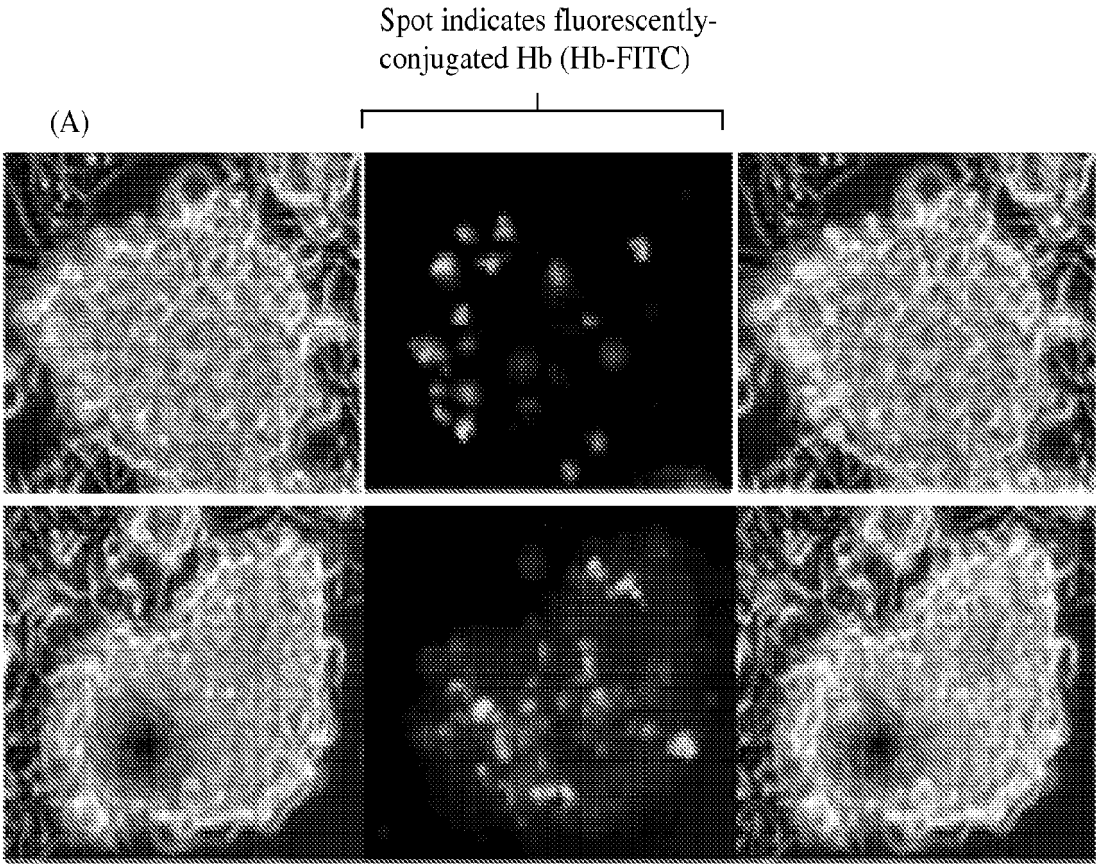

Spot indicates fluorescently-conjugated Hb (Hb-FITC)
(A)

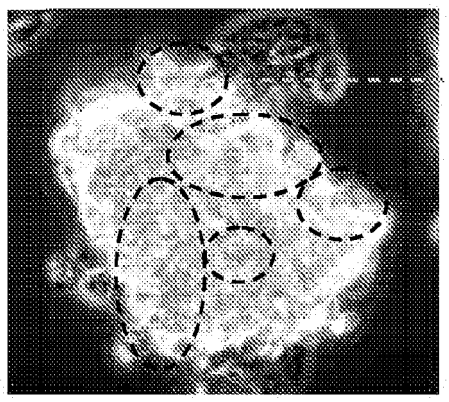

(B)
Dotted circle shows the uptake of fluorescently-conjugated polymerized Hb

FIG. 1

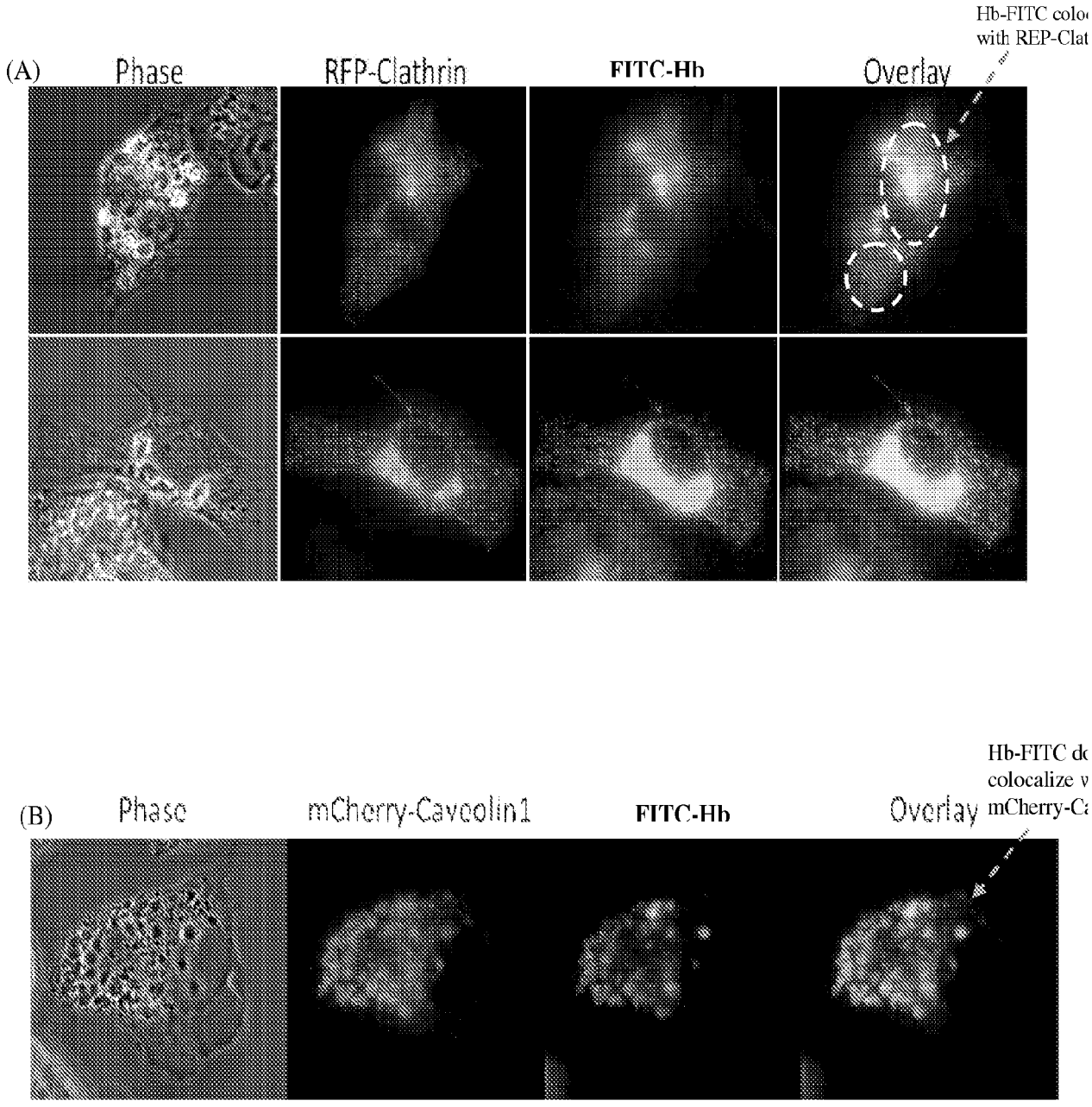

(A)
Phase
RFP-Clathrin
FITC-Hb
Overlay
Hb-FITC colo
with REP-Clat
(B)
Phase
mCherry-Caveolin1
FITC-Hb
Overlay
Hb-FITC d
colocalize v
mCherry-C

FIG. 2

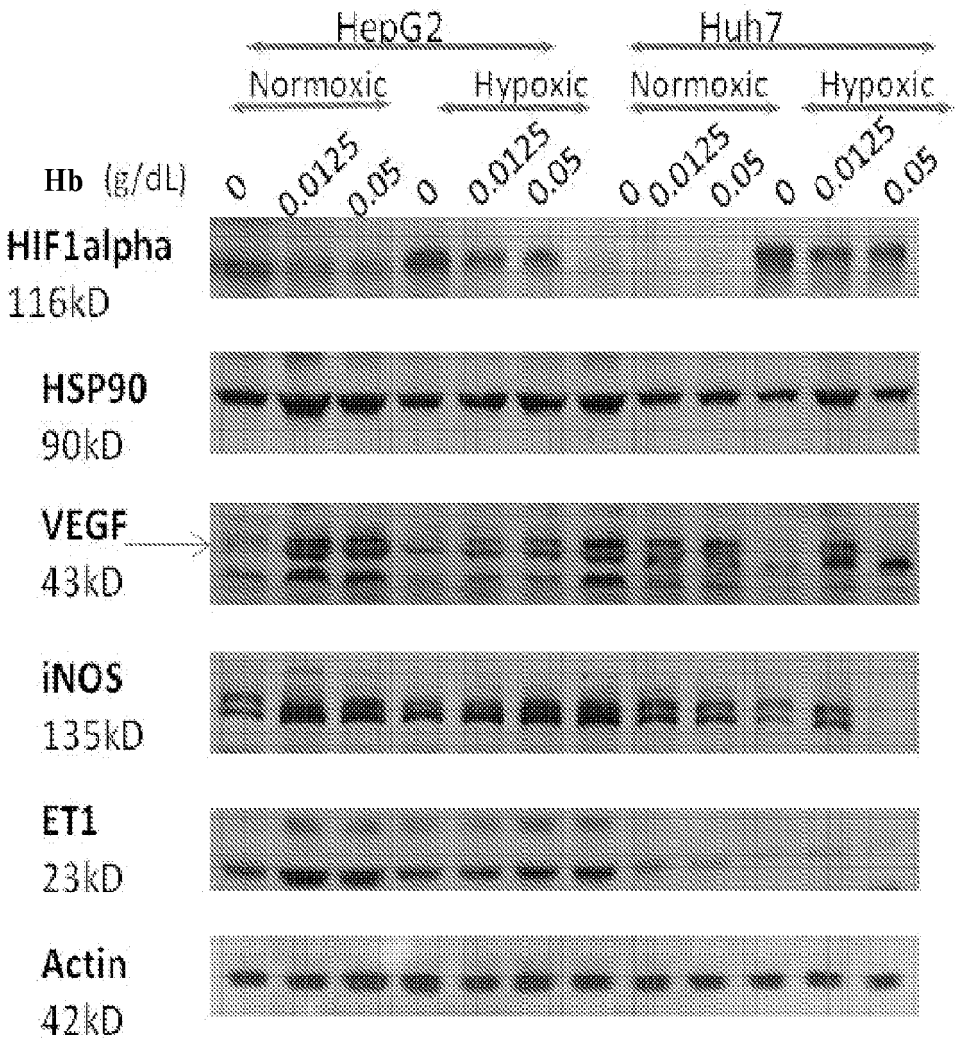

HepG2
Huh7
Normoxic
Hypoxic
Normoxic
Hypoxic
Hb (g/dL)
0
0.0125
0.05
0
0.0125
0.05
0
0.0125
0.05
0
0.0125
0.05
HIF1alpha
116kD
HSP90
90kD
VEGF
43kD
iNOS
135kD
ET1
23kD
Actin
42kD

FIG. 3

HIF1 α

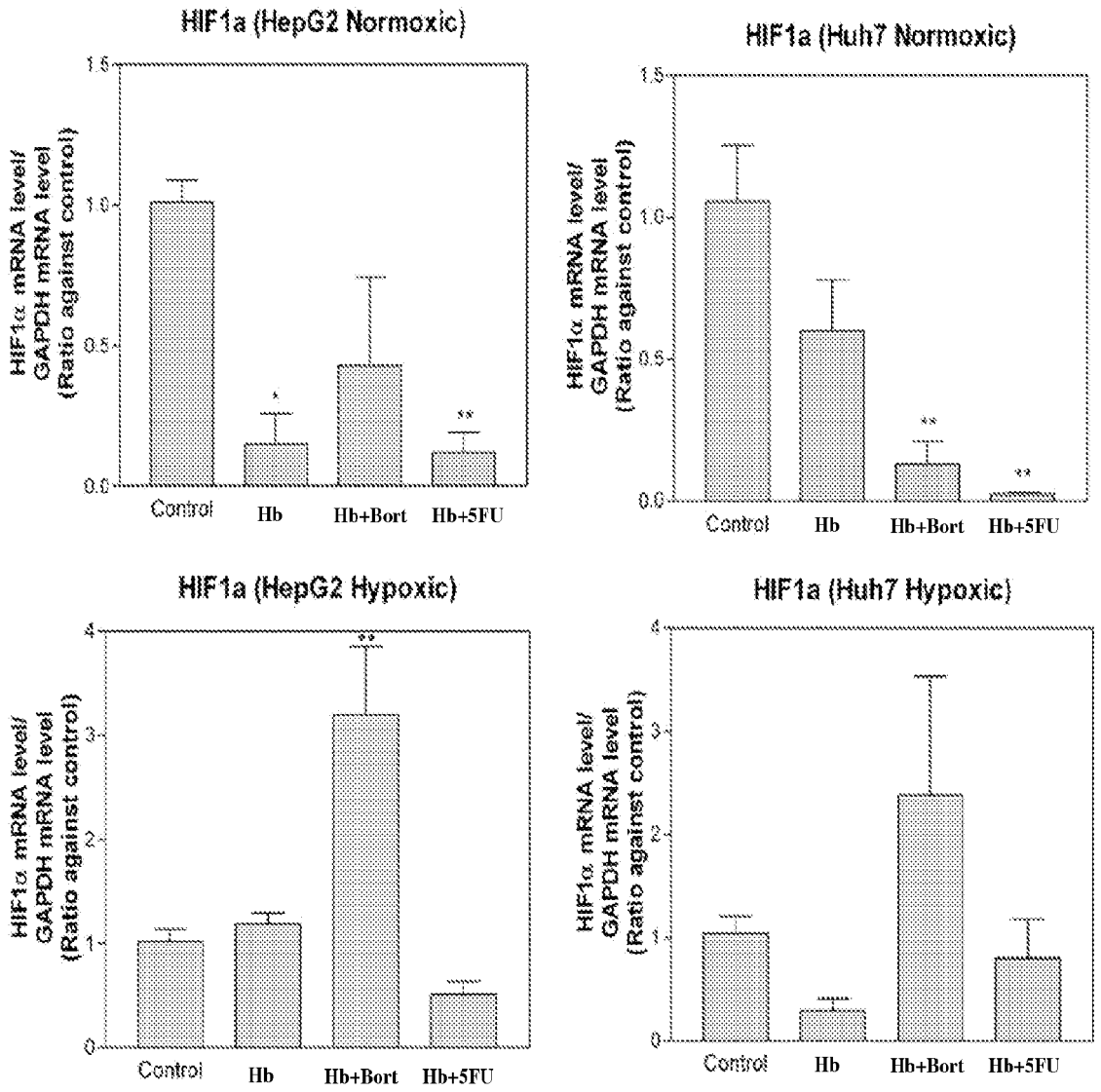

HIF1a (HepG2 Normoxic)
HIF1α mRNA level/ GAPDH mRNA level (Ratio against control)
1.5
1.0
0.5
0.0
*
**
Control
Hb
Hb+Bort
Hb+5FU
HIF1a (Huh7 Normoxic)
HIF1α mRNA level/ GAPDH mRNA level (Ratio against control)
1.5
1.0
0.5
0.0
**
**
Control
Hb
Hb+Bort
Hb+5FU
HIF1a (HepG2 Hypoxic)
HIF1α mRNA level/ GAPDH mRNA level (Ratio against control)
4
3
2
1
0
**
Control
Hb
Hb+Bort
Hb+5FU
HIF1a (Huh7 Hypoxic)
HIF1α mRNA level/ GAPDH mRNA level (Ratio against control)
4
3
2
1
0
Control
Hb
Hb+Bort
Hb+5FU

