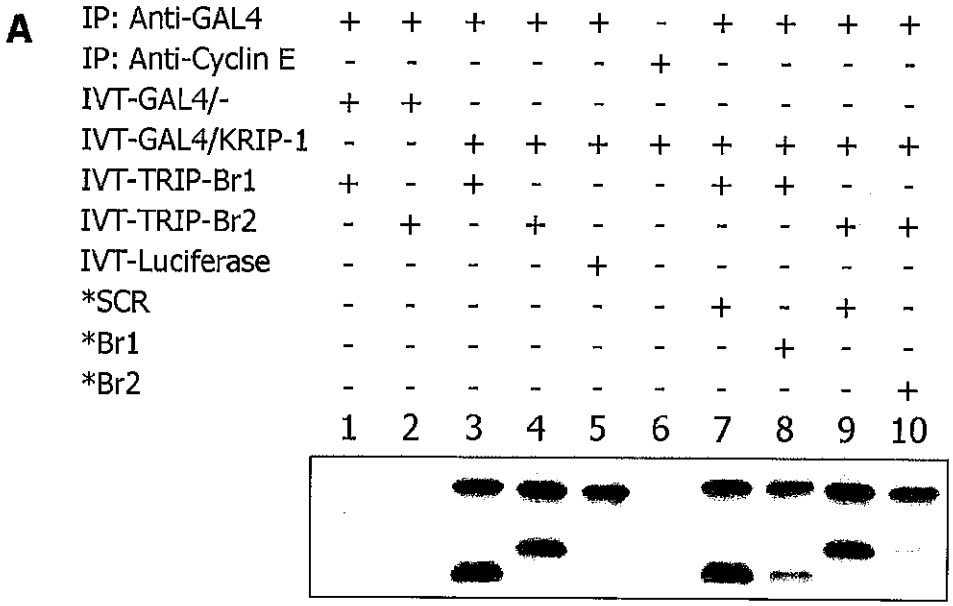

A
IP: Anti-GAL4 + + + + + - + + + +
IP: Anti-Cyclin E - - - - - + - - - -
IVT-GAL4/- + + - - - - - - - -
IVT-GAL4/KRIP-1 - - + + + + + + + +
IVT-TRIP-Br1 + - + - - - + + - -
IVT-TRIP-Br2 - + - + - - - - + +
IVT-Luciferase - - - - + - - - - -
*SCR - - - - - - + - + -
*Br1 - - - - - - - + - -
*Br2 - - - - - - - - - +
1 2 3 4 5 6 7 8 9 10

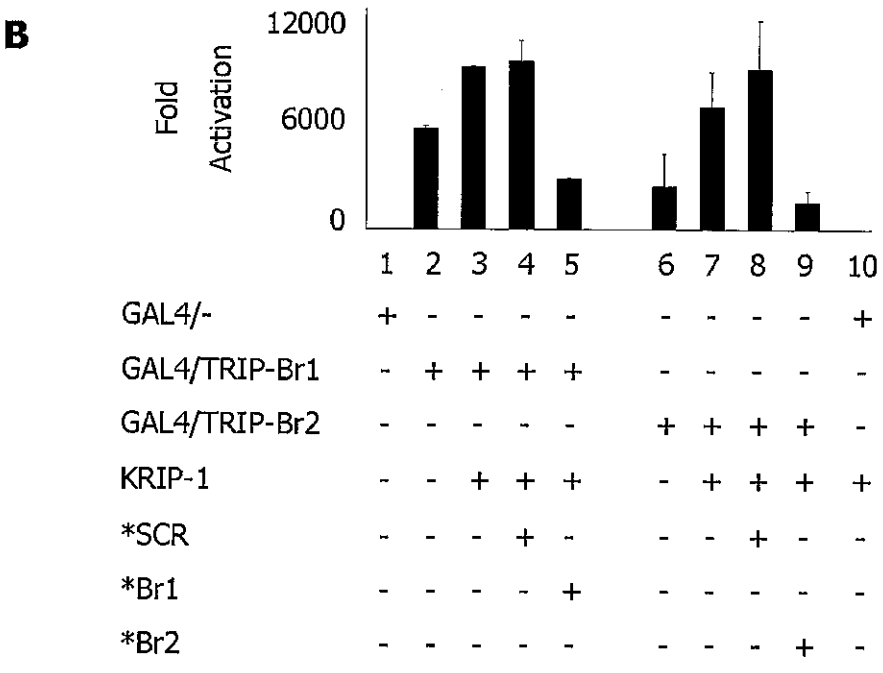

B
Fold Activation
12000
6000
0
1 2 3 4 5 6 7 8 9 10
GAL4/- + - - - - - - - - +
GAL4/TRIP-Br1 - + + + + - - - - -
GAL4/TRIP-Br2 - - - - - + + + + -
KRIP-1 - - + + + - + + + +
*SCR - - - + - - - + - -
*Br1 - - - - + - - - - -
*Br2 - - - - - - - - + -

FIGURE 1

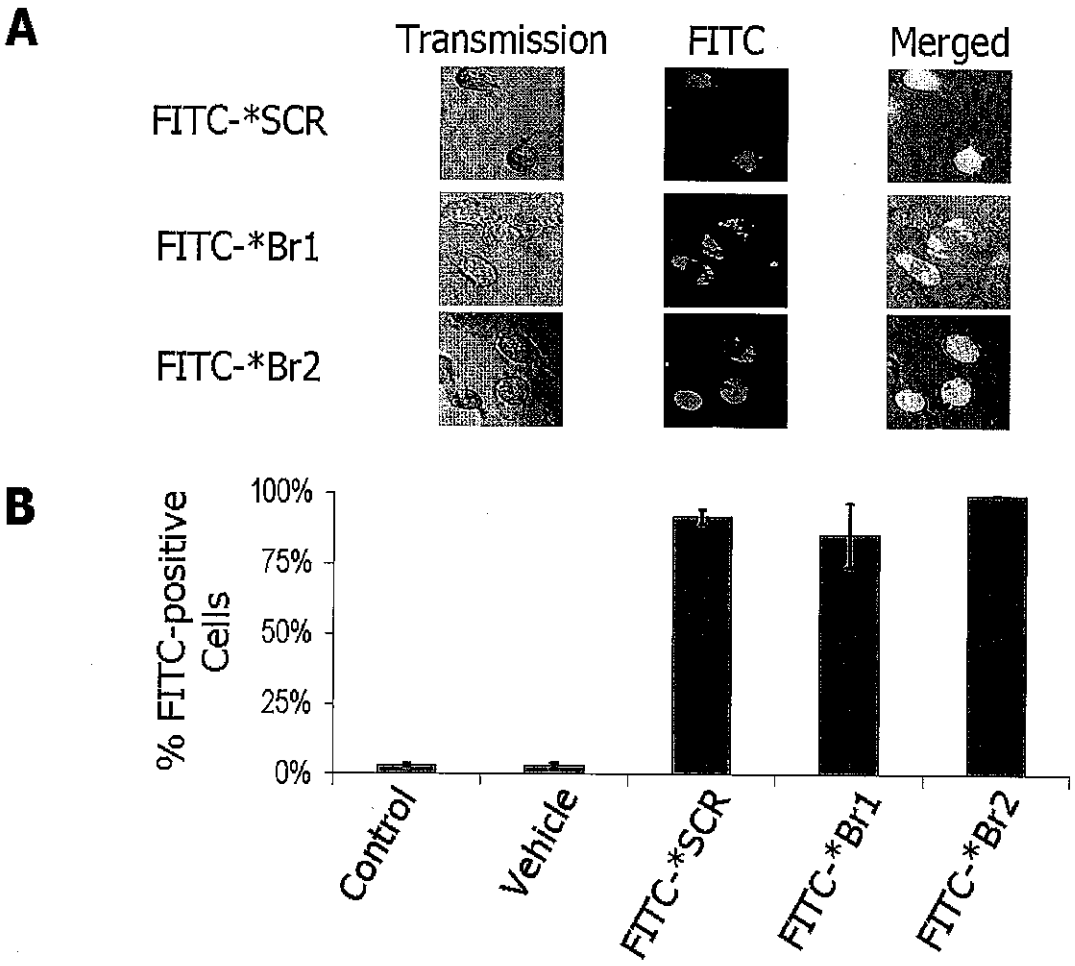

A
Transmission
FITC
Merged
FITC-*SCR
FITC-*Br1
FITC-*Br2
B
% FITC-positive Cells
100%
75%
50%
25%
0%
Control
Vehicle
FITC-*SCR
FITC-*Br1
FITC-*Br2

