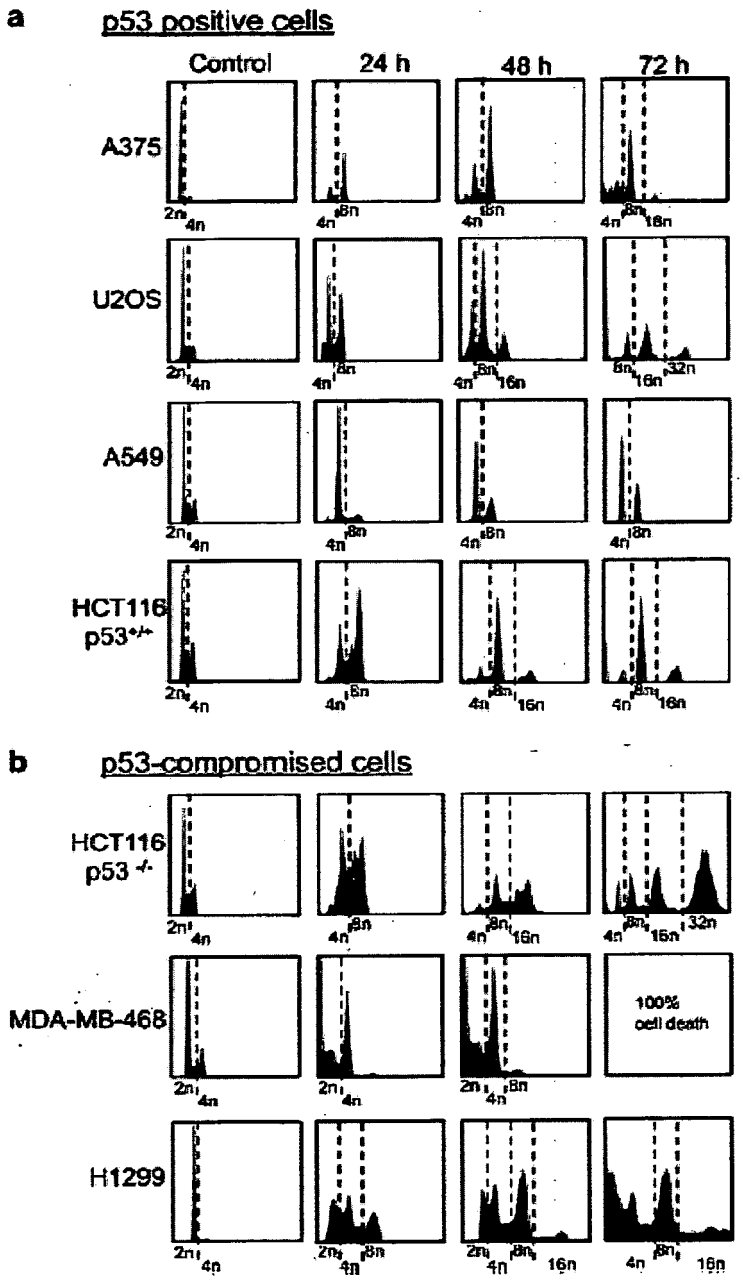

a
p53 positive cells
Control
24 h
48 h
72 h
A375
U2OS
A549
HCT116 p53+/+
b
p53-compromised cells
HCT116 p53 -/-
MDA-MB-468
100% cell death
H1299

Figure 1

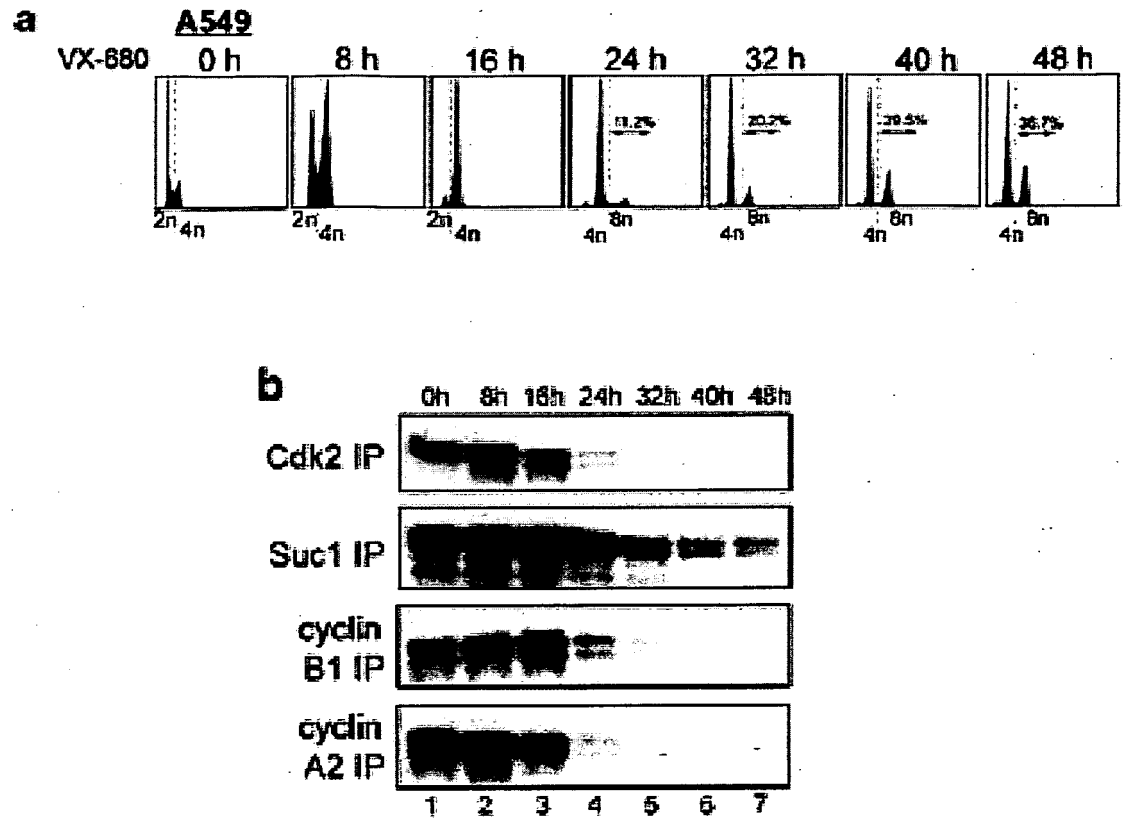

a
A549
VX-680
0 h
8 h
16 h
24 h
32 h
40 h
48 h
2n
4n
8n
b
0h 8h 16h 24h 32h 40h 48h
Cdk2 IP
Suc1 IP
cyclin B1 IP
cyclin A2 IP
1 2 3 4 5 6 7

Figure 2

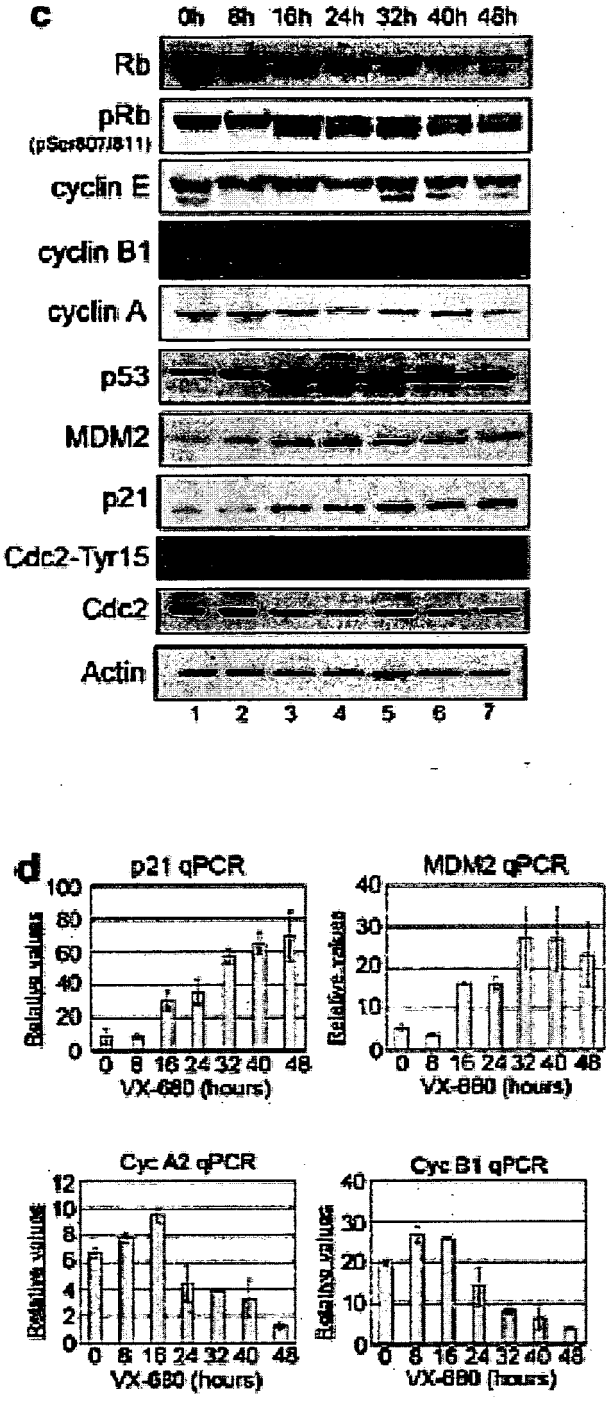

c
0h 8h 16h 24h 32h 40h 48h
Rb
pRb
(pSer807/811)
cyclin E
cyclin B1
cyclin A
p53
MDM2
p21
Cdc2-Tyr15
Cdc2
Actin
1 2 3 4 5 6 7
d
p21 qPCR
Relative values
VX-680 (hours)
MDM2 qPCR
Relative values
VX-680 (hours)
Cyc A2 qPCR
Relative values
VX-680 (hours)
Cyc B1 qPCR
Relative values
VX-680 (hours)

