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Supplementary Materials for

Inhibition of sphingolipid de novo synthesis counteracts muscular dystrophy

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Other Supplementary Material for this manuscript includes the following:

Table S1

Supplementary figures

Figure S1. Sphinganine (left), dihydroceramide (middle), and ceramide (right) levels in quadriceps muscle of (**A**) 4 week old and (**B**) 10 week old C57/BL10 and *mdx* mice.

Figure S2. (**A**) Proportion of variance explained by each principal component of the sphingolipid *de novo* synthesis pathway in skeletal muscle of symptomatic DMD patients and controls (E-GEOD-38417). Levels of alanine aminotransferase (**B**) and aspartate aminotransferase (**C**) in WT mice, *mdx* mice, and *mdx* mice treated with myriocin. (**D**) Quantification of developed force at 25s (middle) of the fatigue protocol in EDL muscle of mice. Results are expressed relative to the developed force at the 1st tetanus. (**E**) Quantification of force drop during the 50s fatigue protocol in EDL muscle from WT (C57/BL10), *mdx*, and *mdx* mice treated with myriocin (force quantified every 10th tetanus). (**F**) Quantification of force drop during eccentric EDL muscle contractions in WT (C57/BL10), *mdx*, and *mdx* mice treated with myriocin. (**G**) Representative traces of cytosolic Ca^{2+} transients in isolated FDB muscle fibers upon 2.5 mM caffeine stimulation (SR Ca^{2+} store) and after 2 mM $CaCl_2$ (SOCE). (**H**) Ca^{2+} amplitude (SR Ca^{2+} store) upon 2.5 mM caffeine stimulation as percentage of C57/BL10. (**I**) Ca^{2+} amplitude (SR Ca^{2+} store) upon 40 μM histamine stimulation as percentage of C57/BL10. (**J**) Sarcoplasmic reticulum Ca^{2+} uptake after 2.5 mM caffeine stimulation as percentage of Ca^{2+} peak. All data are shown as mean ± SEM. Statistical significance is calculated using Student's two-tailed T test with BH adjustment for FDR. *BH FDR < 0.05, **BH FDR < 0.01, and ***BH FDR < 0.001.

Figure S3. Sphinganine (**A**), dihydroceramide (**B**), and ceramide (**C**) levels in primary bone marrow derived macrophages of C57/BL10 or *mdx* mice. For **A-C**, statistical significance is calculated using Student's two-tailed T test with BH adjustment for FDR. All data are shown as mean ± SEM. *BH FDR < 0.05, **BH FDR < 0.01, and ***BH FDR < 0.001.

Figure S4. Sphinganine (left), dihydroceramide (middle), and ceramide (right) levels in (**A**) diaphragm and (**B**) heart tissue of C57/BL10 or *mdx* mice. For **A-B**, statistical significance is calculated using Student's two-tailed T test with BH adjustment for FDR. All data are shown as mean ± SEM. *BH FDR < 0.05, **BH FDR < 0.01, and ***BH FDR < 0.001.

Figure S5. (**A**) Immunostaining of PDGFR, a marker of FAPs, and DAPI in diaphragm. Scale bar, 50 μm. (**B**) Immunostaining of PDGFR, and DAPI in TA. Scale bar, 50 μm.

