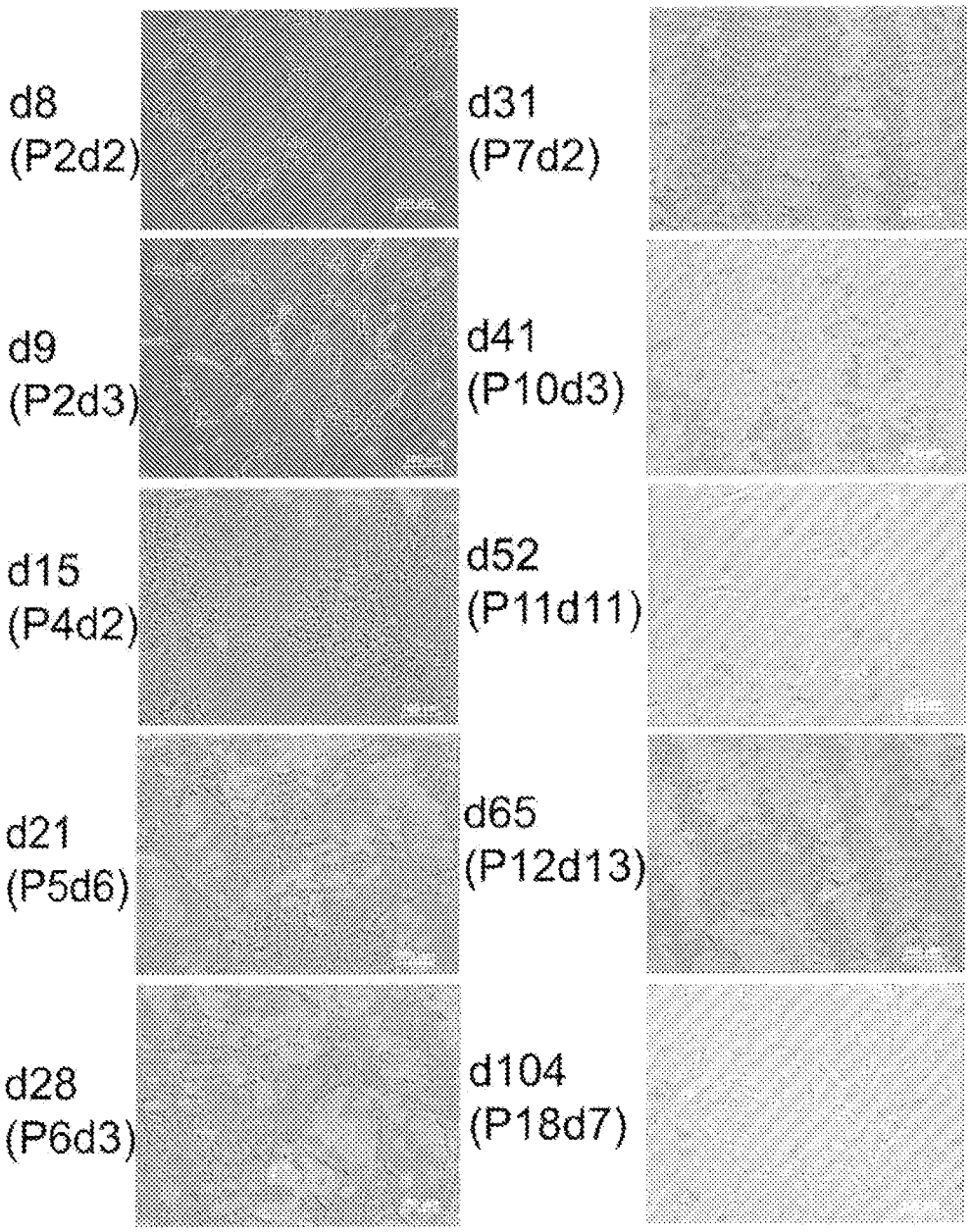

d8
(P2d2)
d31
(P7d2)
d9
(P2d3)
d41
(P10d3)
d15
(P4d2)
d52
(P11d11)
d21
(P5d6)
d65
(P12d13)
d28
(P6d3)
d104
(P18d7)

FIGURE 1

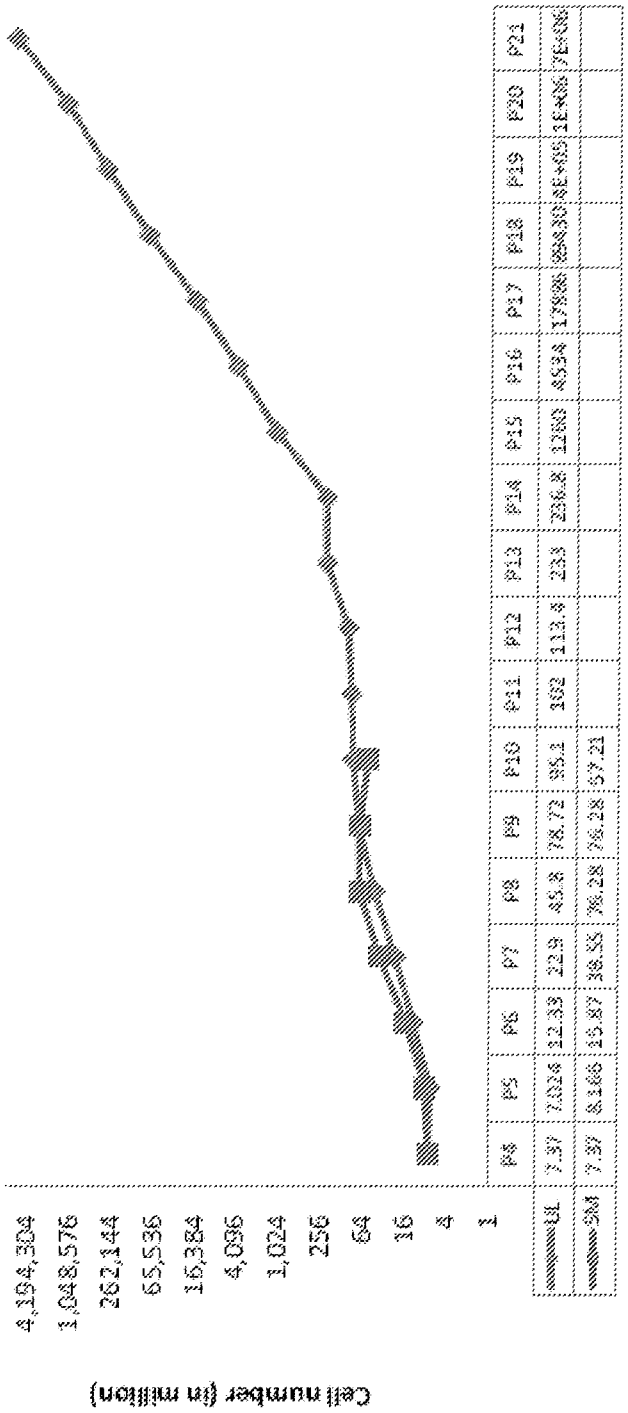

Growth curve
Cell number (in million)
4,194,304
1,048,576
262,144
65,536
16,384
4,096
1,024
256
64
16
4
1
P4 P5 P6 P7 P8 P9 P10 P11 P12 P13 P14 P15 P16 P17 P18 P19 P20 P21
GL 7.37 7.024 12.33 22.9 45.8 78.72 95.2 102 112.8 233 236.8 1260 4524 17592 6E+05 1E+06 7E+06
SM 7.37 8.166 15.87 36.55 76.28 76.28 97.21

FIGURE 2

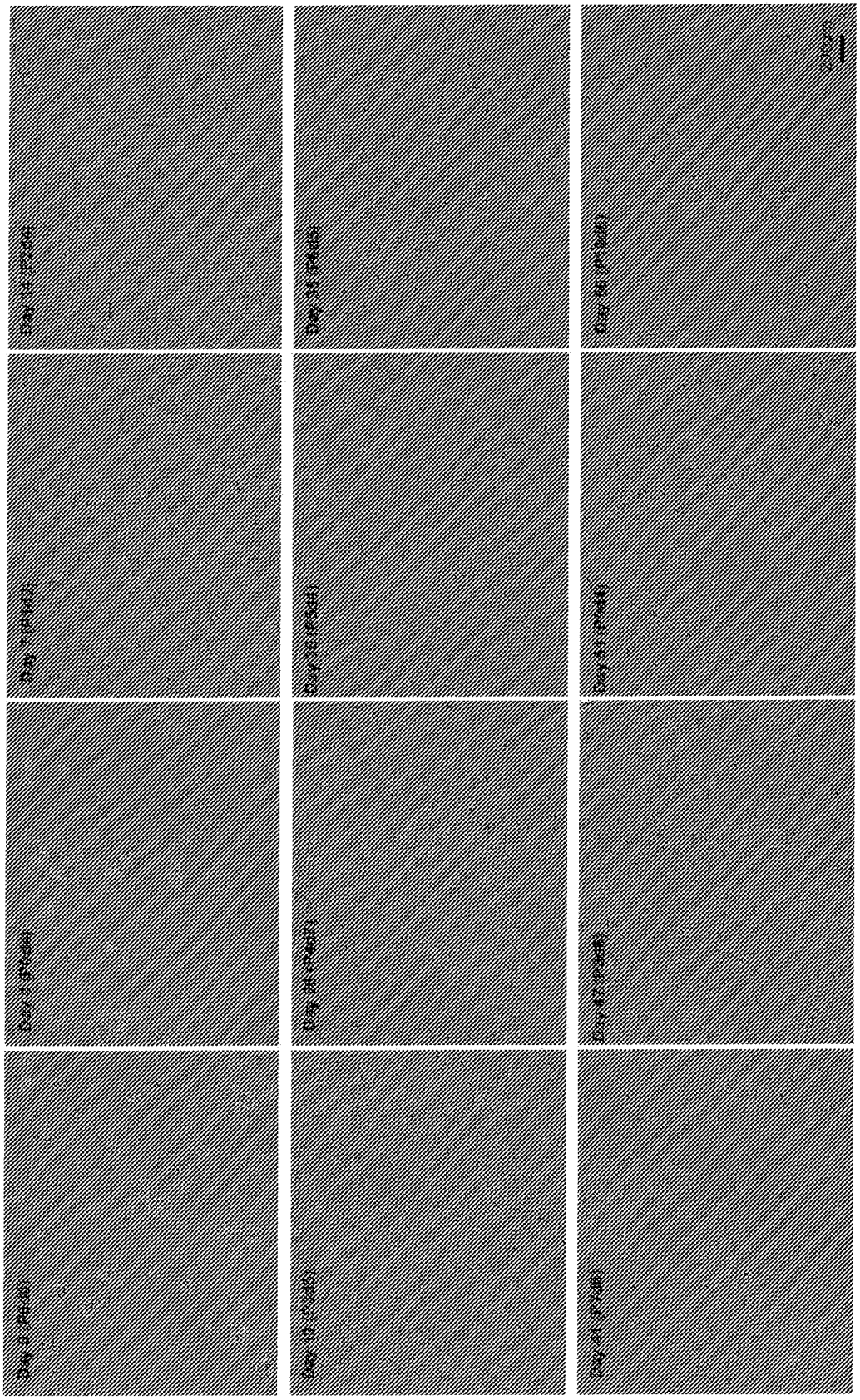
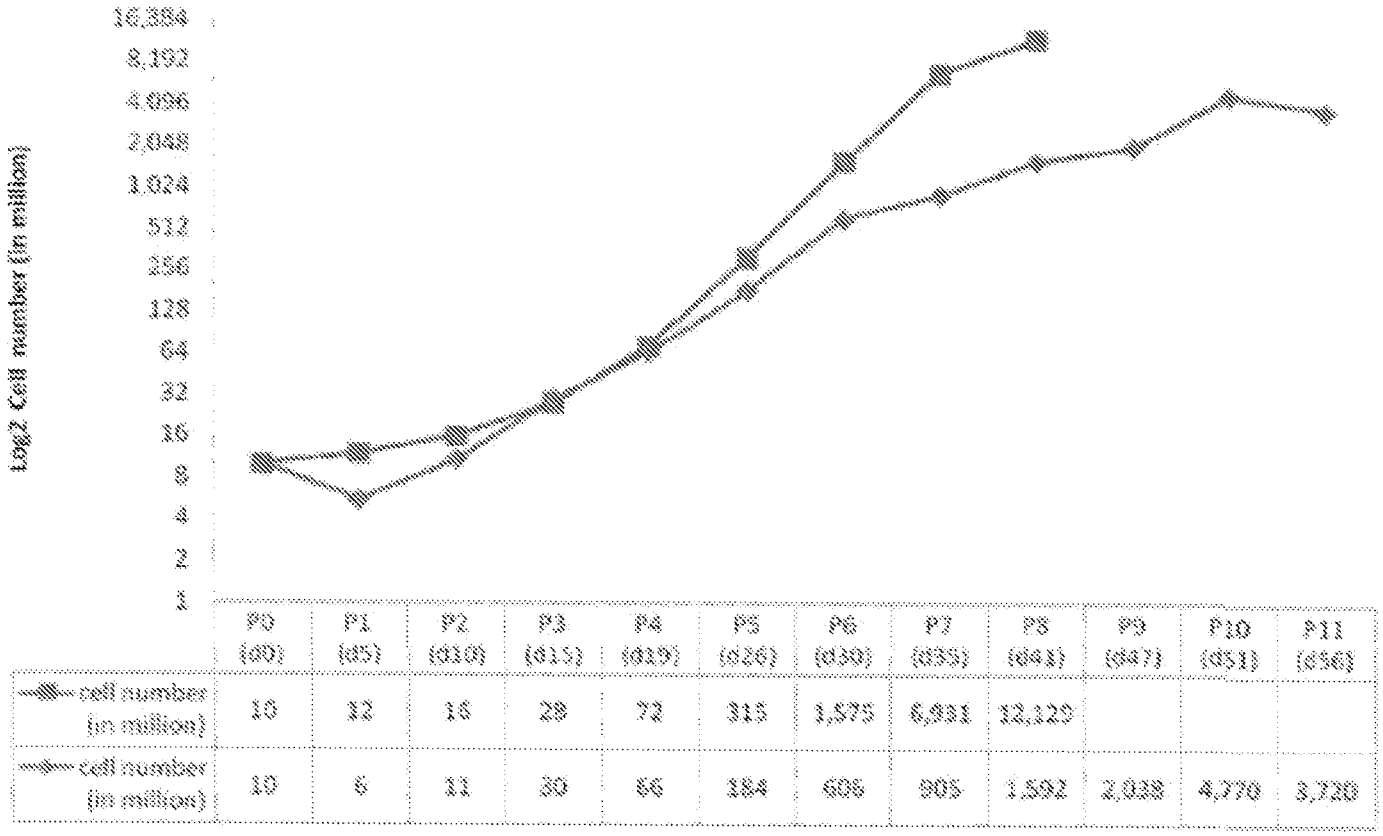

Day 7 (P24)
Day 14 (P31)
Day 28 (P45)
Day 35 (P52)
Day 42 (P59)
Day 49 (P66)
Day 56 (P73)
Day 63 (P80)
Day 70 (P87)
Day 77 (P94)
Day 84 (P101)
Day 91 (P108)
200 µm

FIGURE 3



	P0 (d0)	P1 (d5)	P2 (d10)	P3 (d15)	P4 (d19)	P5 (d26)	P6 (d30)	P7 (d35)	P8 (d41)	P9 (d47)	P10 (d51)	P11 (d56)
cell number (in million)	10	12	16	29	72	315	1,575	6,931	12,129			
cell number (in million)	10	6	11	30	66	184	606	905	1,502	2,038	4,770	3,720

The blue donation was used in patients at ~P5, resulting in visual improvements

FIGURE 4

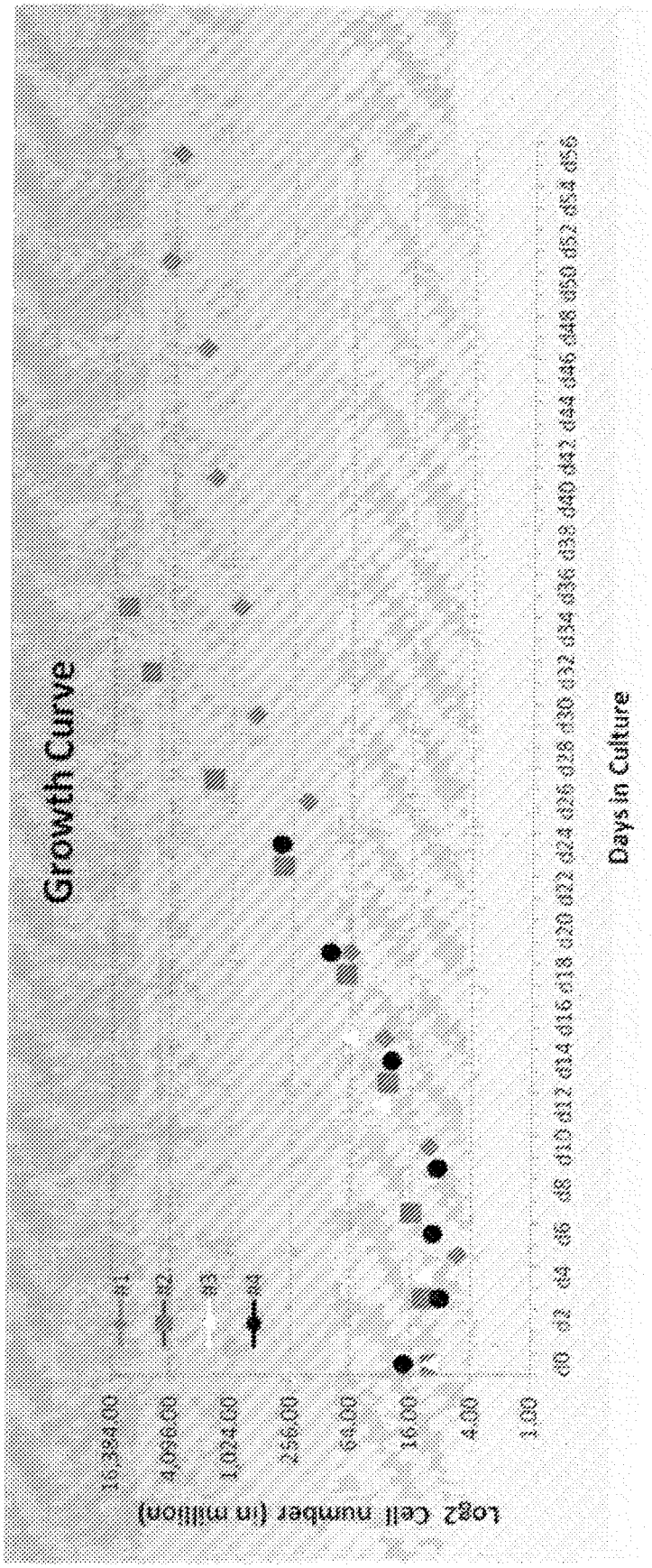
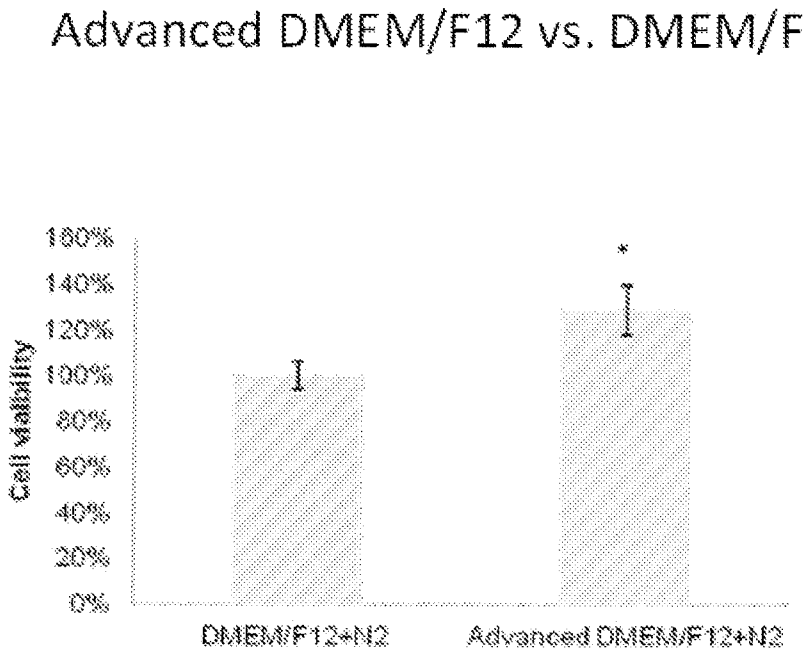

Growth Curve
log2 Cell number (in million)
16,384.00
4,096.00
1,024.00
256.00
64.00
16.00
4.00
1.00
#1
#2
#3
#4
d0 d2 d4 d6 d8 d10 d12 d14 d16 d18 d20 d22 d24 d26 d28 d30 d32 d34 d36 d38 d40 d42 d44 d46 d48 d50 d52 d54 d56
Days in Culture

FIGURE 5

A


Advanced DMEM/F12 vs. DMEM/F12
Cell Viability
180%
140%
120%
100%
80%
60%
40%
20%
0%
*
DMEM/F12+N2
Advanced DMEM/F12+N2

B

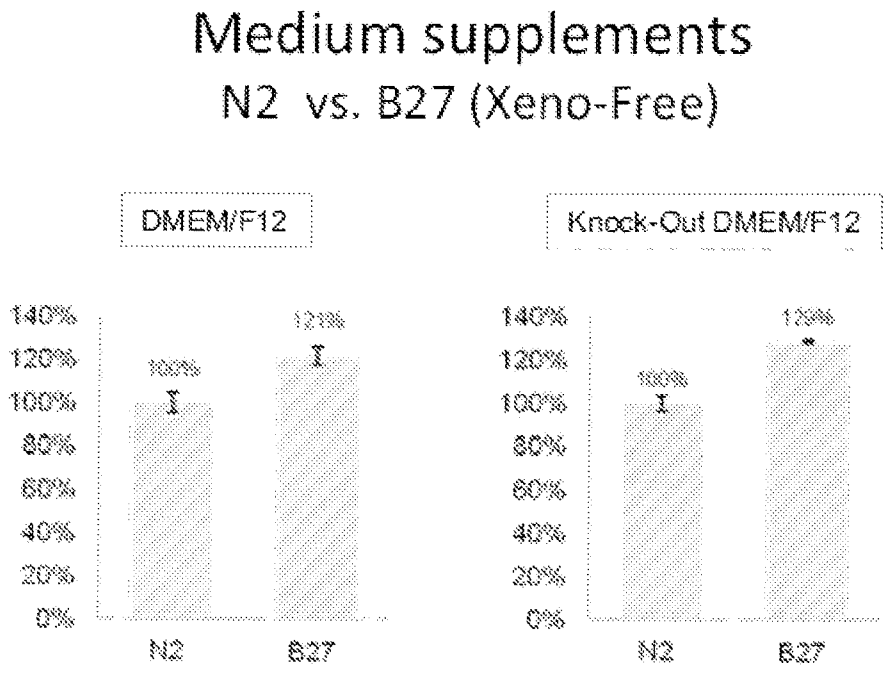
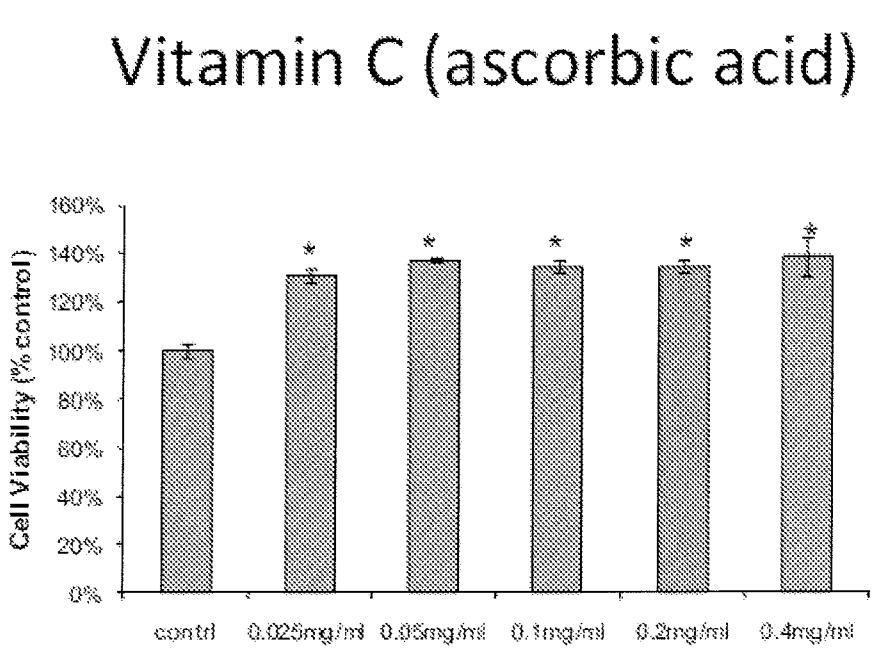
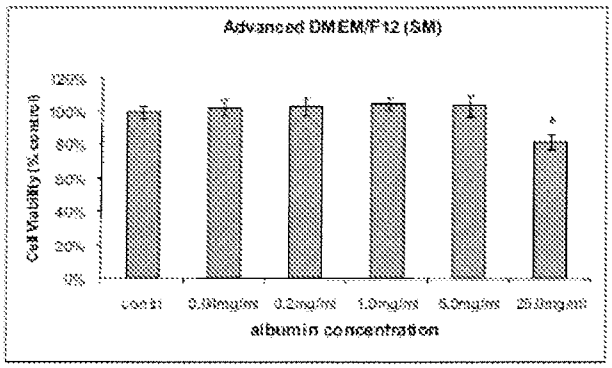

Medium supplements
N2 vs. B27 (Xeno-Free)
DMEM/F12
Knock-Out DMEM/F12
140%
120%
100%
80%
60%
40%
20%
0%
100%
121%
*
N2
B27

