FGF19

RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEI
KAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKH
RLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDP
FGLVTGLEAVRSPSFEK

FGF21

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQKALK
PGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLP
GNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYA
S

M5

HPIPDSSPLLQFGGQVRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVA
LRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVS
LSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVT
GLEAVRSPSFEK

M71

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALK
PGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHSLPLHLP
GNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYA
S

M72

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALK
PGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLP
GNKSPHRDPAPRGPARFLPLPGLPPAPPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYA
S

M73

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKALK
PGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLP
GNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVVQDELQGVGG
EGCHMHPENCKTLLTDIDRTHTEKPVWDGITGE

M1

RPLAFSDASPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEI
KAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKH
RLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDP
FGLVTGLEAVRSPSFEK

FIG. 1

M2

RPLAFSDSSPLVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEI
KAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKH
RLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDP
FGLVTGLEAVRSPSFEK

M69

RDSSPLVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVA
LRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVS
LSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVT
GLEAVRSPSFEK

M3

RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEI
KAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEILEDGYNVYRSEKHR
LPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPF
GLVTGLEAVRSPSFEK

M48

RDSSPLLQFGGQVRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRT
VAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSS
AKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGL
EAVRSPSFEK

M49

RPLAFSDSSPLLQFGGQVRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKA
VALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLP
VSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGL
VTGLEAVRSPSFEK

M50

RHPIPDSSPLLQFGDQVRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAV
ALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEILEDGYNVYRSEKHRLPV
SLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLV
TGLEAVRSPSFEK

FIG. 1 (CONTINUED)

M51

RHPIPDSSPLLQFGGNVRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAV
ALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPV
SLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLV
TGLEAVRSPSFEK

M52

RDSSPLLQWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRT
VAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSS
AKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGL
EAVRSPSFEK

M53

MDSSPLLQWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAVALRT
VAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPVSLSS
AKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLVTGL
EAVRSPSFEK

M70

MRDSSPLVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSAHSLLEIKAV
ALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRPDGYNVYRSEKHRLPV
SLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDPFGLV
TGLEAVRSPSFEK

FIG. 1 (CONTINUED)

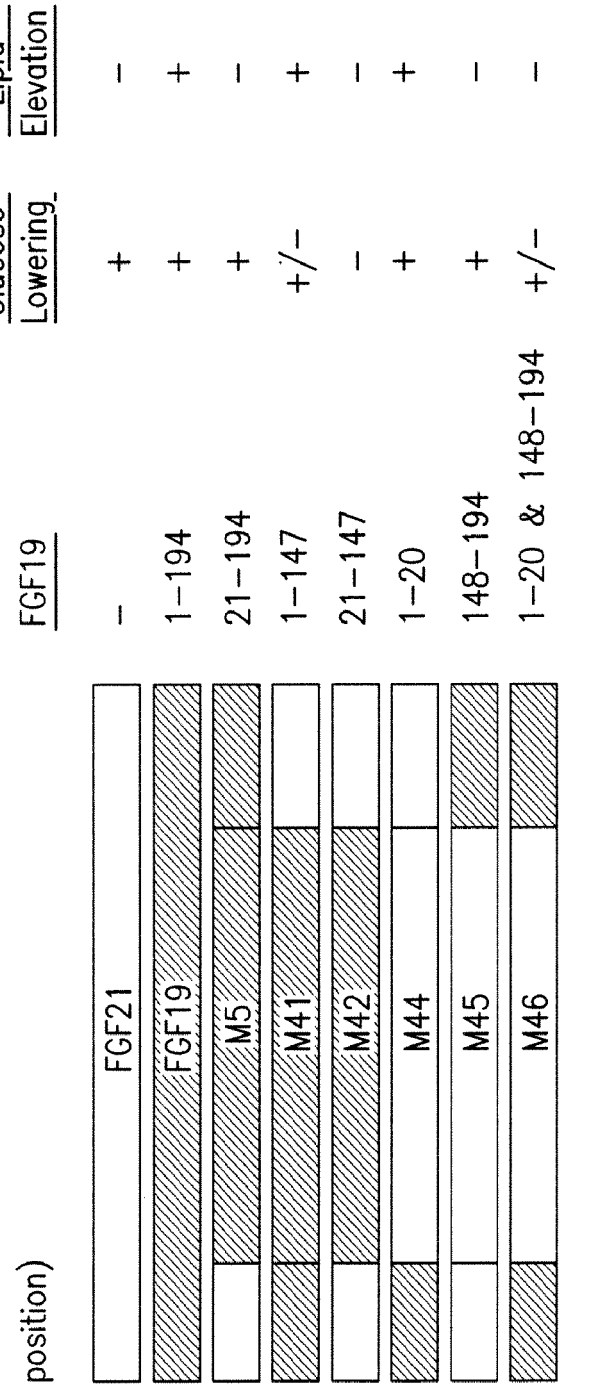

FGF21 (amino acid position)
FGF19
Glucose Lowering
Lipid Elevation
1–181
FGF21
–
+
–
–
FGF19
1–194
+
+
1–16
M5
21–194
+
–
147–181
M41
1–147
+/–
+
1–16 & 147–181
M42
21–147
–
–
17–181
M44
1–20
+
+
1–146
M45
148–194
+
–
17–146
M46
1–20 & 148–194
+/–
–

FIG. 2

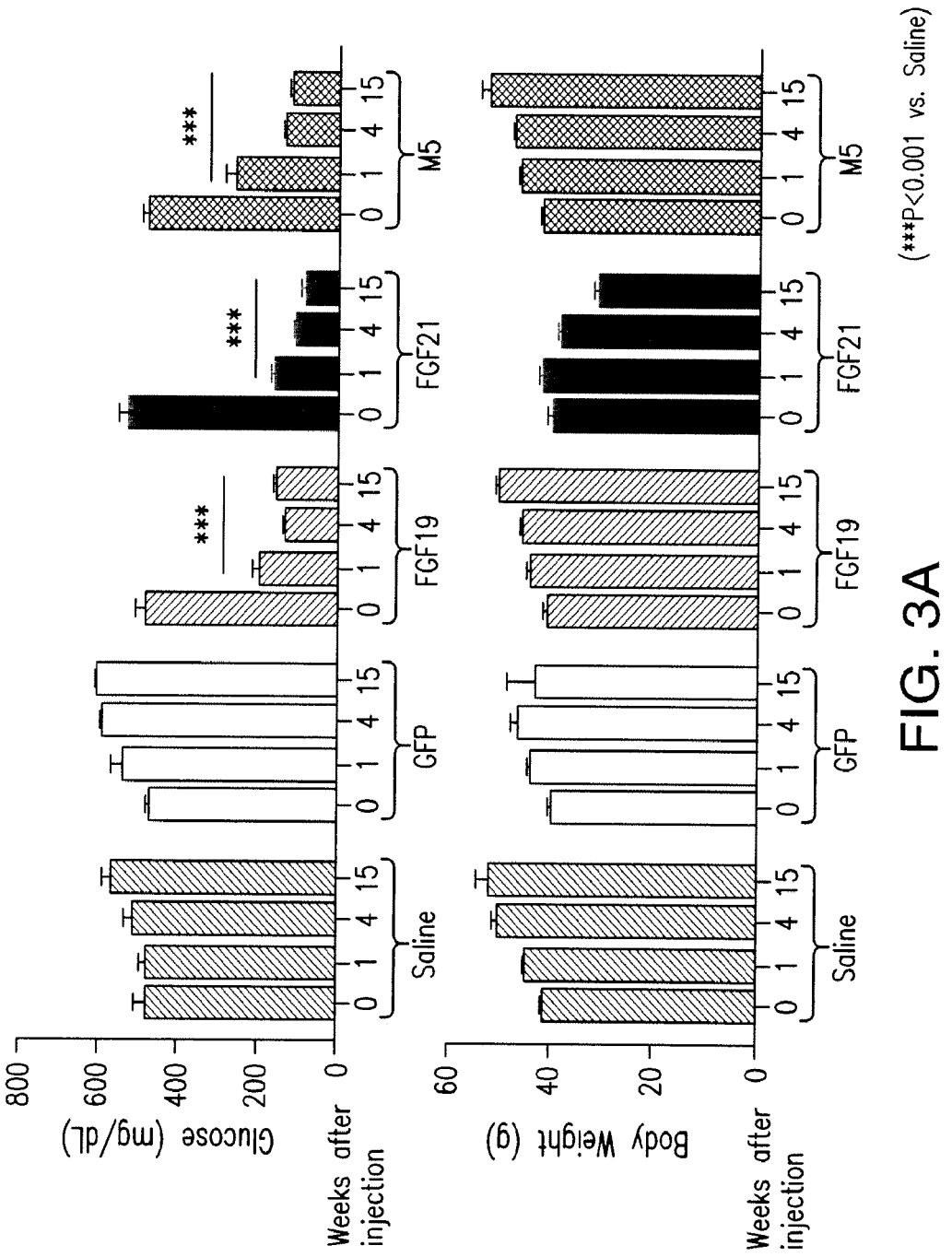

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 1 4 15
Saline
GFP
FGF19
FGF21
M5
***
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3A

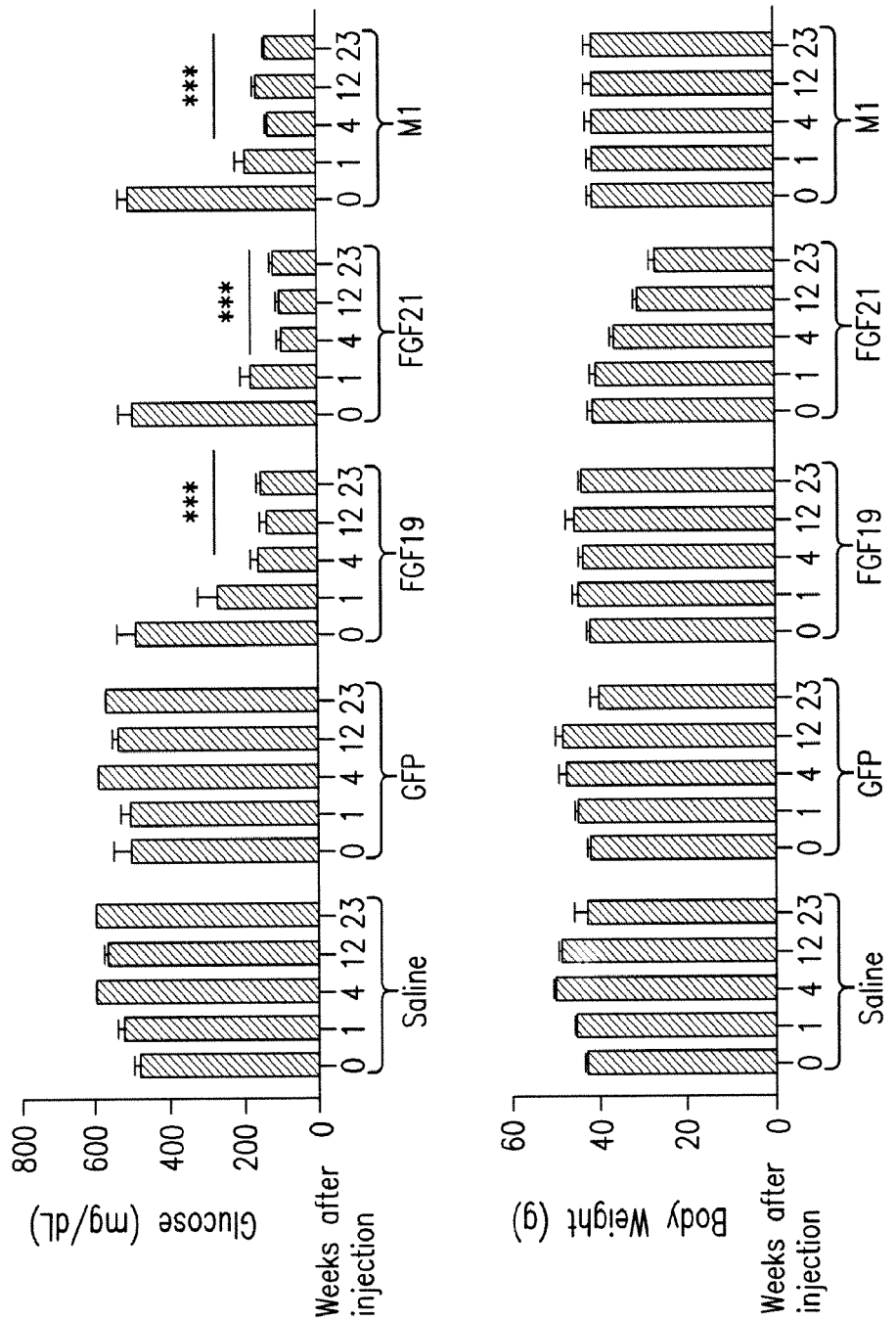

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 1 4 12 23
Saline
GFP
FGF19
FGF21
M1
***
Body Weight (g)
60
40
20
0

FIG. 3B

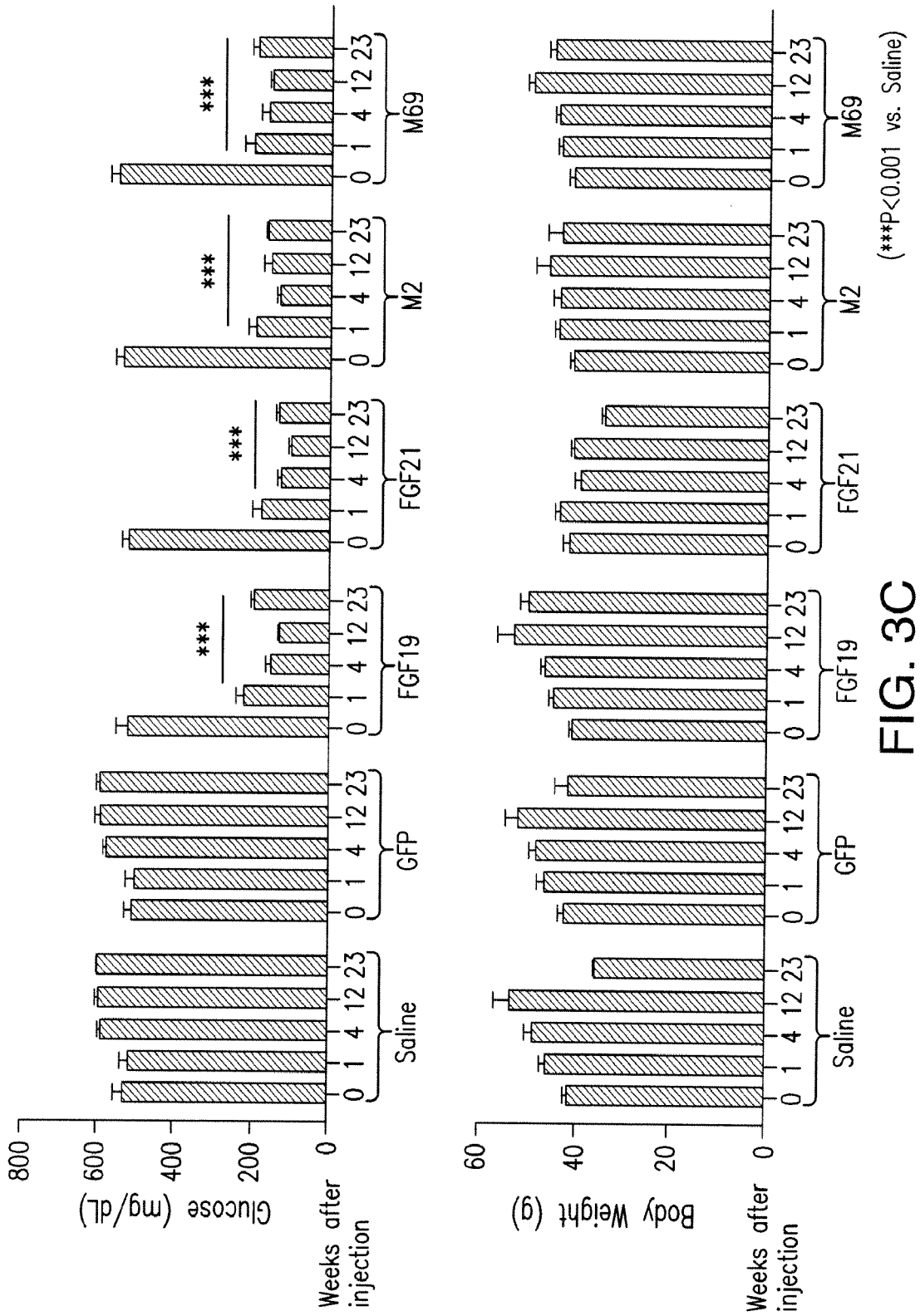

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 1 4 12 23
Saline
GFP
FGF19
FGF21
M2
M69
***
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3C

