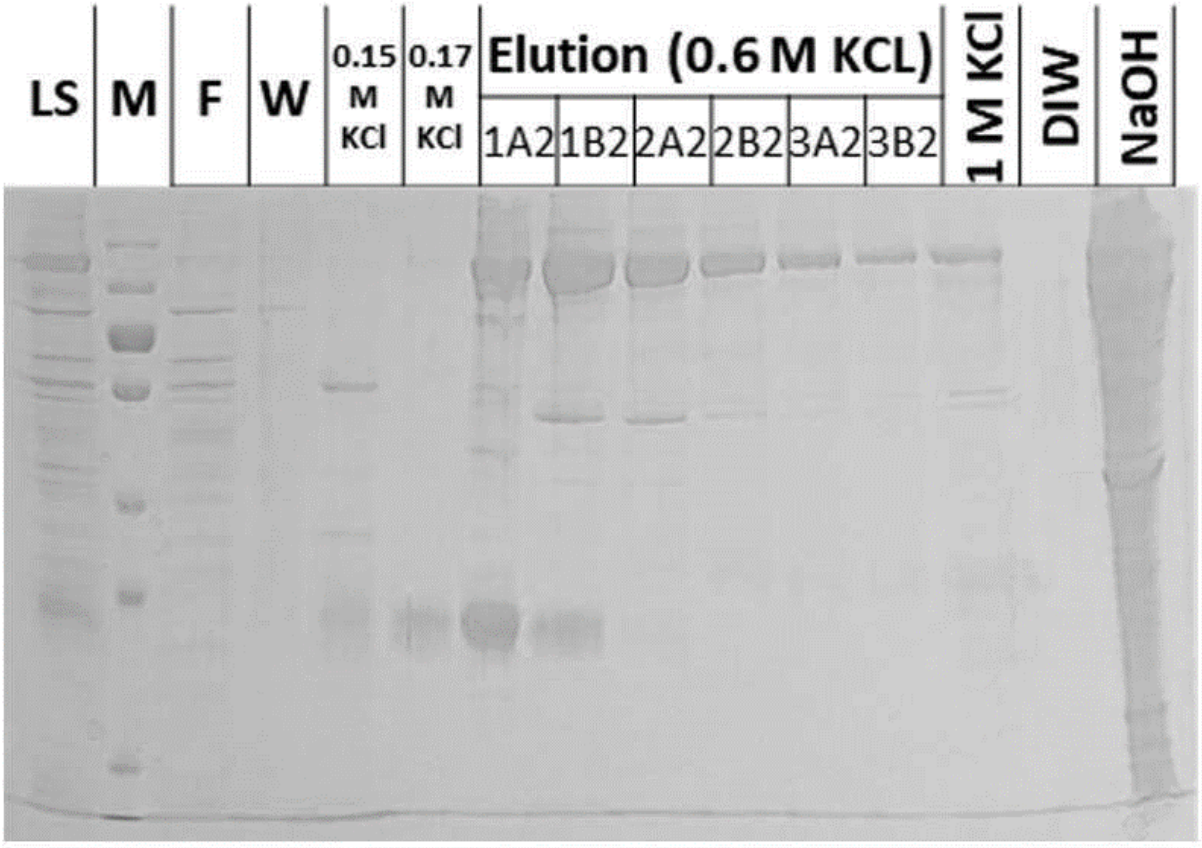
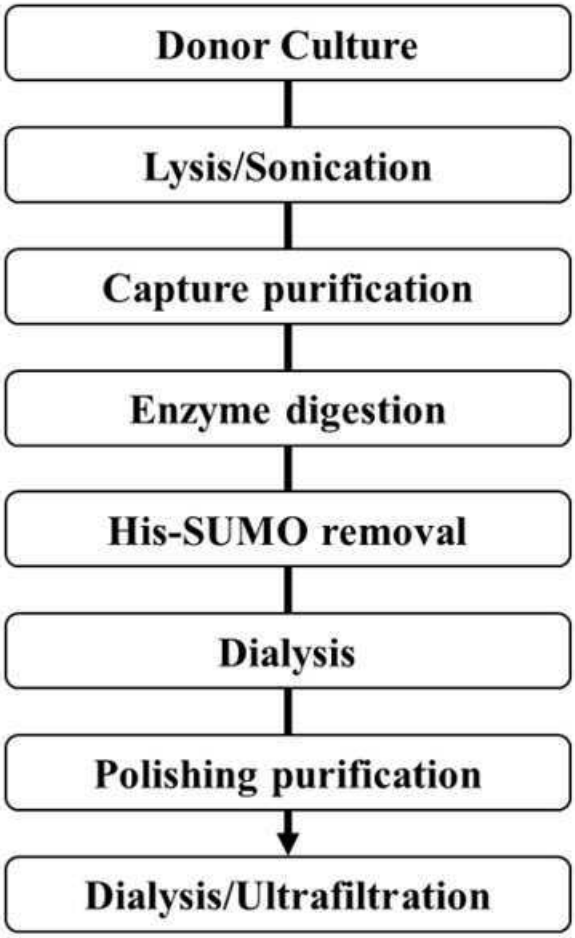
【 Drawings】

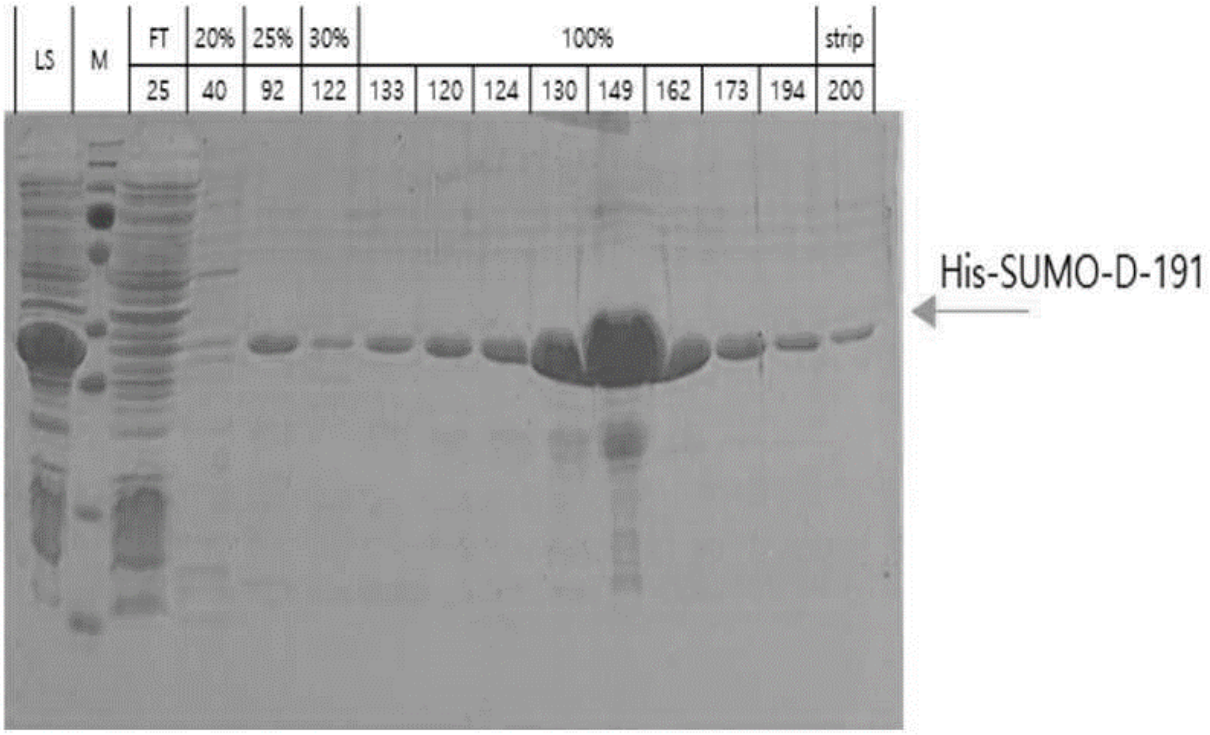
【Fig. 1】



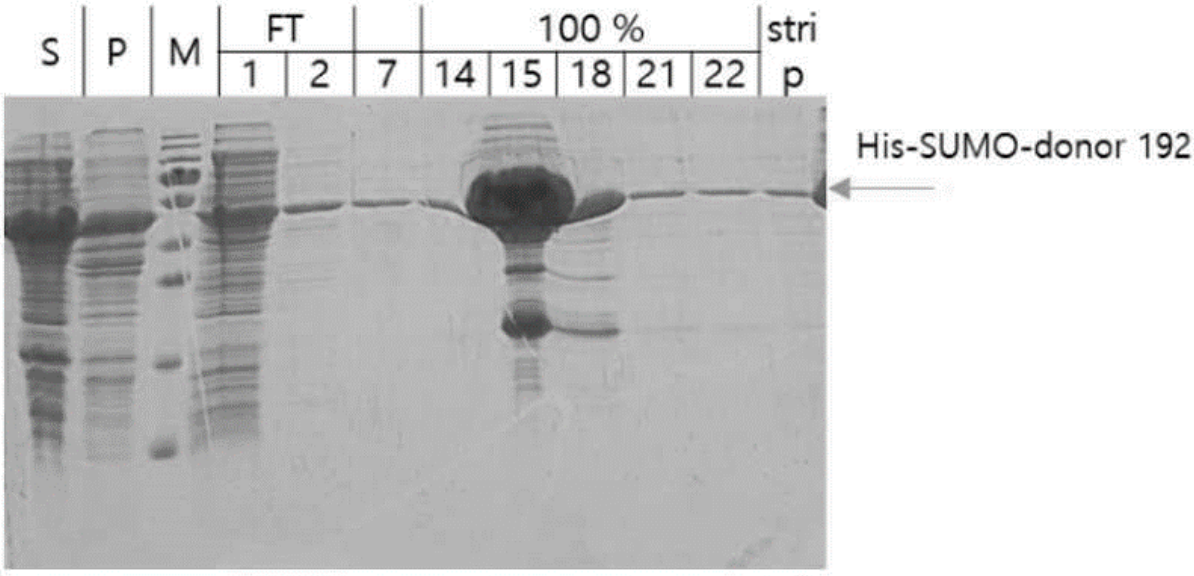
【Fig. 2】


Donor Culture
Lysis/Sonication
Capture purification
Enzyme digestion
His-SUMO removal
Dialysis
Polishing purification
Dialysis/Ultrafiltration

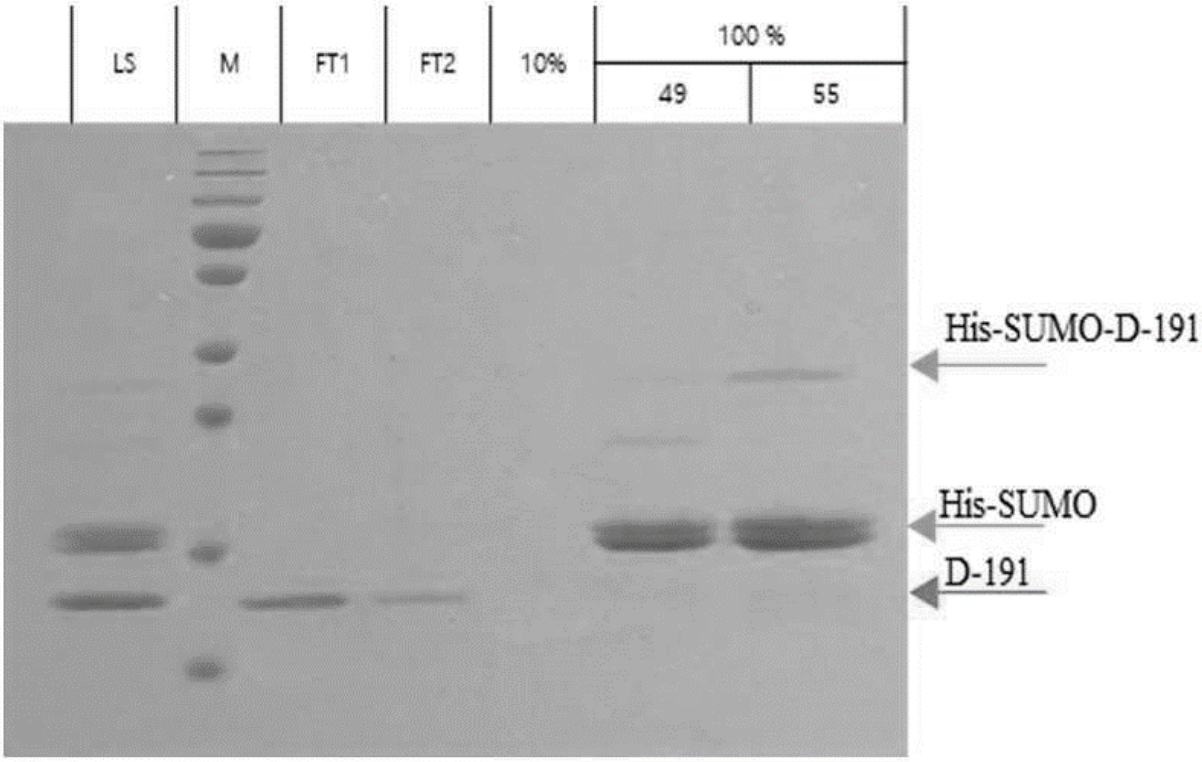
【Fig. 3】


LS
M
FT
20%
25%
30%
100%
strip
25
40
92
122
133
120
124
130
149
162
173
194
200
His-SUMO-D-191

