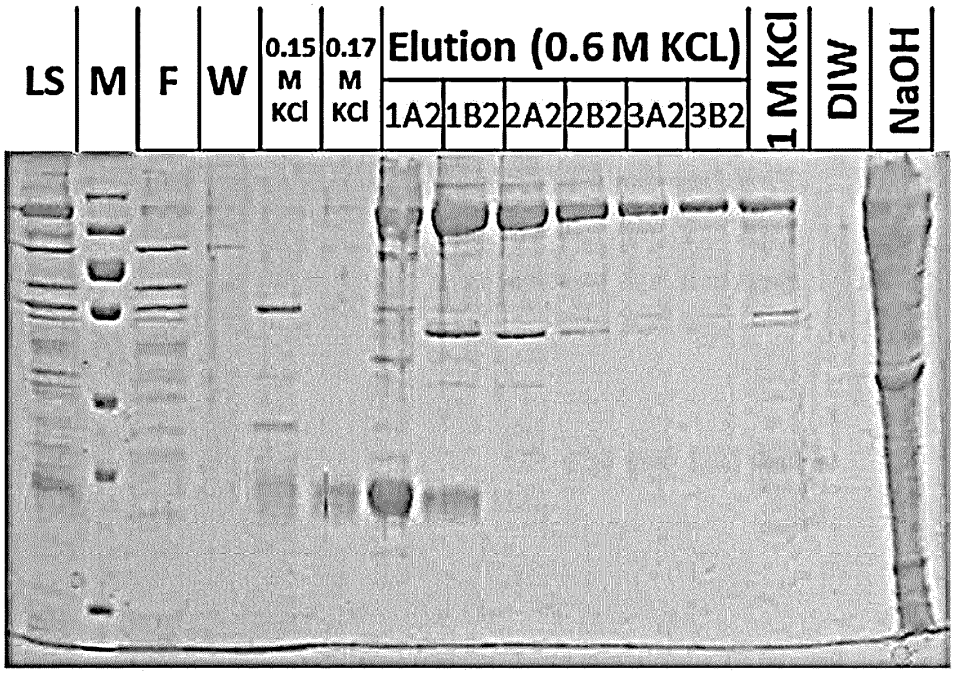
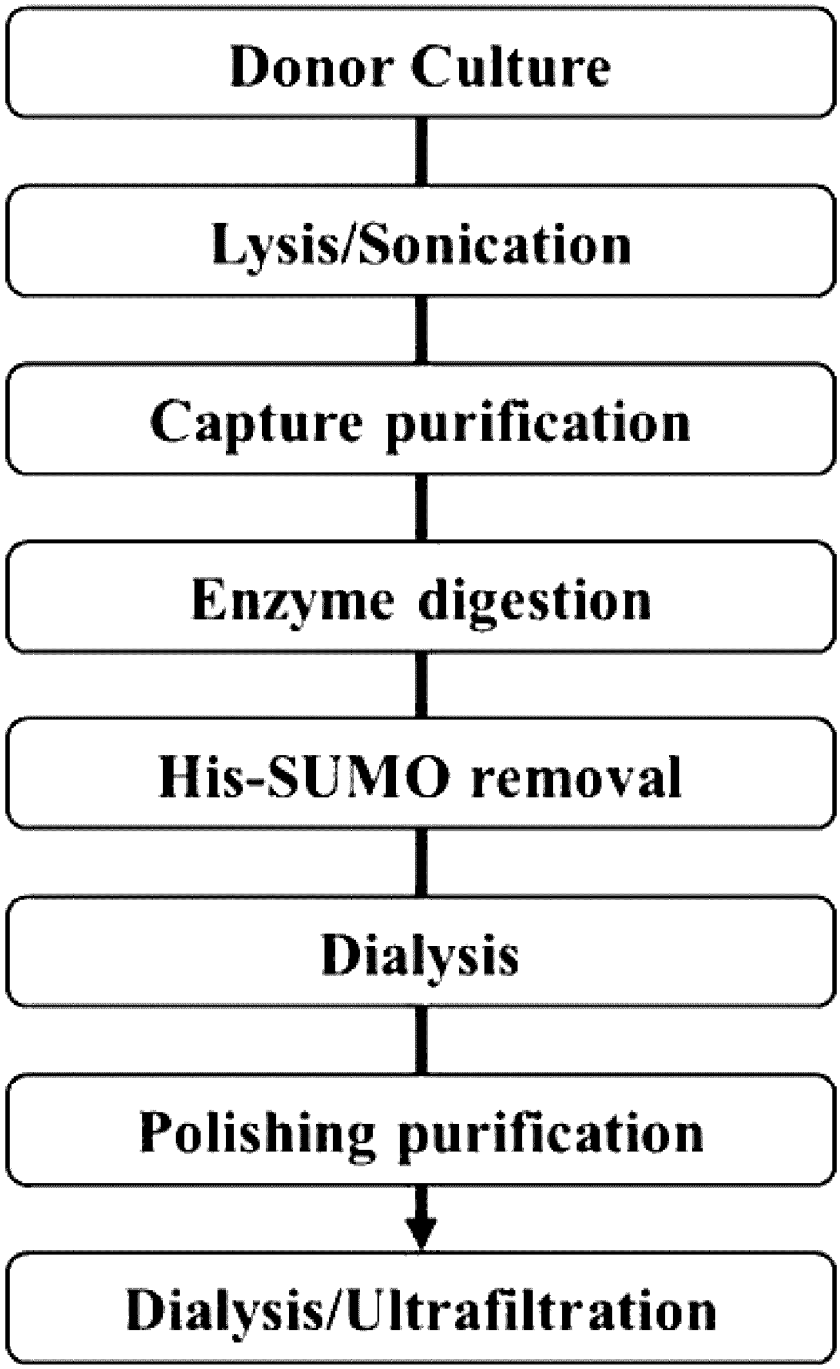
WO 2021/221482 PCT/KR2021/005481

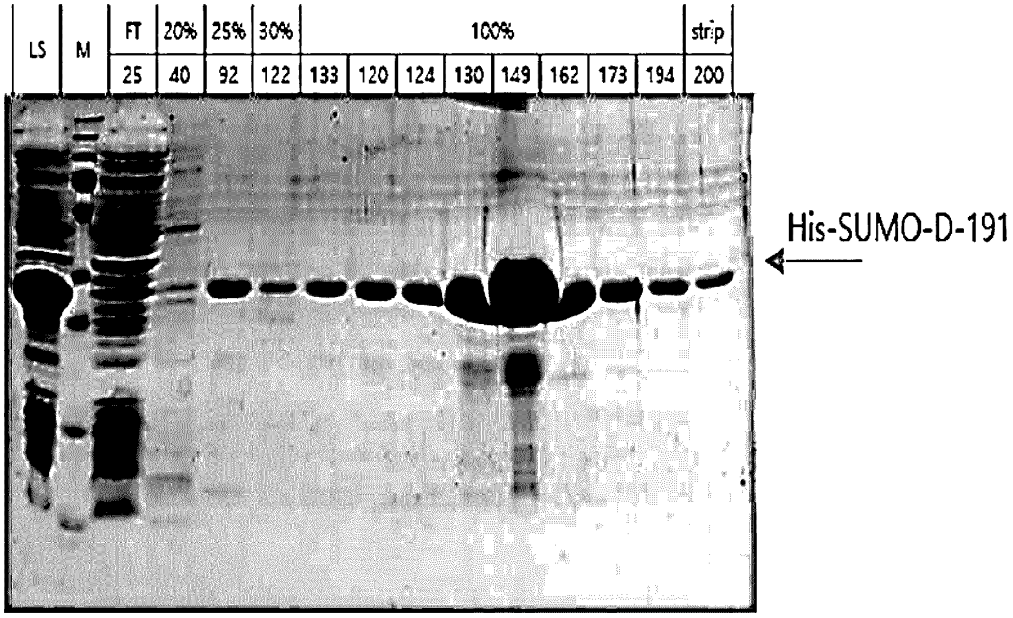
[도1]



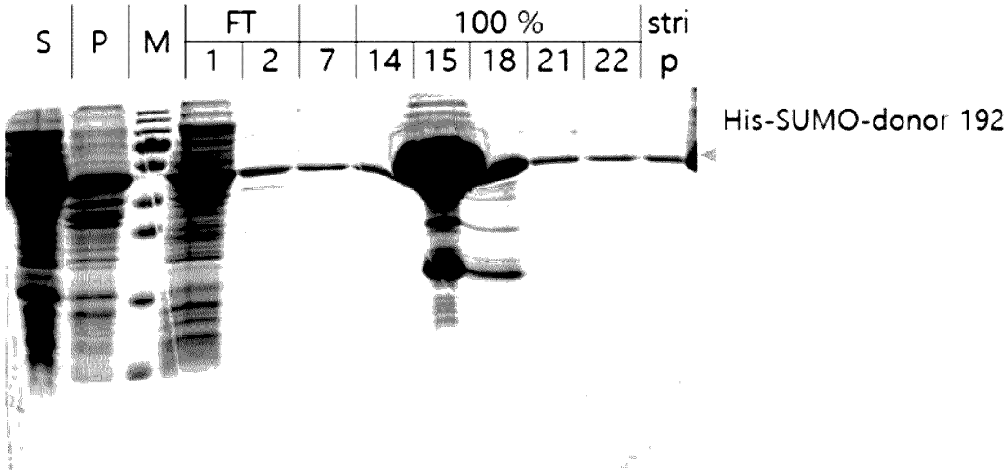
[도2]


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

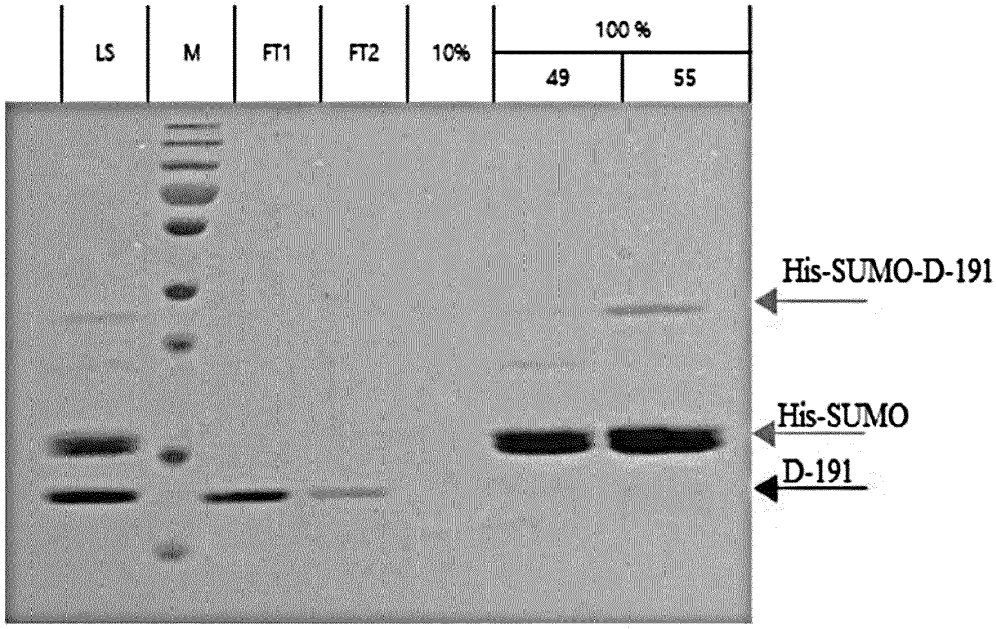
[도3]


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

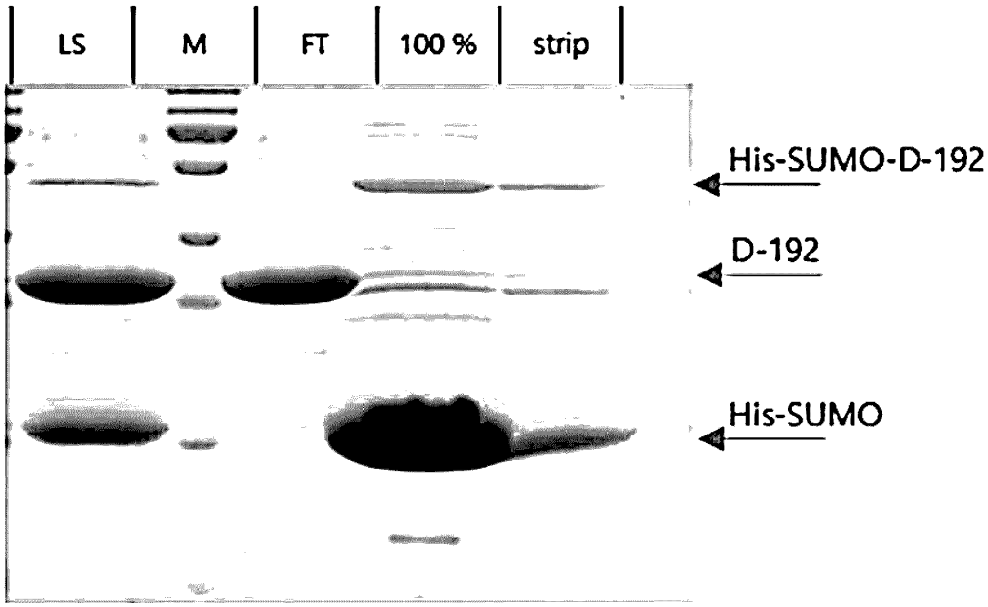
[도4]


S
P
M
FT
100 %
stri
1
2
7
14
15
18
21
22
p
His-SUMO-donor 192

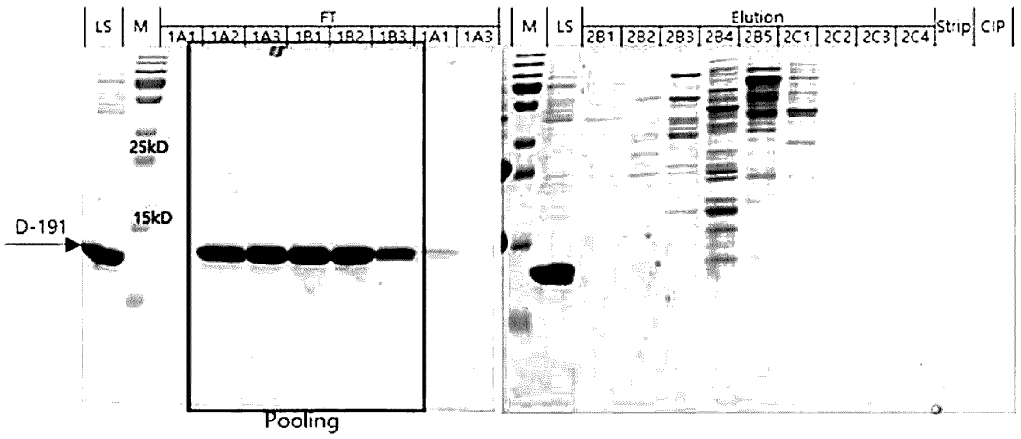
[도5]


