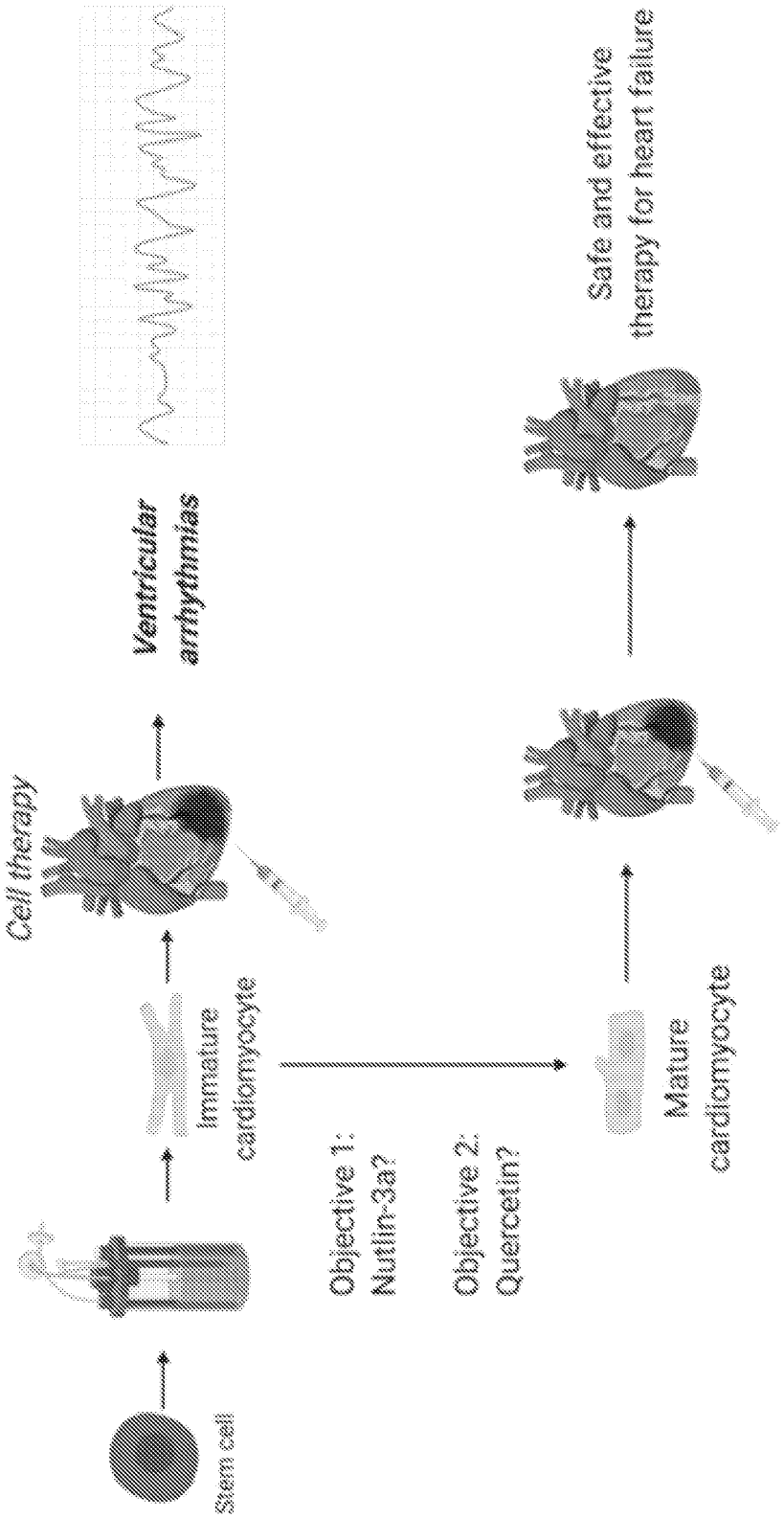

Stem cell
Immature cardiomyocyte
Cell therapy
Ventricular arrhythmias
Objective 1: Nutlin-3a?
Objective 2: Quercetin?
Mature cardiomyocyte
Safe and effective therapy for heart failure

FIG. 1

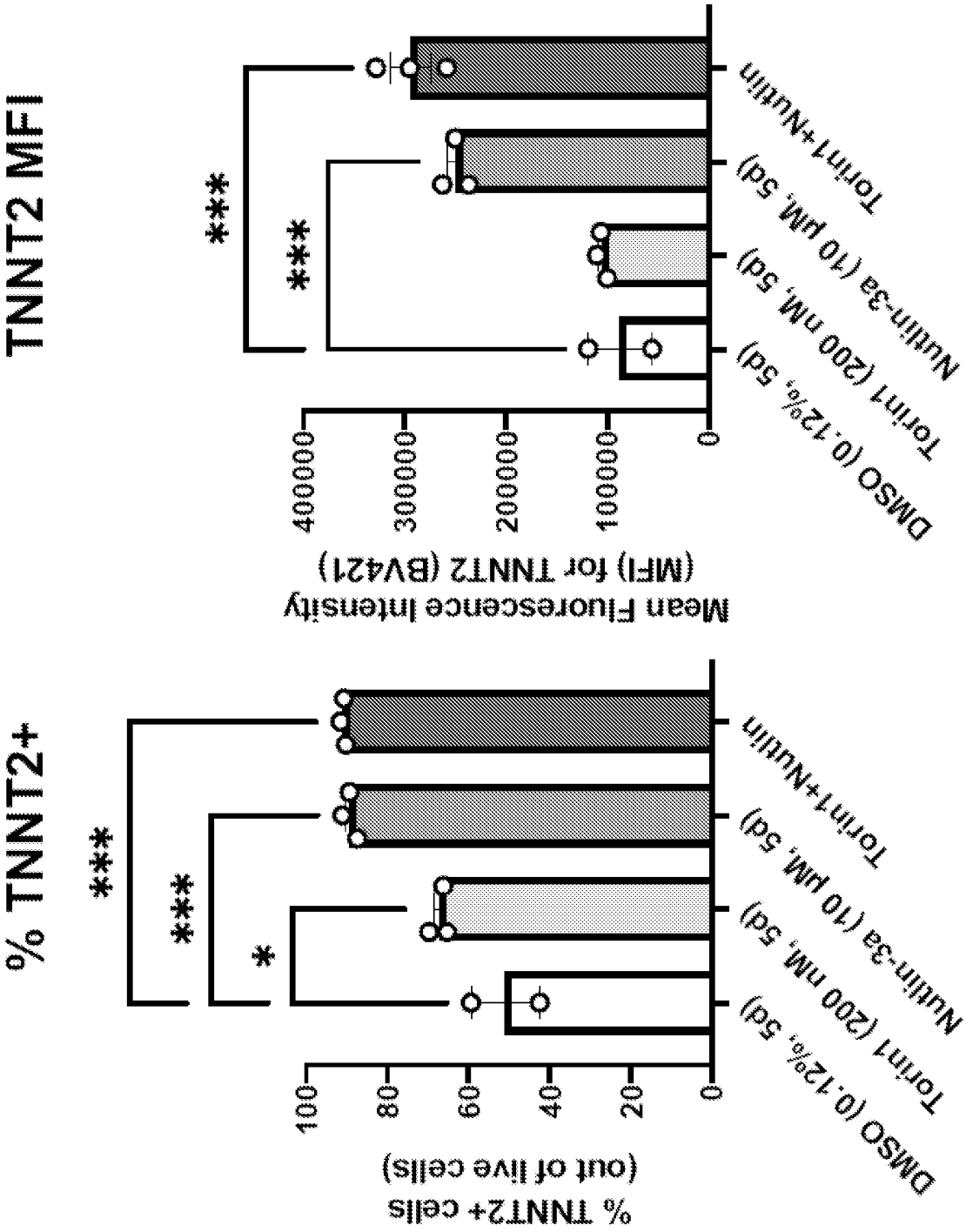

% TNNT2+
% TNNT2+ cells
(out of live cells)
100
80
60
40
20
0
*
****
***
DMSO (0.12%, 5d)
Torin1 (200 nM, 5d)
Nutlin-3a (10 μM, 5d)
Torin1+Nutlin
TNNT2 MFI
Mean Fluorescence Intensity
(MFI) for TNNT2 (BV421)
400000
300000
200000
100000
0
***
***
DMSO (0.12%, 5d)
Torin1 (200 nM, 5d)
Nutlin-3a (10 μM, 5d)
Torin1+Nutlin

FIG. 2A

FIG. 2B

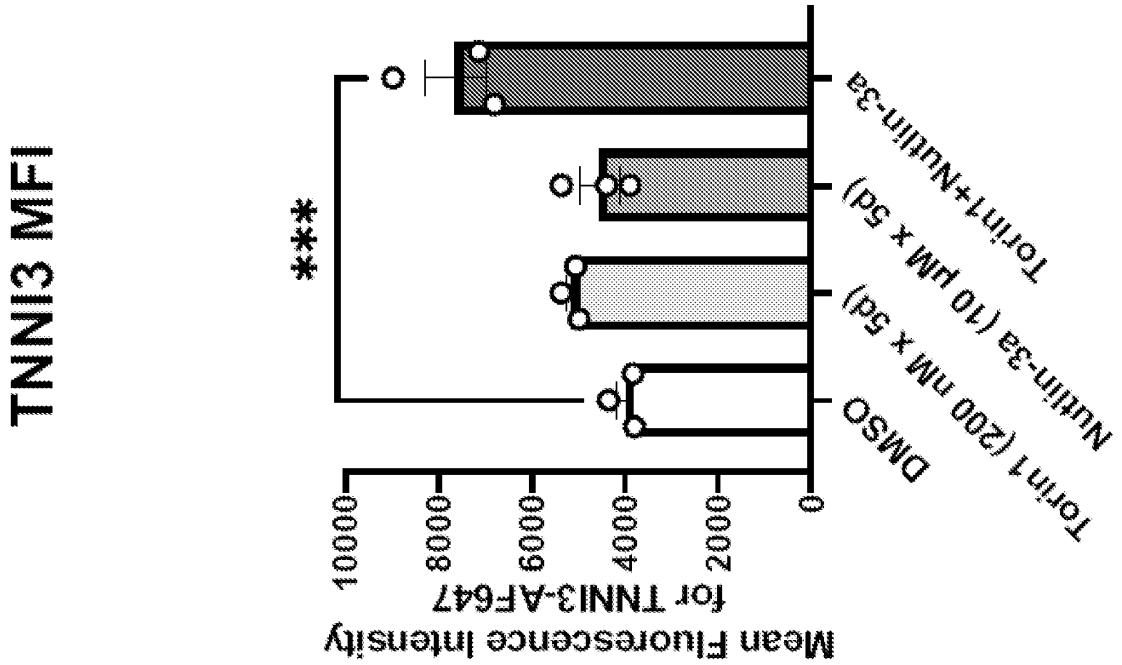

TNNI3 MFI
Mean Fluorescence Intensity
for TNNI3-AF647
10000
8000
6000
4000
2000
0
***
DMSO
Torin 1 (200 nM x 5d)
Nutlin-3a (10 μM x 5d)
Torin1+Nutlin-3a

FIG. 2D

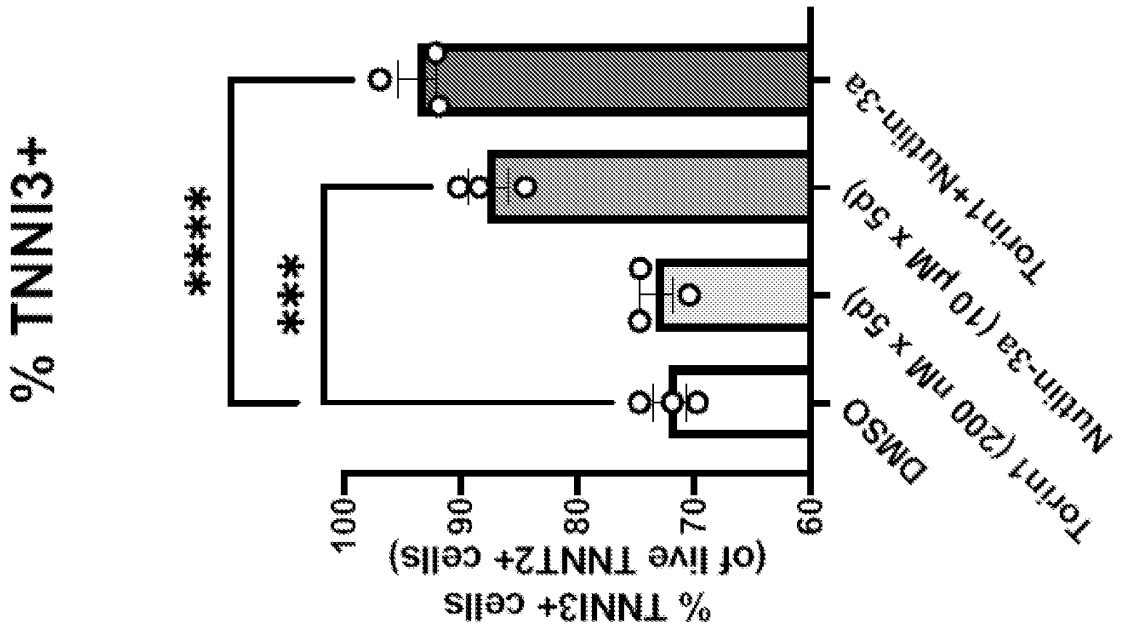

% TNNI3+
% TNNI3+ cells
(of live TNNT2+ cells)
100
90
80
70
60
***
*****
DMSO
Torin 1 (200 nM x 5d)
Nutlin-3a (10 μM x 5d)
Torin1+Nutlin-3a

FIG. 2C

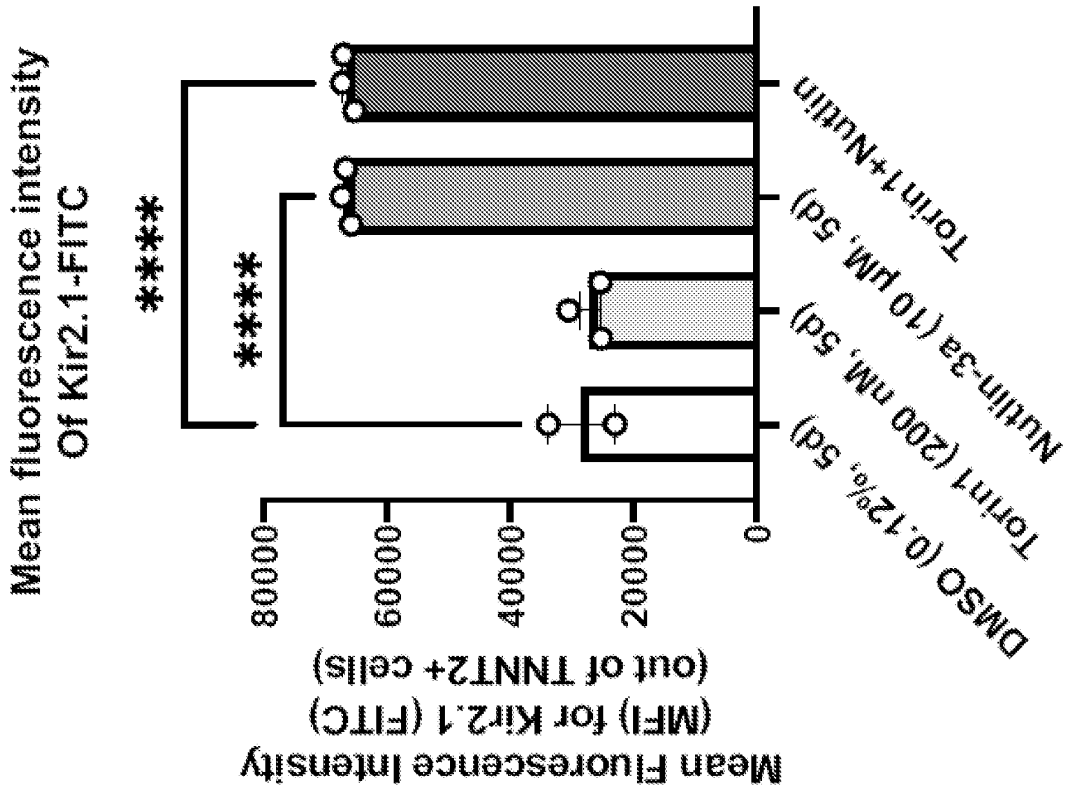

Mean fluorescence intensity
Of Kir2.1-FITC
****
****
80000
60000
40000
20000
0
DMSO (0.12%, 5d)
Torin1 (200 nM, 5d)
Nutlin-3a (10 μM, 5d)
Torin1+Nutlin

FIG. 2F

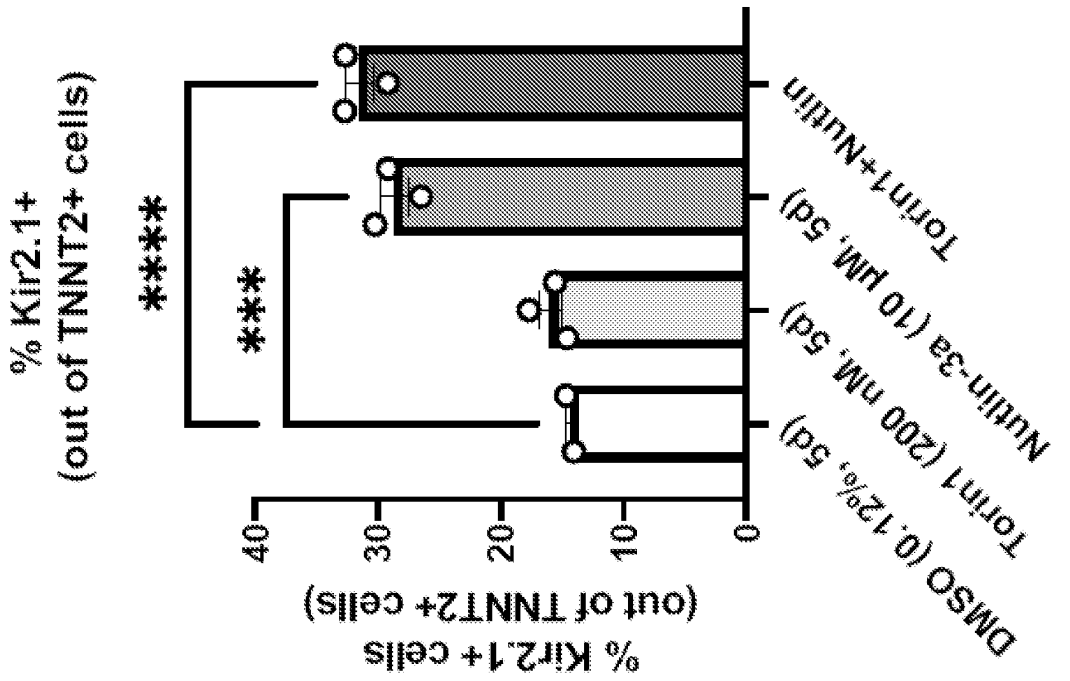

% Kir2.1+
(out of TNNT2+ cells)
*****
***
40
30
20
10
0
DMSO (0.12%, 5d)
Torin1 (200 nM, 5d)
Nutlin-3a (10 μM, 5d)
Torin1+Nutlin

FIG. 2E

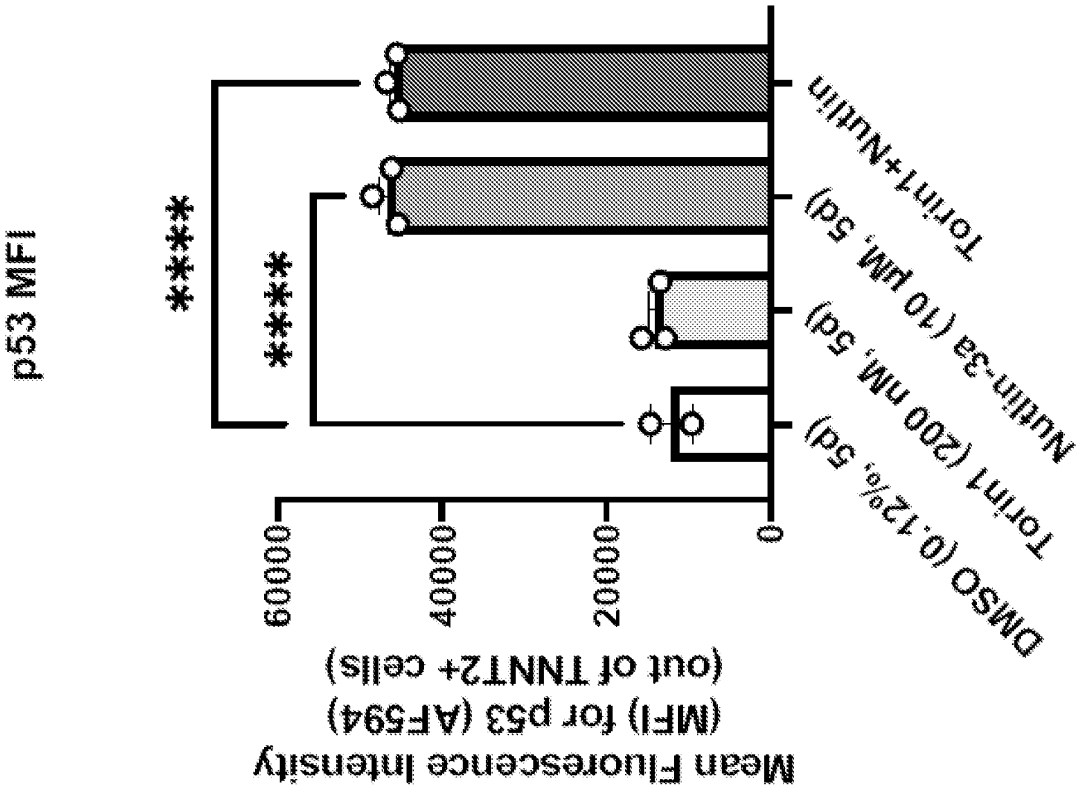

p53 MFI
*****
*****
60000
40000
20000
0
Mean Fluorescence Intensity
(MFI) for p53 (AF594)
(out of TNNT2+ cells)
DMSO (0.12%, 5d)
Torin1 (200 nM, 5d)
Nutlin-3a (10 μM, 5d)
Torin1+Nutlin

FIG. 2H

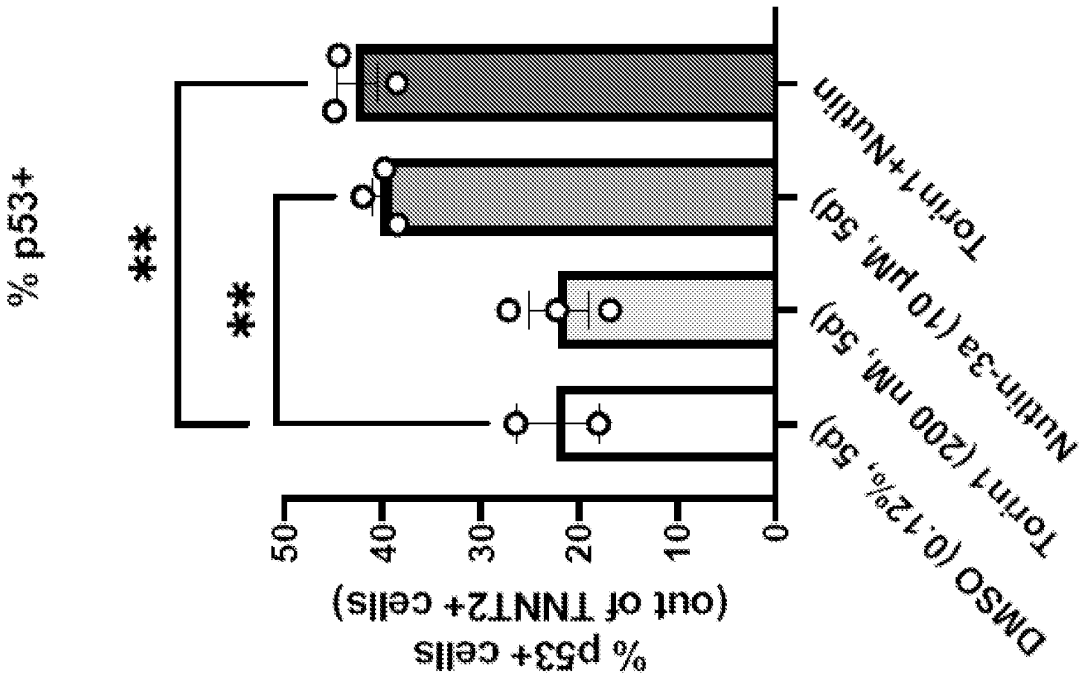

% p53+
**
**
50
40
30
20
10
0
% p53+ cells
(out of TNNT2+ cells)
DMSO (0.12%, 5d)
Torin1 (200 nM, 5d)
Nutlin-3a (10 μM, 5d)
Torin1+Nutlin

FIG. 2G

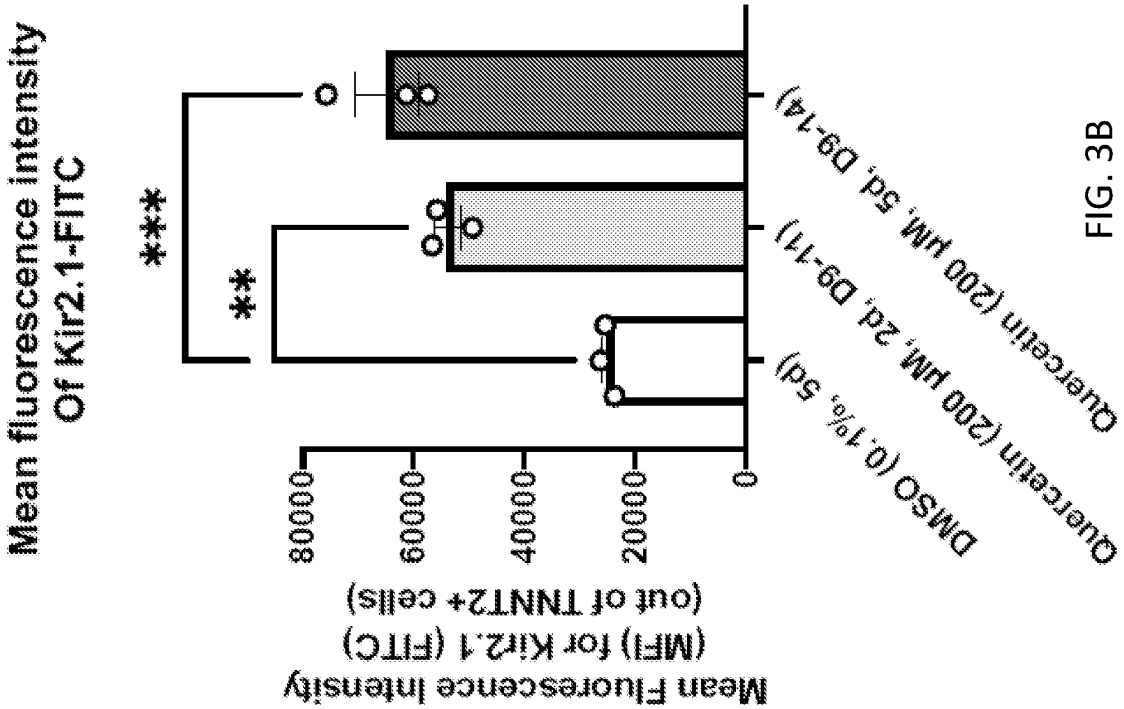

Mean fluorescence intensity
Of Kir2.1-FITC
***
**
80000
60000
40000
20000
0
Mean Fluorescence Intensity
(MFI) for Kir2.1 (FITC)
(out of TNNT2+ cells)
DMSO (0.1%, 5d)
Quercetin (200 μM, 2d, D9-11)
Quercetin (200 μM, 5d, D9-14)