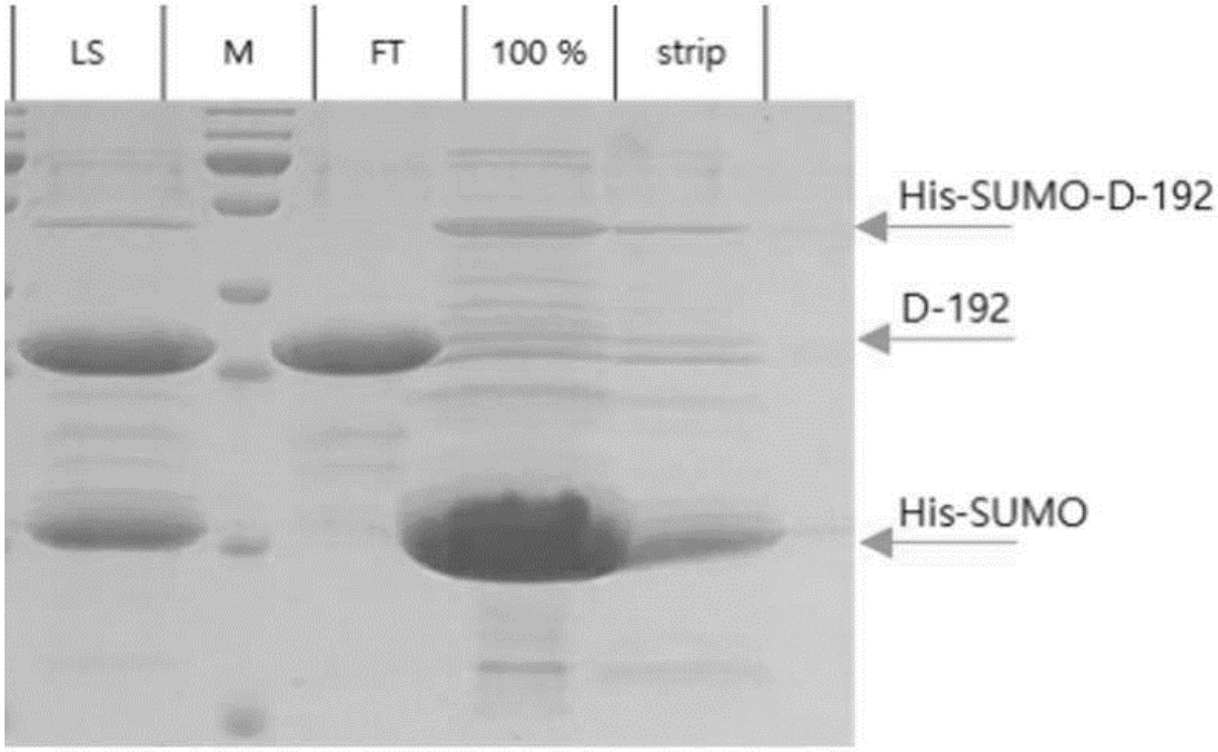
【Fig. 4】



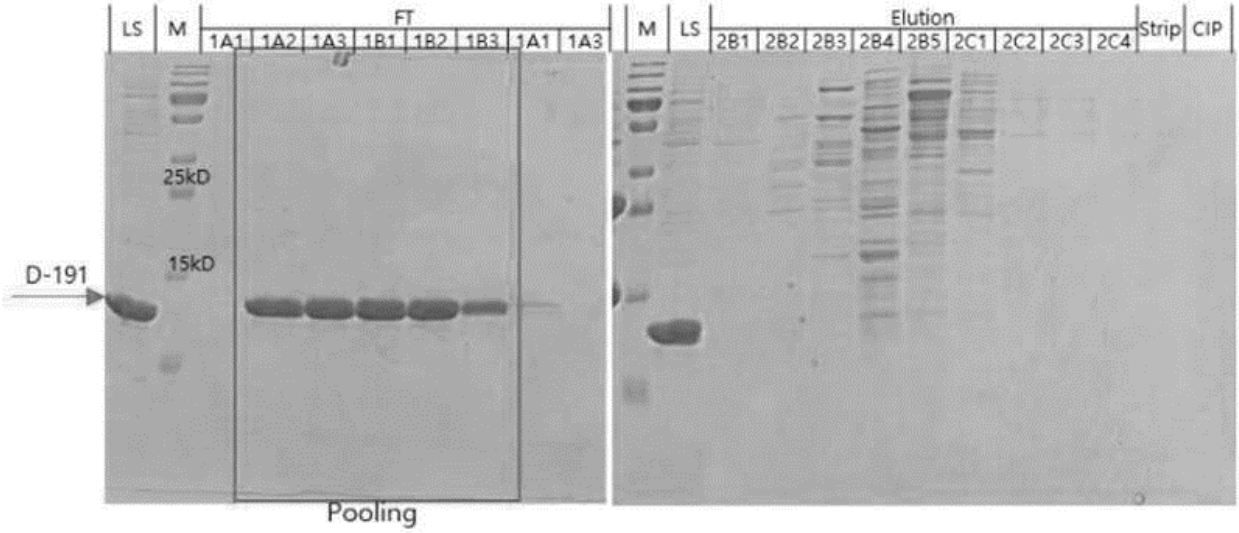
【Fig. 5】



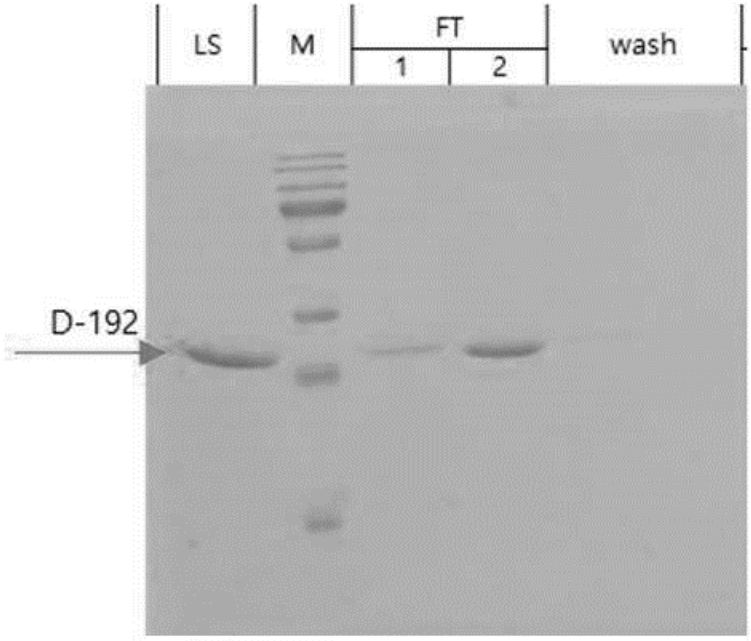
【Fig. 6】



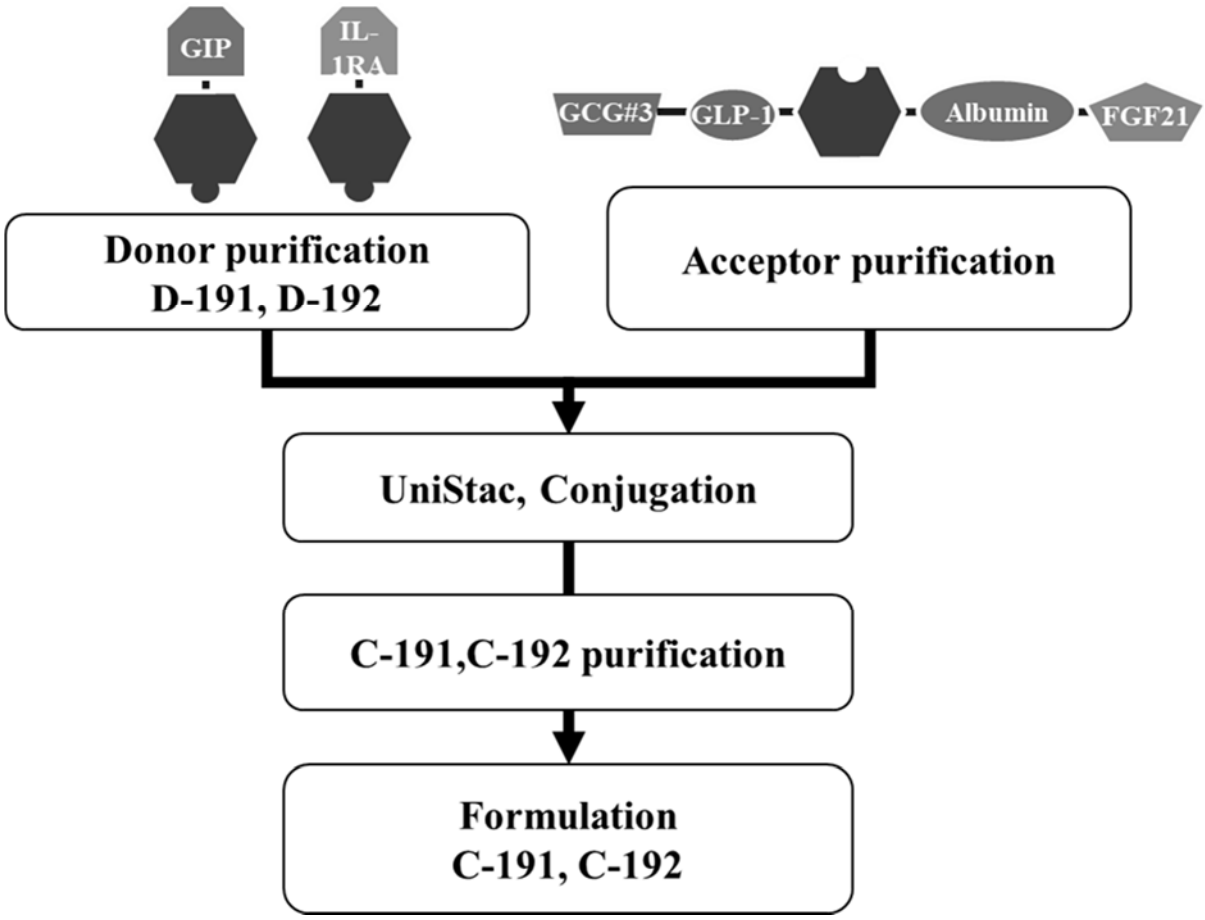
【Fig. 7】


LS
M
FT
1A1
1A2
1A3
1B1
1B2
1B3
1A1
1A3
M
LS
Elution
2B1
2B2
2B3
2B4
2B5
2C1
2C2
2C3
2C4
Strip
CIP
25kD
15kD
D-191
Pooling

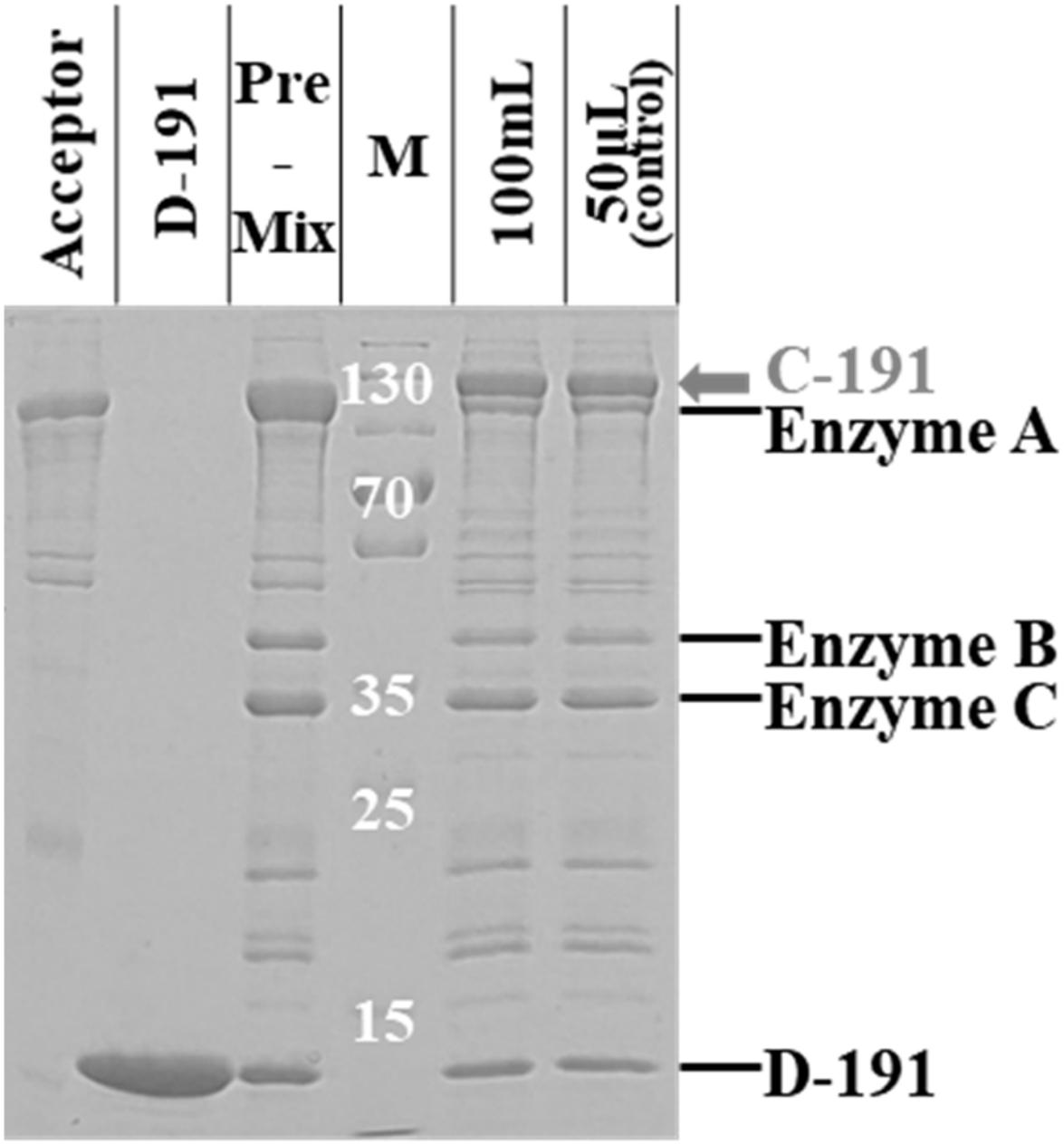
【Fig. 8】



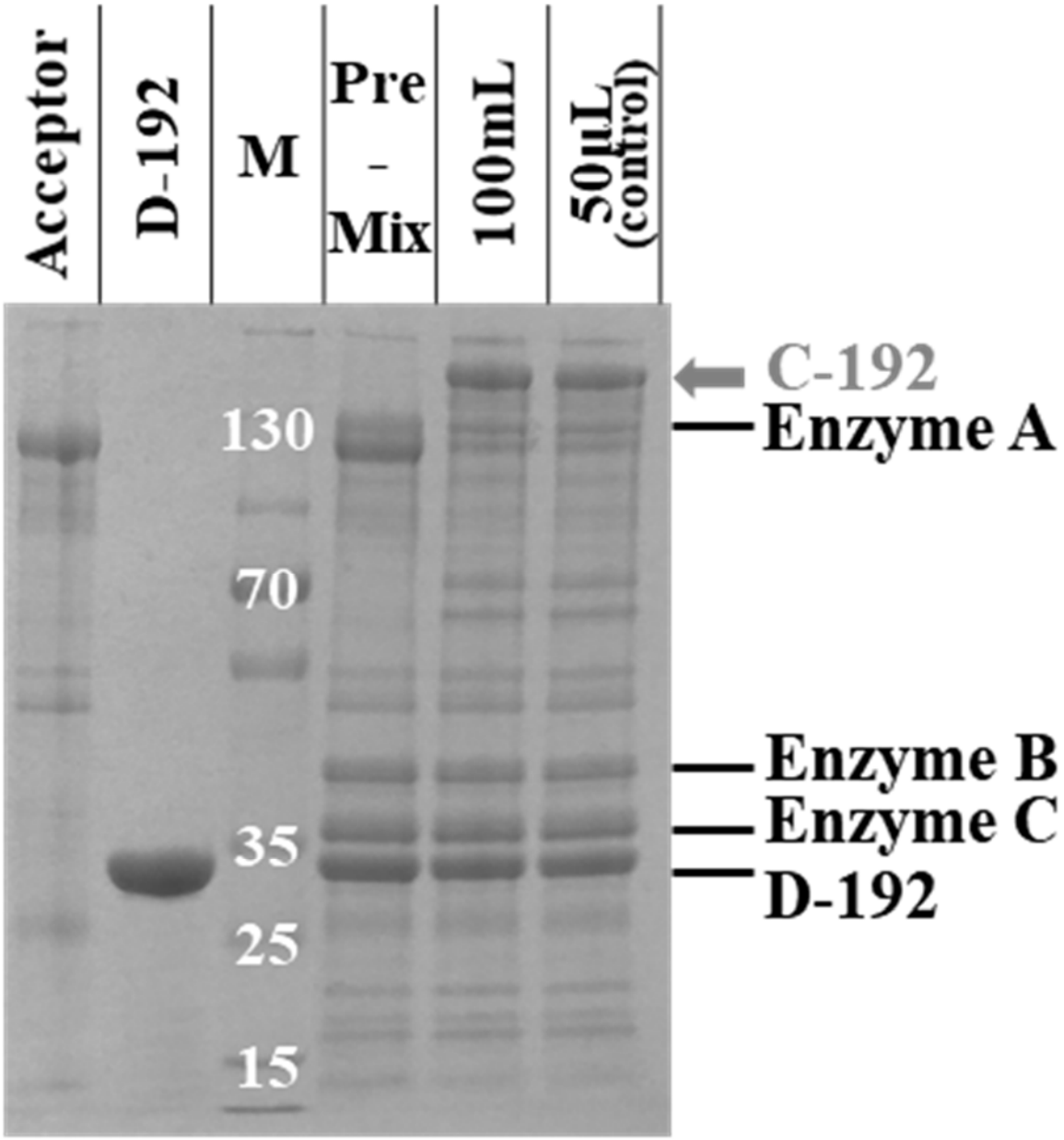
【Fig. 9】


GIP
IL-1RA
GCG#3
GLP-1
Albumin
FGF21
Donor purification
D-191, D-192
Acceptor purification
UniStac, Conjugation
C-191,C-192 purification
Formulation
C-191, C-192

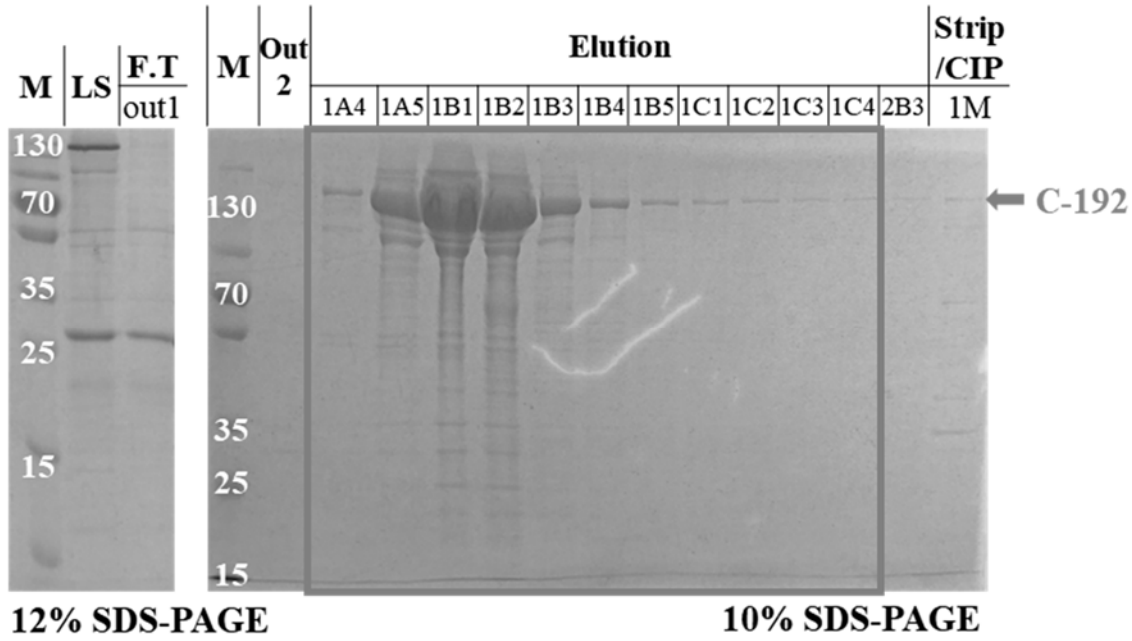
【Fig. 10】


Acceptor
D-191
Pre - Mix
M
100mL
50μL (control)
130
70
35
25
15
C-191
Enzyme A
Enzyme B
Enzyme C
D-191

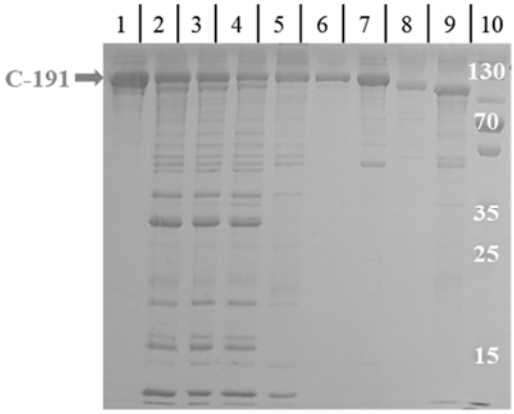
【Fig. 11】


Acceptor
D-192
M
Pre - Mix
100mL
50μL (control)
130
70
35
25
15
C-192
Enzyme A
Enzyme B
Enzyme C
D-192

【Fig. 12】

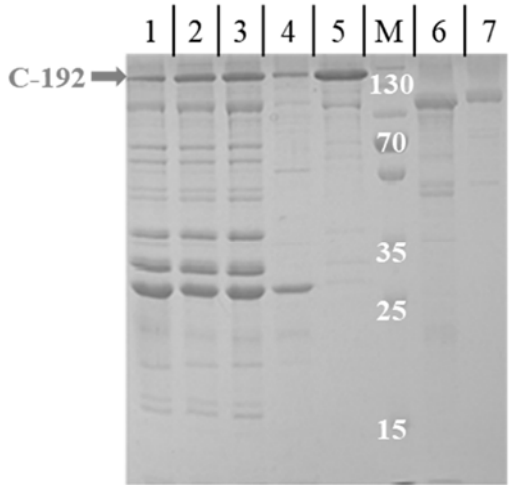

M
LS
F.T
out1
M
Out
2
Elution
Strip
/CIP
1A4
1A5
1B1
1B2
1B3
1B4
1B5
1C1
1C2
1C3
1C4
2B3
1M
130
70
35
25
15
130
70
35
25
15
C-192
12% SDS-PAGE
10% SDS-PAGE

