FIG. 1

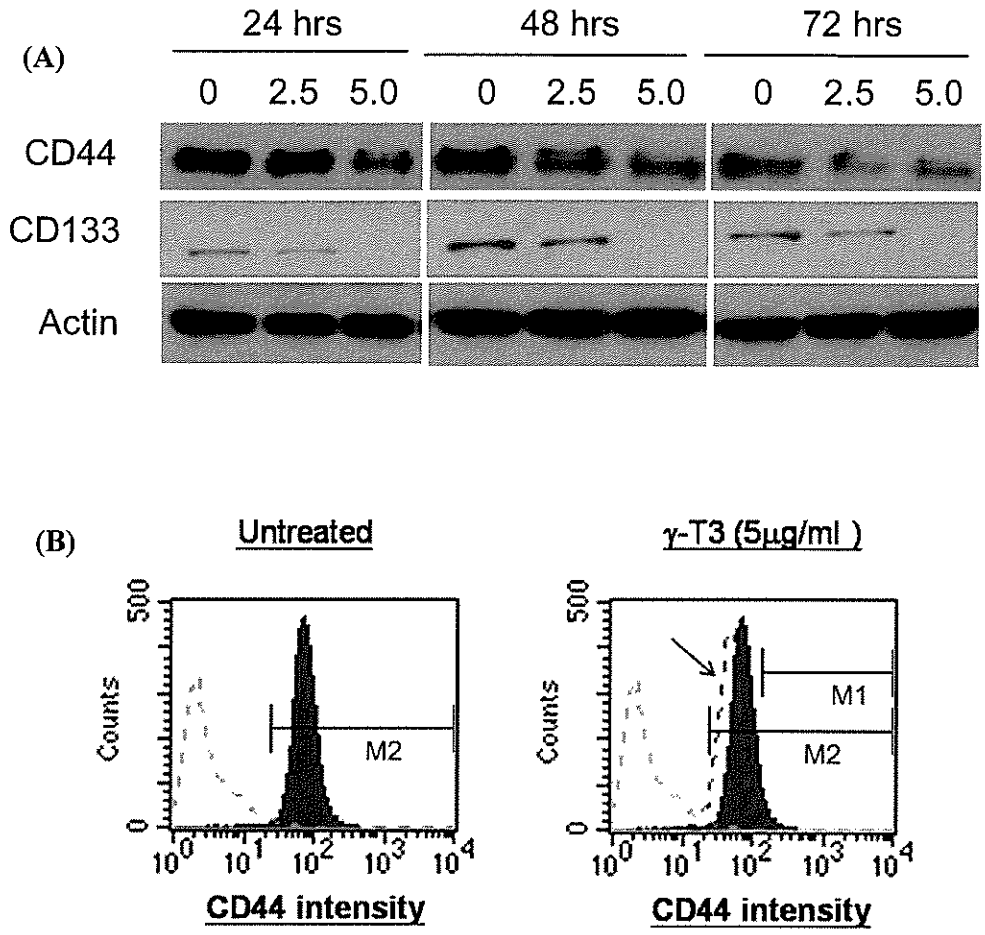
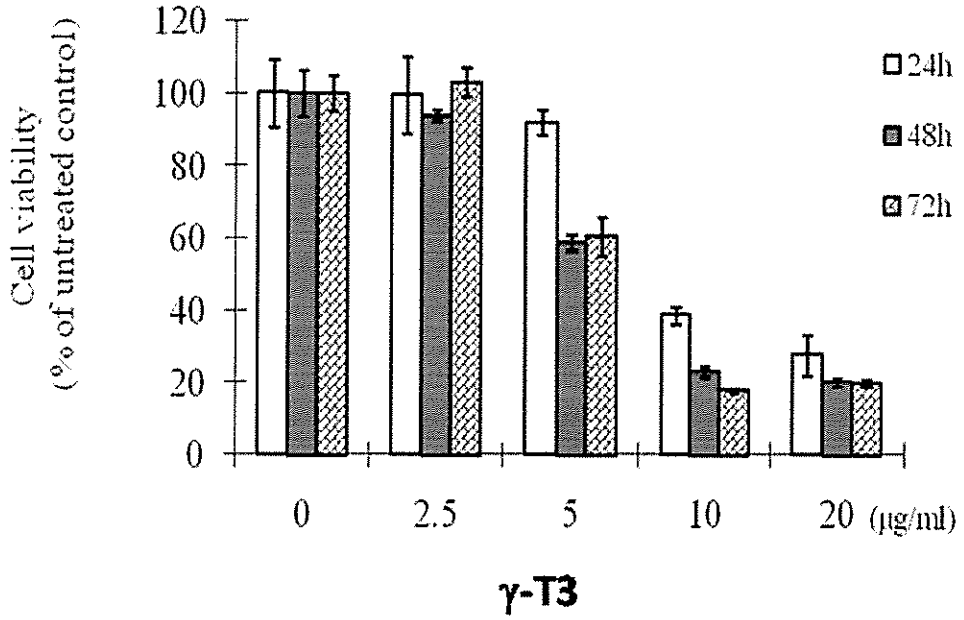

(A)
24 hrs
48 hrs
72 hrs
0 2.5 5.0 0 2.5 5.0 0 2.5 5.0
CD44
CD133
Actin
(B)
Untreated
γ-T3 (5μg/ml)
Counts
500
0
M1
M2
CD44 intensity
(C)
24hr
48hr
72hr
γ-T3 (μg/ml) 0 2.5 5.0 0 2.5 5.0 0 2.5 5.0
CD44
CD133
GAPDH

FIG. 1 (continued)

(D)



(E)

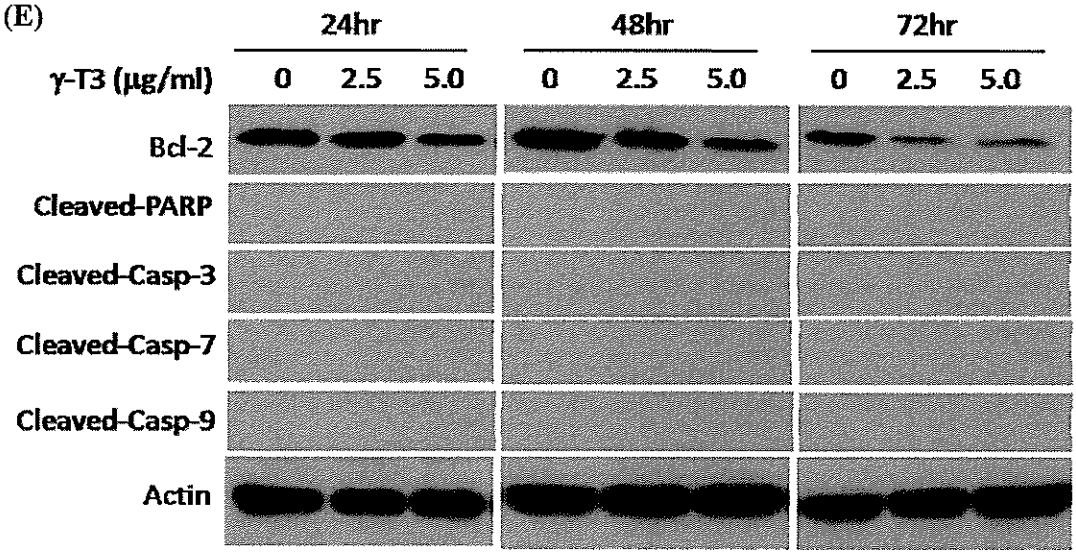
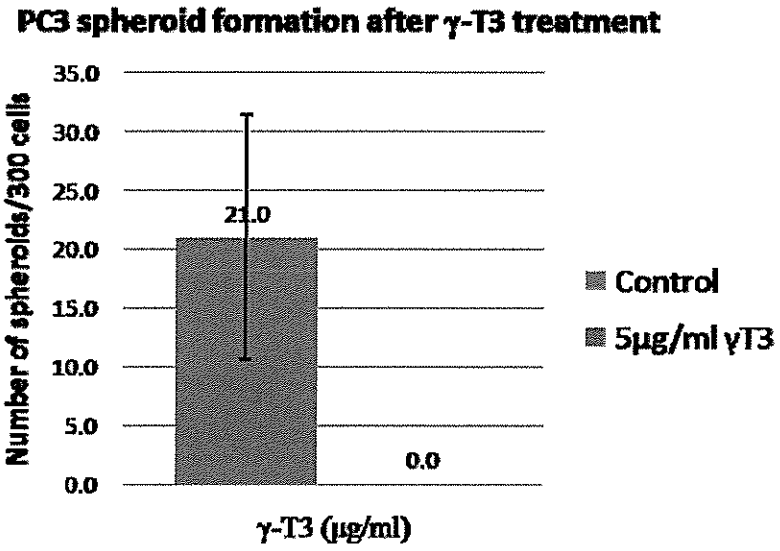


FIG. 2

(A)


PC3 spheroid formation after γ-T3 treatment
Number of spheroids/300 cells
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
21.0
0.0
γ-T3 (μg/ml)
Control
5μg/ml γT3

(B)

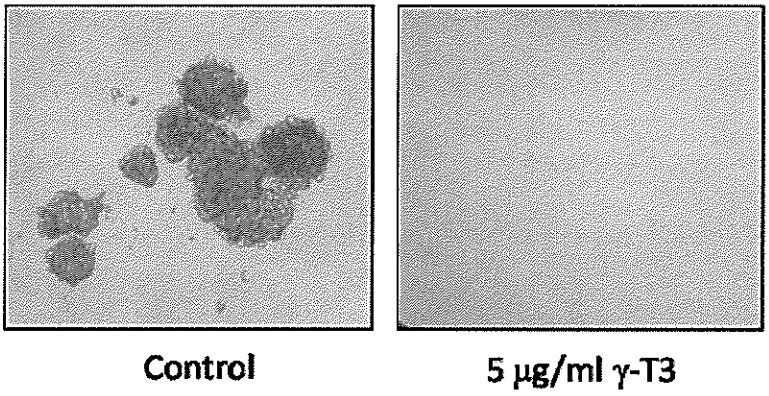
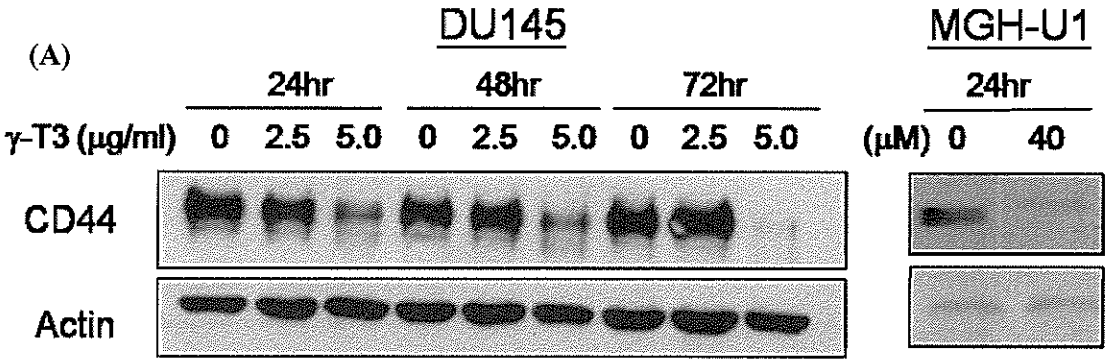

Control
5 μg/ml γ-T3

FIG. 3


(A)
DU145
MGH-U1
24hr
48hr
72hr
24hr
γ-T3 (μg/ml) 0 2.5 5.0 0 2.5 5.0 0 2.5 5.0
(μM) 0 40
CD44
Actin

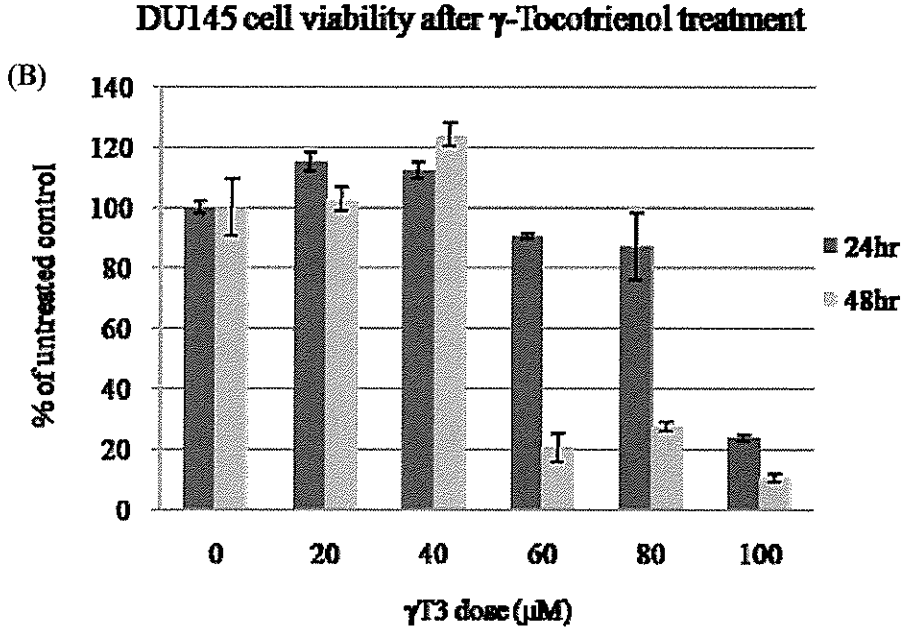
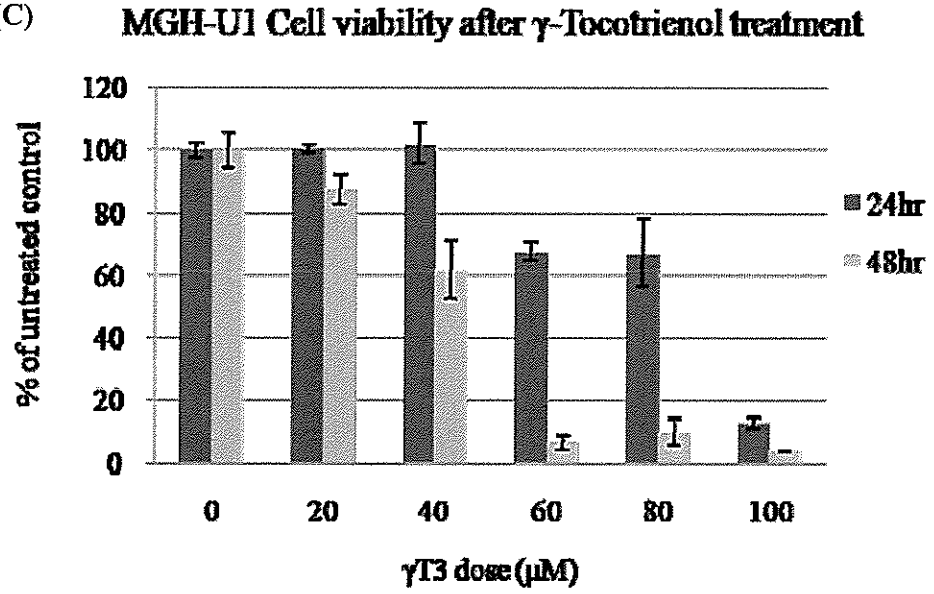
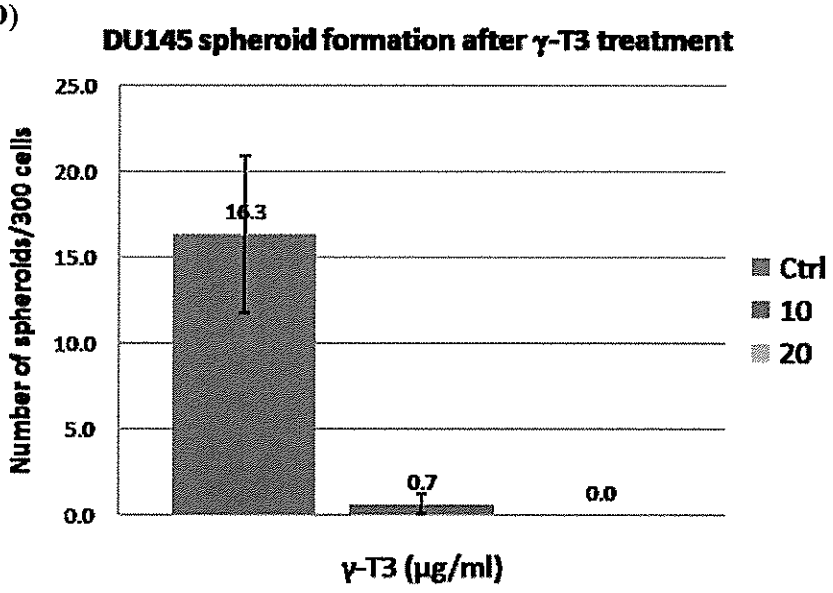

DU145 cell viability after γ-Tocotrienol treatment
(B)
% of untreated control
140
120
100
80
60
40
20
0
0
20
40
60
80
100
γT3 dose (μM)
24hr
48hr

FIG. 3 (continued)

(C)



(D)



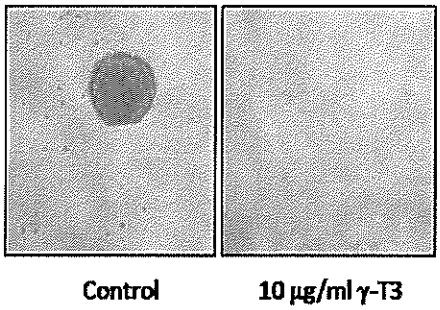


FIG. 3 (continued)

(E)

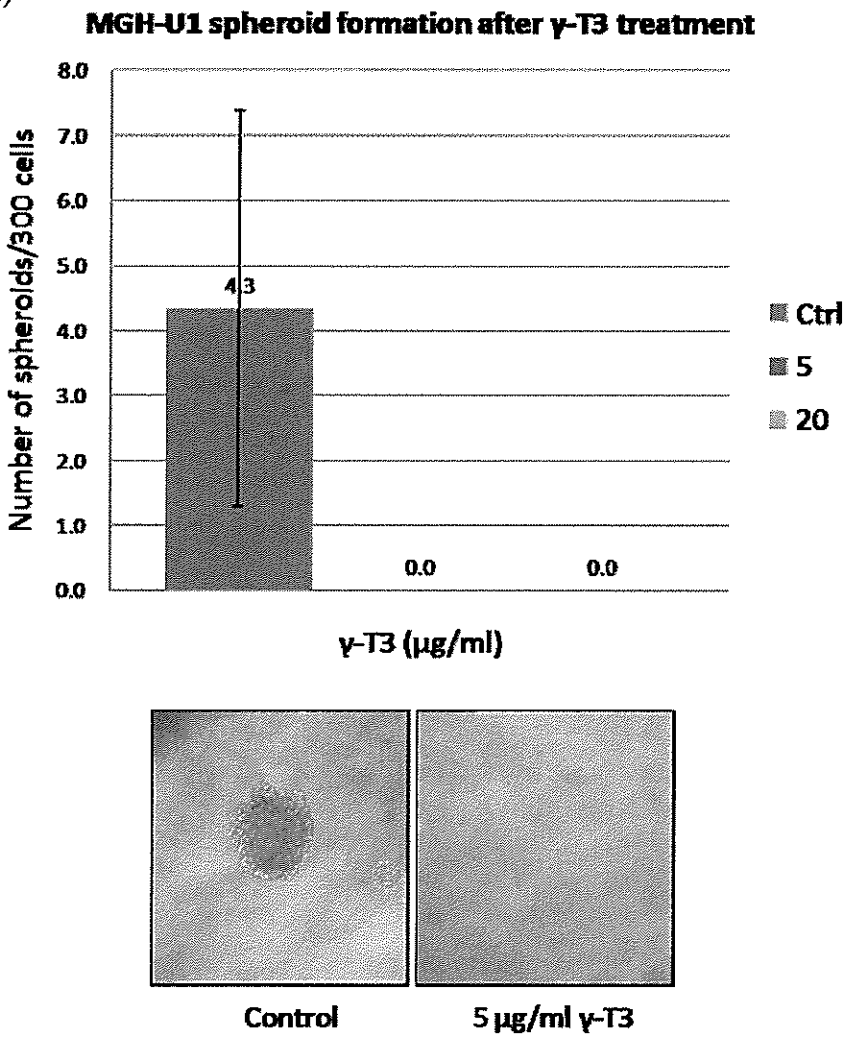
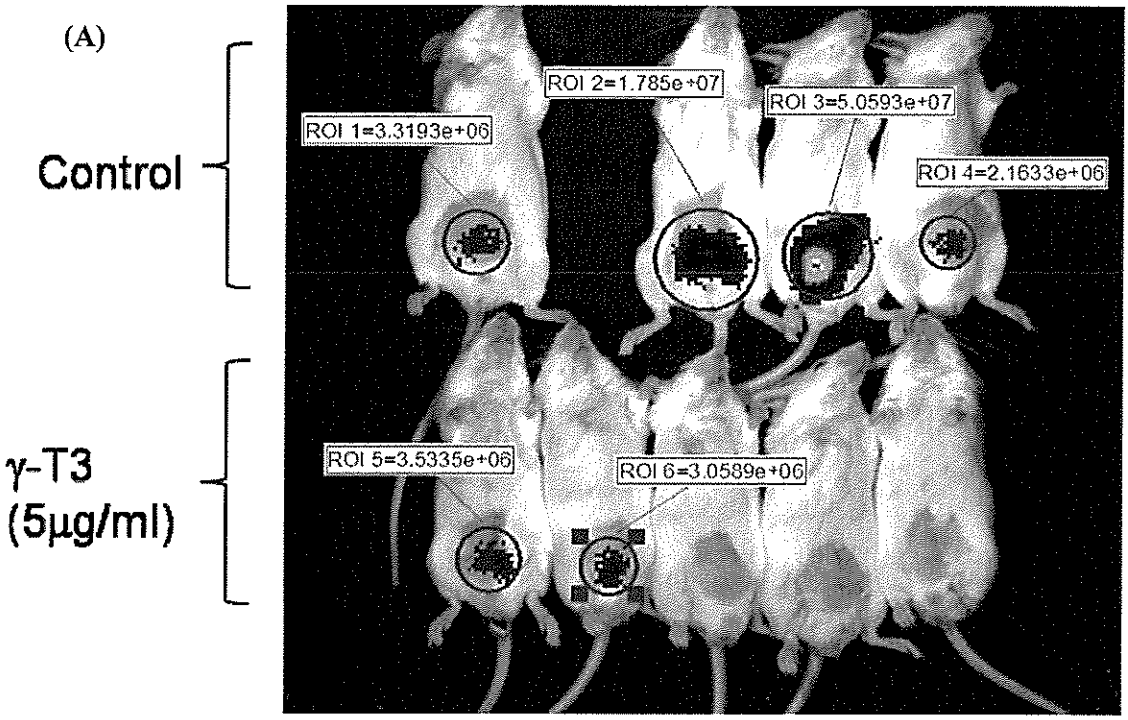


FIG. 4

(A)

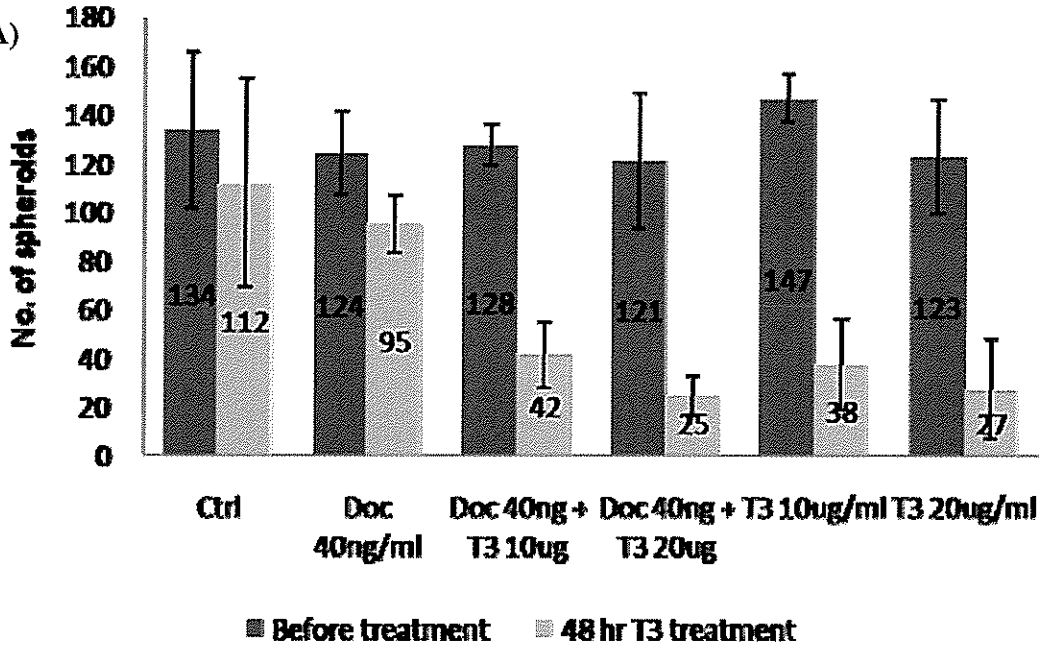


(B)