FIG. 4

VEGF

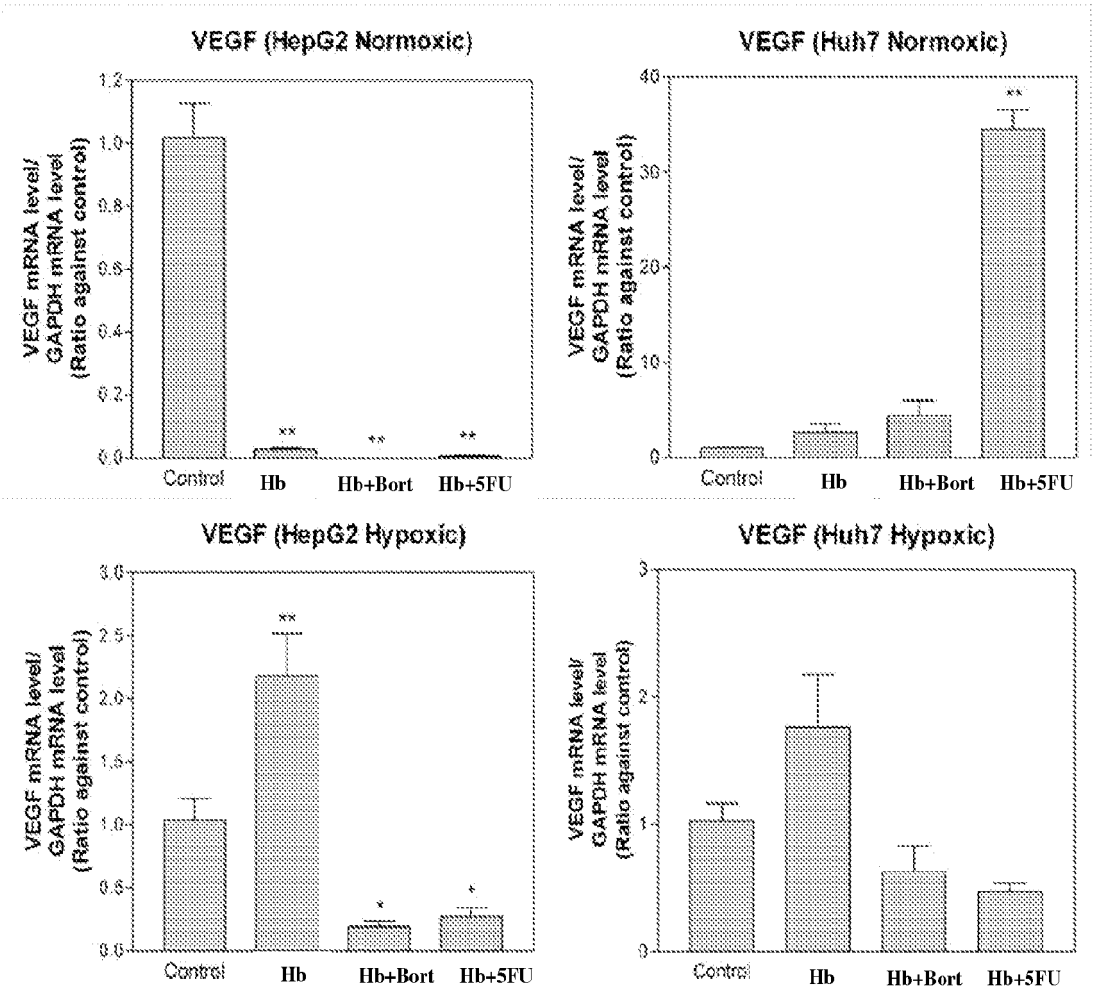

VEGF (HepG2 Normoxic)
VEGF mRNA level/ GAPDH mRNA level (Ratio against control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Control
Hb
Hb+Bort
Hb+5FU
VEGF (Huh7 Normoxic)
VEGF mRNA level/ GAPDH mRNA level (Ratio against control)
40
30
20
10
0
**
Control
Hb
Hb+Bort
Hb+5FU
VEGF (HepG2 Hypoxic)
VEGF mRNA level/ GAPDH mRNA level (Ratio against control)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
*
*
Control
Hb
Hb+Bort
Hb+5FU
VEGF (Huh7 Hypoxic)
VEGF mRNA level/ GAPDH mRNA level (Ratio against control)
3
2
1
0
Control
Hb
Hb+Bort
Hb+5FU

FIG. 5

ET1

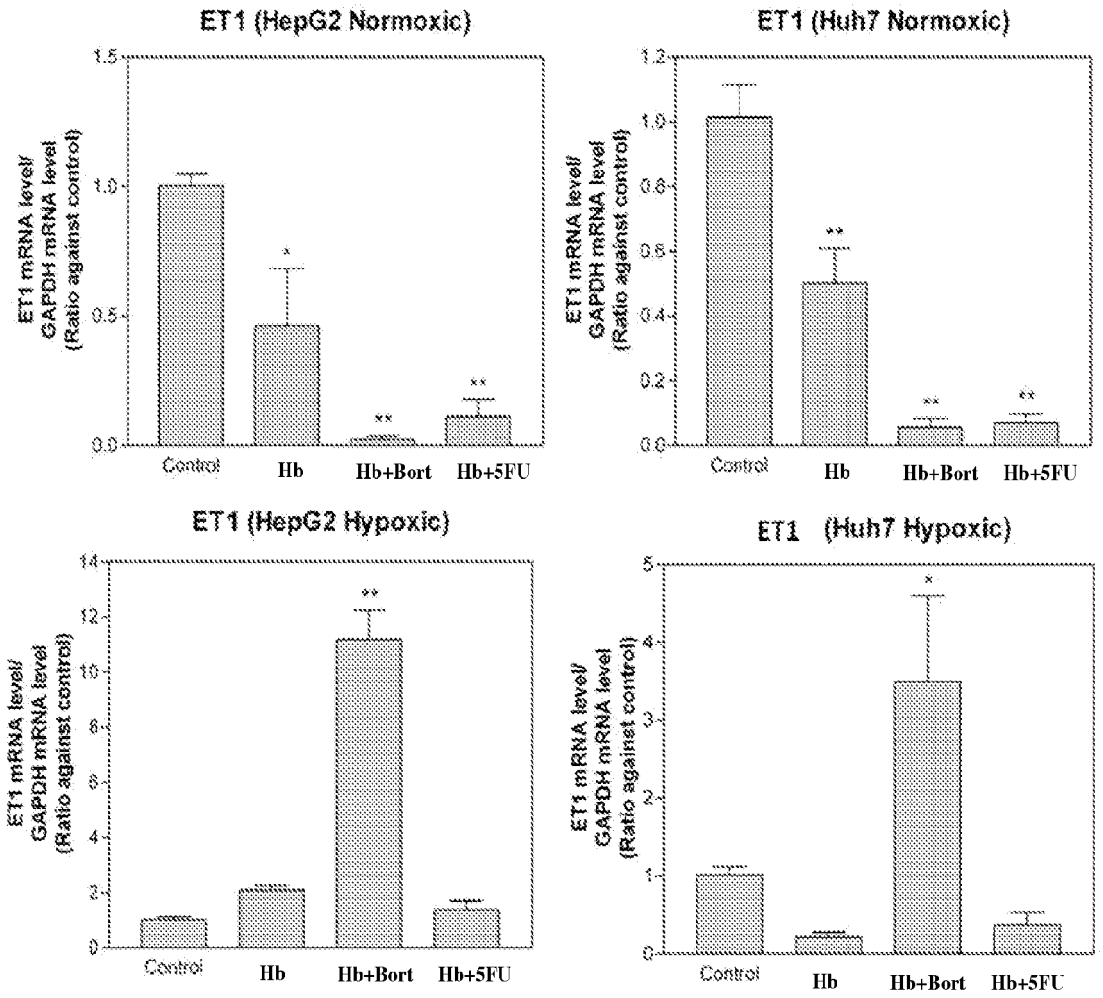

ET1 (HepG2 Normoxic)
ET1 (Huh7 Normoxic)
ET1 (HepG2 Hypoxic)
ET1 (Huh7 Hypoxic)
ET1 mRNA level/ GAPDH mRNA level (Ratio against control)
Control
Hb
Hb+Bort
Hb+5FU

FIG. 6

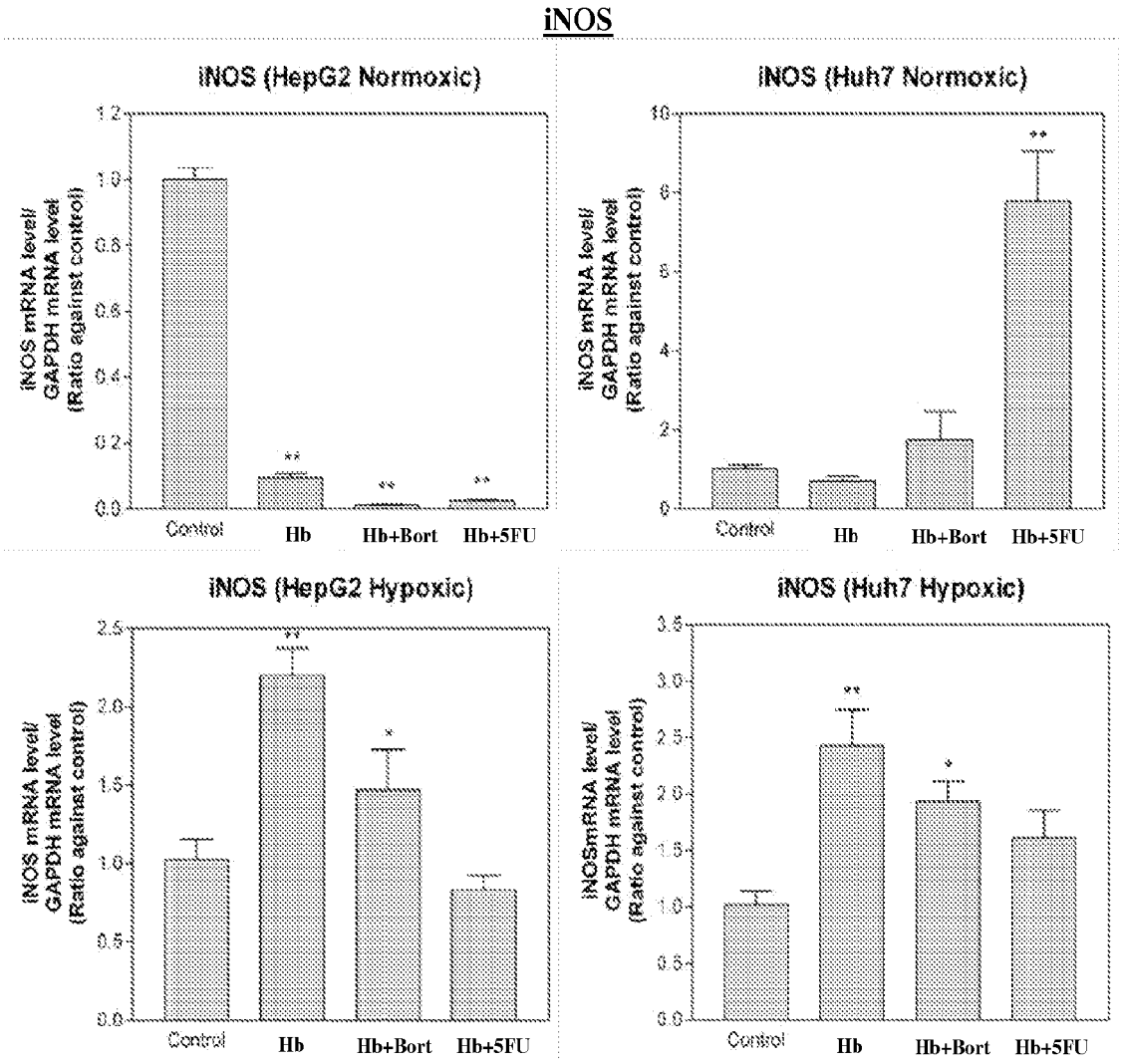

iNOS
iNOS (HepG2 Normoxic)
iNOS (Huh7 Normoxic)
iNOS (HepG2 Hypoxic)
iNOS (Huh7 Hypoxic)
iNOS mRNA level/ GAPDH mRNA level (Ratio against control)
iNOSmRNA level/ GAPDH mRNA level (Ratio against control)
Control
Hb
Hb+Bort
Hb+5FU

