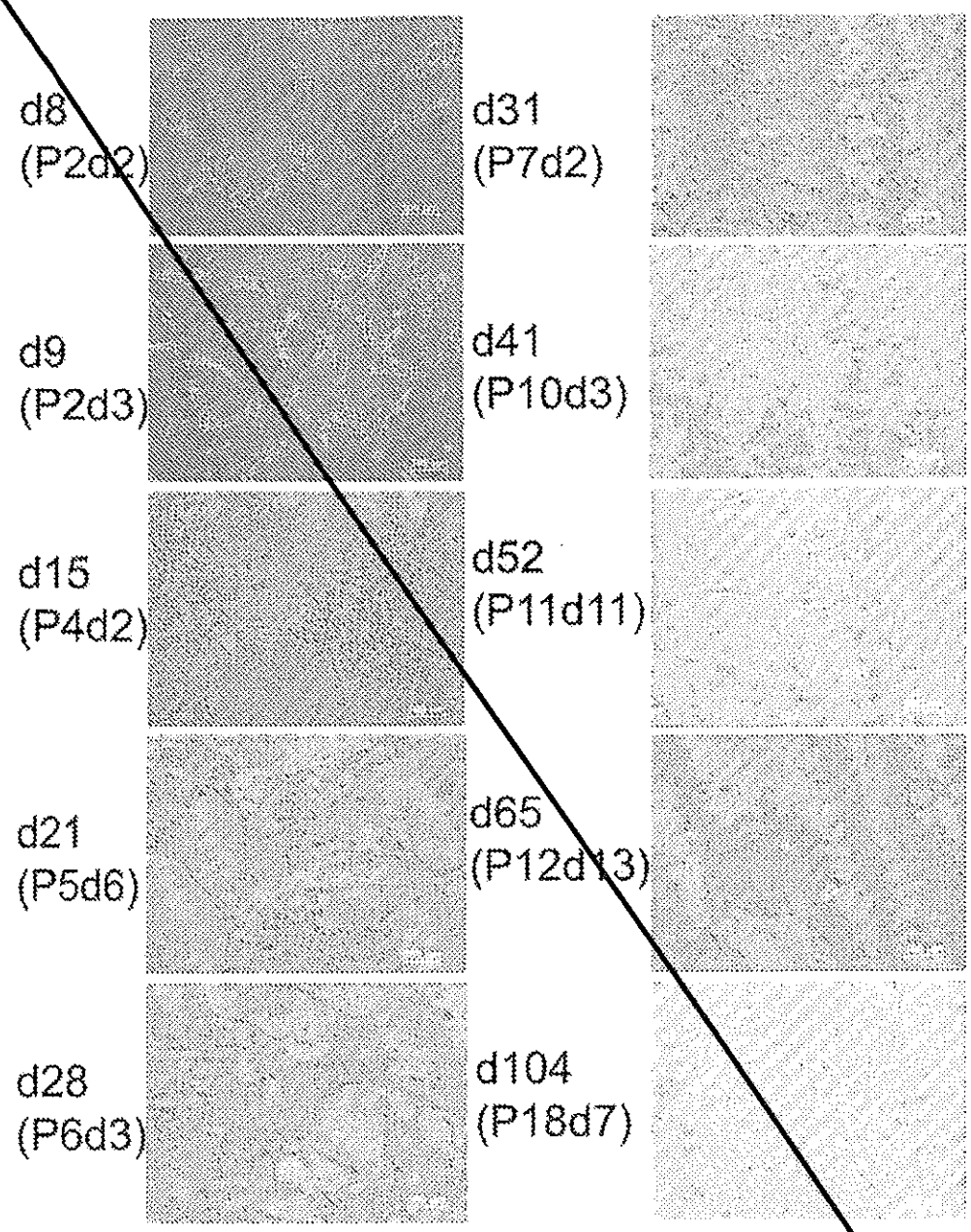

d8
(P2d2)
d31
(P7d2)
d9
(P2d3)
d41
(P10d3)
d15
(P4d2)
d52
(P11d11)
d21
(P5d6)
d65
(P12d13)
d28
(P6d3)
d104
(P18d7)

FIGURE 1

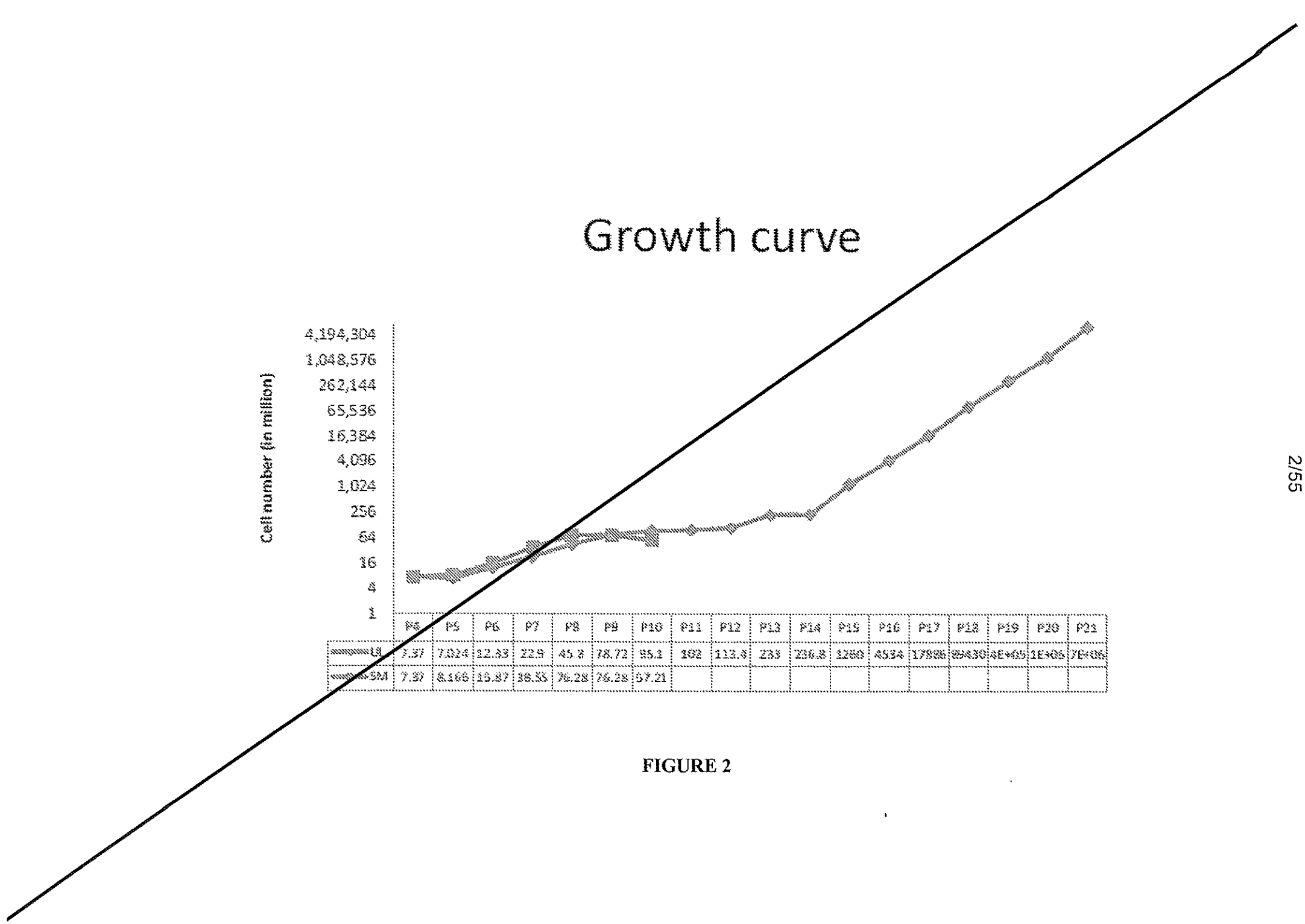
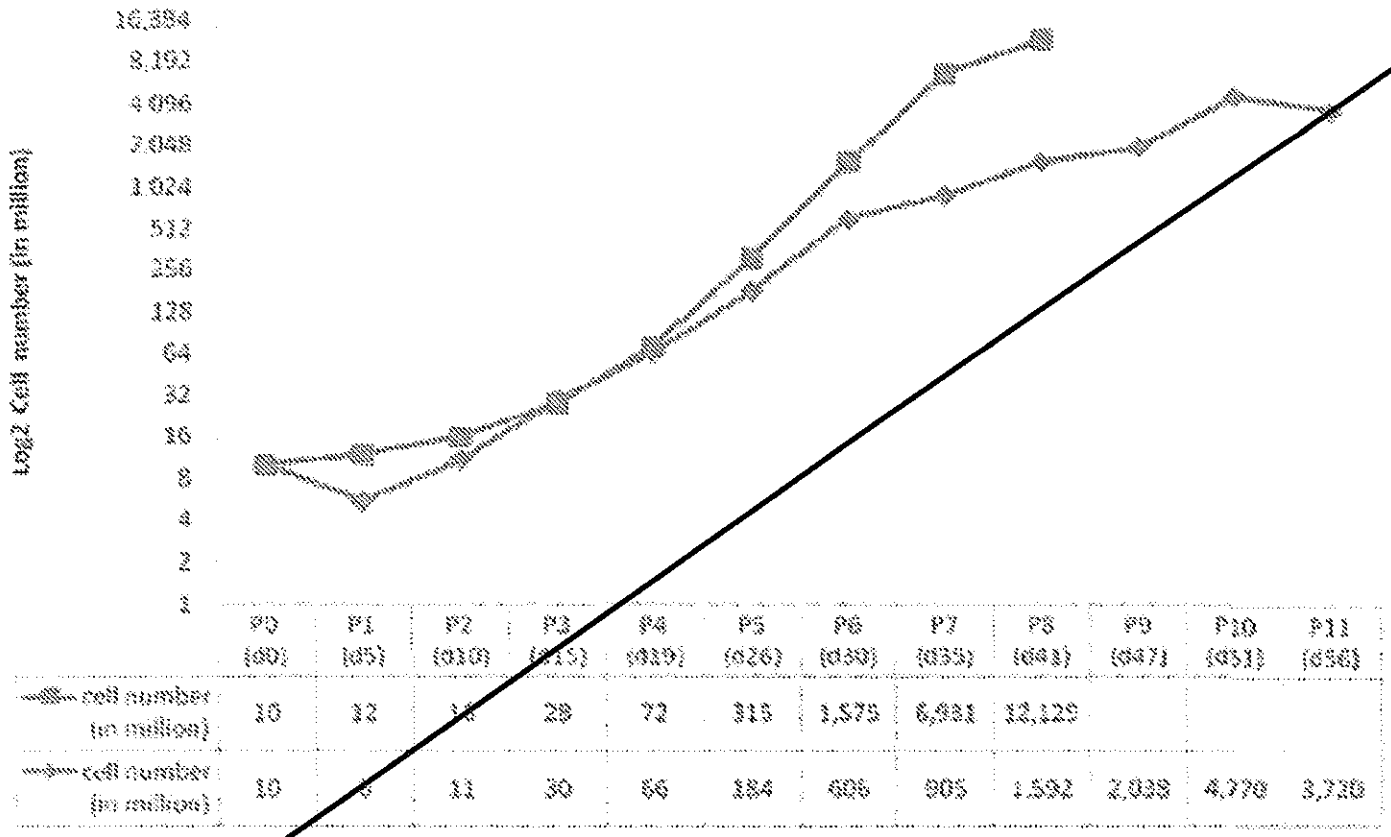

Growth curve
Cell number (in million)
4,194,304
1,048,576
262,144
65,536
16,384
4,096
1,024
256
64
16
4
1
P4 P5 P6 P7 P8 P9 P10 P11 P12 P13 P14 P15 P16 P17 P18 P19 P20 P21
UI 7.37 7.824 12.83 22.9 45.8 78.72 85.1 102 113.8 233 236.8 1260 4554 17586 8E+06 6E+06 1E+06 7E+06
SM 7.37 8.166 15.87 38.55 76.28 76.28 67.21

FIGURE 2

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	14	29	72	315	1,575	6,881	12,129			
cell number (in million)	10	9	11	30	66	184	606	905	1,592	2,039	4,770	3,730

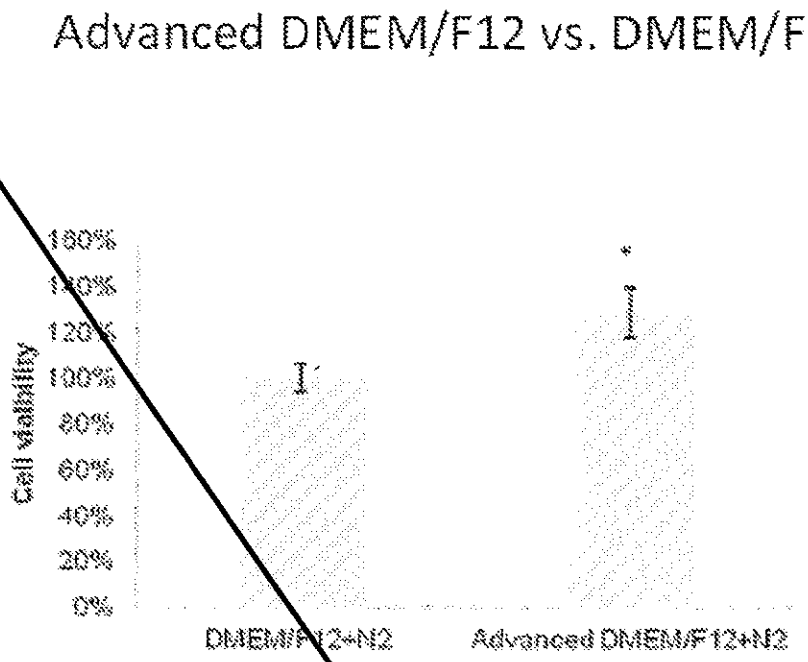
The blue donation was used in patients at ~P5, resulting in visual improvements

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
Days in Culture

FIGURE 5

A


Advanced DMEM/F12 vs. DMEM/F12
Cell viability
160%
140%
120%
100%
80%
60%
40%
20%
0%
*
DMEM/F12+N2
Advanced DMEM/F12+N2

B

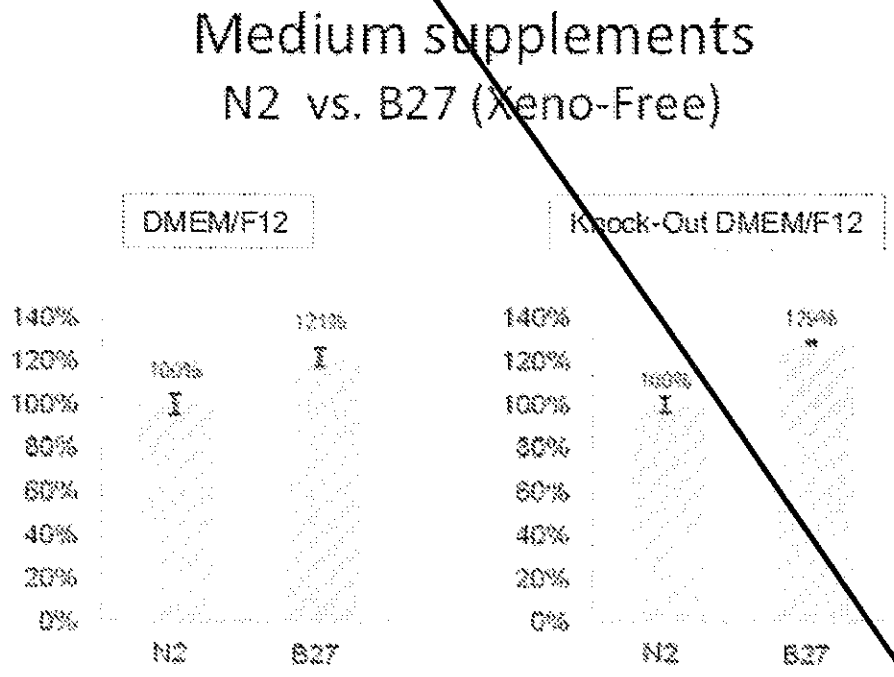
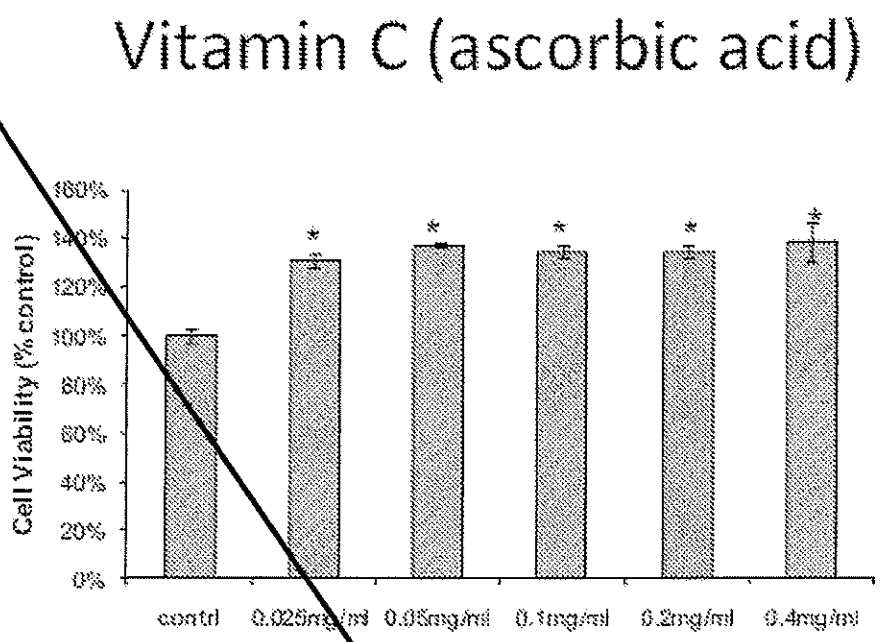
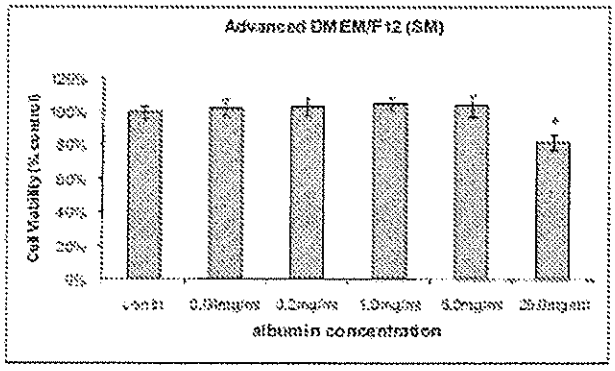

Medium supplements
N2 vs. B27 (Xeno-Free)
DMEM/F12
Knock-Out DMEM/F12
140%
120%
100%
80%
60%
40%
20%
0%
100%
121%
100%
104%
**
N2
B27
N2
B27

FIGURE 6

A


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

B


Advanced DMEM/F12 (SM)
albumin concentration

C

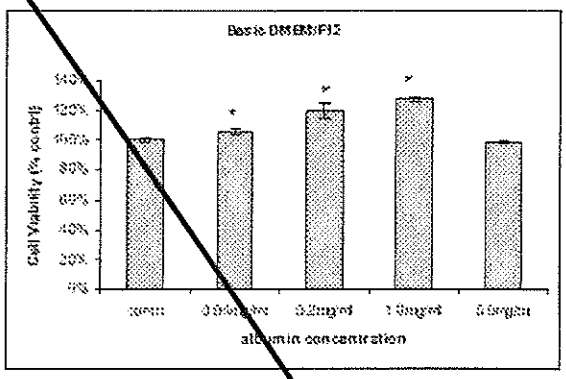

albumin concentration

FIGURE 7

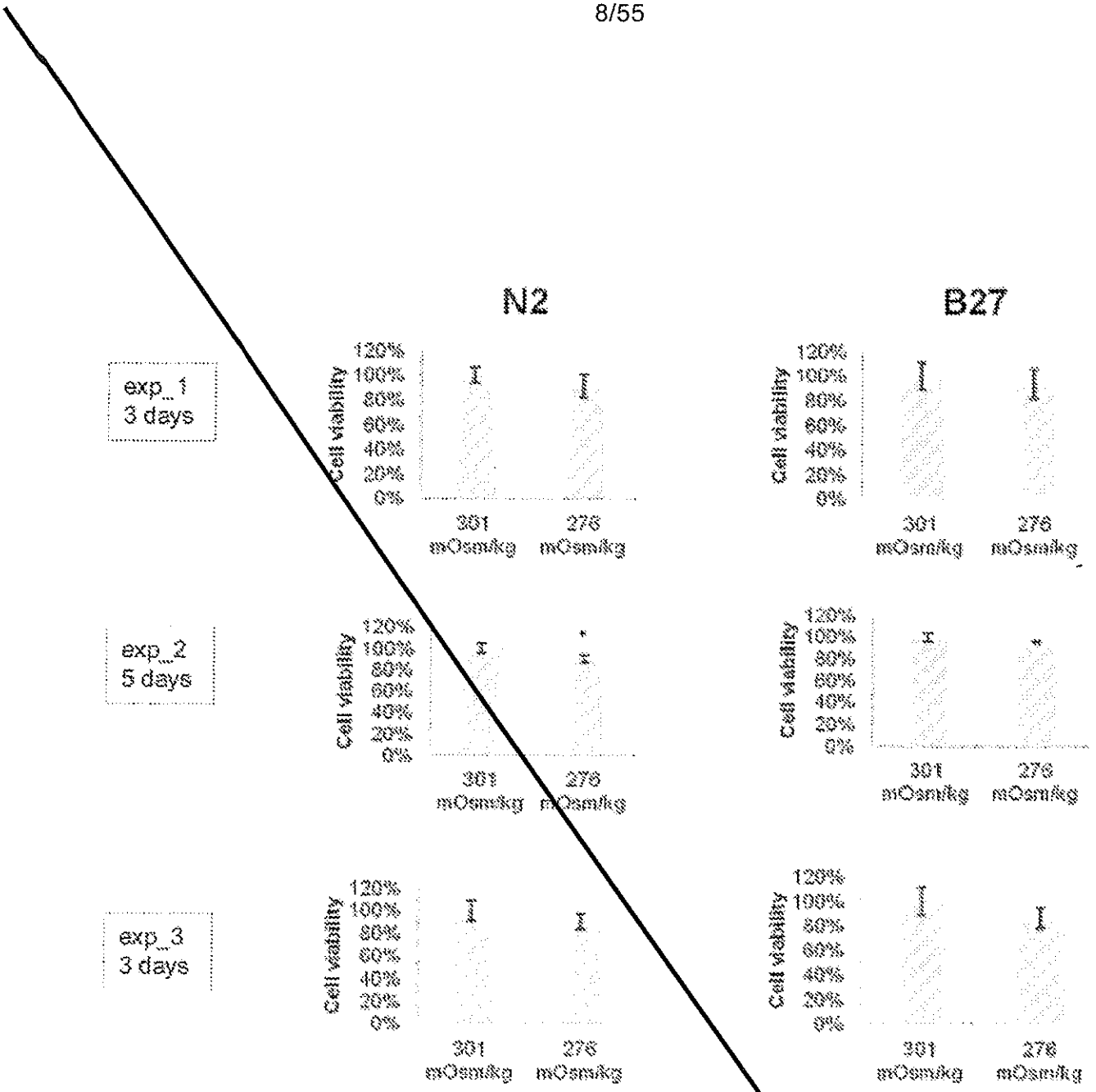
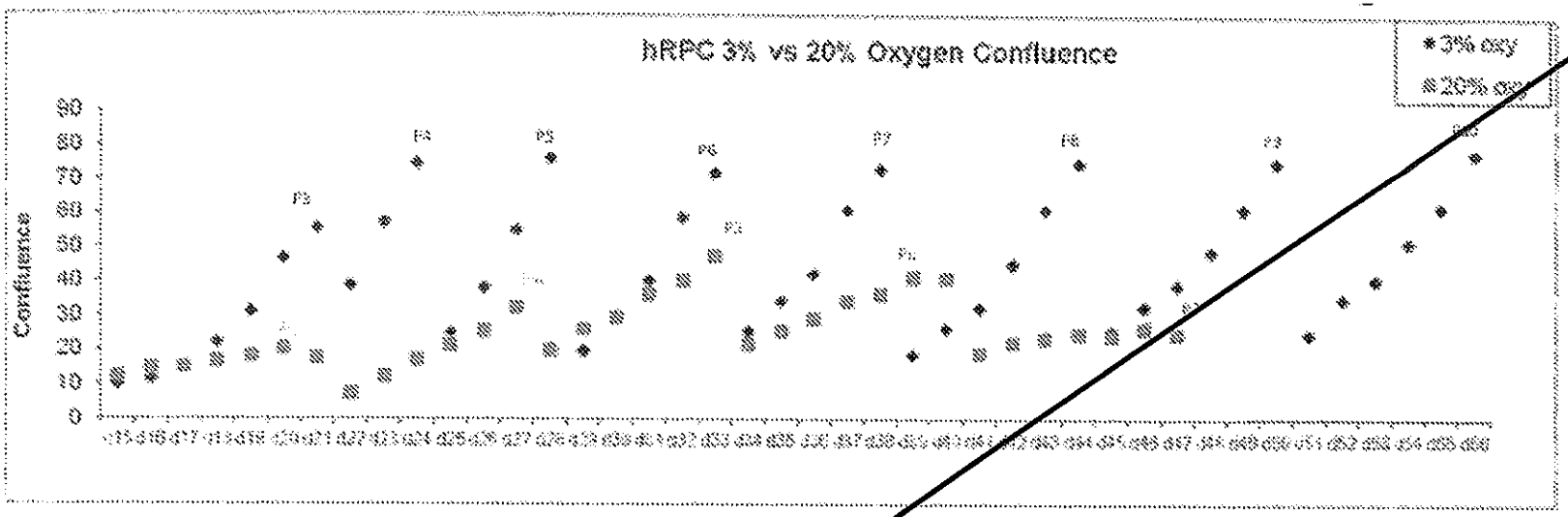

N2
B27
exp_1
3 days
exp_2
5 days
exp_3
3 days
Cell viability
120%
100%
80%
60%
40%
20%
0%
301
mOsm/kg
276
mOsm/kg
*

FIGURE 8


hRPC 3% vs 20% Oxygen Confluence
3% oxy
20% oxy
Confluence
0
10
20
30
40
50
60
70
80
90
P3
P4
P5
P6
P7
P8
P9

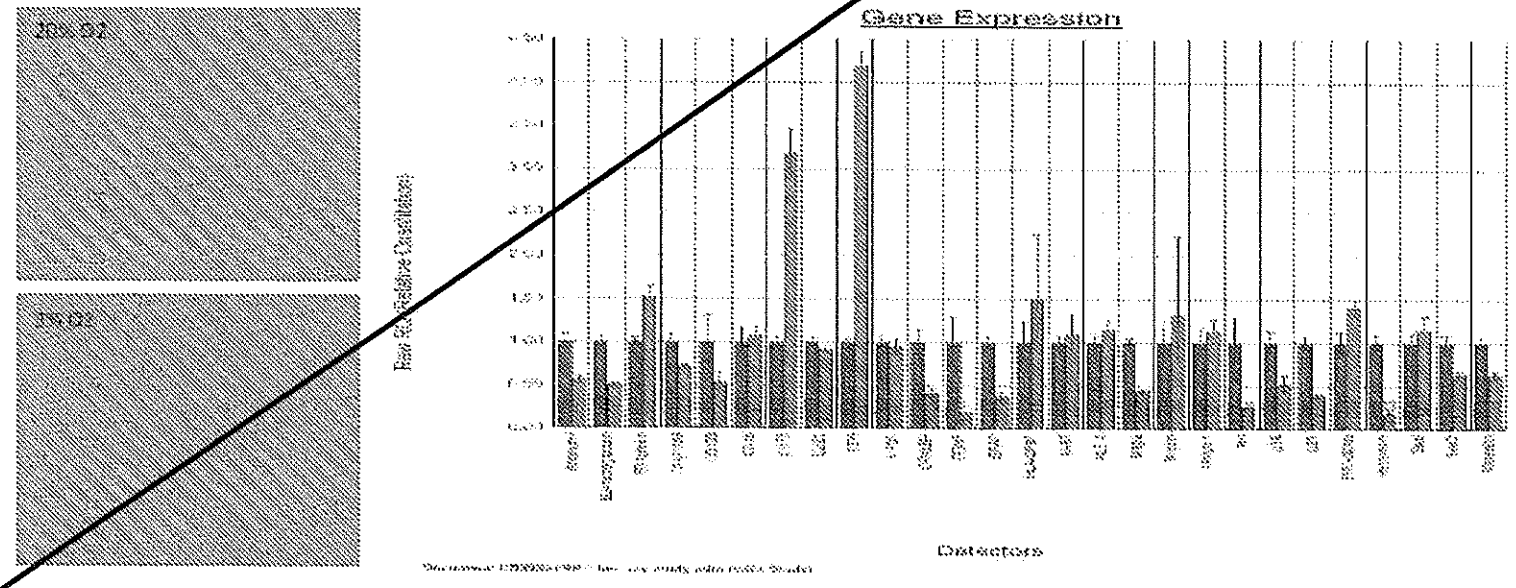
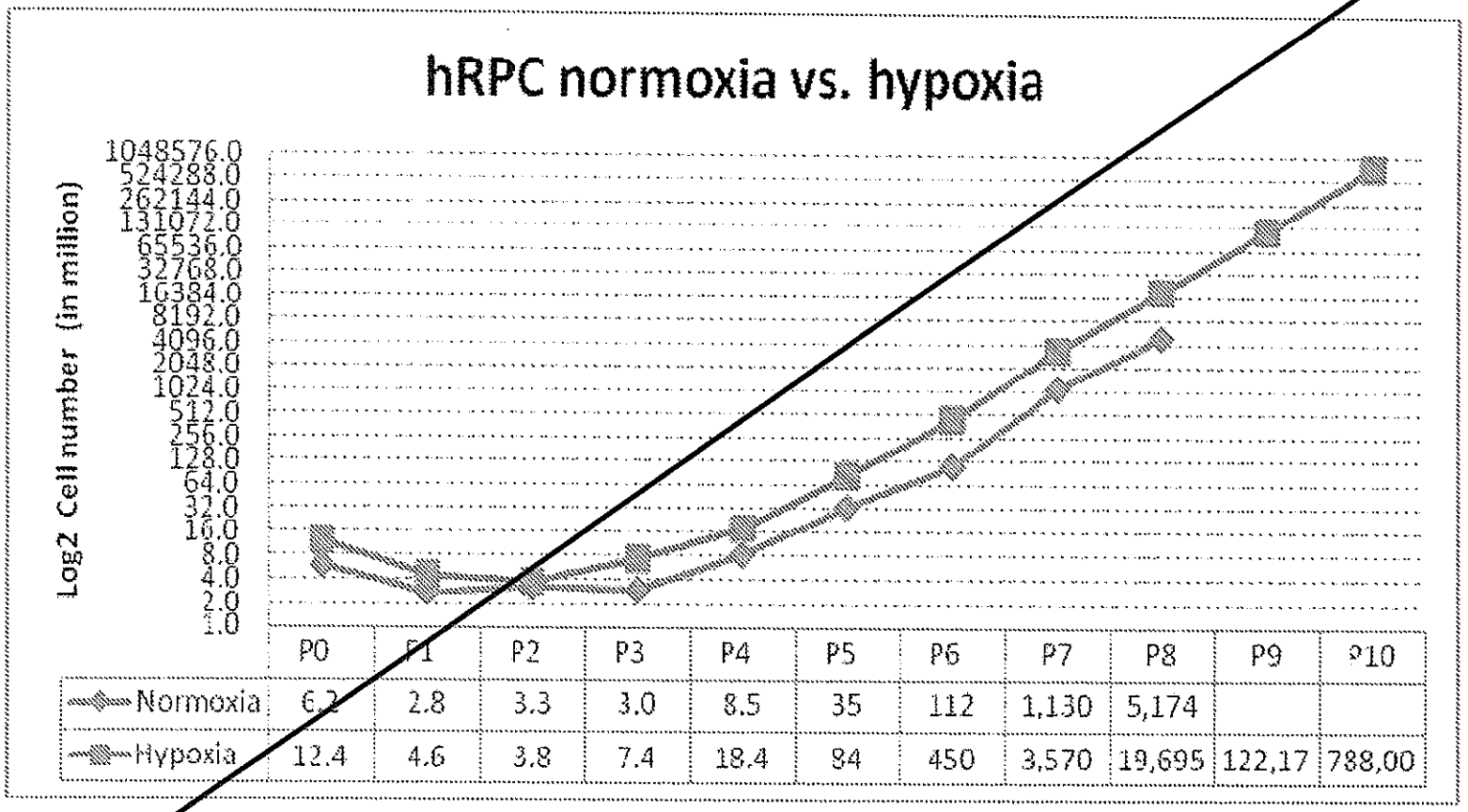

20% O2
3% O2
Gene Expression
Detectors

FIGURE 9



	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

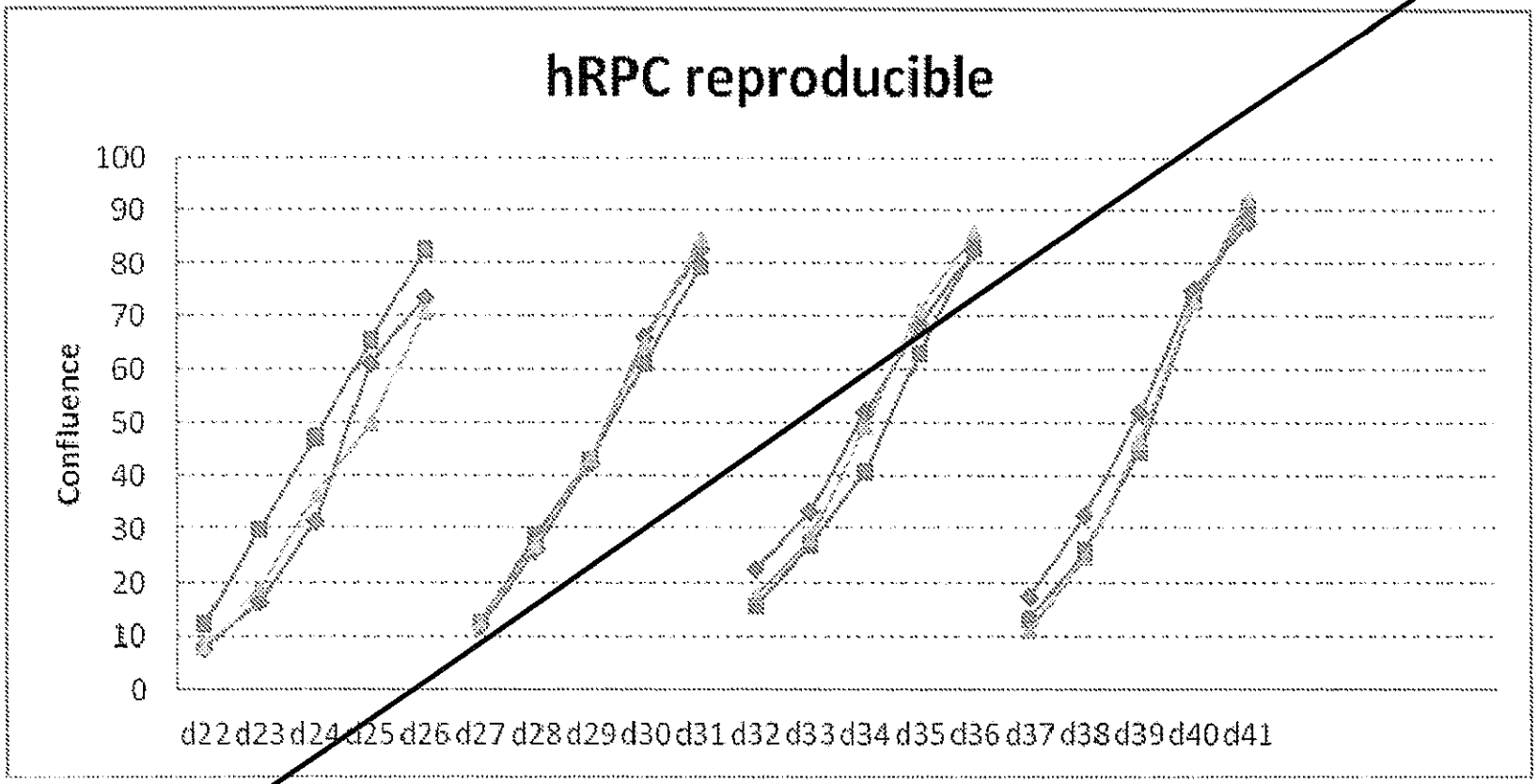

hRPC reproducible
Confluence
100
90
80
70
60
50
40
30
20
10
0
d22 d23 d24 d25 d26 d27 d28 d29 d30 d31 d32 d33 d34 d35 d36 d37 d38 d39 d40 d41

FIGURE 11

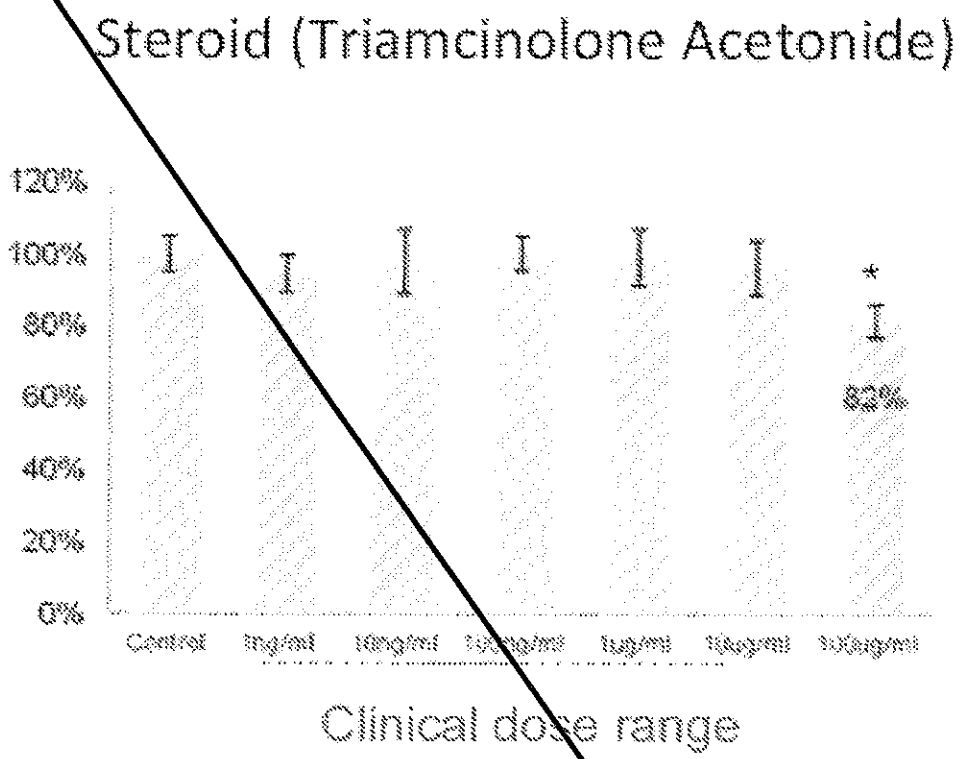

Steroid (Triamcinolone Acetonide)
120%
100%
80%
60%
40%
20%
0%
*
82%
Control
Clinical dose range

FIGURE 12

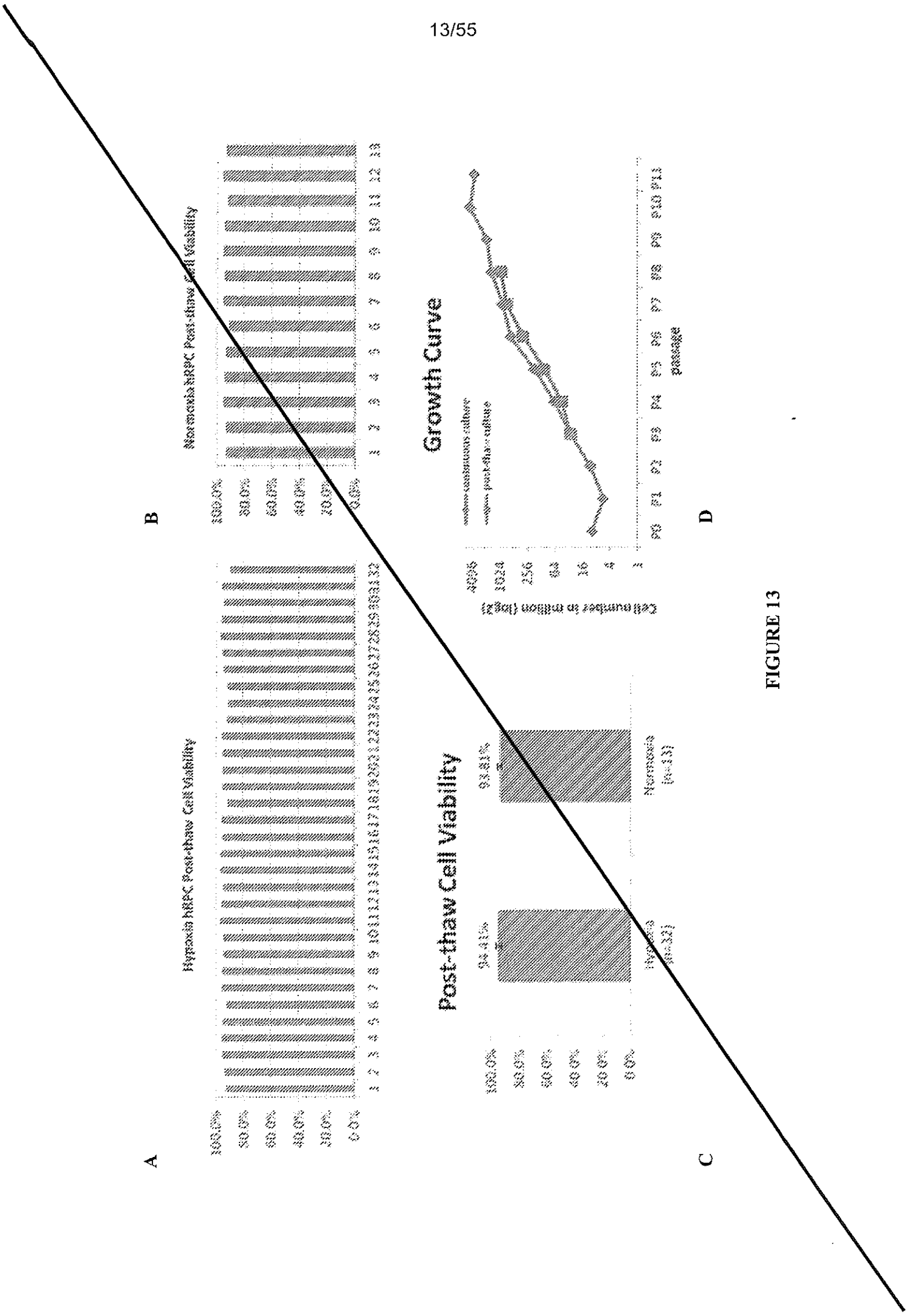

A
Hypoxia hREC Post-thaw Cell Viability
B
Normoxia hREC Post-thaw Cell Viability
C
Post-thaw Cell Viability
94.81%
Normoxia (n=13)
D
Growth Curve
cell number in million (log2)
passage

FIGURE 13

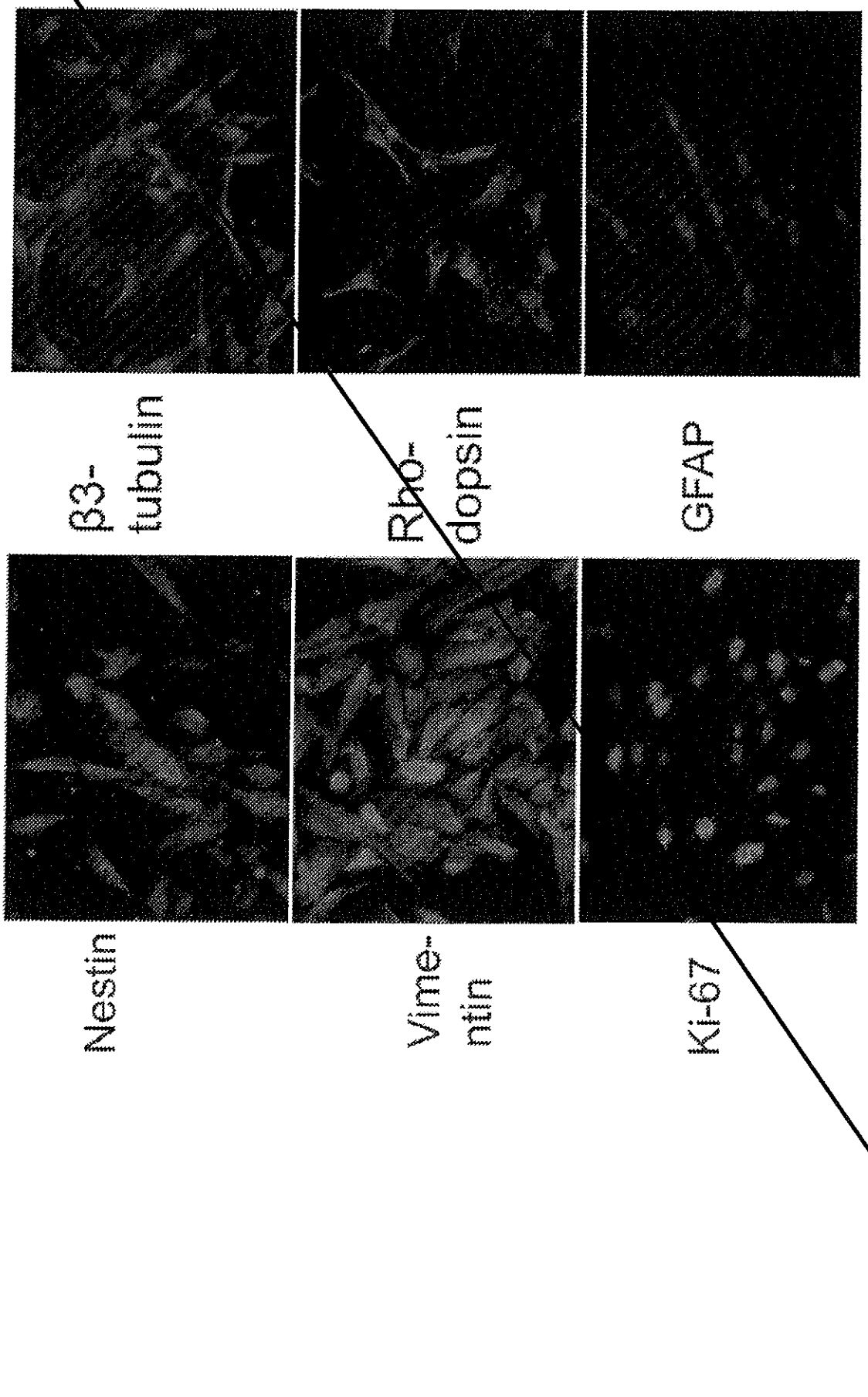


FIGURE 14

Gene profile by time point

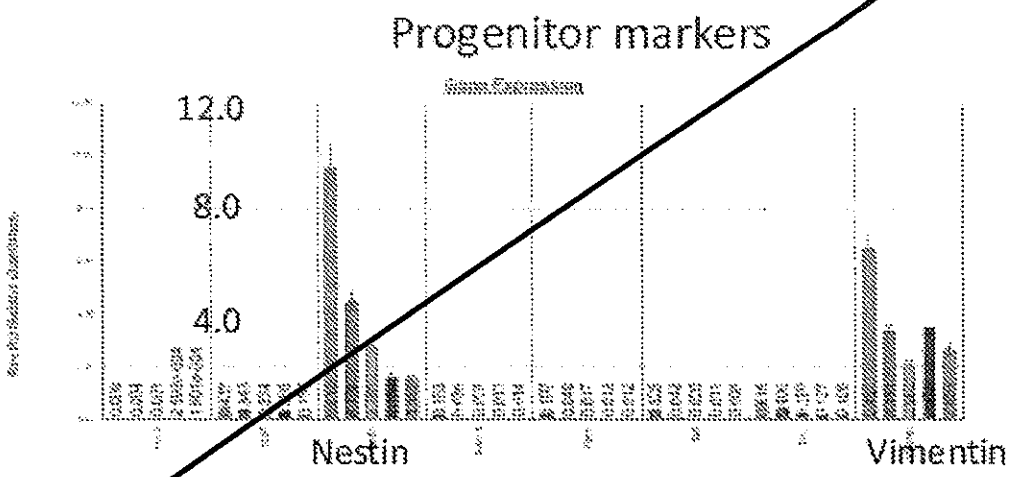
In UL

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)



