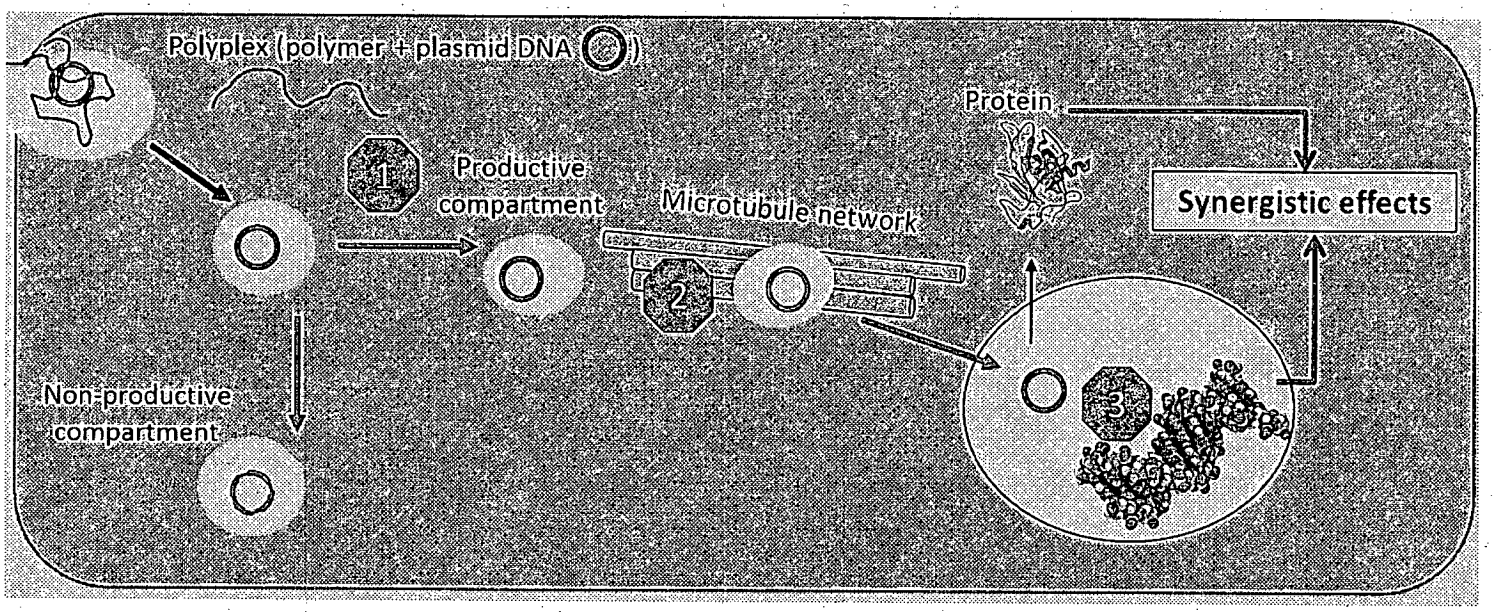


TrafEn Site of Actions

1. Re-routing of plasmid
2. Stabilization of microtubular network
3. Modify genome activity

DOPE/CHEMS

HDACi

Results:

1. Increased transfection
2. Stabilization of microtubular network
3. Modify genome activity through epigenetic modification

Stem cell differentiation
Somatic cell reprogramming
and etc...

FIG. 1

FIG. 2

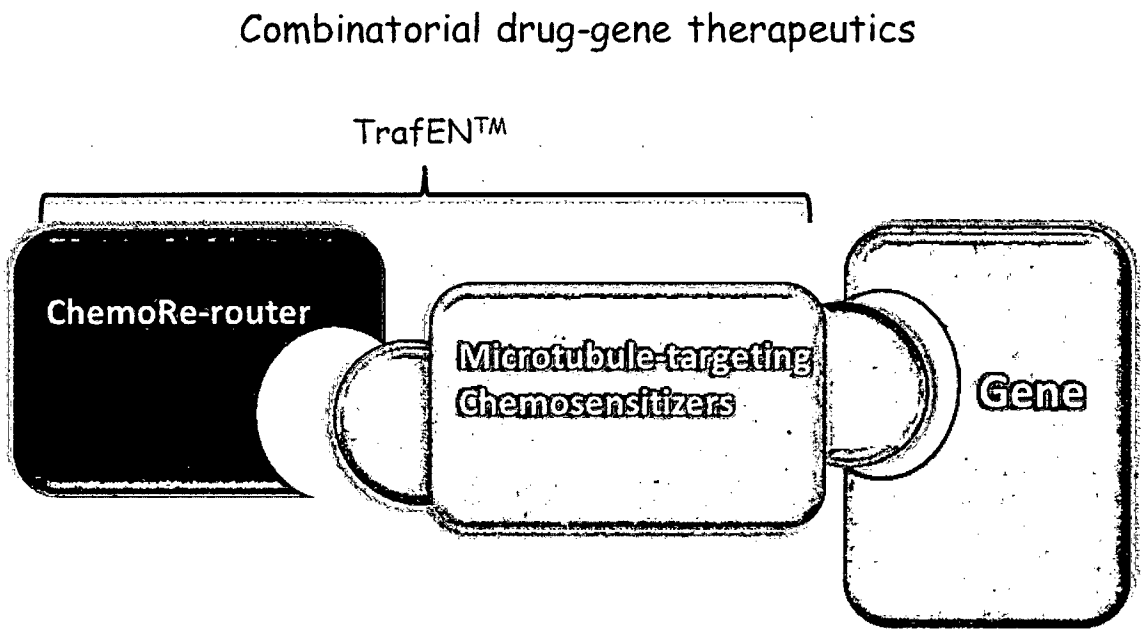
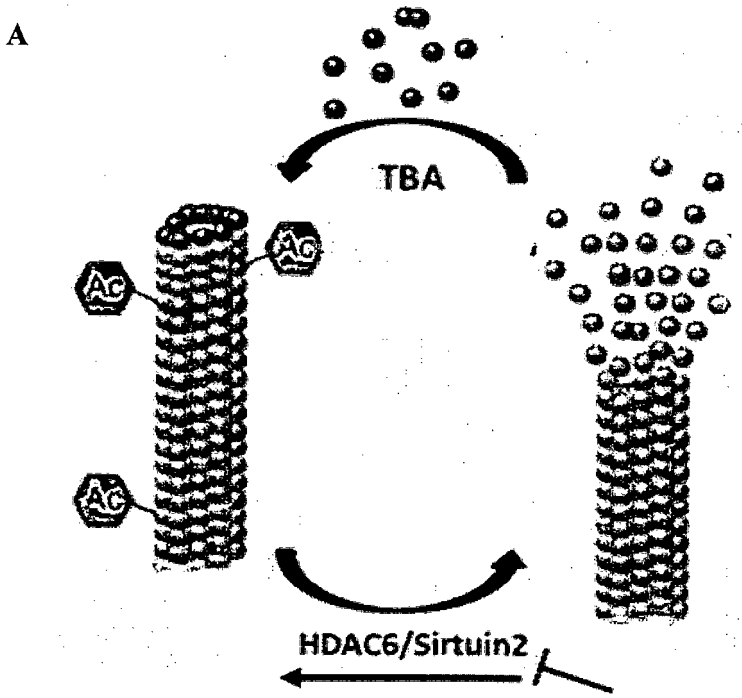
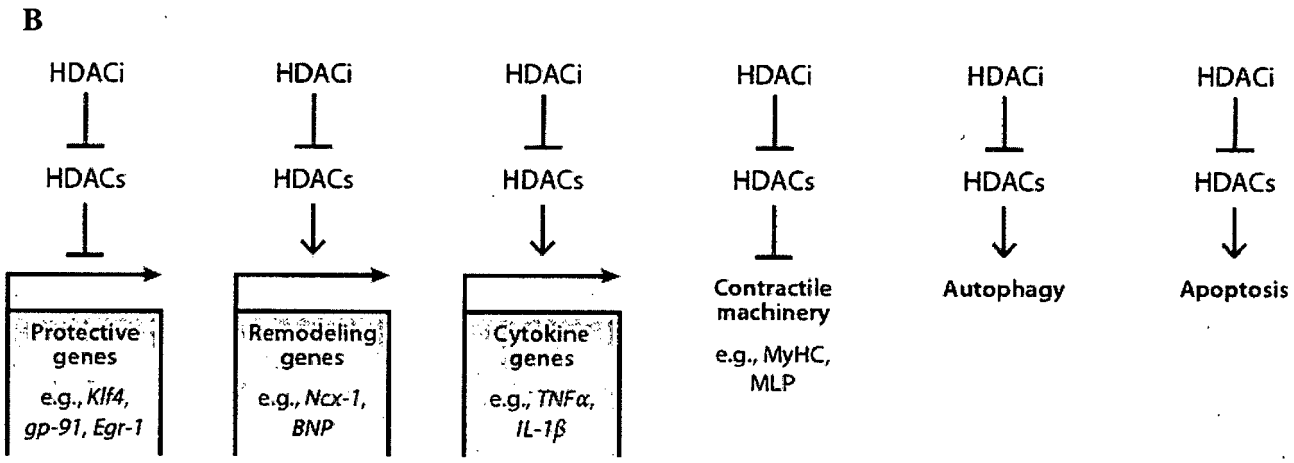

Combinatorial drug-gene therapeutics
TrafEN™
ChemoRe-router
Microtubule-targeting Chemosensitizers
Gene

FIG. 3


A
TBA
Ac
Ac
Ac
HDAC6/Sirtuin2


B
HDACi
HDACs
Protective genes
e.g., Klf4, gp-91, Egr-1
HDACi
HDACs
Remodeling genes
e.g., Ncx-1, BNP
HDACi
HDACs
Cytokine genes
e.g., TNFα, IL-1β
HDACi
HDACs
Contractile machinery
e.g., MyHC, MLP
HDACi
HDACs
Autophagy
HDACi
HDACs
Apoptosis

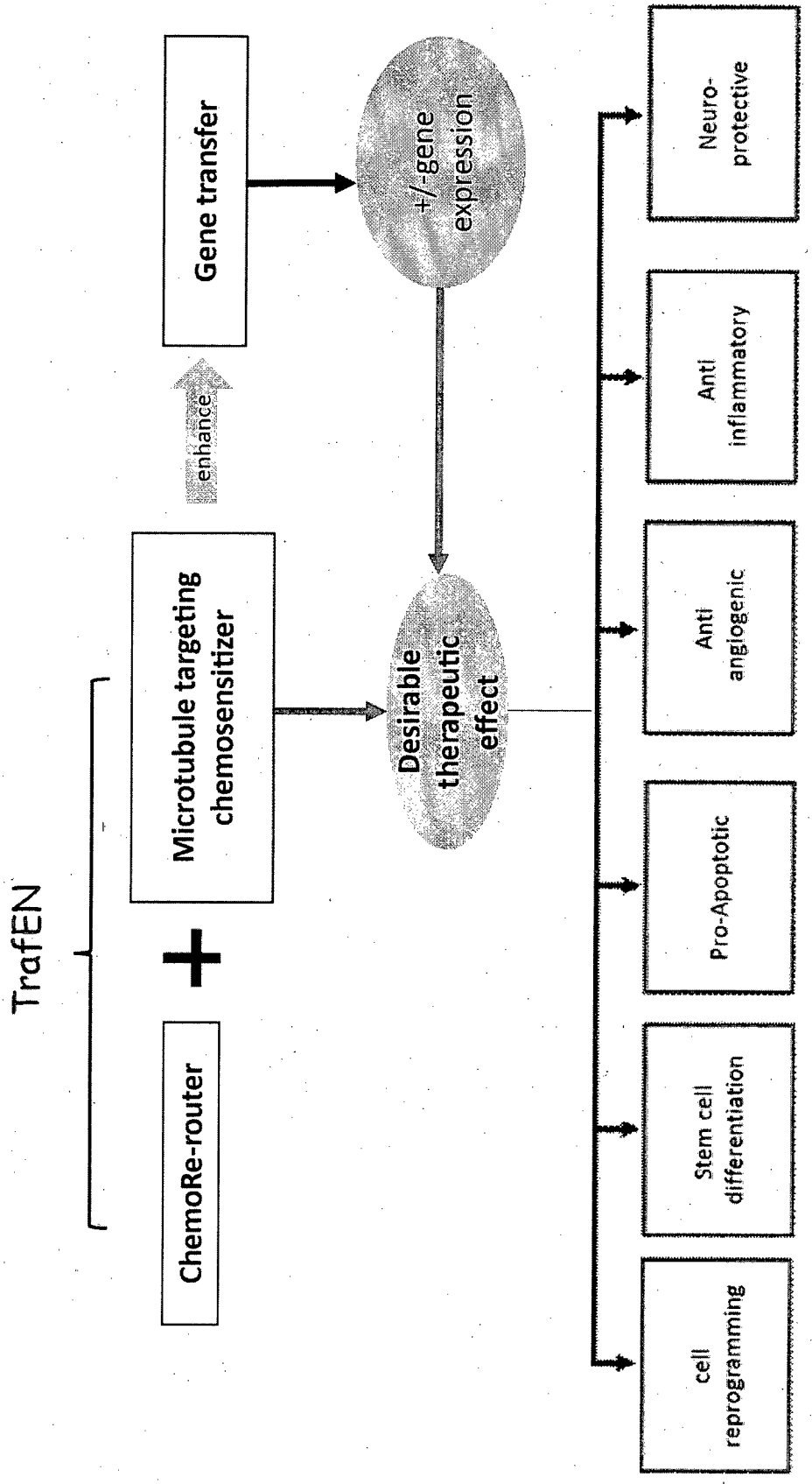
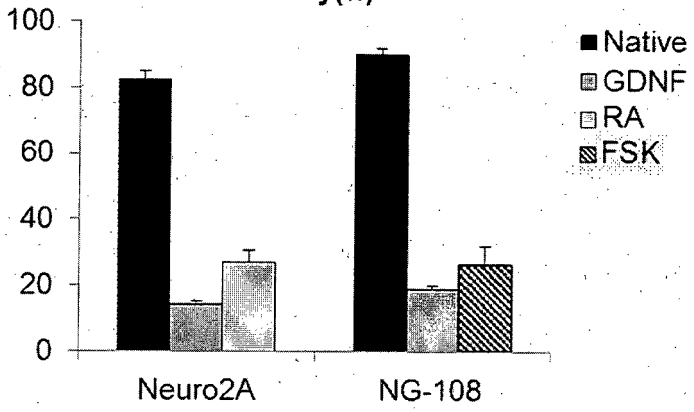

TrafEN
ChemoRe-router
+
Microtubule targeting chemosensitizer
enhance
Gene transfer
+/-gene expression
Desirable therapeutic effect
cell reprogramming
Stem cell differentiation
Pro-Apoptotic
Anti angiogenic
Anti inflammatory
Neuro-protective

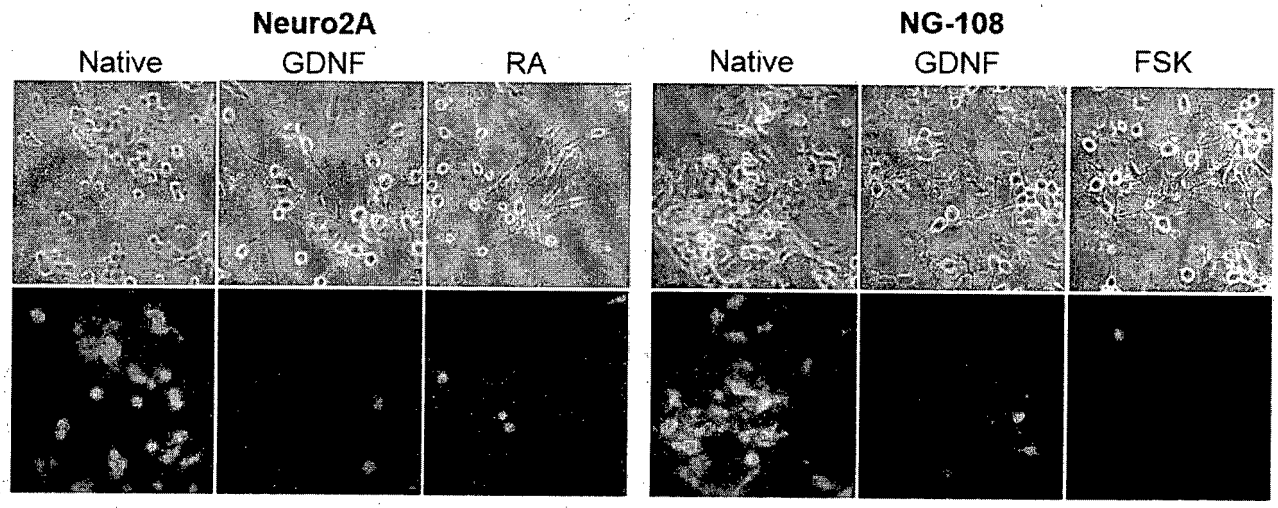
FIG. 4

FIG. 5

a.


Transfection Efficiency(%)
100
80
60
40
20
0
Native
GDNF
RA
FSK
Neuro2A
NG-108

b.


Neuro2A
Native
GDNF
RA
NG-108
Native
GDNF
FSK

c.

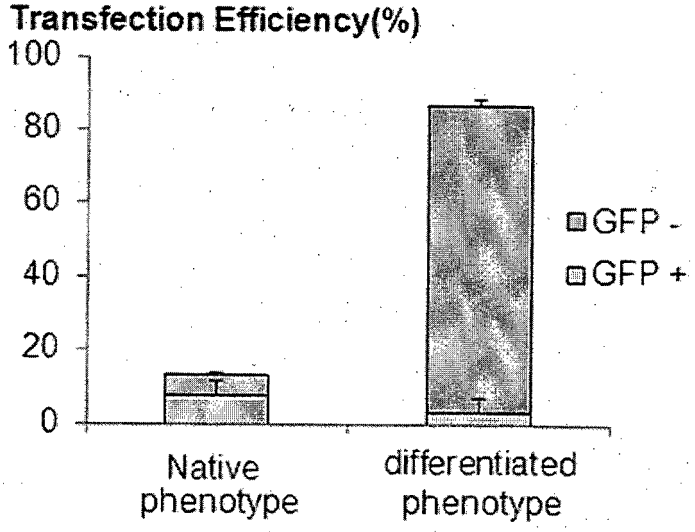
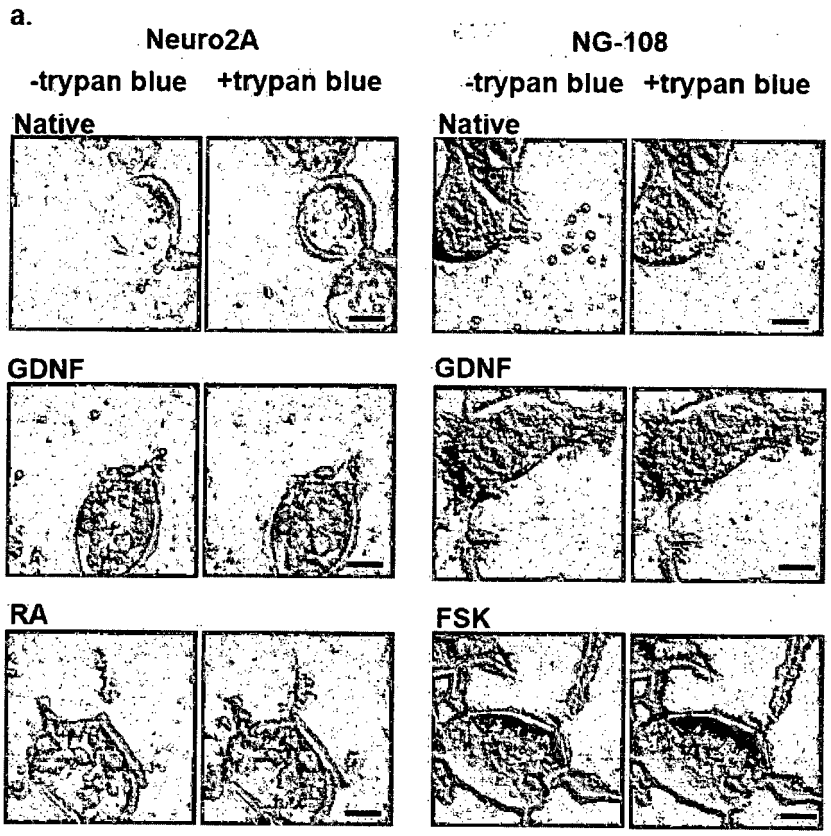

Transfection Efficiency(%)
100
80
60
40
20
0
GFP -
GFP +
Native phenotype
differentiated phenotype

FIG. 6


a.
Neuro2A
-trypan blue
+trypan blue
NG-108
-trypan blue
+trypan blue
Native
GDNF
RA
Native
GDNF
FSK

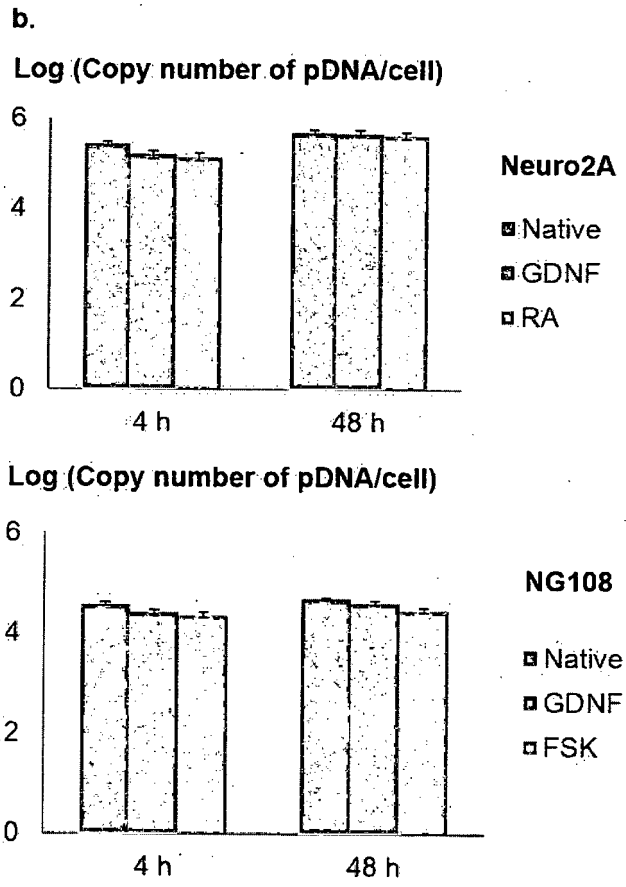

b.
Log (Copy number of pDNA/cell)
6
4
2
0
4 h
48 h
Neuro2A
Native
GDNF
RA
Log (Copy number of pDNA/cell)
6
4
2
0
4 h
48 h
NG108
Native
GDNF
FSK

FIG. 7

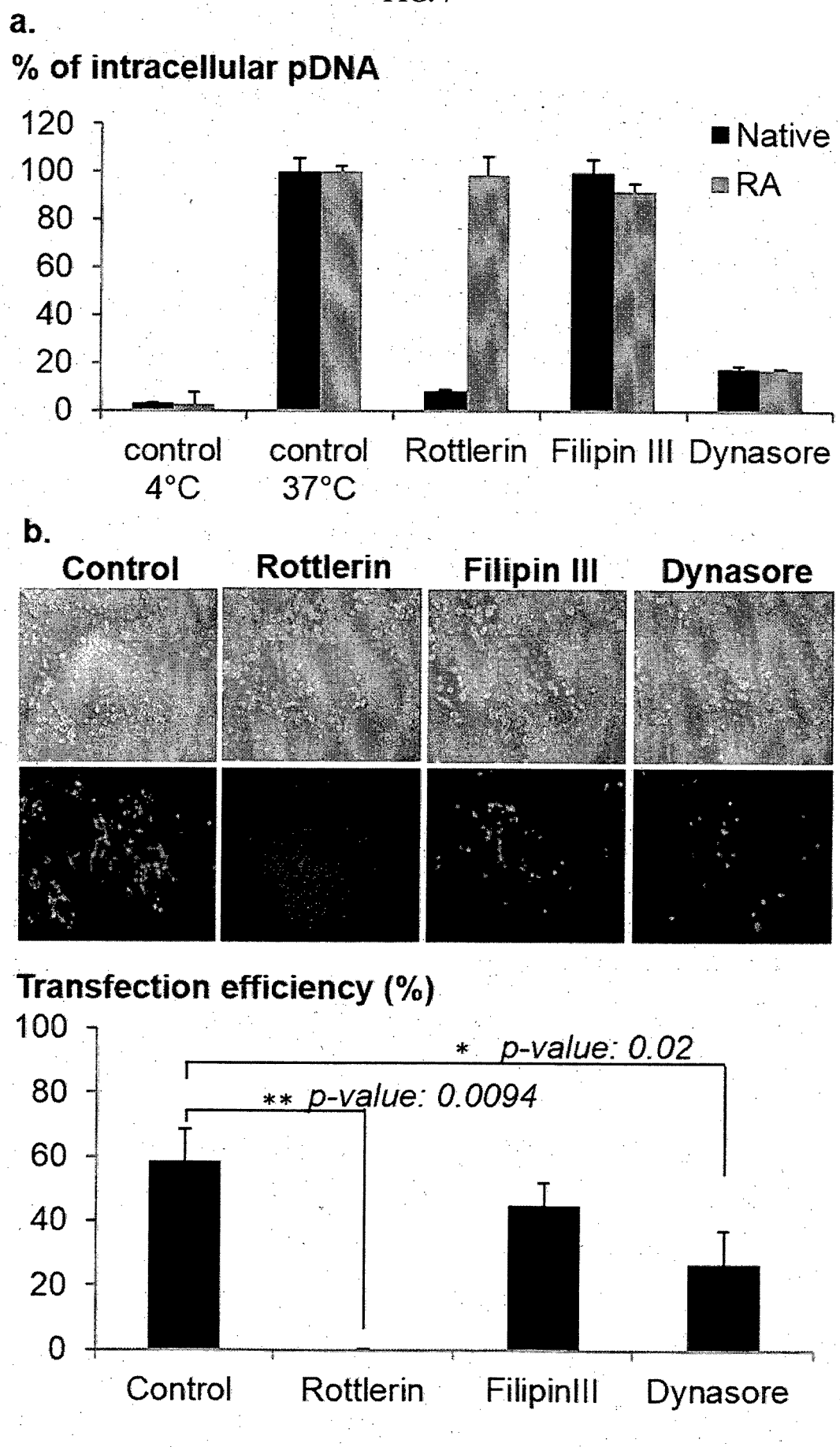
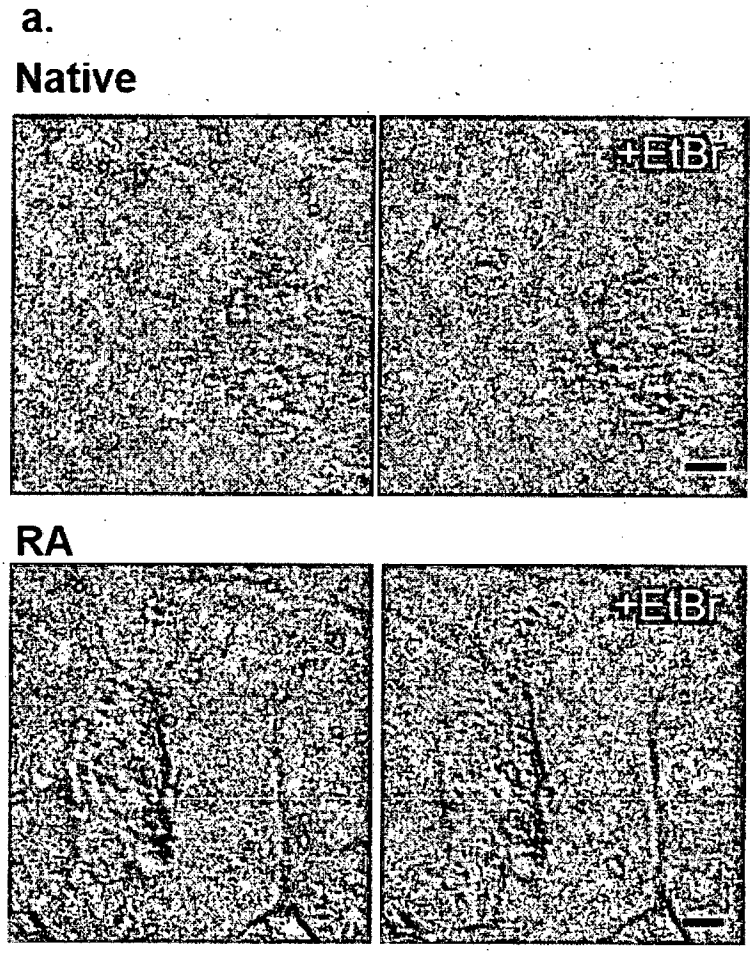

a.
% of intracellular pDNA
120
100
80
60
40
20
0
Native
RA
control 4°C
control 37°C
Rottlerin
Filipin III
Dynasore
b.
Control
Rottlerin
Filipin III
Dynasore
Transfection efficiency (%)
100
80
60
40
20
0
* p-value: 0.02
** p-value: 0.0094
Control
Rottlerin
FilipinIII
Dynasore

FIG. 8


a.
Native
+EtBr
RA
+EtBr

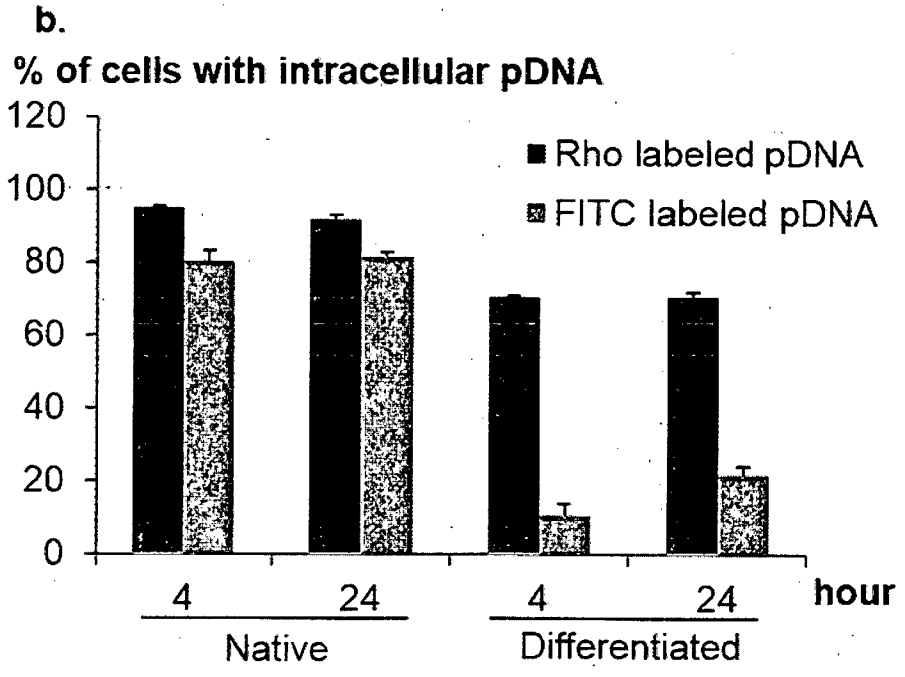

b.
% of cells with intracellular pDNA
120
100
80
60
40
20
0
Rho labeled pDNA
FITC labeled pDNA
4
24
4
24
hour
Native
Differentiated