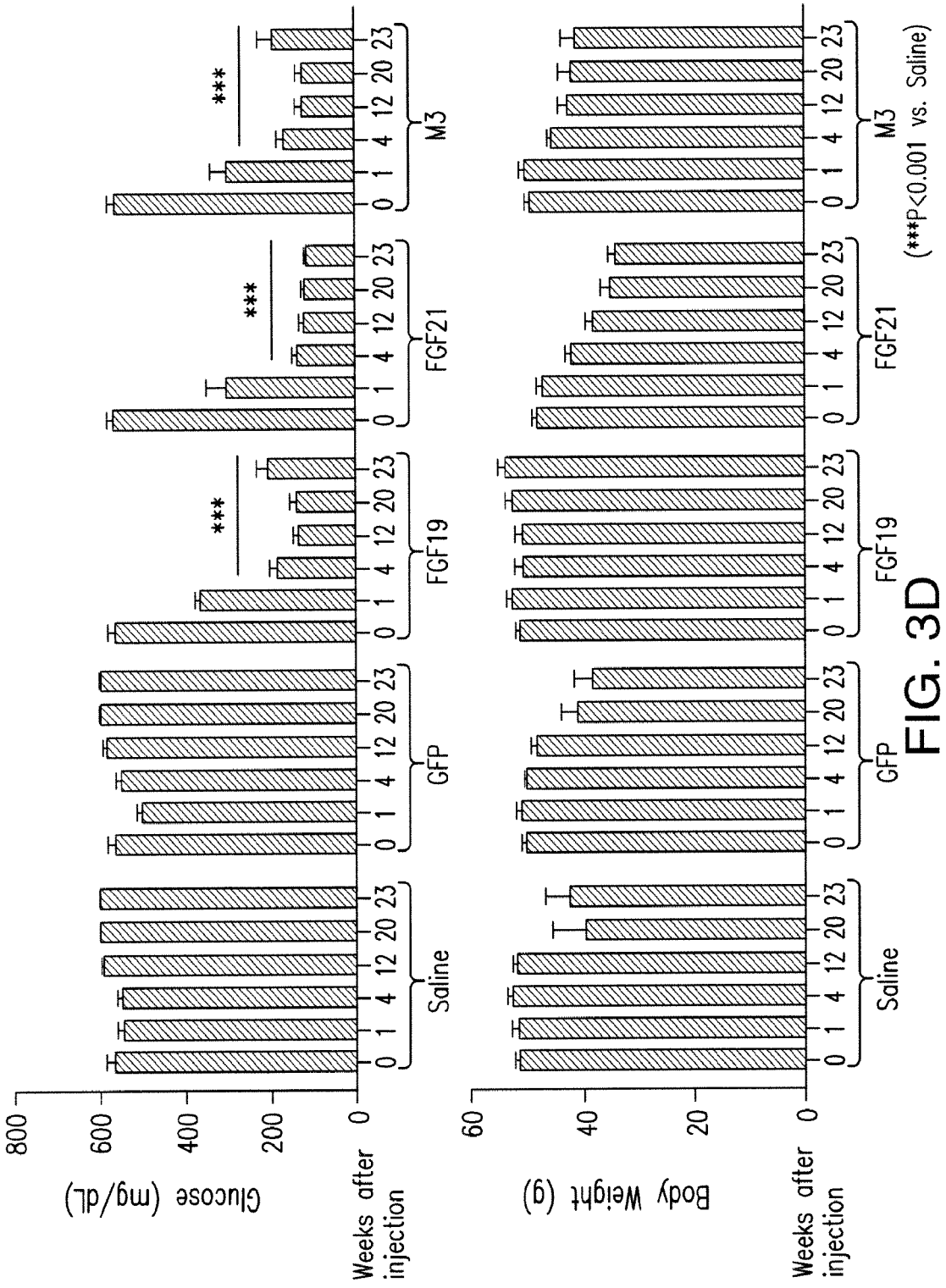

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 1 4 12 20 23
Saline
GFP
FGF19
FGF21
M3
***
***
***
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3D

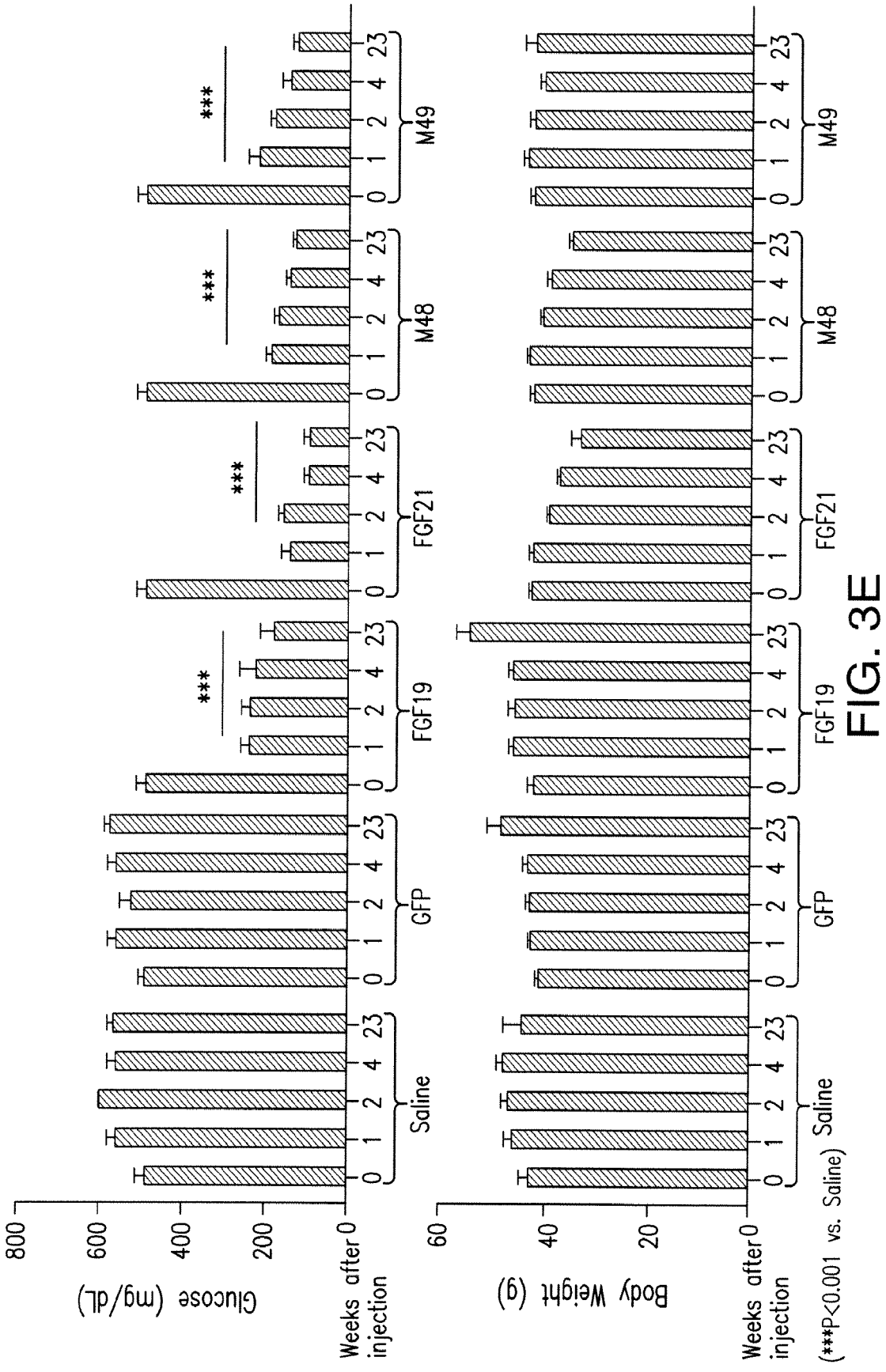

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 1 2 4 23
Saline
GFP
FGF19
FGF21
M48
M49
***
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3E

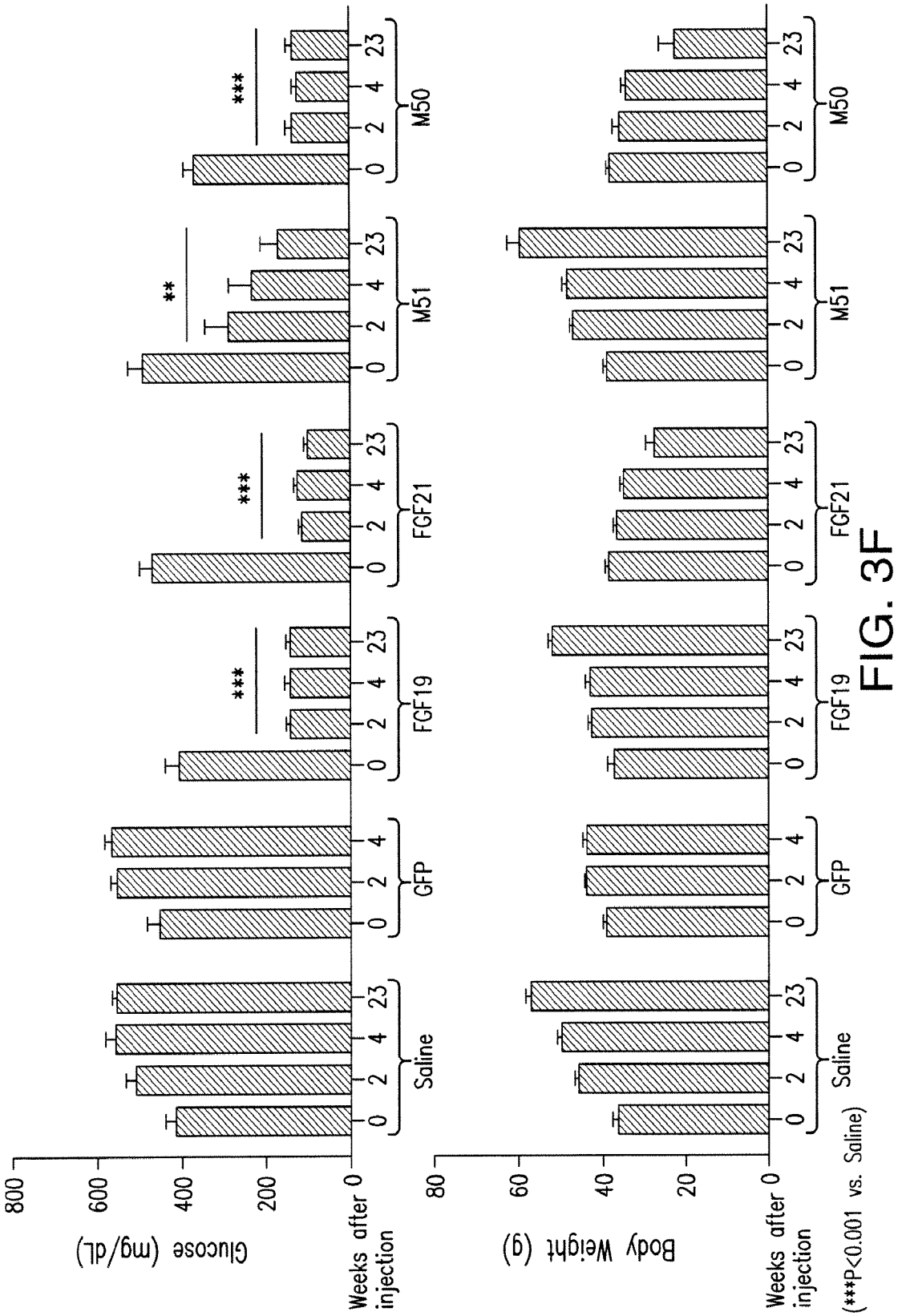

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 2 4 23
Saline
0 2 4
GFP
0 2 4 23
FGF19
0 2 4 23
FGF21
0 2 4 23
M51
0 2 4 23
M50
***
***
**
***
Body Weight (g)
80
60
40
20
0
Weeks after injection
(***P<0.001 vs. Saline)