LS
M
FT1
FT2
10%
100 %
49
55
His-SUMO-D-191
His-SUMO
D-191

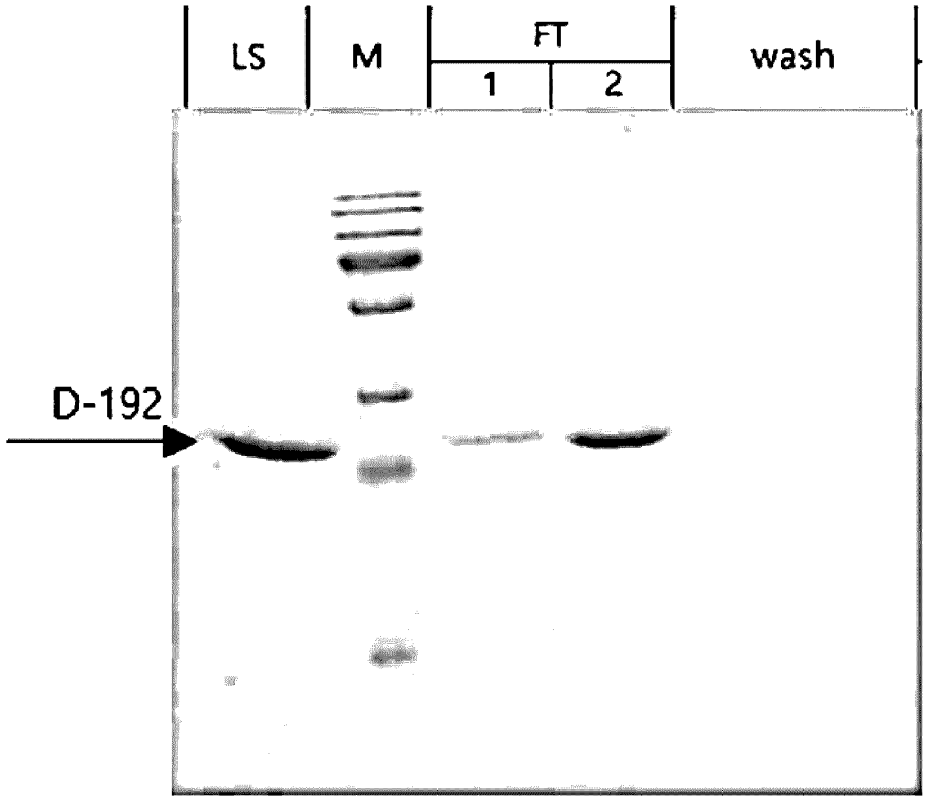
[도6]


LS
M
FT
100 %
strip
His-SUMO-D-192
D-192
His-SUMO

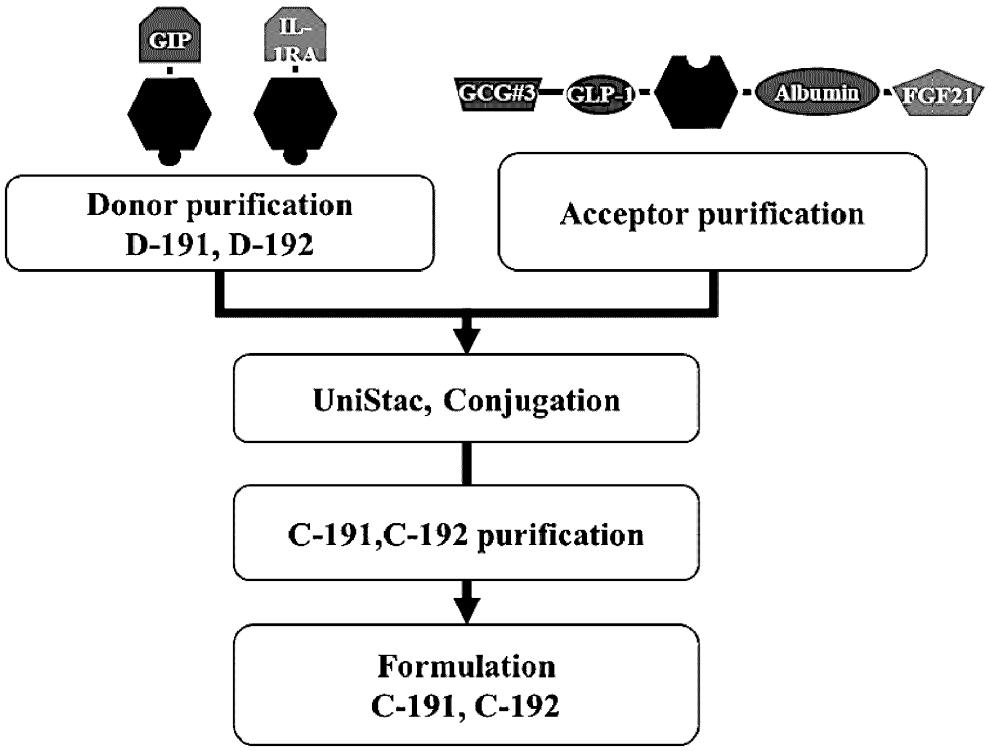
[도7]


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

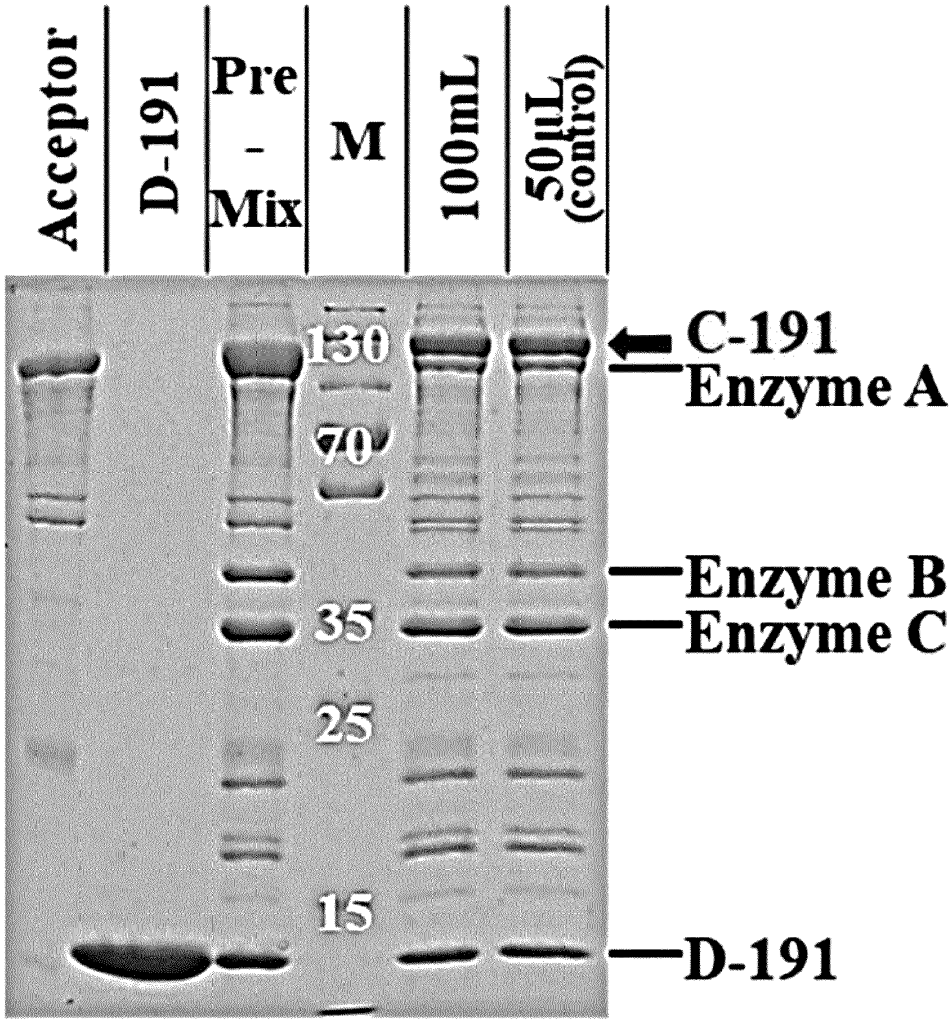
[도8]


LS
M
FT
1
2
wash
D-192

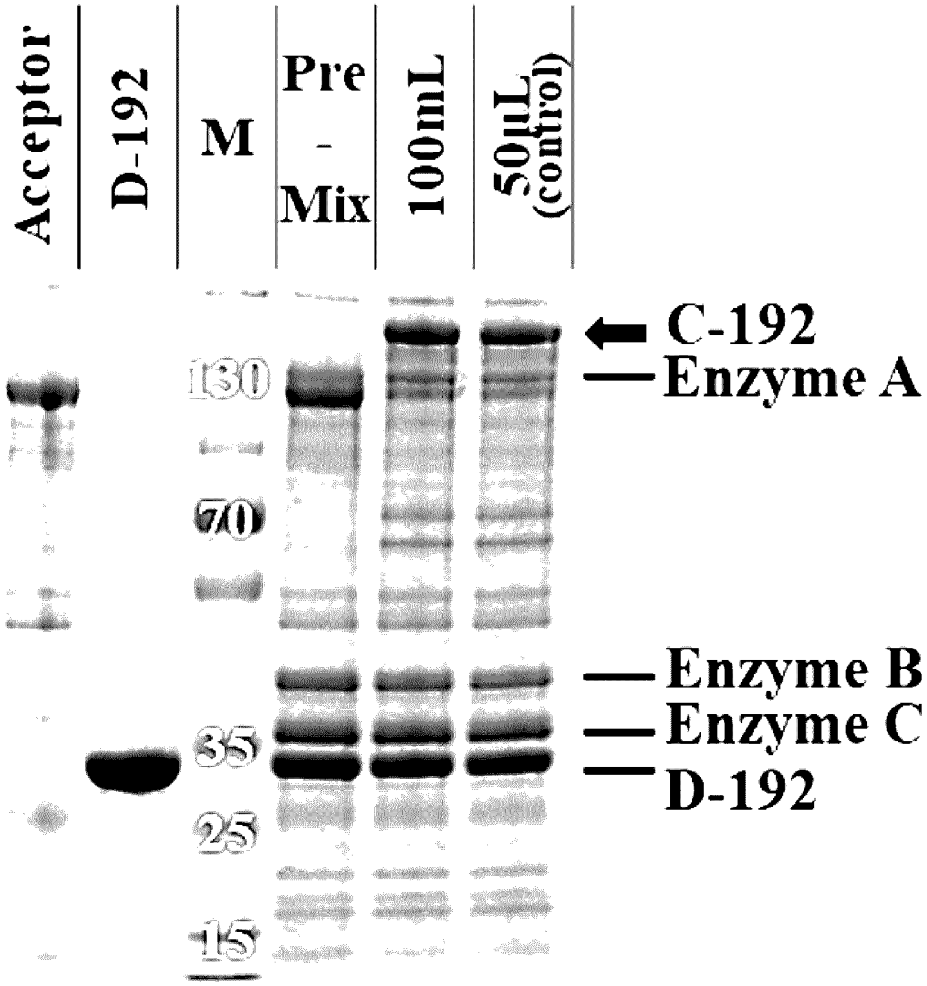
[도9]


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

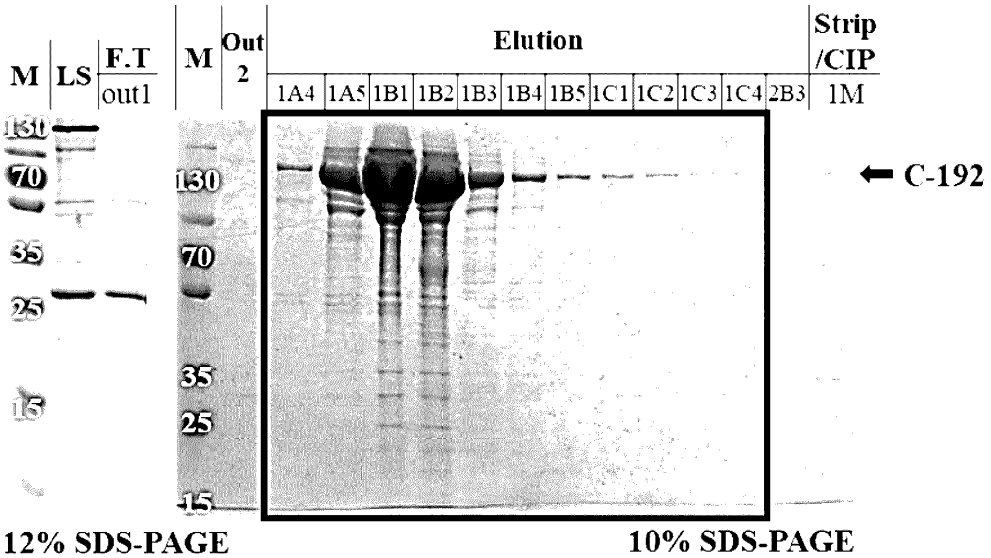
[도10]


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

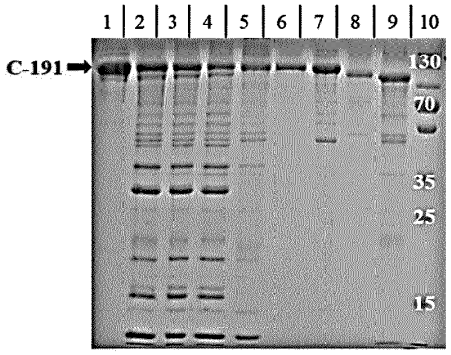
[도11]


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

[도12]

