Fig.1

(a)


Day
0
2
4
7
14
DOXORUBICIN
(INTRAVENOUSLY ADMINISTERED
FROM TAIL VEIN, 6mg/kg)
sacrifice
echocardiography

(b)

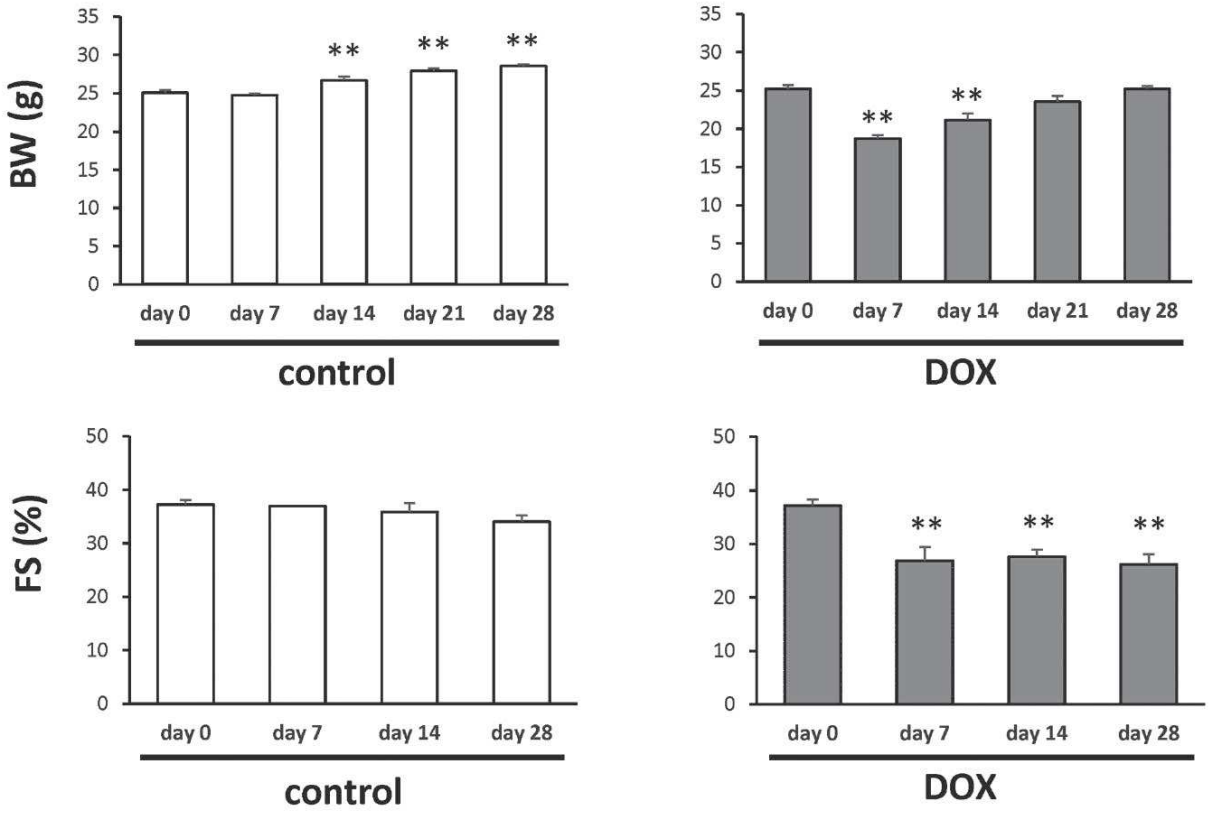
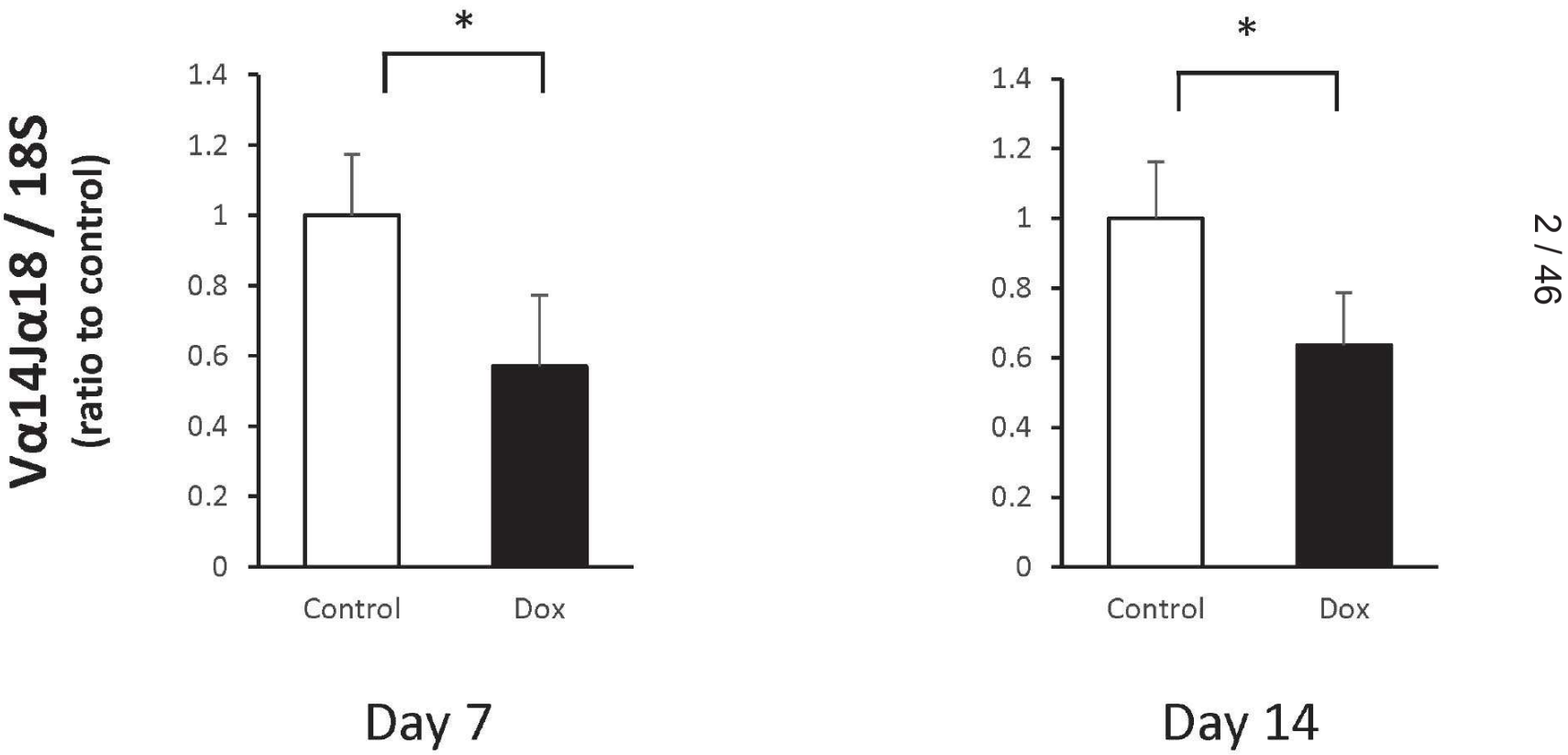

BW (g)
FS (%)
day 0
day 7
day 14
day 21
day 28
control
DOX
**

Fig.2


Vα14Jα18 / 18S
(ratio to control)
*
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
Dox
Day 7
*
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
Dox
Day 14

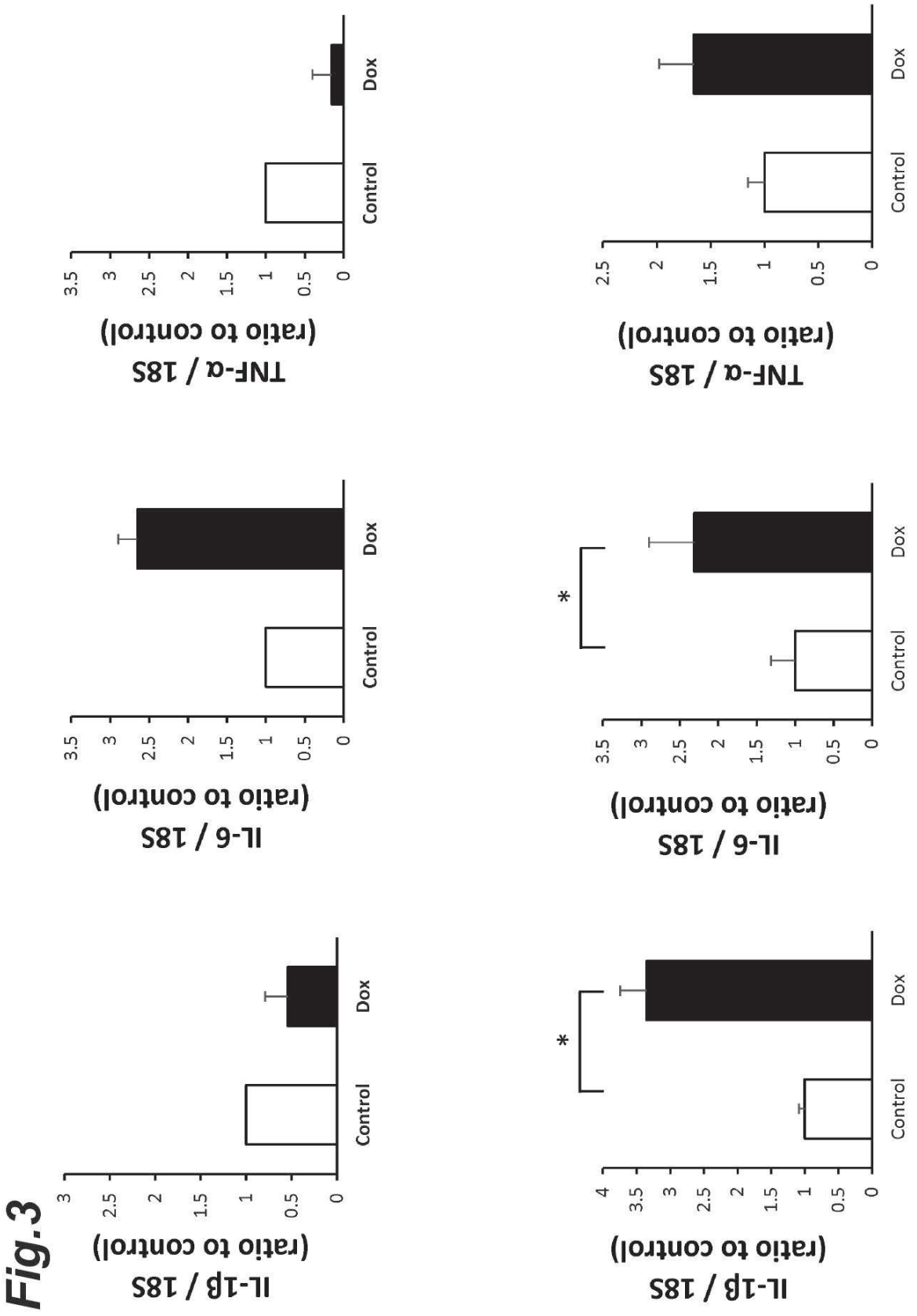
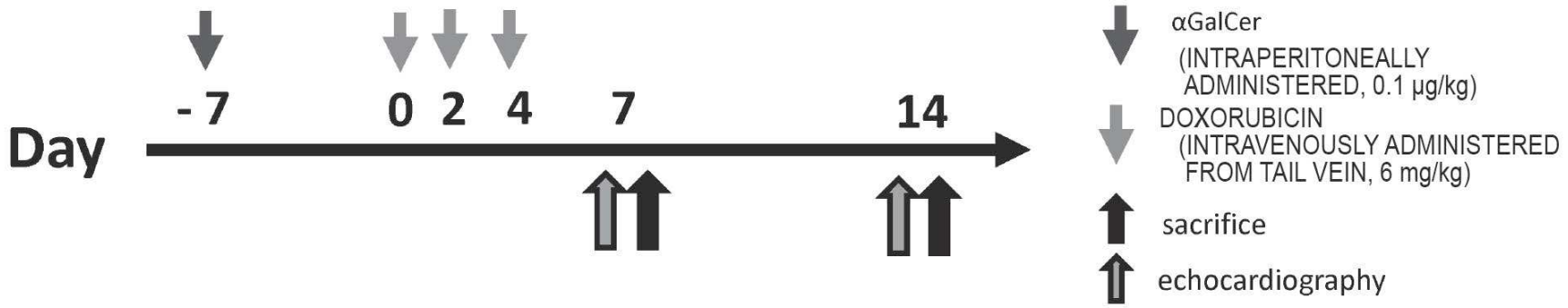

IL-1β / 18S
(ratio to control)
IL-6 / 18S
(ratio to control)
TNF-α / 18S
(ratio to control)
Control
Dox
*

Fig.3

Fig.4

(a)


Day
- 7
0
2
4
7
14
αGalCer
(INTRAPERITONEALLY ADMINISTERED, 0.1 μg/kg)
DOXORUBICIN
(INTRAVENOUSLY ADMINISTERED FROM TAIL VEIN, 6 mg/kg)
sacrifice
echocardiography

(b)

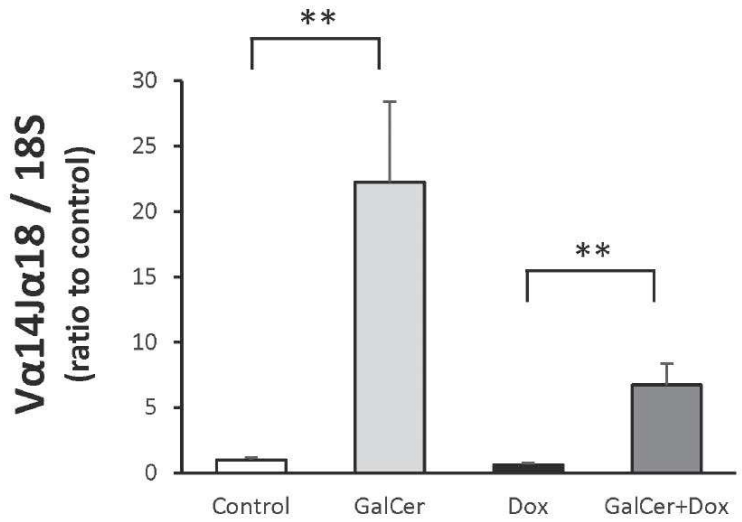
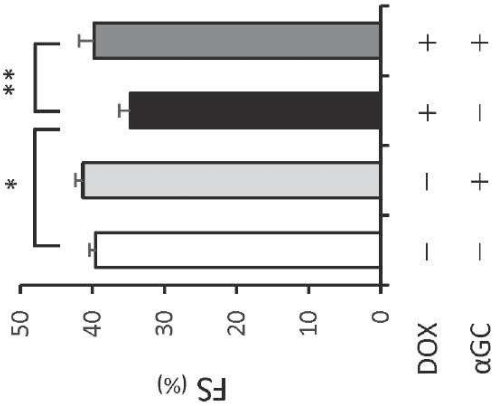
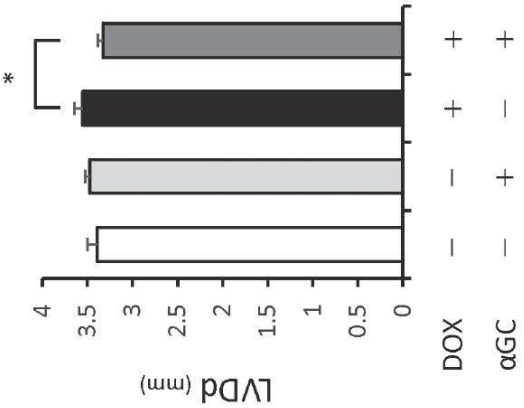
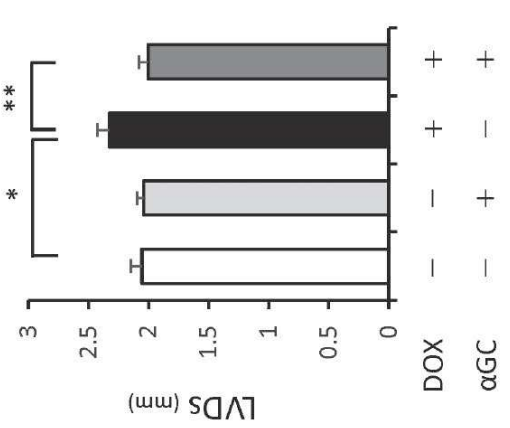

**
**
Vα14Jα18 / 18S
(ratio to control)
30
25
20
15
10
5
0
Control
GalCer
Dox
GalCer+Dox

Fig.5







Control	n=8
GalCer	n=9
Dox	n=9
GalCer/Dox	n=10

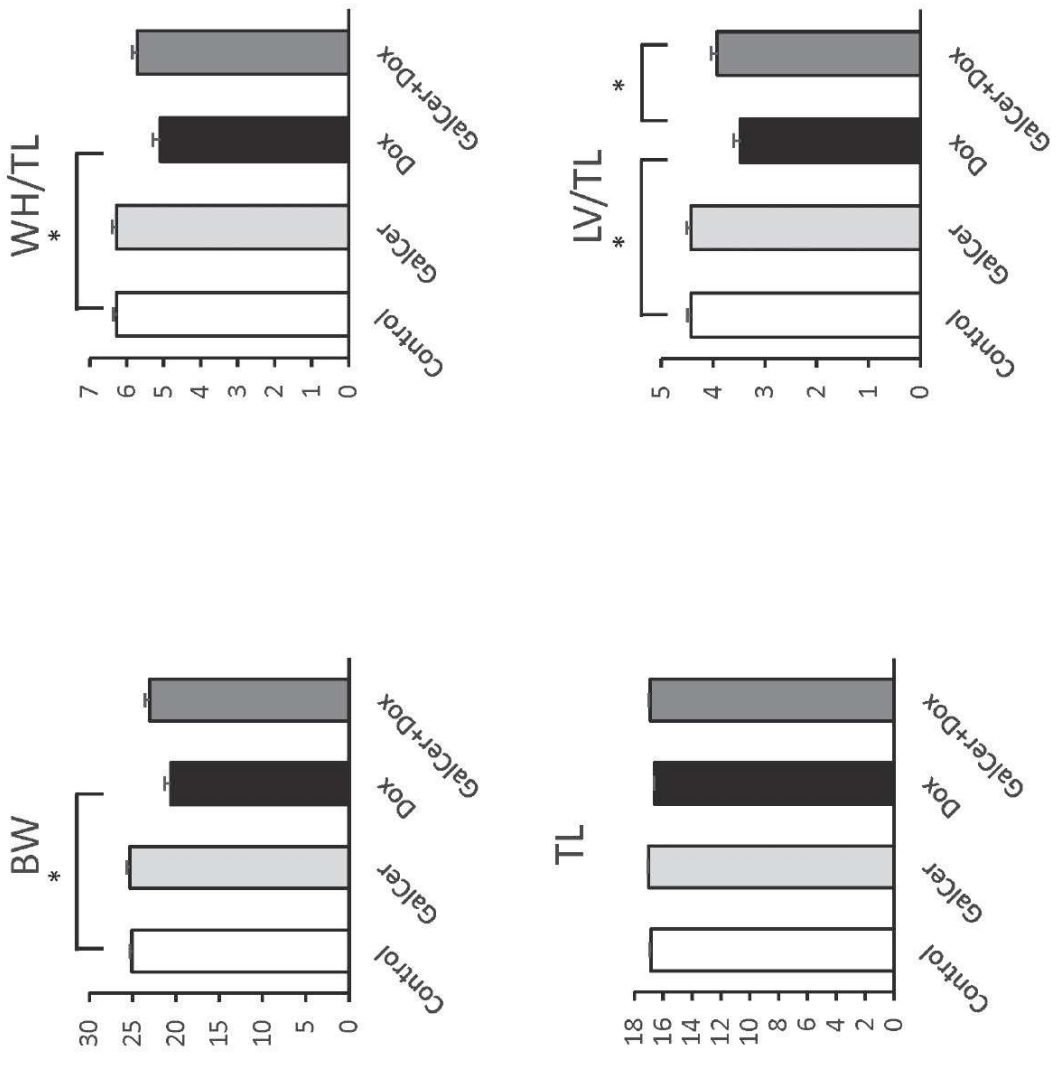

WH/TL
*
7
6
5
4
3
2
1
0
Control
Galcer
Dox
Galcer+Dox
LV/TL
*
*
5
4
3
2
1
0
Control
Galcer
Dox
Galcer+Dox
BW
*
30
25
20
15
10
5
0
Control
Galcer
Dox
Galcer+Dox
TL
18
16
14
12
10
8
6
4
2
0
Control
Galcer
Dox
Galcer+Dox