FIG. 3F

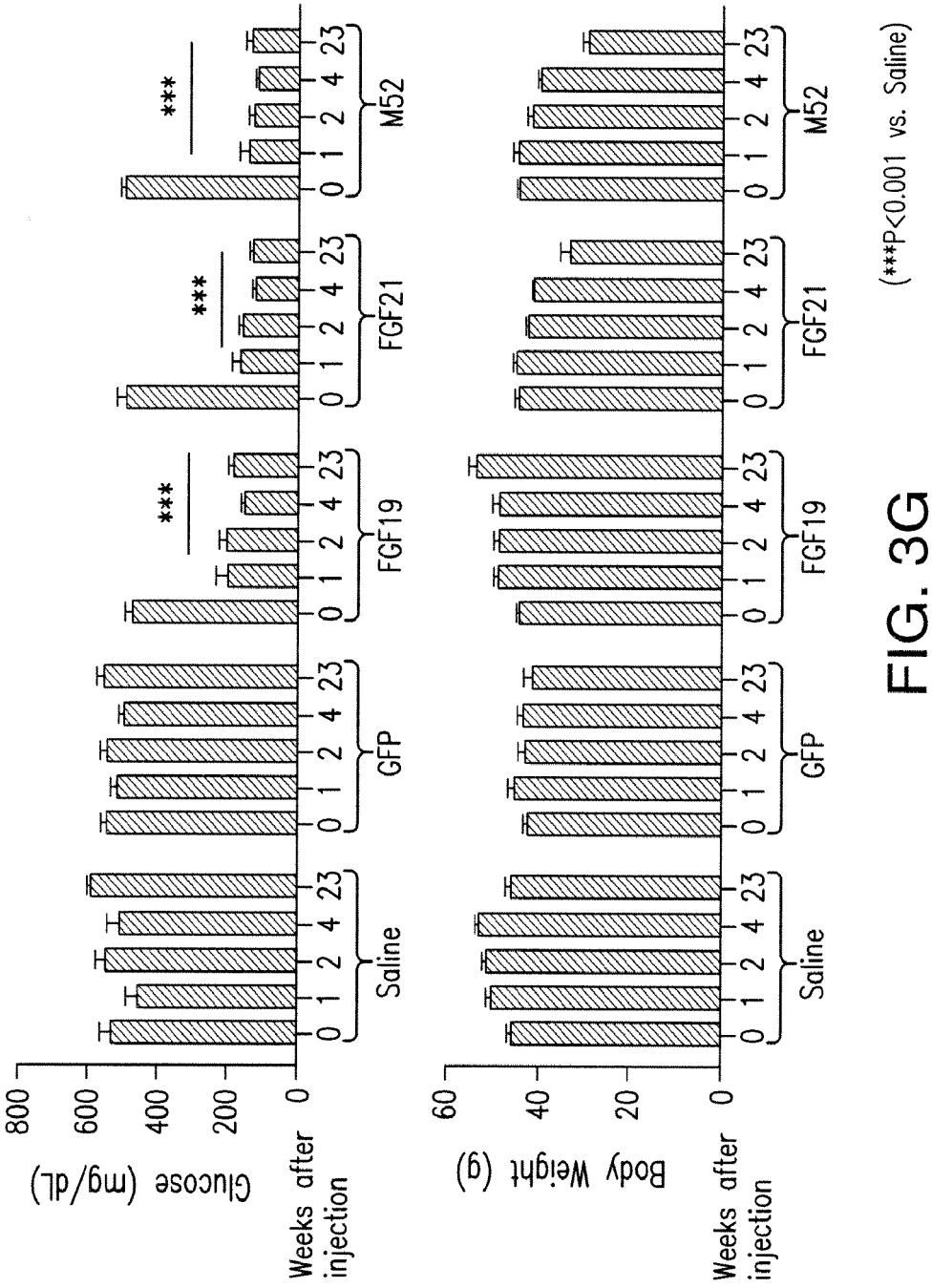

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 1 2 4 23
Saline
GFP
FGF19
FGF21
M52
***
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3G

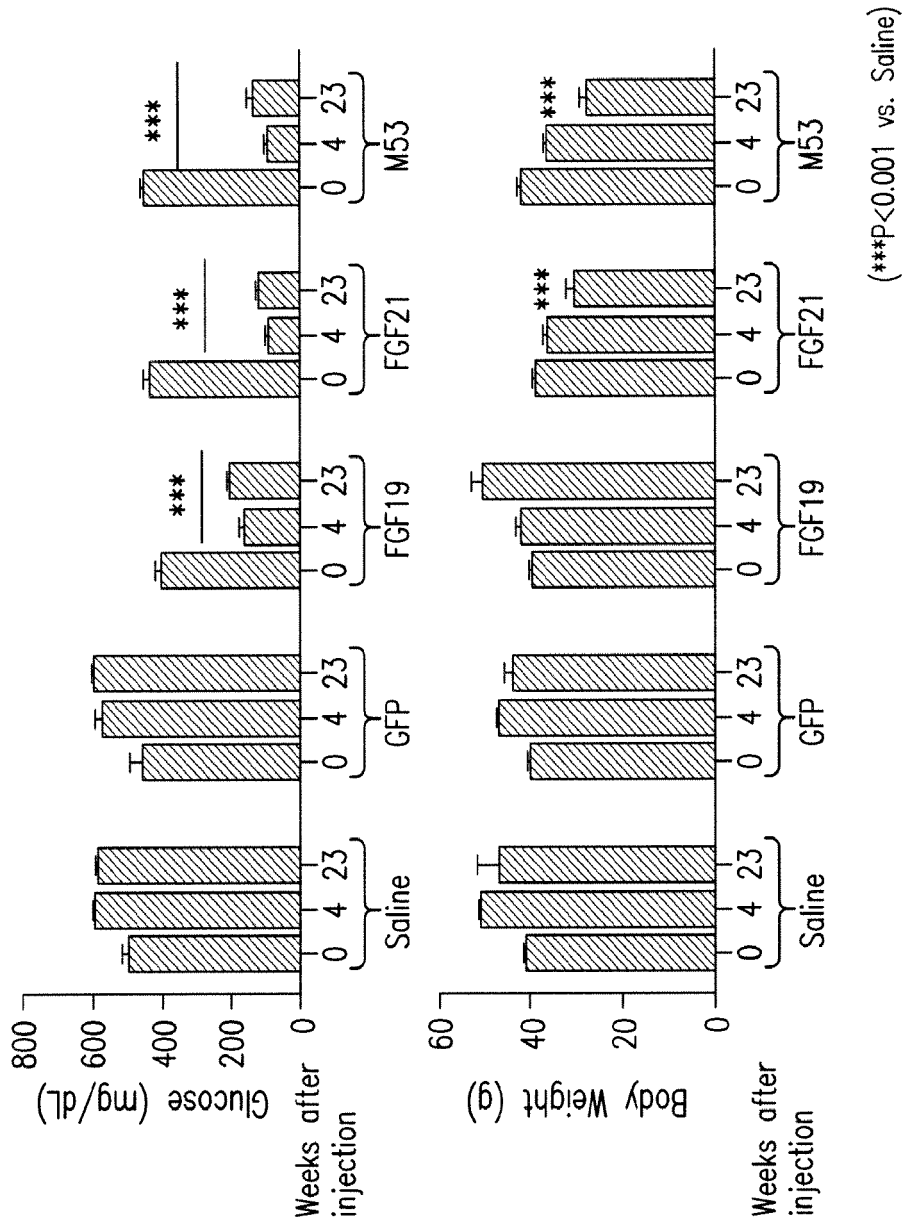

Glucose (mg/dL)
800
600
400
200
0
***
***
***
Weeks after injection
0 4 23
Saline
GFP
FGF19
FGF21
M53
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3H

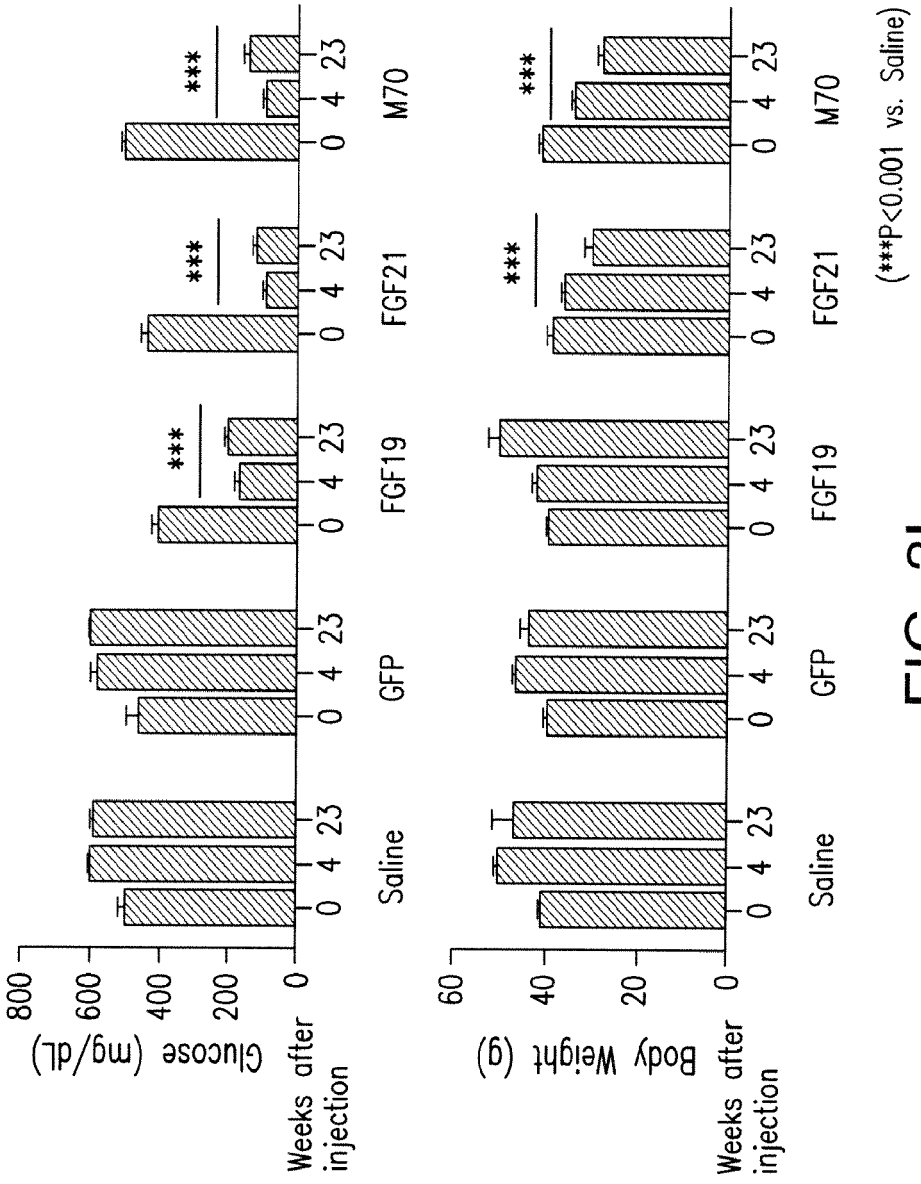

Glucose (mg/dL)
800
600
400
200
0
Weeks after injection
0 4 23
Saline
GFP
FGF19
FGF21
M70
***
Body Weight (g)
60
40
20
0
(***P<0.001 vs. Saline)

FIG. 3I

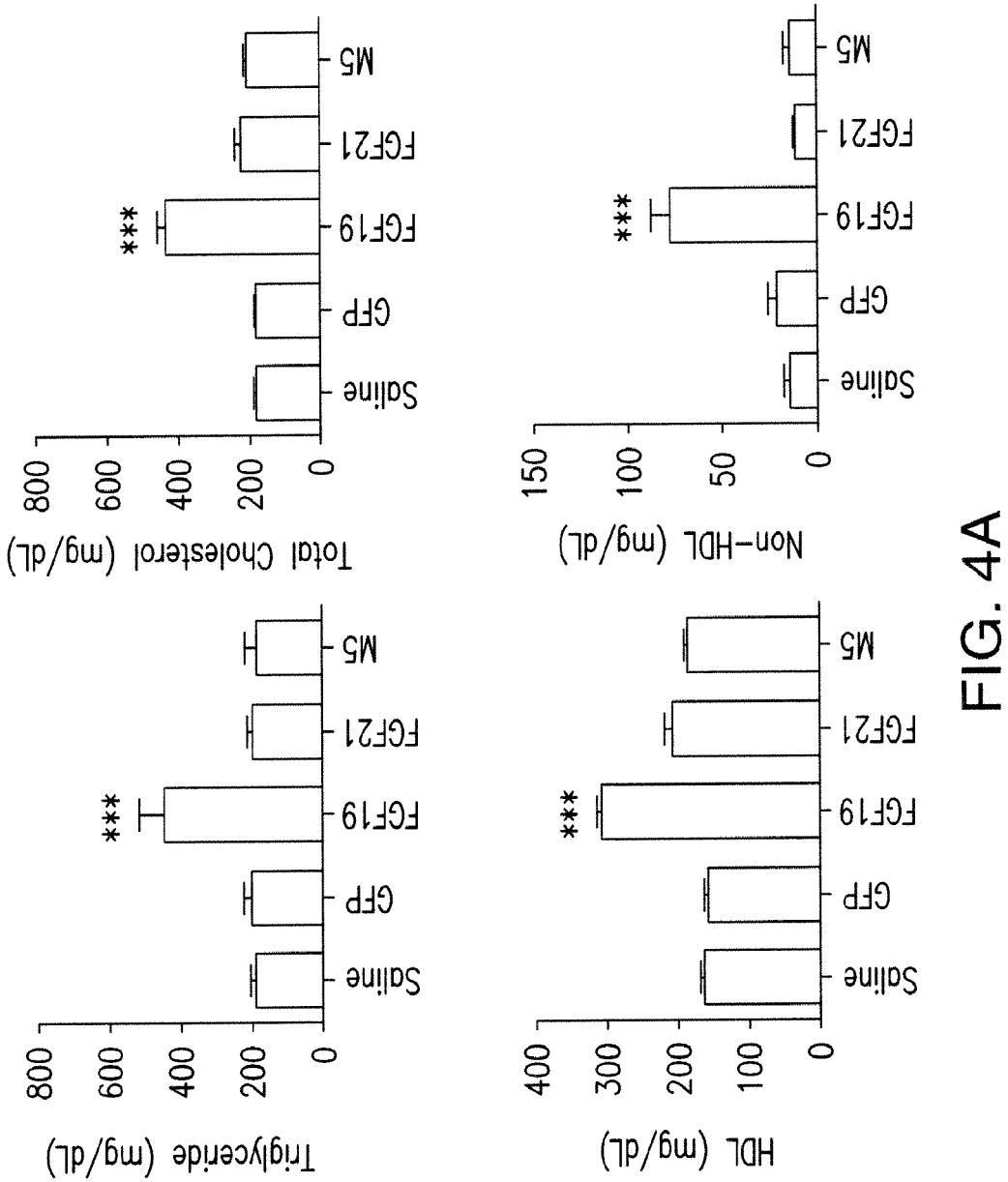

Triglyceride (mg/dL)
800
600
400
200
0
Saline
GFP
FGF19
FGF21
M5
***
HDL (mg/dL)
400
300
200
100
0
Saline
GFP
FGF19
FGF21
M5
***
Total Cholesterol (mg/dL)
800
600
400
200
0
Saline
GFP
FGF19
FGF21
M5
***
Non-HDL (mg/dL)
150
100
50
0
Saline
GFP
FGF19
FGF21
M5
***