Figure 2

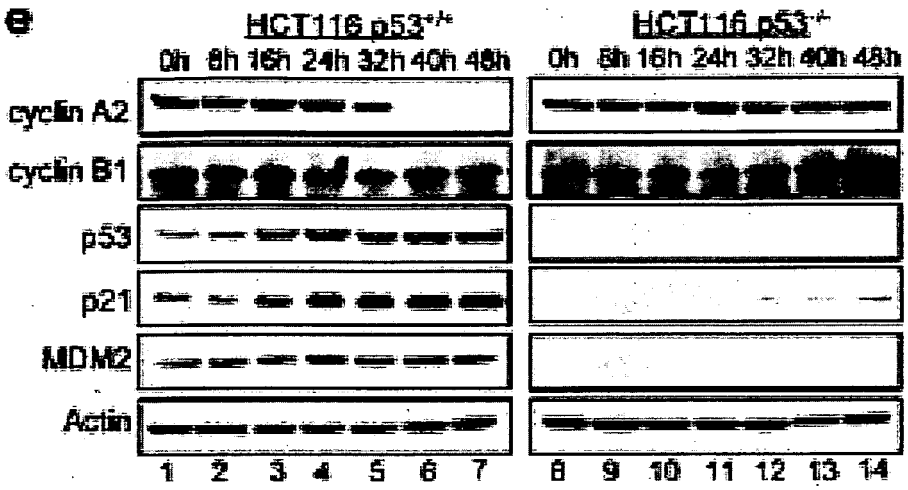

e
HCT116 p53+/+
HCT116 p53-/-
0h 8h 16h 24h 32h 40h 48h
0h 8h 16h 24h 32h 40h 48h
cyclin A2
cyclin B1
p53
p21
MDM2
Actin
1 2 3 4 5 6 7
8 9 10 11 12 13 14

Figure 2

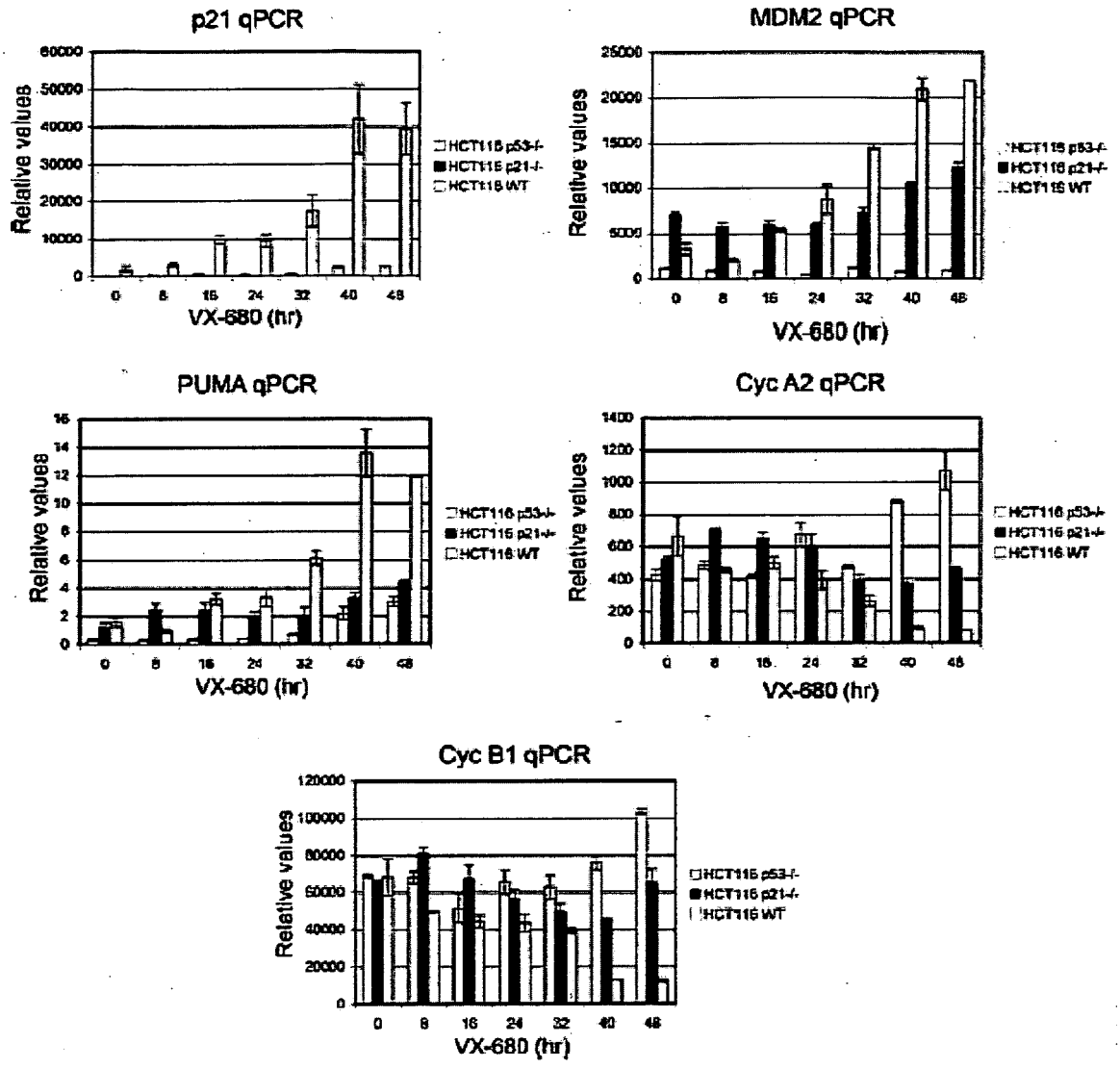

p21 qPCR
Relative values
VX-680 (hr)
HCT116 p53-/-
HCT116 p21-/-
HCT116 WT
MDM2 qPCR
Relative values
VX-680 (hr)
HCT116 p53-/-
HCT116 p21-/-
HCT116 WT
PUMA qPCR
Relative values
VX-680 (hr)
HCT116 p53-/-
HCT116 p21-/-
HCT116 WT
Cyc A2 qPCR
Relative values
VX-680 (hr)
HCT116 p53-/-
HCT116 p21-/-
HCT116 WT
Cyc B1 qPCR
Relative values
VX-680 (hr)
HCT116 p53-/-
HCT116 p21-/-
HCT116 WT

