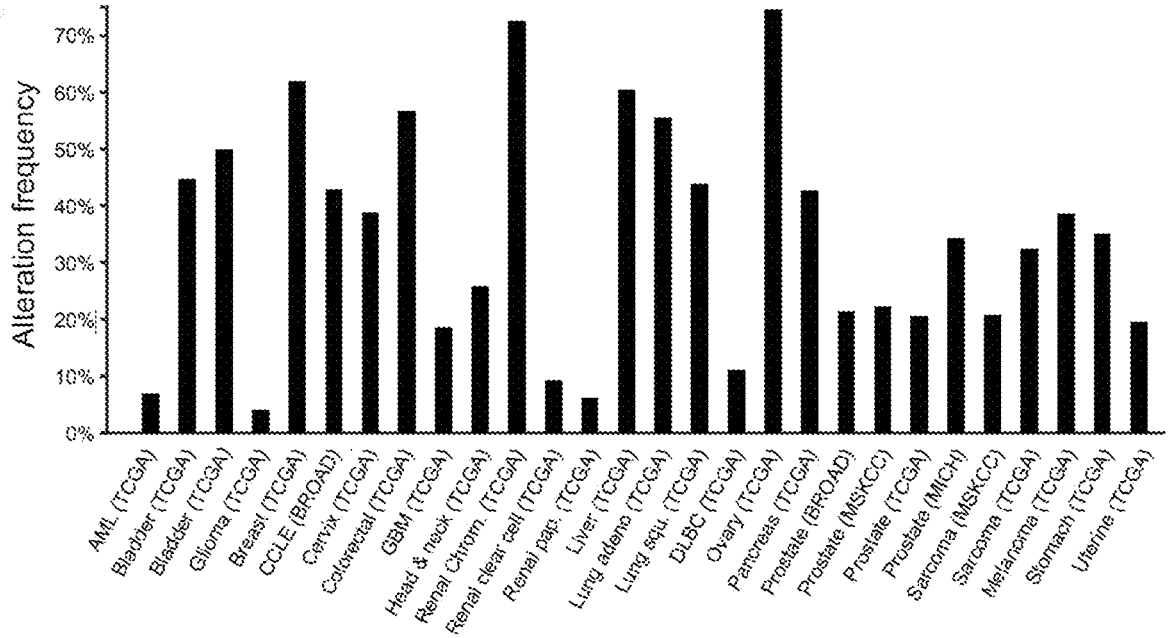

Alteration frequency
70%
60%
50%
40%
30%
20%
10%
0%
AML (TCGA)
Bladder (TCGA)
Bladder (TCGA)
Glioma (TCGA)
Breast (TCGA)
CCLE (BROAD)
Cervix (TCGA)
Colorectal (TCGA)
GBM (TCGA)
Head & neck (TCGA)
Renal Chrom. (TCGA)
Renal clear cell (TCGA)
Renal pap. (TCGA)
Liver (TCGA)
Lung adeno (TCGA)
Lung squ. (TCGA)
DLBC (TCGA)
Ovary (TCGA)
Pancreas (TCGA)
Prostate (BROAD)
Prostate (MSKCC)
Prostate (TCGA)
Prostate (MICH)
Sarcoma (MSKCC)
Sarcoma (TCGA)
Melanoma (TCGA)
Stomach (TCGA)
Uterine (TCGA)

FIG. 1A

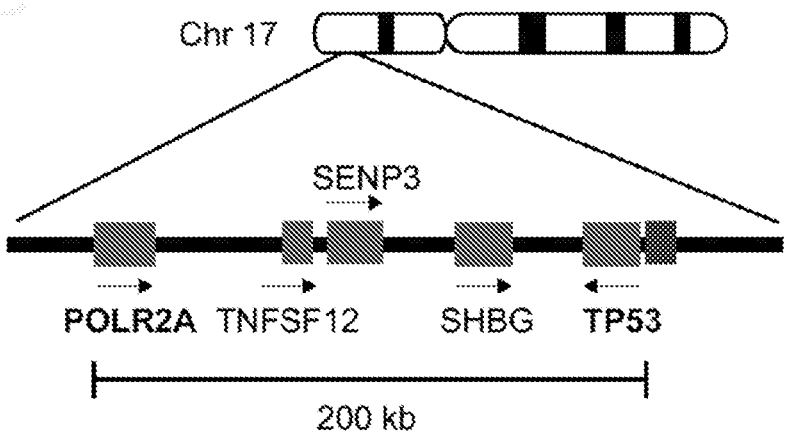

Chr 17
SENP3
POLR2A
TNFSF12
SHBG
TP53
200 kb

FIG. 1B

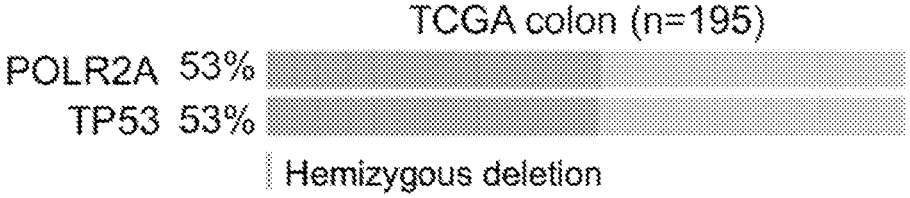

TCGA colon (n=195)
POLR2A 53%
TP53 53%
Hemizygous deletion

FIG. 1C

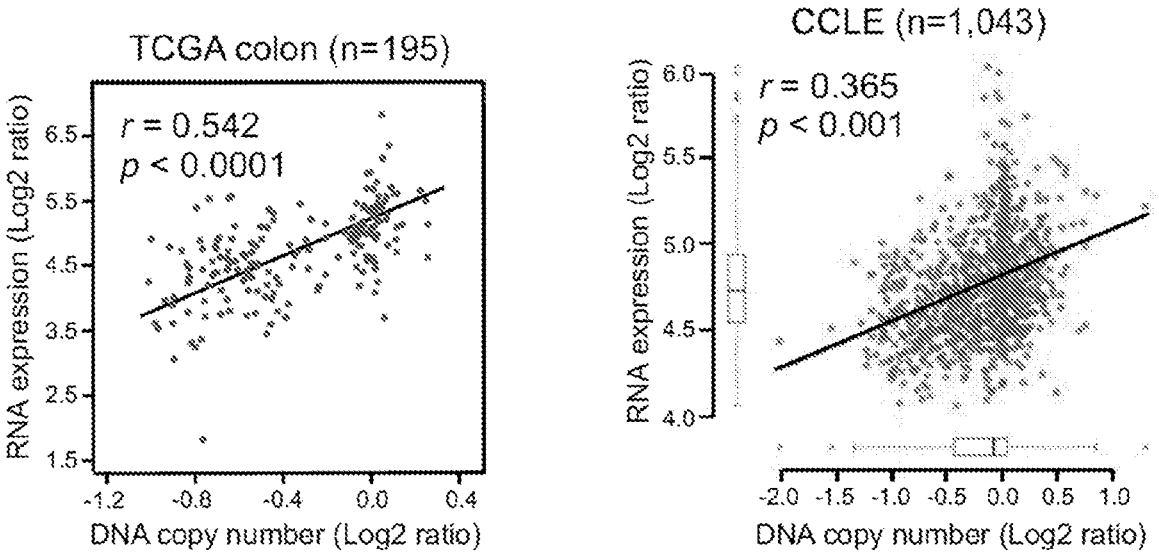


FIG. 1D

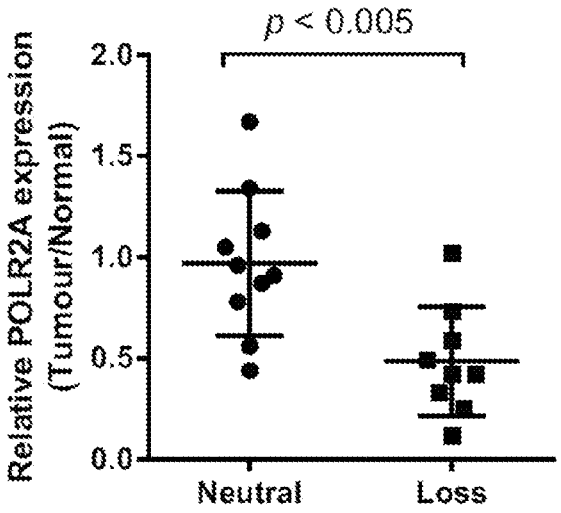


FIG. 1E

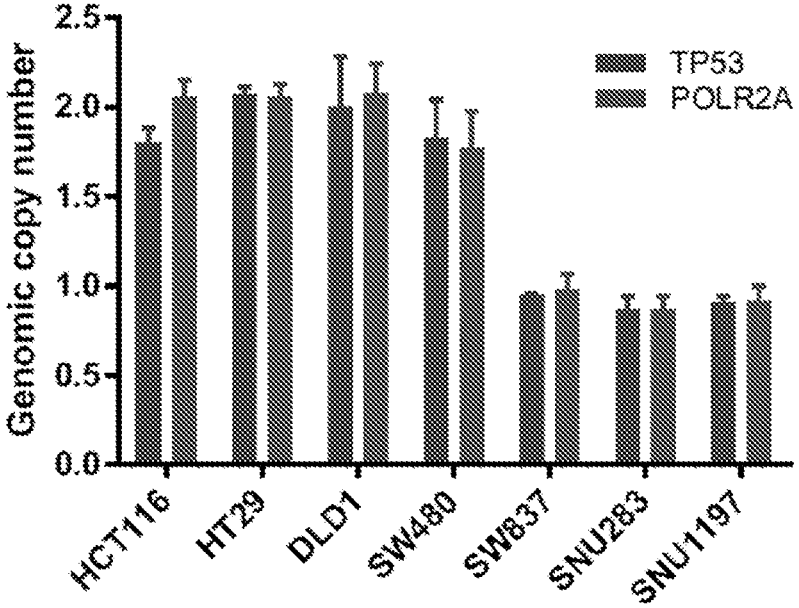

Genomic copy number
2.5
2.0
1.5
1.0
0.5
0.0
TP53
POLR2A
HCT116
HT29
DLD1
SW480
SW837
SNU283
SNU1197

FIG. 1F

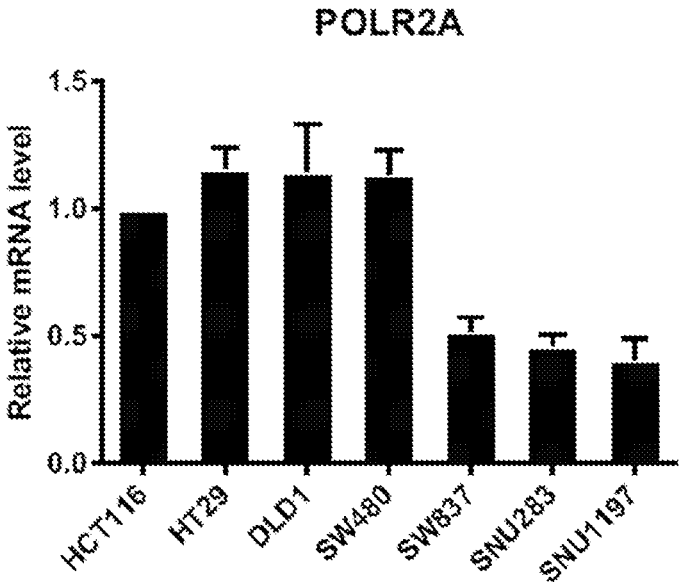

POLR2A
Relative mRNA level
1.5
1.0
0.5
0.0
HCT116
HT29
DLD1
SW480
SW837
SNU283
SNU1197

FIG. 1G

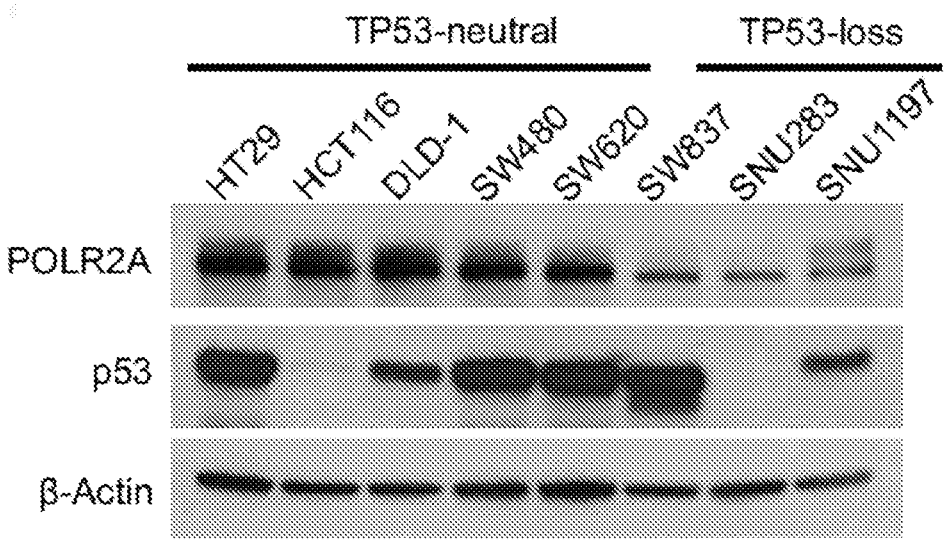

TP53-neutral
TP53-loss
HT29
HCT116
DLD-1
SW480
SW620
SW837
SNU283
SNU1197
POLR2A
p53
β-Actin

FIG. 1H

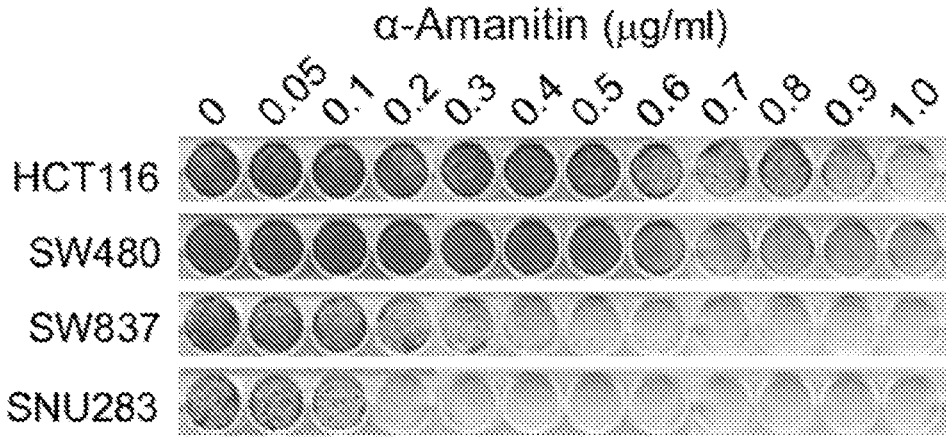

α-Amanitin (μg/ml)
0
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
HCT116
SW480
SW837
SNU283

FIG. 2A

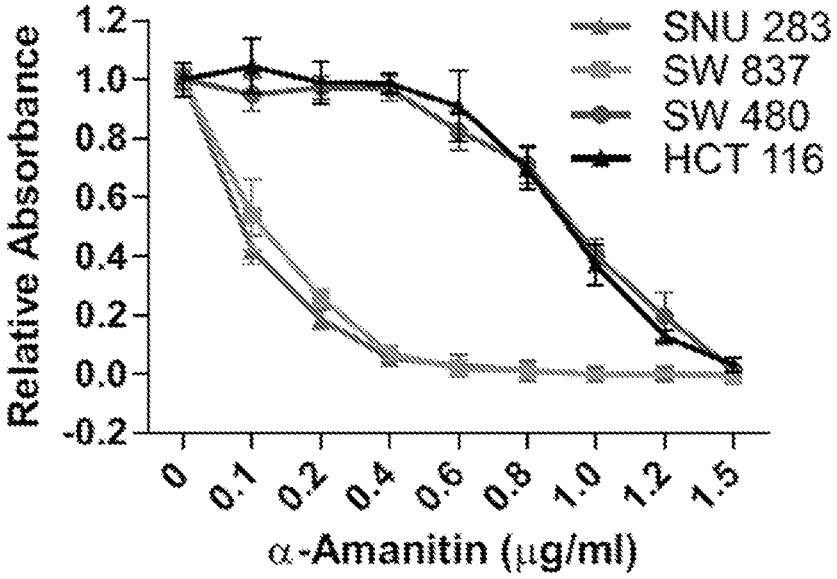

Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.1
0.2
0.4
0.6
0.8
1.0
1.2
1.5
α-Amanitin (μg/ml)
SNU 283
SW 837
SW 480
HCT 116