FIG. 8 continued

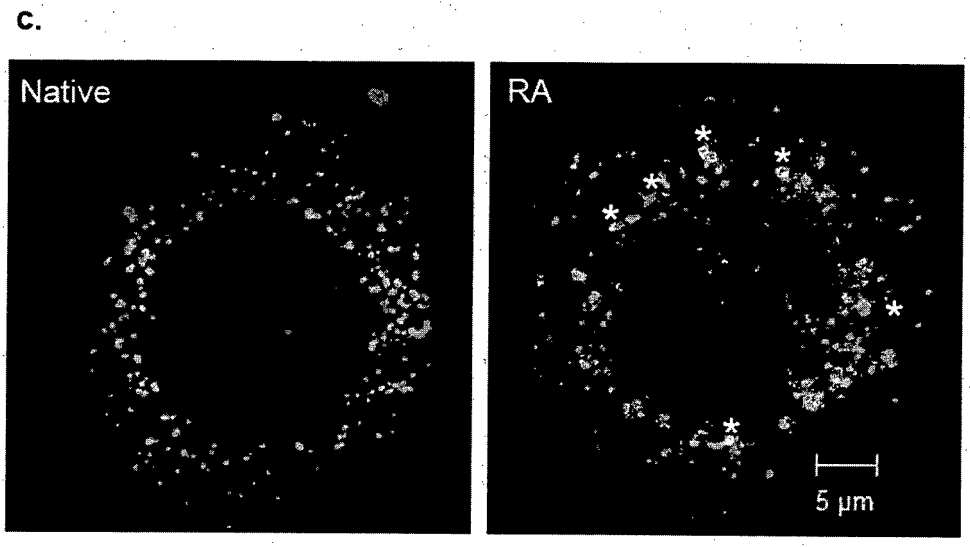


FIG. 9

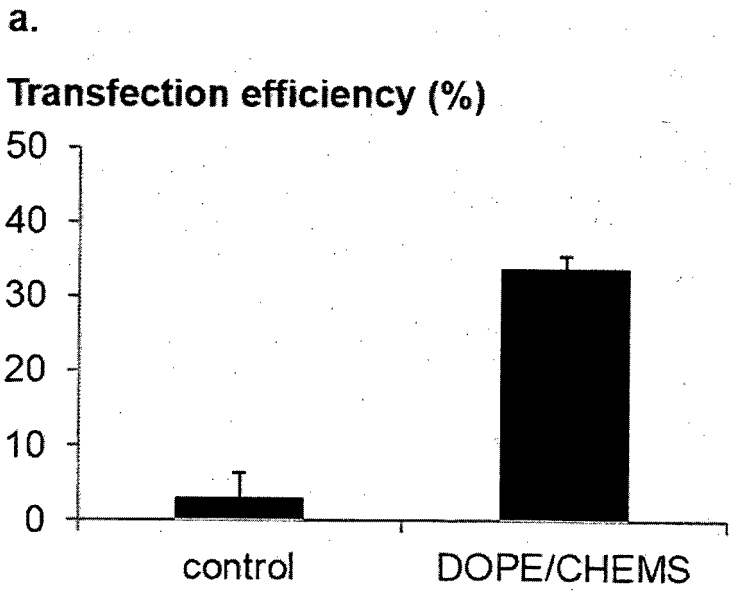
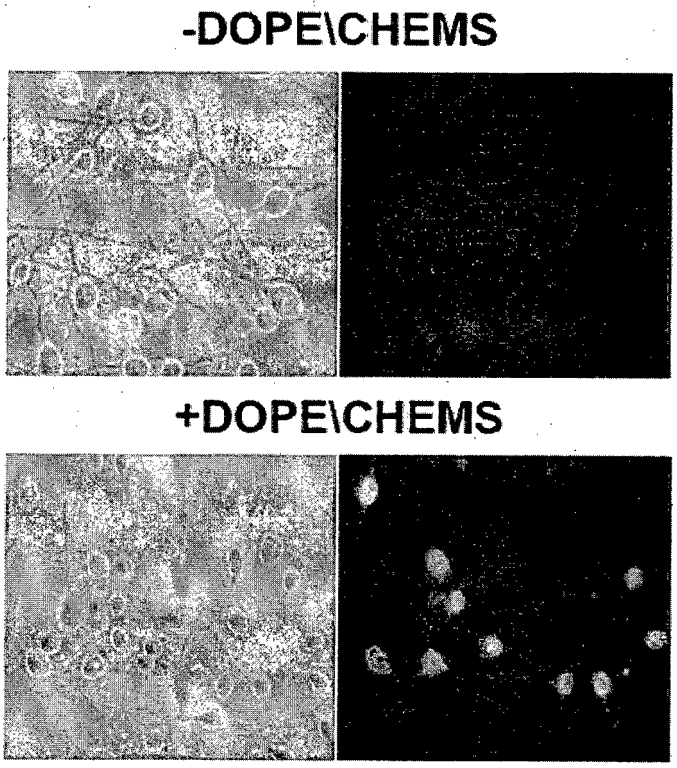
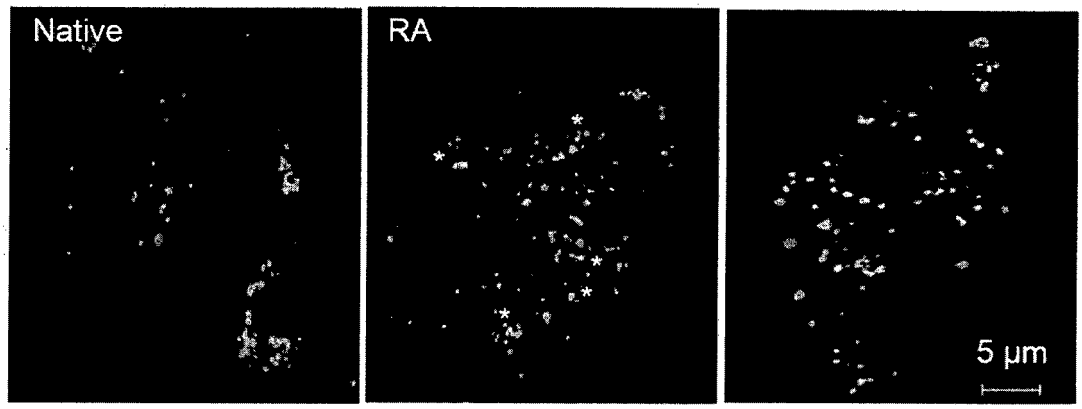


FIG. 9 continued

b.



c.



d.

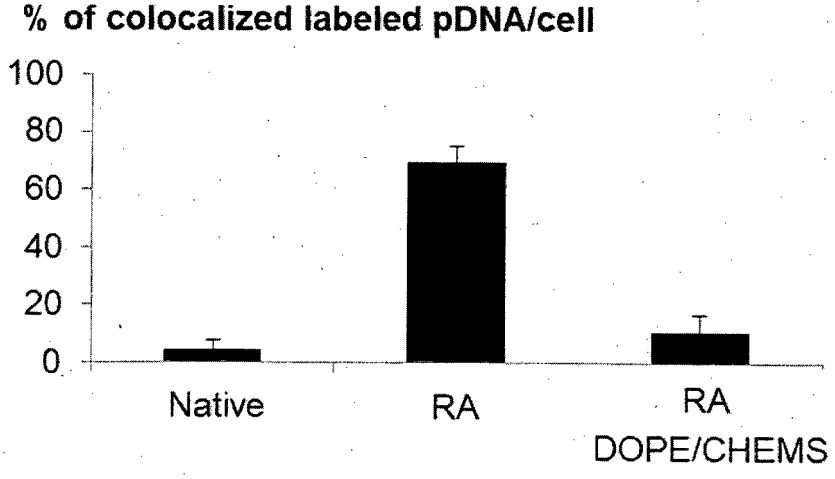


FIG. 10

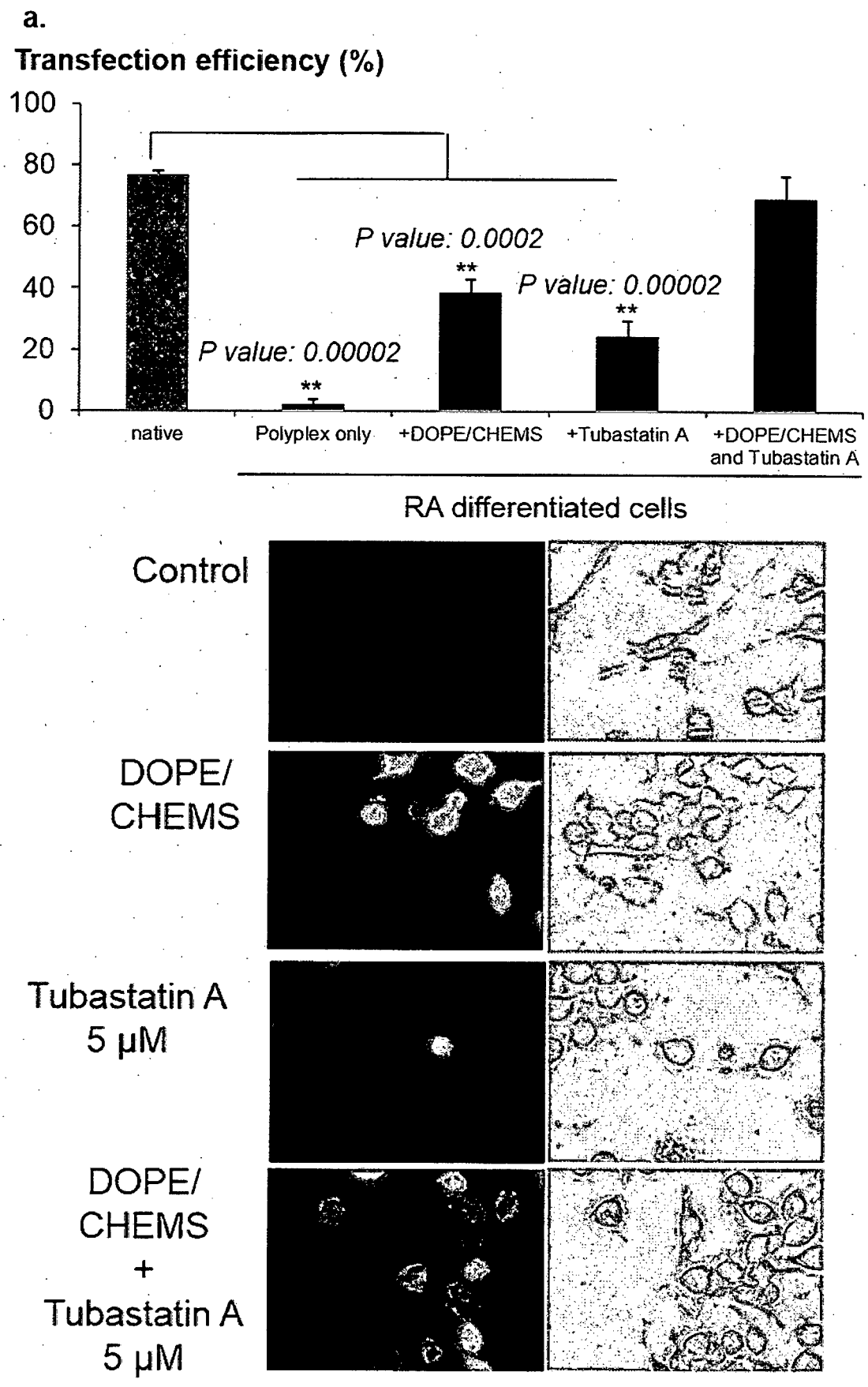

a.
Transfection efficiency (%)
100
80
60
40
20
0
P value: 0.0002
P value: 0.00002
P value: 0.00002
**
**
**
native
Polyplex only
+DOPE/CHEMS
+Tubastatin A
+DOPE/CHEMS and Tubastatin A
RA differentiated cells
Control
DOPE/ CHEMS
Tubastatin A 5 µM
DOPE/ CHEMS + Tubastatin A 5 µM

FIG. 10 continued

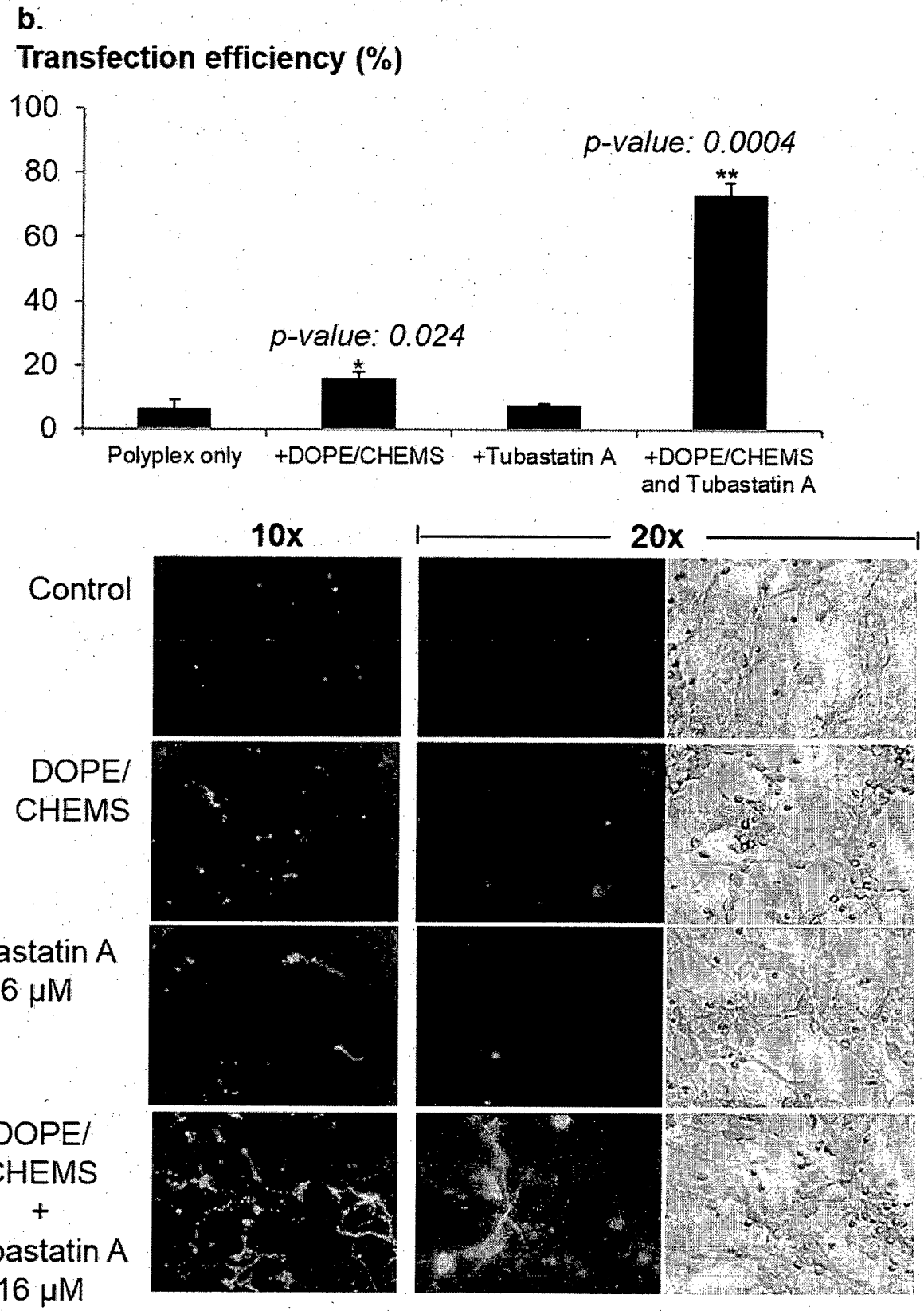


FIG.11

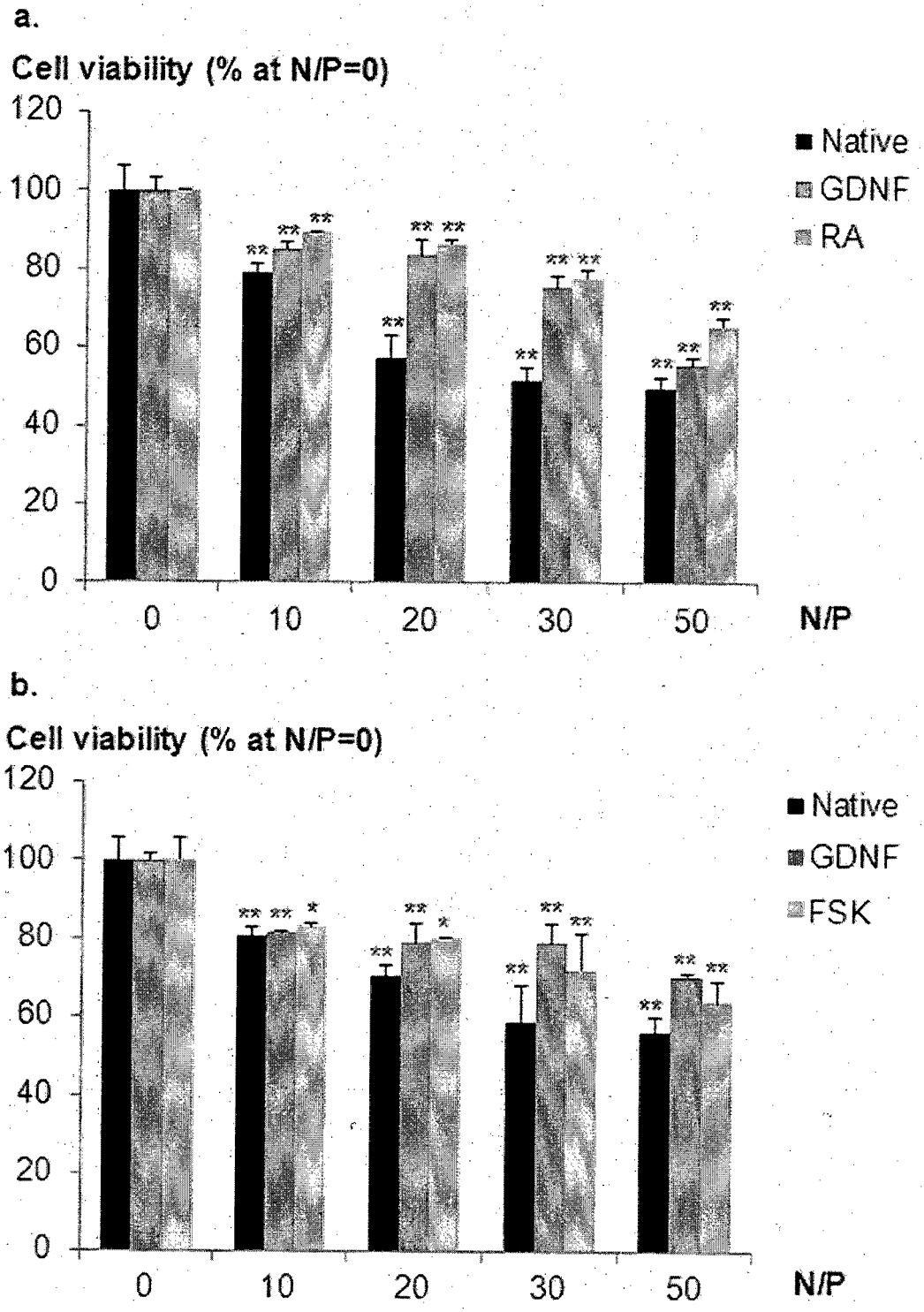

a.
Cell viability (% at N/P=0)
120
100
80
60
40
20
0
0
10
20
30
50
N/P
Native
GDNF
RA
b.
Cell viability (% at N/P=0)
120
100
80
60
40
20
0
0
10
20
30
50
N/P
Native
GDNF
FSK

FIG. 12

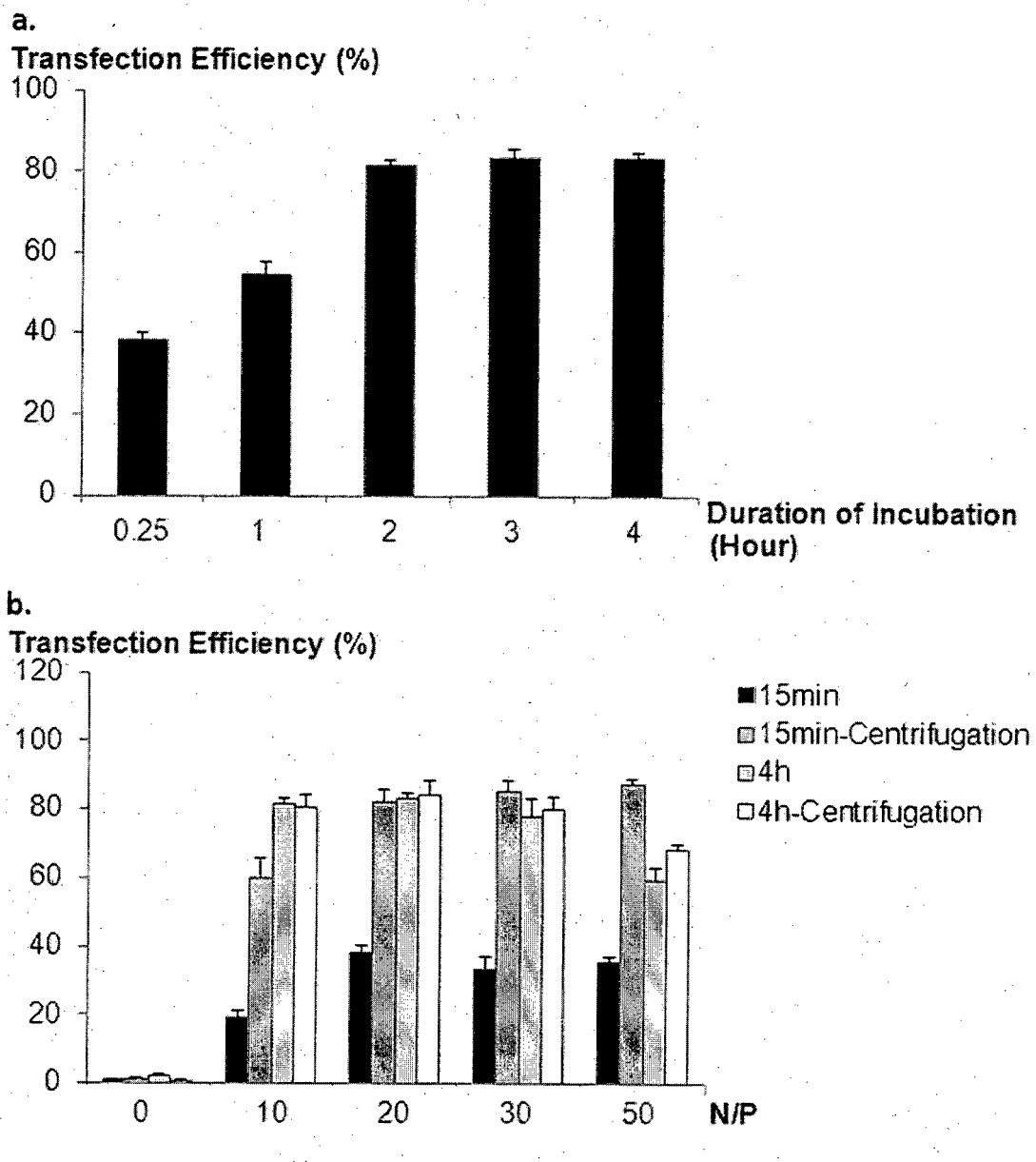

a.
Transfection Efficiency (%)
100
80
60
40
20
0
0.25
1
2
3
4
Duration of Incubation (Hour)
b.
Transfection Efficiency (%)
120
100
80
60
40
20
0
0
10
20
30
50
N/P
15min
15min-Centrifugation
4h
4h-Centrifugation

FIG. 13

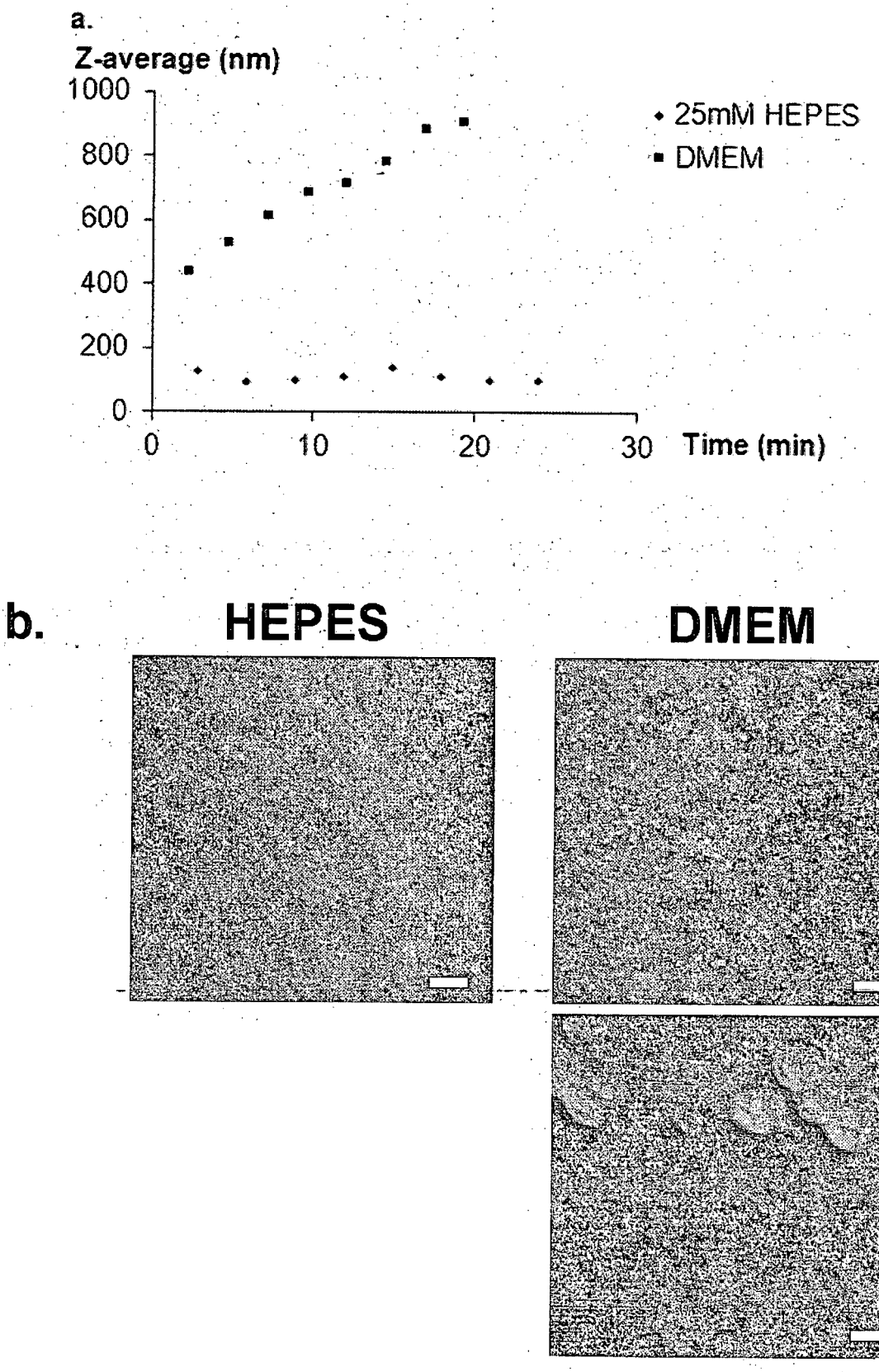
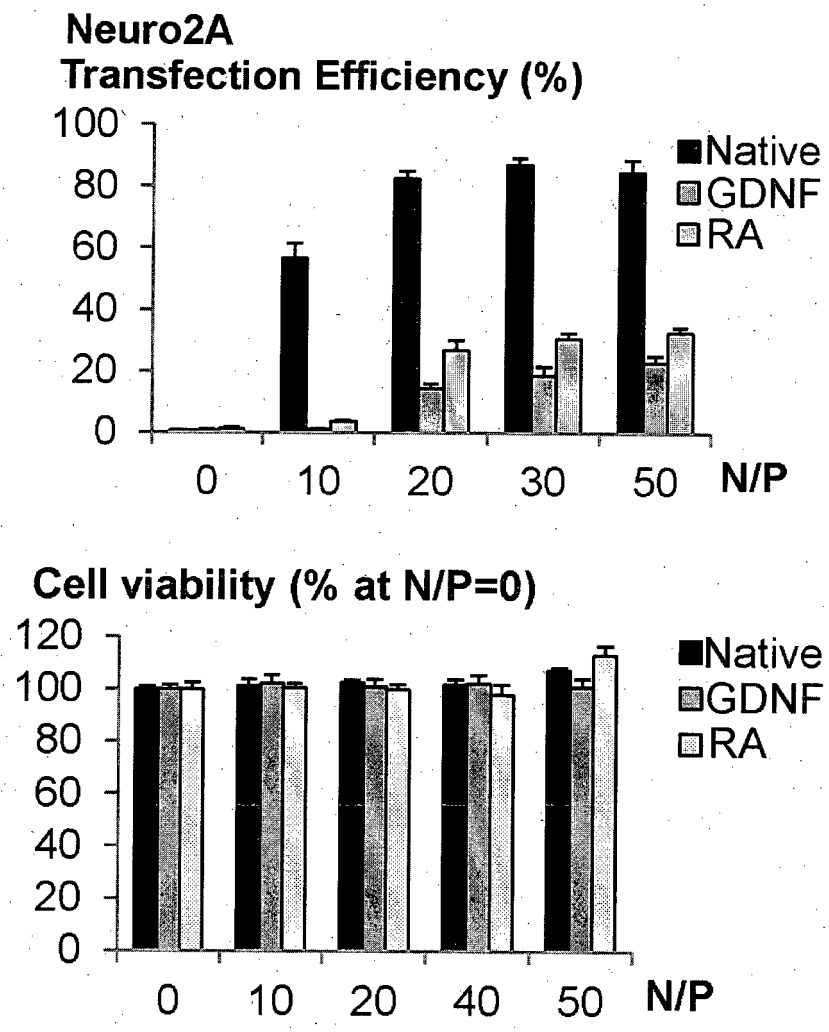

a.
Z-average (nm)
1000
800
600
400
200
0
0
10
20
30
Time (min)
25mM HEPES
DMEM
b.
HEPES
DMEM

FIG. 14

a.


Neuro2A
Transfection Efficiency (%)
100
80
60
40
20
0
0
10
20
30
50
N/P
Native
GDNF
RA
Cell viability (% at N/P=0)
120
100
80
60
40
20
0
0
10
20
40
50
N/P
Native
GDNF
RA

b.