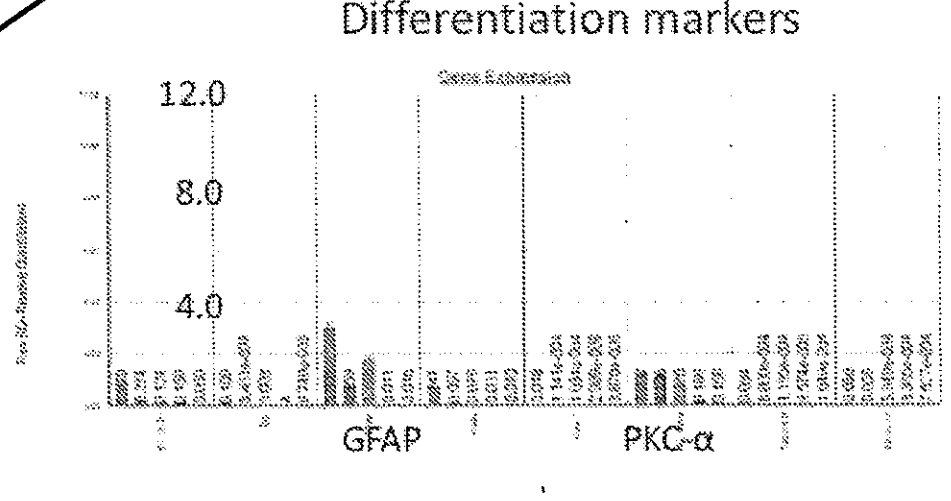
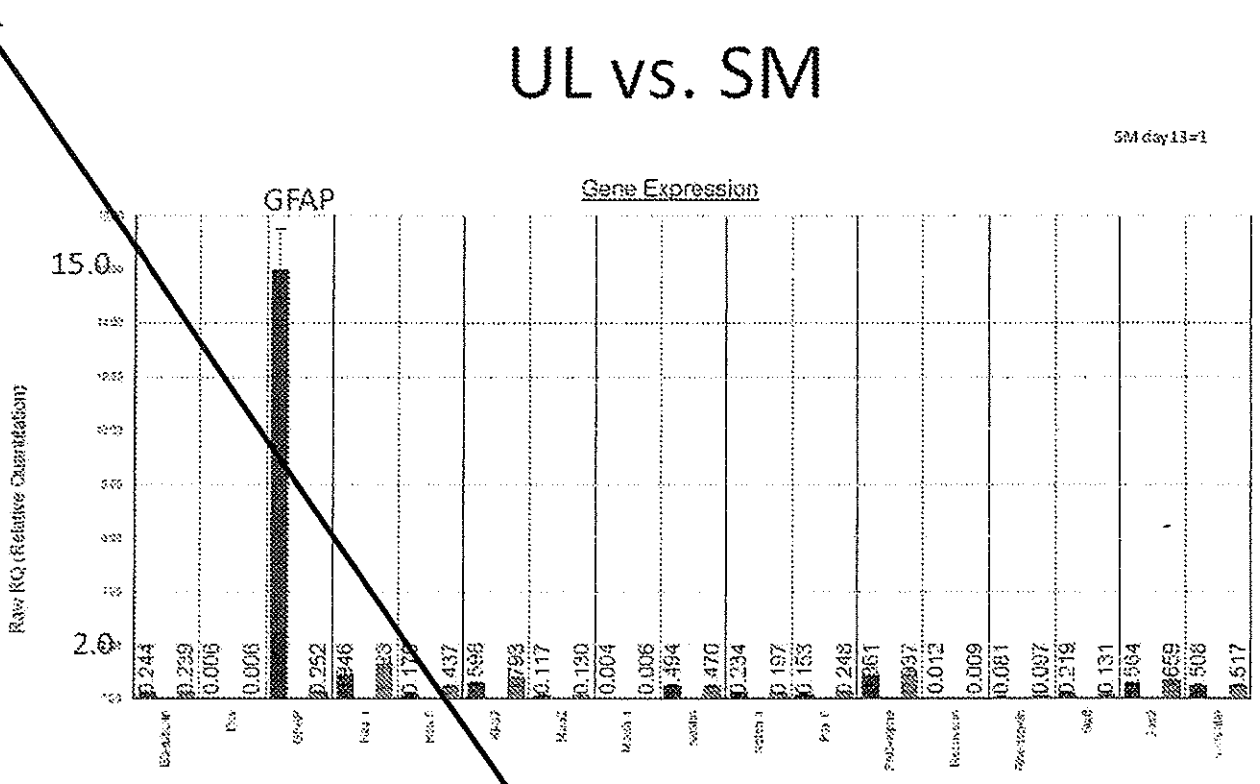


FIGURE 15

A


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

B

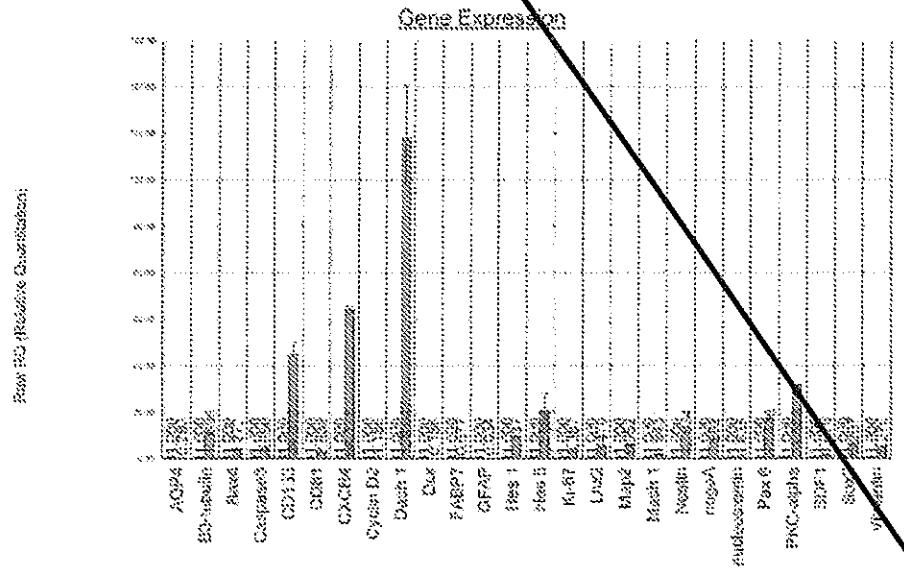

cRPC vs. cBPC
Gene Expression
Raw KQ (Relative Quantitation)

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

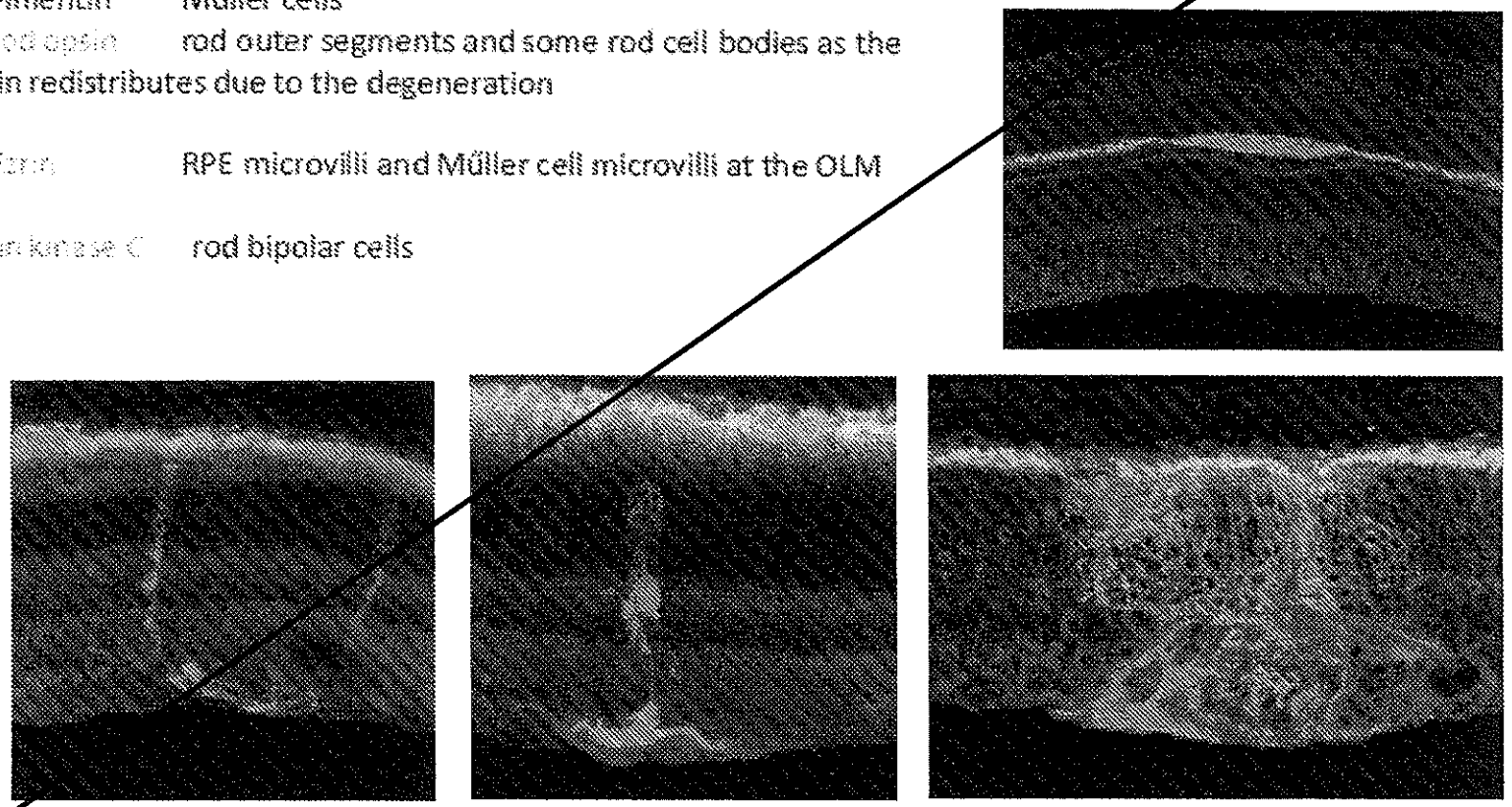


FIGURE 17

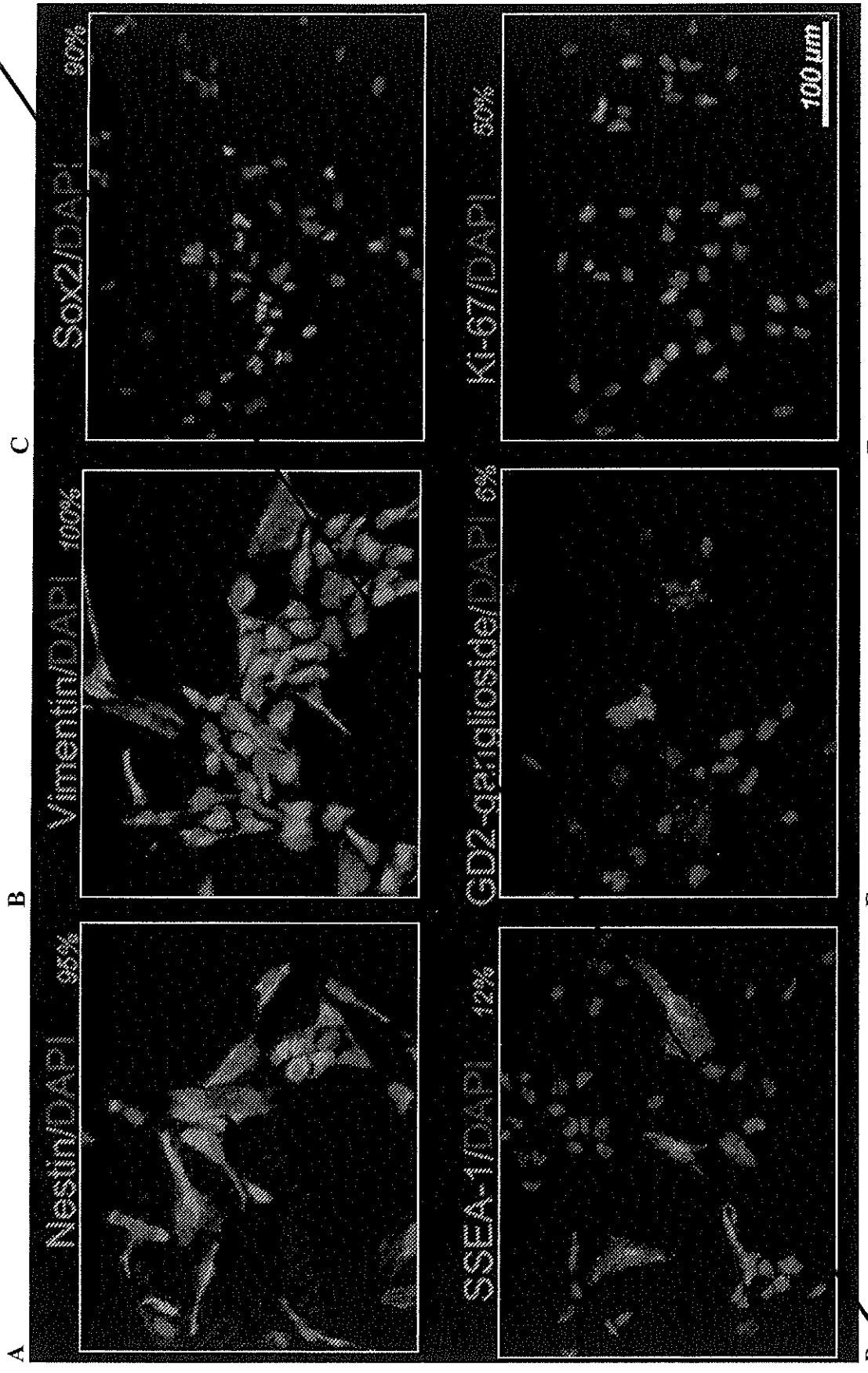

A
Nestin/DAPI 95%
B
Vimentin/DAPI 100%
C
Sox2/DAPI 99%
D
SSEA-1/DAPI 12%
E
GD2-ganglioside/DAPI 6%
F
Ki-67/DAPI 60%
100 um

FIGURE 18

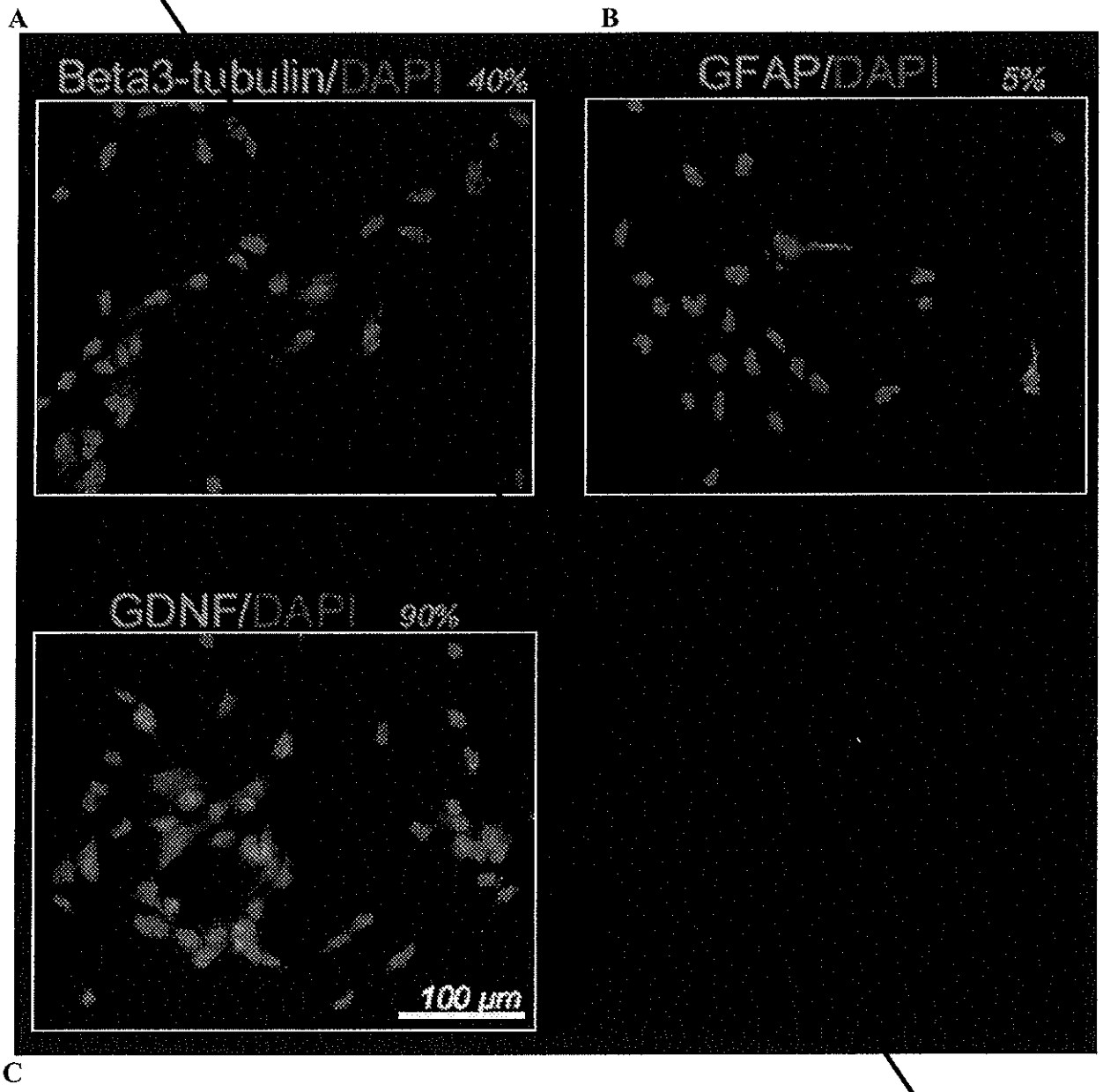


FIGURE 19

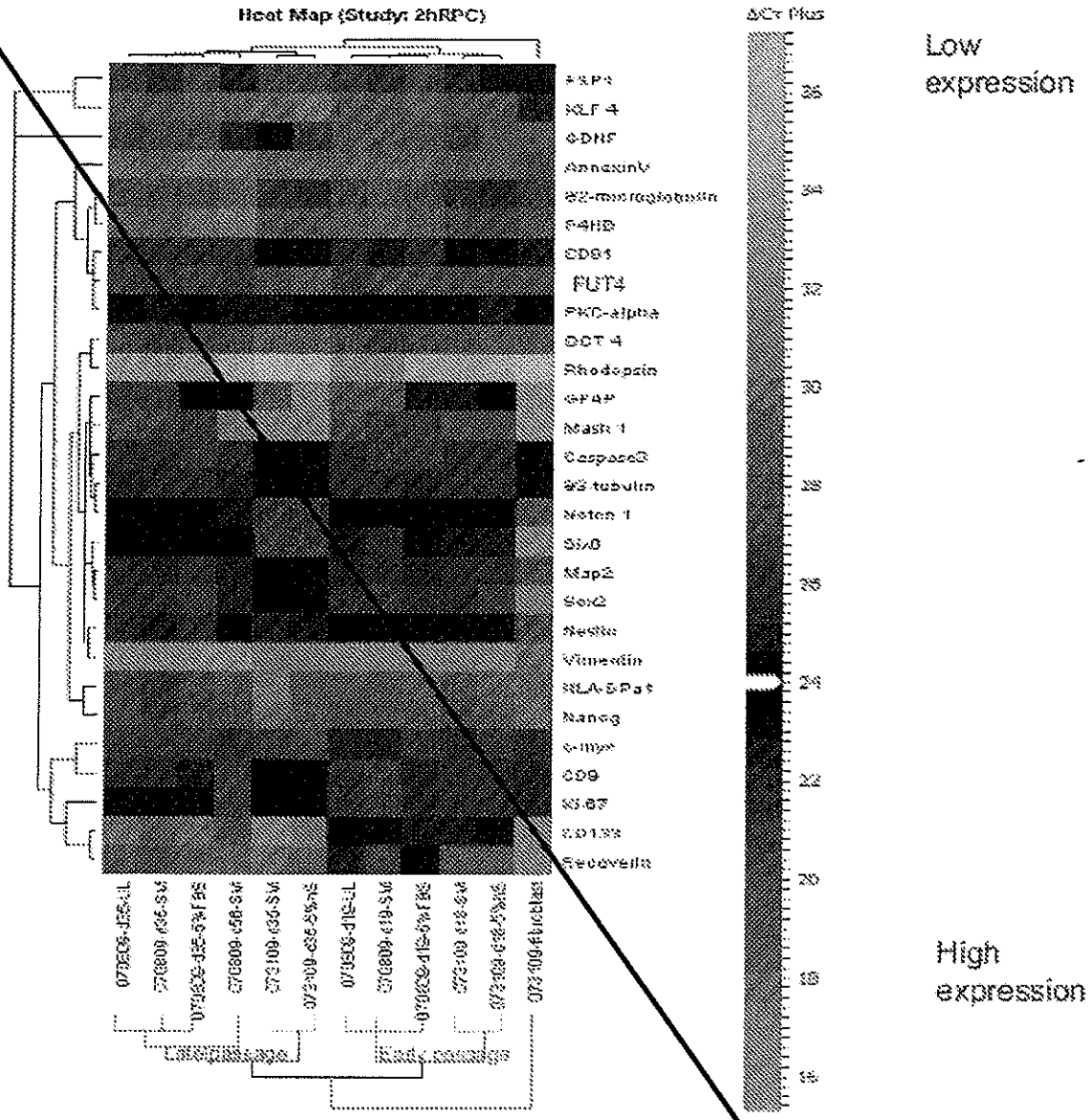
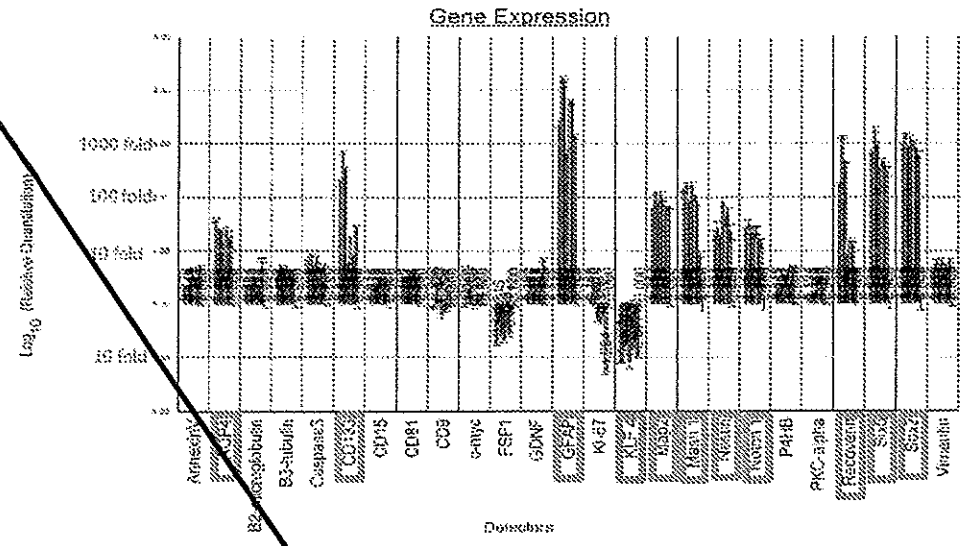
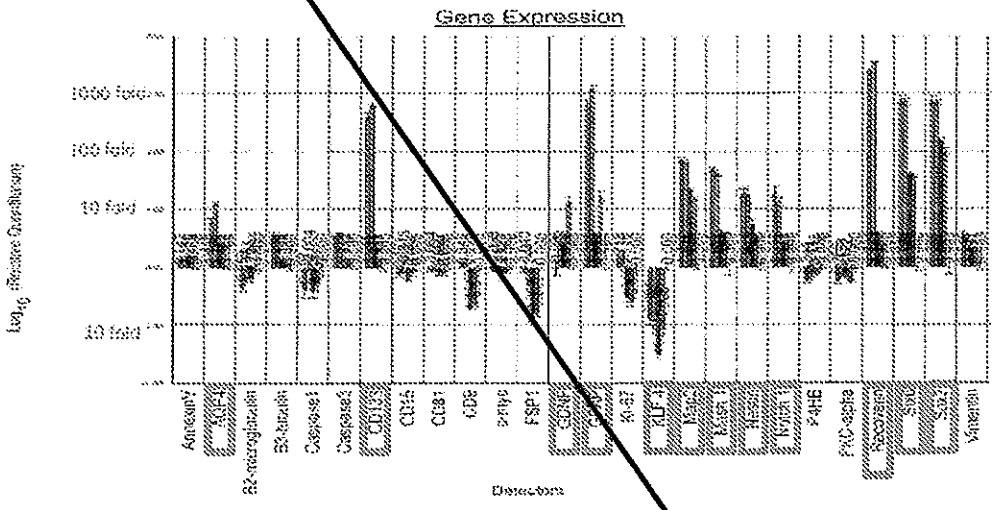

Heat Map (Study: 2hRPC)
ΔCt Plus
Low expression
High expression
FSP4
KLF 4
c-DNF
Annexin V
β2-microglobulin
P4HB
CD91
FUT4
PKC-alpha
OCT 4
Rhodopsin
GFAP
Mash 1
Caspase3
βIII-tubulin
Neten 1
SMI
Map2
Sox2
Nestin
Vimentin
NLA-6Pat
Nanog
c-myc
CD9
Ki-67
CD133
Recoverin

FIGURE 20

A



B



C

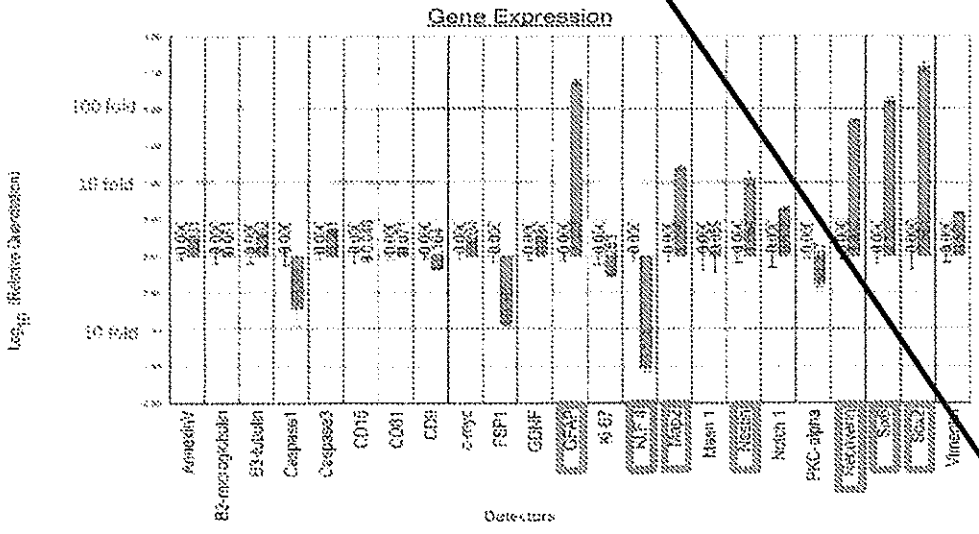


FIGURE 21

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

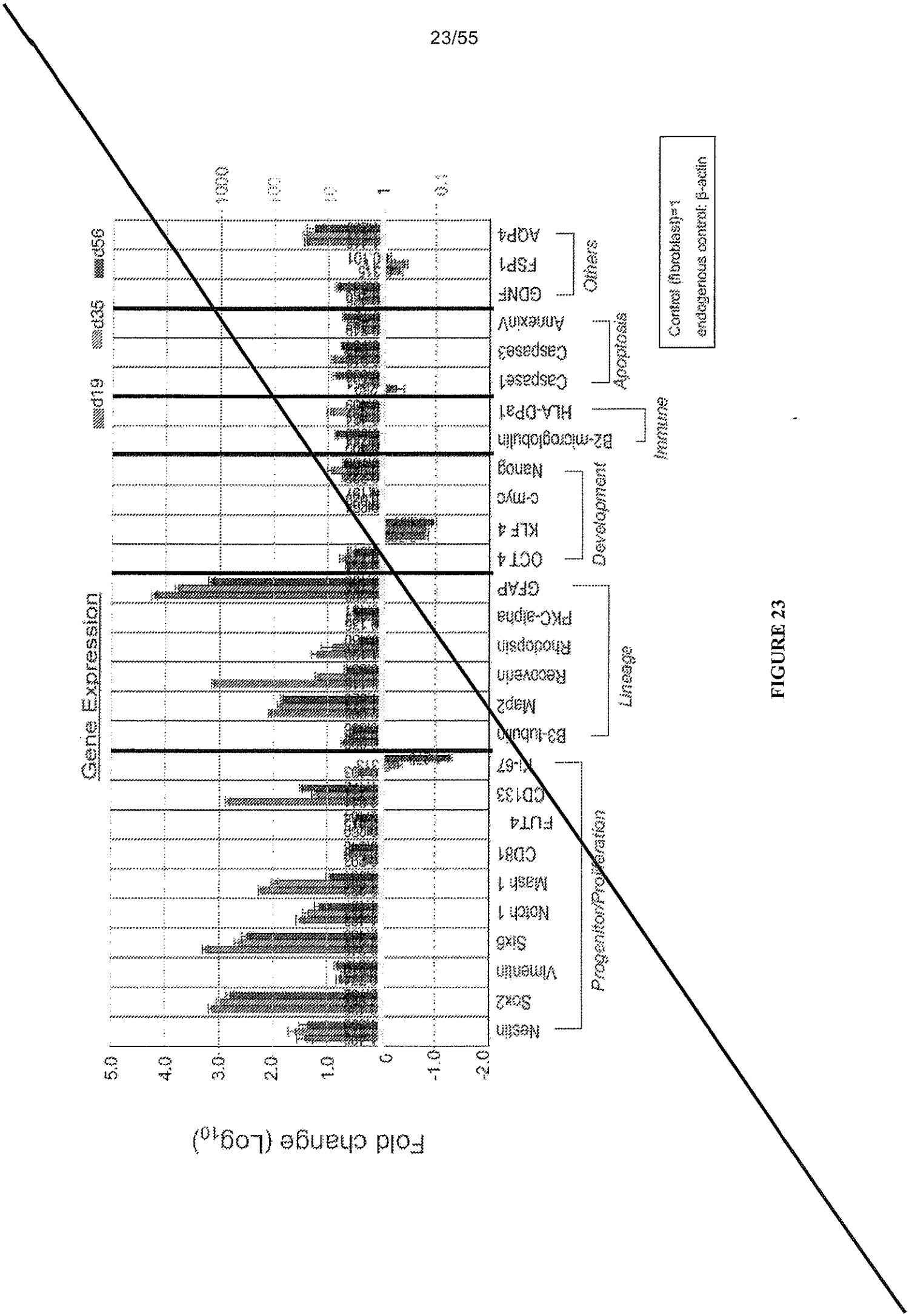

Gene Expression
Fold change (Log10)
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
1000
100
10
1
0.1
d19
d35
d56
Nestin
Sox2
Vimentin
Six6
Notch 1
Mash 1
CD81
FUT4
CD133
Ki-67
Progenitor/Proliferation
B3-tubulin
Map2
Recoverin
Rhodopsin
PKC-alpha
GFAP
Lineage
OCT 4
KLF 4
c-myc
Nanog
Development
B2-microglobulin
HLA-DPa1
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(63.3)
Sox2 fold change(4)
Notch 1
Annexin V
Bmi1

FIGURE 24

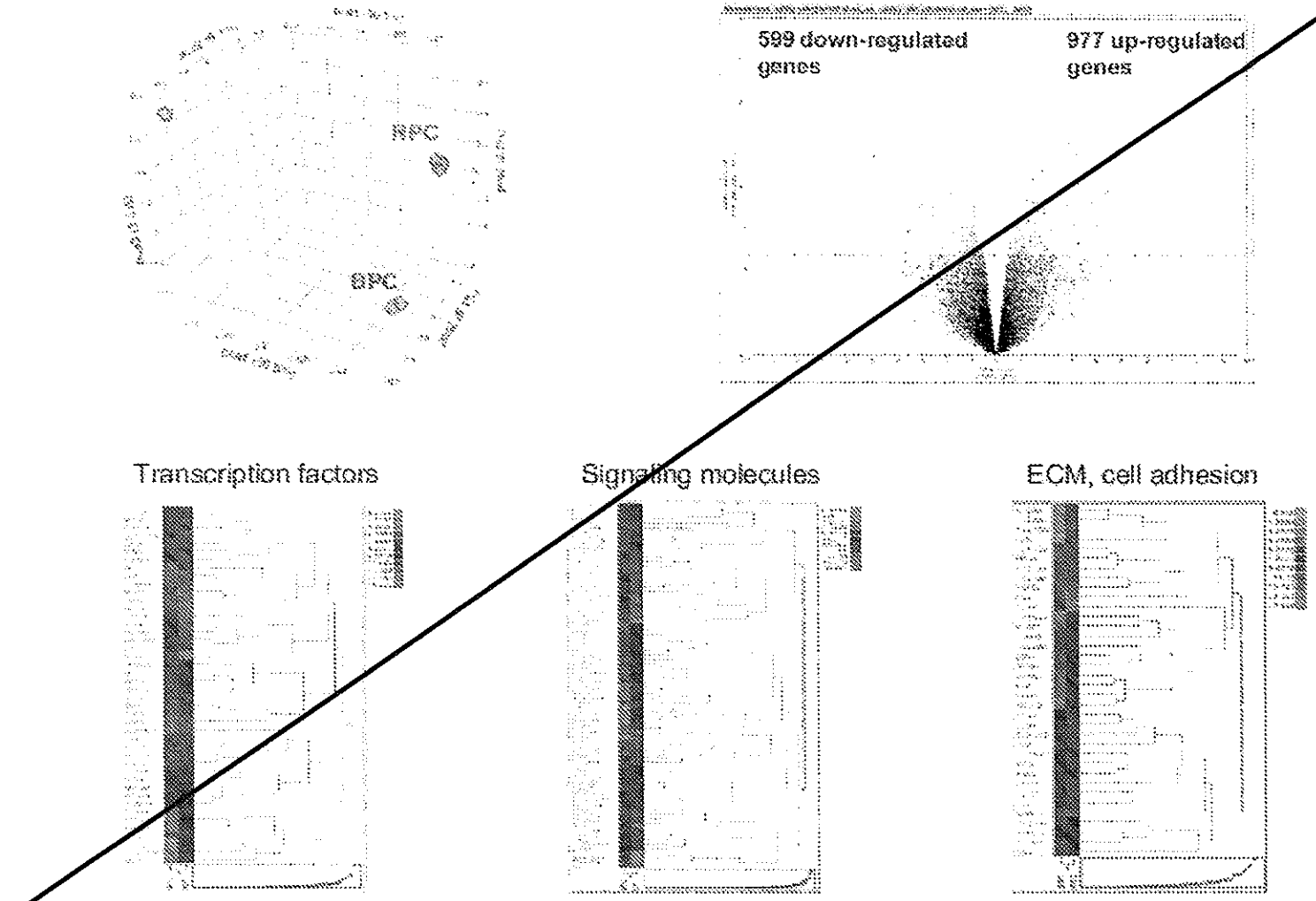

Princ. Component Analysis (PCA)
RPC
BPC
RPC vs. BPC
599 down-regulated genes
977 up-regulated genes
Transcription factors
Signaling molecules
ECM, cell adhesion

FIGURE 25

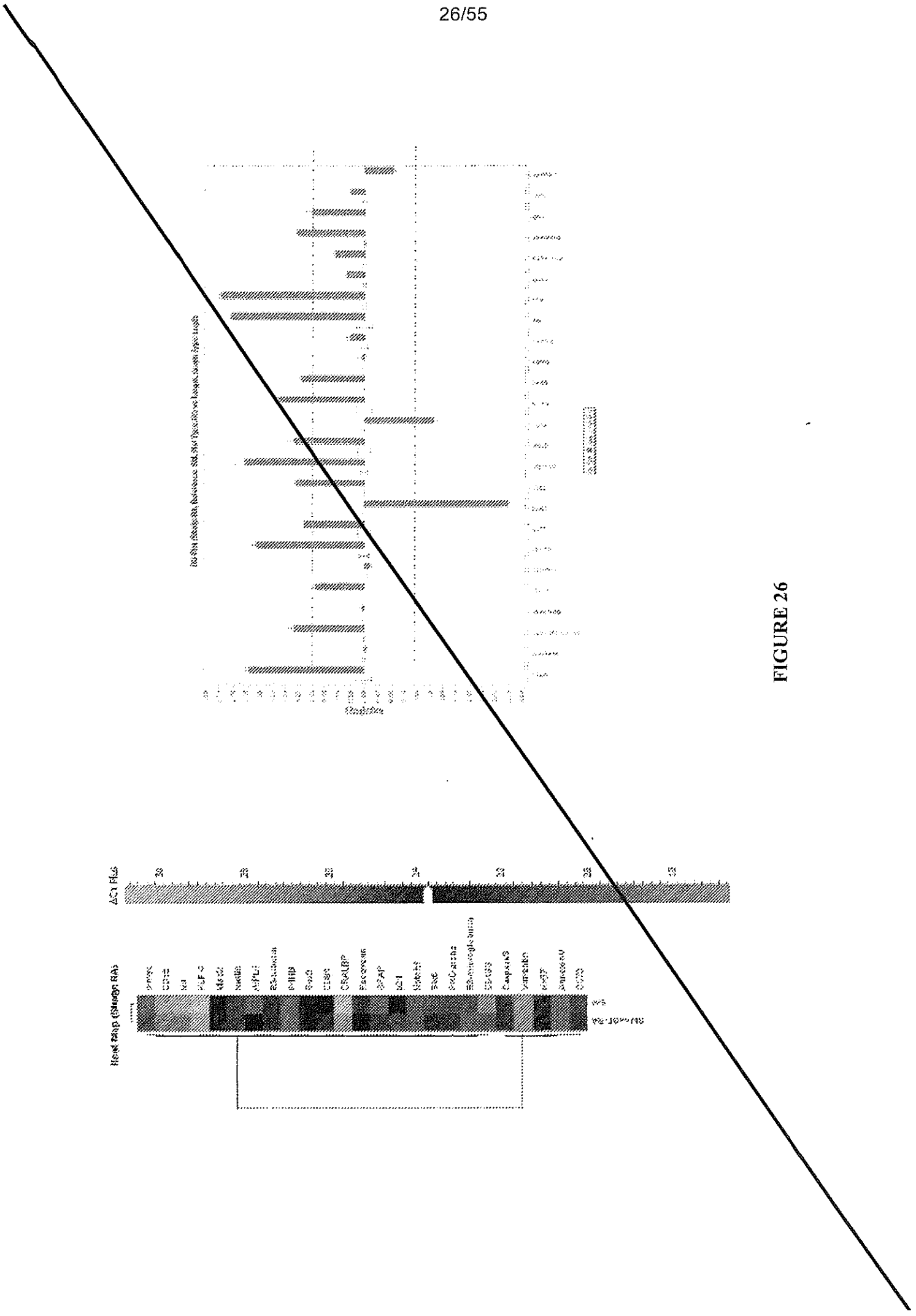


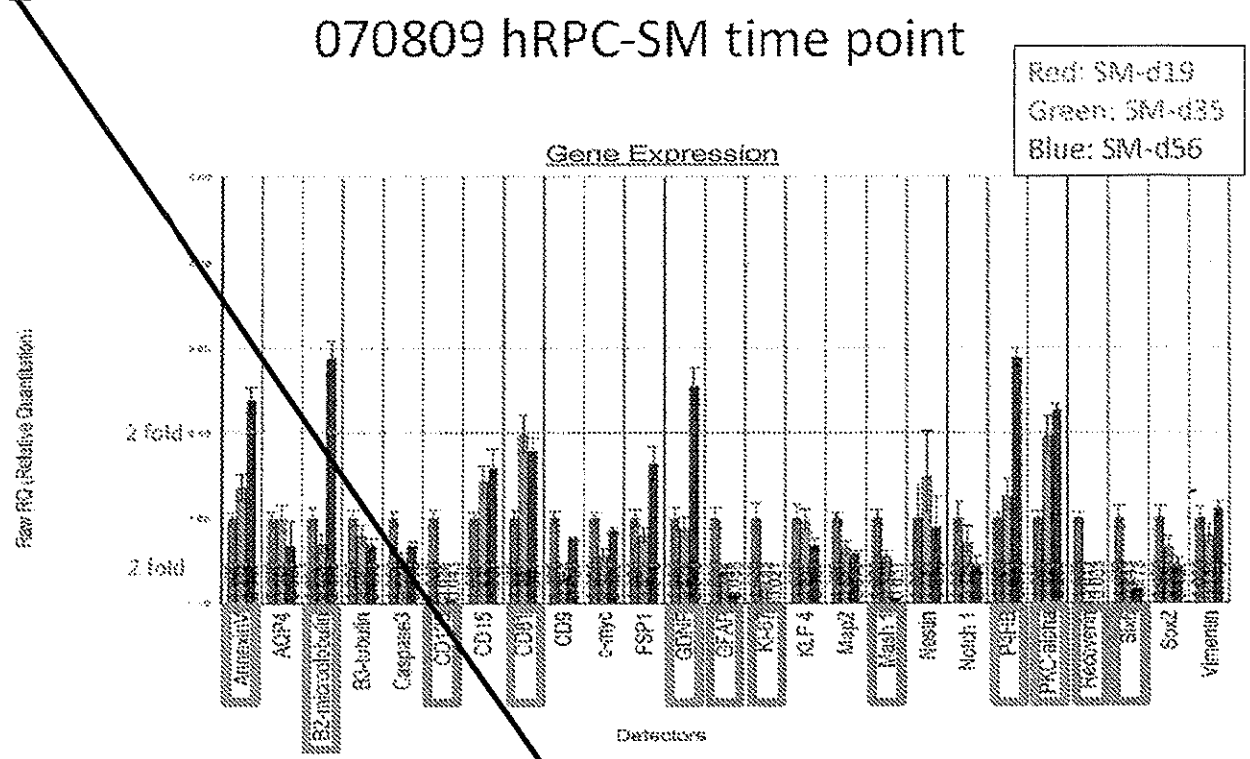
FIGURE 26

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
Detectors

B

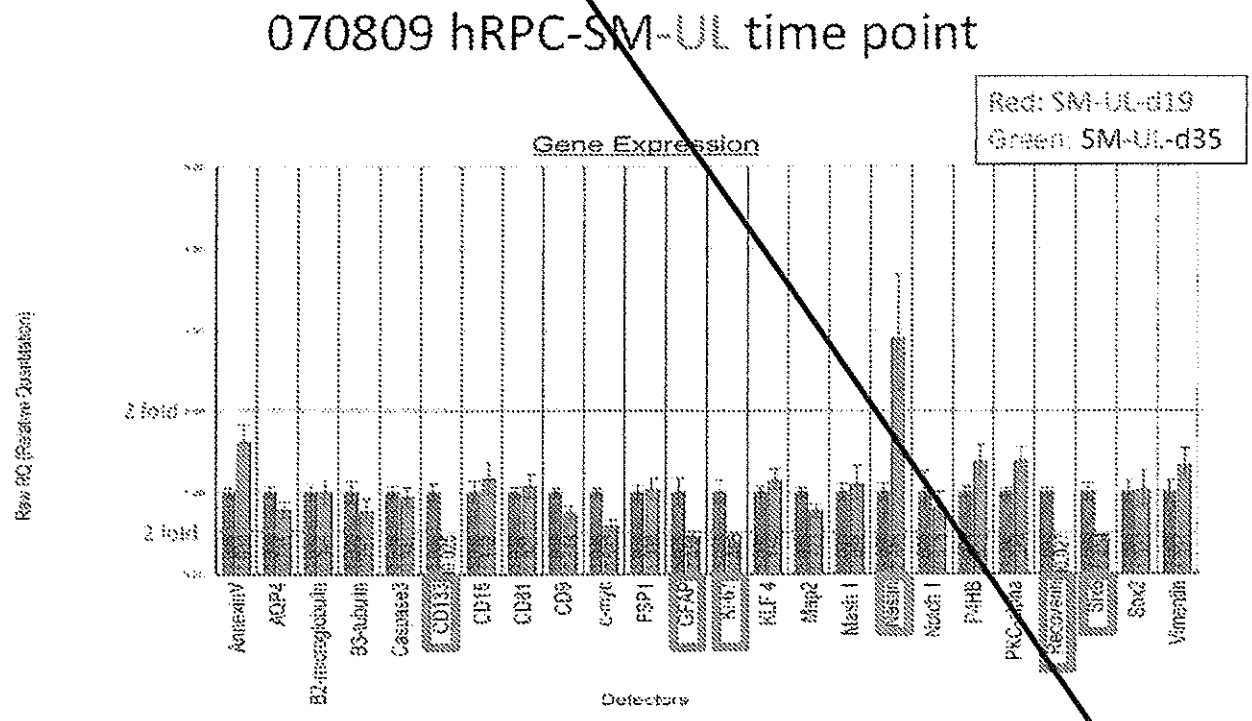
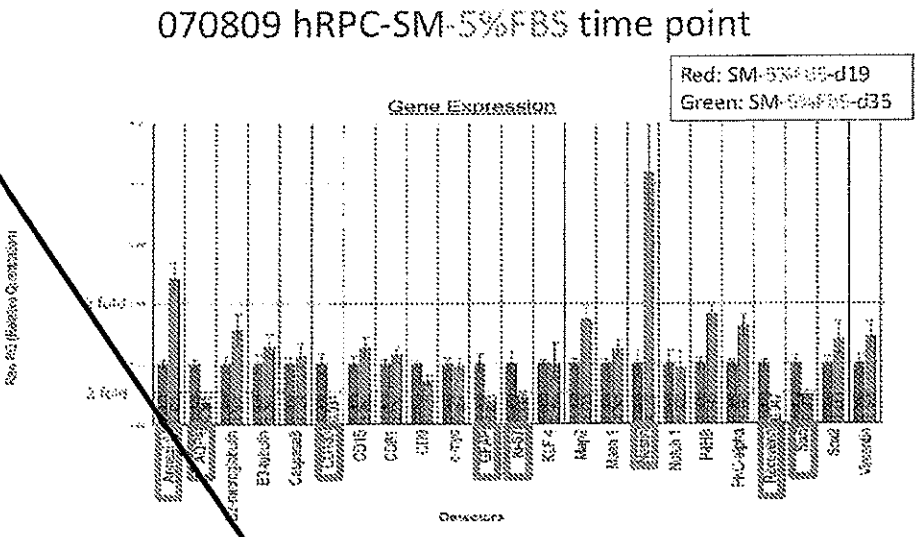
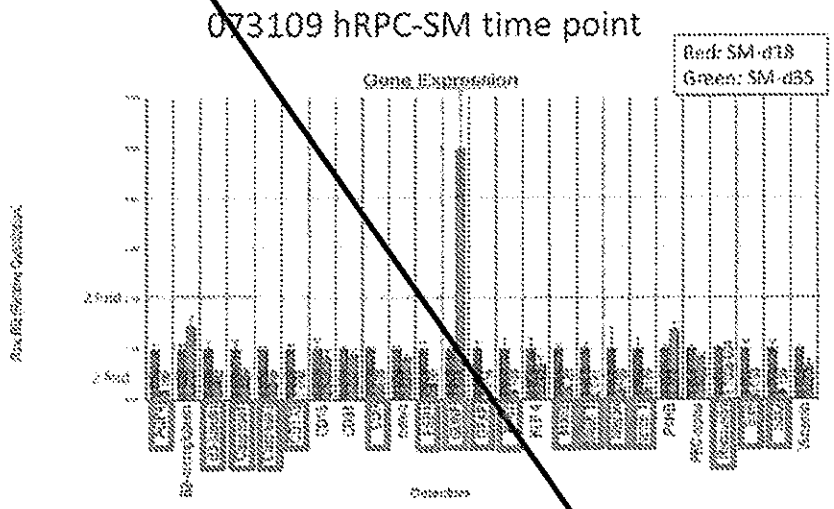

070809 hRPC-SM-UL time point
Red: SM-UL-d19
Green: SM-UL-d35
Gene Expression
Raw RQ (Relative Quantitation)
2 fold
Detectors