FIG. 4A

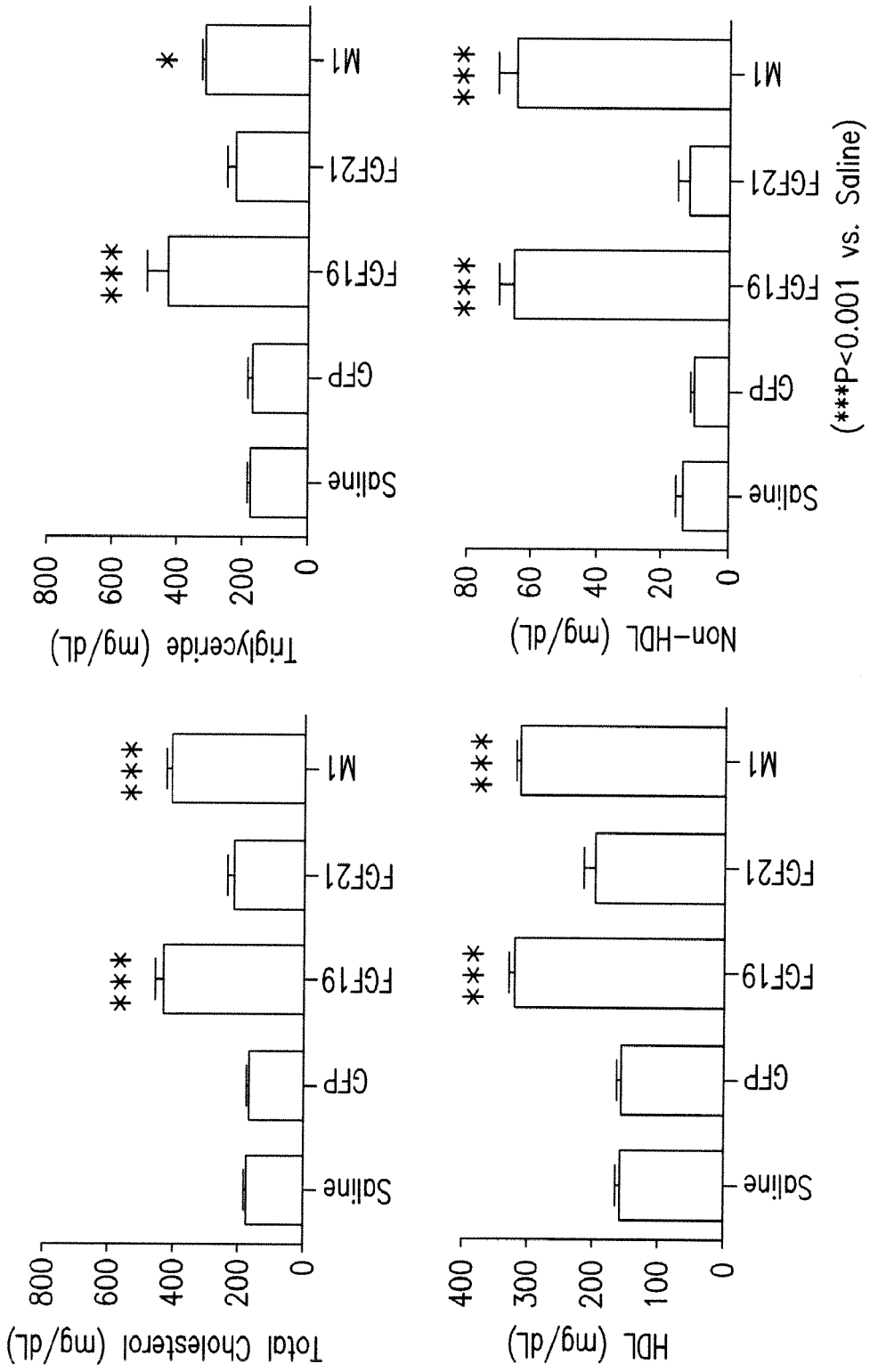

Triglyceride (mg/dL)
800
600
400
200
0
Saline
GFP
FGF19
FGF21
M1
***
*
Non-HDL (mg/dL)
80
60
40
20
0
***
***
(***P<0.001 vs. Saline)
Total Cholesterol (mg/dL)
800
600
400
200
0
***
***
HDL (mg/dL)
400
300
200
100
0
***
***

FIG. 4B

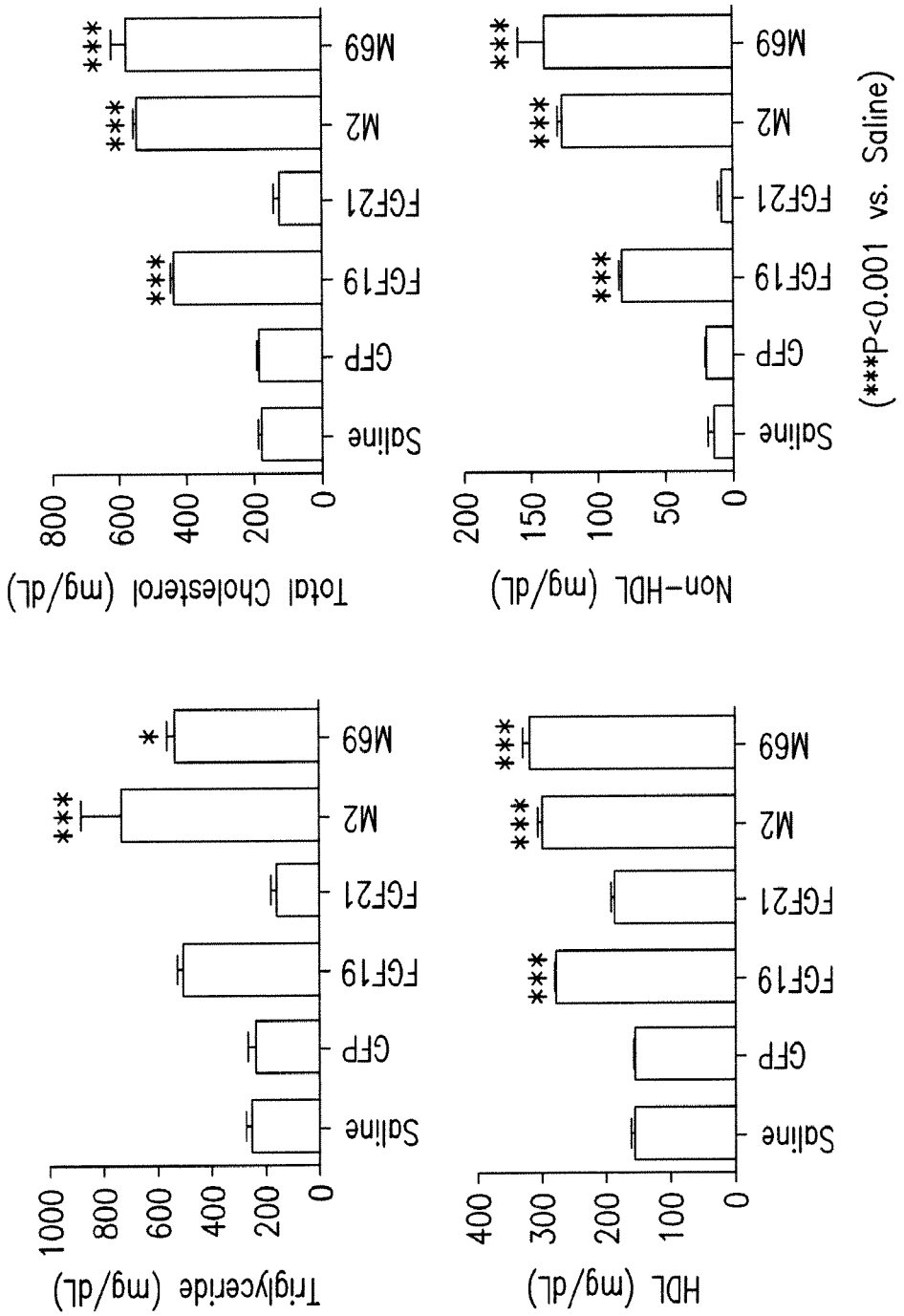

Total Cholesterol (mg/dL)
800
600
400
200
0
Saline
GFP
FGF19
FGF21
M2
M69
***
Non-HDL (mg/dL)
200
150
100
50
0
(***P<0.001 vs. Saline)
Triglyceride (mg/dL)
1000
800
600
400
200
0
*
HDL (mg/dL)
400
300
200
100
0

FIG. 4C

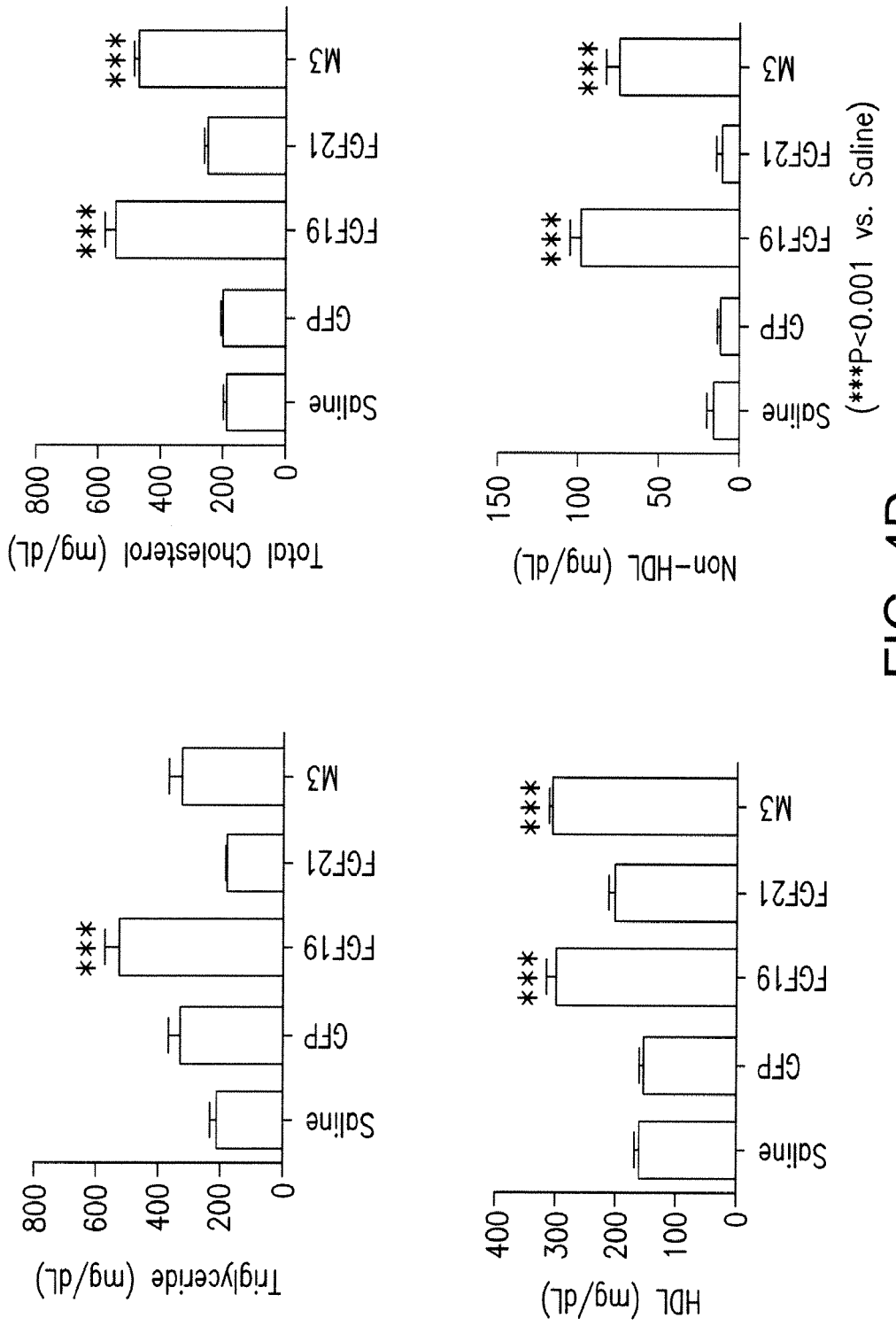

Total Cholesterol (mg/dL)
800
600
400
200
0
Saline
GFP
FGF19
FGF21
M3
***
Non-HDL (mg/dL)
150
100
50
0
(***P<0.001 vs. Saline)
Triglyceride (mg/dL)
HDL (mg/dL)
400
300
200
100

FIG. 4D

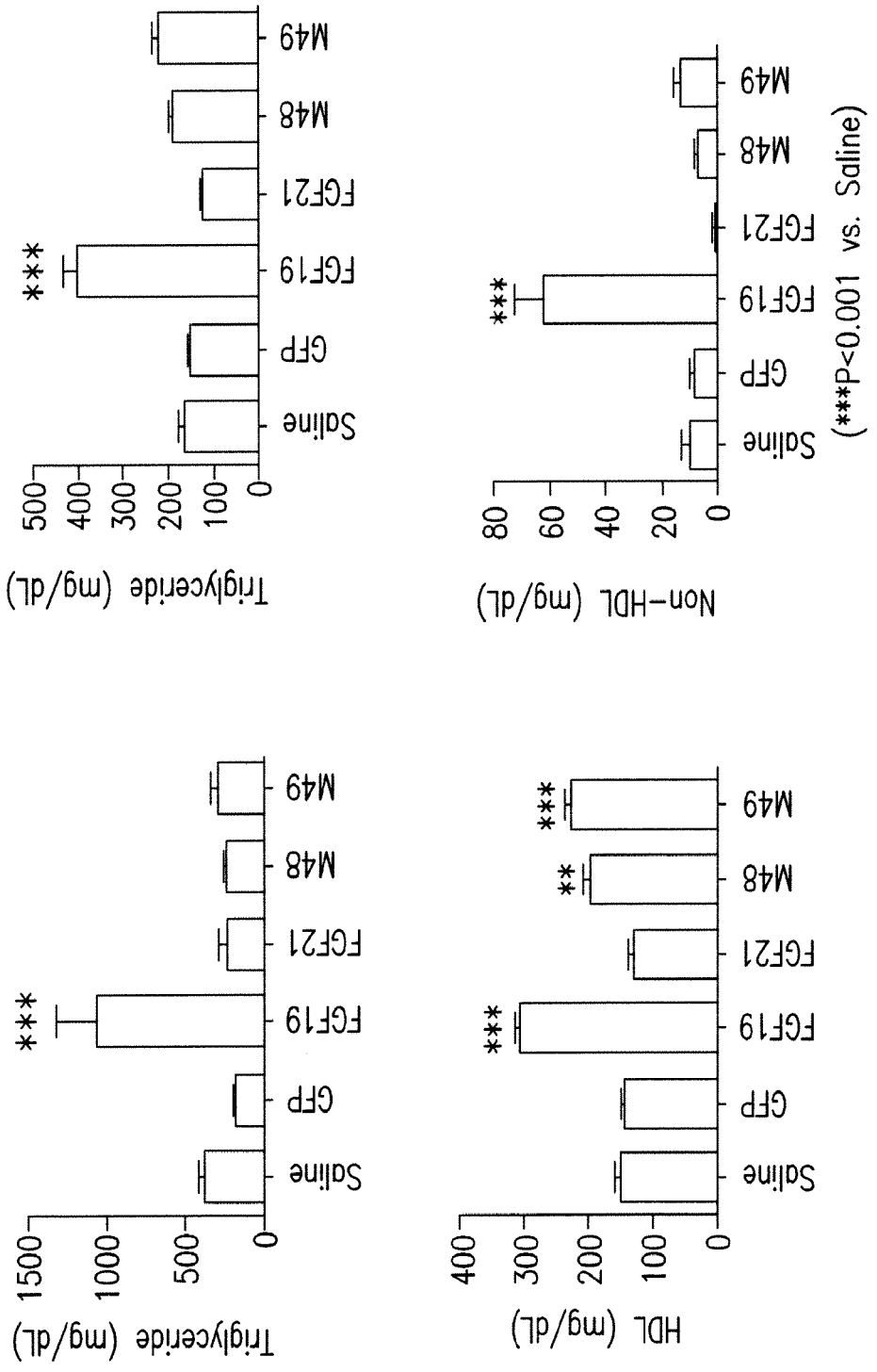

Triglyceride (mg/dL)
500
400
300
200
100
0
***
Saline
GFP
FGF19
FGF21
M48
M49
Non-HDL (mg/dL)
80
60
40
20
0
***
(***P<0.001 vs. Saline)
HDL (mg/dL)
400
300
200
100
0
***
**
***