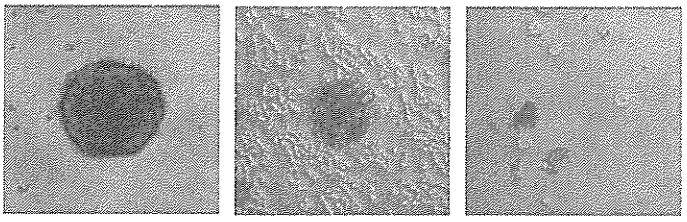
	Control	γT3 treated
Total no. of mice	7	9
Mice with visible tumor at week 2	7	4
% of tumor initiation	100%	44.4%

FIG. 5

(A)



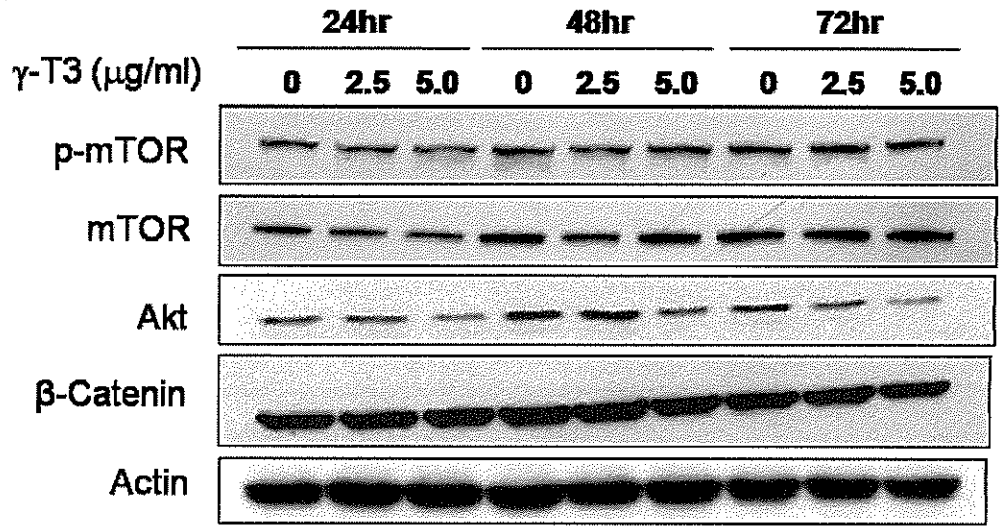
(B)



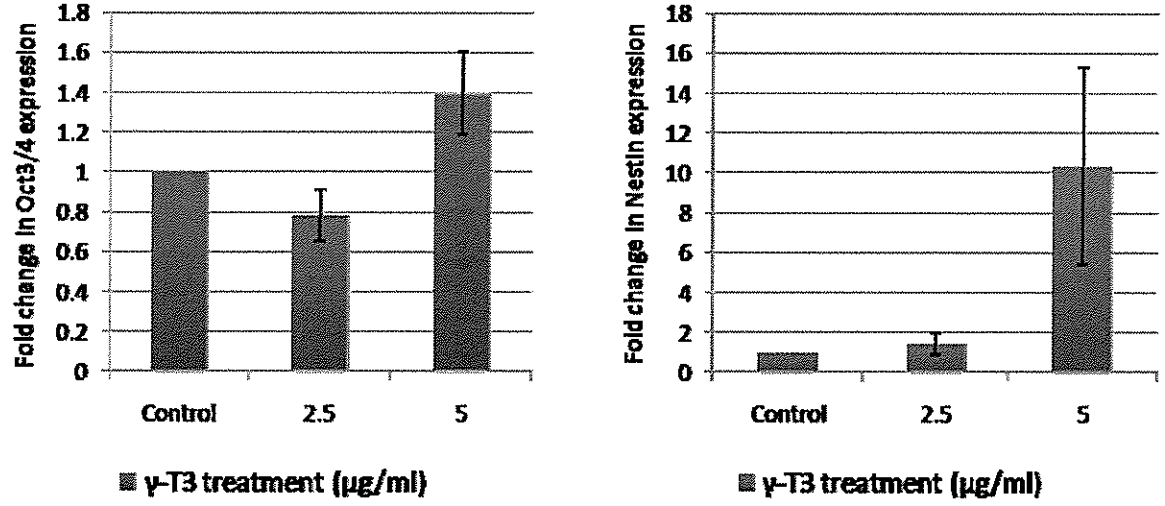
Vehicle | Docetaxel 40 ng/ml | γ-T3 10 μg/ml

FIG. 6

(A)


24hr
48hr
72hr
γ-T3 (μg/ml)
0 2.5 5.0 0 2.5 5.0 0 2.5 5.0
p-mTOR
mTOR
Akt
β-Catenin
Actin

(B)


Fold change in Oct3/4 expression
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
2.5
5
γ-T3 treatment (μg/ml)
Fold change in Nestin expression
18
16
14
12
10
8
6
4
2
0
Control
2.5
5
γ-T3 treatment (μg/ml)

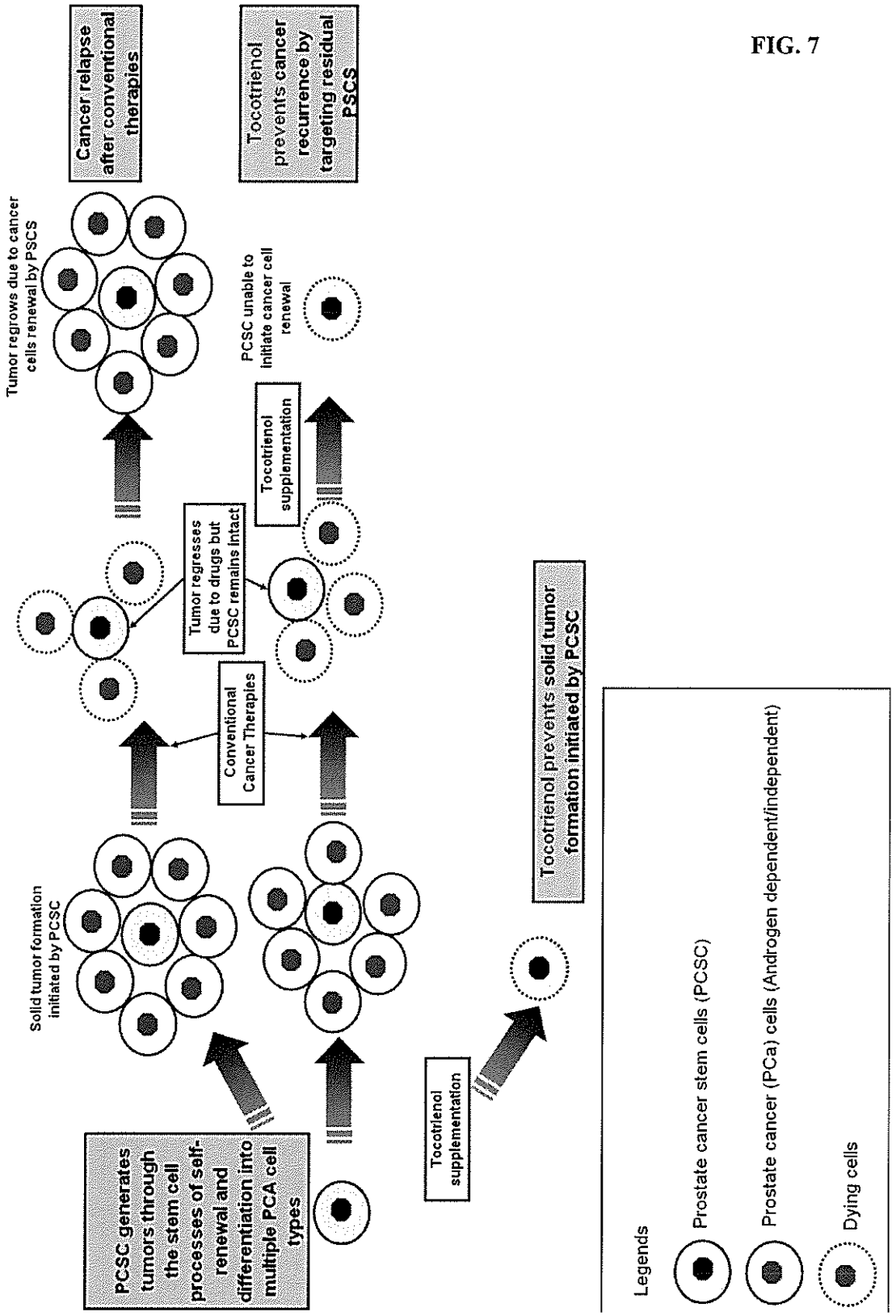

PCSC generates tumors through the stem cell processes of self-renewal and differentiation into multiple PCA cell types
Solid tumor formation initiated by PCSC
Conventional Cancer Therapies
Tumor regresses due to drugs but PCSC remains intact
Tocotrienol supplementation
PCSC unable to initiate cancer cell renewal
Tumor regrows due to cancer cells renewal by PSCS
Cancer relapse after conventional therapies
Tocotrienol prevents cancer recurrence by targeting residual PSCS
Tocotrienol supplementation
Tocotrienol prevents solid tumor formation initiated by PCSC
Legends
Prostate cancer stem cells (PCSC)
Prostate cancer (PCa) cells (Androgen dependent/independent)
Dying cells

FIG. 7

FIG. 8

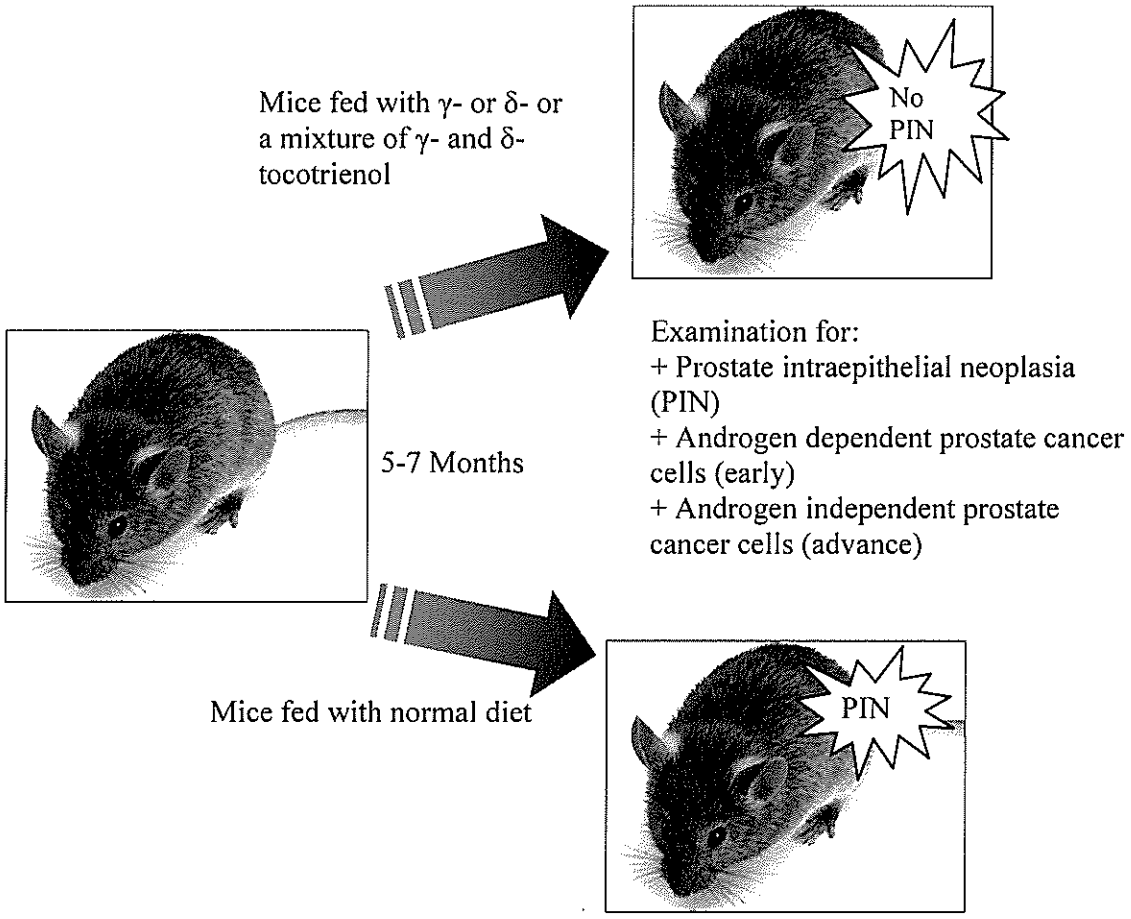

Mice fed with γ- or δ- or a mixture of γ- and δ- tocotrienol
No PIN
Examination for:
+ Prostate intraepithelial neoplasia (PIN)
+ Androgen dependent prostate cancer cells (early)
+ Androgen independent prostate cancer cells (advance)
5-7 Months
Mice fed with normal diet
PIN

FIG.9(A)

Mean cell viability in percentage (relative to control)

Tocopherols (T)

	α				β				γ				δ			
Incubation (hrs)	24		48		24		48		24		48		24		48	
Dose (μM)	20	80	20	80	20	80	20	80	20	80	20	80	20	80	20	80
LNCaP	106±19	92±35	102±13	77±17	91±8	75±24	103±25	72±17	81±26	67±17	81±4	52±7	103±16	93±18	84±14	83±11
PC-3	96±7	93±5	94±6	91±10	98±9	95±8	86±12	97±11	91±8	83±7	88±11	77±14	94±4	87±5	98±15	85±18
PZ-HPV7	108±8	98±5	103±15	104±16	103±8	99±11	96±12	96±8	99±6	100±8	97±8	99±14	84±11	90±10	90±8	94±13

Tocotrienols (T3)

	α					β				γ					δ				
Incubation (hrs)	24			48		24			48		24			48		24			48	
Dose (μM)	20	80	IC_{50}	20	80	20	80	IC_{50}	20	80	20	80	IC_{50}	20	80	20	80	IC_{50}	20	80
LNCaP	98±15	90±14	UD	96±14	94±20	93±10	91±7	UD	97±10	89±19	93±16	68±28	92	89±11	47±13	96±8	47±15	75	89±15	42±9
PC-3	105±8	98±8	UD	99±17	106±19	78±7	31±9	54	69±10	8±1	63±8	4±2	32	51±5	7±2	73±8	10±4	41	61±5	15±1
PZ-HPV7	98±7	105±9	UD	101±13	101±11	98±8	91±5	UD	93±9	91±10	94±9	91±10	UD	88±12	86±9	97±5	96±8	UD	89±10	86±10

FIG.9(B)

Mean cell preliferation in percentage (relative to time 0^{th})

	Control						100 μM α-T3						IC_{50} β-T3					
Incubation (hrs)	0	24	48	72	120	144	0	24	48	72	120	144	0	24	48	72	120	144
LNCaP	100	101±5	121±8	137±6	123±11	157±13	100	95±5	87±6	107±5	113±8	122±10	100	79±8	64±8	44±10	59±12	48±9
PC-3	100	102±7	106±5	108±9	155±8	174±10	100	102±7	106±9	118±6	136±9	144±9	100	82±8	43±6	48±5	26±4	22±5

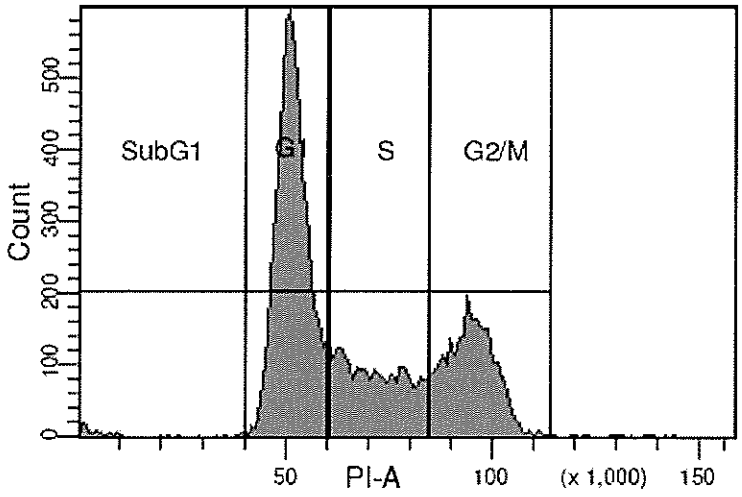
	IC_{50} γ-T3						IC_{50} δ-T3					
Incubation (hrs)	0	24	48	72	120	144	0	24	48	72	120	144
LNCaP	100	59±8	49±5	45±5	37±6	60±9	100	57±8	53±7	34±10	15±5	17±3
PC-3	100	53±6	10±3	8±3	2±2	3±2	100	59±5	18±6	9±2	5±2	3±2

FIG. 10

Control

(A)

PC-3



LNCaP

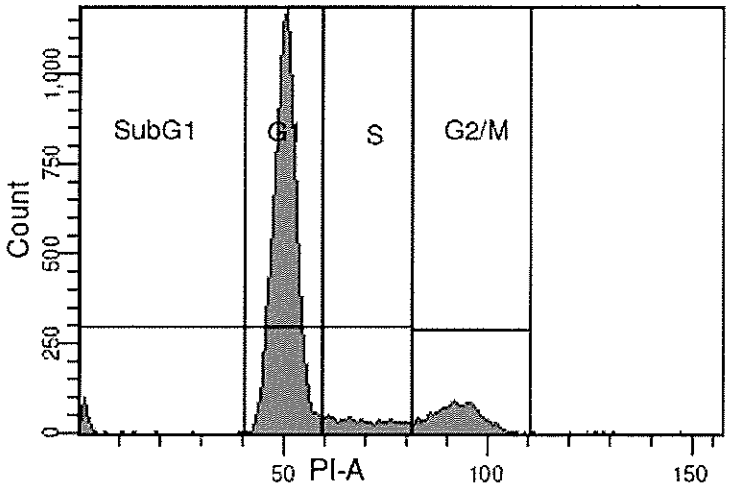
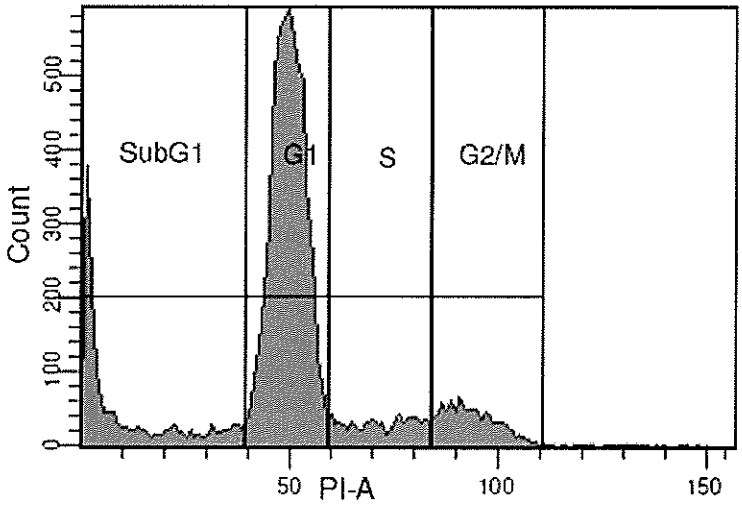


FIG. 10 (continued)

IC50 gamma Tocotrienol

(A)

PC-3



LCaP

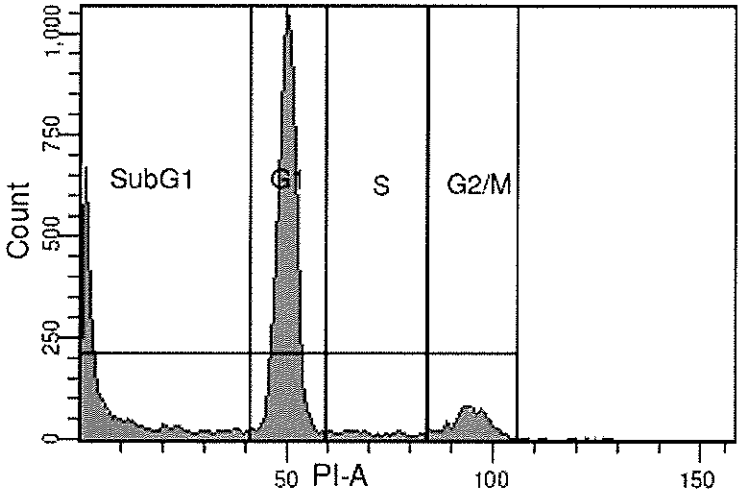
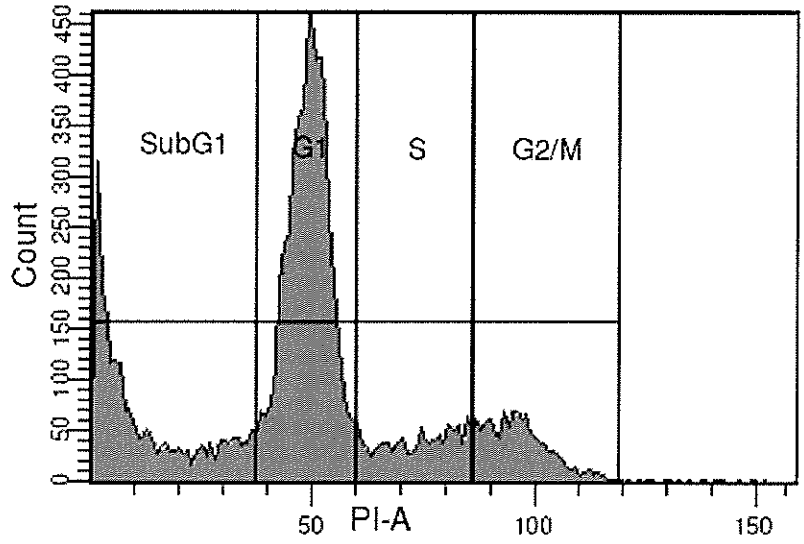


FIG. 10 (continued)

IC75 gamma Tocotrienol

(A)

PC-3



LCaP

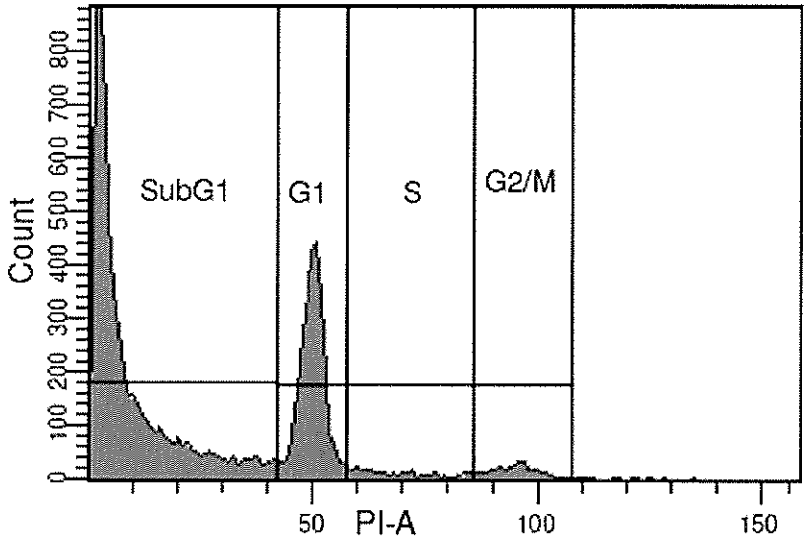


FIG. 10 (continued)

IC95 gamma Tocotrienol

(A)