Fig.6

Fig.7

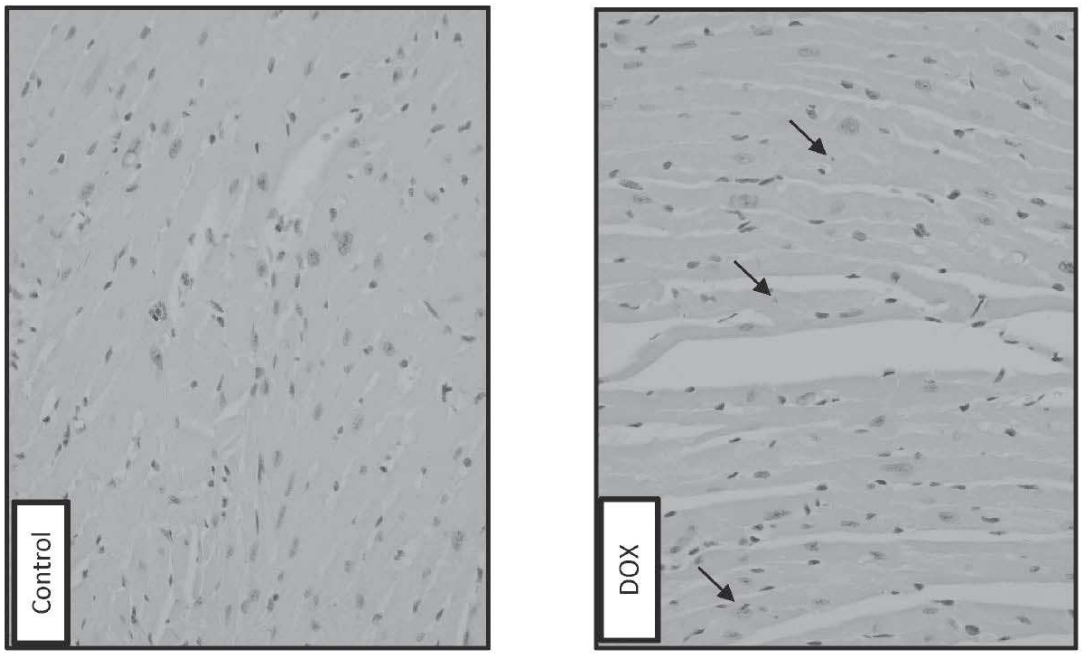
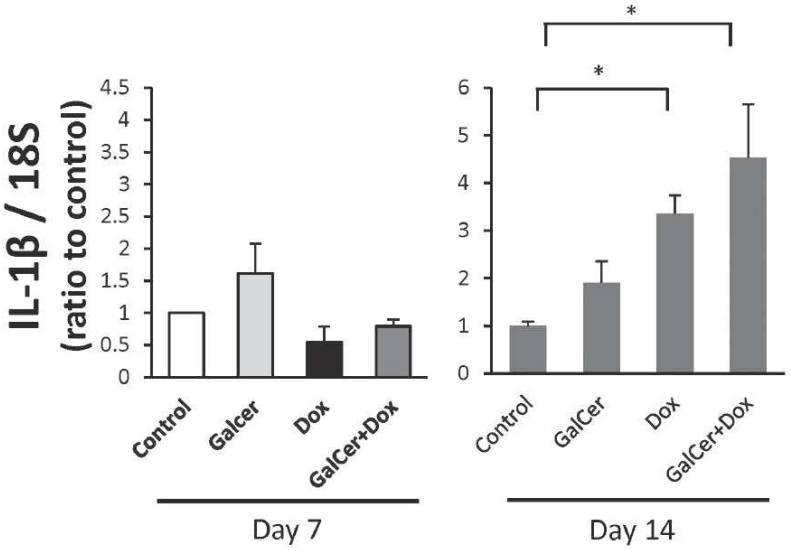
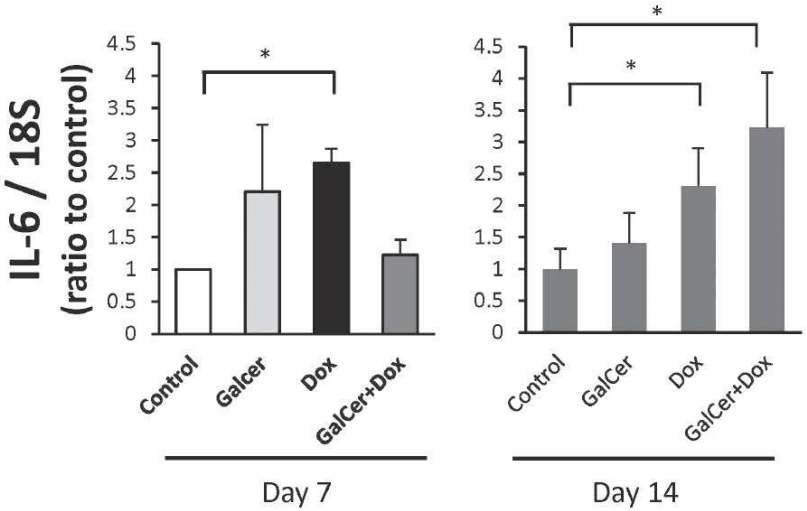


Fig.8


IL-1β / 18S
(ratio to control)
Control
Galcer
Dox
GalCer+Dox
Day 7
Control
GalCer
Dox
GalCer+Dox
Day 14


IL-6 / 18S
(ratio to control)
Control
Galcer
Dox
GalCer+Dox
Day 7
Control
GalCer
Dox
GalCer+Dox
Day 14

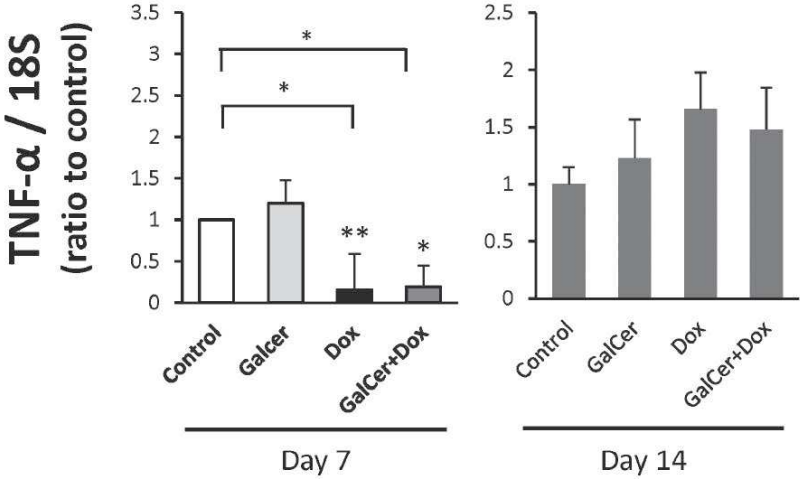
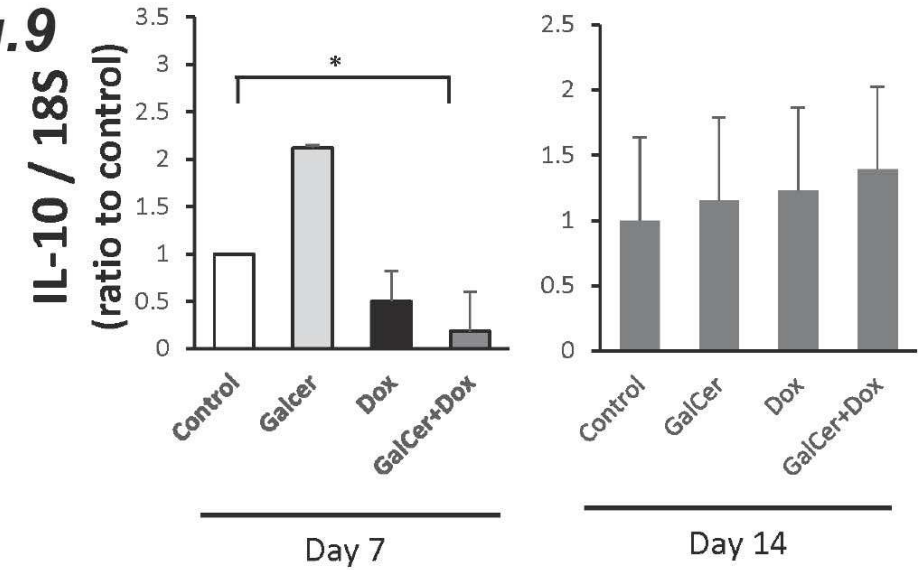
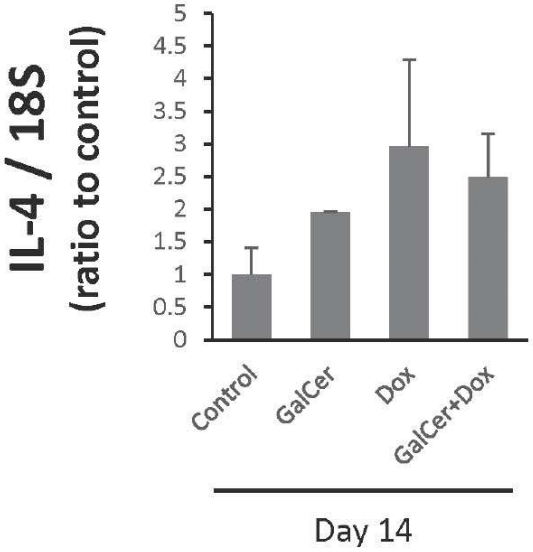
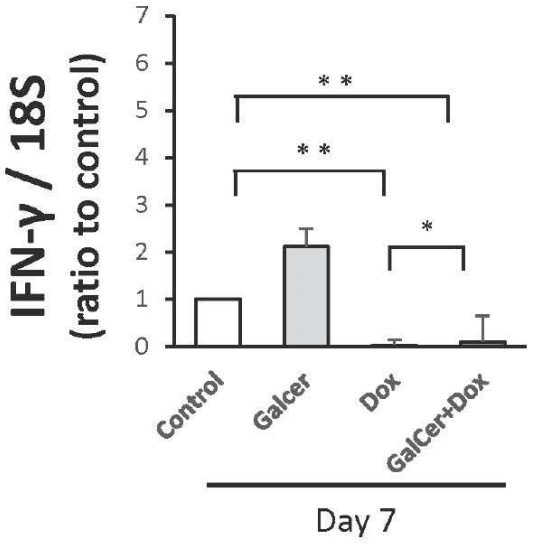

TNF-α / 18S
(ratio to control)
Control
Galcer
Dox
GalCer+Dox
Day 7
Control
GalCer
Dox
GalCer+Dox
Day 14

Fig.9


IL-10 / 18S
(ratio to control)
3.5
3
2.5
2
1.5
1
0.5
0
*
Control
Galcer
Dox
GalCer+Dox
Day 7

2.5
2
1.5
1
0.5
0
Control
GalCer
Dox
GalCer+Dox
Day 14


IL-4 / 18S
(ratio to control)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
GalCer
Dox
GalCer+Dox
Day 14


IFN-γ / 18S
(ratio to control)
7
6
5
4
3
2
1
0
* *
* *
*
Control
Galcer
Dox
GalCer+Dox
Day 7

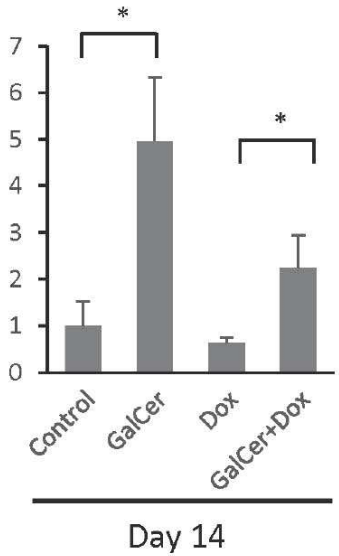
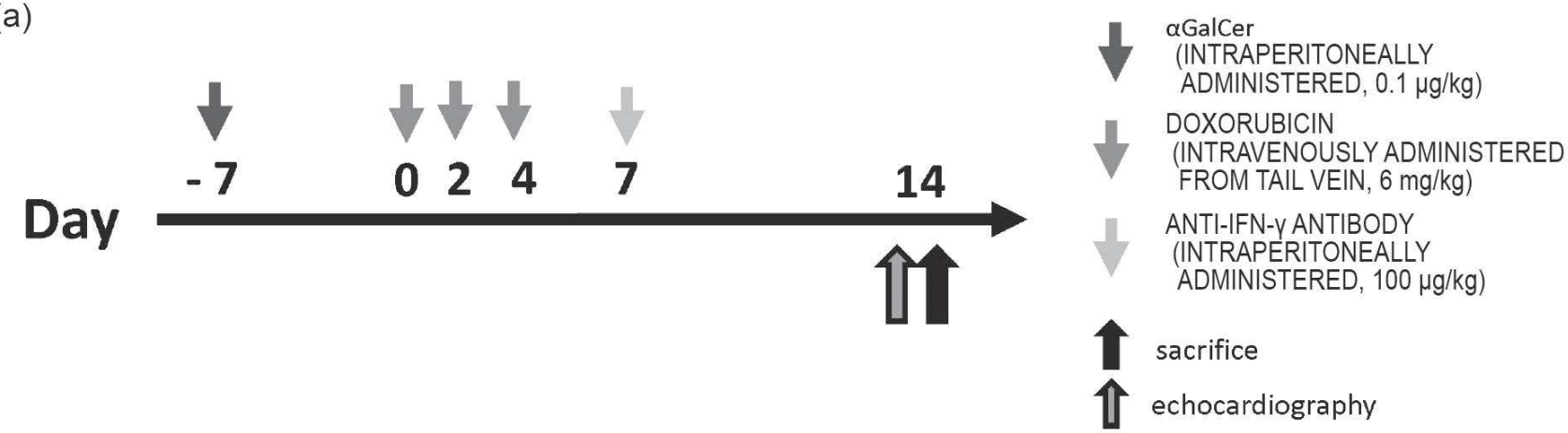

7
6
5
4
3
2
1
0
*
*
Control
GalCer
Dox
GalCer+Dox
Day 14

Fig.10

(a)


- 7
0 2 4
7
14
Day
αGalCer
(INTRAPERITONEALLY
ADMINISTERED, 0.1 μg/kg)
DOXORUBICIN
(INTRAVENOUSLY ADMINISTERED
FROM TAIL VEIN, 6 mg/kg)
ANTI-IFN-γ ANTIBODY
(INTRAPERITONEALLY
ADMINISTERED, 100 μg/kg)
sacrifice
echocardiography

(b)

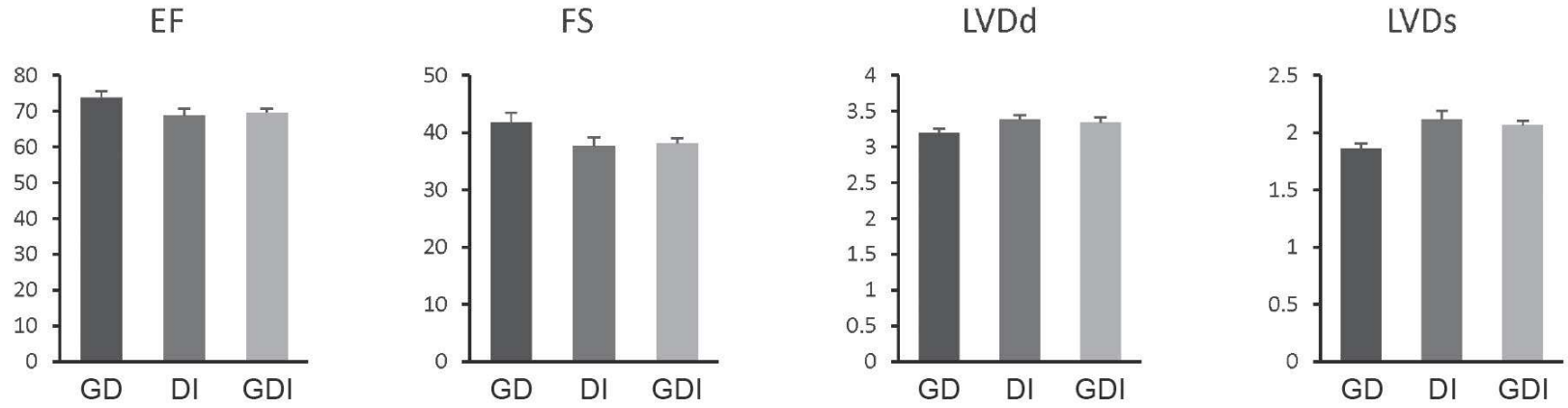
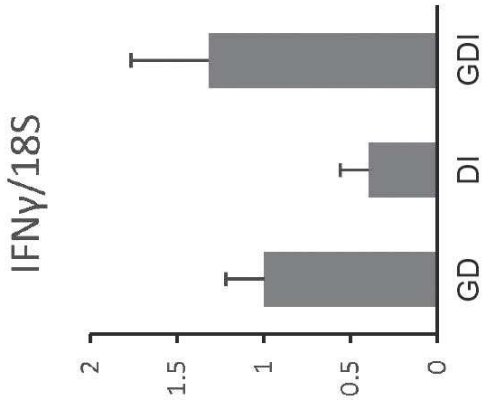

EF
80
70
60
50
40
30
20
10
0
GD
DI
GDI
FS
50
40
30
20
10
0
GD
DI
GDI
LVDd
4
3.5
3
2.5
2
1.5
1
0.5
0
GD
DI
GDI
LVDs
2.5
2
1.5
1
0.5
0
GD
DI
GDI

Fig.11



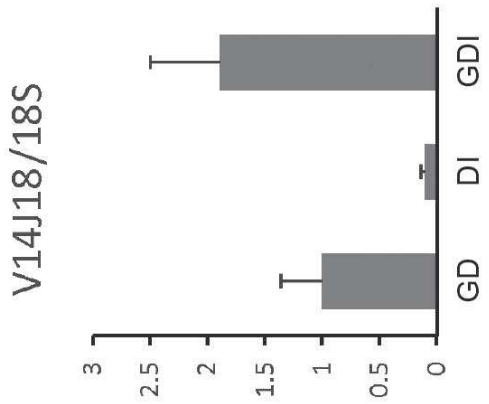


Fig.12

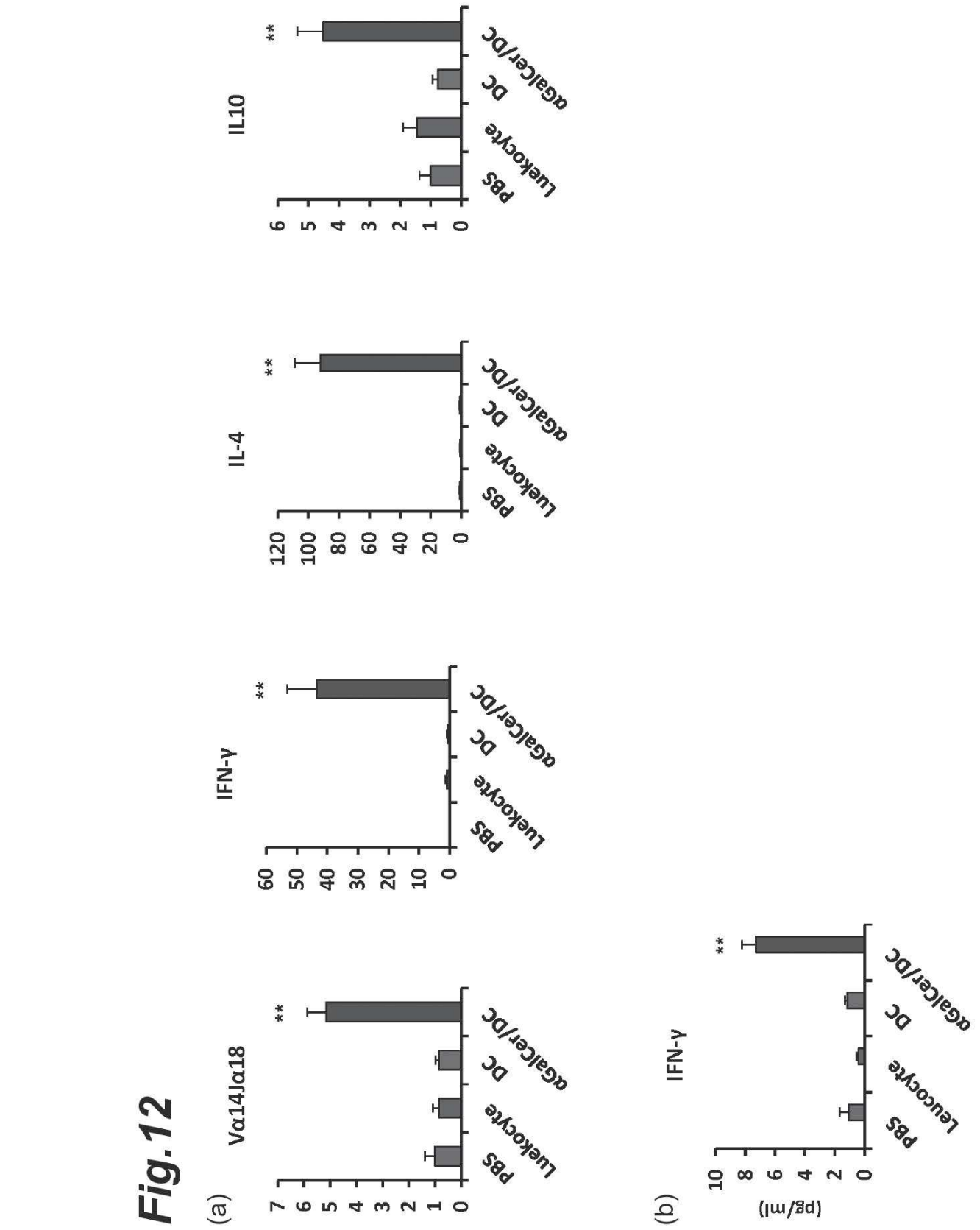

(a)
Vα14Jα18
IFN-γ
IL-4
IL10
PBS
Leukocyte
DC
αgalCer/DC
**
(b)
IFN-γ
(pg/ml)
PBS
Leucocyte
DC
αgalcer/DC
**

