FIG. 1A

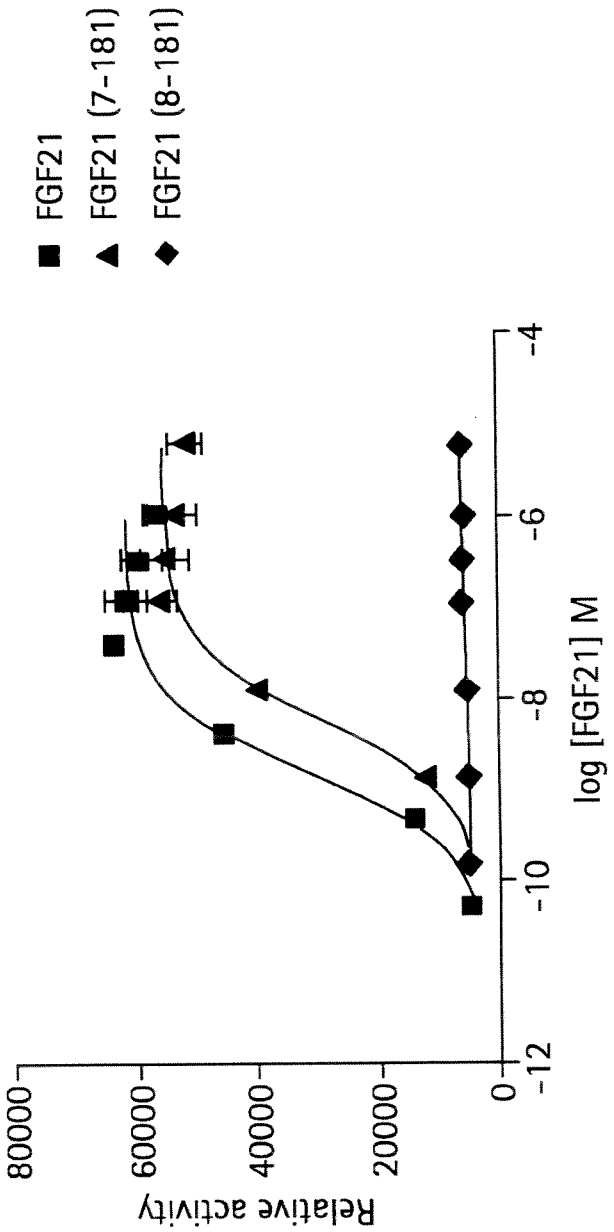

FGF21
FGF21 (7-181)
FGF21 (8-181)
Relative activity
80000
60000
40000
20000
0
-12
-10
-8
-6
-4
log [FGF21] M

FIG. 1B

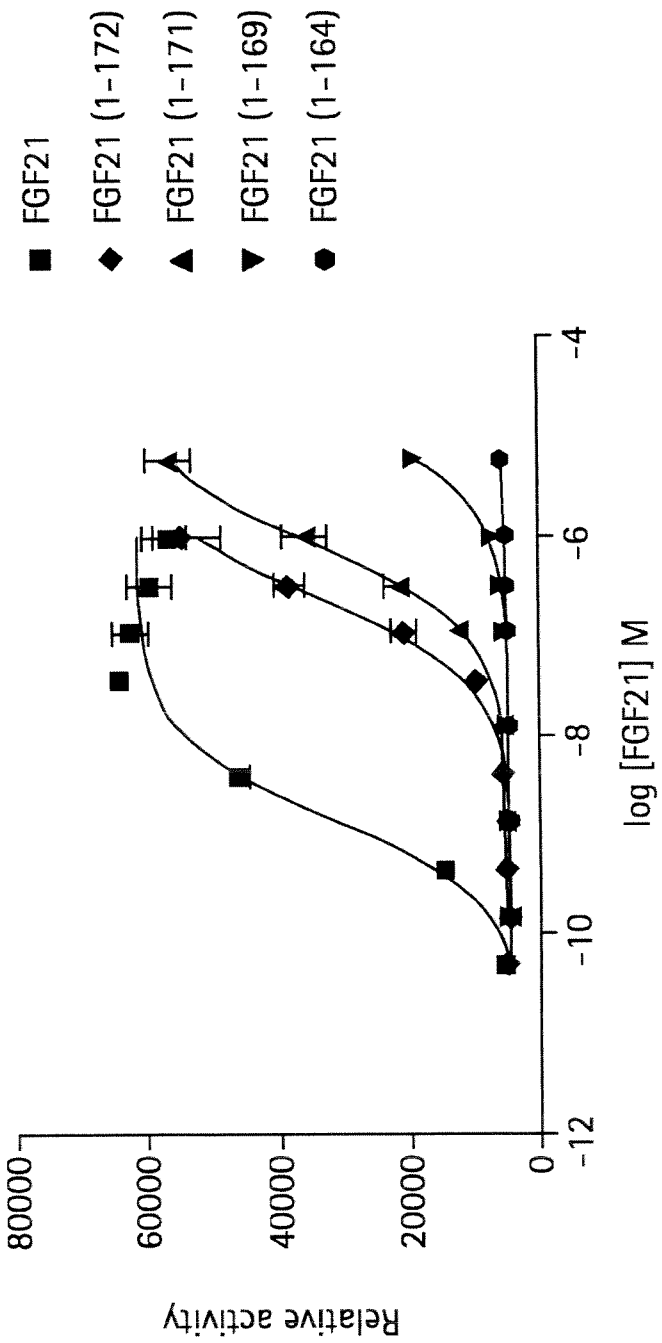

FGF21
FGF21 (1-172)
FGF21 (1-171)
FGF21 (1-169)
FGF21 (1-164)
Relative activity
80000
60000
40000
20000
0
-12
-10
-8
-6
-4
log [FGF21] M

FIG. 2

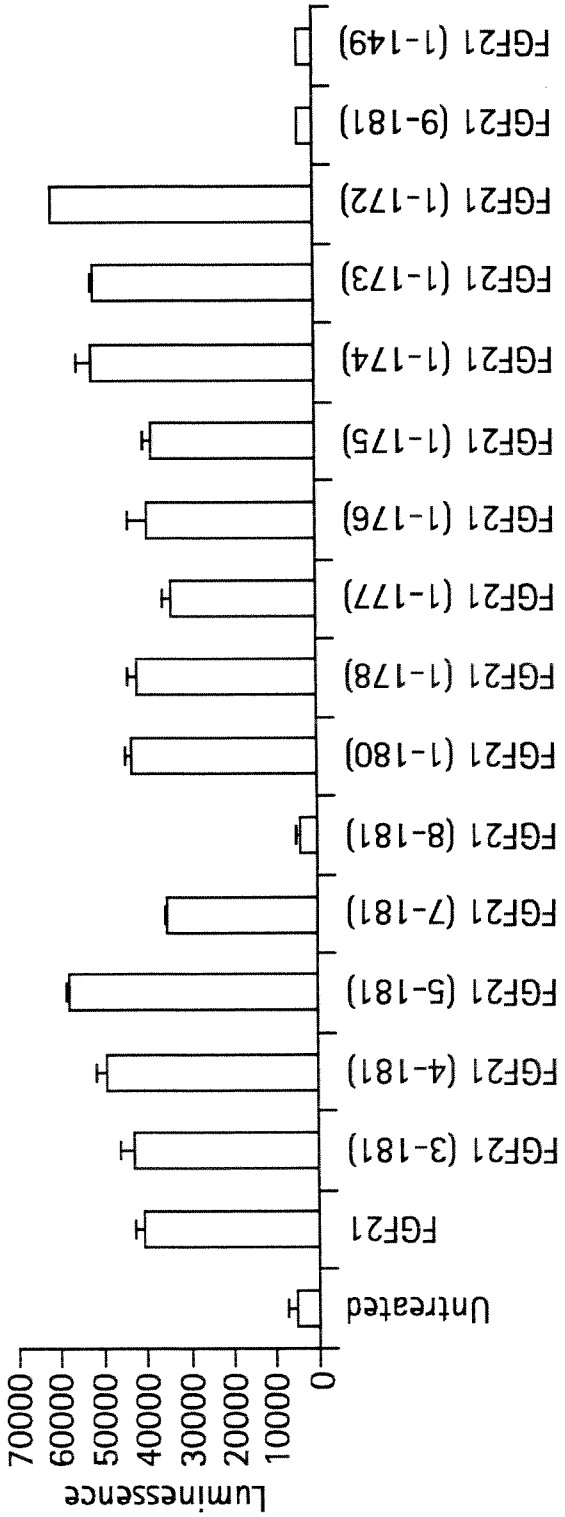

Luminescence
70000
60000
50000
40000
30000
20000
10000
0
Untreated
FGF21
FGF21 (3-181)
FGF21 (4-181)
FGF21 (5-181)
FGF21 (7-181)
FGF21 (8-181)
FGF21 (1-180)
FGF21 (1-178)
FGF21 (1-177)
FGF21 (1-176)
FGF21 (1-175)
FGF21 (1-174)
FGF21 (1-173)
FGF21 (1-172)
FGF21 (9-181)
FGF21 (1-149)

FIG. 3

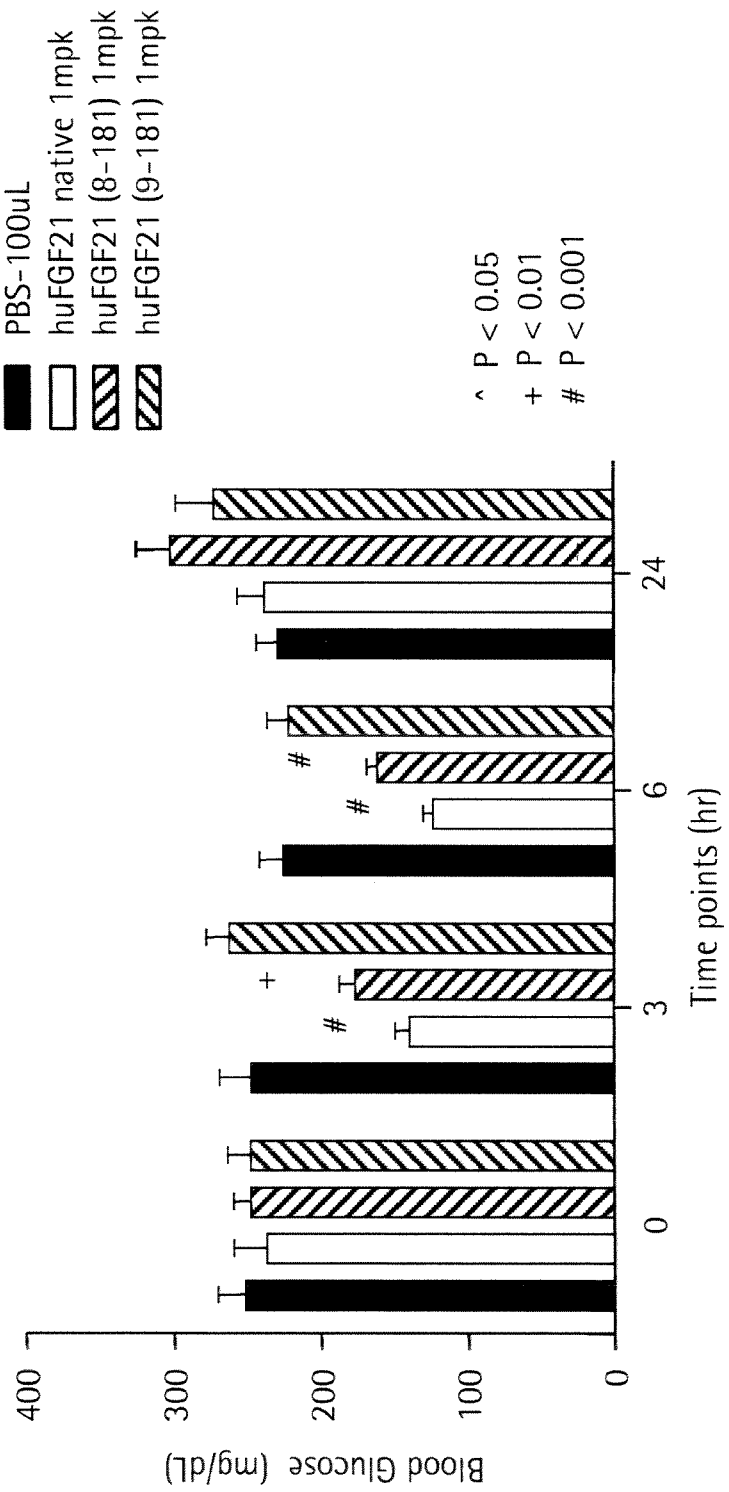

PBS-100uL
huFGF21 native 1mpk
huFGF21 (8-181) 1mpk
huFGF21 (9-181) 1mpk
^ P < 0.05
+ P < 0.01
# P < 0.001
Blood Glucose (mg/dL)
0
100
200
300
400
0
3
6
24
Time points (hr)
+
#
#
#

FIG. 4

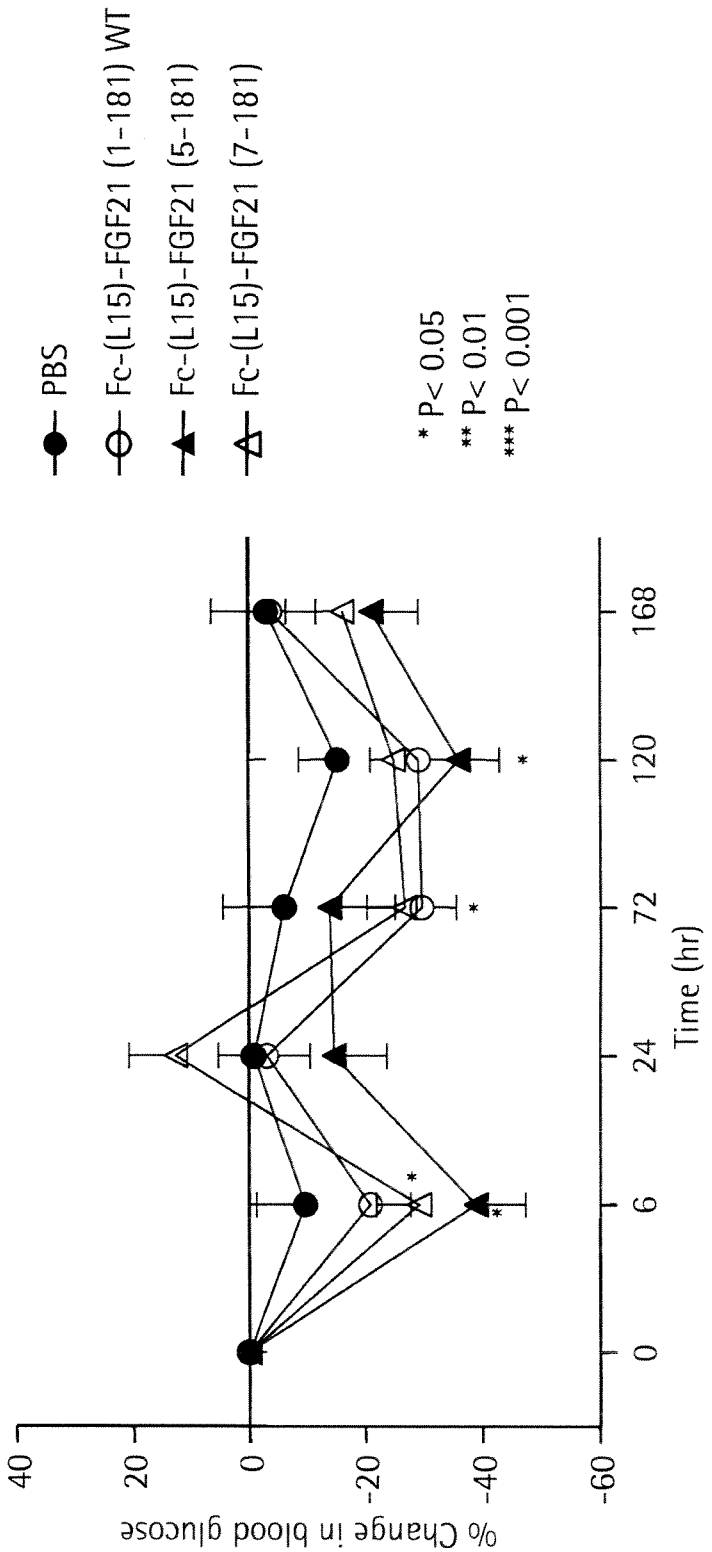

PBS
Fc-(L15)-FGF21 (1-181) WT
Fc-(L15)-FGF21 (5-181)
Fc-(L15)-FGF21 (7-181)
* P< 0.05
** P< 0.01
*** P< 0.001
% Change in blood glucose
40
20
0
-20
-40
-60
0
6
24
72
120
168
Time (hr)

FIG. 5

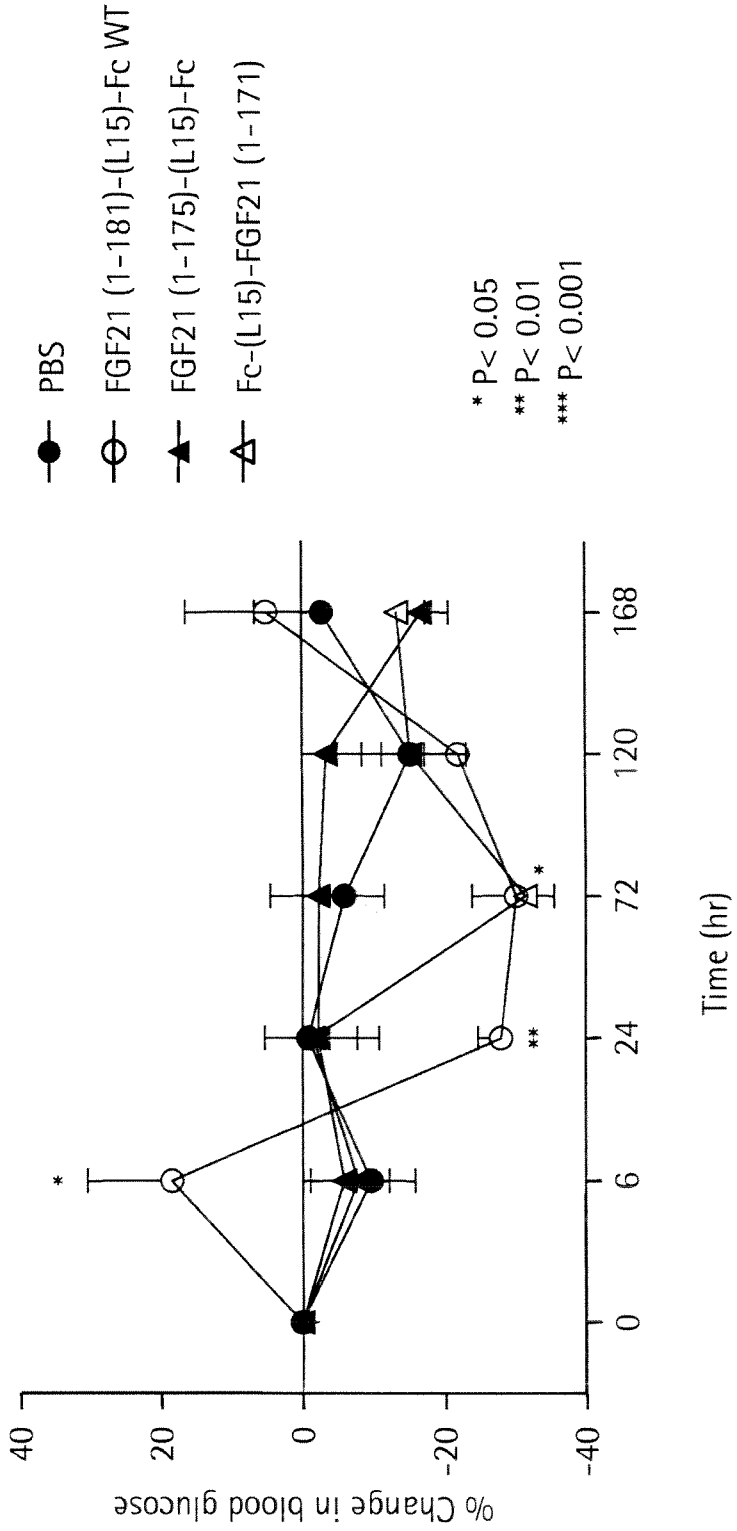

PBS
FGF21 (1-181)-(L15)-Fc WT
FGF21 (1-175)-(L15)-Fc
Fc-(L15)-FGF21 (1-171)
* P< 0.05
** P< 0.01
*** P< 0.001
% Change in blood glucose
40
20
0
-20
-40
0
6
24
72
120
168
Time (hr)
*
**
*

FIG. 6A

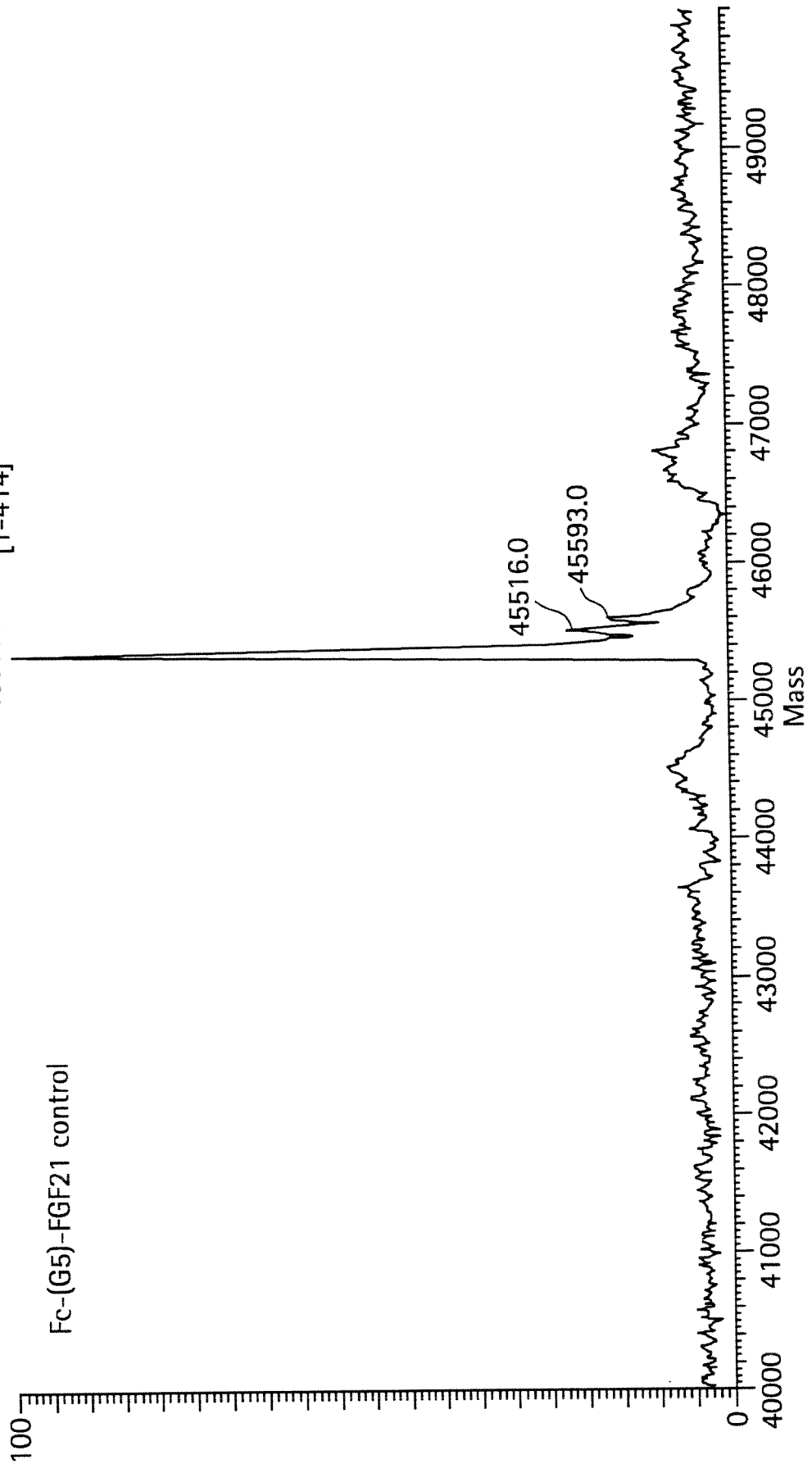

Fc-(G5)-FGF21 control
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass
45390.0
[1-414]
45516.0
45593.0

FIG. 6B

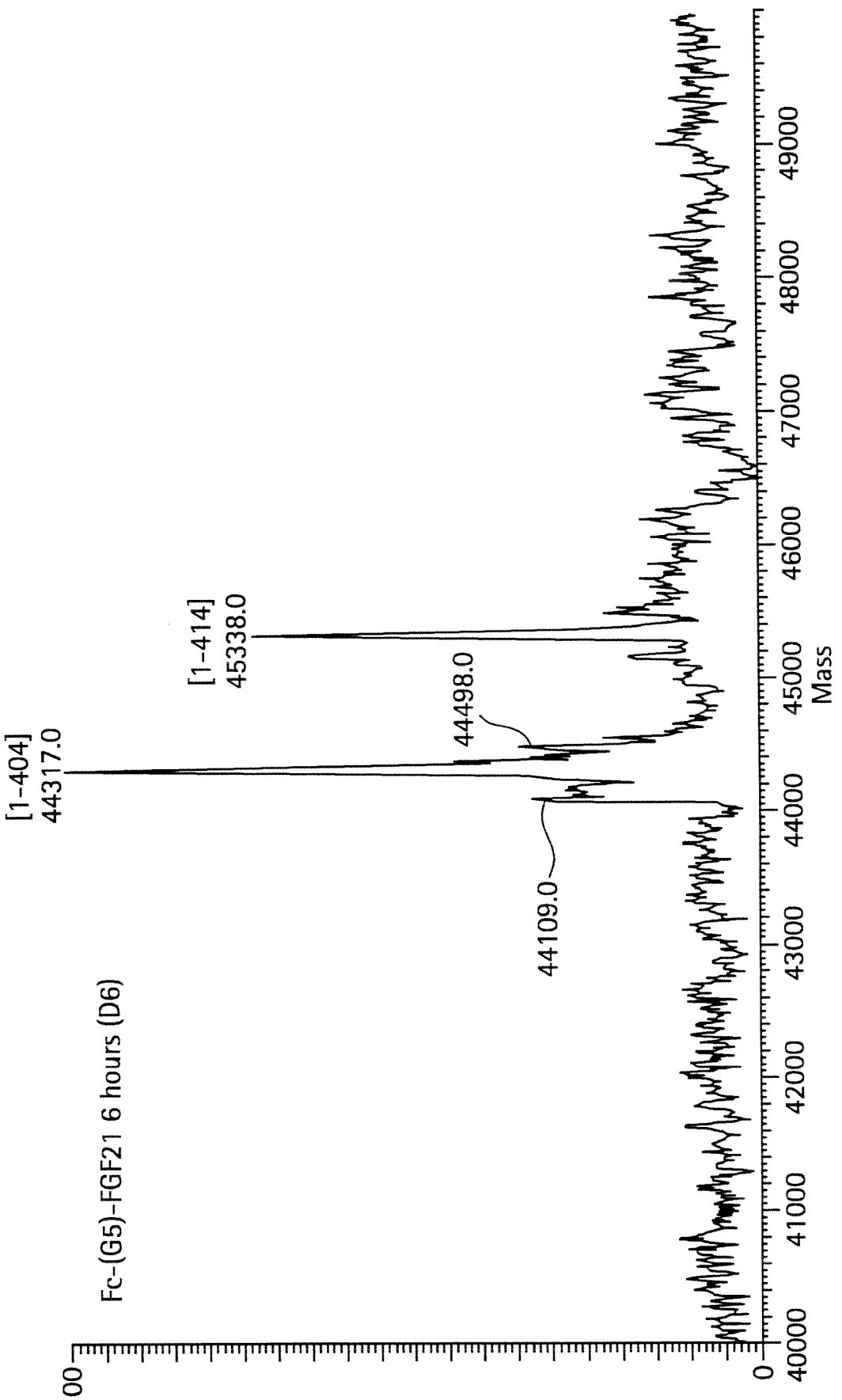

Fc-(G5)-FGF21 6 hours (D6)
[1-404]
44317.0
[1-414]
45338.0
44498.0
44109.0
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 6C

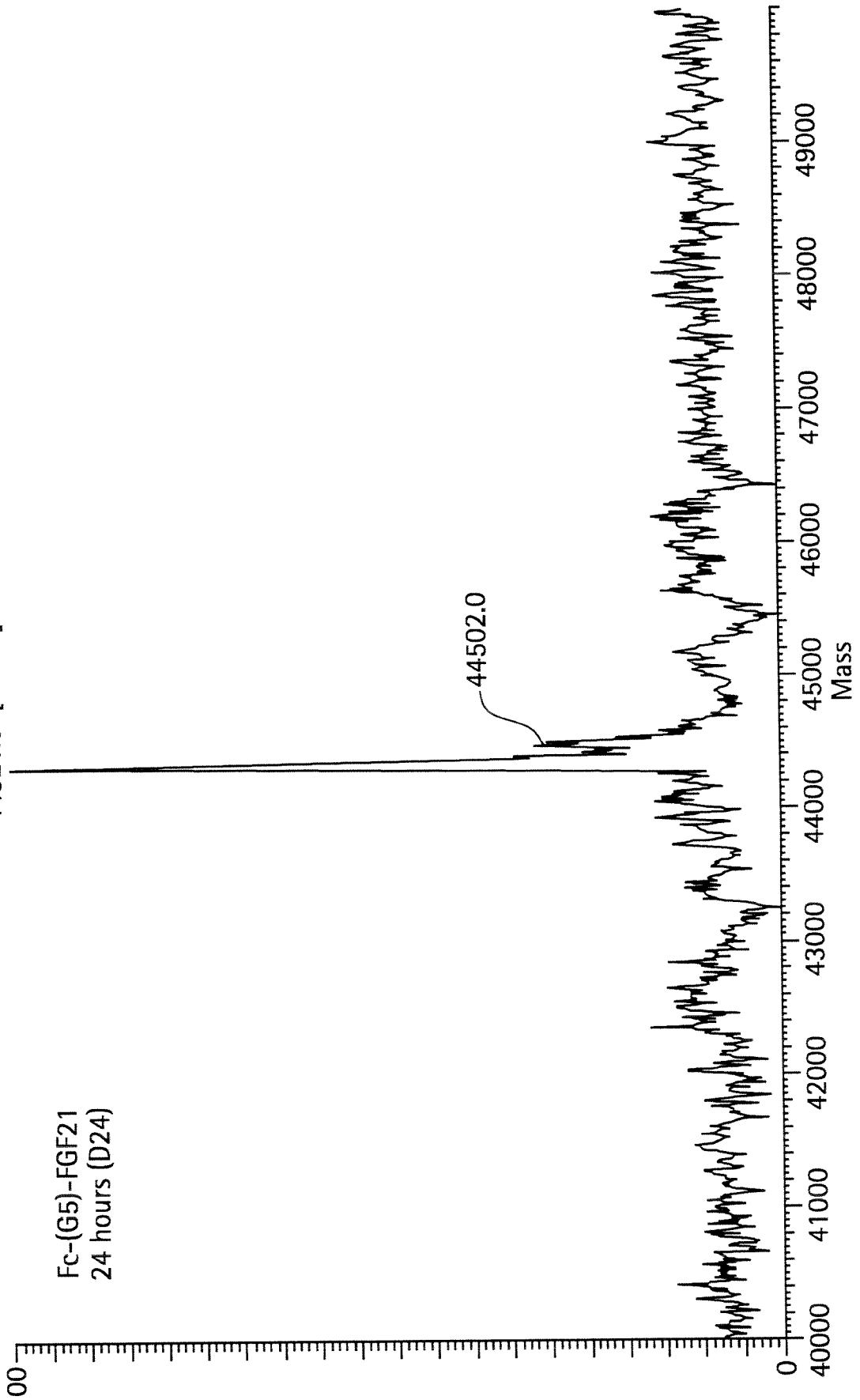

44321.0 [1-404]
44502.0
Fc-(G5)-FGF21
24 hours (D24)
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 6D

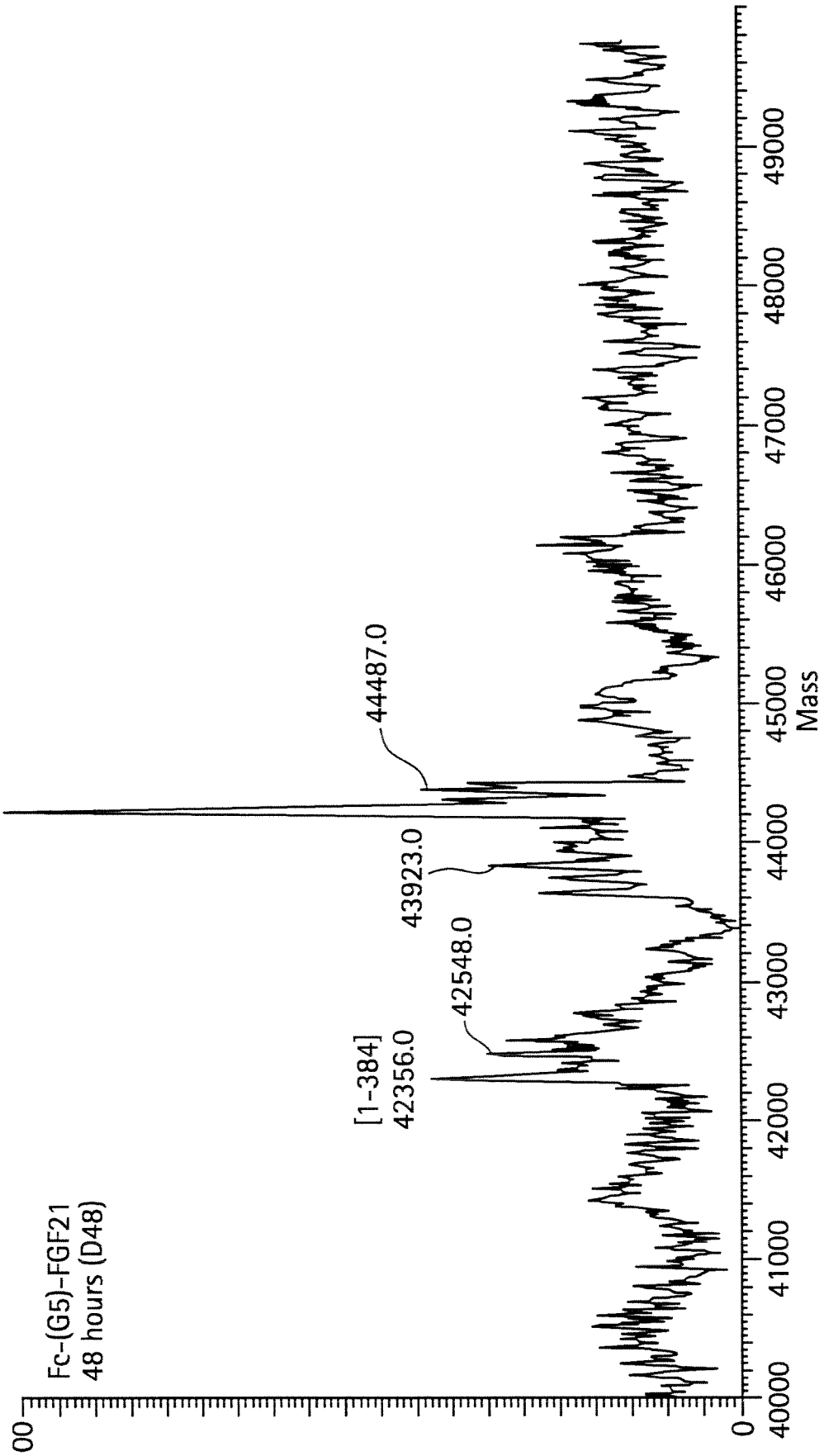

Fc-(G5)-FGF21
48 hours (D48)
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass
44327.0 [1-404]
44487.0
43923.0
42548.0
[1-384]
42356.0

FIG. 7A

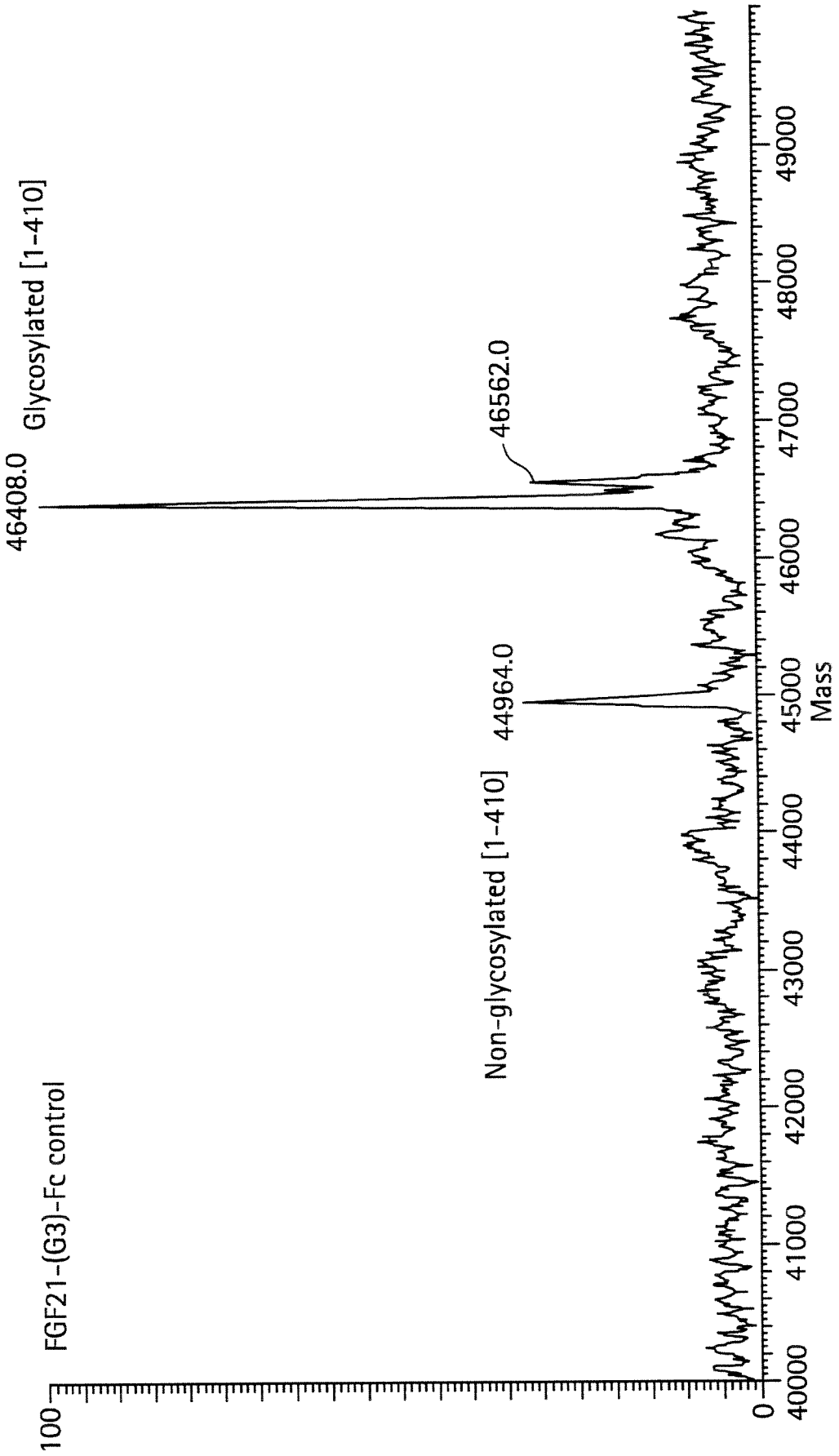

FGF21-(G3)-Fc control
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass
46408.0
Glycosylated [1-410]
46562.0
44964.0
Non-glycosylated [1-410]

FIG. 7B

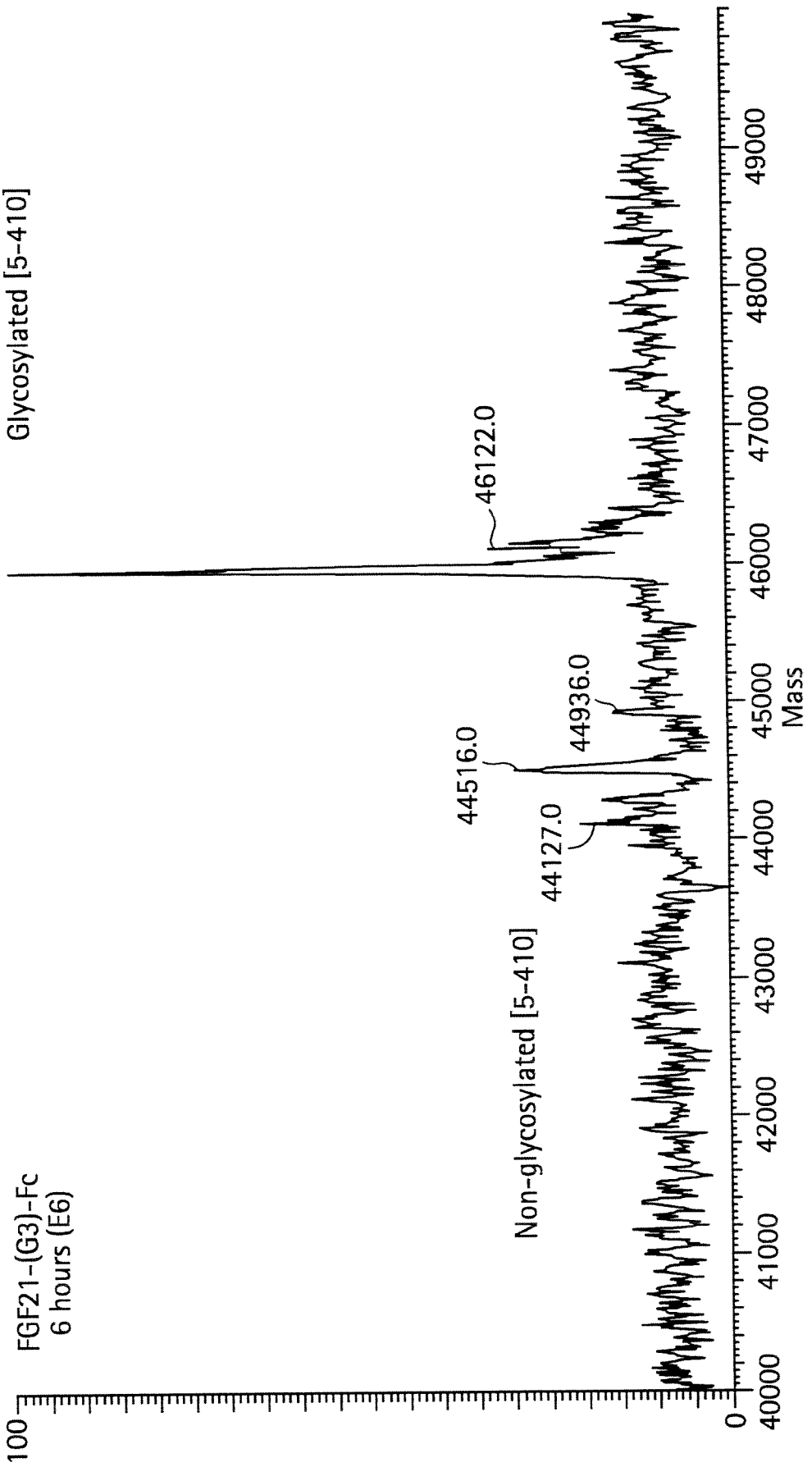

FGF21-(G3)-Fc
6 hours (E6)
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass
45963.0
Glycosylated [5-410]
46122.0
44936.0
44516.0
44127.0
Non-glycosylated [5-410]

FIG. 7C

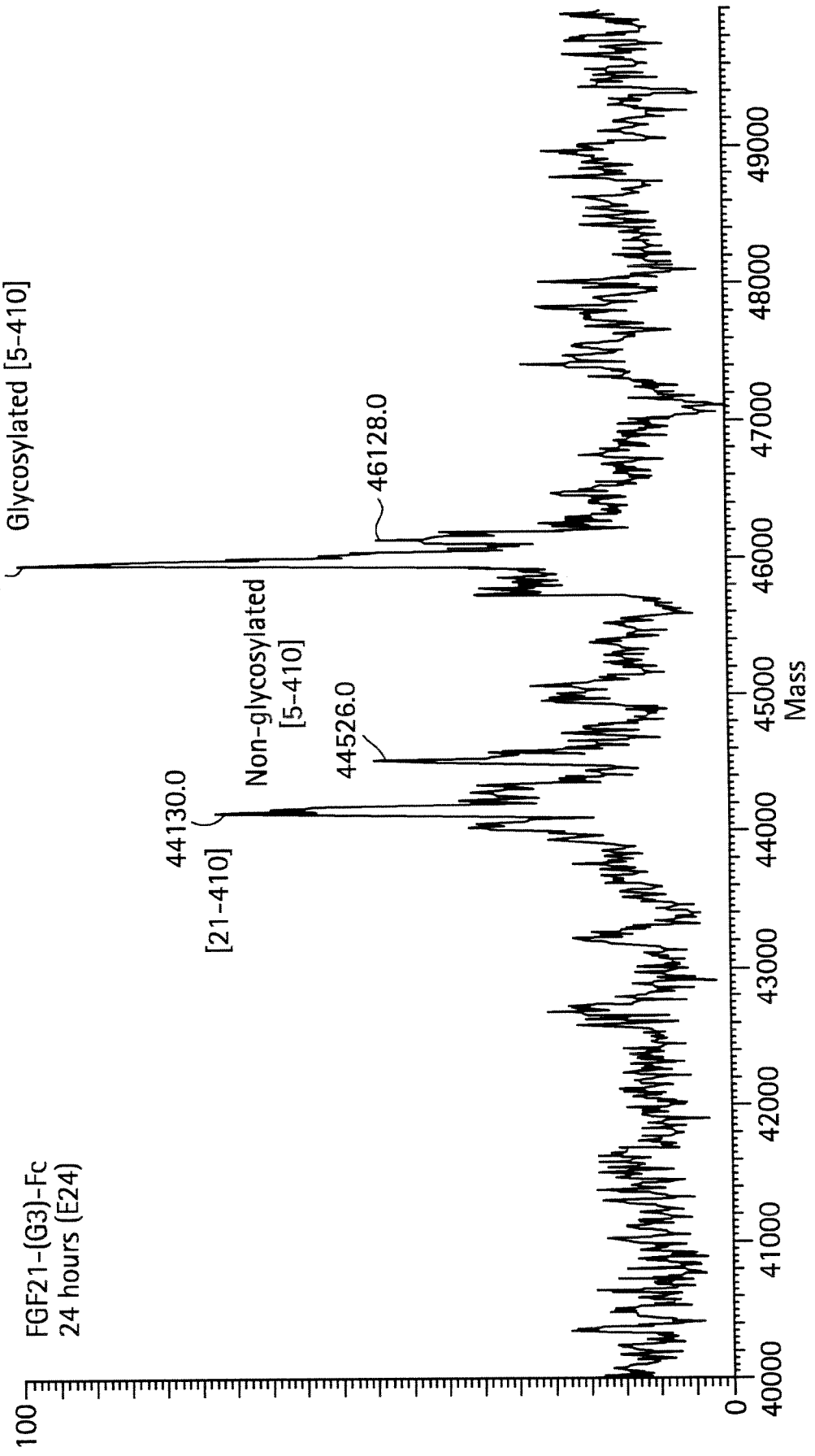

FGF21-(G3)-Fc
24 hours (E24)
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass
45963.0
Glycosylated [5-410]
46128.0
44130.0
[21-410]
Non-glycosylated
[5-410]
44526.0

FIG. 7D

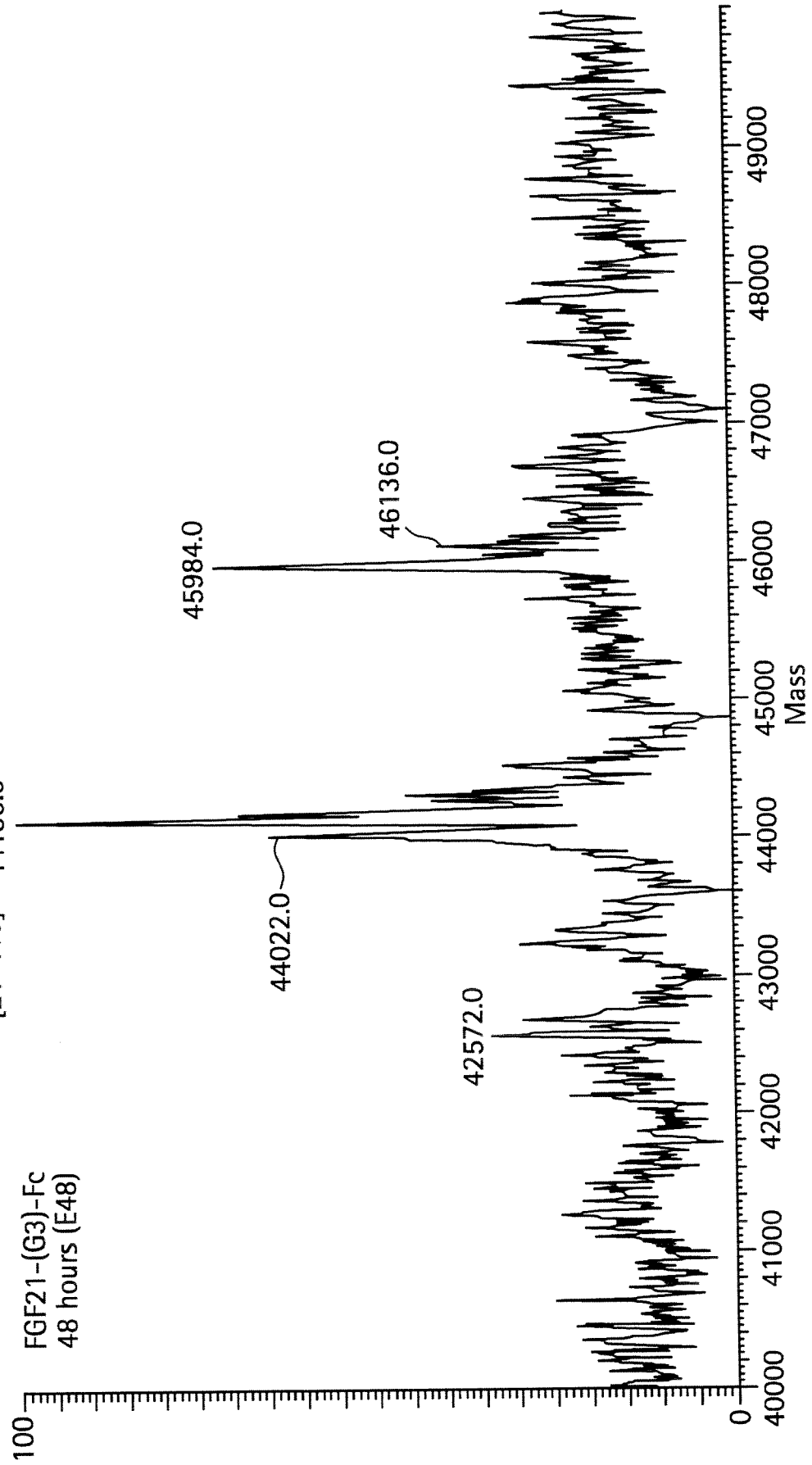

FGF21-(G3)-Fc
48 hours (E48)
[21-410] 44130.0
44022.0
42572.0
45984.0
46136.0
100
0
40000
41000
42000
43000
44000
45000
46000
47000
48000
49000
Mass