FIG. 2B

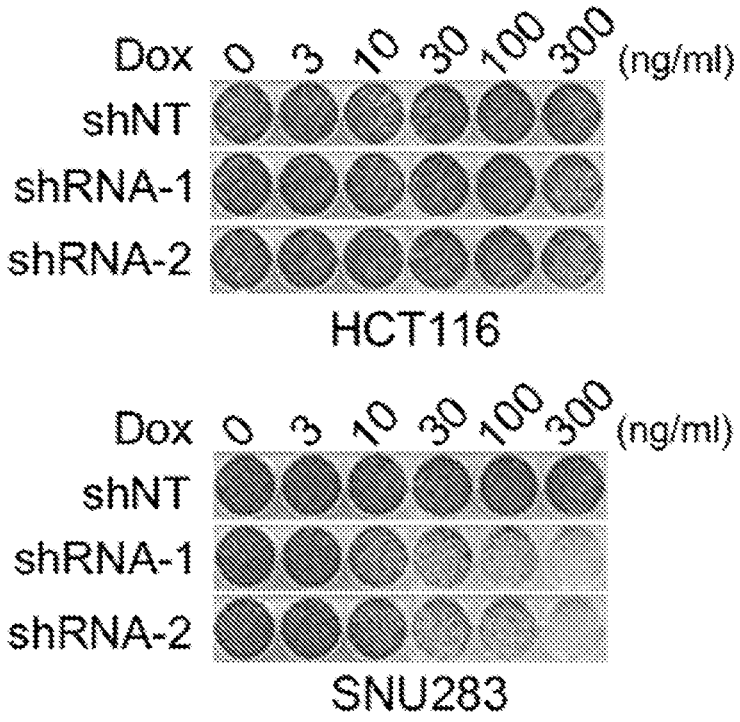

Dox 0 3 10 30 100 300 (ng/ml)
shNT
shRNA-1
shRNA-2
HCT116
Dox 0 3 10 30 100 300 (ng/ml)
shNT
shRNA-1
shRNA-2
SNU283

FIG. 2C

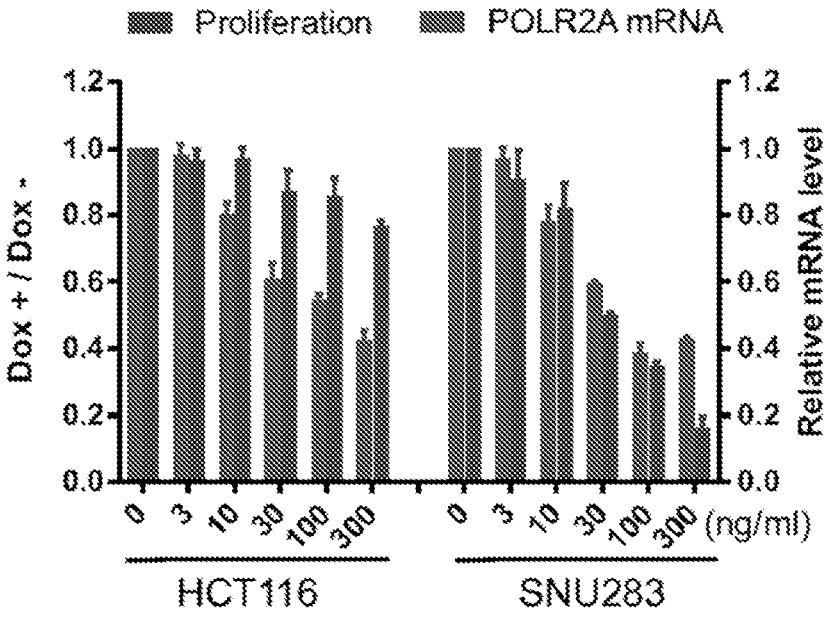

Proliferation
POLR2A mRNA
Dox + / Dox -
Relative mRNA level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 3 10 30 100 300
0 3 10 30 100 300 (ng/ml)
HCT116
SNU283

FIG. 2D

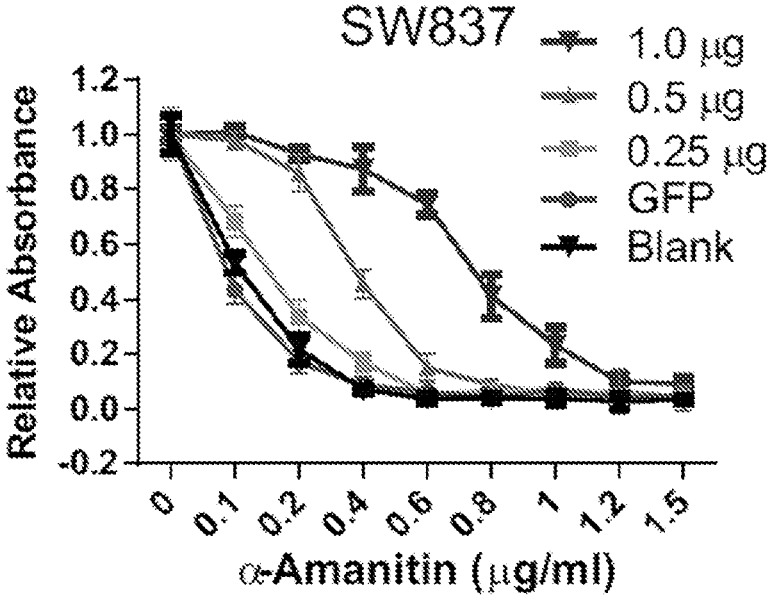

SW837
1.0 μg
0.5 μg
0.25 μg
GFP
Blank
Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.1
0.2
0.4
0.6
0.8
1
1.2
1.5
α-Amanitin (μg/ml)

FIG. 2E

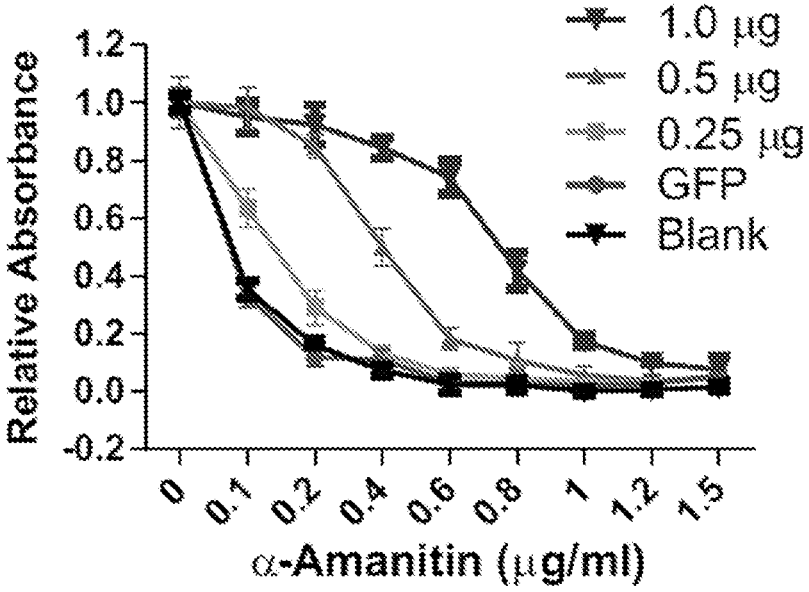

1.0 μg
0.5 μg
0.25 μg
GFP
Blank
Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.1
0.2
0.4
0.6
0.8
1
1.2
1.5
α-Amanitin (μg/ml)

FIG. 2F

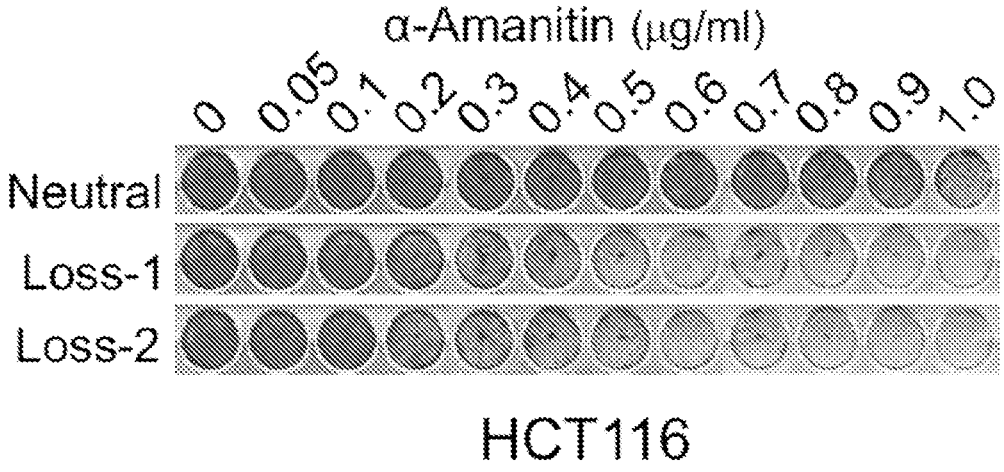

α-Amanitin (μg/ml)
0
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Neutral
Loss-1
Loss-2
HCT116

FIG. 2G

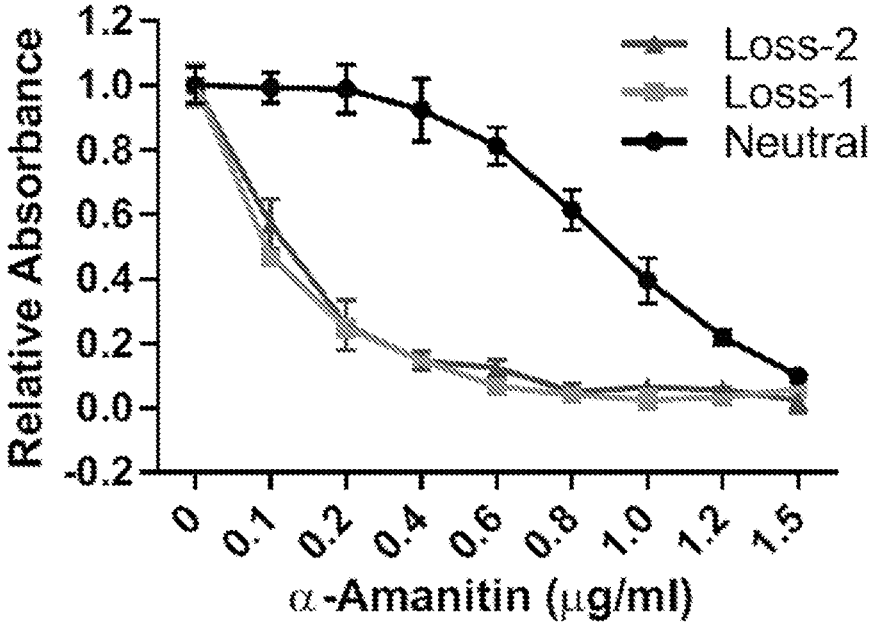

Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Loss-2
Loss-1
Neutral
0
0.1
0.2
0.4
0.6
0.8
1.0
1.2
1.5
α-Amanitin (μg/ml)

FIG. 2H

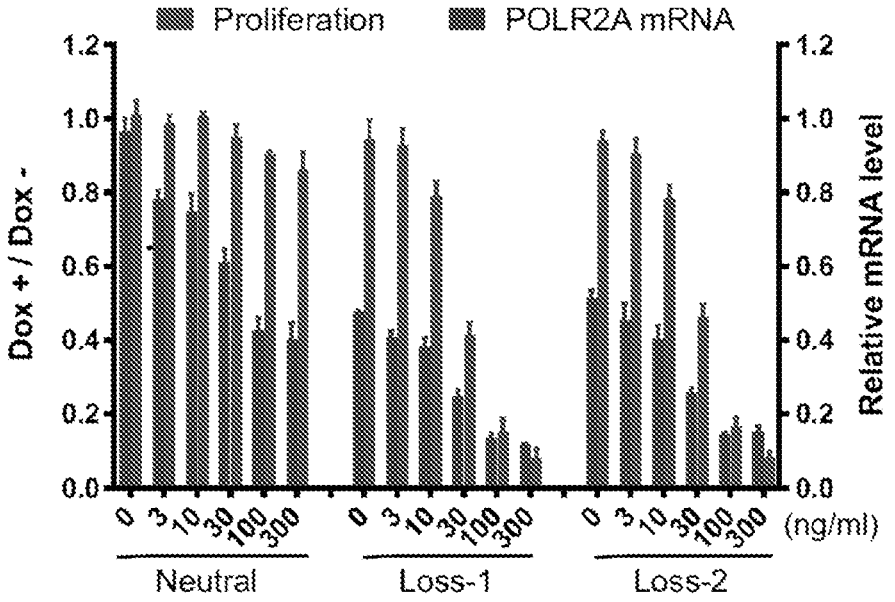

Proliferation
POLR2A mRNA
Dox + / Dox -
Relative mRNA level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 3 10 30 100 300
(ng/ml)
Neutral
Loss-1
Loss-2

FIG. 2I

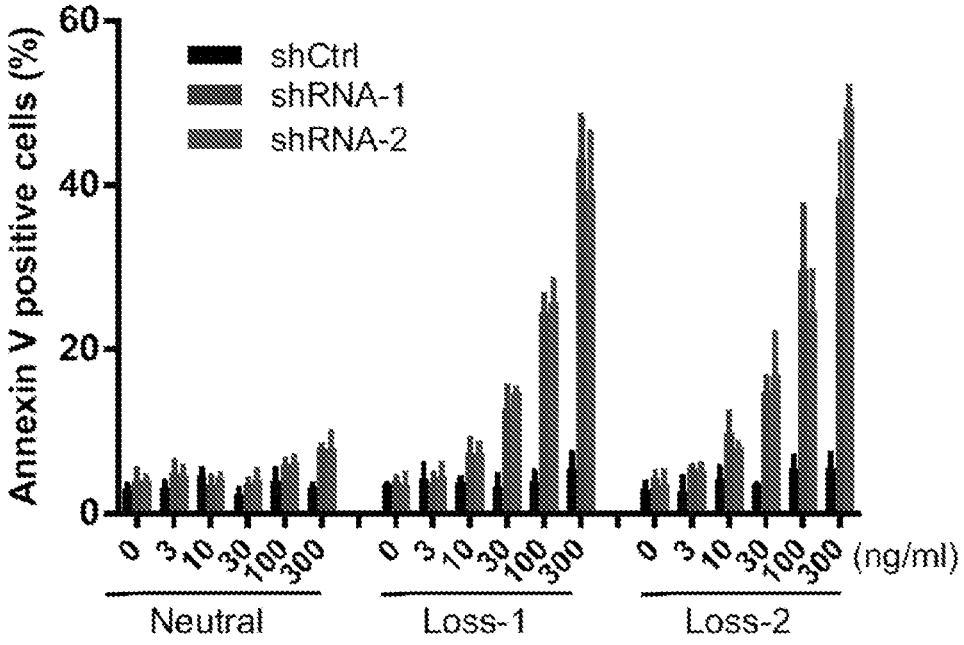

shCtrl
shRNA-1
shRNA-2
Annexin V positive cells (%)
60
40
20
0
0 3 10 30 100 300
(ng/ml)
Neutral
Loss-1
Loss-2

