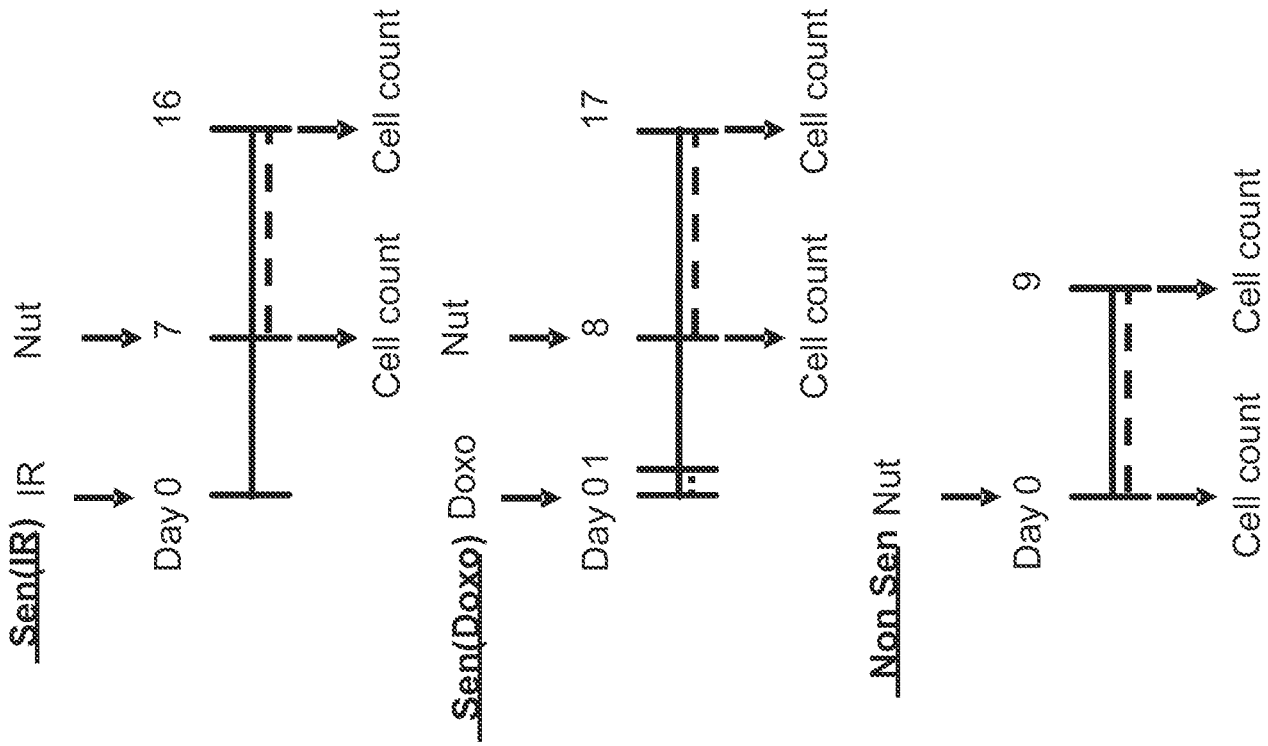

Sen(IR) IR
Nut
Day 0
7
16
Cell count
Cell count
Sen(Doxo) Doxo
Nut
Day 0 1
8
17
Cell count
Cell count
Non Sen Nut
Day 0
9
Cell count
Cell count

FIG. 1

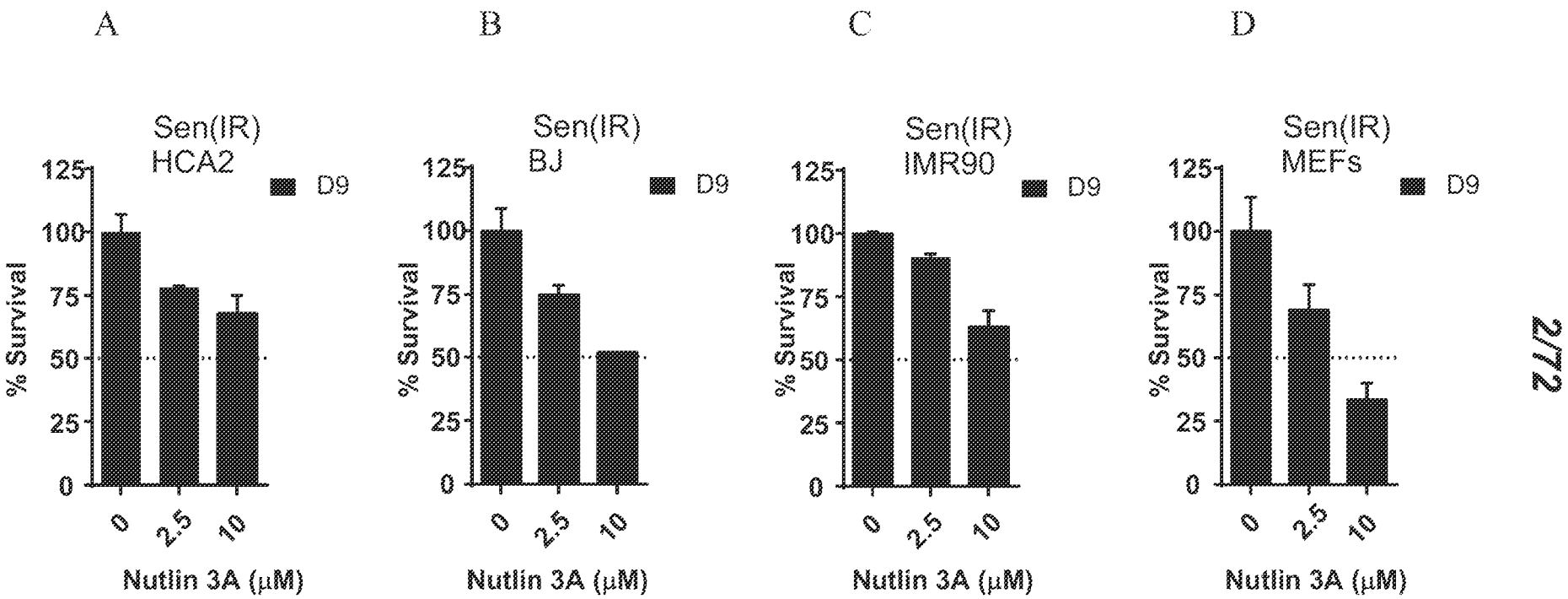

A
B
C
D
Sen(IR)
HCA2
Sen(IR)
BJ
Sen(IR)
IMR90
Sen(IR)
MEFs
D9
% Survival
125
100
75
50
25
0
0
2.5
10
Nutlin 3A (μM)

FIG. 2

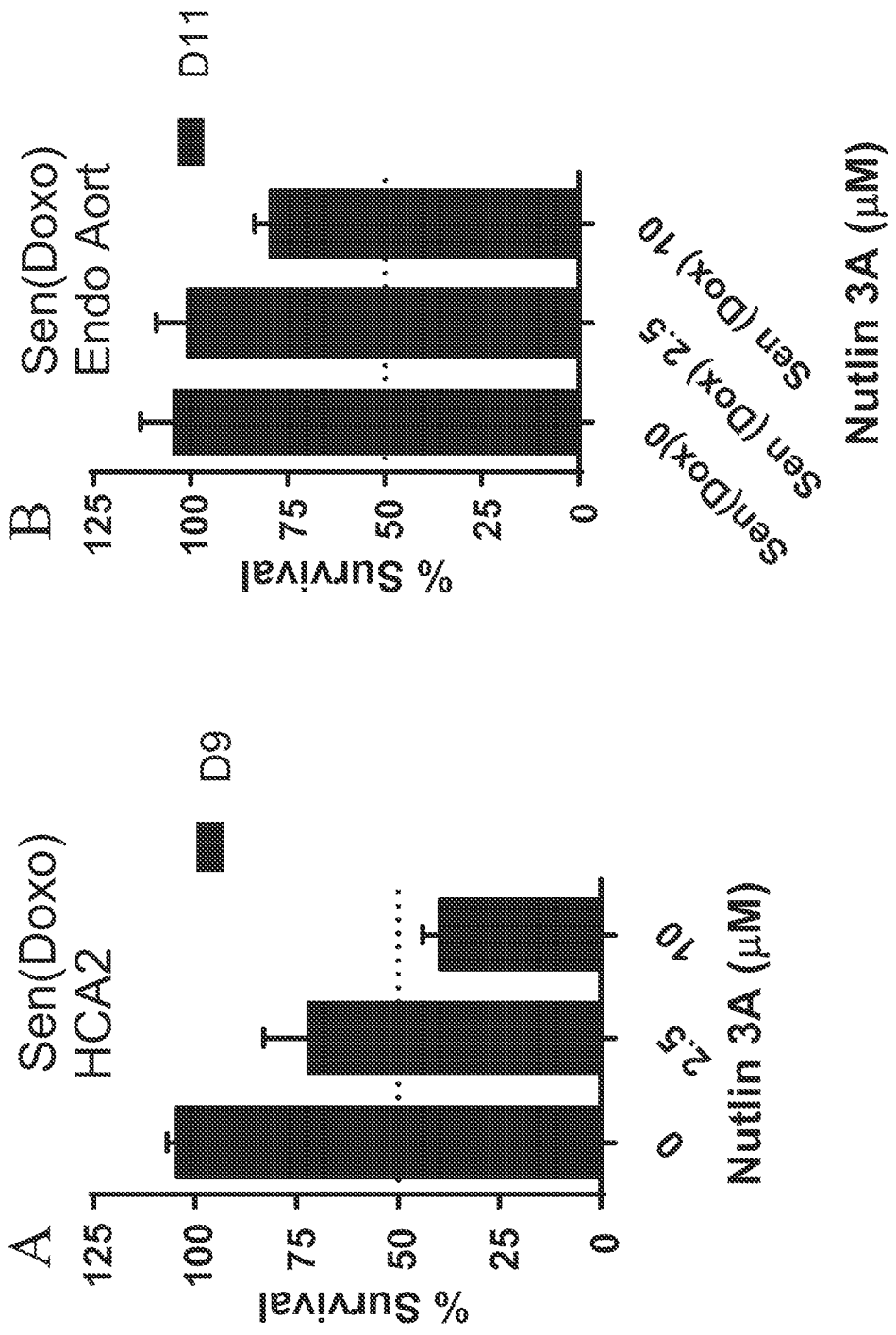

A
Sen(Doxo)
HCA2
D9
% Survival
125
100
75
50
25
0
0
2.5
10
Nutlin 3A (μM)
B
Sen(Doxo)
Endo Aort
D11
% Survival
125
100
75
50
25
0
Sen(Dox)0
Sen (Dox) 2.5
Sen (Dox) 10
Nutlin 3A (μM)

FIG. 3

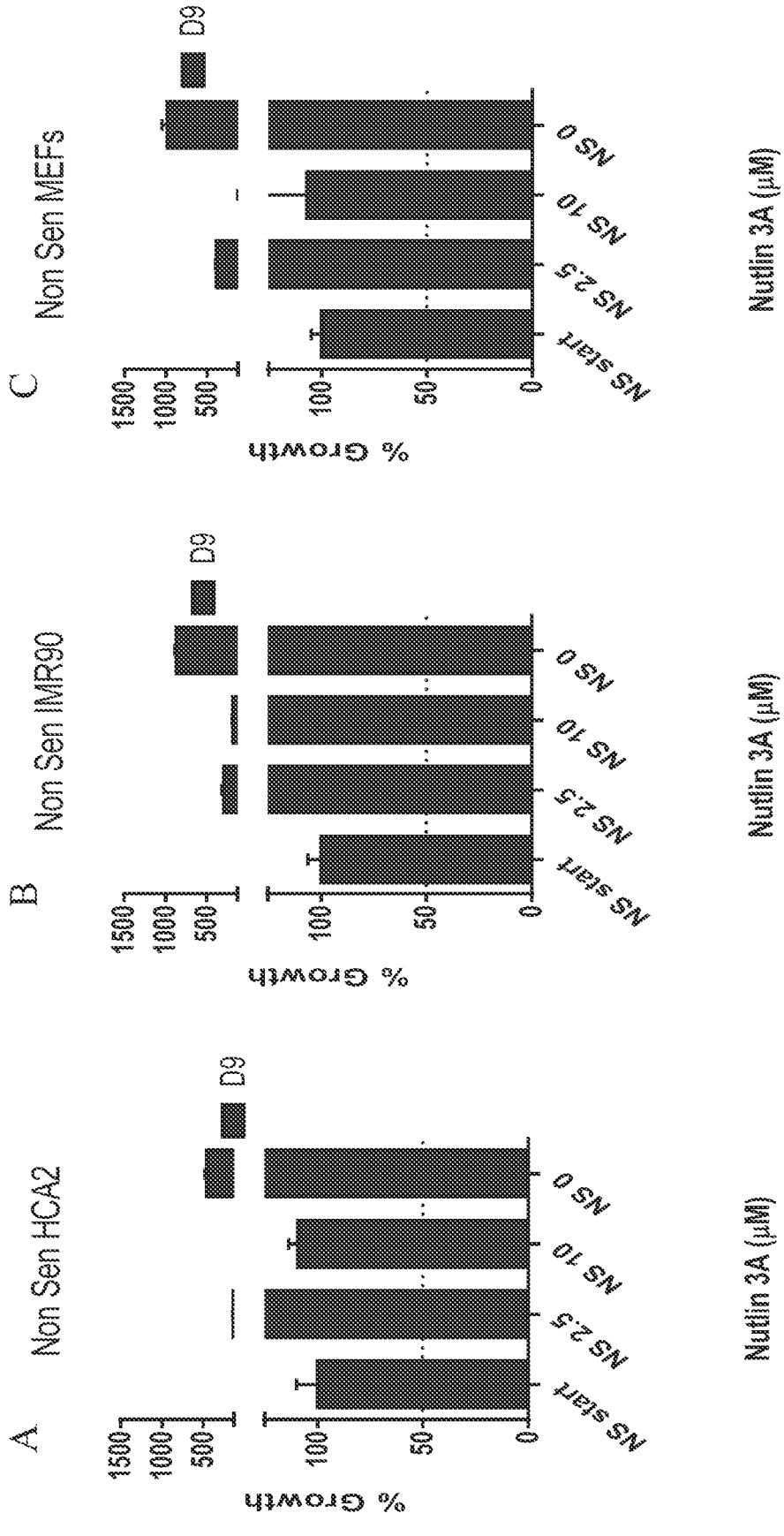

A
Non Sen HCA2
D9
% Growth
1500
1000
500
100
50
0
NS start
NS 2.5
NS 10
NS 0
Nutlin 3A (μM)
B
Non Sen IMR90
D9
% Growth
1500
1000
500
100
50
0
NS start
NS 2.5
NS 10
NS 0
Nutlin 3A (μM)
C
Non Sen MEFs
D9
% Growth
1500
1000
500
100
50
0
NS start
NS 2.5
NS 10
NS 0
Nutlin 3A (μM)

FIG. 4

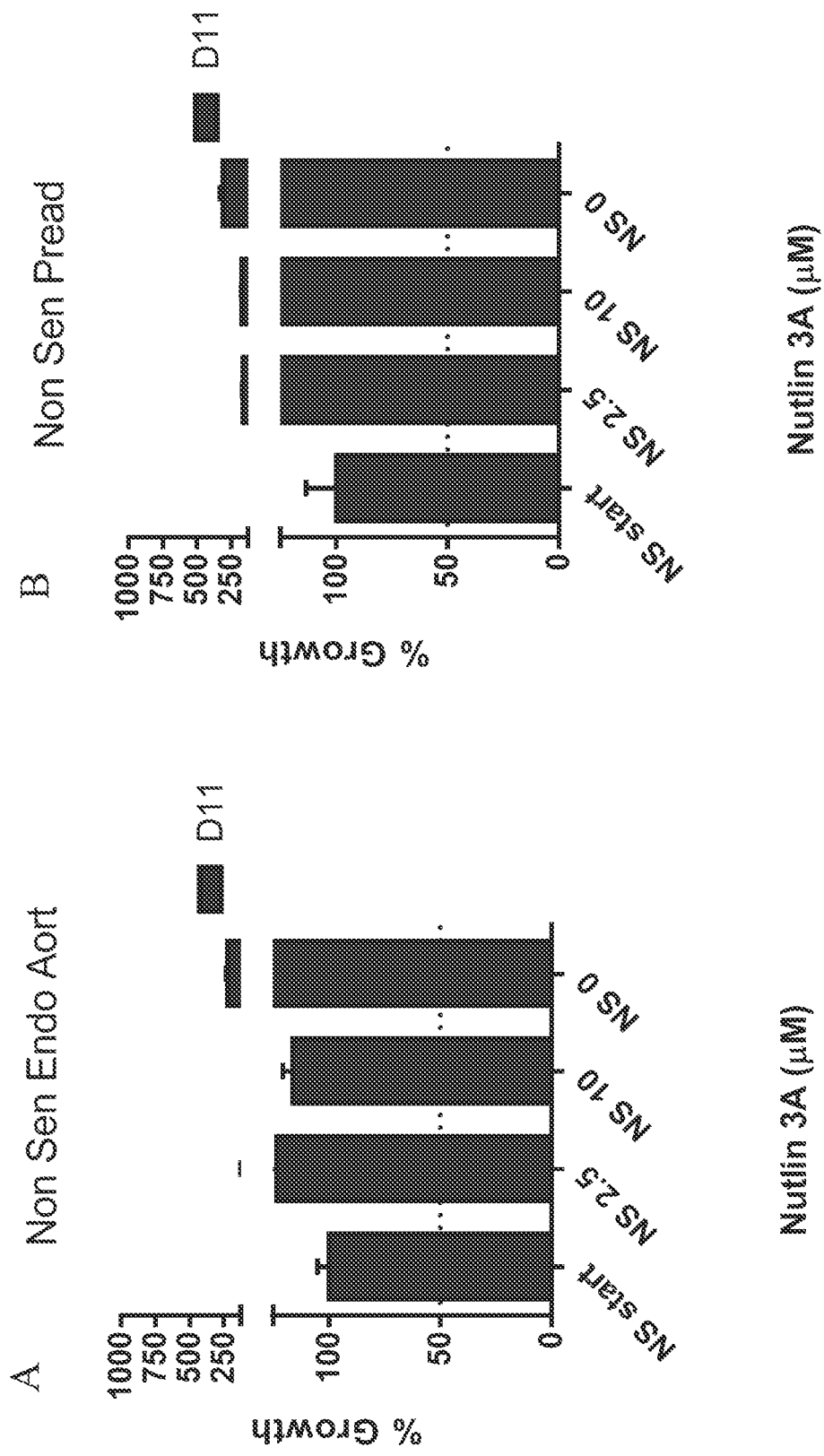
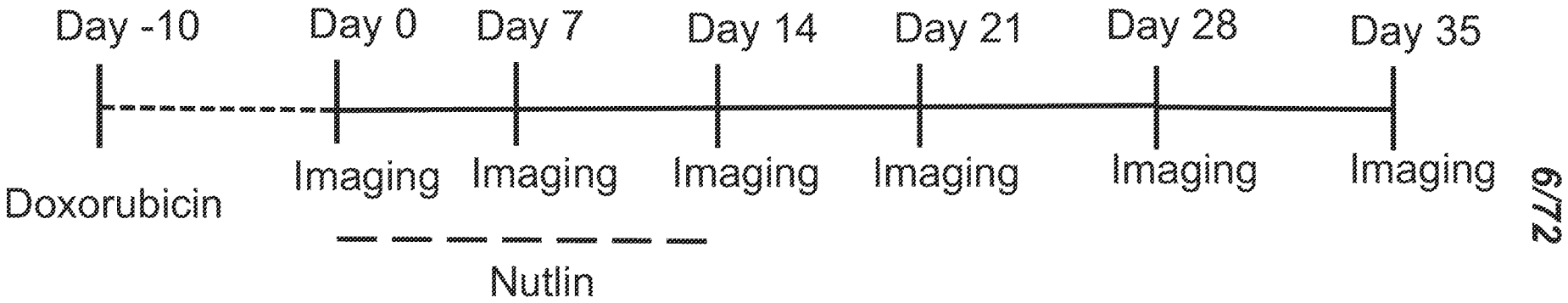

A
Non Sen Endo Aort
D11
% Growth
1000
750
500
250
100
50
0
NS start
NS 2.5
NS 10
NS 0
Nutlin 3A (μM)
B
Non Sen Pread
D11
% Growth
1000
750
500
250
100
50
0
NS start
NS 2.5
NS 10
NS 0
Nutlin 3A (μM)

FIG. 5


Day -10
Day 0
Day 7
Day 14
Day 21
Day 28
Day 35
Doxorubicin
Imaging
Imaging
Imaging
Imaging
Imaging
Imaging
Nutlin

6/72

FIG. 6

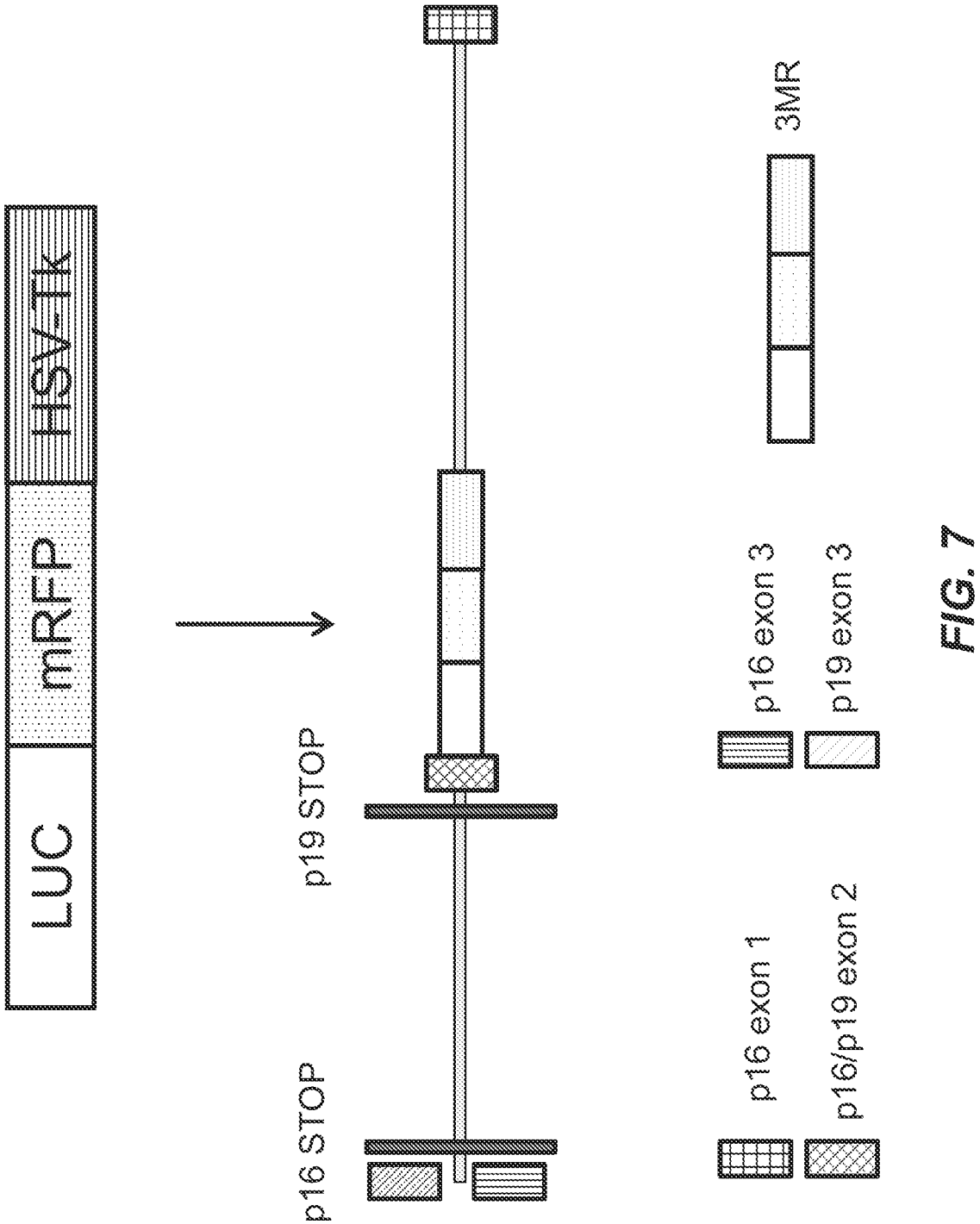

LUC
mRFP
HSV-Tk
p19 STOP
p16 STOP
3MR
p16 exon 3
p19 exon 3
p16 exon 1
p16/p19 exon 2

FIG. 7

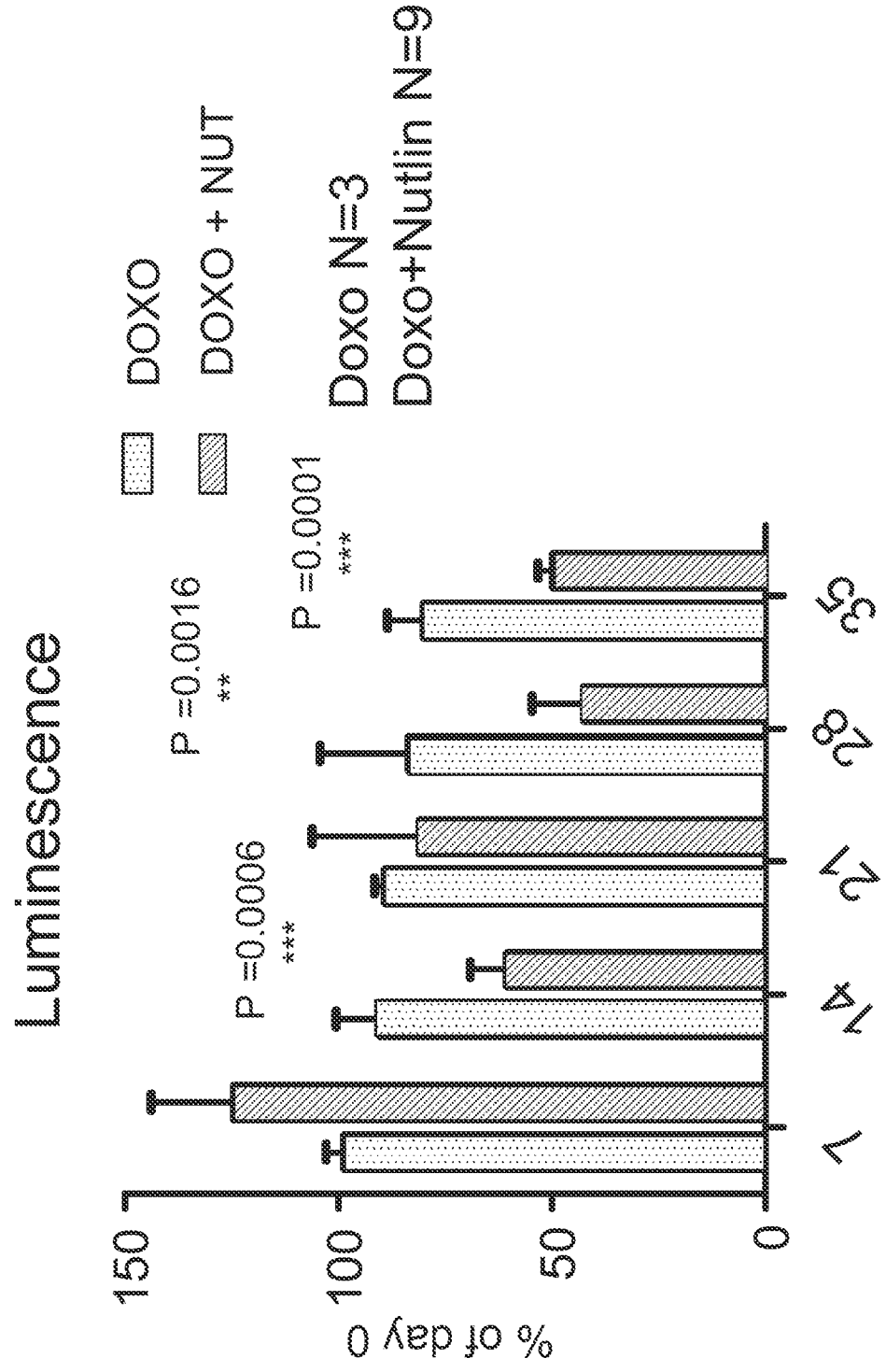

Luminescence
DOXO
DOXO + NUT
Doxo N=3
Doxo+Nutlin N=9
P =0.0016
**
P =0.0006
***
P =0.0001
***
% of day 0
150
100
50
0
7
14
21
28
35

FIG. 8

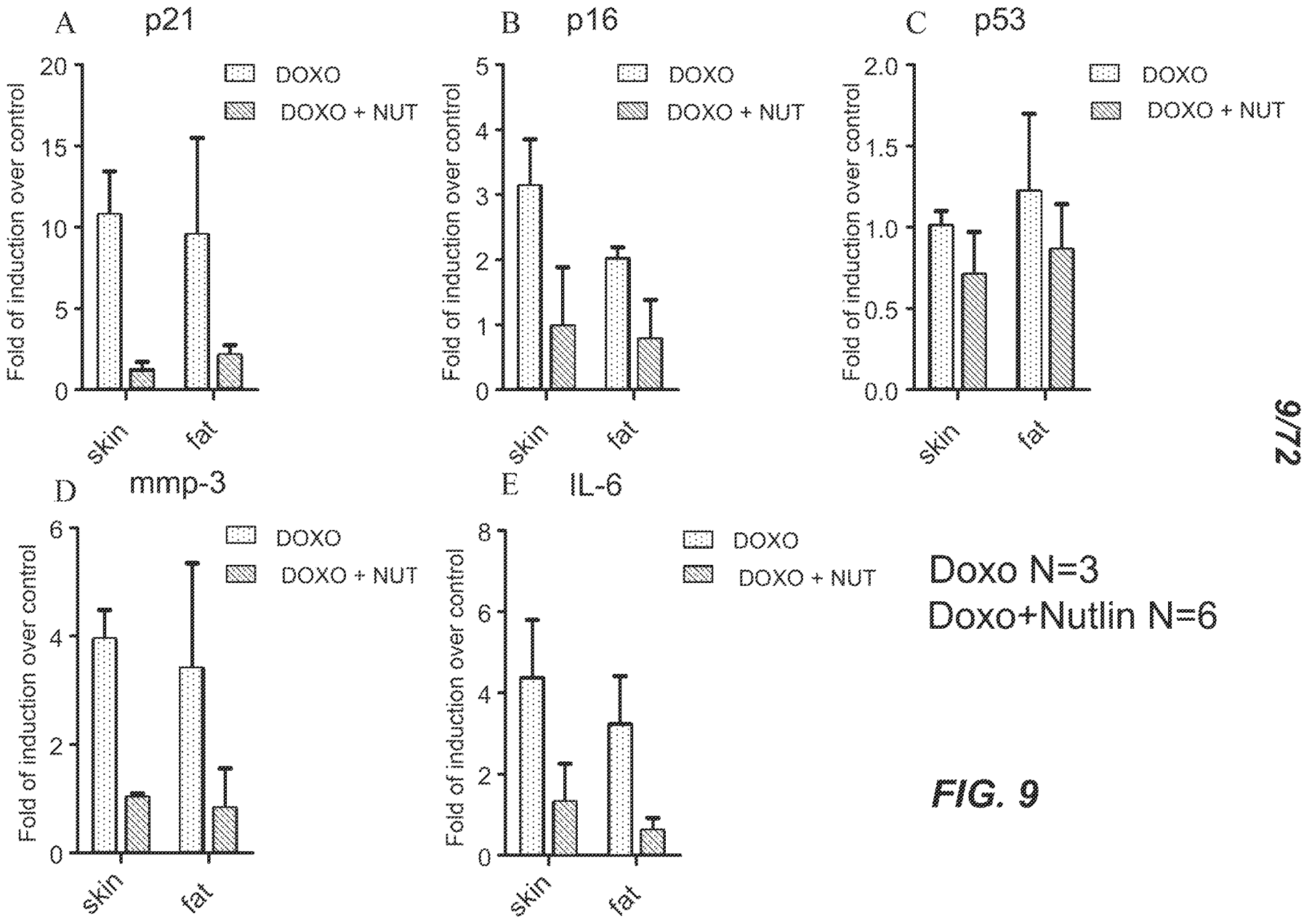

A
p21
B
p16
C
p53
D
mmp-3
E
IL-6
DOXO
DOXO + NUT
Fold of induction over control
skin
fat
Doxo N=3
Doxo+Nutlin N=6

