FIG. 1A

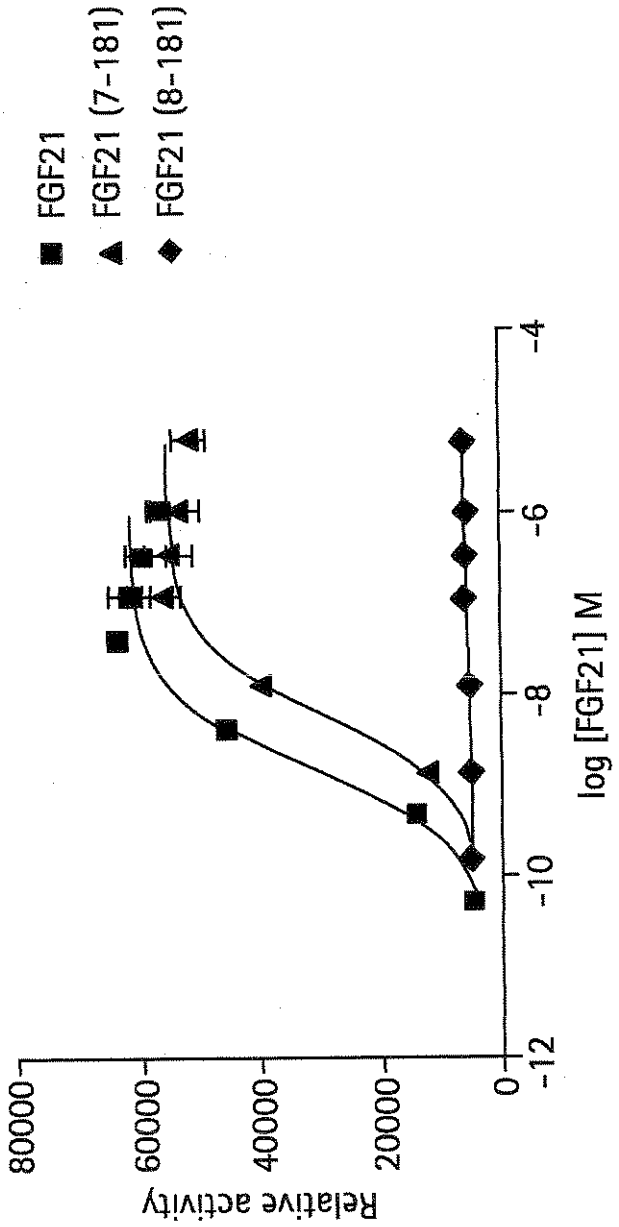

FGF21
FGF21 (7-181)
FGF21 (8-181)
Relative activity
80000
60000
40000
20000
0
-12
-10
-8
-6
-4
log [FGF21] M

FIG. 1B

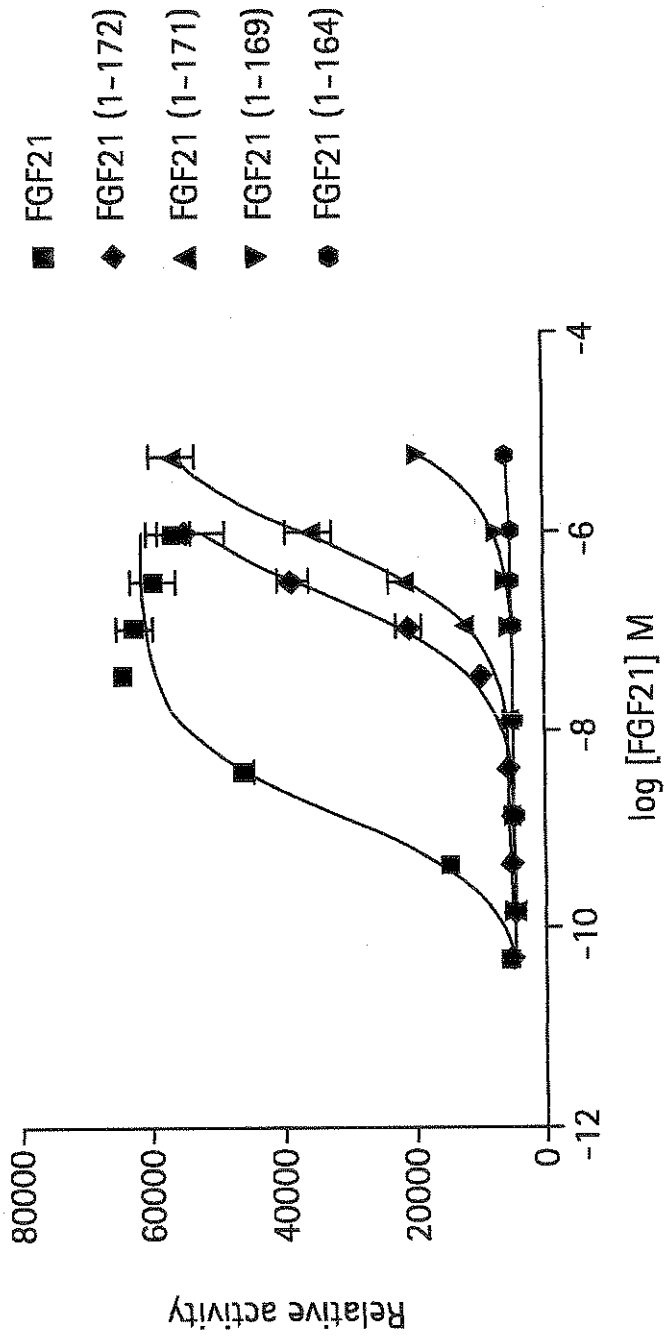

FGF21
FGF21 (1-172)
FGF21 (1-171)
FGF21 (1-169)
FGF21 (1-164)
Relative activity
80000
60000
40000
20000
0
-12
-10
-8
-6
-4
log [FGF21] M

FIG. 2

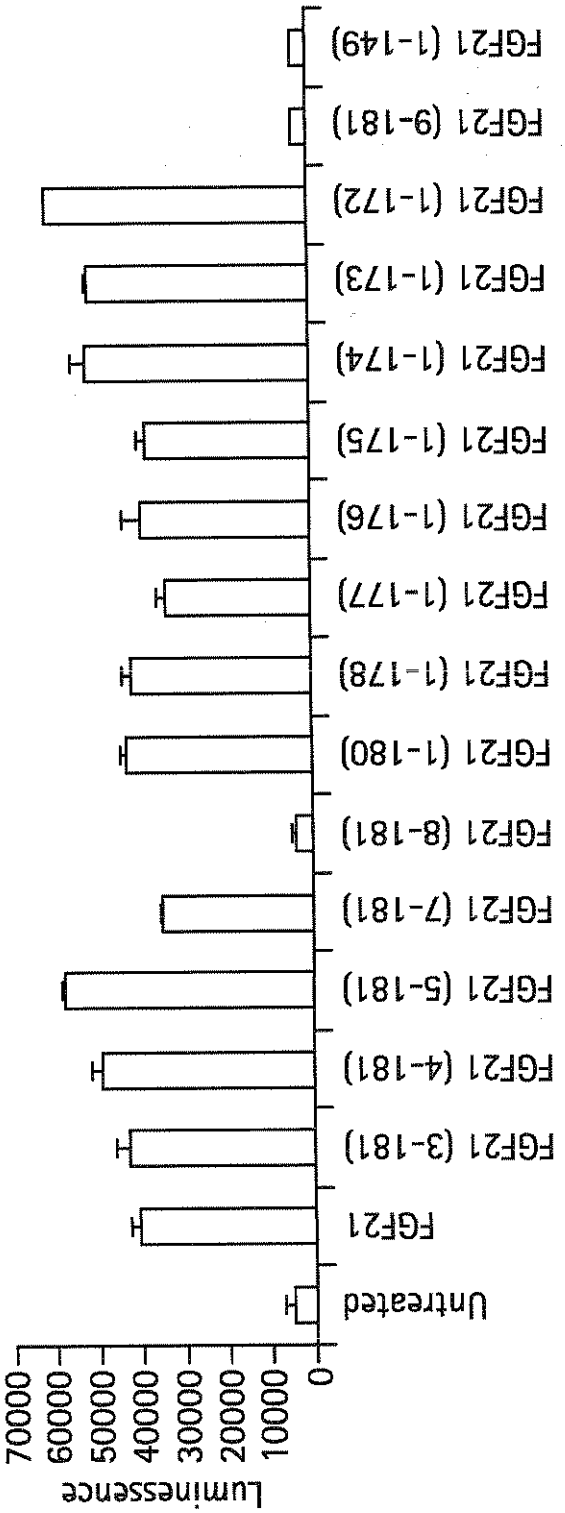

Luminescence
70000
60000
50000
40000
30000
20000
10000
0
Untreated
FGF21
FGF21 (3-181)
FGF21 (4-181)
FGF21 (5-181)
FGF21 (7-181)
FGF21 (8-181)
FGF21 (1-180)
FGF21 (1-178)
FGF21 (1-177)
FGF21 (1-176)
FGF21 (1-175)
FGF21 (1-174)
FGF21 (1-173)
FGF21 (1-172)
FGF21 (9-181)
FGF21 (1-149)

FIG. 3

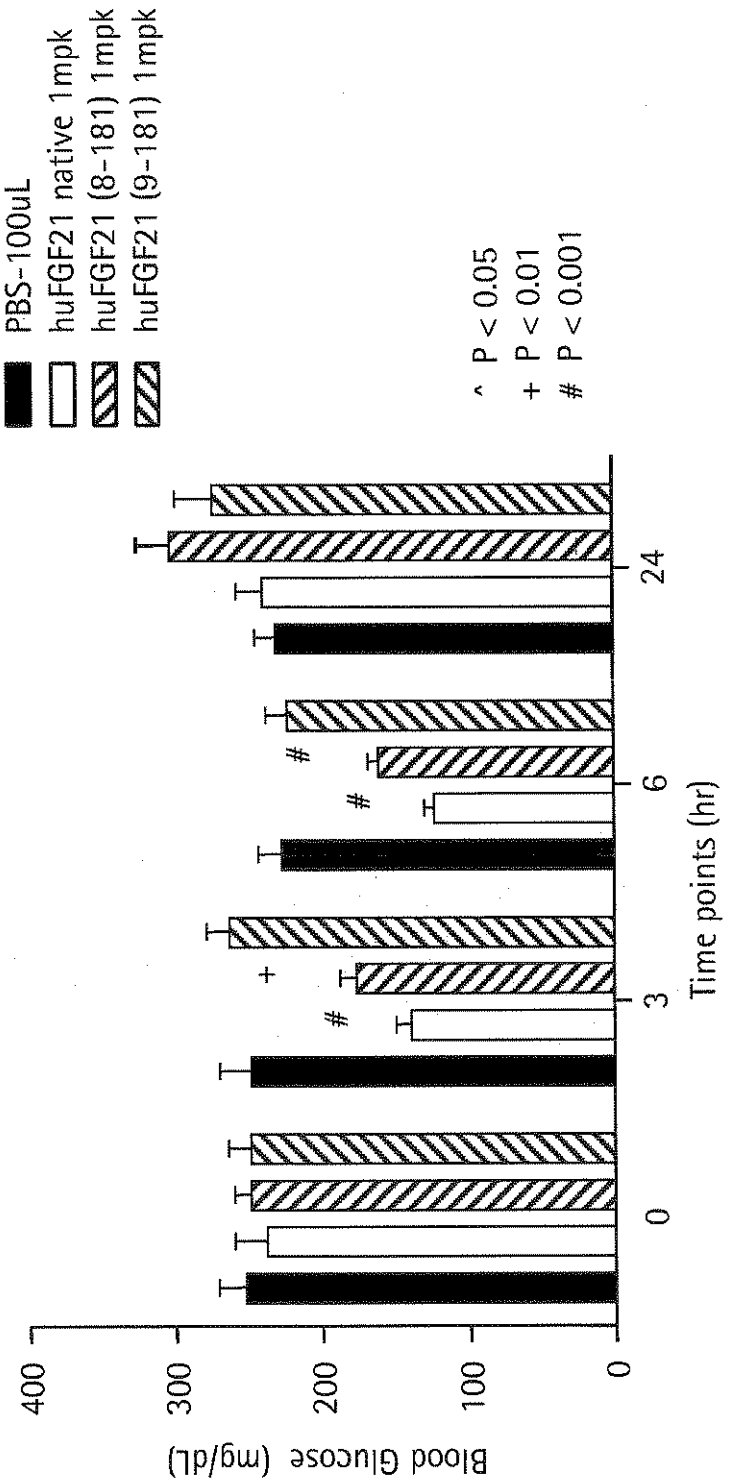

PBS-100uL
huFGF21 native 1mpk
huFGF21 (8-181) 1mpk
huFGF21 (9-181) 1mpk
^ P < 0.05
+ P < 0.01
# P < 0.001
Blood Glucose (mg/dL)
0
100
200
300
400
0
3
6
24
Time points (hr)

FIG. 4

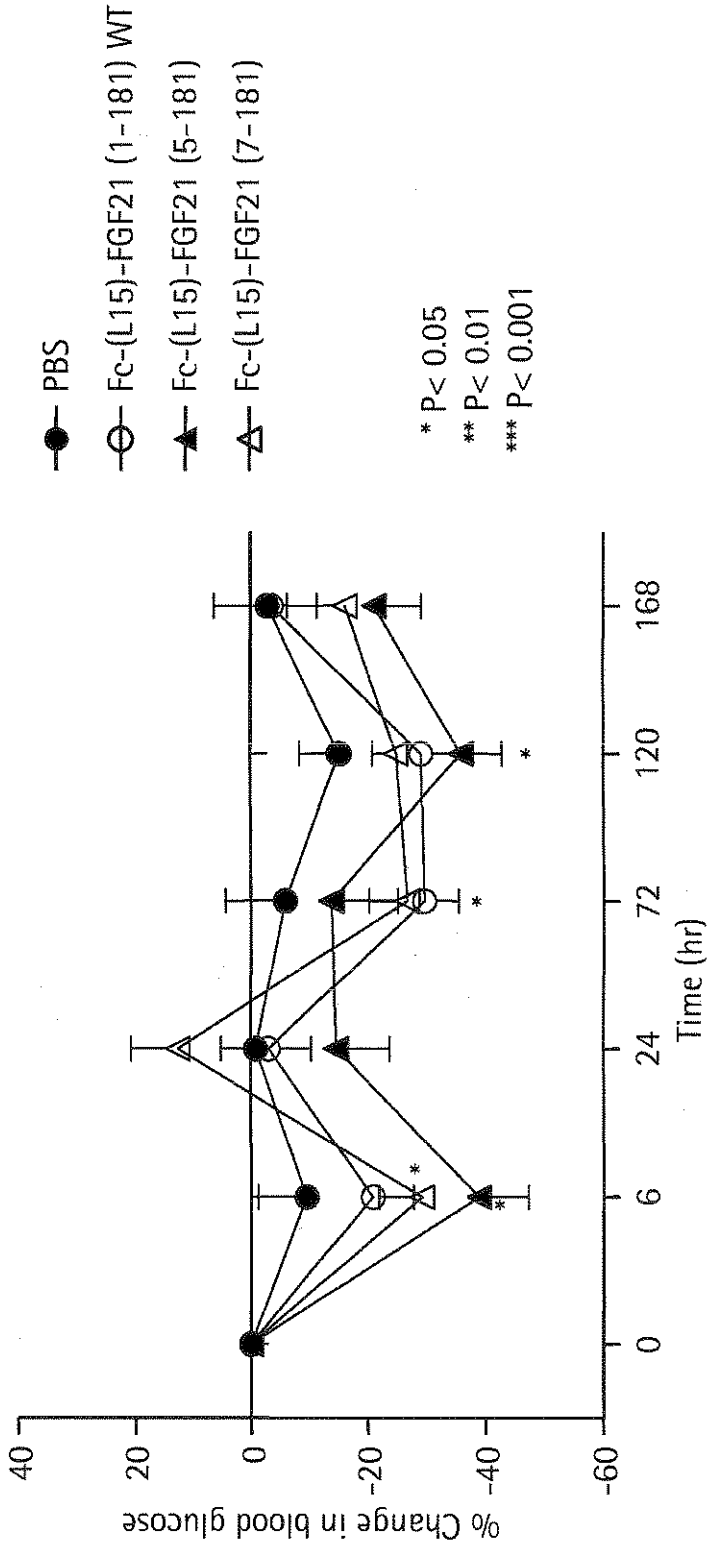

PBS
Fc-(L15)-FGF21 (1-181) WT
Fc-(L15)-FGF21 (5-181)
Fc-(L15)-FGF21 (7-181)
* P< 0.05
** P< 0.01
*** P< 0.001
% Change in blood glucose
40
20
0
-20
-40
-60
0
6
24
72
120
168
Time (hr)

FIG. 5

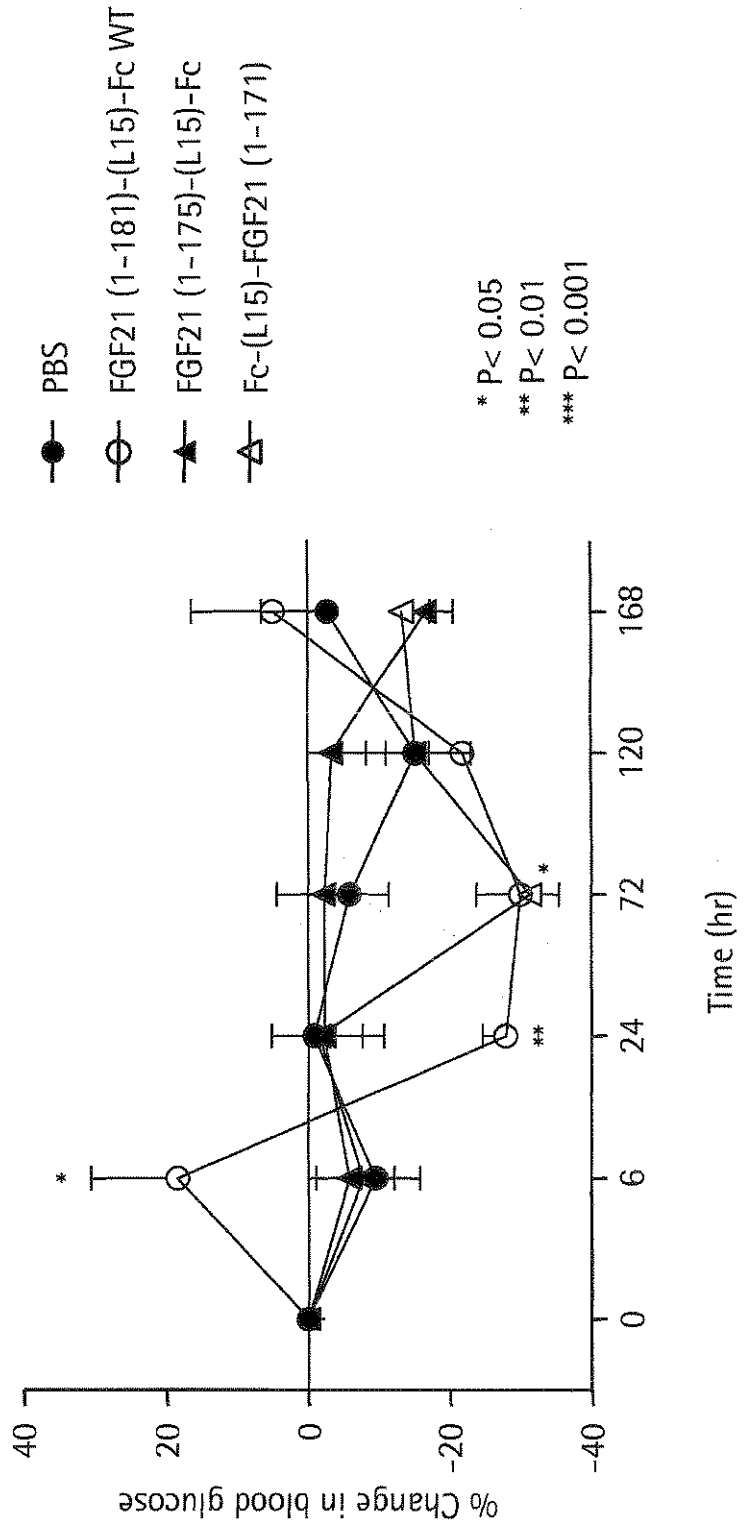

PBS
FGF21 (1-181)-(L15)-Fc WT
FGF21 (1-175)-(L15)-Fc
Fc-(L15)-FGF21 (1-171)
* P< 0.05
** P< 0.01
*** P< 0.001
% Change in blood glucose
40
20
0
-20
-40
0
6
24
72
120
168
Time (hr)
*
**
*

FIG. 6A

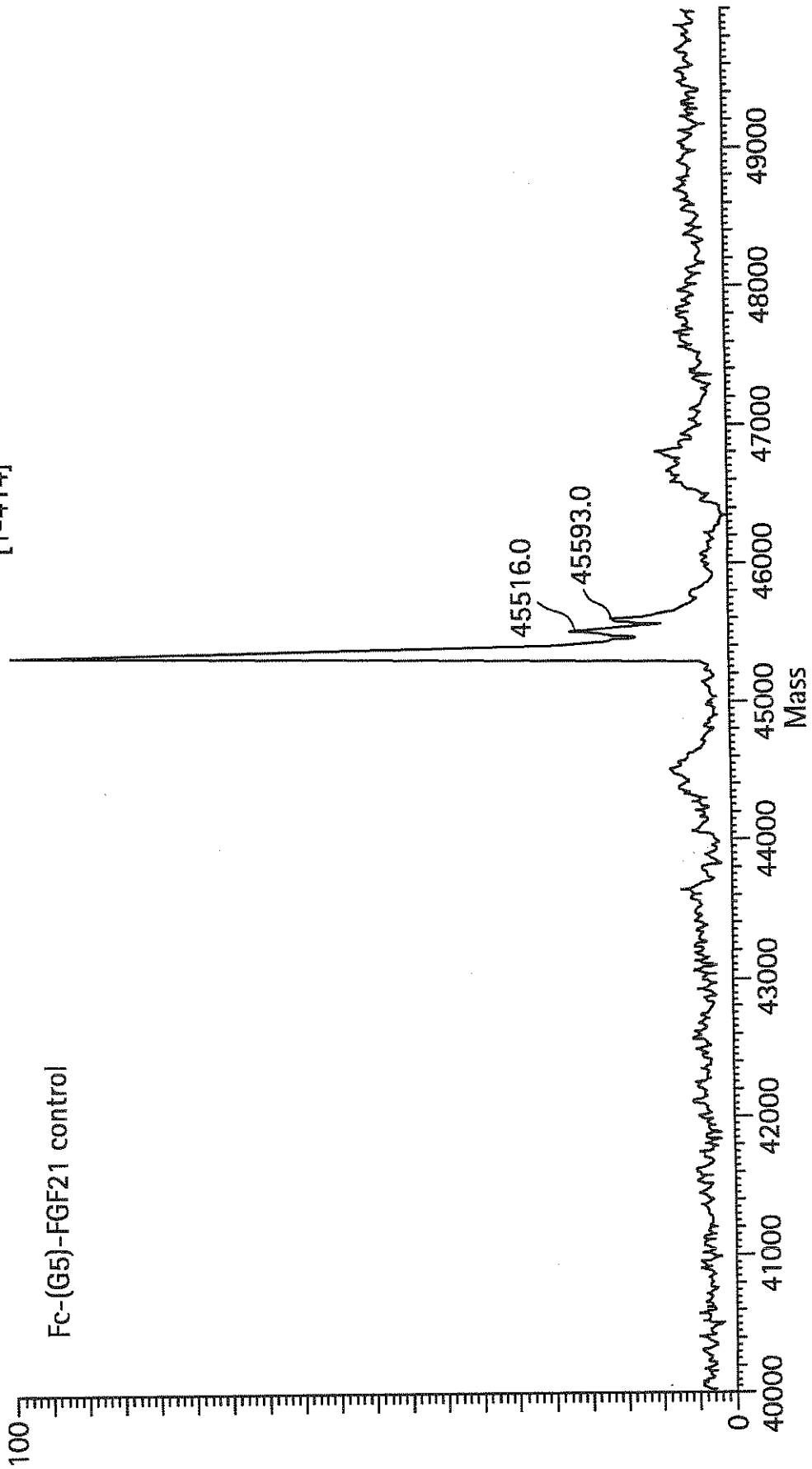

Fc-(G5)-FGF21 control
453390.0
[1-414]
45516.0
45593.0
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 6B

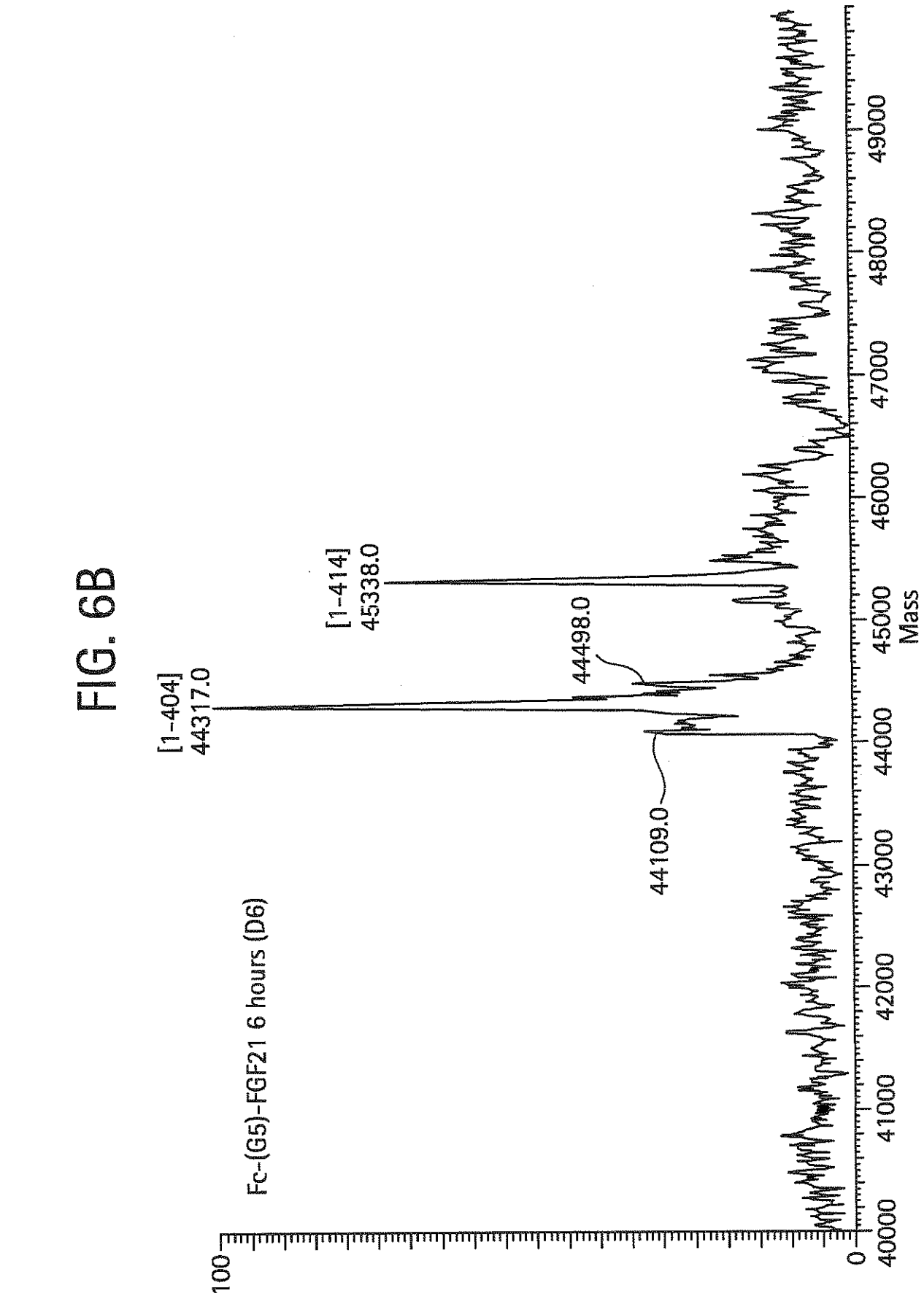

Fc-(G5)-FGF21 6 hours (D6)
[1-404]
44317.0
[1-414]
45338.0
44498.0
44109.0
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 6C

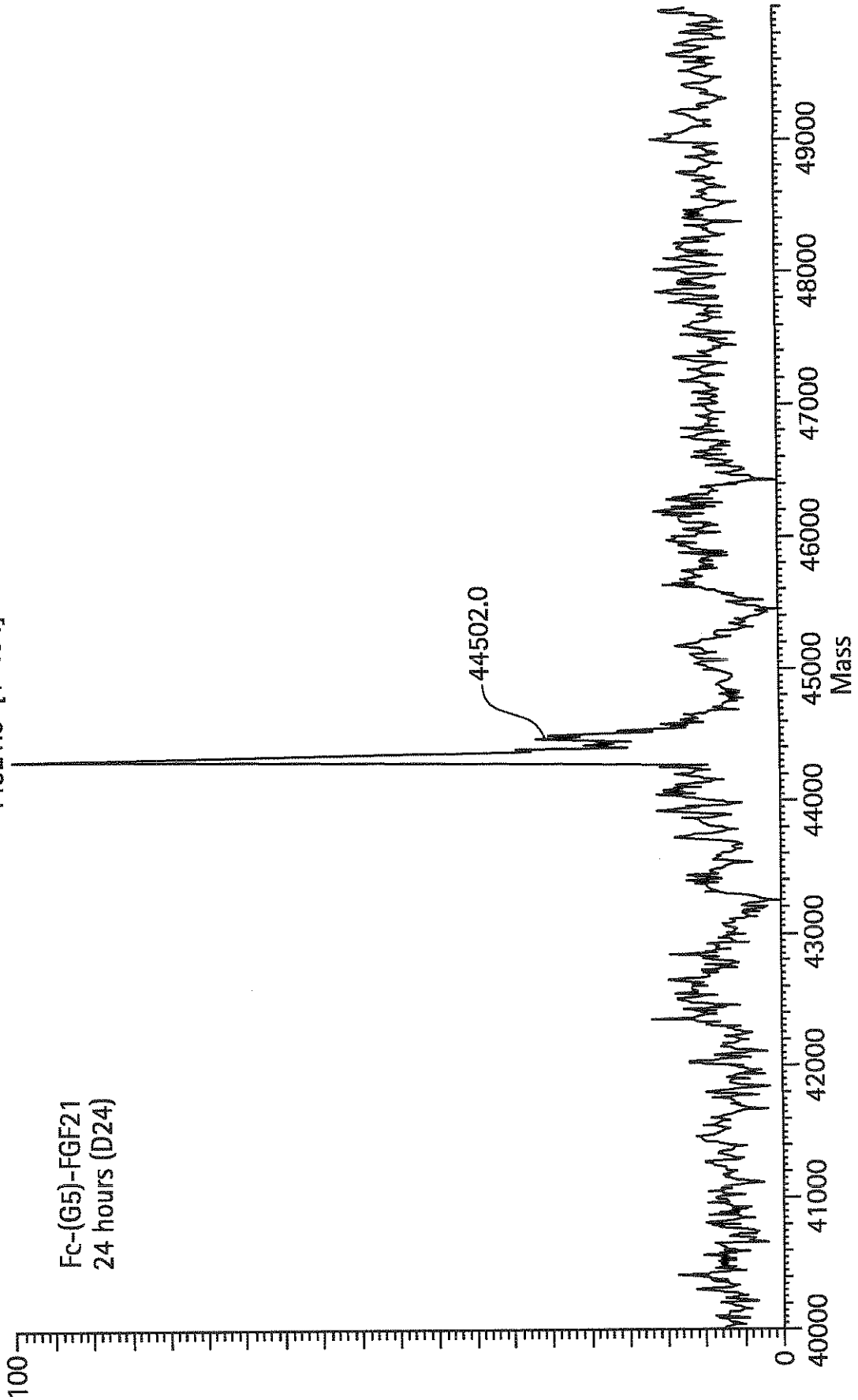

44321.0 [1-404]
44502.0
Fc-(G5)-FGF21
24 hours (D24)
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 6D

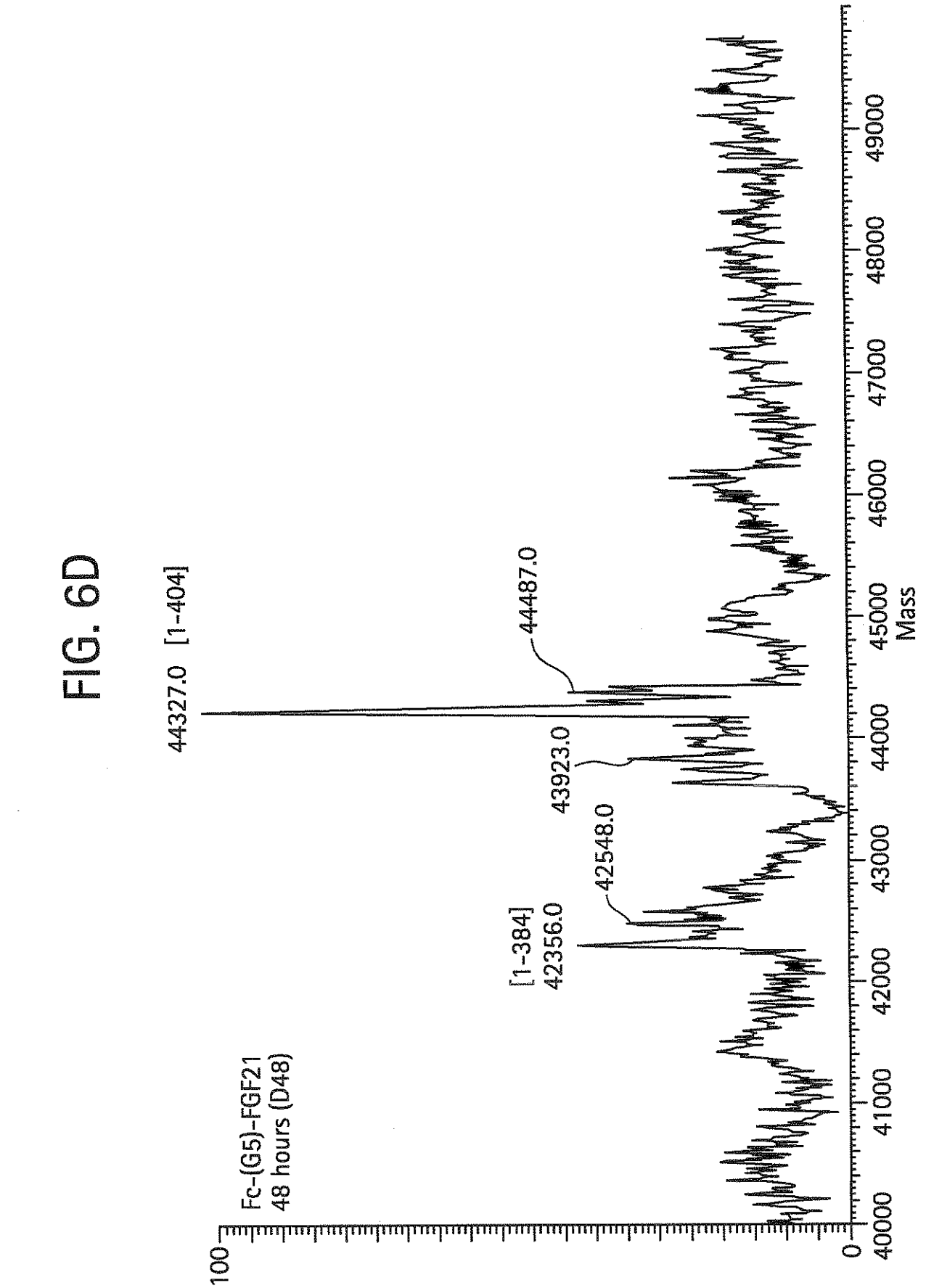

Fc-(G5)-FGF21
48 hours (D48)
100
0
44327.0 [1-404]
44487.0
43923.0
42548.0
[1-384]
42356.0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 7A

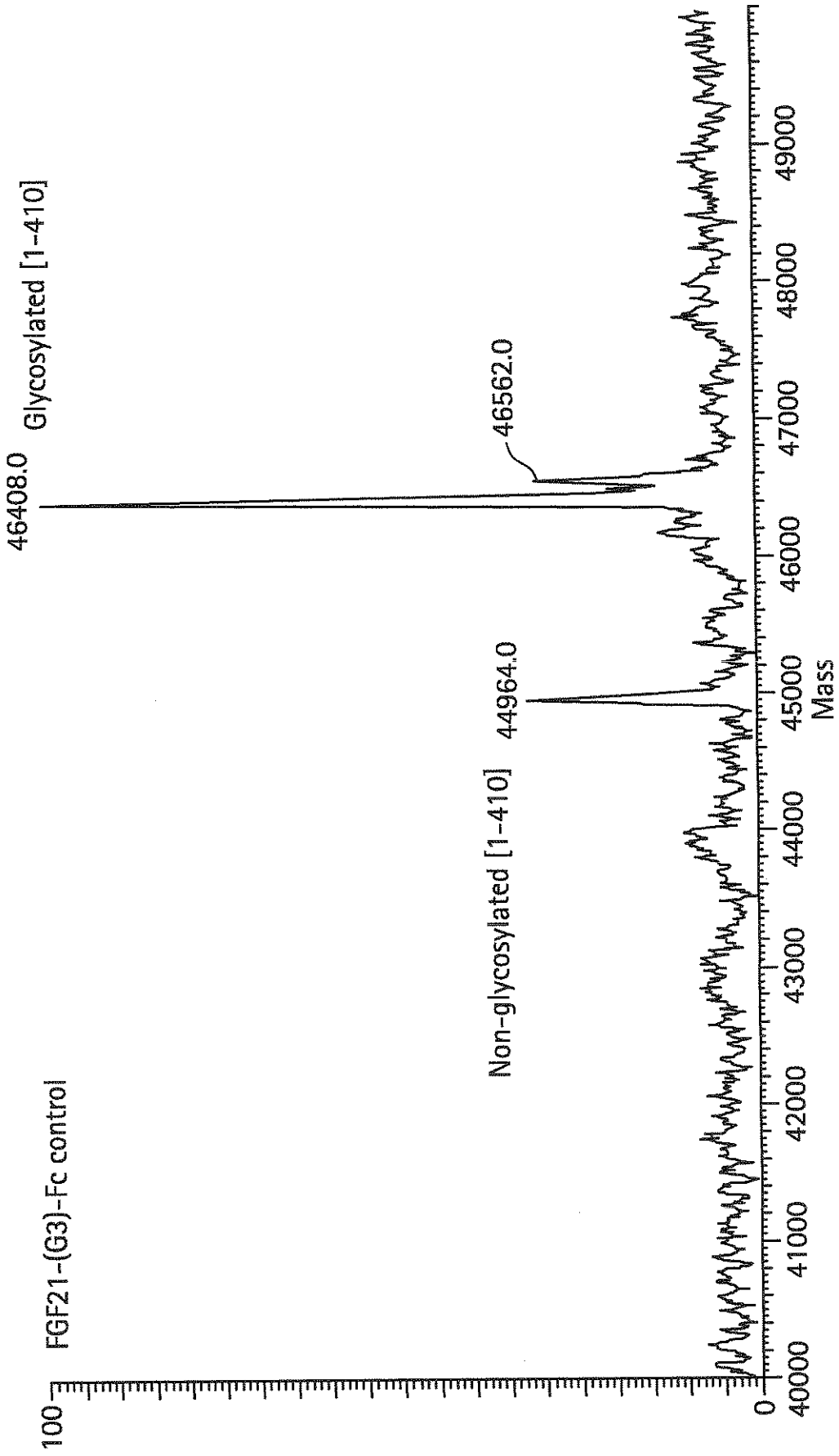

FGF21-(G3)-Fc control
100
0
Glycosylated [1-410]
46408.0
46562.0
Non-glycosylated [1-410]
44964.0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 7B

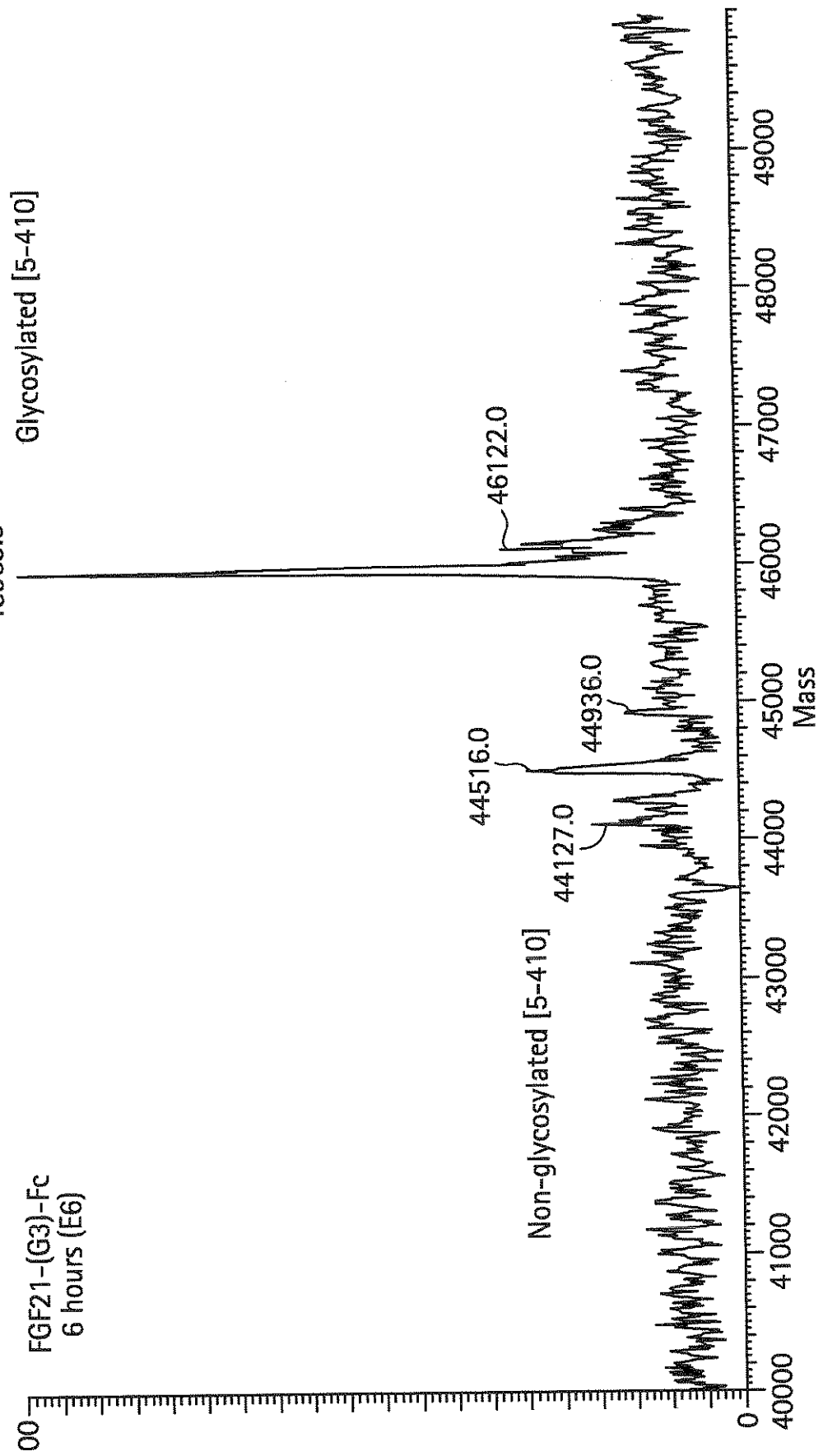

FGF21-(G3)-Fc
6 hours (E6)
100
0
Glycosylated [5-410]
Non-glycosylated [5-410]
45963.0
46122.0
44936.0
44516.0
44127.0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 7C

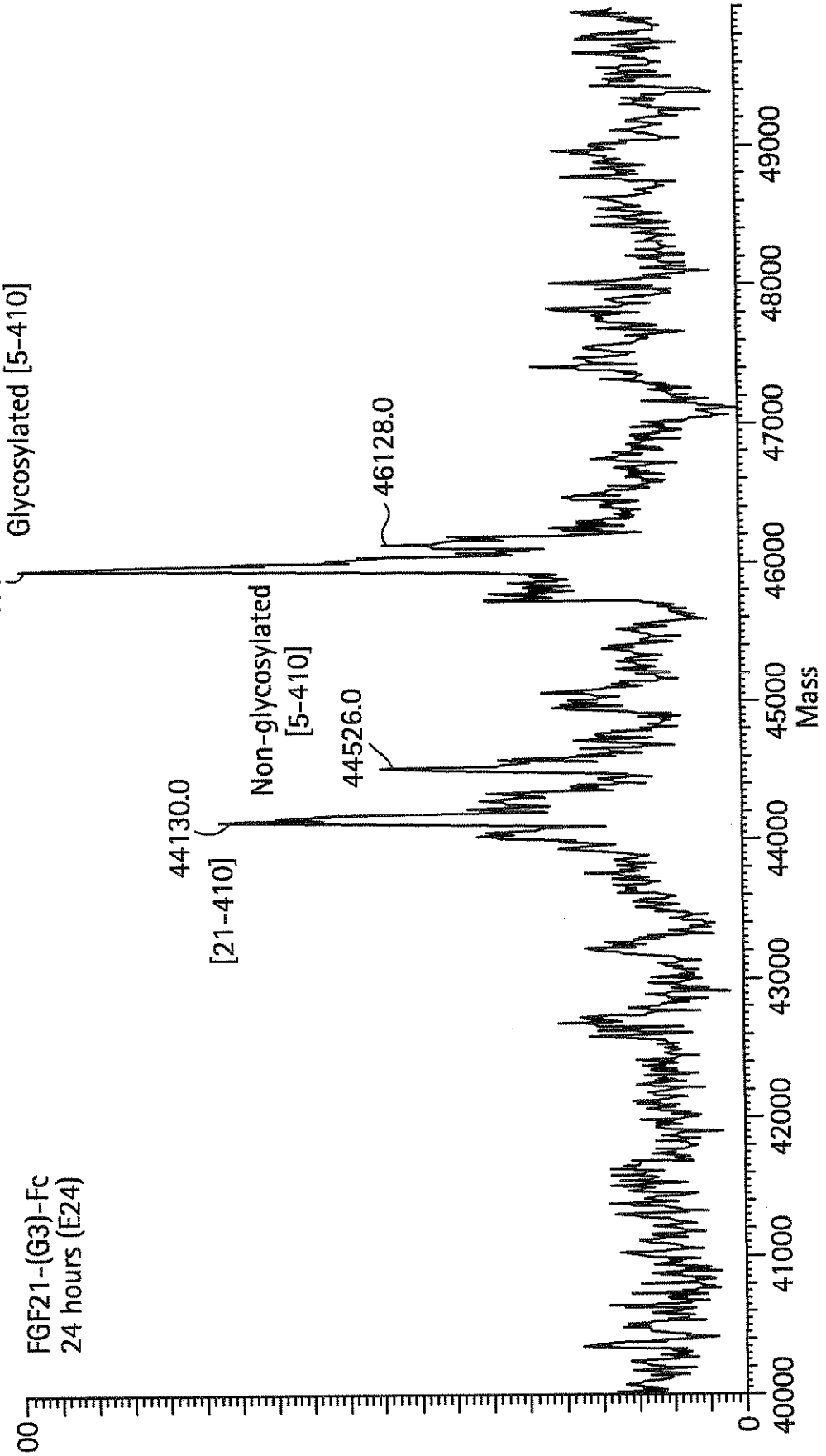

FGF21-(G3)-Fc
24 hours (E24)
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass
45963.0
Glycosylated [5-410]
46128.0
44130.0
[21-410]
Non-glycosylated
[5-410]
44526.0

FIG. 7D

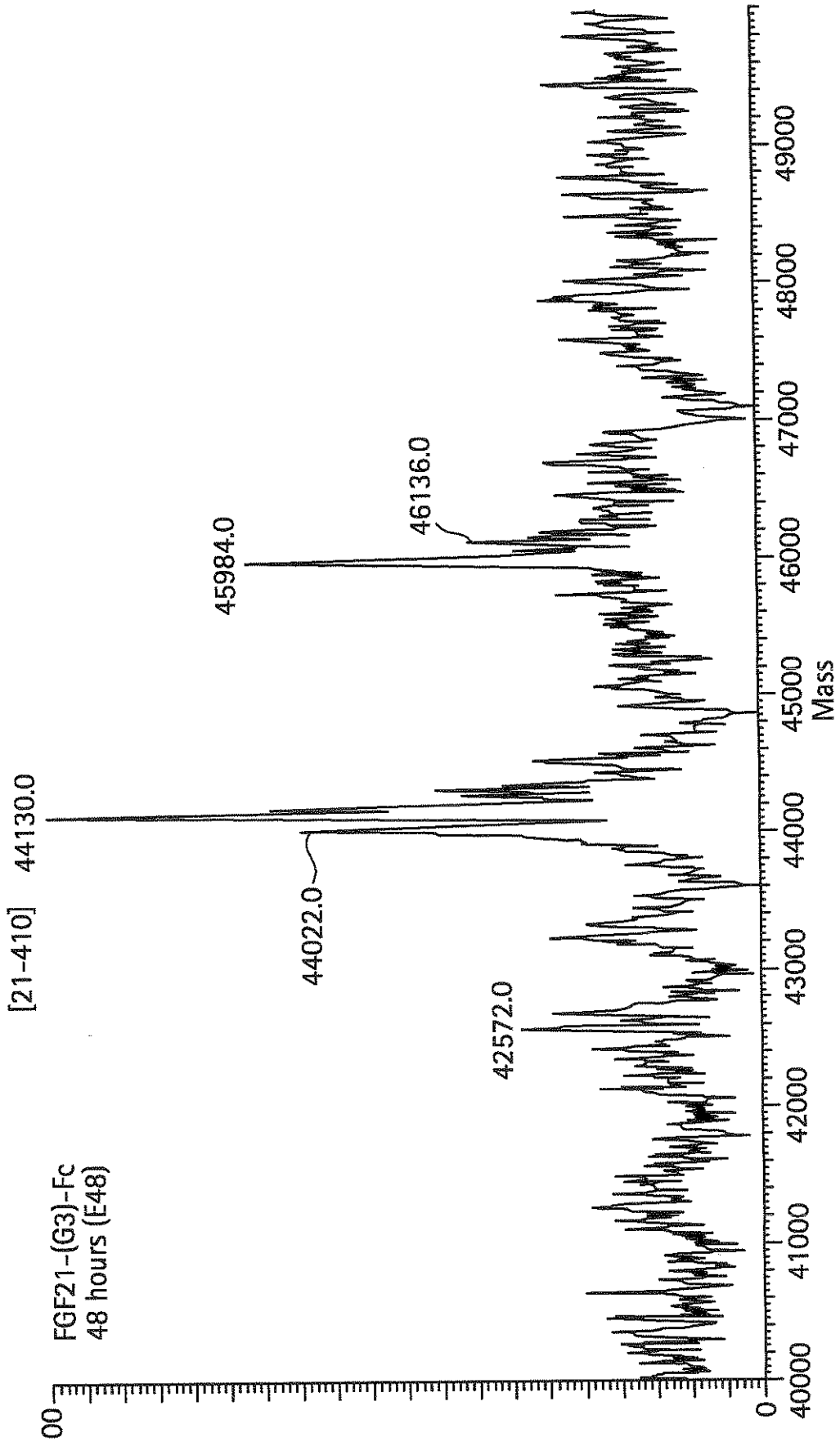

FGF21-(G3)-Fc
48 hours (E48)
[21-410] 44130.0
44022.0
42572.0
45984.0
46136.0
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 8A