【Fig. 13】



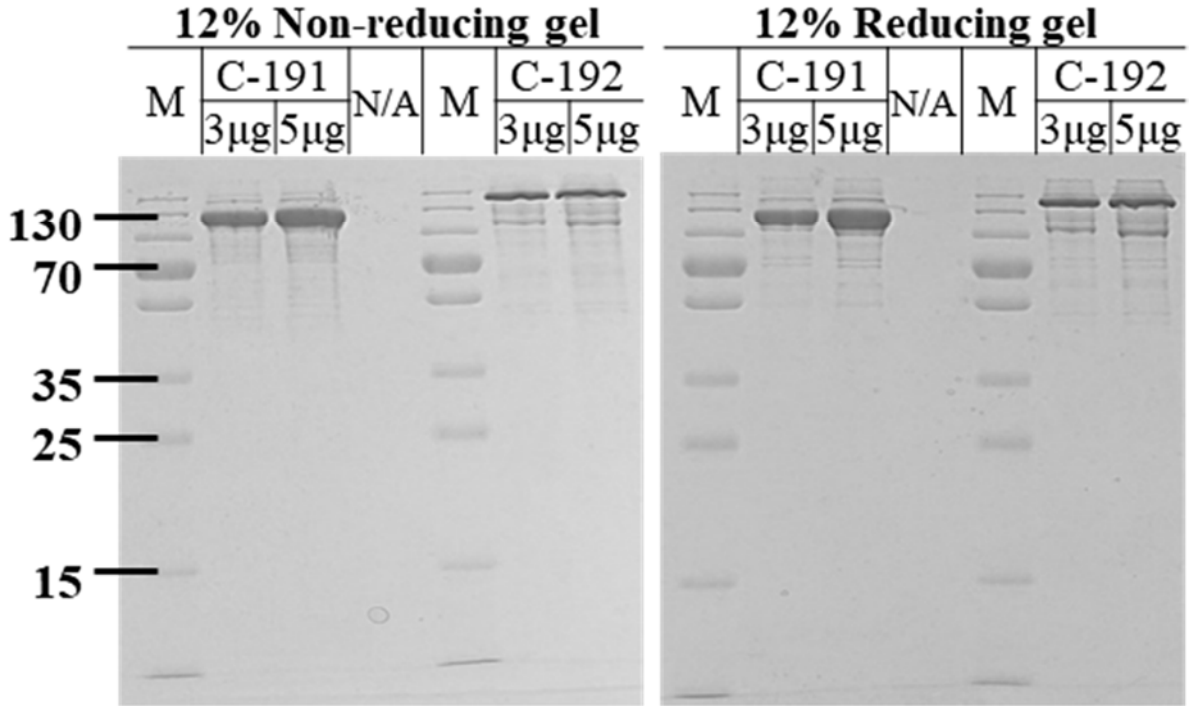
Lane 1: RD191-003-DS, positive control (Lot. No.: S191031)
2: RD191-004-C-01
3: RD191-004-C-02
4: RD191-004-C-03
5: RD191-004-C-NF-01
6: RD191-004-C-AX-02
7: RD191-004-C-NB-01
8: UCTE1 (S191109-01)
9: Acceptor (RD191-A-004-AF-06)
10: Marker (thermo fisher, #26619)

【Fig. 14】

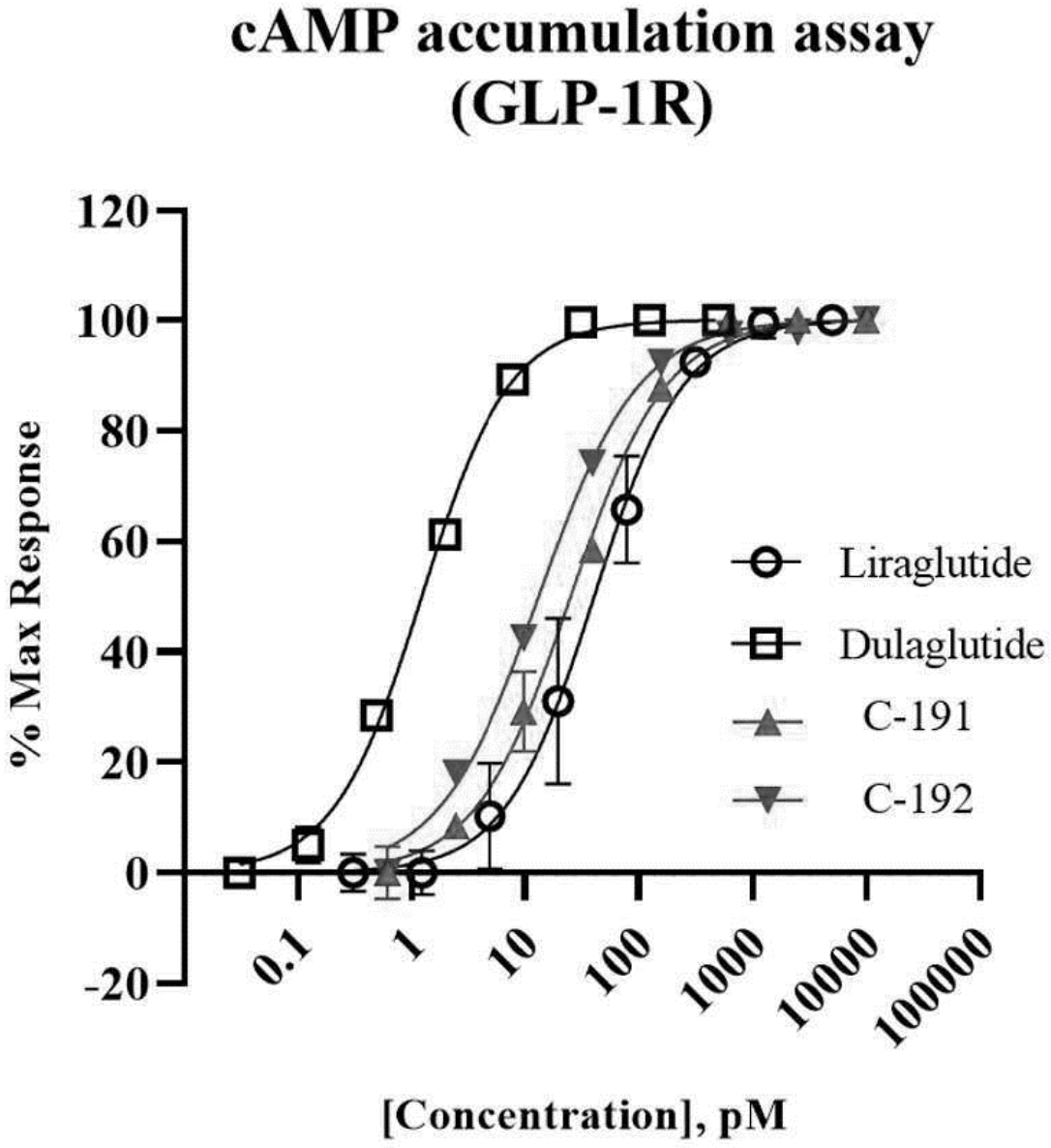


Lane 1: RD192-001-C-03
2: RD192-001-C-02
3: RD192-001-C-01
4: RD192-001-C-NF-01
5: RD192-001-C-AX-01
M: Marker (thermo fisher, #26619)
6: Acceptor (RD191-A-004-AF-06)
7: Enzyme 1 (Lot. No: S191109-01)

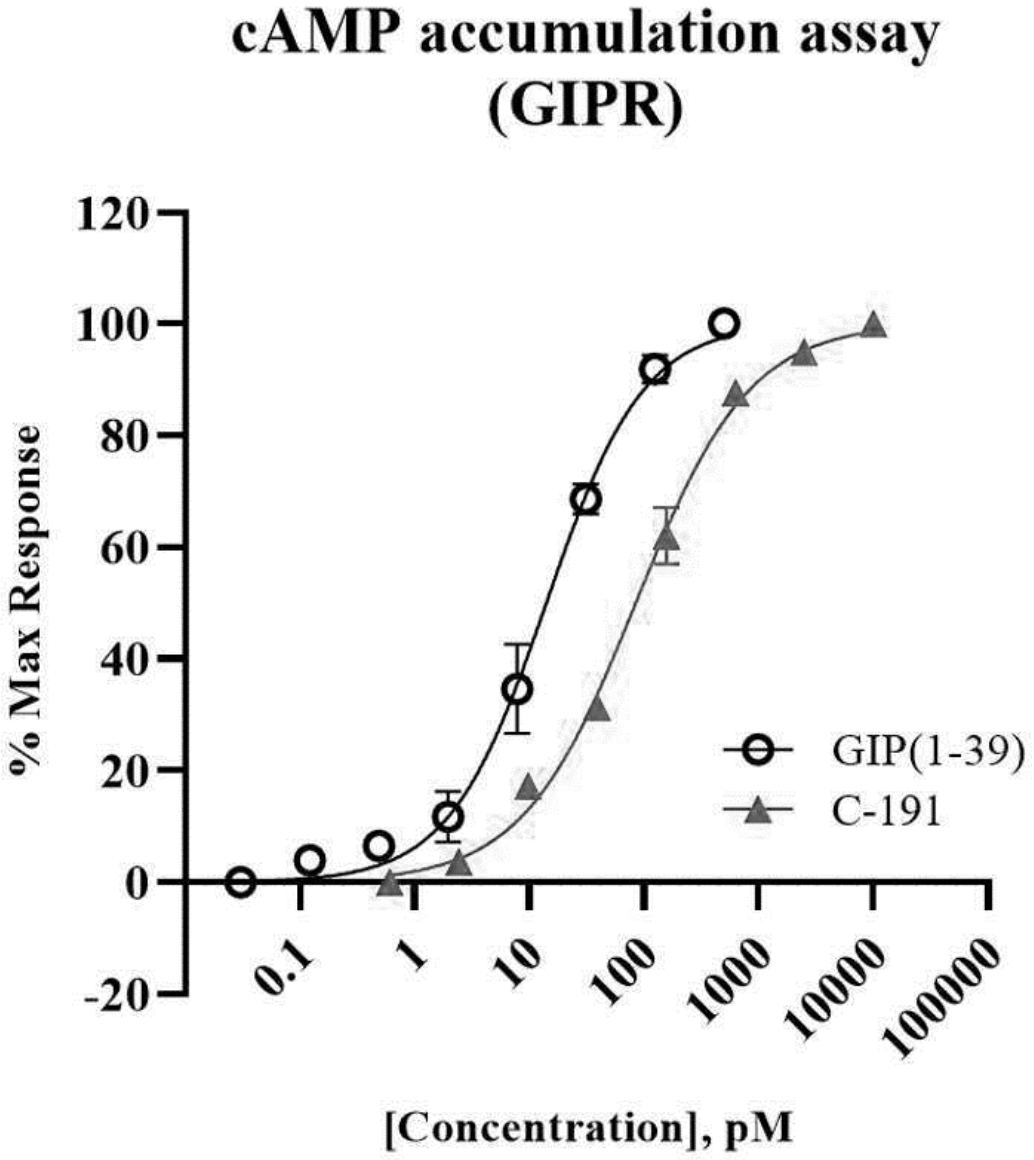
【Fig. 15】


12% Non-reducing gel
12% Reducing gel
M
C-191
3μg
5μg
N/A
M
C-192
3μg
5μg
130
70
35
25
15

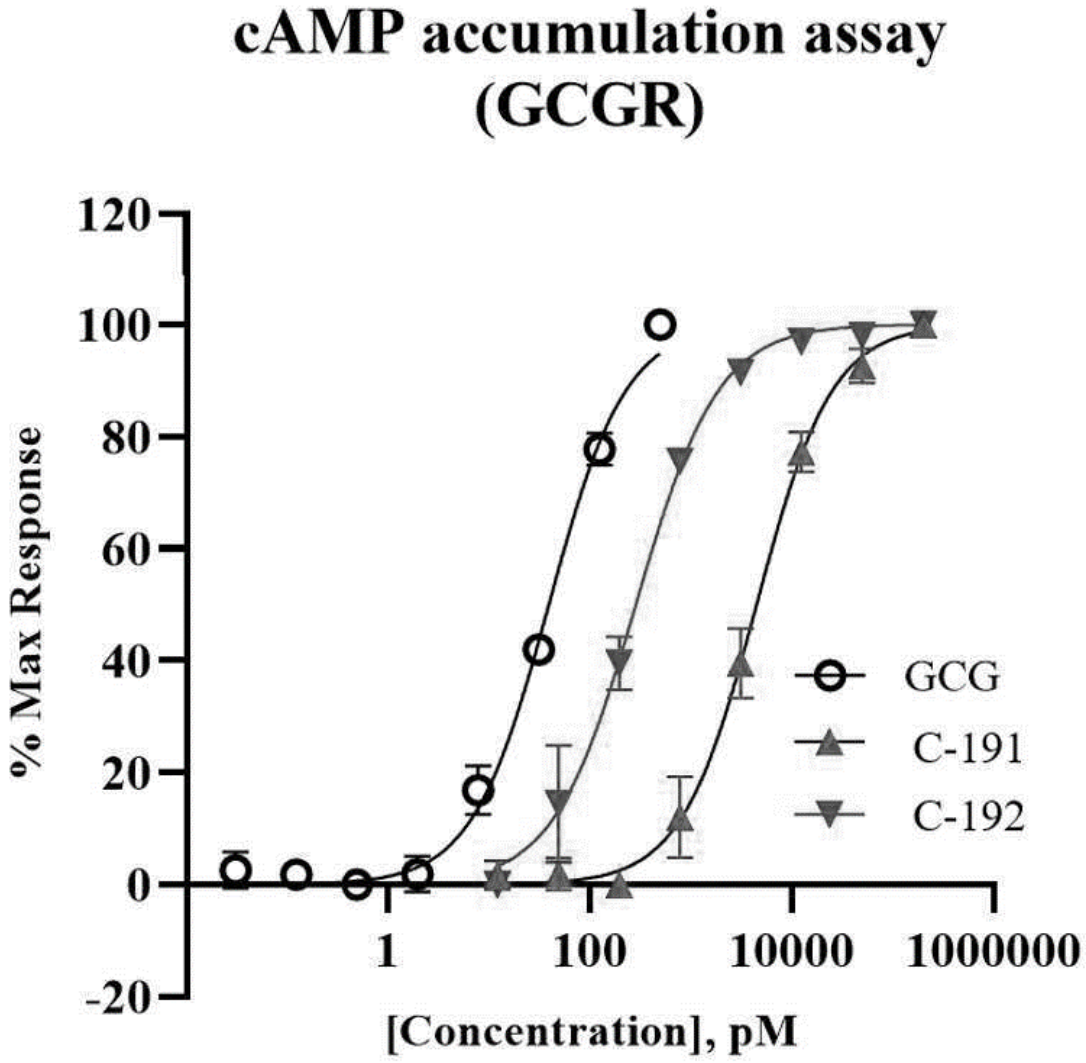
【Fig. 16】


cAMP accumulation assay
(GLP-1R)
% Max Response
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
100000
[Concentration], pM
Liraglutide
Dulaglutide
C-191
C-192

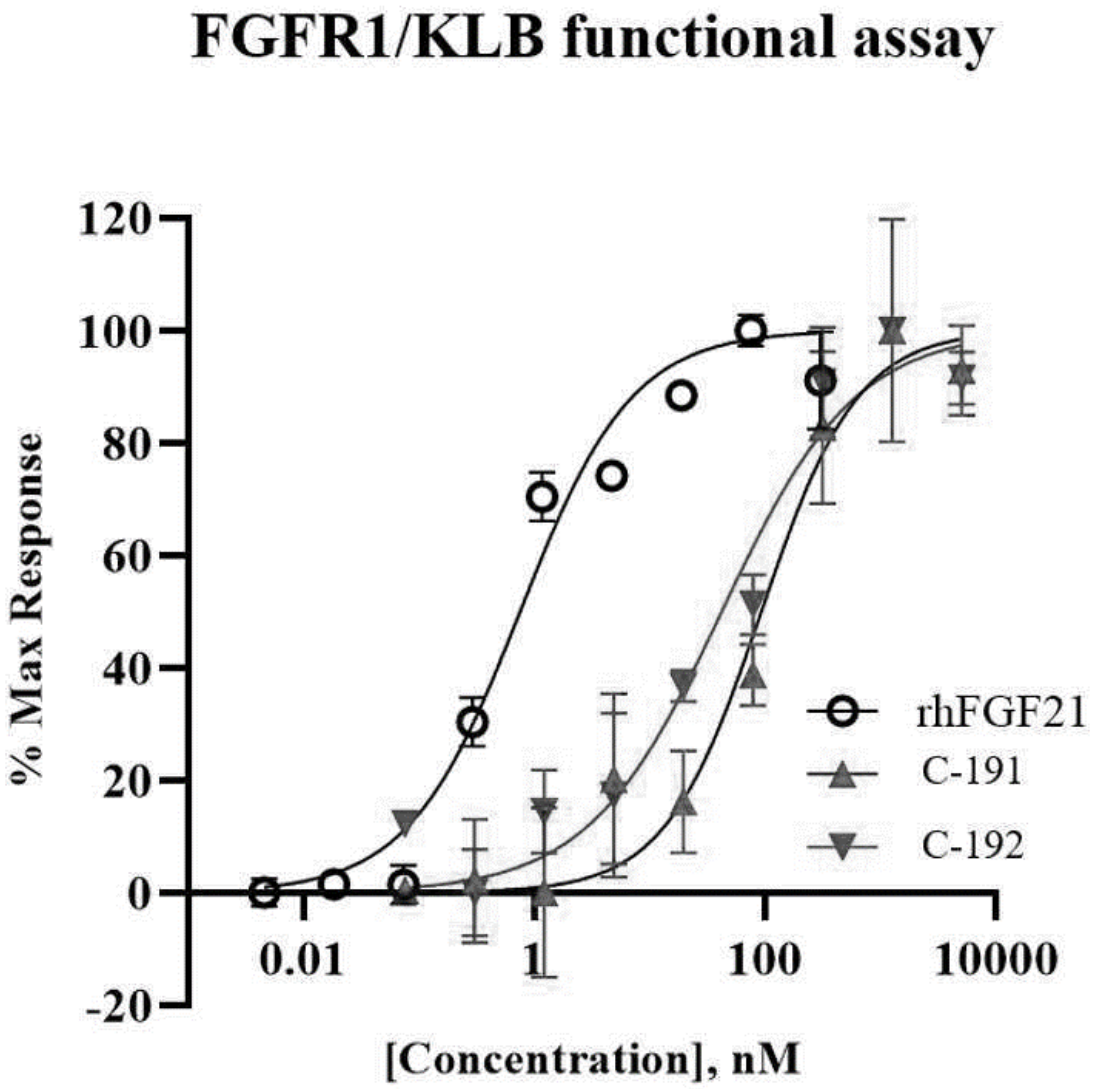
【Fig. 17】


cAMP accumulation assay
(GIPR)
% Max Response
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
10000
100000
[Concentration], pM
GIP(1-39)
C-191