FIG. 9

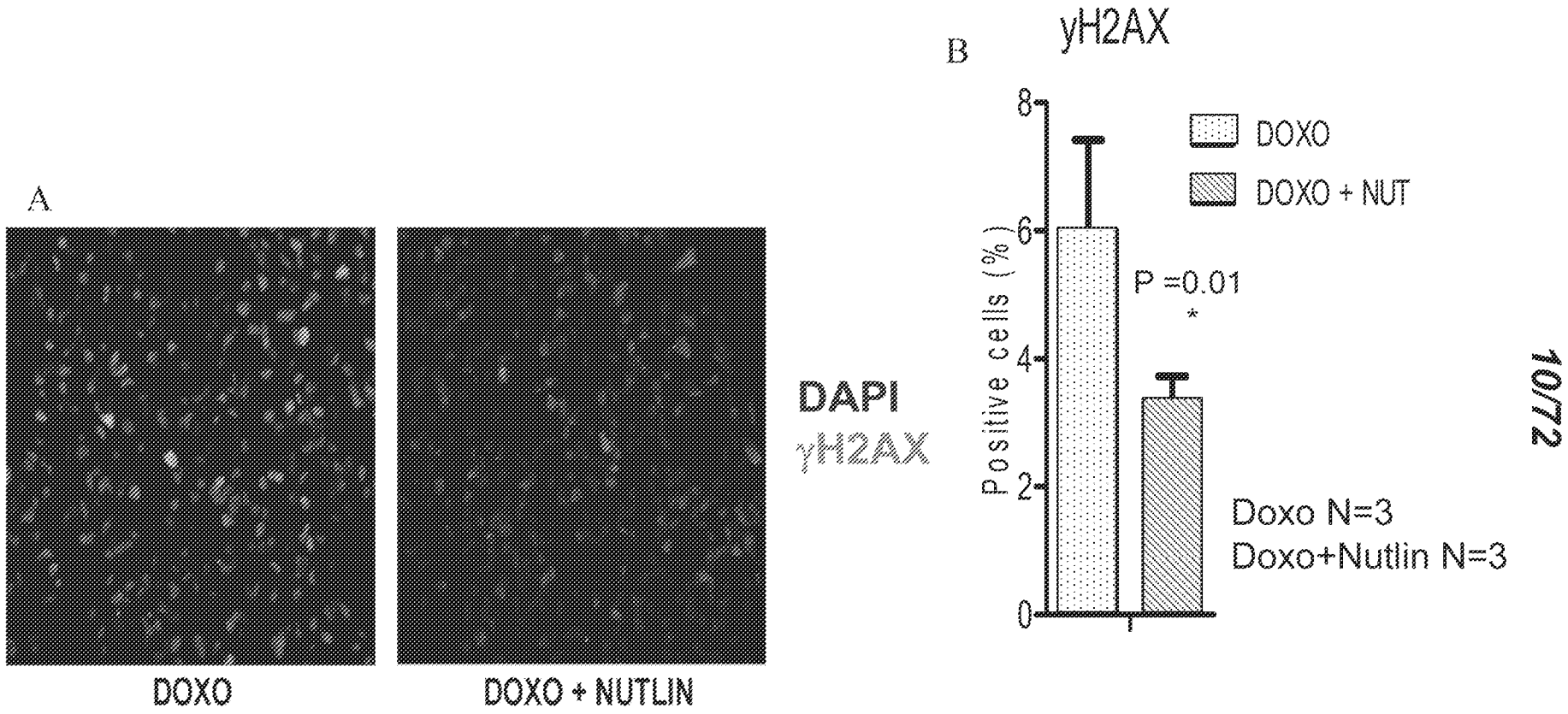

A
DOXO
DOXO + NUTLIN
DAPI
γH2AX
B
γH2AX
DOXO
DOXO + NUT
Positive cells (%)
P =0.01
*
Doxo N=3
Doxo+Nutlin N=3
0
2
4
6
8

FIG. 10

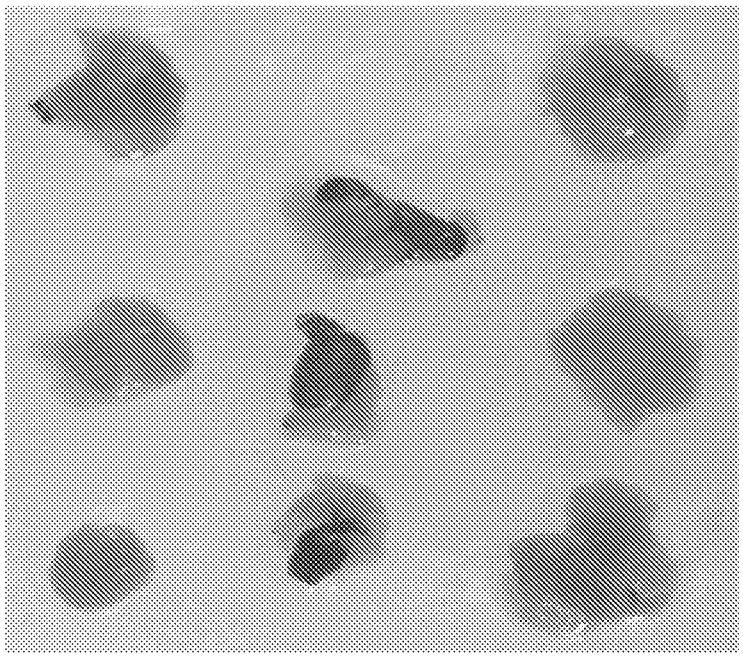


FIG. 11

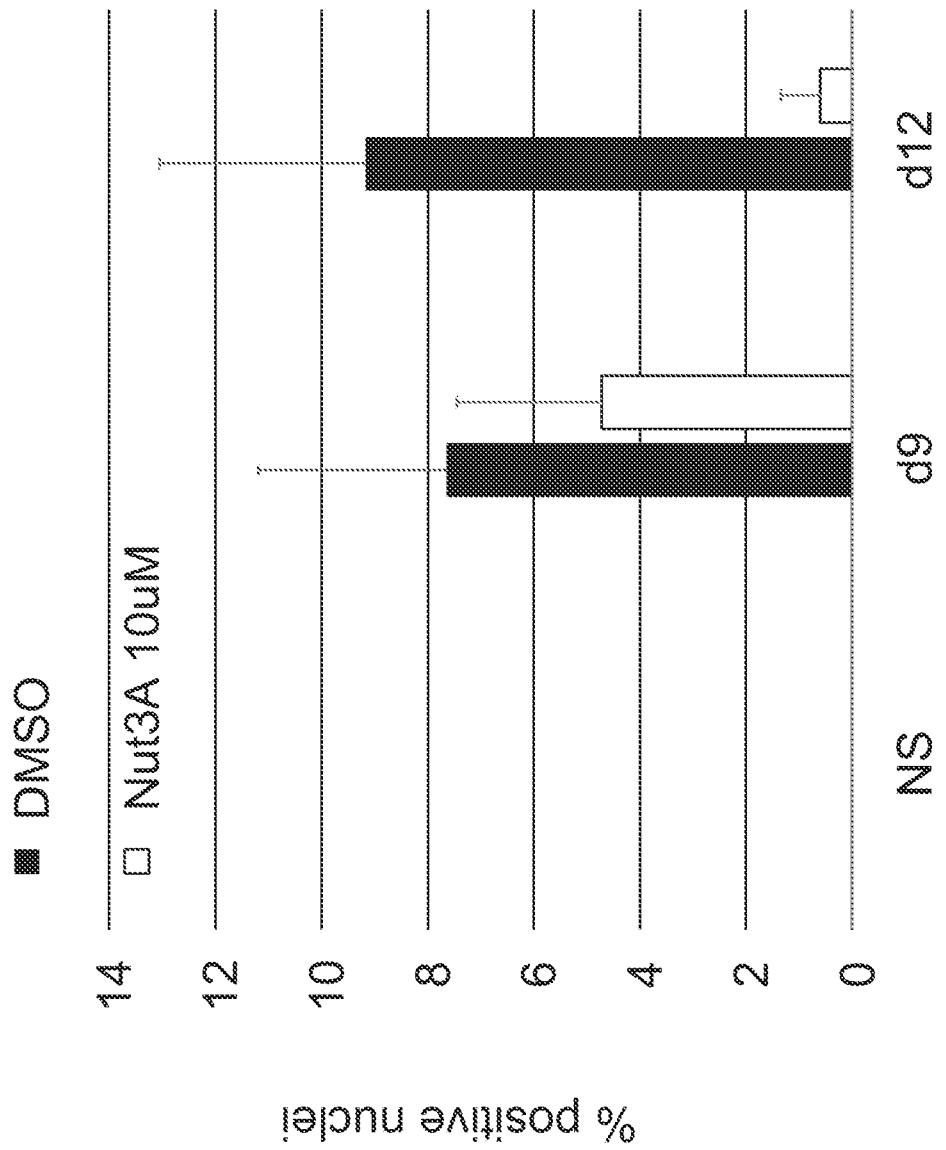


FIG. 12A

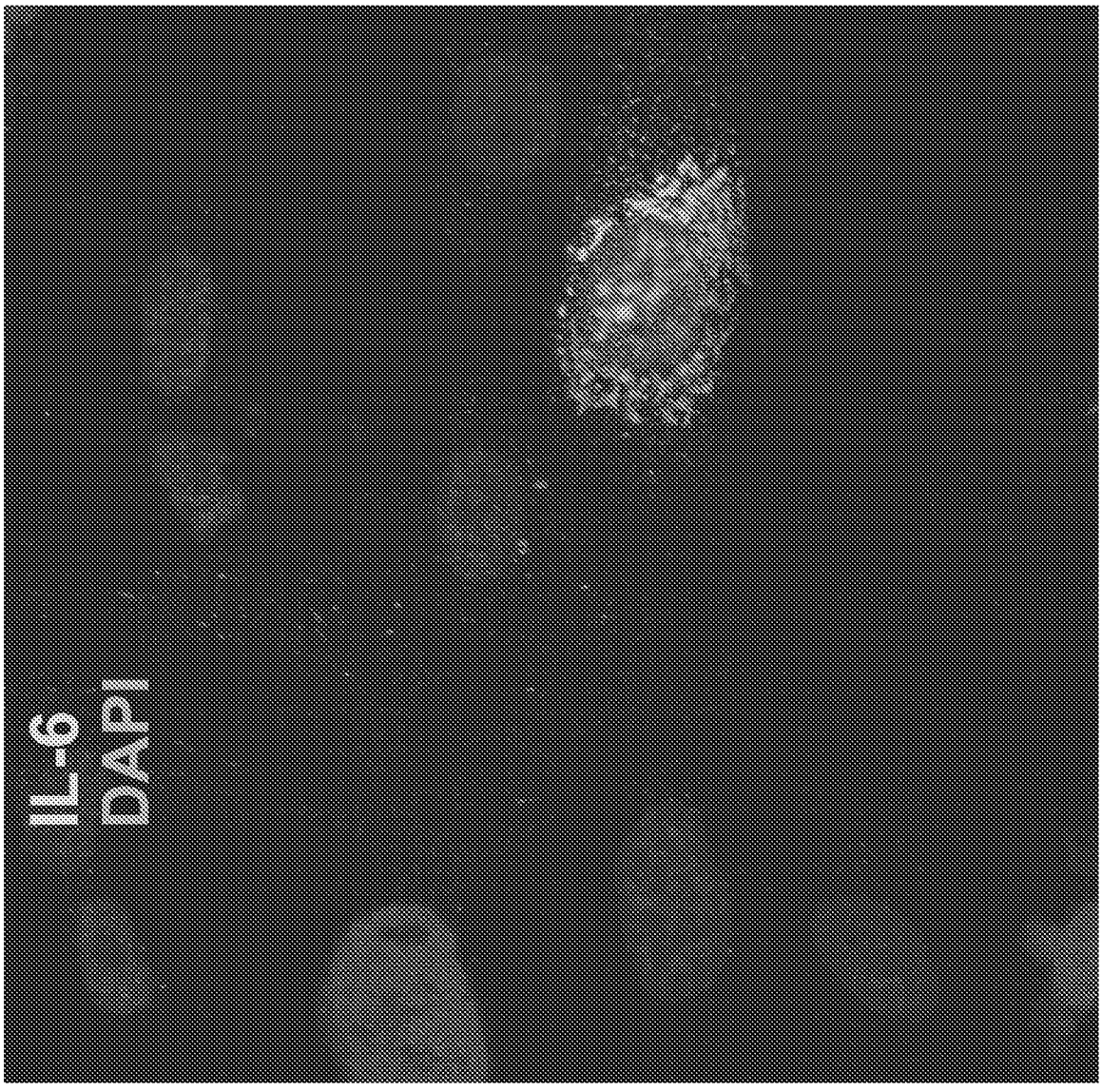


FIG. 12B

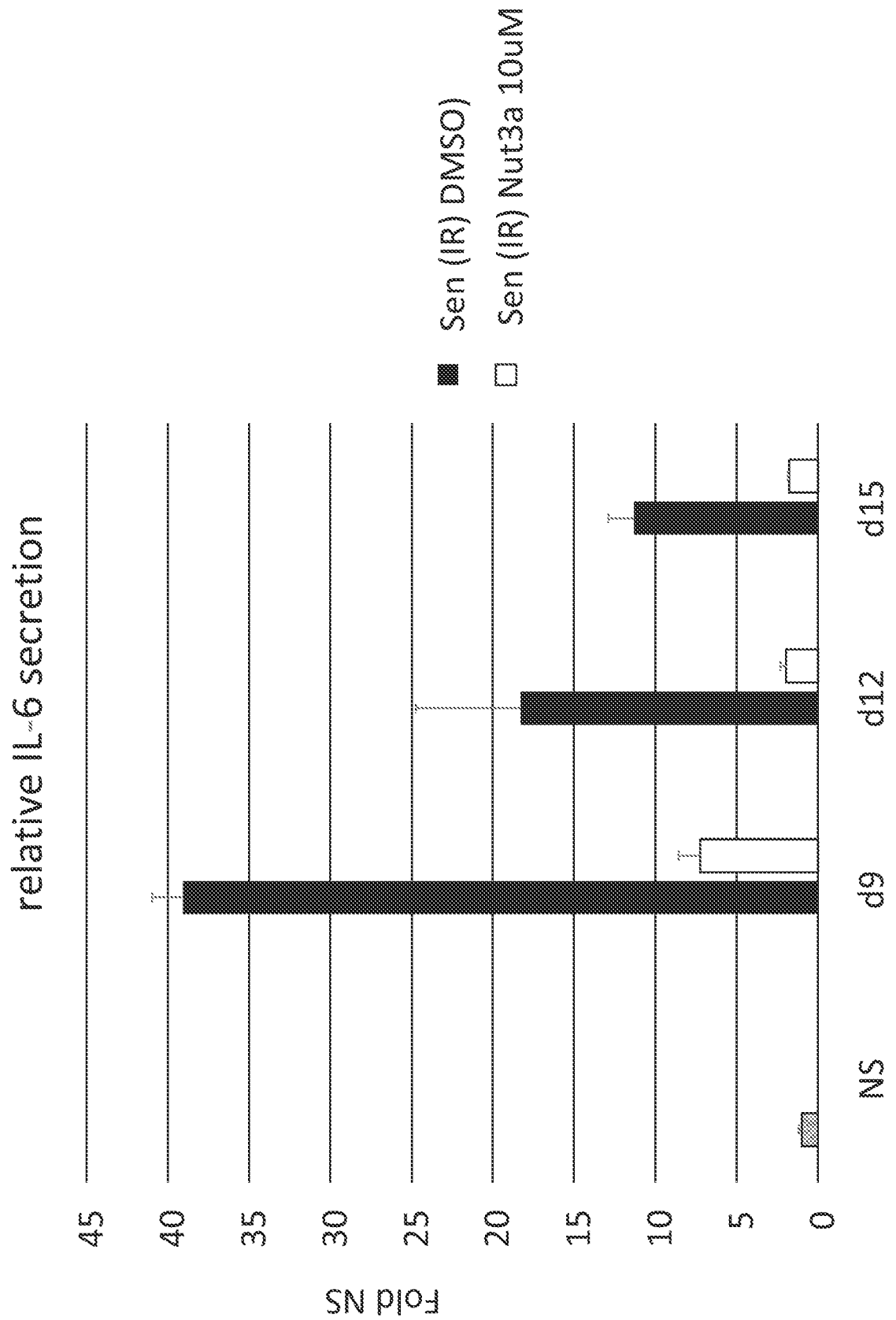

relative IL-6 secretion
Sen (IR) DMSO
Sen (IR) Nut3a 10uM
fold NS
45
40
35
30
25
20
15
10
5
0
NS
d9
d12
d15

FIG. 12C

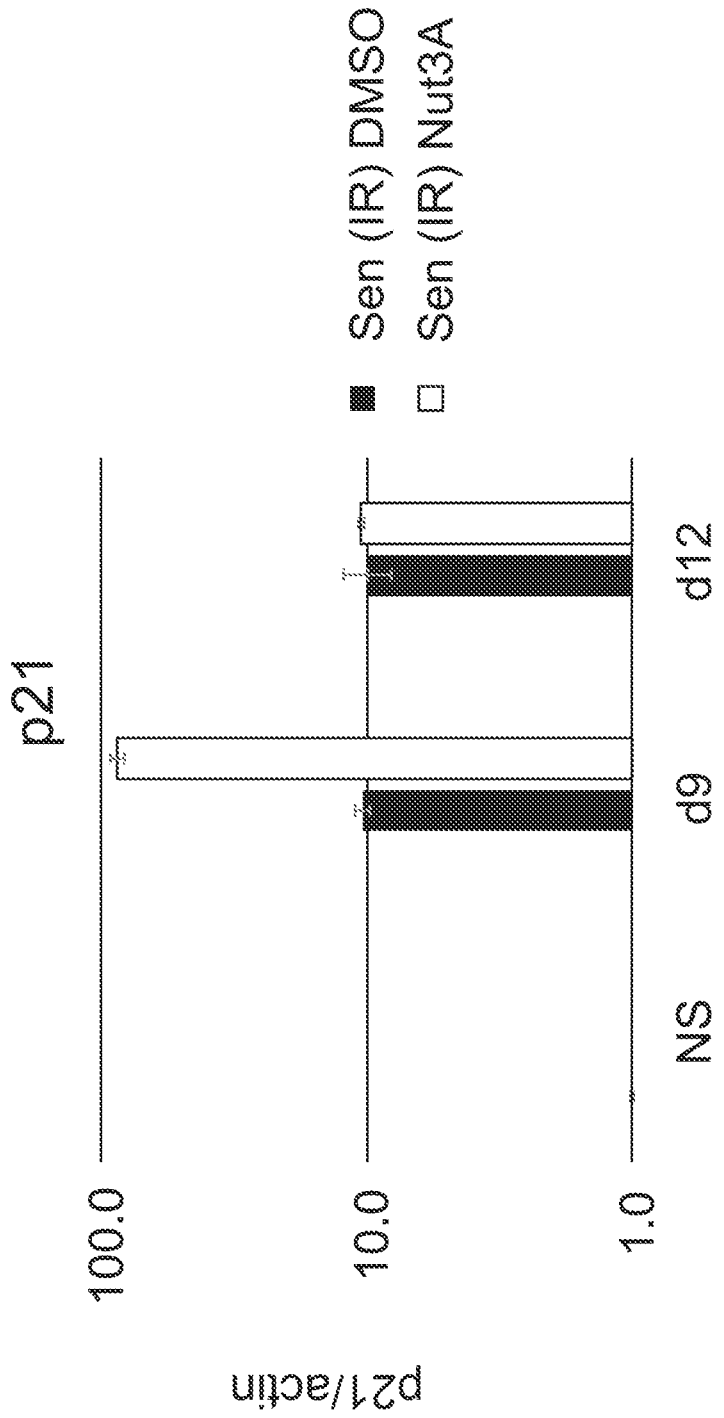


FIG. 13A

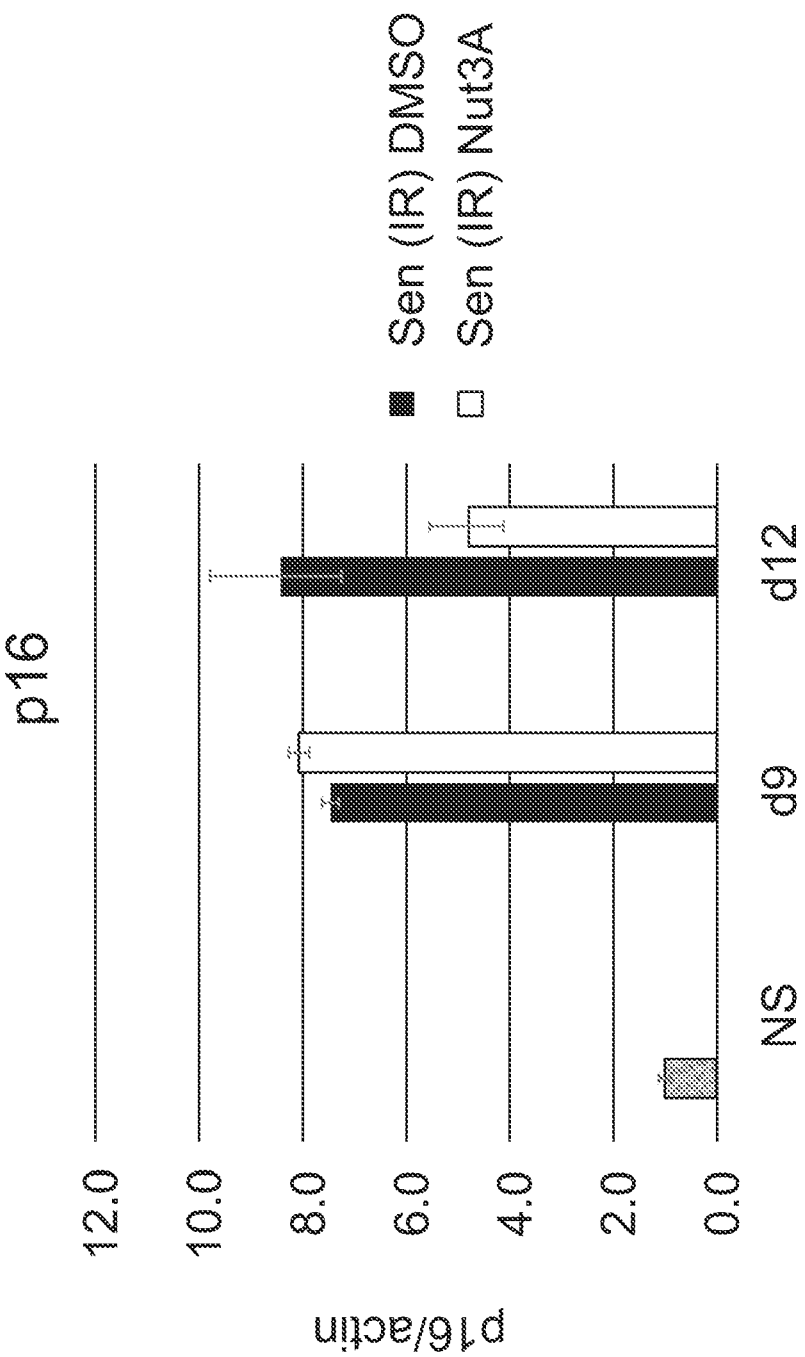

p16
Sen (IR) DMSO
Sen (IR) Nut3A
p16/actin
12.0
10.0
8.0
6.0
4.0
2.0
0.0
NS
d9
d12

FIG. 13B

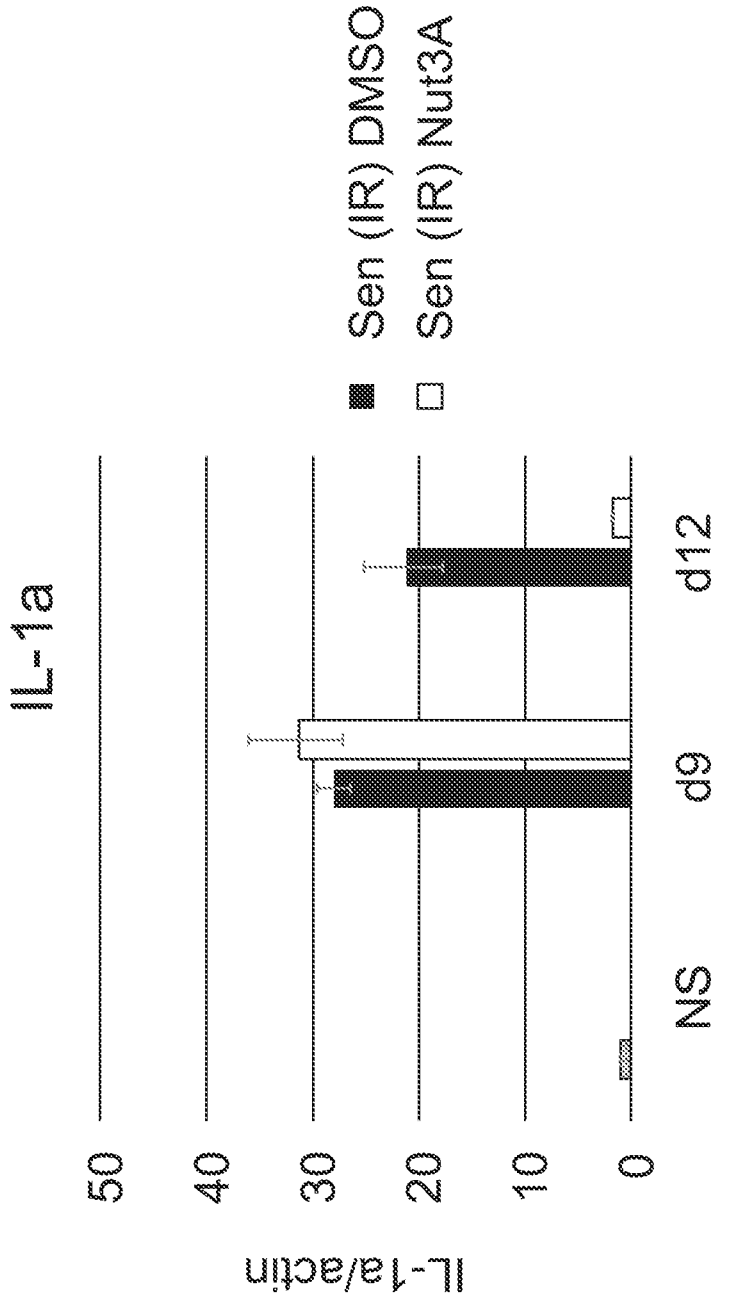

IL-1a
Sen (IR) DMSO
Sen (IR) Nut3A
IL-1a/actin
50
40
30
20
10
0
NS
d9
d12

FIG. 13C

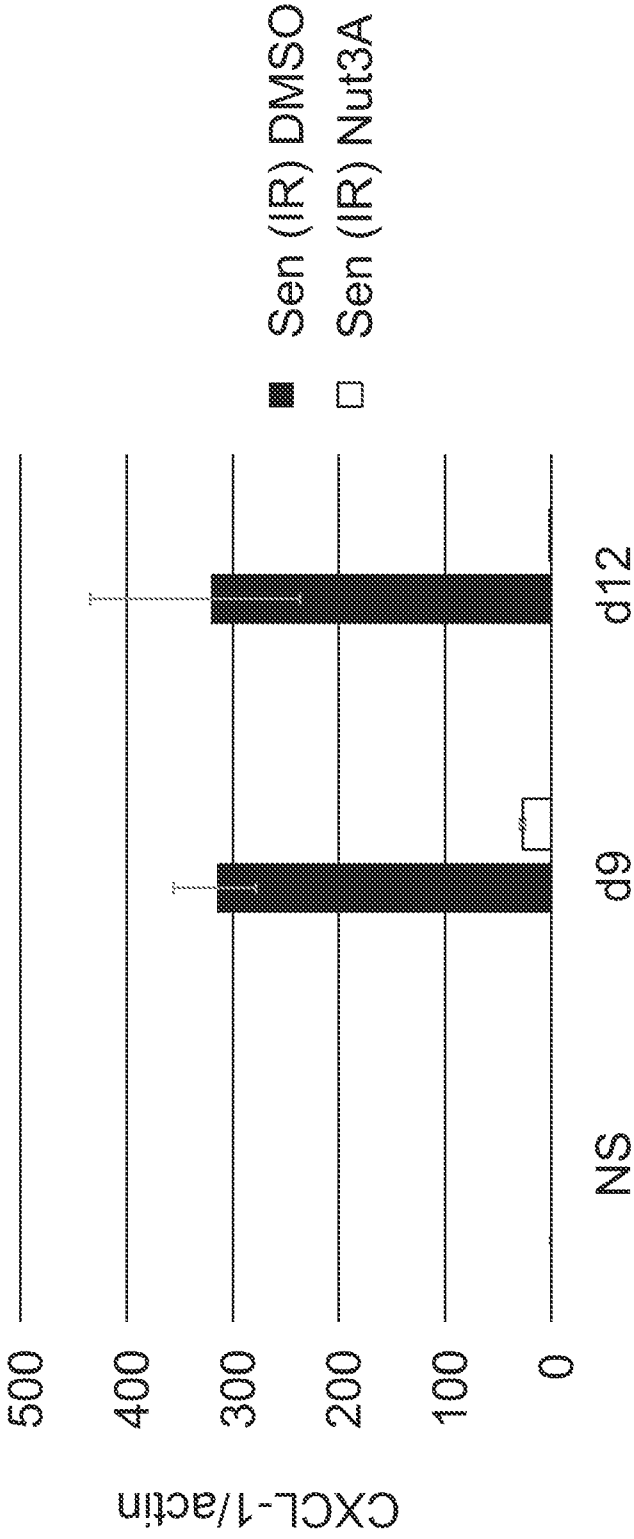

CXCL-1
Sen (IR) DMSO
Sen (IR) Nut3A
CXCL-1/actin
500
400
300
200
100
0
NS
d9
d12

FIG. 13D

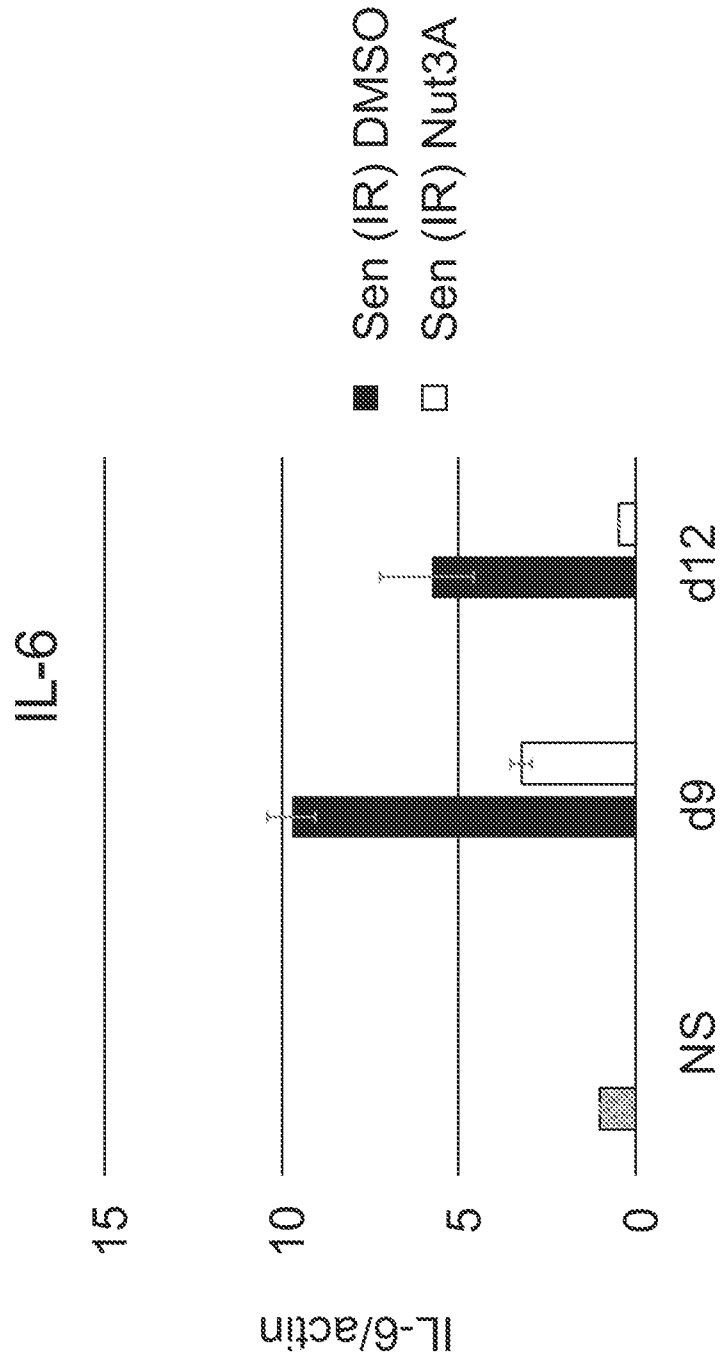


FIG. 13E

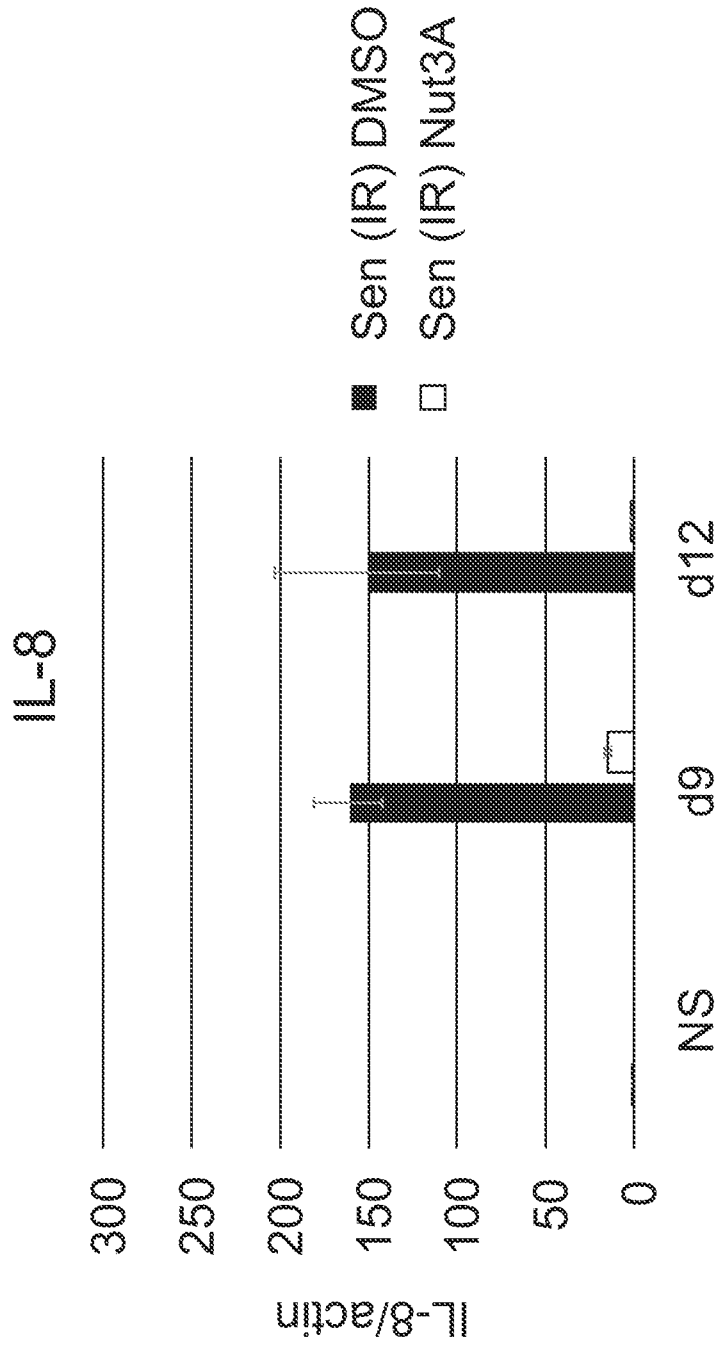

IL-8
Sen (IR) DMSO
Sen (IR) Nut3A
IL-8/actin
300
250
200
150
100
50
0
NS
d9
d12

FIG. 13F

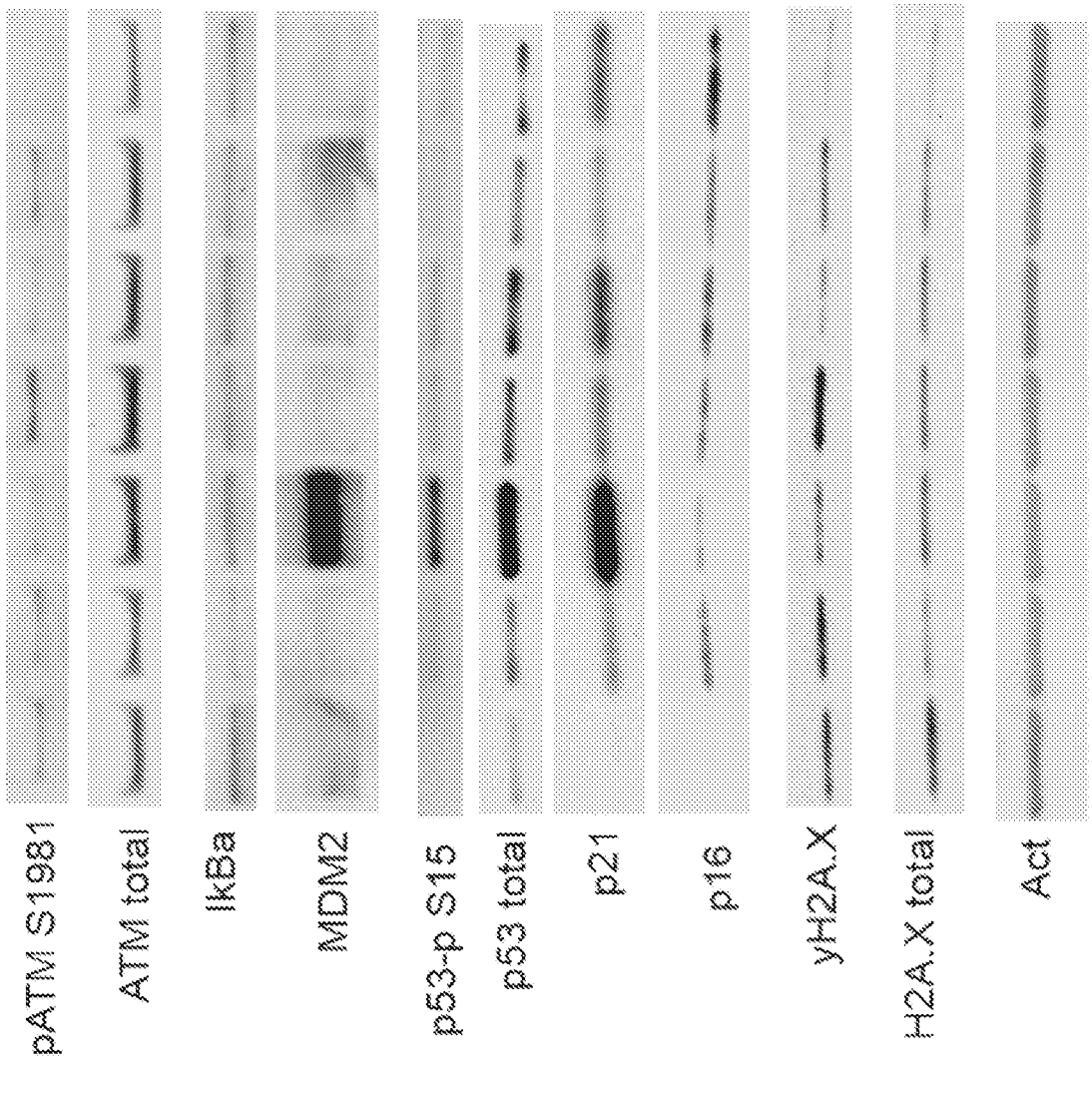
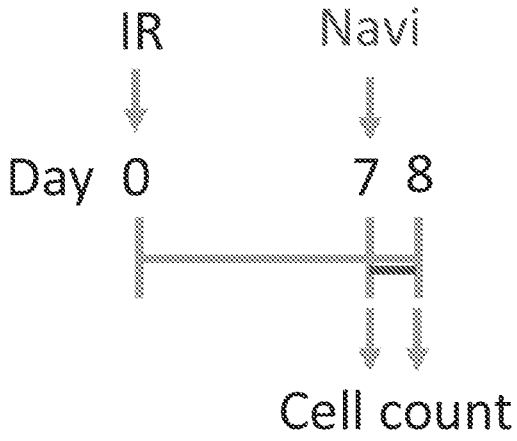

pATM S1981
ATM total
IkBa
MDM2
p53-p S15
p53 total
p21
p16
γH2A.X
H2A.X total
Act
10uM Nutlin:
-
-
+
-
+
-
+
NS
Xd9
Xd12
Xd15