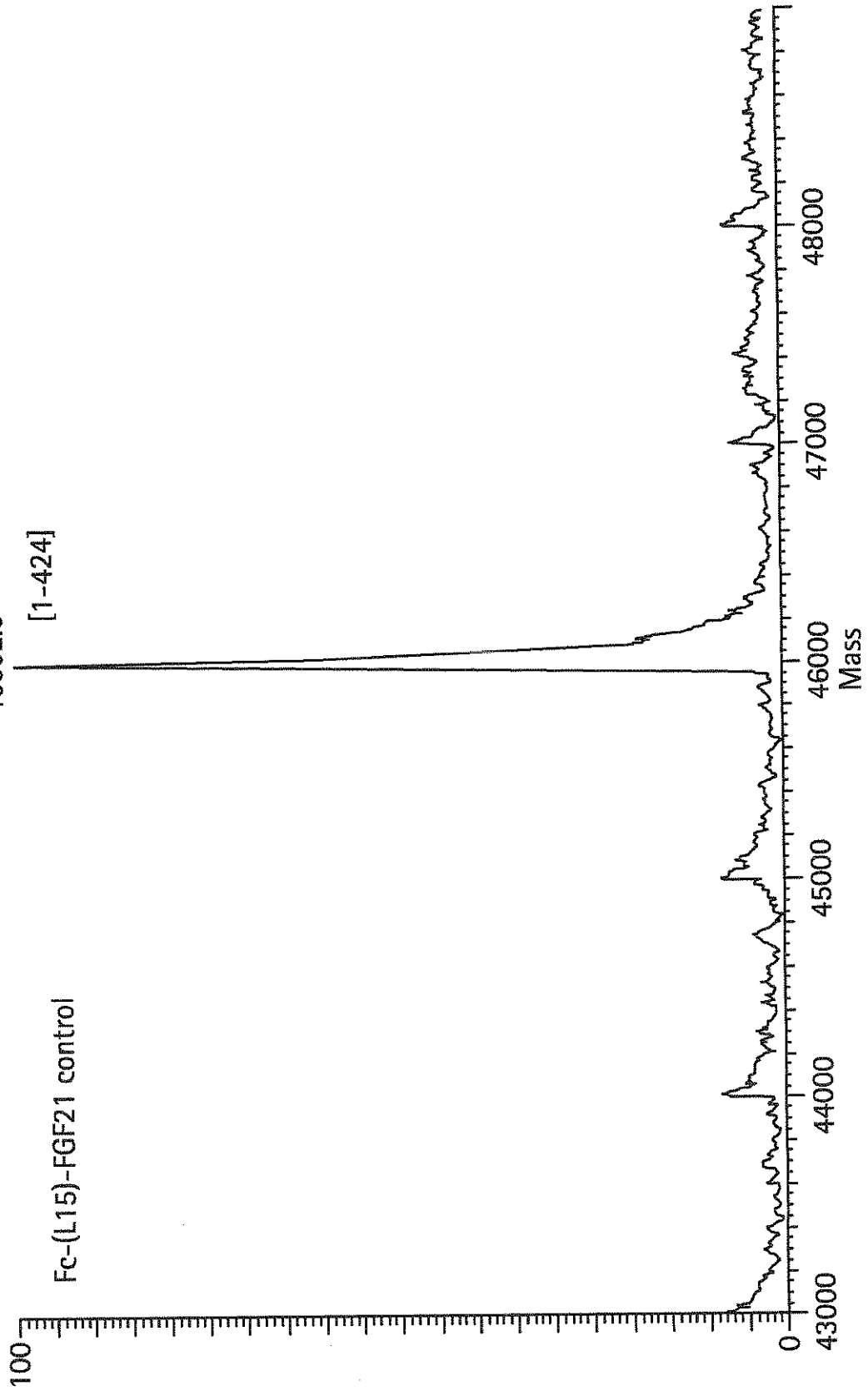

Fc-(L15)-FGF21 control
46002.0
[1-424]
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 8B

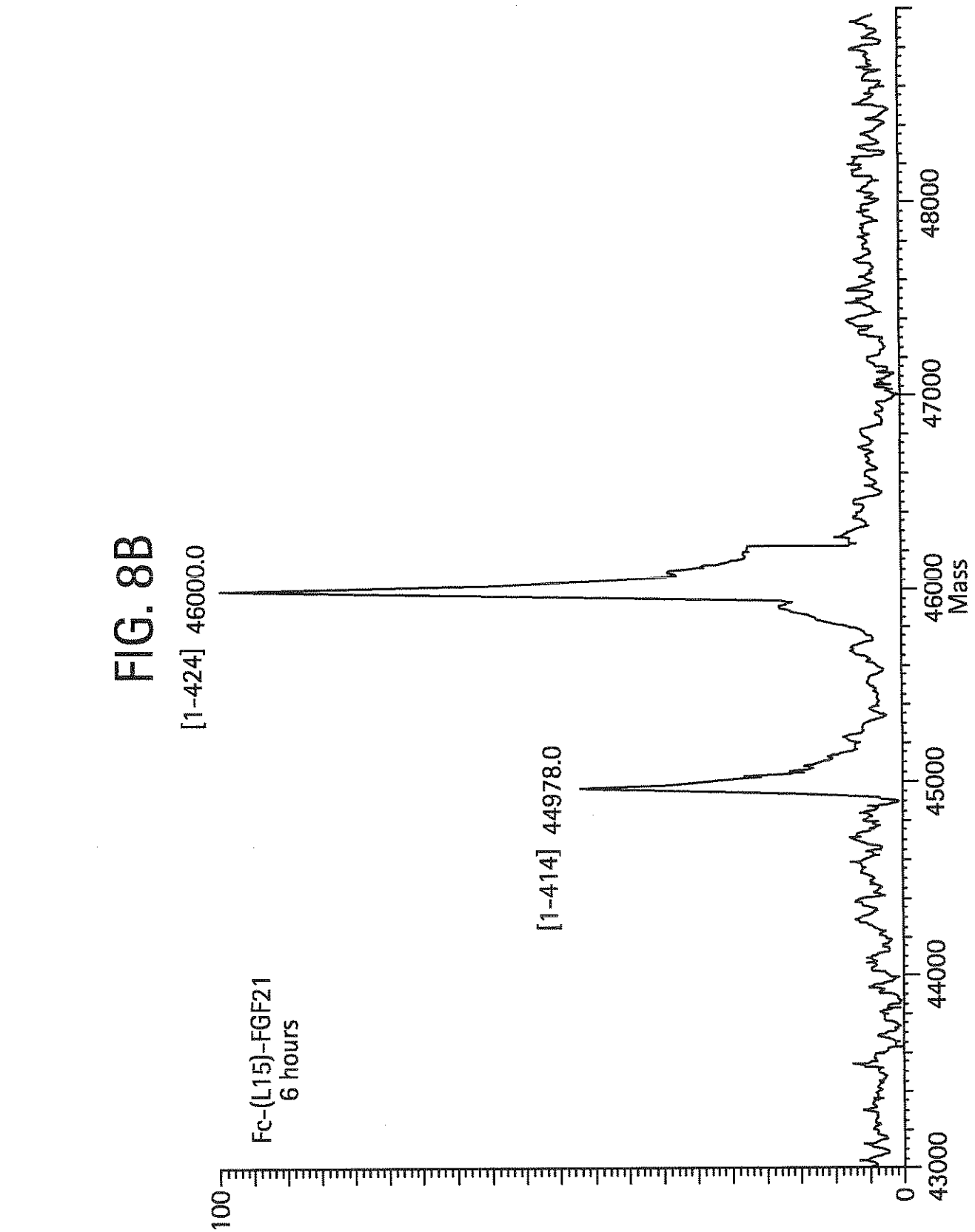

Fc-(L15)-FGF21
6 hours
[1-424] 46000.0
[1-414] 44978.0
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 8C

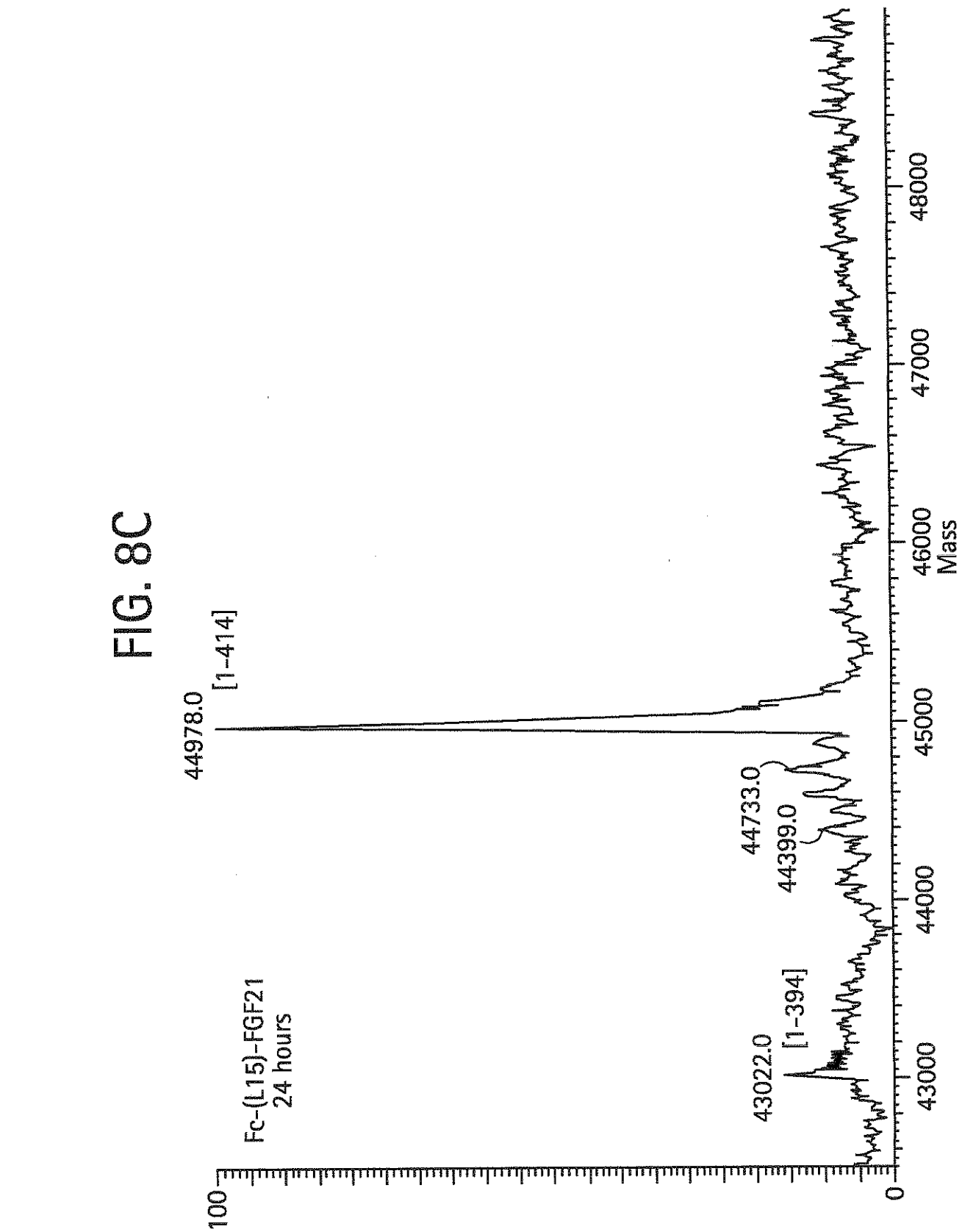

Fc-(L15)-FGF21
24 hours
100
0
44978.0
[1-414]
44733.0
44399.0
43022.0
[1-394]
43000
44000
45000
46000
47000
48000
Mass

FIG. 8D

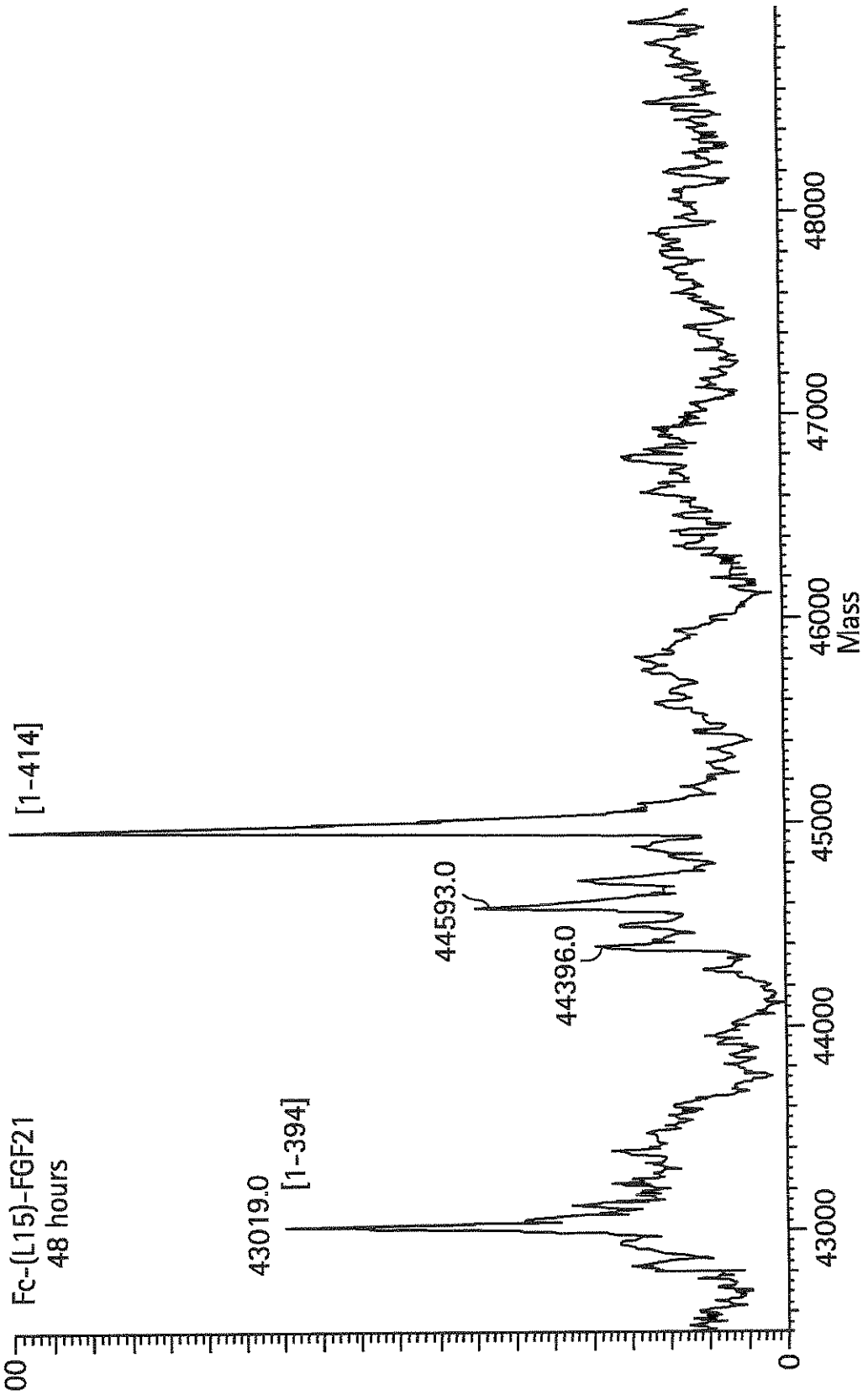

Fc-(L15)-FGF21
48 hours
100
0
43019.0
[1-394]
44976.0
[1-414]
44593.0
44396.0
43000
44000
45000
46000
47000
48000
Mass

FIG. 9A

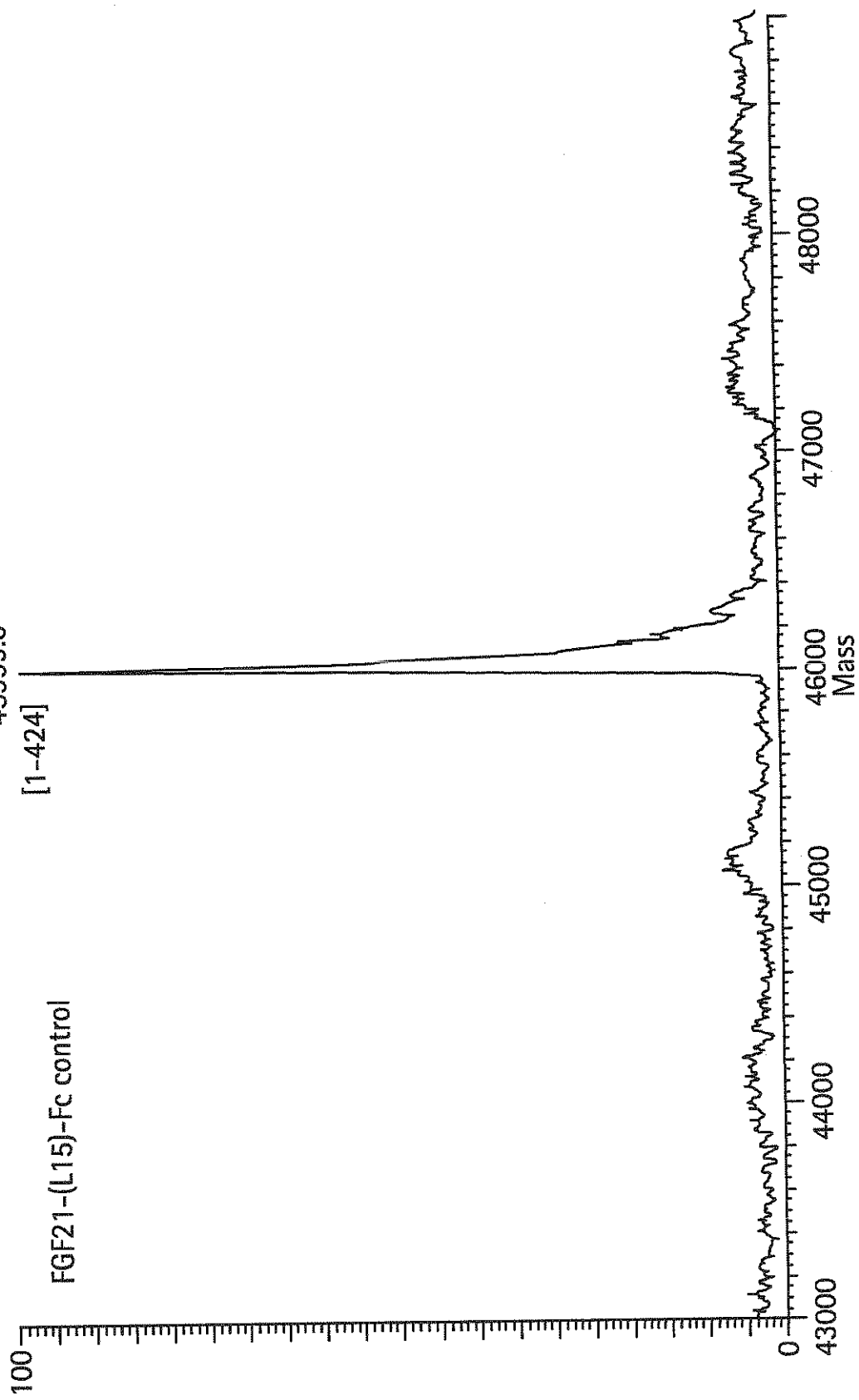

45999.0
[1-424]
FGF21-(L15)-Fc control
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 9B

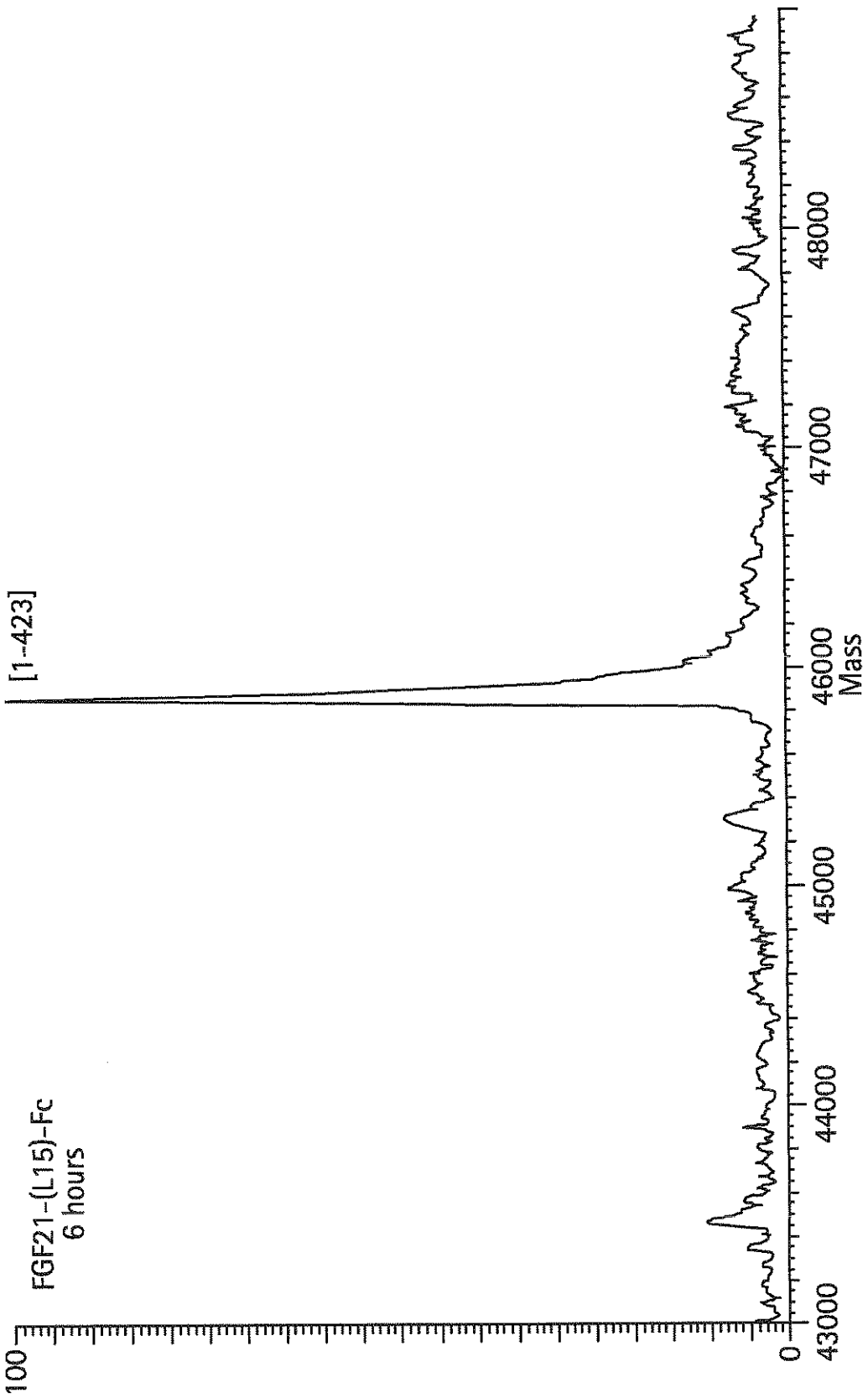

45870.0
[1-423]
FGF21-(L15)-Fc
6 hours
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 9C

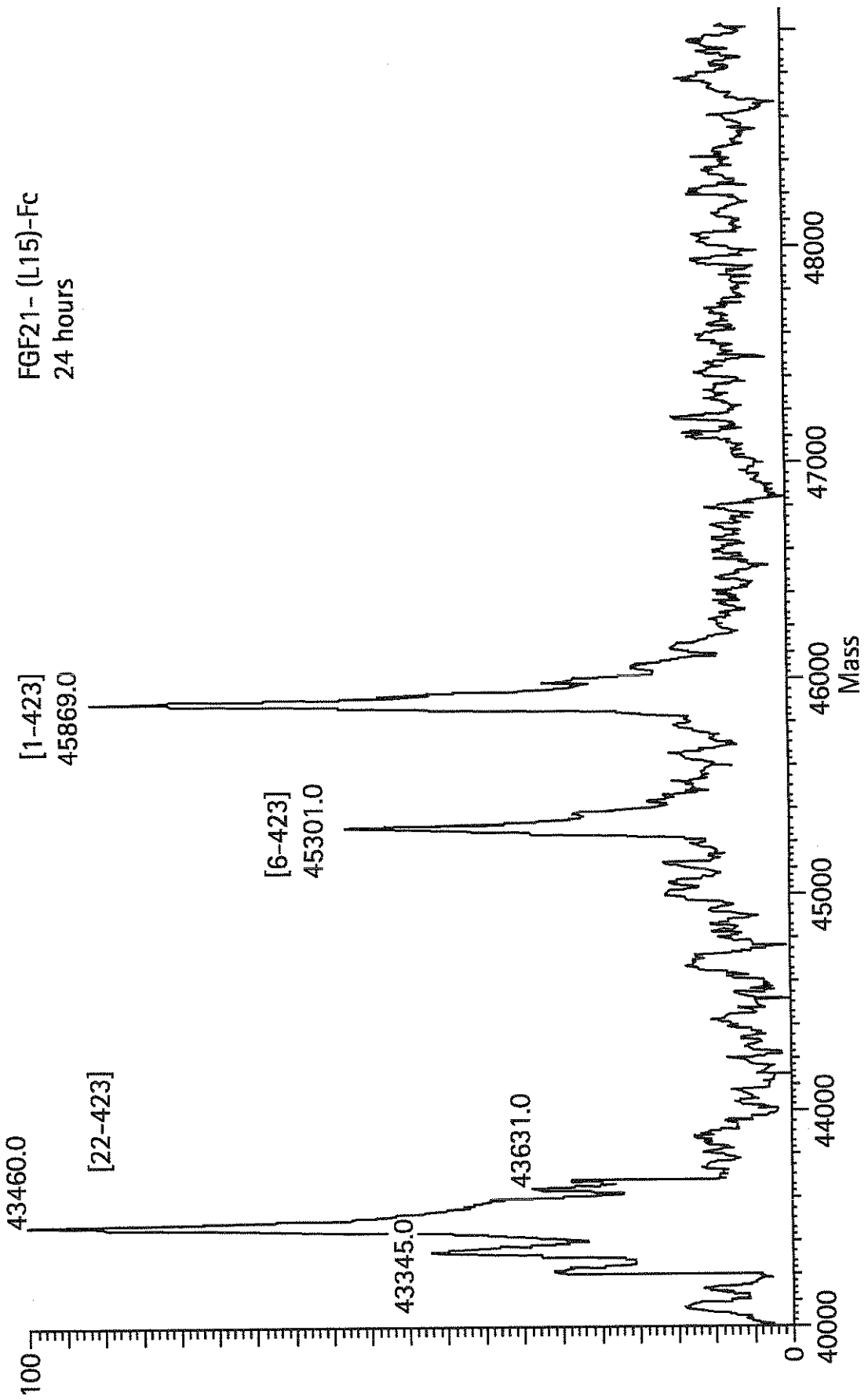

FGF21- (L15)-Fc
24 hours
[1-423]
45869.0
[6-423]
45301.0
[22-423]
43460.0
43631.0
43345.0
100
0
40000
44000
45000
46000
47000
48000
Mass

FIG. 9D

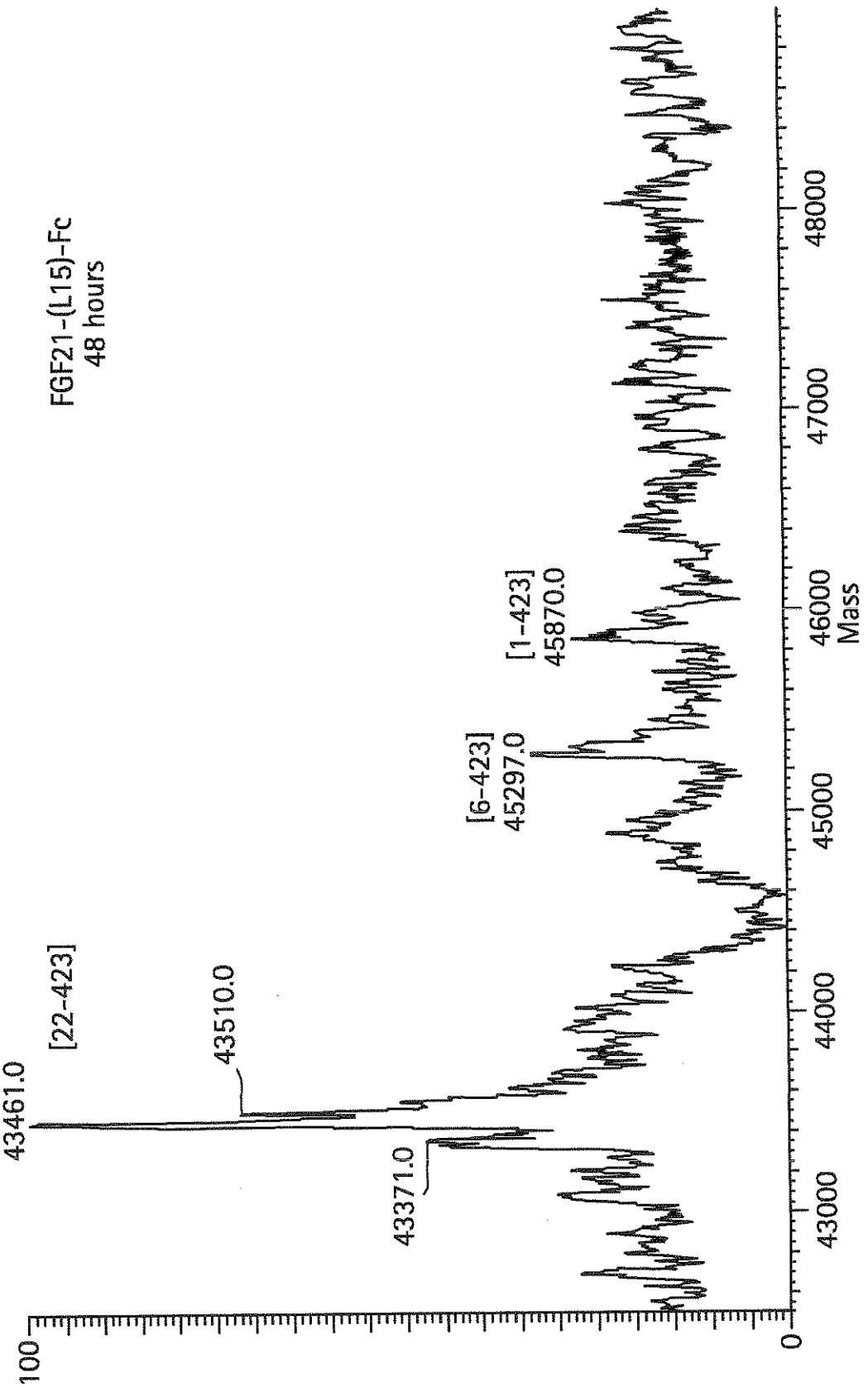

FGF21-(L15)-Fc
48 hours
43461.0
[22-423]
43510.0
43371.0
[6-423]
45297.0
[1-423]
45870.0
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 10A

Fc-(L15)-FGF21

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGSGG*
*GG*SHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVGPSQGRSPSYAS

6 hrs (35%)
24 hrs (85%) [1-414]
48 hrs (60%)

24 hrs (15%)
48 hrs (40%) [1-394]

FIG. 10B

Fc-(L15)-FGF21

24 hrs (35%)
48 hrs (20%) [6-423]

24 hrs (25%)
48 hrs (65%) [22-423]

6 hrs (100%) [1-423]

MHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPD
GALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQPPD
VGSSDPLSMVGPSQGRSPSYAS*GGGGGSGGGGSGGGGS*DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

FIG. 11

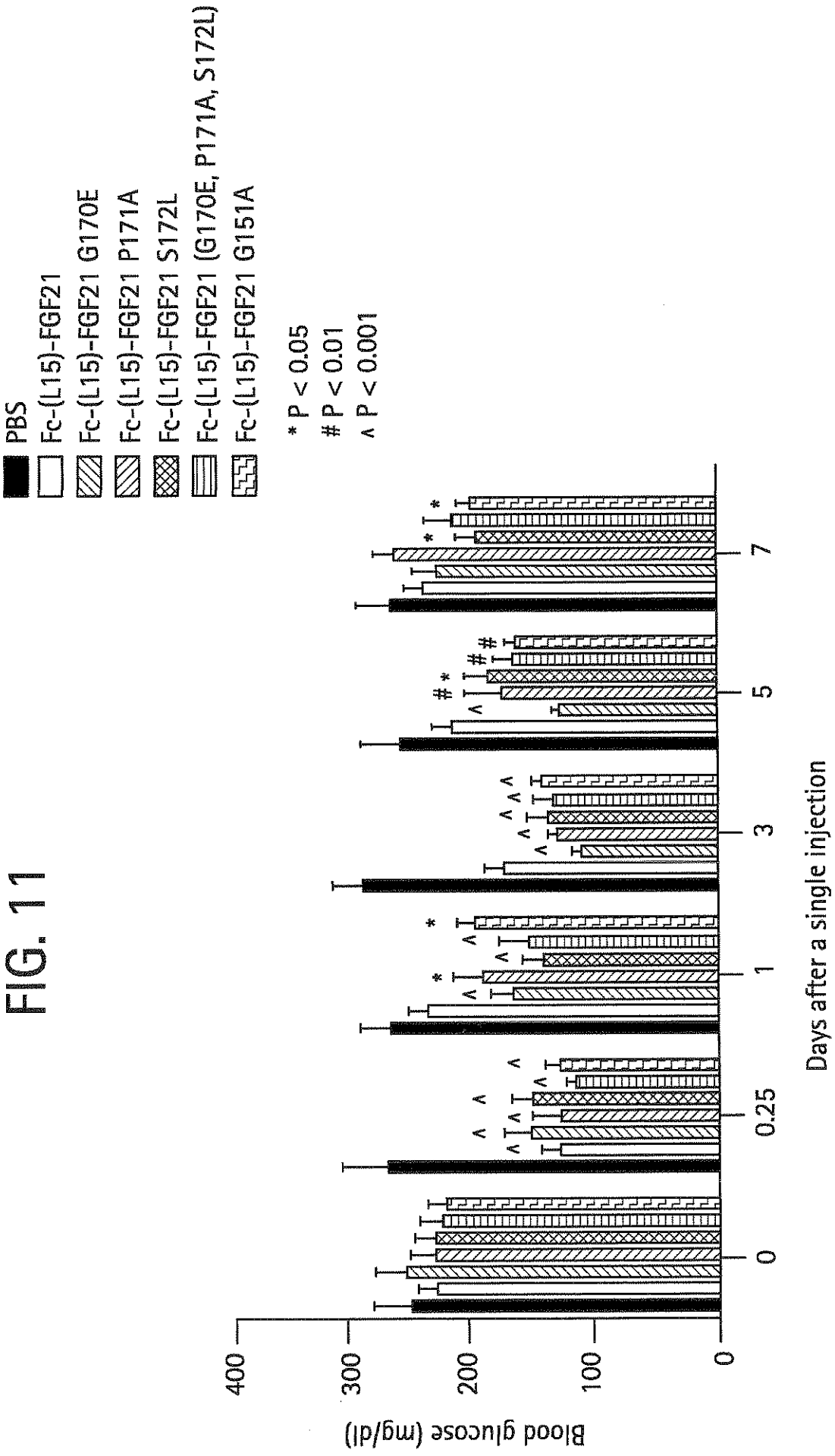

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171A
Fc-(L15)-FGF21 S172L
Fc-(L15)-FGF21 (G170E, P171A, S172L)
Fc-(L15)-FGF21 G151A
* P < 0.05
# P < 0.01
^ P < 0.001
Blood glucose (mg/dl)
400
300
200
100
0
0
0.25
1
3
5
7
Days after a single injection

FIG. 12

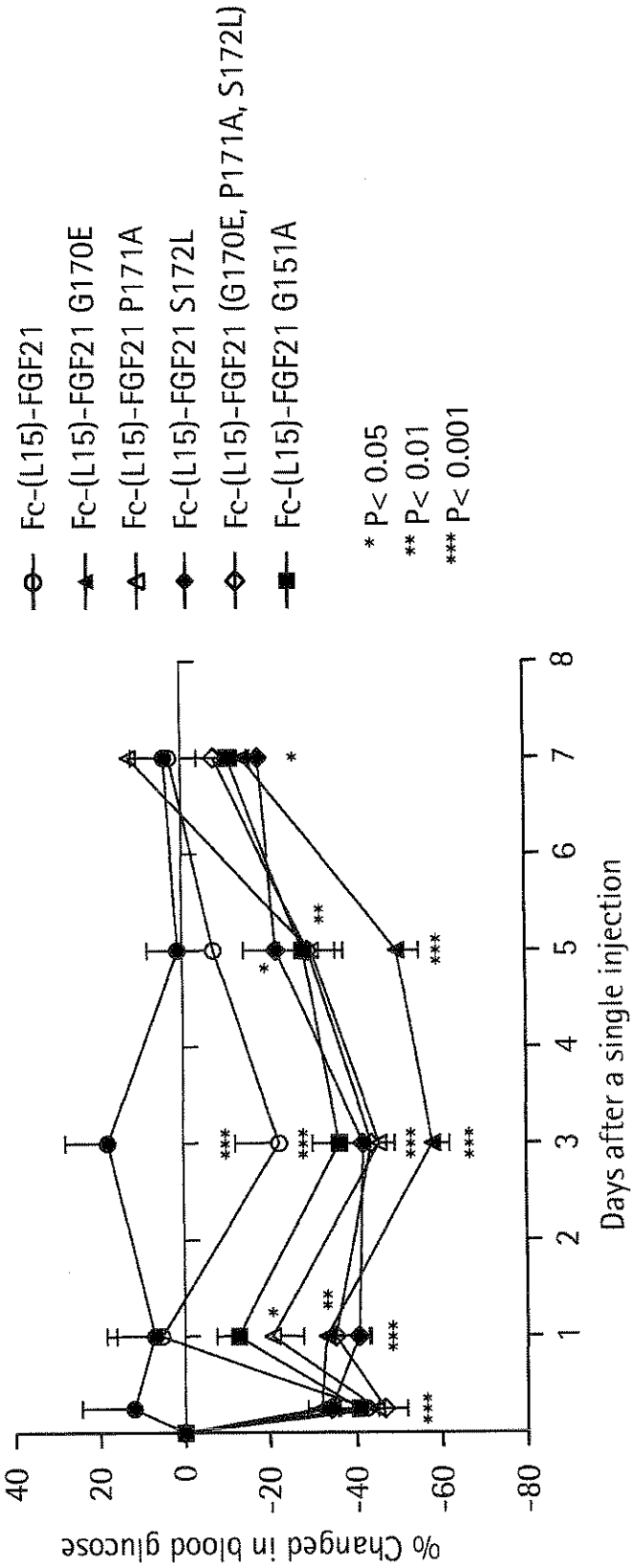

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171A
Fc-(L15)-FGF21 S172L
Fc-(L15)-FGF21 (G170E, P171A, S172L)
Fc-(L15)-FGF21 G151A
* P< 0.05
** P< 0.01
*** P< 0.001
% Changed in blood glucose
40
20
0
-20
-40
-60
-80
1
2
3
4
5
6
7
8
Days after a single injection

FIG. 13

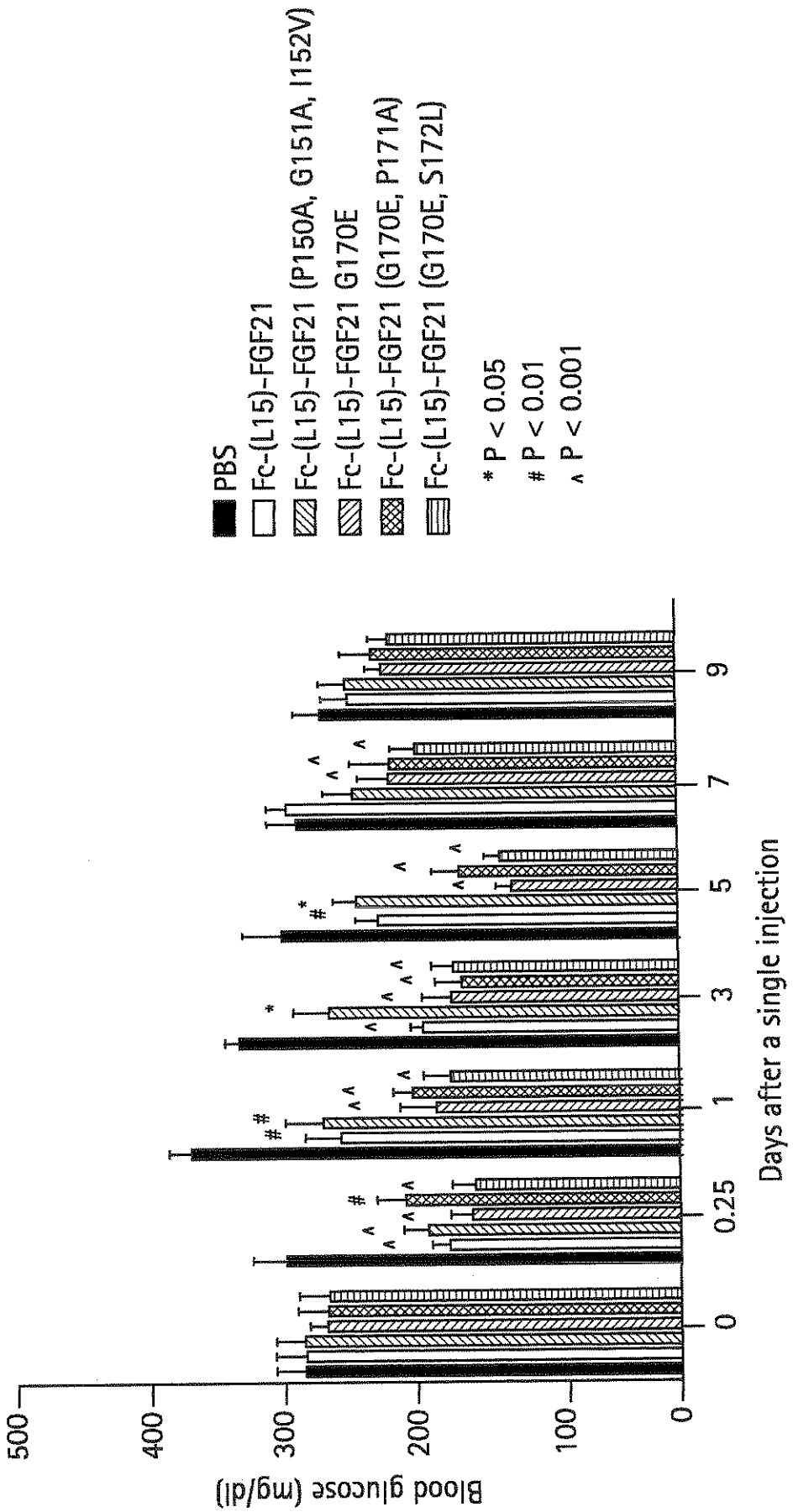

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (P150A, G151A, I152V)
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 (G170E, P171A)
Fc-(L15)-FGF21 (G170E, S172L)
* P < 0.05
# P < 0.01
^ P < 0.001
Blood glucose (mg/dl)
0
100
200
300
400
500
Days after a single injection
0
0.25
1
3
5
7
9

FIG. 14

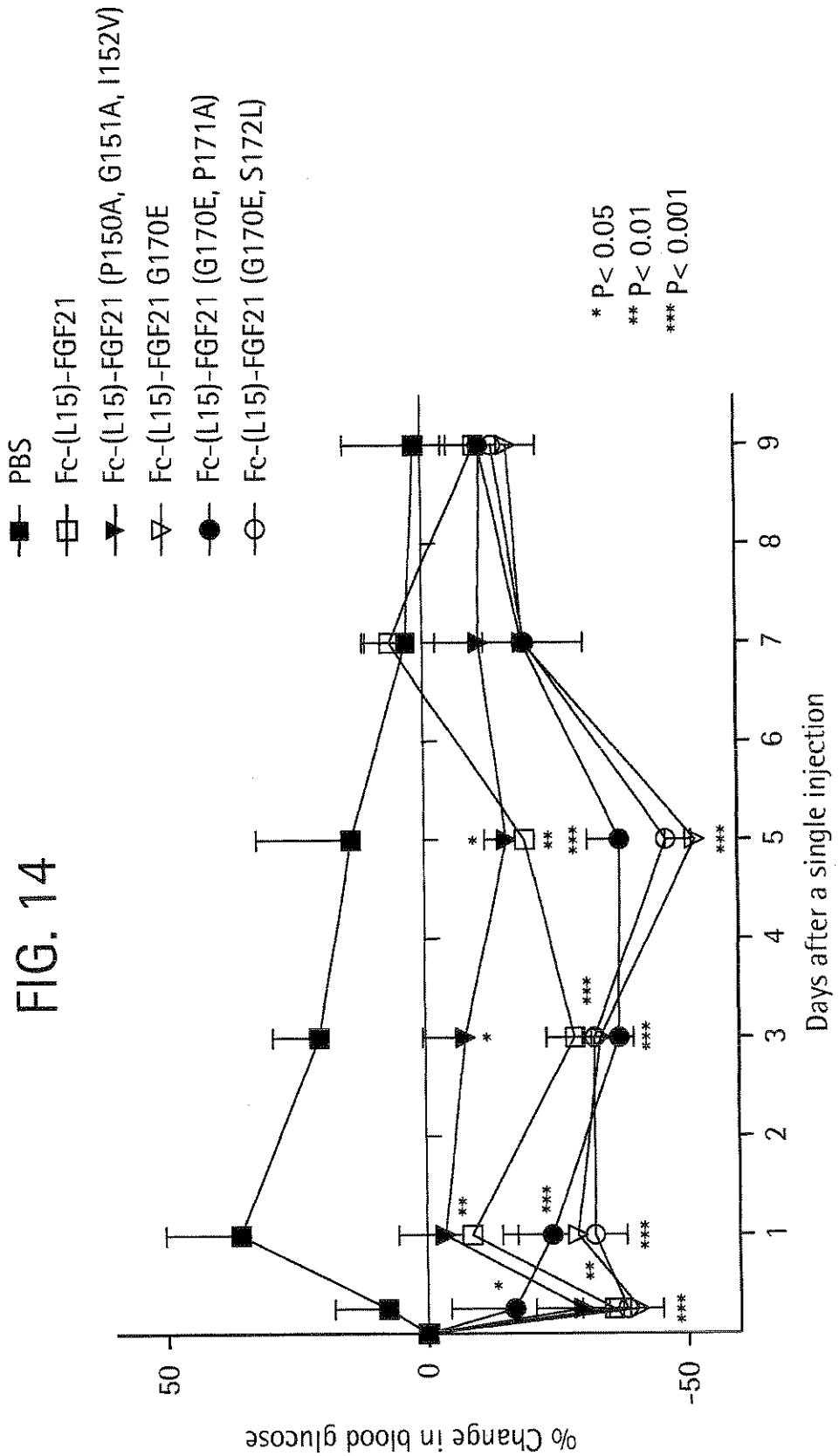

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (P150A, G151A, I152V)
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 (G170E, P171A)
Fc-(L15)-FGF21 (G170E, S172L)
* P< 0.05
** P< 0.01
*** P< 0.001
% Change in blood glucose
50
0
-50
1
2
3
4
5
6
7
8
9
Days after a single injection

FIG. 15

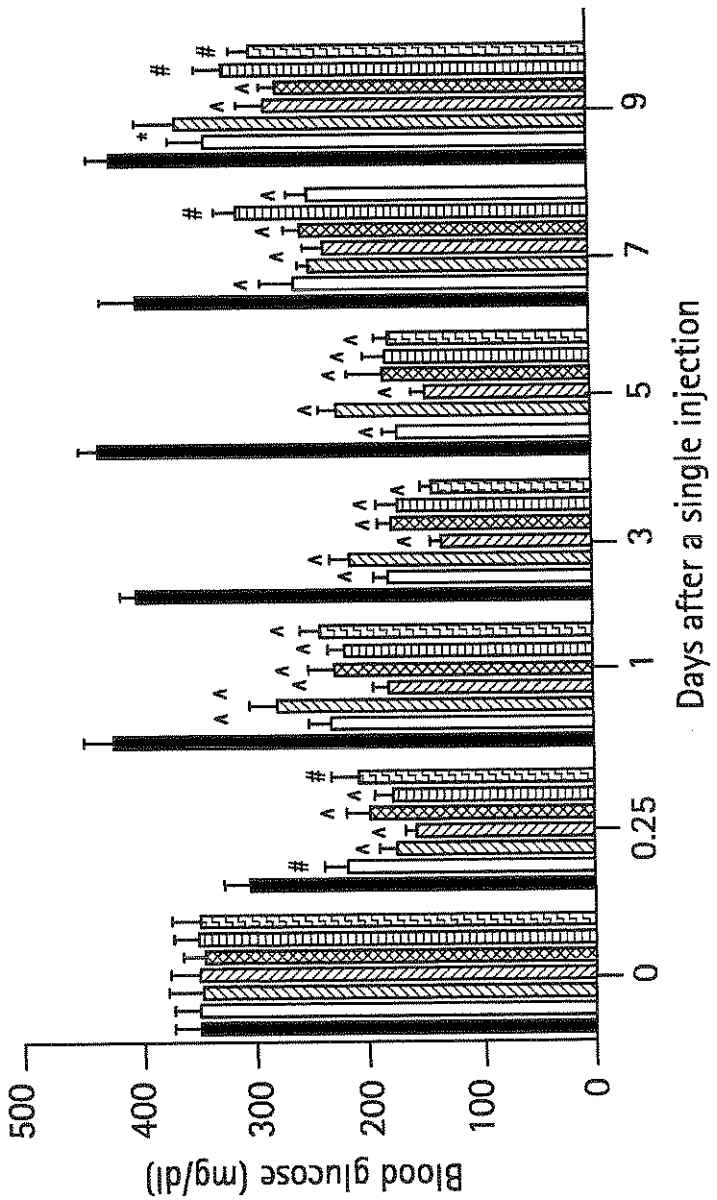

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 G170A
Fc-(L15)-FGF21 G170C
Fc-(L15)-FGF21 G170D
Fc-(L15)-FGF21 G170N
Fc-(L15)-FGF21 G170S
* P < 0.05
# P < 0.01
^ P < 0.001
Treatment vs. PBS
Blood glucose (mg/dl)
0
100
200
300
400
500
0
0.25
1
3
5
7
9
Days after a single injection