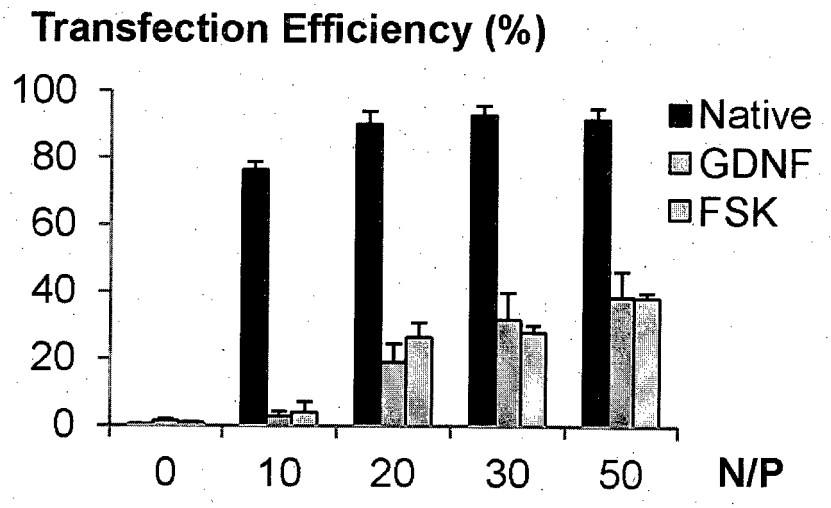

NG-108
Transfection Efficiency (%)
100
80
60
40
20
0
0
10
20
30
50
N/P
Native
GDNF
FSK

FIG. 14 continued

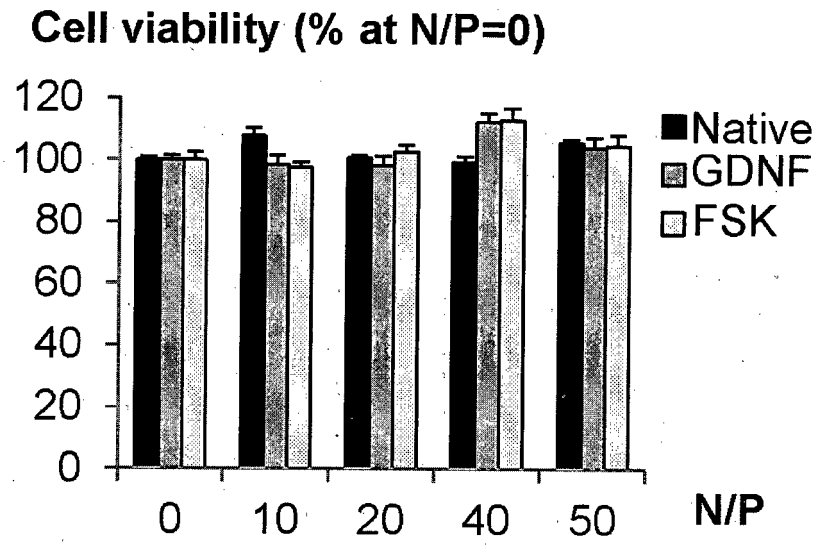


FIG. 15

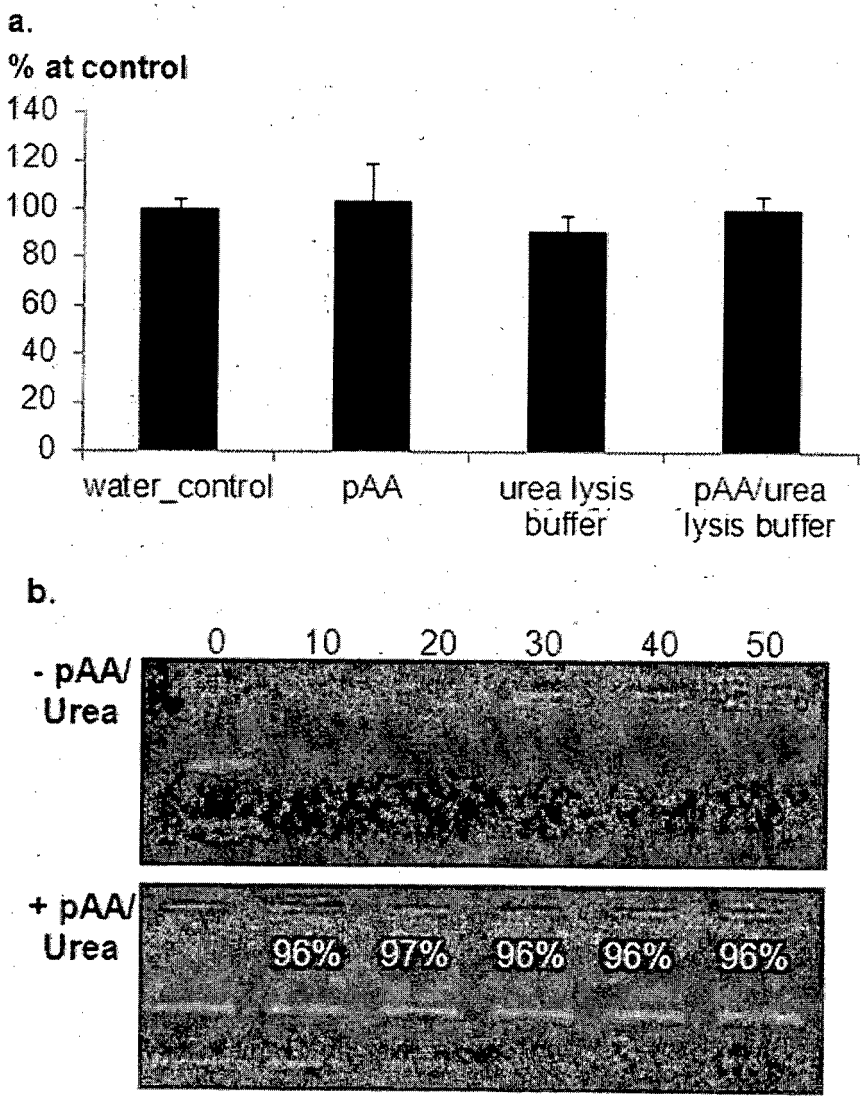


FIG. 15 continued

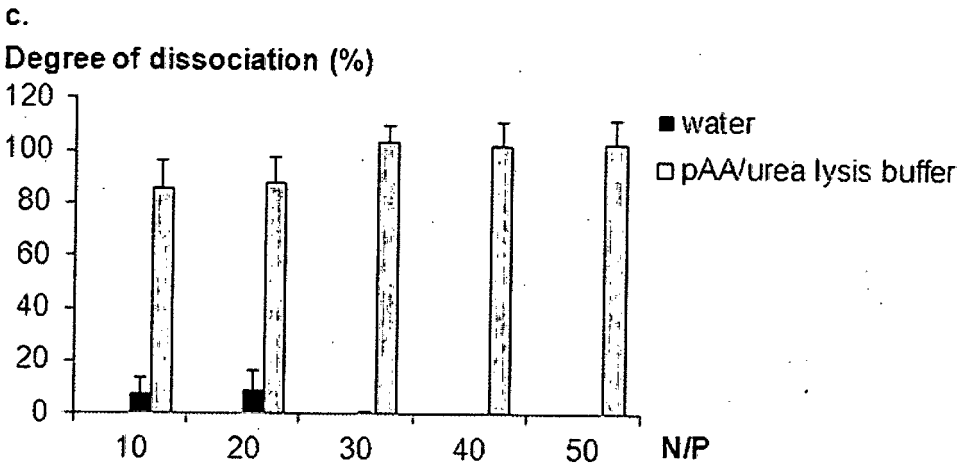


FIG. 16

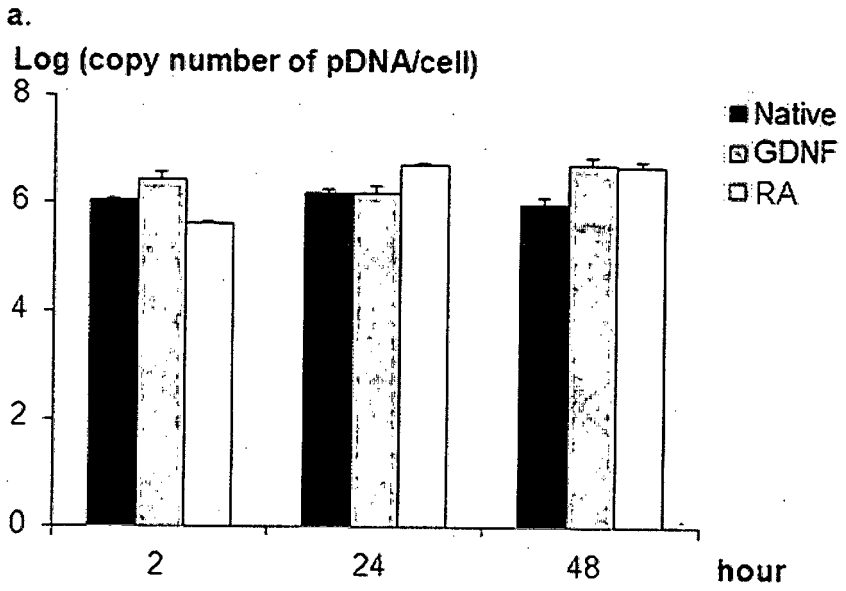


FIG. 16 continued

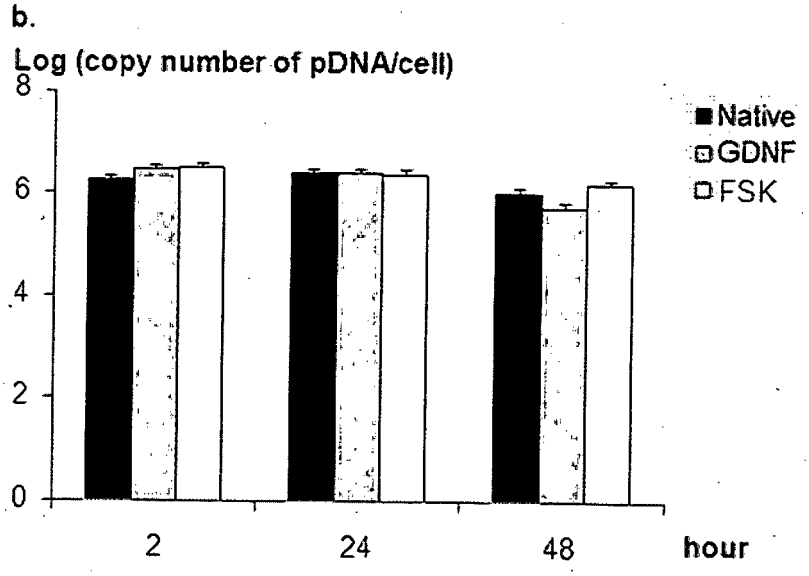


FIG. 17

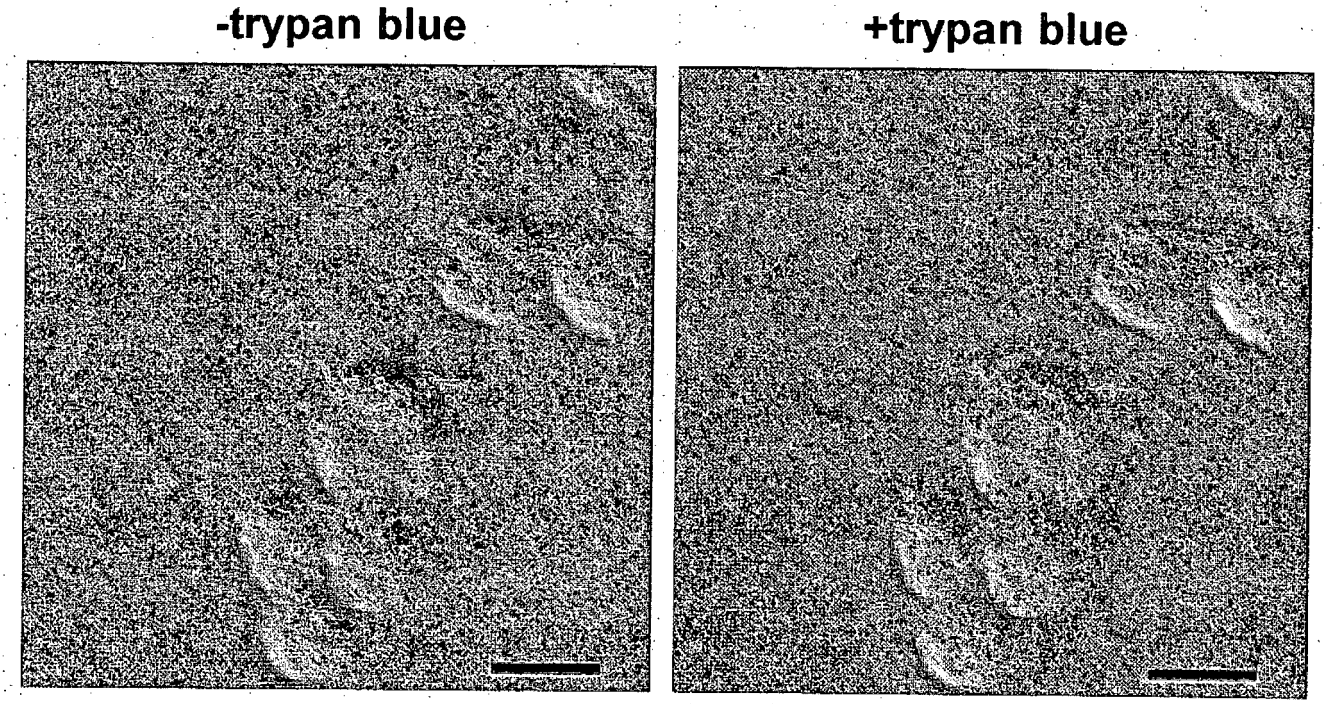

-trypan blue
+trypan blue

FIG. 18

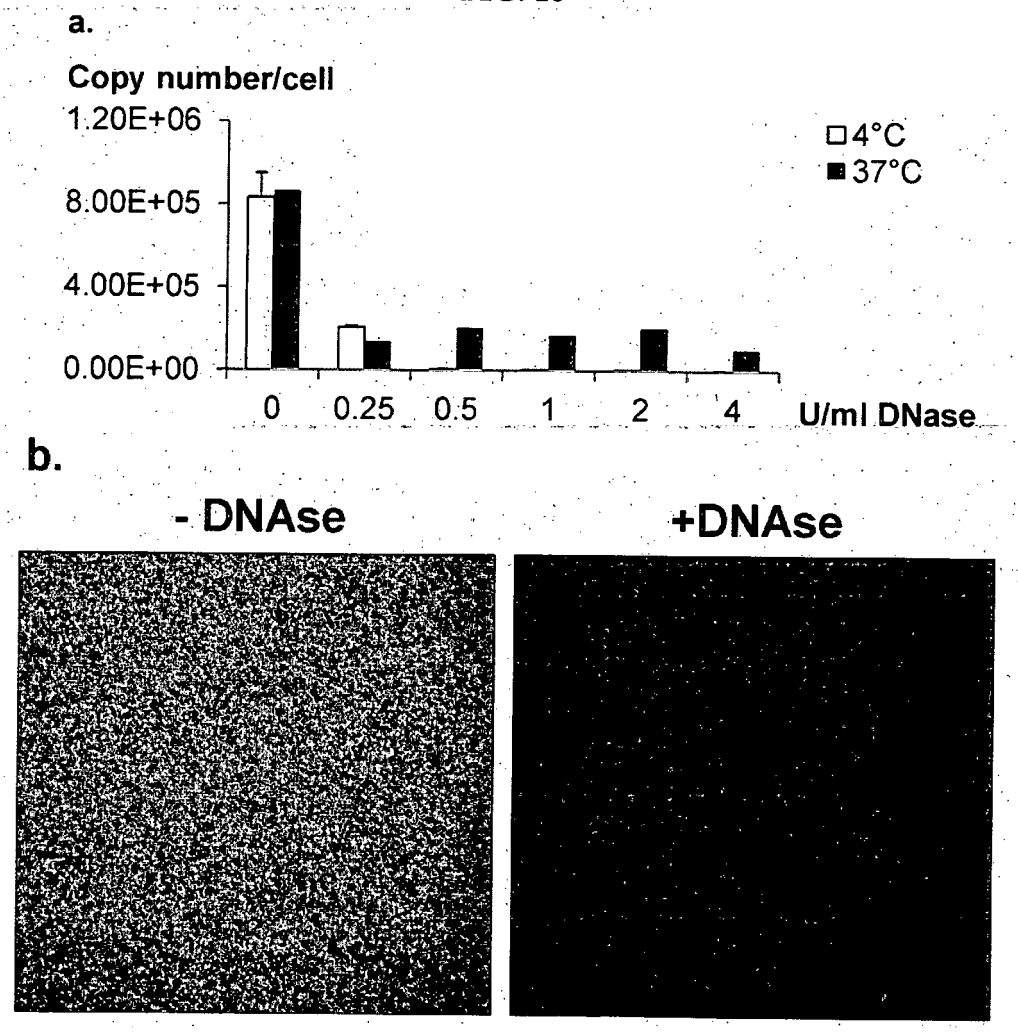

a.
Copy number/cell
1.20E+06
8.00E+05
4.00E+05
0.00E+00
4°C
37°C
0
0.25
0.5
1
2
4
U/ml DNase
b.
- DNAse
+DNAse

FIG. 19

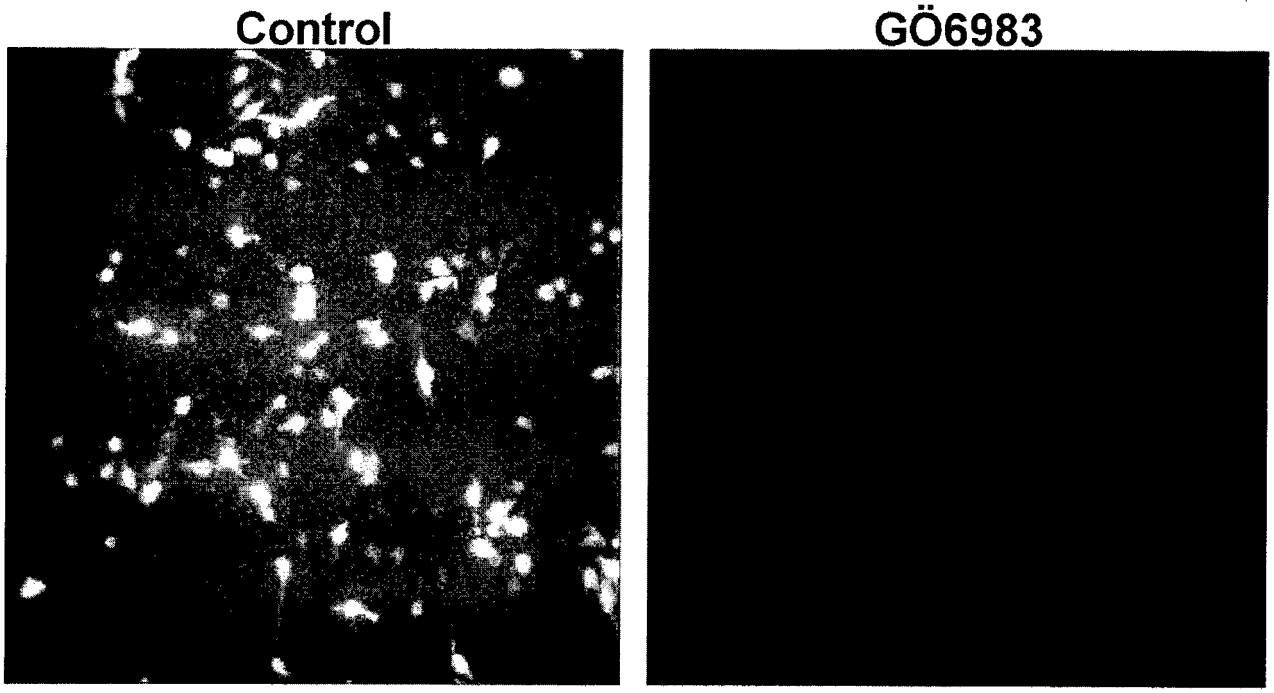

Control
GÖ6983

FIG. 20

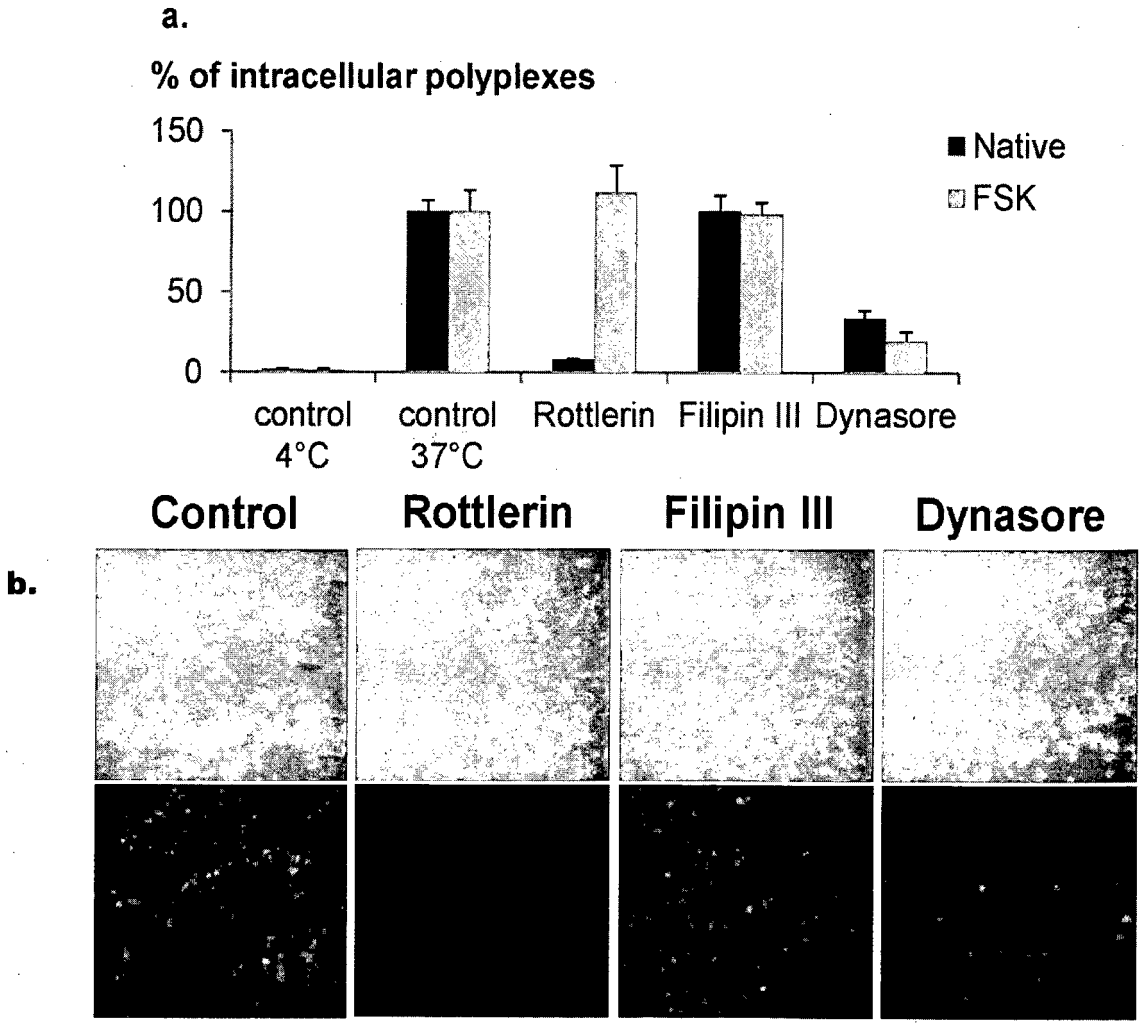

a.
% of intracellular polyplexes
150
100
50
0
Native
FSK
control 4°C
control 37°C
Rottlerin
Filipin III
Dynasore
b.
Control
Rottlerin
Filipin III
Dynasore

FIG. 20 continued

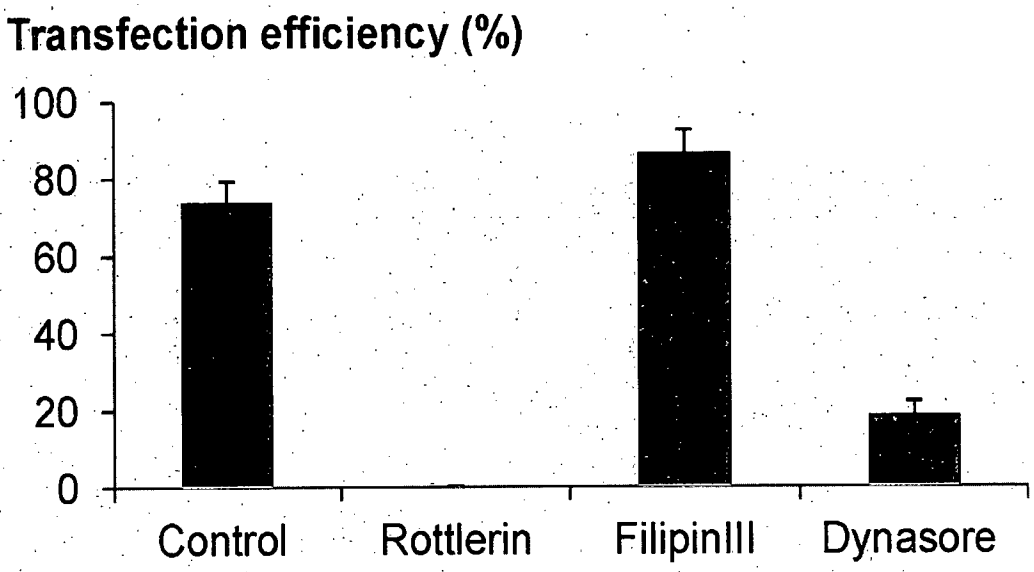


FIG. 21

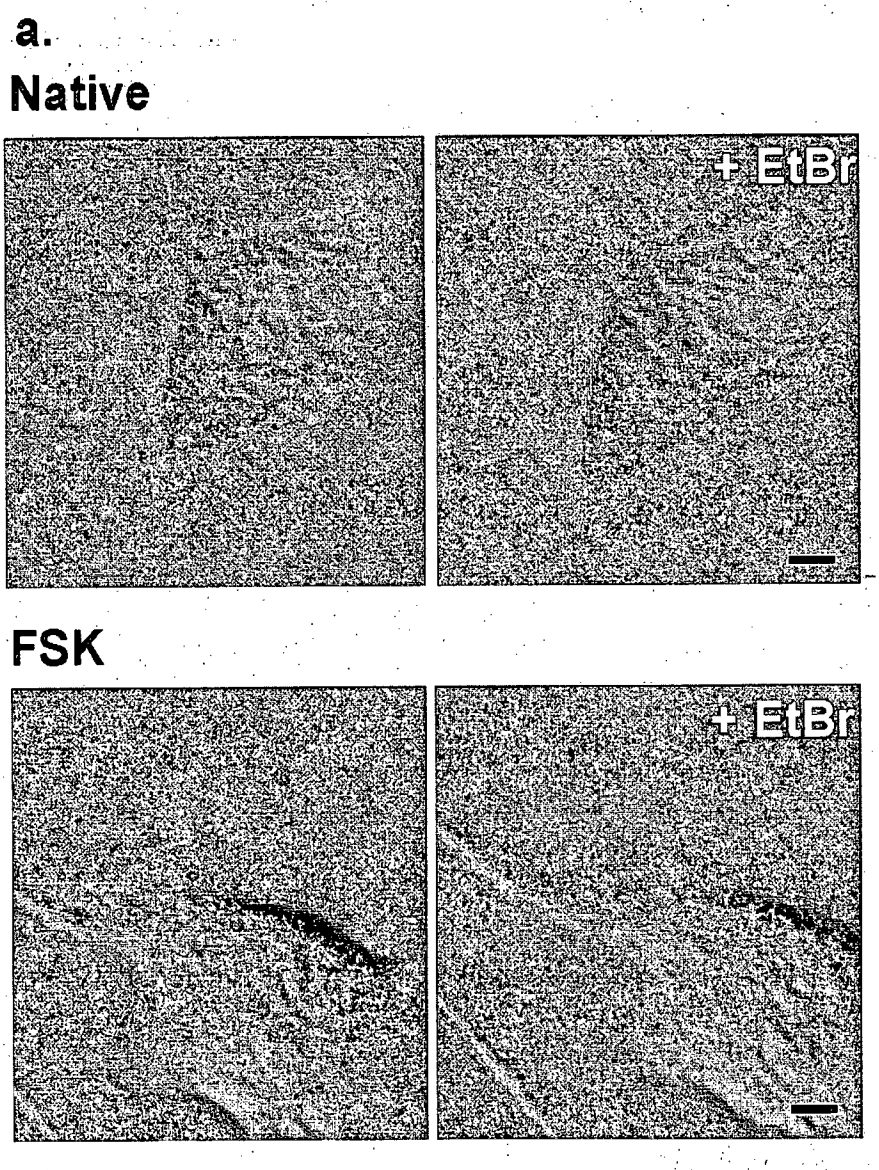


FIG. 21 continued

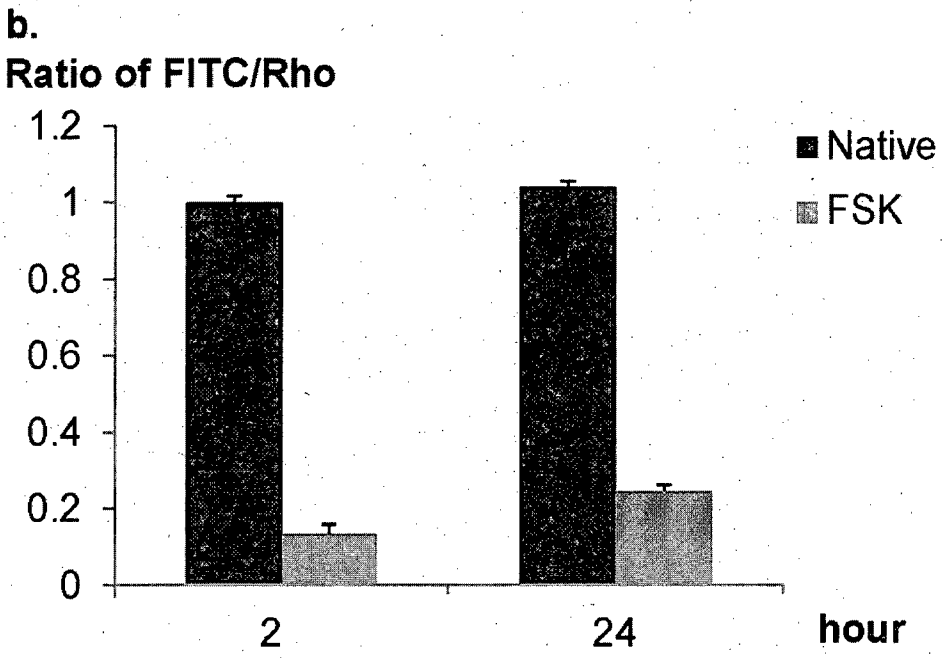
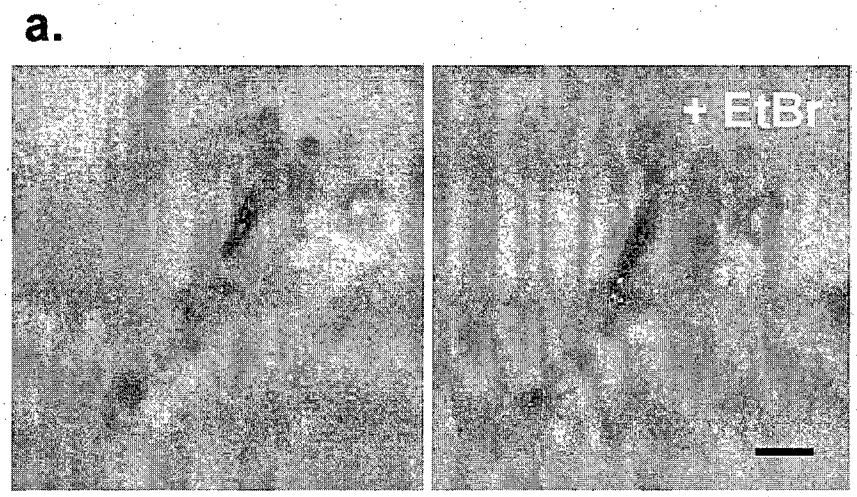