Figure 3

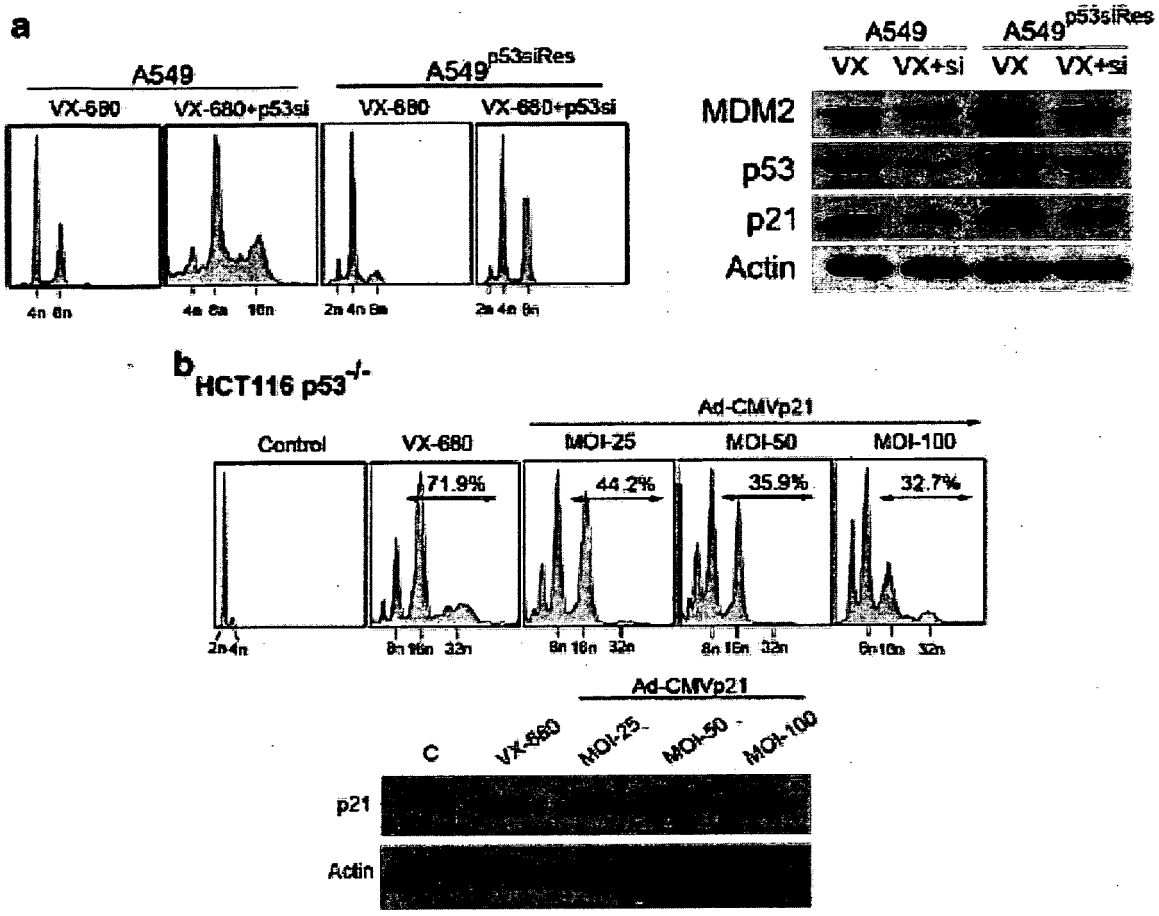

a
A549
A549p53siRes
VX-680
VX-680+p53si
VX-680
VX-680+p53si
4n 8n
4n 8n 16n
2n 4n 8n
2n 4n 8n
A549
A549p53siRes
VX VX+si VX VX+si
MDM2
p53
p21
Actin
b
HCT116 p53-/-
Ad-CMVp21
Control
VX-680
MOI-25
MOI-50
MOI-100
71.9%
44.2%
35.9%
32.7%
2n 4n
8n 16n 32n
8n 16n 32n
8n 16n 32n
8n 16n 32n
Ad-CMVp21
C
VX-680
MOI-25
MOI-50
MOI-100
p21
Actin

Figure 4

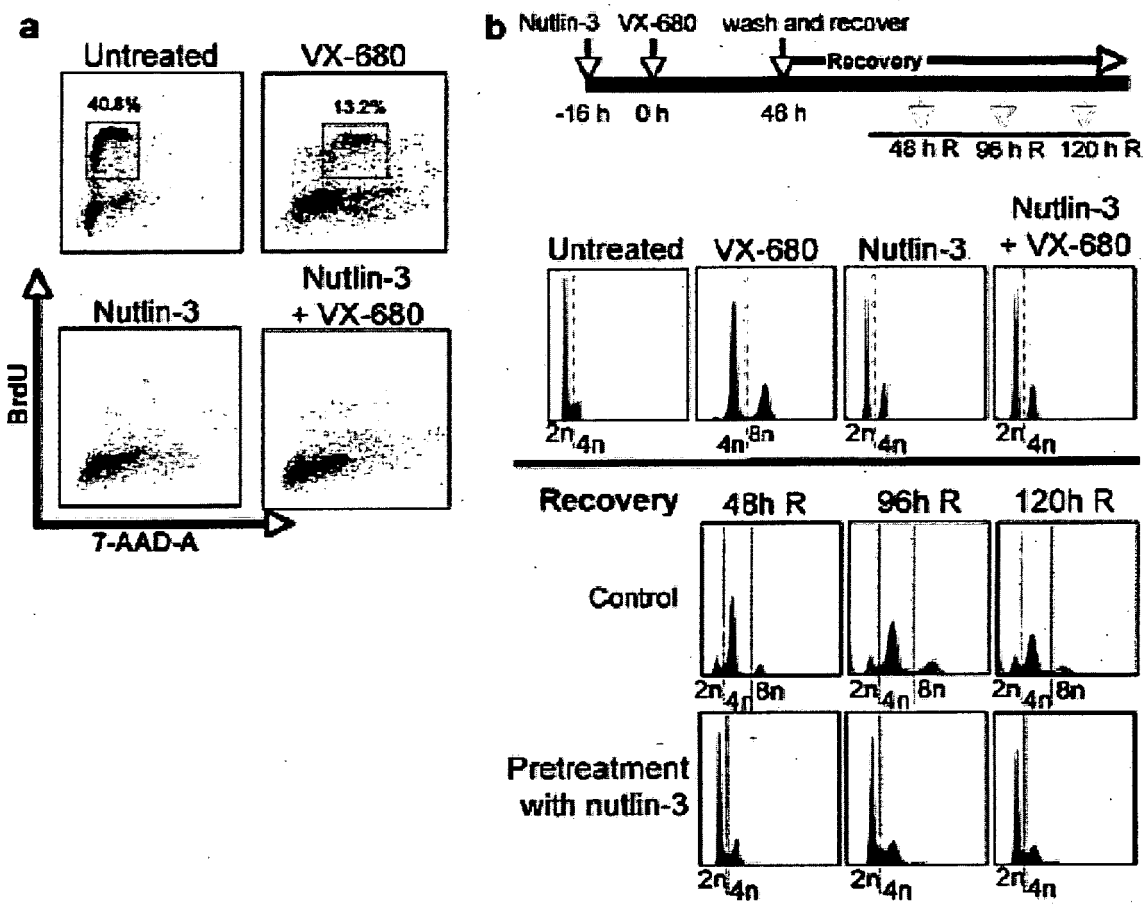

a
Untreated
VX-680
40.8%
13.2%
Nutlin-3
Nutlin-3
+ VX-680
BrdU
7-AAD-A
b
Nutlin-3
VX-680
wash and recover
Recovery
-16 h
0 h
48 h
48 h R
96 h R
120 h R
Untreated
VX-680
Nutlin-3
Nutlin-3
+ VX-680
2n
4n
8n
Recovery
48h R
96h R
120h R
Control
Pretreatment
with nutlin-3