FIG. 16

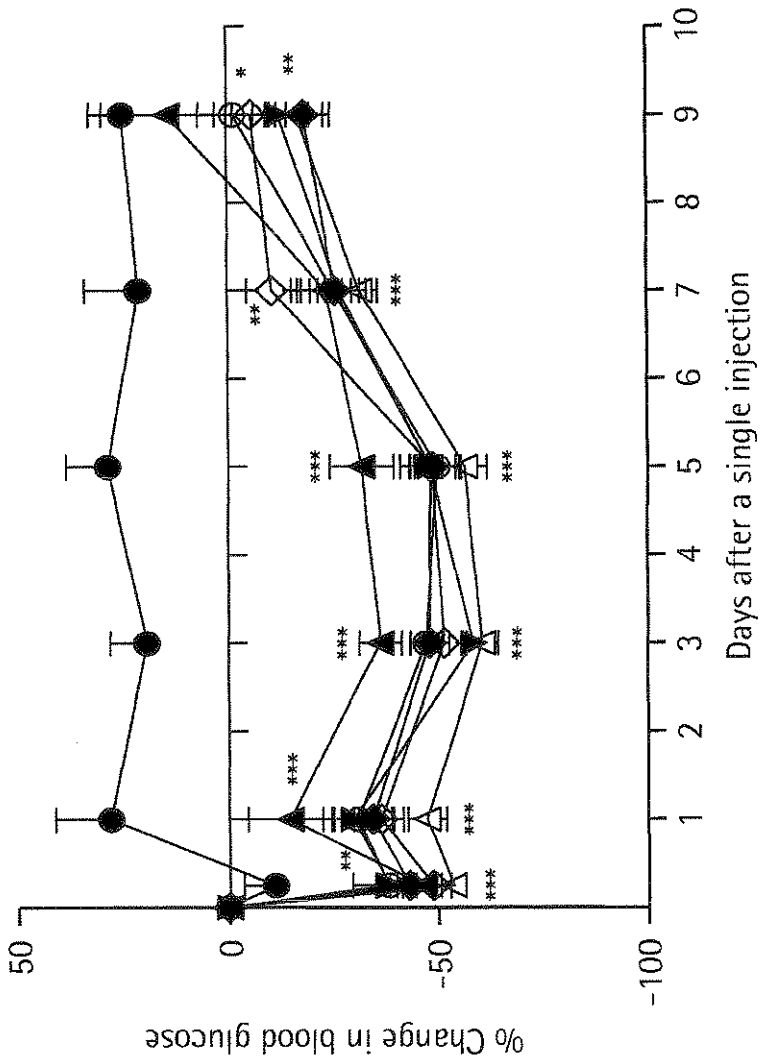

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 G170A
Fc-(L15)-FGF21 G170C
Fc-(L15)-FGF21 G170D
Fc-(L15)-FGF21 G170N
Fc-(L15)-FGF21 G170S
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. PBS
% Change in blood glucose
50
0
-50
-100
1
2
3
4
5
6
7
8
9
10
Days after a single injection

FIG. 17

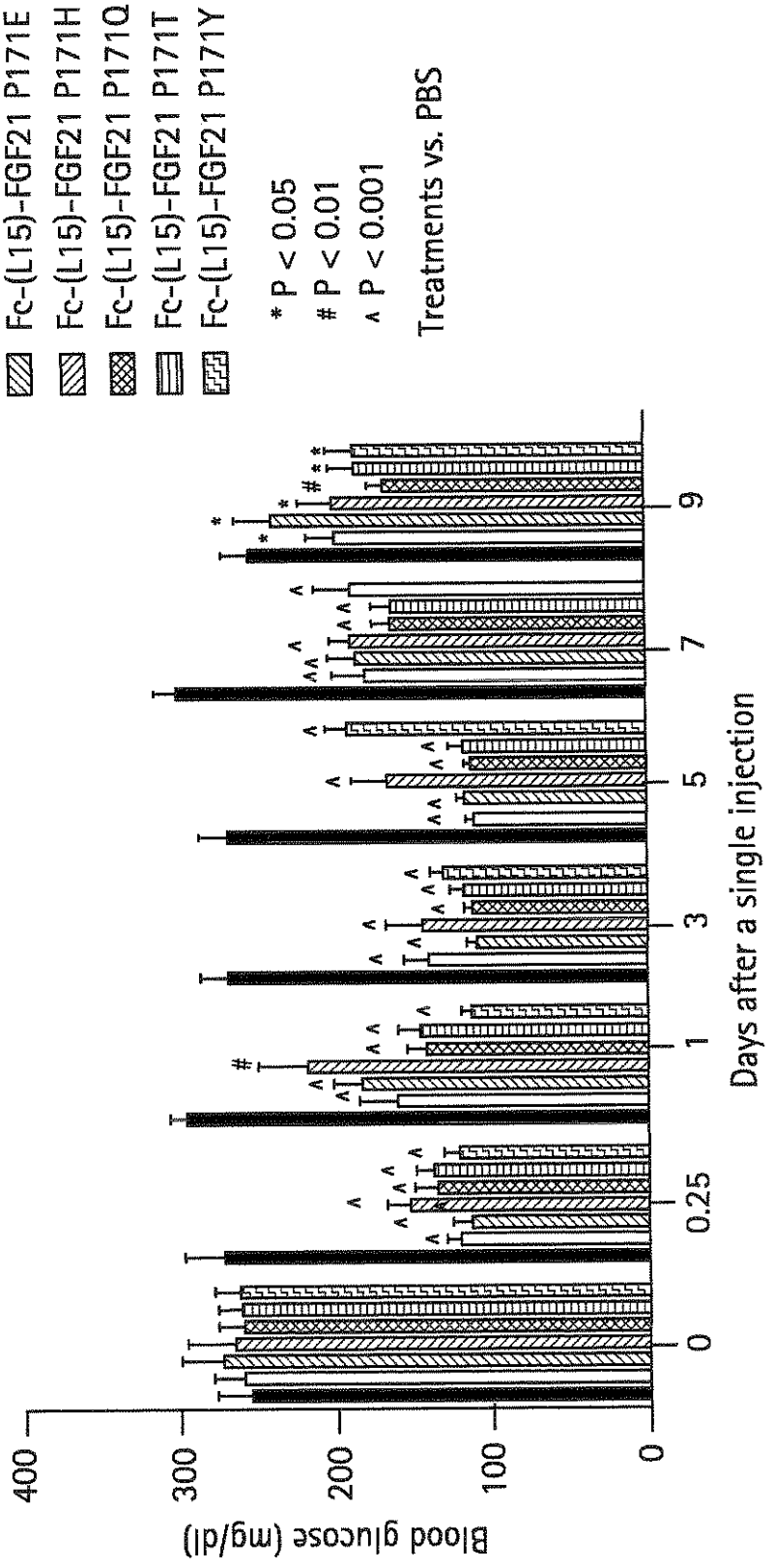

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 P171H
Fc-(L15)-FGF21 P171Q
Fc-(L15)-FGF21 P171T
Fc-(L15)-FGF21 P171Y
* P < 0.05
# P < 0.01
^ P < 0.001
Treatments vs. PBS
Blood glucose (mg/dl)
0
100
200
300
400
0
0.25
1
3
5
7
9
Days after a single injection

FIG. 18

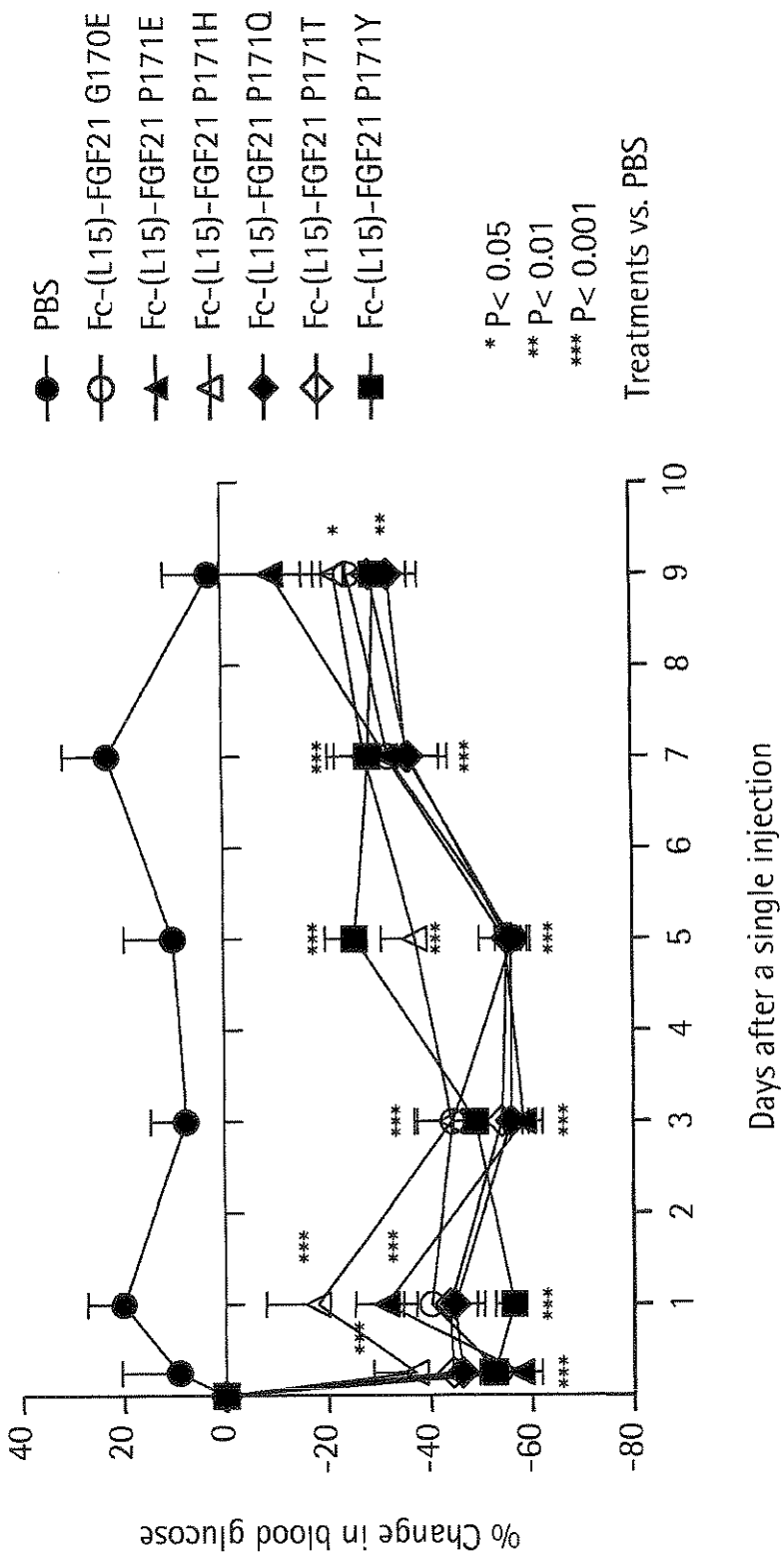

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 P171H
Fc-(L15)-FGF21 P171Q
Fc-(L15)-FGF21 P171T
Fc-(L15)-FGF21 P171Y
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. PBS
% Change in blood glucose
Days after a single injection

FIG. 19A

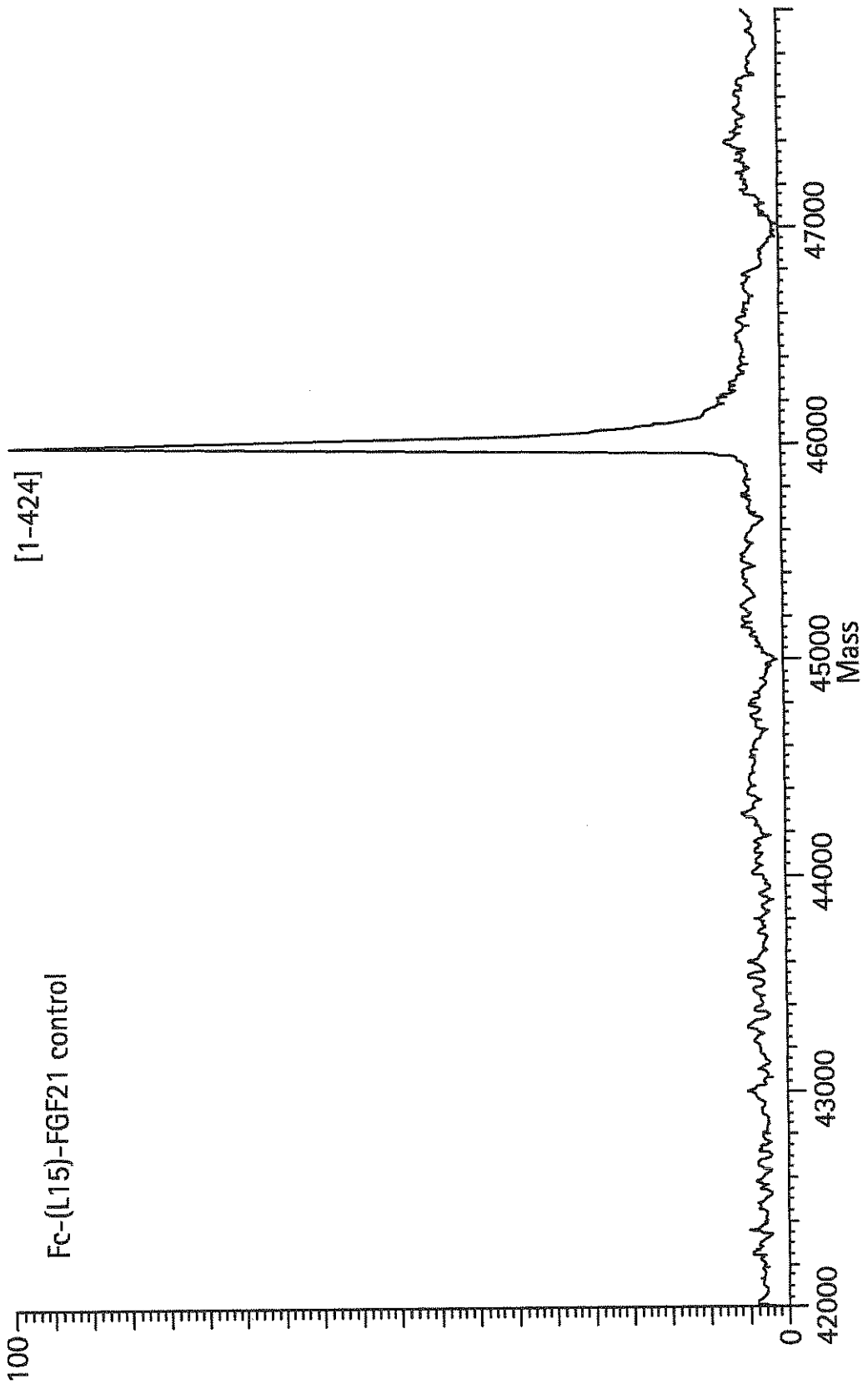

45994.0
[1-424]
Fc-(L15)-FGF21 control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 19B

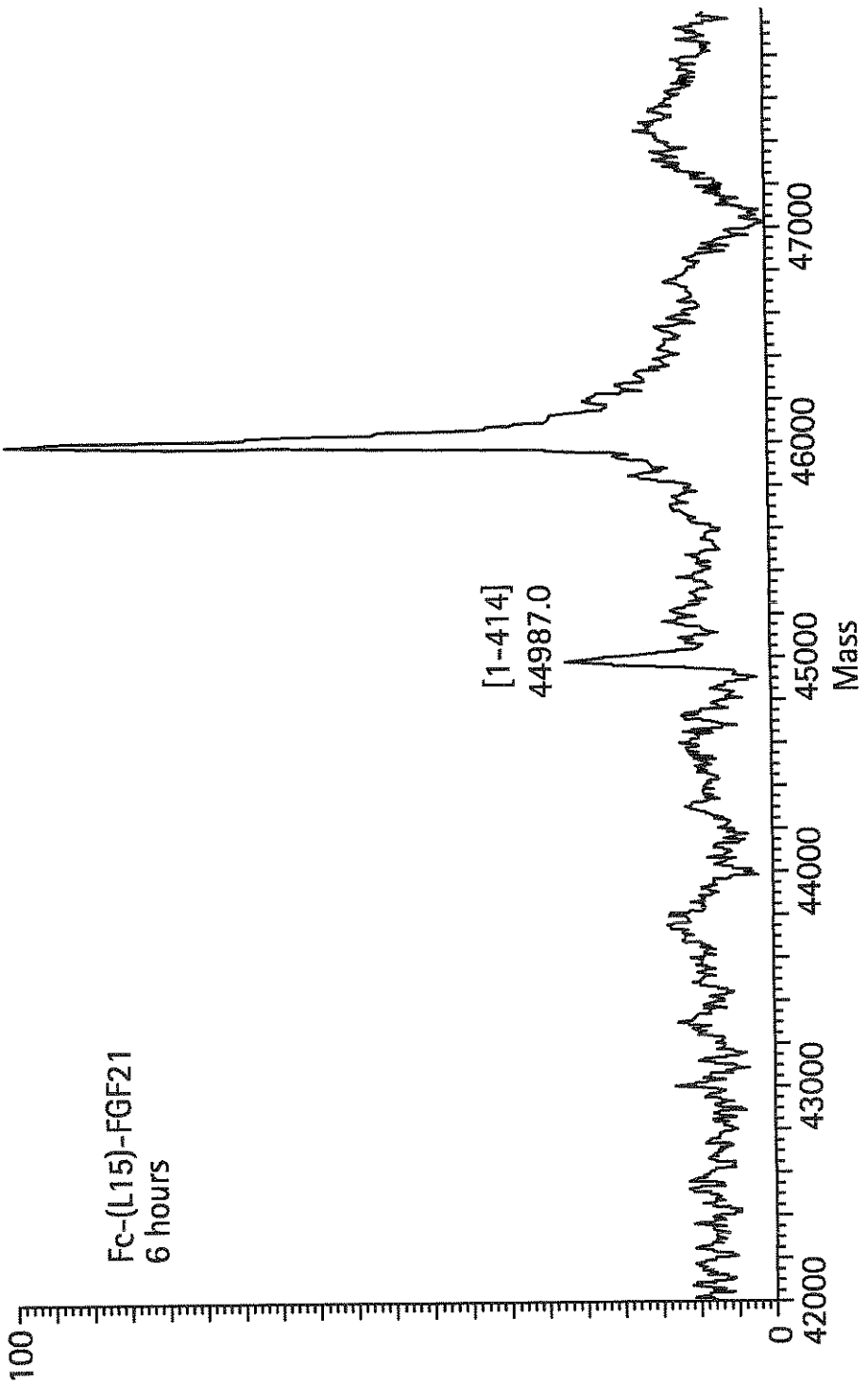

[1-424]
46001.0
[1-414]
44987.0
Fc-(L15)-FGF21
6 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 19C

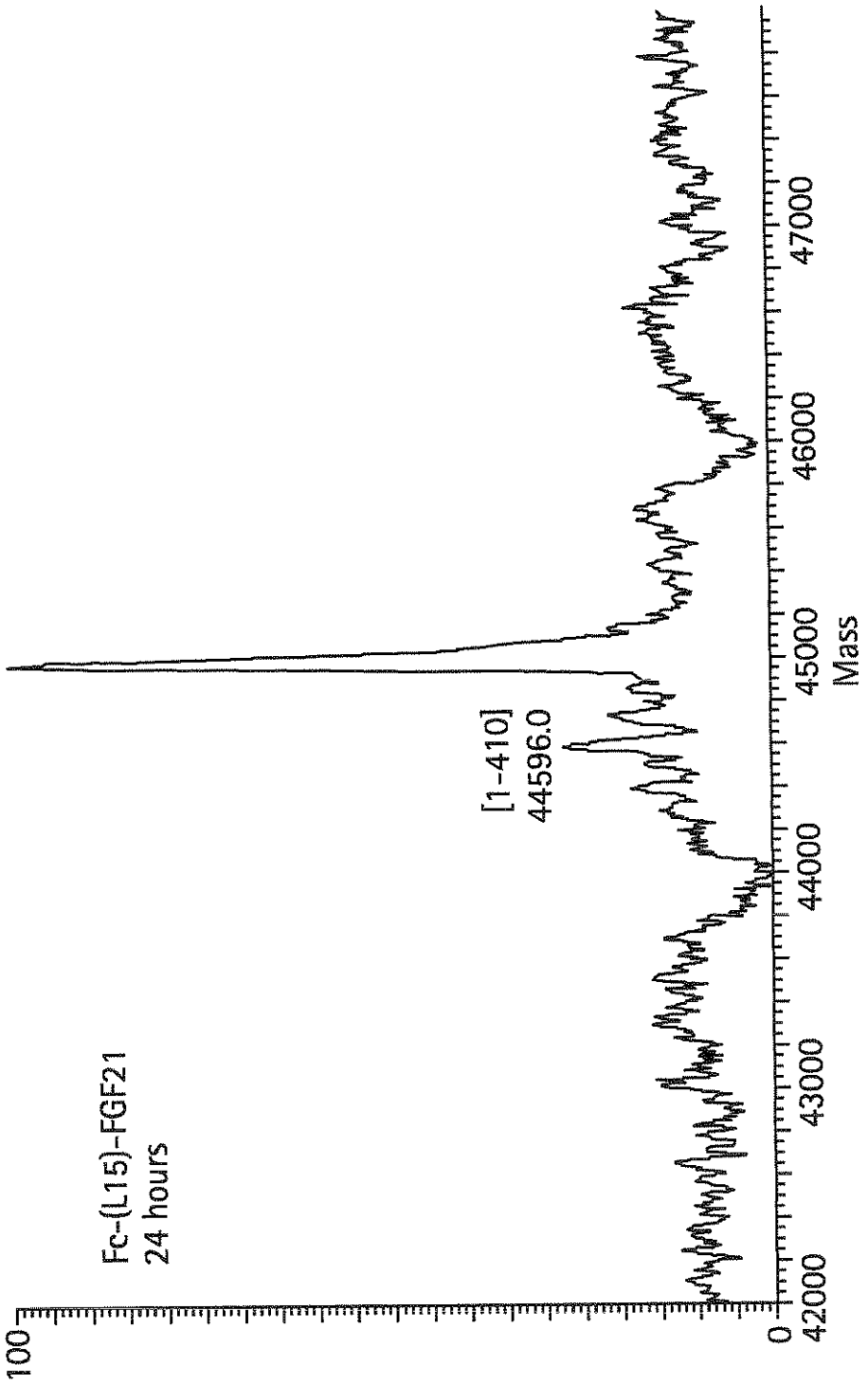

[1-414]
44979.0
[1-410]
44596.0
Fc-(L15)-FGF21
24 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 19D

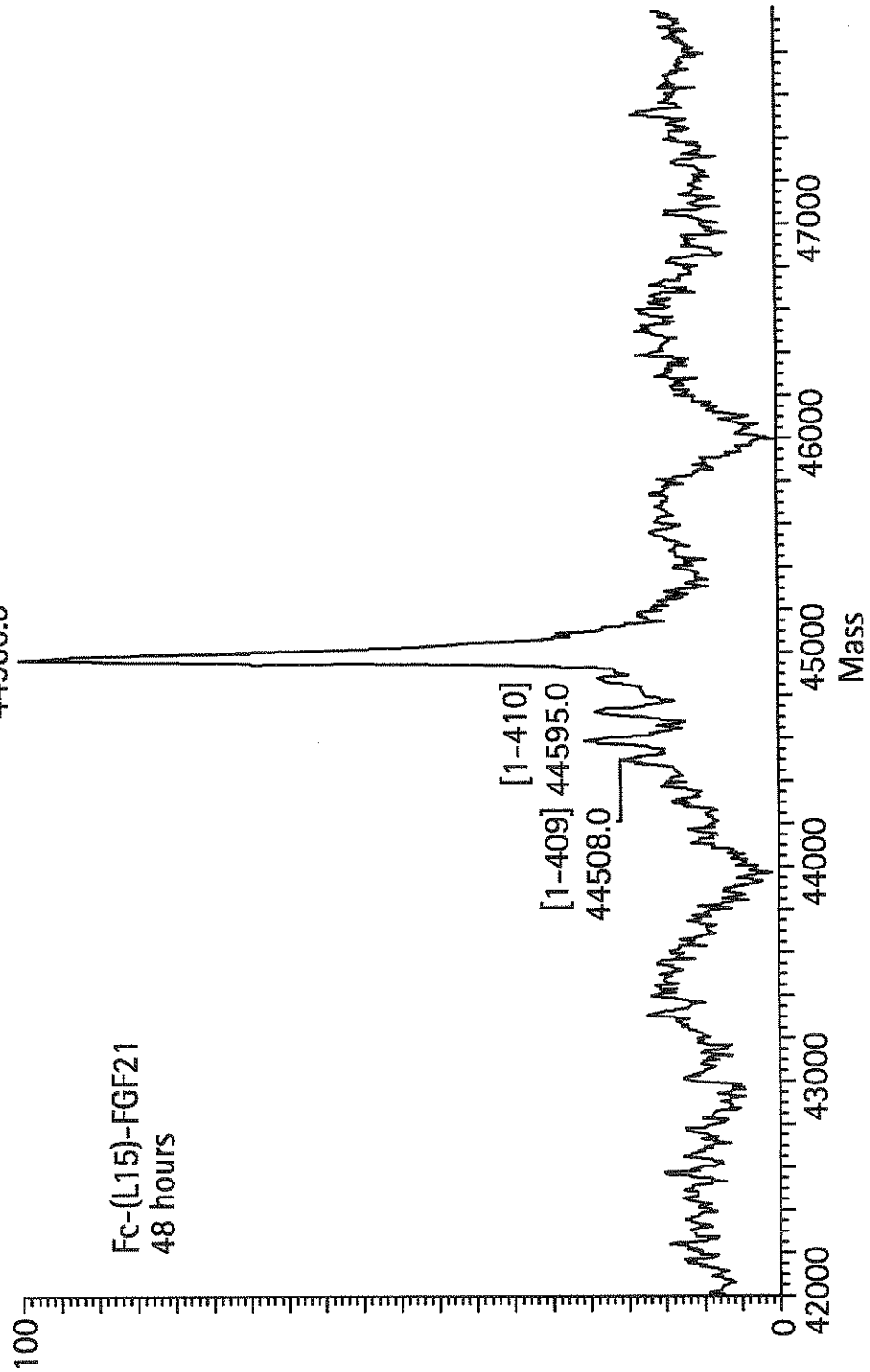

Fc-(L15)-FGF21
48 hours
[1-414]
44980.0
[1-410]
44595.0
[1-409]
44508.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20A

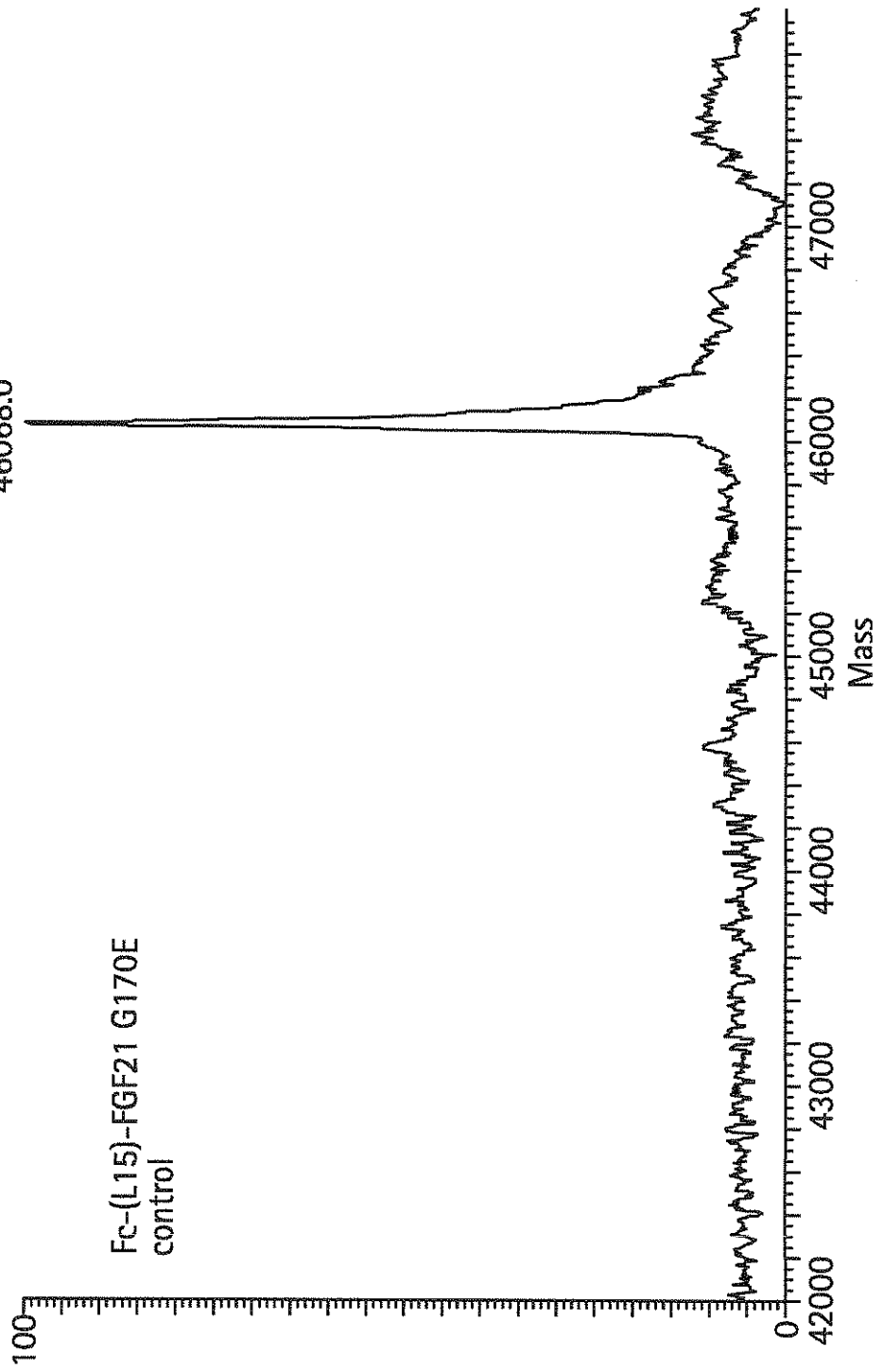

[1-424]
46068.0
Fc-(L15)-FGF21 G170E
control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20B

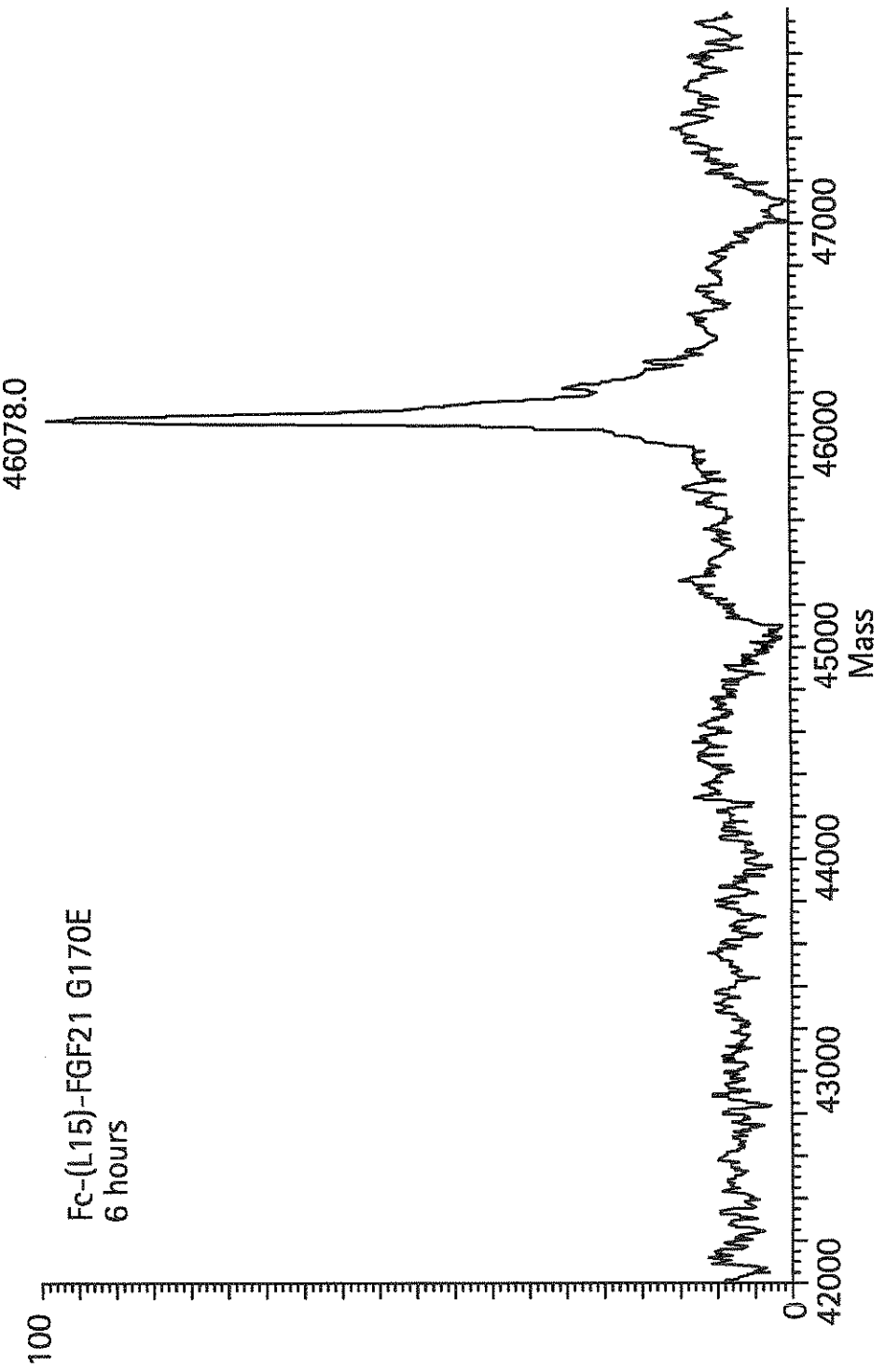

[1-424]
46078.0
Fc-(L15)-FGF21 G170E
6 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20C

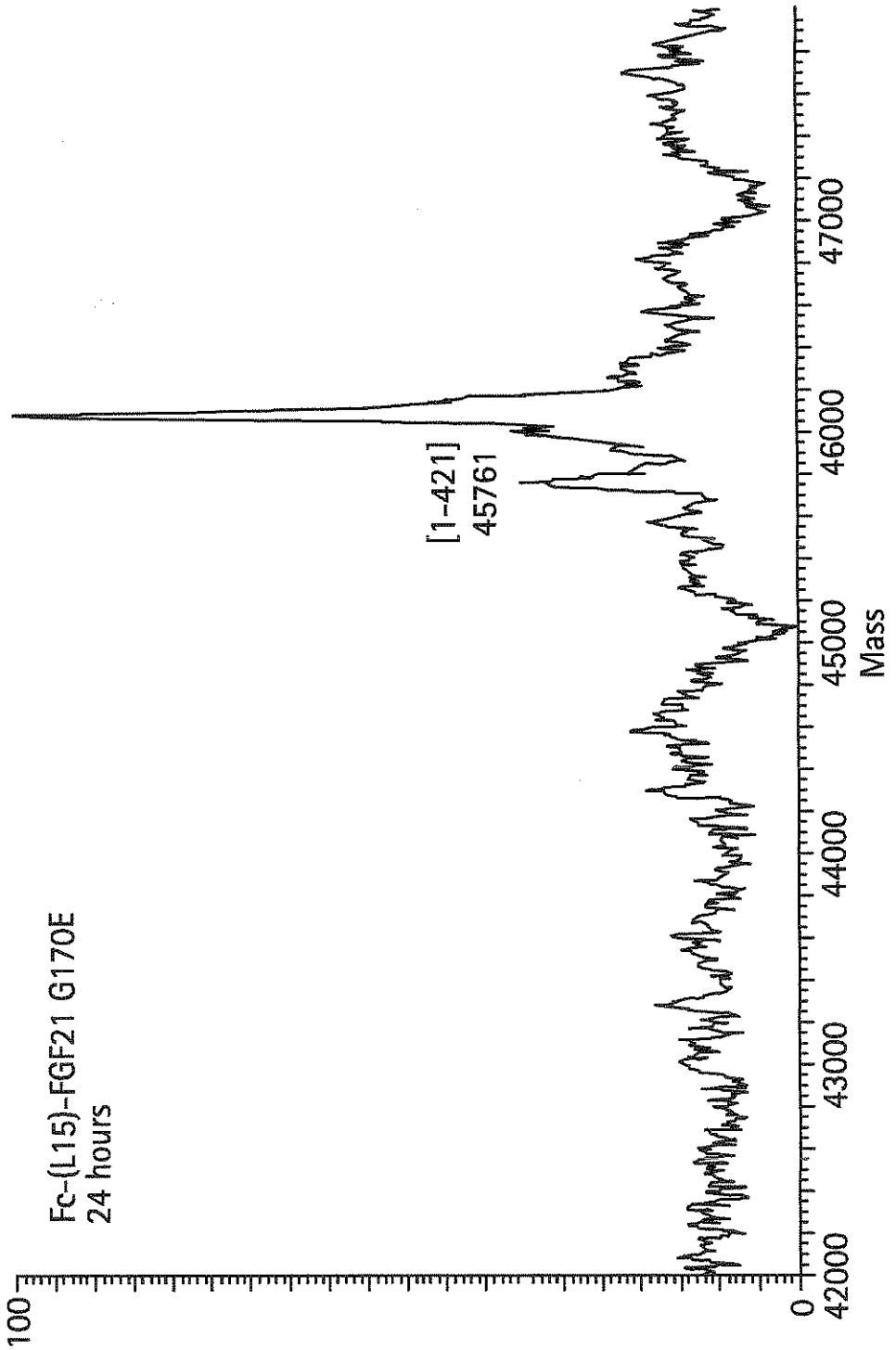

Fc-(L15)-FGF21 G170E
24 hours
[1-424]
46074.0
[1-421]
45761
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20D

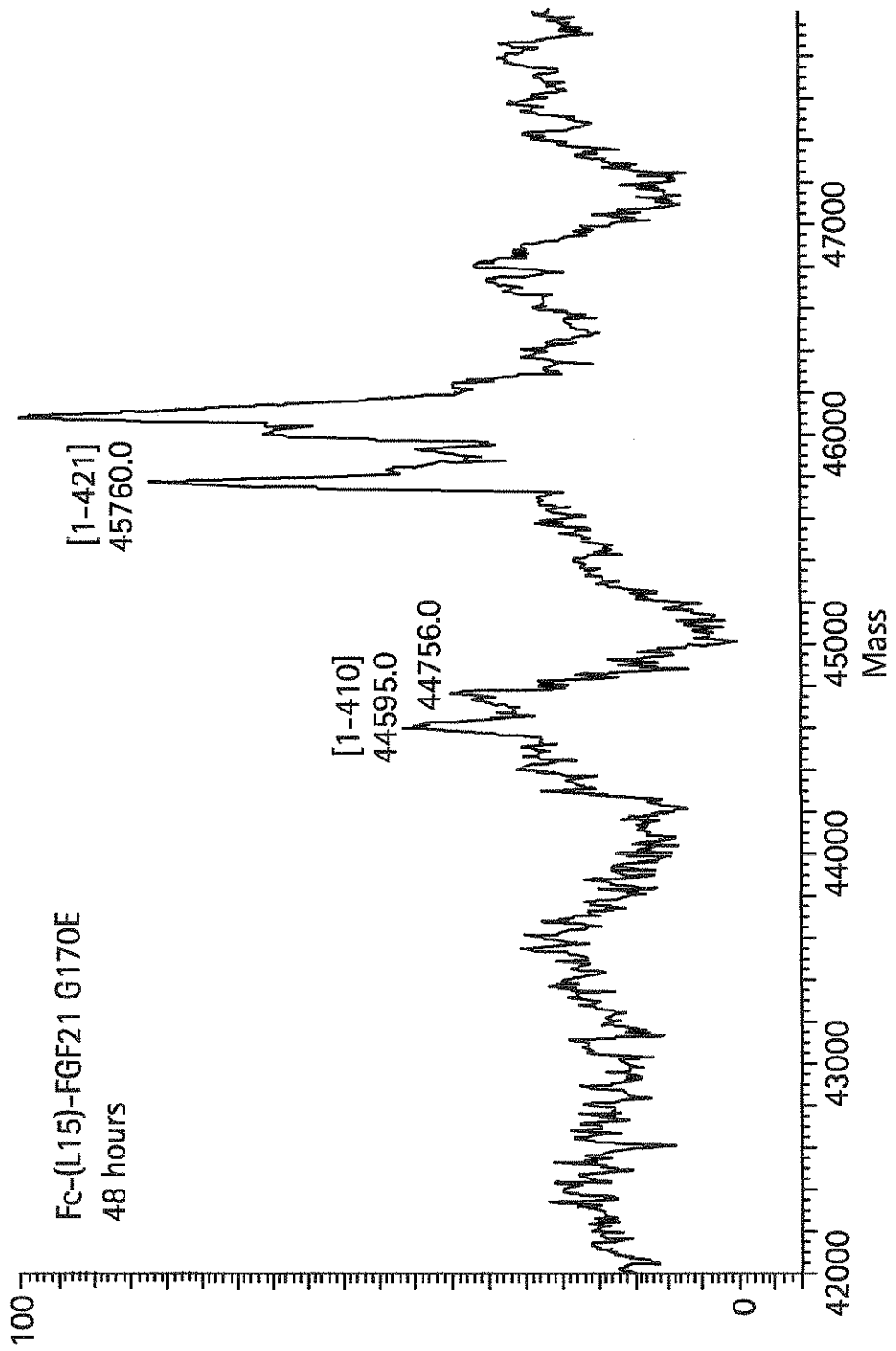

Fc-(L15)-FGF21 G170E
48 hours
[1-424]
46072.0
[1-421]
45760.0
[1-410]
44595.0
44756.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21A

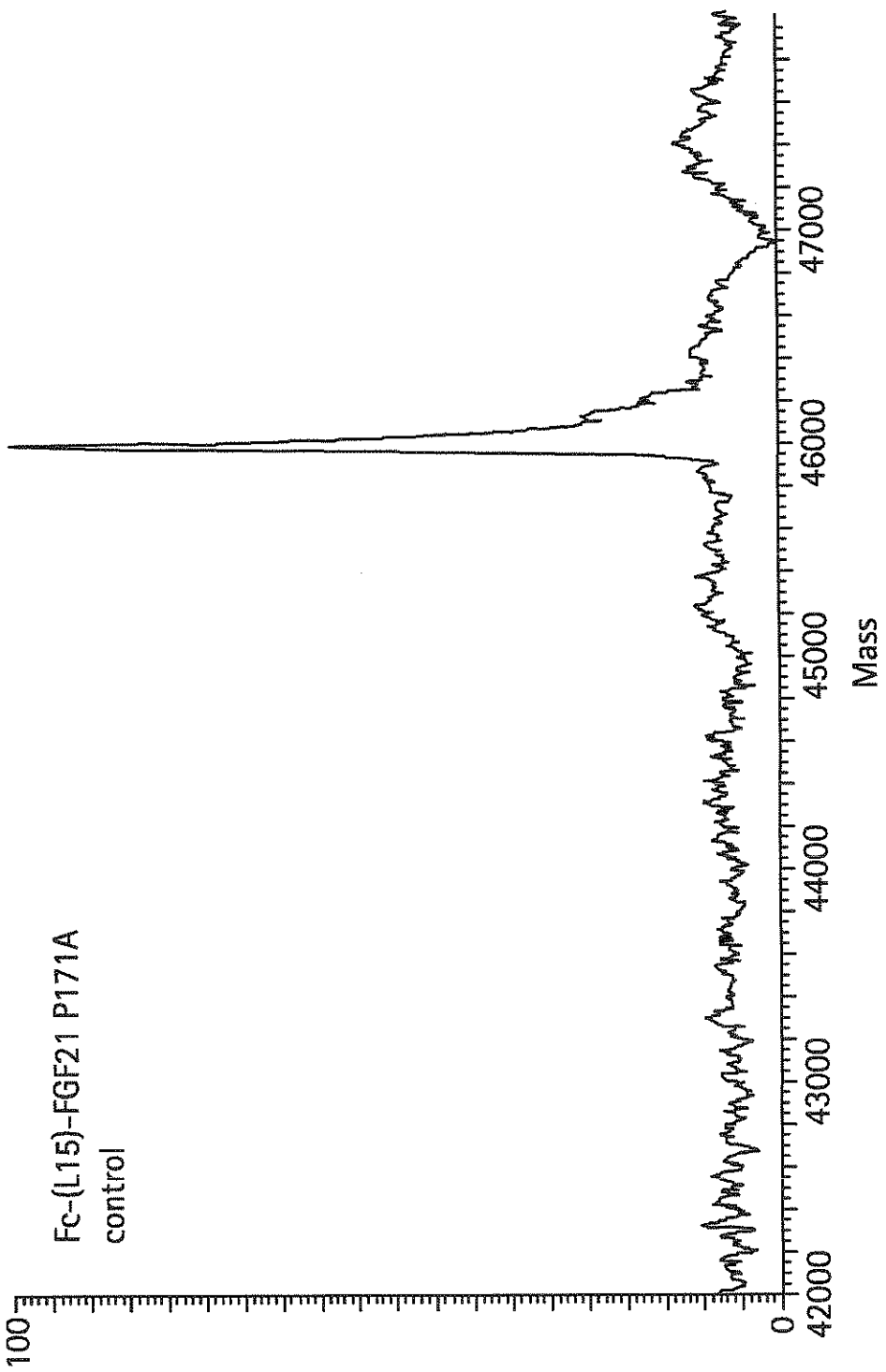

[1-424]
45970.0
Fc-(L15)-FGF21 P171A
control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21B

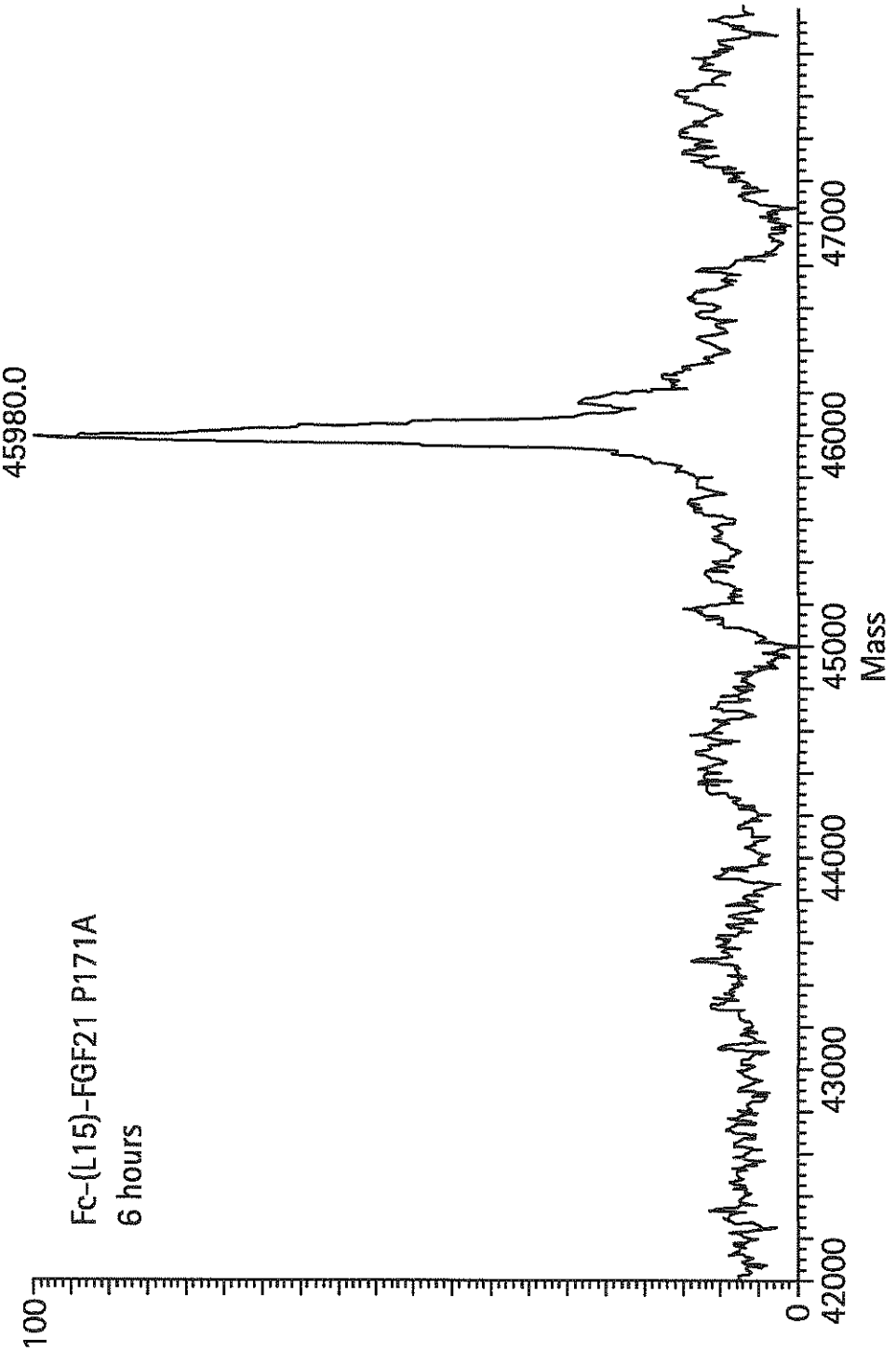

[1-424]
45980.0
Fc-(L15)-FGF21 P171A
6 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21C

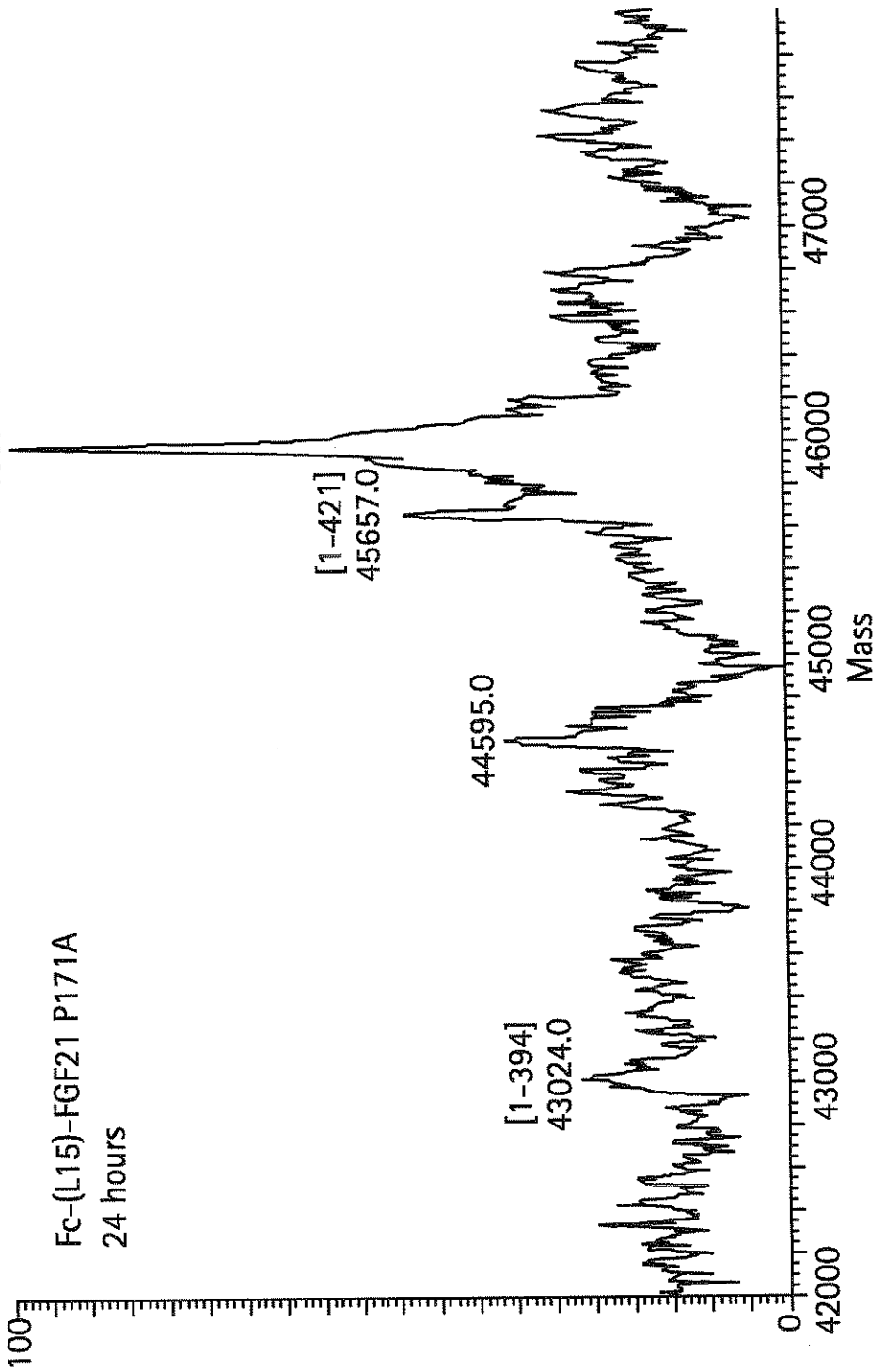

Fc-(L15)-FGF21 P171A
24 hours
[1-424]
45973.0
[1-421]
45657.0
44595.0
[1-394]
43024.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21D