FIGURE 2

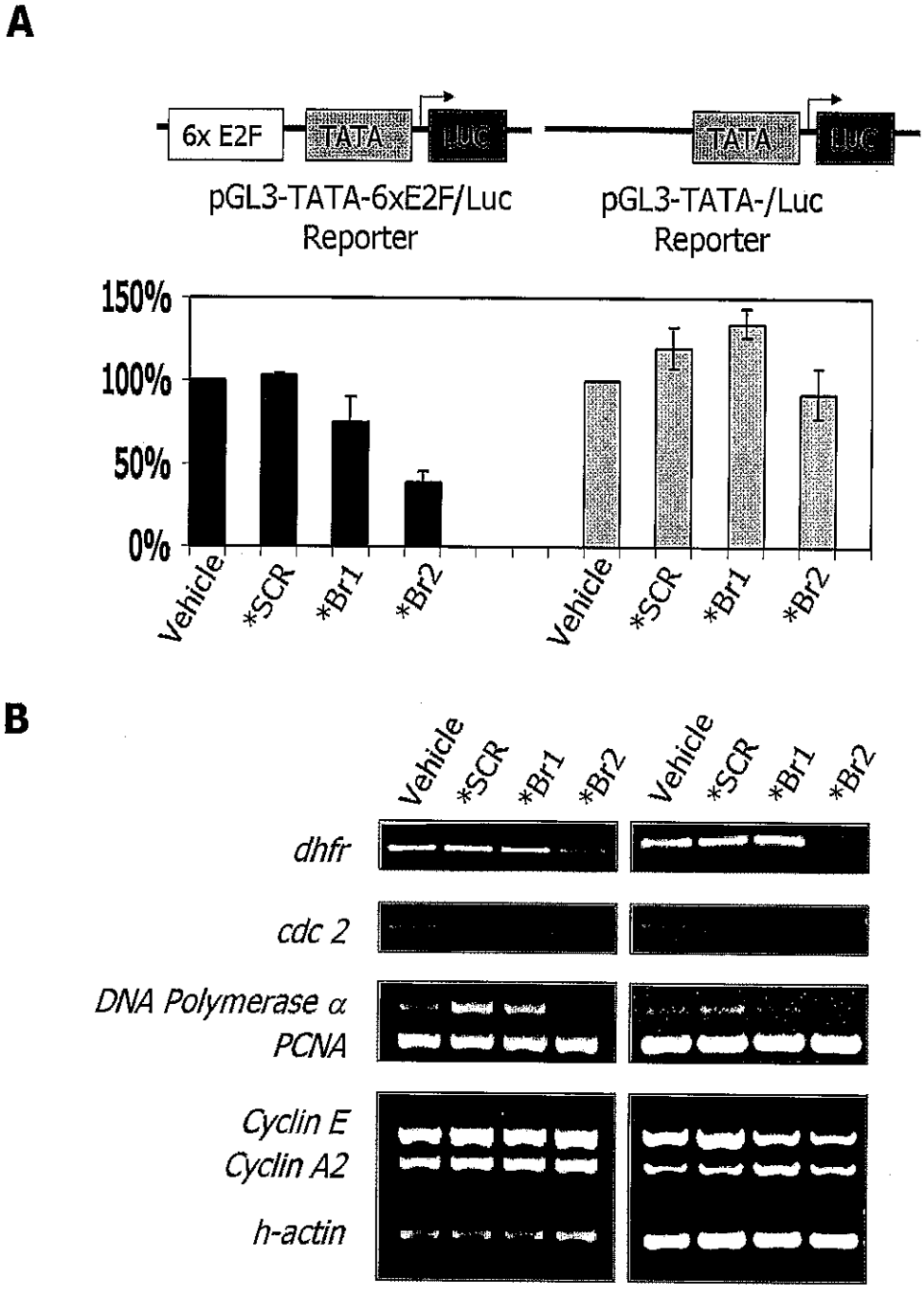

A
6x E2F
TATA
LUC
TATA
LUC
pGL3-TATA-6xE2F/Luc Reporter
pGL3-TATA-/Luc Reporter
150%
100%
50%
0%
Vehicle
*SCR
*Br1
*Br2
Vehicle
*SCR
*Br1
*Br2
B
Vehicle
*SCR
*Br1
*Br2
Vehicle
*SCR
*Br1
*Br2
dhfr
cdc 2
DNA Polymerase α
PCNA
Cyclin E
Cyclin A2
h-actin

FIGURE 3

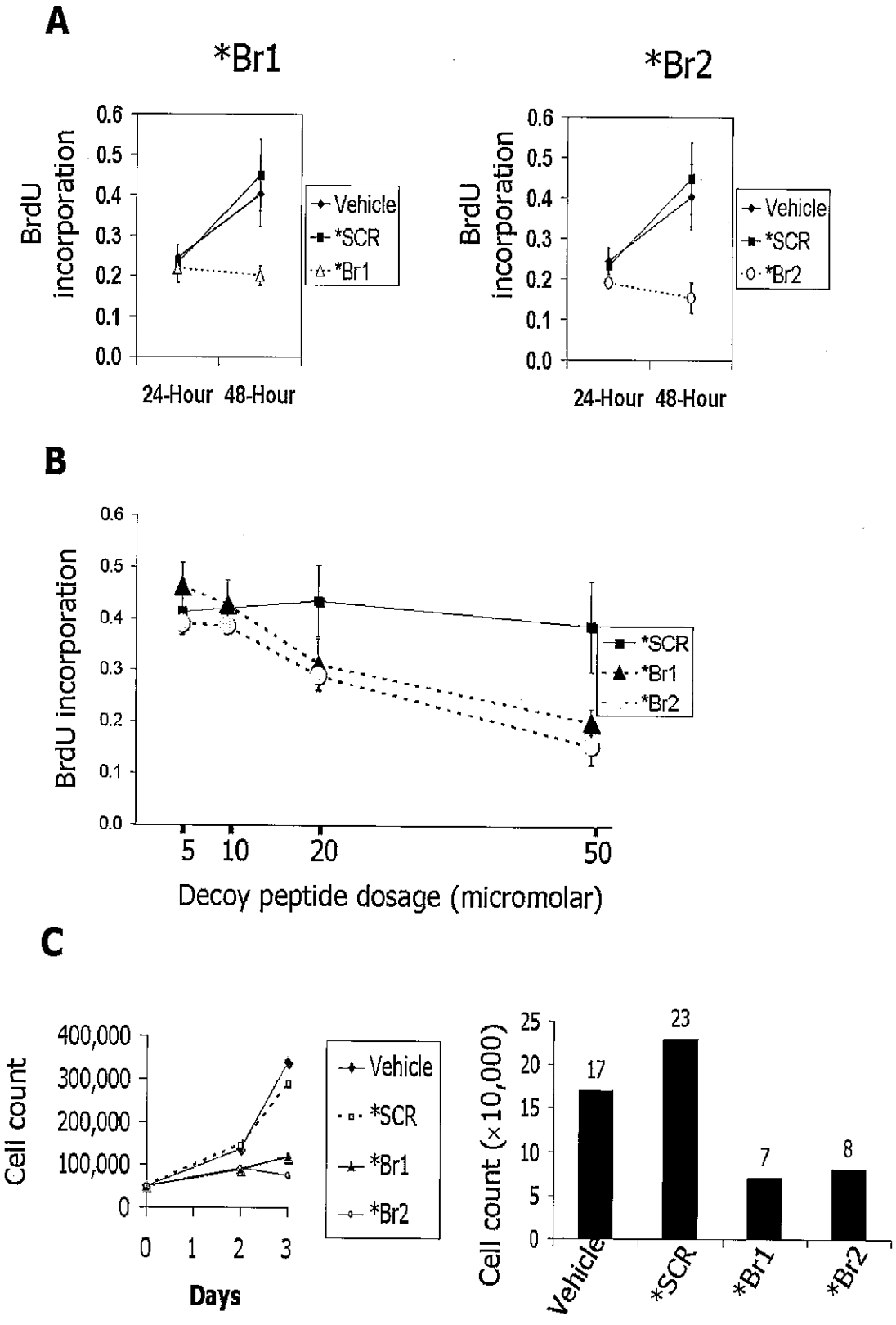
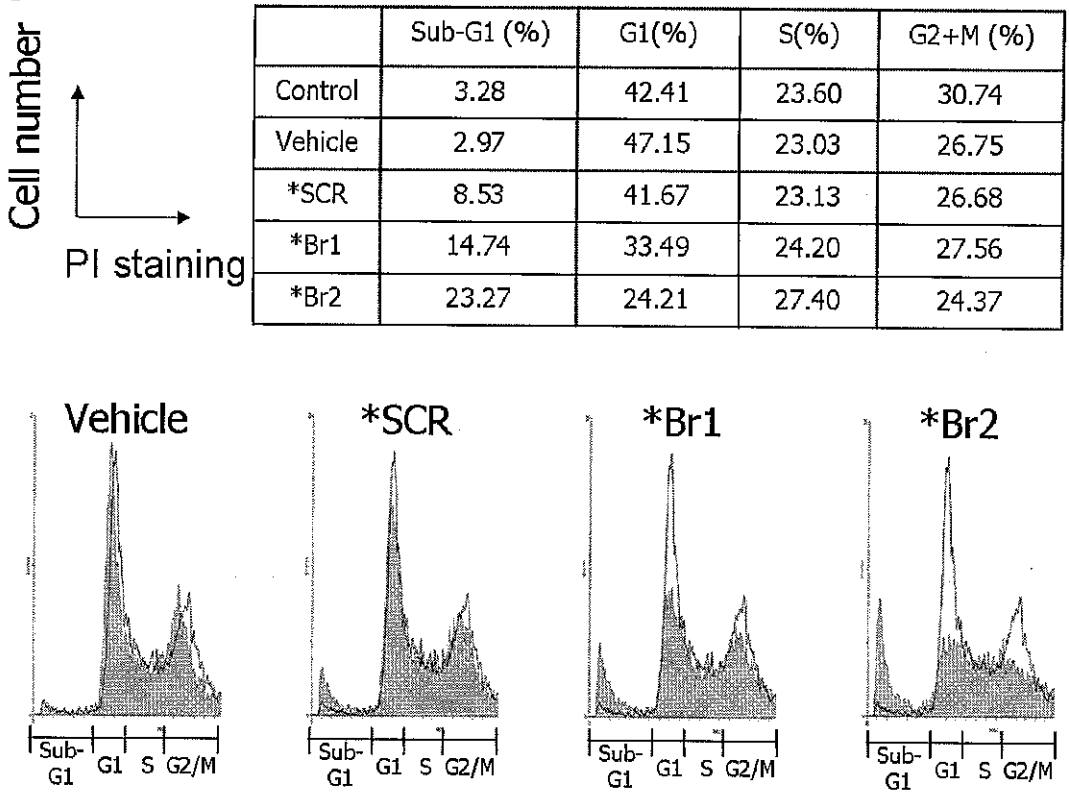

A
*Br1
*Br2
BrdU incorporation
0.6
0.5
0.4
0.3
0.2
0.1
0.0
24-Hour 48-Hour
Vehicle
*SCR
*Br1
*Br2
B
BrdU incorporation
5 10 20 50
Decoy peptide dosage (micromolar)
*SCR
*Br1
*Br2
C
Cell count
400,000
300,000
200,000
100,000
0
0 1 2 3
Days
Vehicle
*SCR
*Br1
*Br2
Cell count (×10,000)
25
20
15
10
5
0
17
23
7
8
Vehicle
*SCR
*Br1
*Br2

FIGURE 4

A

	Sub-G1 (%)	G1(%)	S(%)	G2+M (%)
Control	3.28	42.41	23.60	30.74
Vehicle	2.97	47.15	23.03	26.75
*SCR	8.53	41.67	23.13	26.68
*Br1	14.74	33.49	24.20	27.56
*Br2	23.27	24.21	27.40	24.37



