relative mRNA levels of key metabolic genes in the livers from mice in the indicated groups. n = 6 per group.

(H) Serum levels of inflammatory cytokines of mice in the indicated groups. n = 7 per group.

(I) Relative mRNA levels of inflammatory genes in liver and eWAT from mice in the indicated groups. n = 6 per group.

Data are mean ± SEM and representative of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, unpaired Student's t-test (A-C, E-I), two-way analysis of variance (ANOVA) (D).

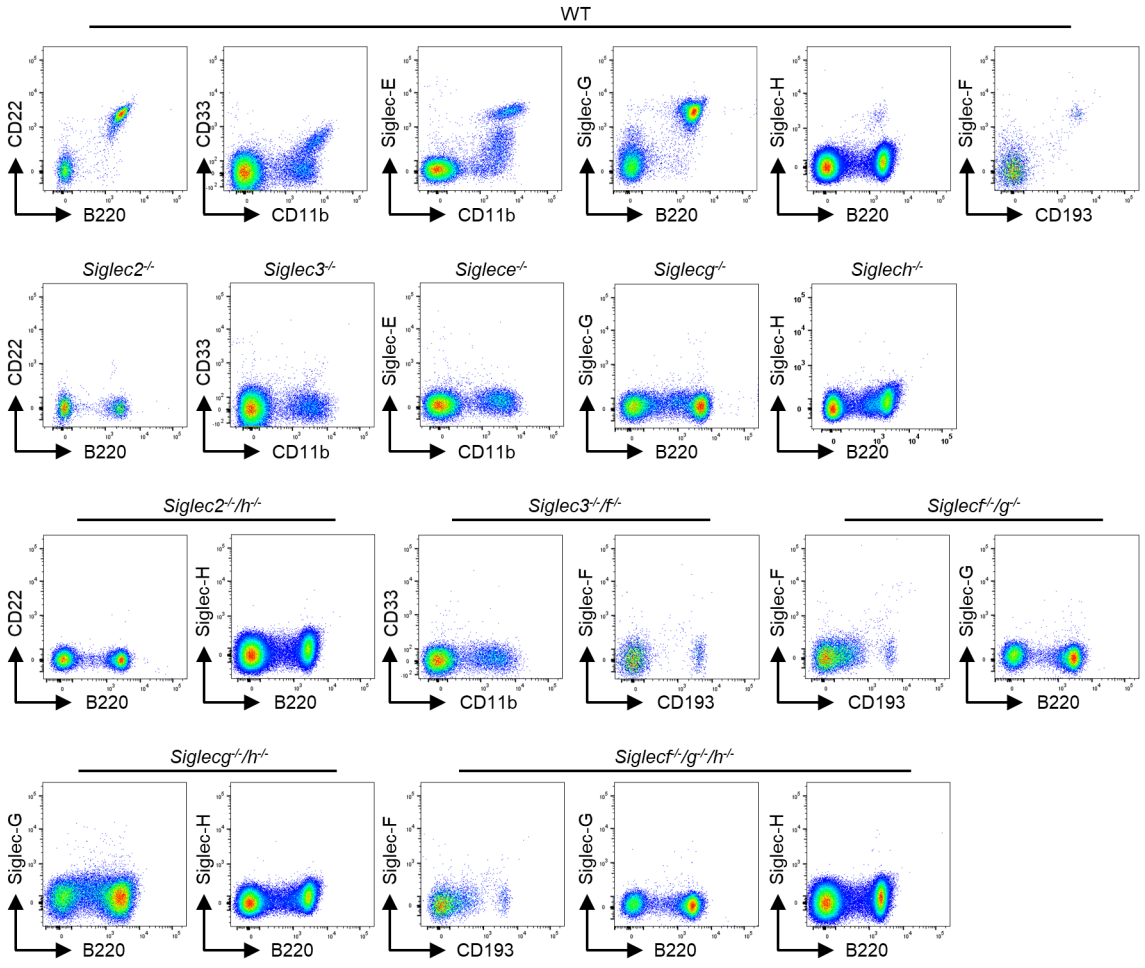

Figure S3. *Siglecs* mutations abrogate their cell surface expression, related to Figure 3.

Mice with mutations of *Siglec* genes were generated by CRISPR/Cas9 system. Siglec mutations were confirmed by flow cytometry of indicated strains. X-axis represents cell population and Y-axis represents Siglec expression.