Figure S1

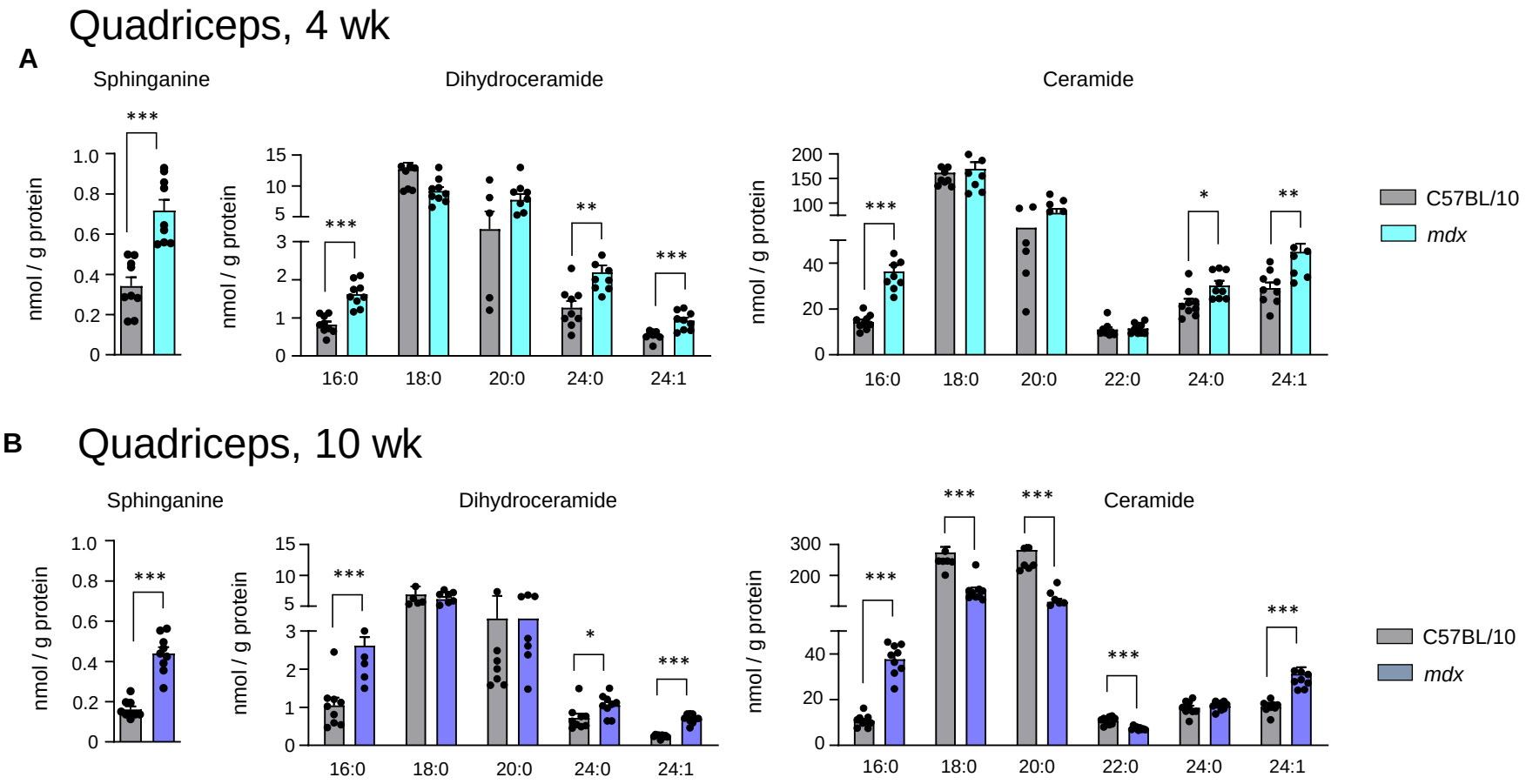
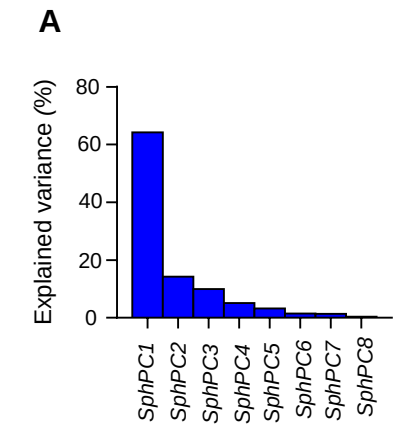
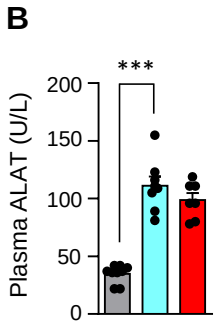
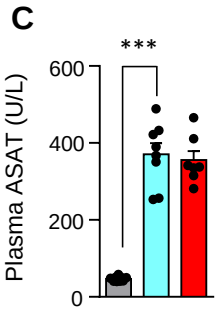
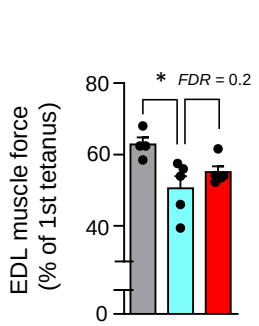

