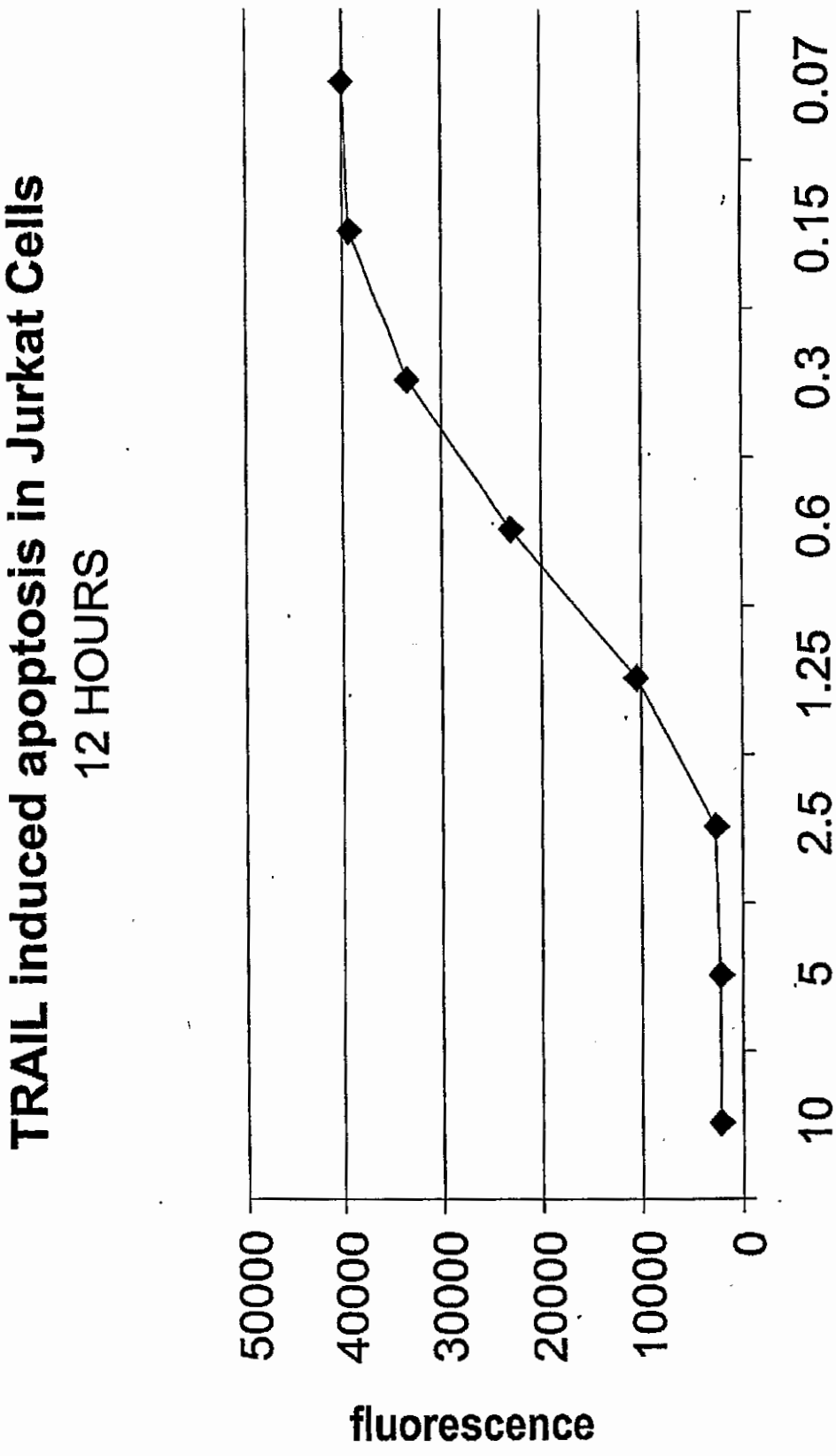
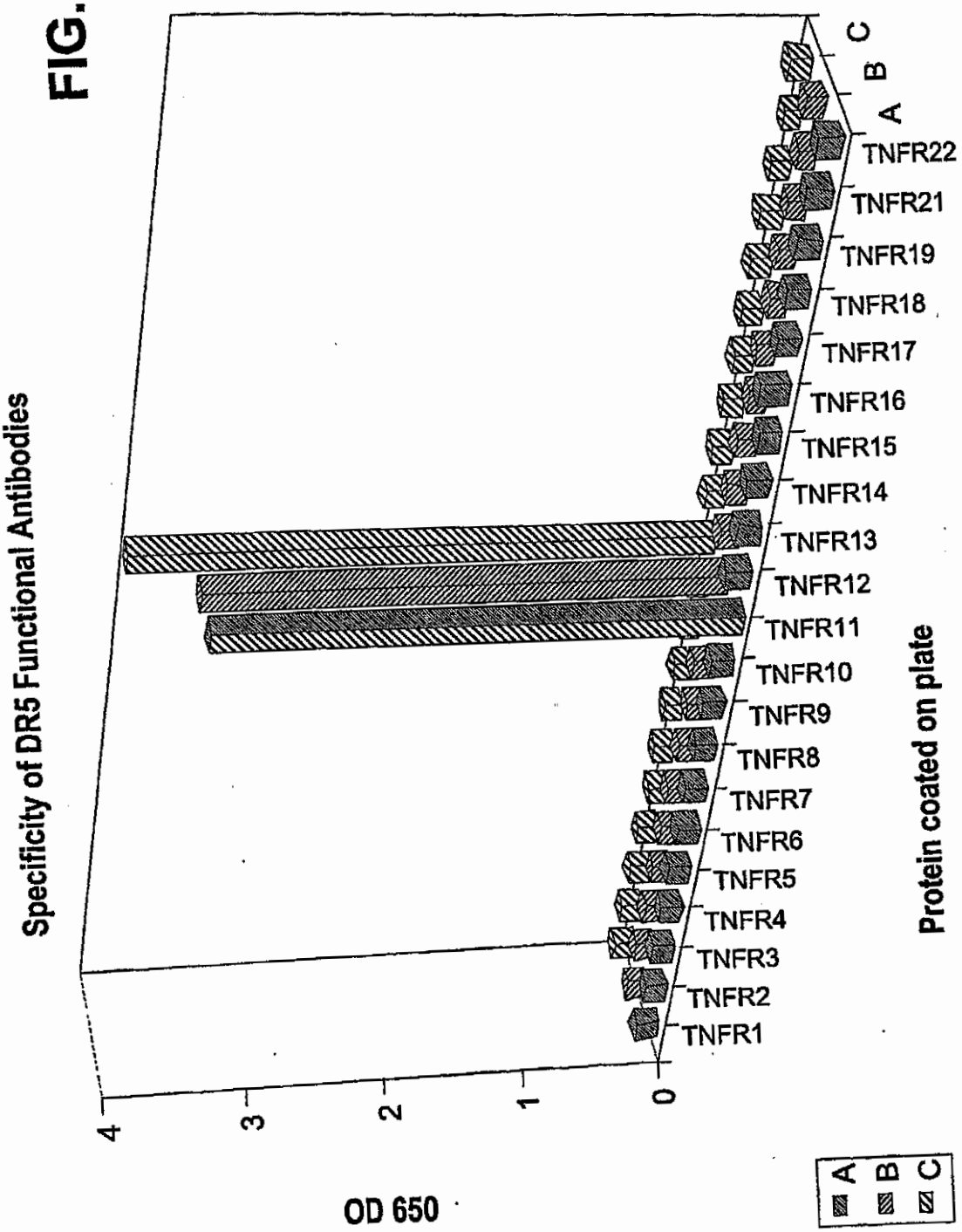

TRAIL induced apoptosis in Jurkat Cells
12 HOURS
fluorescence
50000
40000
30000
20000
10000
0
10
5
2.5
1.25
0.6
0.3
0.15
0.07
Thio-TRAIL (ug/ml)

FIG. 1

FIG. 2


Specificity of DR5 Functional Antibodies
Protein coated on plate
A
B
C
TNFR22
TNFR21
TNFR19
TNFR18
TNFR17
TNFR16
TNFR15
TNFR14
TNFR13
TNFR12
TNFR11
TNFR10
TNFR9
TNFR8
TNFR7
TNFR6
TNFR5
TNFR4
TNFR3
TNFR2
TNFR1
0
1
2
3
4
OD 650
A
B
C

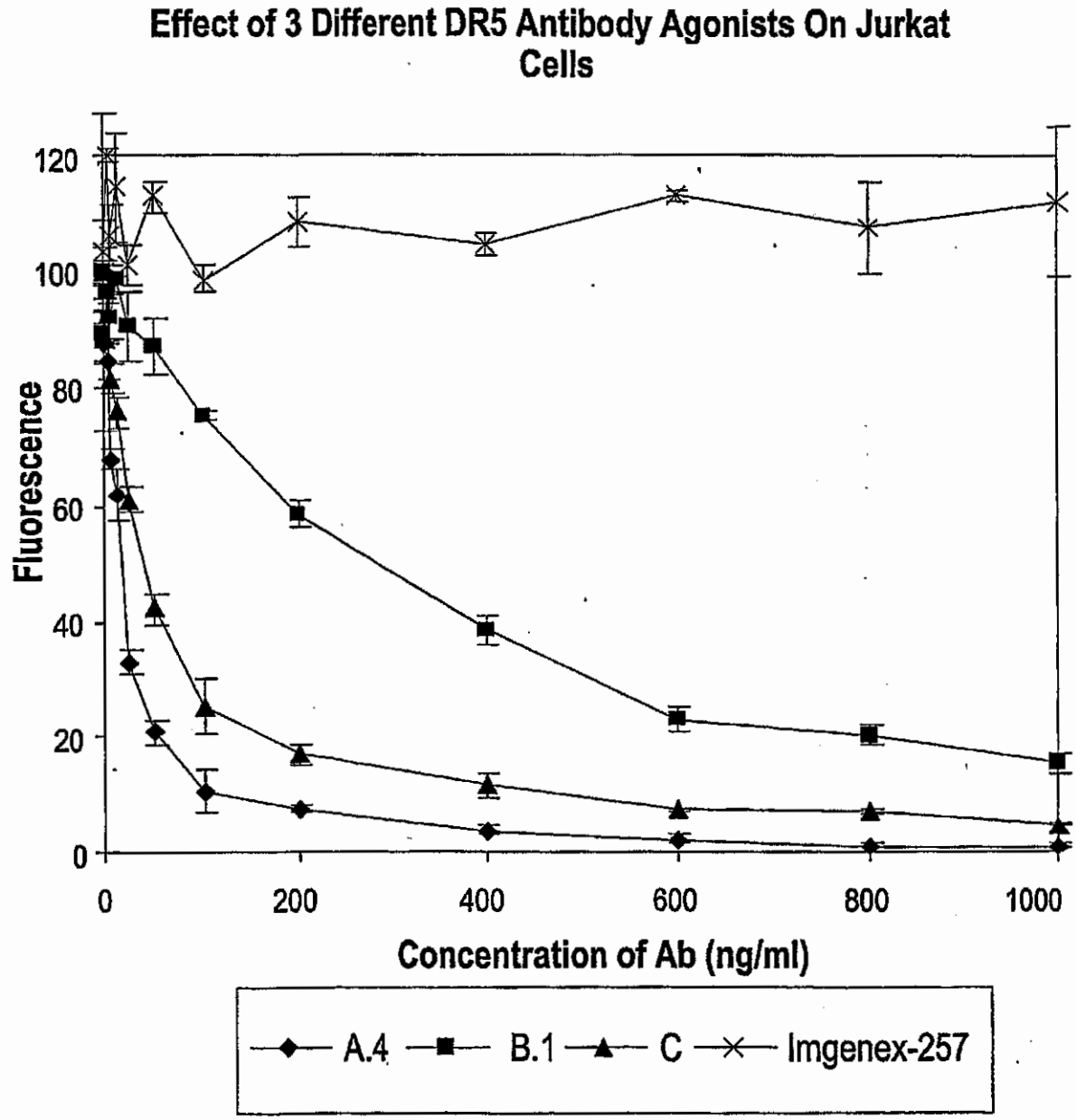

Effect of 3 Different DR5 Antibody Agonists On Jurkat Cells
Fluorescence
120
100
80
60
40
20
0
0
200
400
600
800
1000
Concentration of Ab (ng/ml)
A.4
B.1
C
Imgenex-257

FIG. 3

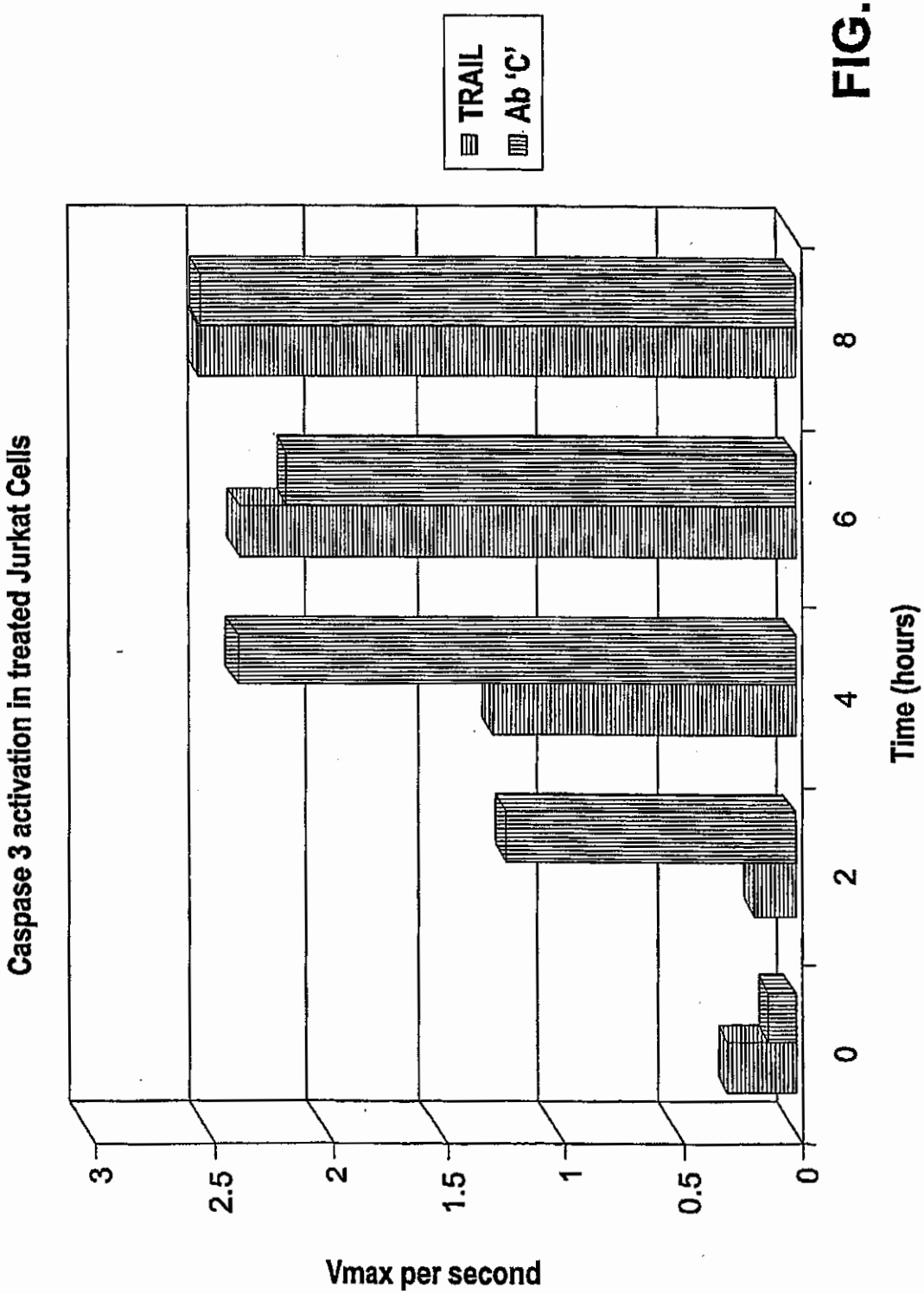

TRAIL
Ab 'C'
Caspase 3 activation in treated Jurkat Cells
Vmax per second
0
0.5
1
1.5
2
2.5
3
0
2
4
6
8
Time (hours)