FIG. 2J

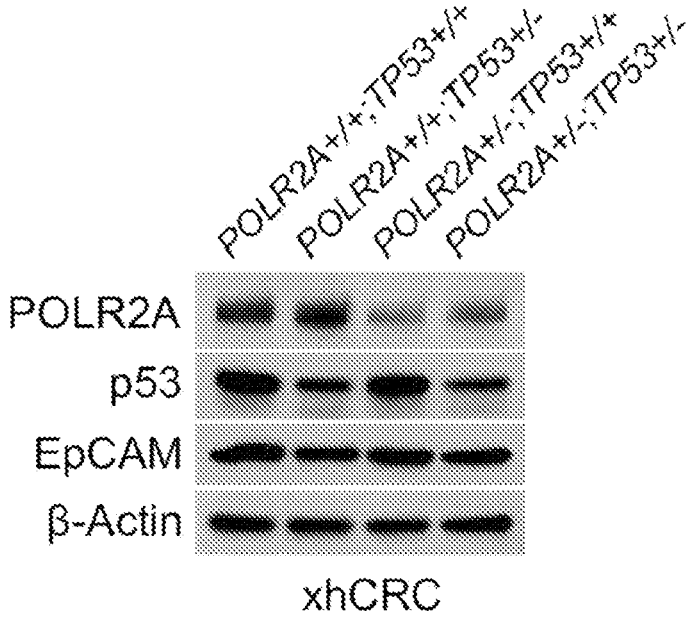

POLR2A+/+;TP53+/+
POLR2A+/+;TP53+/-
POLR2A+/-;TP53+/+
POLR2A+/-;TP53+/-
POLR2A
p53
EpCAM
β-Actin
xhCRC

FIG. 3A

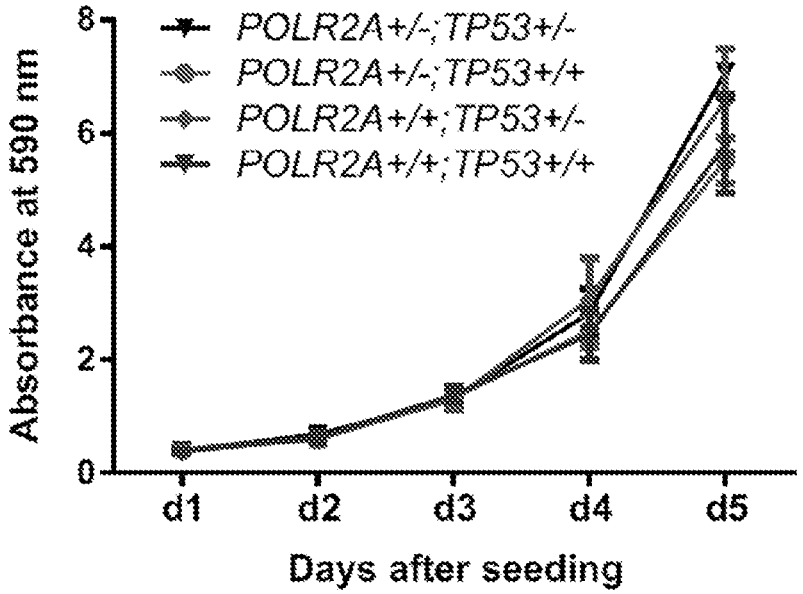

POLR2A+/-;TP53+/-
POLR2A+/-;TP53+/+
POLR2A+/+;TP53+/-
POLR2A+/+;TP53+/+
Absorbance at 590 nm
0
2
4
6
8
d1
d2
d3
d4
d5
Days after seeding

FIG. 3B

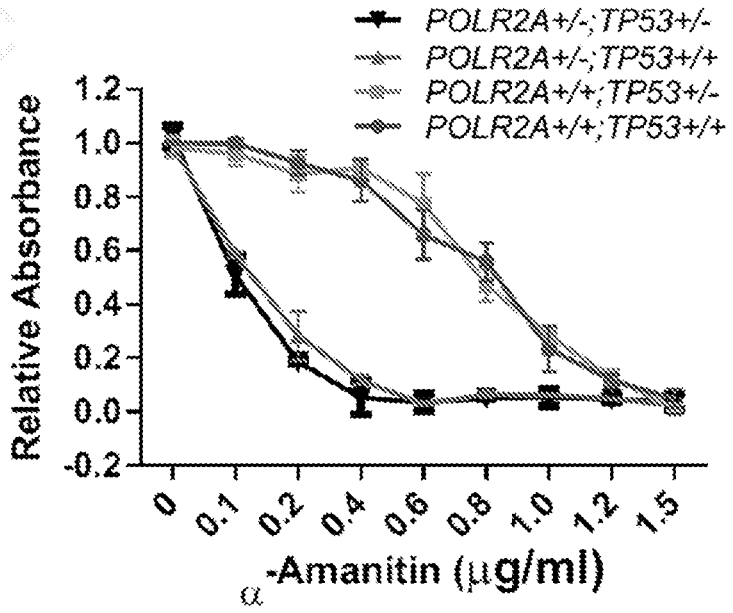

POLR2A+/-;TP53+/-
POLR2A+/-;TP53+/+
POLR2A+/+;TP53+/-
POLR2A+/+;TP53+/+
Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.1
0.2
0.4
0.6
0.8
1.0
1.2
1.5
α-Amanitin (μg/ml)

FIG. 3C

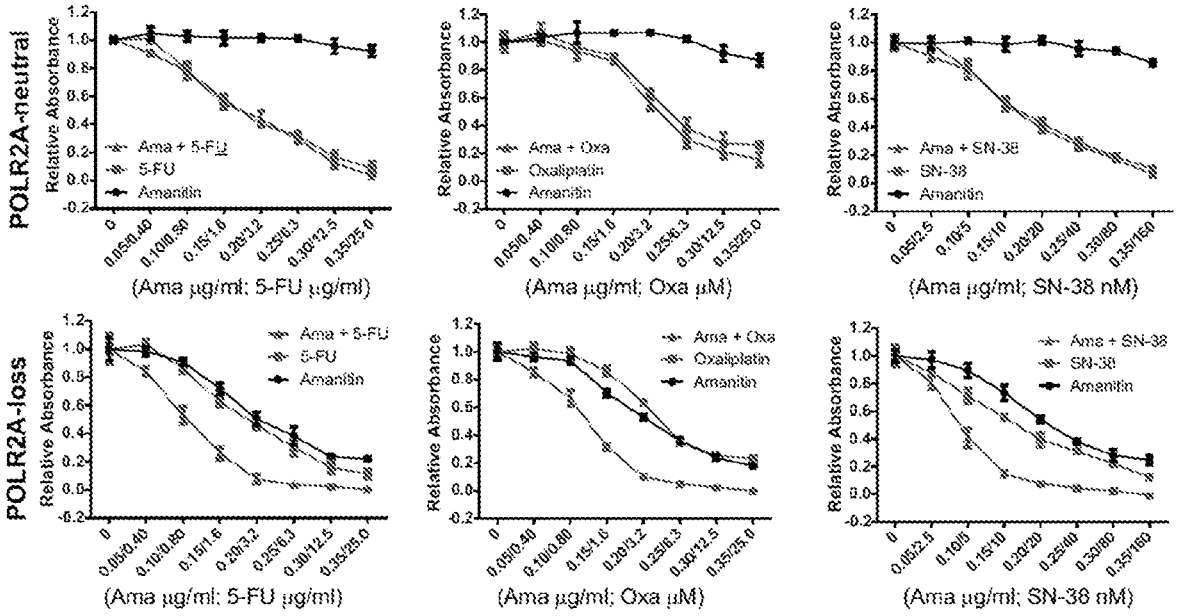

POLR2A-neutral
POLR2A-loss
Relative Absorbance
Ama + 5-FU
5-FU
Amanitin
Ama + Oxa
Oxaliplatin
Ama + SN-38
SN-38
(Ama μg/ml; 5-FU μg/ml)
(Ama μg/ml; Oxa μM)
(Ama μg/ml; SN-38 nM)

FIG. 3D

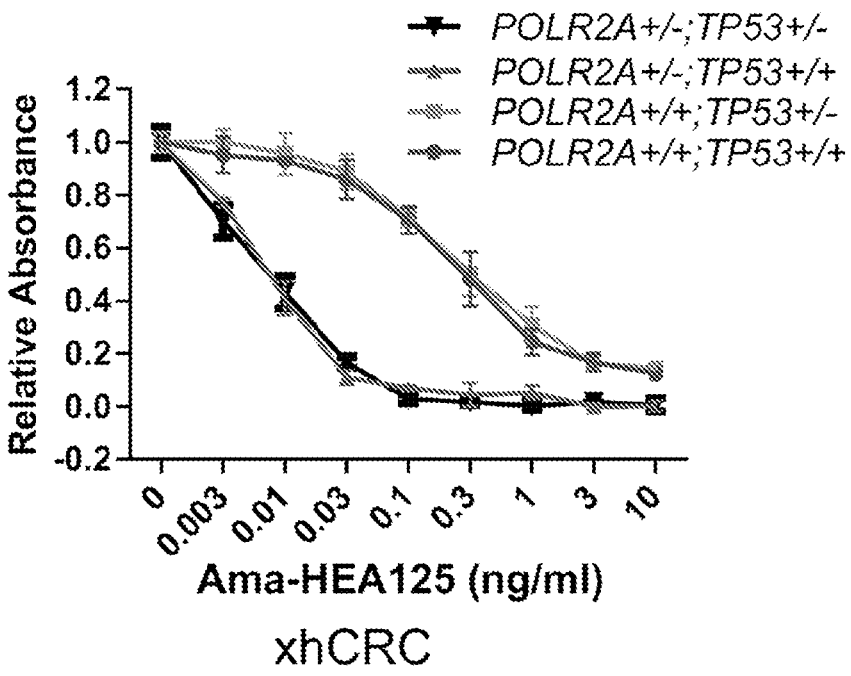

POLR2A+/-;TP53+/-
POLR2A+/-;TP53+/+
POLR2A+/+;TP53+/-
POLR2A+/+;TP53+/+
Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.003
0.01
0.03
0.1
0.3
1
3
10
Ama-HEA125 (ng/ml)
xhCRC

FIG. 3E

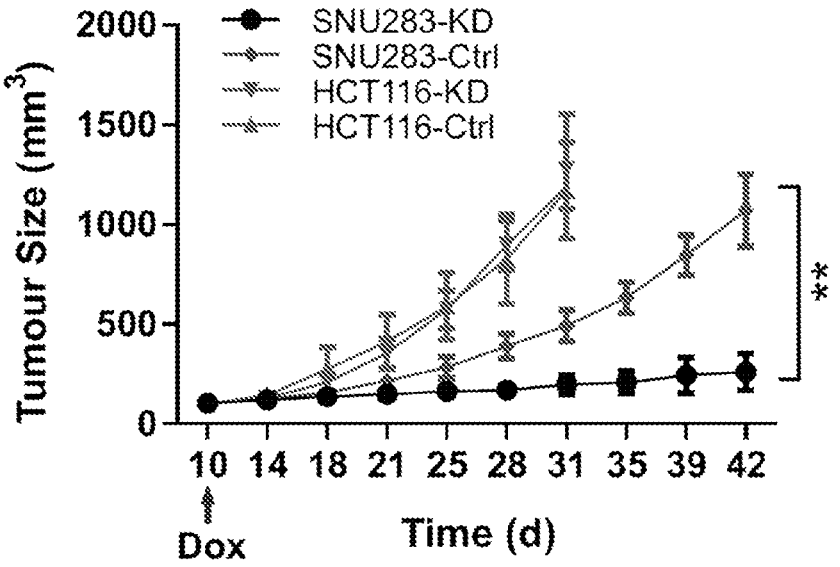

SNU283-KD
SNU283-Ctrl
HCT116-KD
HCT116-Ctrl
Tumour Size (mm3)
2000
1500
1000
500
0
10 14 18 21 25 28 31 35 39 42
Dox
Time (d)
**

FIG. 4A

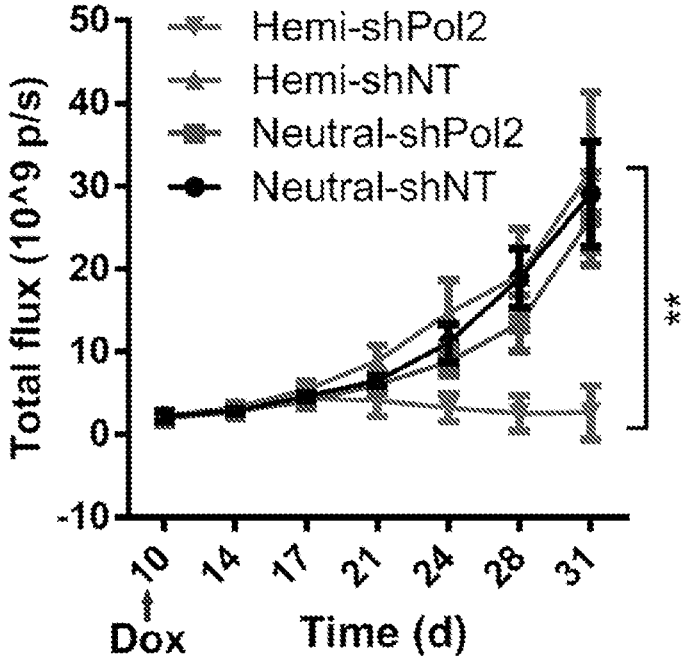

Hemi-shPol2
Hemi-shNT
Neutral-shPol2
Neutral-shNT
Total flux (10^9 p/s)
50
40
30
20
10
0
-10
10 14 17 21 24 28 31
Dox
Time (d)
**

FIG. 4B

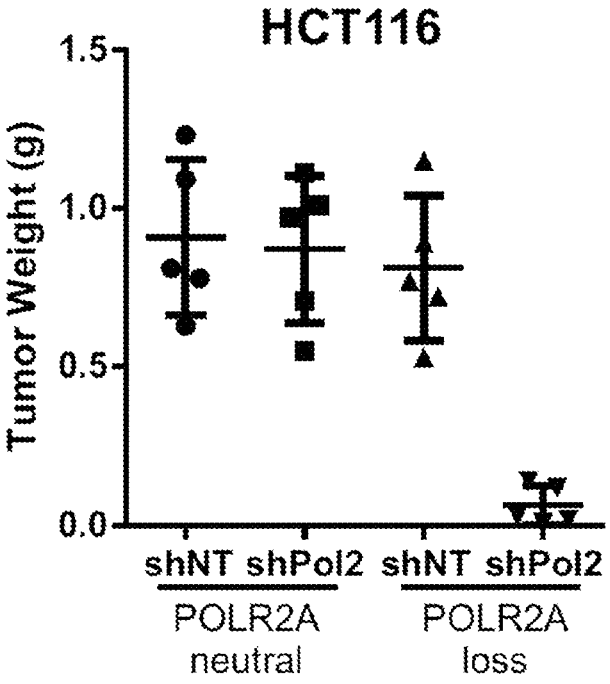

HCT116
Tumor Weight (g)
1.5
1.0
0.5
0.0
shNT shPol2 shNT shPol2
POLR2A neutral
POLR2A loss

FIG. 4C

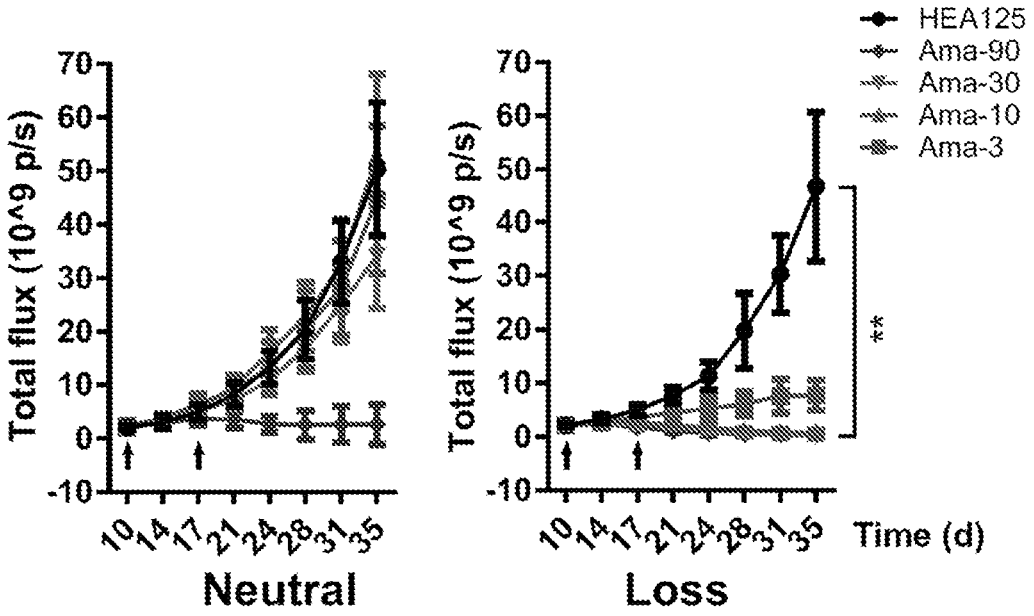

HEA125
Ama-90
Ama-30
Ama-10
Ama-3
Total flux (10^9 p/s)
70
60
50
40
30
20
10
0
-10
10 14 17 21 24 28 31 35
Time (d)
Neutral
Loss
**