FIG. 7

VHL

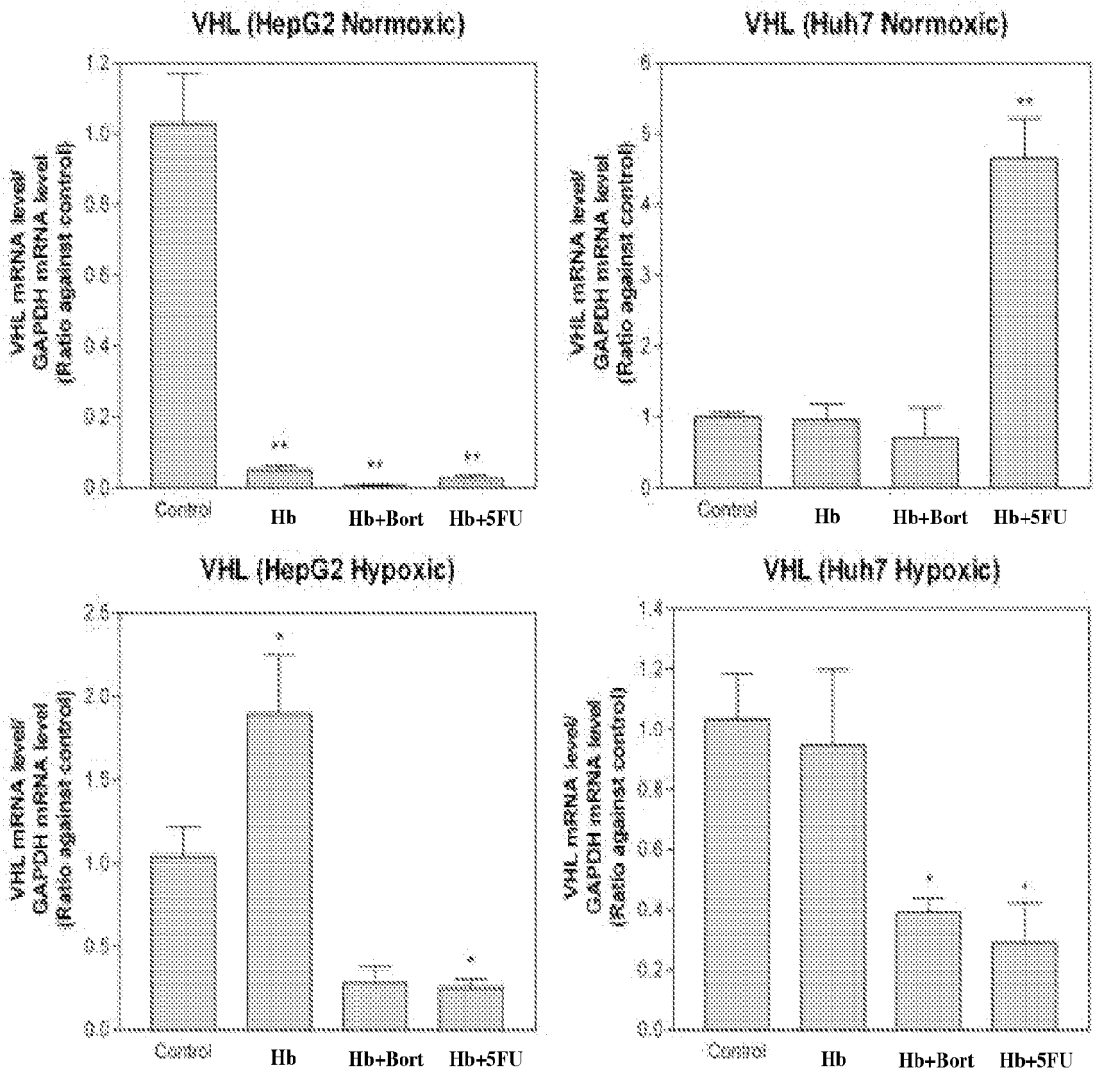

VHL (HepG2 Normoxic)
VHL (Huh7 Normoxic)
VHL (HepG2 Hypoxic)
VHL (Huh7 Hypoxic)
VHL mRNA level/ GAPDH mRNA level (Ratio against control)
Control
Hb
Hb+Bort
Hb+5FU

FIG. 8

HSP90

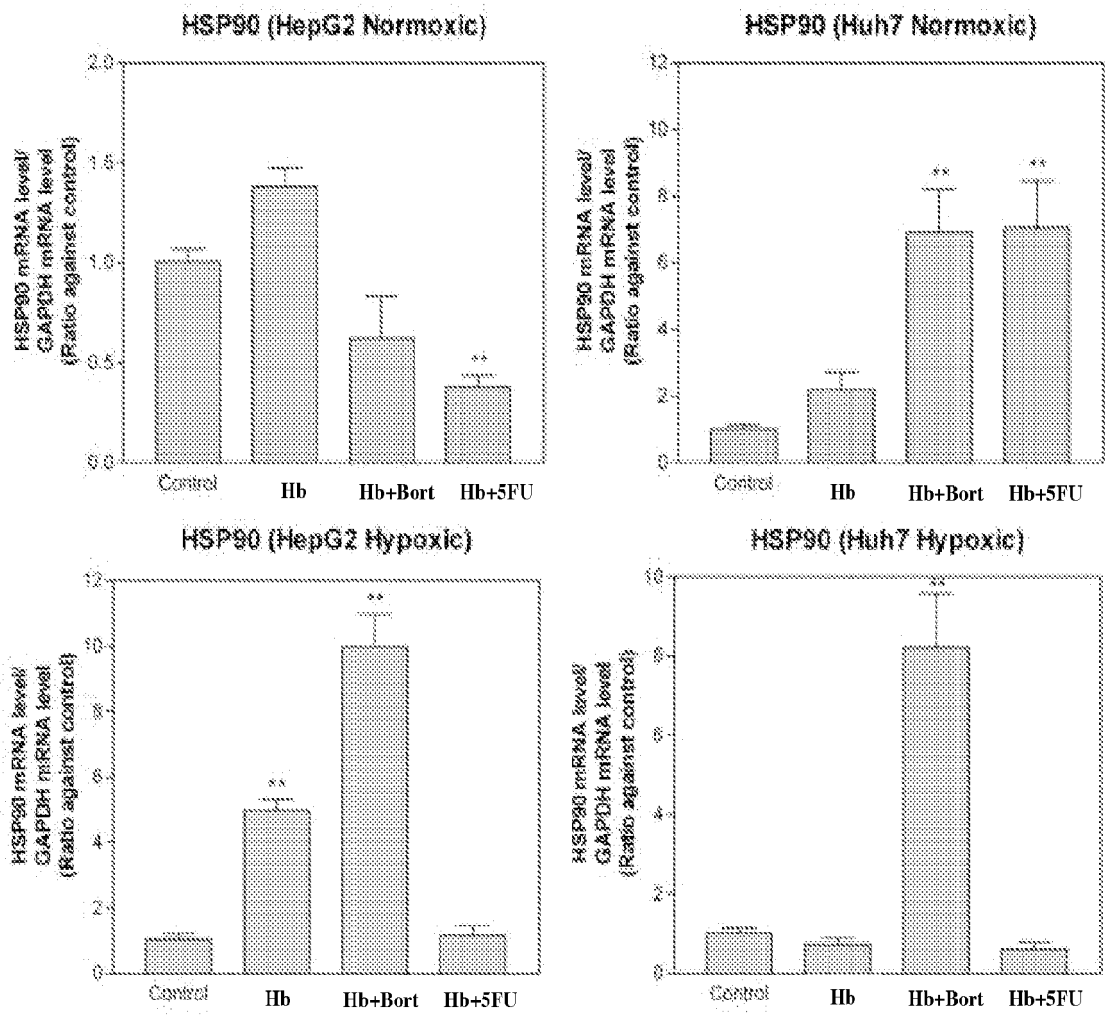

HSP90 (HepG2 Normoxic)
HSP90 (Huh7 Normoxic)
HSP90 (HepG2 Hypoxic)
HSP90 (Huh7 Hypoxic)
HSP90 mRNA level/ GAPDH mRNA level (Ratio against control)
Control
Hb
Hb+Bort
Hb+5FU

FIG. 9

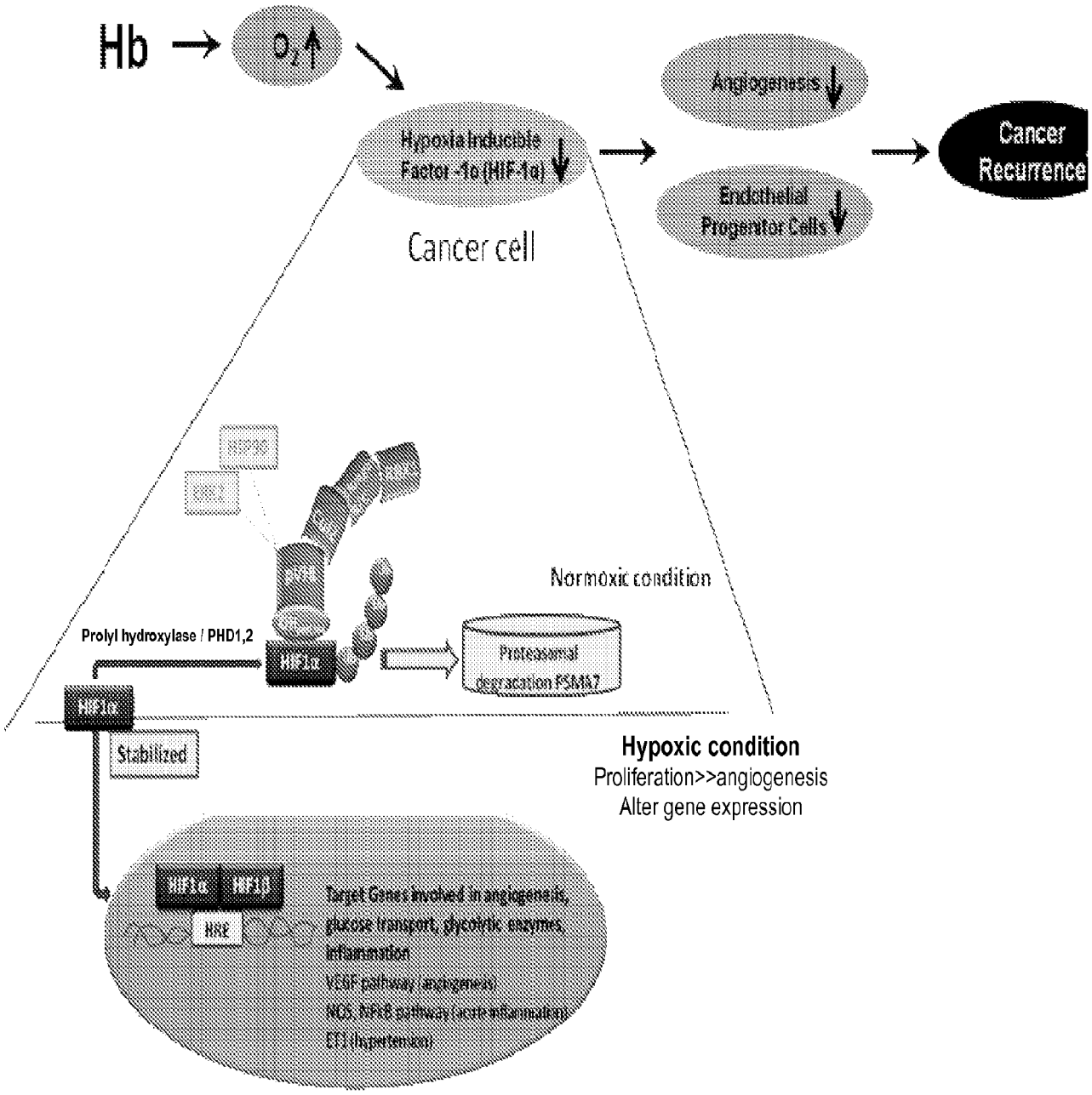

Hb
O_2
Hypoxia Inducible Factor -1α (HIF-1α)
Angiogenesis
Endothelial Progenitor Cells
Cancer Recurrence
Cancer cell
Normoxic condition
Prolyl hydroxylase / PHD1,2
Proteasomal degradation PSMA7
Stabilized
Hypoxic condition
Proliferation>>angiogenesis
Alter gene expression
HRE
Target Genes involved in angiogenesis, glucose transport, glycolytic enzymes, inflammation
VEGF pathway (angiogenesis)
ET1 (hypertension)

FIG. 10

HSP7C

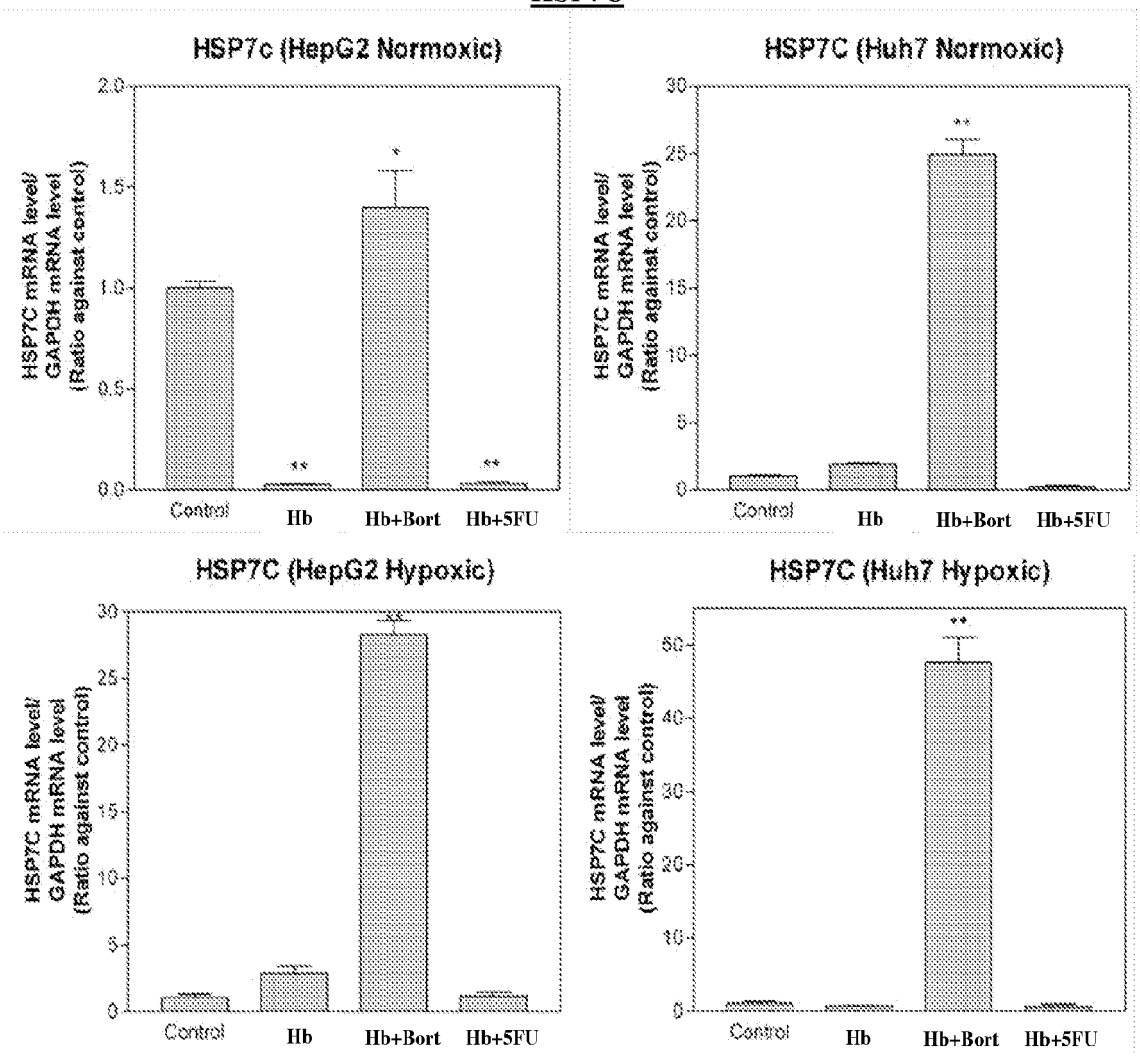

HSP7c (HepG2 Normoxic)
HSP7C (Huh7 Normoxic)
HSP7C (HepG2 Hypoxic)
HSP7C (Huh7 Hypoxic)
HSP7C mRNA level/ GAPDH mRNA level (Ratio against control)
Control
Hb
Hb+Bort
Hb+5FU