FIG. 3B

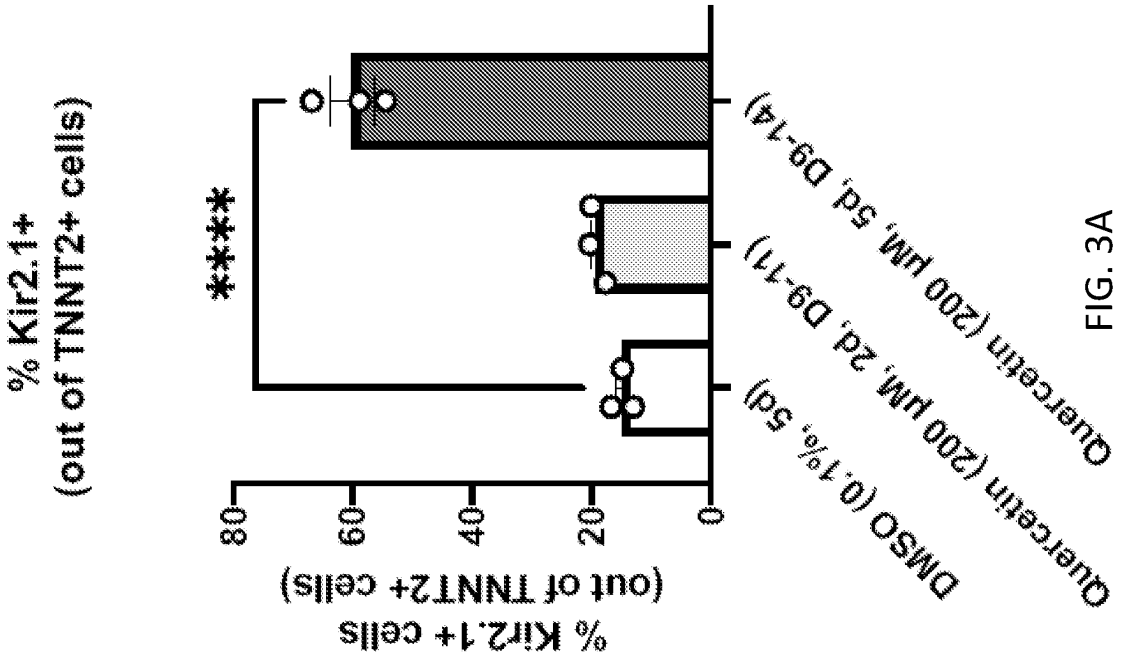

% Kir2.1+
(out of TNNT2+ cells)
****
80
60
40
20
0
% Kir2.1+ cells
(out of TNNT2+ cells)
DMSO (0.1%, 5d)
Quercetin (200 μM, 2d, D9-11)
Quercetin (200 μM, 5d, D9-14)

FIG. 3A

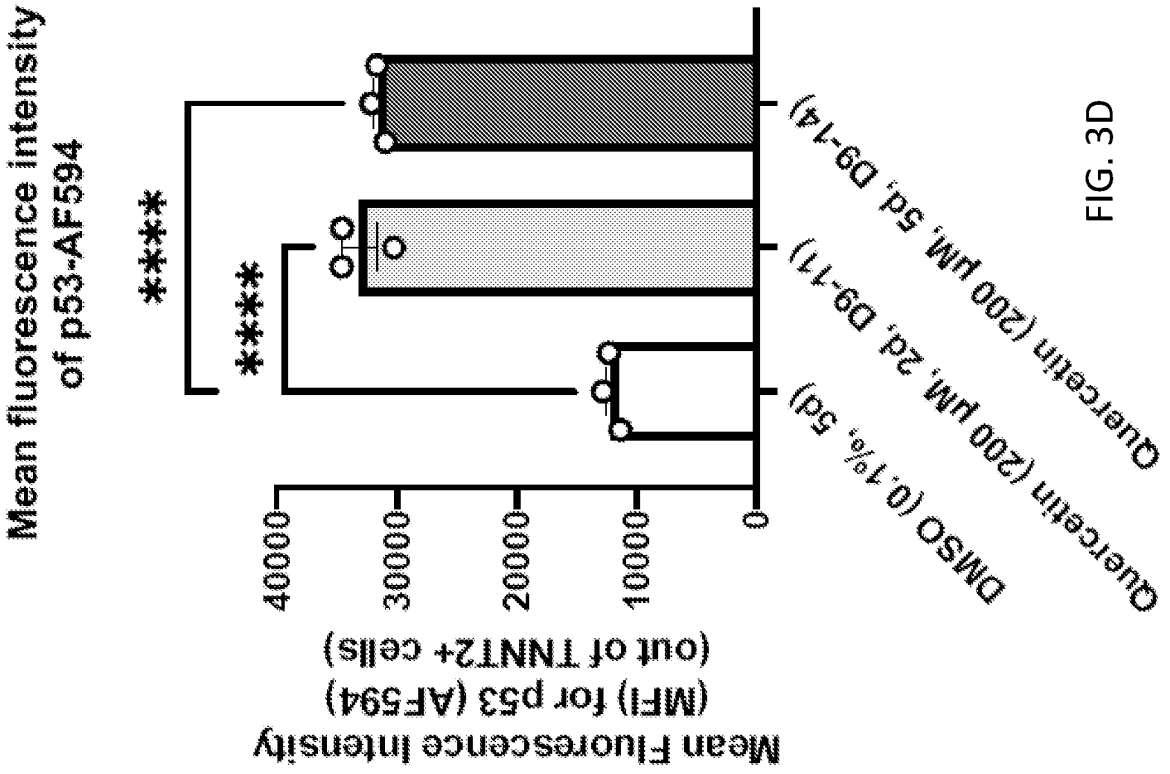

Mean fluorescence intensity
of p53-AF594
****
****
****
40000
30000
20000
10000
0
Mean Fluorescence Intensity
(MFI) for p53 (AF594)
(out of TNNT2+ cells)
DMSO (0.1%, 5d)
Quercetin (200 uM, 2d, D9-11)
Quercetin (200 uM, 5d, D9-14)

FIG. 3D

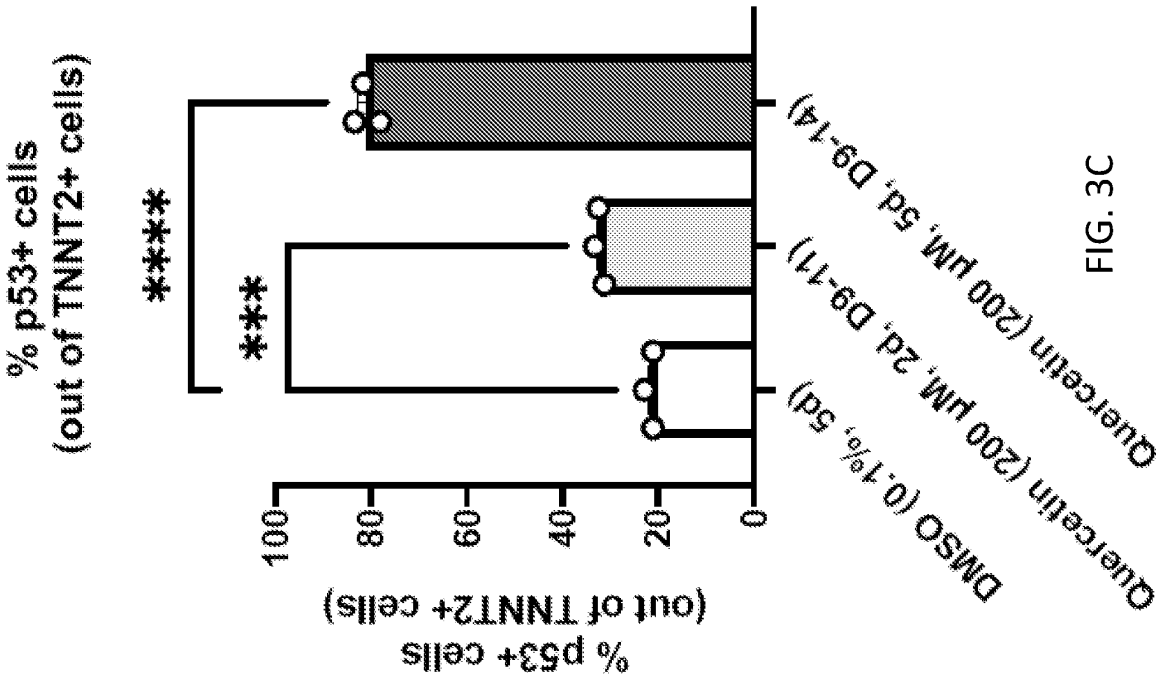

% p53+ cells
(out of TNNT2+ cells)
****
***
100
80
60
40
20
0
% p53+ cells
(out of TNNT2+ cells)
DMSO (0.1%, 5d)
Quercetin (200 uM, 2d, D9-11)
Quercetin (200 uM, 5d, D9-14)

FIG. 3C

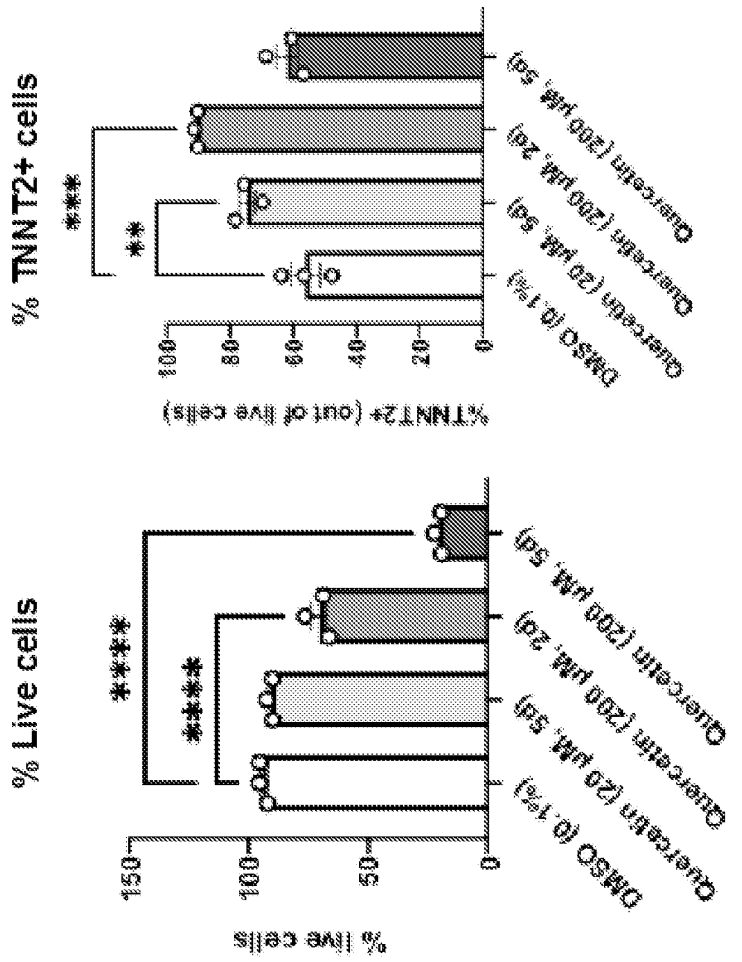

% Live cells
% live cells
150
100
50
0
****
****
DMSO (0.1%)
Quercetin (20 µM, 5d)
Quercetin (200 µM, 2d)
Quercetin (200 µM, 5d)
% TNNT2+ cells
%TNNT2+ (out of live cells)
***
**
100
80
60
40
20
0
DMSO (0.1%)
Quercetin (20 µM, 5d)
Quercetin (200 µM, 2d)
Quercetin (200 µM, 5d)

FIG. 4A

FIG. 4B

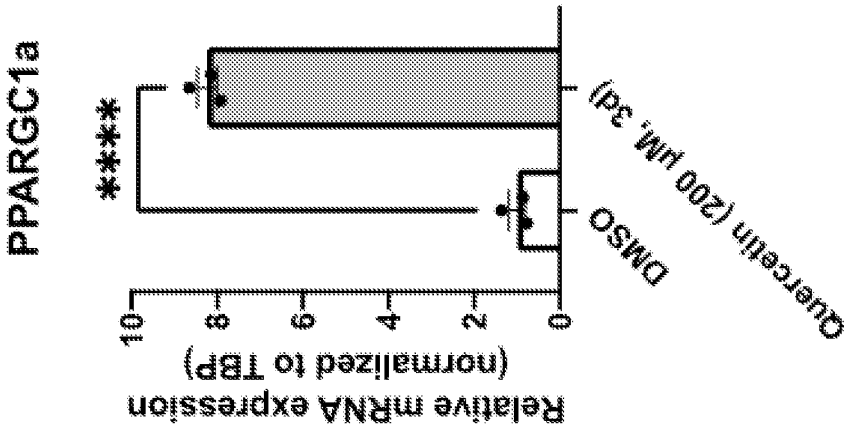

PPARGC1a
****
Relative mRNA expression
(normalized to TBP)
10
8
6
4
2
0
DMSO
Quercetin (200 μM, 3d)

FIG. 5

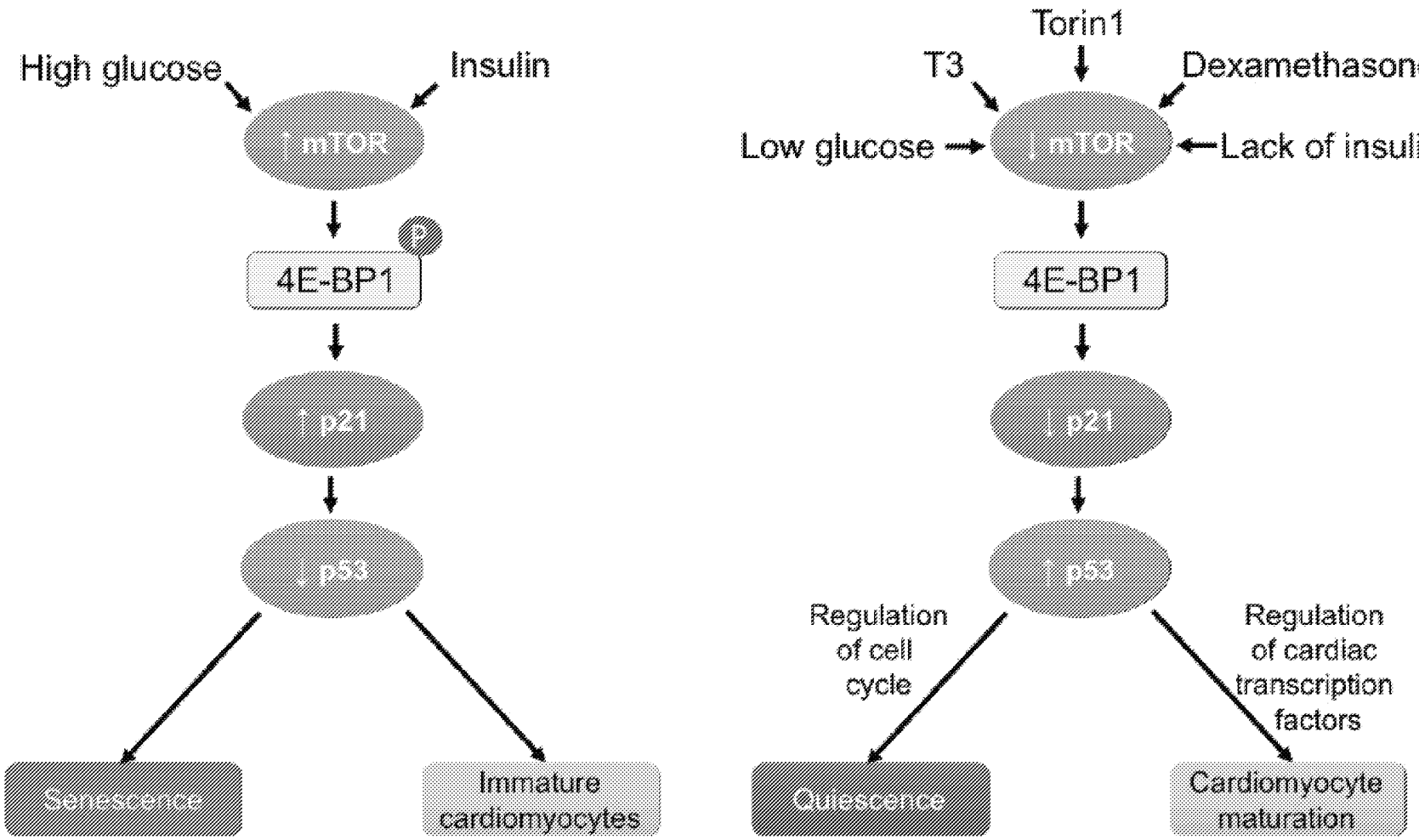

High glucose
Insulin
↑ mTOR
P
4E-BP1
↑ p21
↓ p53
Senescence
Immature cardiomyocytes
Torin1
T3
Dexamethasone
Low glucose
↓ mTOR
Lack of insulin
4E-BP1
↓ p21
↑ p53
Regulation of cell cycle
Regulation of cardiac transcription factors
Quiescence
Cardiomyocyte maturation

FIG. 6A

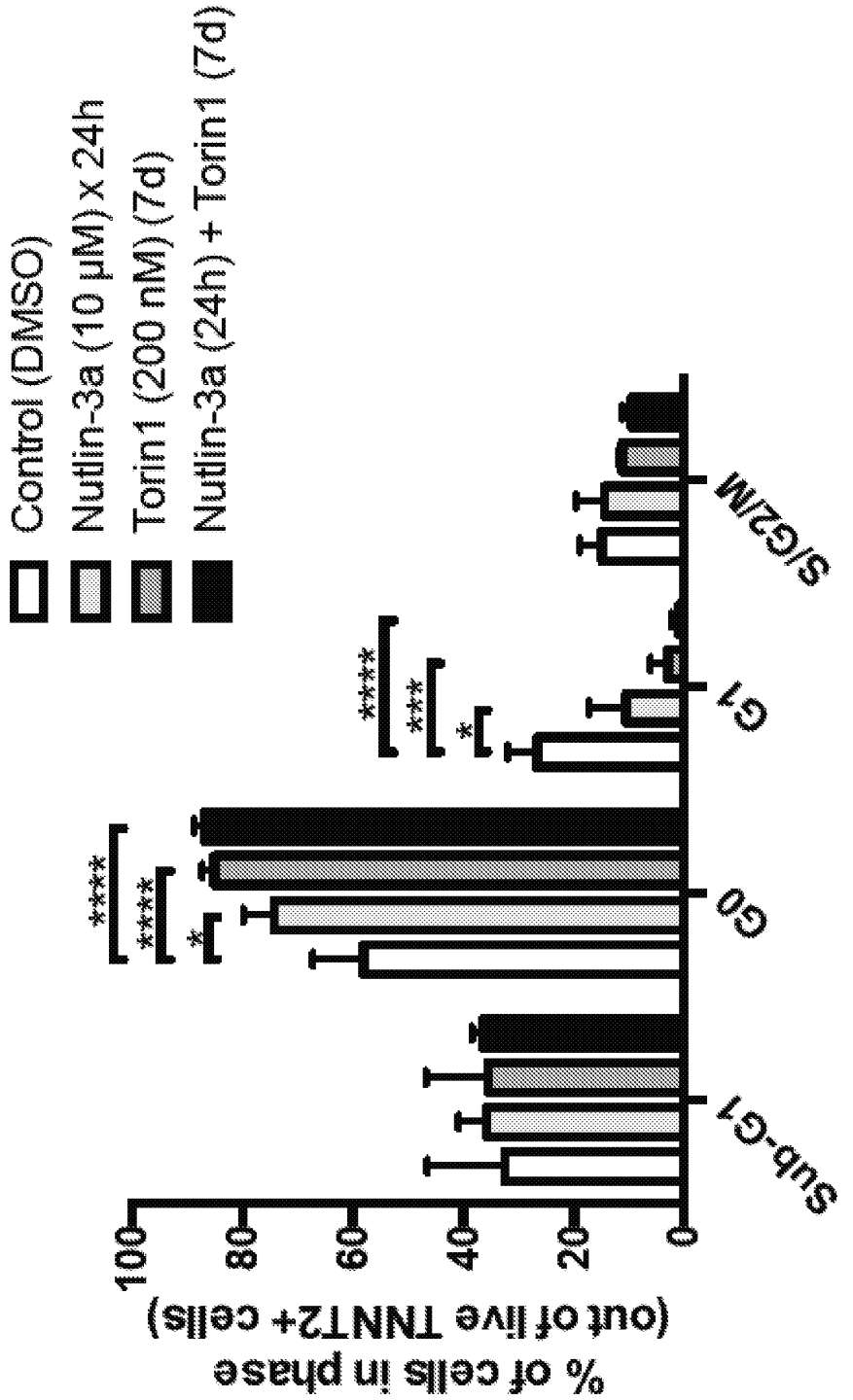

Control (DMSO)
Nutlin-3a (10 µM) x 24h
Torin1 (200 nM) (7d)
Nutlin-3a (24h) + Torin1 (7d)
% of cells in phase
(out of live TNNT2+ cells)
100
80
60
40
20
0
Sub-G1
G0
G1
S/G2M
Cell cycle phase
*
****
****
*
***
****

FIG. 6B

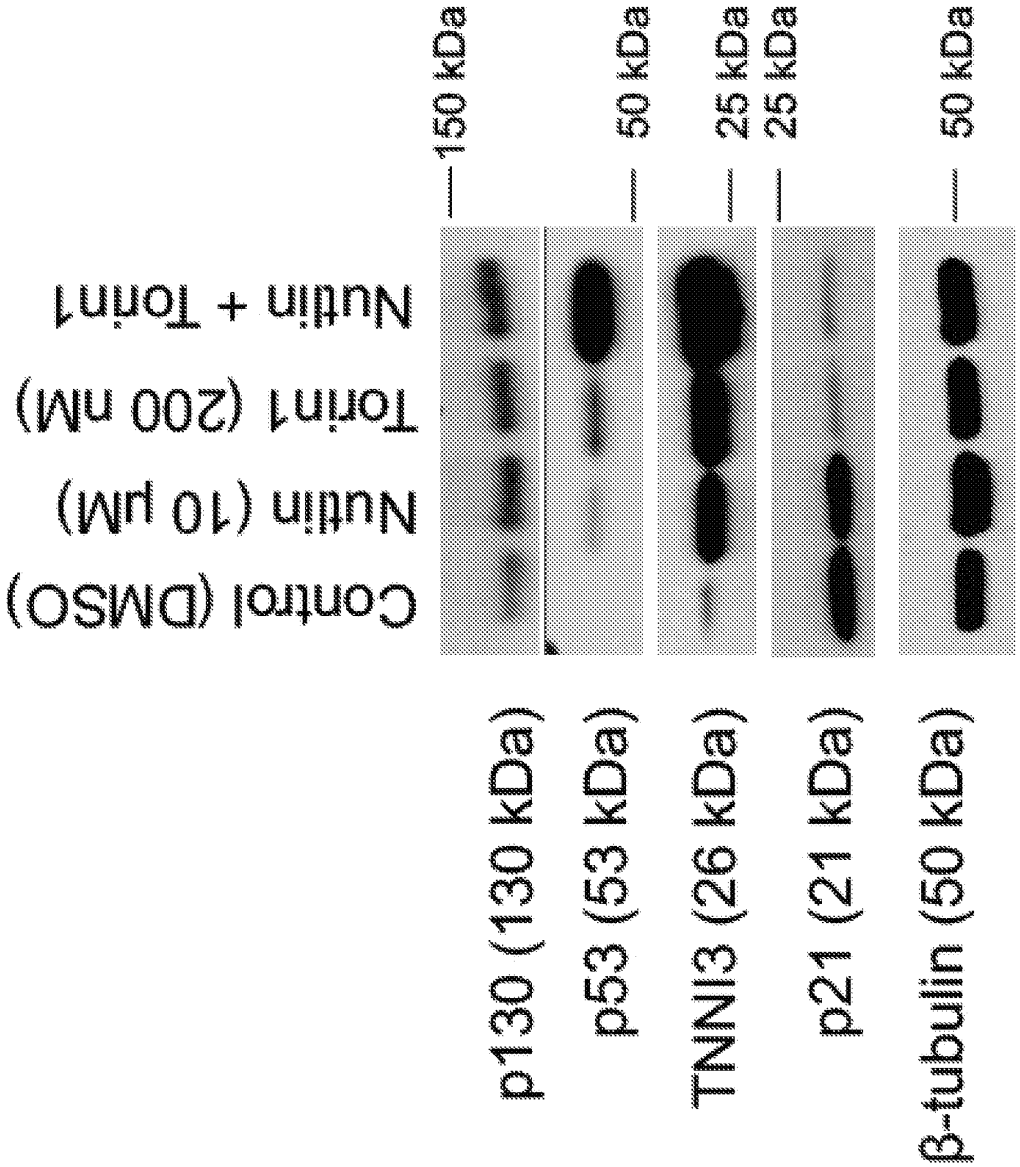

Control (DMSO)
Nutlin (10 μM)
Torin1 (200 nM)
Nutlin + Torin1
150 kDa
50 kDa
25 kDa
25 kDa
50 kDa
p130 (130 kDa)
p53 (53 kDa)
TNNI3 (26 kDa)
p21 (21 kDa)
β-tubulin (50 kDa)