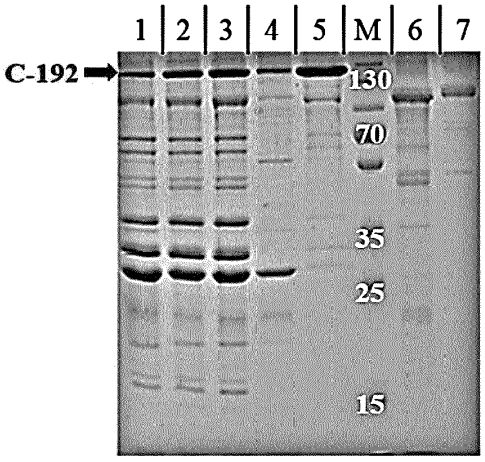

M
LS
F.T
out1
M
Out
2
Elution
1A4
1A5
1B1
1B2
1B3
1B4
1B5
1C1
1C2
1C3
1C4
2B3
Strip
/CIP
1M
130
70
35
25
15
C-192
12% SDS-PAGE
10% SDS-PAGE

[도13]



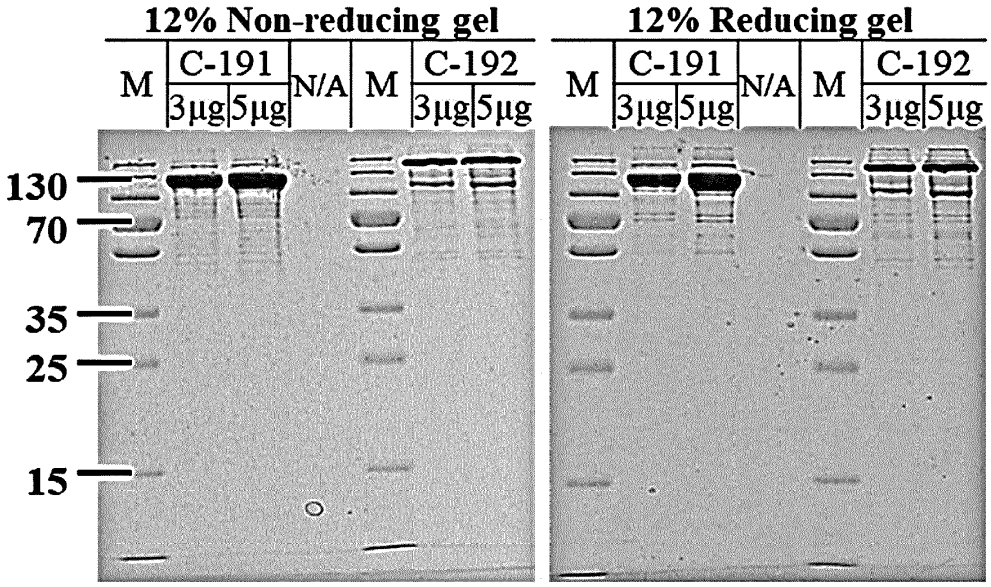
Lane 1: RD191-003-DS, positive control (Lot. No.: S191031)
2: RD191-004-C-01
3: RD191-004-C-02
4: RD191-004-C-03
5: RD191-004-C-NF-01
6: RD191-004-C-AX-02
7: RD191-004-C-NB-01
8: UCTE1 (S191109-01)
9: Acceptor (RD191-A-004-AF-06)
10: Marker (thermo fisher, #26619)

[도14]

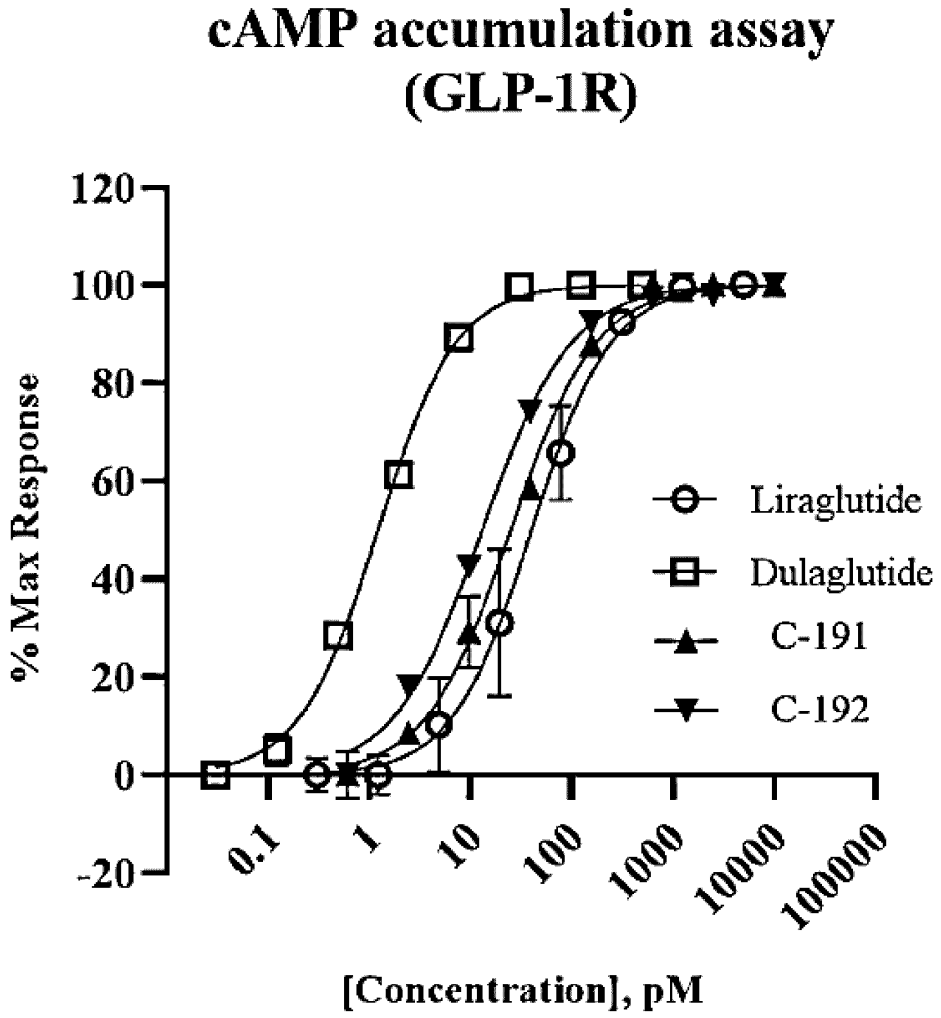


Lane 1: RD192-001-C-03
2: RD192-001-C-02
3: RD192-001-C-01
4: RD192-001-C-NF-01
5: RD192-001-C-AX-01
M: Marker (thermo fisher, #26619)
6: Acceptor (RD191-A-004-AF-06)
7: Enzyme 1 (Lot. No: S191109-01)

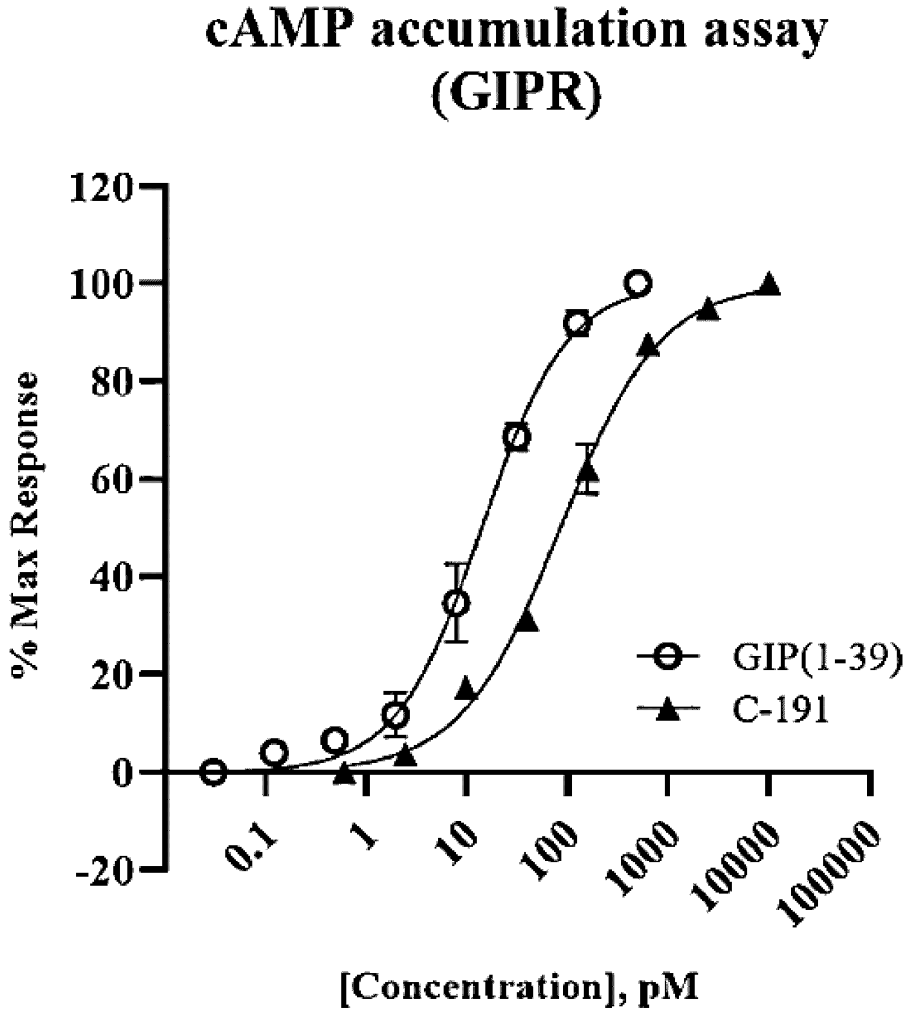
[도15]



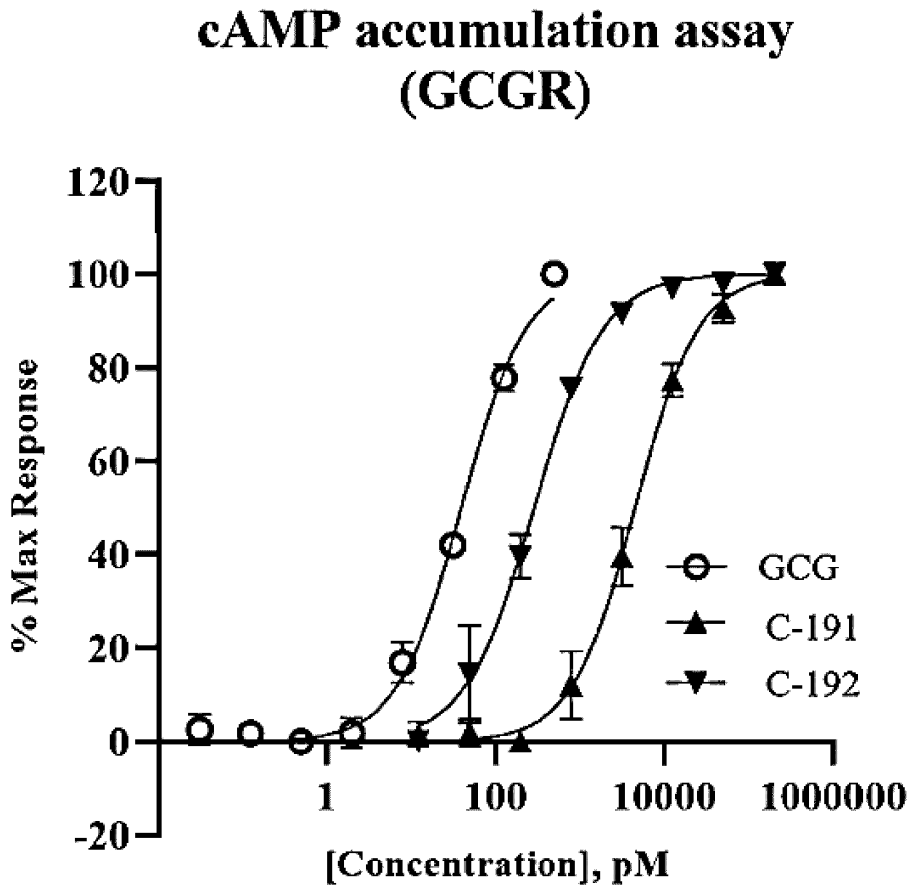
[도16]


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

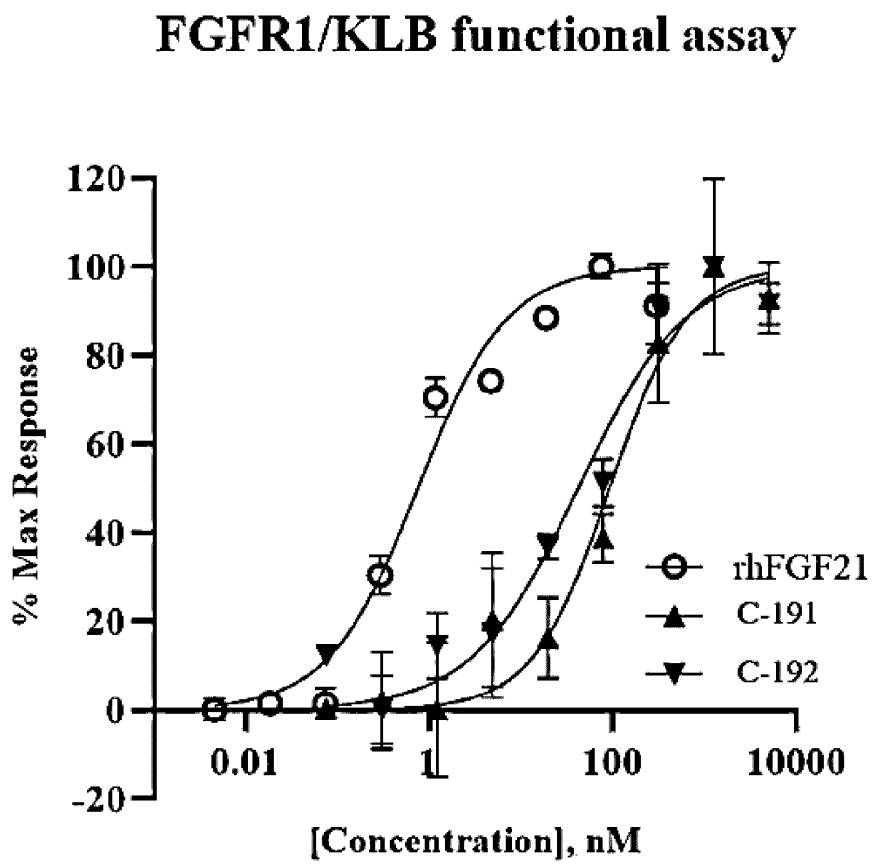
[도17]