FIG. 11

HMGB3

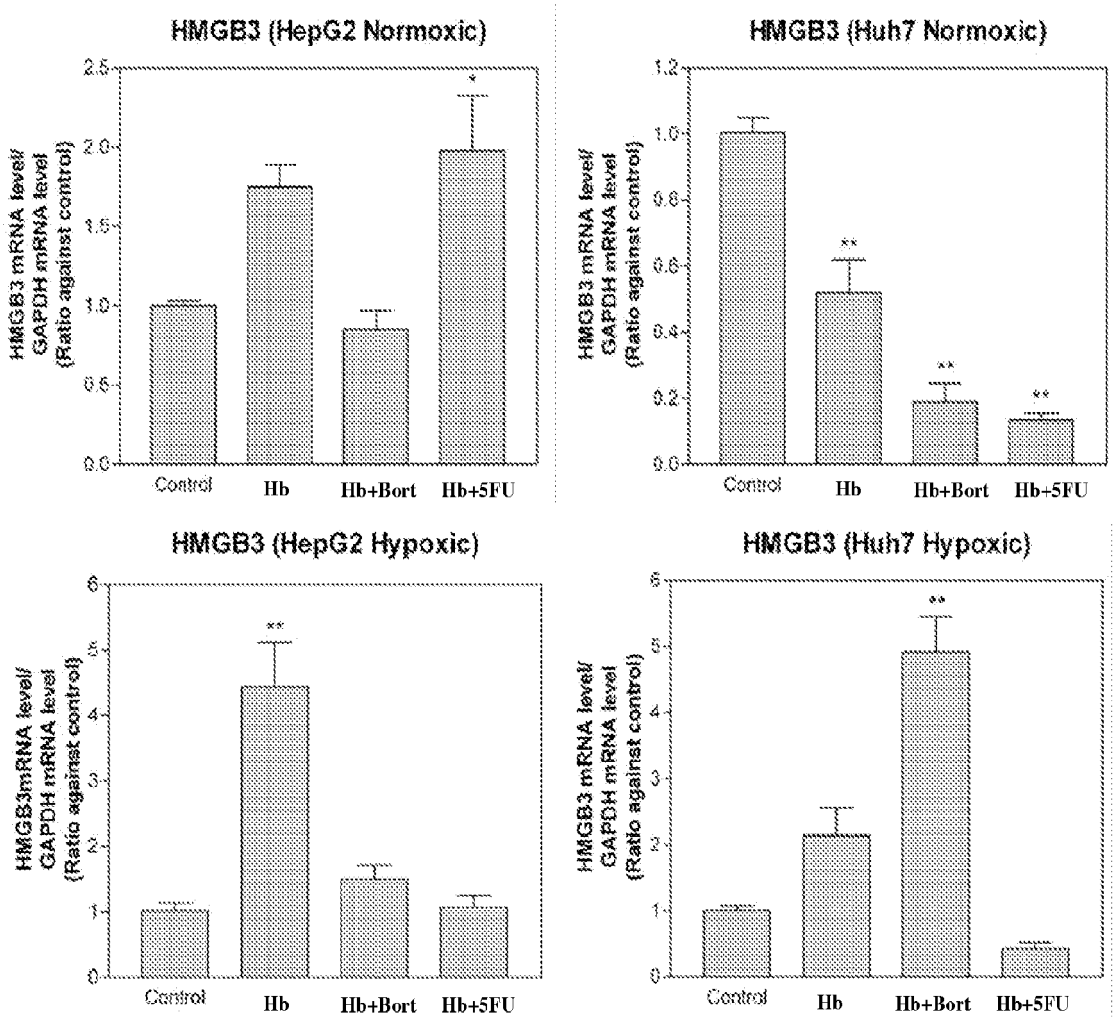

HMGB3 (HepG2 Normoxic)
HMGB3 mRNA level/ GAPDH mRNA level (Ratio against control)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Hb
Hb+Bort
Hb+5FU
HMGB3 (Huh7 Normoxic)
HMGB3 mRNA level/ GAPDH mRNA level (Ratio against control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Control
Hb
Hb+Bort
Hb+5FU
HMGB3 (HepG2 Hypoxic)
HMGB3mRNA level/ GAPDH mRNA level (Ratio against control)
6
5
4
3
2
1
0
**
Control
Hb
Hb+Bort
Hb+5FU
HMGB3 (Huh7 Hypoxic)
HMGB3 mRNA level/ GAPDH mRNA level (Ratio against control)
6
5
4
3
2
1
0
**
Control
Hb
Hb+Bort
Hb+5FU

FIG. 12

RFC1

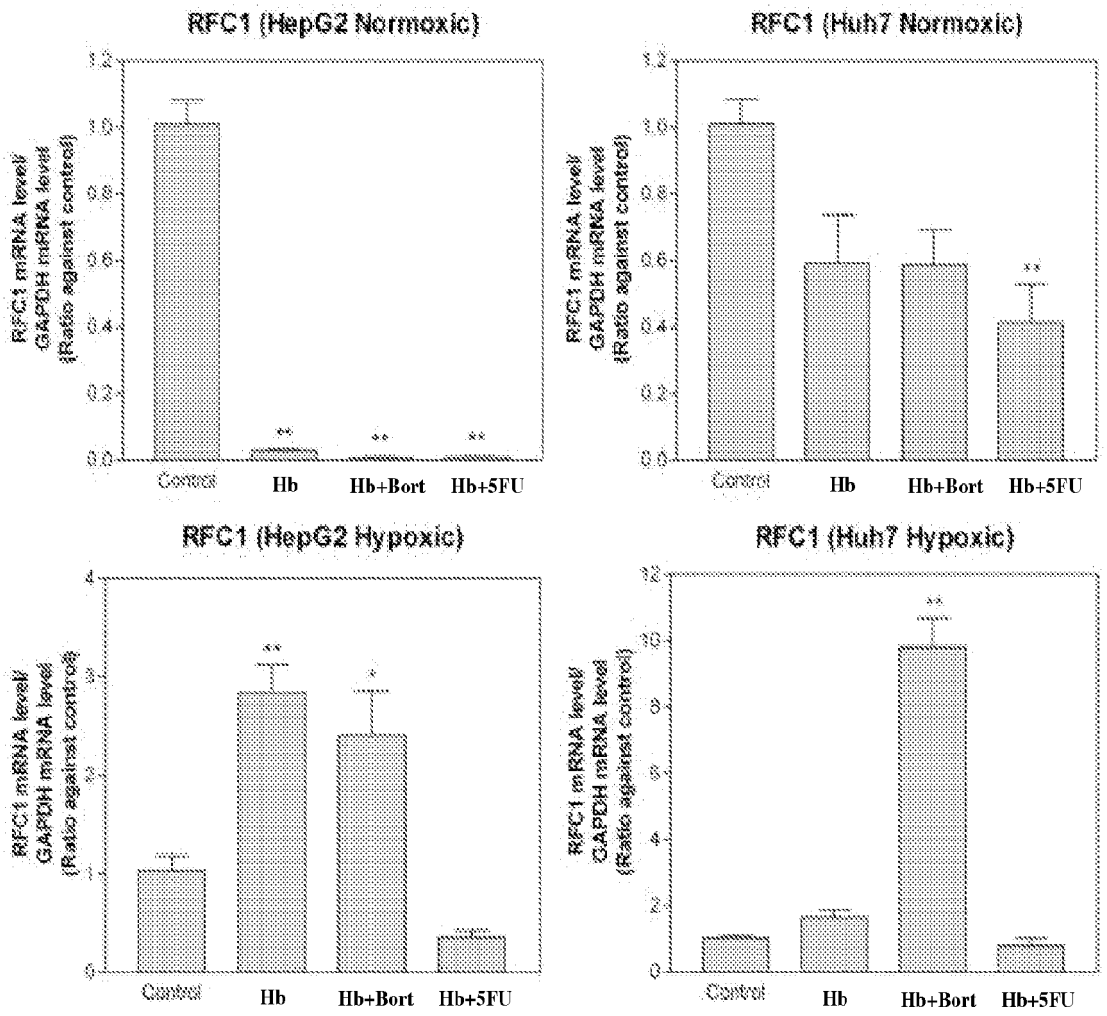

RFC1 (HepG2 Normoxic)
RFC1 (Huh7 Normoxic)
RFC1 (HepG2 Hypoxic)
RFC1 (Huh7 Hypoxic)
RFC1 mRNA level/ GAPDH mRNA level (Ratio against control)
Control
Hb
Hb+Bort
Hb+5FU

FIG. 13

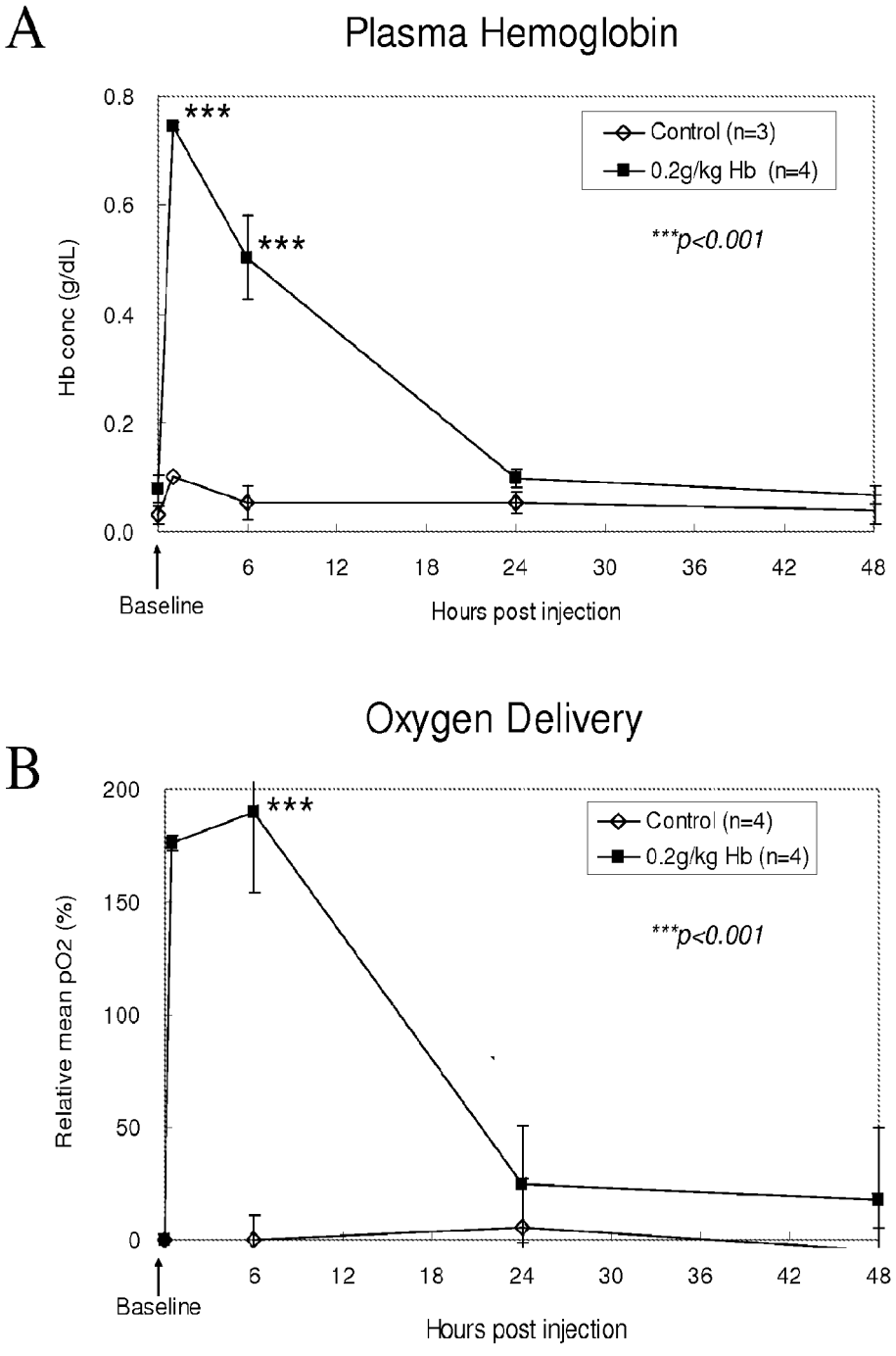

A
Plasma Hemoglobin
Hb conc (g/dL)
0.8
0.6
0.4
0.2
0.0
***
***
Control (n=3)
0.2g/kg Hb (n=4)
***p<0.001
6
12
18
24
30
36
42
48
Baseline
Hours post injection
B
Oxygen Delivery
Relative mean pO2 (%)
200
150
100
50
0
***
Control (n=4)
0.2g/kg Hb (n=4)
***p<0.001
6
12
18
24
30
36
42
48
Baseline
Hours post injection