FIG. 4E

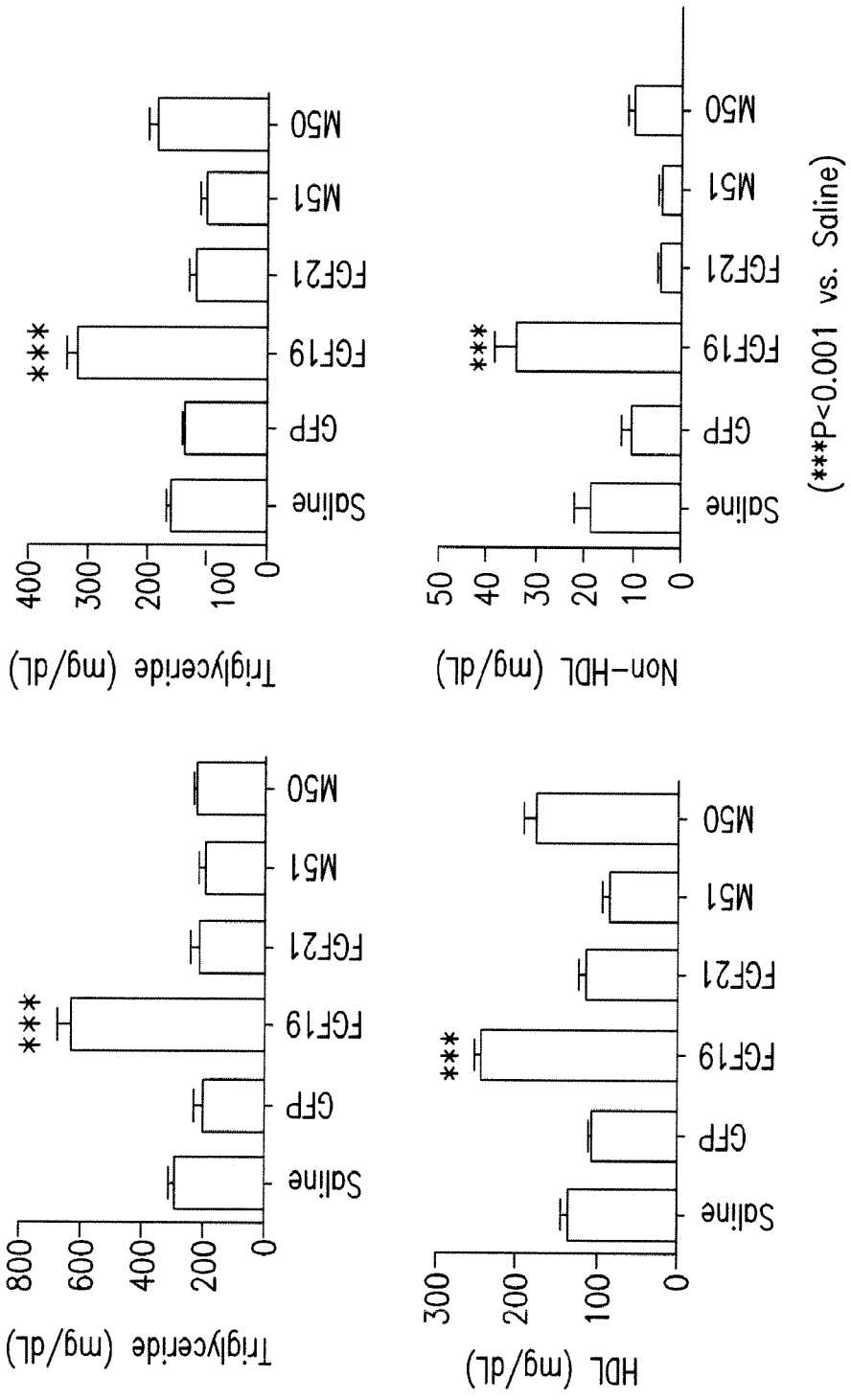

Triglyceride (mg/dL)
0
100
200
300
400
***
Saline
GFP
FGF19
FGF21
M51
M50
Non-HDL (mg/dL)
0
10
20
30
40
50
***
Saline
GFP
FGF19
FGF21
M51
M50
(***P<0.001 vs. Saline)
Triglyceride (mg/dL)
0
200
400
600
800
***
Saline
GFP
FGF19
FGF21
M51
M50
HDL (mg/dL)
0
100
200
300
***
Saline
GFP
FGF19
FGF21
M51
M50

FIG. 4F

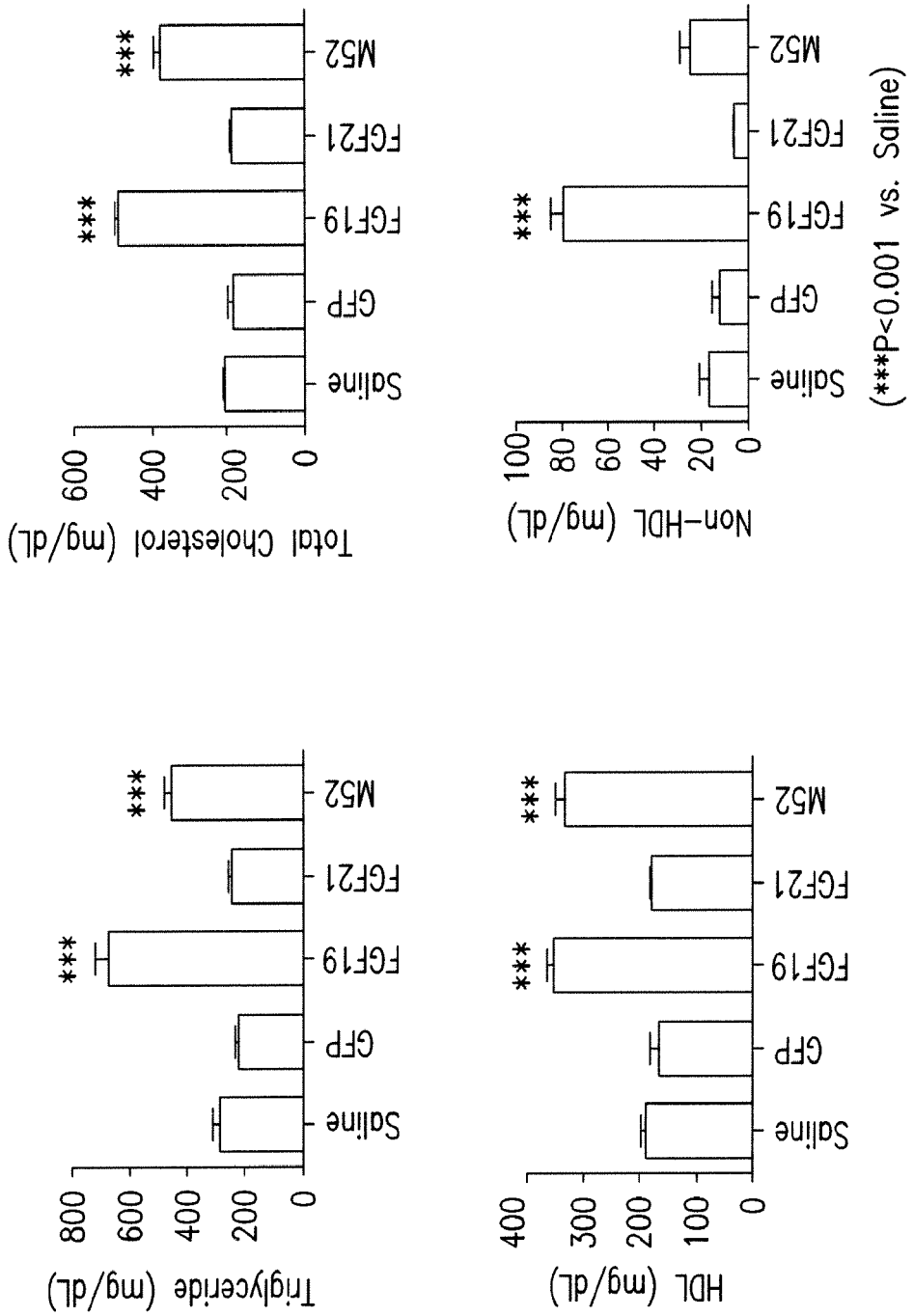

Total Cholesterol (mg/dL)
0
200
400
600
Saline
GFP
FGF19
FGF21
M52
***
Non-HDL (mg/dL)
0
20
40
60
80
100
(***P<0.001 vs. Saline)
Triglyceride (mg/dL)
0
200
400
600
800
HDL (mg/dL)
0
100
200
300
400

FIG. 4G

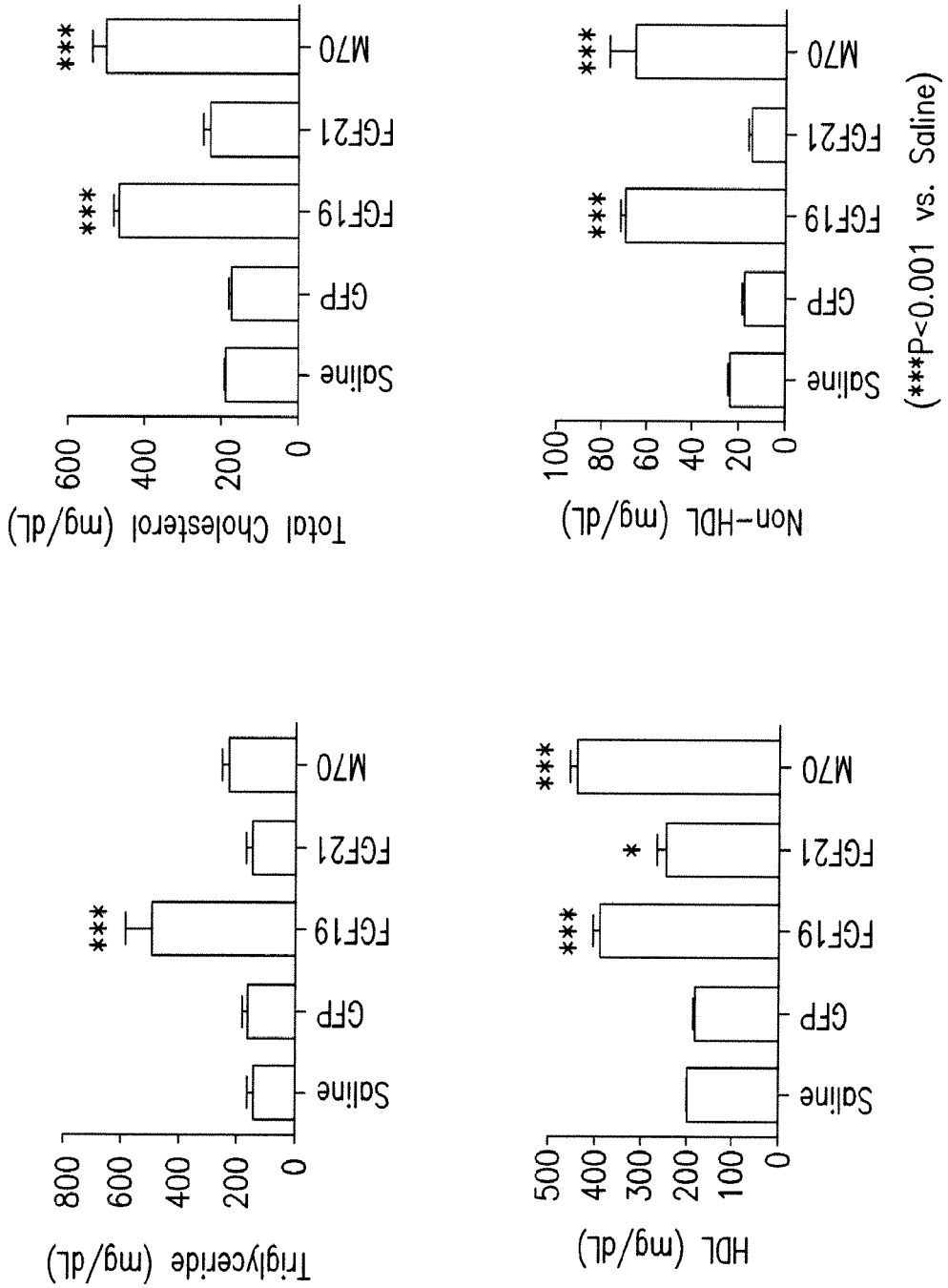

Total Cholesterol (mg/dL)
0
200
400
600
Saline
GFP
FGF19
FGF21
M70
***
***
Non-HDL (mg/dL)
0
20
40
60
80
100
Saline
GFP
FGF19
FGF21
M70
***
***
(***P<0.001 vs. Saline)
Triglyceride (mg/dL)
0
200
400
600
800
Saline
GFP
FGF19
FGF21
M70
***
HDL (mg/dL)
0
100
200
300
400
500
Saline
GFP
FGF19
FGF21
M70
***
***
*