FIG. 14

Sen(IR)



Non Sen

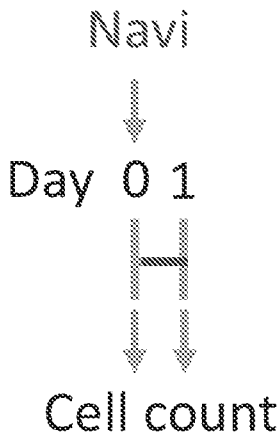


FIG. 15

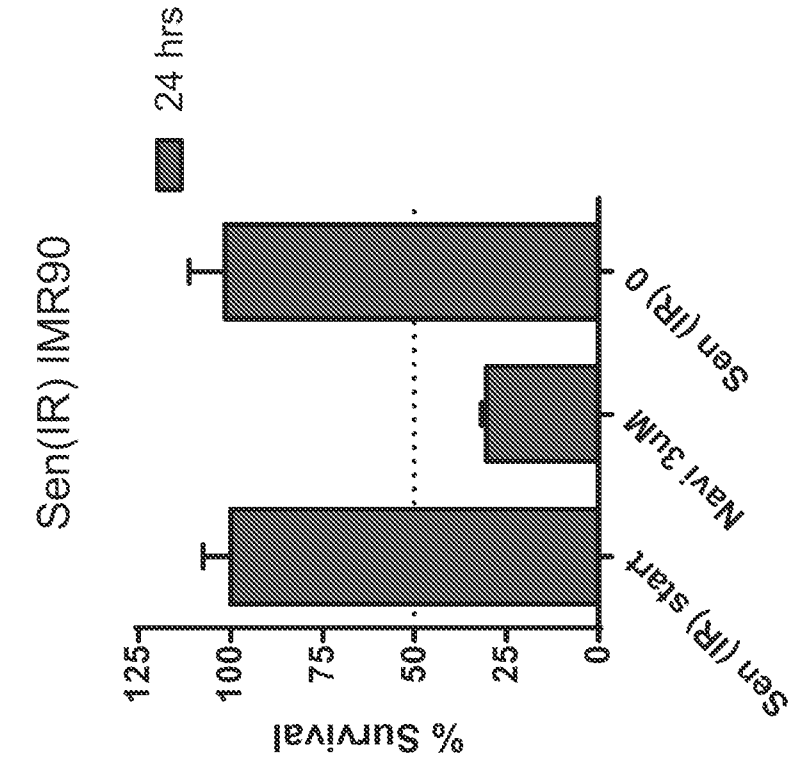

Sen(IR) IMR90
24 hrs
% Survival
125
100
75
50
25
0
Sen (IR) 0
Navi 3uM
Sen (IR) 5hrt

FIG. 17

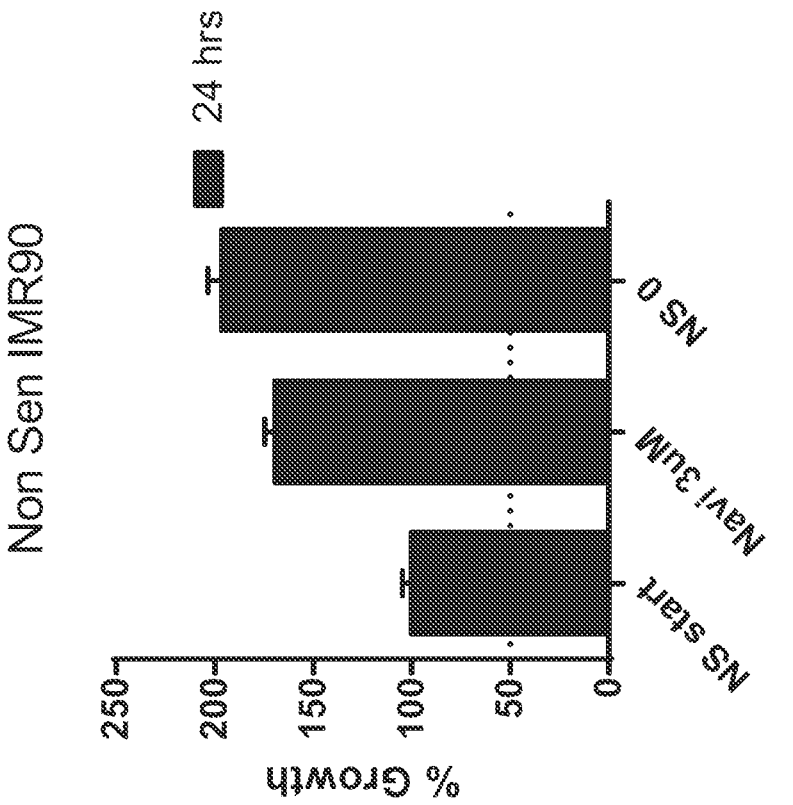

Non Sen IMR90
24 hrs
% Growth
250
200
150
100
50
0
NS 0
Navi 3uM
NS 5hrt

FIG. 16

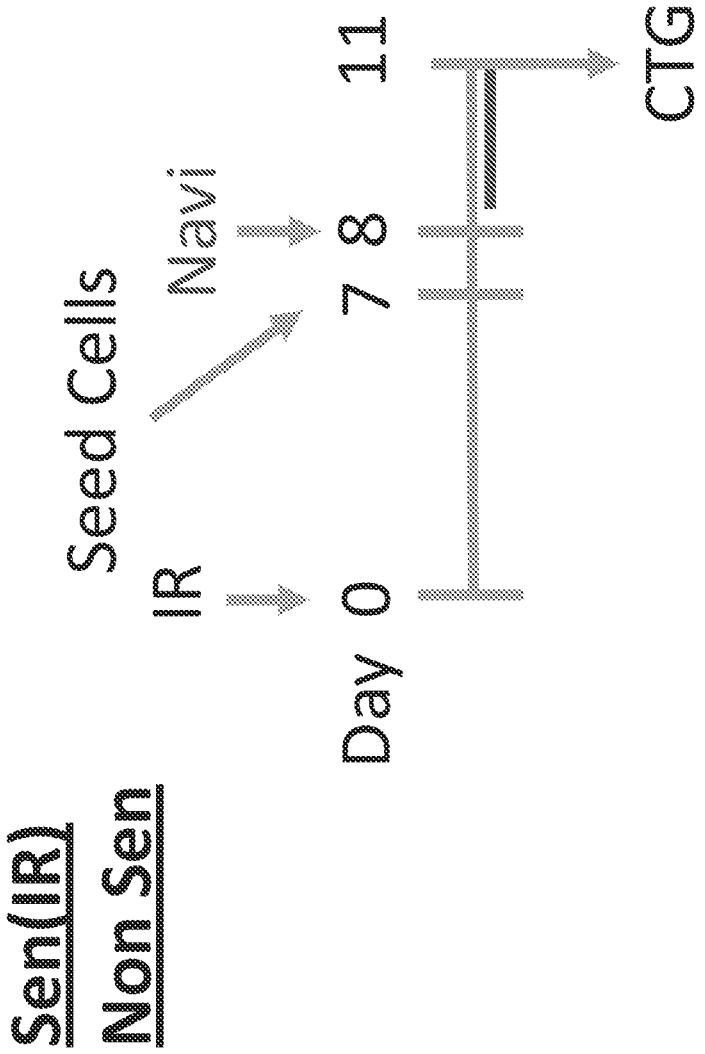


FIG. 18

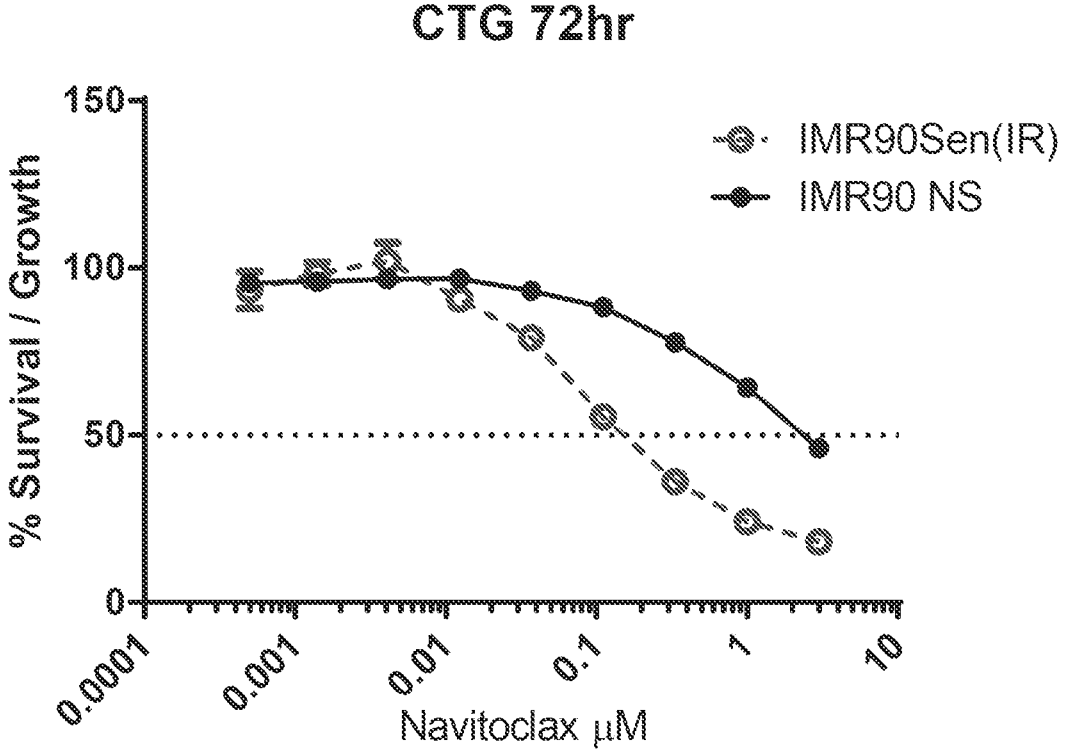

CTG 72hr
150
100
50
0
% Survival / Growth
IMR90Sen(IR)
IMR90 NS
0.0001
0.001
0.01
0.1
1
10
Navitoclax μM

FIG. 19

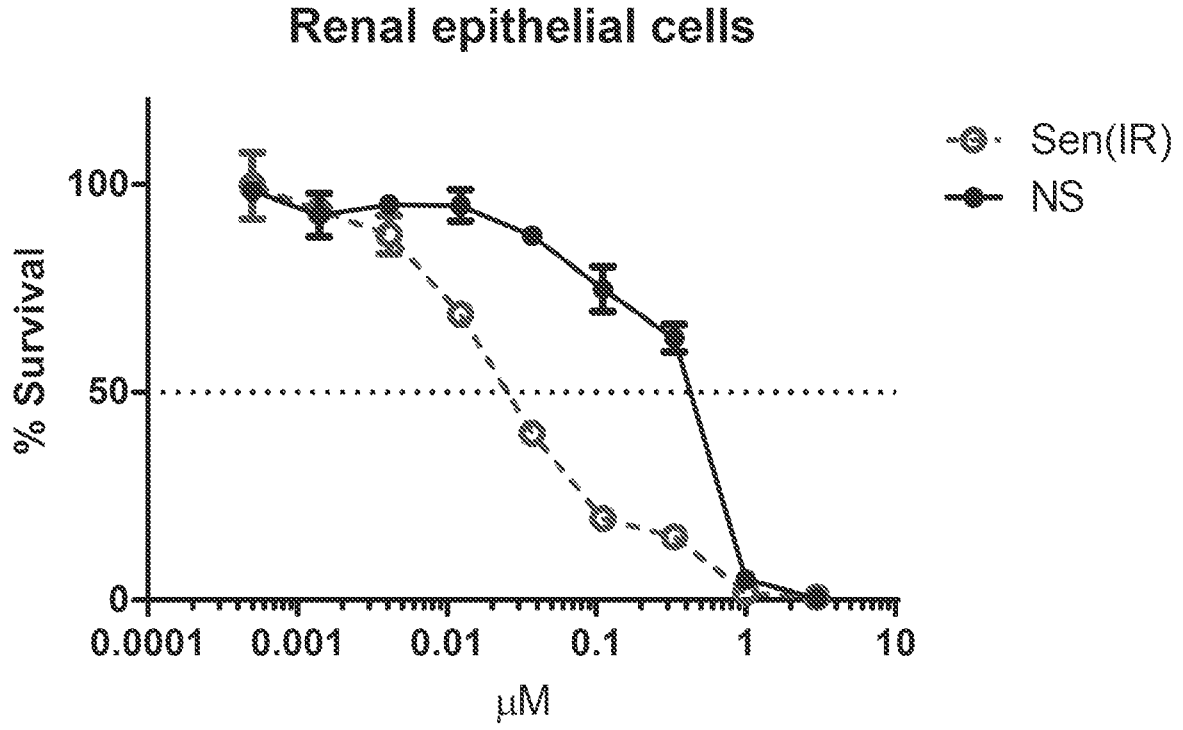

Renal epithelial cells
100
50
0
% Survival
Sen(IR)
NS
0.0001
0.001
0.01
0.1
1
10
μM

FIG. 20

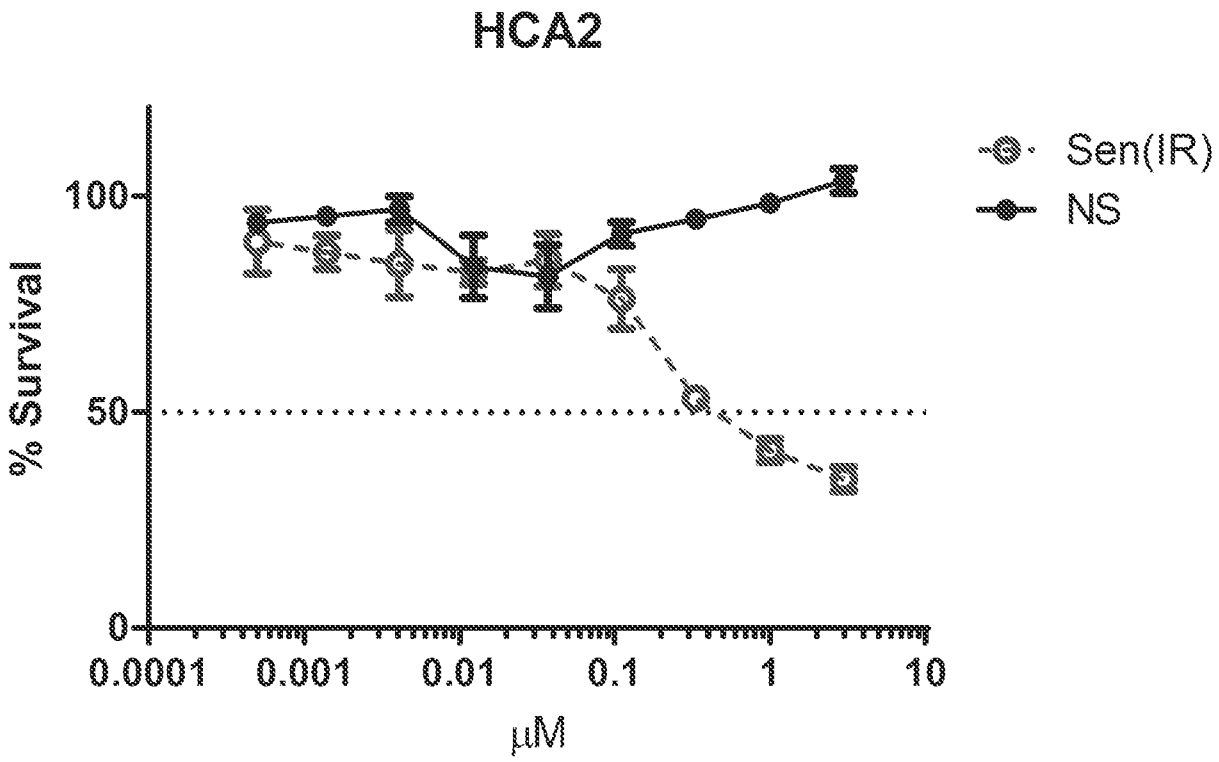

HCA2
Sen(IR)
NS
% Survival
100
50
0
0.0001
0.001
0.01
0.1
1
10
μM

FIG. 21

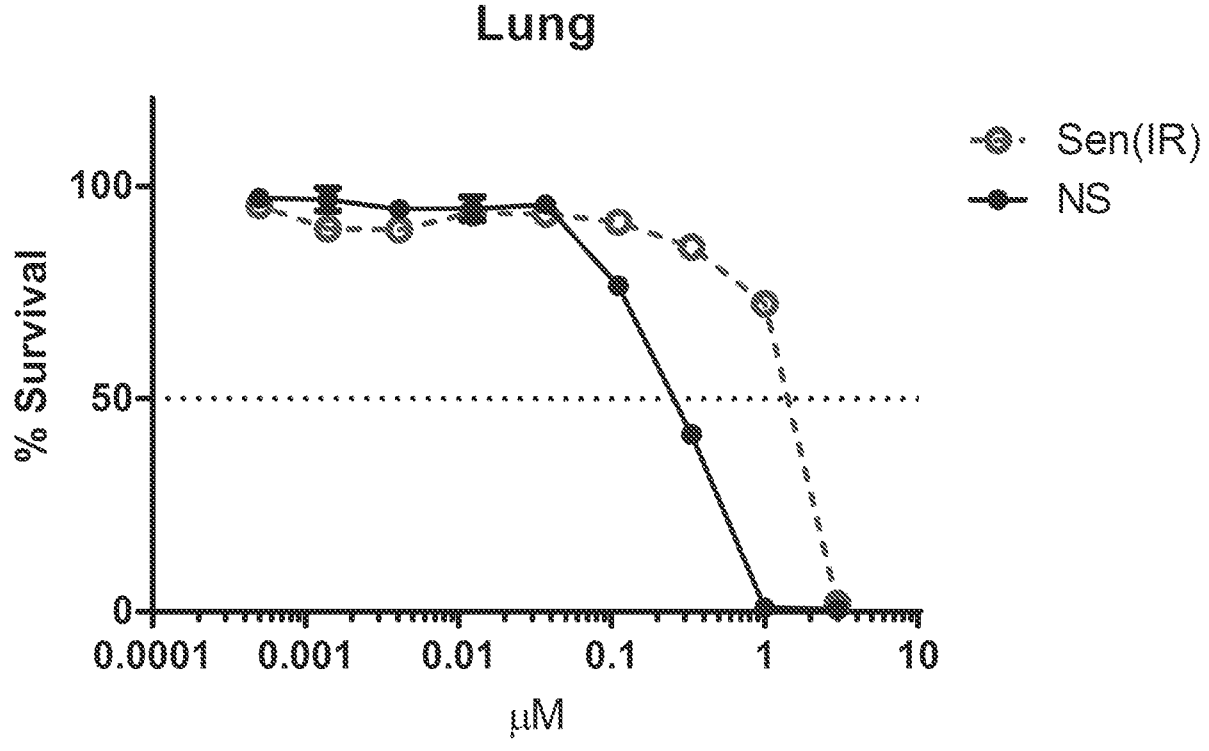

Lung
Sen(IR)
NS
% Survival
100
50
0
0.0001
0.001
0.01
0.1
1
10
μM

FIG. 22

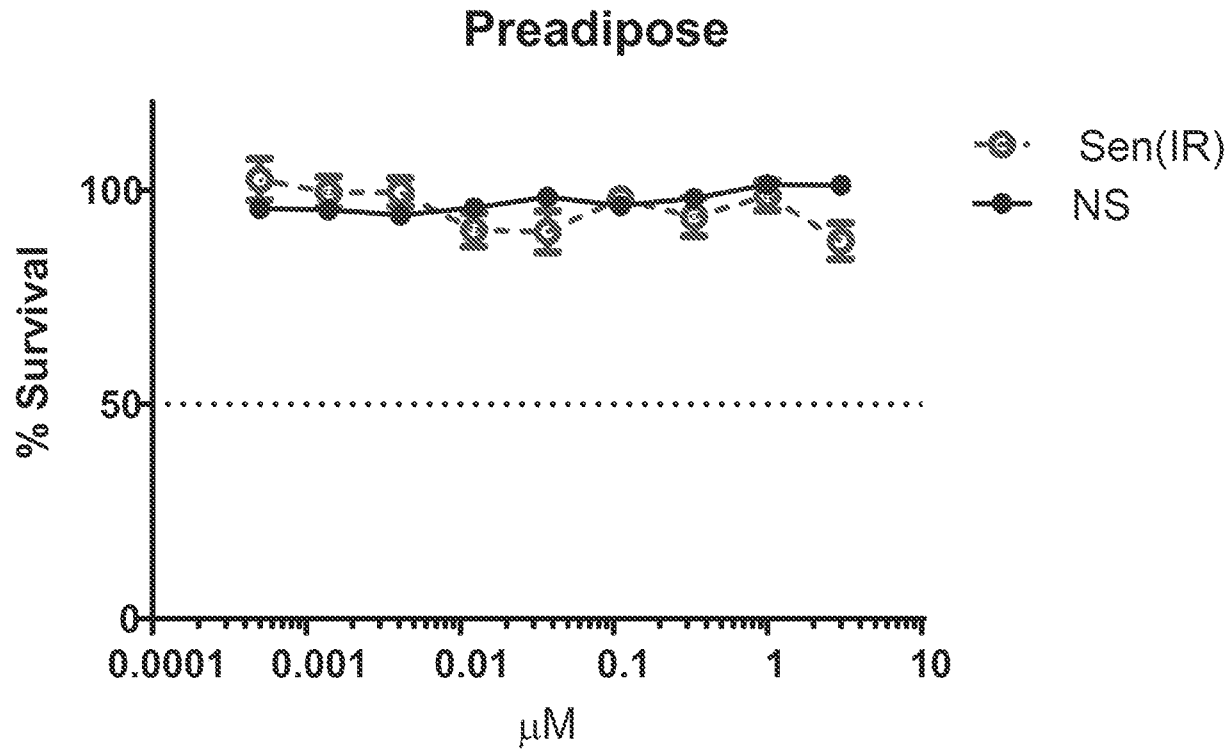

Preadipose
Sen(IR)
NS
% Survival
100
50
0
0.0001
0.001
0.01
0.1
1
10
μM

FIG. 23

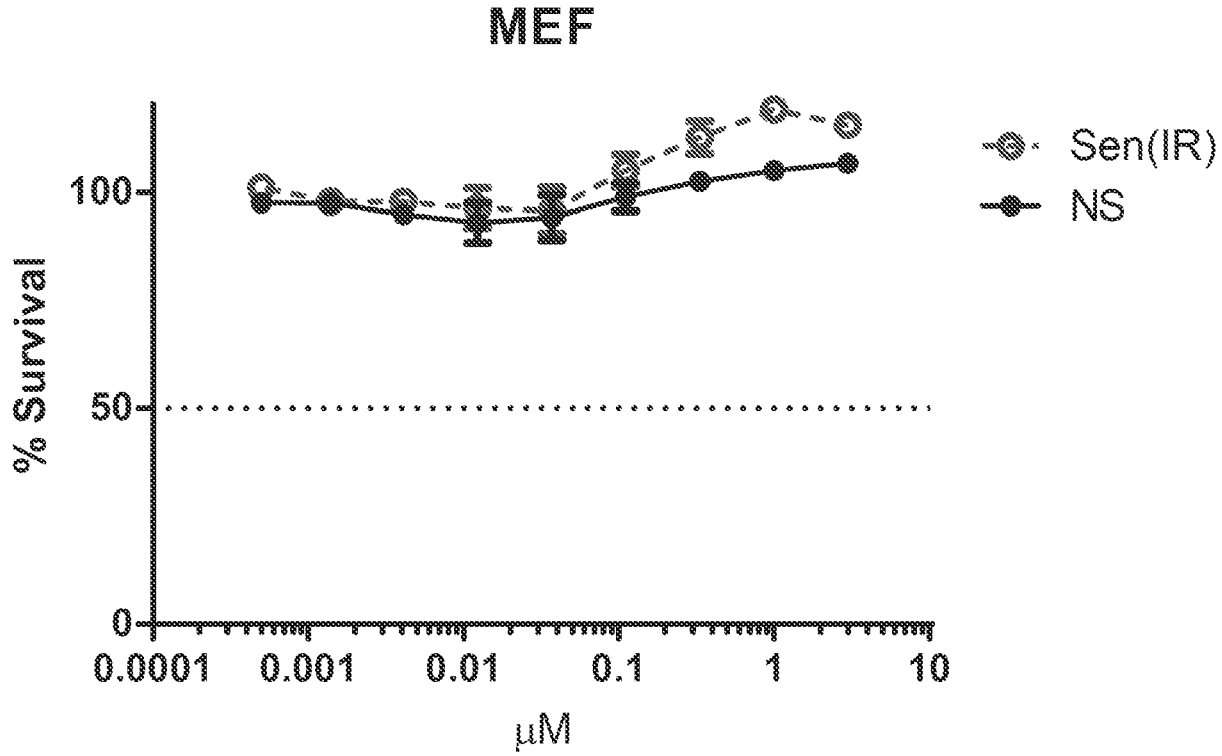

MEF
Sen(IR)
NS
% Survival
100
50
0
0.0001
0.001
0.01
0.1
1
10
μM

FIG. 24

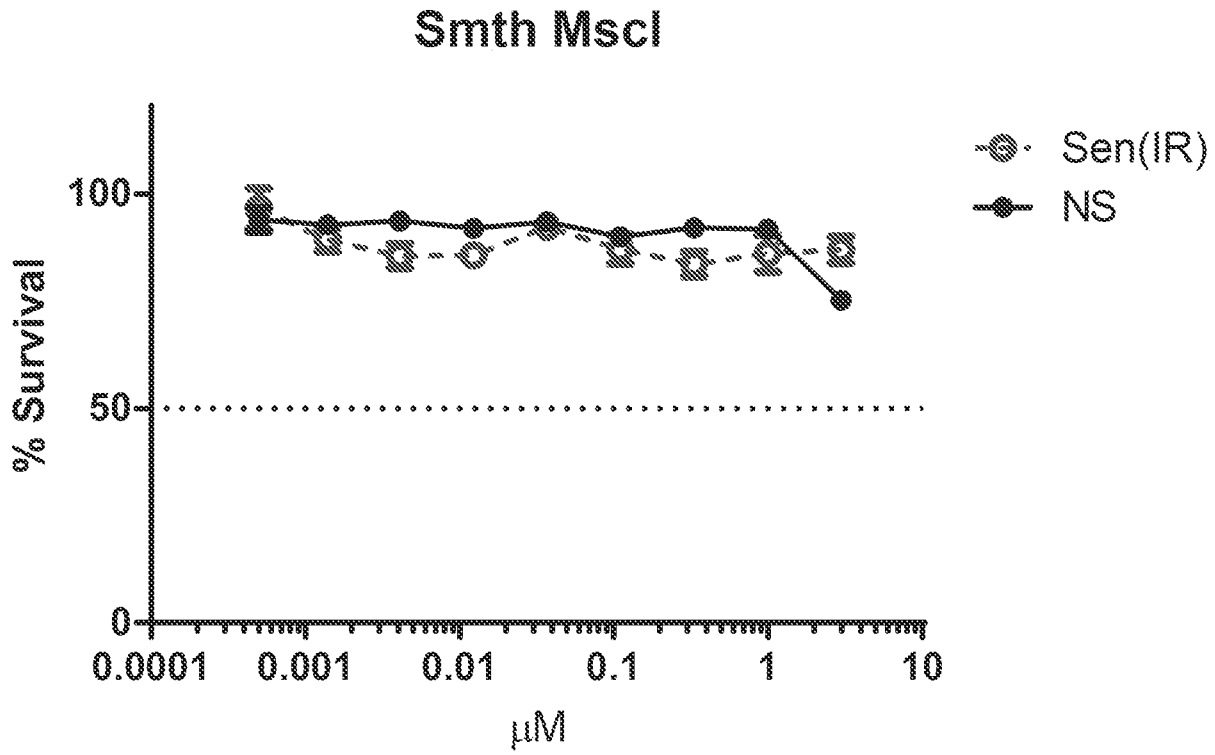

Smth Mscl
Sen(IR)
NS
100
50
0
% Survival
0.0001
0.001
0.01
0.1
1
10
μM

FIG. 25

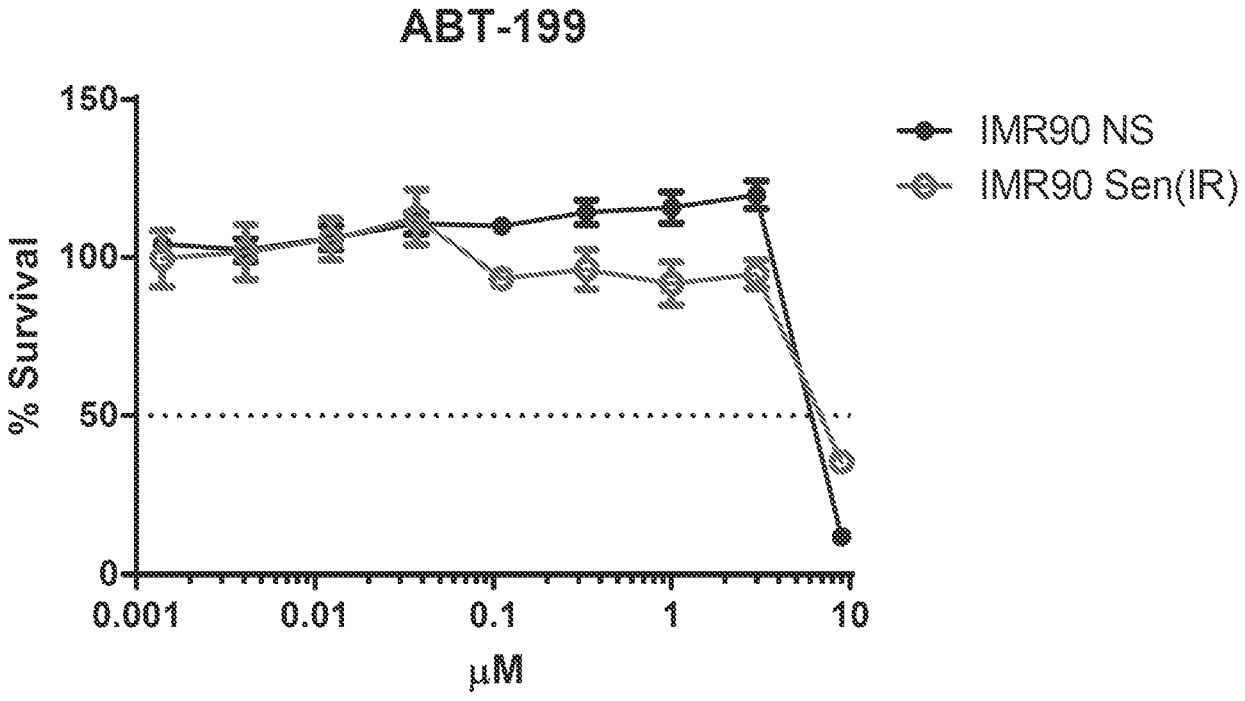

ABT-199
IMR90 NS
IMR90 Sen(IR)
150
100
50
0
% Survival
0.001
0.01
0.1
1
10
μM

FIG. 26

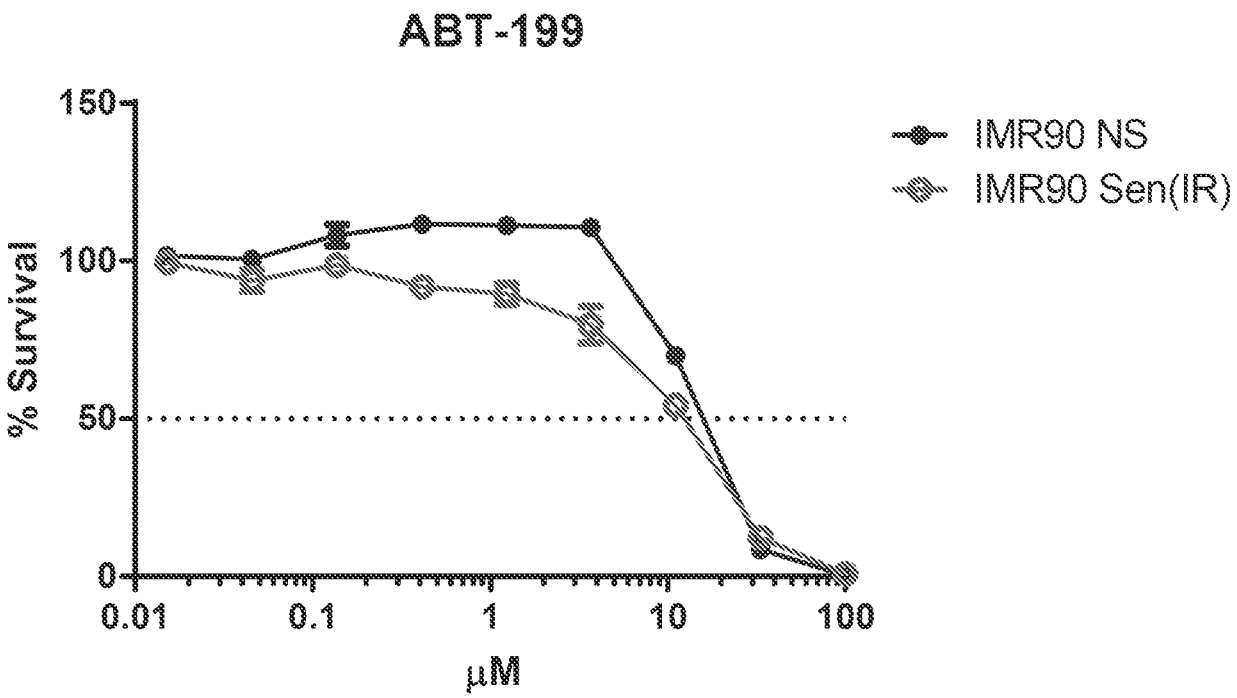

ABT-199
150
100
50
0
% Survival
0.01
0.1
1
10
100
μM
IMR90 NS
IMR90 Sen(IR)