FIG. 4D

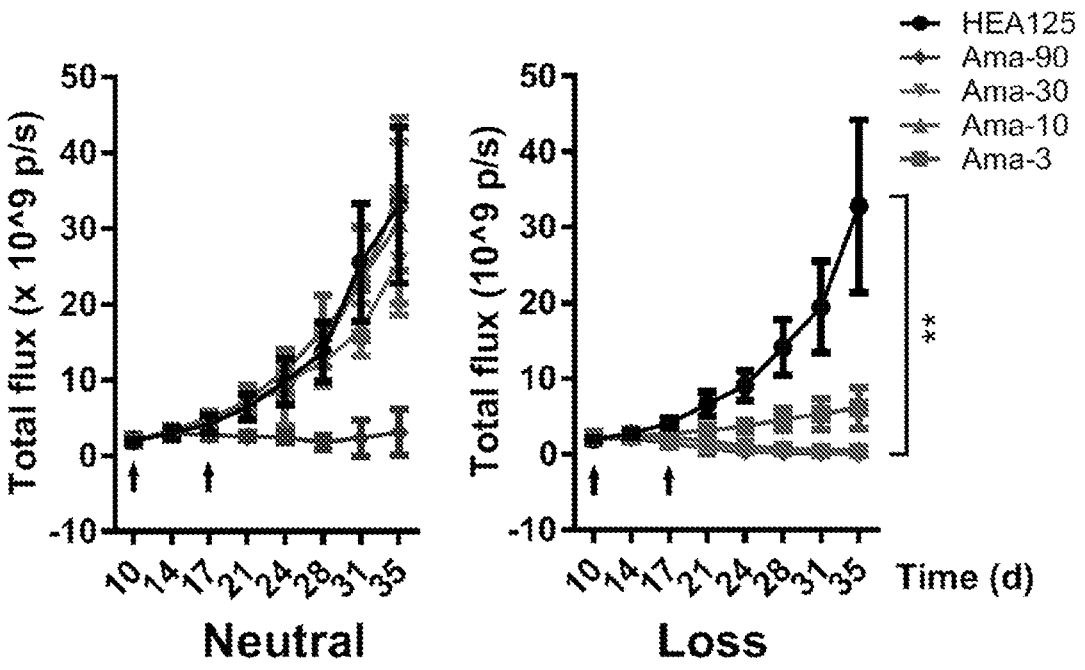

HEA125
Ama-90
Ama-30
Ama-10
Ama-3
Total flux (x 10^9 p/s)
Total flux (10^9 p/s)
50
40
30
20
10
0
-10
10
14
17
21
24
28
31
35
Time (d)
**
Neutral
Loss

FIG. 4E

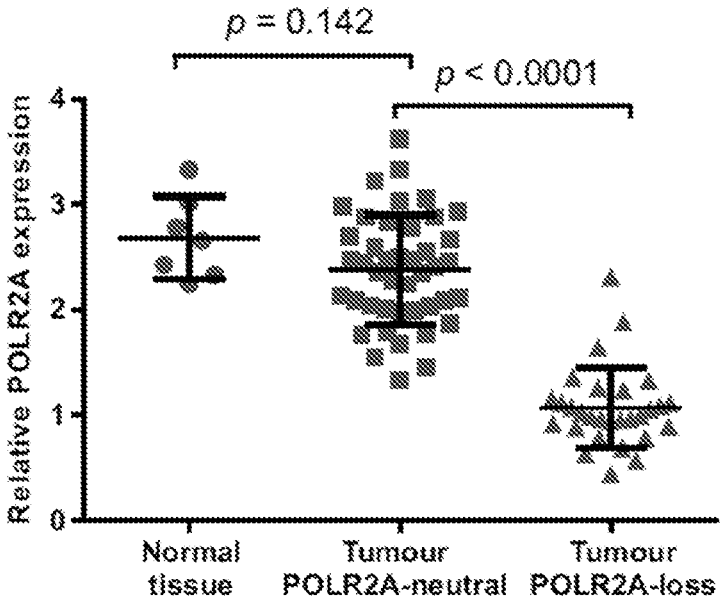

p = 0.142
p < 0.0001
Relative POLR2A expression
0
1
2
3
4
Normal tissue
Tumour POLR2A-neutral
Tumour POLR2A-loss

FIG. 5A

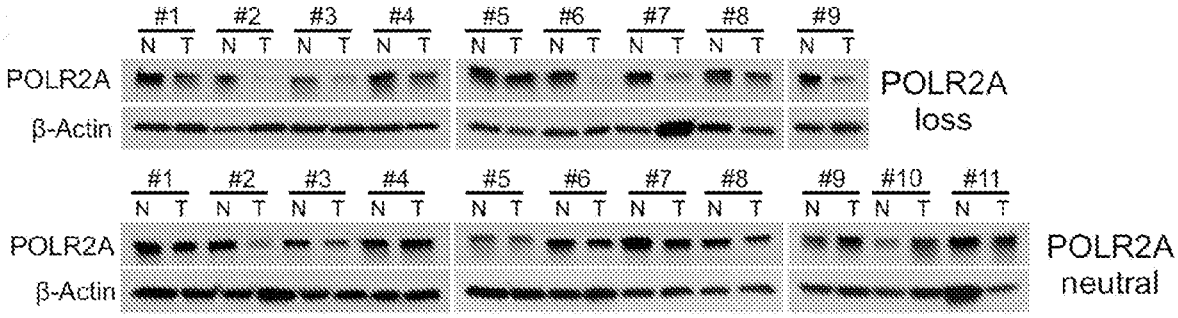

#1 #2 #3 #4 #5 #6 #7 #8 #9
N T N T N T N T N T N T N T N T N T
POLR2A
β-Actin
POLR2A loss
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11
N T N T N T N T N T N T N T N T N T N T N T
POLR2A
β-Actin
POLR2A neutral

FIG. 5B

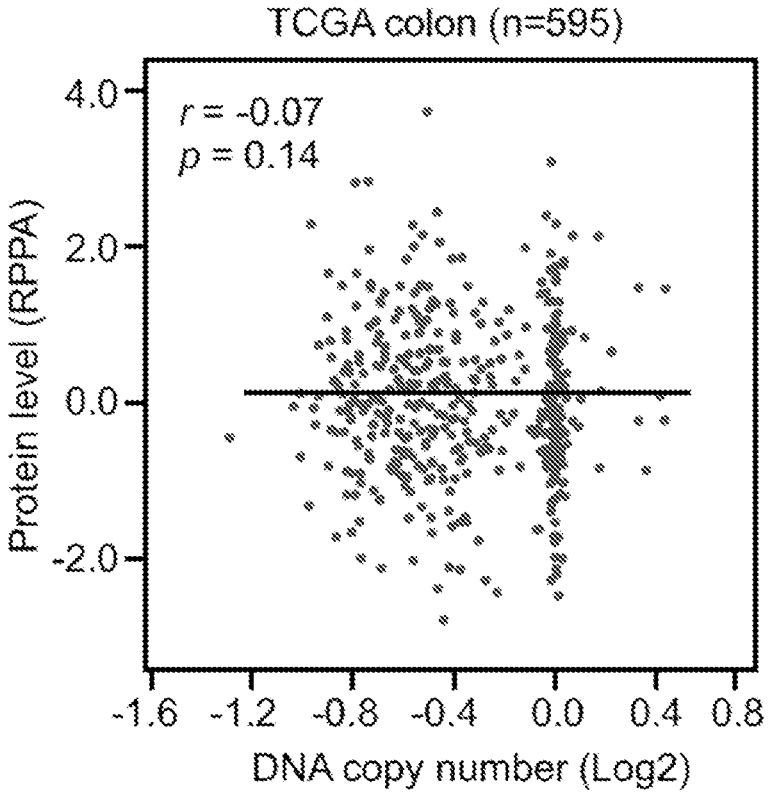

TCGA colon (n=595)
r = -0.07
p = 0.14
Protein level (RPPA)
4.0
2.0
0.0
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
DNA copy number (Log2)

FIG. 6A

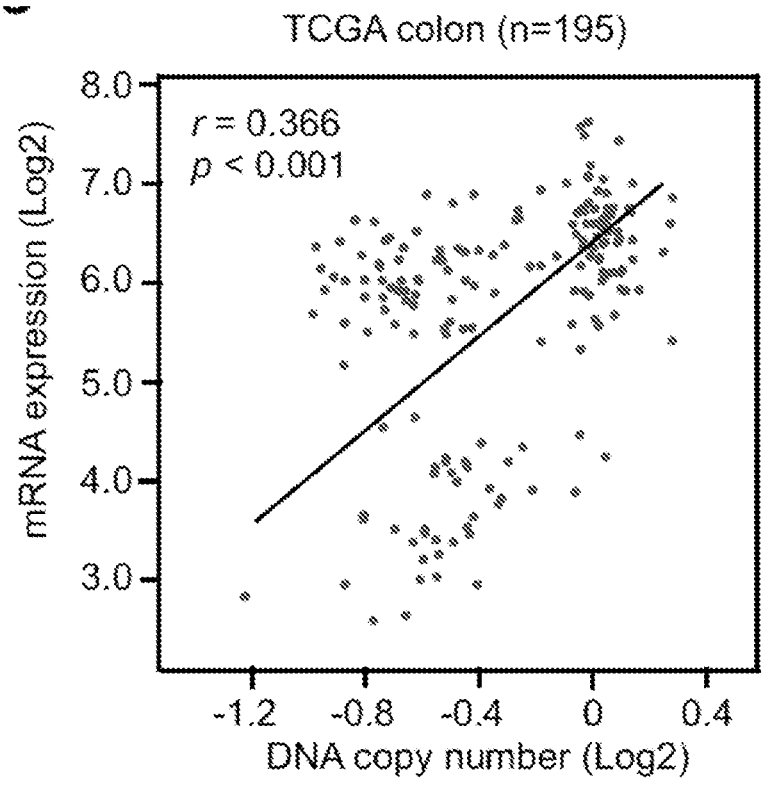

TCGA colon (n=195)
r = 0.366
p < 0.001
mRNA expression (Log2)
8.0
7.0
6.0
5.0
4.0
3.0
-1.2
-0.8
-0.4
0
0.4
DNA copy number (Log2)

FIG. 6B

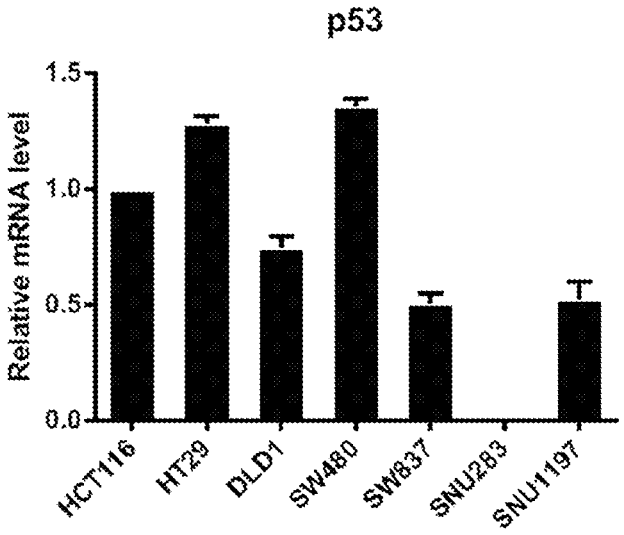

p53
Relative mRNA level
1.5
1.0
0.5
0.0
HCT116
HT29
DLD1
SW480
SW837
SNU283
SNU1197

FIG. 6C

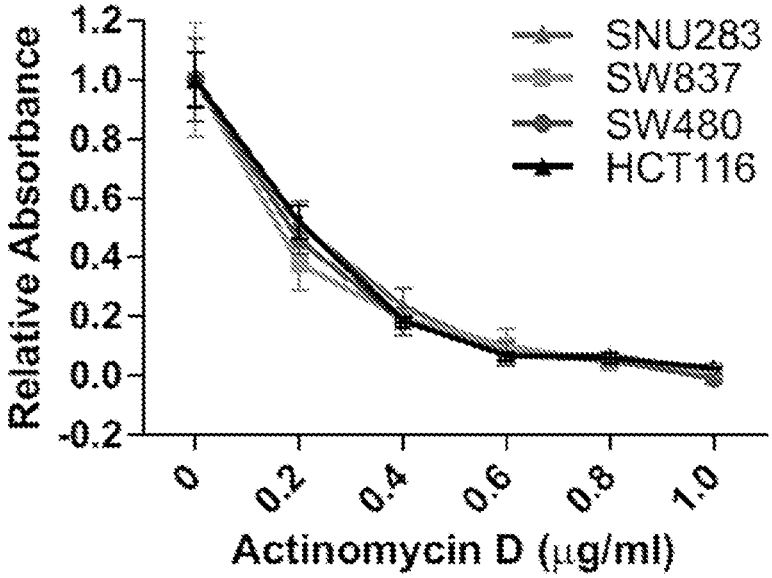

Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.2
0.4
0.6
0.8
1.0
Actinomycin D (μg/ml)
SNU283
SW837
SW480
HCT116

FIG. 7A

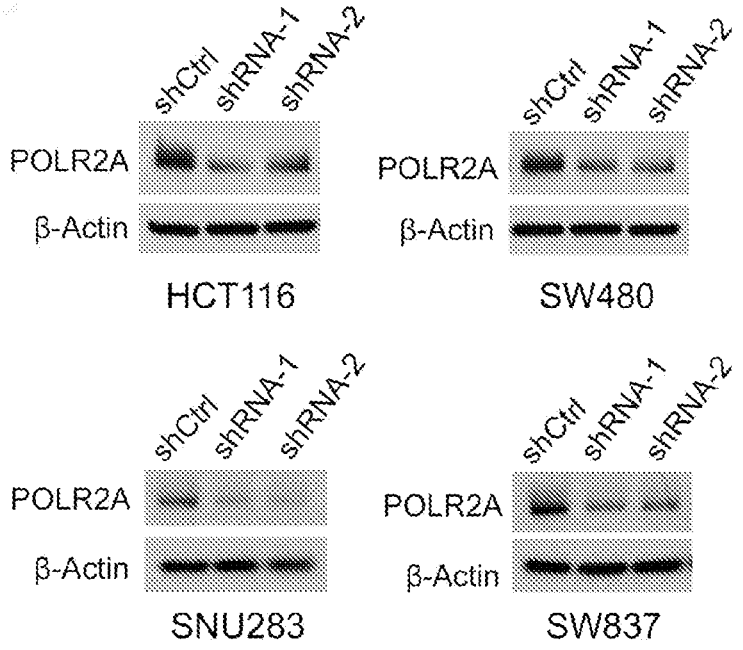

shCtrl
shRNA-1
shRNA-2
POLR2A
β-Actin
HCT116
SW480
SNU283
SW837

FIG. 7B

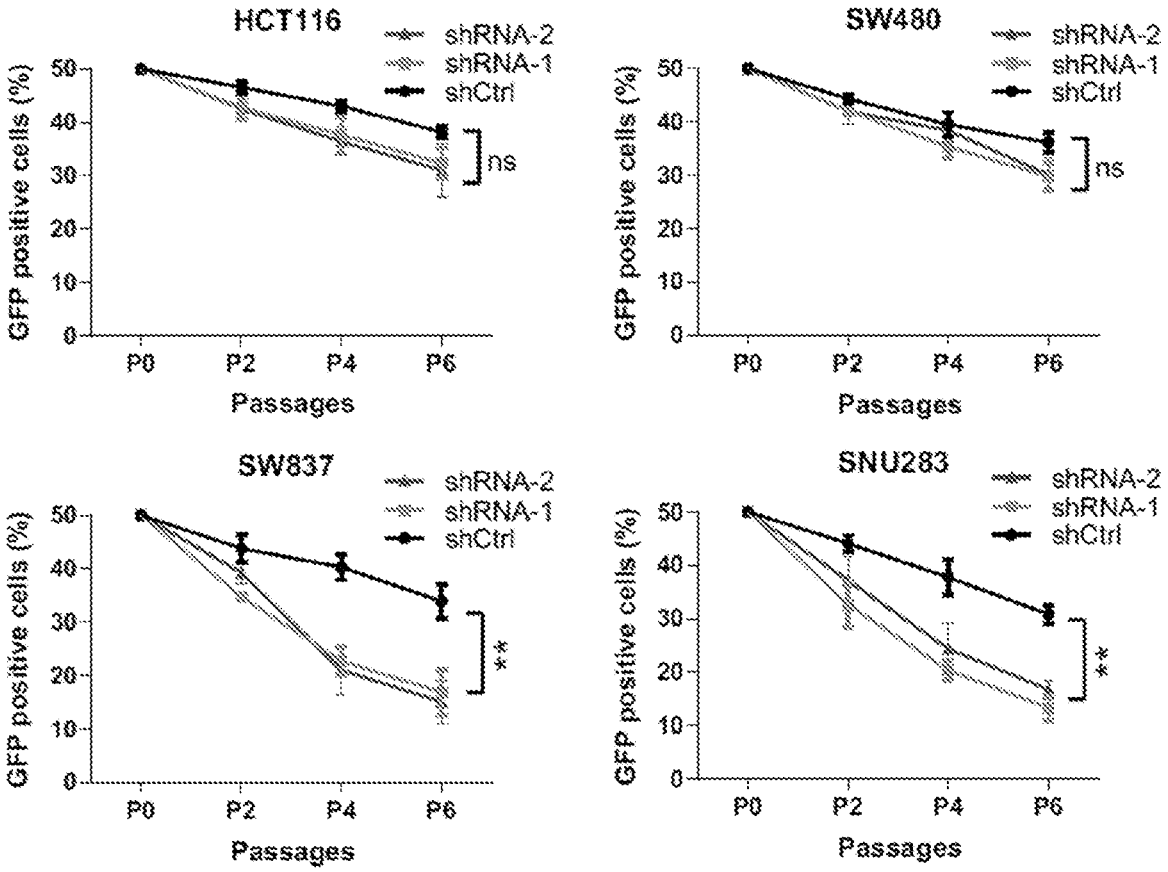

HCT116
shRNA-2
shRNA-1
shCtrl
GFP positive cells (%)
50
40
30
20
10
0
ns
P0
P2
P4
P6
Passages
SW480
shRNA-2
shRNA-1
shCtrl
GFP positive cells (%)
ns
Passages
SW837
shRNA-2
shRNA-1
shCtrl
GFP positive cells (%)
**
Passages
SNU283
shRNA-2
shRNA-1
shCtrl
GFP positive cells (%)
**
Passages