Figure 5

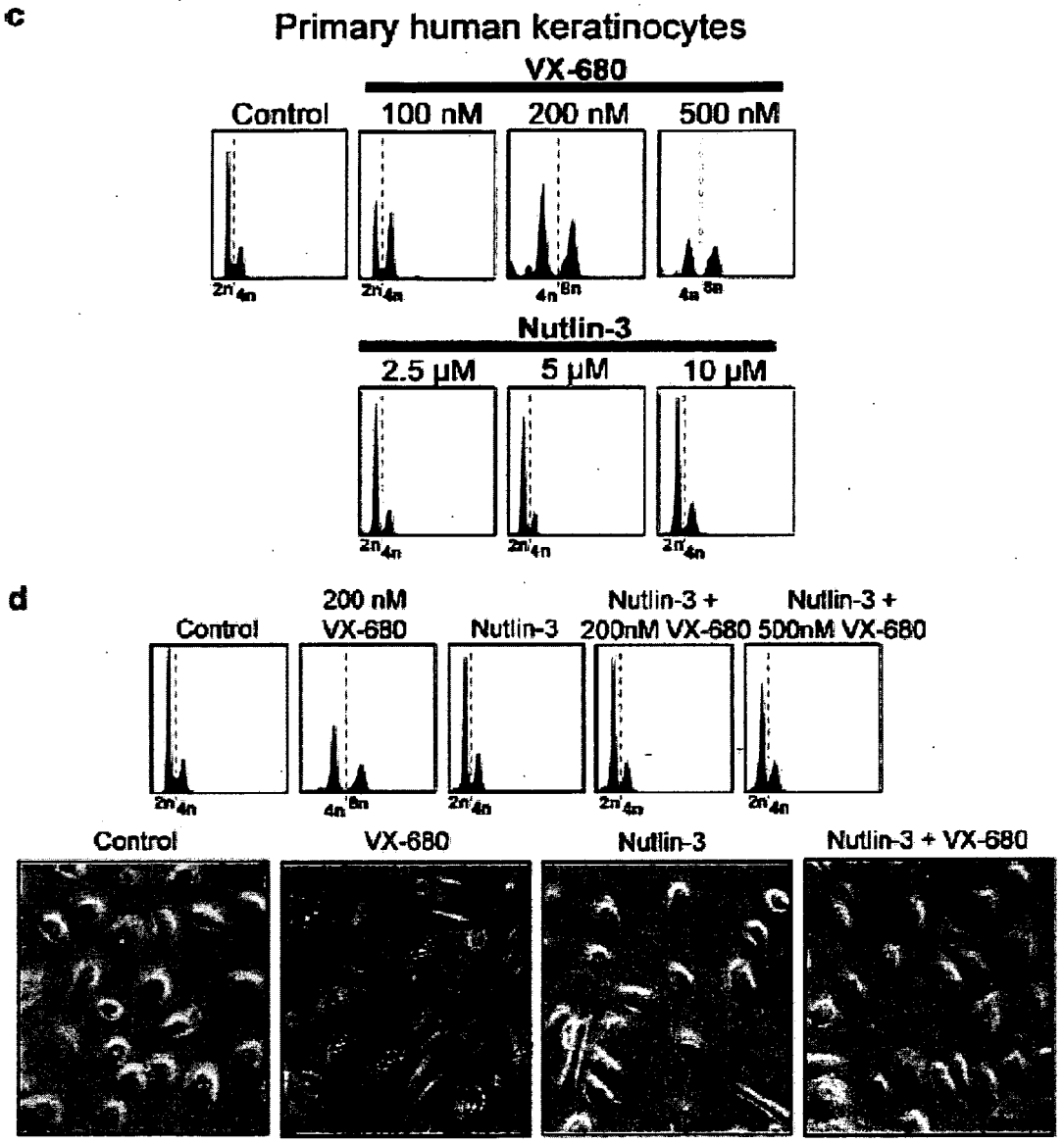

c
Primary human keratinocytes
VX-680
Control
100 nM
200 nM
500 nM
Nutlin-3
2.5 μM
5 μM
10 μM
d
Control
200 nM VX-680
Nutlin-3
Nutlin-3 + 200nM VX-680
Nutlin-3 + 500nM VX-680
Control
VX-680
Nutlin-3
Nutlin-3 + VX-680

Figure 5

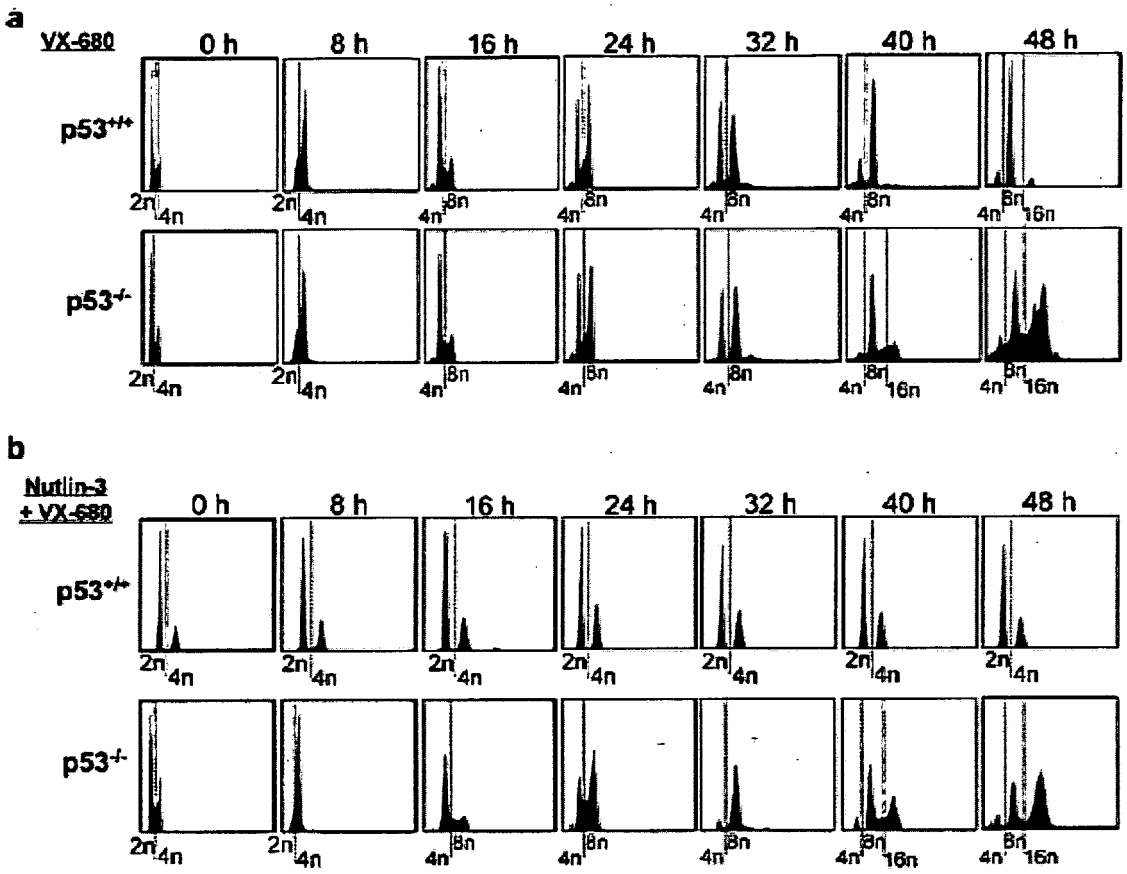

a
VX-680
0 h
8 h
16 h
24 h
32 h
40 h
48 h
p53+/+
p53-/-
2n
4n
8n
16n
b
Nutlin-3
+ VX-680
0 h
8 h
16 h
24 h
32 h
40 h
48 h
p53+/+
p53-/-
2n
4n
8n
16n

Figure 6

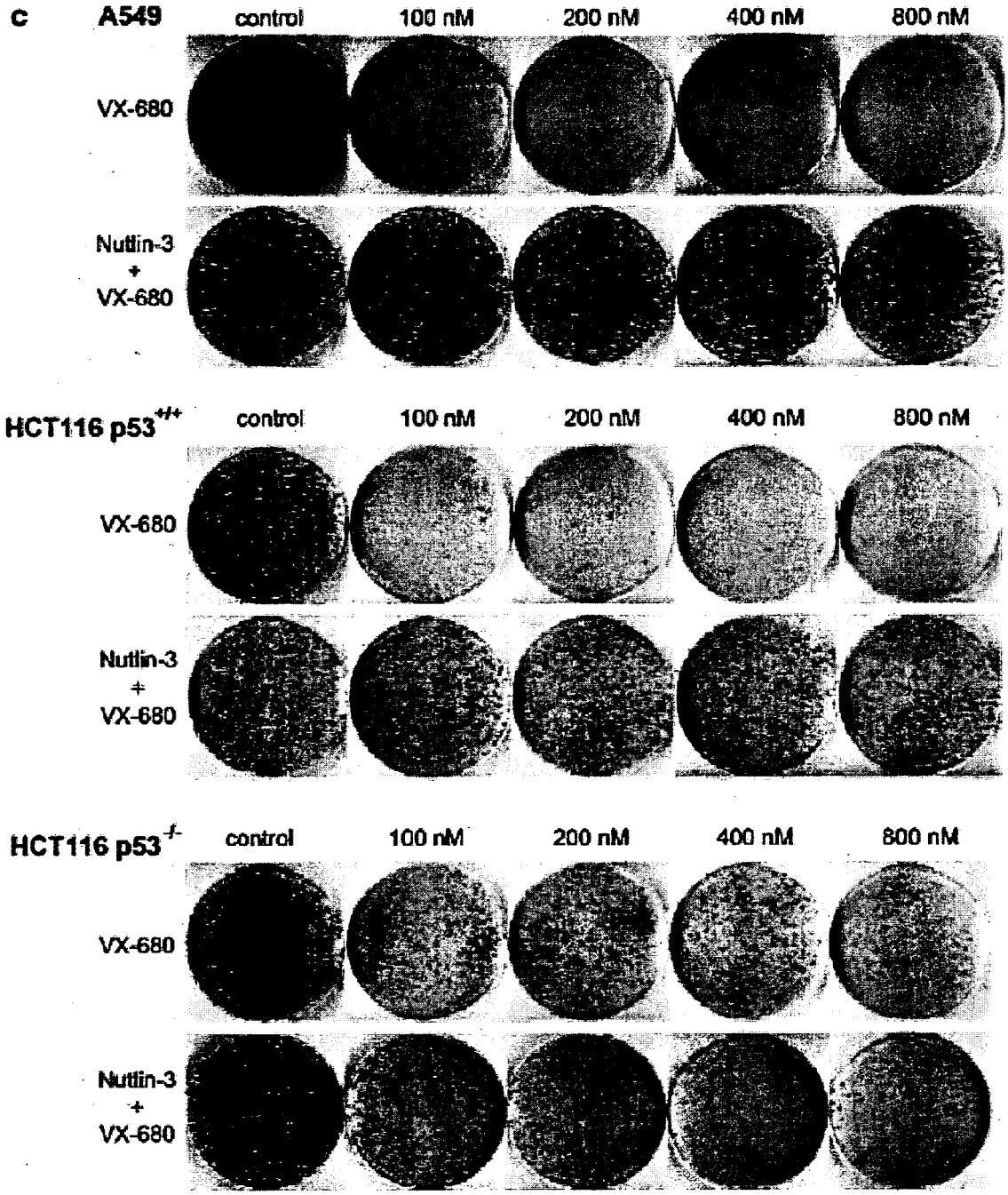

c
A549
control
100 nM
200 nM
400 nM
800 nM
VX-680
Nutlin-3
+
VX-680
HCT116 p53+/+
control
100 nM
200 nM
400 nM
800 nM
VX-680
Nutlin-3
+
VX-680
HCT116 p53-/-
control
100 nM
200 nM
400 nM
800 nM
VX-680
Nutlin-3
+
VX-680

