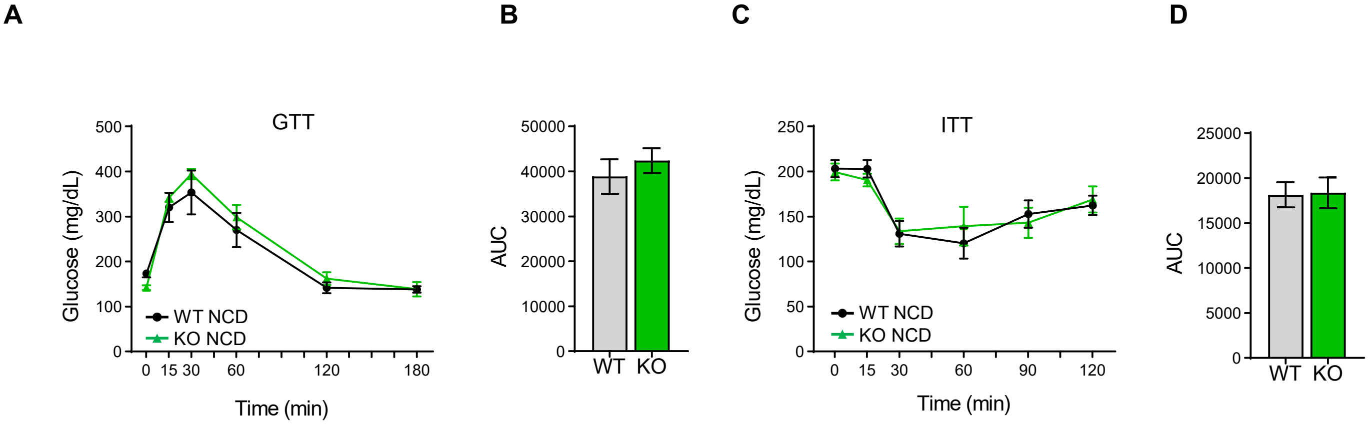
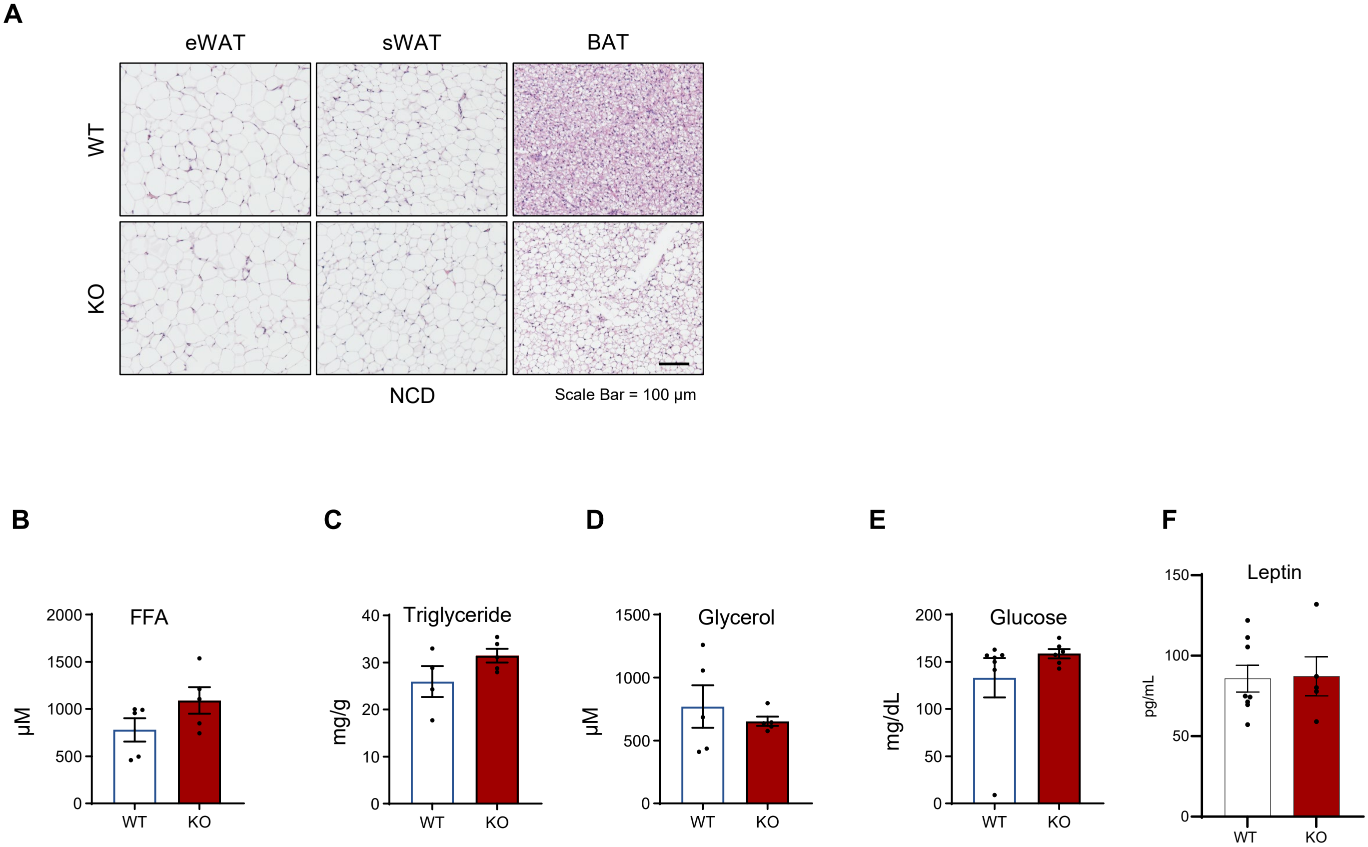
Supplementary Figure 1