FIG. 4

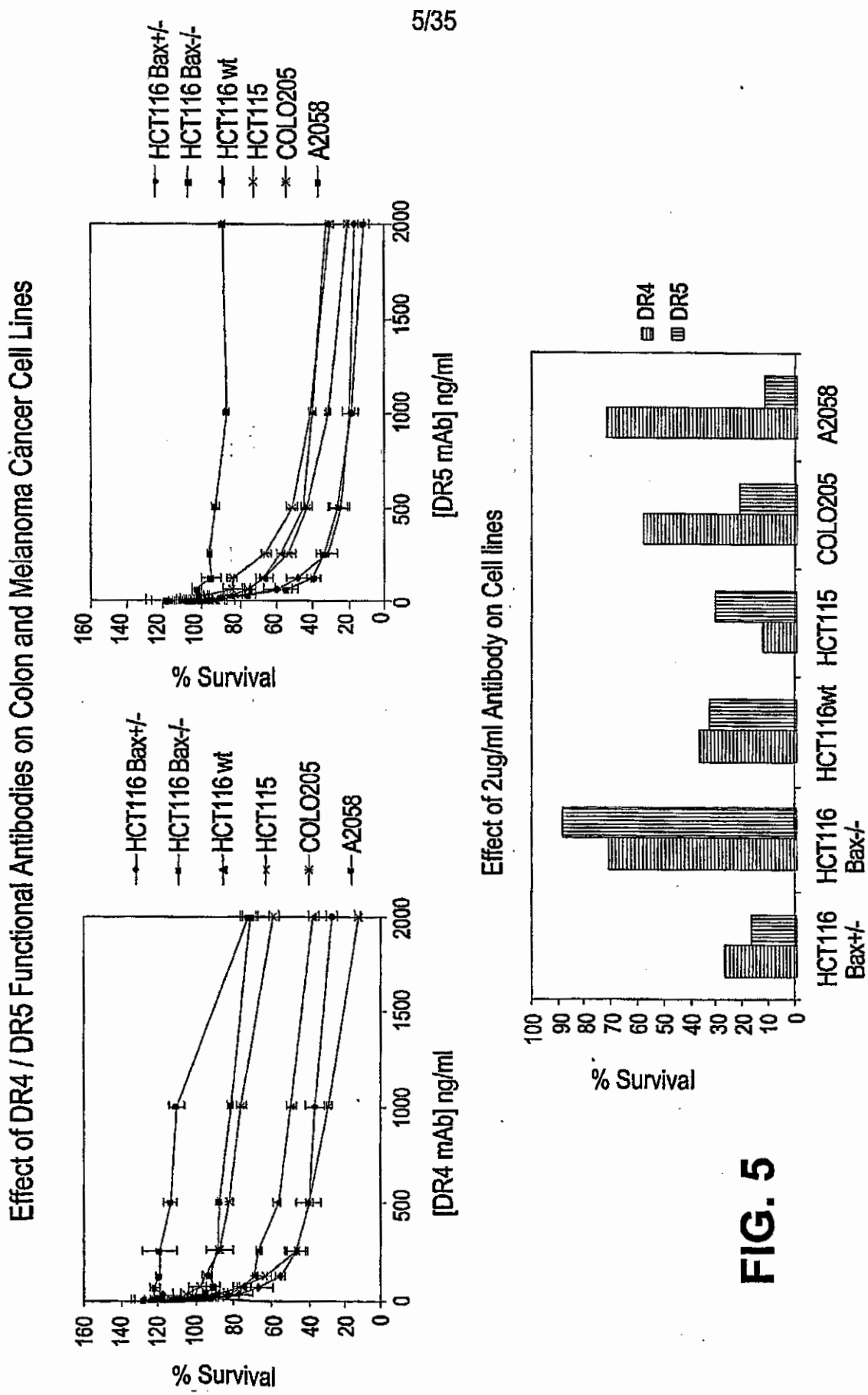

Effect of DR4 / DR5 Functional Antibodies on Colon and Melanoma Cancer Cell Lines
HCT116 Bax+/-
HCT116 Bax-/-
HCT116 wt
HCT115
COLO205
A2058
% Survival
[DR4 mAb] ng/ml
[DR5 mAb] ng/ml
Effect of 2ug/ml Antibody on Cell lines
DR4
DR5
HCT116 Bax+/-
HCT116 Bax-/-
HCT116wt
HCT115
COLO205
A2058

FIG. 5

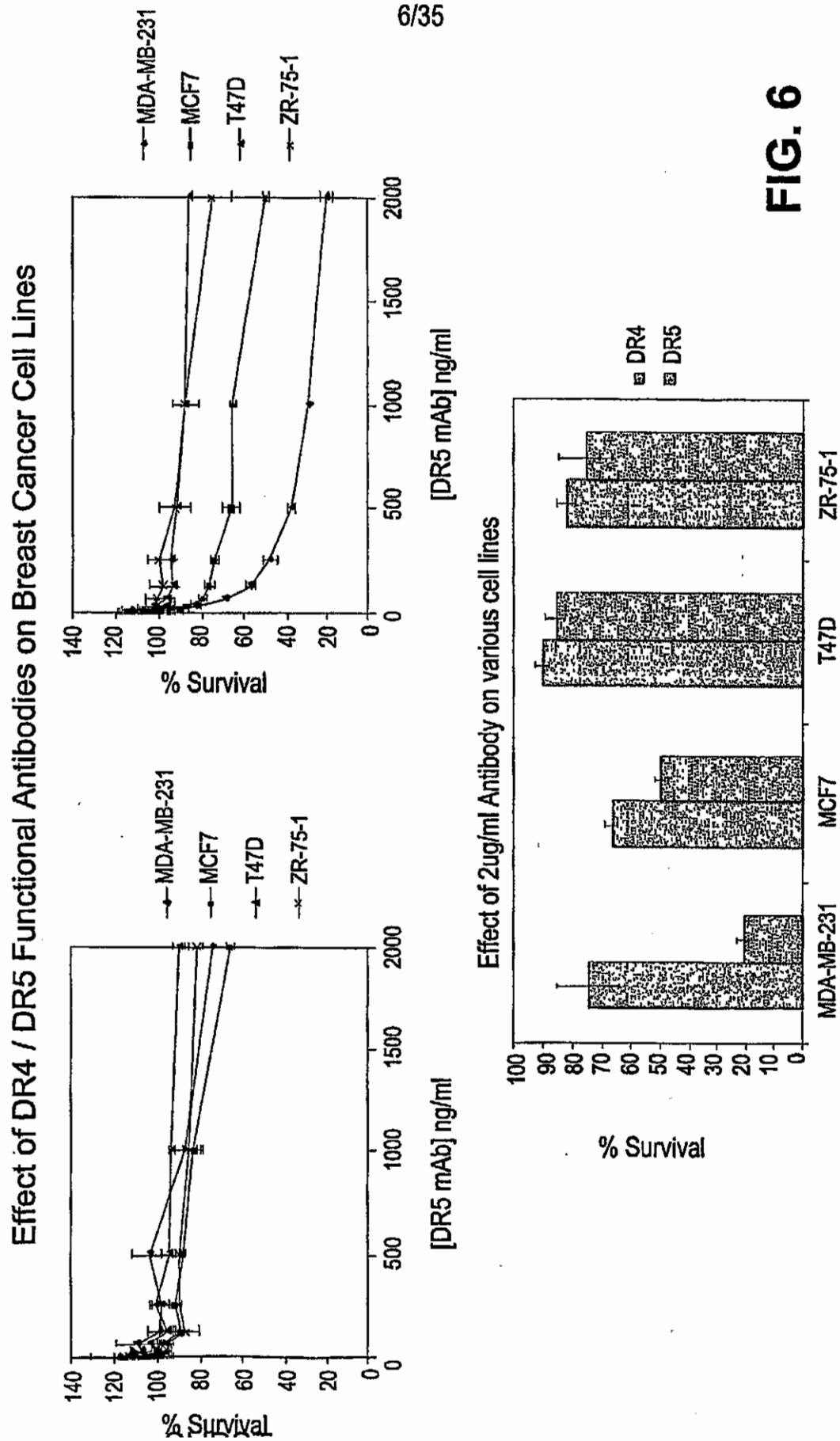

Effect of DR4 / DR5 Functional Antibodies on Breast Cancer Cell Lines
MDA-MB-231
MCF7
T47D
ZR-75-1
% Survival
[DR5 mAb] ng/ml
Effect of 2ug/ml Antibody on various cell lines
DR4
DR5
MDA-MB-231
MCF7
T47D
ZR-75-1
% Survival

FIG. 6

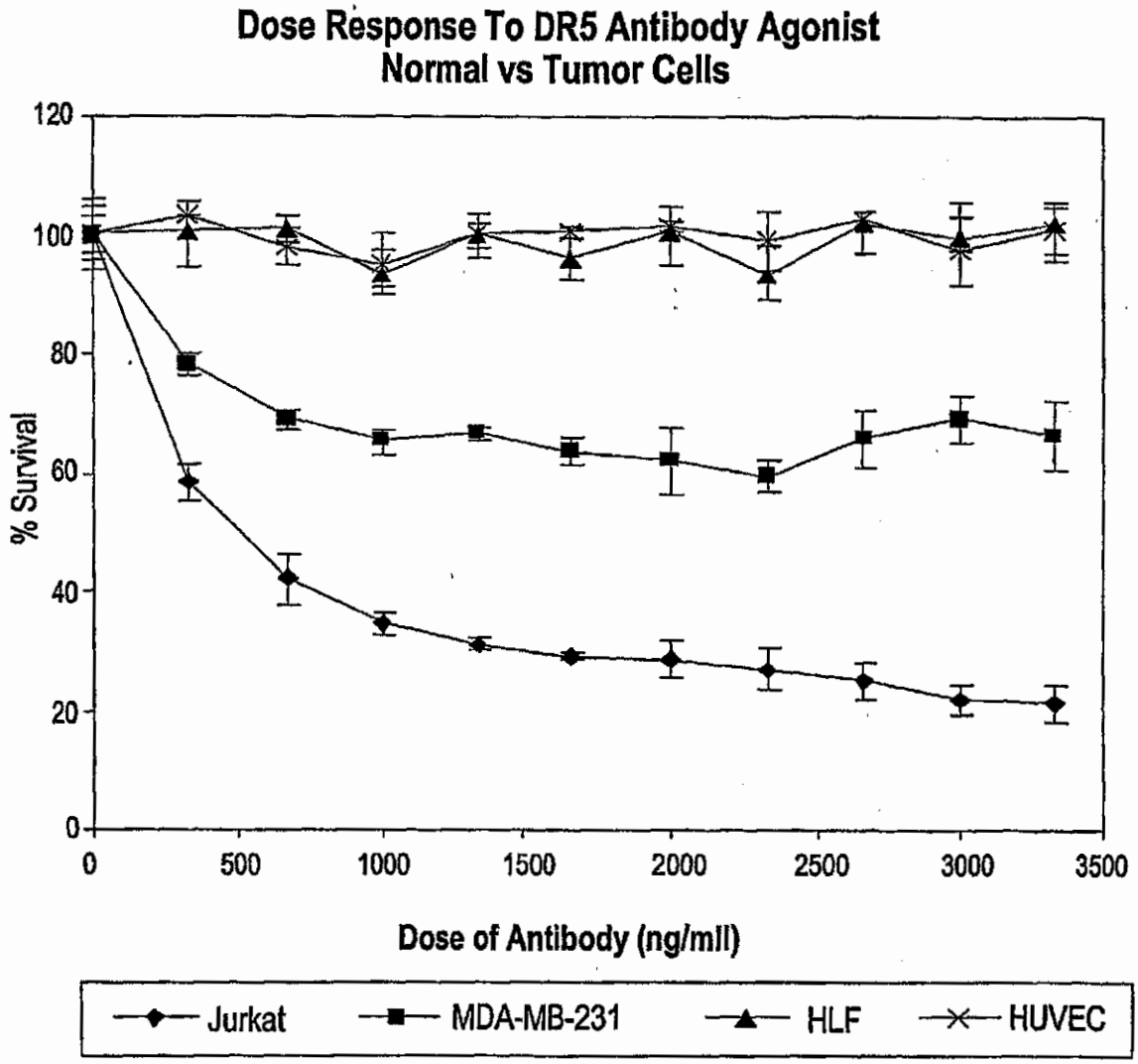

Dose Response To DR5 Antibody Agonist
Normal vs Tumor Cells
120
100
80
60
40
20
0
% Survival
0
500
1000
1500
2000
2500
3000
3500
Dose of Antibody (ng/ml)
Jurkat
MDA-MB-231
HLF
HUVEC

FIG. 7

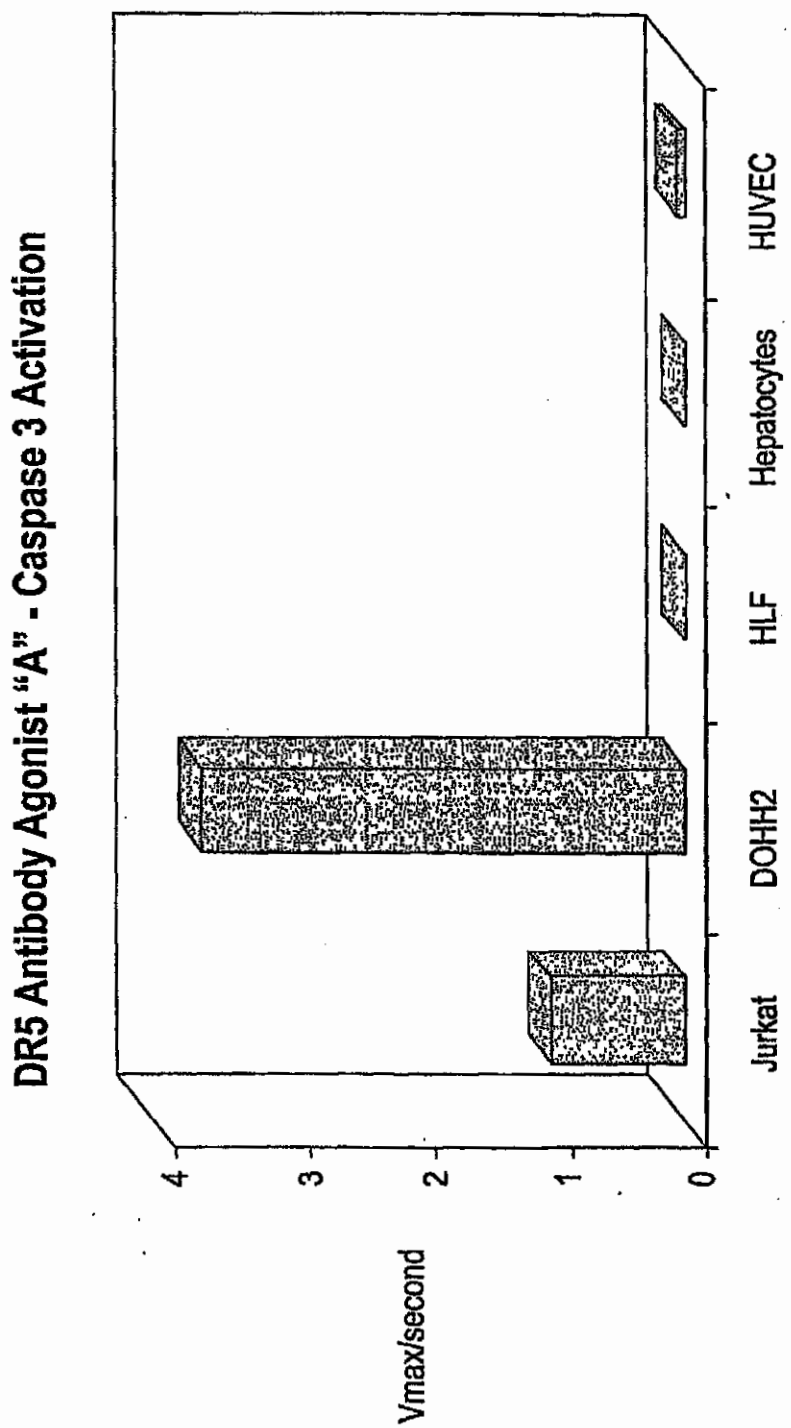

DR5 Antibody Agonist "A" - Caspase 3 Activation
Vmax/second
0
1
2
3
4
Jurkat
DOHH2
HLF
Hepatocytes
HUVEC