FIG. 27

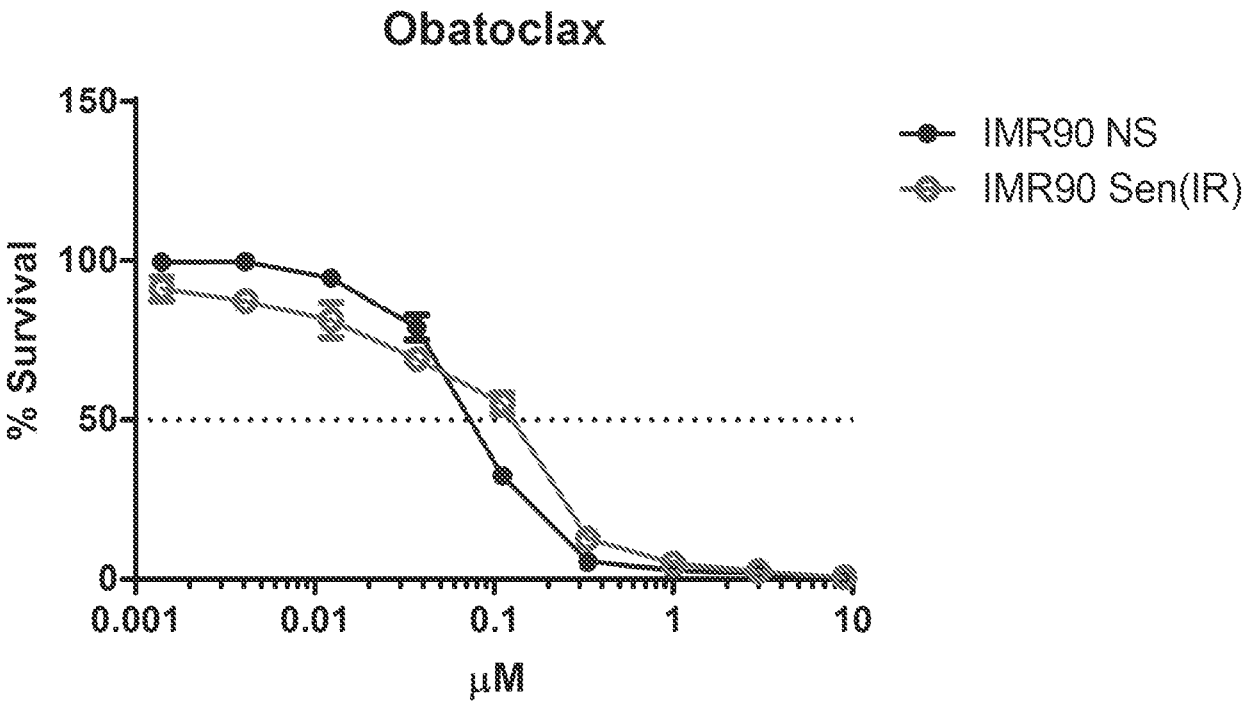

Obatoclax
150
100
50
0
% Survival
0.001
0.01
0.1
1
10
μM
IMR90 NS
IMR90 Sen(IR)

FIG. 28

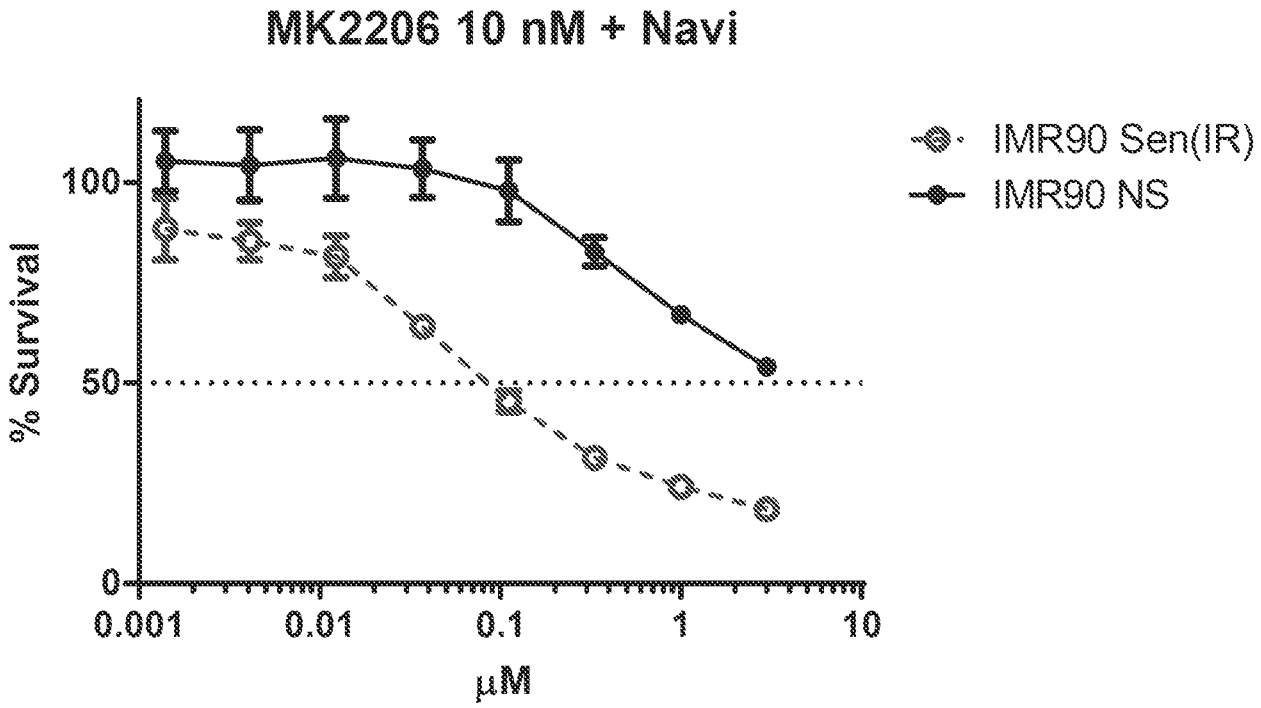


FIG. 29A

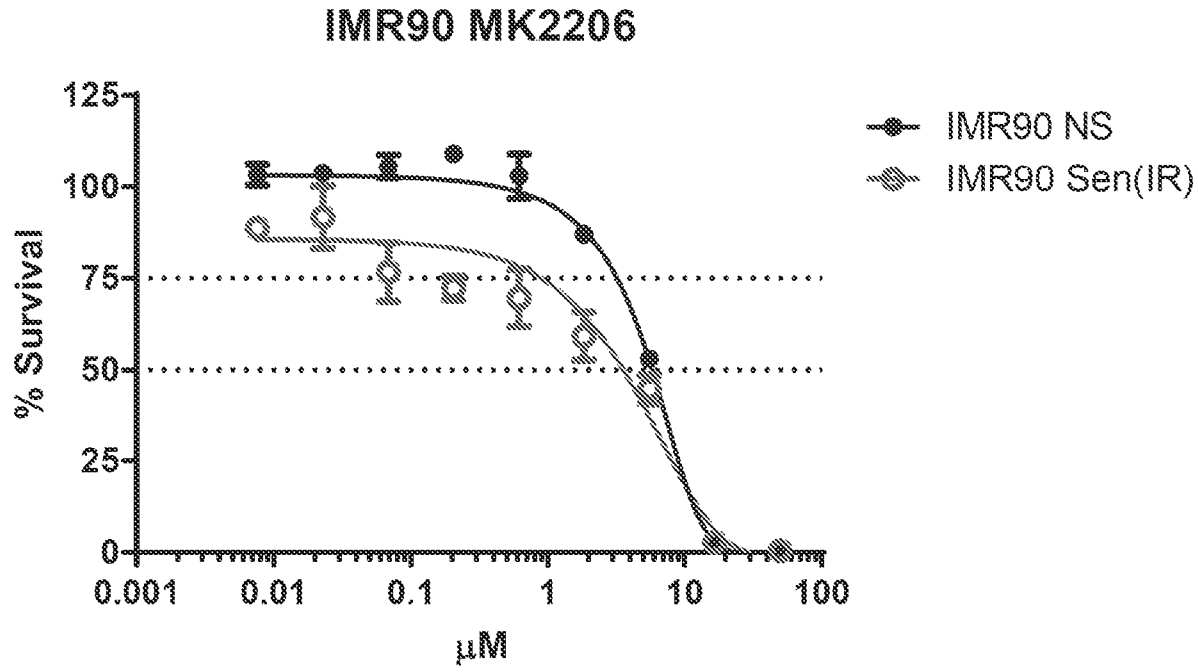


FIG. 29B

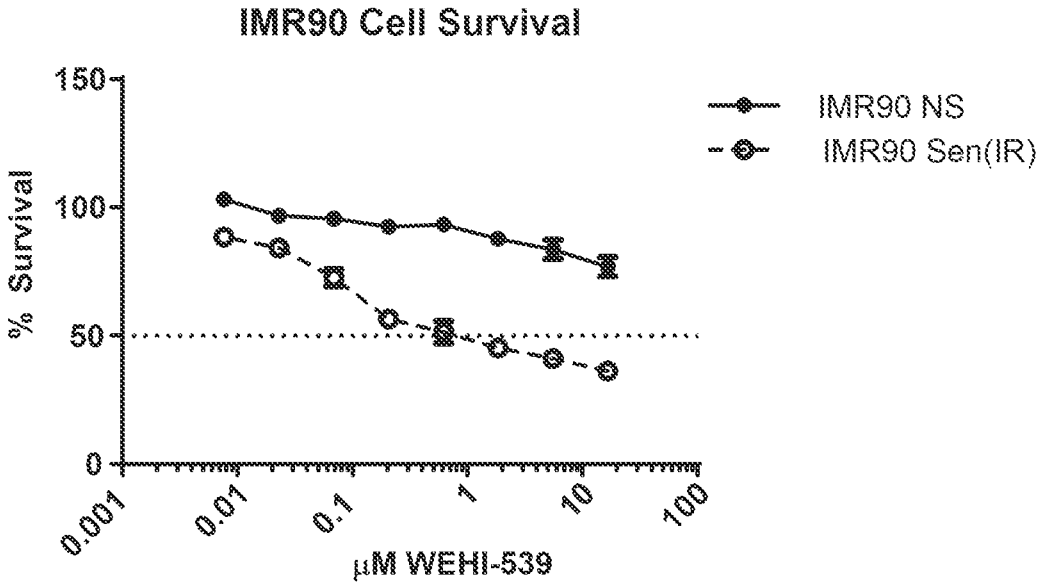


FIG. 30A

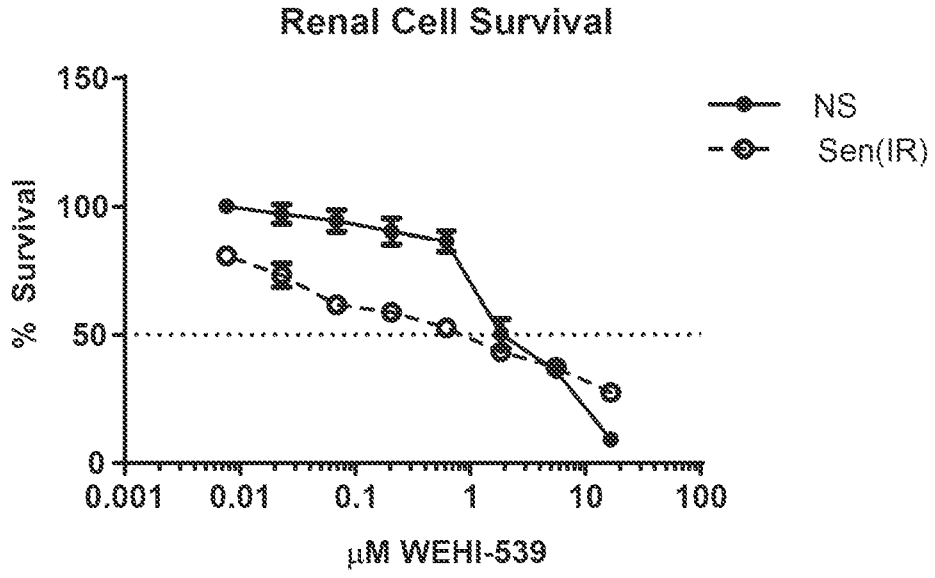


FIG. 30B

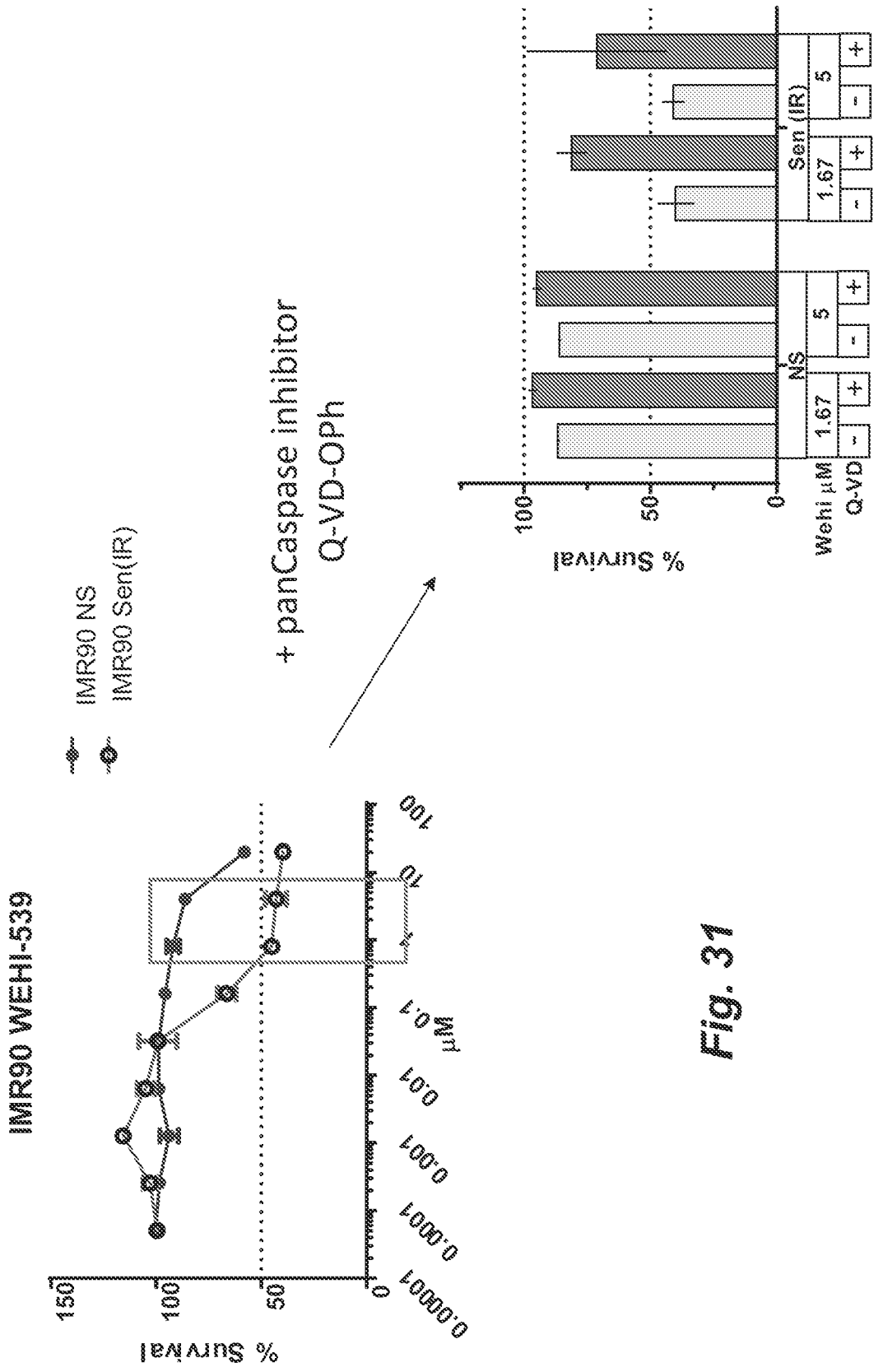

IMR90 WEHI-539
IMR90 NS
IMR90 Sen(IR)
% Survival
150
100
50
0
100
10
1
0.1
0.01
0.001
0.0001
0.00001
μM
+ panCaspase inhibitor
Q-VD-OPh
% Survival
100
50
0
Wehi μM
Q-VD
NS
Sen (IR)
1.67
5
1.67
5
-
+
-
+
-
+
-
+

Fig. 31

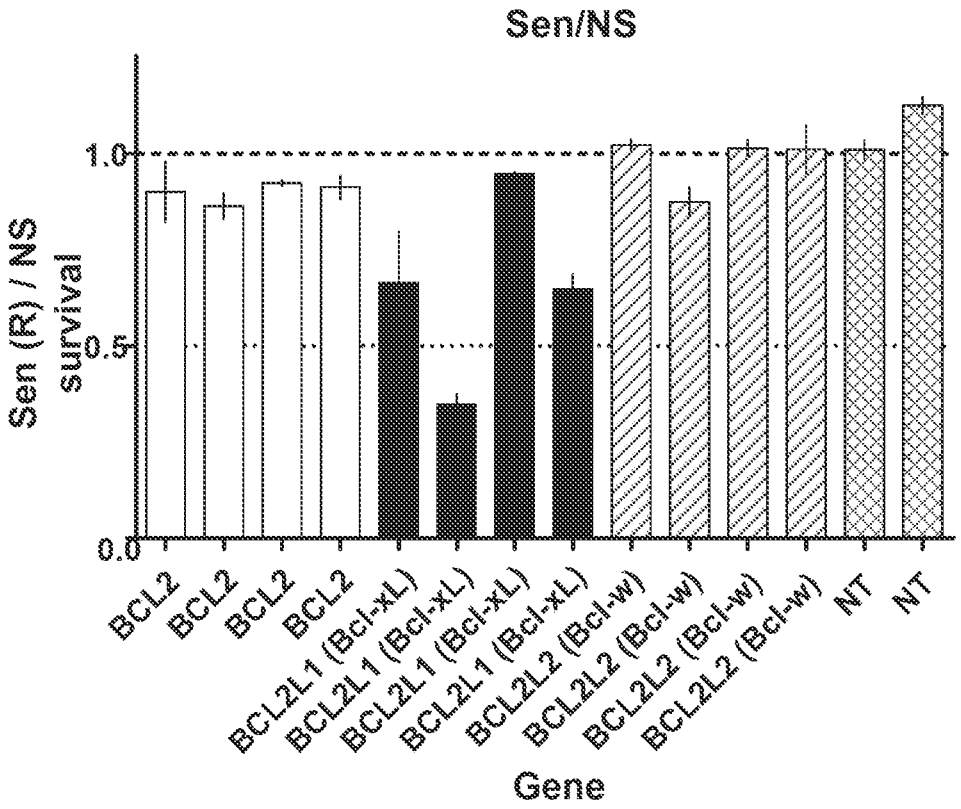

Sen/NS
Sen (R) / NS survival
1.0
0.5
0.0
BCL2
BCL2
BCL2
BCL2
BCL2L1 (Bcl-xL)
BCL2L1 (Bcl-xL)
BCL2L1 (Bcl-xL)
BCL2L1 (Bcl-xL)
BCL2L2 (Bcl-w)
BCL2L2 (Bcl-w)
BCL2L2 (Bcl-w)
BCL2L2 (Bcl-w)
NT
NT
Gene

FIG. 32

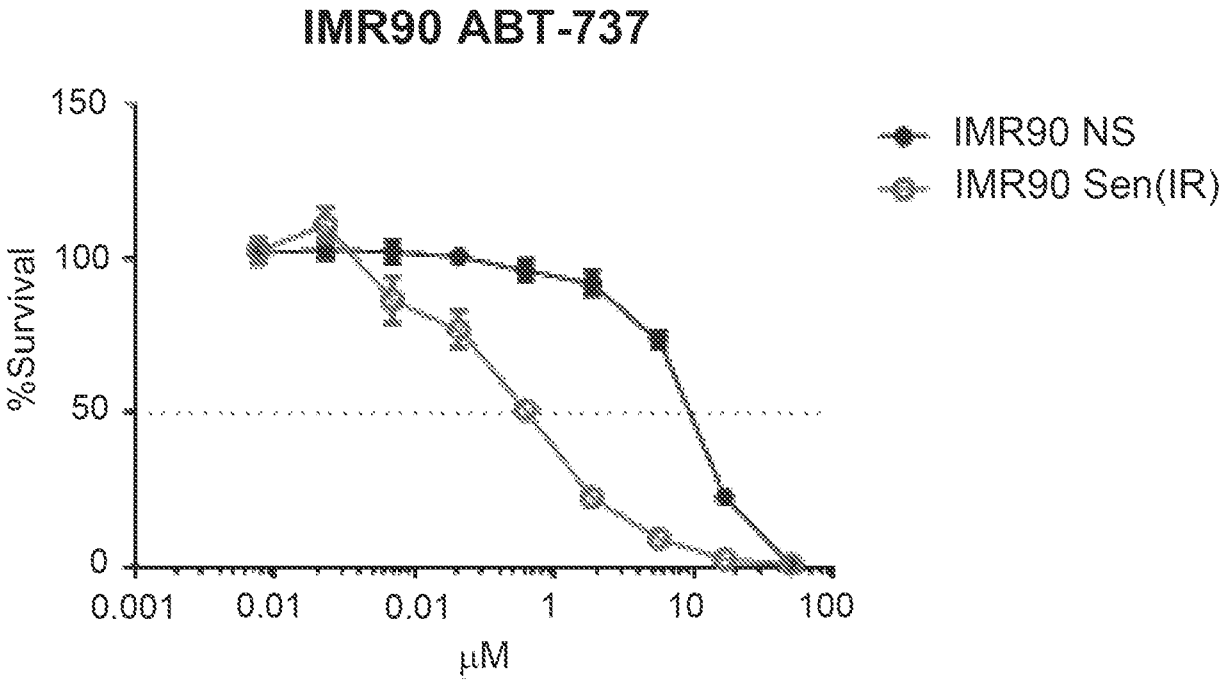

IMR90 ABT-737
150
100
50
0
%Survival
0.001
0.01
0.01
1
10
100
μM
IMR90 NS
IMR90 Sen(IR)

FIG. 33

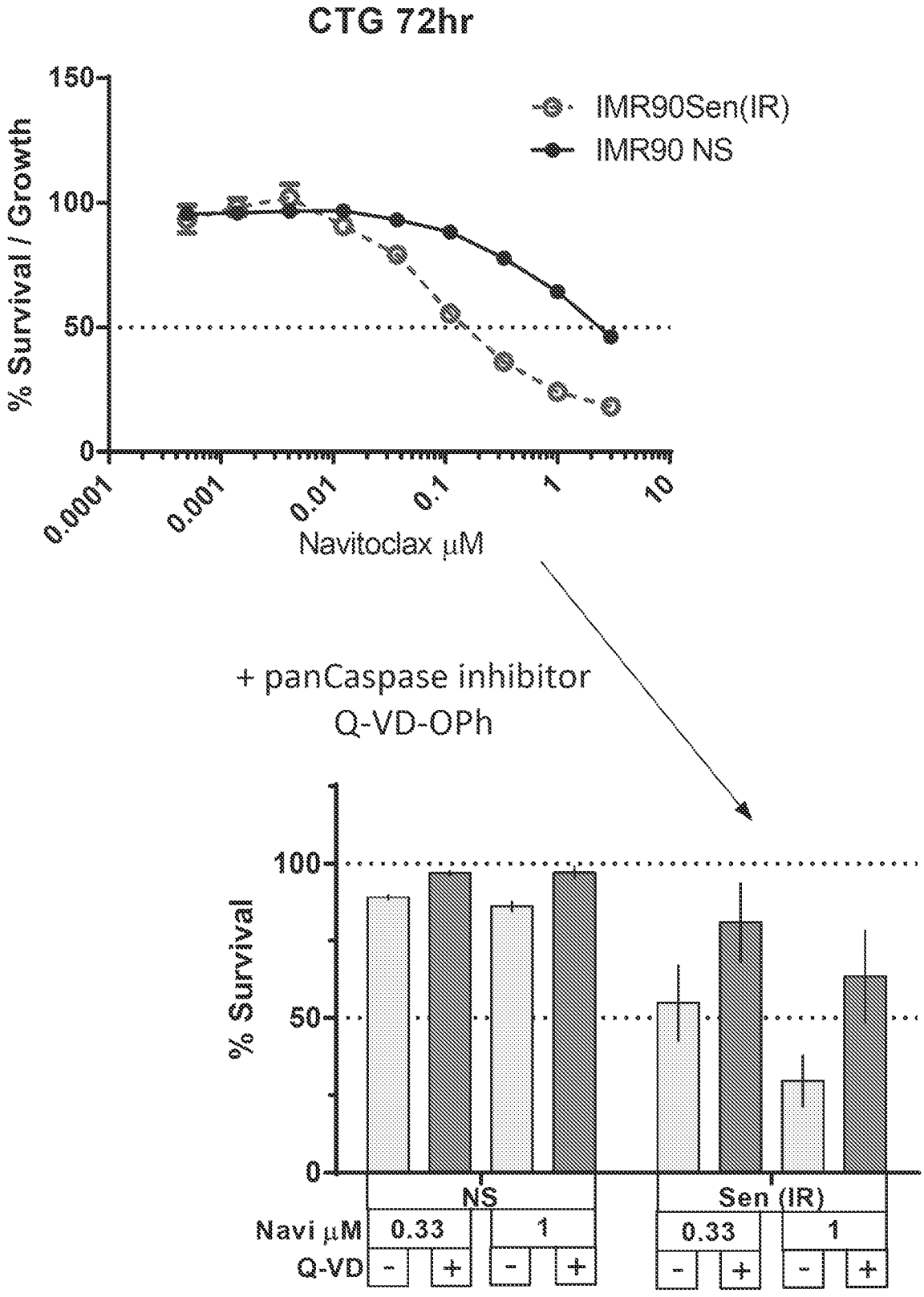
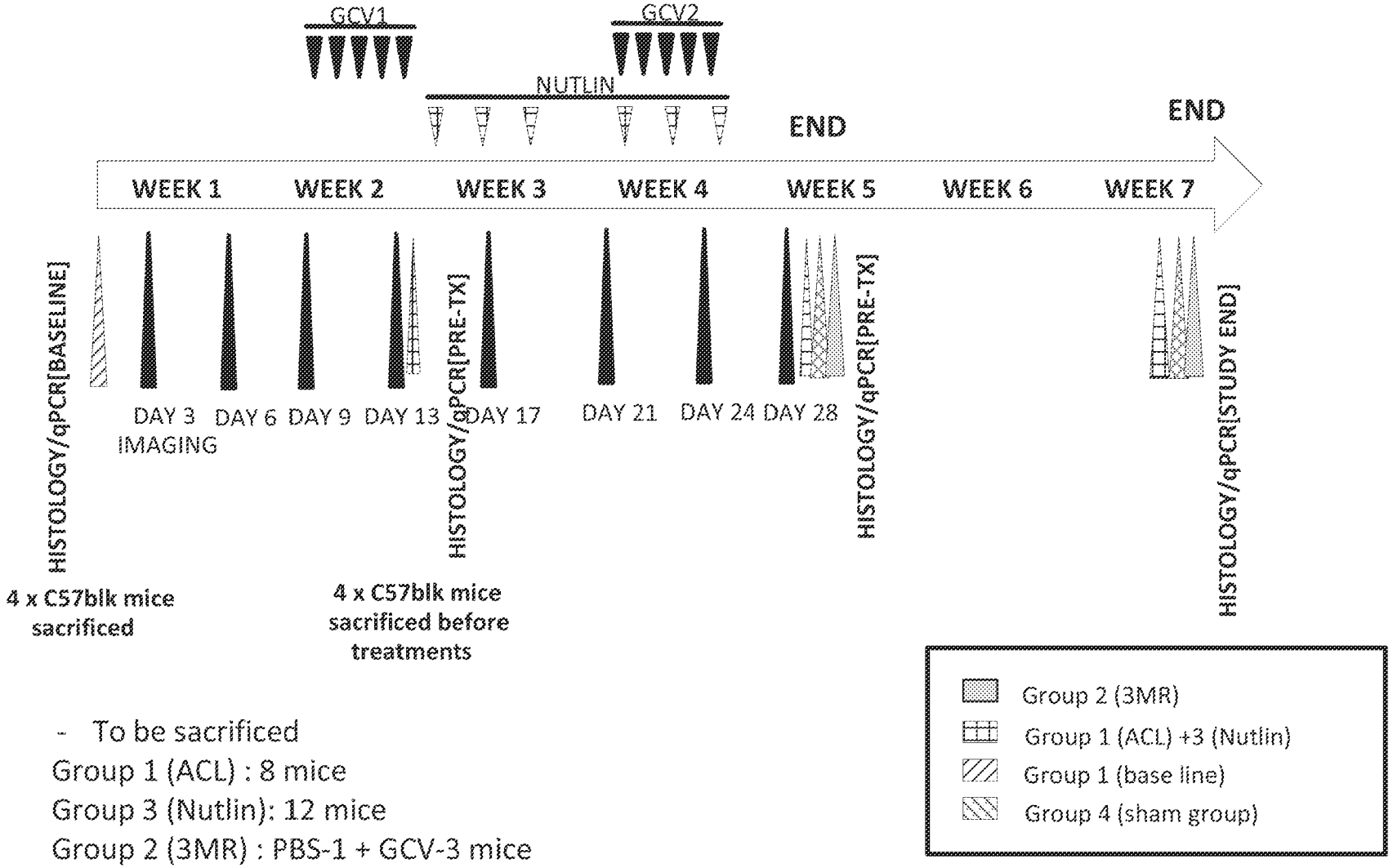