FIGURE 6

A


Vitamin C (ascorbic acid)
Cell Viability (% control)
160%
140%
120%
100%
80%
60%
40%
20%
0%
contrl
0.025mg/ml
0.05mg/ml
0.1mg/ml
0.2mg/ml
0.4mg/ml

B


Advanced DMEM/F12 (SM)
albumin concentration

C

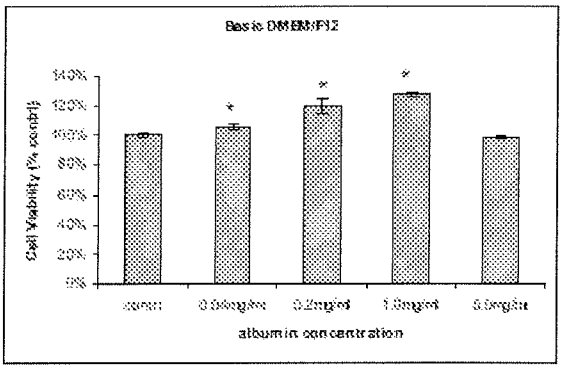

Basic DMEM/F12
albumin concentration

FIGURE 7

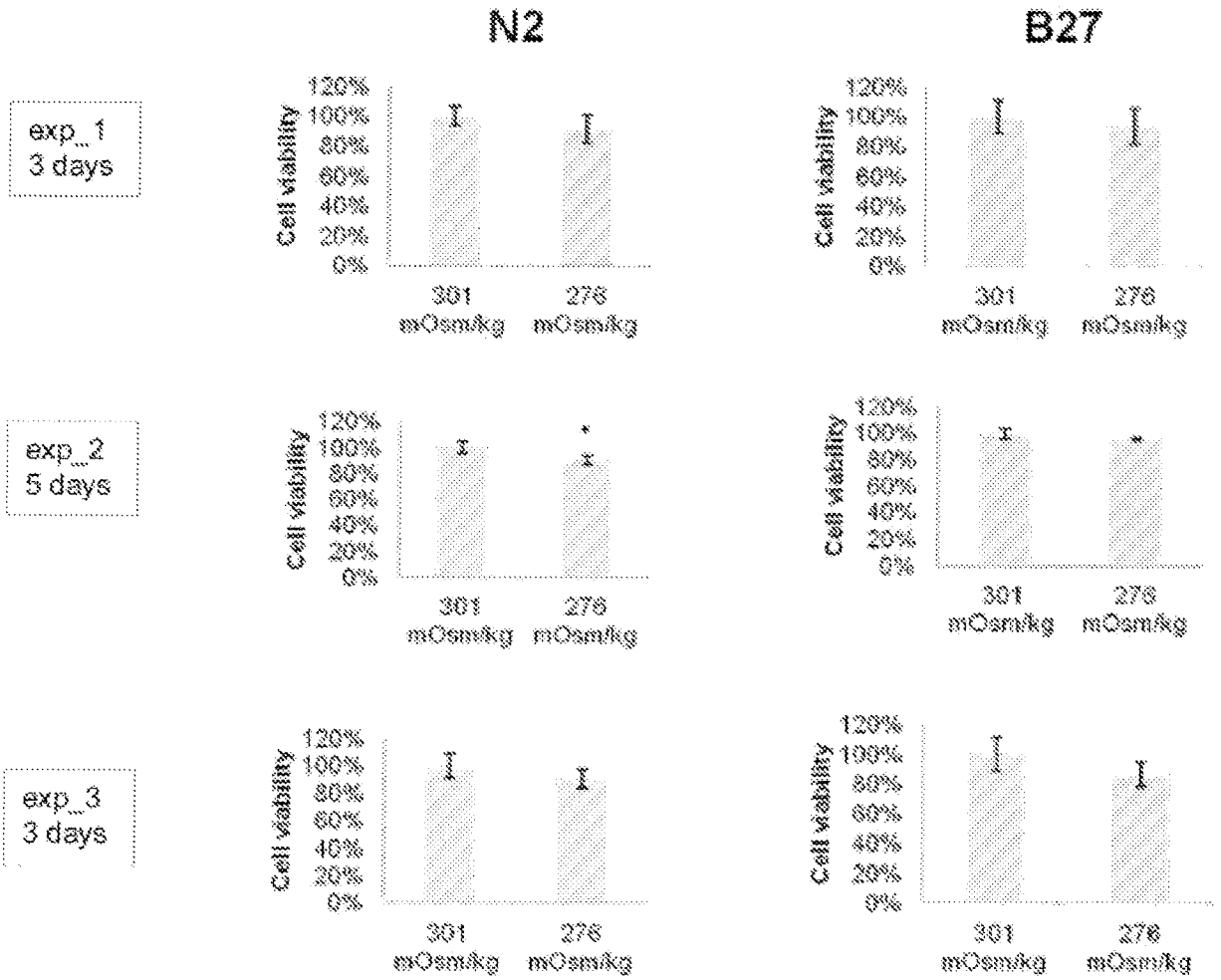

N2
B27
exp_1
3 days
exp_2
5 days
exp_3
3 days
Cell viability
120%
100%
80%
60%
40%
20%
0%
301
mOsm/kg
276
mOsm/kg
*

FIGURE 8

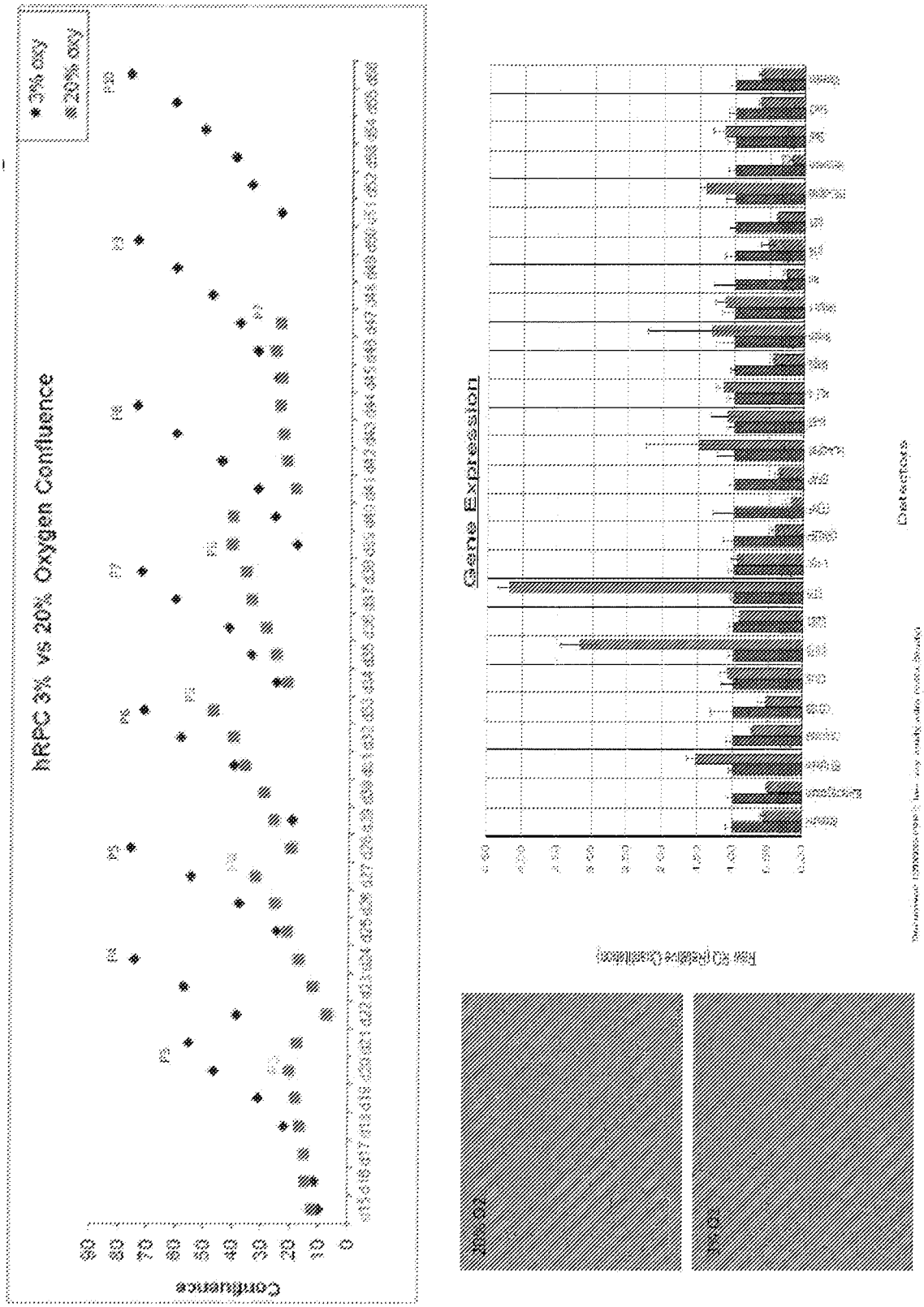

hRPC 3% vs 20% Oxygen Confluence
3% oxy
20% oxy
Confluence
Gene Expression
20% O2
3% O2

FIGURE 9

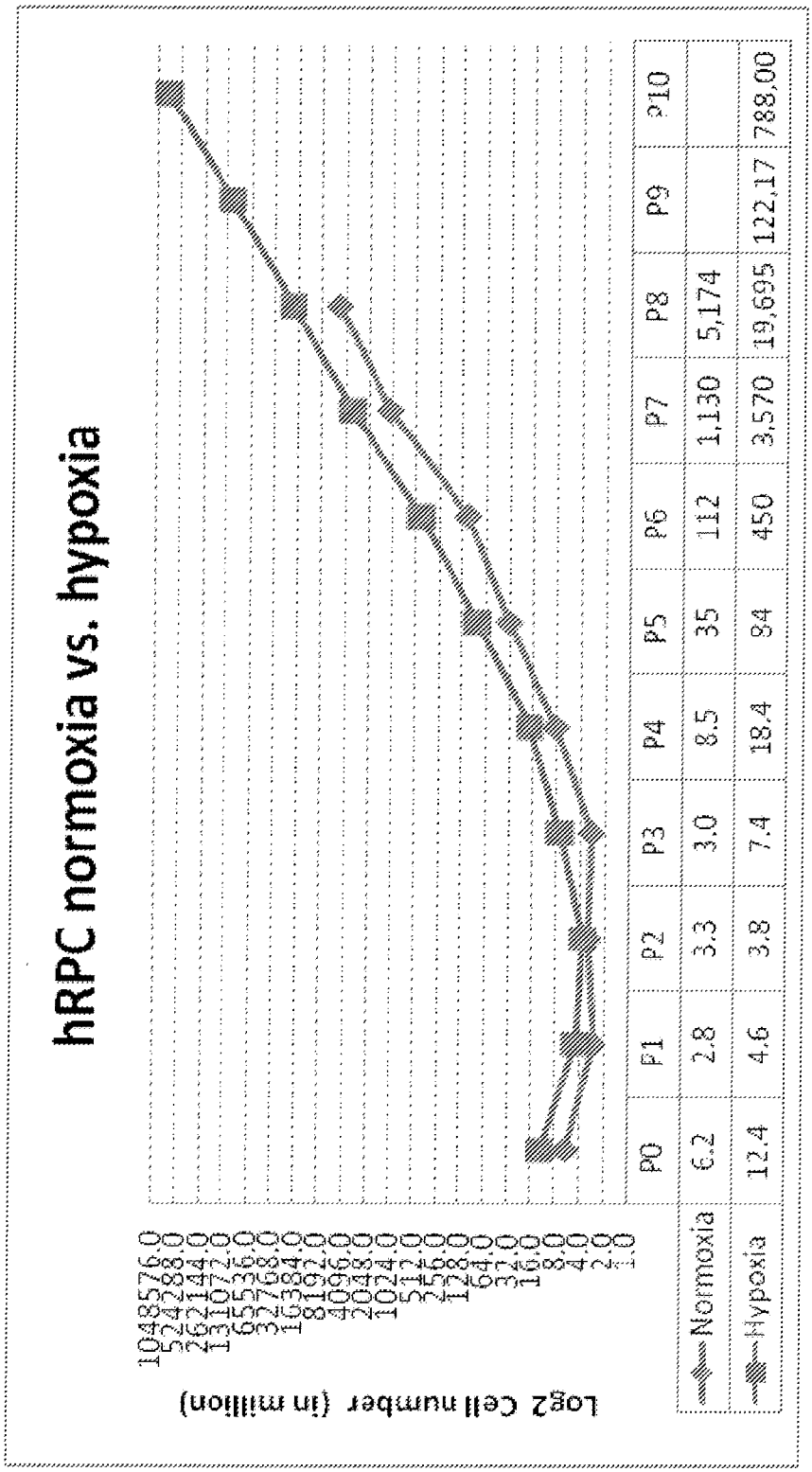

hRPC normoxia vs. hypoxia
log2 Cell number (in million)
1048576,00
524288,00
262144,00
131072,00
65536,00
32768,00
16384,00
8192,00
4096,00
2048,00
1024,00
512,00
256,00
128,00
64,00
32,00
16,00
8,00
4,00
2,00
1,00
P0 P1 P2 P3 P4 P5 P6 P7 P8 P9 P10
Normoxia 6.2 2.8 3.3 3.0 8.5 35 112 1,130 5,174
Hypoxia 12.4 4.6 3.8 7.4 18.4 84 450 3,570 19,695 122,17 788,00

FIGURE 10

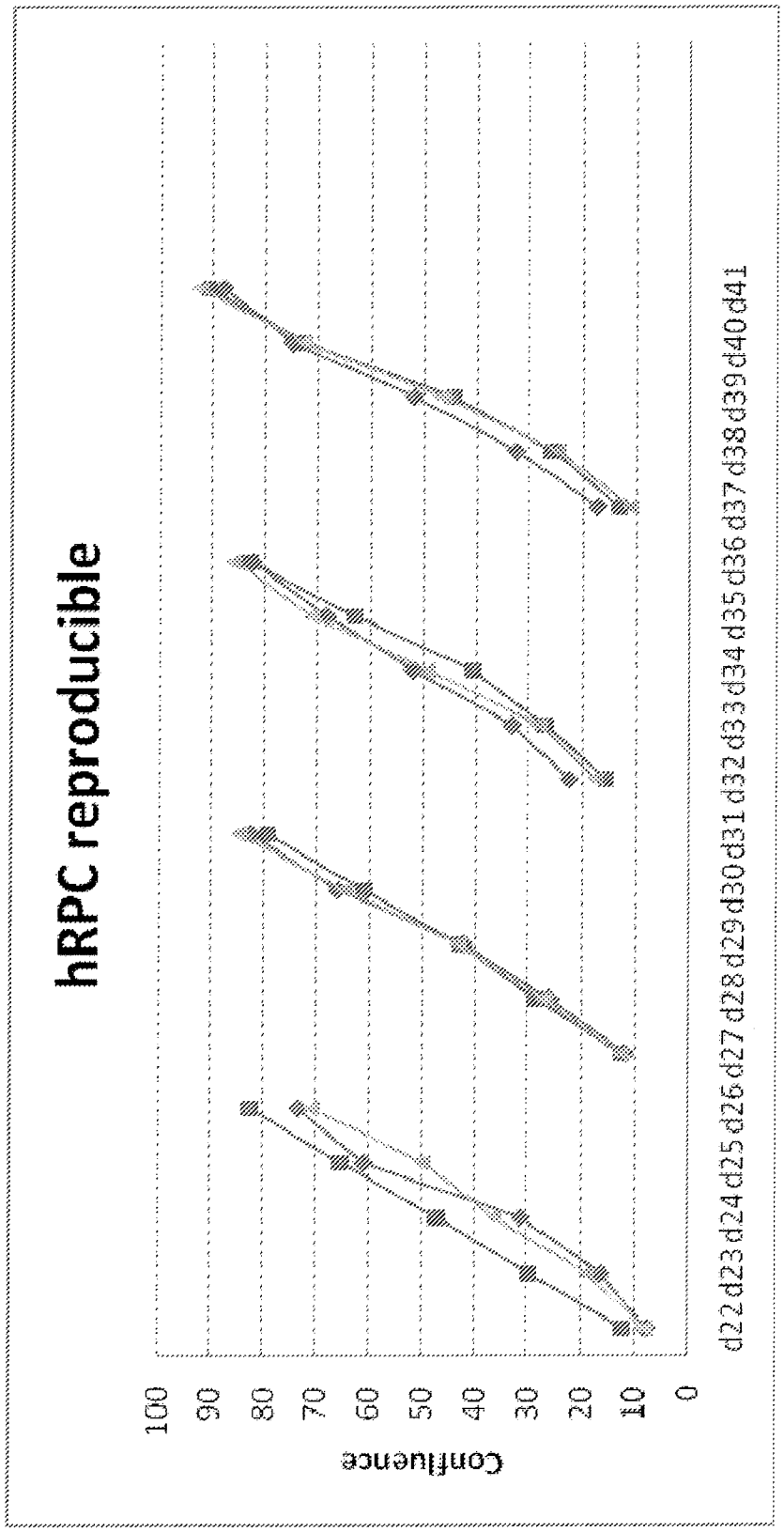

hRPC reproducible
Confluence
100
90
80
70
60
50
40
30
20
10
0
d22 d23 d24 d25 d26 d27 d28 d29 d30 d31 d32 d33 d34 d35 d36 d37 d38 d39 d40 d41

FIGURE 11

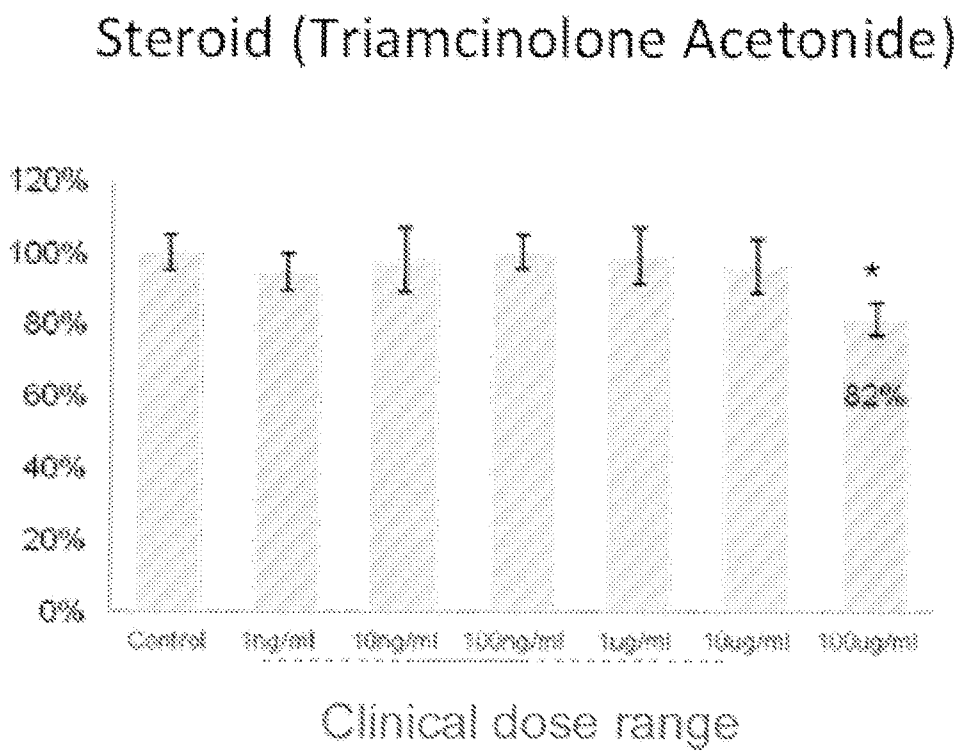
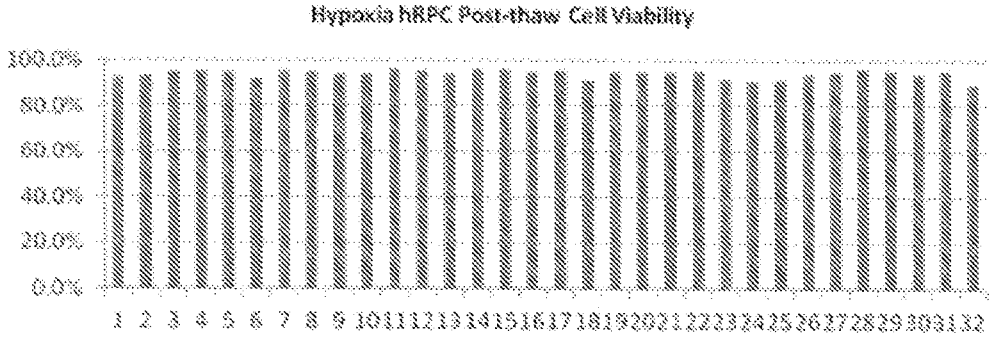
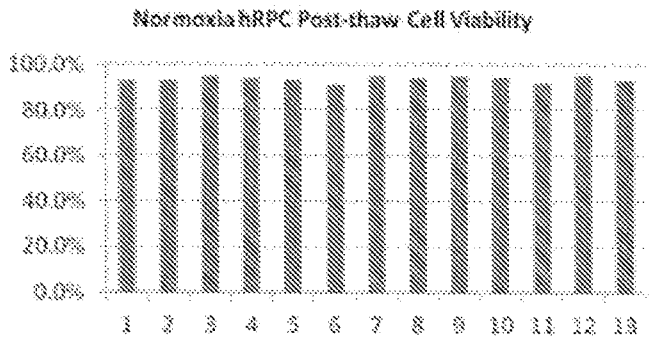

Steroid (Triamcinolone Acetonide)
120%
100%
80%
60%
40%
20%
0%
*
82%
Control
1ng/ml
10ng/ml
100ng/ml
1ug/ml
10ug/ml
100ug/ml
Clinical dose range

FIGURE 12

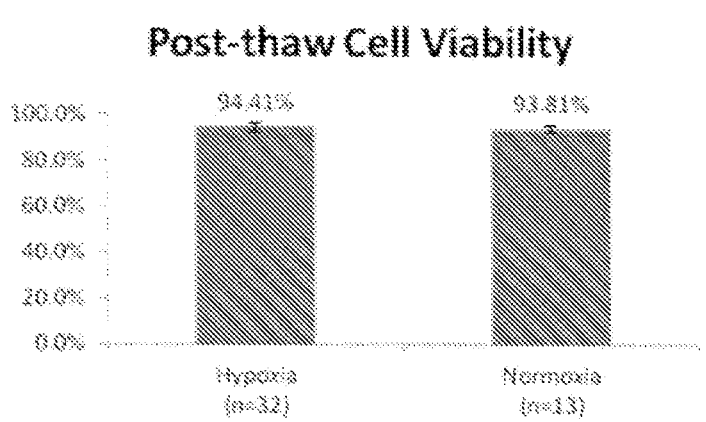
A



B



C



D

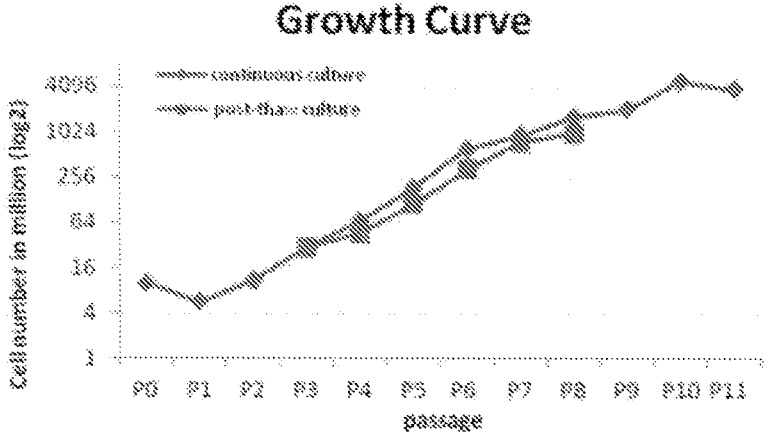


FIGURE 13

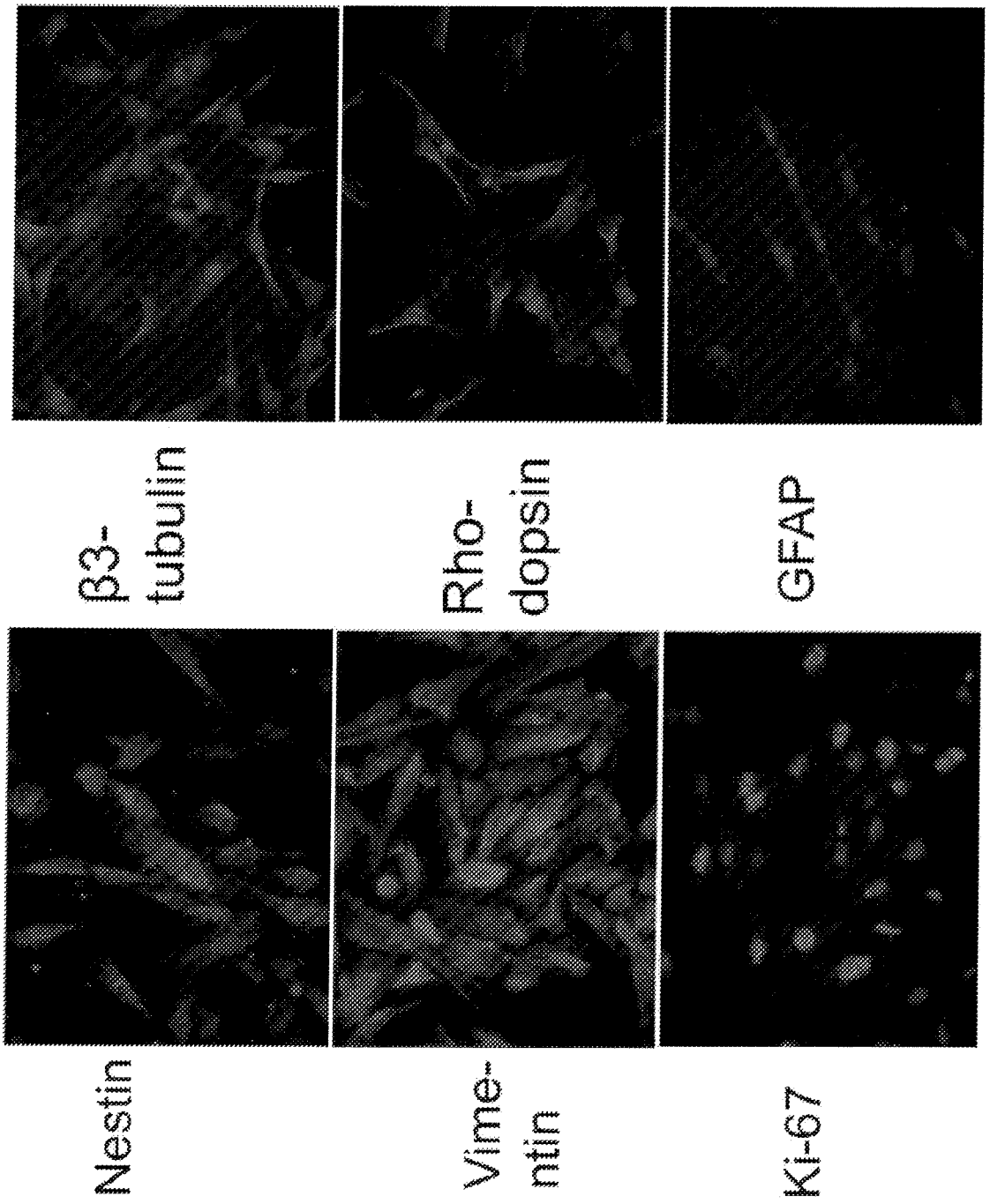


FIGURE 14

Gene profile by time point

In UL

D10=1 (as control)