FIG. 8

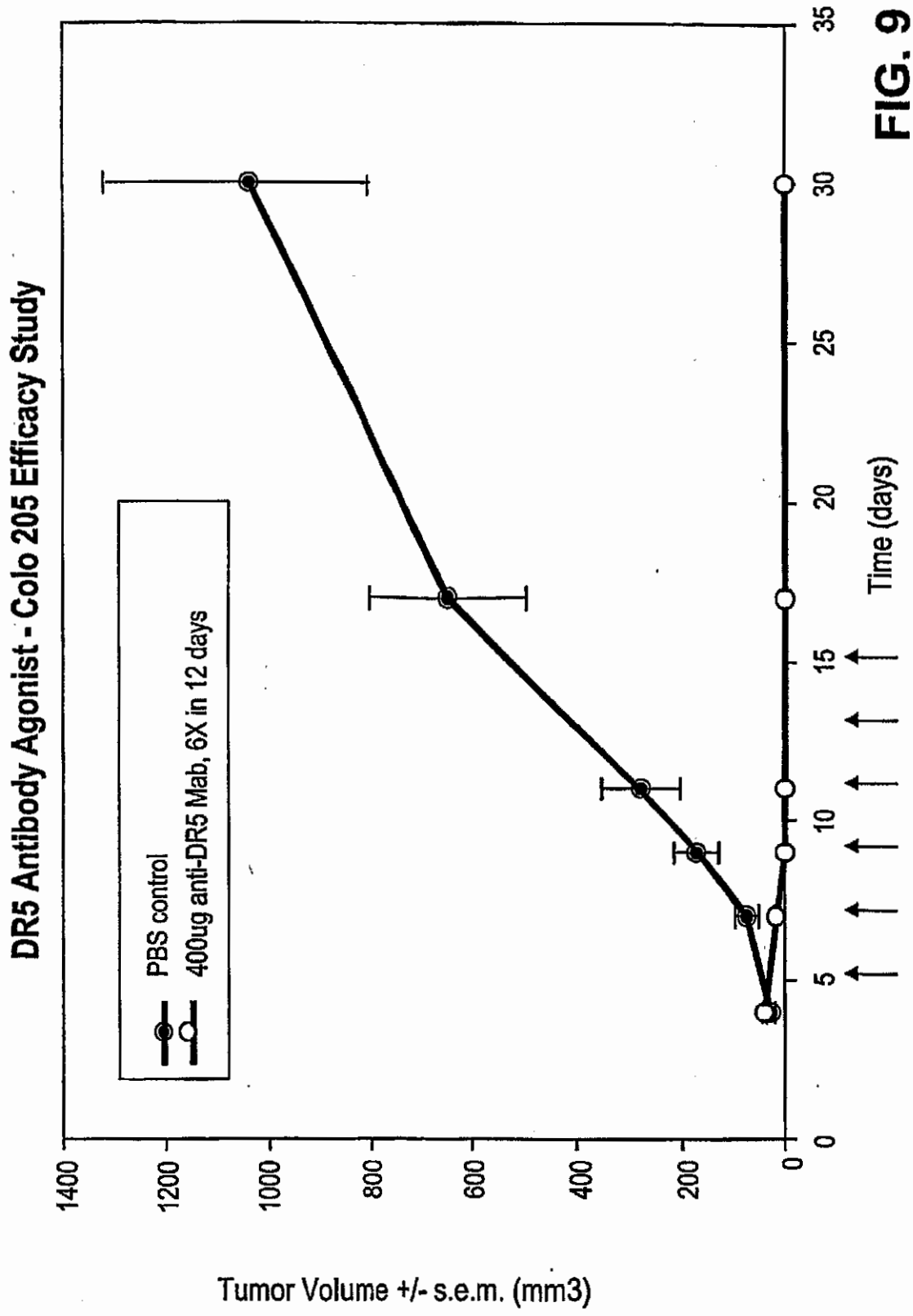

DR5 Antibody Agonist - Colo 205 Efficacy Study
PBS control
400ug anti-DR5 Mab, 6X in 12 days
Tumor Volume +/- s.e.m. (mm3)
Time (days)
0
200
400
600
800
1000
1200
1400
0
5
10
15
20
25
30
35

FIG. 9

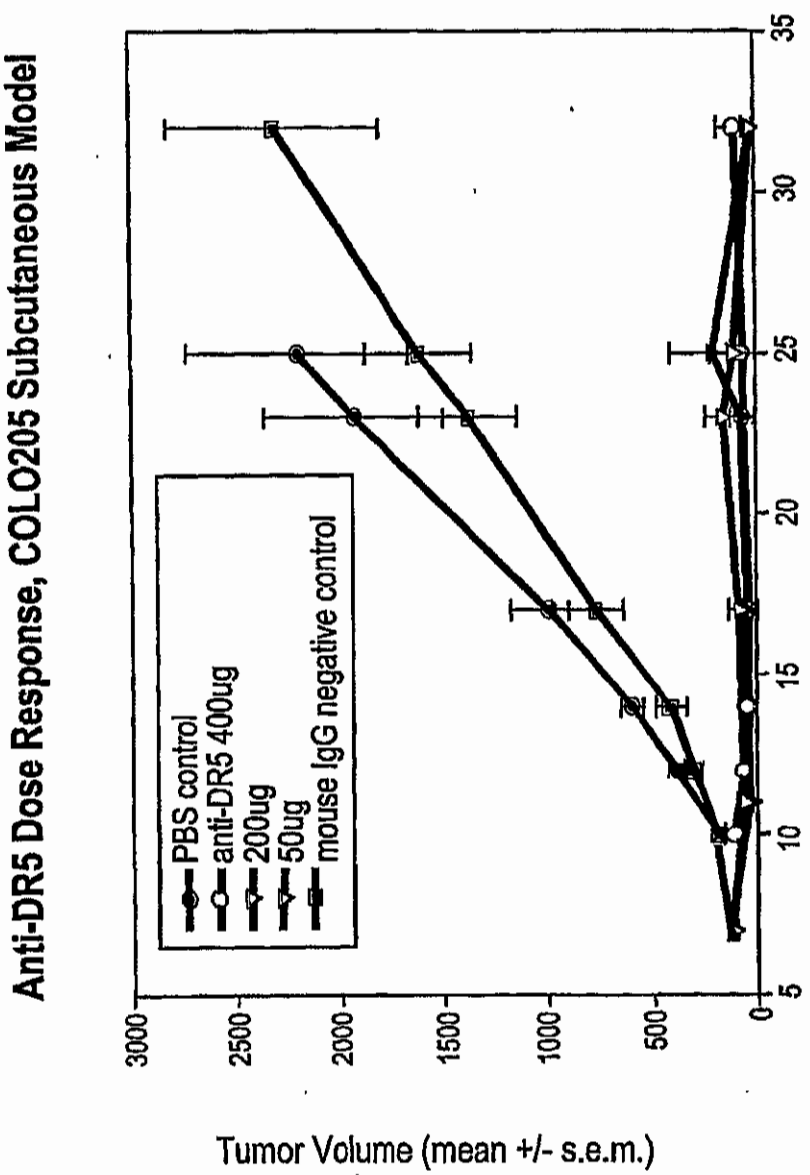

Anti-DR5 Dose Response, COLO205 Subcutaneous Model
PBS control
anti-DR5 400ug
200ug
50ug
mouse IgG negative control
Tumor Volume (mean +/- s.e.m.)
Time (days)
0
500
1000
1500
2000
2500
3000
5
10
15
20
25
30
35

FIG. 10

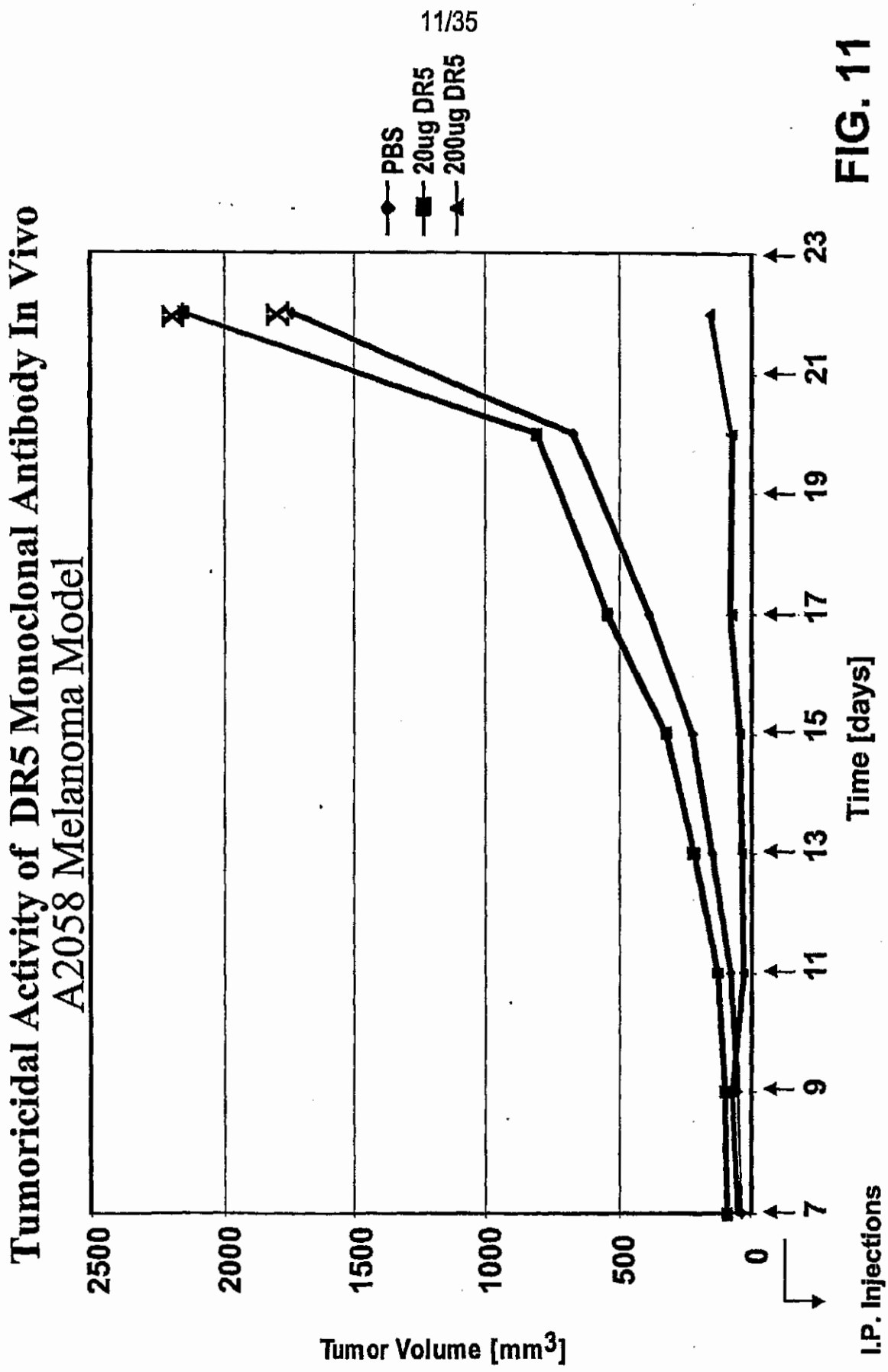

Tumoricidal Activity of DR5 Monoclonal Antibody In Vivo
A2058 Melanoma Model
PBS
20ug DR5
200ug DR5
Tumor Volume [mm3]
2500
2000
1500
1000
500
0
Time [days]
7
9
11
13
15
17
19
21
23
I.P. Injections