Fig.13

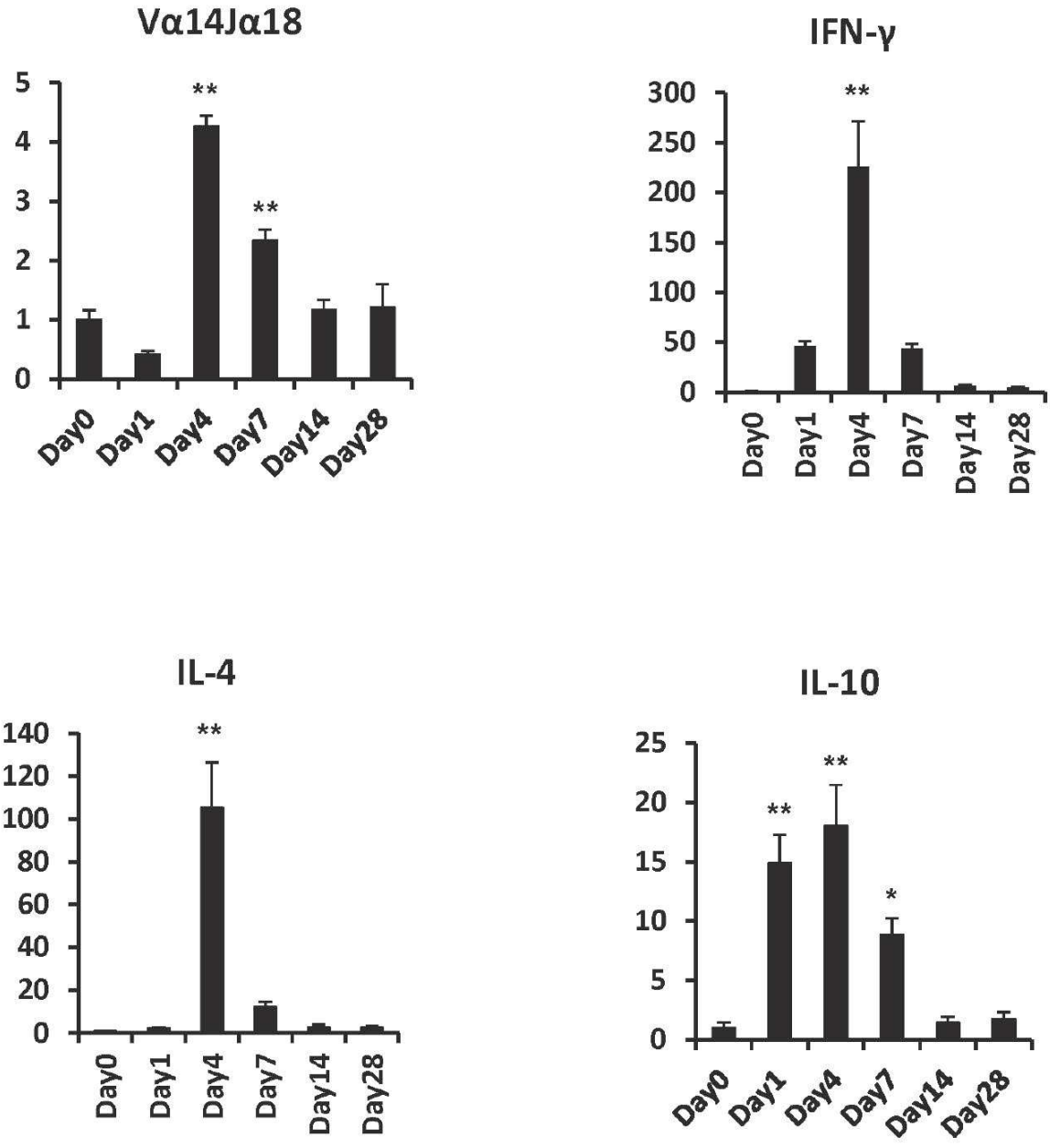
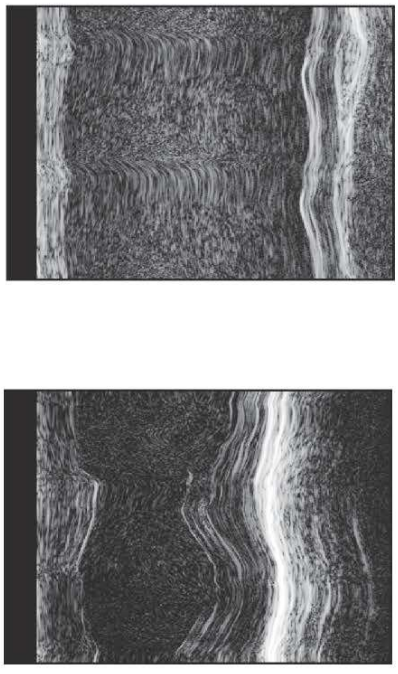
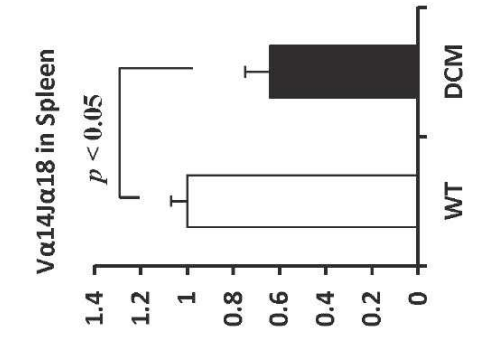

Vα14Jα18
5
4
3
2
1
0
**
**
Day0
Day1
Day4
Day7
Day14
Day28
IFN-γ
300
250
200
150
100
50
0
**
Day0
Day1
Day4
Day7
Day14
Day28
IL-4
140
120
100
80
60
40
20
0
**
Day0
Day1
Day4
Day7
Day14
Day28
IL-10
25
20
15
10
5
0
**
**
*
Day0
Day1
Day4
Day7
Day14
Day28

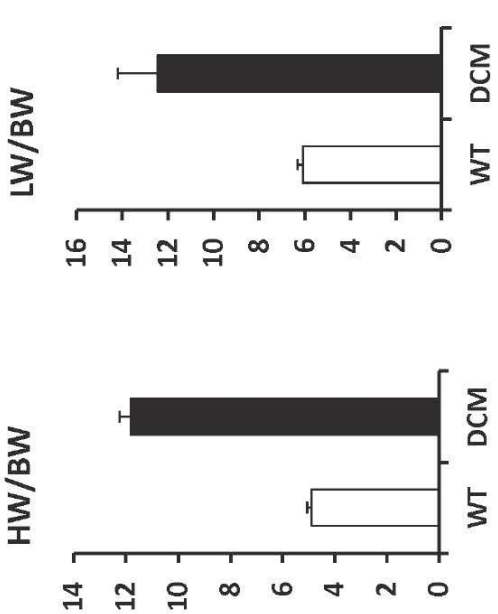
Fig.14

(a)


WT
DCM

(b)

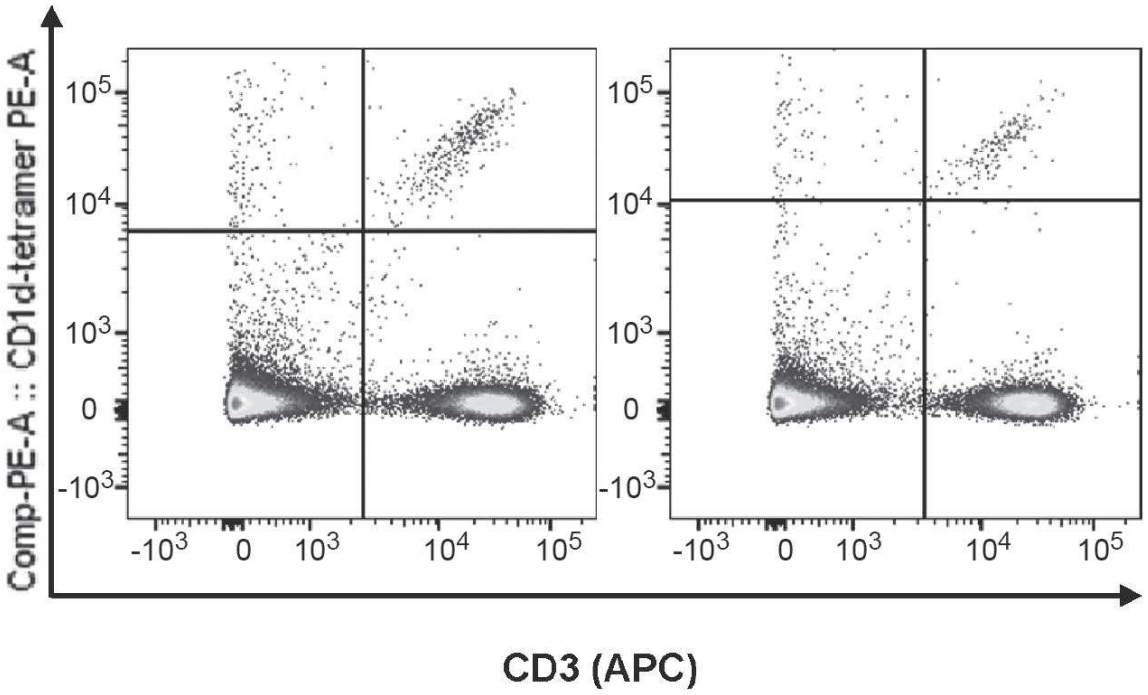

Vα14Jα18 in Spleen
$p < 0.05$
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT
DCM


LW/BW
16
14
12
10
8
6
4
2
0
WT
DCM

HW/BW
14
12
10
8
6
4
2
0
WT
DCM

Fig.15

(a)


Comp-PE-A :: CD1d-tetramer PE-A
10^5
10^4
10^3
0
-10^3
-10^3
0
10^3
10^4
10^5
CD3 (APC)

(b)

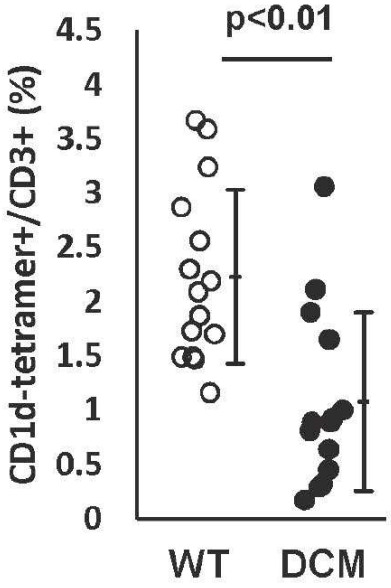
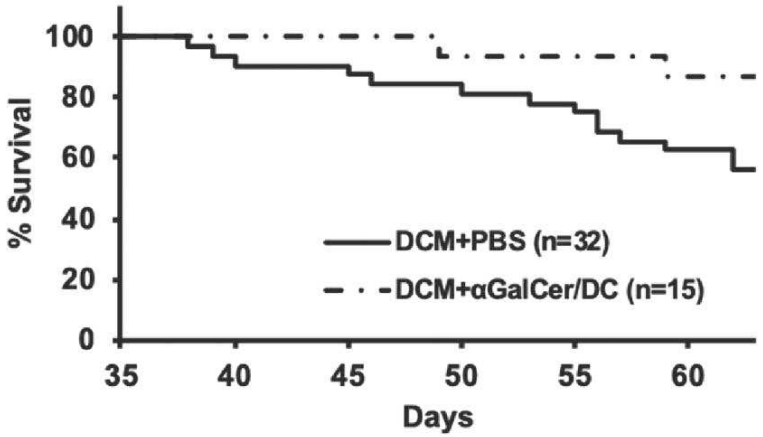
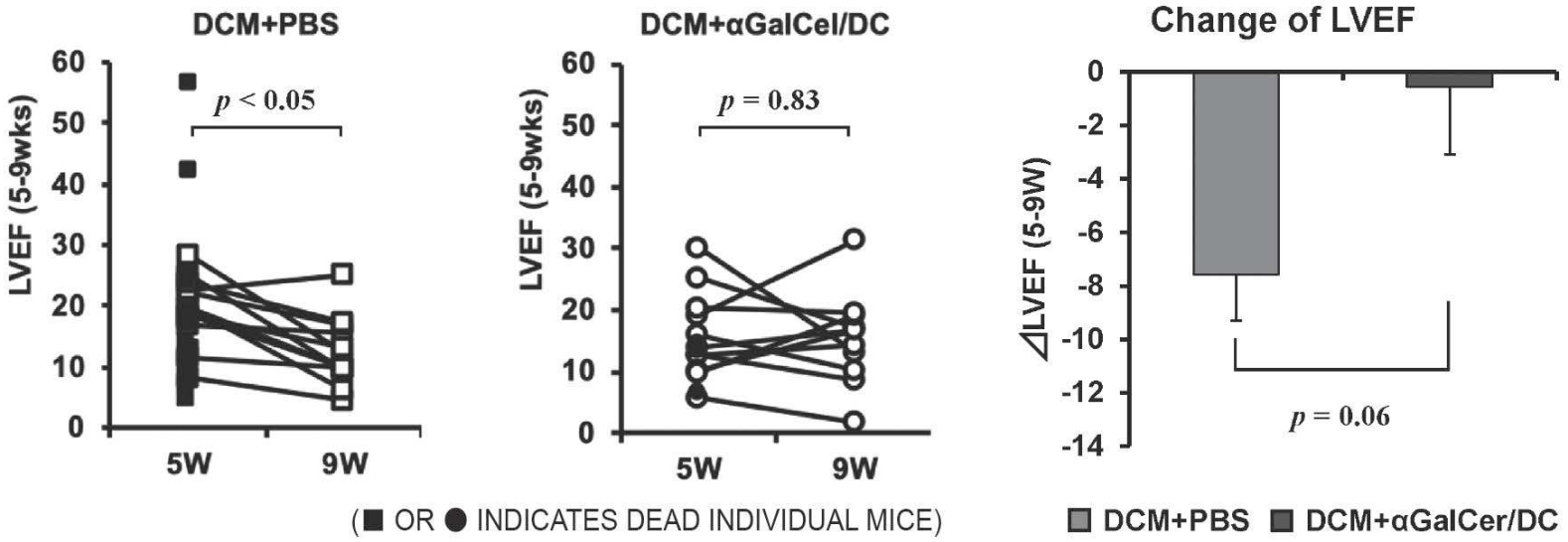

p<0.01
CD1d-tetramer+/CD3+ (%)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
WT
DCM

Fig.16

(a)


100
80
60
40
20
0
% Survival
DCM+PBS (n=32)
DCM+αGalCer/DC (n=15)
35
40
45
50
55
60
Days

(b)


DCM+PBS
DCM+αGalCel/DC
LVEF (5-9wks)
p < 0.05
p = 0.83
5W
9W
(■ OR ● INDICATES DEAD INDIVIDUAL MICE)
Change of LVEF
⊿LVEF (5-9W)
p = 0.06
DCM+PBS
DCM+αGalCer/DC

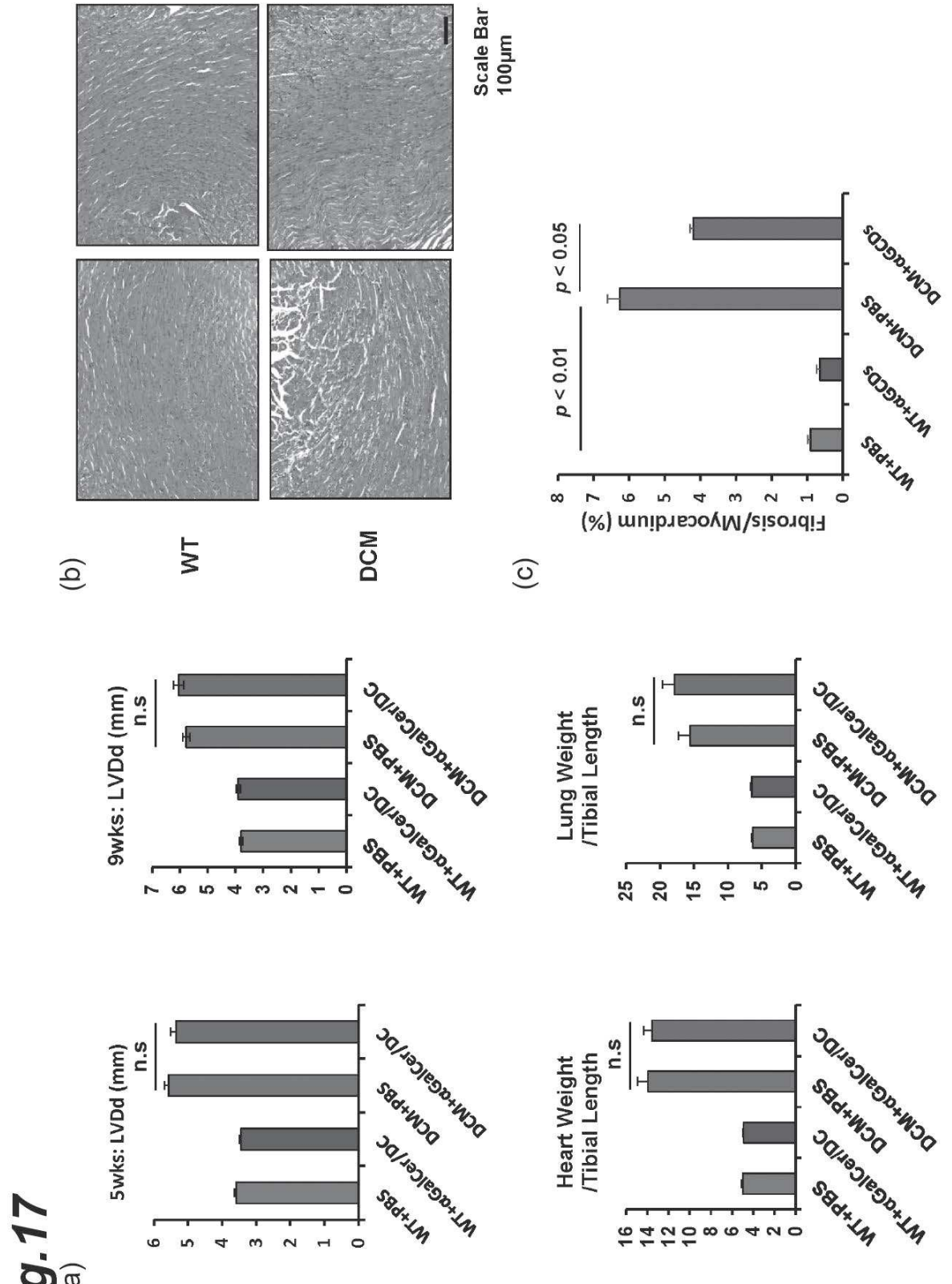

Fig.17
(a)
5wks: LVDd (mm)
n.s
9wks: LVDd (mm)
n.s
Heart Weight /Tibial Length
n.s
Lung Weight /Tibial Length
n.s
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
(b)
WT
DCM
Scale Bar 100μm
(c)
Fibrosis/Myocardium (%)
p < 0.01
p < 0.05
WT+PBS
WT+αGCDS
DCM+PBS
DCM+αGCDS