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

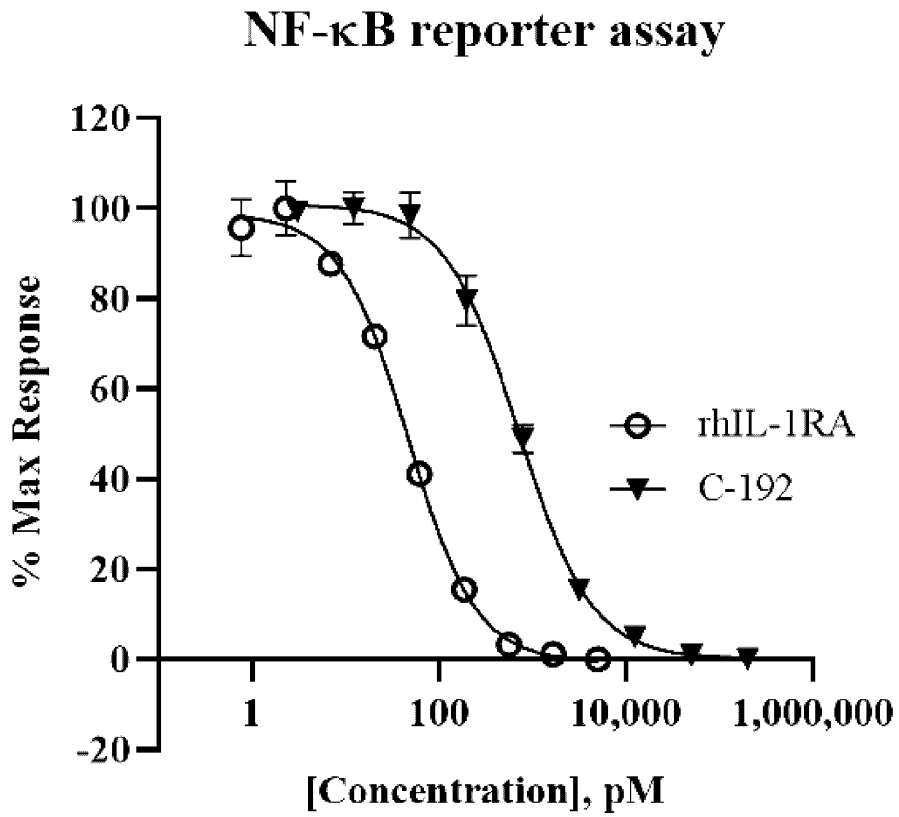
[도18]


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

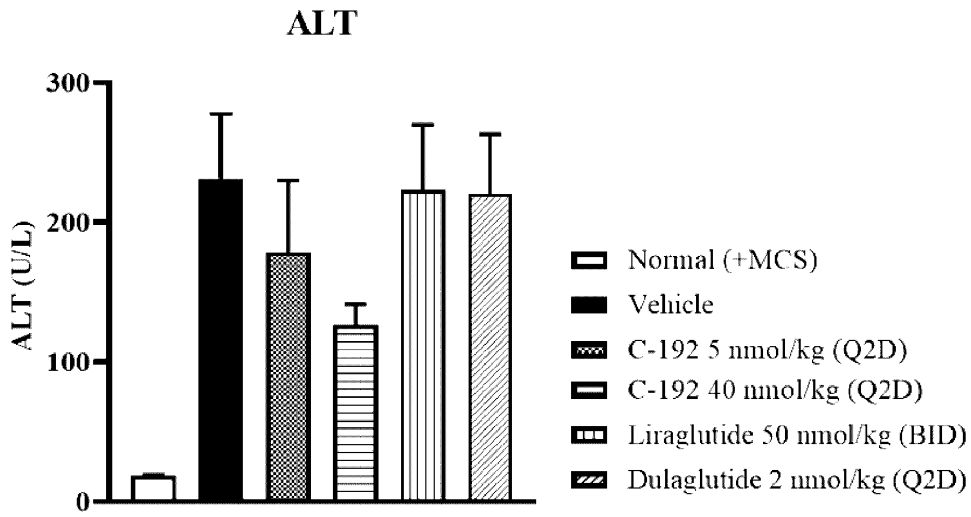
[도19]


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

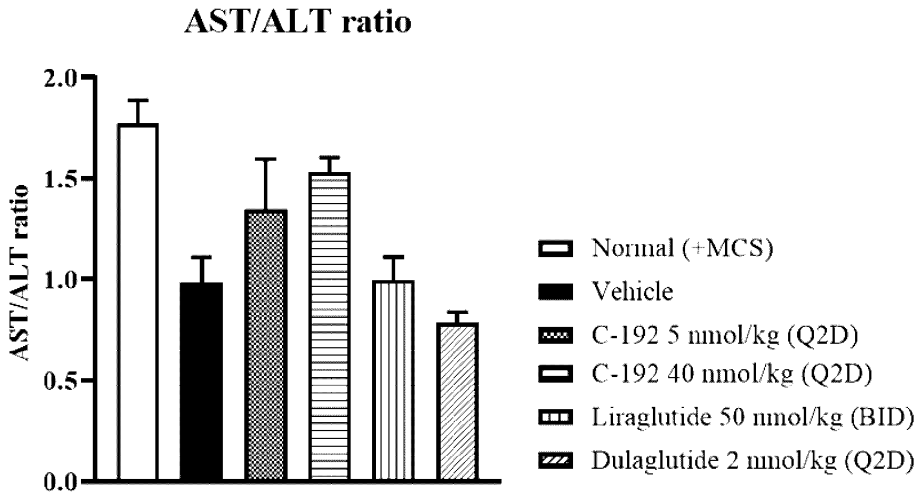
[도20]


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

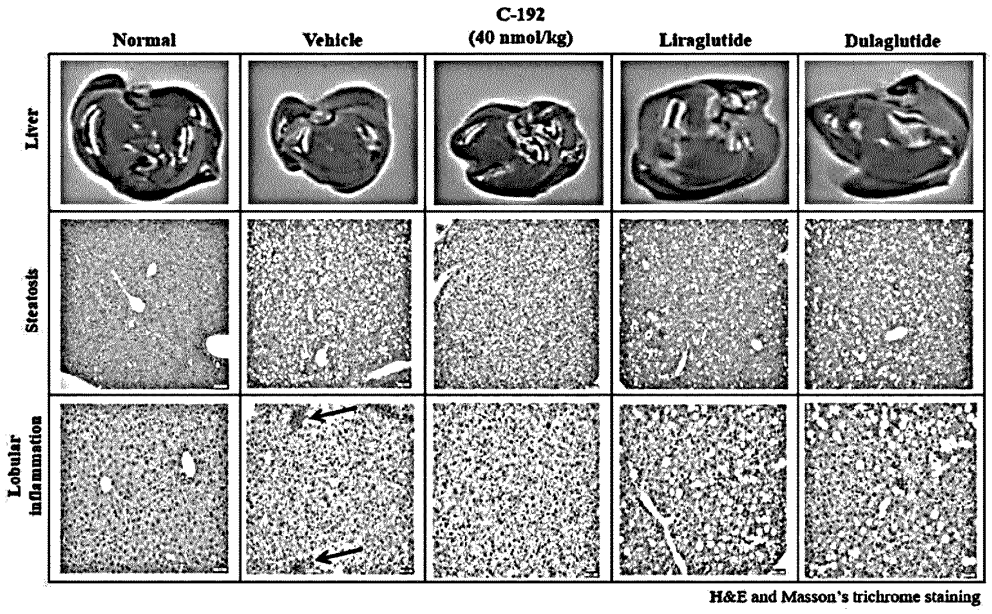
[도21]


ALT
300
200
100
0
ALT (U/L)
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)

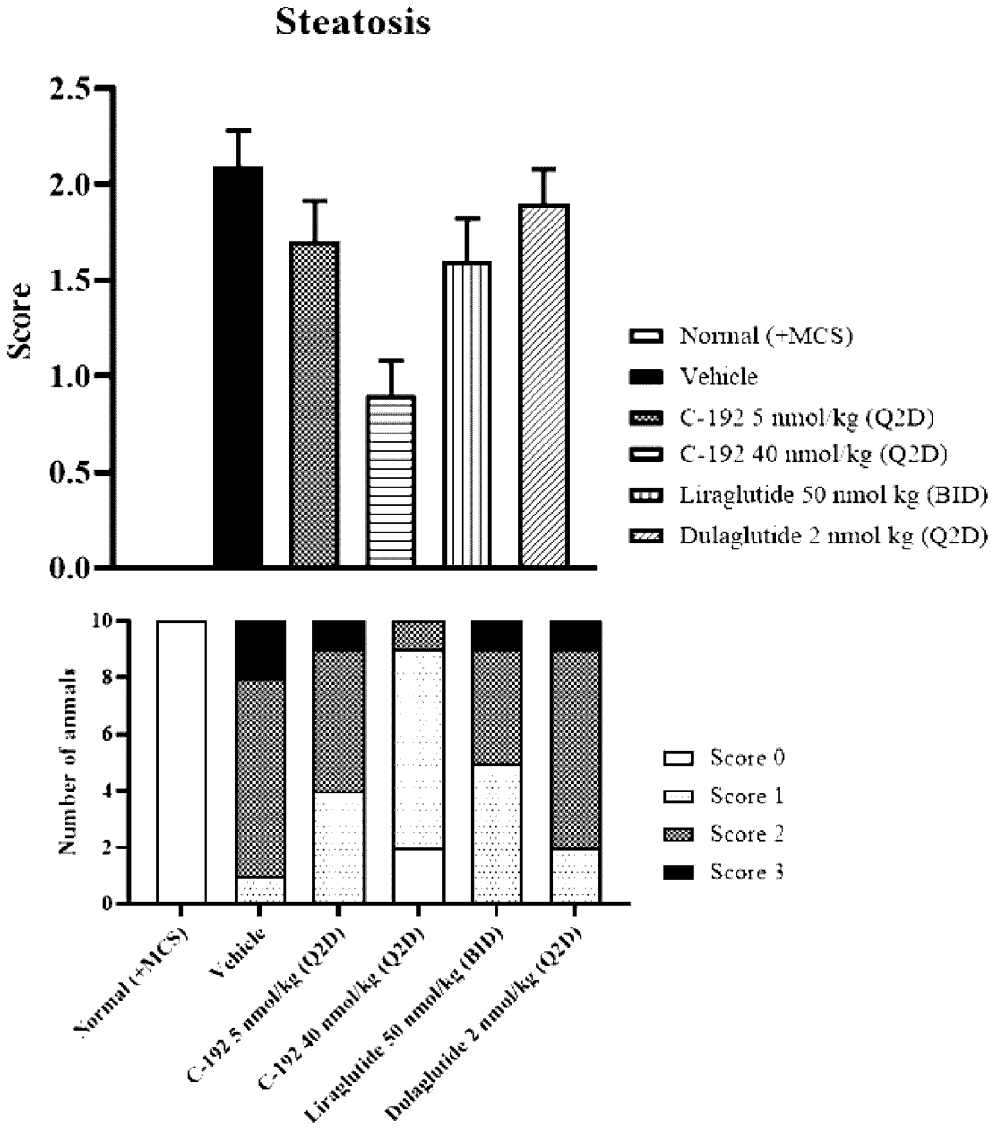
[도22]


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)

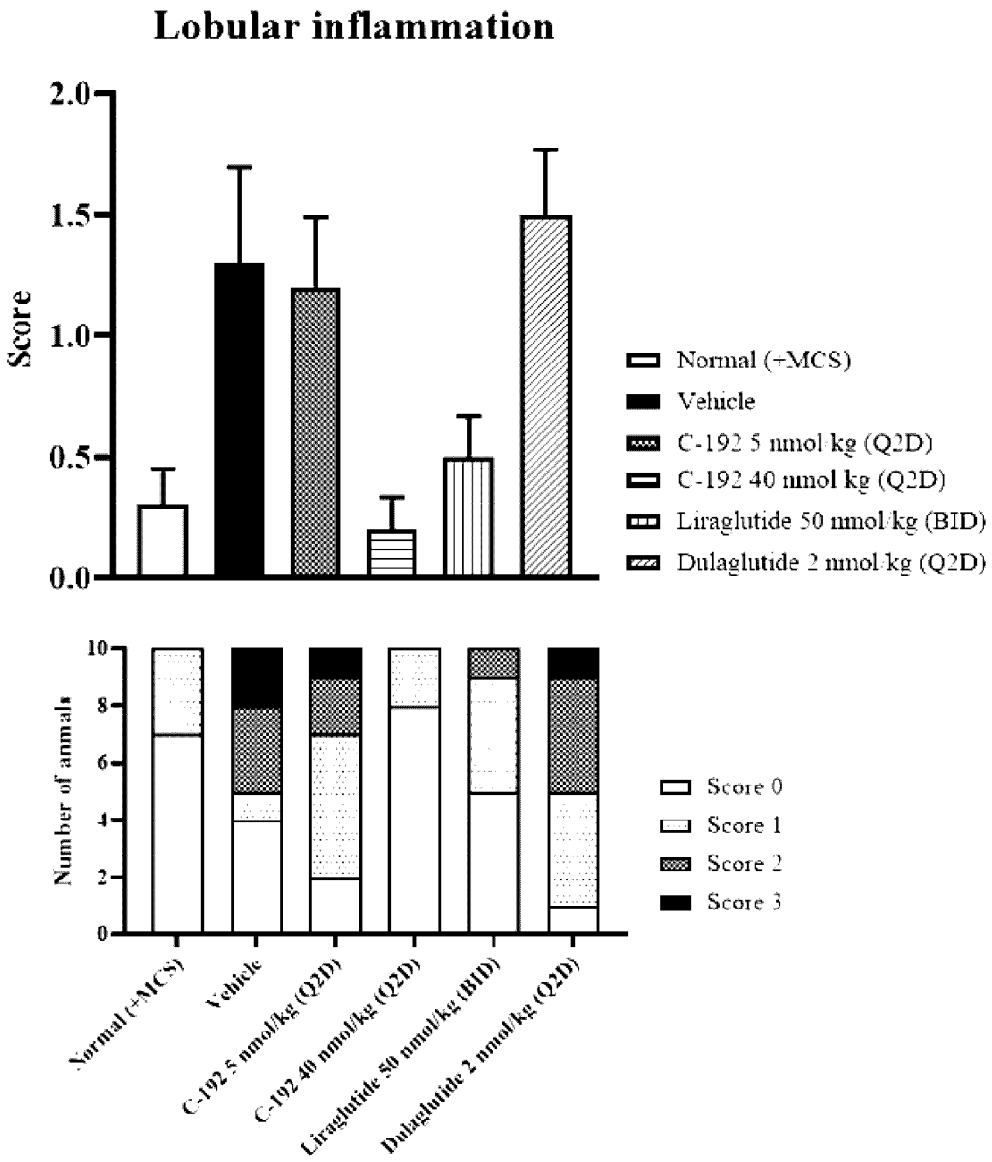
[도23]