FIG. 6C

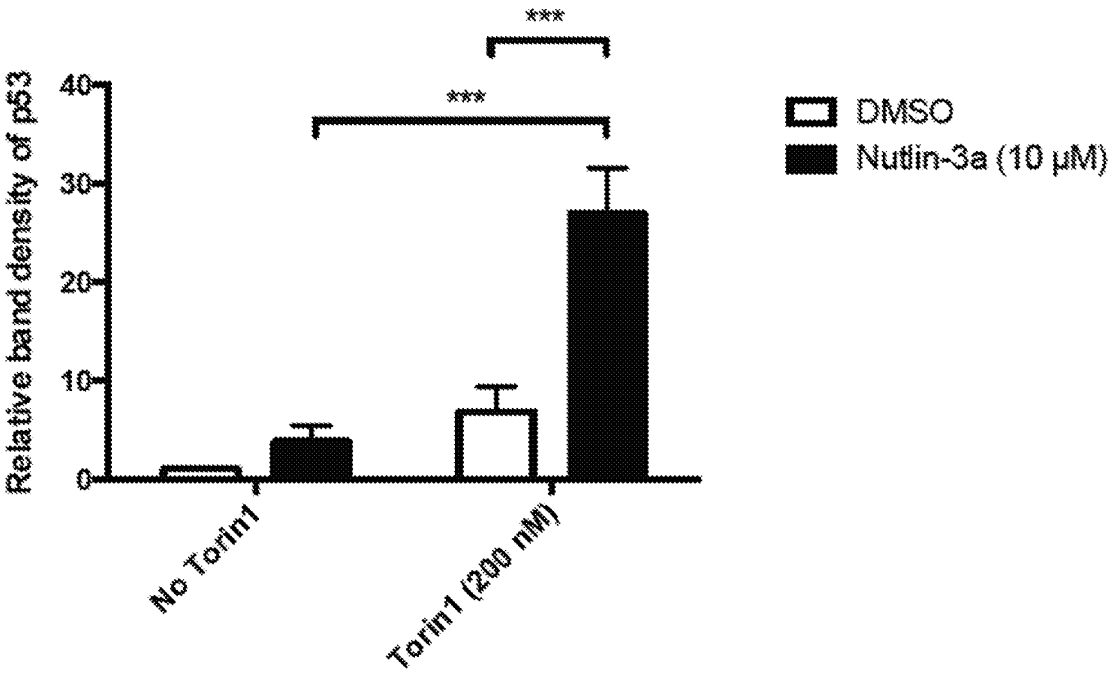

***
***
Relative band density of p53
40
30
20
10
0
No Torin1
Torin1 (200 nM)
DMSO
Nutlin-3a (10 μM)

FIG. 6D

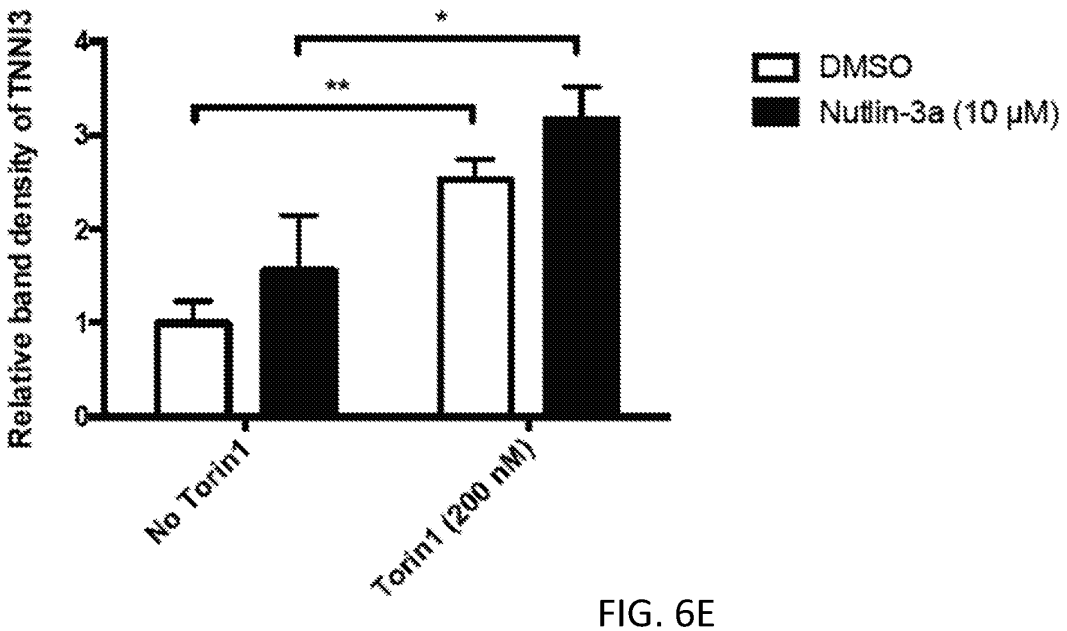

*
**
Relative band density of TNNI3
4
3
2
1
0
No Torin1
Torin1 (200 nM)
DMSO
Nutlin-3a (10 μM)

FIG. 6E

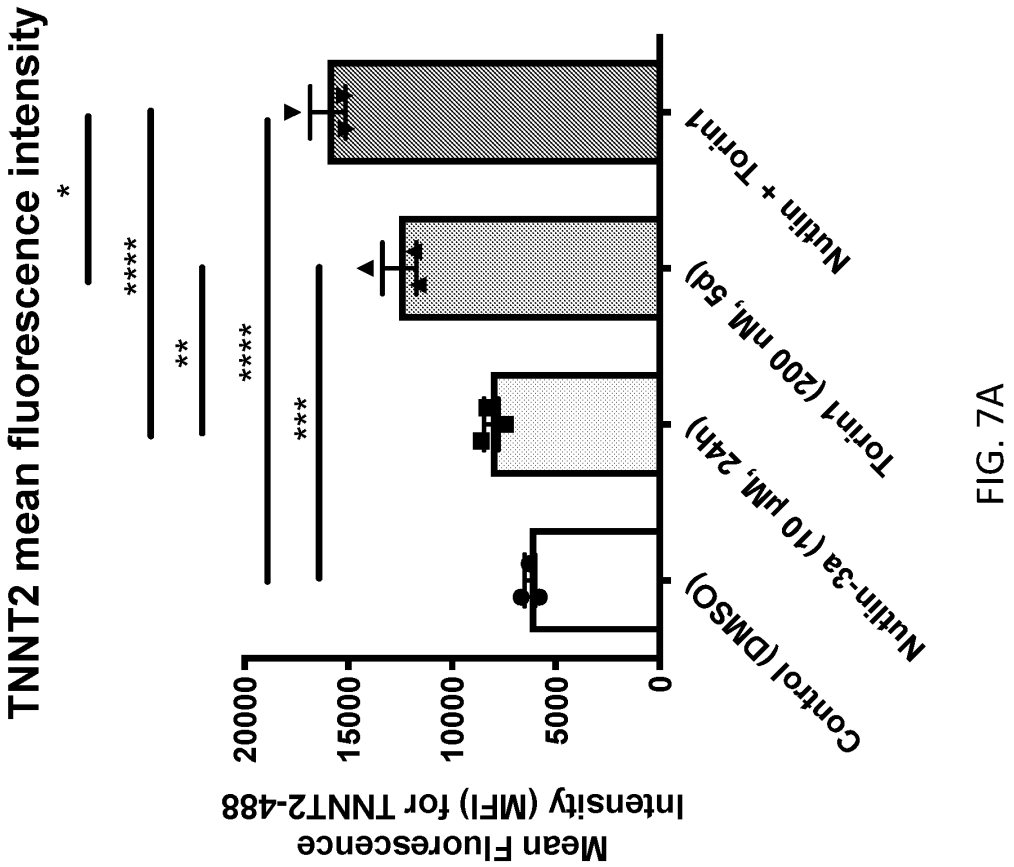

TNNT2 mean fluorescence intensity
Mean Fluorescence Intensity (MFI) for TNNT2-488
20000
15000
10000
5000
0
Control (DMSO)
Nutlin-3a (10 μM, 24h)
Torin1 (200 nM, 5d)
Nutlin + Torin1
*
****
**
***
****

FIG. 7A

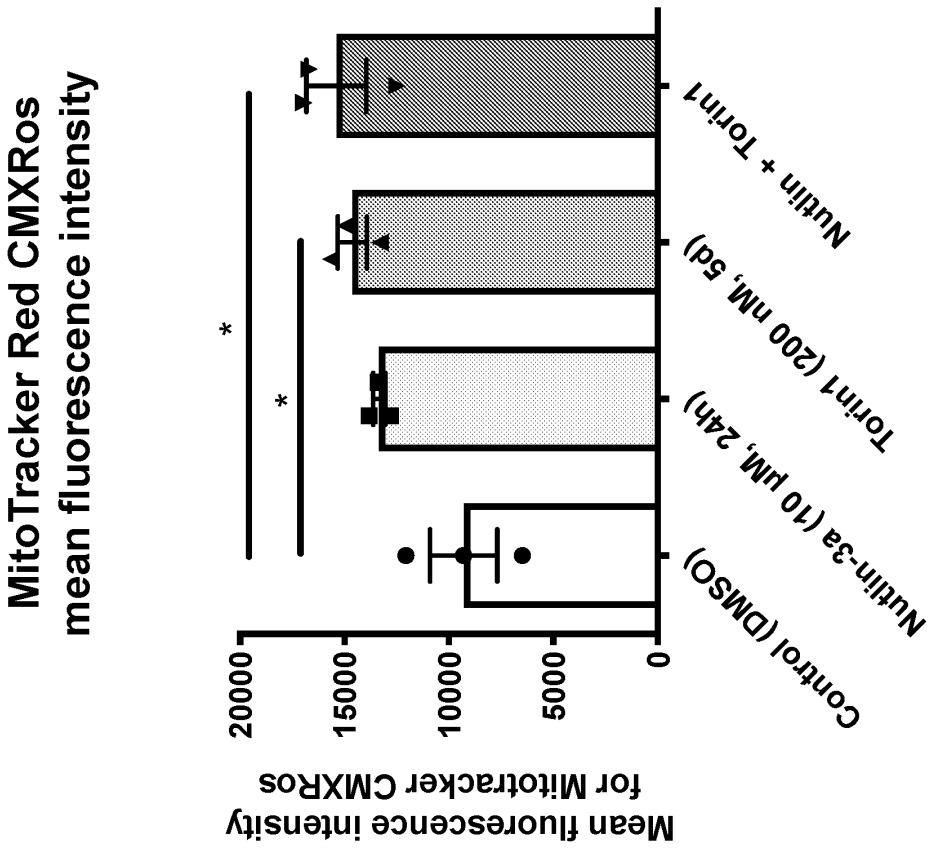

MitoTracker Red CMXRos
mean fluorescence intensity
Mean fluorescence intensity
for Mitotracker CMXRos
20000
15000
10000
5000
0
*
*
Control (DMSO)
Nutlin-3a (10 μM, 24h)
Torin1 (200 nM, 5d)
Nutlin + Torin1

FIG. 7B

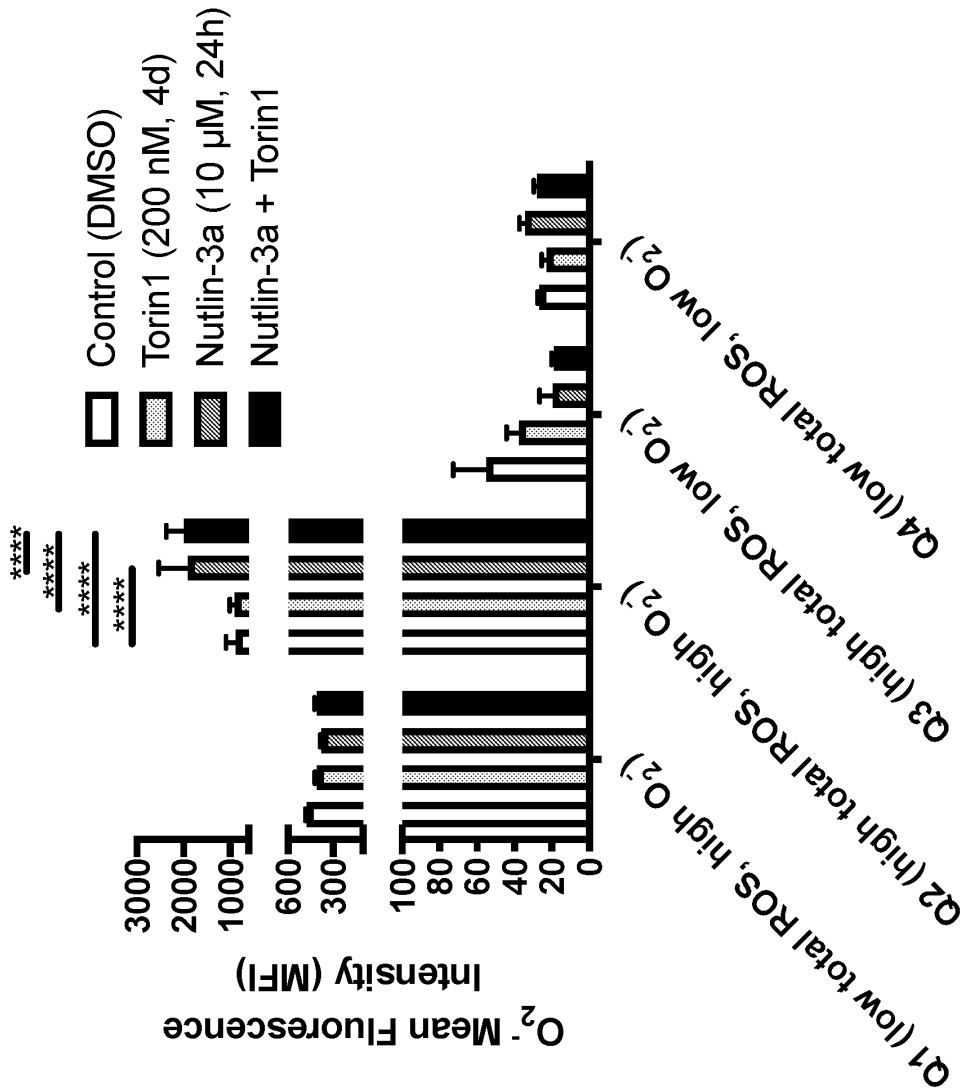
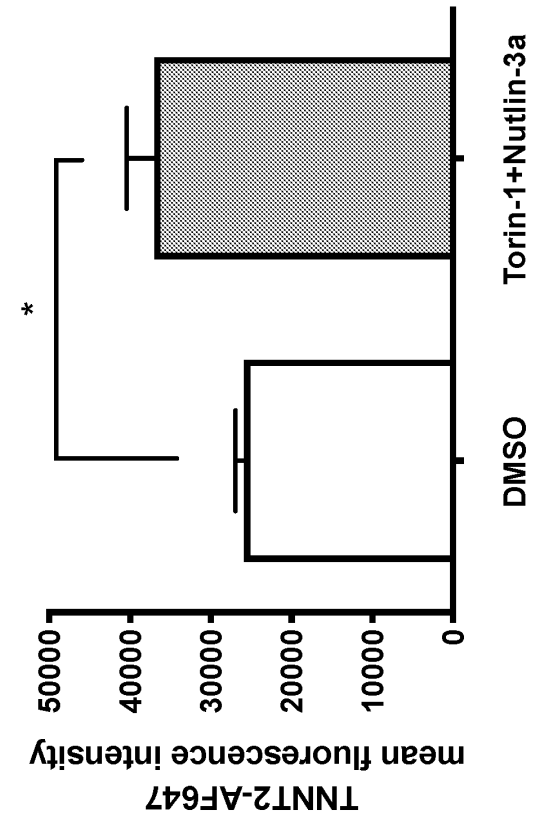
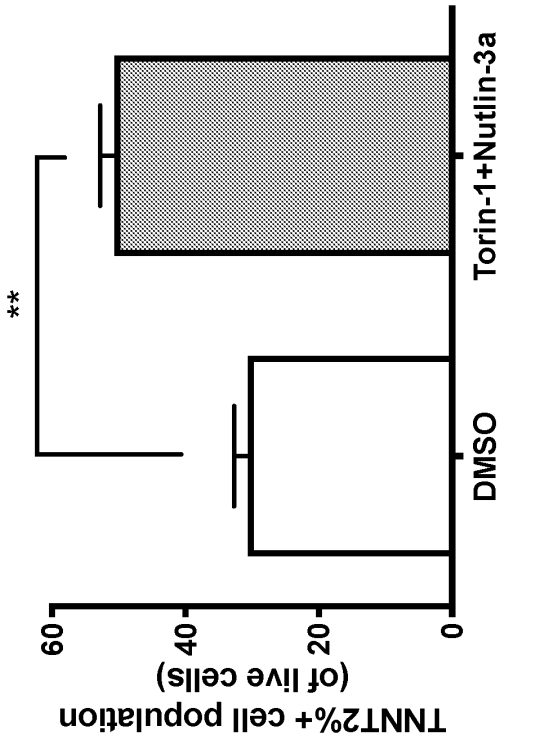

Control (DMSO)
Torin1 (200 nM, 4d)
Nutlin-3a (10 μM, 24h)
Nutlin-3a + Torin1
****
****
****
****
3000
2000
1000
600
300
100
80
60
40
20
0
O2- Mean Fluorescence Intensity (MFI)
Q1 (low total ROS, high O2-)
Q2 (high total ROS, high O2-)
Q3 (high total ROS, low O2-)
Q4 (low total ROS, low O2-)

FIG. 8



*p<0.05 by unpaired t-test

FIG. 9B



**p<0.01 by unpaired t-test

FIG. 9A

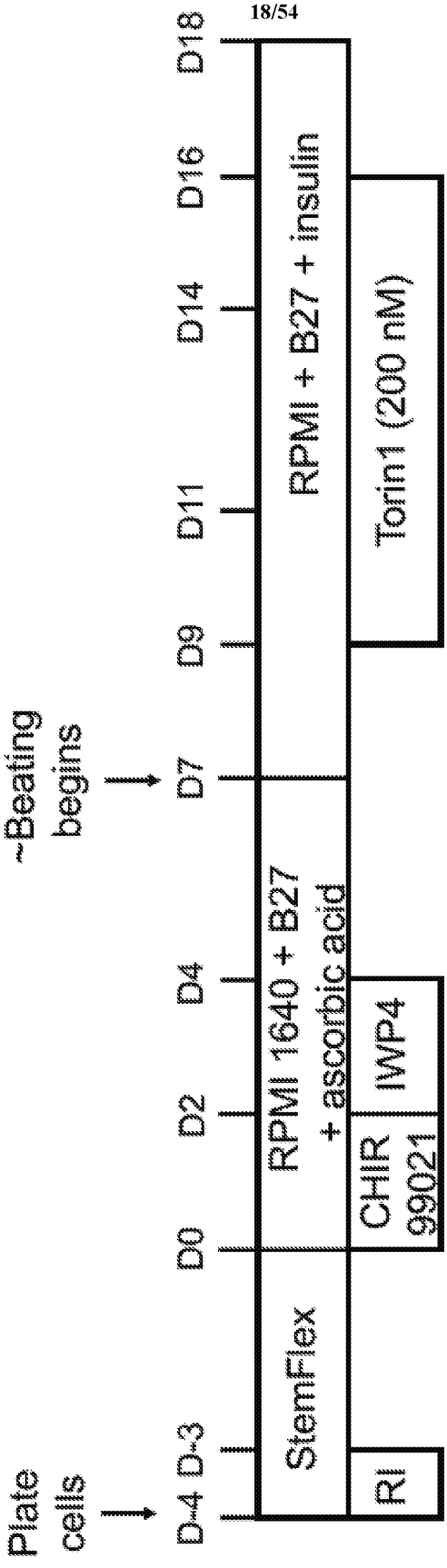


Plate cells
~Beating begins
D-4
D-3
D0
D2
D4
D7
D9
D11
D14
D16
D18
RI
StemFlex
CHIR 99021
IWP4
RPMI 1640 + B27 + ascorbic acid
Torin1 (200 nM)
RPMI + B27 + insulin

FIG. 10A

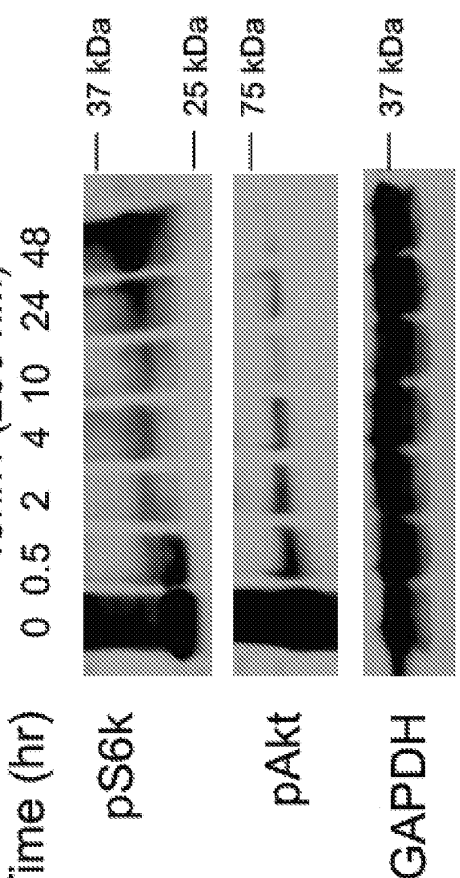


FIG. 10B

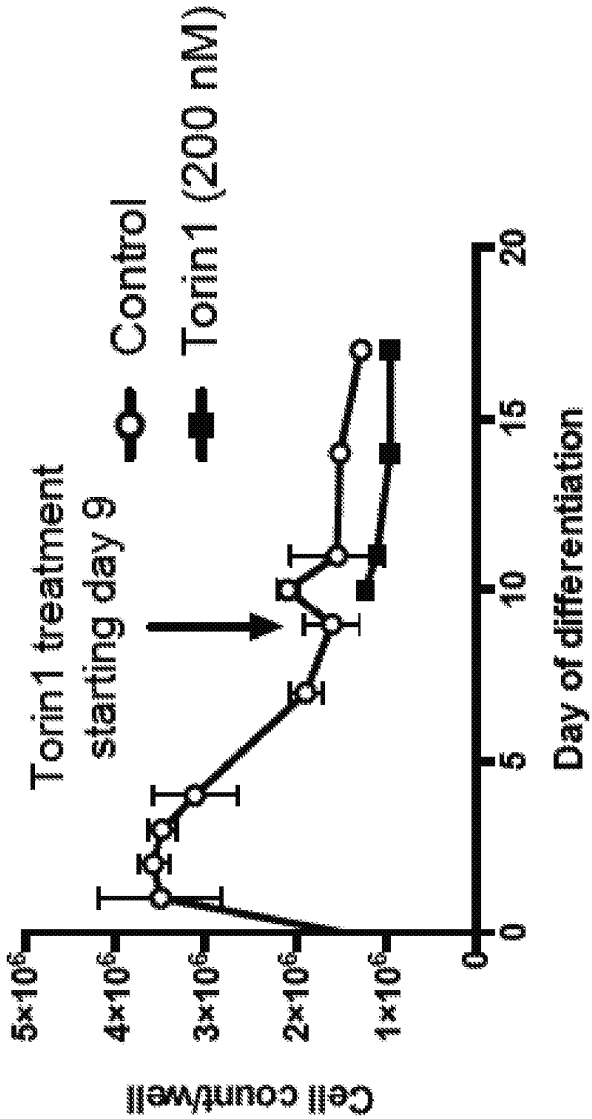


FIG. 10C

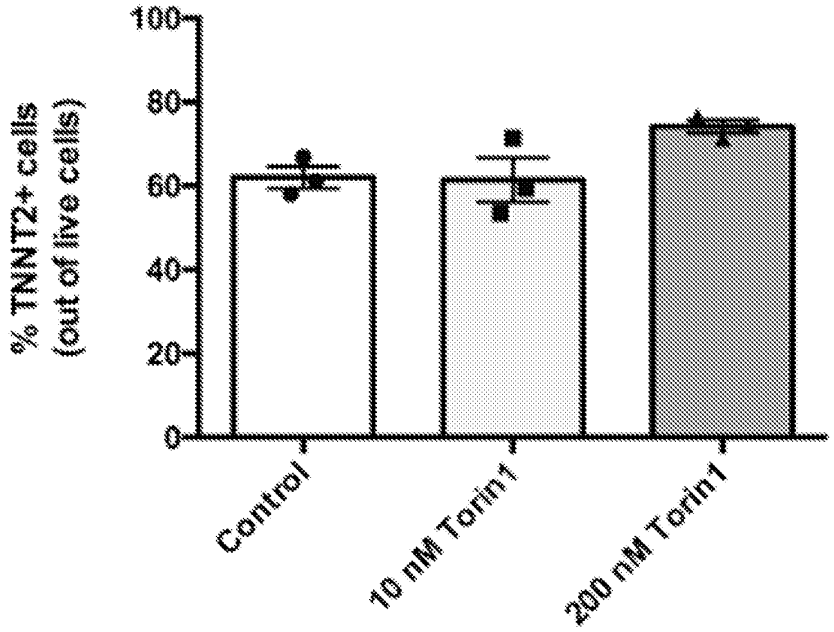


FIG. 10D

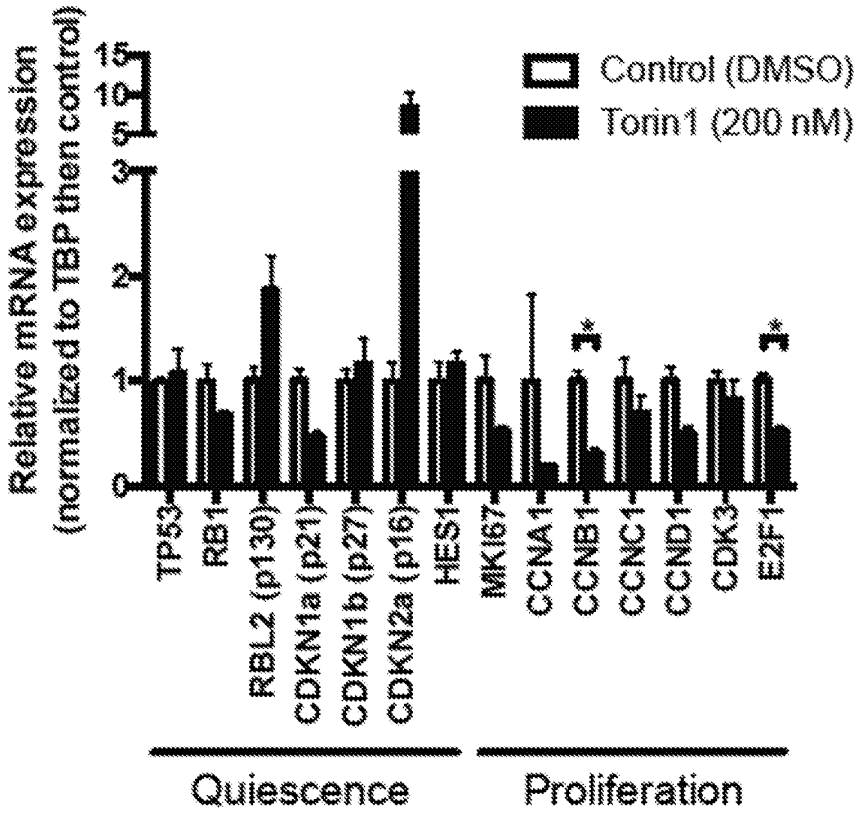


FIG. 10E

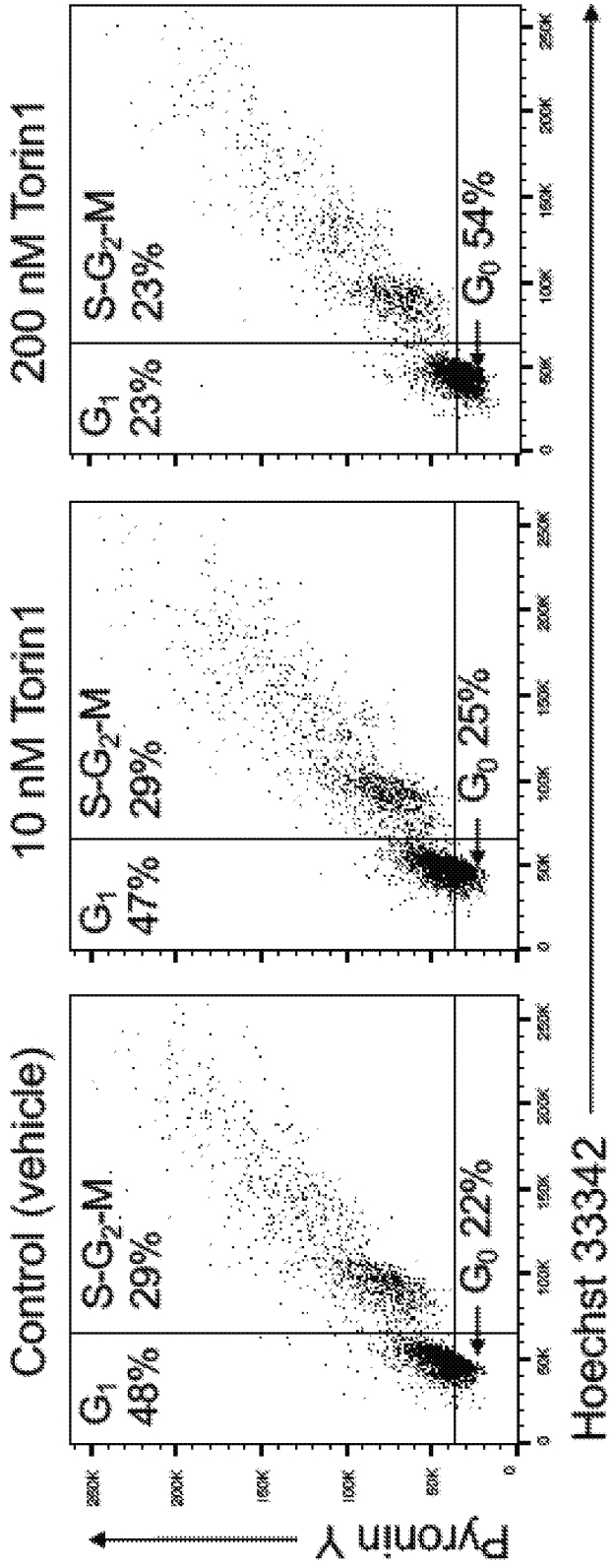

Control (vehicle)
10 nM Torin1
200 nM Torin1
G1 48%
S-G2-M 29%
G0 22%
G1 47%
S-G2-M 29%
G0 25%
G1 23%
S-G2-M 23%
G0 54%
Pyronin Y
Hoechst 33342