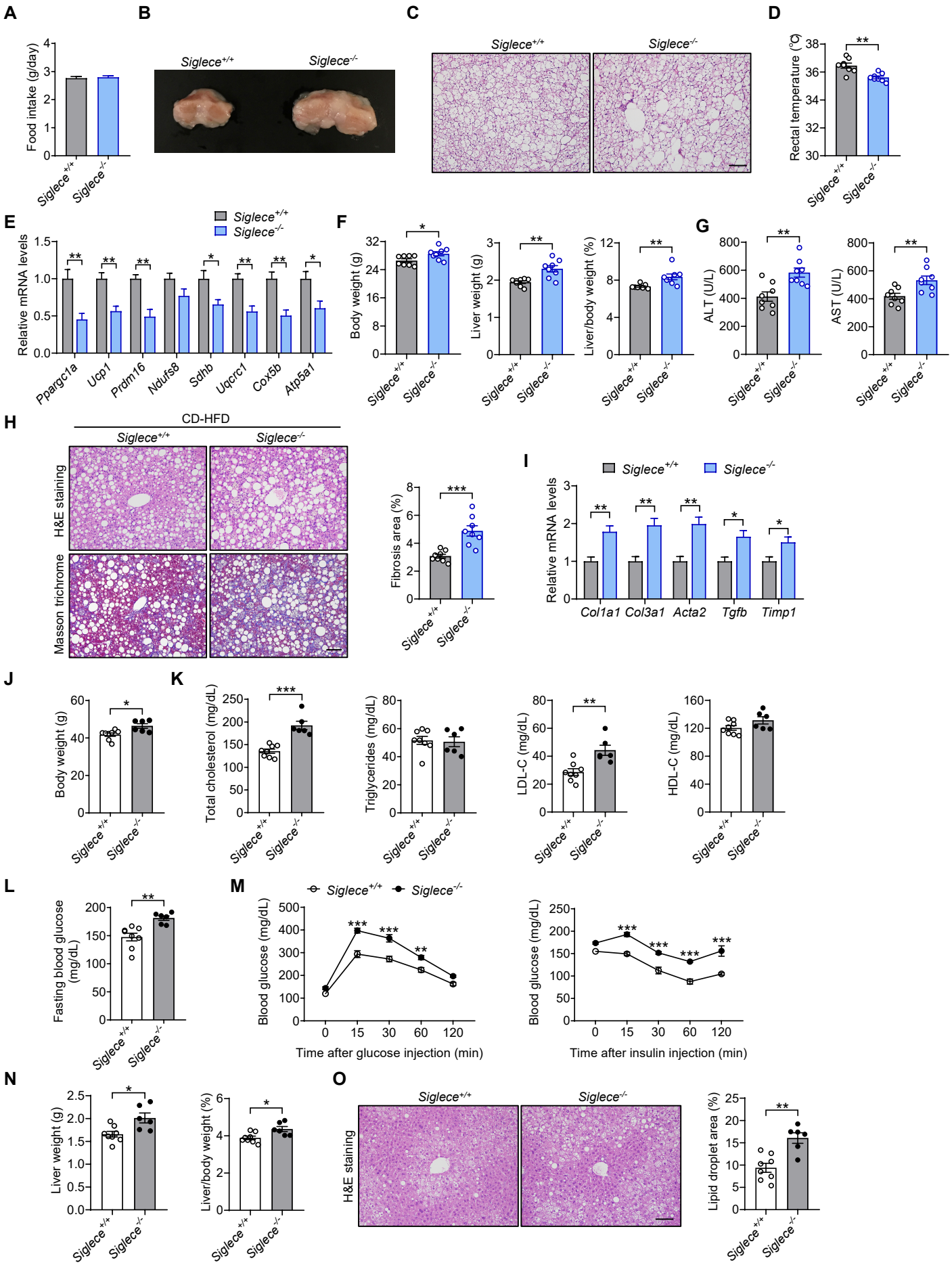
A
Food intake (g/day)
Siglece+/+
Siglece-/-
B
Siglece+/+
Siglece-/-
C
Siglece+/+
Siglece-/-
D
Rectal temperature (°C)
E
Siglece+/+
Siglece-/-
Relative mRNA levels
Ppargc1a
Ucp1
Prdm16
Ndufs8
Sdhb
Uqcrc1
Cox5b
Atp5a1
F
Body weight (g)
Liver weight (g)
Liver/body weight (%)
G
ALT (U/L)
AST (U/L)
H
CD-HFD
H&E staining
Masson trichrome
Fibrosis area (%)
I
Relative mRNA levels
Col1a1
Col3a1
Acta2
Tgfb
Timp1
J
Body weight (g)
K
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
LDL-C (mg/dL)
HDL-C (mg/dL)
L
Fasting blood glucose (mg/dL)
M
Blood glucose (mg/dL)
Time after glucose injection (min)
Time after insulin injection (min)
N
Liver weight (g)
Liver/body weight (%)
O
H&E staining
Lipid droplet area (%)

Figure S4. Metabolic phenotypes of *Siglece*$^{-/-}$ mice, related to Figure 3.