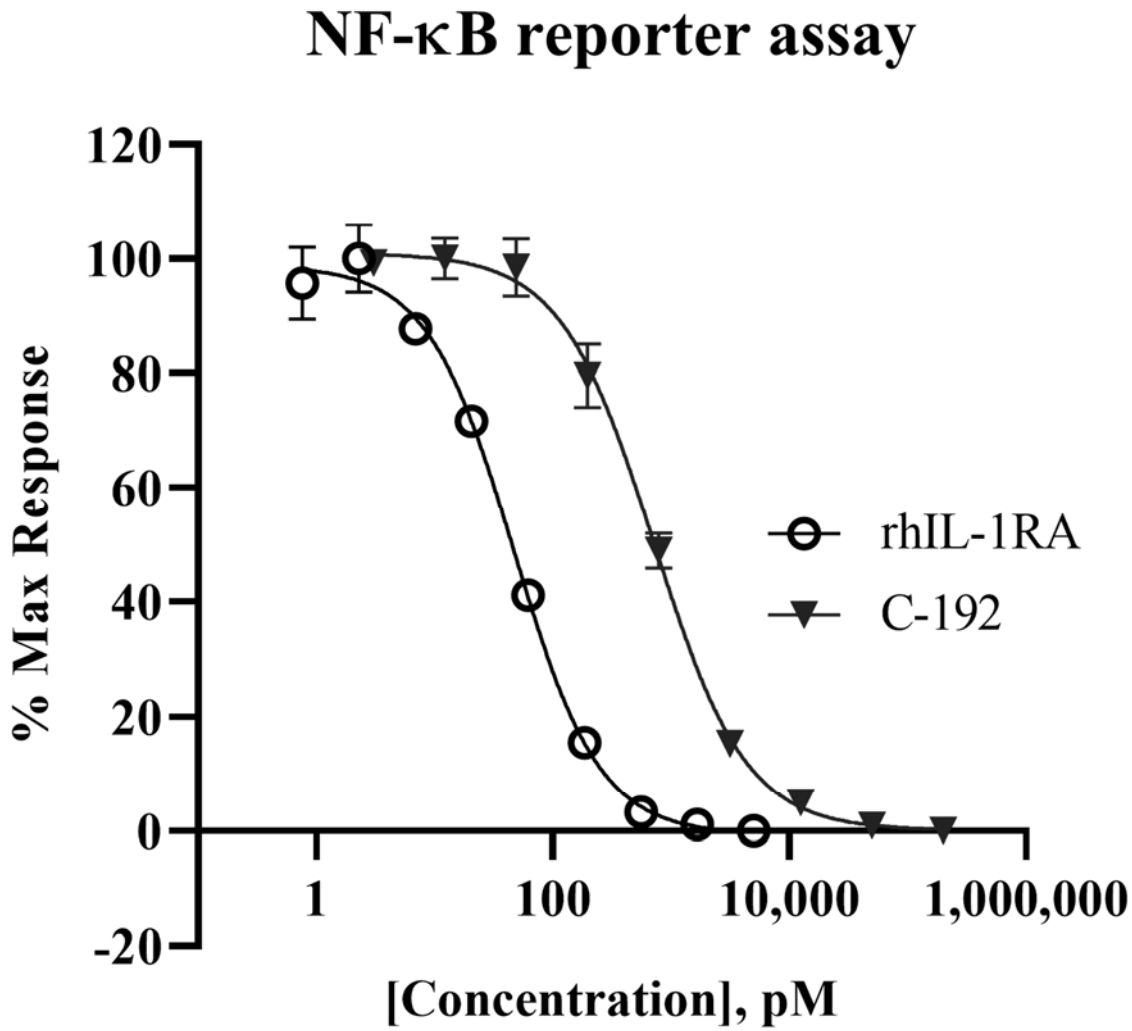
【Fig. 18】


cAMP accumulation assay
(GCGR)
% Max Response
120
100
80
60
40
20
0
-20
1
100
10000
1000000
[Concentration], pM
GCG
C-191
C-192

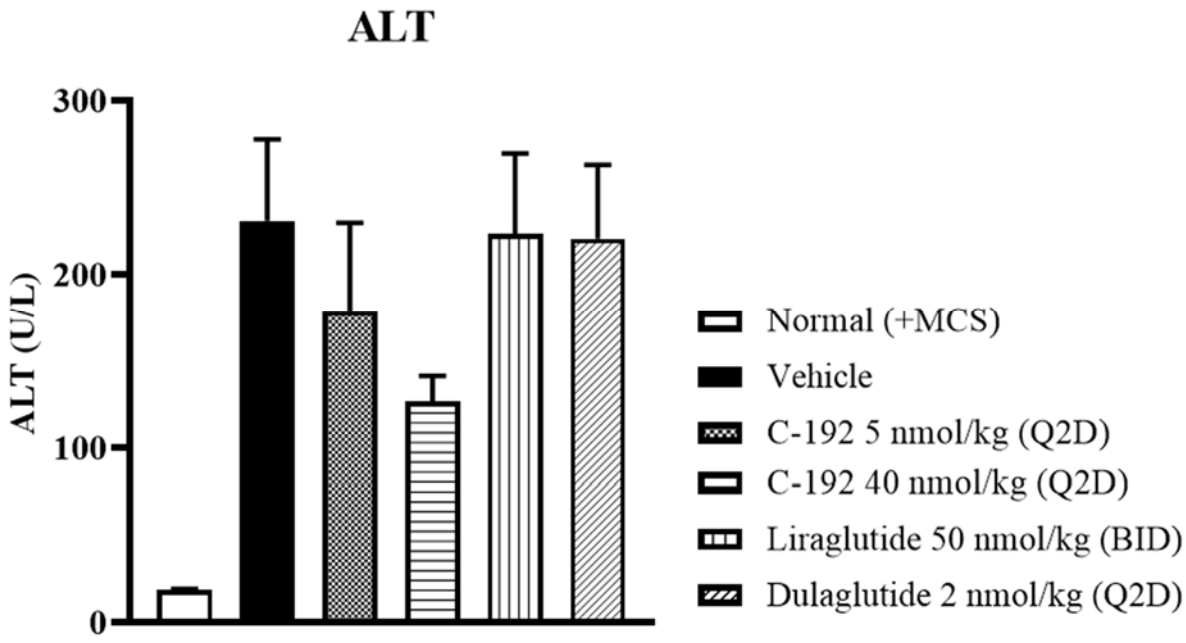
【Fig. 19】


FGFR1/KLB functional assay
% Max Response
120
100
80
60
40
20
0
-20
0.01
1
100
10000
[Concentration], nM
rhFGF21
C-191
C-192

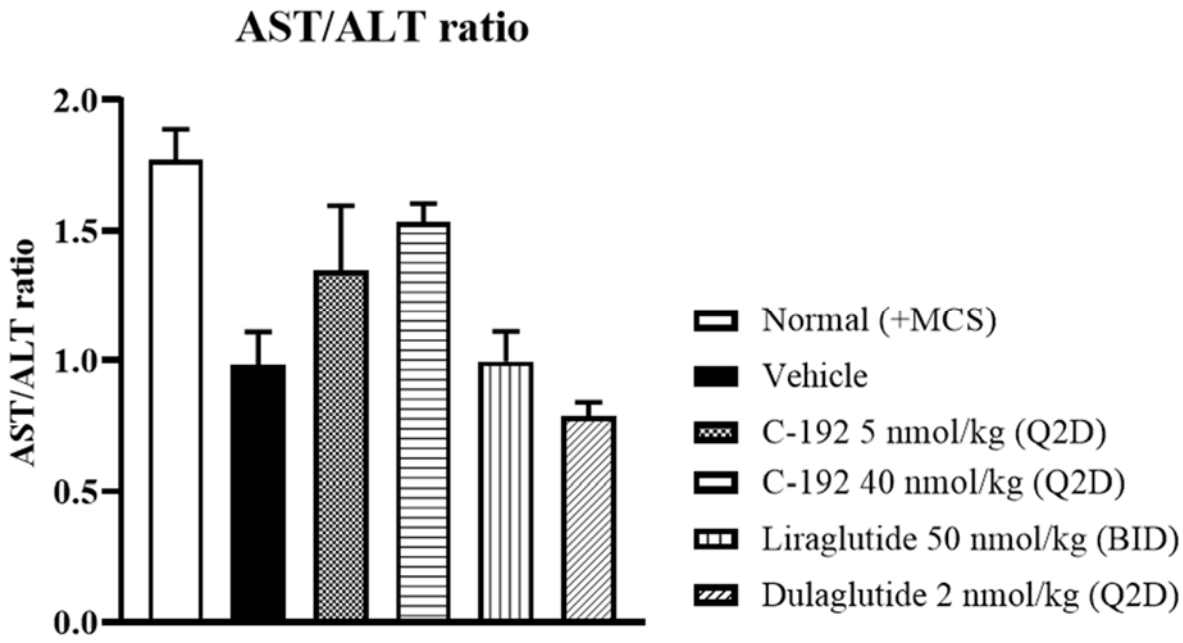
【Fig. 20】


NF-κB reporter assay
120
100
80
60
40
20
0
-20
% Max Response
1
100
10,000
1,000,000
[Concentration], pM
rhIL-1RA
C-192

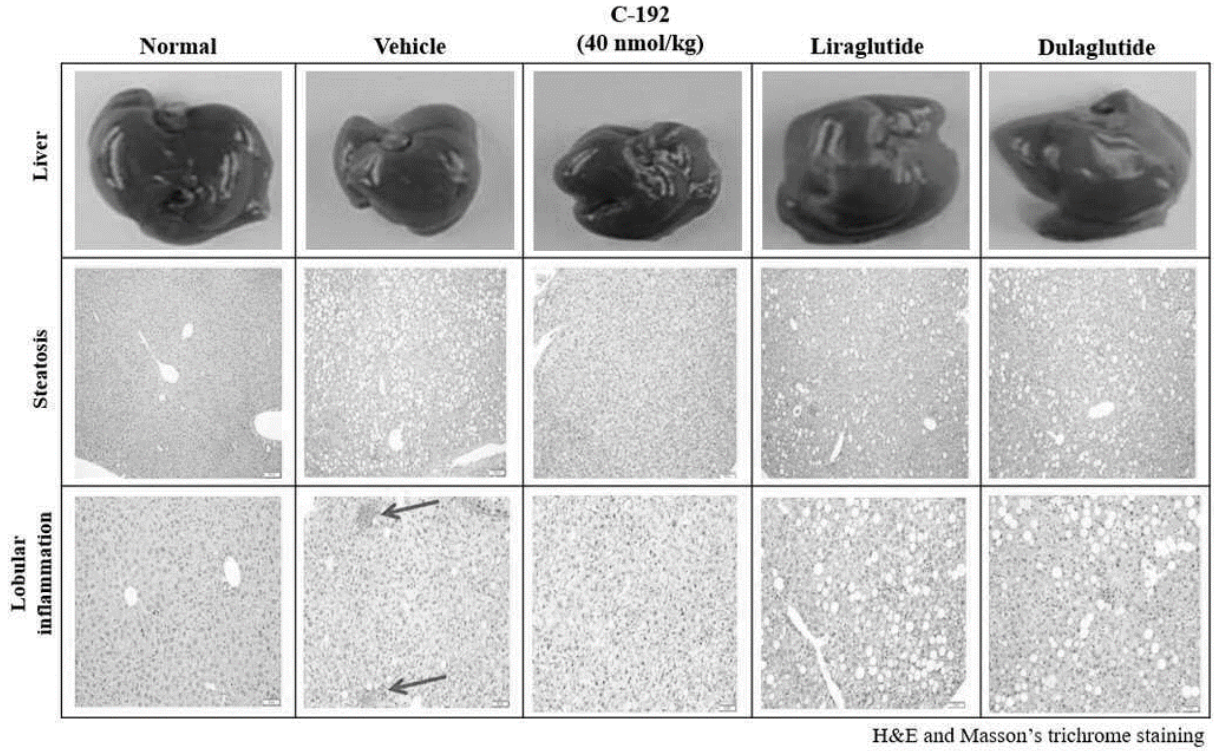
【Fig. 21】


ALT
ALT (U/L)
300
200
100
0
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)

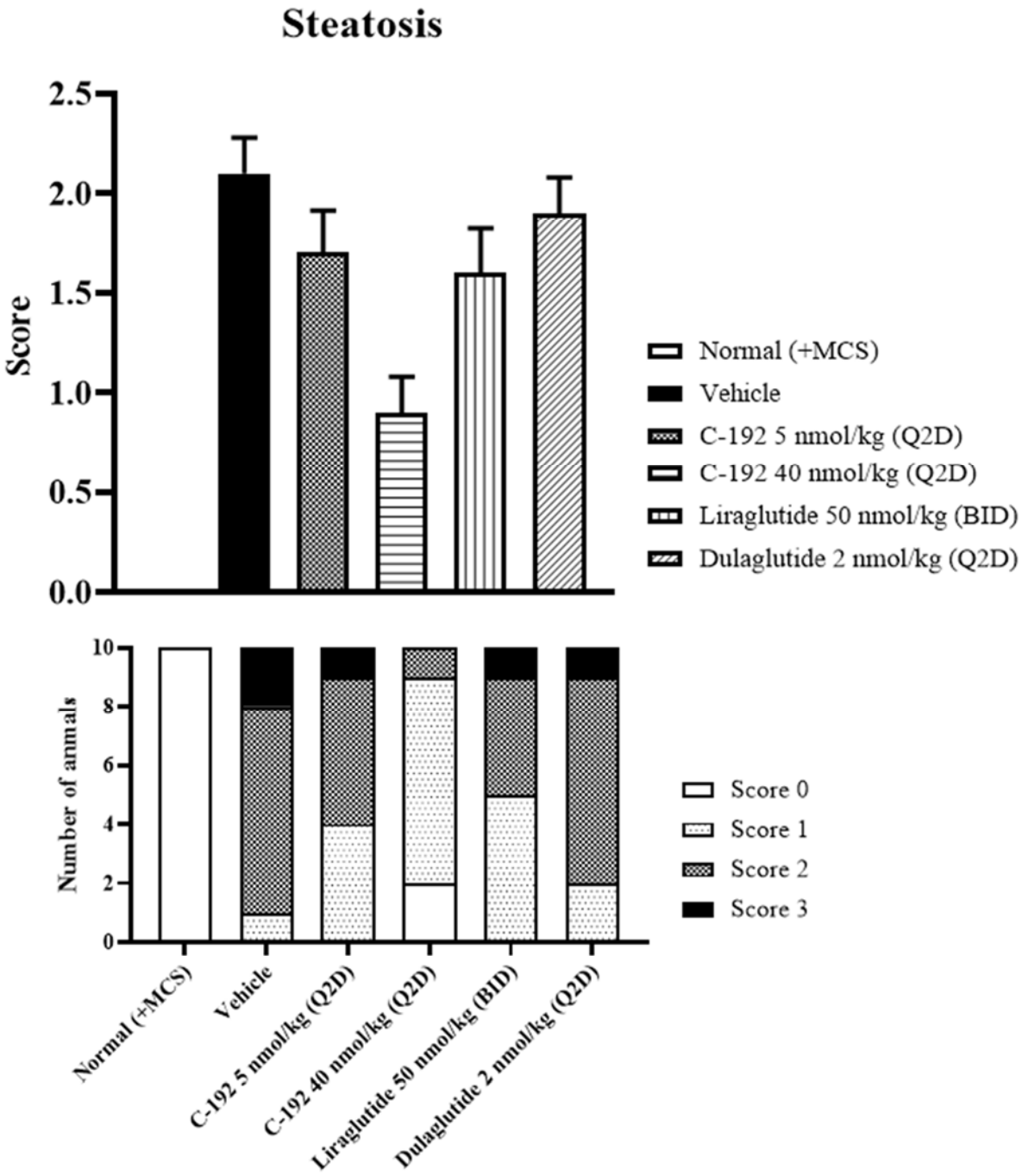
【Fig. 22】


AST/ALT ratio
AST/ALT ratio
2.0
1.5
1.0
0.5
0.0
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)

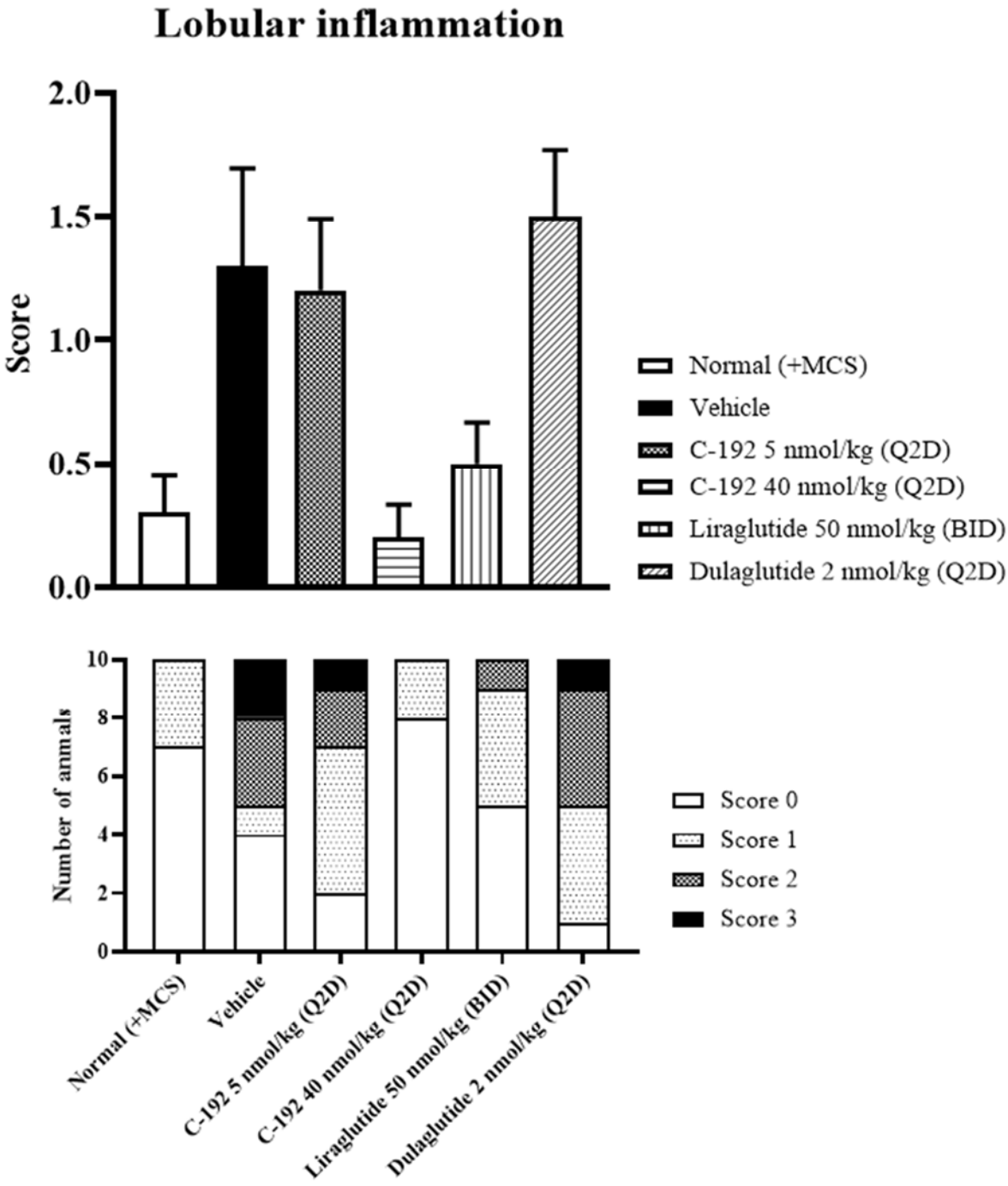
【Fig. 23】


Normal
Vehicle
C-192
(40 nmol/kg)
Liraglutide
Dulaglutide
Liver
Steatosis
Lobular inflammation
H&E and Masson's trichrome staining