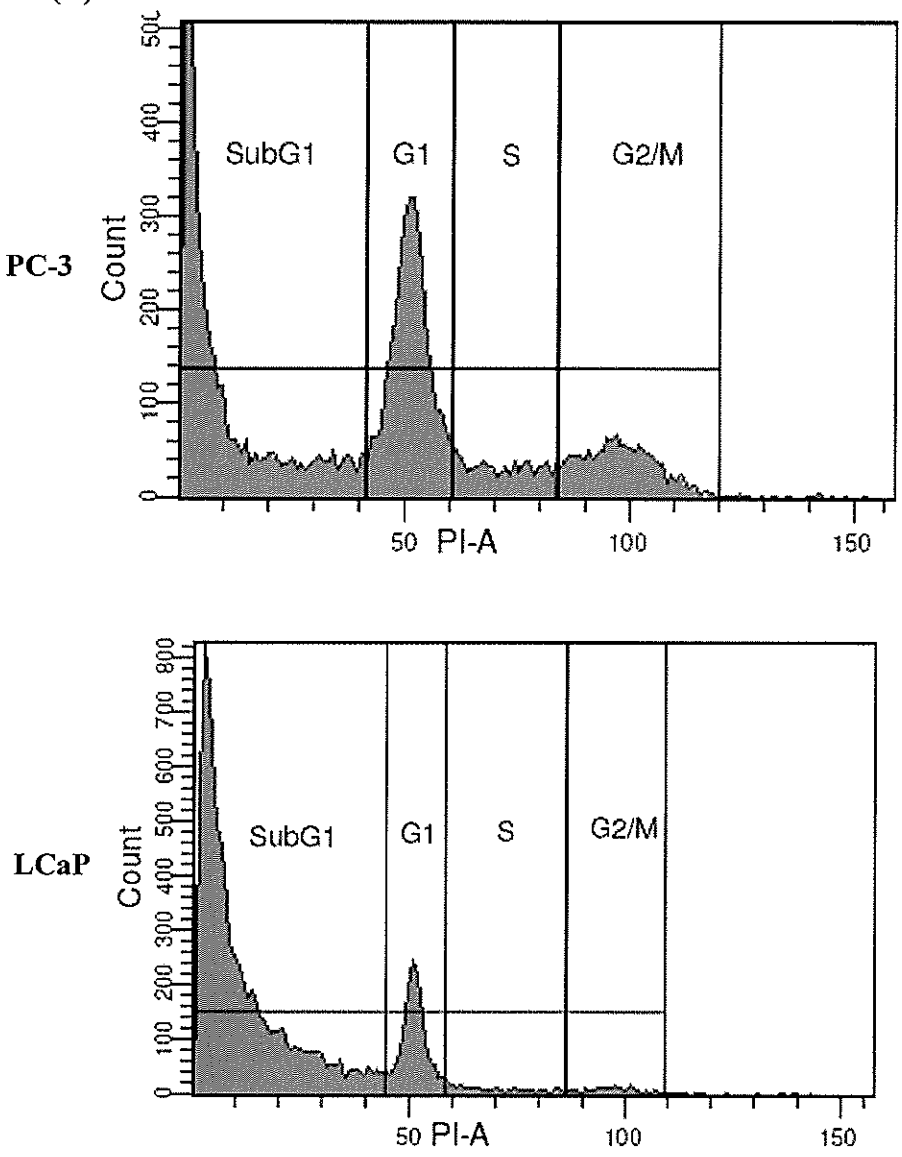


FIG. 10 (continued)

(A)

Mean percentage of cell population/%

	PC-3				LNCaP			
	sub G1	G1	S	G2	sub G1	G1	S	G2
Ctrl	0.9	51.1	21.8	35.2	2.1	77.4	7.7	12.6
IC_{50}	18.4	64.1	7.6	9.2	29.1	57.1	3.8	9.0
IC_{75}	24.7	52.5	10.9	11.7	66.1	26.8	3.3	3.6
IC_{95}	47.3	31.1	7.8	13.0	81.0	14.2	2.6	1.9

(B)

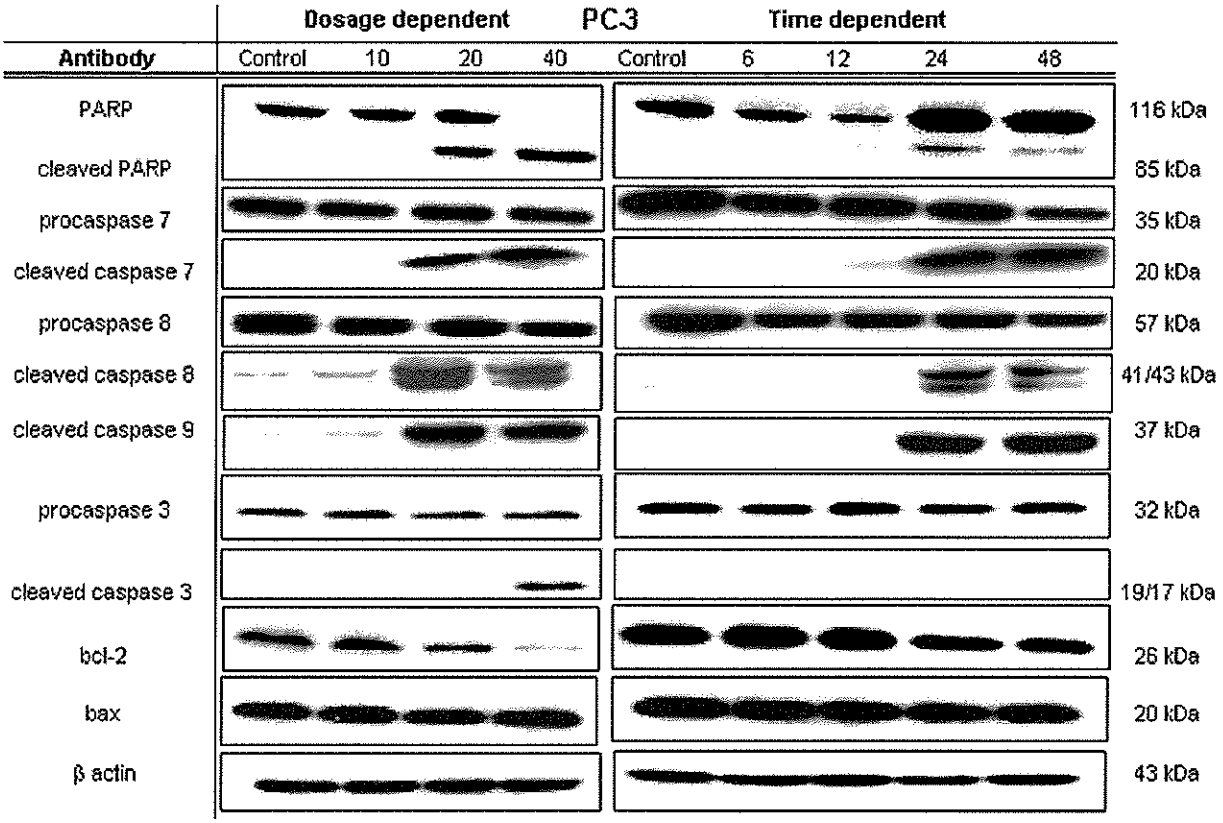
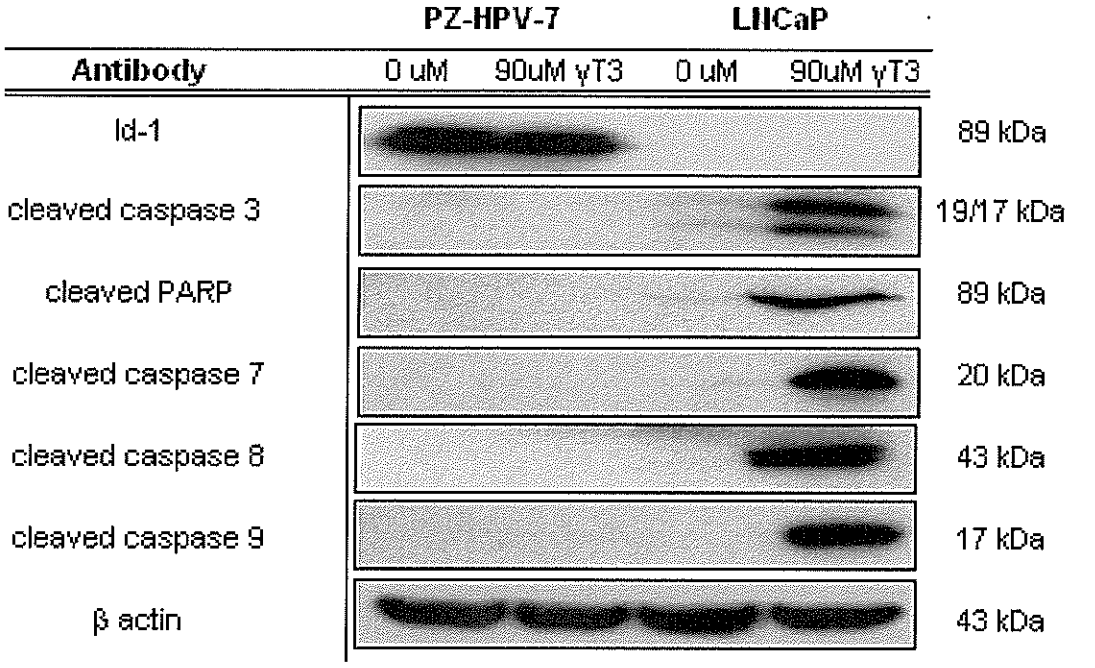


FIG. 10 (continued)

(C)



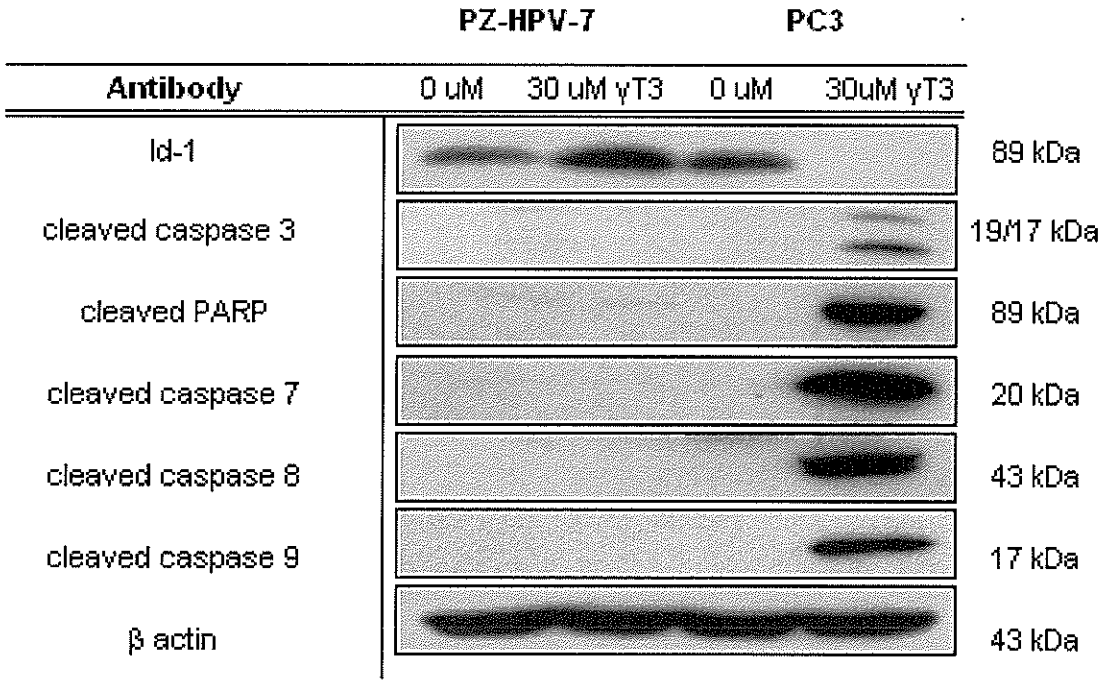
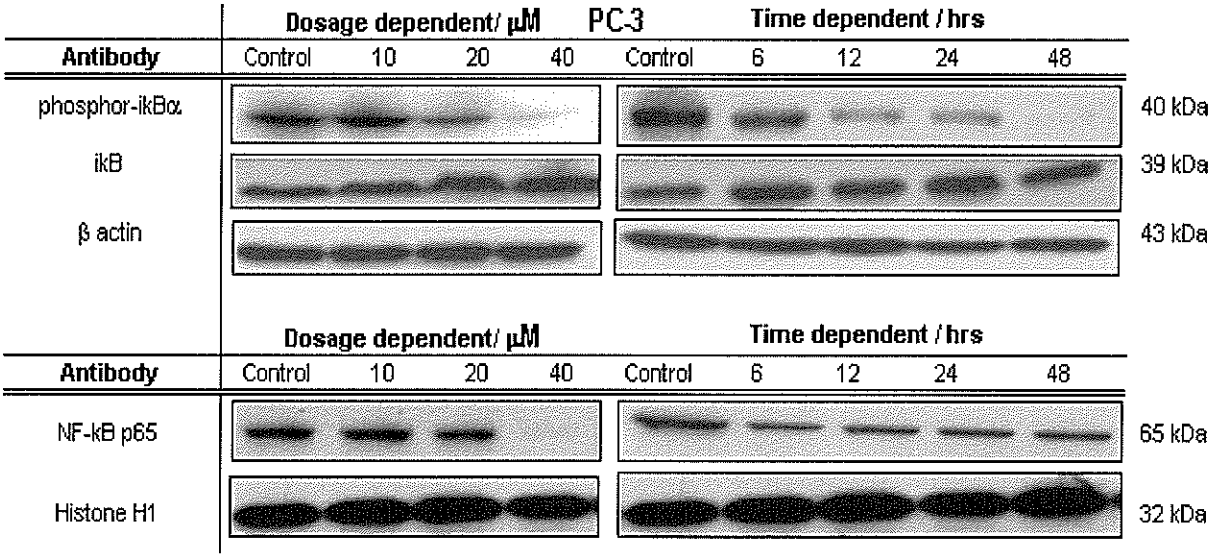


FIG. 11

(A)


Dosage dependent/ μM
PC-3
Time dependent / hrs
Antibody
Control
10
20
40
Control
6
12
24
48
phosphor-ikBα
ikB
β actin
40 kDa
39 kDa
43 kDa
Dosage dependent/ μM
Time dependent / hrs
Antibody
Control
10
20
40
Control
6
12
24
48
NF-kB p65
Histone H1
65 kDa
32 kDa

(B)

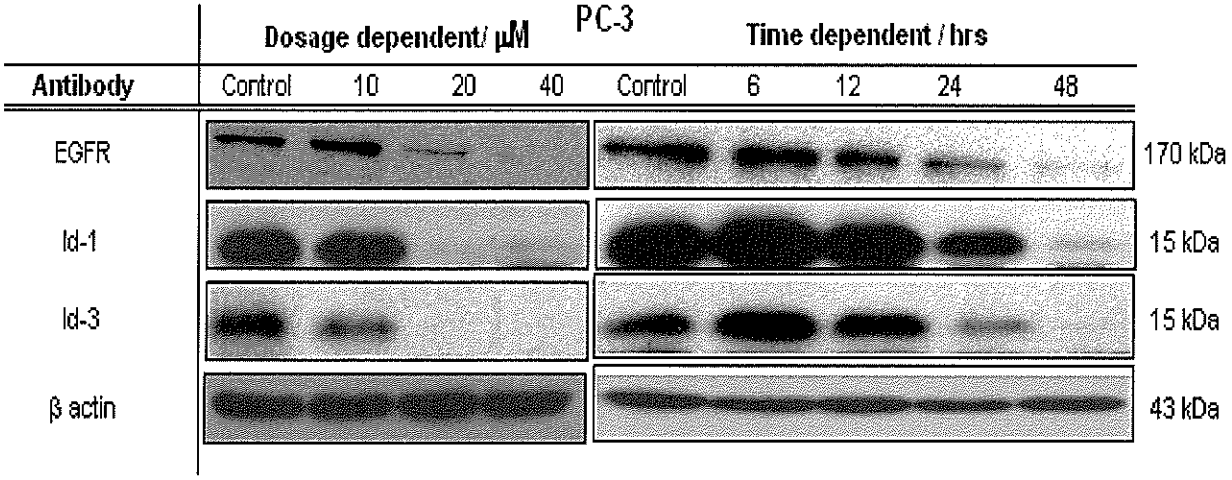
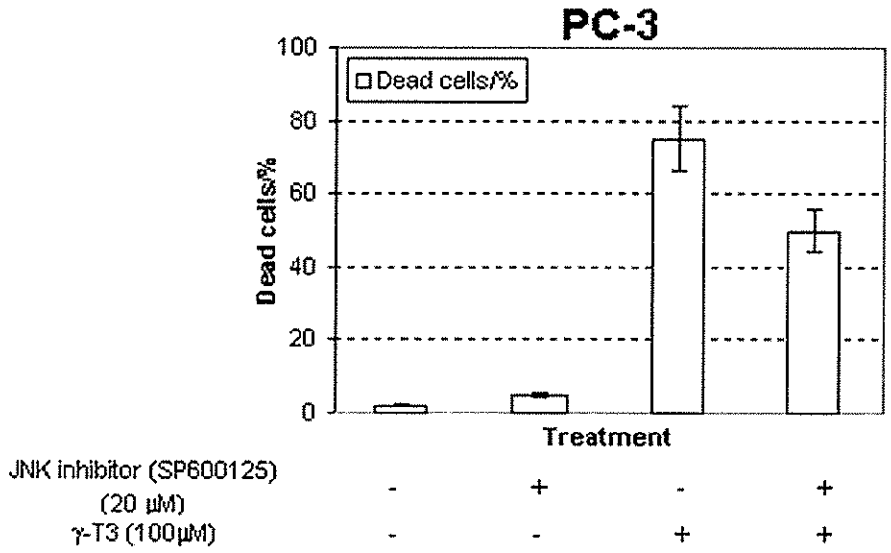

Dosage dependent/ μM
PC-3
Time dependent / hrs
Antibody
Control
10
20
40
Control
6
12
24
48
EGFR
Id-1
Id-3
β actin
170 kDa
15 kDa
15 kDa
43 kDa

FIG. 12

(A)


PC-3
Dead cells/%
100
80
60
40
20
0
Dead cells/%
Treatment
JNK inhibitor (SP600125)
(20 μM)
γ-T3 (100μM)
- + - +
- - + +

(B)

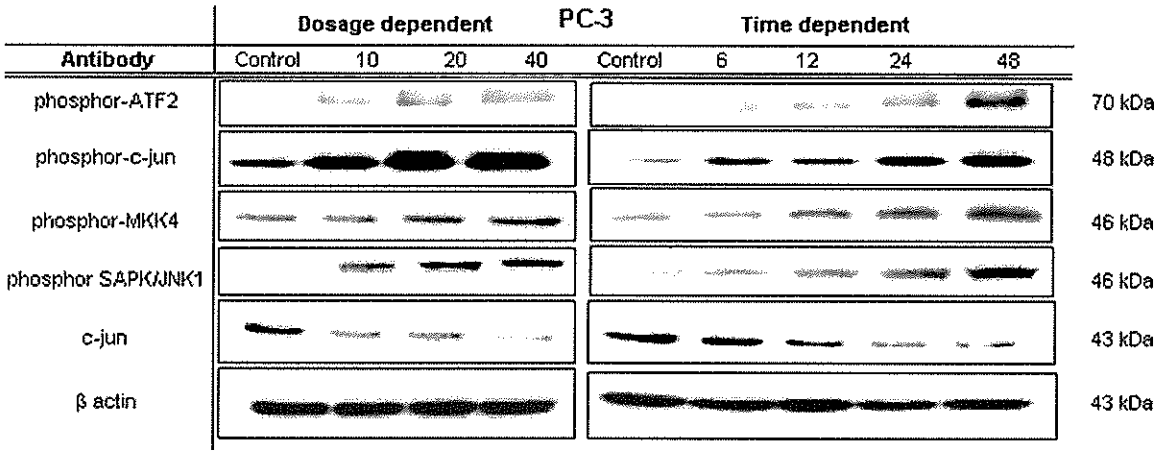
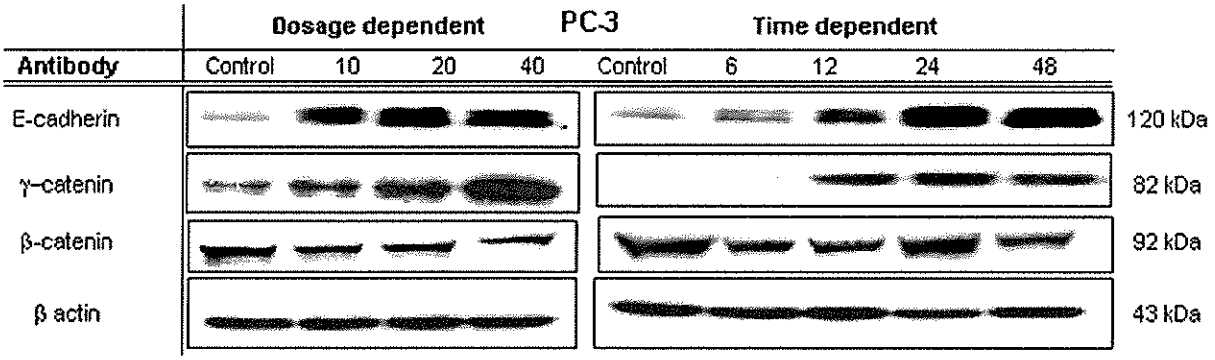

PC-3
Dosage dependent
Time dependent
Antibody
Control 10 20 40 Control 6 12 24 48
phosphor-ATF2
phosphor-c-jun
phosphor-MKK4
phosphor SAPK/JNK1
c-jun
β actin
70 kDa
48 kDa
46 kDa
46 kDa
43 kDa
43 kDa

FIG. 13

(A)


Dosage dependent
PC-3
Time dependent
Antibody
Control
10
20
40
Control
6
12
24
48
E-cadherin
γ-catenin
β-catenin
β actin
120 kDa
82 kDa
92 kDa
43 kDa

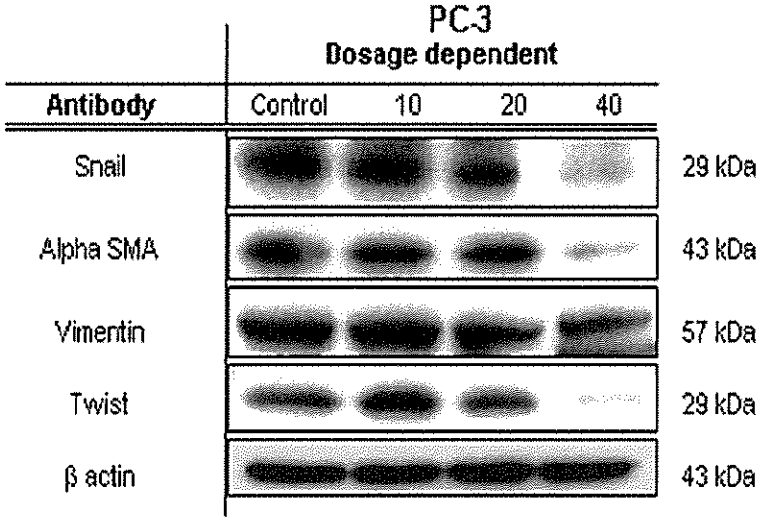

PC-3
Dosage dependent
Antibody
Control
10
20
40
Snail
Alpha SMA
Vimentin
Twist
β actin
29 kDa
43 kDa
57 kDa
29 kDa
43 kDa

FIG. 13 (continued)