(A-E) *Siglece*$^{-/-}$ mice and WT littermate controls were fed a HFD for 12 weeks.

(A) Food intake of mice in the indicated groups. n = 5 per group.

(B) Representative images of interscapular BAT of mice in the indicated groups.

(C) Representative images of H&E staining of BAT sections. Scale bar, 100μm.

(D) Rectal temperature of mice in the indicated groups. n = 8 per group.

(E) Relative mRNA levels of thermogenic and mitochondrial genes in BAT from mice in the indicated groups. n = 6 per group.

(F-I) *Siglece*$^{-/-}$ mice and WT littermates were fed a CD-HFD for 12 weeks.

(F) Body weight, liver weight and liver/body weight ratio of mice in the indicated groups. n = 8 per group.

(G) Serum levels of ALT and AST in the indicated groups of mice. n = 8 per group.

(H) H&E and Masson's trichrome staining of liver sections. Scale bar, 100 μm. Graph shows the quantitation of liver fibrosis area. n = 8 per group.

(I) Relative mRNA levels of profibrotic genes in the livers from mice in the indicated groups. n = 6 per group.

(J-O) *Siglece*$^{-/-}$ mice and WT littermates were maintained on a normal chow diet for 15 months.

(J) Body weight of mice in the indicated groups. n = 6-8 per group.

(K) TC, TG, LDL-C and HDL-C levels in the indicated groups. n = 6-8 per group.

(L) Fasting blood glucose levels of mice in the indicated groups. n = 6-8 per group.

(M) GTT and ITT results of mice in the indicated groups. n = 7-8 per group.

(N) Liver weight and liver/body weight ratio of mice in the indicated groups. n = 6-8 per group.

(O) Representative images of H&E staining of liver sections. Scale bar, 100 μm. The lipid droplet area was quantified. n = 6-8 per group.

Data are mean ± SEM and representative of two or three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, unpaired Student's t-test (A, D-L, N, O) or two-way analysis of variance (ANOVA) (M).

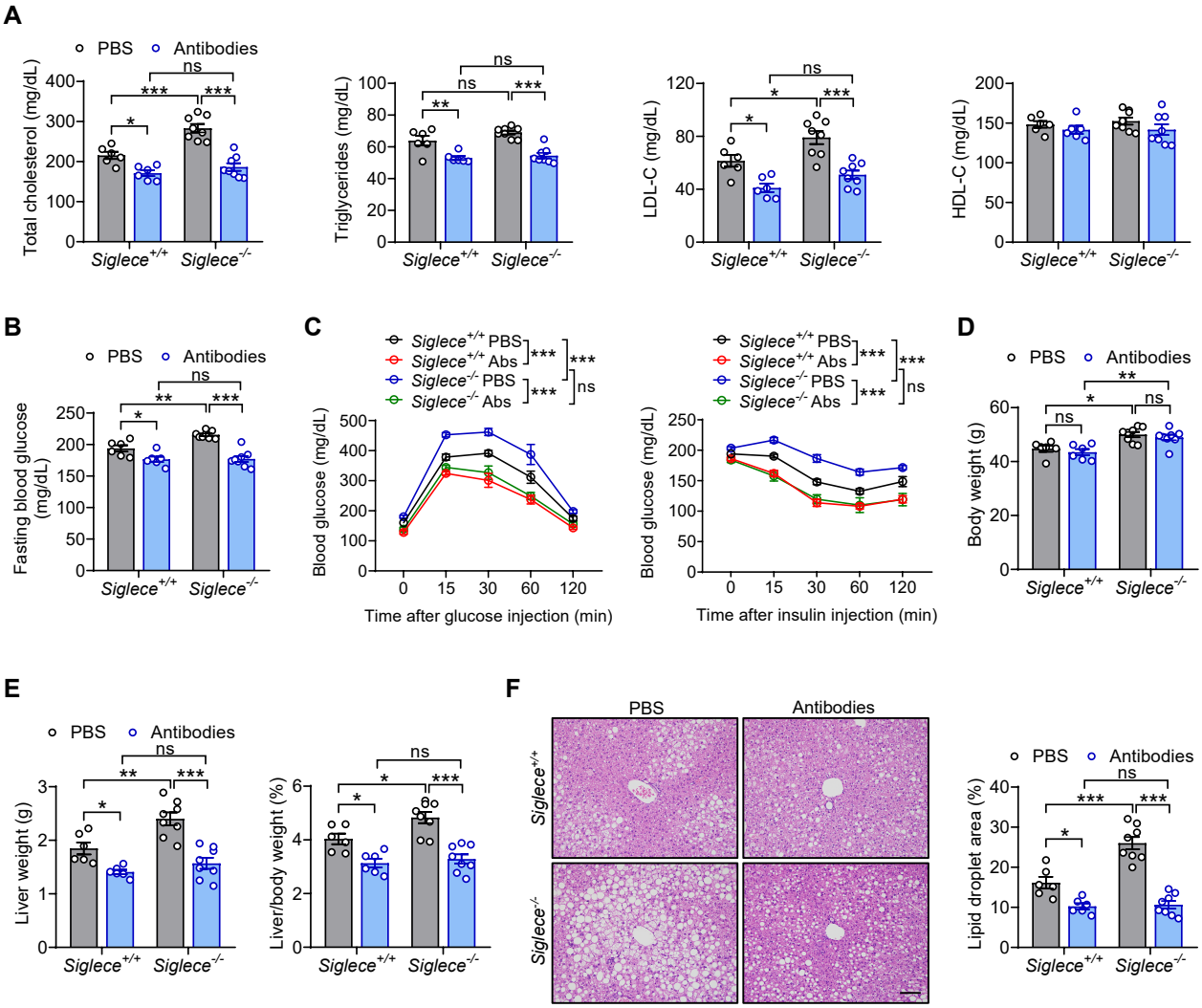
A
PBS
Antibodies
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
LDL-C (mg/dL)
HDL-C (mg/dL)
Siglece+/+
Siglece-/-
B
Fasting blood glucose (mg/dL)
C
Siglece+/+ PBS
Siglece+/+ Abs
Siglece-/- PBS
Siglece-/- Abs
Blood glucose (mg/dL)
Time after glucose injection (min)
Time after insulin injection (min)
D
Body weight (g)
E
Liver weight (g)
Liver/body weight (%)
F
Lipid droplet area (%)
ns