FIG. 11

FIG. 12

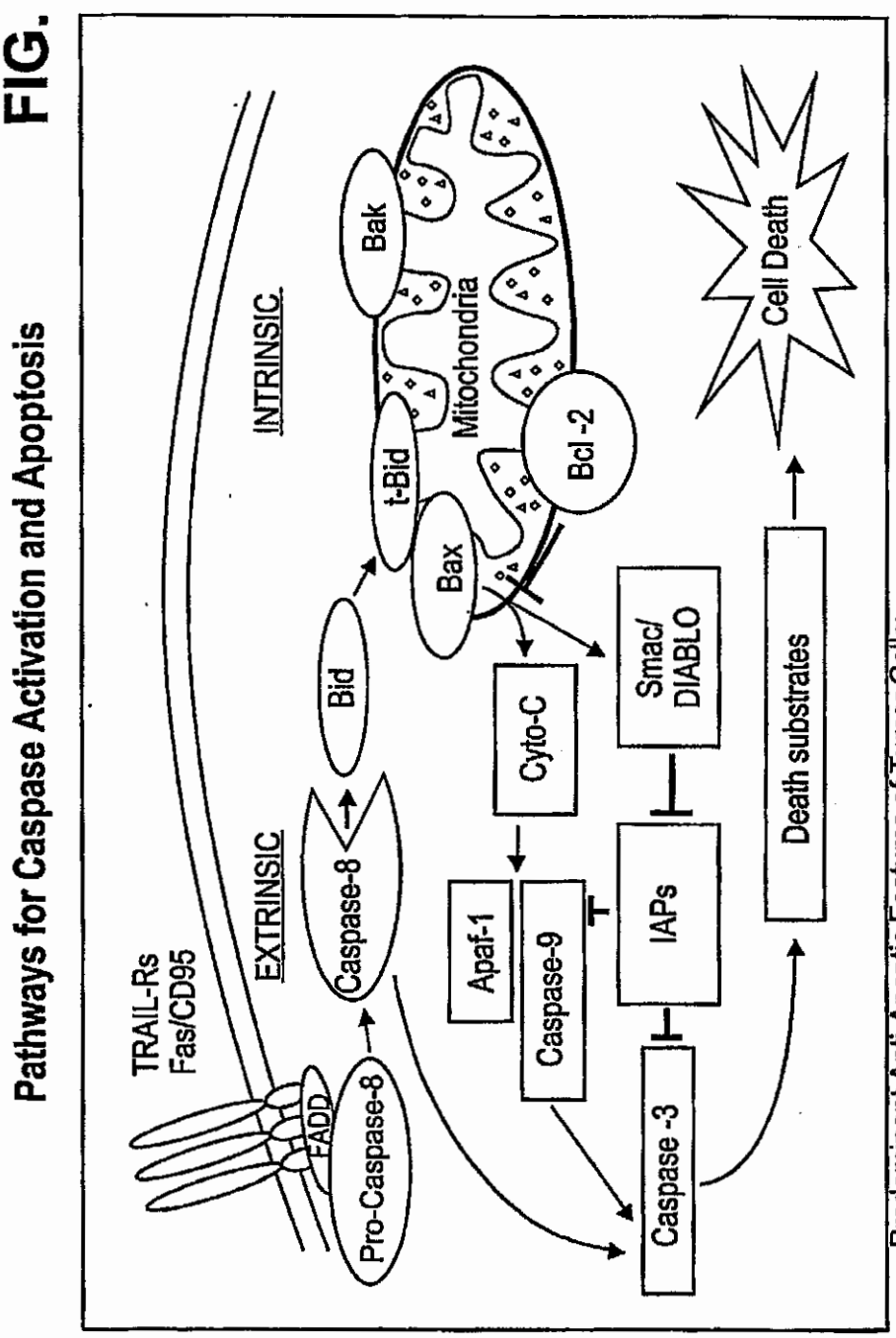
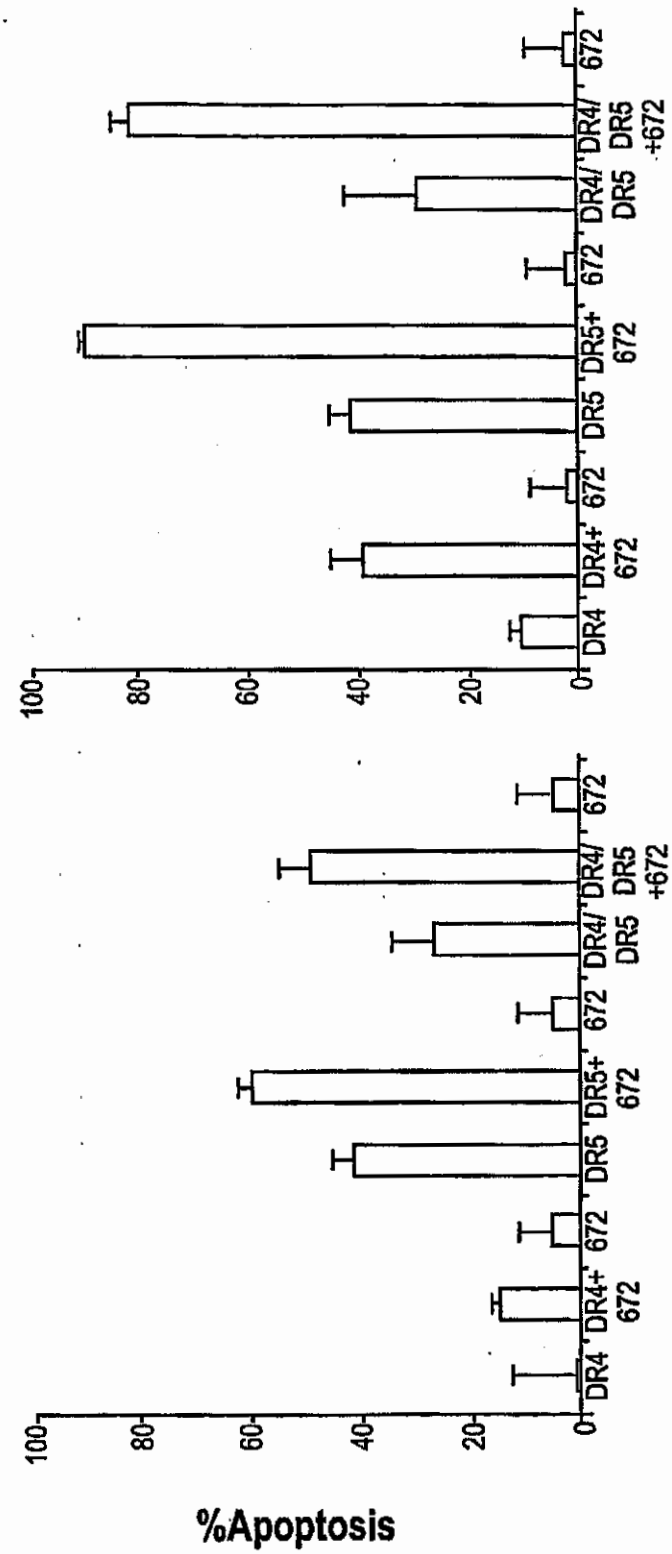

Pathways for Caspase Activation and Apoptosis
TRAIL-Rs
Fas/CD95
FADD
Pro-Caspase-8
EXTRINSIC
Caspase-8
Bid
INTRINSIC
t-Bid
Bak
Bax
Mitochondria
Bcl -2
Cyto-C
Apaf-1
Caspase-9
Smac/
DIABLO
IAPs
Caspase -3
Death substrates
Cell Death
Predominant Anti-Apoptic Features of Tumor Cells:
Over-expression of Bcl 2
Increased Levels of IAP's
Mutations in Bax

FIG. 13

Anti-DR4 or DR5-induced apoptosis in A2058 cells in the absence of presence of 0.5 uM LBP 672



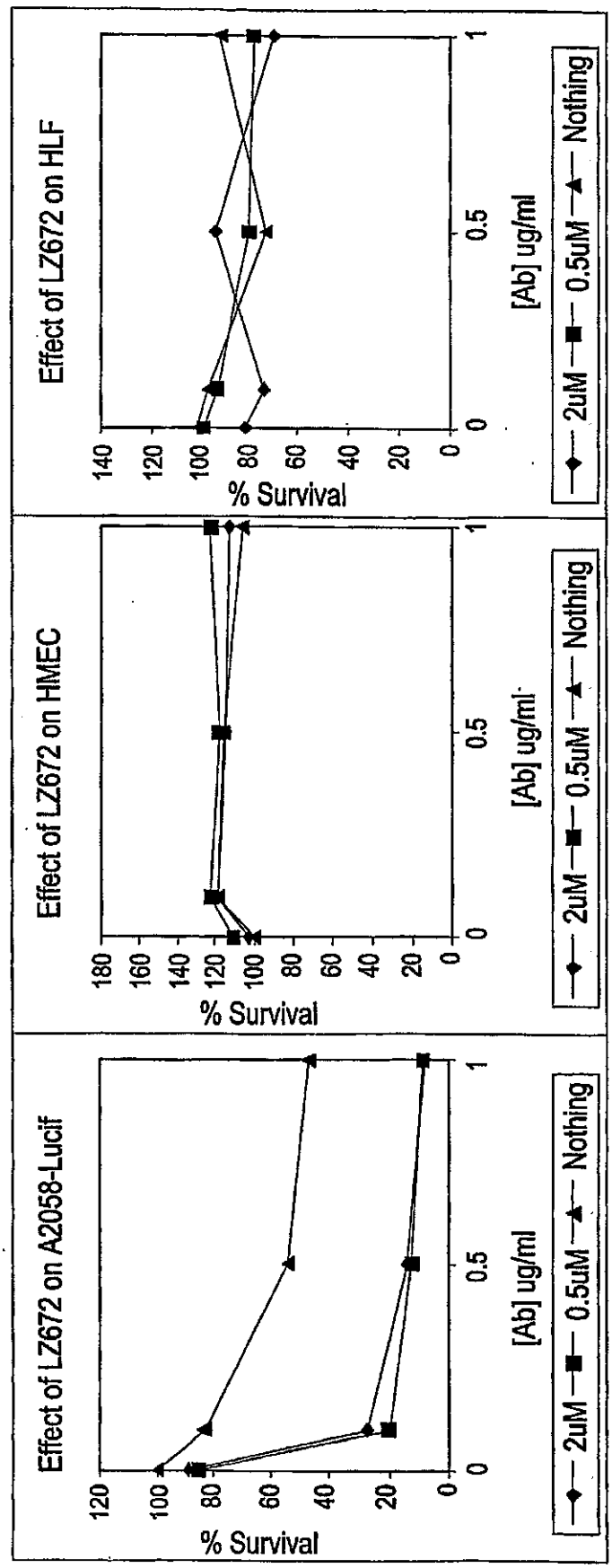
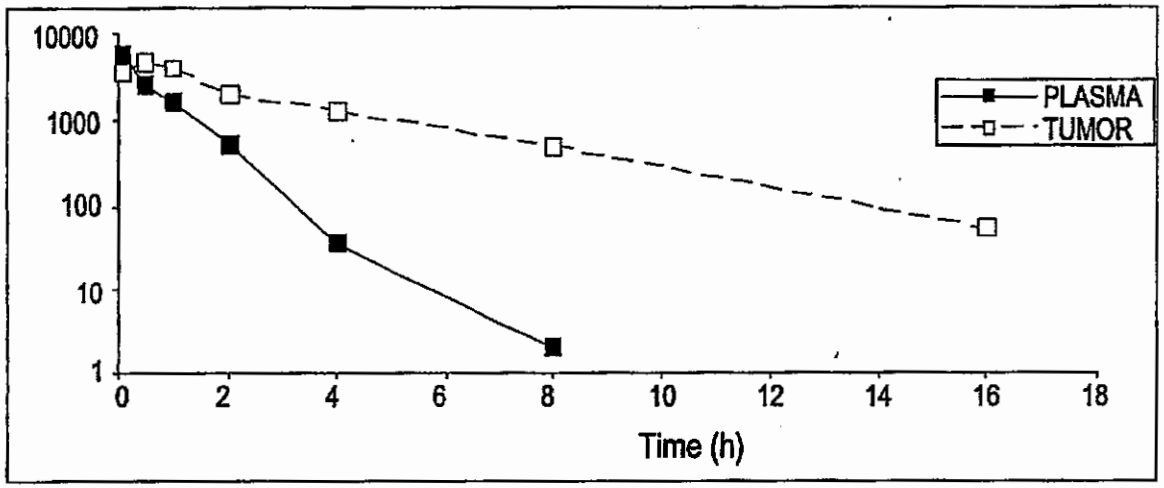
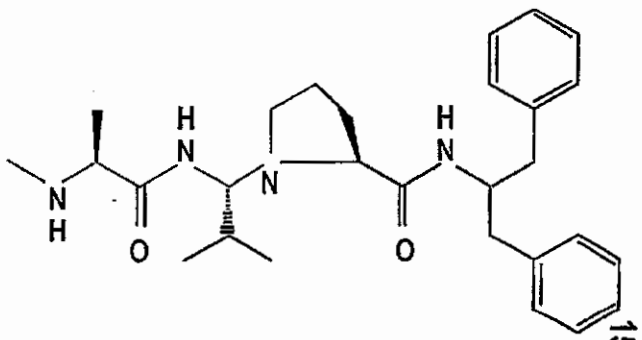

Effect of LB 672 On Normal And Tumor Cells
Effect of LZ672 on A2058-Lucif
% Survival
[Ab] ug/ml
2uM
0.5uM
Nothing
Effect of LZ672 on HMEC
% Survival
[Ab] ug/ml
2uM
0.5uM
Nothing
Effect of LZ672 on HLF
% Survival
[Ab] ug/ml
2uM
0.5uM
Nothing

FIG. 14

PK and PD Study of Smac Mimetic LBP672 In Nude Mice Bearing HCT116 Tumors





NVP-LBP672	
M.W. = 492.67 as a free base	

PK SUMMARY

Model: Athymic nude mice bearing subcutaneous HCT116 tumors

Dose: single 25 mg/kg i.v. trifluoroacetate salt (20.3 mg/kg free base) in D5W.

LBP672 rapidly absorbed by tumor Tmax @ 0.5 h. Mean tumor Cmax @ 4620ng/g (9.38 uM).

At 16 h. post dose LBP672 mean tumor [co] @ 55.1 ng/g (110 nM).

FIG. 15

NFkB Activation By The Proteosome

Viruses, growth factors, radiation or chemotherapeutic drugs activate pathways that lead to the degradation of IkB by the proteosome. NFkB activates transcription of genes that protect the cell from apoptosis.