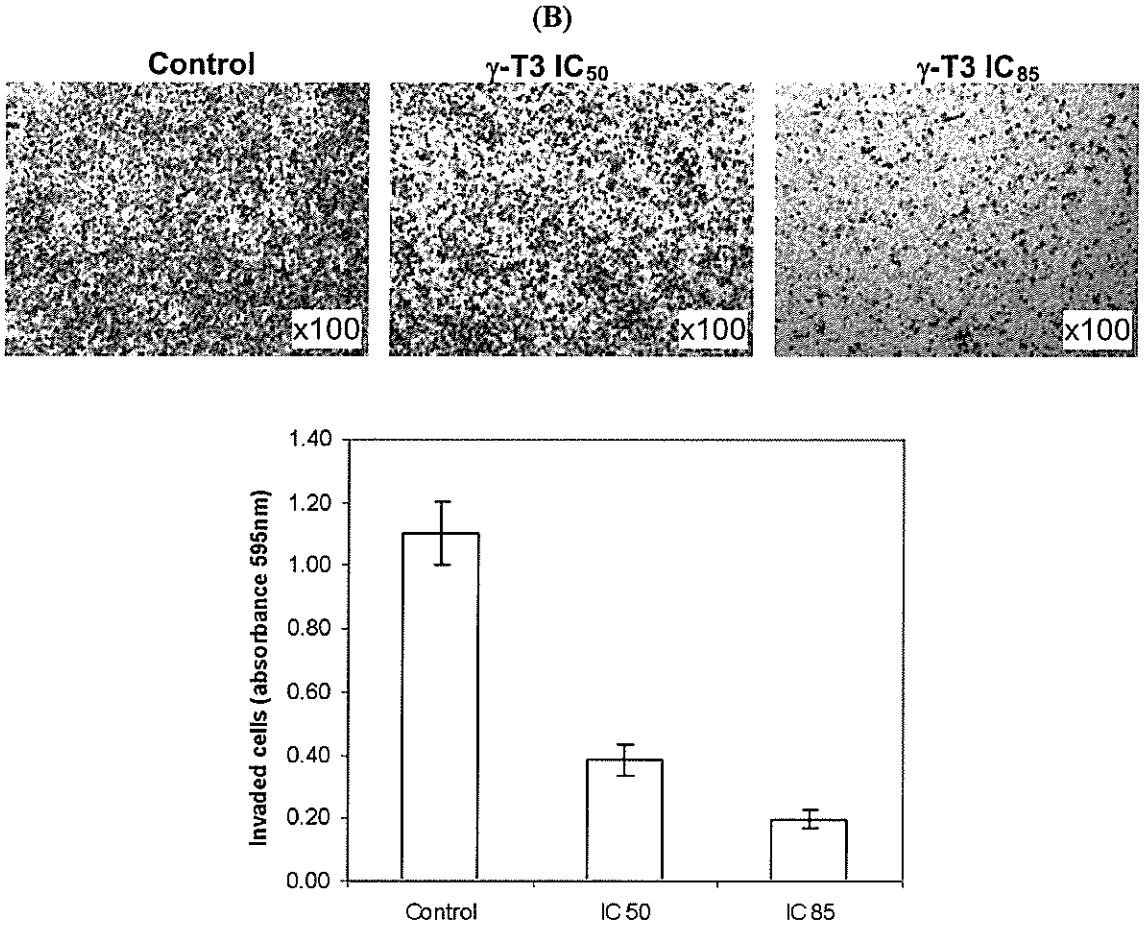


FIG. 14

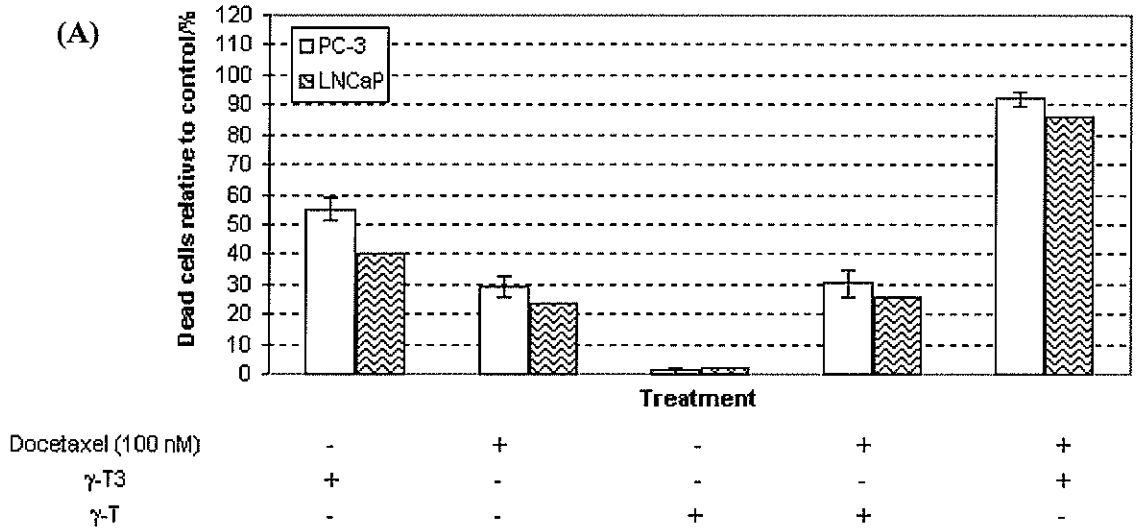
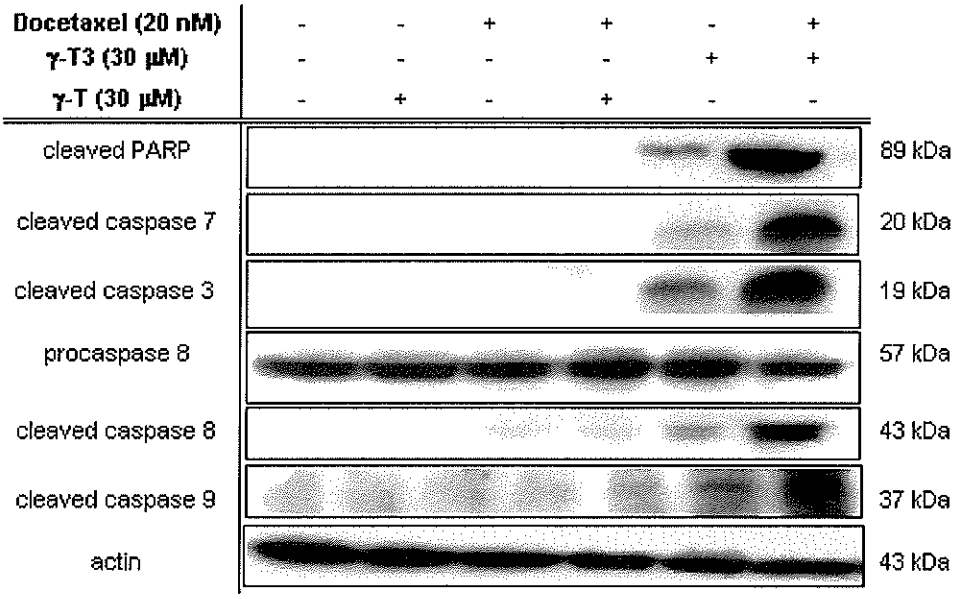


FIG. 14 (continued)

(B)



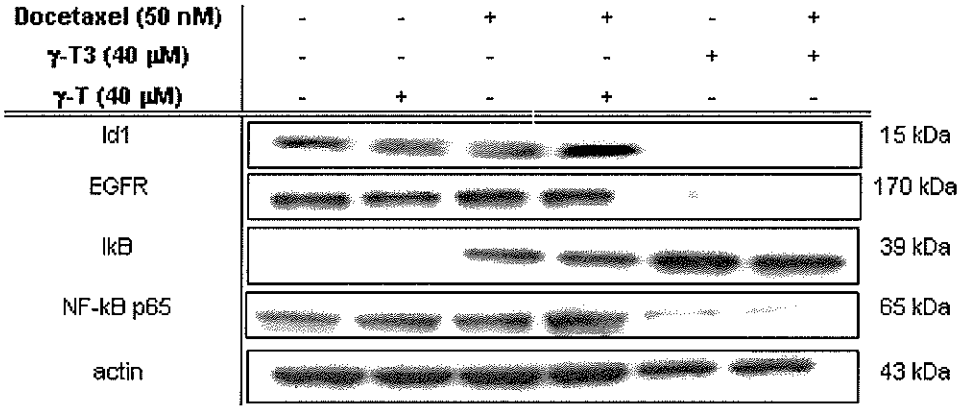


FIG. 14 (continued)

(C)

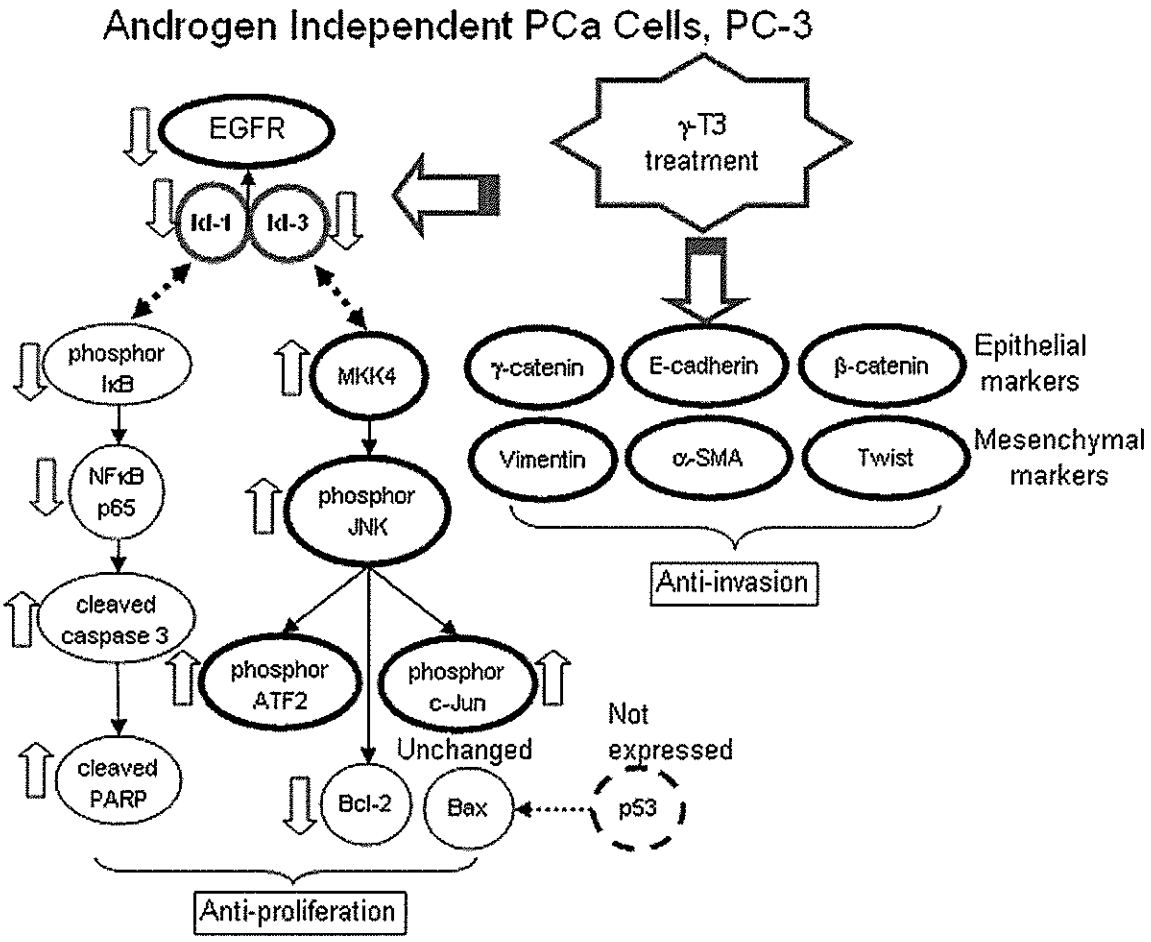


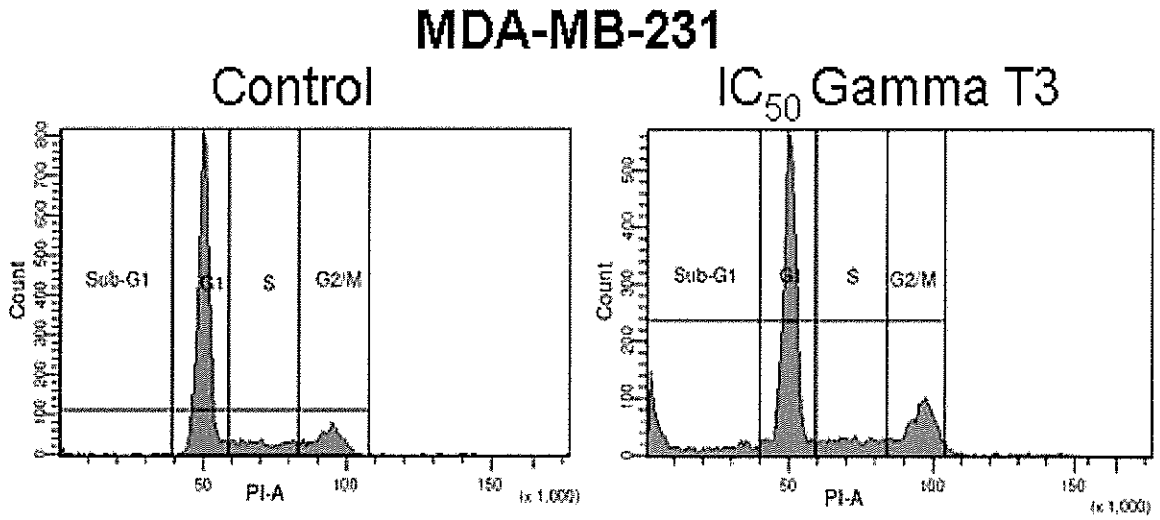
FIG. 15

(A)

Dose (uM)	24 Hours Treatment			
	α-TP	β-TP	γ-TP	δ-TP
	IC_{50}	IC_{50}	IC_{50}	IC_{50}
MCF10A MCF7 MDA-MB231	>80 μM			

Dose (uM)	24 Hours Treatment			
	α-T3	β-T3	γ-T3	δ-T3
	IC_{50}	IC_{50}	IC_{50}	IC_{50}
MCF10A	>80 μM			
MCF7		75±4	67±4	80±7
MDA-MB231		57±6	51±4	62±7

(B)



Population %	Ctrl	IC_{50}	IC_{75}	IC_{90}
Sub-G1	1.6	16.2	21.9	26
G1	62.9	52.2	51	47.8
S	10.3	10.6	10.6	10.1
G2/M	24	18.1	15.3	13.6

FIG. 15 (continued)

(C)

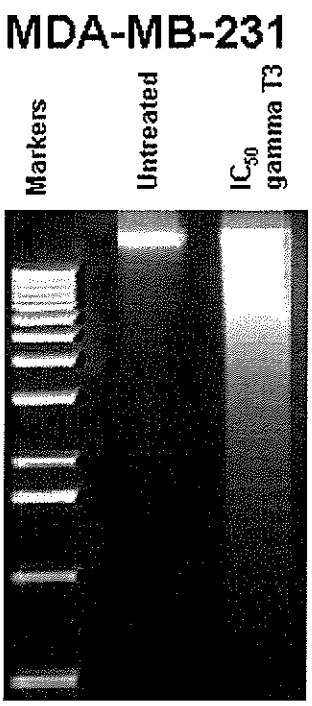
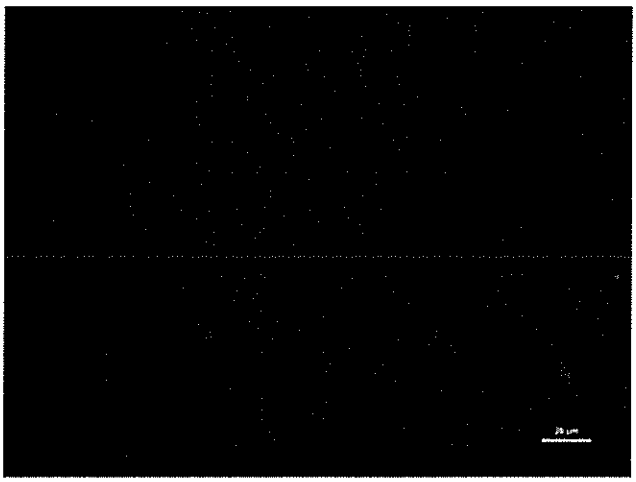


FIG. 15 (continued)

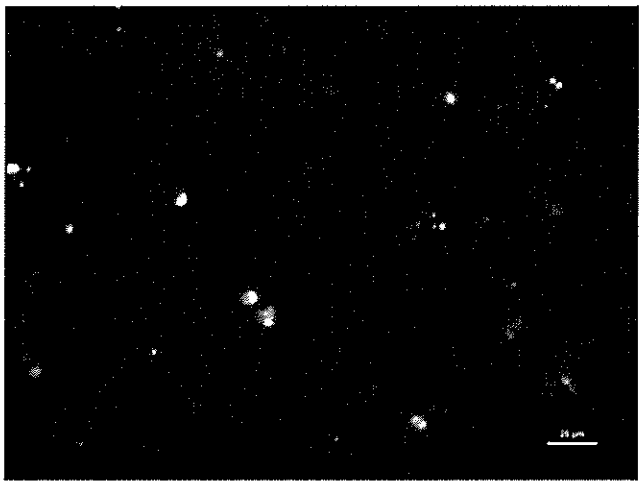
FIG. 15(D)

MDA-MB-231

Untreated,
Control 100 x,
green



20 μM gamma T3, 100x



40 μM gamma T3, 100x

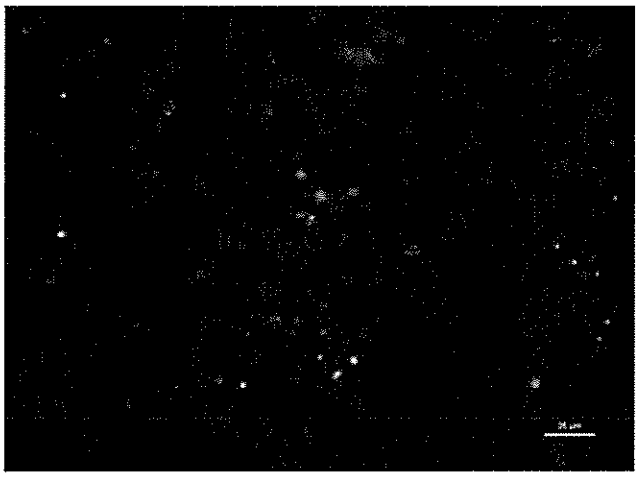
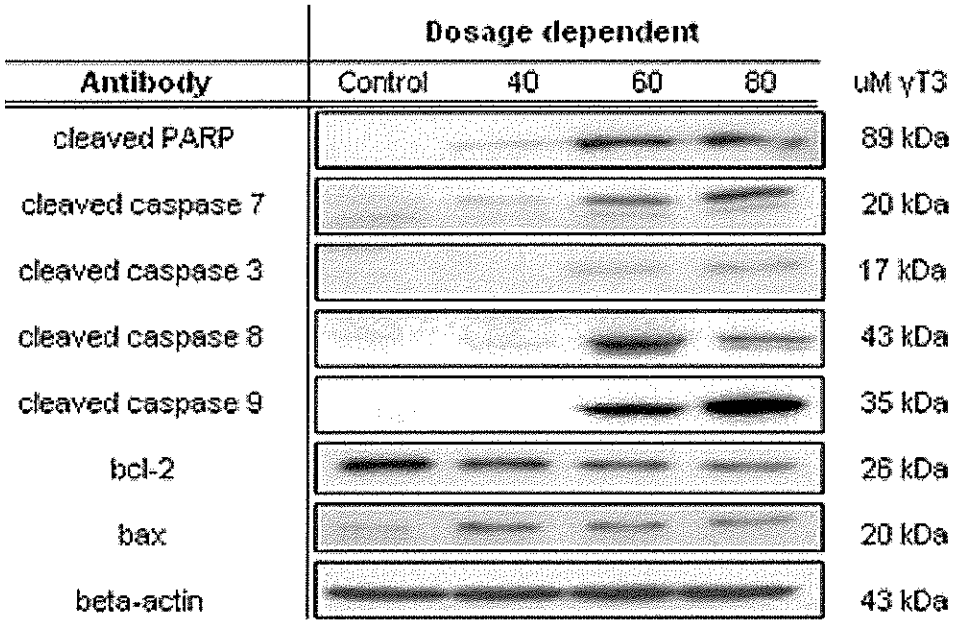


FIG. 16

(A)


Dosage dependent
Antibody
Control
40
60
80
uM γT3
cleaved PARP
89 kDa
cleaved caspase 7
20 kDa
cleaved caspase 3
17 kDa
cleaved caspase 8
43 kDa
cleaved caspase 9
35 kDa
bcl-2
26 kDa
bax
20 kDa
beta-actin
43 kDa

(B)

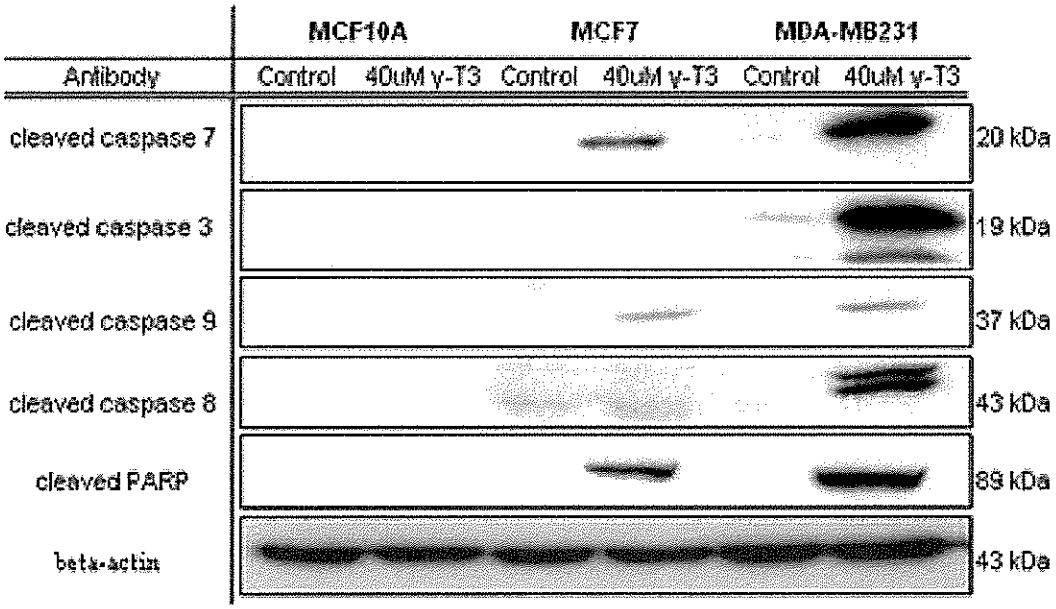
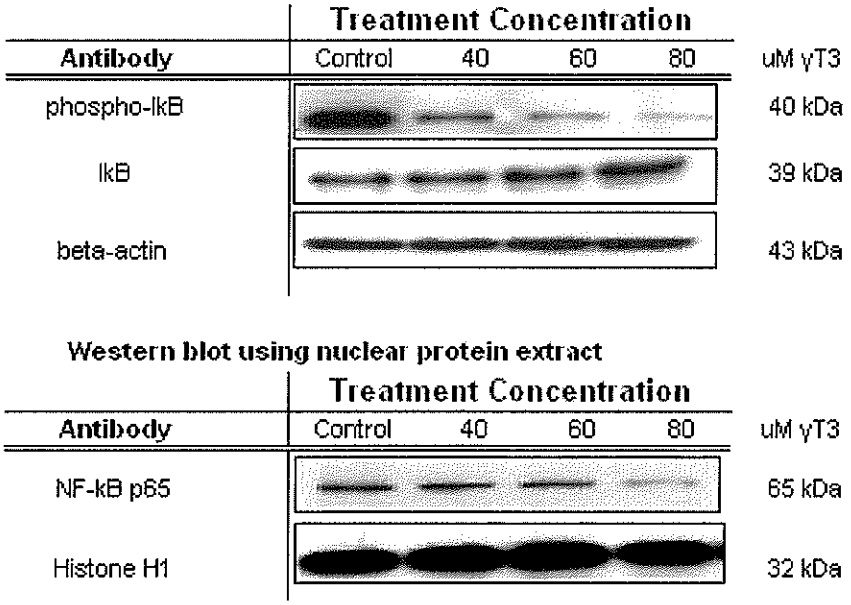

MCF10A
MCF7
MDA-MB231
Antibody
Control
40uM γ-T3
Control
40uM γ-T3
Control
40uM γ-T3
cleaved caspase 7
20 kDa
cleaved caspase 3
19 kDa
cleaved caspase 9
37 kDa
cleaved caspase 8
43 kDa
cleaved PARP
89 kDa
beta-actin
43 kDa

FIG. 17

(A)


Treatment Concentration
Antibody
Control
40
60
80
uM γT3
phospho-IkB
IkB
beta-actin
40 kDa
39 kDa
43 kDa
Western blot using nuclear protein extract
Treatment Concentration
Antibody
Control
40
60
80
uM γT3
NF-kB p65
Histone H1
65 kDa
32 kDa

(B)