FIG. 4H

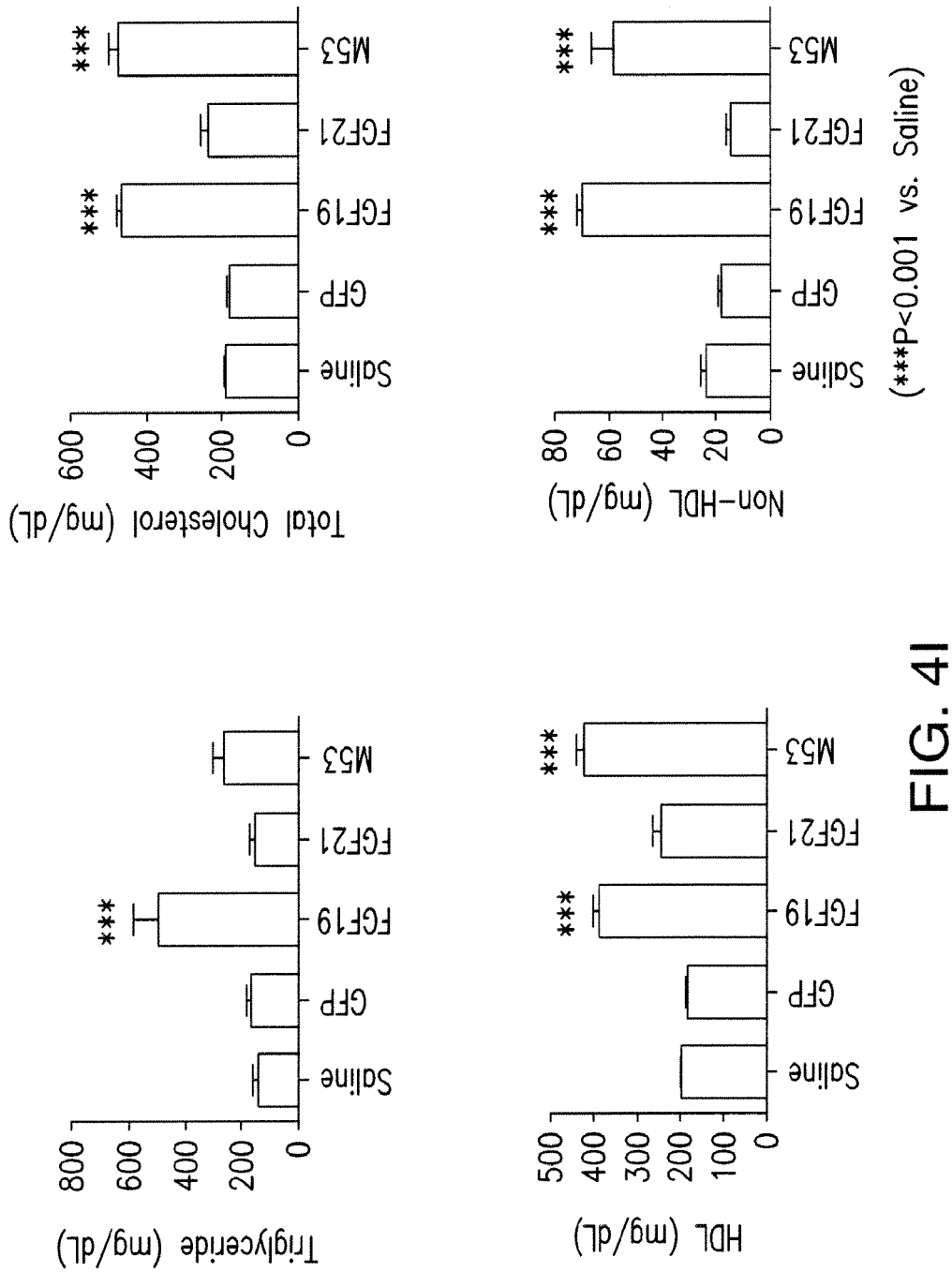

Total Cholesterol (mg/dL)
600
400
200
0
Saline
GFP
FGF19
FGF21
M53
***
***
Non-HDL (mg/dL)
80
60
40
20
0
Saline
GFP
FGF19
FGF21
M53
***
***
(***P<0.001 vs. Saline)
Triglyceride (mg/dL)
800
600
400
200
0
Saline
GFP
FGF19
FGF21
M53
***
HDL (mg/dL)
500
400
300
200
100
0
Saline
GFP
FGF19
FGF21
M53
***
***

FIG. 4I

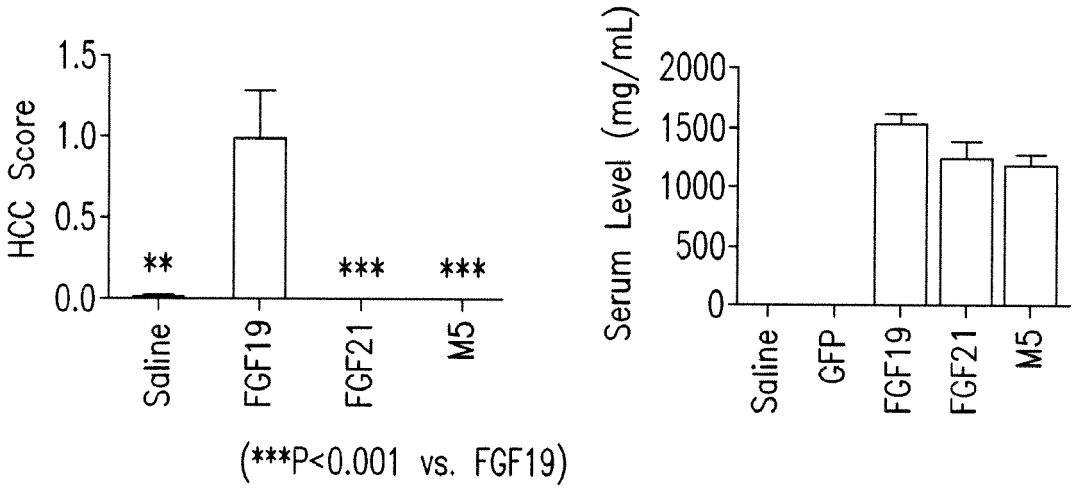

HCC Score
1.5
1.0
0.5
0.0
**
***
***
Saline
FGF19
FGF21
M5
(***P<0.001 vs. FGF19)
Serum Level (mg/mL)
2000
1500
1000
500
0
Saline
GFP
FGF19
FGF21
M5

FIG. 5A

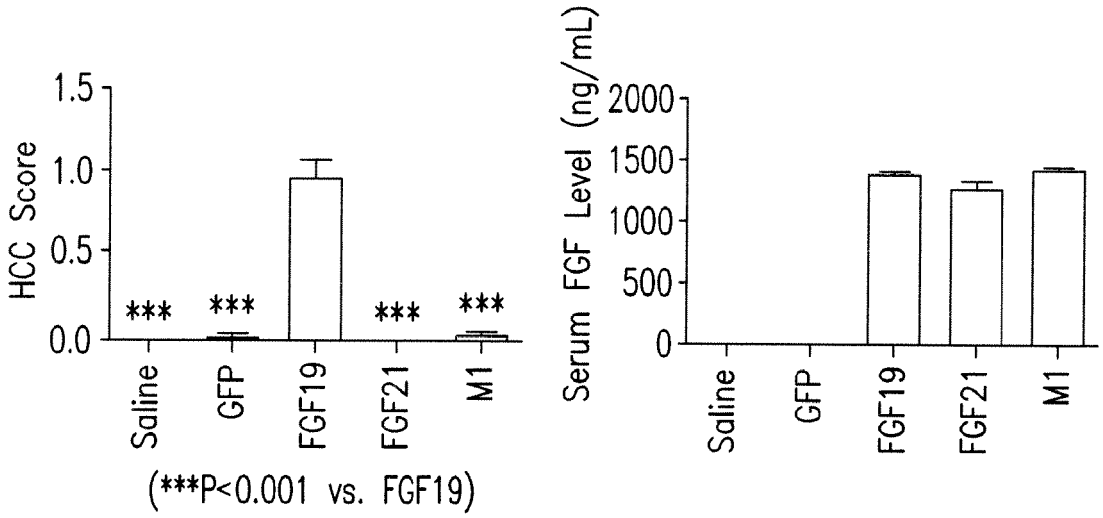

HCC Score
1.5
1.0
0.5
0.0
***
***
***
***
Saline
GFP
FGF19
FGF21
M1
(***P<0.001 vs. FGF19)
Serum FGF Level (ng/mL)
2000
1500
1000
500
0
Saline
GFP
FGF19
FGF21
M1

FIG. 5B

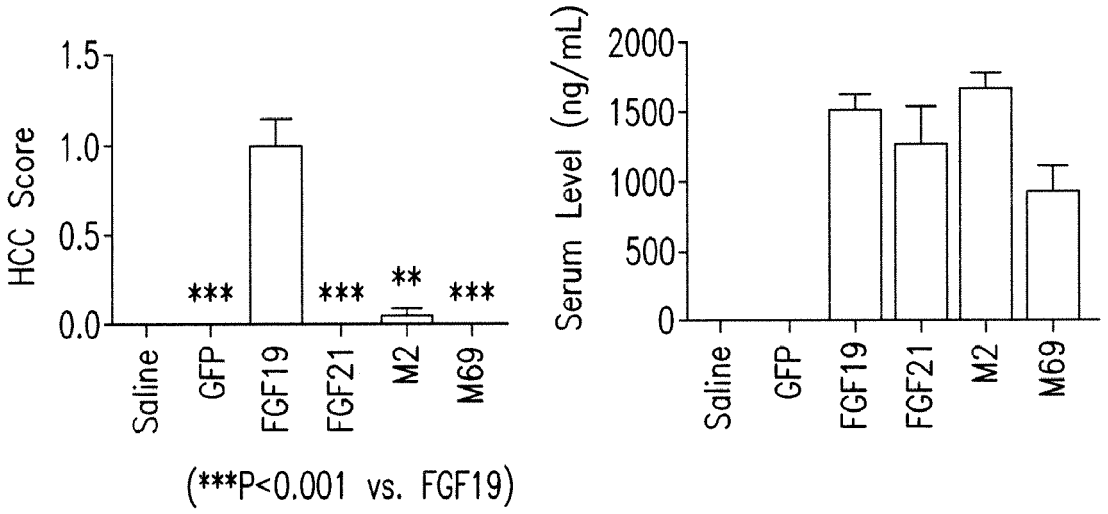

HCC Score
1.5
1.0
0.5
0.0
***
***
**
***
Saline
GFP
FGF19
FGF21
M2
M69
(***P<0.001 vs. FGF19)
Serum Level (ng/mL)
2000
1500
1000
500
0
Saline
GFP
FGF19
FGF21
M2
M69

FIG. 5C

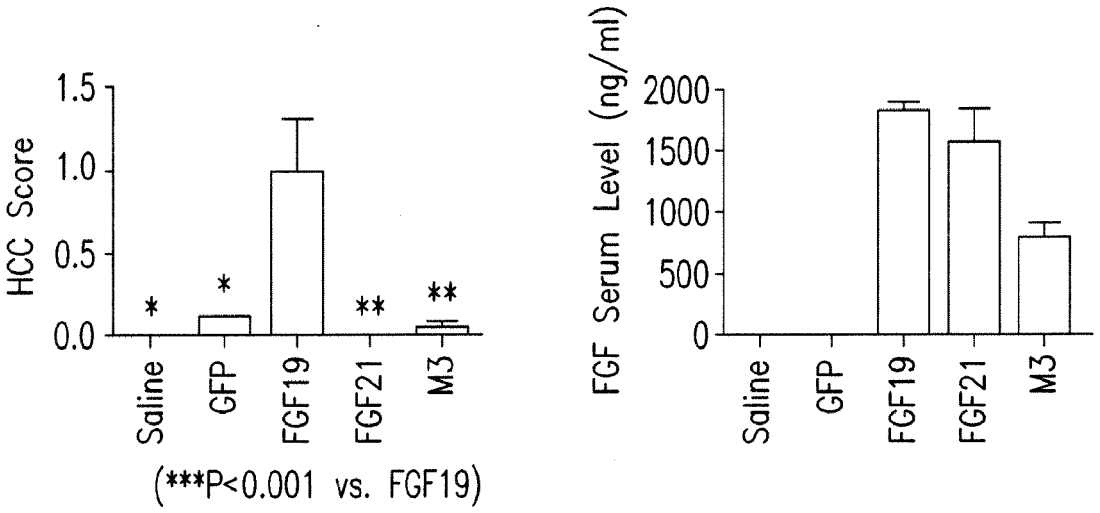

HCC Score
1.5
1.0
0.5
0.0
*
*
**
**
Saline
GFP
FGF19
FGF21
M3
(***P<0.001 vs. FGF19)
FGF Serum Level (ng/ml)
2000
1500
1000
500
0
Saline
GFP
FGF19
FGF21
M3

FIG. 5D

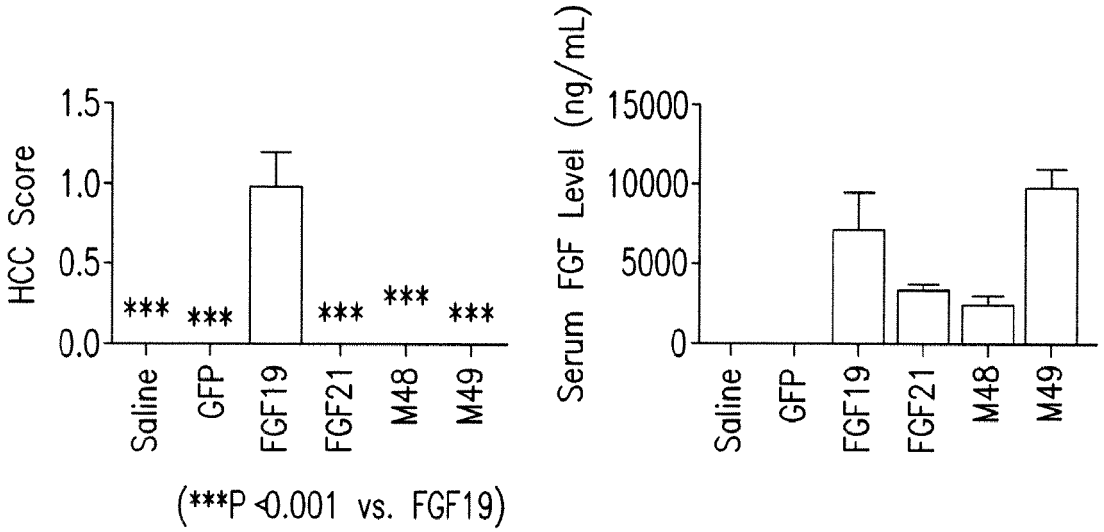

HCC Score
1.5
1.0
0.5
0.0
***
***
***
***
***
Saline
GFP
FGF19
FGF21
M48
M49
(***P <0.001 vs. FGF19)
Serum FGF Level (ng/mL)
15000
10000
5000
0
Saline
GFP
FGF19
FGF21
M48
M49

FIG. 5E

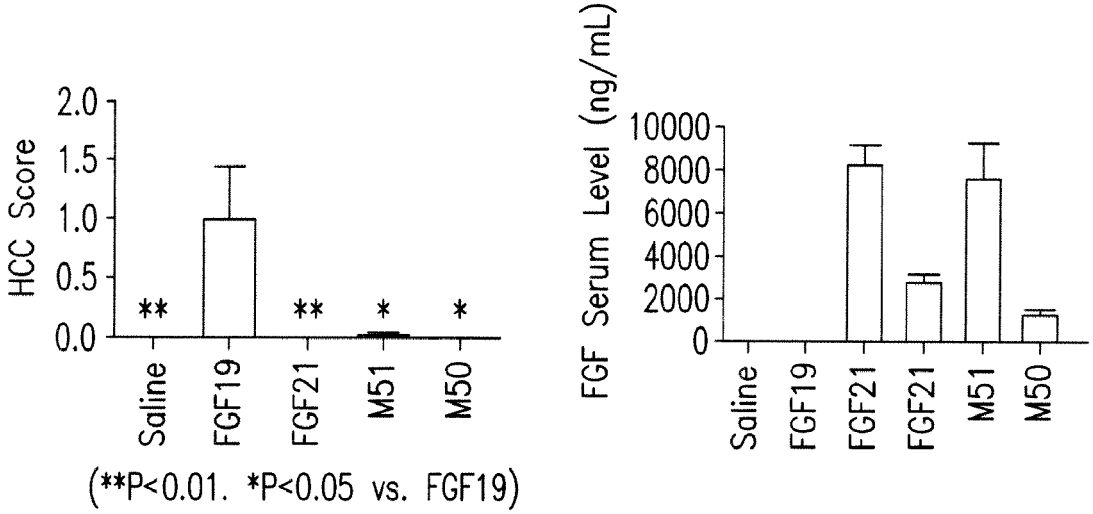

HCC Score
2.0
1.5
1.0
0.5
0.0
**
**
*
*
Saline
FGF19
FGF21
M51
M50
(**P<0.01. *P<0.05 vs. FGF19)
FGF Serum Level (ng/mL)
10000
8000
6000
4000
2000
0
Saline
FGF19
FGF21
FGF21
M51
M50