FIG. 22



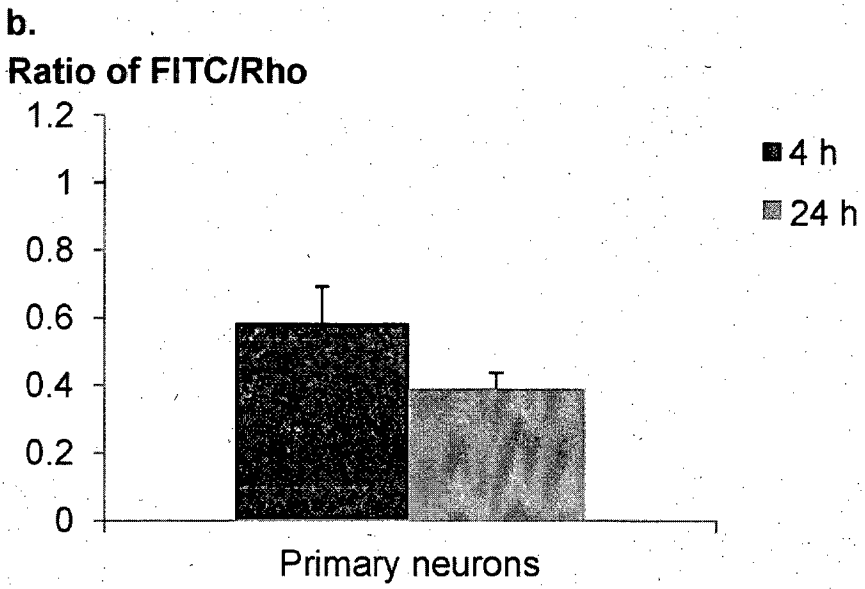


FIG. 23

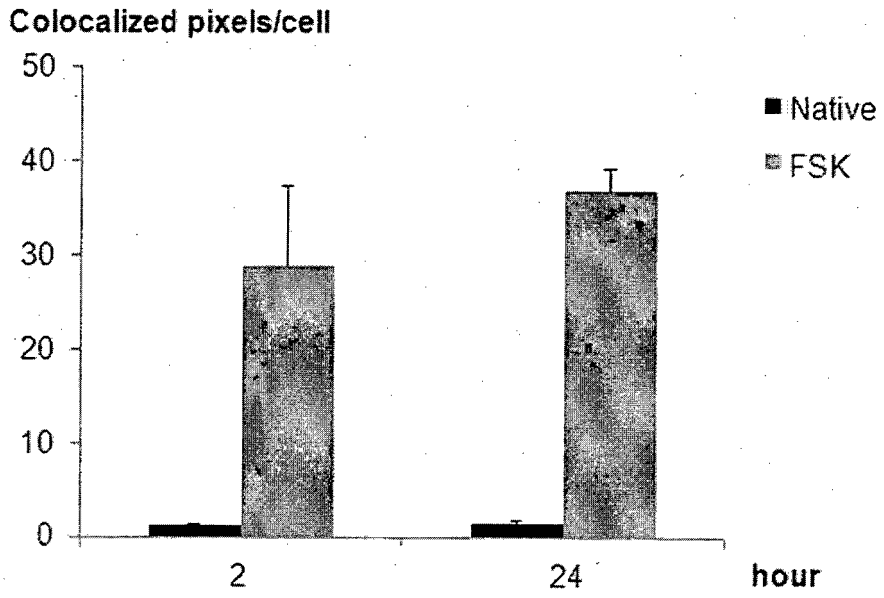

Colocalized pixels/cell
50
40
30
20
10
0
2
24
hour
Native
FSK

FIG. 24

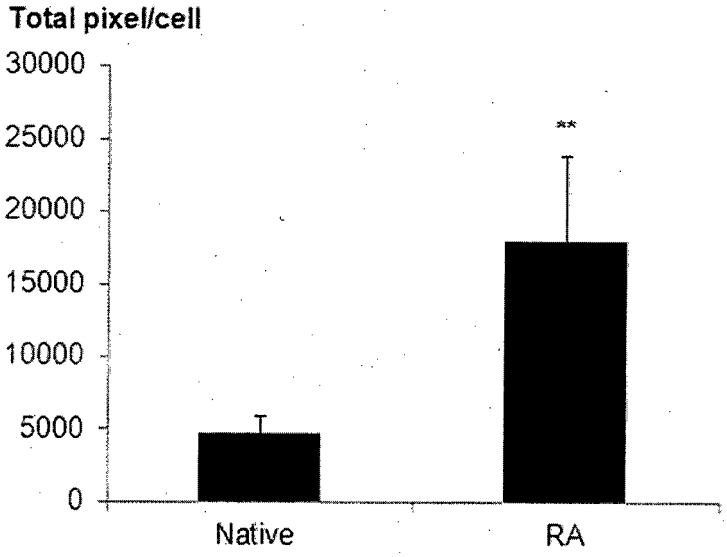

Total pixel/cell
30000
25000
20000
15000
10000
5000
0
**
Native
RA

FIG. 25

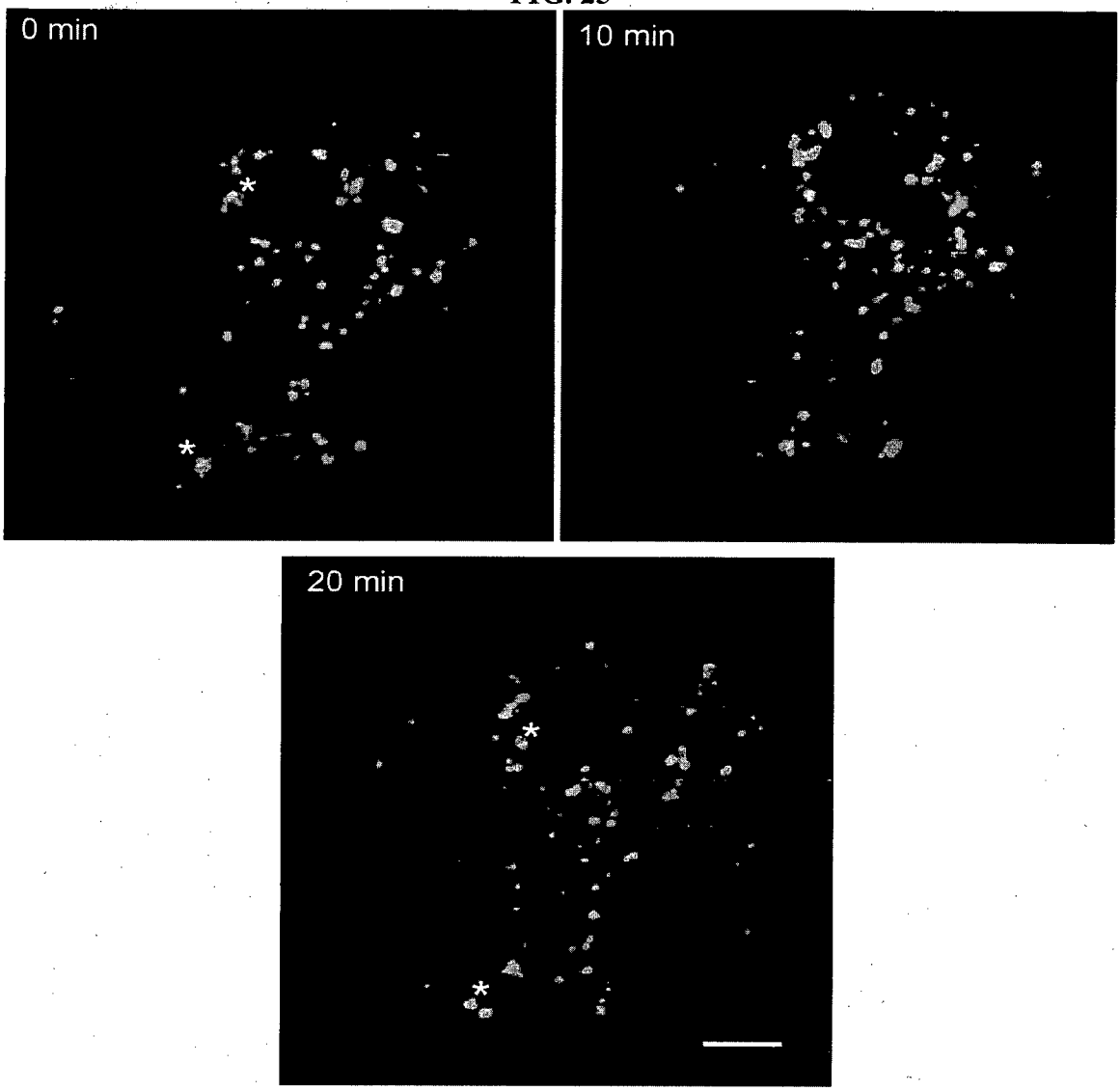

0 min
10 min
20 min

FIG. 26

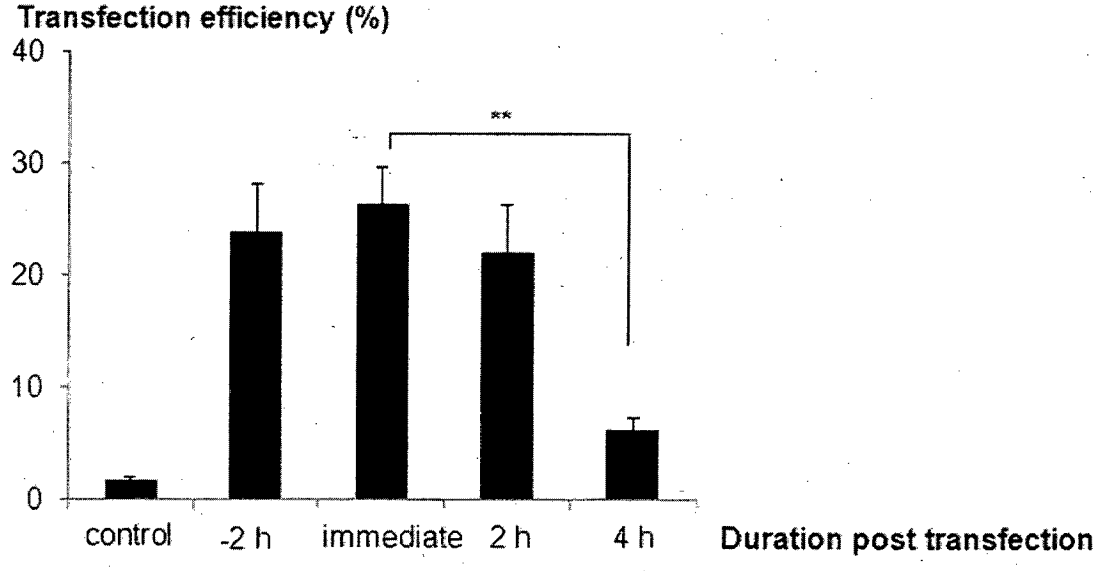

Transfection efficiency (%)
40
30
20
10
0
**
control
-2 h
immediate
2 h
4 h
Duration post transfection

FIG. 27

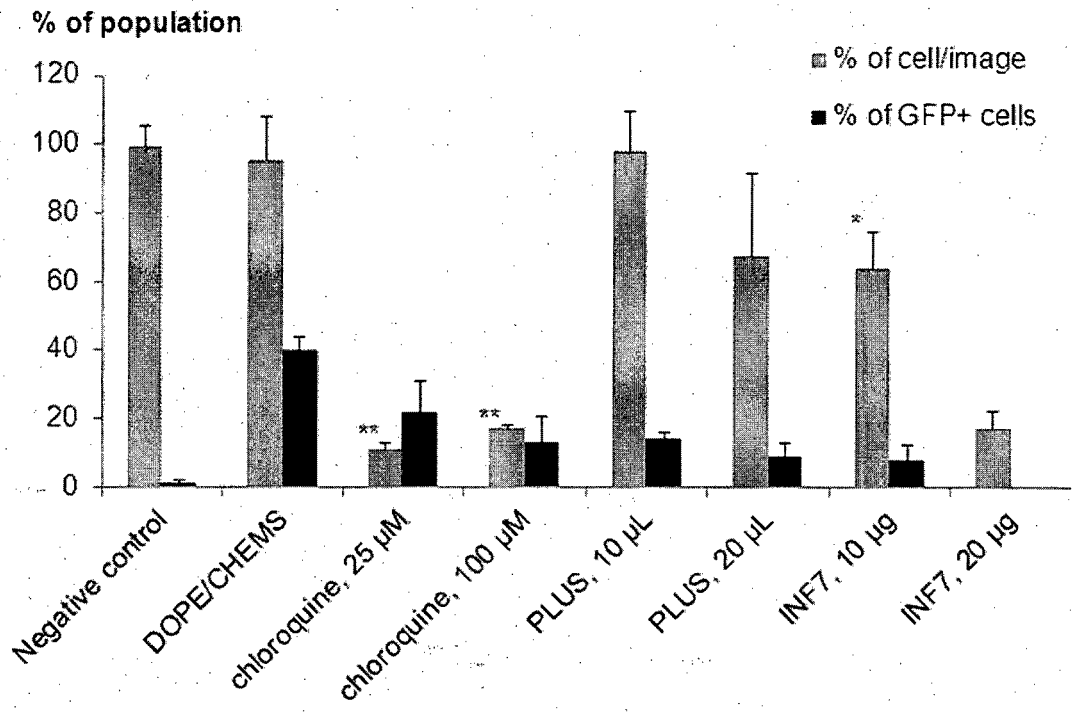

% of population
% of cell/image
% of GFP+ cells
120
100
80
60
40
20
0
**
**
*
Negative control
DOPE/CHEMS
chloroquine, 25 µM
chloroquine, 100 µM
PLUS, 10 µL
PLUS, 20 µL
INF7, 10 µg
INF7, 20 µg

FIG. 28

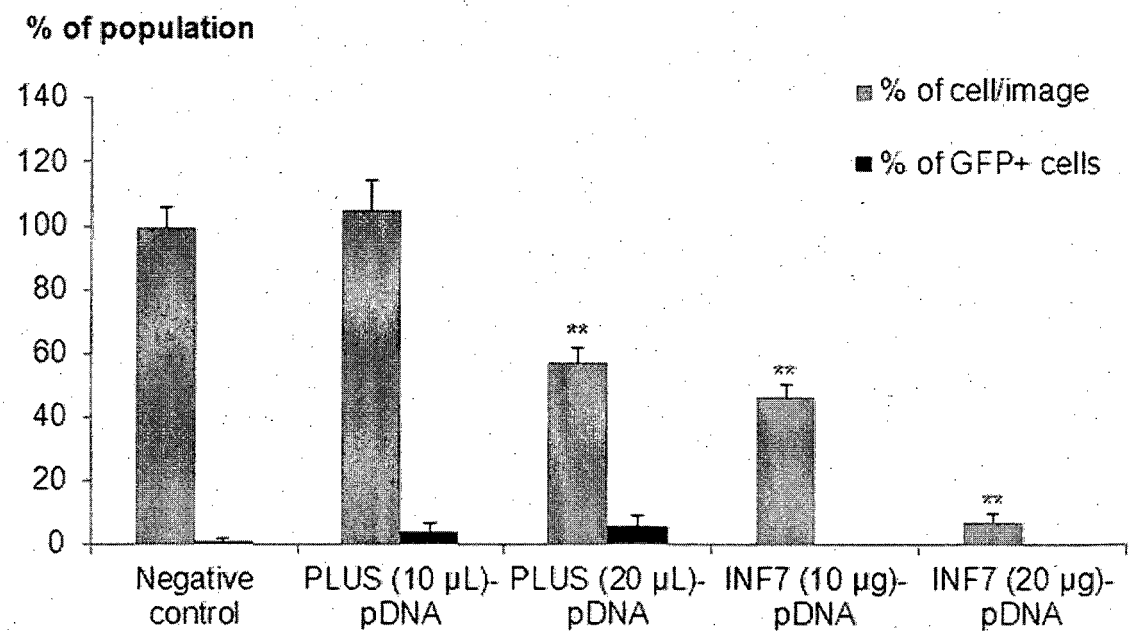

% of population
% of cell/image
% of GFP+ cells
140
120
100
80
60
40
20
0
**
**
**
Negative control
PLUS (10 µL)-pDNA
PLUS (20 µL)-pDNA
INF7 (10 µg)-pDNA
INF7 (20 µg)-pDNA

FIG. 29

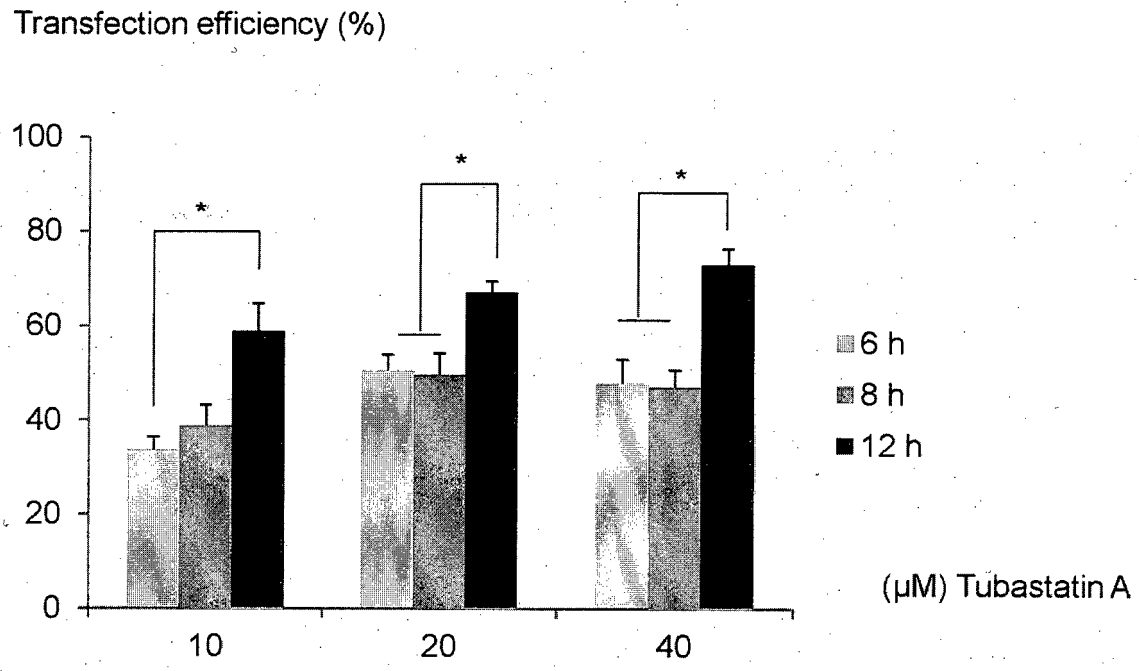

Transfection efficiency (%)
100
80
60
40
20
0
*
*
*
10
20
40
6 h
8 h
12 h
(μM) Tubastatin A

FIG. 30

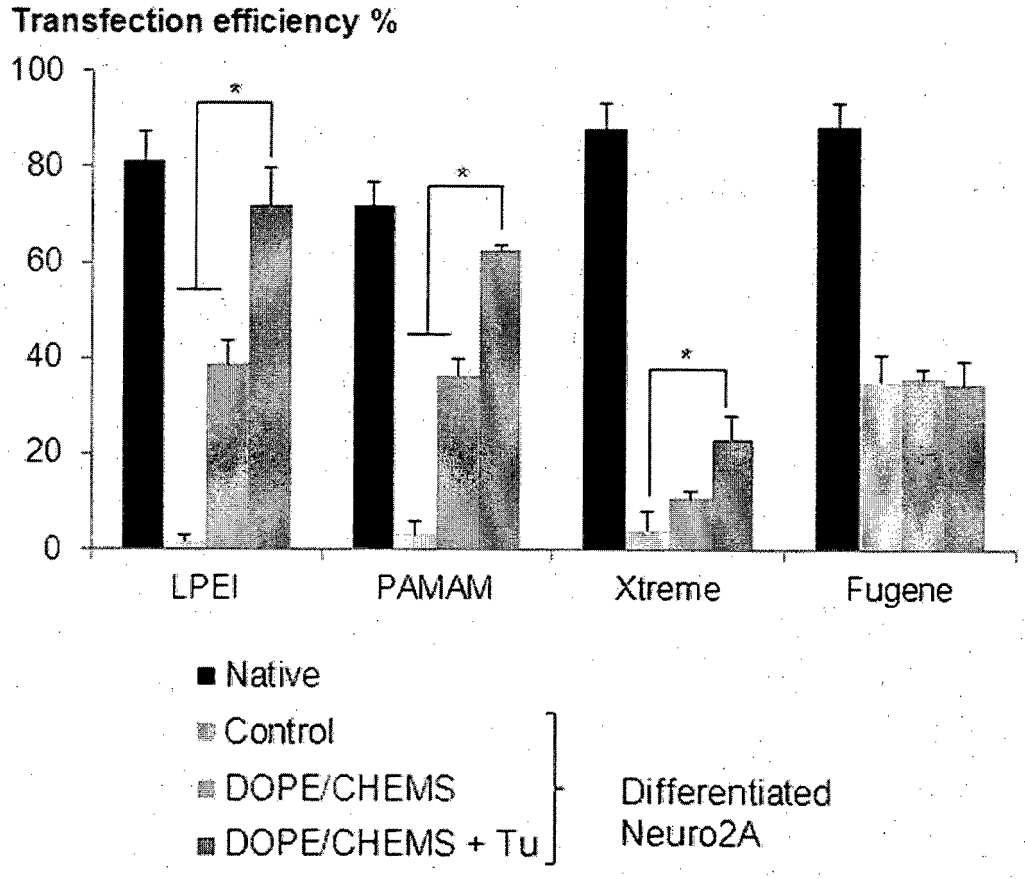

Transfection efficiency %
100
80
60
40
20
0
*
*
*
LPEI
PAMAM
Xtreme
Fugene
Native
Control
DOPE/CHEMS
DOPE/CHEMS + Tu
Differentiated
Neuro2A

FIG. 31

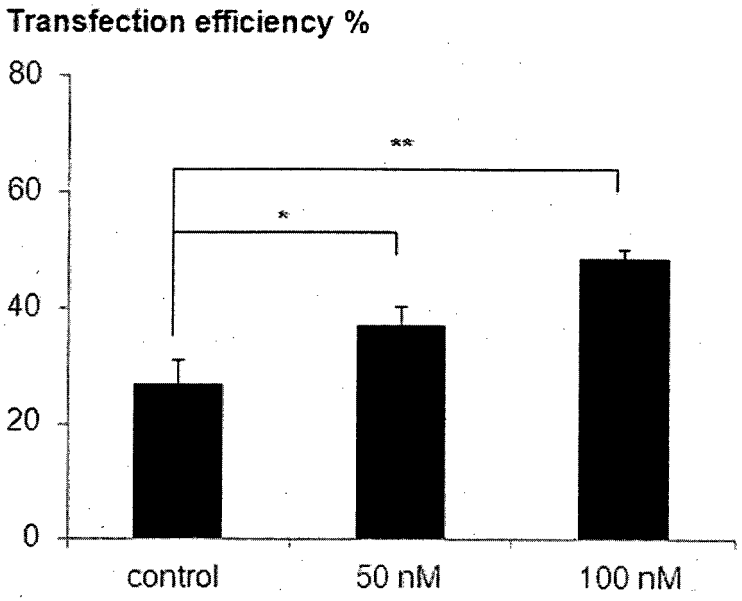
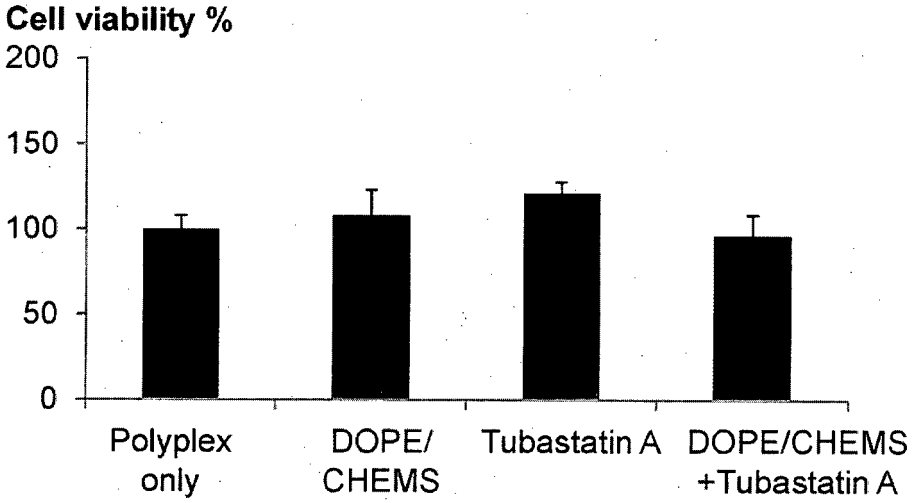
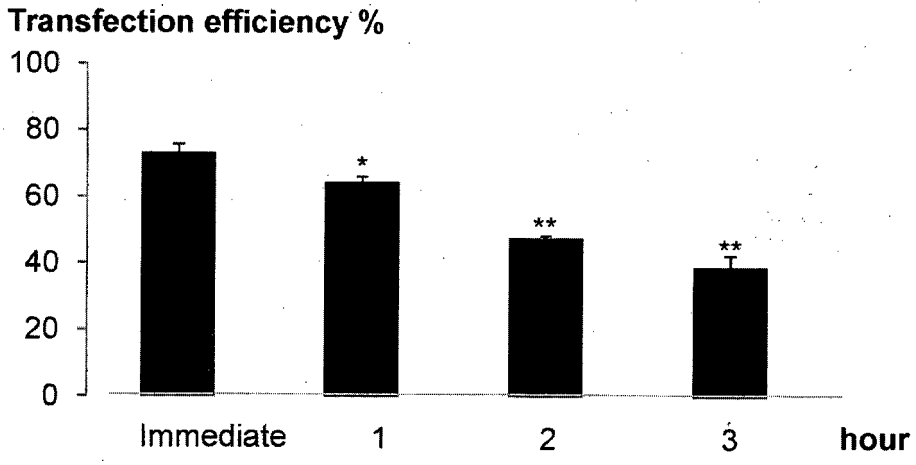


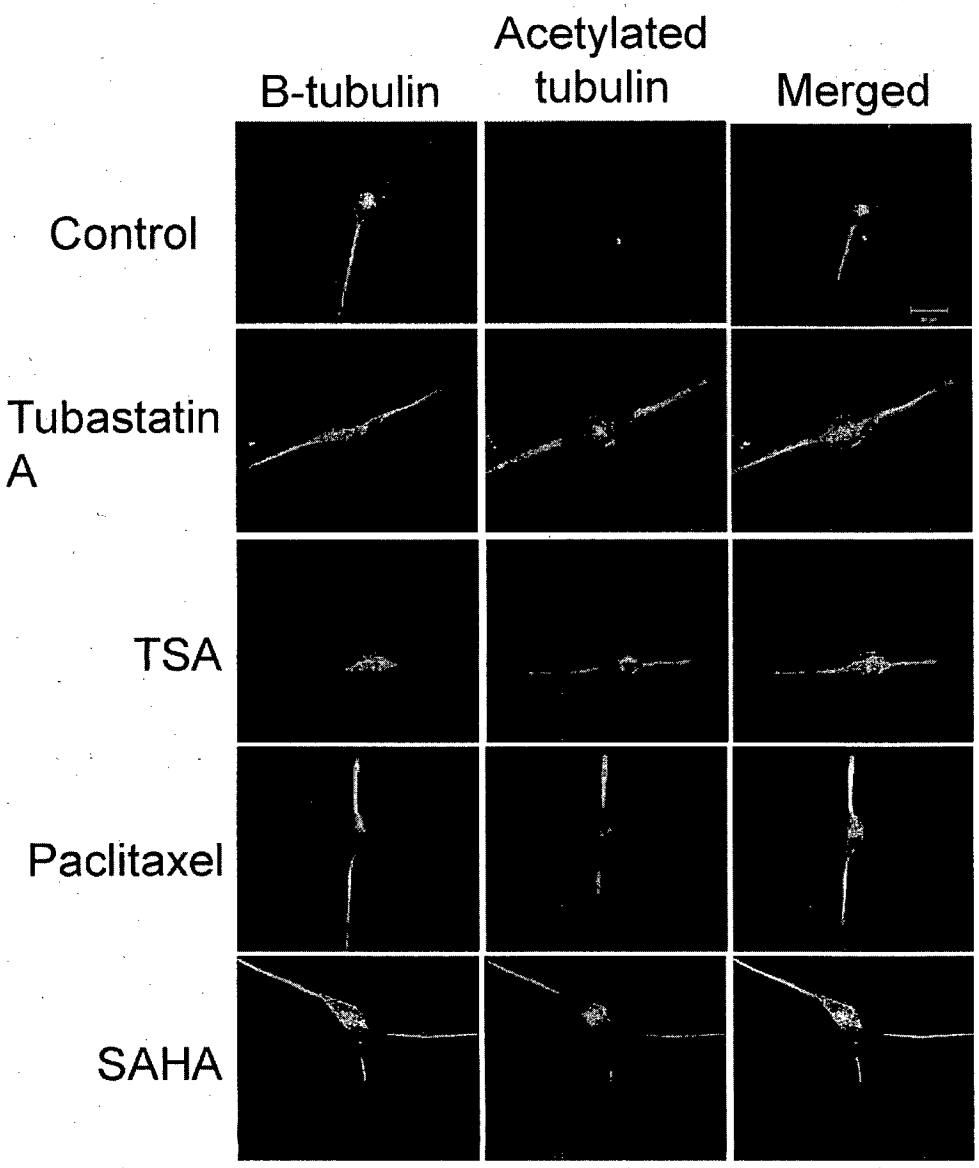
FIG. 32

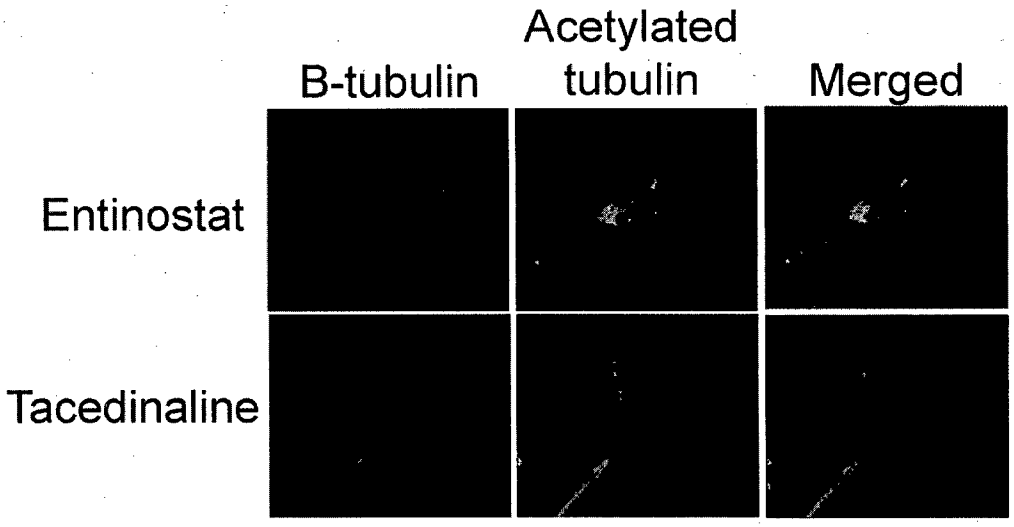
a.



b.