FIG. 8A

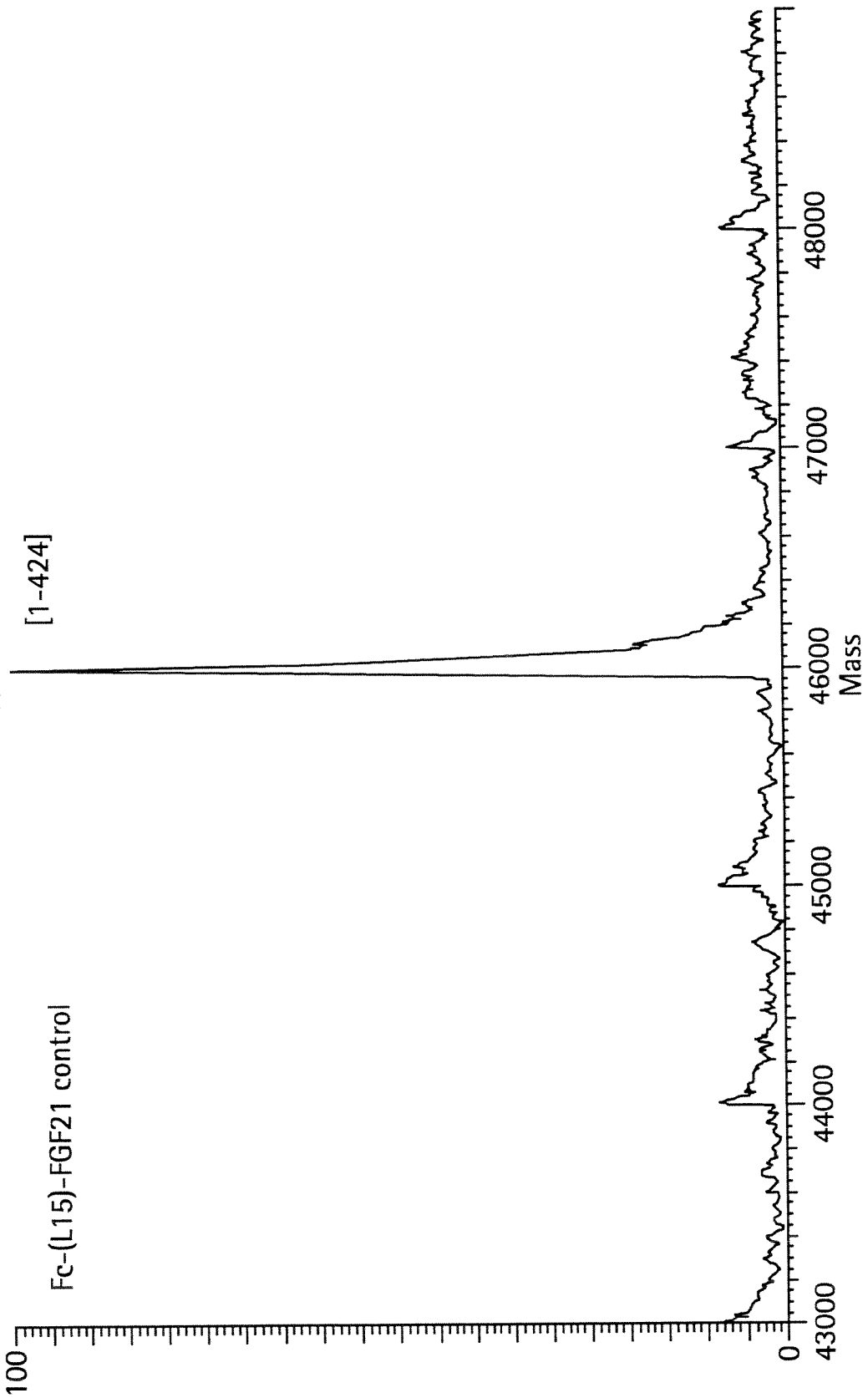

Fc-(L15)-FGF21 control
[1-424]
46002.0
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 8B

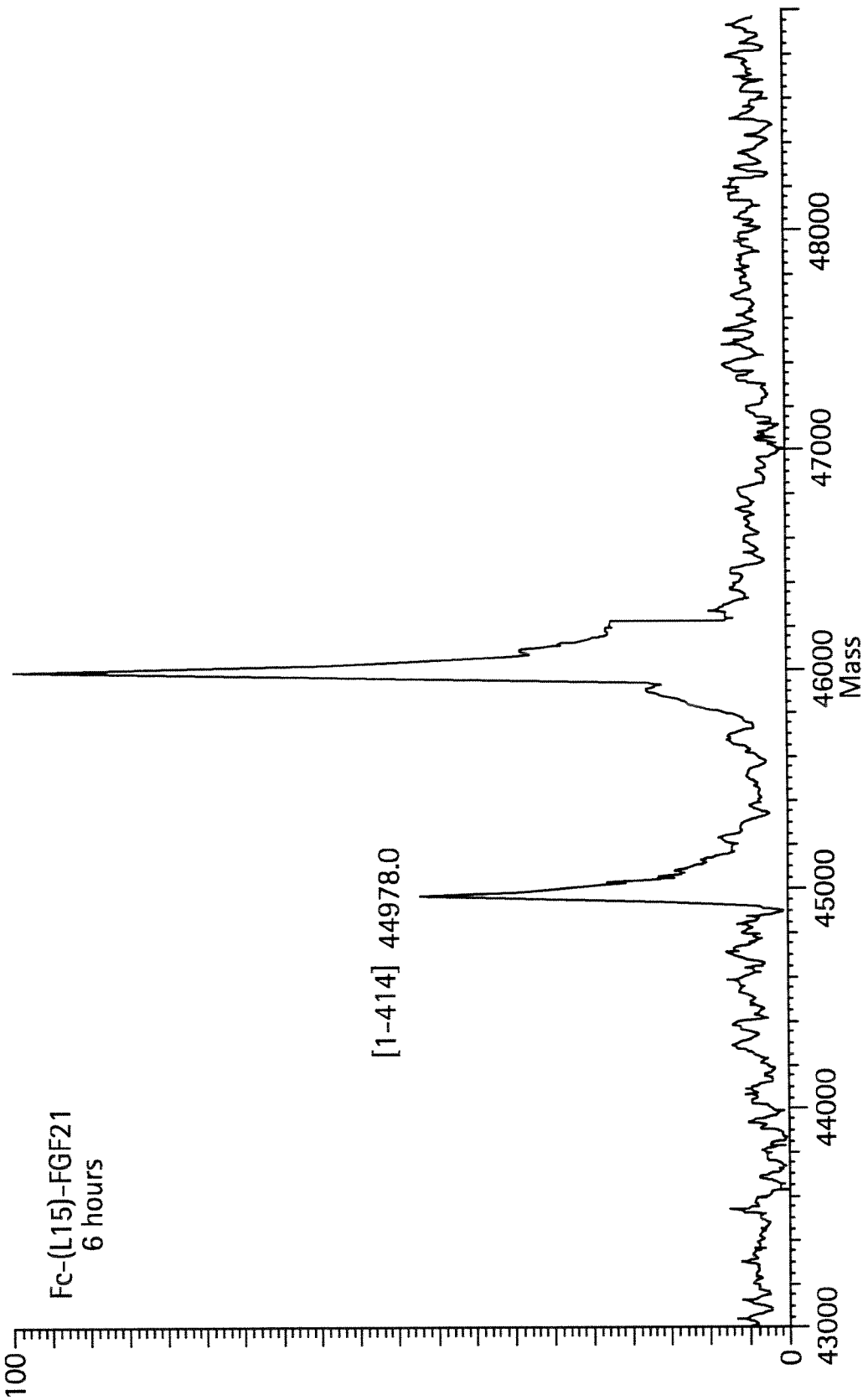

Fc-(L15)-FGF21
6 hours
[1-424] 46000.0
[1-414] 44978.0
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 8C

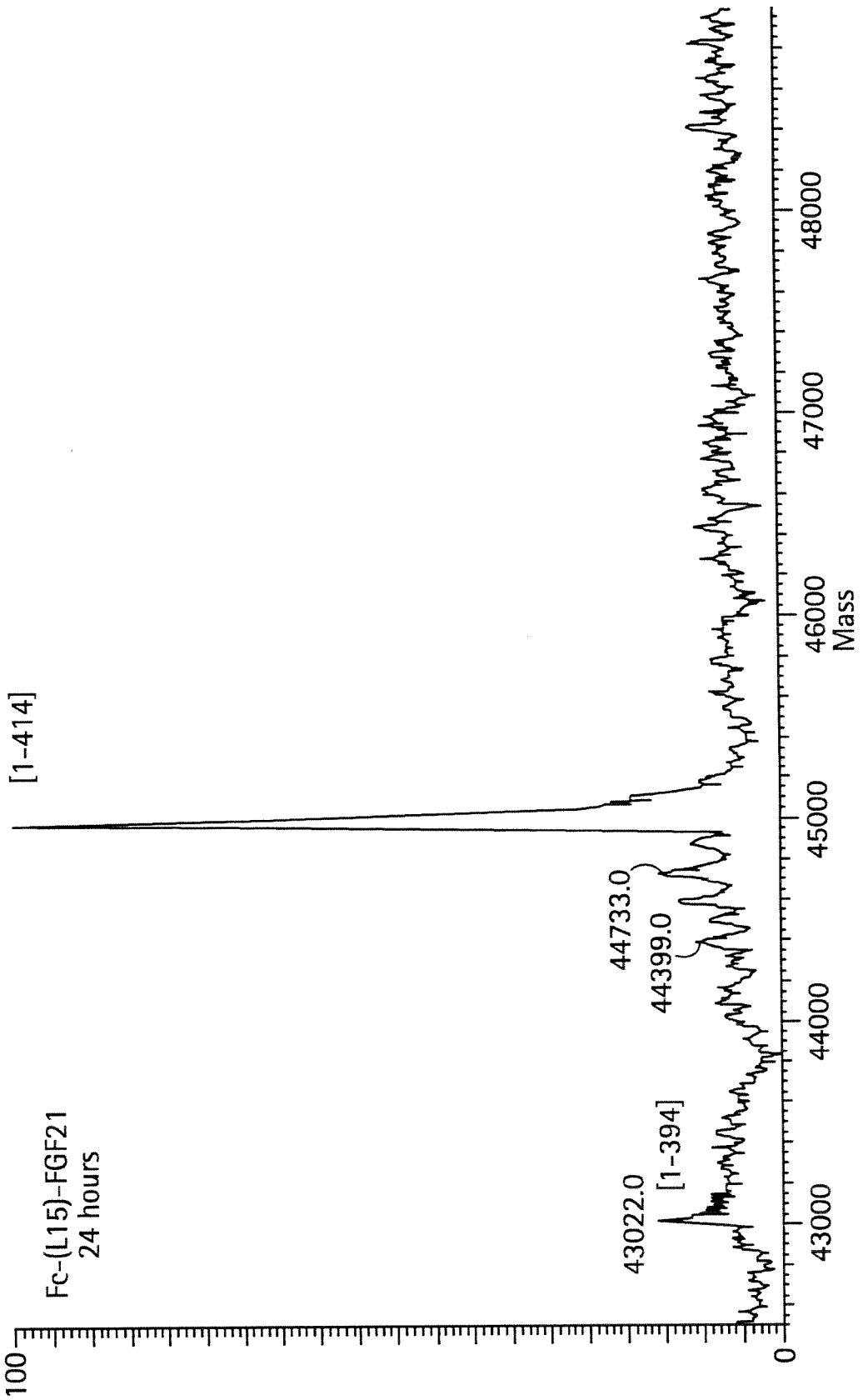

Fc-(L15)-FGF21
24 hours
44978.0
[1-414]
44733.0
44399.0
43022.0
[1-394]
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 8D

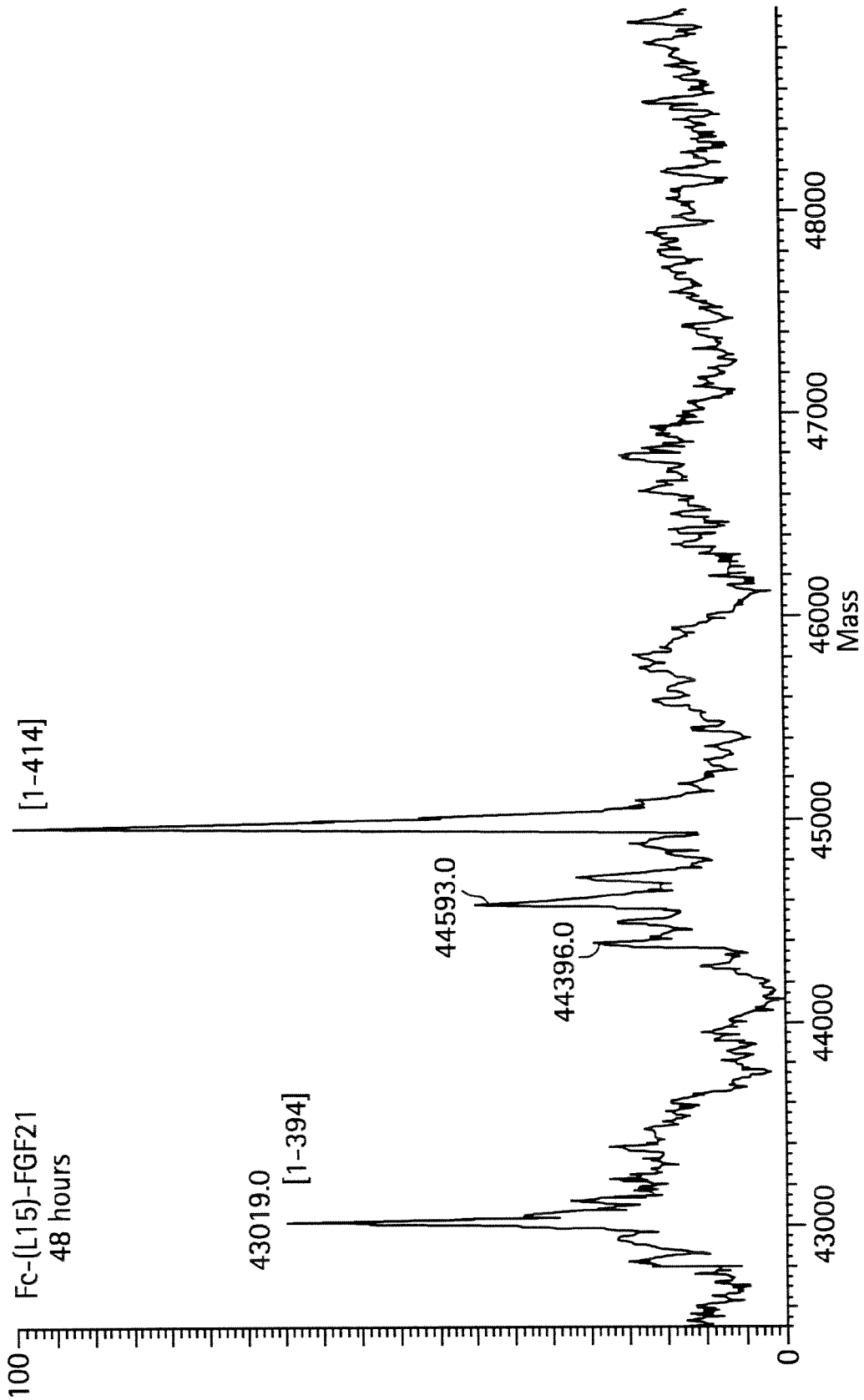

Fc-(L15)-FGF21
48 hours
100
0
43019.0
[1-394]
44396.0
44593.0
44976.0
[1-414]
43000
44000
45000
46000
47000
48000
Mass

FIG. 9A

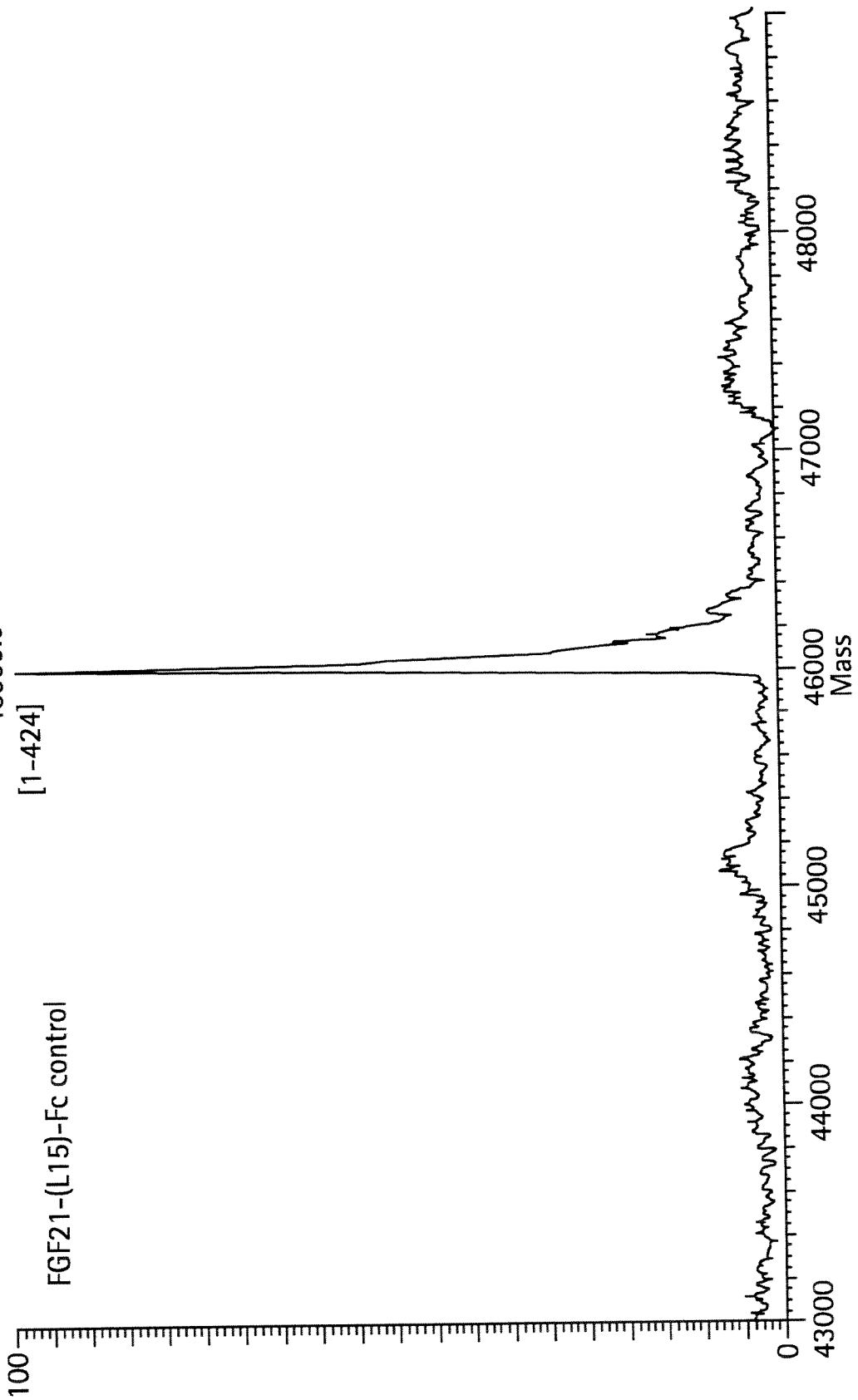

45999.0
[1-424]
FGF21-(L15)-Fc control
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 9B

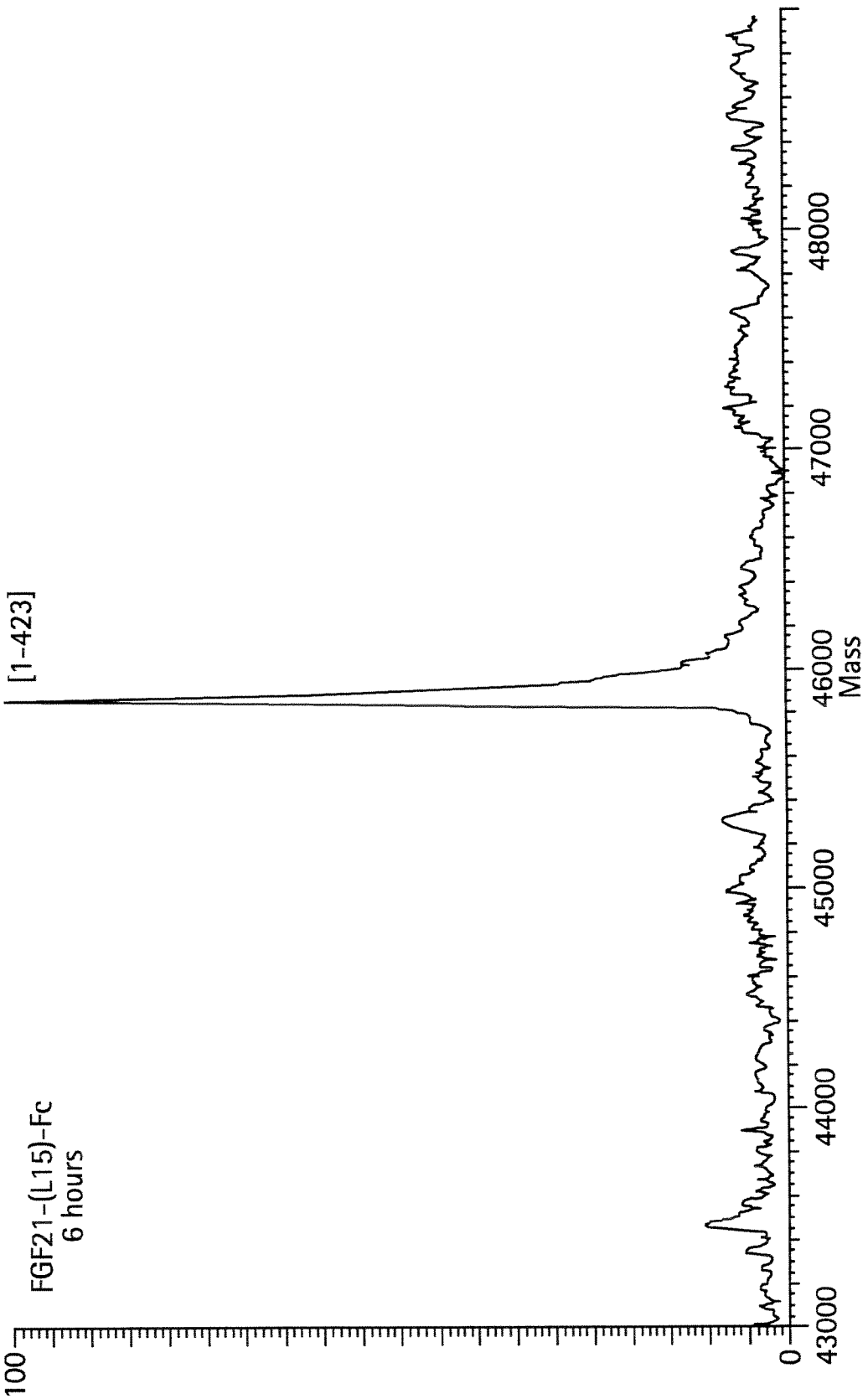

FGF21-(L15)-Fc
6 hours
45870.0
[1-423]
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 9C

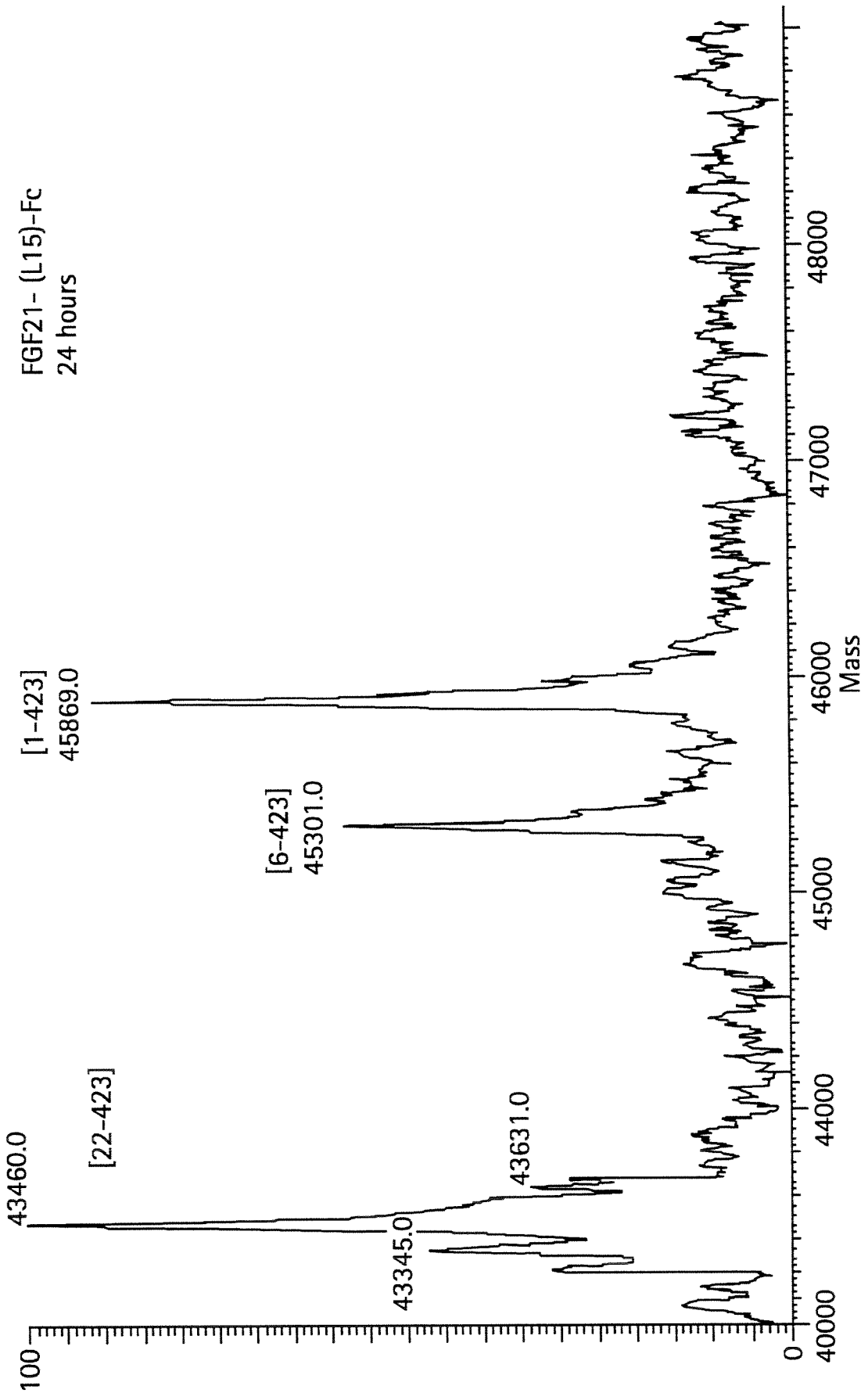

FGF21- (L15)-Fc
24 hours
[1-423]
45869.0
[6-423]
45301.0
[22-423]
43460.0
43345.0
43631.0
100
0
40000
44000
45000
46000
47000
48000
Mass

FIG. 9D

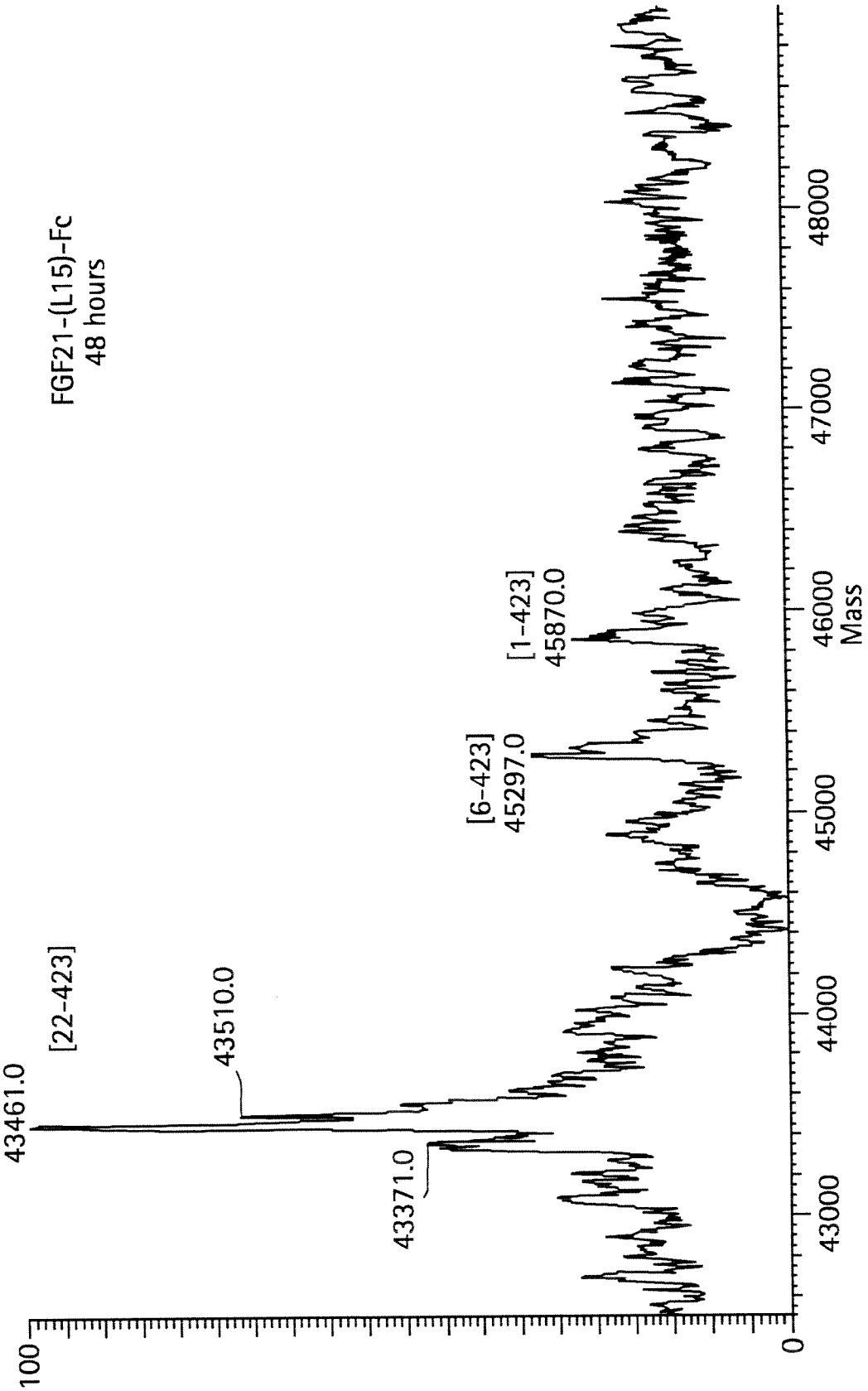

FGF21-(L15)-Fc
48 hours
43461.0
[22-423]
43510.0
43371.0
[6-423]
45297.0
[1-423]
45870.0
100
0
43000
44000
45000
46000
47000
48000
Mass

FIG. 10A

Fc-(L15)-FGF21

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGSGG*
*GGSH*IPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
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*GGGGSGGGSGGGGSGGGS*DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

FIG. 11

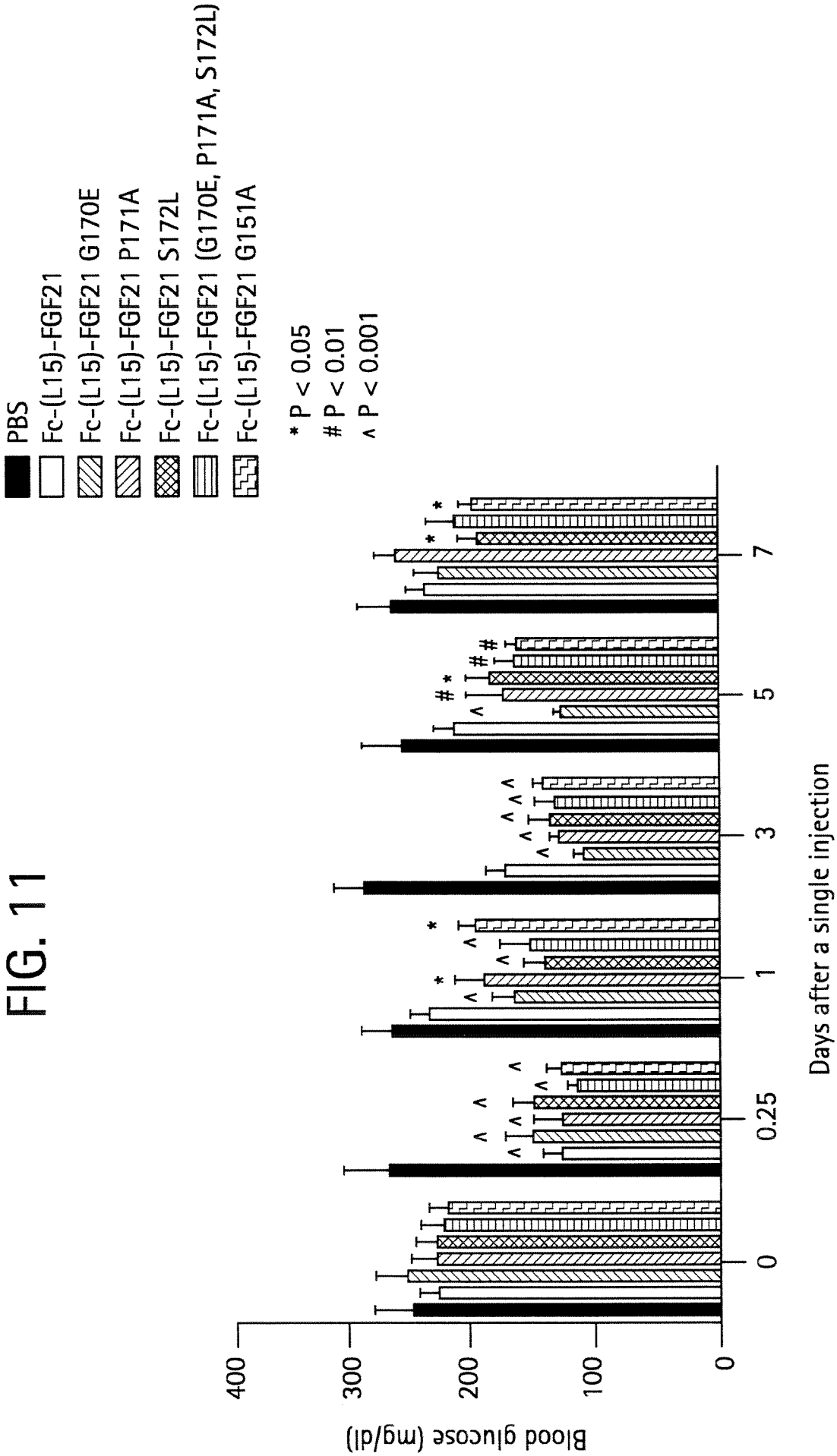

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171A
Fc-(L15)-FGF21 S172L
Fc-(L15)-FGF21 (G170E, P171A, S172L)
Fc-(L15)-FGF21 G151A
* P < 0.05
# P < 0.01
^ P < 0.001
Blood glucose (mg/dl)
0
100
200
300
400
0
0.25
1
3
5
7
Days after a single injection

FIG. 12

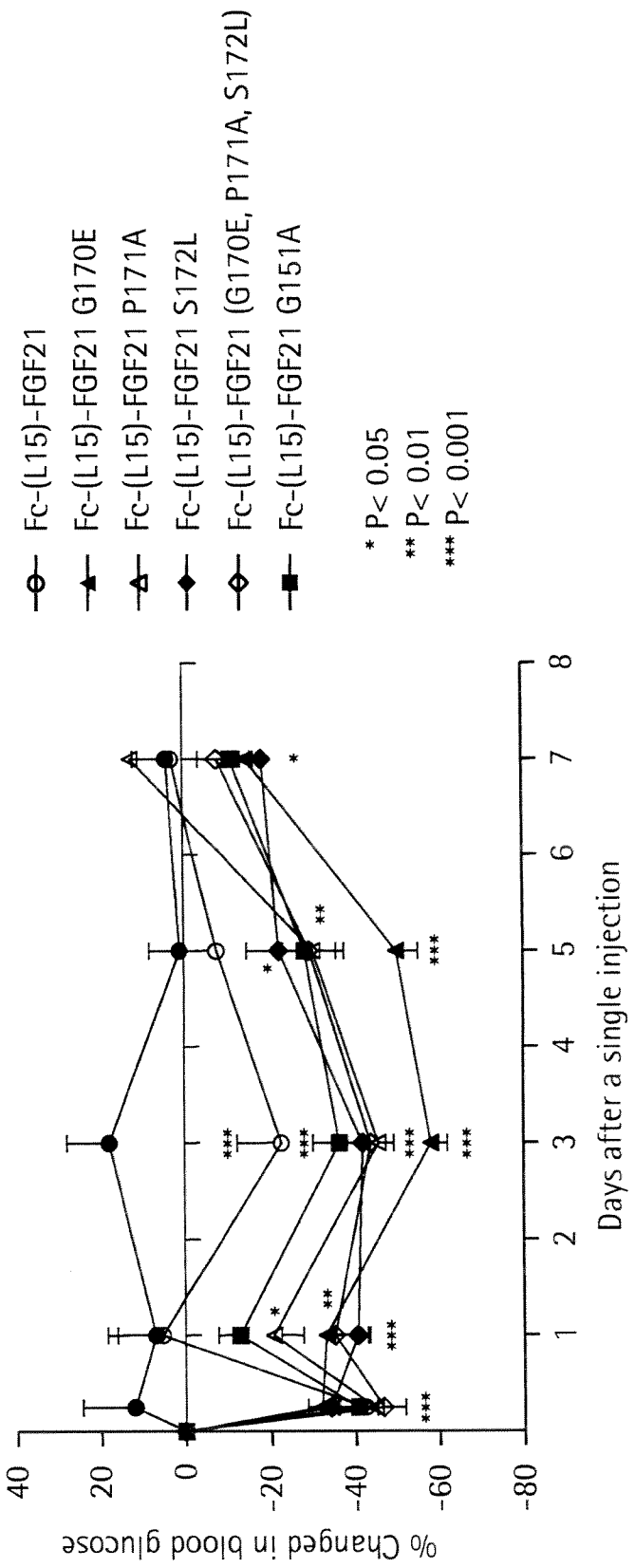

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171A
Fc-(L15)-FGF21 S172L
Fc-(L15)-FGF21 (G170E, P171A, S172L)
Fc-(L15)-FGF21 G151A
* P< 0.05
** P< 0.01
*** P< 0.001
% Changed in blood glucose
40
20
0
-20
-40
-60
-80
1
2
3
4
5
6
7
8
Days after a single injection

FIG. 13

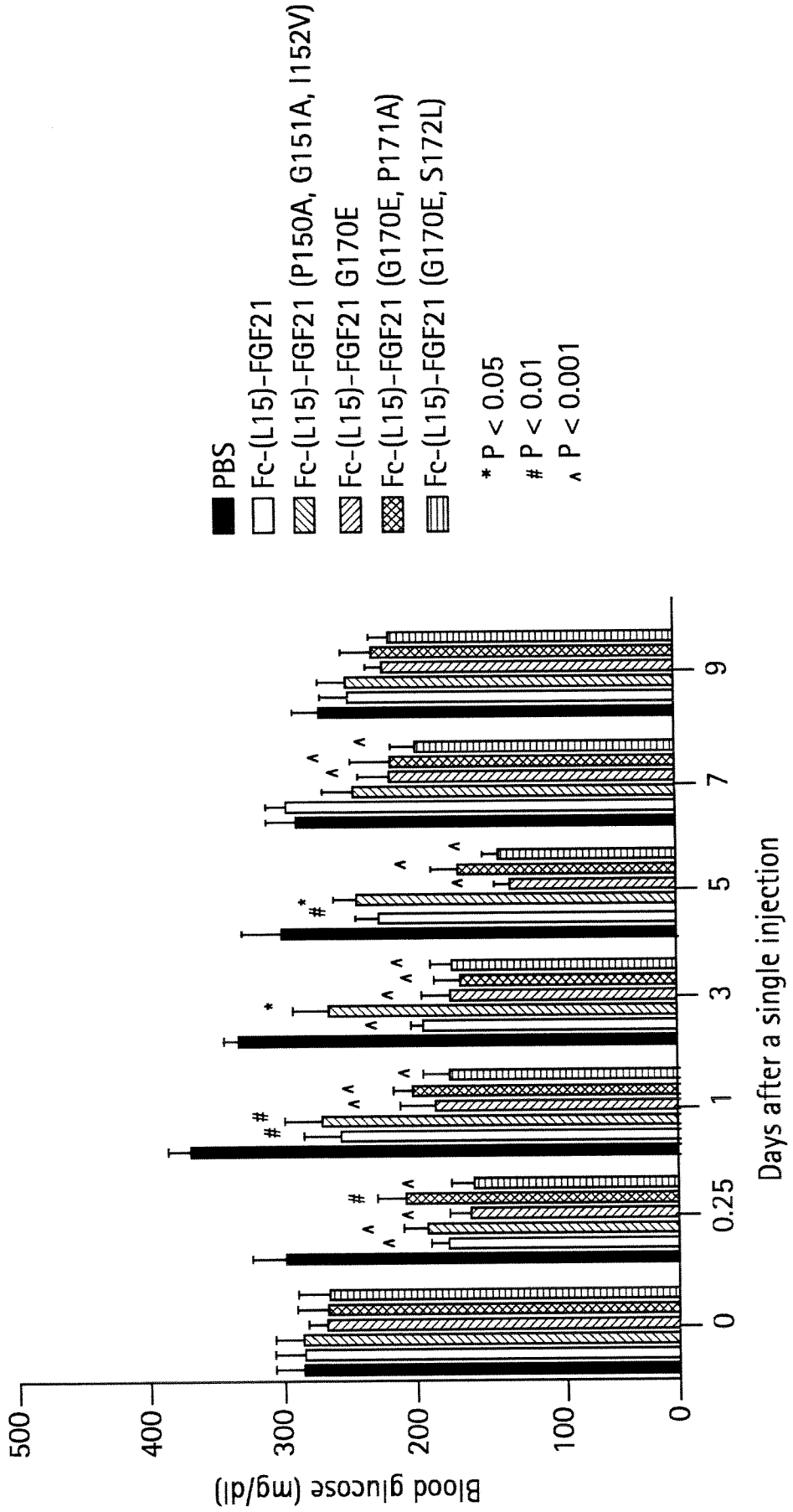

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (P150A, G151A, I152V)
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 (G170E, P171A)
Fc-(L15)-FGF21 (G170E, S172L)
* P < 0.05
# P < 0.01
^ P < 0.001
Blood glucose (mg/dl)
0
100
200
300
400
500
0
0.25
1
3
5
7
9
Days after a single injection

FIG. 14

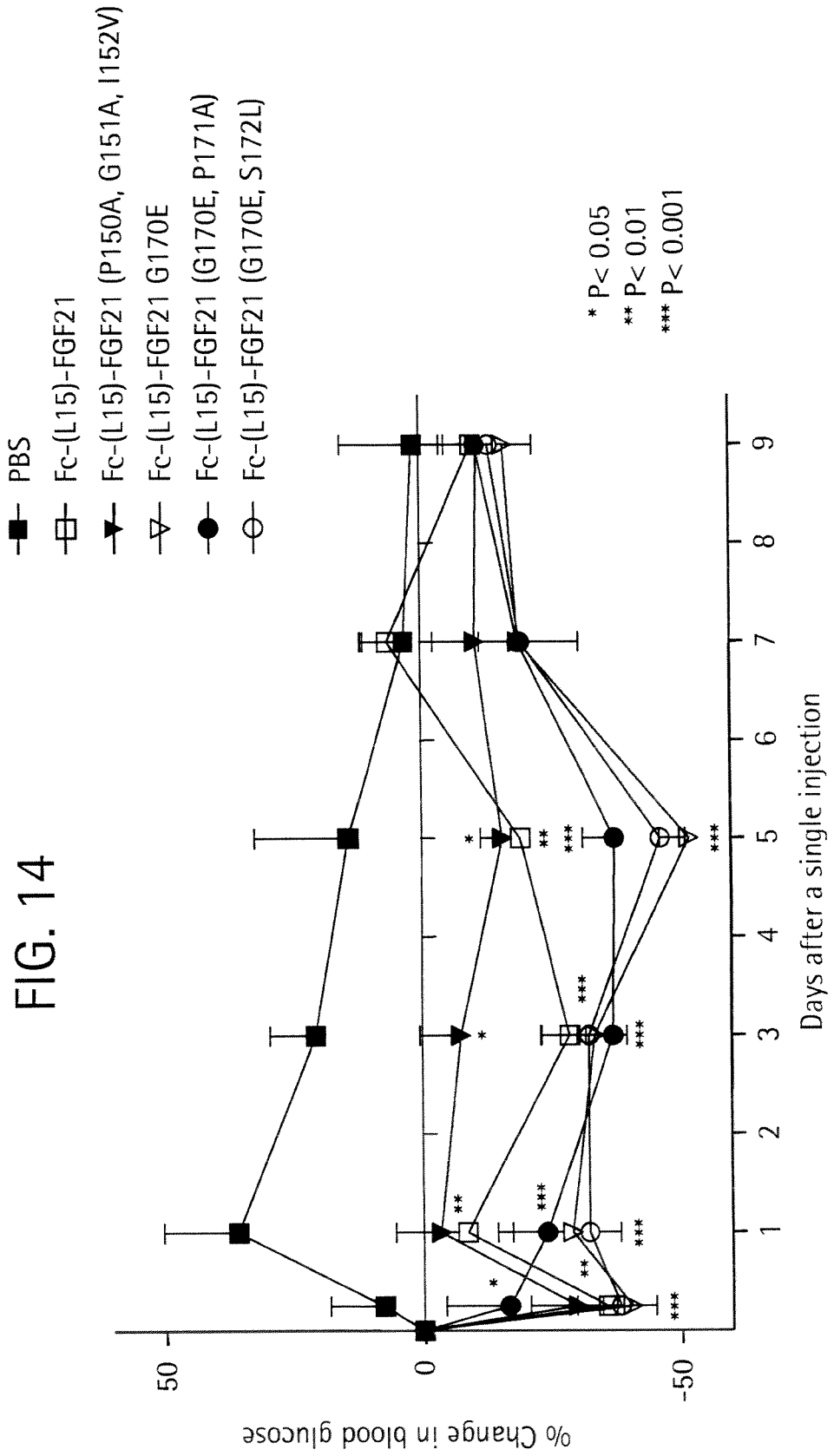

PBS
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (P150A, G151A, I152V)
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 (G170E, P171A)
Fc-(L15)-FGF21 (G170E, S172L)
* P< 0.05
** P< 0.01
*** P< 0.001
% Change in blood glucose
50
0
-50
1
2
3
4
5
6
7
8
9
Days after a single injection

FIG. 15

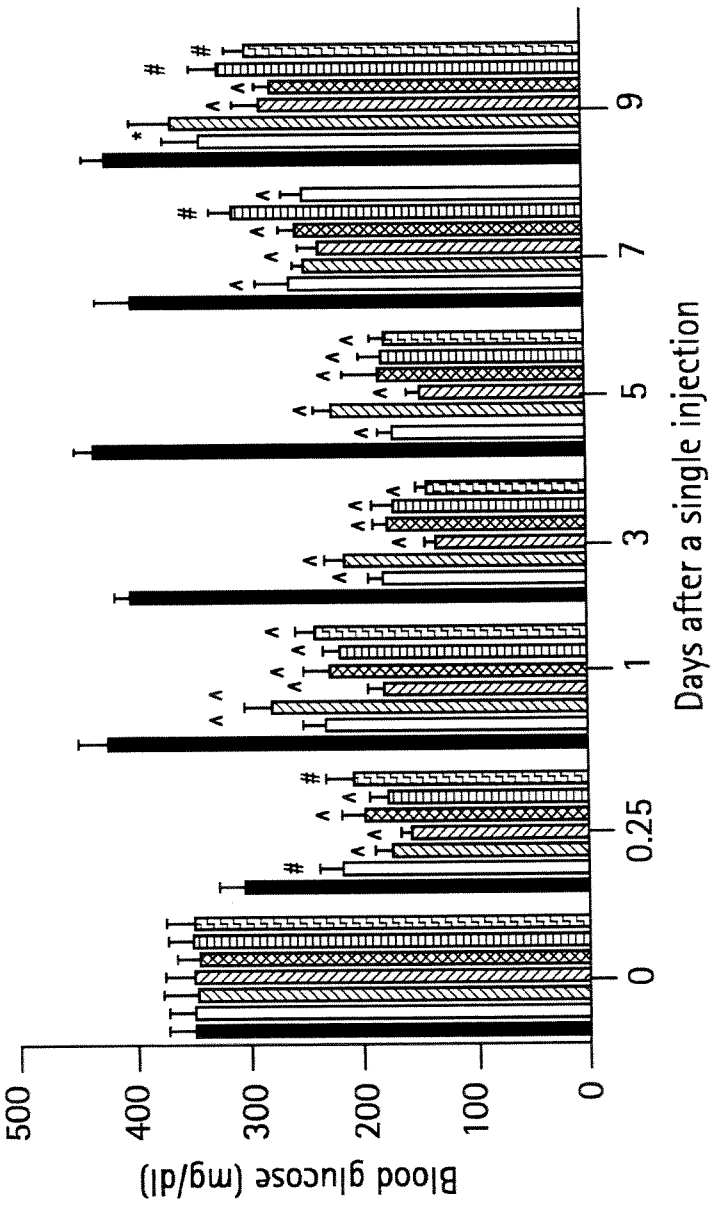

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 G170A
Fc-(L15)-FGF21 G170C
Fc-(L15)-FGF21 G170D
Fc-(L15)-FGF21 G170N
Fc-(L15)-FGF21 G170S
* P < 0.05
# P < 0.01
^ P < 0.001
Treatment vs. PBS
Blood glucose (mg/dl)
0
100
200
300
400
500
0
0.25
1
3
5
7
9
Days after a single injection