Fig.18

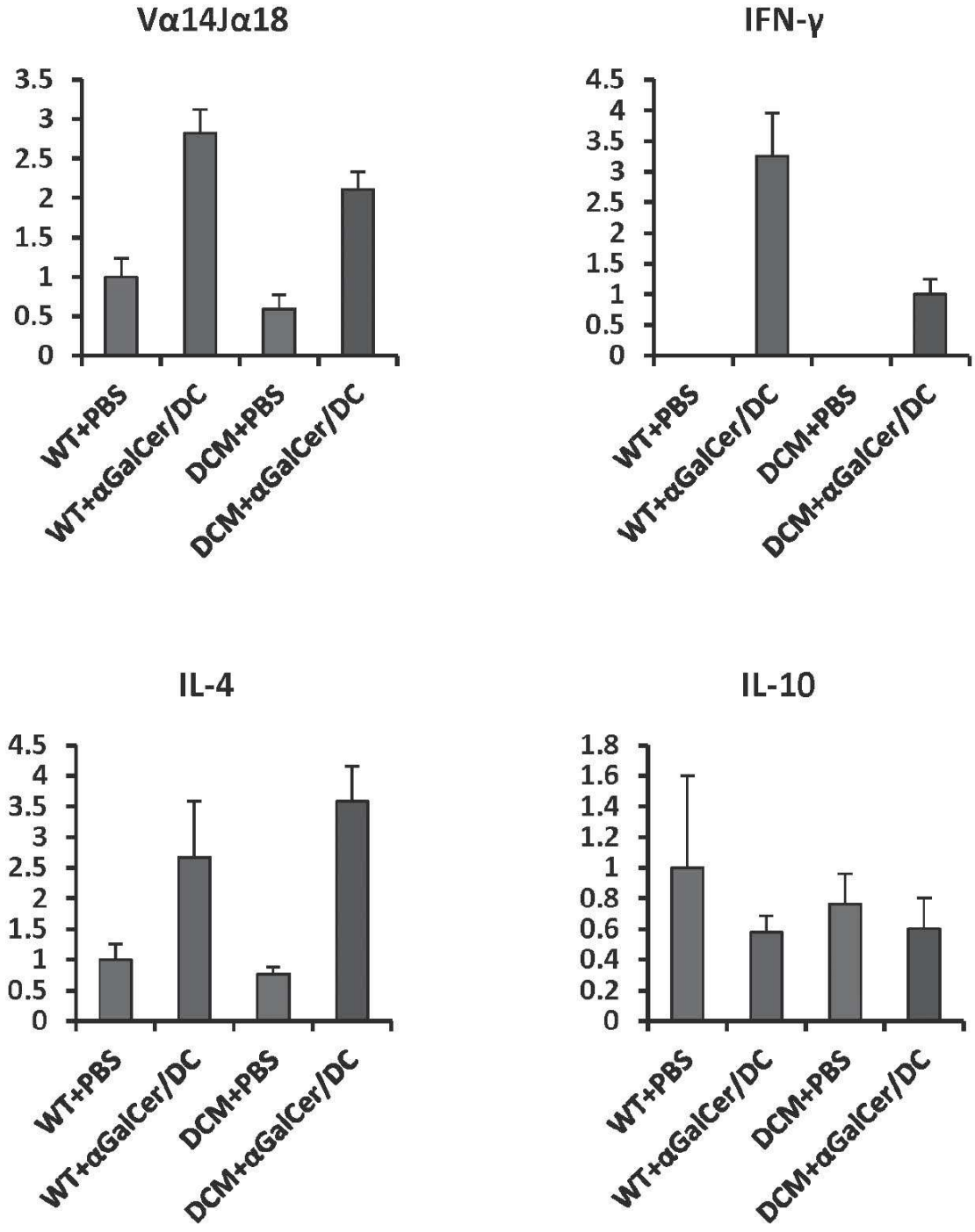
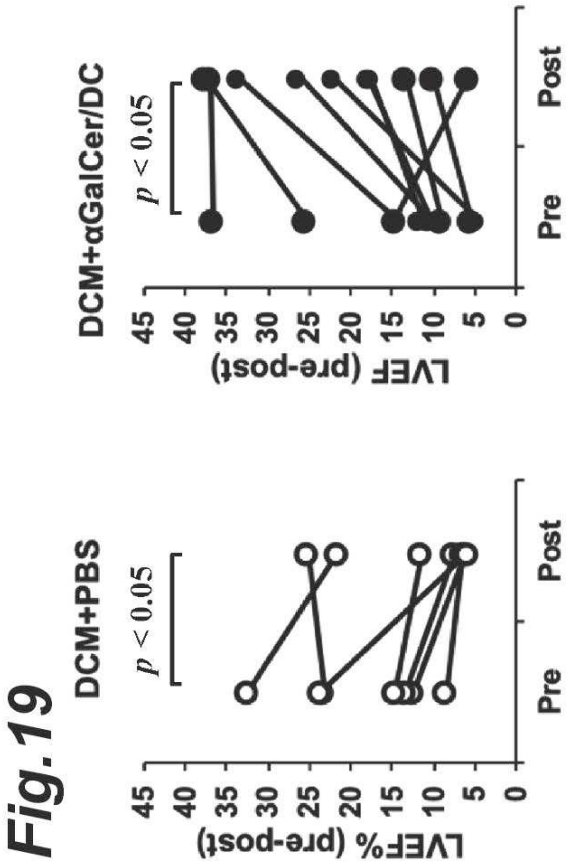
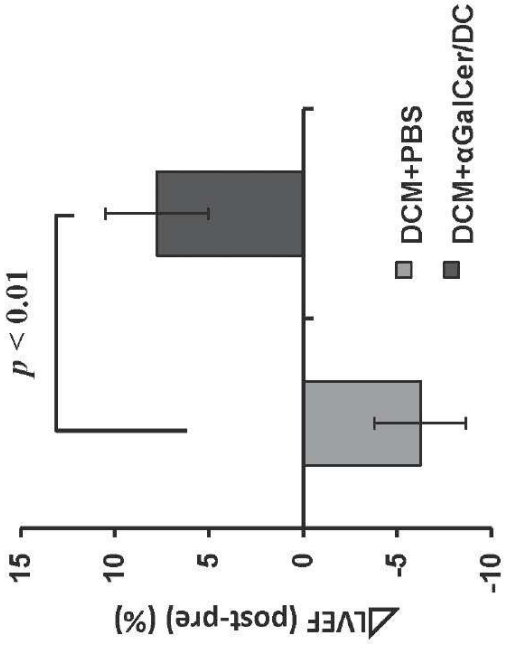

Vα14Jα18
3.5
3
2.5
2
1.5
1
0.5
0
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
IFN-γ
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
IL-4
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
IL-10
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC

Fig.19


DCM+PBS
$p < 0.05$
LVEF% (pre-post)
45
40
35
30
25
20
15
10
5
0
Pre
Post

DCM+αGalCer/DC
$p < 0.05$
LVEF (pre-post)
45
40
35
30
25
20
15
10
5
0
Pre
Post


$p < 0.01$
ΔLVEF (post-pre) (%)
15
10
5
0
-5
-10
DCM+PBS
DCM+αGalCer/DC

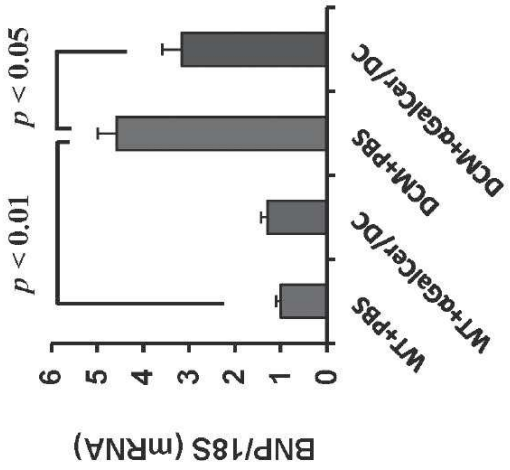

$p < 0.01$
$p < 0.05$
BNP/18S (mRNA)
6
5
4
3
2
1
0
WT+PBS
WT+αgalcer/DC
DCM+PBS
DCM+αgalcer/DC

Fig.20

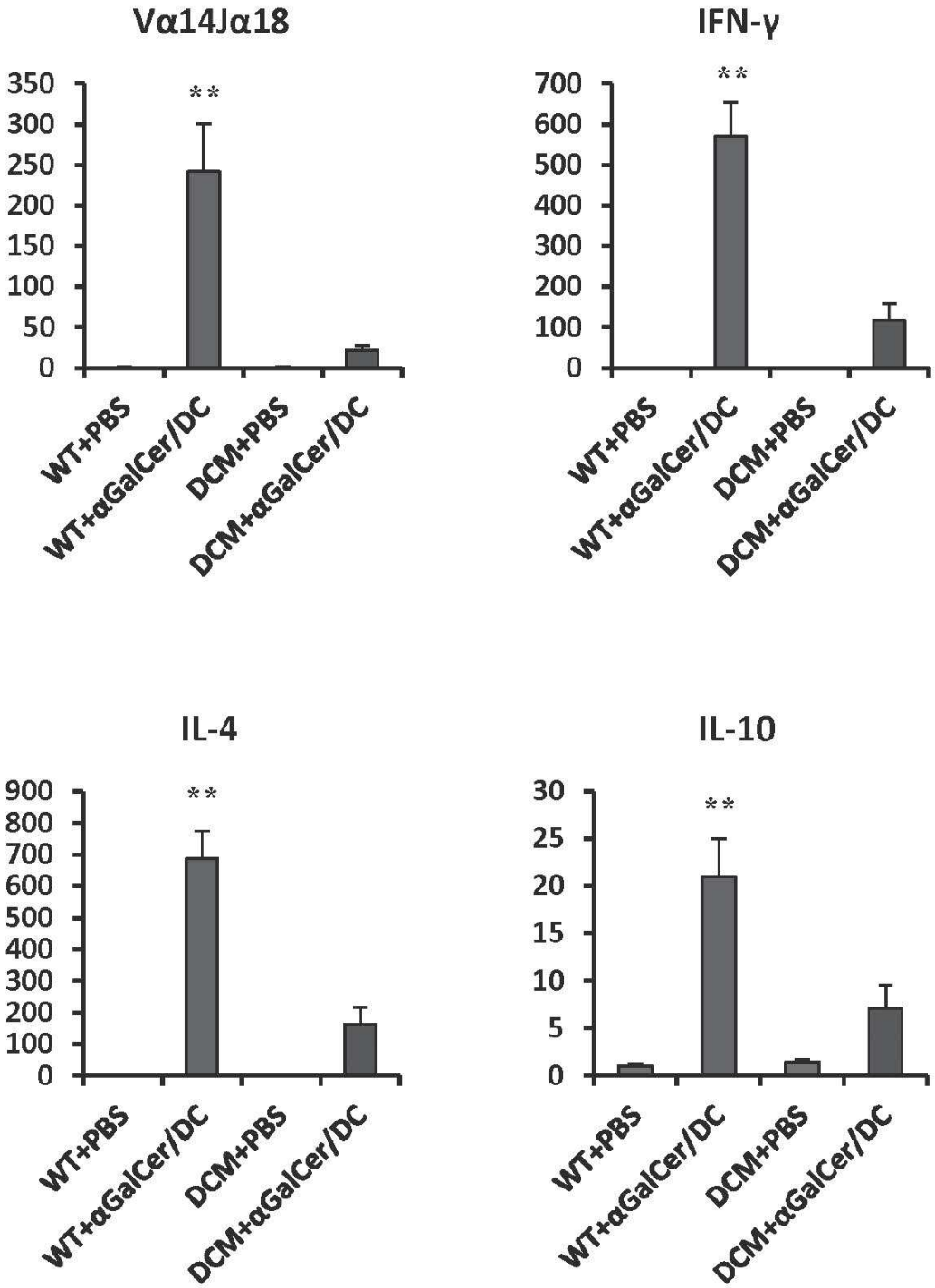
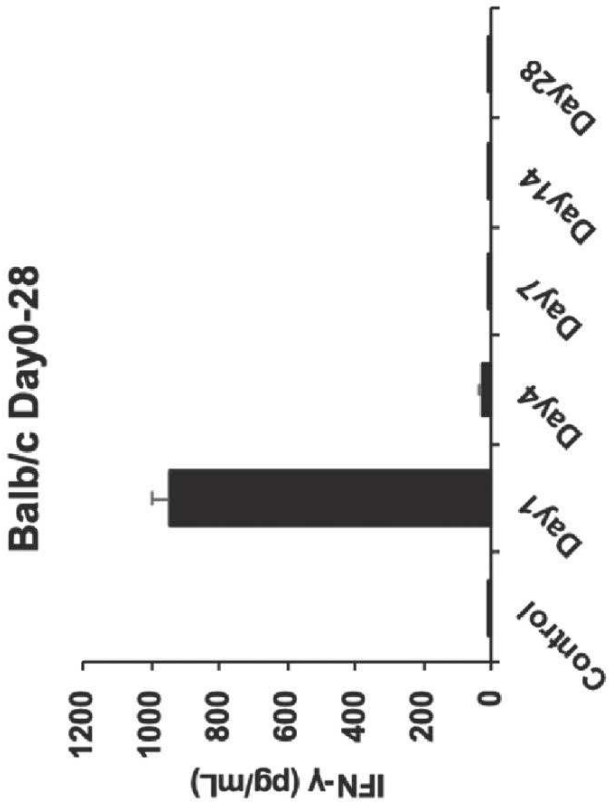

Vα14Jα18
350
300
250
200
150
100
50
0
**
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
IFN-γ
700
600
500
400
300
200
100
0
**
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
IL-4
900
800
700
600
500
400
300
200
100
0
**
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC
IL-10
30
25
20
15
10
5
0
**
WT+PBS
WT+αGalCer/DC
DCM+PBS
DCM+αGalCer/DC

Fig.21


Balb/c Day0-28
IFN-γ (pg/mL)
0
200
400
600
800
1000
1200
Control
Day1
Day4
Day7
Day14
Day28

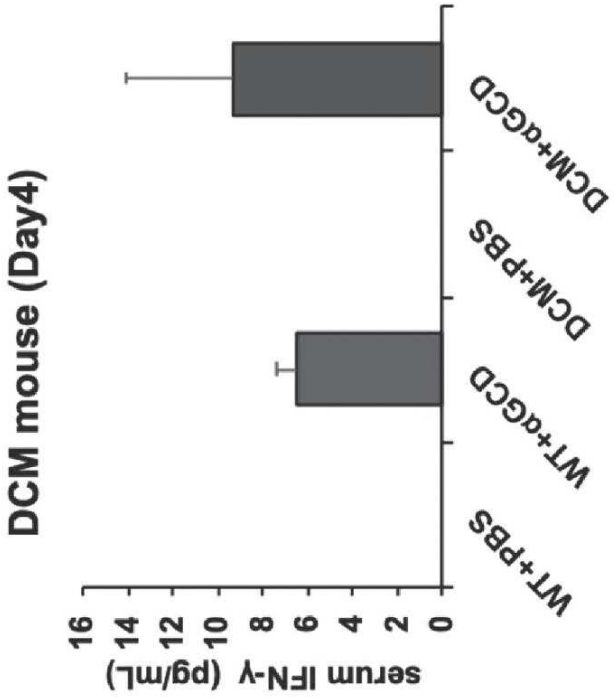
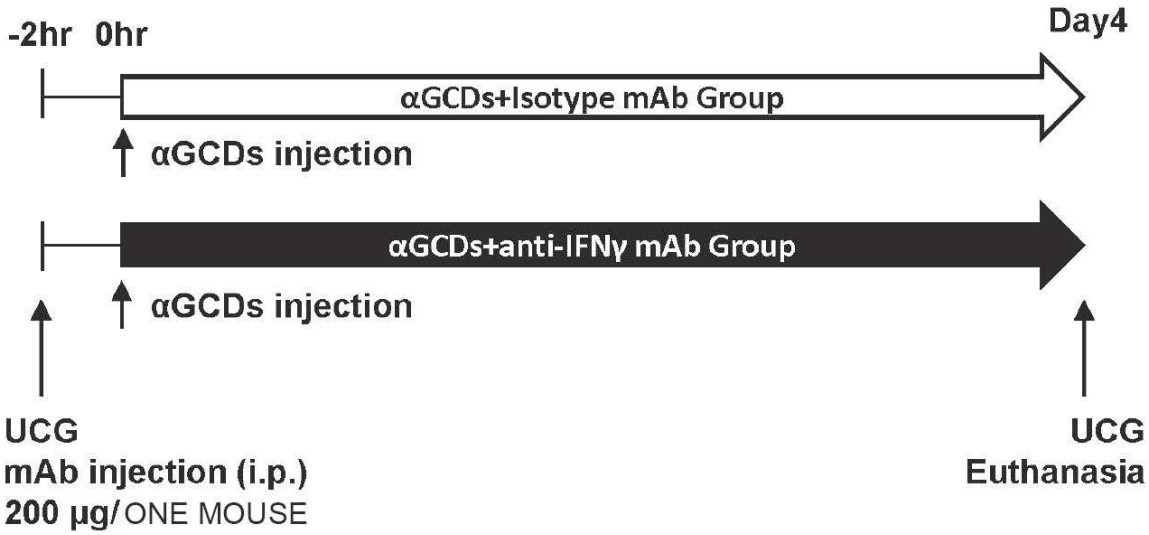

DCM mouse (Day4)
serum IFN-γ (pg/mL)
0
2
4
6
8
10
12
14
16
WT+PBS
WT+αGCD
DCM+PBS
DCM+αGCD

Fig.22

(a)


-2hr 0hr
Day4
αGCDs+Isotype mAb Group
αGCDs injection
αGCDs+anti-IFNγ mAb Group
αGCDs injection
UCG
mAb injection (i.p.)
200 μg/ONE MOUSE
UCG
Euthanasia

(b)

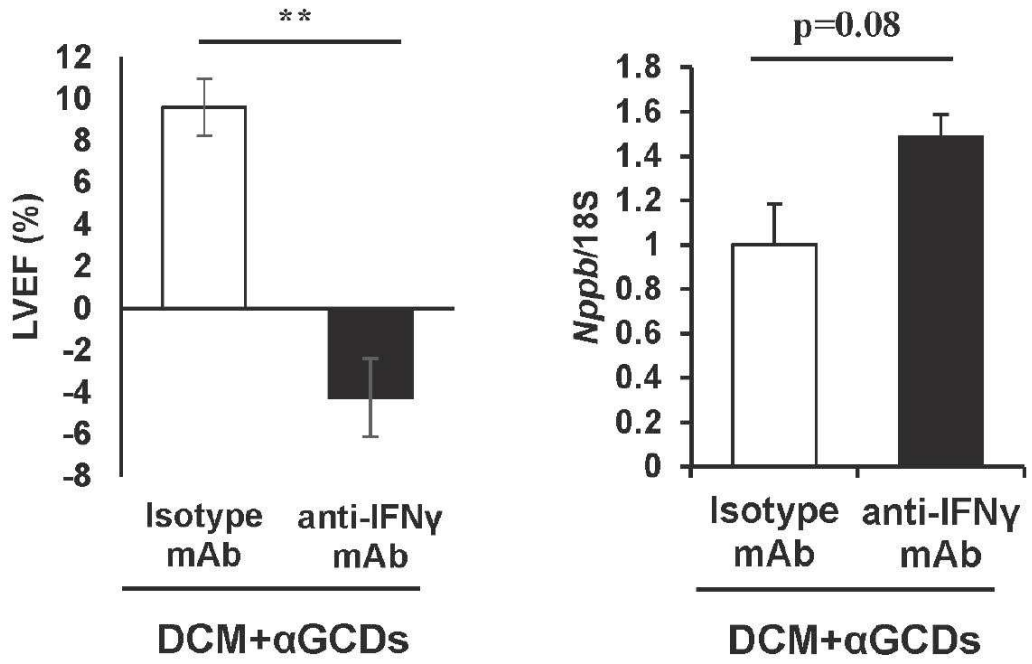

**
LVEF (%)
12
10
8
6
4
2
0
-2
-4
-6
-8
Isotype mAb
anti-IFNγ mAb
DCM+αGCDs
p=0.08
Nppb/18S
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Isotype mAb
anti-IFNγ mAb
DCM+αGCDs

Fig.23

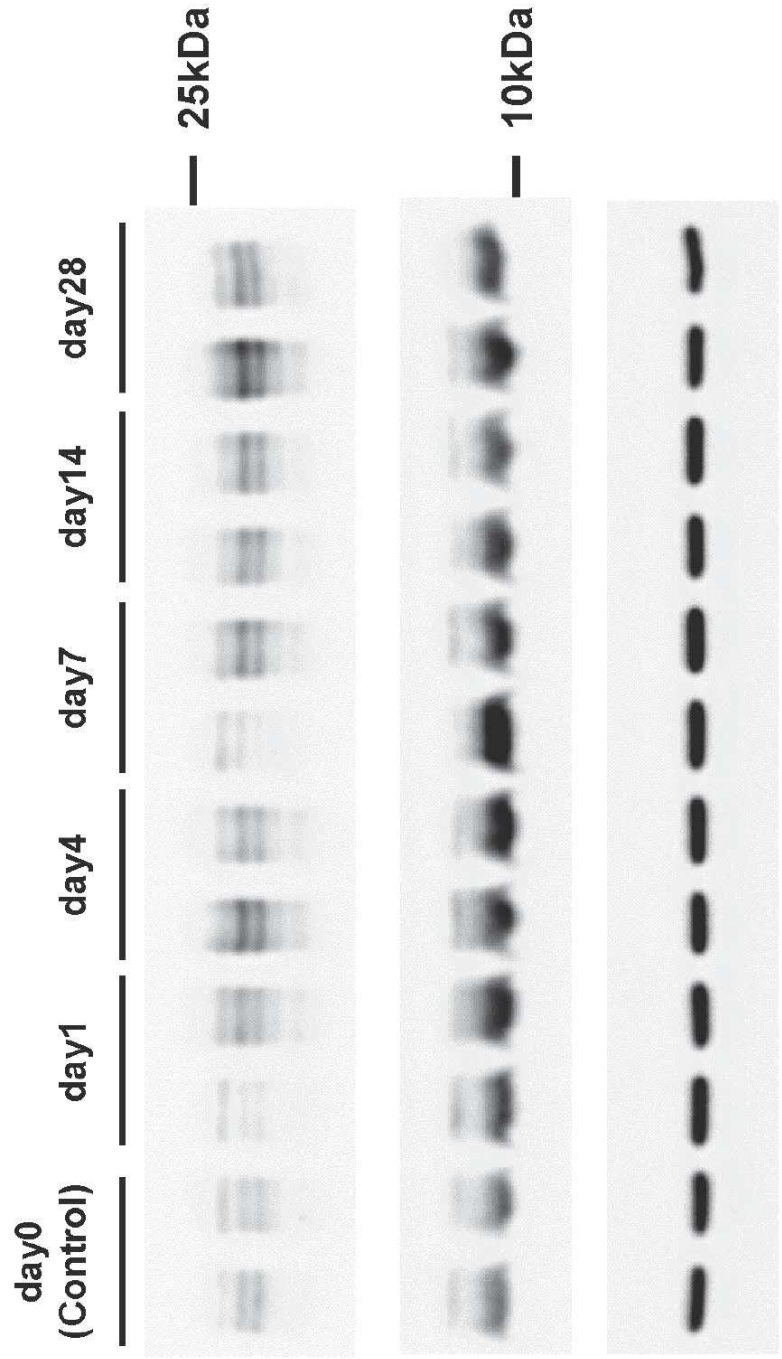

day0 (Control)
day1
day4
day7
day14
day28
25kDa
10kDa
pPLN (ser16) long exp.
pPLN (ser16)
GAPDH