(A, B) Glucose tolerance test (GTT) with the area under the curve (AUC) in NCD-fed WT and KO mice (n = 6/group).

(C, D) Insulin tolerance test (ITT) with AAC in NCD-fed WT and KO mice (n = 6/group). Unpaired Student's *t*-test.

Supplementary Figure 2



(A) Representative H&E staining of sWAT, eWAT, and BAT in the WT and KO mice fed on NCD. Scale bar = 100 μm.

(B) The levels of free fatty acids (FFA) in livers from chow-fed mice (n = 5 for WT, n = 5 for KO).

(C) The levels of triglyceride (TAG) in fasted serum from chow-fed mice (n = 5 for WT, n = 5 for KO).

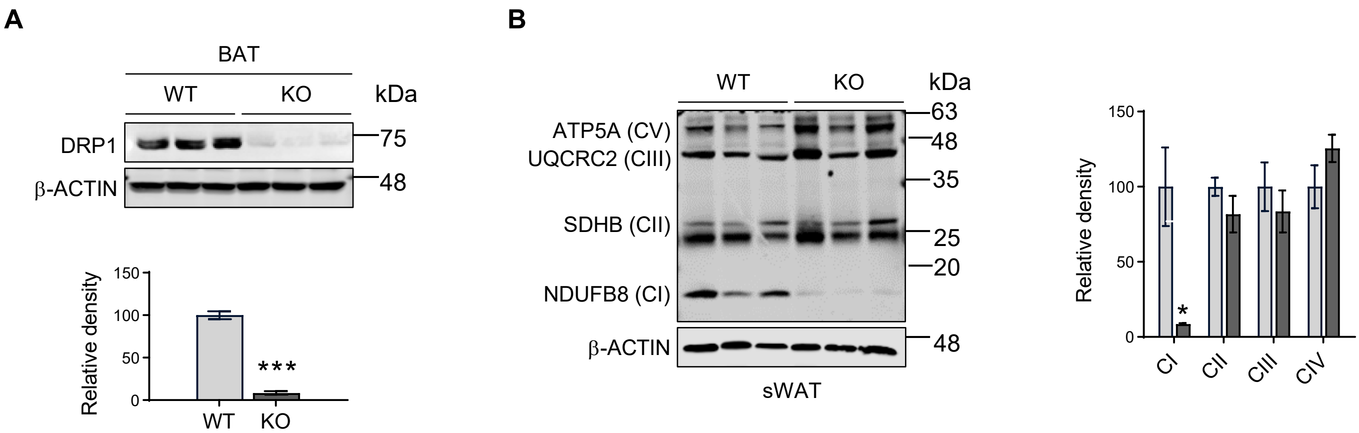
(D) The levels of glycerol in the liver of chow-fed mice (n = 5 for WT, n = 5 for KO).

(E) The levels of circulating glucose in chow-fed mice (n = 5 for WT, n = 5 for KO)

(F) The levels of leptin in the serum of chow-fed mice (n = 5 for WT, n=5 for KO).

For **(B)-(F):** unpaired student t-test, no statistical significance.

Supplementary Figure 3

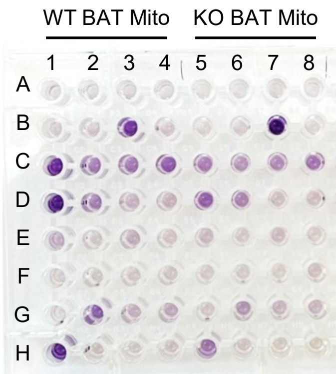


(A) Representative western blots and densitometric analysis of DRP1 in the BAT of HFD-fed WT and KO mice (n = 3/group).