FIG. 14

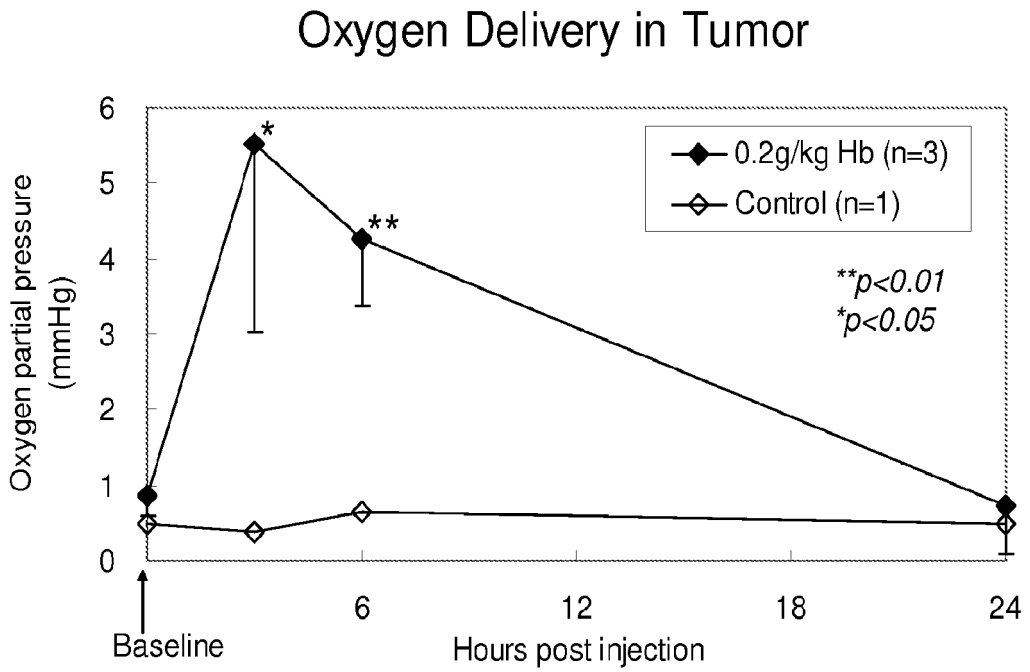
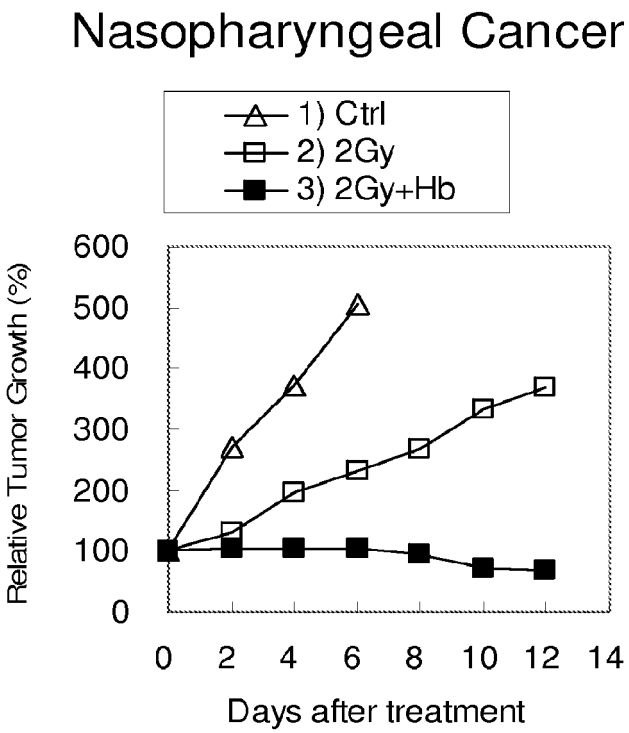

Oxygen Delivery in Tumor
Oxygen partial pressure (mmHg)
0
1
2
3
4
5
6
*
**
0.2g/kg Hb (n=3)
Control (n=1)
**p<0.01
*p<0.05
Baseline
6
12
18
24
Hours post injection

FIG. 15

A


Nasopharyngeal Cancer
1) Ctrl
2) 2Gy
3) 2Gy+Hb
Relative Tumor Growth (%)
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14
Days after treatment

B

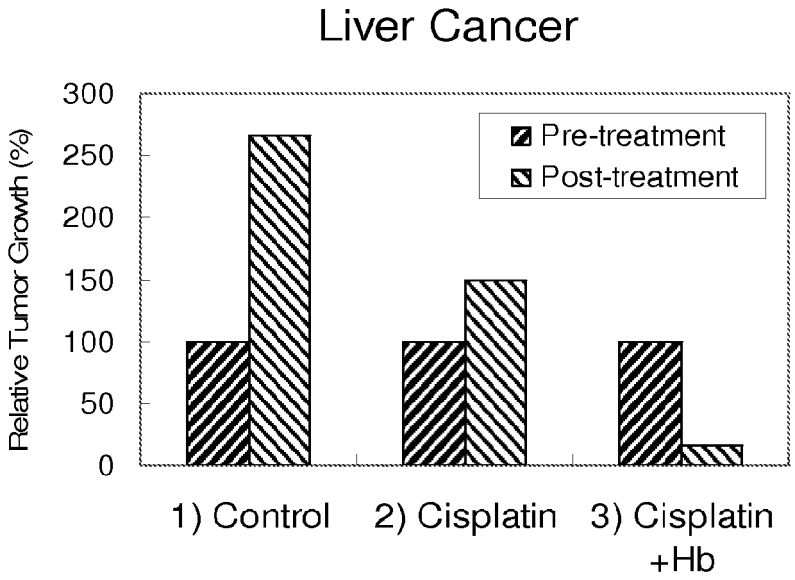
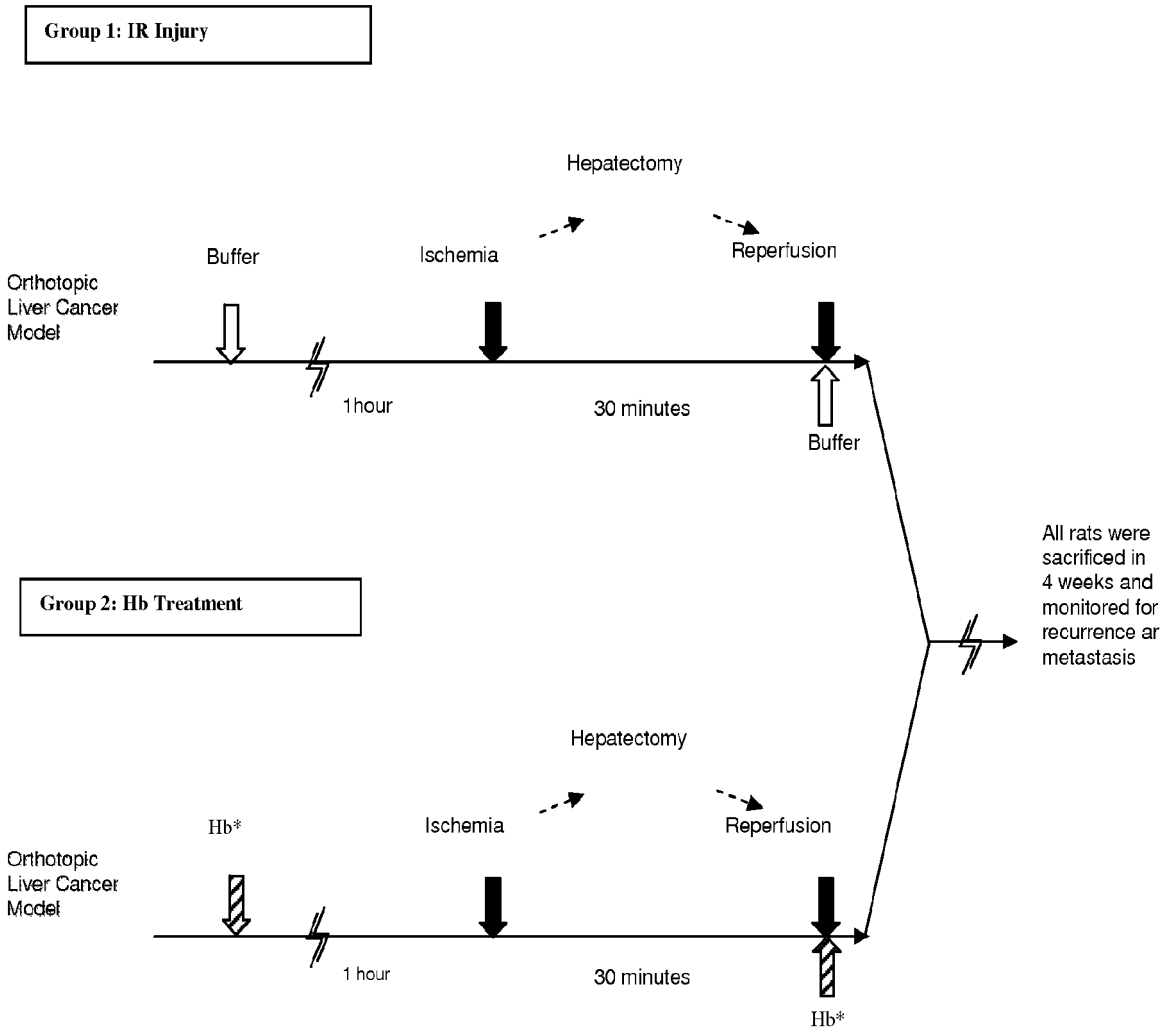

Liver Cancer
Pre-treatment
Post-treatment
Relative Tumor Growth (%)
300
250
200
150
100
50
0
1) Control
2) Cisplatin
3) Cisplatin +Hb