FIG. 10F

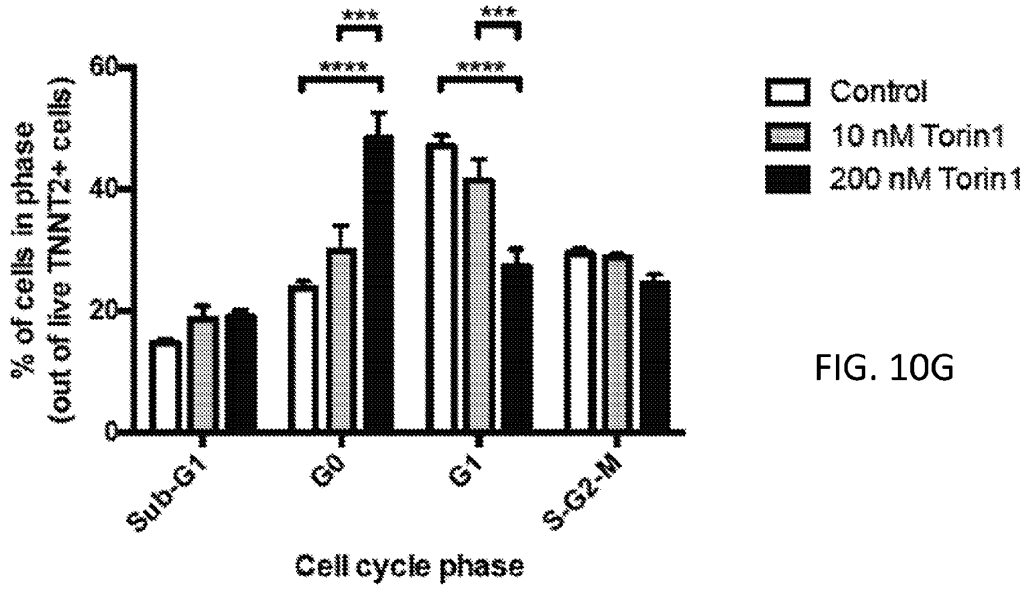


FIG. 10G

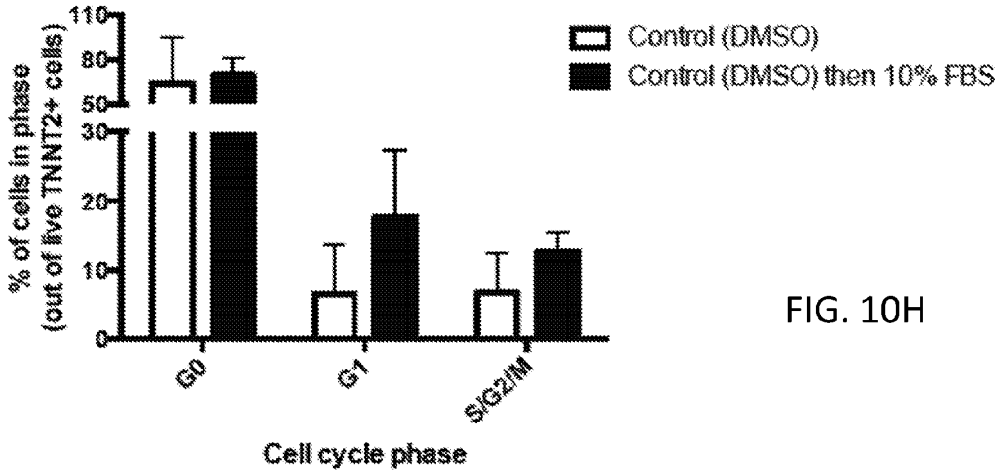


FIG. 10H

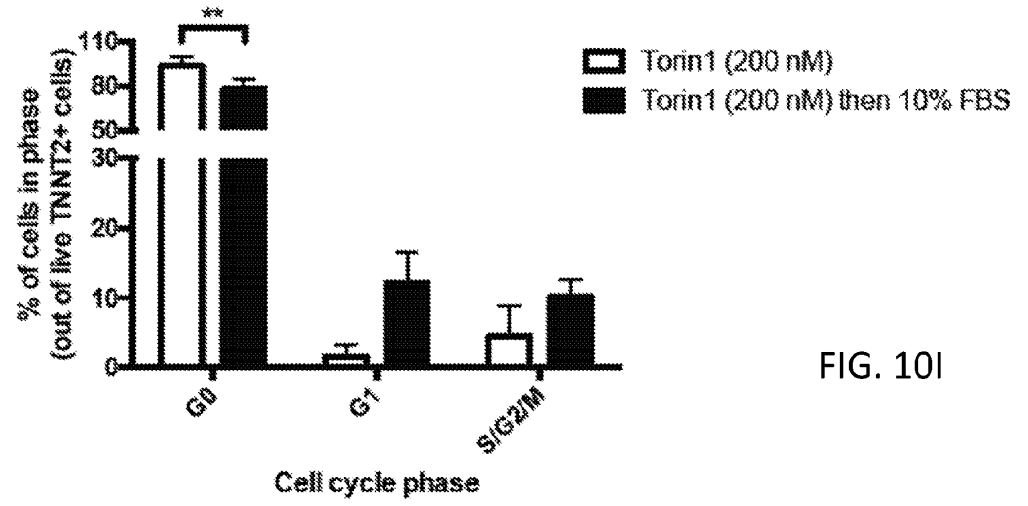


FIG. 10I

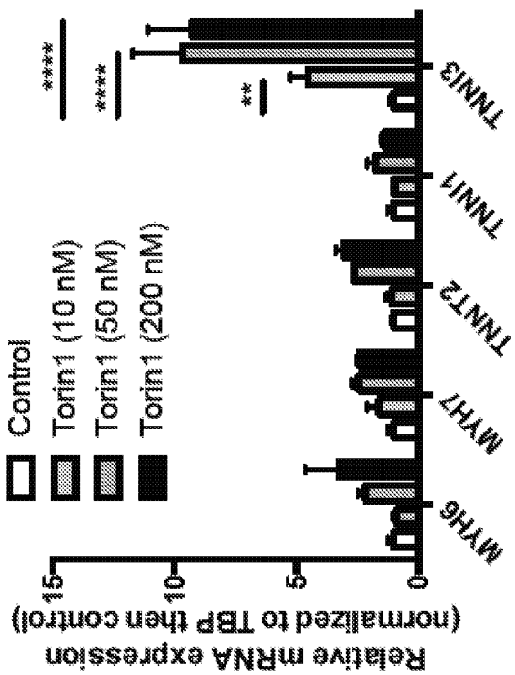


FIG. 11A

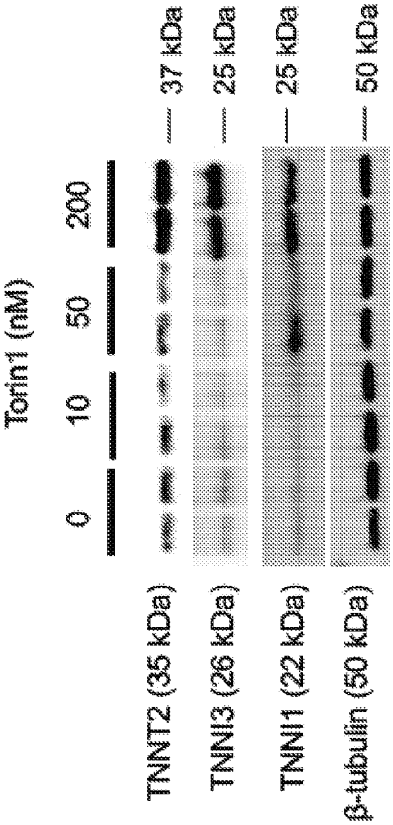


FIG. 11B

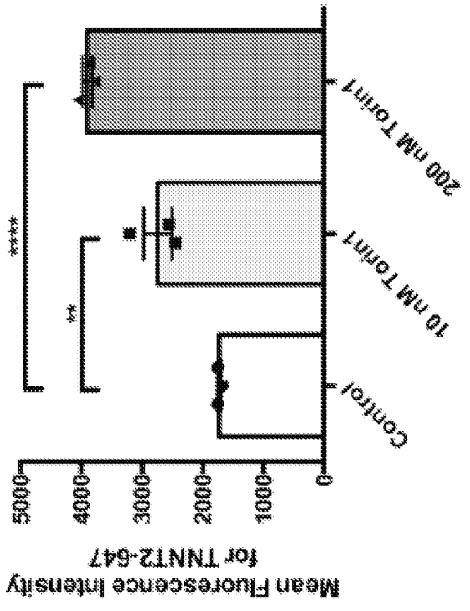


FIG. 11C

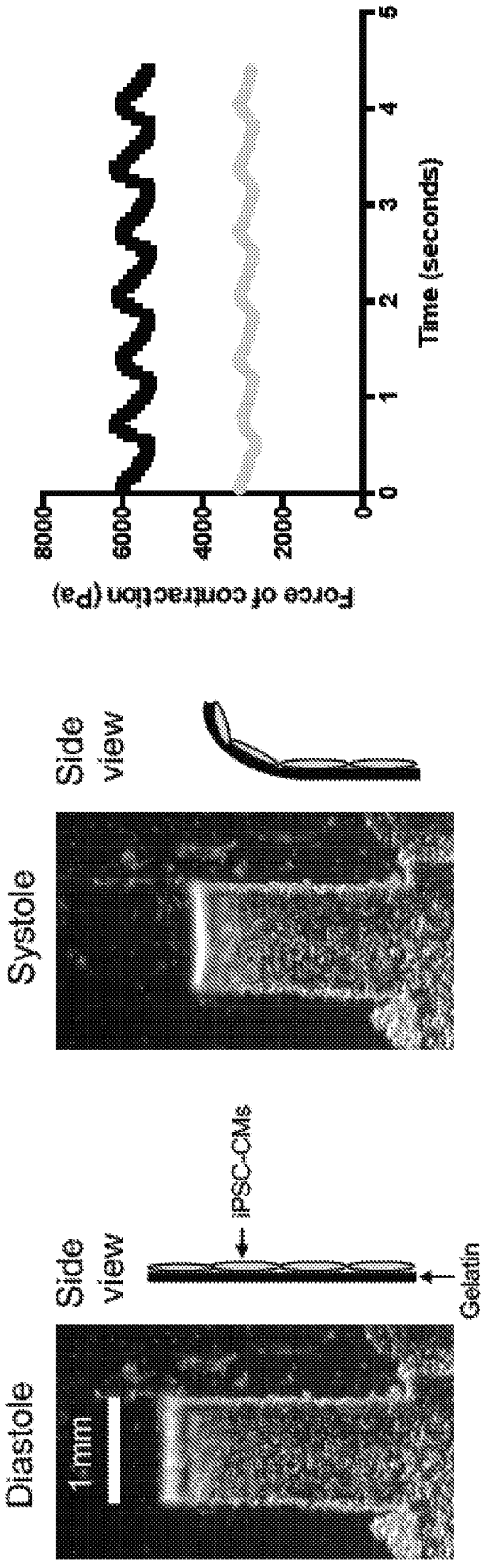


FIG. 11D

FIG. 11E

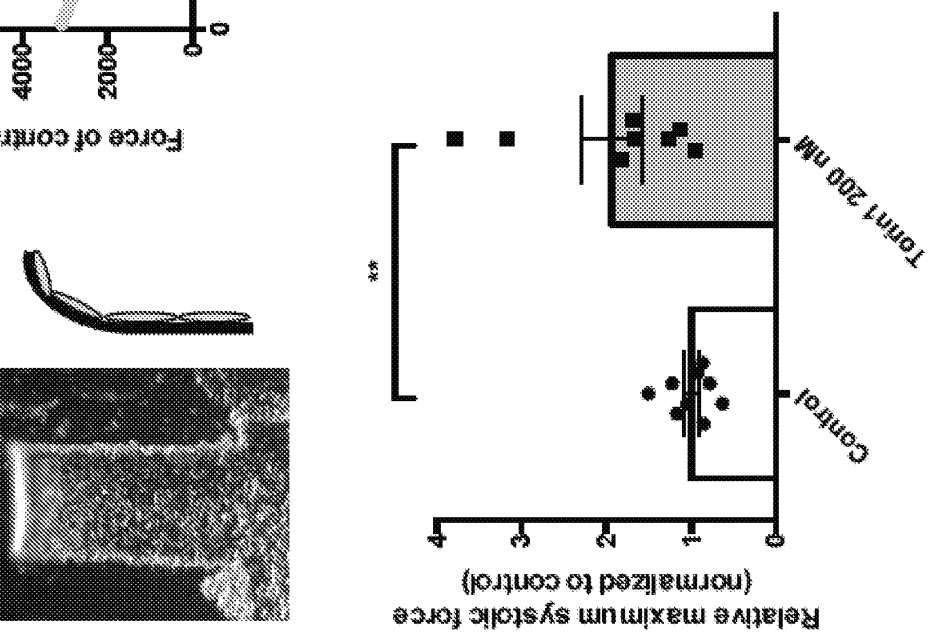


FIG. 11F

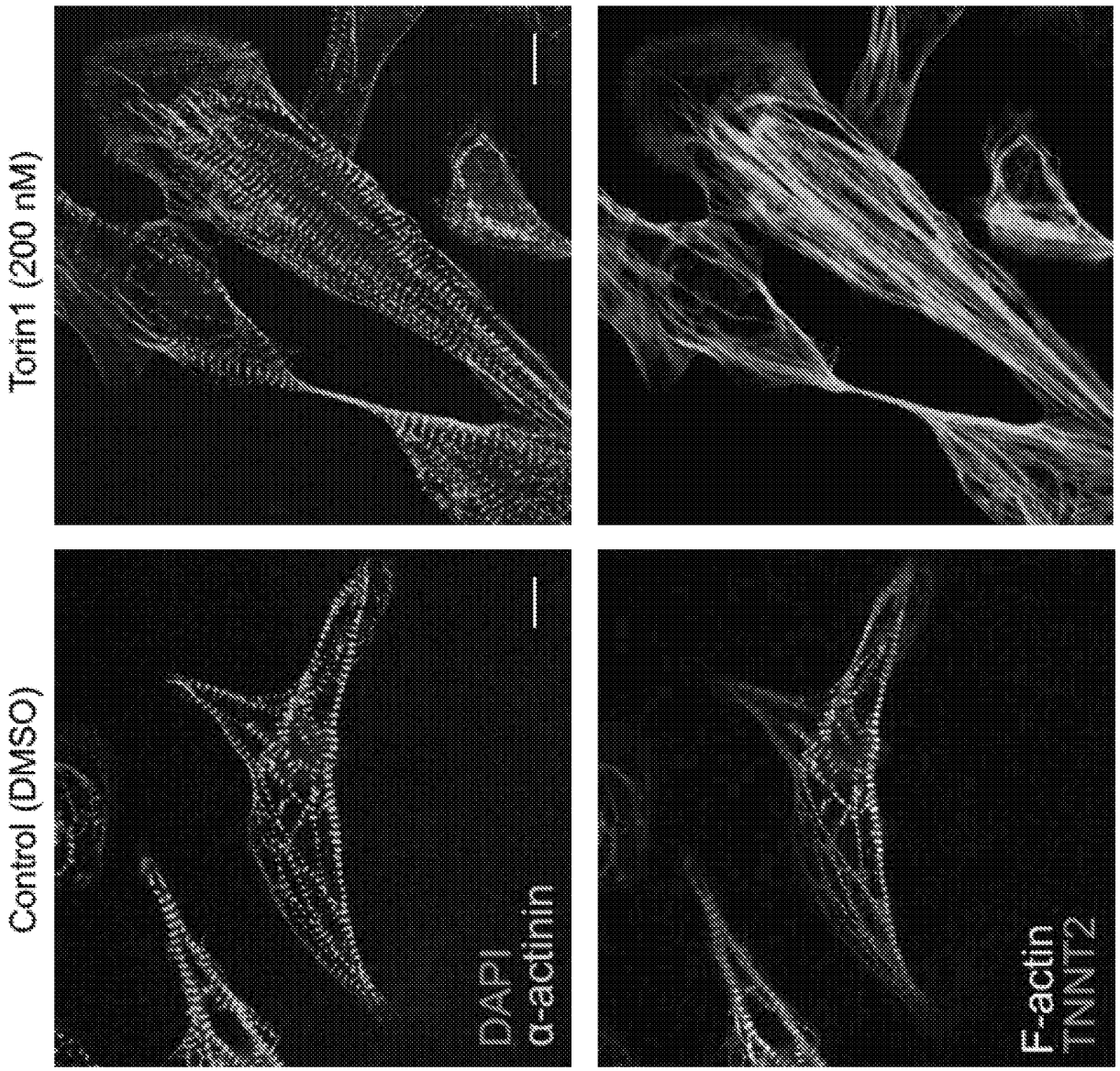


FIG. 11G

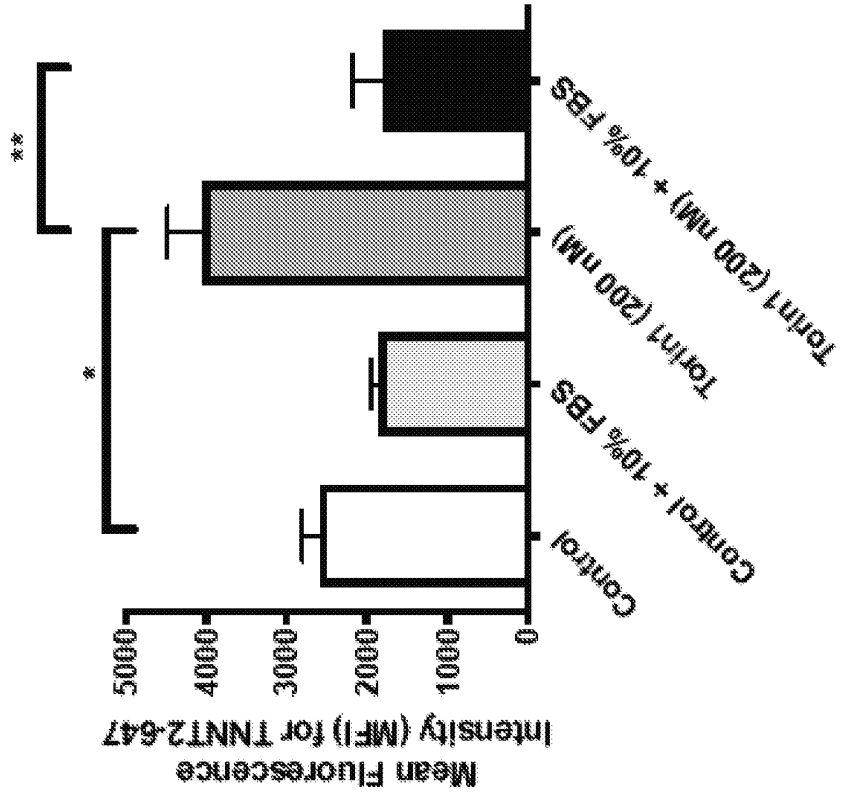


FIG. 11I

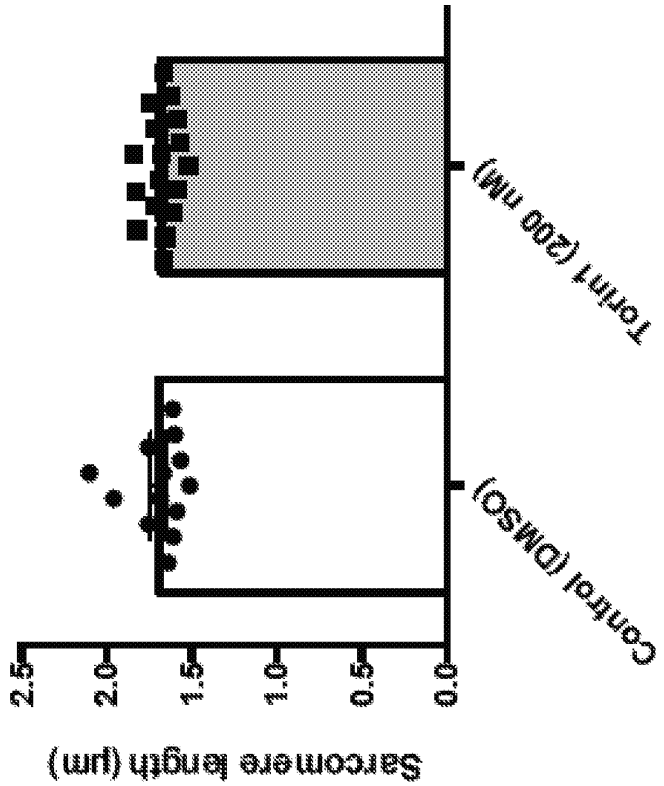


FIG. 11H

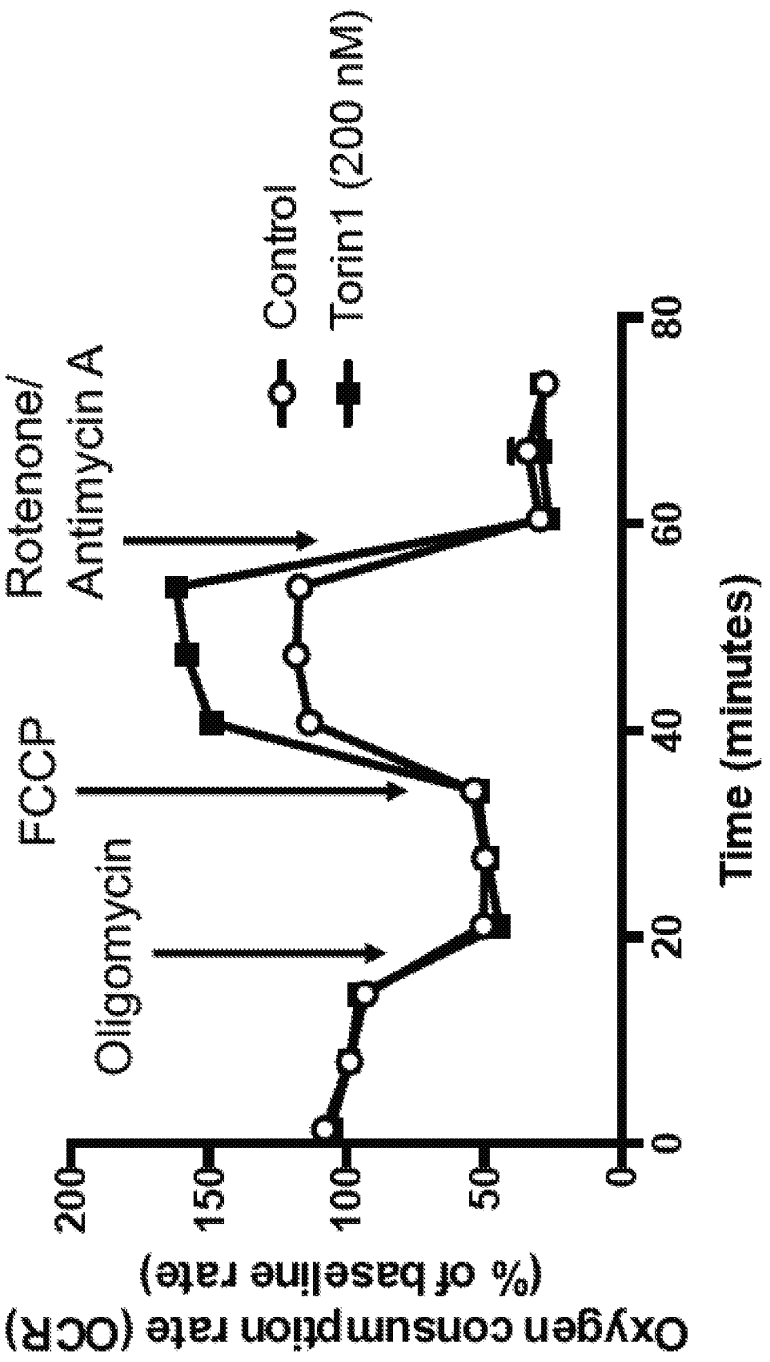

Oxygen consumption rate (OCR)
(% of baseline rate)
200
150
100
50
0
0
20
40
60
80
Time (minutes)
Oligomycin
FCCP
Rotenone/
Antimycin A
Control
Torin1 (200 nM)

FIG. 12A

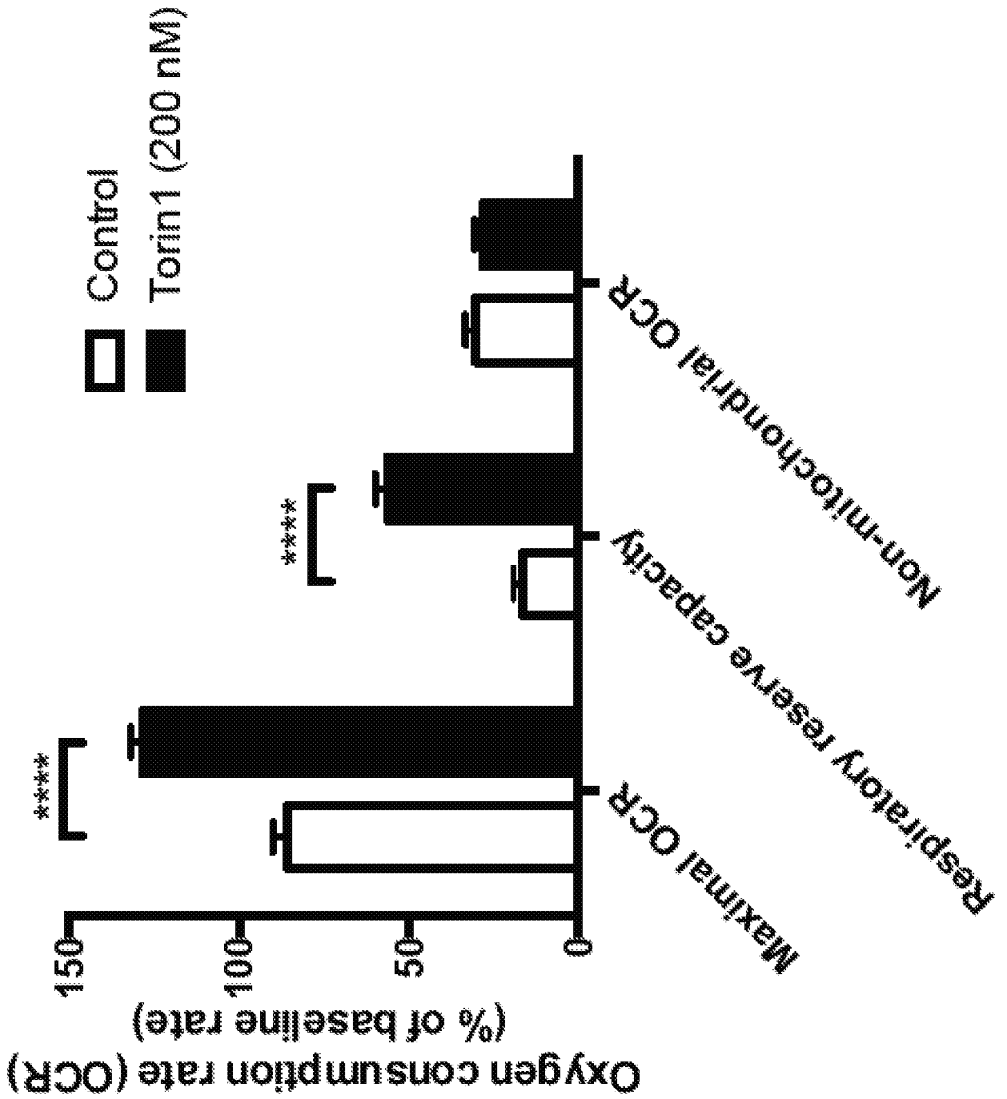

Control
Torin1 (200 nM)
Oxygen consumption rate (OCR)
(% of baseline rate)
150
100
50
0
****
****
Maximal OCR
Respiratory reserve capacity
Non-mitochondrial OCR