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

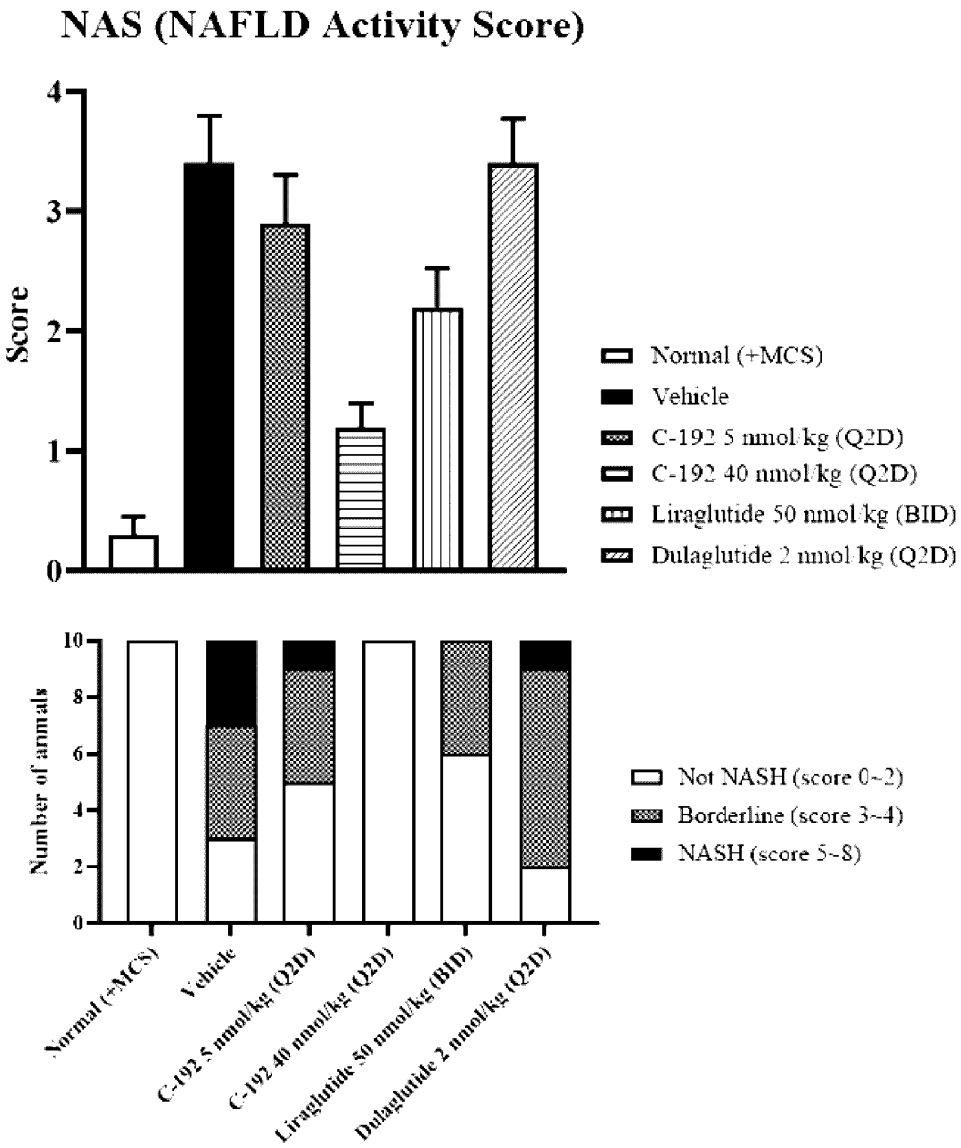
[도24]


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)

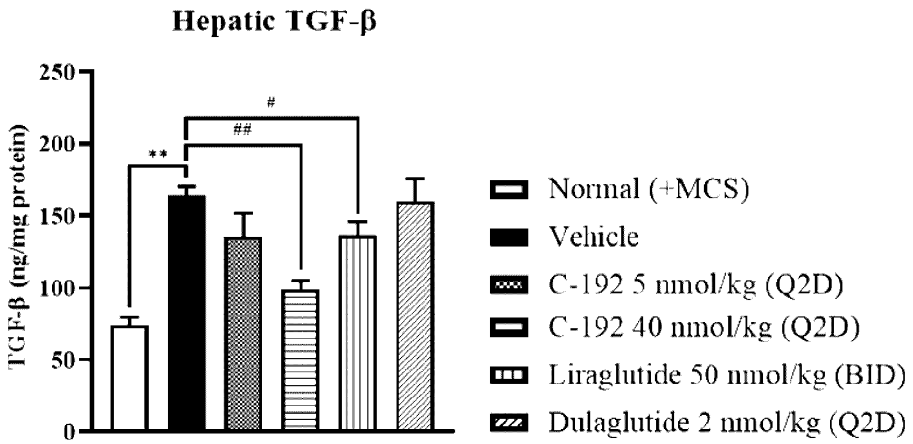
[도25]


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 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)

[도26]

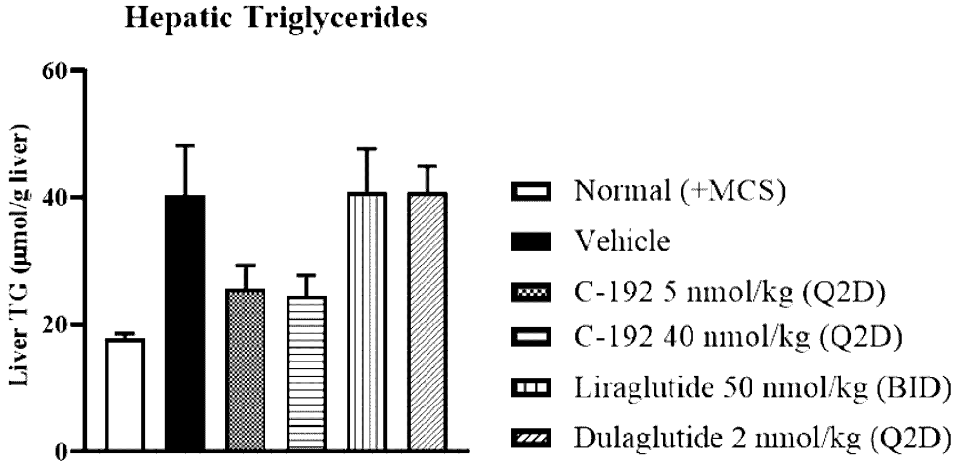

NAS (NAFLD Activity Score)
Score
4
3
2
1
0
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)
C-192 40 nmol/kg (Q2D)
Liraglutide 50 nmol/kg (BID)
Dulaglutide 2 nmol/kg (Q2D)
Number of animals
10
8
6
4
2
0
Not NASH (score 0~2)
Borderline (score 3~4)
NASH (score 5~8)
Normal (+MCS)
Vehicle
C-192 5 nmol/kg (Q2D)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

[도27]

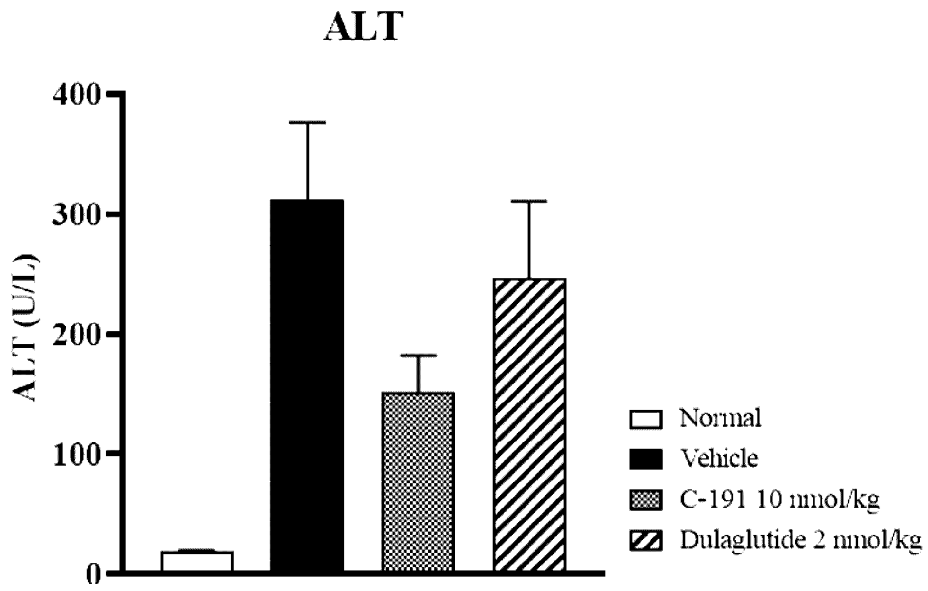


** p < 0.01. Significant difference from the normal control by Student t-test.
# p < 0.05. Significant difference from the negative control by Steel's t-test.
p < 0.01. Significant difference from the negative control by Steel's t-test.

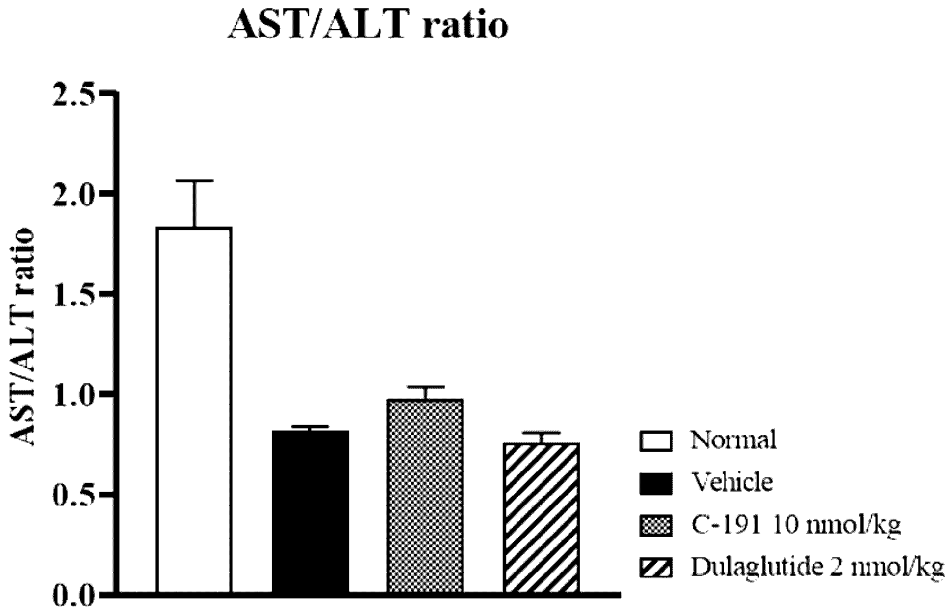
[도28]



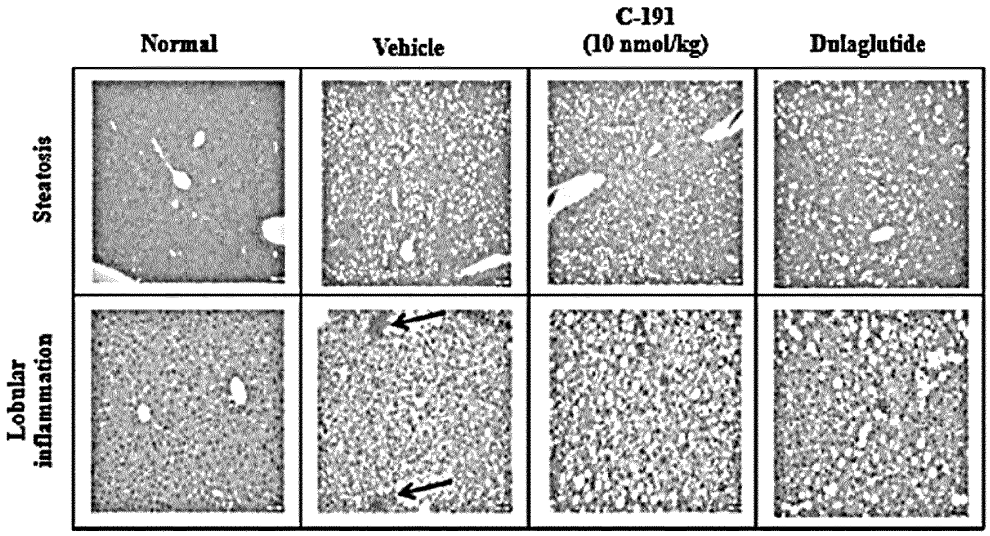
[도29]