FIGURE 28

A



B



C

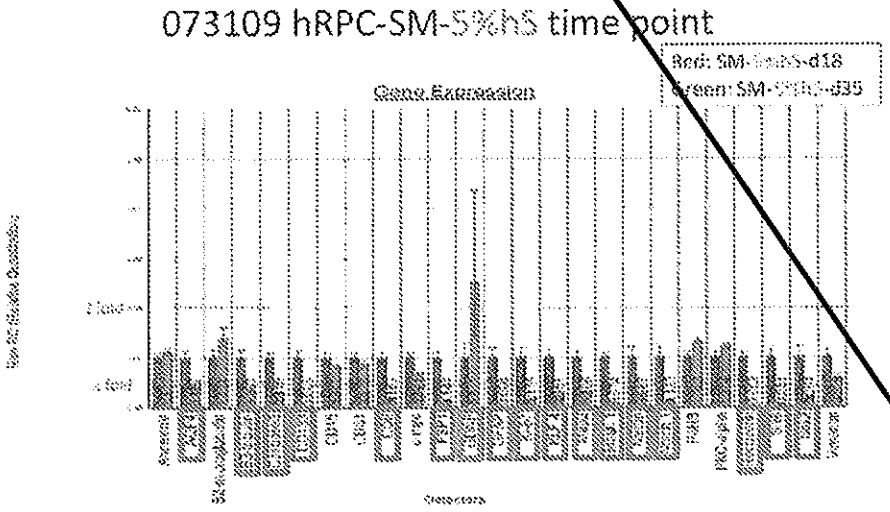


FIGURE 29

hRPC time point comparison

Red ↓ green ↑

0520hRPC		0604hRPC			0730hRPC	
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-3	caspase-3				caspase-3	
caspase-3	caspase-3				caspase-3	caspase-3
CD9	CD9				CD9	CD9
ESP1	ESP1				ESP1	ESP1
		CD133	CD133	CD133	CD133	CD133
CDS1	CDS1	CDS1				
GDNF	GDNF	GDNF	GDNF ?	GDNF	GDNF	GDNF
GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP
KI67	KI67	KI67	KI67	KI67	KI67	KI67
KI8	KI8					KI8
Map2	Map2				Map2	Map2
Nestin	Nestin		Nestin	Nestin	Nestin	Nestin
		Mash1			Mash1	Mash1
Notch1	Notch1				Notch1	Notch1
		PAX6				
PAX-6?		PAX-6				
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 V	Annexin V	Annexin V		Annexin V		

FIGURE 30

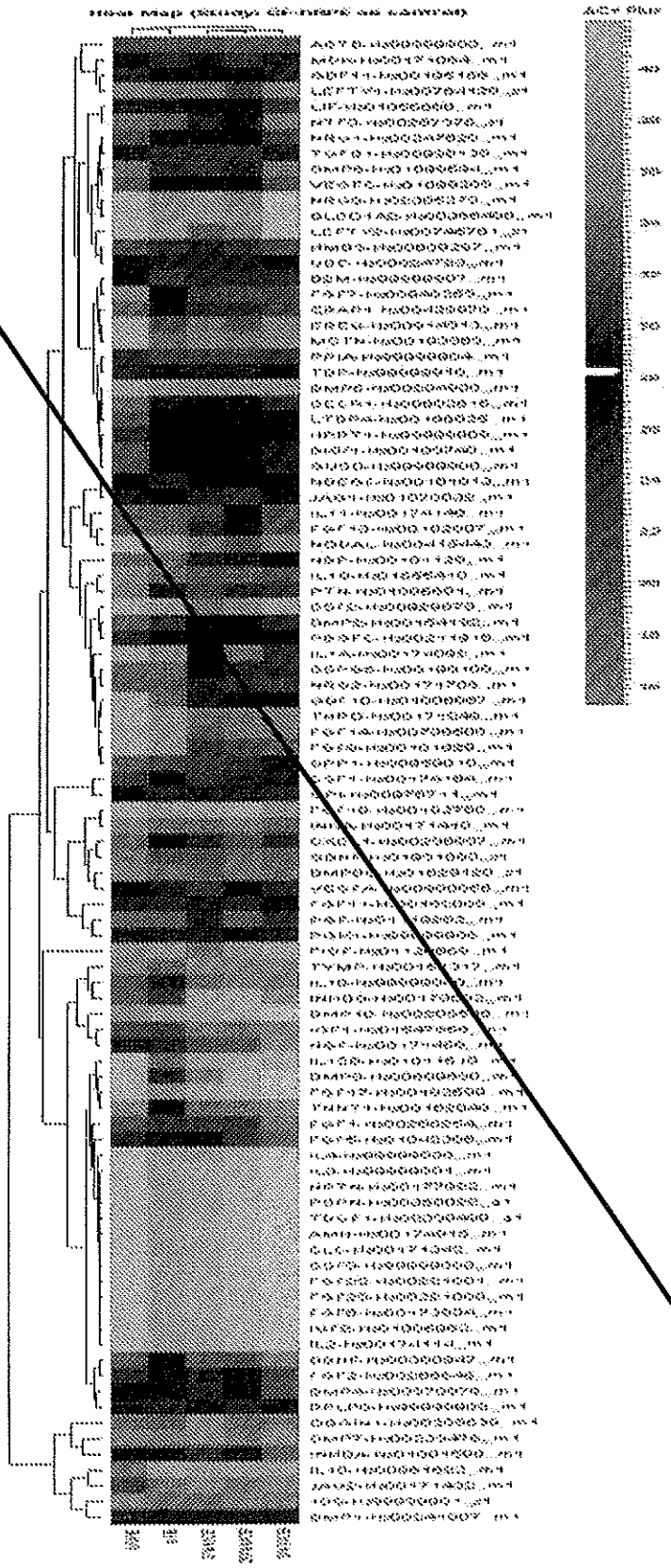


FIGURE 31

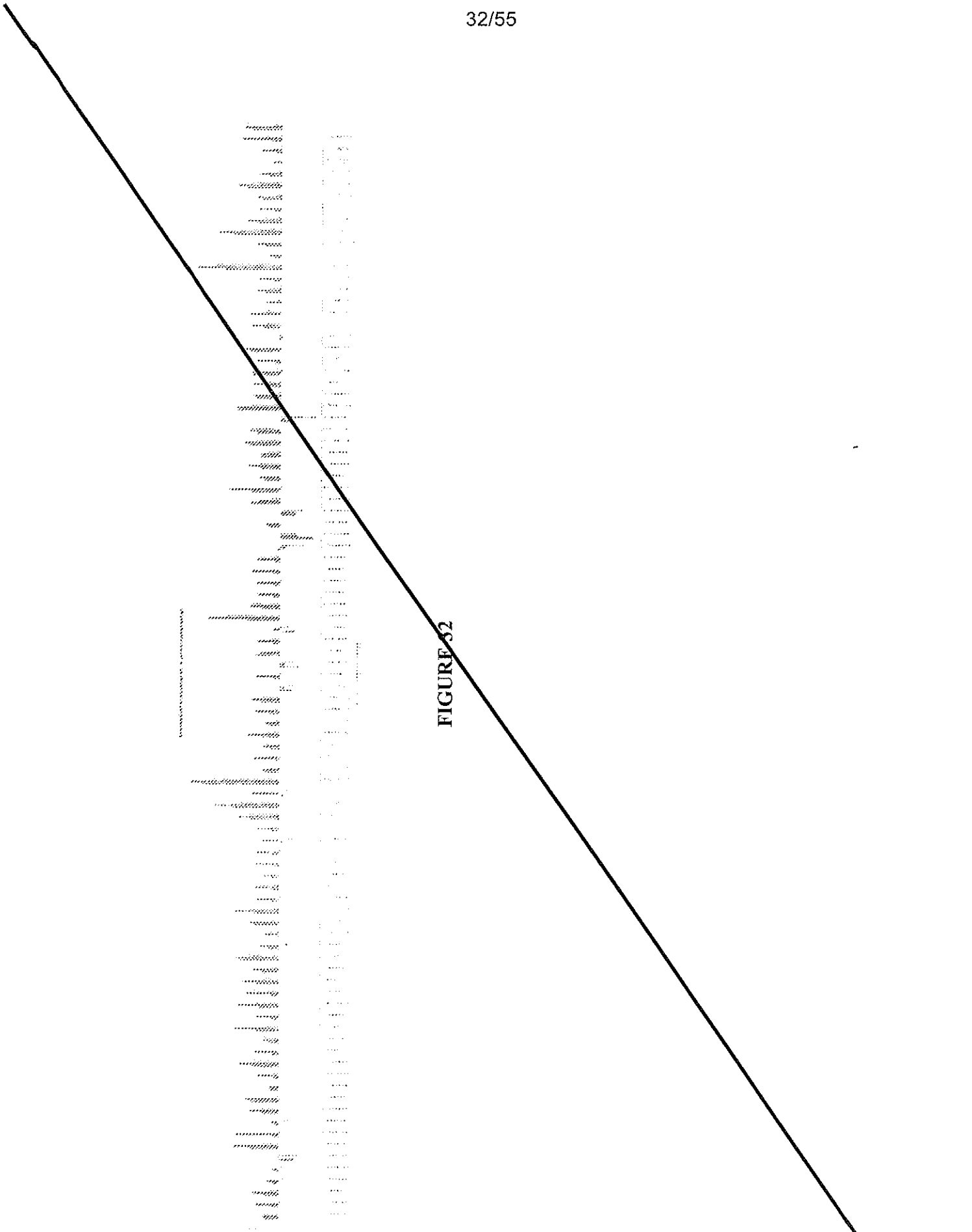
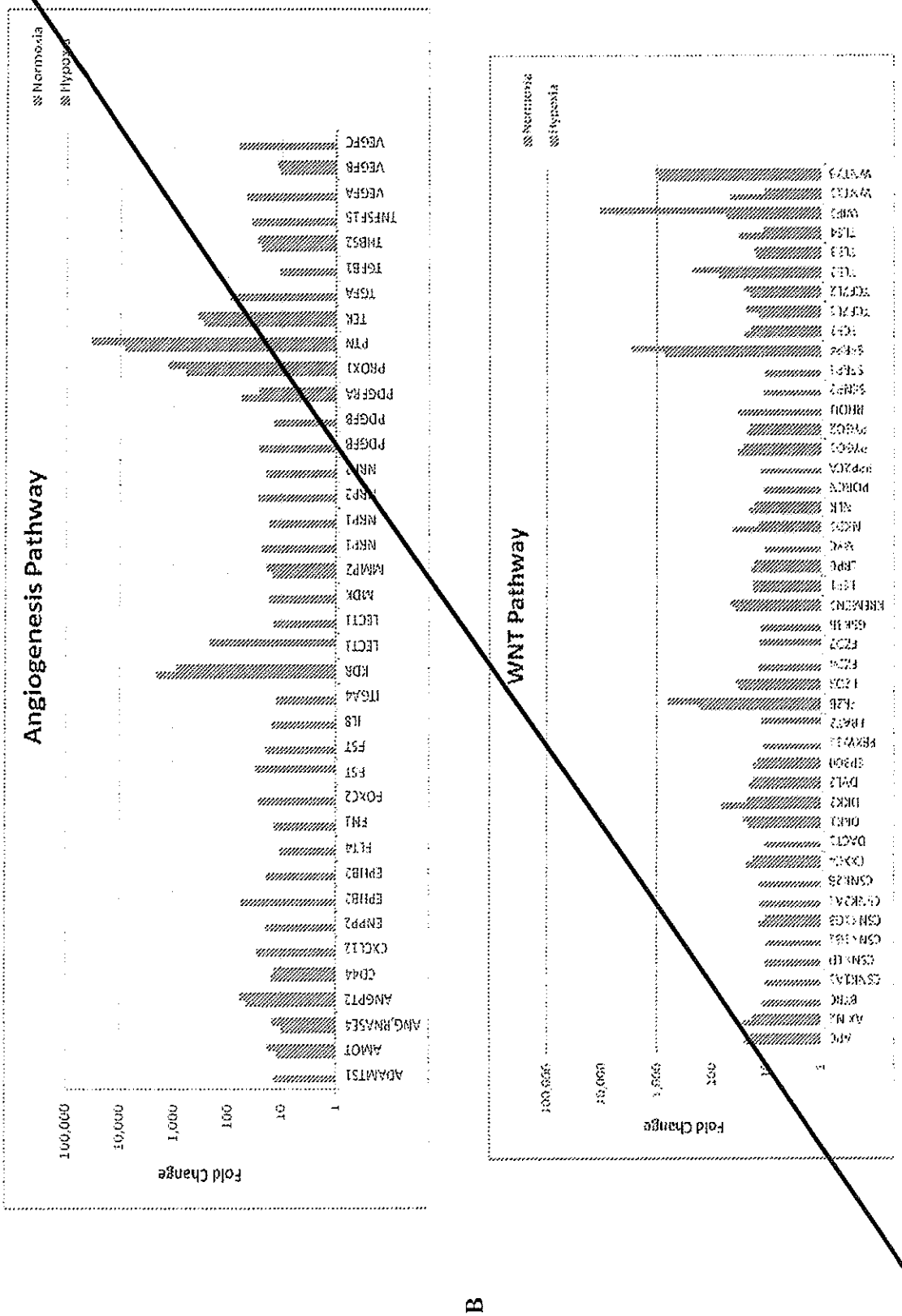
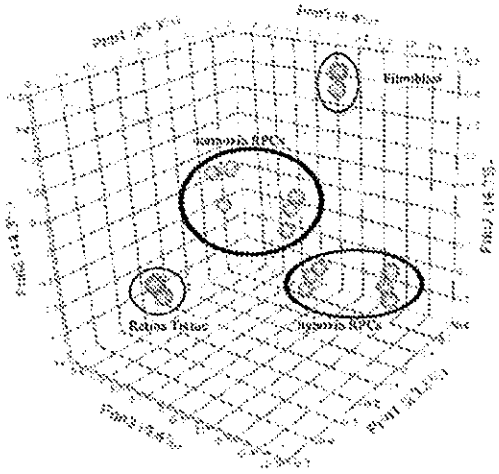


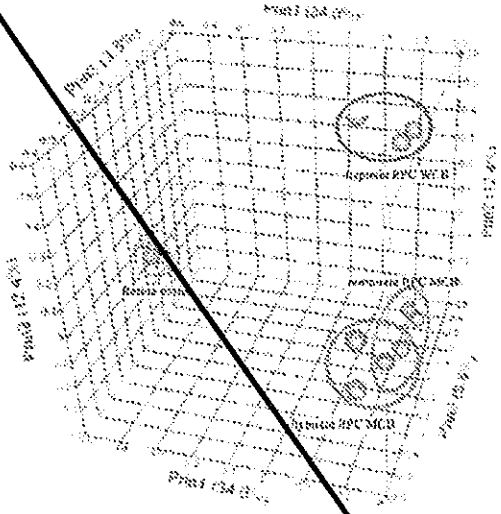
FIGURE 52


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
EPHB3
EPHB4
FLT4
FN1
FOXC2
FST
FST
IL8
ITGA4
KDR
LECT1
LECT1
MDK
NRP2
NRP1
NRP1
NRP2
PDGFB
PDGFB
PDGFRA
PROX1
PTN
TEK
TGFA
TGFB1
TNFRSF12
VEGFA
VEGFB
VEGFC
B
WNT Pathway
Normoxia
Hypoxia
Fold Change
100,000
10,000
1,000
100
10
1
AXIN1
AXIN2
BTRC
CSNK1A1
CSNK1D
CSNK1E
CSNK2A1
CSNK2B
CTBP1
DACT1
DKK1
DKK2
DVL2
EP300
FBXW11
FRAT2
HLA-B
LEF1
FZD4
FZD7
GSK3B
KREMEN2
LRP6
MYC
NCOR1
NLK
PORCN
RUVBL1
RUVBL2
SFRP1
SFRP2
SFRP4
SMAD4
TCF7
TCF7L1
TCF7L2
TLE3
TLE4
WIF1
WNT2
WNT3A
FIGURE 33

A



B



C

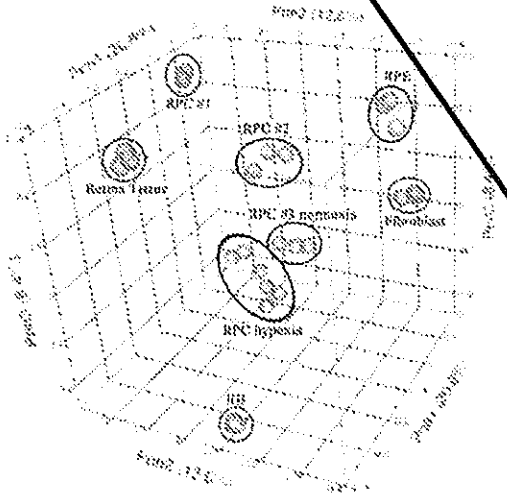


FIGURE 34

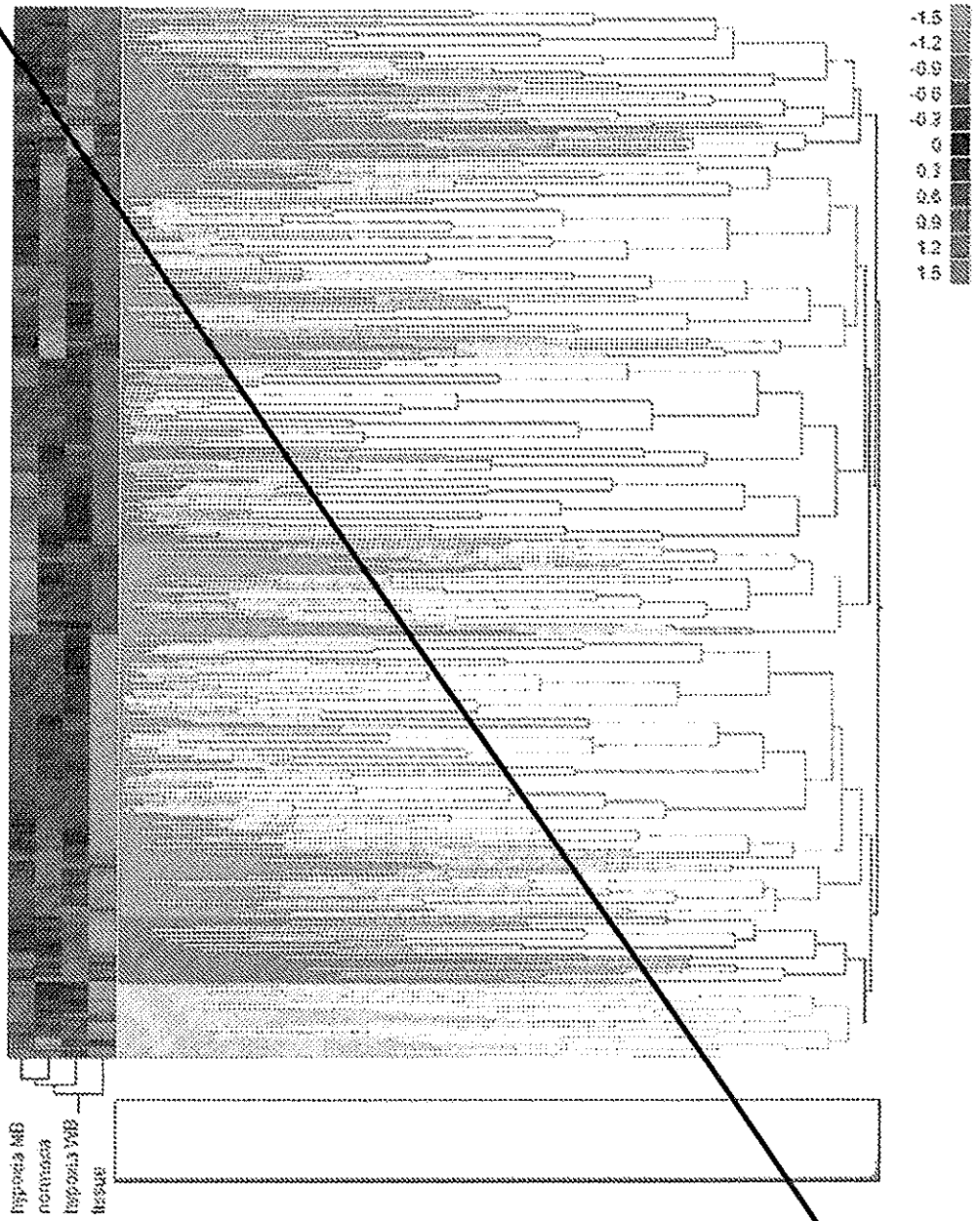


FIGURE 35

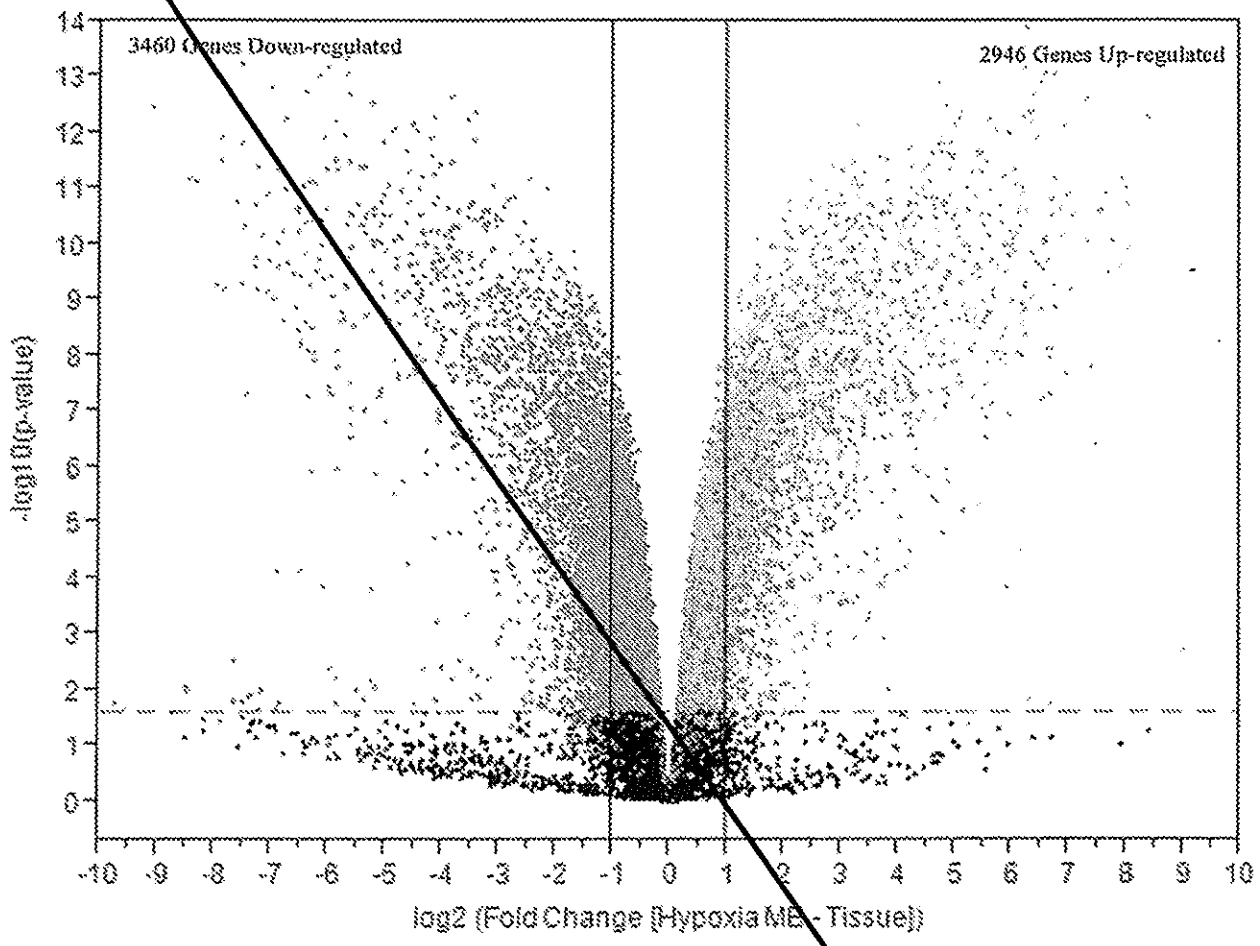
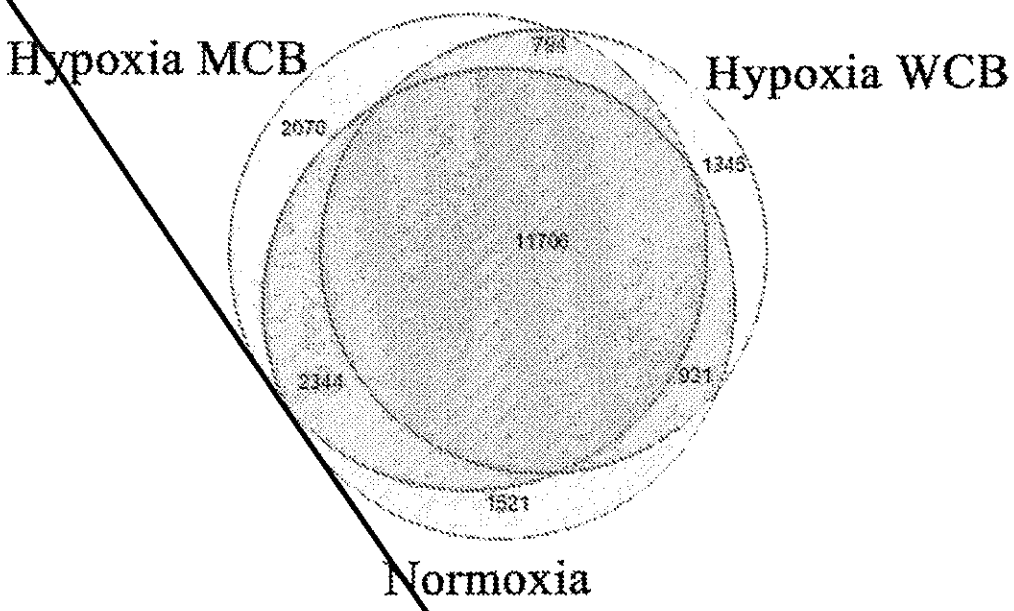

3460 Genes Down-regulated
2946 Genes Up-regulated
-log1 0(p-value)
log2 (Fold Change (Hypoxia ME - Tissue))

FIGURE 36

A


Hypoxia MCB
Hypoxia WCB
2670
1345
11796
2344
931
1521
Normoxia

B

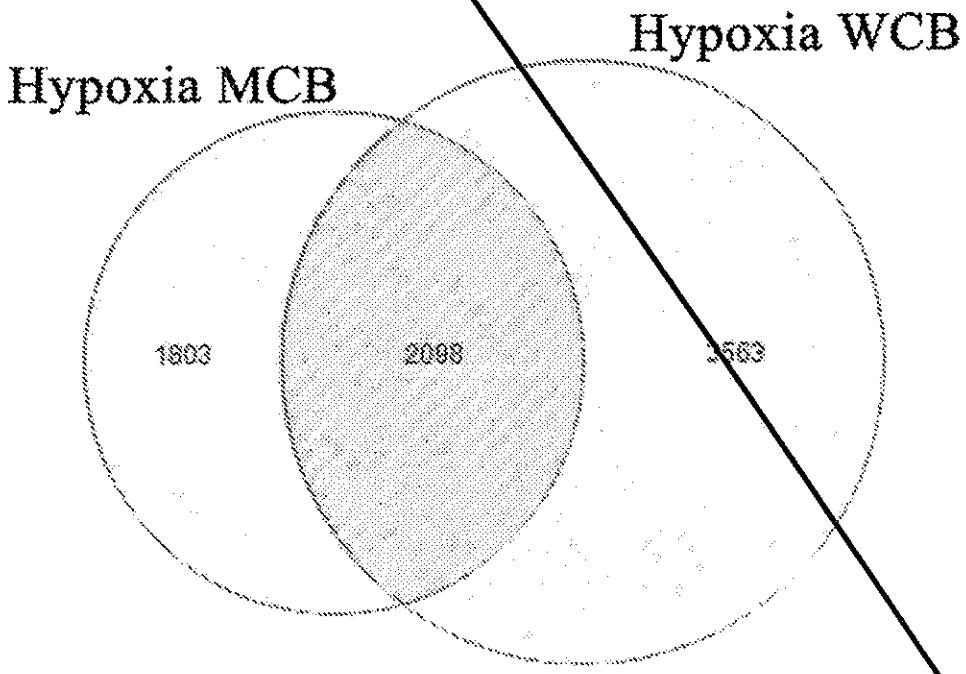
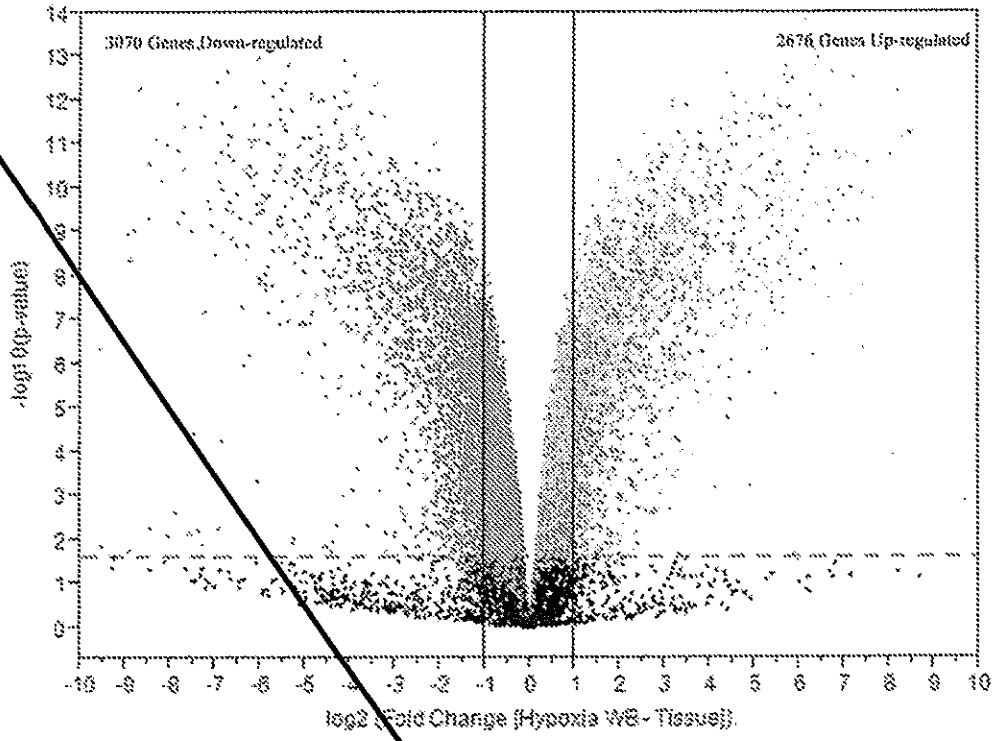

Hypoxia WCB
Hypoxia MCB
1803
2093
3563

FIGURE 37

A


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

B

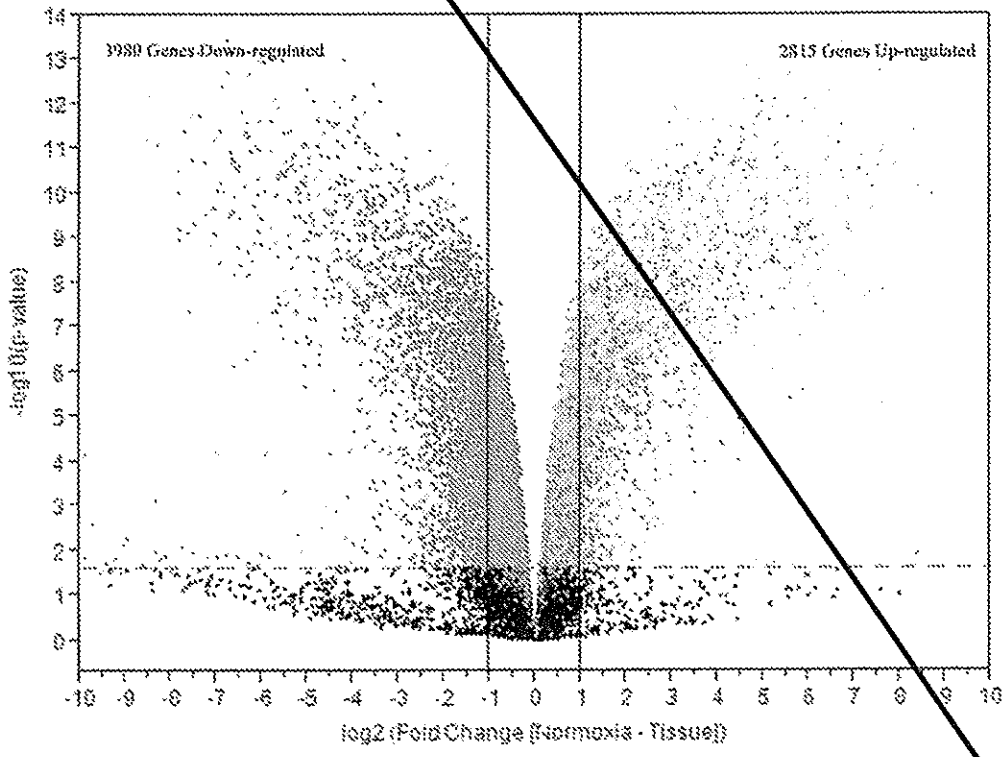
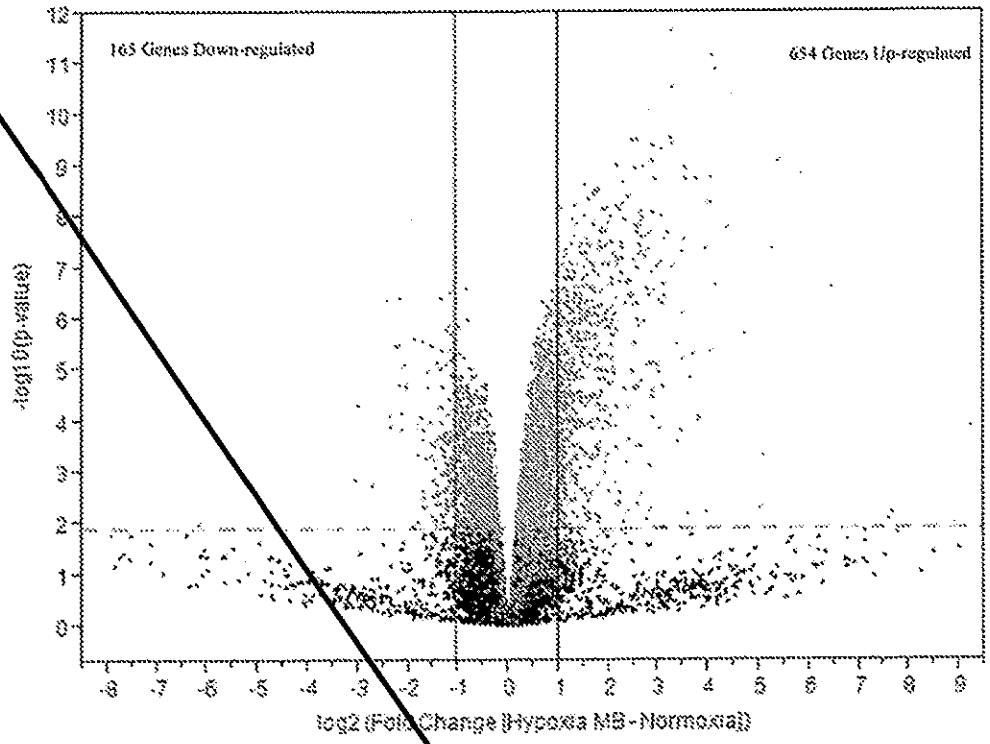

3980 Genes Down-regulated
2815 Genes Up-regulated
-log10 (p-value)
log2 (Fold Change [Normoxia - Tissue])

FIGURE 38

A


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

B

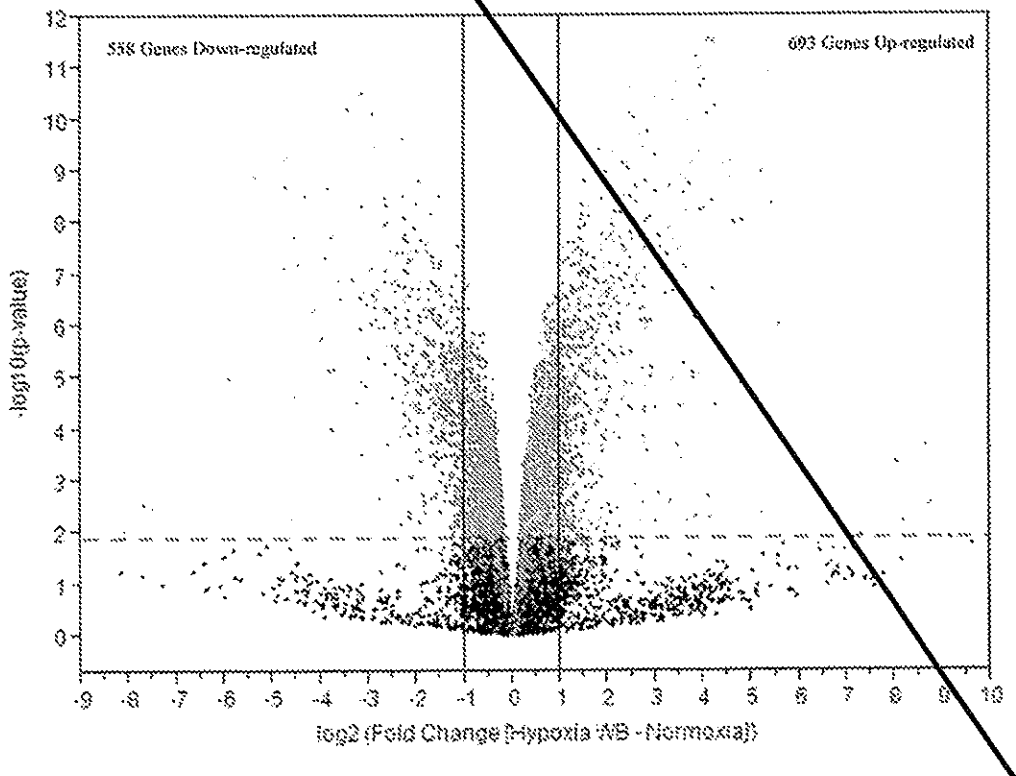

558 Genes Down-regulated
693 Genes Up-regulated
log2 (Fold Change [Hypoxia MB - Normoxia])

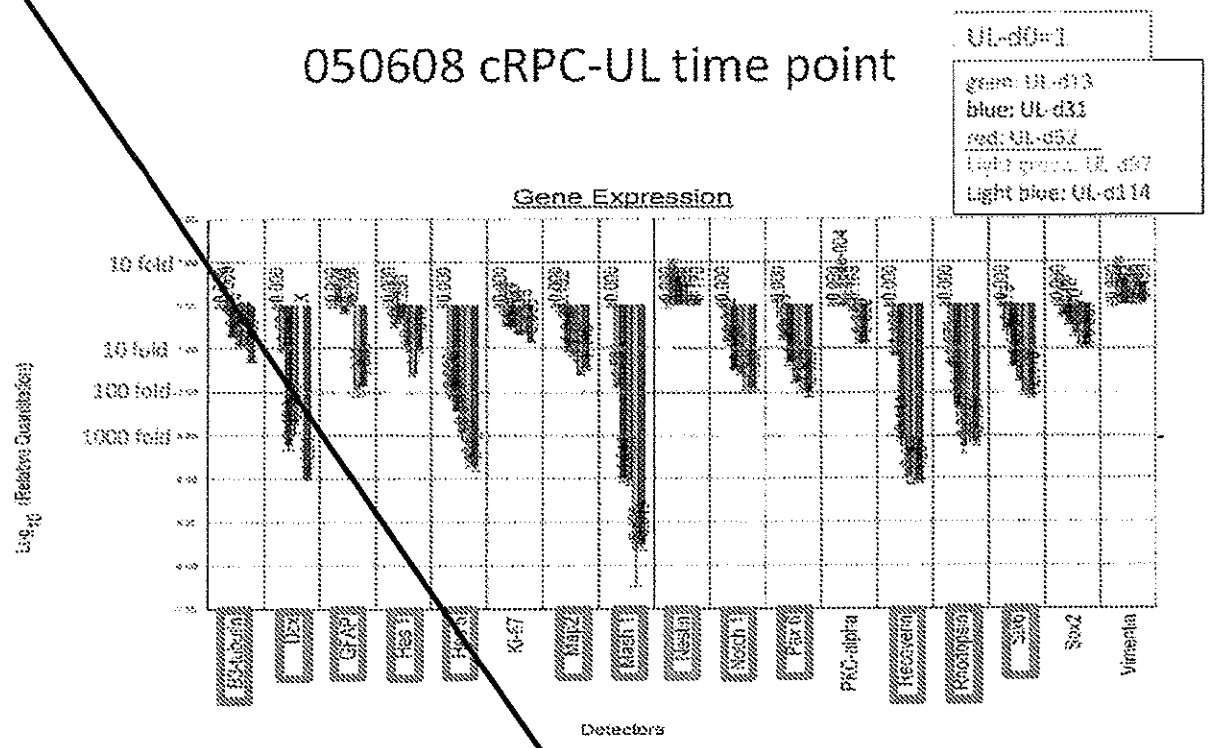
FIGURE 39

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
Gene Expression
10 fold
100 fold
1000 fold
Detectors

B

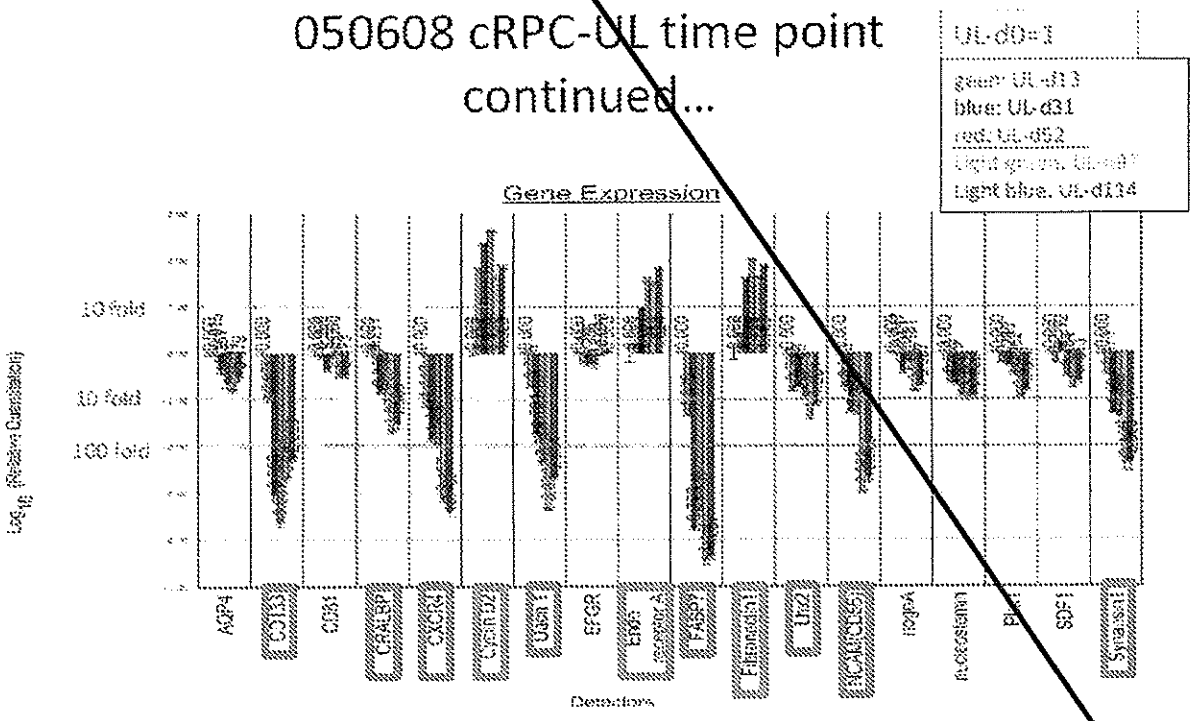
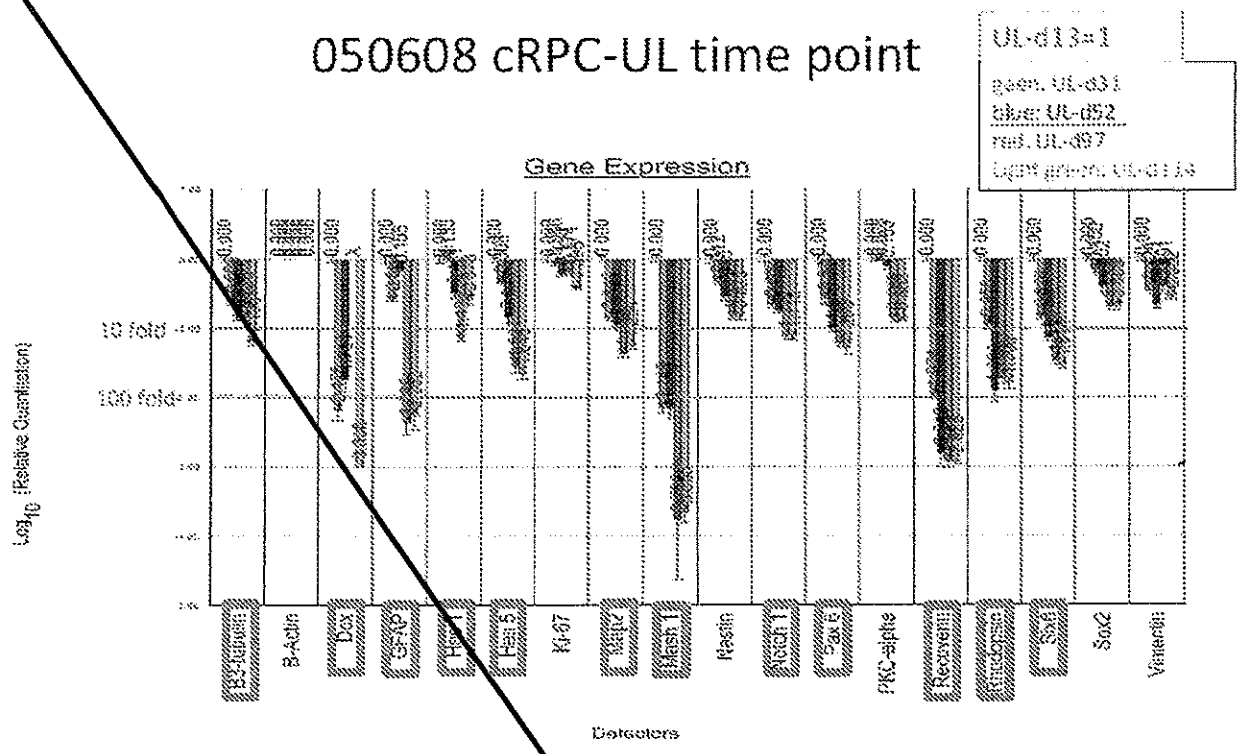

050608 cRPC-UL time point
continued...
UL-d0=1
blue: UL-d31
Gene Expression
10 fold
100 fold
Detectors