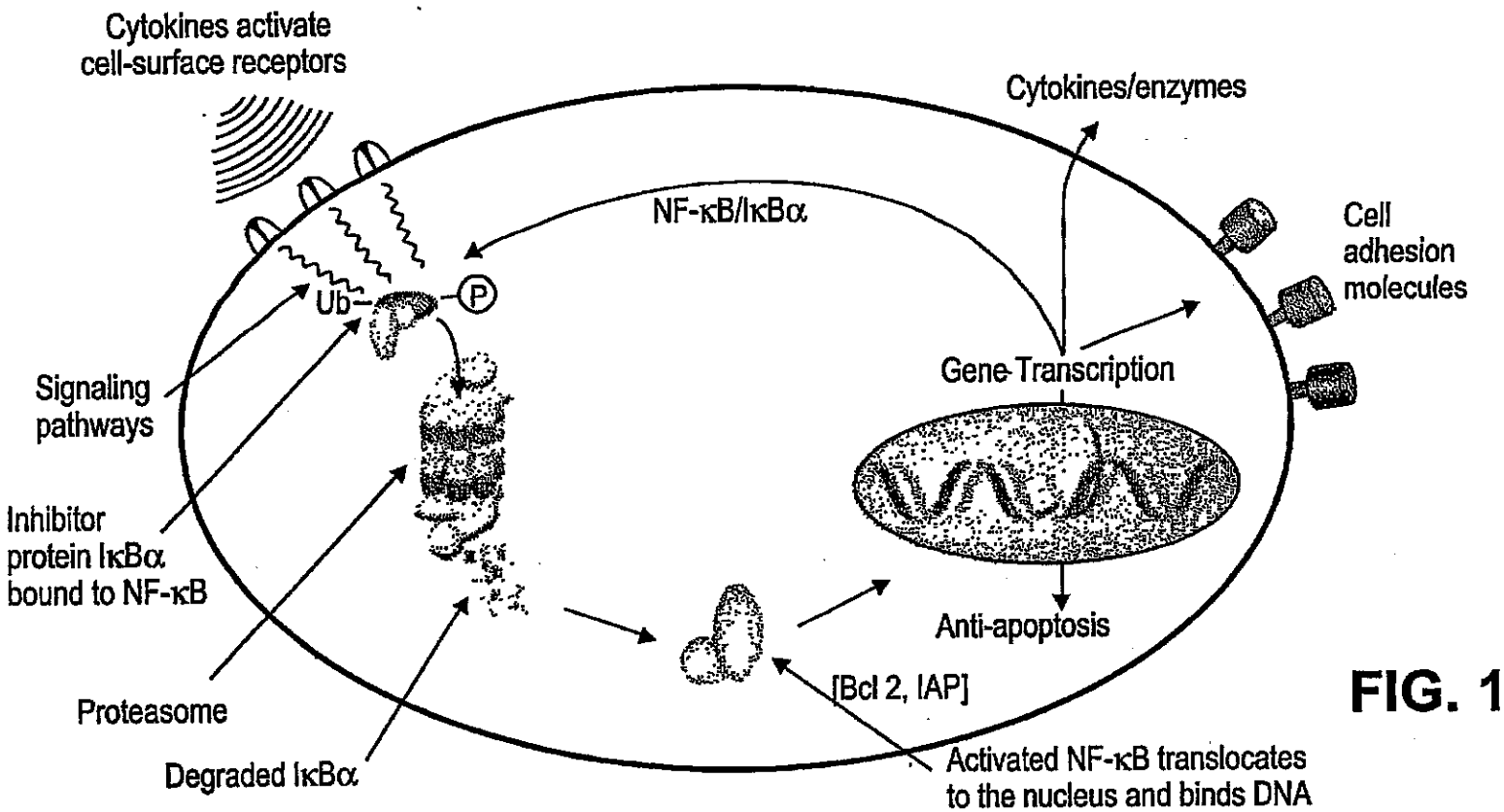
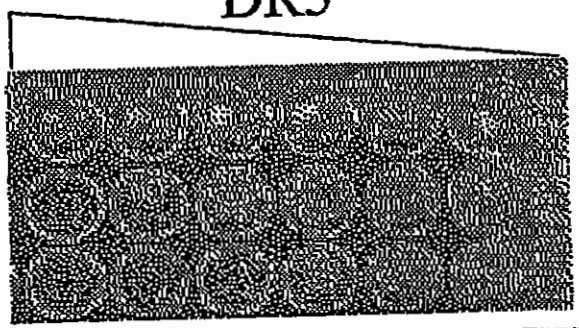
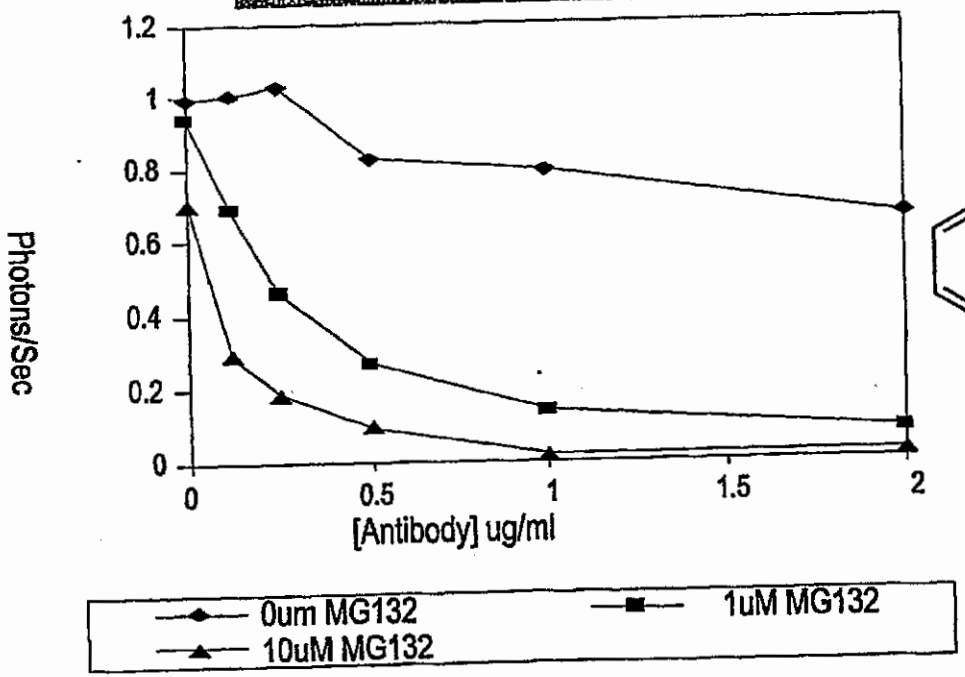


FIG. 16

Proteosome Inhibitor MG132 Enhances DR5 Antibody Induced Apoptosis of SW 480 Human Colon Carcinoma Cells





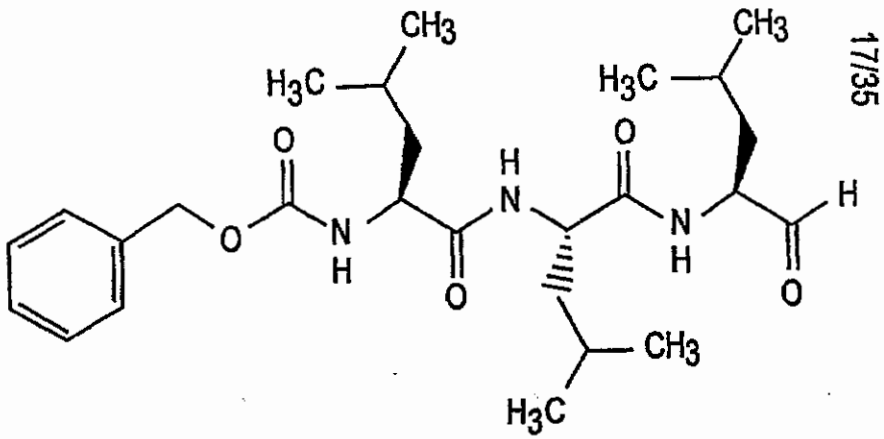


FIG. 17

20S Proteosome Inhibitors –[Chymotryptic Activity]

Compound	Description	Selectivity	IC50	MTD	Sol.
NVP-AFB340-NX	*Boronate*	*Good*	*<1nM*	*3mg/kg-1x*	*Good*
NVP-AFD314-NX	*Boronate*	*Good*	*<1nM*	*3mg/kg-1x*	*Good*
NVP-AEV273-NX	*Beta-Lactame*	*Excellent*	*3nM*	*20 mg/kg-2x*	*Poor*

NVP-AFB340-NX

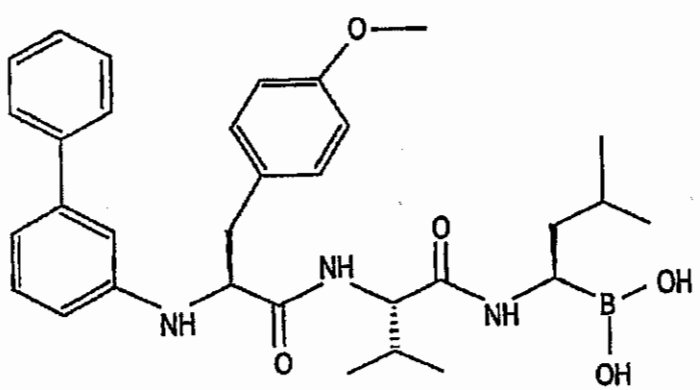
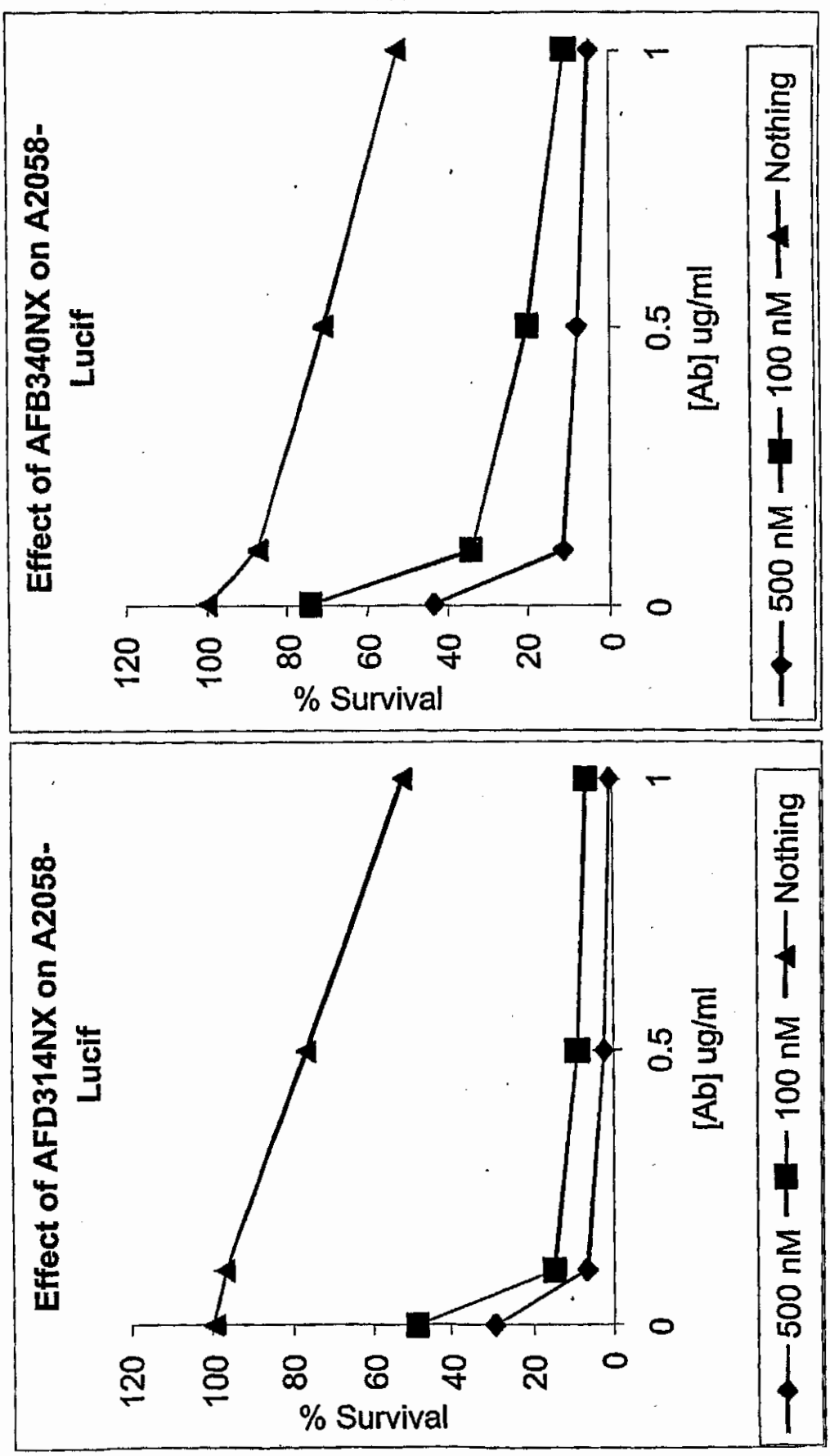


FIG. 18

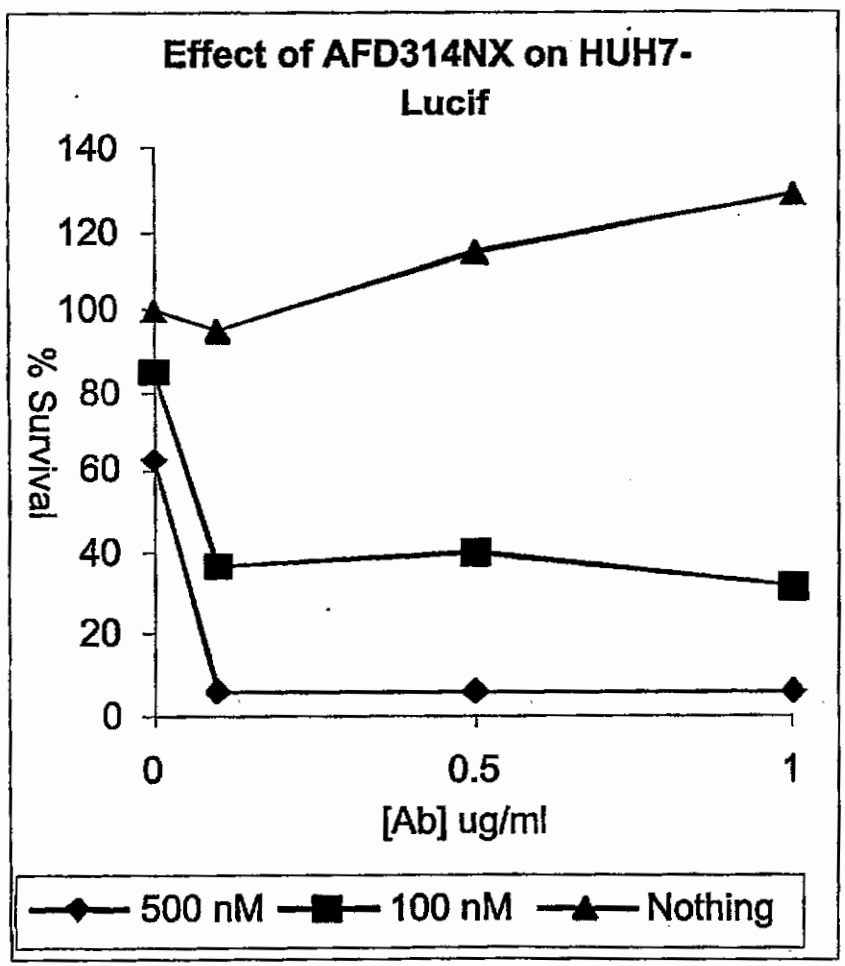
FIG. 19

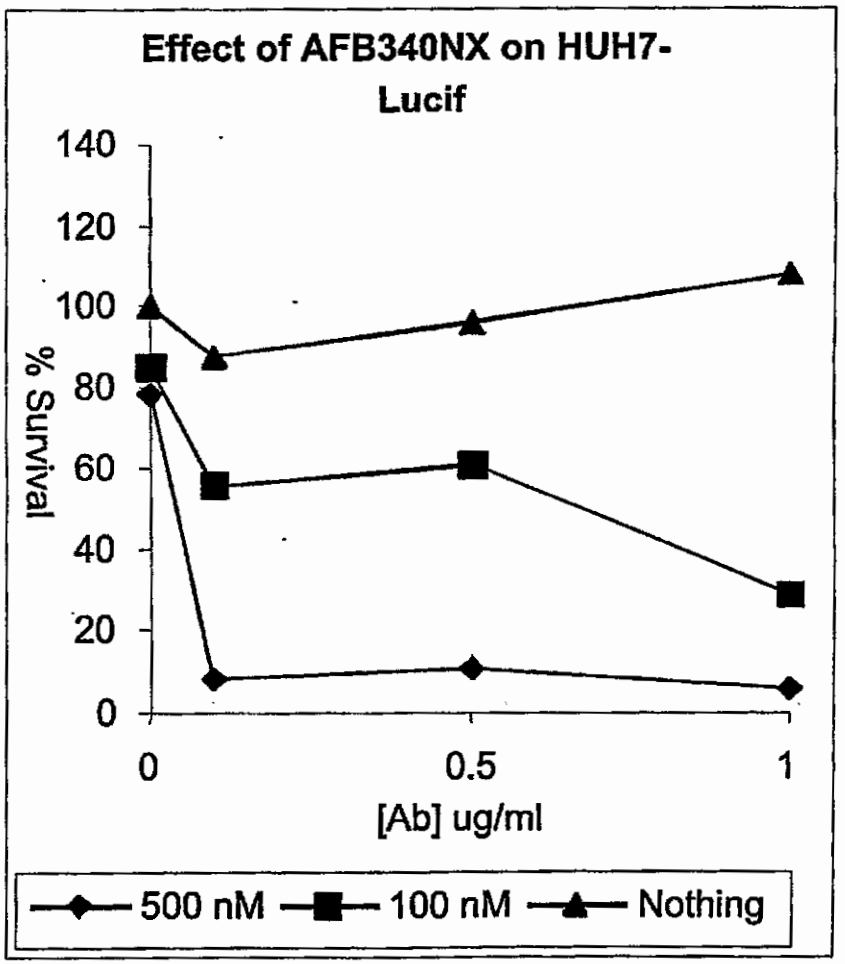
Effect of Proteosome Inhibitors On A2058 -Luc