Endogenous control: β-actin

Time point:

- D13 (0.5 month)
- D31 (1 month)
- D52 (2 months)
- D97 (3 months)
- D114 (4 months)

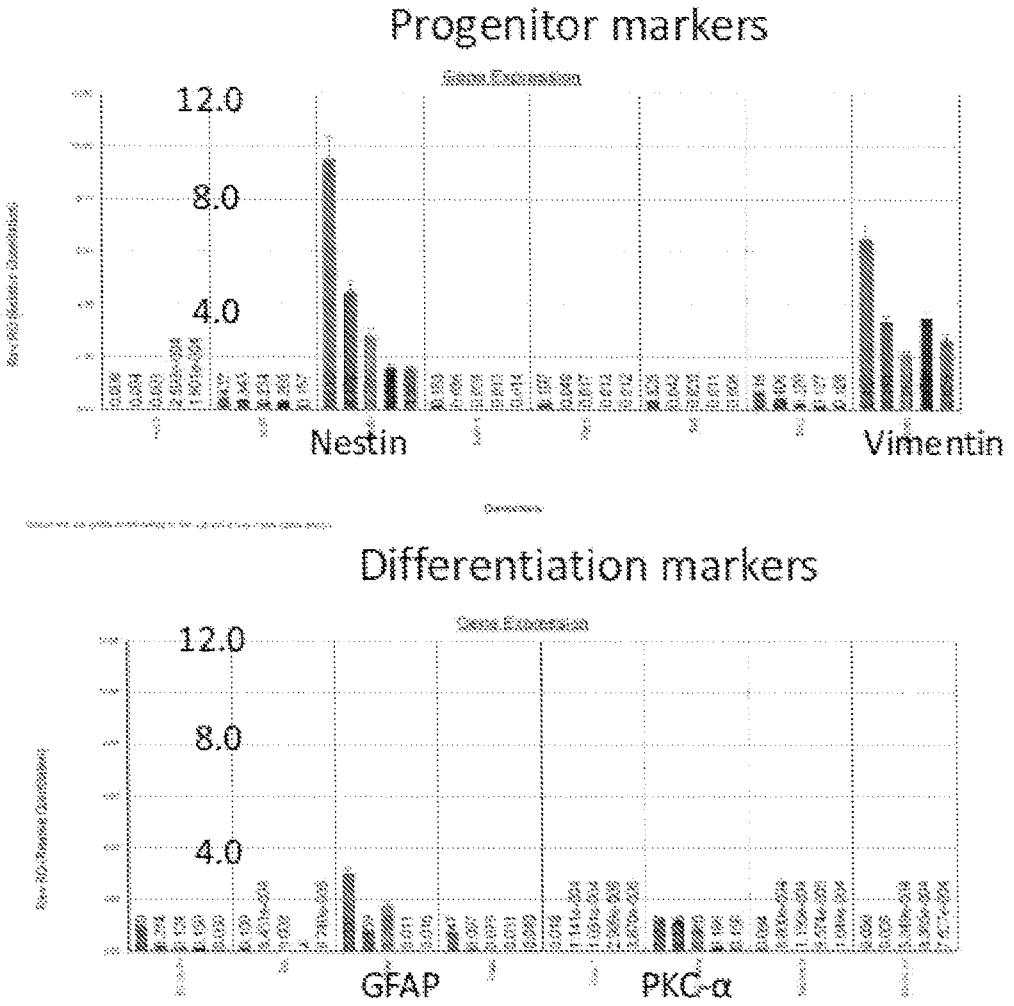
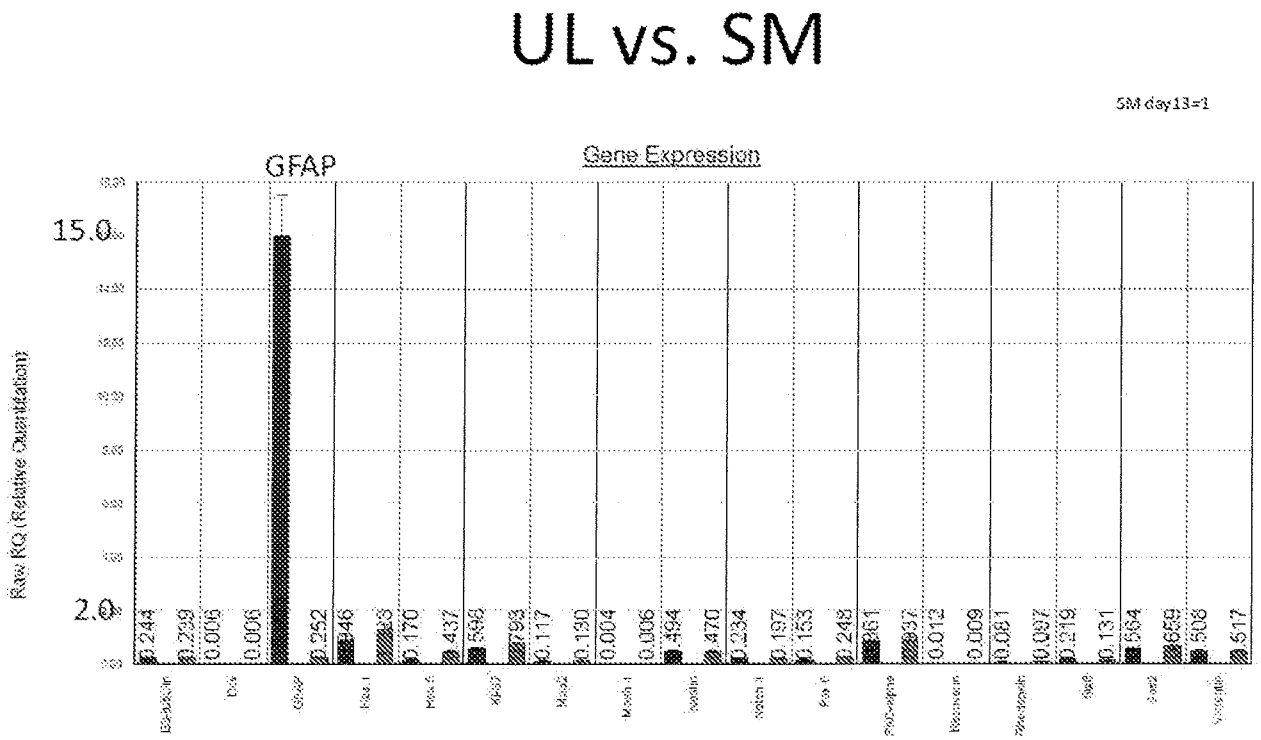


FIGURE 15

A


UL vs. SM
SM day13=1
GFAP
Gene Expression
15.0
2.0
Raw RQ (Relative Quantitation)

B

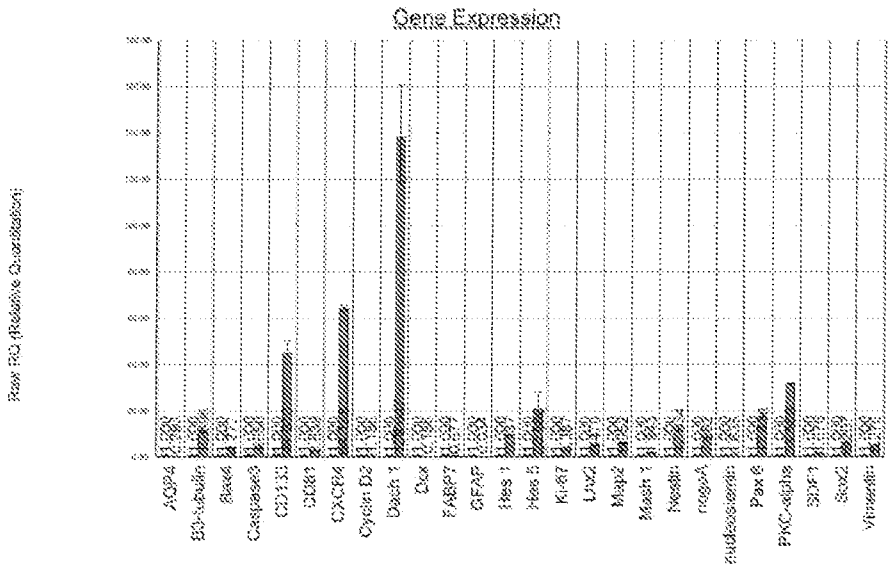

cRPC vs. cBPC
Gene Expression
Raw RQ (Relative Quantitation)

FIGURE 16

ANTIBODIES USED Labeling

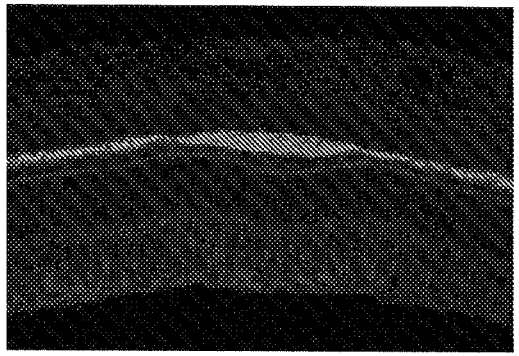
Anti-RFP	injected cells
Anti-vimentin	Müller cells
Anti-rod opsin	rod outer segments and some rod cell bodies as the protein redistributes due to the degeneration

OR

Anti-Ezrin	RPE microvilli and Müller cell microvilli at the OLM

OR

Protein kinase C	rod bipolar cells



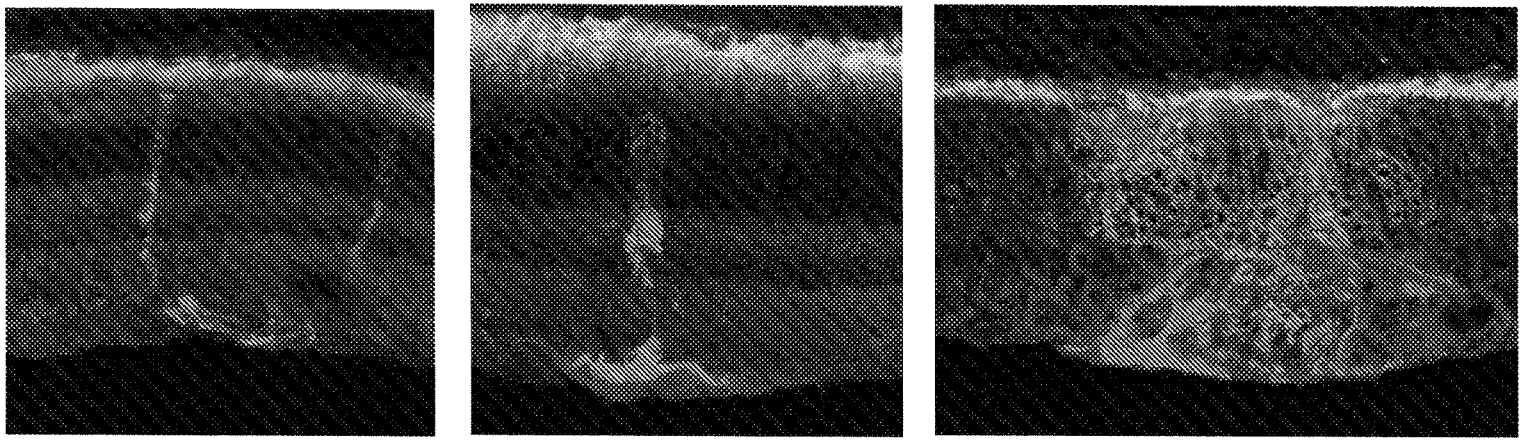


FIGURE 17

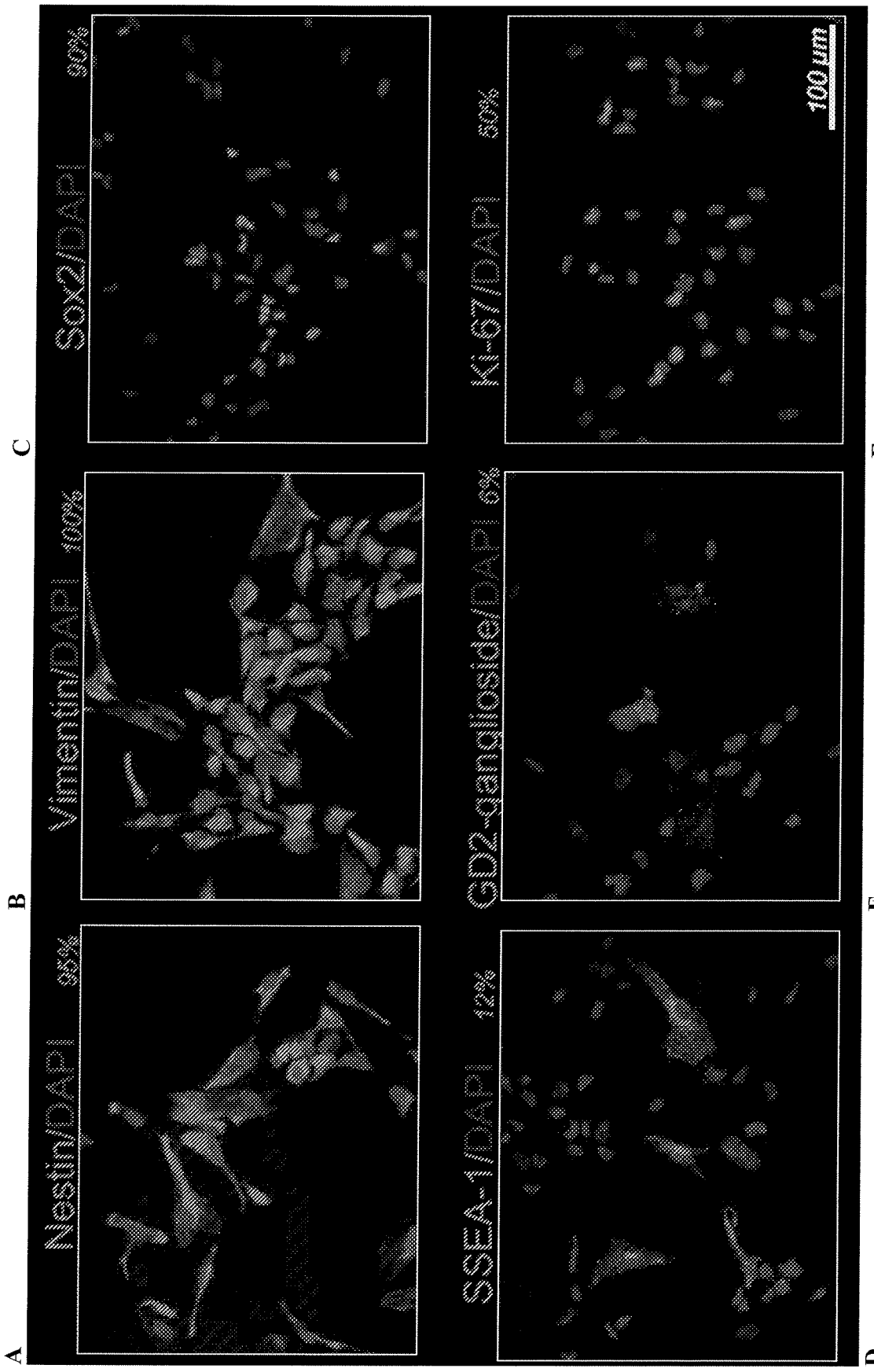

A
Nestin/DAPI 95%
B
Vimentin/DAPI 100%
C
Sox2/DAPI 90%
D
SSEA-1/DAPI 12%
E
GD2-ganglioside/DAPI 6%
F
Ki-67/DAPI 60%
100 um

FIGURE 18

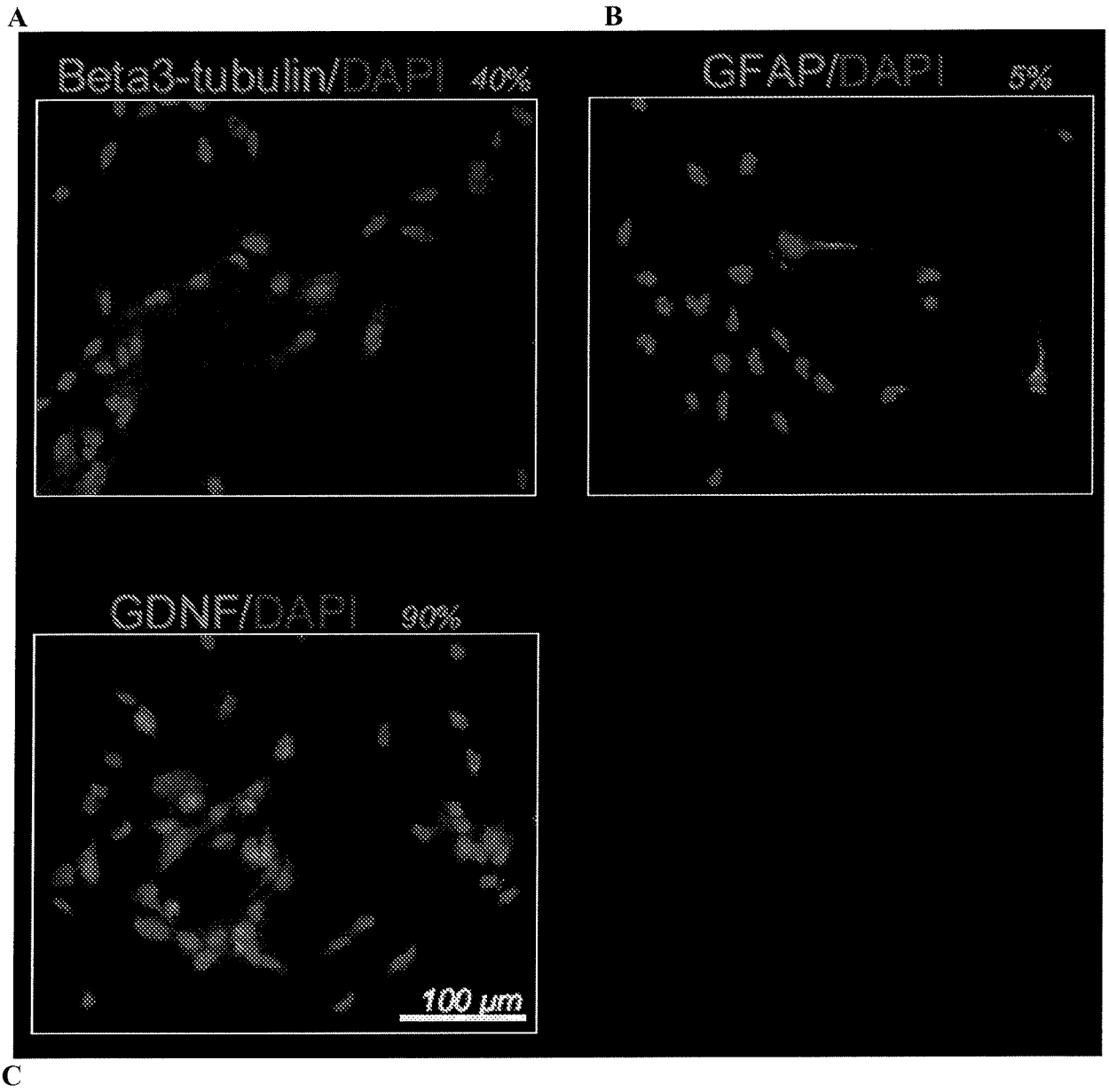


FIGURE 19

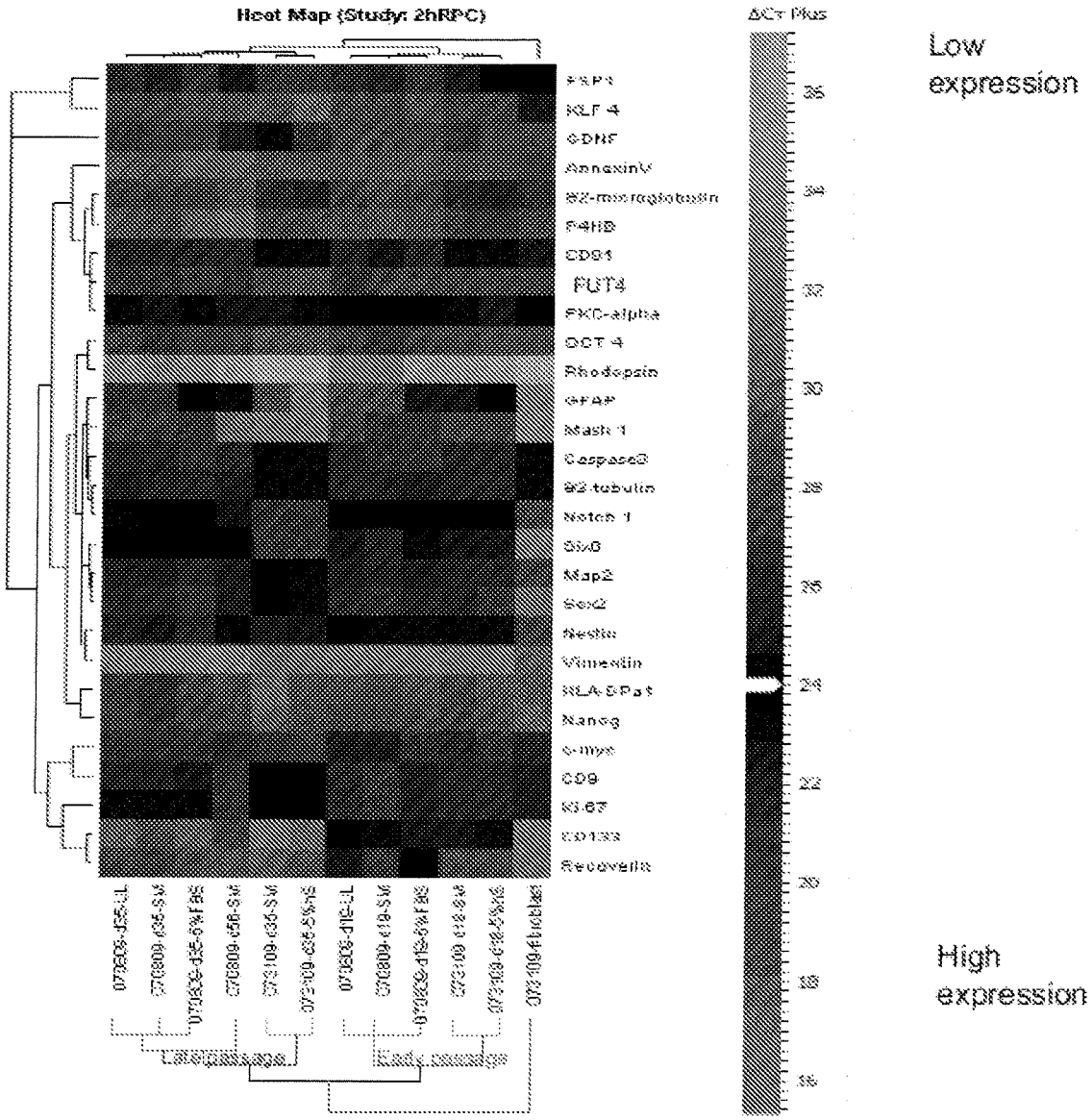
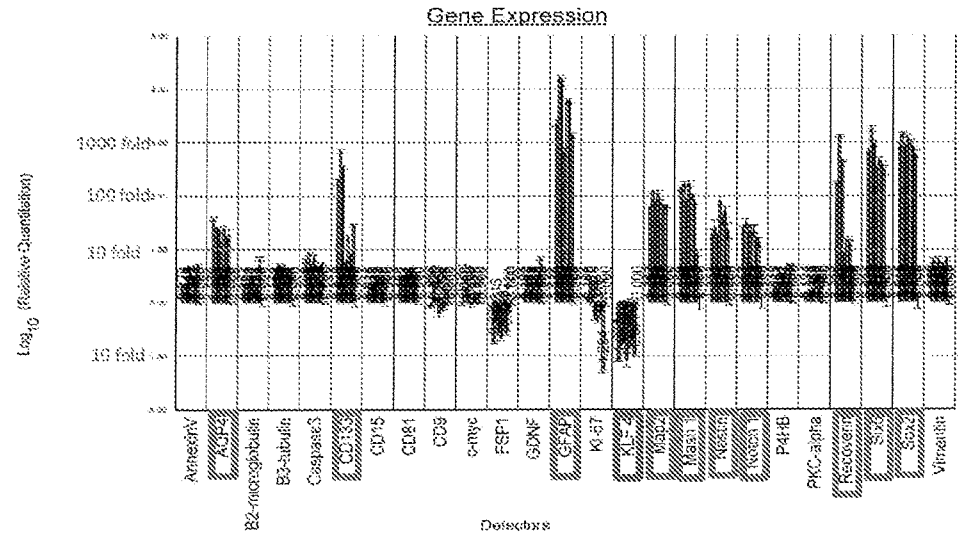
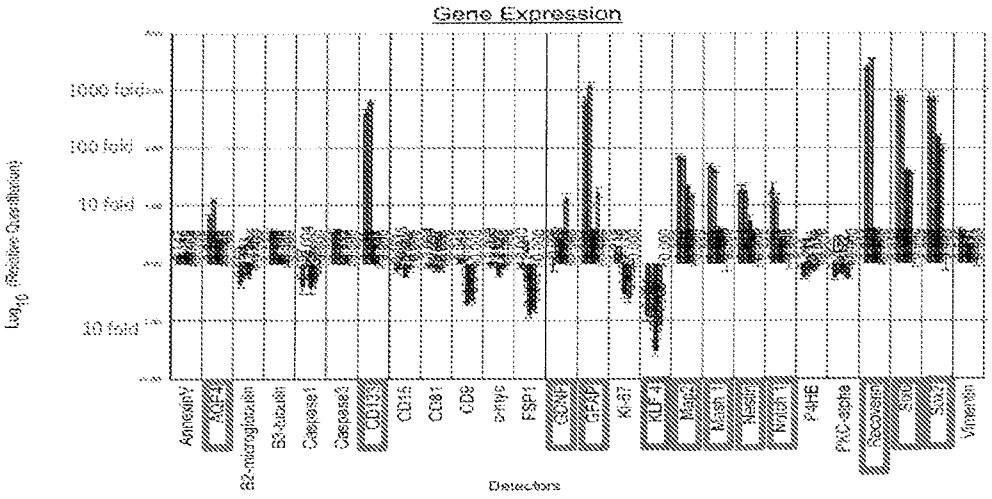