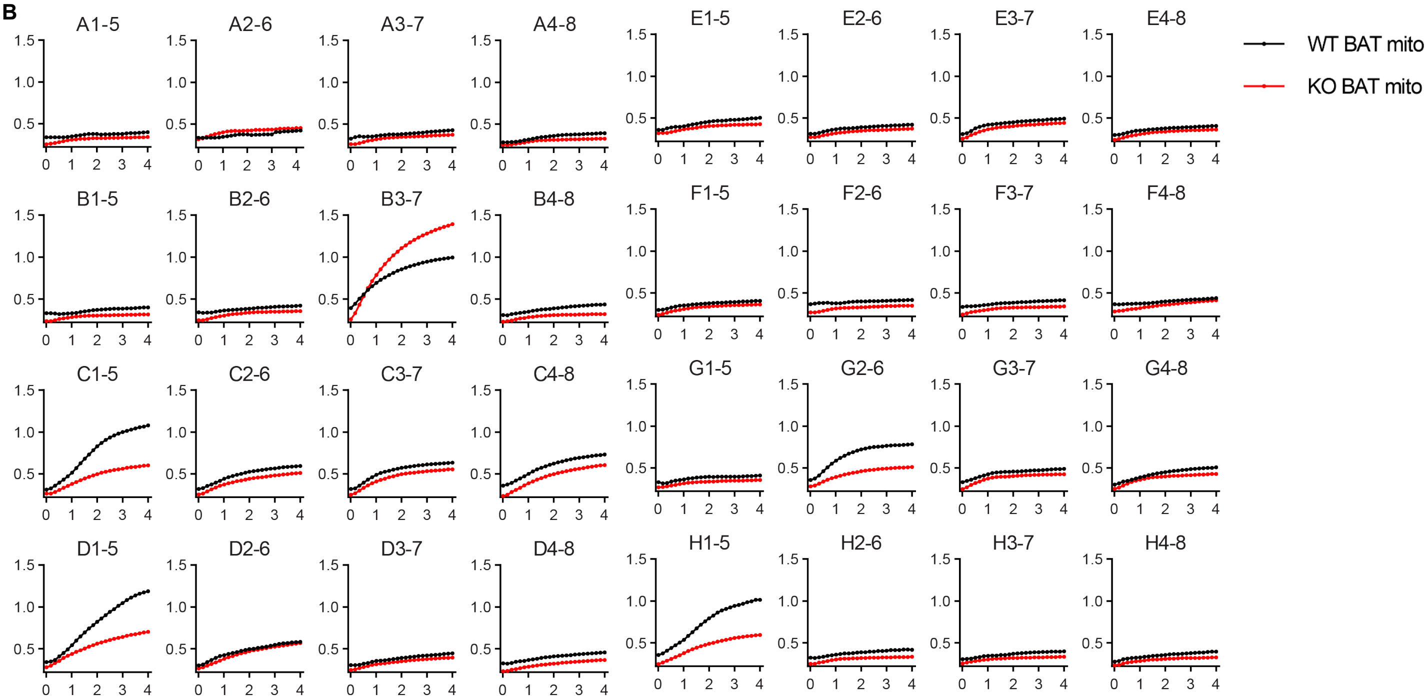
(B) Representative western blots and densitometric analysis of oxidative phosphorylation (OXPHOS) protein complexes in the sWAT of HFD-fed WT and KO mice (n = 3/group).
*P <0.05, ***P <0.001, unpaired Student's *t*-test.