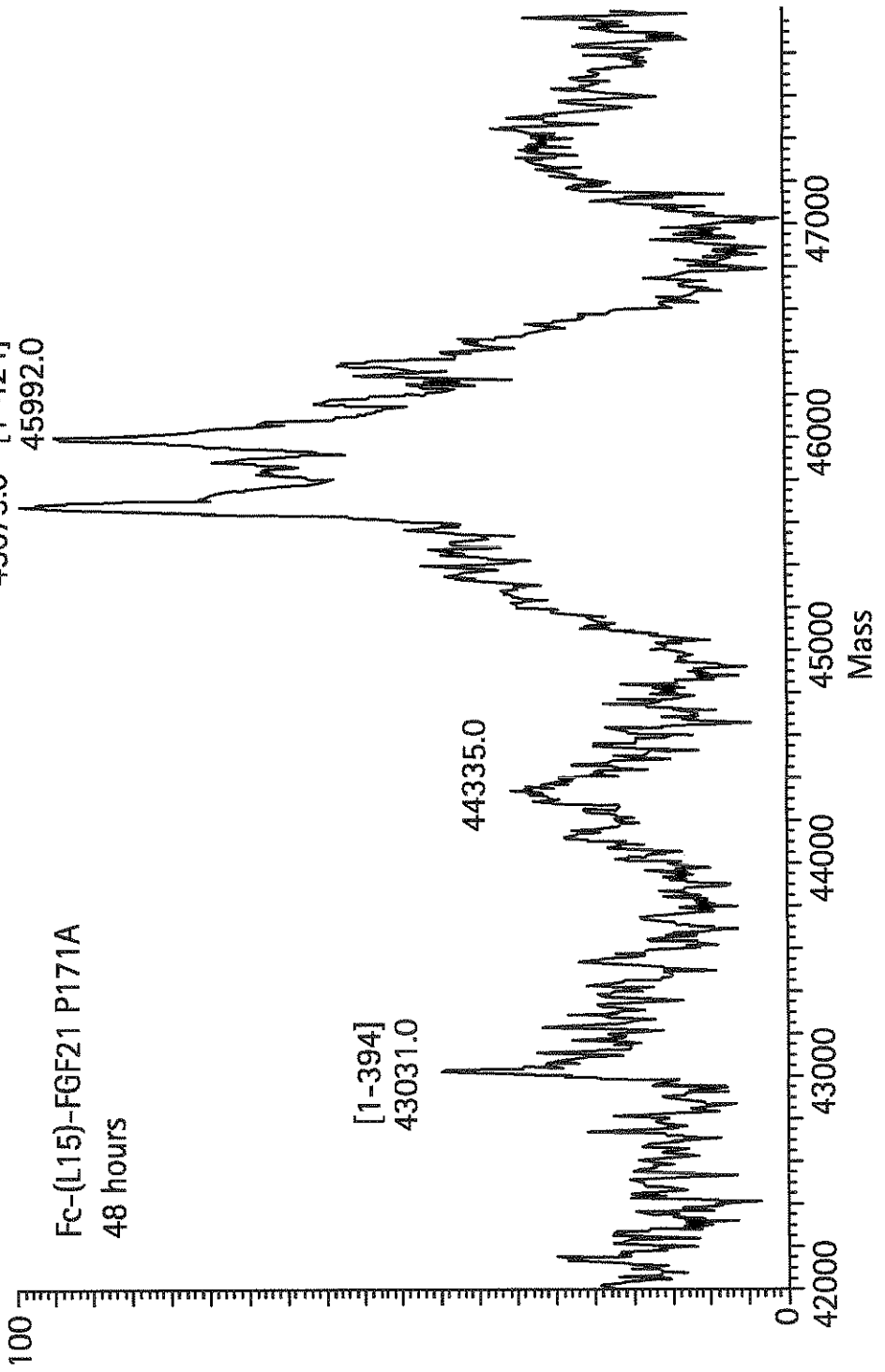

Fc-(L15)-FGF21 P171A
48 hours
[1-394]
43031.0
44335.0
[1-421]
45673.0
[1-424]
45992.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22A

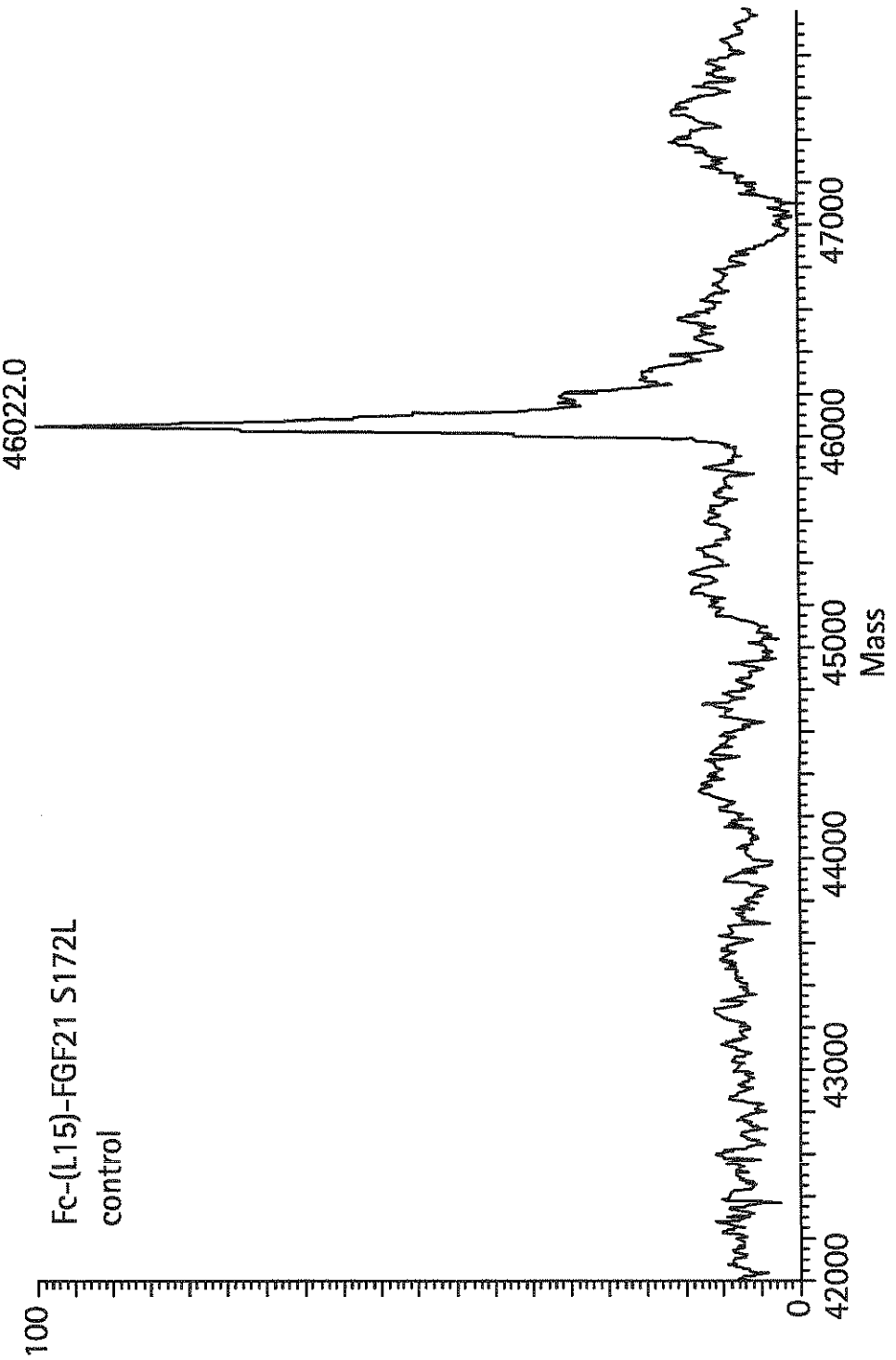

Fc-(L15)-FGF21 S172L
control
[1-424]
46022.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22B

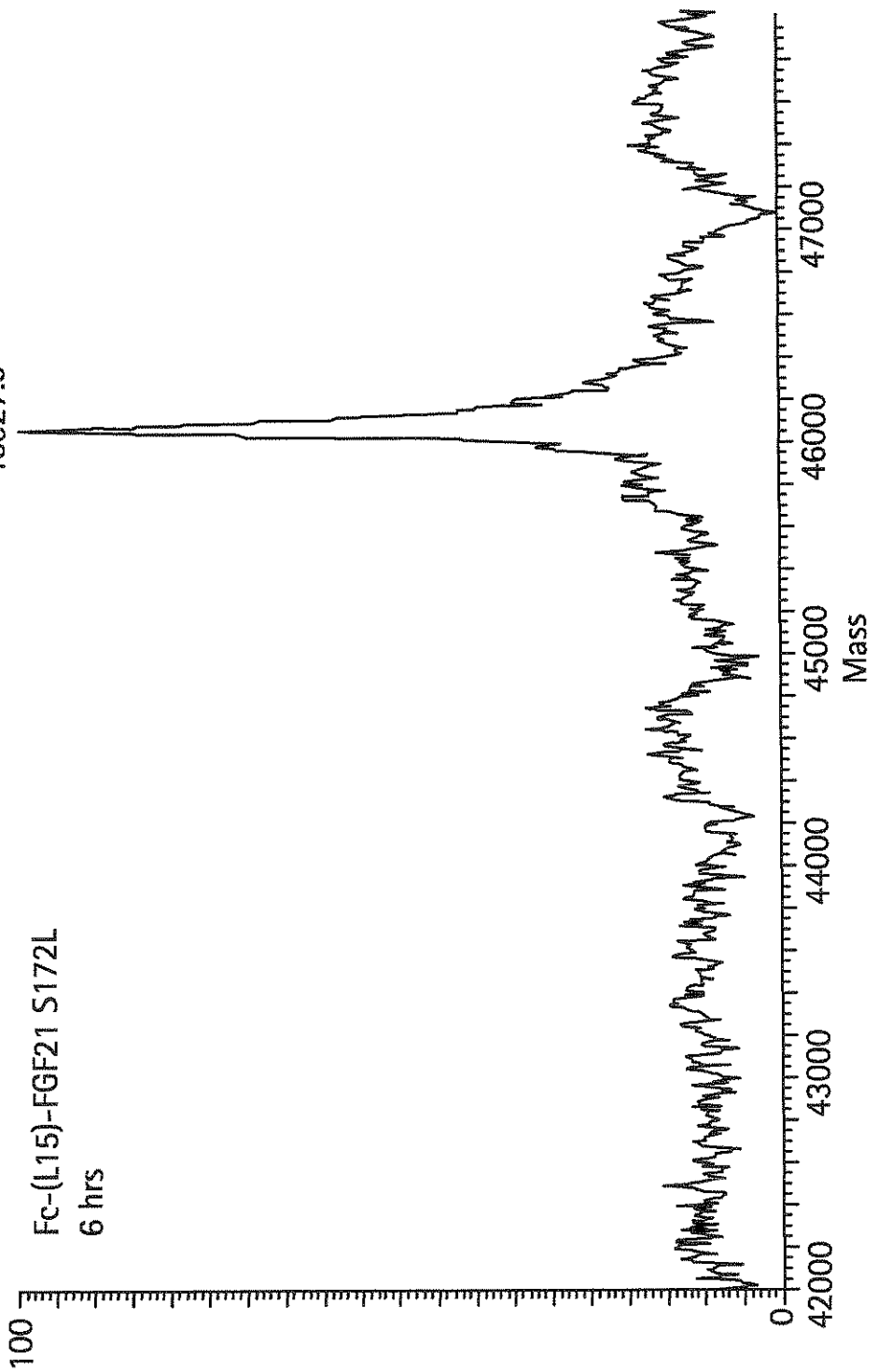

Fc-(L15)-FGF21 S172L
6 hrs
[1-424]
46027.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22C

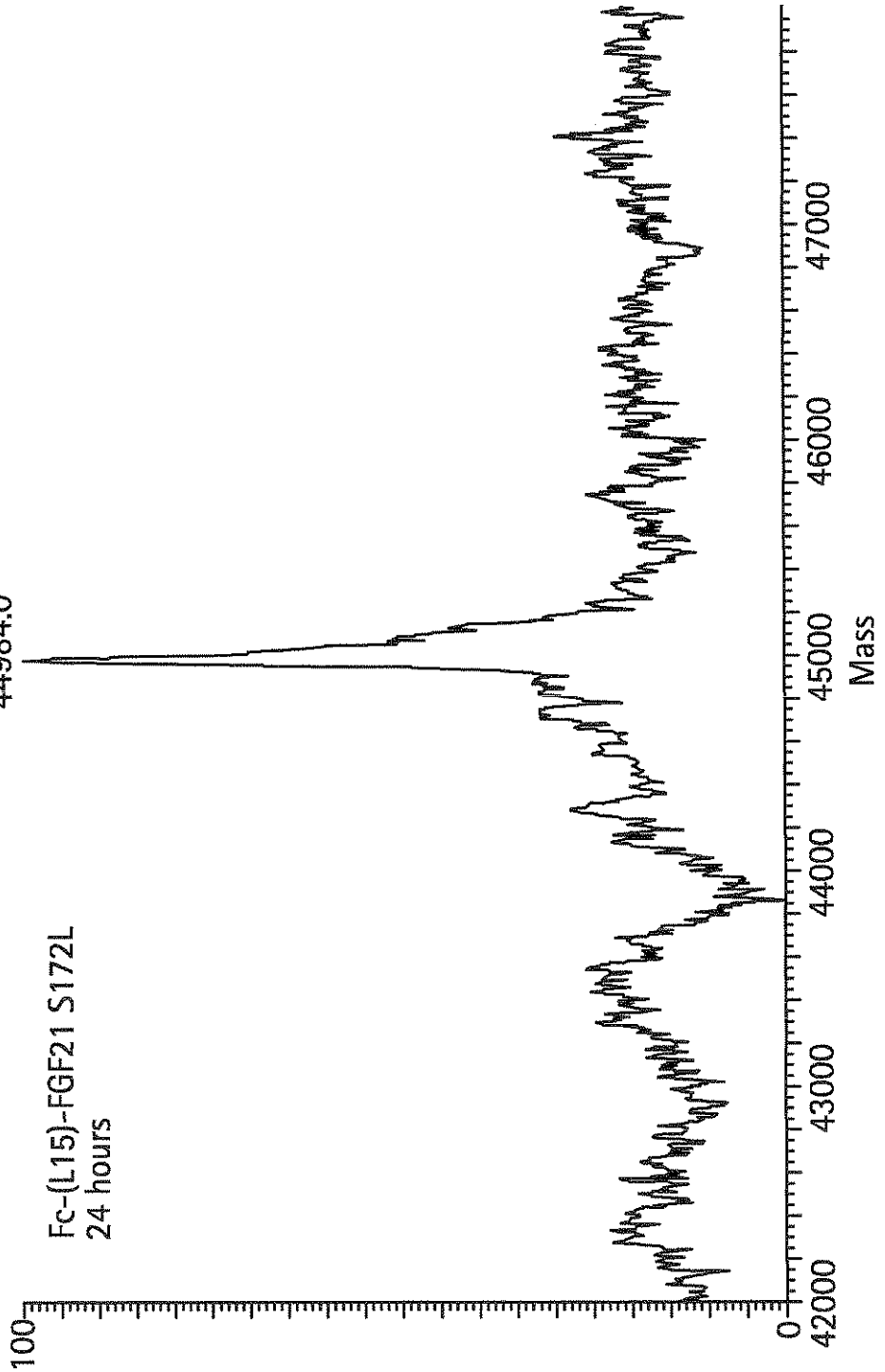

[1-414]
44984.0
Fc-(L15)-FGF21 S172L
24 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22D

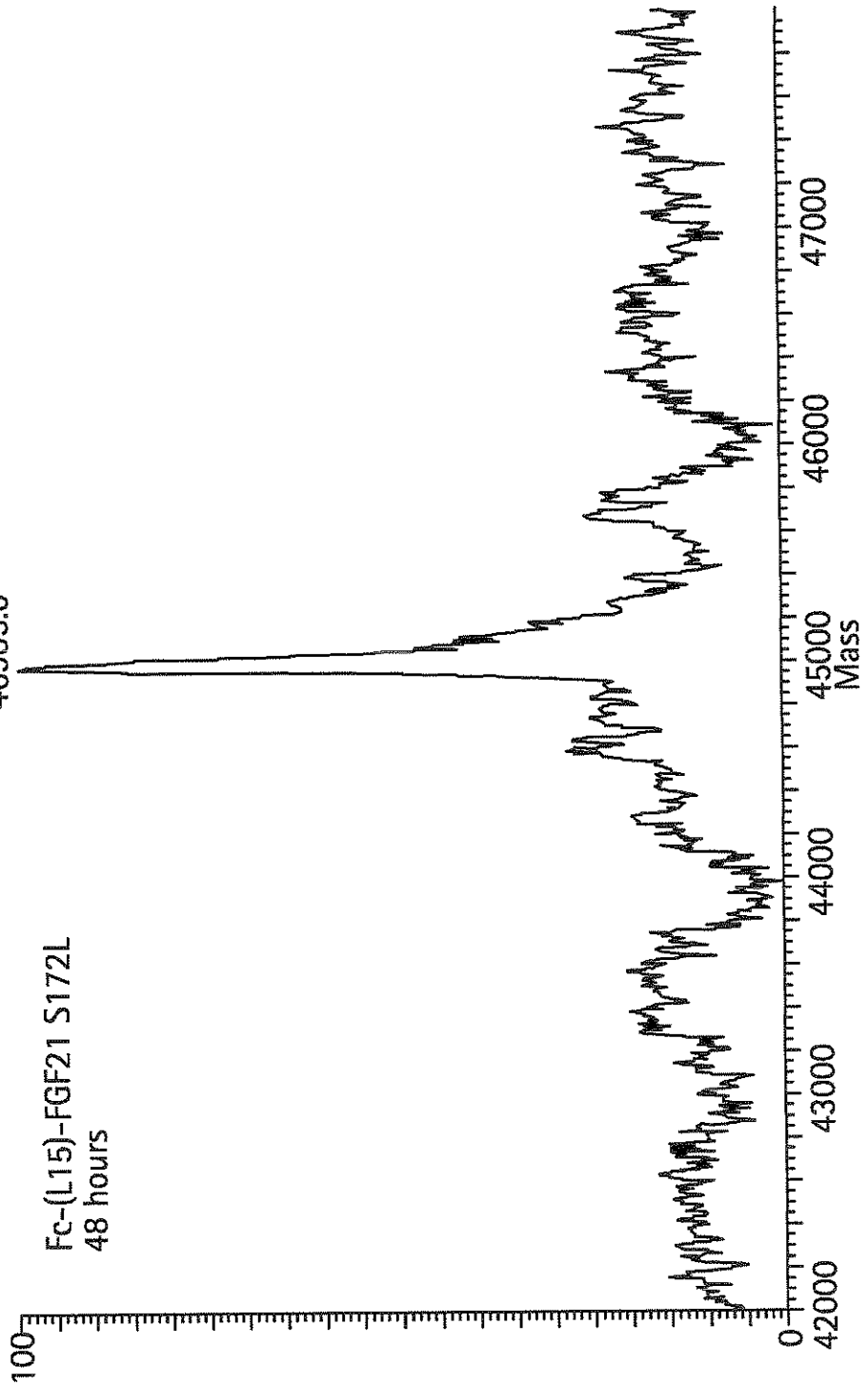

Fc-(L15)-FGF21 S172L
48 hours
[1-414]
46985.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 23A

Fc-(L15)-FGF21

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGGSGGGSGG*
*GGS*HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVGPSQGRSPSYAS

6 hrs (20%)
24 hrs (100%) [1-414]
48 hrs (~100%)

FIG. 23B

Fc-(L15)-FGF21 G170E

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGGSGGGSGG*
*GGS*HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVEPSQGRSPSYAS

6 hrs (0%)
24 hrs (20%) [1-442]
48 hrs (~40%)

FIG. 23C

Fc-(L15)-FGF21 P171A

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGSGG*
*GGSH*PIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVGASQGRSPSYAS

6 hrs (0%)
24 hrs (~30%) [1-421]
48 hrs (~50%)

FIG. 23D

Fc-(L15)-FGF21 S172L

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELYKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGGSGGGSGG*
*GGS*HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVGPLQGRSPSYAS

6 hrs (0%)
24 hrs (100%) [1-414]
48 hrs (100%)

FIG. 24A

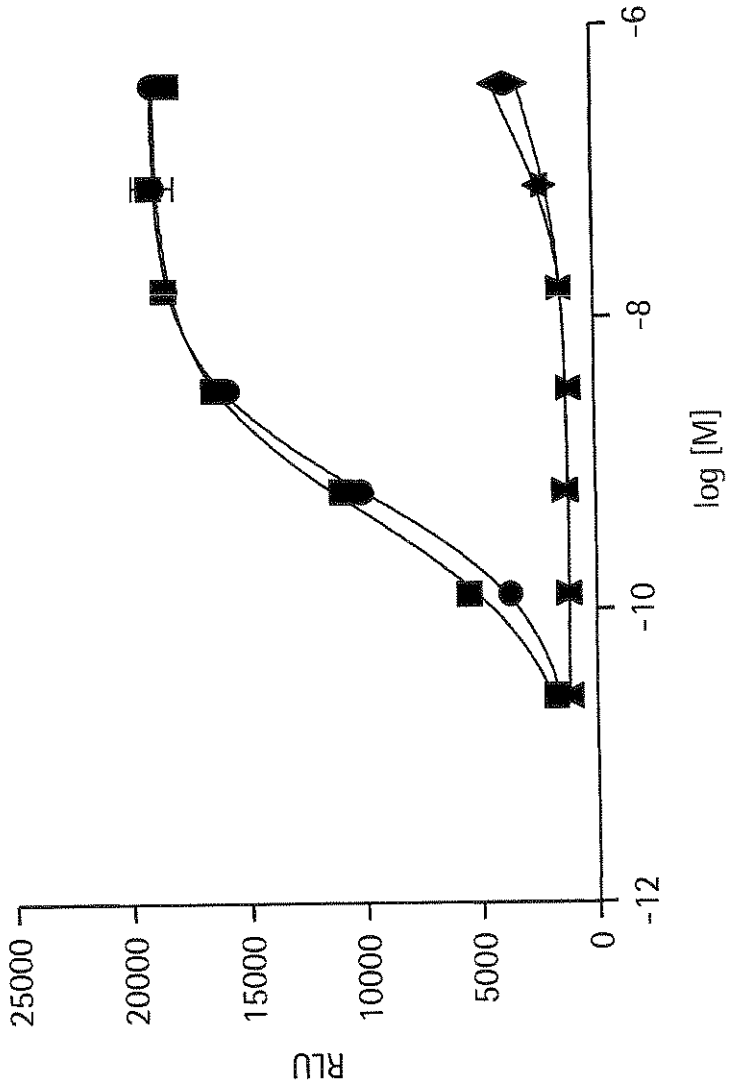

FGF21
FGF21 L99R
FGF21 L99D
FGF21 A111T
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
log [M]

FIG. 24B

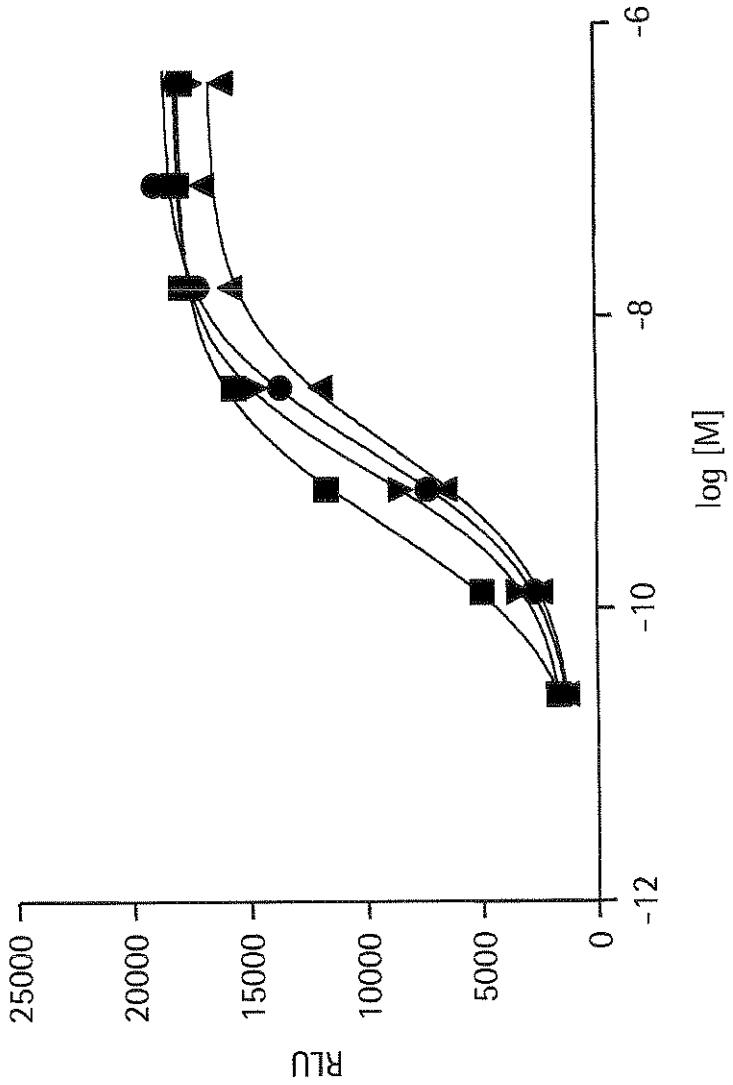

FGF21
FGF21 A129D
FGF21 A129Q
FGF21 A134K
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
log [M]

FIG. 24C

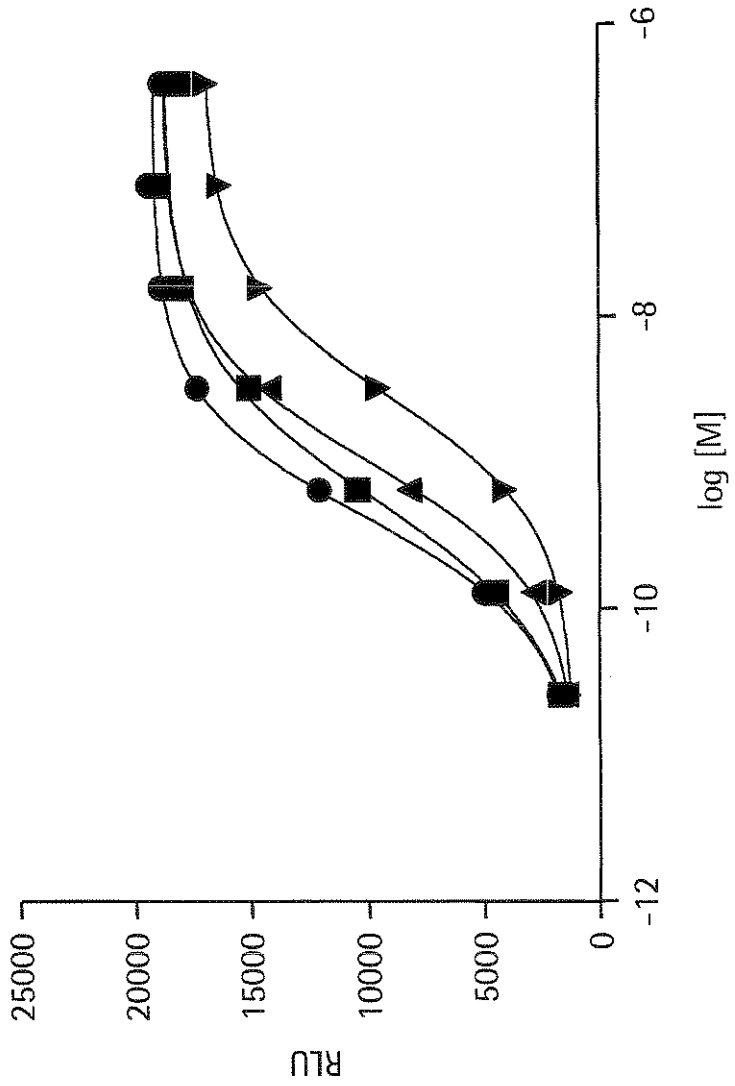

FGF21
FGF21 A134Y
FGF21 A134E
FGF21 A129K
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
log [M]

FIG. 25A

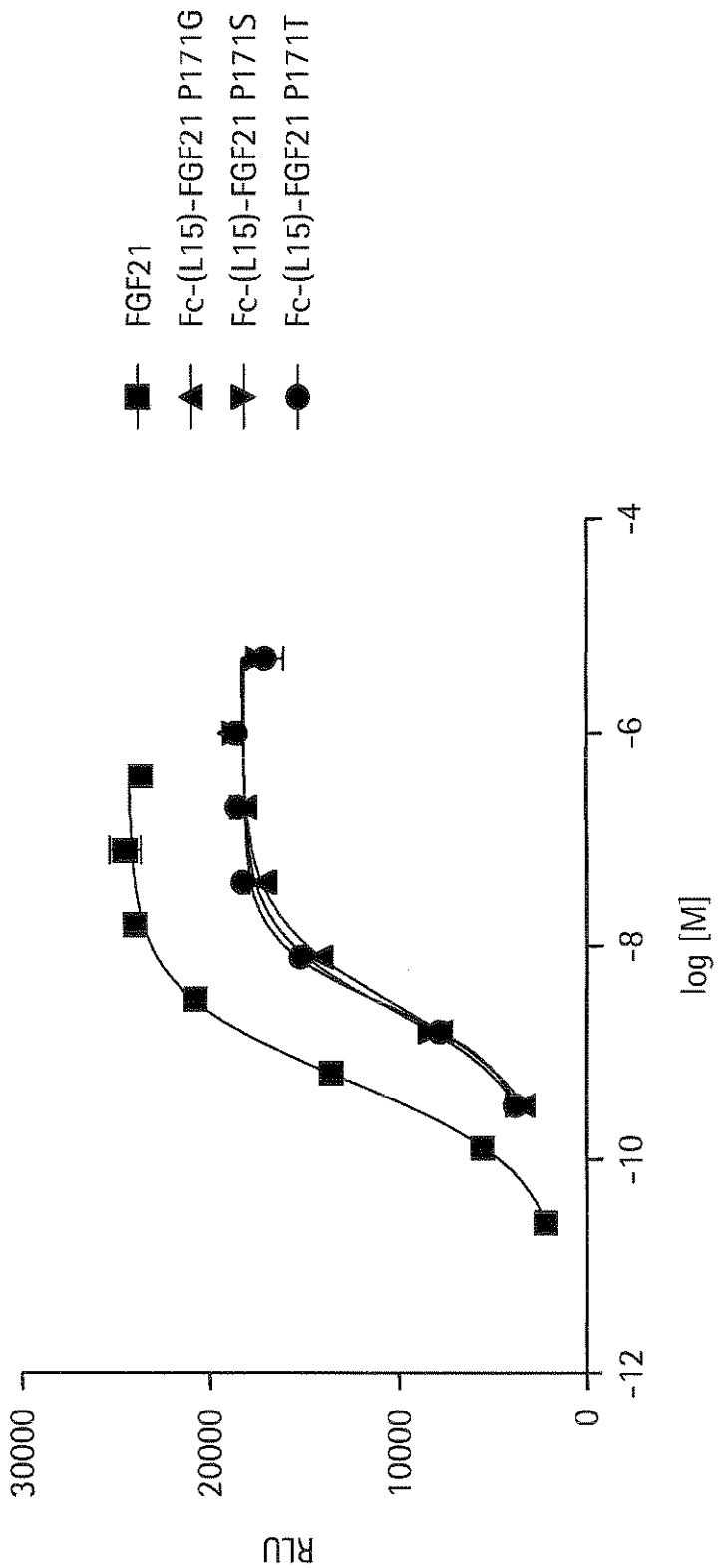

FGF21
Fc-(L15)-FGF21 P171G
Fc-(L15)-FGF21 P171S
Fc-(L15)-FGF21 P171T
RLU
30000
20000
10000
0
-12
-10
-8
-6
-4
log [M]

FIG. 25B

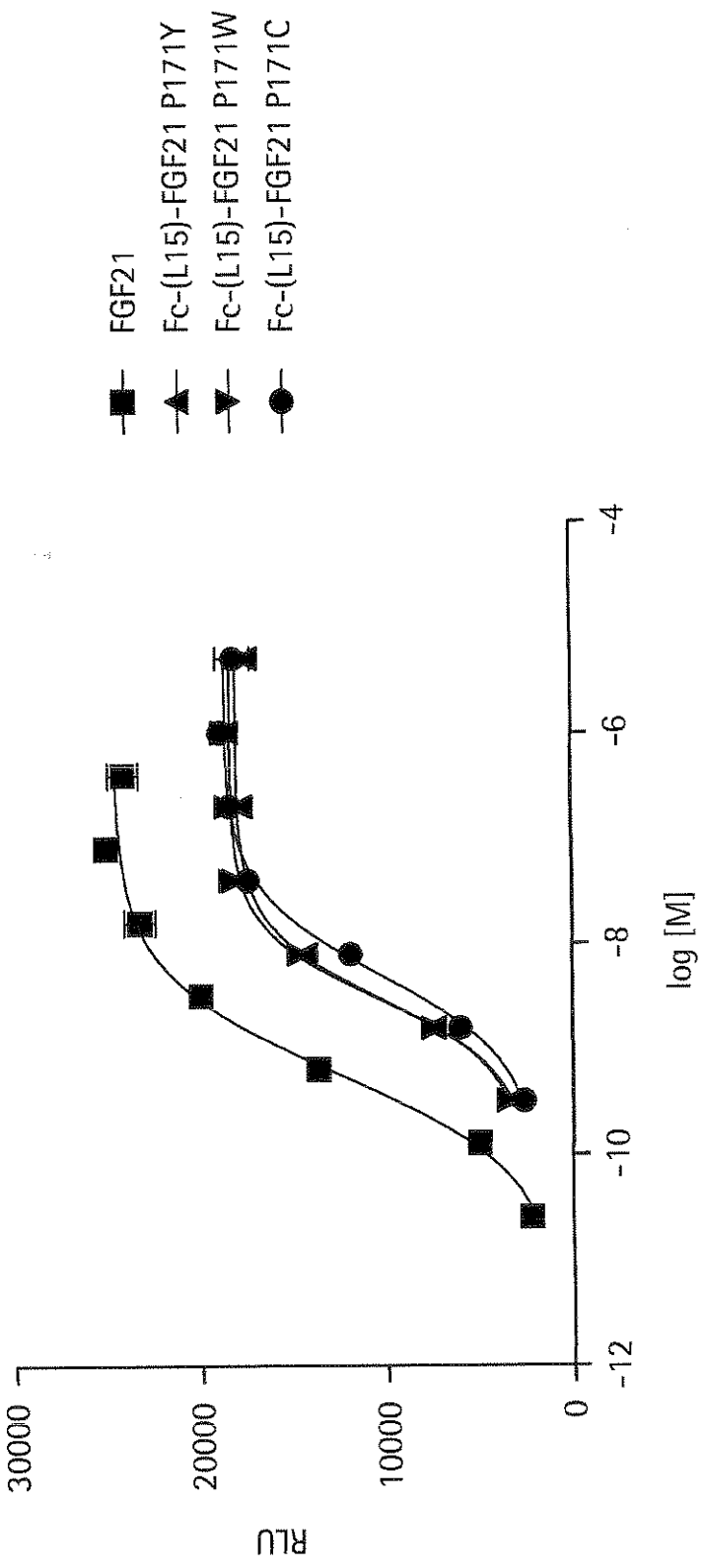

FGF21
Fc-(L15)-FGF21 P171Y
Fc-(L15)-FGF21 P171W
Fc-(L15)-FGF21 P171C
RLU
30000
20000
10000
0
-12
-10
-8
-6
-4
log [M]

FIG. 25C

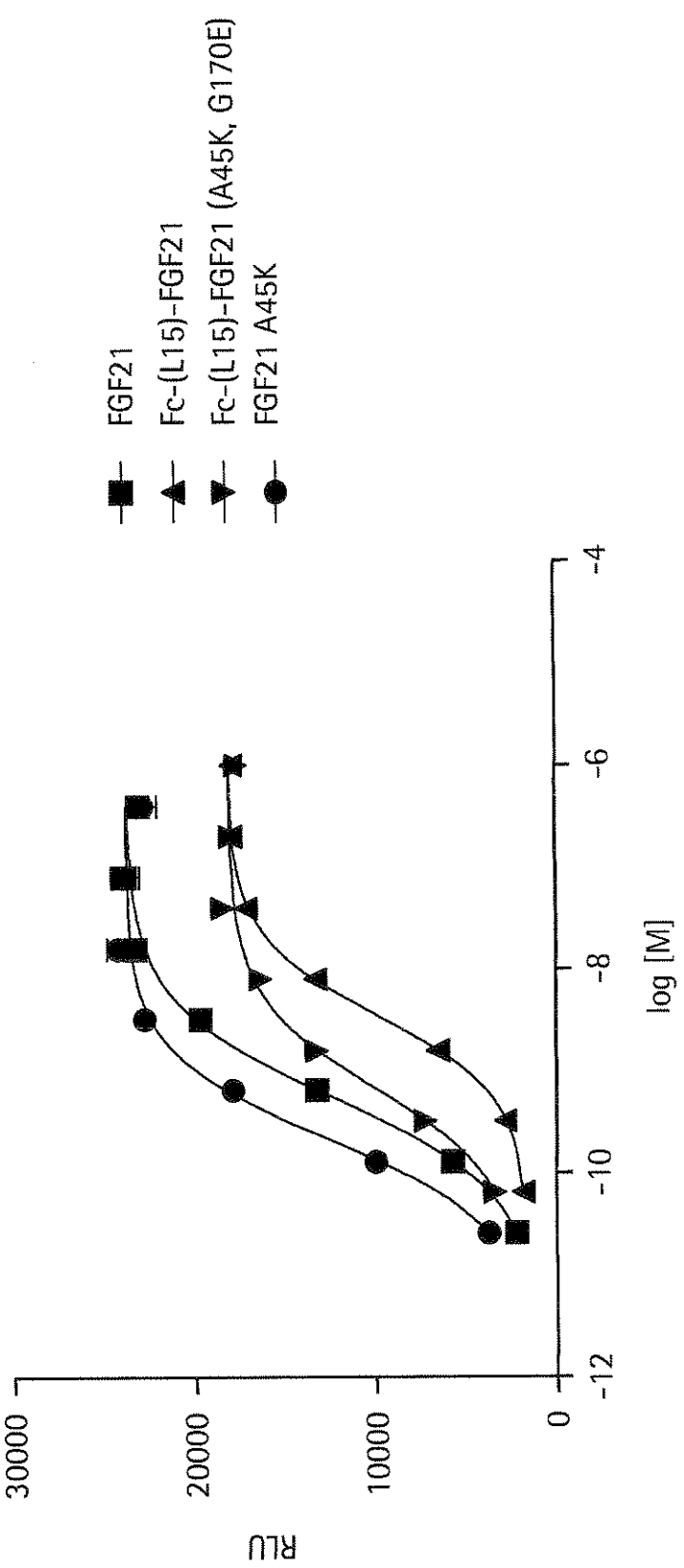

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (A45K, G170E)
FGF21 A45K
RLU
30000
20000
10000
0
-12
-10
-8
-6
-4
log [M]

FIG. 25D

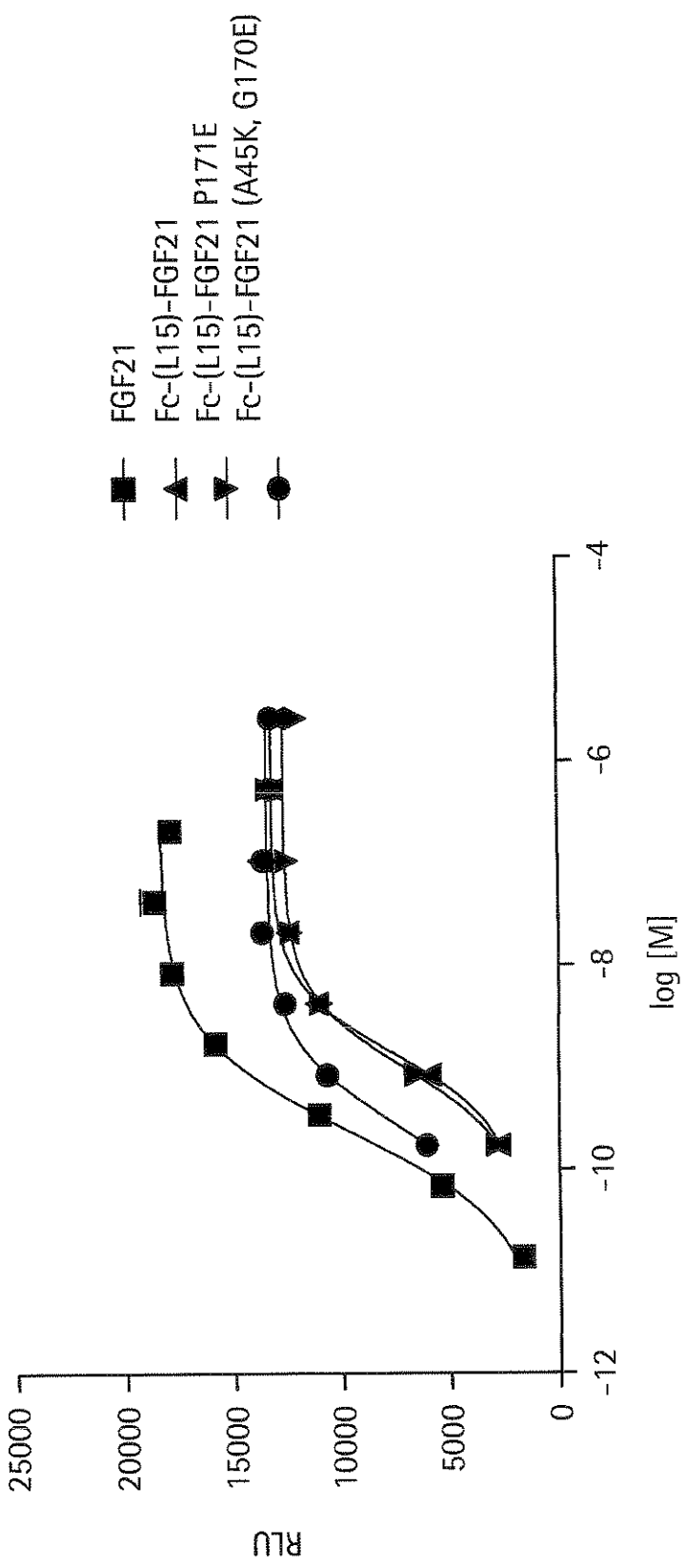

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 (A45K, G170E)
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
-4
log [M]

FIG. 26A

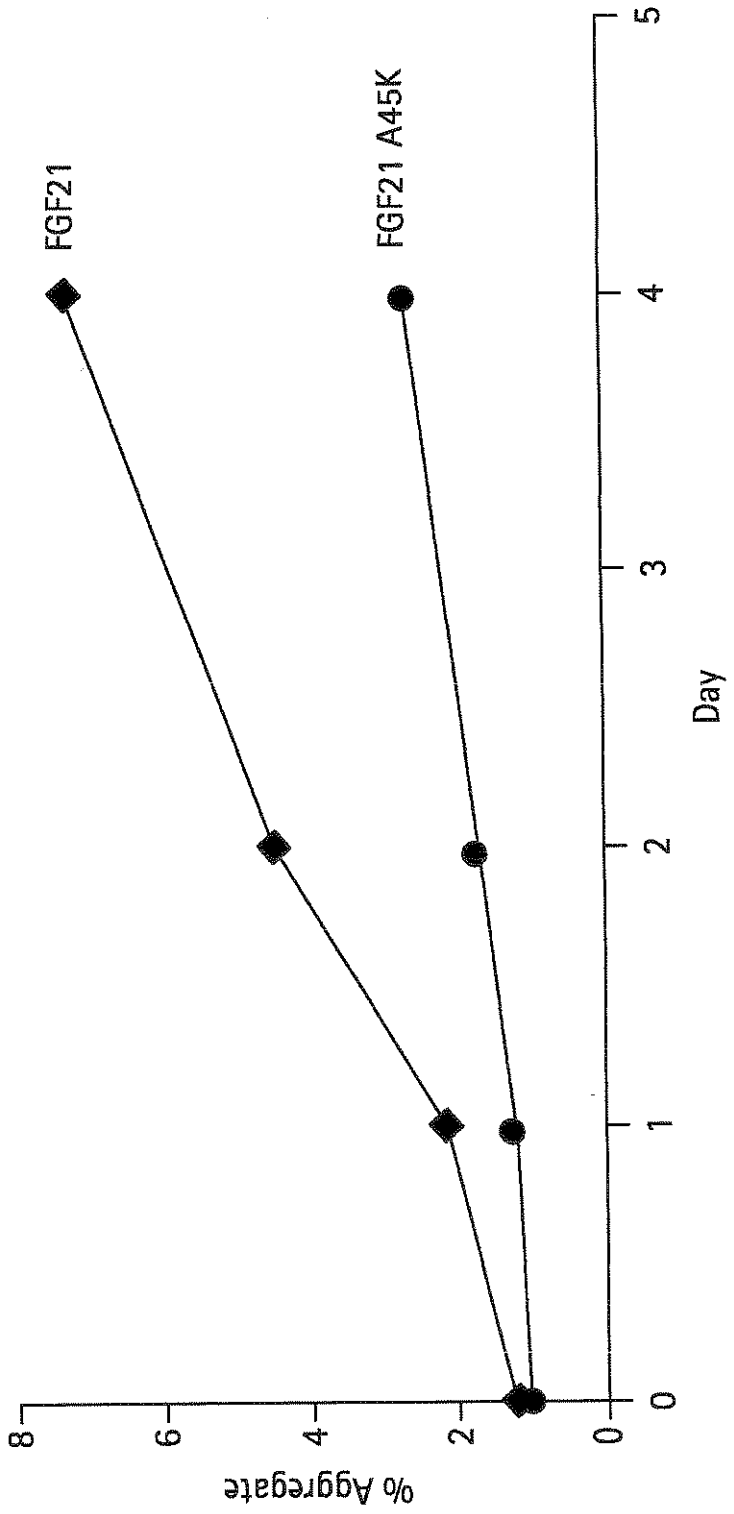


FIG. 26B

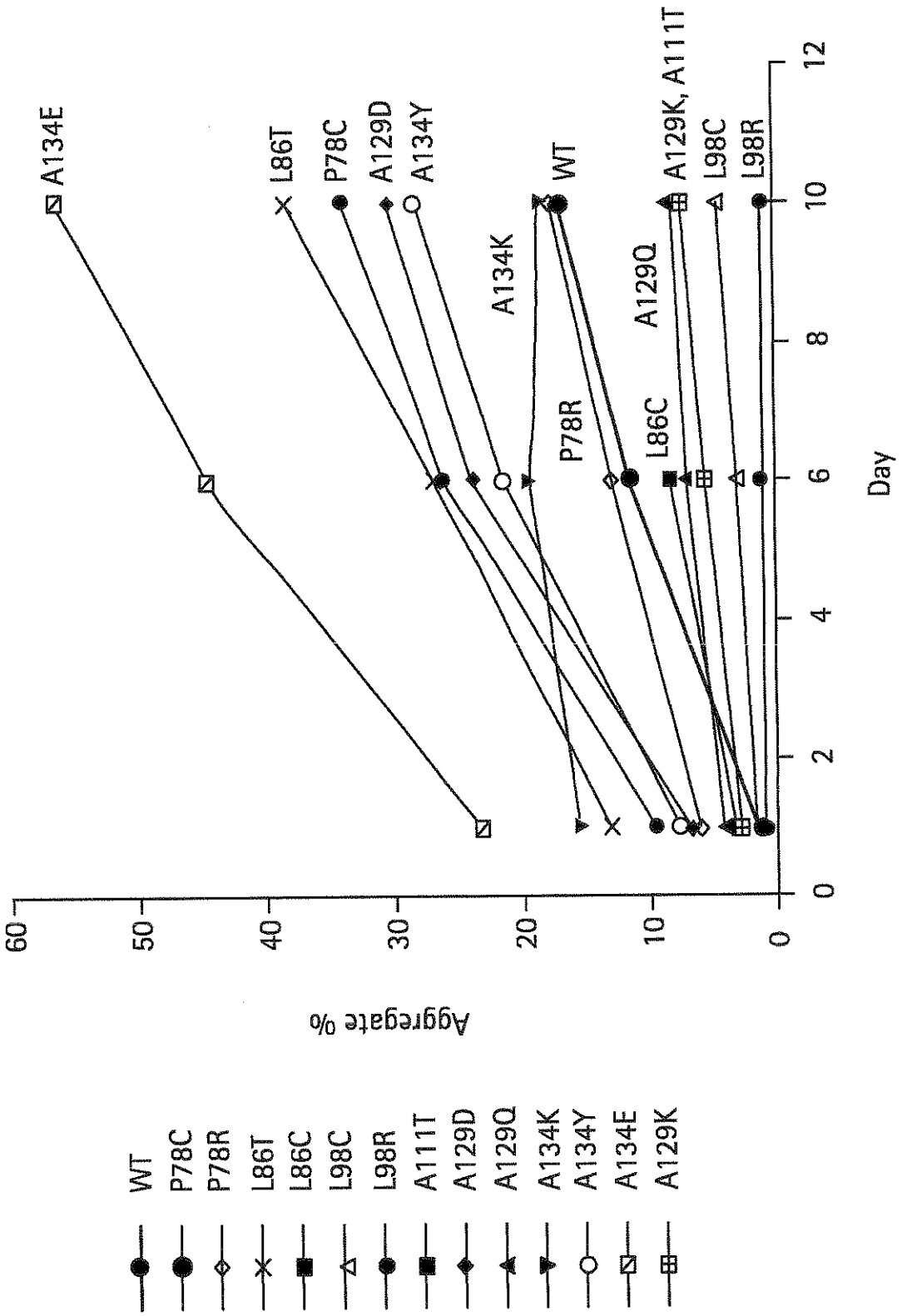

A134E
L86T
P78C
A129D
A134Y
A134K
WT
P78R
L86C
A129Q
A129K, A111T
L98C
L98R
Aggregate %
Day
0
10
20
30
40
50
60
0
2
4
6
8
10
12
WT
P78C
P78R
L86T
L86C
L98C
L98R
A111T
A129D
A129Q
A134K
A134Y
A134E
A129K