B

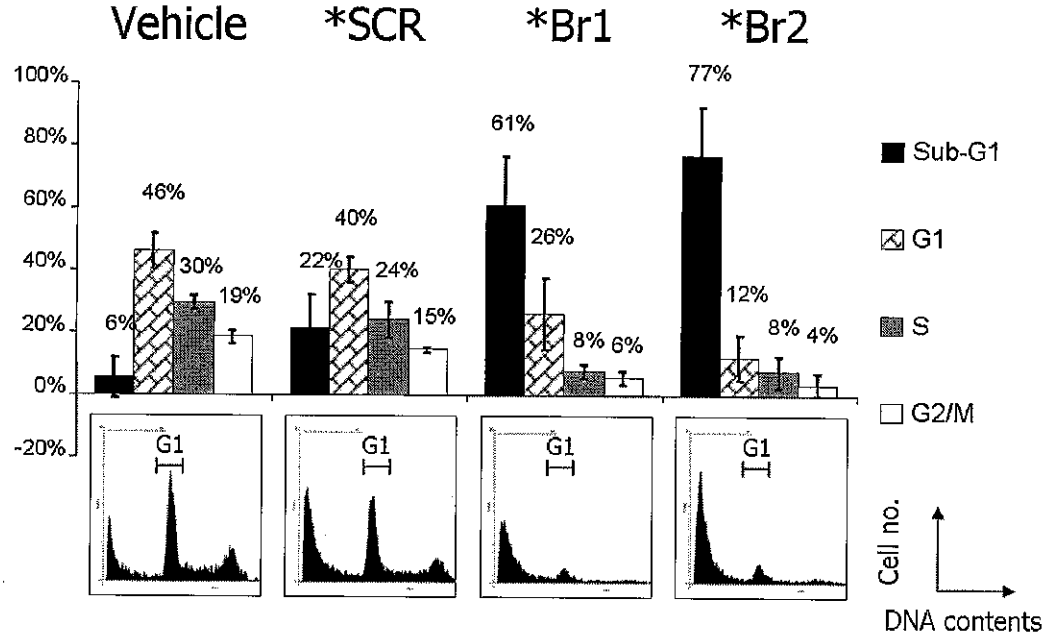
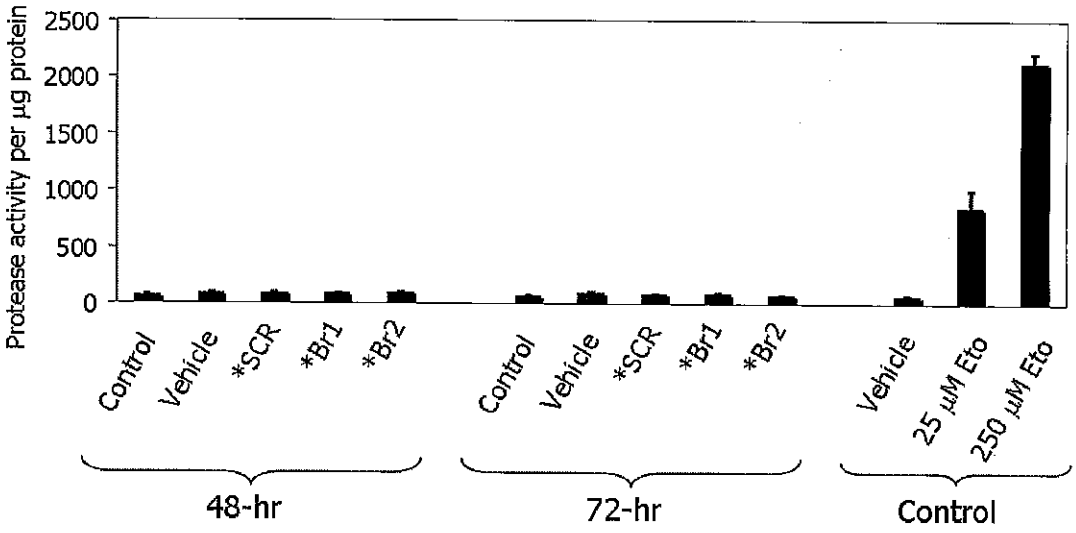


FIGURE 5

A


Protease activity per µg protein
2500
2000
1500
1000
500
0
Control
Vehicle
*SCR
*Br1
*Br2
Control
Vehicle
*SCR
*Br1
*Br2
Vehicle
25 µM Eto
250 µM Eto
48-hr
72-hr
Control

B

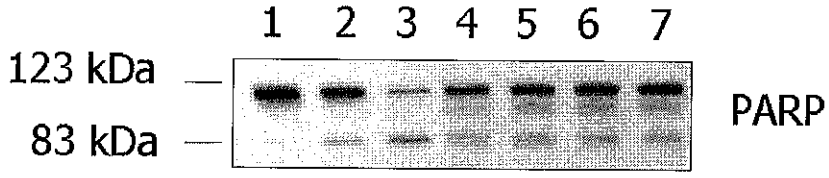

1 2 3 4 5 6 7
123 kDa
83 kDa
PARP

FIGURE 6

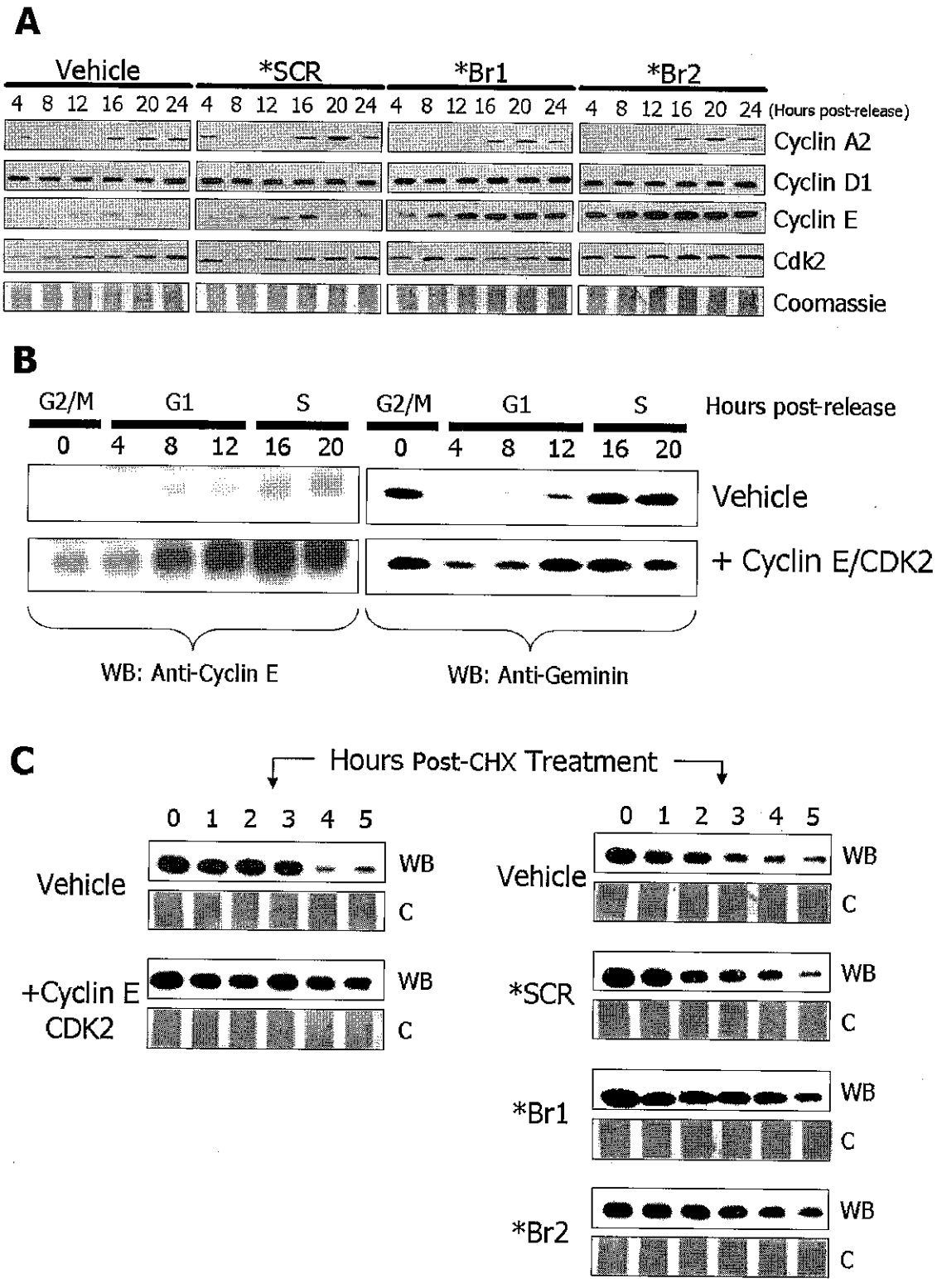

A
Vehicle
*SCR
*Br1
*Br2
4 8 12 16 20 24 4 8 12 16 20 24 4 8 12 16 20 24 4 8 12 16 20 24 (Hours post-release)
Cyclin A2
Cyclin D1
Cyclin E
Cdk2
Coomassie
B
G2/M G1 S G2/M G1 S Hours post-release
0 4 8 12 16 20 0 4 8 12 16 20
Vehicle
+ Cyclin E/CDK2
WB: Anti-Cyclin E
WB: Anti-Geminin
C
Hours Post-CHX Treatment
0 1 2 3 4 5
Vehicle
WB
C
+Cyclin E CDK2
WB
C
0 1 2 3 4 5
Vehicle
WB
C
*SCR
WB
C
*Br1
WB
C
*Br2
WB
C