FIGURE 41

A


050608 cRPC-UL time point
Gene Expression
10 fold
100 fold
Detectors

B

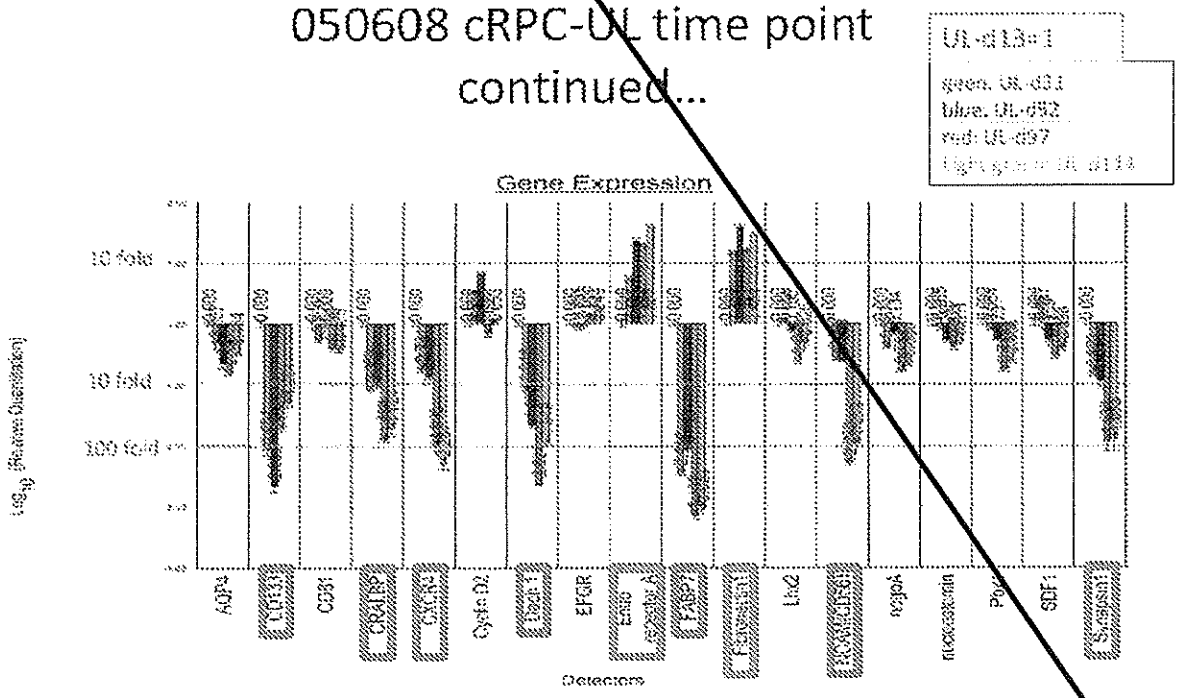
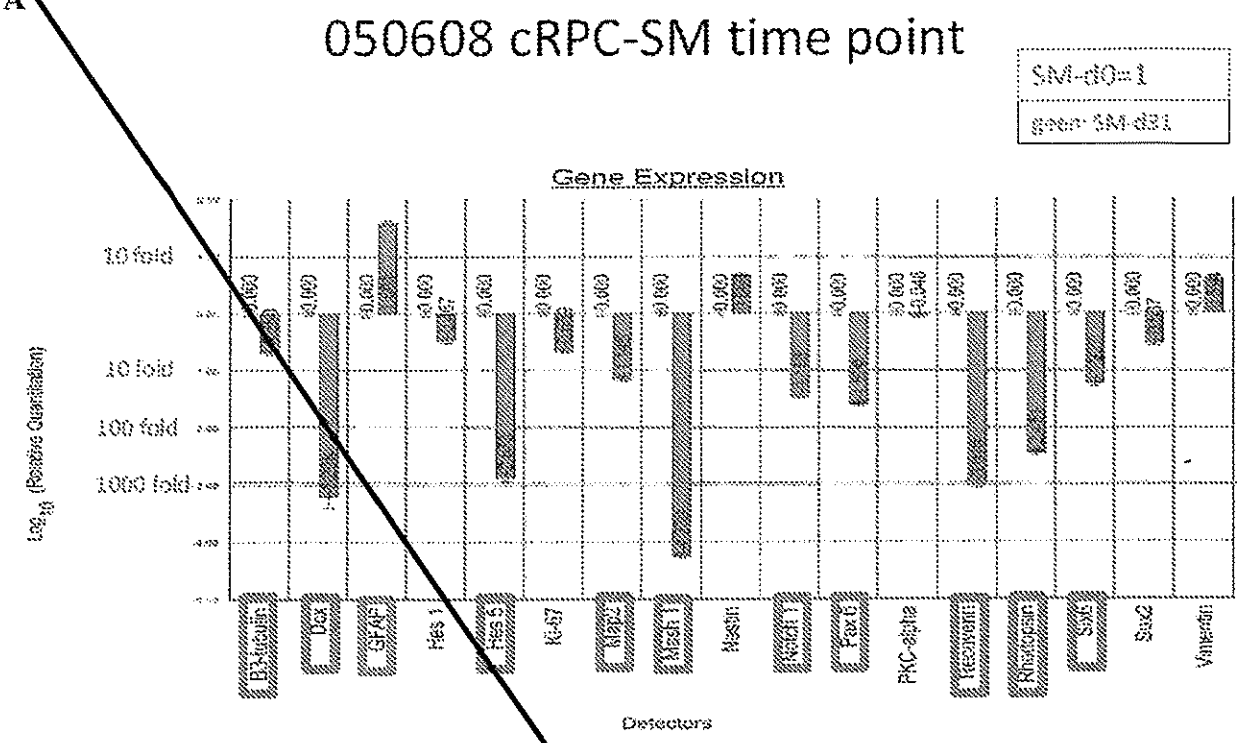

050608 cRPC-UL time point continued...
Gene Expression
10 fold
100 fold
Detectors

FIGURE 42

A


050608 cRPC-SM time point
SM-d0=1
green SM-d31
Gene Expression
10 fold
1 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
Detectors
B7-tubulin
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Musashi 1
Nestin
Notch 1
Pax6
PKC-alpha
Vimentin
Nucleostemin
Synaptophysin
Sox2
Vimentin

B

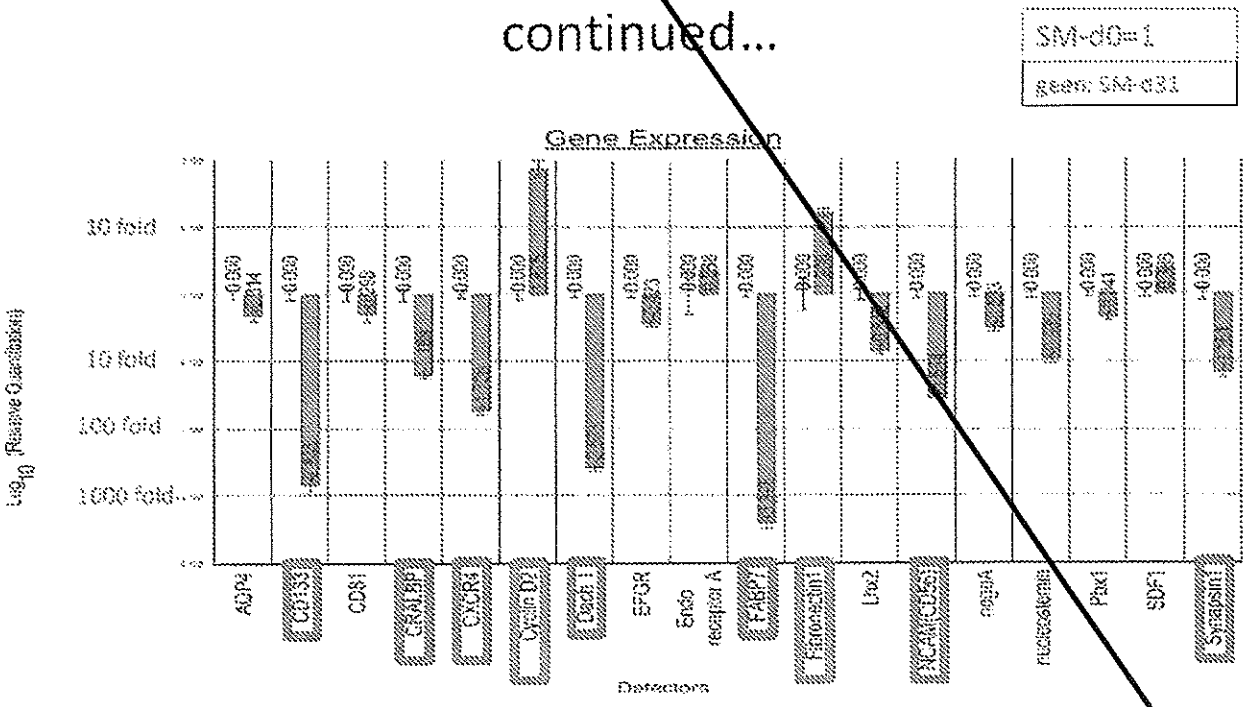

050608 cRPC-SM time point continued...
SM-d0=1
green SM-d31
Gene Expression
10 fold
1 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
Detectors
ADRa2
CD133
CD81
CNR1
CXCR4
Cyclin D2
Dach 1
EPOR
Ephrin receptor A
FABP7
Fibronectin
Lhx2
NCAM-CD56
nestin
nucleostemin
Pax6
SDF1
Synaptophysin

FIGURE 43

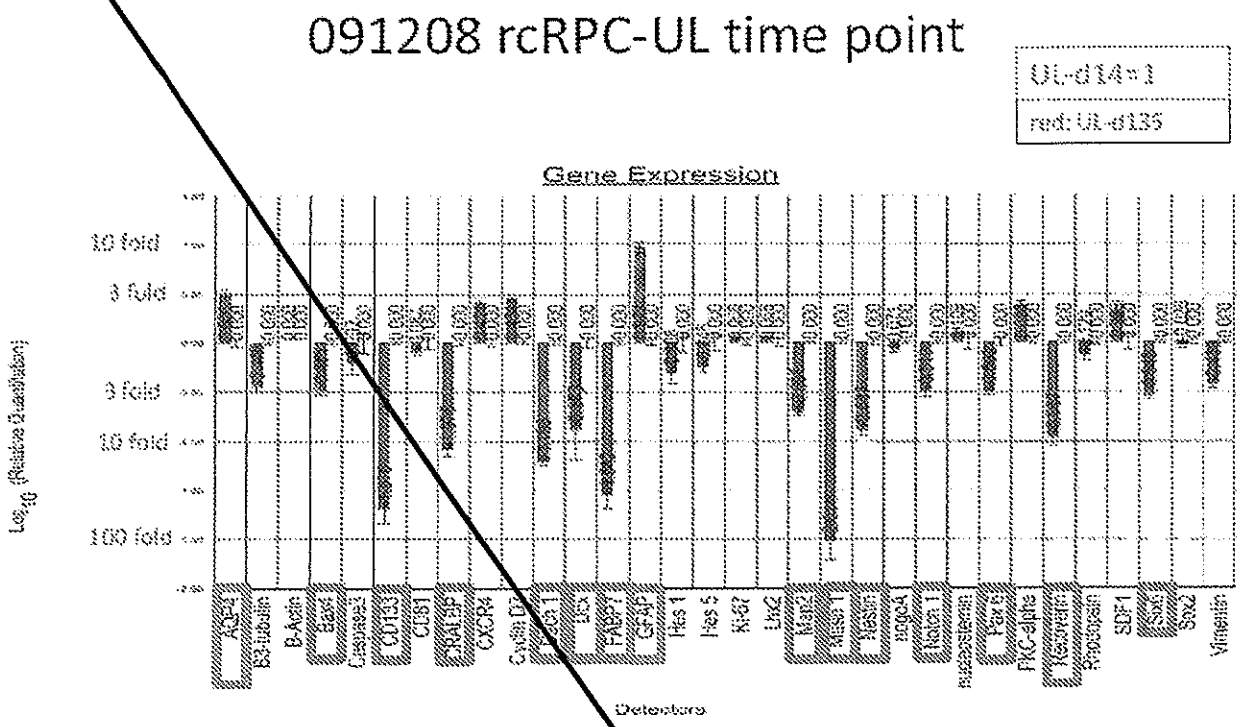

091208 rcRPC-UL time point
UL-d14=1
red: UL-d135
Gene Expression
10 fold
3 fold
3 fold
10 fold
100 fold
ADRP
B3-tubulin
B-Actin
Ecad
Caspase3
CD133
CD81
CRABP
CXCR4
Cyclin D1
Ncam 1
ICS
FABP7
GFAP
Hes 1
Hes 5
Ki-67
Lhx2
Nanog
Musashi 1
Nestin
nogoA
Notch 1
Pax6
PKC-alpha
Tenascin
Synaptophysin
SDF1
Sox1
Sox2
Vimentin
Osteopontin

FIGURE 44

cRPC time point

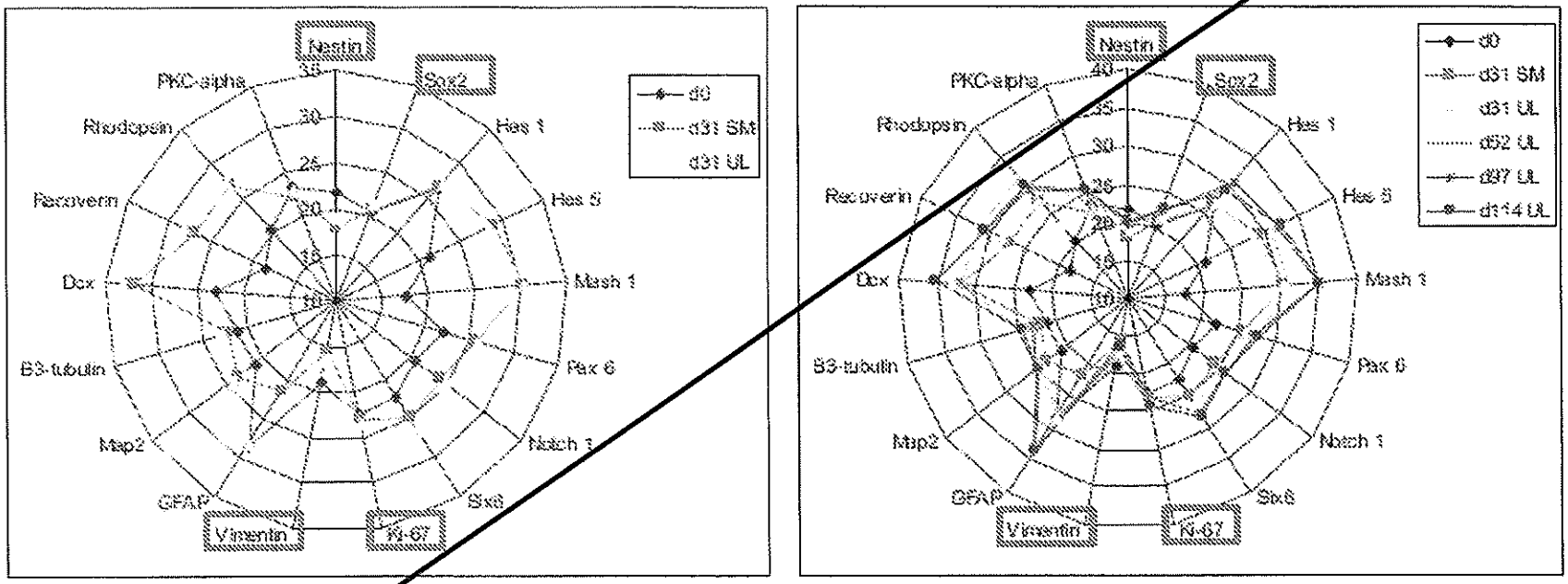


FIGURE 45

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	
Lhx2	Lhx2	Lhx2	Lhx2	
NCAM	NCAM	NCAM	NCAM	
Synapsin1	Synapsin1	Synapsin1	Synapsin1	
			Six6	

FIGURE 46

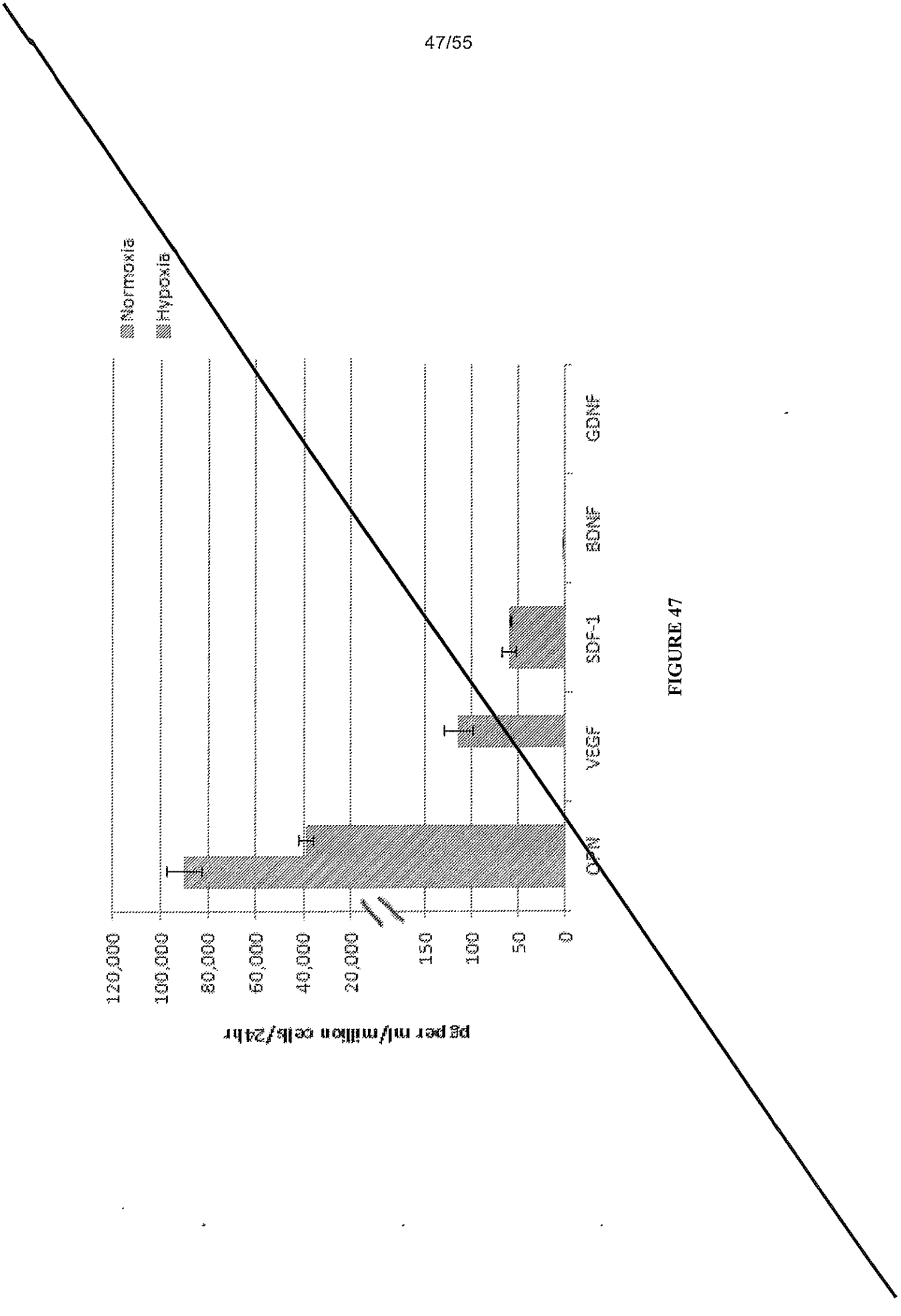

Normoxia
Hypoxia
pg per ml/million cells/24hr
120,000
100,000
80,000
60,000
40,000
20,000
150
100
50
0
OSN
VEGF
SDF-1
BDNF
GDNF

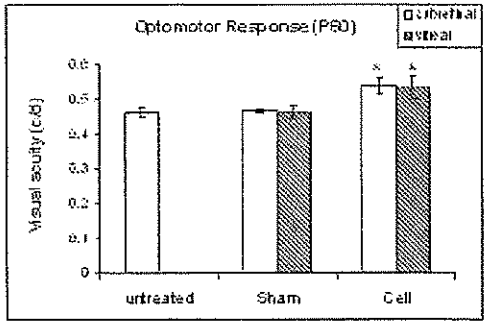
FIGURE 47

	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

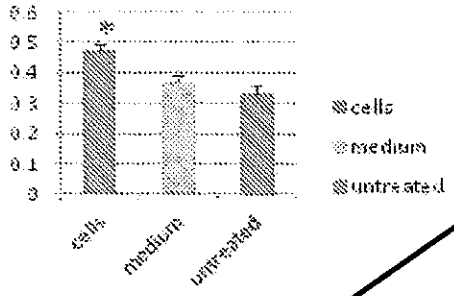
A

Optomotor response P60



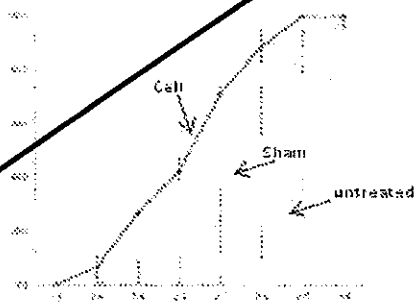
B

Optomotor response P90



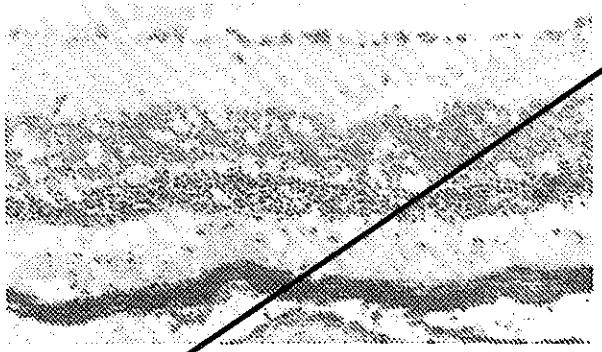
C

Luminance threshold response P90



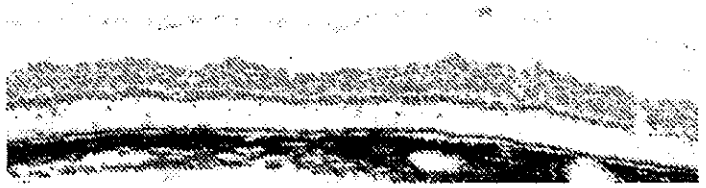
D

Histology P60 (intravitreal injection)



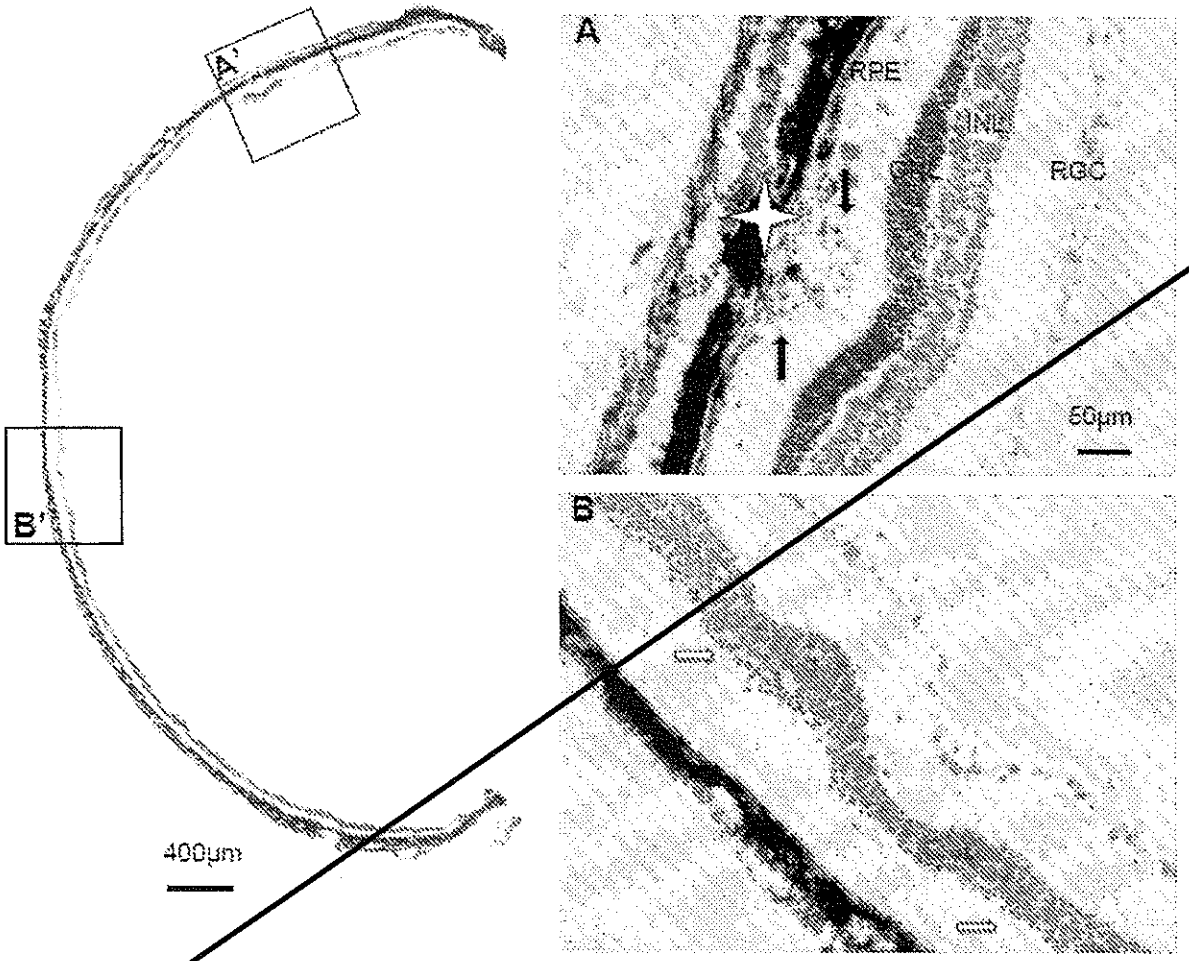
E

Histology P90 (intravitreal injection)



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

FIGURE 49



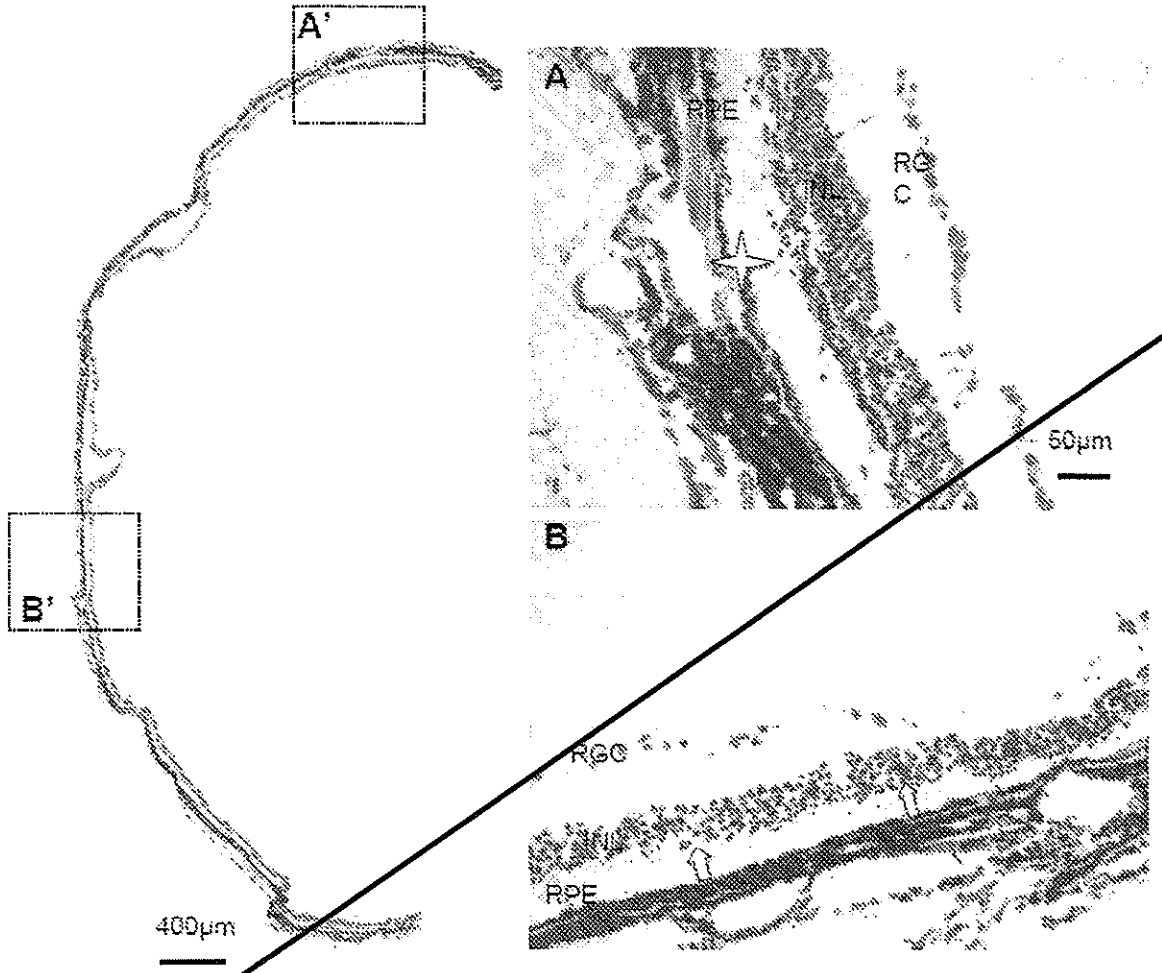
Subretinal Injection of hRPCs

A. Substantial local rescue (dark blue layer = nuclei in ONL; the arrows point to grafted cells)

B. But no rescue away from the injection site (only scattered nuclei in ONL, open arrows)

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



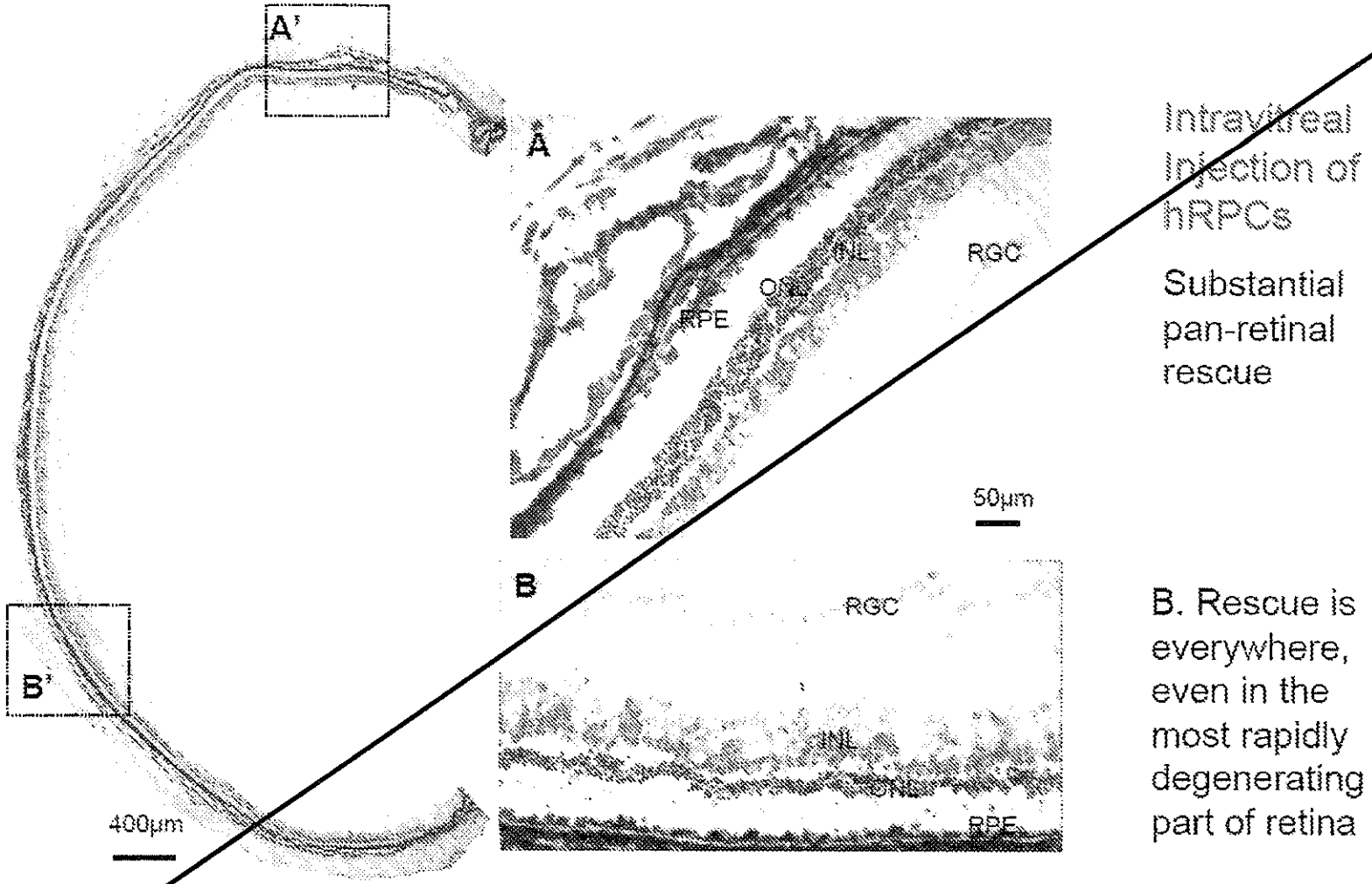
Subretinal Injection of vehicle (sham)

A. Also shows local rescue (moderate)

B. No rescue away from the site

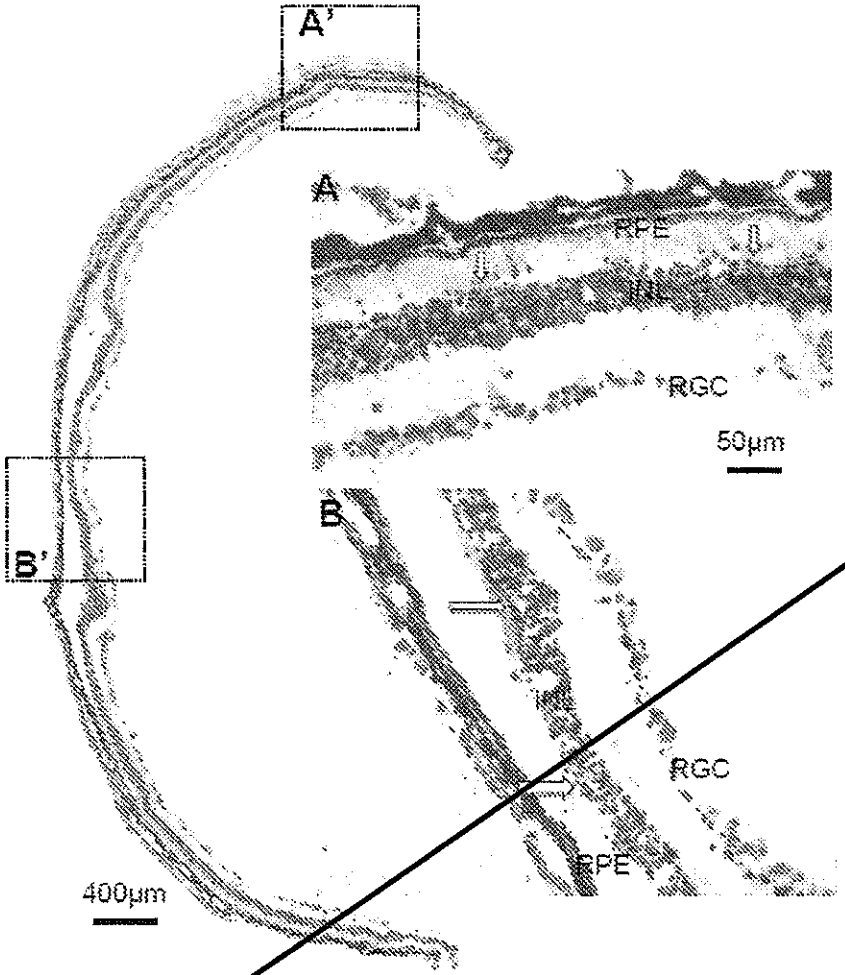
Subretinal injection of vehicle in RCS rat, viewed at P100. Left-montage image of cresyl violet stained section. A, high power image of outline A' showing injection site (star) and localized ONL rescue. B, high power image of outline B, showing a single layer of ONL (arrows) at area away from injection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



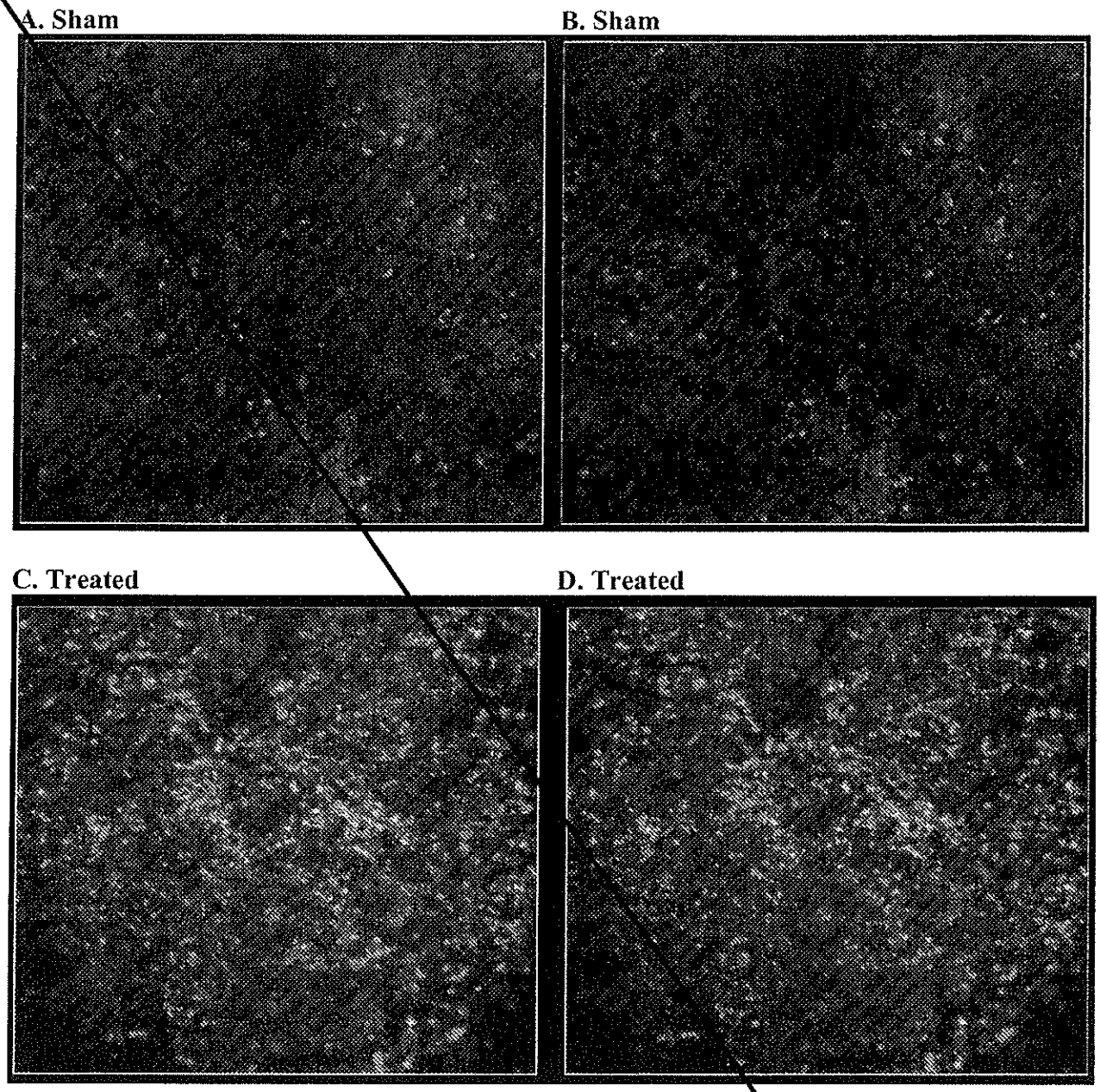
Intravitreal Injection of vehicle (sham)

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



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

FIGURE 54

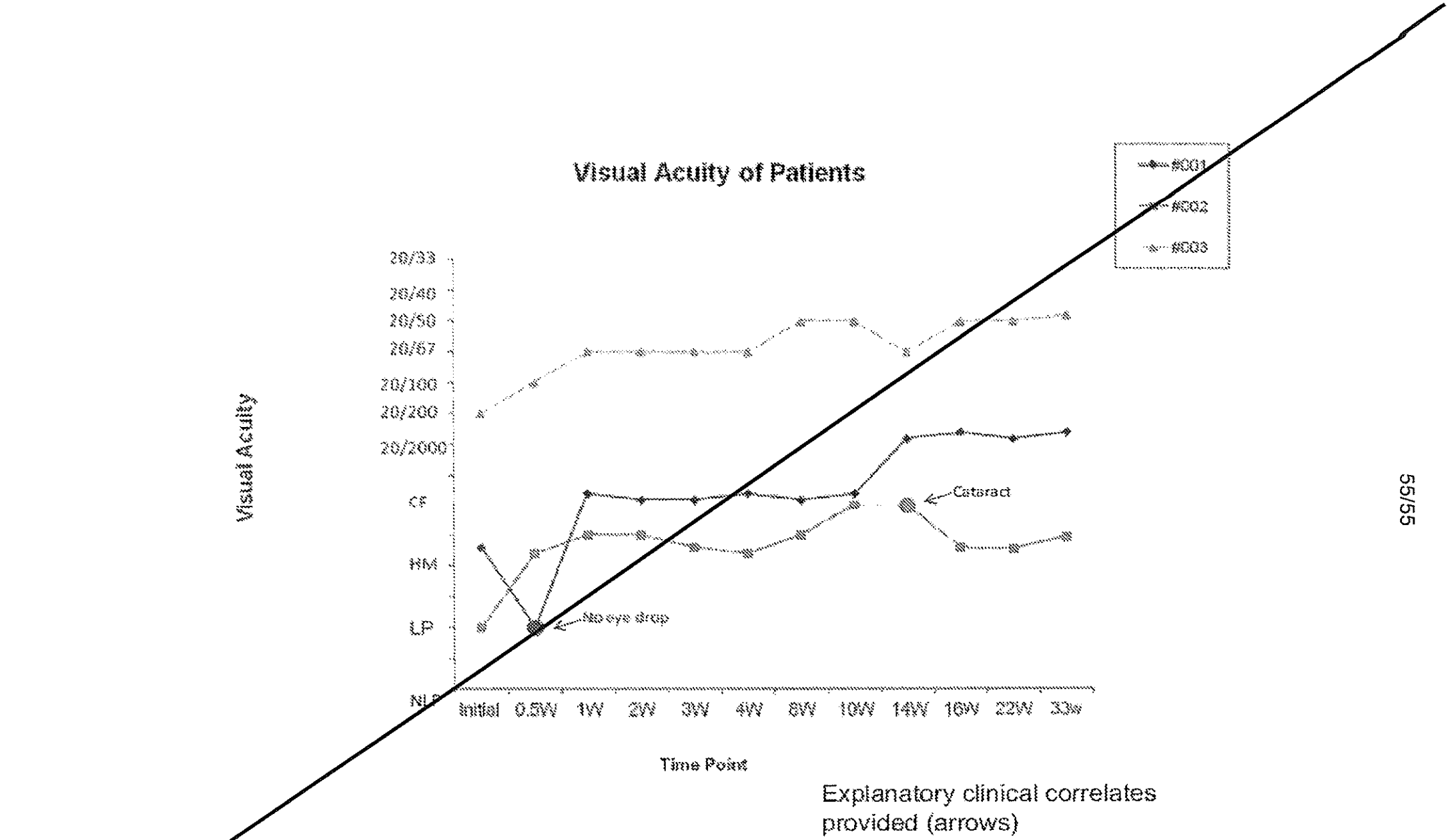

Visual Acuity of Patients
#001
#002
#003
20/33
20/40
20/50
20/67
20/100
20/200
20/2000
CF
HM
LP
NLP
Visual Acuity
Initial
0.5W
1W
2W
3W
4W
8W
10W
14W
16W
22W
33w
Time Point
No eye drop
Cataract
Explanatory clinical correlates provided (arrows)

FIGURE 55

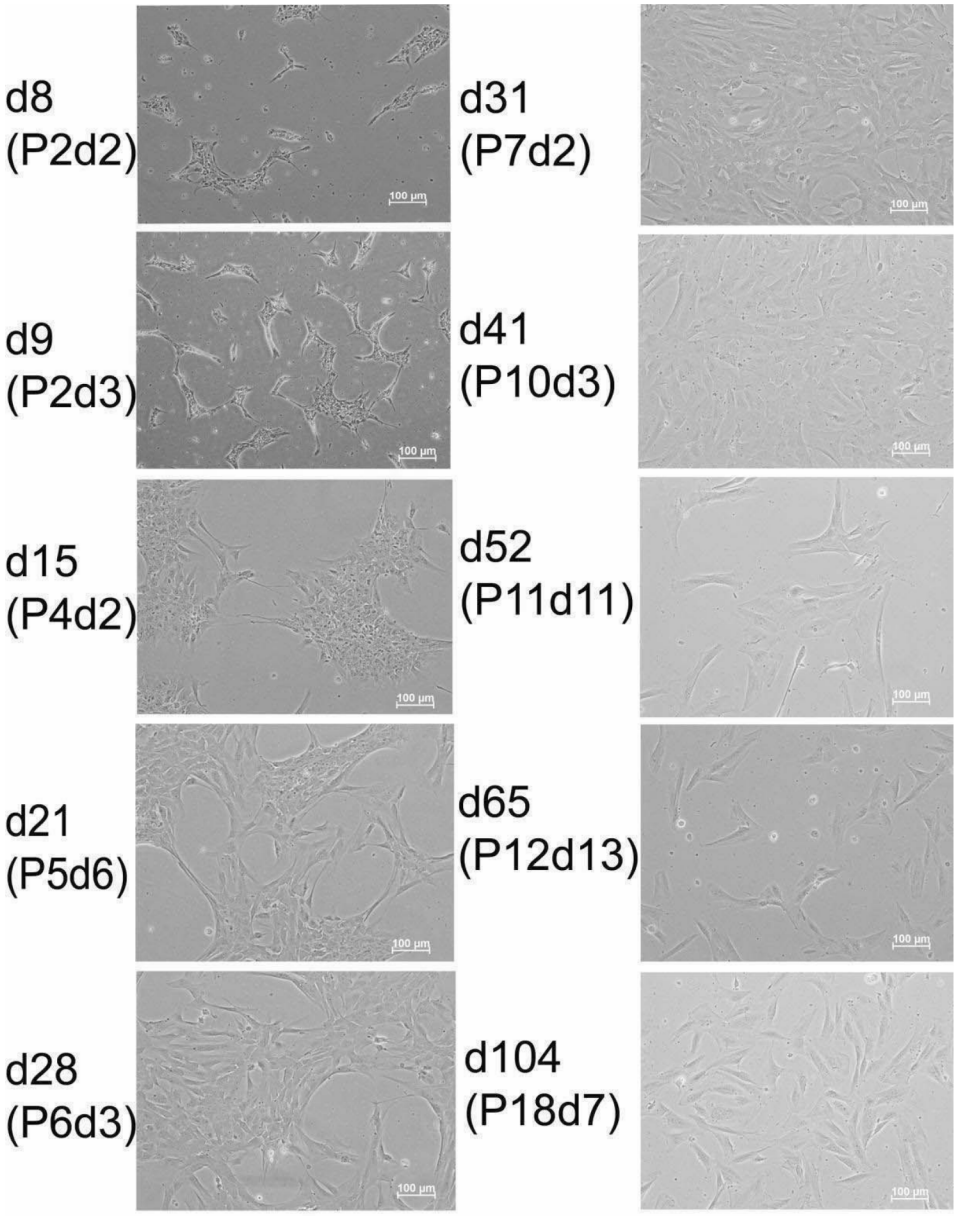
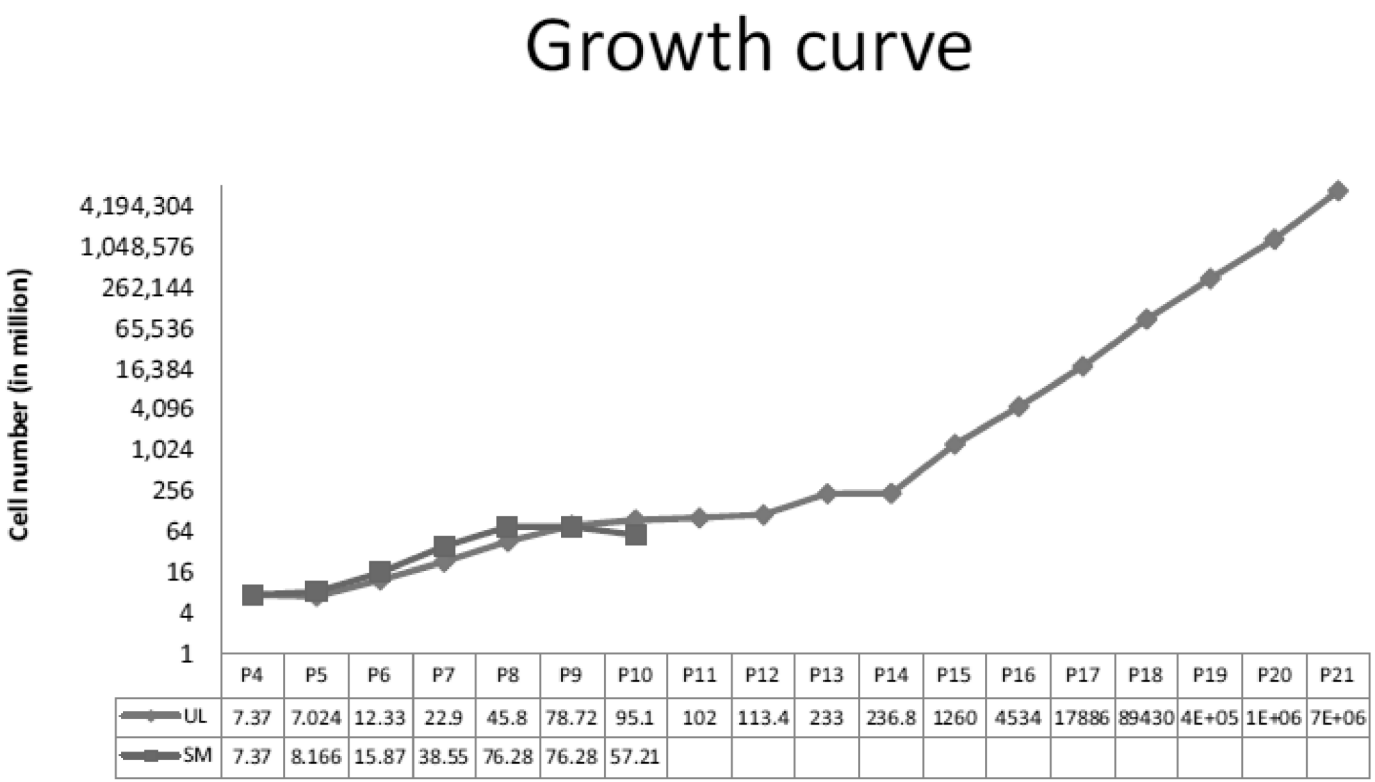

d8
(P2d2)
d31
(P7d2)
d9
(P2d3)
d41
(P10d3)
d15
(P4d2)
d52
(P11d11)
d21
(P5d6)
d65
(P12d13)
d28
(P6d3)
d104
(P18d7)
100 µm