FIG. 16

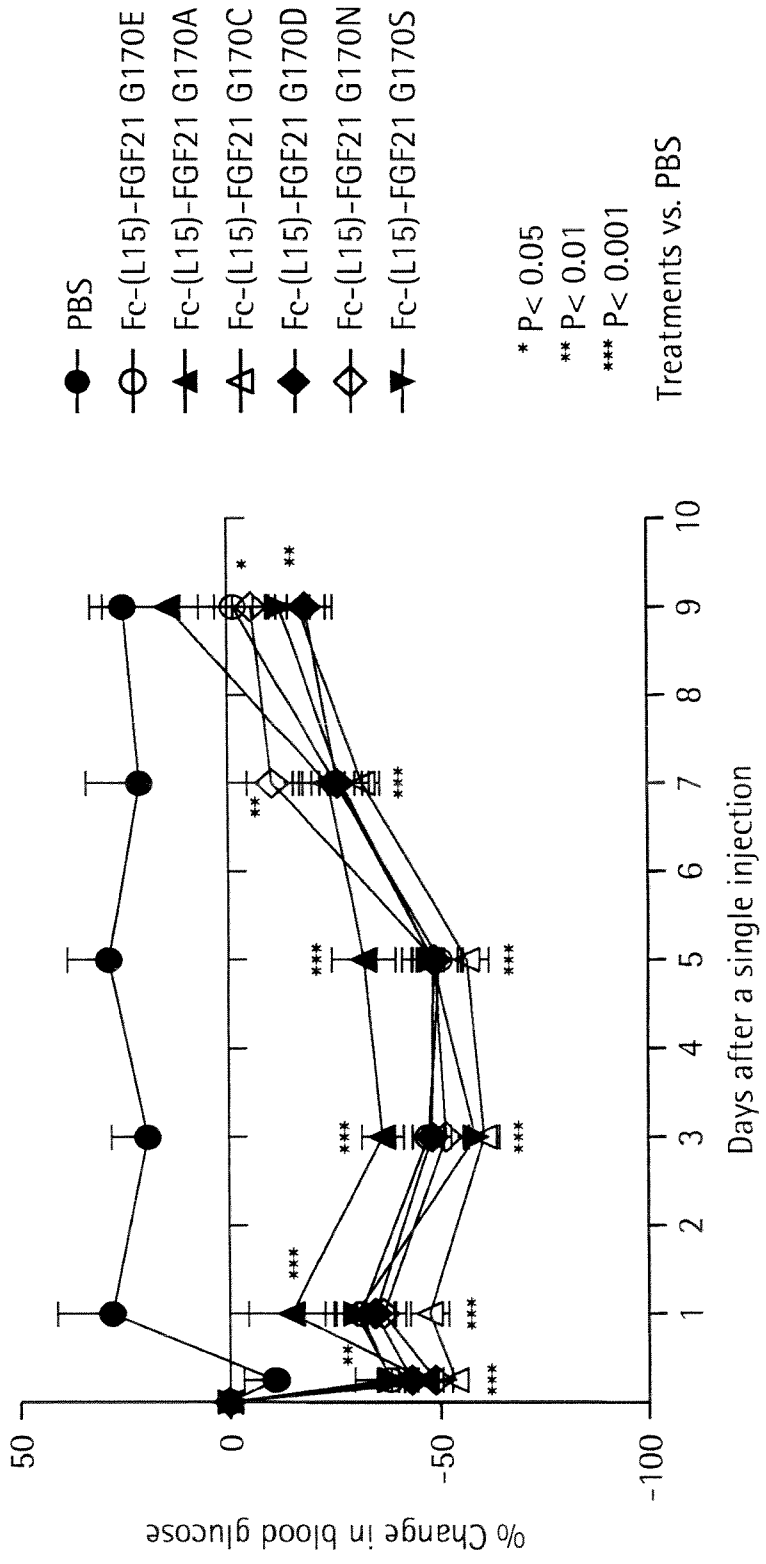

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 G170A
Fc-(L15)-FGF21 G170C
Fc-(L15)-FGF21 G170D
Fc-(L15)-FGF21 G170N
Fc-(L15)-FGF21 G170S
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. PBS
% Change in blood glucose
50
0
-50
-100
1
2
3
4
5
6
7
8
9
10
Days after a single injection

FIG. 17

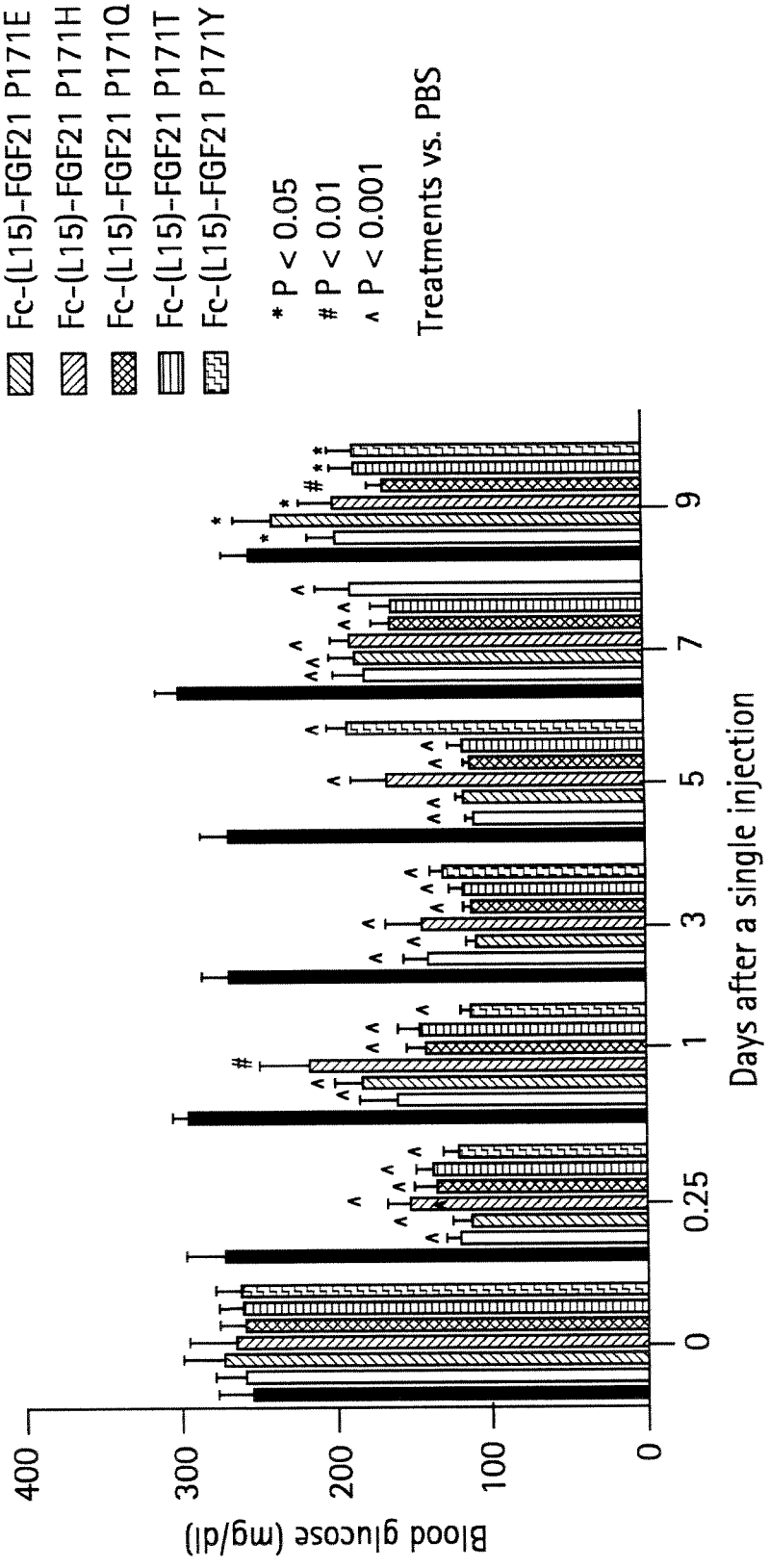

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 P171H
Fc-(L15)-FGF21 P171Q
Fc-(L15)-FGF21 P171T
Fc-(L15)-FGF21 P171Y
* P < 0.05
# P < 0.01
^ P < 0.001
Treatments vs. PBS
Blood glucose (mg/dl)
0
100
200
300
400
0
0.25
1
3
5
7
9
Days after a single injection

FIG. 18

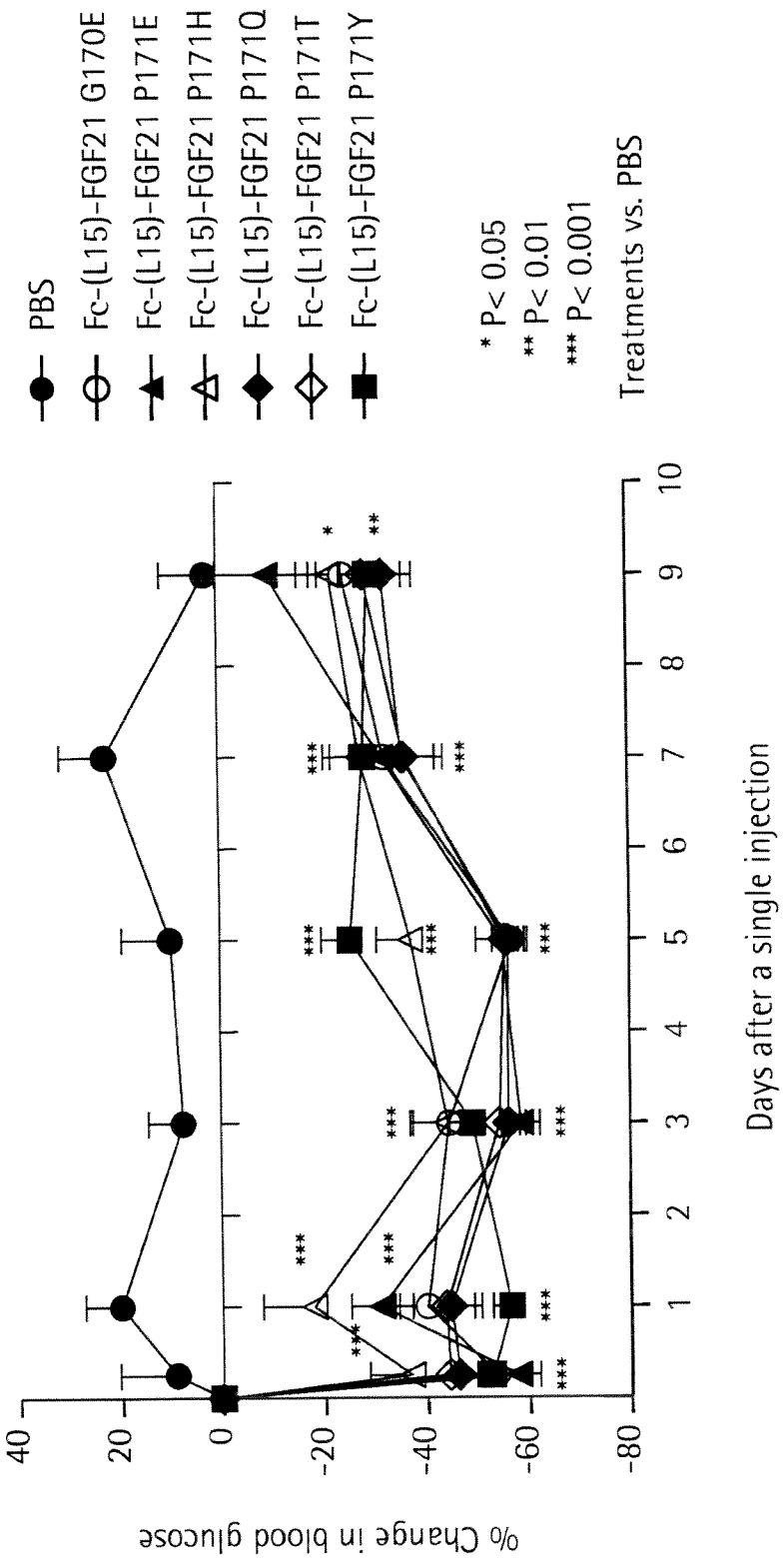

PBS
Fc-(L15)-FGF21 G170E
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 P171H
Fc-(L15)-FGF21 P171Q
Fc-(L15)-FGF21 P171T
Fc-(L15)-FGF21 P171Y
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. PBS
% Change in blood glucose
40
20
0
-20
-40
-60
-80
1
2
3
4
5
6
7
8
9
10
Days after a single injection

FIG. 19A

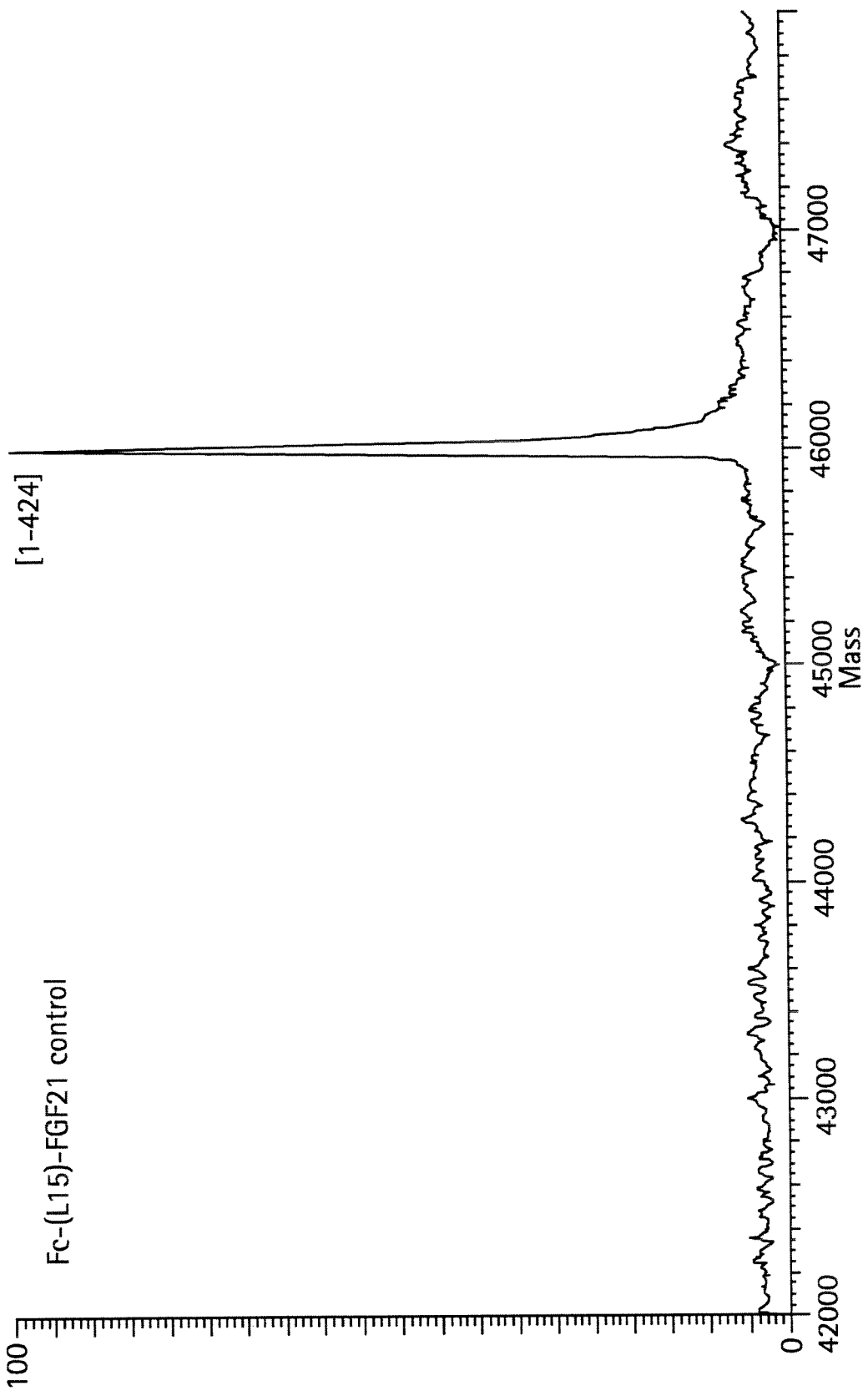

45994.0
[1-424]
Fc-(L15)-FGF21 control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 19B

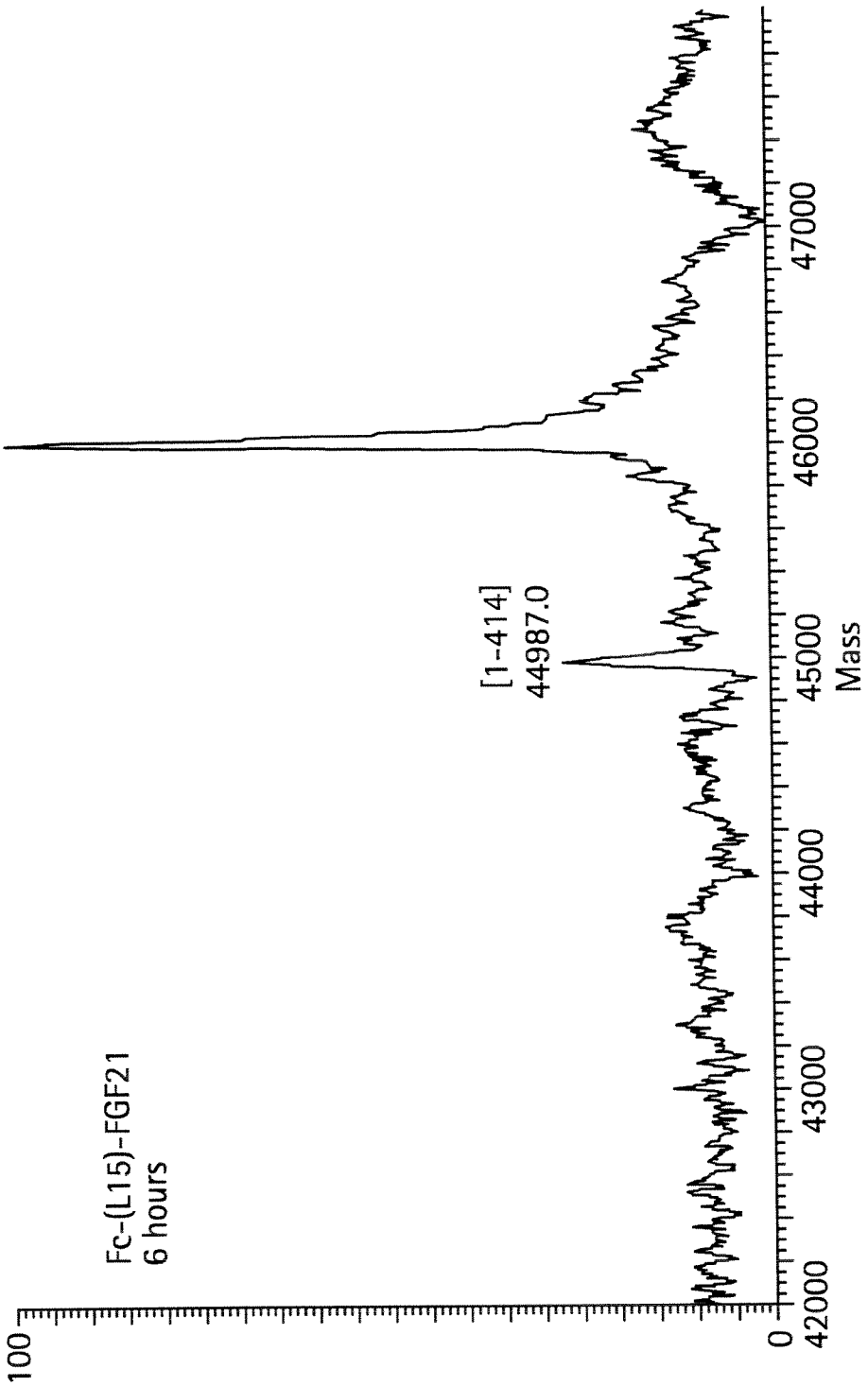

Fc-(L15)-FGF21
6 hours
[1-424]
46001.0
[1-414]
44987.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 19C

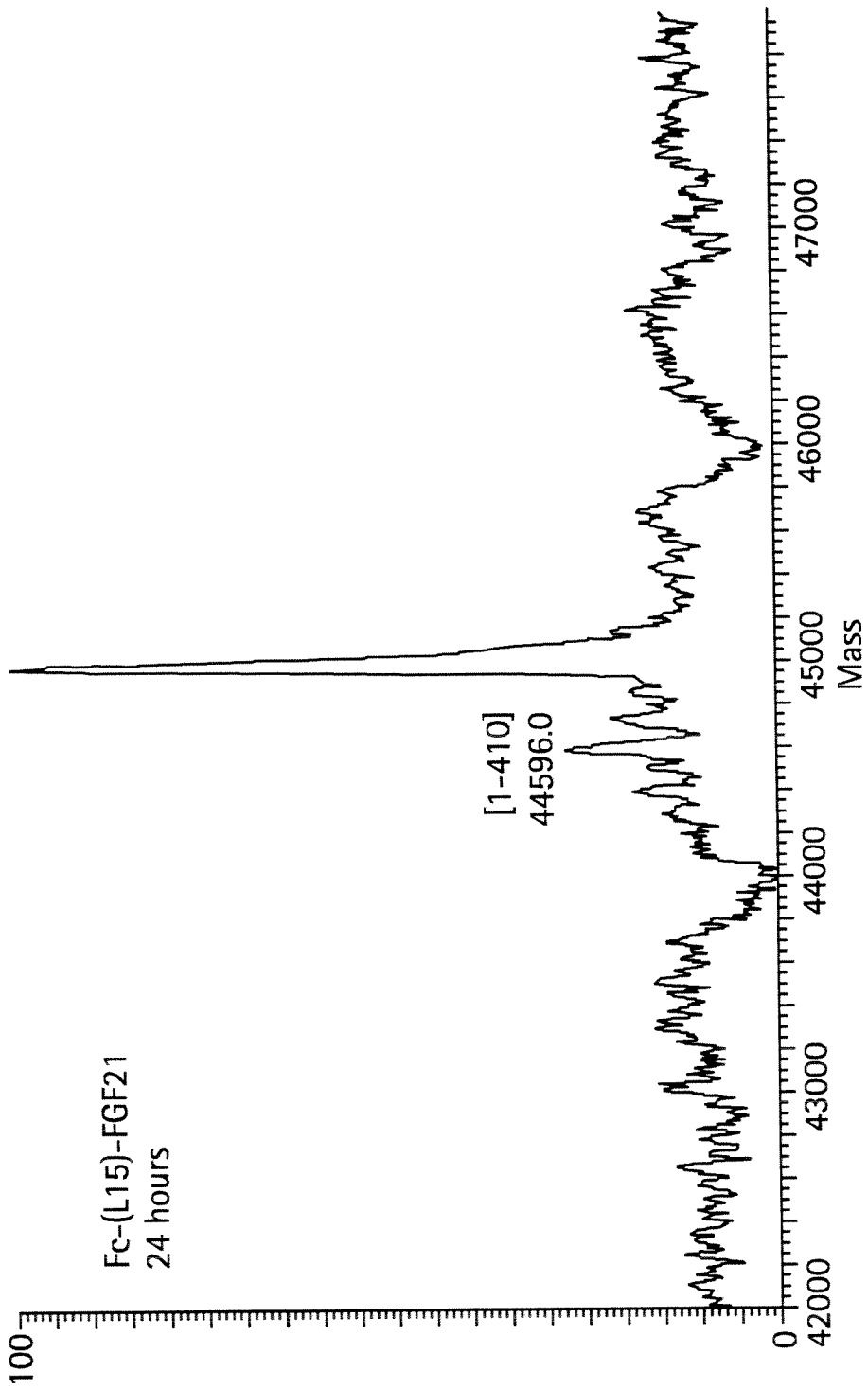

Fc-(L15)-FGF21
24 hours
[1-414]
44979.0
[1-410]
44596.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 19D

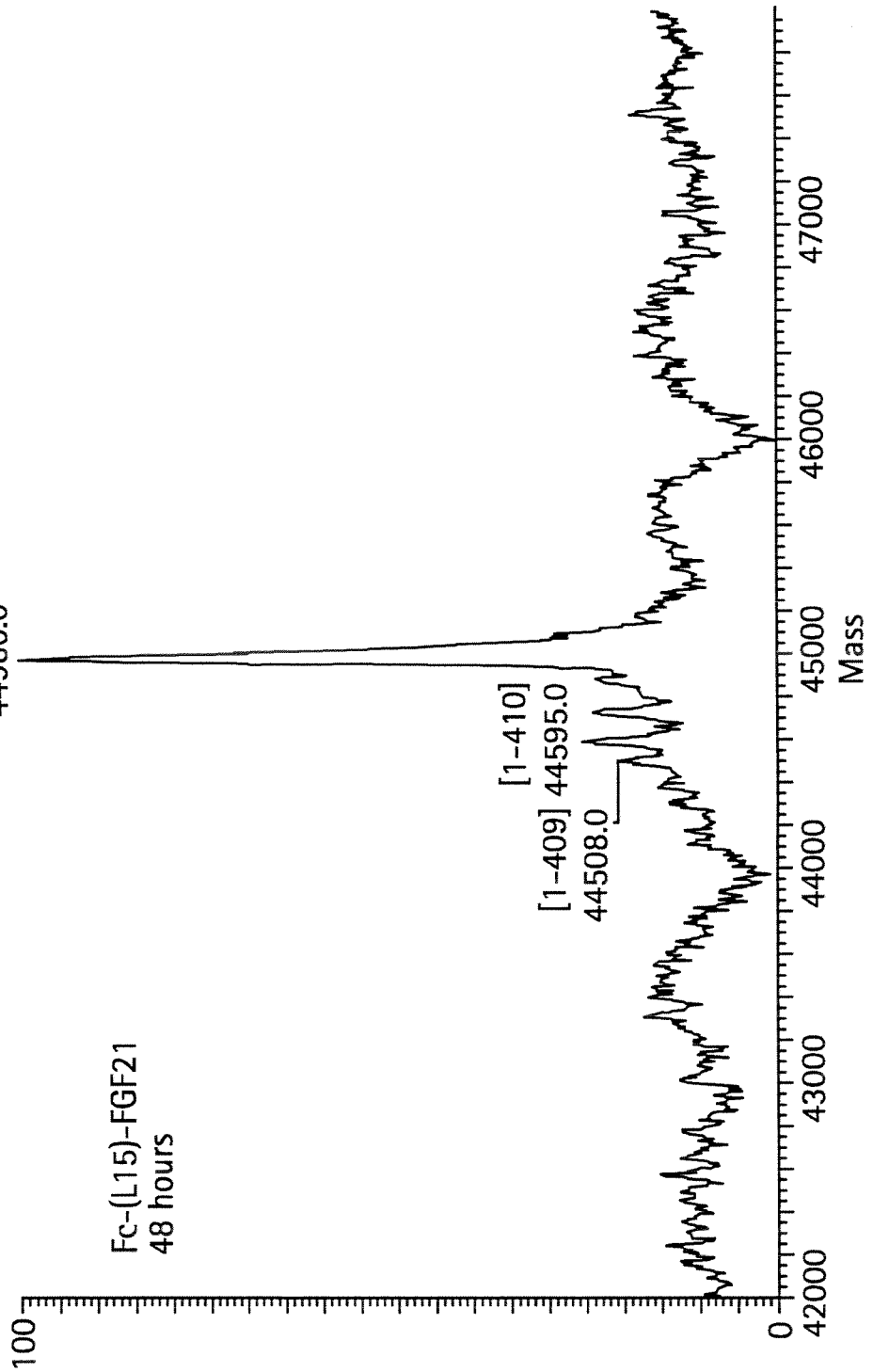

Fc-(L15)-FGF21
48 hours
[1-414]
44980.0
[1-410]
[1-409] 44595.0
44508.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20A

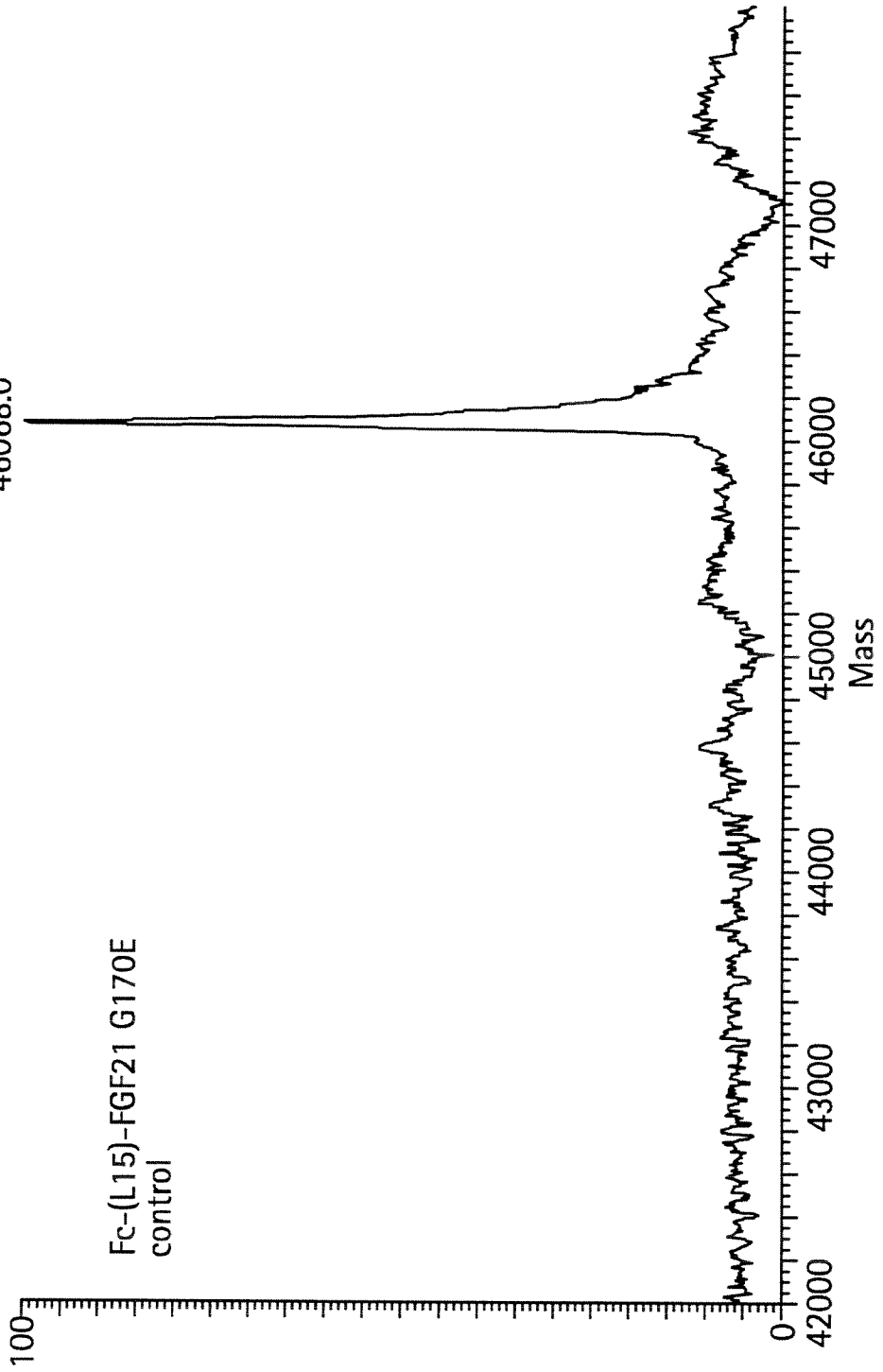

[1-424]
46068.0
Fc-(L15)-FGF21 G170E
control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20B

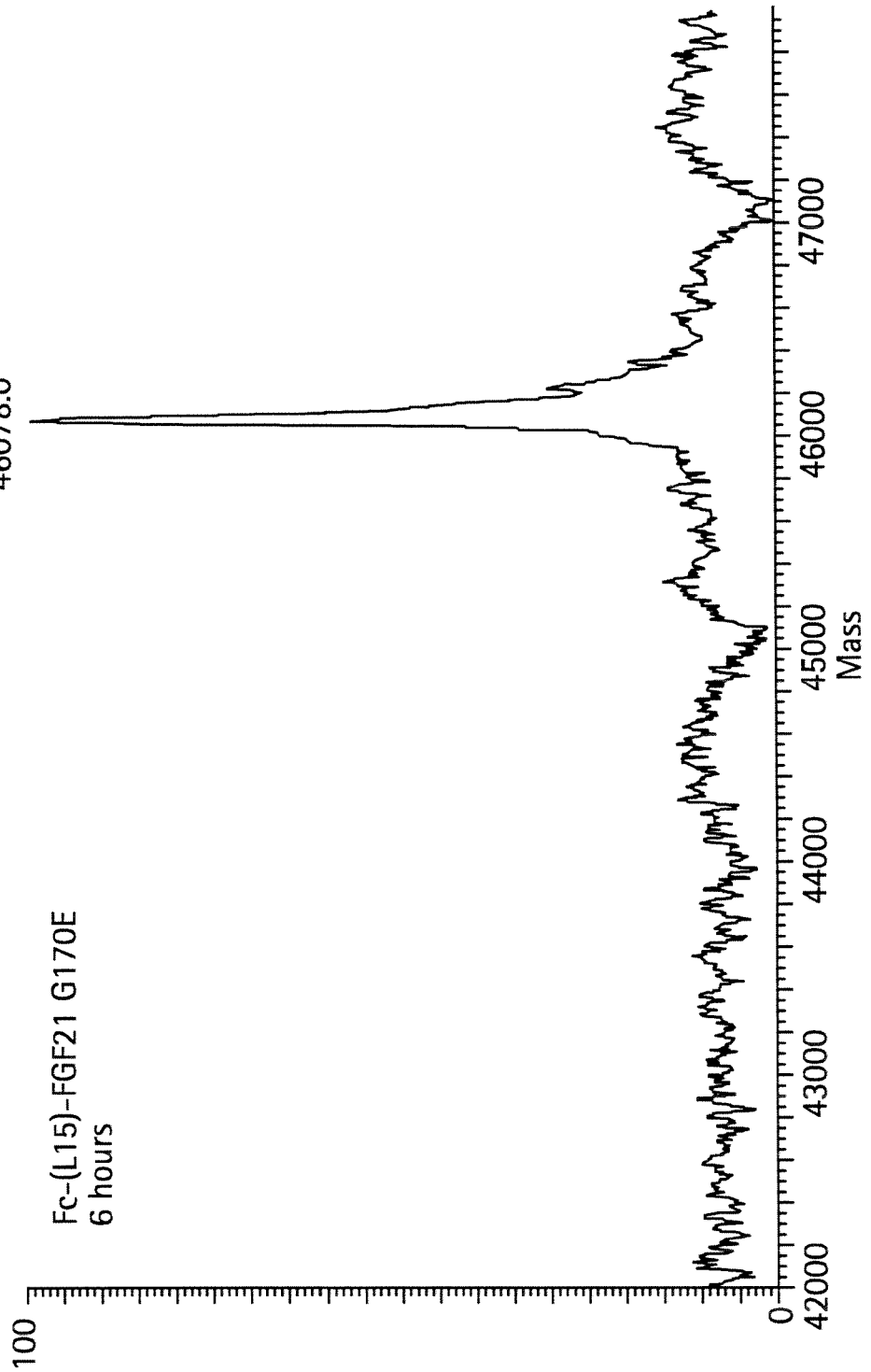

[1-424]
46078.0
Fc-(L15)-FGF21 G170E
6 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20C

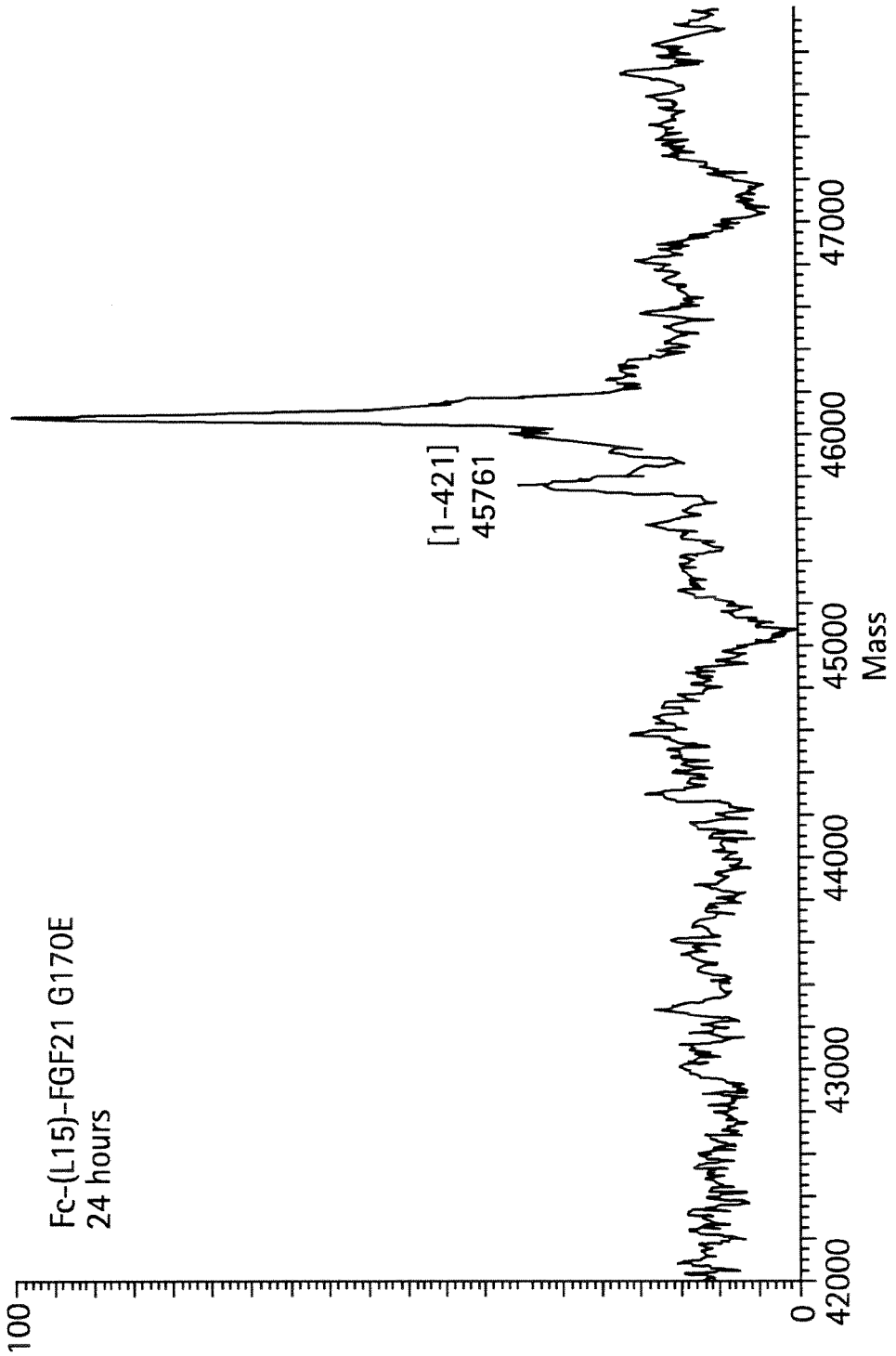

Fc-(L15)-FGF21 G170E
24 hours
[1-424]
46074.0
[1-421]
45761
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 20D

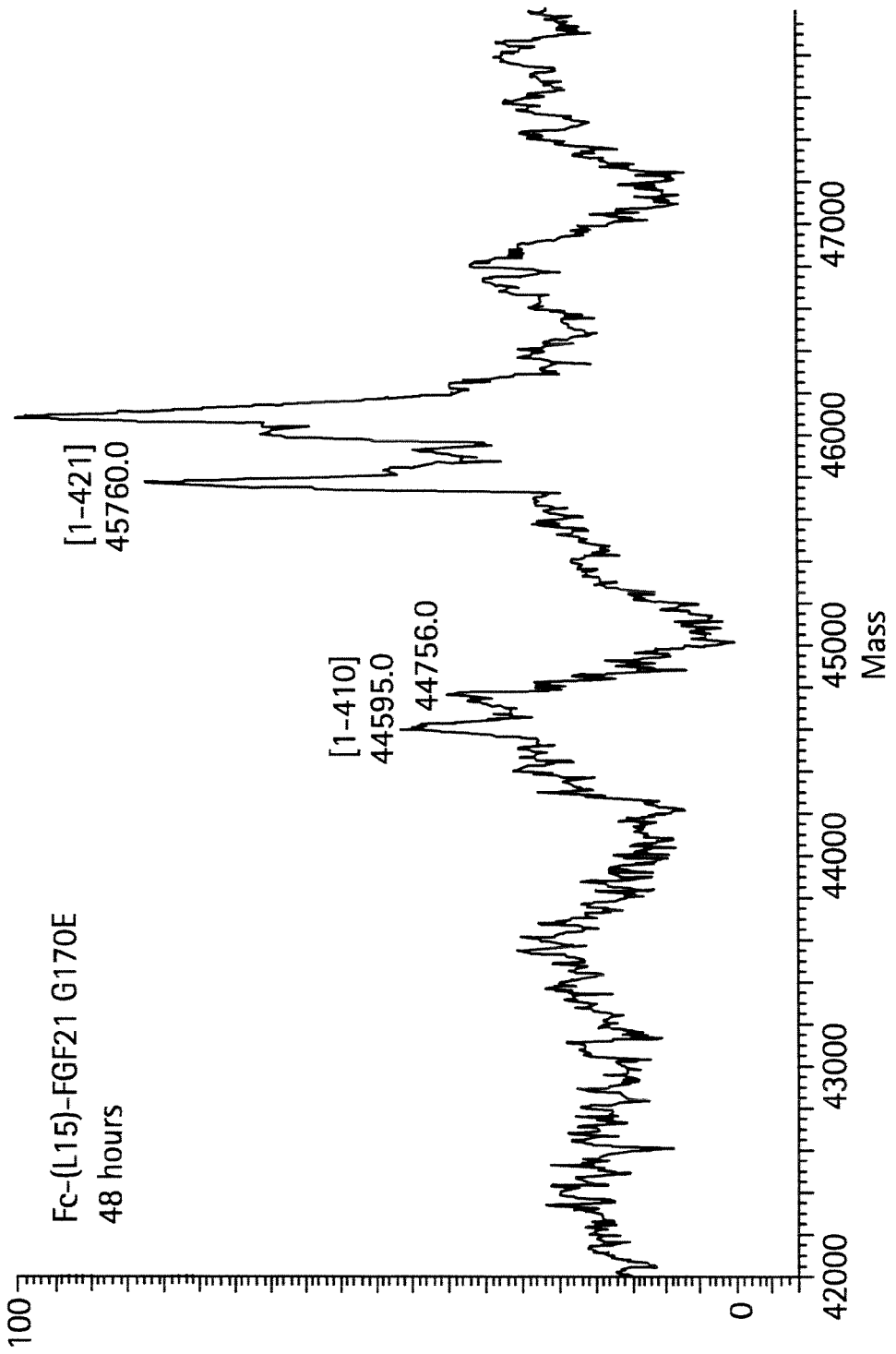

Fc-(L15)-FGF21 G170E
48 hours
[1-424]
46072.0
[1-421]
45760.0
[1-410]
44595.0
44756.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21A

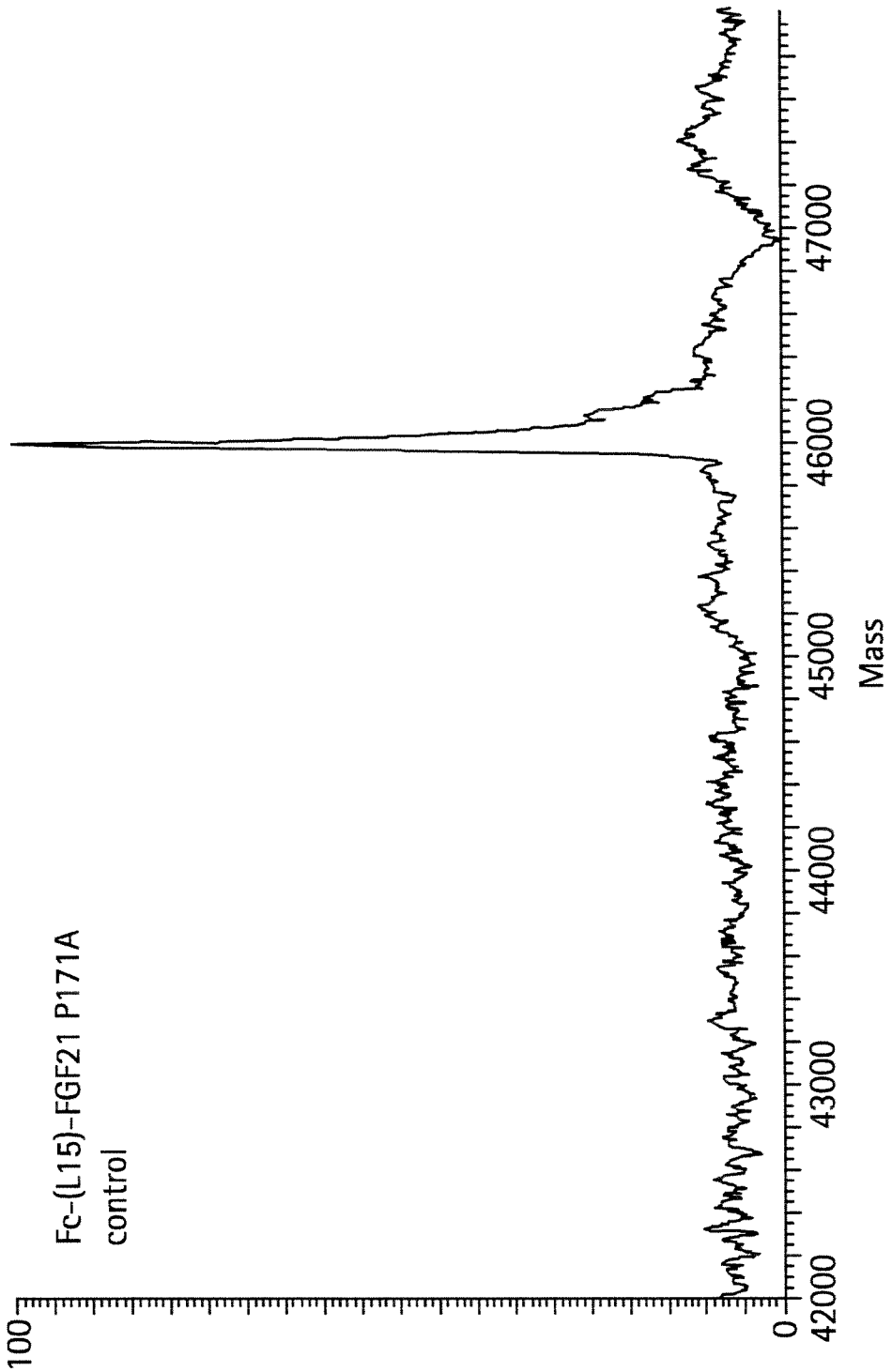

[1-424]
45970.0
Fc-(L15)-FGF21 P171A
control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21B

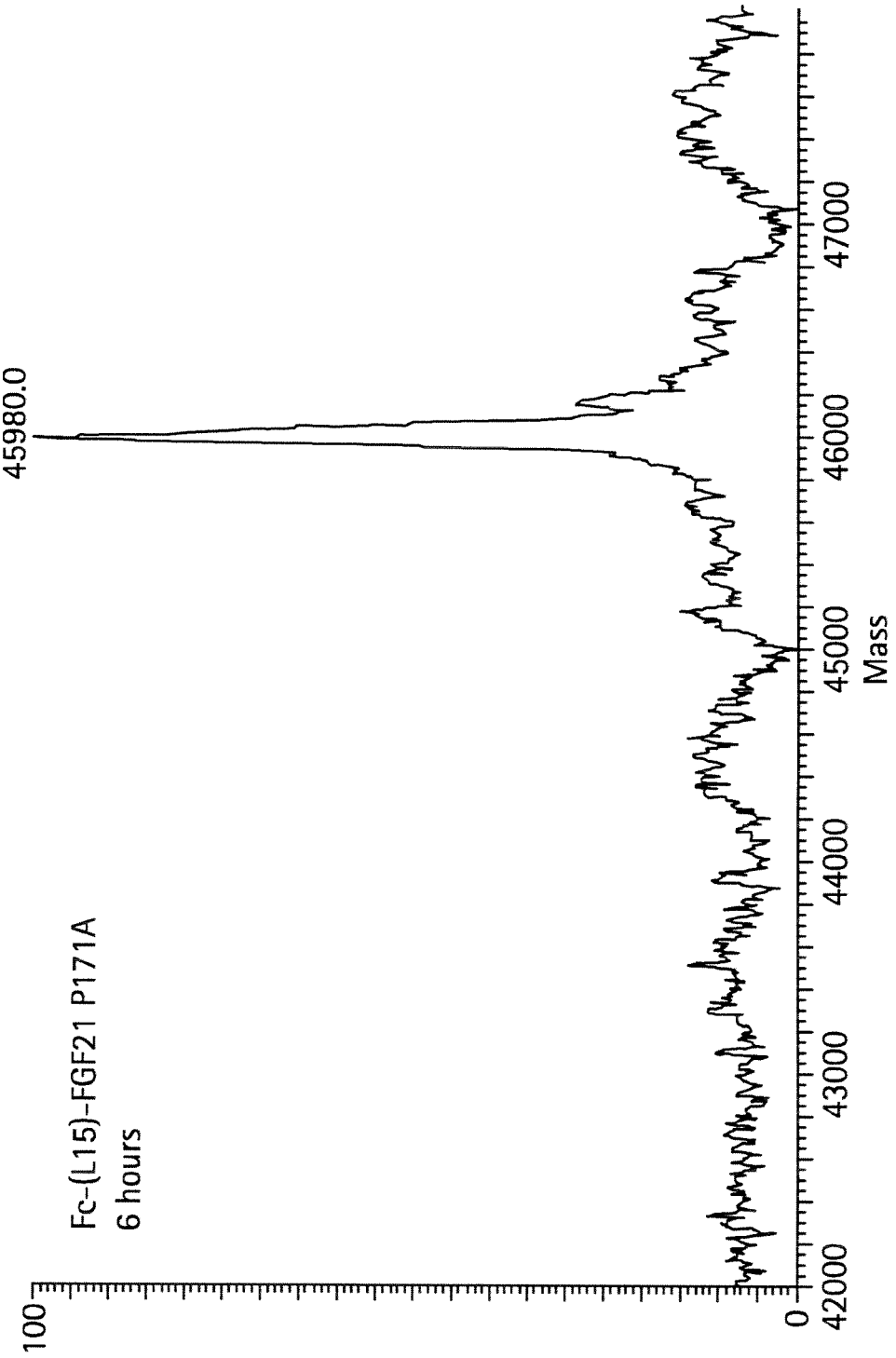

[1-424]
45980.0
Fc-(L15)-FGF21 P171A
6 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 21C