FIG. 12B

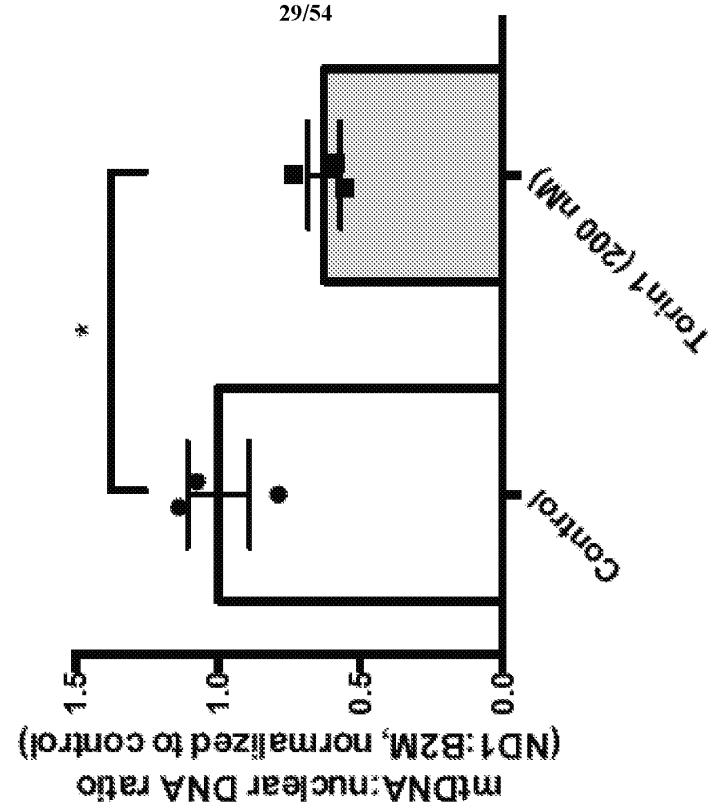


FIG. 12D

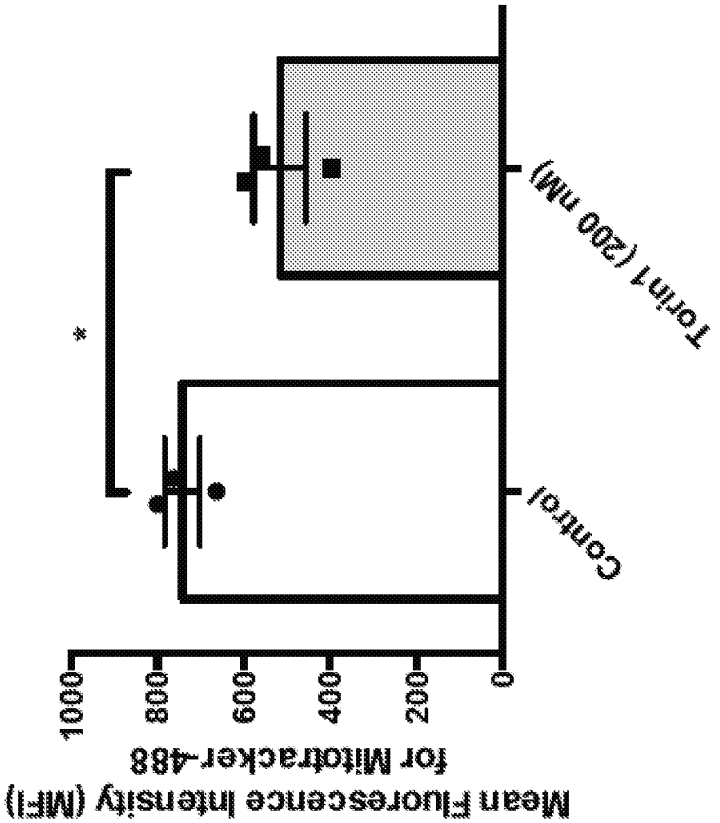


FIG. 12C

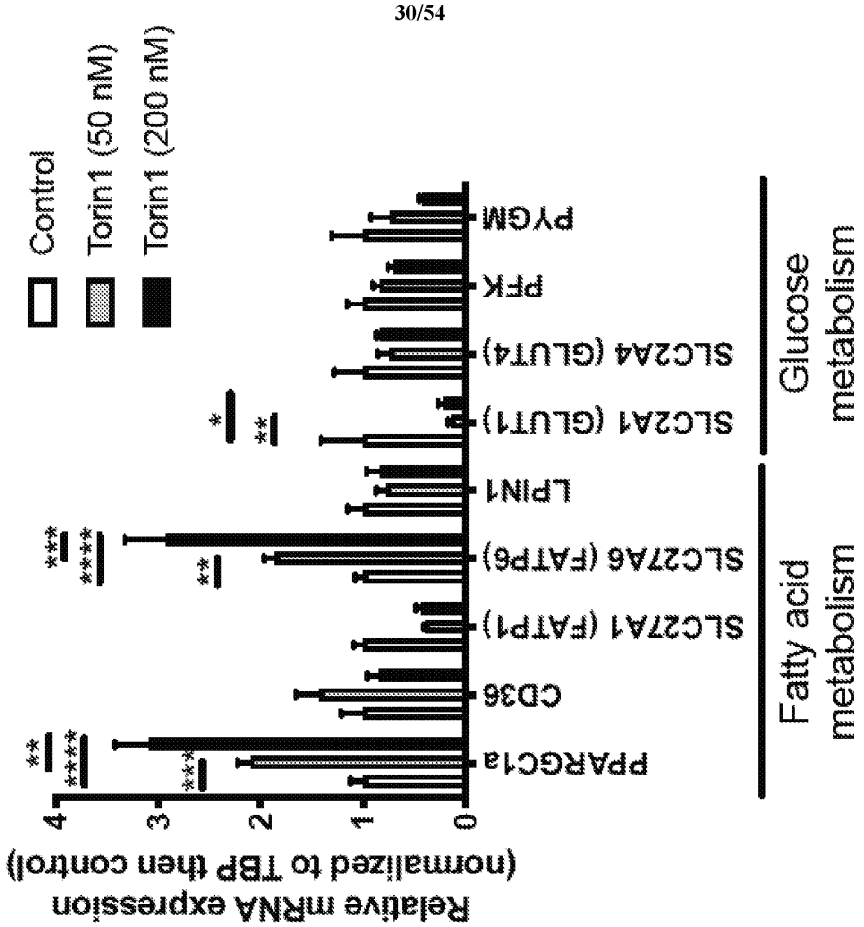


FIG. 12F

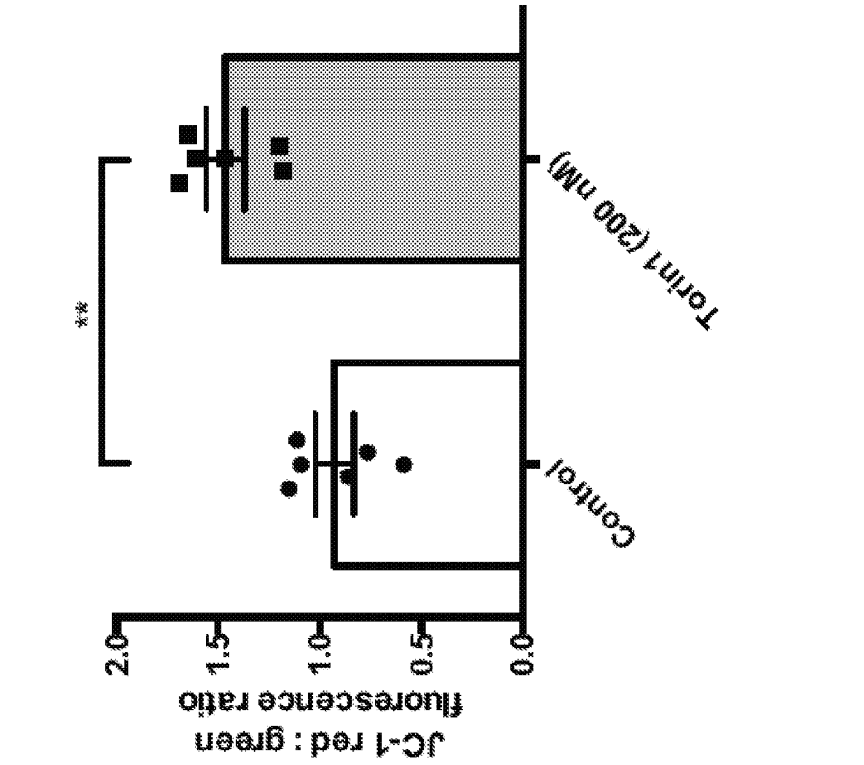


FIG. 12E

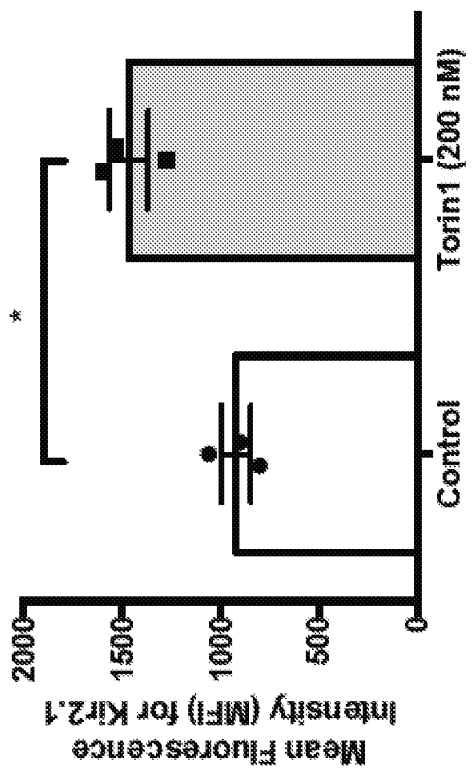


FIG. 13E

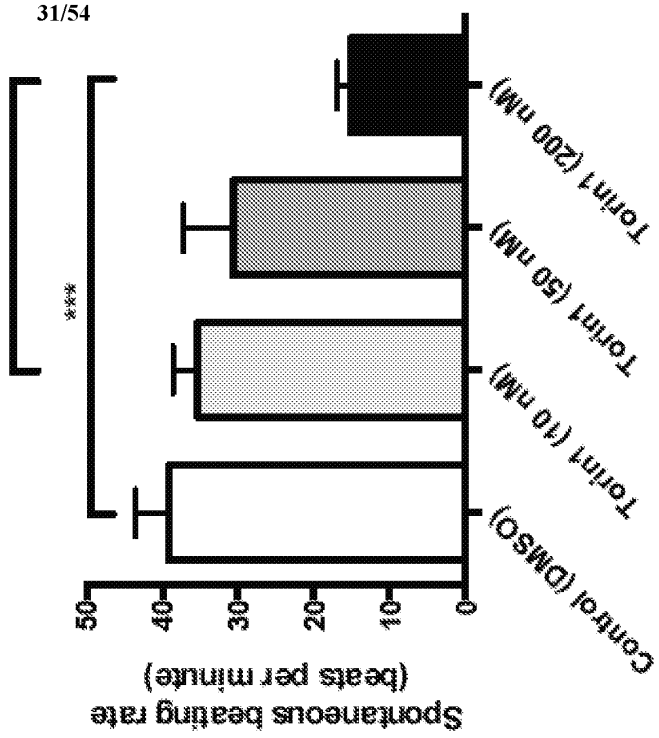


FIG. 13C

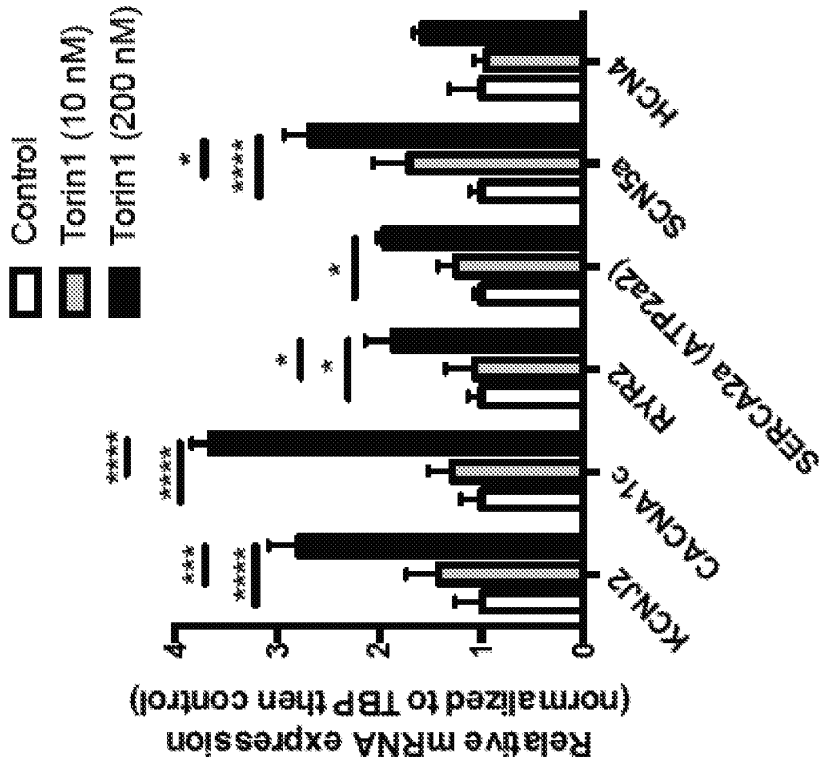


FIG. 13A

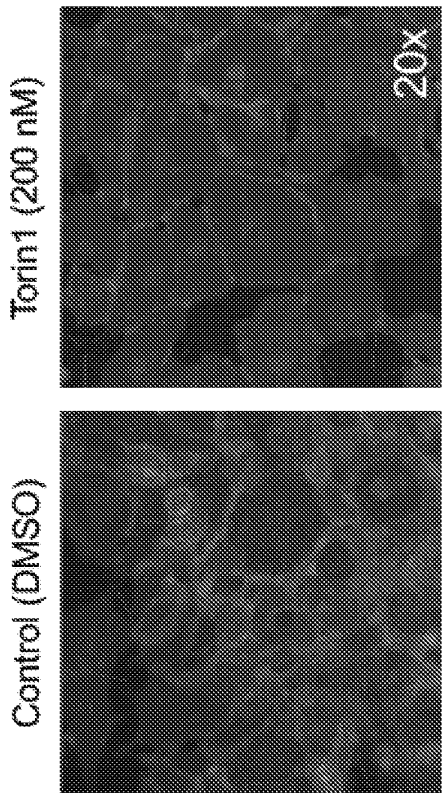


FIG. 13D

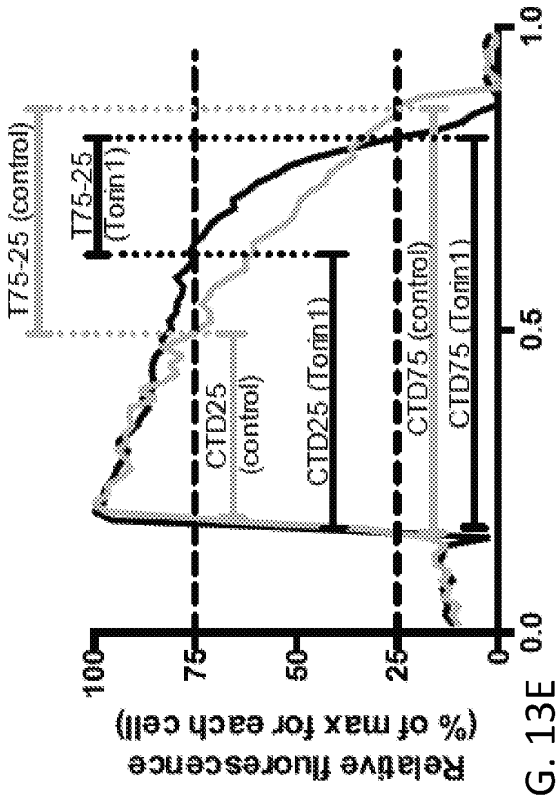


FIG. 13E

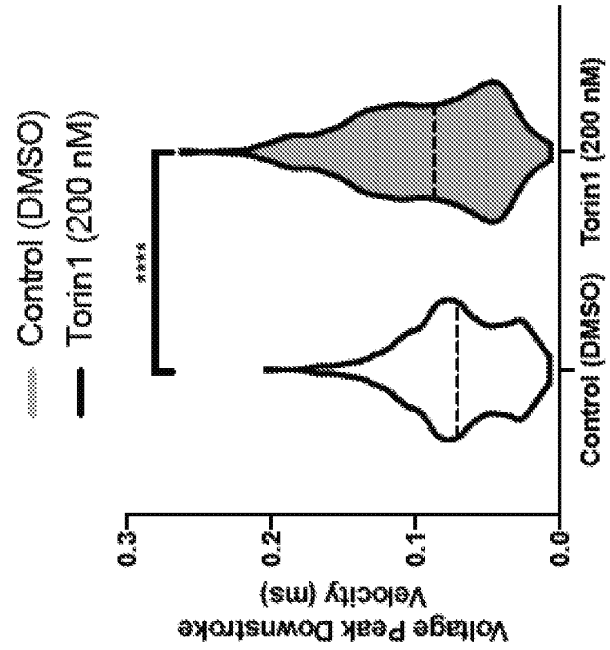


FIG. 13G

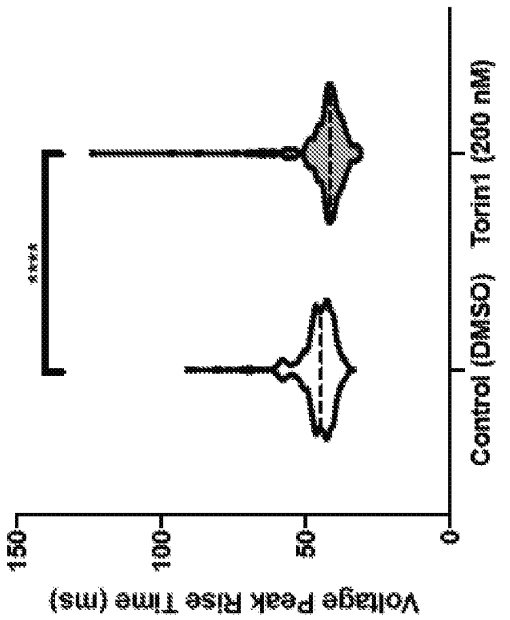


FIG. 13F

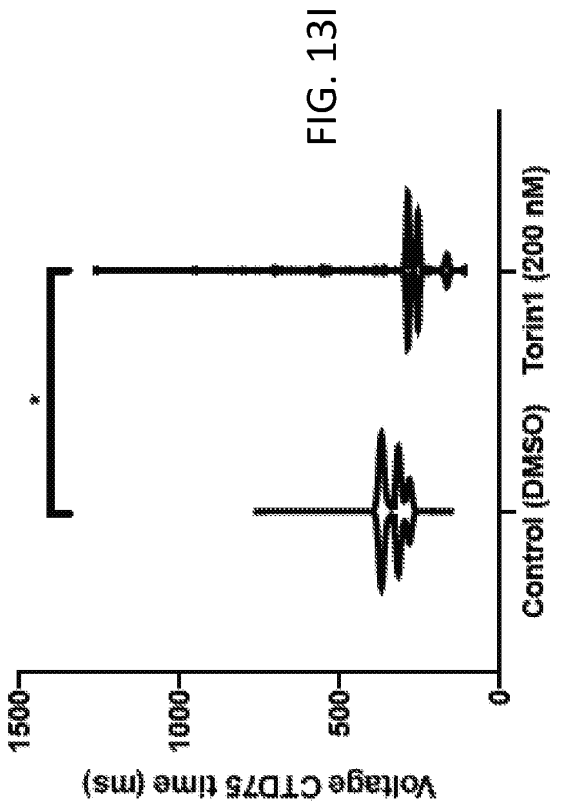


FIG. 13I

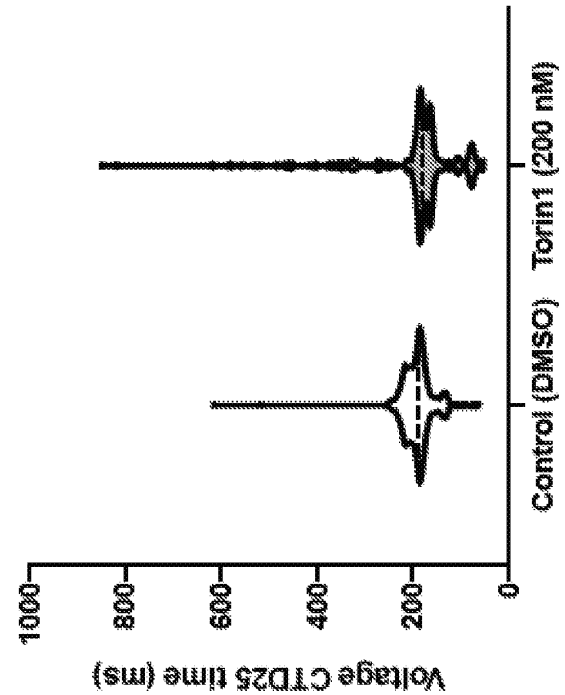


FIG. 13H

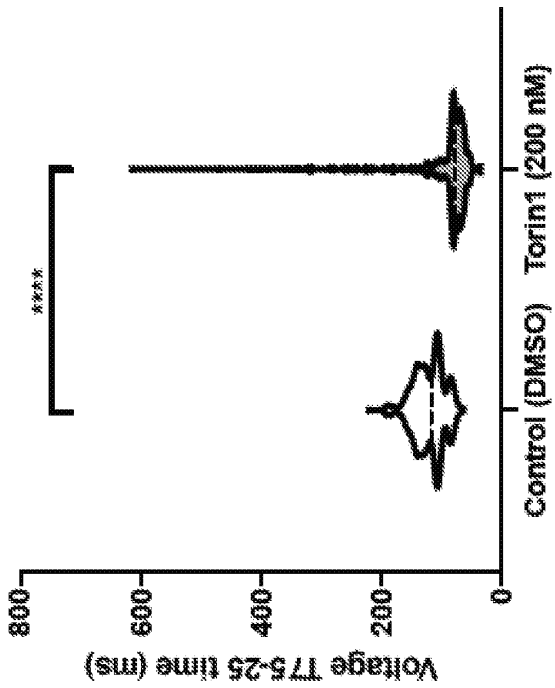


FIG. 13J

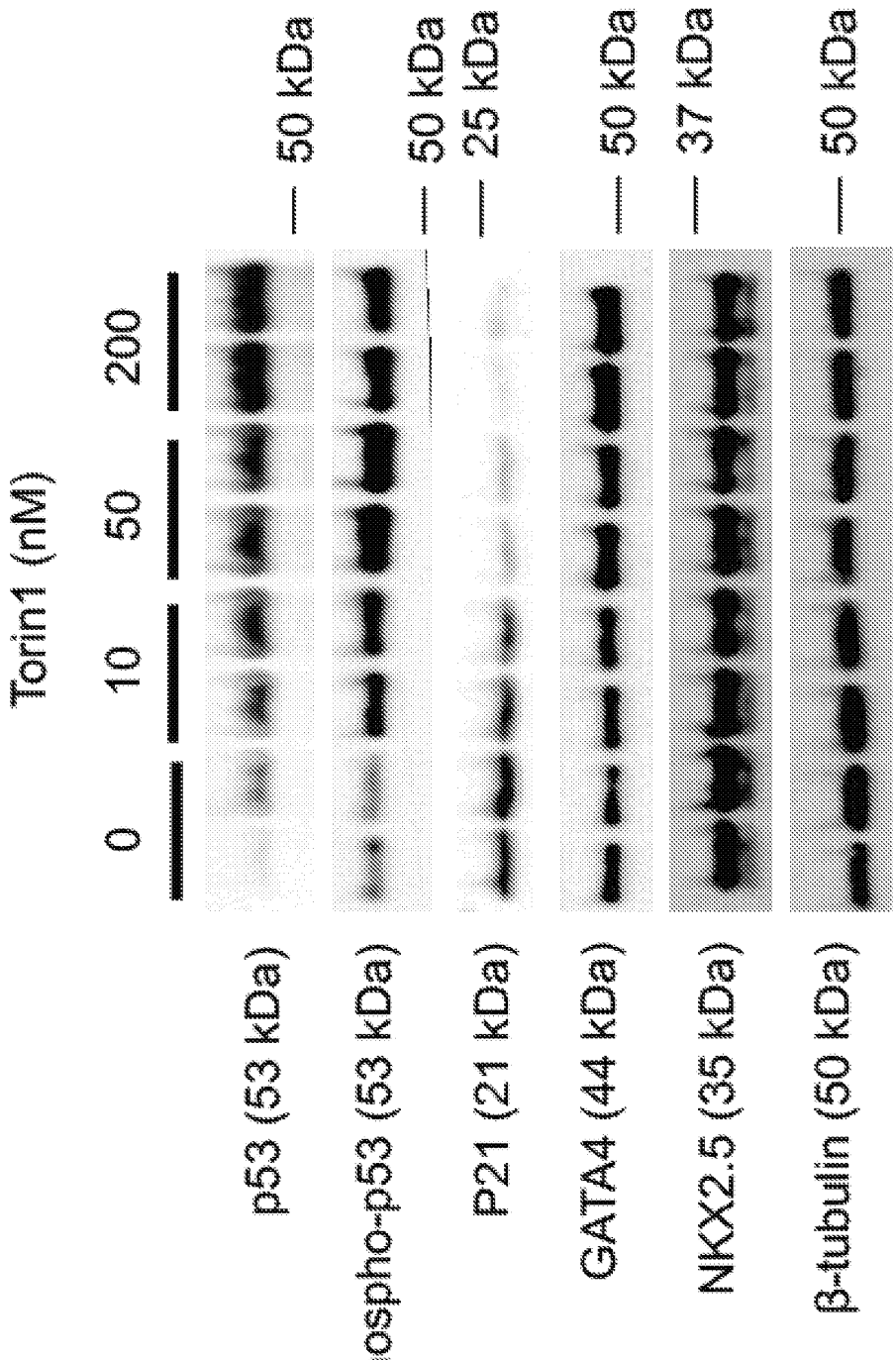

Torin1 (nM)
0
10
50
200
p53 (53 kDa)
50 kDa
Phospho-p53 (53 kDa)
50 kDa
P21 (21 kDa)
25 kDa
GATA4 (44 kDa)
50 kDa
NKX2.5 (35 kDa)
37 kDa
β-tubulin (50 kDa)
50 kDa