FIGURE 7

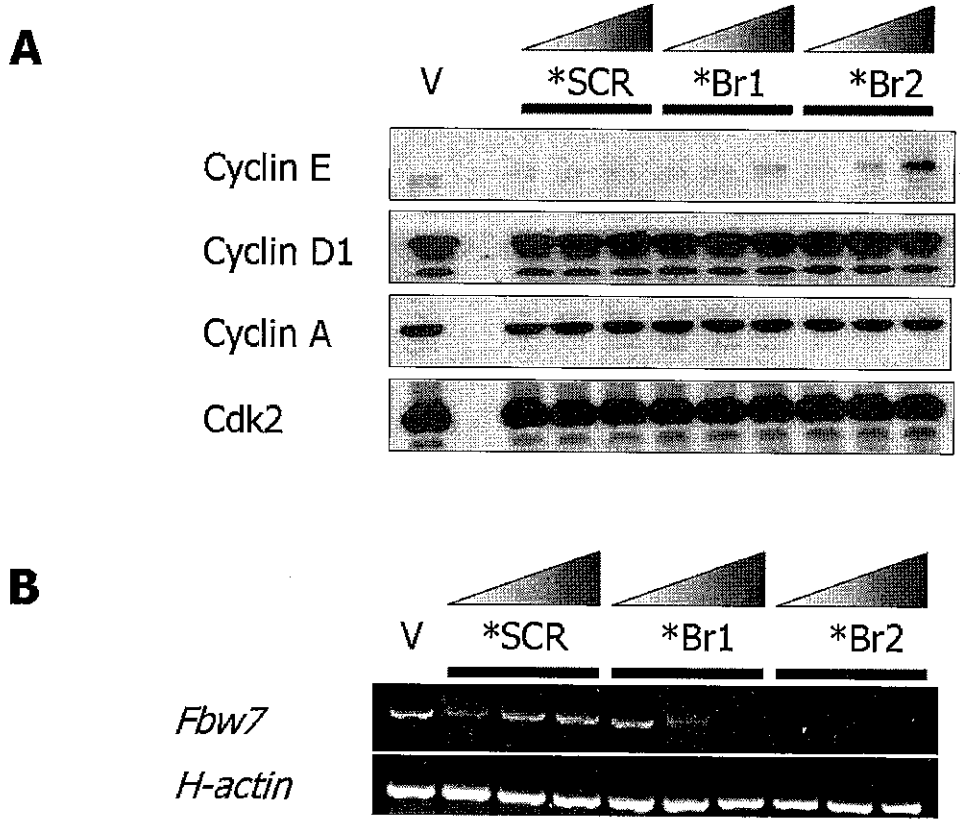

A
V
*SCR
*Br1
*Br2
Cyclin E
Cyclin D1
Cyclin A
Cdk2
B
V
*SCR
*Br1
*Br2
Fbw7
H-actin
C
- + + OHT
- - + CHX
1 2 3
Cyclin-E
H-actin
Fbxw7
H-actin

FIGURE 8

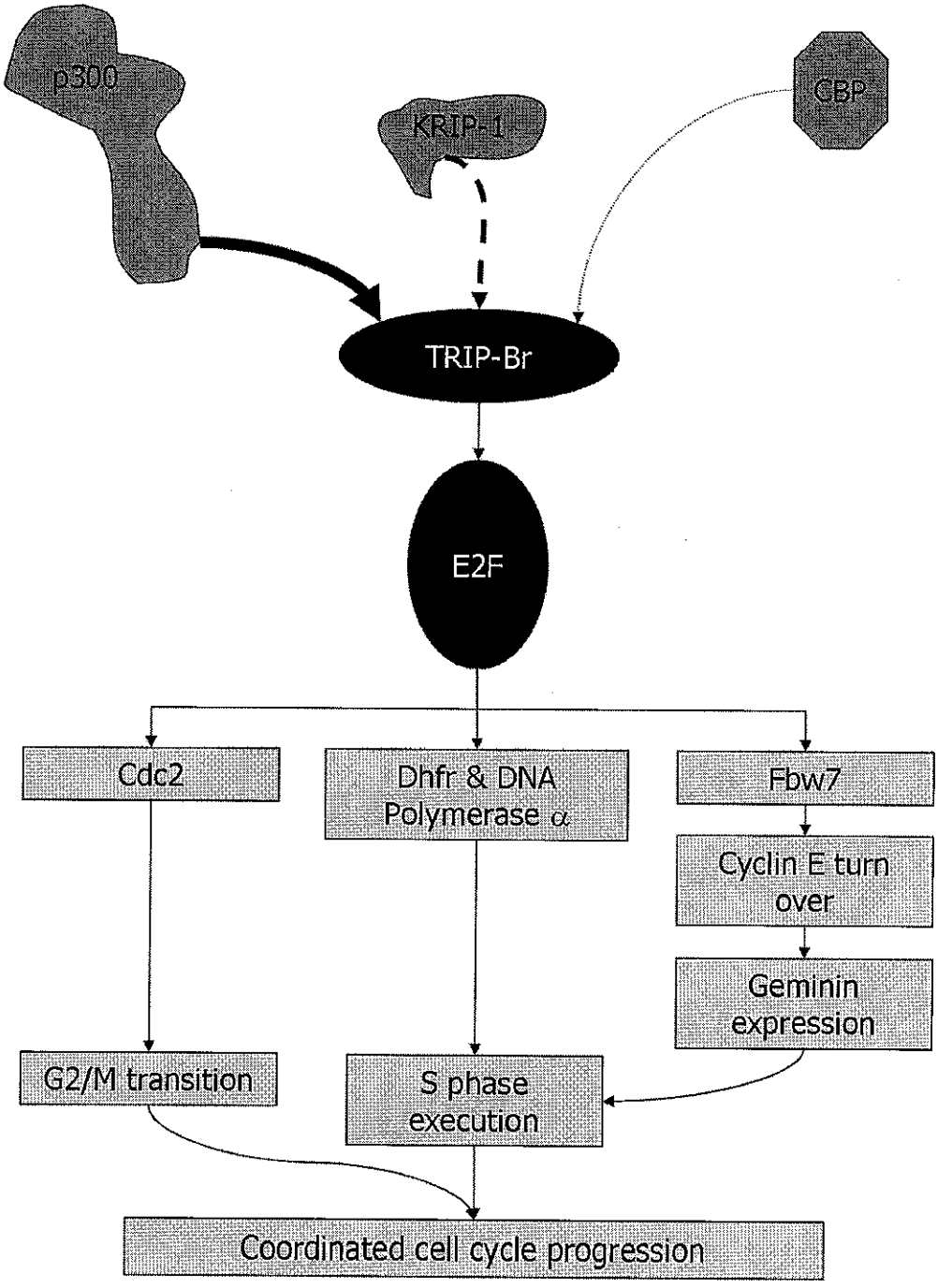
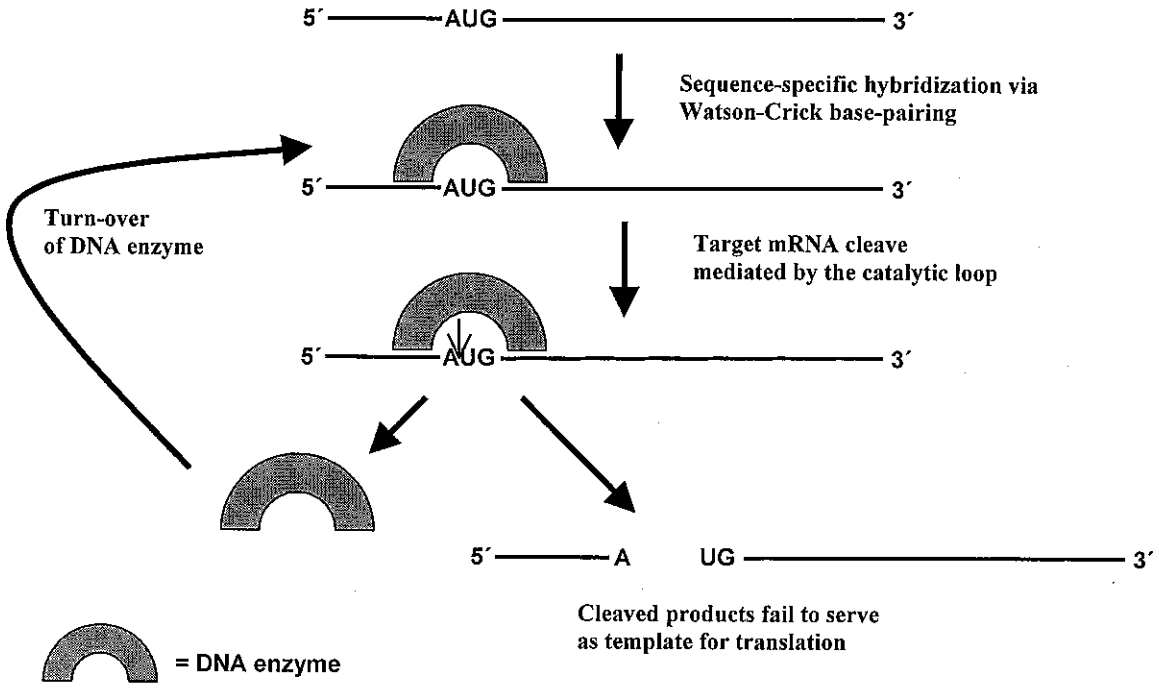

p300
KRIP-1
CBP
TRIP-Br
E2F
Cdc2
Dhfr & DNA Polymerase α
Fbw7
Cyclin E turn over
Geminin expression
G2/M transition
S phase execution
Coordinated cell cycle progression

FIGURE 9

A



B

Cleavage of hTRIP-Br2 mRNA by E-Br2:

5'-.....AUG UGA UAU AUG UUG GGU AAA.....-3'

3'-AC ACT ATA AC AAC CCA TT-5'

E-SCR:

3'-TA CTG TCC TG TCA TCG AC-5'

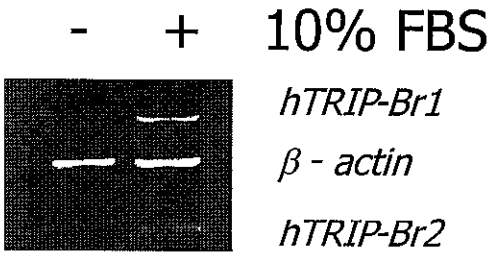
Cleavage of hTRIP-Br1 mRNA by E-Br1:

5'-.....GUG AGC AAG AUG CUG AGC AAG.....-3'

3'-AC TCG TTC AC GAC TCG TT-5'