FIG. 16



*Hb= heat stable tetrameric hemoglobin

FIG. 17

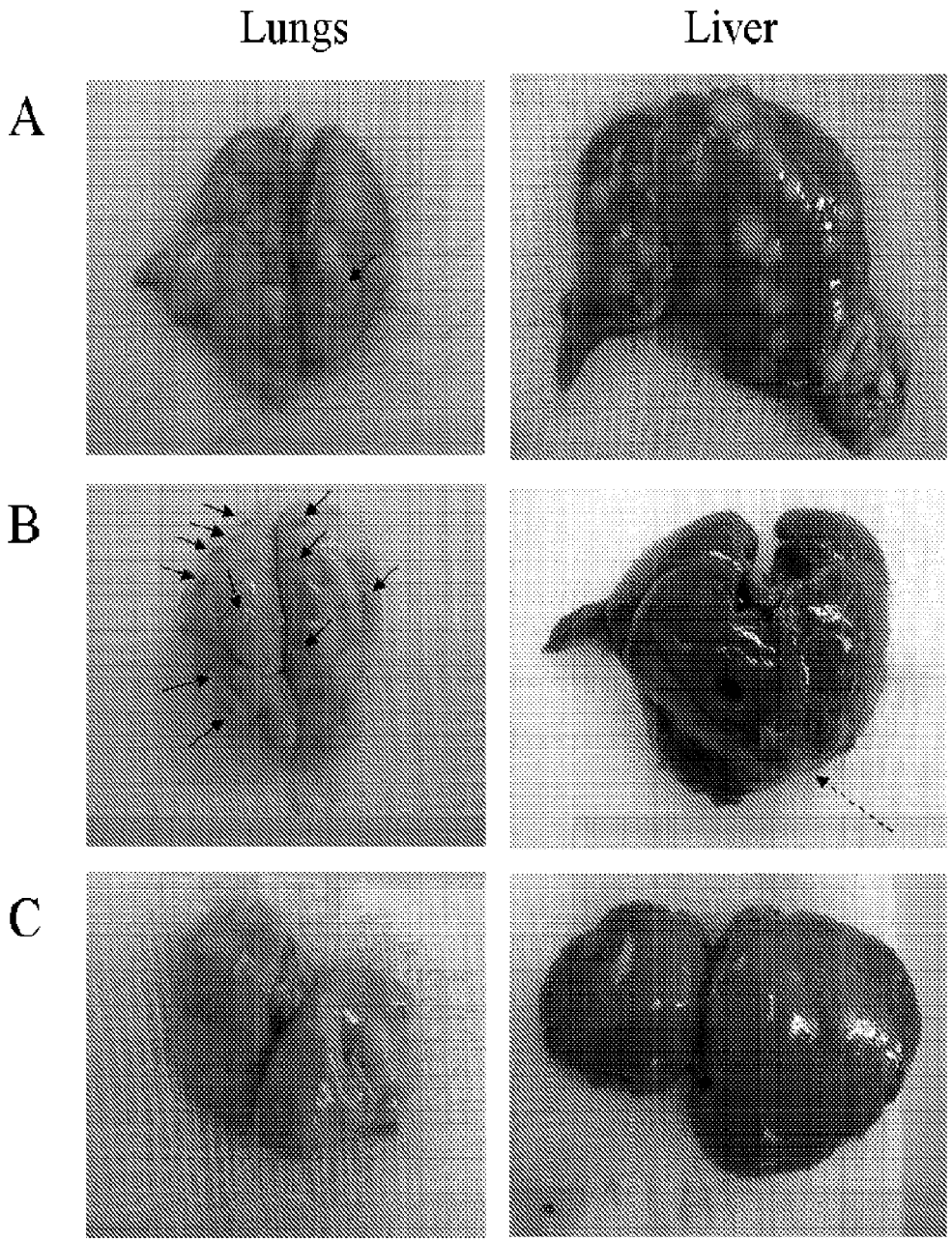


FIG. 18

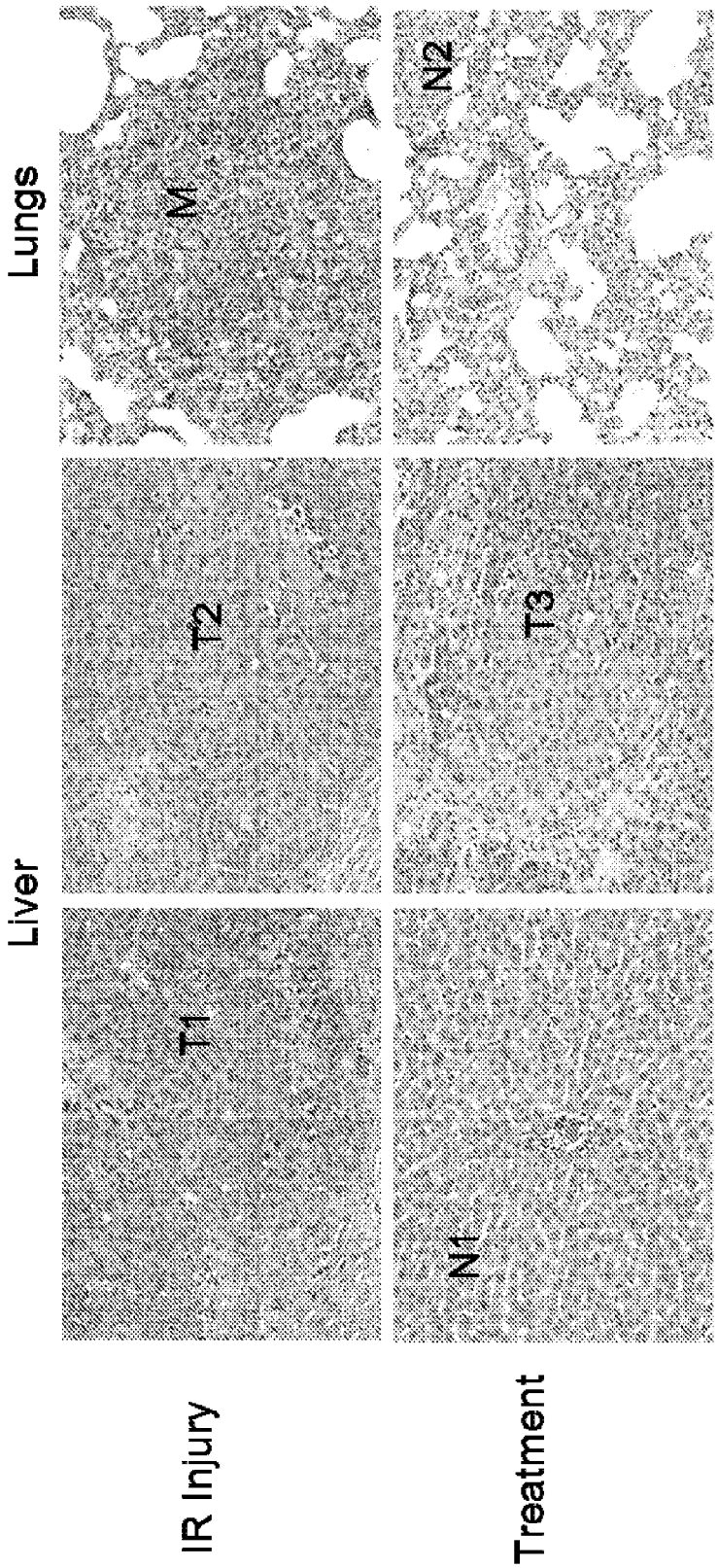
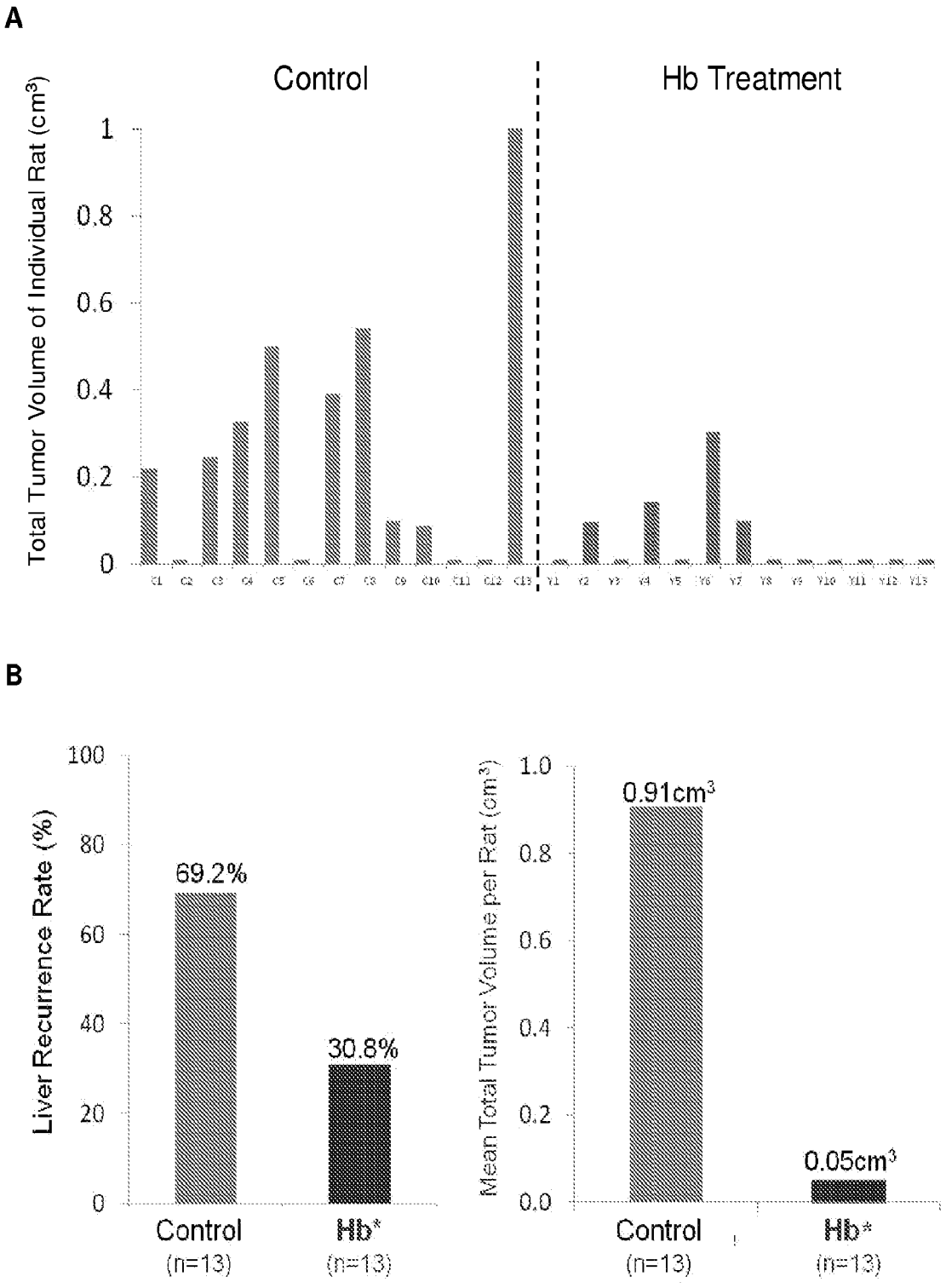


Fig. 19



*Hb= heat stable tetrameric hemoglobin

FIG. 20

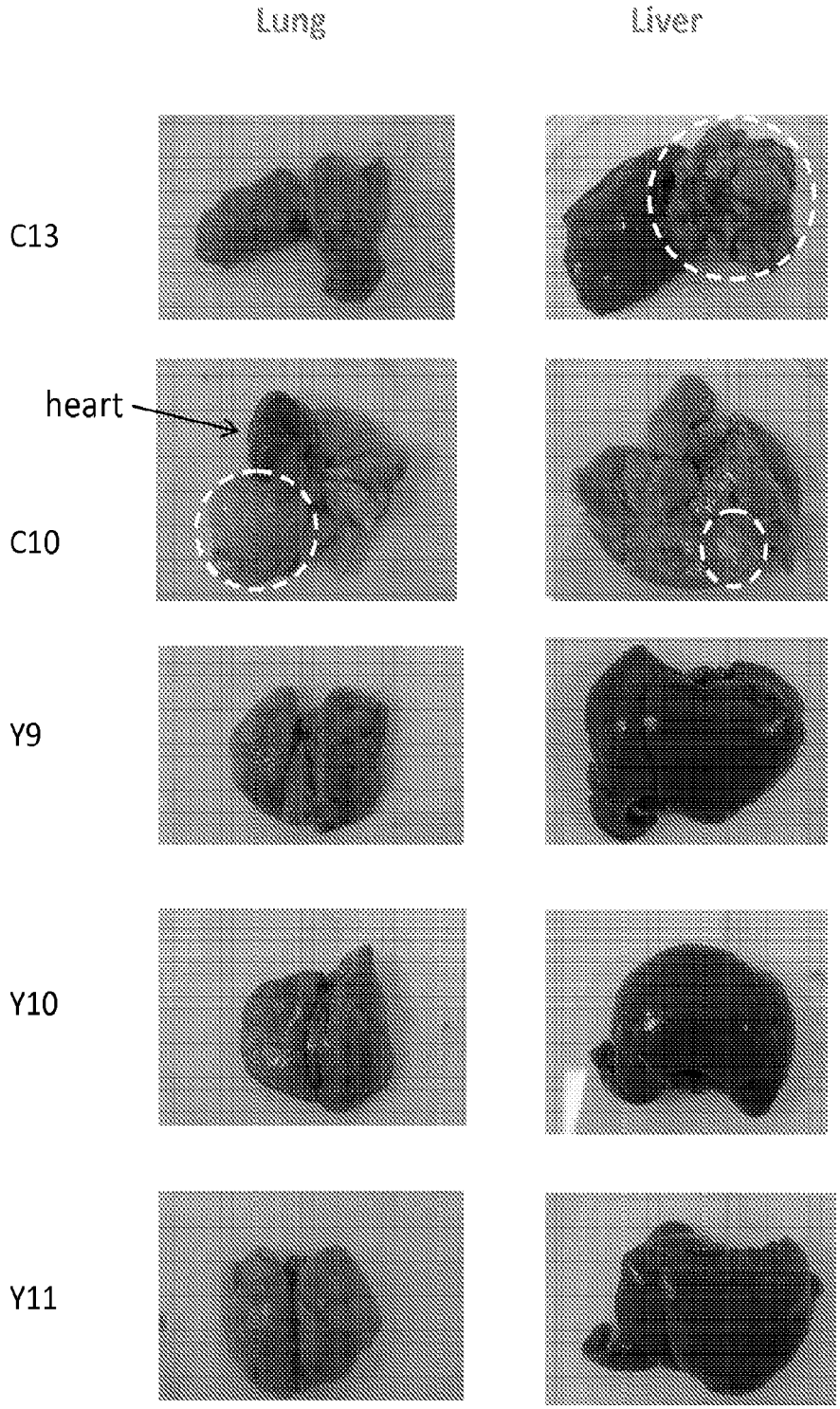


FIG. 21

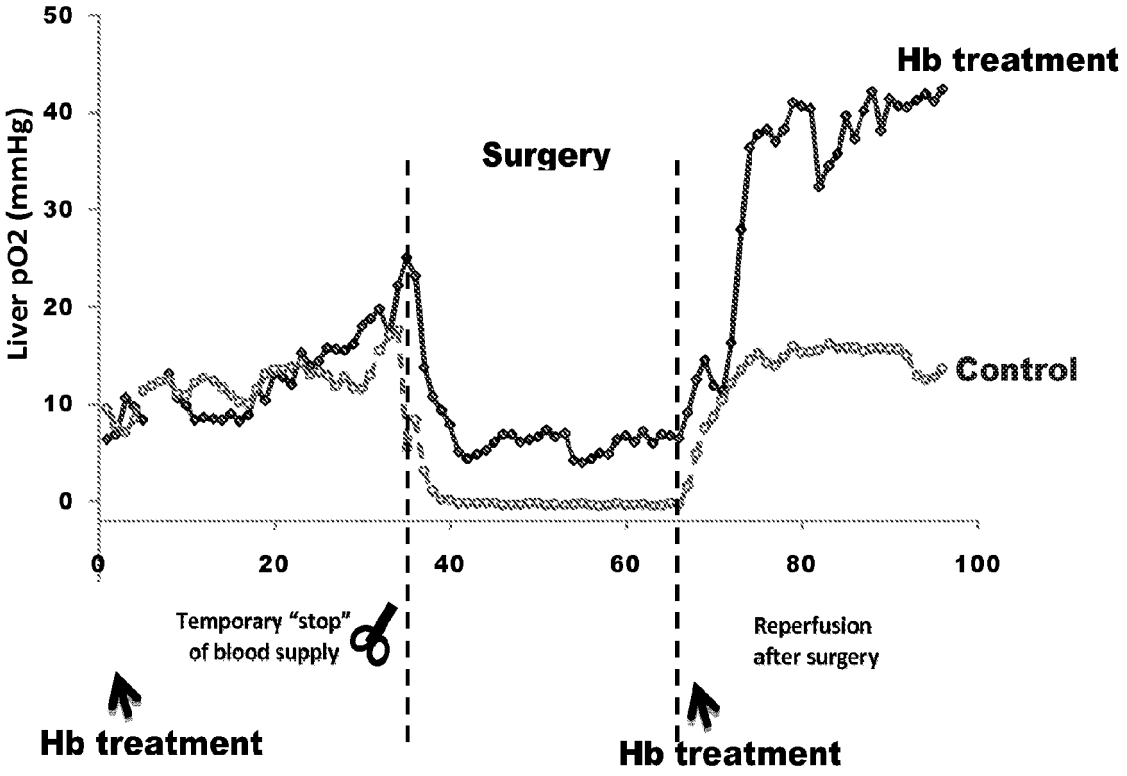

Liver pO2 (mmHg)
50
40
30
20
10
0
0
20
40
60
80
100
Hb treatment
Surgery
Control
Temporary "stop" of blood supply
Reperfusion after surgery
Hb treatment
Hb treatment

FIG. 22

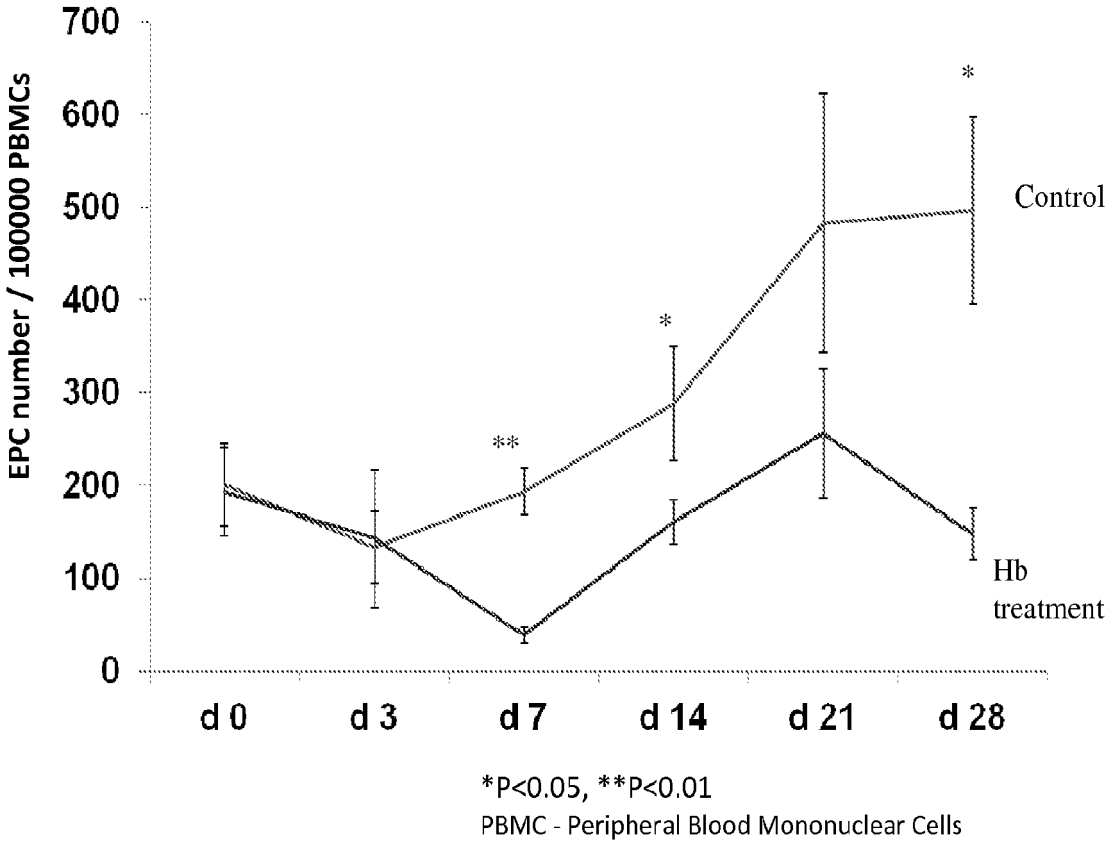

EPC number / 100000 PBMCs
700
600
500
400
300
200
100
0
d 0
d 3
d 7
d 14
d 21
d 28
**
*
*
Control
Hb
treatment
*P<0.05, **P<0.01
PBMC - Peripheral Blood Mononuclear Cells