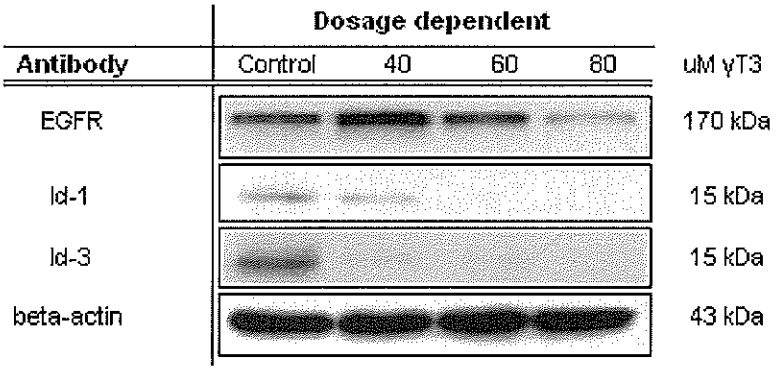
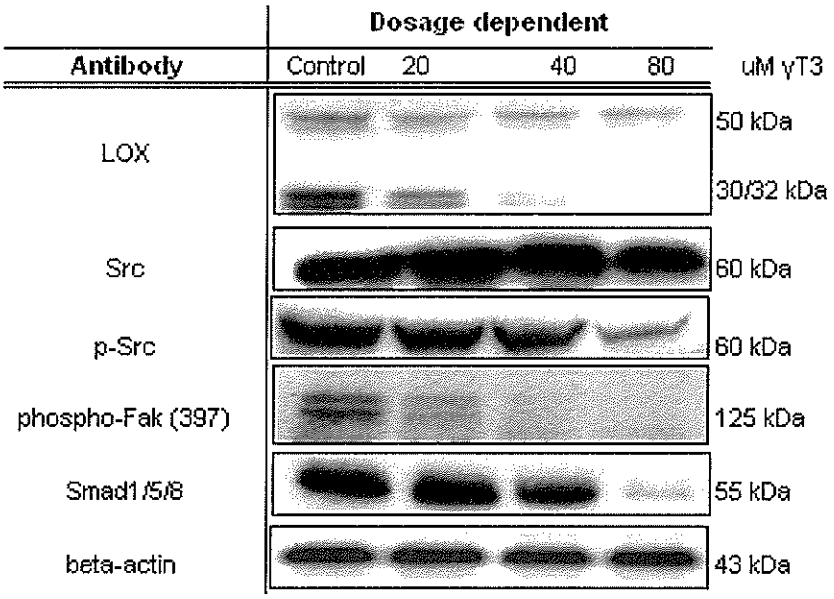

Dosage dependent
Antibody
Control
40
60
80
uM γT3
EGFR
Id-1
Id-3
beta-actin
170 kDa
15 kDa
15 kDa
43 kDa

FIG. 17 (continued)

(C)



(D)

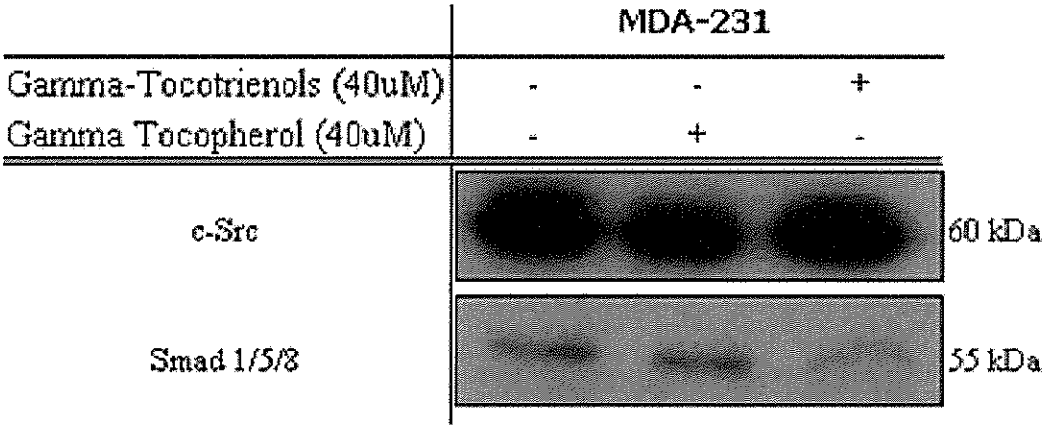
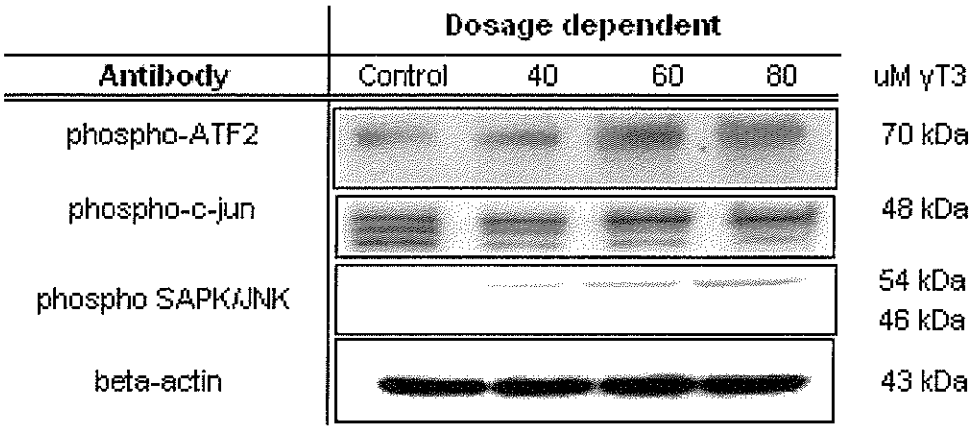


FIG. 18

(A)


Dosage dependent
Antibody
Control
40
60
80
uM γT3
phospho-ATF2
70 kDa
phospho-c-jun
48 kDa
phospho SAPK/JNK
54 kDa
46 kDa
beta-actin
43 kDa

(B)

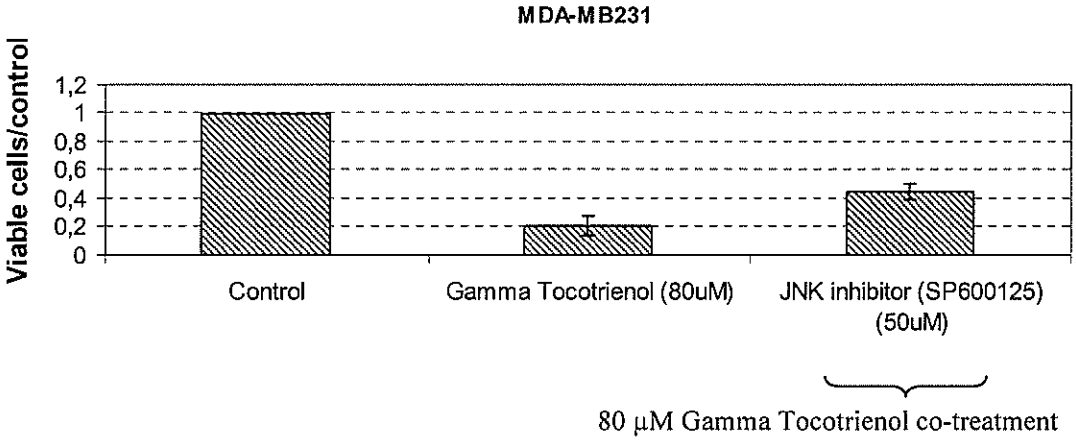
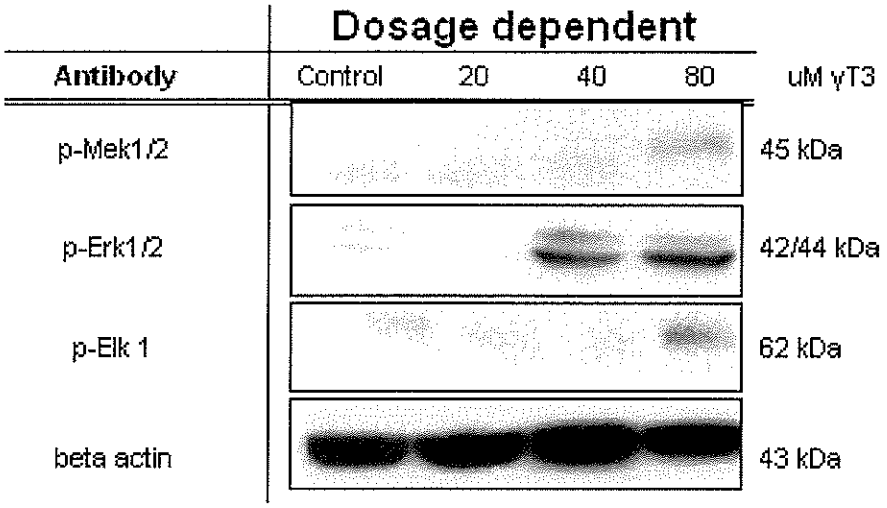

MDA-MB231
Viable cells/control
1,2
1
0,8
0,6
0,4
0,2
0
Control
Gamma Tocotrienol (80uM)
JNK inhibitor (SP600125) (50uM)
80 μM Gamma Tocotrienol co-treatment

FIG. 18 (continued)

(C)



(D)

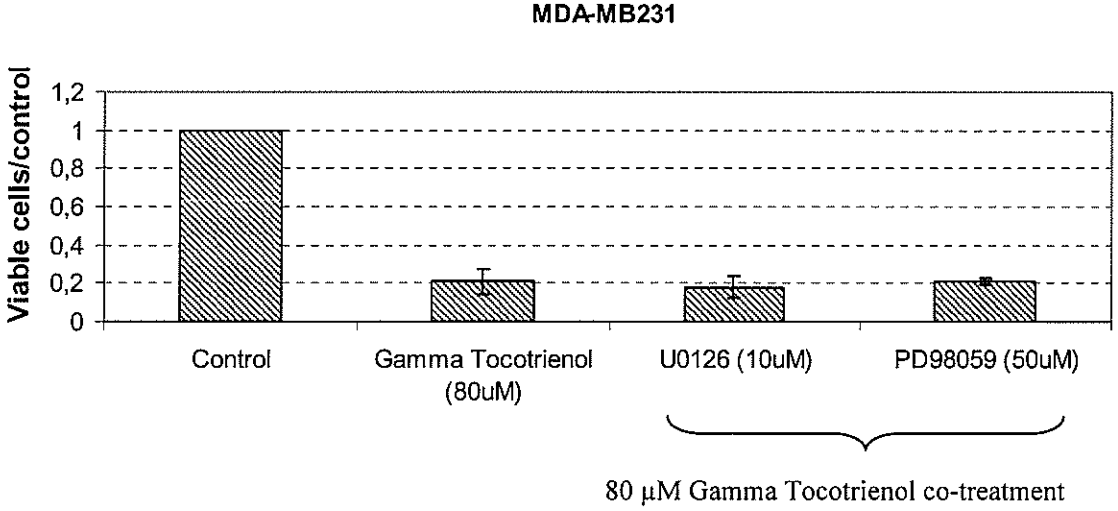
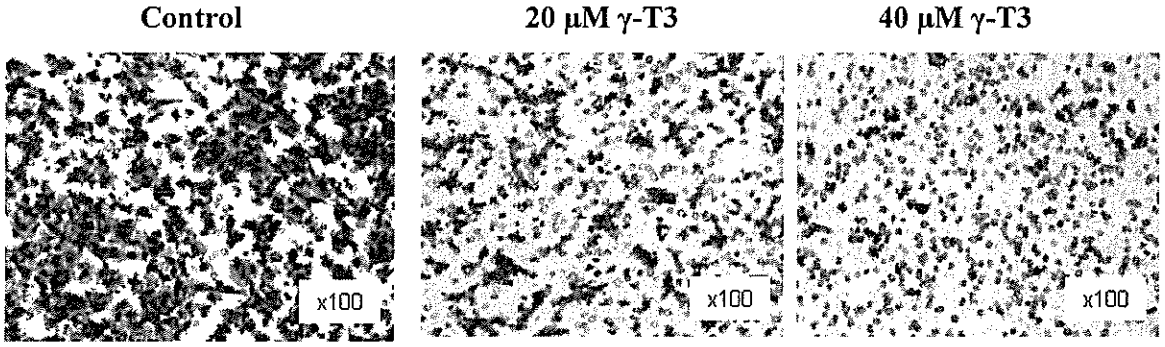


FIG. 19

(A)


Control
20 μM γ-T3
40 μM γ-T3
x100
x100
x100

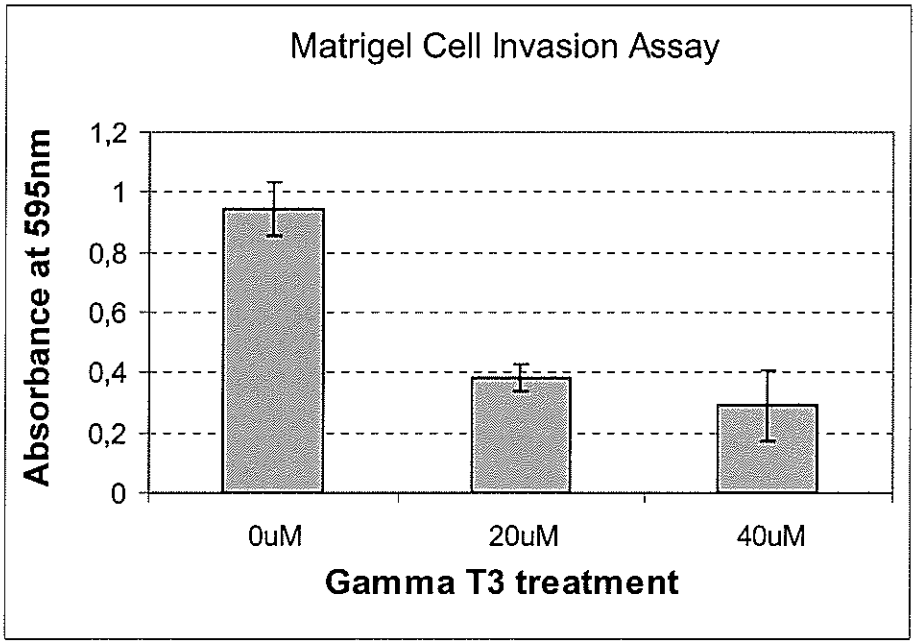

Matrigel Cell Invasion Assay
Absorbance at 595nm
1,2
1
0,8
0,6
0,4
0,2
0
0uM
20uM
40uM
Gamma T3 treatment

FIG. 19 (continued)

(B)

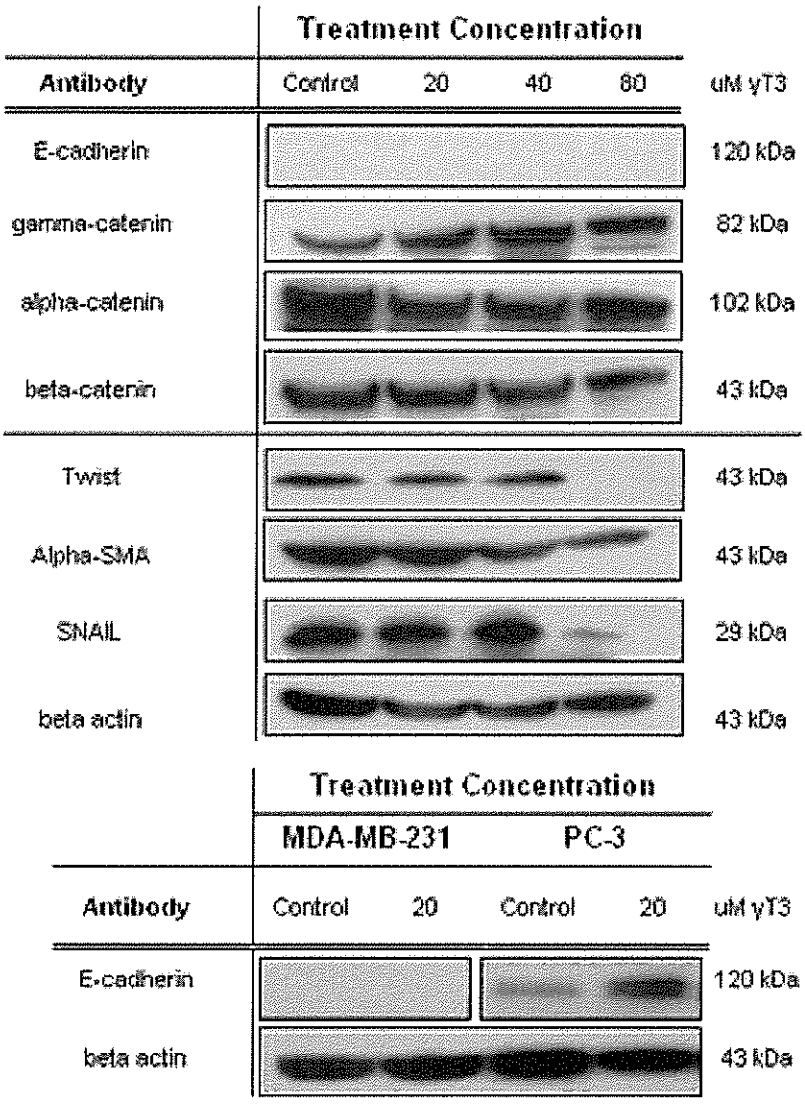


FIG. 20

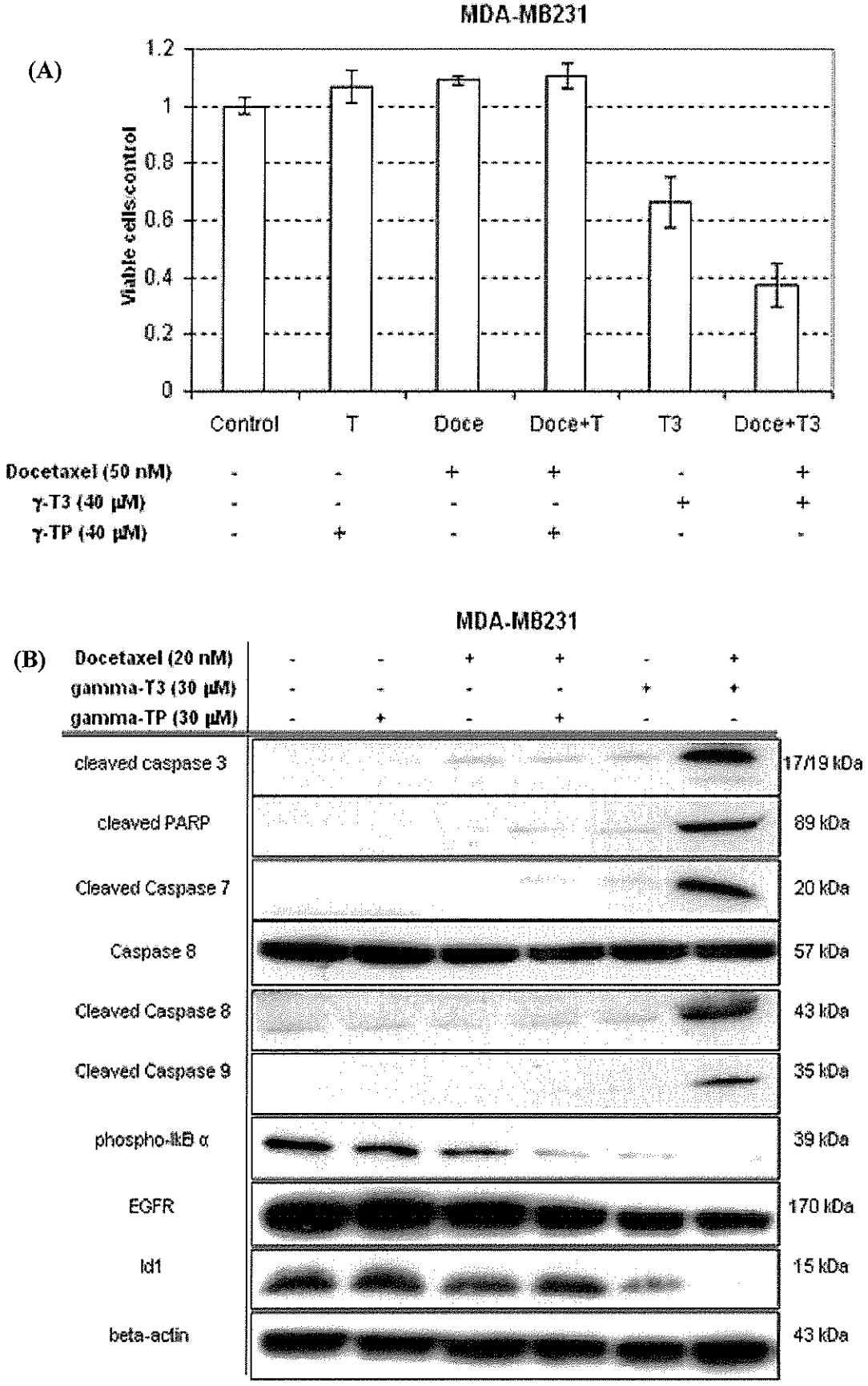
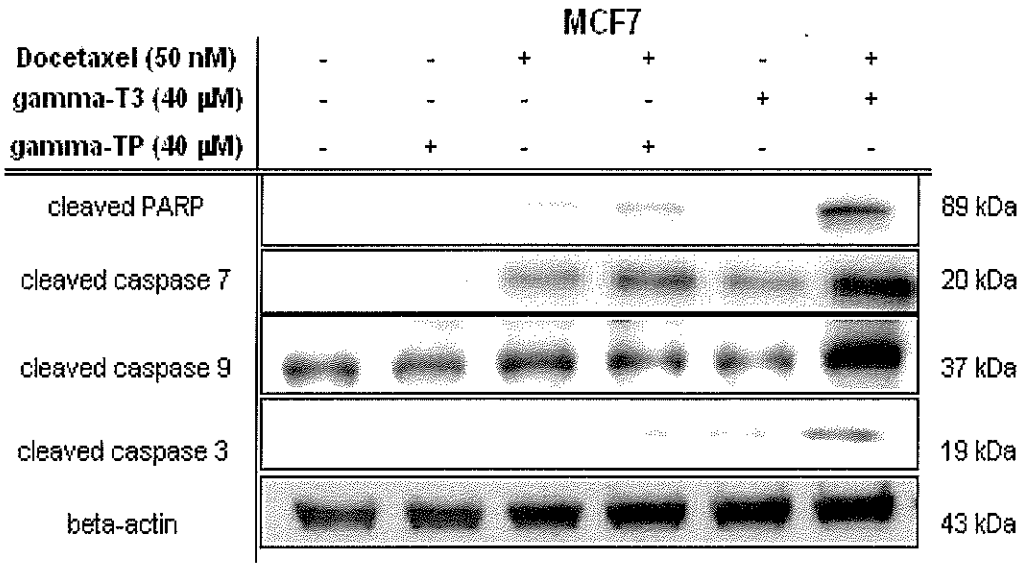

MDA-MB231
(A)
Viable cells/control
1.2
1
0.8
0.6
0.4
0.2
0
Control
T
Doce
Doce+T
T3
Doce+T3
Docetaxel (50 nM) - - + + - +
γ-T3 (40 μM) - - - - + +
γ-TP (40 μM) - + - + - -
MDA-MB231
(B)
Docetaxel (20 nM) - - + + - +
gamma-T3 (30 μM) - - - - + +
gamma-TP (30 μM) - + - + - -
cleaved caspase 3
17/19 kDa
cleaved PARP
89 kDa
Cleaved Caspase 7
20 kDa
Caspase 8
57 kDa
Cleaved Caspase 8
43 kDa
Cleaved Caspase 9
35 kDa
phospho-IkB α
39 kDa
EGFR
170 kDa
Id1
15 kDa
beta-actin
43 kDa

FIG. 20 (continued)

(C)



(D)

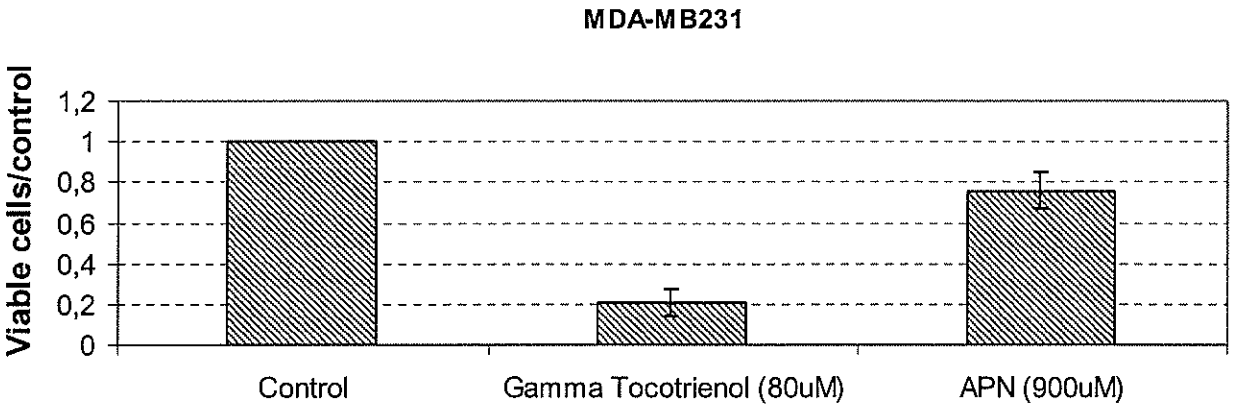


FIG. 20 (continued)