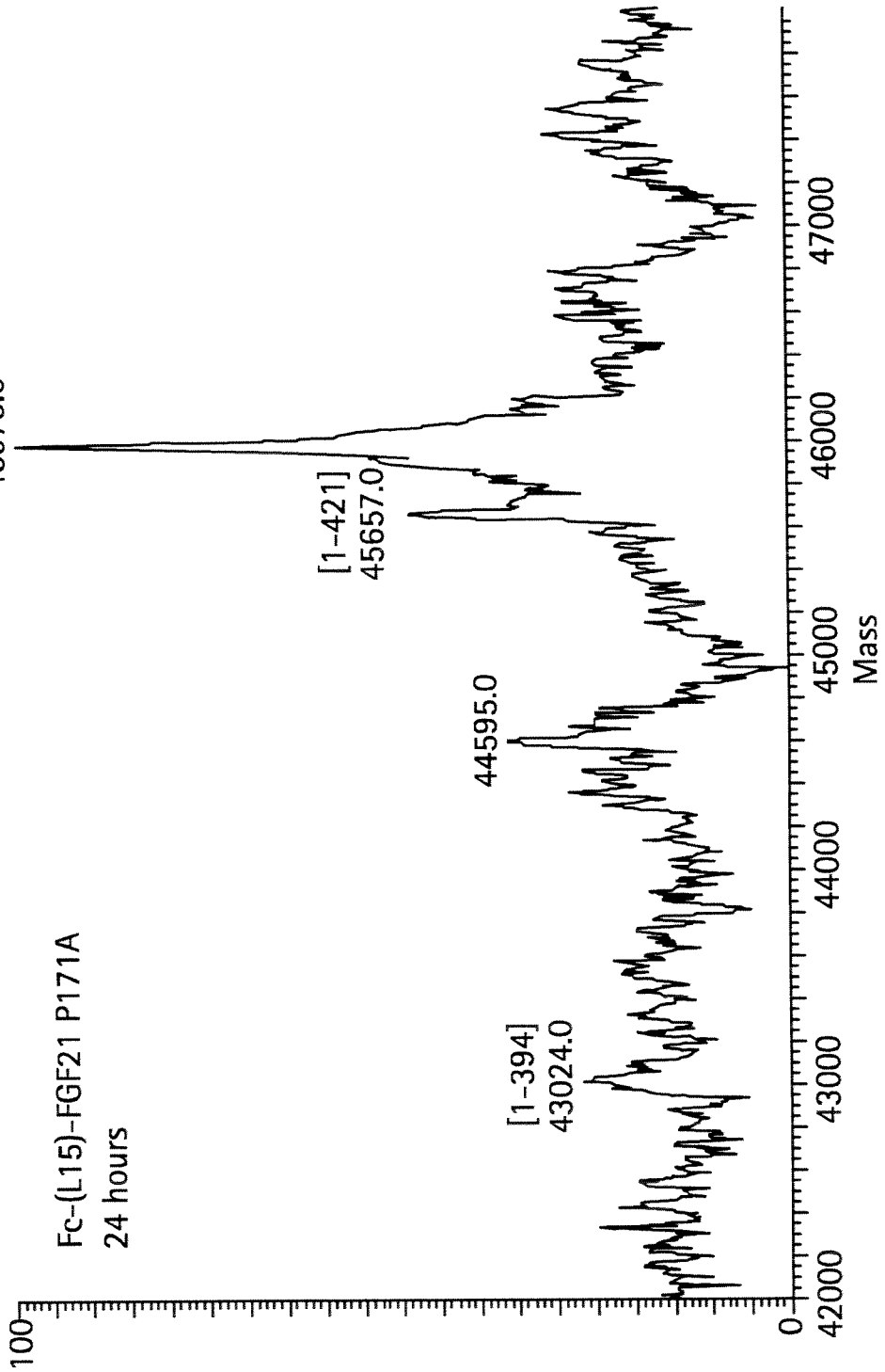

Fc-(L15)-FGF21 P171A
24 hours
100
0
42000
43000
44000
45000
46000
47000
Mass
[1-424]
45973.0
[1-421]
45657.0
44595.0
[1-394]
43024.0

FIG. 21D

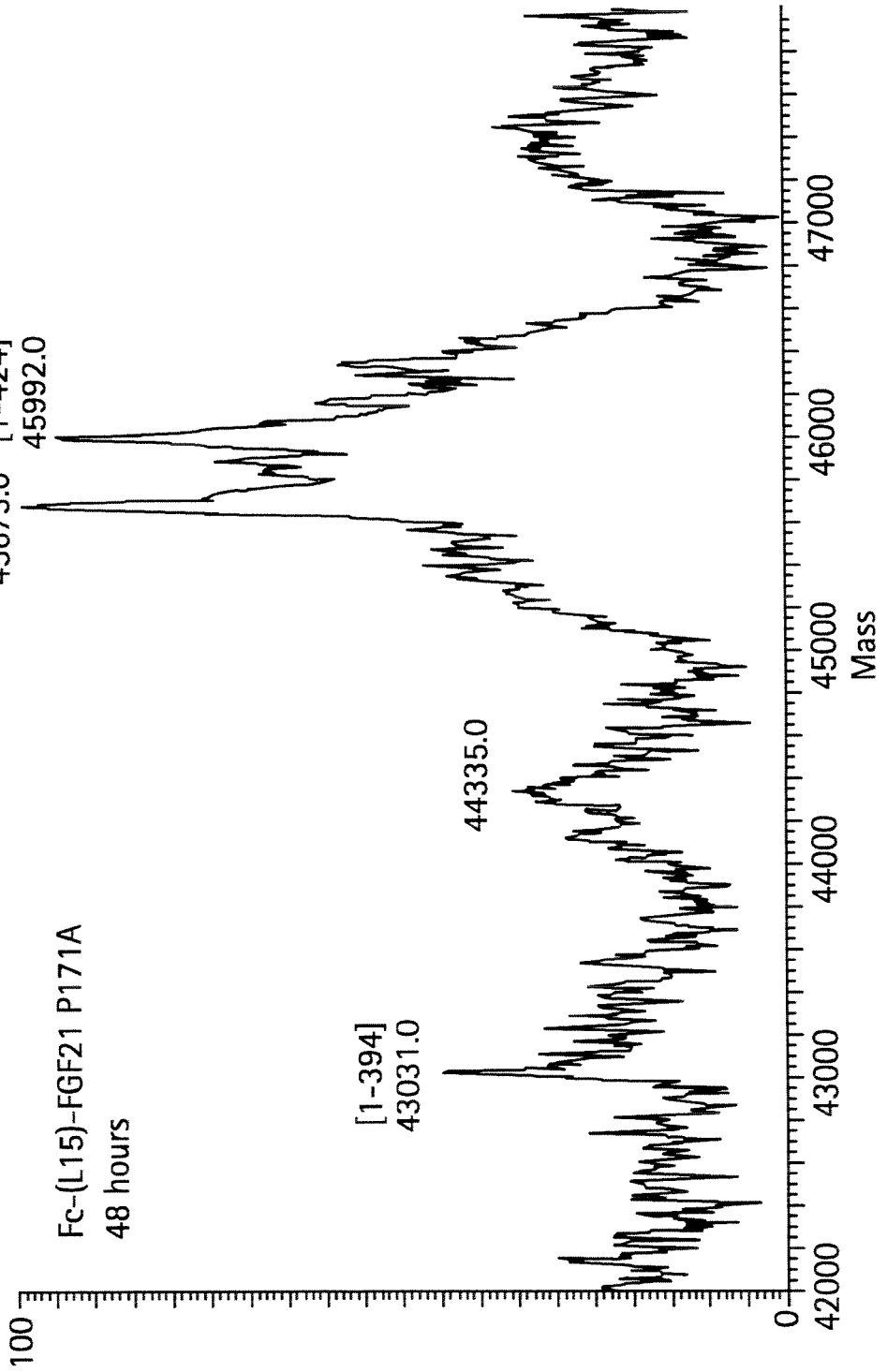

Fc-(L15)-FGF21 P171A
48 hours
[1-421]
45673.0
[1-424]
45992.0
[1-394]
43031.0
44335.0
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22A

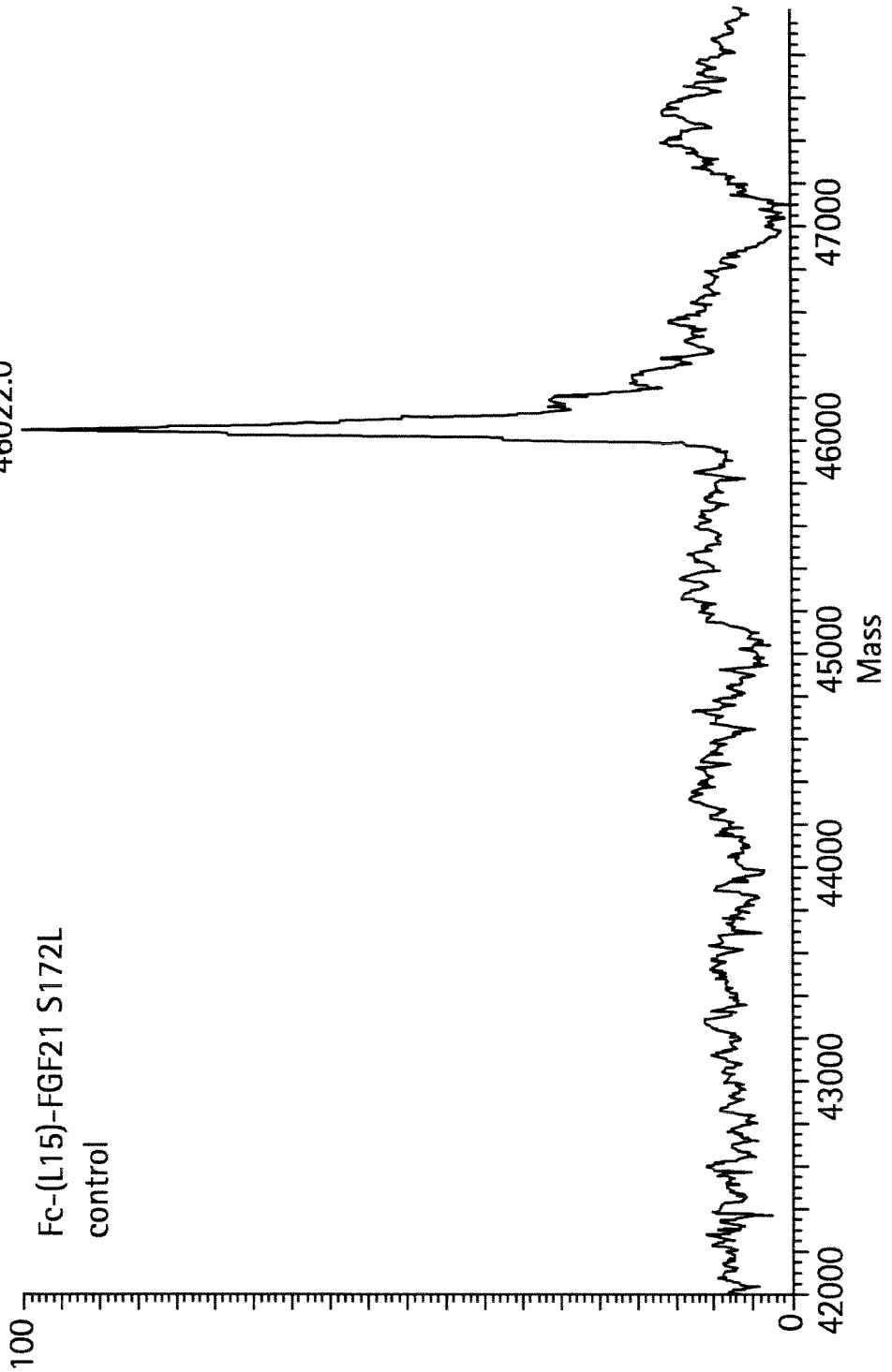

[1-424]
46022.0
Fc-(L15)-FGF21 S172L
control
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22B

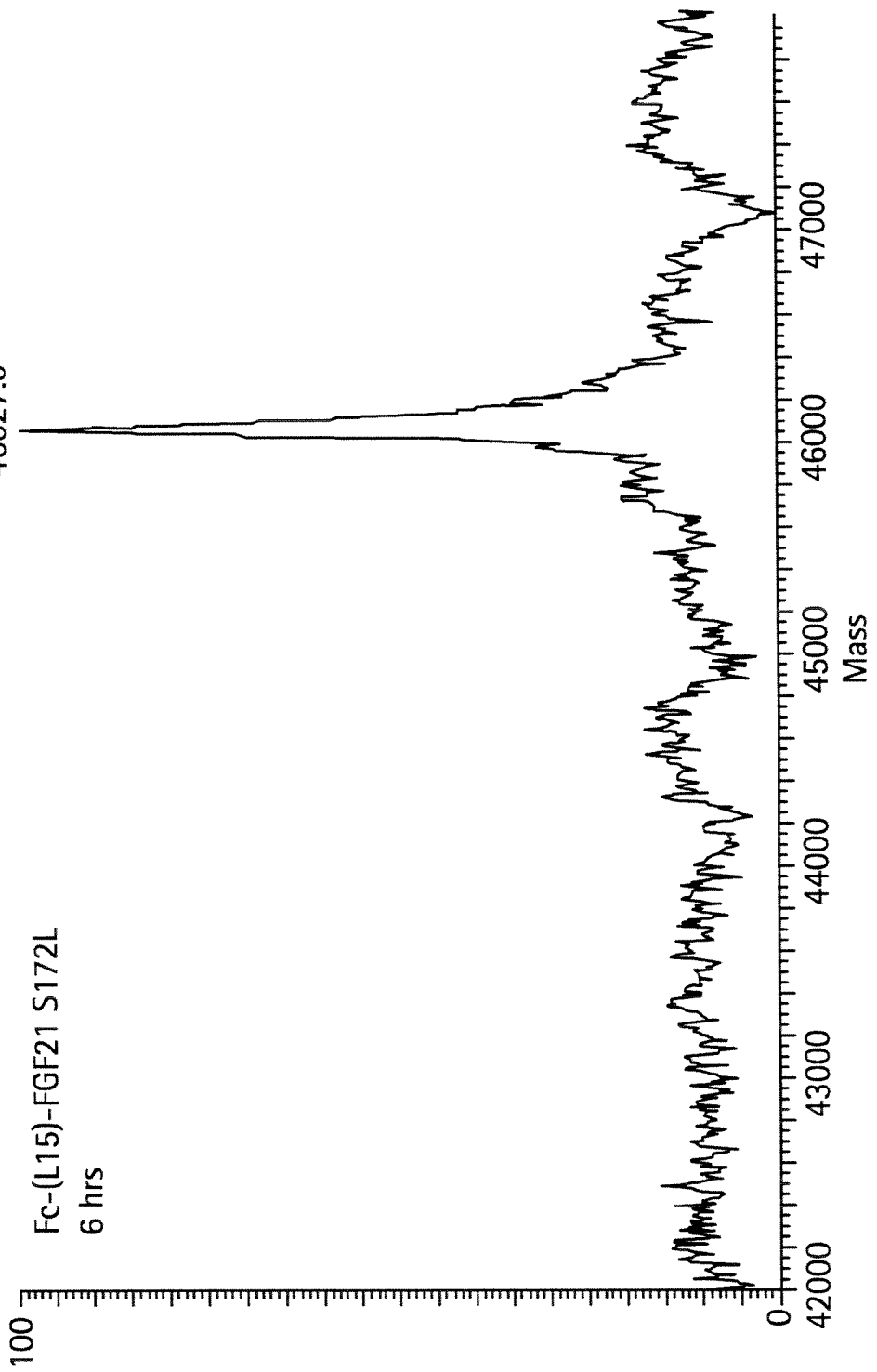

[1-424]
46027.0
Fc-(L15)-FGF21 S172L
6 hrs
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22C

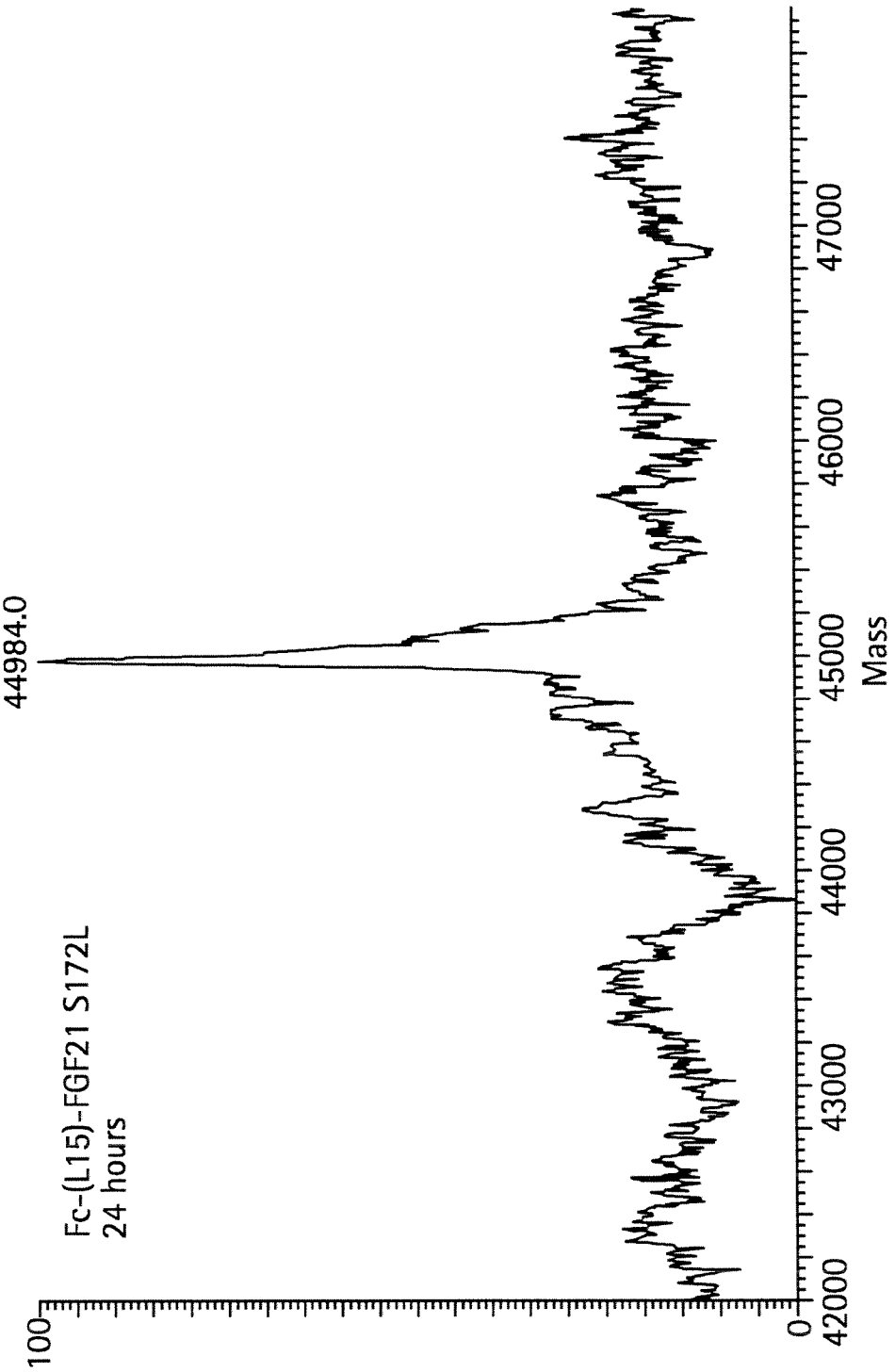

[1-414]
44984.0
Fc-(L15)-FGF21 S172L
24 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 22D

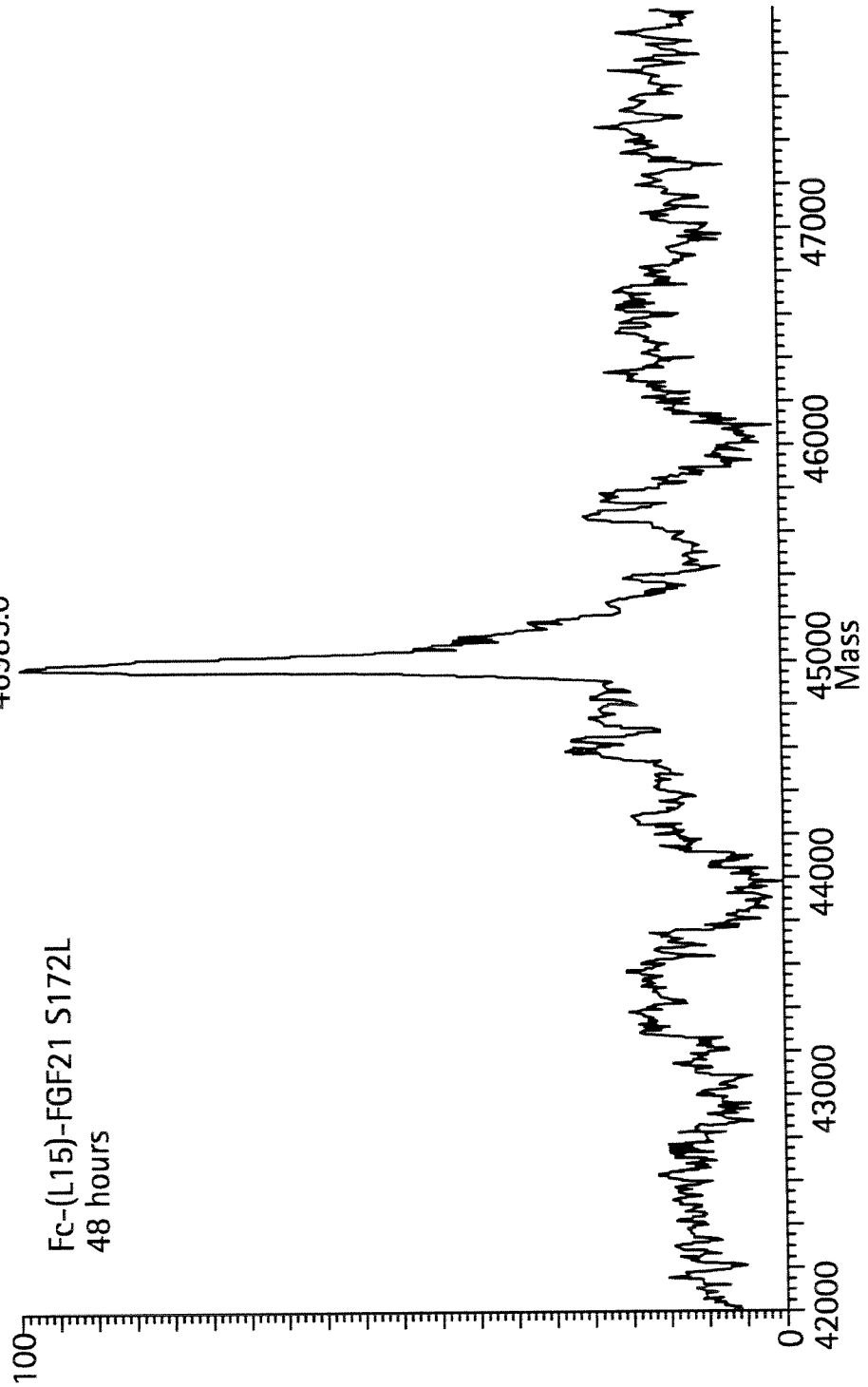

[1-414]
46985.0
Fc-(L15)-FGF21 S172L
48 hours
100
0
42000
43000
44000
45000
46000
47000
Mass

FIG. 23A

Fc-(L15)-FGF21

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

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

FIG. 23D

Fc-(L15)-FGF21 S172L

MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELYKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGSGG*
*GGSH*PIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVGPLQGRSPSYAS

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

FIG. 24A

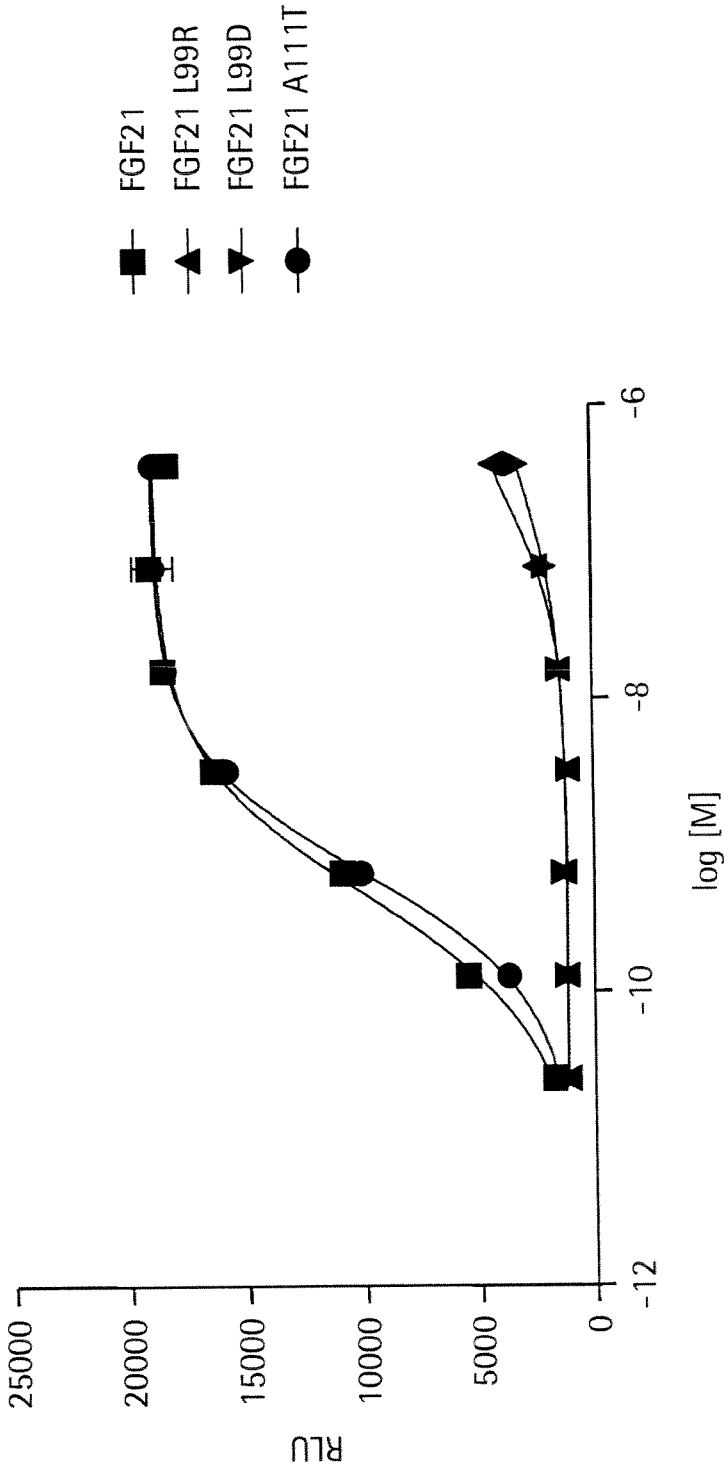

FGF21
FGF21 L99R
FGF21 L99D
FGF21 A111T
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
log [M]

FIG. 24B

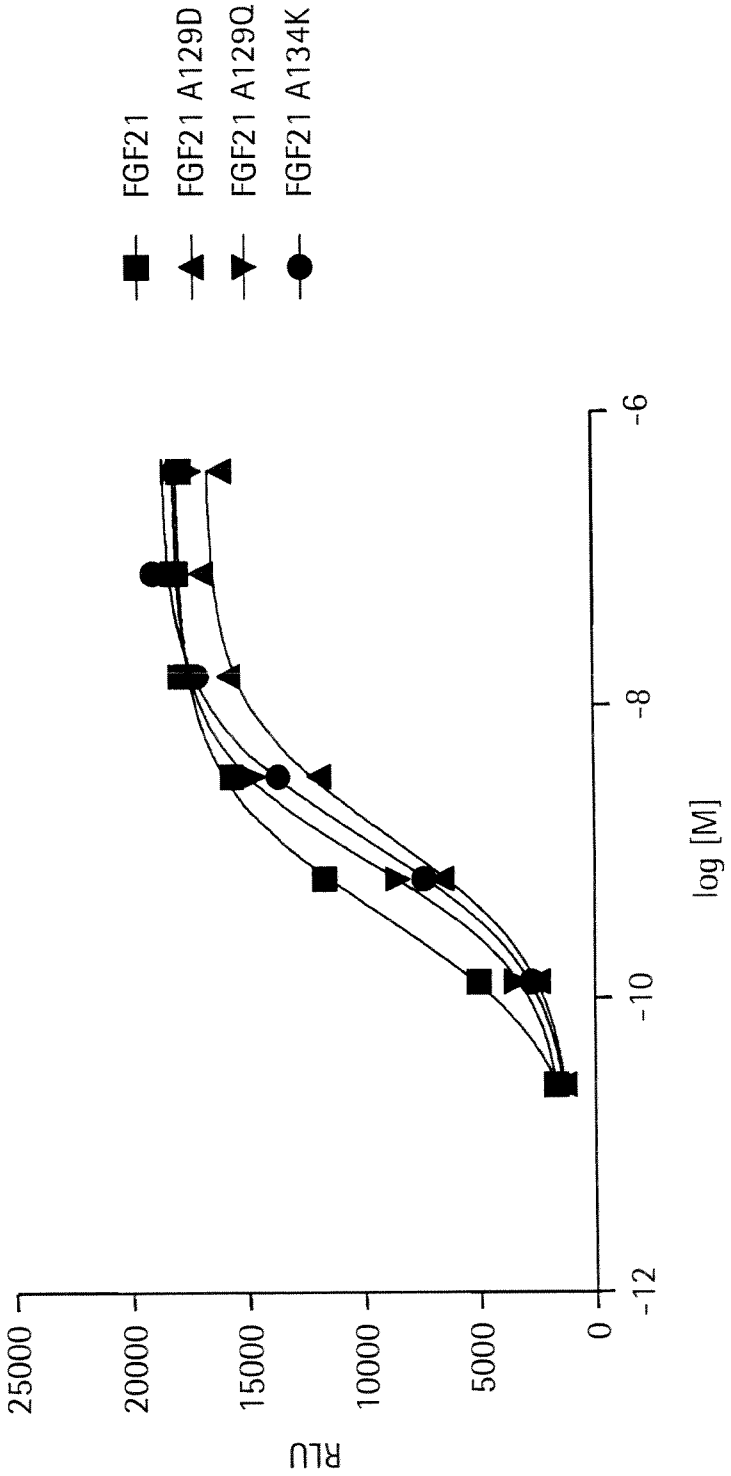

FGF21
FGF21 A129D
FGF21 A129Q
FGF21 A134K
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
log [M]

FIG. 24C

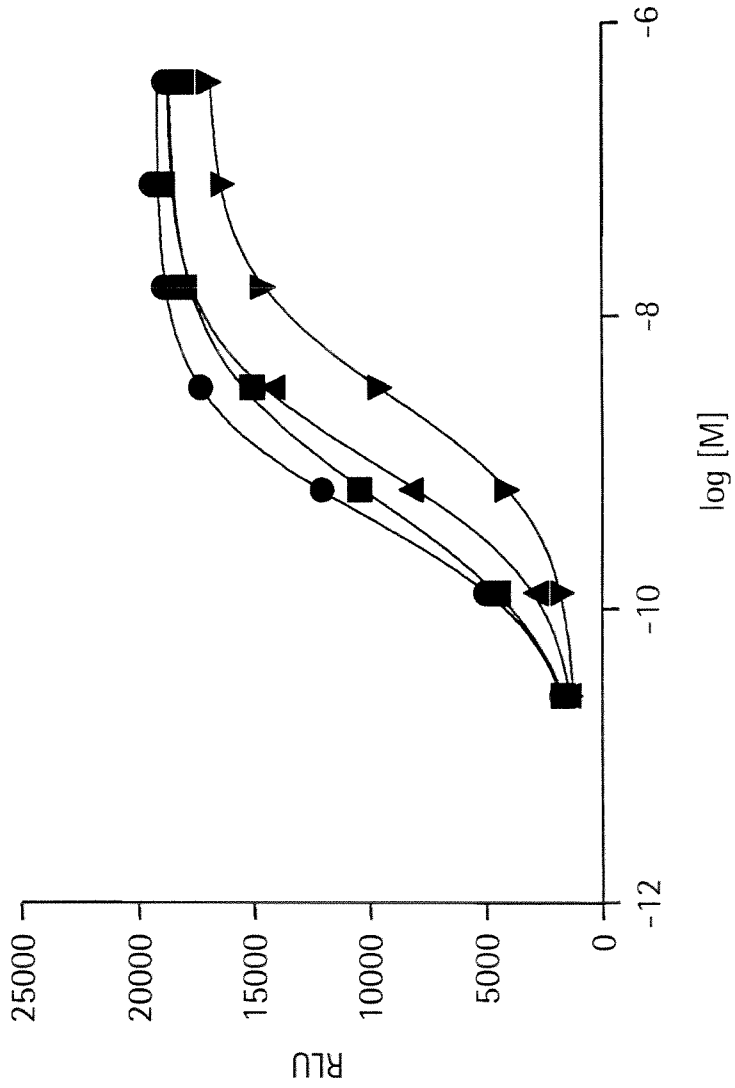

FGF21
FGF21 A134Y
FGF21 A134E
FGF21 A129K
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
log [M]

FIG. 25A

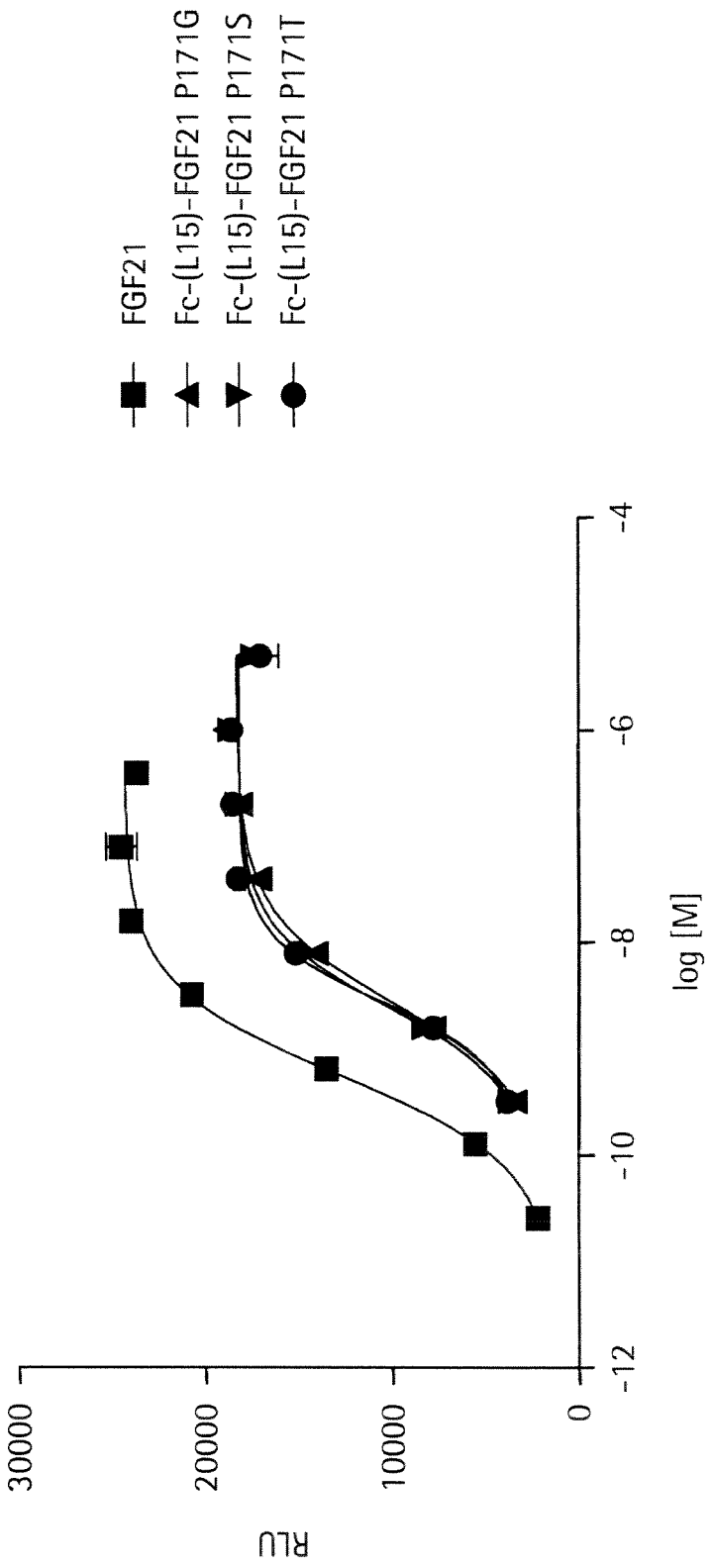

FGF21
Fc-(L15)-FGF21 P171G
Fc-(L15)-FGF21 P171S
Fc-(L15)-FGF21 P171T
RLU
30000
20000
10000
0
-12
-10
-8
-6
-4
log [M]

FIG. 25B

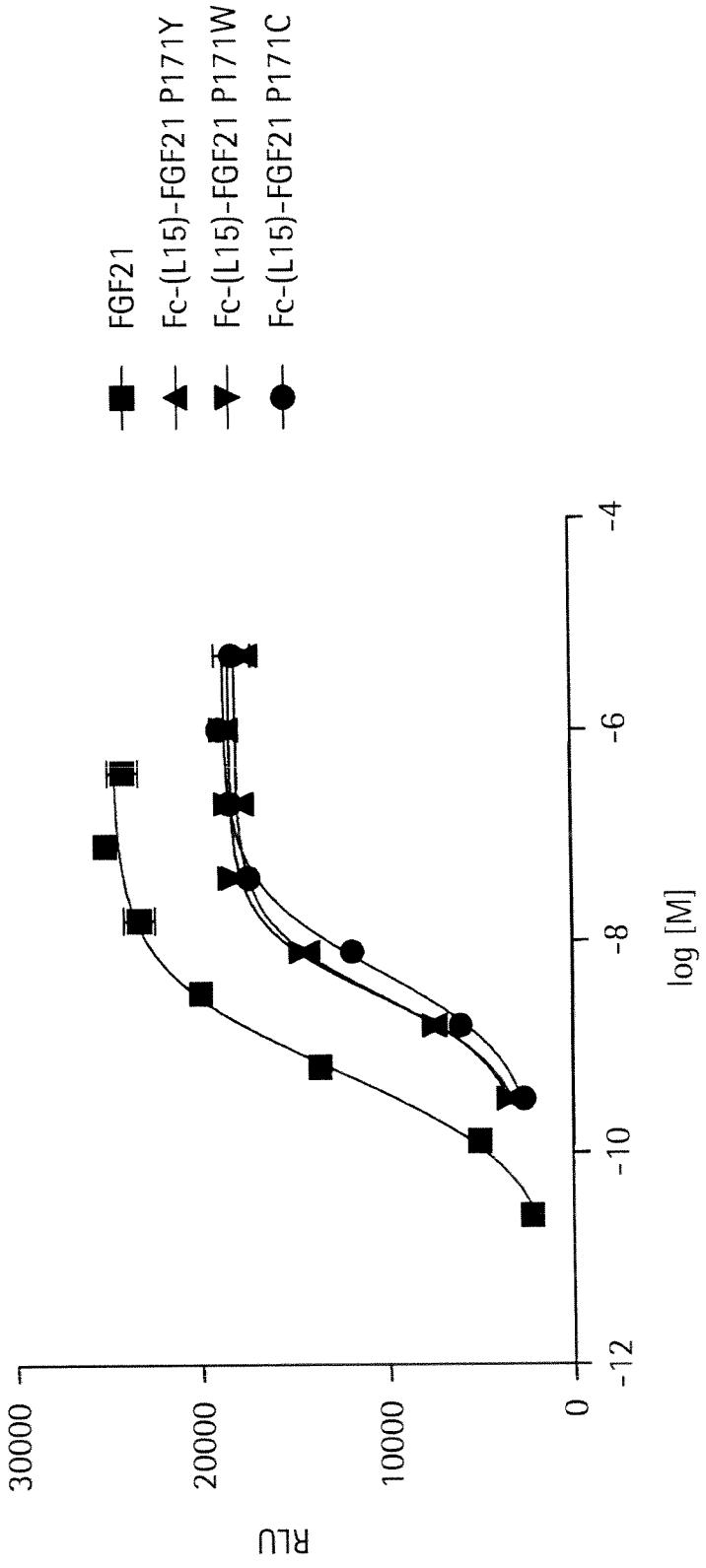

FGF21
Fc-(L15)-FGF21 P171Y
Fc-(L15)-FGF21 P171W
Fc-(L15)-FGF21 P171C
RLU
30000
20000
10000
0
-12
-10
-8
-6
-4
log [M]

FIG. 25C

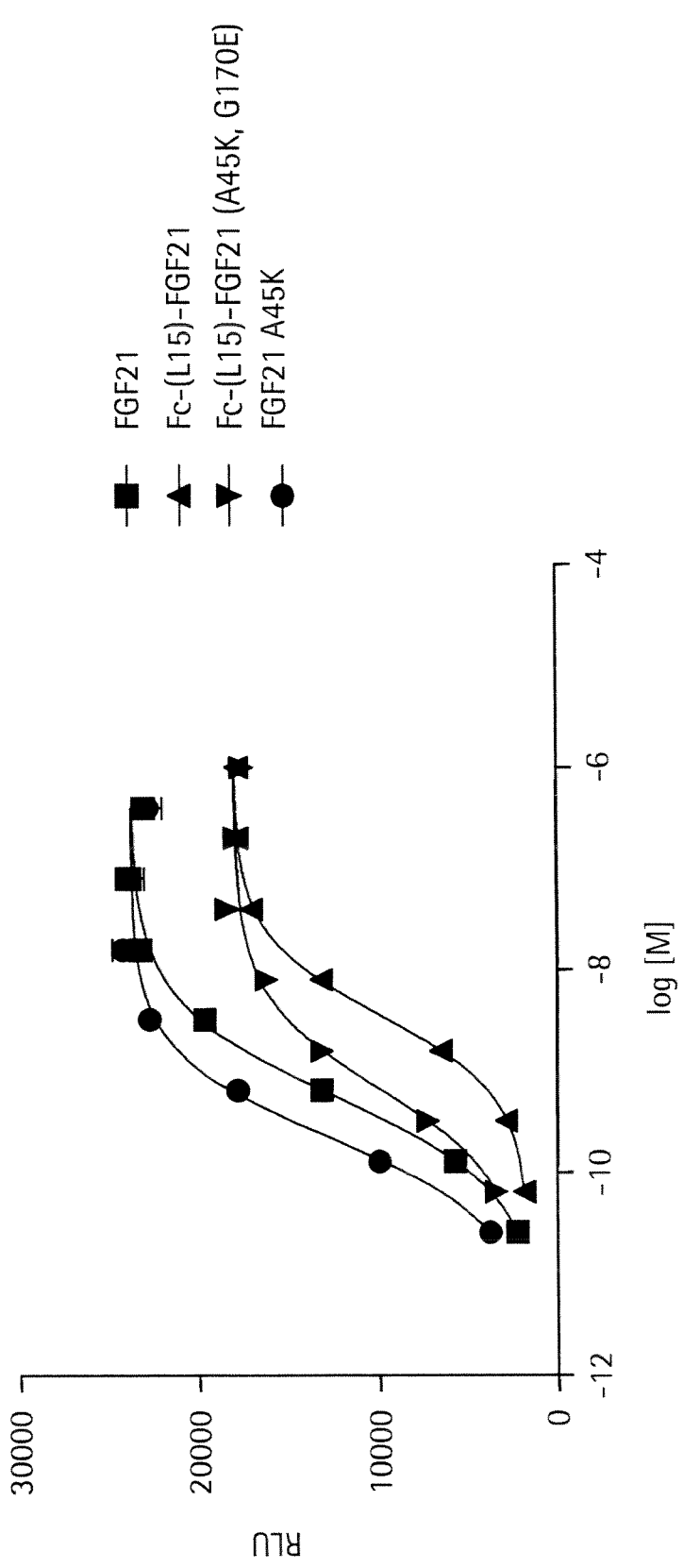

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (A45K, G170E)
FGF21 A45K
RLU
30000
20000
10000
0
-12
-10
-8
-6
-4
log [M]

FIG. 25D

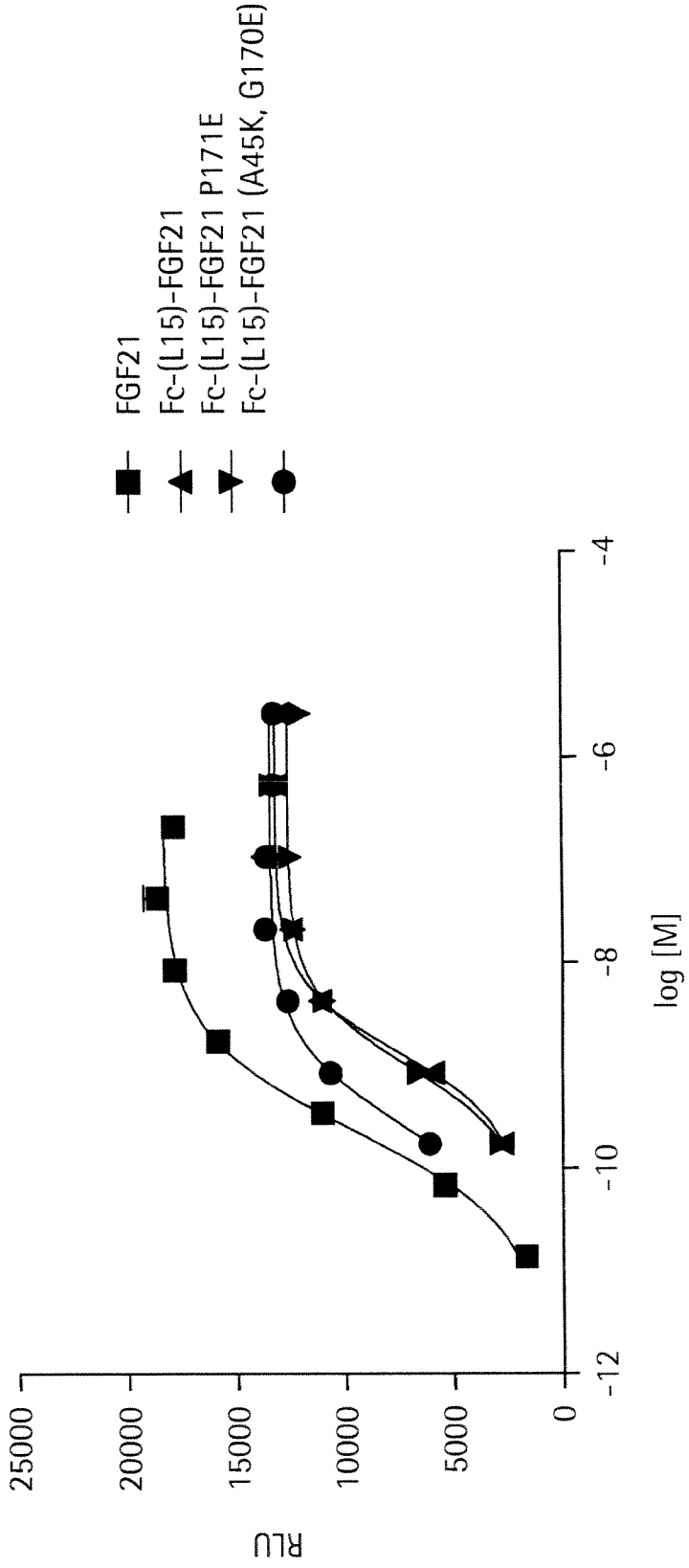

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 (A45K, G170E)
RLU
25000
20000
15000
10000
5000
0
-12
-10
-8
-6
-4
log [M]

FIG. 26A

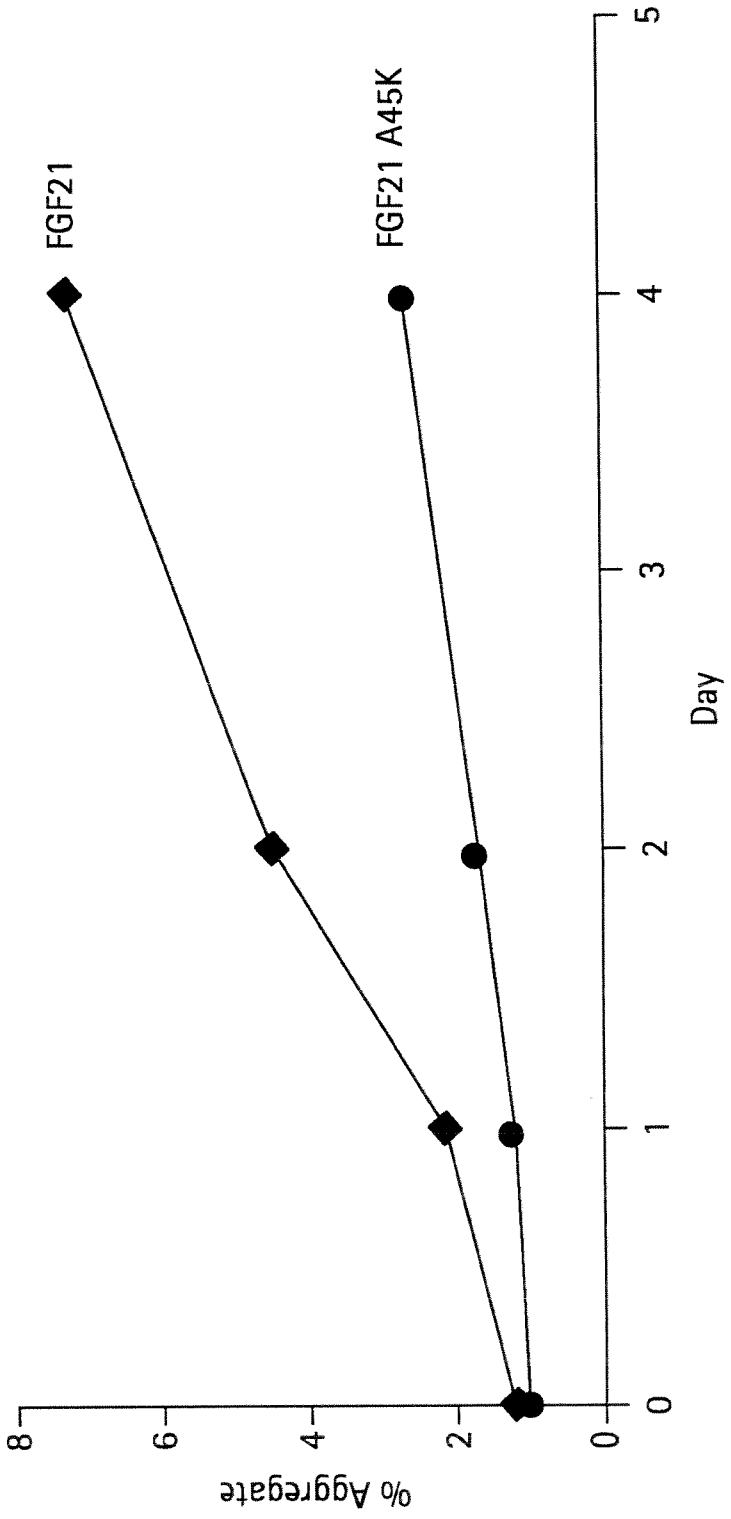


FIG. 26B

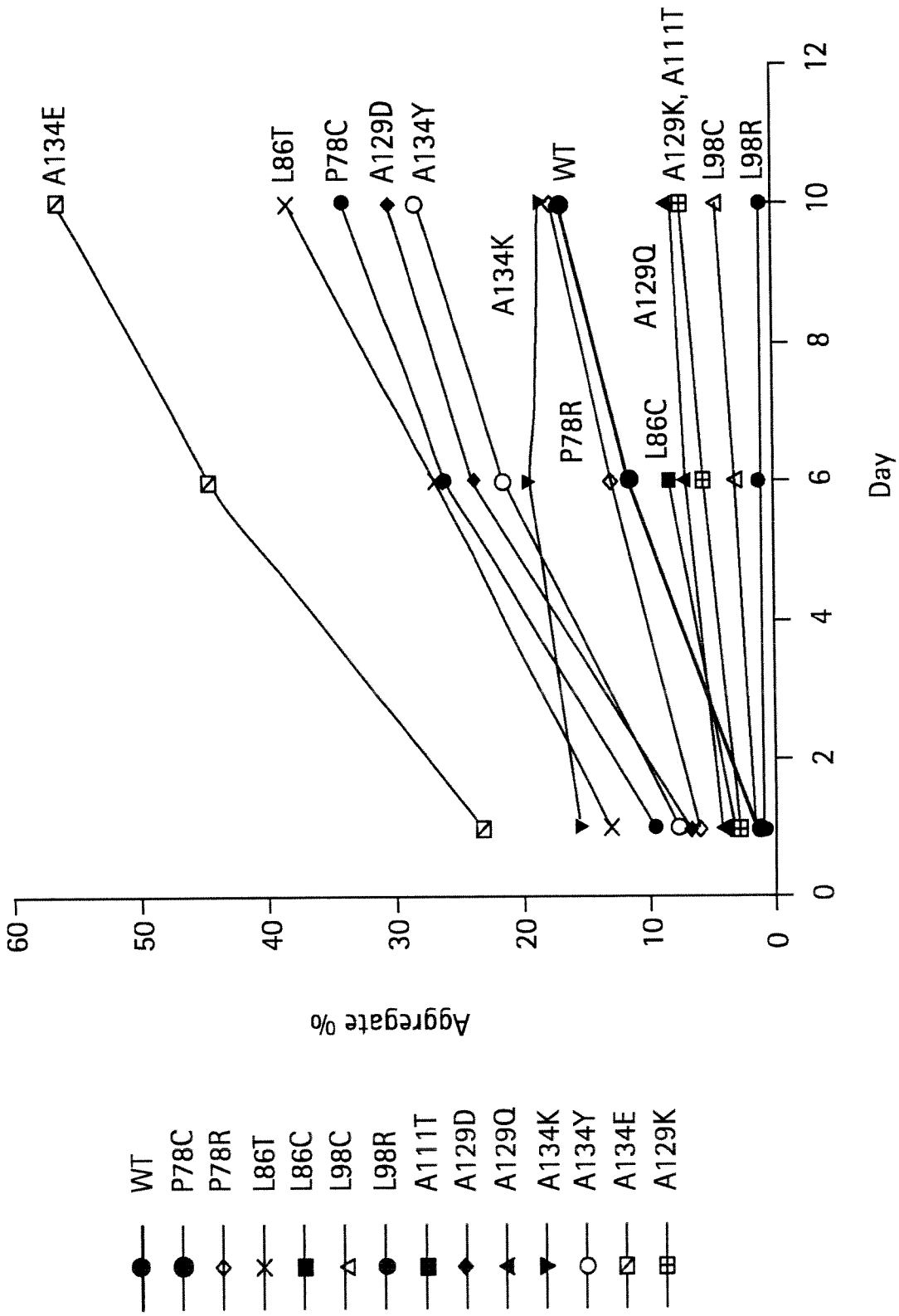

Aggregate %
Day
0
10
20
30
40
50
60
0
2
4
6
8
10
12
A134E
L86T
P78C
A129D
A134Y
A134K
WT
P78R
L86C
A129Q
A129K, A111T
L98C
L98R
WT
P78C
P78R
L86T
L86C
L98C
L98R
A111T
A129D
A129Q
A134K
A134Y
A134E
A129K