FIGURE 10

A


- +
10% FBS
hTRIP-Br1
β - actin
hTRIP-Br2

B

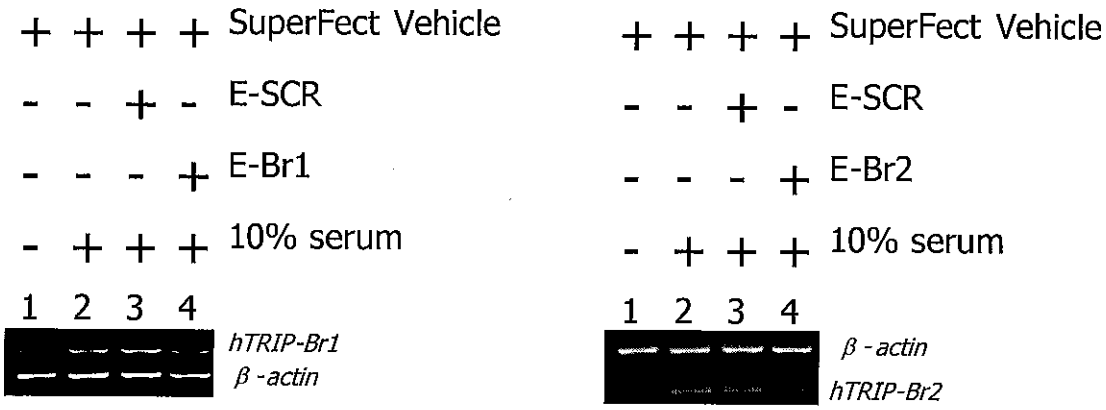
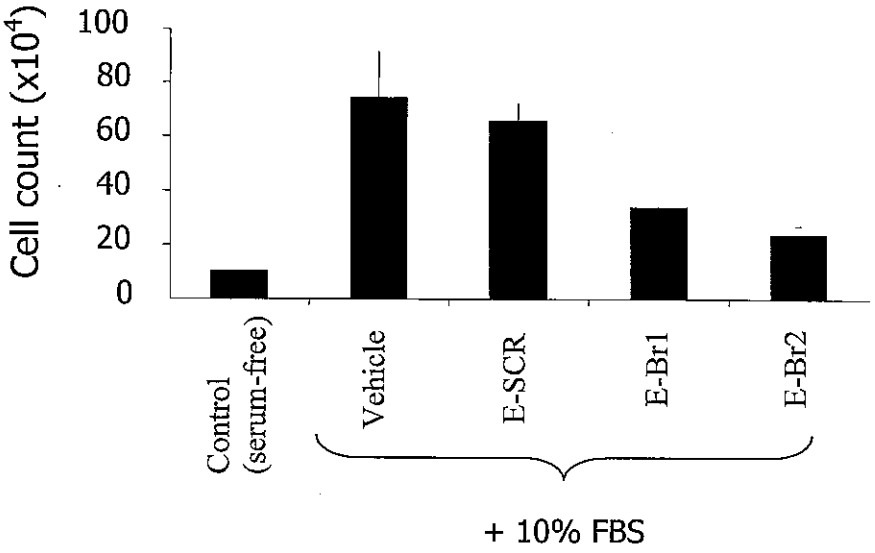

+ + + + SuperFect Vehicle
- - + - E-SCR
- - - + E-Br1
- + + + 10% serum
1 2 3 4
hTRIP-Br1
β -actin
+ + + + SuperFect Vehicle
- - + - E-SCR
- - - + E-Br2
- + + + 10% serum
1 2 3 4
β -actin
hTRIP-Br2

FIGURE 11

A


Cell count (x10⁴)
100
80
60
40
20
0
Control (serum-free)
Vehicle
E-SCR
E-Br1
E-Br2
+ 10% FBS

B

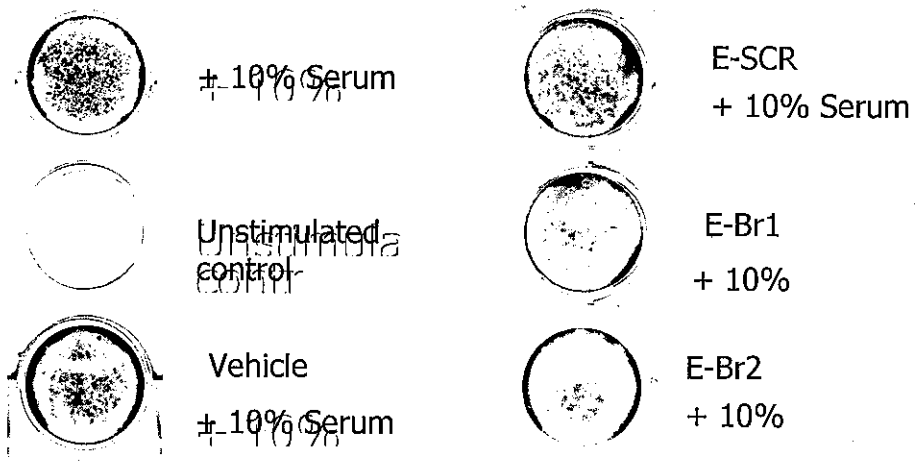

+ 10% Serum
E-SCR
+ 10% Serum
Unstimulated control
E-Br1
+ 10%
Vehicle
+ 10% Serum
E-Br2
+ 10%

FIGURE 12

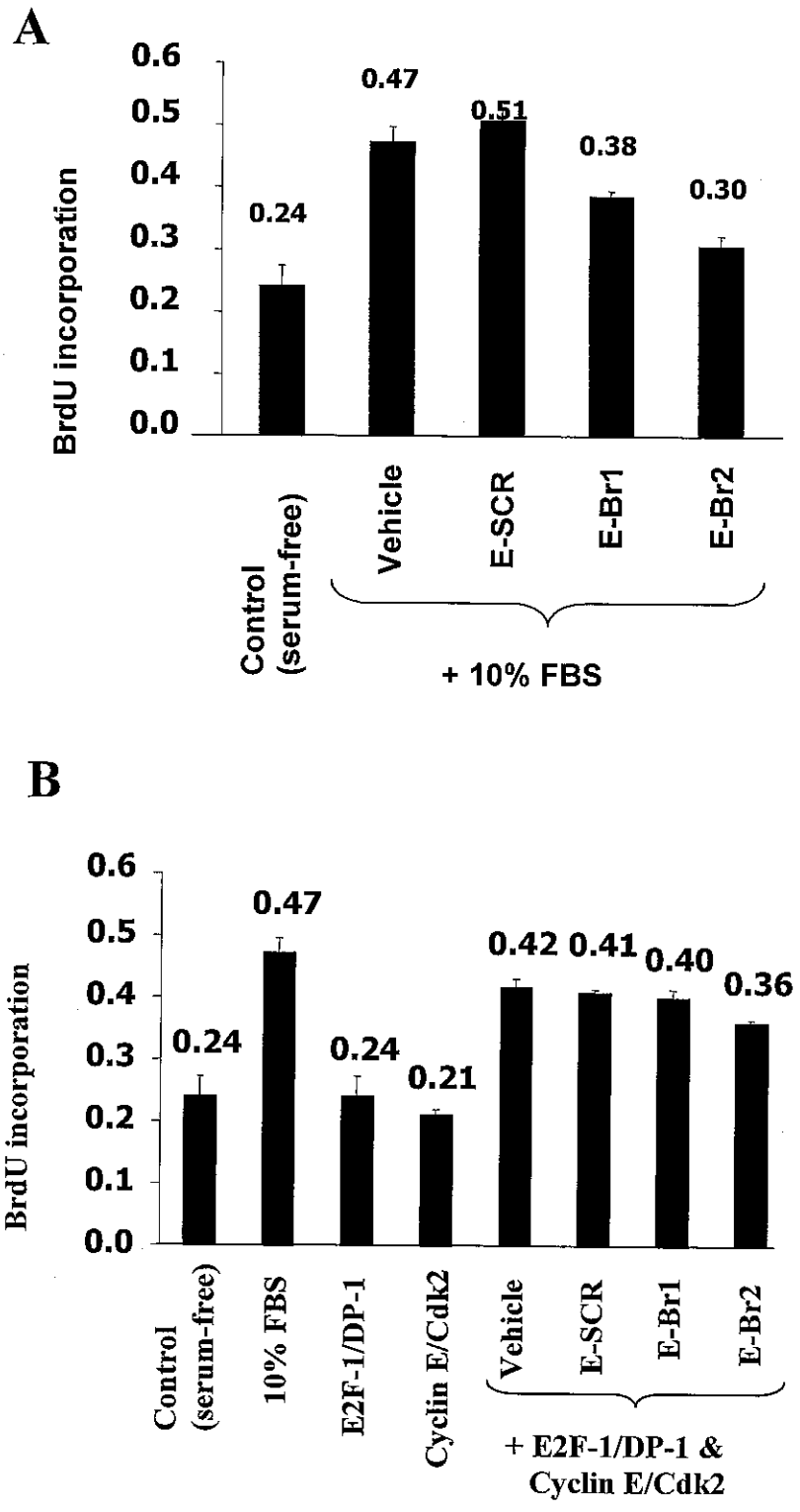

A
BrdU incorporation
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.24
0.47
0.51
0.38
0.30
Control (serum-free)
Vehicle
E-SCR
E-Br1
E-Br2
+ 10% FBS
B
BrdU incorporation
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.24
0.47
0.24
0.21
0.42
0.41
0.40
0.36
Control (serum-free)
10% FBS
E2F-1/DP-1
Cyclin E/Cdk2
Vehicle
E-SCR
E-Br1
E-Br2
+ E2F-1/DP-1 & Cyclin E/Cdk2