Effect of Proteosome Inhibitors On The Hepatocarcinoma Cell Line HUH7-Luc

FIG. 20





Effect of Proteosome Inhibitors On Normal Human Mammary Epithelial Cells (HMEC)

FIG. 21

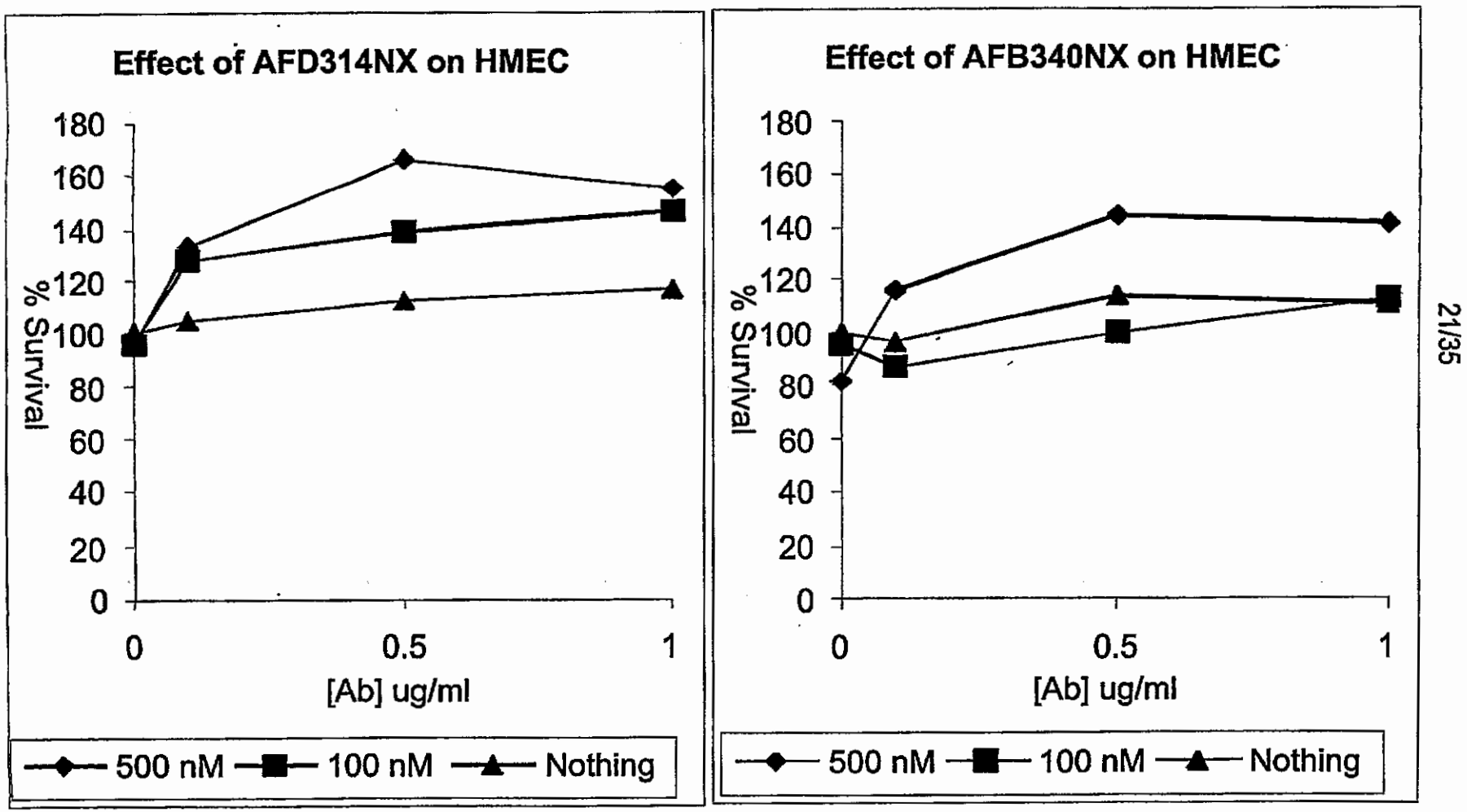
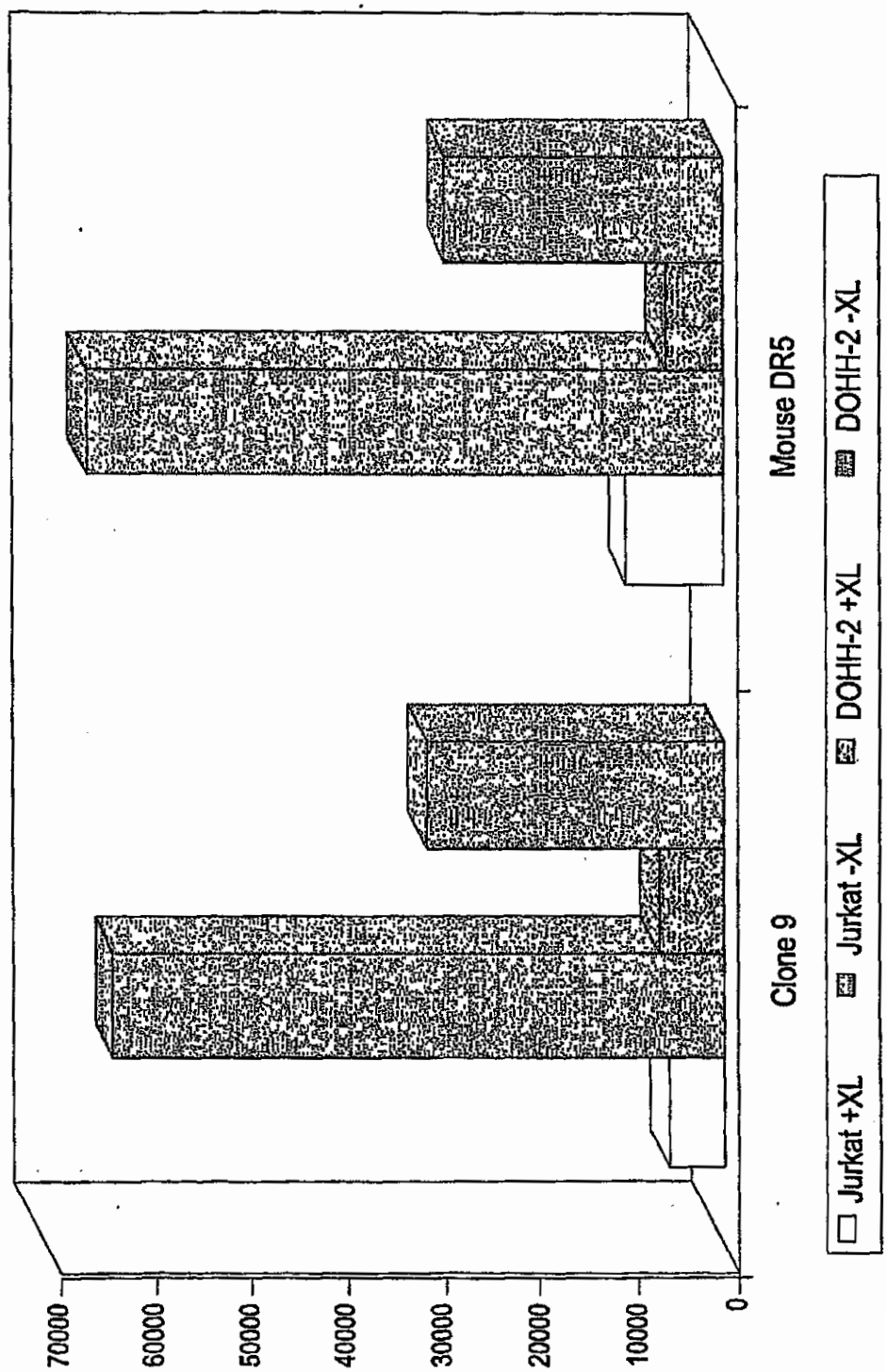


FIG. 22

Expression of Mouse-Human Chimeric DR5 Antibody
Functional Activity



Anti-DR5 DNA Sequence

Light Chain Variable Region

GACATTGCGATGACCCAGTCTCACAAGTTCATGTCCACATTAGTGGGAGACAGGGTCA
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAGCCTGGTATCAACAAAA
ACCAGGGCAATCTCCTAAACTACTGATTTACTGGGCATCCACCCGGCACACTGGAGTC
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTATACTCTCACCATCAGCAGTA
TGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGAGTAGTAACCCGCTCAC
GTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTATCC
ATCTTCCCACC

Heavy Chain Variable Region

CAGGCAAAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAAACCCGGGGCATCA
GTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTTCACTGACTATACTATACACTGGGT
AAAGCAGAGGTCTGGACAGGGTCTTGAGTGGATTGGGTGGTTTTACCCTGGAGGTGGT
TATATAAAATACAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCT
CCAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGGCTCTGCGGTCTATTTC
TGTGCAAGACACGAAGAGGGCATCTATTTTGACTACTGGGGCCAAGGCACCACTCTCA
CAGTCTCCTCA

FIG. 23

DR5 V_H Sequence – Heavy Chain Subgroup 2B

FR1

Lys Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val

CDR1

Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Thr Ile His Trp Val

FR2

Lys Gln Arg Ser Gly Gln Gly Leu Glu Trp Ile Gly Trp Phe Tyr Pro Gly Gly

CDR2

Gly Tyr Ile Lys Tyr Asn Glu Lys Phe Lys Asp Arg Ala Thr Leu Thr Ala Asp

FR3

Lys Ser Ser Asn Thr Val Tyr Met Glu Leu Ser Arg Leu Thr Ser Glu Gly Ser

CDR3

Ala Val Tyr Phe Cys Ala Arg His Glu Glu Gly Ile Tyr Phe Asp Tyr Trp Gly

FR4

Gln Gly Thr Thr Leu Thr Val Ser Ser

FIG. 24

DR5 V_L Sequence– Kappa Light Chain Subgroup 5

FR1

Asp Ile Ala Met Thr Gln Ser His Lys Phe Met Ser Thr Leu Val Gly Asp

CDR1

Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Ile Ala

FR2

Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala

CDR2 *FR3*

Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly

Thr Asp Tyr Thr Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr

CDR3 *FR4*

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr

Lys Leu Glu Leu Lys Arg Ala

FIG. 25

FIG. 26

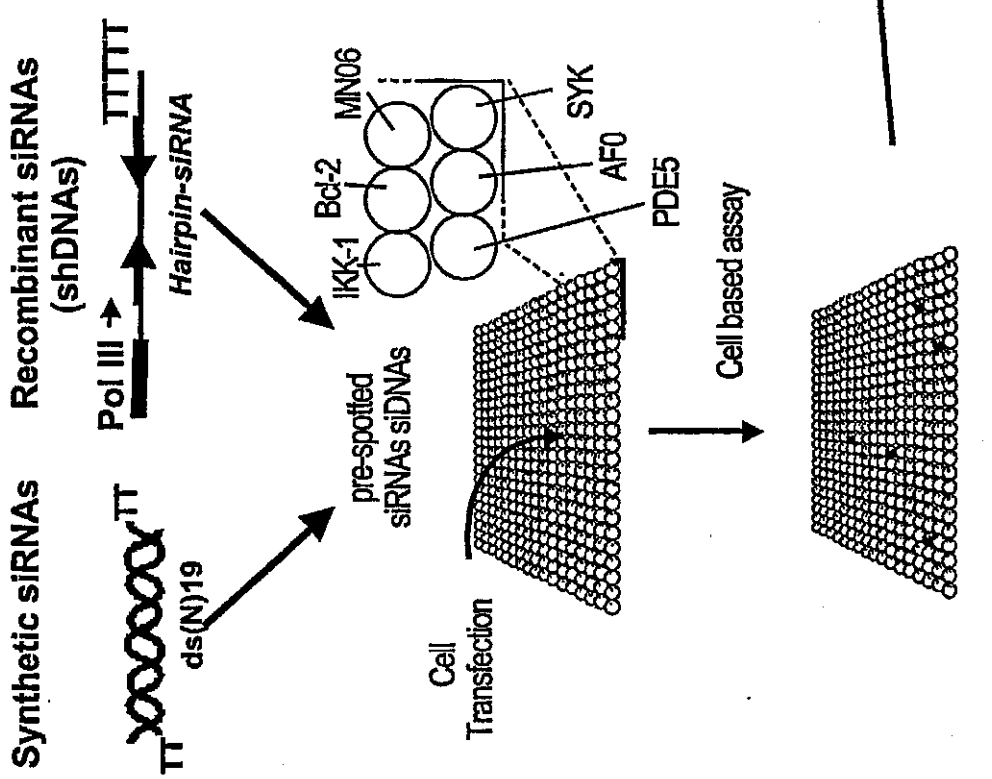
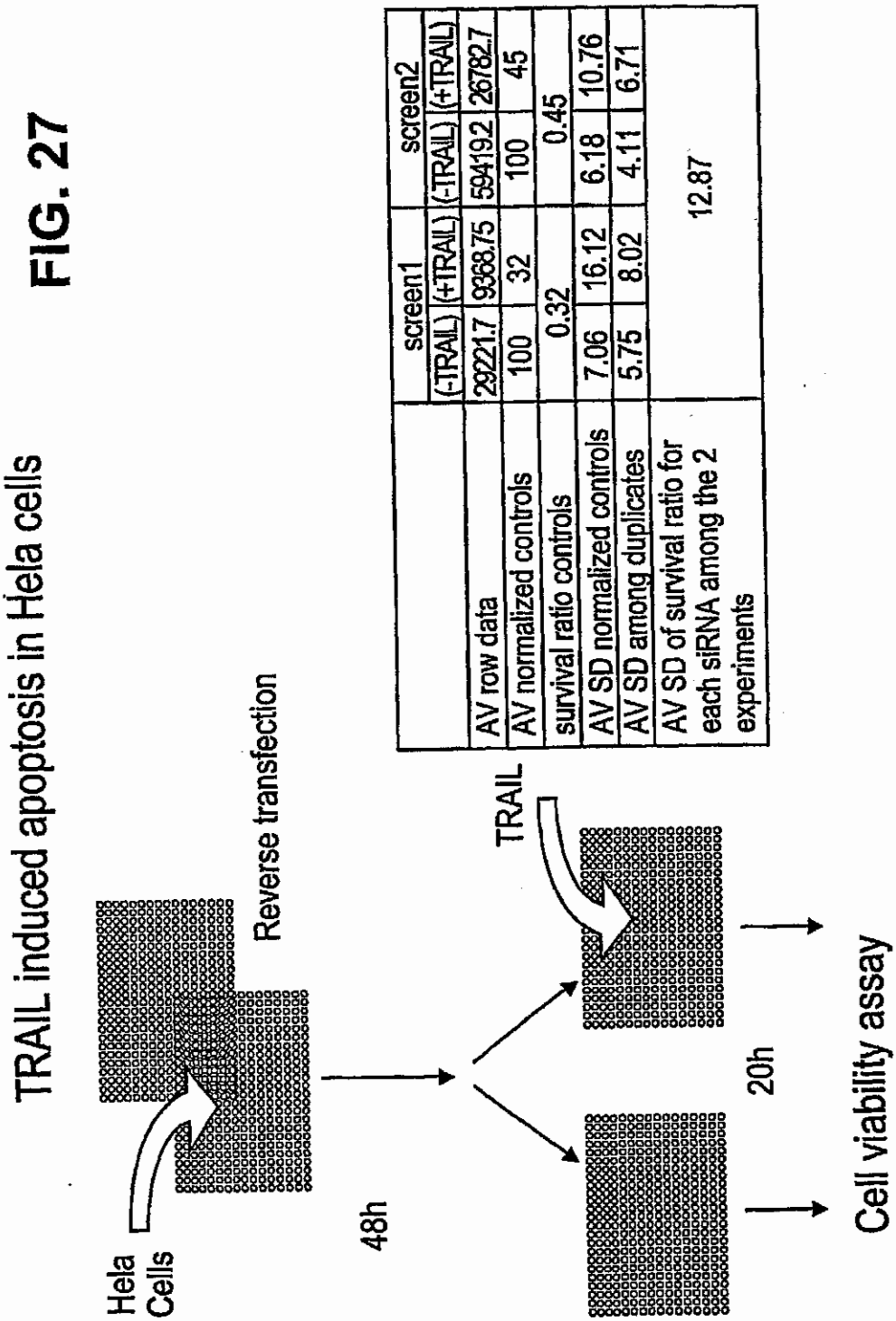
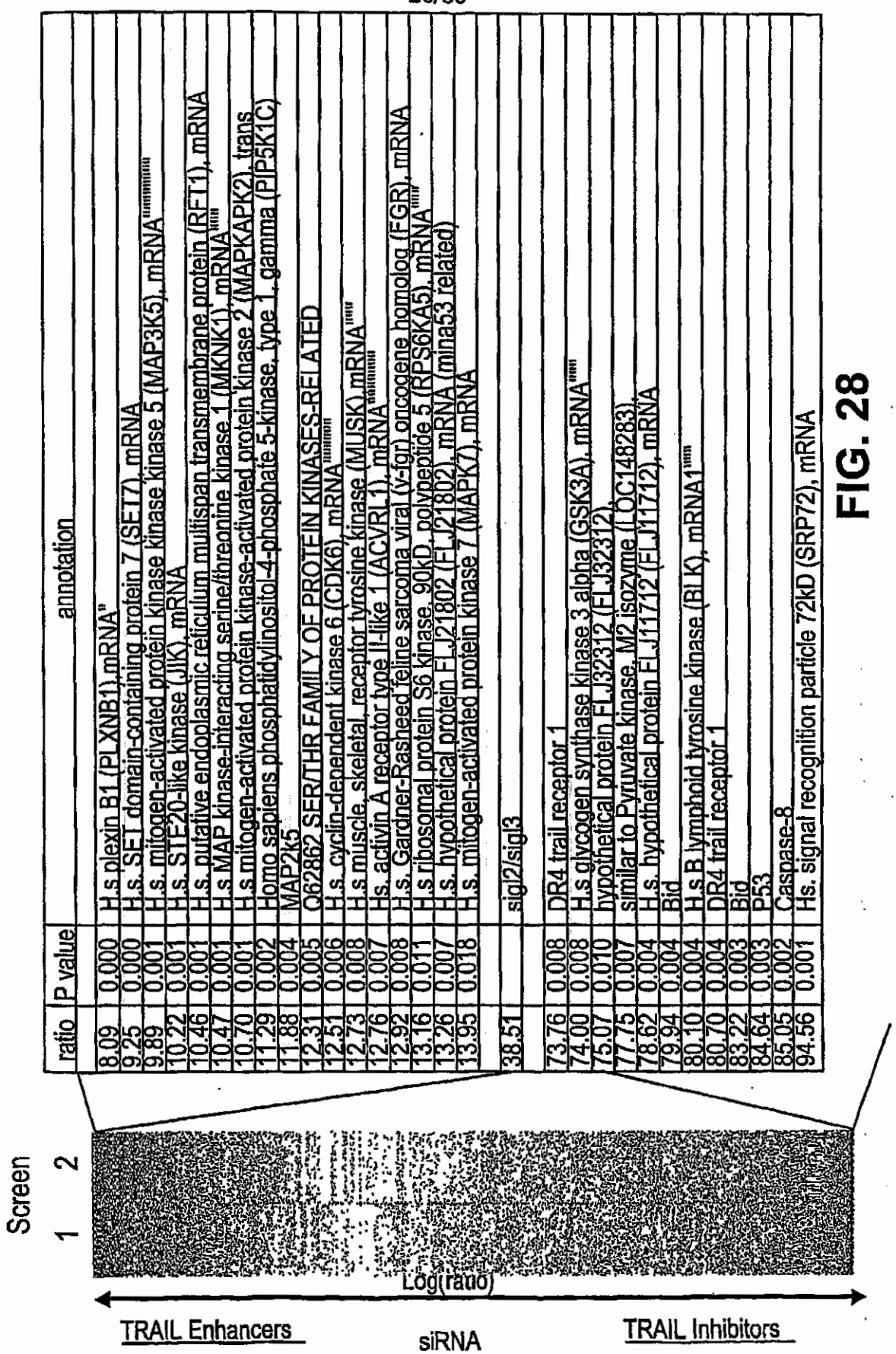