(E)

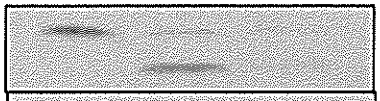
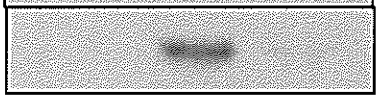
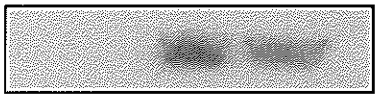
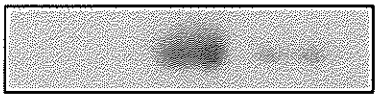
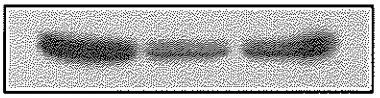
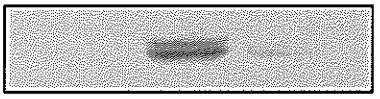
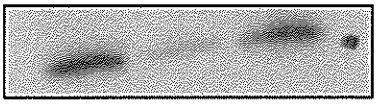
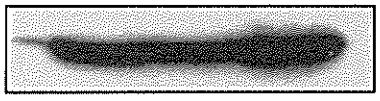
	MDA-231			
Gamma-Tocotrienols (40uM)	-	+	+	
3-aminoproprinitrile (APN) (900uM)	-	-	+	
Cleaved Caspase 3 Asp 175 (17, 19kDa)				17/19 kDa
Cleaved Caspase 9 (Large Fragment, 37kDa)				37 kDa
Cleaved PARP (89kDa)				89 kDa
Cleaved Caspase 7 (20kDa)				20 kDa
Caspase 8 (57kDa)				57 kDa
Cleaved caspase 8 (43kDa)				43 kDa
Id1 (15kDA)				15 kDa
beta-actin				17/19 kDa

FIG. 20 (continued)

(F)

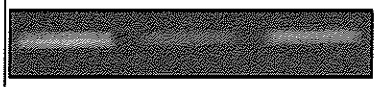
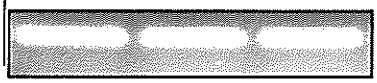
	MDA-231		
Gamma-Tocotrienols (40uM)	-	+	+
APN (900uM)	-	-	+
Id1			
GAPDH			

FIG. 21

(A)

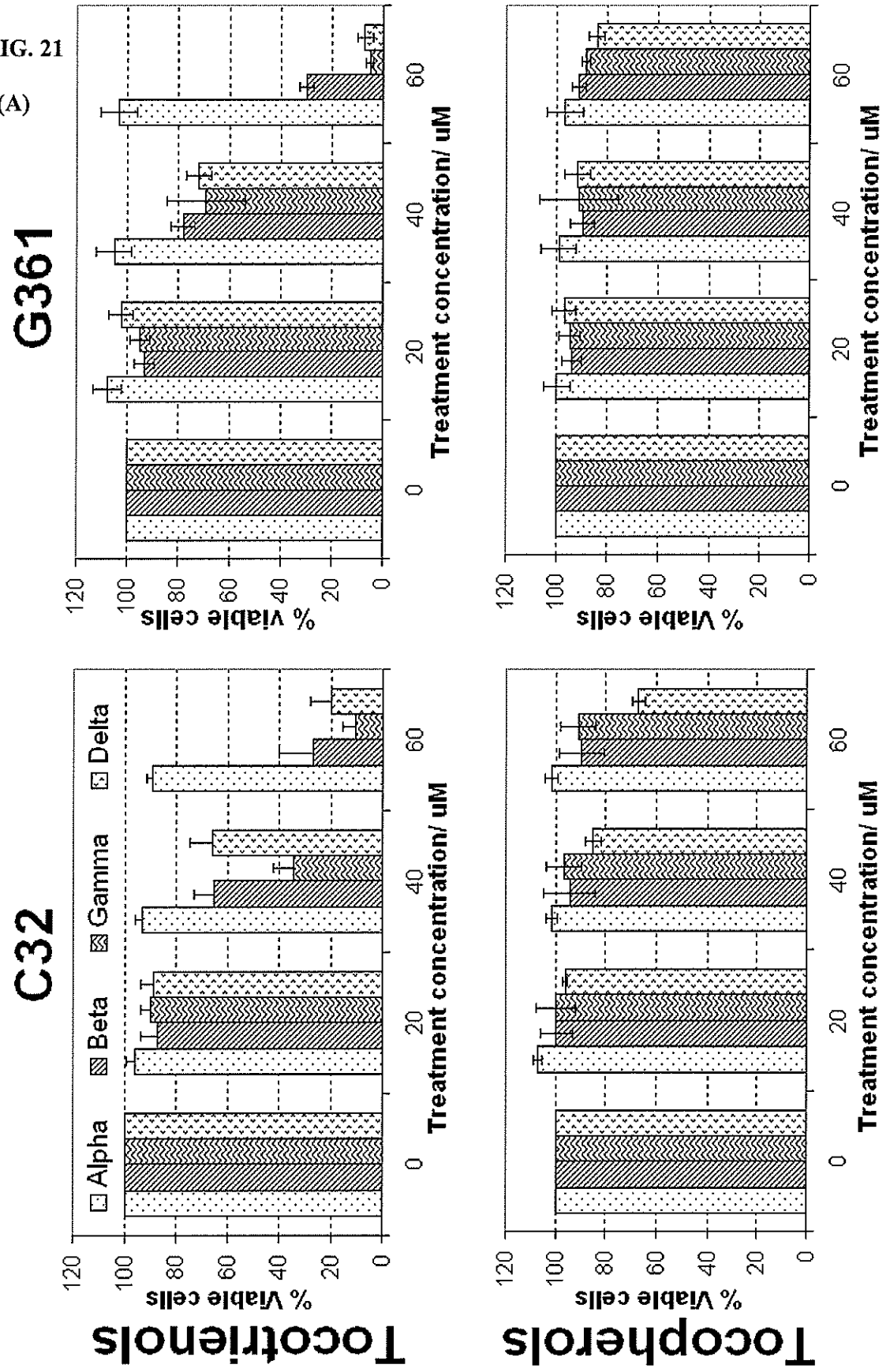

C32
G361
Tocotrienols
Tocopherols
Alpha
Beta
Gamma
Delta
% Viable cells
Treatment concentration/ uM
0
20
40
60
80
100
120

FIG. 21

(B)

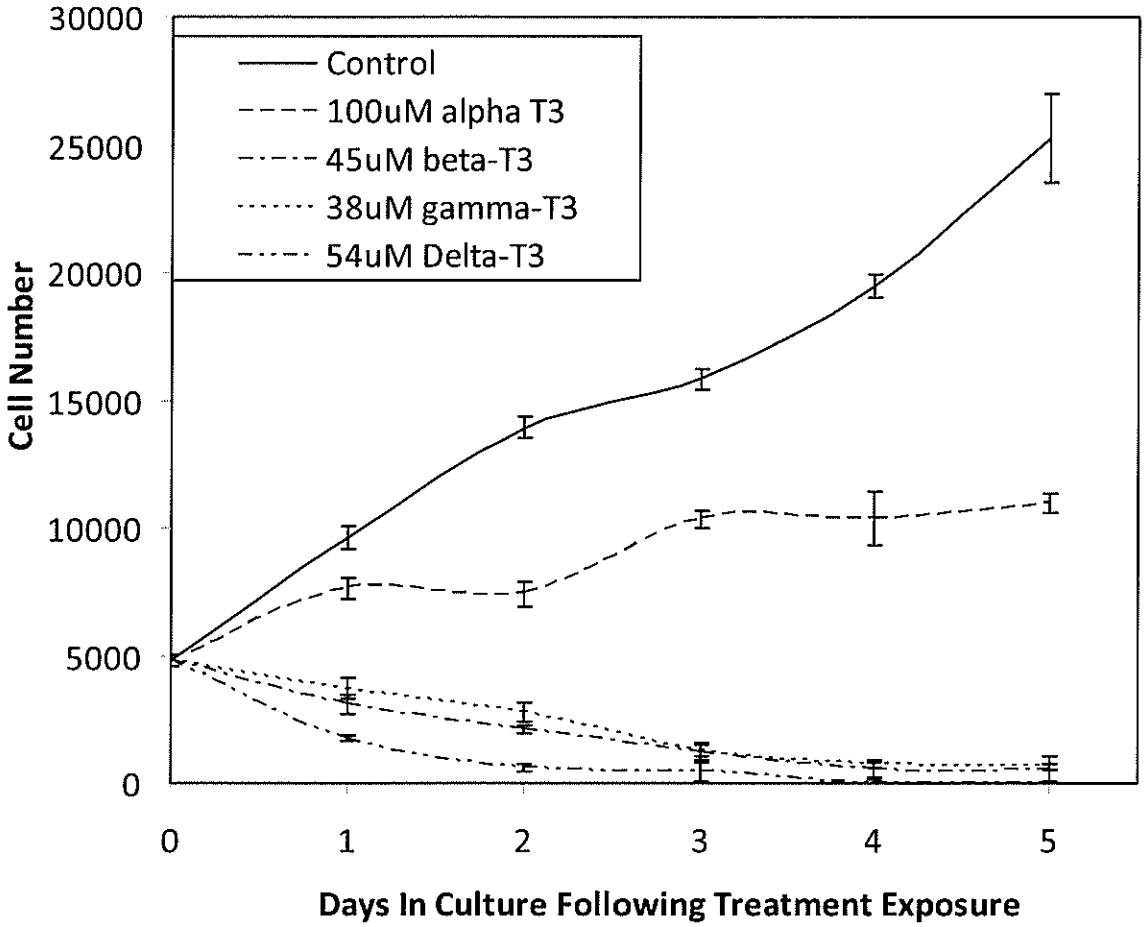
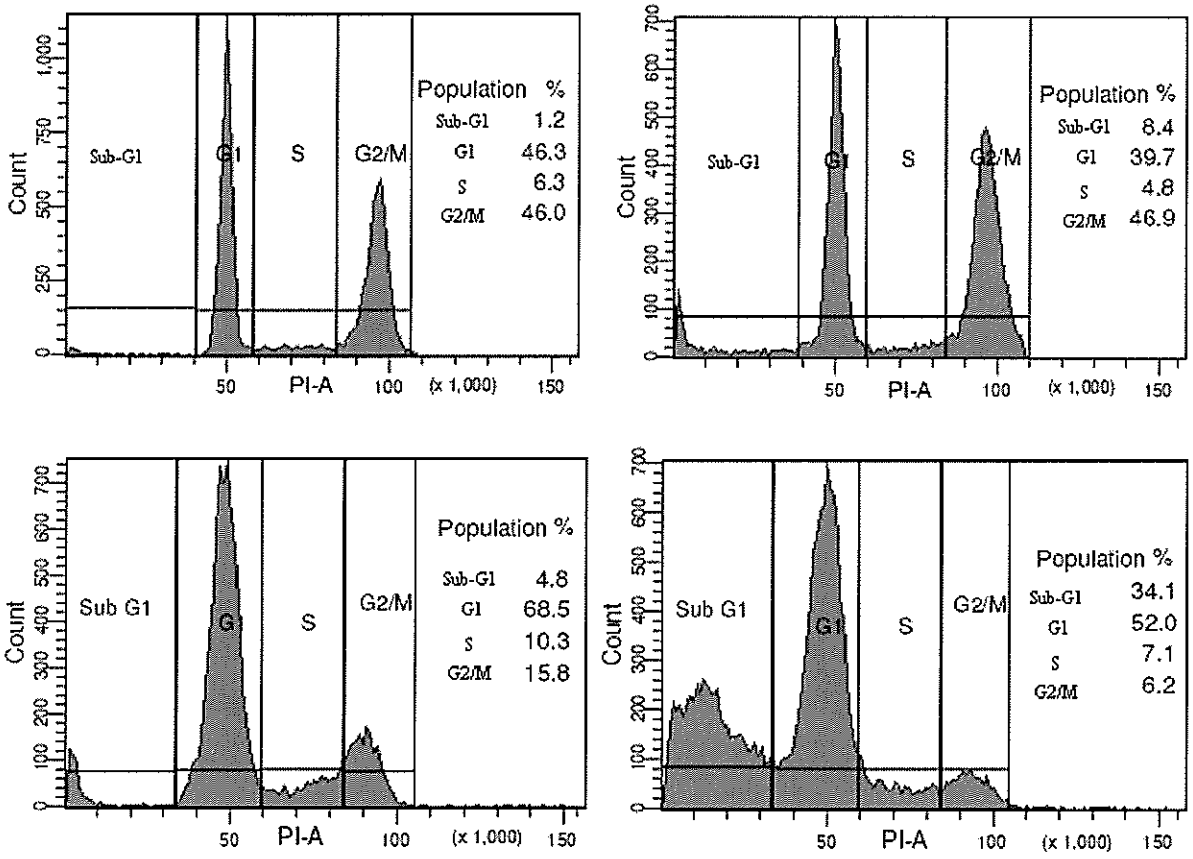

30000
25000
20000
15000
10000
5000
0
Cell Number
Control
100uM alpha T3
45uM beta-T3
38uM gamma-T3
54uM Delta-T3
0
1
2
3
4
5
Days In Culture Following Treatment Exposure

FIG. 22

(A)


Ctrl
60 μM gamma-T3
Count
PI-A
(x 1,000)
Sub-G1
G1
S
G2/M
Population %
Sub-G1 1.2
G1 46.3
S 6.3
G2/M 46.0
Sub-G1 8.4
G1 39.7
S 4.8
G2/M 46.9
Sub G1 4.8
G1 68.5
S 10.3
G2/M 15.8
Sub-G1 34.1
G1 52.0
S 7.1
G2/M 6.2

(B)

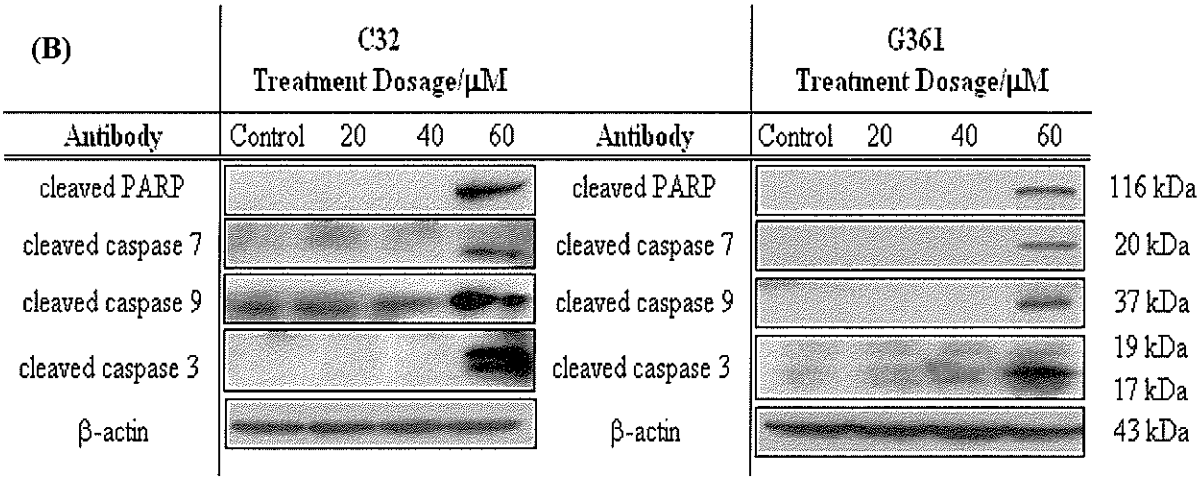
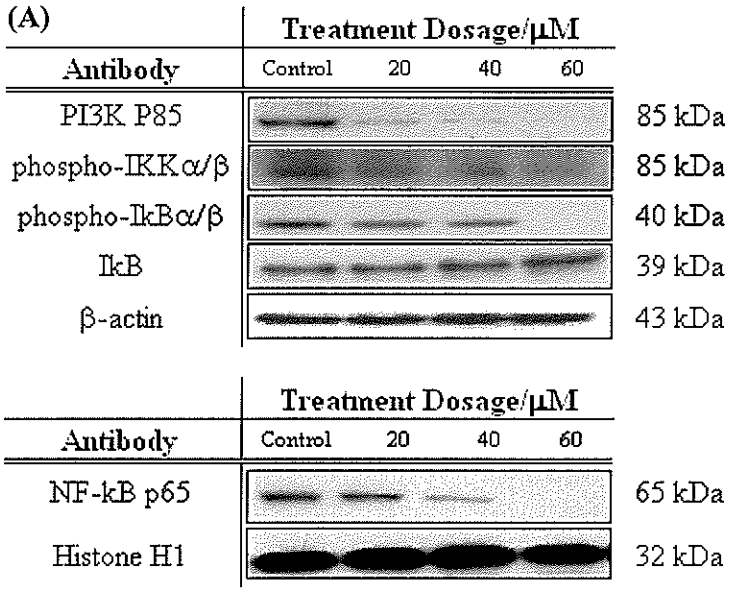

C32
Treatment Dosage/μM
G361
Treatment Dosage/μM
Antibody
Control 20 40 60
cleaved PARP
cleaved caspase 7
cleaved caspase 9
cleaved caspase 3
β-actin
116 kDa
20 kDa
37 kDa
19 kDa
17 kDa
43 kDa

FIG. 23


(A)
Treatment Dosage/μM
Antibody
Control
20
40
60
PI3K P85
85 kDa
phospho-IKKα/β
85 kDa
phospho-IkBα/β
40 kDa
IkB
39 kDa
β-actin
43 kDa
Treatment Dosage/μM
Antibody
Control
20
40
60
NF-kB p65
65 kDa
Histone H1
32 kDa

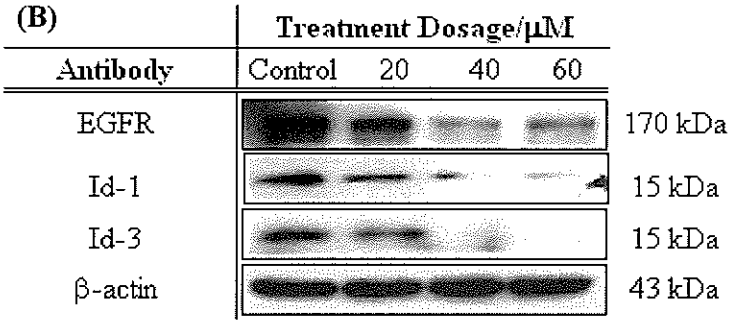
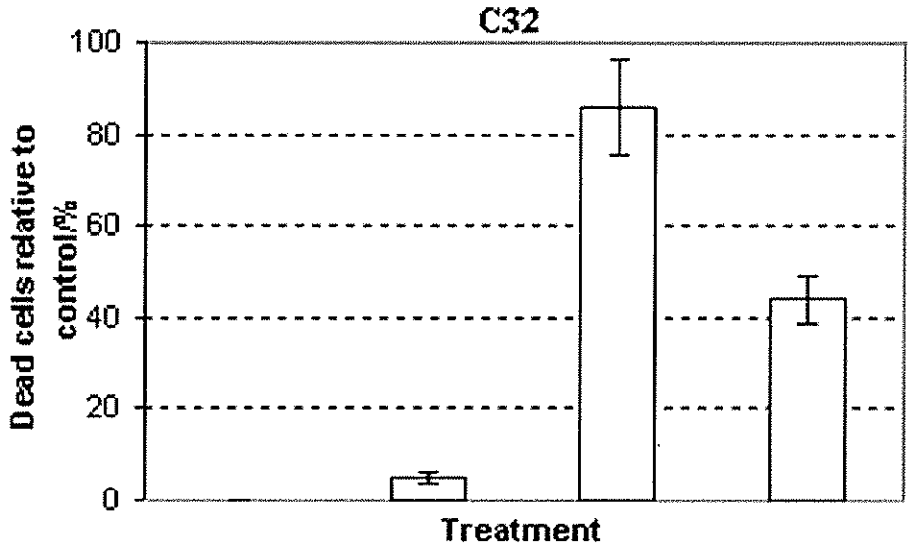

(B)
Treatment Dosage/μM
Antibody
Control
20
40
60
EGFR
170 kDa
Id-1
15 kDa
Id-3
15 kDa
β-actin
43 kDa

FIG. 24

(A)



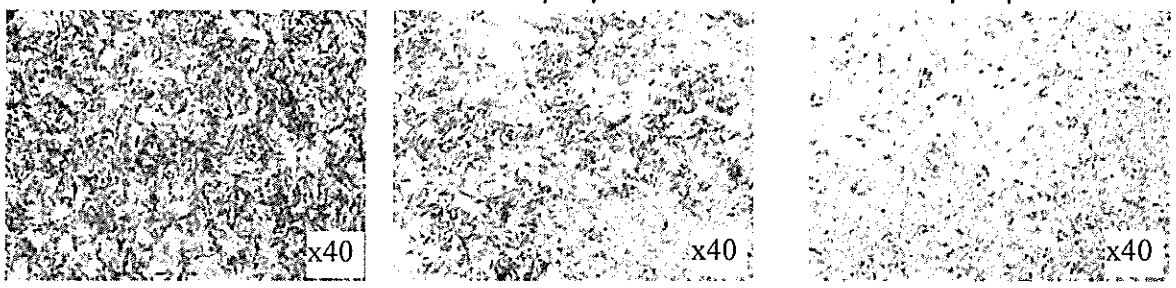
JNK inhibitor (SP600125) (20 μM)	+	-	+
Gamma-T3 (100 μM)	-	+	+

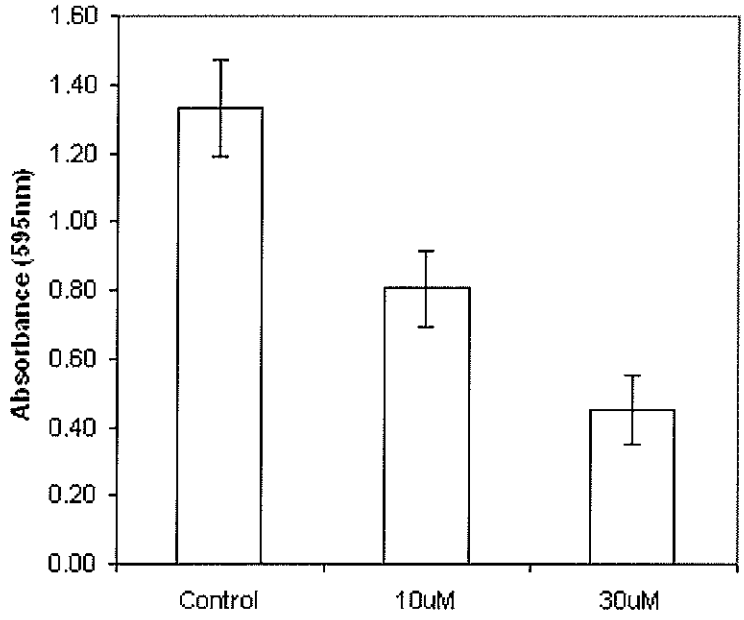
(B)

Antibody	Treatment Dosage/μM: Control	20	40	60	
phospho-ATF2					70 kDa
phospho-c-jun					48 kDa
phospho SAPK/JNK					54 kDa 46 kDa
β-actin					43 kDa

FIG. 25

(A)


Control
10 μM γ-T3
30 μM γ-T3
x40
x40
x40


Absorbance (595nm)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Control
10uM
30uM

(B)