Reagent	Targets	Reaction
Tubastatin A	HDAC6	10 μM
TSA	HDAC1,2,3,6	50 nM
Paclitaxel	Microtubule	25 nM
SAHA	HDAC1,2,3,6	5 μM
Entinostat	HDAC1,2,3,8	10 μM
Tacedinaline	HDAC1,2,3	50 μM

FIG. 33

FIG. 34

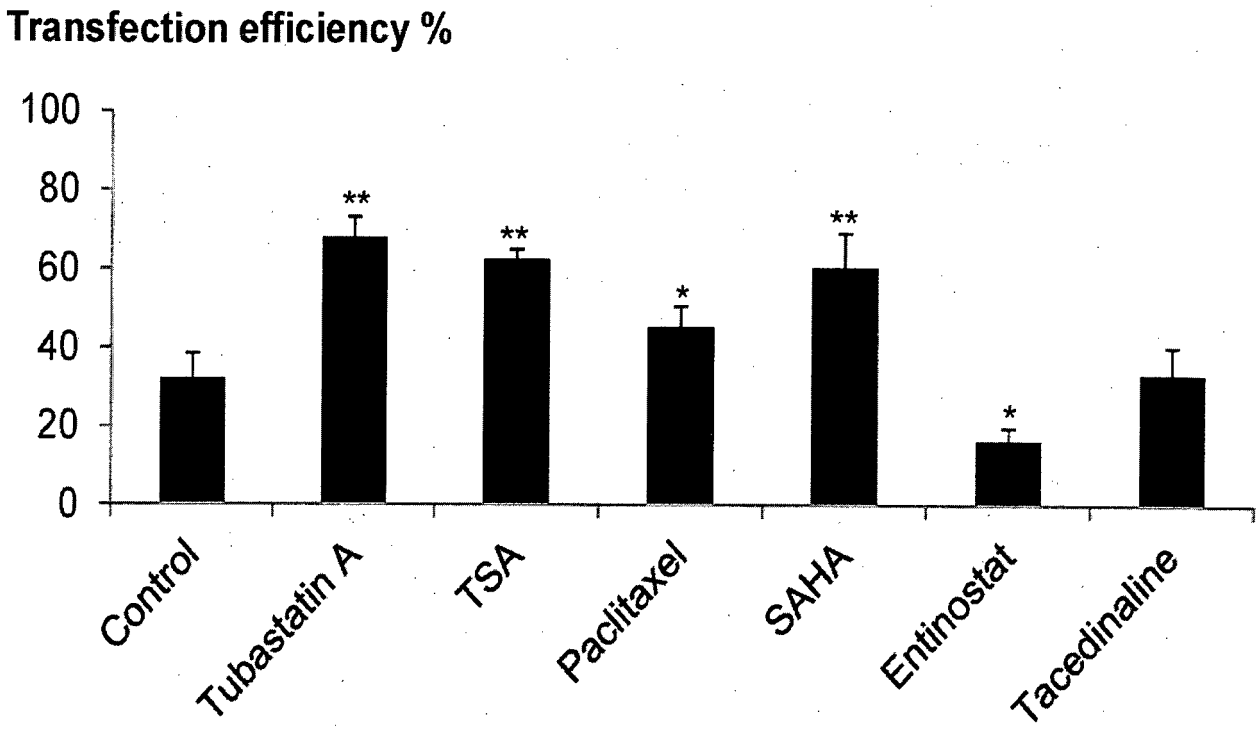

Transfection efficiency %
100
80
60
40
20
0
**
**
*
**
*
Control
Tubastatin A
TSA
Paclitaxel
SAHA
Entinostat
Tacedinaline

FIG. 35

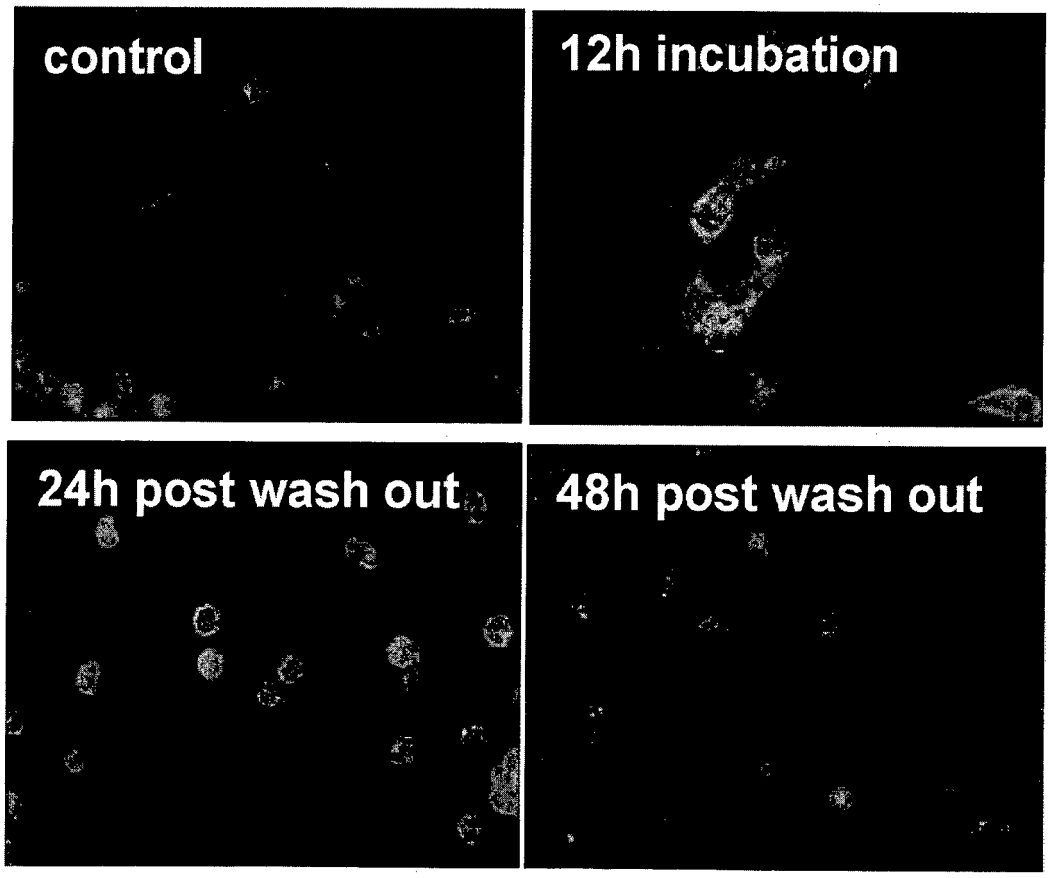

control
12h incubation
24h post wash out
48h post wash out

FIG. 36

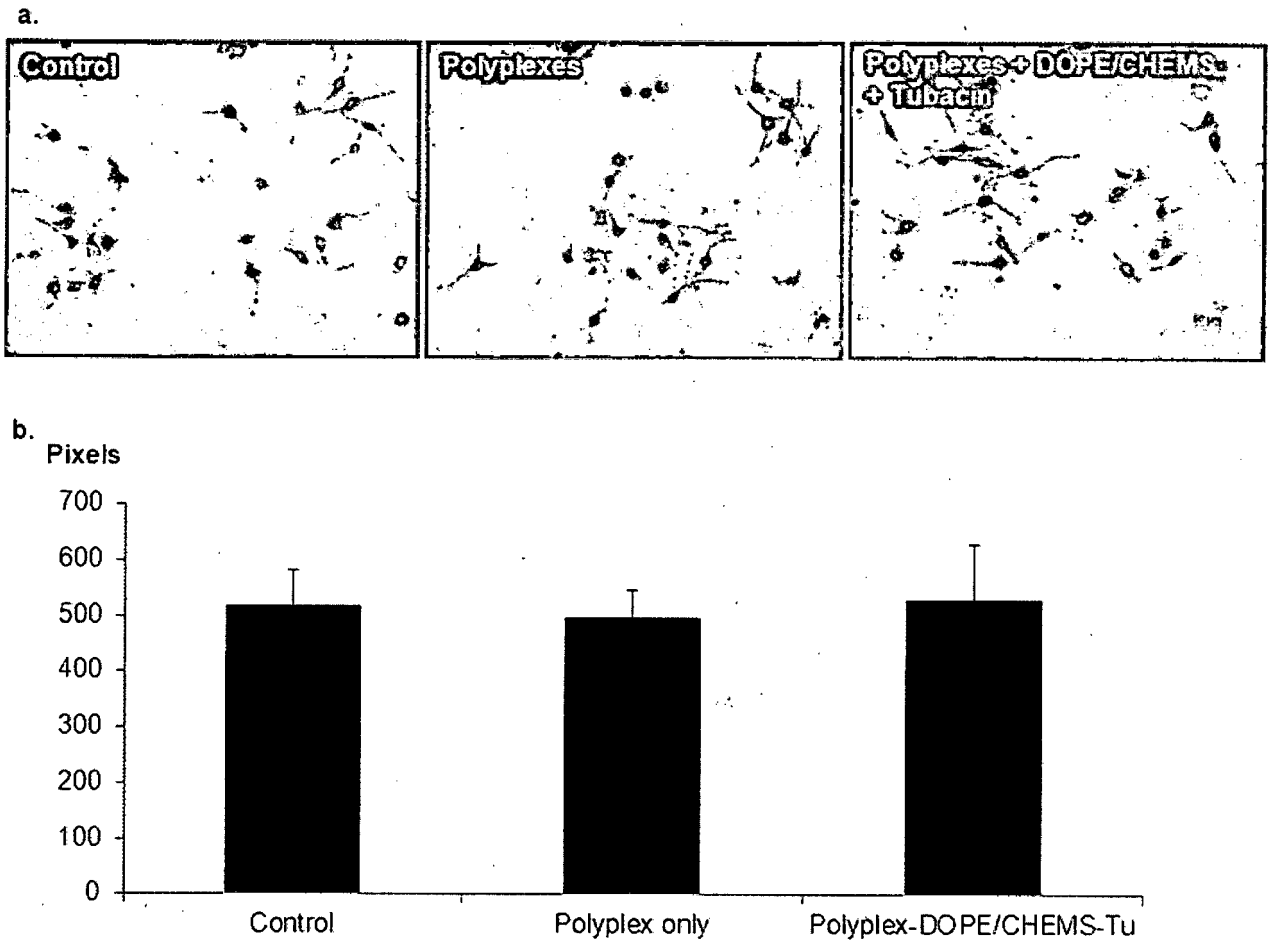

a.
Control
Polyplexes
Polyplexes + DOPE/CHEMS + Tubacin
b.
Pixels
700
600
500
400
300
200
100
0
Control
Polyplex only
Polyplex-DOPE/CHEMS-Tu

FIG. 37

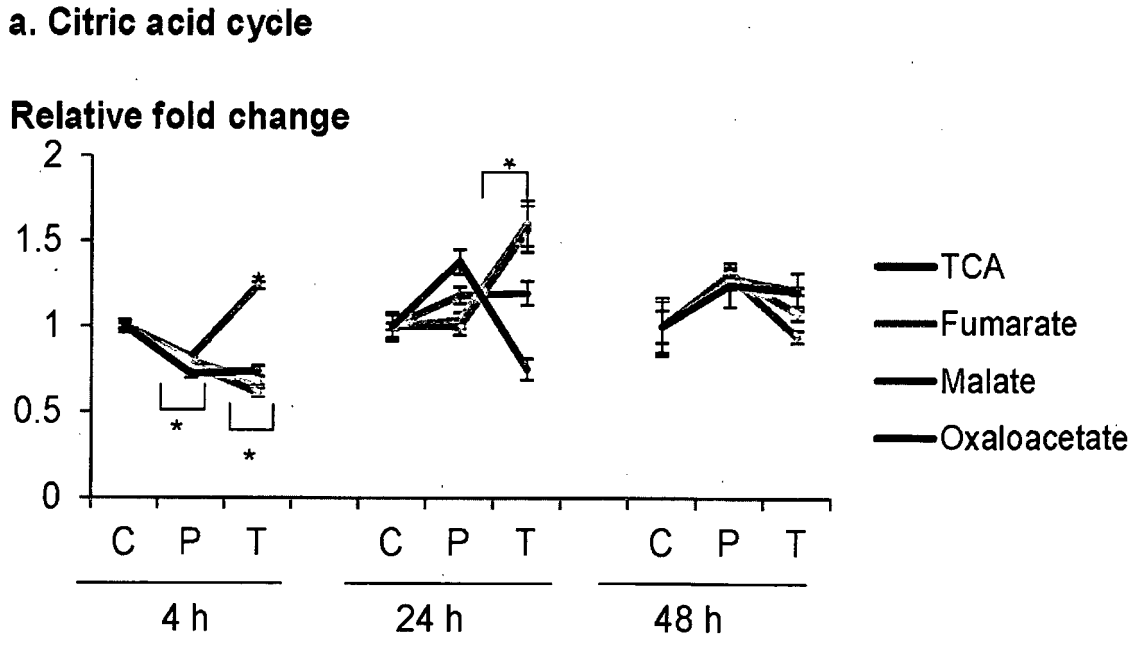

a. Citric acid cycle
Relative fold change
2
1.5
1
0.5
0
C P T C P T C P T
4 h
24 h
48 h
TCA
Fumarate
Malate
Oxaloacetate

FIG. 37 continued

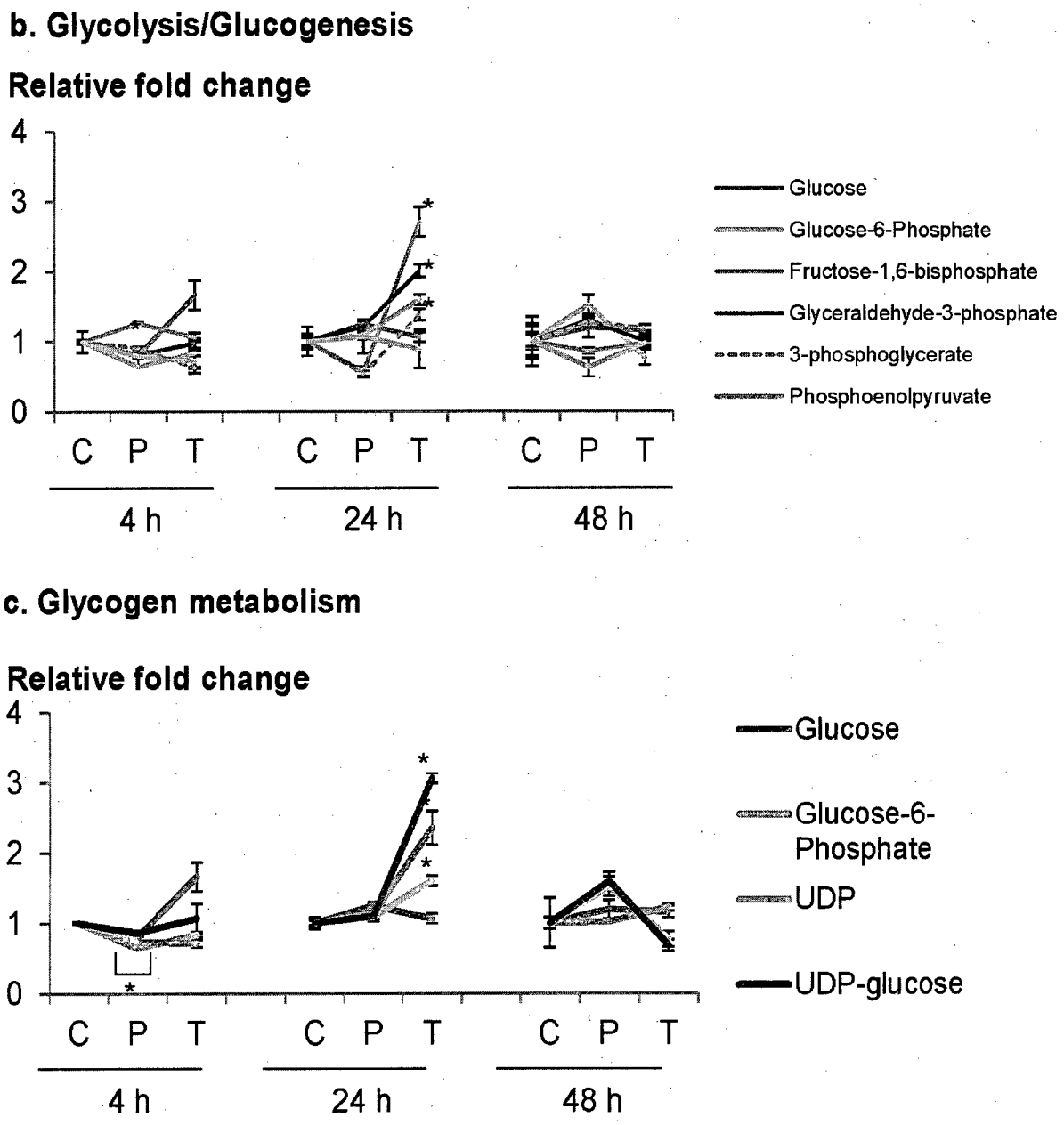
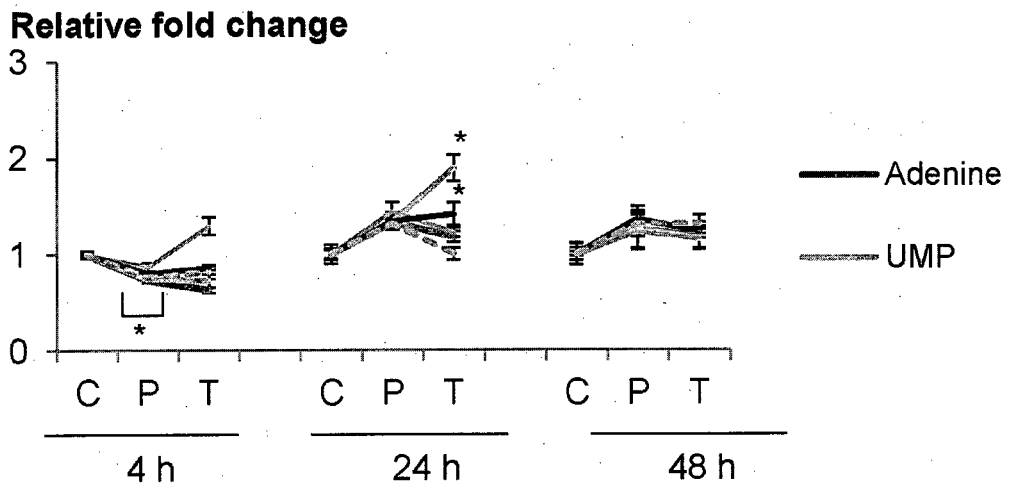
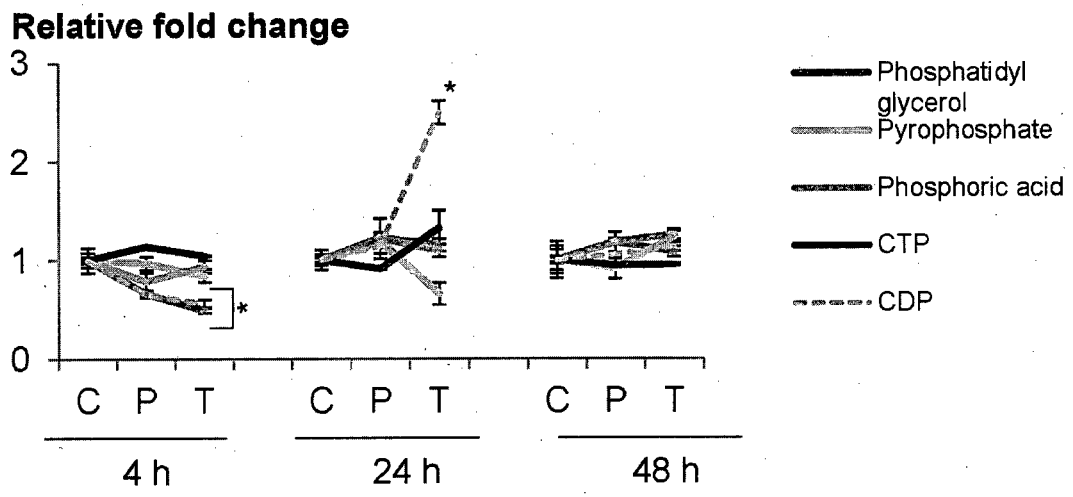


FIG. 37 continued

d. Nucleotide metabolism



e. Phospholipid Biosynthesis



f. Tryptophan metabolism

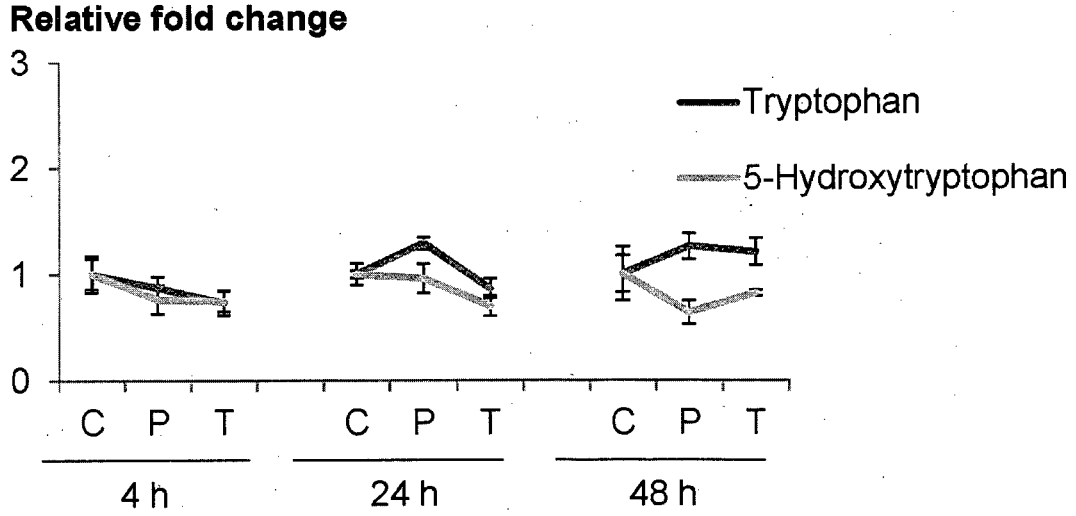


FIG. 38

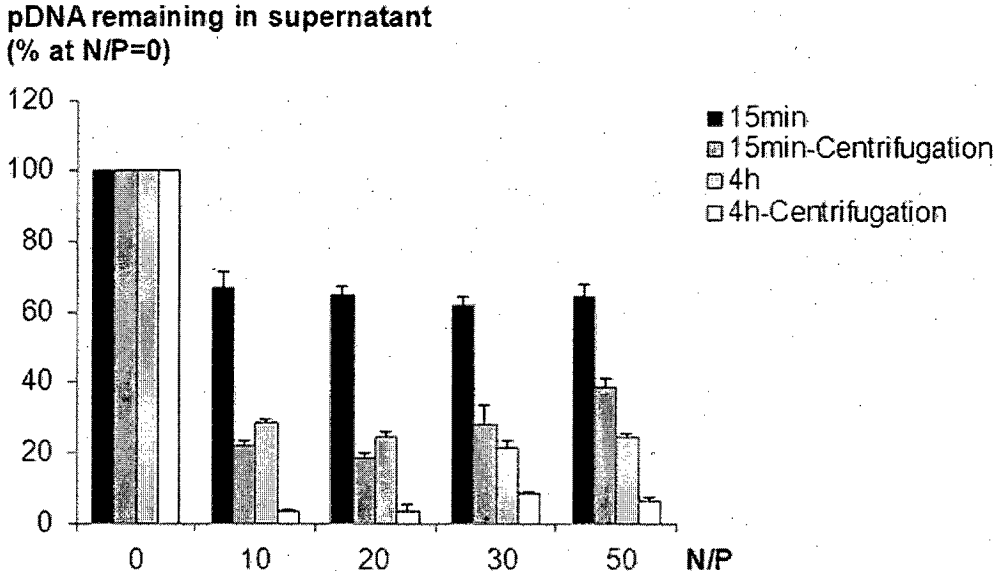

pDNA remaining in supernatant
(% at N/P=0)
120
100
80
60
40
20
0
0
10
20
30
50
N/P
15min
15min-Centrifugation
4h
4h-Centrifugation

FIG. 39

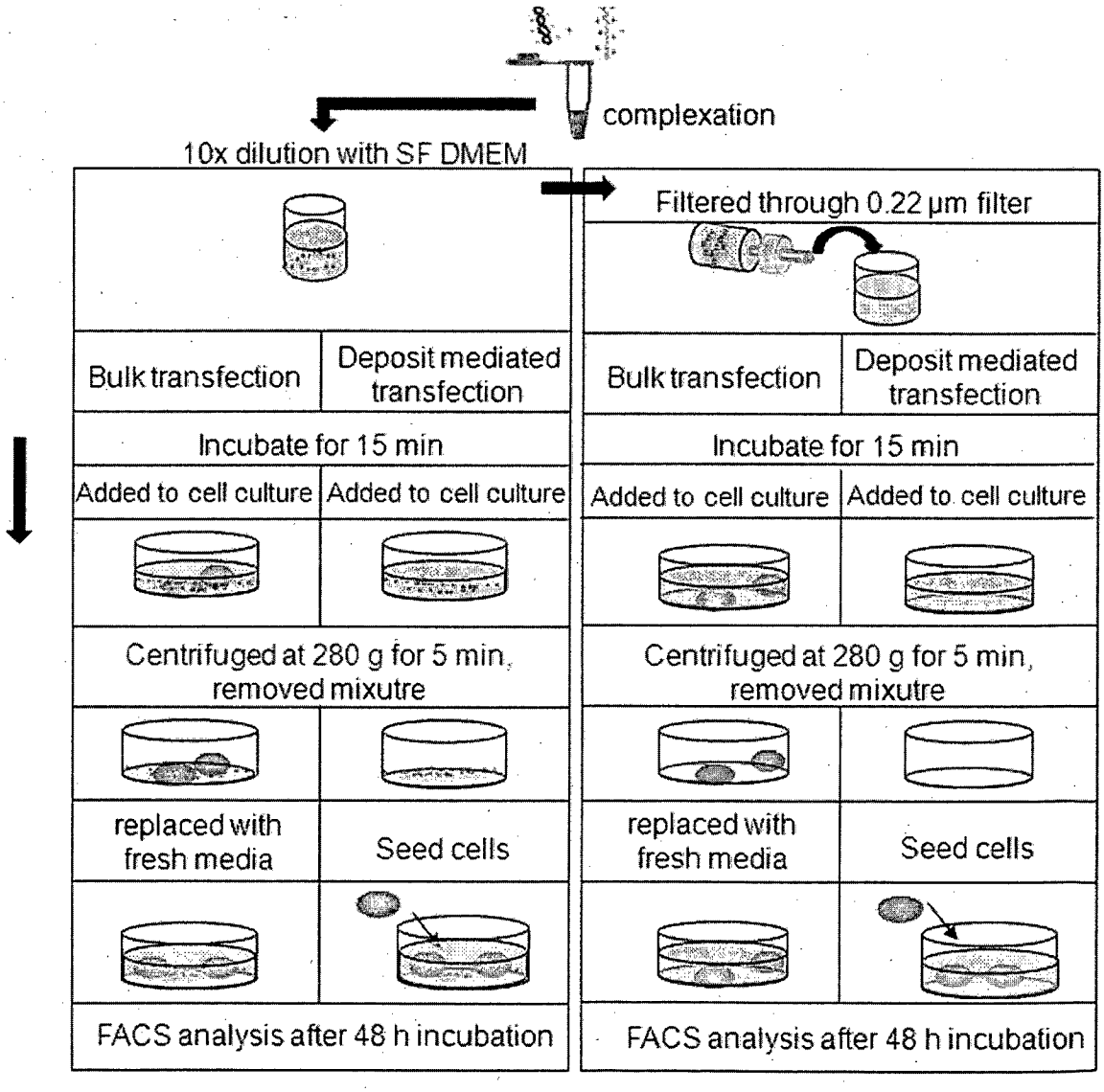

complexation
10x dilution with SF DMEM
Filtered through 0.22 µm filter
Bulk transfection
Deposit mediated transfection
Incubate for 15 min
Added to cell culture
Added to cell culture
Centrifuged at 280 g for 5 min, removed mixutre
replaced with fresh media
Seed cells
FACS analysis after 48 h incubation
Bulk transfection
Deposit mediated transfection
Incubate for 15 min
Added to cell culture
Added to cell culture
Centrifuged at 280 g for 5 min, removed mixutre
replaced with fresh media
Seed cells
FACS analysis after 48 h incubation

FIG. 40

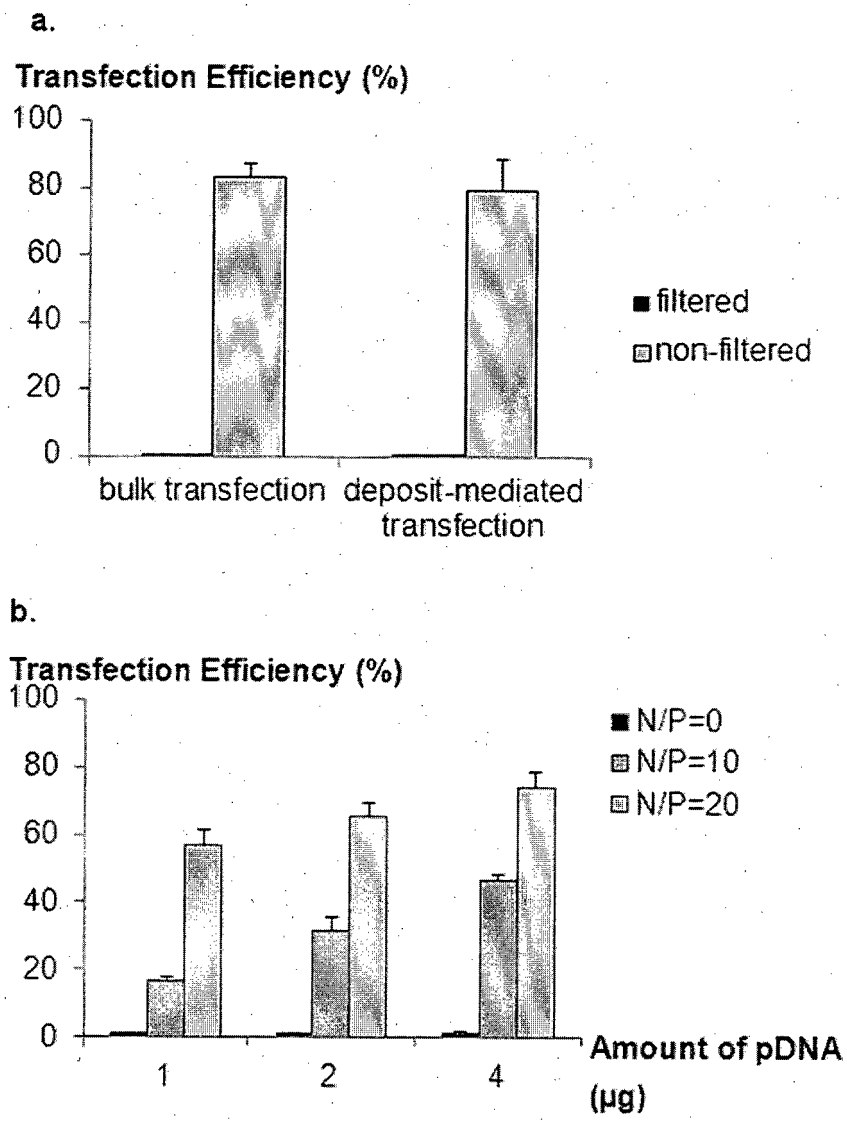

a.
Transfection Efficiency (%)
100
80
60
40
20
0
bulk transfection
deposit-mediated transfection
filtered
non-filtered
b.
Transfection Efficiency (%)
100
80
60
40
20
0
1
2
4
N/P=0
N/P=10
N/P=20
Amount of pDNA (μg)