Heat Map (Study: 2hRPC)
ΔCt Plus
Low expression
High expression
FUT4

FIGURE 20

A



B



C

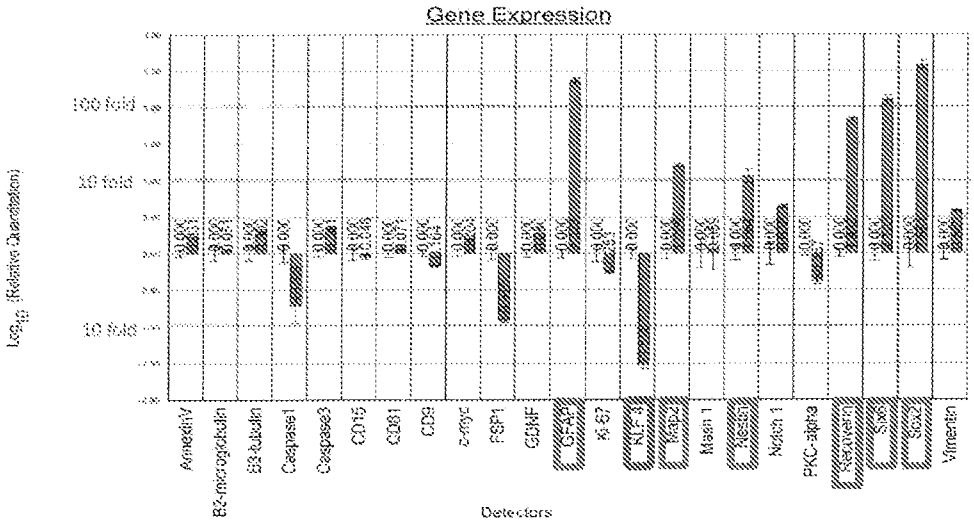


FIGURE 21

Top 10 genes up-regulated >10 fold (hRPC vs h-fibroblast=1)

	070809hRPC	073109hRPC	011310hRPC
1	AQP4	AQP4	
2	CD133	CD133	
3	**GFAP**	**GFAP**	**GFAP**
4 ** (down)	Klf4	Klf4	Klf4
5	Map2	Map2	Map2
6	Mash1	Mash1	
7	Nestin	Nestin	Nestin
8	Notch1	Notch1	
9	**Recoverin**	**Recoverin**	**Recoverin**
10	**six6**	**six6**	**six6**
11	sox2	sox2	sox2

FIGURE 22

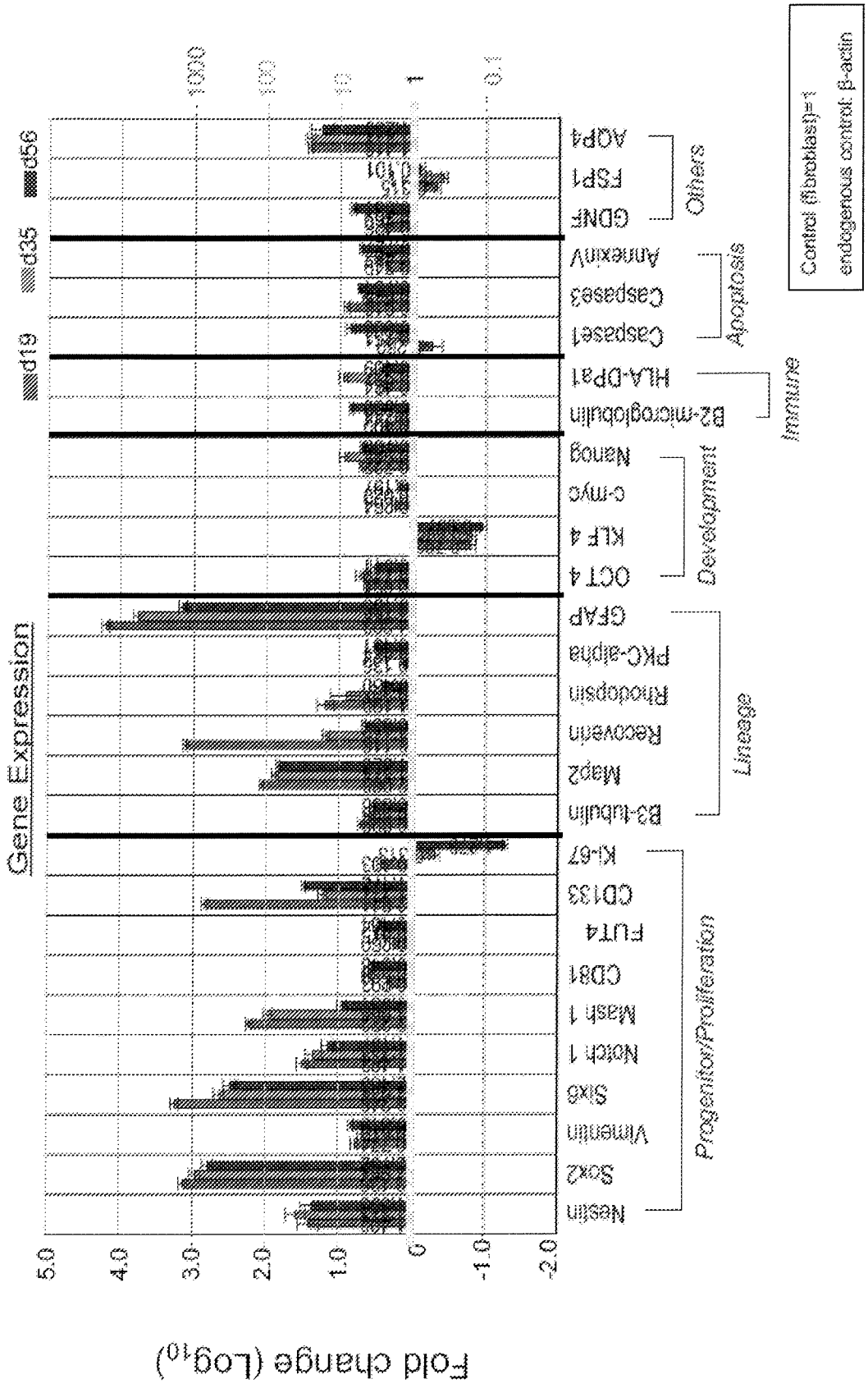

Gene Expression
Fold change (Log10)
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
1000
100
10
1
0.1
d19
d35
d56
Nestin
Sox2
Vimentin
Six6
Notch 1
Mash 1
CD81
FUT4
CD133
Ki-67
Progenitor/Proliferation
B3-tubulin
Map2
Recoverin
Rhodopsin
PKC-alpha
GFAP
Lineage
OCT 4
KLF 4
c-myc
Nanog
Development
B2-microglobulin
HLA-DPa1
Immune
Caspase1
Caspase3
AnnexinV
Apoptosis
GDNF
FSP1
AQP4
Others
Control (fibroblast)=1
endogenous control β-actin

FIGURE 23

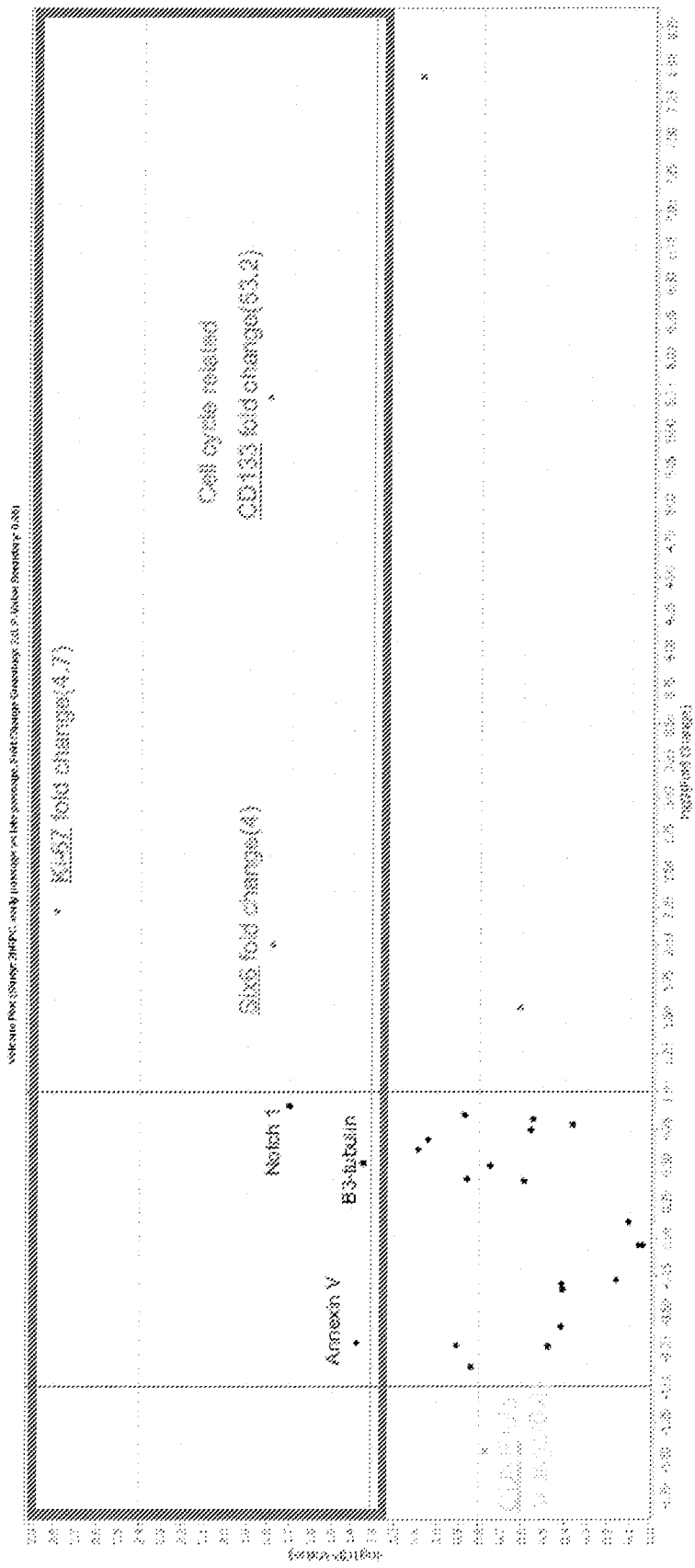

Ki67 fold change(4.7)
Cell cycle related
CD133 fold change(63.2)
Slug fold change(4)
Notch 1
B3-tubulin
Annexin V

FIGURE 24

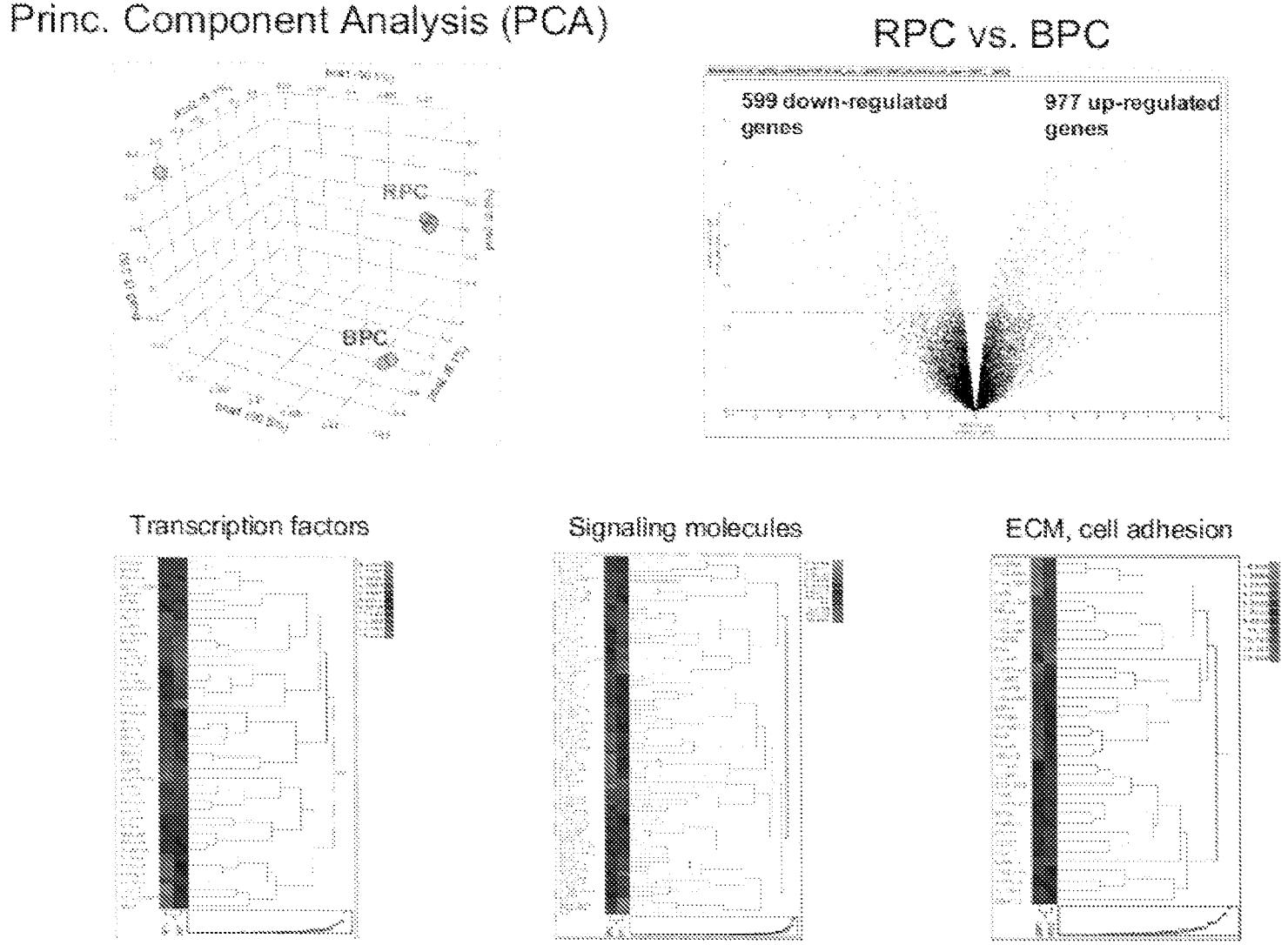

Princ. Component Analysis (PCA)
RPC
BPC
RPC vs. BPC
599 down-regulated genes
977 up-regulated genes
Transcription factors
Signaling molecules
ECM, cell adhesion

FIGURE 25

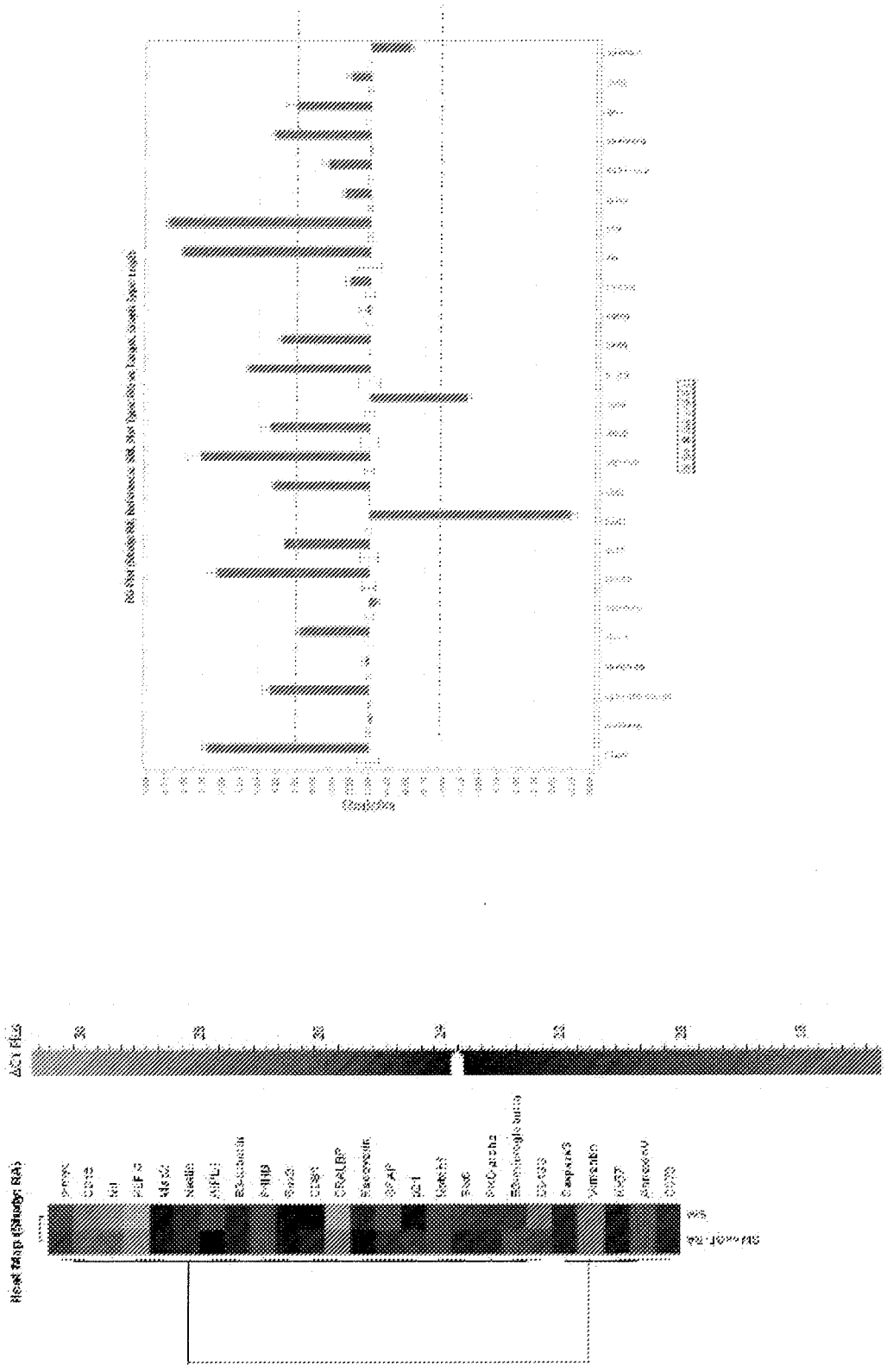


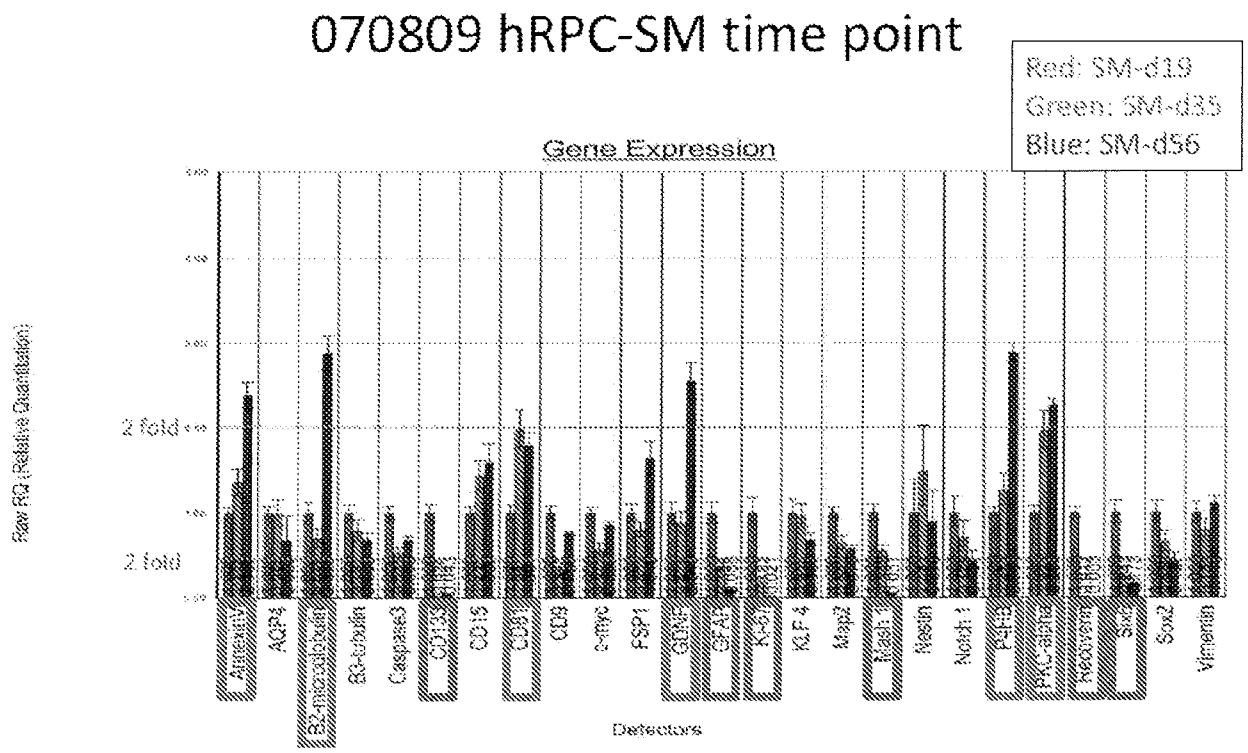
FIGURE 26

hRPC time point design

012208hRPC		070809hRPC			073109hRPC		011310hRPC
SM-d17 -1		SM-d19-1	SM-UL-d19-1	SM-5%FBS-d19-1	SM-d18-1	SM-5%hS-d18-1	SM-d22
SM-d22	UL-d22	SM-d35	SM-UL-d35	SM-5%FBS-d35	SM-d35	SM-5%hS-d35	
SM-d27	UL-d27	SM-d56					
SM-d38	UL-d38						
SM-d48	UL-d48						
SM-d72	UL-d72						
SM-d99	UL-d99						

FIGURE 27

A


070809 hRPC-SM time point
Red: SM-d19
Green: SM-d35
Blue: SM-d56
Gene Expression
Raw RQ (Relative Quantitation)
2 fold
2 fold
Detectors
Annexin V
AQP4
B2-microglobulin
B3-tubulin
Caspase3
CD133
CD15
CD81
CD9
c-myc
FSP1
GFAP
Ki-67
KLF 4
Map2
Mash 1
Nestin
Notch 1
P4HB
PKC-alpha
Recoverin
Sox1
Sox2
Vimentin