Synthetic siRNAs
Recombinant siRNAs (shDNAs)
Pol III
TTTTT
Hairpin-siRNA
TT
TT
ds(N)19
pre-spotted siRNAs siDNAs
IKK-1
Bcl-2
MN06
SYK
AF0
PDE5
Cell Transfection
Cell based assay
Assays:
Viability, cell survival
Reporter assays
Image based

FIG. 27



	screen1		screen2	
	(-TRAIL)	(+TRAIL)	(-TRAIL)	(+TRAIL)
AV row data	29221.7	9368.75	59419.2	26782.7
AV normalized controls	100	32	100	45
survival ratio controls	0.32		0.45	
AV SD normalized controls	7.06	16.12	6.18	10.76
AV SD among duplicates	5.75	8.02	4.11	6.71
AV SD of survival ratio for each siRNA among the 2 experiments	12.87			



ratio	P value	annotation
8.09	0.000	H.s.plexin B1 (PLXNB1) mRNA"
9.25	0.000	H.s. SET domain-containing protein 7 (SET7), mRNA
9.89	0.001	H.s. mitogen-activated protein kinase kinase kinase 5 (MAP3K5), mRNA"""""""""
10.22	0.001	H.s. STE20-like kinase (JIK), mRNA
10.46	0.001	H.s. putative endoplasmic reticulum multispan transmembrane protein (RFT1), mRNA
10.47	0.001	H.s MAP kinase-interacting serine/threonine kinase 1 (MKNK1), mRNA"""""
10.70	0.001	H.s mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), trans
11.29	0.002	Homo sapiens phosphatidylinositol-4-phosphate 5-kinase, type 1, gamma (PIP5K1C)
11.88	0.004	MAP2k5
12.31	0.005	Q62862 SER/THR FAMILY OF PROTEIN KINASES-RELATED
12.51	0.006	H.s. cyclin-dependent kinase 6 (CDK6), mRNA"""""""""
12.73	0.008	H.s muscle, skeletal, receptor tyrosine kinase (MUSK) mRNA"""""
12.76	0.007	Hs. activin A receptor type II-like 1 (ACVRL1), mRNA"""""""""
12.92	0.008	H.s. Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog (FGR), mRNA
13.16	0.011	H.s ribosomal protein S6 kinase, 90kD, polypeptide 5 (RPS6KA5), mRNA"""""
13.26	0.007	H.s. hypothetical protein FLJ21802 (FLJ21802), mRNA (mina53 related)
13.95	0.018	H.s. mitogen-activated protein kinase 7 (MAPK7), mRNA
38.51		sigl2/sigl3
73.76	0.008	DR4 trail receptor 1
74.00	0.008	H.s glycogen synthase kinase 3 alpha (GSK3A), mRNA"""""
75.07	0.010	hypothetical protein FLJ32312 (FLJ32312)
77.75	0.007	similar to Pyruvate kinase, M2 isozyme (LOC148283)
78.62	0.004	H.s. hypothetical protein FLJ11712 (FLJ11712), mRNA
79.94	0.004	Bid
80.10	0.004	H.s B lymphoid tyrosine kinase (BLK), mRNA1"""""
80.70	0.004	DR4 trail receptor 1
83.22	0.003	Bid
84.64	0.003	P53
85.05	0.002	Caspase-8
94.56	0.001	Hs. signal recognition particle 72kD (SRP72), mRNA

FIG. 28

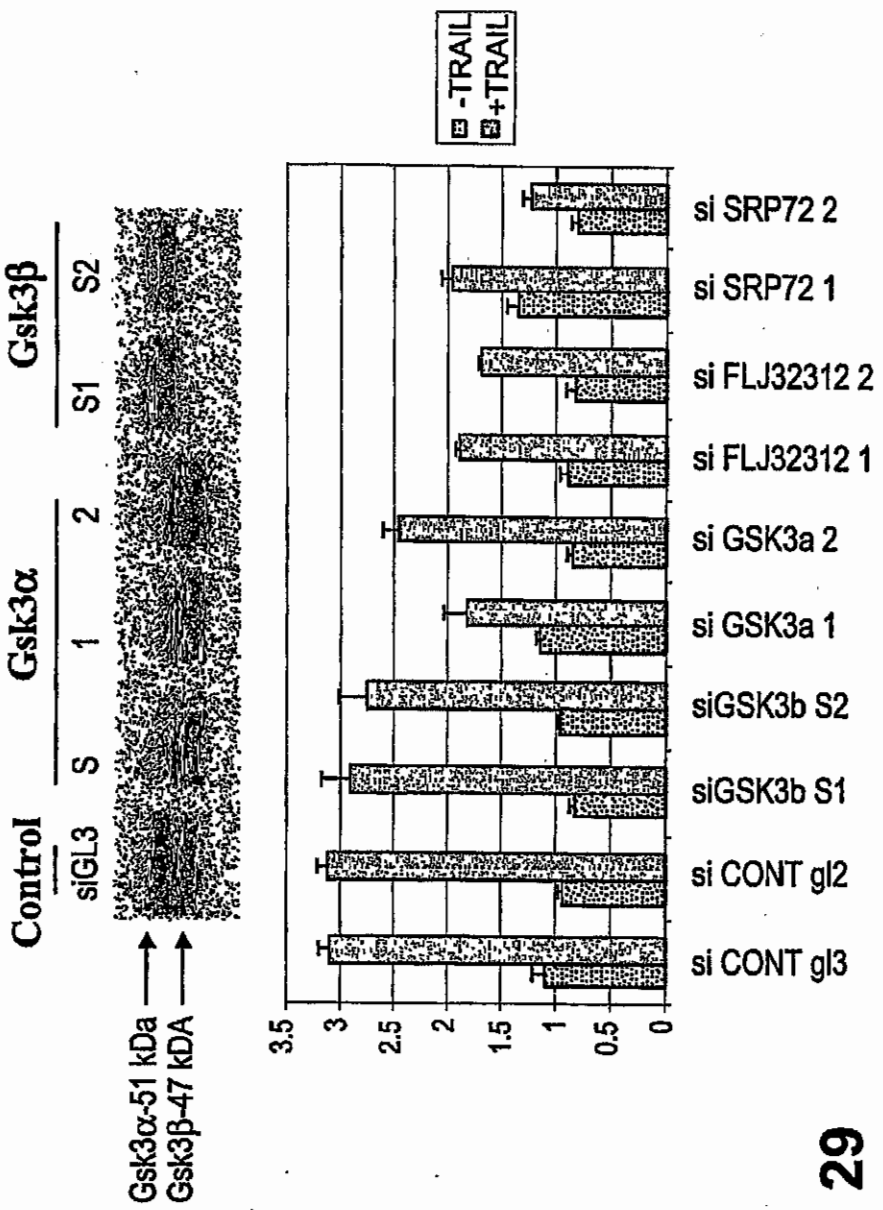

Control
siGL3
Gsk3α
S
1
2
Gsk3β
S1
S2
Gsk3α-51 kDa
Gsk3β-47 kDA
-TRAIL
+TRAIL
si SRP72 2
si SRP72 1
si FLJ32312 2
si FLJ32312 1
si GSK3a 2
si GSK3a 1
siGSK3b S2
siGSK3b S1
si CONT gl2
si CONT gl3
3.5
3
2.5
2
1.5
1
0.5
0

FIG. 29

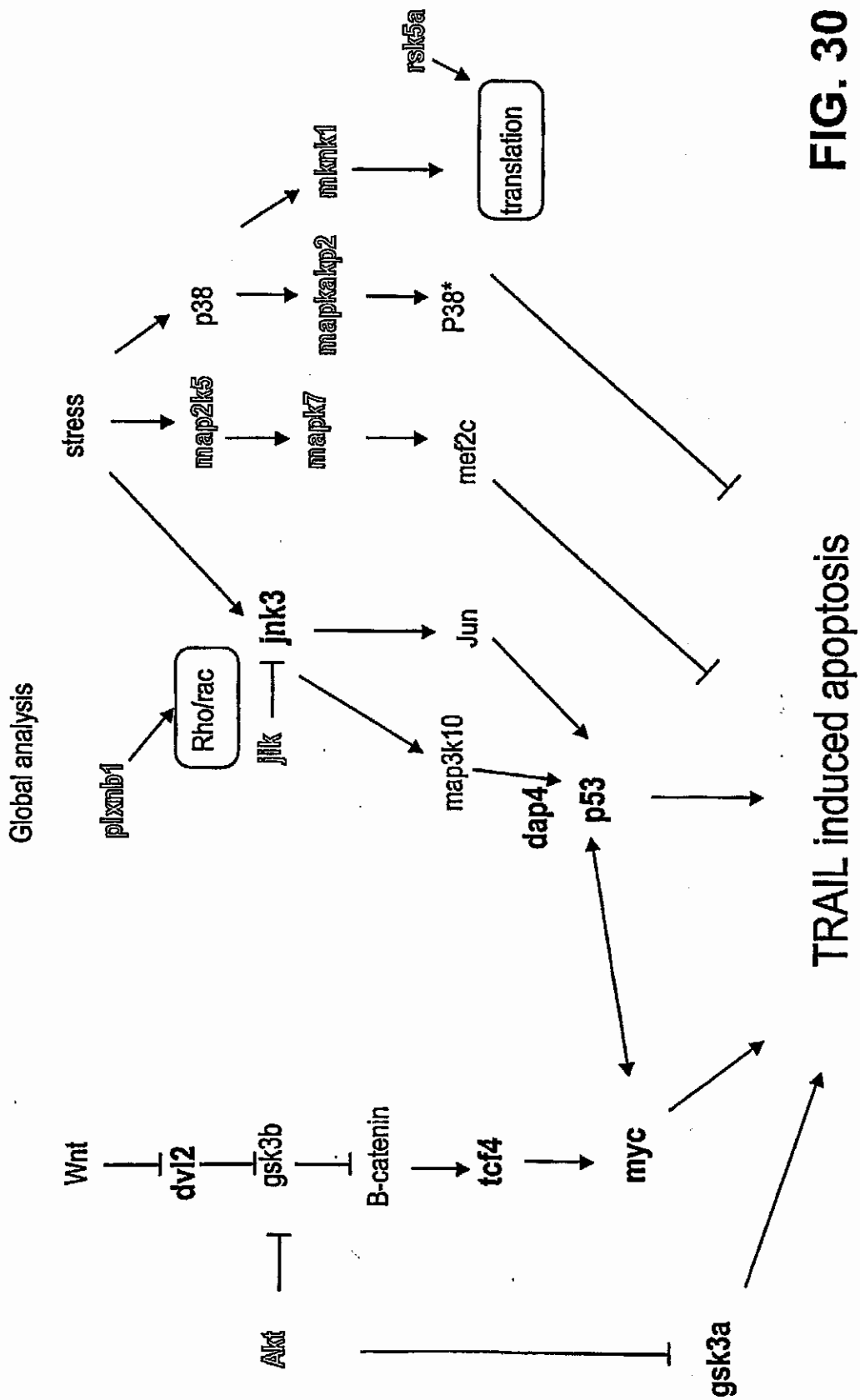

Global analysis
stress
plxnb1
Rho/rac
jnk3
jlk
map2k5
p38
mapkakp2
mknk1
rsk5a
translation
P38*
mapk7
mef2c
Jun
map3k10
dap4
p53
Wnt
dvl2
gsk3b
B-catenin
tcf4
myc
Akt
gsk3a
TRAIL induced apoptosis