CTG 72hr
150
100
50
0
% Survival / Growth
IMR90Sen(IR)
IMR90 NS
0.0001
0.001
0.01
0.1
1
10
Navitoclax μM
+ panCaspase inhibitor
Q-VD-OPh
100
50
0
% Survival
NS
Sen (IR)
Navi μM
0.33
1
0.33
1
Q-VD
-
+
-
+
-
+
-
+

FIG. 34

Groups		Animals	Procedure	Treatment	Readout
1	ACL Control	16 x C57Blk 1 x 3MR	ACL surgery Vehicle IA injections to parallel GCV group [Control group for GCV treatment]	Vehicle (10 µl) qd for 5 days, optional second cycle	qPCR & histology
2	GCV	3 x 3MR	ACL surgery IA injection of GCV	GCV (2.5 µg per knee injection) qd for 5 days, optional second cycle	Luminescence qPCR & histology
3	Nutlin	12 C57Blk	ACL surgery IA injection of nutlin-3a	Nutlin-3a (5.8 µg per knee injection) qod for 2 weeks	qPCR & histology
4	Sham Control	4 C57Blk (n=3 PCR, n=1 histology)	Sham surgery Vehicle IA injections to parallel GCV group [Control group for knee injections]	Vehicle (10 µl) qd for 5 days, optional second cycle	qPCR & histology

FIG. 35



- To be sacrificed

Group 1 (ACL) : 8 mice

Group 3 (Nutlin): 12 mice

Group 2 (3MR) : PBS-1 + GCV-3 mice

Group 4: sham : 4 mice

FIG. 36

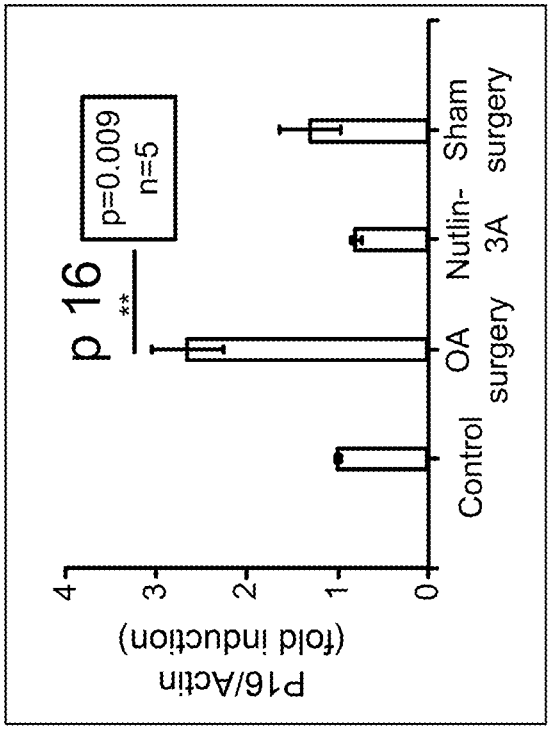


FIG. 37A

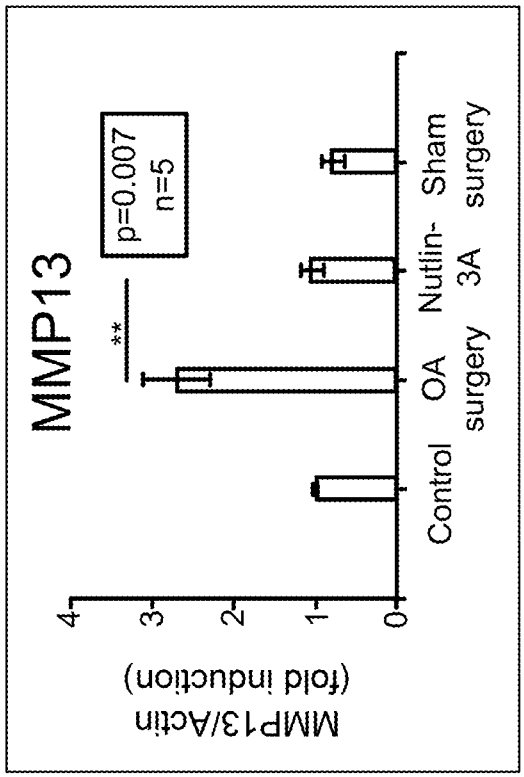


FIG. 37C

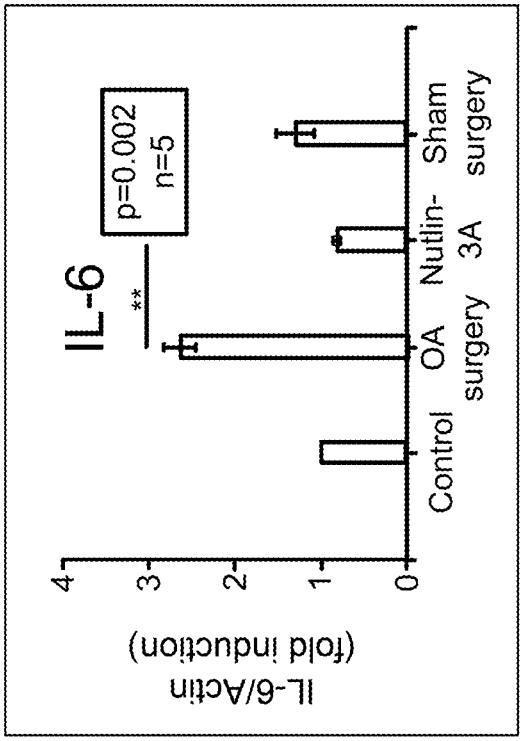


FIG. 37B

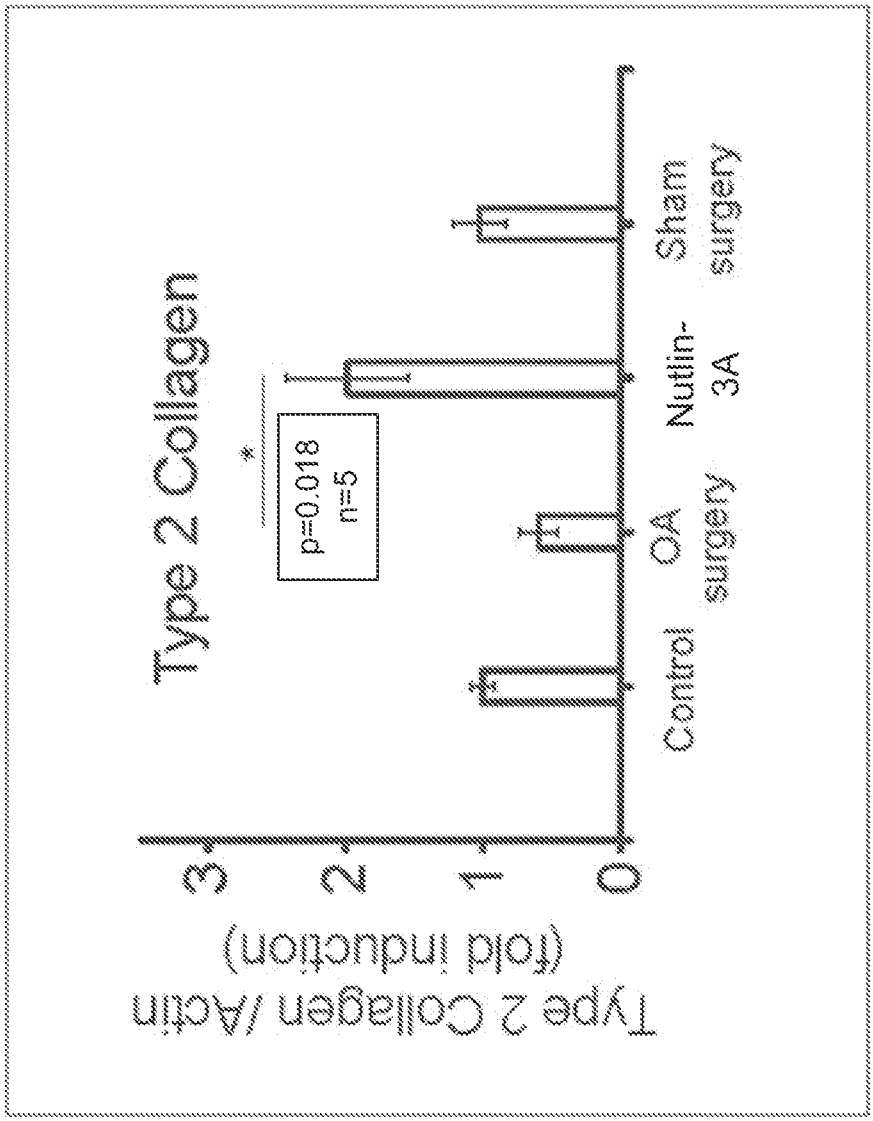

Type 2 Collagen
Type 2 Collagen /Actin
(fold induction)
3
2
1
0
*
p=0.018
n=5
Control
OA surgery
Nutlin-3A
Sham surgery

FIG. 38

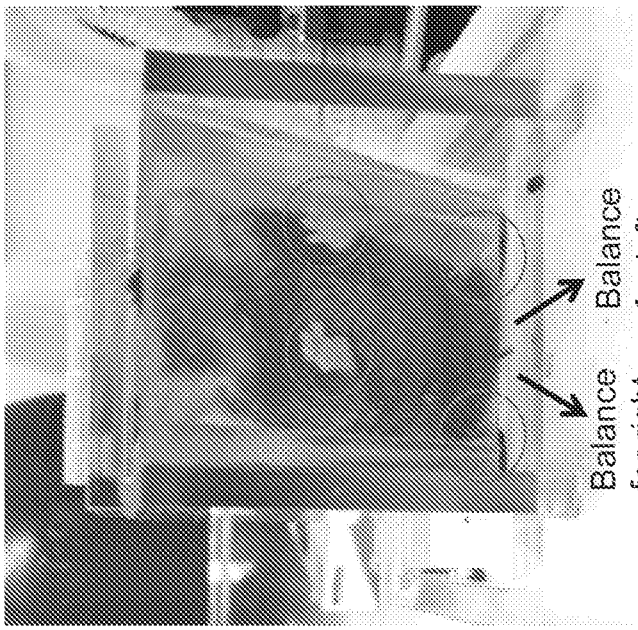




FIG. 39

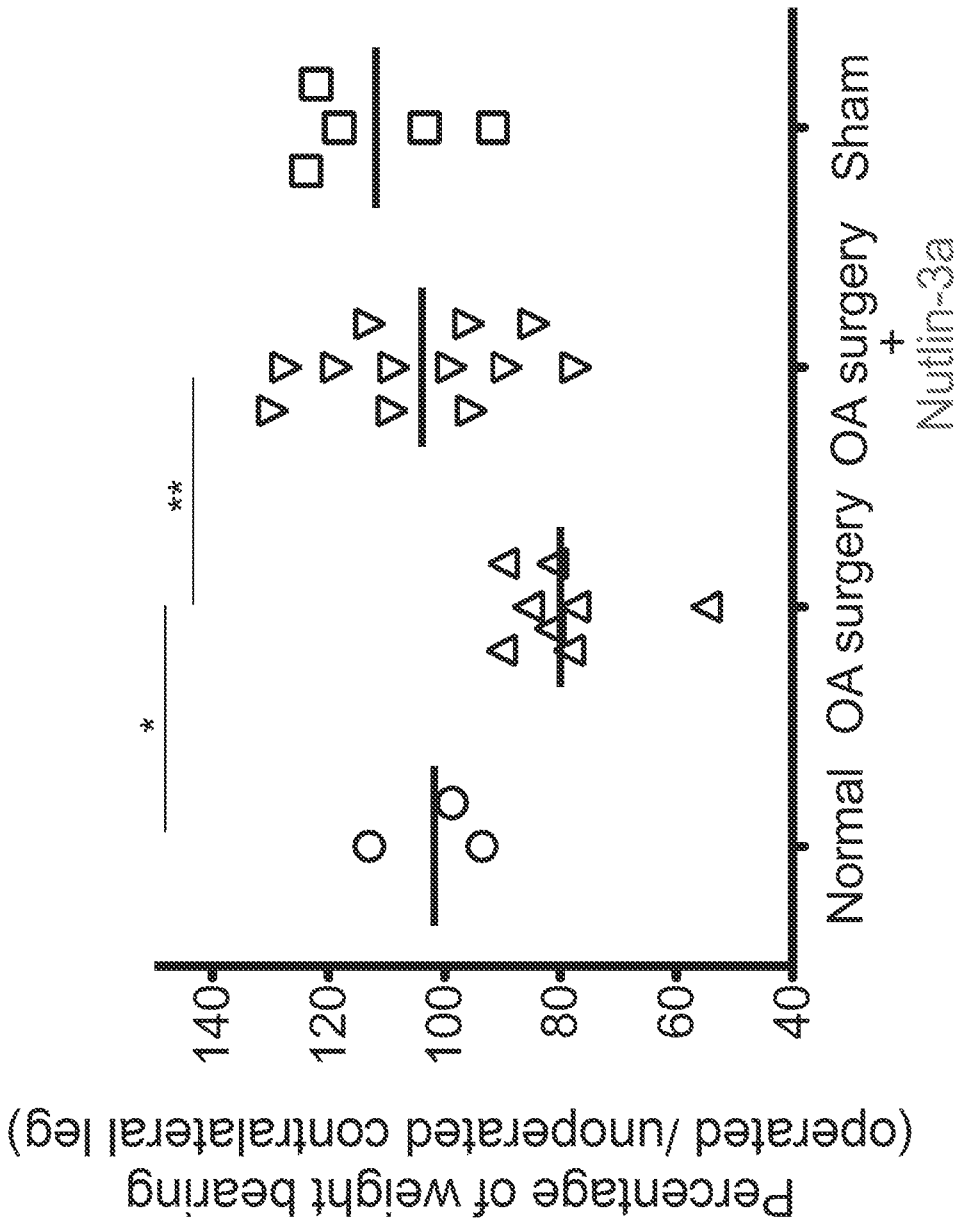

Percentage of weight bearing
(operated /unoperated contralateral leg)
140
120
100
80
60
40
*
**
Normal
OA surgery
OA surgery + Nutlin-3a
Sham

FIG. 40

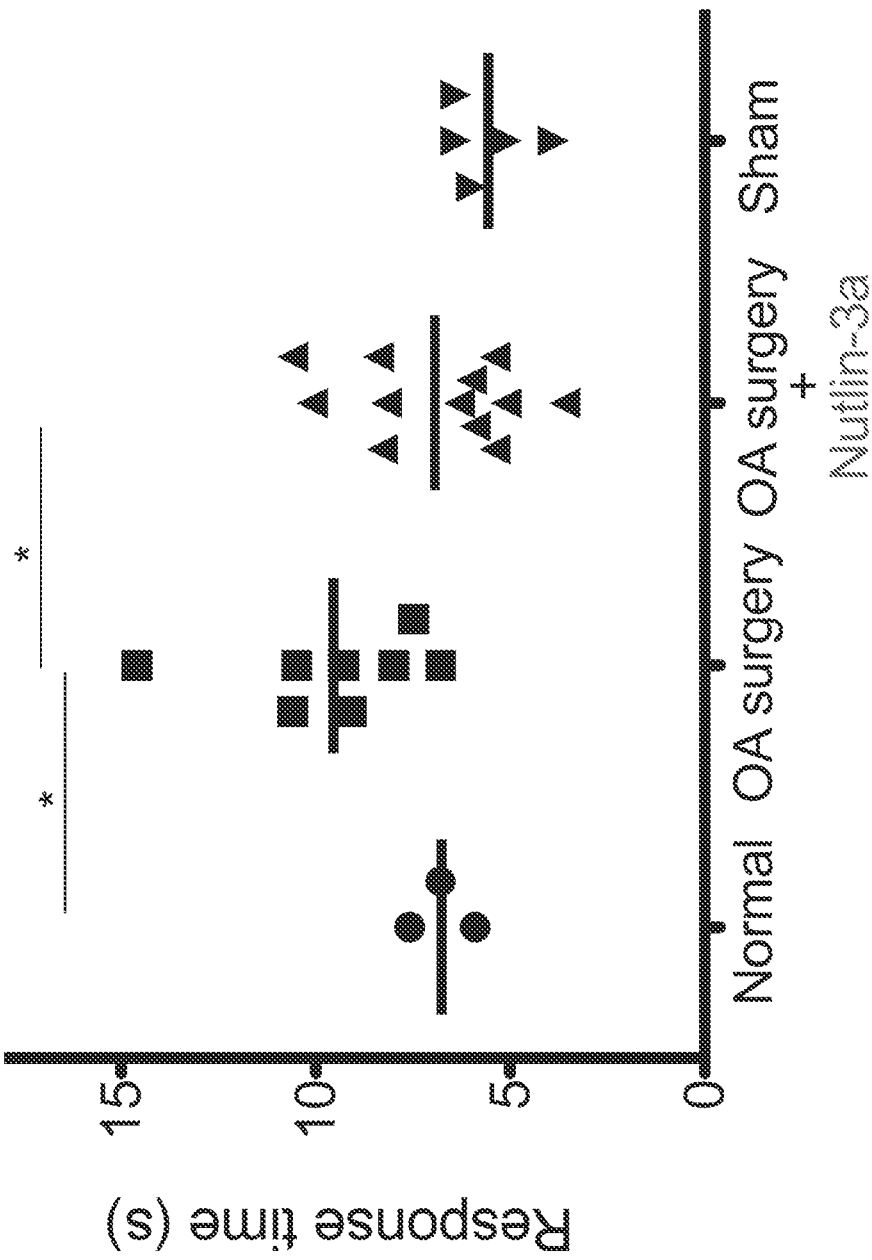

Response time (s)
15
10
5
0
*
*
Normal
OA surgery
OA surgery + Nutlin-3a
Sham

FIG. 41

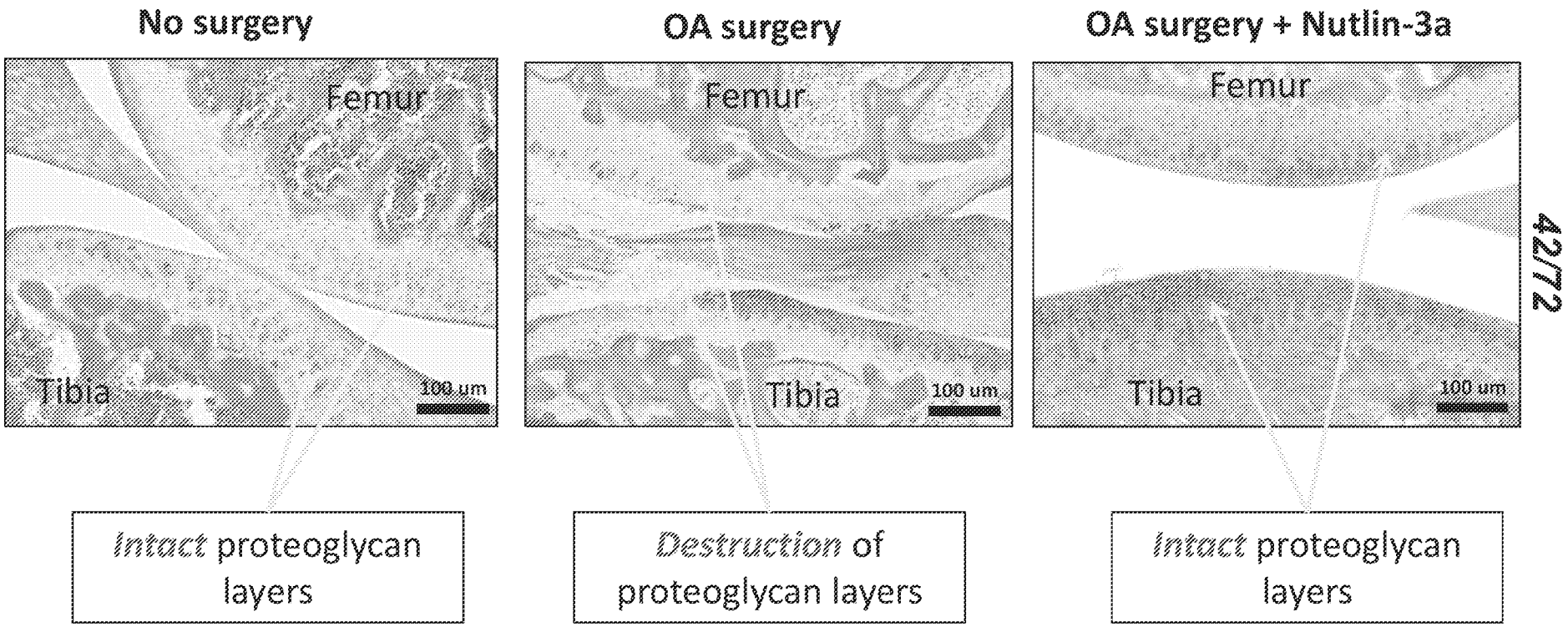
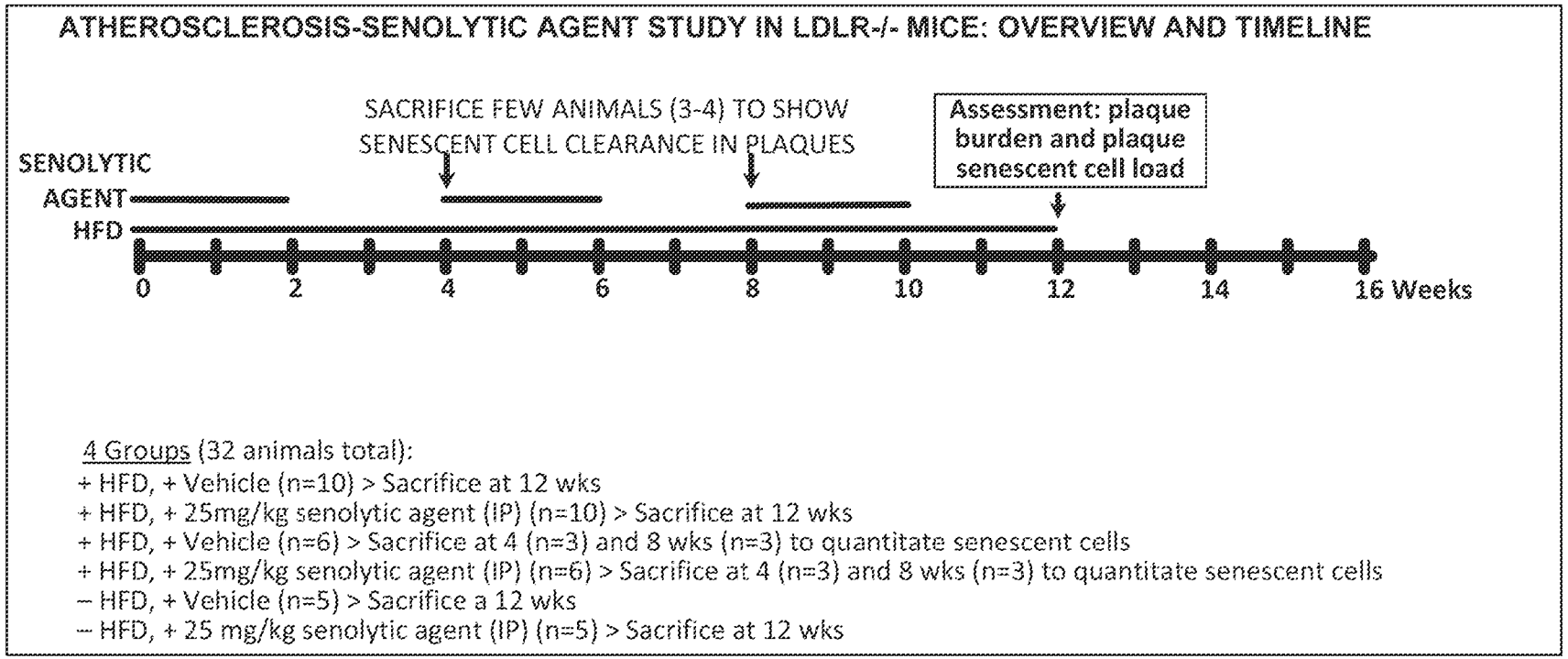

No surgery
OA surgery
OA surgery + Nutlin-3a
Femur
Tibia
100 um
Femur
Tibia
100 um
Femur
Tibia
100 um
Intact proteoglycan layers
Destruction of proteoglycan layers
Intact proteoglycan layers

FIG. 42

A


ATHEROSCLEROSIS-SENOLYTIC AGENT STUDY IN LDLR-/- MICE: OVERVIEW AND TIMELINE
SACRIFICE FEW ANIMALS (3-4) TO SHOW SENESCENT CELL CLEARANCE IN PLAQUES
Assessment: plaque burden and plaque senescent cell load
SENOLYTIC AGENT
HFD
0
2
4
6
8
10
12
14
16 Weeks
4 Groups (32 animals total):
+ HFD, + Vehicle (n=10) > Sacrifice at 12 wks
+ HFD, + 25mg/kg senolytic agent (IP) (n=10) > Sacrifice at 12 wks
+ HFD, + Vehicle (n=6) > Sacrifice at 4 (n=3) and 8 wks (n=3) to quantitate senescent cells
+ HFD, + 25mg/kg senolytic agent (IP) (n=6) > Sacrifice at 4 (n=3) and 8 wks (n=3) to quantitate senescent cells
– HFD, + Vehicle (n=5) > Sacrifice a 12 wks
– HFD, + 25 mg/kg senolytic agent (IP) (n=5) > Sacrifice at 12 wks

B

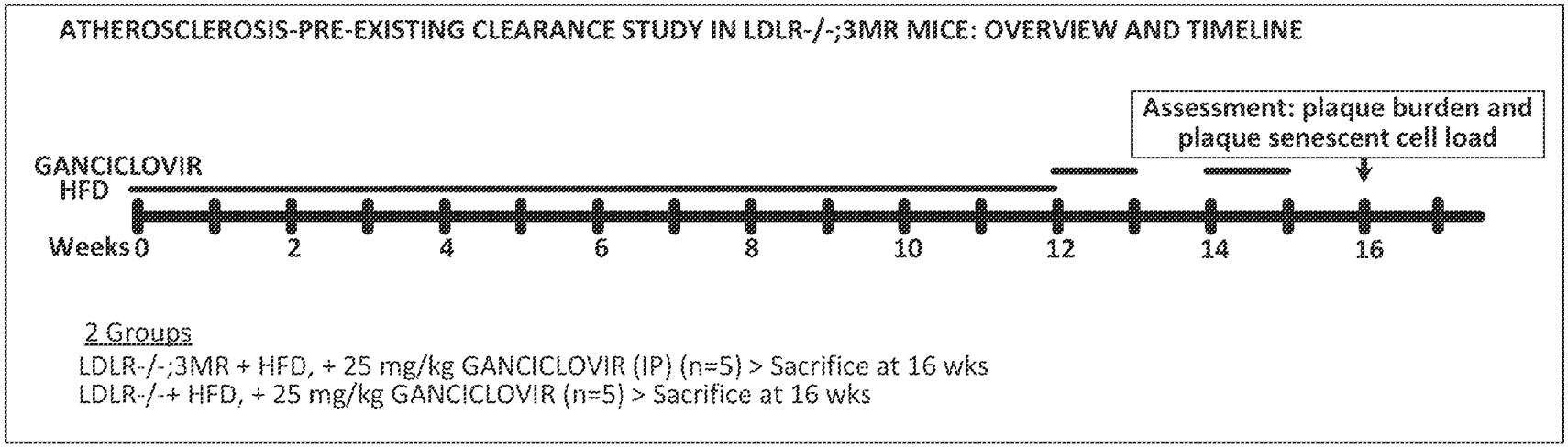
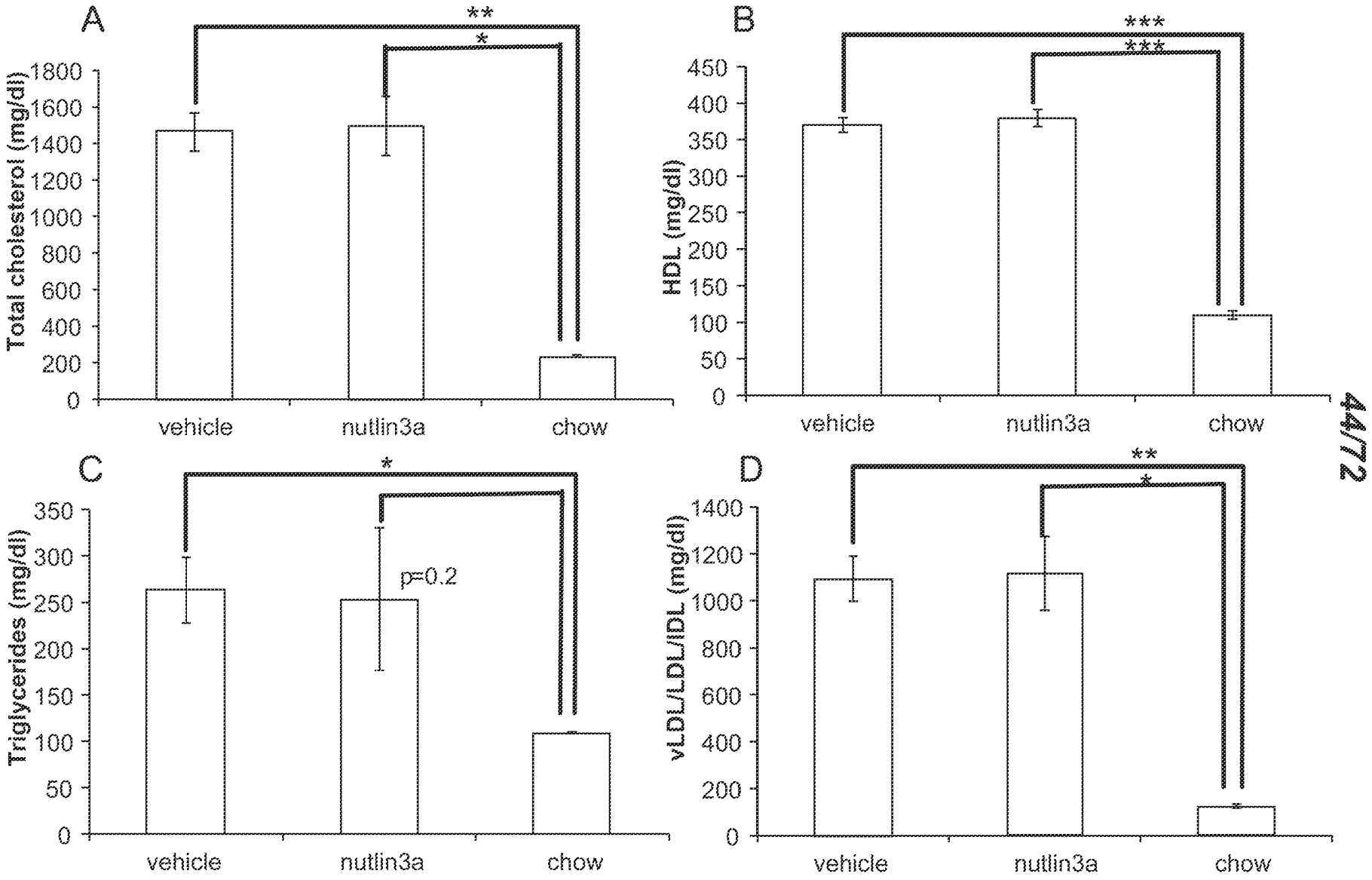
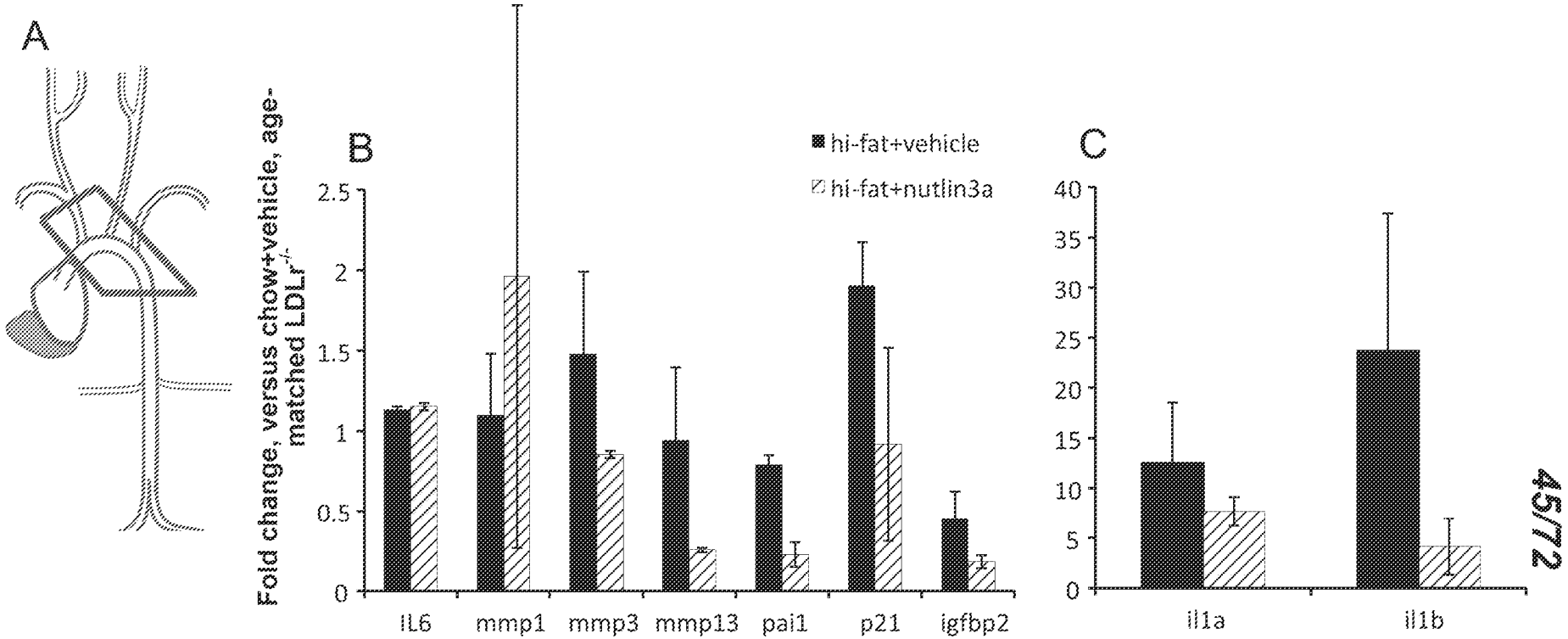