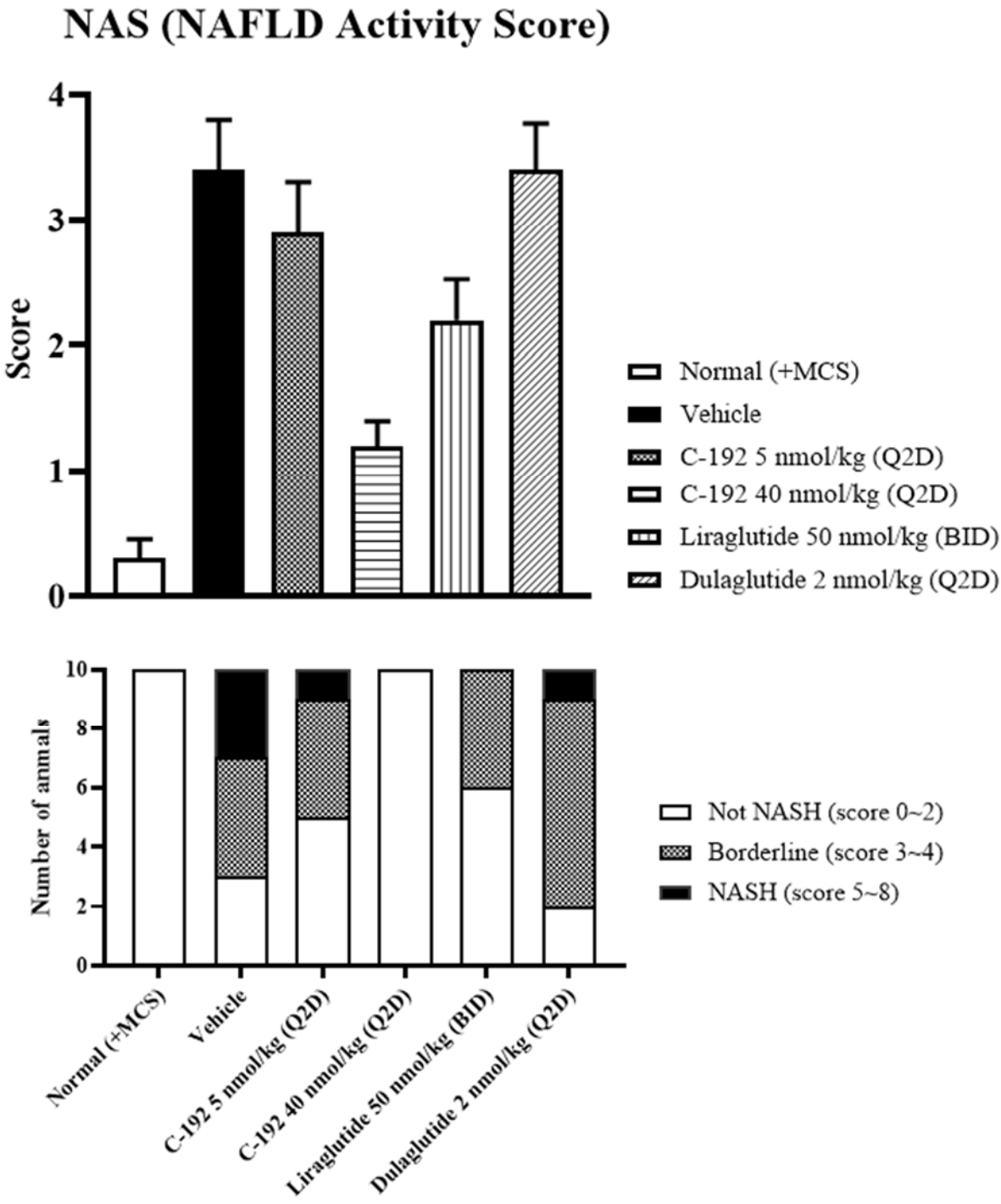
【Fig. 24】


Steatosis
Score
2.5
2.0
1.5
1.0
0.5
0.0
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)
Number of animals
10
8
6
4
2
0
Score 0
Score 1
Score 2
Score 3
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)

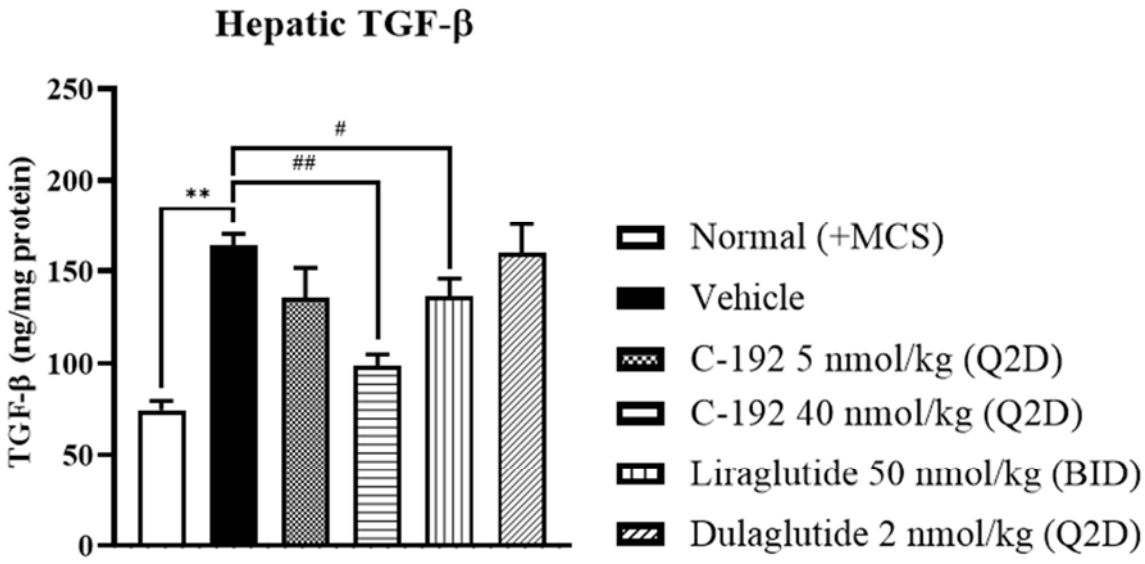
【Fig. 25】


Lobular inflammation
Score
2.0
1.5
1.0
0.5
0.0
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)
Number of anmals
10
8
6
4
2
0
Score 0
Score 1
Score 2
Score 3
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)

【Fig. 26】

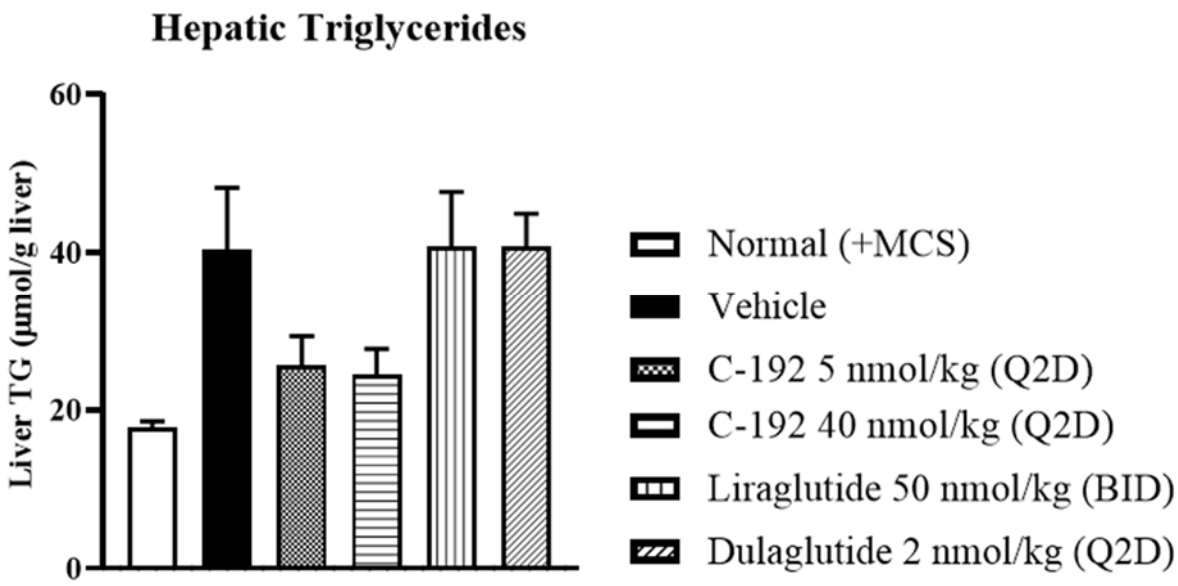

NAS (NAFLD Activity Score)
Score
0
1
2
3
4
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)
Number of animals
0
2
4
6
8
10
Not NASH (score 0~2)
Borderline (score 3~4)
NASH (score 5~8)
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)

【Fig. 27】

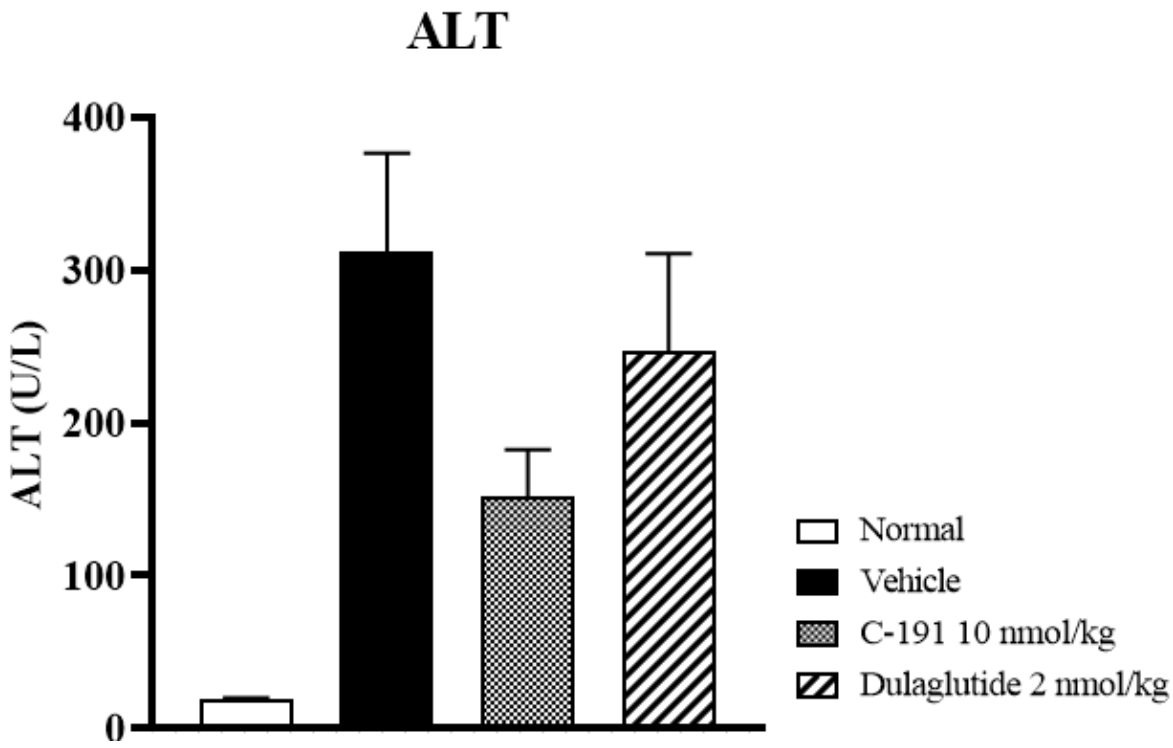


** $p < 0.01$, Significant difference from the normal control by Student t-test.
# $p < 0.05$, Significant difference from the negative control by Steel's t-test.
$p < 0.01$, Significant difference from the negative control by Steel's t-test.

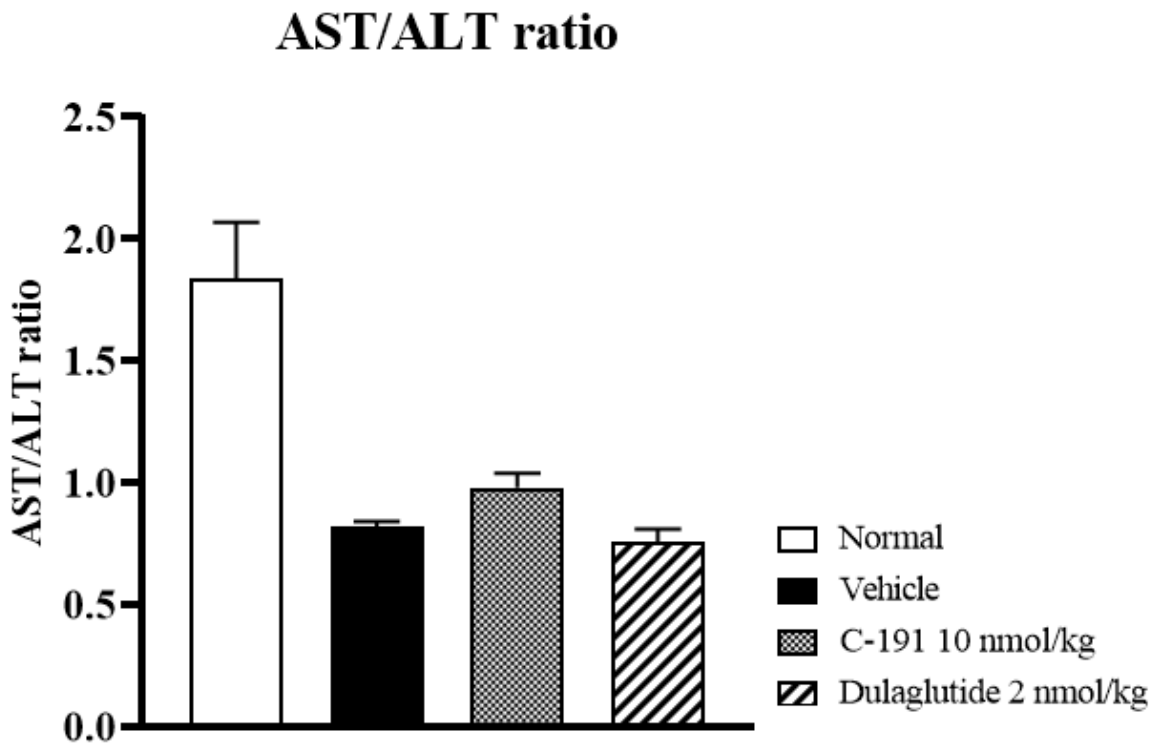
【Fig. 28】


Hepatic Triglycerides
Liver TG (µmol/g liver)
60
40
20
0
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)

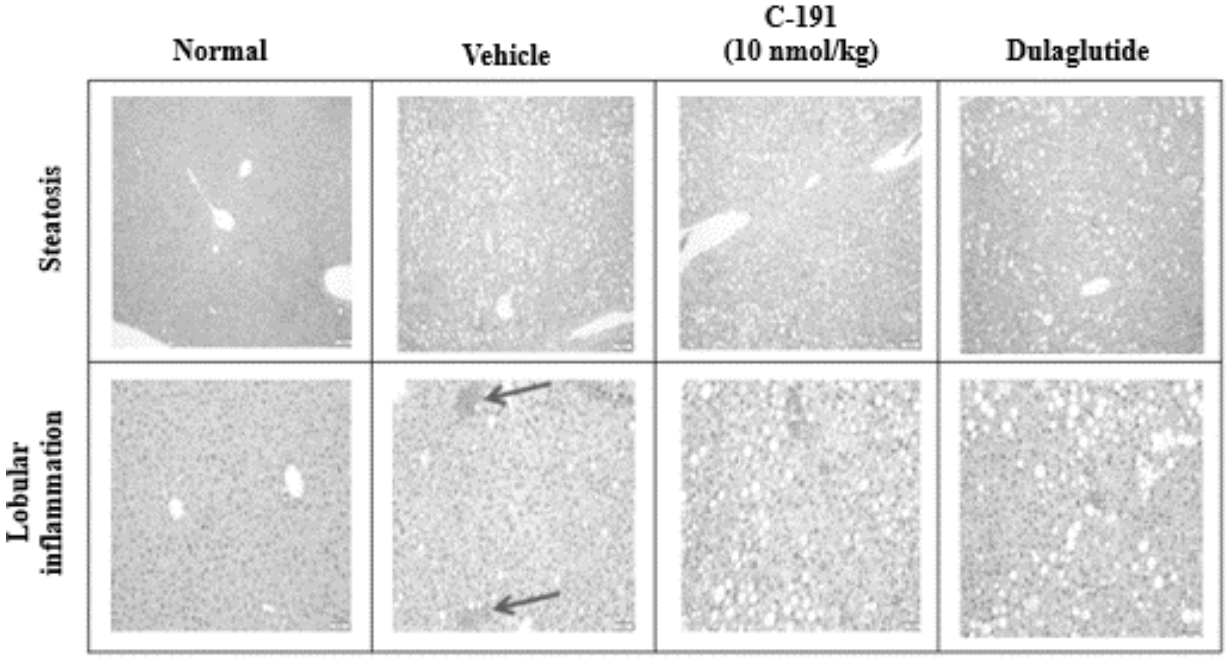
【Fig. 29】


ALT
400
300
200
100
0
ALT (U/L)
Normal
Vehicle
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg

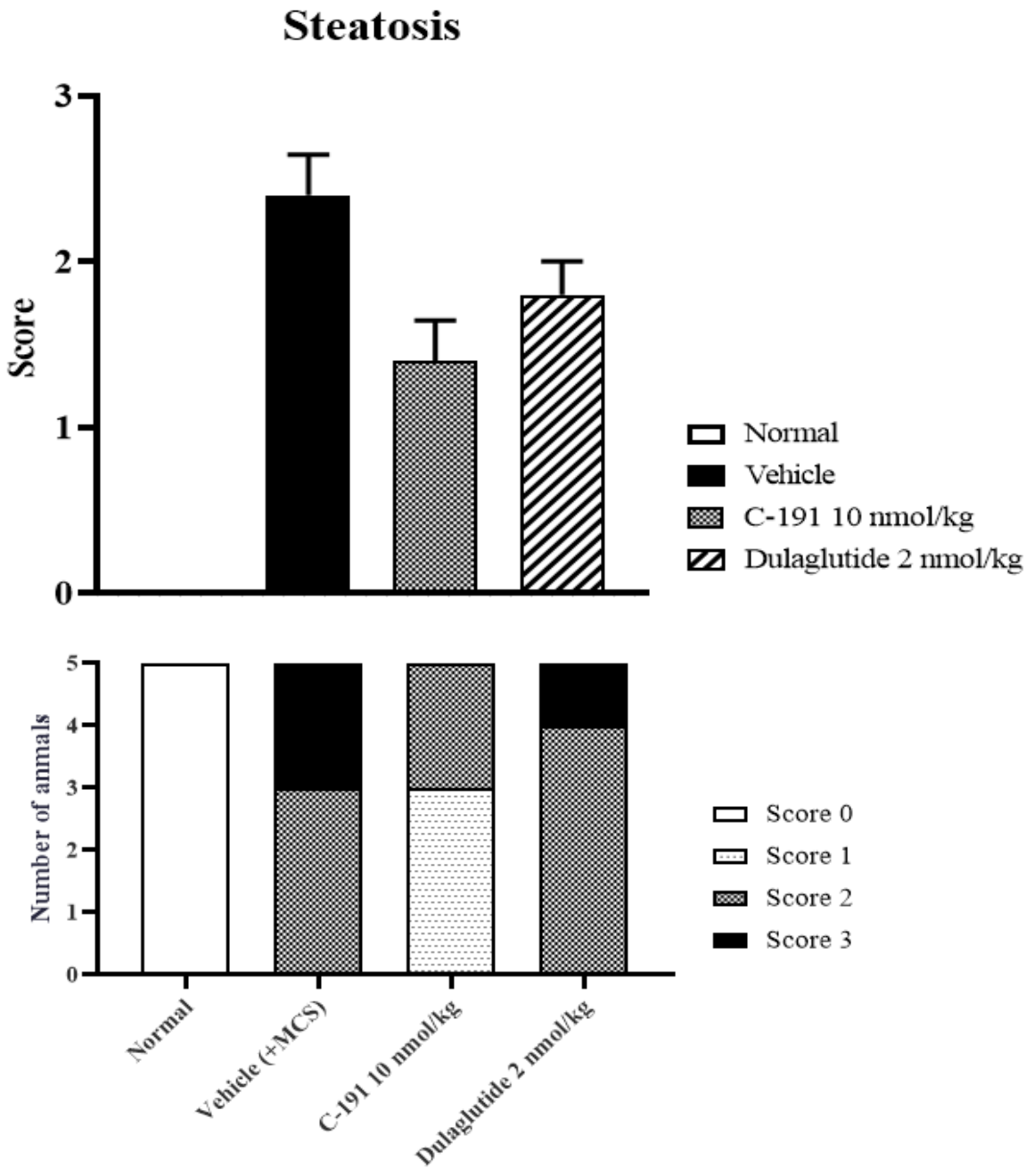
【Fig. 30】


AST/ALT ratio
AST/ALT ratio
2.5
2.0
1.5
1.0
0.5
0.0
Normal
Vehicle
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg

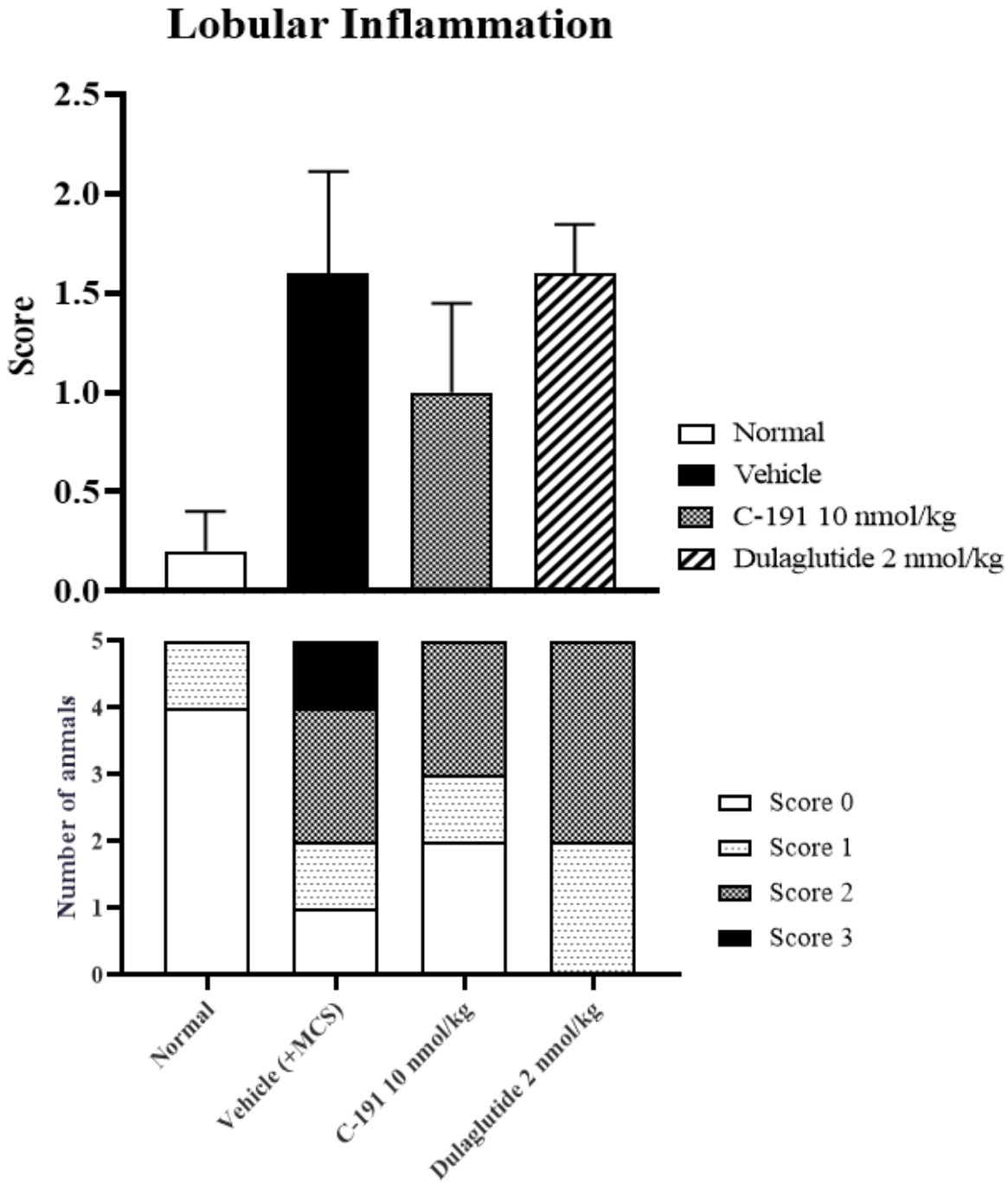
【Fig. 31】


Normal
Vehicle
C-191
(10 nmol/kg)
Dulaglutide
Steatosis
Lobular inflammation

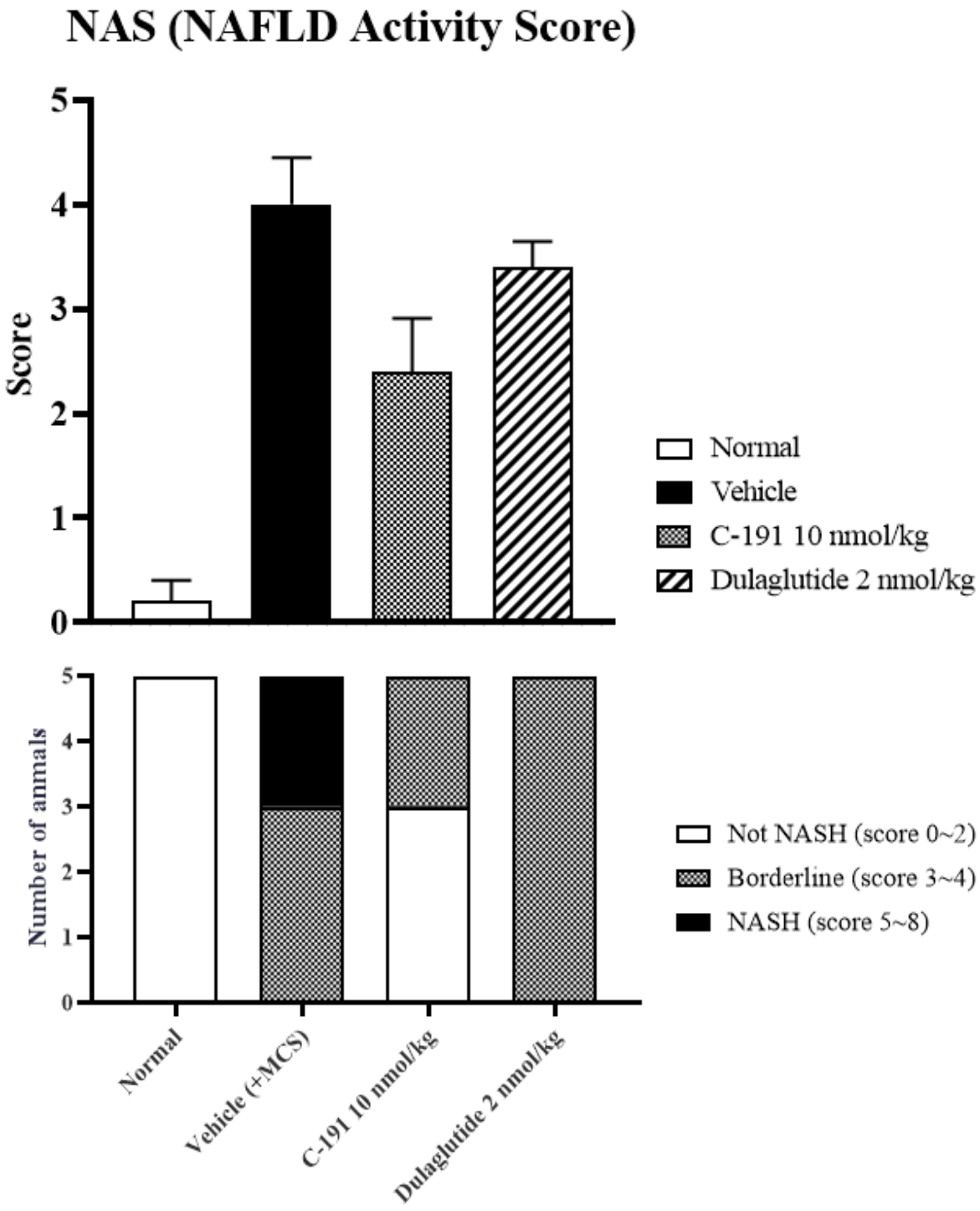
【Fig. 32】


Steatosis
Score
3
2
1
0
Normal
Vehicle
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg
Number of annals
5
4
3
2
1
0
Score 0
Score 1
Score 2
Score 3
Normal
Vehicle (+MCS)
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg

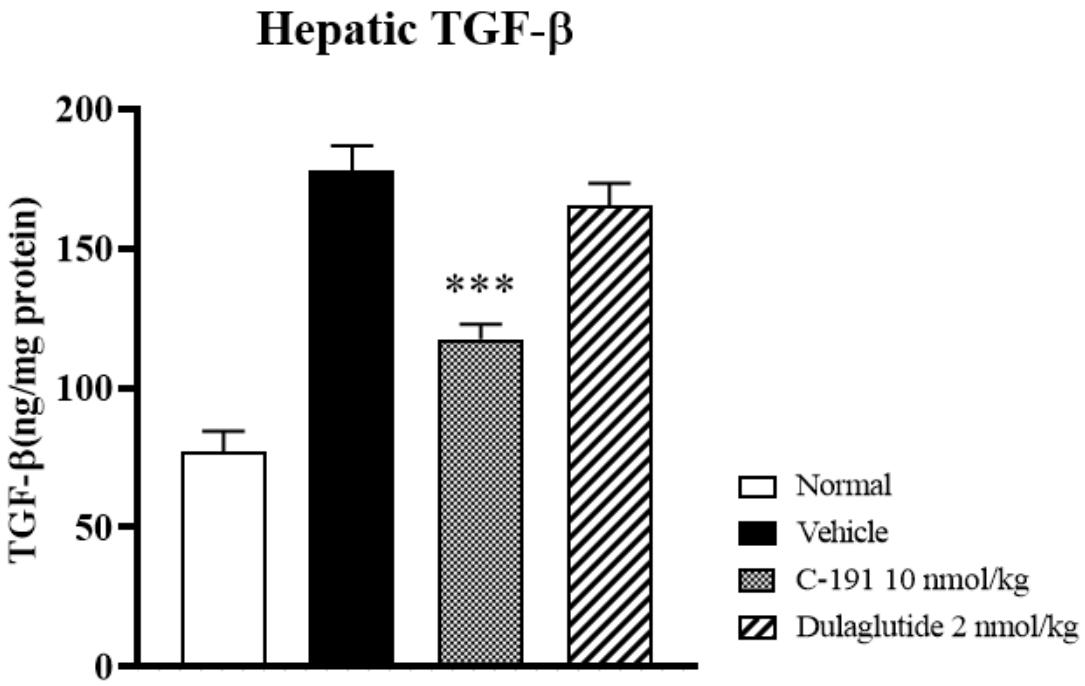
【Fig. 33】


Lobular Inflammation
Score
2.5
2.0
1.5
1.0
0.5
0.0
Normal
Vehicle
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg
Number of annals
5
4
3
2
1
0
Score 0
Score 1
Score 2
Score 3
Normal
Vehicle (+MCS)
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg

【Fig. 34】

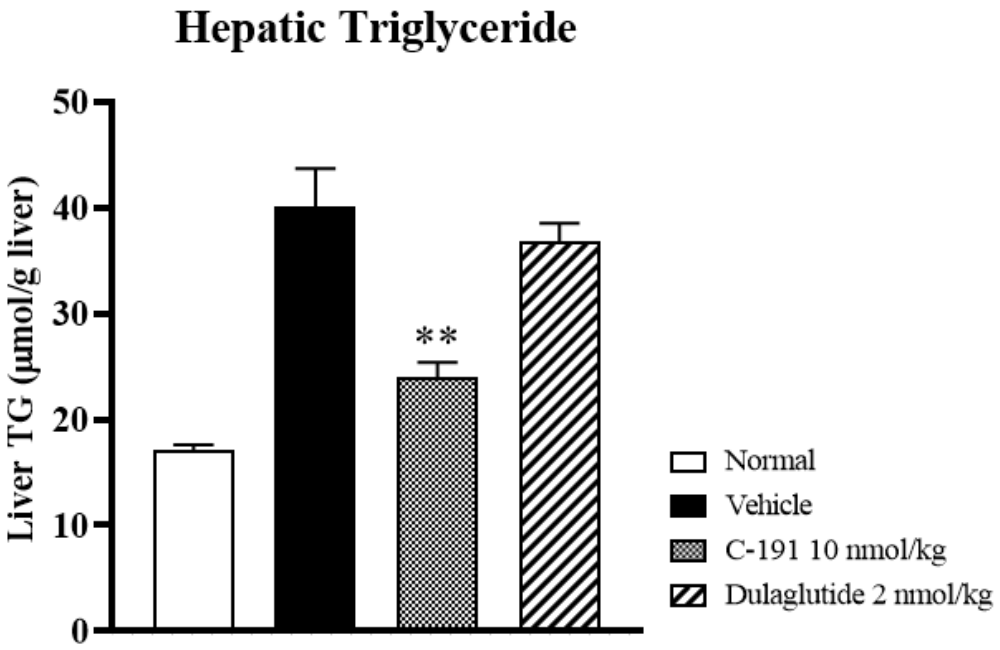

NAS (NAFLD Activity Score)
Score
5
4
3
2
1
0
Normal
Vehicle
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg
Number of annals
5
4
3
2
1
0
Not NASH (score 0~2)
Borderline (score 3~4)
NASH (score 5~8)
Normal
Vehicle (+MCS)
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg

【Fig. 35】



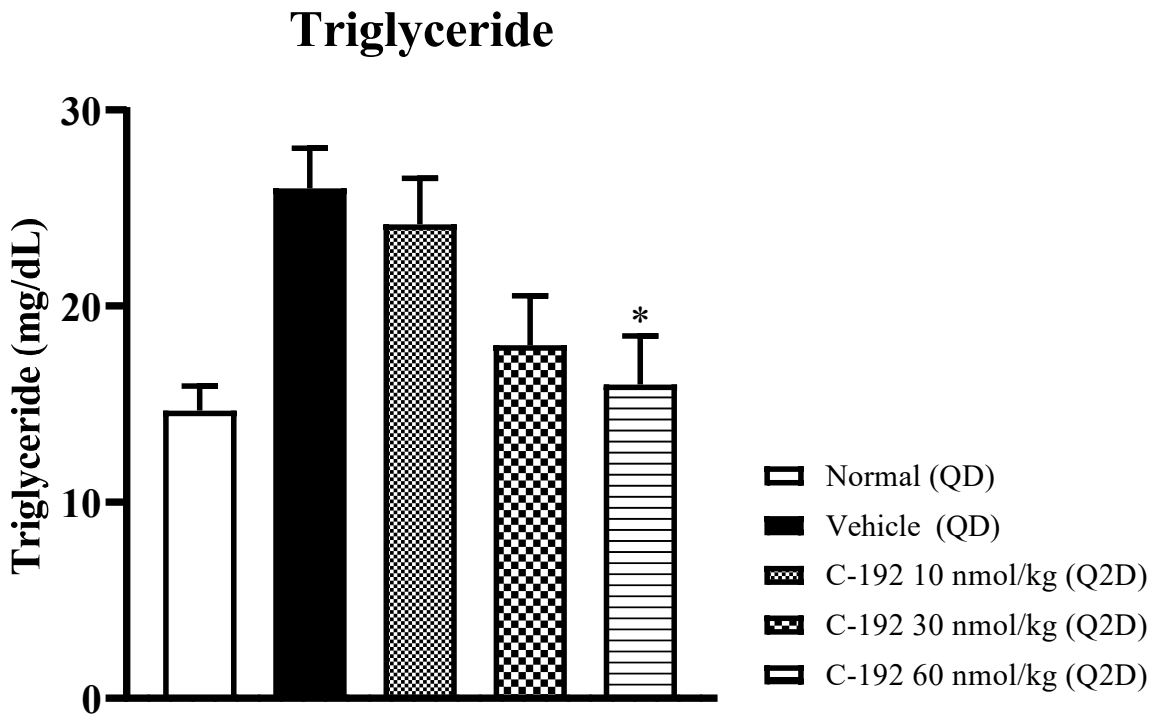
Data were expressed as mean ± SEM. One-way repeated measurement ANOVA and post-hoc Dunnett's test. ***$p < 0.001$, compared to vehicle group.