FIG. 7C

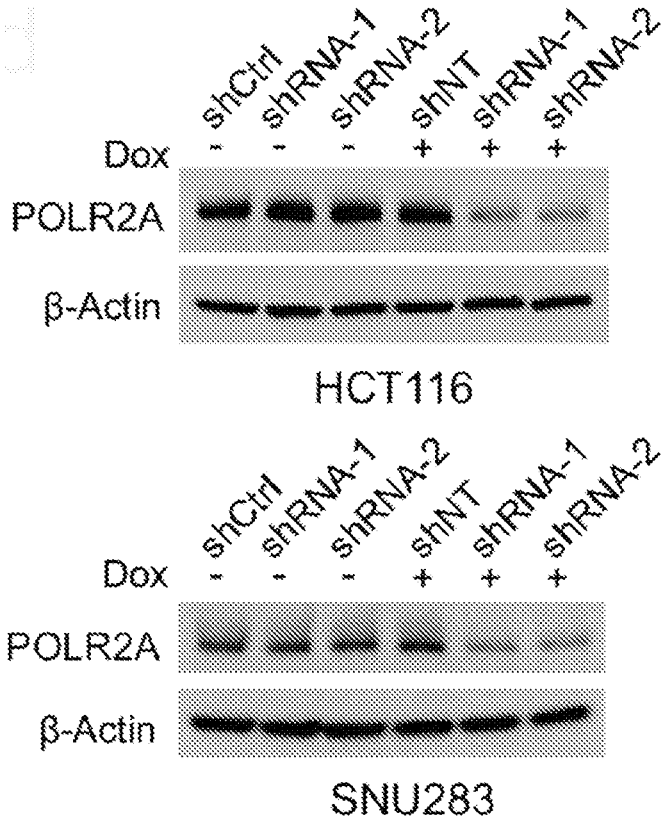

shCtrl
shRNA-1
shRNA-2
shNT
shRNA-1
shRNA-2
Dox
- - - + + +
POLR2A
β-Actin
HCT116
shCtrl
shRNA-1
shRNA-2
shNT
shRNA-1
shRNA-2
Dox
- - - + + +
POLR2A
β-Actin
SNU283

FIG. 7D

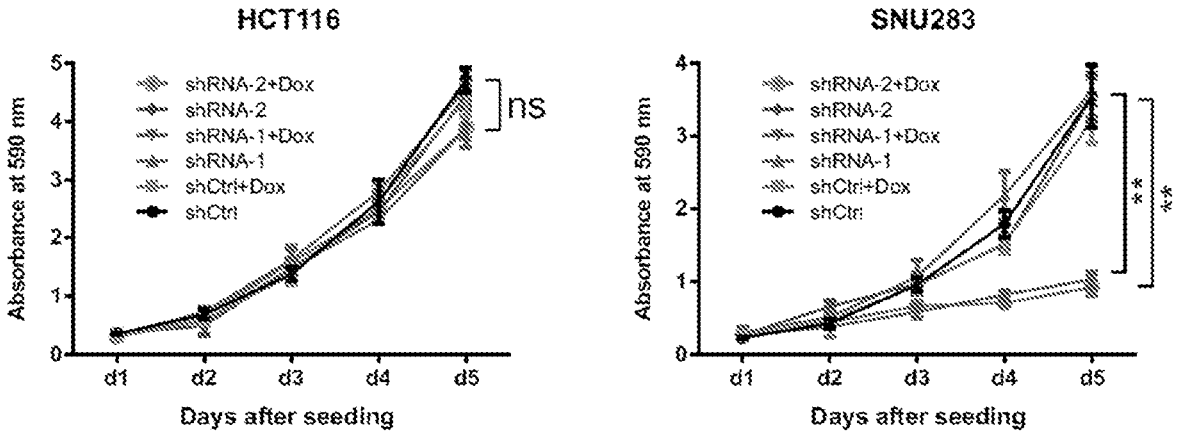

HCT116
shRNA-2+Dox
shRNA-2
shRNA-1+Dox
shRNA-1
shCtrl+Dox
shCtrl
ns
Absorbance at 590 nm
0
1
2
3
4
5
d1
d2
d3
d4
d5
Days after seeding
SNU283
shRNA-2+Dox
shRNA-2
shRNA-1+Dox
shRNA-1
shCtrl+Dox
shCtrl
**
**
Absorbance at 590 nm
0
1
2
3
4
d1
d2
d3
d4
d5
Days after seeding

FIG. 7E

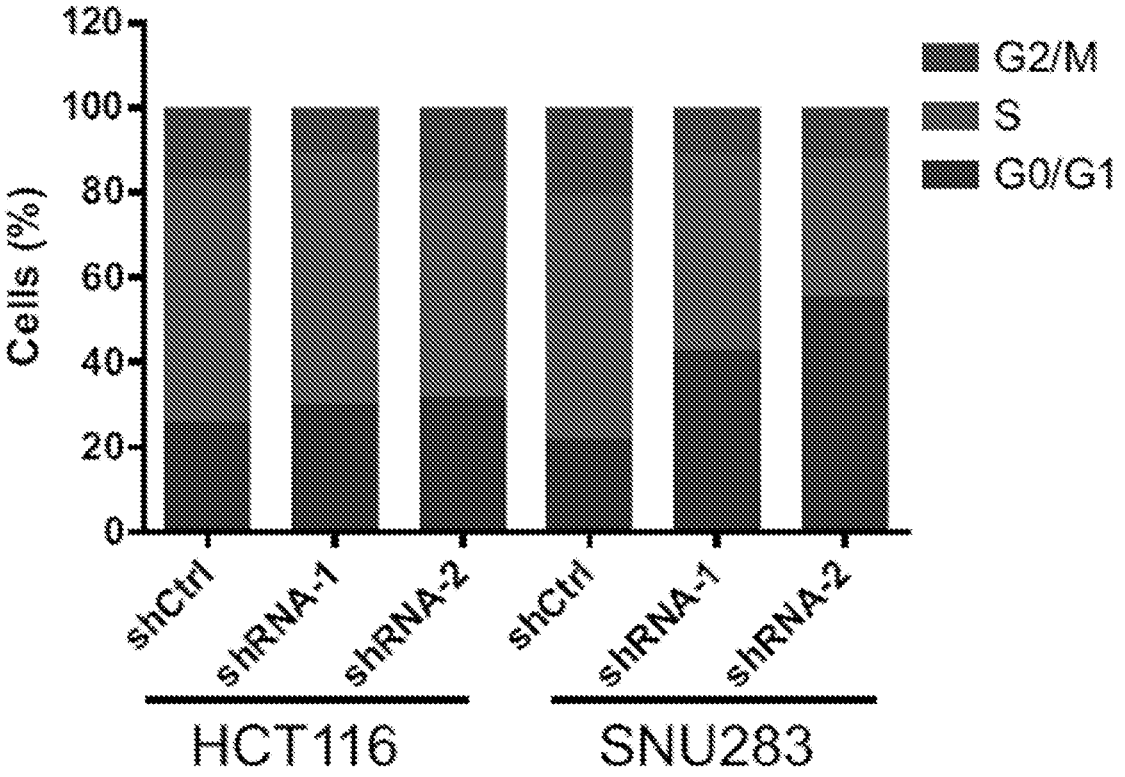

120
100
80
60
40
20
0
Cells (%)
G2/M
S
G0/G1
shCtrl
shRNA-1
shRNA-2
shCtrl
shRNA-1
shRNA-2
HCT116
SNU283

FIG. 7F

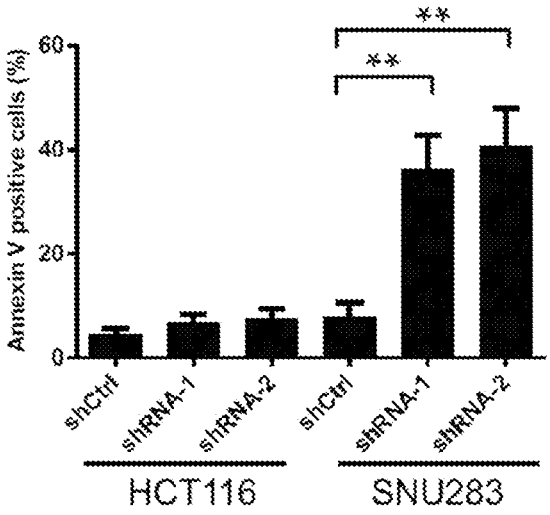

**
**
60
40
20
0
Annexin V positive cells (%)
shCtrl
shRNA-1
shRNA-2
shCtrl
shRNA-1
shRNA-2
HCT116
SNU283

FIG. 7G

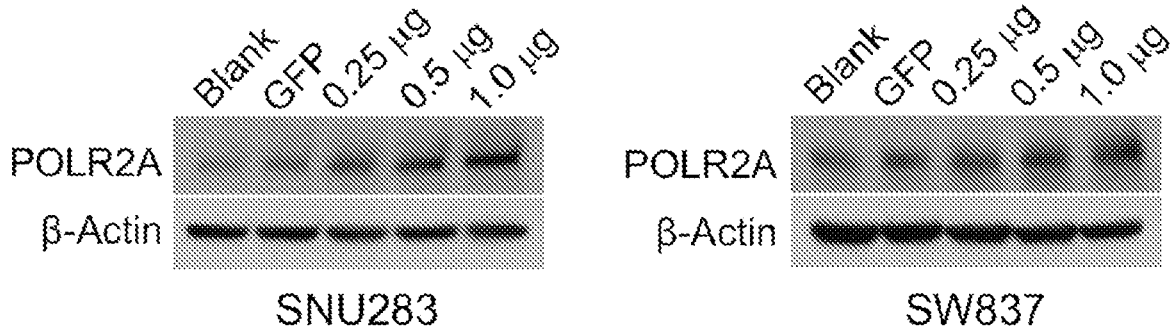

Blank
GFP
0.25 μg
0.5 μg
1.0 μg
POLR2A
β-Actin
SNU283
SW837

FIG. 8A

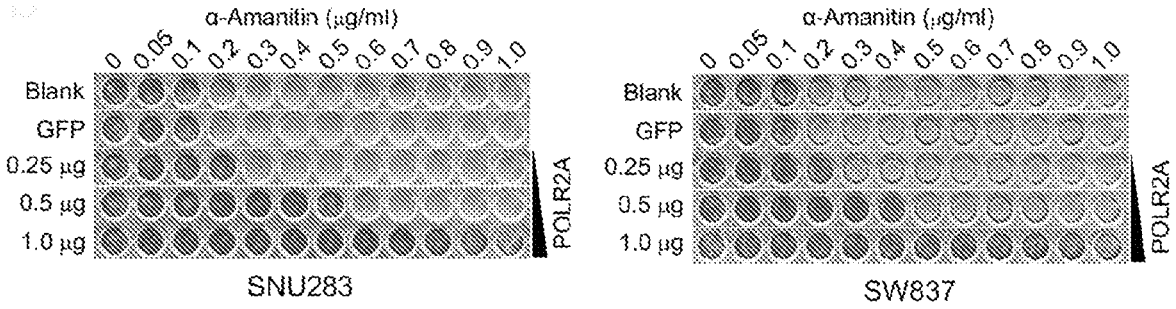

α-Amanitin (μg/ml)
0
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Blank
GFP
0.25 μg
0.5 μg
1.0 μg
POLR2A
SNU283
SW837