Figure S5. Blockade of inflammatory cytokines reverses diet-induced metabolic disorder in *Siglece*$^{-/-}$ mice, related to Figure 5.

Siglece$^{-/-}$ mice and WT littermates were fed a HFD for 8 weeks, followed by injection of neutralizing antibodies or PBS twice a week for 4 weeks while continuing HFD.

(A) TC, TG, LDL-C and HDL-C levels of mice. n = 6-8 per group.

(B) Fasting blood glucose levels of mice in the indicated groups. n = 6-8 per group.

(C) GTT and ITT results of mice in the indicated groups. n = 5-6 per group.

(D) Body weight of mice in the indicated groups. n = 6-8 per group.

(E) Liver weight and liver/body weight ratio of mice in the indicated groups. n = 6-8 per group.

(F) Representative images of H&E staining of liver sections. Scale bar, 100 μm. Graph shows the quantitation of lipid droplet area. n = 6-8 per group.

Data are mean ± SEM and representative of two independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001, two-way ANOVA.

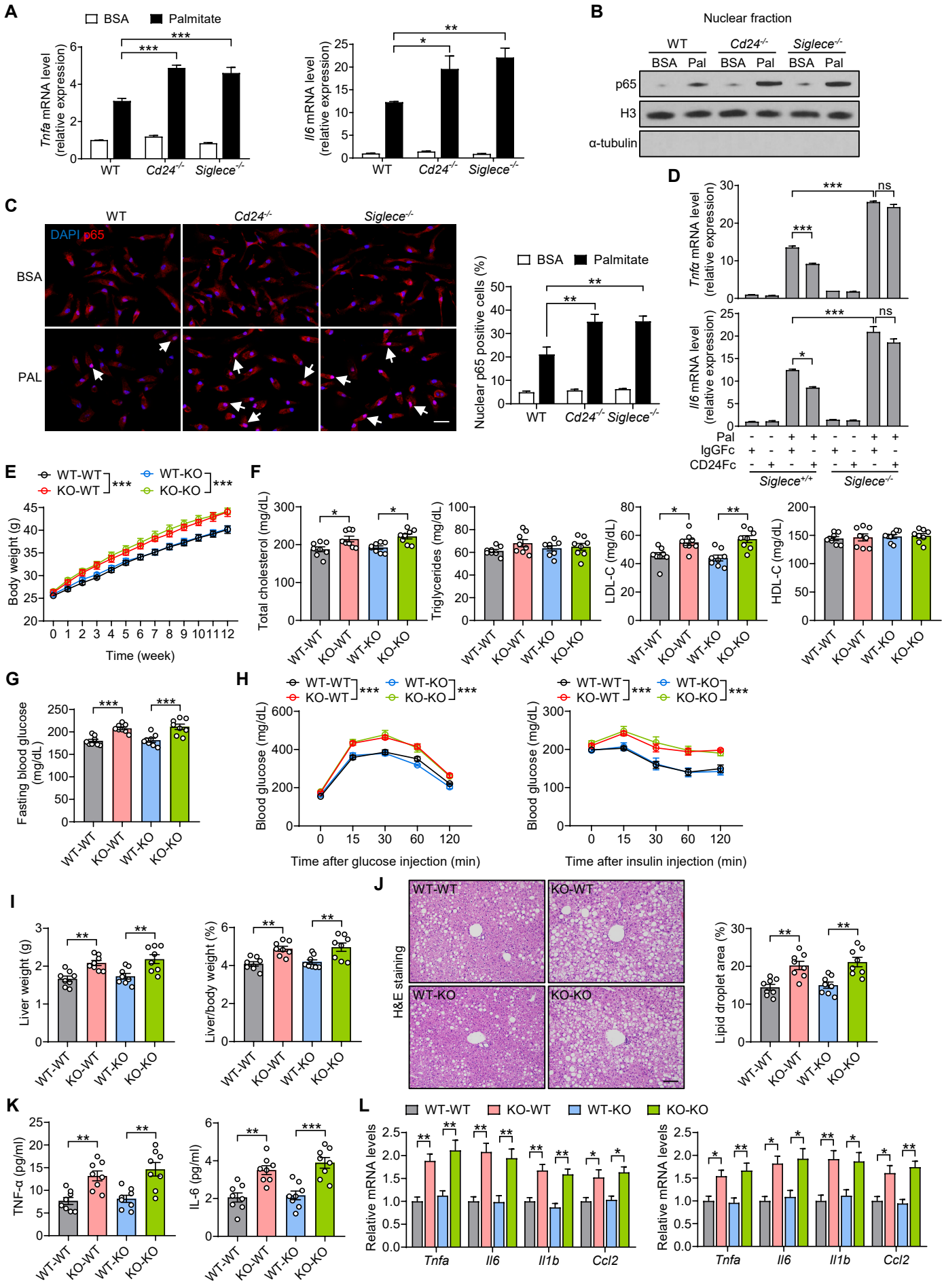
A
BSA
Palmitate
Tnfa mRNA level (relative expression)
Il6 mRNA level (relative expression)
WT
Cd24-/-
Siglece-/-
B
Nuclear fraction
WT
Cd24-/-
Siglece-/-
BSA
Pal
p65
H3
α-tubulin
C
DAPI p65
BSA
PAL
Nuclear p65 positive cells (%)
D
Tnfa mRNA level (relative expression)
Il6 mRNA level (relative expression)
ns
Pal
IgGFc
CD24Fc
Siglece+/+
Siglece-/-
E
WT-WT
KO-WT
WT-KO
KO-KO
Body weight (g)
Time (week)
F
Total cholesterol (mg/dL)
Triglycerides (mg/dL)
LDL-C (mg/dL)
HDL-C (mg/dL)
G
Fasting blood glucose (mg/dL)
H
Blood glucose (mg/dL)
Time after glucose injection (min)
Time after insulin injection (min)