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

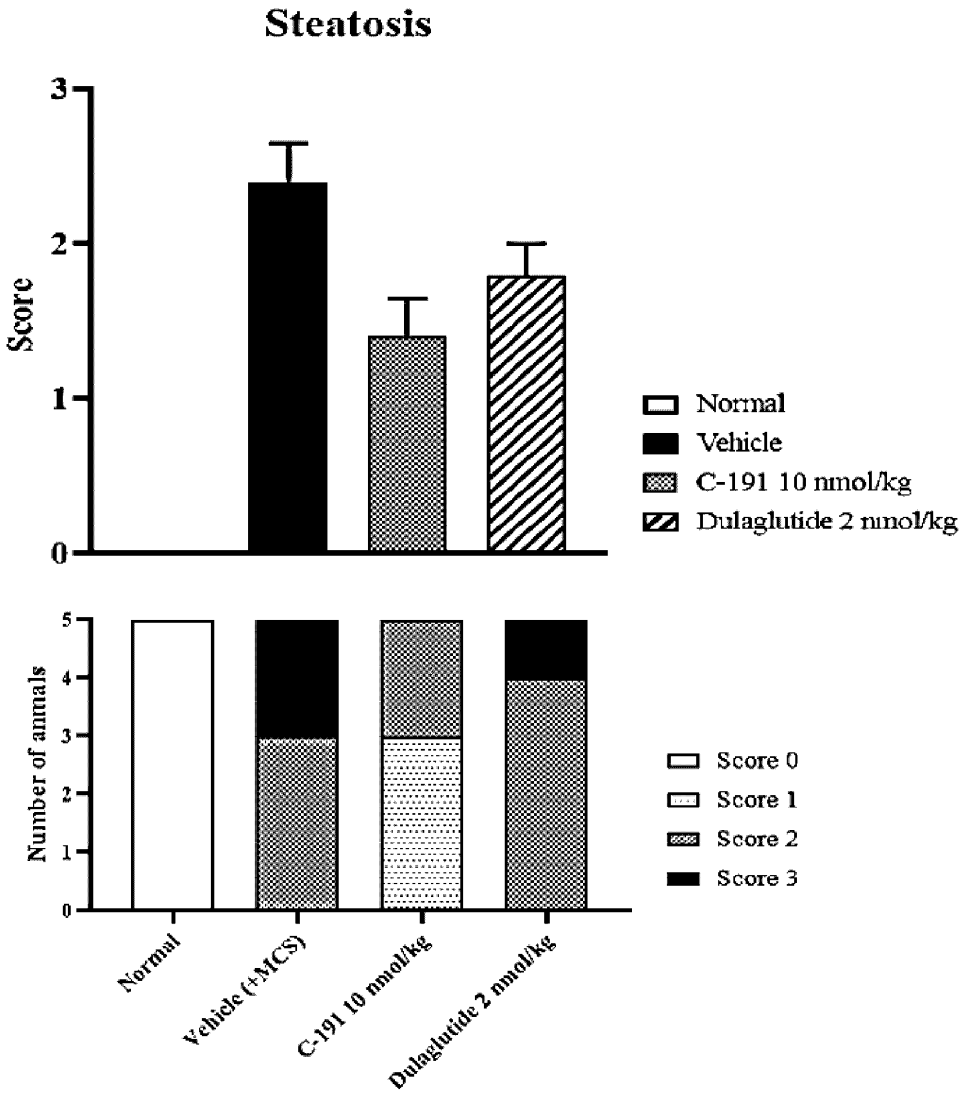
[도30]


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

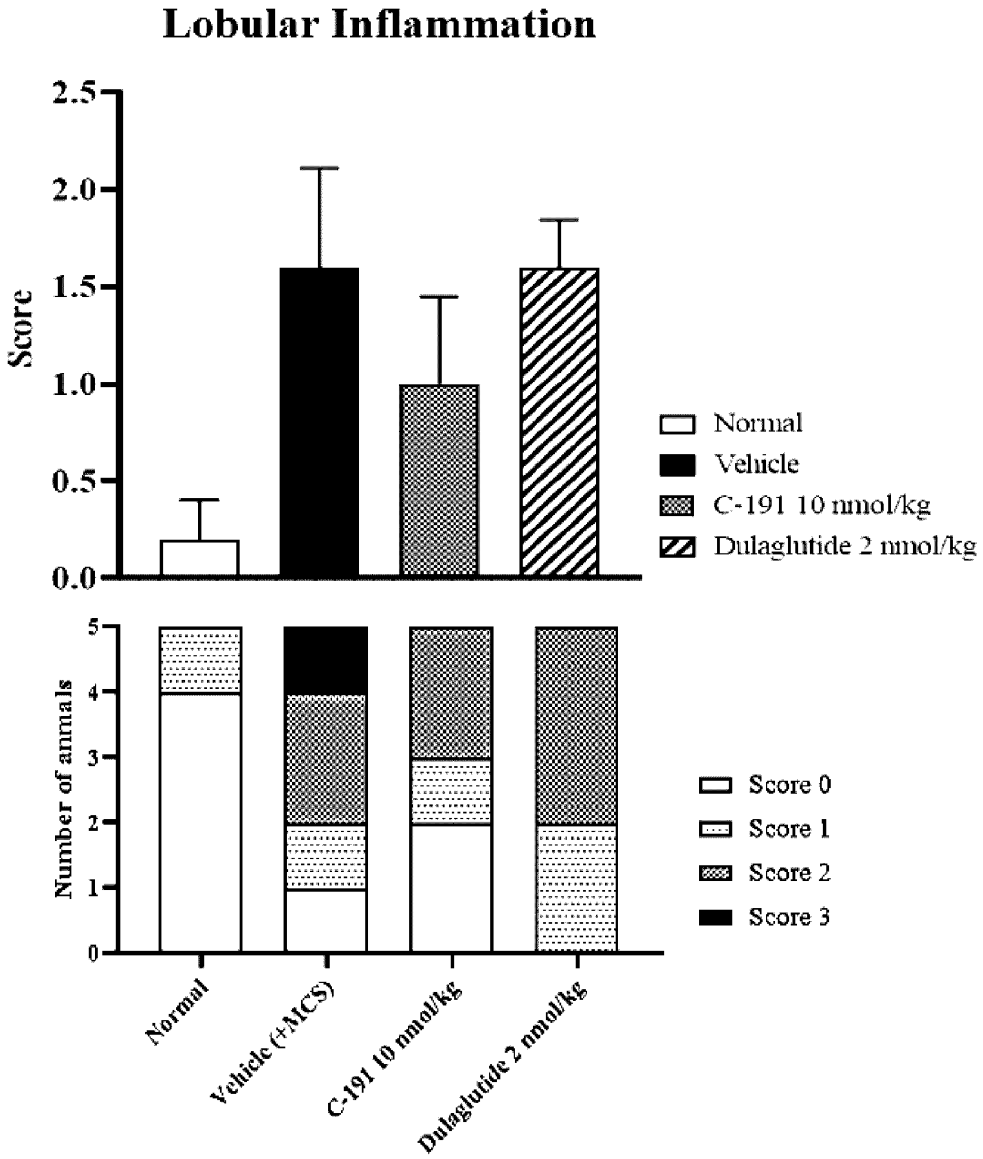
[도31]


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

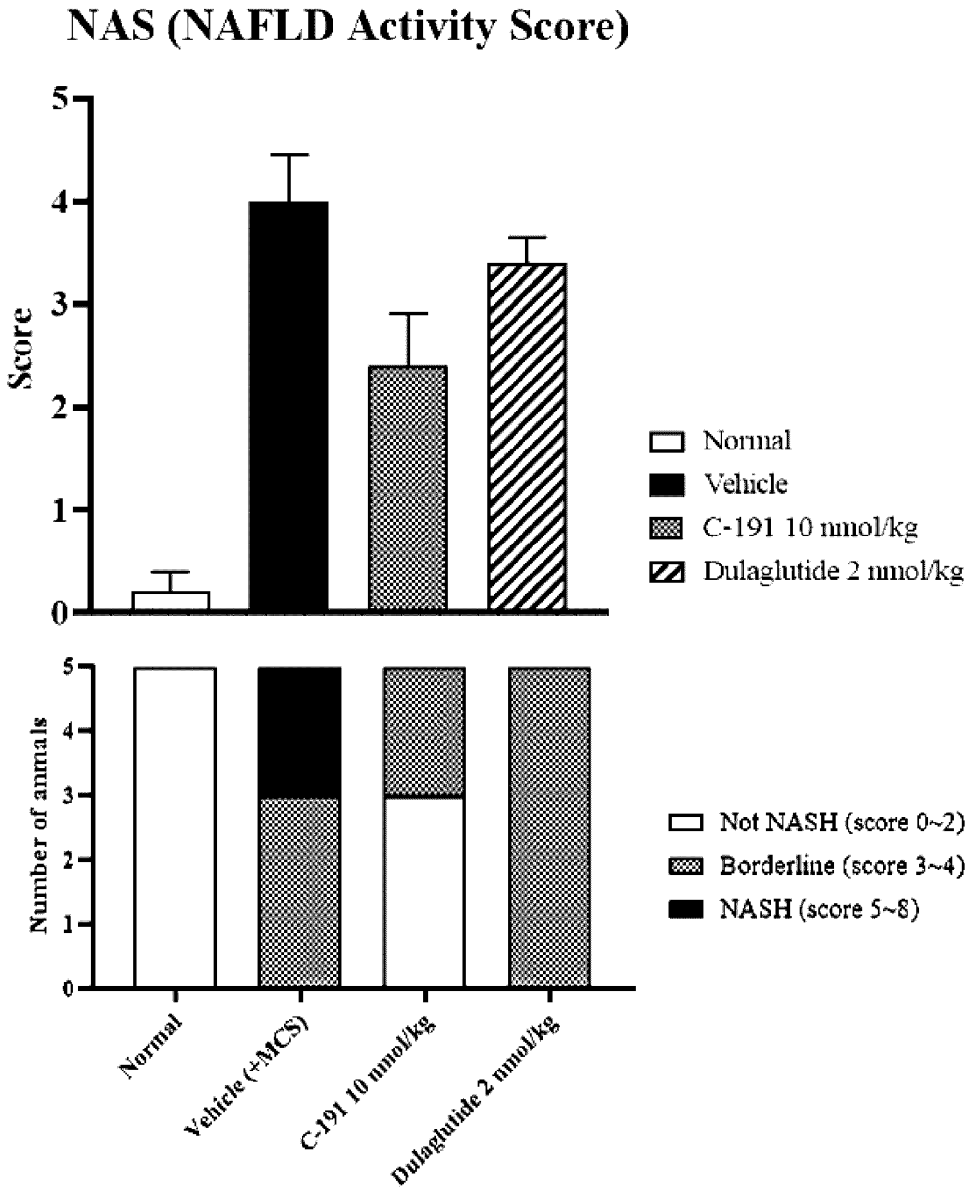
[도32]


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

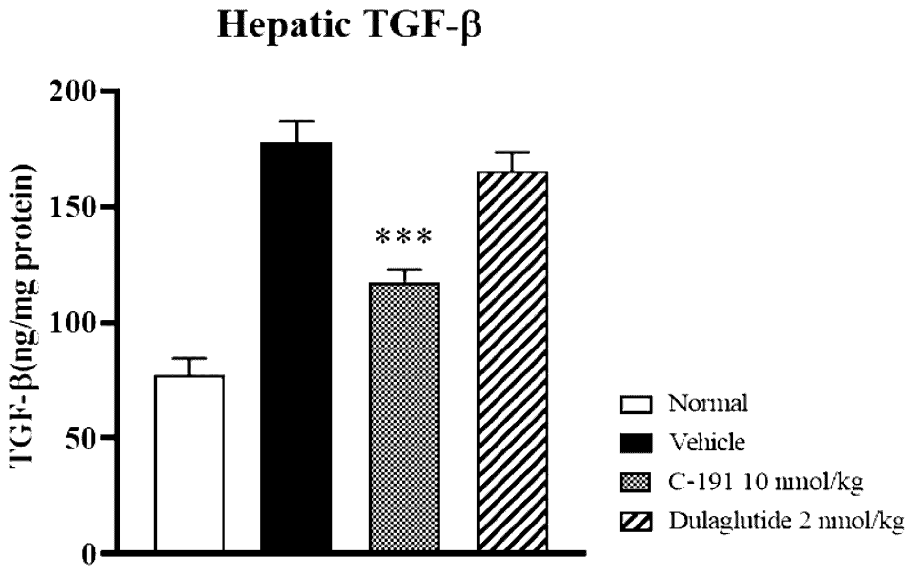
[도33]


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 animals
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

[도34]

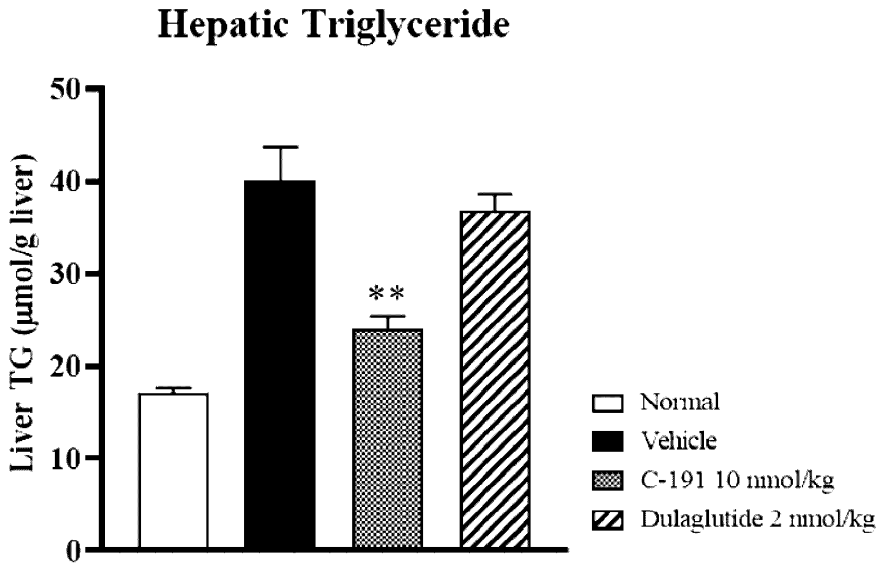

NAS (NAFLD Activity Score)
Score
0
1
2
3
4
5
Normal
Vehicle
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg
Number of animals
0
1
2
3
4
5
Not NASH (score 0~2)
Borderline (score 3~4)
NASH (score 5~8)
Normal
Vehicle (+MCS)
C-191 10 nmol/kg
Dulaglutide 2 nmol/kg

[도35]



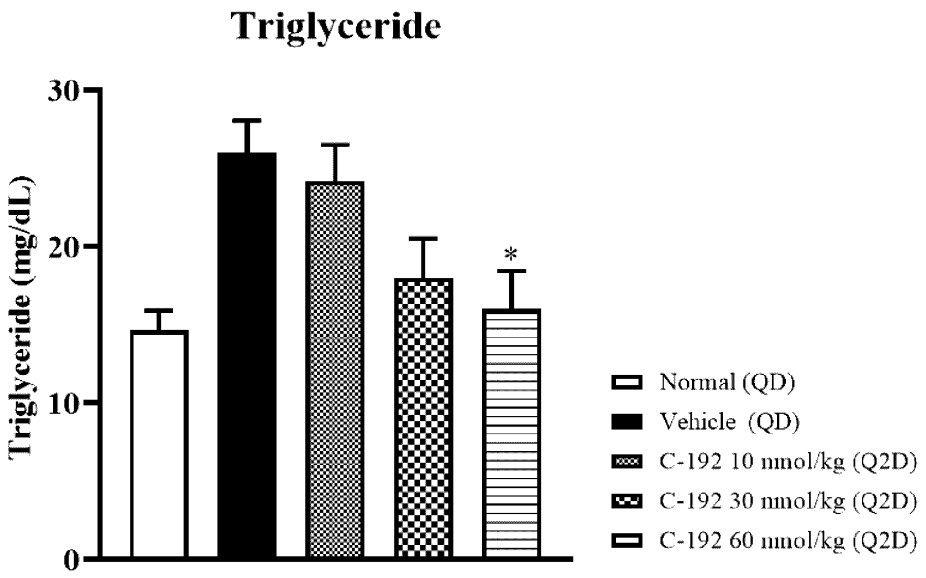
Data were expressed as mean ± SEM. One-way repeated measurement ANOVA and post-hoc Dunnett's test. ***p < 0.001, compared to vehicle group.