Figure 6

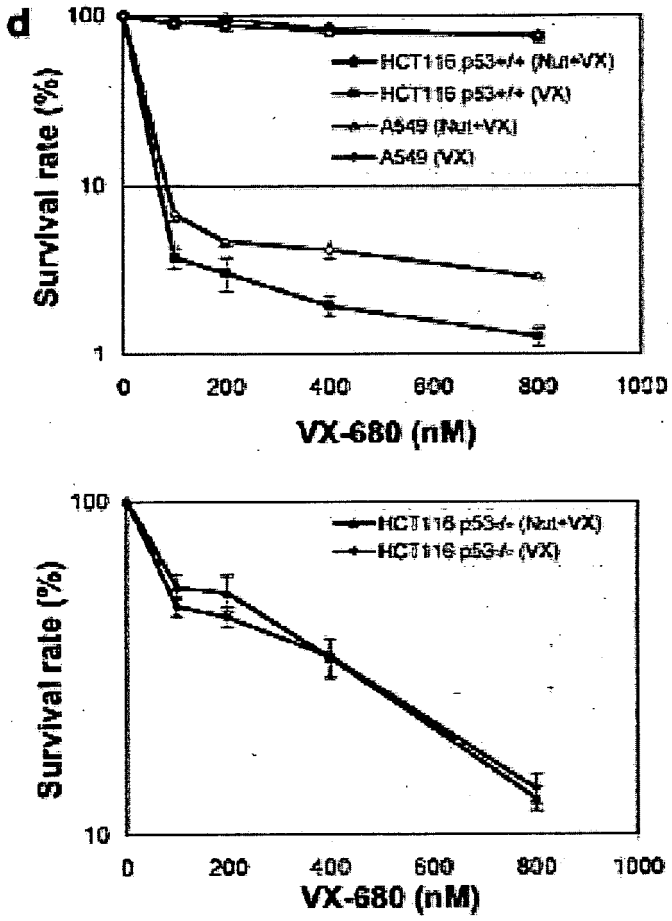

d
100
10
1
Survival rate (%)
HCT116 p53+/+ (Nut+VX)
HCT116 p53+/+ (VX)
A549 (Nut+VX)
A549 (VX)
0
200
400
600
800
1000
VX-680 (nM)
100
10
Survival rate (%)
HCT116 p53-/- (Nut+VX)
HCT116 p53-/- (VX)
0
200
400
600
800
1000
VX-680 (nM)

Figure 6

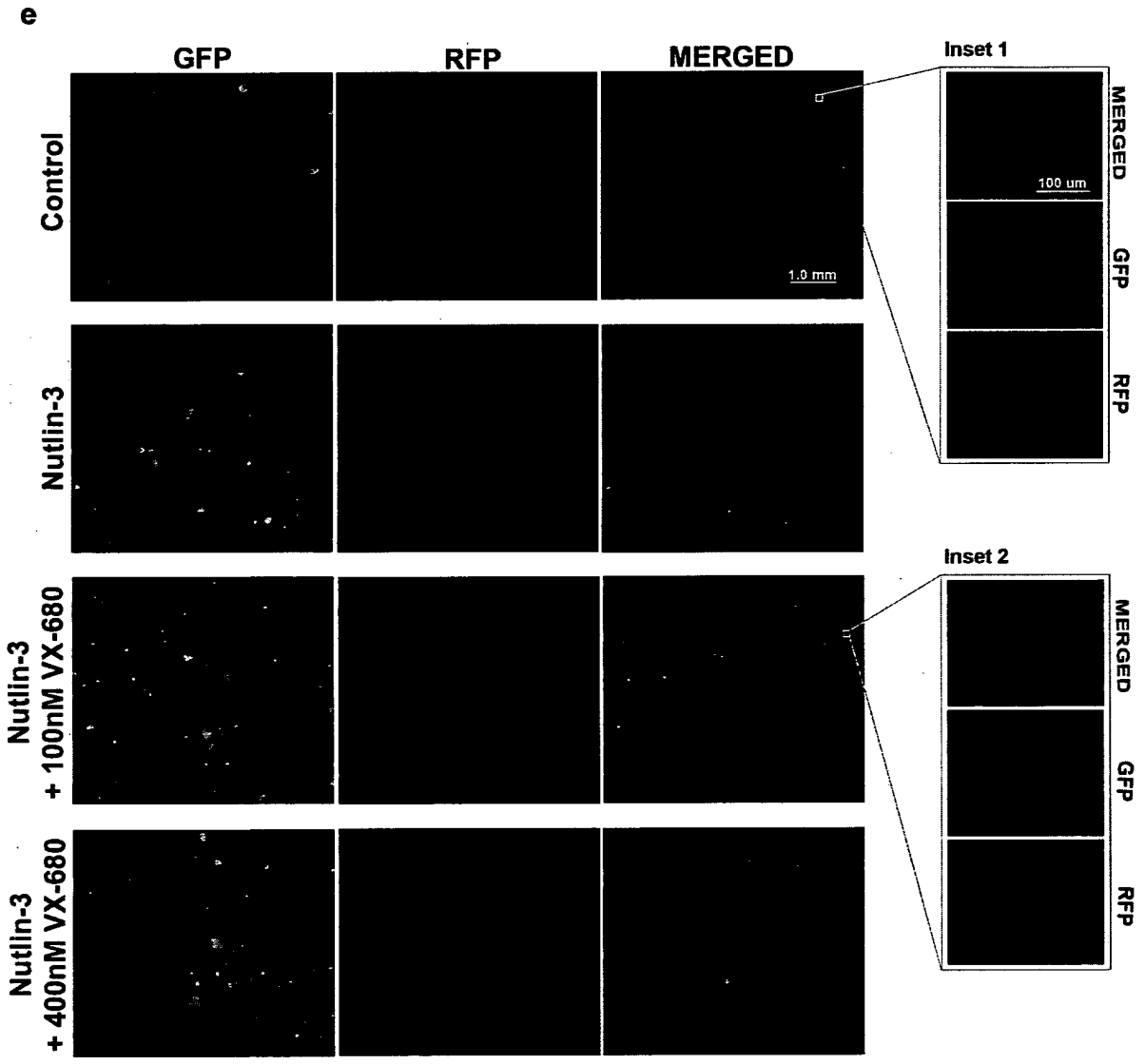

e
GFP
RFP
MERGED
Inset 1
Control
Nutlin-3
Nutlin-3 + 100nM VX-680
Nutlin-3 + 400nM VX-680
1.0 mm
100 um
MERGED
GFP
RFP
Inset 2
MERGED
GFP
RFP