I
Liver weight (g)
Liver/body weight (%)
J
H&E staining
Lipid droplet area (%)
K
TNF-α (pg/ml)
IL-6 (pg/ml)
L
Relative mRNA levels
Tnfa
Il6
Il1b
Ccl2

Figure S6. CD24 expressed in immune cells confers the improvement of metabolic disorder, related to Figure 6.

(A-C) Peritoneal macrophages from WT, *Cd24*$^{-/-}$ and *Siglece*$^{-/-}$ mice were stimulated with palmitate or BSA control for 16 hours before collection. .

(A) Relative mRNA levels of *Tnfa* and *Il6* in macrophages. n = 3 per group.

(B) Macrophages were fractionated to separate nuclear extract. The nuclear fraction of p65 was assessed by immunoblotting. H3 and α-tubulin were used as internal loading control of nuclear and cytoplasmic fractions, respectively.

(C) Representative immunofluorescence images of macrophages co-stained with p65 (red) and DAPI (blue). White arrows indicate co-localization of p65 and DAPI. Scale bar, 50 μm. Graph shows the percentage of nuclear p65 positive cells.

(D) Peritoneal macrophages from WT and *Siglece*$^{-/-}$ mice were stimulated with palmitate or BSA control, concurrently treated with CD24Fc or control IgG for 16 hours. mRNA levels of *Tnfα* and *Il6* were detected. n = 3 per group.

(E-L) Irradiated WT or *Cd24*$^{-/-}$ mice received bone marrow cells from either WT or *Cd24*$^{-/-}$ mice. After a 6-week recovery on a normal diet, mice were fed a HFD for an additional 12 weeks.

(E) Body weight of mice in the indicated groups. n = 8 per group.

(F) TC, LDL-C, HDL-C and TG levels of mice. n = 8 per group.

(G) Fasting blood glucose levels of mice in the indicated groups. n = 8 per group.