Fig.24

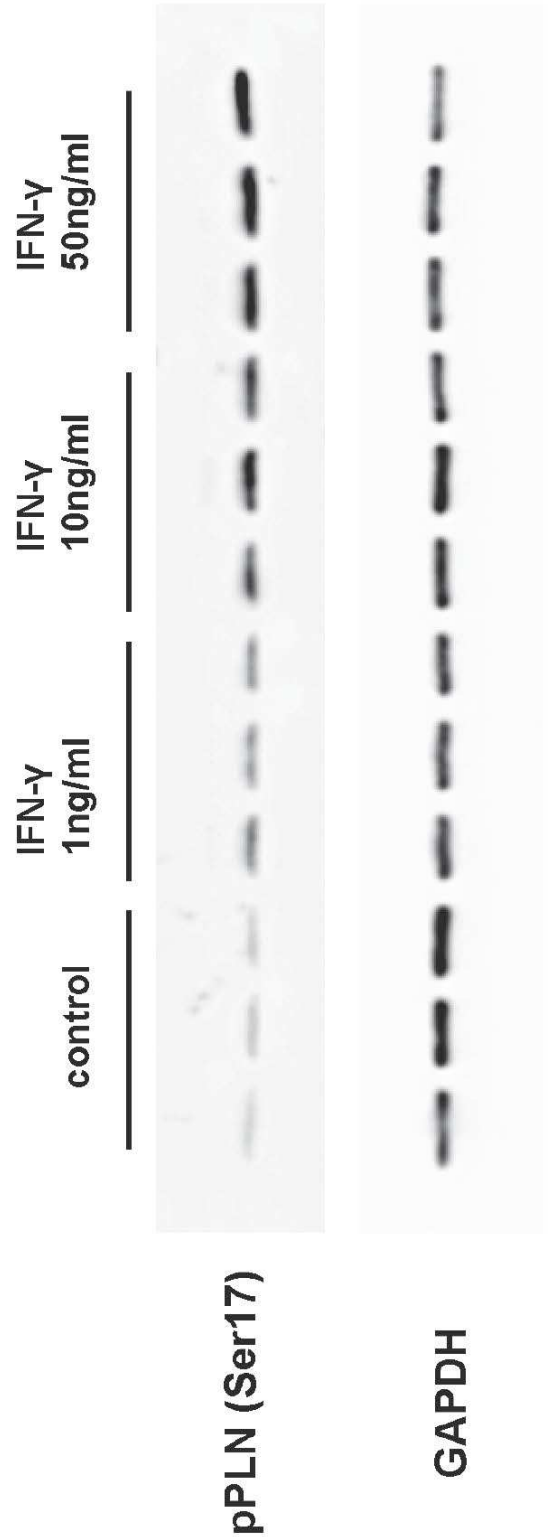

control
IFN-γ 1ng/ml
IFN-γ 10ng/ml
IFN-γ 50ng/ml
pPLN (Ser17)
GAPDH

Fig.25

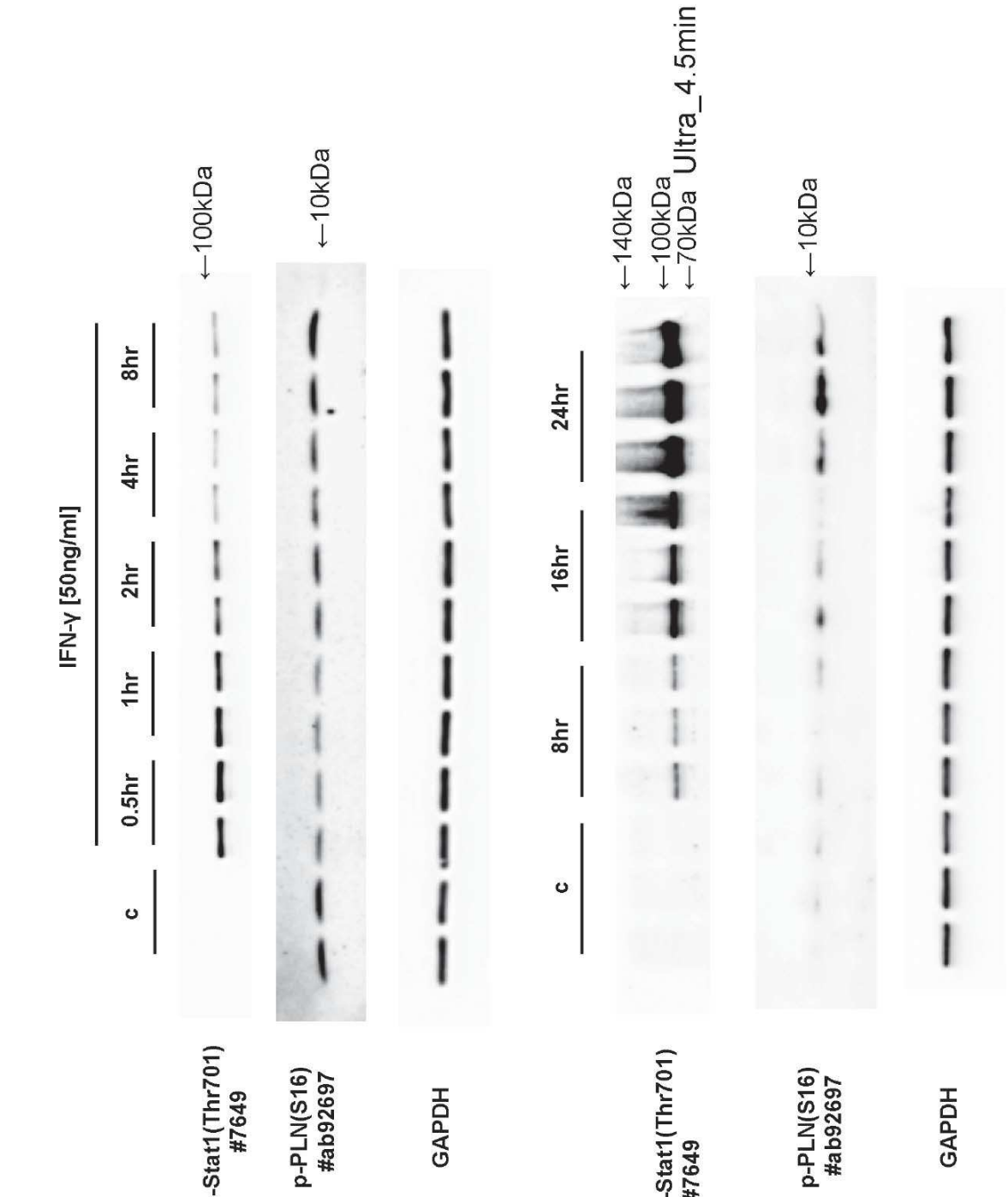
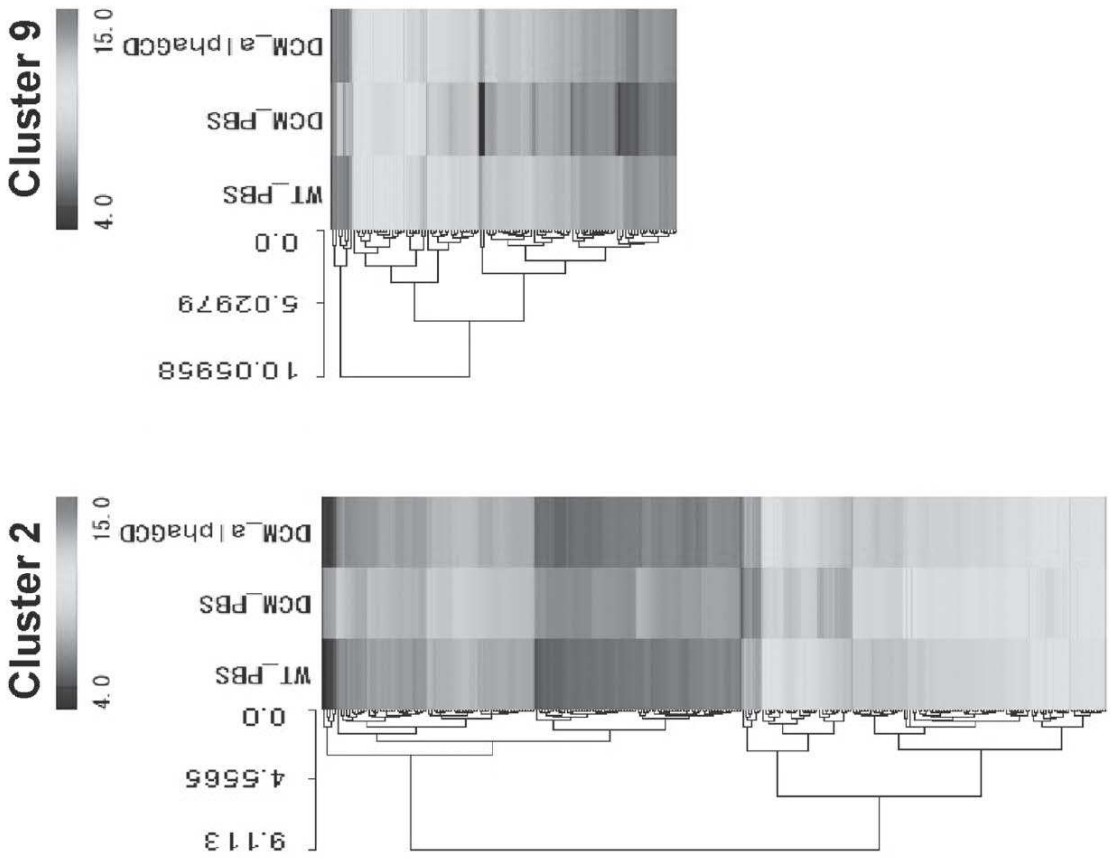

IFN-γ [50ng/ml]
c
0.5hr
1hr
2hr
4hr
8hr
←100kDa
p-Stat1(Thr701)
#7649
←10kDa
p-PLN(S16)
#ab92697
GAPDH
c
8hr
16hr
24hr
←140kDa
←100kDa
←70kDa
Ultra_4.5min
p-Stat1(Thr701)
#7649
←10kDa
p-PLN(S16)
#ab92697
GAPDH

Fig.26



52145 genes expression

→ Grouping genes by expression pattern (K-means)

10 Clusters

→ Genes changing 1.5 folds were picked up.

Cluster 2:
277 Genes, upregulated in DCM+PBS normalized in DCM+αGCD

Cluster 9:
117 Genes, downregulated in DCM+PBS normalized in DCM+αGCD

Fig.27

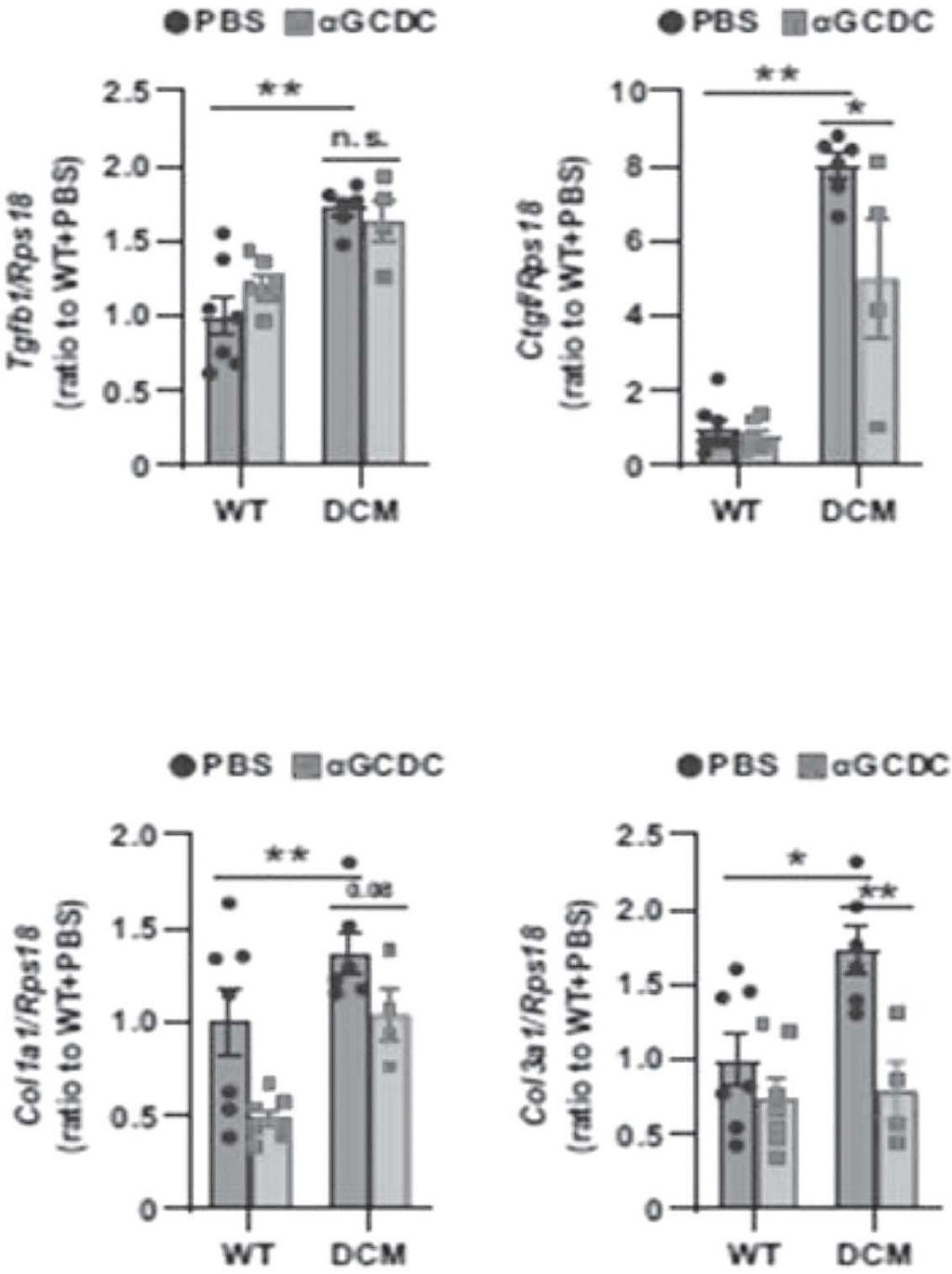
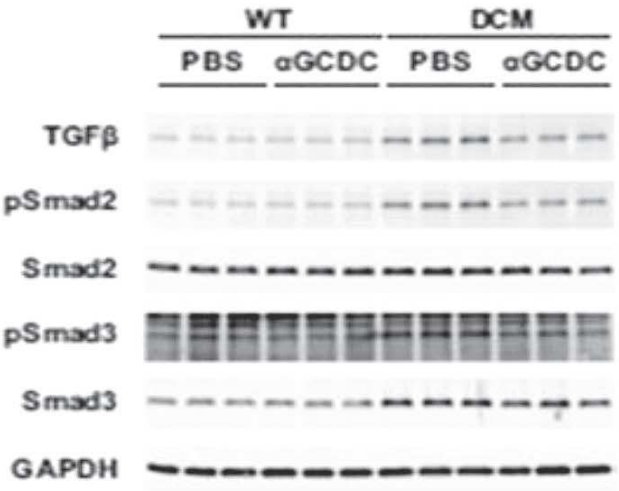

PBS
αGCDC
Tgfb1/Rps18 (ratio to WT+PBS)
Ctgf/Rps18 (ratio to WT+PBS)
Col1a1/Rps18 (ratio to WT+PBS)
Col3a1/Rps18 (ratio to WT+PBS)
**
*
n.s.
0.06
WT
DCM

Fig.28

(a)


WT
DCM
PBS
αGCDC
PBS
αGCDC
TGFβ
pSmad2
Smad2
pSmad3
Smad3
GAPDH

(b)

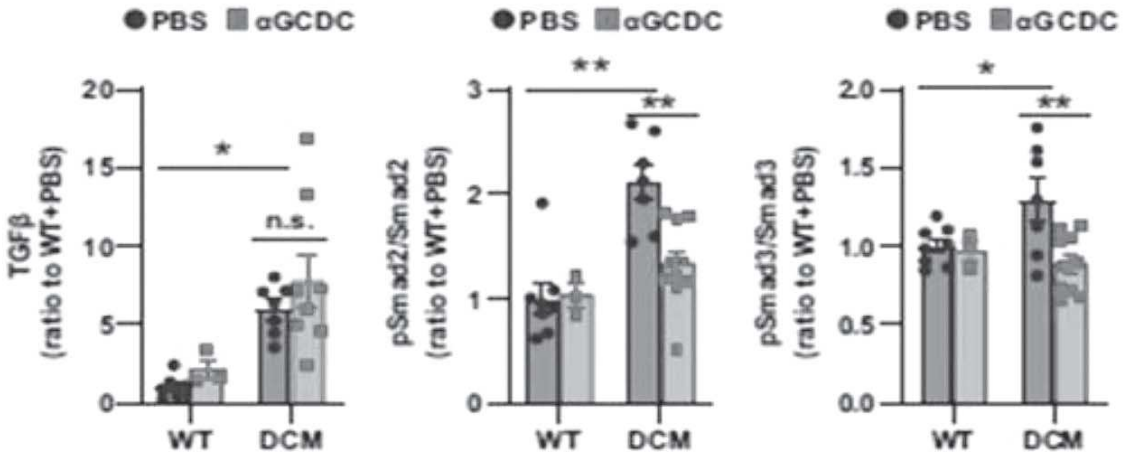

PBS
αGCDC
TGFβ (ratio to WT+PBS)
n.s.
WT
DCM
pSmad2/Smad2 (ratio to WT+PBS)
pSmad3/Smad3 (ratio to WT+PBS)

Fig.29

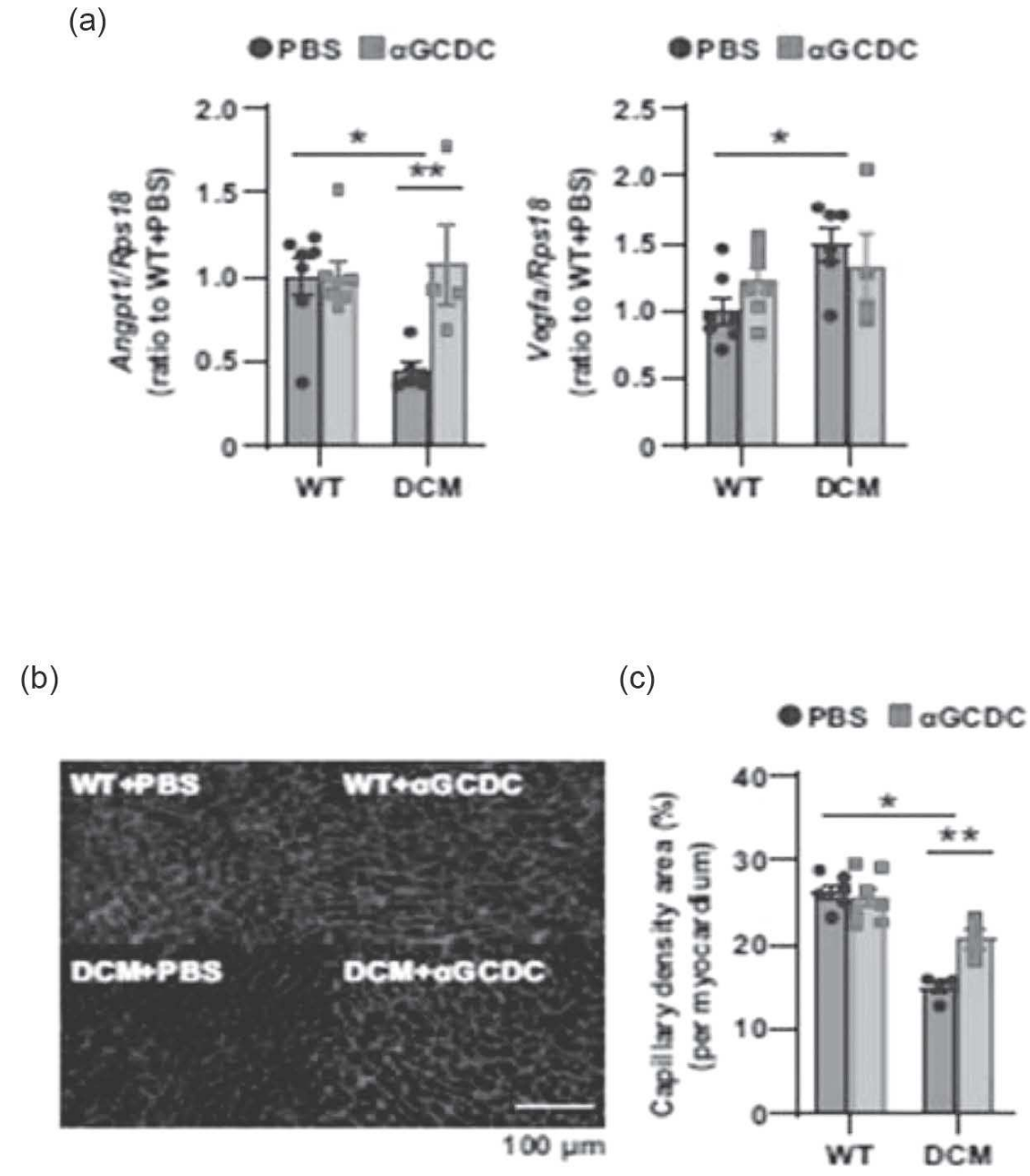

(a)
PBS
αGCDC
Angpt1/Rps18 (ratio to WT+PBS)
2.0
1.5
1.0
0.5
0
*
**
WT
DCM
PBS
αGCDC
Vegfa/Rps18 (ratio to WT+PBS)
2.5
2.0
1.5
1.0
0.5
0
*
WT
DCM
(b)
WT+PBS
WT+αGCDC
DCM+PBS
DCM+αGCDC
100 μm
(c)
PBS
αGCDC
Capillary density area (%) (per myocardium)
40
30
20
10
0
*
**
WT
DCM