FIG. 27

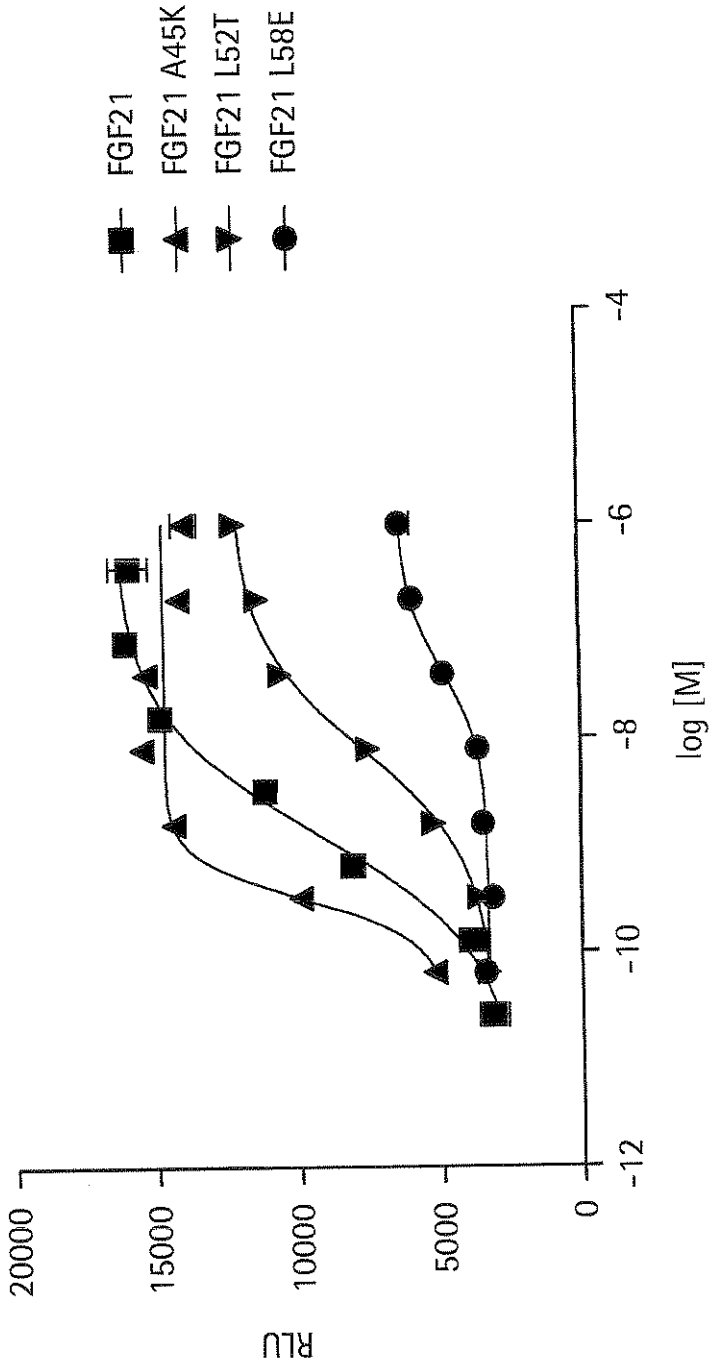

FGF21
FGF21 A45K
FGF21 L52T
FGF21 L58E
RLU
20000
15000
10000
5000
0
-12
-10
-8
-6
-4
log [M]

FIG. 28A

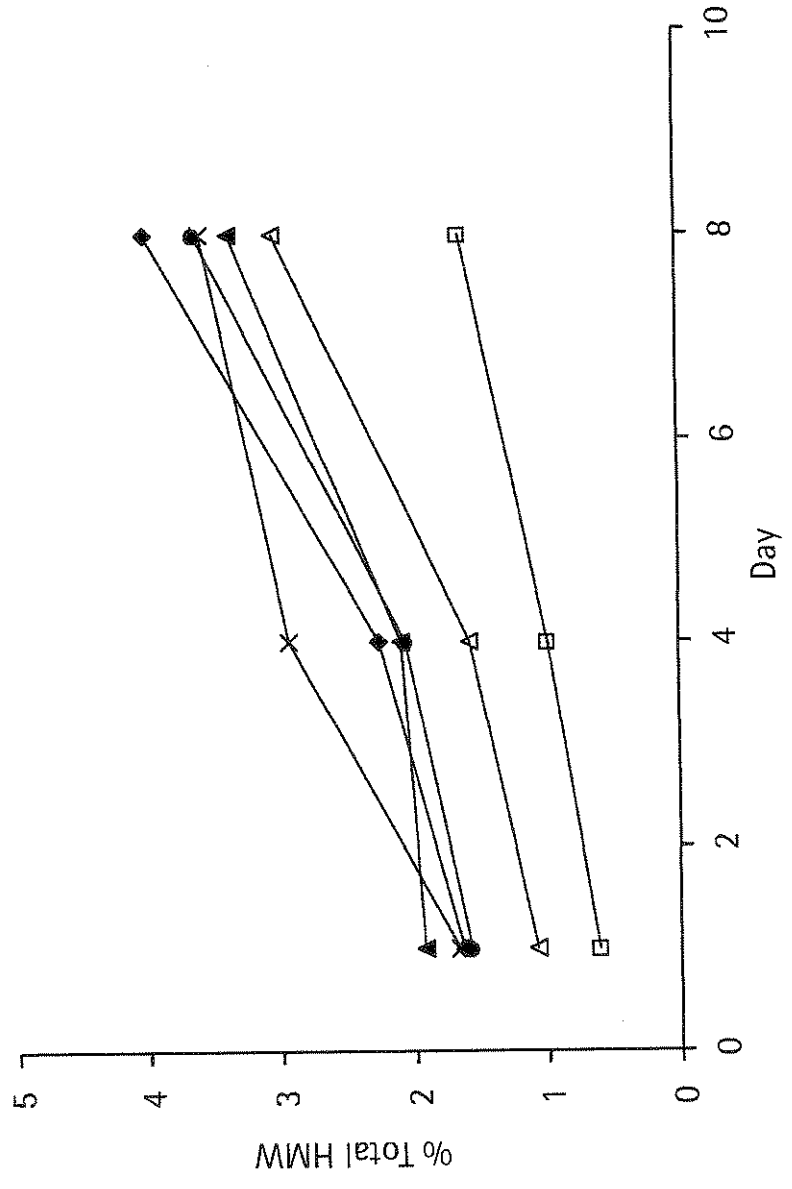

Fc(L15)-FGF21 (6-181, G170E)
Fc(L15)-FGF21 (A45K, G170E)
Fc(L15)-FGF21 P171E
Fc(L15)-FGF21 P171A
Fc(L15)-FGF21 G170E
FGF21
% Total HMW
0
1
2
3
4
5
0
2
4
6
8
10
Day

FIG. 28B

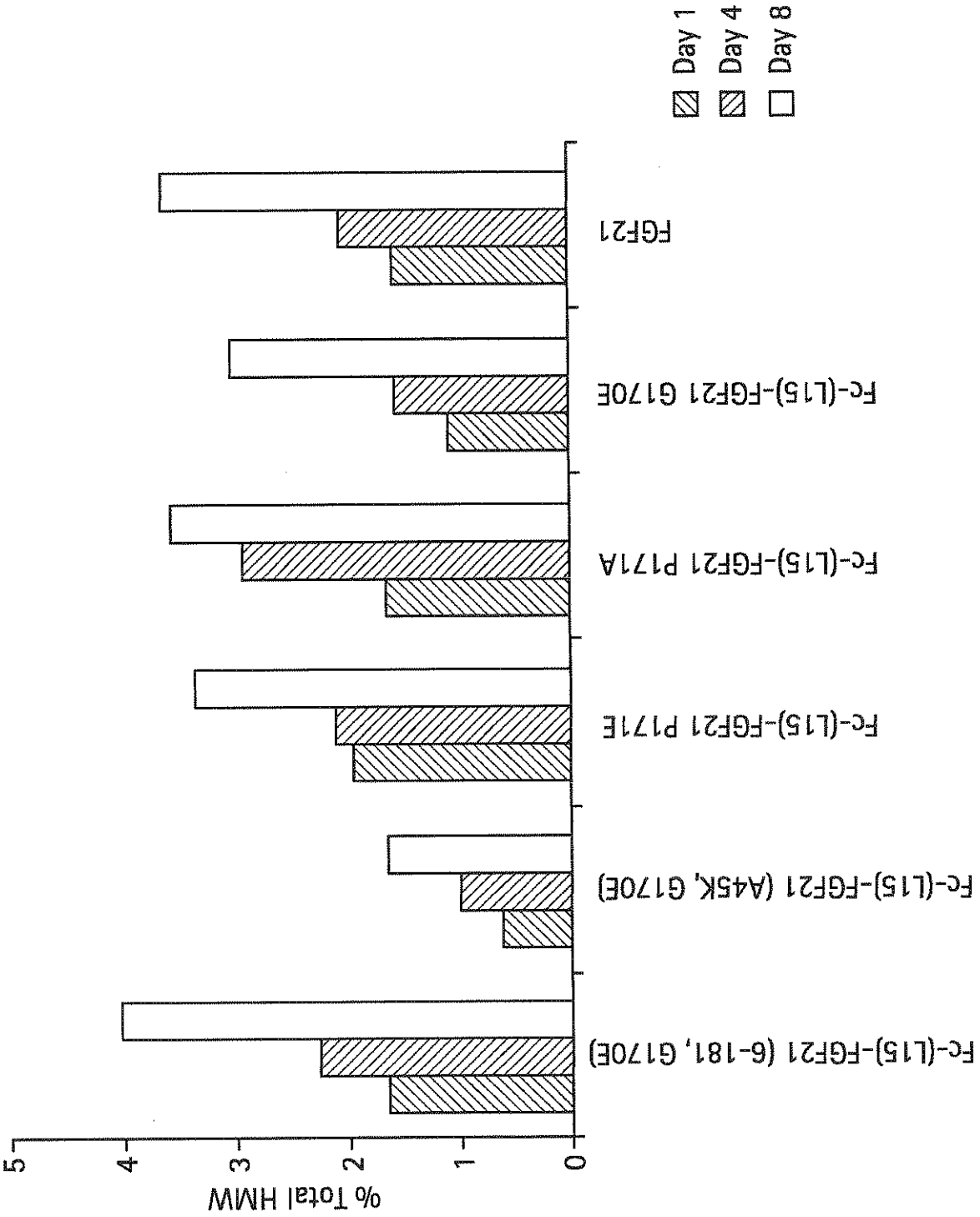

Day 1
Day 4
Day 8
% Total HMW
0
1
2
3
4
5
Fc-(L15)-FGF21 (6-181, G170E)
Fc-(L15)-FGF21 (A45K, G170E)
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 P171A
Fc-(L15)-FGF21 G170E
FGF21

FIG. 29

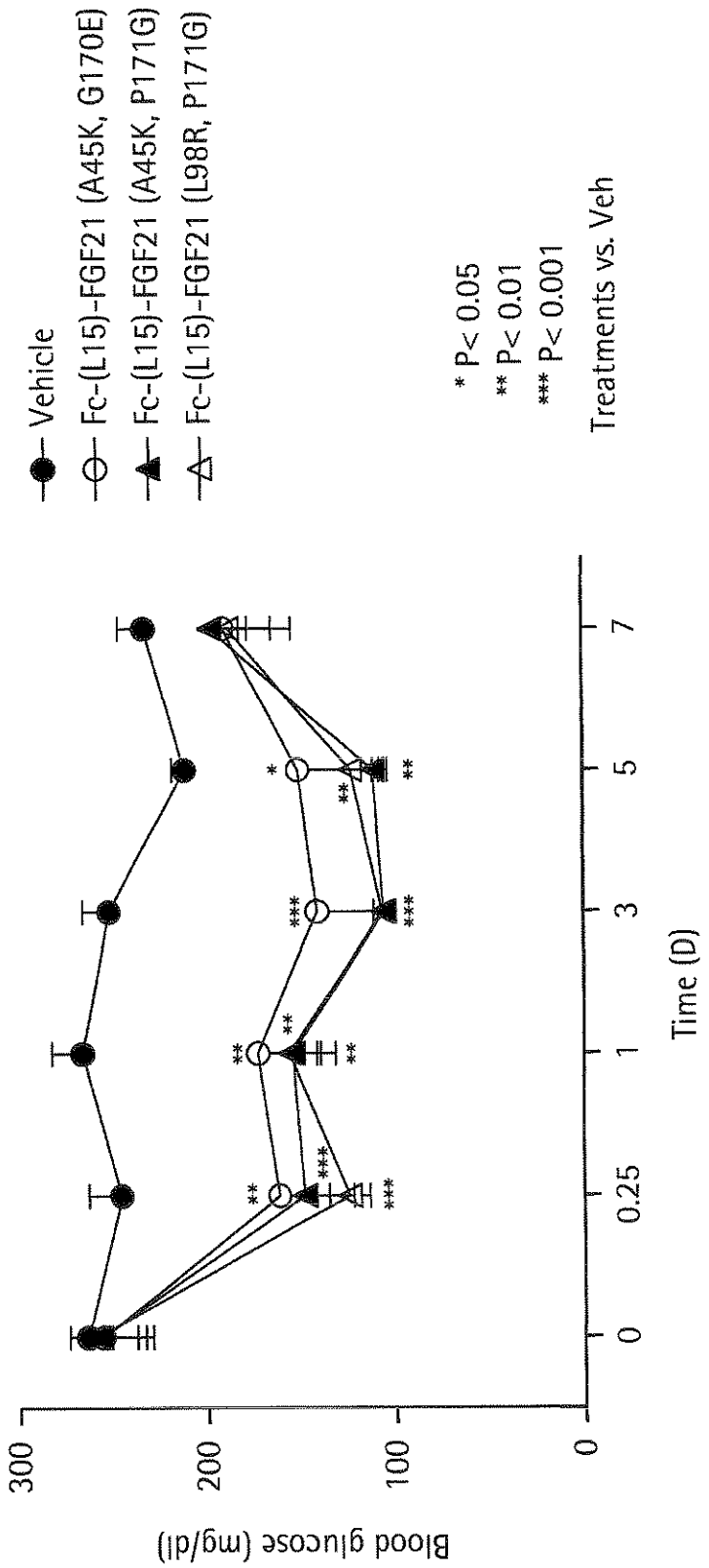

Vehicle
Fc-(L15)-FGF21 (A45K, G170E)
Fc-(L15)-FGF21 (A45K, P171G)
Fc-(L15)-FGF21 (L98R, P171G)
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Blood glucose (mg/dl)
300
200
100
0
0
0.25
1
3
5
7
Time (D)

FIG. 30

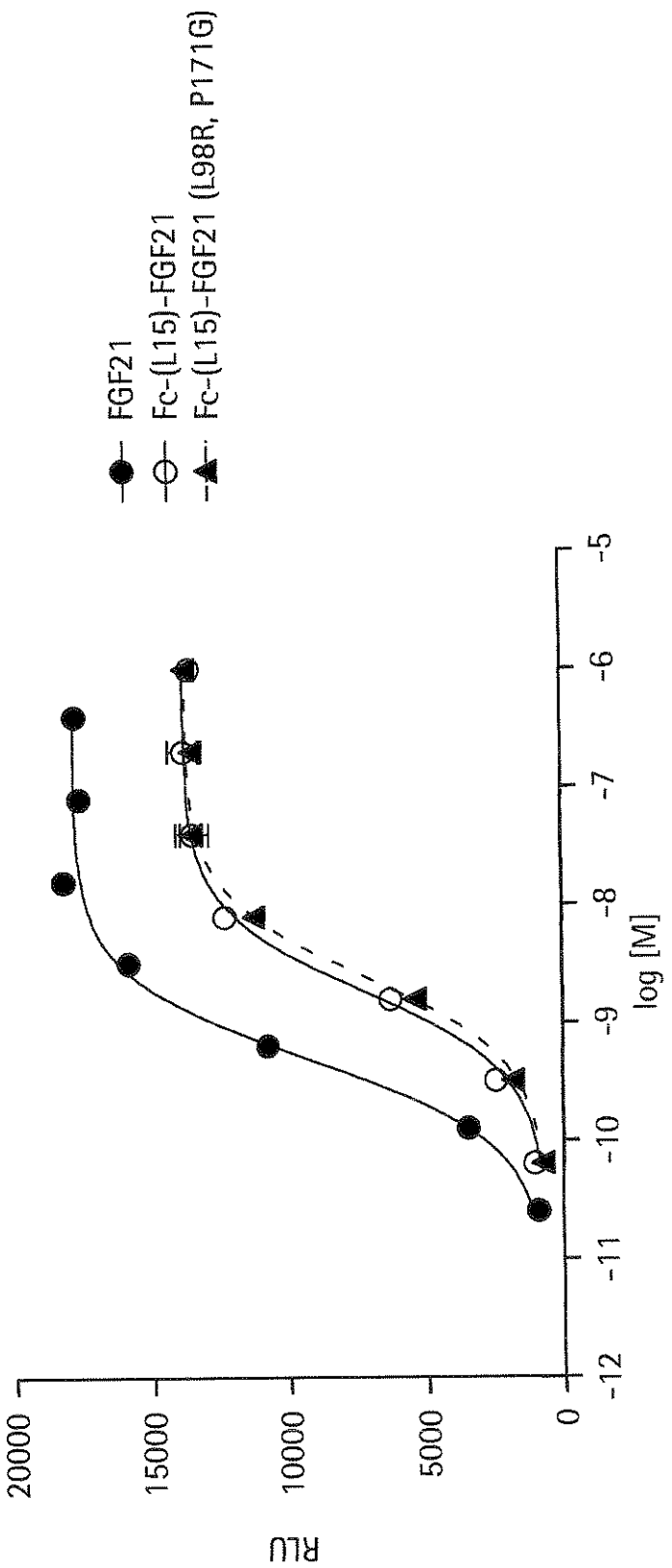

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (L98R, P171G)
RLU
20000
15000
10000
5000
0
-12
-11
-10
-9
-8
-7
-6
-5
log [M]

FIG. 31A

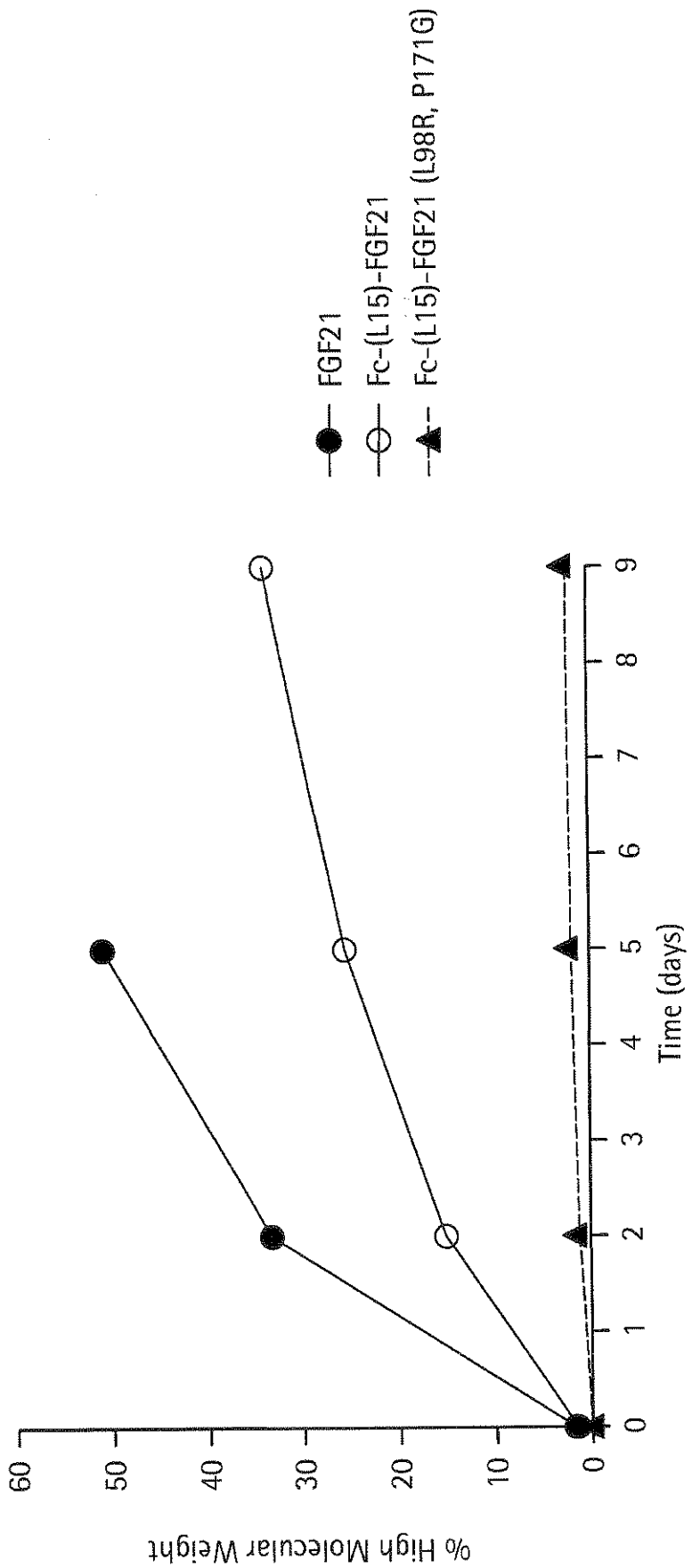

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (L98R, P171G)
% High Molecular Weight
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
9
Time (days)

FIG. 31B

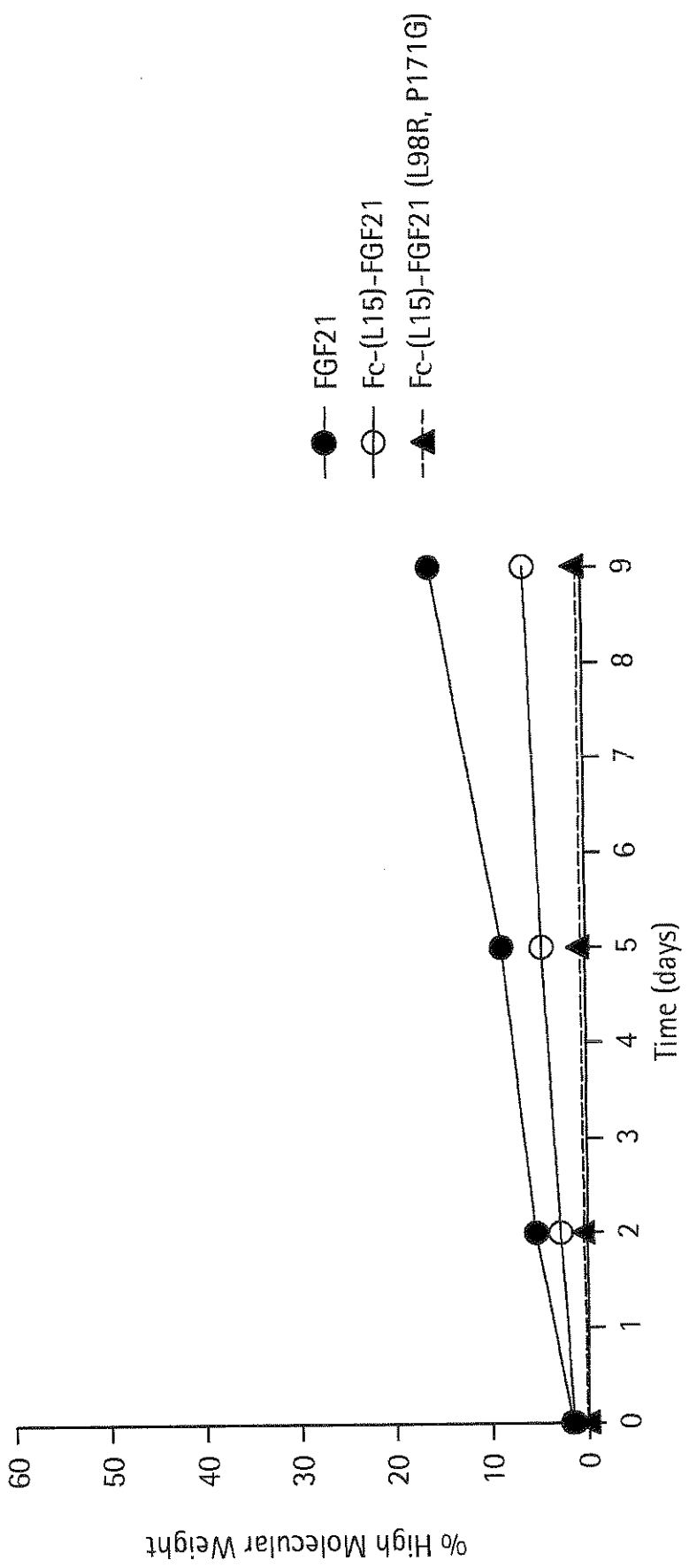

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (L98R, P171G)
% High Molecular Weight
0
10
20
30
40
50
60
Time (days)
0
1
2
3
4
5
6
7
8
9

FIG. 32

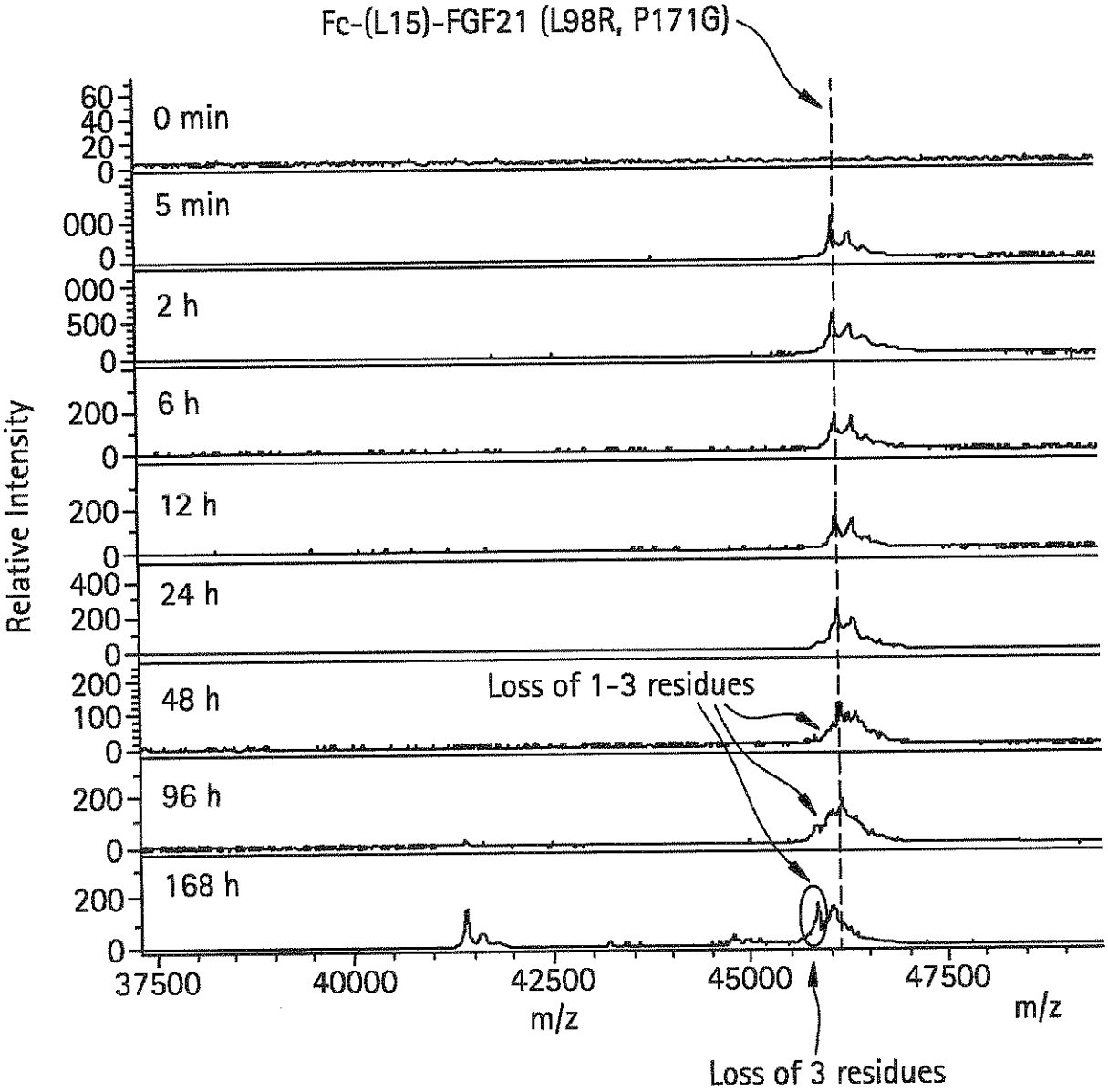

Fc-(L15)-FGF21 (L98R, P171G)
0 min
5 min
2 h
6 h
12 h
24 h
48 h
96 h
168 h
Loss of 1-3 residues
Loss of 3 residues
Relative Intensity
m/z
m/z
37500
40000
42500
45000
47500

FIG. 33

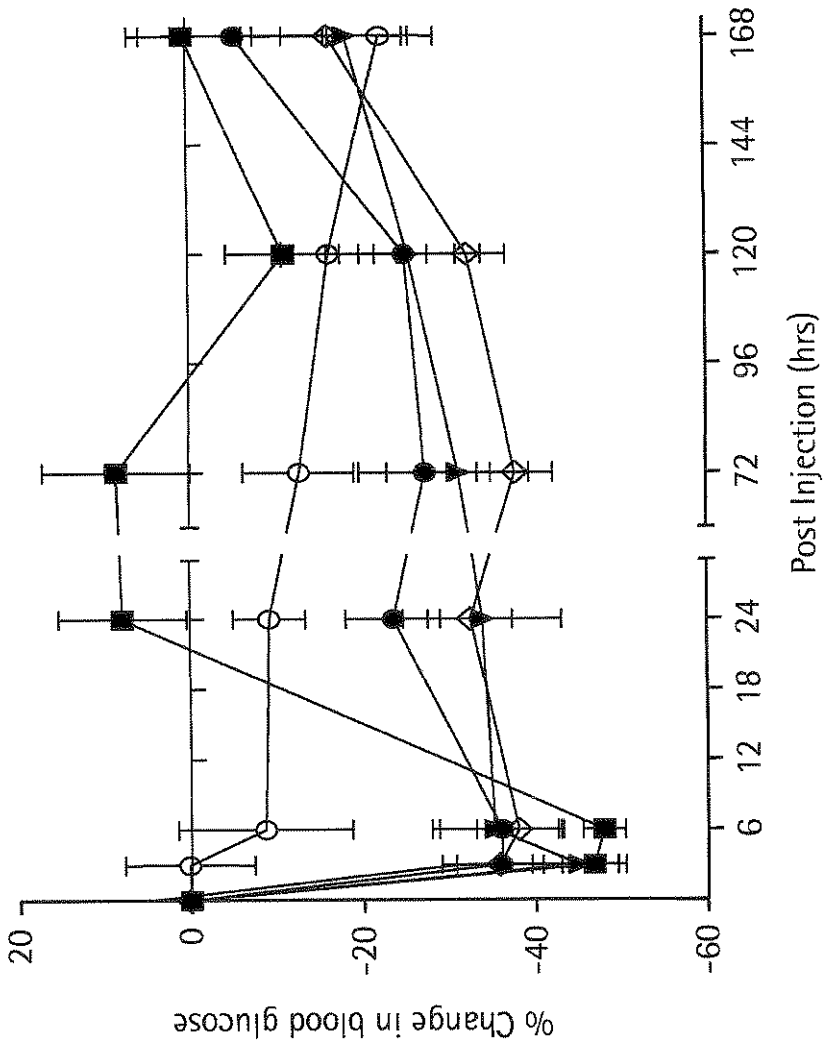

PBS 100uL
FGF21
Fc-(L15)-FGF21 (L98R,P171G)
Fc-(L15)-FGF21 (L98R,P171G,182P)
Fc-(L15)-FGF21 (L98R,P171G,182G)
% Change in blood glucose
20
0
-20
-40
-60
6
12
18
24
72
96
120
144
168
Post Injection (hrs)

FIG. 34

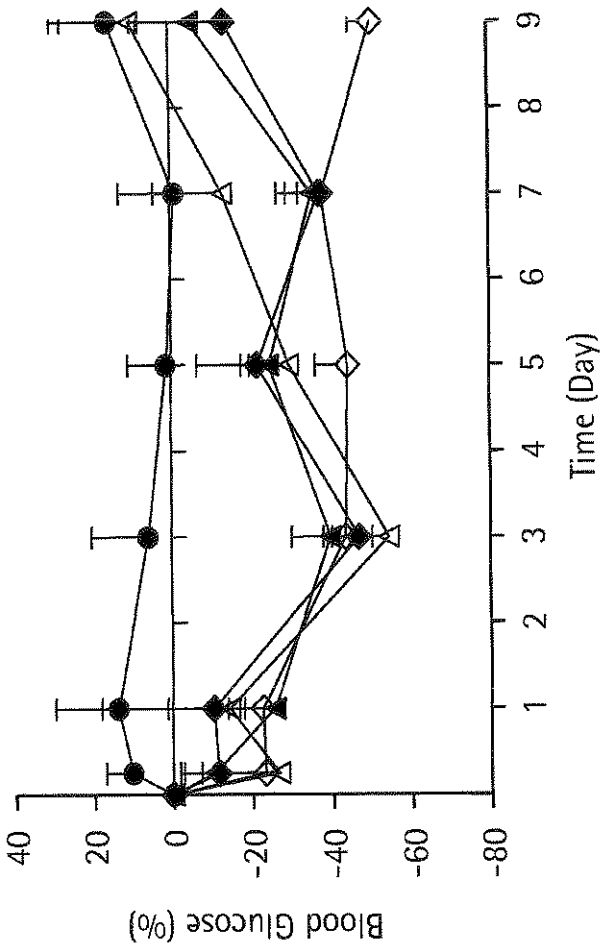

Veh
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(L15)-FGF21 (L98R, P171G, 182G, 183G)
Fc-(L15)-FGF21 (L98R, P171G, 182G)
Fc-(L15)-FGF21 (L98R, P171G, 182P)
Blood Glucose (%)
40
20
0
-20
-40
-60
-80
1
2
3
4
5
6
7
8
9
Time (Day)

FIG. 35

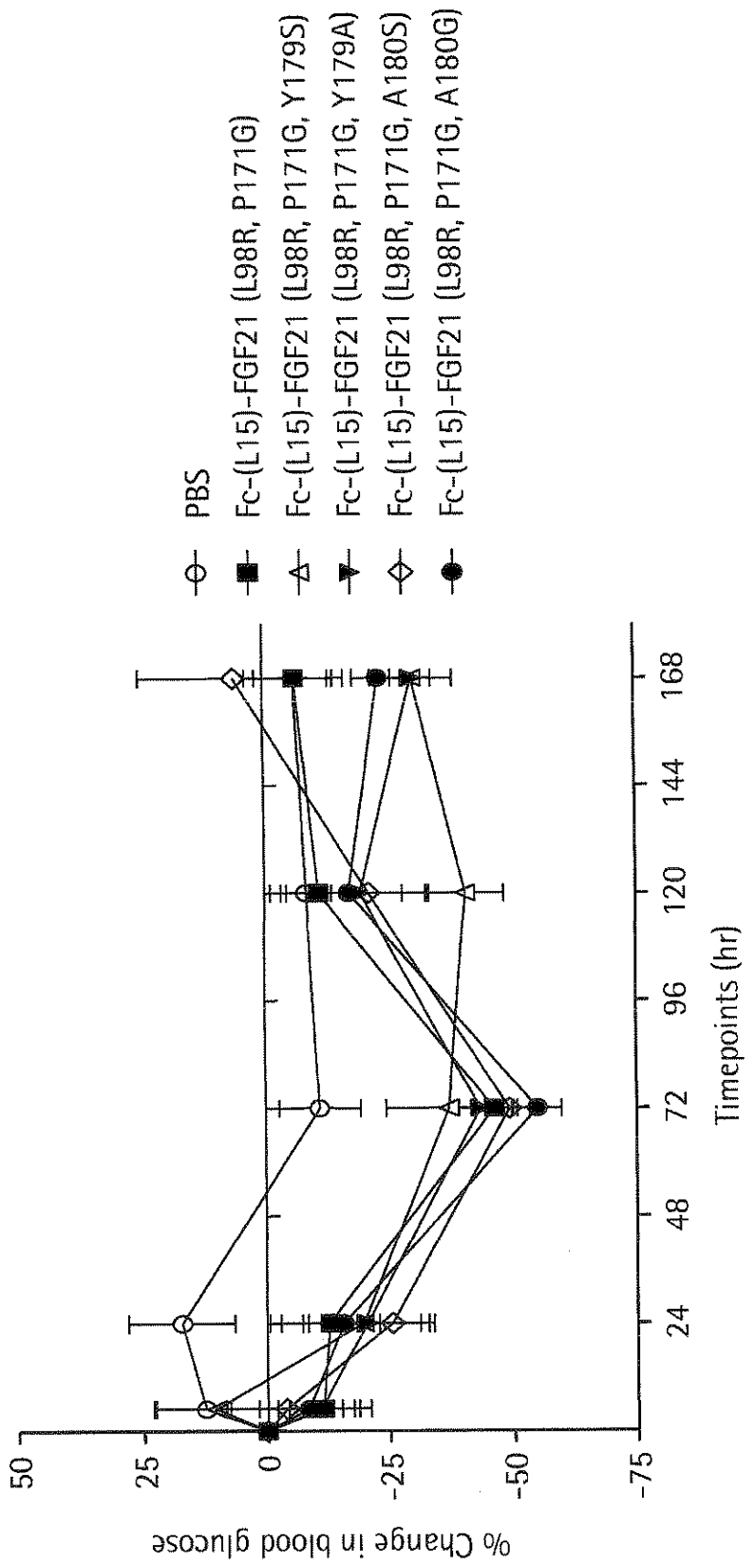

PBS
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(L15)-FGF21 (L98R, P171G, Y179S)
Fc-(L15)-FGF21 (L98R, P171G, Y179A)
Fc-(L15)-FGF21 (L98R, P171G, A180S)
Fc-(L15)-FGF21 (L98R, P171G, A180G)
% Change in blood glucose
50
25
0
-25
-50
-75
24
48
72
96
120
144
168
Timepoints (hr)

FIG. 36

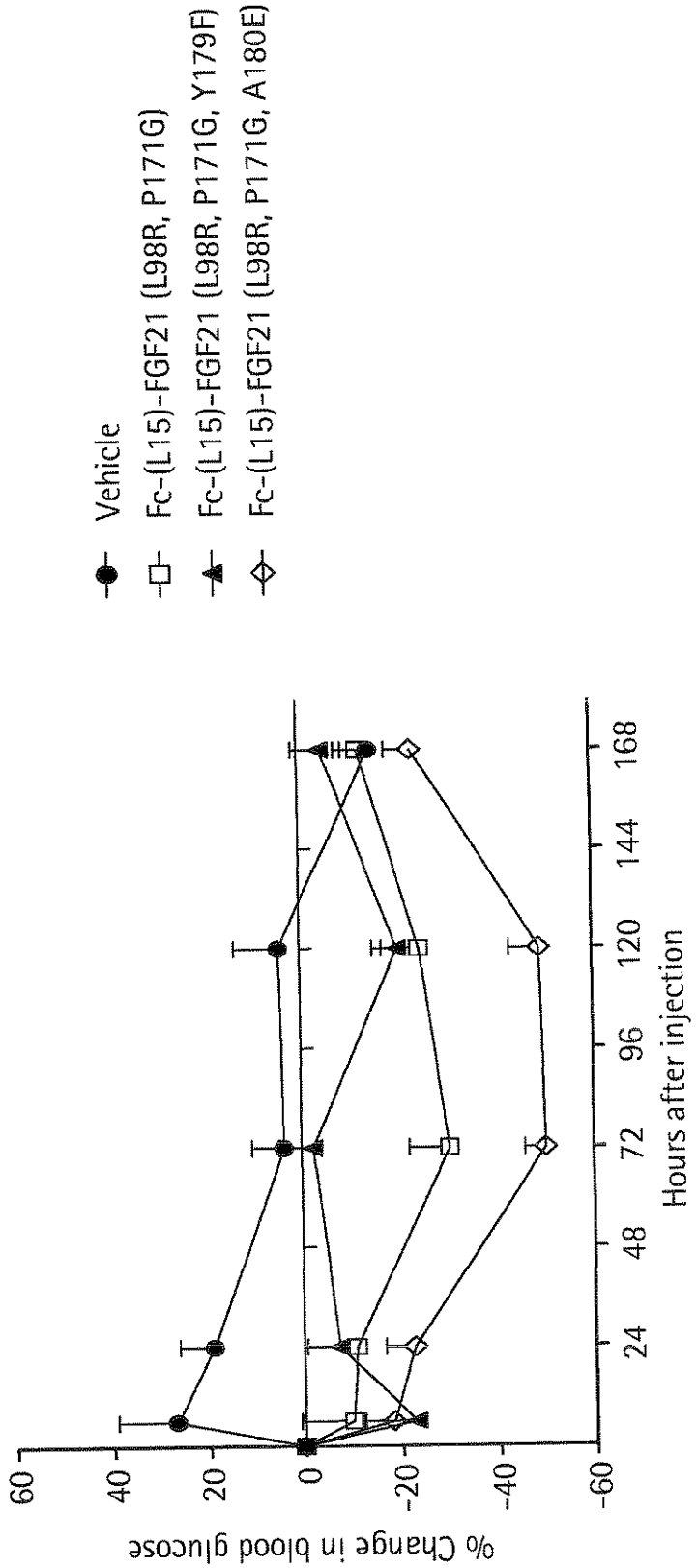

Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(L15)-FGF21 (L98R, P171G, Y179F)
Fc-(L15)-FGF21 (L98R, P171G, A180E)
% Change in blood glucose
60
40
20
0
-20
-40
-60
24
48
72
96
120
144
168
Hours after injection

FIG. 37

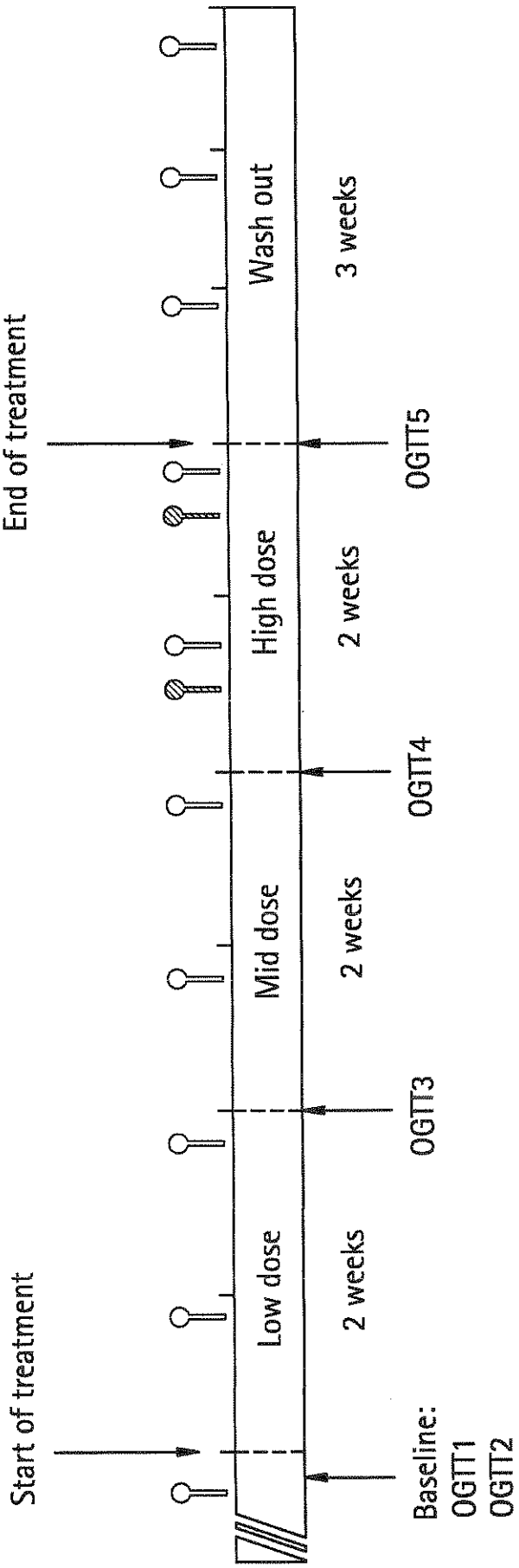

Start of treatment
End of treatment
Low dose
Mid dose
High dose
Wash out
2 weeks
2 weeks
2 weeks
3 weeks
Baseline:
OGTT1
OGTT2
OGTT3
OGTT4
OGTT5

FIG. 38A

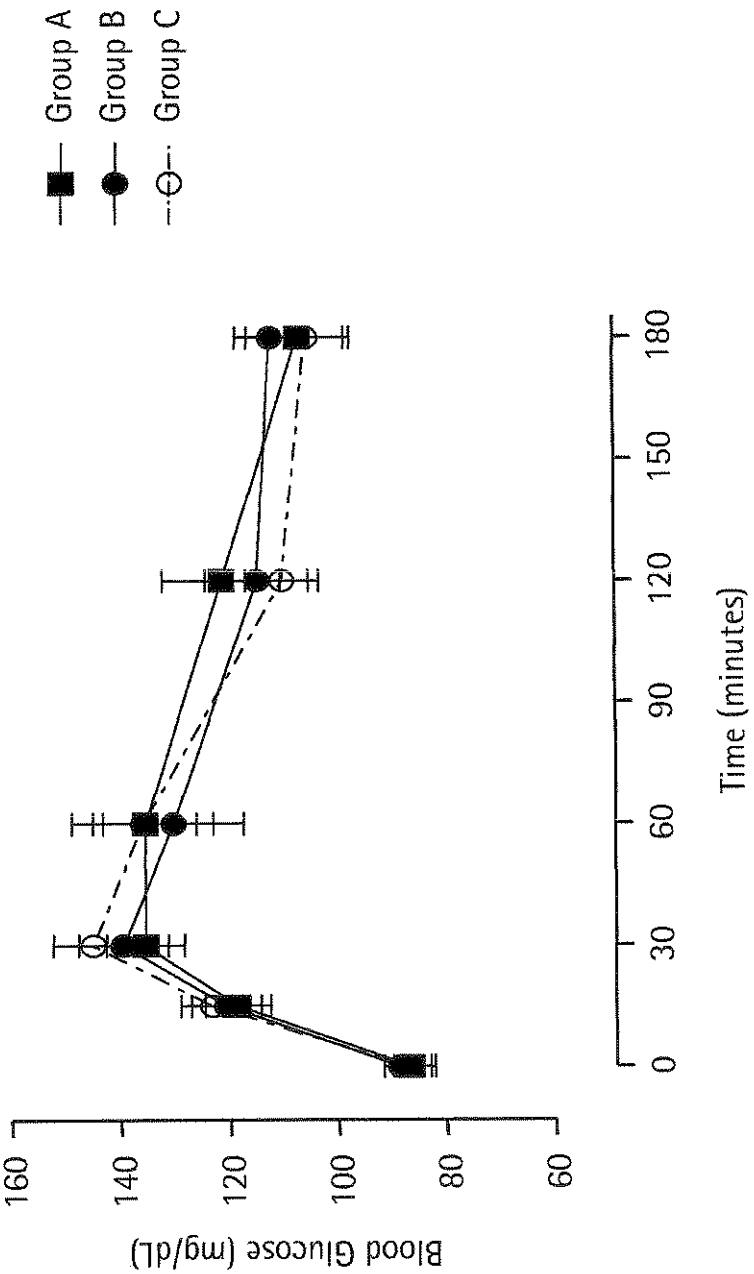

Group A
Group B
Group C
Blood Glucose (mg/dL)
160
140
120
100
80
60
0
30
60
90
120
150
180
Time (minutes)

FIG. 38B

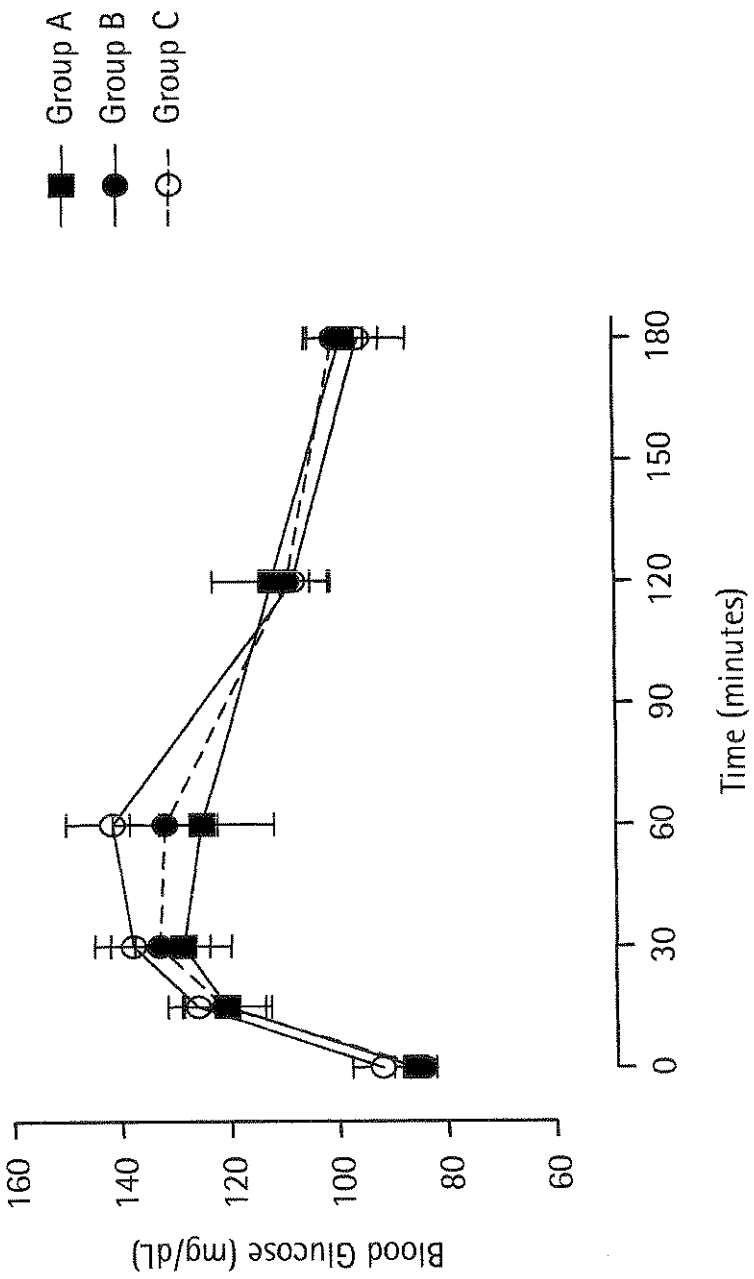

Group A
Group B
Group C
Blood Glucose (mg/dL)
160
140
120
100
80
60
0
30
60
90
120
150
180
Time (minutes)