FIG. 14A

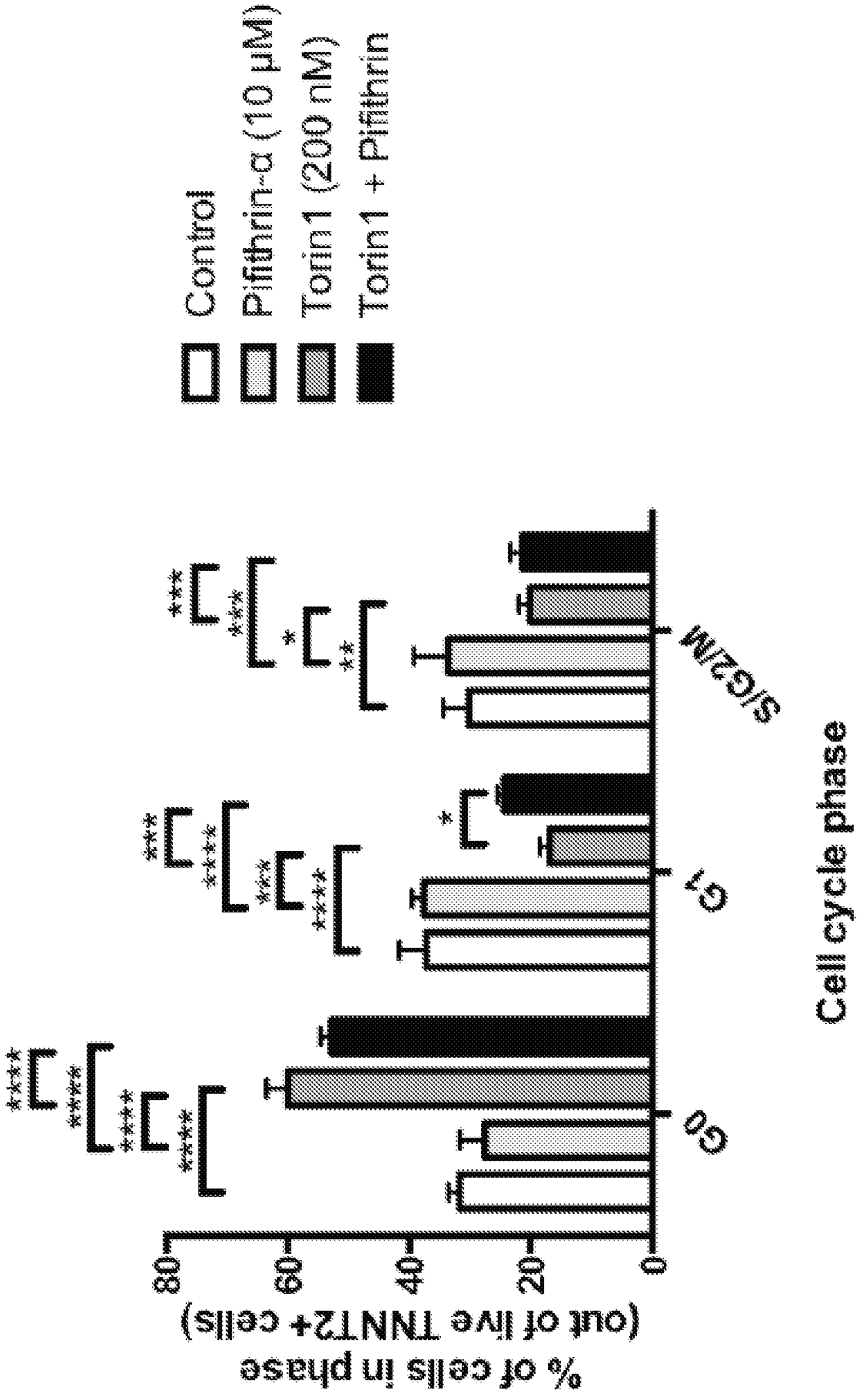

Control
Pifithrin-α (10 μM)
Torin1 (200 nM)
Torin1 + Pifithrin
% of cells in phase
(out of live TNNT2+ cells)
0
20
40
60
80
G0
G1
S/G2/M
Cell cycle phase

FIG. 14B

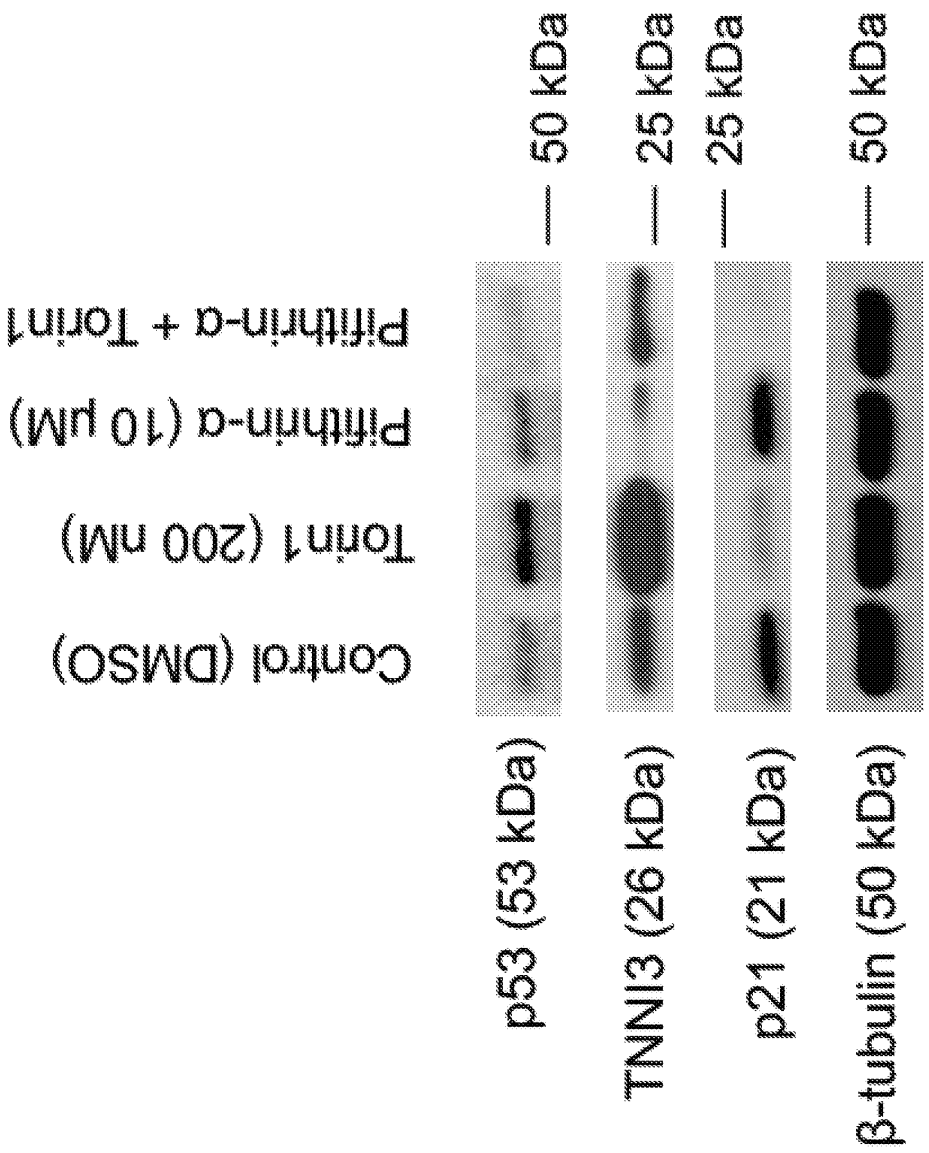

Control (DMSO)
Torin1 (200 nM)
Pifithrin-α (10 μM)
Pifithrin-α + Torin1
p53 (53 kDa)
TNNI3 (26 kDa)
p21 (21 kDa)
β-tubulin (50 kDa)
50 kDa
25 kDa
25 kDa
50 kDa

FIG. 14C

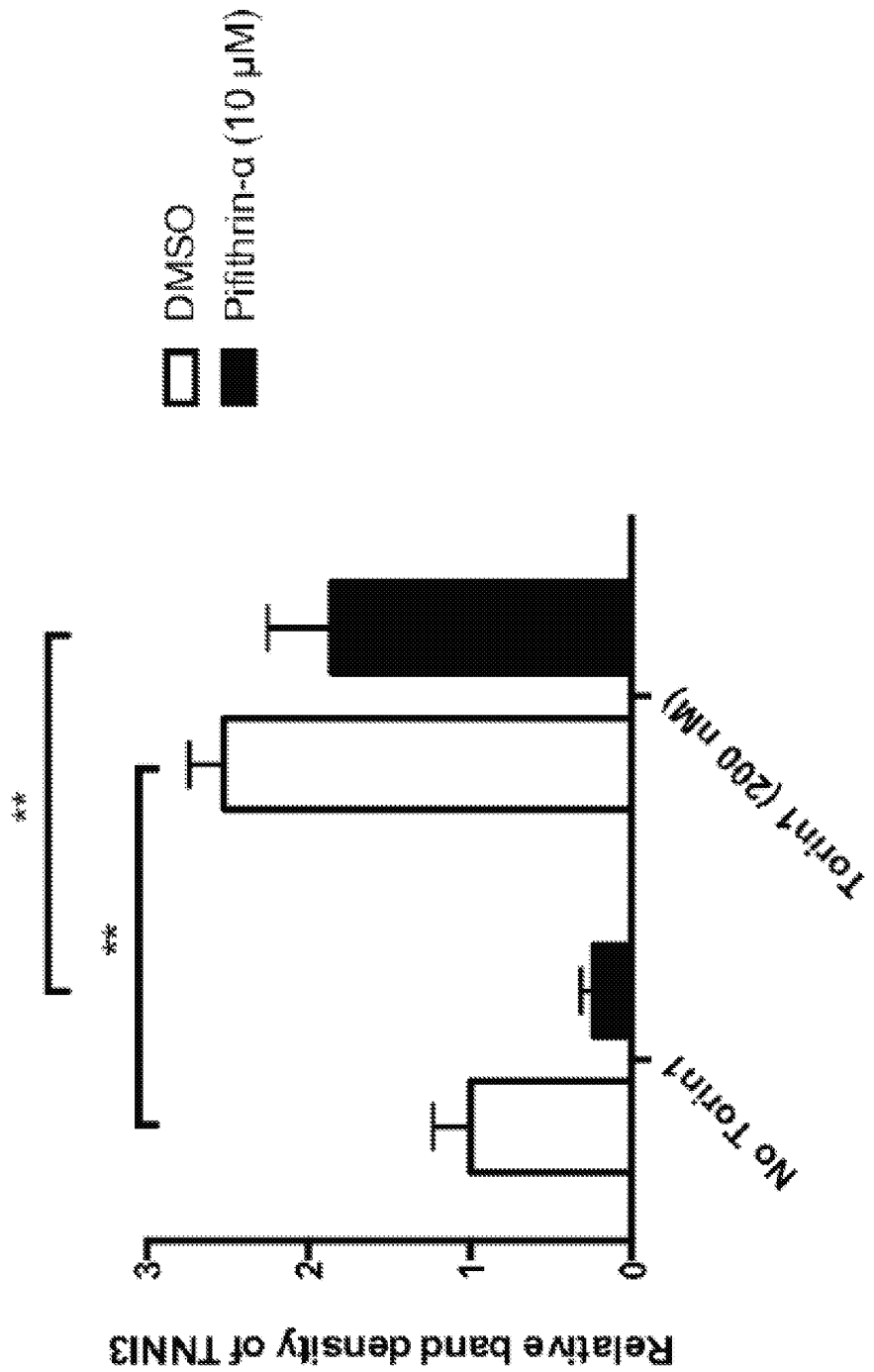

DMSO
Pifithrin-α (10 μM)
Relative band density of TNNI3
0
1
2
3
No Torin1
Torin1 (200 nM)
**
**

FIG. 14D

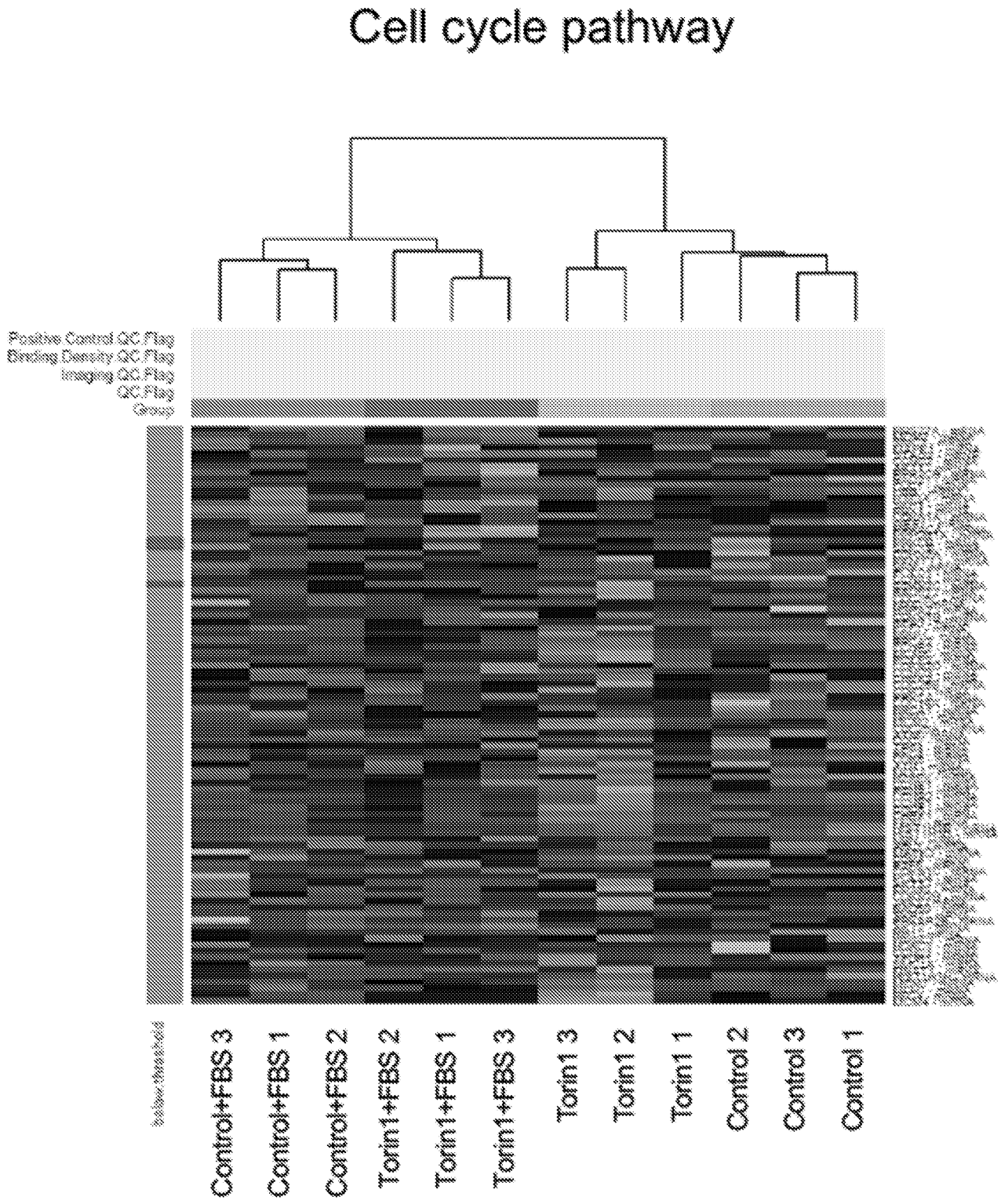

Cell cycle pathway
Positive Control QC Flag
Binding Density QC Flag
Imaging QC Flag
QC Flag
Group
Control+FBS 3
Control+FBS 1
Control+FBS 2
Torin1+FBS 2
Torin1+FBS 1
Torin1+FBS 3
Torin1 3
Torin1 2
Torin1 1
Control 2
Control 3
Control 1

FIG. 15A

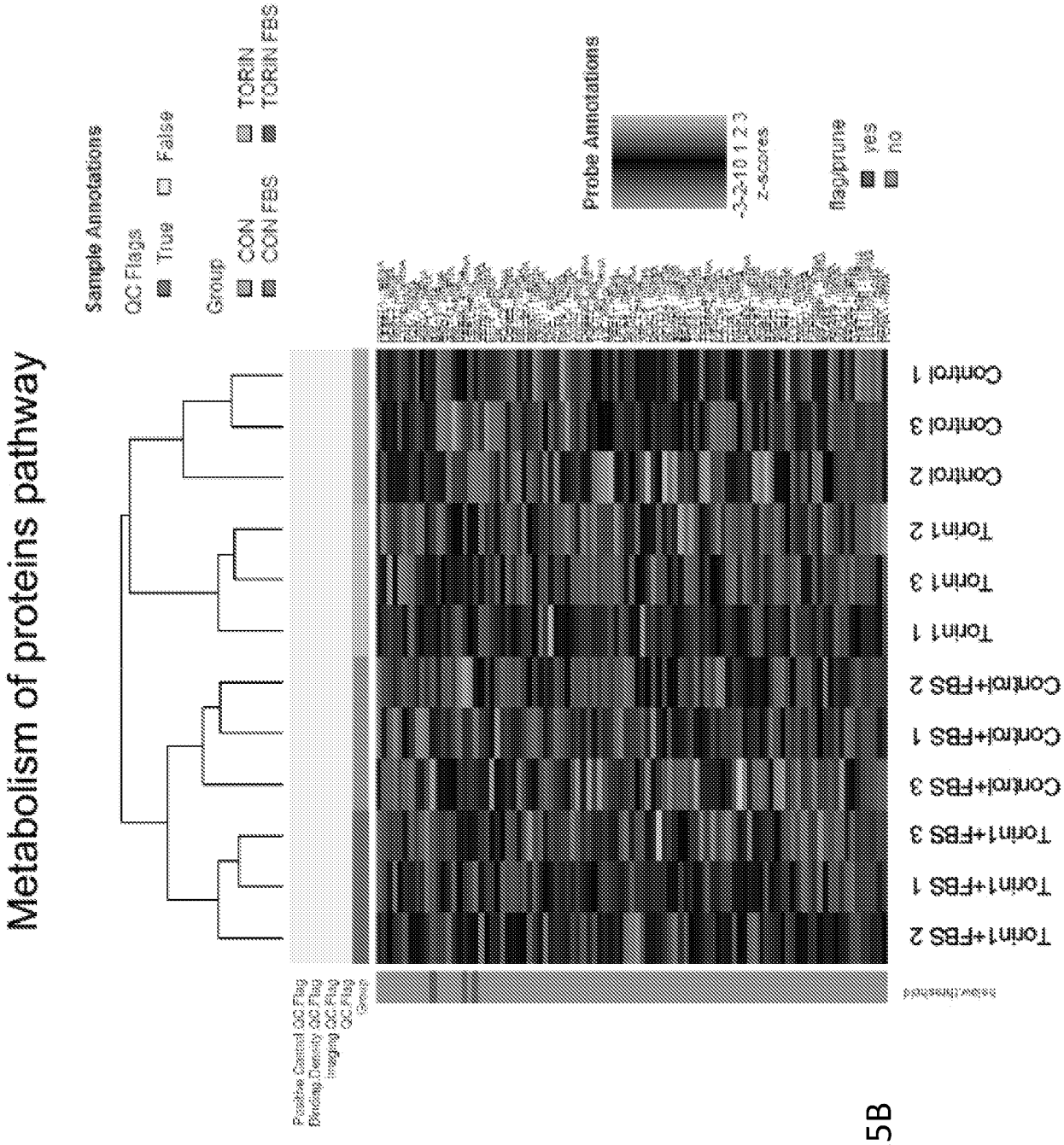

Metabolism of proteins pathway
Sample Annotations
QC Flags
True
False
Group
CON
CON FBS
TORIN
TORIN FBS
Probe Annotations
-3 -2 -1 0 1 2 3
z-scores
flagInPrune
yes
no
Torin1+FBS 2
Torin1+FBS 1
Torin1+FBS 3
Control+FBS 3
Control+FBS 1
Control+FBS 2
Torin1 1
Torin1 3
Torin1 2
Control 2
Control 3
Control 1

FIG. 15B

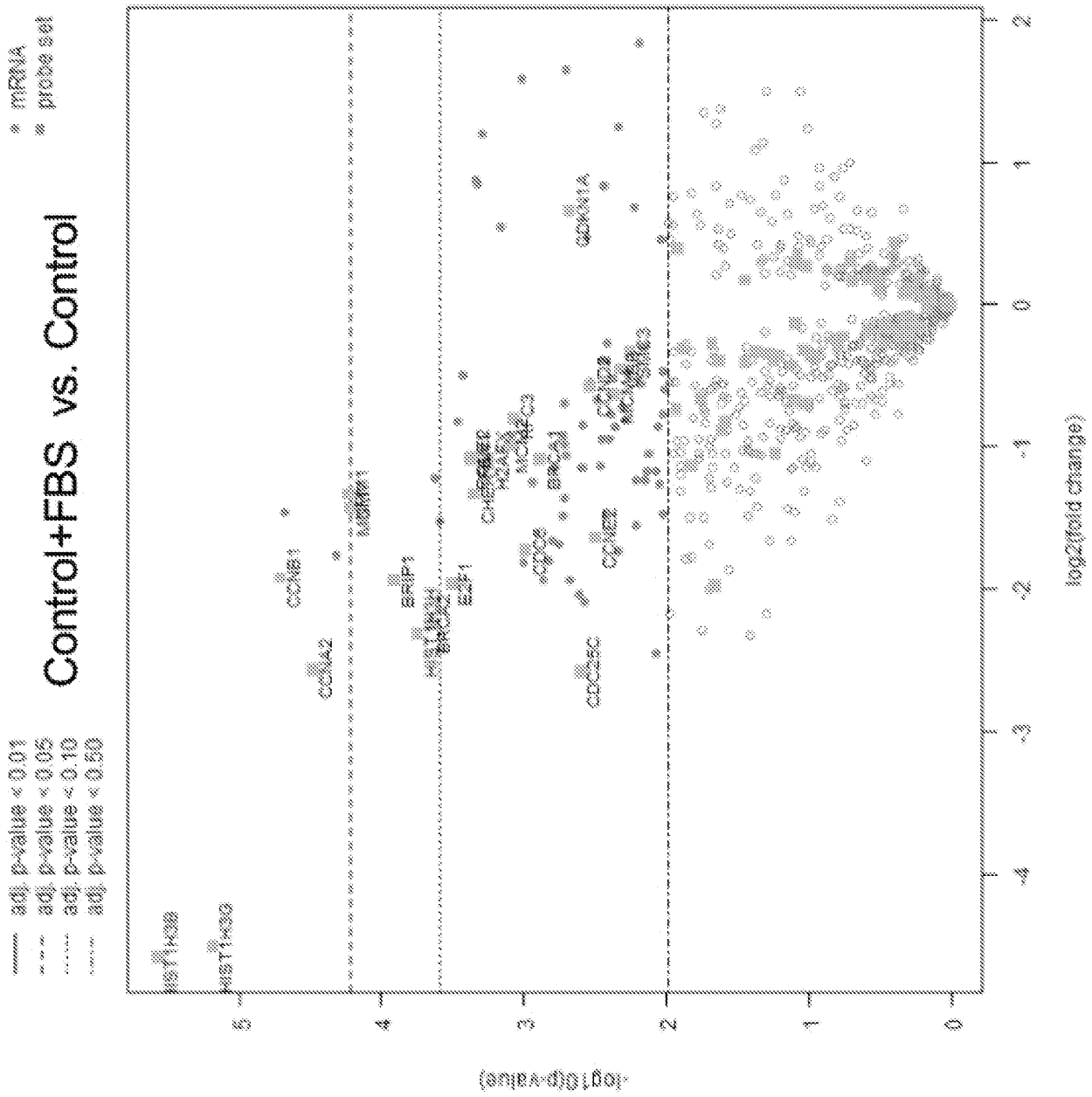

Control+FBS vs. Control
* mRNA
* probe set
adj p-value < 0.01
adj p-value < 0.05
adj p-value < 0.10
adj p-value < 0.50
-log10(p-value)
log2(fold change)

FIG. 15C

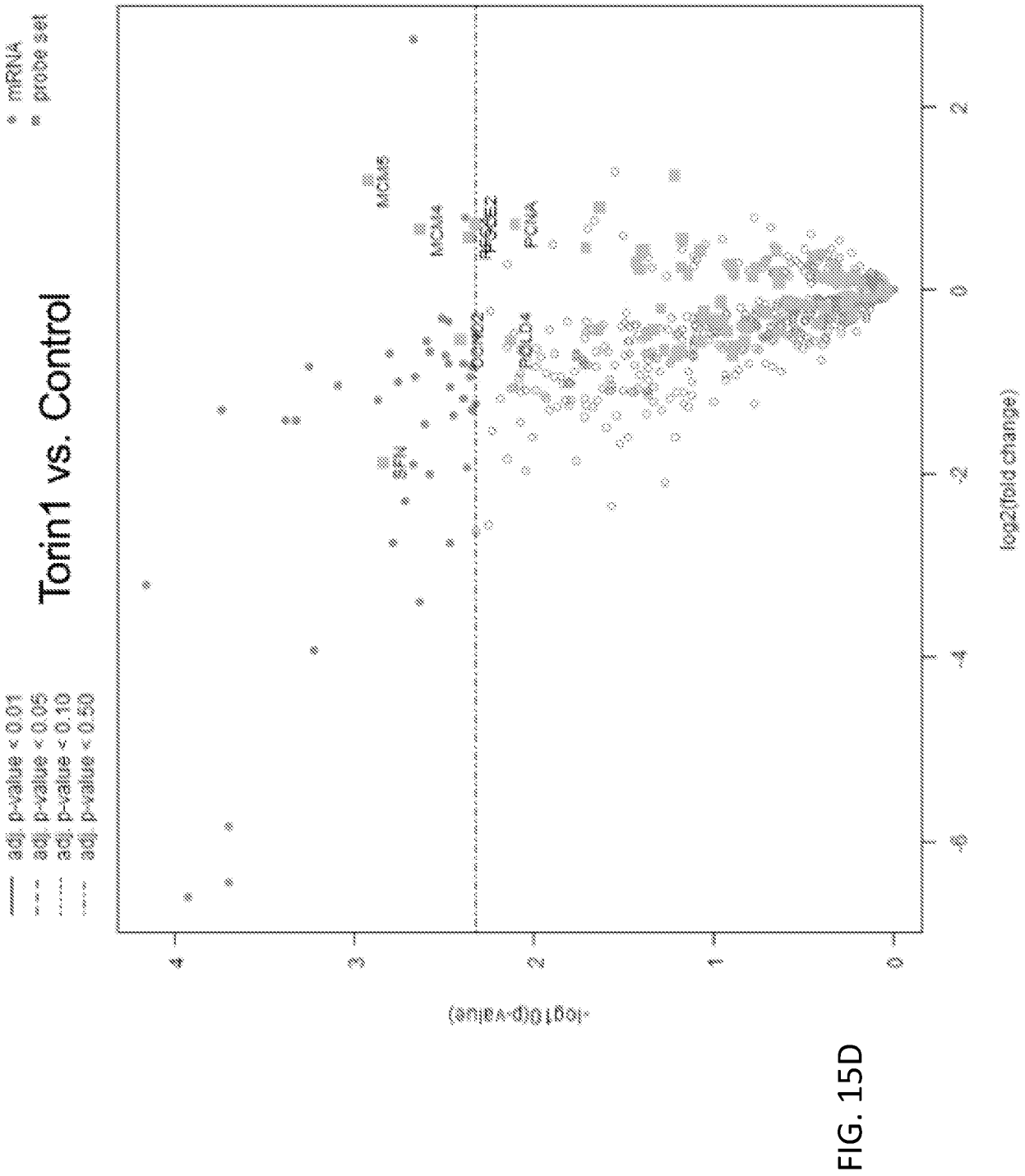

Torin1 vs. Control
* mRNA
* probe set
adj. p-value < 0.01
adj. p-value < 0.05
adj. p-value < 0.10
adj. p-value < 0.50
MCM6
MCM4
PCNA
-log10(p-value)
log2(fold change)