FIG. 41

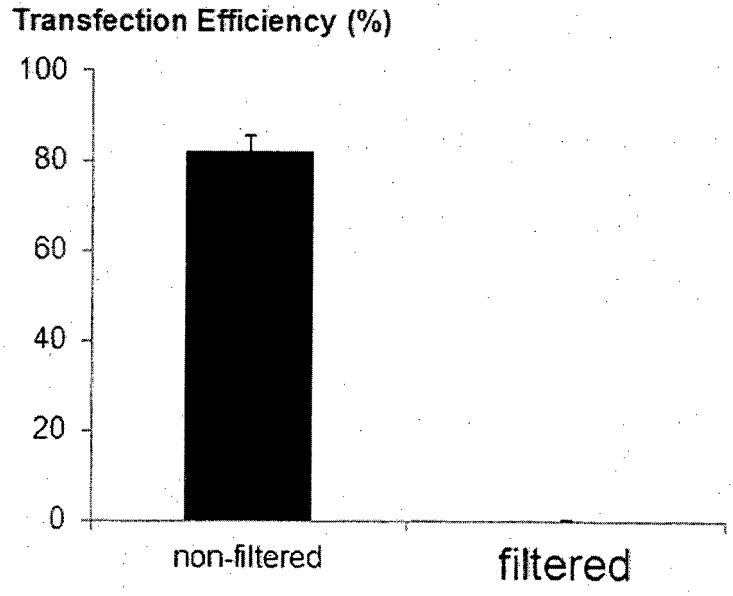

Transfection Efficiency (%)
100
80
60
40
20
0
non-filtered
filtered

FIG. 41 continued

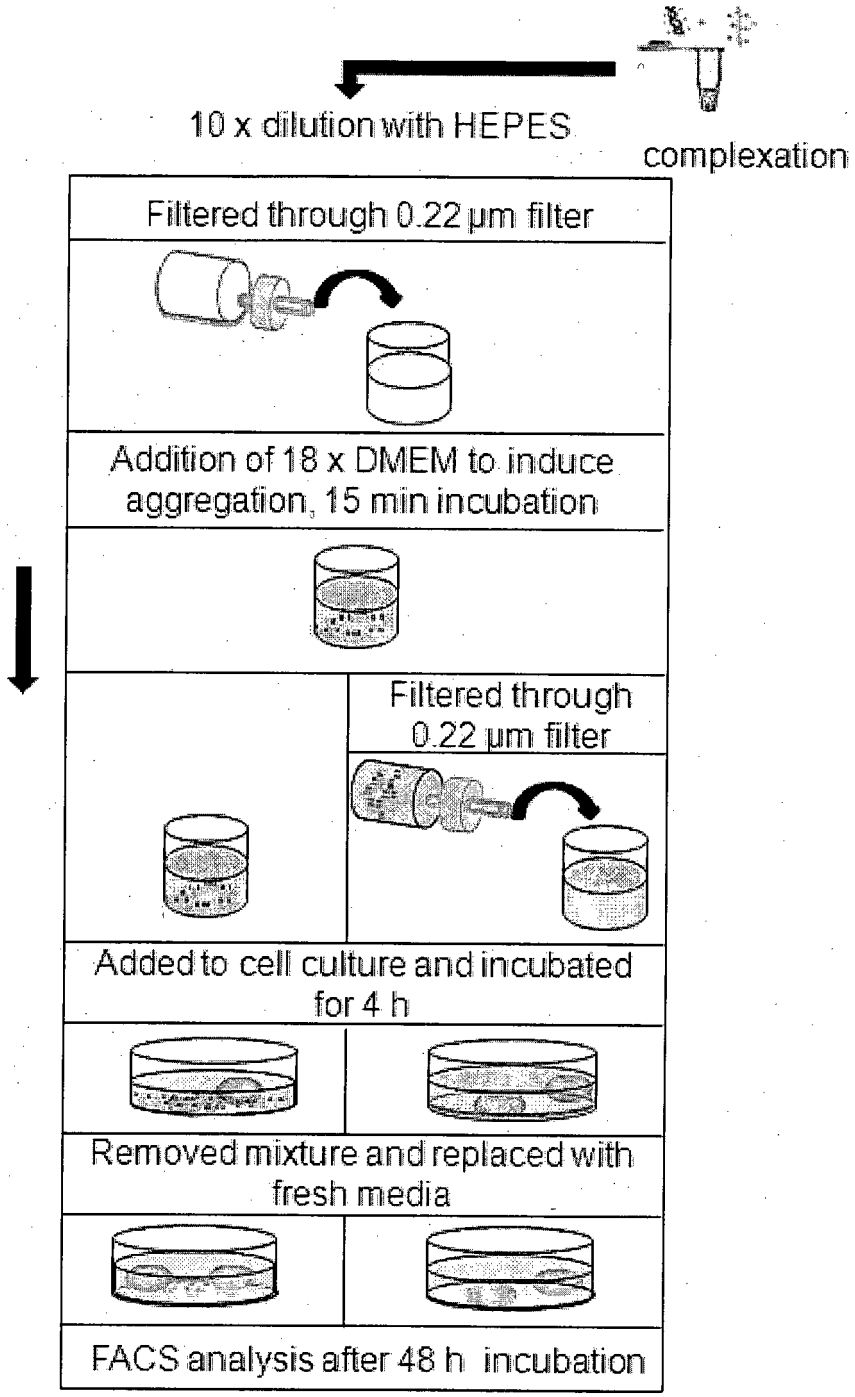


FIG. 42

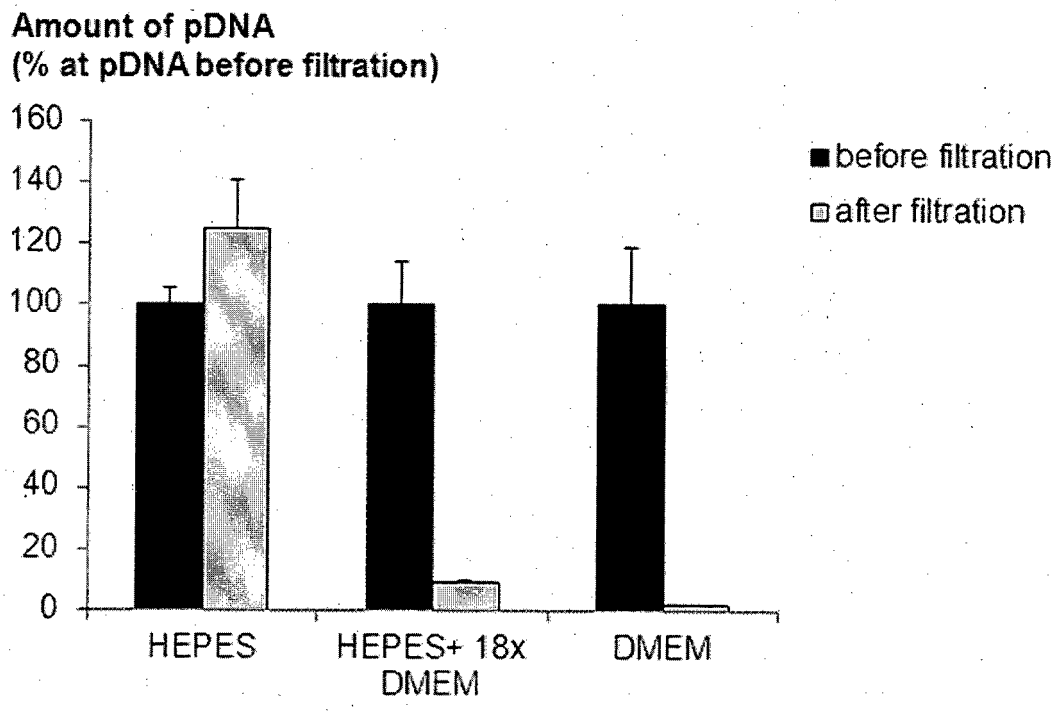
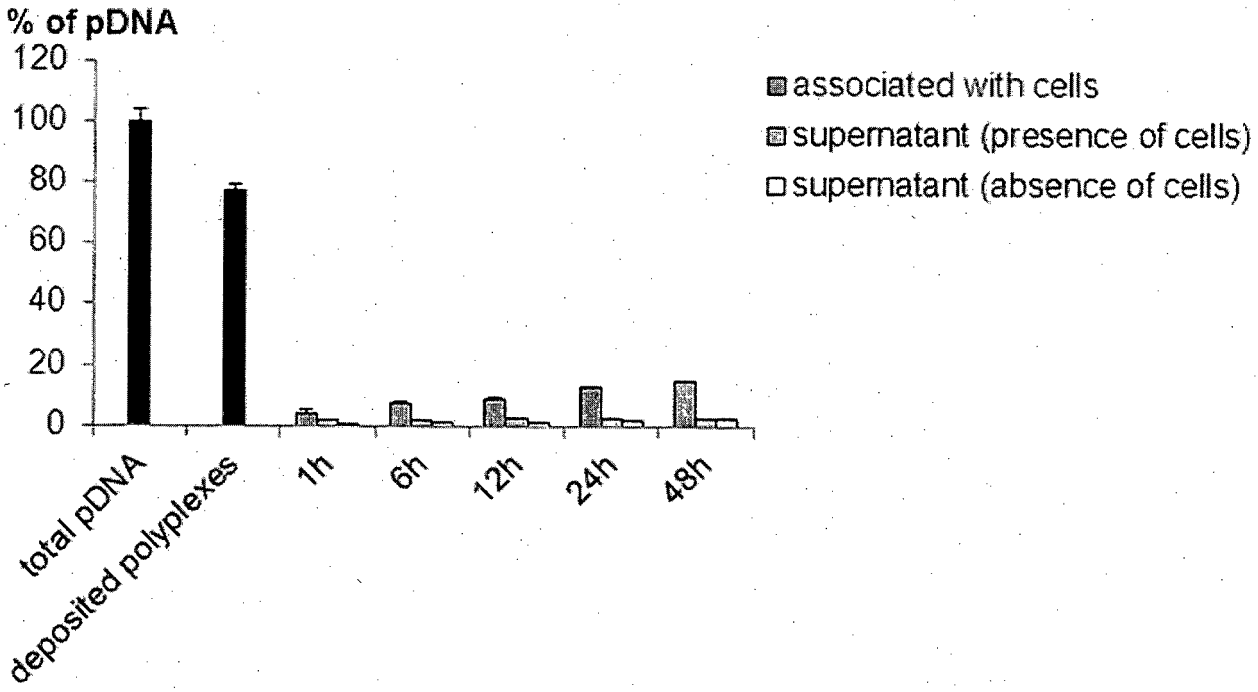

Amount of pDNA
(% at pDNA before filtration)
160
140
120
100
80
60
40
20
0
HEPES
HEPES+ 18x DMEM
DMEM
before filtration
after filtration

FIG. 43


% of pDNA
120
100
80
60
40
20
0
total pDNA
deposited polyplexes
1h
6h
12h
24h
48h
associated with cells
supernatant (presence of cells)
supernatant (absence of cells)

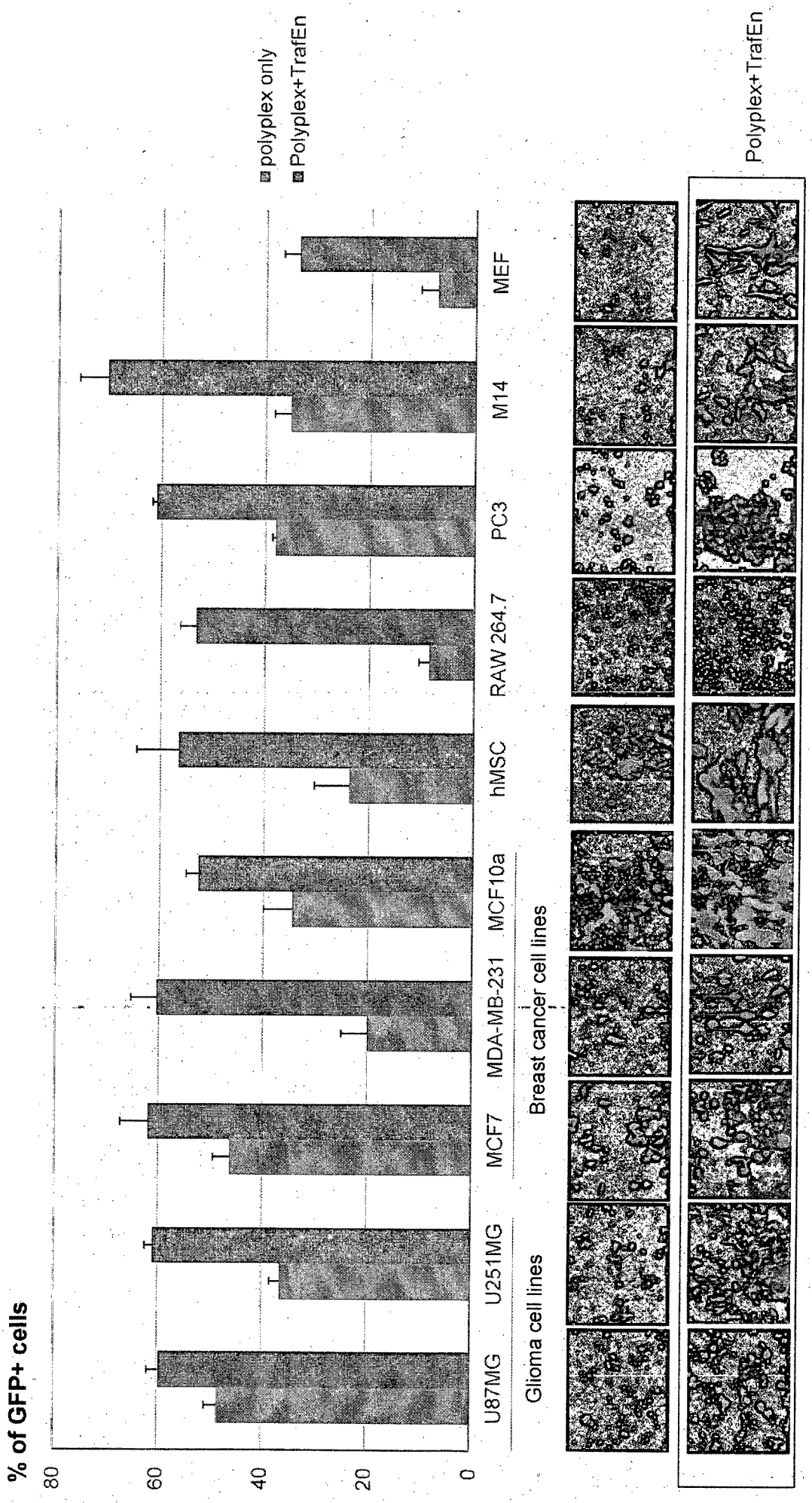

% of GFP+ cells
80
60
40
20
0
polyplex only
Polyplex+TrafEn
U87MG
U251MG
MCF7
MDA-MB-231
MCF10a
hMSC
RAW 264.7
PC3
M14
MEF
Glioma cell lines
Breast cancer cell lines
Polyplex+TrafEn

FIG. 44

FIG. 45

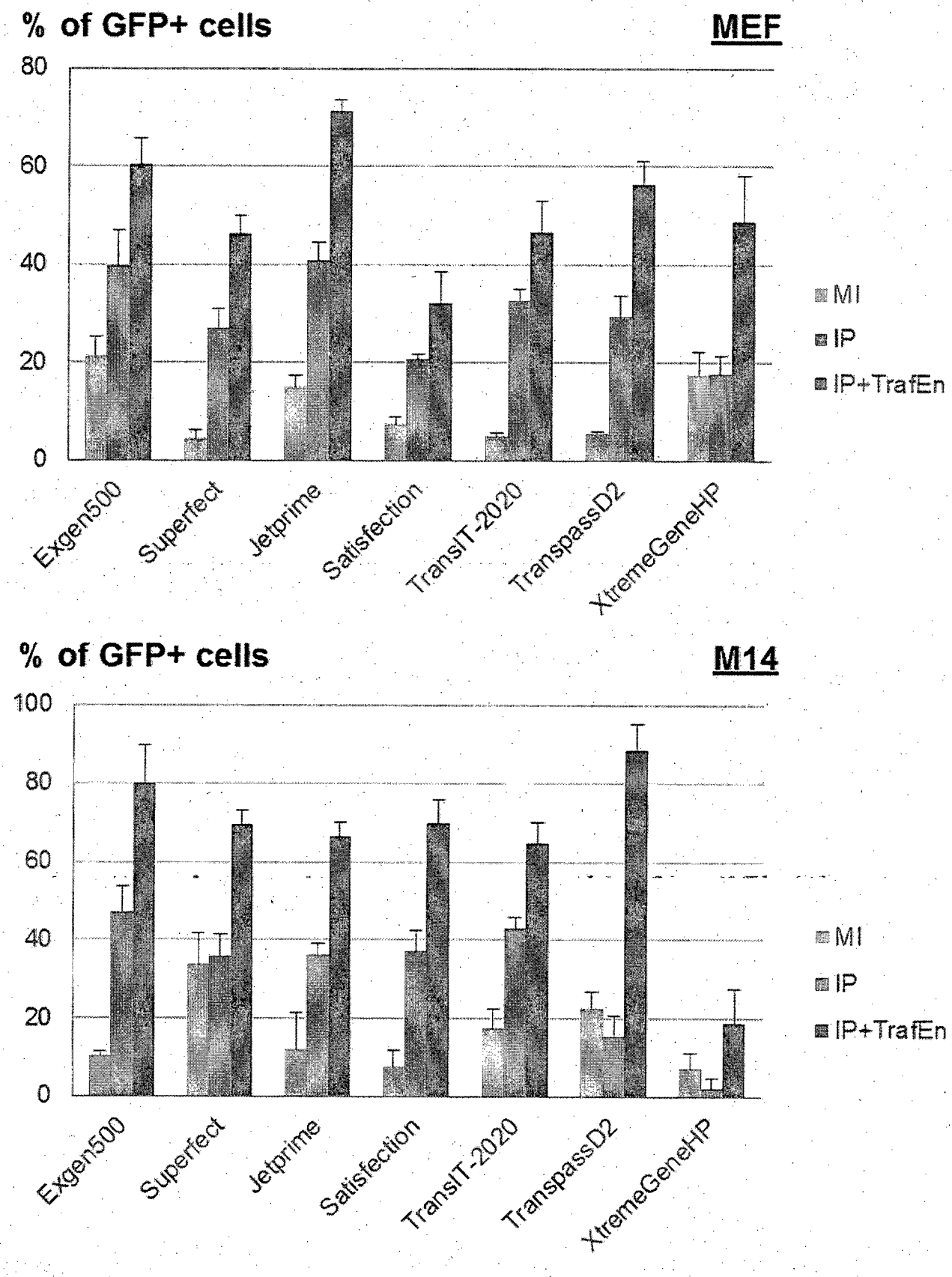
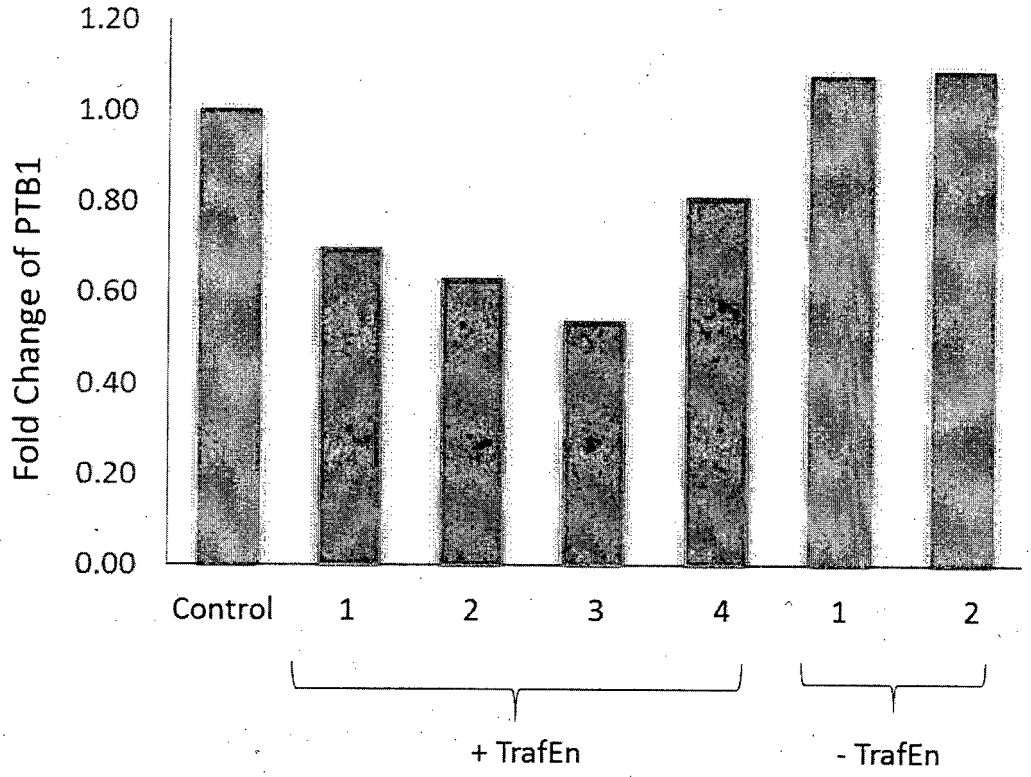

% of GFP+ cells
MEF
80
60
40
20
0
MI
IP
IP+TrafEn
Exgen500
Superfect
Jetprime
Satisfection
TransIT-2020
TranspassD2
XtremeGeneHP
% of GFP+ cells
M14
100
80
60
40
20
0
MI
IP
IP+TrafEn
Exgen500
Superfect
Jetprime
Satisfection
TransIT-2020
TranspassD2
XtremeGeneHP

FIG. 46


1.20
1.00
0.80
0.60
0.40
0.20
0.00
Fold Change of PTB1
Control
1
2
3
4
1
2
+ TrafEn
- TrafEn

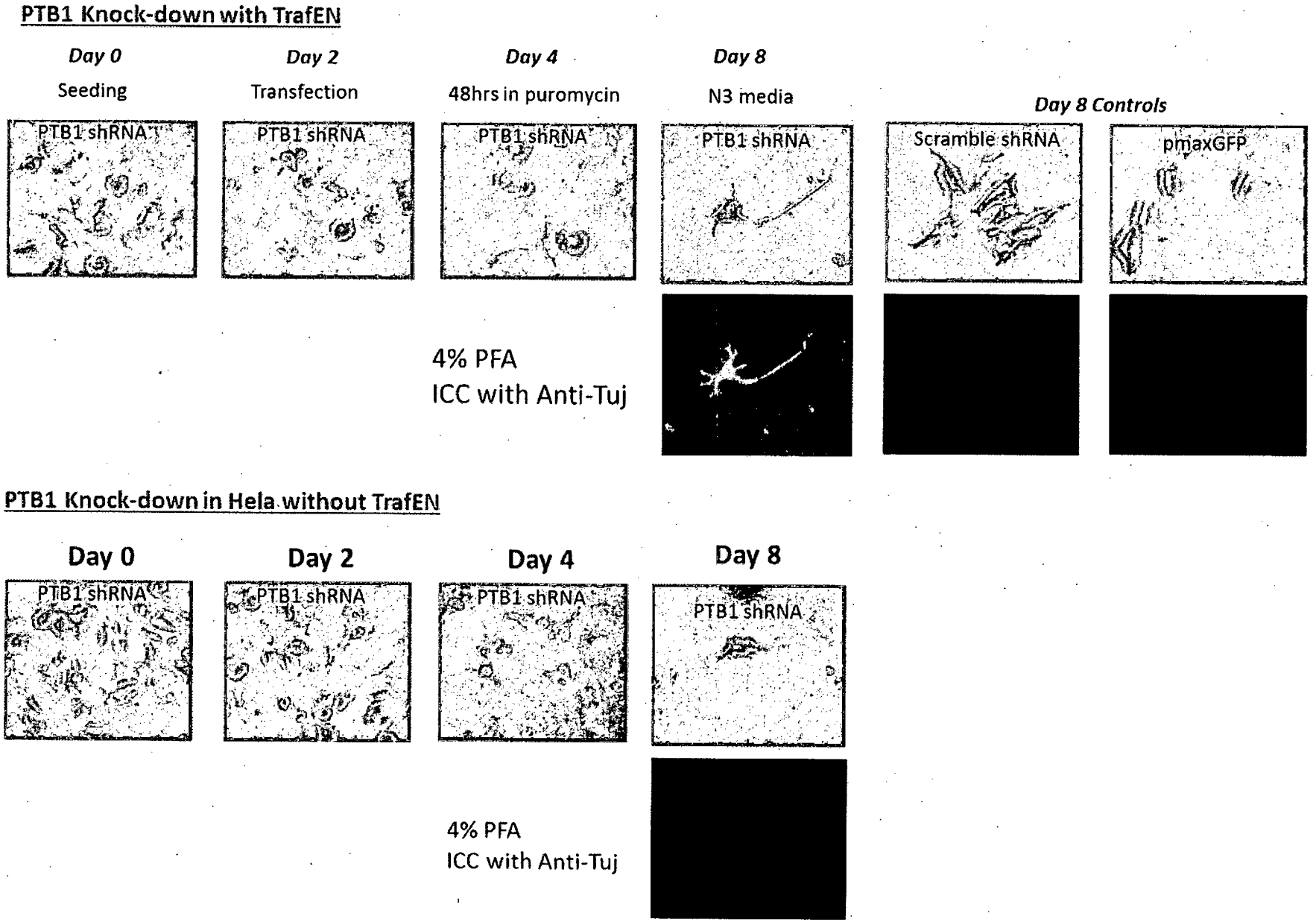

PTB1 Knock-down with TrafEN
Day 0
Seeding
Day 2
Transfection
Day 4
48hrs in puromycin
Day 8
N3 media
Day 8 Controls
PTB1 shRNA
PTB1 shRNA
PTB1 shRNA
PTB1 shRNA
Scramble shRNA
pmaxGFP
4% PFA
ICC with Anti-Tuj
PTB1 Knock-down in Hela without TrafEN
Day 0
Day 2
Day 4
Day 8
PTB1 shRNA
PTB1 shRNA
PTB1 shRNA
PTB1 shRNA
4% PFA
ICC with Anti-Tuj

FIG. 47

FIG. 48

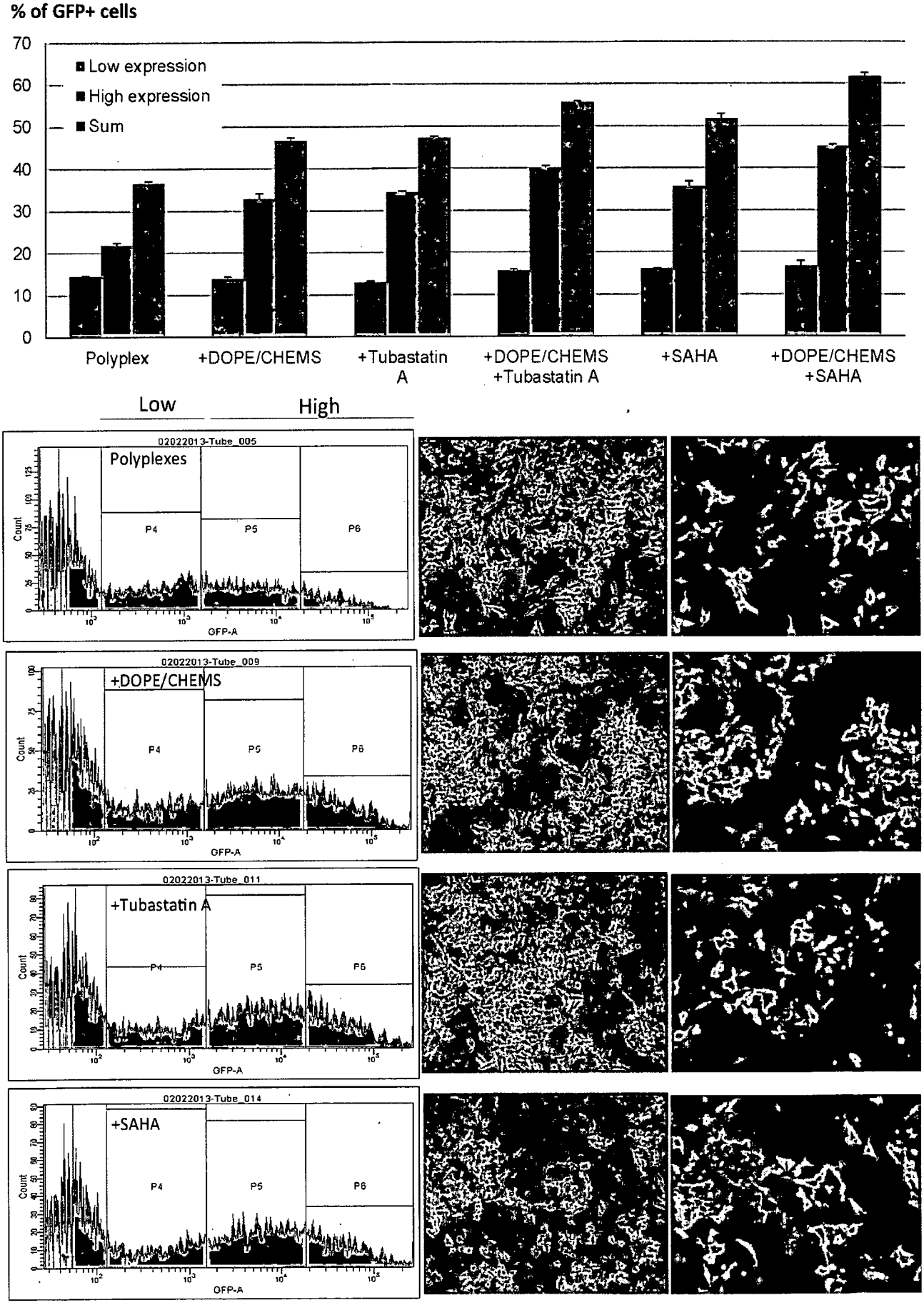
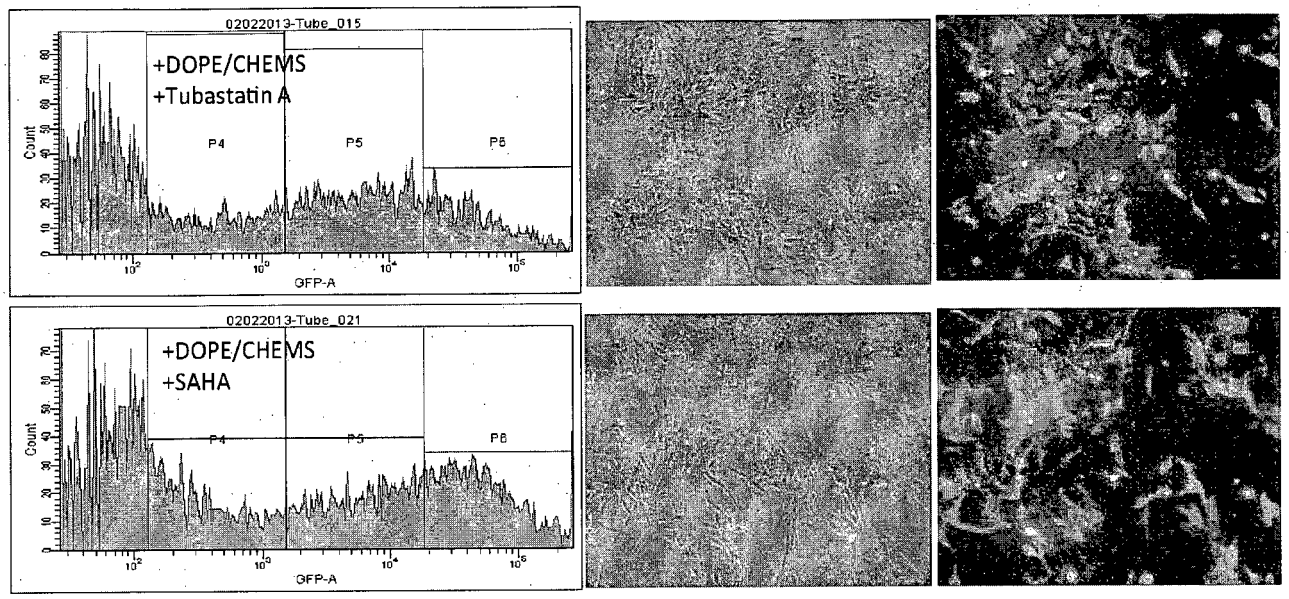

% of GFP+ cells
70
60
50
40
30
20
10
0
Low expression
High expression
Sum
Polyplex
+DOPE/CHEMS
+Tubastatin A
+DOPE/CHEMS +Tubastatin A
+SAHA
+DOPE/CHEMS +SAHA
Low
High
02022013-Tube_005
Polyplexes
P4
P5
P6
Count
GFP-A
02022013-Tube_009
+DOPE/CHEMS
02022013-Tube_011
+Tubastatin A
02022013-Tube_014
+SAHA

FIG. 48 continued



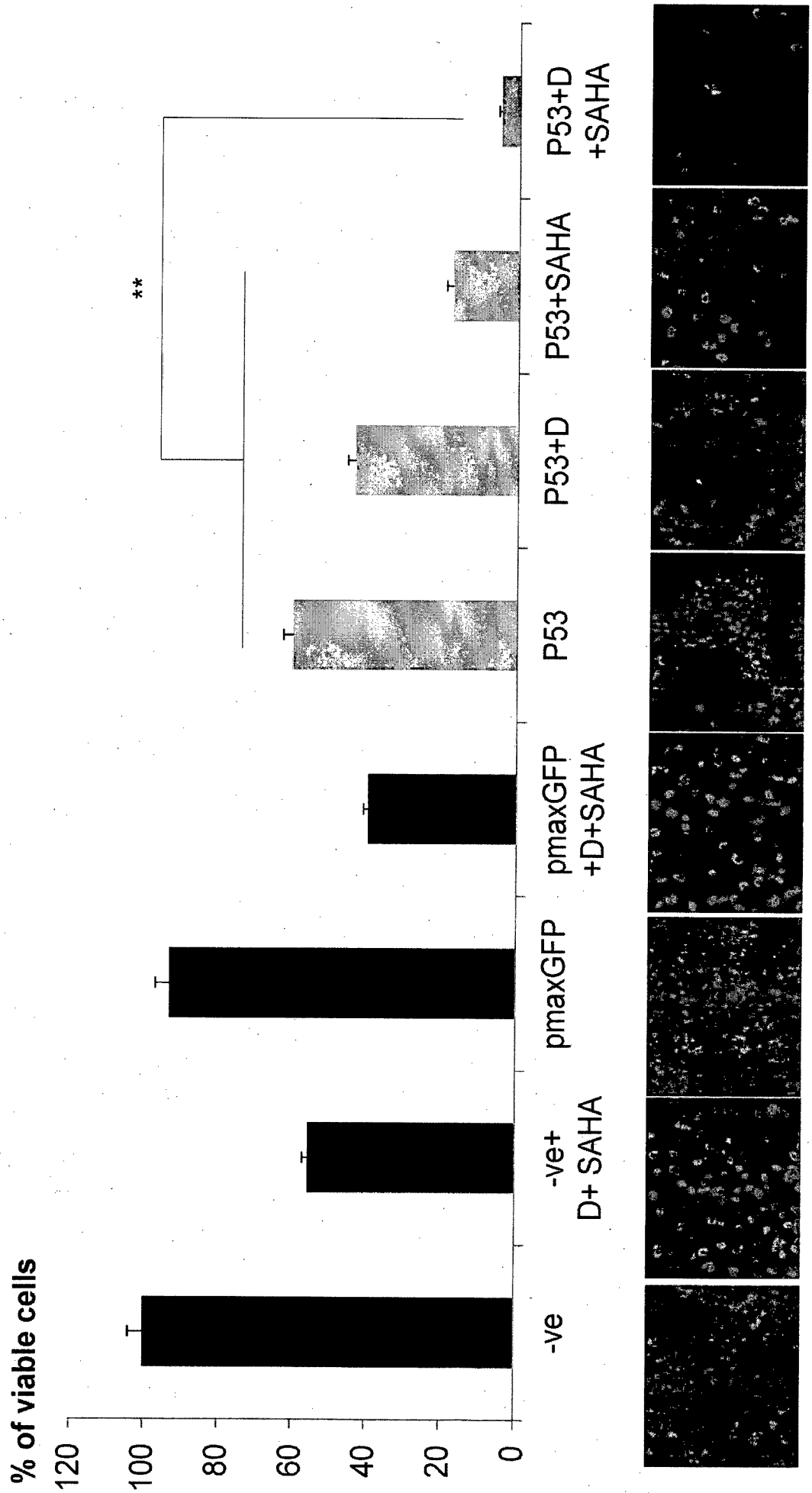

% of viable cells
120
100
80
60
40
20
0
**
-ve
-ve+ D+ SAHA
pmaxGFP
pmaxGFP +D+SAHA
P53
P53+D
P53+SAHA
P53+D +SAHA