(H) GTT and ITT results of mice in the indicated groups. n = 6 per group.

(I) Liver weight and liver/body weight ratio of mice. n = 8 per group.

(J) Representative images of H&E staining of liver sections. Scale bar, 100 μm. Graph shows the quantitation of lipid droplet area. n = 8 per group.

(K) Serum concentrations of inflammatory cytokines of mice. n = 8 per group.

(L) Relative mRNA levels of inflammatory genes in liver (left) and eWAT (right) from mice in the indicated groups. n = 6 per group.

Data are mean ± SEM and representative of two or three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, one-way ANOVA (F, G, I-L) or two-way ANOVA (A, C-E, H).

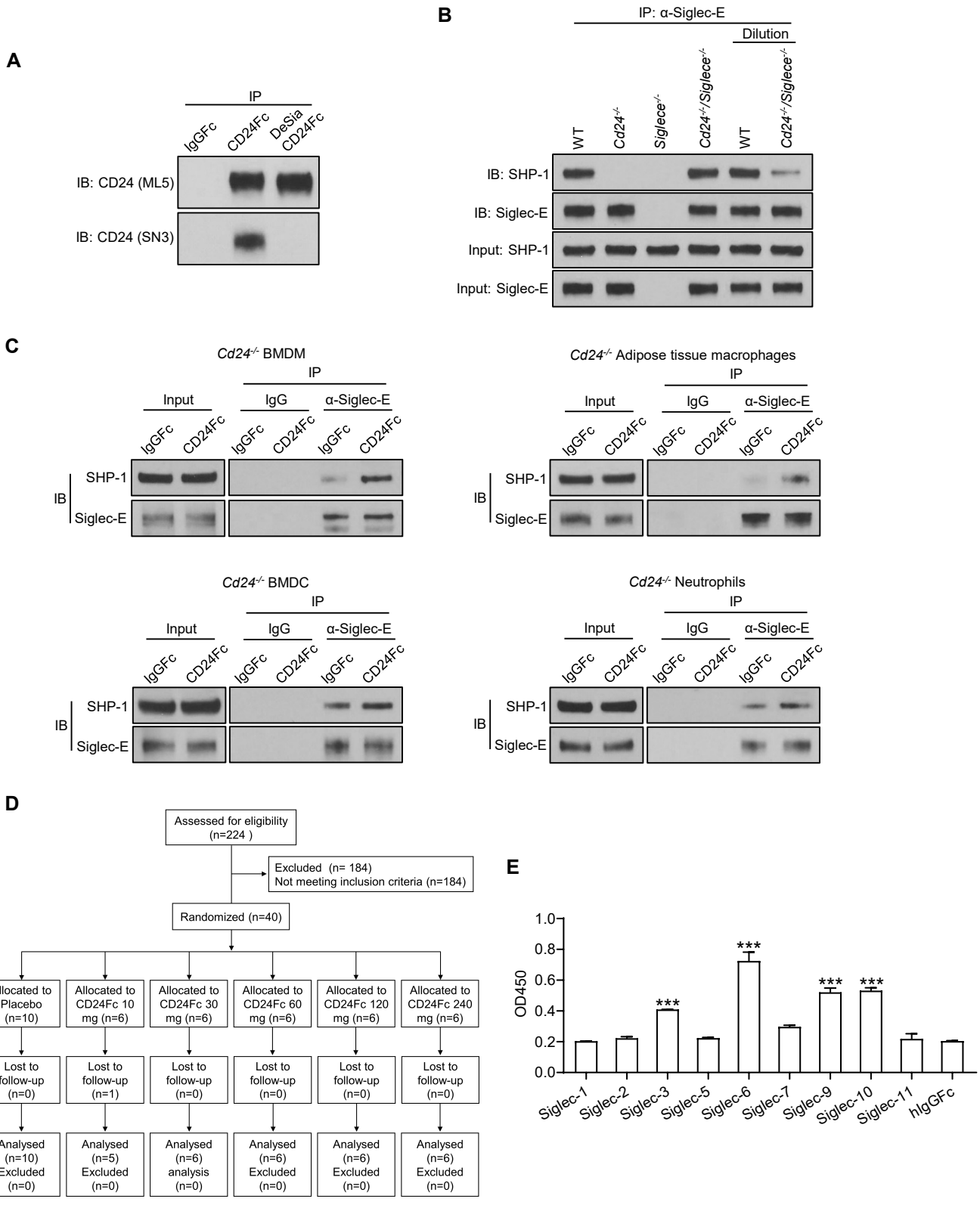
A
IP
IgGFc
CD24Fc
DeSia CD24Fc
IB: CD24 (ML5)
IB: CD24 (SN3)
B
IP: α-Siglec-E
Dilution
WT
Cd24-/-
Siglece-/-
Cd24-/-/Siglece-/-
WT
Cd24-/-/Siglece-/-
IB: SHP-1
IB: Siglec-E
Input: SHP-1
Input: Siglec-E
C
Cd24-/- BMDM
Cd24-/- Adipose tissue macrophages
Cd24-/- BMDC
Cd24-/- Neutrophils
IP
Input
IgG
α-Siglec-E
IgGFc
CD24Fc
IB
SHP-1
Siglec-E
D
Assessed for eligibility (n=224)
Excluded (n= 184)
Not meeting inclusion criteria (n=184)
Randomized (n=40)
Allocated to Placebo (n=10)
Allocated to CD24Fc 10 mg (n=6)
Allocated to CD24Fc 30 mg (n=6)
Allocated to CD24Fc 60 mg (n=6)
Allocated to CD24Fc 120 mg (n=6)
Allocated to CD24Fc 240 mg (n=6)
Lost to follow-up (n=0)
Lost to follow-up (n=1)
Lost to follow-up (n=0)
Lost to follow-up (n=0)
Lost to follow-up (n=0)
Lost to follow-up (n=0)
Analysed (n=10) Excluded (n=0)
Analysed (n=5) Excluded (n=0)
Analysed (n=6) analysis (n=0)
Analysed (n=6) Excluded (n=0)
Analysed (n=6) Excluded (n=0)
Analysed (n=6) Excluded (n=0)
E
OD450
1.0
0.8
0.6
0.4
0.2
0.0
***
Siglec-1
Siglec-2
Siglec-3
Siglec-5
Siglec-6
Siglec-7
Siglec-9
Siglec-10
Siglec-11
hIgGFc