Figure 6

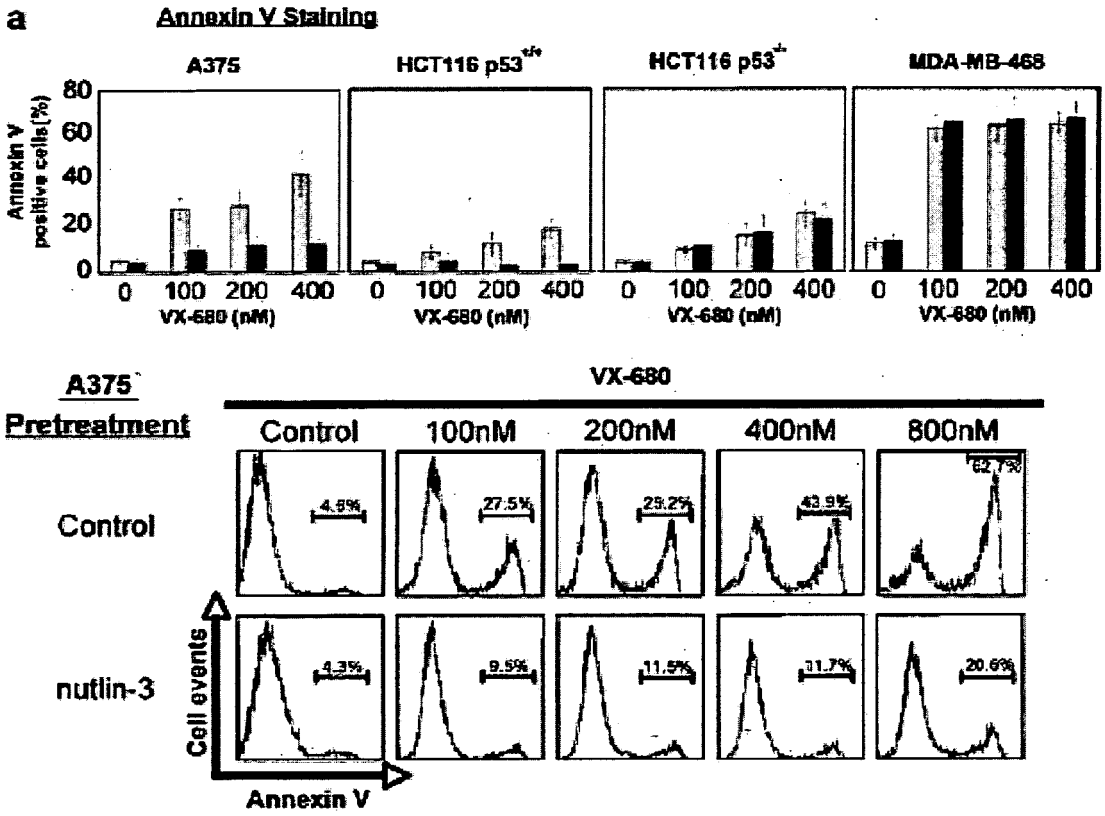

a
Annexin V Staining
A375
HCT116 p53+/+
HCT116 p53-/-
MDA-MB-468
Annexin V positive cells(%)
80
60
40
20
0
0 100 200 400
VX-680 (nM)
A375
Pretreatment
VX-680
Control
100nM
200nM
400nM
800nM
Control
nutlin-3
4.9%
27.5%
25.2%
43.9%
62.7%
4.3%
9.5%
11.5%
31.7%
20.6%
Cell events
Annexin V

Figure 7

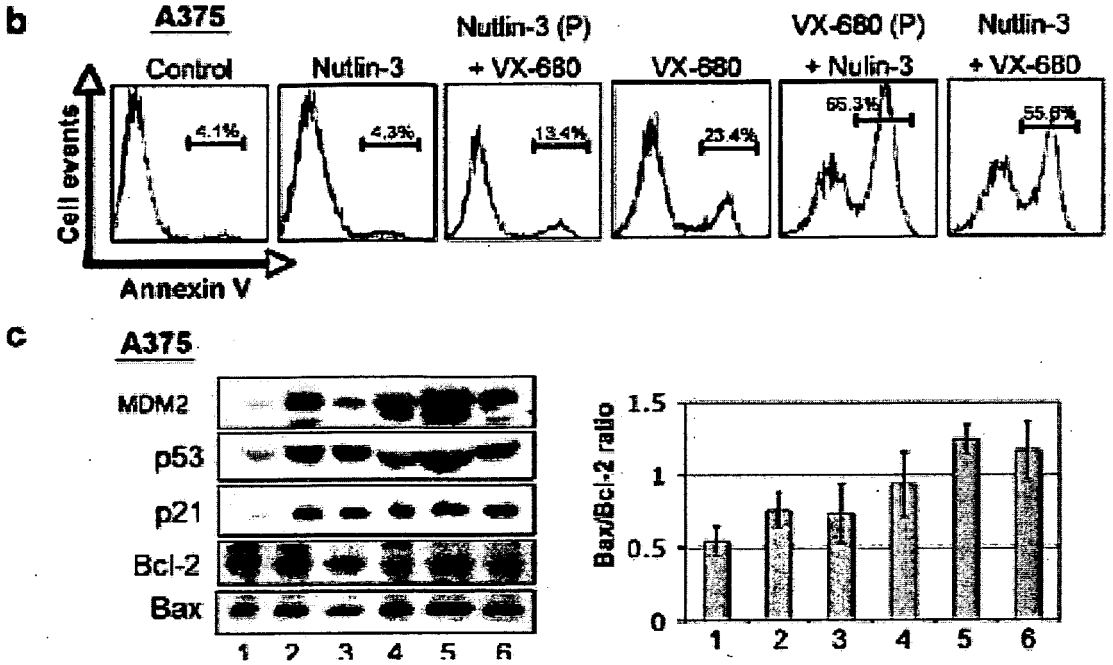
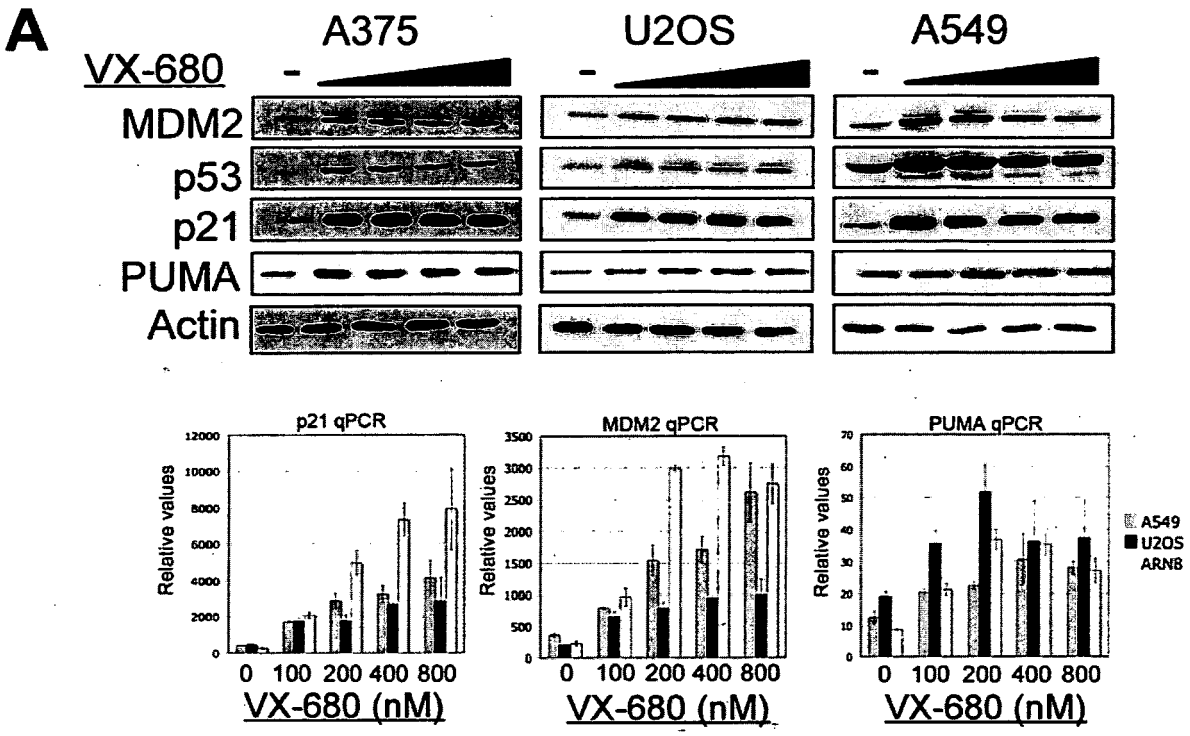

b
A375
Control
Nutlin-3
Nutlin-3 (P)
+ VX-680
VX-680
VX-680 (P)
+ Nulin-3
Nutlin-3
+ VX-680
4.1%
4.3%
13.4%
23.4%
65.3%
55.8%
Cell events
Annexin V
c
A375
MDM2
p53
p21
Bcl-2
Bax
1 2 3 4 5 6
Bax/Bcl-2 ratio
1.5
1
0.5
0
1 2 3 4 5 6

Figure 7


A
A375
U2OS
A549
VX-680
-
MDM2
p53
p21
PUMA
Actin
p21 qPCR
MDM2 qPCR
PUMA qPCR
Relative values
0 100 200 400 800
VX-680 (nM)
A549
U2OS
ARN8