FIG. 5F

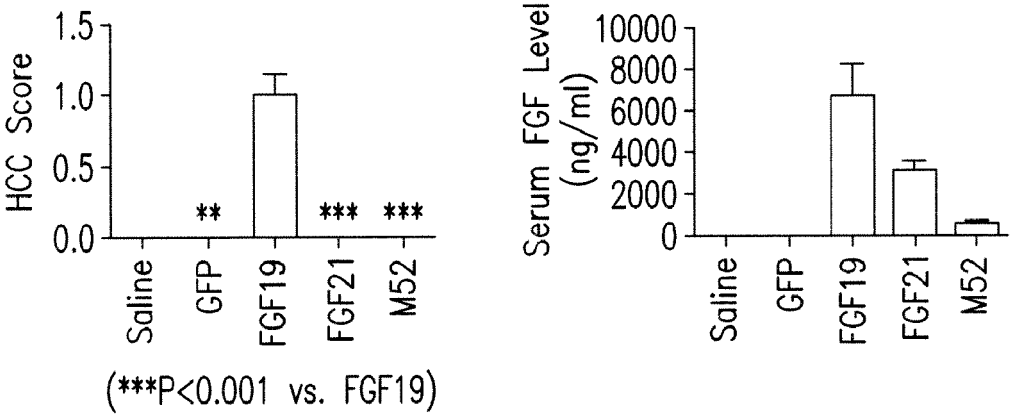

HCC Score
1.5
1.0
0.5
0.0
**
***
***
Saline
GFP
FGF19
FGF21
M52
(***P<0.001 vs. FGF19)
Serum FGF Level (ng/ml)
10000
8000
6000
4000
2000
0
Saline
GFP
FGF19
FGF21
M52

FIG. 5G

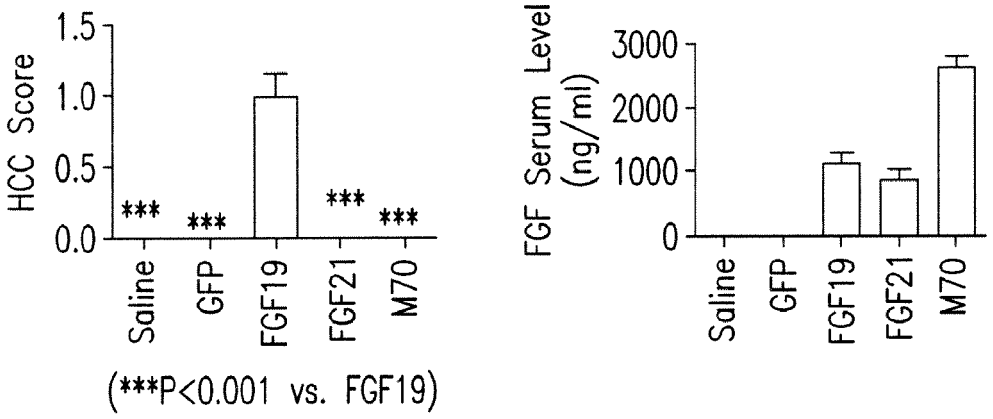

HCC Score
1.5
1.0
0.5
0.0
***
***
***
***
Saline
GFP
FGF19
FGF21
M70
(***P<0.001 vs. FGF19)
FGF Serum Level (ng/ml)
3000
2000
1000
0
Saline
GFP
FGF19
FGF21
M70

FIG. 5H

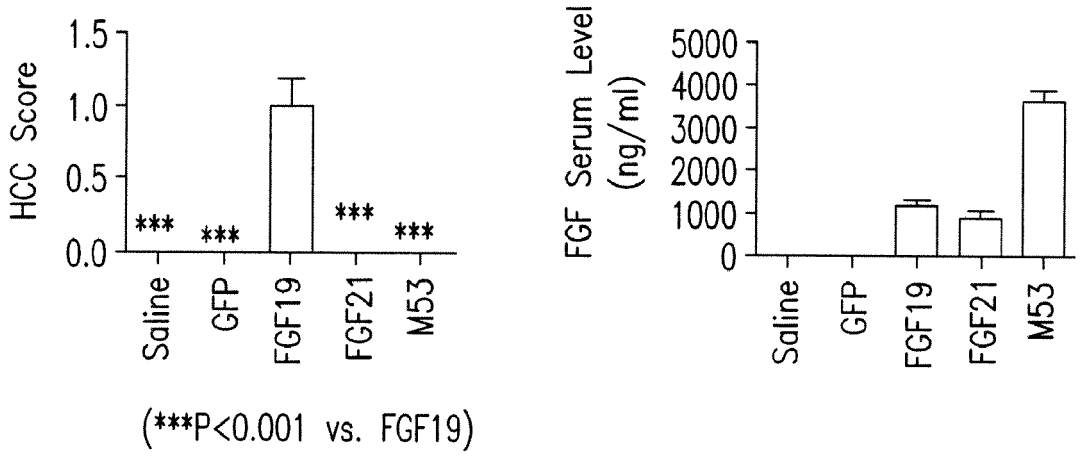

HCC Score
1.5
1.0
0.5
0.0
***
***
***
***
Saline
GFP
FGF19
FGF21
M53
(***P<0.001 vs. FGF19)
FGF Serum Level (ng/ml)
5000
4000
3000
2000
1000
0
Saline
GFP
FGF19
FGF21
M53

FIG. 5I

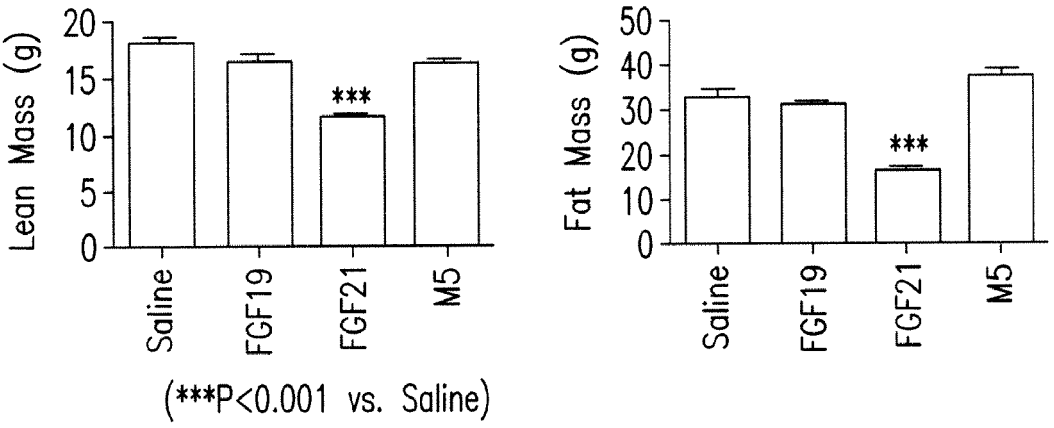

Lean Mass (g)
20
15
10
5
0
***
Saline
FGF19
FGF21
M5
(***P<0.001 vs. Saline)
Fat Mass (g)
50
40
30
20
10
0
***
Saline
FGF19
FGF21
M5

FIG. 6A

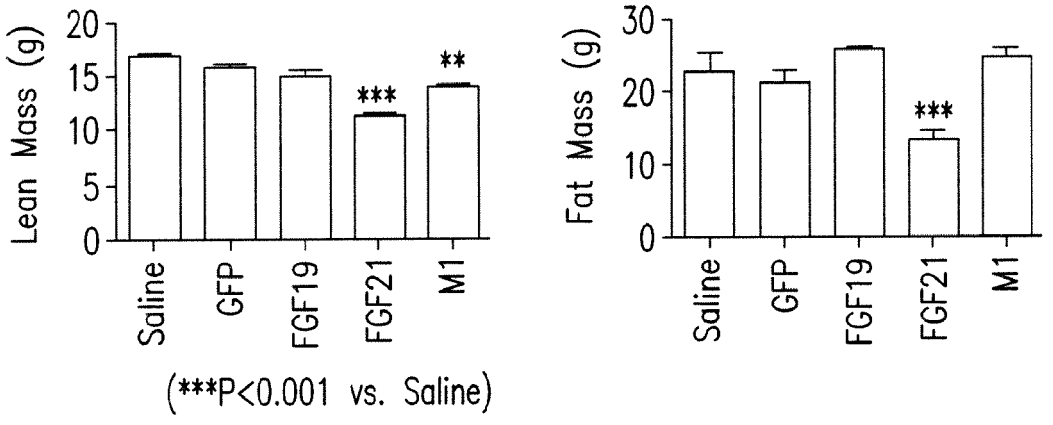

Lean Mass (g)
20
15
10
5
0
***
**
Saline
GFP
FGF19
FGF21
M1
(***P<0.001 vs. Saline)
Fat Mass (g)
30
20
10
0
***
Saline
GFP
FGF19
FGF21
M1

FIG. 6B

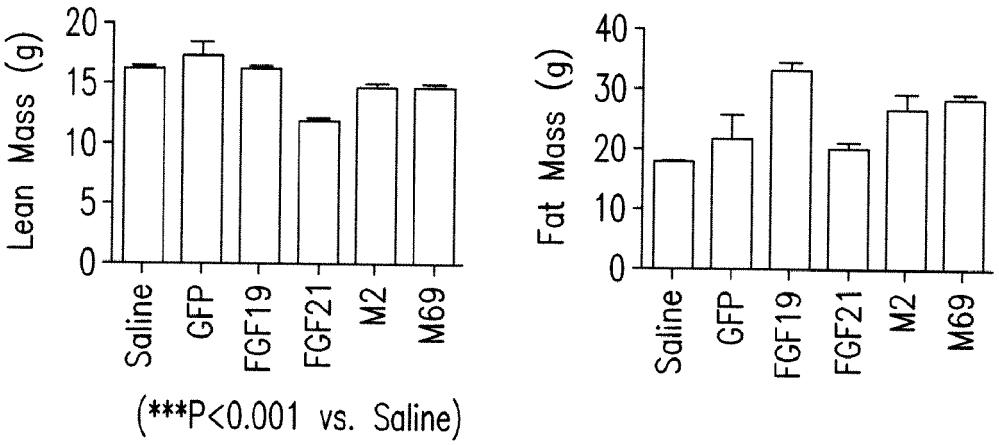

Lean Mass (g)
20
15
10
5
0
Saline
GFP
FGF19
FGF21
M2
M69
(***P<0.001 vs. Saline)
Fat Mass (g)
40
30
20
10
0
Saline
GFP
FGF19
FGF21
M2
M69

FIG. 6C

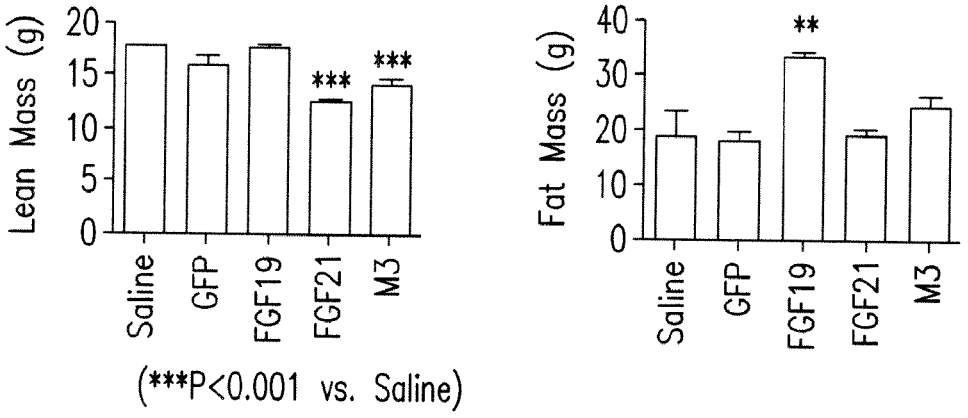

Lean Mass (g)
20
15
10
5
0
***
***
Saline
GFP
FGF19
FGF21
M3
(***P<0.001 vs. Saline)
Fat Mass (g)
40
30
20
10
0
**
Saline
GFP
FGF19
FGF21
M3

FIG. 6D

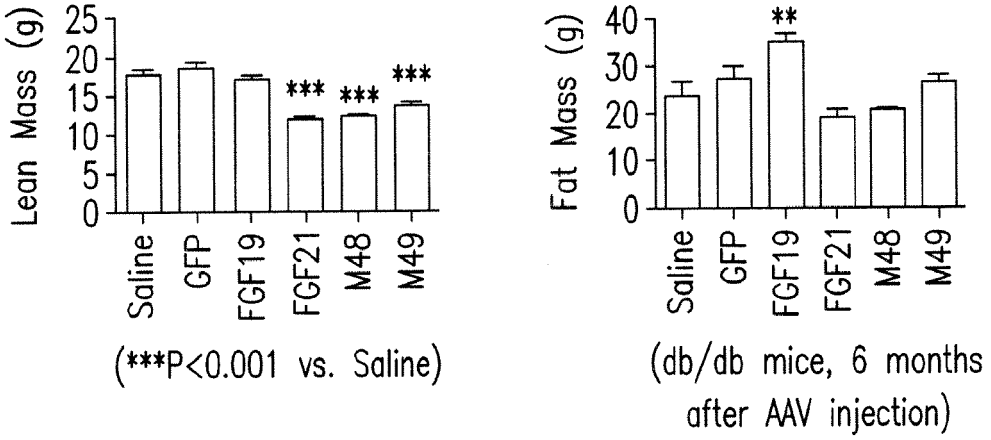

Lean Mass (g)
25
20
15
10
5
0
***
***
***
Saline
GFP
FGF19
FGF21
M48
M49
(***P<0.001 vs. Saline)
Fat Mass (g)
40
30
20
10
0
**
Saline
GFP
FGF19
FGF21
M48
M49
(db/db mice, 6 months after AAV injection)