FIG. 8B

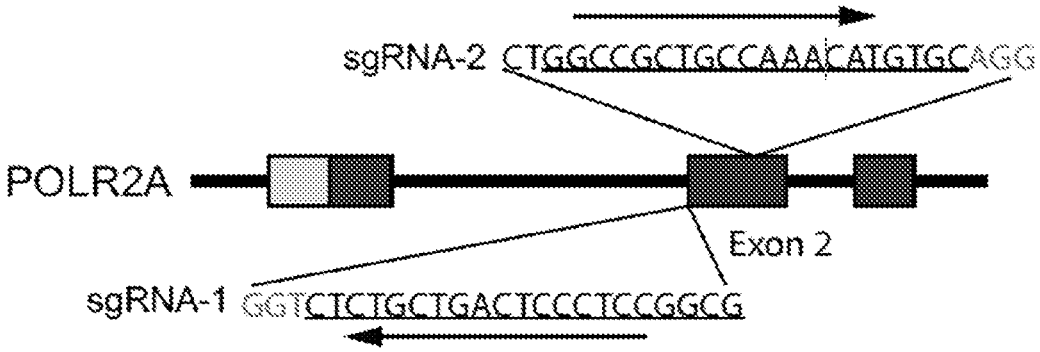


FIG. 9A

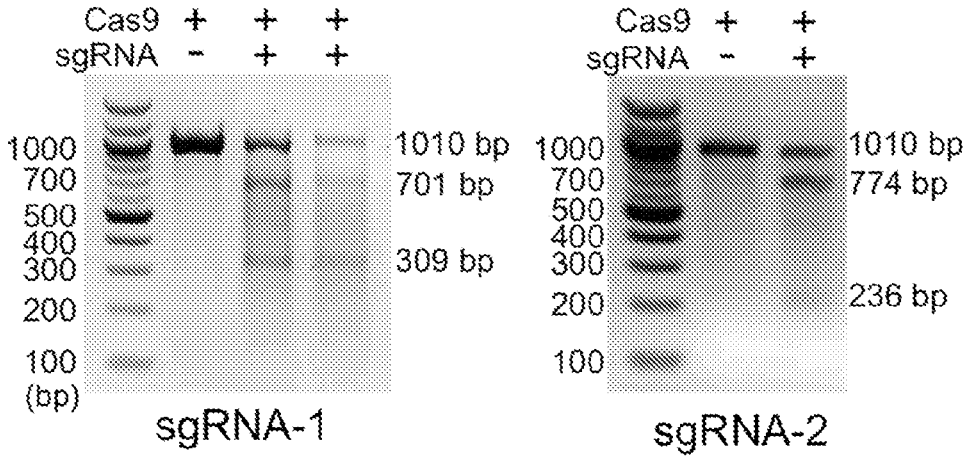


FIG. 9B

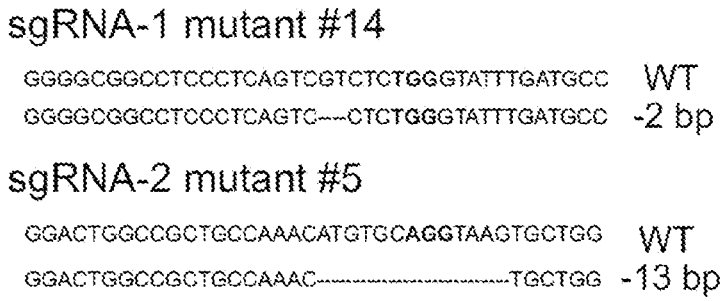


FIG. 9C

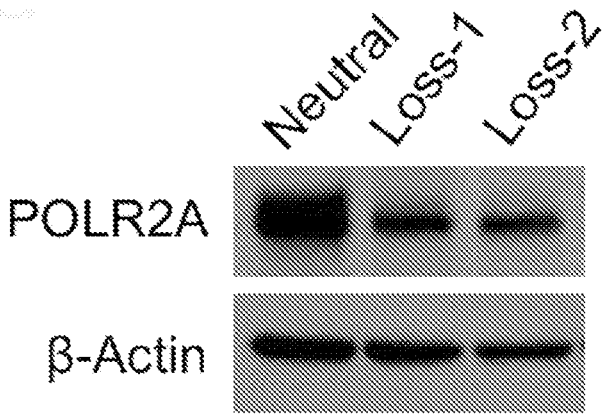

Neutral
Loss-1
Loss-2
POLR2A
β-Actin

FIG. 9D

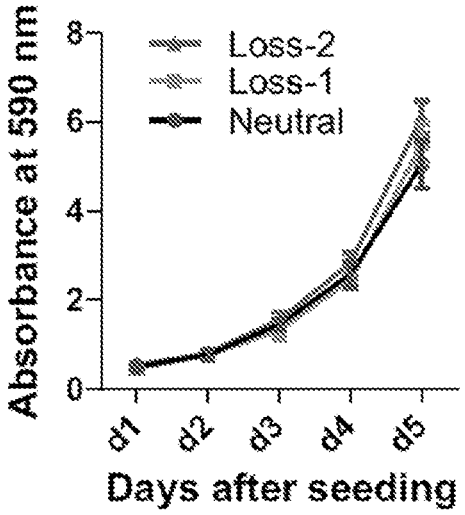

Absorbance at 590 nm
0
2
4
6
8
Loss-2
Loss-1
Neutral
d1
d2
d3
d4
d5
Days after seeding

FIG. 9E

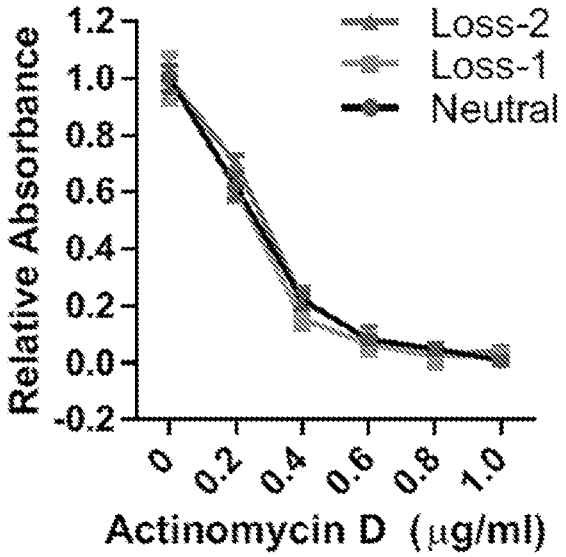


FIG. 9F

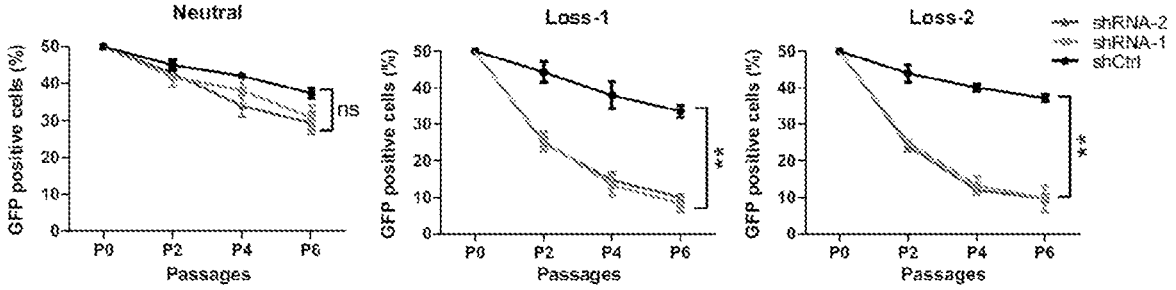


FIG. 9G

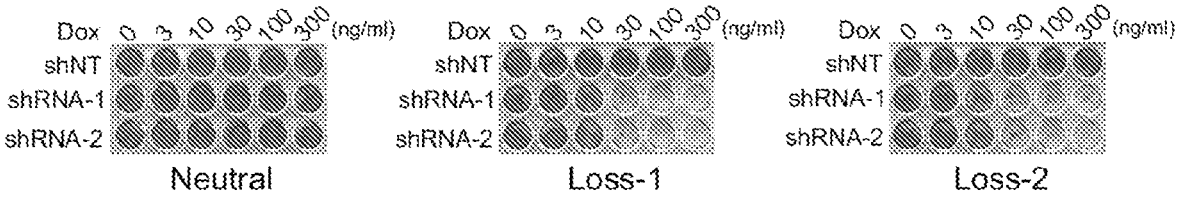


FIG. 9H

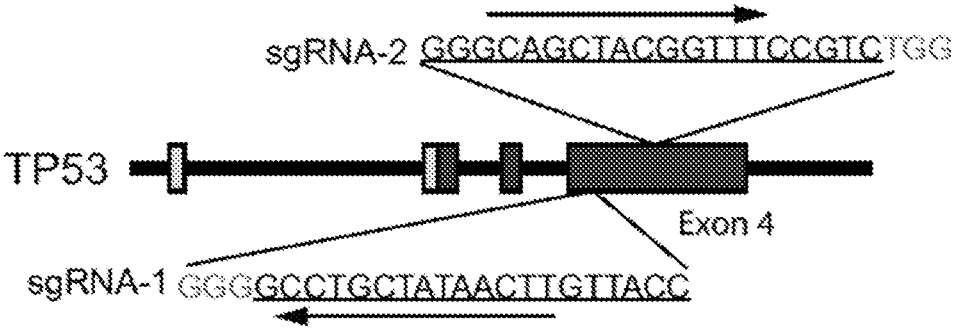


FIG. 10A

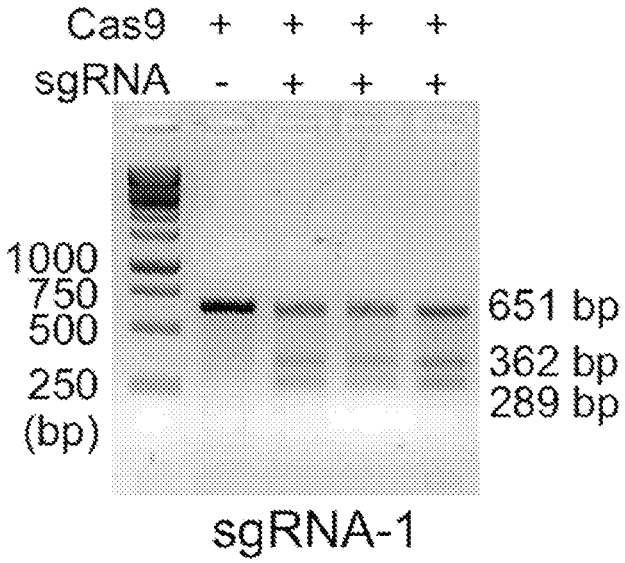


FIG. 10B

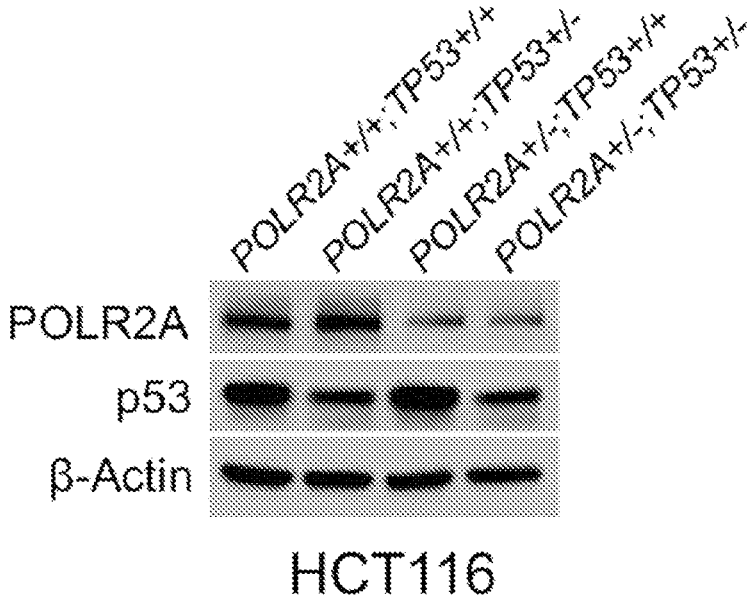


FIG. 10C

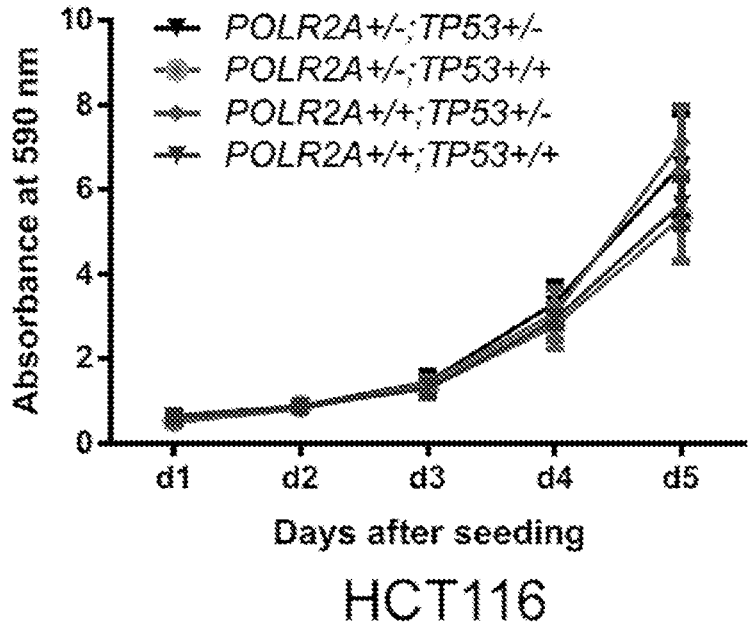


FIG. 10D

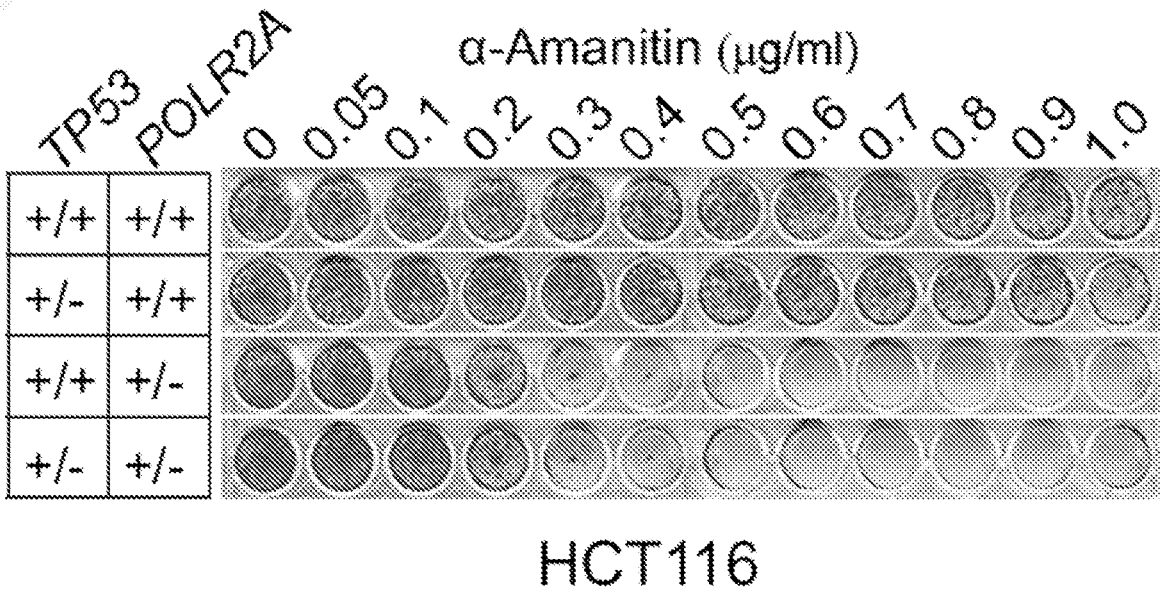


FIG. 10E

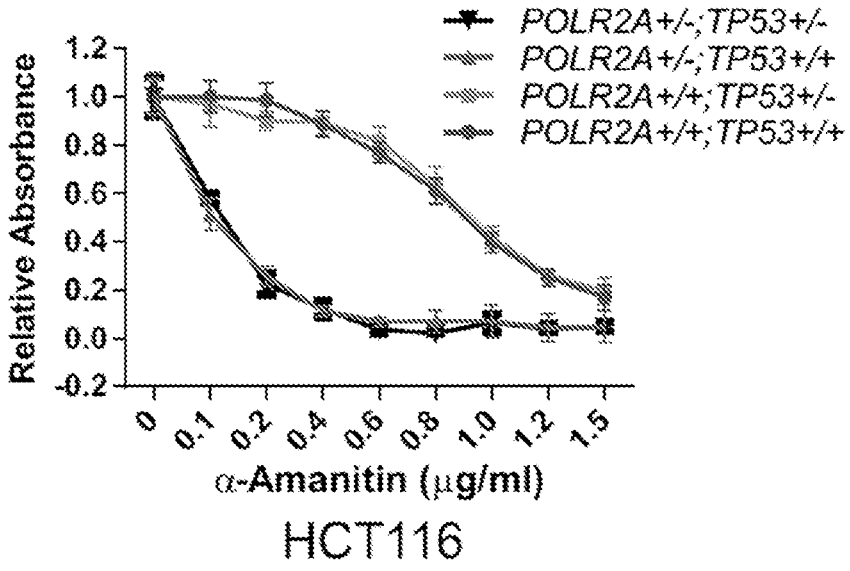


FIG. 10F

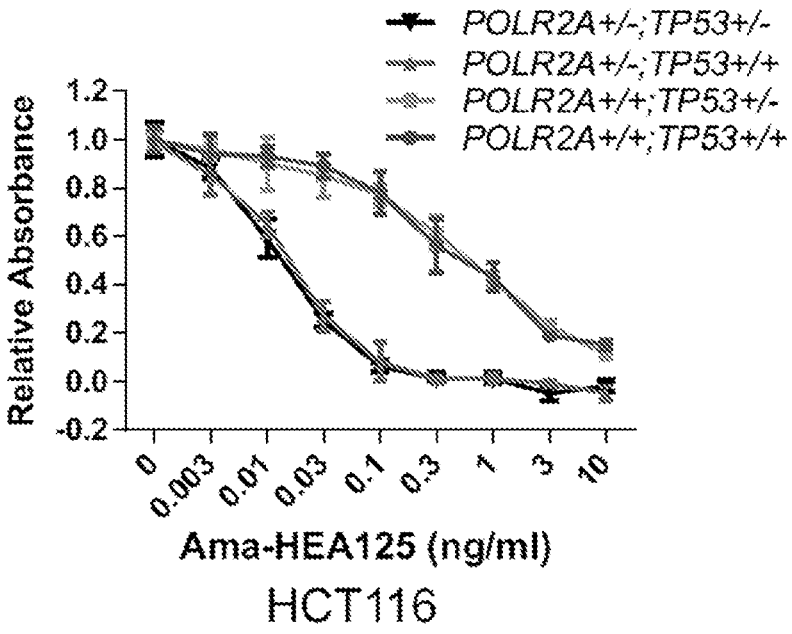

POLR2A+/-;TP53+/-
POLR2A+/-;TP53+/+
POLR2A+/+;TP53+/-
POLR2A+/+;TP53+/+
Relative Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
0.003
0.01
0.03
0.1
0.3
1
3
10
Ama-HEA125 (ng/ml)
HCT116