FIGURE 13

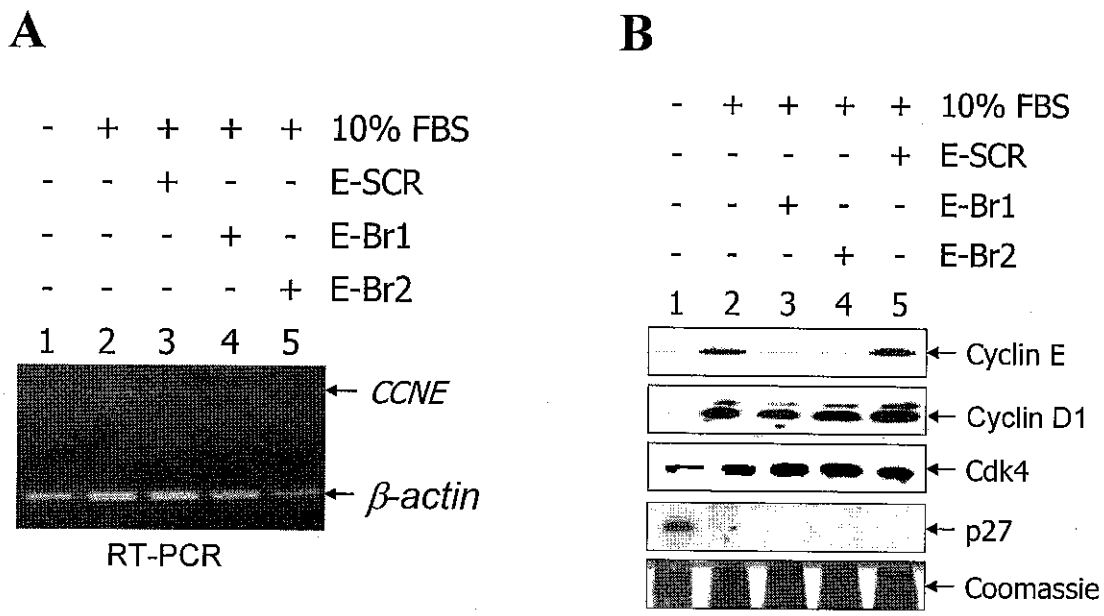

A
- + + + + 10% FBS
- - + - - E-SCR
- - - + - E-Br1
- - - - + E-Br2
1 2 3 4 5
CCNE
β-actin
RT-PCR
B
- + + + + 10% FBS
- - - - + E-SCR
- - + - - E-Br1
- - - + - E-Br2
1 2 3 4 5
Cyclin E
Cyclin D1
Cdk4
p27
Coomassie

FIGURE 14

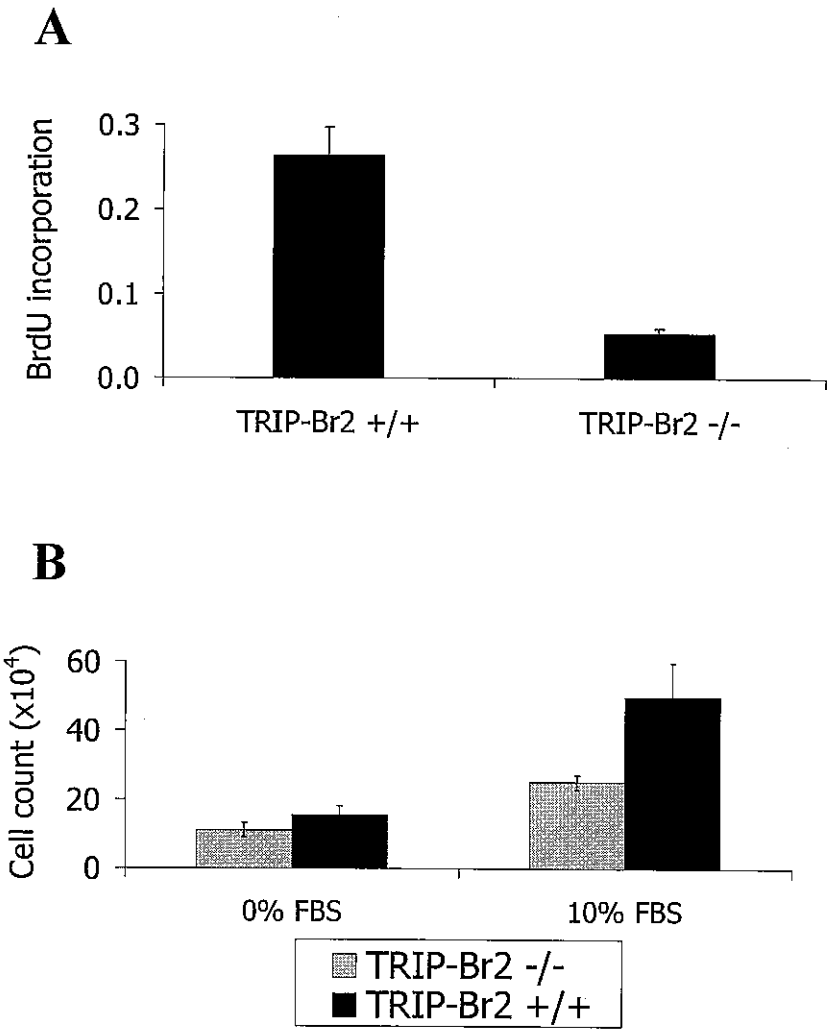

A
BrdU incorporation
0.3
0.2
0.1
0.0
TRIP-Br2 +/+
TRIP-Br2 -/-
B
Cell count (x10^4)
60
40
20
0
0% FBS
10% FBS
TRIP-Br2 -/-
TRIP-Br2 +/+
C
+
-
β-Actin
CCNE

FIGURE 15

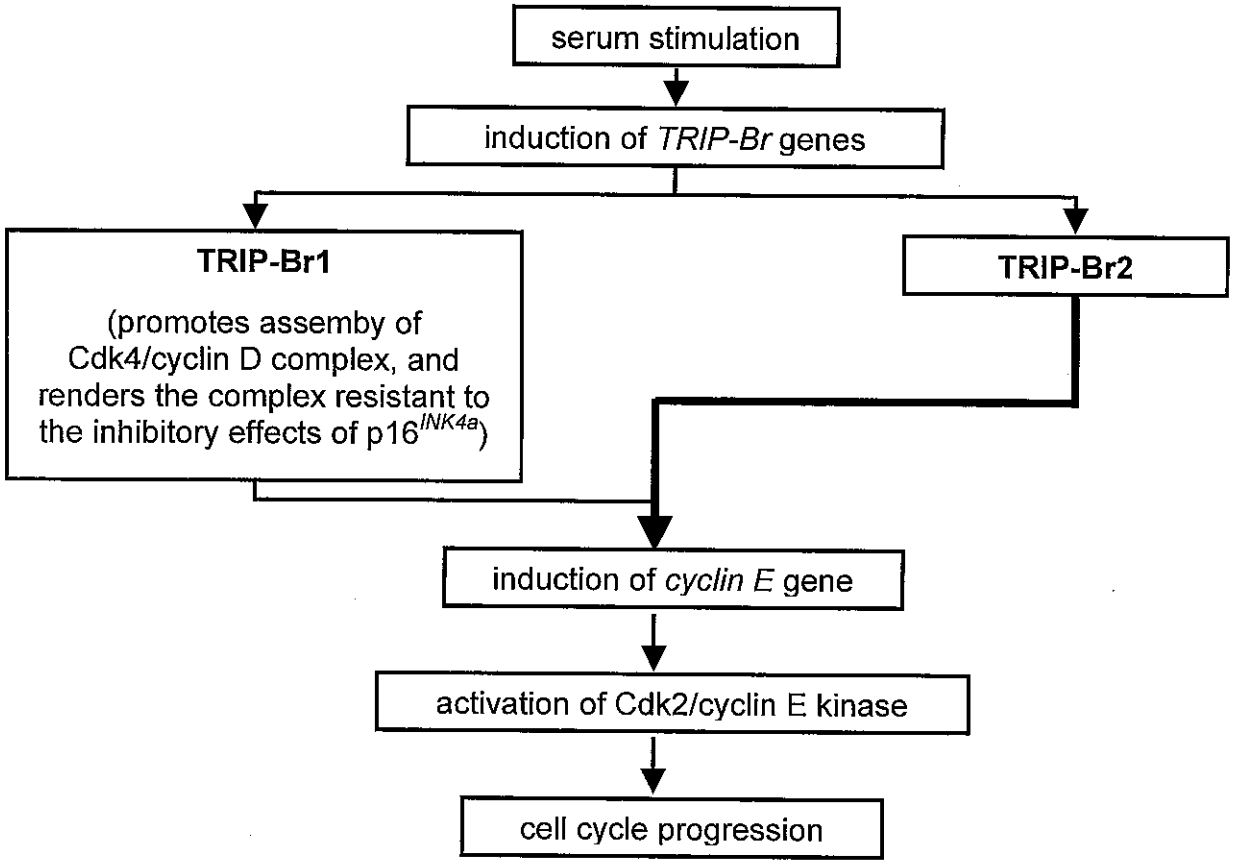

serum stimulation
induction of *TRIP-Br* genes
TRIP-Br1
(promotes assemby of Cdk4/cyclin D complex, and renders the complex resistant to the inhibitory effects of $p16^{INK4a}$)
TRIP-Br2
induction of *cyclin E* gene
activation of Cdk2/cyclin E kinase
cell cycle progression