Supplementary Figure 4

A



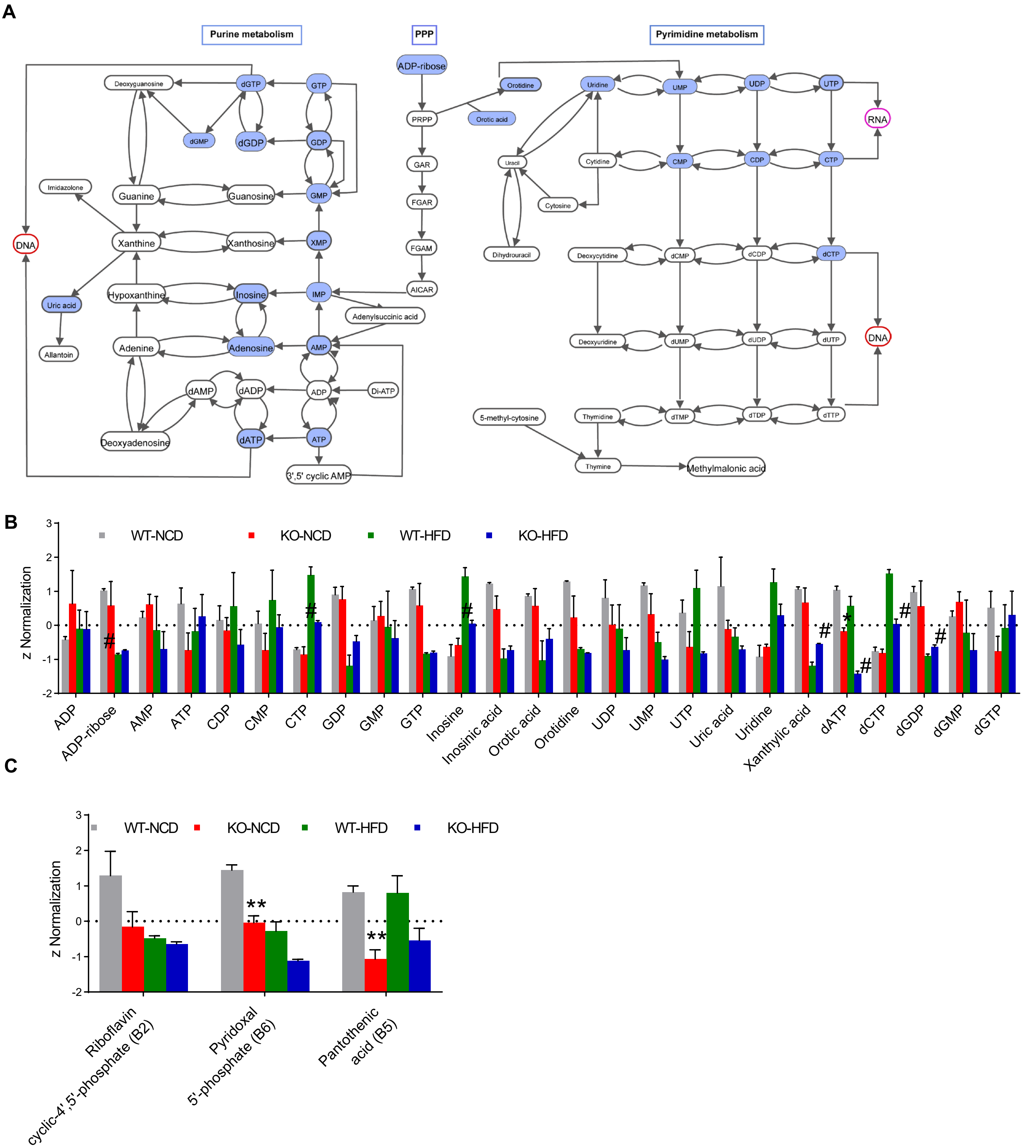
B



(A) Image of the color-reaction plates (Figure 4. X) after a 4-hour incubation. Redox Dye turned purple upon receiving the electrons from ETC when mitochondria utilized the indicated substrates. The information about precoated substrates for each well is attached in Table 1.

(B) Kinetics of electron transport in the isolated mitochondria in the presence of mitochondrial substrates. The reading for the entire analysis plate was shown. Mitochondria were isolated from the BAT of WT and KO mice fed on NCD.

Supplementary Figure 5



(A) Diagram of purine and pyrimidine metabolism pathways. Solid blue boxes indicate the metabolites detected.

(B) The levels of detected metabolites that are involved in purine and pyrimidine metabolism.

(C) Comparison of the levels of vitamin B2, B6, and B5 in WT and KO mice. n = 3/group, *P <0.05, **P <0.01, ***P <0.001, $^{\#}$P <0.05. KO v.s. WT (under HFD), one-way ANOVA followed by Turkey's post hoc test.

Supplementary Figure 6: List of substrates incubated with mitochondria from WT and KO