ATHEROSCLEROSIS-PRE-EXISTING CLEARANCE STUDY IN LDLR-/-;3MR MICE: OVERVIEW AND TIMELINE
Assessment: plaque burden and plaque senescent cell load
GANCICLOVIR
HFD
Weeks 0
2
4
6
8
10
12
14
16
2 Groups
LDLR-/-;3MR + HFD, + 25 mg/kg GANCICLOVIR (IP) (n=5) > Sacrifice at 16 wks
LDLR-/-+ HFD, + 25 mg/kg GANCICLOVIR (n=5) > Sacrifice at 16 wks

FIG. 43


A
**
*
Total cholesterol (mg/dl)
1800
1600
1400
1200
1000
800
600
400
200
0
vehicle
nutlin3a
chow
B
***
***
HDL (mg/dl)
450
400
350
300
250
200
150
100
50
0
vehicle
nutlin3a
chow
C
*
p=0.2
Triglycerides (mg/dl)
350
300
250
200
150
100
50
0
vehicle
nutlin3a
chow
D
**
*
vLDL/LDL/IDL (mg/dl)
1400
1200
1000
800
600
400
200
0
vehicle
nutlin3a
chow

44/72

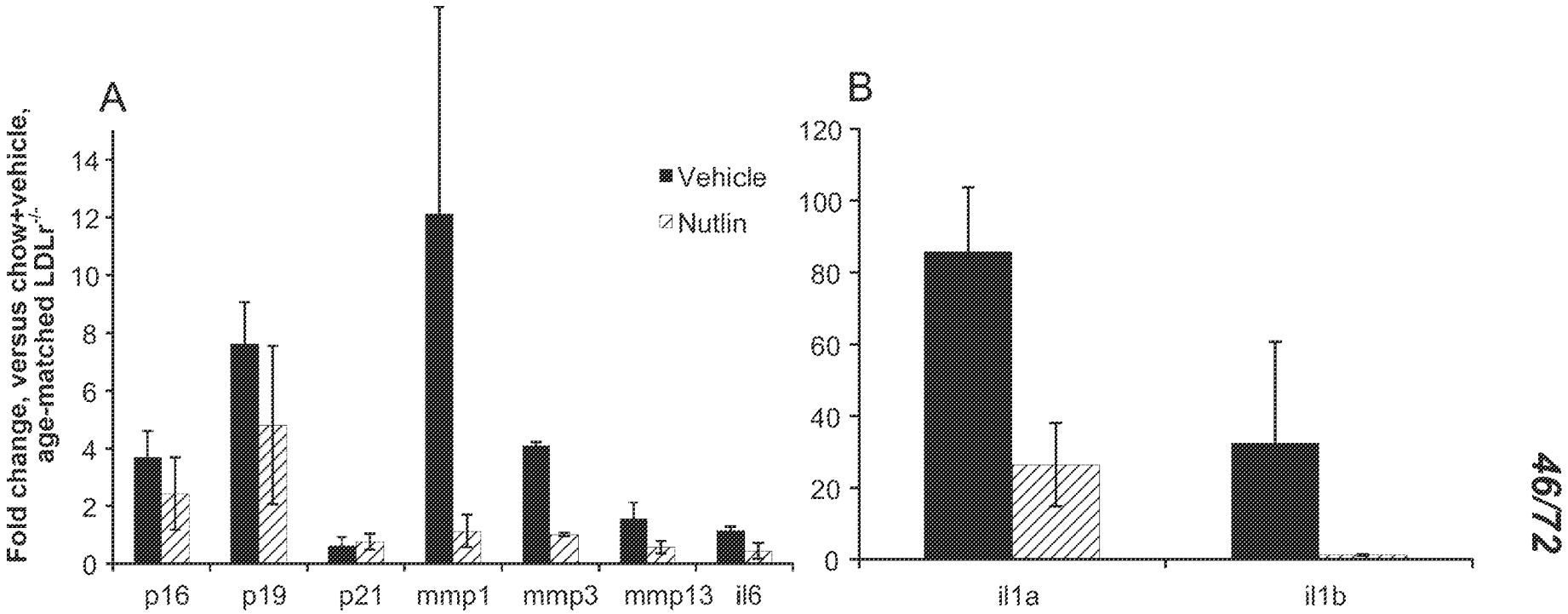
FIG. 44



D

	Group	IL6	mmp1	mmp3	mmp13	pai1	p21	igfbp2	il1a	il1b
hi-fat+nutlin3a	versus hi-fat + vehicle	**0.055**	0.632	0.448	**0.064**	0.129	0.902	0.158	**0.041**	0.379
	versus chow + vehicle	0.532	0.664	0.348	0.270	**0.005**	0.238	0.244	0.505	0.290
hi-fat+vehicle	versus chow + vehicle	**0.077**	0.878	0.455	0.914	0.575	**0.072**	0.279	0.193	0.241

FIG. 45



C

	Group	p16	p19	p21	il1a	il1b	mmp1	mmp3	mmp13	il6
hi-fat + Nutlin3A	versus hi- fat + vehicle	0.4643	0.4307	0.7635	0.0583	0.3803	0.2631	0.0002	0.2066	0.1048
	versus chow + vehicle	0.3754	0.2989	0.5981	0.1617	0.4710	0.3416	0.8363	0.2459	0.4106
hi-fat + Vehicle	versus chow + vehicle	**0.0833**	**0.0355**	0.4376	**0.0414**	0.3787	0.2594	**0.0003**	0.4303	0.7756

FIG. 46

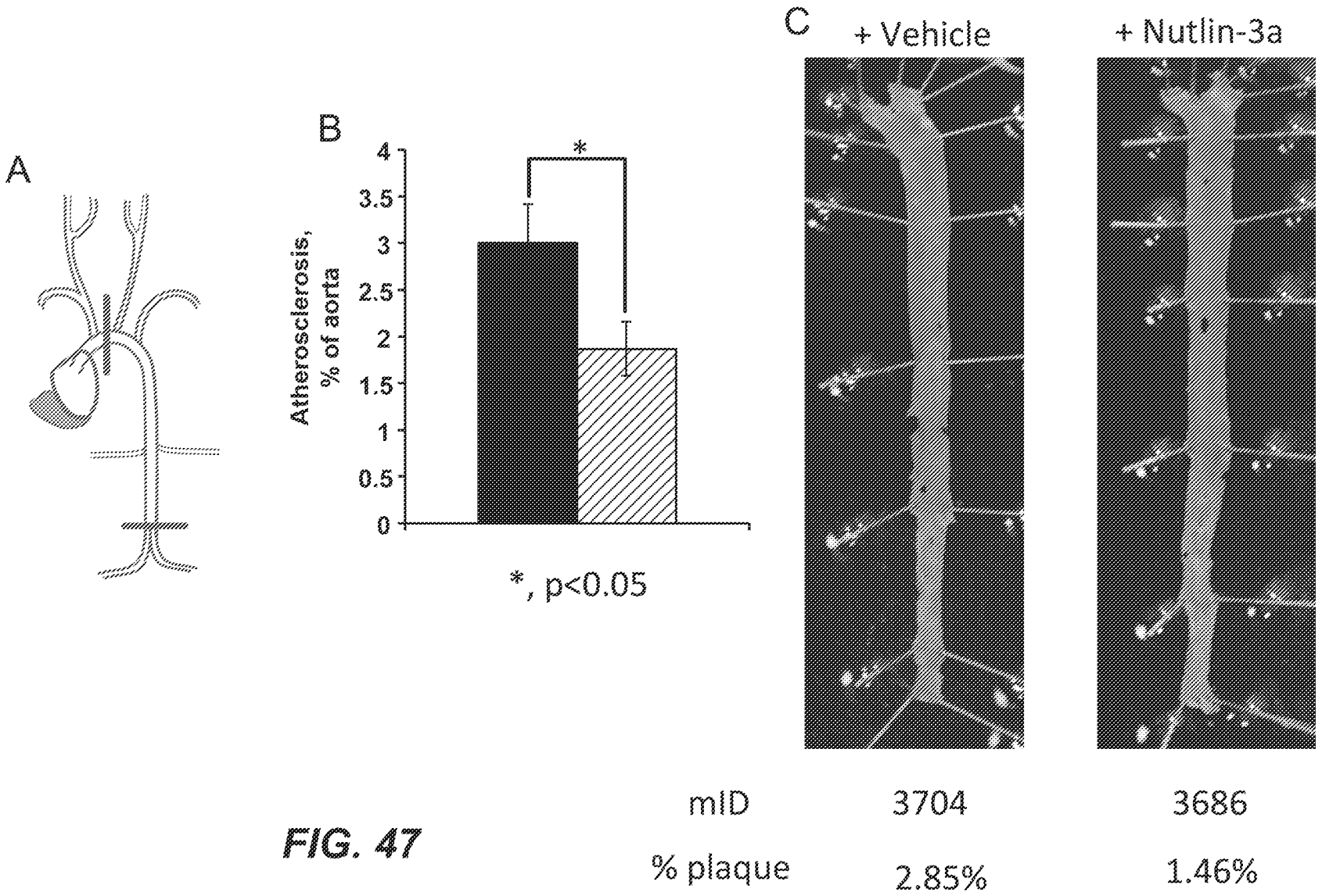

A
B
Atherosclerosis, % of aorta
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*, p<0.05
C
+ Vehicle
+ Nutlin-3a
mID
3704
3686
% plaque
2.85%
1.46%

FIG. 47

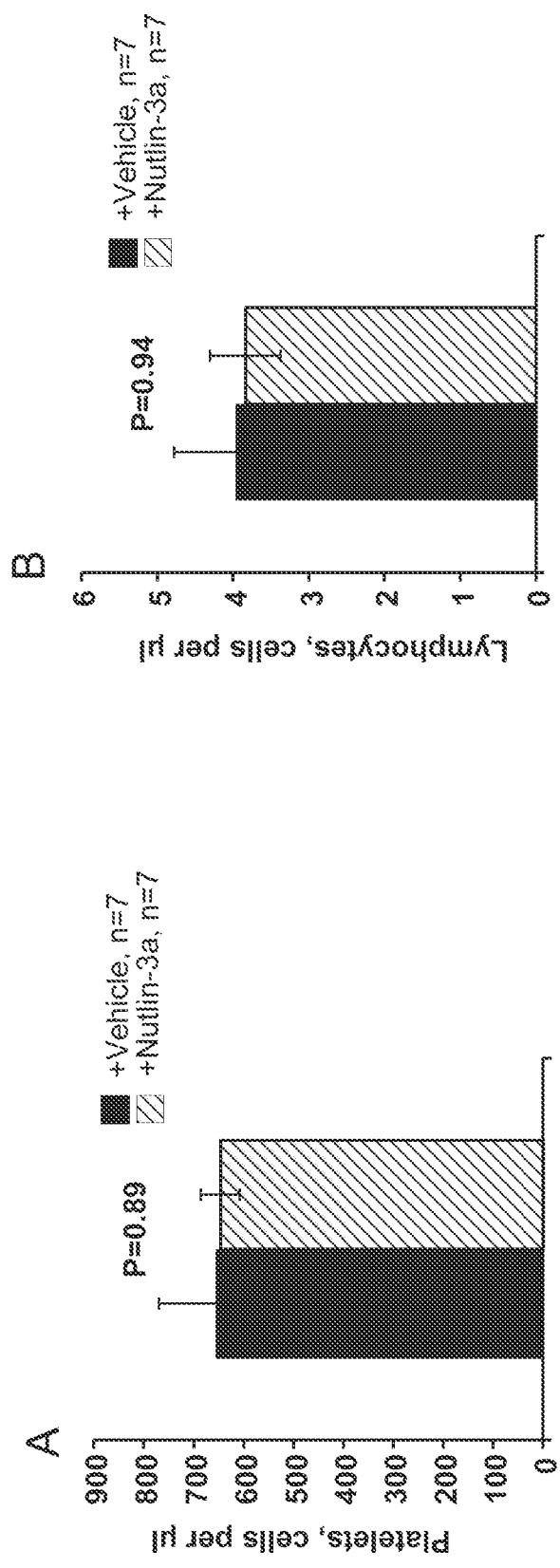

A
Platelets, cells per µl
900
800
700
600
500
400
300
200
100
0
P=0.89
+Vehicle, n=7
+Nutlin-3a, n=7
B
Lymphocytes, cells per µl
6
5
4
3
2
1
0
P=0.94
+Vehicle, n=7
+Nutlin-3a, n=7

FIG. 48

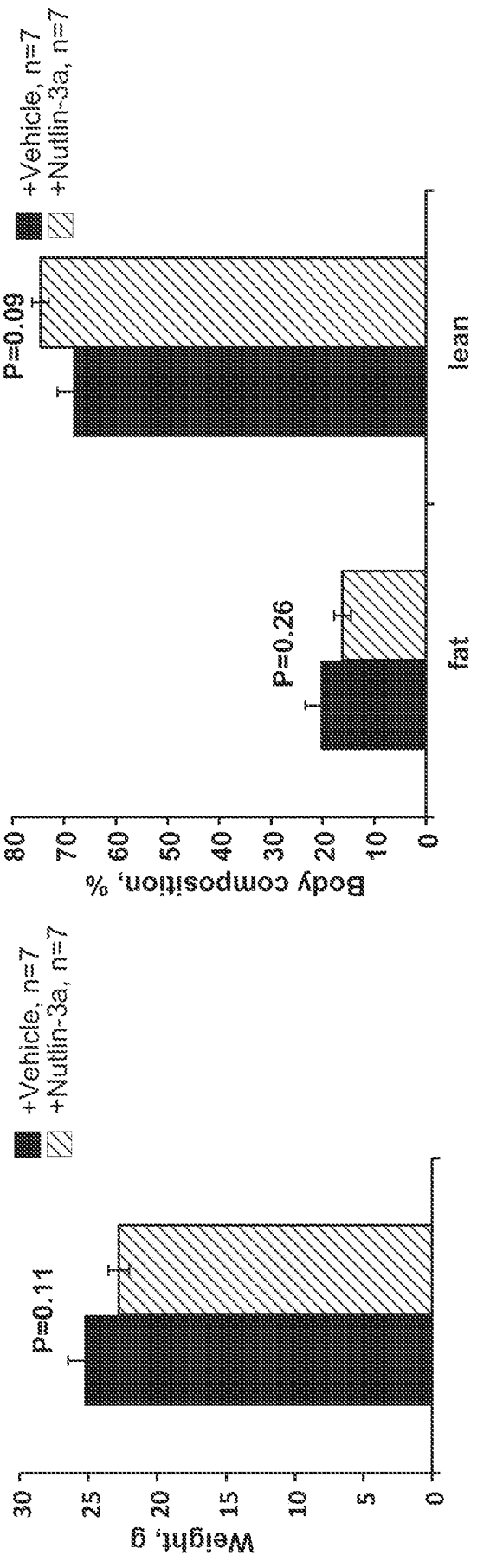

+Vehicle, n=7
+Nutlin-3a, n=7
P=0.09
P=0.26
lean
fat
Body composition, %
0
10
20
30
40
50
60
70
80
+Vehicle, n=7
+Nutlin-3a, n=7
P=0.11
Weight, g
0
5
10
15
20
25
30

FIG. 49

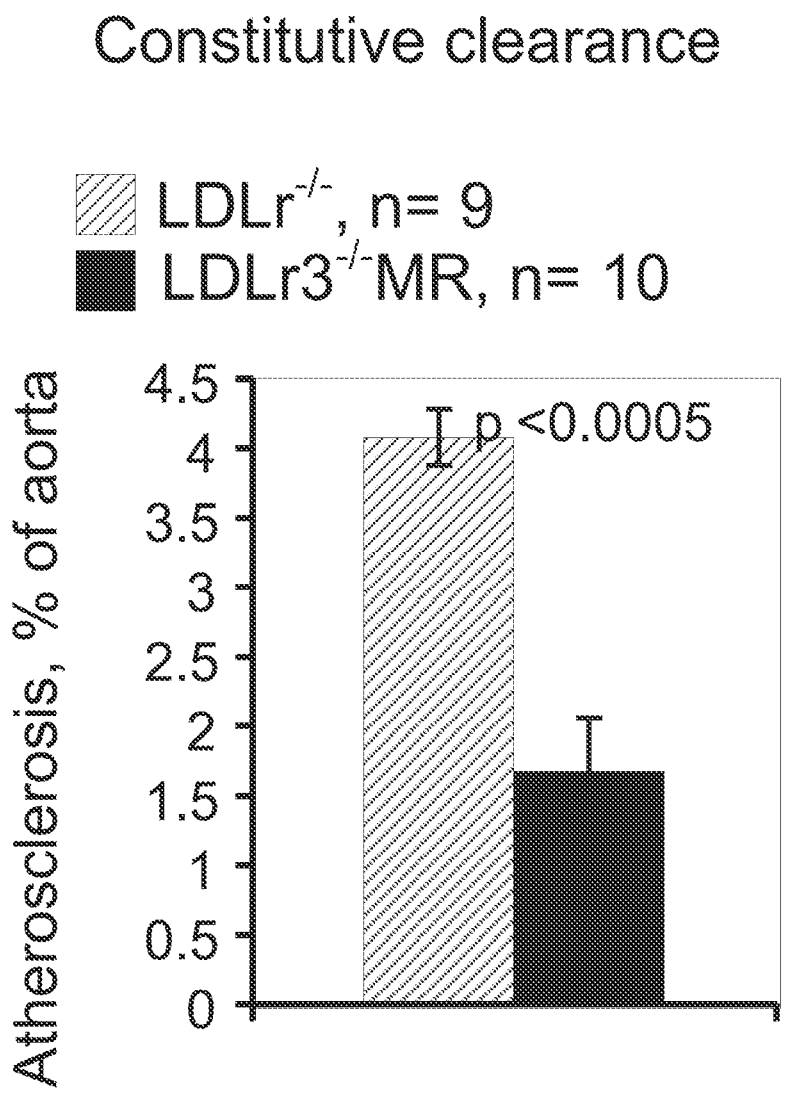

Constitutive clearance
LDLr-/-, n= 9
LDLr3-/-MR, n= 10
Atherosclerosis, % of aorta
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p <0.0005

FIG. 50

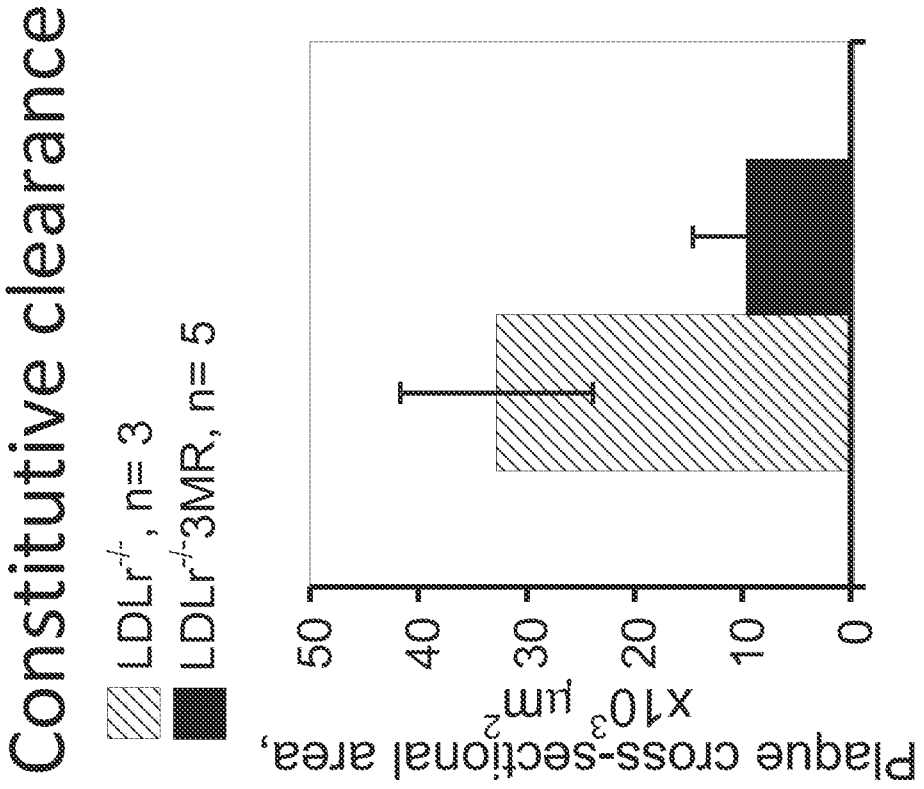

Constitutive clearance
LDLr-/-, n= 3
LDLr-/-3MR, n= 5
Plaque cross-sectional area, x10³ μm²
50
40
30
20
10
0

FIG. 51

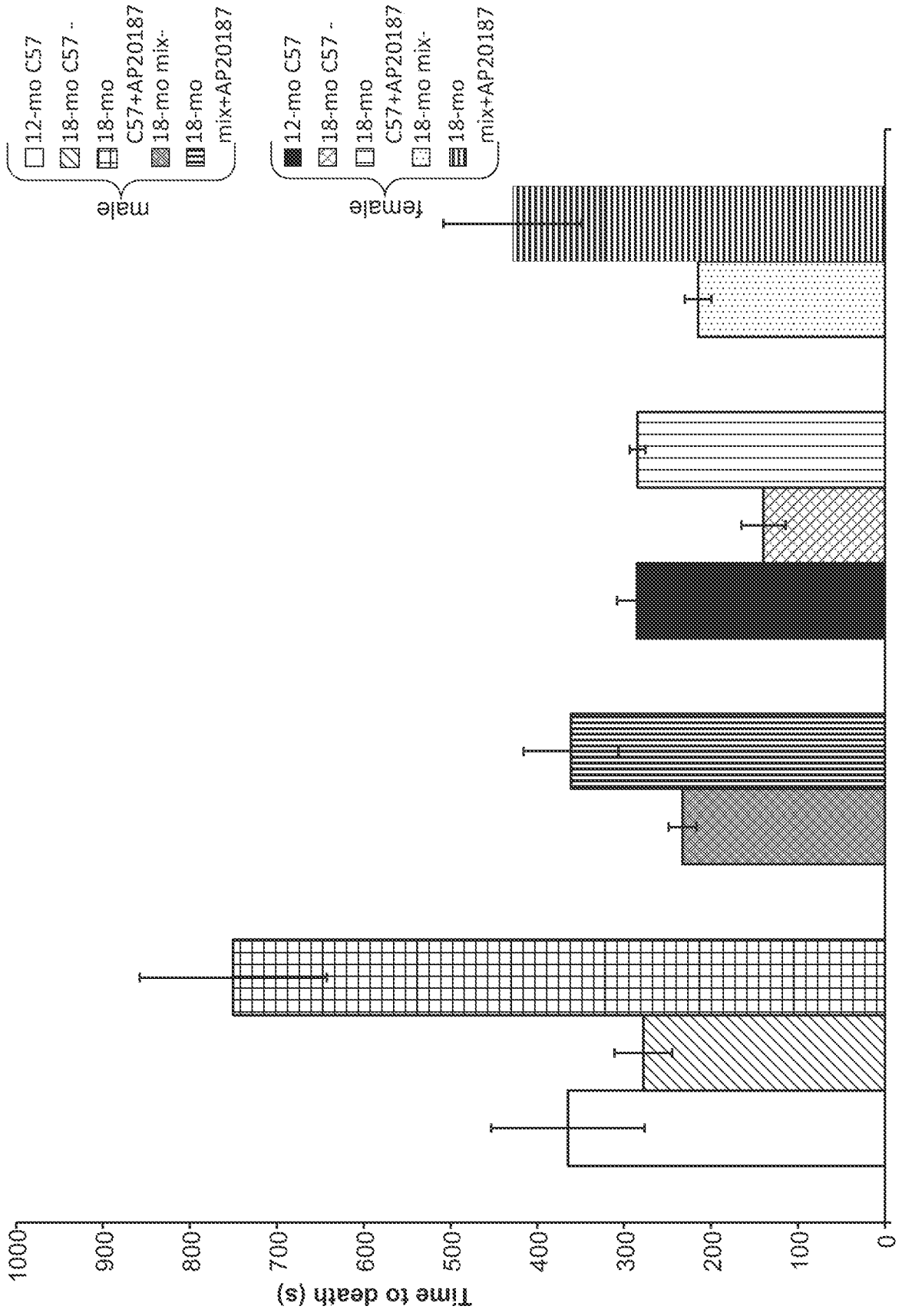

Time to death (s)
1000
900
800
700
600
500
400
300
200
100
0
male
12-mo C57
18-mo C57 -
18-mo C57+AP20187
18-mo mix-
18-mo mix+AP20187
female
12-mo C57
18-mo C57 -
18-mo C57+AP20187
18-mo mix-
18-mo mix+AP20187

FIG. 52

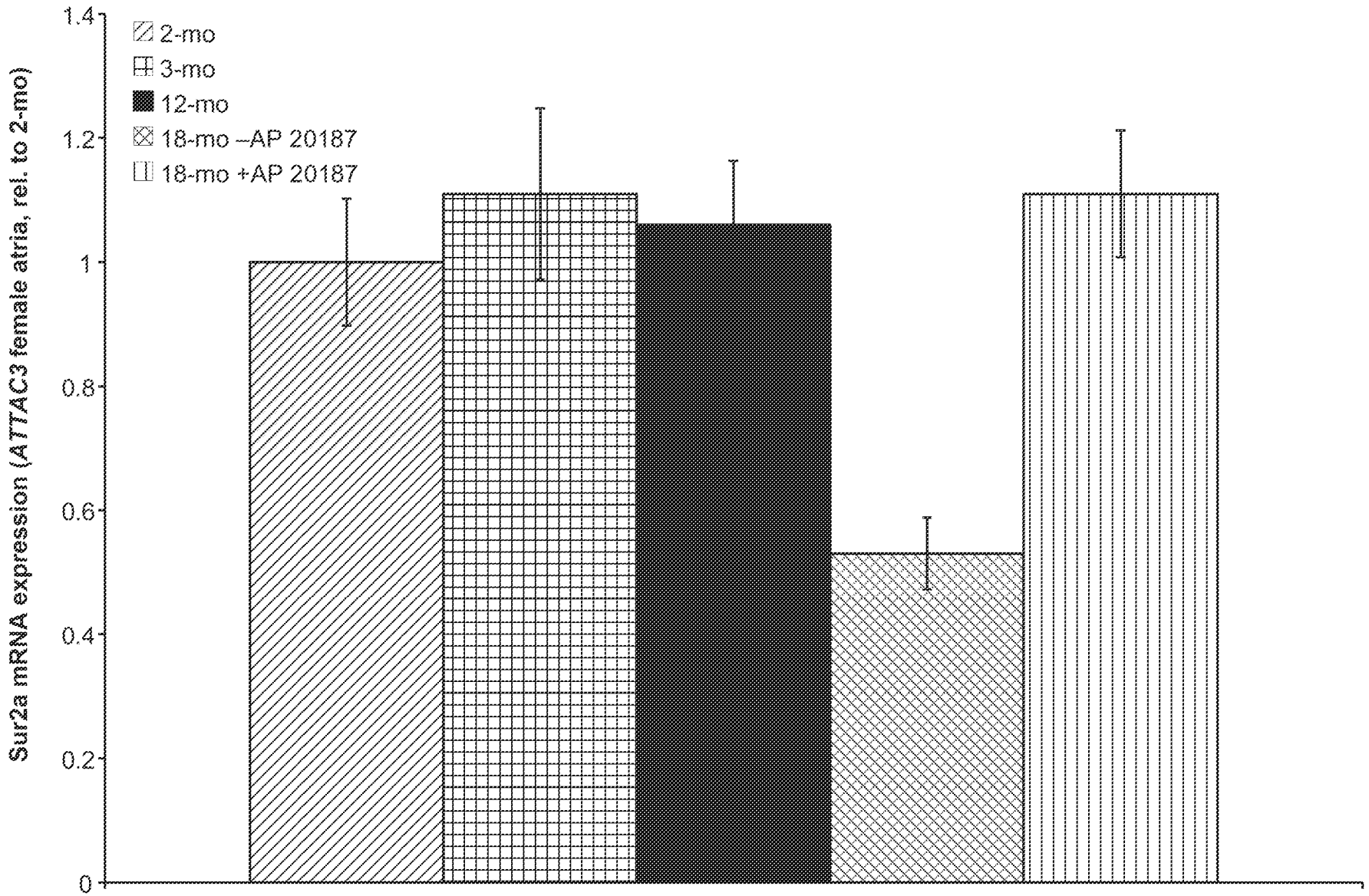

Sur2a mRNA expression (ATTAC3 female atria, rel. to 2-mo)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2-mo
3-mo
12-mo
18-mo –AP 20187
18-mo +AP 20187

FIG. 53

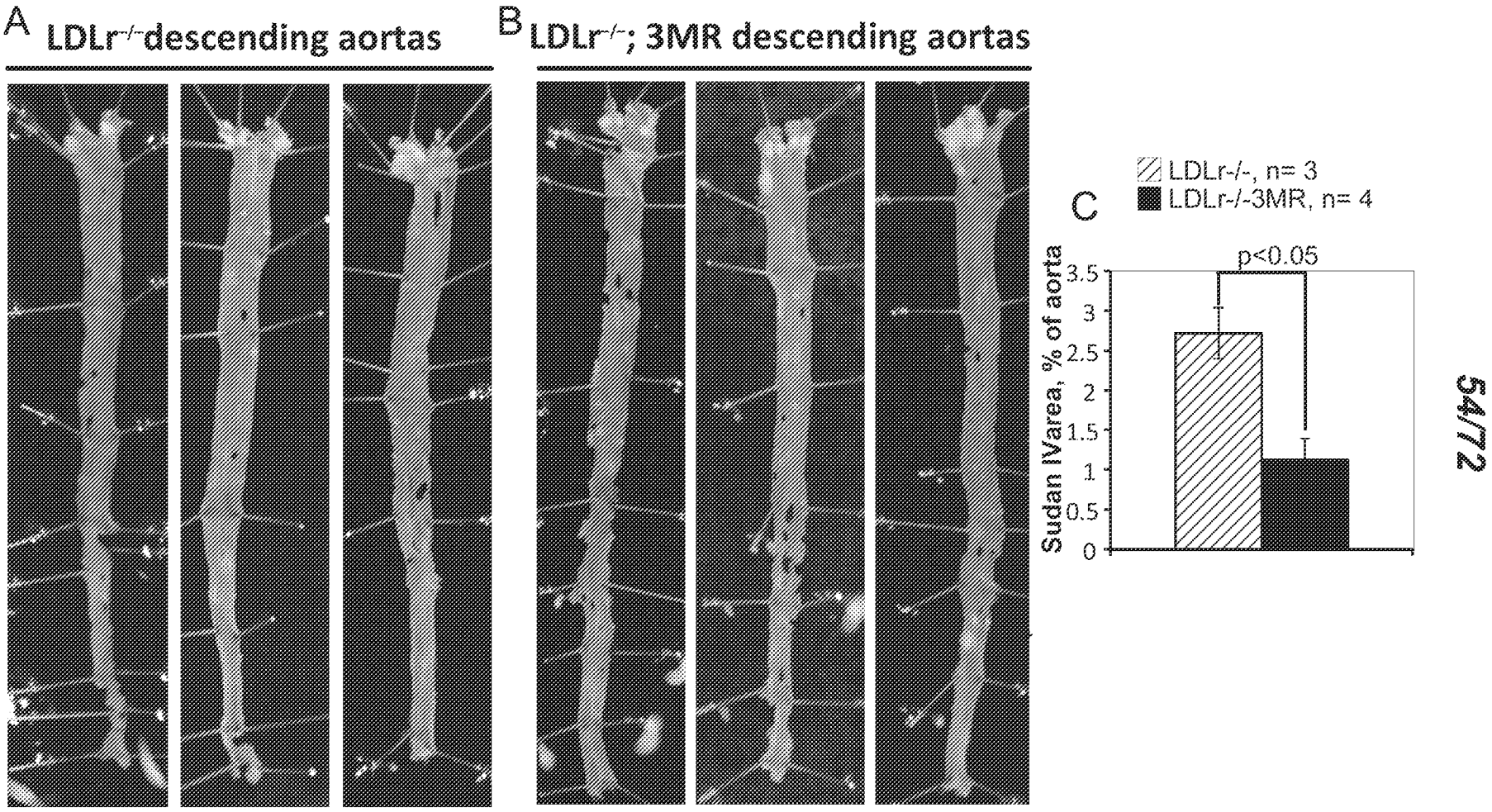

A LDLr-/-descending aortas
B LDLr-/-; 3MR descending aortas
C
LDLr-/-, n= 3
LDLr-/-3MR, n= 4
p<0.05
Sudan IVarea, % of aorta
3.5
3
2.5
2
1.5
1
0.5
0