B

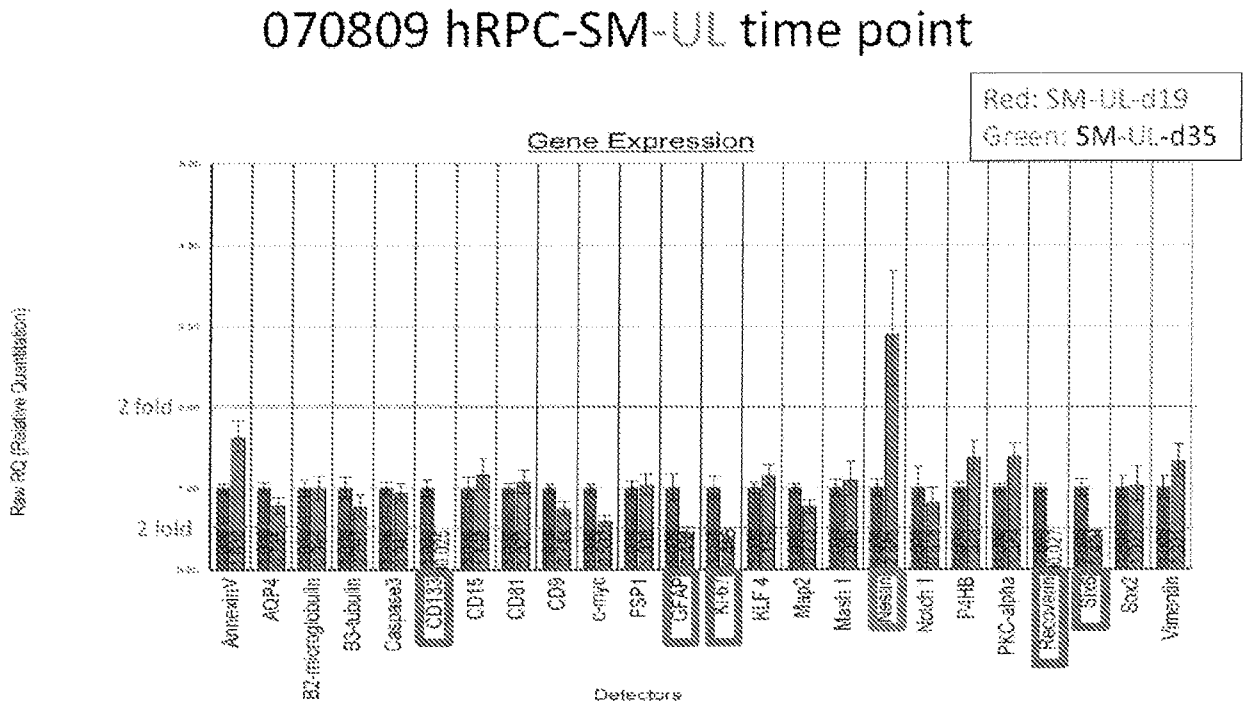
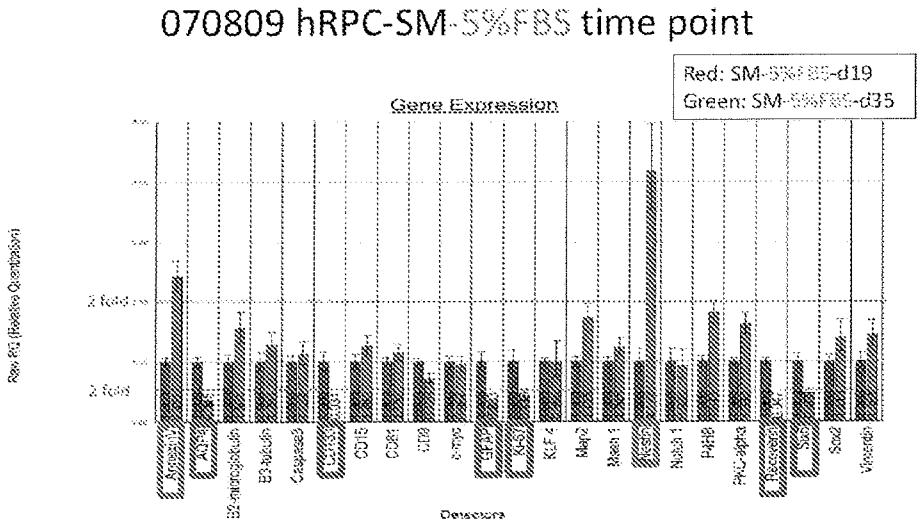
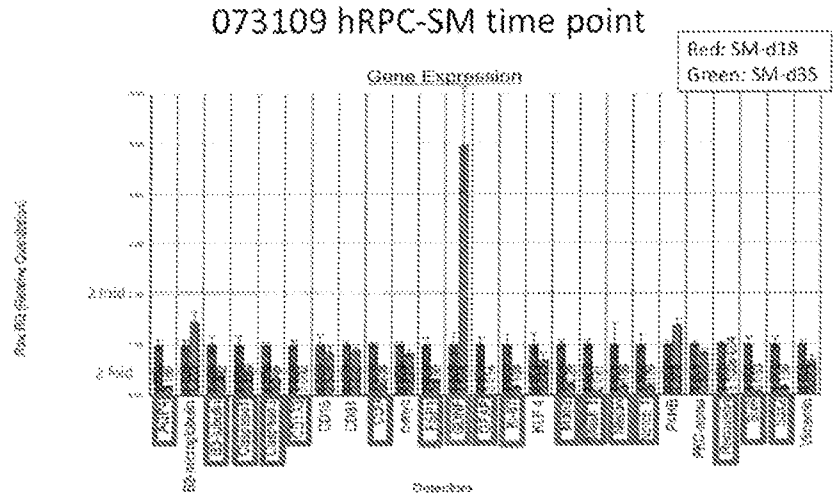

070809 hRPC-SM-UL time point
Red: SM-UL-d19
Green: SM-UL-d35
Gene Expression
Raw RQ (Relative Quantitation)
2 fold
2 fold
Detectors
Annexin V
AQP4
B2-microglobulin
B3-tubulin
Caspase3
CD133
CD15
CD81
CD9
c-myc
FSP1
GFAP
Ki-67
KLF 4
Map2
Mash 1
Nestin
Notch 1
P4HB
PKC-alpha
Recoverin
Sox1
Sox2
Vimentin

FIGURE 28

A



B



C

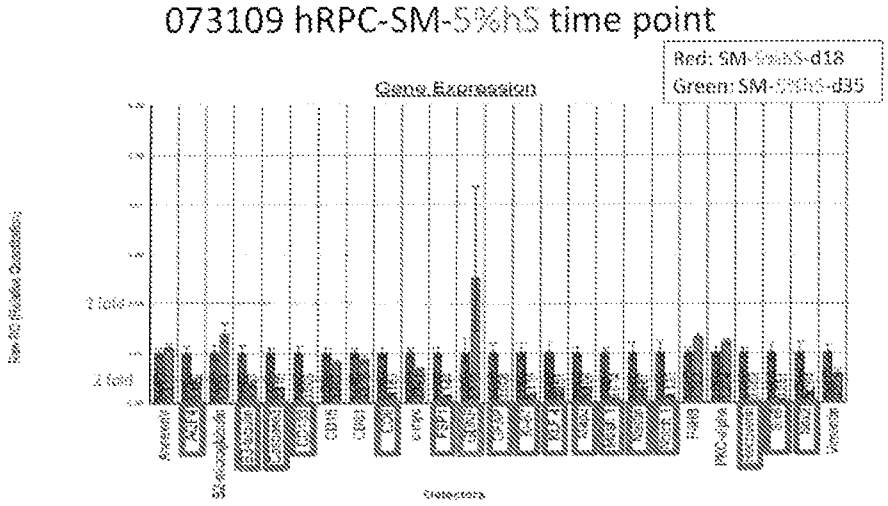


FIGURE 29

hRPC time point comparison

Red ↓ green ↑

01.22dhRPC		07.03dhRPC			07.10dhRPC	
SM	UL	SM	SM-UL	SM-5%FBS	SM	SM-5%hS
AQP4	AQP4			AQP4	AQP4	AQP4
b3-tubulin?	b3-tubulin				b3-tubulin	b3-tubulin
caspase-1	caspase-1				caspase-1	x
caspase-3	caspase-3				caspase-3	caspase-3
CD9	CD9				CD9	CD9
FSP1	FSP1				FSP1	FSP1
		CD133	CD133	CD133	CD133	CD133
CD81	CD81	CD81				
GDNF	GDNF	GDNF	GDNF?	GDNF?	GDNF	GDNF
GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP
KI67	KI67	KI67	KI67	KI67	KI67	KI67
KIT	KIT					KIT
Map2	Map2				Map2	Map2
Nestin	Nestin		Nestin	Nestin	Nestin	Nestin
		Mash1			Mash1	Mash1
Notch1	Notch1				Notch1	Notch1
		PAHB				
PECAM		PECAM				
Recoverin	Recoverin	Recoverin	Recoverin	Recoverin	Recoverin	Recoverin
Six6	Six6	Six6	Six6	Six6	Six6	Six6
Sox2	Sox2				Sox2	Sox2
vimentin	vimentin					
	CD15?					
b2-microglobulin	b2-microglobulin	b2-microglobulin				
c-myc	c-myc					
Annexin	Annexin	Annexin		Annexin		

FIGURE 30

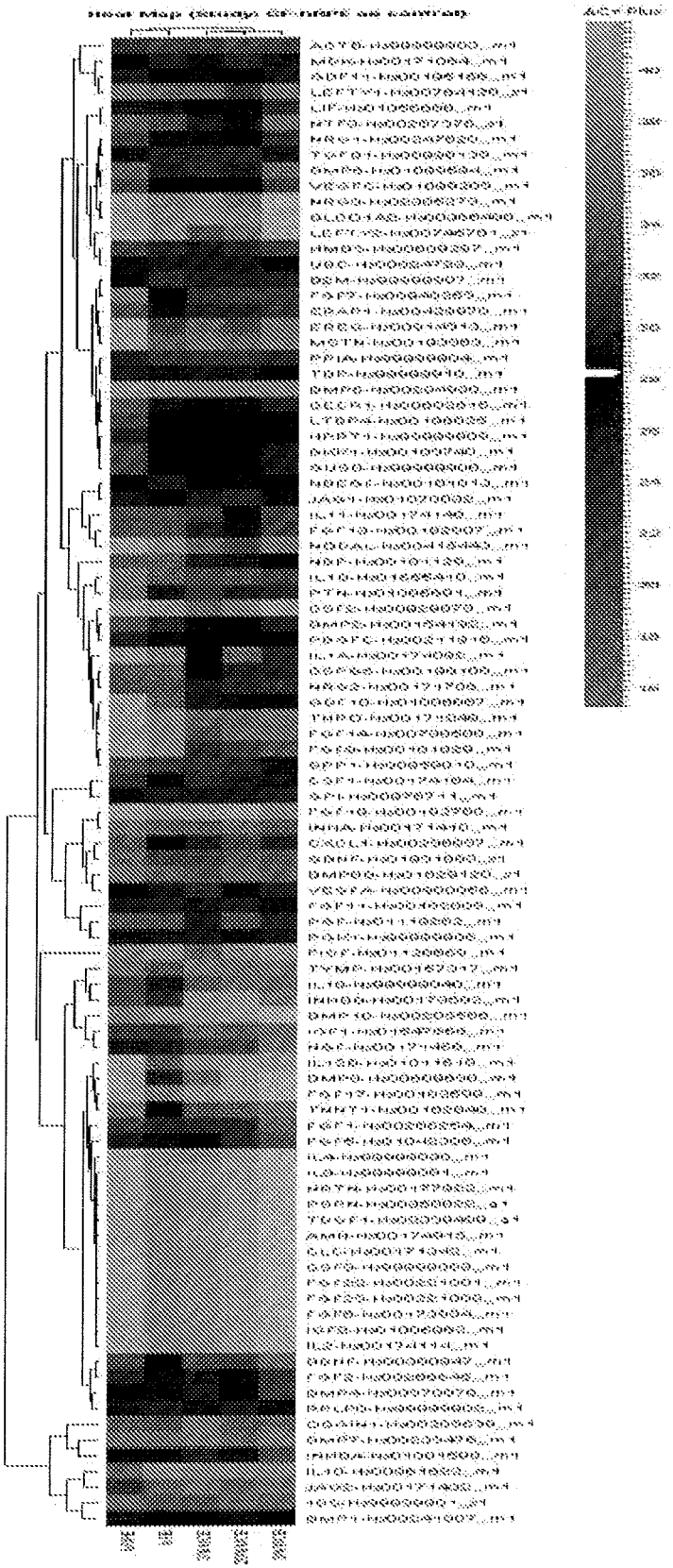


FIGURE 31

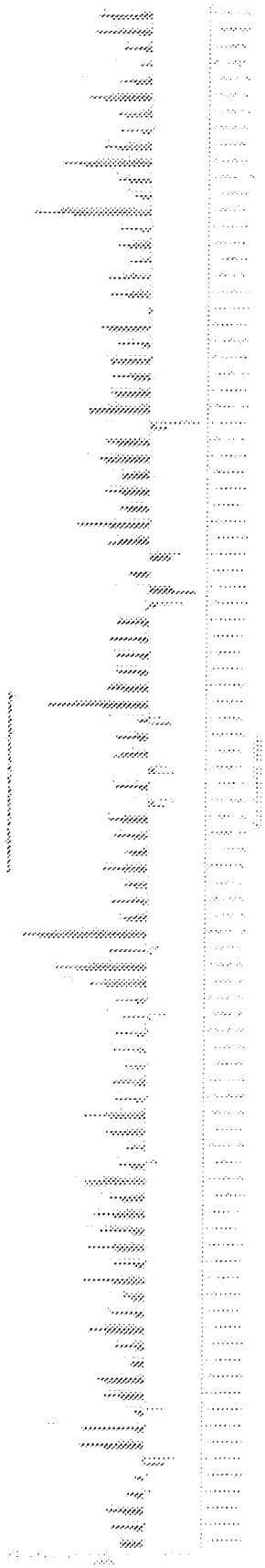


FIGURE 32

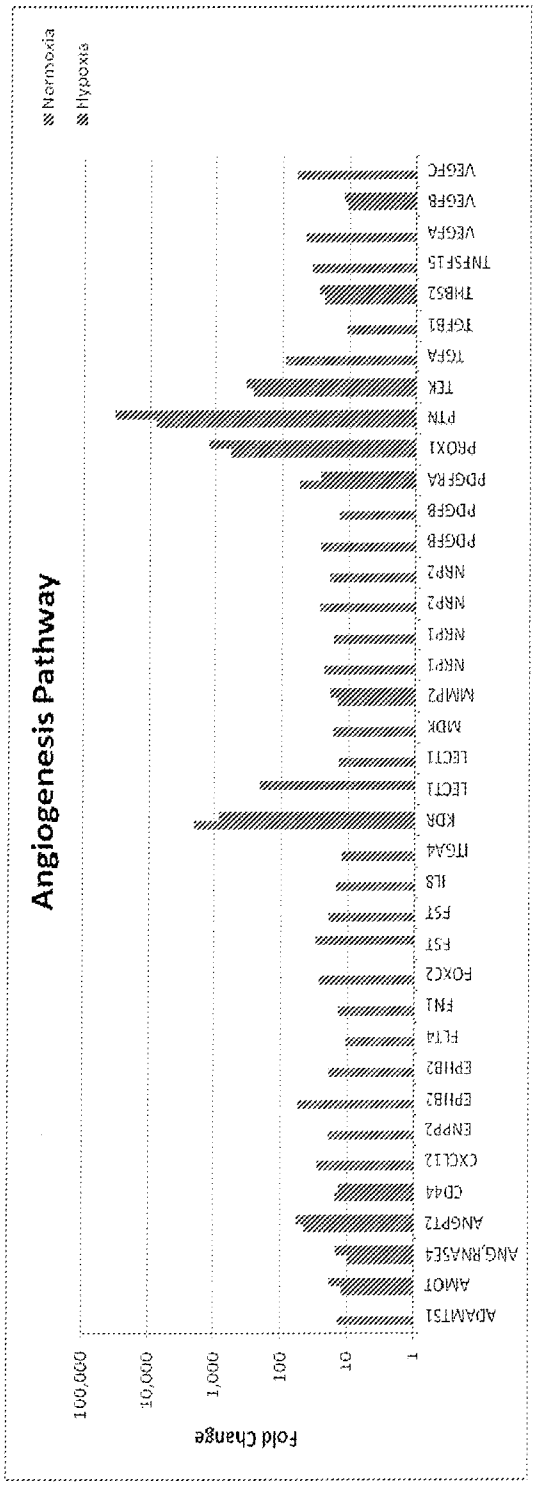
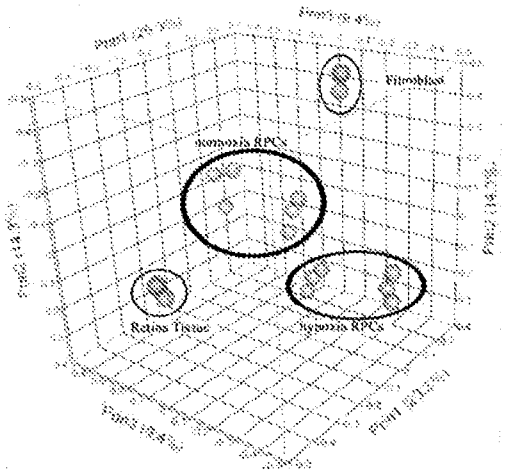
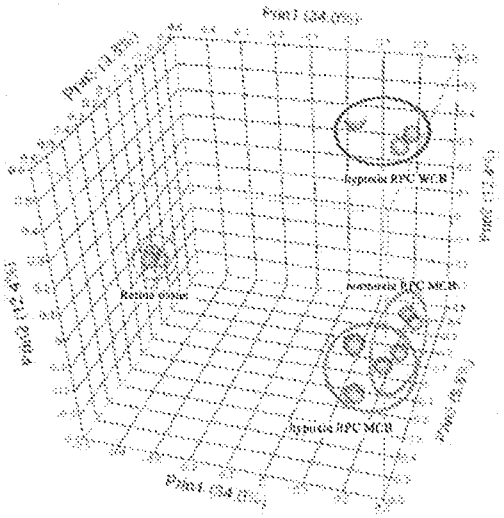

A
Angiogenesis Pathway
Normoxia
Hypoxia
Fold Change
100,000
10,000
1,000
100
10
1
ADAMTS1
AMOT
ANG,RNASE4
ANGPT2
CD44
CXCL12
ENPP2
EPHB2
EPHB2
FLT4
FN1
FOXC2
FST
FST
IL8
ITGA4
KDR
LECT1
LECT1
MDK
NRP2
NRP1
NRP1
NRP2
NRP2
PDGFB
PDGFB
PDGFRA
PROX1
PTN
TEK
TGFA
TGFB1
THBS2
TNFSF15
VEGFA
VEGFB
VEGFC
B
WNT Pathway
Normoxia
Hypoxia
Fold Change
100,000
10,000
1,000
100
10
1
AFC
AXIN2
BTRC
CSNK1A1
CSNK1D
CSNK1E1
CSNK1G3
CTNNB1
CSNK2A1
CXXC4
DACT1
DKK1
DKK3
DVL2
EP300
FBXW11
FRAT2
FZD5
FZD7
FZD8
GSK3B
HDAC1
LEF1
SFRP1
WNT
NKD2
PLK
PORCN
PPP2CA
PYGO1
PYGO2
RHOU
SIAH2
SFRP4
TCF7
TCF7L1
TCF7L2
TLE2
TLE3
TLE4
WIF1
WNT10
WNT3A

FIGURE 33

A



B



C

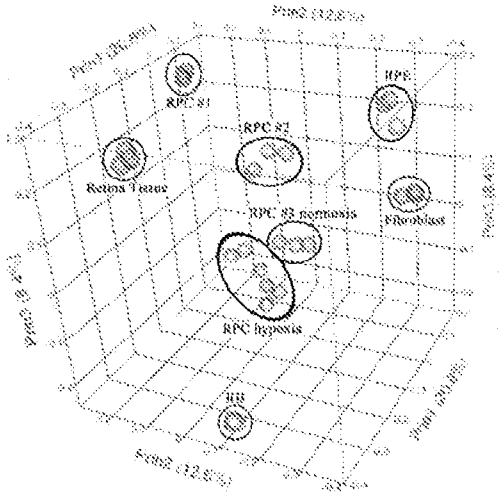


FIGURE 34

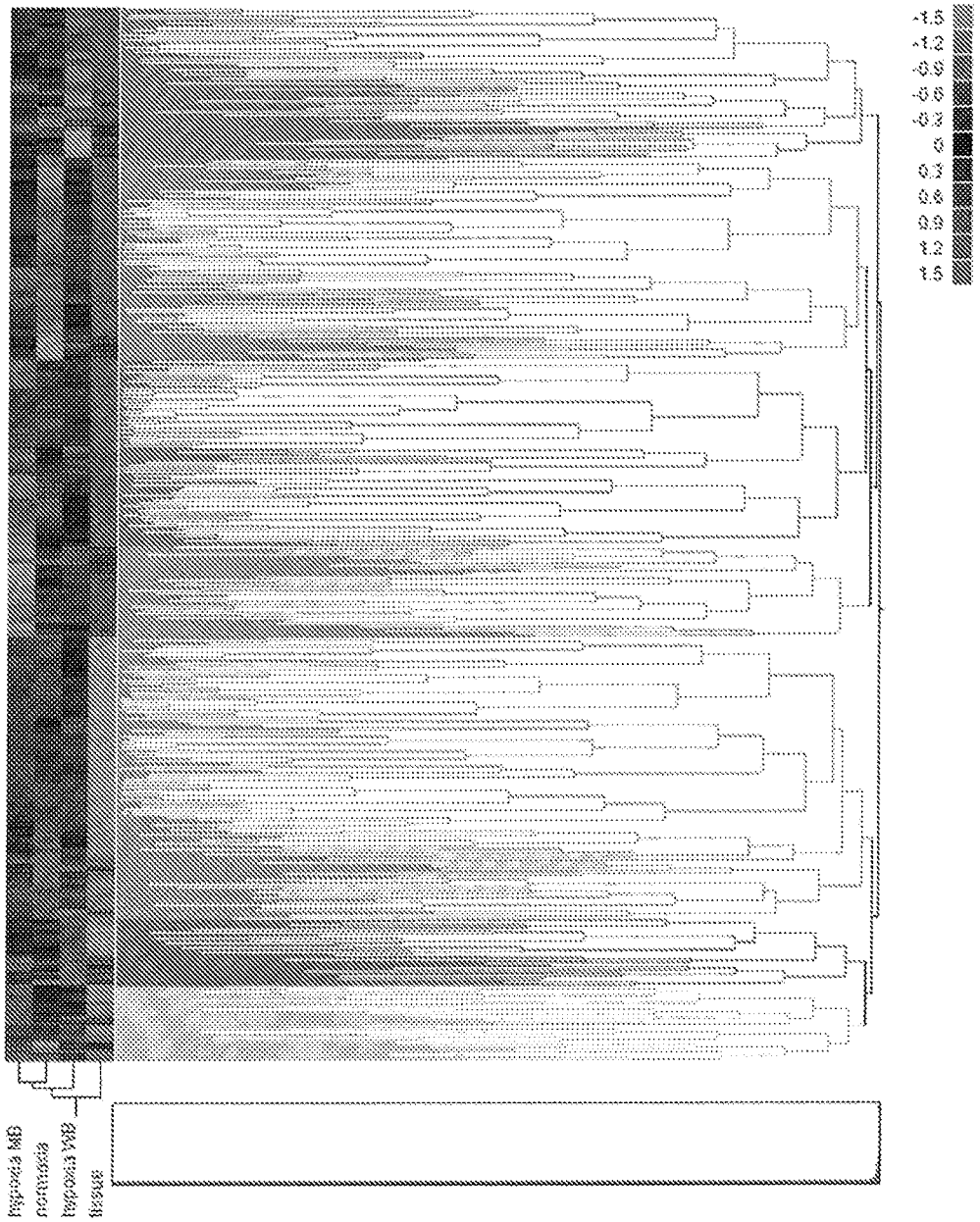


FIGURE 35

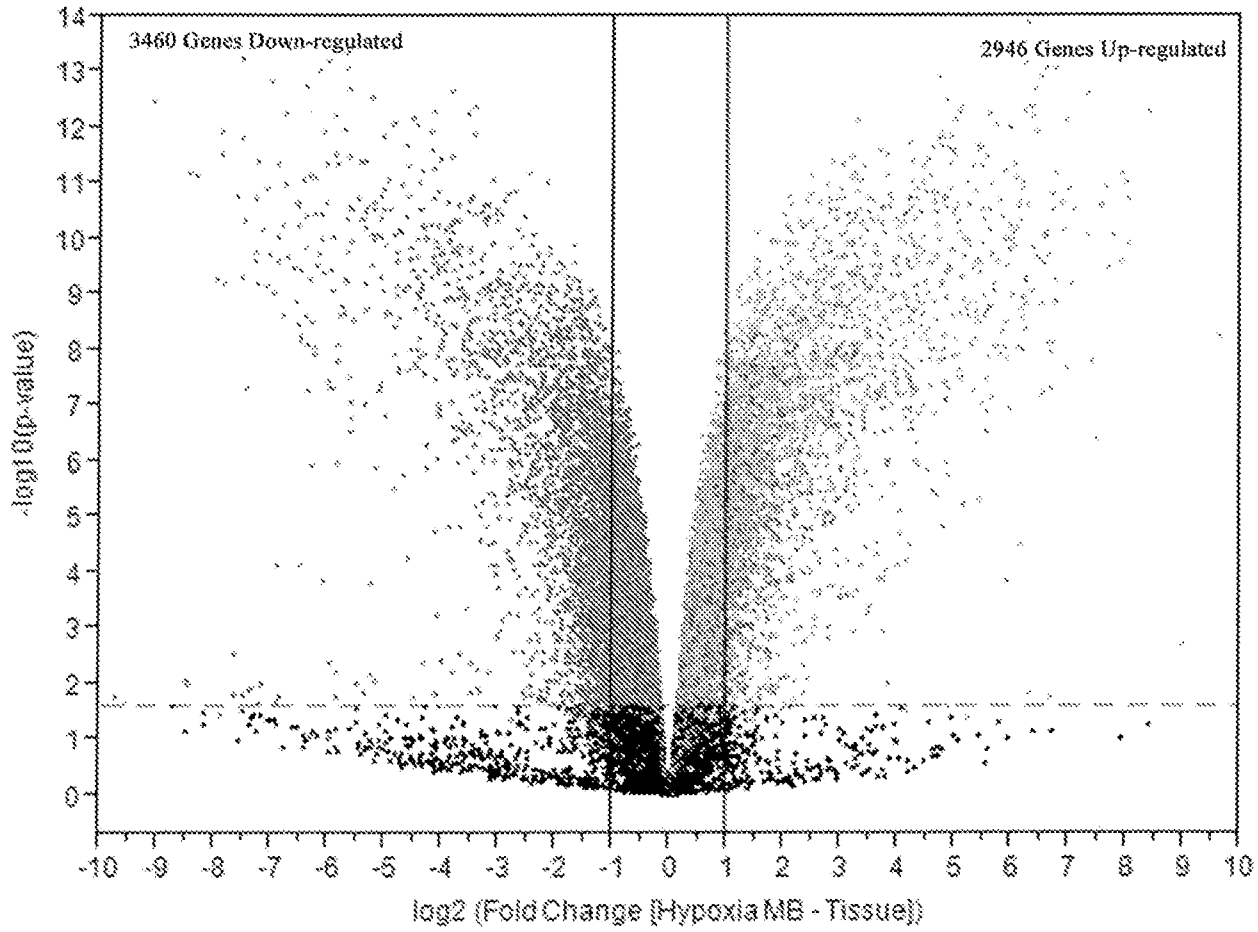
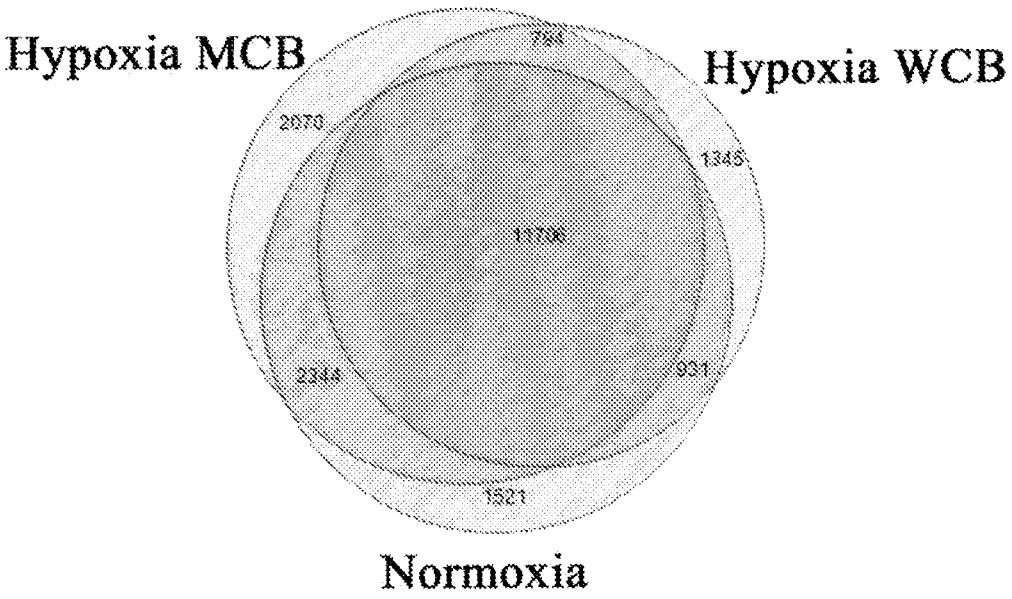