FIGURE 16

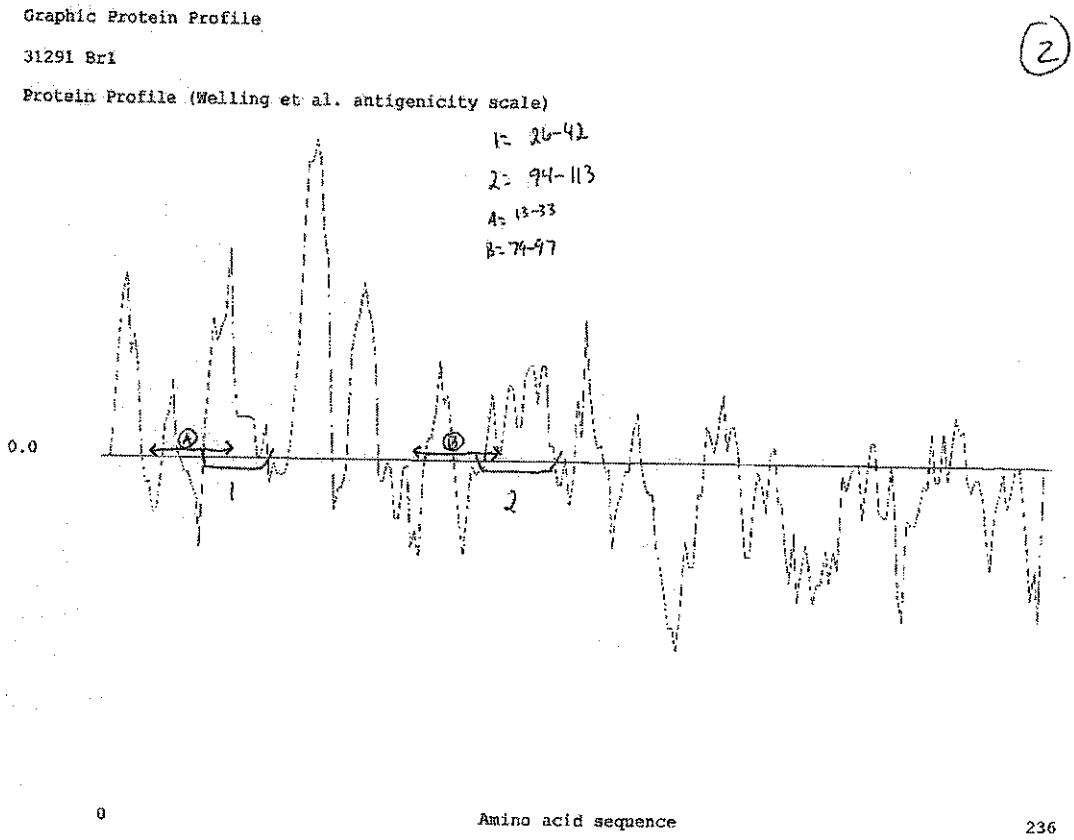
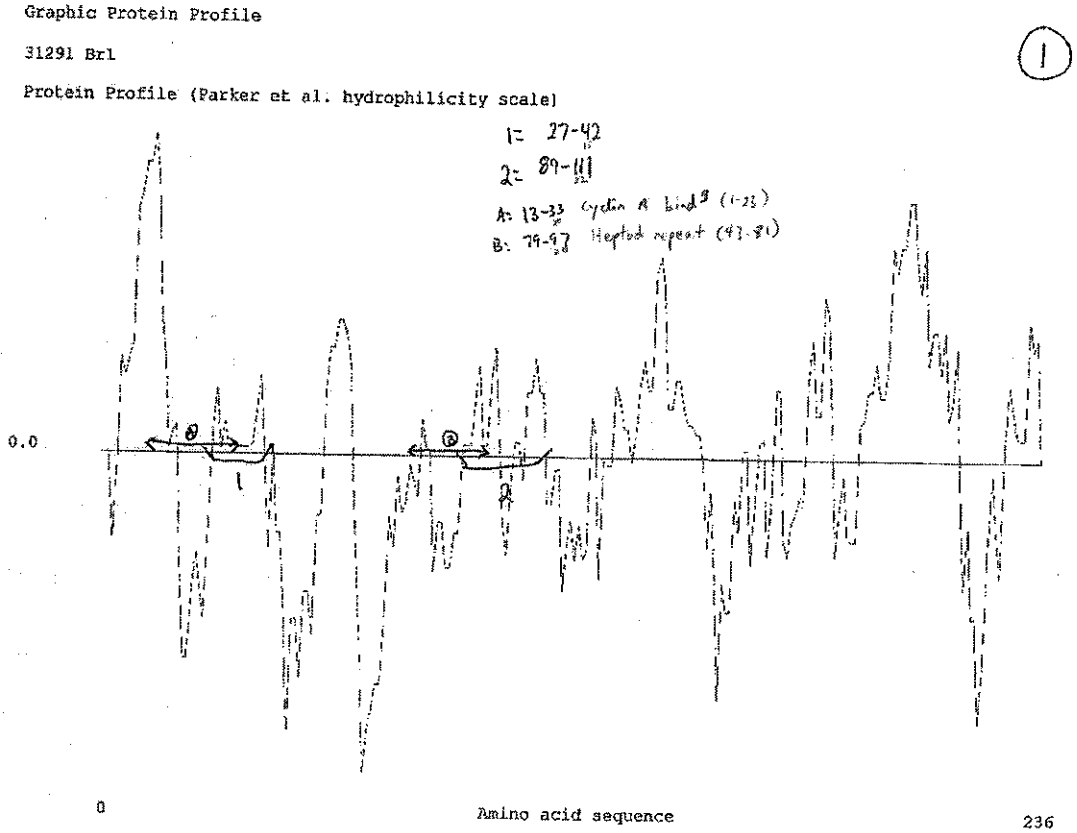

Graphic Protein Profile
31291 Br1
Protein Profile (Welling et al. antigenicity scale)
1= 26-42
2= 94-113
A= 13-33
B= 74-97
(2)
0.0
A
B
1
2
0
Amino acid sequence
236
02/05/2003 18:47 2185519544 ALPHA DIAGNOSTIC INT PAGE 03

FIGURE 17



02/05/2003 10:47 2105619544 ALPHA DIAGNOSTIC INT PAGE 02

FIGURE 18

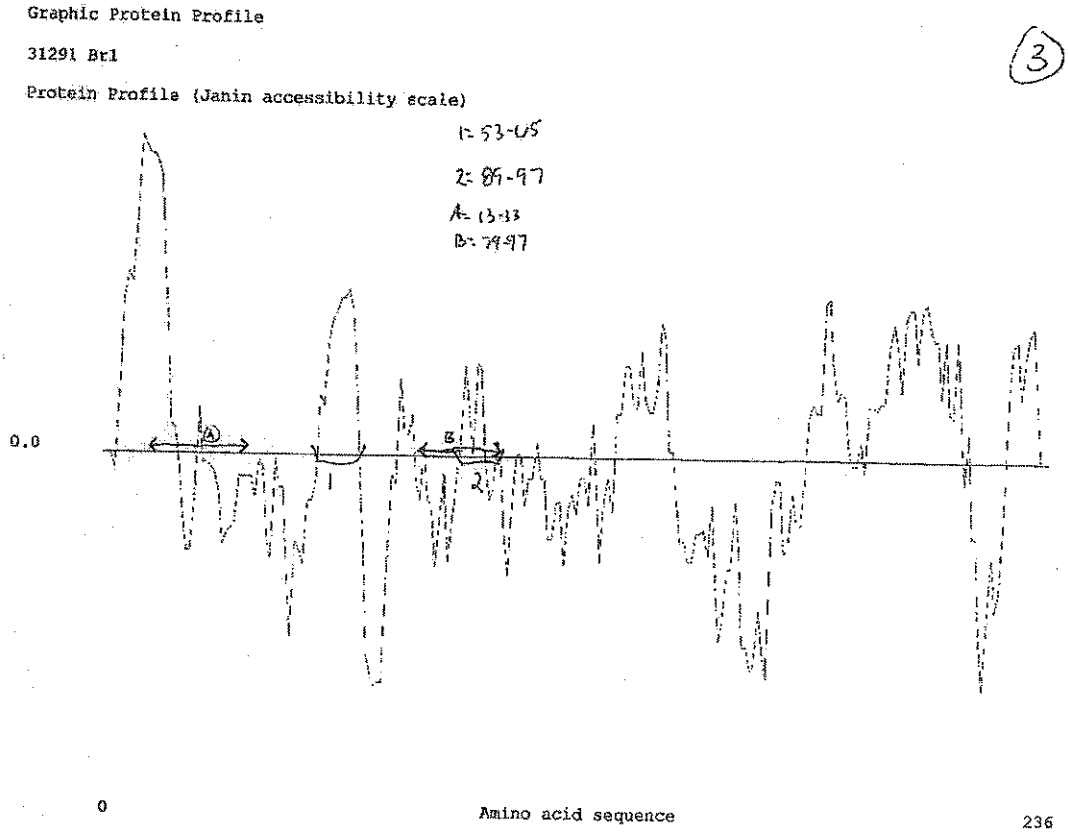
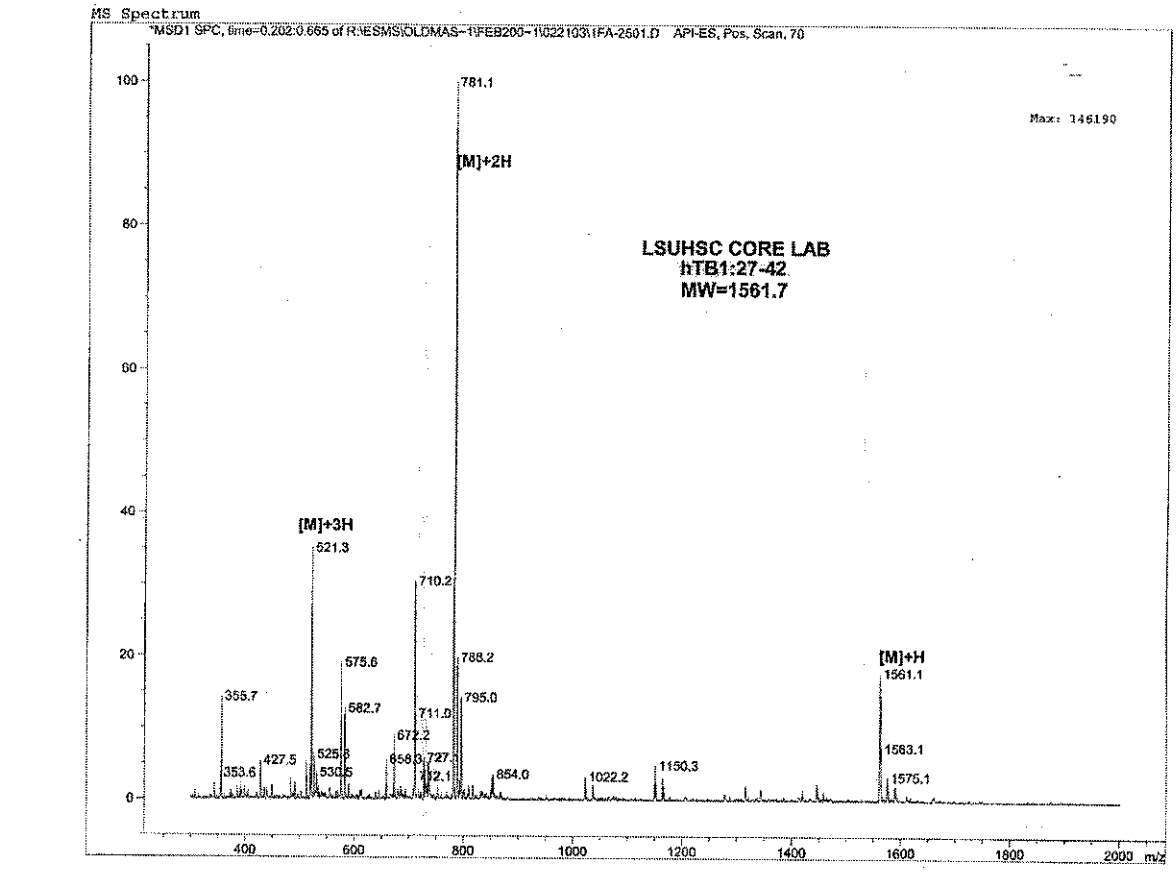