Figure S7. Sialylated CD24 regulates Siglec-E-SHP-1 signaling, related to Figures 6 and 7.

(A) Recombinant CD24Fc was incubated with NanA or vehicle. The desialylation of CD24Fc was confirmed by immunoblotting with anti-CD24 antibodies that recognize total CD24 (ML5) or sialylated CD24 (SN3) respectively.

(B) Co-immunoprecipitation of Siglec-E and SHP-1 in WT, *Cd24*$^{-/-}$, *Siglece*$^{-/-}$ and *Cd24*$^{-/-}$*/Siglece*$^{-/-}$ co-culture spleen cells. In the dilution groups, WT and *Cd24*$^{-/-}$*/Siglece*$^{-/-}$ co-culture cells were diluted 200 times.

(C) Co-immunoprecipitation of Siglec-E and SHP-1 in *Cd24*$^{-/-}$ immune cells treated with CD24Fc or control IgGFc. The immune cells include bone marrow derived macrophage (BMDM), adipose tissue macrophages, bone marrow derived DC (BMDC) and neutrophils.

(D) CONSORT flow diagram. A total of 40 subjects were randomized, and 39 subjects completed the study. Ten subjects were randomized in the placebo group, and 6 subjects each were randomized in the CD24Fc 10 mg, 30 mg, 60 mg, 120 mg, and 240 mg groups. One subject in the CD24Fc 10 mg group withdrew early from the study due to other reasons.

(E) Direct interactions between CD24Fc and recombinant human Siglecs. n = 3 per group. Data are mean ± SEM and representative of two independent experiments. ***$p < 0.001$, one-way ANOVA.

Table S1. Summary of mutations in Siglec KO strains, related to Figures 3 and S3.

Mouse strain	Mutation (out-of-frame mutation)	Nucleotide alteration	Protein alteration
Siglec2$^{-/-}$	9 bp deletion, 1 bp insertion	c.386_394 deletion, C insertion	p.Ser22ArgfsTer26
Siglec3$^{-/-}$	1bp deletion	c.351 C deletion	p.Asp94ThrfsTer125
Siglech$^{-/-}$	5 bp deletion	c.209_213 deletion	p.Cys53ProfsTer55
Siglec2$^{-/-}$/*h*$^{-/-}$	Siglec2: 17 bp deletion	c.385_401 deletion	p.Ser22ArgfsTer23
	Siglech: 6 bp deletion, 2 bp insertion	c.209_214 deletion, GC insertion	p.Cys53ProfsTer59
Siglec3$^{-/-}$/*f*$^{-/-}$	Siglec3: 2bp deletion	c.351_352 CT deletion	p.Leu92TrpfsTer98
	Siglecf: 1bp insertion	c.127 A insertion	p.Glu32GlyfsTer72
Siglecf$^{-/-}$/*g*$^{-/-}$	Siglecf: 1bp insertion	c.130 A insertion	p.Glu32GlyfsTer72
	Siglecg: 1bp deletion	c.559 C deletion	p.Gln137LysfsTer141
Siglecg$^{-/-}$/*h*$^{-/-}$	Siglecg: 1bp insertion	c.450 T insertion	p.Gln137LeufsTer151
	Siglech: 1 bp deletion	c.210 C deletion	p.His54ThrfsTer60
Siglecf$^{-/-}$/*g*$^{-/-}$/*h*$^{-/-}$	Siglecf: 1bp insertion	c.130 A insertion	p.Glu32GlyfsTer72
	Siglecg: 2 bp deletion, 1 bp insertion	c.387_388 deletion, G insertion	p.Ser129ArgfsTer141
	Siglech: 1 bp deletion	c.210 C deletion	p.His54ThrfsTer60