Fig.30

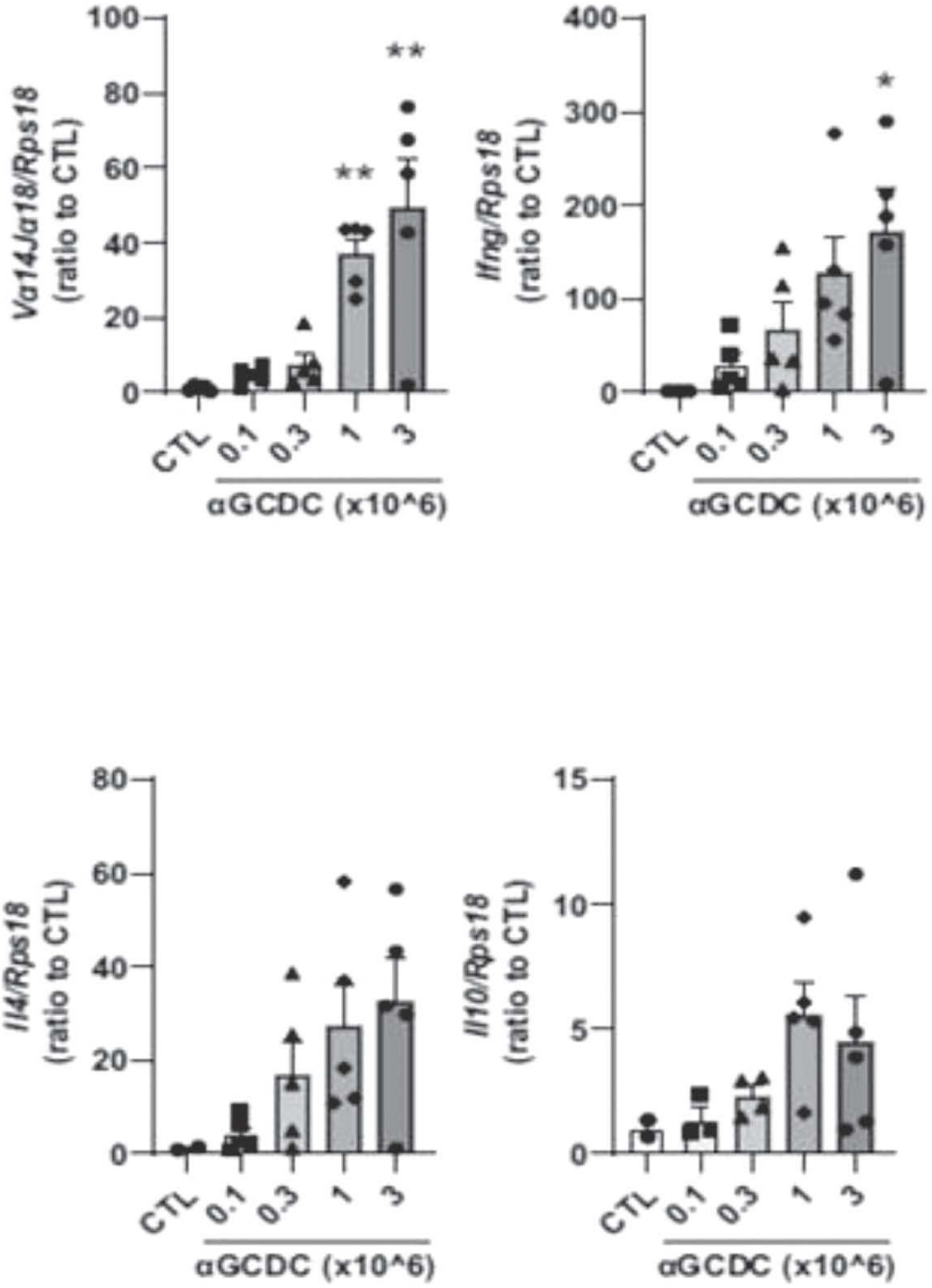
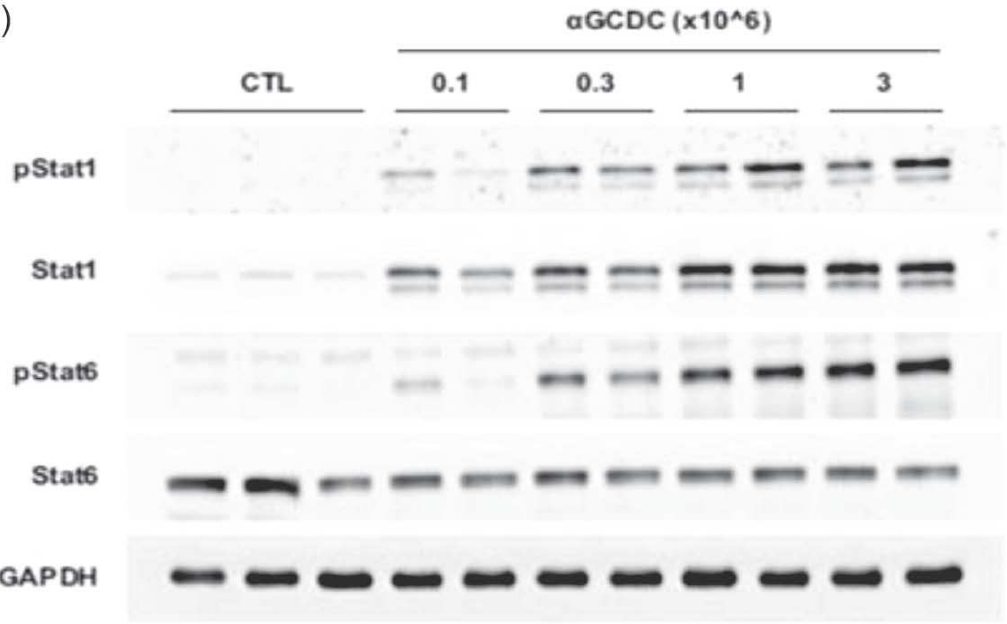

Vα14Jα18/Rps18
(ratio to CTL)
100
80
60
40
20
0
**
**
CTL
0.1
0.3
1
3
αGCDC (x10^6)
Ifng/Rps18
(ratio to CTL)
400
300
200
100
0
*
CTL
0.1
0.3
1
3
αGCDC (x10^6)
Il4/Rps18
(ratio to CTL)
80
60
40
20
0
CTL
0.1
0.3
1
3
αGCDC (x10^6)
Il10/Rps18
(ratio to CTL)
15
10
5
0
CTL
0.1
0.3
1
3
αGCDC (x10^6)

Fig.31

(a)


αGCDC (x10^6)
CTL
0.1
0.3
1
3
pStat1
Stat1
pStat6
Stat6
GAPDH

(b)

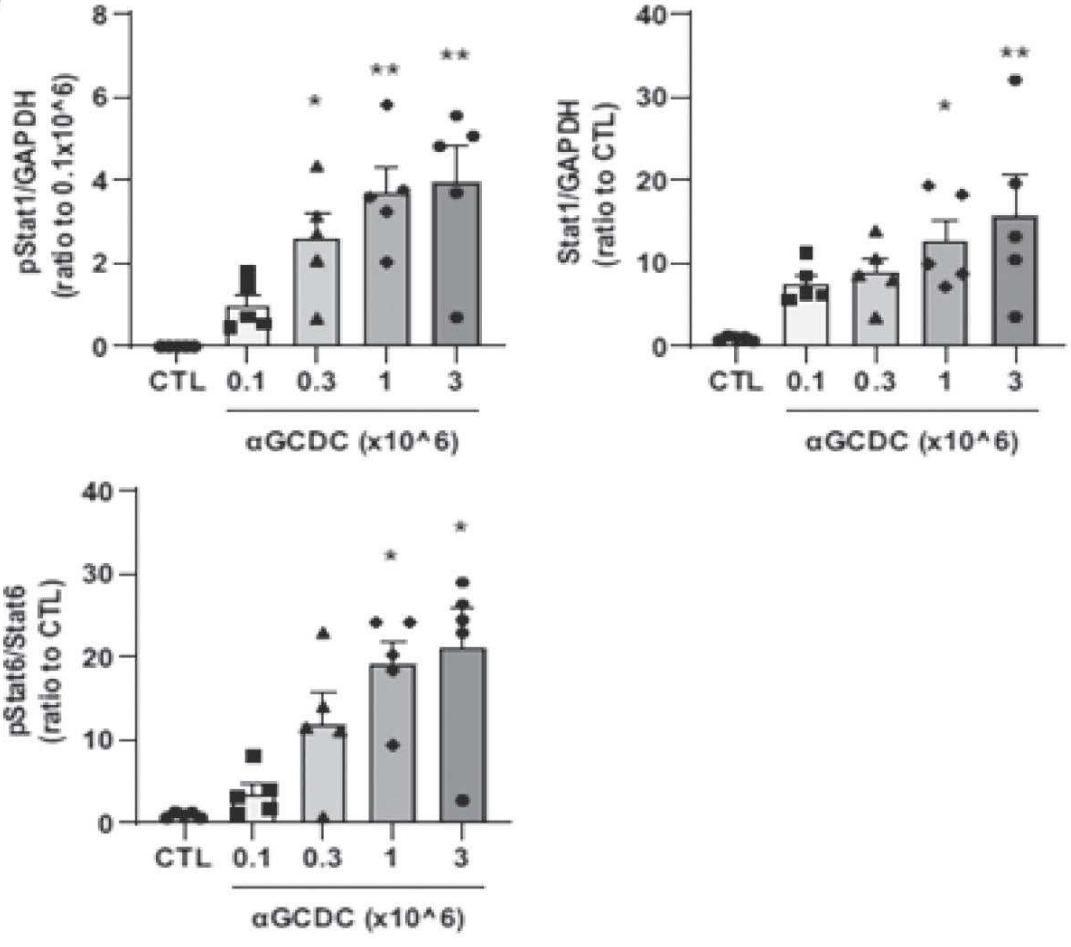

pStat1/GAPDH (ratio to 0.1x10^6)
Stat1/GAPDH (ratio to CTL)
pStat6/Stat6 (ratio to CTL)
CTL
0.1
0.3
1
3
αGCDC (x10^6)

Fig.32

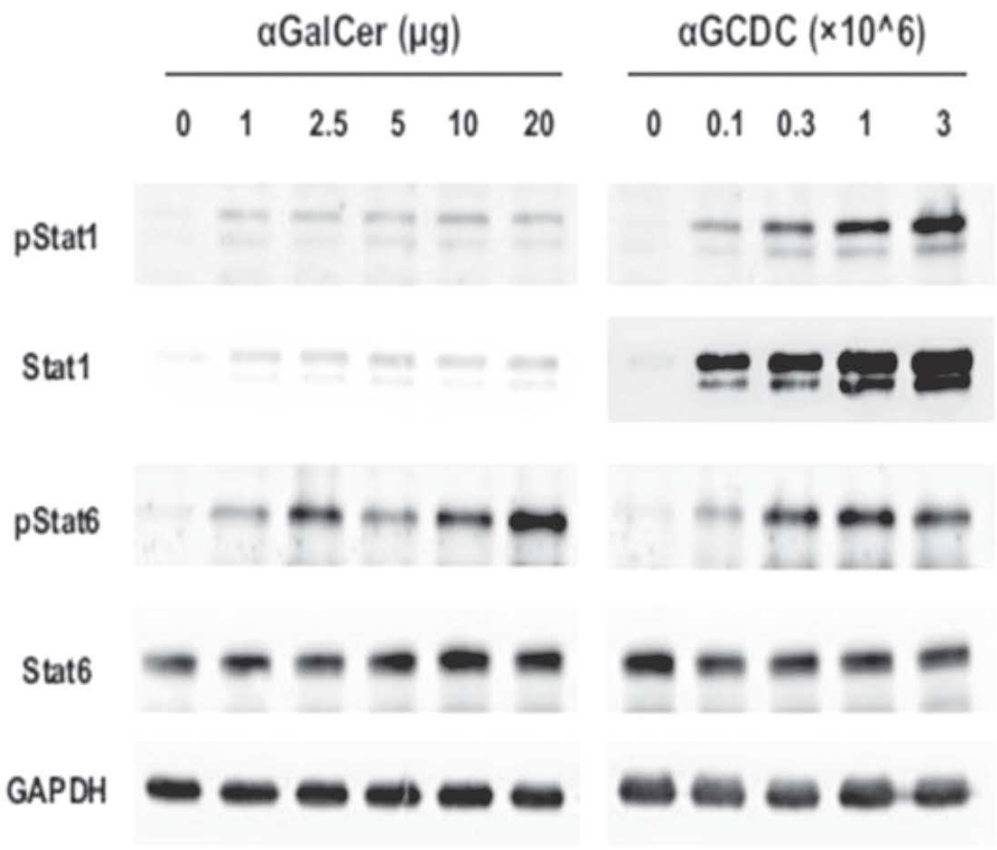

αGalCer (μg)
αGCDC (×10^6)
0 1 2.5 5 10 20
0 0.1 0.3 1 3
pStat1
Stat1
pStat6
Stat6
GAPDH

Fig.33

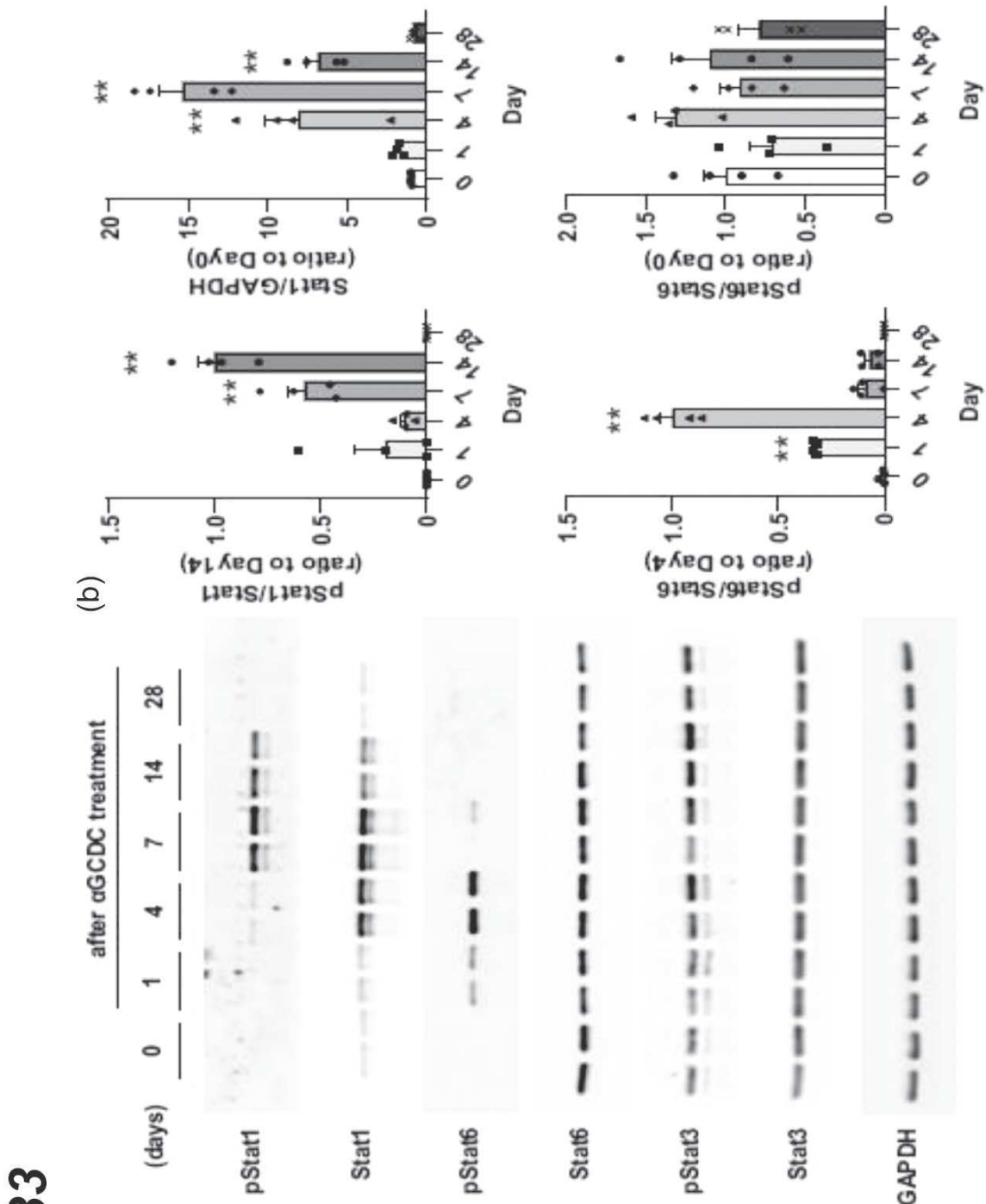

(a)
after αGCDC treatment
(days) 0 1 4 7 14 28
pStat1
Stat1
pStat6
Stat6
pStat3
Stat3
GAPDH
(b)
pStat1/Stat1 (ratio to Day14)
Stat1/GAPDH (ratio to Day0)
pStat6/Stat6 (ratio to Day4)
pStat6/Stat6 (ratio to Day0)
Day

Fig.34

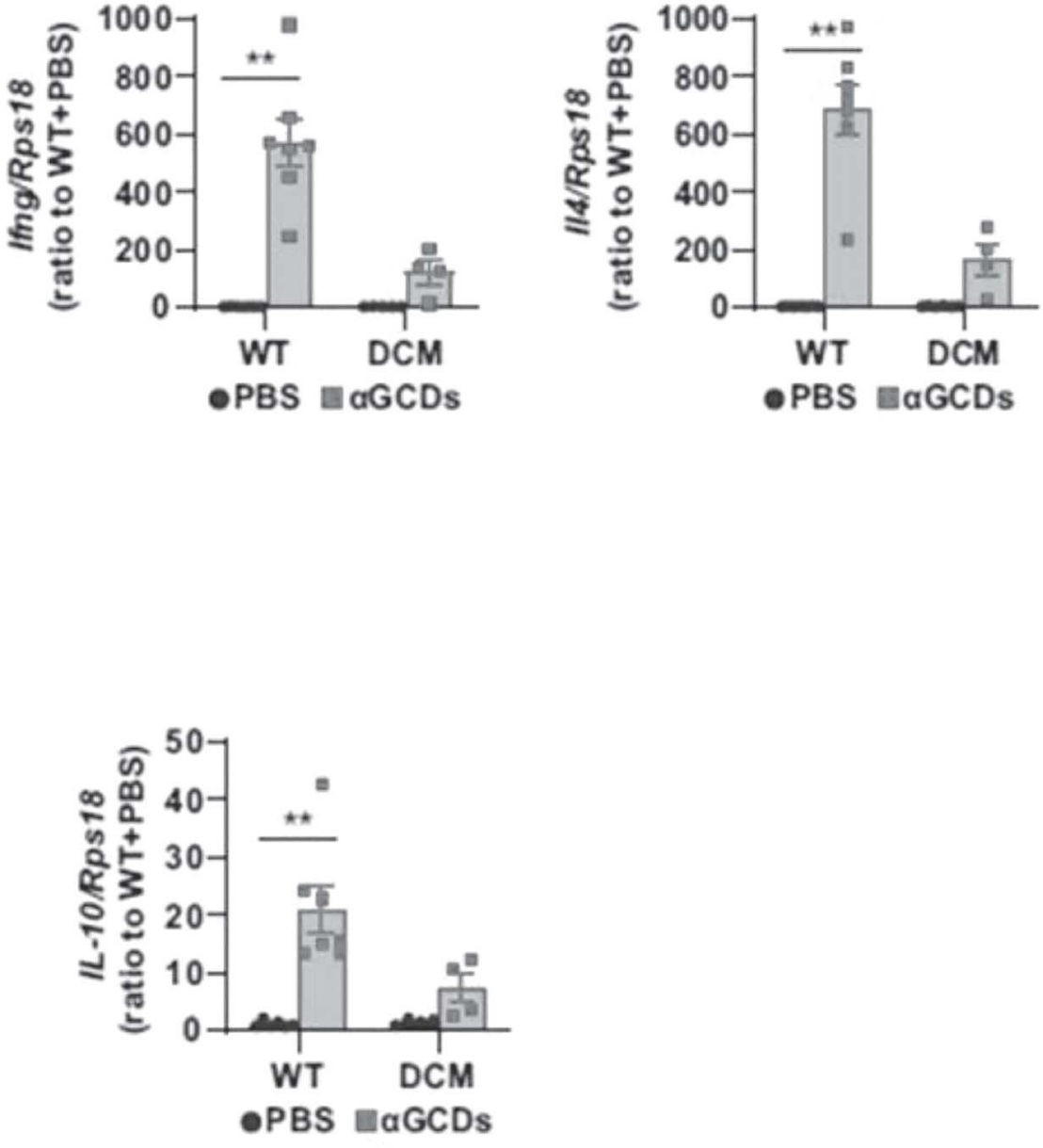

Ifng/Rps18
(ratio to WT+PBS)
1000
800
600
400
200
0
**
WT
DCM
PBS
αGCDs
Il4/Rps18
(ratio to WT+PBS)
1000
800
600
400
200
0
**
WT
DCM
PBS
αGCDs
IL-10/Rps18
(ratio to WT+PBS)
50
40
30
20
10
0
**
WT
DCM
PBS
αGCDs