Graphic Protein Profile
31291 Brl
Protein Profile (Janin accessibility scale)
1: 53-65
2: 89-97
A: 13-33
B: 79-97
3
0.0
A
B
1
2
0
Amino acid sequence
236
02/05/2003 18:47 2105519544 ALPHA DIAGNOSTIC INT PAGE 04

FIGURE 19


hTRIP-Br2 MLGKGGKRKFDEHEDGLEGKIVSPCDGPSKVSYTLQRQTIFNISLMKLYNHRPLTEPSLQ 60
mTRIP-Br2 ----------
hTRIP-Br1 ----------
mTRIP-Br1 ----------
hTRIP-Br2 KTVLINNMLRRIQEELKQEGSLRPMFTPSSQPTTEPSDSYREAPPAFSHLASPSSHPCDL 120
mTRIP-Br2 ----------
hTRIP-Br1 ----------MLSKGLKRKREEEEEKEPLAVDSWWLDPGHAAVAQAPPAVASSSL 45
mTRIP-Br1 ----------MLSKGLKRKREEEETMEALSVDSCWLDPSHPAVAQTPPTVASSSL 45
hTRIP-Br2 GSTTPLEACLTPASLLEDDDDTFCTSQAMQPTAPTKLSPPALLPEKDSFSSALDEIEELC 180
mTRIP-Br2 ----------LEDDNDDTFFTFQAVHSAAPTRLSSAALPAEKDSFSSALDEIEELC 46
hTRIP-Br1 FDLSVLKLHHSLQQSEPDLRHLVLVVNTLRRIQASMAPAAALPPVPSPPAAPSVADNLLA 105
mTRIP-Br1 FDLSVVKLHHSLRQSEPDLRHLVLVVNTLRRIQASMEPAPVLPPEPIQPPAPSVADSLLA 105
hTRIP-Br2 PTSTSTEAA-TAATDSVKGTSSEAGTQKLDGPQESRADDSKLMDSLPGNFEIT-TSTGFL 238
mTRIP-Br2 PTSTSTEAAHTAAPEGPKGTSSESSVQKPEGPEEGRTDDSRFMDSLPGNFEIT-TSTGFL 105
hTRIP-Br1 SSDAALSASMASLLEDLSHIEGLSQAPQPLADEGPPGRSIGGAAPSLGALDLLGPATGCL 165
mTRIP-Br1 SSDAGLSASMASLLEDLNHIEDLNQAPQPQADEGPPGRSIGGISPNLGALDLLGPATGCL 165
hTRIP-Br2 TDLTLDDILFADIDTSMYDFDPCTSSSGTASKMAPVSADDLLKTLAPYSSQPVTPSQPFK 298
mTRIP-Br2 TDLTLDDILFADIDTSMYDFDPCTSASGTASKMAPVSADDLLKTLAPYSNQPVAPSQPFK 165
hTRIP-Br1 LDDGLEG-LFEDIDTSMYDNELWAPAS----------EGLKPGPEDGPGKEEAPE 209
mTRIP-Br1 LDDGLEG-LFEDIDTSMYDSELWLPAS----------EGLKPGPENGPAKEEPPE 209
hTRIP-Br2 MDLTELDHIMEVLVGS---------- 314
mTRIP-Br2 MDLTELDHIMEVLVGS---------- 181
hTRIP-Br1 LDEAELDYLMDVLVGTQALERPPGPGR 236
mTRIP-Br1 LDEAELDYLMDVLVGTQALERPPGPGR 236

FIGURE 20

Print of window 79: MS Spectrum



Instrument 1 04/10/2003 3:15:37 PM

Page 1 of 1

FIGURE 21

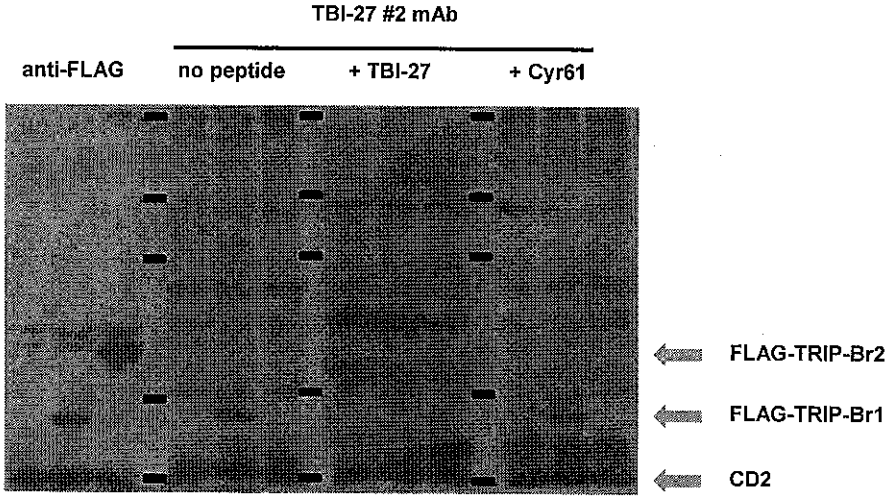


FIGURE 22

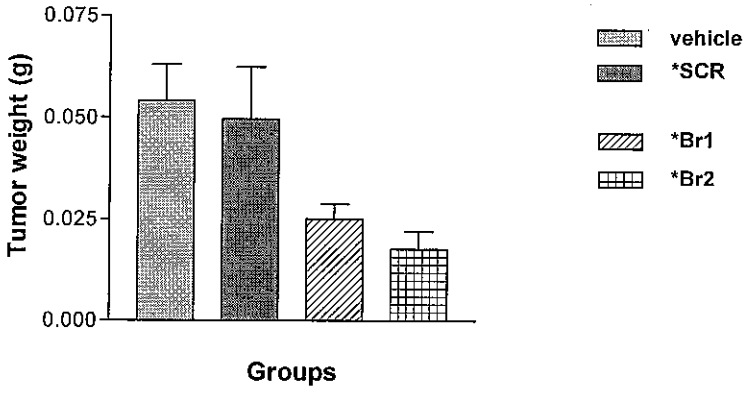


FIGURE 24

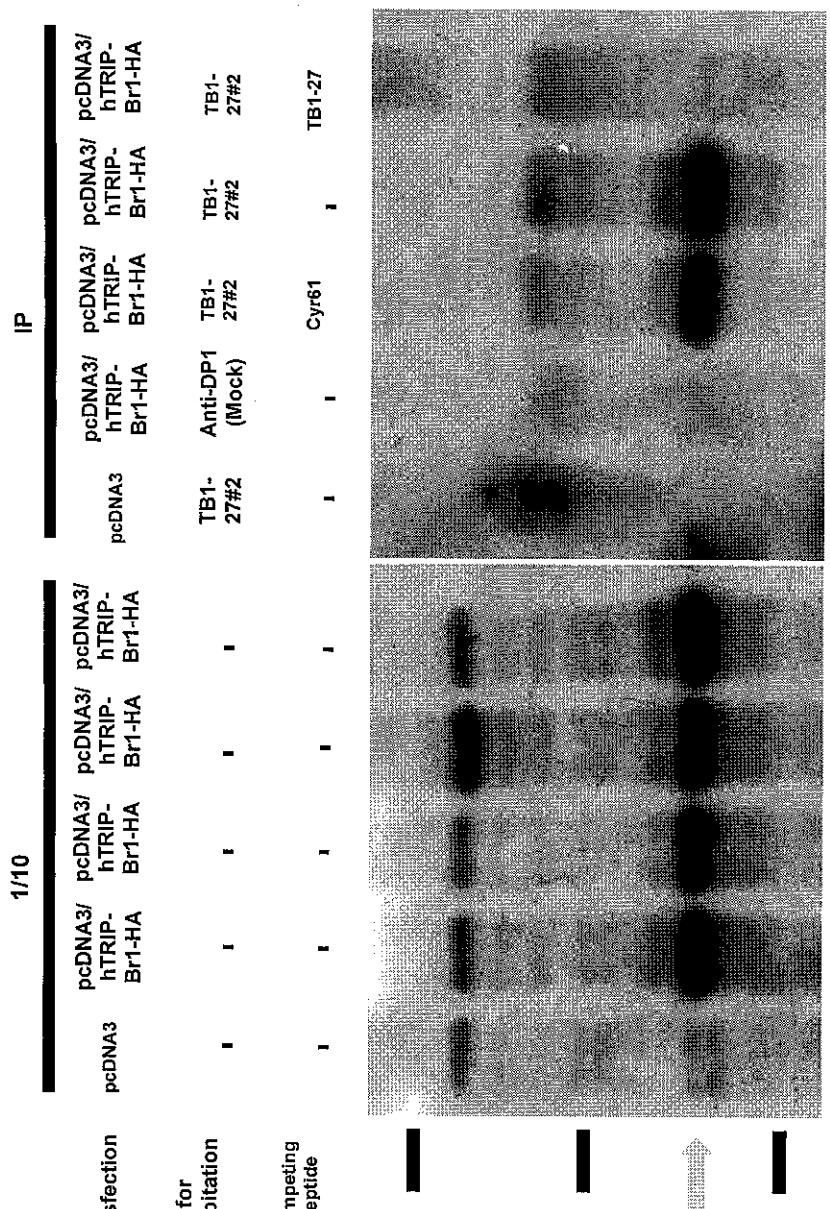

1/10
IP
Transfection
pcDNA3
pcDNA3/hTRIP-Br1-HA
pcDNA3/hTRIP-Br1-HA
pcDNA3/hTRIP-Br1-HA
pcDNA3/hTRIP-Br1-HA
pcDNA3
pcDNA3/hTRIP-Br1-HA
pcDNA3/hTRIP-Br1-HA
pcDNA3/hTRIP-Br1-HA
Antibody for immunoprecipitation
TB1-27#2
Anti-DP1 (Mock)
TB1-27#2
TB1-27#2
TB1-27#2
Competing peptide
Cyr61
TB1-27

FIGURE 23