FIG. 27

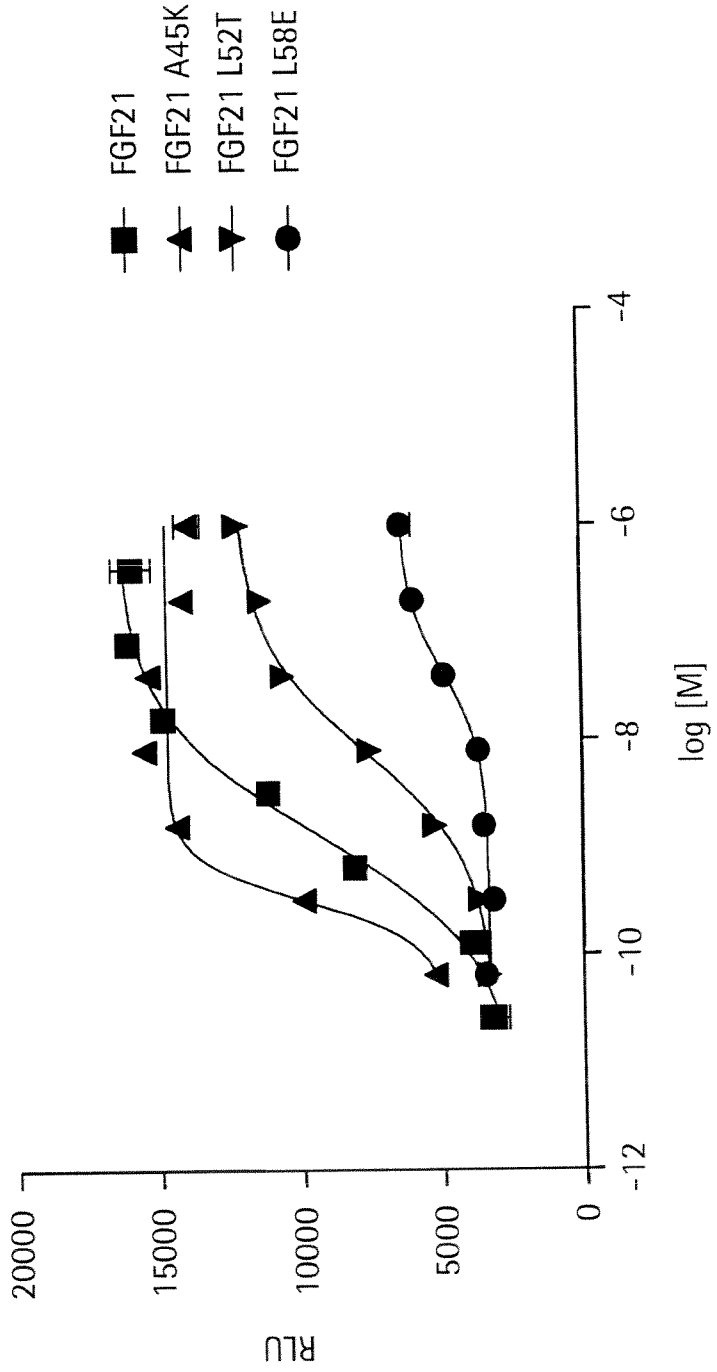

FGF21
FGF21 A45K
FGF21 L52T
FGF21 L58E
RLU
20000
15000
10000
5000
0
-12
-10
-8
-6
-4
log [M]

FIG. 28A

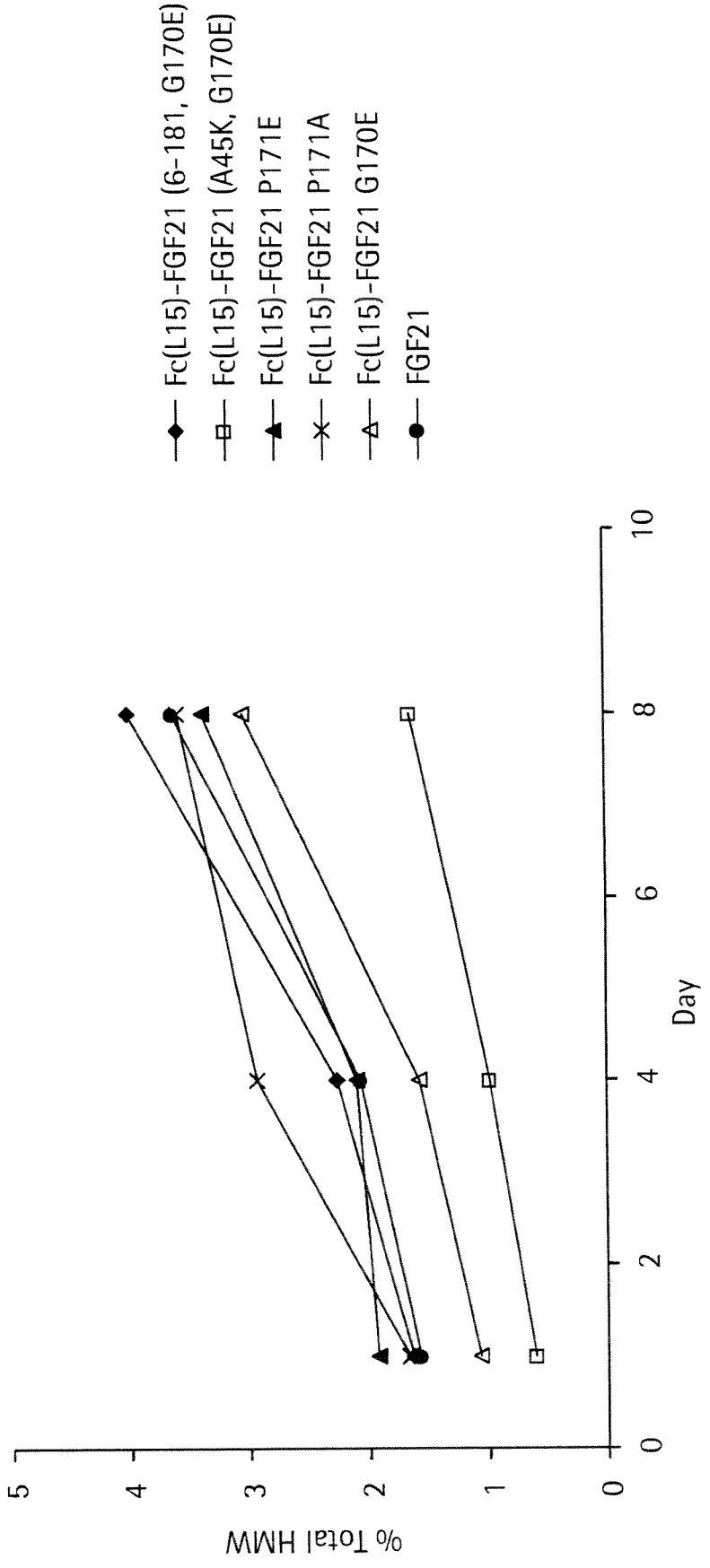

Fc(L15)-FGF21 (6-181, G170E)
Fc(L15)-FGF21 (A45K, G170E)
Fc(L15)-FGF21 P171E
Fc(L15)-FGF21 P171A
Fc(L15)-FGF21 G170E
FGF21
% Total HMW
0
1
2
3
4
5
0
2
4
6
8
10
Day

FIG. 28B

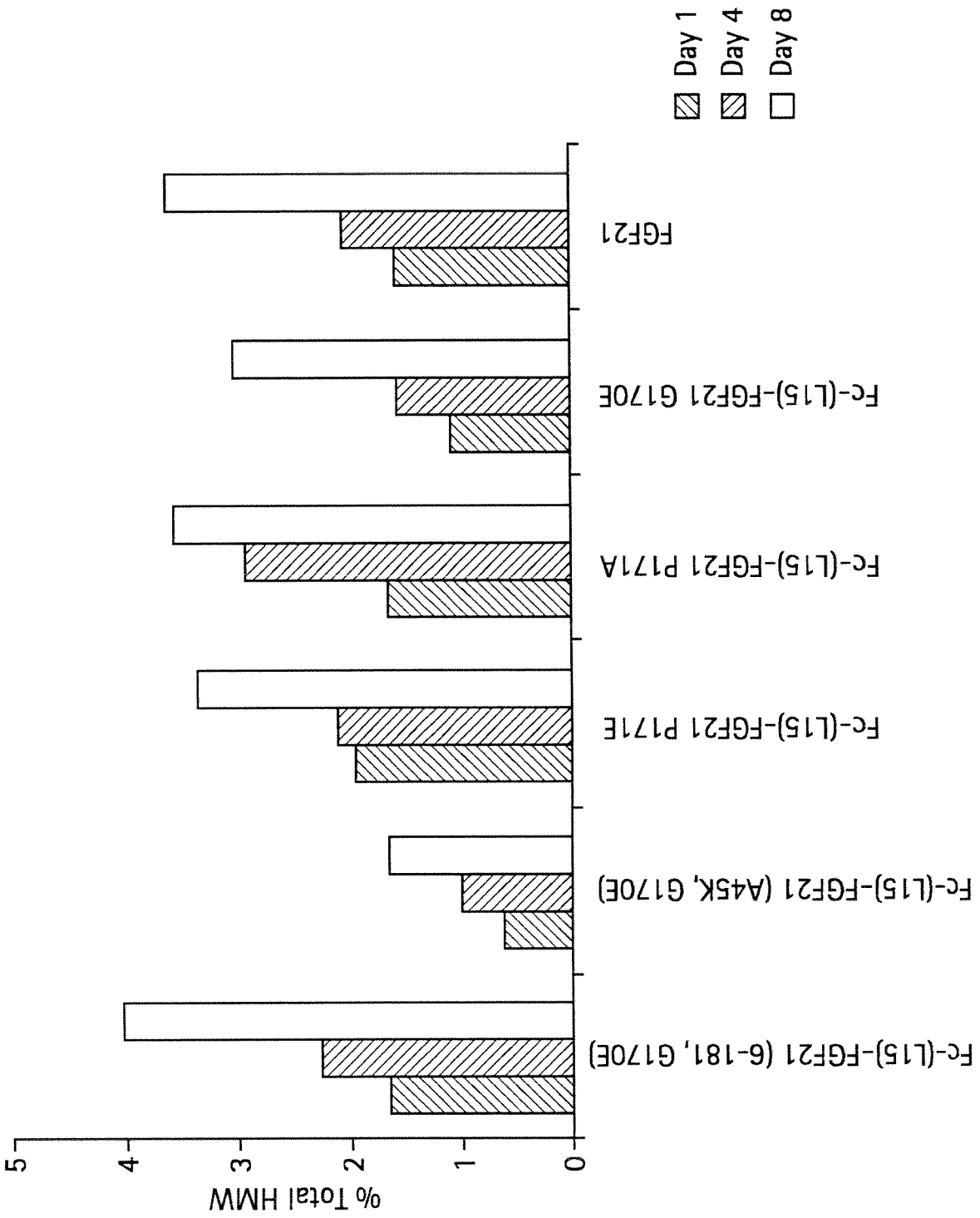

Day 1
Day 4
Day 8
% Total HMW
0
1
2
3
4
5
Fc-(L15)-FGF21 (6-181, G170E)
Fc-(L15)-FGF21 (A45K, G170E)
Fc-(L15)-FGF21 P171E
Fc-(L15)-FGF21 P171A
Fc-(L15)-FGF21 G170E
FGF21

FIG. 29

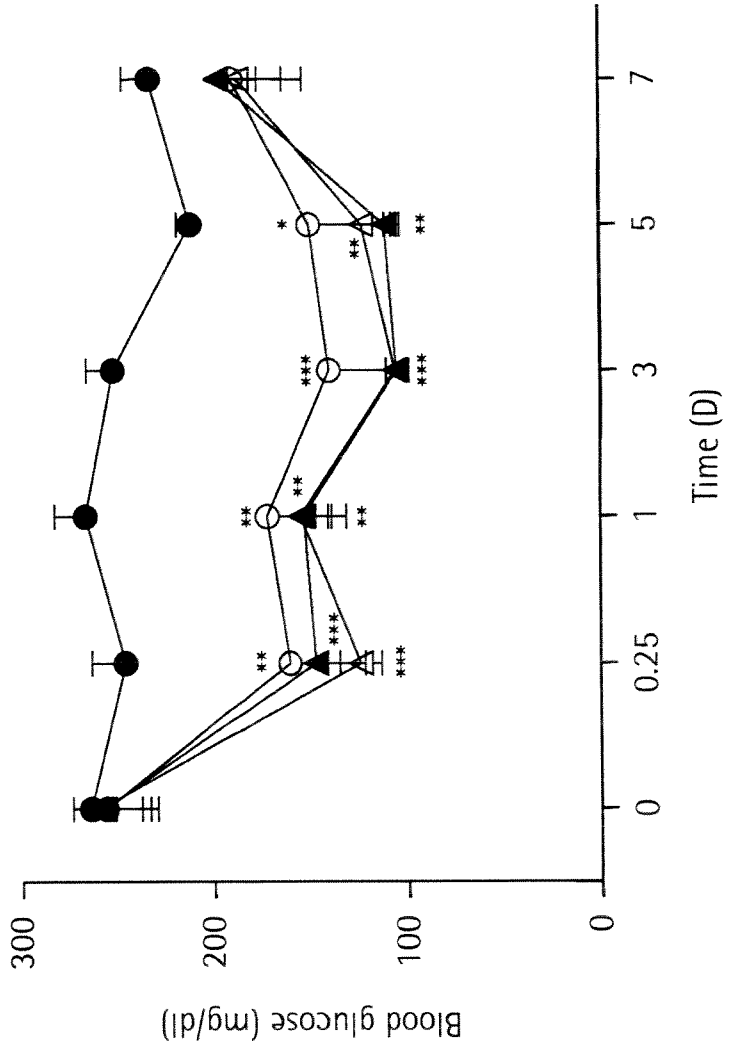

Vehicle
Fc-(L15)-FGF21 (A45K, G170E)
Fc-(L15)-FGF21 (A45K, P171G)
Fc-(L15)-FGF21 (L98R, P171G)
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Blood glucose (mg/dl)
300
200
100
0
0
0.25
1
3
5
7
Time (D)

FIG. 30

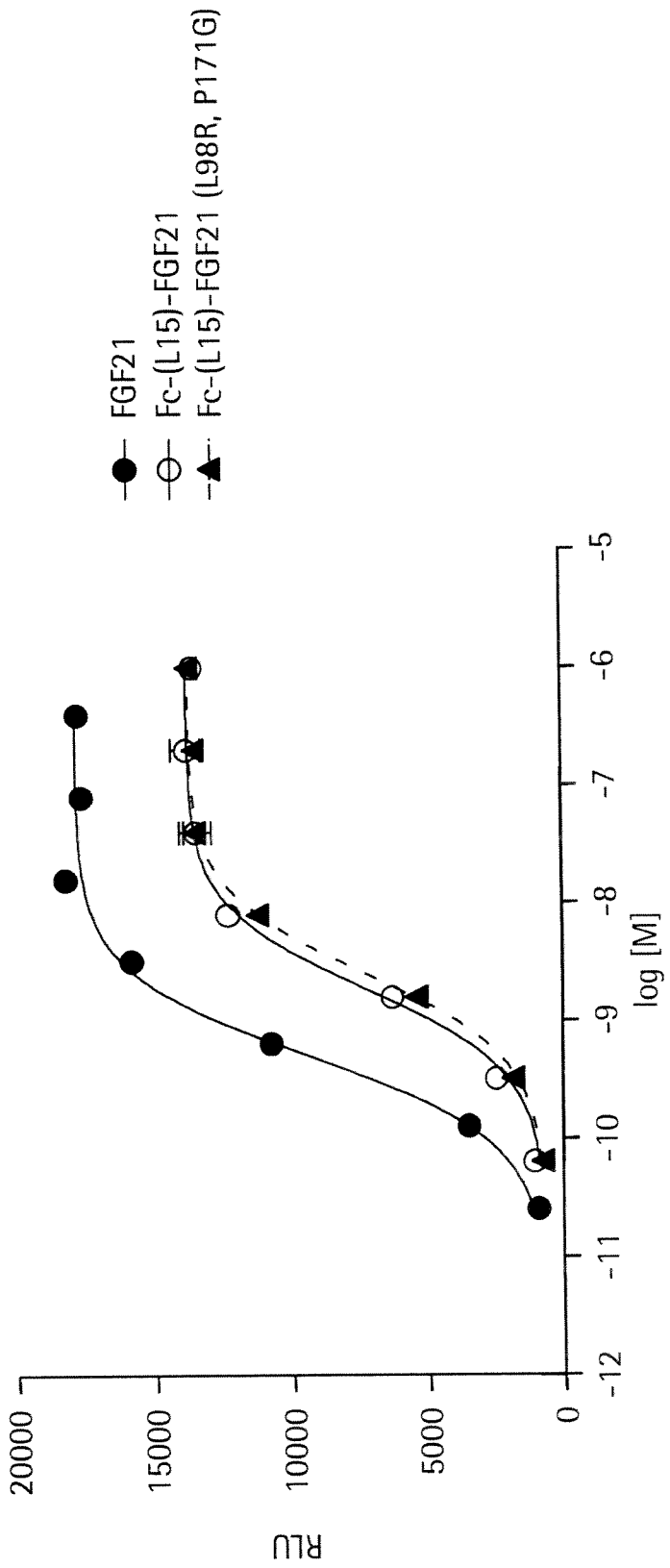

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (L98R, P171G)
RLU
20000
15000
10000
5000
0
-12
-11
-10
-9
-8
-7
-6
-5
log [M]

FIG. 31A

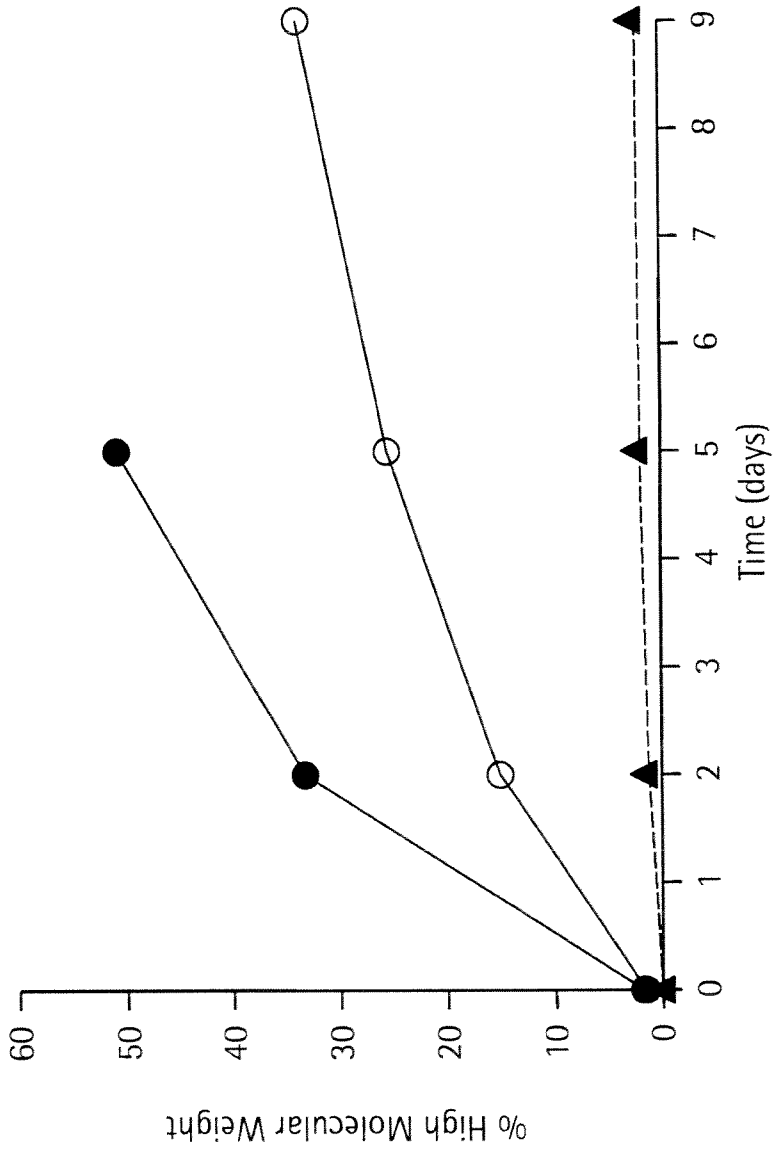

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (L98R, P171G)
% High Molecular Weight
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
9
Time (days)

FIG. 31B

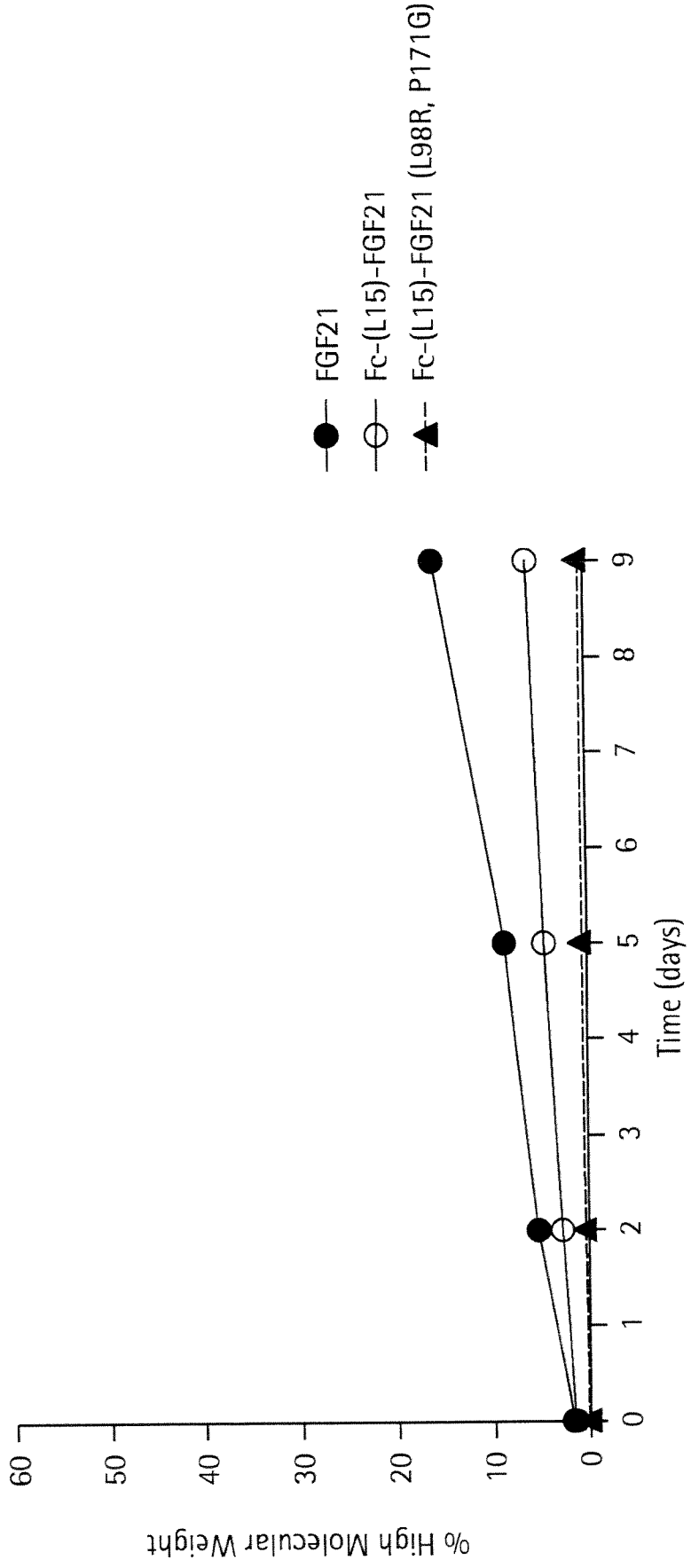

FGF21
Fc-(L15)-FGF21
Fc-(L15)-FGF21 (L98R, P171G)
% High Molecular Weight
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
9
Time (days)

FIG. 32

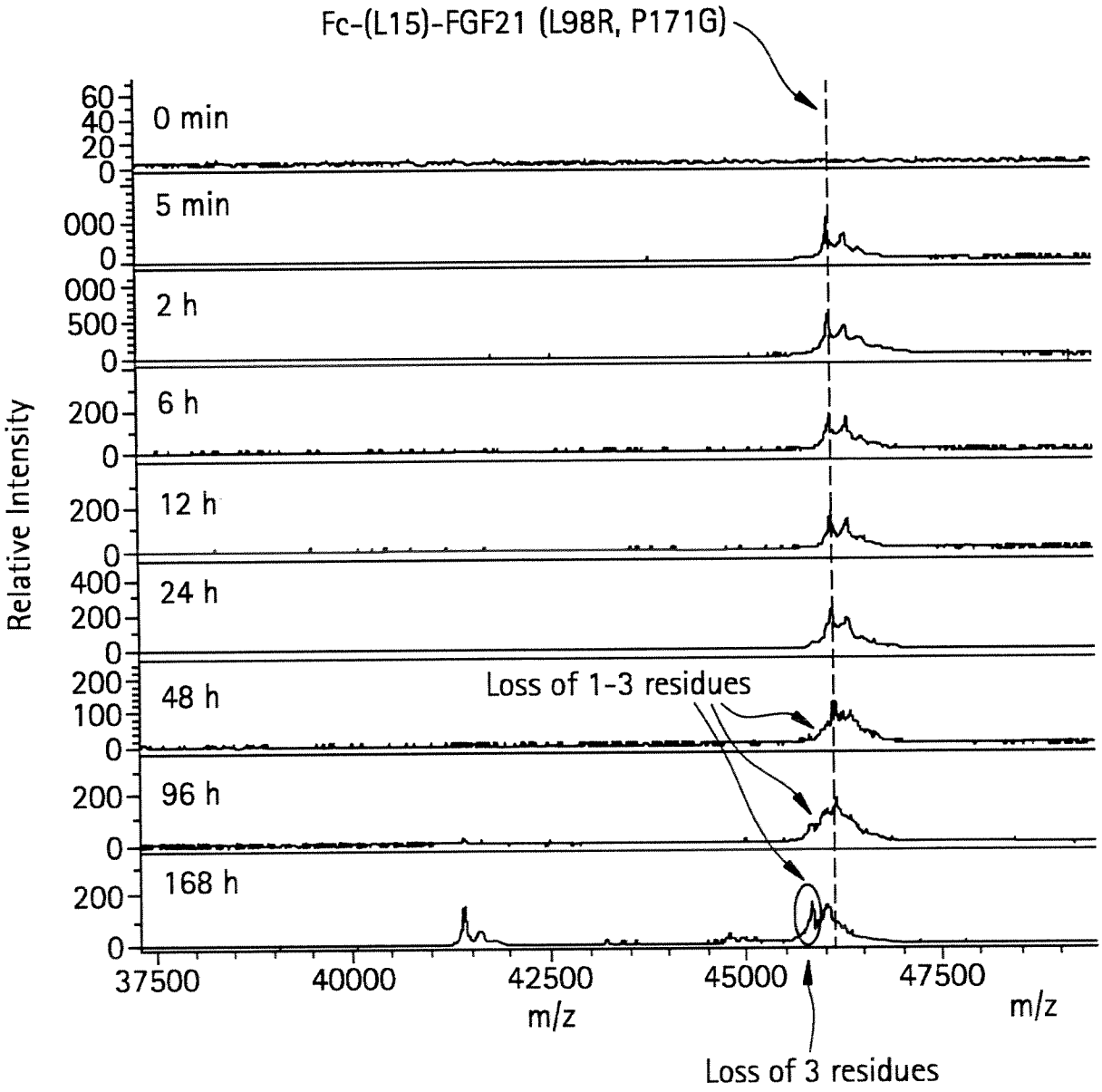

Fc-(L15)-FGF21 (L98R, P171G)
0 min
5 min
2 h
6 h
12 h
24 h
48 h
96 h
168 h
Relative Intensity
Loss of 1-3 residues
Loss of 3 residues
37500
40000
42500
45000
47500
m/z
m/z

FIG. 33

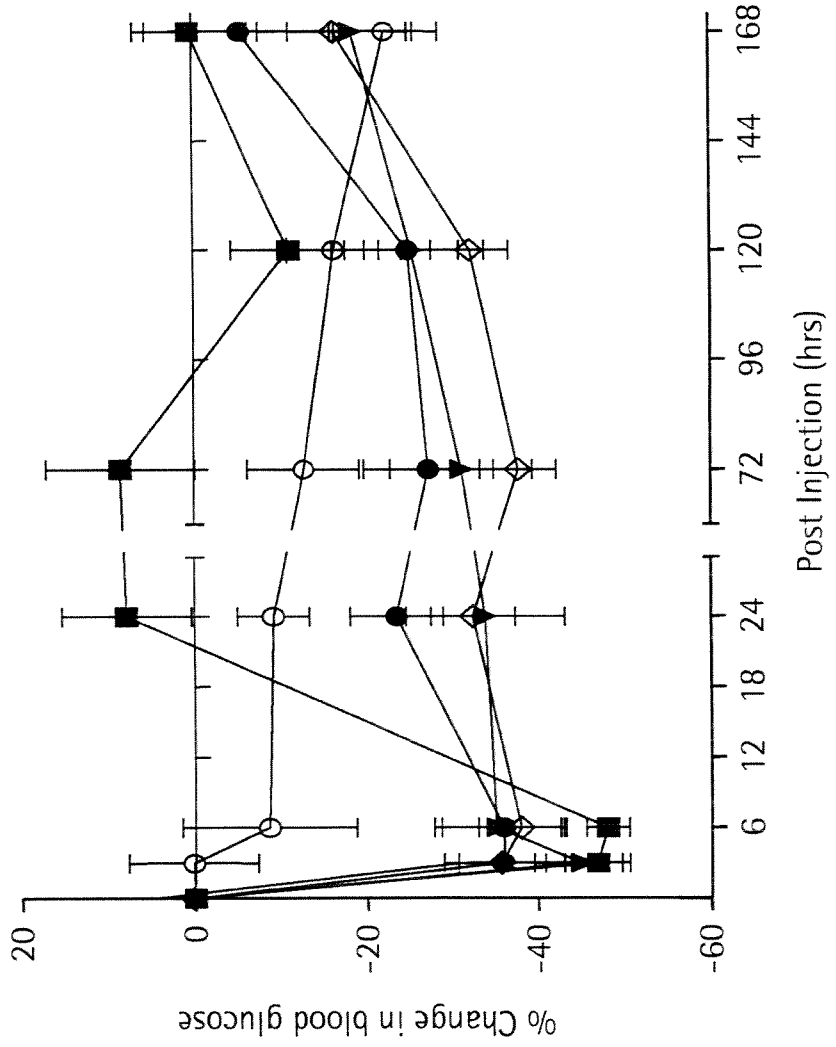

PBS 100uL
FGF21
Fc-(L15)-FGF21 (L98R,P171G)
Fc-(L15)-FGF21 (L98R,P171G,182P)
Fc-(L15)-FGF21 (L98R,P171G,182G)
% Change in blood glucose
20
0
-20
-40
-60
6
12
18
24
72
96
120
144
168
Post Injection (hrs)

FIG. 34

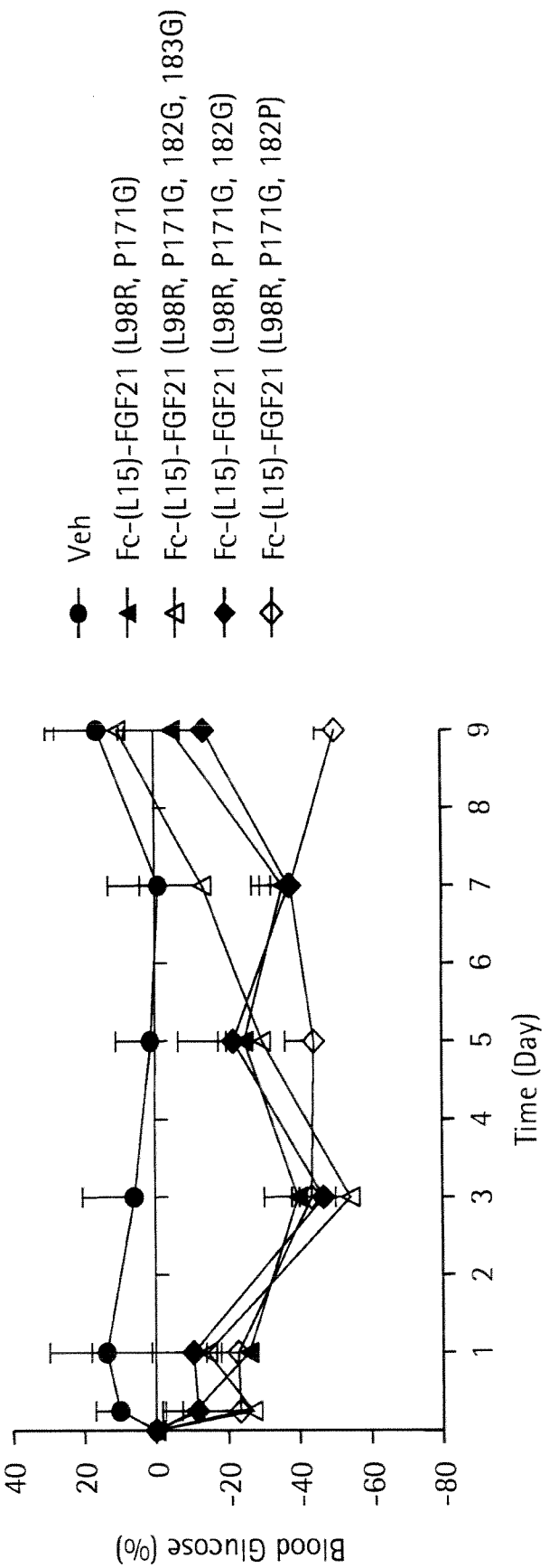

Veh
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(L15)-FGF21 (L98R, P171G, 182G, 183G)
Fc-(L15)-FGF21 (L98R, P171G, 182G)
Fc-(L15)-FGF21 (L98R, P171G, 182P)
Blood Glucose (%)
40
20
0
-20
-40
-60
-80
1
2
3
4
5
6
7
8
9
Time (Day)

FIG. 35

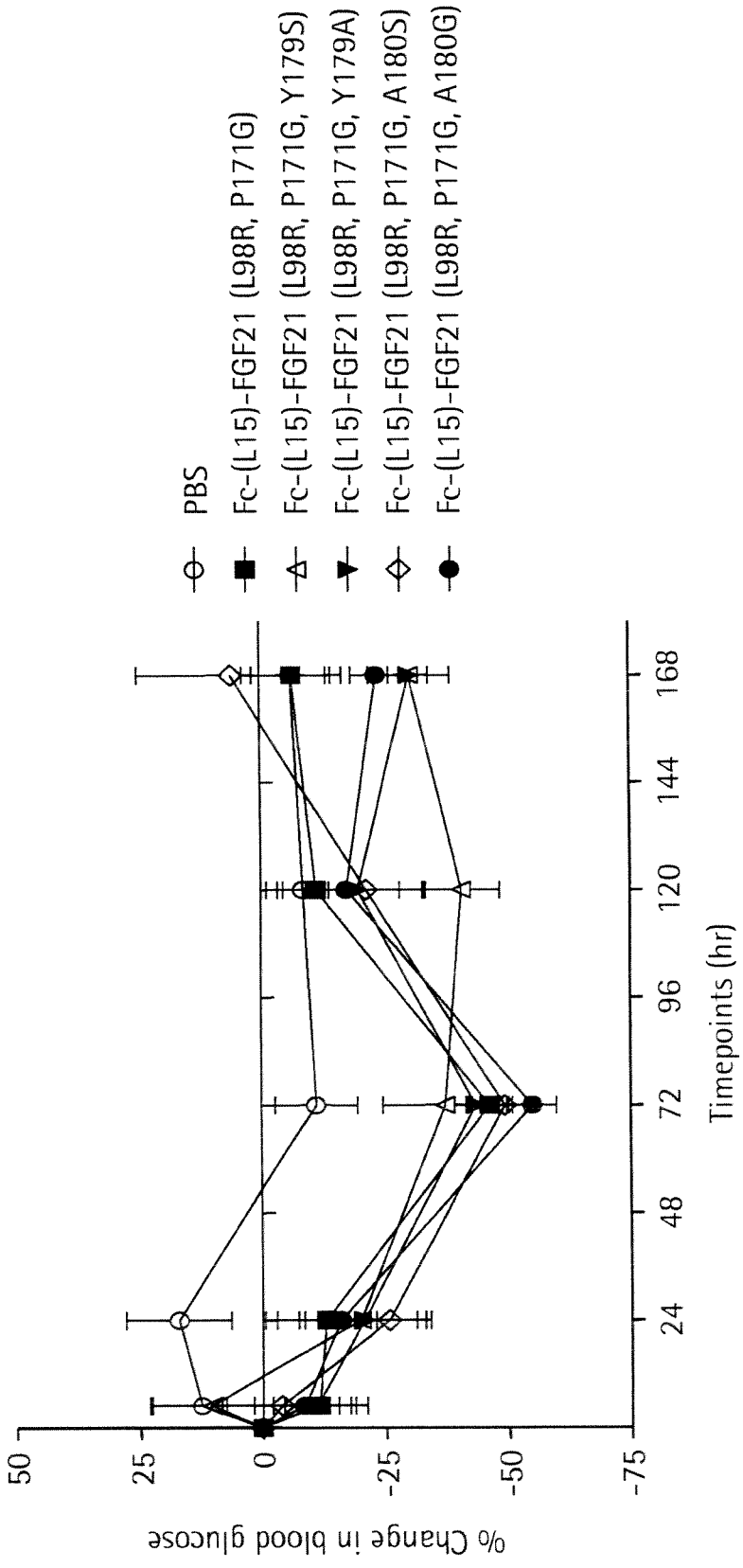

PBS
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(L15)-FGF21 (L98R, P171G, Y179S)
Fc-(L15)-FGF21 (L98R, P171G, Y179A)
Fc-(L15)-FGF21 (L98R, P171G, A180S)
Fc-(L15)-FGF21 (L98R, P171G, A180G)
% Change in blood glucose
50
25
0
-25
-50
-75
24
48
72
96
120
144
168
Timepoints (hr)

FIG. 36

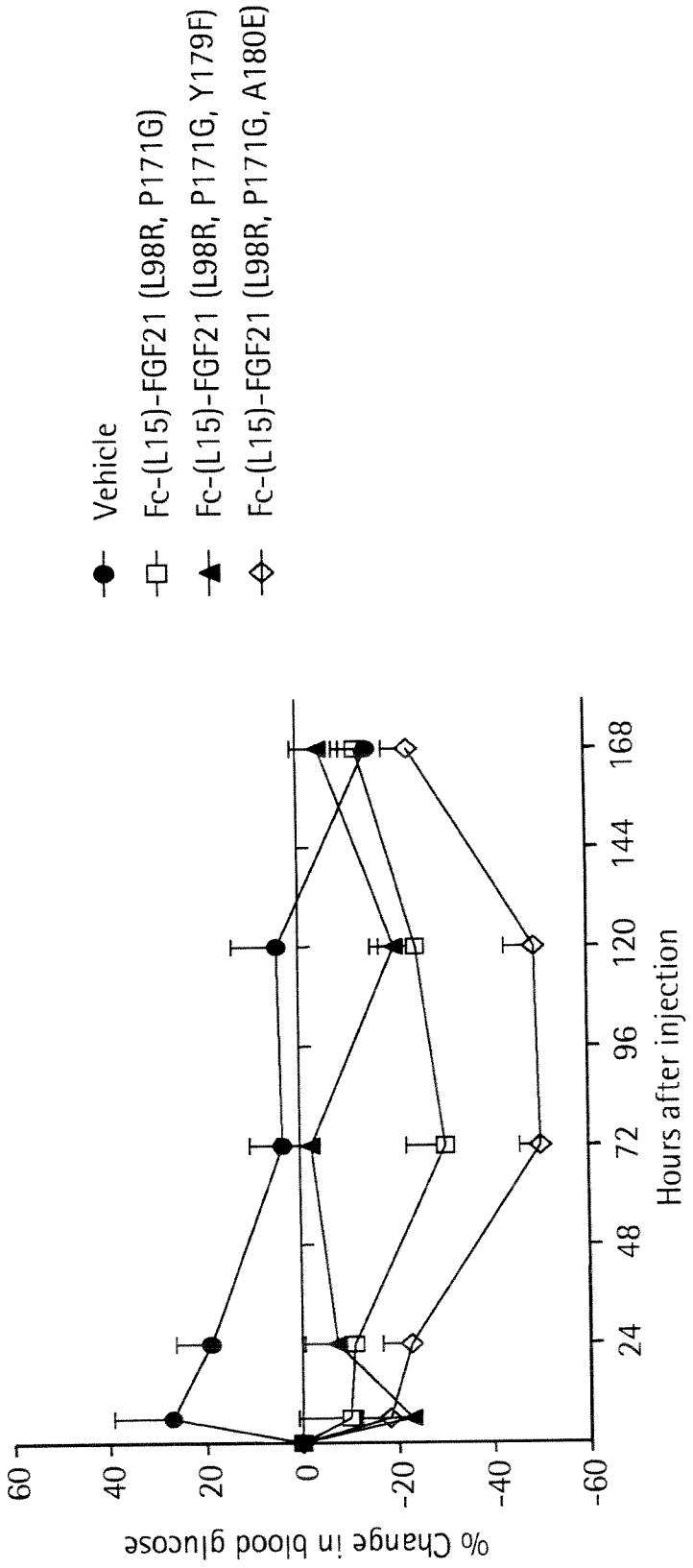

Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(L15)-FGF21 (L98R, P171G, Y179F)
Fc-(L15)-FGF21 (L98R, P171G, A180E)
% Change in blood glucose
60
40
20
0
-20
-40
-60
24
48
72
96
120
144
168
Hours after injection

FIG. 37

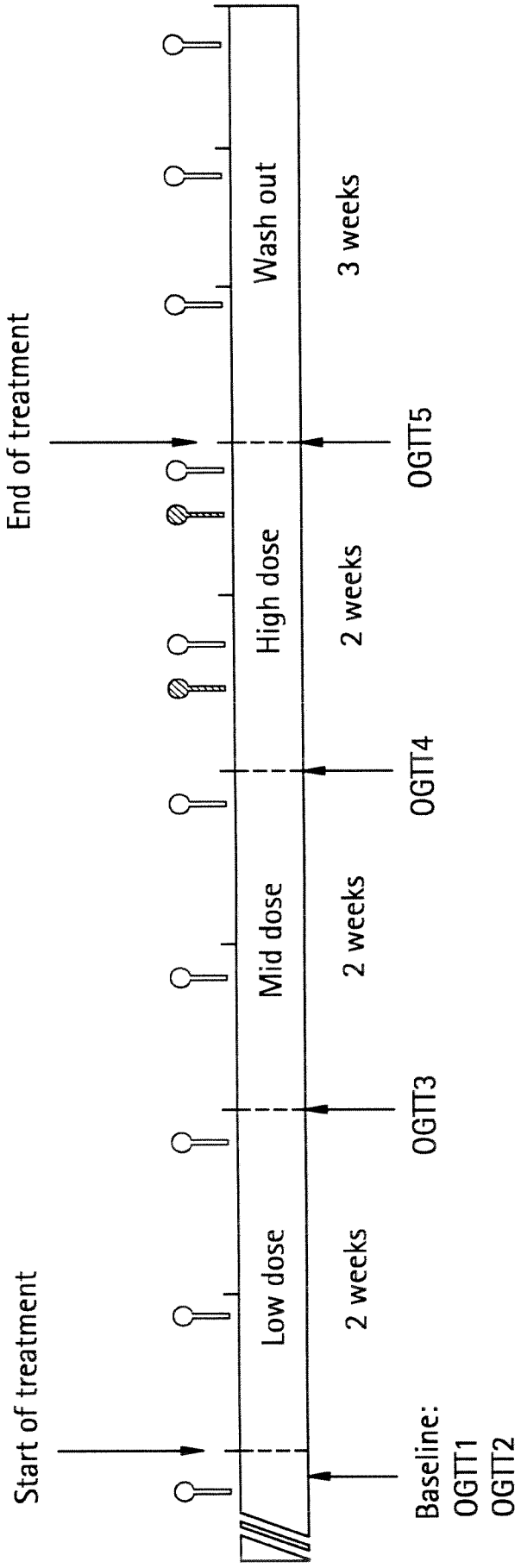

Start of treatment
End of treatment
Low dose
Mid dose
High dose
Wash out
2 weeks
2 weeks
2 weeks
3 weeks
Baseline:
OGTT1
OGTT2
OGTT3
OGTT4
OGTT5

FIG. 38A

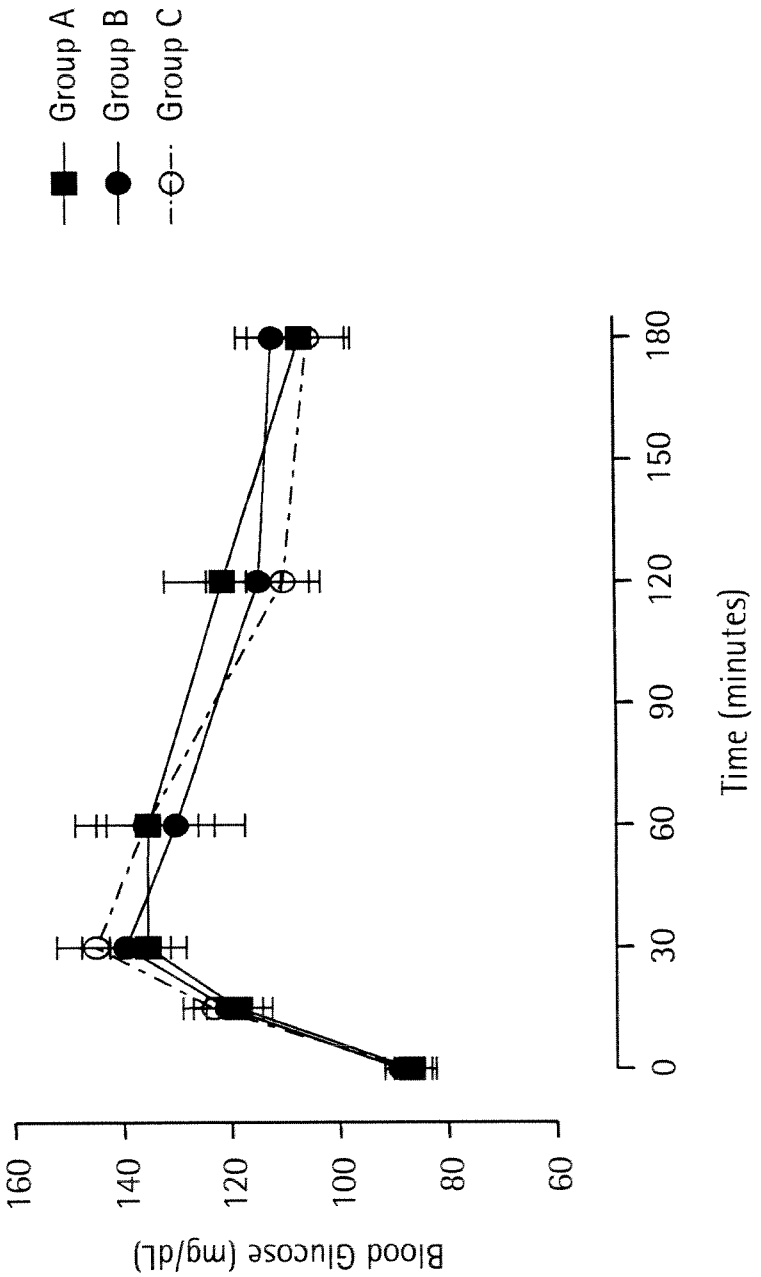

Group A
Group B
Group C
Blood Glucose (mg/dL)
160
140
120
100
80
60
0
30
60
90
120
150
180
Time (minutes)