[도36]



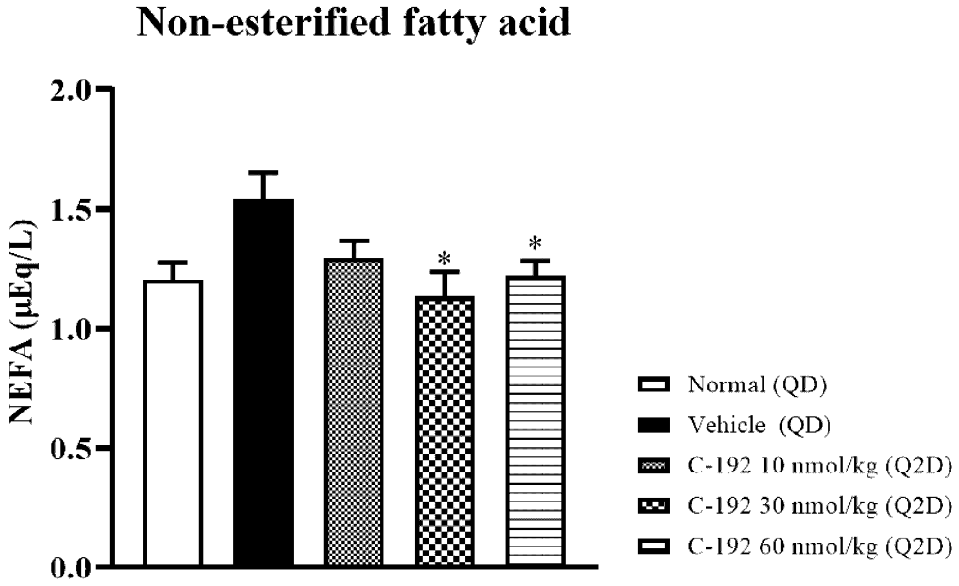
Data were expressed as mean ± SEM. One-way repeated measurement ANOVA and post-hoc Dunnett's test. **p < 0.05, compared to vehicle group.

[도37]



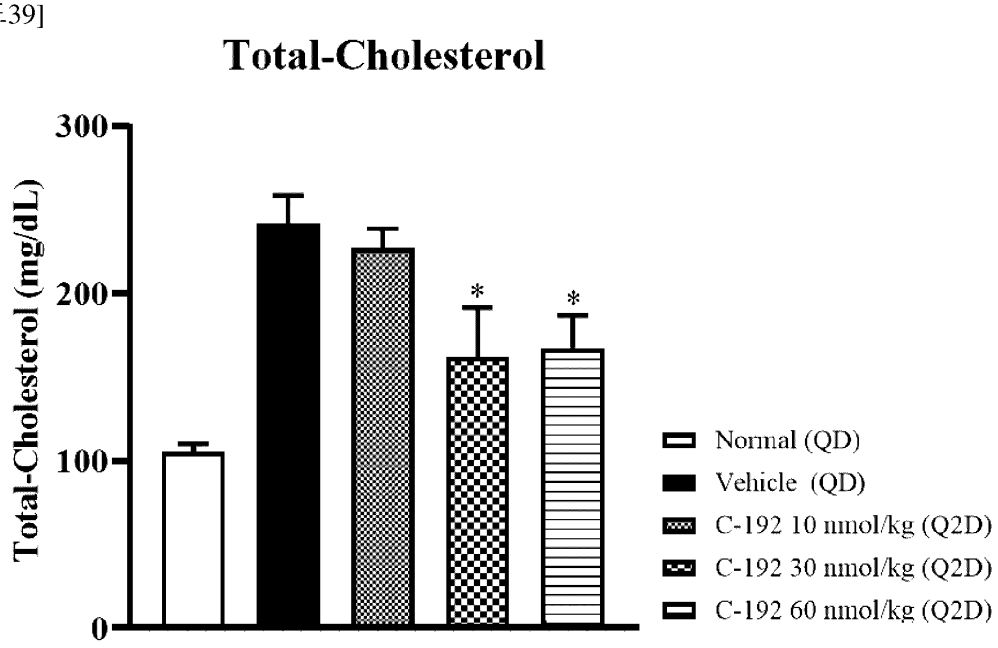
Data were expressed as mean ± SEM of tyiglyceride. One-way ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. n = 6.

[도38]



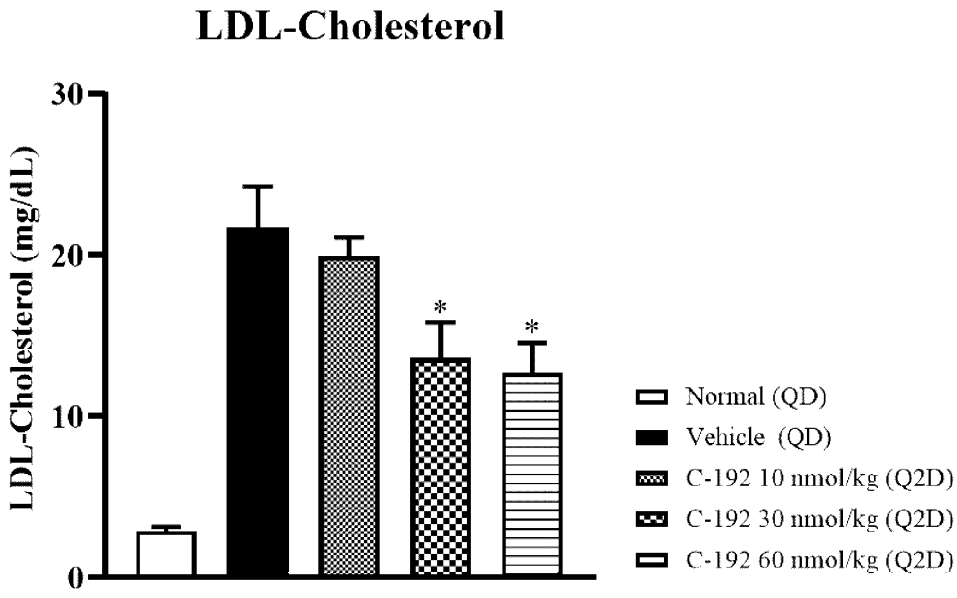
Data were expressed as mean ± SEM of non-esterified fatty acid. One-way ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. n = 6.

[도39]



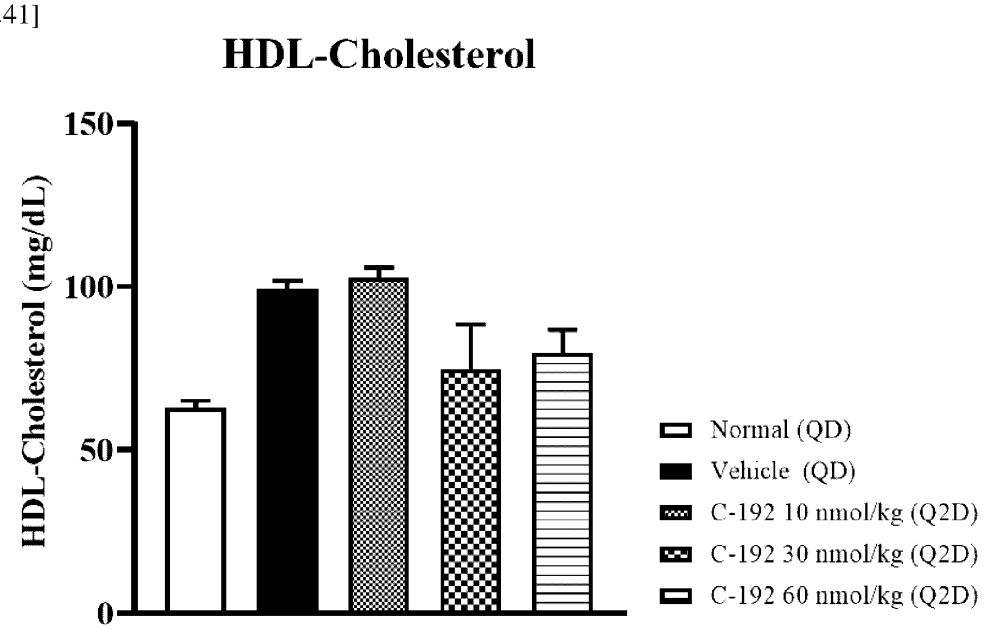
Data were expressed as mean ± SEM of total-cholesterol. One-way ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. n = 6.

[도40]



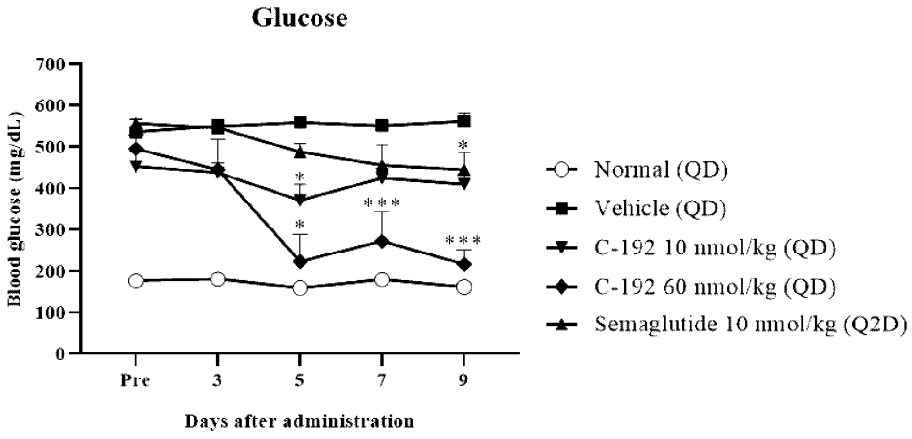
Data were expressed as mean ± SEM of LDL-cholesterol. One-way ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. n = 6.

[도41]



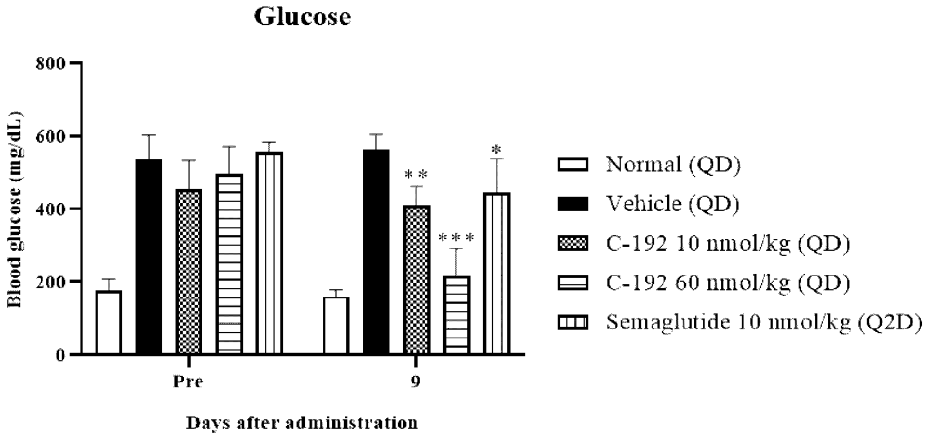
Data were expressed as mean ± SEM of HDL-cholesterol. n = 6.