FIG. 54

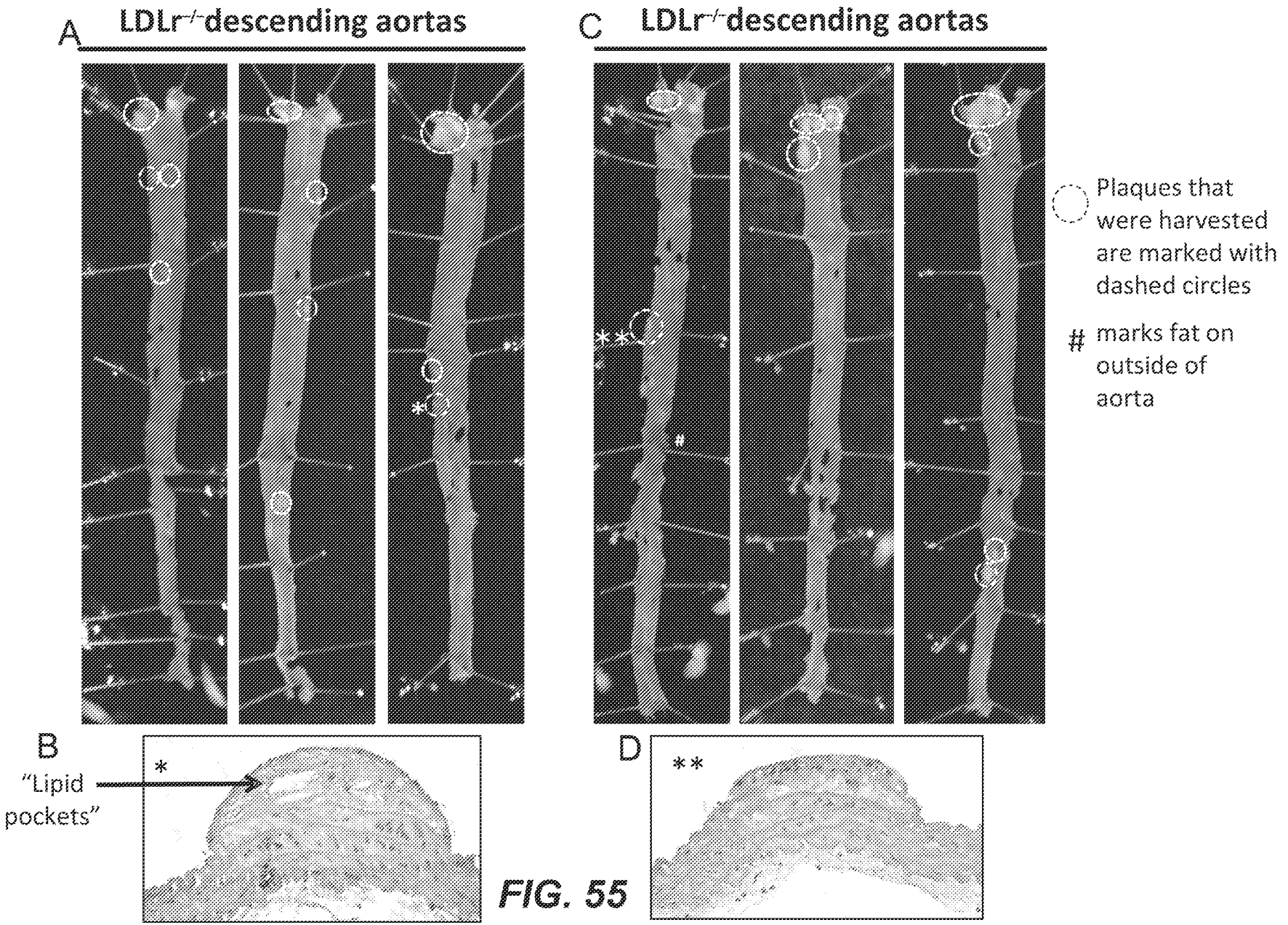

A
LDLr-/- descending aortas
C
LDLr-/- descending aortas
Plaques that were harvested are marked with dashed circles
marks fat on outside of aorta
B
"Lipid pockets"
*
D
**

FIG. 55

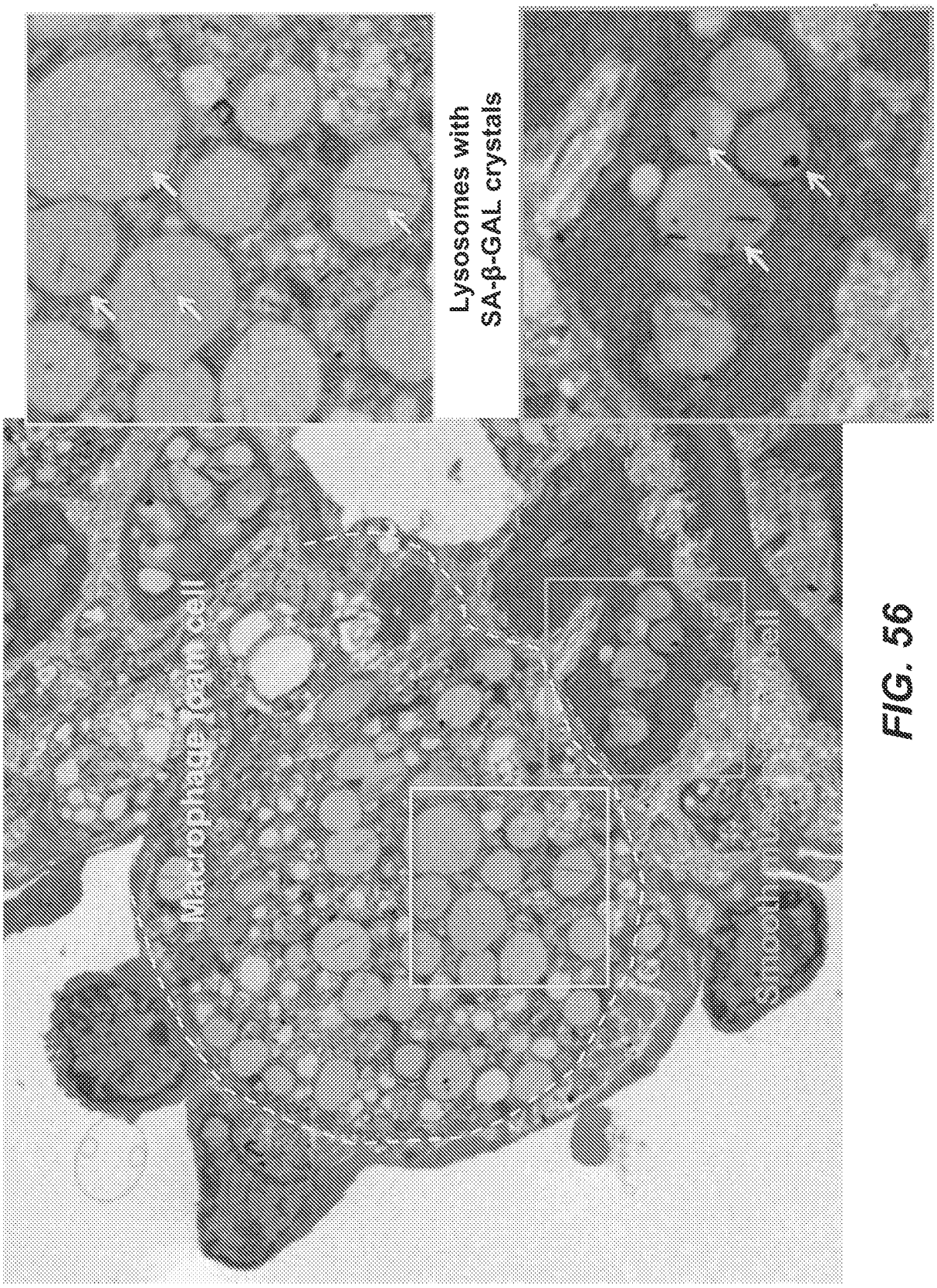
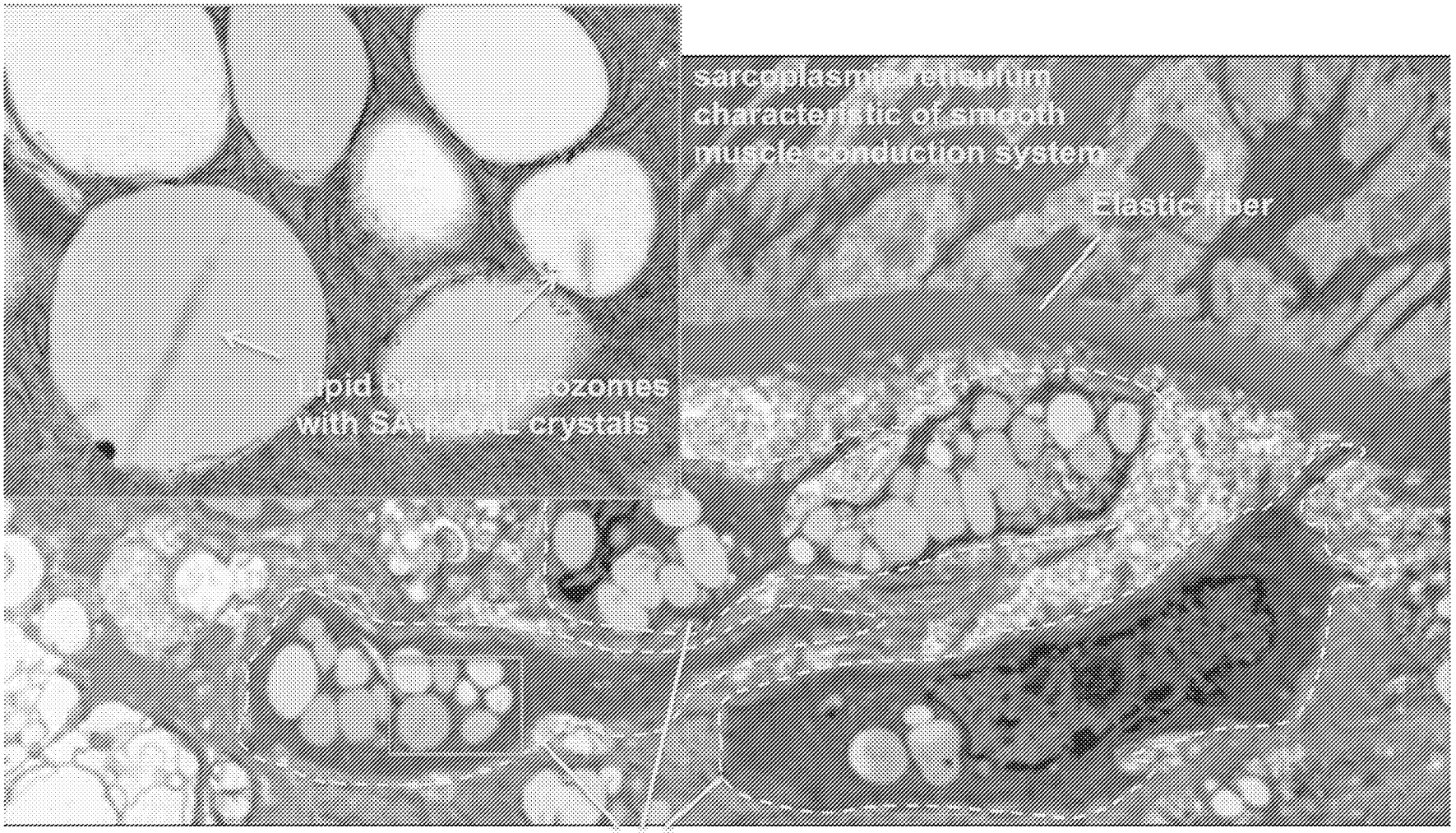


FIG. 56



FIG. 57



Senescent (lipid-bearing)
smooth muscle foam cells

FIG. 58

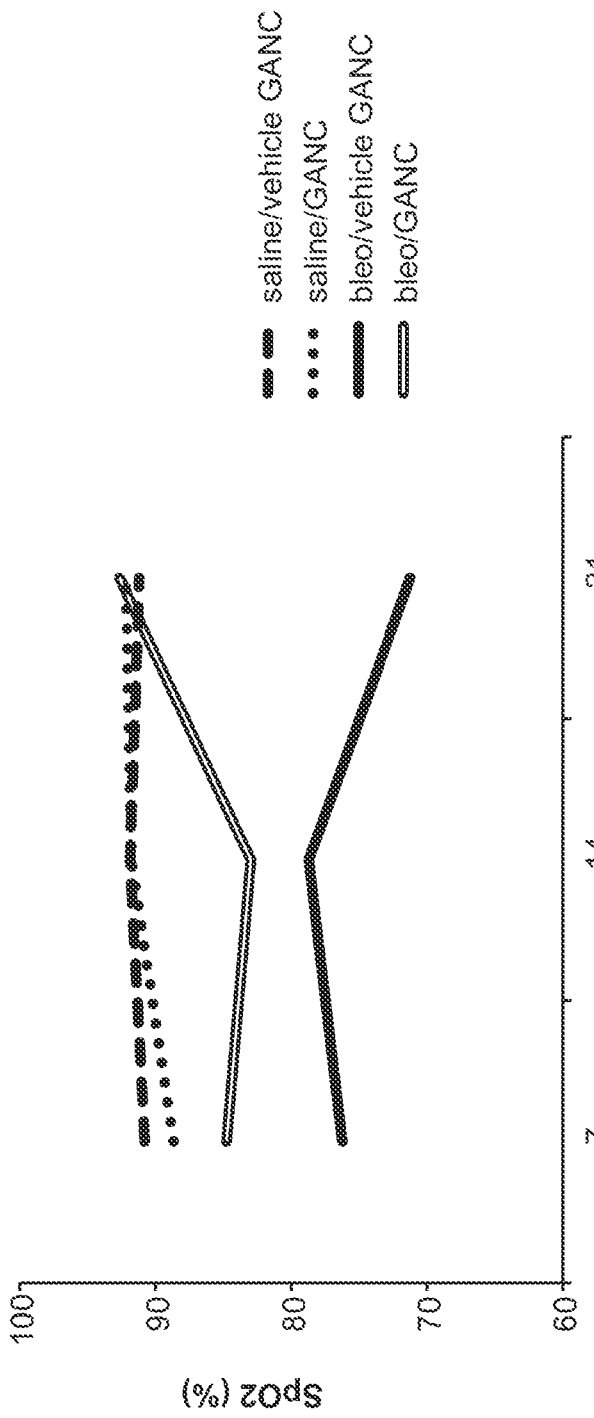

saline/vehicle GANC
saline/GANC
bleo/vehicle GANC
bleo/GANC
100
90
80
70
60
sp02 (%)
7
14
21
Days post bleo

FIG. 59

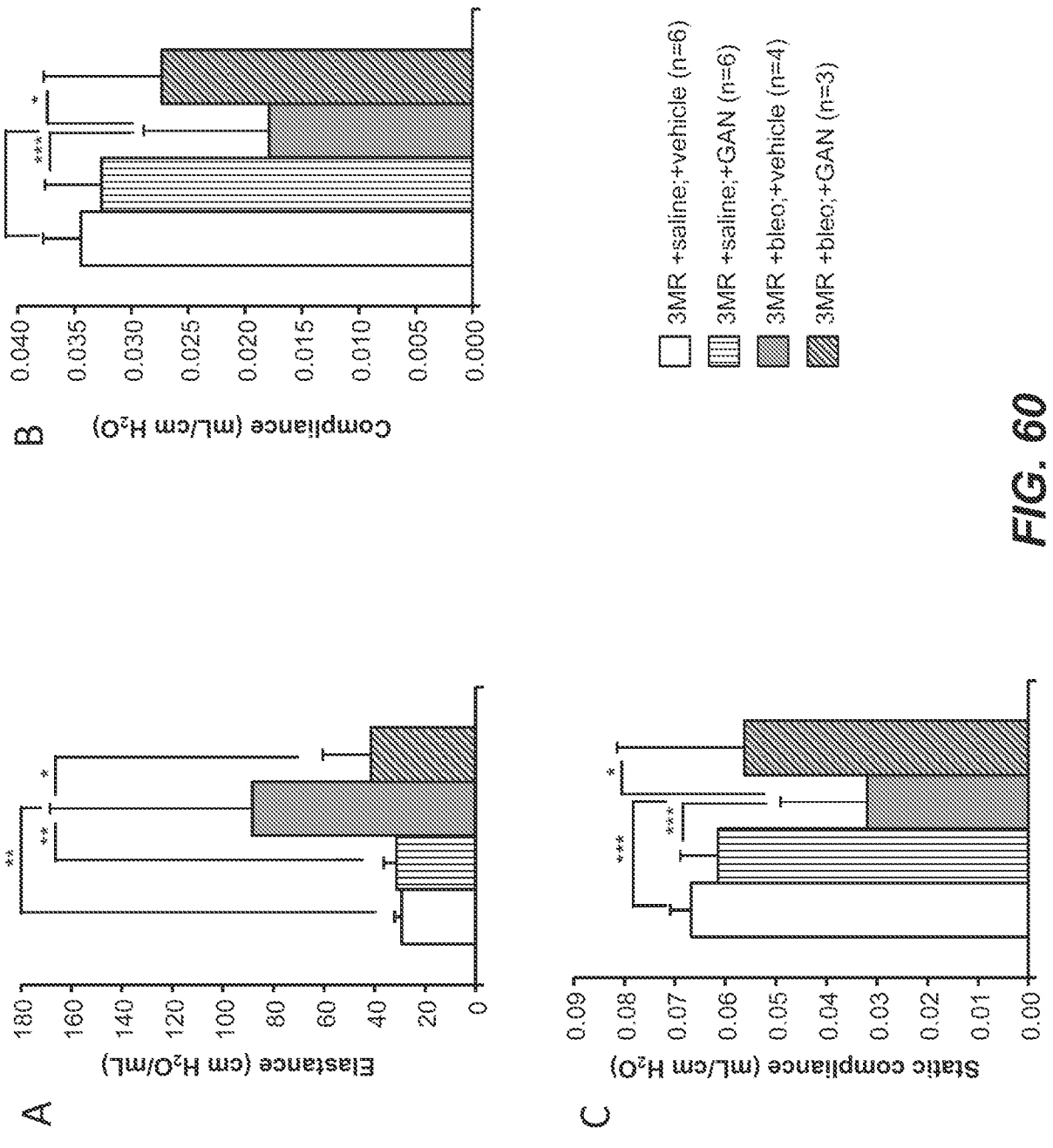

A
Elastance (cm H2O/mL)
180
160
140
120
100
80
60
40
20
0
**
**
*
B
Compliance (mL/cm H2O)
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
***
***
*
C
Static compliance (mL/cm H2O)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
***
***
*
3MR +saline;+vehicle (n=6)
3MR +saline;+GAN (n=6)
3MR +bleo;+vehicle (n=4)
3MR +bleo;+GAN (n=3)

FIG. 60

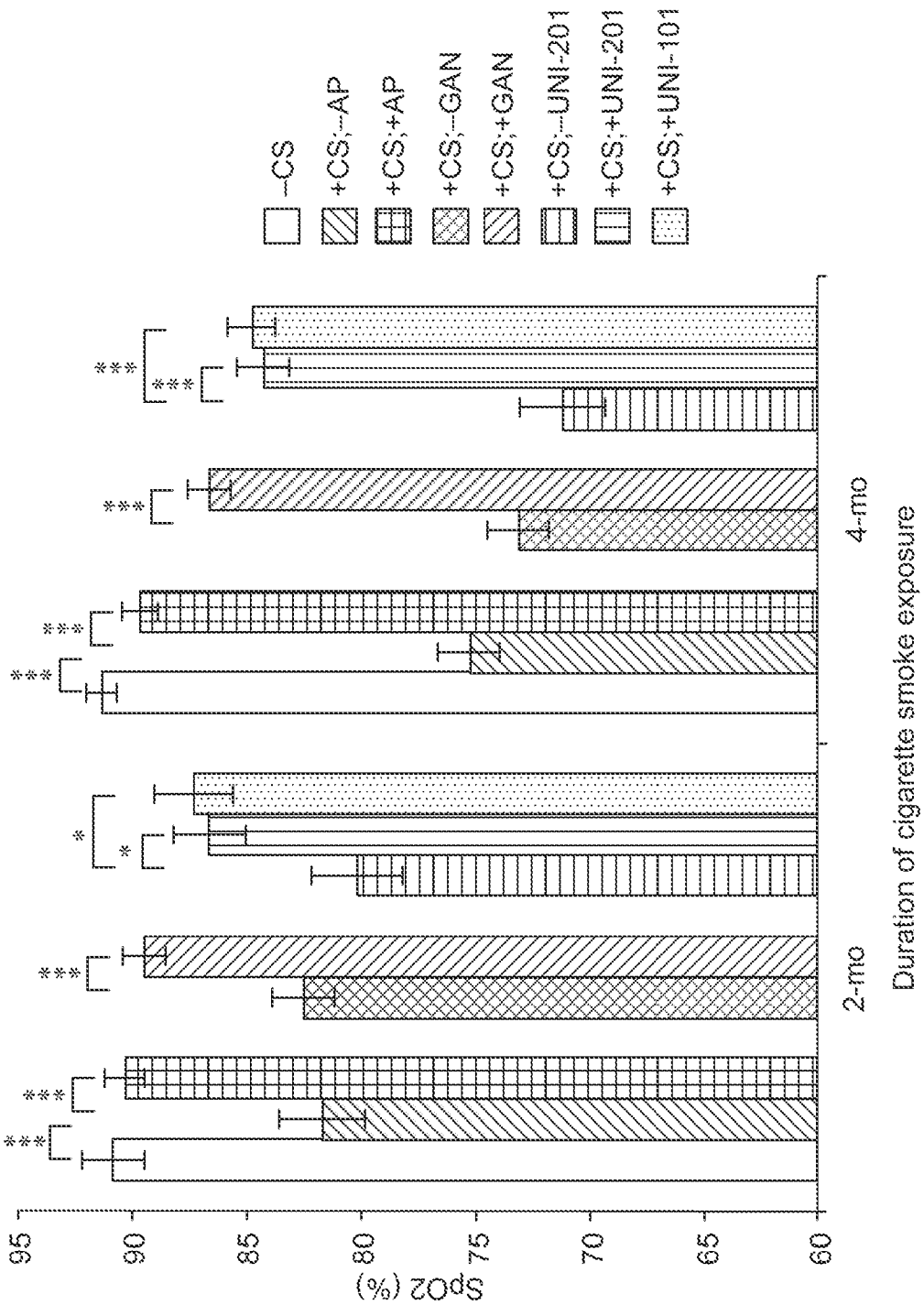
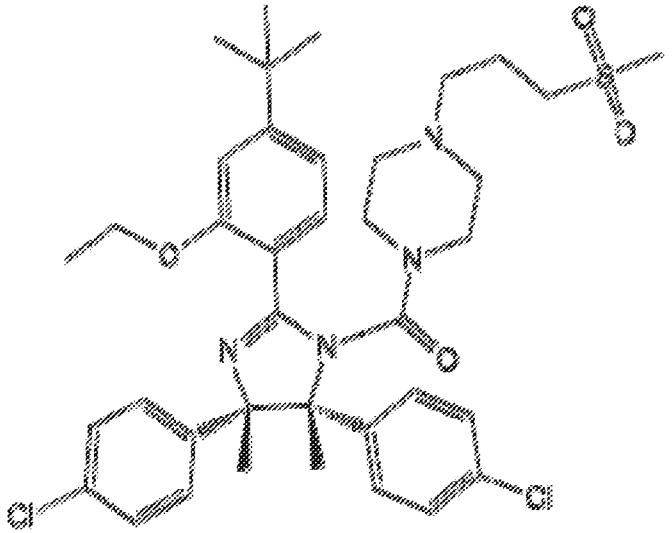
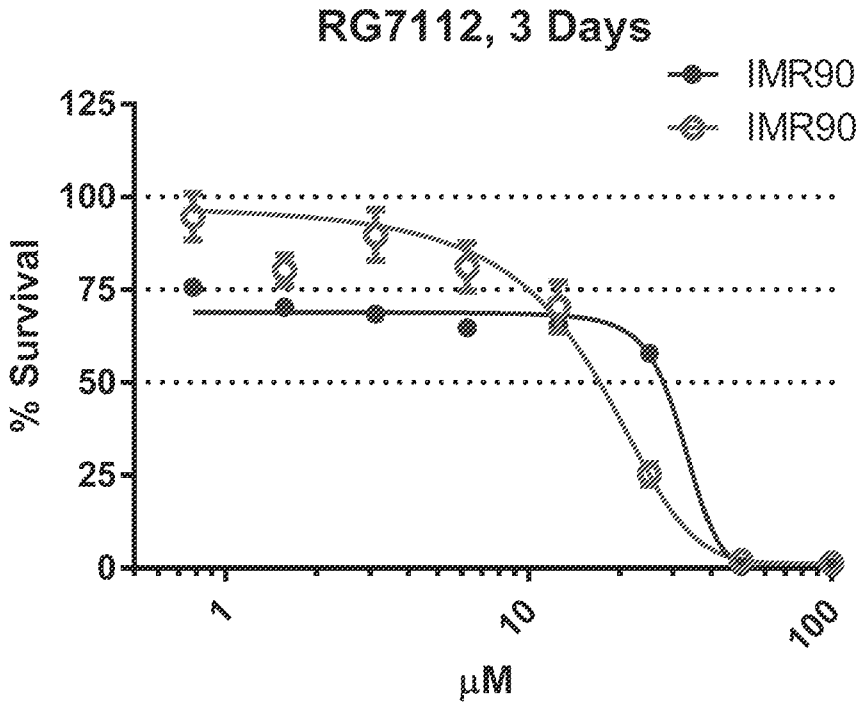

−CS
+CS;−AP
+CS;+AP
+CS;−GAN
+CS;+GAN
+CS;−UNI-201
+CS;+UNI-201
+CS;+UNI-101
SpO2 (%)
95
90
85
80
75
70
65
60
2-mo
4-mo
Duration of cigarette smoke exposure
***
*

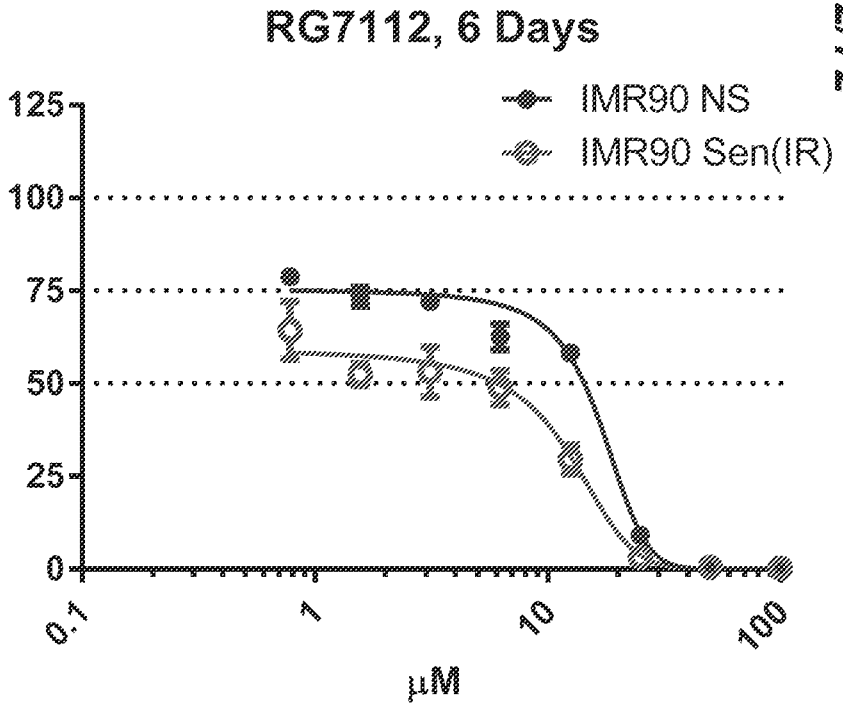
FIG. 61

FIG. 62

A


O
O
S
O
N
N
N
N
O
O
Cl
Cl


RG7112, 3 Days
IMR90 NS
IMR90 Sen(IR)
% Survival
125
100
75
50
25
0
1
10
100
μM


RG7112, 6 Days
IMR90 NS
IMR90 Sen(IR)
125
100
75
50
25
0
0.1
1
10
100
μM

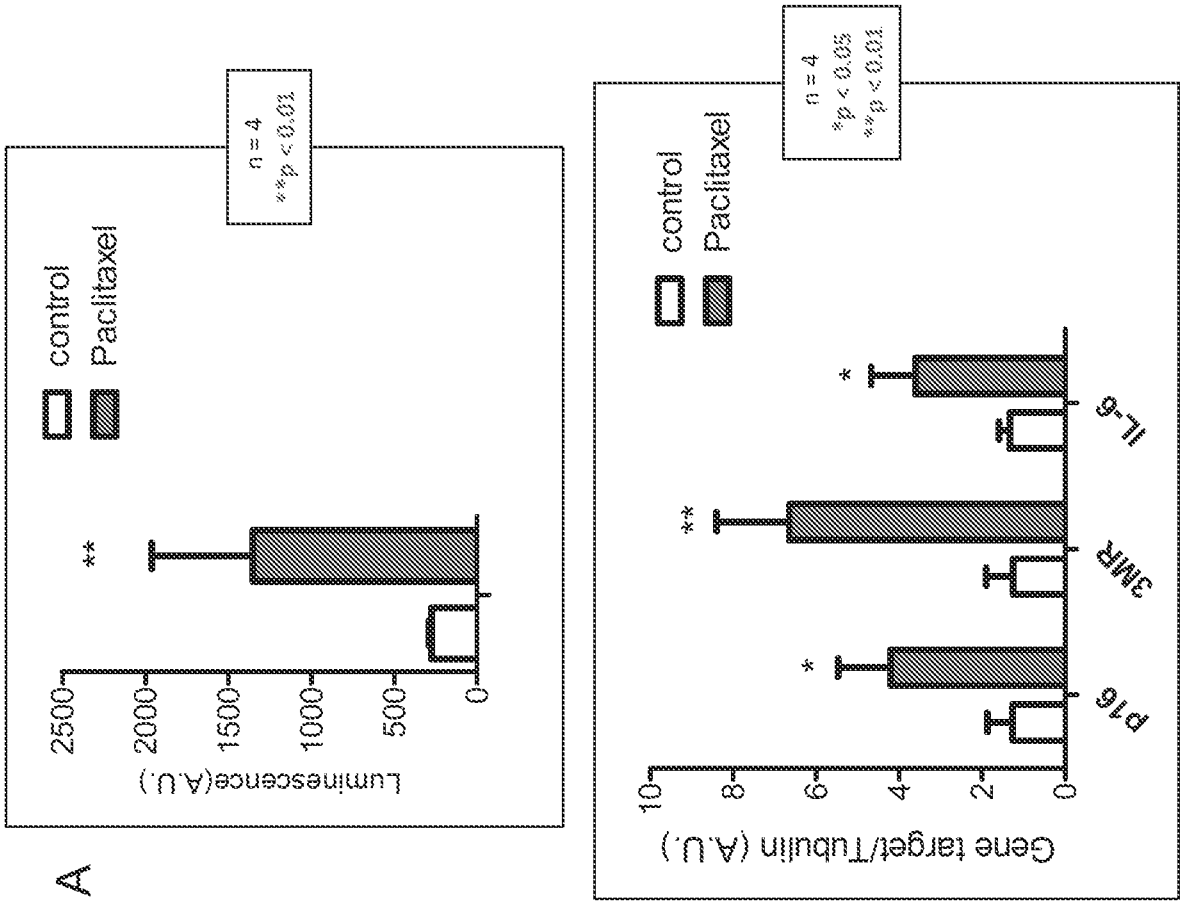
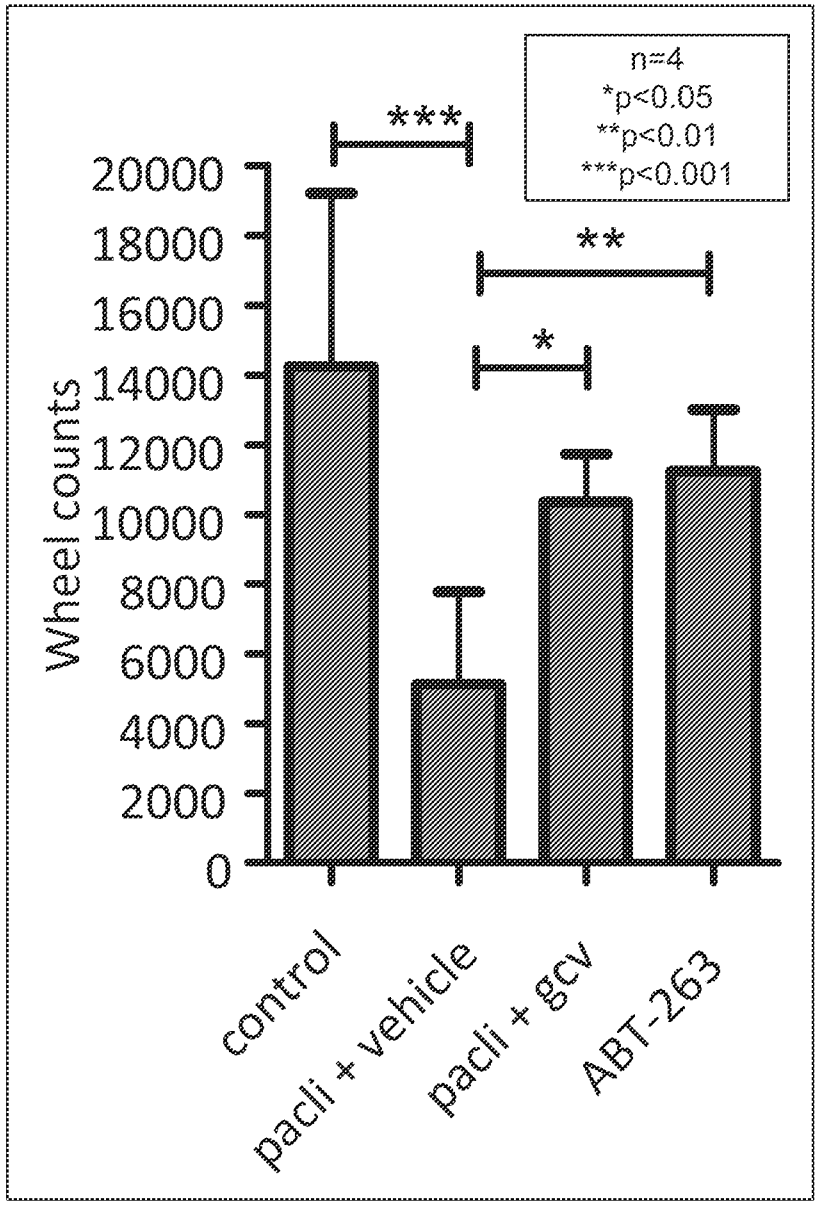

A
control
Paclitaxel
n = 4
**p < 0.01
Luminescence(A.U.)
2500
2000
1500
1000
500
0
**
B
control
Paclitaxel
n = 4
*p < 0.05
**p < 0.01
Gene target/Tubulin (A.U.)
10
8
6
4
2
0
*
**
*
p16
3hIR
IL-6

FIG. 63


n=4
*p<0.05
**p<0.01
***p<0.001
***
**
*
Wheel counts
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
control
pacli + vehicle
pacli + gcv
ABT-263