FIG. 38B

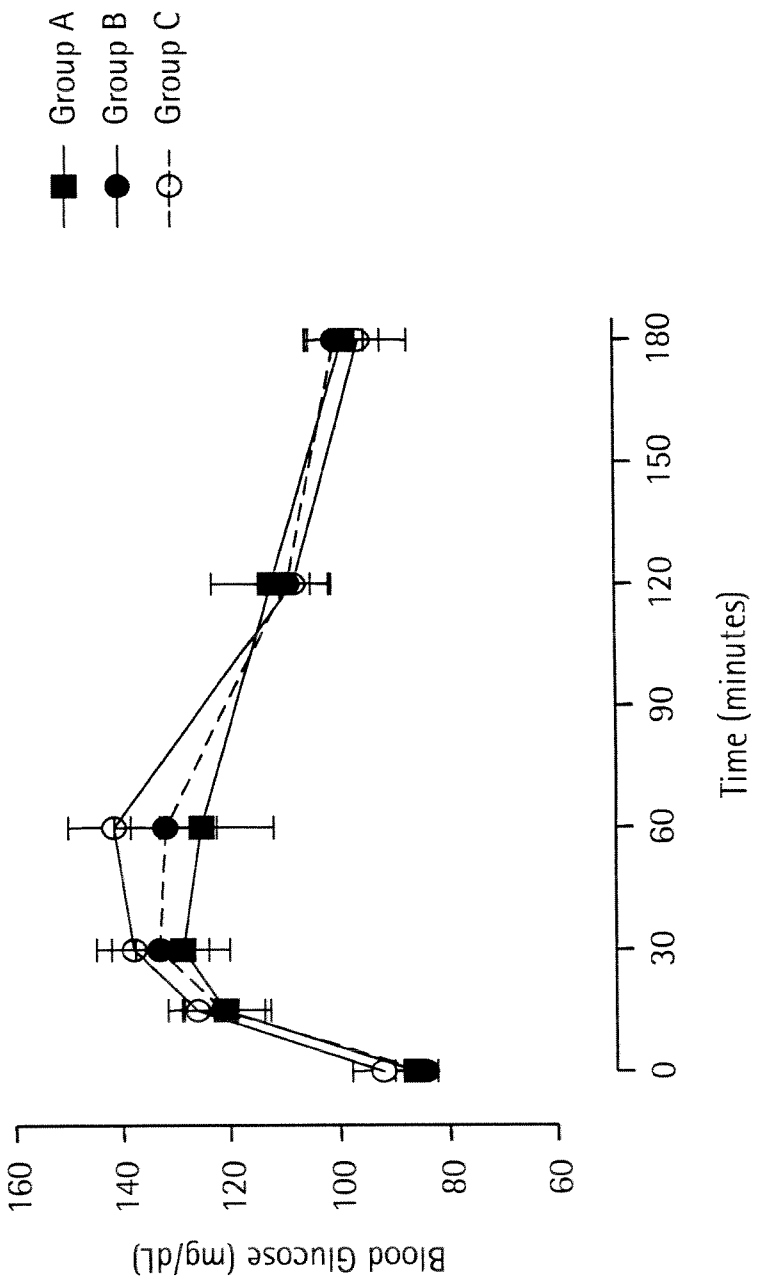

Group A
Group B
Group C
Blood Glucose (mg/dL)
160
140
120
100
80
60
0
30
60
90
120
150
180
Time (minutes)

FIG. 38C

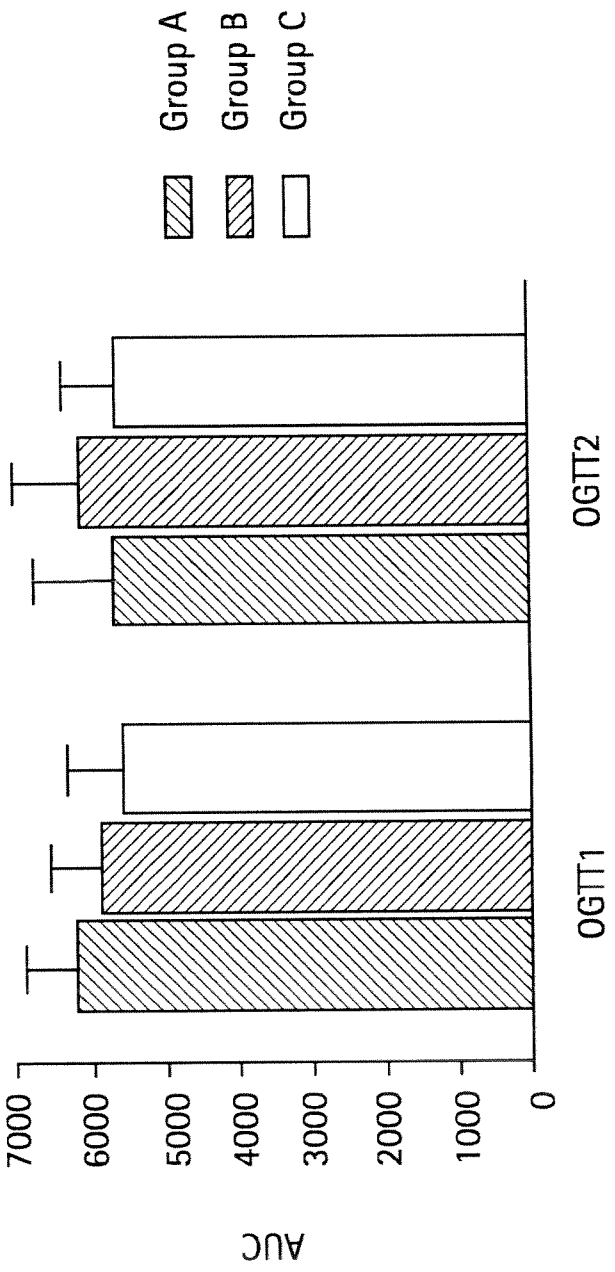

Group A
Group B
Group C
AUC
7000
6000
5000
4000
3000
2000
1000
0
OGTT1
OGTT2

FIG. 38D

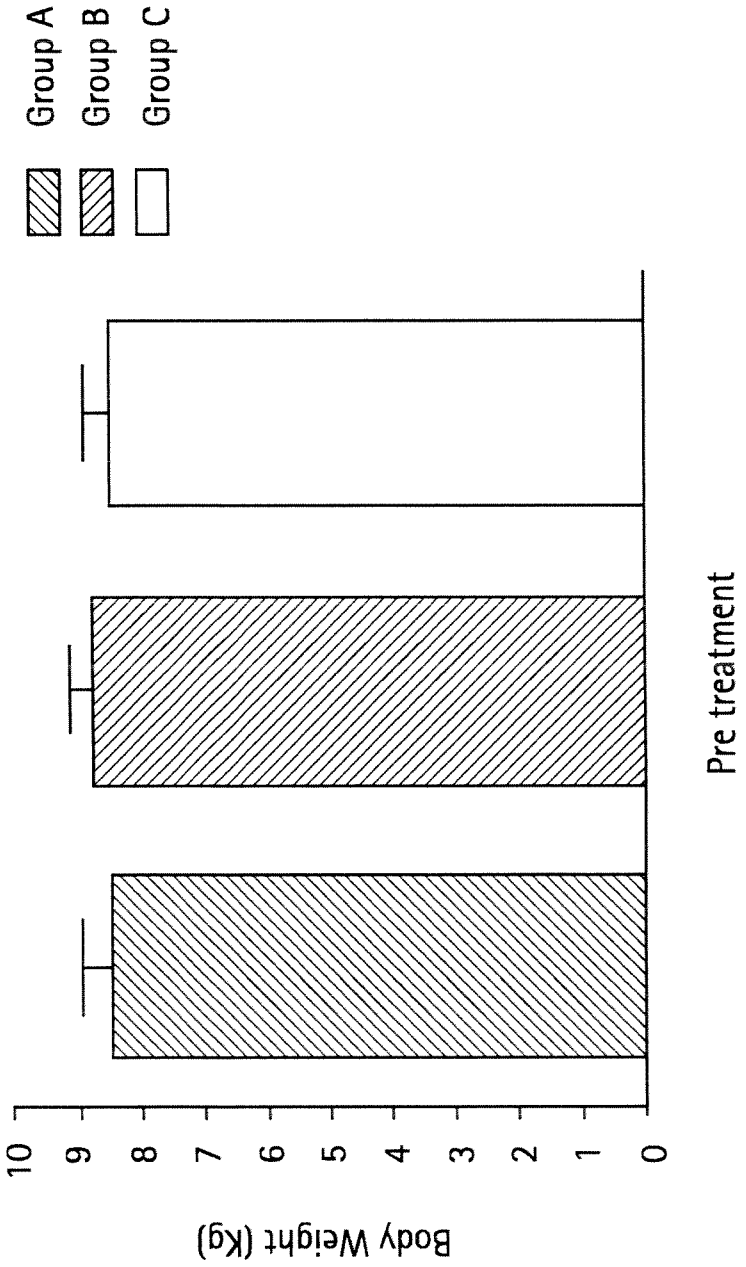

Group A
Group B
Group C
Body Weight (Kg)
10
9
8
7
6
5
4
3
2
1
0
Pre treatment

FIG. 39

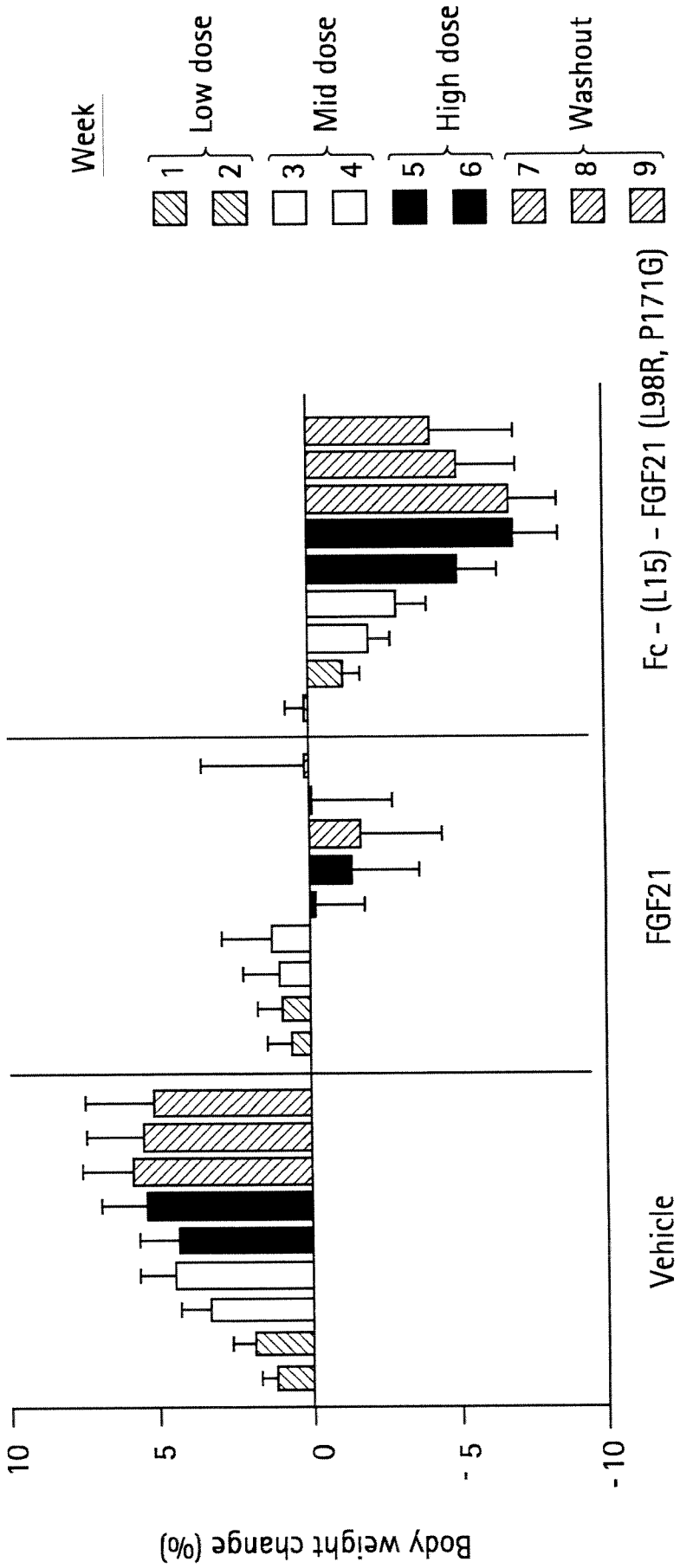

Week
1
2
Low dose
3
4
Mid dose
5
6
High dose
7
8
9
Washout
Body weight change (%)
10
5
0
- 5
- 10
Vehicle
FGF21
Fc - (L15) - FGF21 (L98R, P171G)

FIG. 40

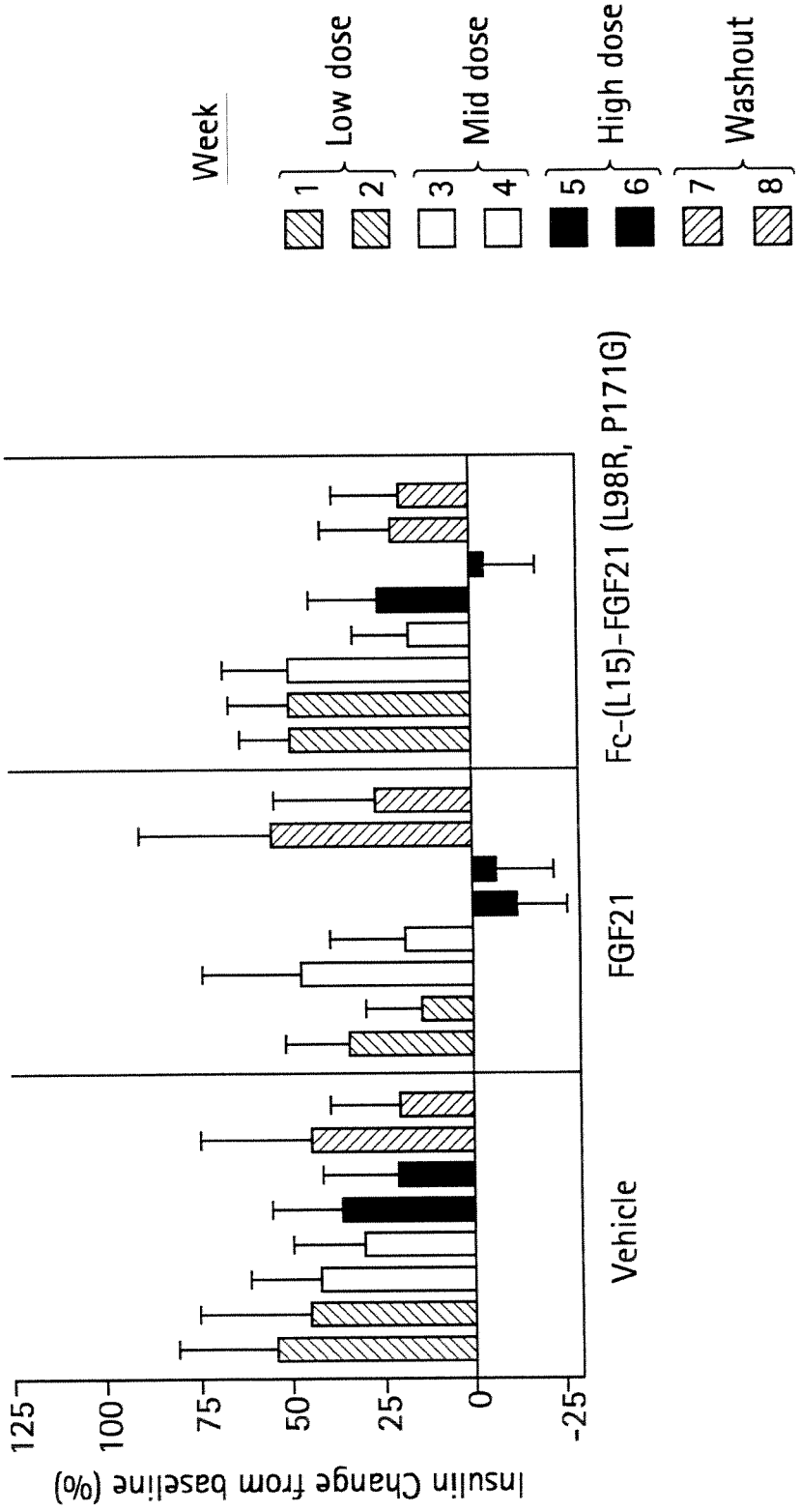

Week
1
2
Low dose
3
4
Mid dose
5
6
High dose
7
8
Washout
Insulin Change from baseline (%)
125
100
75
50
25
0
-25
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 41

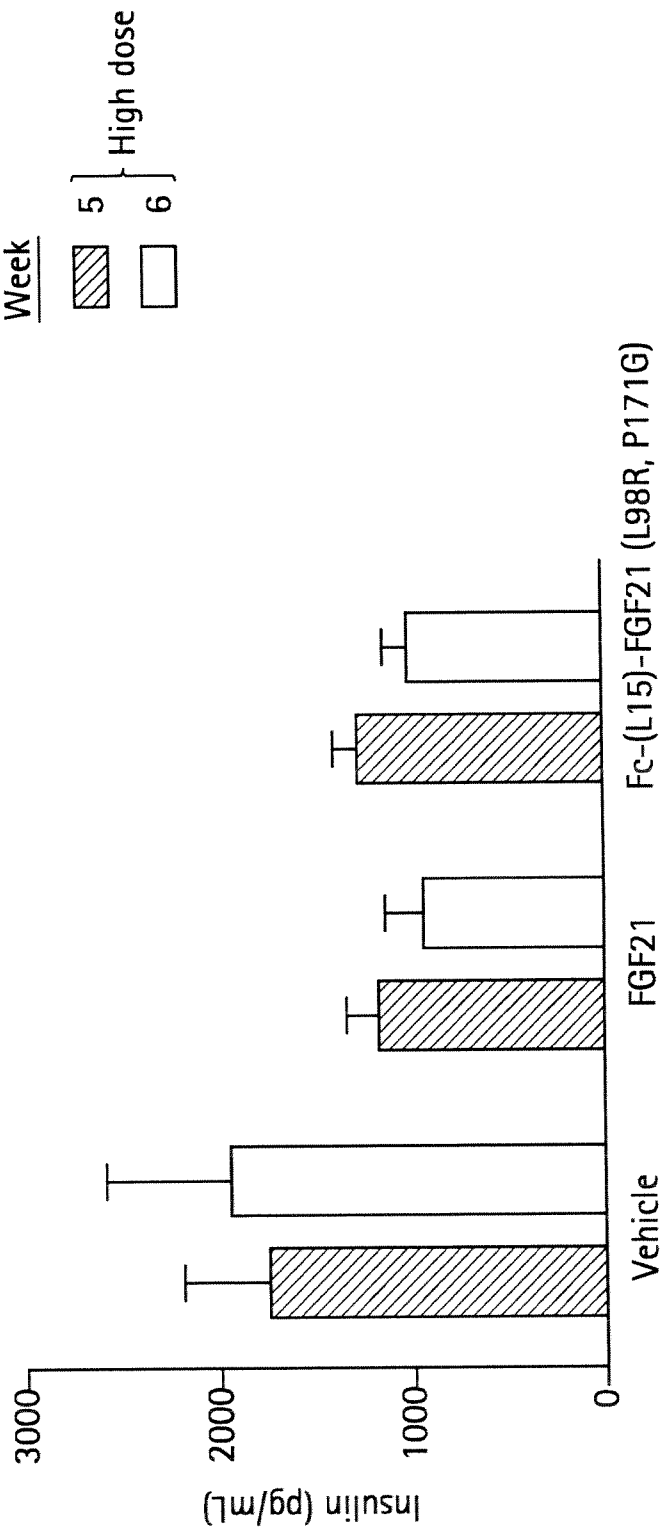

Week
5
6
High dose
Insulin (pg/mL)
3000
2000
1000
0
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 42

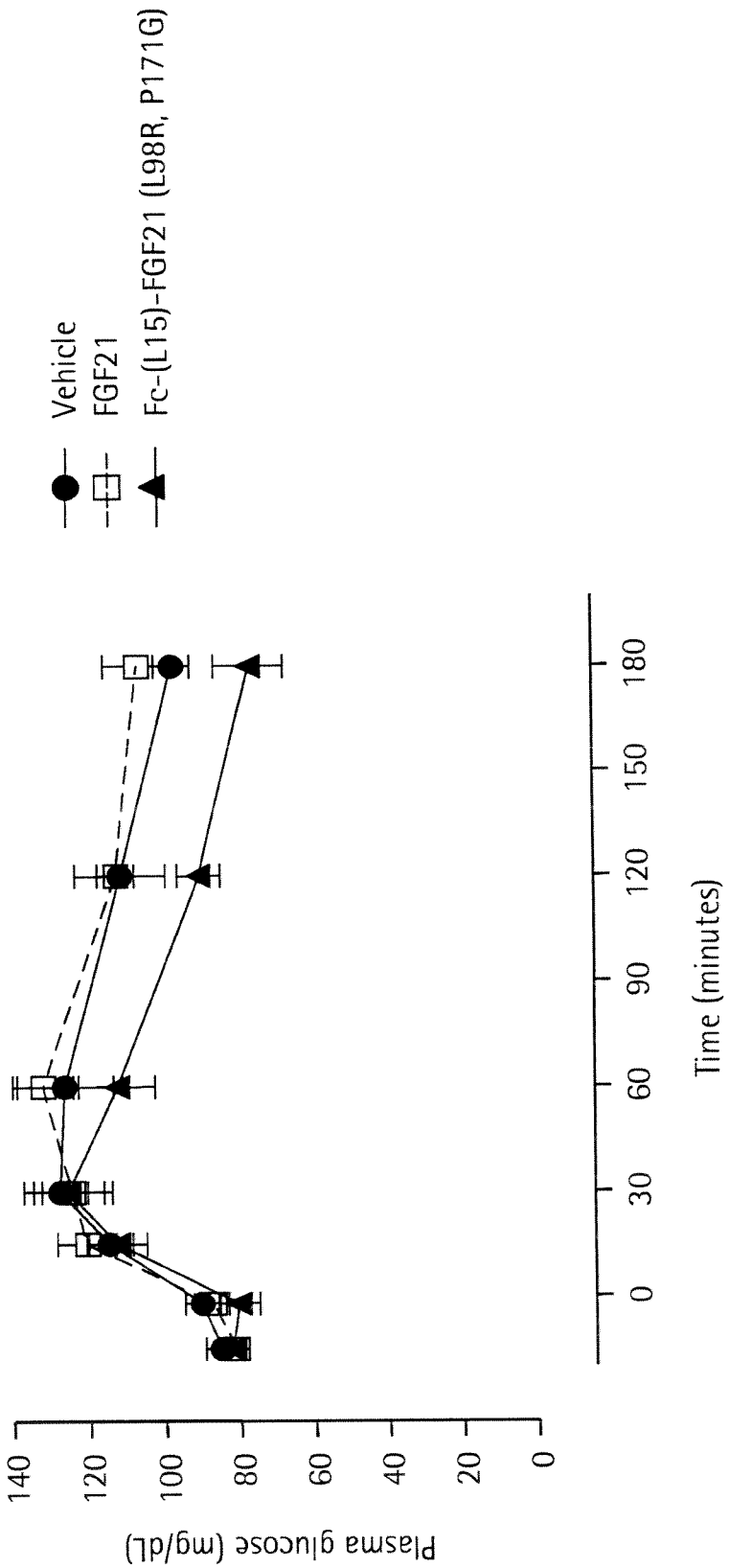

Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)
Plasma glucose (mg/dL)
0
20
40
60
80
100
120
140
Time (minutes)
0
30
60
90
120
150
180

FIG. 43

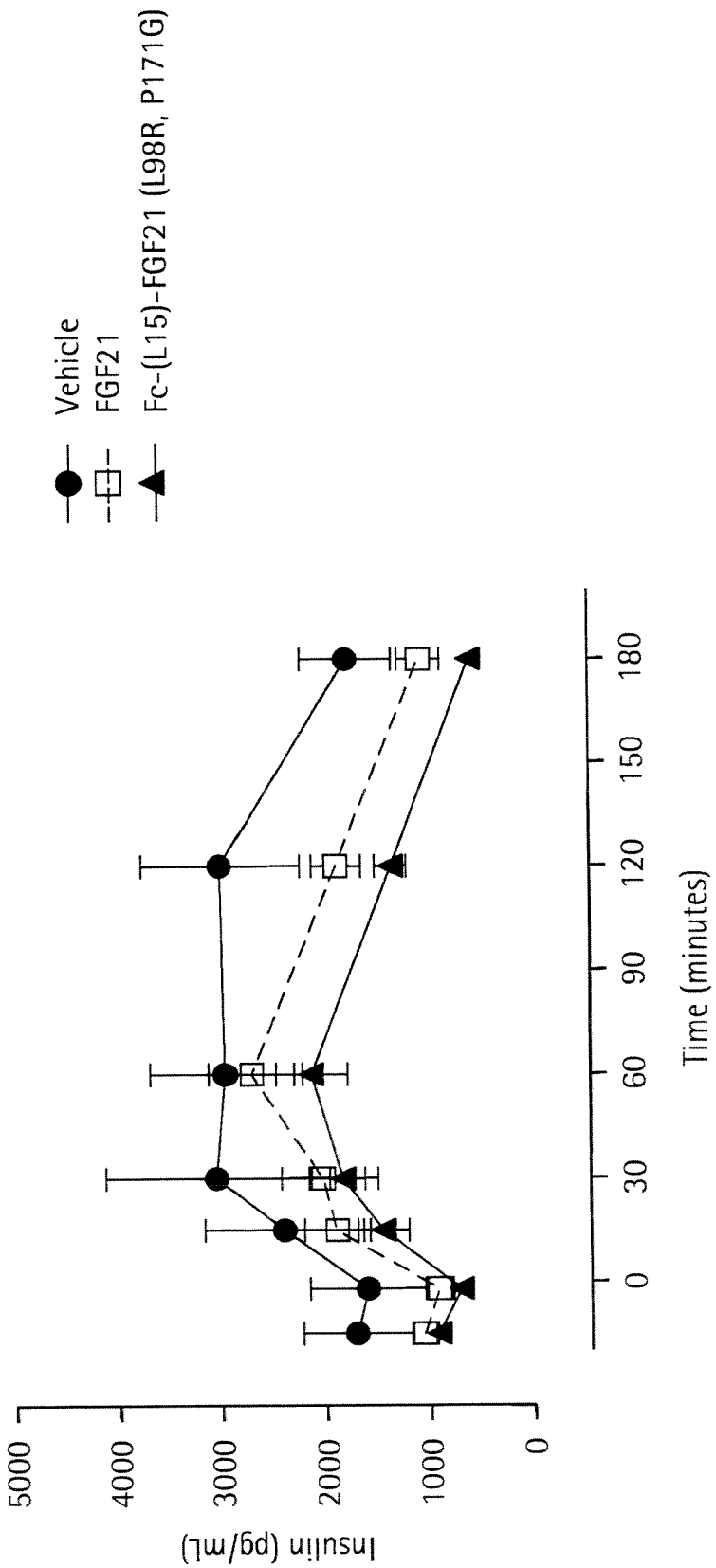

Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)
Insulin (pg/mL)
5000
4000
3000
2000
1000
0
0
30
60
90
120
150
180
Time (minutes)

FIG. 44

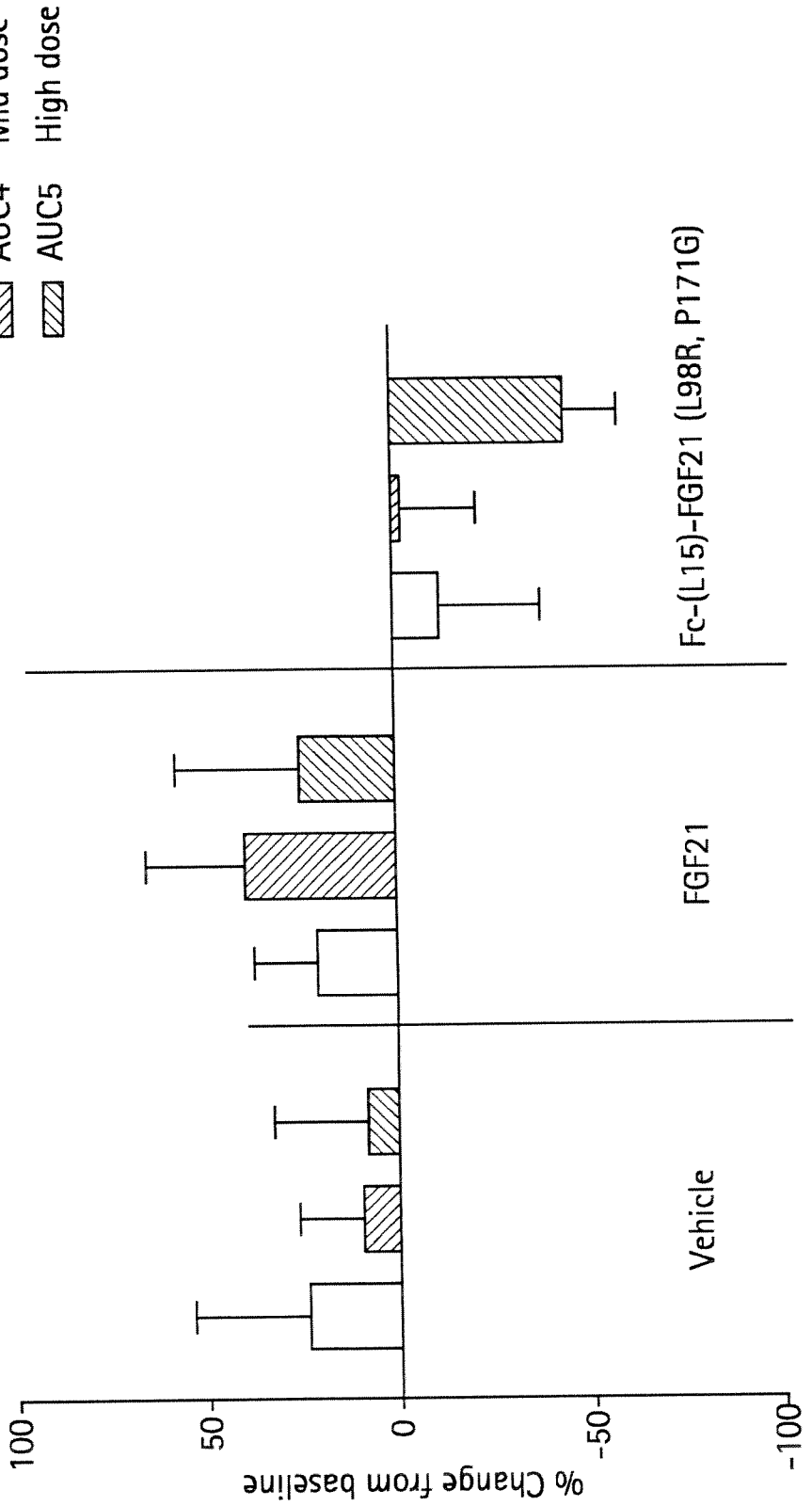

AUC3 Low dose
AUC4 Mid dose
AUC5 High dose
% Change from baseline
100
50
0
-50
-100
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 45

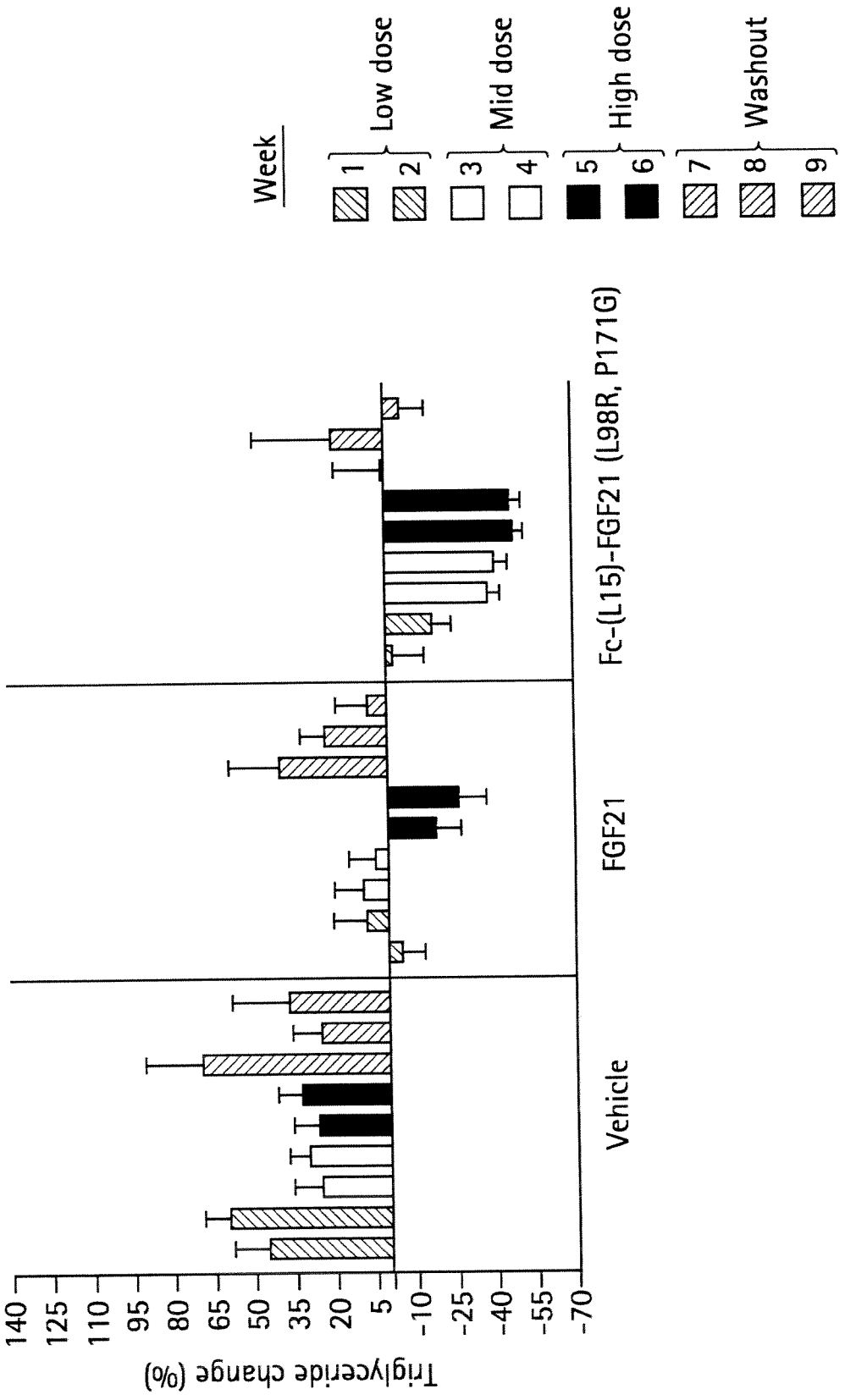

Week
1
2
Low dose
3
4
Mid dose
5
6
High dose
7
8
9
Washout
Triglyceride change (%)
140
125
110
95
80
65
50
35
20
5
-10
-25
-40
-55
-70
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)

FIG. 46

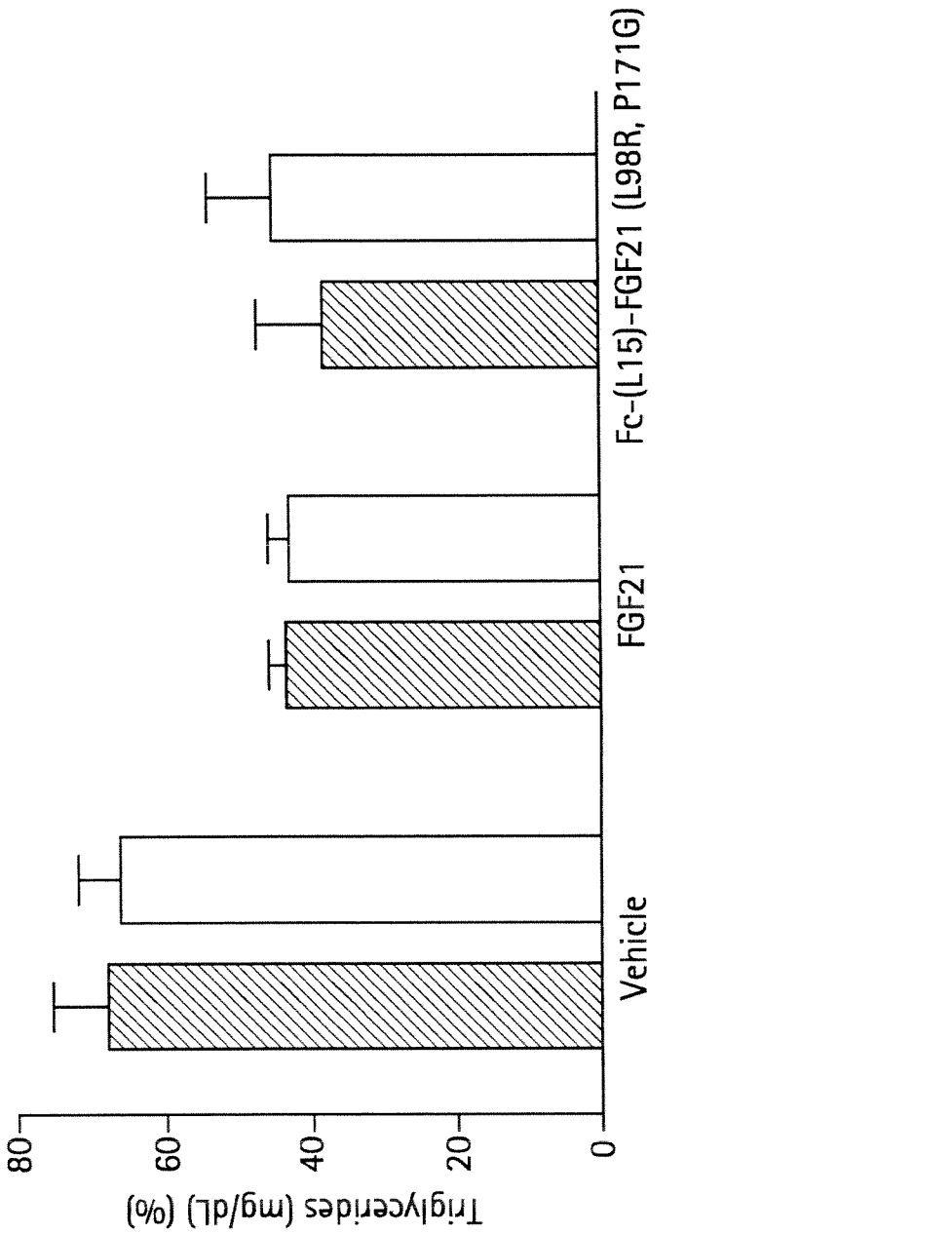

Triglycerides (mg/dL) (%)
0
20
40
60
80
Vehicle
FGF21
Fc-(L15)-FGF21 (L98R, P171G)
Week
5
6

FIG. 47

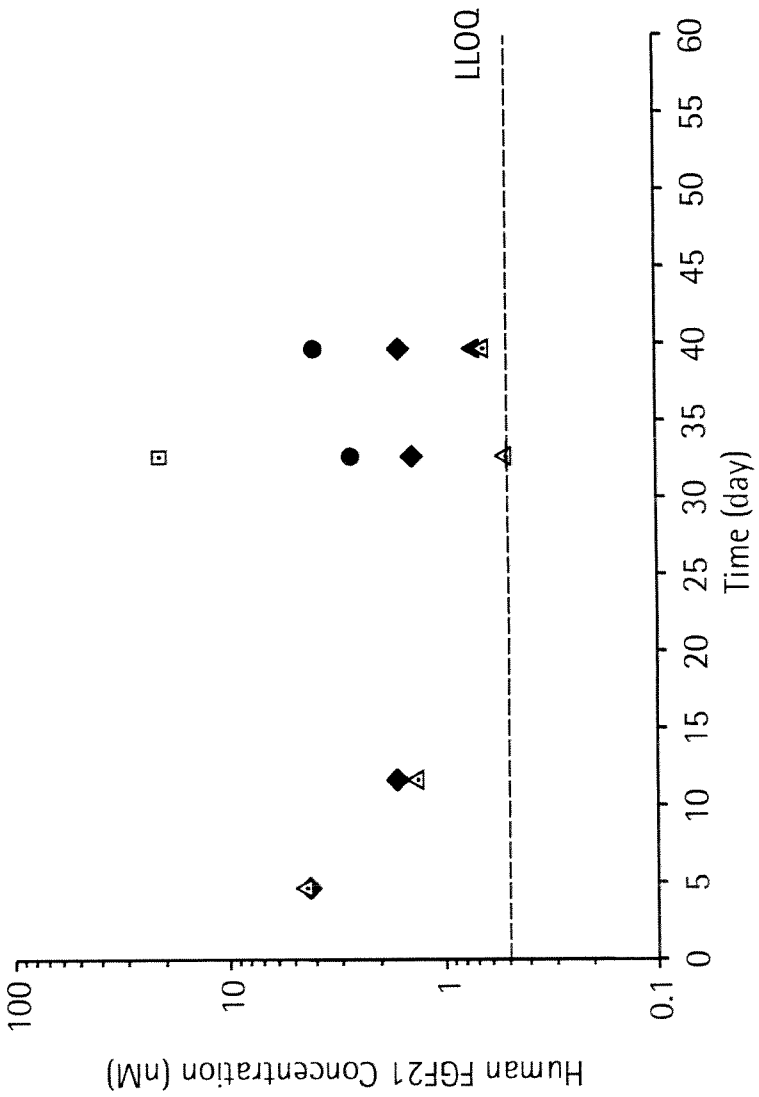

Human FGF21 Concentration (nM)
100
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (day)
LLOQ

FIG. 48

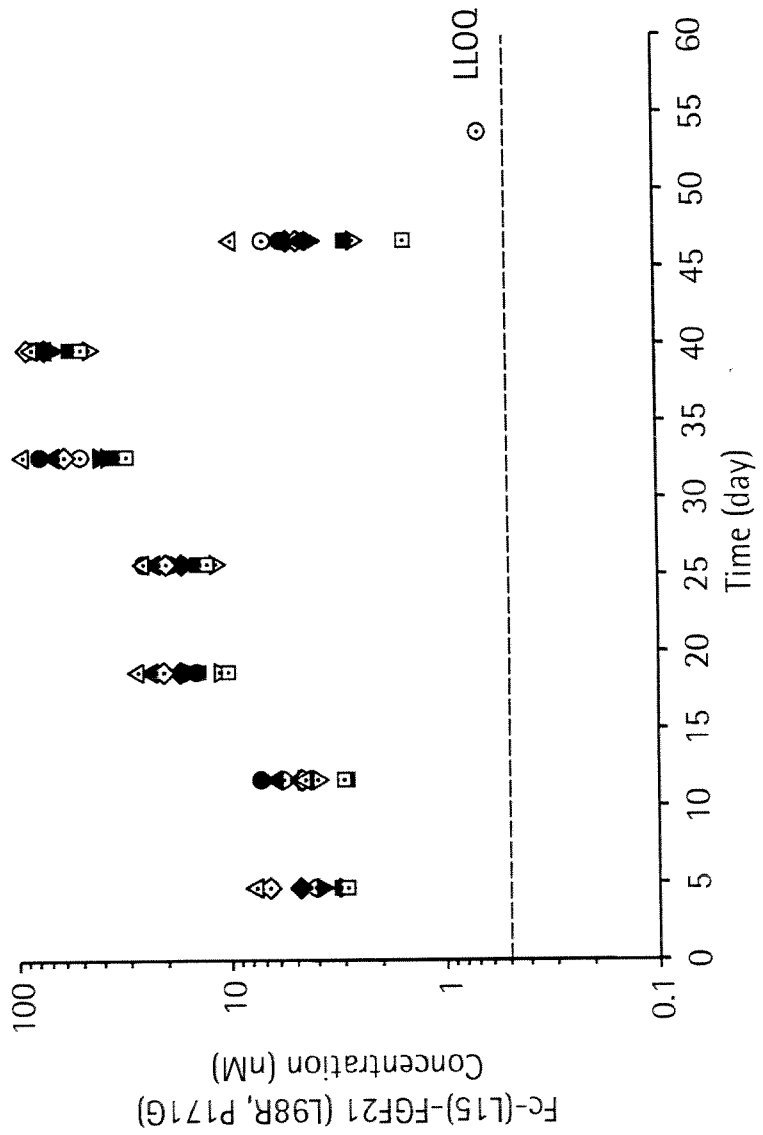

Fc-(L15)-FGF21 (L98R, P171G)
Concentration (nM)
100
10
1
0.1
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (day)
LLOQ

FIG. 49

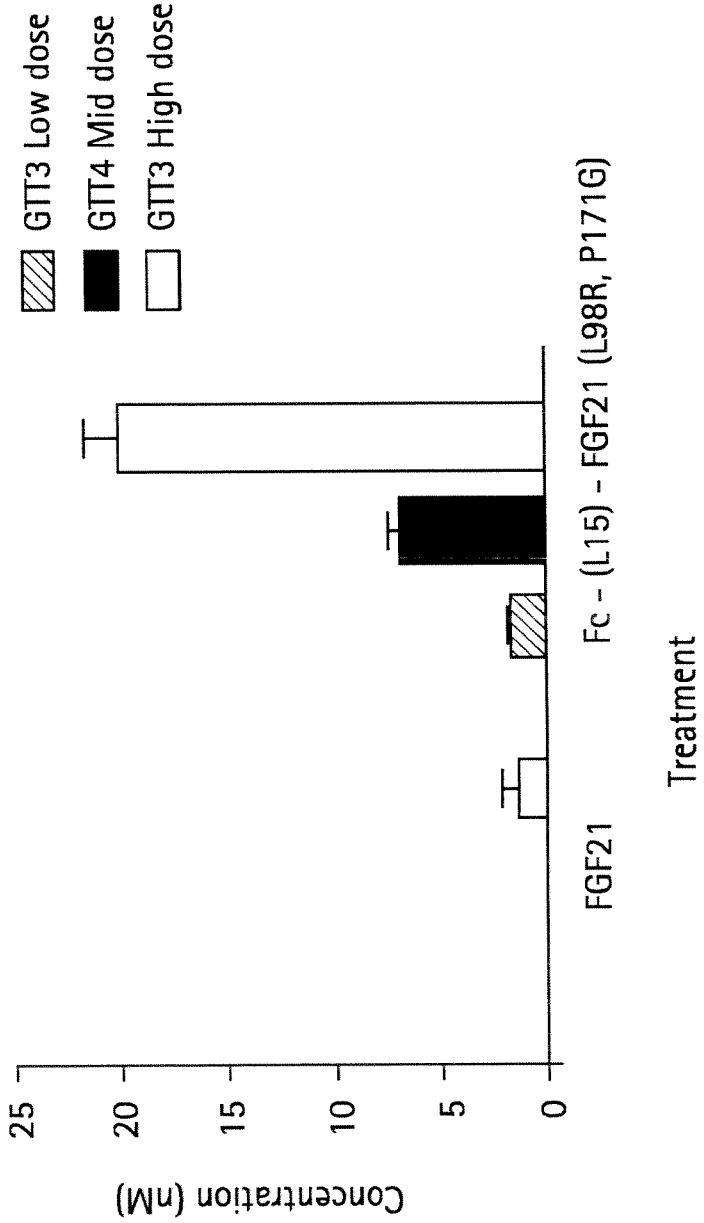
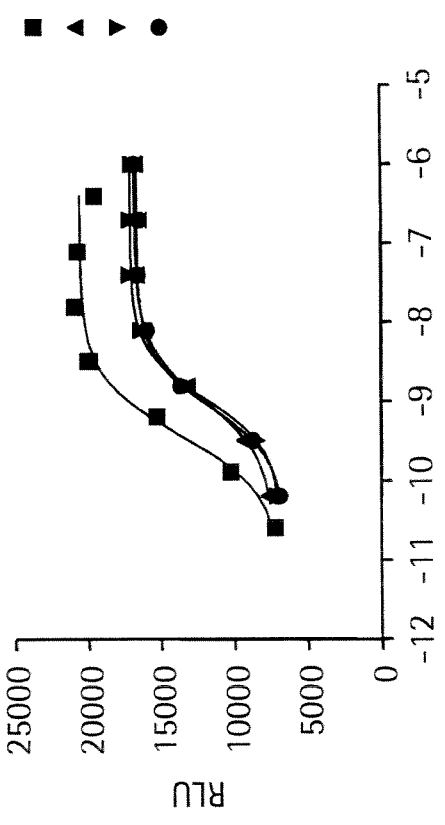

GTT3 Low dose
GTT4 Mid dose
GTT3 High dose
25
20
15
10
5
0
Concentration (nM)
FGF21
Fc - (L15) - FGF21 (L98R, P171G)
Treatment

FIG. 50

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

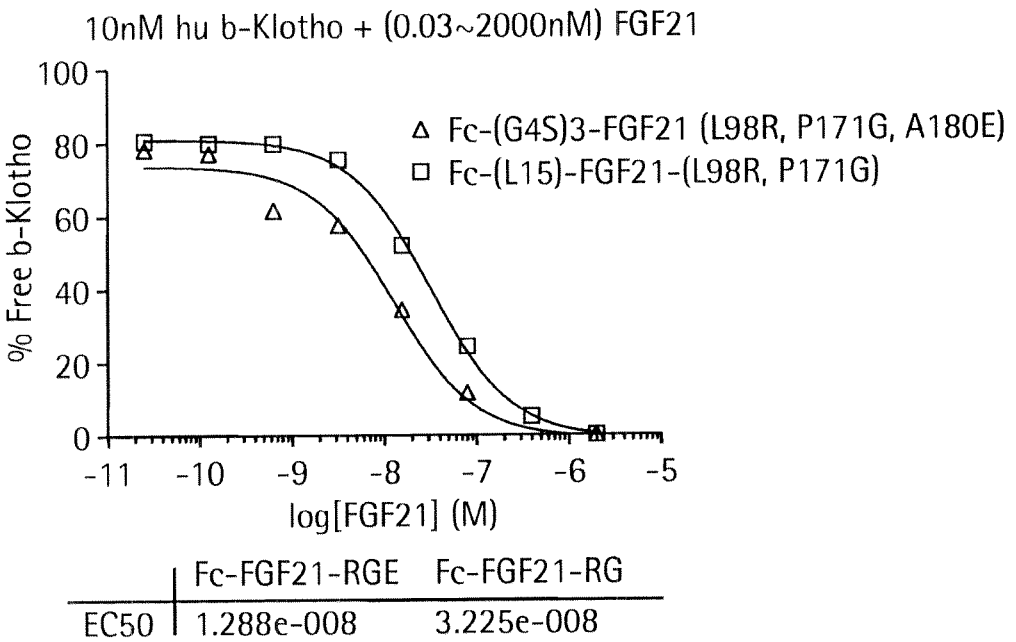
MDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGG*
*GGSH*IPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQR
PDGALYGSLHFDPEACSFRE**R**LLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQP
PDVGSSDPLSMVG**G**SQGRSPSY**E**S

FIG. 51

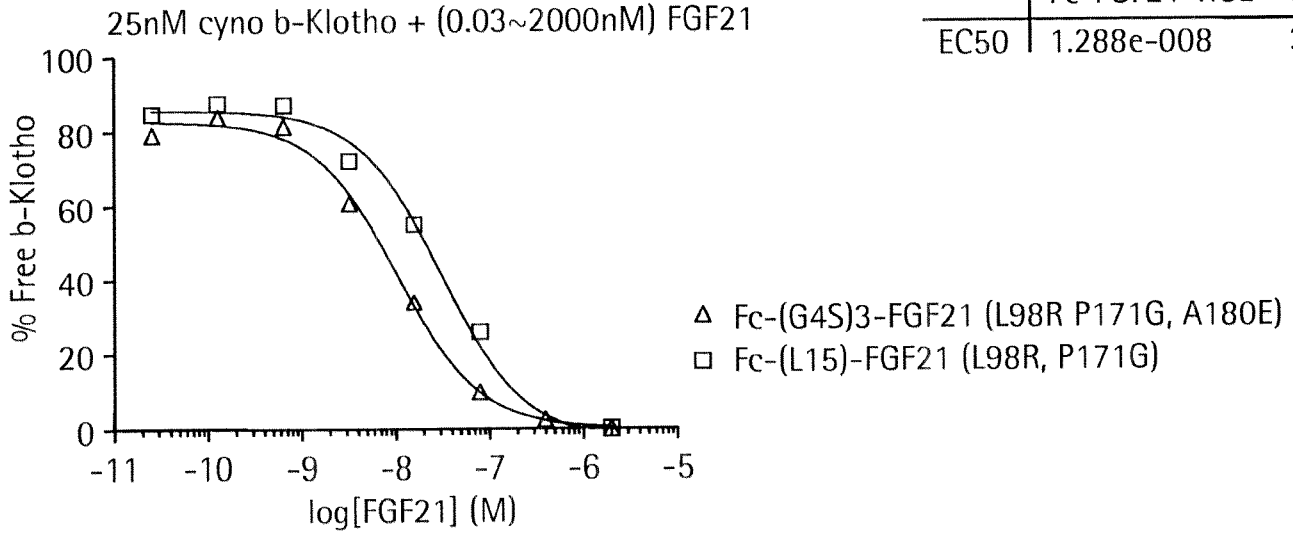


EC50	FGF21	Fc-(L15)-FGF21	Fc-L15-FGF21(L98R/P171G/A180E)	Fc-G4S-FGF21(L98R/P171G/A180E)
	3.831e-010	1.034e-009	1.095e-009	8.515e-010