FIGURE 1



	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21
UL	7.37	7.024	12.33	22.9	45.8	78.72	95.1	102	113.4	233	236.8	1260	4534	17886	89430	4E+05	1E+06	7E+06
SM	7.37	8.166	15.87	38.55	76.28	76.28	57.21											

FIGURE 2

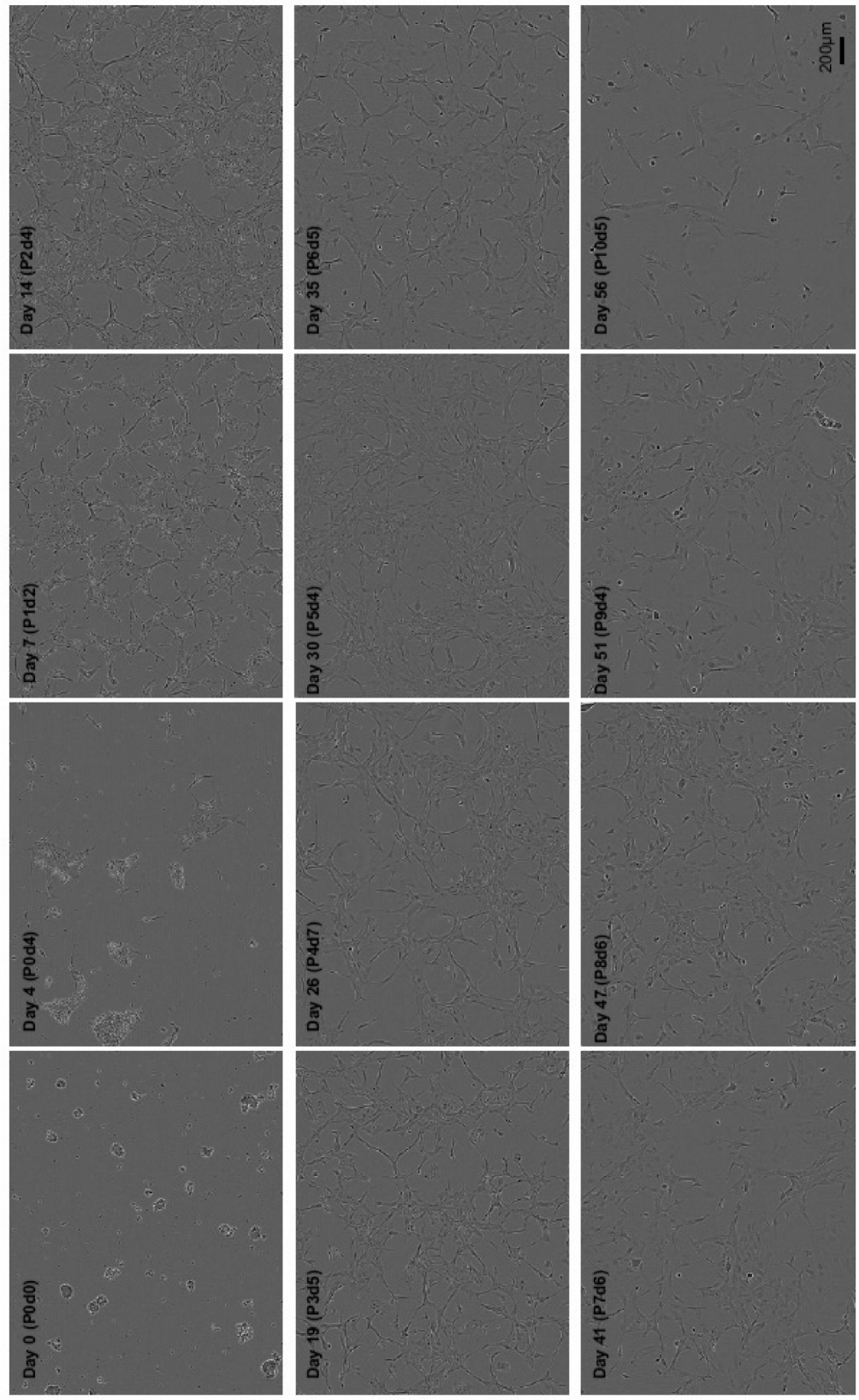
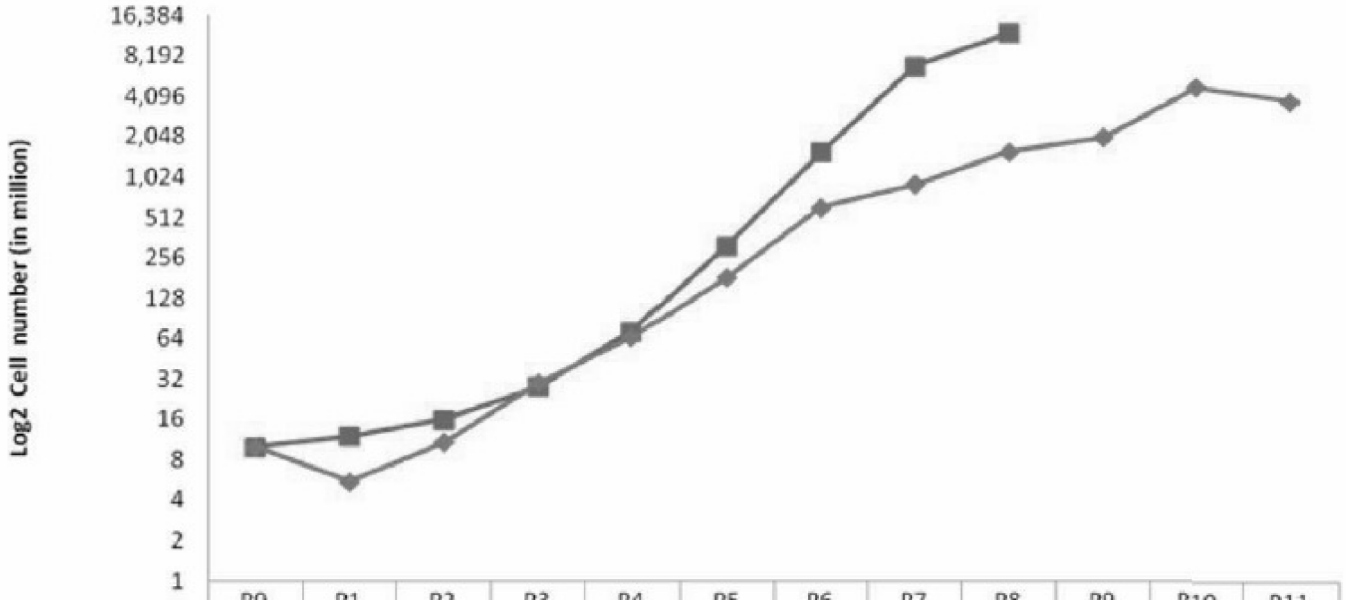

Day 0 (P0d0)
Day 4 (P0d4)
Day 7 (P1d2)
Day 14 (P2d4)
Day 19 (P3d5)
Day 26 (P4d7)
Day 30 (P5d4)
Day 35 (P6d5)
Day 41 (P7d6)
Day 47 (P8d6)
Day 51 (P9d4)
Day 56 (P10d5)
200µm

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	28	72	315	1,575	6,931	12,129			
cell number (in million)	10	6	11	30	66	184	606	905	1,592	2,038	4,770	3,720

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

FIGURE 4

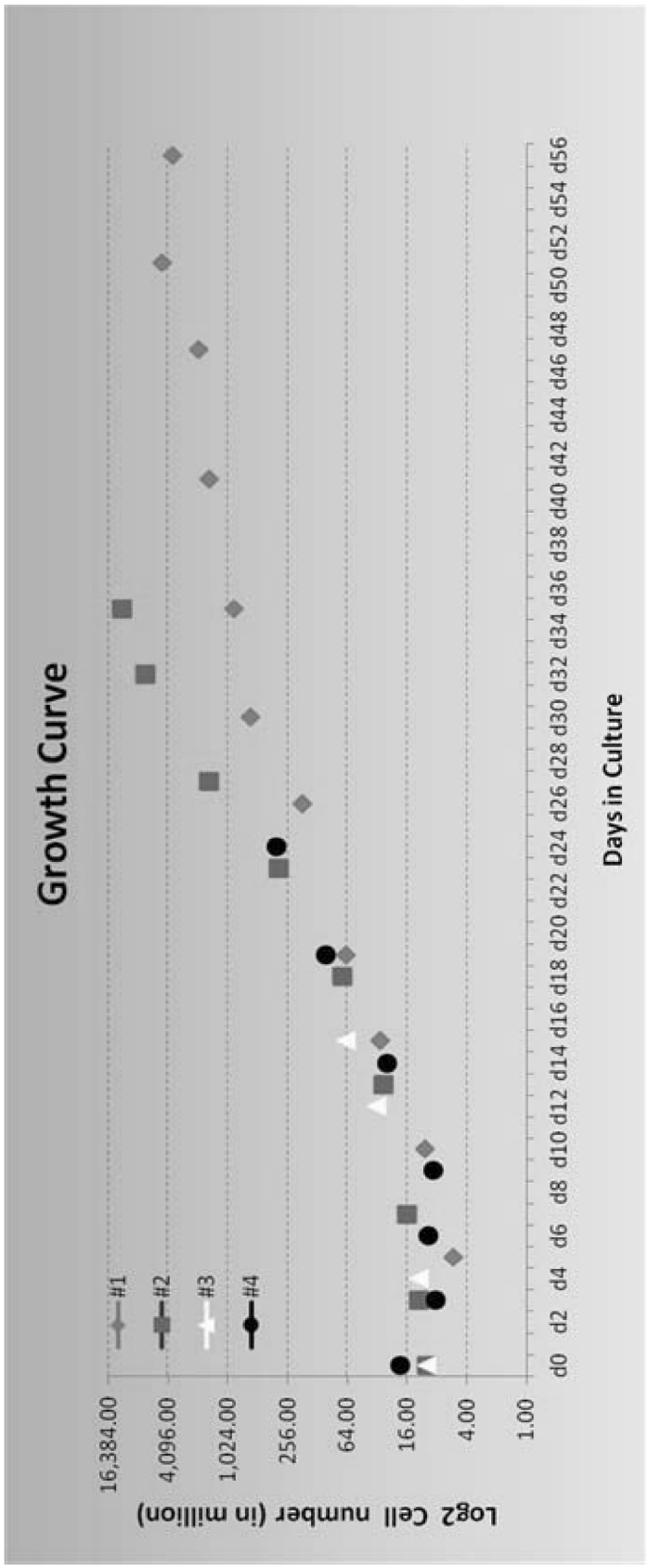
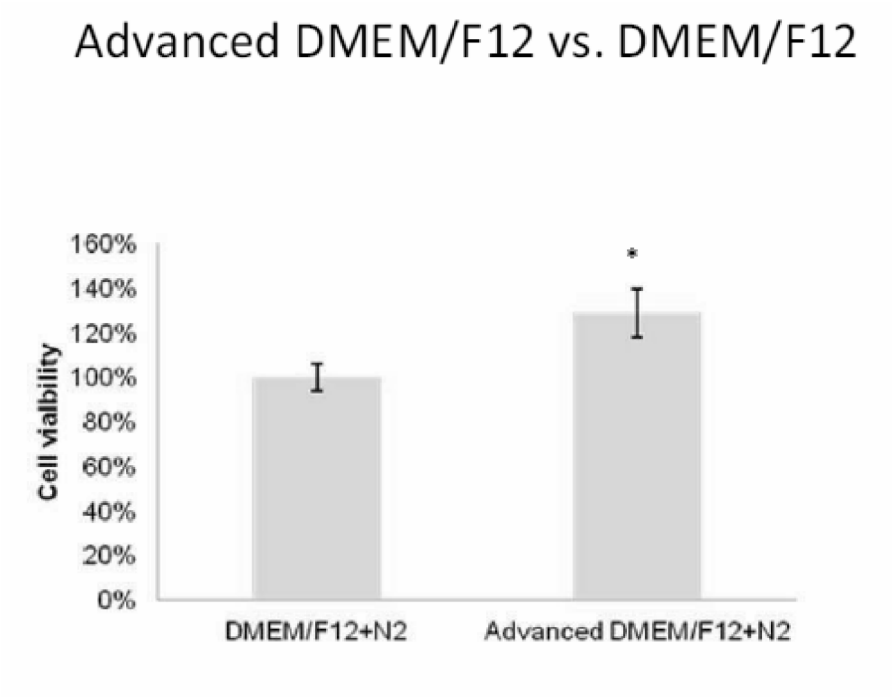

Growth Curve
#1
#2
#3
#4
16,384.00
4,096.00
1,024.00
256.00
64.00
16.00
4.00
1.00
Log2 Cell number (in million)
d0 d2 d4 d6 d8 d10 d12 d14 d16 d18 d20 d22 d24 d26 d28 d30 d32 d34 d36 d38 d40 d42 d44 d46 d48 d50 d52 d54 d56
Days in Culture

FIGURE 5

A


Advanced DMEM/F12 vs. DMEM/F12
Cell viability
160%
140%
120%
100%
80%
60%
40%
20%
0%
*
DMEM/F12+N2
Advanced DMEM/F12+N2

B

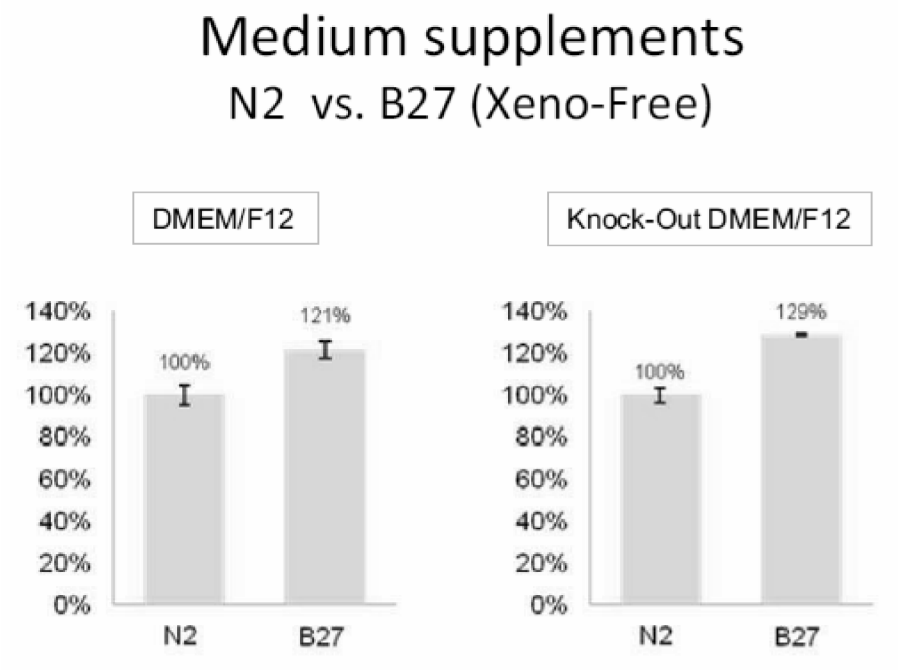
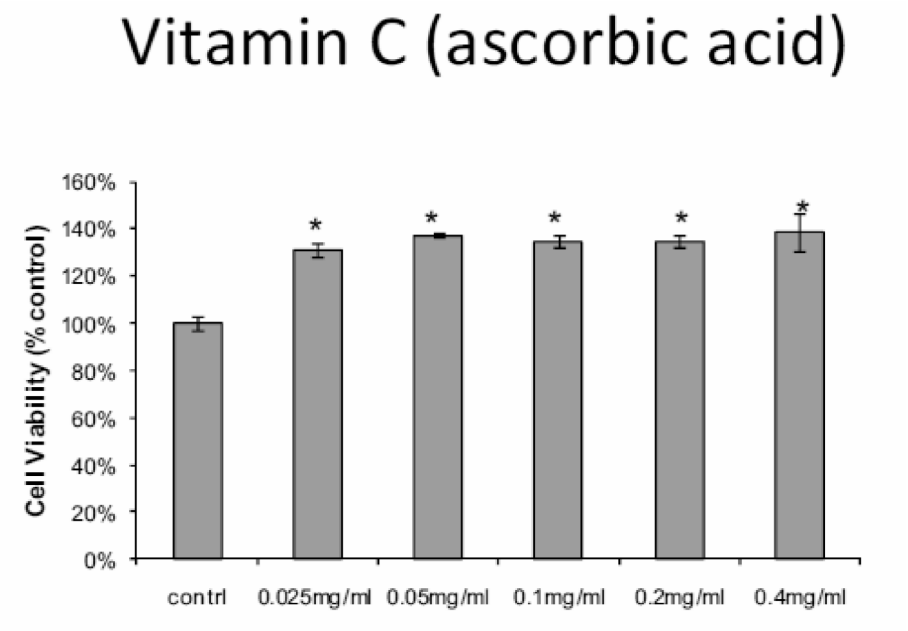
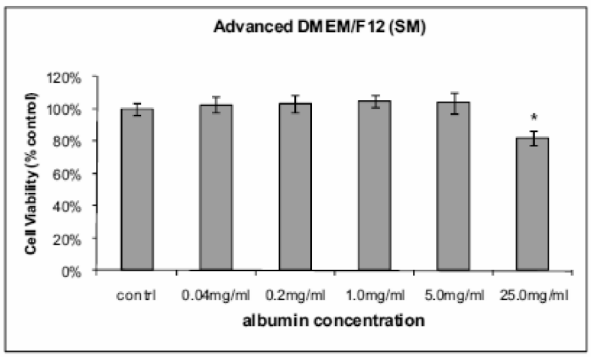

Medium supplements
N2 vs. B27 (Xeno-Free)
DMEM/F12
Knock-Out DMEM/F12
140%
120%
100%
80%
60%
40%
20%
0%
100%
121%
129%
N2
B27

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)
Cell Viability (% control)
120%
100%
80%
60%
40%
20%
0%
contrl
0.04mg/ml
0.2mg/ml
1.0mg/ml
5.0mg/ml
25.0mg/ml
albumin concentration

C

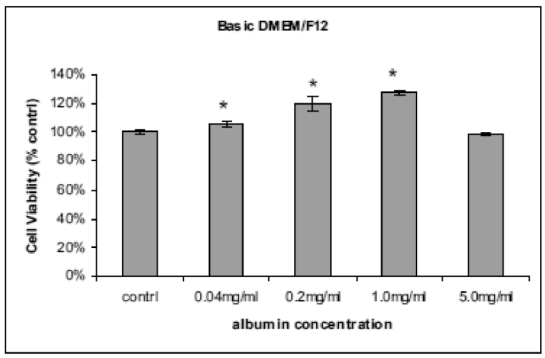

Basic DMEM/F12
Cell Viability (% contrl)
140%
120%
100%
80%
60%
40%
20%
0%
contrl
0.04mg/ml
0.2mg/ml
1.0mg/ml
5.0mg/ml
albumin concentration

FIGURE 7

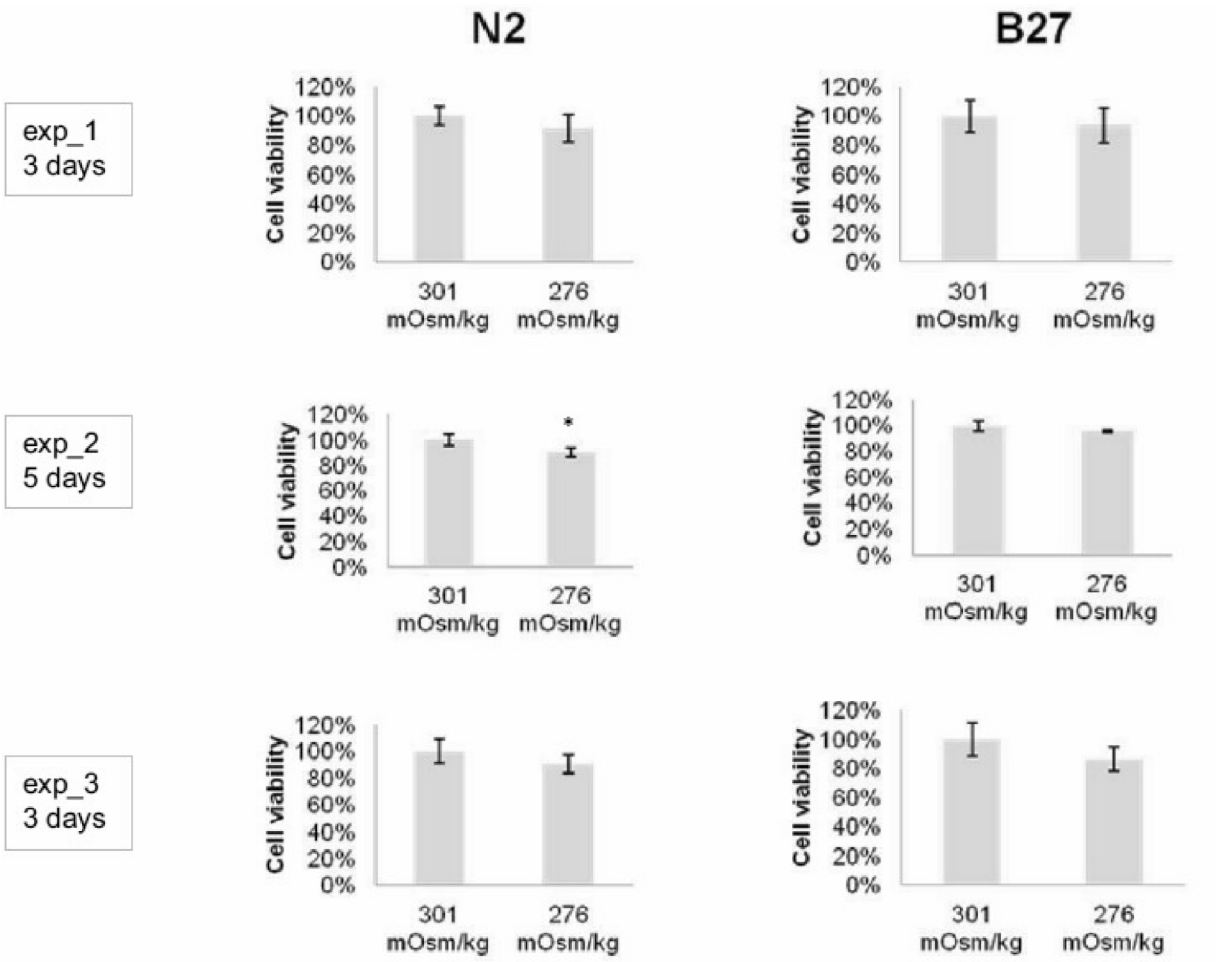

N2
B27
exp_1
3 days
exp_2
5 days
exp_3
3 days
Cell viability
120%
100%
80%
60%
40%
20%
0%
301
mOsm/kg
276
mOsm/kg
*

FIGURE 8

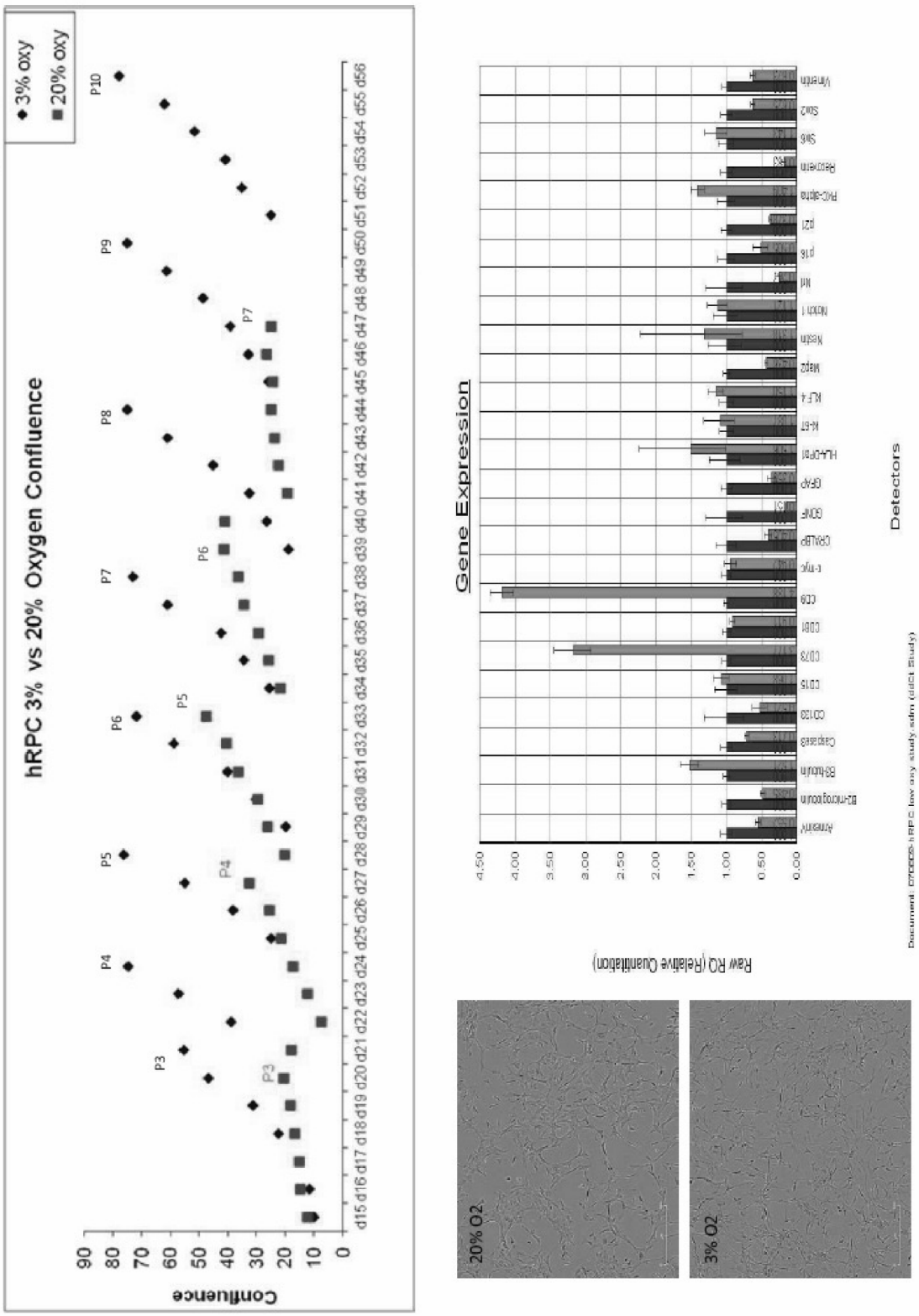
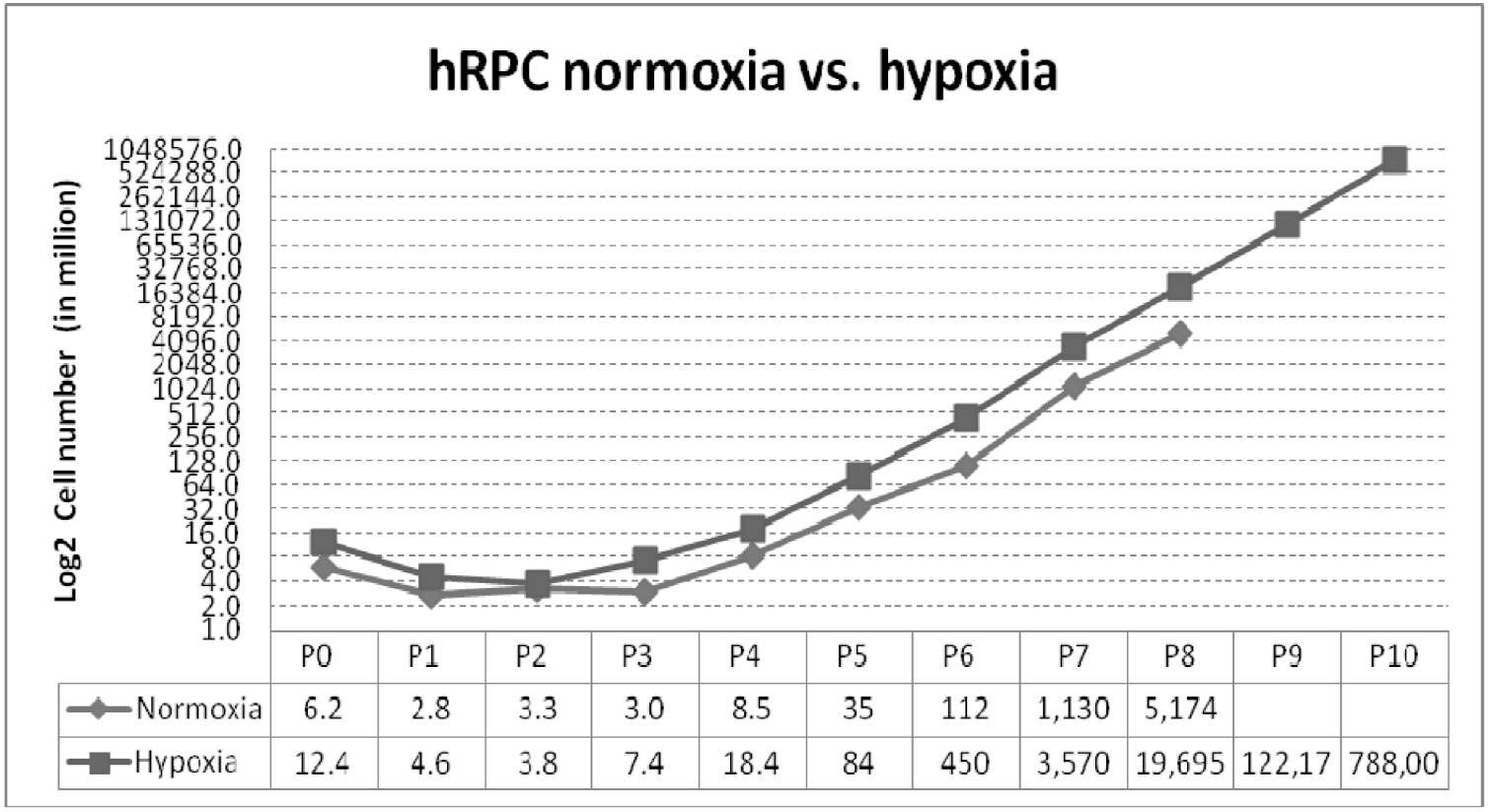

hRPC 3% vs 20% Oxygen Confluence
3% oxy
20% oxy
Confluence
P3
P4
P5
P6
P7
P8
P9
P10
Gene Expression
Raw RQ (Relative Quantitation)
Detectors
20% O2
3% O2

FIGURE 9



	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

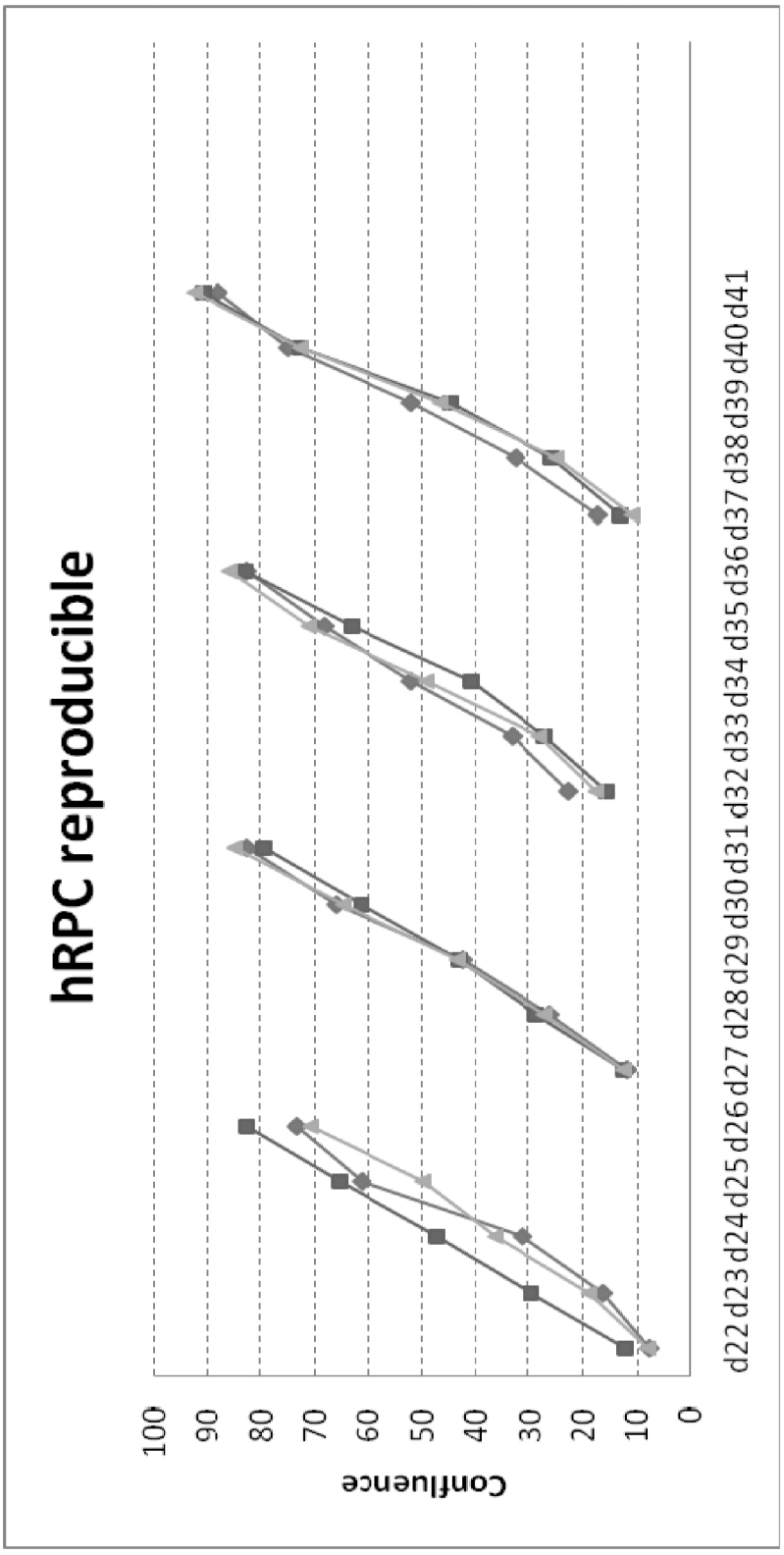

hRPC reproducible
Confluence
100
90
80
70
60
50
40
30
20
10
0
d22 d23 d24 d25 d26 d27 d28 d29 d30 d31 d32 d33 d34 d35 d36 d37 d38 d39 d40 d41

FIGURE 11

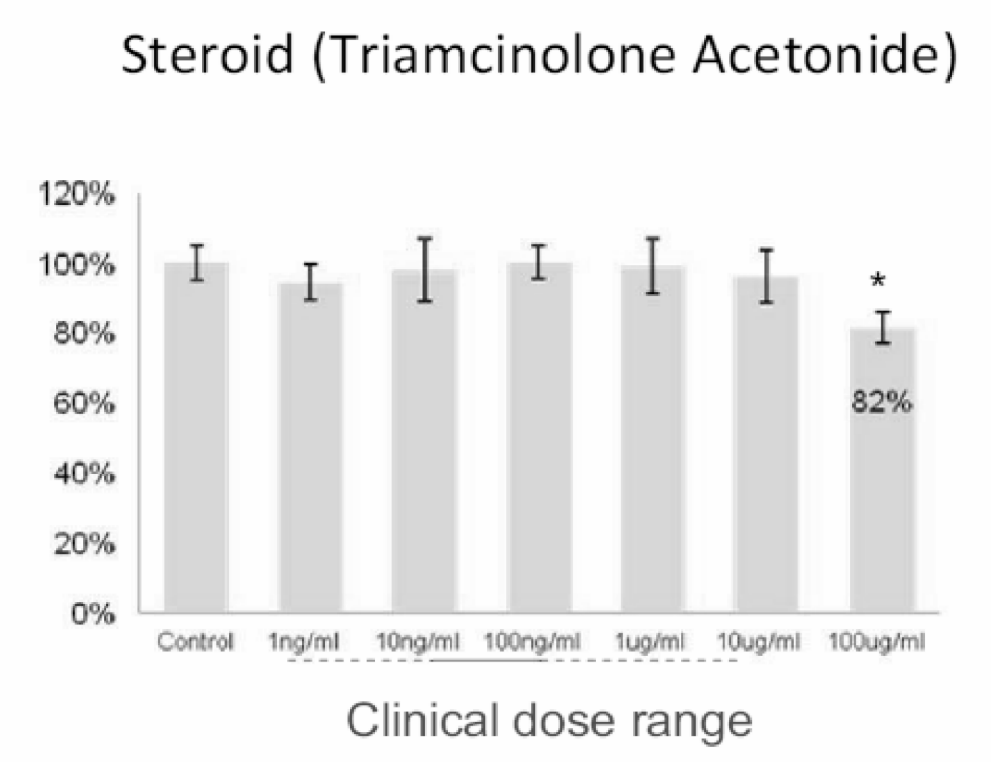

Steroid (Triamcinolone Acetonide)
120%
100%
80%
60%
40%
20%
0%
*
82%
Control
1ng/ml
10ng/ml
100ng/ml
1ug/ml
10ug/ml
100ug/ml
Clinical dose range

FIGURE 12

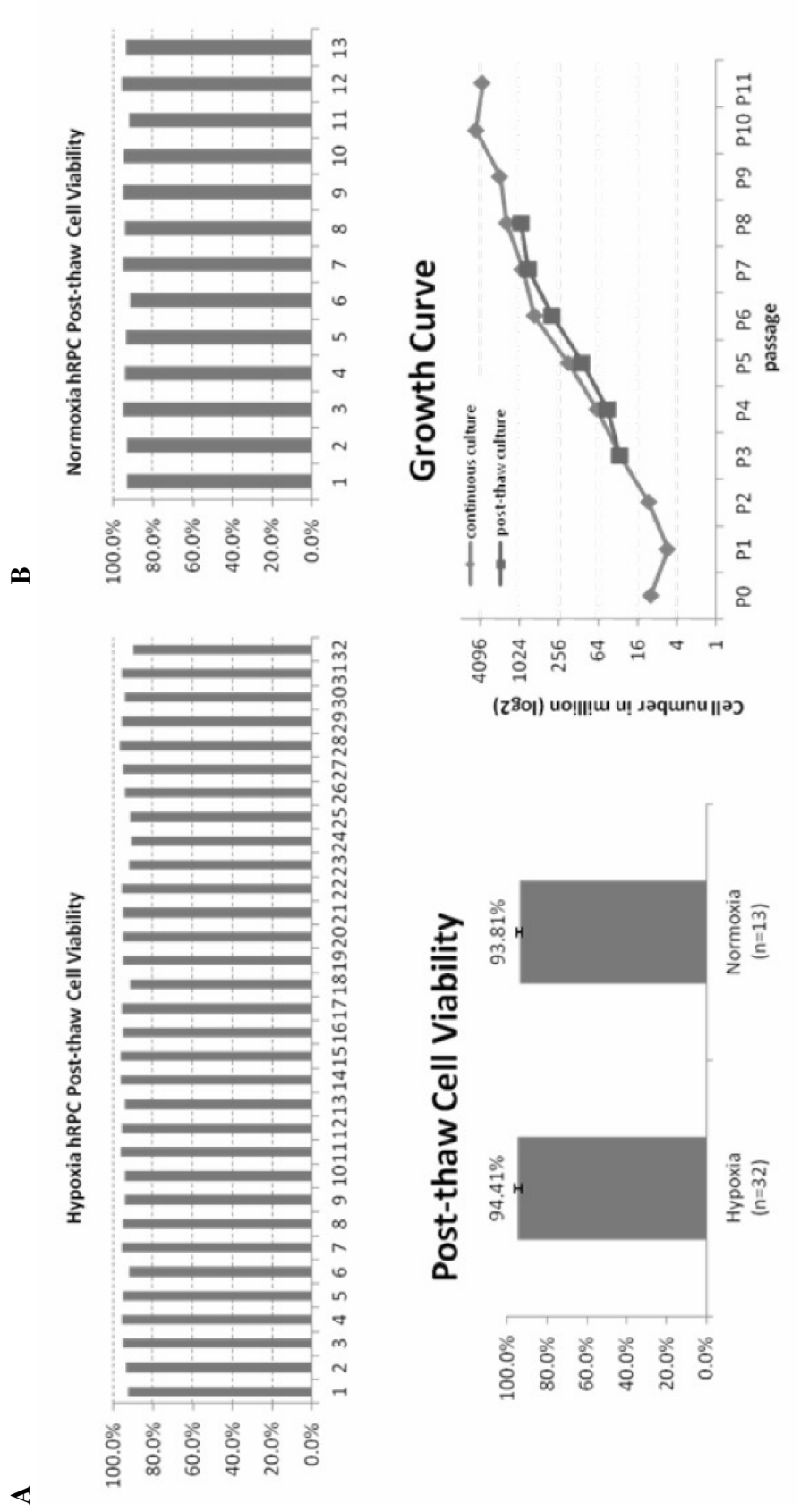

A
Hypoxia hRPC Post-thaw Cell Viability
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
B
Normoxia hRPC Post-thaw Cell Viability
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
1 2 3 4 5 6 7 8 9 10 11 12 13
C
Post-thaw Cell Viability
94.41%
93.81%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Hypoxia (n=32)
Normoxia (n=13)
D
Growth Curve
continuous culture
post-thaw culture
Cell number in million (log2)
4096
1024
256
64
16
4
1
P0 P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11
passage

FIGURE 13

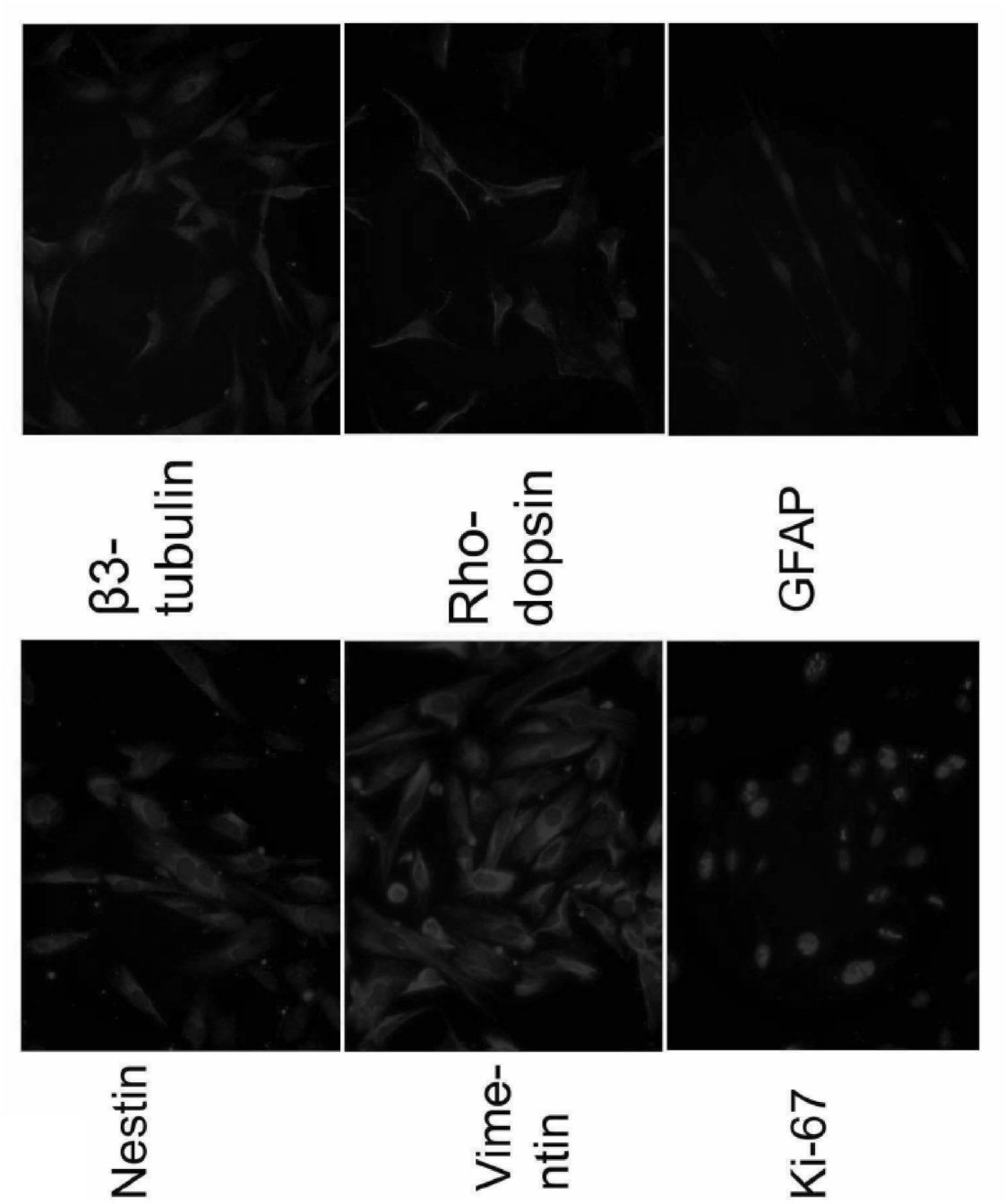


FIGURE 14

In UL

Gene profile by time point

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)