3460 Genes Down-regulated
2946 Genes Up-regulated
-log10 (p-value)
log2 (Fold Change [Hypoxia MB - Tissue])

FIGURE 36

A


Hypoxia MCB
Hypoxia WCB
2070
1345
2344
931
1821
Normoxia

B

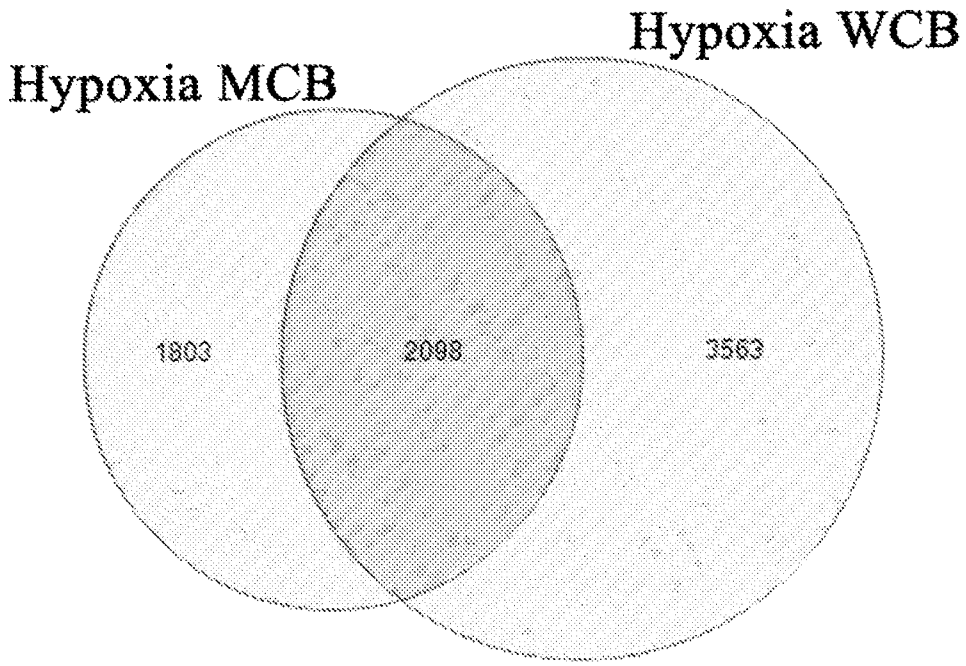
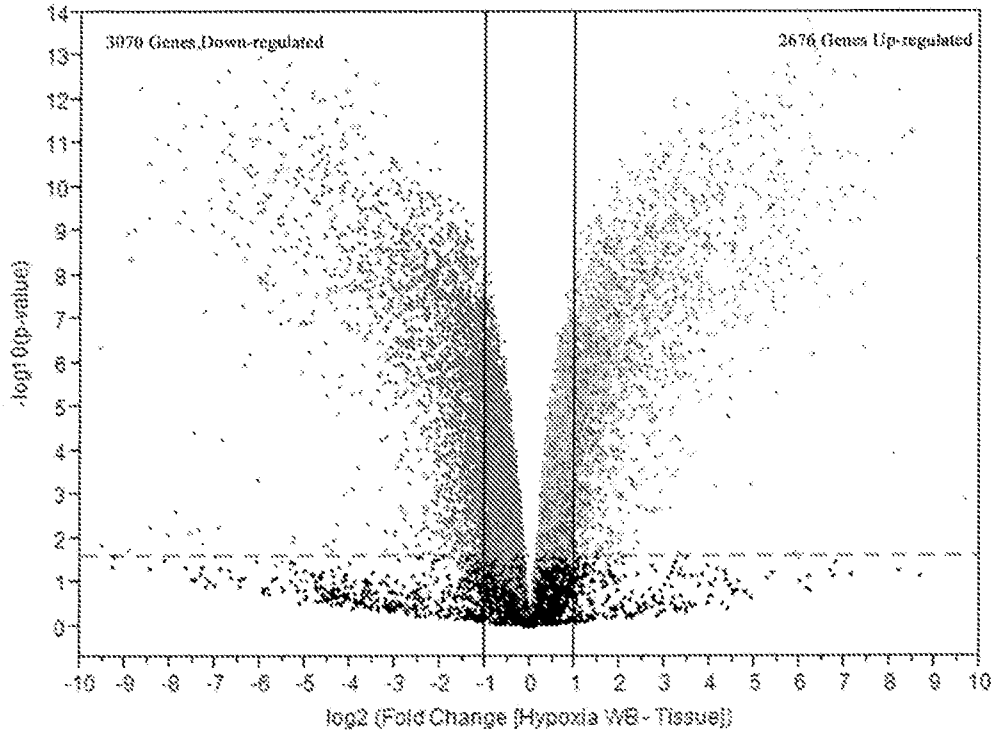

Hypoxia WCB
Hypoxia MCB
1803
2098
3563

FIGURE 37

A


3070 Genes Down-regulated
2676 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Hypoxia WB - Tissue])

B

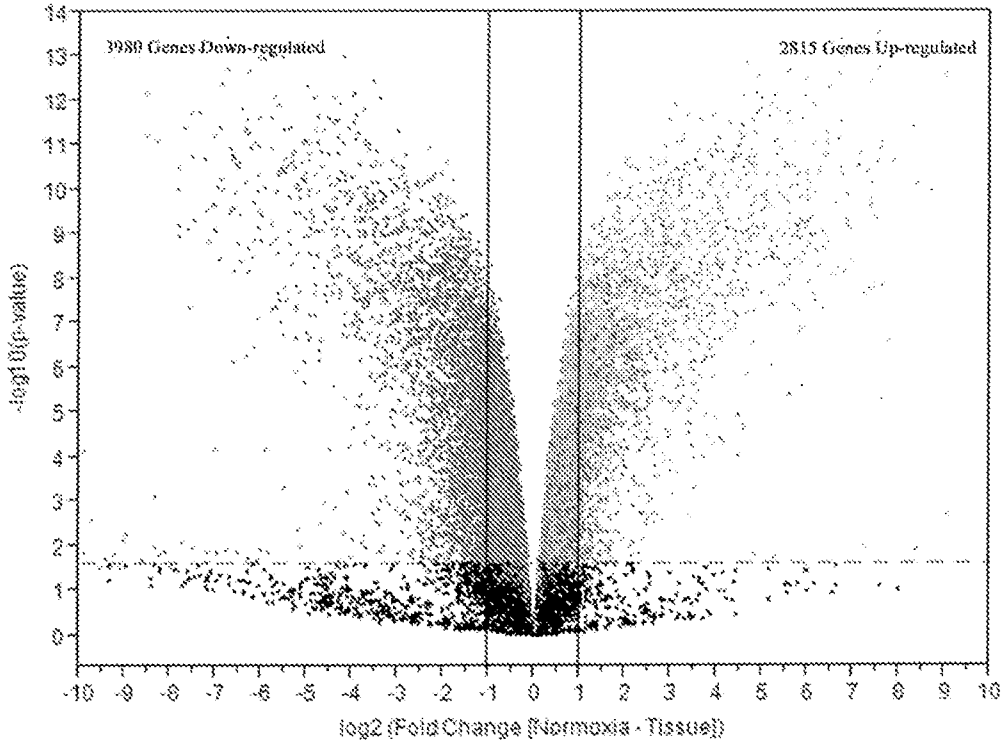
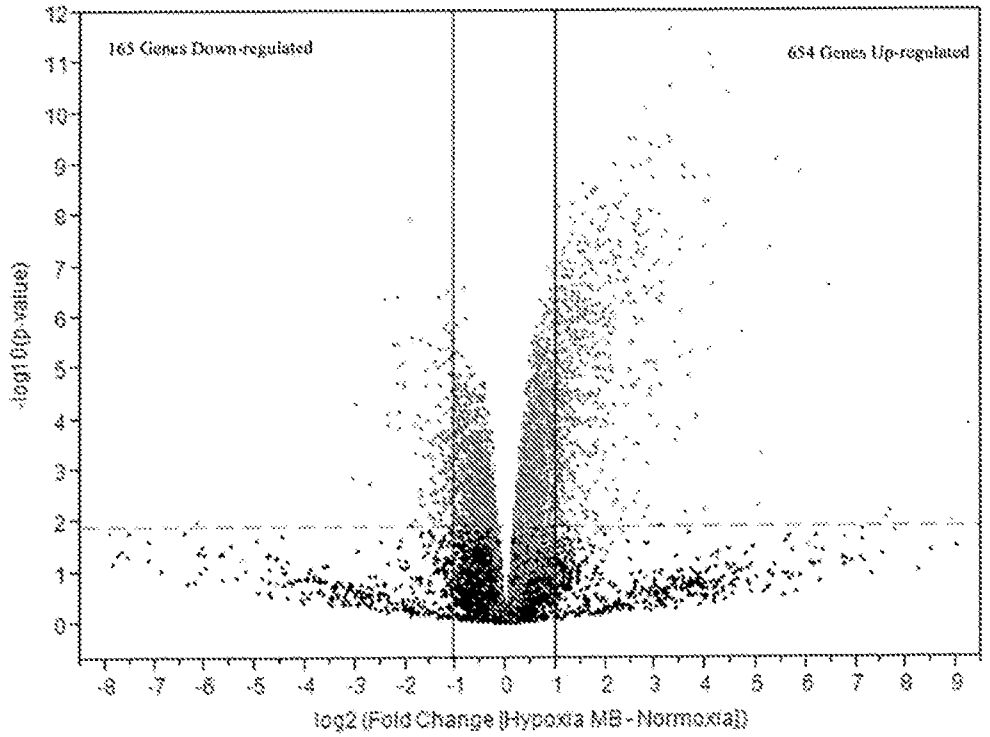

3980 Genes Down-regulated
2815 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Normoxia - Tissue])

FIGURE 38

A


165 Genes Down-regulated
654 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Hypoxia MB - Normoxia])

B

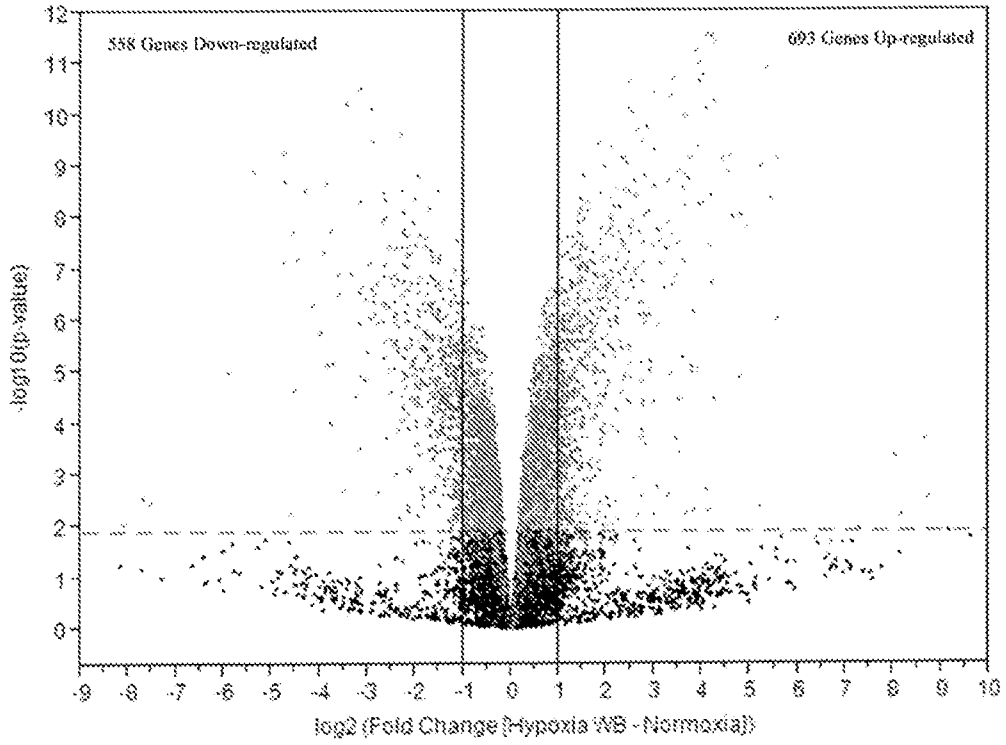

558 Genes Down-regulated
693 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Hypoxia WB - Normoxia])

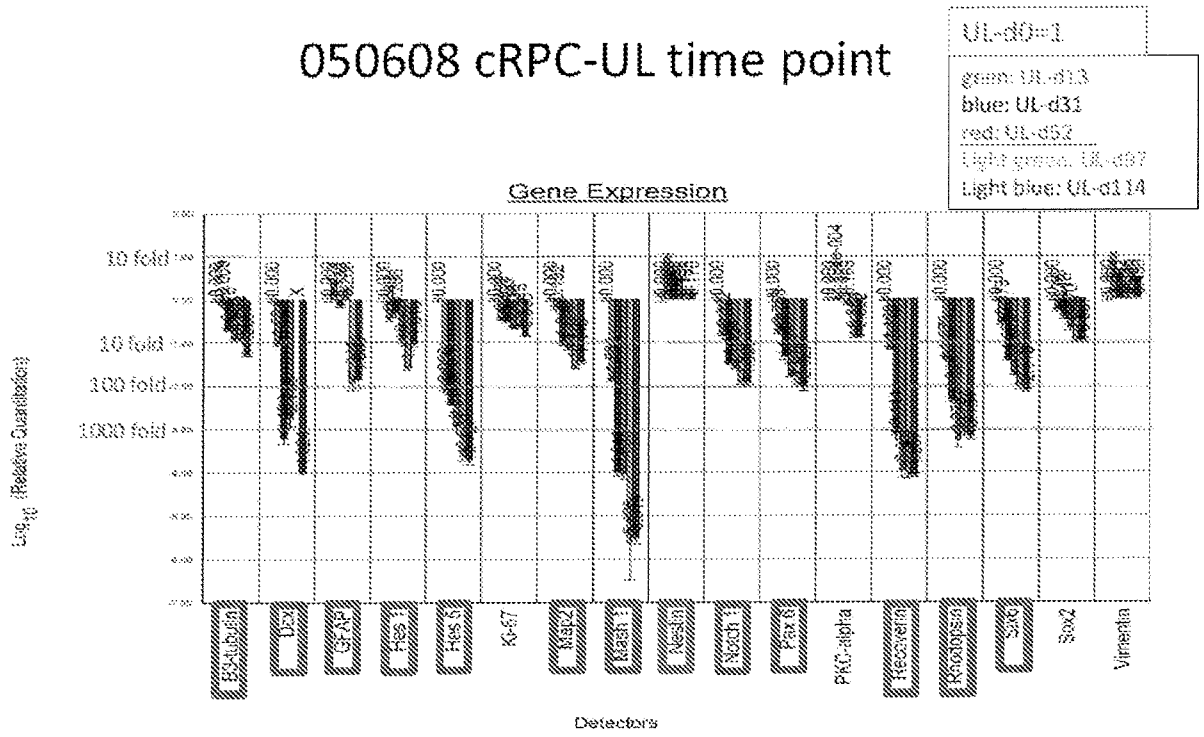
FIGURE 39

cRPC time point design

112707cRPC		050608cRPC		090808rcRPC	091208rcRPC
SM-d24	UL-d24	SM-d0=1	UL-d0=1	UL-d20	UL-d14=1
			UL-d13=1		UL-d135
		SM-d31	UL-d31		
			UL-d52		
			UL-d76		
			UL-d97		
			UL-d114		

FIGURE 40

A


050608 cRPC-UL time point
UL-d0=1
blue: UL-d31
red: UL-d52
Light blue: UL-d114
Gene Expression
10 fold
10 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
Detectors
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Nestin
Notch 1
Pax 6
PKC-alpha
Synaptophysin
Sox2
Vimentin

B

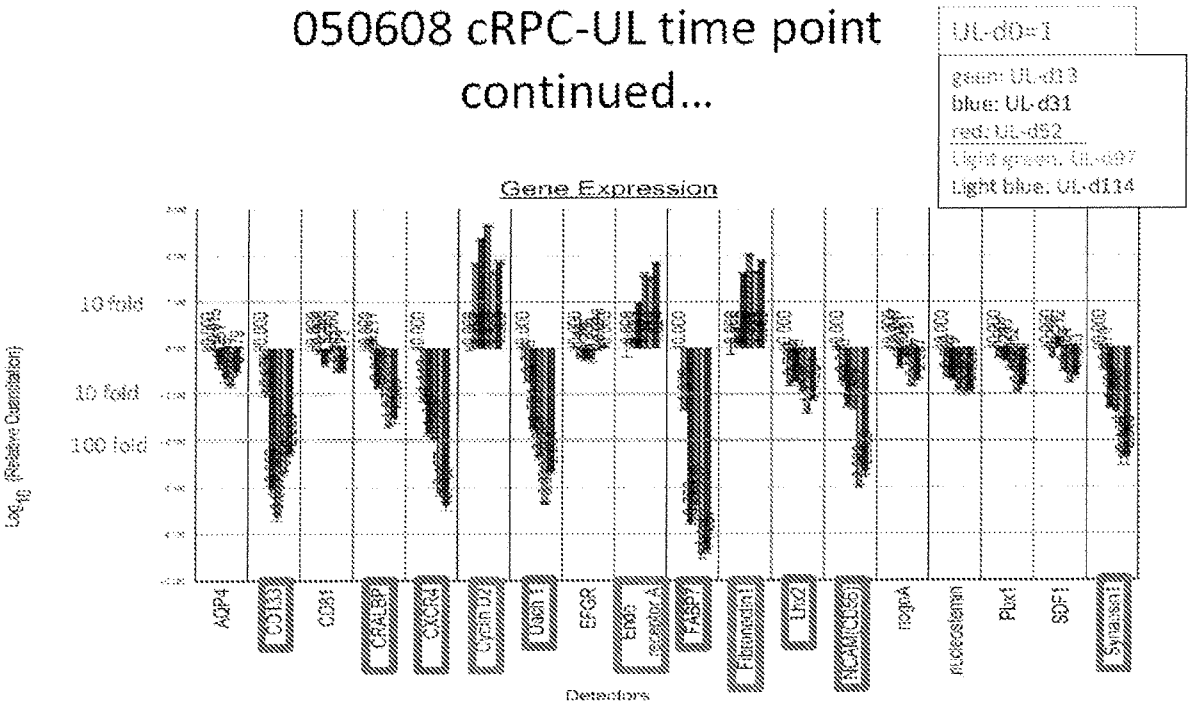
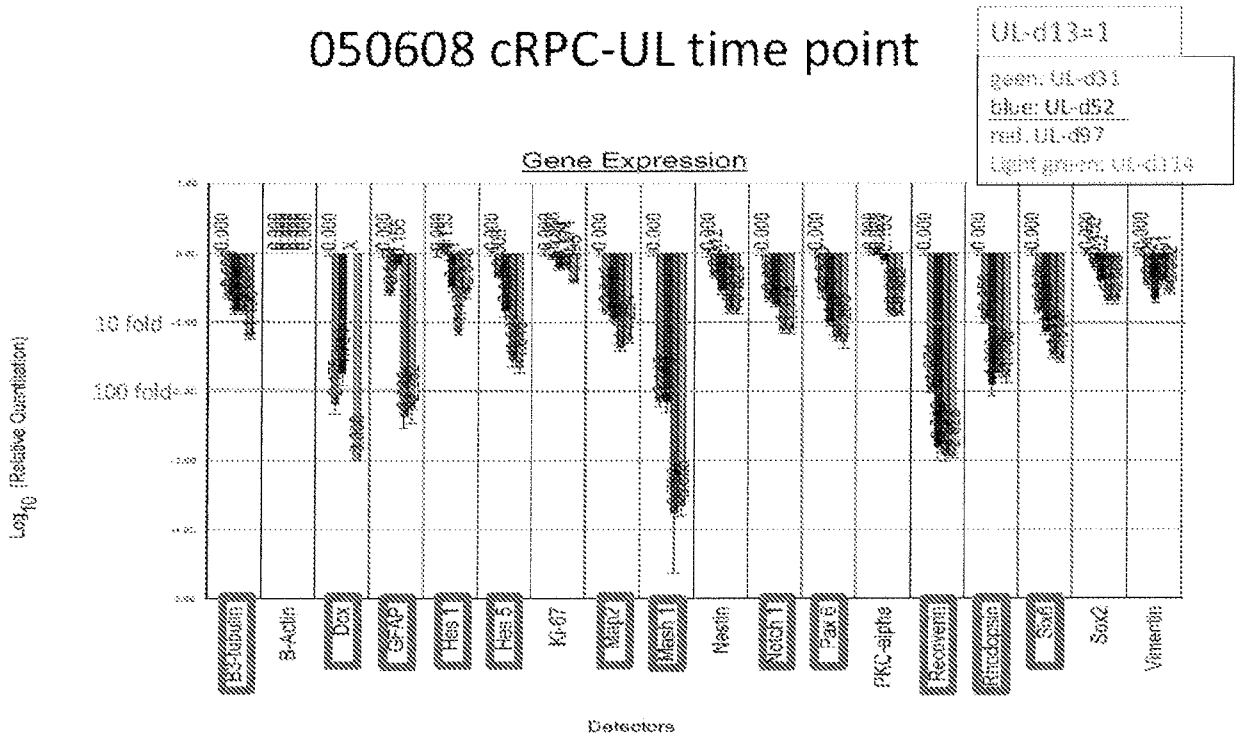

050608 cRPC-UL time point
continued...
UL-d0=1
blue: UL-d31
red: UL-d52
Light blue: UL-d114
Gene Expression
10 fold
10 fold
100 fold
Log10 (Relative Quantitation)
Detectors
AQP4
CD133
CD31
CHI3L2
CXCR4
Cyclin D2
EGFR
EMC
HIF1A
Fibronectin
Lhx2
N-CAM (CD56)
Nestin
Osteopontin
Plxd1
SDF1
Synaptin

FIGURE 41

A


050608 cRPC-UL time point
UL-d13=1
green: UL-d31
blue: UL-d52
red: UL-d97
Gene Expression
10 fold
100 fold
Log10 (Relative Quantitation)
B-Tubulin
B-Actin
Desmin
GFAP
Hes 1
Hes 5
Ki-67
Musashi 1
Nestin
Notch 1
Pax 6
PKC-alpha
Reelin
Synaptophysin
Sox1
Sox2
Vimentin
Detectors