FIG. 52



	Fc-FGF21-RGE	Fc-FGF21-RG
EC50	1.288e-008	3.225e-008



	Fc-(L15)-FGF21 (L98R P171G, AE	Fc-(L15)-FGF21 (L98R, P171G)
EC50	1.038e-008	3.081e-008

FIG. 53A

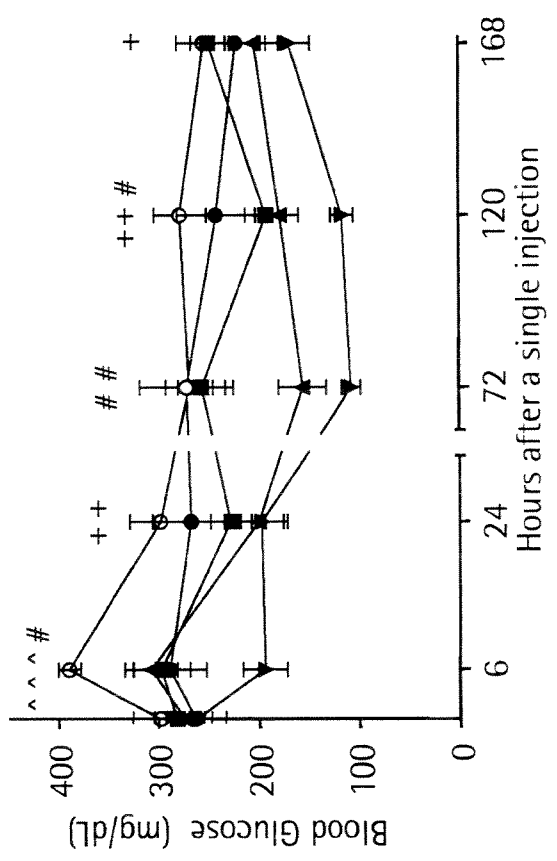


FIG. 53B

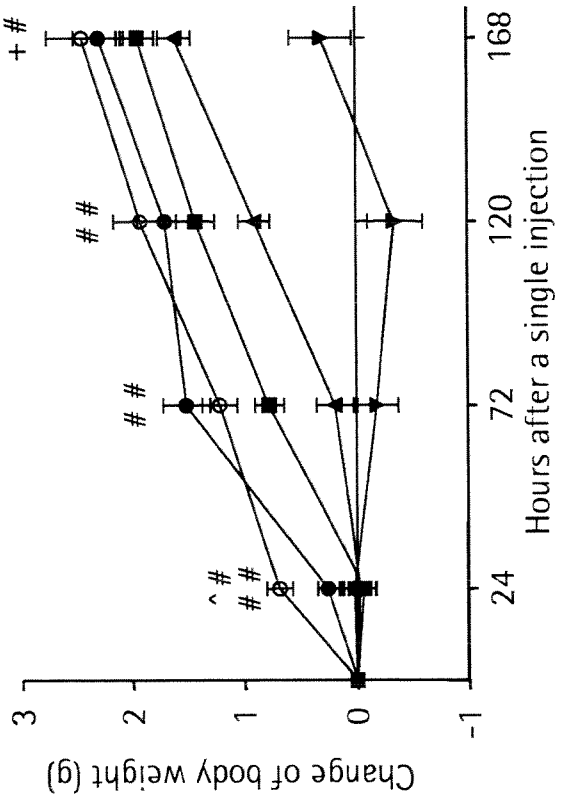


FIG. 54

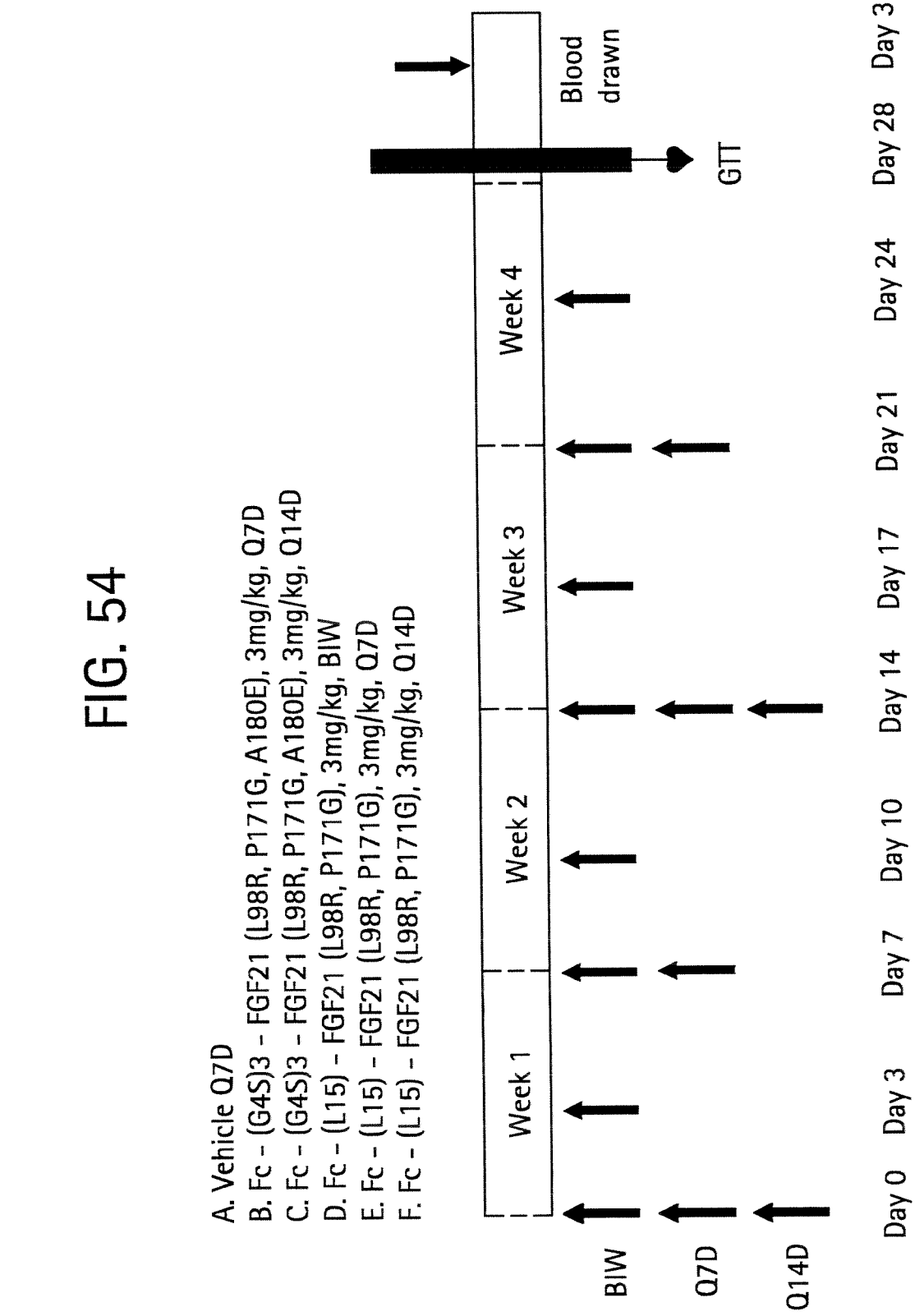

A. Vehicle Q7D
B. Fc - (G4S)3 - FGF21 (L98R, P171G, A180E), 3mg/kg, Q7D
C. Fc - (G4S)3 - FGF21 (L98R, P171G, A180E), 3mg/kg, Q14D
D. Fc - (L15) - FGF21 (L98R, P171G), 3mg/kg, BIW
E. Fc - (L15) - FGF21 (L98R, P171G), 3mg/kg, Q7D
F. Fc - (L15) - FGF21 (L98R, P171G), 3mg/kg, Q14D
Week 1
Week 2
Week 3
Week 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

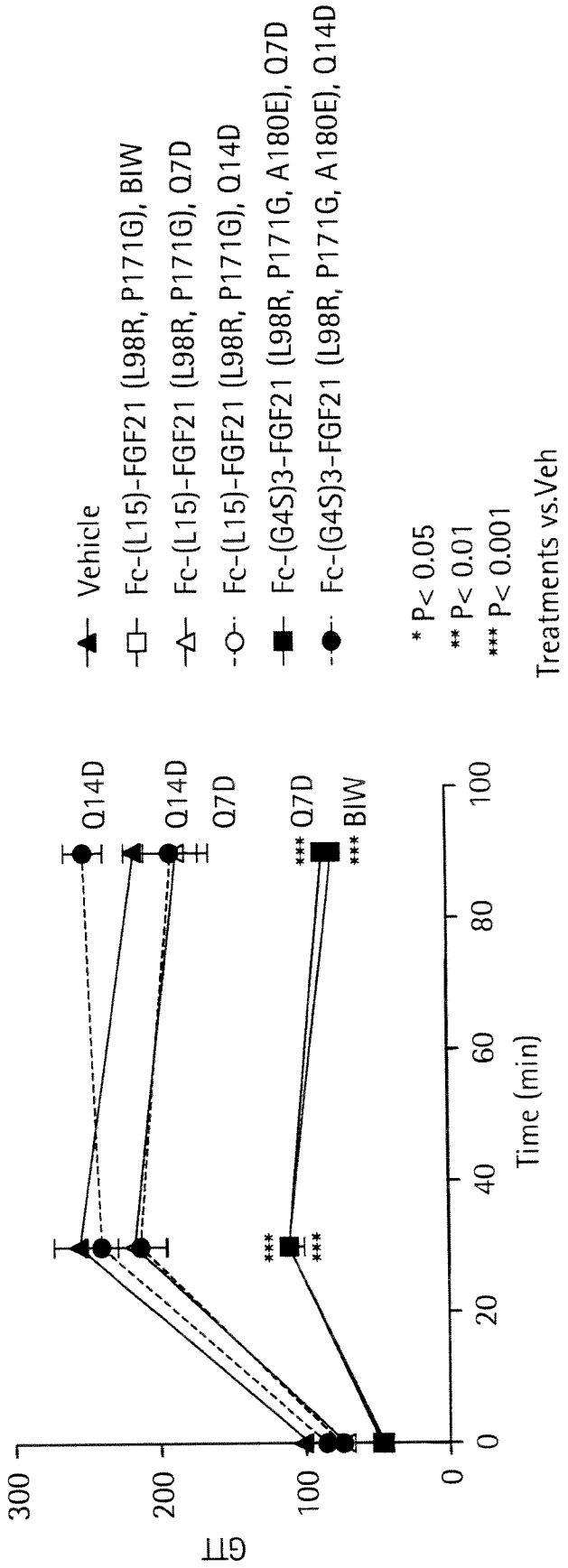

Vehicle
Fc-(L15)-FGF21 (L98R, P171G), BIW
Fc-(L15)-FGF21 (L98R, P171G), Q7D
Fc-(L15)-FGF21 (L98R, P171G), Q14D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q7D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q14D
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs.Veh
GTT
300
200
100
0
0
20
40
60
80
100
Time (min)
Q14D
Q14D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

FIG. 56

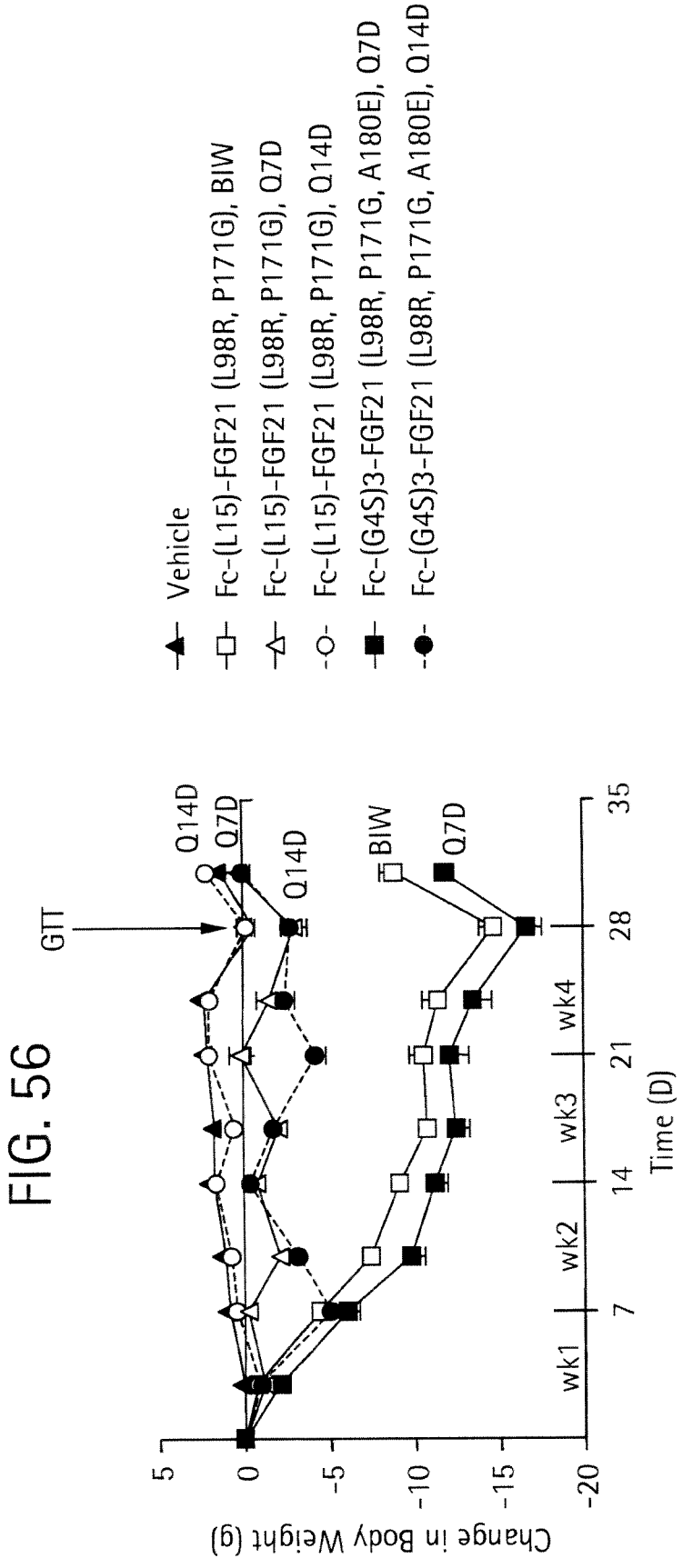

Vehicle
Fc-(L15)-FGF21 (L98R, P171G), BIW
Fc-(L15)-FGF21 (L98R, P171G), Q7D
Fc-(L15)-FGF21 (L98R, P171G), Q14D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q7D
Fc-(G4S)3-FGF21 (L98R, P171G, A180E), Q14D
GTT
Q14D
Q7D
Q14D
BIW
Q7D
5
0
-5
-10
-15
-20
Change in Body Weight (g)
wk1
wk2
wk3
wk4
7
14
21
28
35
Time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

FIG. 57

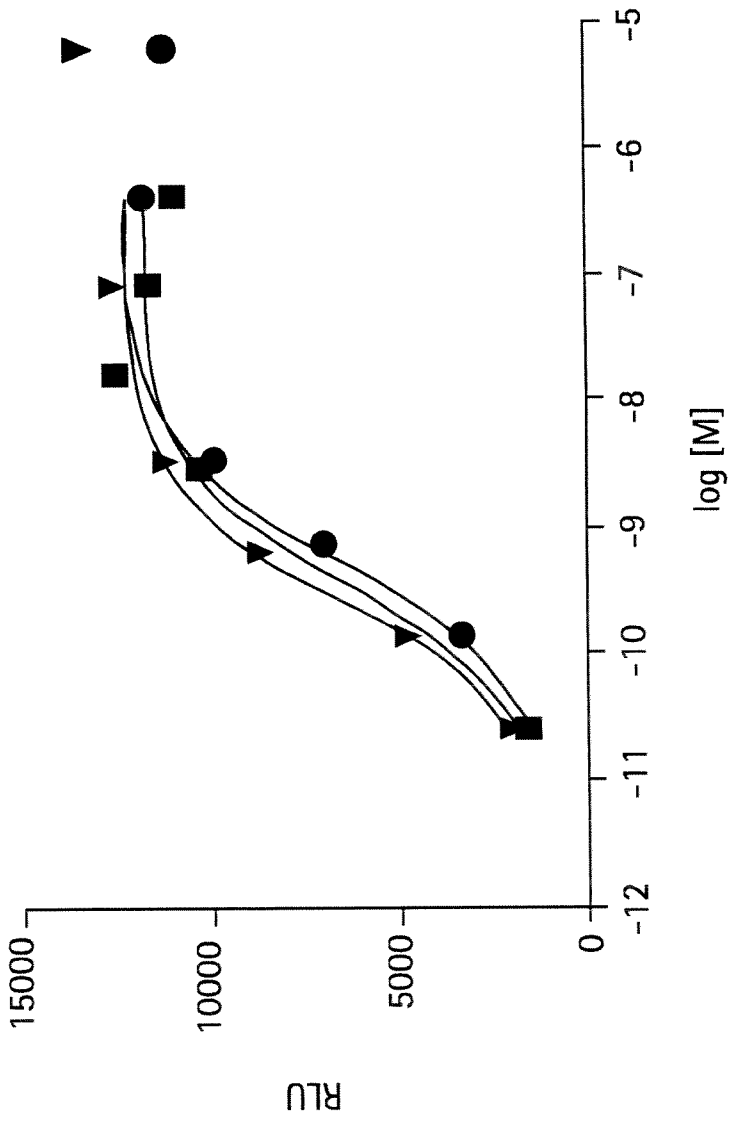

Fc - (L15) - FGF21 (L98R, P171G) ctrl
FGF21 hydrogel 16-10
FGF21 hydrogel 24-10
FGF21 hydrogel 32-10
RLU
15000
10000
5000
0
-12
-11
-10
-9
-8
-7
-6
-5
log [M]

FIG. 58

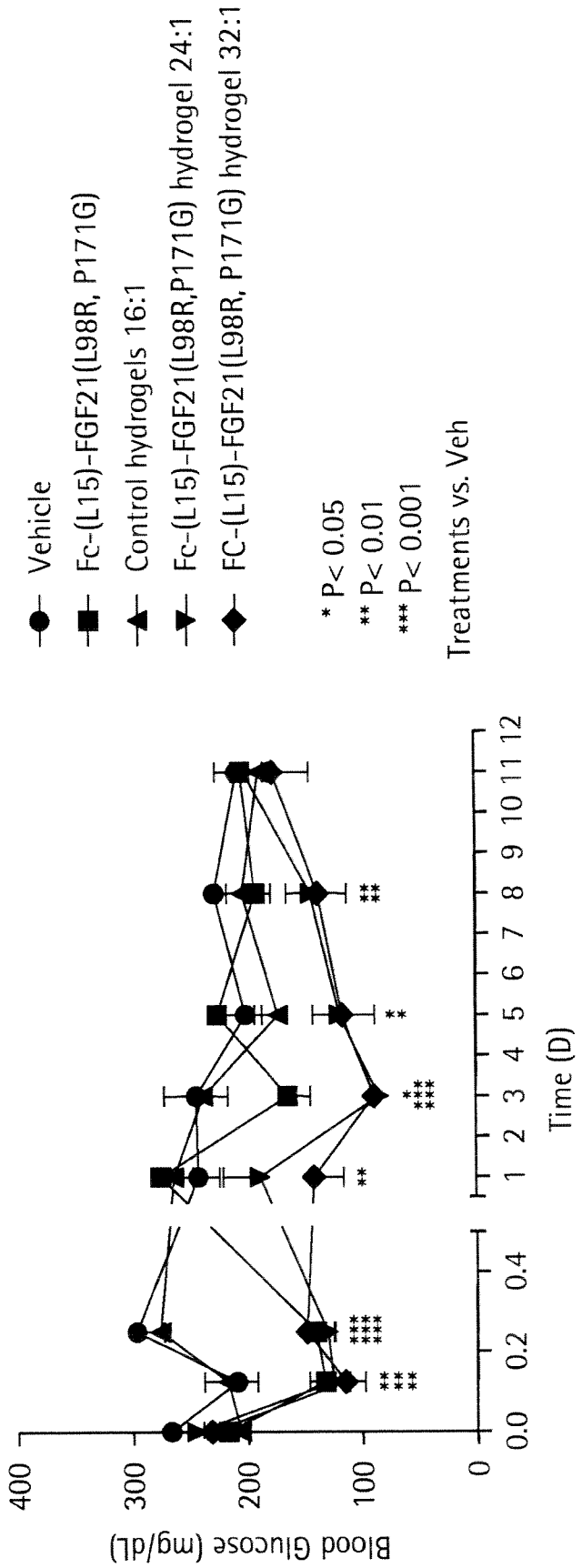

Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Control hydrogels 16:1
Fc-(L15)-FGF21(L98R,P171G) hydrogel 24:1
FC-(L15)-FGF21(L98R, P171G) hydrogel 32:1
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Blood Glucose (mg/dL)
400
300
200
100
0
0.0
0.2
0.4
1 2 3 4 5 6 7 8 9 10 11 12
Time (D)

FIG. 59

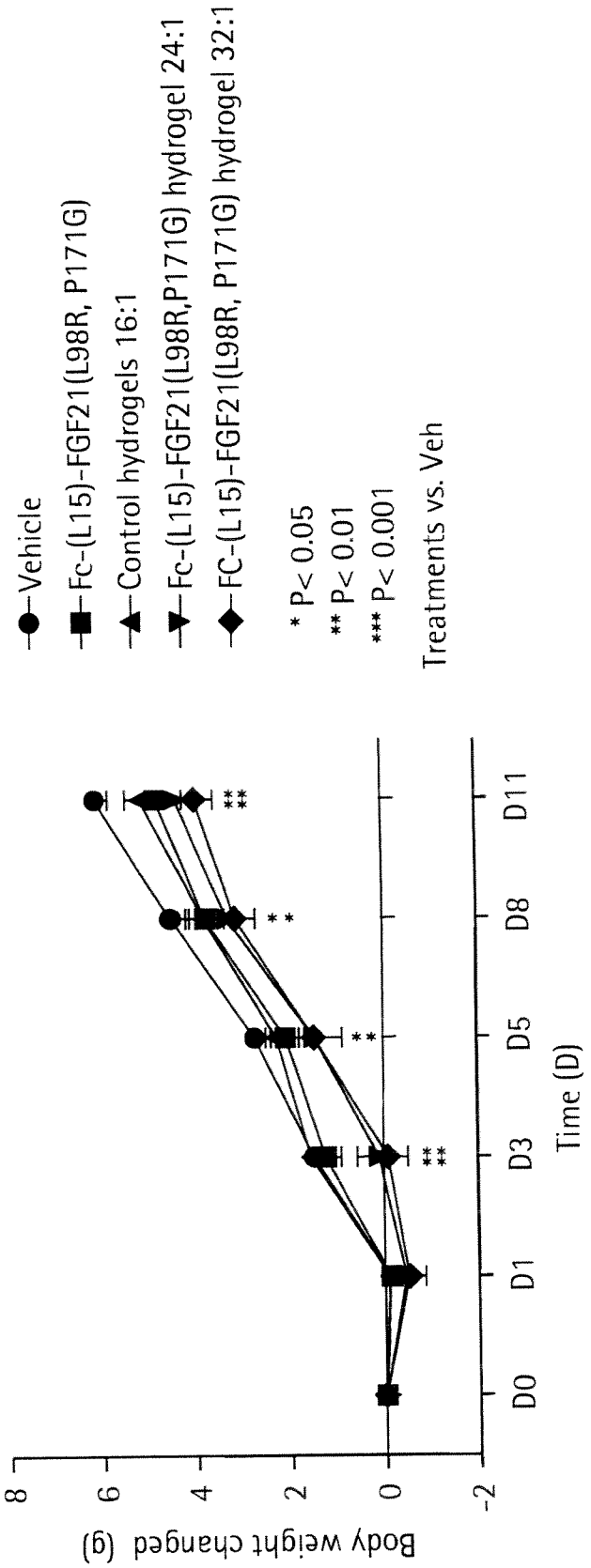

Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Control hydrogels 16:1
Fc-(L15)-FGF21(L98R,P171G) hydrogel 24:1
FC-(L15)-FGF21(L98R, P171G) hydrogel 32:1
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Body weight changed (g)
8
6
4
2
0
-2
D0
D1
D3
D5
D8
D11
Time (D)

FIG. 60

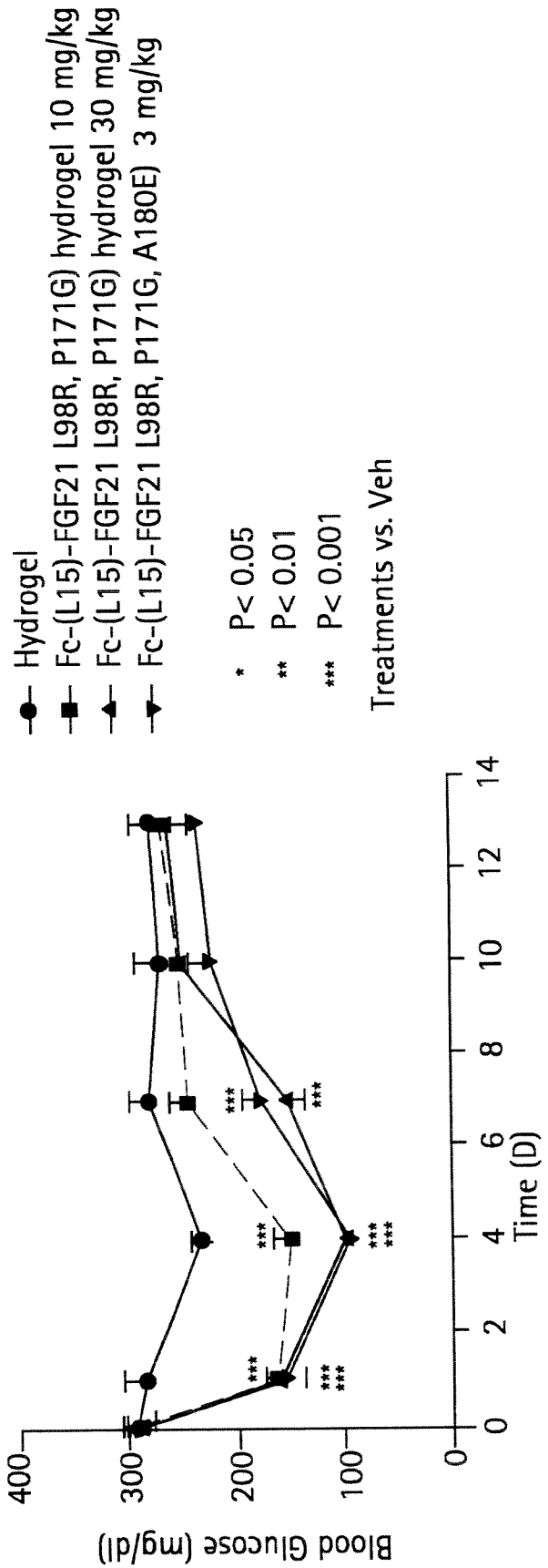

Hydrogel
Fc-(L15)-FGF21 L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21 L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 L98R, P171G, A180E) 3 mg/kg
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Blood Glucose (mg/dl)
400
300
200
100
0
0
2
4
6
8
10
12
14
Time (D)

FIG. 61

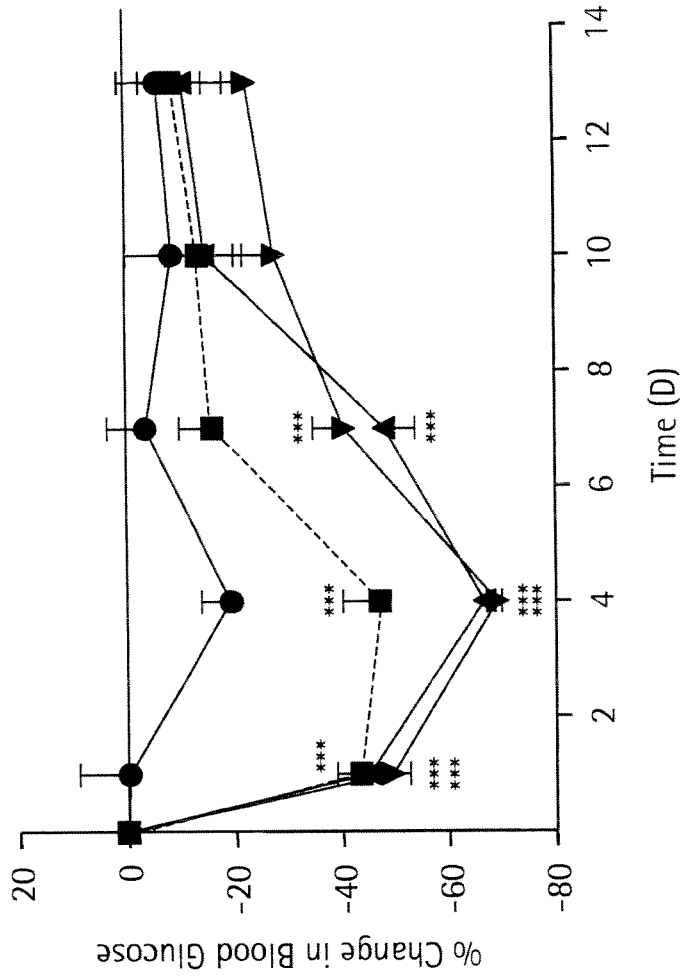

Hydrogel
Fc-(L15)-FGF21(L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21(L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 (L98R, P171G, A180E) 3mg/kg
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
% Change in Blood Glucose
20
0
-20
-40
-60
-80
2
4
6
8
10
12
14
Time (D)

FIG. 62

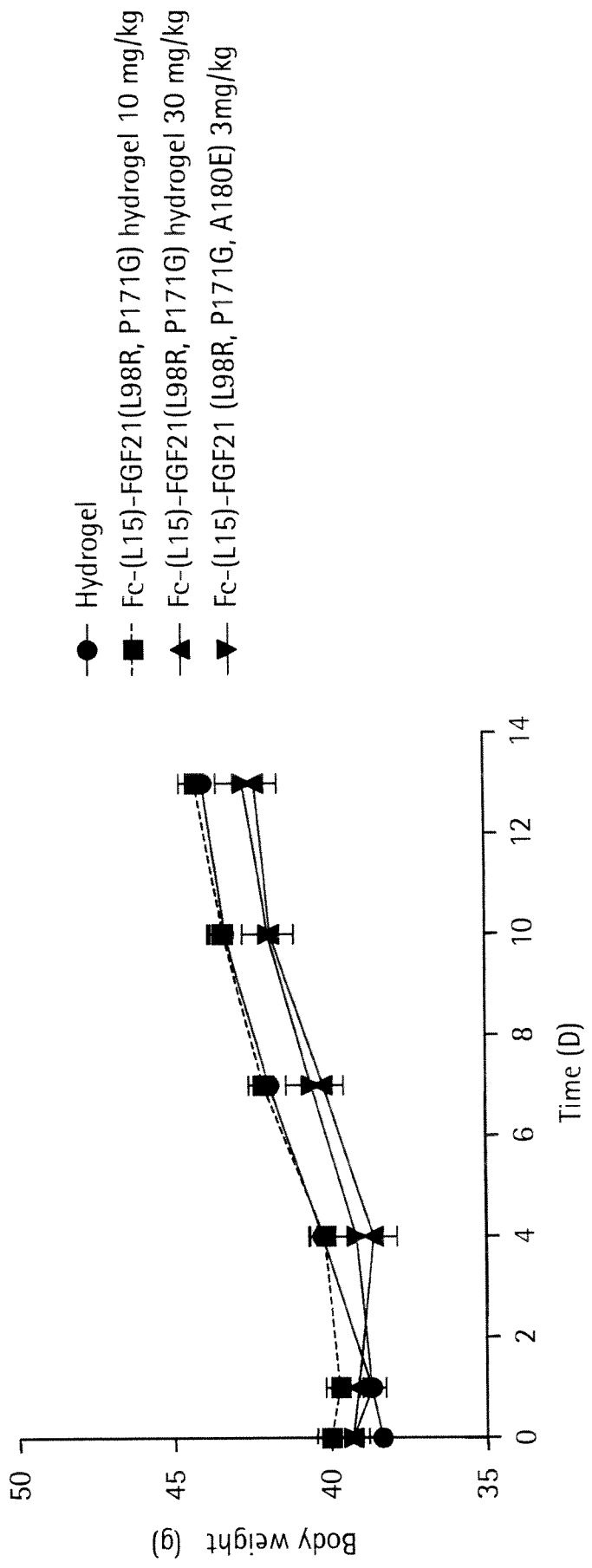

Hydrogel
Fc-(L15)-FGF21(L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21(L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 (L98R, P171G, A180E) 3mg/kg
Body weight (g)
50
45
40
35
0
2
4
6
8
10
12
14
Time (D)

FIG. 63

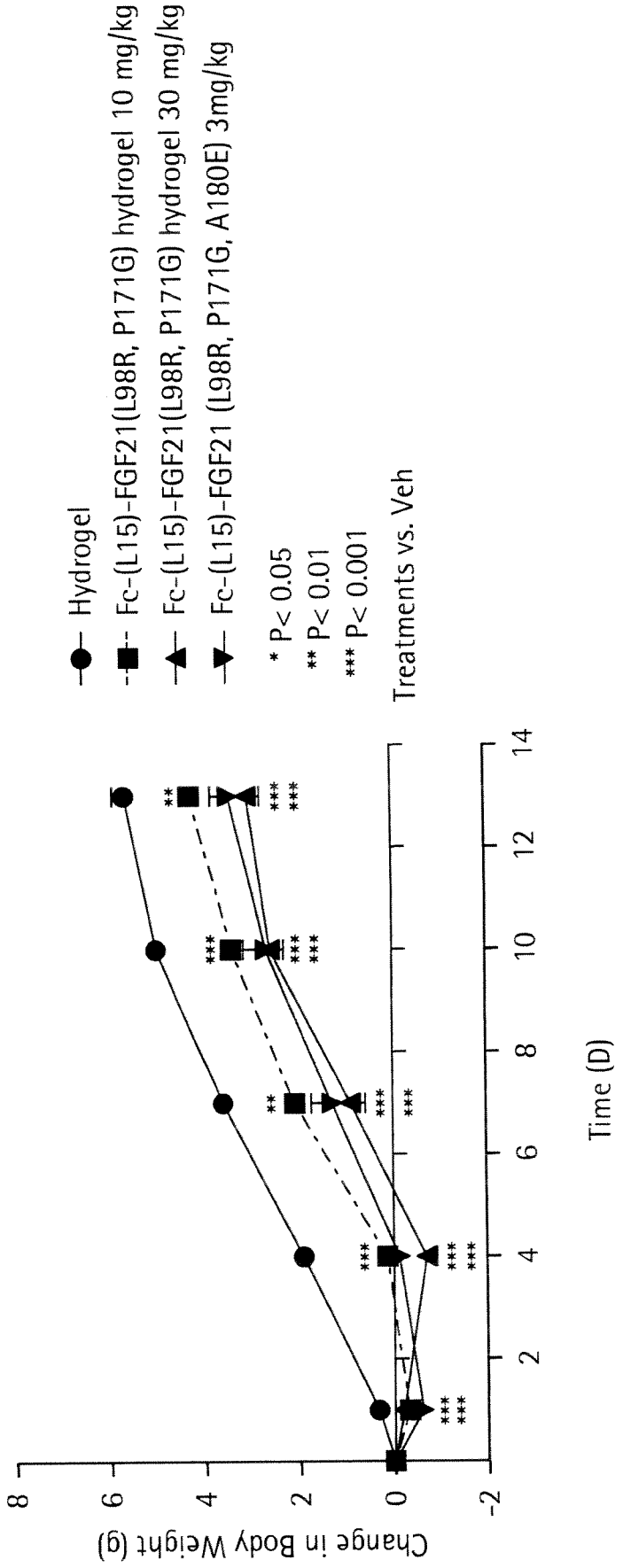

Hydrogel
Fc-(L15)-FGF21(L98R, P171G) hydrogel 10 mg/kg
Fc-(L15)-FGF21(L98R, P171G) hydrogel 30 mg/kg
Fc-(L15)-FGF21 (L98R, P171G, A180E) 3mg/kg
* P< 0.05
** P< 0.01
*** P< 0.001
Treatments vs. Veh
Change in Body Weight (g)
8
6
4
2
0
-2
2
4
6
8
10
12
14
Time (D)

FIG. 64

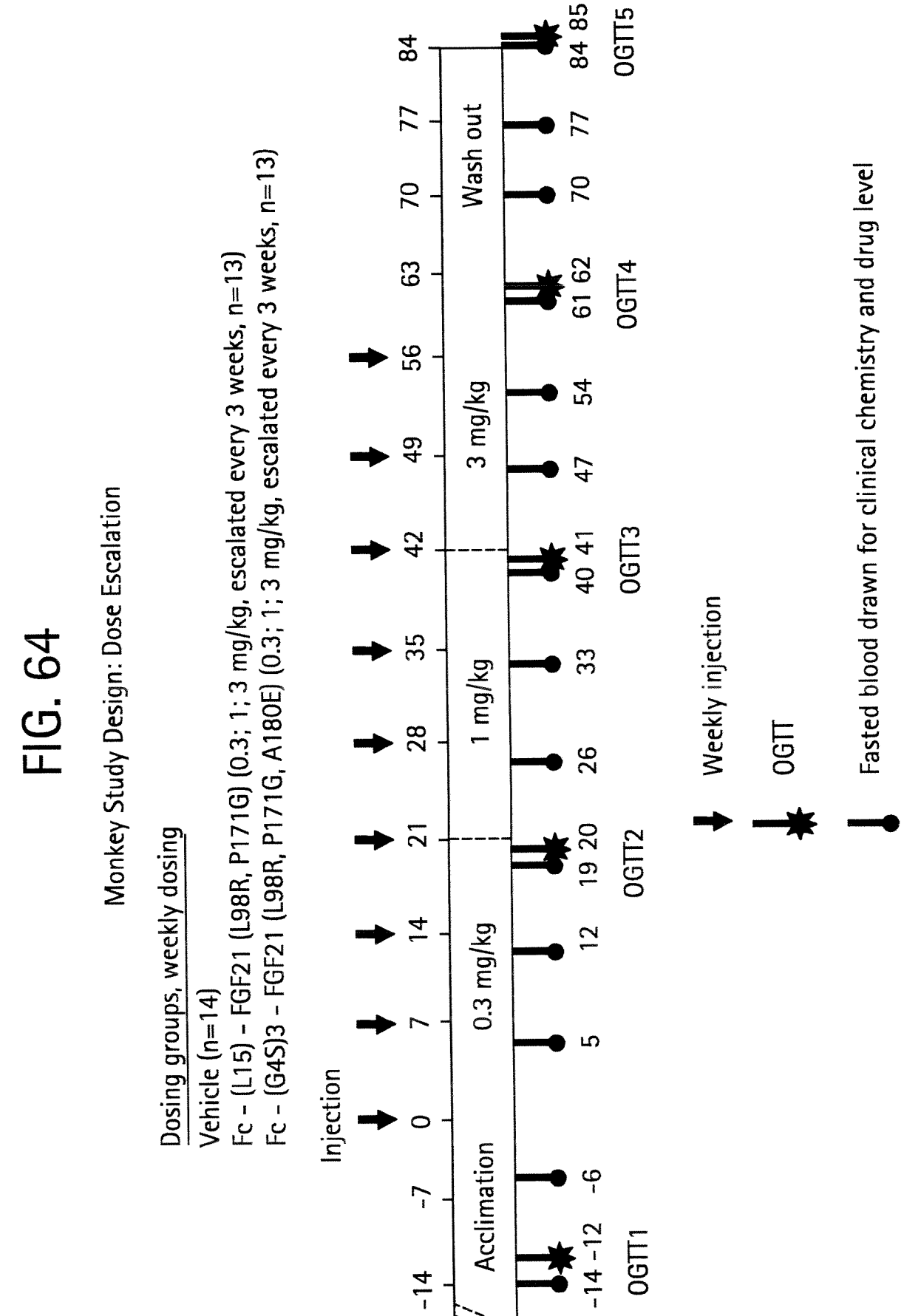

Monkey Study Design: Dose Escalation
Dosing groups, weekly dosing
Vehicle (n=14)
Fc - (L15) - FGF21 (L98R, P171G) (0.3; 1; 3 mg/kg, escalated every 3 weeks, n=13)
Fc - (G4S)3 - FGF21 (L98R, P171G, A180E) (0.3; 1; 3 mg/kg, escalated every 3 weeks, n=13)
Injection
-14 -7 0 7 14 21 28 35 42 49 56 63 70 77 84
Acclimation
0.3 mg/kg
1 mg/kg
3 mg/kg
Wash out
-14 -12 -6 5 12 19 20 26 33 40 41 47 54 61 62 70 77 84 85
OGTT1
OGTT2
OGTT3
OGTT4
OGTT5
Weekly injection
OGTT
Fasted blood drawn for clinical chemistry and drug level

FIG. 65

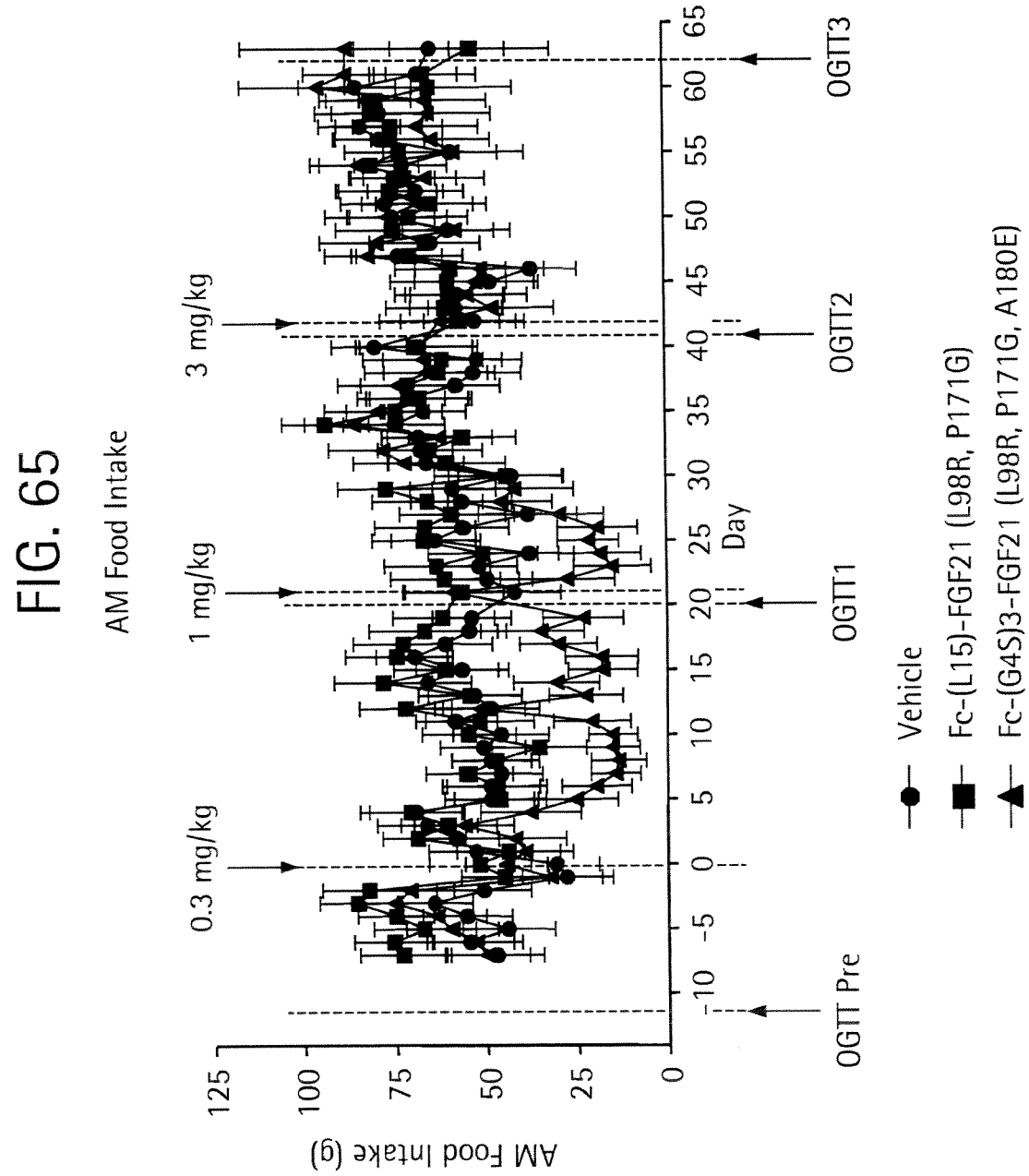

AM Food Intake
AM Food Intake (g)
125
100
75
50
25
0
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Day
0.3 mg/kg
1 mg/kg
3 mg/kg
OGTT Pre
OGTT1
OGTT2
OGTT3
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 66

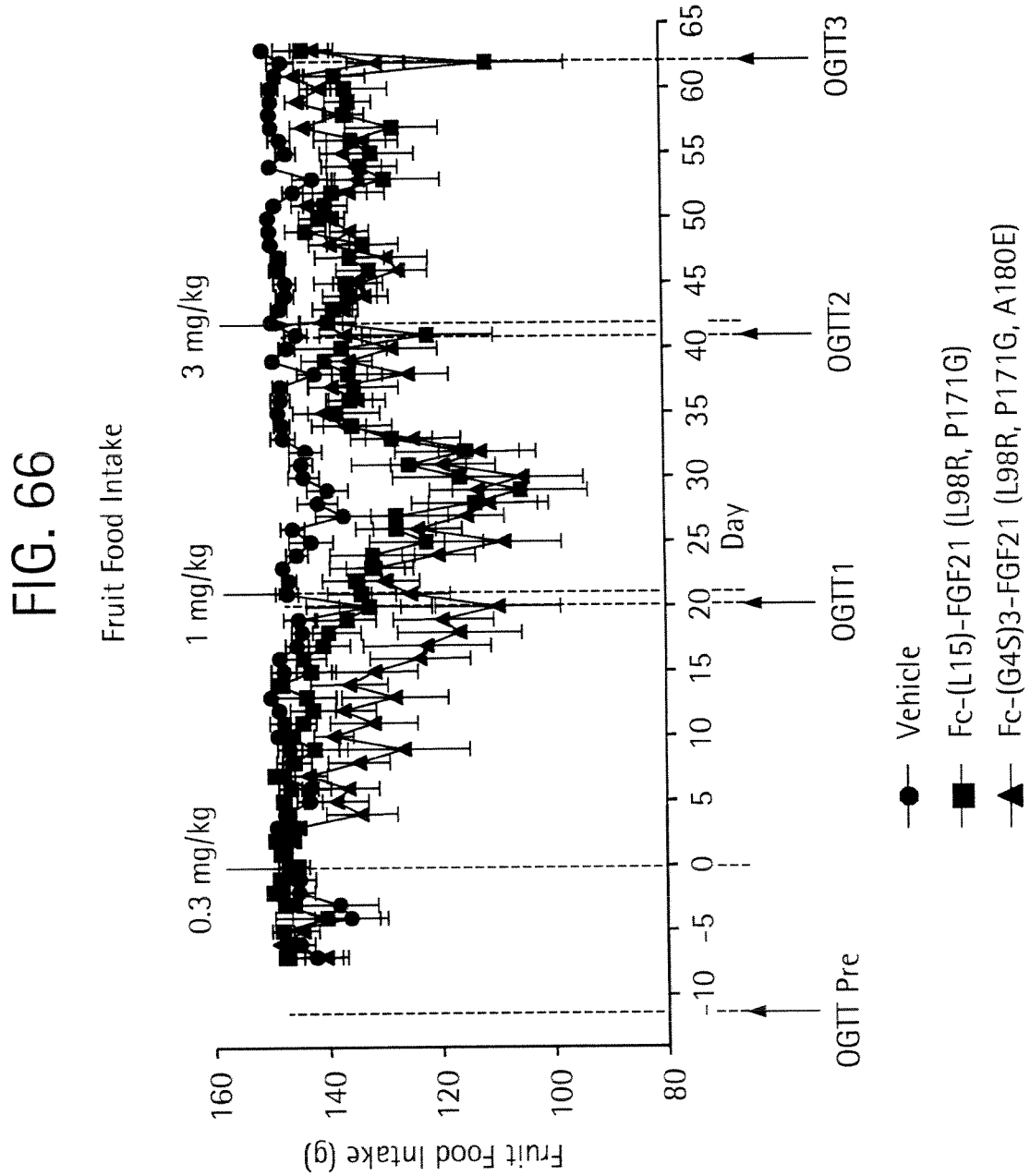

Fruit Food Intake
0.3 mg/kg
1 mg/kg
3 mg/kg
OGTT Pre
OGTT1
OGTT2
OGTT3
Day
Fruit Food Intake (g)
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 67