[도42]



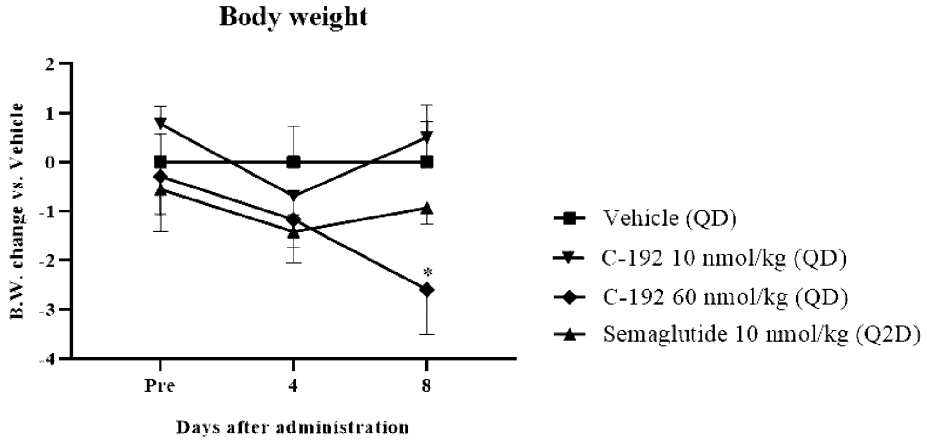
Data were expressed as mean ± SEM of blood glucose. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

[도43]



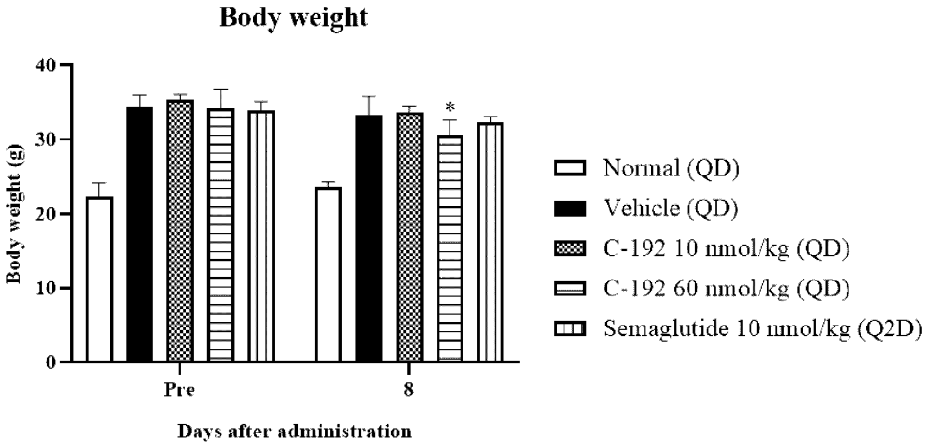
Data were expressed as mean ± SEM of blood glucose. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

[도44]



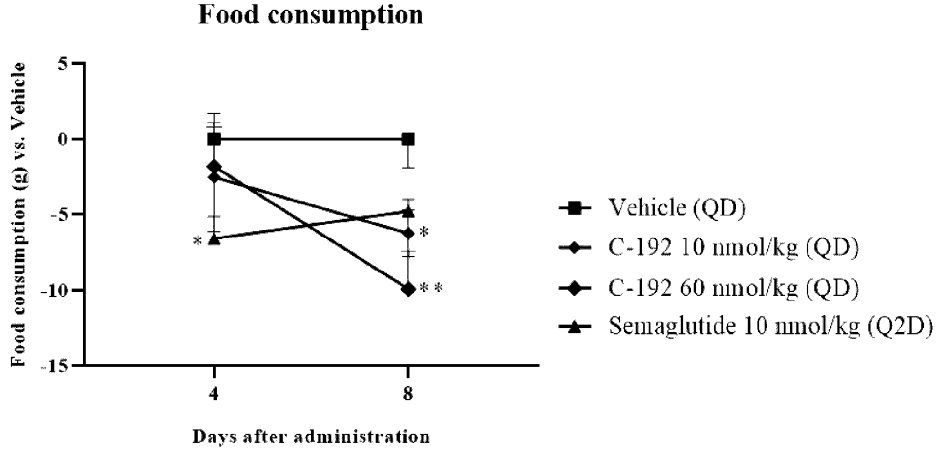
Data were expressed as mean ± SEM of body weight change verse vehicle. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. n = 5.

[도45]



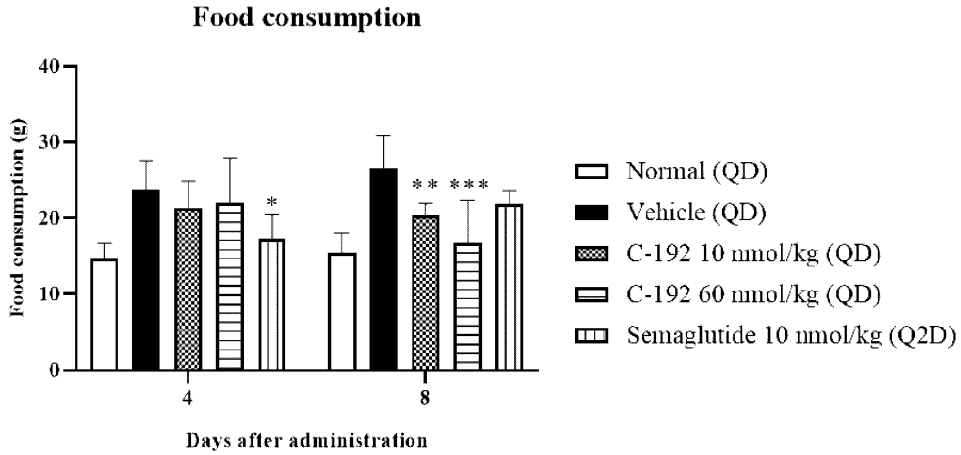
Data were expressed as mean ± SEM of body weight change verse vehicle. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. n = 5.

[도46]



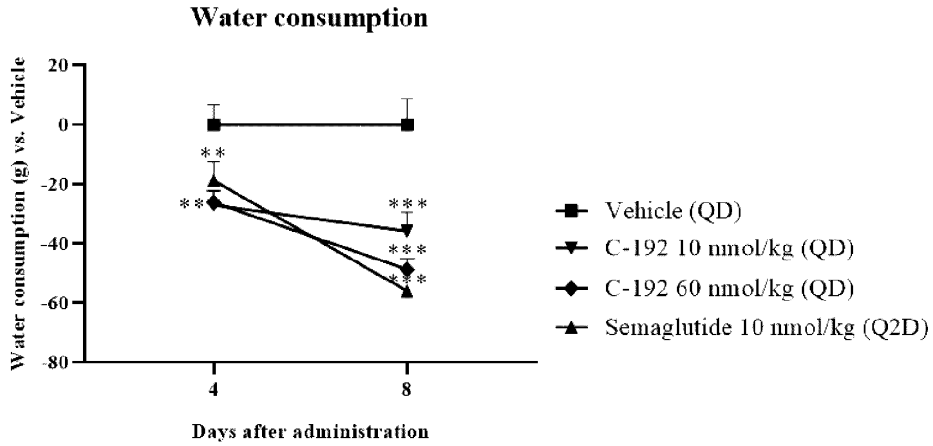
Data were expressed as mean ± SEM of food consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. **p < 0.01, compared to vehicle group. n = 5.

[도47]



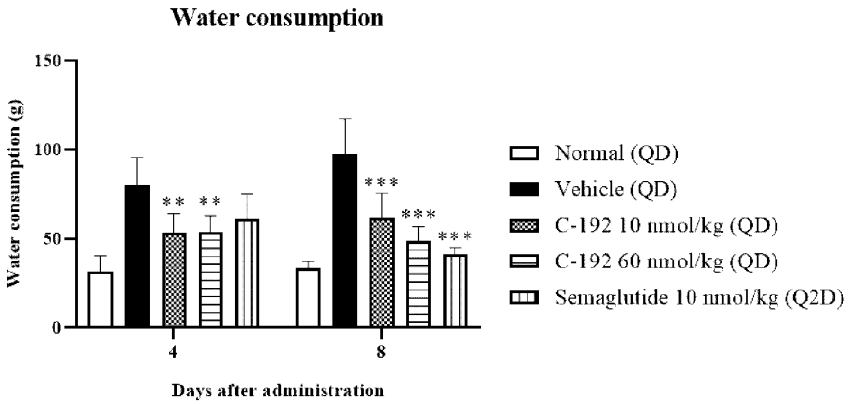
Data were expressed as mean ± SEM of food consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. *p < 0.05, compared to vehicle group. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

[도48]



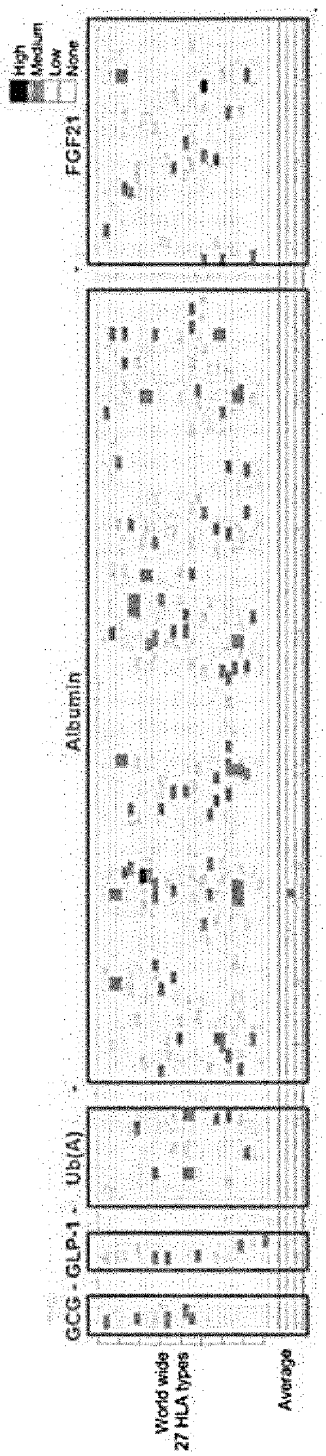
Data were expressed as mean ± SEM of water consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

[도49]

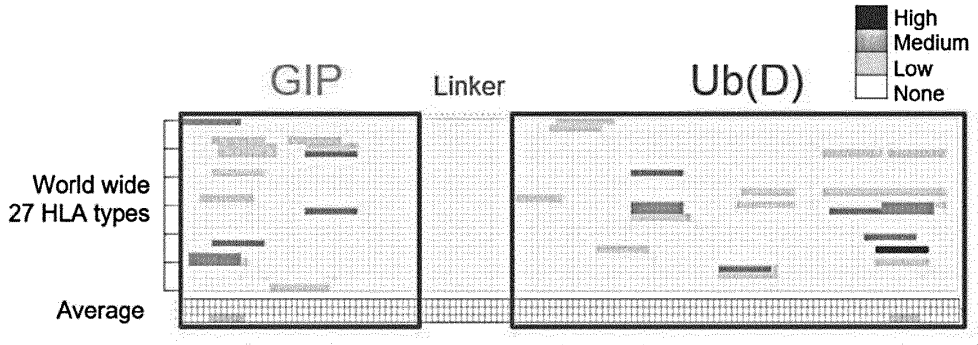


Data were expressed as mean ± SEM of water consumption. Two-way repeated measurement ANOVA and post-hoc Dunnett's test. **p < 0.01, compared to vehicle group. ***p < 0.001, compared to vehicle group. n = 5.

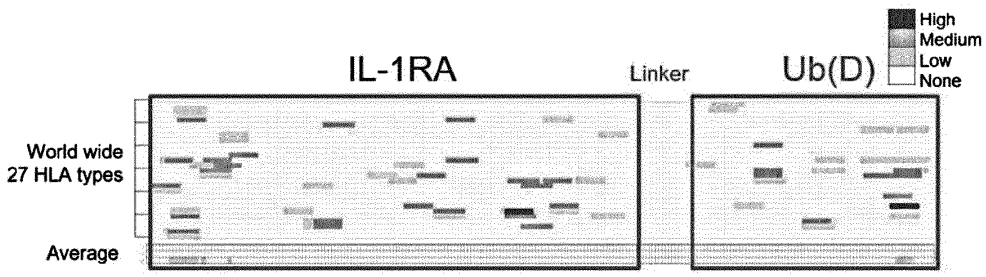
[도50]


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

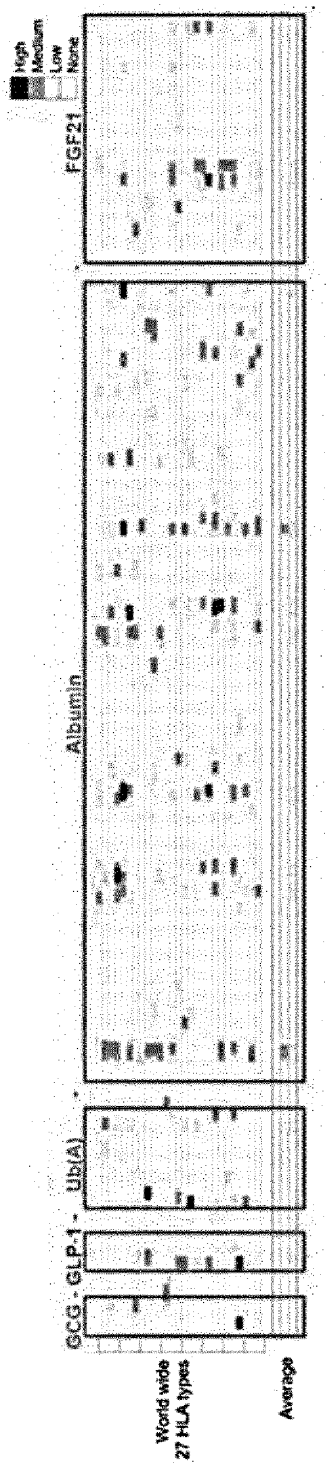
[도51]


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

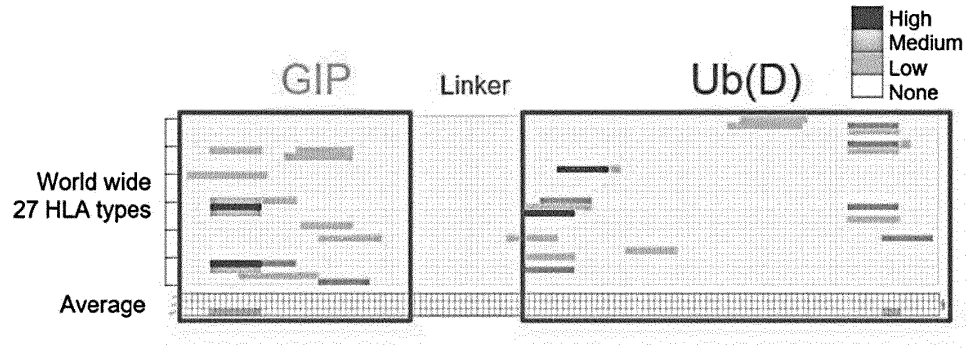
[도52]


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

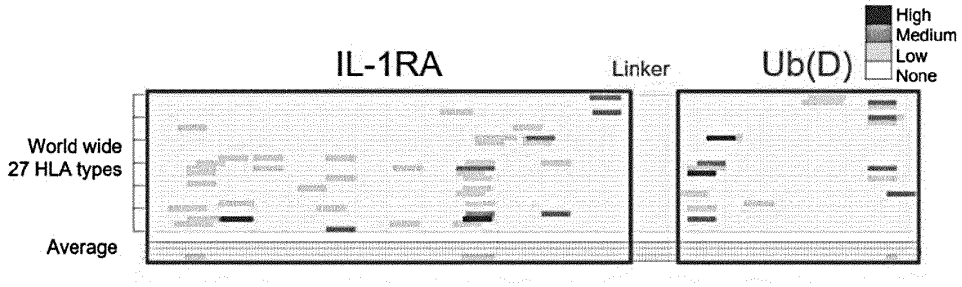
[도53]


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

[도54]


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

[도55]


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