B

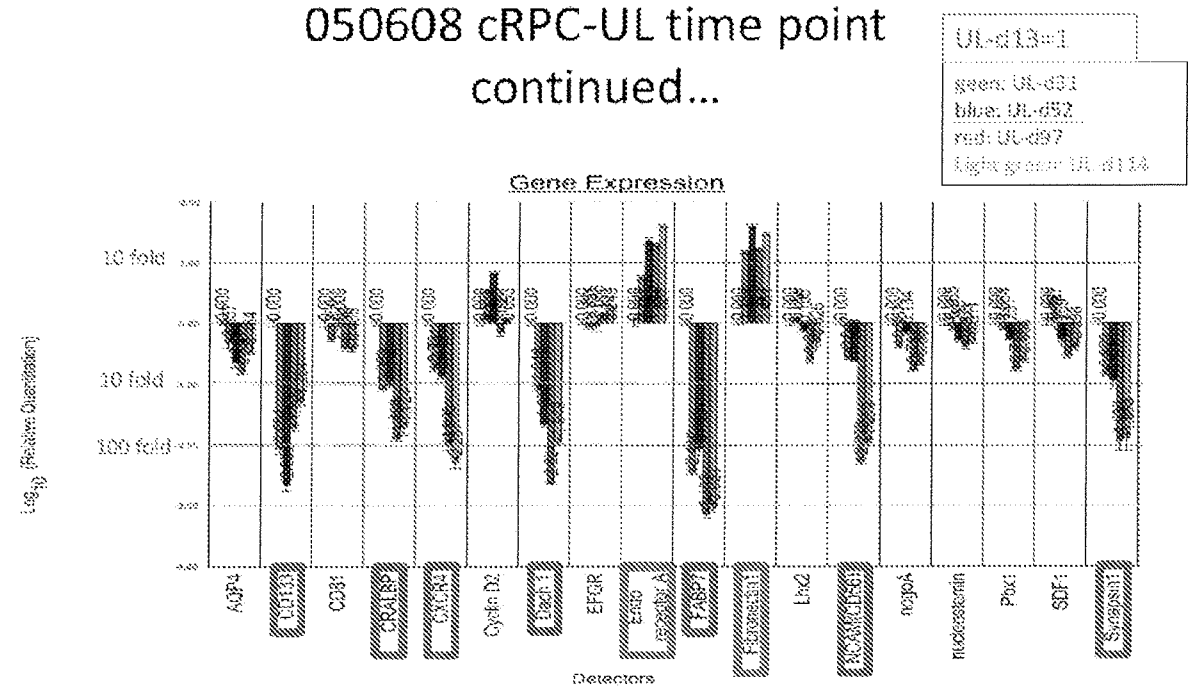
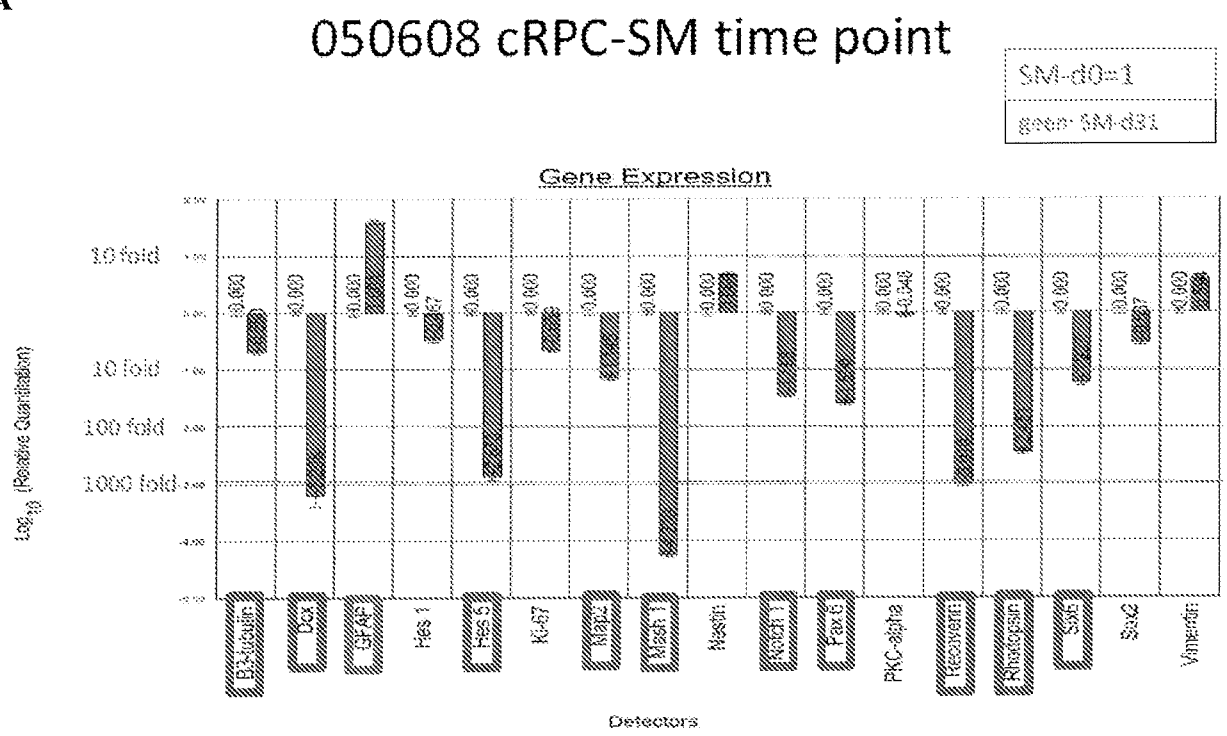

050608 cRPC-UL time point continued...
UL-d13=1
green: UL-d31
blue: UL-d52
red: UL-d97
Gene Expression
10 fold
10 fold
100 fold
Log10 (Relative Quantitation)
AQP4
CD133
CD31
CRALBP
CXCR4
Cyclin D2
Dach 1
EFGR
Ephrin A2
FABP7
Fibronectin
Lin2
NCAM(CD56)
nestin
osteocalcin
Pax6
SDF1
Synapsin1
Detectors

FIGURE 42

A


050608 cRPC-SM time point
SM-d0=1
geen SM-d31
Gene Expression
10 fold
10 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
Detectors
B3-tubulin
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Mash 1
Nestin
Notch 1
Pax 6
PKC-alpha
Tau
Tenascin
Rhakopsin
Sox1
Sox2
Vimentin

B

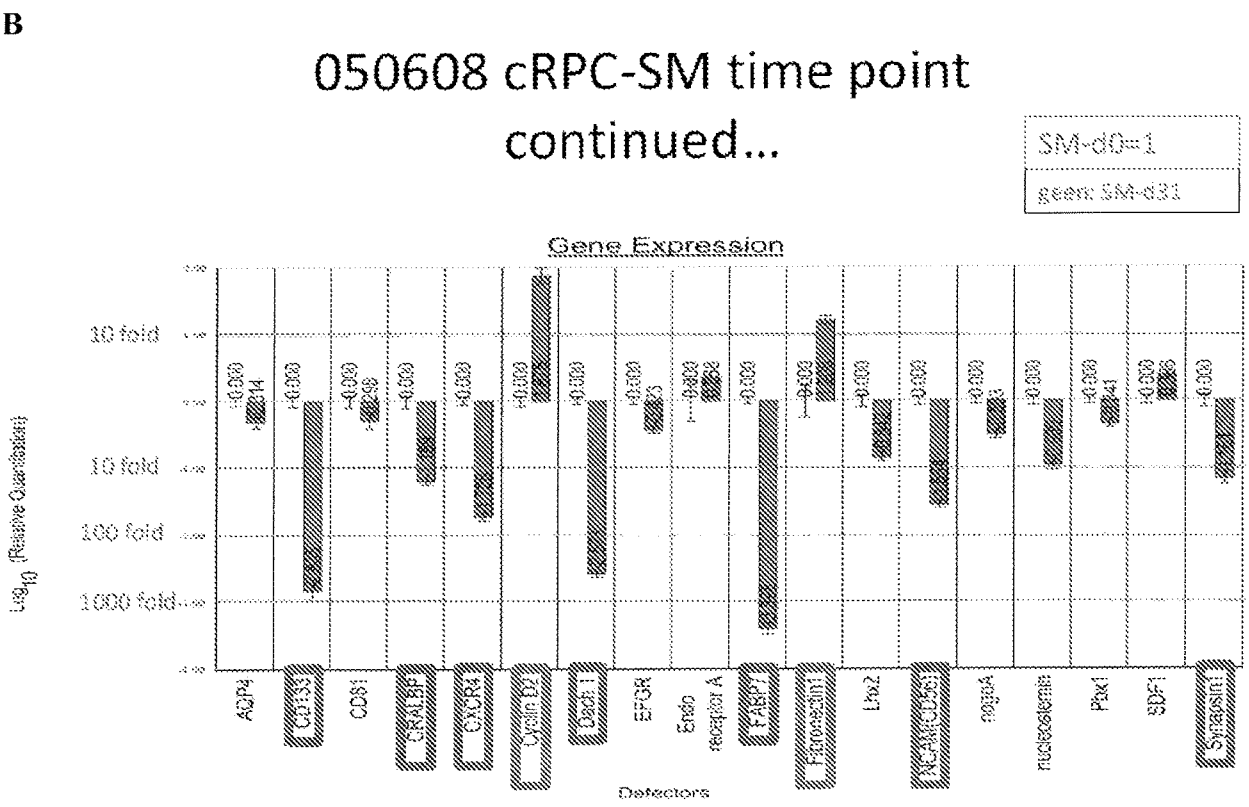

050608 cRPC-SM time point
continued...
SM-d0=1
geen SM-d31
Gene Expression
10 fold
10 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
Detectors
AQP4
CD133
CD31
CRABP
CXCR4
Cyclin D2
Dach 1
EPOR
Endo receptor A
FABP7
Fibronectin
Lhx2
NCAM(CD56)
ngn4
nucleostemin
Pax1
SDF1
Synapsin I

FIGURE 43

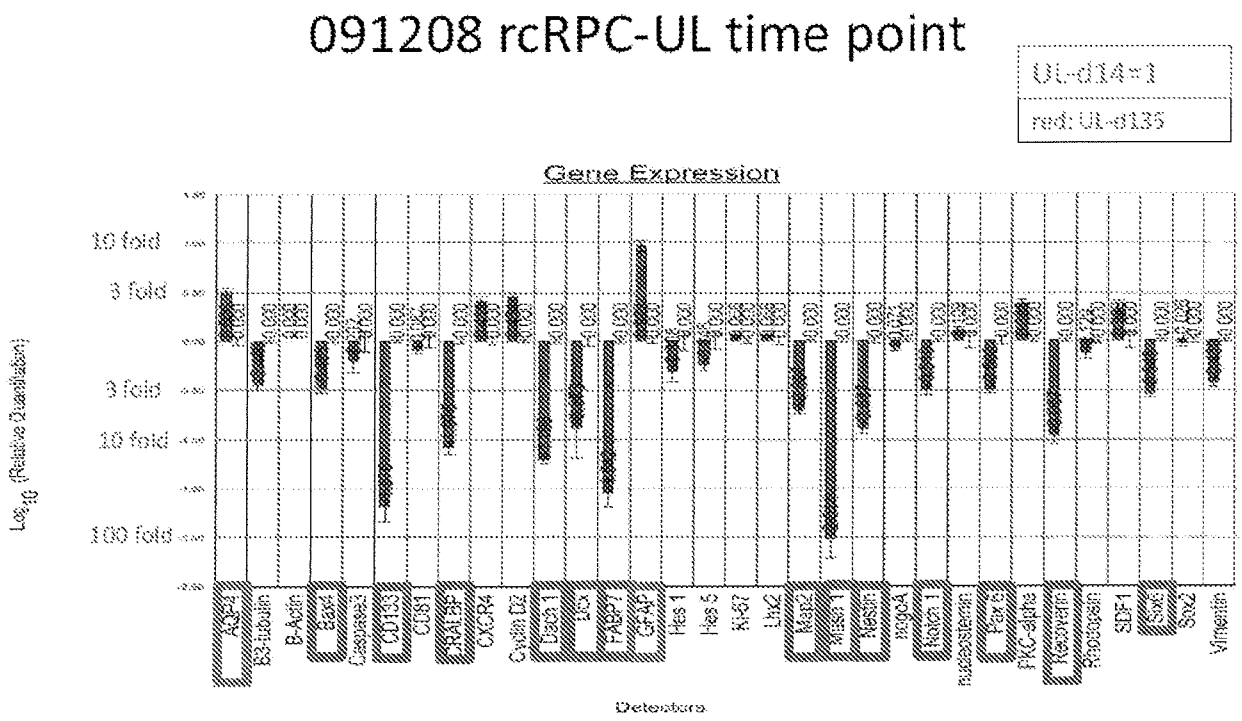

091208 rcRPC-UL time point
UL-d14=1
red: UL-d135
Gene Expression
10 fold
3 fold
3 fold
10 fold
100 fold
AQP4
B3-tubulin
B-Actin
Ecad
Caspase3
CD133
CD81
CRALBP
CXCR4
Cyclin D2
Dach 1
Dcx
FABP7
GFAP
Hes 1
Hes 5
Ki-67
Lhx2
Map2
Msi1
Nestin
nrgo4
Notch 1
nucleostemin
Pax6
PKC-alpha
Recoverin
Rhodopsin
SDF1
Sox1
Sox2
Vimentin
Detectors

FIGURE 44

cRPC time point

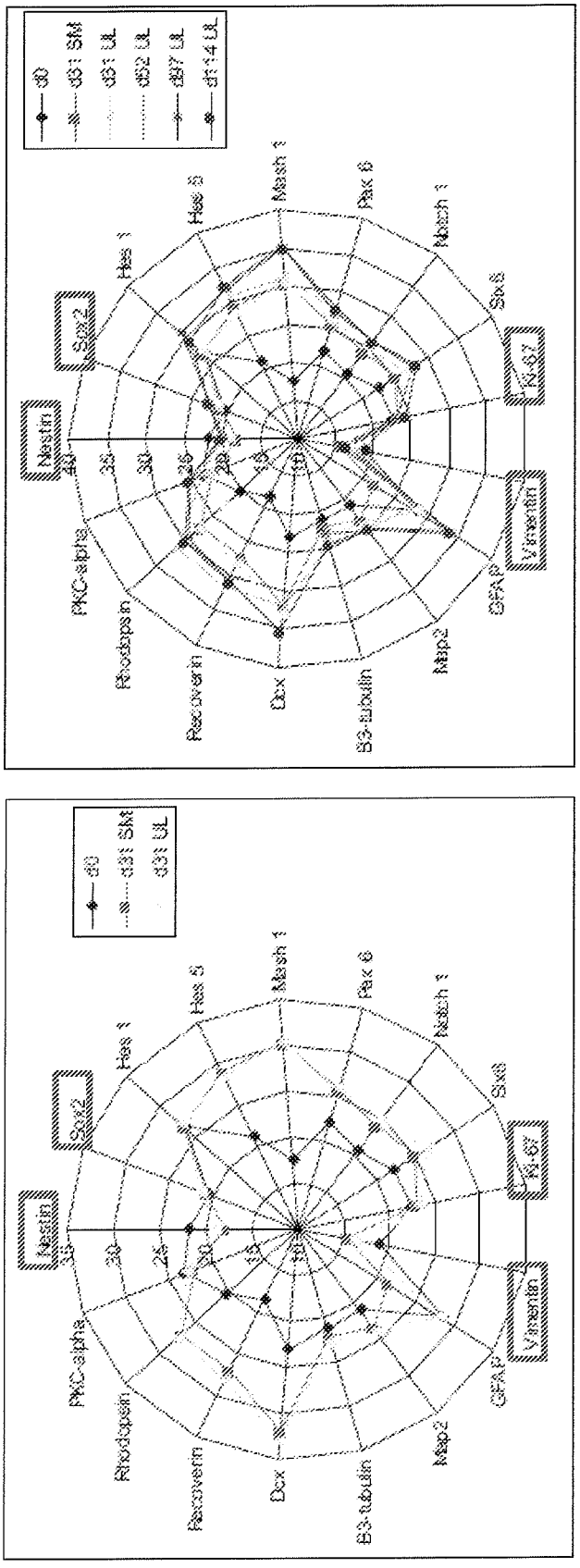
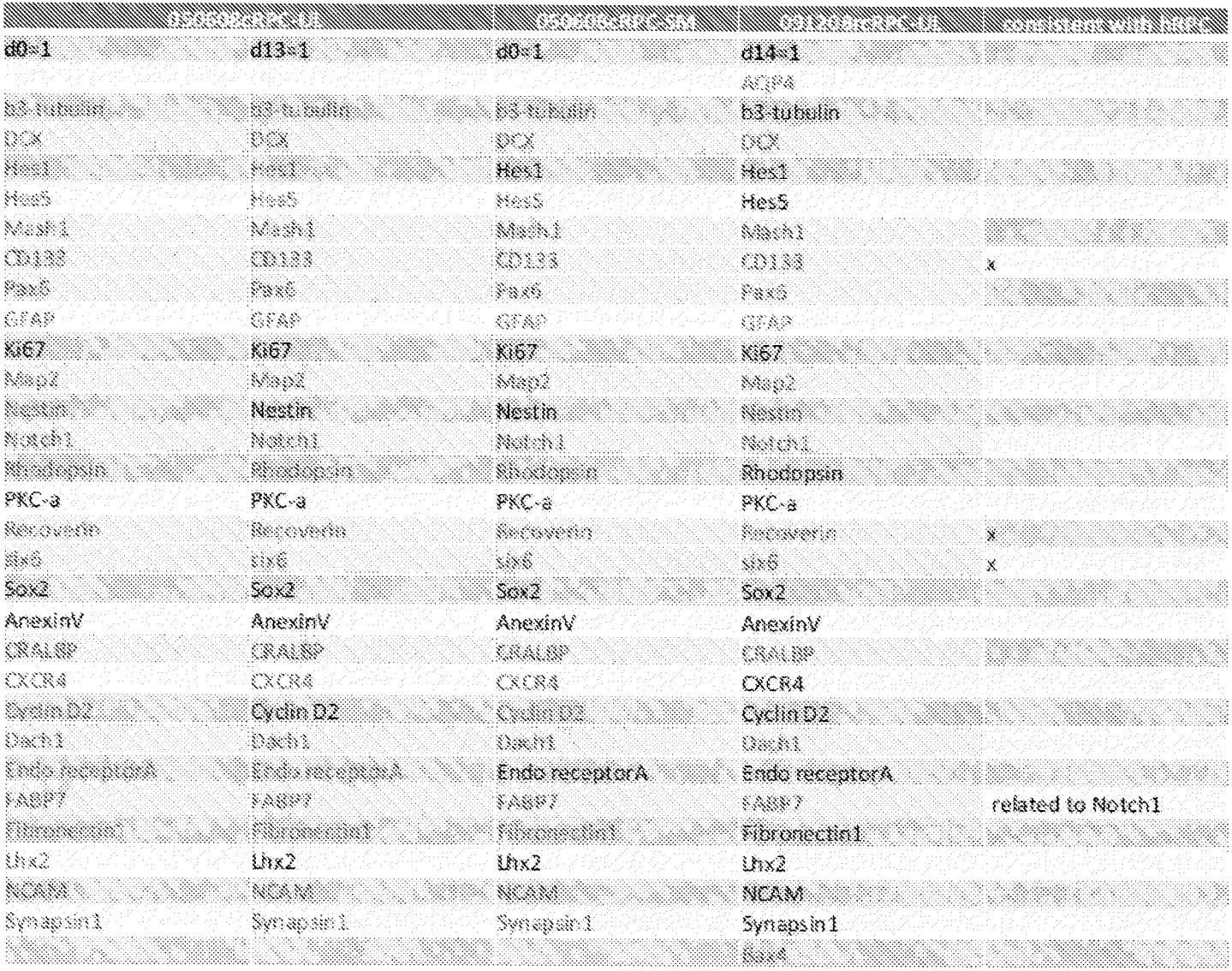


FIGURE 45



Retinoblastoma cell		Retinoblastoma cell	Retinoblastoma cell	correlation with Notch
d0~1	d13~1	d0~1	d14~1	
			AQP4	
b3-tubulin	b3-tubulin	b3-tubulin	b3-tubulin	
DCX	DCX	DCX	DCX	
Hes1	Hes1	Hes1	Hes1	
Hes5	Hes5	Hes5	Hes5	
Mash1	Mash1	Mash1	Mash1	
CD133	CD133	CD133	CD133	x
Pax6	Pax6	Pax6	Pax6	
GFAP	GFAP	GFAP	GFAP	
Ki67	Ki67	Ki67	Ki67	
Map2	Map2	Map2	Map2	
Nestin	Nestin	Nestin	Nestin	
Notch1	Notch1	Notch1	Notch1	
Rhodopsin	Rhodopsin	Rhodopsin	Rhodopsin	
PKC-a	PKC-a	PKC-a	PKC-a	
Recoverin	Recoverin	Recoverin	Recoverin	x
six6	six6	six6	six6	x
Sox2	Sox2	Sox2	Sox2	
AnexinV	AnexinV	AnexinV	AnexinV	
CRALBP	CRALBP	CRALBP	CRALBP	
CXCR4	CXCR4	CXCR4	CXCR4	
Cyclin D2	Cyclin D2	Cyclin D2	Cyclin D2	
Dach1	Dach1	Dach1	Dach1	
Endo receptorA	Endo receptorA	Endo receptorA	Endo receptorA	
FABP7	FABP7	FABP7	FABP7	related to Notch1
Fibronectin1	Fibronectin1	Fibronectin1	Fibronectin1	
Unx2	Unx2	Unx2	Unx2	
NCAM	NCAM	NCAM	NCAM	
Synapsin1	Synapsin1	Synapsin1	Synapsin1	
			Sox4	

FIGURE 46

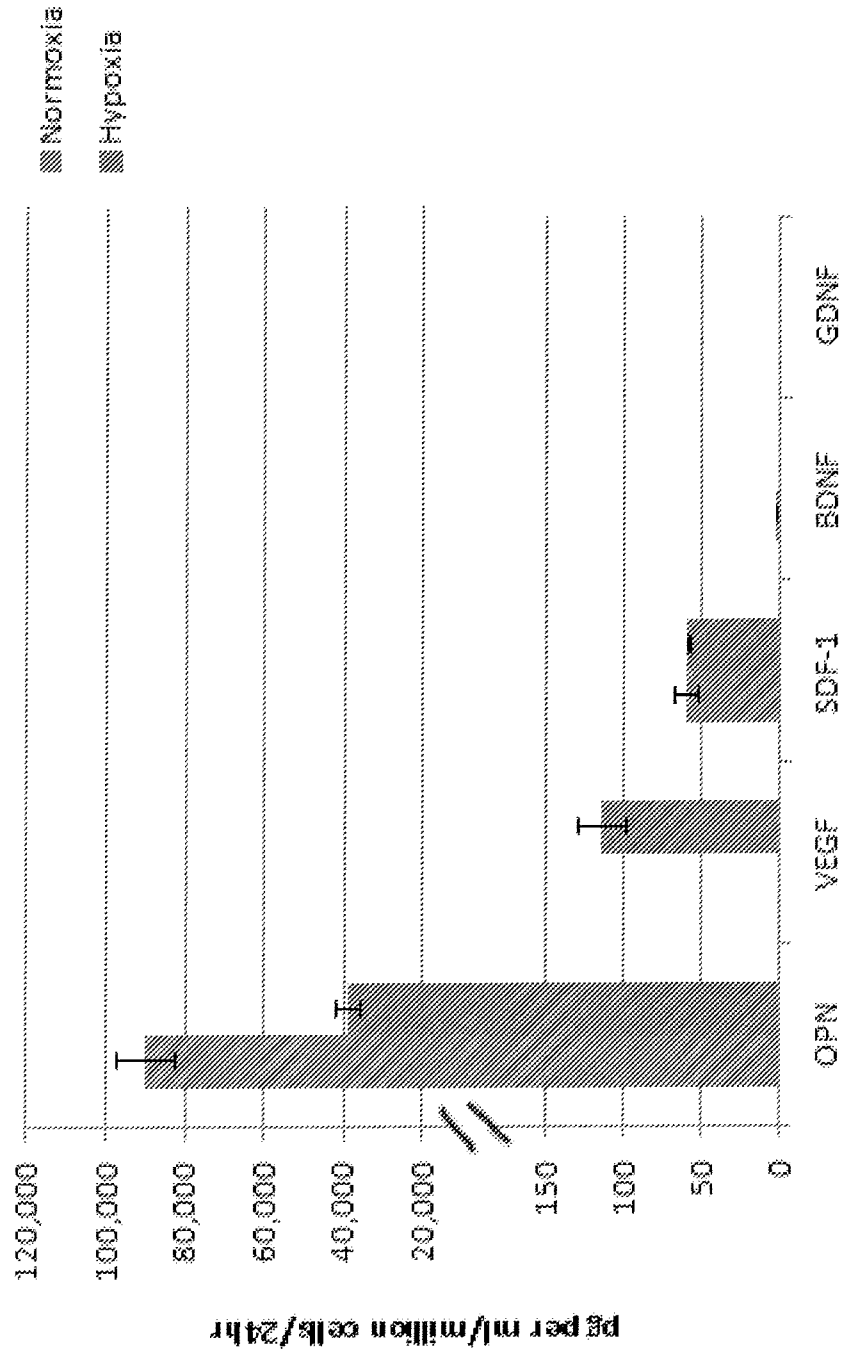

Normoxia
Hypoxia
pg per ml/million cells/24hr
120,000
100,000
80,000
60,000
40,000
20,000
150
100
50
0
OPN
VEGF
SDF-1
BDNF
GDNF

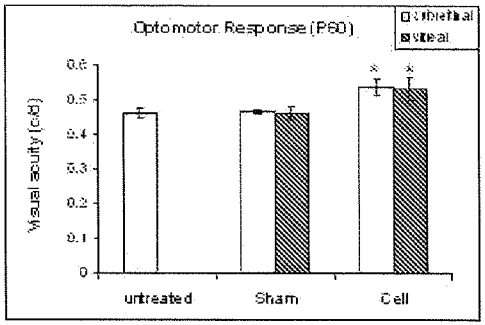
FIGURE 47

	retina	cultured hRPC		
	retina tissue	**Normoxia hRPC**	**Hypoxia hRPC**	**ICC confirmed**
Nestin	86%	99%	99%	95%
Sox2	70%	90-99%	90-99%	90%
Ki67				40-50%
GFAP	0.5%	5-10%	5-10%	5%
MHC I	2%	90%	90%	
MHC II	0%	1-3%	0-1%	
FAS (CD95)	0%	40-60%	40-60%	
CXCR4	80%	5-30%	90%	
CD15	4-8%	4-8%	15-35%	12%
GD2	2%	2-4%	15%	6%