Fig.35

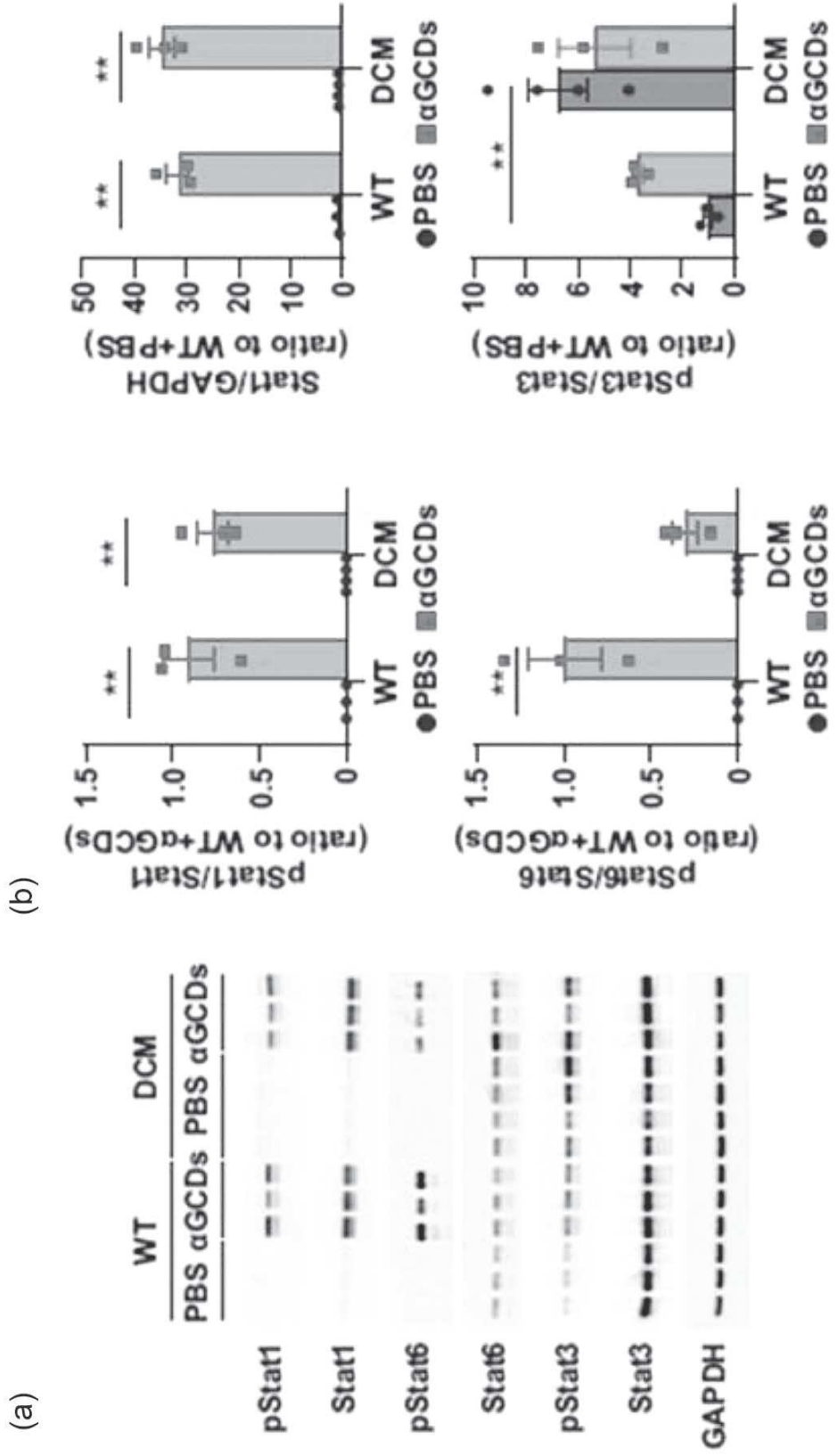

(a)
WT
DCM
PBS αGCDs PBS αGCDs
pStat1
Stat1
pStat6
Stat6
pStat3
Stat3
GAPDH
(b)
pStat1/Stat1 (ratio to WT+αGCDs)
pStat6/Stat6 (ratio to WT+αGCDs)
Stat1/GAPDH (ratio to WT+PBS)
pStat3/Stat3 (ratio to WT+PBS)
WT
DCM
●PBS ■αGCDs
**

Fig.36

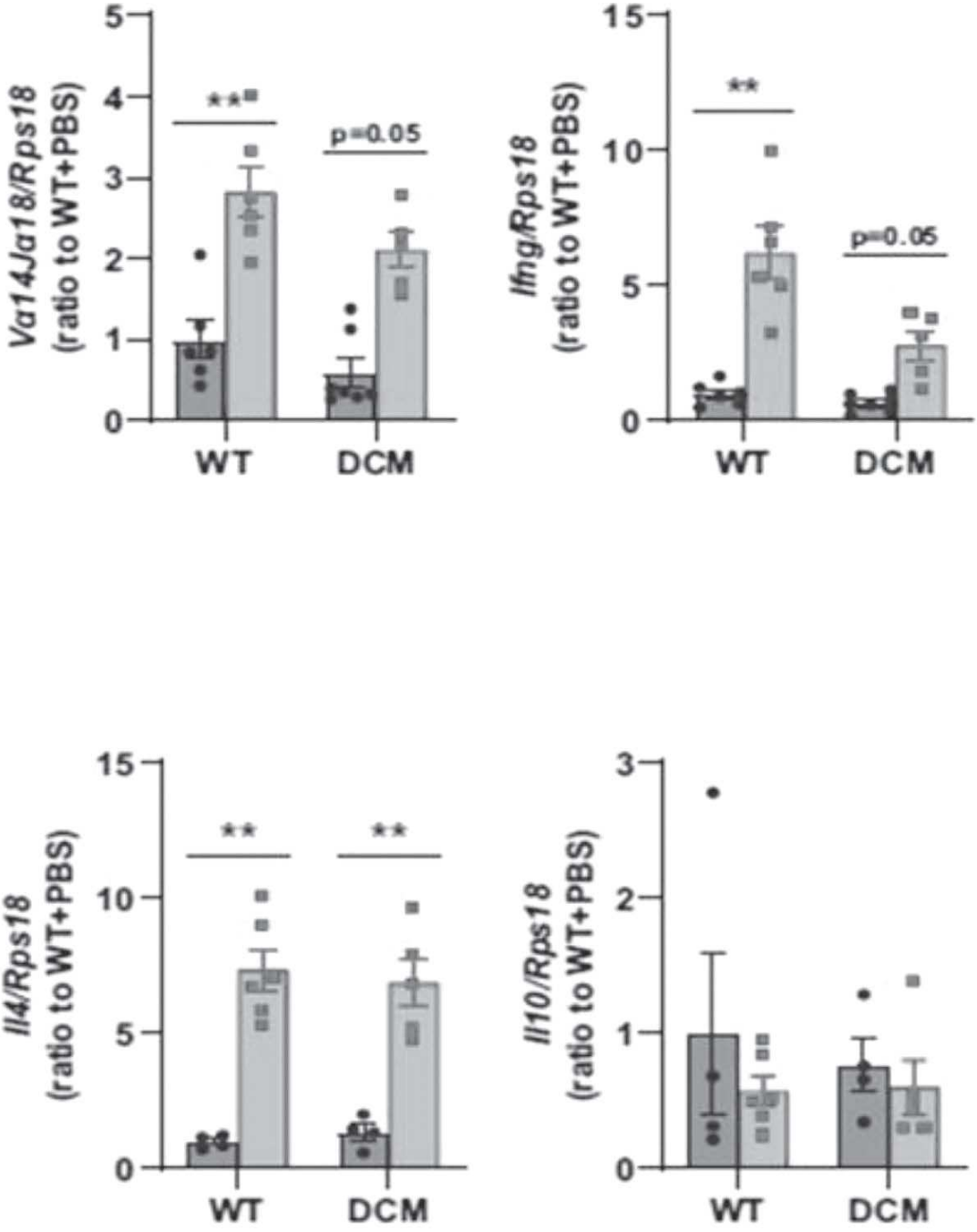
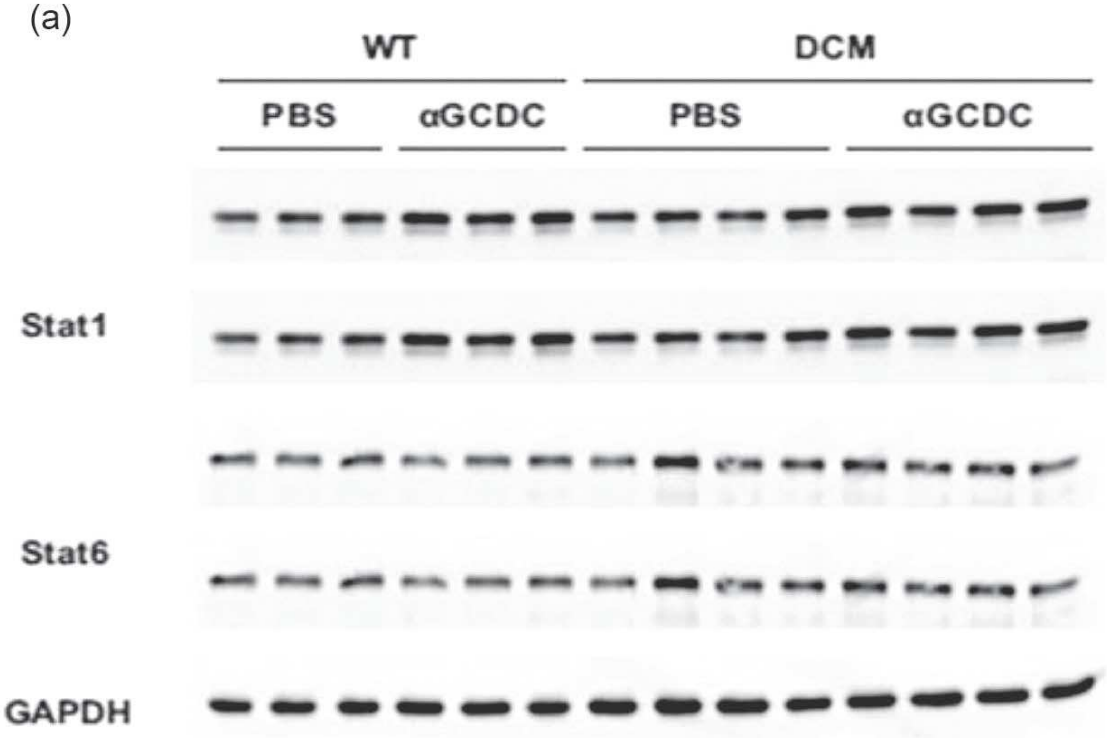

Vα14Jα18/Rps18
(ratio to WT+PBS)
5
4
3
2
1
0
**
p=0.05
WT
DCM
Ifng/Rps18
(ratio to WT+PBS)
15
10
5
0
**
p=0.05
WT
DCM
Il4/Rps18
(ratio to WT+PBS)
15
10
5
0
**
**
WT
DCM
Il10/Rps18
(ratio to WT+PBS)
3
2
1
0
WT
DCM

Fig.37

(a)


WT
DCM
PBS
αGCDC
PBS
αGCDC
Stat1
Stat6
GAPDH

(b)

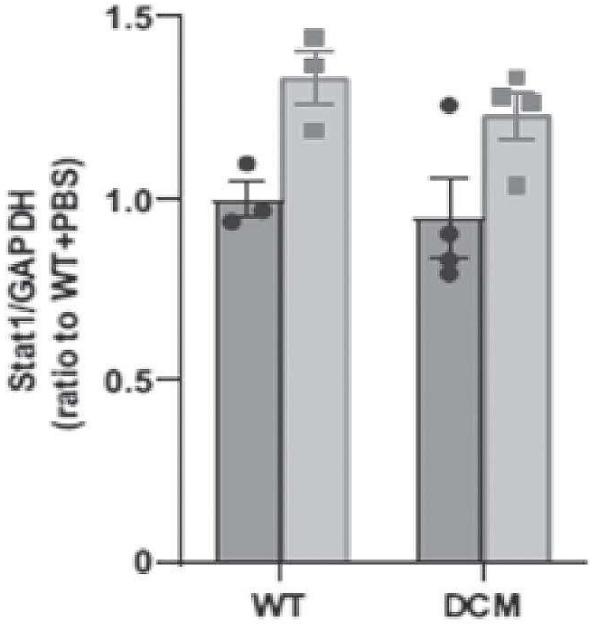

Stat1/GAPDH
(ratio to WT+PBS)
1.5
1.0
0.5
0
WT
DCM

Fig.38

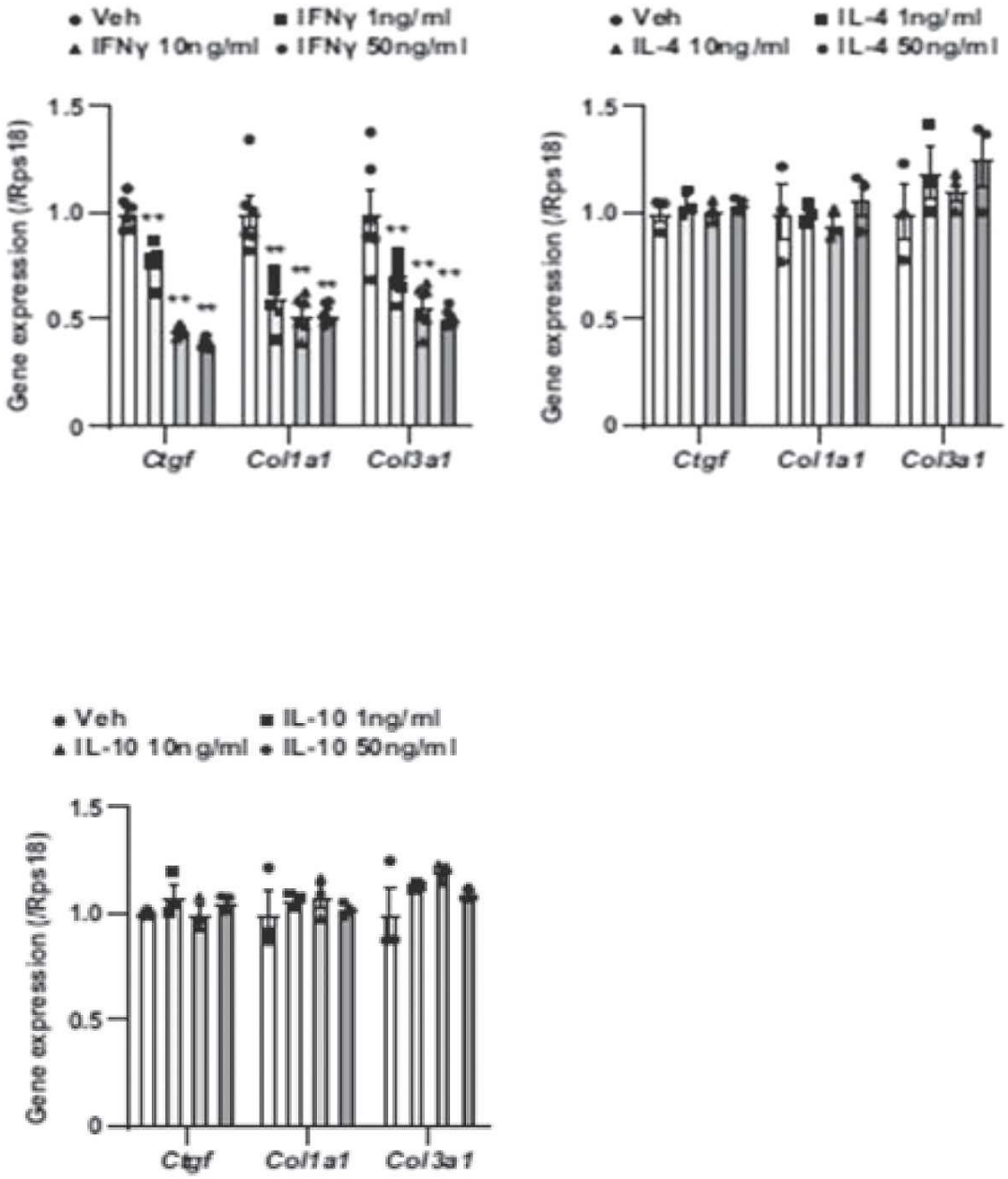

● Veh
■ IFNγ 1ng/ml
▲ IFNγ 10ng/ml
● IFNγ 50ng/ml
Gene expression (/Rps18)
1.5
1.0
0.5
0
Ctgf
Col1a1
Col3a1
● Veh
■ IL-4 1ng/ml
▲ IL-4 10ng/ml
● IL-4 50ng/ml
Gene expression (/Rps18)
1.5
1.0
0.5
0
Ctgf
Col1a1
Col3a1
● Veh
■ IL-10 1ng/ml
▲ IL-10 10ng/ml
● IL-10 50ng/ml
Gene expression (/Rps18)
1.5
1.0
0.5
0
Ctgf
Col1a1
Col3a1

Fig.39

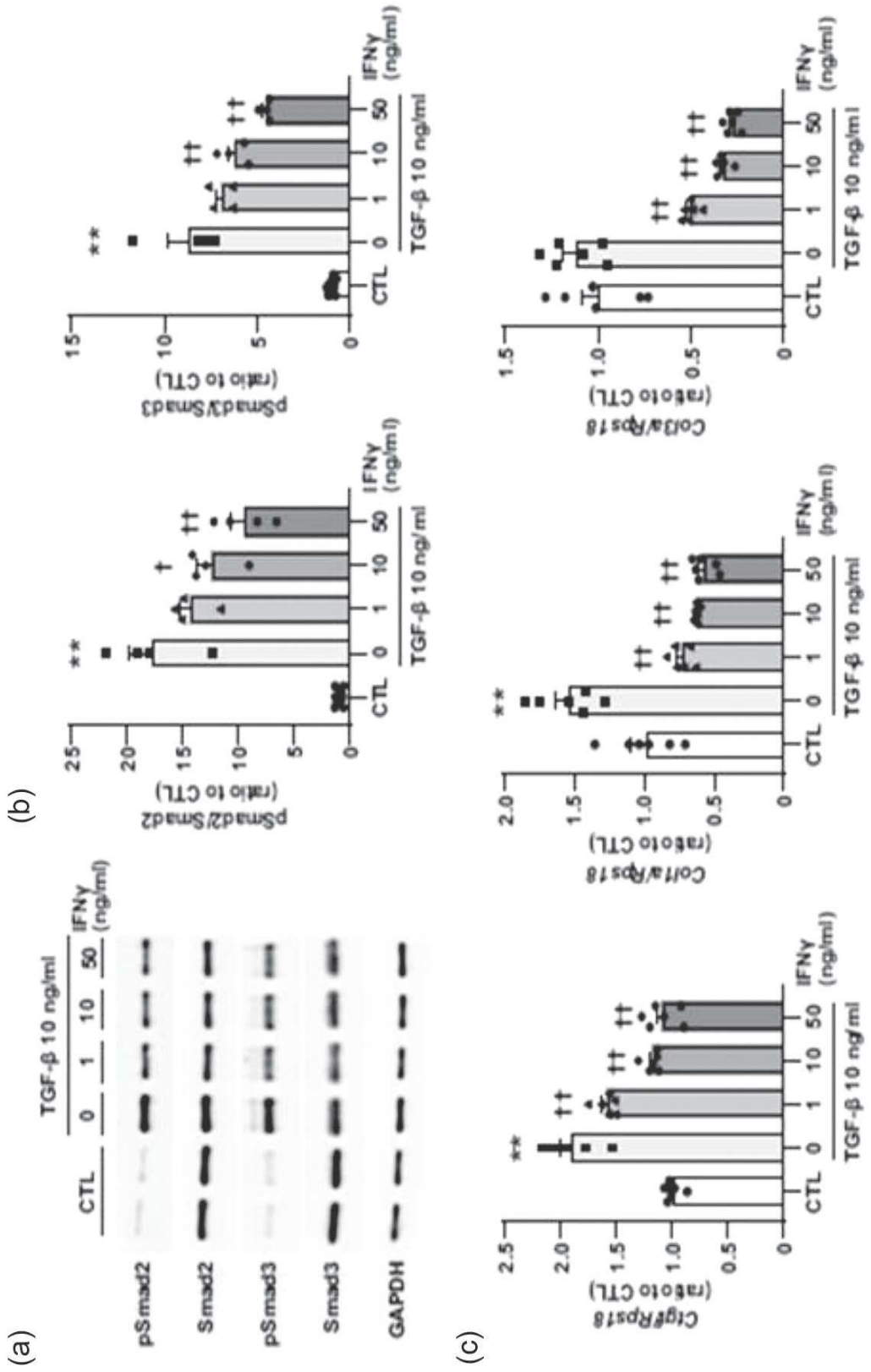
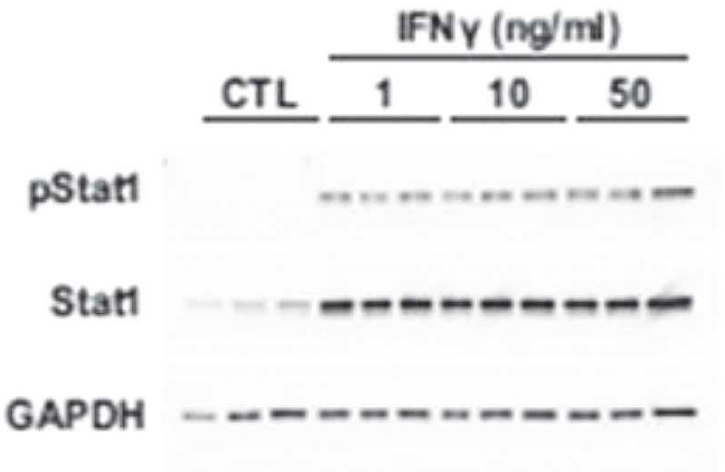