Table S2. Primers for qPCR, related to Figures 1-3, 5, 7, S1, S2, S4 and S6.

Human		
Gene	**Forward primer**	**Reverse primer**
TNFα	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
IL6	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
CCL2	TTCTGTGCCTGCTGCTCAT	GGGGCATTGATTGCATCT
CCL3	GCAACCAGTTCTCTGCATCA	TGGCTGCTCGTCTCAAAGTA
CCL4	GCTTTTCTTACACCGCGAGGA	CCAGGATTCACTGGGATCAG
CCL5	ACACCAGTGGCAAGTGCTC	ACACACTTGGCGGTTCTTTC
CXCL4	TCCTGCCACTTGTGGTCGCCT	CCTTGATCACCTCCAGGCTGG
CXCL5	CAGACCACGCAAGGAGTTCATC	TTCCTTCCCGTTCTTCAGGGAG
ACTB	GACTTCGAGCAAGAGATGGCC	TGAAGGTAGTTTCGTGGATGCC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
Mouse		
Tnfα	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
Il6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
Il1b	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
Ccl2	GCTACAAGAGGATCACCAGCAG	GTCTGGACCCATTCCTTCTTGG
Ccl3	TGCCCTTGCTGTTCTTCTCTG	AGGCTGCTGGTTTCAAAATAGTC
Ccl5	CCTGCTGCTTTGCCTACCTCTC	ACACACTTGGCGGTTCCTTCGA
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Gapdh	CCGTAGACAAAATGGTGAAGGT	AACAATCTCCACTTTGCCACTG
Fas	CGGCTGCTGTTGGAAGTCA	TGCCTCTGAACCACTCACACC
Scd1	GCAAGCTCTACACCTGCCTCTT	CGTGCCTTGTAAGTTCTGTGGC
Acc1	GTTCTGTTGGACAACGCCTTCAC	GGAGTCACAGAAGCAGCCCATT
Hmgcs	GGAAATGCCAGACCTACAGGTG	TACTCGGAGAGCATGTCAGGCT
Hmgcr	GCTCGTCTACAGAAACTCCACG	GCTTCAGCAGTGCTTTCTCCGT
Ppara	TCCACGAAGCCTACCTGAAGA	AAGCGTCTTCTCGGCCATAC
Cpt1a	GCTGGCTTATCGTGGTGGT	CGCCACTCACGATGTTCTTC
Cpt2	AGAAGCCTCTCTTGAATGACAGC	TTCTGTTTATCCTGAGCGAGCA

Mcad	AGCAGAGAAGAAGGGTGACGAG	GGCTTCCACAATGAATCCAGTA
Lcad	TTCCTCGGAGCATGACATTTT	TGATGCCAAGCAAGCCCT
Col1a1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
Col3a1	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCAC
Acta2	TGCTGACAGAGGCACCACTGAA	CAGTTGTACGTCCAGAGGCATAG
Tgfb	TGATACGCCTGAGTGGCTGTCT	CACAAGAGCAGTGAGCGCTGAA
Timp1	TCTTGGTTCCCTGGCGTACTCT	GTGAGTGTCACTCTCCAGTTTGC
Ppargc1a	CACCAAACCCACAGAAAACAG	GGGTCAGAGGAAGAGATAAAGTTG
Ucp1	ACTCAGGATTGGCCTCTACGAC	AATGAACACTGCCACACCTCC
Prdm16	ATCCACAGCACGGTGAAGCCAT	ACATCTGCCCACAGTCCTTGCA
Ndufs8	GTTCATAGGGTCAGAGGTCAAG	TCCATTAAGATGTCCTGTGCG
Sdhb	ACCCCTTCTCTGTCTACCG	AATGCTCGCTTCTCCTTGTAG
Uqcrc1	ATCAAGGCACTGTCCAAGG	TCATTTTCCTGCATCTCCCG
Cox5b	ACCCTAATCTAGTCCCGTCC	CAGCCAAAACCAGATGACAG
Atp5a1	CATTGGTGATGGTATTGCGC	TCCCAAACACGACAACTCC