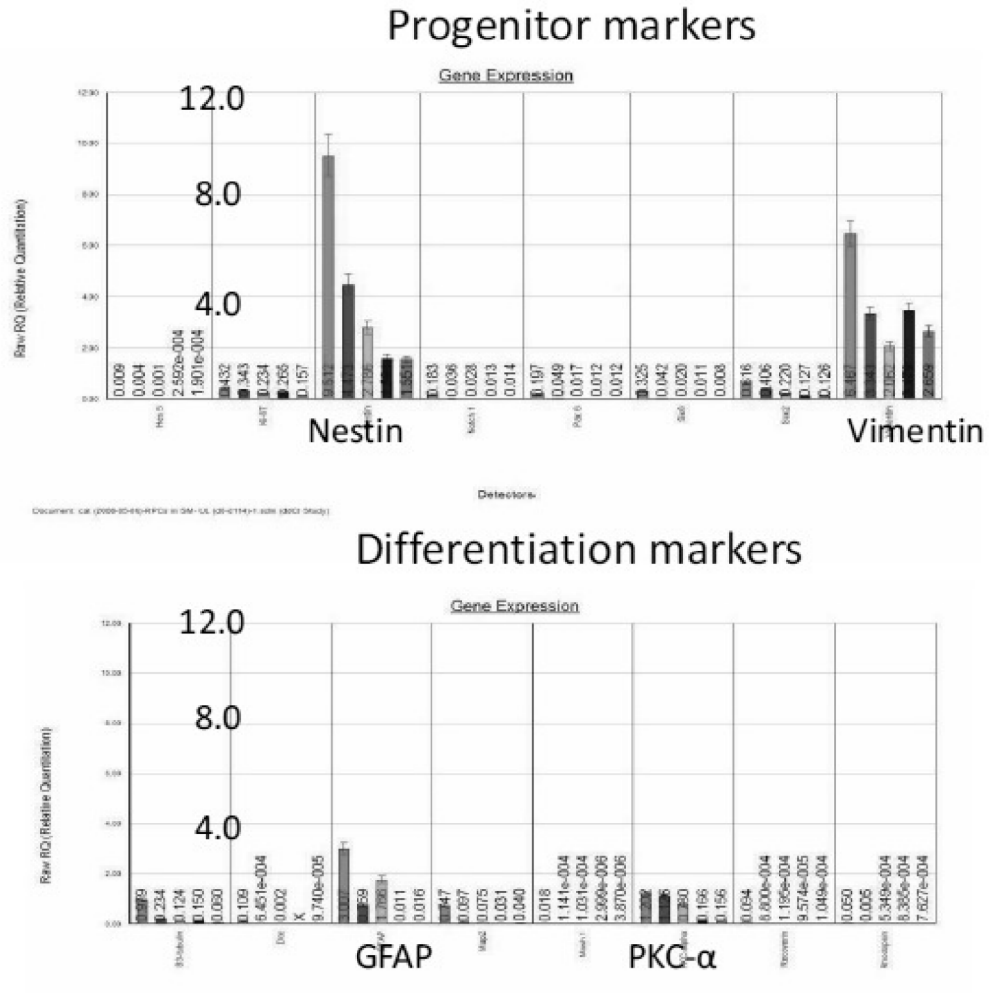
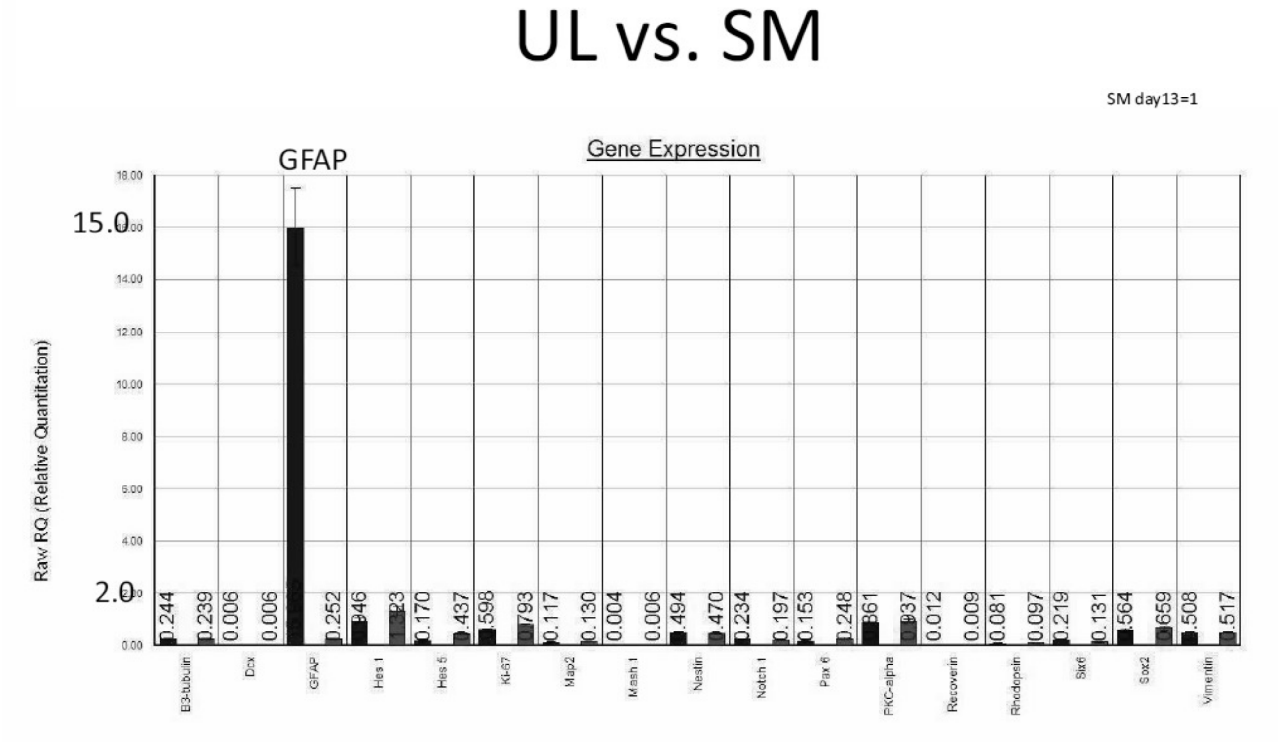


FIGURE 15

A


UL vs. SM
SM day13=1
Gene Expression
GFAP
Raw RQ (Relative Quantitation)
15.0
2.0
B3-tubulin
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Mash 1
Nestin
Notch 1
Pax 6
PKC-alpha
Recoverin
Rhodopsin
Six6
Sox2
Vimentin

B

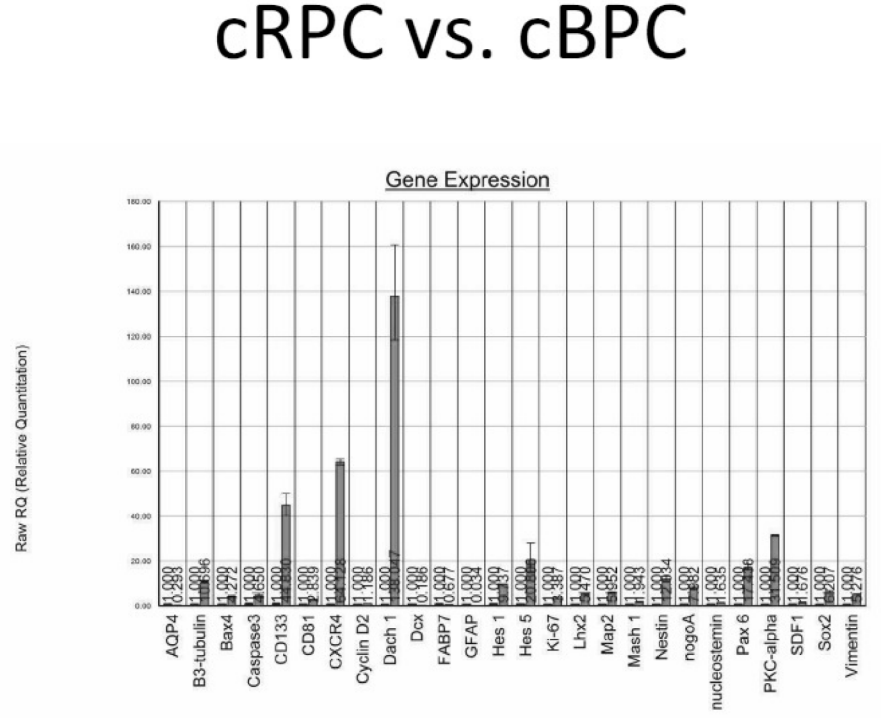

cRPC vs. cBPC
Gene Expression
Raw RQ (Relative Quantitation)
AQP4
B3-tubulin
Bax4
Caspase3
CD133
CD81
CXCR4
Cyclin D2
Dach 1
Dcx
FABP7
GFAP
Hes 1
Hes 5
Ki-67
Lhx2
Map2
Mash 1
Nestin
nogoA
nucleostemin
Pax 6
PKC-alpha
SDF1
Sox2
Vimentin

FIGURE 16

ANTIBODIES USED Labeling

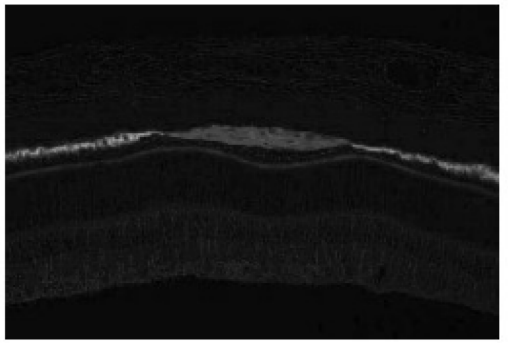
Anti-RFP	injected cells
Anti-vimentin	Műller cells
Anti-rod opsin	rod outer segments and some rod cell bodies as the protein redistributes due to the degeneration

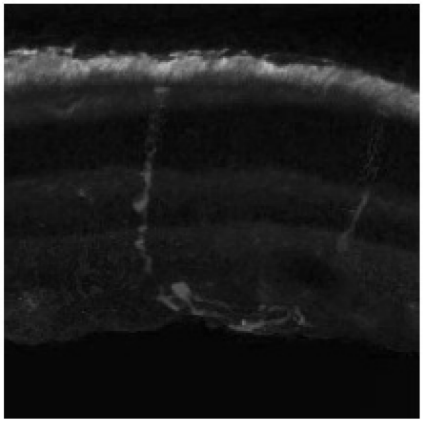
OR

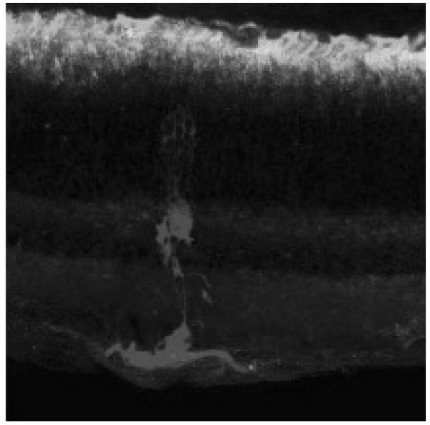
Anti-Ezrin	RPE microvilli and Műller cell microvilli at the OLM

OR

Protein kinase C	rod bipolar cells







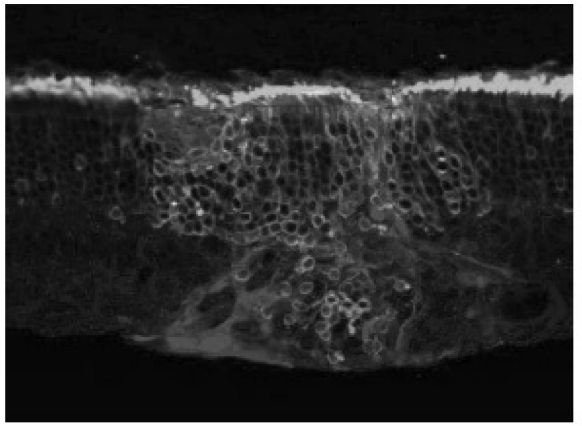


FIGURE 17

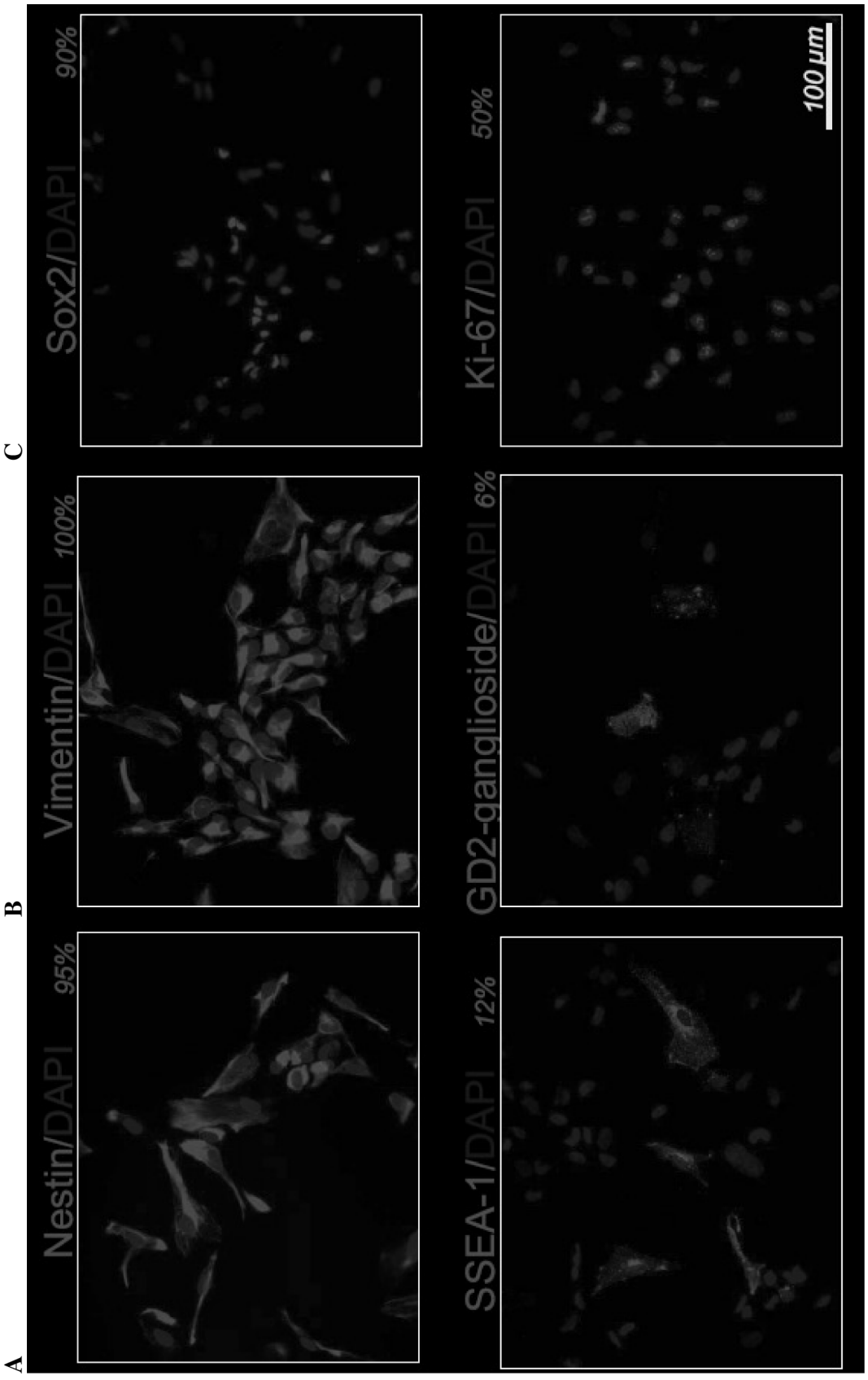

A
Nestin/DAPI 95%
B
Vimentin/DAPI 100%
C
Sox2/DAPI 90%
D
SSEA-1/DAPI 12%
E
GD2-ganglioside/DAPI 6%
F
Ki-67/DAPI 50%
100 µm

FIGURE 18

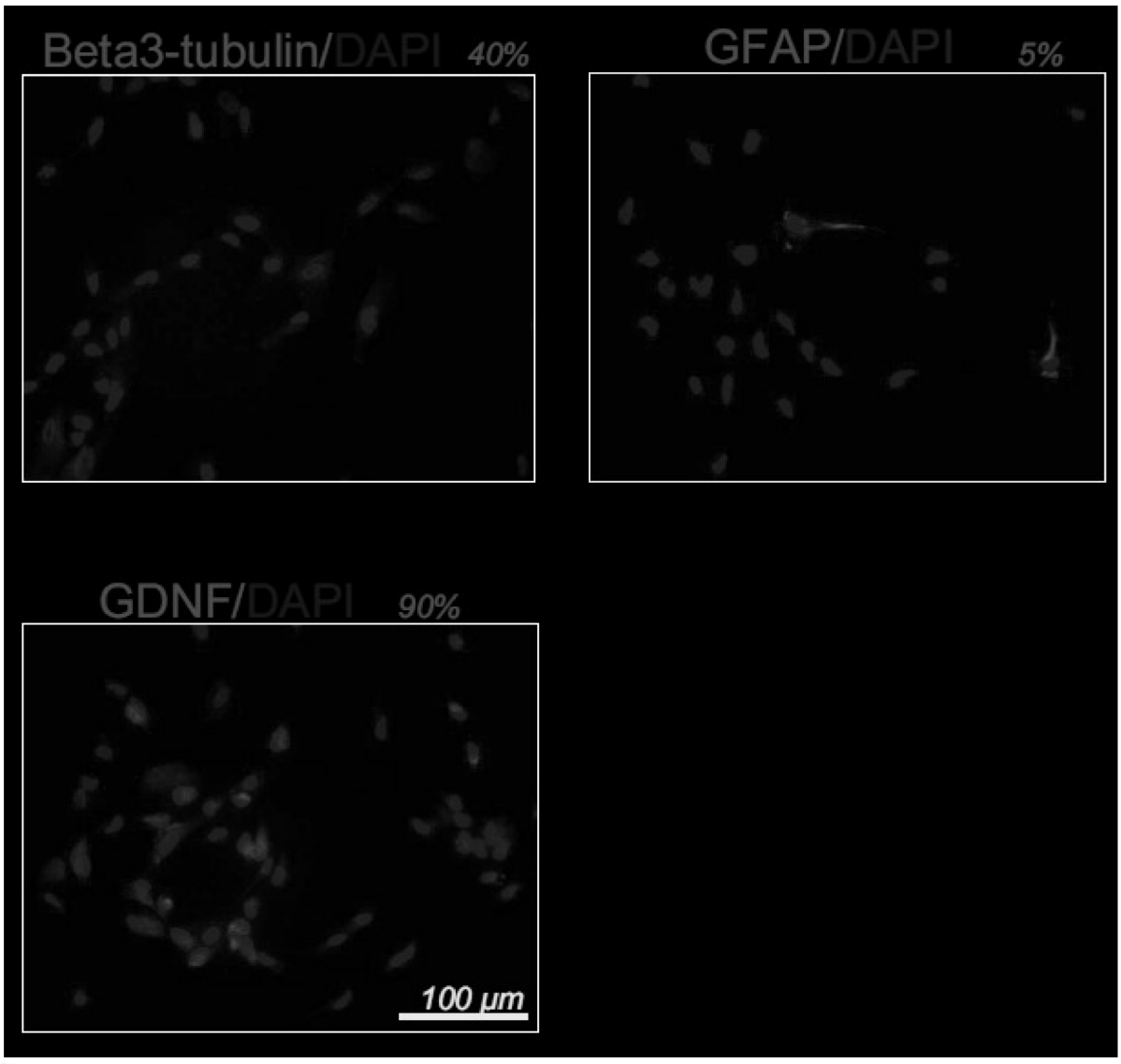


FIGURE 19

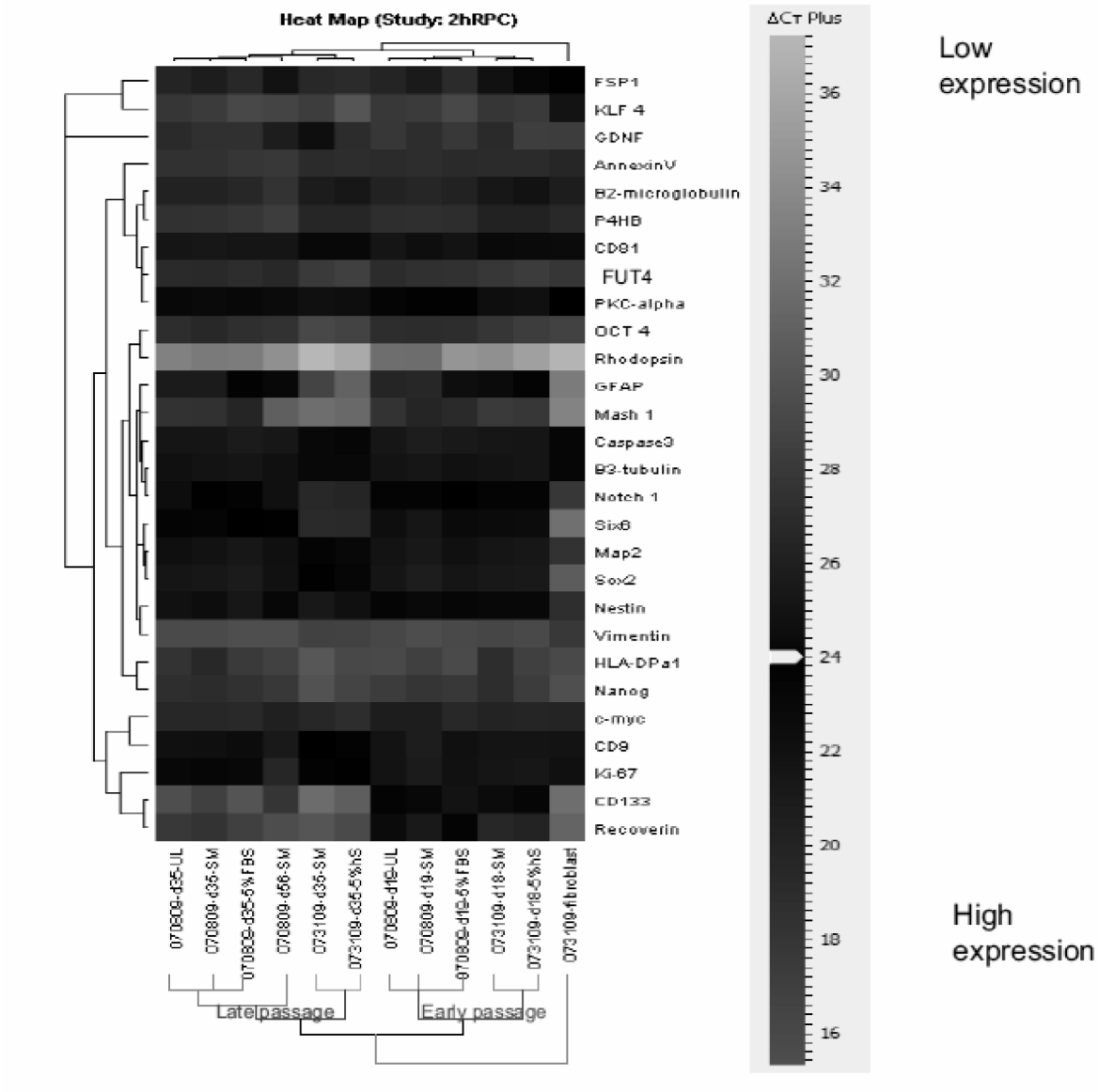
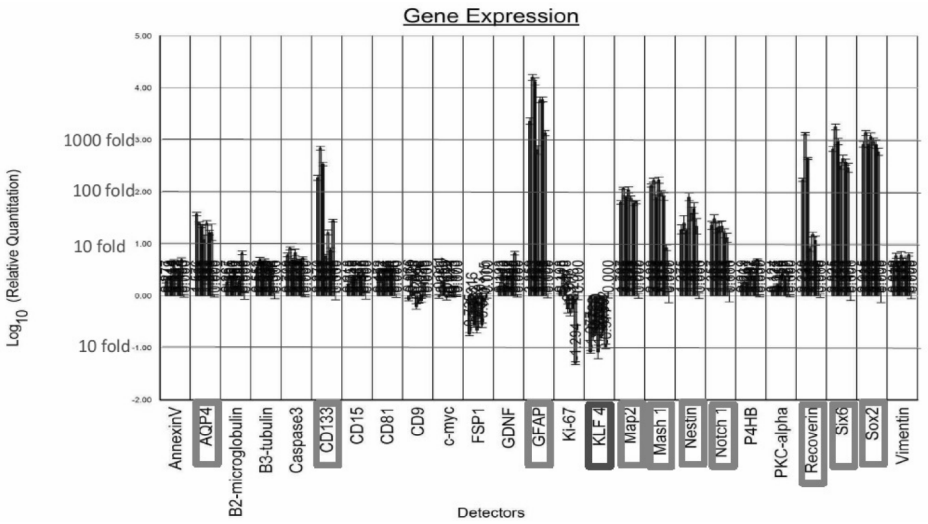
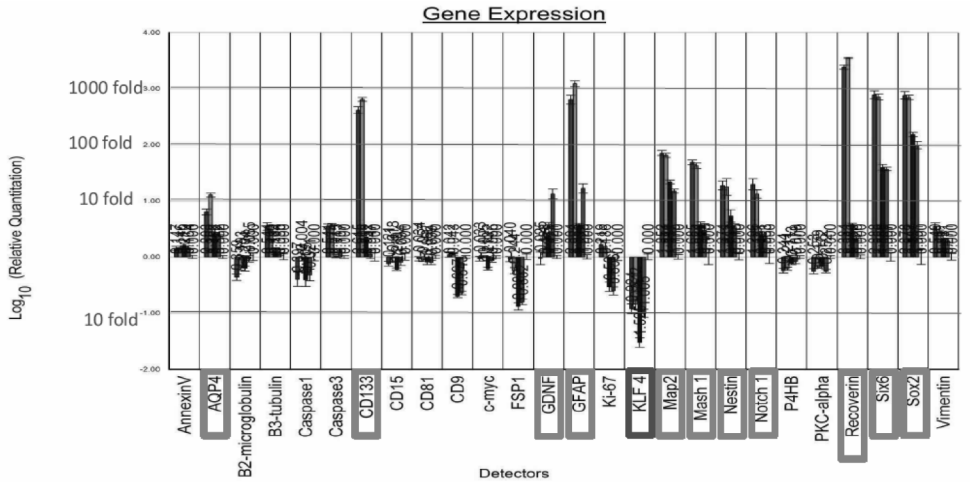

Heat Map (Study: 2hRPC)
ΔCT Plus
Low expression
High expression
FSP1
KLF 4
GDNF
AnnexinV
B2-microglobulin
P4HB
CD81
FUT4
PKC-alpha
OCT 4
Rhodopsin
GFAP
Mash 1
Caspase3
B3-tubulin
Notch 1
Six6
Map2
Sox2
Nestin
Vimentin
HLA-DPa1
Nanog
c-myc
CD9
Ki-67
CD133
Recoverin
070809-d35-UL
070809-d35-SM
070809-d35-5%FBS
070809-d56-SM
073109-d35-SM
073109-d35-5%hS
070809-d19-UL
070809-d19-SM
070809-d19-5%FBS
073109-d18-SM
073109-d18-5%hS
073109-fibroblast
Late passage
Early passage
36
34
32
30
28
26
24
22
20
18
16

FIGURE 20

A



B



C

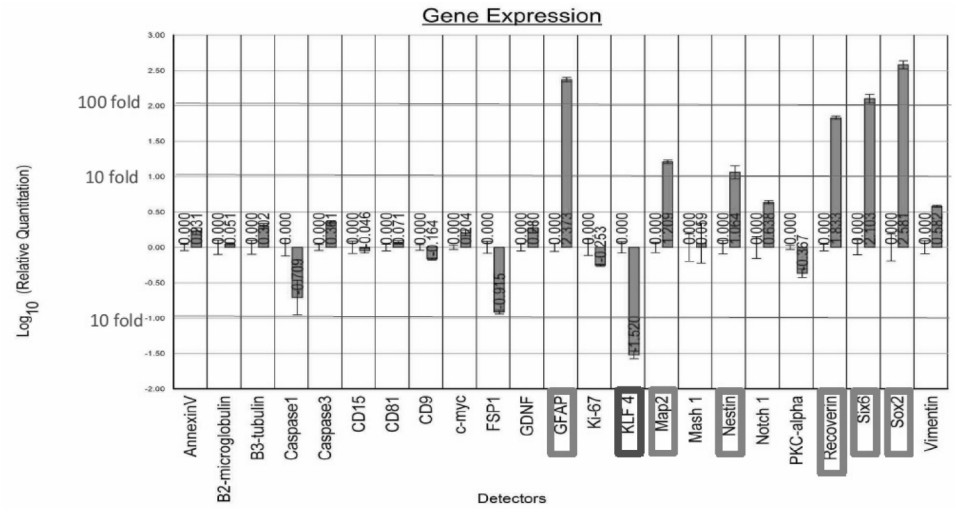


FIGURE 21

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

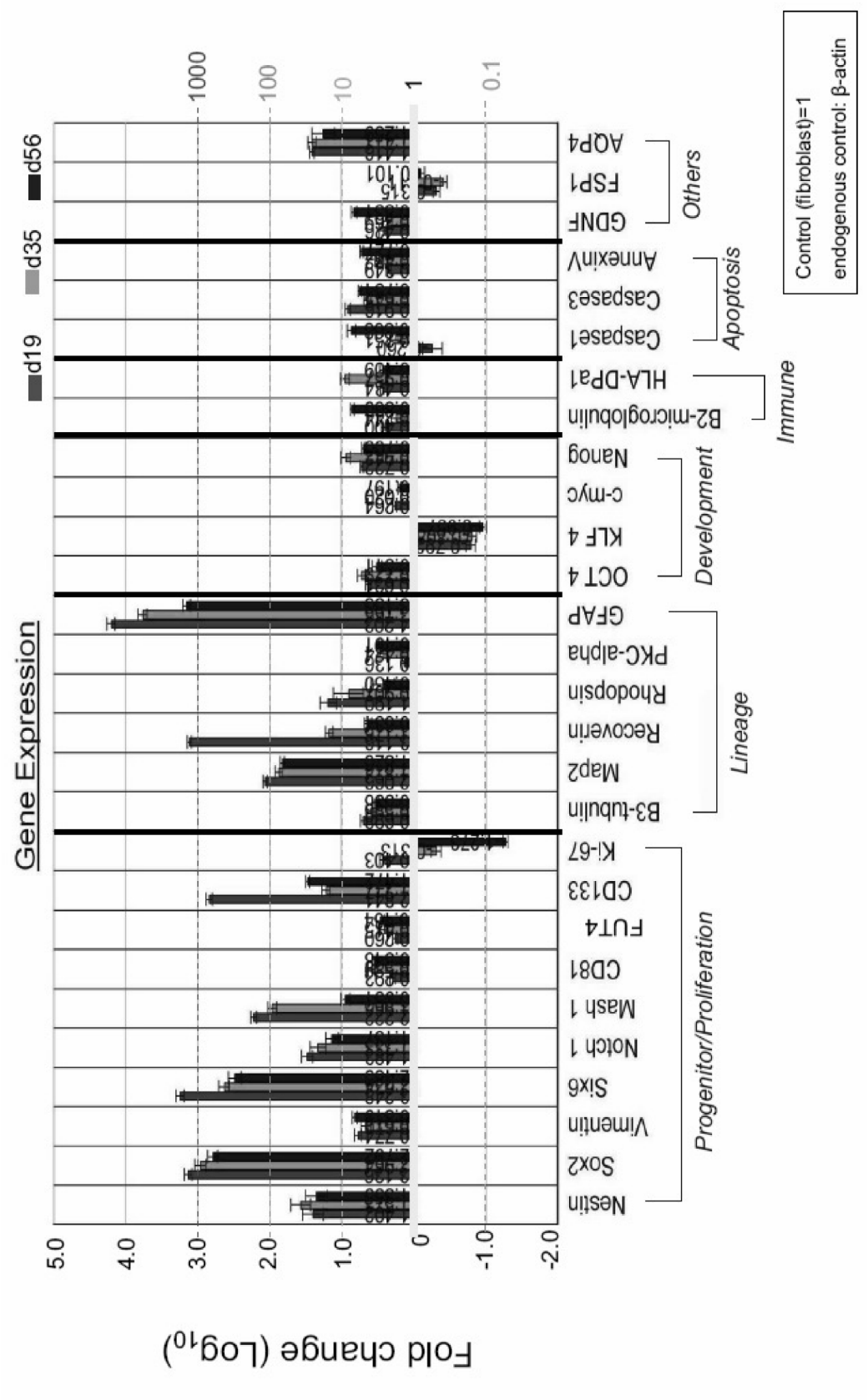

Gene Expression
Fold change (Log10)
d19
d35
d56
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
1000
100
10
1
0.1
Nestin
Sox2
Vimentin
Six6
Notch 1
Mash 1
CD81
FUT4
CD133
Ki-67
B3-tubulin
Map2
Recoverin
Rhodopsin
PKC-alpha
GFAP
OCT 4
KLF 4
c-myc
Nanog
B2-microglobulin
HLA-DPa1
Caspase1
Caspase3
AnnexinV
GDNF
FSP1
AQP4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

FIGURE 23

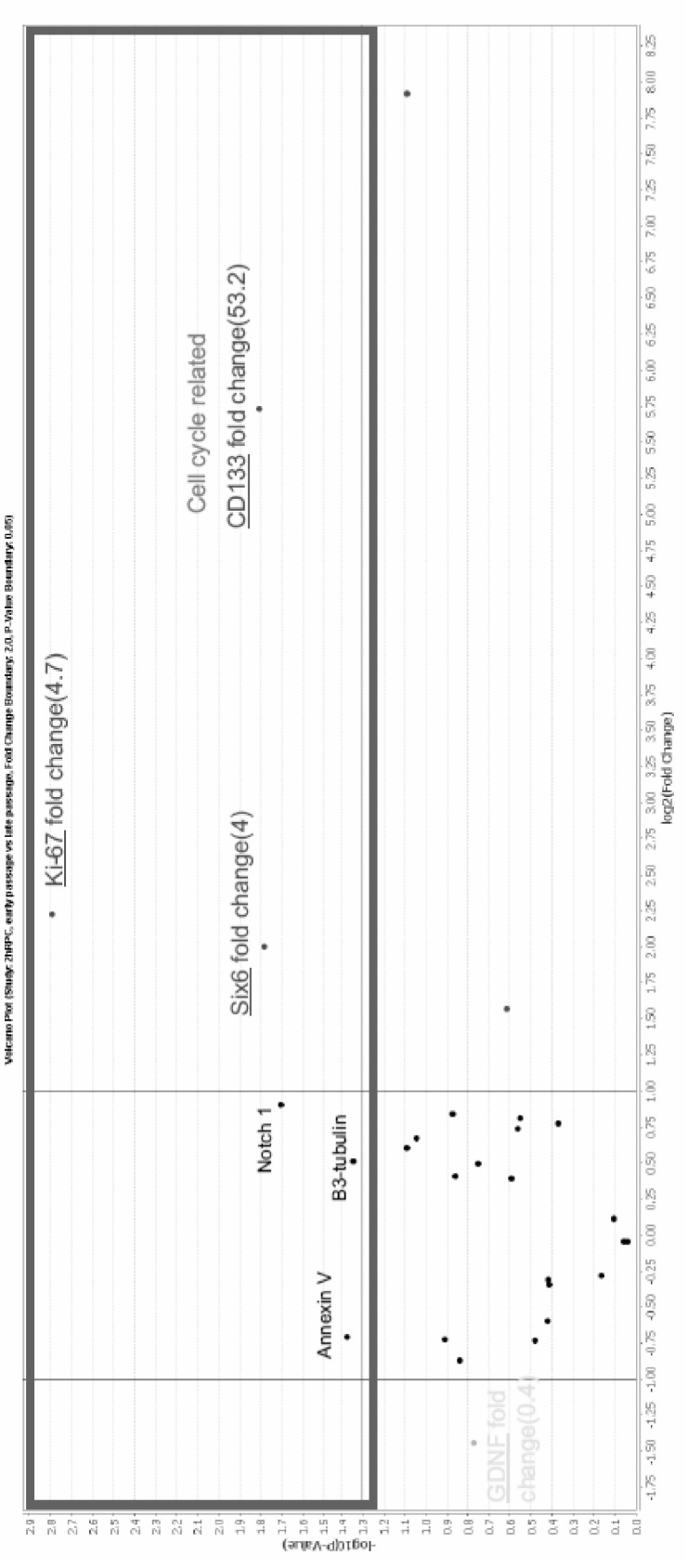

Volcano Plot (Study: 2hRPC, early passage vs late passage, Fold Change Boundary: 2.0, P-Value Boundary: 0.05)
Ki-67 fold change(4.7)
Cell cycle related
Six6 fold change(4)
CD133 fold change(53.2)
Notch 1
B3-tubulin
Annexin V
GDNF fold change(0.4)
-log10(P-Value)
log2(Fold Change)

FIGURE 24

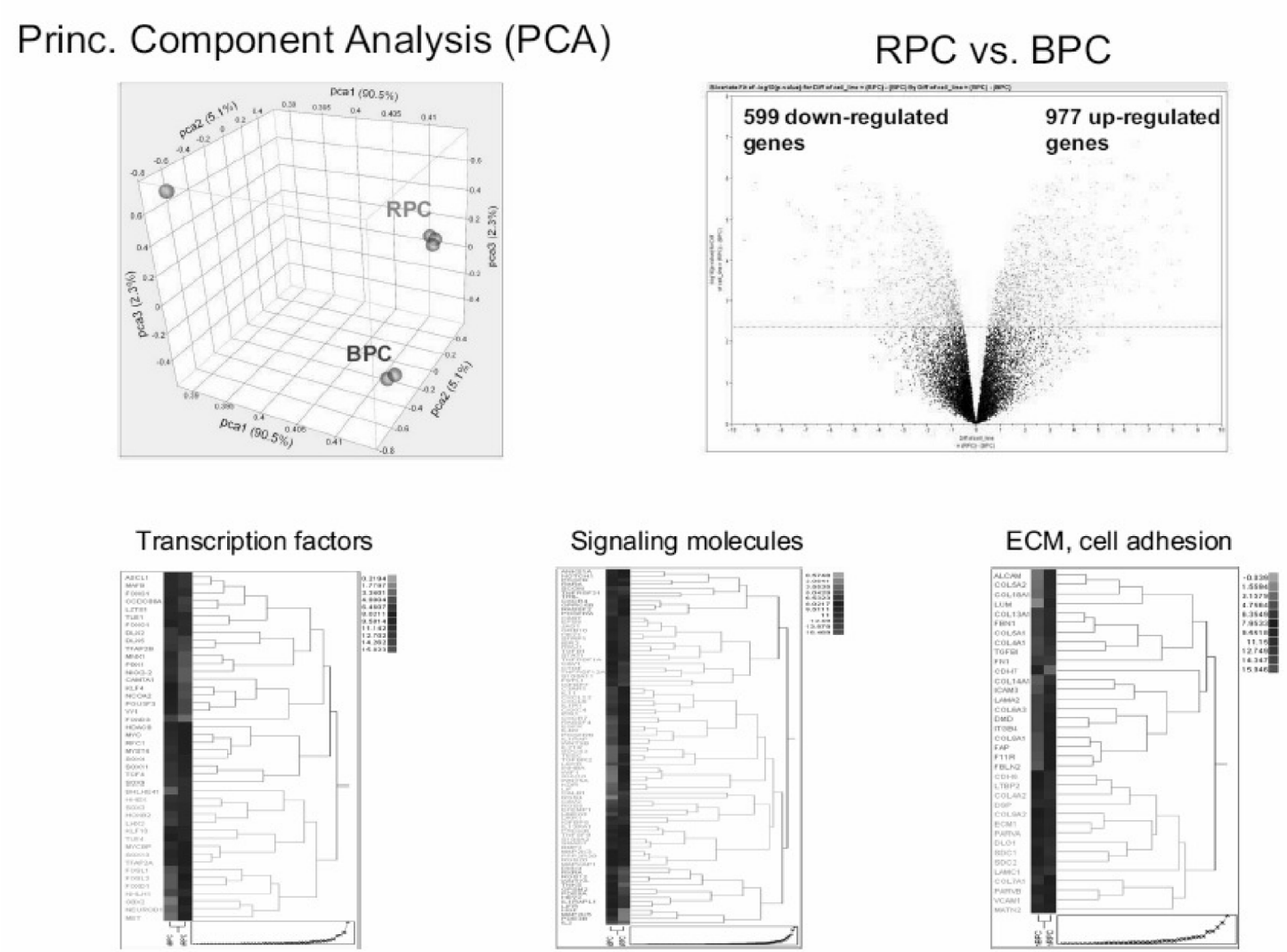

Princ. Component Analysis (PCA)
pca1 (90.5%)
pca2 (5.1%)
pca3 (2.3%)
RPC
BPC
RPC vs. BPC
599 down-regulated genes
977 up-regulated genes
Transcription factors
Signaling molecules
ECM, cell adhesion

FIGURE 25

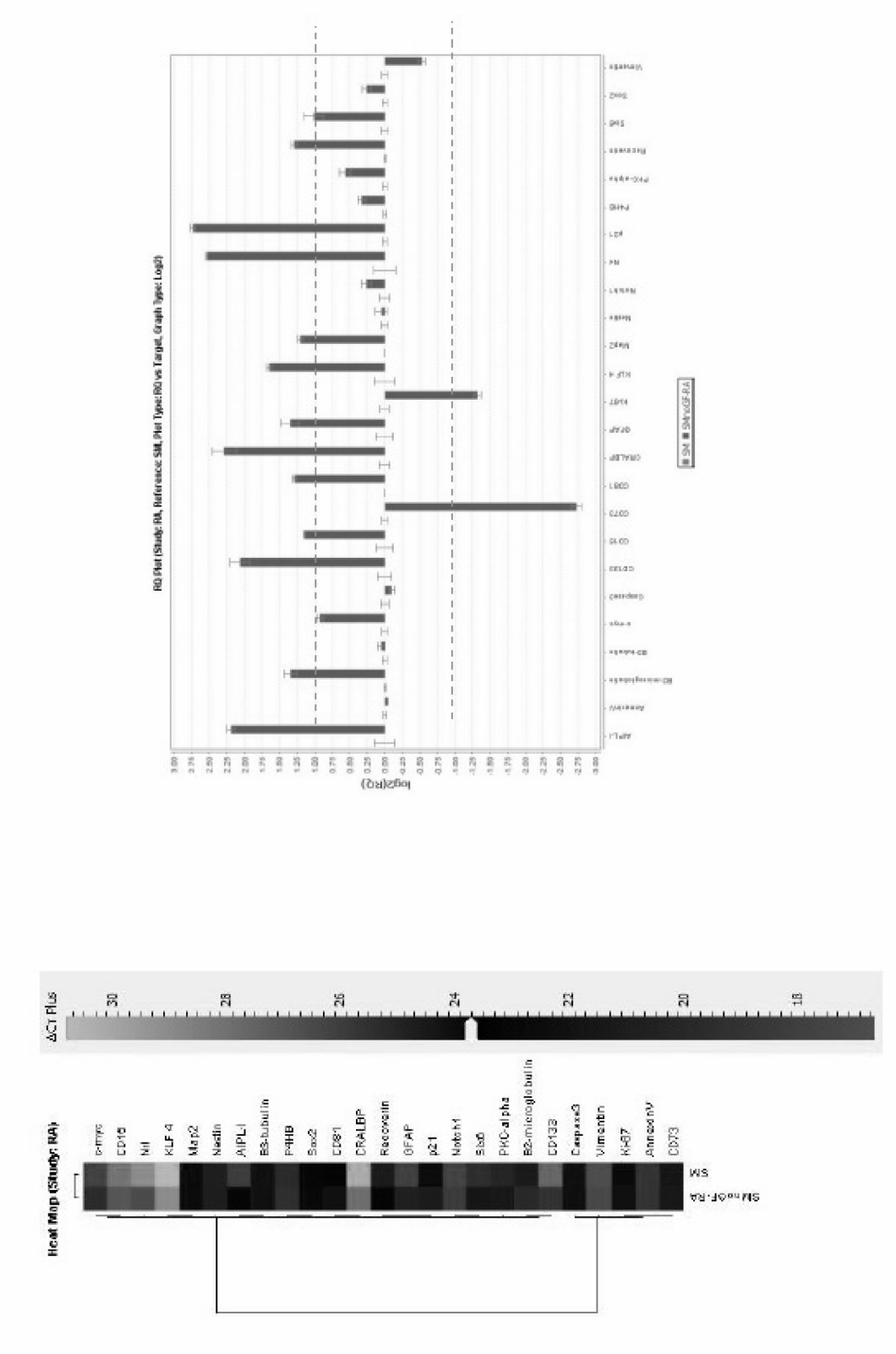

RQ Plot (Study: RA, Reference: SM, Plot Type: RQ vs Target, Graph Type: Log2)
log2(RQ)
SM
SM+hOF-RA
Heat Map (Study: RA)
ΔCT Plus
30
28
26
24
22
20
18
c-myc
CD15
Nil
KLF 4
Map2
Nestin
AIPL1
Bß-tubulin
PHB
Sox2
CD81
CRALBP
Recoverin
GFAP
p21
Notch1
Sox6
PKC-alpha
Bß-microglobulin
CD133
Caspase3
Vimentin
Ki-67
Annexin V
CD73
SM
SM+hOF-RA

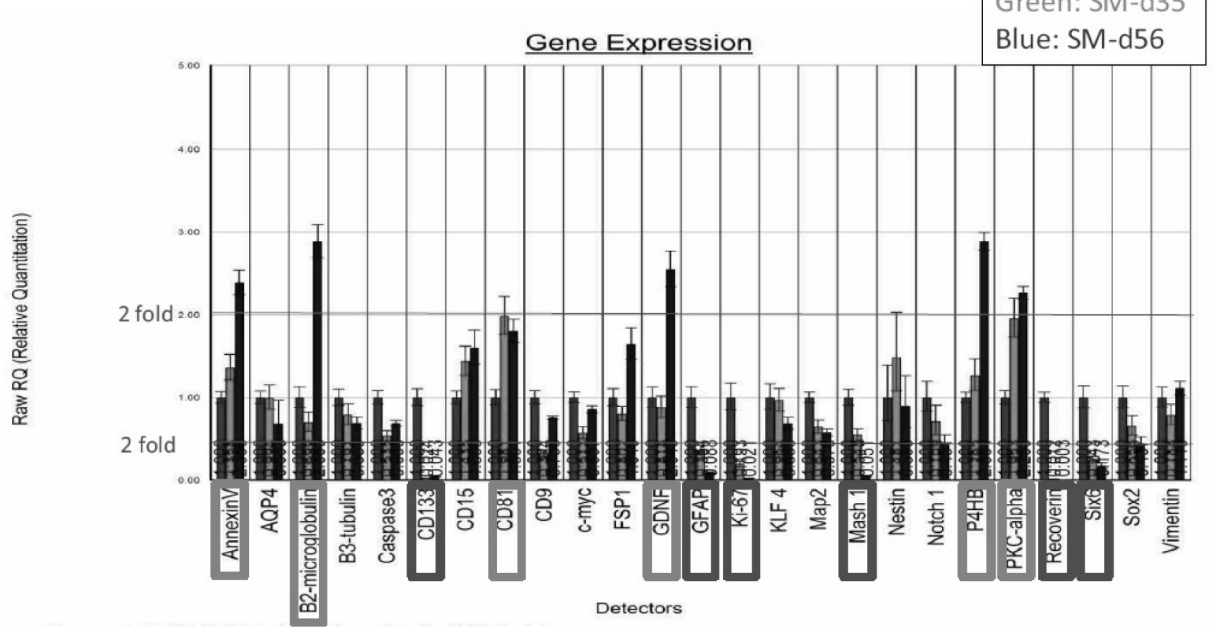
FIGURE 26

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
AnnexinV
AQP4
B2-microglobulin
B3-tubulin
Caspase3
CD133
CD15
CD81
CD9
c-myc
FSP1
GDNF
GFAP
Ki-67
KLF 4
Map2
Mash 1
Nestin
Notch 1
P4HB
PKC-alpha
Recoverin
Six6
Sox2
Vimentin