FIG. 15D

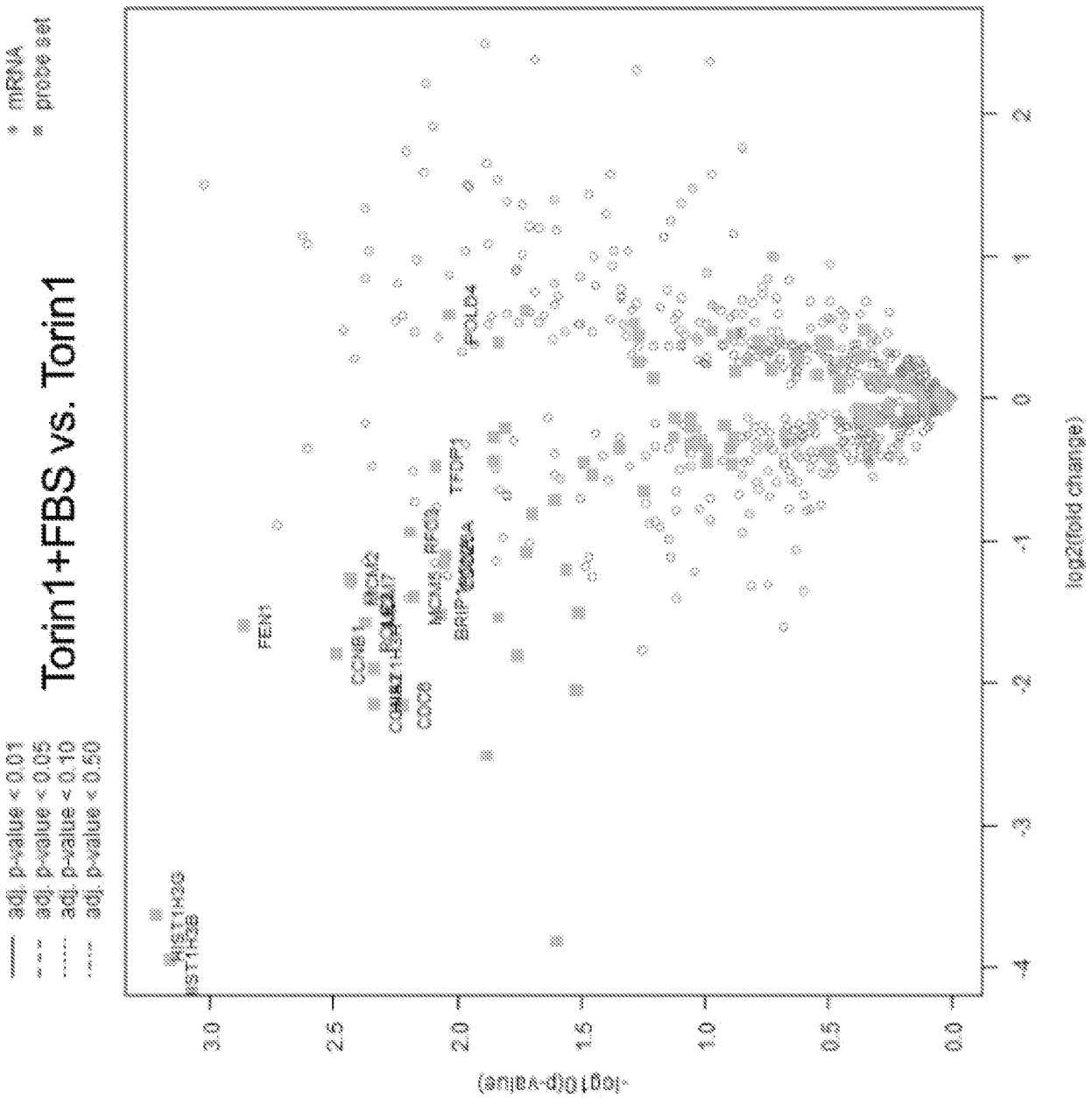

Torin1+FBS vs. Torin1
adj. p-value < 0.01
adj. p-value < 0.05
adj. p-value < 0.10
adj. p-value < 0.50
mRNA
probe set
-log10(p-value)
log2(fold change)
FEN1
CCNB1
CDC6
TFDP1
POLD4
HIST1H3G
HIST1H3B

FIG. 15E

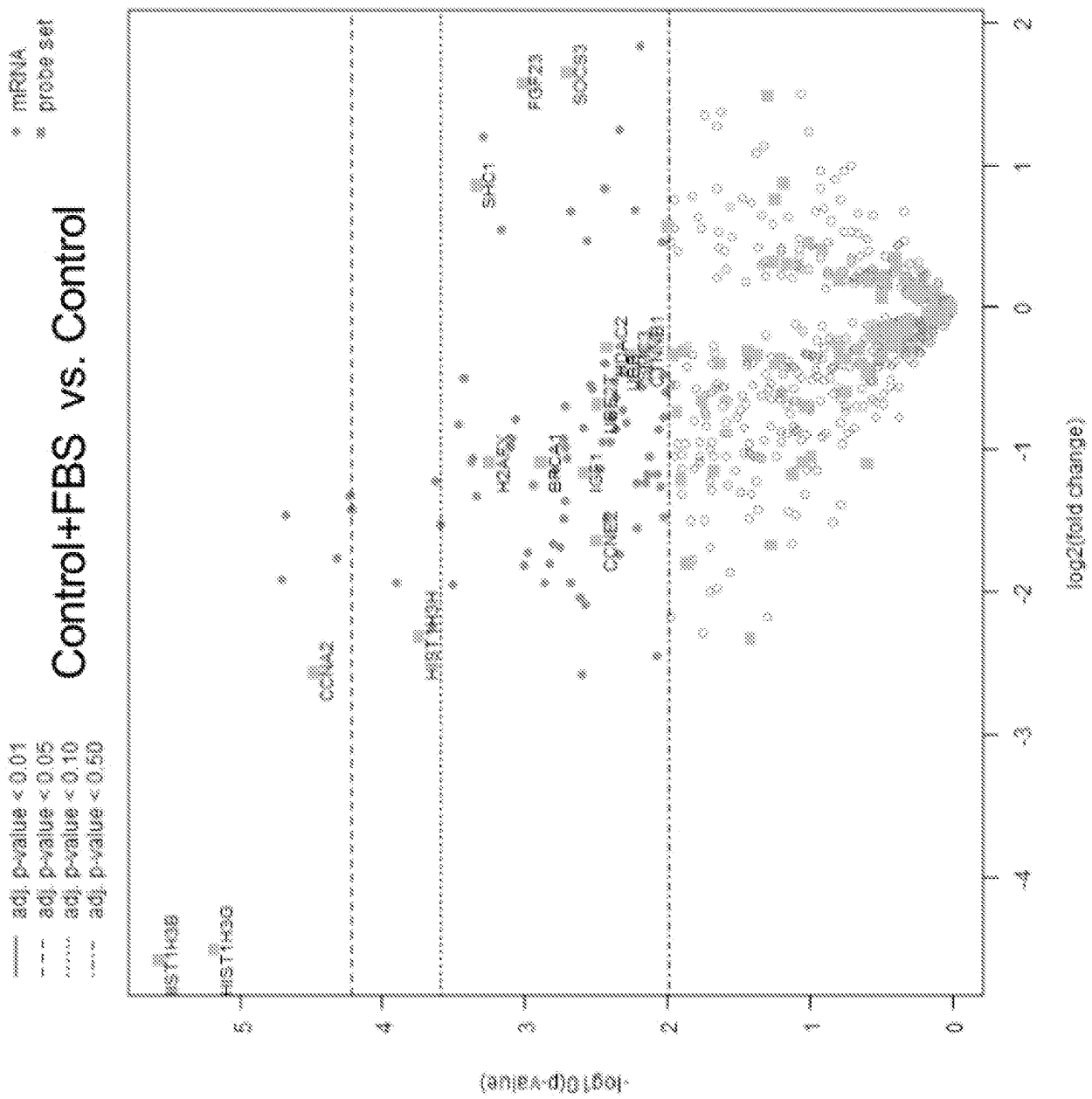

Control+FBS vs. Control
adj. p-value < 0.01
adj. p-value < 0.05
adj. p-value < 0.10
adj. p-value < 0.50
mRNA
probe set
-log10(p-value)
log2(fold change)
HIST1H3B
HIST1H3G
CCNA2
HIST1H3H
H2AFX
BRCA1
CCNE2
SHC1
FGF23
SOCS3

FIG. 15F

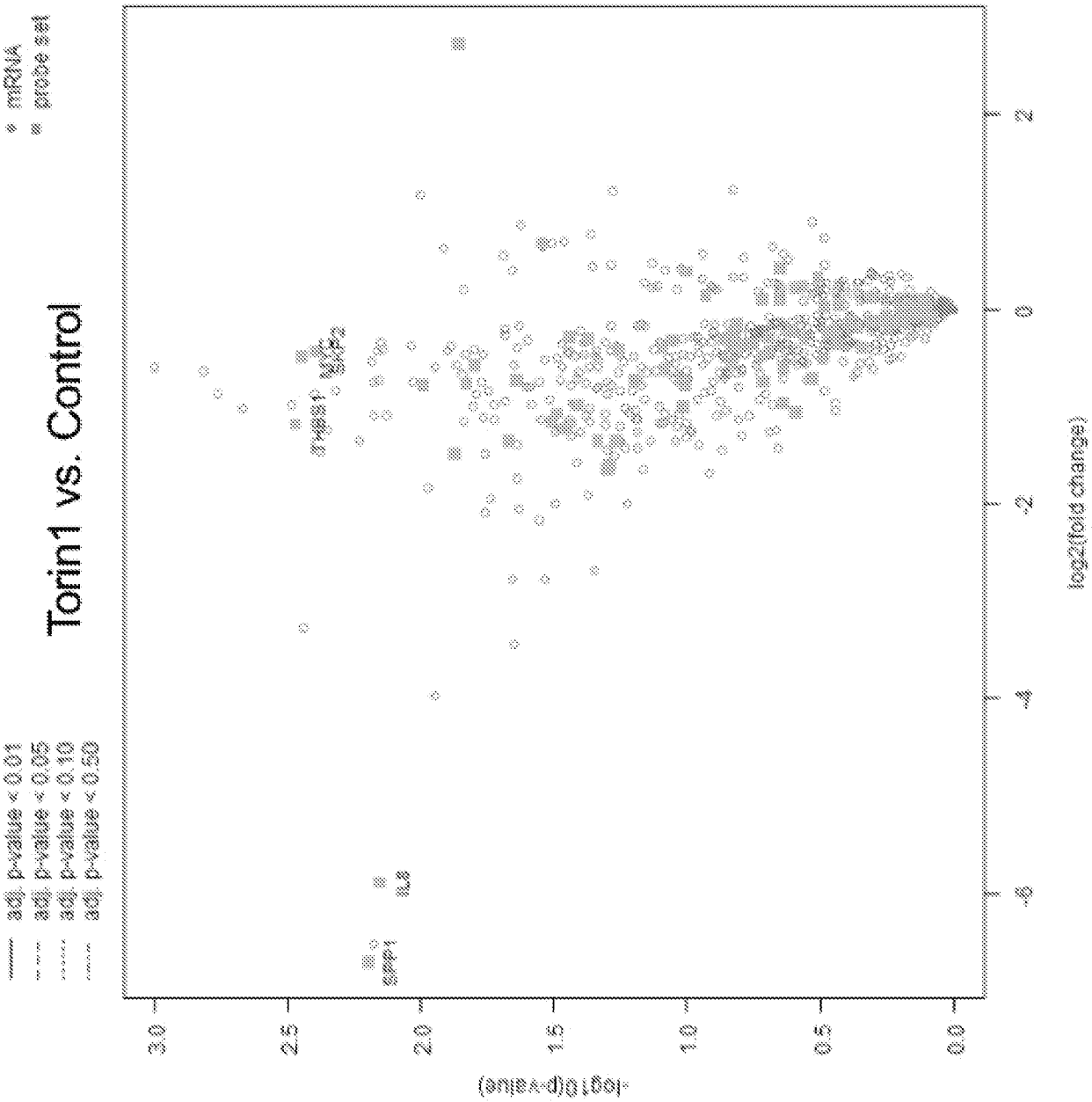

Torin1 vs. Control
adj. p-value < 0.01
adj. p-value < 0.05
adj. p-value < 0.10
adj. p-value < 0.50
mRNA
probe set
-log10(p-value)
log2(fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-6
-4
-2
0
2
SPP1
IL8
THBS1
MMP2

FIG. 15G

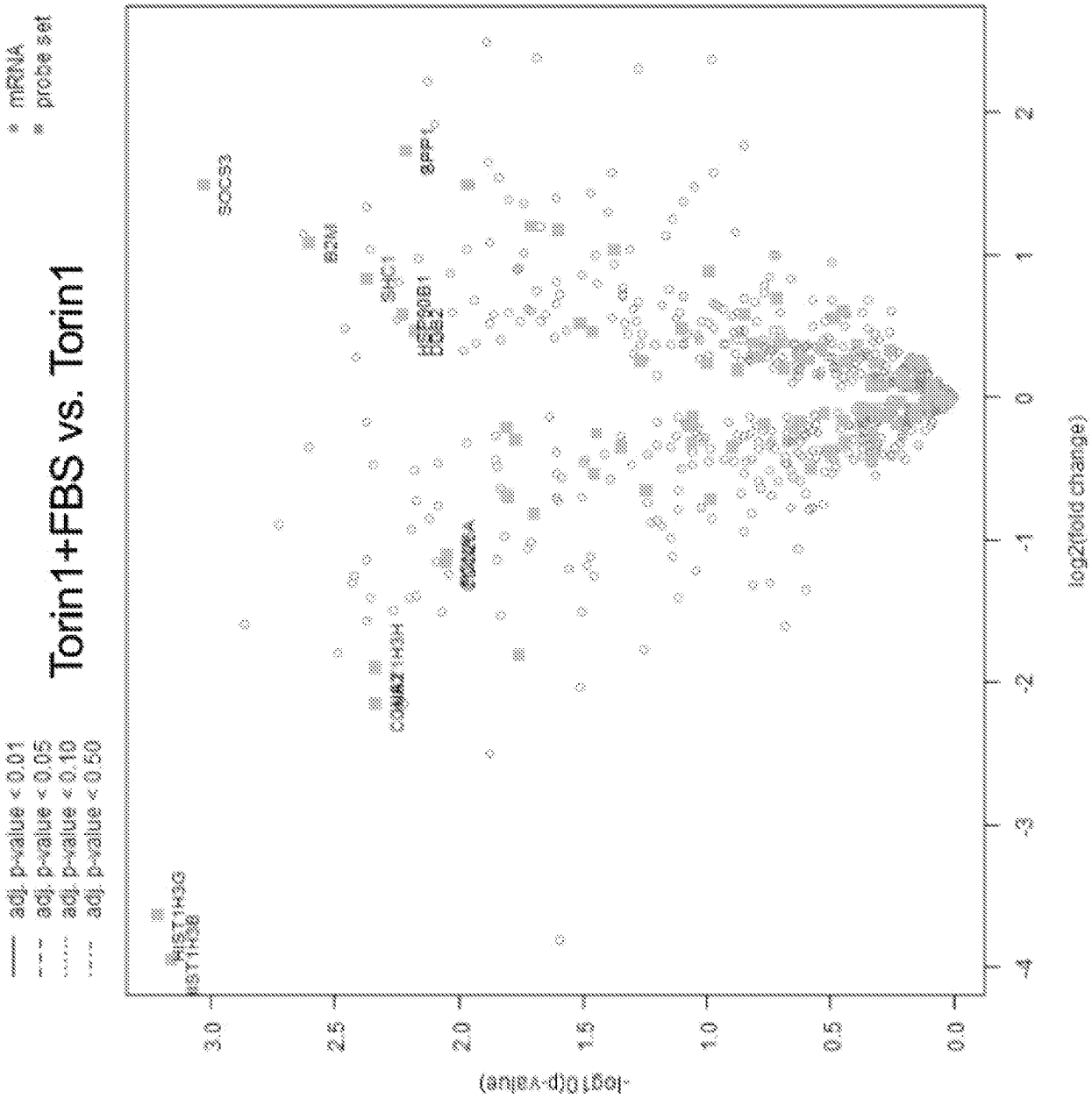

Torin1+FBS vs. Torin1
adj. p-value < 0.01
adj. p-value < 0.05
adj. p-value < 0.10
adj. p-value < 0.50
mRNA
probe set
SOCS3
B2M
SHC1
SPP1
-log10(p-value)
log2(fold change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-4
-3
-2
-1
0
1
2