FIG. 38C

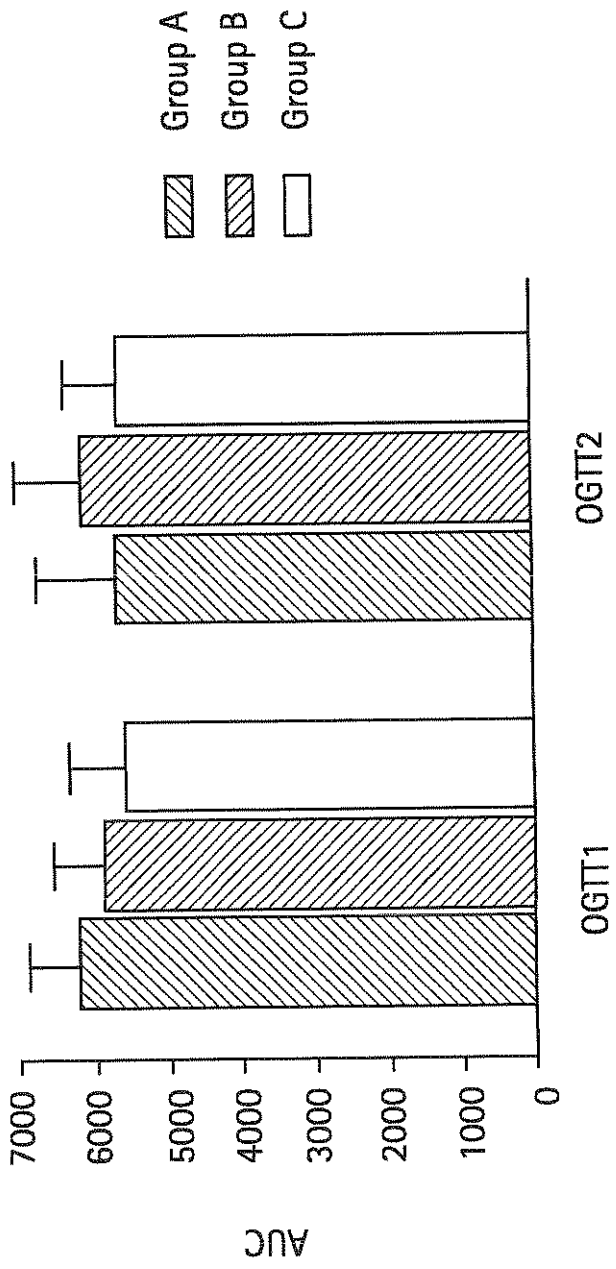

Group A
Group B
Group C
AUC
7000
6000
5000
4000
3000
2000
1000
0
OGTT1
OGTT2

FIG. 38D

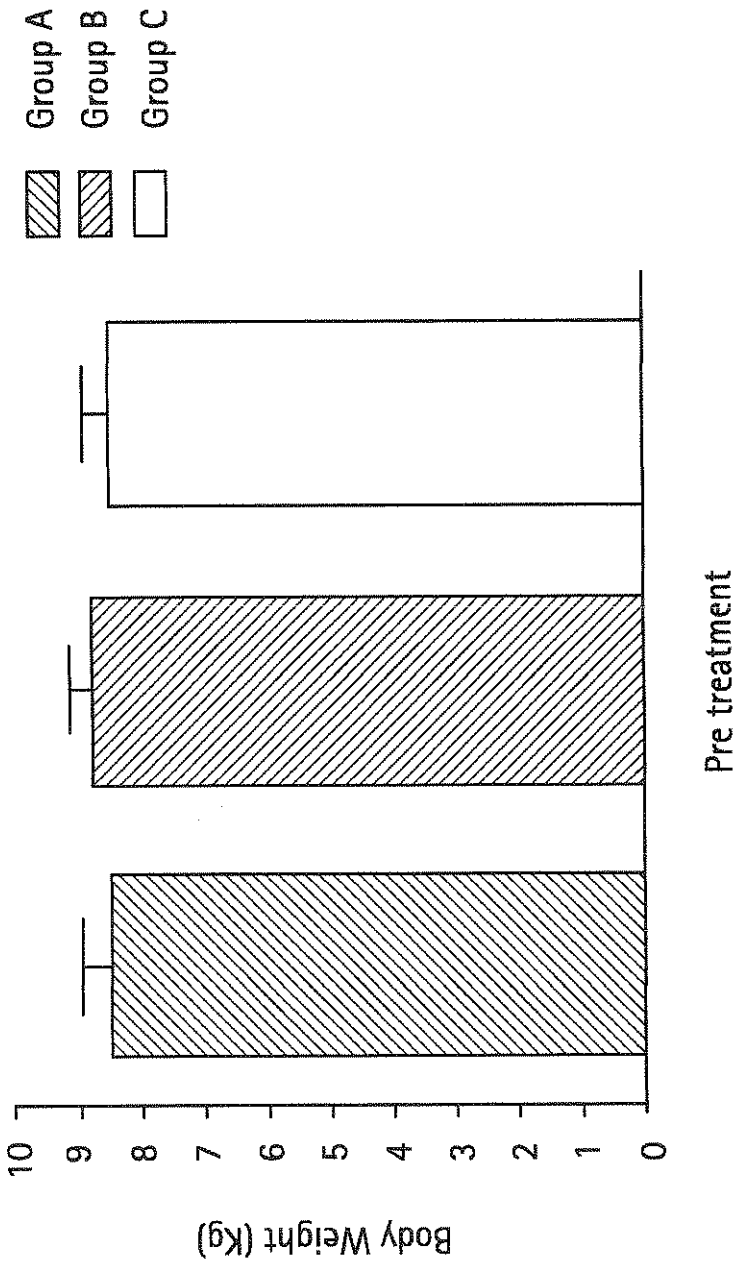

Group A
Group B
Group C
Body Weight (Kg)
0
1
2
3
4
5
6
7
8
9
10
Pre treatment

FIG. 39

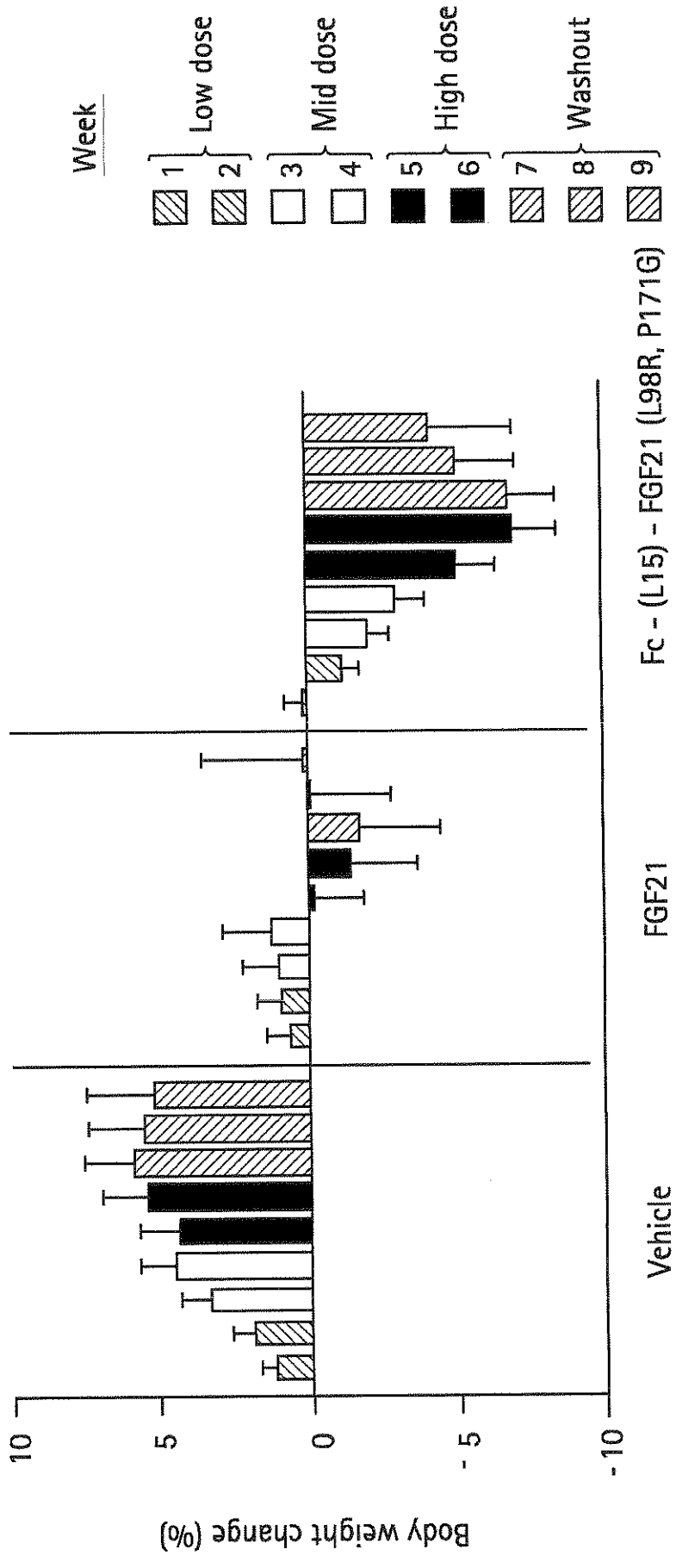

Week
1
2
Low dose
3
4
Mid dose
5
6
High dose
7
8
9
Washout
Vehicle
FGF21
Fc - (L15) - FGF21 (L98R, P171G)
10
5
0
- 5
- 10
Body weight change (%)

FIG. 40

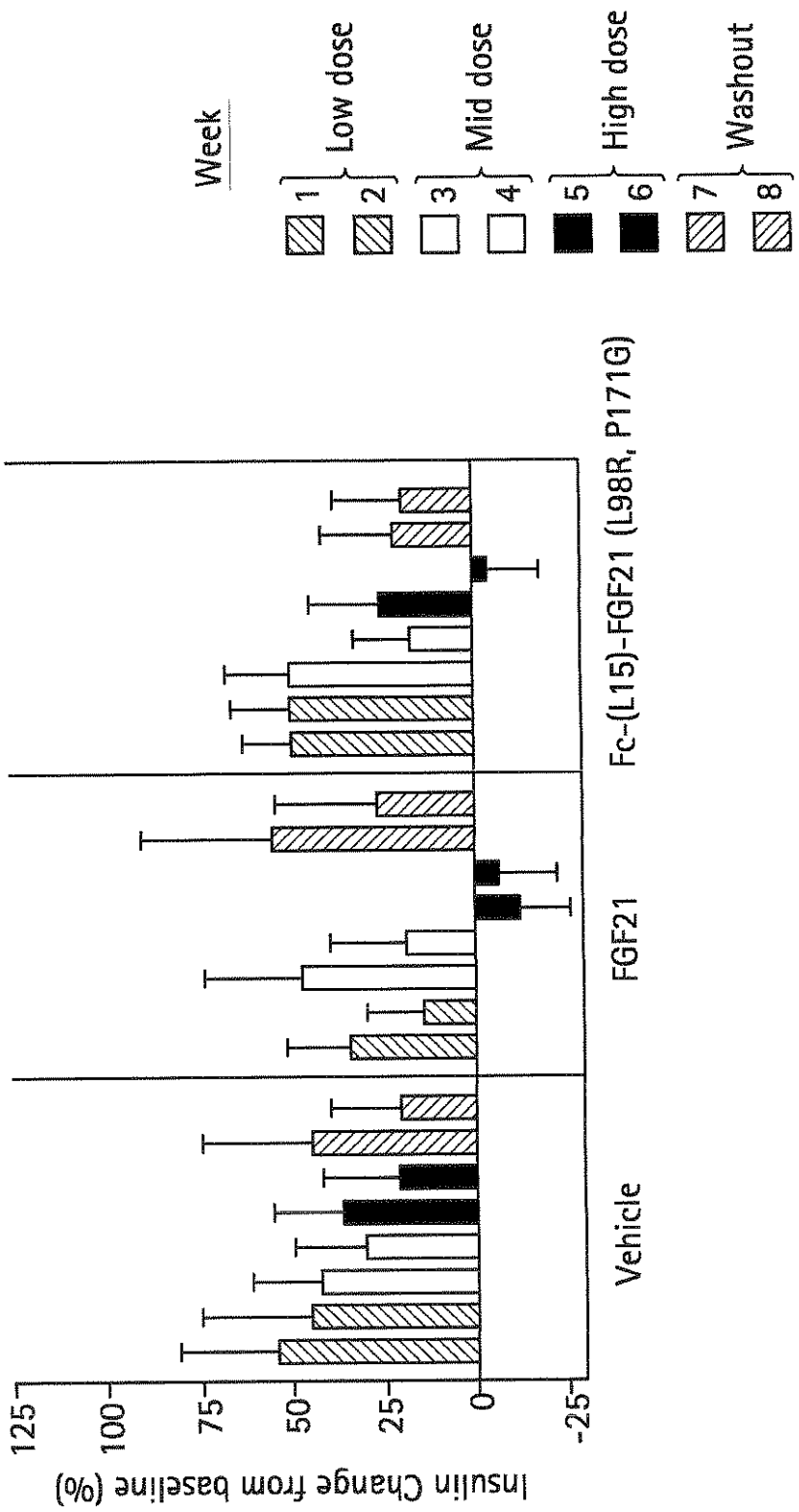

Week
1
2
Low dose
3
4
Mid dose
5
6
High dose
7
8
Washout
Insulin Change from baseline (%)
125
100
75
50
25
0
-25
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 41

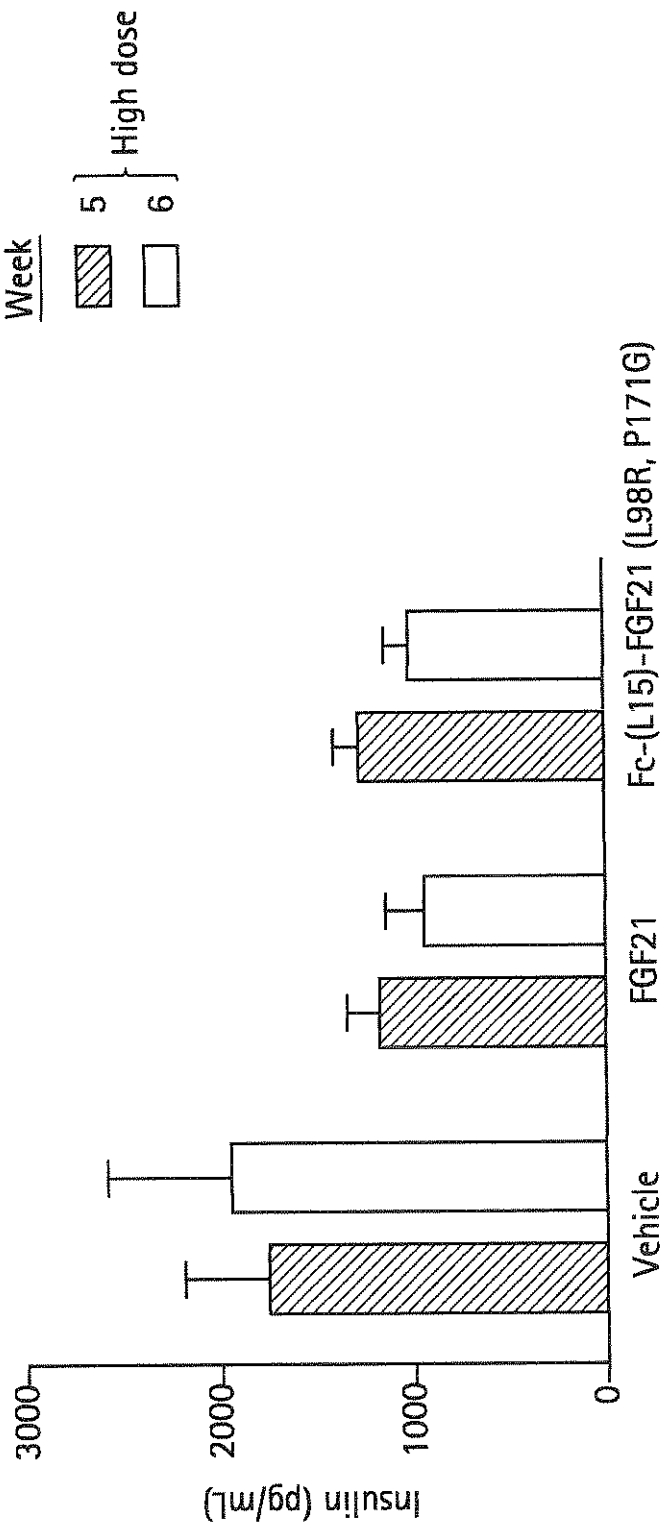

Week
5
6
High dose
Insulin (pg/mL)
3000
2000
1000
0
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 42

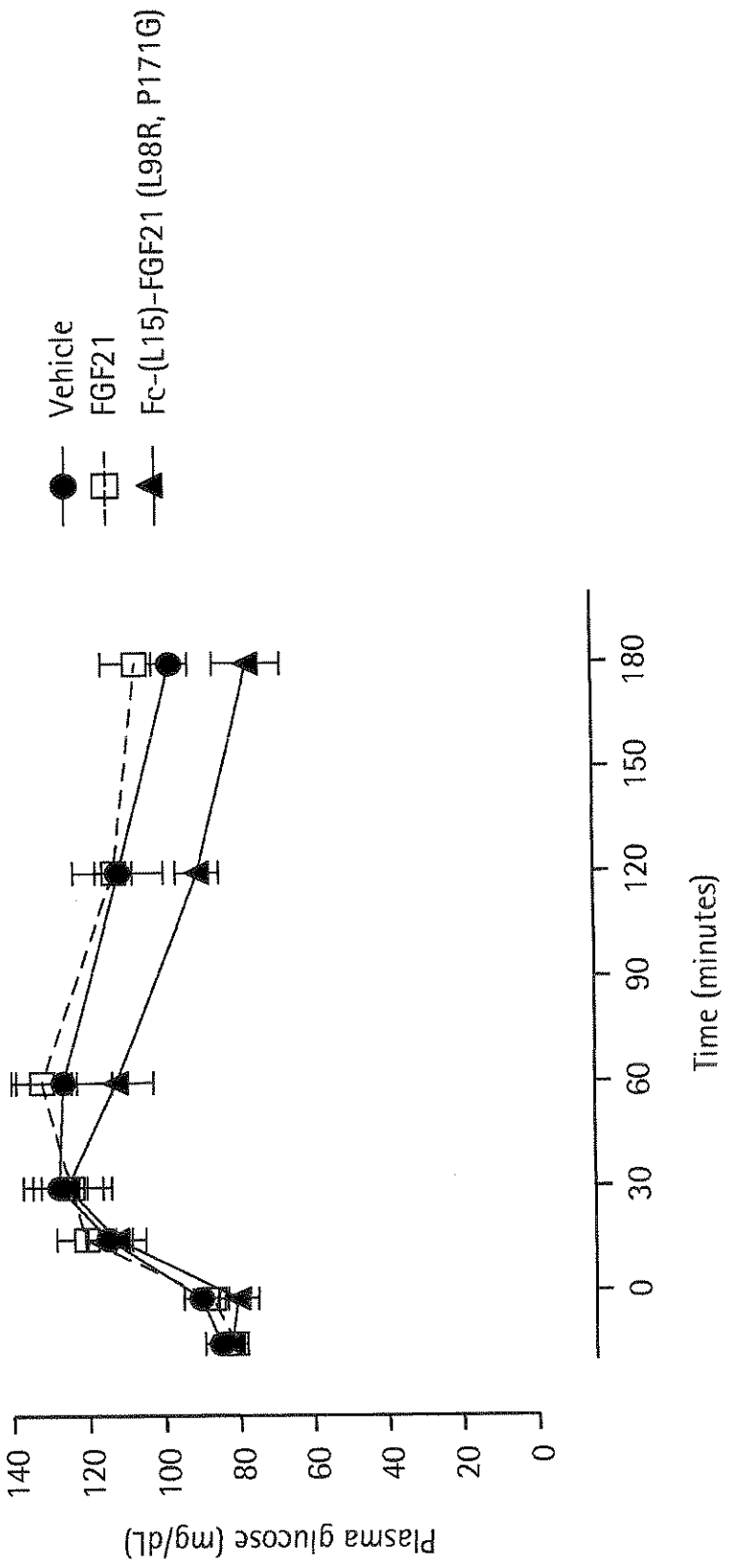

Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)
Plasma glucose (mg/dL)
0
20
40
60
80
100
120
140
Time (minutes)
0
30
60
90
120
150
180

FIG. 43

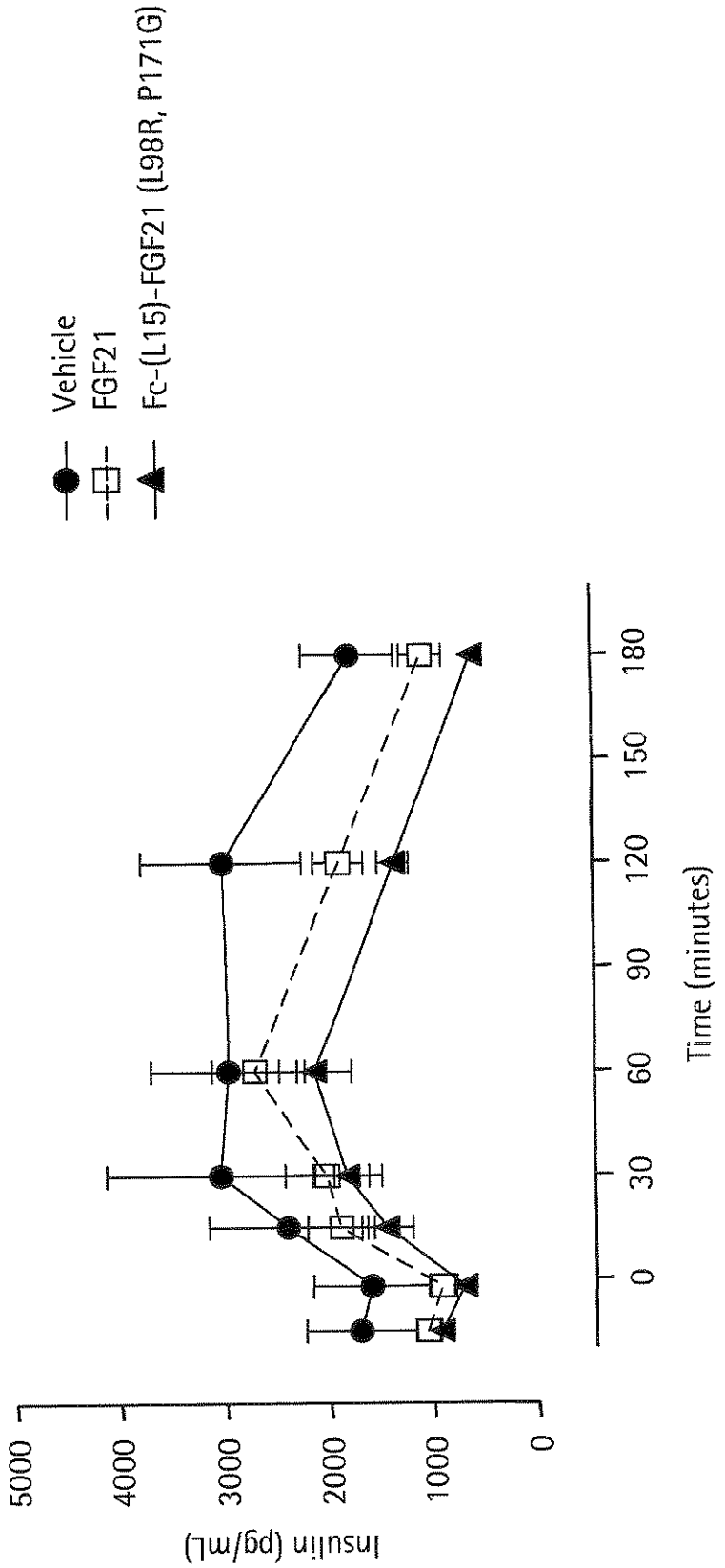

Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)
Insulin (pg/mL)
5000
4000
3000
2000
1000
0
0
30
60
90
120
150
180
Time (minutes)

FIG. 44

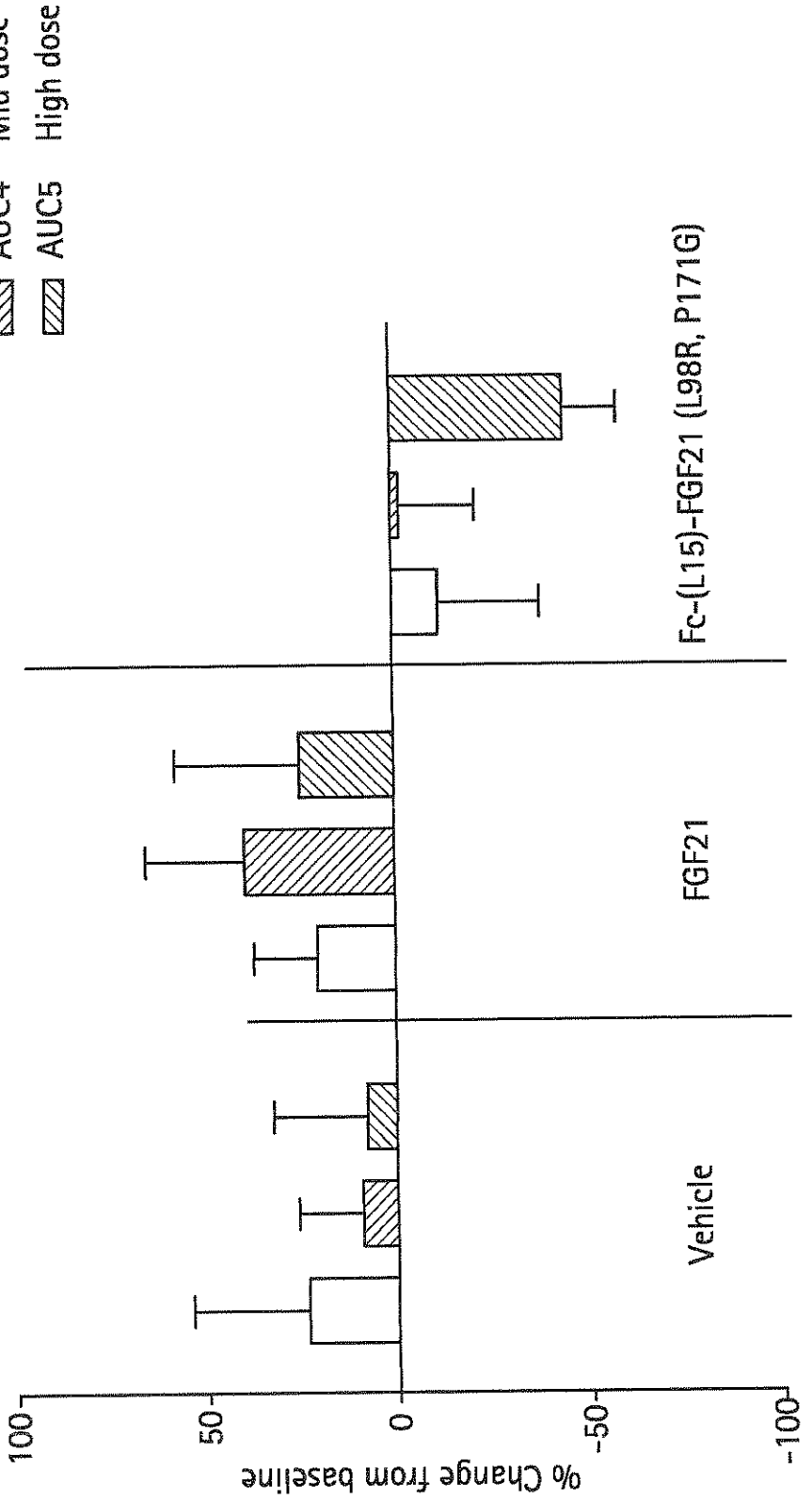

AUC3 Low dose
AUC4 Mid dose
AUC5 High dose
% Change from baseline
100
50
0
-50
-100
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 45

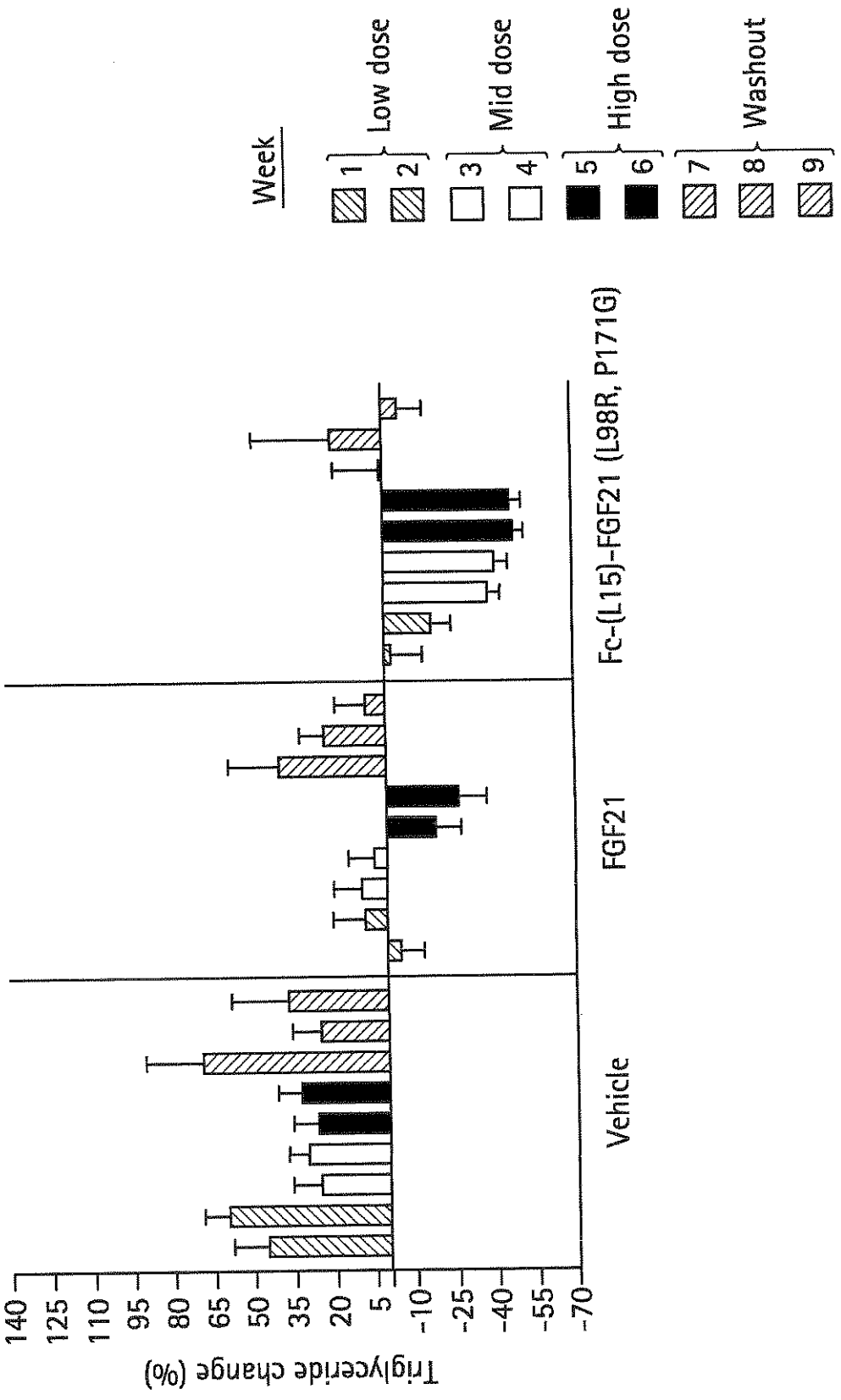

Week
1
2
Low dose
3
4
Mid dose
5
6
High dose
7
8
9
Washout
Triglyceride change (%)
140
125
110
95
80
65
50
35
20
5
-10
-25
-40
-55
-70
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 46

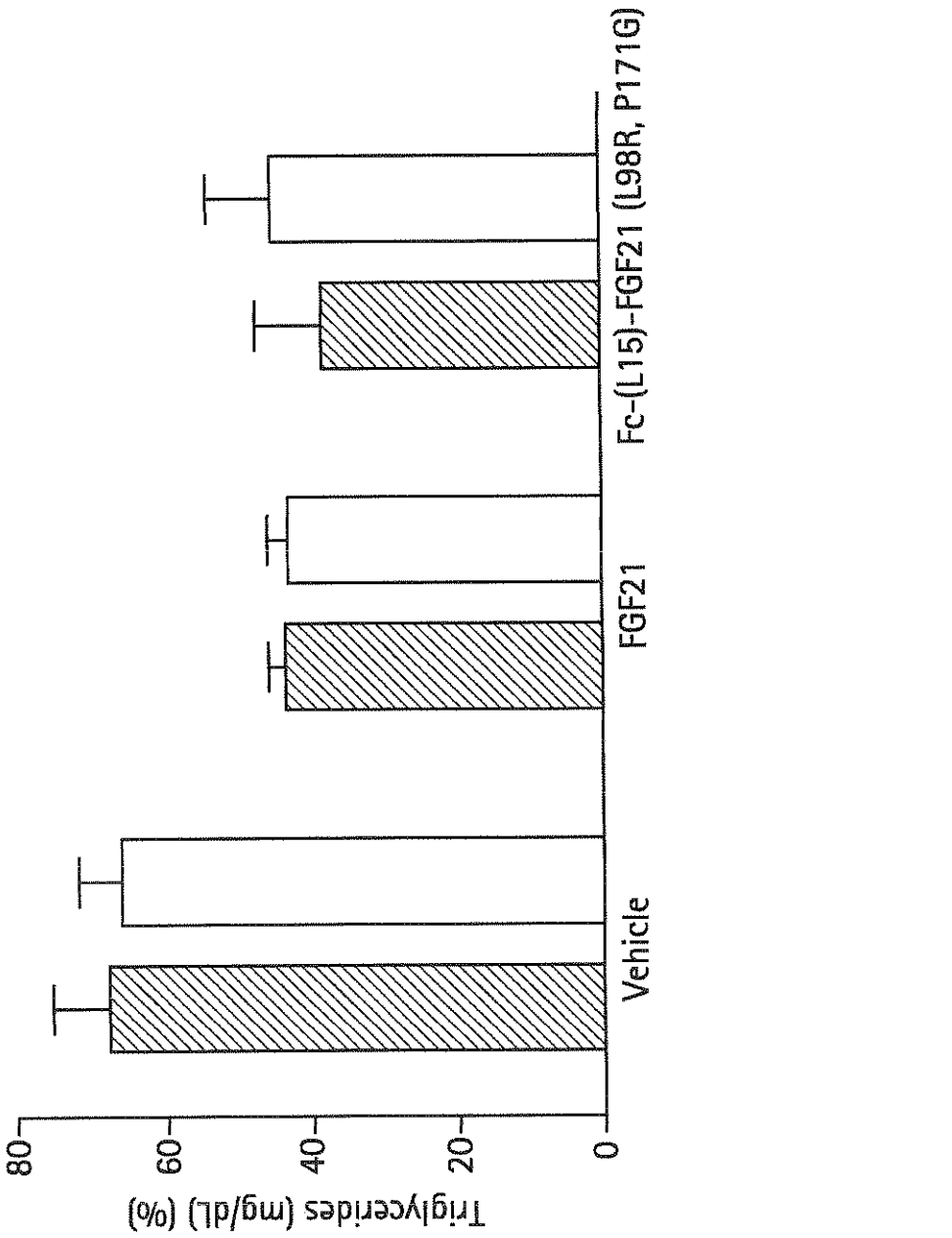

Week
5
6
Triglycerides (mg/dL) (%)
0
20
40
60
80
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 47

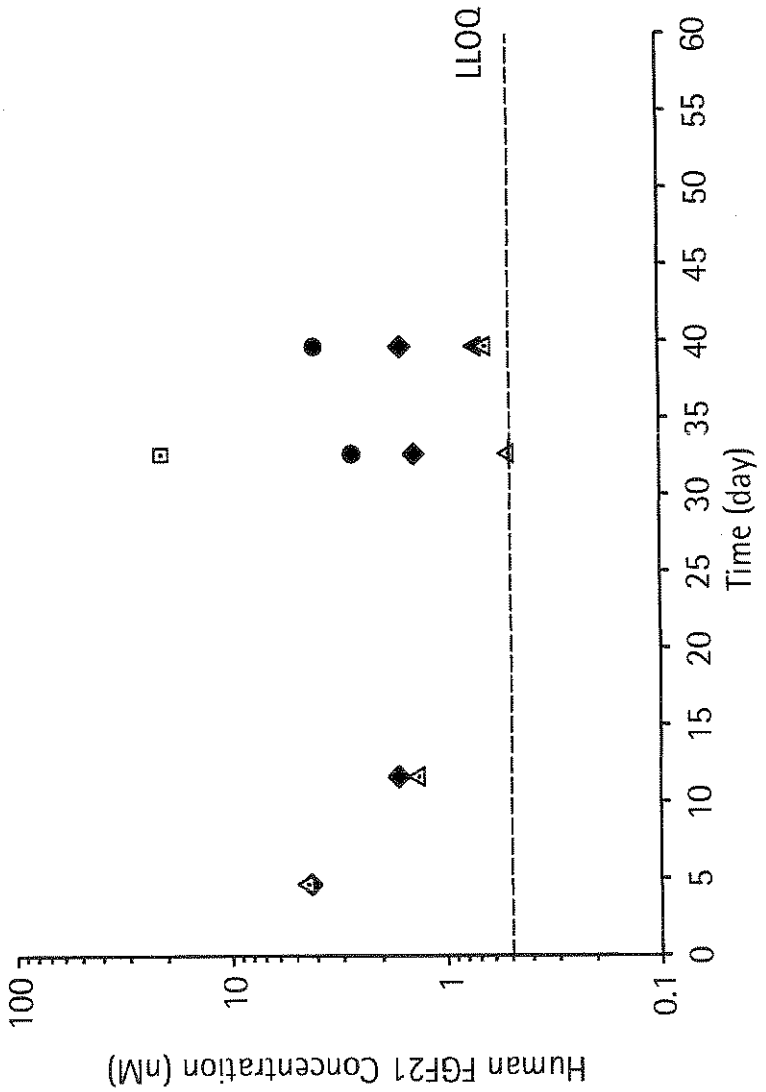

Human FGF21 Concentration (nM)
100
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (day)
LLOQ

FIG. 48

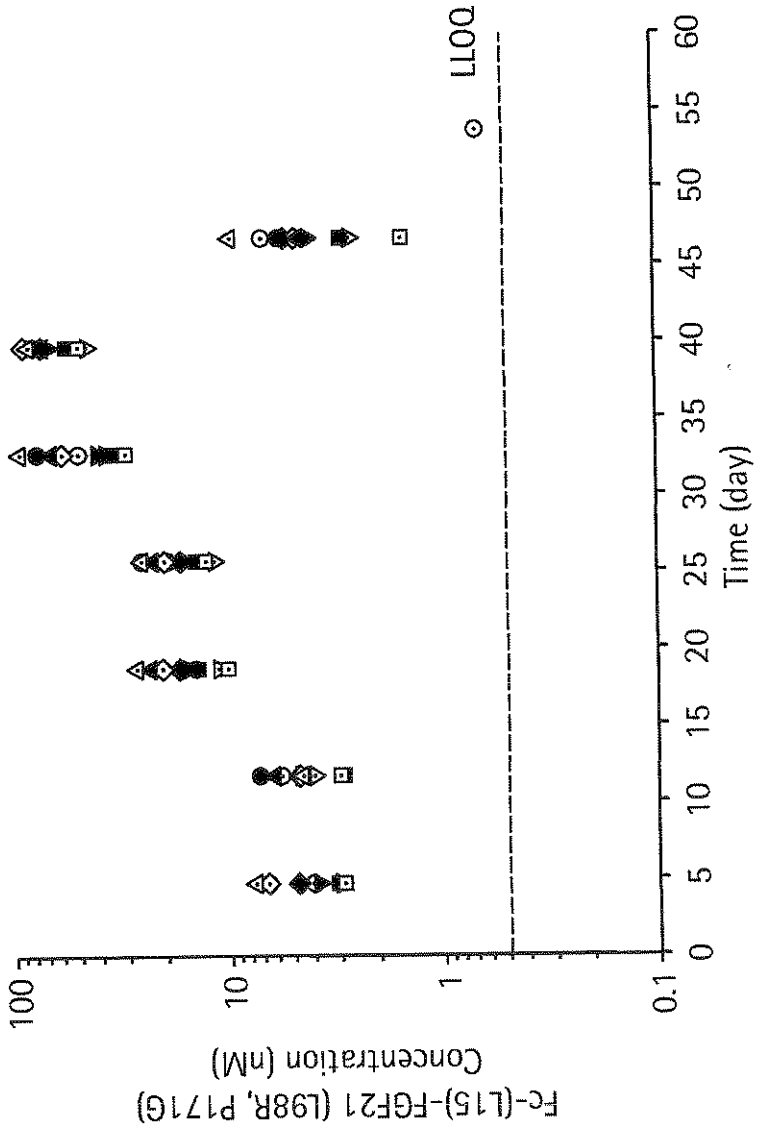

Fc-(L15)-FGF21 (L98R, P171G)
Concentration (nM)
100
10
1
0.1
LLOQ
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (day)

FIG. 49

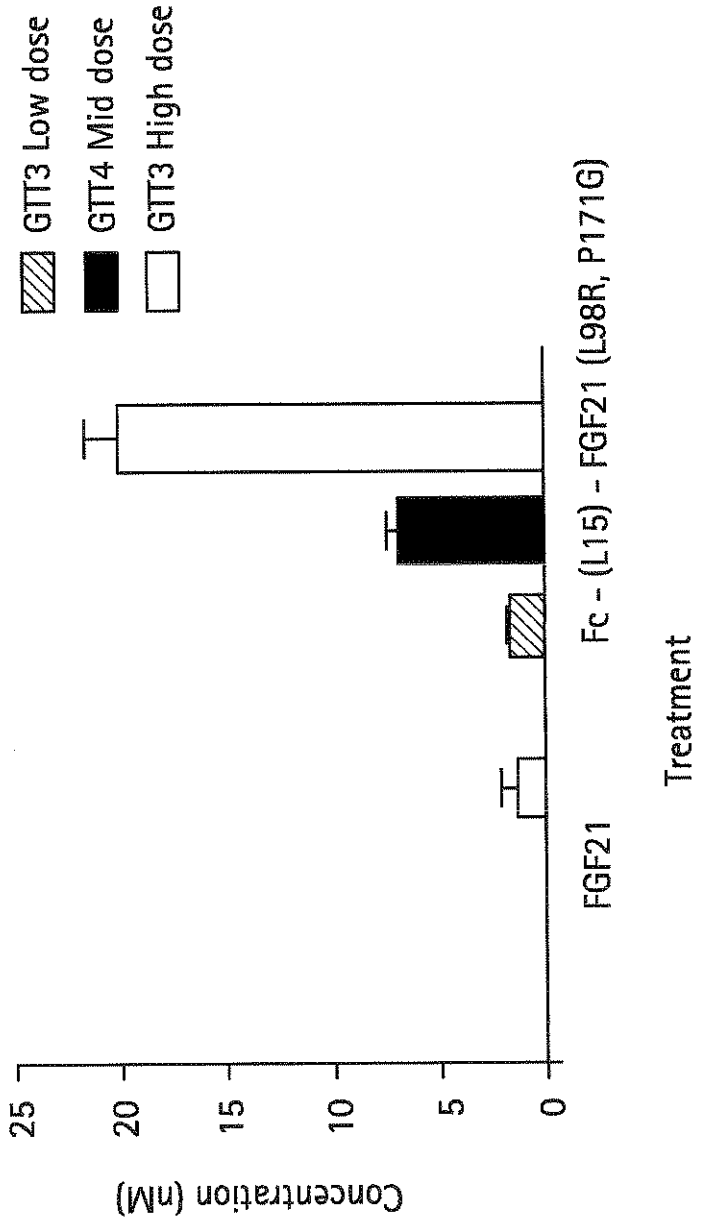
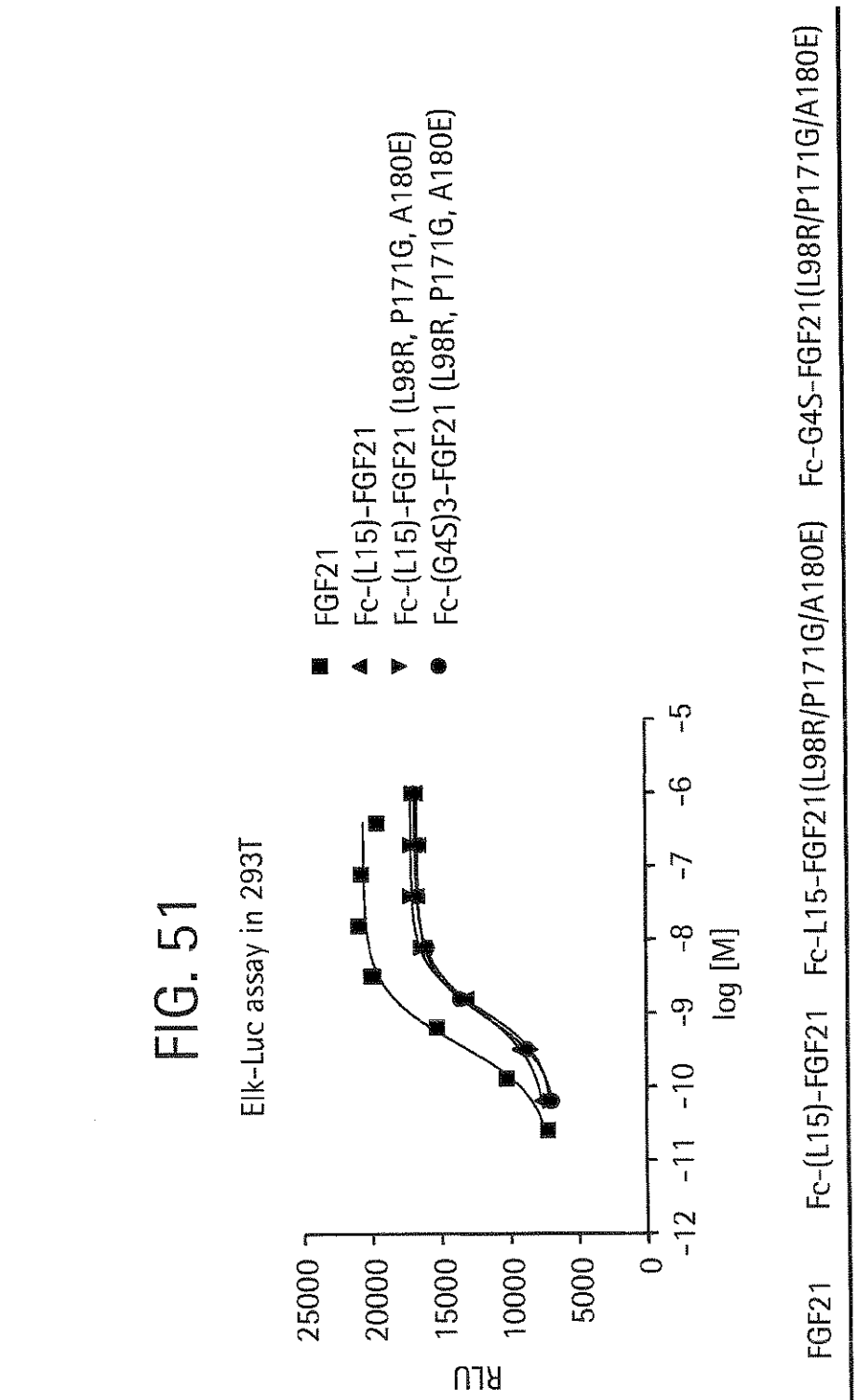

GTT3 Low dose
GTT4 Mid dose
GTT3 High dose
Concentration (nM)
25
20
15
10
5
0
FGF21
Fc - (L15) - FGF21 (L98R, P171G)
Treatment

FIG. 50

Fc-(G4S)3-FGF21 (L98R, P171G, A190E)

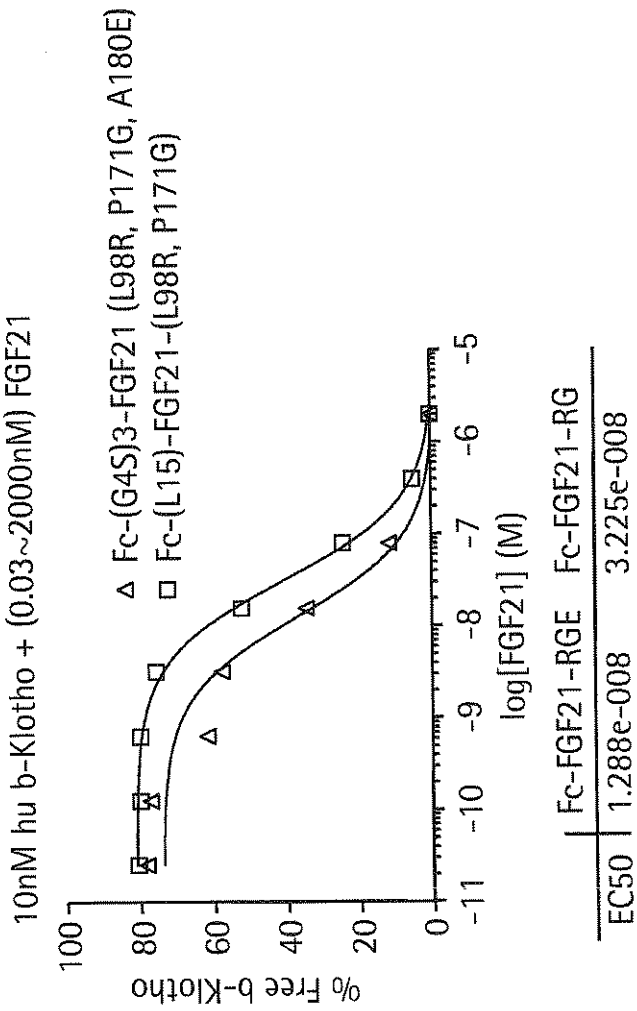
MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGG*
*GGSH*PIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRE**R**LLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVG**G**SQGRSPSYE**S**

FIG. 51



	FGF21	Fc-(L15)-FGF21	Fc-L15-FGF21(L98R/P171G/A180E)	Fc-G4S-FGF21(L98R/P171G/A180E)
EC50	3.831e-010	1.034e-009	1.095e-009	8.515e-010

FIG. 52


10nM hu b-Klotho + (0.03~2000nM) FGF21
Δ Fc-(G4S)3-FGF21 (L98R, P171G, A180E)
□ Fc-(L15)-FGF21-(L98R, P171G)
% Free b-Klotho
log[FGF21] (M)
Fc-FGF21-RGE Fc-FGF21-RG
EC50 1.288e-008 3.225e-008

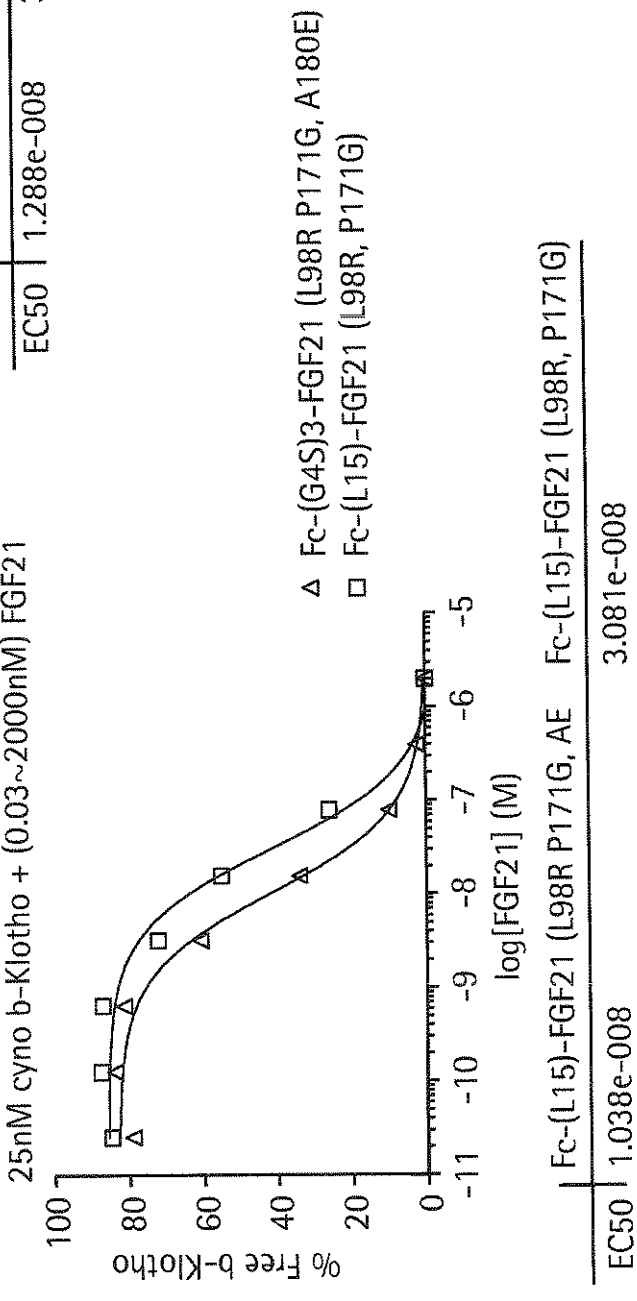

25nM cyno b-Klotho + (0.03~2000nM) FGF21
Δ Fc-(G4S)3-FGF21 (L98R P171G, A180E)
□ Fc-(L15)-FGF21 (L98R, P171G)
% Free b-Klotho
log[FGF21] (M)
Fc-(L15)-FGF21 (L98R P171G, AE Fc-(L15)-FGF21 (L98R, P171G)
EC50 1.038e-008 3.081e-008