FIG. 10G

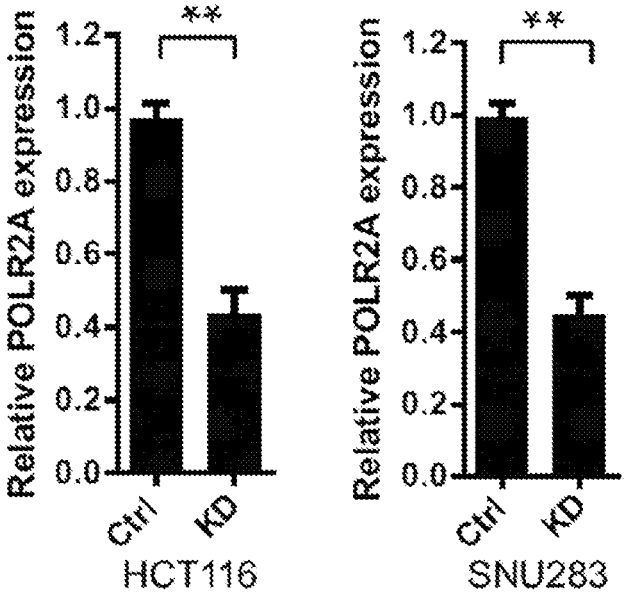


FIG. 11A

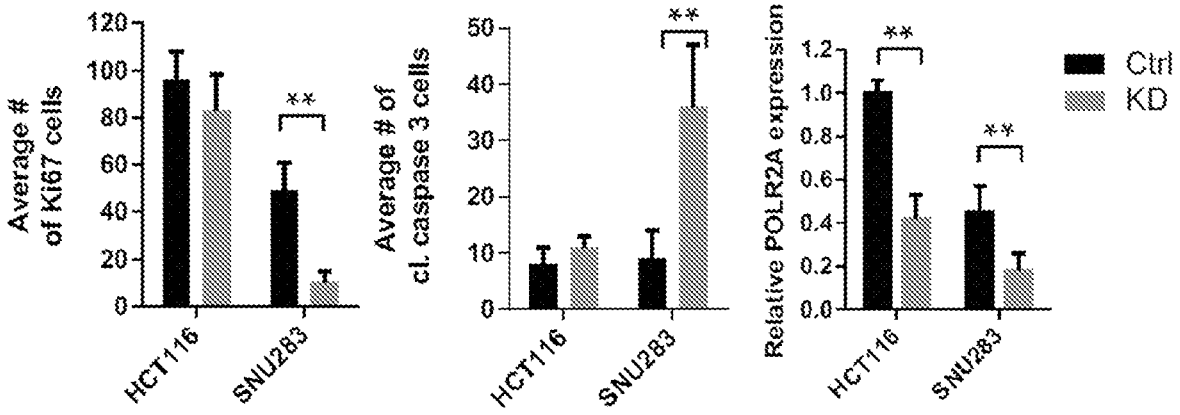


FIG. 11B

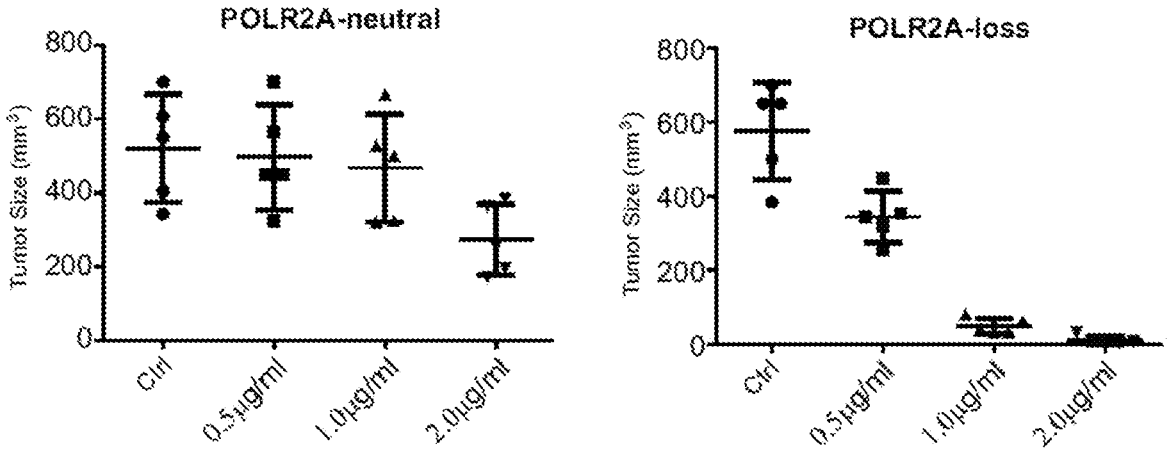

POLR2A-neutral
Tumor Size (mm³)
800
600
400
200
0
Ctrl
0.5μg/ml
1.0μg/ml
2.0μg/ml
POLR2A-loss
Tumor Size (mm³)
800
600
400
200
0
Ctrl
0.5μg/ml
1.0μg/ml
2.0μg/ml

FIG. 11C

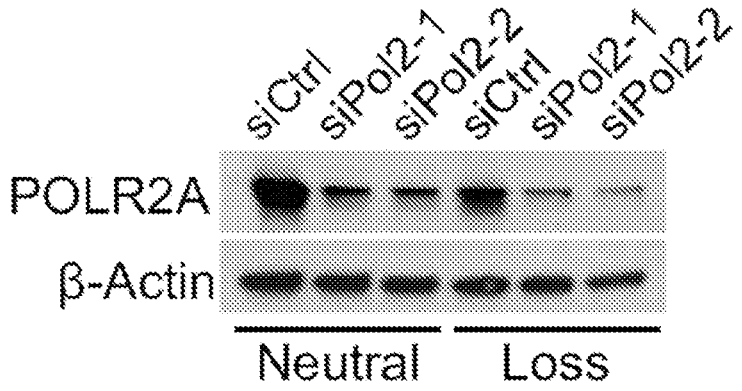


FIG. 12A

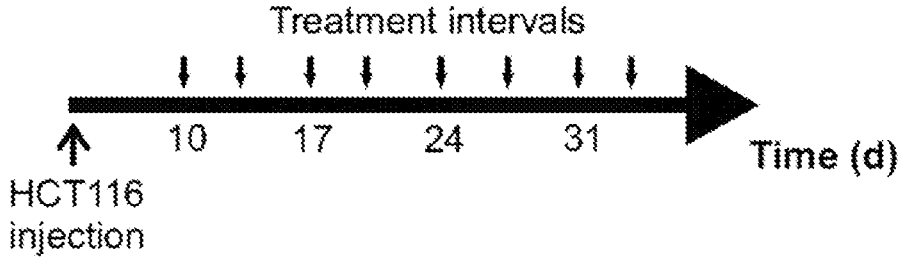


FIG. 12B

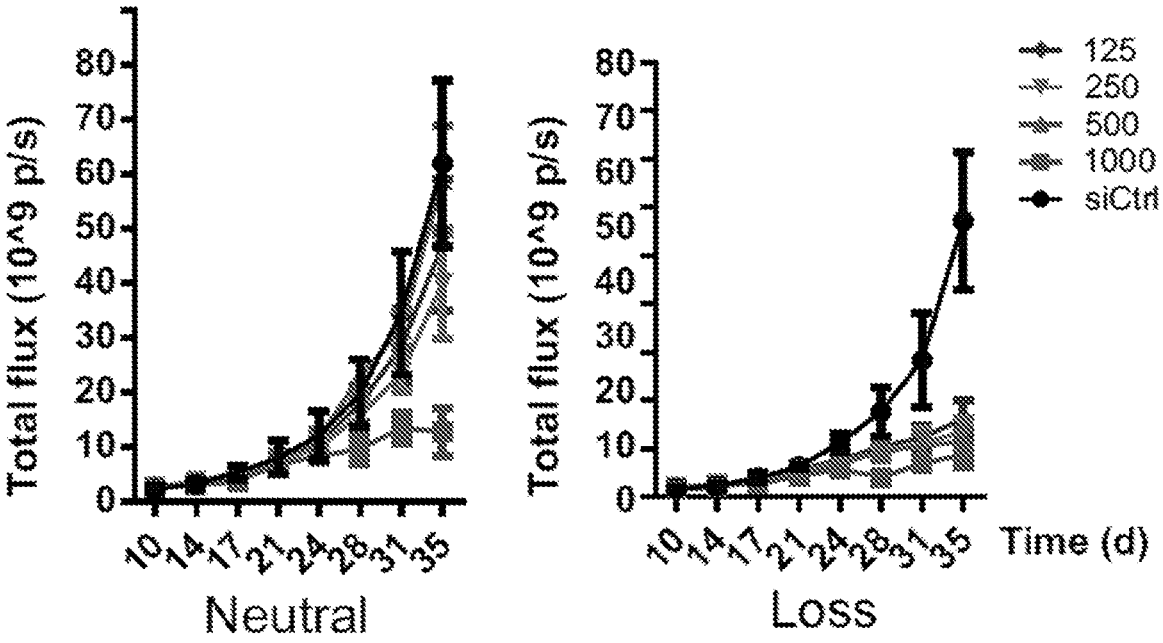


FIG. 12C

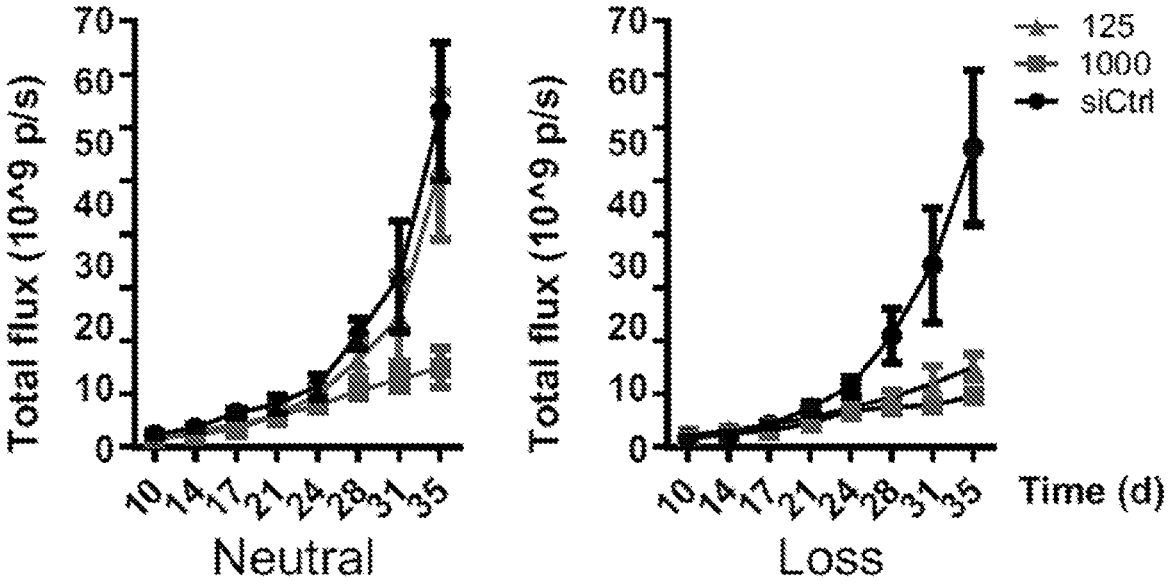


FIG. 12D

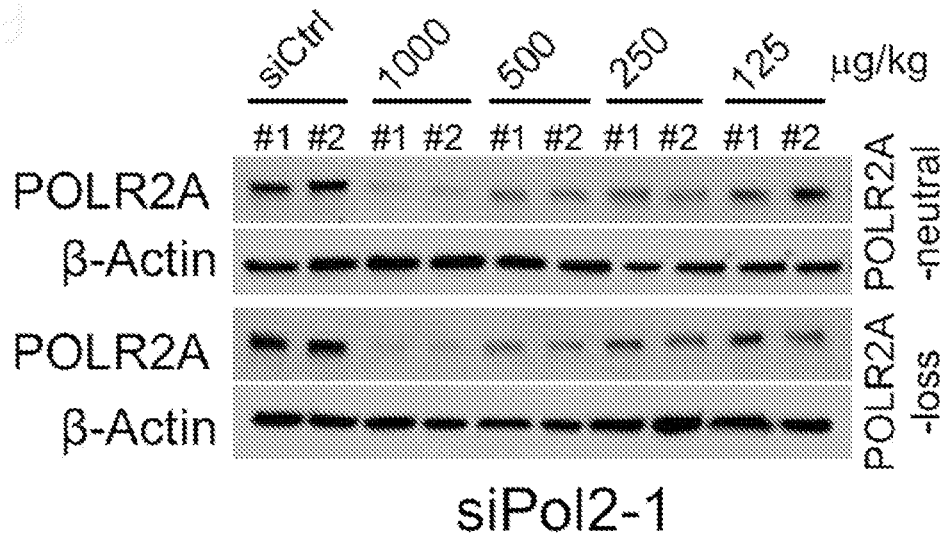


FIG. 12E

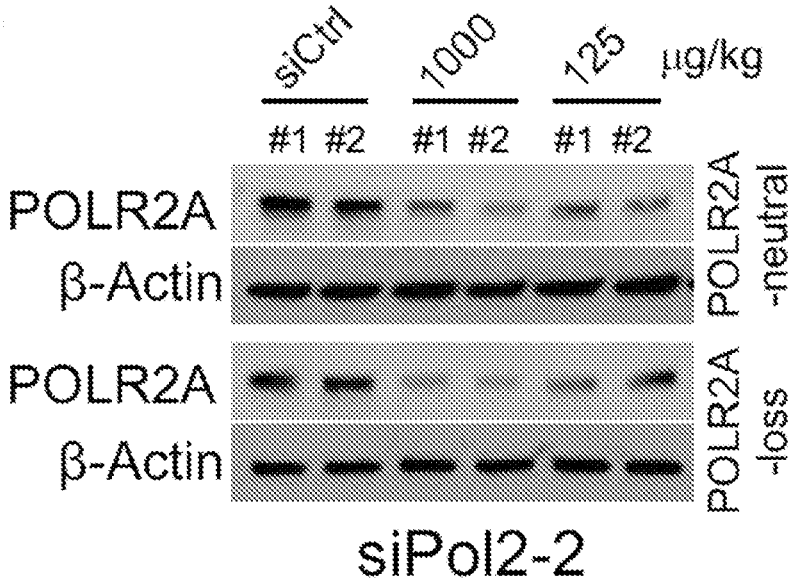

siCtrl
1000
125
µg/kg
#1 #2 #1 #2 #1 #2
POLR2A
β-Actin
POLR2A -neutral
POLR2A
β-Actin
POLR2A -loss
siPol2-2