FIG. 23

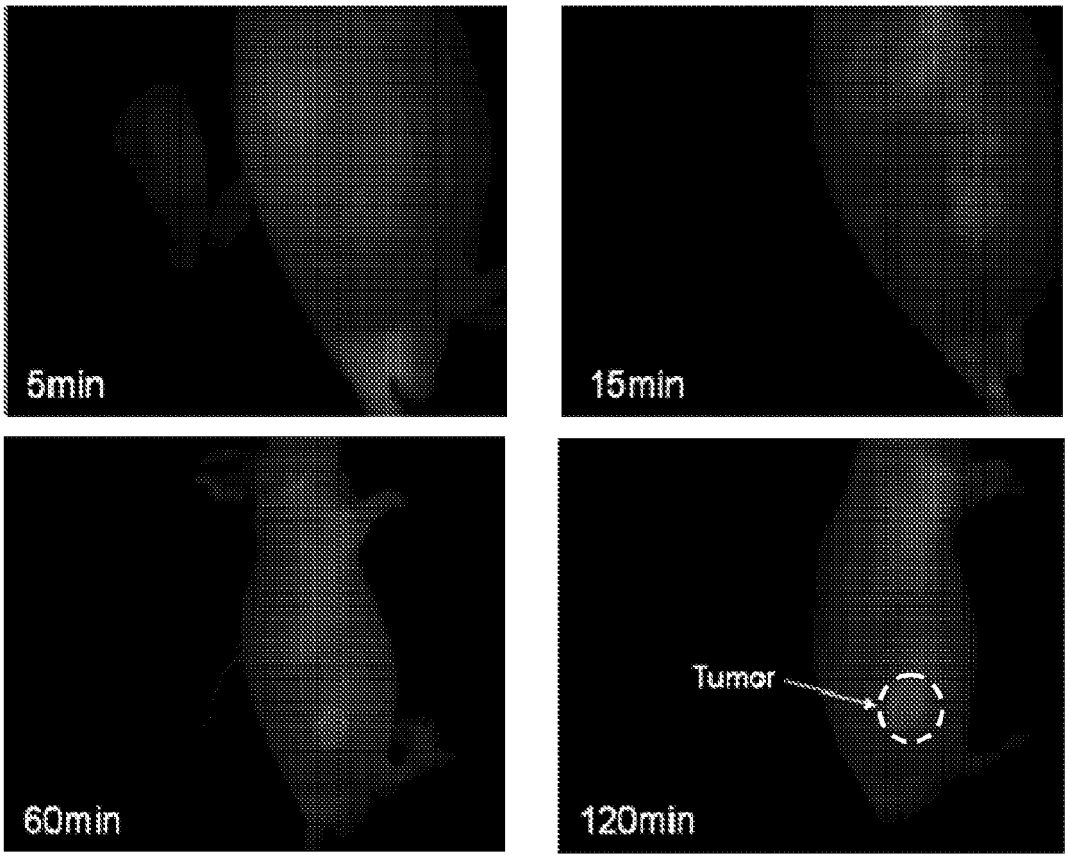


FIG. 24

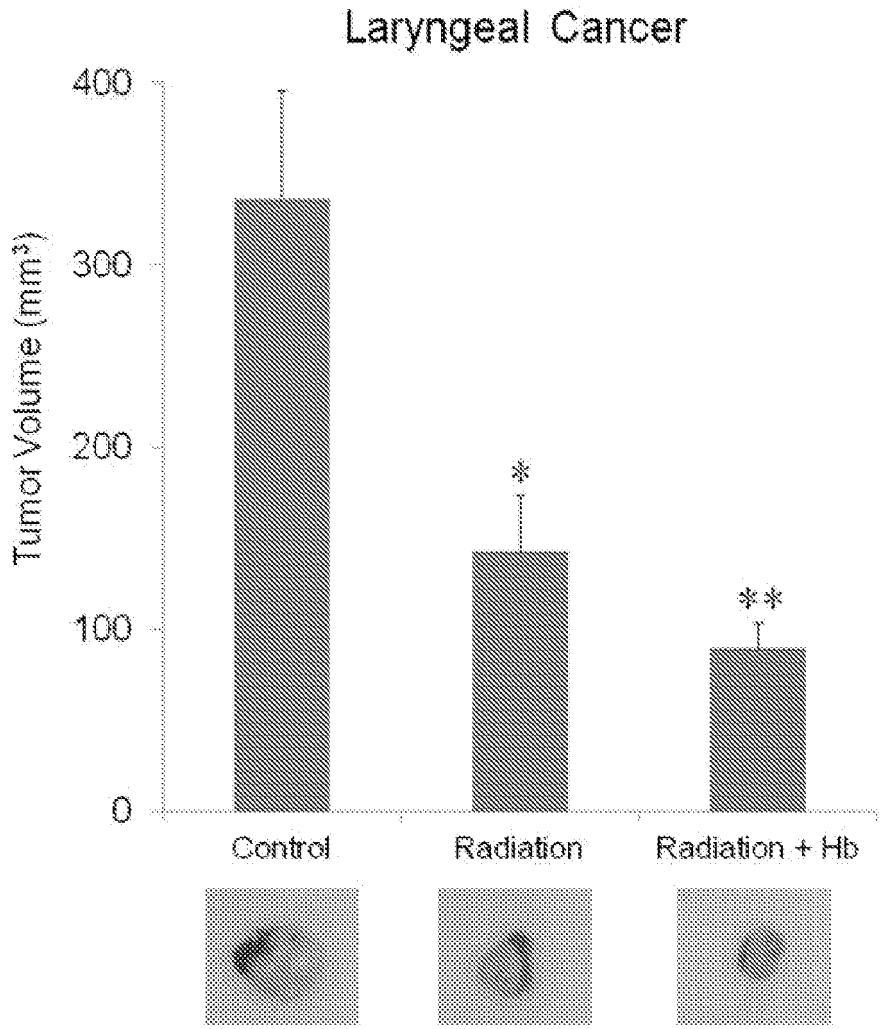

Laryngeal Cancer
Tumor Volume (mm³)
400
300
200
100
0
*
**
Control
Radiation
Radiation + Hb

FIG. 25

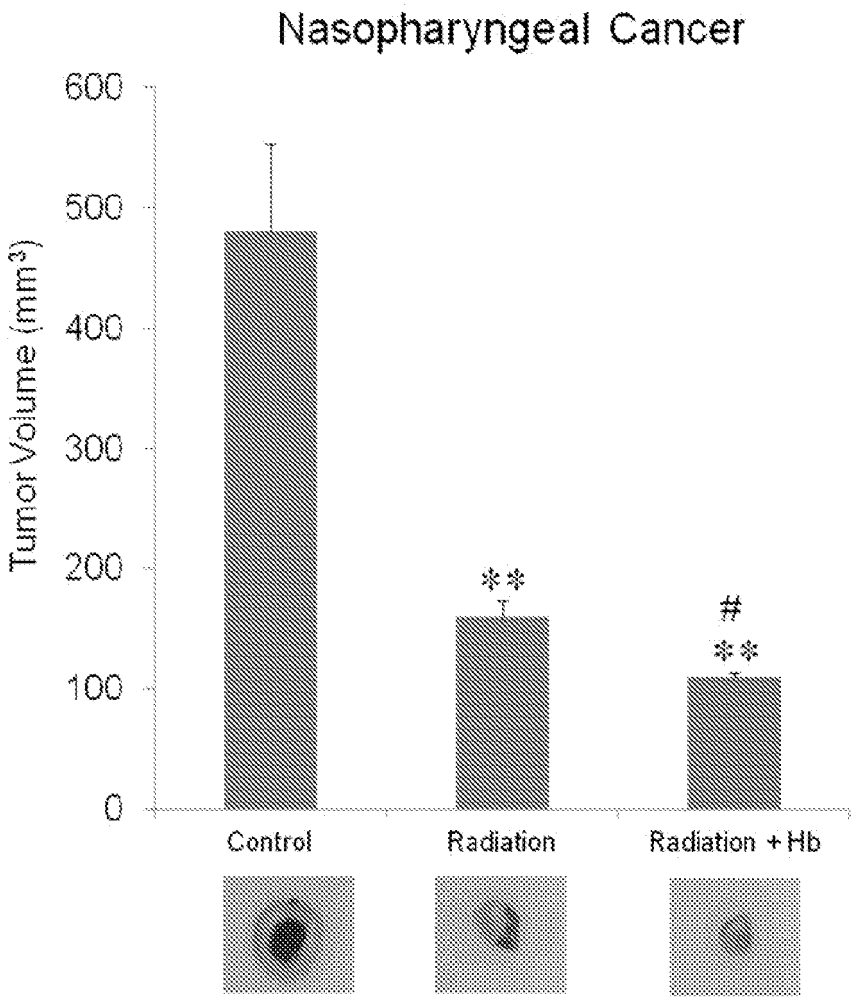

Nasopharyngeal Cancer
Tumor Volume (mm3)
600
500
400
300
200
100
0
**
#
**
Control
Radiation
Radiation + Hb

FIG. 26

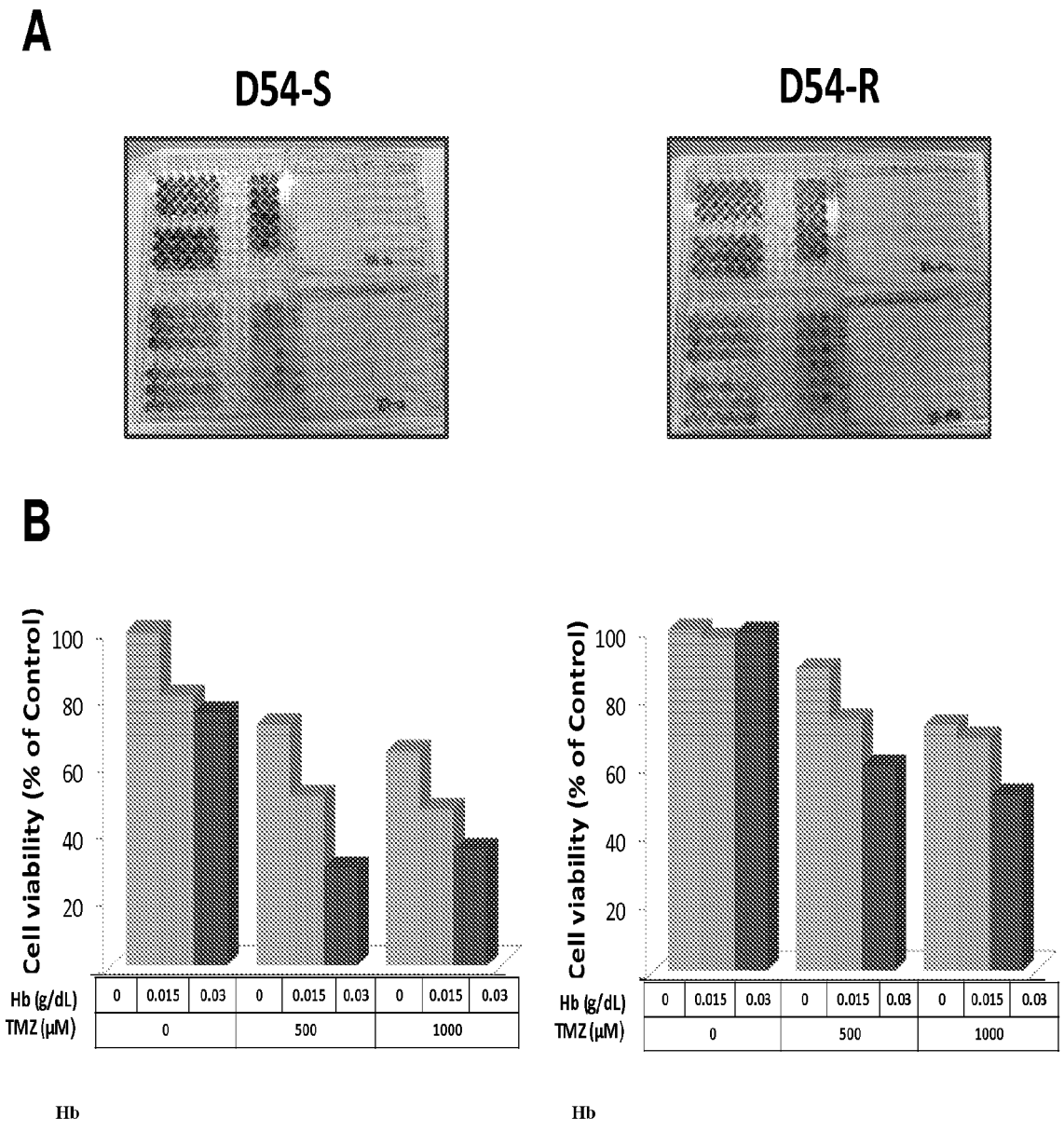

A
D54-S
D54-R
B
Cell viability (% of Control)
100
80
60
40
20
Hb (g/dL)
TMZ (μM)
0
0.015
0.03
0
0.015
0.03
0
0.015
0.03
0
500
1000
Hb
Cell viability (% of Control)
100
80
60
40
20
Hb (g/dL)
TMZ (μM)
0
0.015
0.03
0
0.015
0.03
0
0.015
0.03
0
500
1000
Hb