FIG. 49

FIG. 50

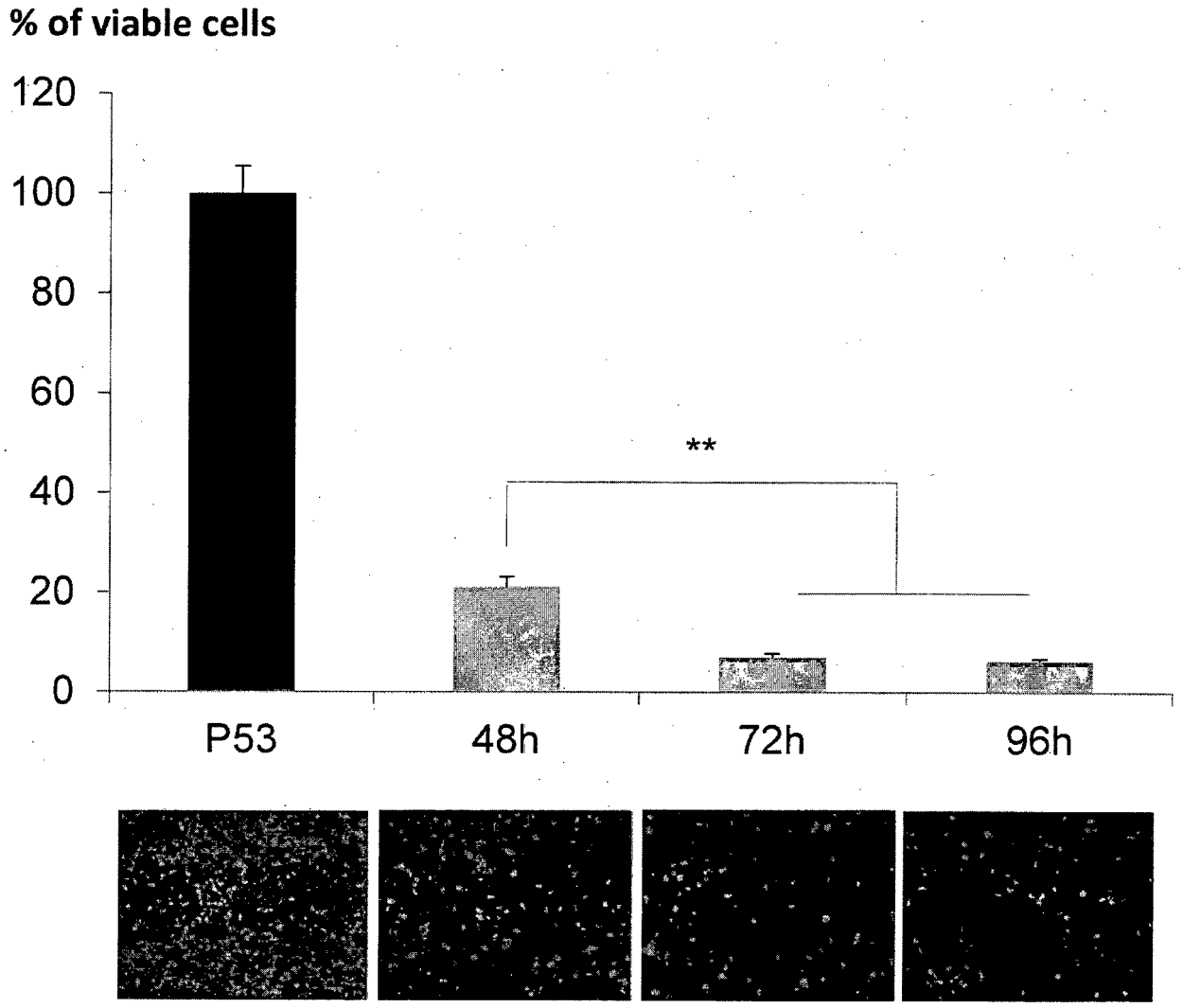
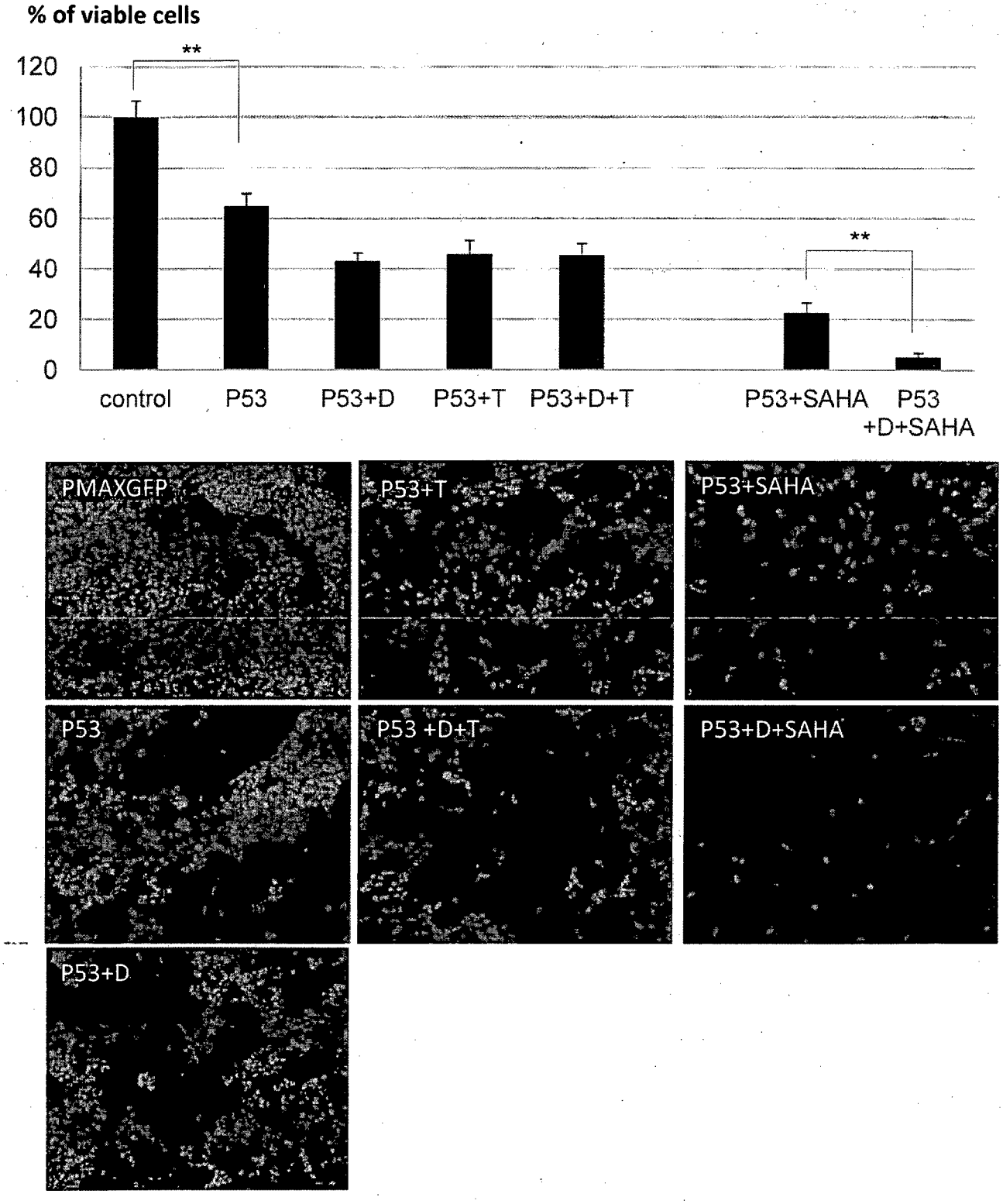


FIG. 51


% of viable cells
120
100
80
60
40
20
0
**
**
control
P53
P53+D
P53+T
P53+D+T
P53+SAHA
P53 +D+SAHA
PMAXGFP
P53+T
P53+SAHA
P53
P53 +D+T
P53+D+SAHA
P53+D

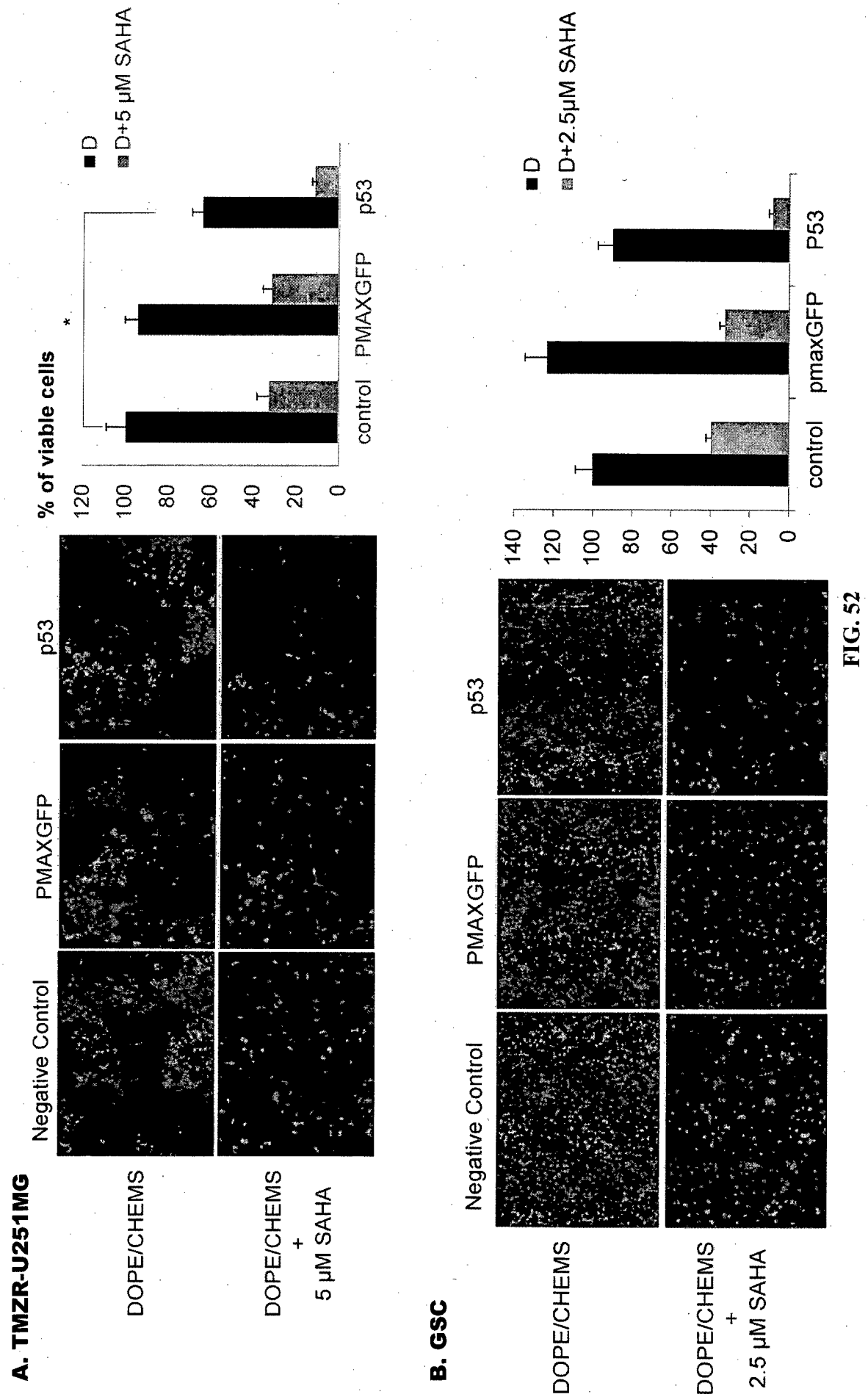
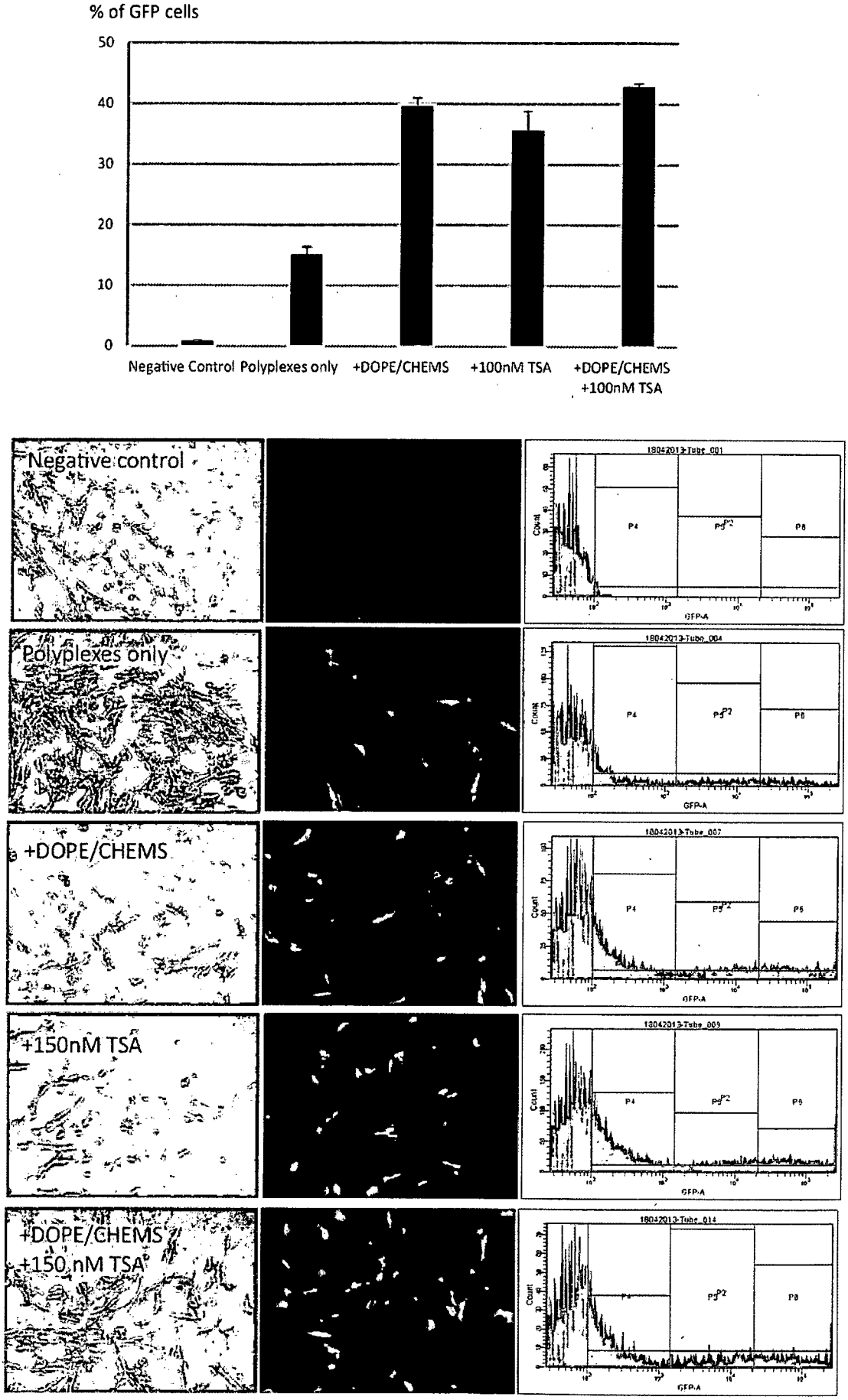

A. TMZR-U251MG
Negative Control
PMAXGFP
p53
DOPE/CHEMS
DOPE/CHEMS + 5 μM SAHA
% of viable cells
120
100
80
60
40
20
0
*
D
D+5 μM SAHA
control
PMAXGFP
p53
B. GSC
Negative Control
PMAXGFP
p53
DOPE/CHEMS
DOPE/CHEMS + 2.5 μM SAHA
140
120
100
80
60
40
20
0
D
D+2.5μM SAHA
control
pmaxGFP
P53

FIG. 52

FIG. 53


% of GFP cells
50
40
30
20
10
0
Negative Control
Polyplexes only
+DOPE/CHEMS
+100nM TSA
+DOPE/CHEMS +100nM TSA
Negative control
Polyplexes only
+DOPE/CHEMS
+150nM TSA
+DOPE/CHEMS +150 nM TSA
P4
P5
P6
Count
GFP-A

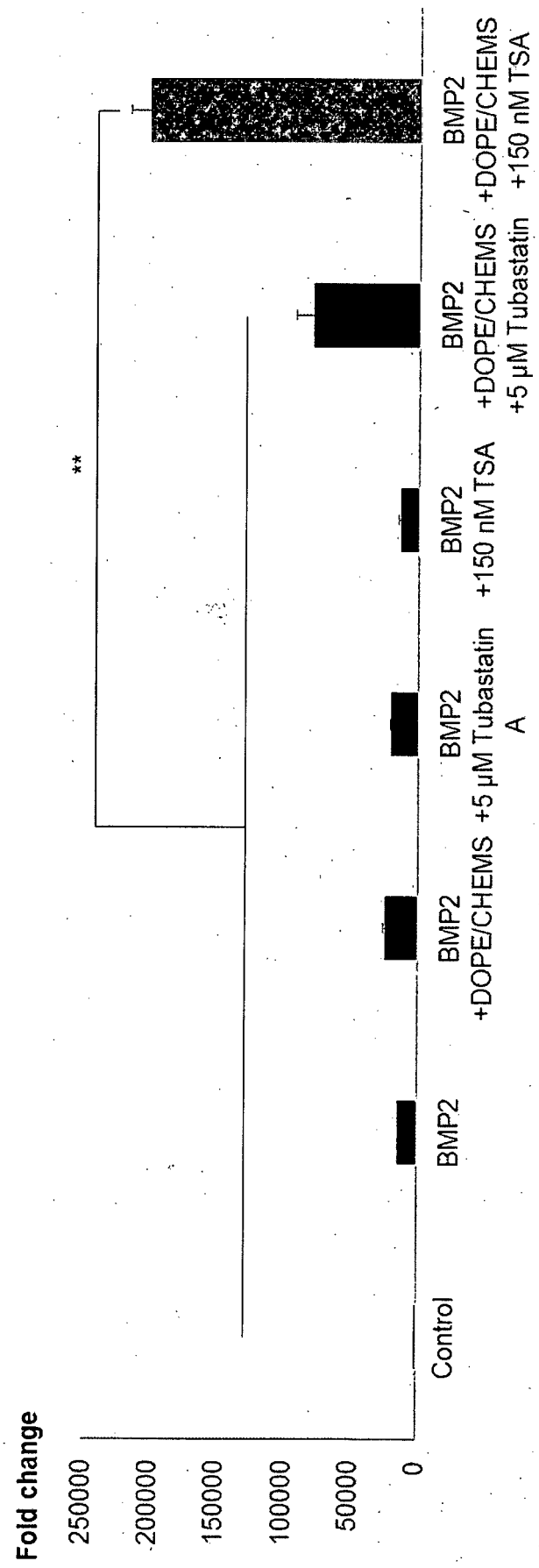
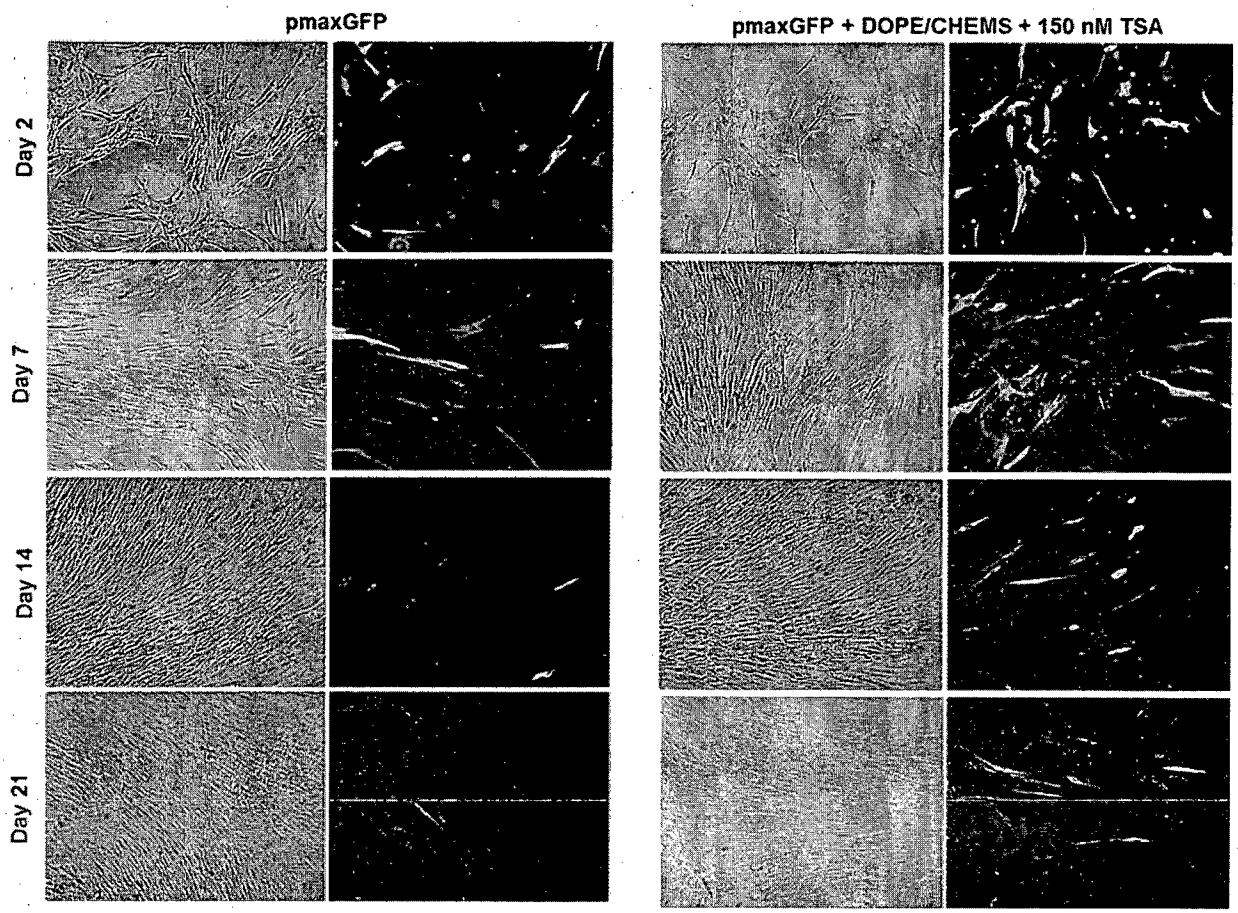

Fold change
250000
200000
150000
100000
50000
0
**
Control
BMP2
BMP2 +DOPE/CHEMS
BMP2 +5 µM Tubastatin A
BMP2 +150 nM TSA
BMP2 +DOPE/CHEMS +5 µM Tubastatin A
BMP2 +DOPE/CHEMS +150 nM TSA

FIG. 54

FIG. 55



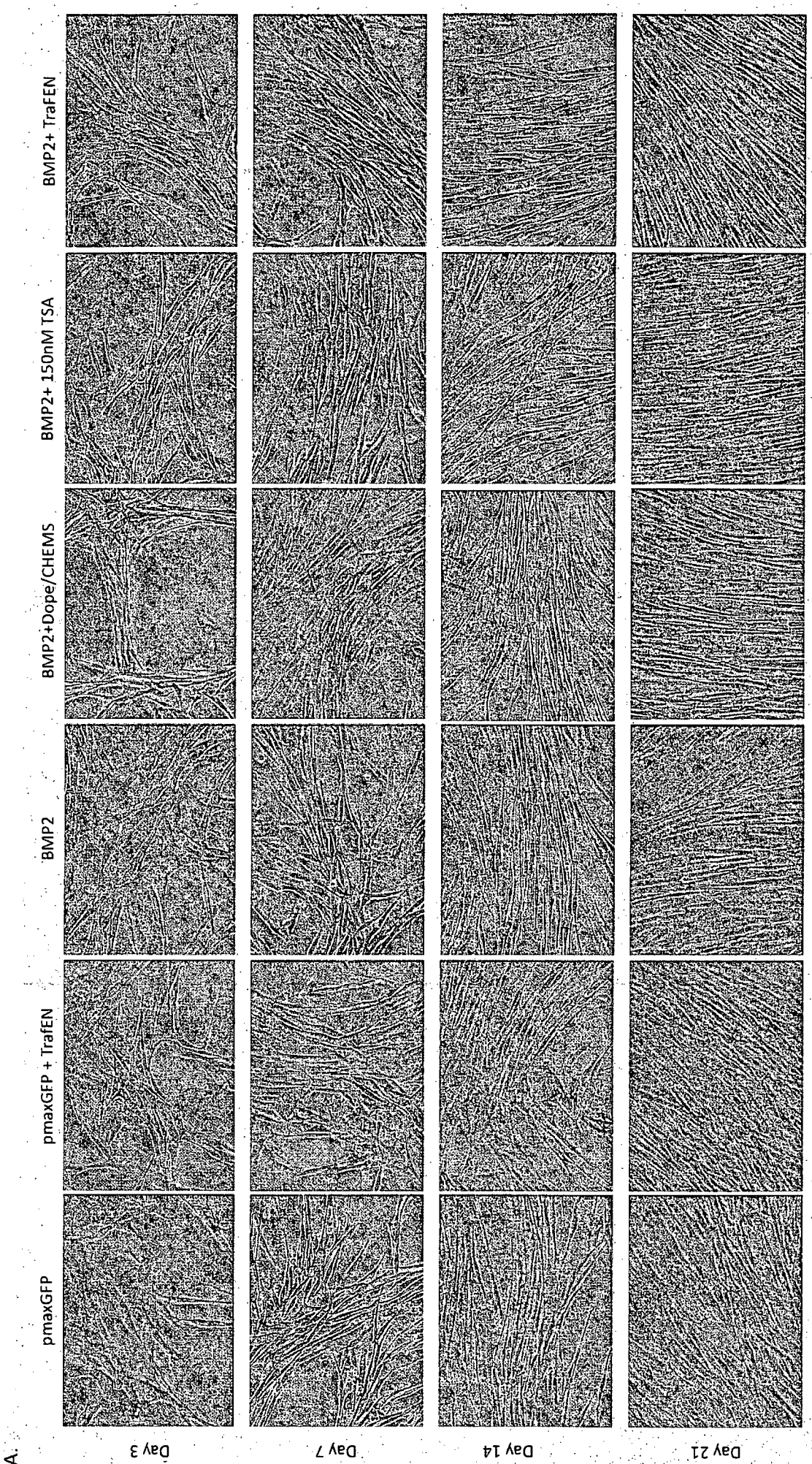

A.
pmaxGFP
pmaxGFP + TrafEN
BMP2
BMP2+Dope/CHEMS
BMP2+ 150nM TSA
BMP2+ TraFEN
Day 3
Day 7
Day 14
Day 21

FIG. 56

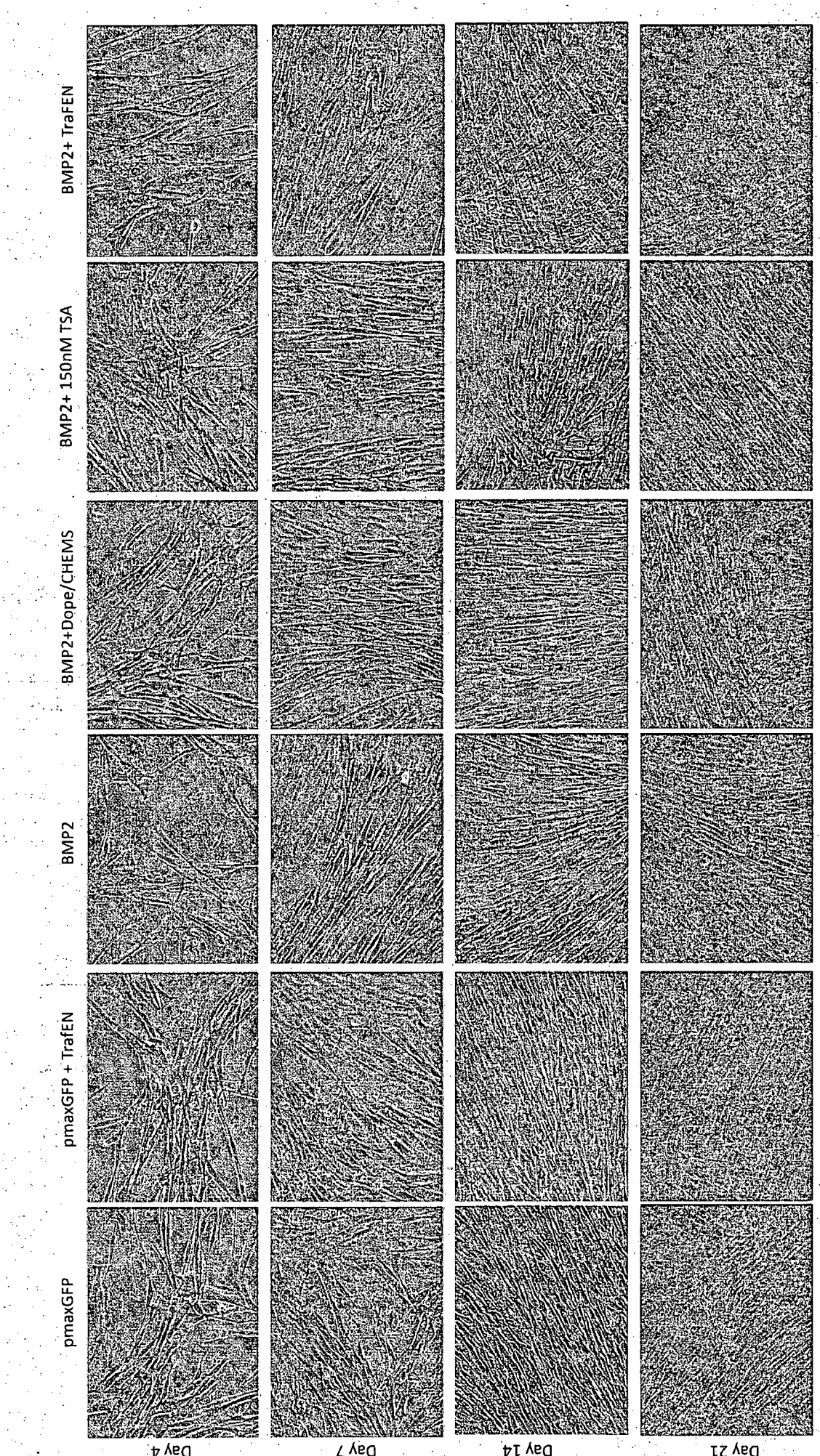
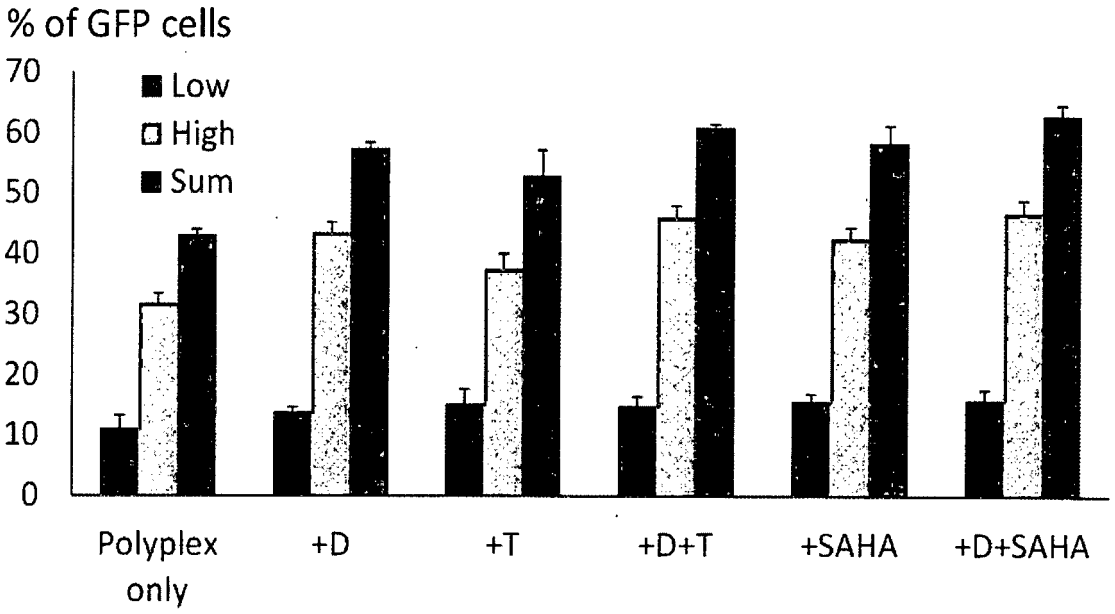