【Fig. 36】



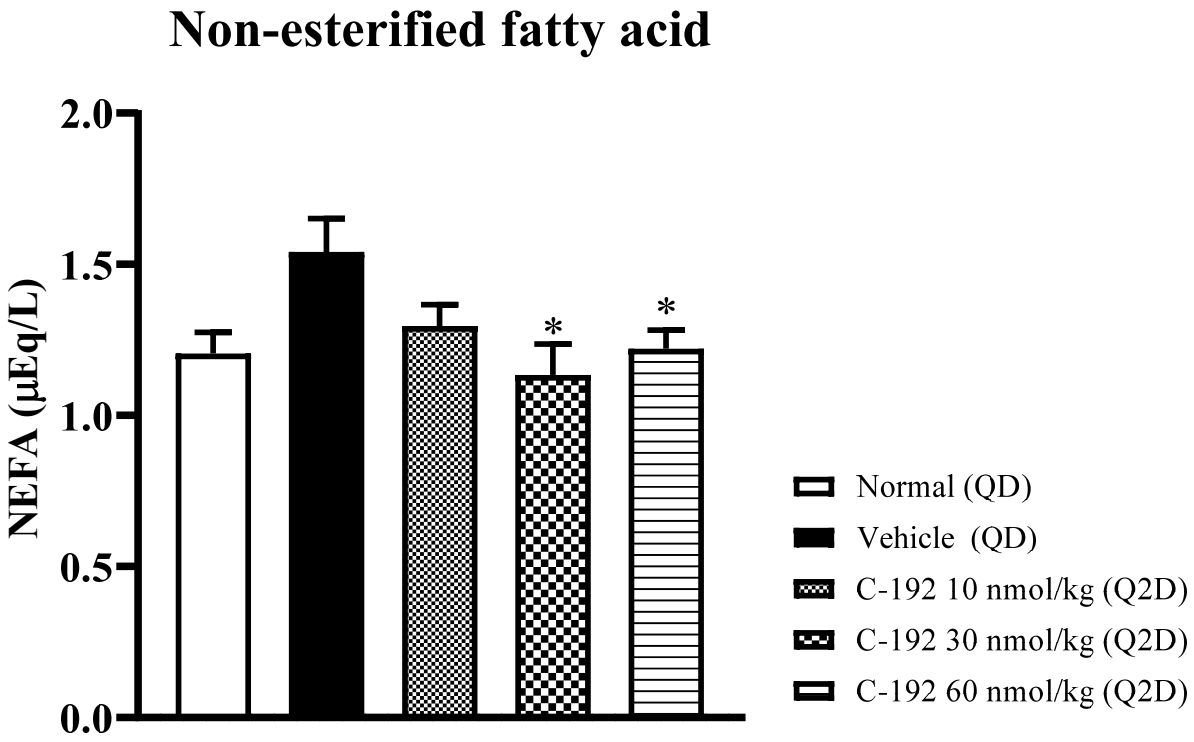
Data were expressed as mean ± SEM. One-way repeated measurement ANOVA and post-hoc Dunnett's test. **$p < 0.05$, compared to vehicle group.

【Fig. 37】



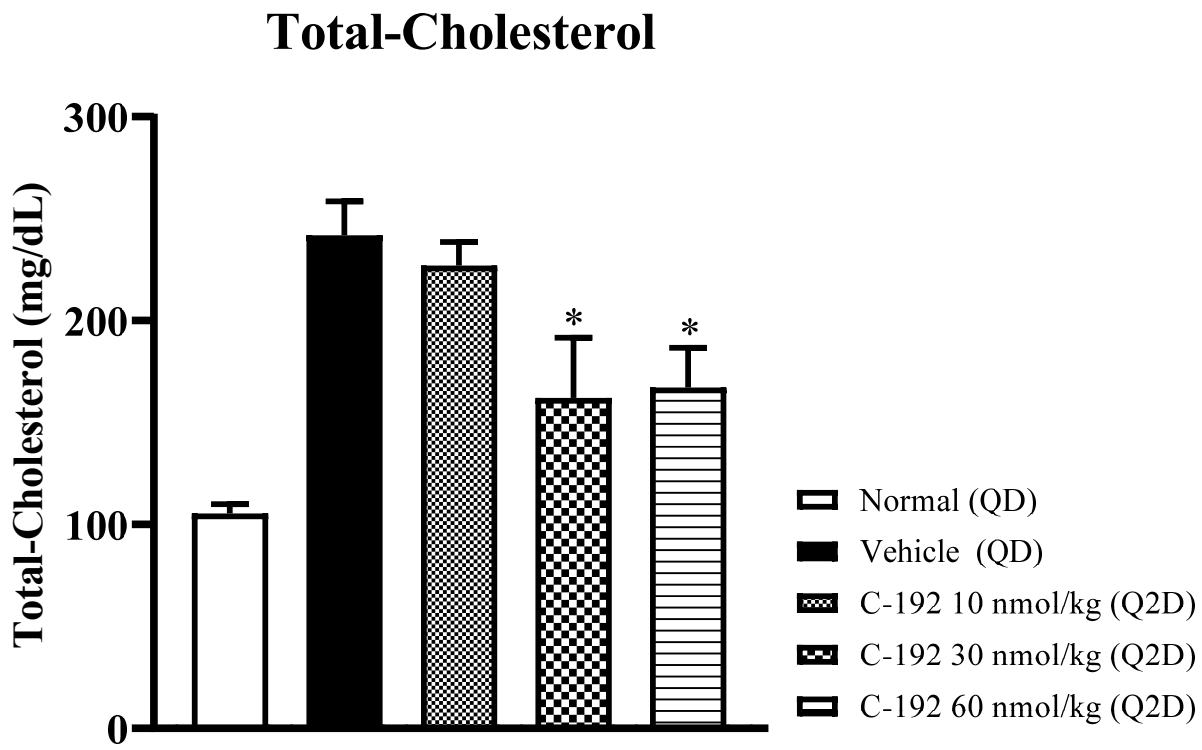
Data were expressed as mean ± SEM of tyiglyceride. One-way ANOVA and post-hoc Dunnett's test. *$p < 0.05$, compared to vehicle group. $n = 6$.

【Fig. 38】



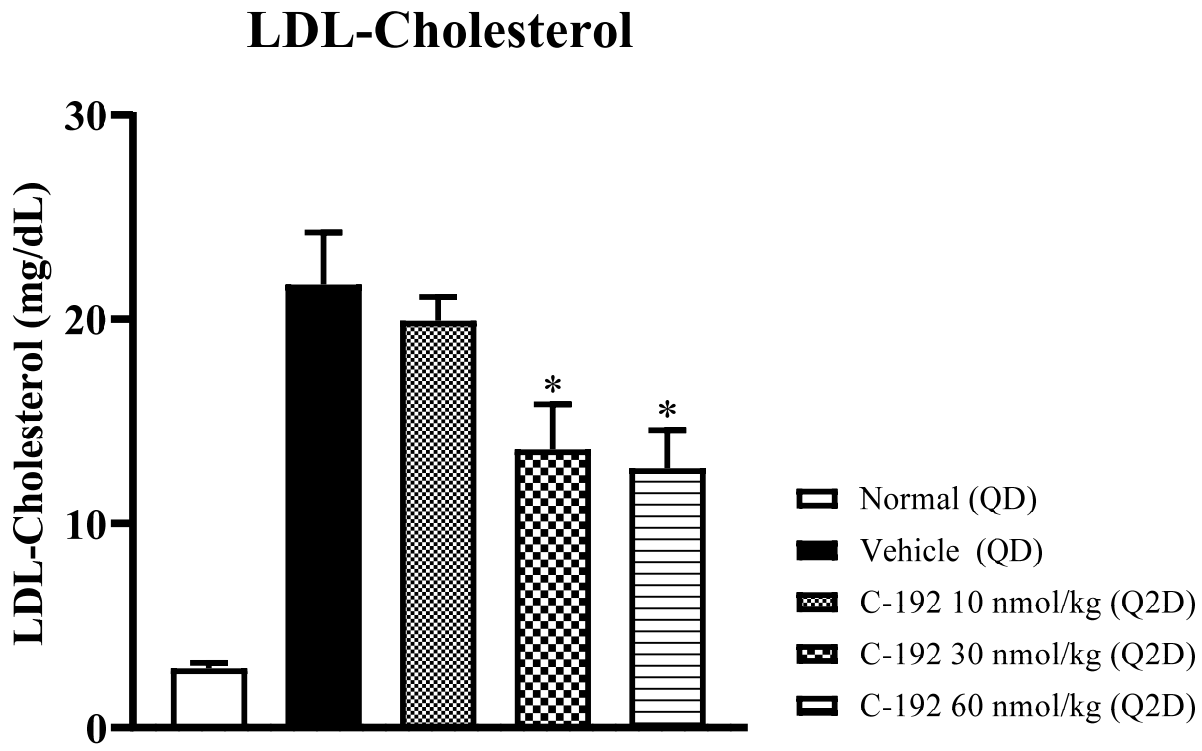
Data were expressed as mean ± SEM of non-esterified fatty acid. One-way ANOVA and post-hoc Dunnett's test. *$p < 0.05$, compared to vehicle group. $n = 6$.

【Fig. 39】



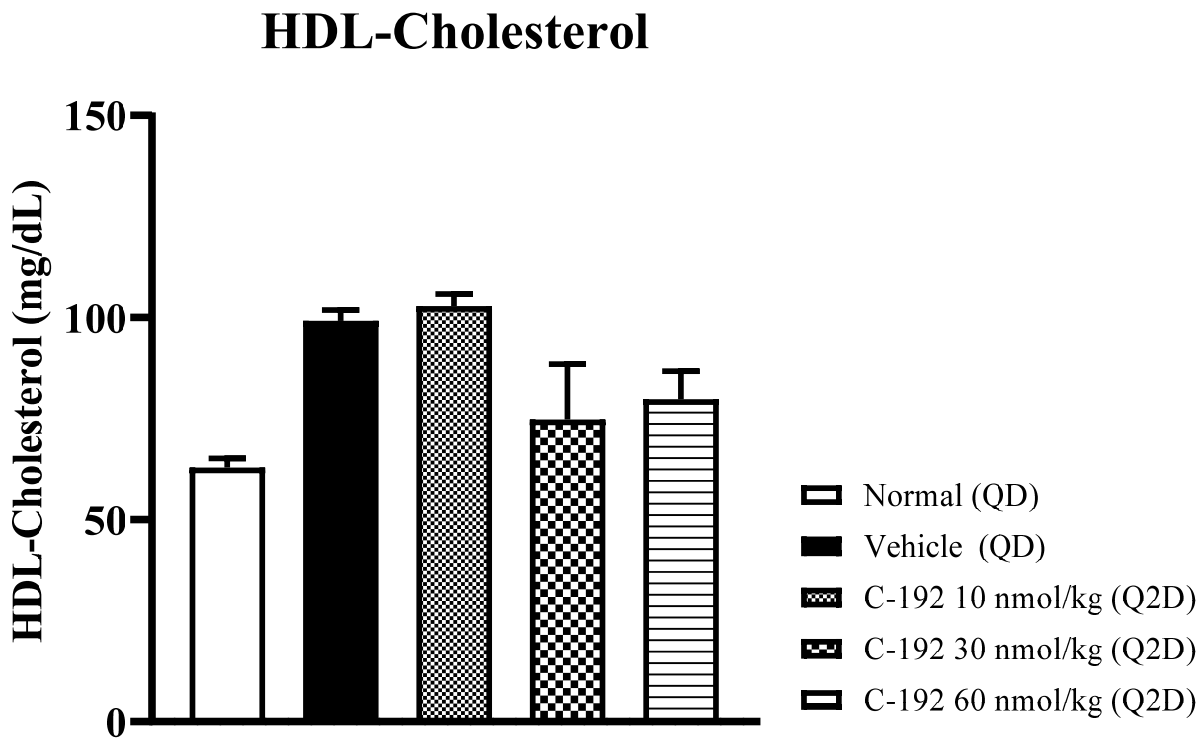
Data were expressed as mean ± SEM of total-cholesterol. One-way ANOVA and post-hoc Dunnett's test. *$p < 0.05$, compared to vehicle group. $n = 6$.

【Fig. 40】



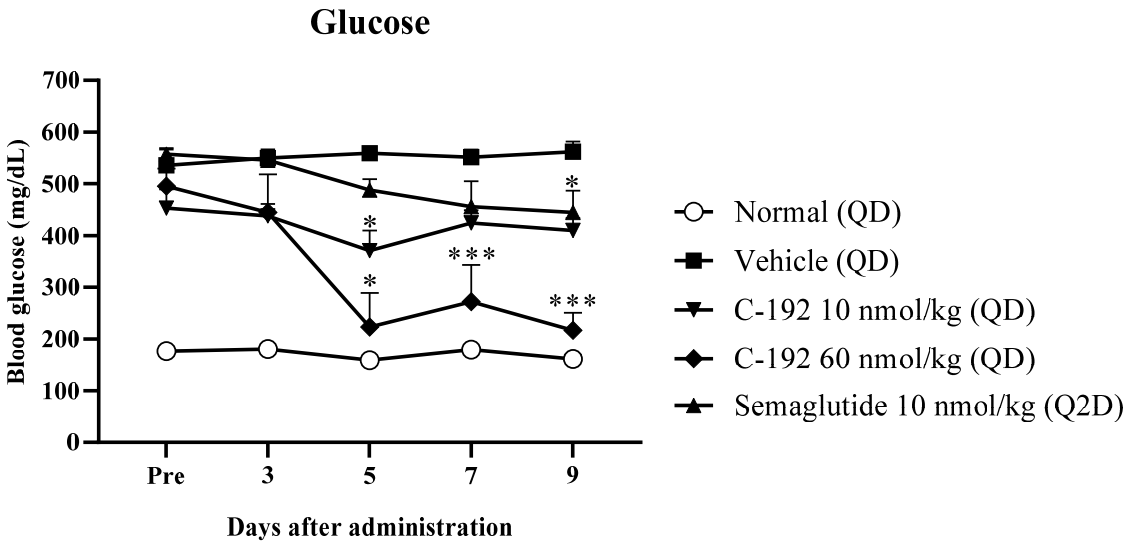
Data were expressed as mean ± SEM of LDL-cholesterol. One-way ANOVA and post-hoc Dunnett's test. *$p < 0.05$, compared to vehicle group. $n = 6$.

【Fig. 41】



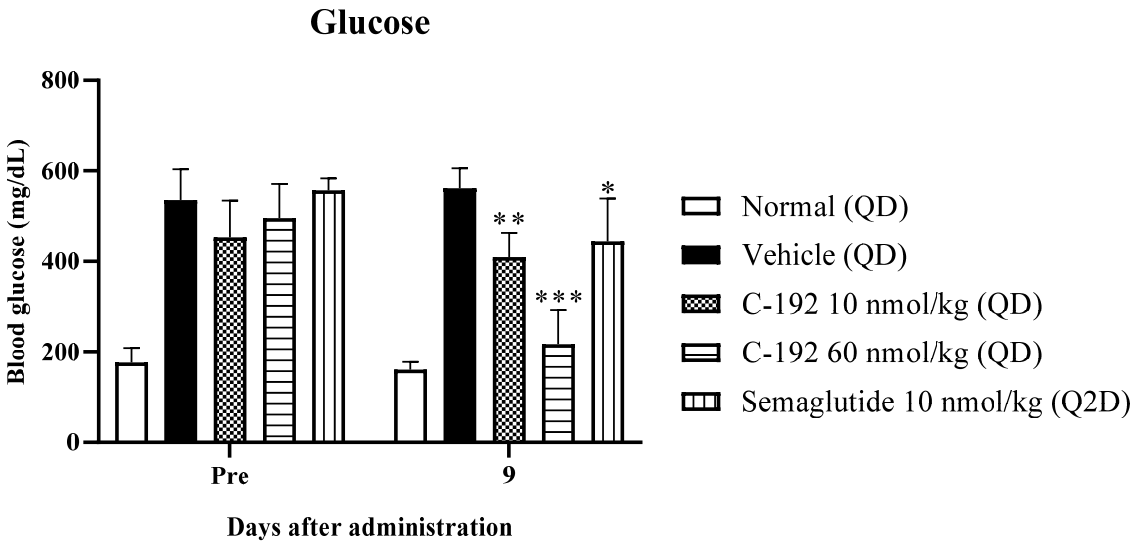
Data were expressed as mean ± SEM of HDL-cholesterol. n = 6.

【Fig. 42】



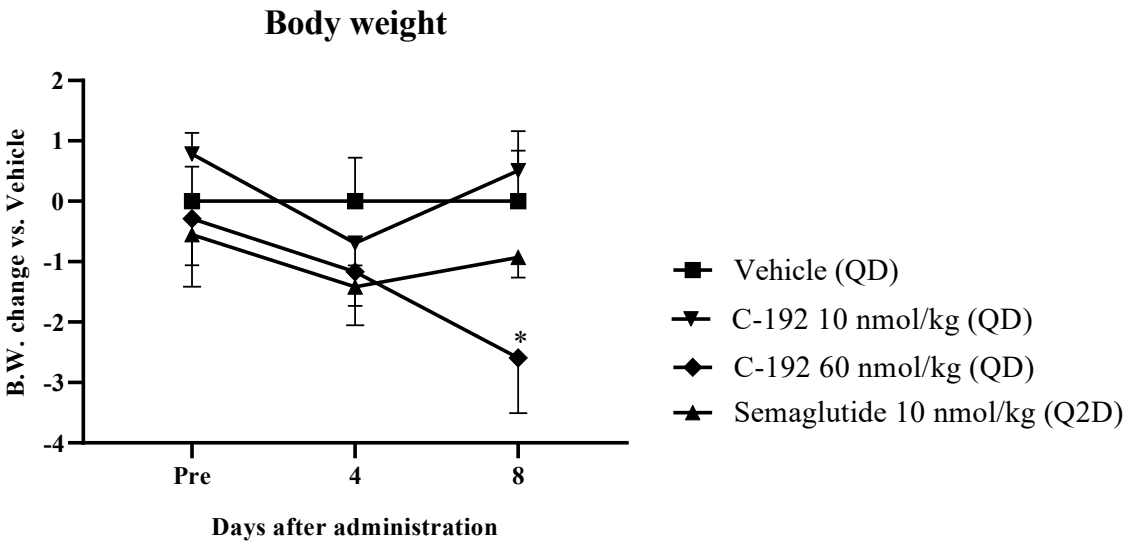
Data were expressed as mean ± SEM of blood glucose. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

【Fig. 43】



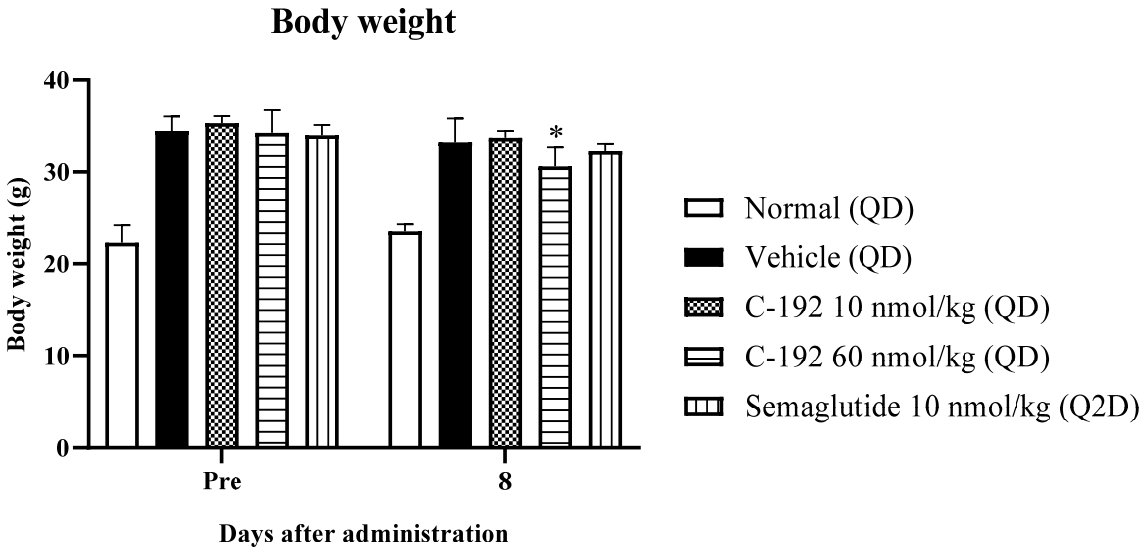
Data were expressed as mean ± SEM of blood glucose. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