(a)
CTL
TGF-β 10 ng/ml
0
1
10
50
IFNγ (ng/ml)
pSmad2
Smad2
pSmad3
Smad3
GAPDH
(b)
pSmad2/Smad2 (ratio to CTL)
pSmad3/Smad3 (ratio to CTL)
**
††
†
CTL
TGF-β 10 ng/ml
IFNγ (ng/ml)
(c)
Ctgf/Rps18 (ratio to CTL)
Col1a/Rps18 (ratio to CTL)
Col3a/Rps18 (ratio to CTL)

Fig.40

(a)


IFNγ (ng/ml)
CTL
1
10
50
pStat1
Stat1
GAPDH

(b)

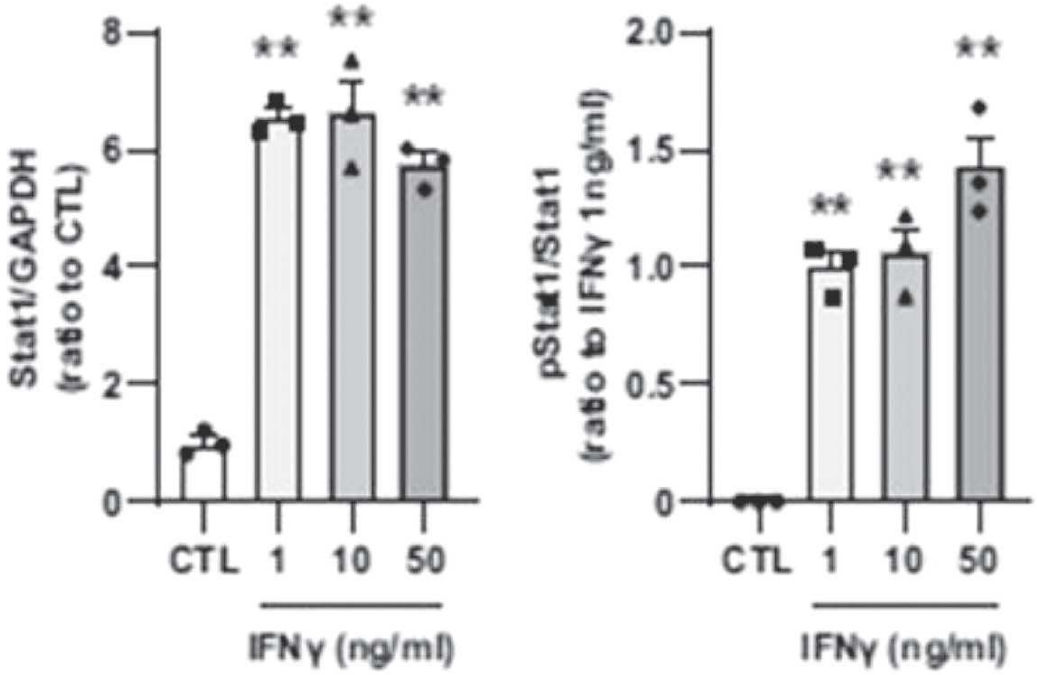
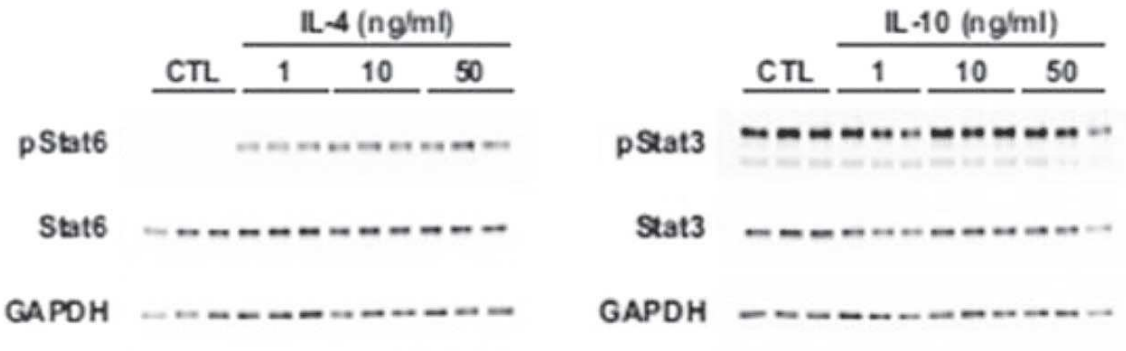

Stat1/GAPDH (ratio to CTL)
8
6
4
2
0
**
**
**
CTL
1
10
50
IFNγ (ng/ml)
pStat1/Stat1 (ratio to IFNγ 1ng/ml)
2.0
1.5
1.0
0.5
0
**
**
**
CTL
1
10
50
IFNγ (ng/ml)

Fig.41

(a)


IL-4 (ng/ml)
CTL
1
10
50
pStat6
Stat6
GAPDH
IL-10 (ng/ml)
CTL
1
10
50
pStat3
Stat3
GAPDH

(b)

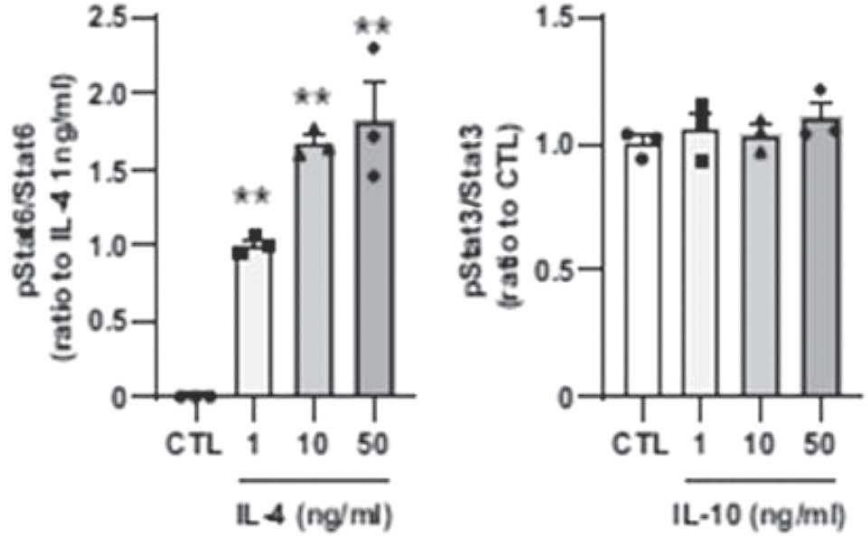

pStat6/Stat6
(ratio to IL-4 1ng/ml)
2.5
2.0
1.5
1.0
0.5
0
**
**
**
CTL
1
10
50
IL-4 (ng/ml)
pStat3/Stat3
(ratio to CTL)
1.5
1.0
0.5
0
CTL
1
10
50
IL-10 (ng/ml)

Fig.42

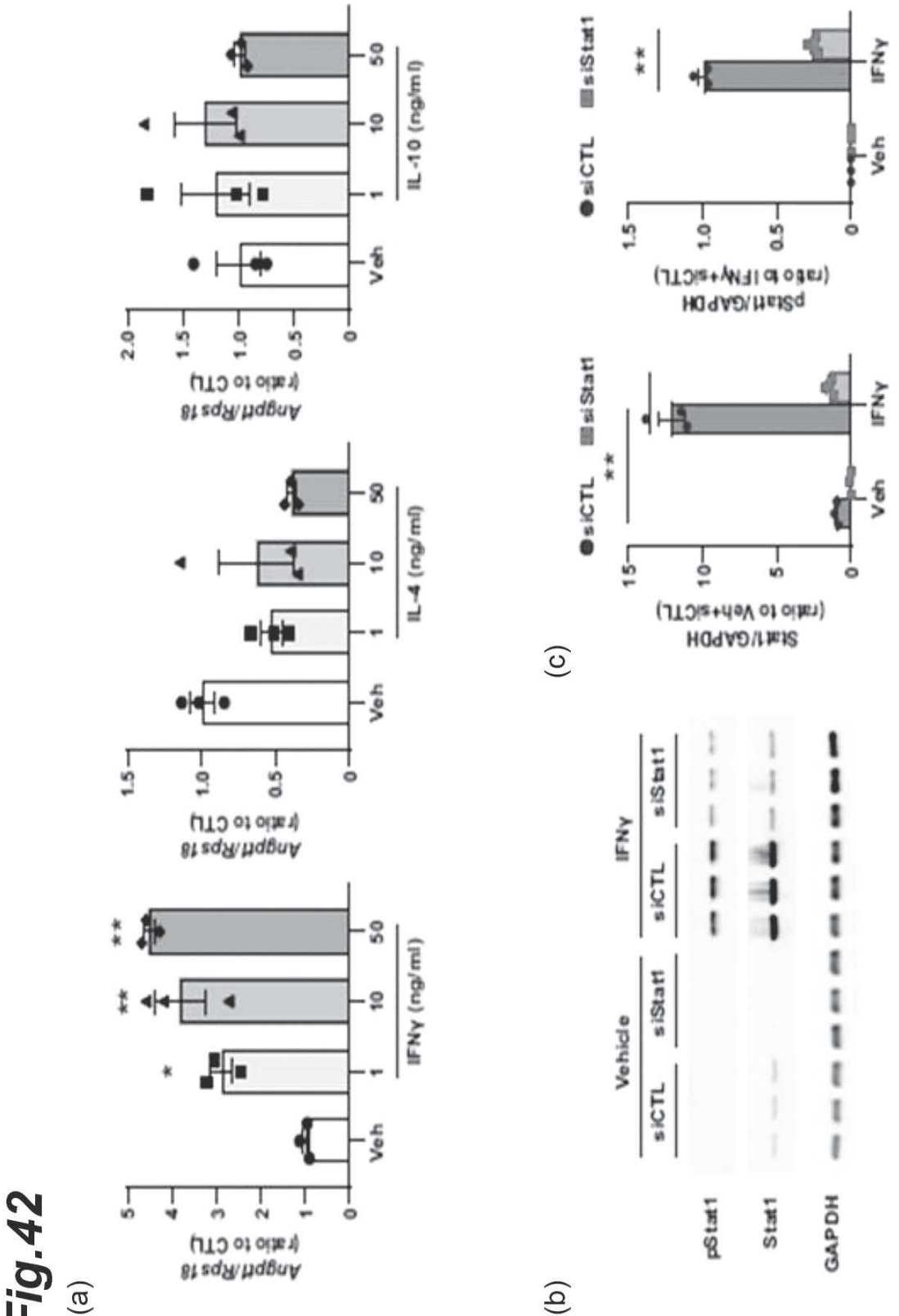

(a)
Angptl4/Rps18 (ratio to CTL)
IFNγ (ng/ml)
IL-4 (ng/ml)
IL-10 (ng/ml)
Veh
1
10
50
(b)
Vehicle
IFNγ
siCTL
siStat1
pStat1
Stat1
GAPDH
(c)
pStat1/GAPDH (ratio to Veh+siCTL)
pStat1/GAPDH (ratio to IFNγ+siCTL)
IFNγ

Fig.43

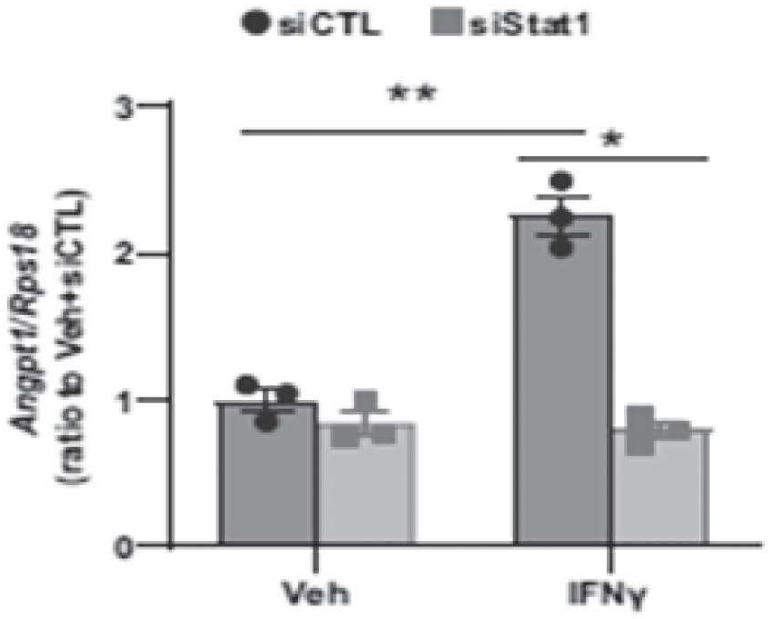
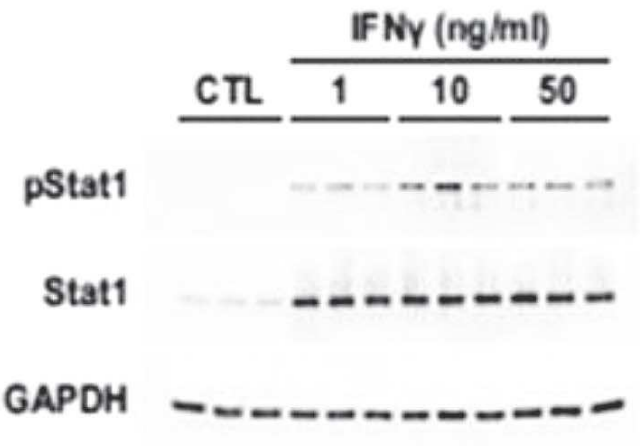


Fig.44

(a)


IFNγ (ng/ml)
CTL
1
10
50
pStat1
Stat1
GAPDH

(b)

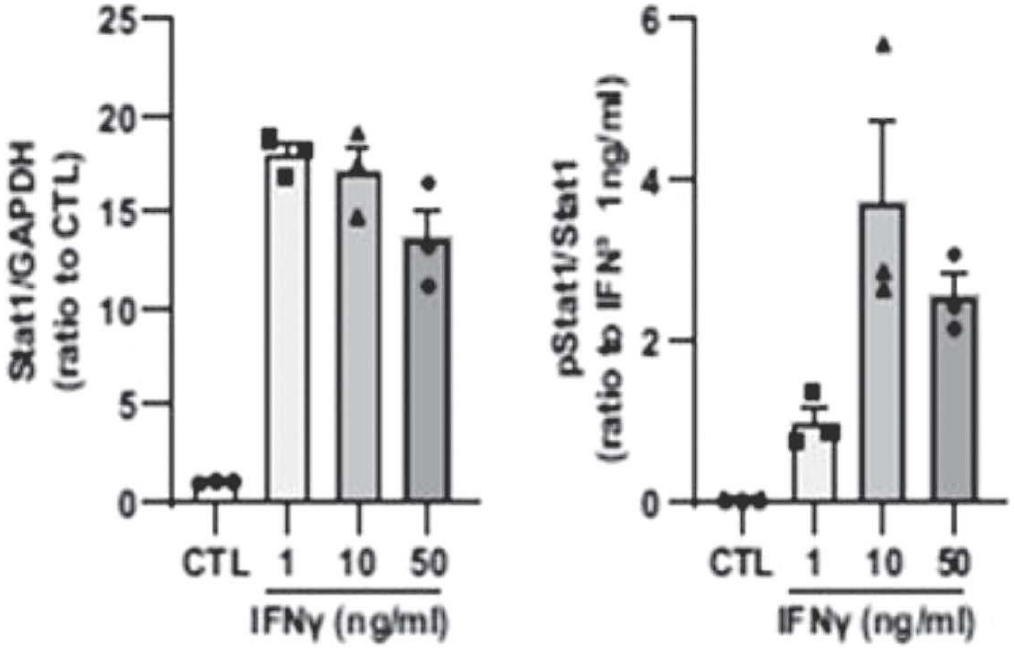
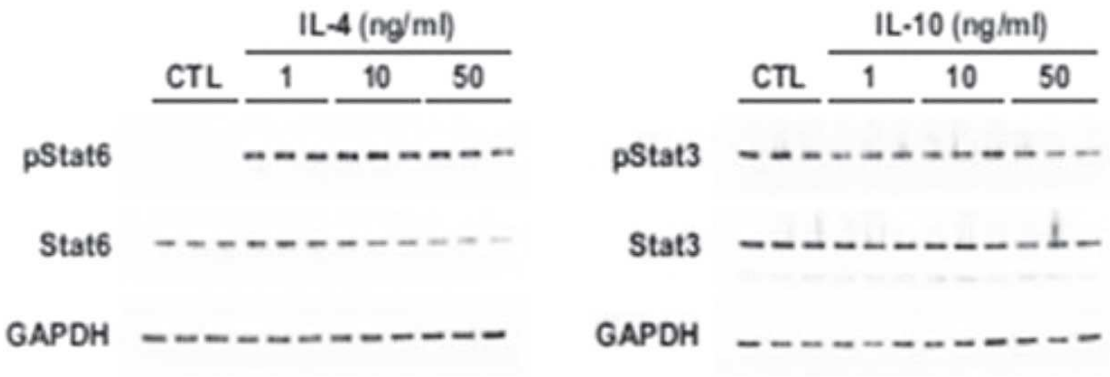

Stat1/GAPDH (ratio to CTL)
25
20
15
10
5
0
CTL
1
10
50
IFNγ (ng/ml)
pStat1/Stat1 (ratio to IFNγ 1ng/ml)
6
4
2
0
CTL
1
10
50
IFNγ (ng/ml)

Fig.45

(a)


IL-4 (ng/ml)
CTL
1
10
50
pStat6
Stat6
GAPDH
IL-10 (ng/ml)
CTL
1
10
50
pStat3
Stat3
GAPDH

(b)

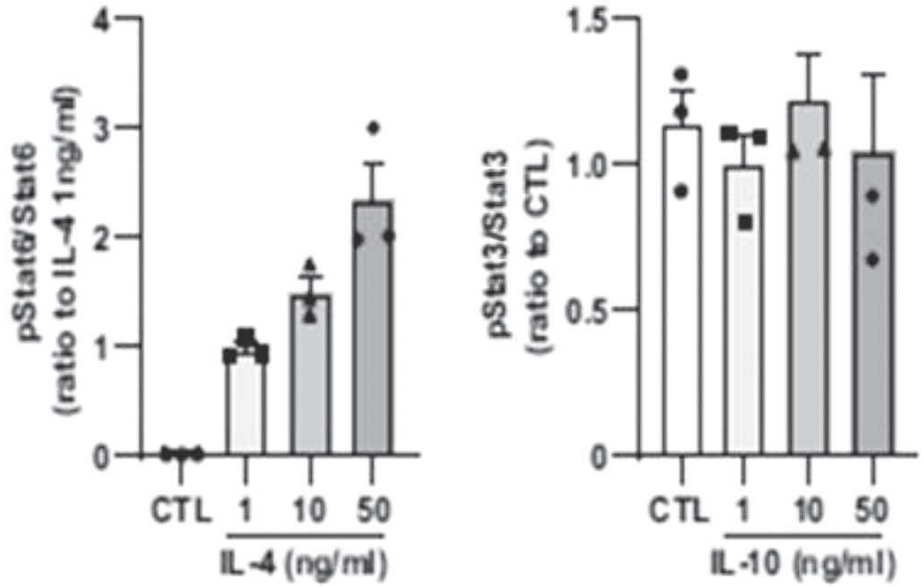

pStat6/Stat6
(ratio to IL-4 1ng/ml)
4
3
2
1
0
CTL
1
10
50
IL-4 (ng/ml)
pStat3/Stat3
(ratio to CTL)
1.5
1.0
0.5
0
CTL
1
10
50
IL-10 (ng/ml)

Fig.46


MLWHF
70
60
50
40
30
20
10
0
BEFORE ADMINISTRATION
AFTER 4 WEEKS
KCCQ (Quality of Life Score)
120
100
80
60
40
20
0
BEFORE ADMINISTRATION
AFTER 4 WEEKS
KCCQ (Overall Summary Score)
120
100
80
60
40
20
0
BEFORE ADMINISTRATION
AFTER 4 WEEKS