FIG. 56 continued

FIG. 57


% of GFP cells
70
60
50
40
30
20
10
0
Low
High
Sum
Polyplex only
+D
+T
+D+T
+SAHA
+D+SAHA

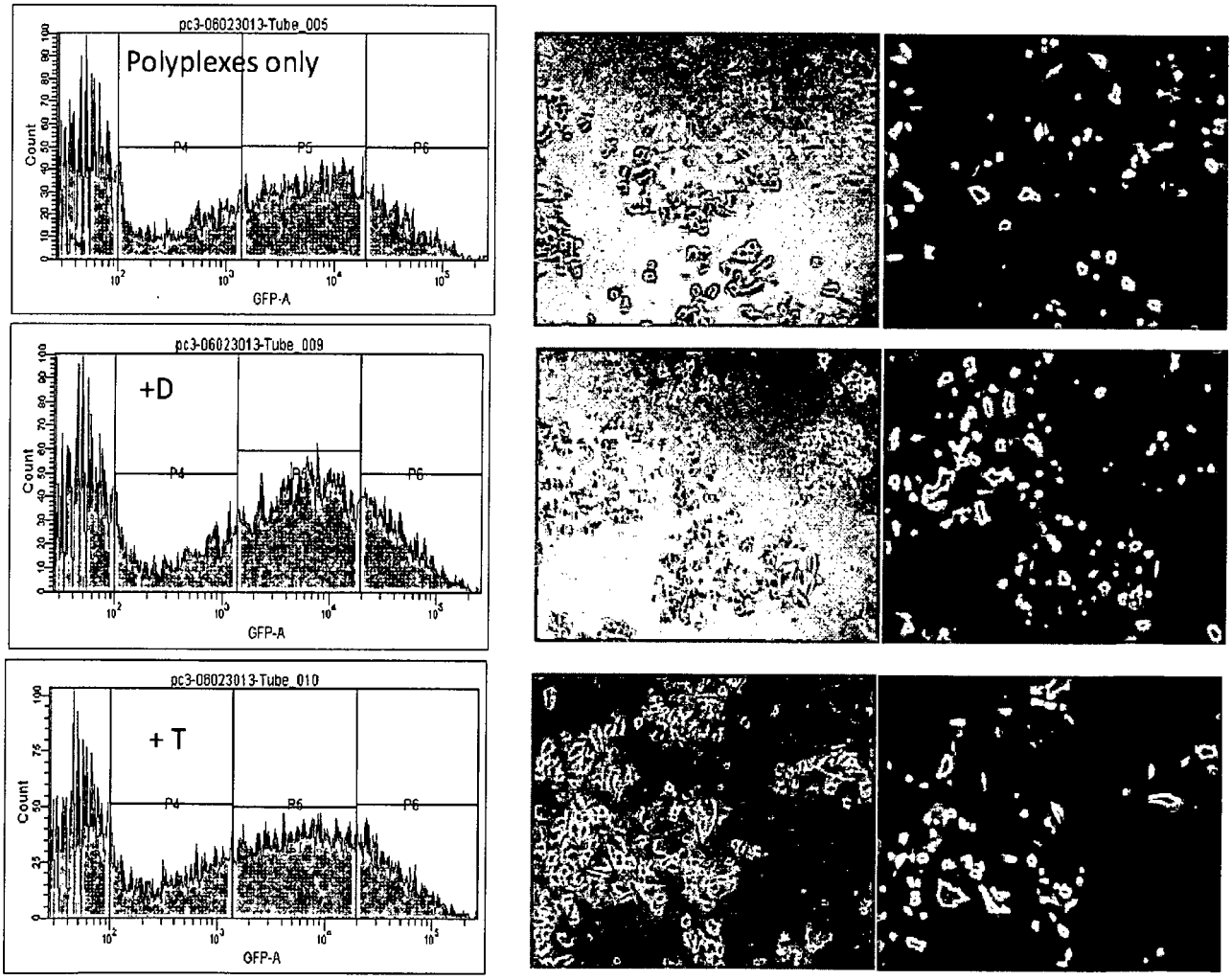

pc3-08023013-Tube_005
Polyplexes only
Count
GFP-A
pc3-08023013-Tube_009
+D
pc3-08023013-Tube_010
+T

FIG. 57 continued

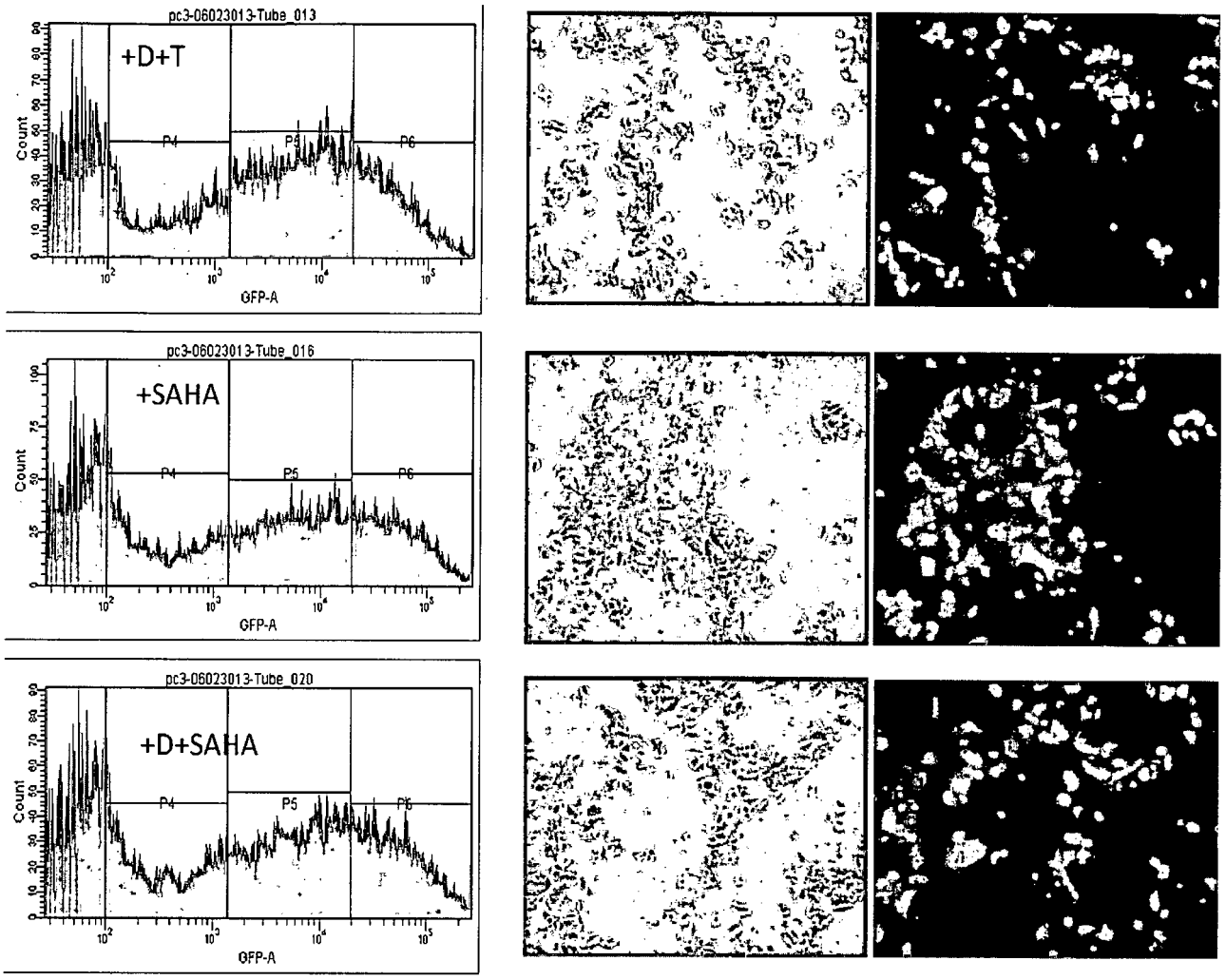


FIG. 58

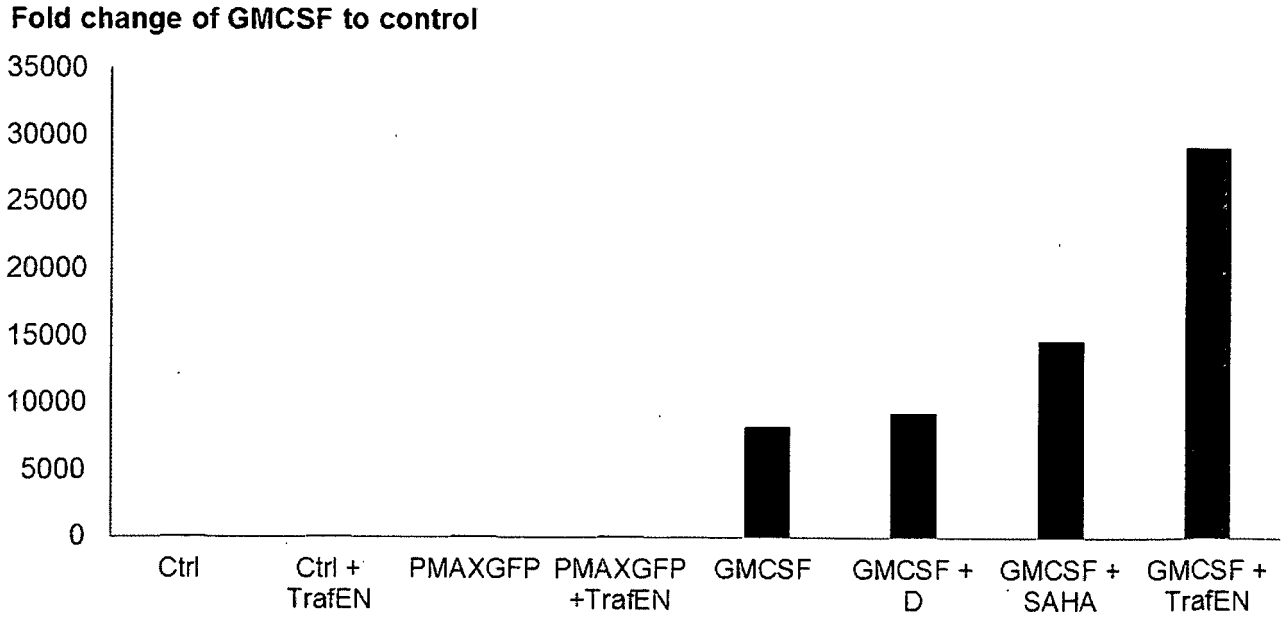


FIG. 59

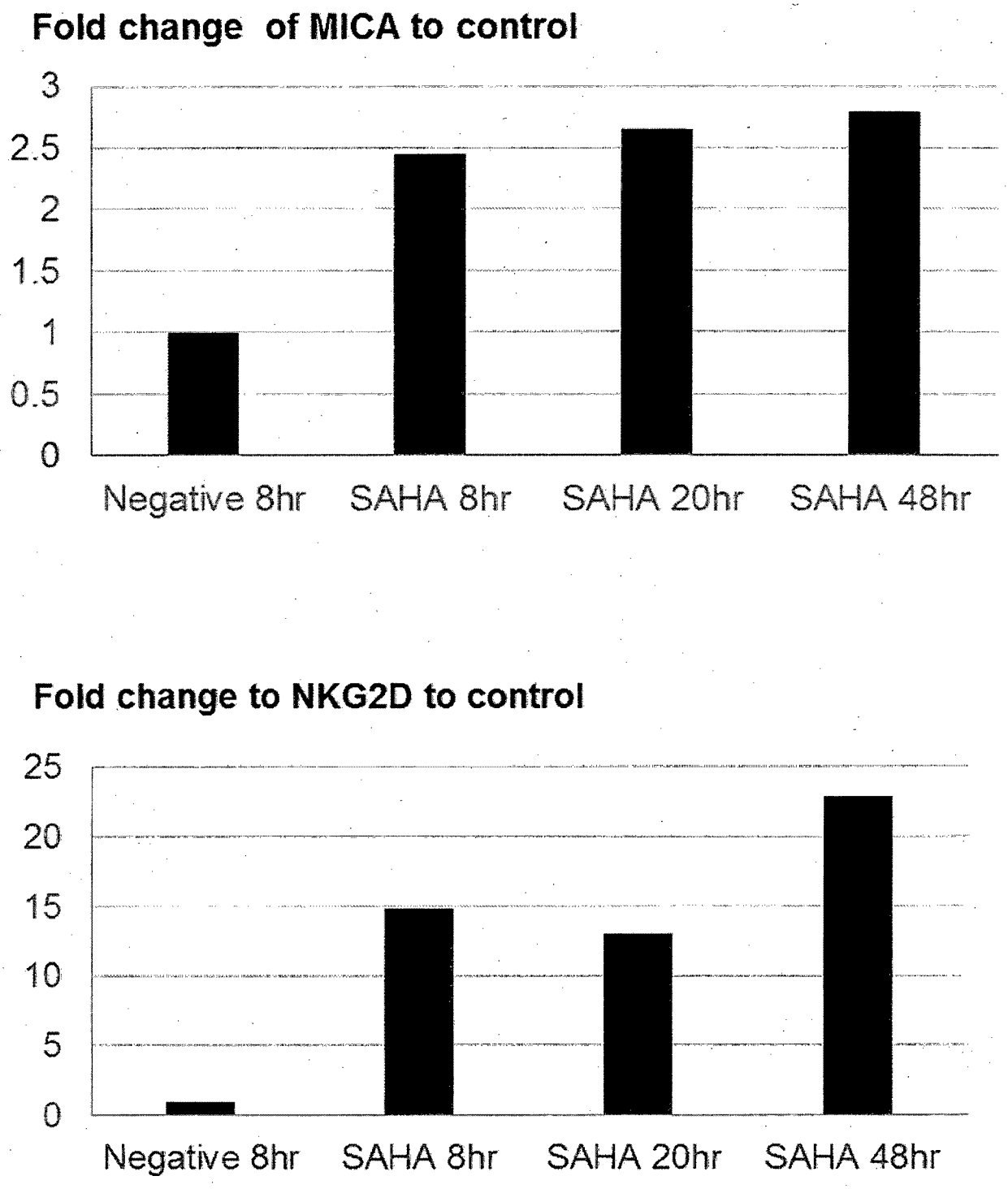
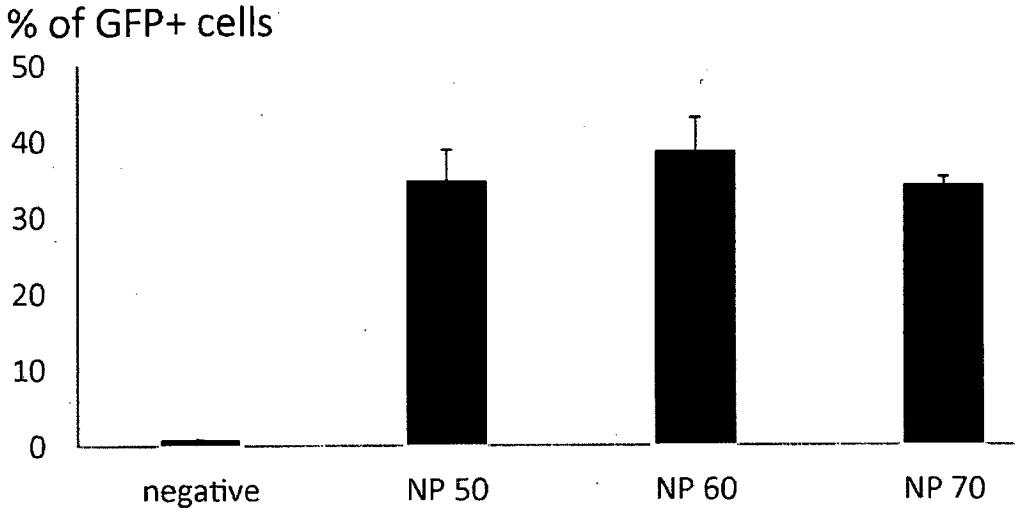

Fold change of MICA to control
3
2.5
2
1.5
1
0.5
0
Negative 8hr
SAHA 8hr
SAHA 20hr
SAHA 48hr
Fold change to NKG2D to control
25
20
15
10
5
0
Negative 8hr
SAHA 8hr
SAHA 20hr
SAHA 48hr

FIG. 60


% of GFP+ cells
50
40
30
20
10
0
negative
NP 50
NP 60
NP 70

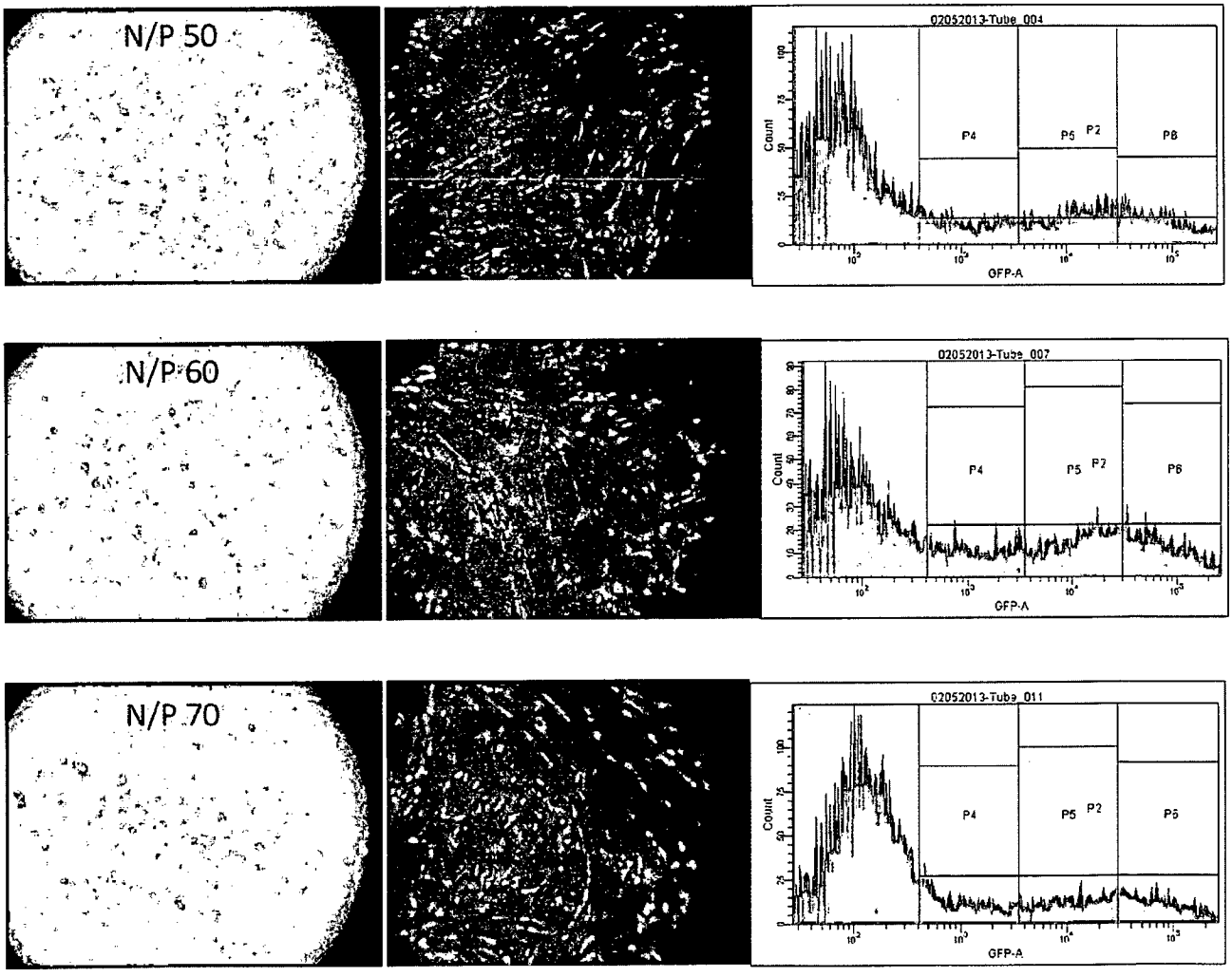
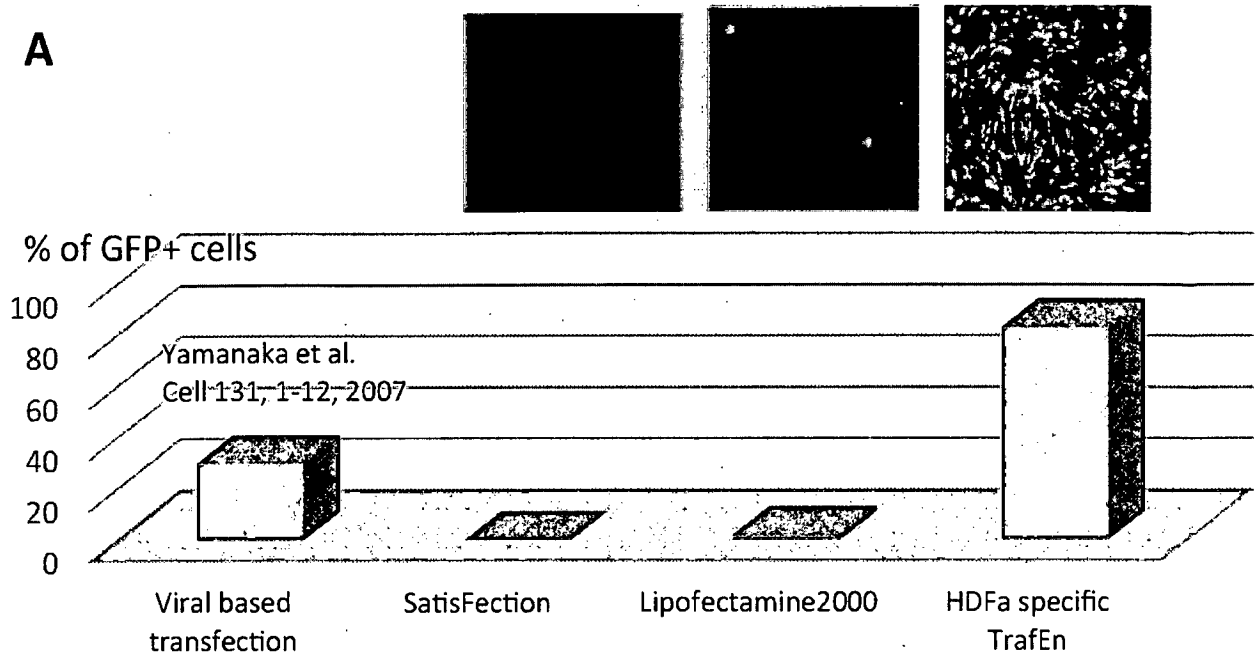

N/P 50
02052013-Tube_004
Count
P4
P5
P2
P6
GFP-A
N/P 60
02052013-Tube_007
Count
P4
P5
P2
P6
GFP-A
N/P 70
02052013-Tube_011
Count
P4
P5
P2
P6
GFP-A

FIG. 61


A
% of GFP+ cells
100
80
60
40
20
0
Yamanaka et al.
Cell 131, 1-12, 2007
Viral based transfection
SatisFection
Lipofectamine2000
HDFa specific TrafEn

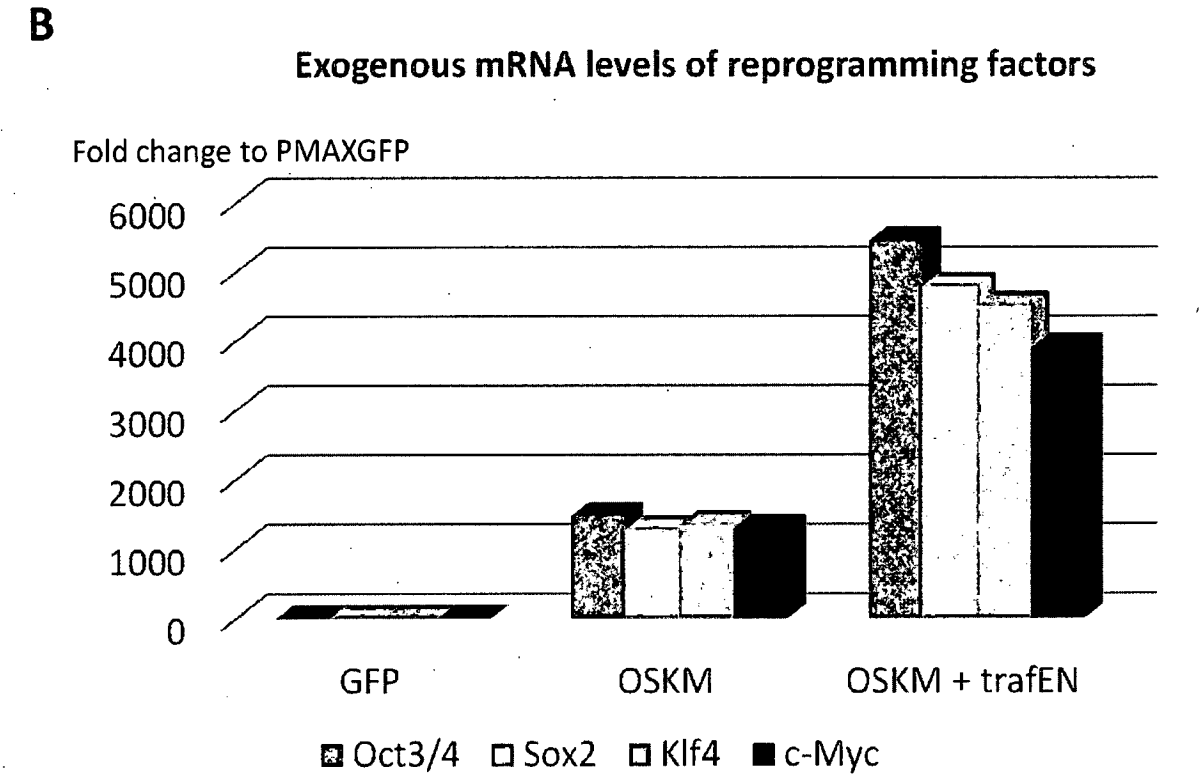

B
Exogenous mRNA levels of reprogramming factors
Fold change to PMAXGFP
6000
5000
4000
3000
2000
1000
0
GFP
OSKM
OSKM + trafEN
Oct3/4
Sox2
Klf4
c-Myc

FIG. 61 continued

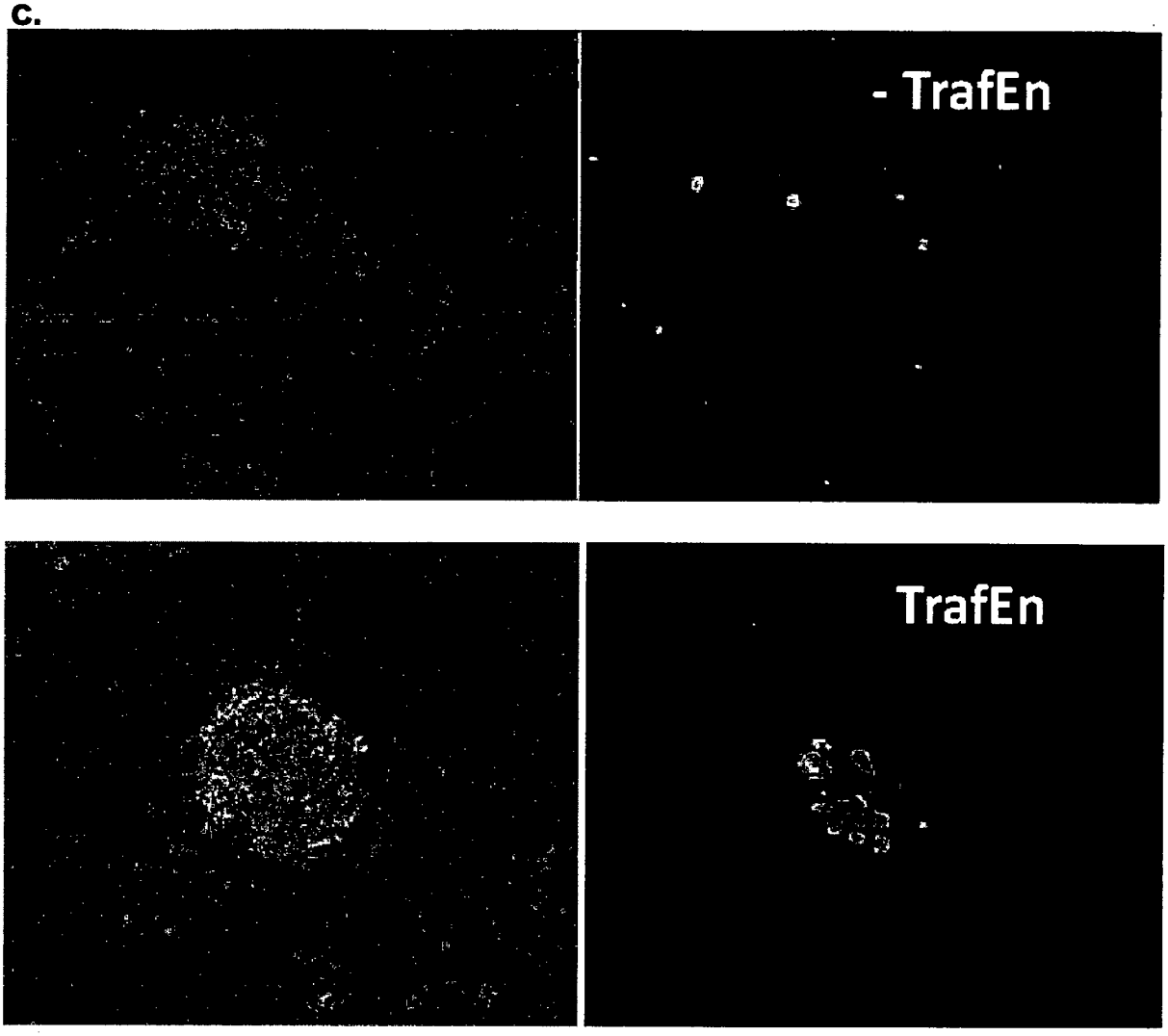


FIG. 62

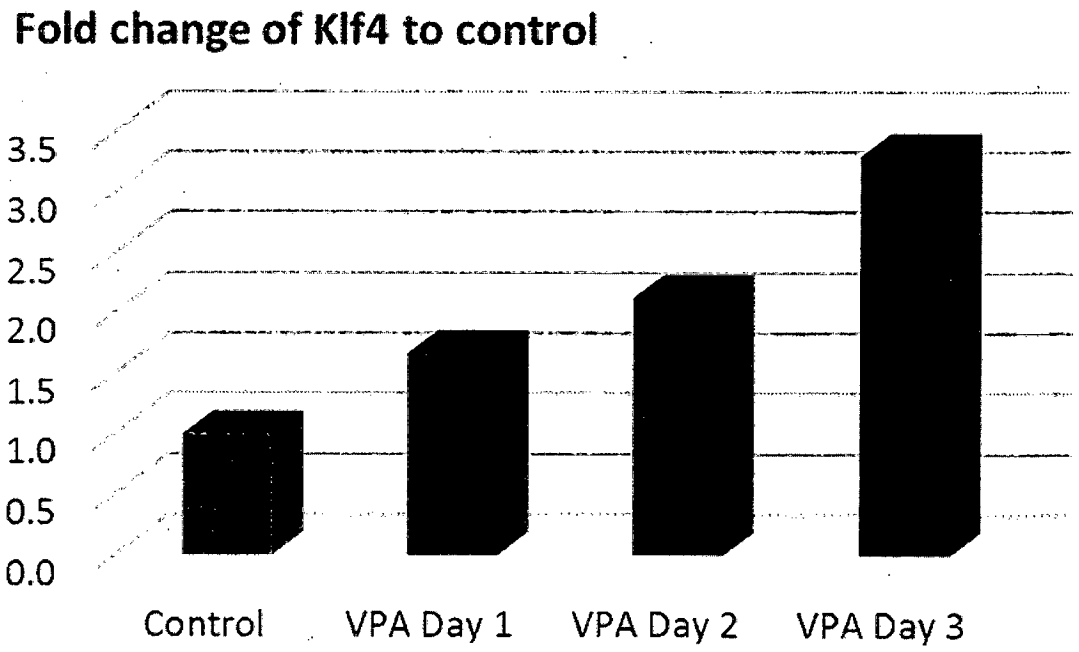


FIG. 62 continued