FIG. 53A

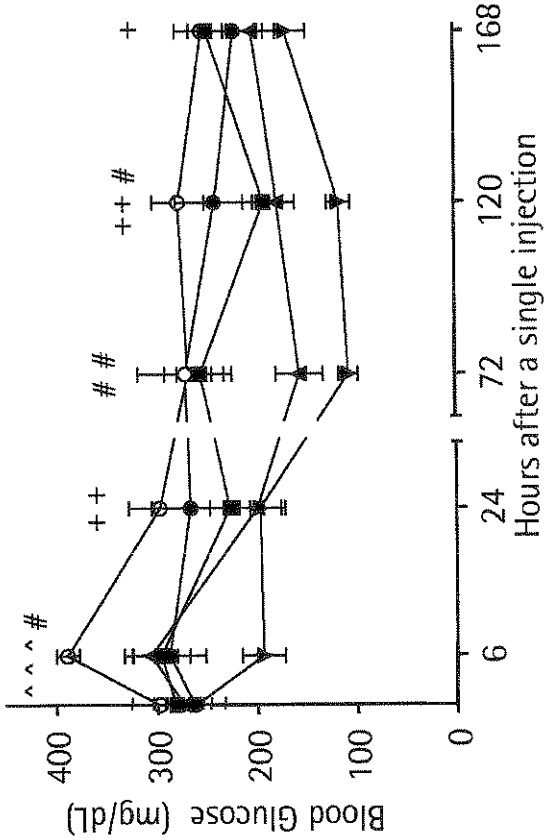


FIG. 53B

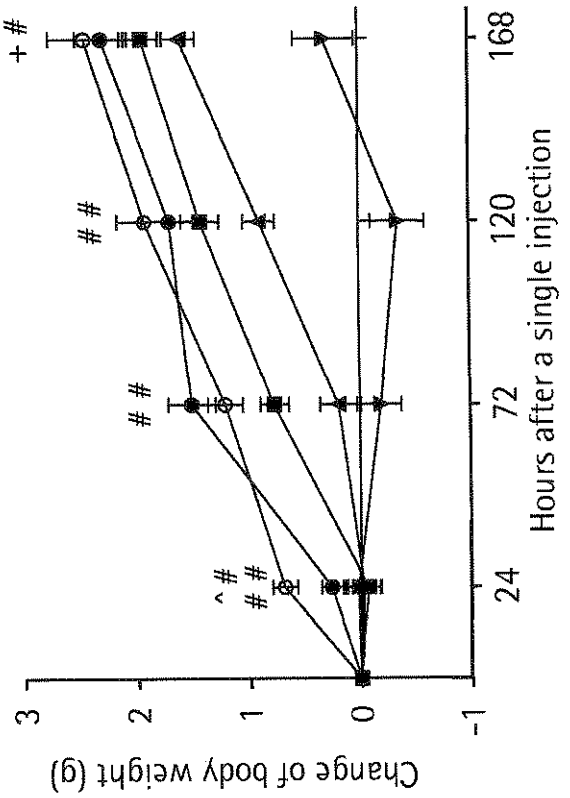


FIG. 54

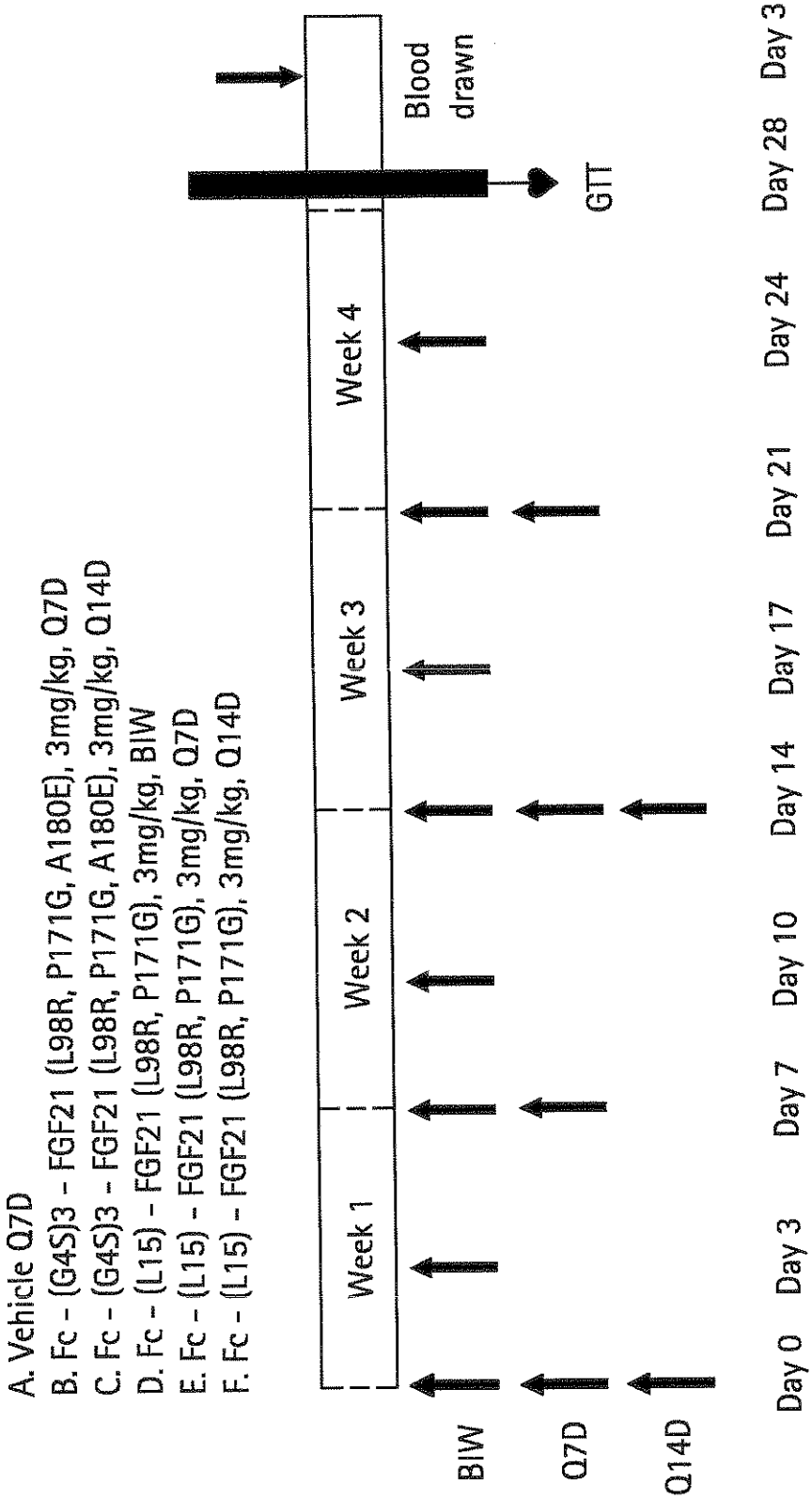

A. Vehicle Q7D
B. Fc - (G4S)3 - FGF21 (L98R, P171G, A180E), 3mg/kg, Q7D
C. Fc - (G4S)3 - FGF21 (L98R, P171G, A180E), 3mg/kg, Q14D
D. Fc - (L15) - FGF21 (L98R, P171G), 3mg/kg, BIW
E. Fc - (L15) - FGF21 (L98R, P171G), 3mg/kg, Q7D
F. Fc - (L15) - FGF21 (L98R, P171G), 3mg/kg, Q14D
Week 1
Week 2
Week 3
Week 4
Blood drawn
GTT
BIW
Q7D
Q14D
Day 0 Day 3 Day 7 Day 10 Day 14 Day 17 Day 21 Day 24 Day 28 Day 31
Buffer: 10mM Tris, 2.2% sucrose, 3.3% Sorbitol; pH 8.5

FIG. 55

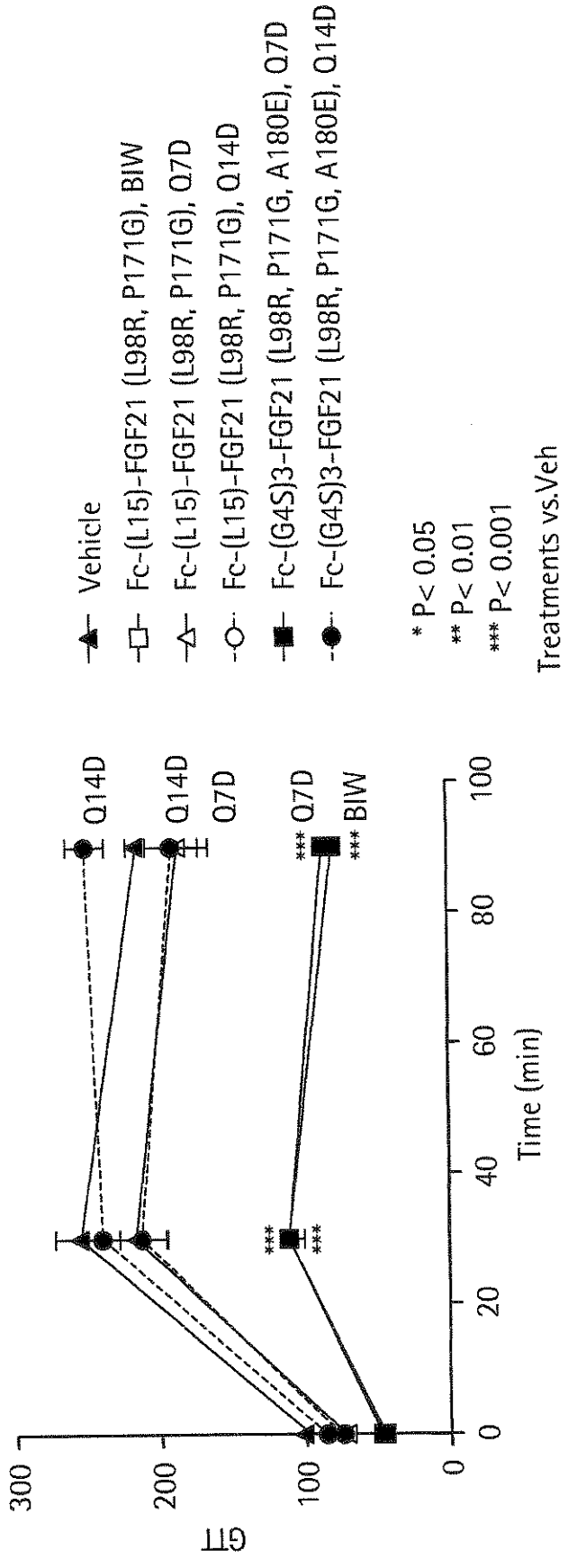

Vehicle
Fc-(L15)-FGF21 (L98R, P171G), BIW
Fc-(L15)-FGF21 (L98R, P171G), Q7D
Fc-(L15)-FGF21 (L98R, P171G), Q14D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q7D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q14D
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs.Veh
GTT
300
200
100
0
0
20
40
60
80
100
Time (min)
Q14D
Q14D
Q7D
*** Q7D
*** BIW
***
***

FIG. 56

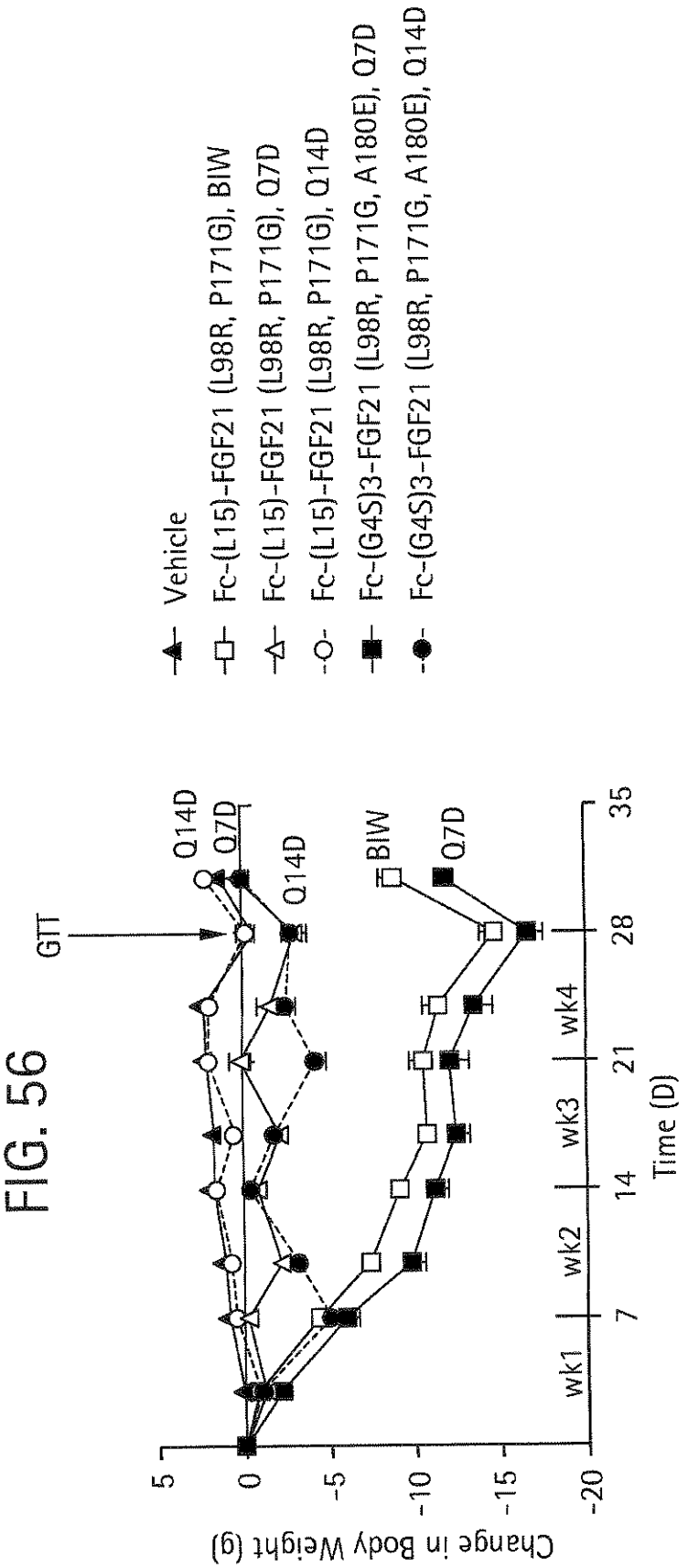

Vehicle
Fc-(L15)-FGF21 (L98R, P171G), BIW
Fc-(L15)-FGF21 (L98R, P171G), Q7D
Fc-(L15)-FGF21 (L98R, P171G), Q14D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q7D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q14D
GTT
Q14D
Q7D
Q14D
BIW
Q7D
5
0
-5
-10
-15
-20
wk1
wk2
wk3
wk4
7
14
21
28
35
Time (D)
Change in Body Weight (g)

FIG. 57

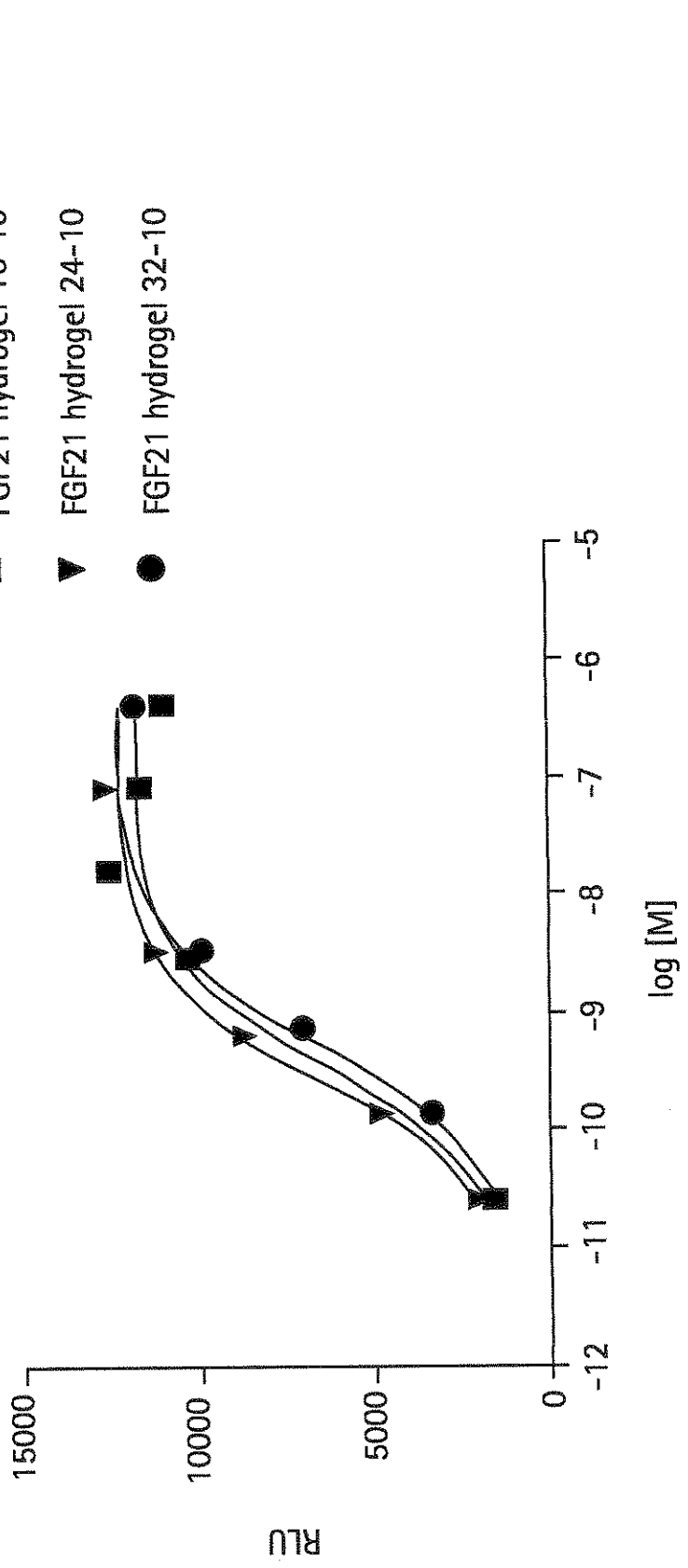

Fc - (L15) - FGF21 (L98R, P171G) ctrl
FGF21 hydrogel 16-10
FGF21 hydrogel 24-10
FGF21 hydrogel 32-10
RLU
15000
10000
5000
0
-12
-11
-10
-9
-8
-7
-6
-5
log [M]

FIG. 58

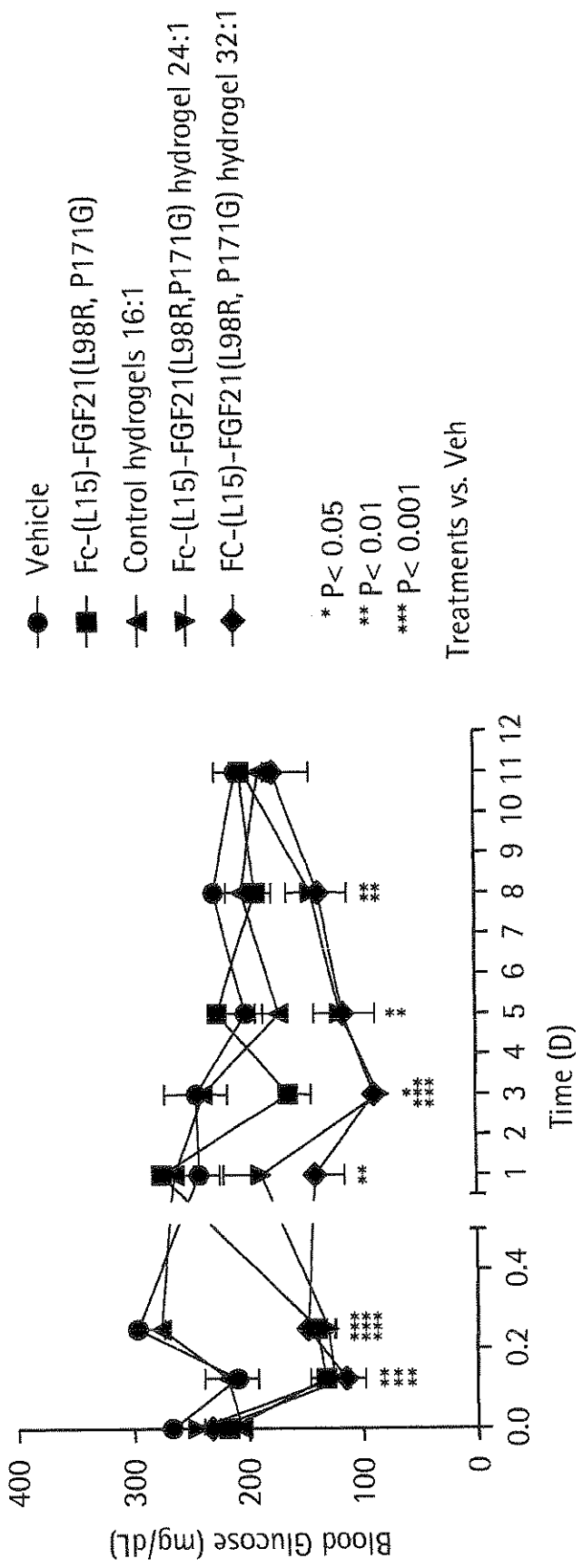

Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Control hydrogels 16:1
Fc-(L15)-FGF21(L98R,P171G) hydrogel 24:1
FC-(L15)-FGF21(L98R, P171G) hydrogel 32:1
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Blood Glucose (mg/dL)
400
300
200
100
0
0.0
0.2
0.4
1 2 3 4 5 6 7 8 9 10 11 12
Time (D)

FIG. 59

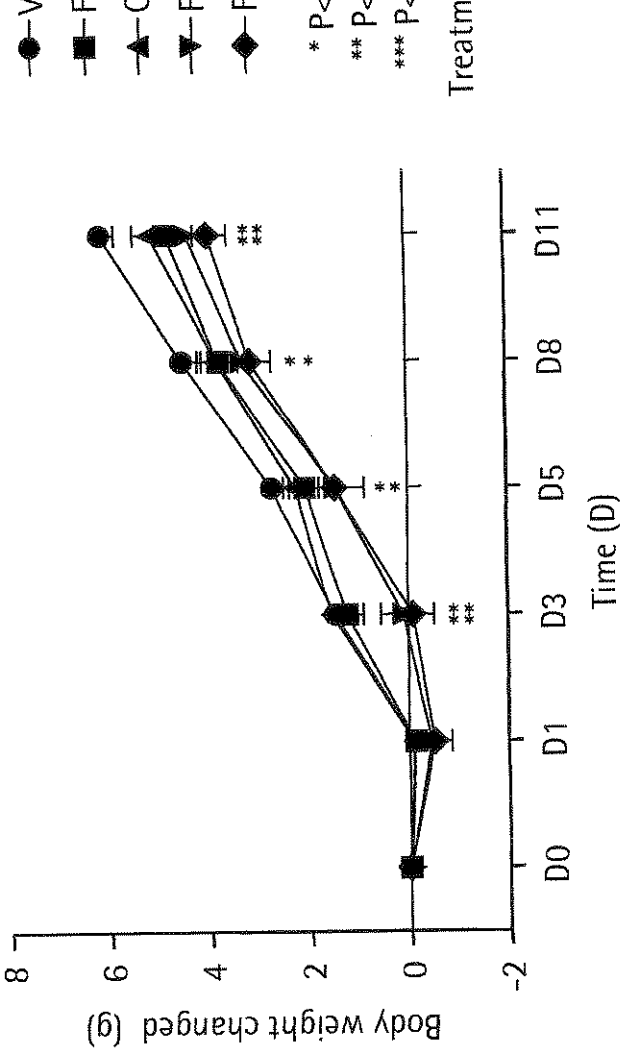

Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Control hydrogels 16:1
Fc-(L15)-FGF21(L98R,P171G) hydrogel 24:1
FC-(L15)-FGF21(L98R, P171G) hydrogel 32:1
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Body weight changed (g)
8
6
4
2
0
-2
D0
D1
D3
D5
D8
D11
Time (D)

FIG. 60

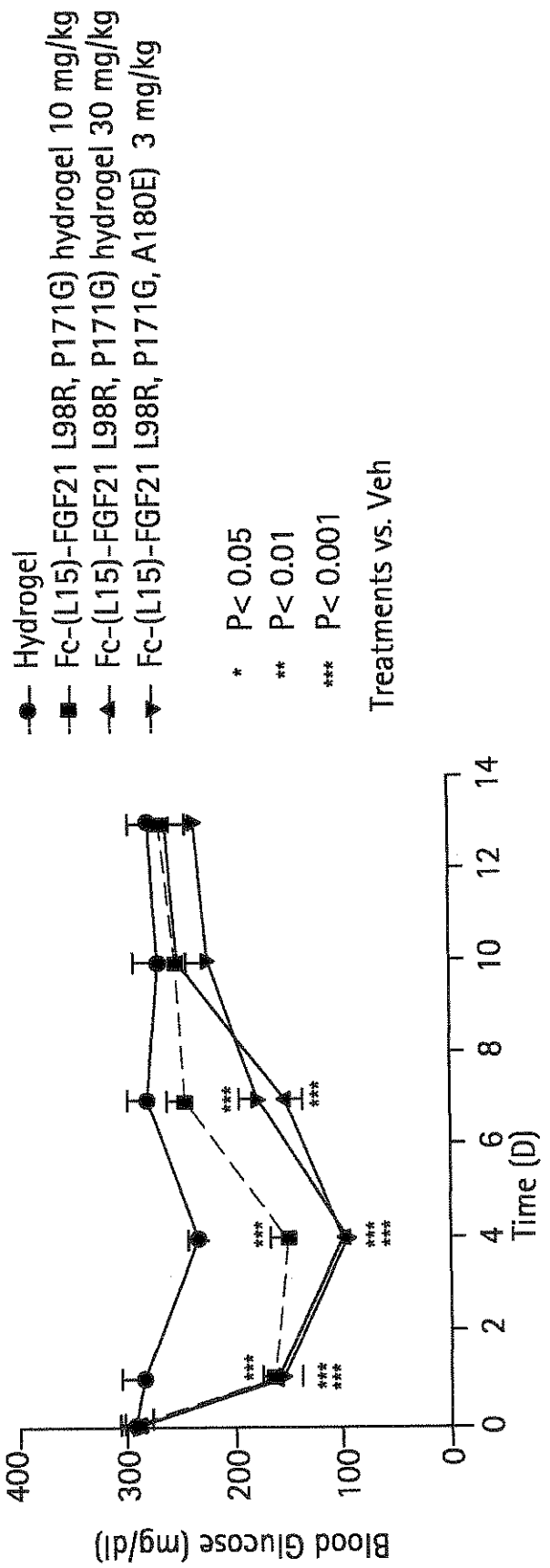

Hydrogel
Fc-(L15)-FGF21 L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21 L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 L98R, P171G, A180E) 3 mg/kg
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Blood Glucose (mg/dl)
400
300
200
100
0
0
2
4
6
8
10
12
14
Time (D)

FIG. 61

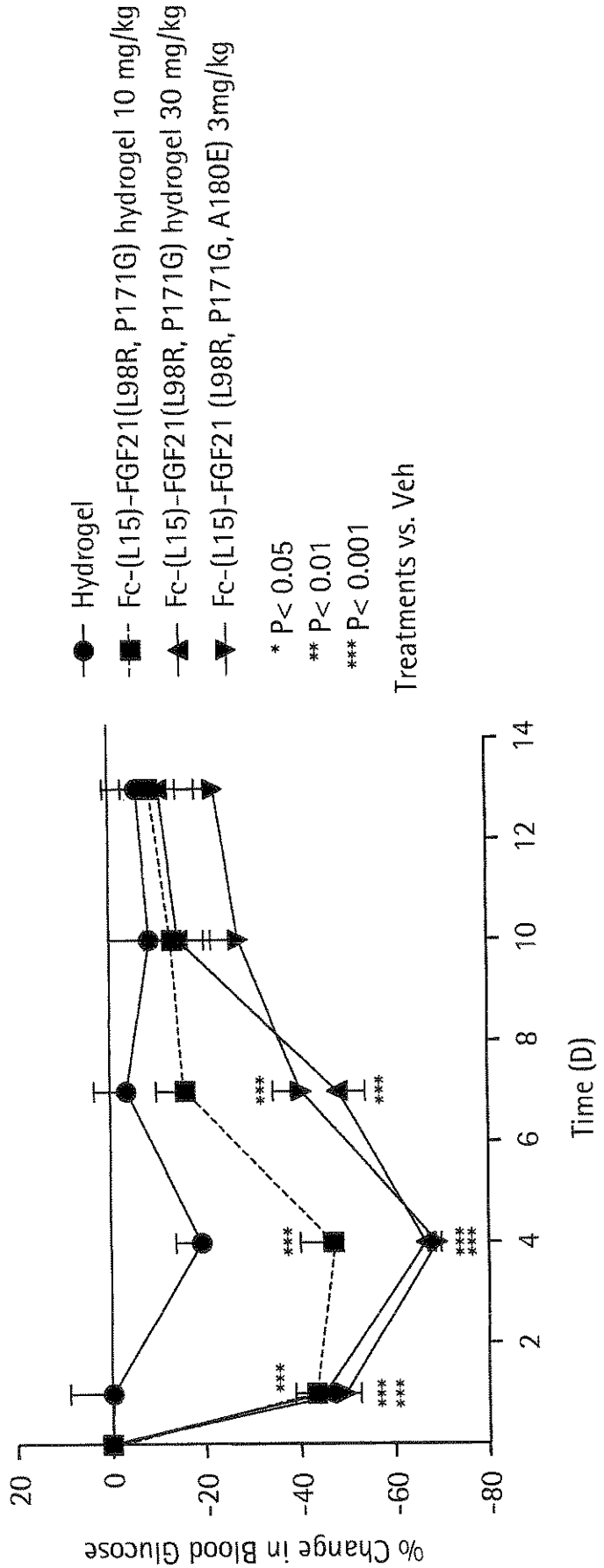

Hydrogel
Fc-(L15)-FGF21(L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21(L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 (L98R, P171G, A180E) 3mg/kg
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
% Change in Blood Glucose
20
0
-20
-40
-60
-80
2
4
6
8
10
12
14
Time (D)

FIG. 62

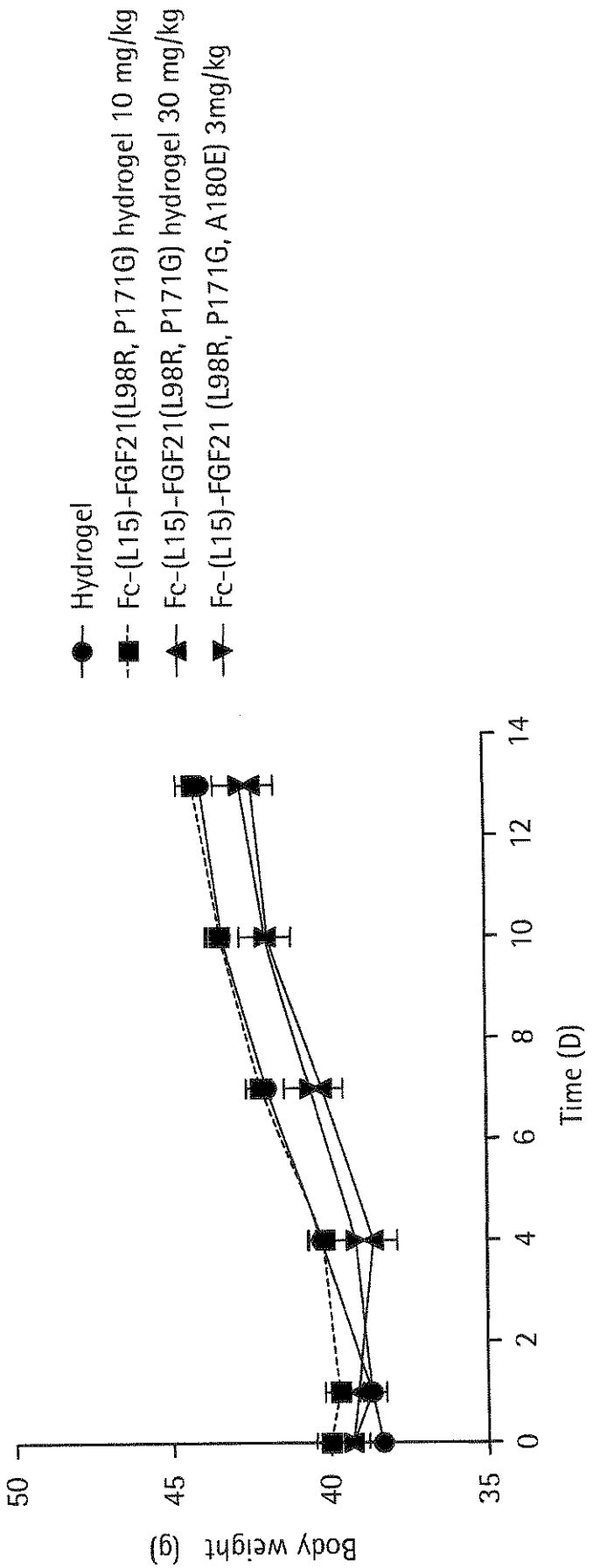

Hydrogel
Fc-(L15)-FGF21(L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21(L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 (L98R, P171G, A180E) 3mg/kg
Body weight (g)
50
45
40
35
0
2
4
6
8
10
12
14
Time (D)

FIG. 63

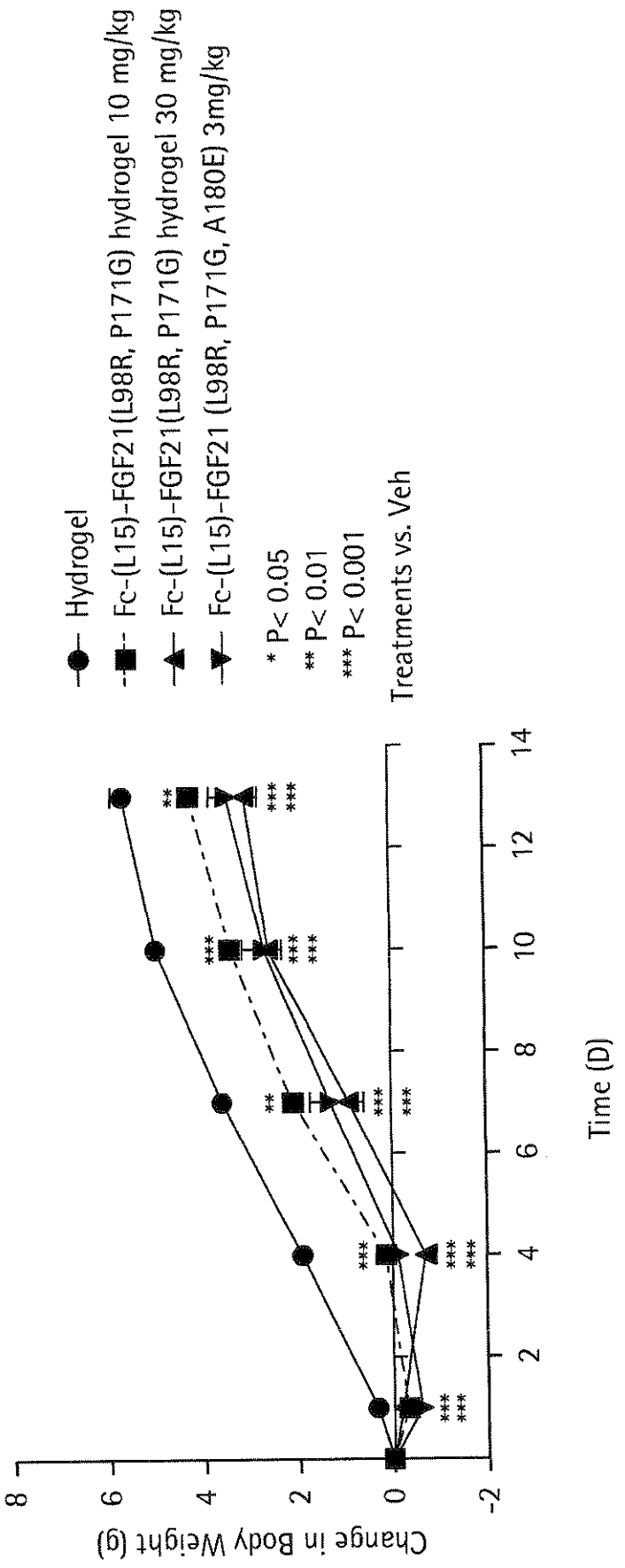

Hydrogel
Fc-(L15)-FGF21(L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21(L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 (L98R, P171G, A180E) 3mg/kg
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Change in Body Weight (g)
8
6
4
2
0
-2
2
4
6
8
10
12
14
Time (D)

FIG. 64

Monkey Study Design: Dose Escalation

Dosing groups, weekly dosing

Vehicle (n=14)

Fc - (L15) - FGF21 (L98R, P171G) (0.3; 1; 3 mg/kg, escalated every 3 weeks, n=13)

Fc - (G4S)3 - FGF21 (L98R, P171G, A180E) (0.3; 1; 3 mg/kg, escalated every 3 weeks, n=13)

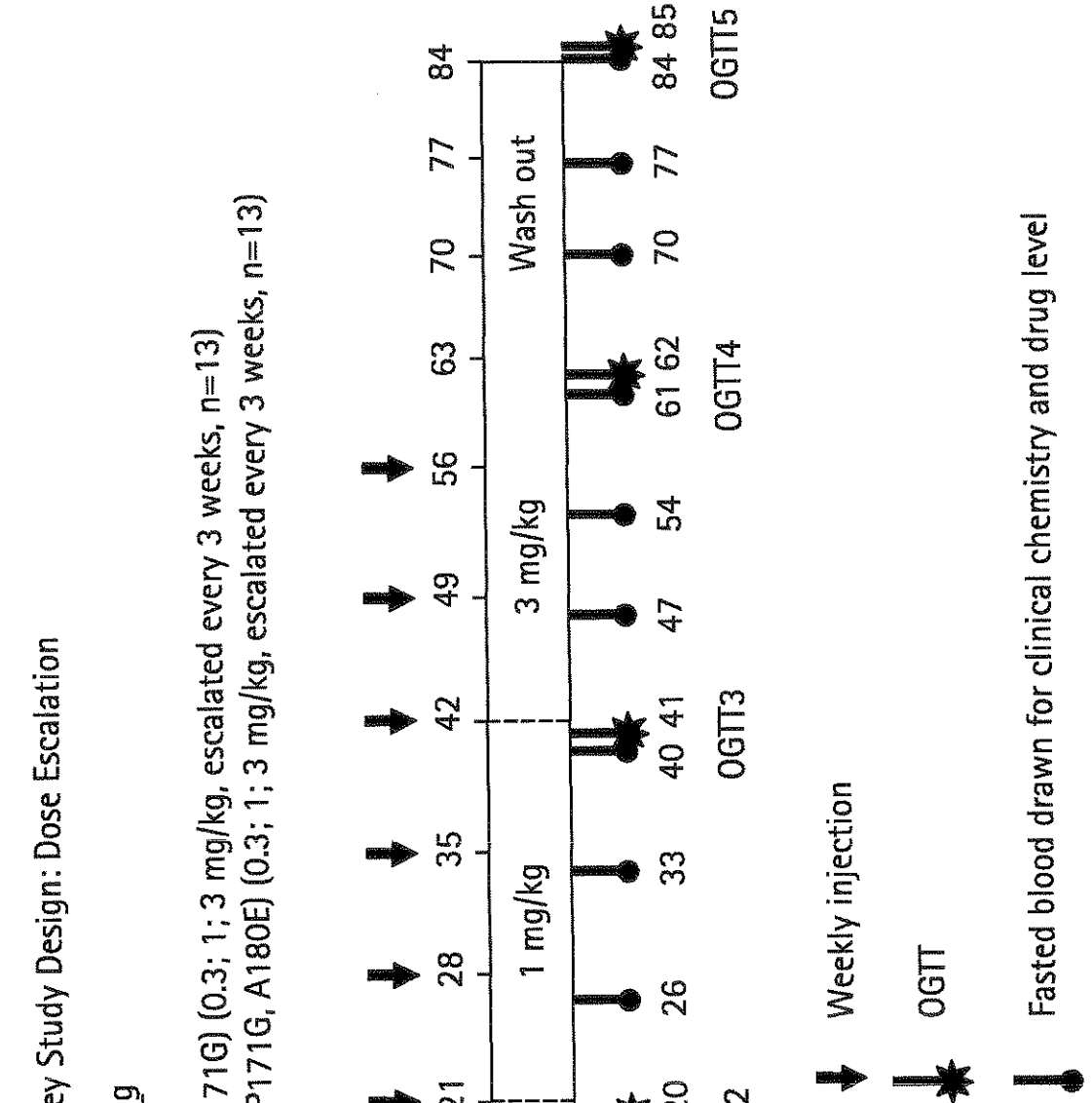


FIG. 65

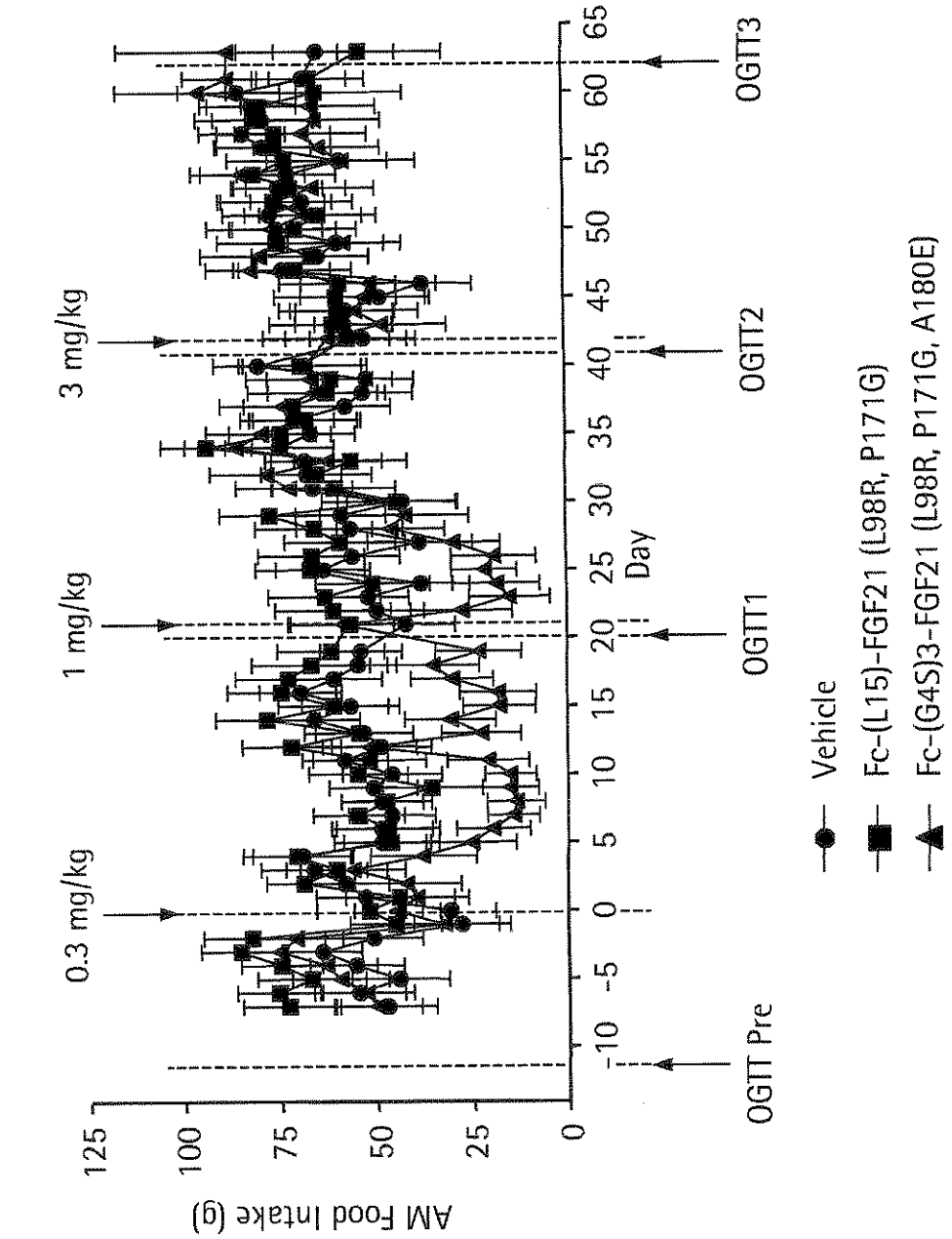

AM Food Intake
0.3 mg/kg
1 mg/kg
3 mg/kg
OGTT Pre
OGTT1
OGTT2
OGTT3
Day
AM Food Intake (g)
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 66

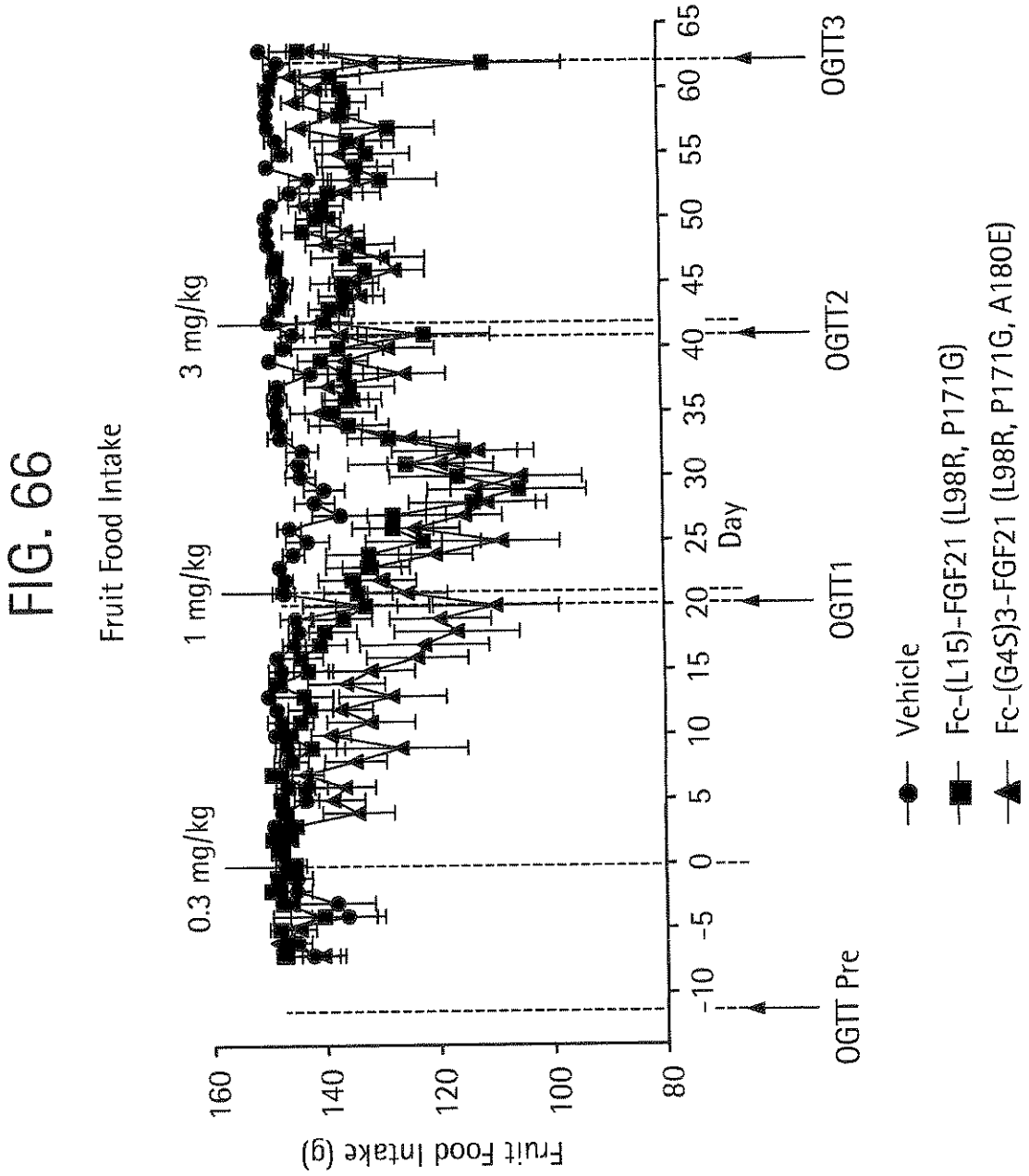

Fruit Food Intake
0.3 mg/kg
1 mg/kg
3 mg/kg
OGTT Pre
OGTT1
OGTT2
OGTT3
Day
Fruit Food Intake (g)
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 67