FIG. 12F

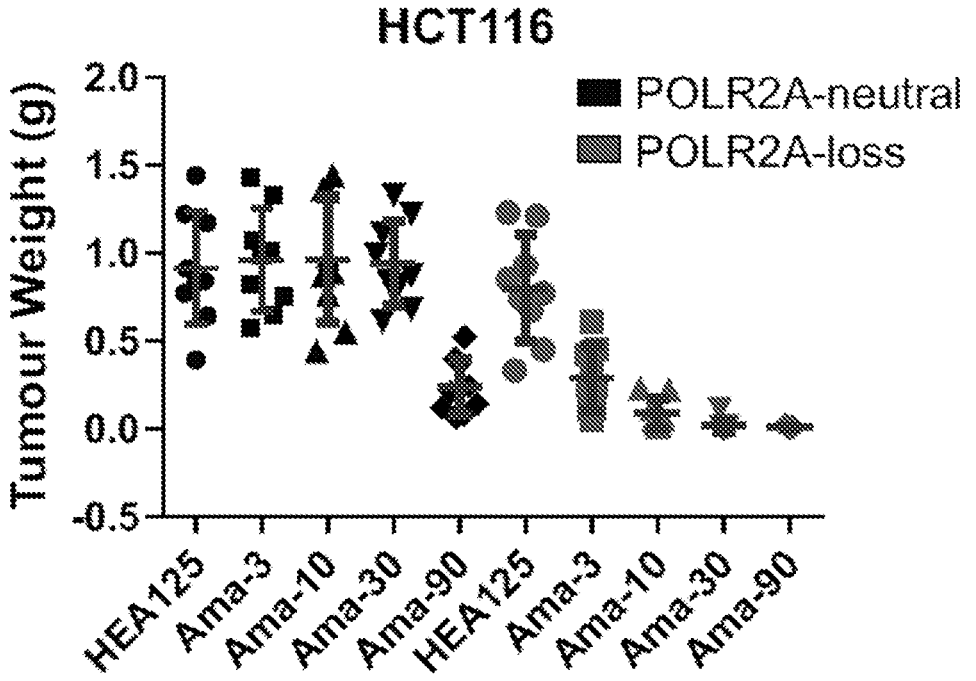


FIG. 13A

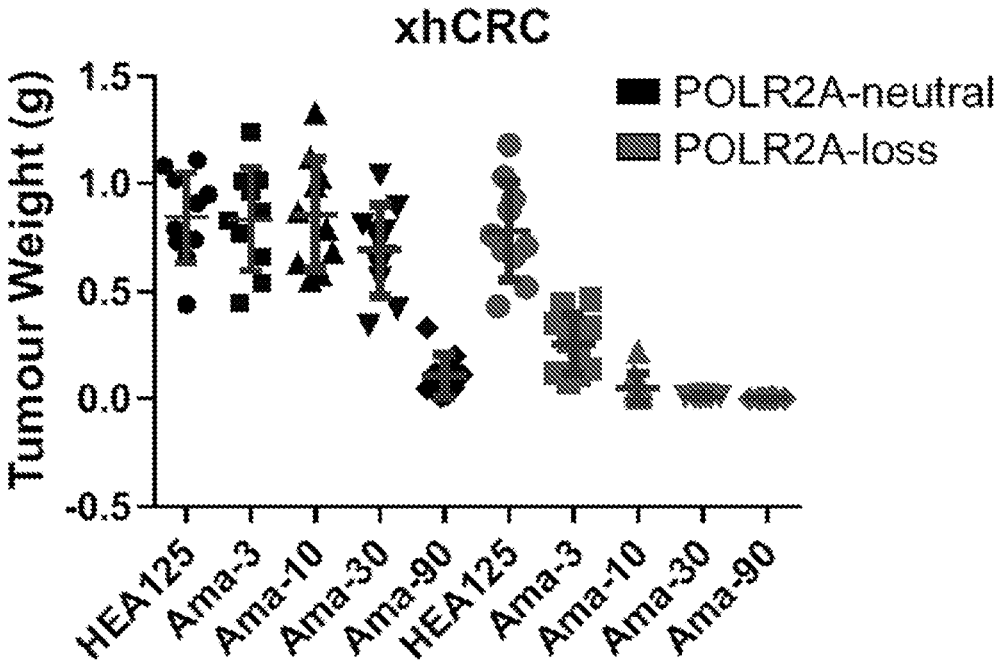


FIG. 13B

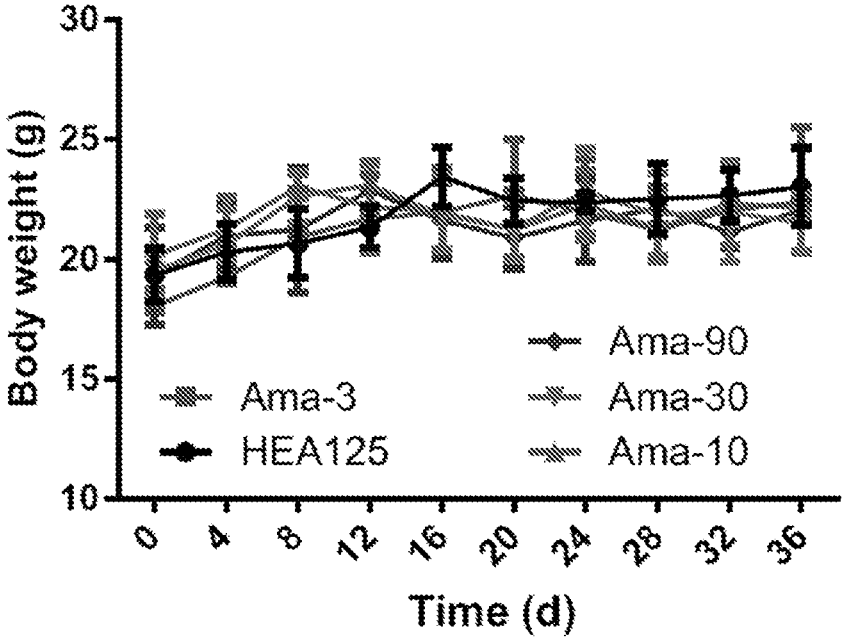


FIG. 13C

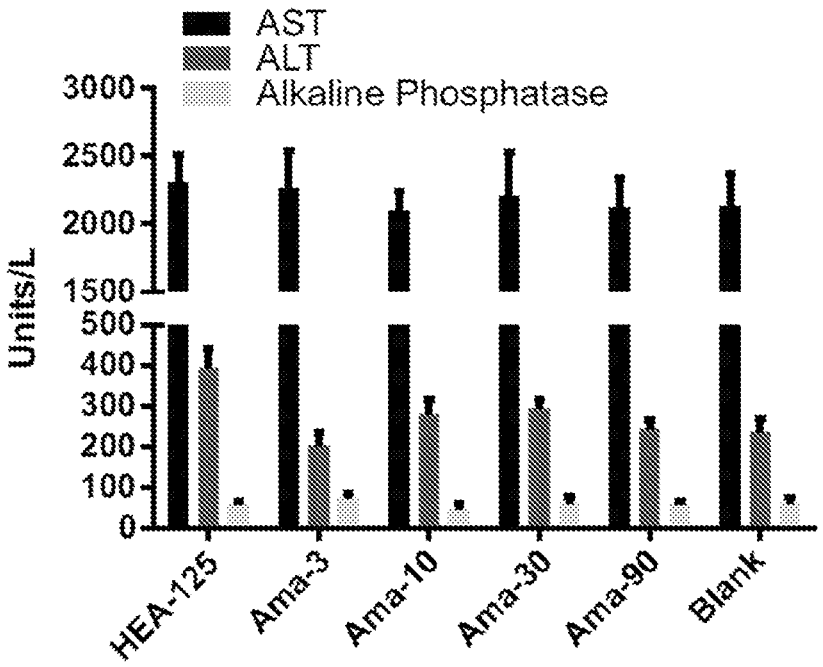


FIG. 13D

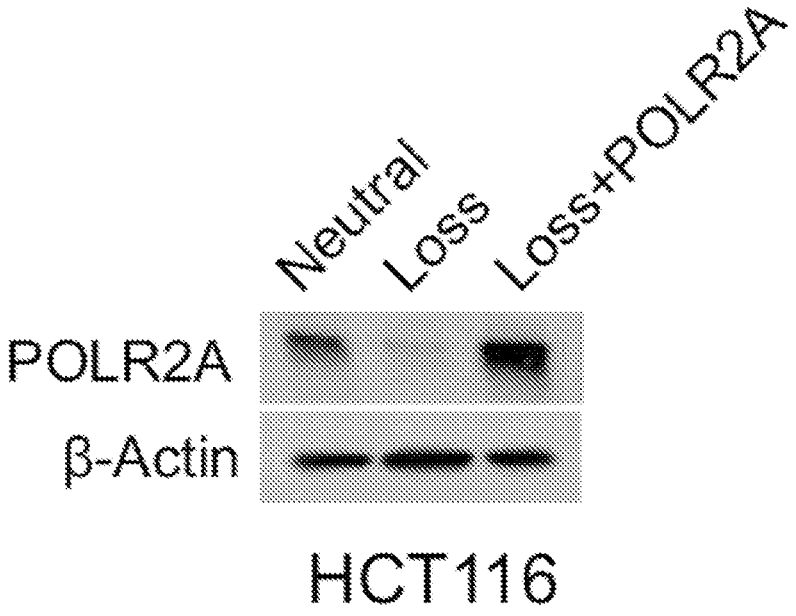


FIG. 14A

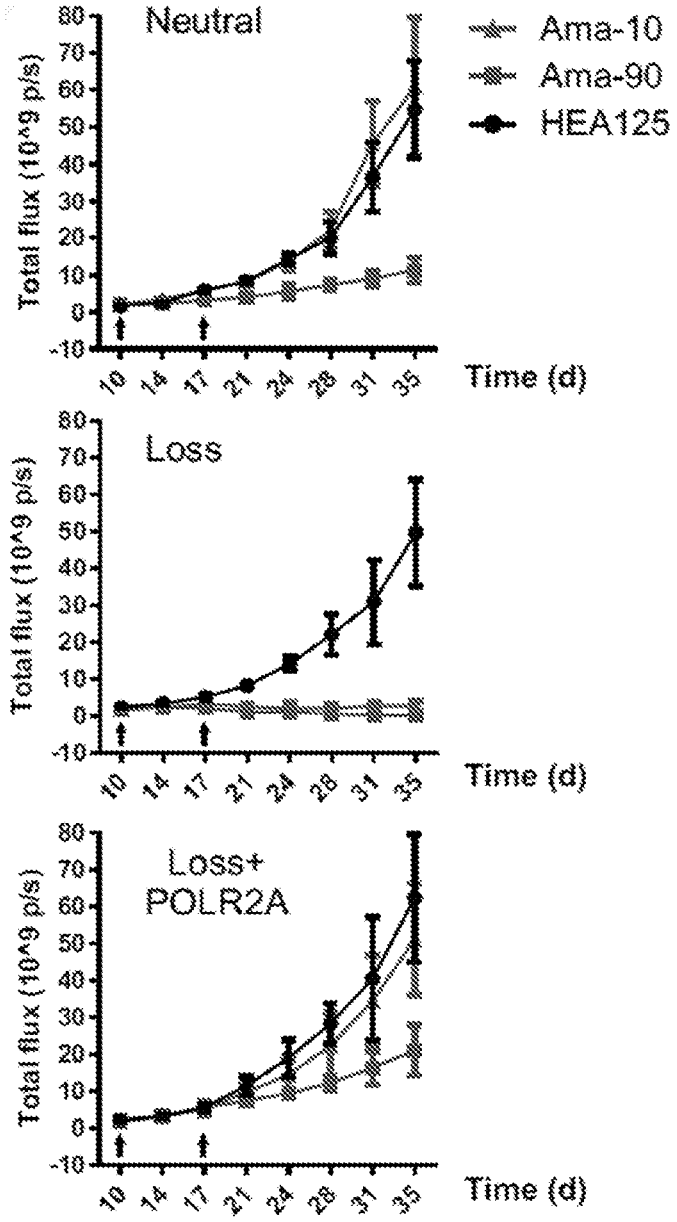

Neutral
Ama-10
Ama-90
HEA125
Total flux (10^9 p/s)
Time (d)
Loss
Loss+
POLR2A

FIG. 14B

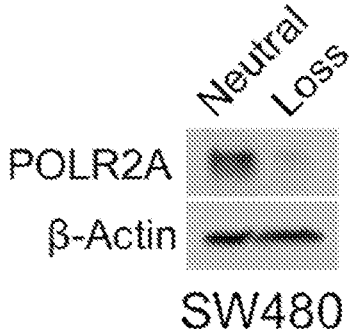

Neutral
Loss
POLR2A
β-Actin
SW480

FIG. 14C

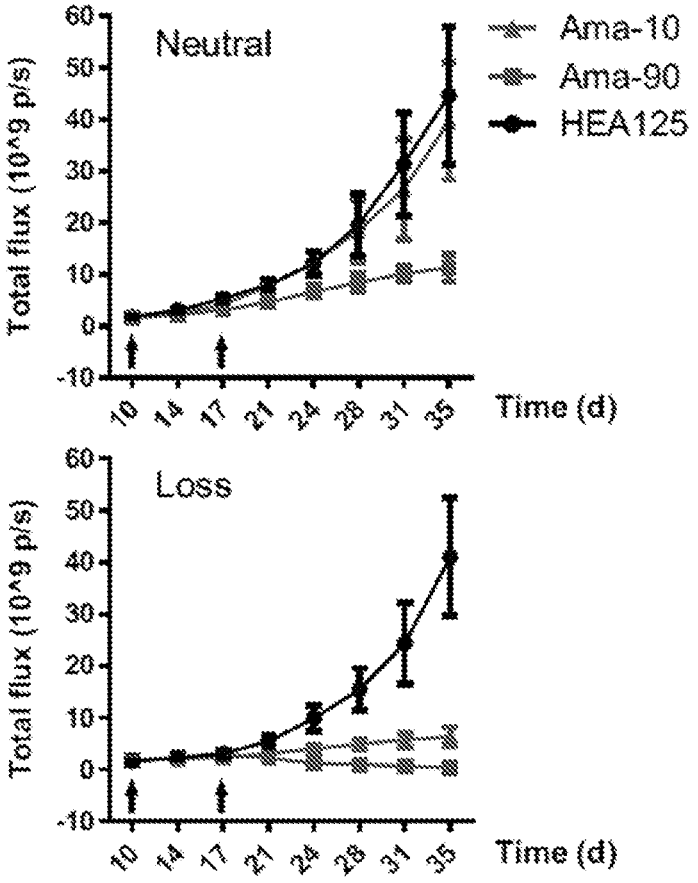


FIG. 14D

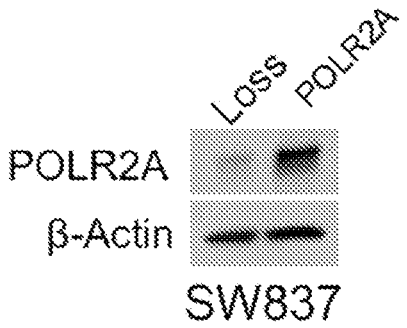


FIG. 14E

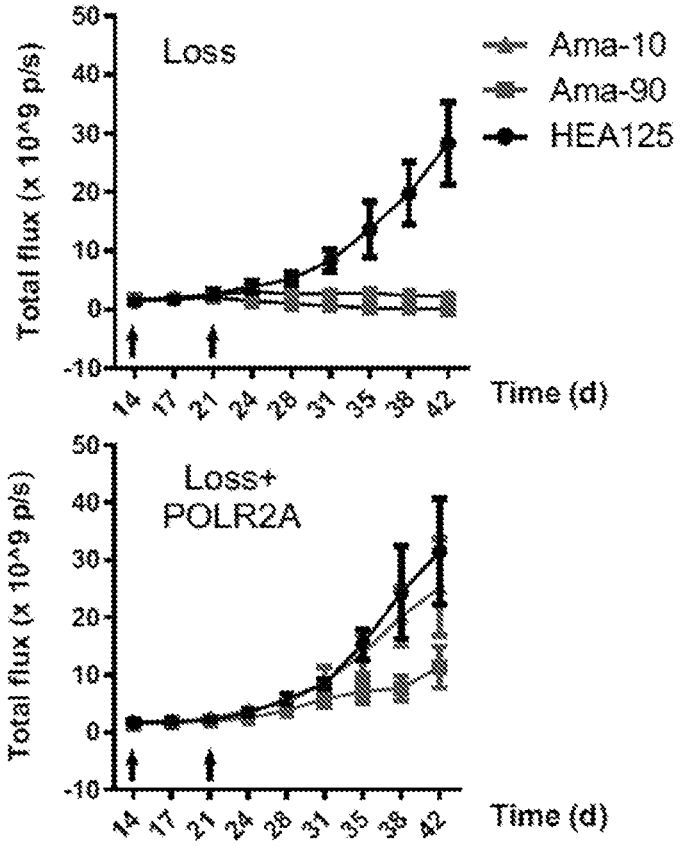

Loss
Ama-10
Ama-90
HEA125
Total flux (x 10^9 p/s)
50
40
30
20
10
0
-10
14
17
21
24
28
31
35
38
42
Time (d)
Loss+
POLR2A
Total flux (x 10^9 p/s)
Time (d)

FIG. 14F