FIG. 27

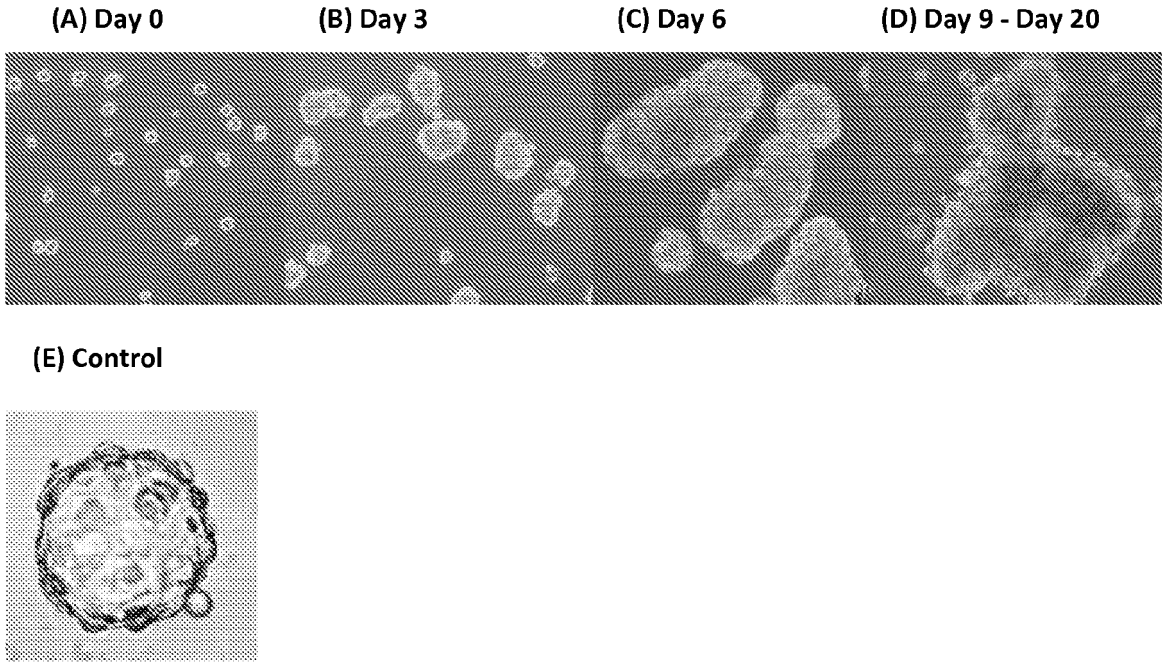


FIG. 28

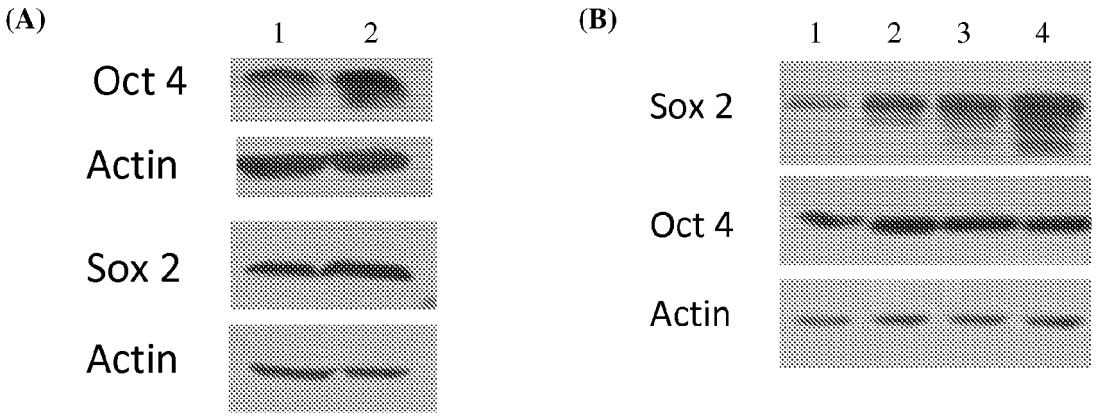


FIG. 29

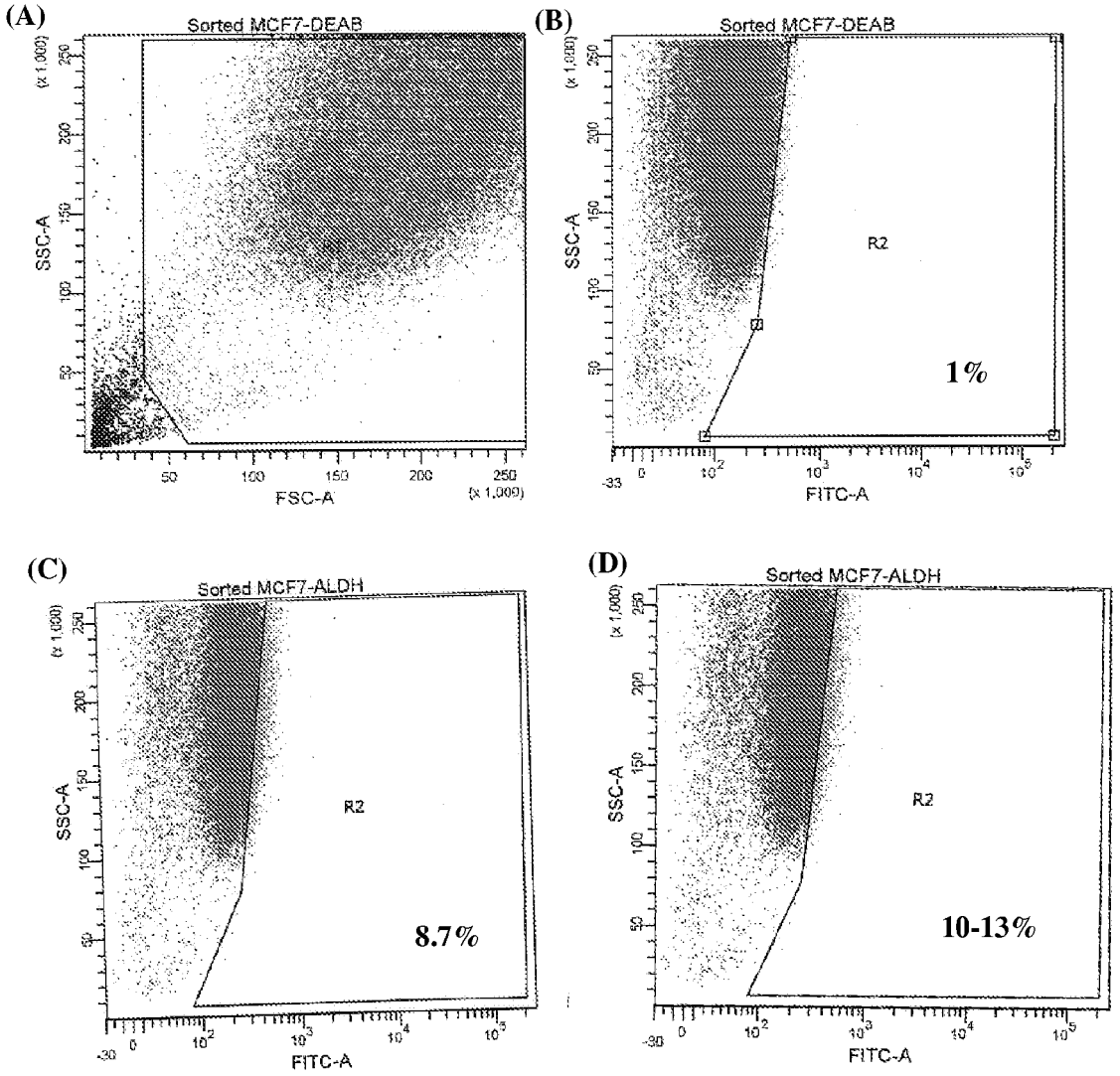

(A)
Sorted MCF7-DEAB
SSC-A
FSC-A
(x 1,000)
(B)
Sorted MCF7-DEAB
SSC-A
FITC-A
R2
1%
(C)
Sorted MCF7-ALDH
SSC-A
FITC-A
R2
8.7%
(D)
Sorted MCF7-ALDH
SSC-A
FITC-A
R2
10-13%

FIG. 30

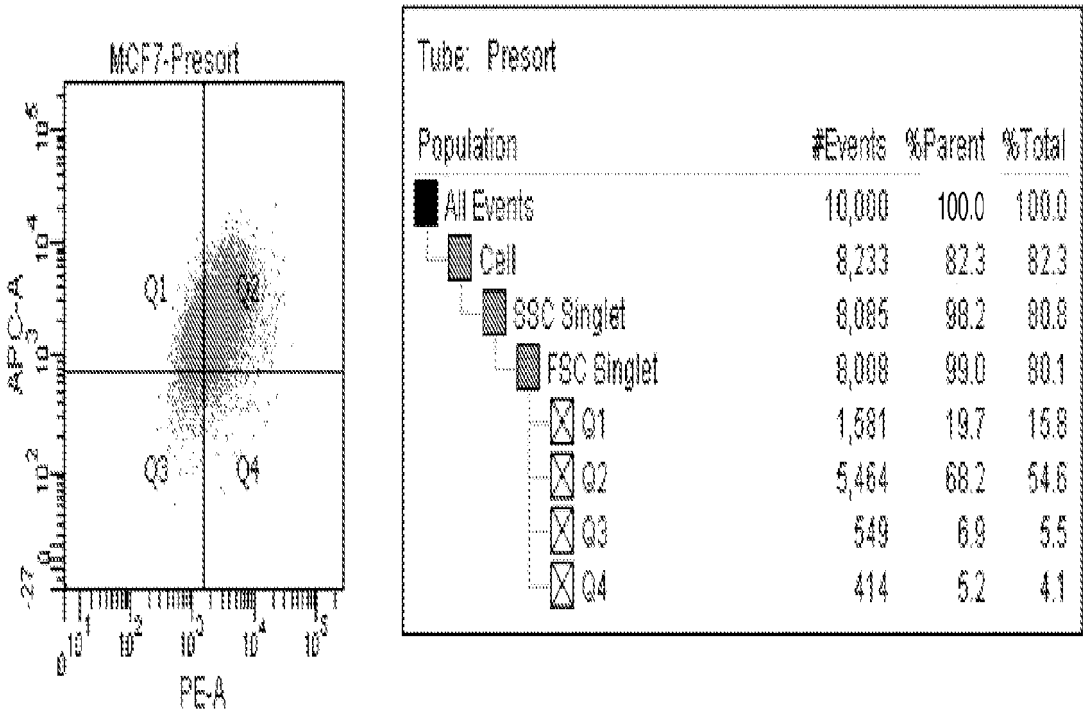

MCF7-Presort
APC-A
PE-A
Q1
Q2
Q3
Q4
Tube: Presort
Population #Events %Parent %Total
All Events 10,000 100.0 100.0
Cell 8,233 82.3 82.3
SSC Singlet 8,085 98.2 80.8
FSC Singlet 8,008 99.0 80.1
Q1 1,581 19.7 15.8
Q2 5,464 68.2 54.6
Q3 549 6.9 5.5
Q4 414 5.2 4.1

FIG. 31

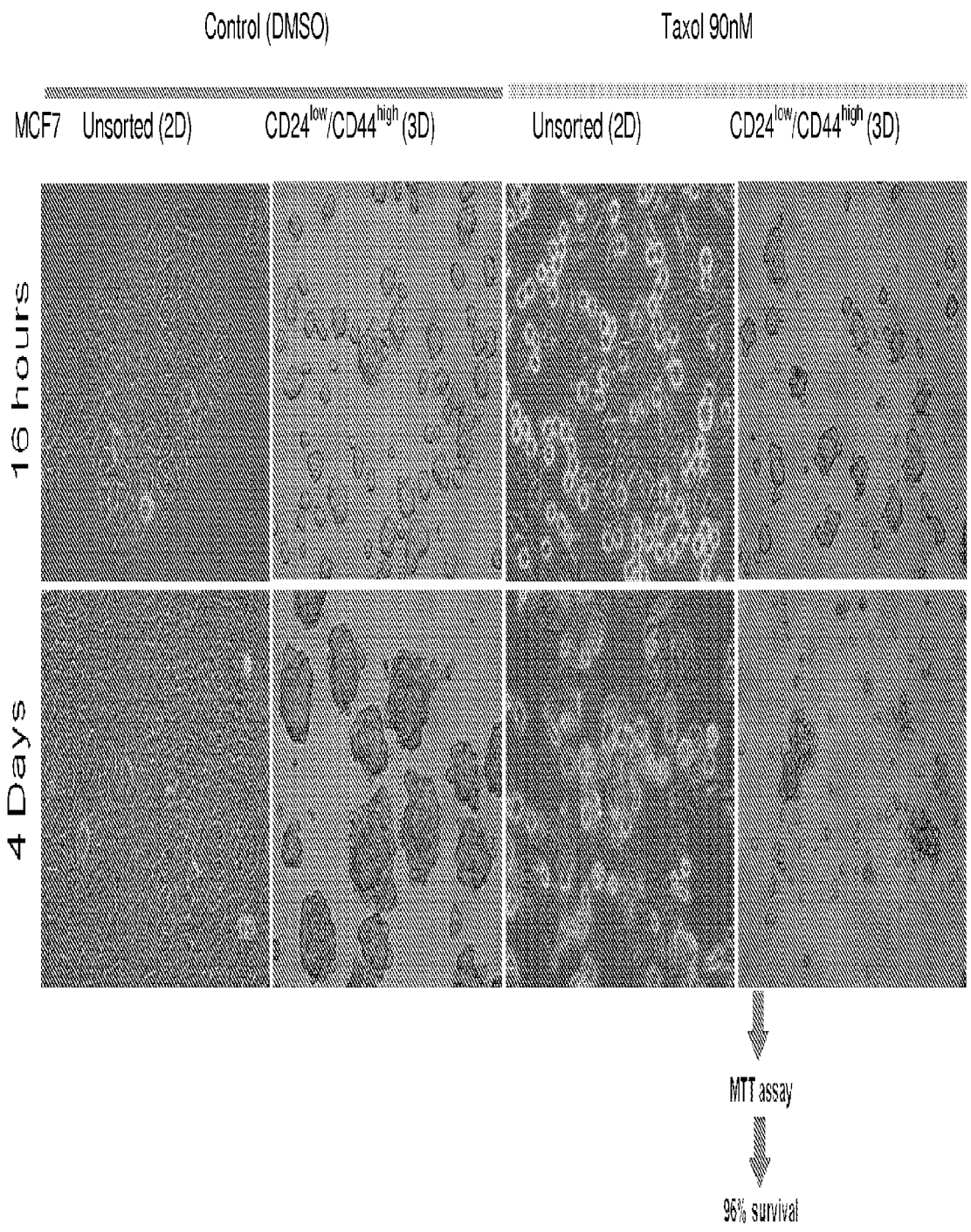

Control (DMSO)
Taxol 90nM
MCF7
Unsorted (2D)
CD24low/CD44high (3D)
Unsorted (2D)
CD24low/CD44high (3D)
16 hours
4 Days
MTT assay
95% survival

FIG. 32