	Isolated mitochondria from the BAT of WT mice				Isolated mitochondria from the BAT of KO mice			
Substrates	1	2	3	4	5	6	7	8
A	No Substrate	α-D-Glucose	Glycogen	D-Glucose-1-PO4	No Substrate	α-D-Glucose	Glycogen	D-Glucose-1-PO4
B	D-Glucose-6-PO4	D-Gluconate-6-PO4	D,L-α-Glycerol-PO4	L-Lactic Acid	D-Glucose-6-PO4	D-Gluconate-6-PO4	D,L-α-Glycerol-PO4	L-Lactic Acid
C	Pyruvic Acid	Citric Acid	D,L-Isocitric Acid	cis-Aconitic Acid	Pyruvic Acid	Citric Acid	D,L-Isocitric Acid	cis-Aconitic Acid
D	α-Keto-Glutaric Acid	Succinic Acid	Fumaric Acid	L-Malic Acid	α-Keto-Glutaric Acid	Succinic Acid	Fumaric Acid	L-Malic Acid
E	α-Keto-Butyric Acid	D,L-β-Hydroxy-Butyric Acid	L-Glutamic Acid	L-Glutamine	α-Keto-Butyric Acid	D,L-β-Hydroxy-Butyric Acid	L-Glutamic Acid	L-Glutamine
F	Ala-Gln	L-Serine	L-Ornithine	Tryptamine	Ala-Gln	L-Serine	L-Ornithine	Tryptamine
G	L-Malic Acid	Acetyl-L-Carnitine + L-Malic Acid	Octanoyl-L-Carnitine + L-Malic Acid	Palmitoyl-D,L-Carnitine + L-Malic Acid	L-Malic Acid	Acetyl-L-Carnitine + L-Malic Acid	Octanoyl-L-Carnitine + L-Malic Acid	Palmitoyl-D,L-Carnitine + L-Malic Acid
H	Pyruvic Acid + L-Malic Acid	γ-Amino-Butyric Acid + L-Malic Acid	A-Keto-Isocaproic Acid + L-Malic Acid	L-Leucine + L-Malic Acid	Pyruvic Acid + L-Malic Acid	γ-Amino-Butyric Acid + L-Malic Acid	A-Keto-Isocaproic Acid + L-Malic Acid	L-Leucine + L-Malic Acid

Supplementary Figure 7: Primers used for the q-PCR analysis

Mouse	Forward (5'-3')	Reverse (5'-3')
β-Actin	GGCACCACACCTTCTACAATG	GGGGTGTTGAAGGTCTCAAAC
Drp1	GGGCACTTAAATTGGGCTCC	TGTATTCTGTTGGCGTGGAAC
Opa1	TCACCTCTGCGTTTATTTGAAGA	GGGTAGAACGGGAGGAAAGG
Mff	AAGTGGCTCTCACCCTAGCA	TGCCCCACTCACCAAATGT
Mfn1	TATCGATGCCTTGCGGAGAT	GGCGAATCACAACACTTCCA
Mfn2	GGAGACCAACAAGGACTGGA	TGCACAGTGACTTTCAACCG
Fis1	GGCTGTCTCCAAGTCCAAATC	GGAGAAAAGGGAAGGCGATG
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Fabp4	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Cd36	AGATGACGTGGCAAAGAACAG	CCTTGGCTAGATAACGAACTCTG
Atgl	ACCACCCTTTCCAACATGCTA	GGCAGAGTATAGGGCACCA
Hsl	TGGCACACCATTTTGACCTG	TTGCGGTTAGAAGCCACATAG
Mgl	ACCATGCTGTGATGCTCTCTG	CAAACGCCTCGGGGATAACC
Acc1	GATGAACCATCTCCGTTGGC	GACCCAATTATGAATCGGGAGTG
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
Scd1	AGATCTCCAGTTCTTACACGACCAC	GACGGATGTCTTCTTCCAGGTG
Dgat1	TTCCGCCTCTGGGCATT	AGAATCGGCCCACAATCCA
Dgat2	AGTGGCAATGCTATCATCATCGT	TCTTCTGGACCCATCGGCCCCAGGA
Il1b	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
Il10	GGGTTGCCAAGCCTTATCG	TCTCACCCAGGGAATTCAAATG
Cd86	TGTTTCCGTGGAGACGCAAG	TTGAGCCTTTGTAAATGGGCA
Cd206	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
Cd163	TCCACACGTCCAGAACAGTC	CCTTGGAAACAGAGACAGGC
Human	**Forward (5'-3')**	**Reverse (5'-3')**
ATF4	GTTCTCCAGCGACAAGGCTA	ATCCTGCTTGCTGTTGTTGG
BIP	TGTTCAACCAATTATCAGCAAACTC	TTCTGCTGTATCCTCTTCACCAGT
CHOP	AGAACCAGGAAACGGAAACAGA	TCTCCTTCATGCGCTGCTTT
uXBP1	CAGCACTCAGACTACGTGCA	ATCCATGGGGAGATGTTCTGG
sXBP1	CTGAGTCCGAATCAGGTGCAG	ATCCATGGGGAGATGTTCTGG
tXBP1	TGGCCGGGTCTGCTGAGTCCG	ATCCATGGGGAGATGTTCTGG