FIGURE 48

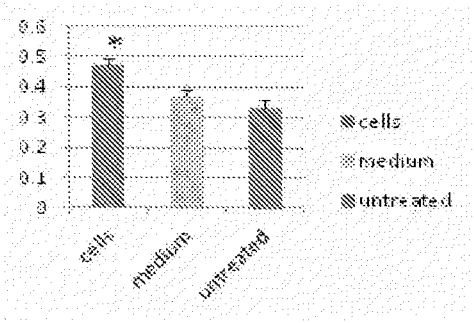
A

Optomotor response P60



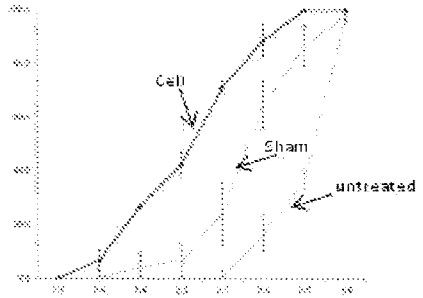
B

Optomotor response P90



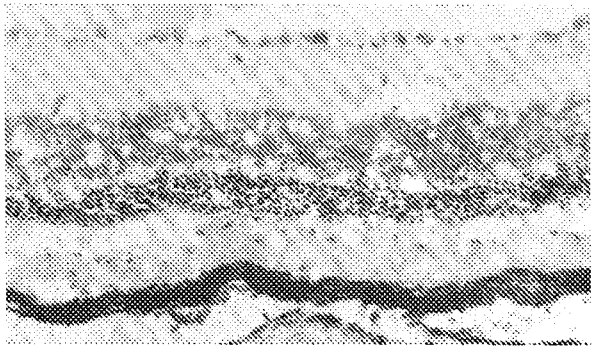
C

Luminance threshold response P90



D

Histology P60 (intravitreal injection)



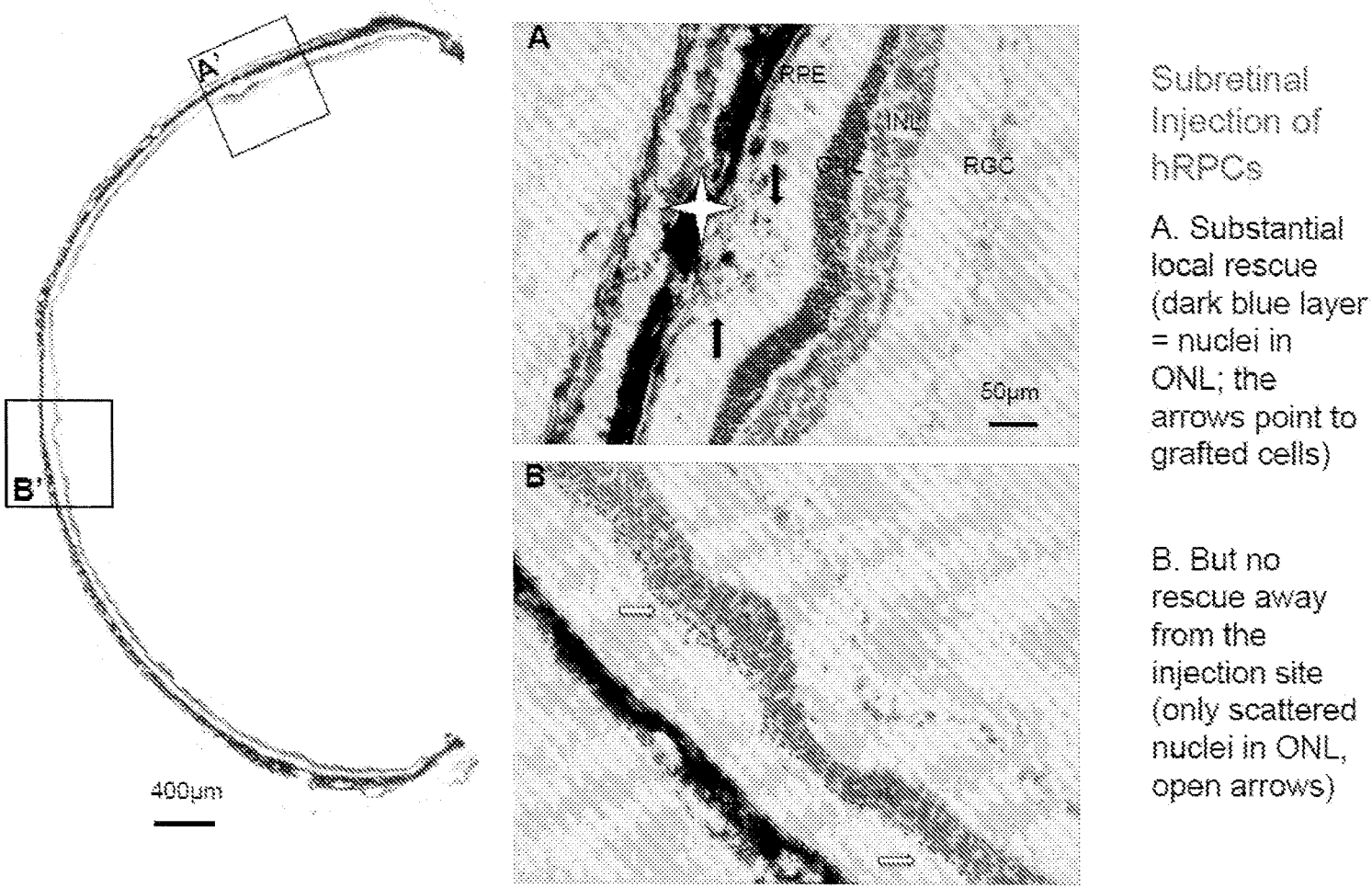
E

Histology P90 (intravitreal injection)



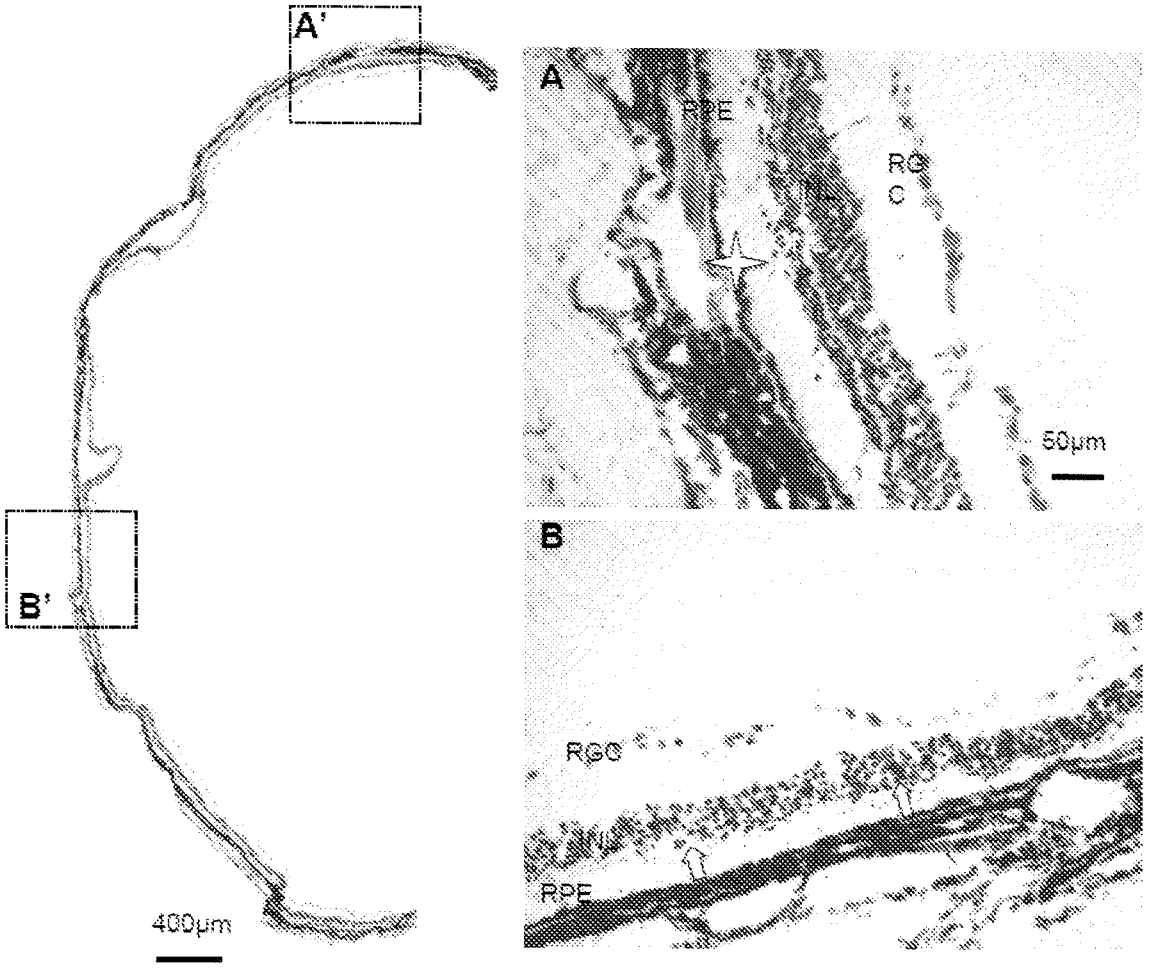
Widespread, persistent rescue of photoreceptor cells in dystrophic retina by hRPCs

FIGURE 49



Subretinal injection of retinal progenitor cells (RPC) in RCS rat (performed at P21, viewed at P100): Left-montage image of cresyl violet stained retinal section.
A: high power image in outline A' showing injection site (star), donor cells (arrows) and ONL preservation. B: high power image in outline B' showing a single layer of ONL (arrows) in area away from the graft.

FIGURE 50



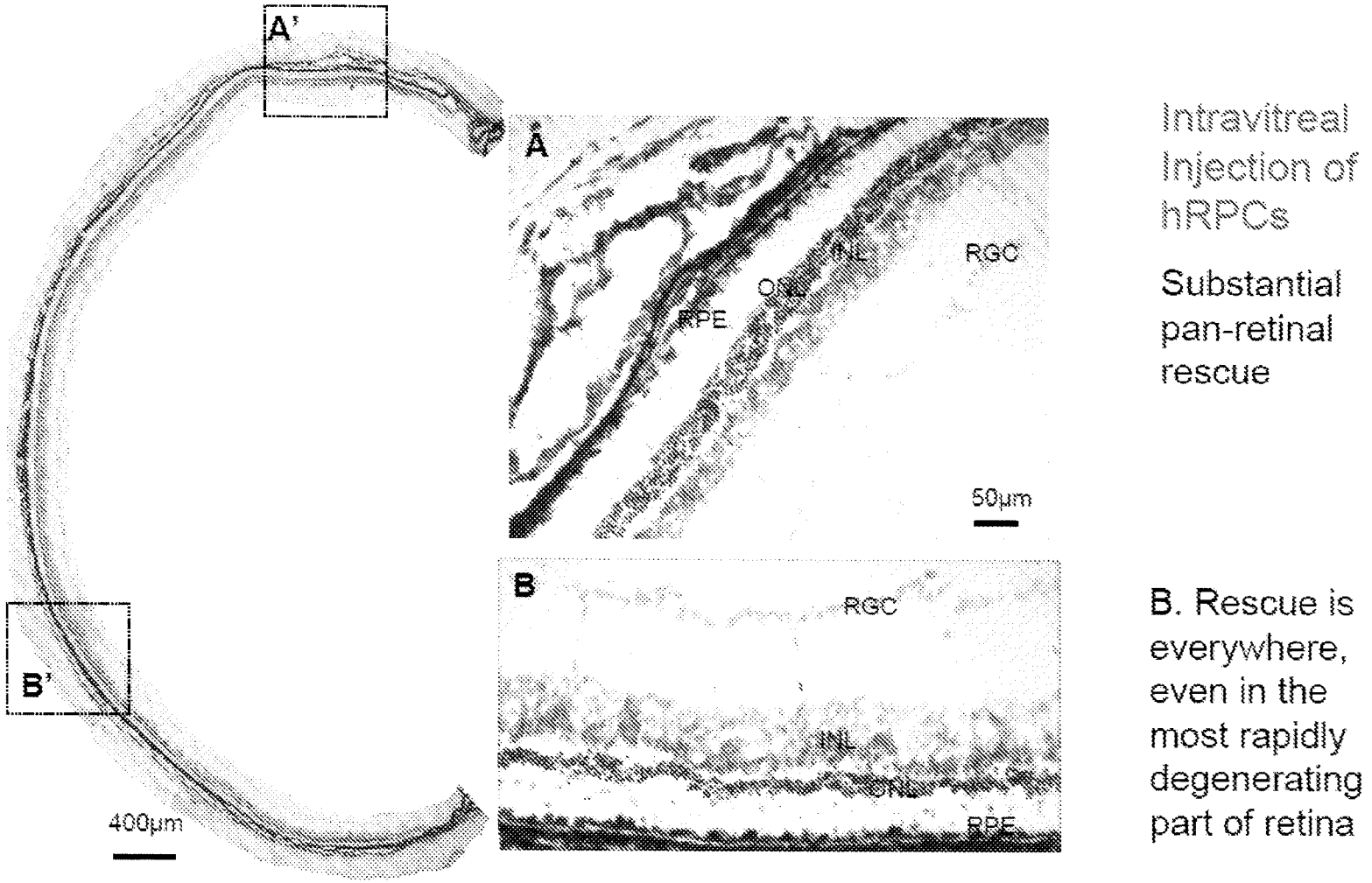
Subretinal Injection of vehicle (sham)

A. Also shows local rescue (moderate)

B. No rescue away from the site

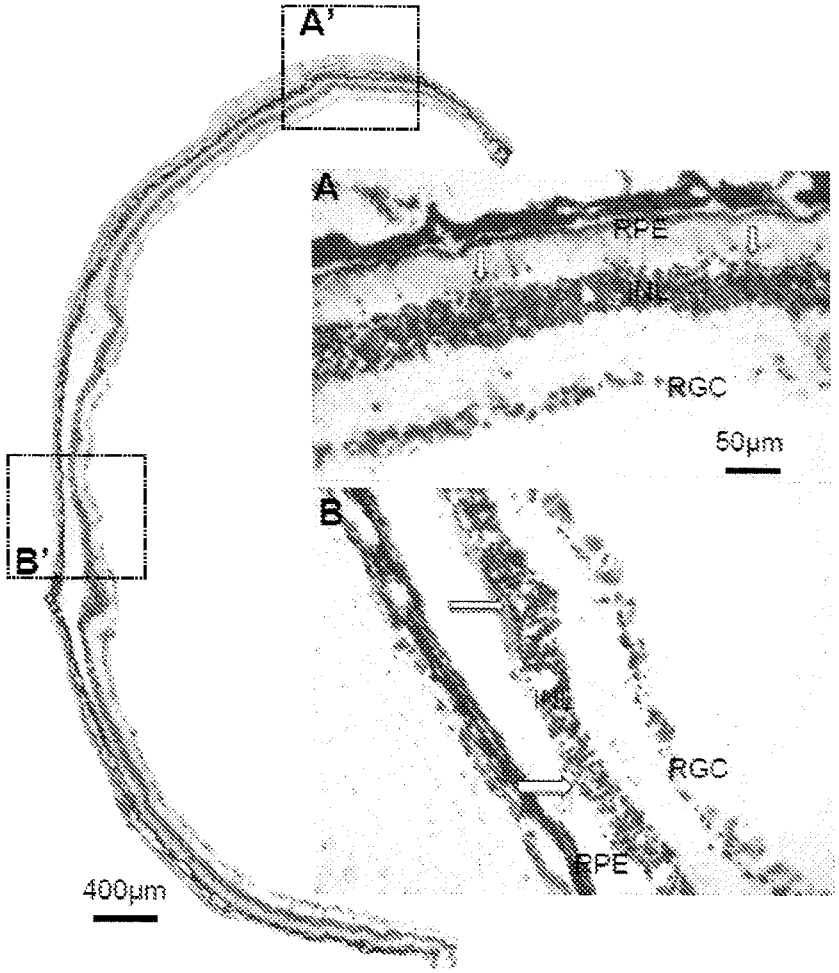
Subretinal injection of vehicle in RCS rat, viewed at P100: Left-montage image of cresyl violet stained section. A: high power image of outline A' showing injection site (star) and localized ONL rescue. B: high power image of outline B; showing a single layer of ONL (arrows) at area away from injection site.

FIGURE 51



Intravitreal injection of RPC in RCS rat, viewed at P100. Left-montage image of cresyl violet stained section showing ONL rescue over the whole retinal. A: high power of outline A' showing 5-6 layers of ONL.B. high power image of outline B' showing ONL preservation.

FIGURE 52



Intravitreal Injection of vehicle (sham)

No discernable rescue*

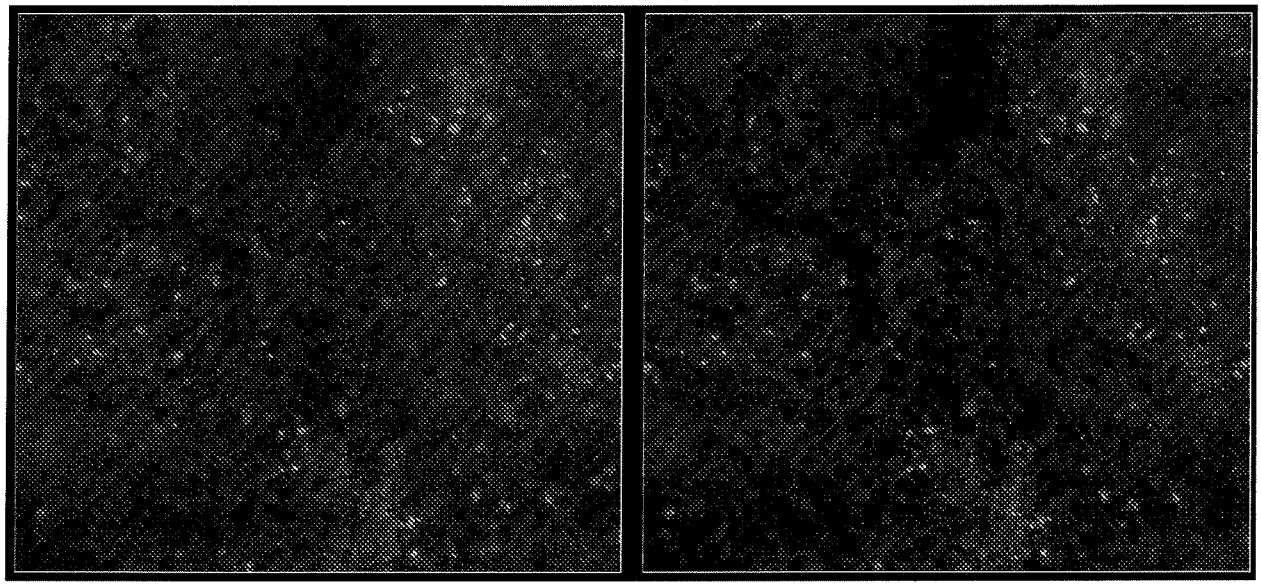
*(There will always be a nonspecific effect at initial needle entry point)

Intravitreal injection of carry medium in RCS rat, viewed at P100. Left-montage image of cresyl violet stained section. A&B: high power of outline A' &B' showing a single layer of photoreceptors (arrows)

FIGURE 53

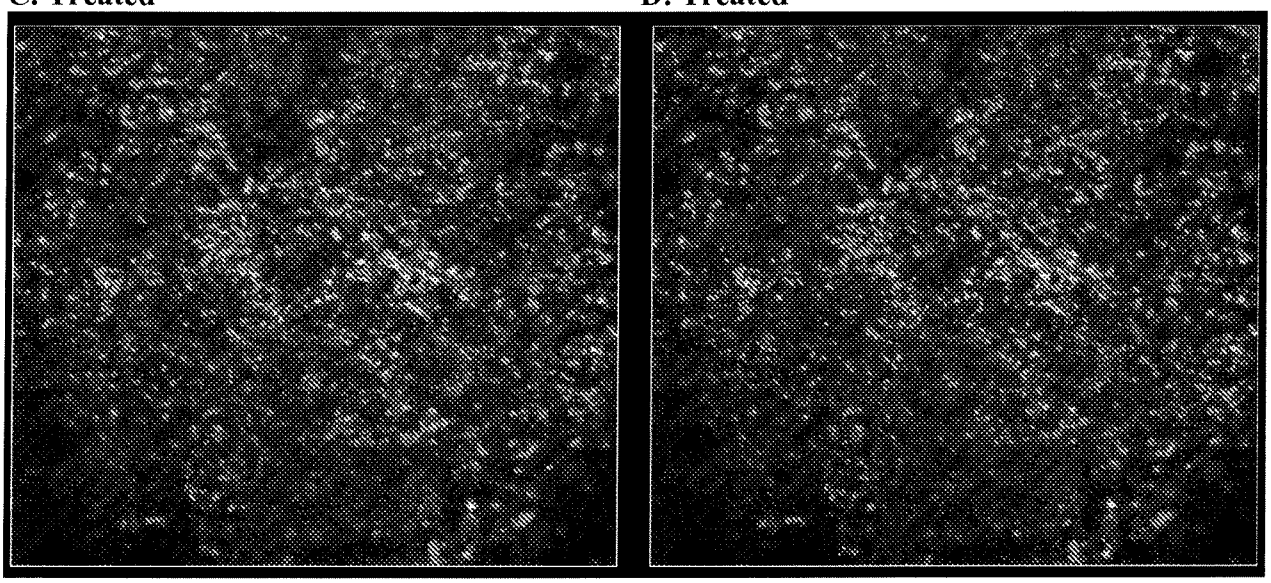
A. Sham

B. Sham



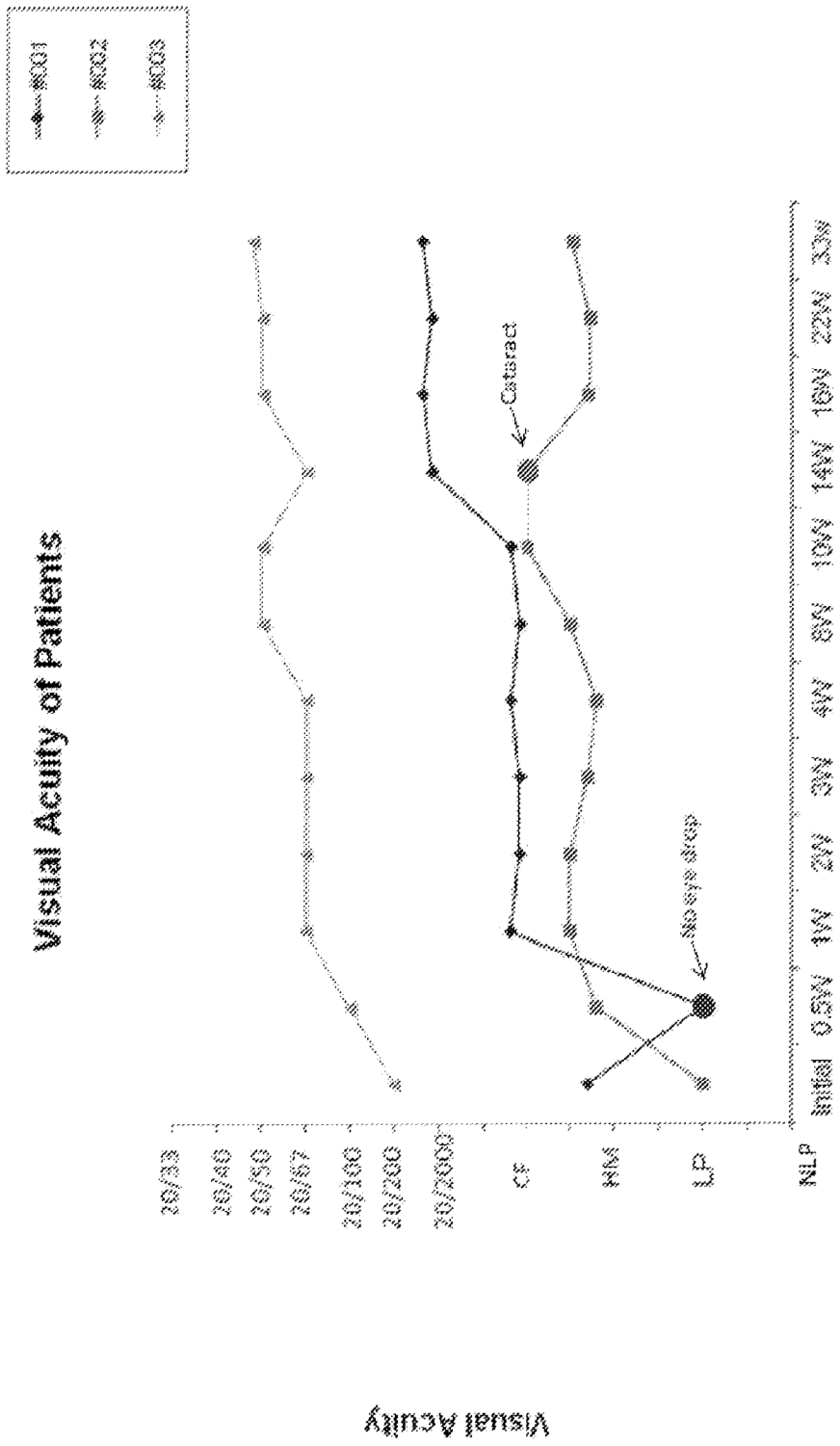
C. Treated

D. Treated



Red: Rod opsin; Green: M-cone opsin; Blue: PNA (M-&S-cone sheaths)

FIGURE 54



Explanatory clinical correlates provided (arrows)

FIGURE 55