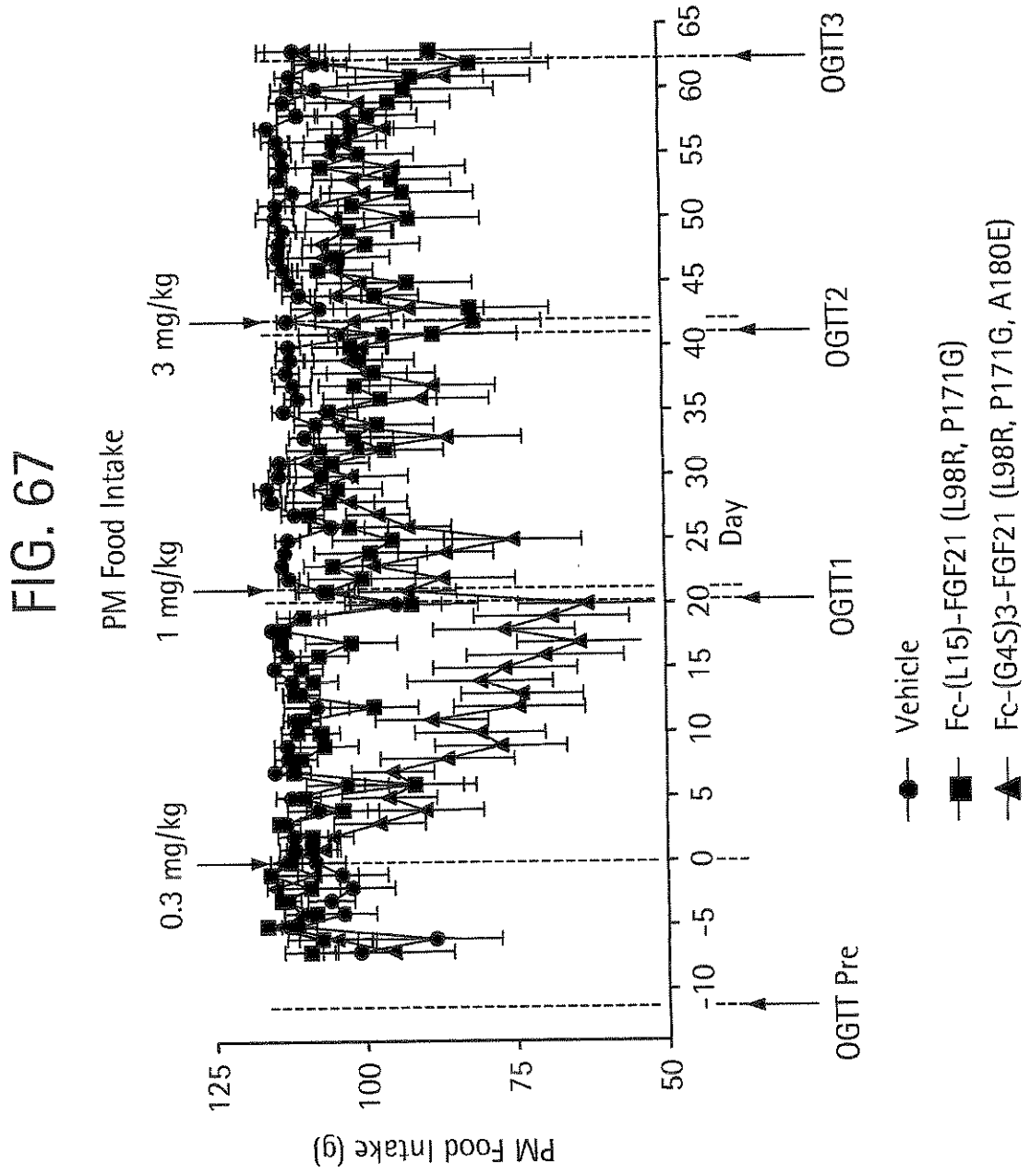

PM Food Intake
0.3 mg/kg
1 mg/kg
3 mg/kg
OGTT Pre
OGTT1
OGTT2
OGTT3
Day
PM Food Intake (g)
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 68

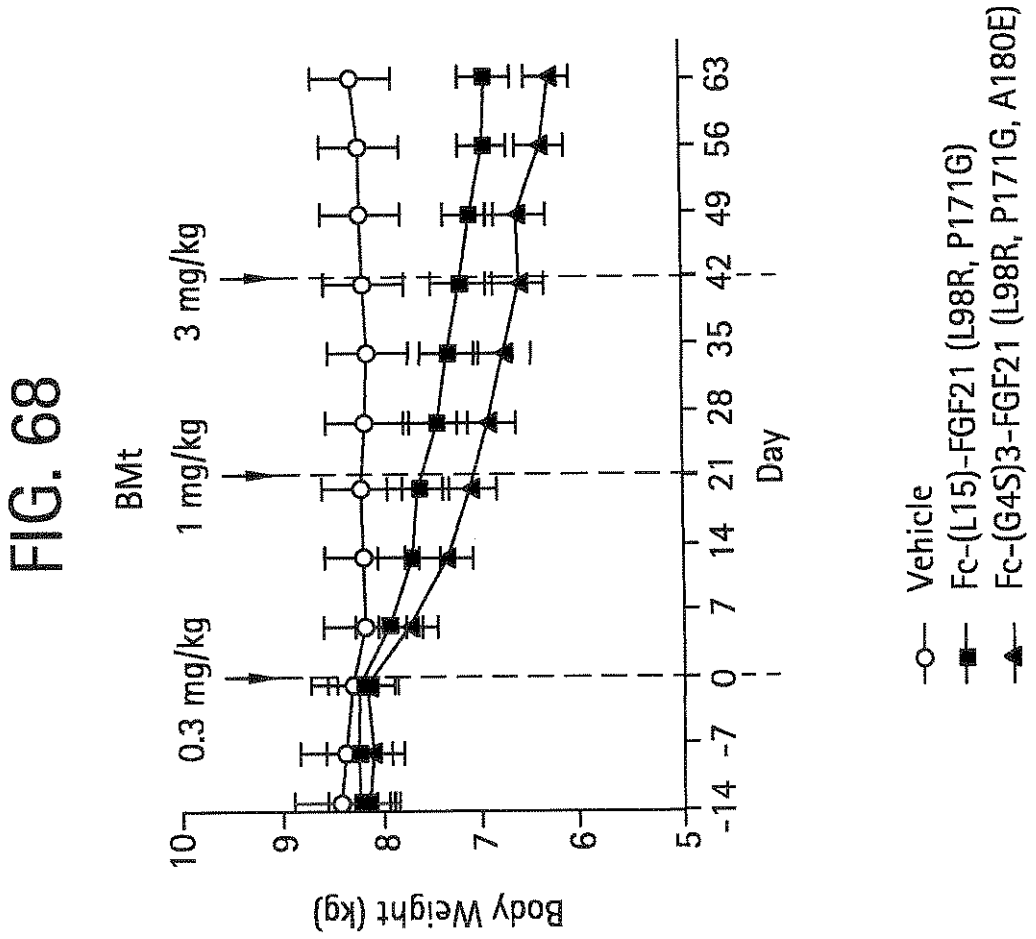

Body Weight (kg)
10
9
8
7
6
5
BMt
0.3 mg/kg
1 mg/kg
3 mg/kg
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 69

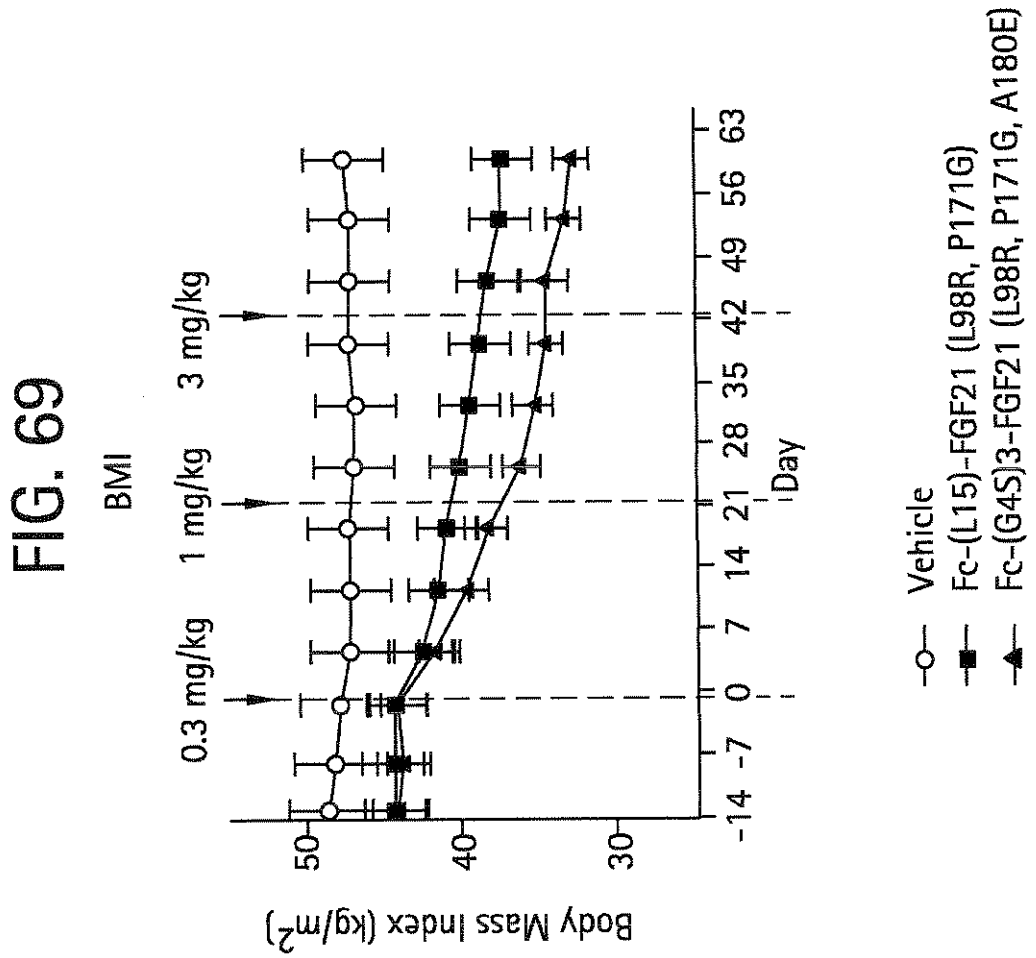

BMI
0.3 mg/kg
1 mg/kg
3 mg/kg
Body Mass Index (kg/m2)
50
40
30
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 70

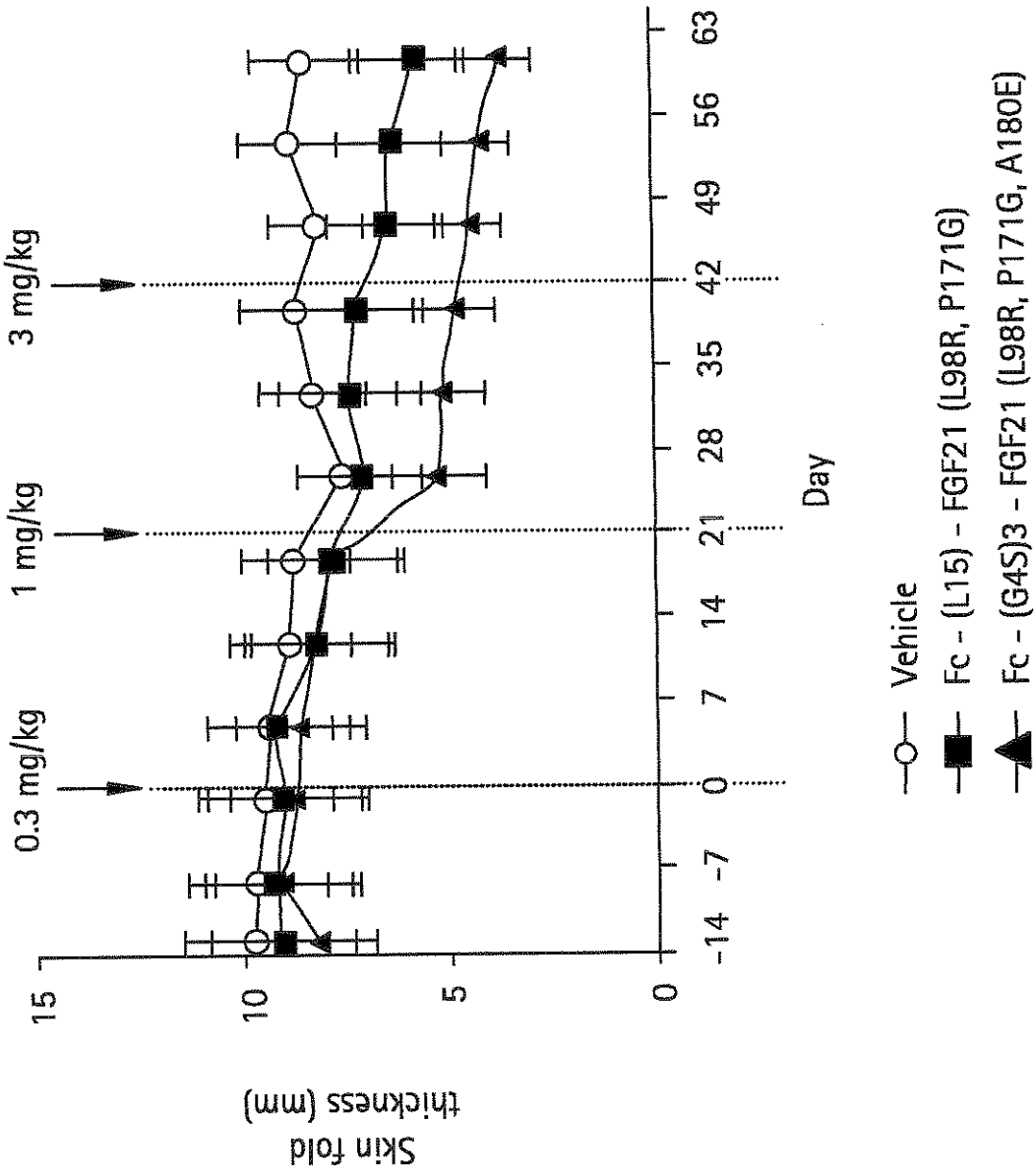

Skin fold thickness (mm)
15
10
5
0
0.3 mg/kg
1 mg/kg
3 mg/kg
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc - (L15) - FGF21 (L98R, P171G)
Fc - (G4S)3 - FGF21 (L98R, P171G, A180E)

FIG. 71

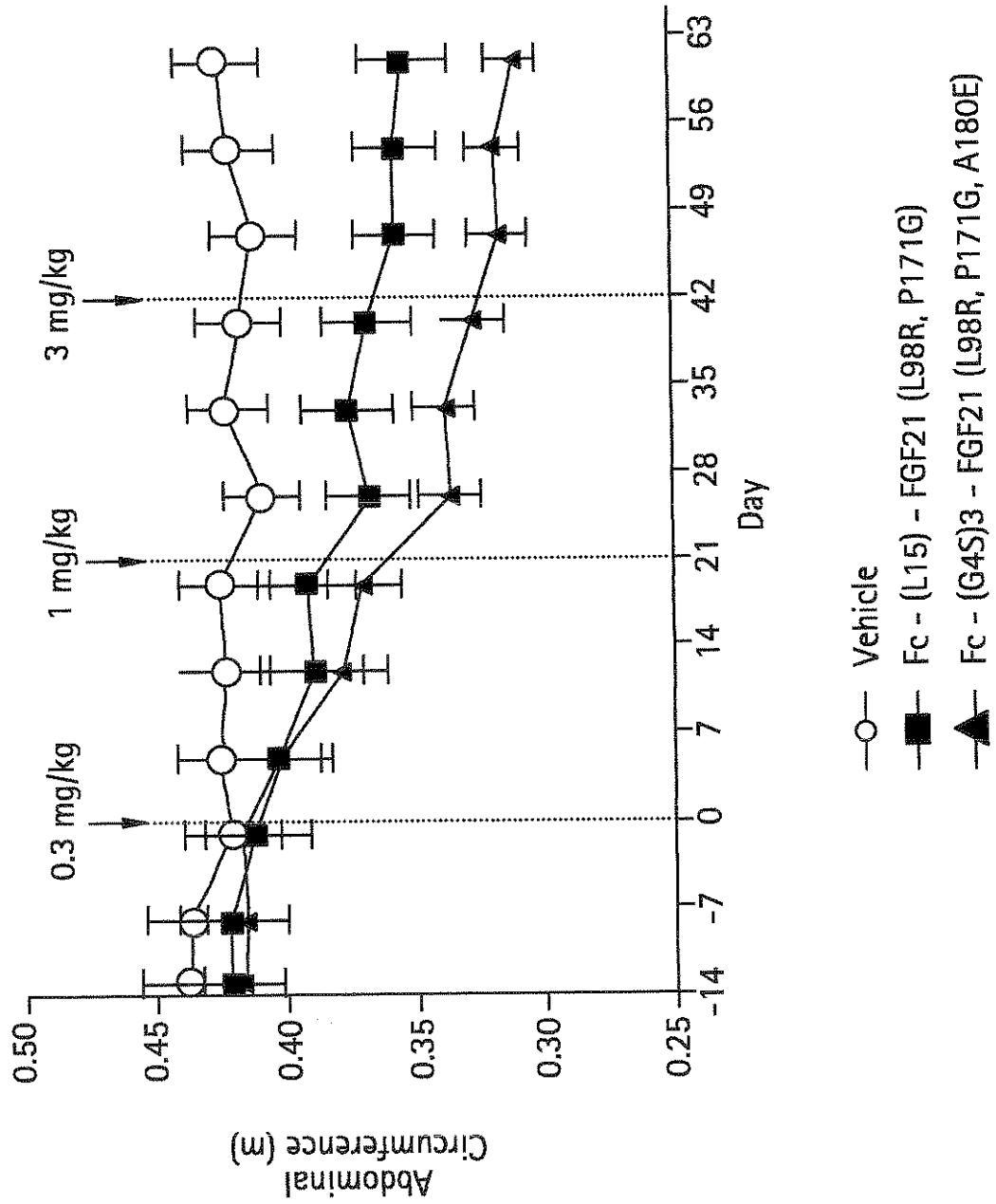

AC
0.3 mg/kg
1 mg/kg
3 mg/kg
Abdominal Circumference (m)
0.50
0.45
0.40
0.35
0.30
0.25
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc - (L15) - FGF21 (L98R, P171G)
Fc - (G4S)3 - FGF21 (L98R, P171G, A180E)

FIG.72

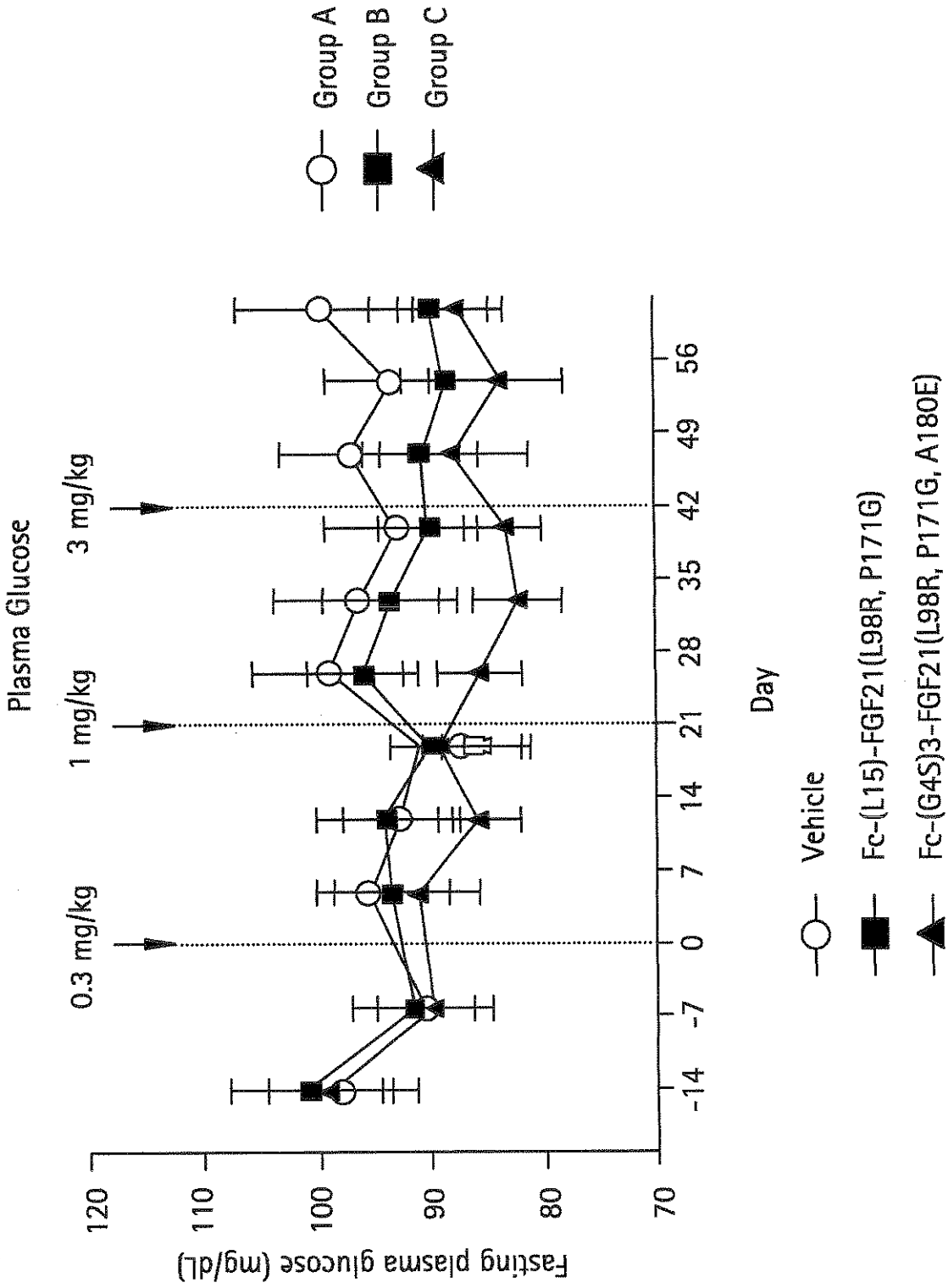

Plasma Glucose
0.3 mg/kg
1 mg/kg
3 mg/kg
Group A
Group B
Group C
Fasting plasma glucose (mg/dL)
120
110
100
90
80
70
-14
-7
0
7
14
21
28
35
42
49
56
Day
Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G, A180E)

FIG. 73

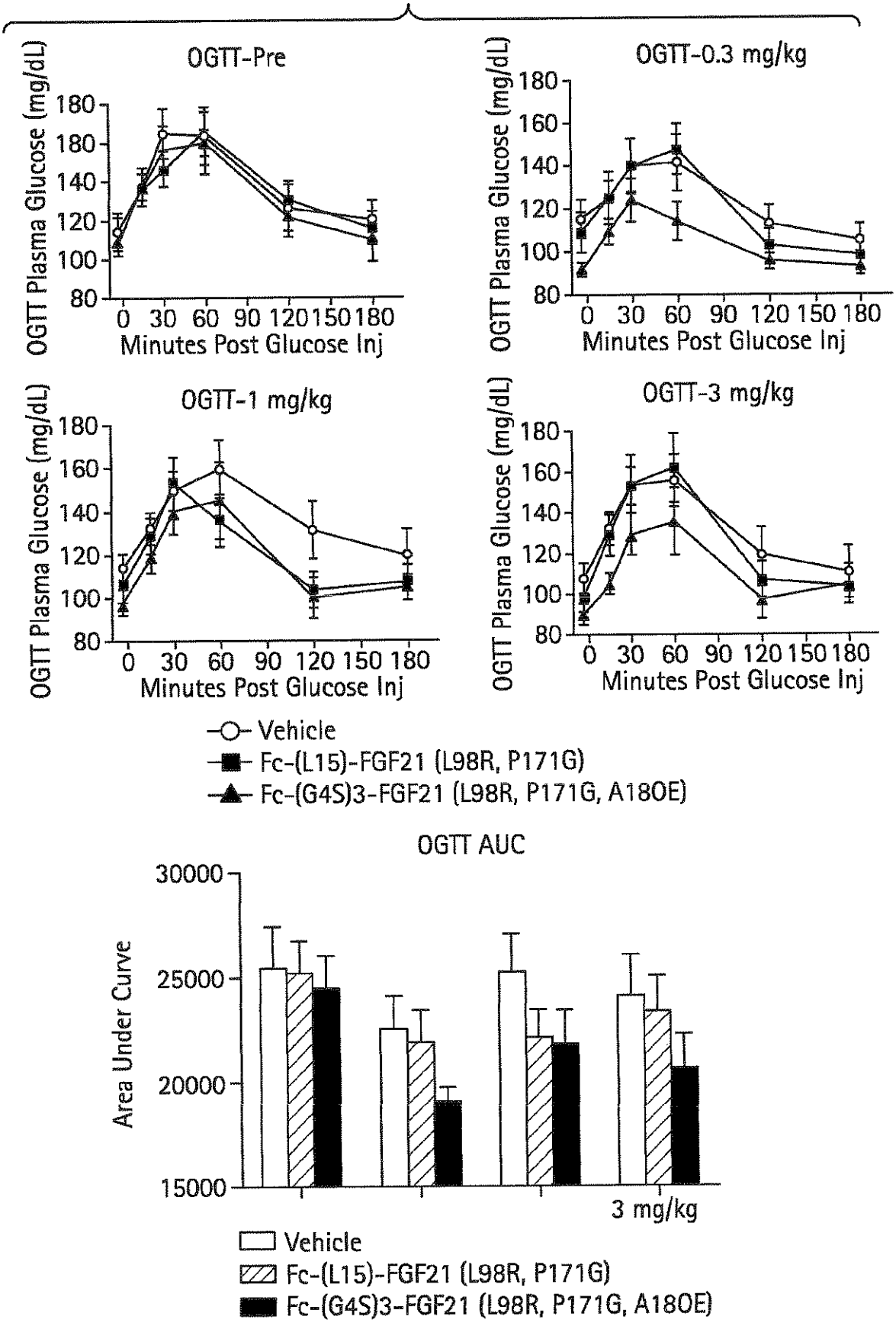

OGTT-Pre
OGTT-0.3 mg/kg
OGTT-1 mg/kg
OGTT-3 mg/kg
OGTT Plasma Glucose (mg/dL)
Minutes Post Glucose Inj
0 30 60 90 120 150 180
80 100 120 140 160 180
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)
OGTT AUC
Area Under Curve
15000 20000 25000 30000
3 mg/kg
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 74

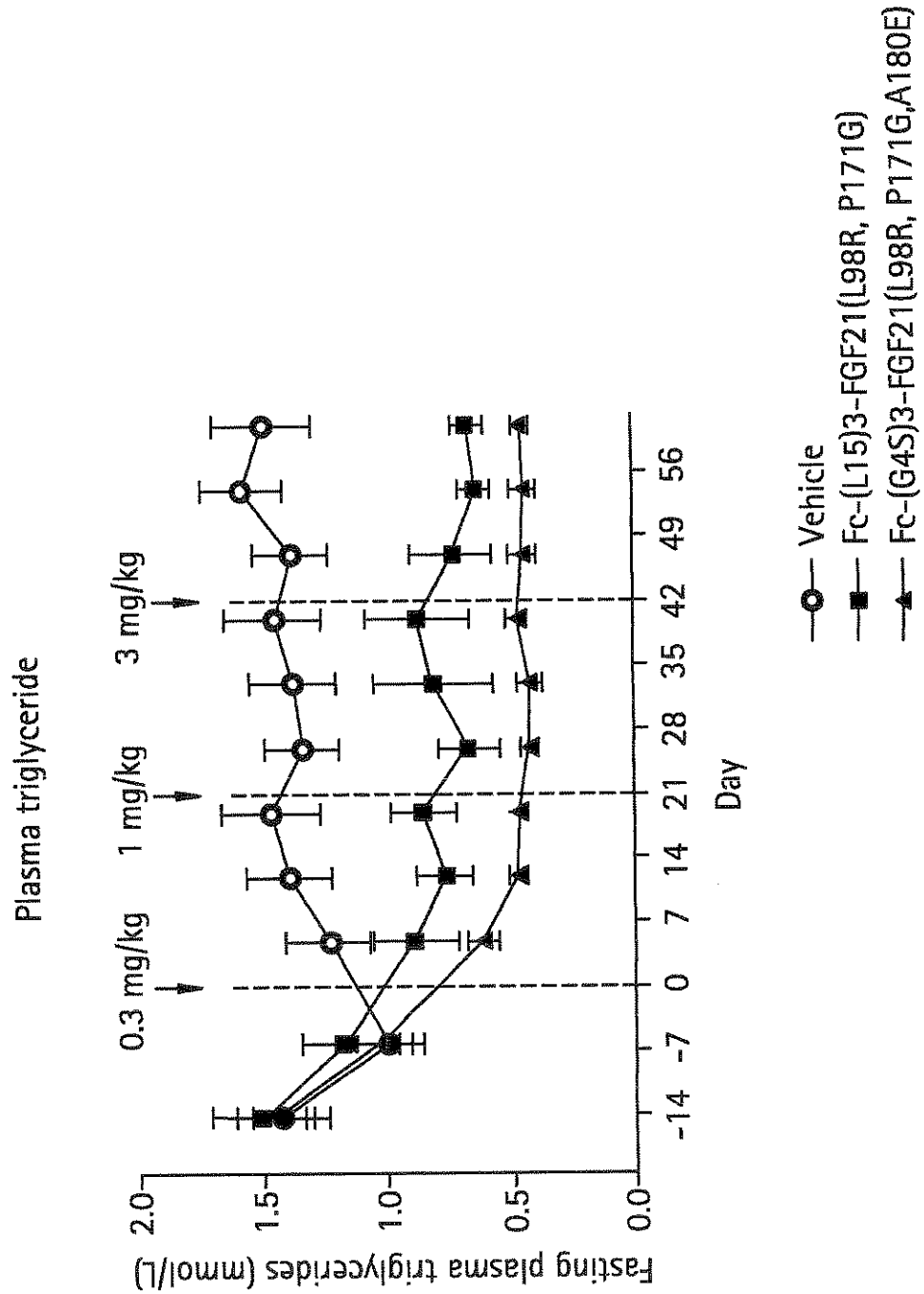

Plasma triglyceride
0.3 mg/kg
1 mg/kg
3 mg/kg
Fasting plasma triglycerides (mmol/L)
2.0
1.5
1.0
0.5
0.0
-14
-7
0
7
14
21
28
35
42
49
56
Day
Vehicle
Fc-(L15)3-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G,A180E)

FIG. 75

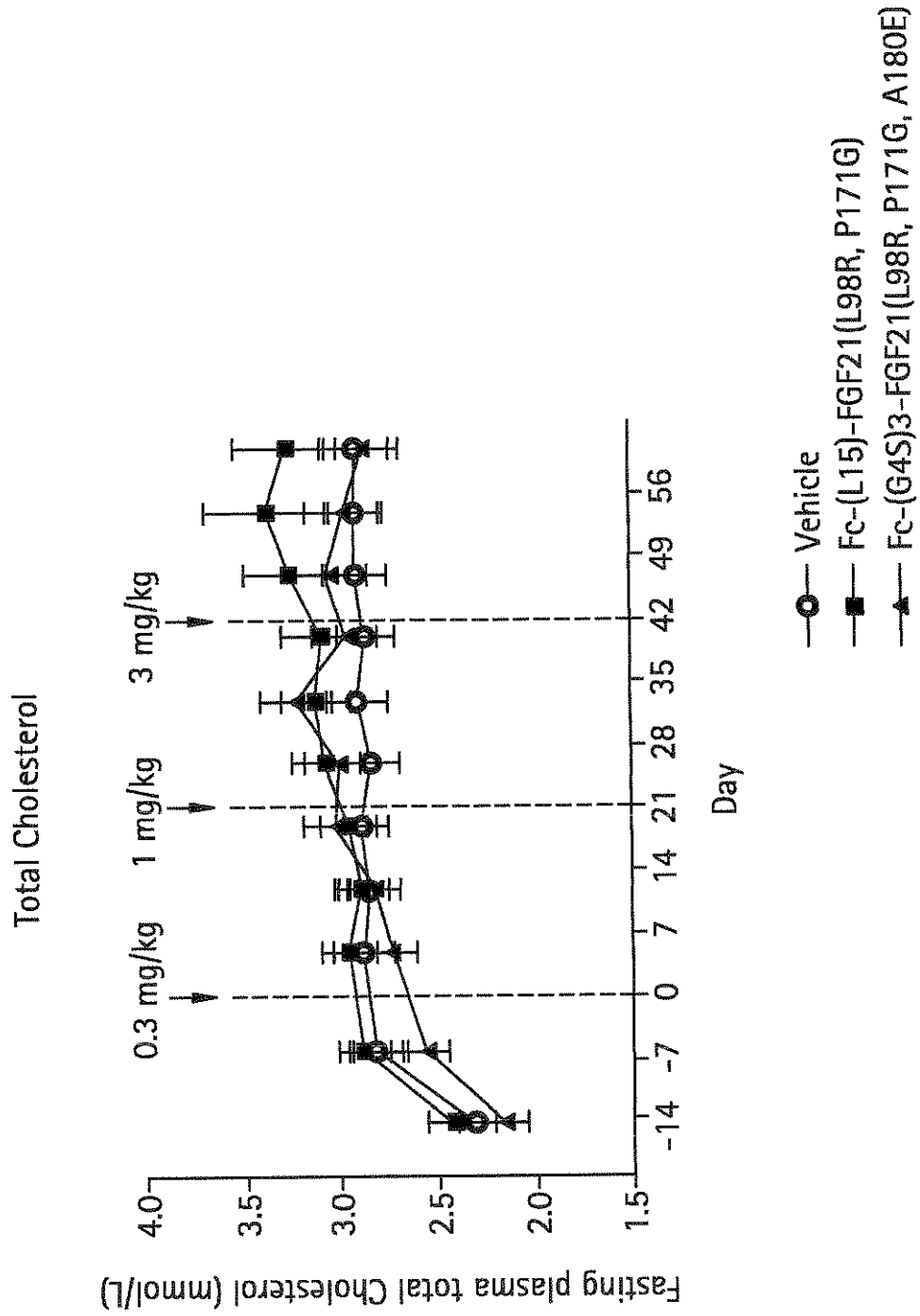

Total Cholesterol
0.3 mg/kg
1 mg/kg
3 mg/kg
Fasting plasma total Cholesterol (mmol/L)
4.0
3.5
3.0
2.5
2.0
1.5
-14
-7
0
7
14
21
28
35
42
49
56
Day
Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G, A180E)

FIG. 76

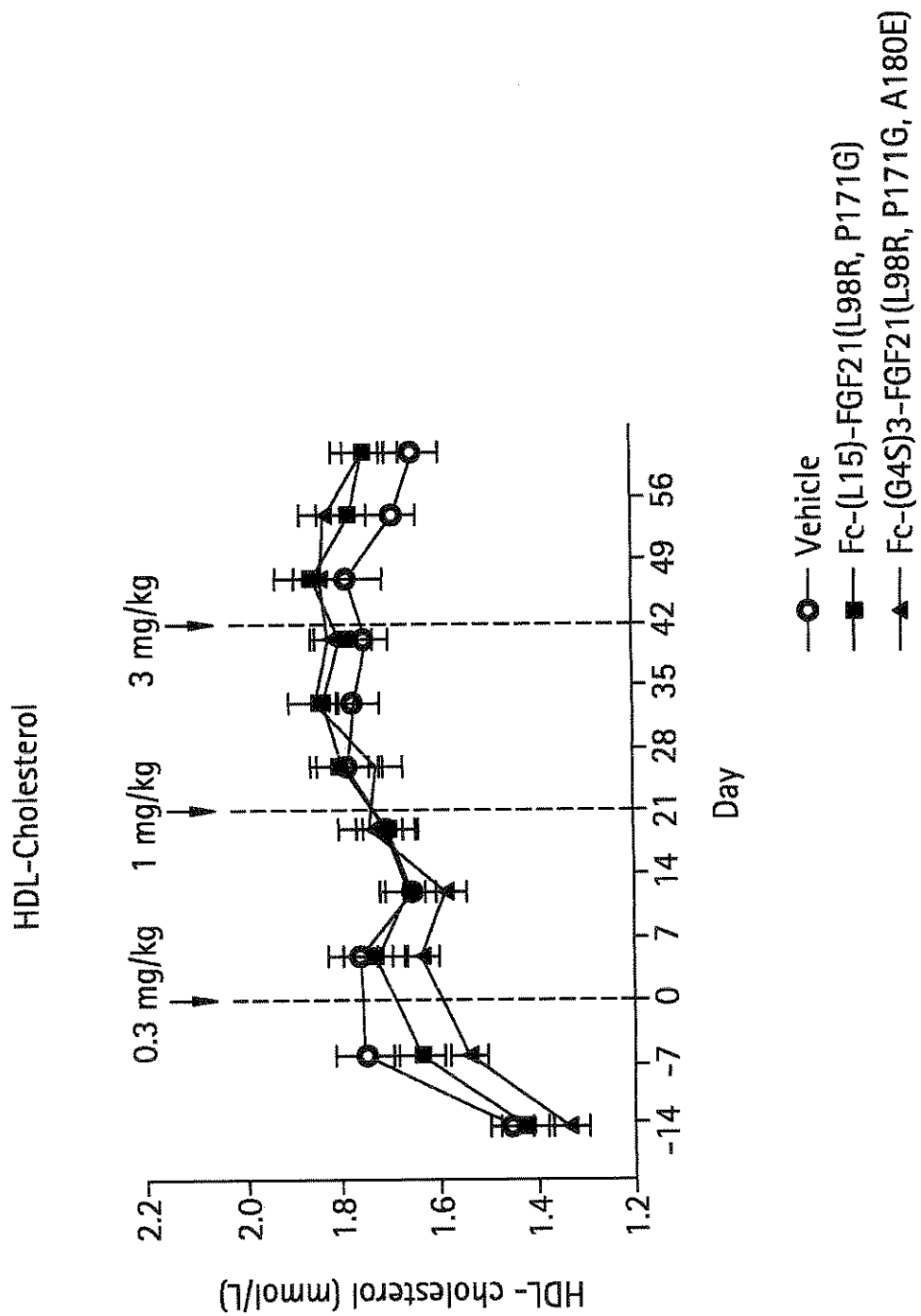

HDL-Cholesterol
0.3 mg/kg
1 mg/kg
3 mg/kg
HDL- cholesterol (mmol/L)
2.2
2.0
1.8
1.6
1.4
1.2
-14
-7
0
7
14
21
28
35
42
49
56
Day
Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G, A180E)

FIG. 77

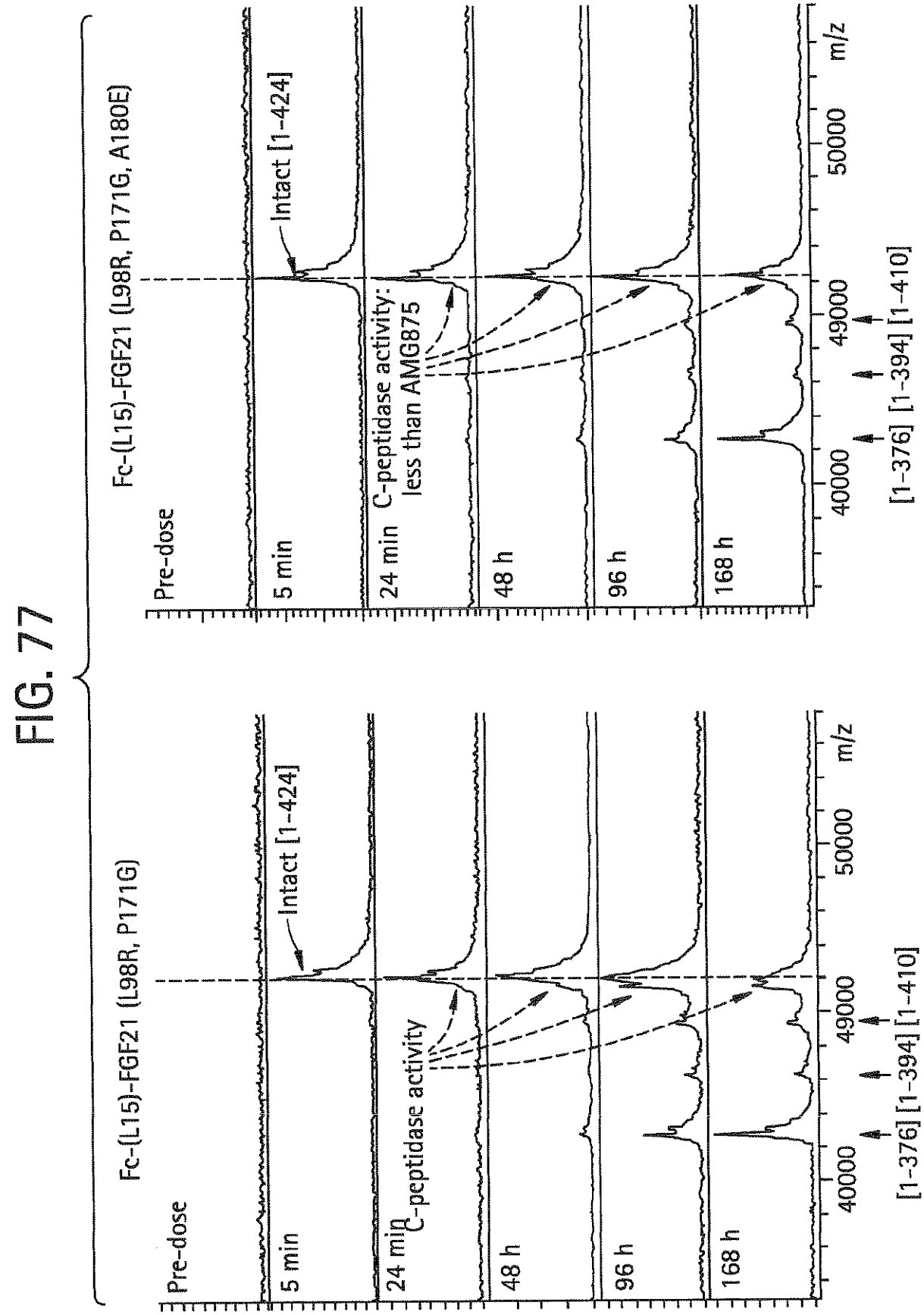

Fc-(L15)-FGF21 (L98R, P171G)
Pre-dose
5 min
24 min
C-peptidase activity
48 h
96 h
168 h
Intact [1-424]
40000
49000
50000
m/z
[1-376] [1-394] [1-410]
Fc-(L15)-FGF21 (L98R, P171G, A180E)
Pre-dose
5 min
24 min
C-peptidase activity:
less than AMG875
48 h
96 h
168 h
Intact [1-424]
40000
49000
50000
m/z
[1-376] [1-394] [1-410]

FIG. 78

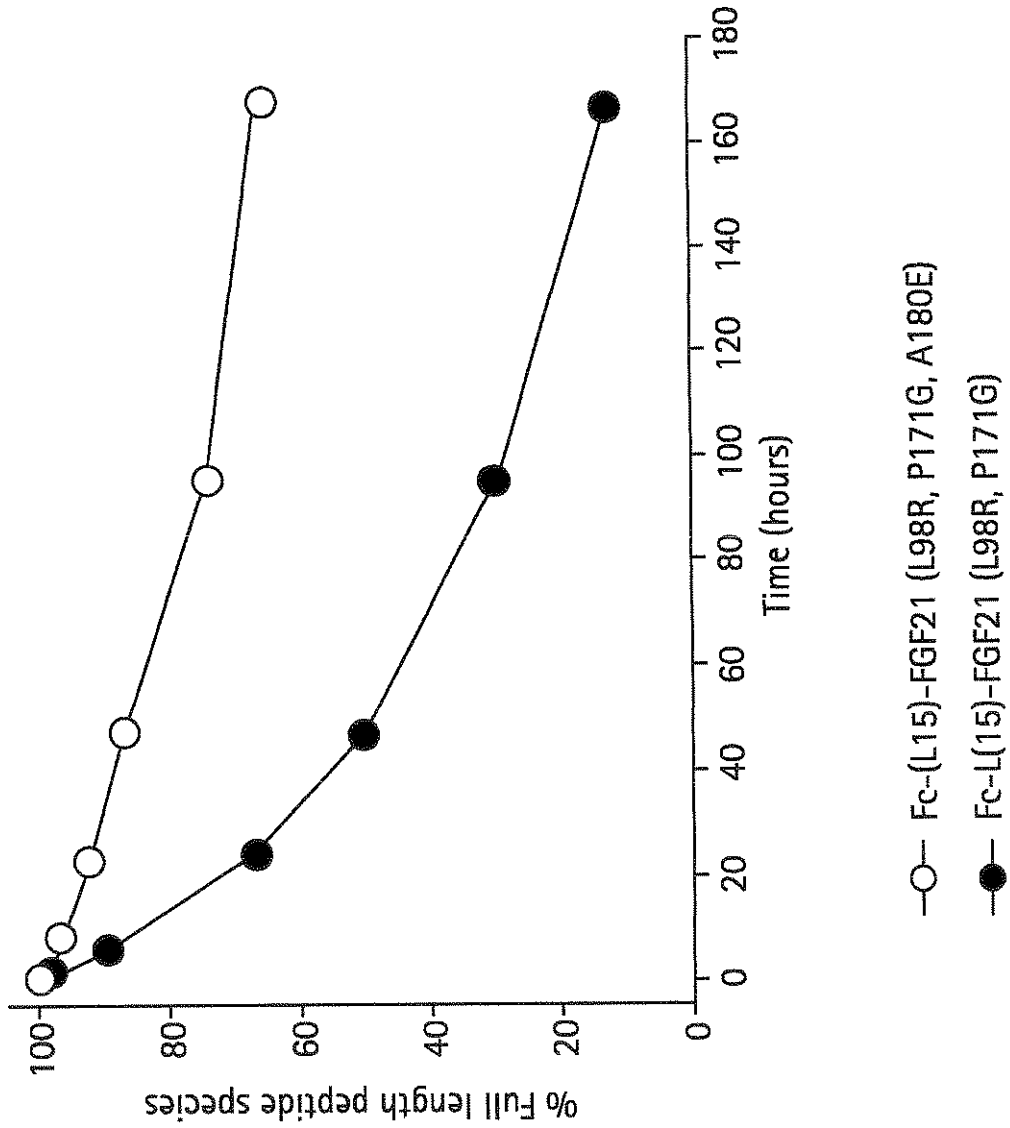

% Full length peptide species
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
Time (hours)
Fc-(L15)-FGF21 (L98R, P171G, A180E)
Fc-L(15)-FGF21 (L98R, P171G)

FIG. 79

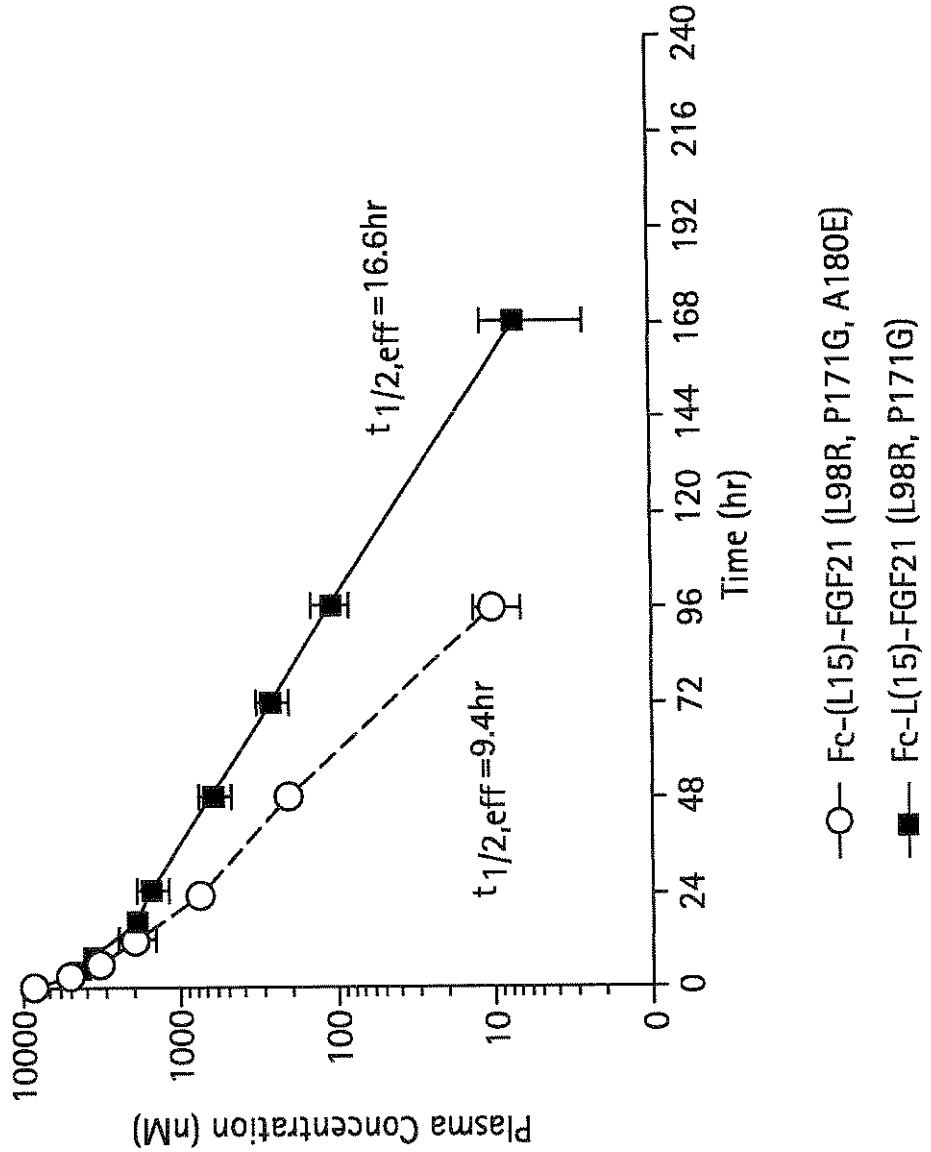
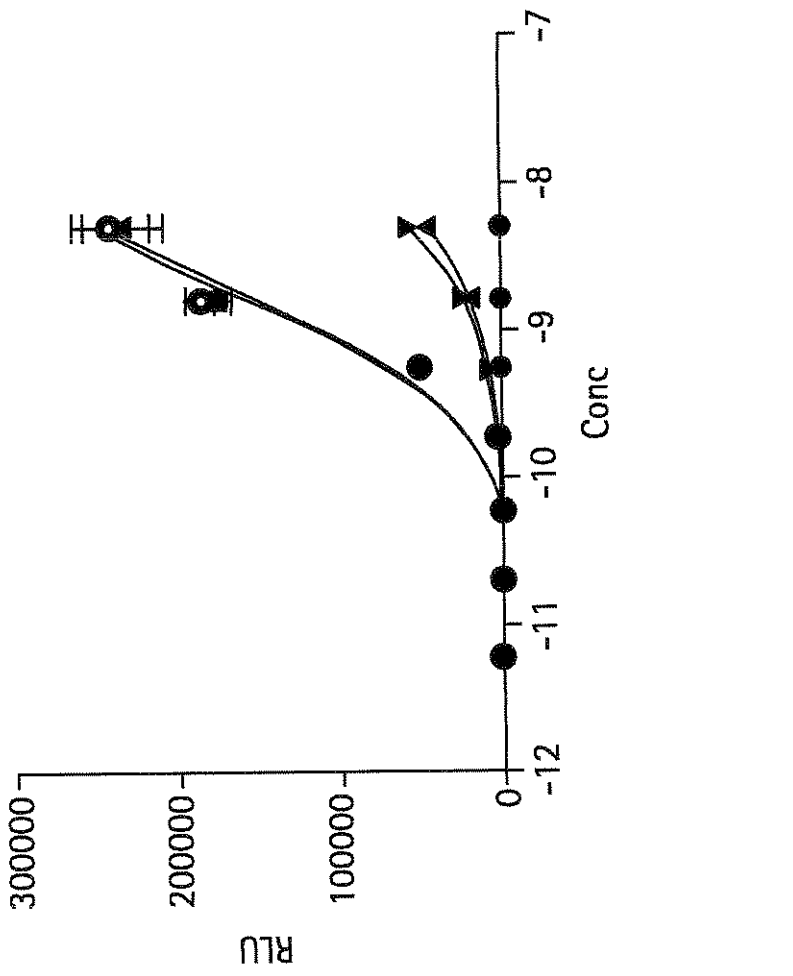

Plasma Concentration (nM)
10000
1000
100
10
0
24
48
72
96
120
144
168
192
216
240
Time (hr)
t 1/2,eff=16.6hr
t 1/2,eff=9.4hr
Fc-(L15)-FGF21 (L98R, P171G, A180E)
Fc-L(15)-FGF21 (L98R, P171G)

FIG. 80


Negative Control
FGF21
FGF21 (Y179N, S181T)
FGF21 Y179N
FGF21 P124S
RLU
300000
200000
100000
0
-12
-11
-10
-9
-8
-7
Conc