A
Quadriceps, 4 wk
Sphinganine
Dihydroceramide
Ceramide
nmol / g protein
16:0
18:0
20:0
22:0
24:0
24:1
C57BL/10
mdx
B
Quadriceps, 10 wk
Sphinganine
Dihydroceramide
Ceramide
nmol / g protein
16:0
18:0
20:0
22:0
24:0
24:1
C57BL/10
mdx

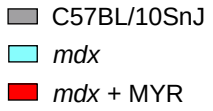
Figure S2

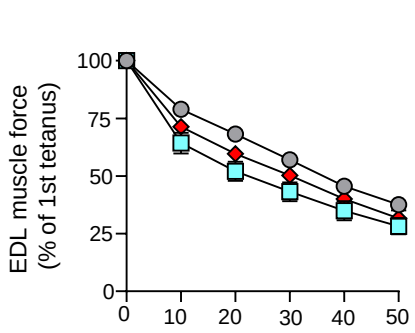

A
Explained variance (%)
80
60
40
20
0
SphPC1
SphPC2
SphPC3
SphPC4
SphPC5
SphPC6
SphPC7
SphPC8

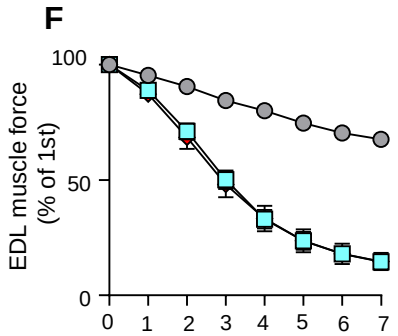

B
Plasma ALAT (U/L)
***
200
150
100
50
0

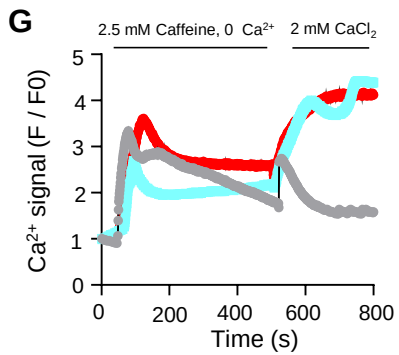

C
Plasma ASAT (U/L)
***
600
400
200
0

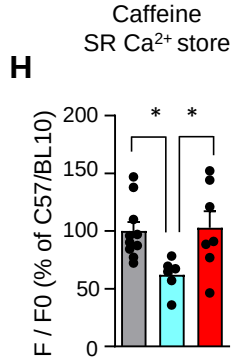

D
EDL muscle force
(% of 1st tetanus)
*
FDR = 0.2
80
60
40
0

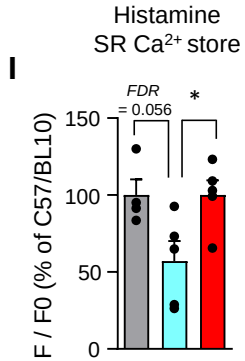

C57BL/10SnJ
mdx
mdx + MYR


E
EDL muscle force
(% of 1st tetanus)
100
75
50
25
0
0
10
20
30
40
50


F
EDL muscle force
(% of 1st)
100
50
0
0
1
2
3
4
5
6
7


G
2.5 mM Caffeine, 0 Ca2+
2 mM CaCl2
Ca2+ signal (F / F0)
5
4
3
2
1
0
0
200
400
600
800
Time (s)