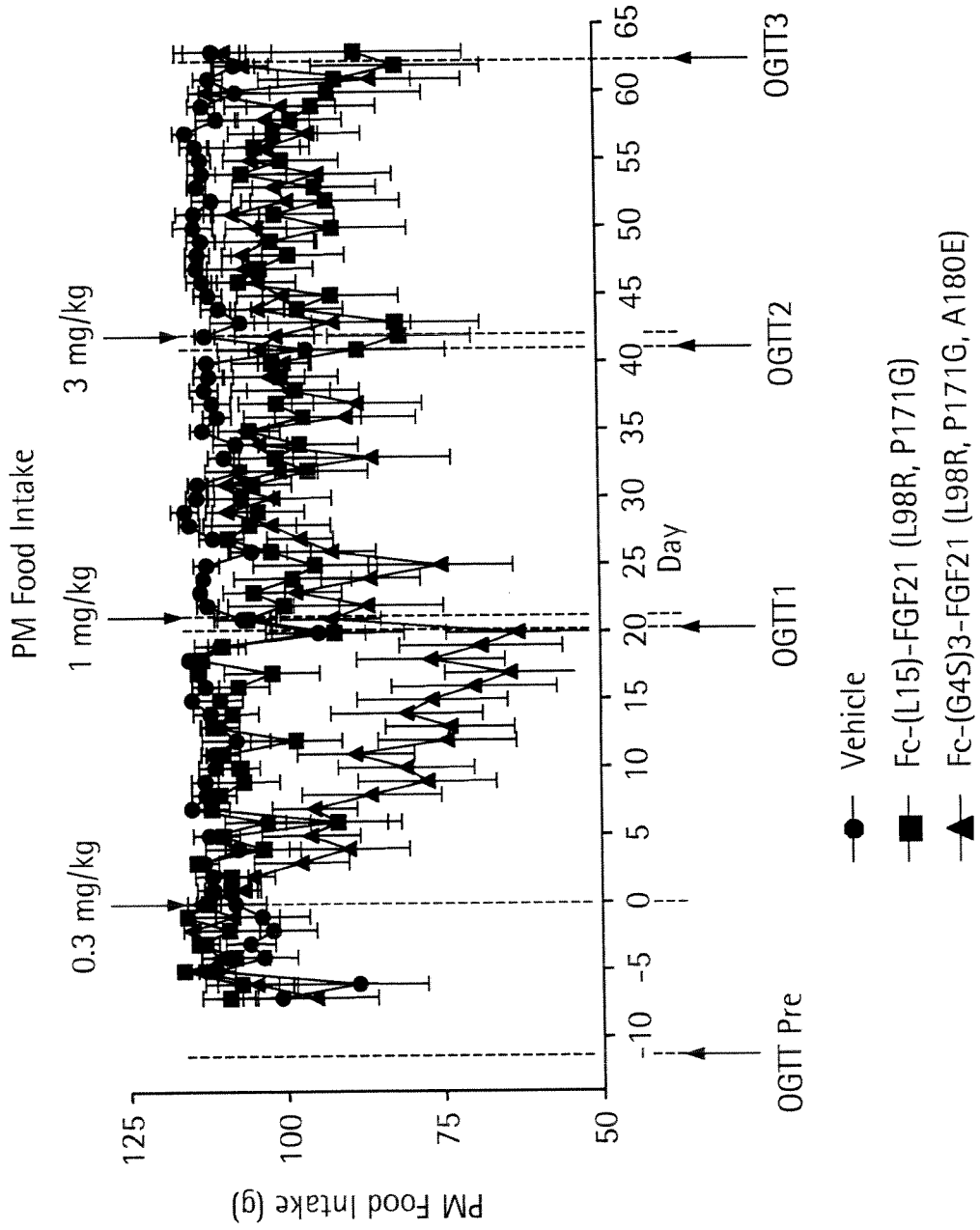

PM Food Intake
0.3 mg/kg
1 mg/kg
3 mg/kg
OGTT Pre
OGTT1
OGTT2
OGTT3
Day
PM Food Intake (g)
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 68

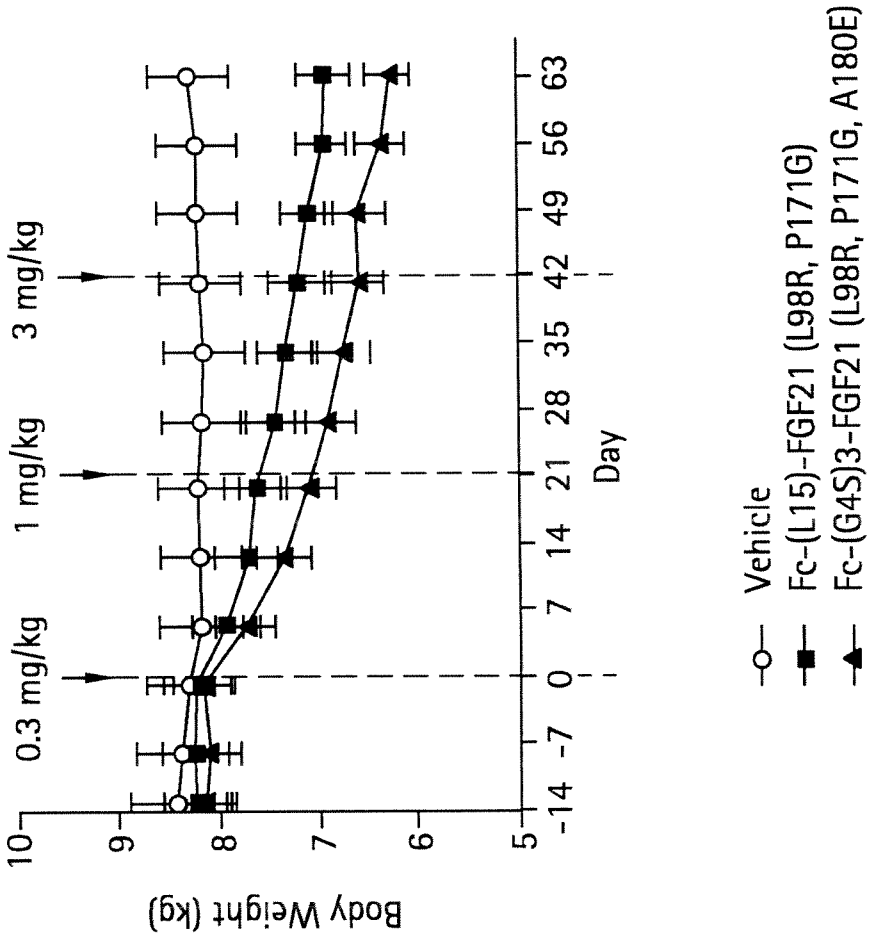

BMt
0.3 mg/kg
1 mg/kg
3 mg/kg
Body Weight (kg)
10
9
8
7
6
5
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 69

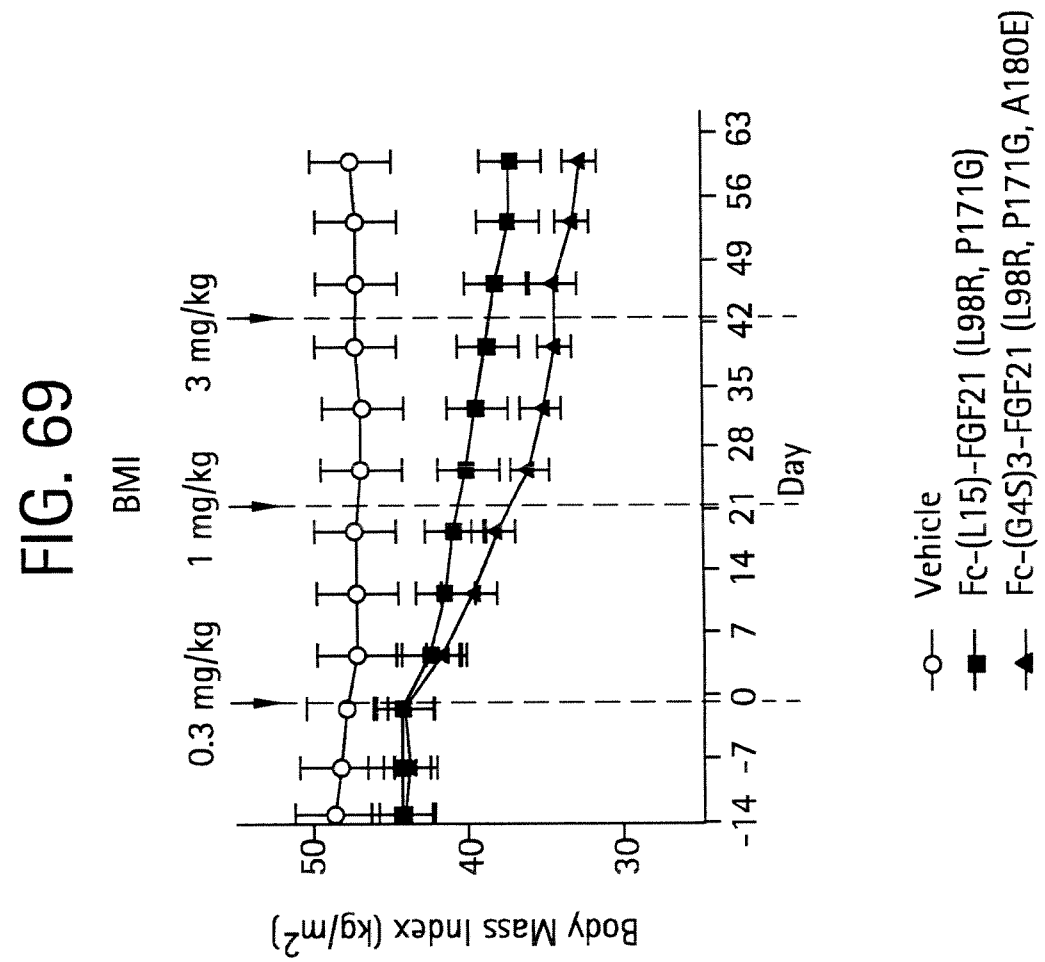

BMI
0.3 mg/kg
1 mg/kg
3 mg/kg
Body Mass Index (kg/m2)
50
40
30
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)

FIG. 70

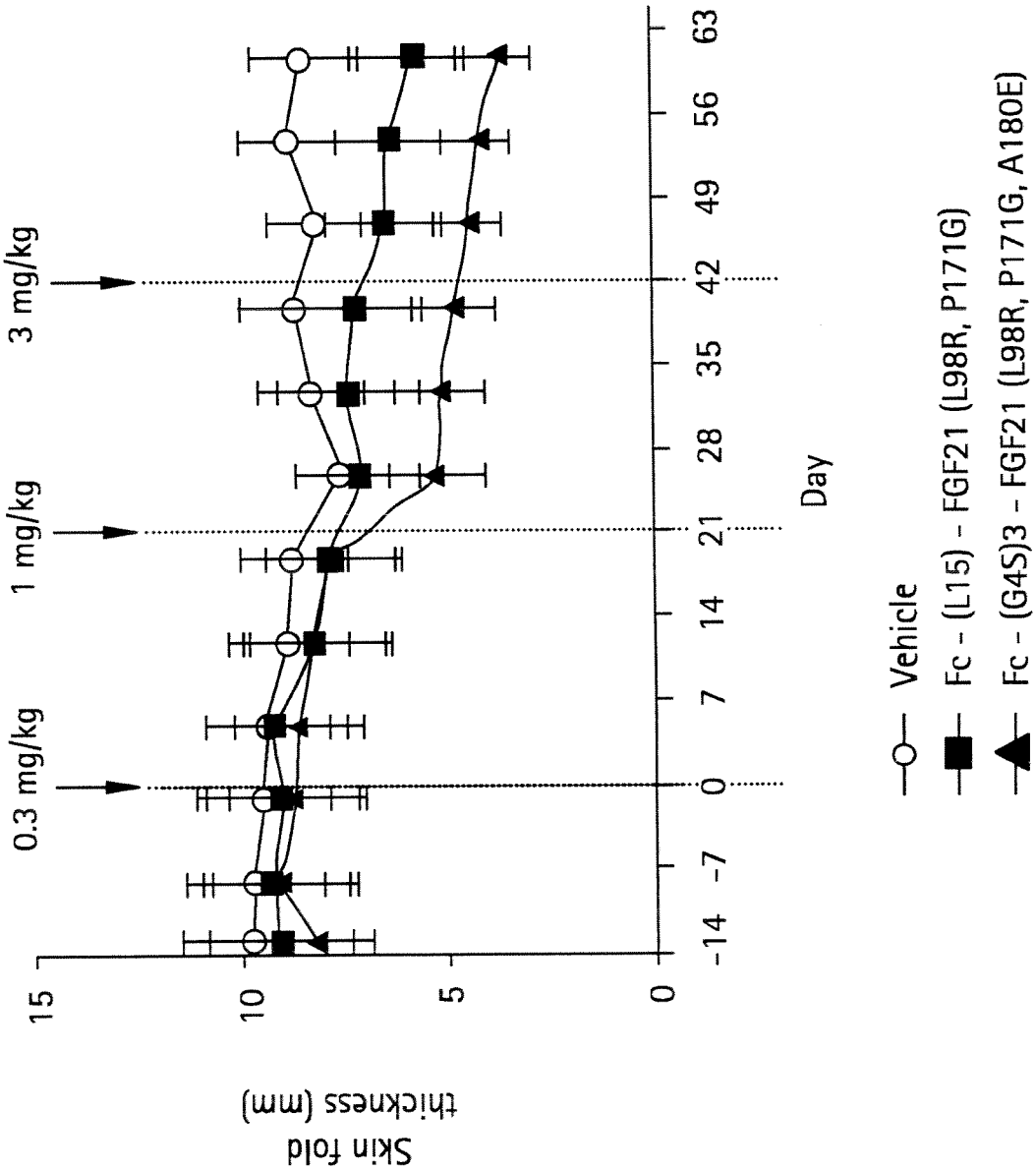

Skin fold thickness (mm)
15
10
5
0
0.3 mg/kg
1 mg/kg
3 mg/kg
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc - (L15) - FGF21 (L98R, P171G)
Fc - (G4S)3 - FGF21 (L98R, P171G, A180E)

FIG. 71

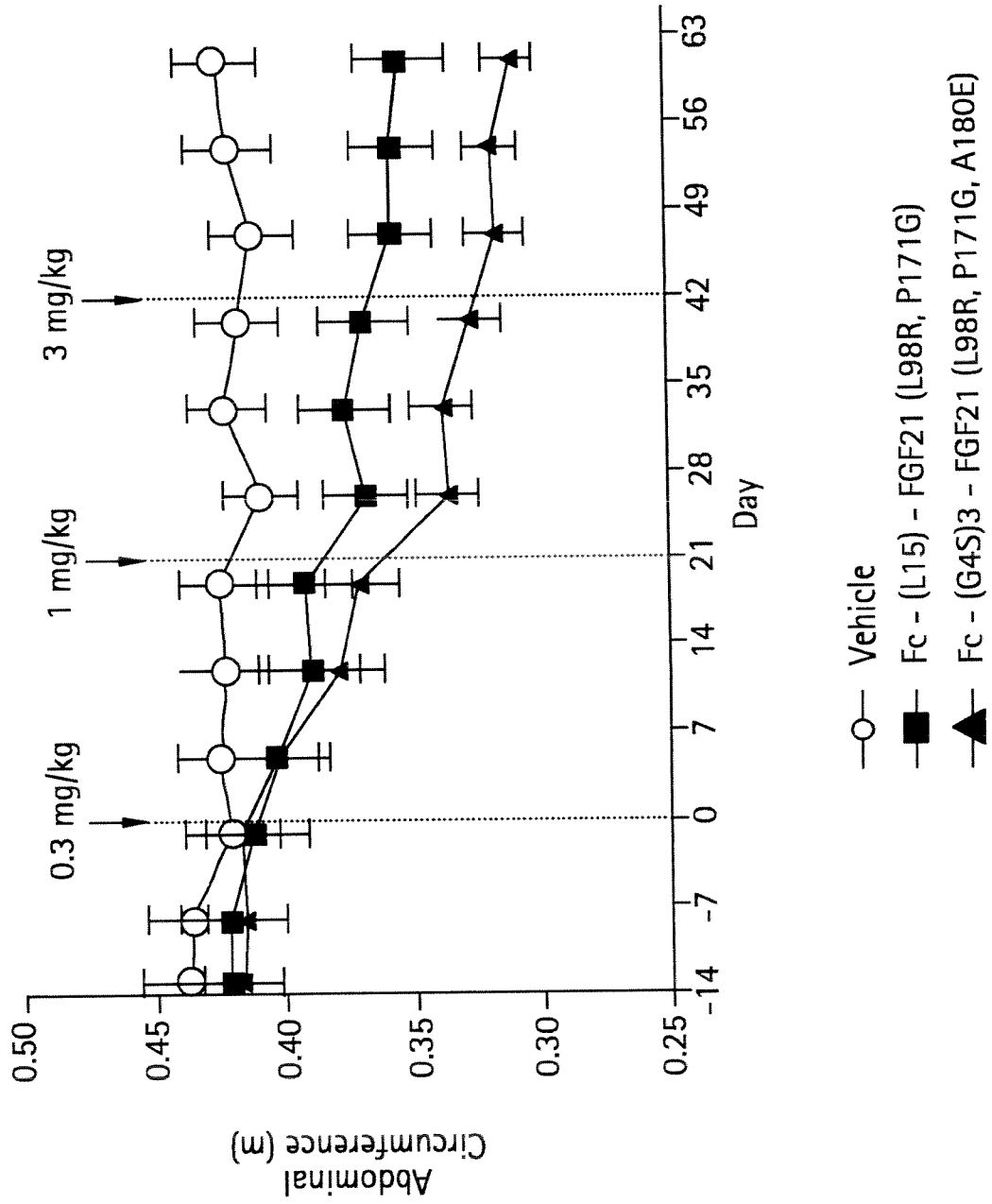

AC
0.3 mg/kg
1 mg/kg
3 mg/kg
Abdominal Circumference (m)
0.50
0.45
0.40
0.35
0.30
0.25
-14
-7
0
7
14
21
28
35
42
49
56
63
Day
Vehicle
Fc - (L15) - FGF21 (L98R, P171G)
Fc - (G4S)3 - FGF21 (L98R, P171G, A180E)

FIG.72

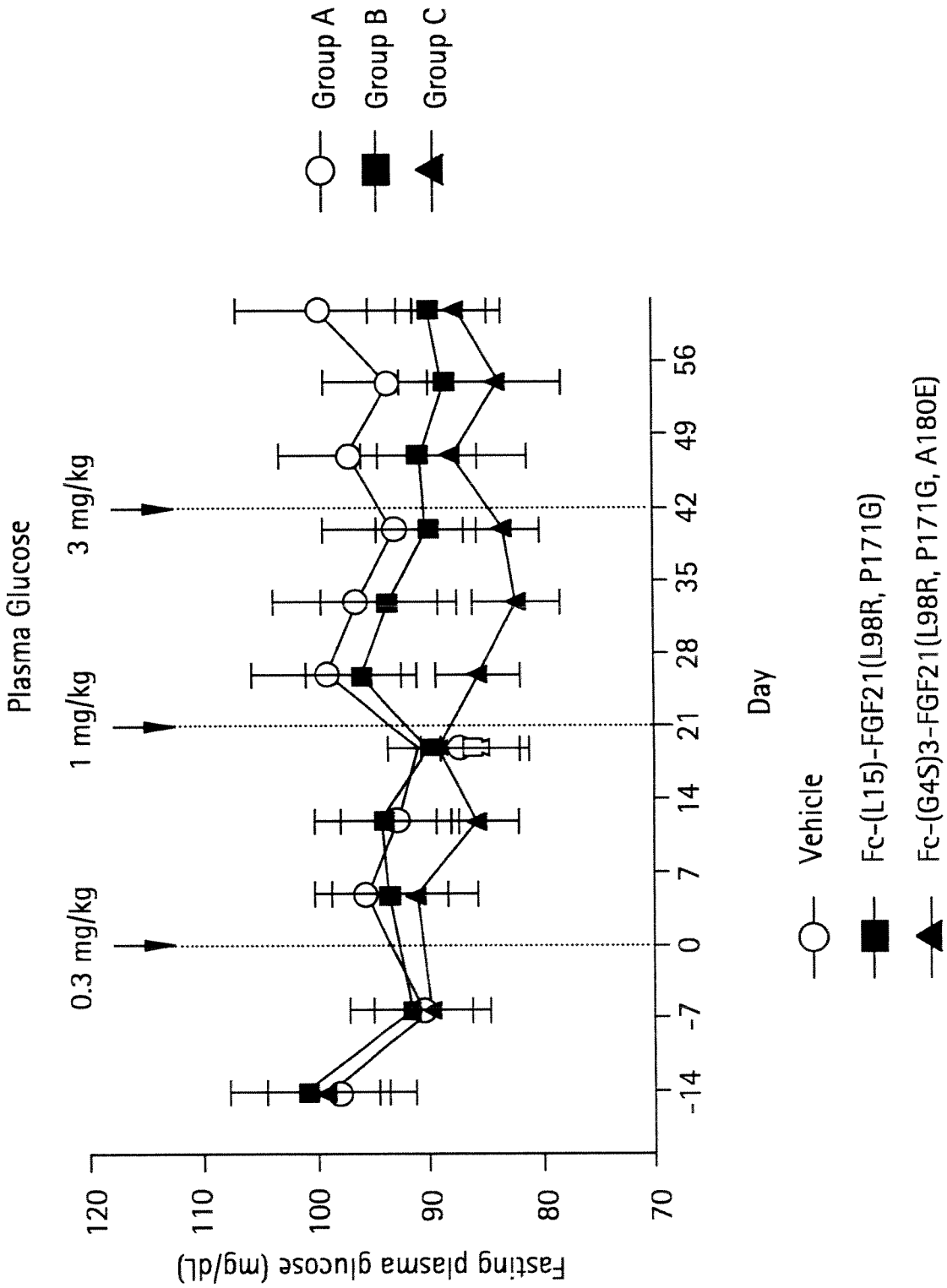

Plasma Glucose
0.3 mg/kg
1 mg/kg
3 mg/kg
Fasting plasma glucose (mg/dL)
120
110
100
90
80
70
-14
-7
0
7
14
21
28
35
42
49
56
Day
Group A
Group B
Group C
Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G, A180E)

FIG. 73

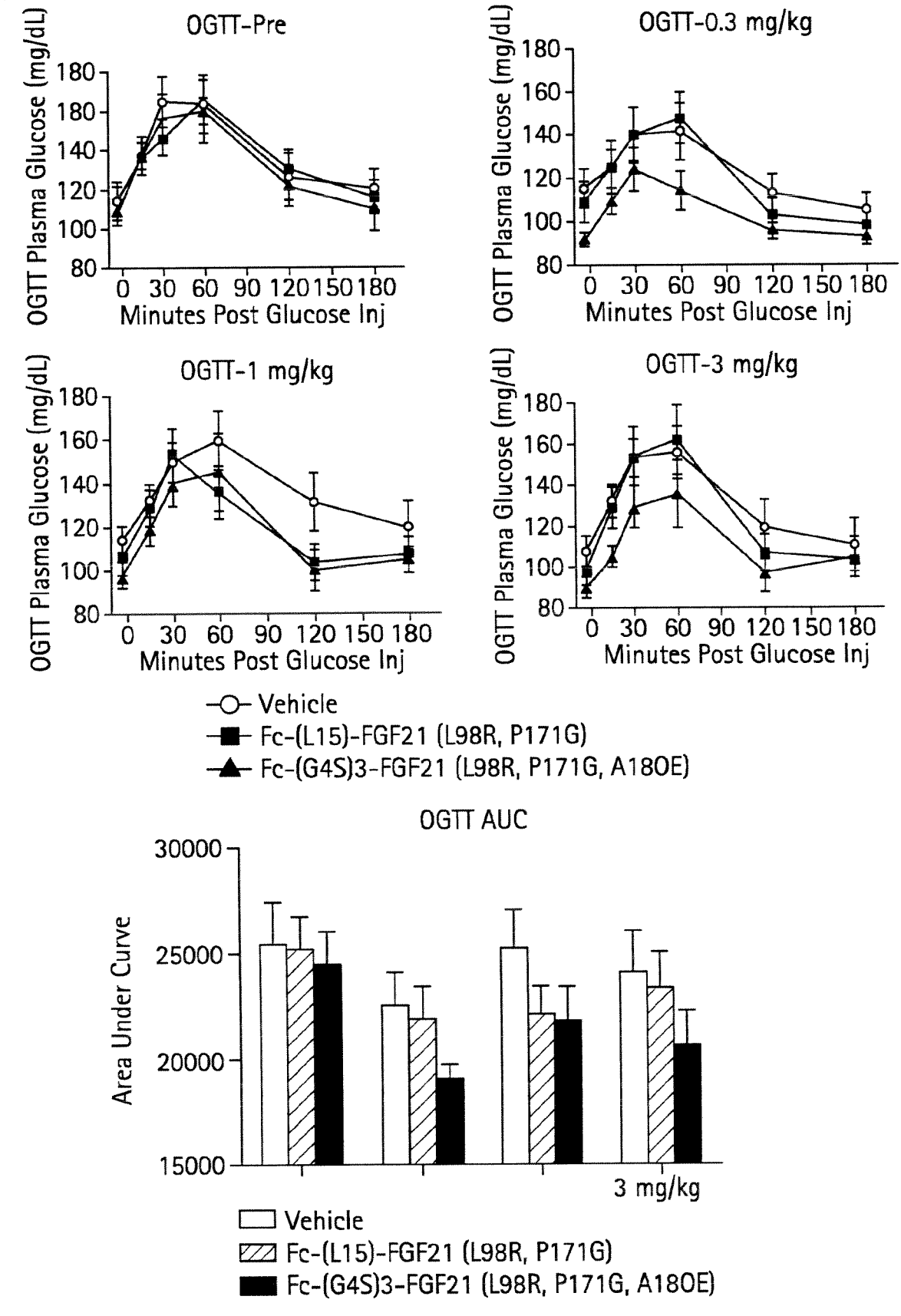

OGTT-Pre
OGTT-0.3 mg/kg
OGTT-1 mg/kg
OGTT-3 mg/kg
OGTT Plasma Glucose (mg/dL)
Minutes Post Glucose Inj
0 30 60 90 120 150 180
80 100 120 140 160 180
Vehicle
Fc-(L15)-FGF21 (L98R, P171G)
Fc-(G4S)3-FGF21 (L98R, P171G, A180E)
OGTT AUC
Area Under Curve
15000 20000 25000 30000
3 mg/kg
Vehicle
Fc-(L15)-FGF21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

FIG. 74

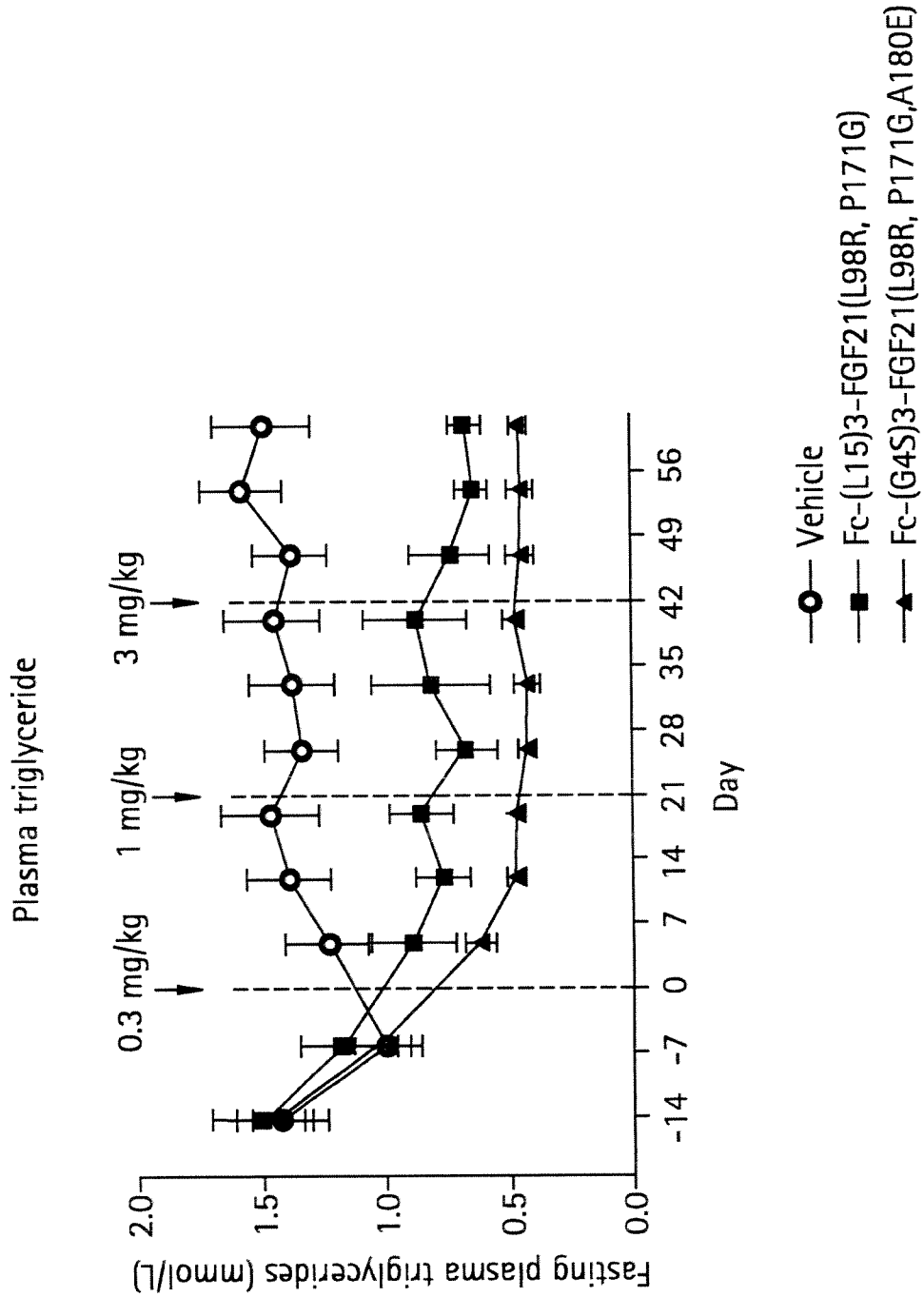

Plasma triglyceride
0.3 mg/kg
1 mg/kg
3 mg/kg
Fasting plasma triglycerides (mmol/L)
2.0
1.5
1.0
0.5
0.0
-14
-7
0
7
14
21
28
35
42
49
56
Day
Vehicle
Fc-(L15)3-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G,A180E)

FIG. 75

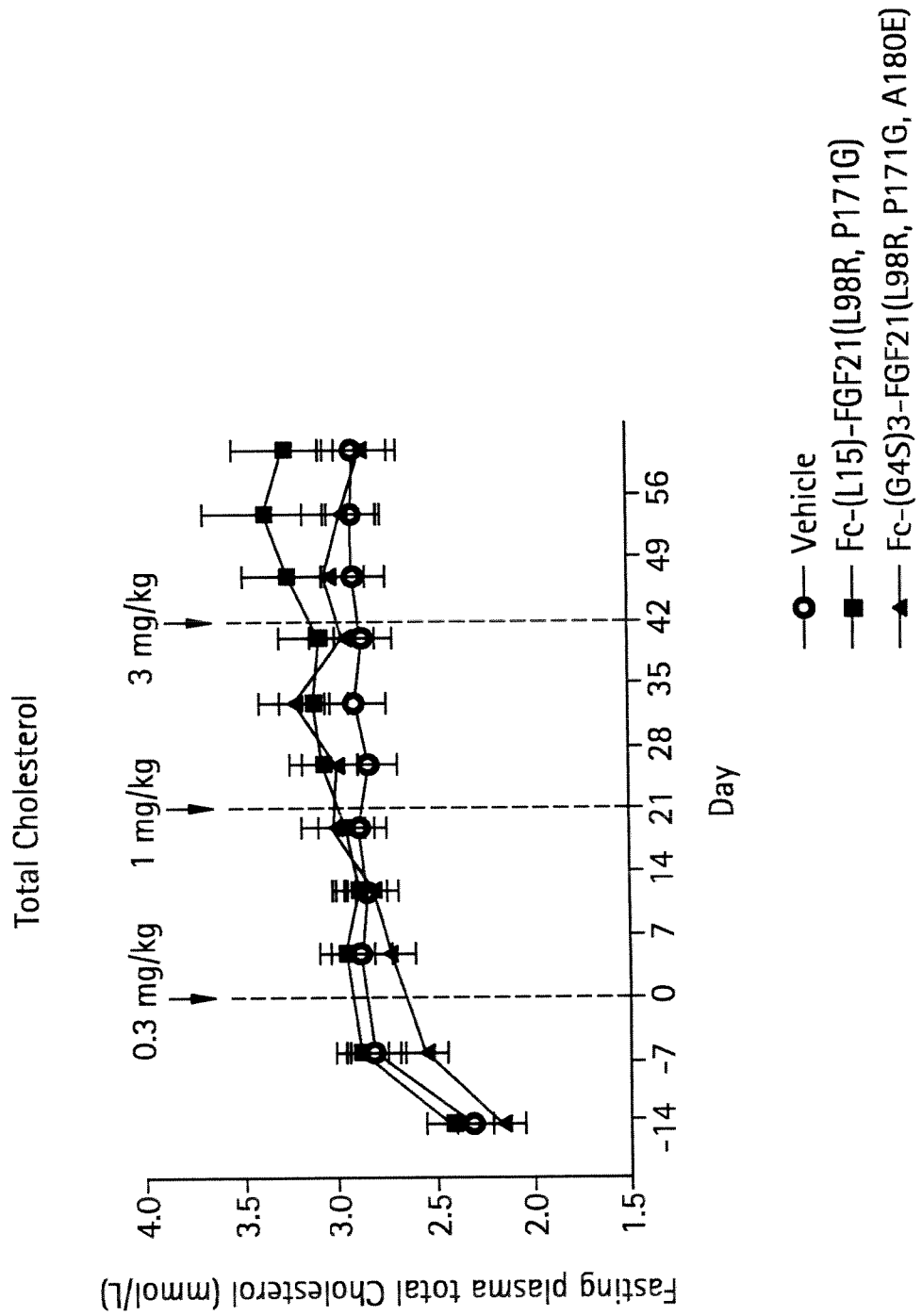

Total Cholesterol
0.3 mg/kg
1 mg/kg
3 mg/kg
Day
-14
-7
0
7
14
21
28
35
42
49
56
4.0
3.5
3.0
2.5
2.0
1.5
Fasting plasma total Cholesterol (mmol/L)
Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G, A180E)

FIG. 76

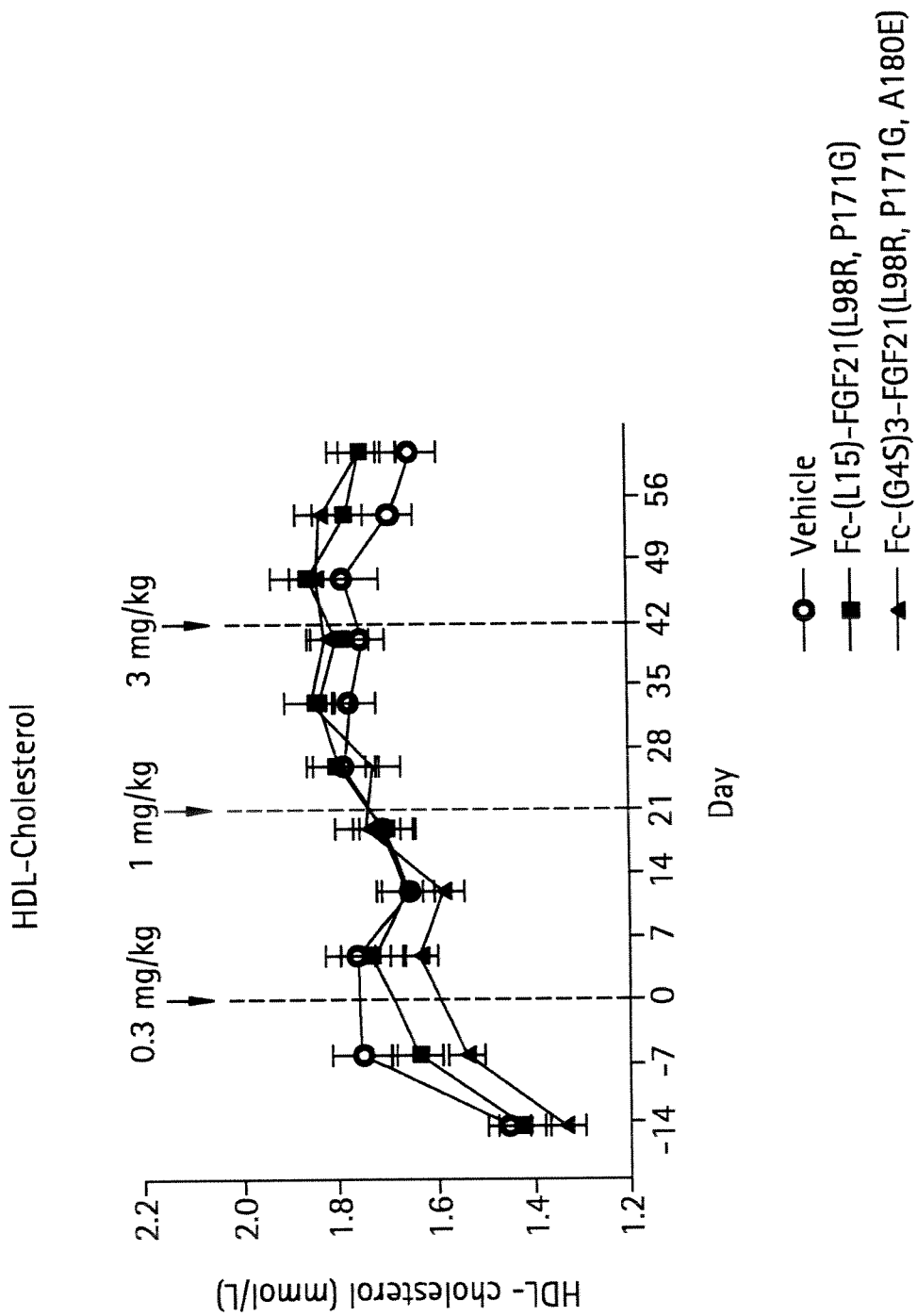

HDL-Cholesterol
0.3 mg/kg
1 mg/kg
3 mg/kg
HDL- cholesterol (mmol/L)
2.2
2.0
1.8
1.6
1.4
1.2
-14
-7
0
7
14
21
28
35
42
49
56
Day
Vehicle
Fc-(L15)-FGF21(L98R, P171G)
Fc-(G4S)3-FGF21(L98R, P171G, A180E)

FIG. 77

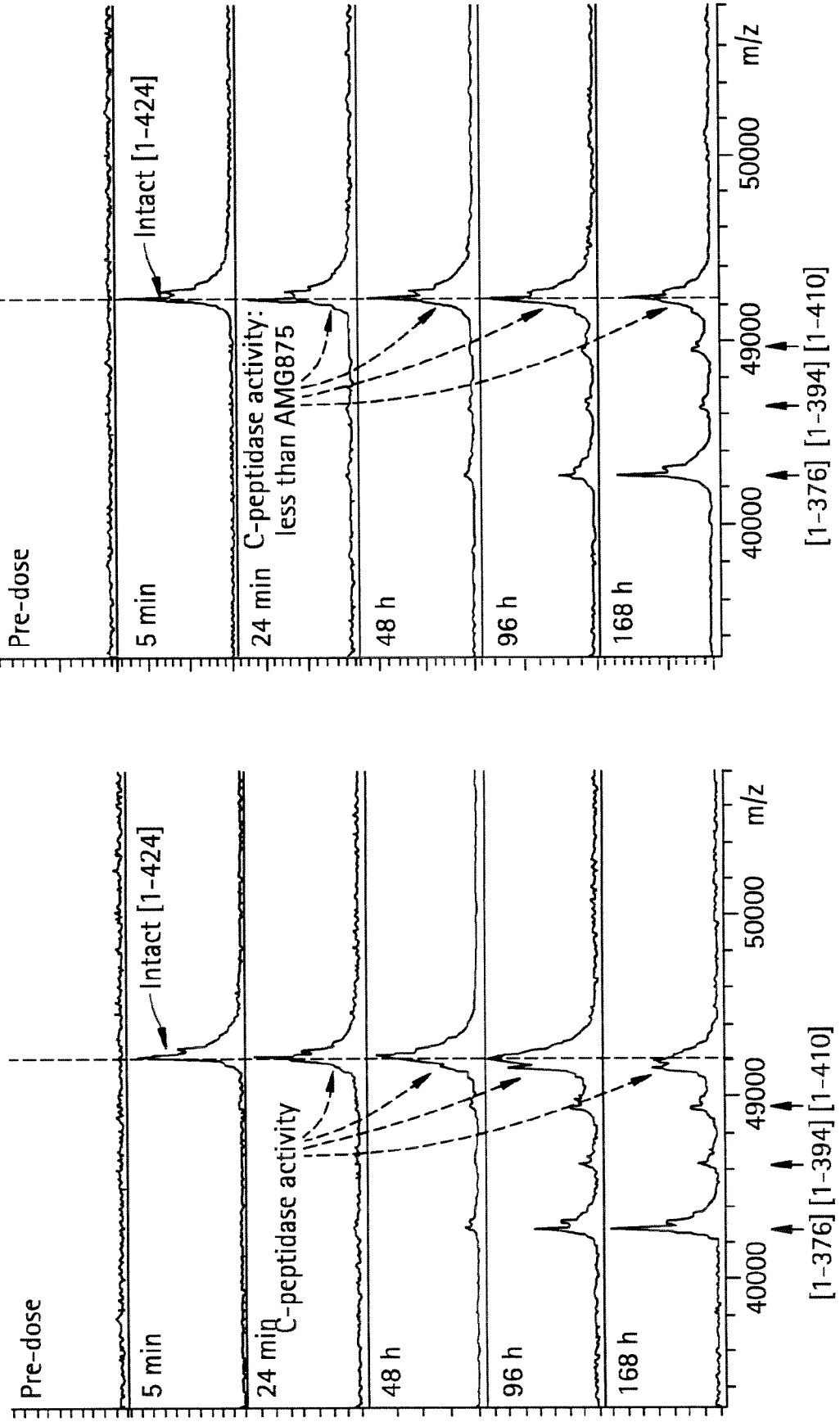

Fc-(L15)-FGF21 (L98R, P171G)
Pre-dose
5 min
24 min
C-peptidase activity
48 h
96 h
168 h
Intact [1-424]
40000
49000
50000
m/z
[1-376] [1-394] [1-410]
Fc-(L15)-FGF21 (L98R, P171G, A180E)
Pre-dose
5 min
24 min
C-peptidase activity:
less than AMG875
48 h
96 h
168 h
Intact [1-424]
40000
49000
50000
m/z
[1-376]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

FIG. 78

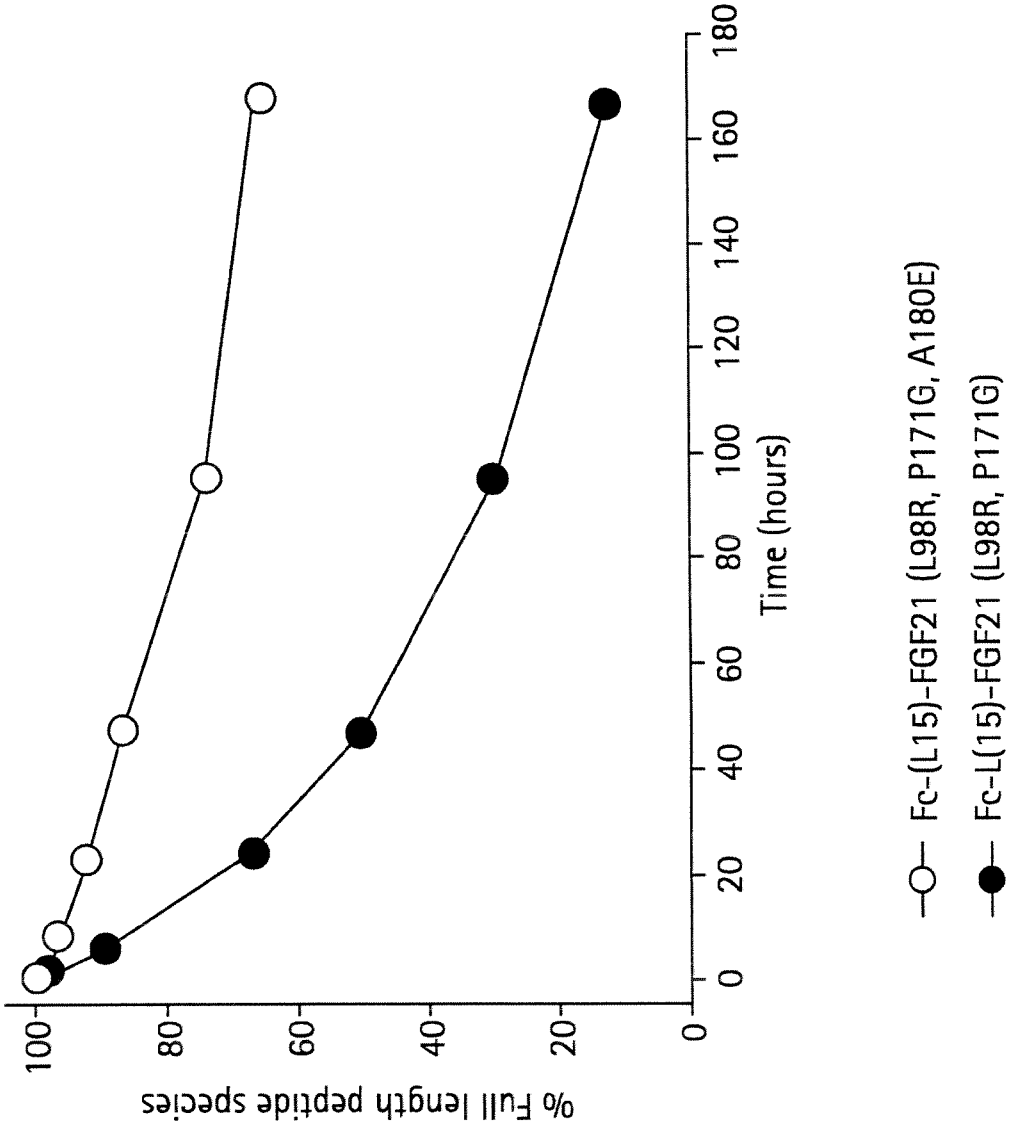

% Full length peptide species
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
Time (hours)
Fc-(L15)-FGF21 (L98R, P171G, A180E)
Fc-L(15)-FGF21 (L98R, P171G)

FIG. 79

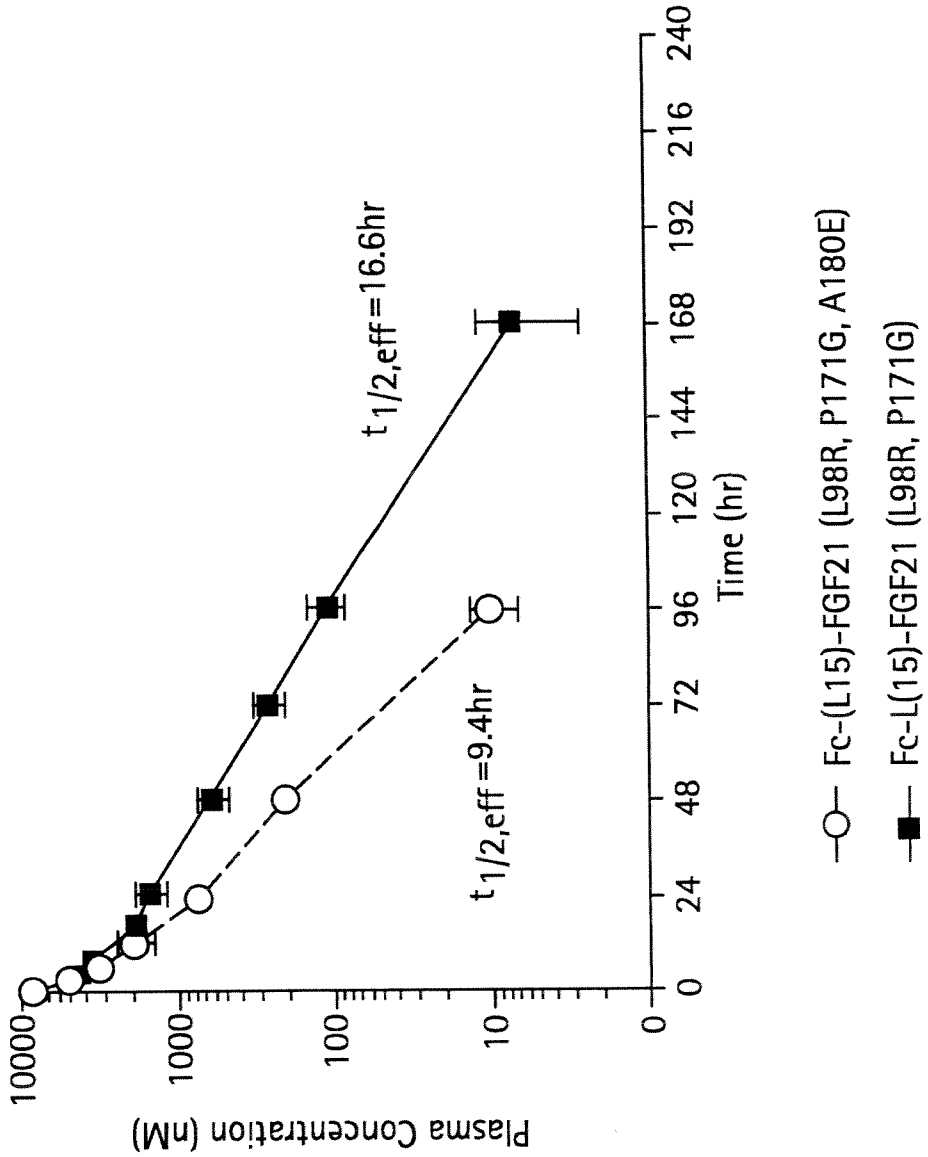
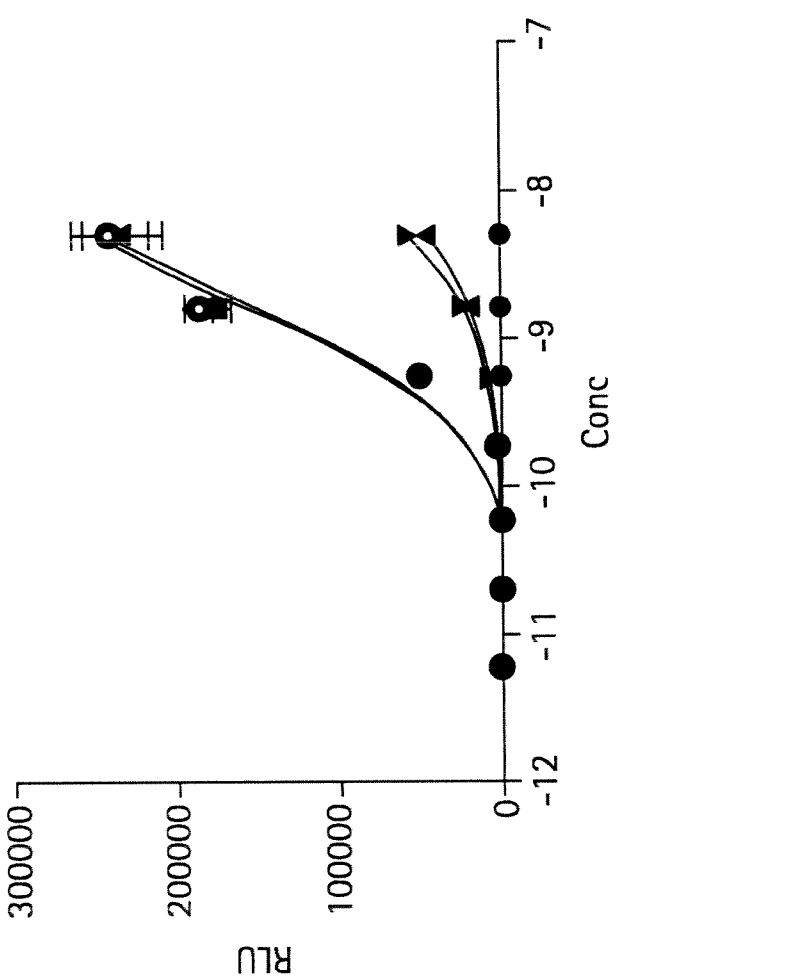

Plasma Concentration (nM)
10000
1000
100
10
0
0
24
48
72
96
120
144
168
192
216
240
Time (hr)
t 1/2,eff =16.6hr
t 1/2,eff =9.4hr
Fc-(L15)-FGF21 (L98R, P171G, A180E)
Fc-L(15)-FGF21 (L98R, P171G)

FIG. 80


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