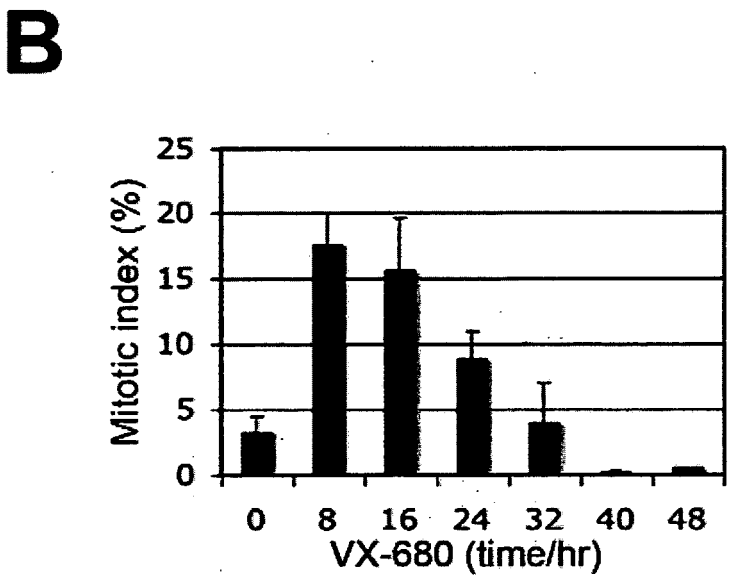
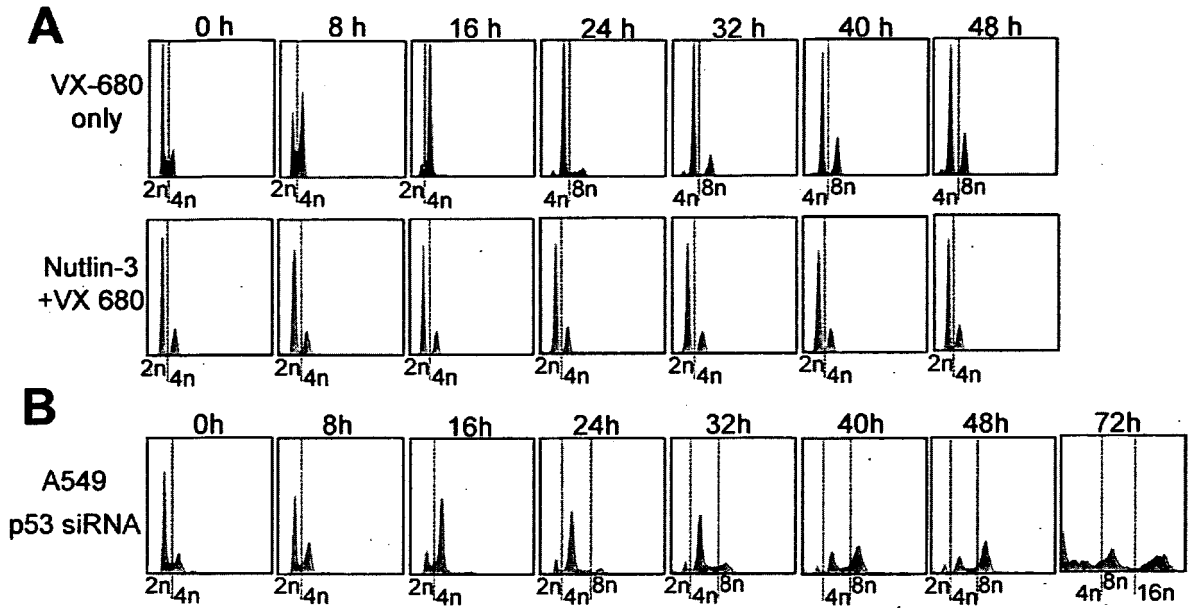

B
Mitotic index (%)
0 5 10 15 20 25
0 8 16 24 32 40 48
VX-680 (time/hr)

Figure 8


A
0 h
8 h
16 h
24 h
32 h
40 h
48 h
VX-680 only
Nutlin-3 +VX 680
2n 4n
4n 8n
B
0h
8h
16h
24h
32h
40h
48h
72h
A549 p53 siRNA
2n 4n
4n 8n
4n 8n 16n

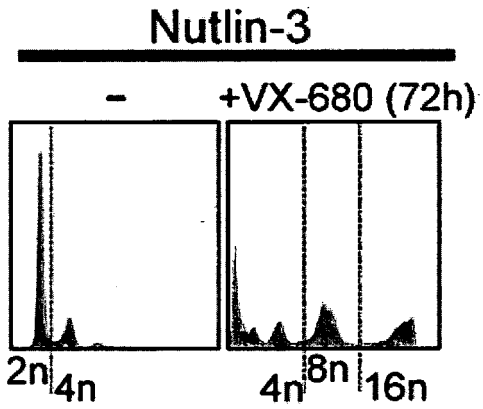

Nutlin-3
-
+VX-680 (72h)
2n 4n
4n 8n 16n

Figure 9

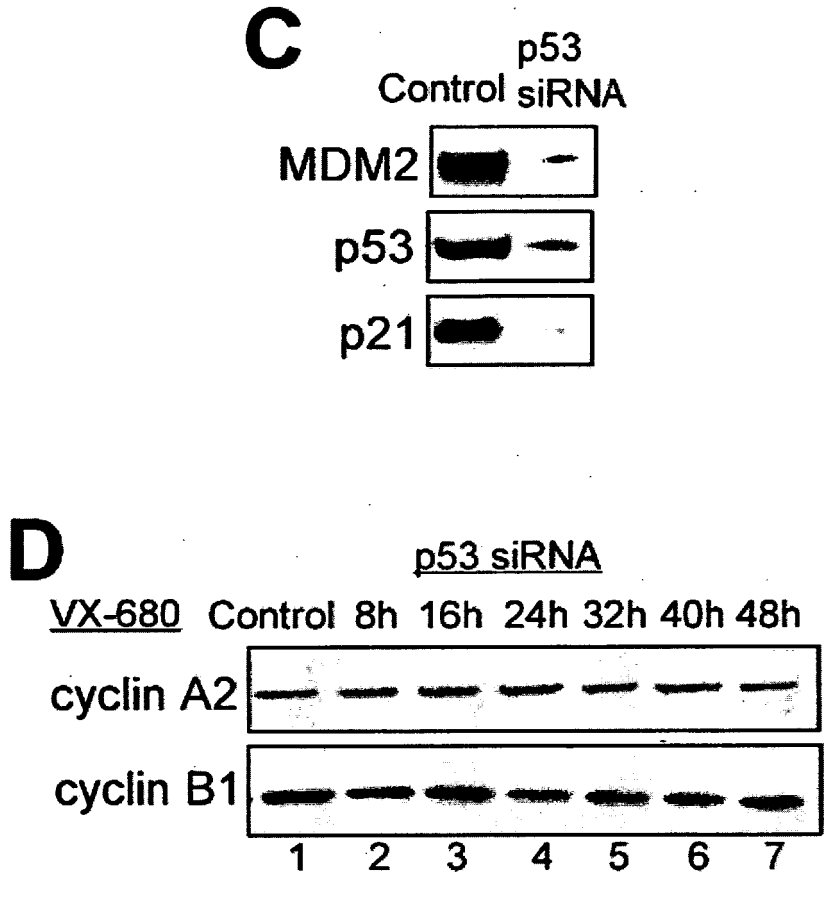
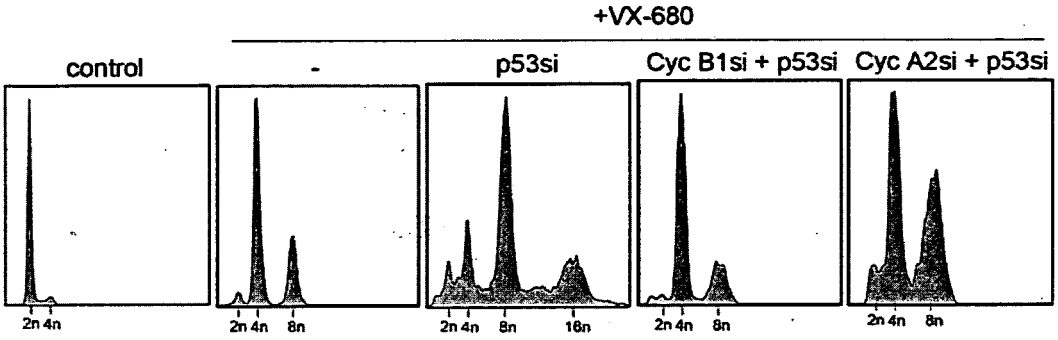

C
Control
p53 siRNA
MDM2
p53
p21
D
p53 siRNA
VX-680
Control
8h
16h
24h
32h
40h
48h
cyclin A2
cyclin B1
1
2
3
4
5
6
7

Figure 9

A


+VX-680
control
-
p53si
Cyc B1si + p53si
Cyc A2si + p53si
2n 4n
2n 4n 8n
2n 4n 8n 16n
2n 4n 8n
2n 4n 8n