H
Caffeine
SR Ca2+ store
F / F0 (% of C57/BL10)
*
*
200
150
100
50
0


I
Histamine
SR Ca2+ store
FDR
= 0.056
*
F / F0 (% of C57/BL10)
150
100
50
0

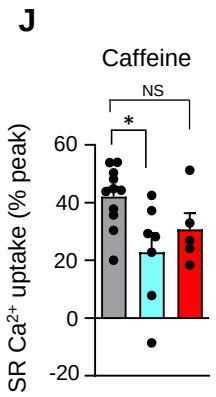

J
Caffeine
NS
*
SR Ca2+ uptake (% peak)
60
40
20
0
-20

Figure S3

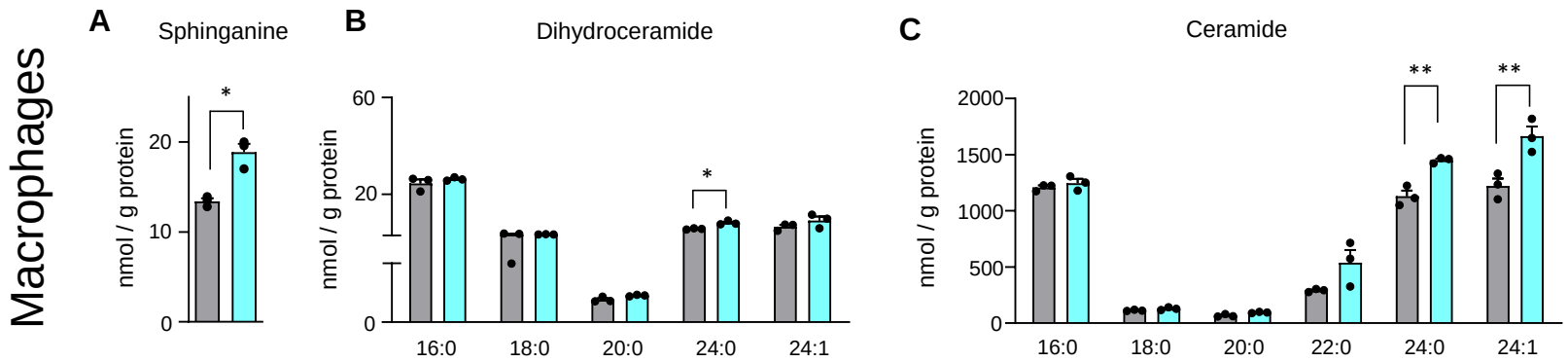

Macrophages
A
Sphinganine
nmol / g protein
0
10
20
*
B
Dihydroceramide
nmol / g protein
0
20
60
*
16:0
18:0
20:0
24:0
24:1
C
Ceramide
nmol / g protein
0
500
1000
1500
2000
**
**
16:0
18:0
20:0
22:0
24:0
24:1