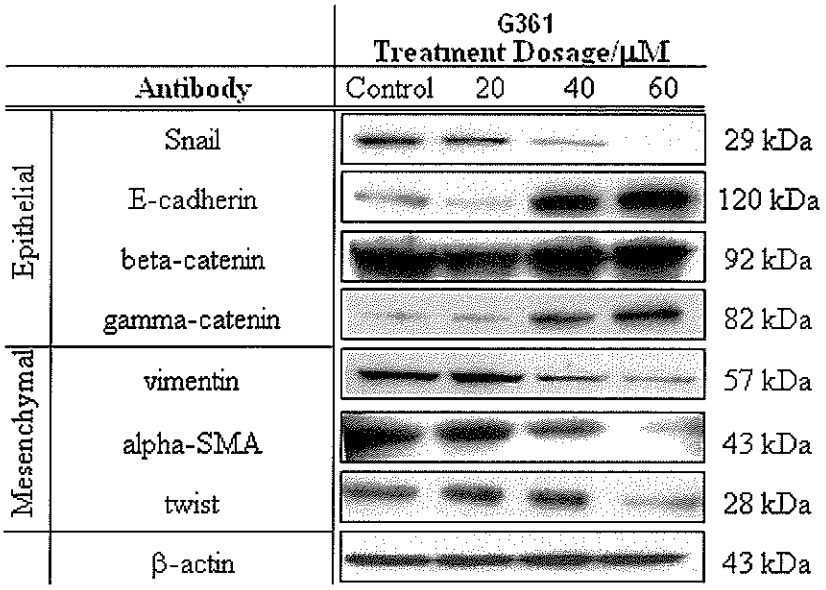

G361
Treatment Dosage/μM
Antibody
Control
20
40
60
Epithelial
Mesenchymal
Snail
E-cadherin
beta-catenin
gamma-catenin
vimentin
alpha-SMA
twist
β-actin
29 kDa
120 kDa
92 kDa
82 kDa
57 kDa
43 kDa
28 kDa
43 kDa

FIG. 26

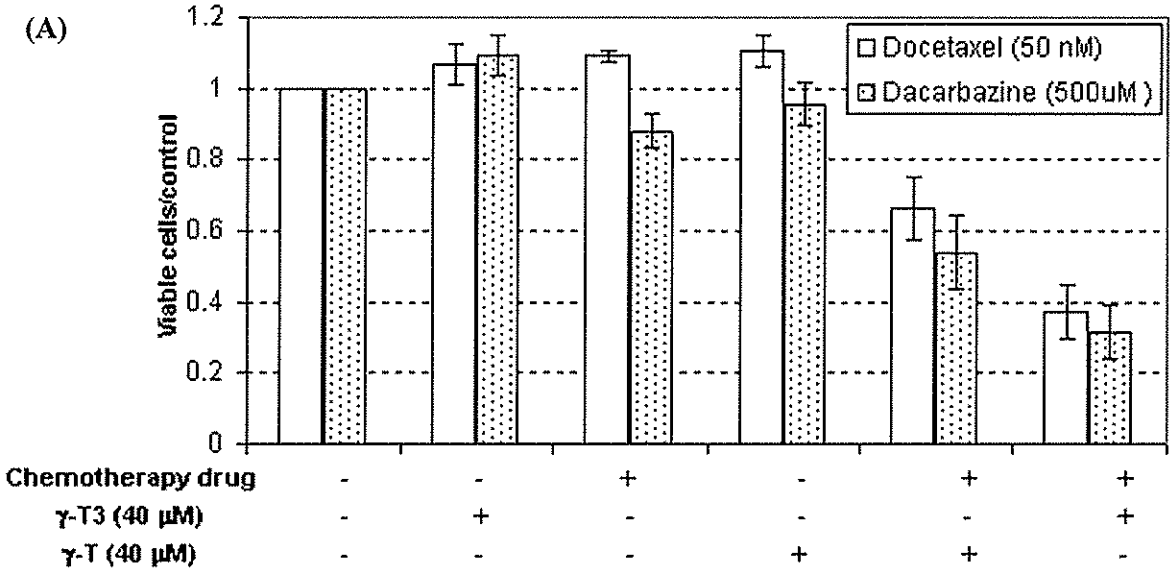

(A)
1.2
1
0.8
0.6
0.4
0.2
0
Viable cells/control
Docetaxel (50 nM)
Dacarbazine (500uM)
Chemotherapy drug - - + - + +
γ-T3 (40 μM) - + - - - +
γ-T (40 μM) - - - + + -

FIG. 26 (continued)

(B)

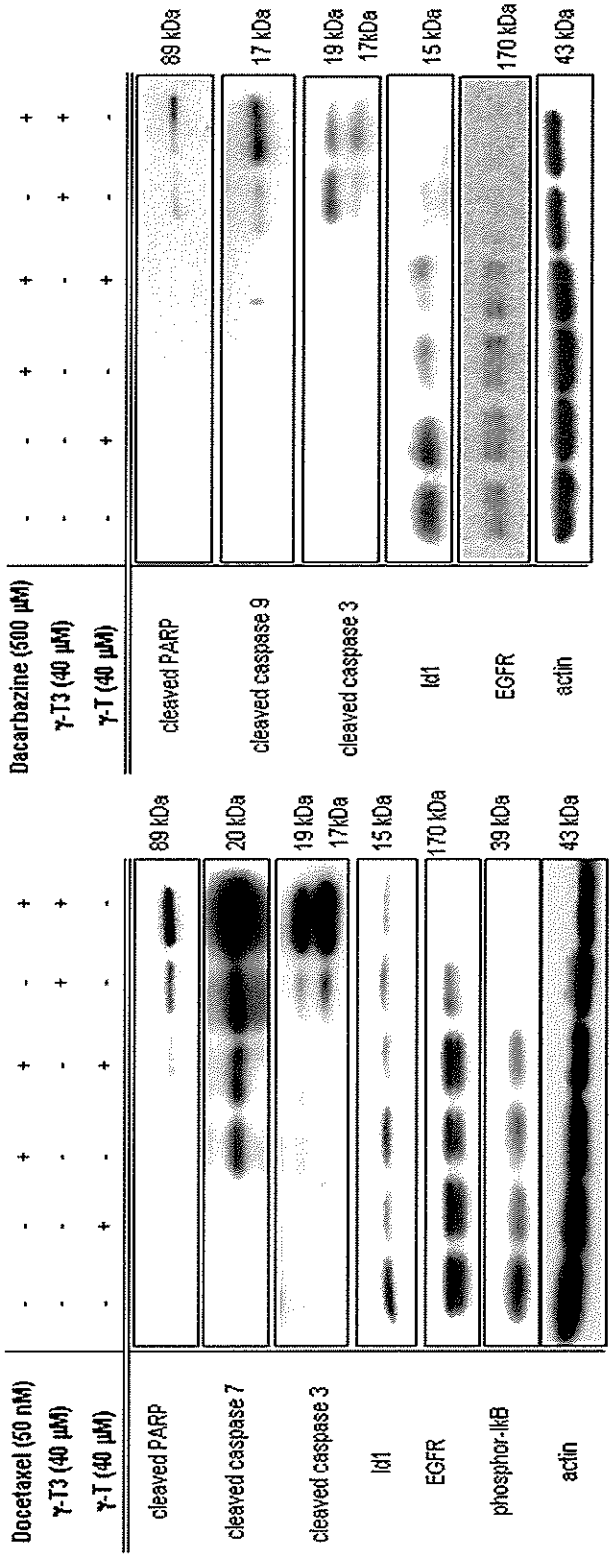


FIG. 26 (continued)

(C)

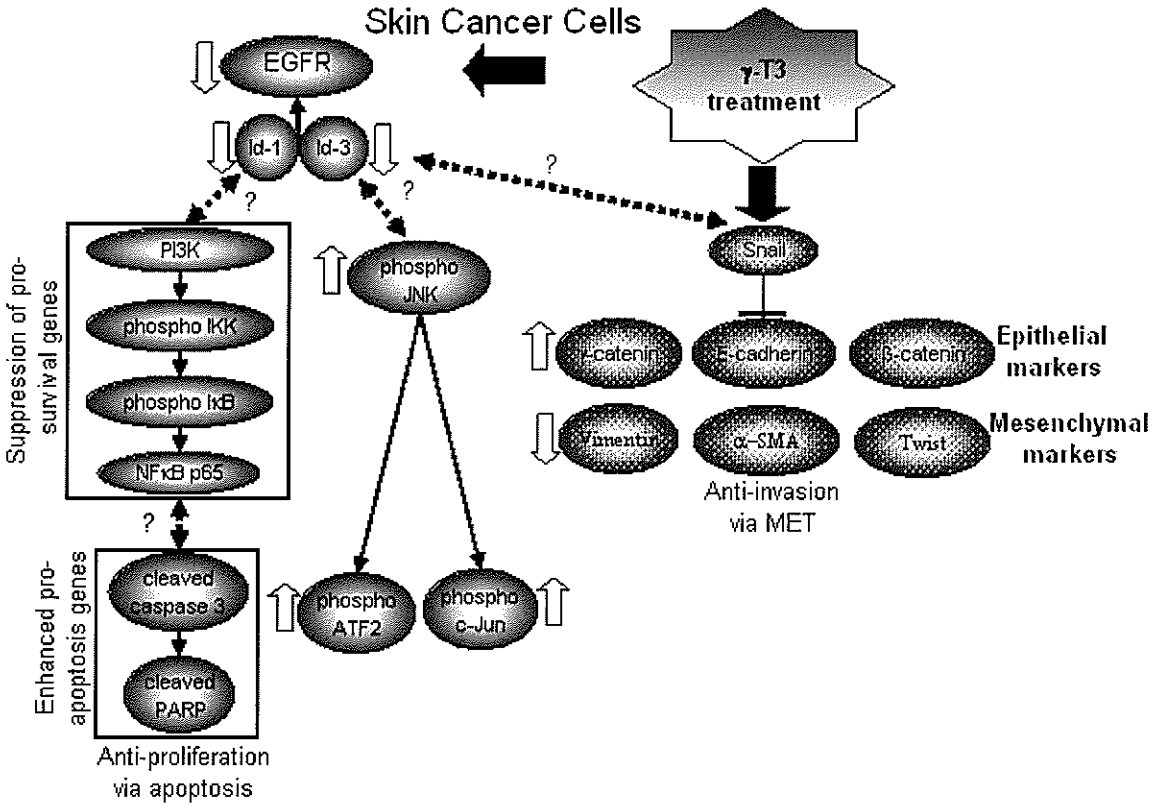
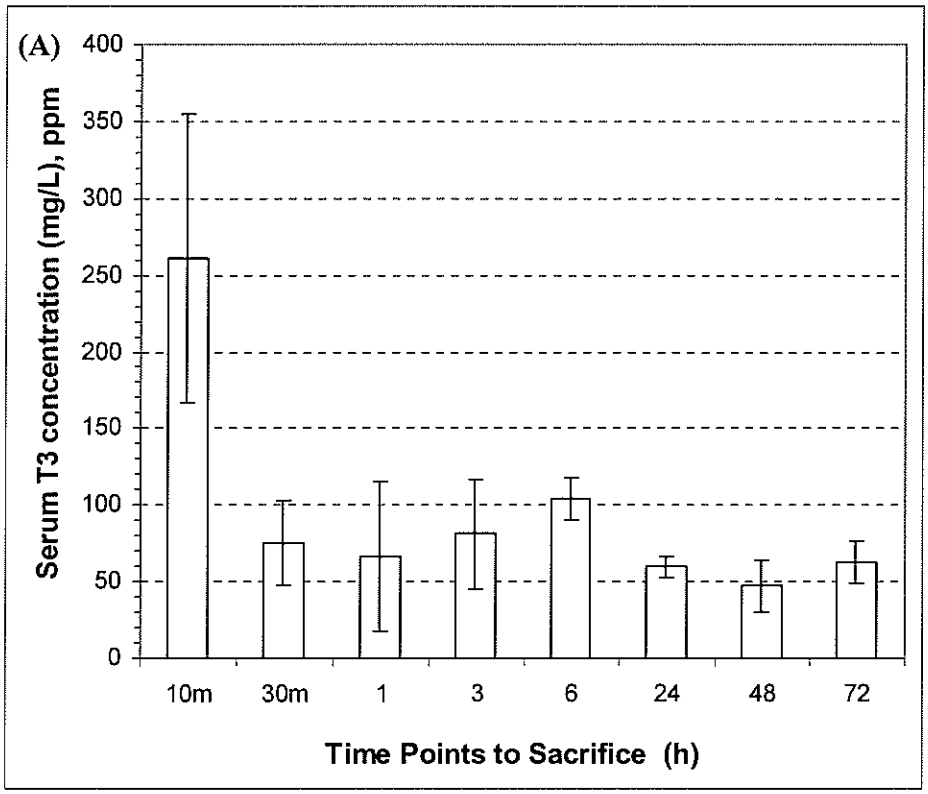


FIG. 27


(A)
Serum T3 concentration (mg/L), ppm
400
350
300
250
200
150
100
50
0
10m
30m
1
3
6
24
48
72
Time Points to Sacrifice (h)

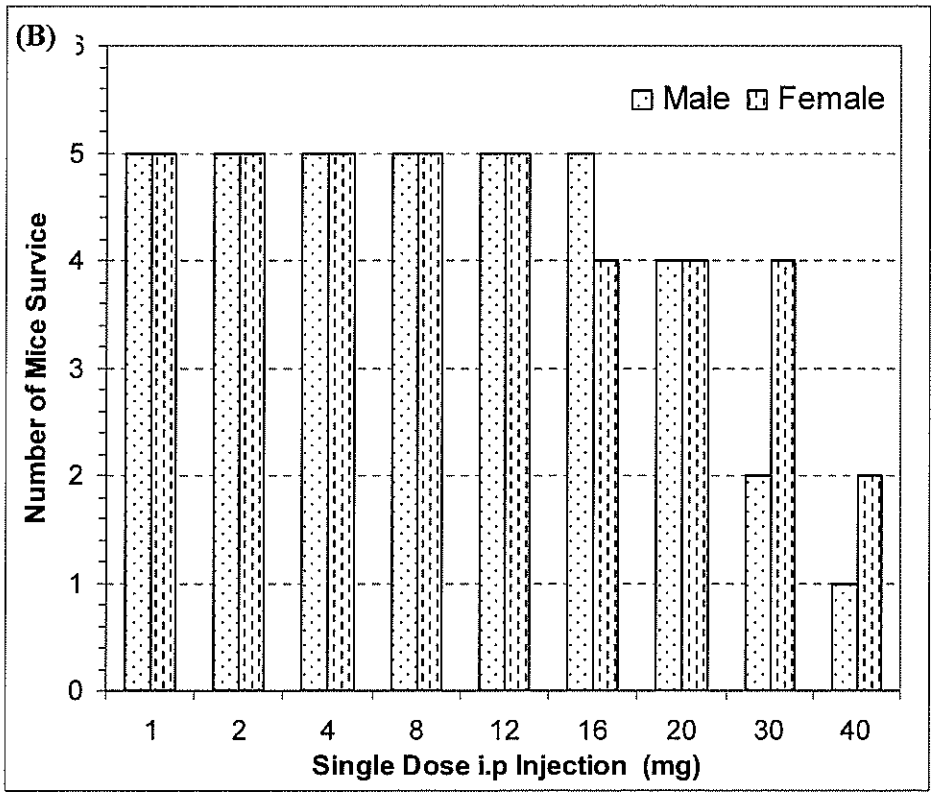

(B)
Male
Female
Number of Mice Survice
5
4
3
2
1
0
1
2
4
8
12
16
20
30
40
Single Dose i.p Injection (mg)

FIG. 27 (continued)

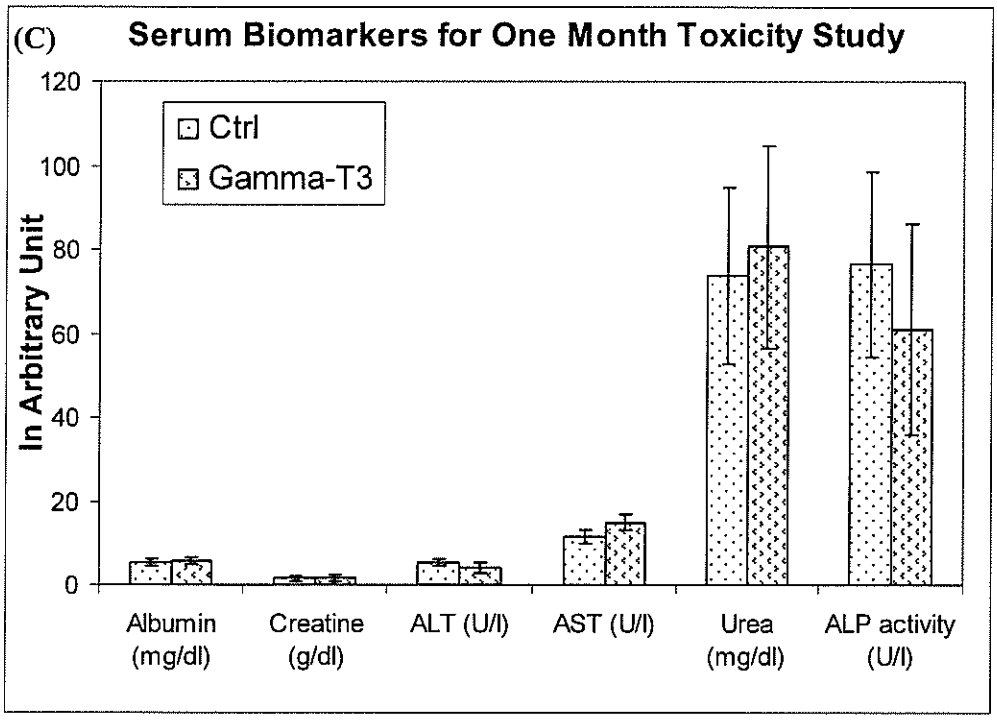


FIG. 28

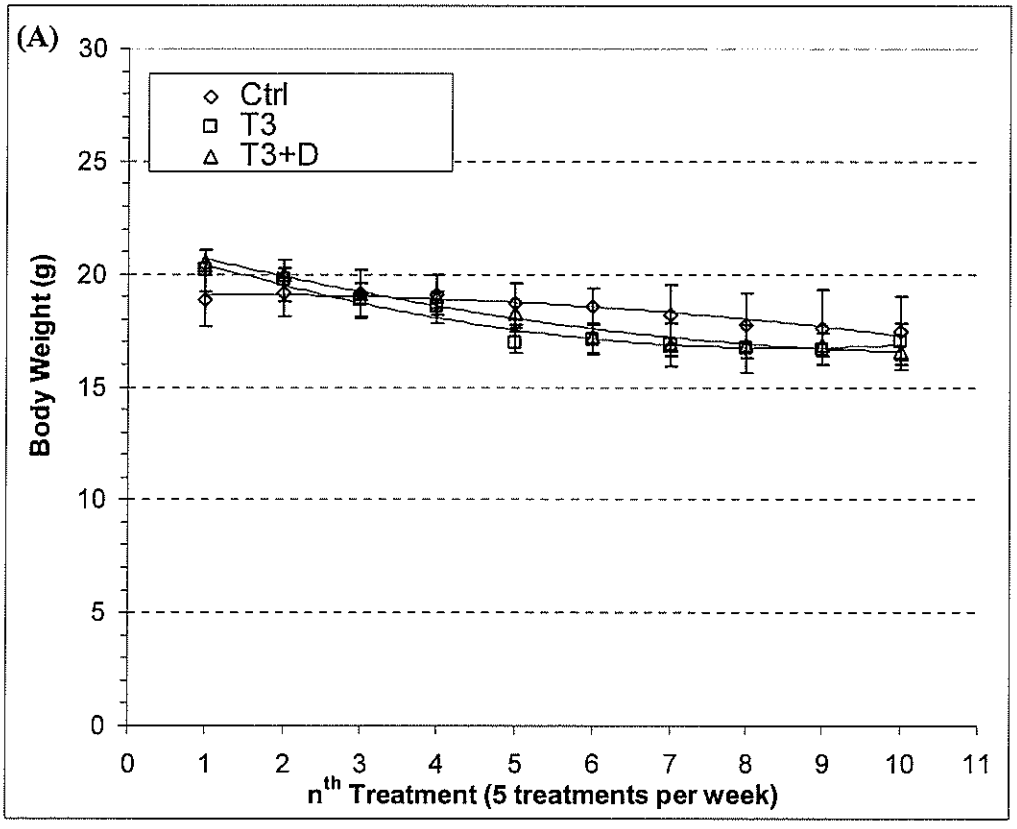
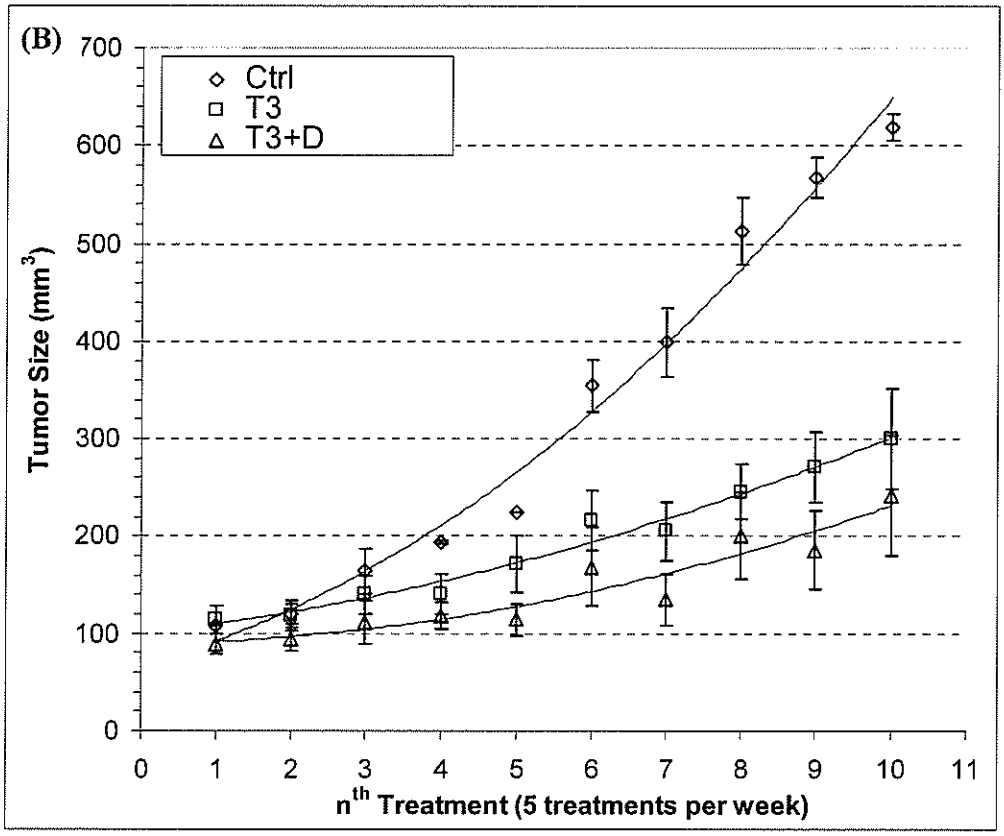


FIG. 28 (continued)