FIG. 15H

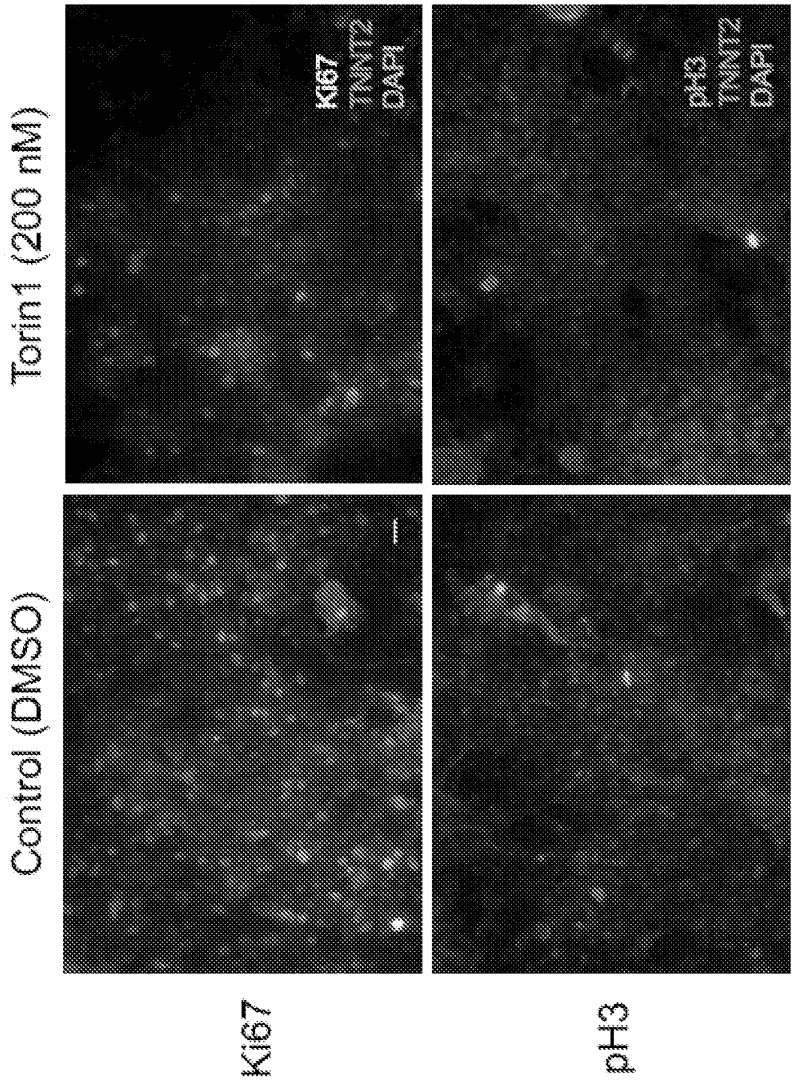

Control (DMSO)
Torin1 (200 nM)
Ki67
pH3
Ki67
TNNT2
DAPI
pH3
TNNT2
DAPI

FIG. 16A

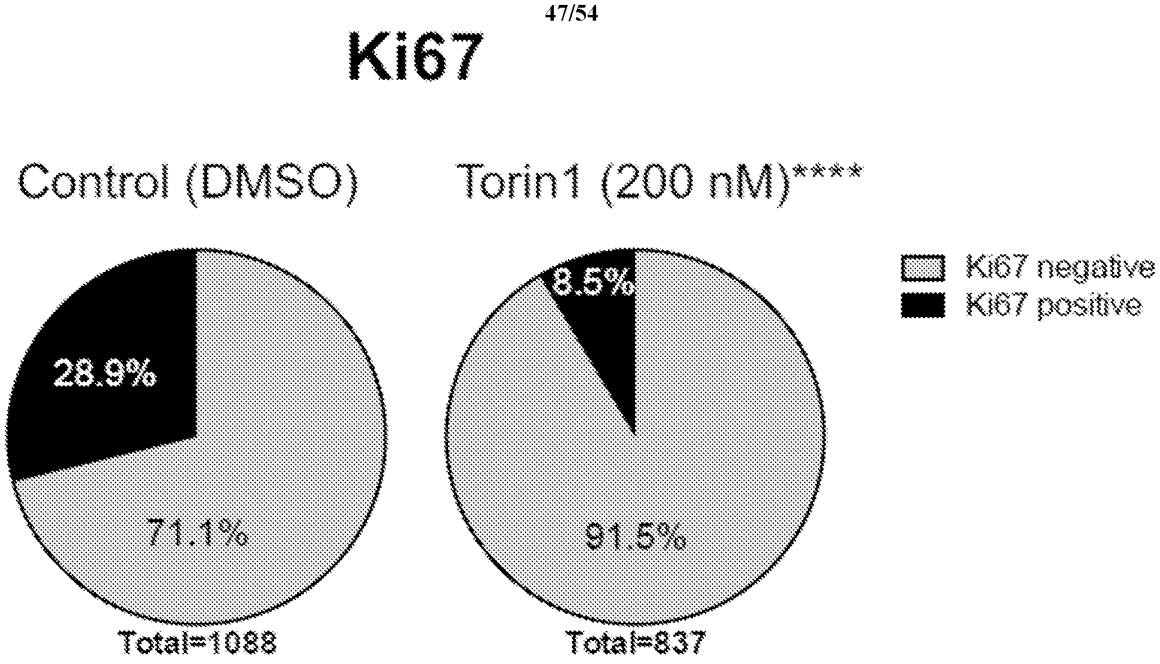


FIG. 16B

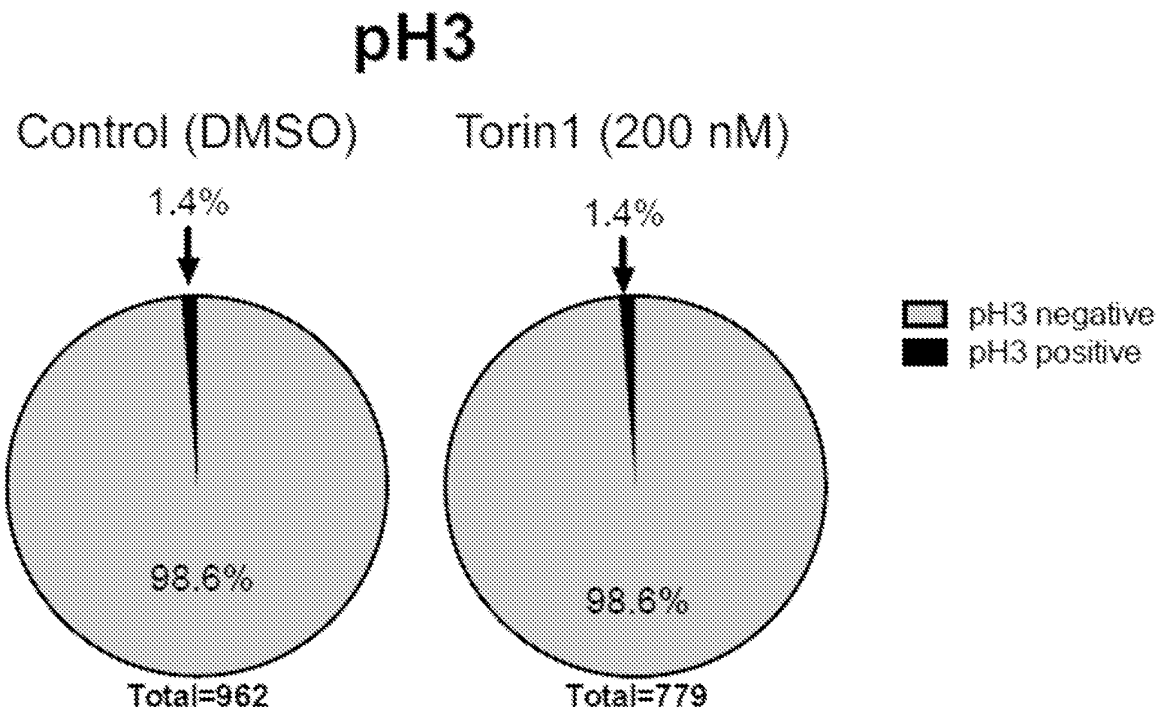


FIG. 16C

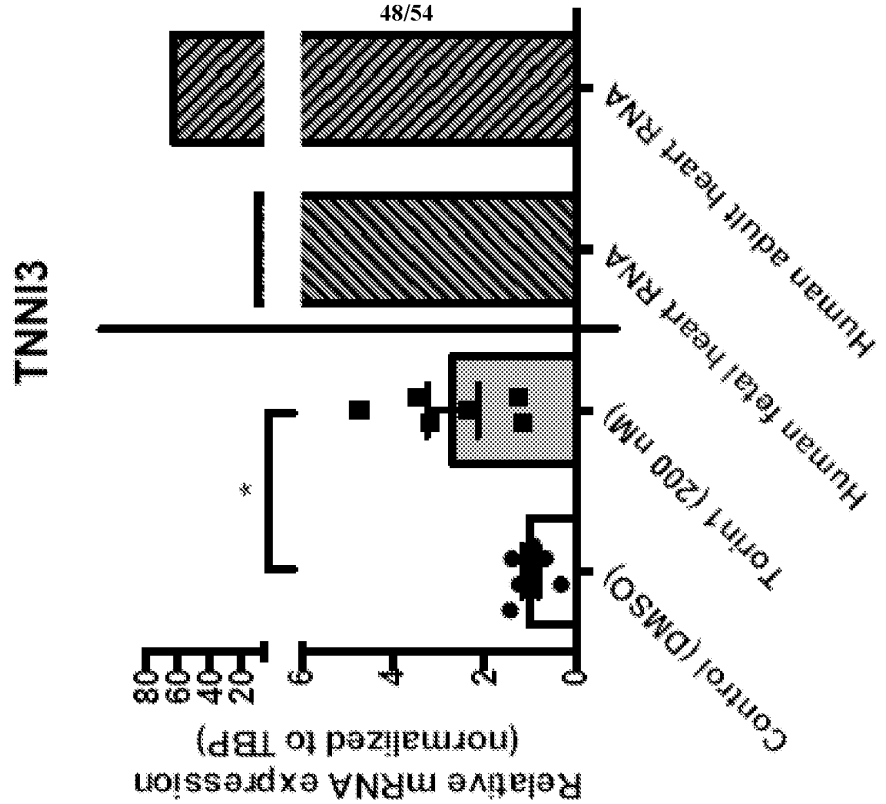


FIG. 17B

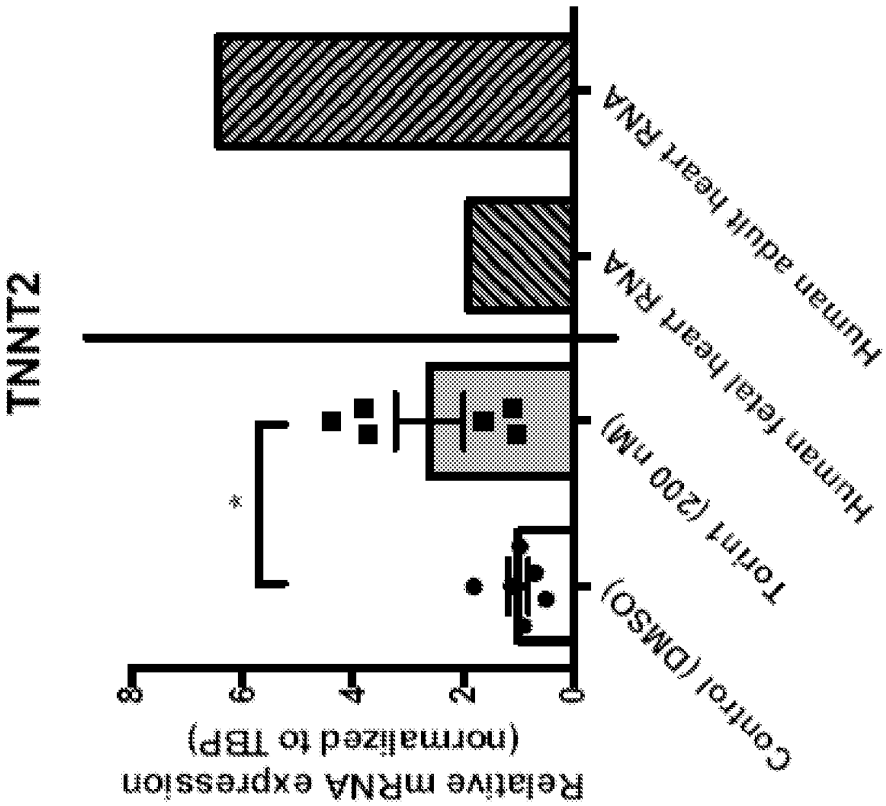


FIG. 17A

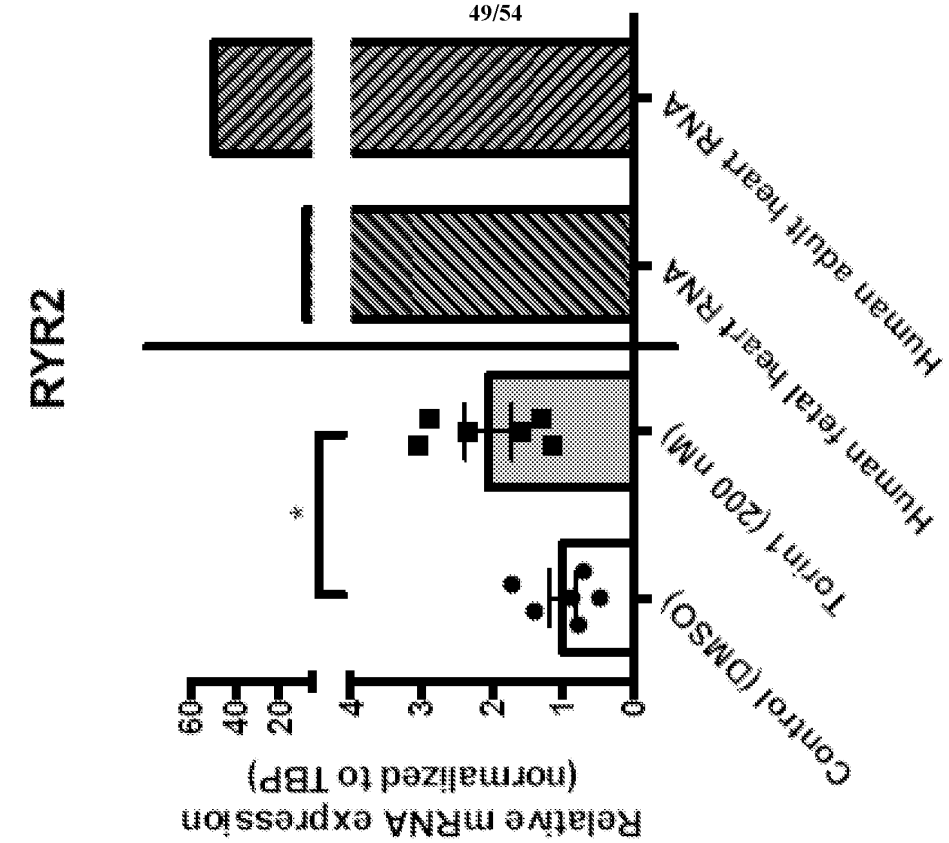


FIG. 17D

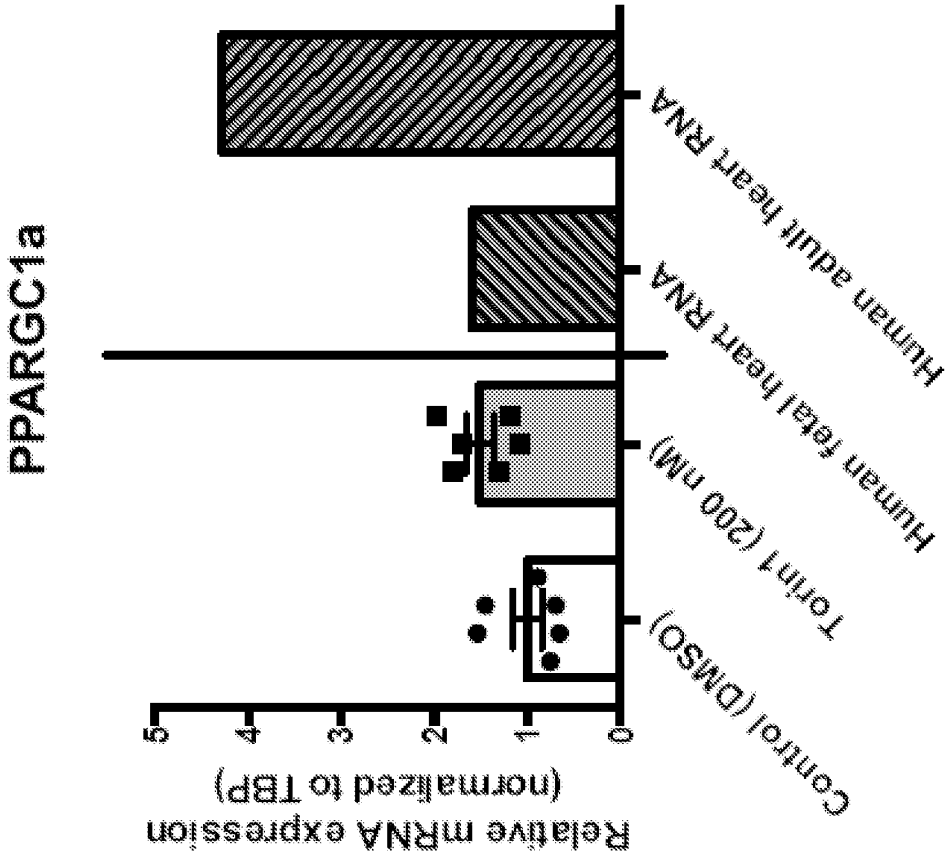


FIG. 17C

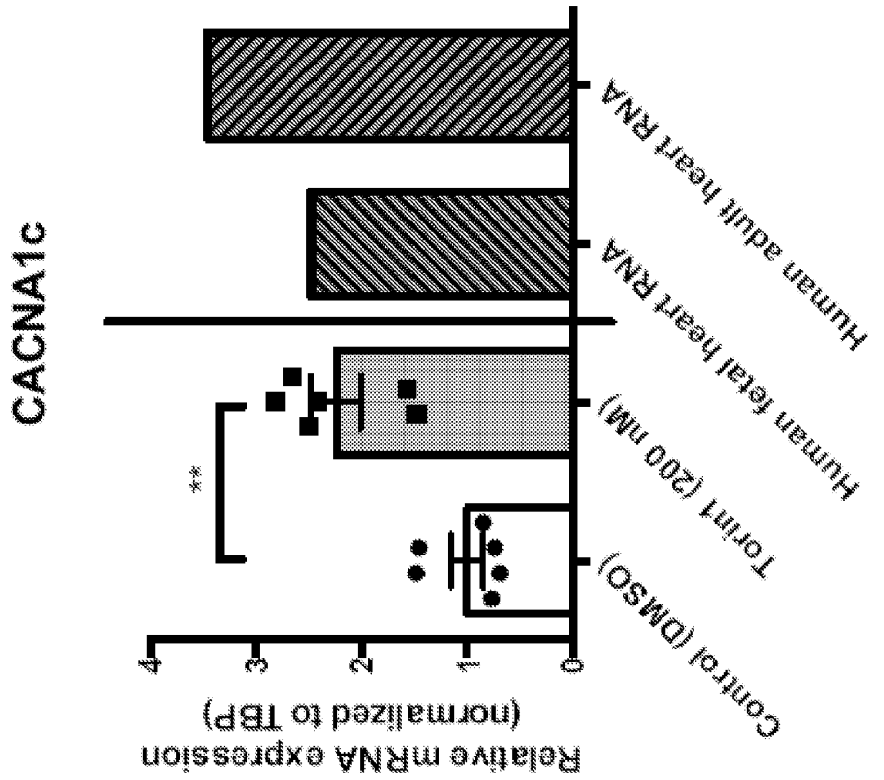


FIG. 17F

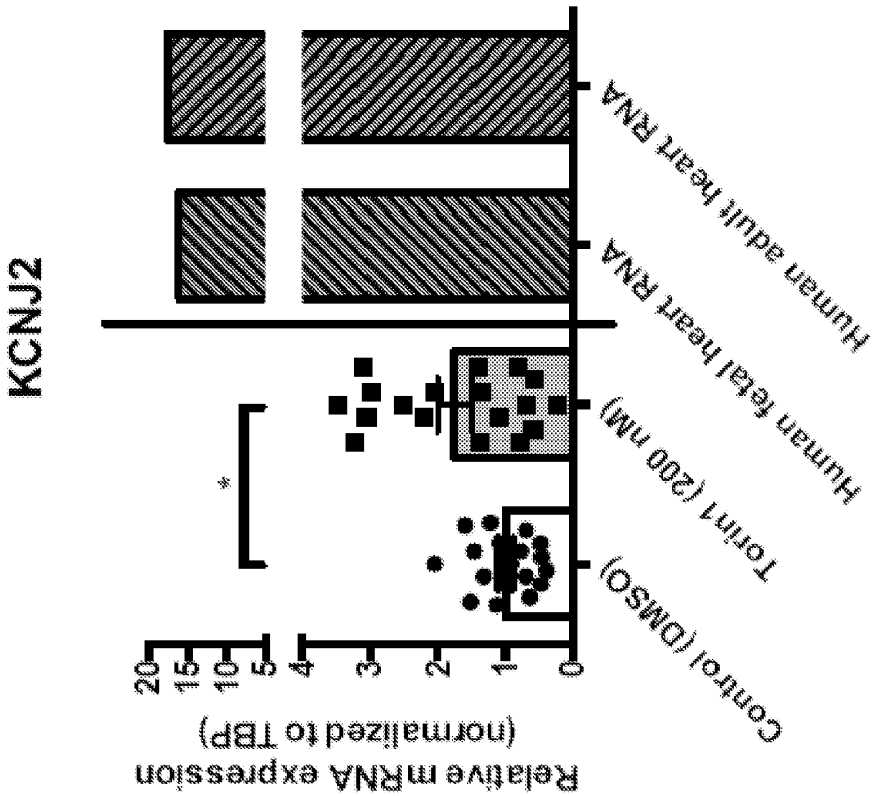


FIG. 17E

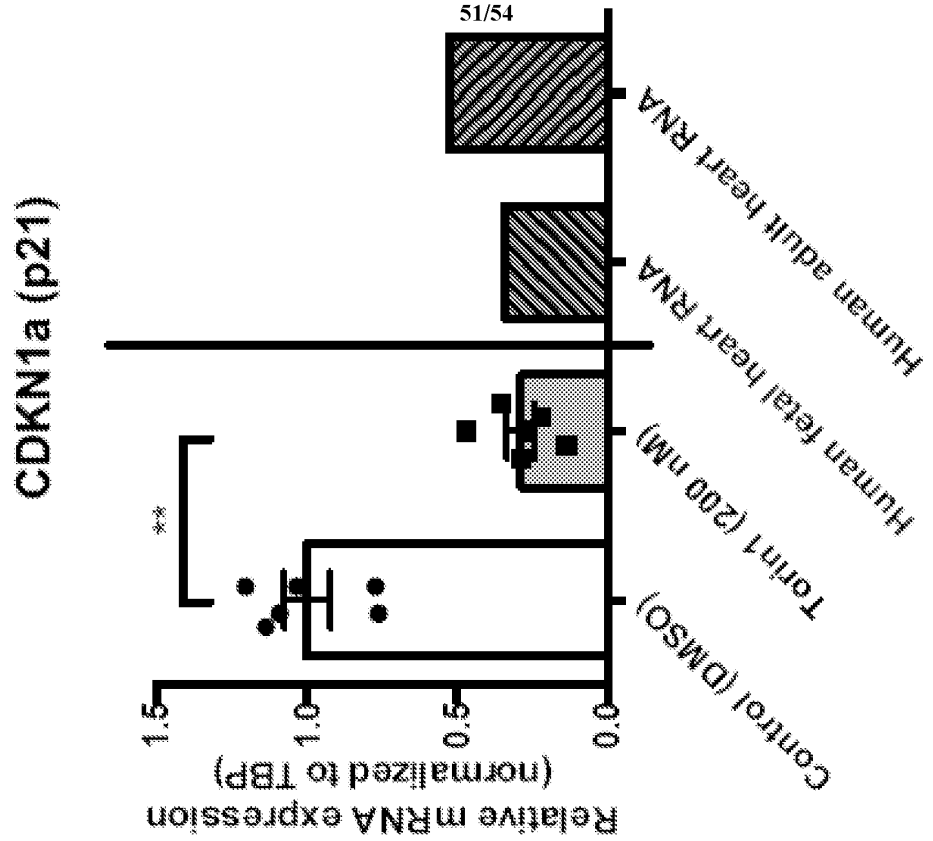


FIG. 17H

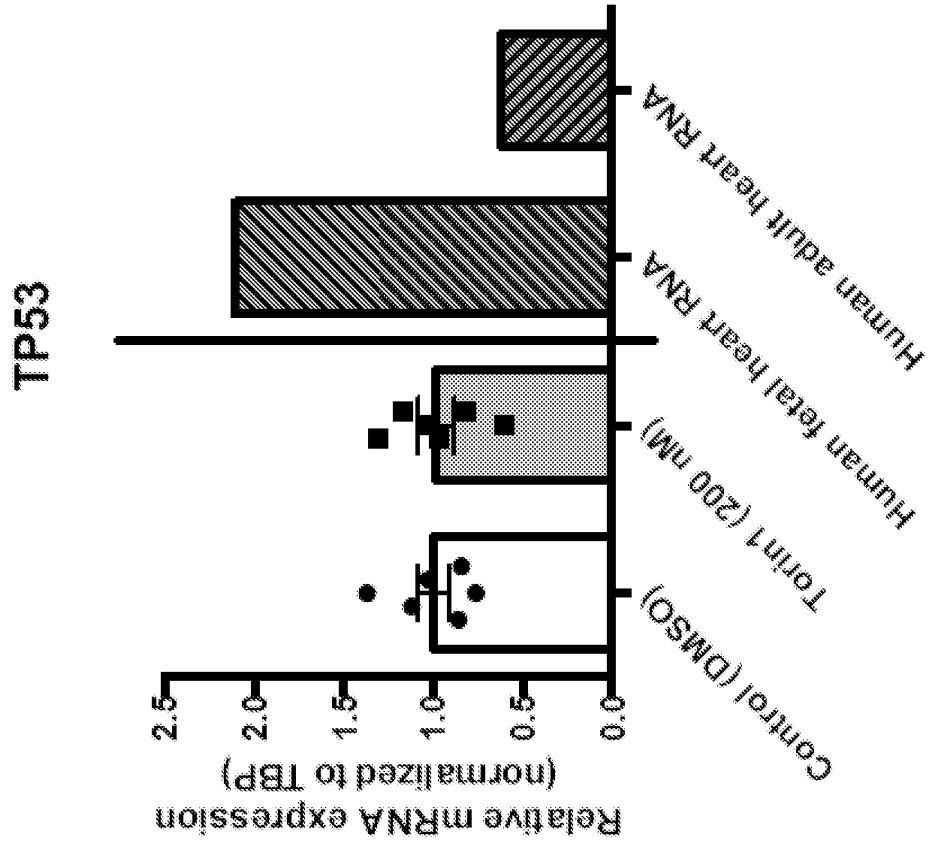


FIG. 17G

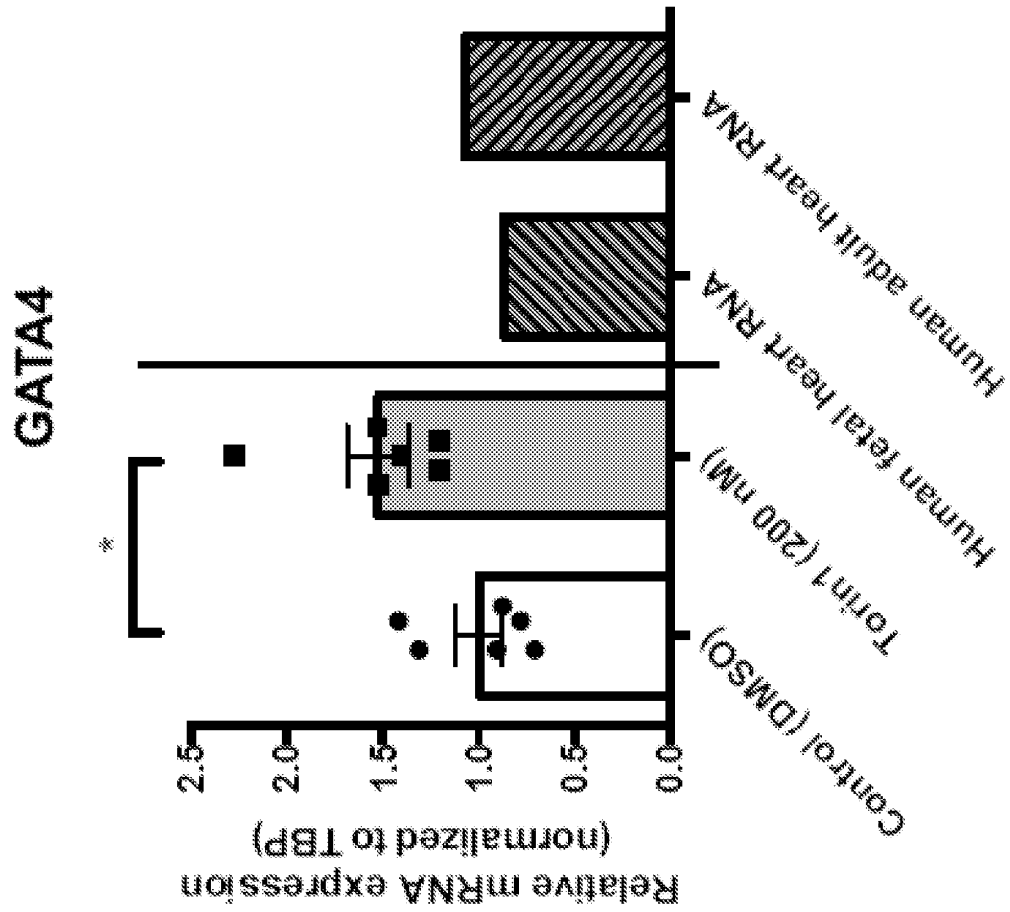

GATA4
Relative mRNA expression
(normalized to TBP)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control (DMSO)
Tonin1 (200 nM)
Human fetal heart RNA
Human adult heart RNA

FIG. 17I

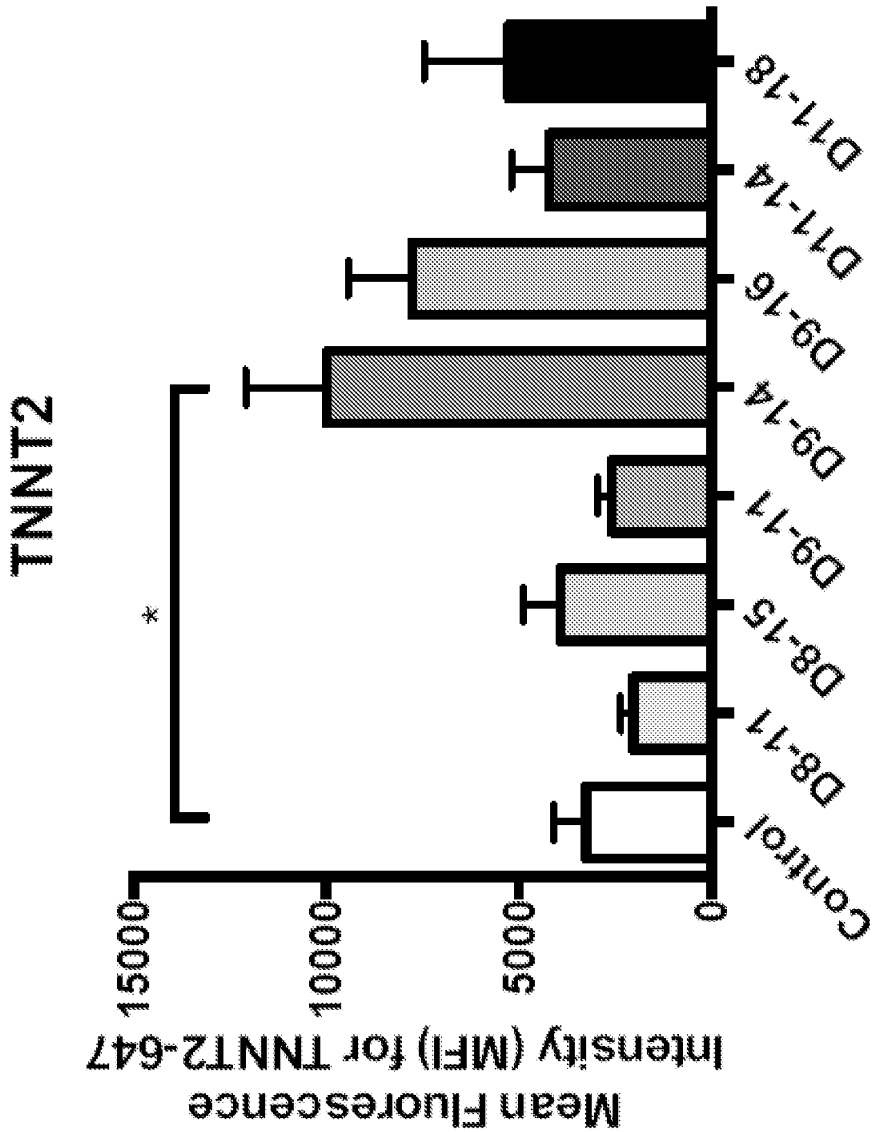

TNNT2
*
Mean Fluorescence Intensity (MFI) for TNNT2-647
15000
10000
5000
0
Control
D8-11
D8-15
D9-11
D9-14
D9-16
D11-14
D11-18
Days of Torin1 treatment
(days after onset of differentiation)

FIG. 18A

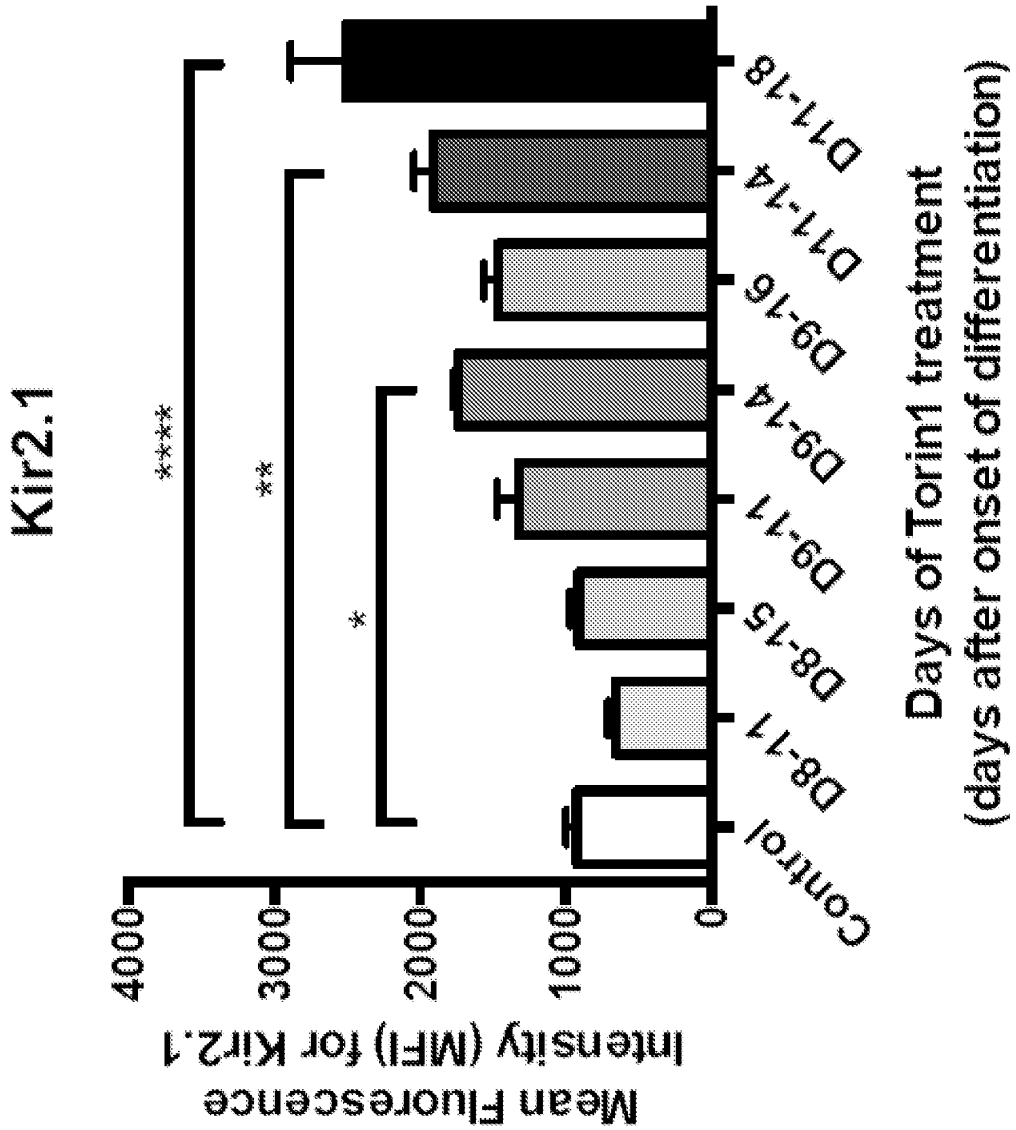

Kir2.1
Mean Fluorescence Intensity (MFI) for Kir2.1
4000
3000
2000
1000
0
****
**
*
Control
D8-11
D8-15
D9-11
D9-14
D9-16
D11-14
D11-18
Days of Torin1 treatment
(days after onset of differentiation)

FIG. 18B