【Fig. 44】



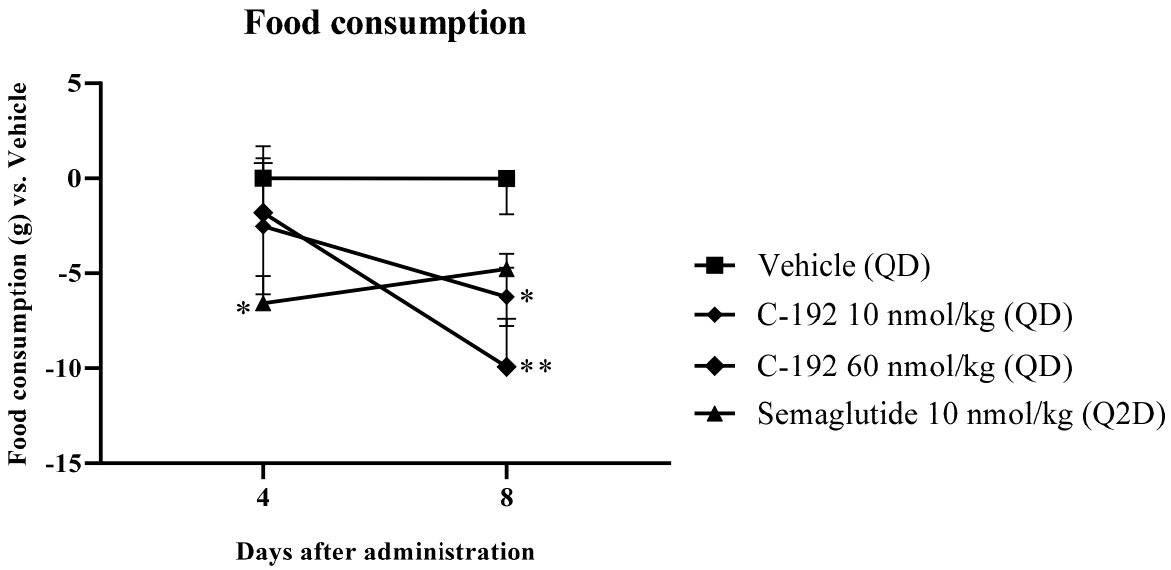
Data were expressed as mean ± SEM of body weight change verse vehicle. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *$p < 0.05$, compared to vehicle group. $n = 5$.

【Fig. 45】



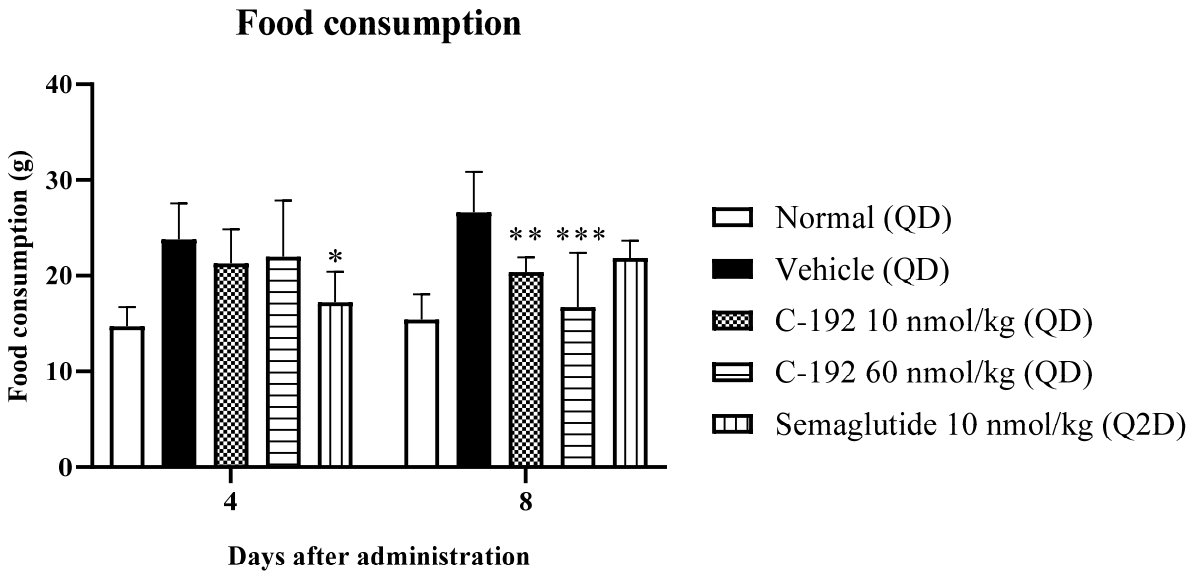
Data were expressed as mean ± SEM of body weight change verse vehicle. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *$p < 0.05$, compared to vehicle group. n = 5.

【Fig. 46】



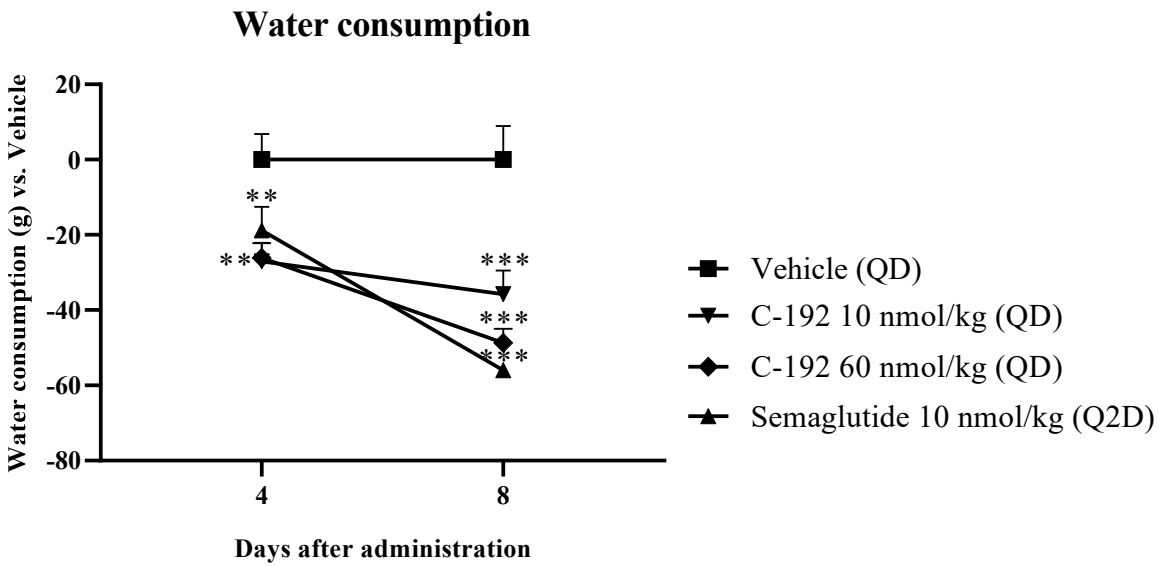
Data were expressed as mean ± SEM of food consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. **p < 0.01, compared to vehicle group. n = 5.

【Fig. 47】



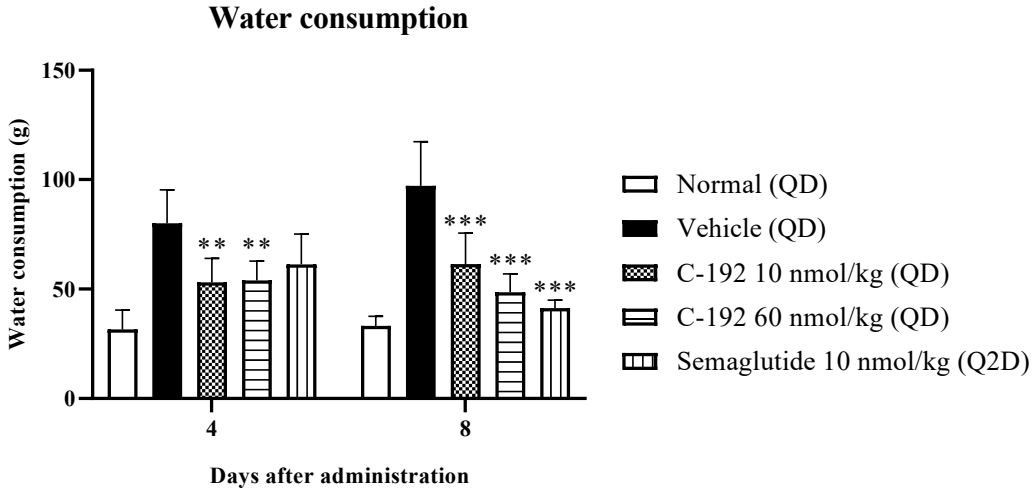
Data were expressed as mean ± SEM of food consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

【Fig. 48】



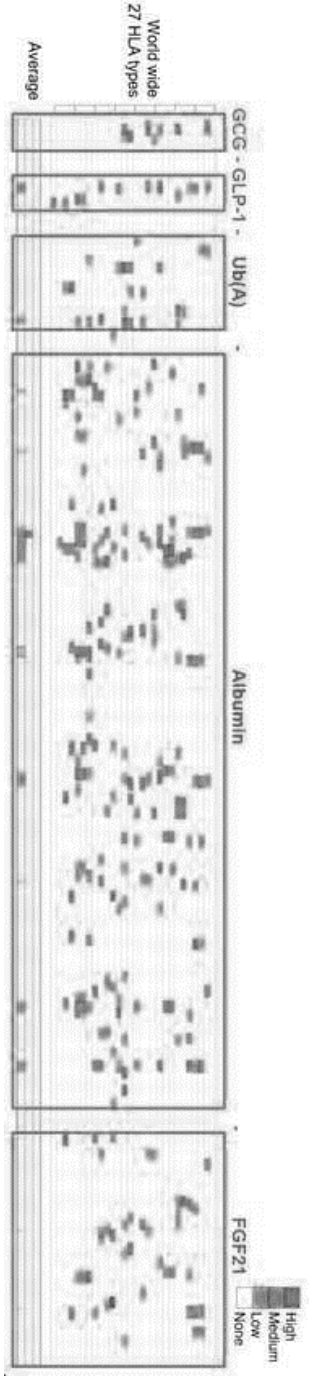
Data were expressed as mean ± SEM of water consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

【Fig. 49】

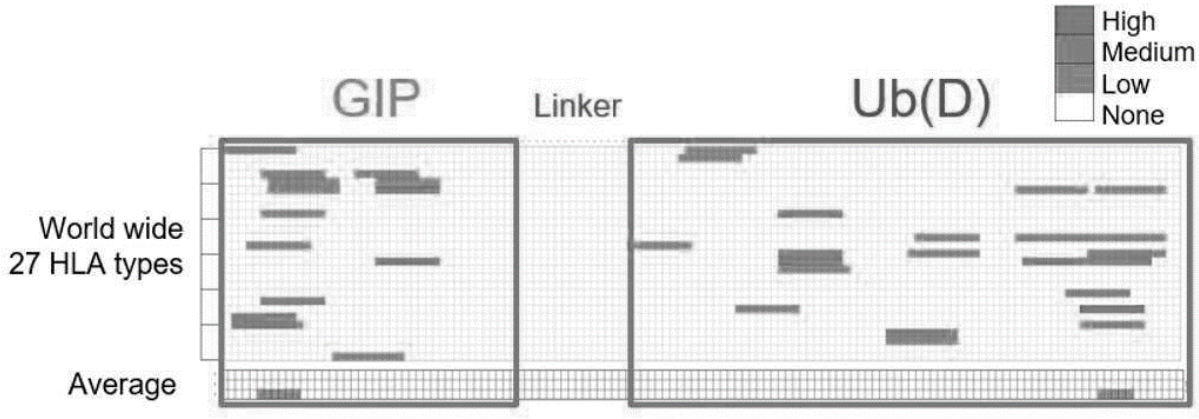


Data were expressed as mean ± SEM of water consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

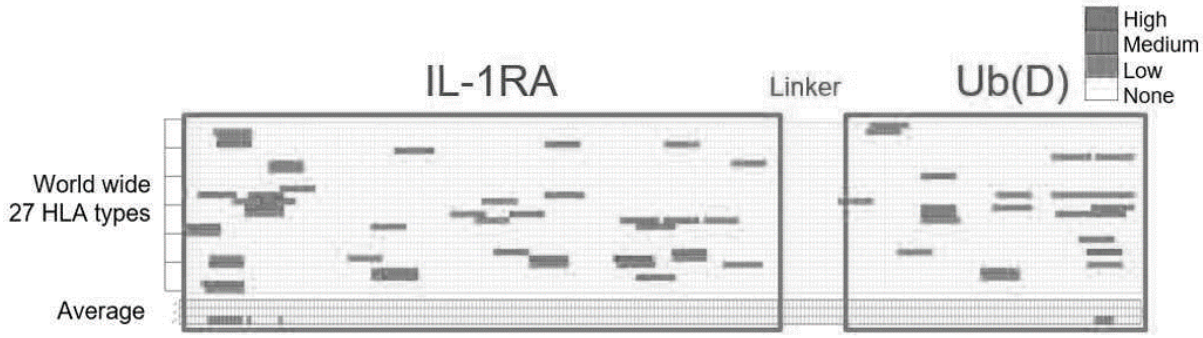
【Fig. 50】


Average
World wide 27 HLA types
GCG - GLP-1 - Ub(A)
Albumin
FGF21
High
Medium
Low
None

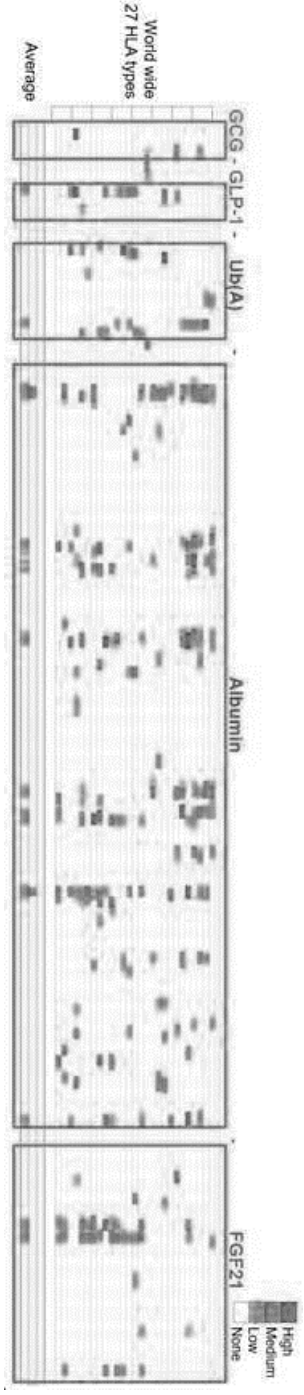
【Fig. 51】


High
Medium
Low
None
GIP
Linker
Ub(D)
World wide
27 HLA types
Average

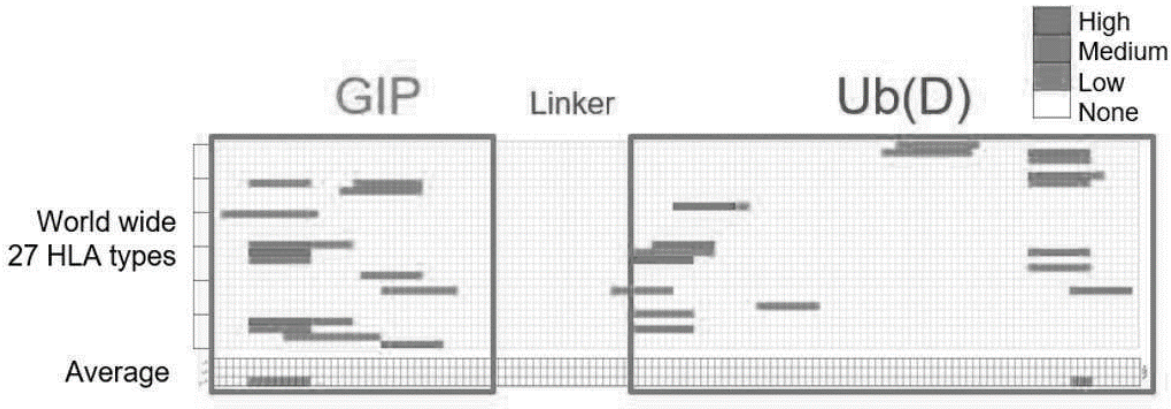
【Fig. 52】


High
Medium
Low
None
IL-1RA
Linker
Ub(D)
World wide
27 HLA types
Average

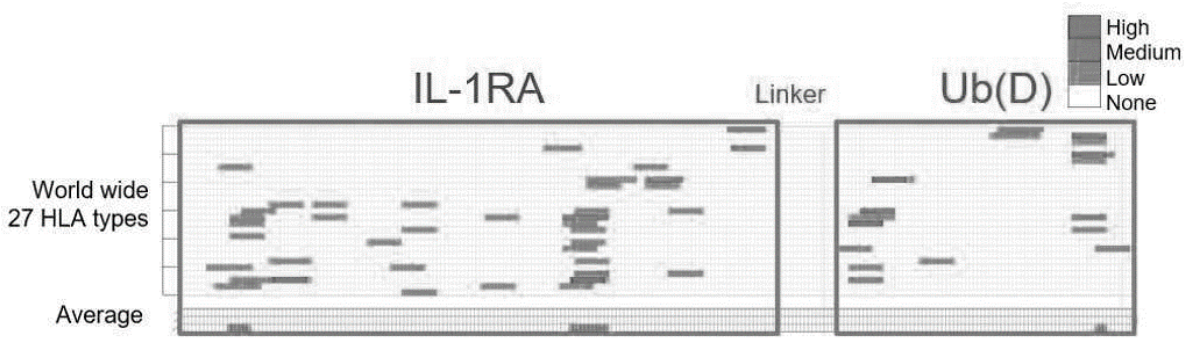
【Fig. 53】


Average
World wide
27 HLA types
GCG - GLP-1 -
Ub(A)
Albumin
FGF21
High
Medium
Low
None

【Fig. 54】


High
Medium
Low
None
GIP
Linker
Ub(D)
World wide
27 HLA types
Average

【Fig. 55】


High
Medium
Low
None
IL-1RA
Linker
Ub(D)
World wide
27 HLA types
Average