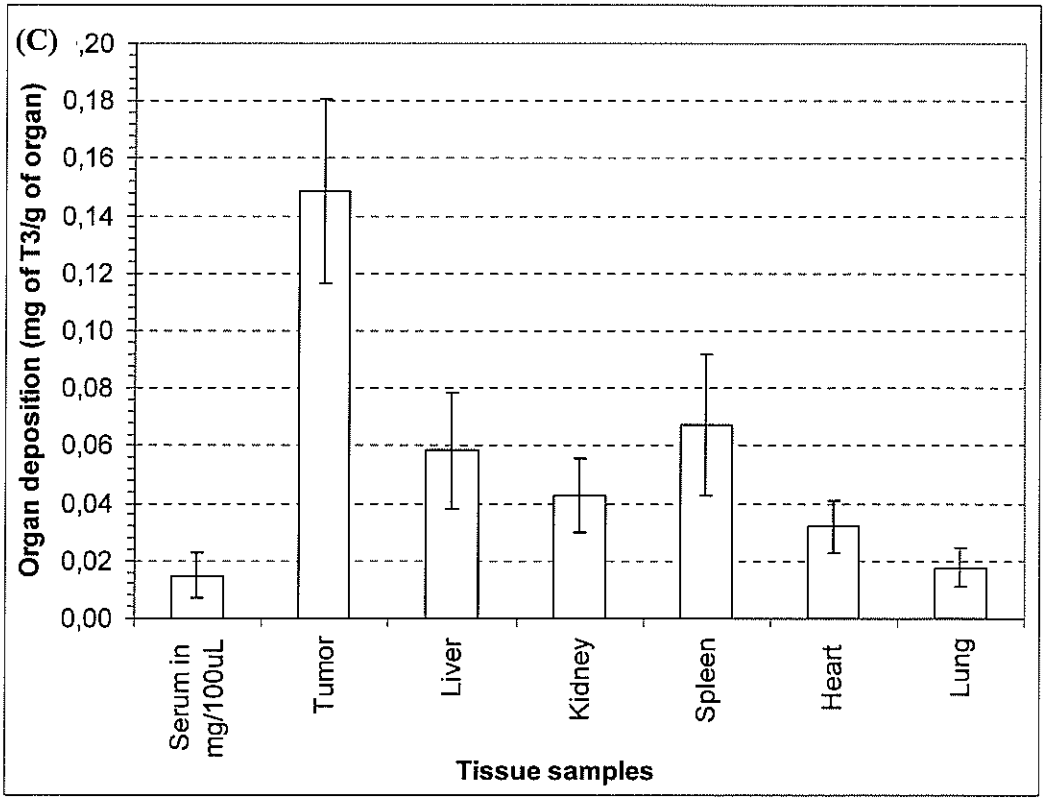
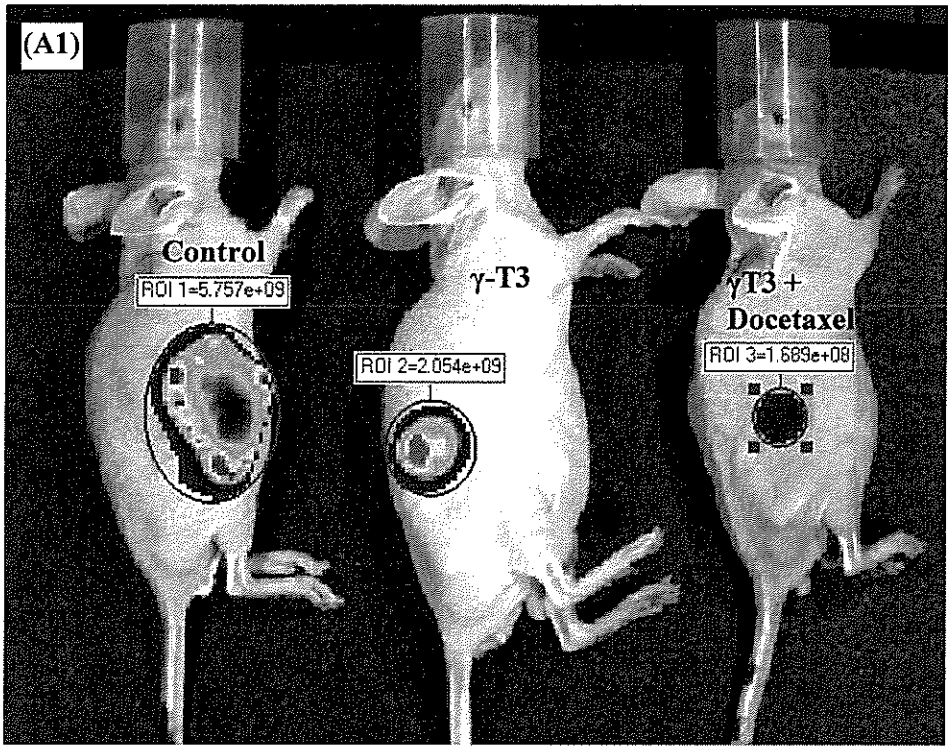


FIG. 29

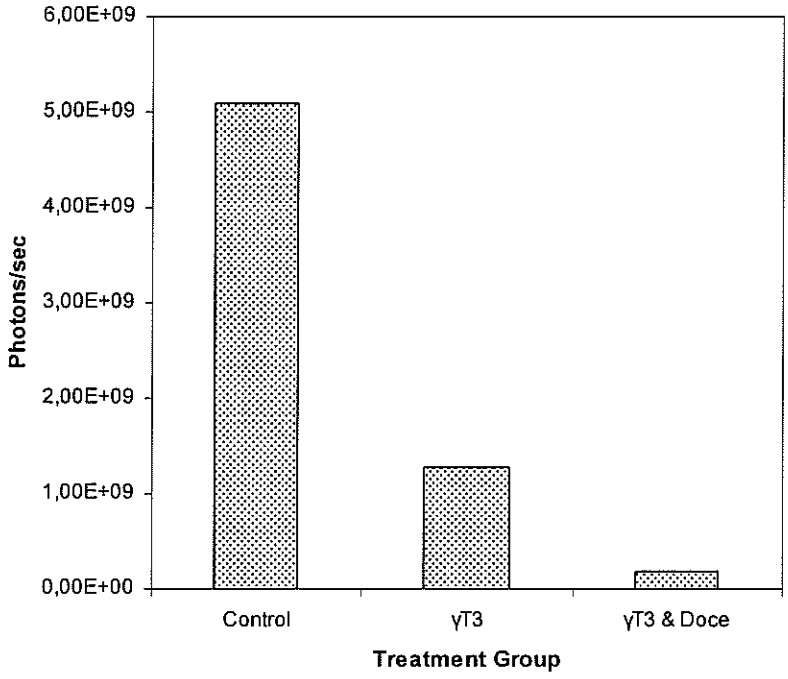


3 mice were used for in vivo imaging system:

Group	Body Weight	Tumor Size
(1) Control	14.8g	2000 mm^3
(2) Gamma T3	18.9g	288 mm^3
(3) Gamma T3 + Docetaxel	14.5g	161 mm^3

FIG. 29 (continued)

(A2)



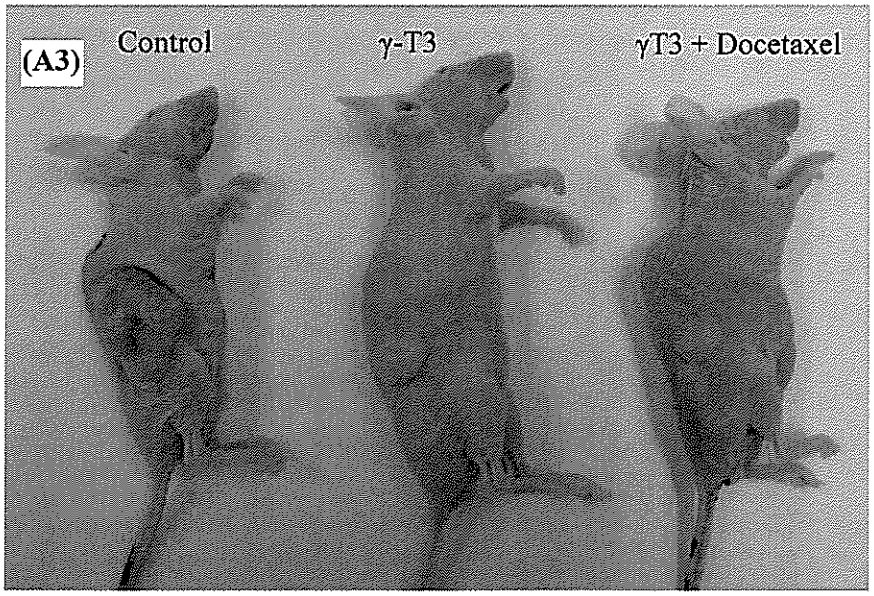


FIG. 29 (continued)

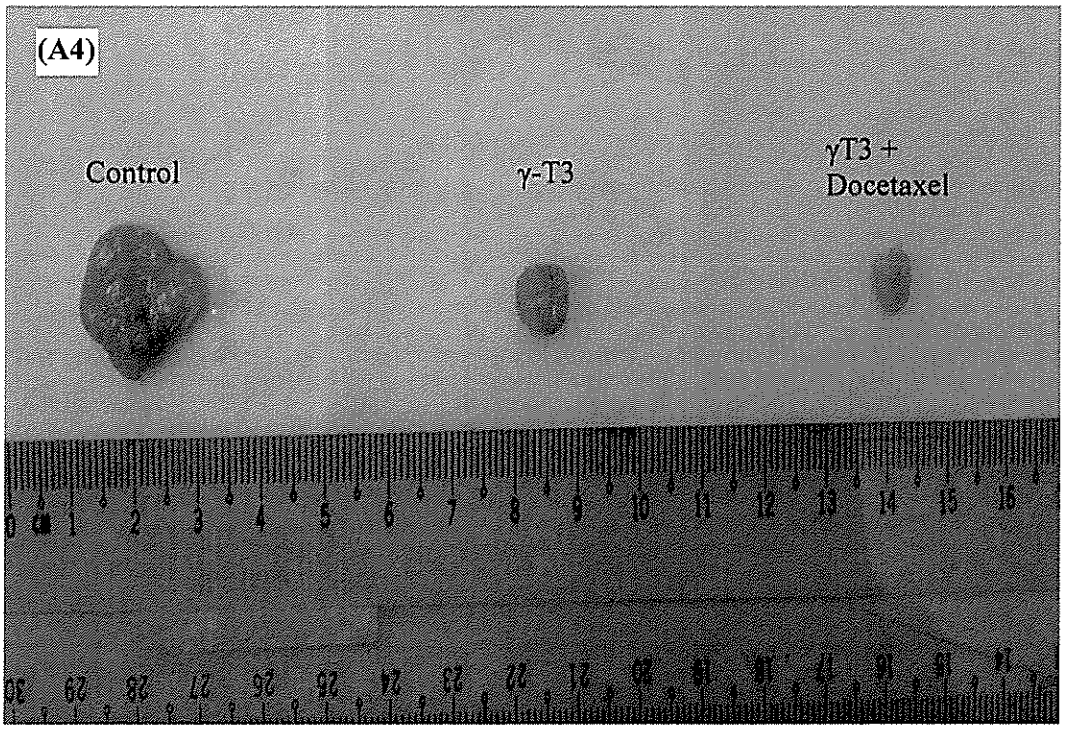
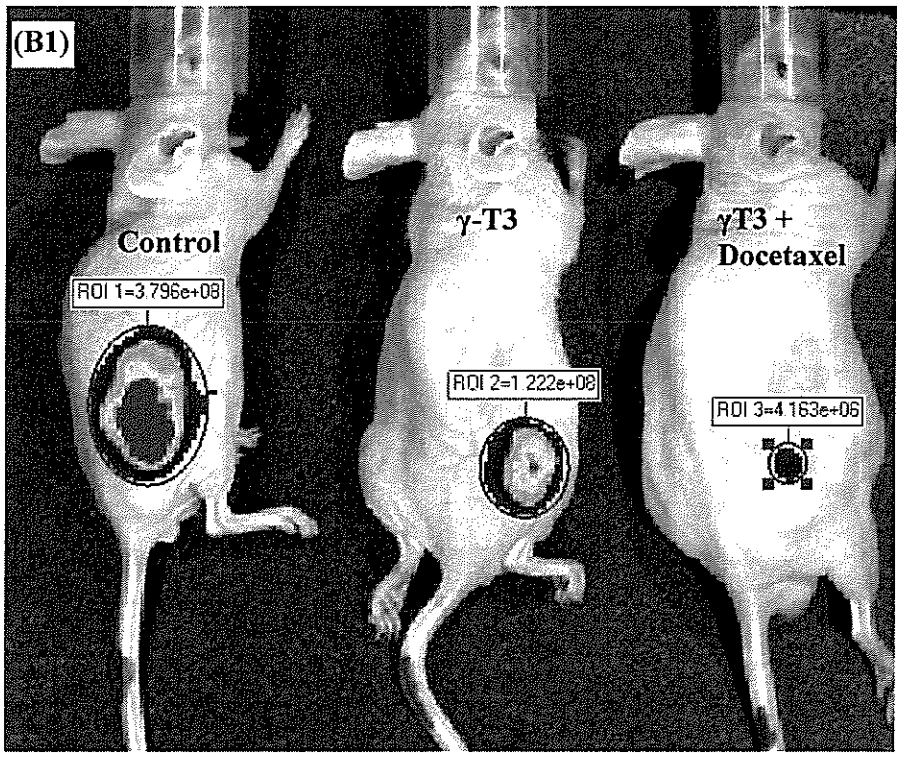


FIG. 29 (continued)

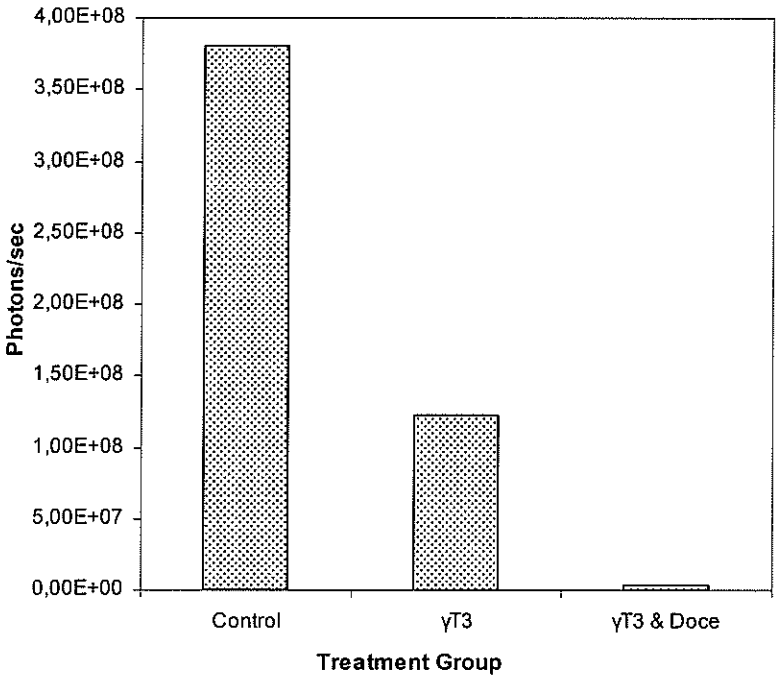


3 mice were used for in vivo imaging system:

Group	Body Weight	Tumor Size
(1) Control	15,3	2000 mm^3
(2) Gamma T3	18,3	288 mm^3
(3) Gamma T3 + Docetaxel	27,3	161 mm^3

FIG. 29 (continued)

(B2)



(B3)

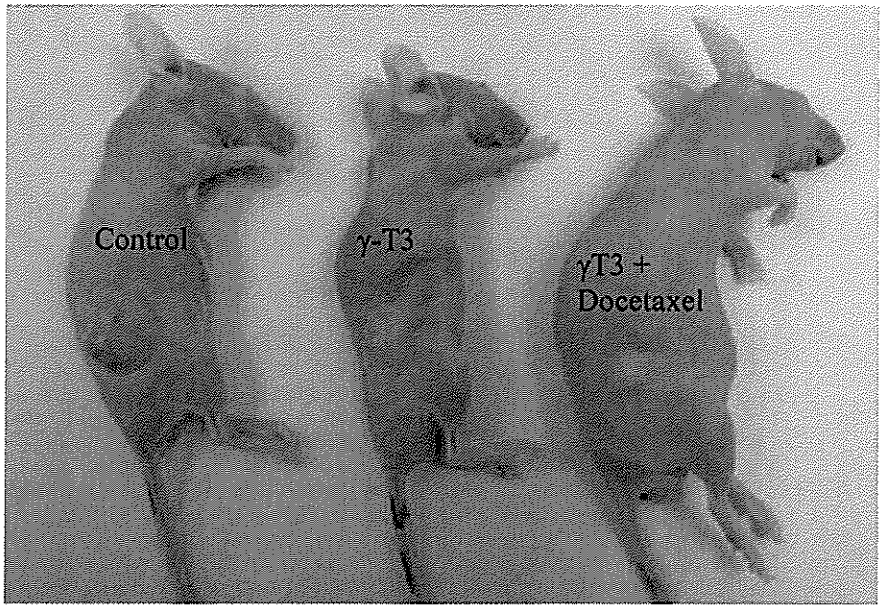


FIG. 29 (continued)

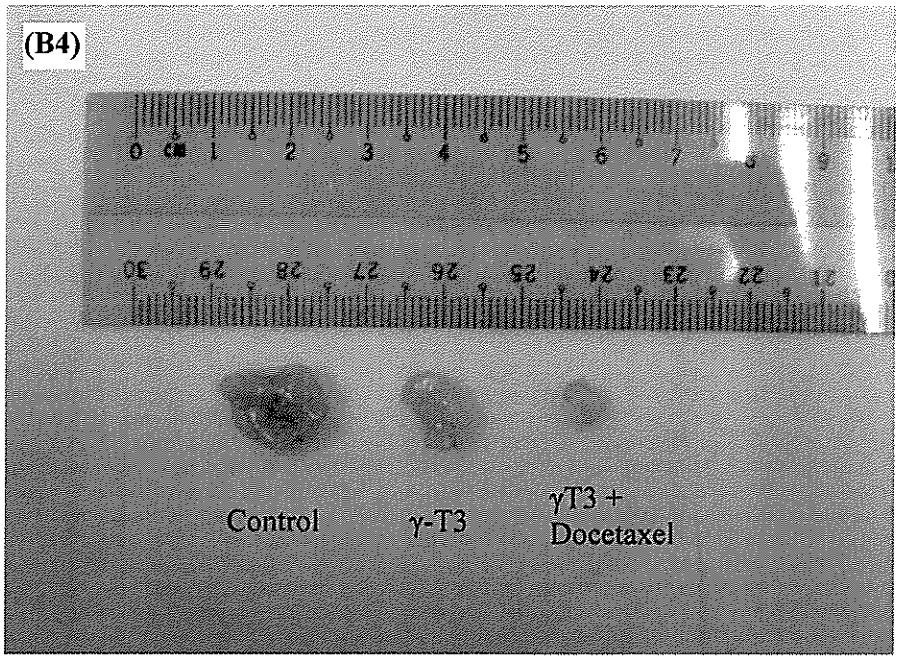


FIG. 30

Tumor Cell Proliferation Markers

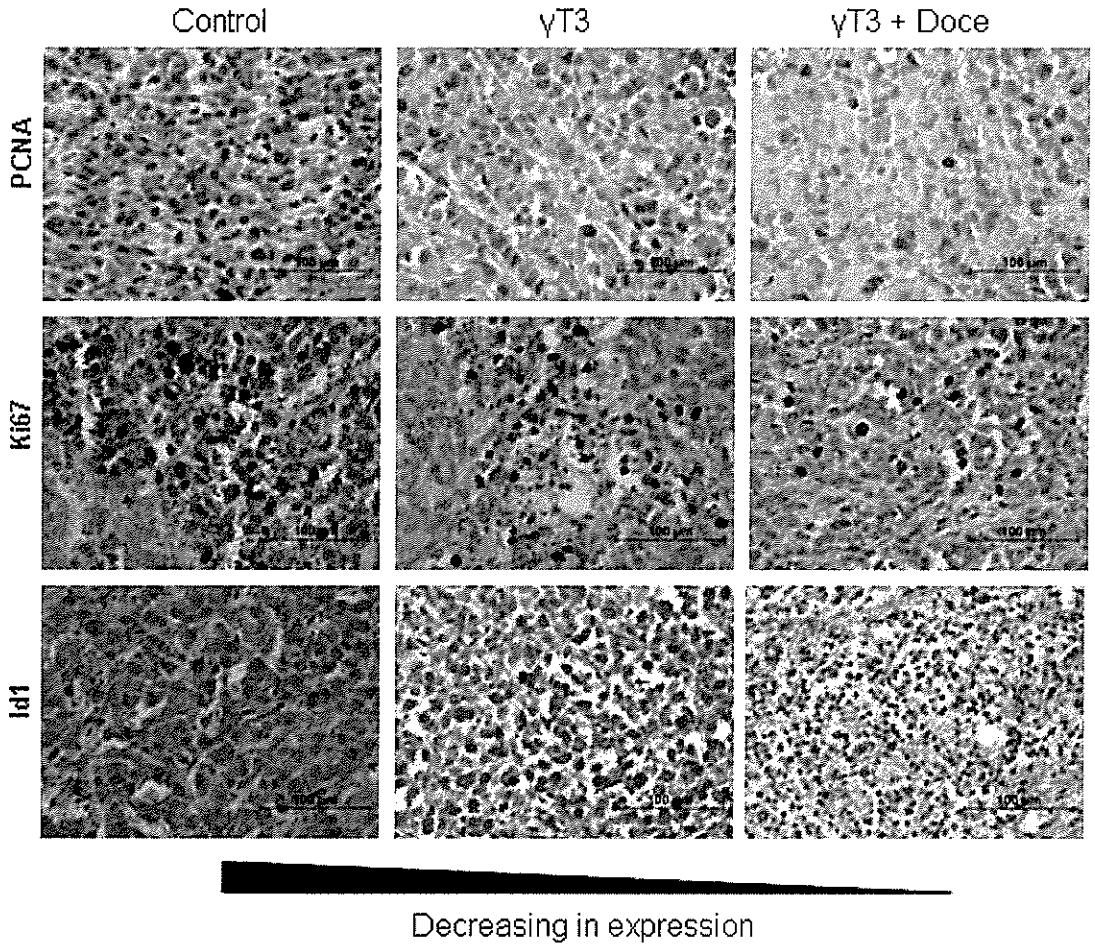


FIG. 31

Apoptotic Markers

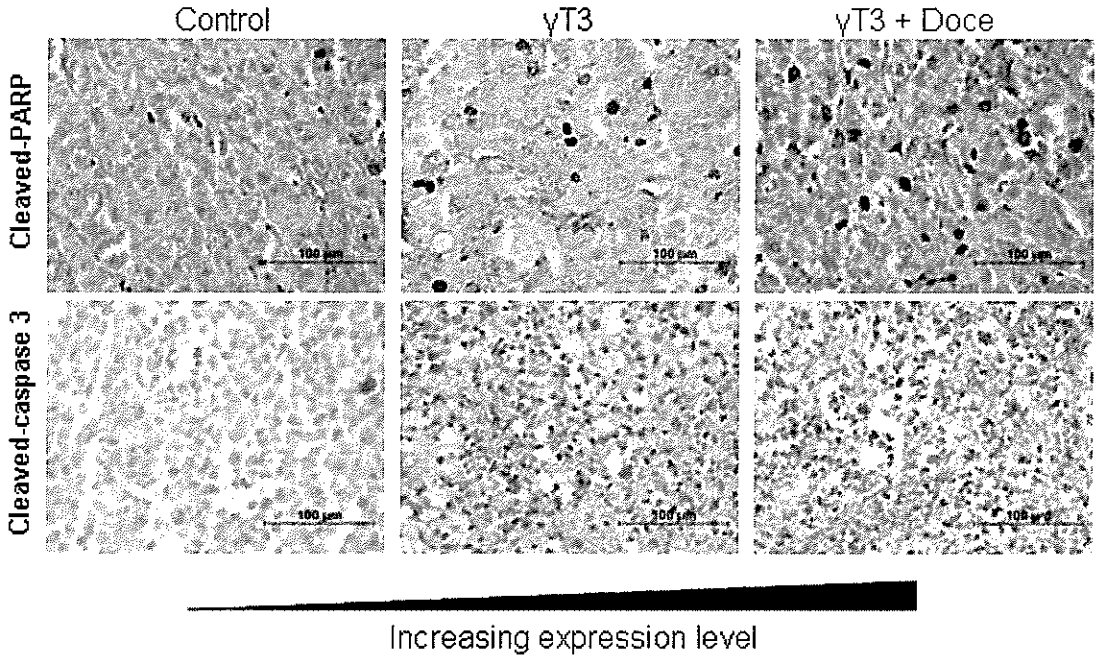


FIG. 32

(A) Tumor Suppressor Gene

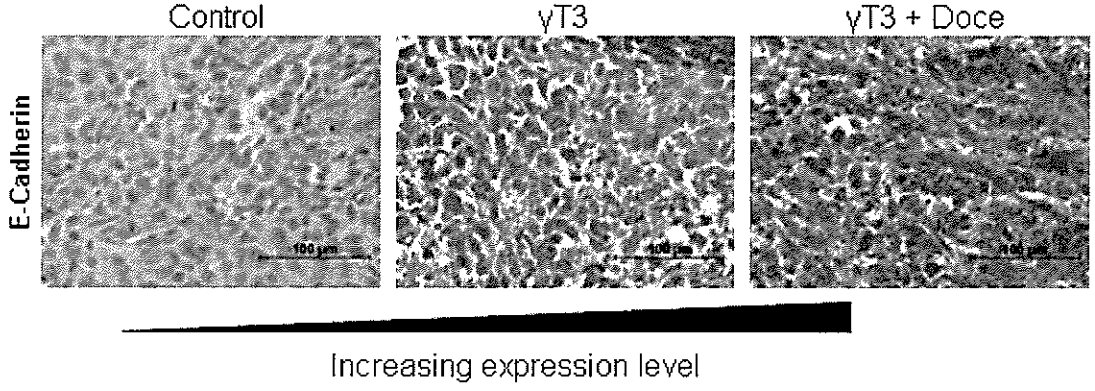


FIG. 32 (continued)

(B) Repressor of E-cadherine