B

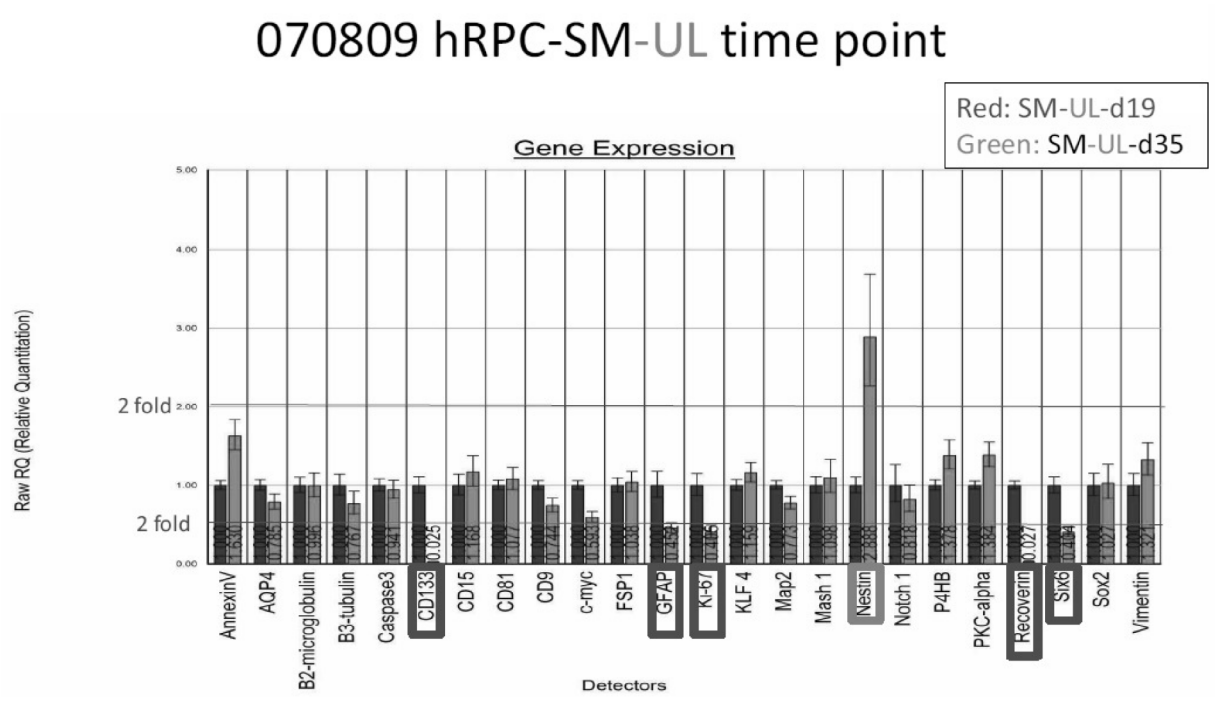
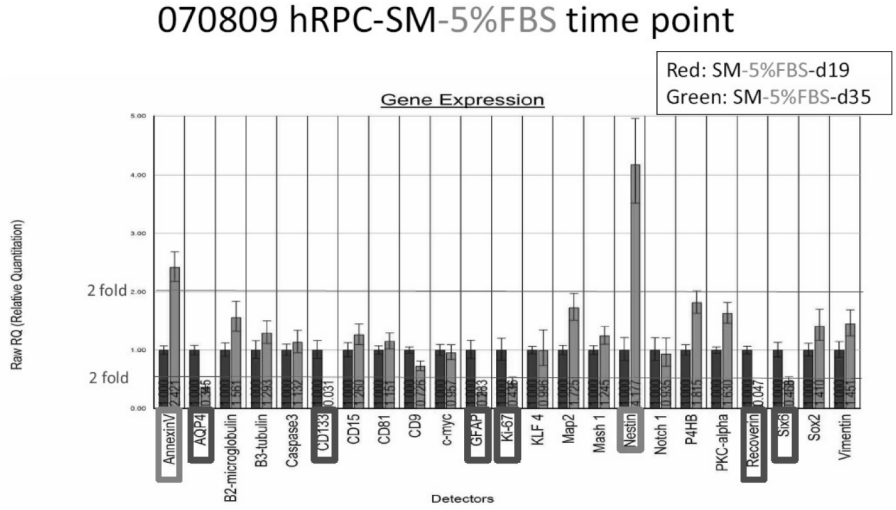
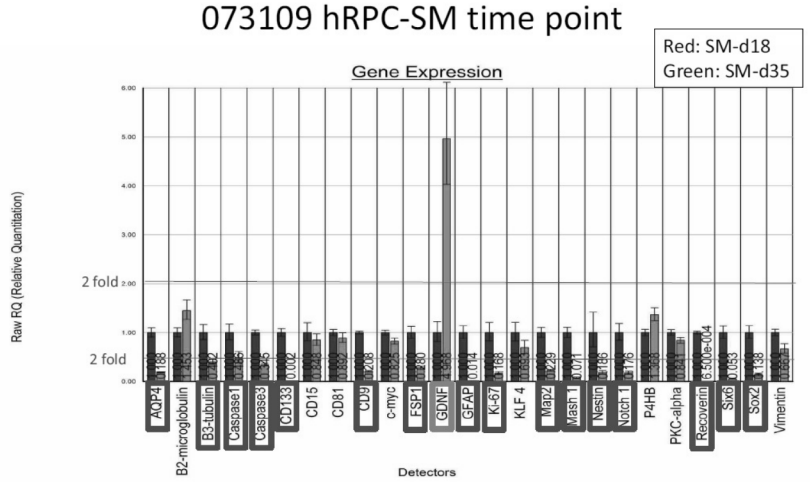

070809 hRPC-SM-UL time point
Red: SM-UL-d19
Green: SM-UL-d35
Gene Expression
Raw RQ (Relative Quantitation)
2 fold
2 fold
Detectors
AnnexinV
AQP4
B2-microglobulin
B3-tubulin
Caspase3
CD133
CD15
CD81
CD9
c-myc
FSP1
GFAP
Ki-67
KLF 4
Map2
Mash 1
Nestin
Notch 1
P4HB
PKC-alpha
Recoverin
Six6
Sox2
Vimentin

FIGURE 28

A



B



C

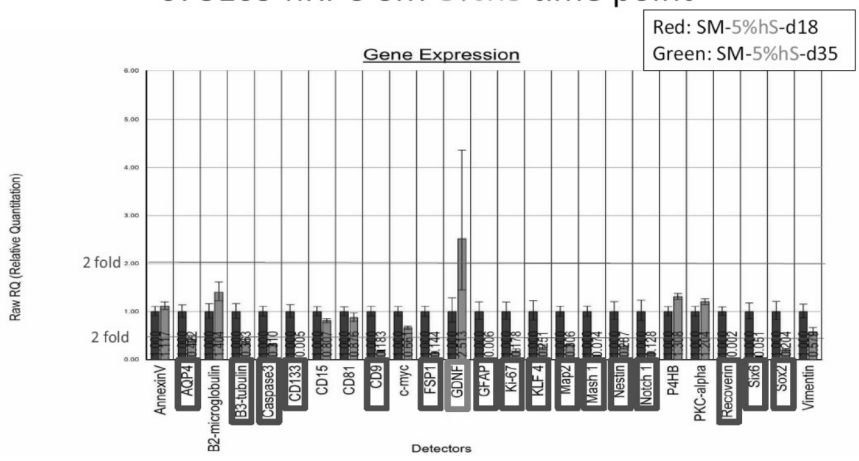


FIGURE 29

hRPC time point comparison

Red ↓ green ↑

012208hRPC		070809hRPC			073109hRPC	
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
Klf4	Klf4					Klf4
Map2	Map2				Map2	Map2
Nestin	Nestin		Nestin	Nestin	Nestin	Nestin
		Mash1			Mash1	Mash1
Notch1	Notch1				Notch1	Notch1
		P4HB				
PKC-a?		PKC-a				
Recoverin	Recoverin	Recoverin	Recoverin	Recoverin	Recoverin	Recoverin
six6	six6	Six6	Six6	Six6	Six6	Six6
Sox2	Sox2				Sox2	Sox2
vimintin	vimintin					
	CD15?					
b2-microglobulin	b2-microglobulin	b2-microglobulin				
c-myc	c-myc					
AnexinV	AnexinV	AnexinV		AnexinV		

FIGURE 30

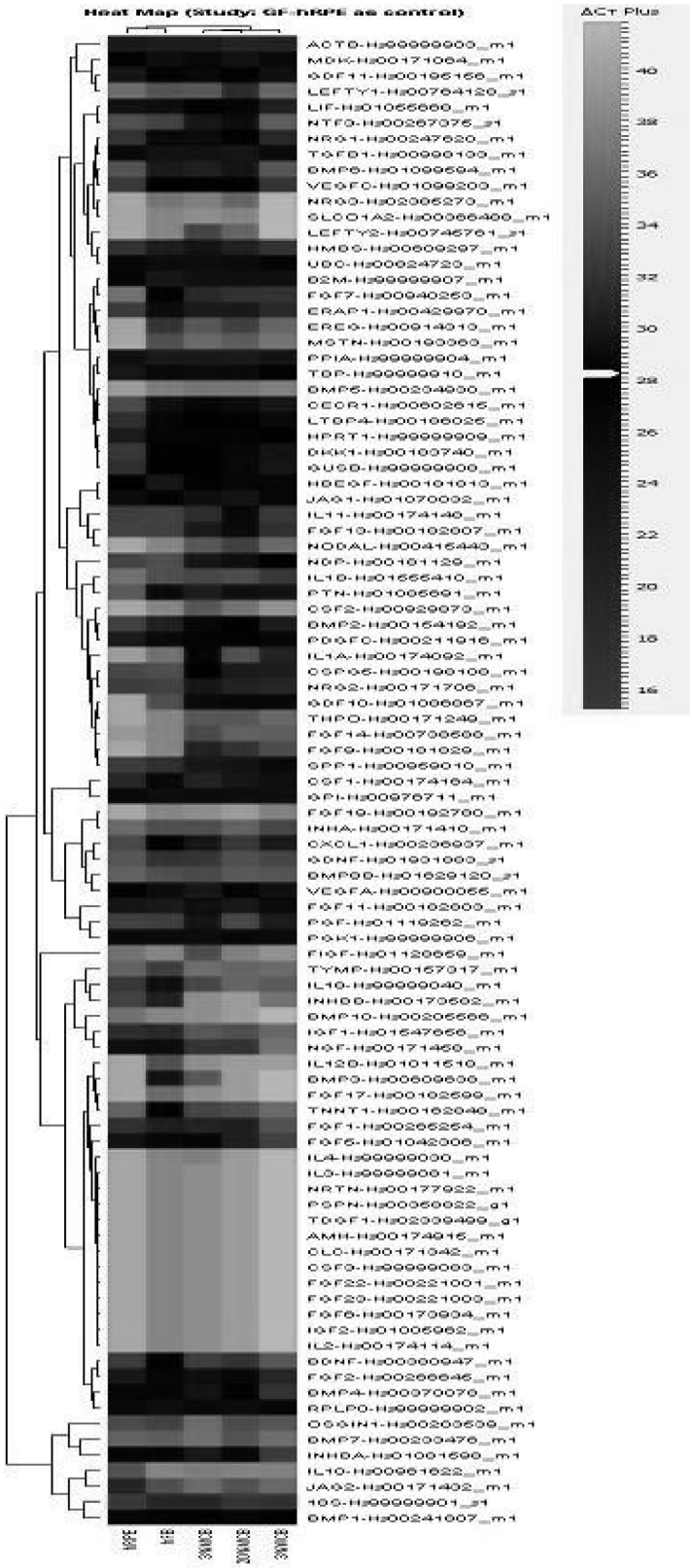

Heat Map (Study: GF-hRPE as control)
ACTB-Hs99999903_m1
MDK-Hs00171064_m1
GDF11-Hs00195156_m1
LEFTY1-Hs00764120_g1
LIF-Hs01055660_m1
NTF3-Hs00267076_g1
NR4A1-Hs00247620_m1
TGFB1-Hs00998133_m1
BMP6-Hs01099594_m1
VEGFC-Hs01099203_m1
NR4A3-Hs02386270_m1
SLC01A2-Hs00864460_m1
LEFTY2-Hs00746761_g1
HMBS-Hs00609297_m1
UBC-Hs00824723_m1
B2M-Hs99999907_m1
FGF7-Hs00940260_m1
ERAP1-Hs00429970_m1
ERCC6-Hs00914013_m1
MSTN-Hs00193963_m1
PPIA-Hs99999904_m1
TBP-Hs99999910_m1
BMP5-Hs00234033_m1
CCR1-Hs00602615_m1
LTBP4-Hs00186025_m1
HPRT1-Hs99999909_m1
DKK1-Hs00183740_m1
GUSB-Hs99999908_m1
HDGF-Hs00191613_m1
JAG1-Hs01070032_m1
IL11-Hs00174148_m1
FGF10-Hs00182807_m1
NODAL-Hs00415443_m1
NGF-Hs00181129_m1
IL1B-Hs01555410_m1
PTN-Hs01066691_m1
CSF2-Hs00929070_m1
BMP2-Hs00154192_m1
PDGFC-Hs00211918_m1
IL1A-Hs00174092_m1
CSF3R-Hs00180108_m1
NR4A2-Hs00171706_m1
GDF10-Hs01086067_m1
THPO-Hs00171249_m1
FGF14-Hs00736660_m1
FGF9-Hs00181629_m1
SPP1-Hs00959010_m1
CSF1-Hs00174164_m1
GPI-Hs00976711_m1
FGF19-Hs00192780_m1
INHA-Hs00171410_m1
CXCL1-Hs00236937_m1
GDNF-Hs01901909_g1
BMP8B-Hs01629120_g1
VEGFA-Hs00900055_m1
FGF11-Hs00182603_m1
PGF-Hs01119262_m1
PGK1-Hs99999906_m1
FIGF-Hs01120659_m1
TYMP-Hs00157017_m1
IL10-Hs00961040_m1
INHBB-Hs00170502_m1
BMP10-Hs00205566_m1
IGF1-Hs01547656_m1
NGF-Hs00171458_m1
IL12B-Hs01011518_m1
BMP3-Hs00609638_m1
FGF17-Hs00182599_m1
TNNT1-Hs00182040_m1
FGF1-Hs00266264_m1
FGF5-Hs01042006_m1
IL4-Hs99999030_m1
IL3-Hs99999001_m1
NRTN-Hs00177922_m1
PSPN-Hs00360922_g1
TGFB1-Hs02599499_g1
AMH-Hs00174915_m1
CLC-Hs00171342_m1
CSF3-Hs99999033_m1
FGF22-Hs00221001_m1
FGF20-Hs00221003_m1
FGF6-Hs00170904_m1
IGF2-Hs01005962_m1
IL2-Hs00174114_m1
BDNF-Hs00380947_m1
FGF2-Hs00266646_m1
BMP4-Hs00370078_m1
RPLP0-Hs99999902_m1
OSGIN1-Hs00205639_m1
BMP7-Hs00233476_m1
INHBA-Hs01081590_m1
IL10-Hs00961622_m1
JAG2-Hs00171402_m1
TGFB-Hs99999901_g1
BMP1-Hs00241807_m1
ACT Plus
40
38
36
34
32
30
28
26
24
22
20
18
16
3 GF/R
GF/R
3 GF/RMSC
GF/RMSC
3 GF/RMSC

FIGURE 31

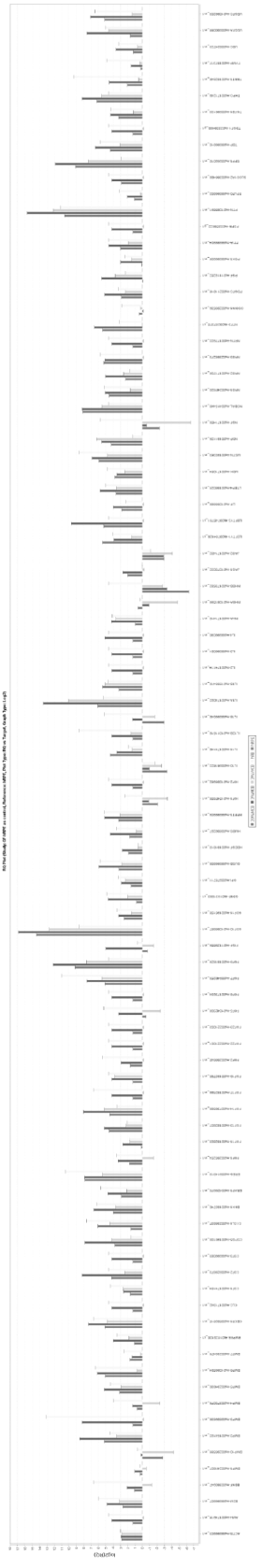
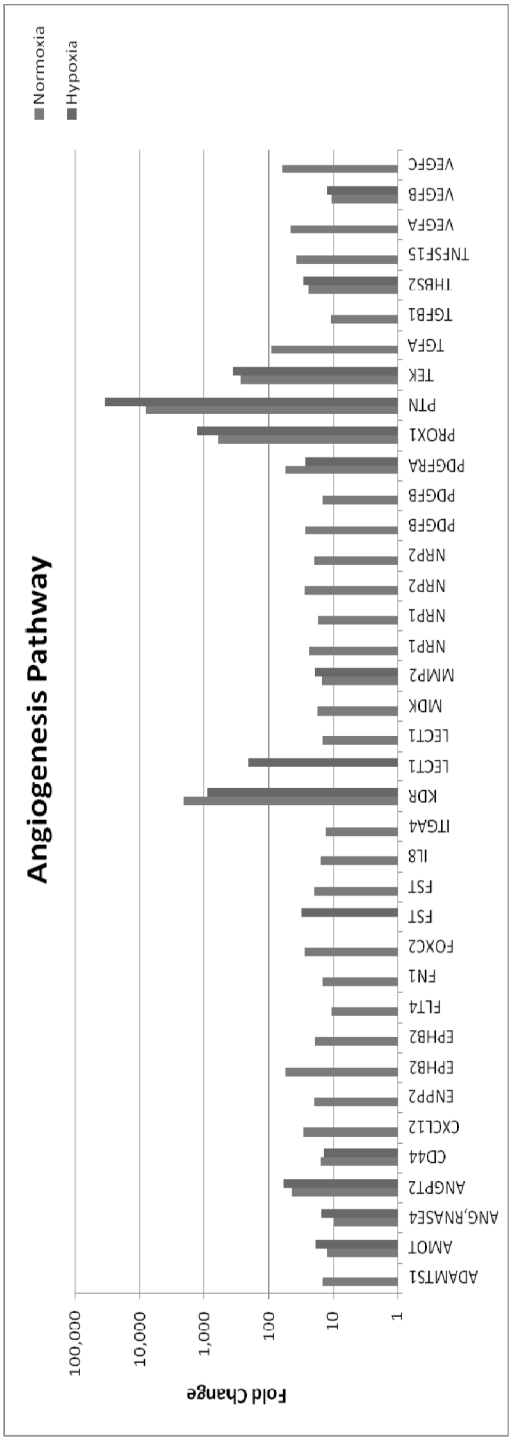


FIGURE 32


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
MMP2
NRP1
NRP1
NRP2
NRP2
PDGFB
PDGFB
PDGFRA
PROX1
PTN
TEK
TGFA
TGFB1
THBS2
TNFSF15
VEGFA
VEGFB
VEGFC

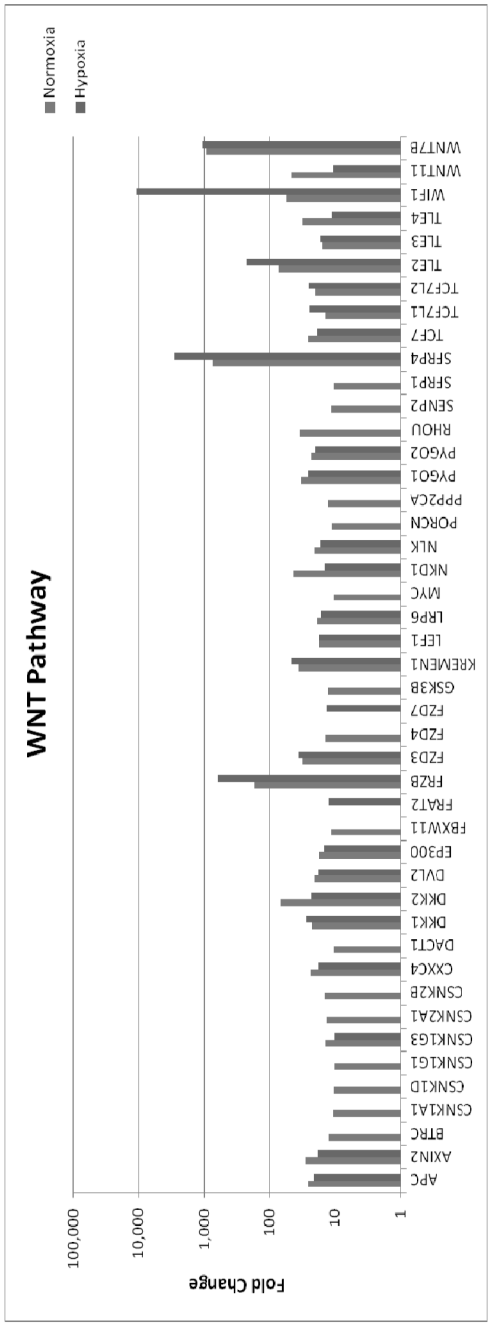
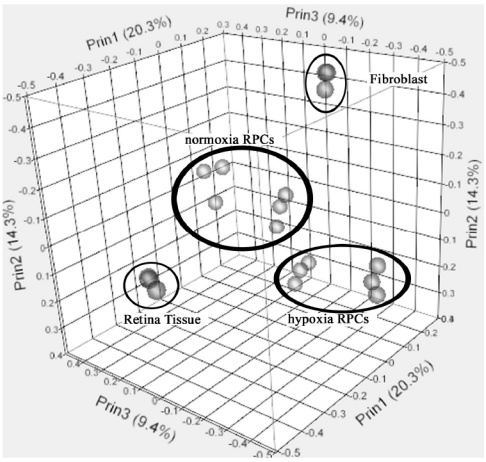
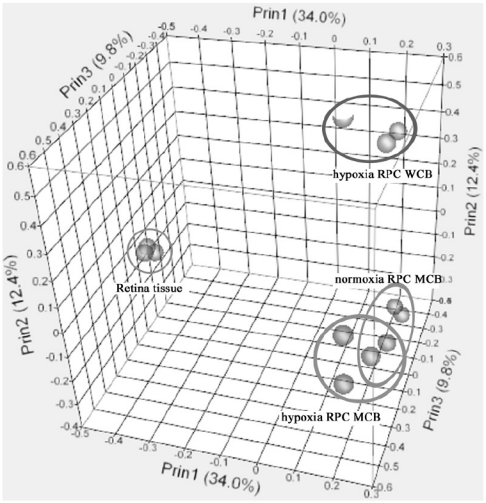

B
WNT Pathway
Normoxia
Hypoxia
Fold Change
100,000
10,000
1,000
100
10
1
APC
AXIN2
BTRC
CSNK1A1
CSNK1D
CSNK1G1
CSNK1G3
CSNK2A1
CSNK2B
CXXC4
DACT1
DKK1
DKK2
DVL2
EP300
FBXW11
FRAT2
FRZB
FZD3
FZD4
FZD7
GSK3B
KREMEN1
LEF1
LRP6
MYC
NKD1
NLK
PORCN
PPP2CA
PYGO1
PYGO2
RHOU
SENP2
SFRP1
SFRP4
TCF7
TCF7L1
TCF7L2
TLE2
TLE3
TLE4
WIF1
WNT11
WNT7B

FIGURE 33

A



B



C

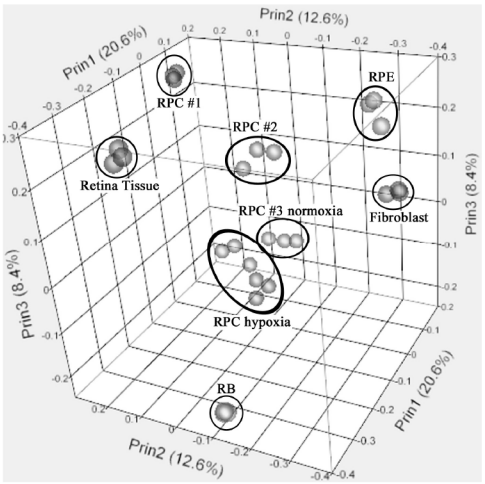


FIGURE 34

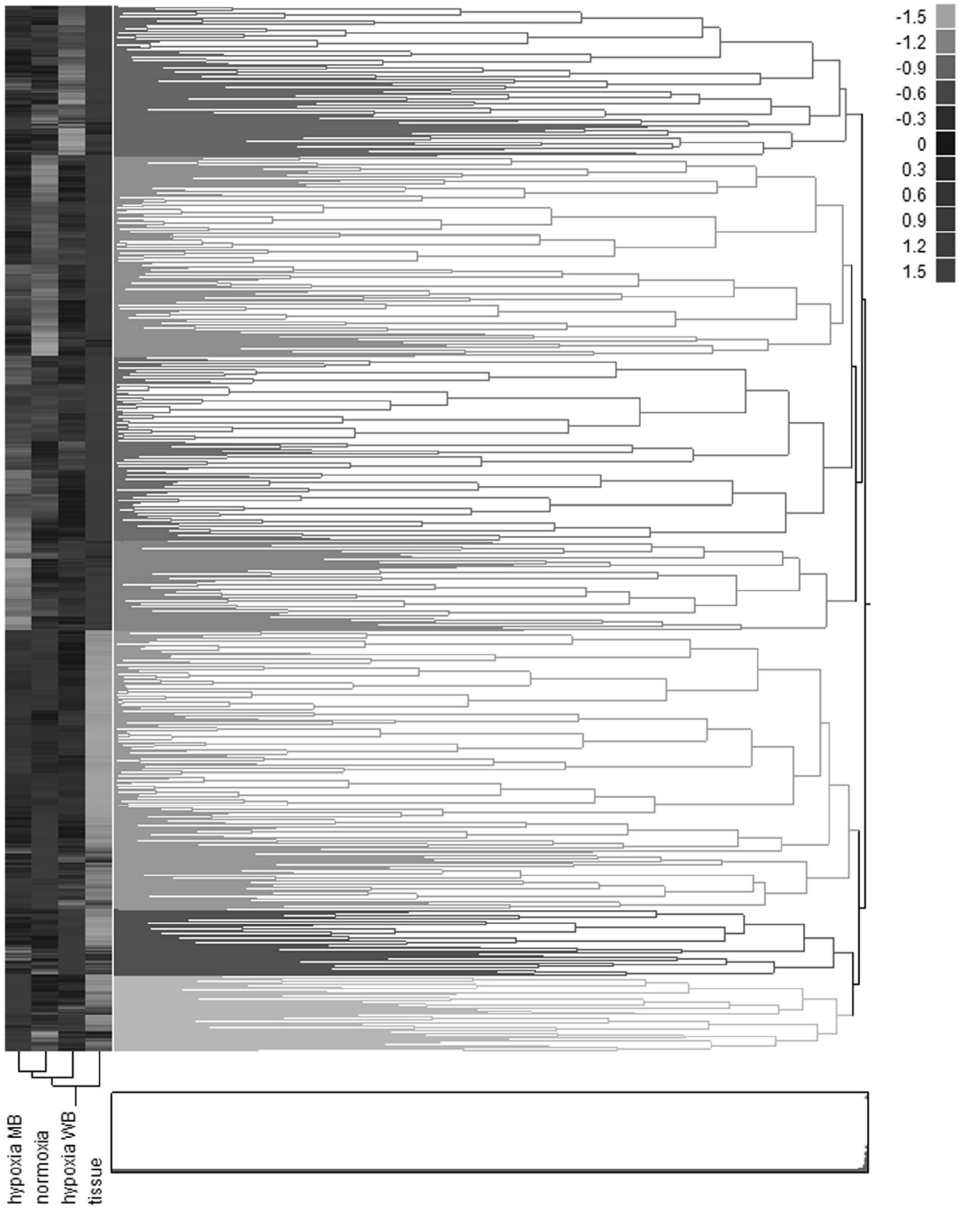

-1.5
-1.2
-0.9
-0.6
-0.3
0
0.3
0.6
0.9
1.2
1.5
hypoxia MB
normoxia
hypoxia WB
tissue

FIGURE 35

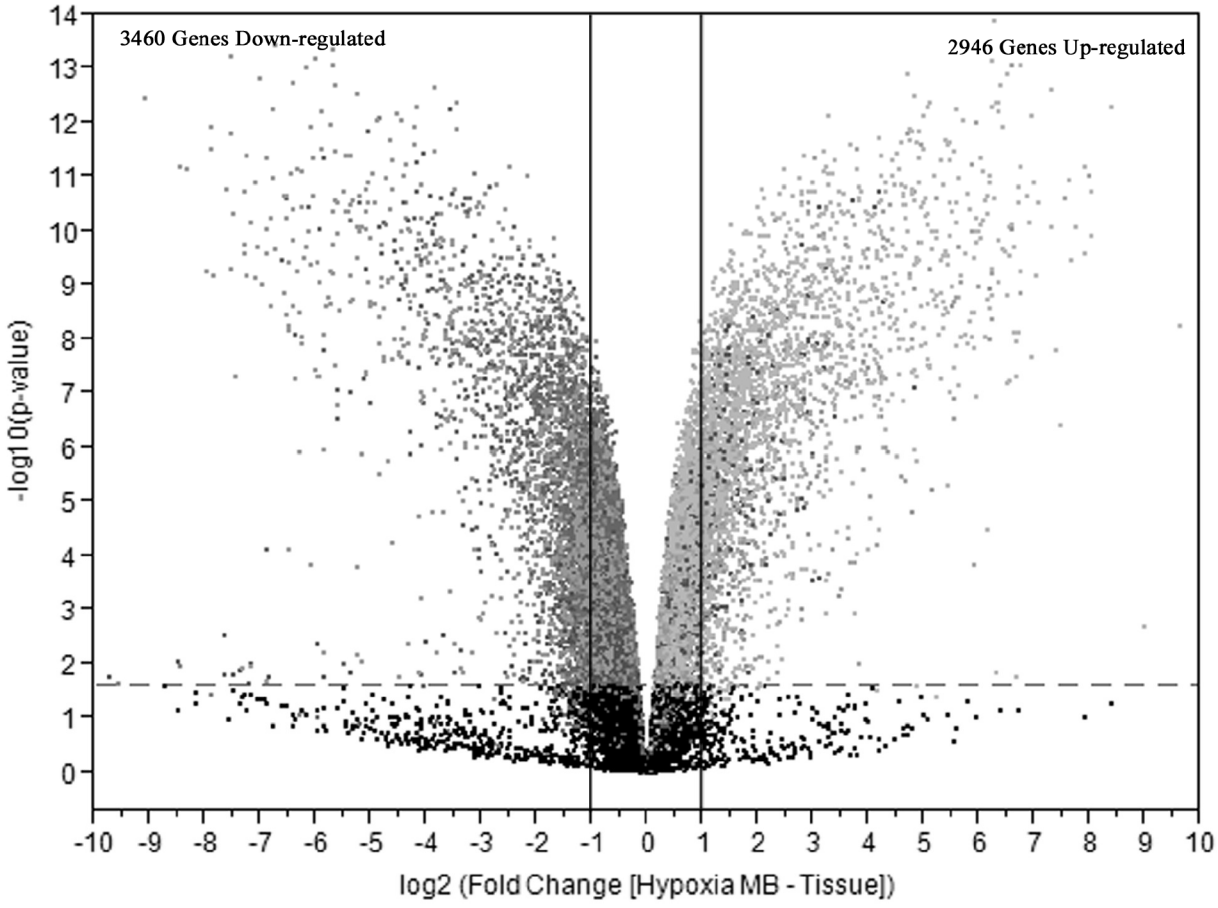
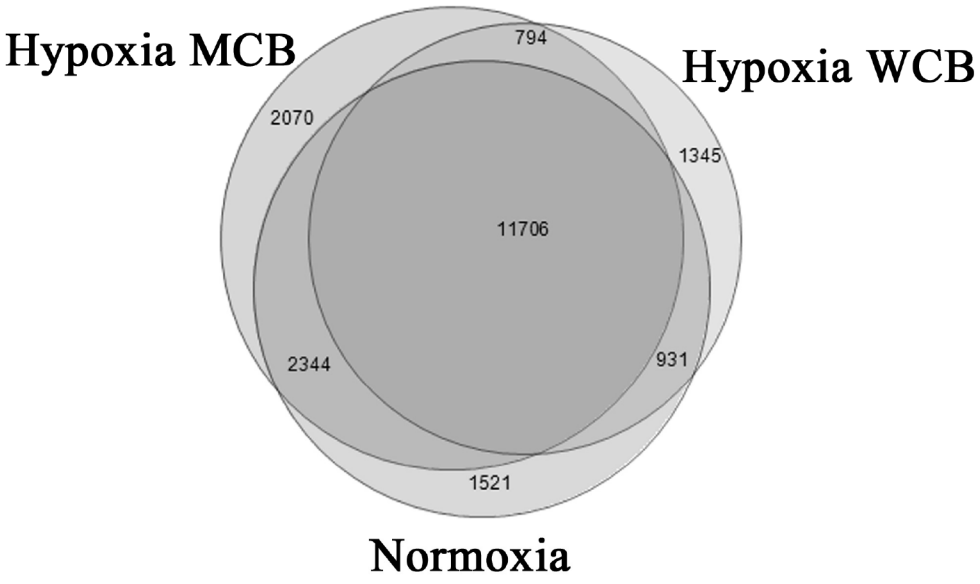

3460 Genes Down-regulated
2946 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Hypoxia MB - Tissue])
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10

FIGURE 36

A


Hypoxia MCB
Hypoxia WCB
Normoxia
794
2070
1345
11706
2344
931
1521

B

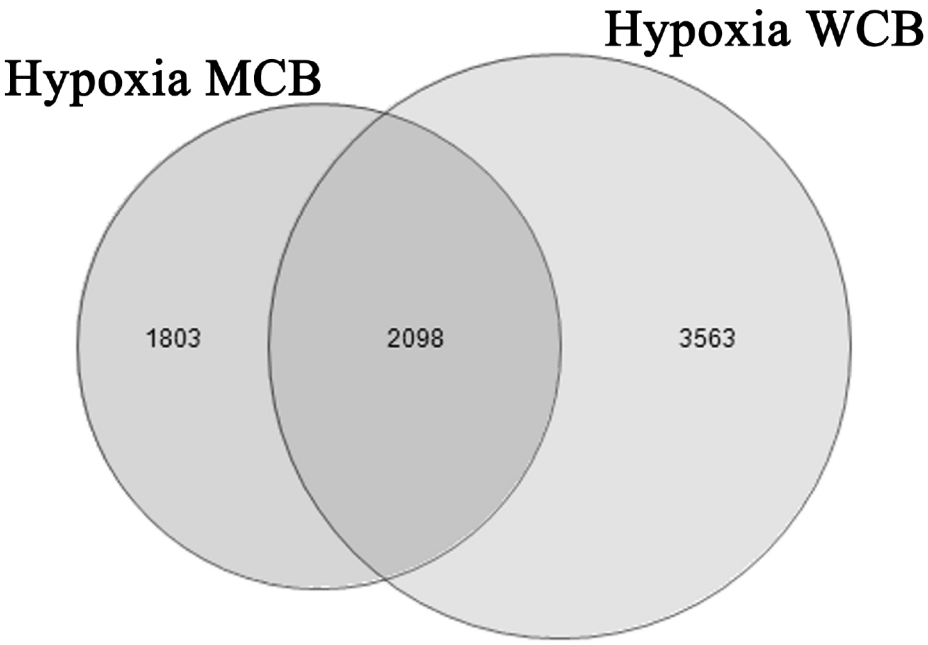
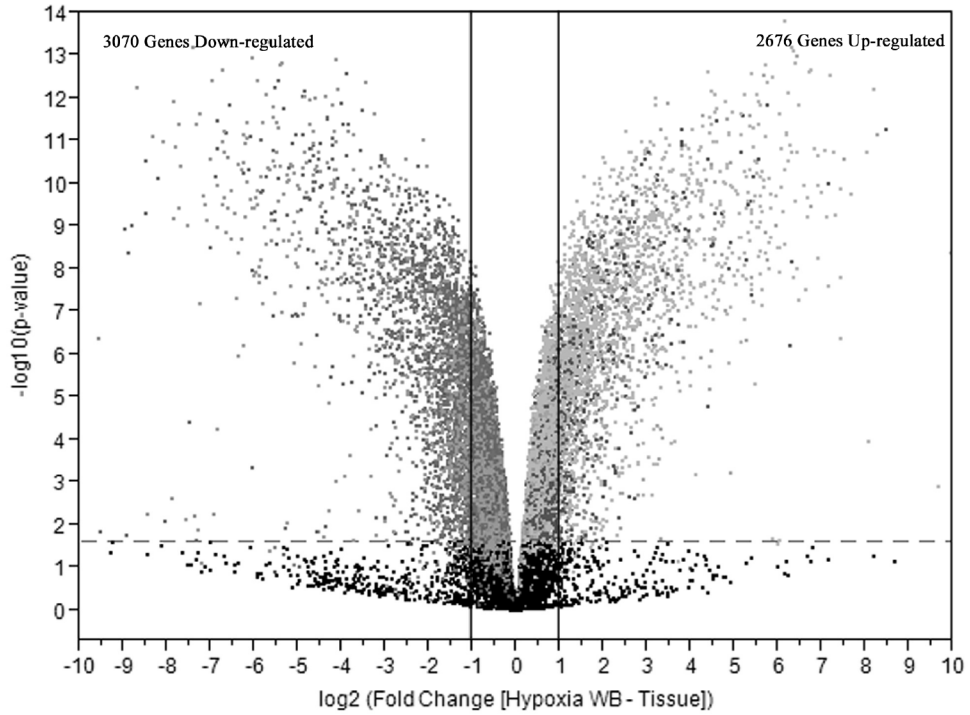

Hypoxia WCB
Hypoxia MCB
1803
2098
3563

FIGURE 37

A


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

B

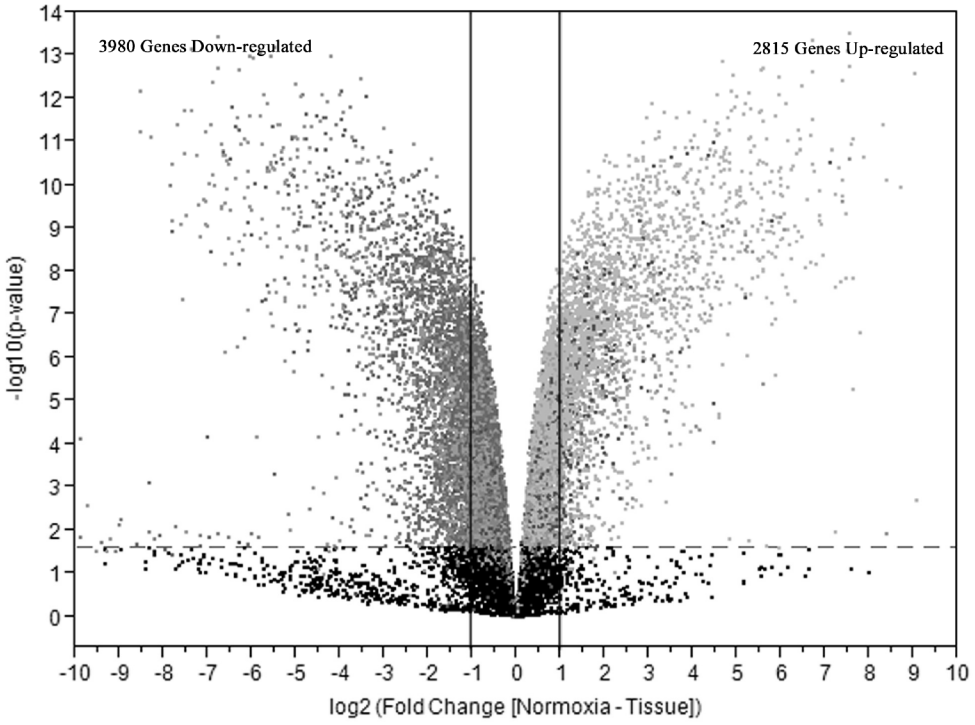
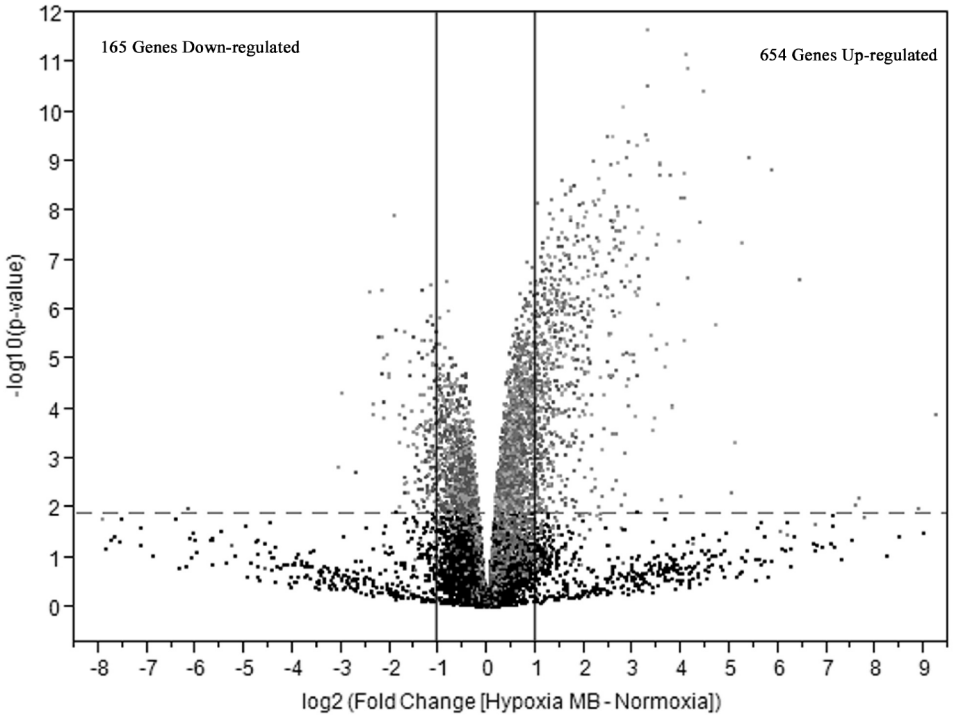

3980 Genes Down-regulated
2815 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Normoxia - Tissue])

FIGURE 38

A


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

B

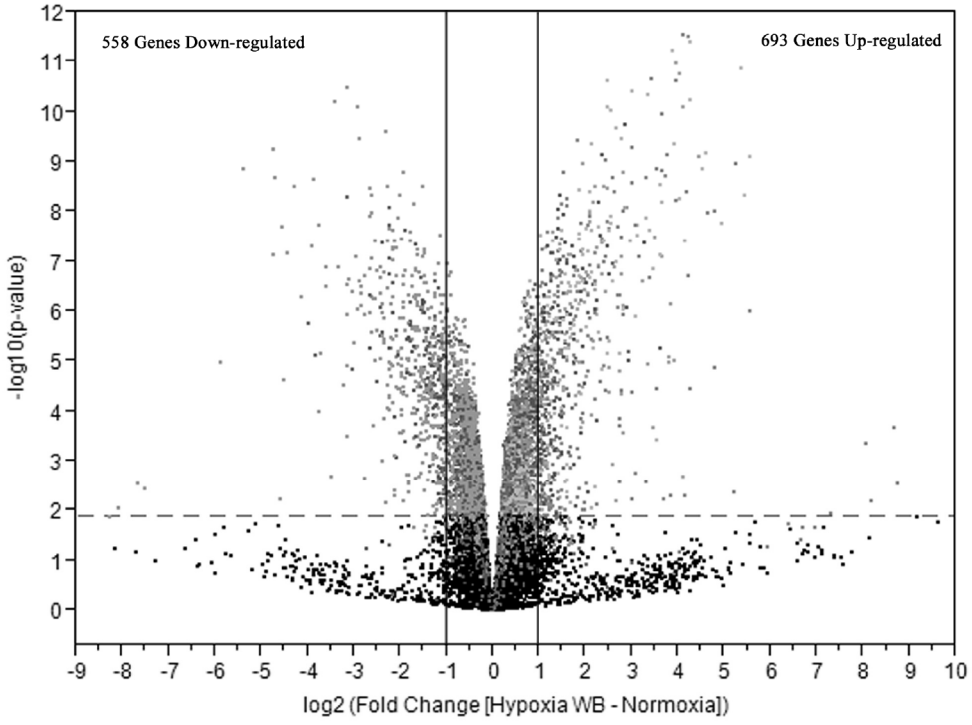

558 Genes Down-regulated
693 Genes Up-regulated
-log10(p-value)
log2 (Fold Change [Hypoxia WB - Normoxia])

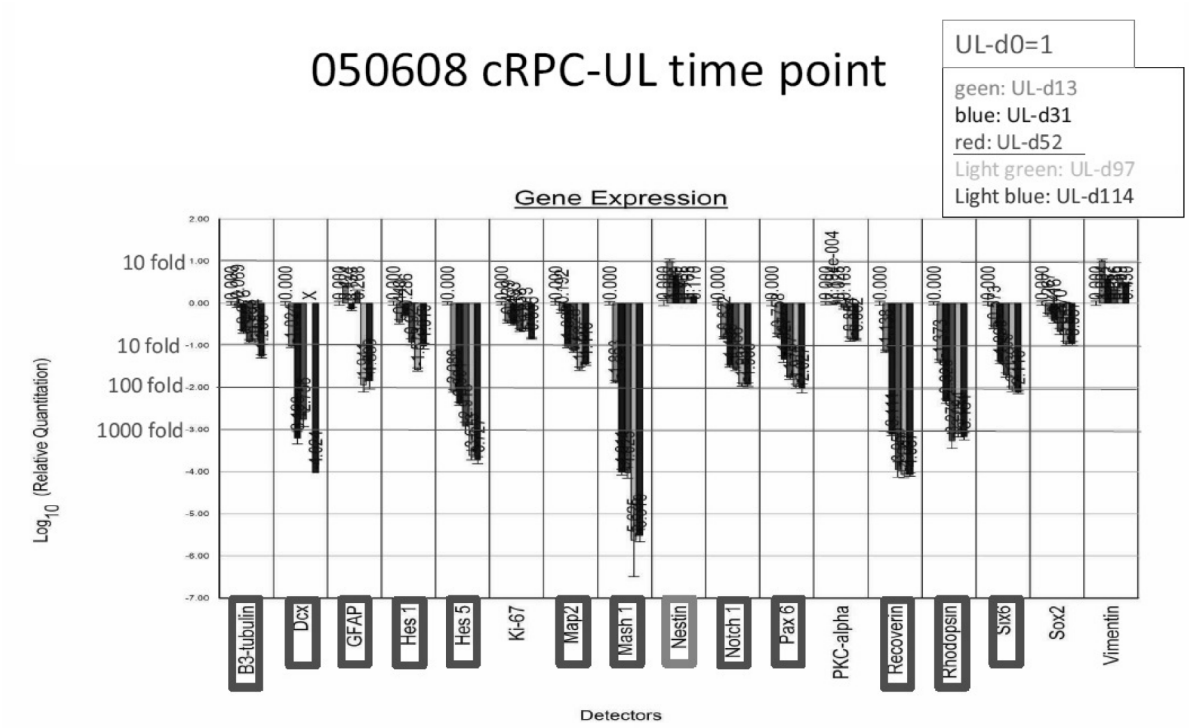
FIGURE 39

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
geen: UL-d13
blue: UL-d31
red: UL-d52
Light green: UL-d97
Light blue: UL-d114
Gene Expression
10 fold
10 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
B3-tubulin
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Mash 1
Nestin
Notch 1
Pax 6
PKC-alpha
Recoverin
Rhodopsin
Six6
Sox2
Vimentin
Detectors

B

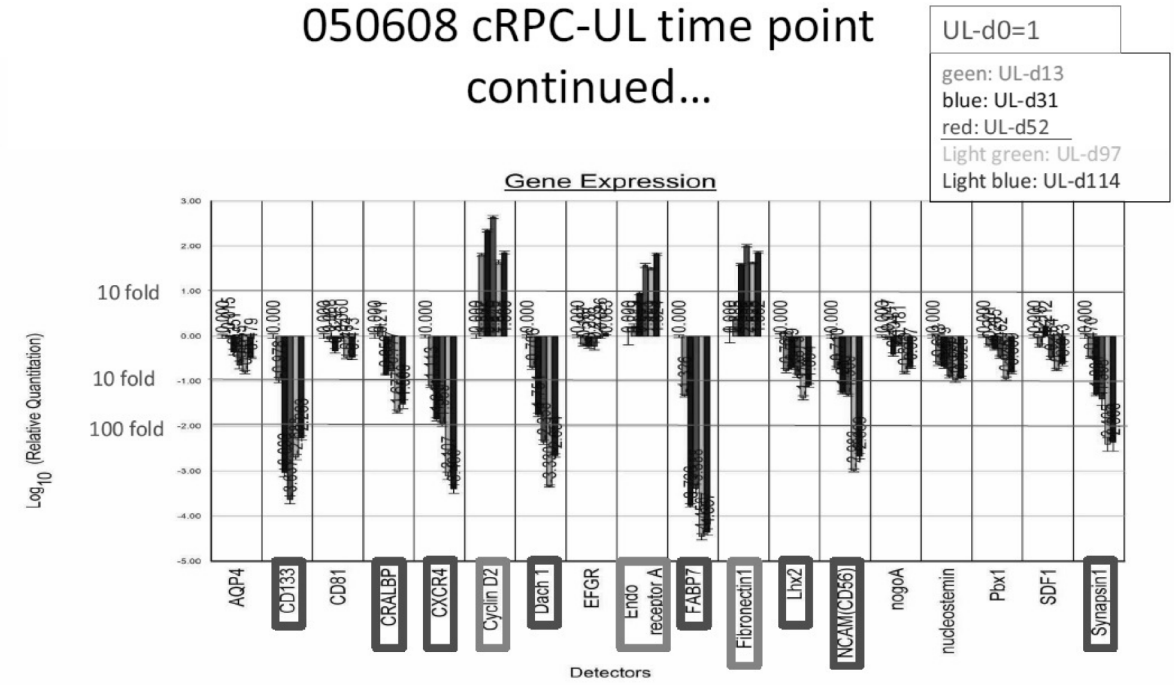
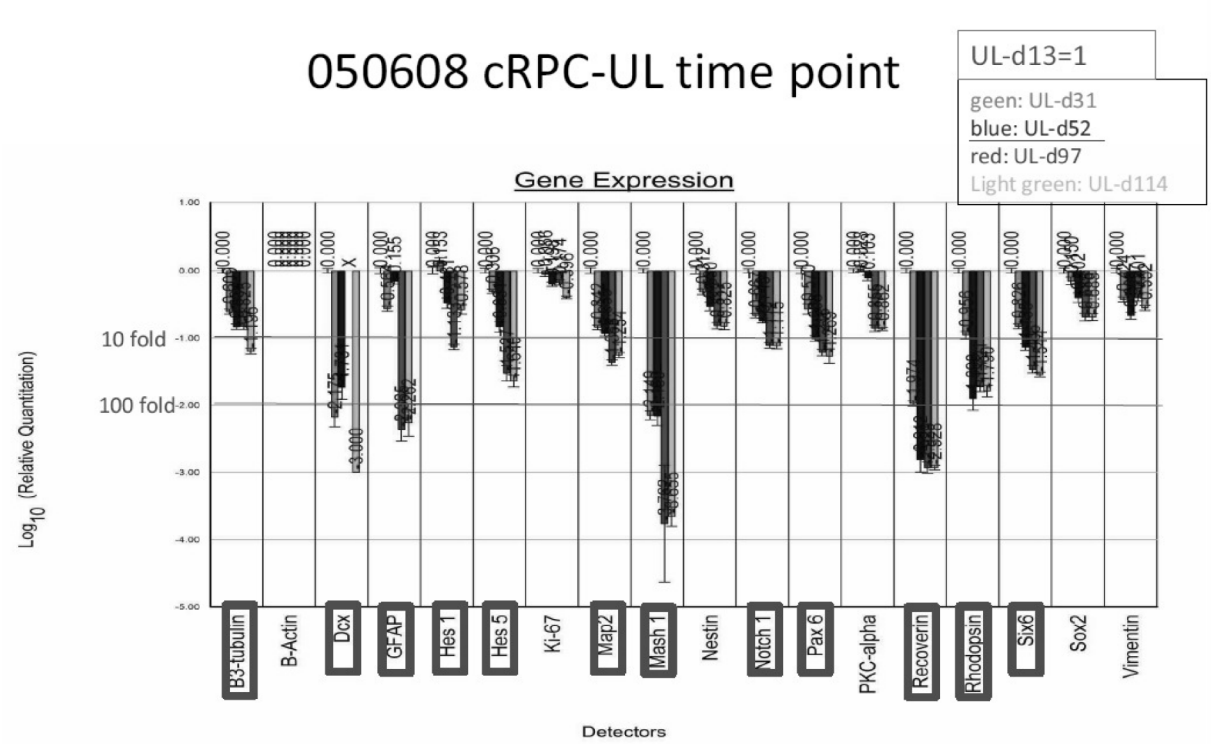