Figure S4

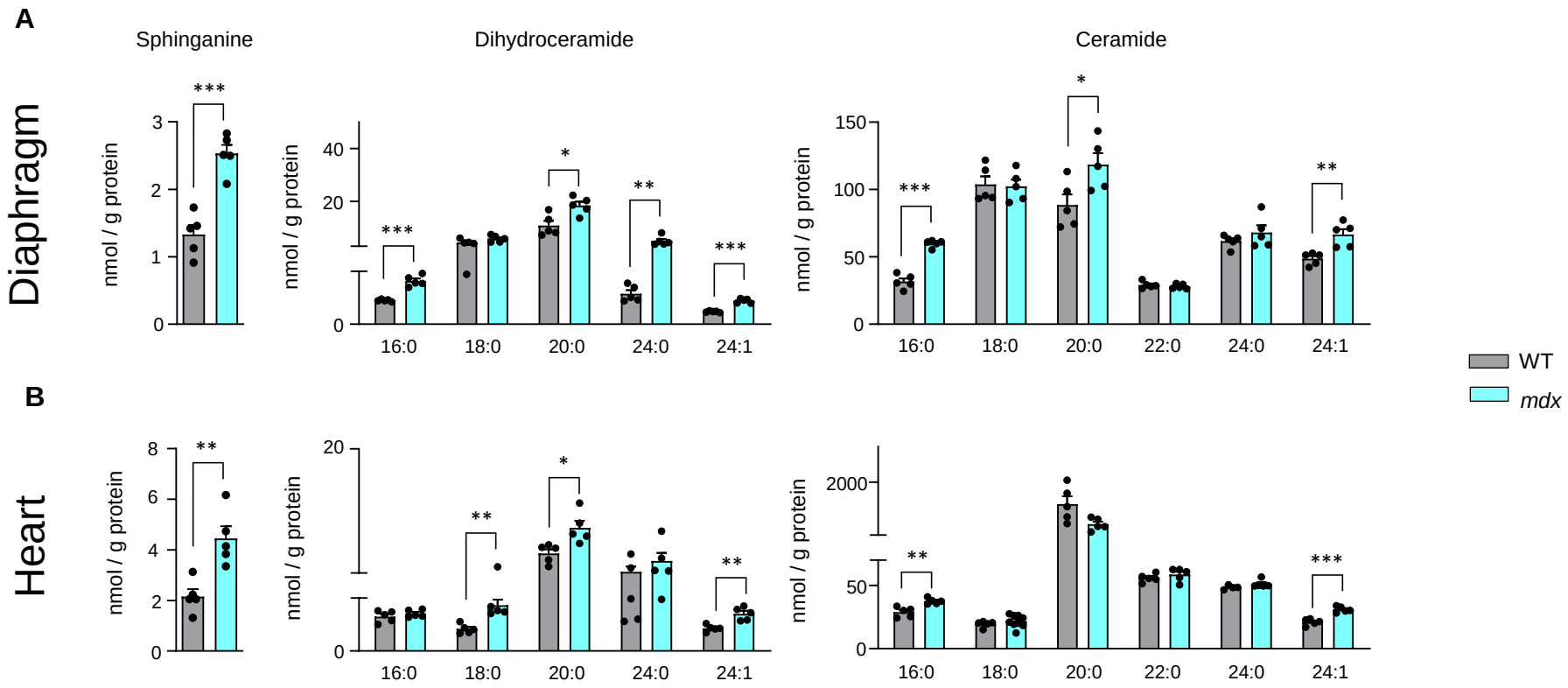
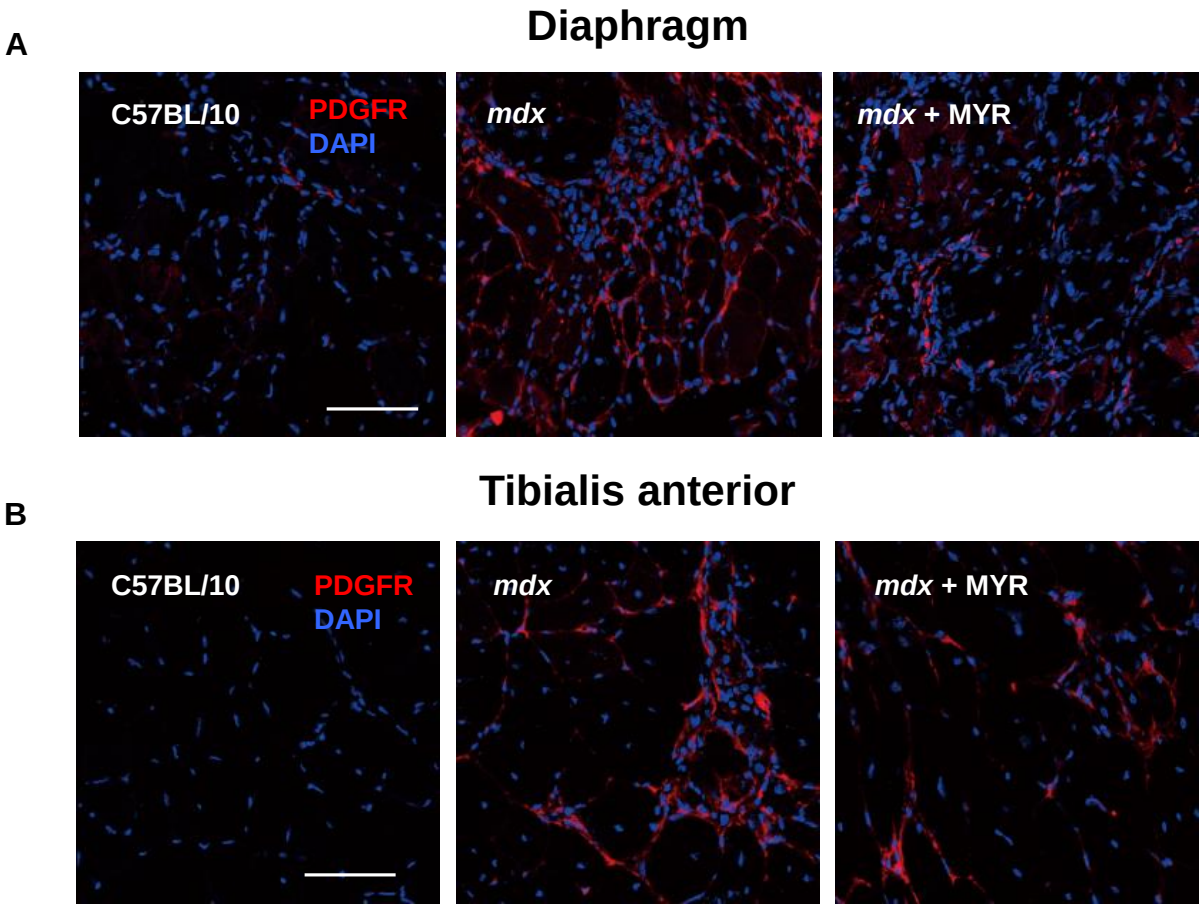