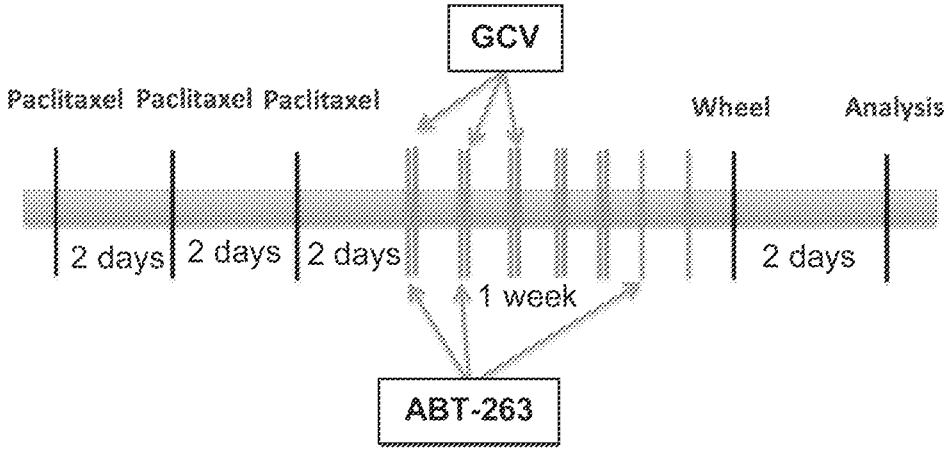

GCV
Paclitaxel
Paclitaxel
Paclitaxel
Wheel
Analysis
2 days
2 days
2 days
2 days
1 week
ABT-263

FIG. 64

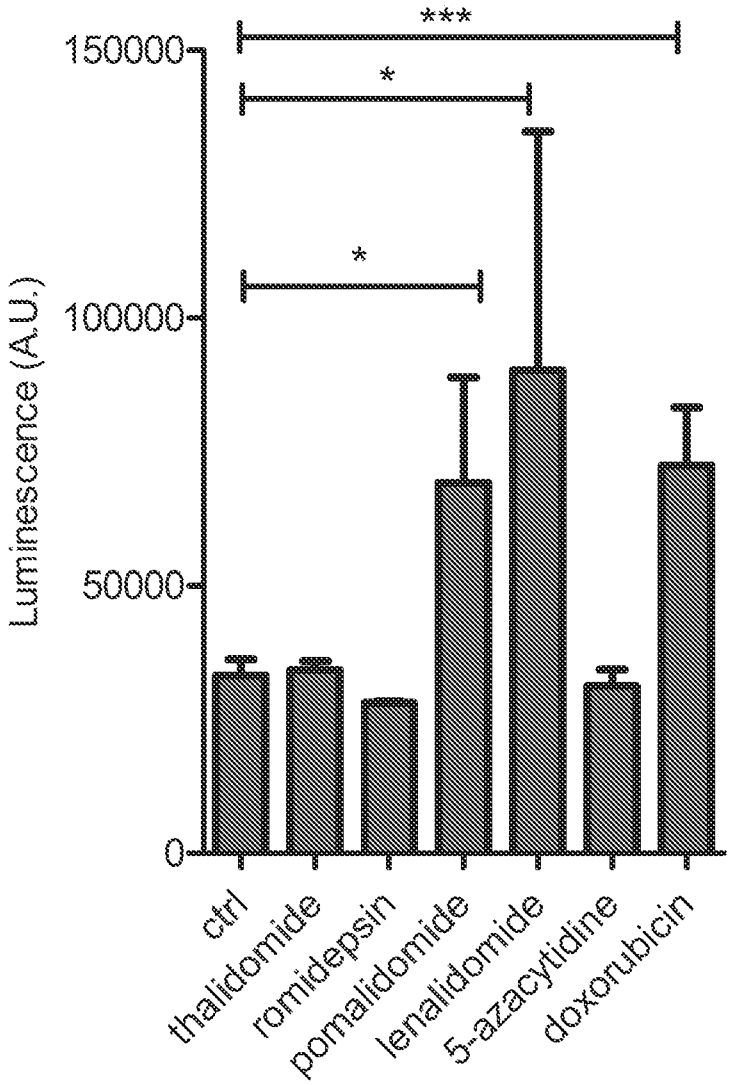

***
*
*
150000
100000
50000
0
Luminescence (A.U.)
ctrl
thalidomide
romidepsin
pomalidomide
lenalidomide
5-azacytidine
doxorubicin

FIG. 65

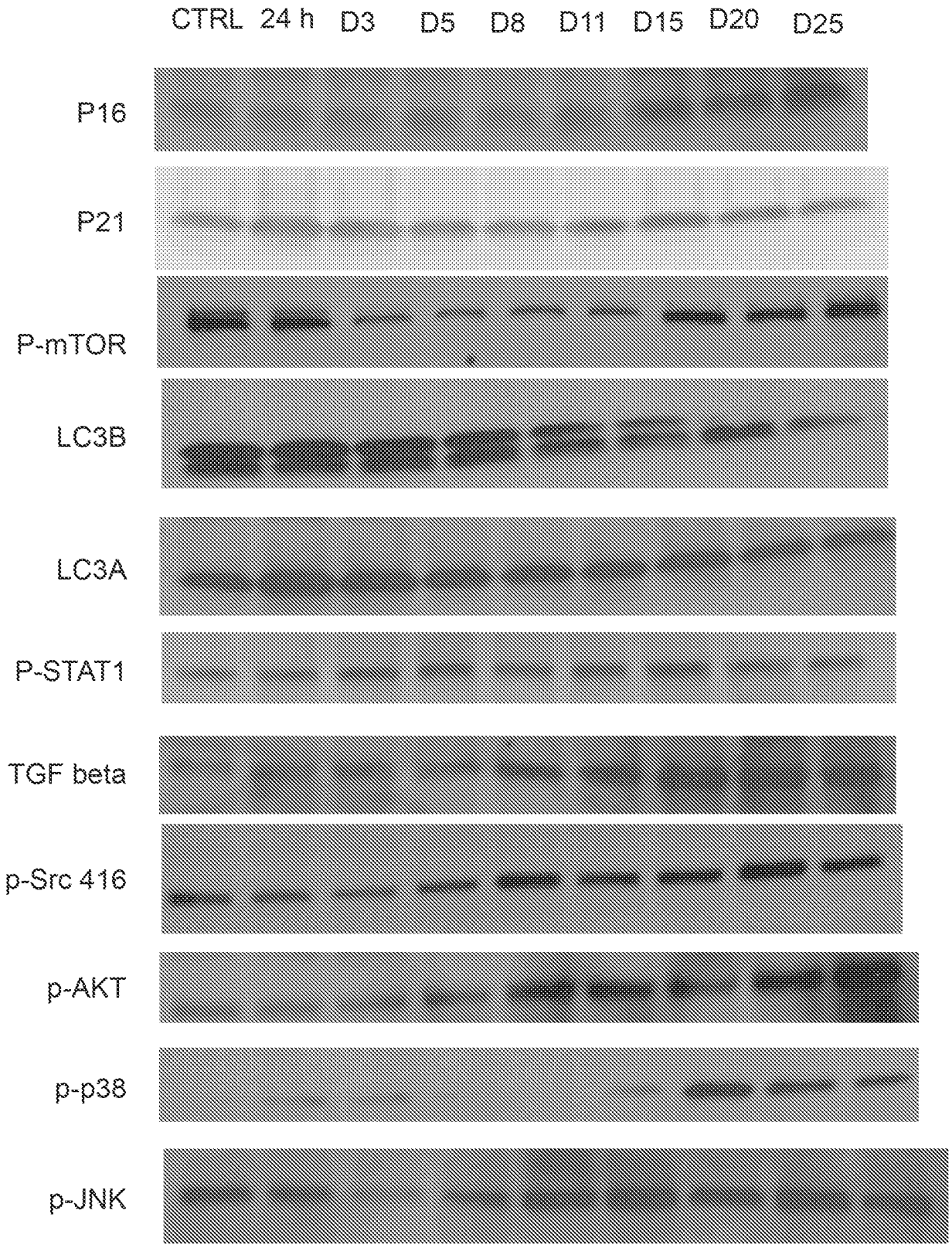

CTRL
24 h
D3
D5
D8
D11
D15
D20
D25
P16
P21
P-mTOR
LC3B
LC3A
P-STAT1
TGF beta
p-Src 416
p-AKT
p-p38
p-JNK

FIG. 66

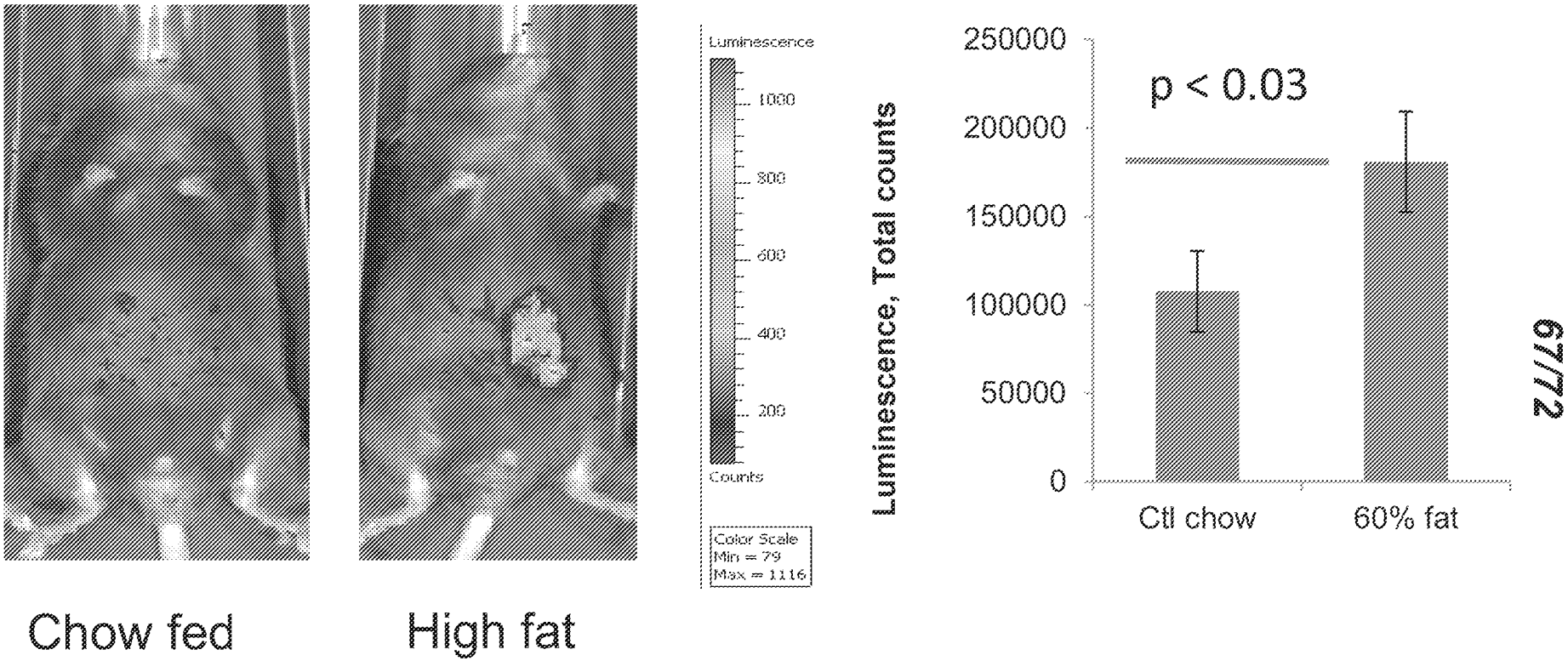

Chow fed
High fat
Luminescence
1000
800
600
400
200
Counts
Color Scale
Min = 79
Max = 1116
Luminescence, Total counts
250000
200000
150000
100000
50000
0
p < 0.03
Ctl chow
60% fat

FIG. 67

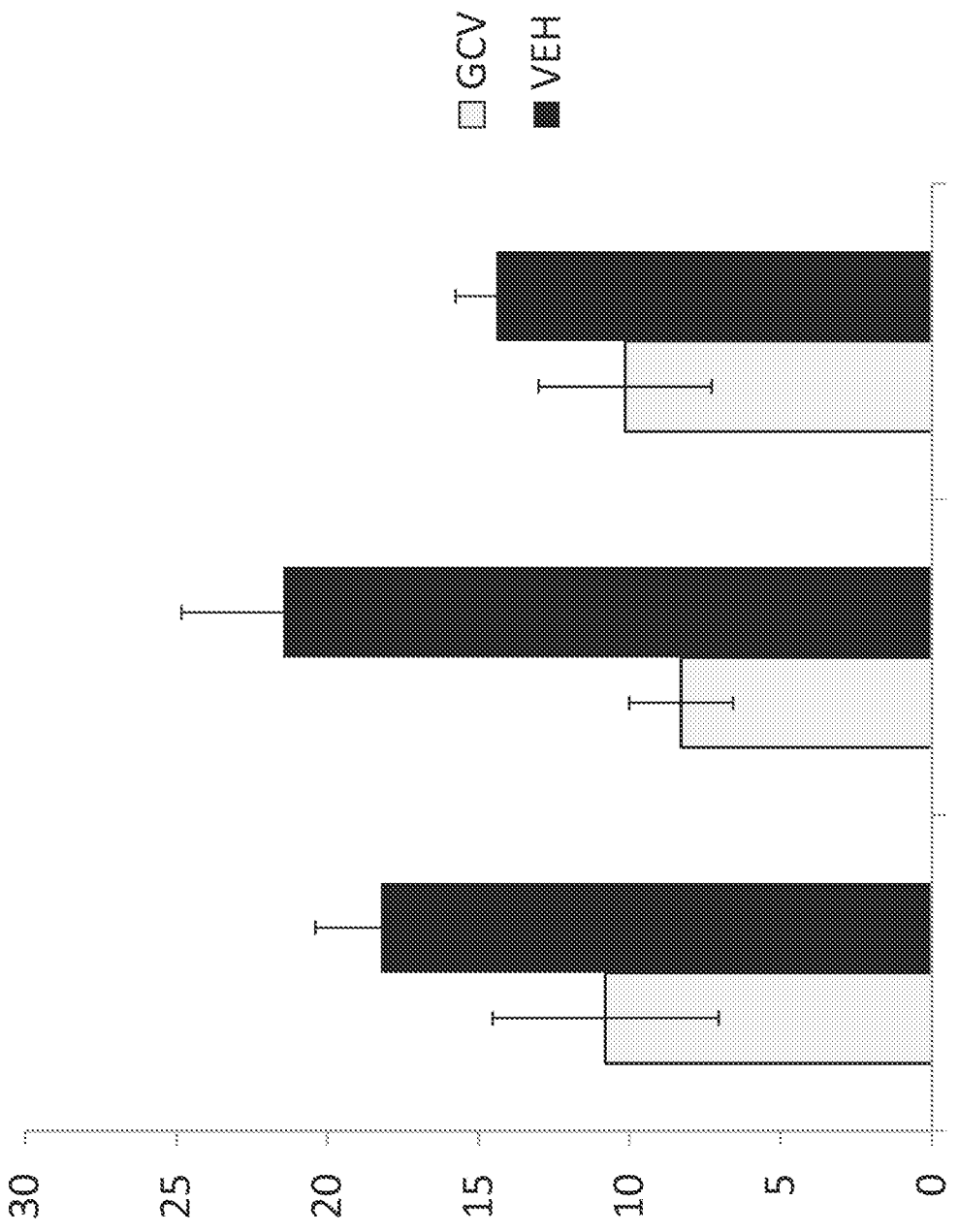


FIG. 68

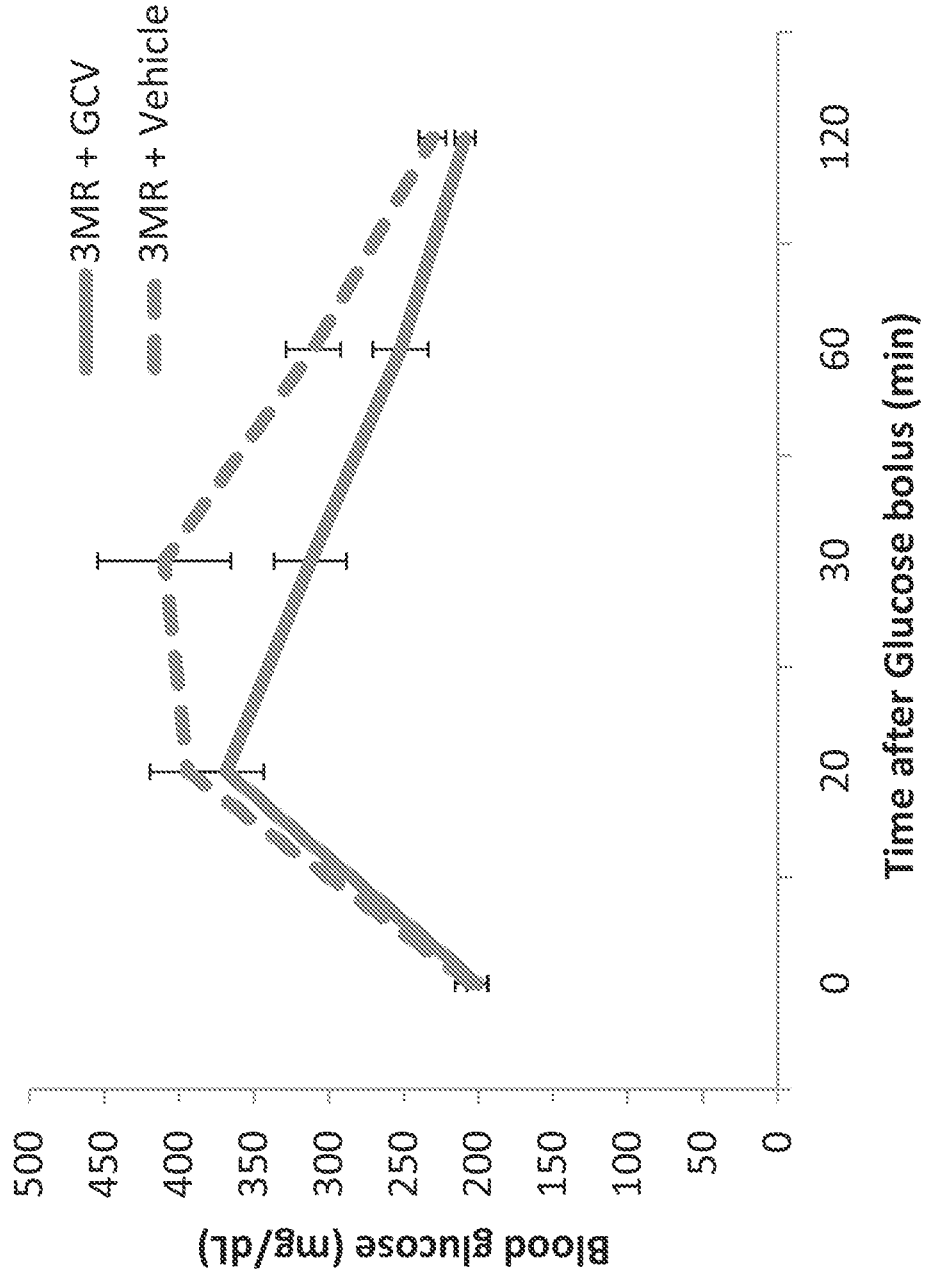

3MR + GCV
3MR + Vehicle
Blood glucose (mg/dL)
500
450
400
350
300
250
200
150
100
50
0
0
20
30
60
120
Time after Glucose bolus (min)

FIG. 69A

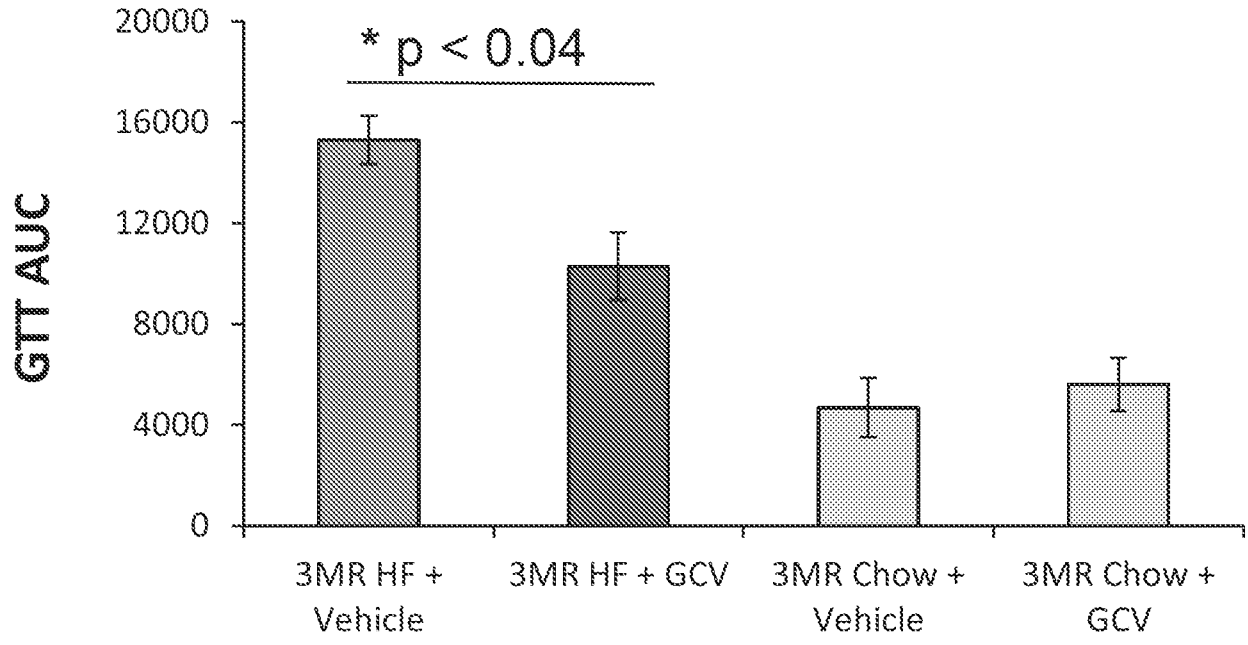


FIG. 69B

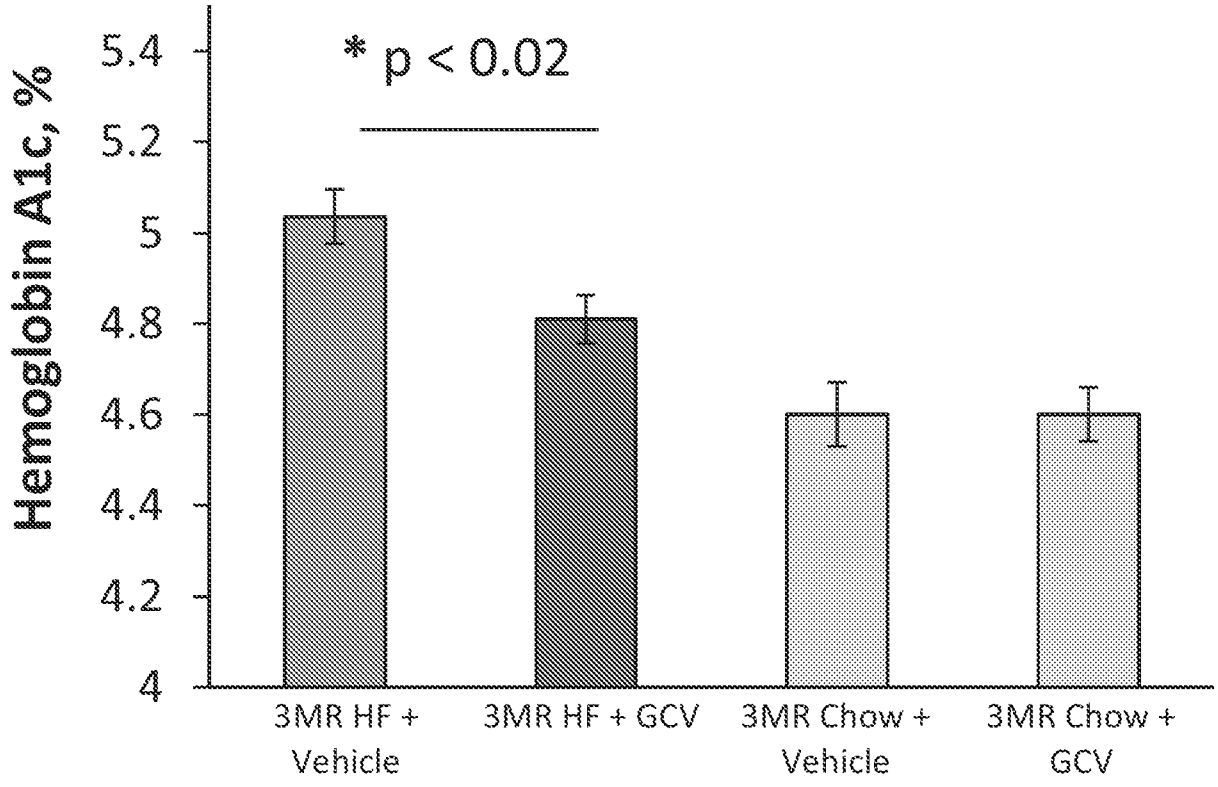


FIG. 69C

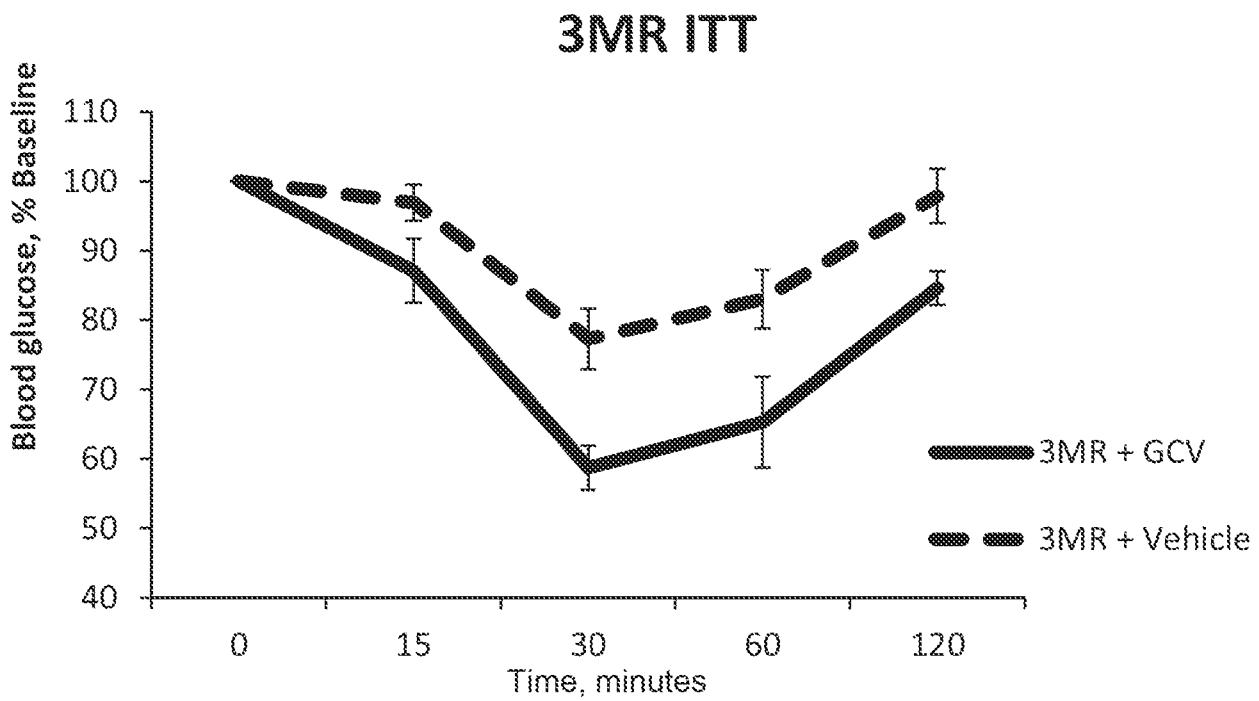


FIG. 70A

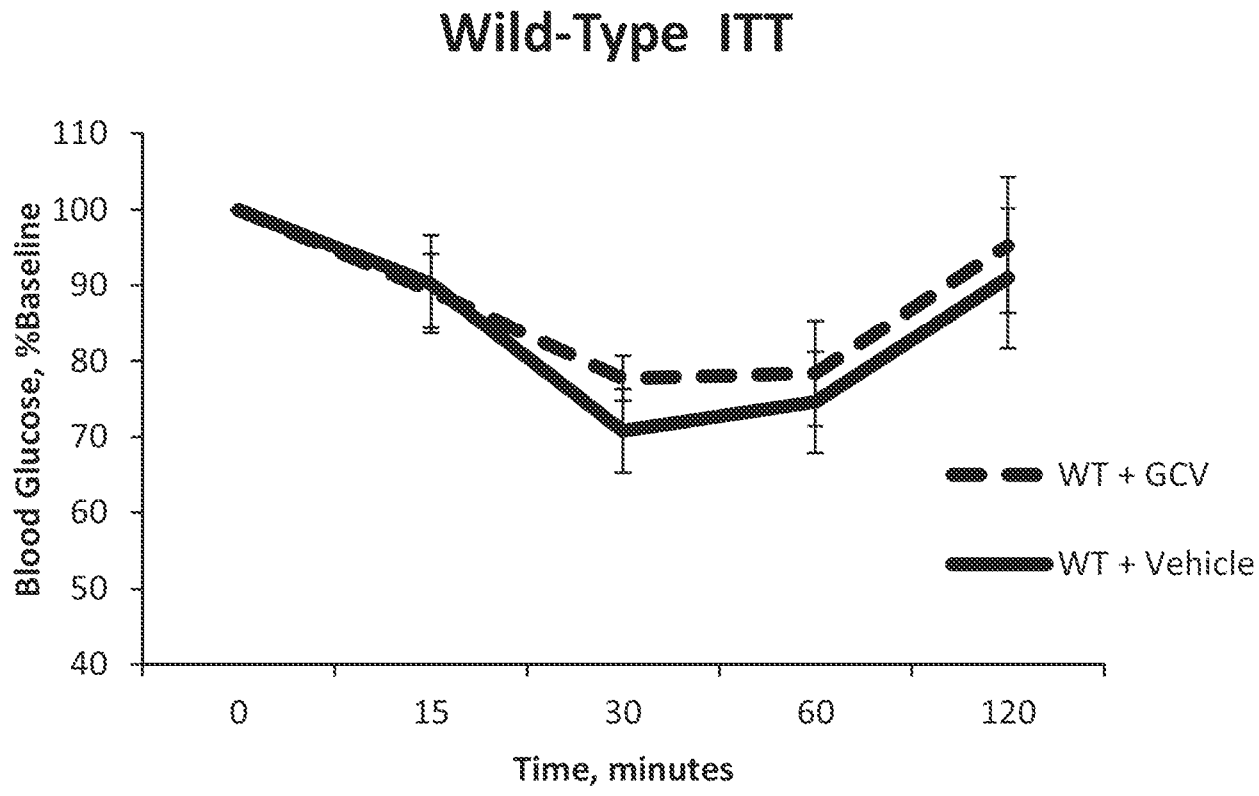


FIG. 70B

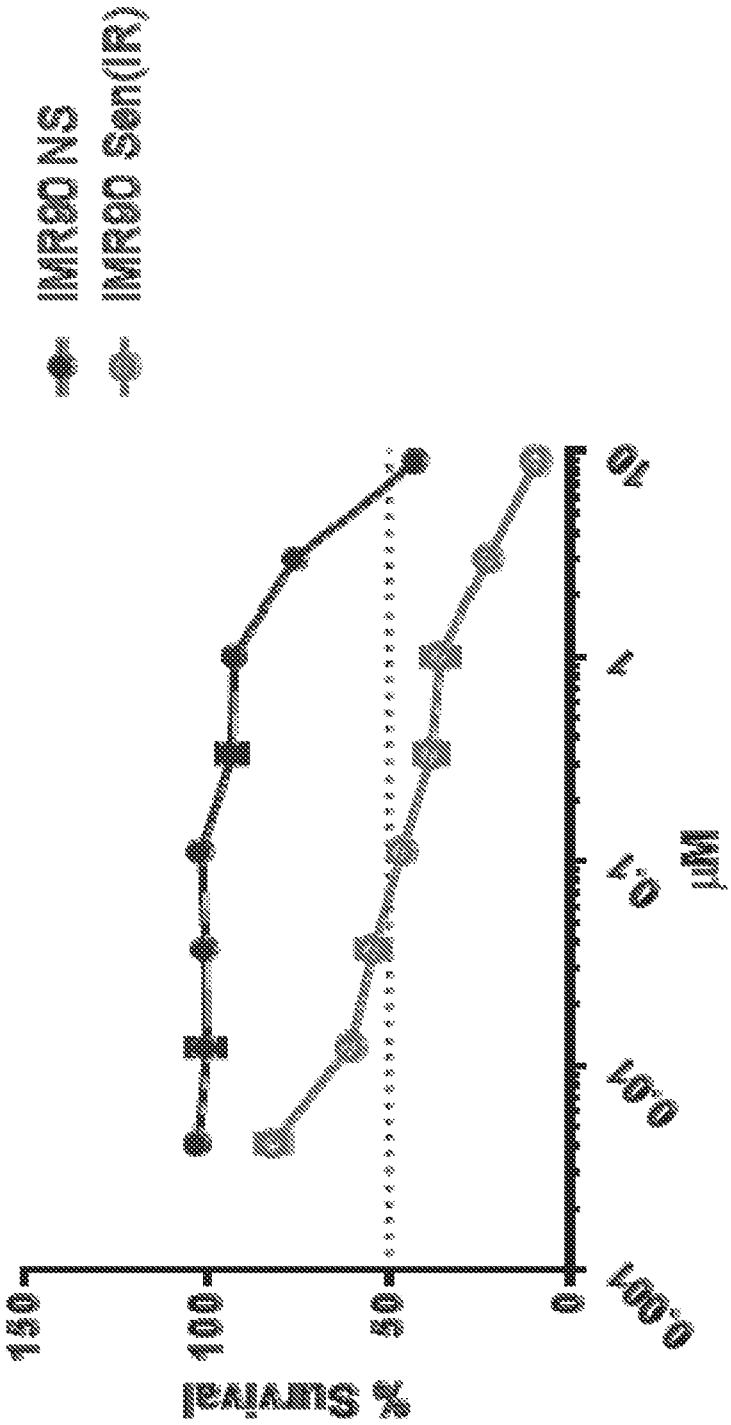

A-1155463
IMR90 NS
IMR90 Sen(IR)
% Survival
150
100
50
0
0.01
0.1
1
10
100
μM

FIG. 71