050608 cRPC-UL time point continued...
UL-d0=1
geen: UL-d13
blue: UL-d31
red: UL-d52
Light green: UL-d97
Light blue: UL-d114
Gene Expression
10 fold
10 fold
100 fold
Log10 (Relative Quantitation)
AQP4
CD133
CD81
CRALBP
CXCR4
Cyclin D2
Dach 1
EFGR
Endo receptor A
FABP7
Fibronectin1
Lhx2
NCAM(CD56)
nogoA
nucleostemin
Pbx1
SDF1
Synapsin1
Detectors

FIGURE 41

A


050608 cRPC-UL time point
UL-d13=1
geen: UL-d31
blue: UL-d52
red: UL-d97
Light green: UL-d114
Gene Expression
10 fold
100 fold
Log10 (Relative Quantitation)
B3-tubulin
B-Actin
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Mash 1
Nestin
Notch 1
Pax 6
PKC-alpha
Recoverin
Rhodopsin
Six6
Sox2
Vimentin
Detectors

B

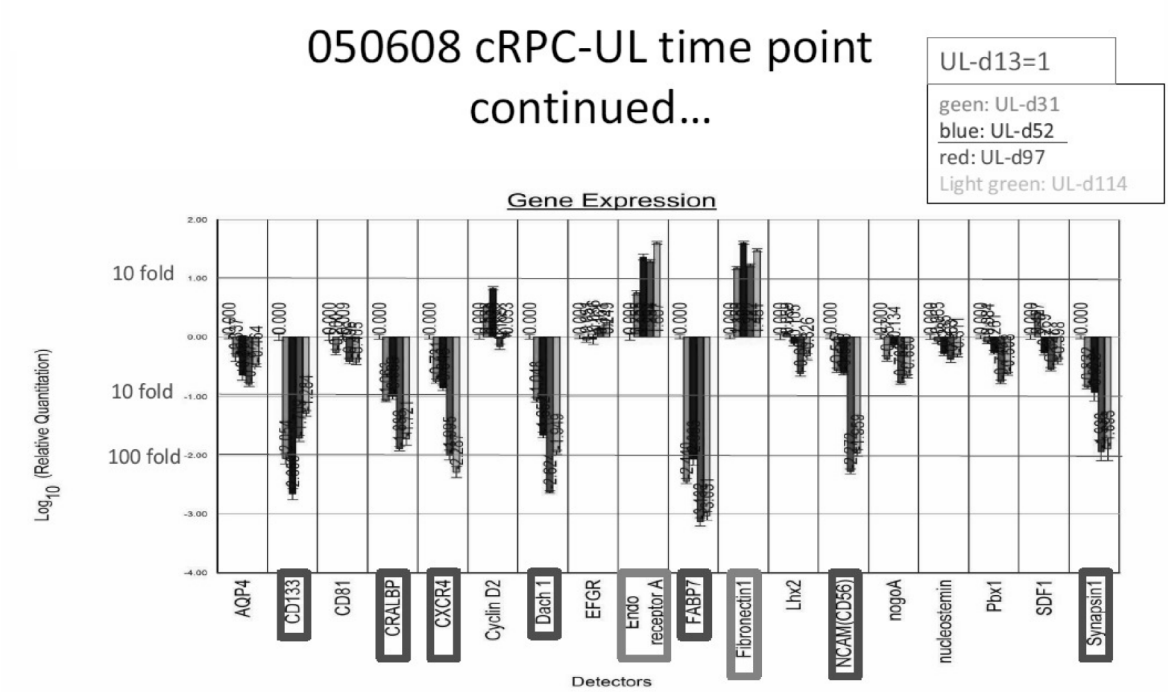
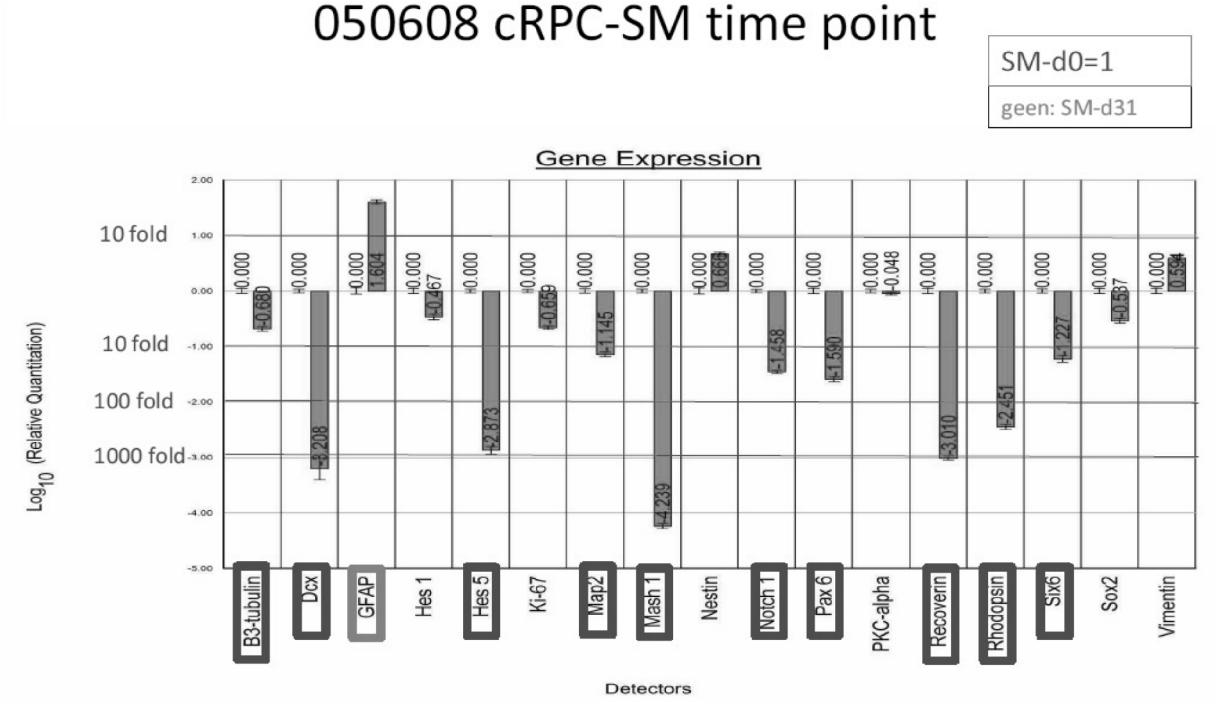

050608 cRPC-UL time point continued...
UL-d13=1
geen: UL-d31
blue: UL-d52
red: UL-d97
Light green: UL-d114
Gene Expression
10 fold
10 fold
100 fold
Log10 (Relative Quantitation)
AQP4
CD133
CD81
CRALBP
CXCR4
Cyclin D2
Dach 1
EFGR
Endo receptor A
FABP7
Fibronectin1
Lhx2
NCAM(CD56)
nogoA
nucleostemin
Pbx1
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)
B3-tubulin
Dcx
GFAP
Hes 1
Hes 5
Ki-67
Map2
Mash 1
Nestin
Notch 1
Pax 6
PKC-alpha
Recoverin
Rhodopsin
Six6
Sox2
Vimentin
Detectors

B

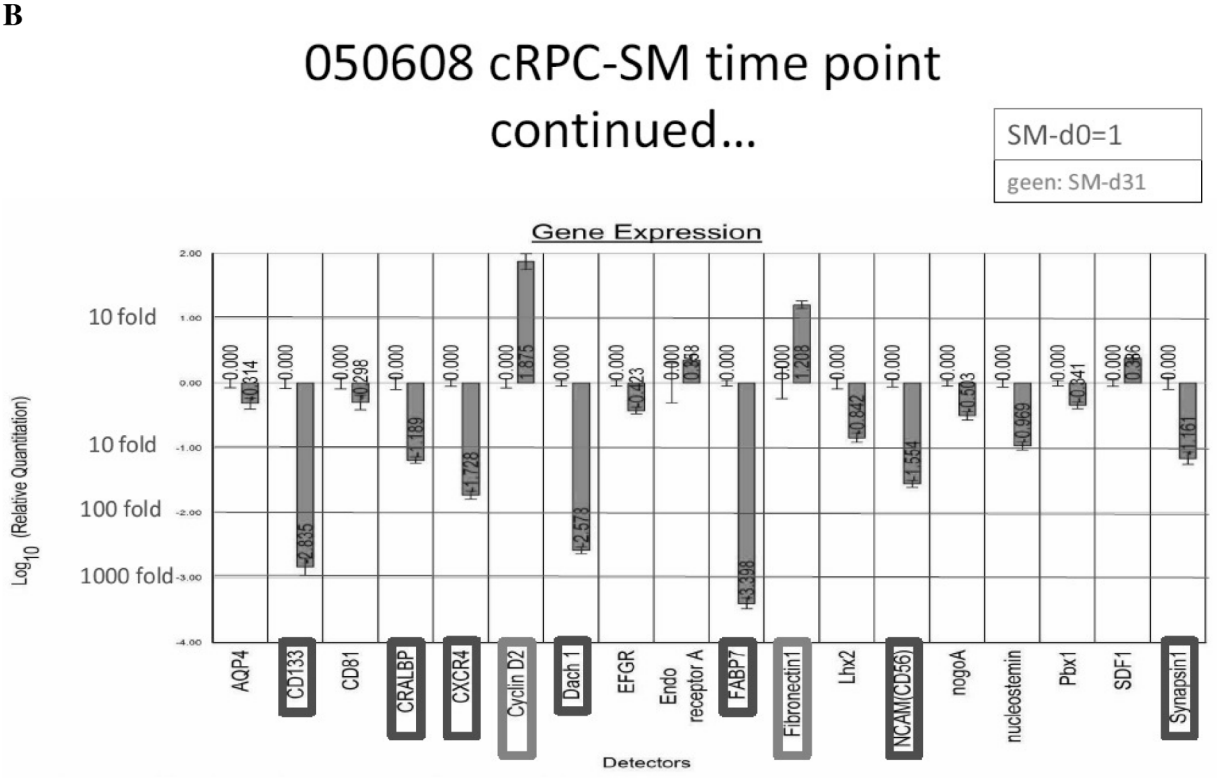

050608 cRPC-SM time point
continued...
SM-d0=1
geen: SM-d31
Gene Expression
10 fold
10 fold
100 fold
1000 fold
Log10 (Relative Quantitation)
AQP4
CD133
CD81
CRALBP
CXCR4
Cyclin D2
Dach 1
EFGR
Endo receptor A
FABP7
Fibronectin1
Lhx2
NCAM(CD56)
nogoA
nucleostemin
Pbx1
SDF1
Synapsin1
Detectors

FIGURE 43

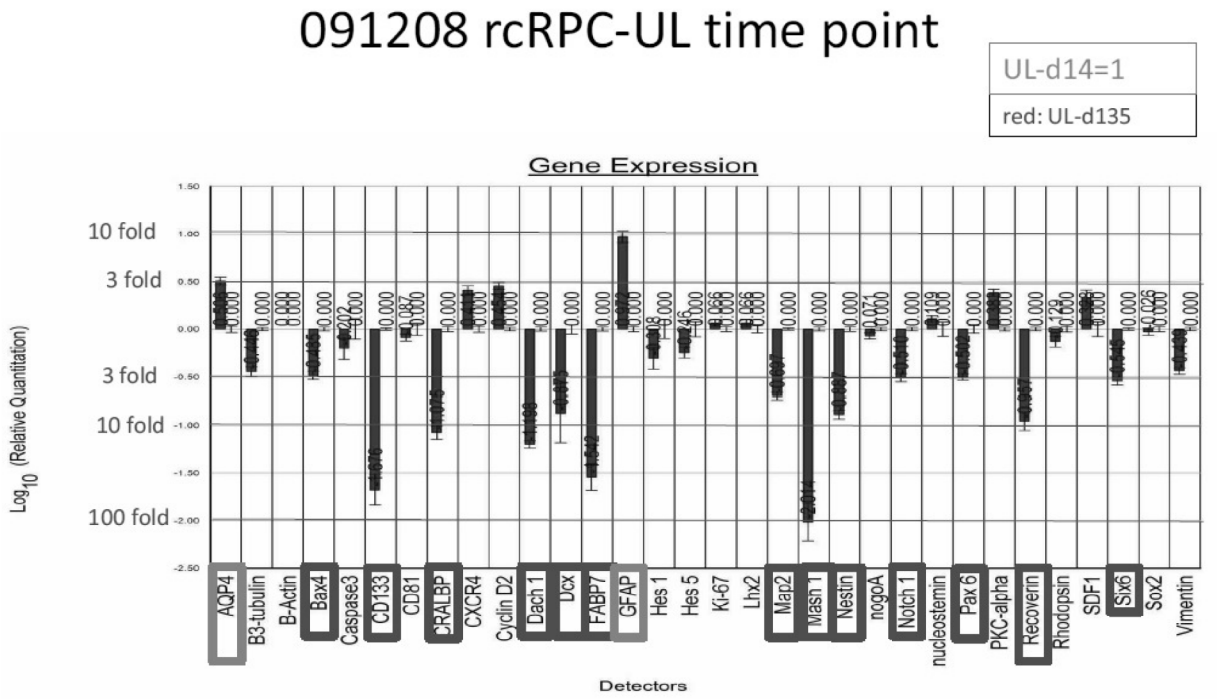

091208 rcRPC-UL time point
UL-d14=1
red: UL-d135
Gene Expression
10 fold
3 fold
3 fold
10 fold
100 fold
Log10 (Relative Quantitation)
AQP4
B3-tubulin
B-Actin
Bax4
Caspase3
CD133
CD81
CRALBP
CXCR4
Cyclin D2
Dach 1
Dcx
FABP7
GFAP
Hes 1
Hes 5
Ki-67
Lhx2
Map2
Masn 1
Nestin
nogoA
Notch 1
nucleostemin
Pax 6
PKC-alpha
Recoverin
Rhodopsin
SDF1
Six6
Sox2
Vimentin
Detectors

FIGURE 44

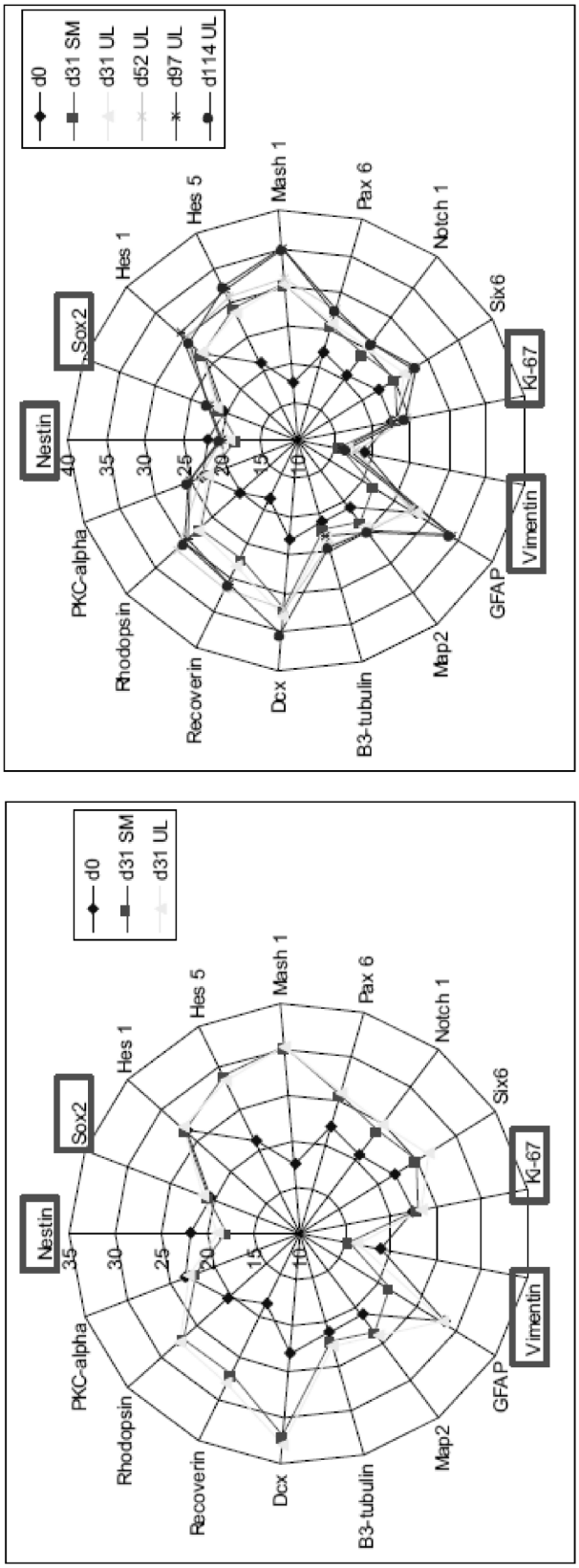

cRPC time point
d0
d31 SM
d31 UL
d52 UL
d97 UL
d114 UL
Nestin
Sox2
Hes 1
Hes 5
Mash 1
Pax 6
Notch 1
Six6
Ki-67
Vimentin
GFAP
Map2
B3-tubulin
Dcx
Recoverin
Rhodopsin
PKC-alpha
5
10
15
20
25
30
35
40

FIGURE 45

050608cRPC-UL		050608cRPC-SM	091208rcRPC-UL	consistent with hRPC
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	
Lhx2	Lhx2	Lhx2	Lhx2	
NCAM	NCAM	NCAM	NCAM	
Synapsin1	Synapsin1	Synapsin1	Synapsin1	
			Bax4	

FIGURE 46

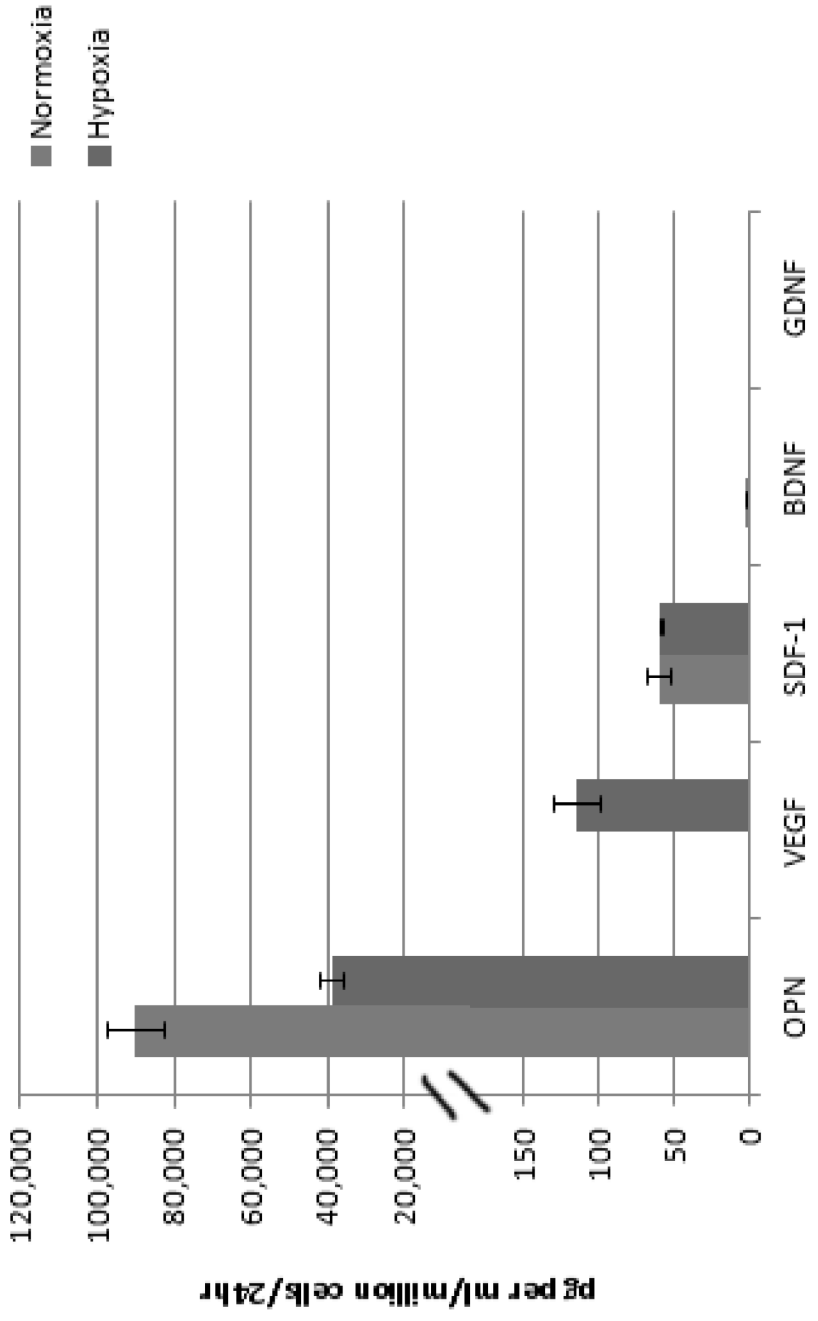

Normoxia
Hypoxia
pg per ml/million cells/24hr
120,000
100,000
80,000
60,000
40,000
20,000
150
100
50
0
OPN
VEGF
SDF-1
BDNF
GDNF

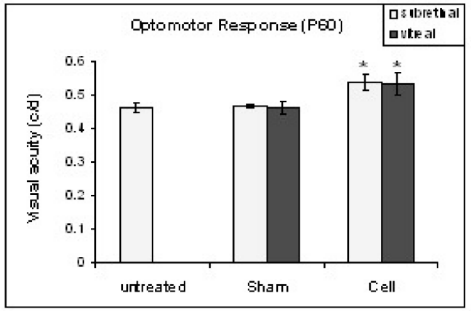
FIGURE 47

	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

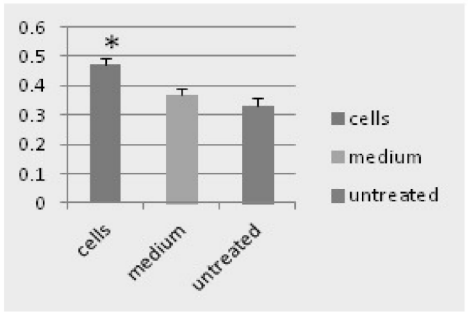
A

Optomotor response P60



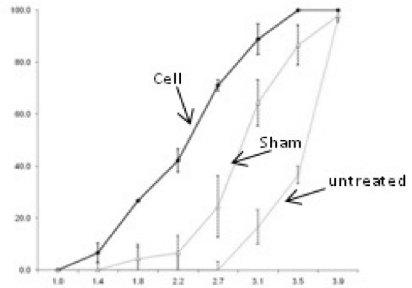
B

Optomotor response P90



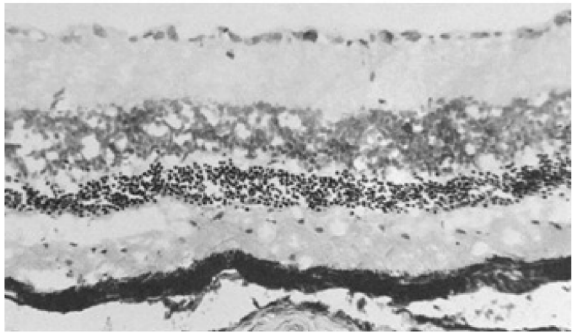
C

Luminance threshold response P90



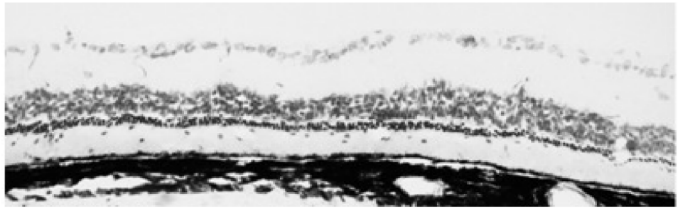
D

Histology P60 (intravitreal injection)



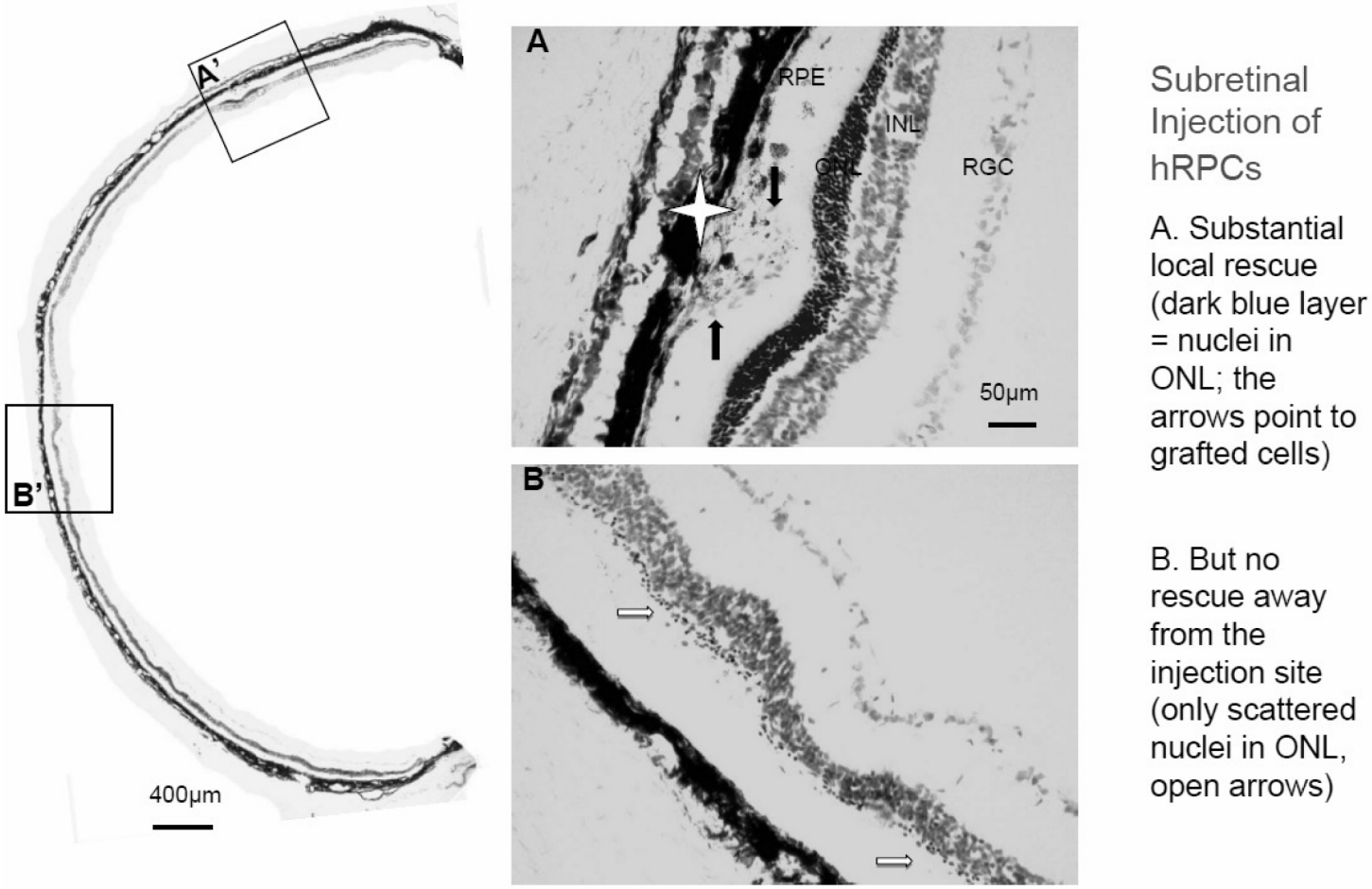
E

Histology P90 (intravitreal injection)



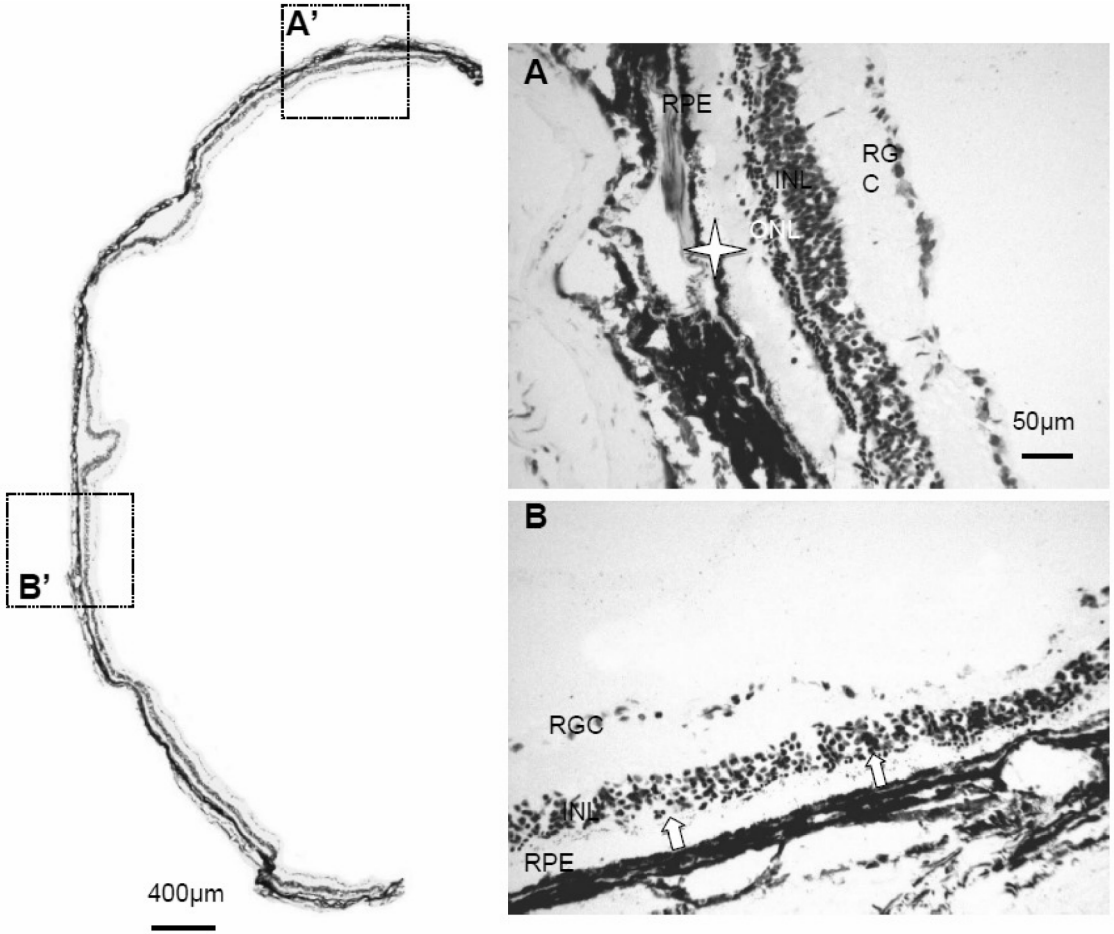
Widespread, persistent rescue of photoreceptor cells in dystrophic retina by hRPCs

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



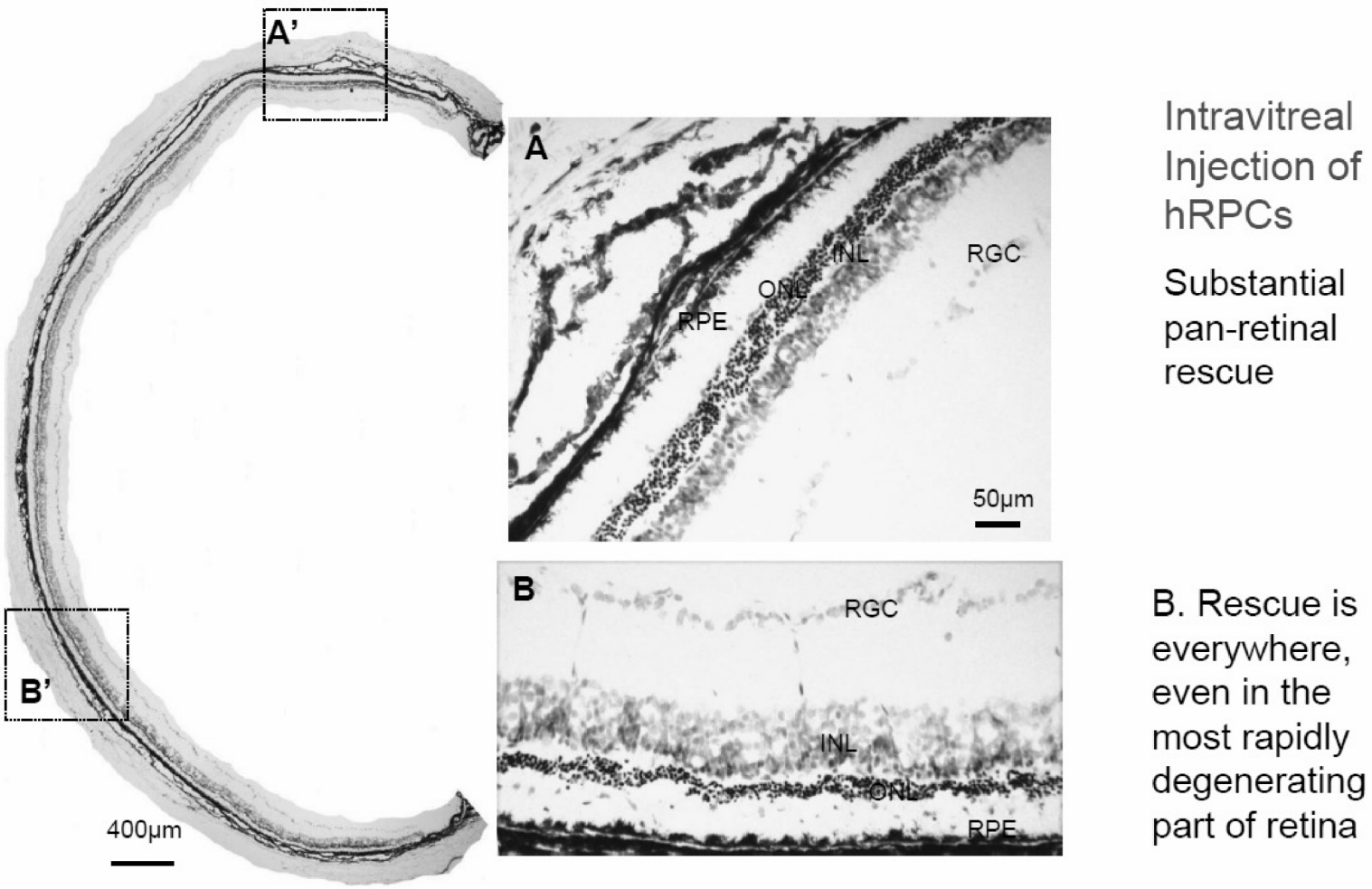
Subretinal Injection of vehicle (sham)

A. Also shows local rescue (moderate)

B. No rescue away from the site

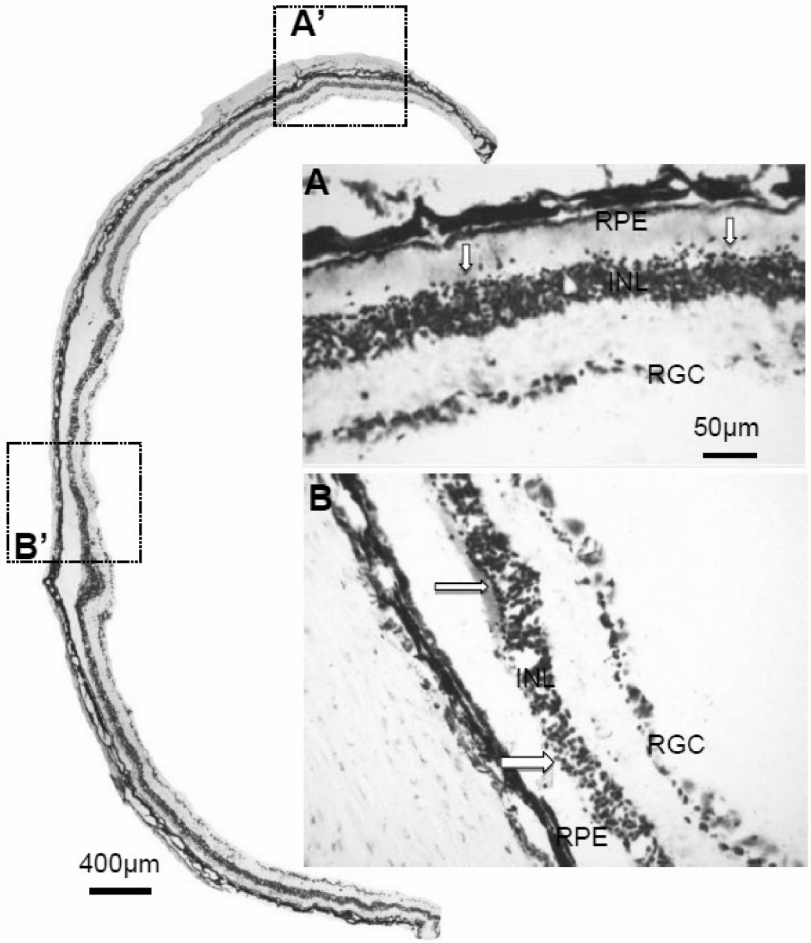
Subretinal injection of vehicle in RCS rat, viewed at P100: Left-montage image of cresyl violet stained section. A: high power Image of outline A' showing injection site (star) and localized ONL rescue. B: high power image of outline B; showing a single layer of ONL (arrows) at area away from injection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

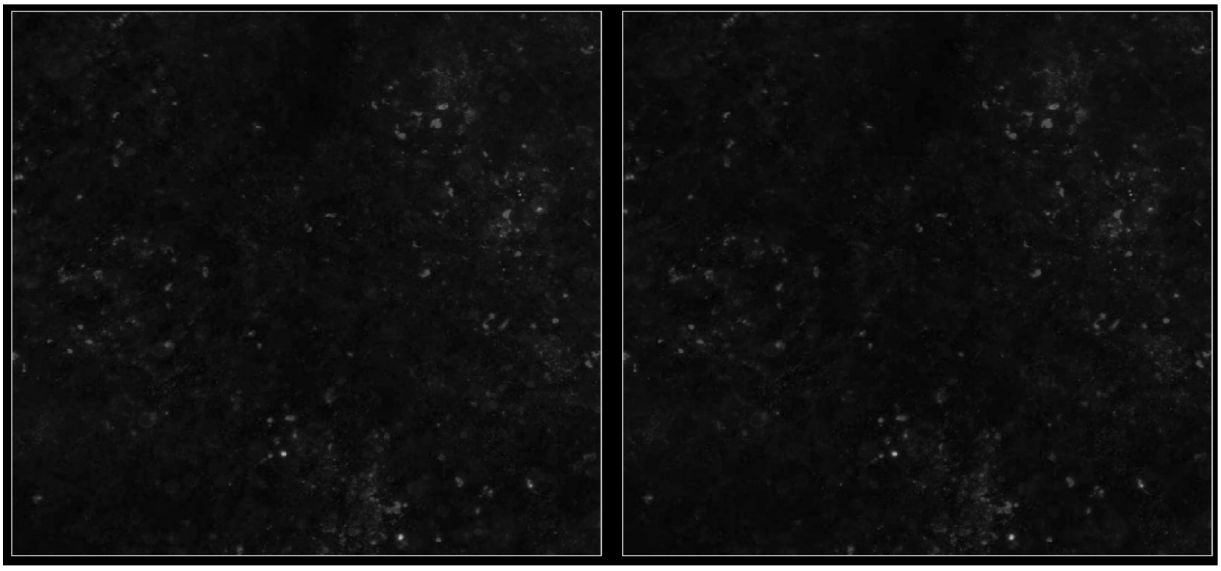
No discernable rescue*

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

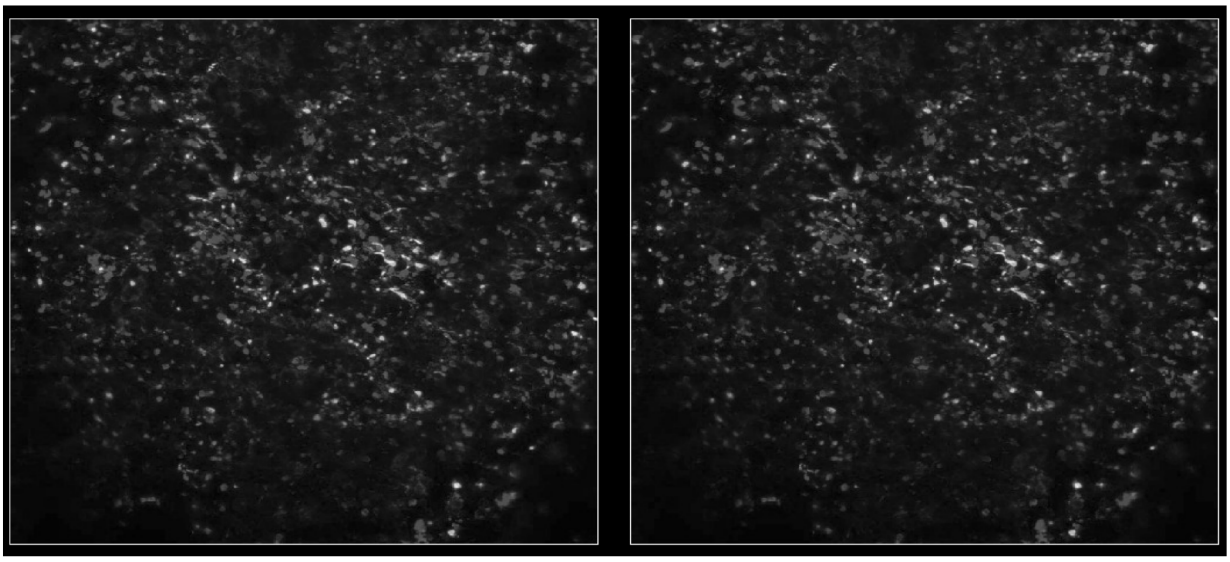
Intravitreal injection of carry medium in RCS rat, viewed at P100. Left-montage image of cresyl violet stained section. A&B: high power of outline A' &B' showing a single layer of photoreceptors (arrows)

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

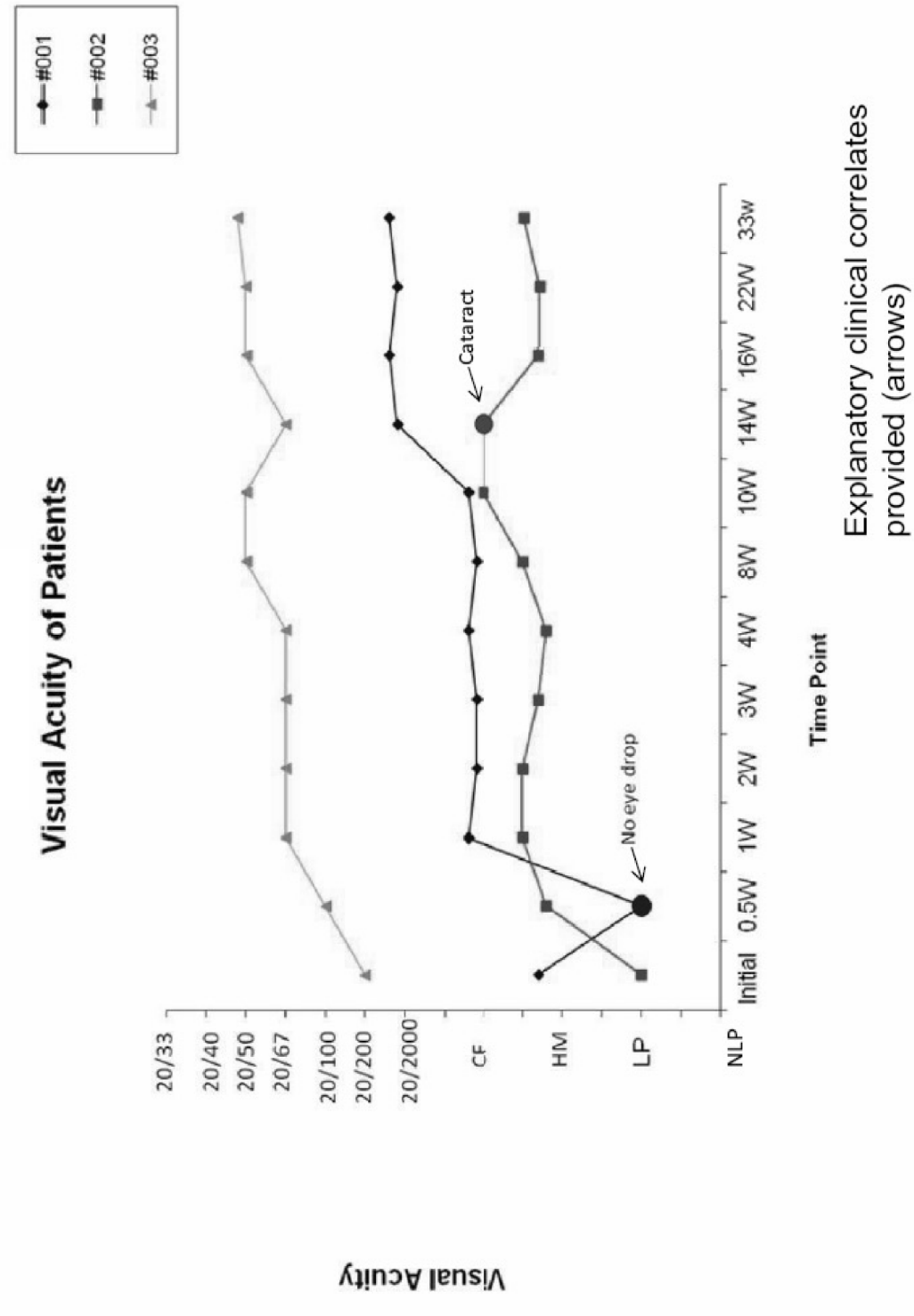

Visual Acuity of Patients
#001
#002
#003
Visual Acuity
20/33
20/40
20/50
20/67
20/100
20/200
20/2000
CF
HM
LP
NLP
Initial
0.5W
1W
2W
3W
4W
8W
10W
14W
16W
22W
33w
Time Point
Cataract
No eye drop
Explanatory clinical correlates provided (arrows)

FIGURE 55