FIG. 6E

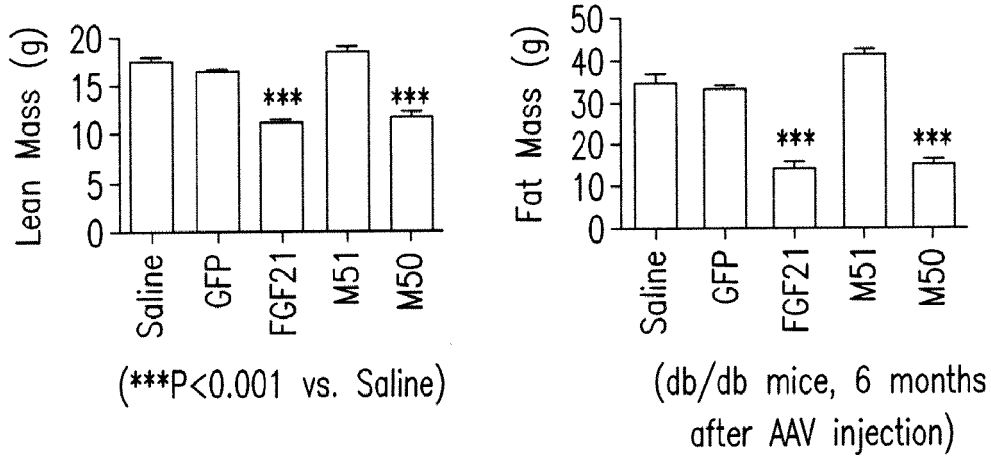

Lean Mass (g)
20
15
10
5
0
***
***
Saline
GFP
FGF21
M51
M50
(***P<0.001 vs. Saline)
Fat Mass (g)
50
40
30
20
10
0
***
***
Saline
GFP
FGF21
M51
M50
(db/db mice, 6 months after AAV injection)

FIG. 6F

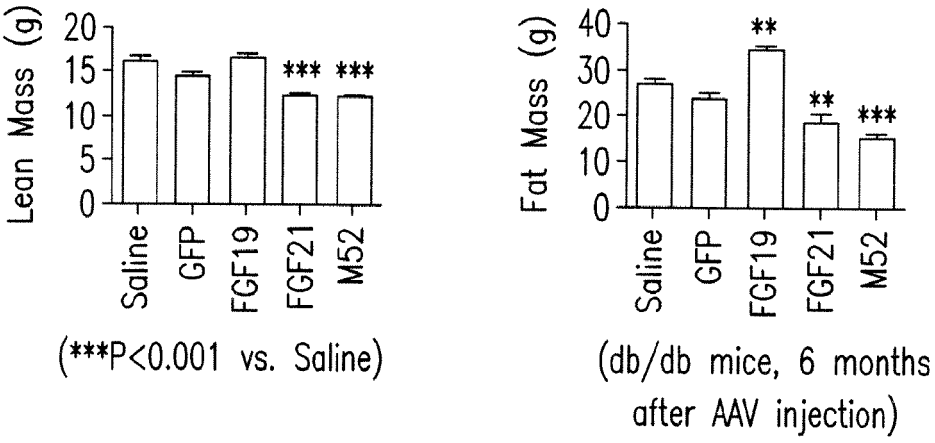

Lean Mass (g)
20
15
10
5
0
***
***
Saline
GFP
FGF19
FGF21
M52
(***P<0.001 vs. Saline)
Fat Mass (g)
40
30
20
10
0
**
**
***
Saline
GFP
FGF19
FGF21
M52
(db/db mice, 6 months after AAV injection)

FIG. 6G

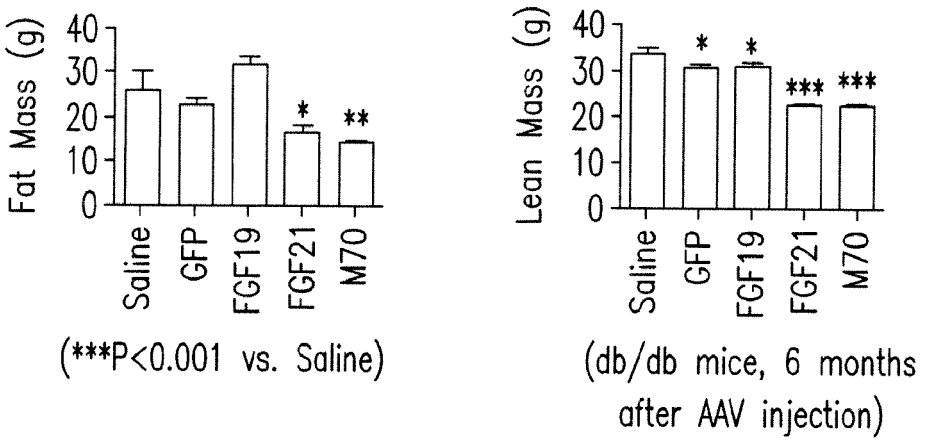
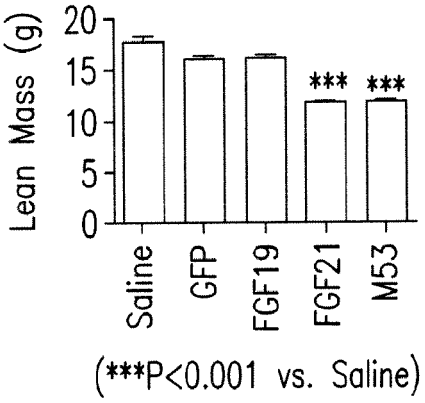

Fat Mass (g)
40
30
20
10
0
*
**
Saline
GFP
FGF19
FGF21
M70
(***P<0.001 vs. Saline)
Lean Mass (g)
40
30
20
10
0
*
*
***
***
Saline
GFP
FGF19
FGF21
M70
(db/db mice, 6 months after AAV injection)

FIG. 6H


Lean Mass (g)
20
15
10
5
0
***
***
Saline
GFP
FGF19
FGF21
M53
(***P<0.001 vs. Saline)

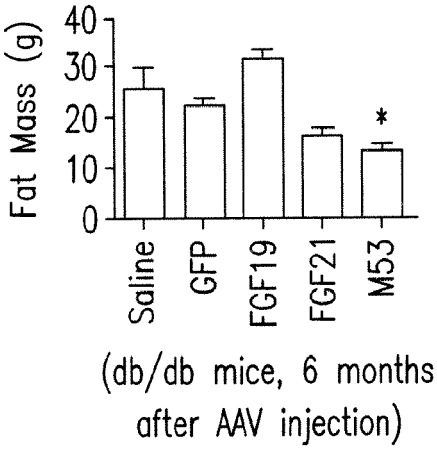

Fat Mass (g)
40
30
20
10
0
*
Saline
GFP
FGF19
FGF21
M53
(db/db mice, 6 months after AAV injection)

FIG. 6I

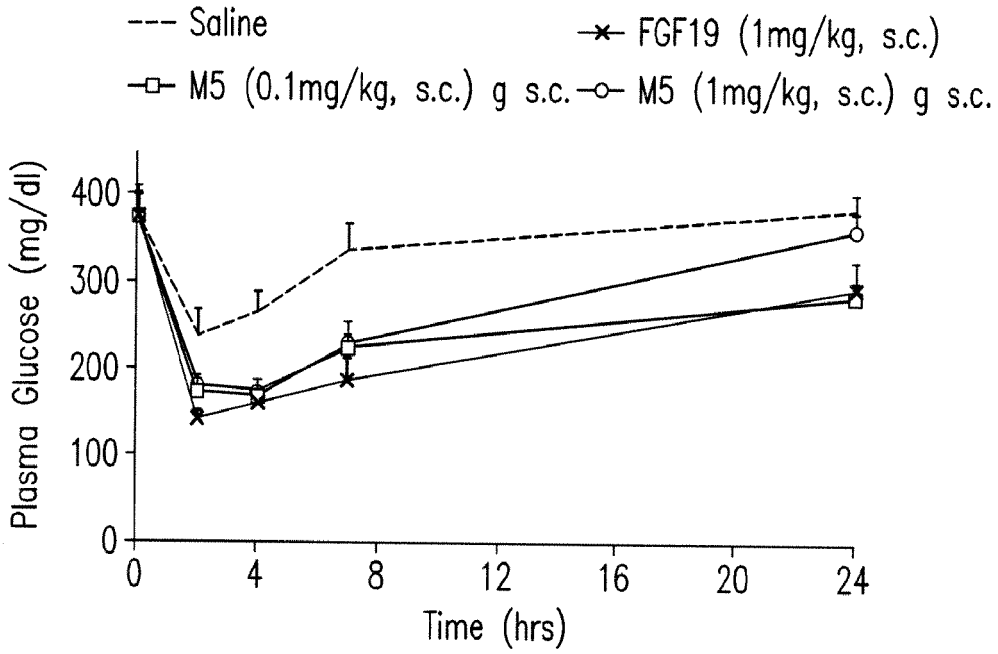

--- Saline
FGF19 (1mg/kg, s.c.)
M5 (0.1mg/kg, s.c.) g s.c.
M5 (1mg/kg, s.c.) g s.c.
Plasma Glucose (mg/dl)
400
300
200
100
0
0
4
8
12
16
20
24
Time (hrs)

FIG. 7A

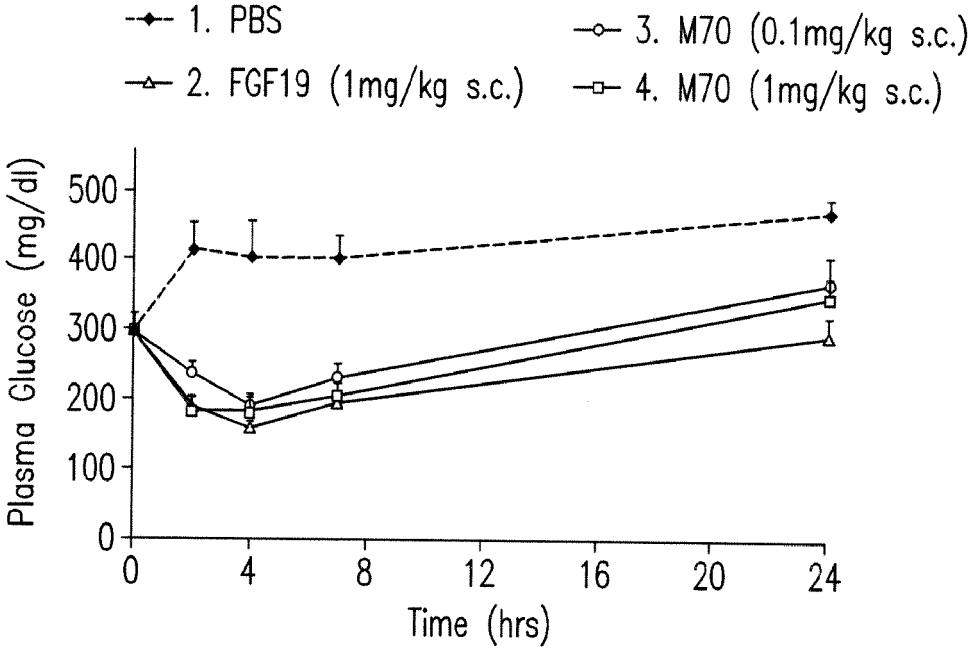

1. PBS
3. M70 (0.1mg/kg s.c.)
2. FGF19 (1mg/kg s.c.)
4. M70 (1mg/kg s.c.)
Plasma Glucose (mg/dl)
500
400
300
200
100
0
0
4
8
12
16
20
24
Time (hrs)

FIG. 7B

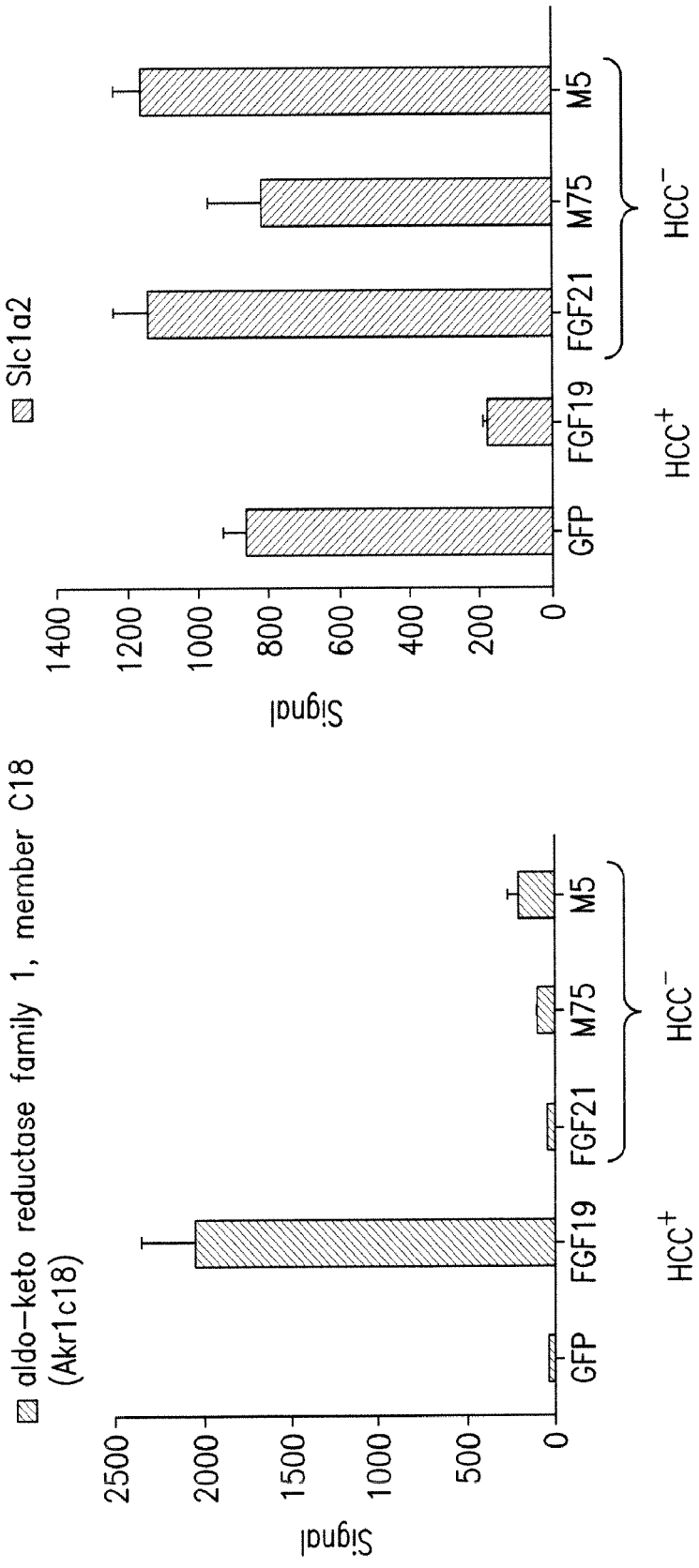

Slc1a2
Signal
1400
1200
1000
800
600
400
200
0
GFP
FGF19
FGF21
M75
M5
HCC+
HCC−
aldo−keto reductase family 1, member C18
(Akr1c18)
Signal
2500
2000
1500
1000
500
0
GFP
FGF19
FGF21
M75
M5
HCC+
HCC−

FIG. 8