A
Diaphragm
Sphinganine
Dihydroceramide
Ceramide
nmol / g protein
16:0
18:0
20:0
22:0
24:0
24:1
B
Heart
WT
mdx

Figure S5


A
Diaphragm
C57BL/10
PDGFR
DAPI
mdx
mdx + MYR
B
Tibialis anterior
C57BL/10
PDGFR
DAPI
mdx
mdx + MYR

Supplementary Table 1. Pathways correlated with the first principal component of sphingolipid *de novo* synthesis pathway (please see separate file).

Supplementary Table 2. List of primers.

Gene symbol (mouse)	**Forward**	**Reverse**
Cd163	TCCACACGTCCAGAACAGTC	CCTTGGAAACAGAGACAGGC
Retnla	ACCTTTCCTGAGATTCTGCCCC	CAGTGGTCCAGTCAACGAGTAAGC
Il10	TGAATTCCCTGGGTGAGAAGCTGA	TGGCCTTGTAGACACCTTGGTCTT
Il6	GCCTTCTTGGGACTGATGCT	TGCCATTGCACAACTCTTTTCT
Il1b	TGCCATTGCACAACTCTTTTCT	GGTGGAGAGCTTTCAGCTCATAT
iNOS	CCCTTCAATGGTTGGTACATGG	ACATTGATCTCCGTGACAGCC
B2m	TTCTGGTGCTTGTCTCACTG	TATGTTCGGCTTCCCATTCT