FIG. 30

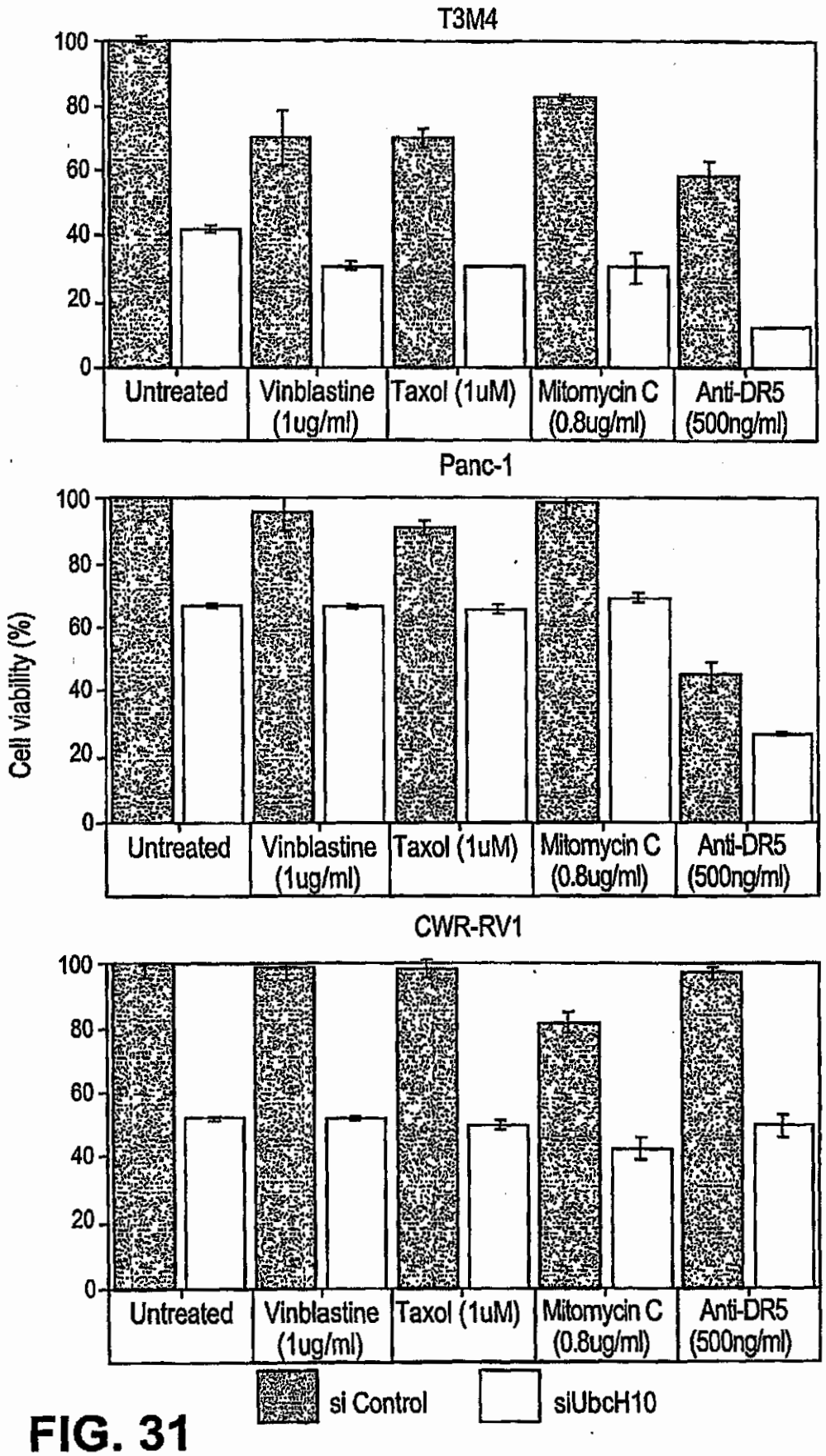

T3M4
Panc-1
CWR-RV1
Cell viability (%)
100
80
60
40
20
0
Untreated
Vinblastine (1ug/ml)
Taxol (1uM)
Mitomycin C (0.8ug/ml)
Anti-DR5 (500ng/ml)
si Control
siUbcH10

FIG. 31

FIG. 32

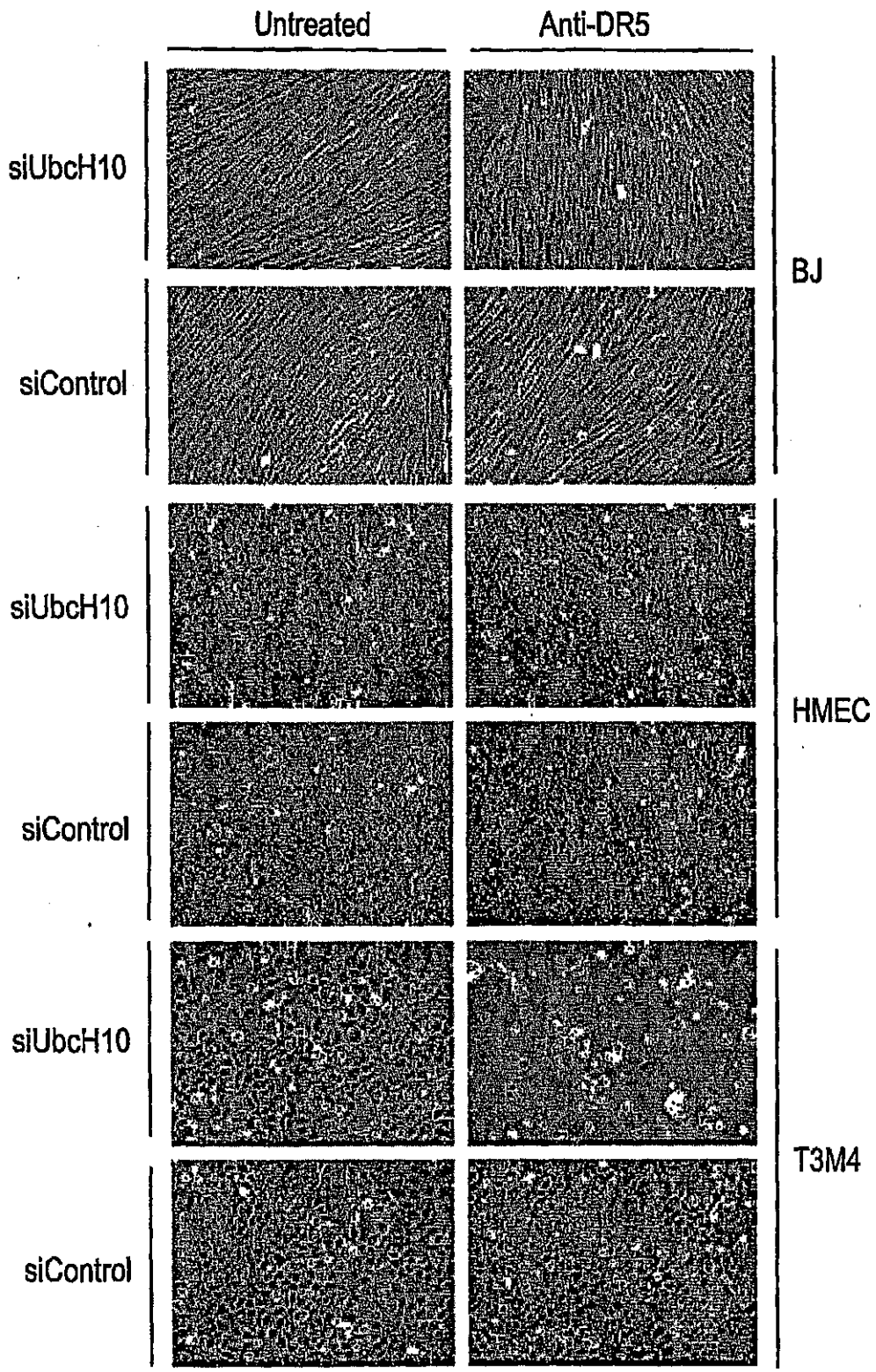

Untreated
Anti-DR5
siUbcH10
siControl
siUbcH10
siControl
siUbcH10
siControl
BJ
HMEC
T3M4

FIG. 33

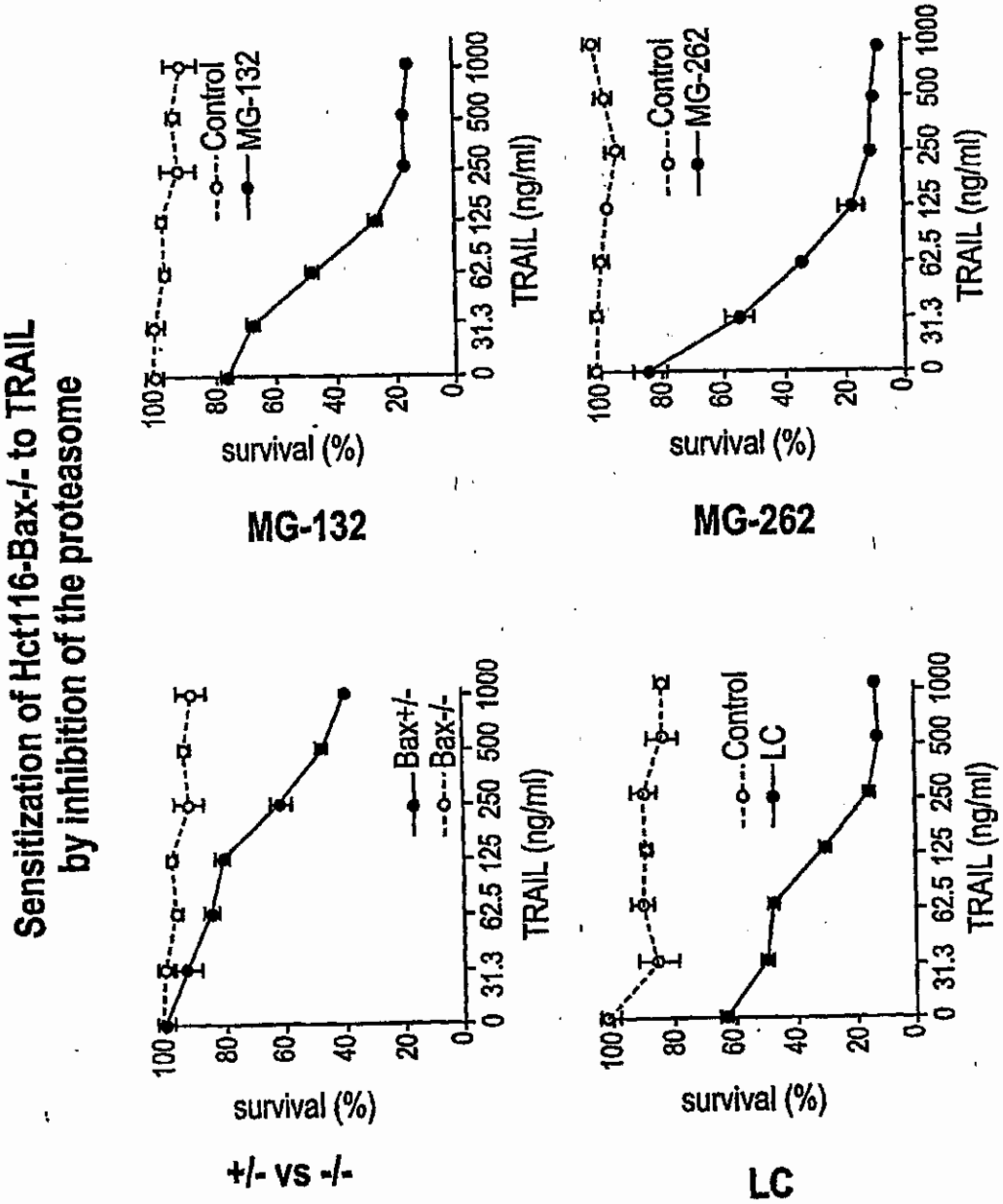

Sensitization of Hct116-Bax-/- to TRAIL
by inhibition of the proteasome
MG-132
MG-262
+/- vs -/-
LC
Control
MG-132
MG-262
LC
Bax+/-
Bax-/-
survival (%)
TRAIL (ng/ml)
0 31.3 62.5 125 250 500 1000
0 20 40 60 80 100
Measurement after 24 h, 5 µM LC, 1 µM MG-132, 1 nM MG-262

FIG. 34

MG-262 restores the mitochondrial apoptosis pathway
Stimulus (h) 0 1 2 4 T 1 2 4 T+M 1 2 4 M
Casp8
Bid
Casp9
Casp3
XIAP
Bcl-xL
T: 1µg/ml TRAIL
M:1 nM MG-262
▼ full length-form
▽ processed form
Trail
Fadd
Casp-8
Casp-8
Fadd
Bid
Bax
Cyto-c
Apaf-1
Casp-9
Casp-3
IAP
SMAC
Viable cells
Cell death

Sequence of DR5'A' heavy chain variable region

AAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTGAAACCCGGGGCATCAGTGAA
GCTGTCCTGCAAGGCTTCTGGCTACACCTTCACTGACTATACTATACACTGGGTAAA
GCAGAGGTCTGGACAGGGTCTTGAGTGGATTGGGTGGTTTTACCCTGGAGGTGGTTA
TATAAAATACAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCTC
CAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGACTCTGCGGTCTATTTC
TGTGCAAGACACGAAGAGGGCATCTATTTTGACTACTGGGGCCAAGGCACCACTCTC
ACAGTCTCCTCA

Amino acid sequence of VH

KVQLQQSGAELVKPGASVKLSCKASGYTFTDYTIHWVKQRSGQGLEWIGWFYPGGGYIK
YNEKFKDRATLTADKSSNTVYMELSRLTSEDSAVYFCARHEEGIYFDYWGQGTTLTVSS

DNA Sequence of DR5'A' light chain variable region

GACATTGTGATGACCCAGTCTCACAAGTTCATGTCCACATCAGTGGGAGACAGGGTCA
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAGCCTGGTATCAACAAAA
ACCAGGGCAATCTCCTAAACTACTGATTTACTGGGCATCCACCCGGCACACTGGAGTC
CCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTATACTCTCACCATCAGCAGTGT
GCAGGCTGAAGACCTGGCACTTTATTACTGTCAGCAACATTATACCACTCCATTCACGT
TCGGCTCGGGGACAAAGTTG

Amino acid sequence of VL

DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAIAWYQQKPGQSPKLLIYWASTRHTGVPDR
FTGSGSGTDYTLTISSVQAEDLALYYCQQHYTTPFTFGSGTKL

FIG. 35