B

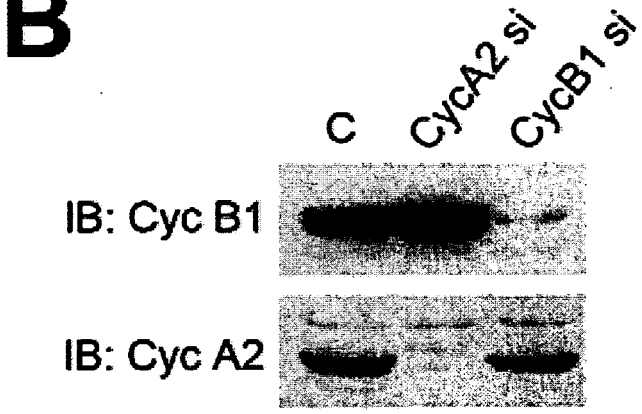

C
CycA2 si
CycB1 si
IB: Cyc B1
IB: Cyc A2

Figure 10

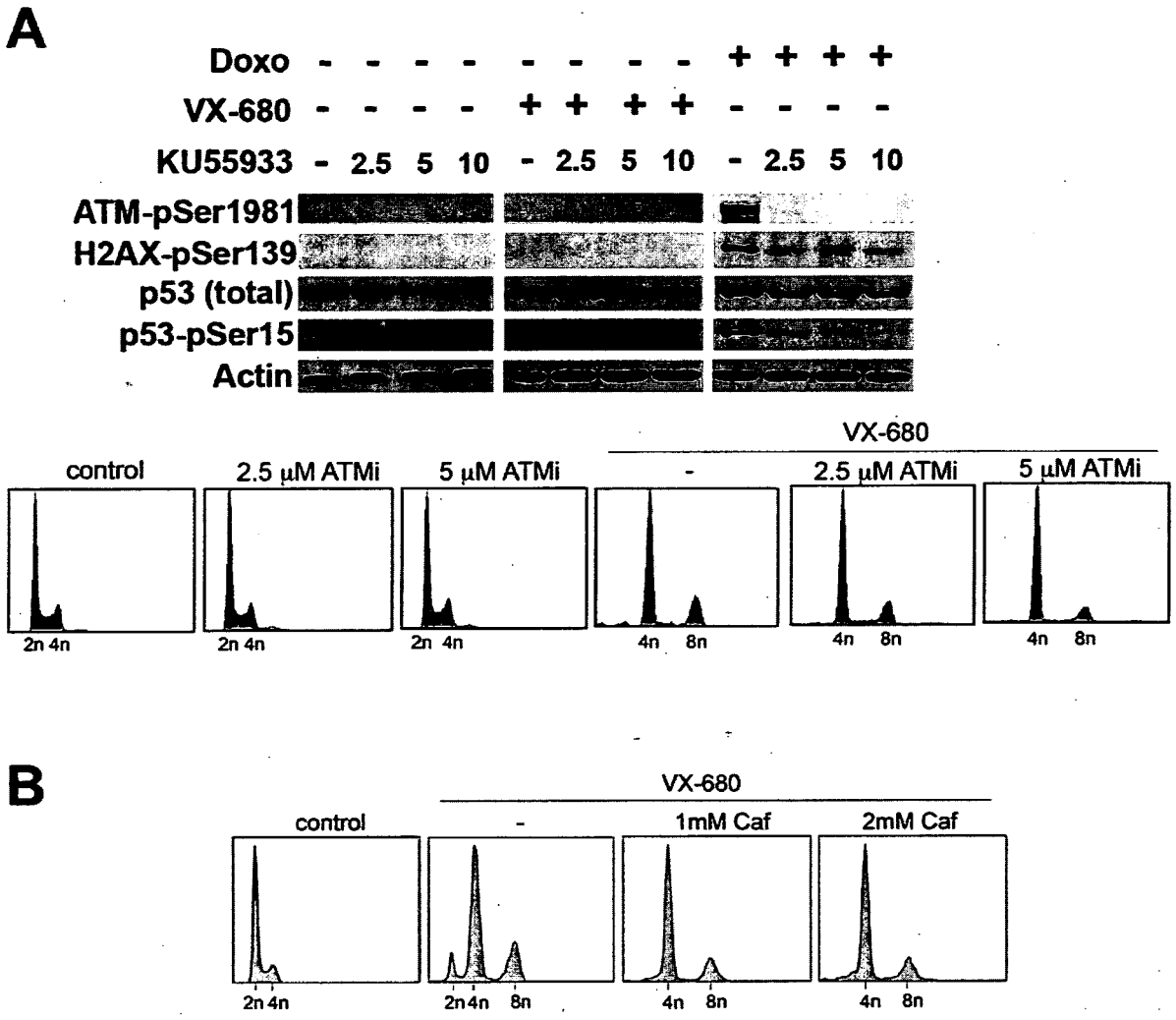
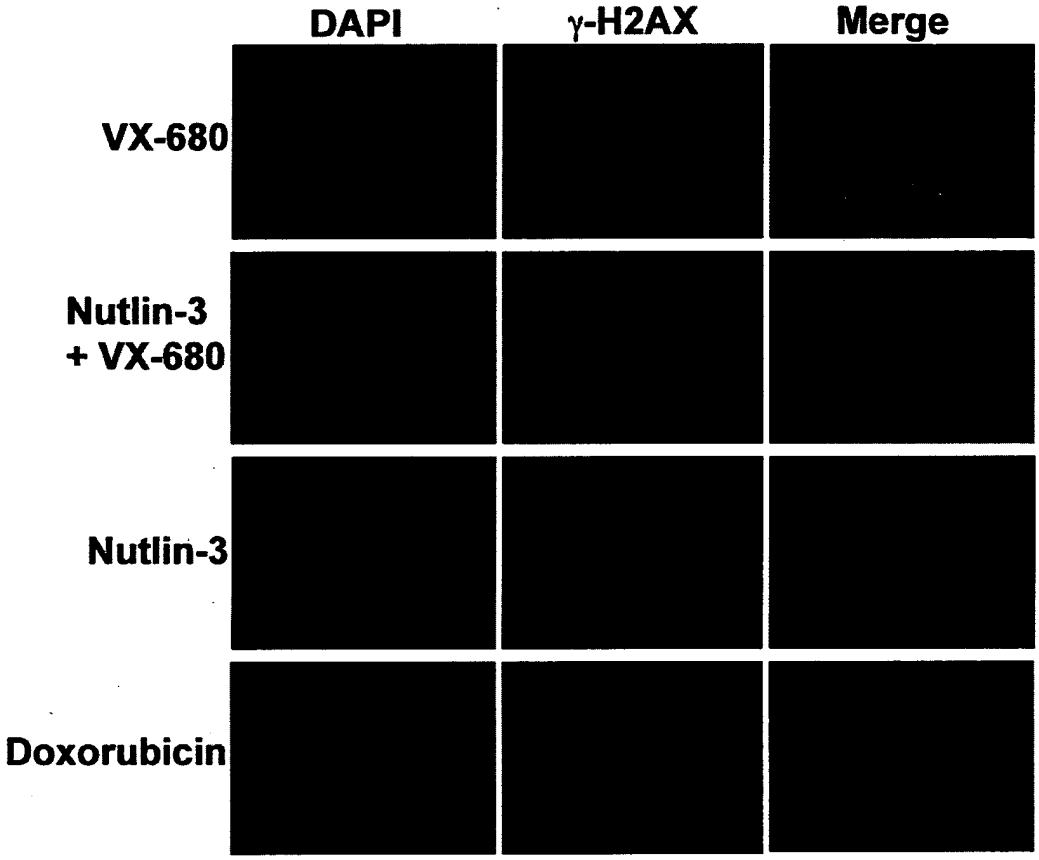

A
Doxo - - - - - - - - + + + +
VX-680 - - - - + + + + - - - -
KU55933 - 2.5 5 10 - 2.5 5 10 - 2.5 5 10
ATM-pSer1981
H2AX-pSer139
p53 (total)
p53-pSer15
Actin
VX-680
control
2.5 μM ATMi
5 μM ATMi
-
2.5 μM ATMi
5 μM ATMi
2n 4n
2n 4n
2n 4n
4n 8n
4n 8n
4n 8n
B
VX-680
control
-
1mM Caf
2mM Caf
2n 4n
2n 4n 8n
4n 8n
4n 8n

Figure 11


DAPI
γ-H2AX
Merge
VX-680
Nutlin-3 + VX-680
Nutlin-3
Doxorubicin

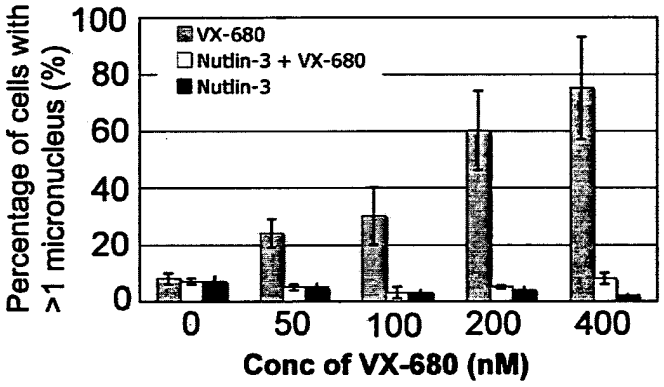

Percentage of cells with >1 micronucleus (%)
100
80
60
40
20
0
VX-680
Nutlin-3 + VX-680
Nutlin-3
0
50
100
200
400
Conc of VX-680 (nM)

Figure 12