FIG. 1/55

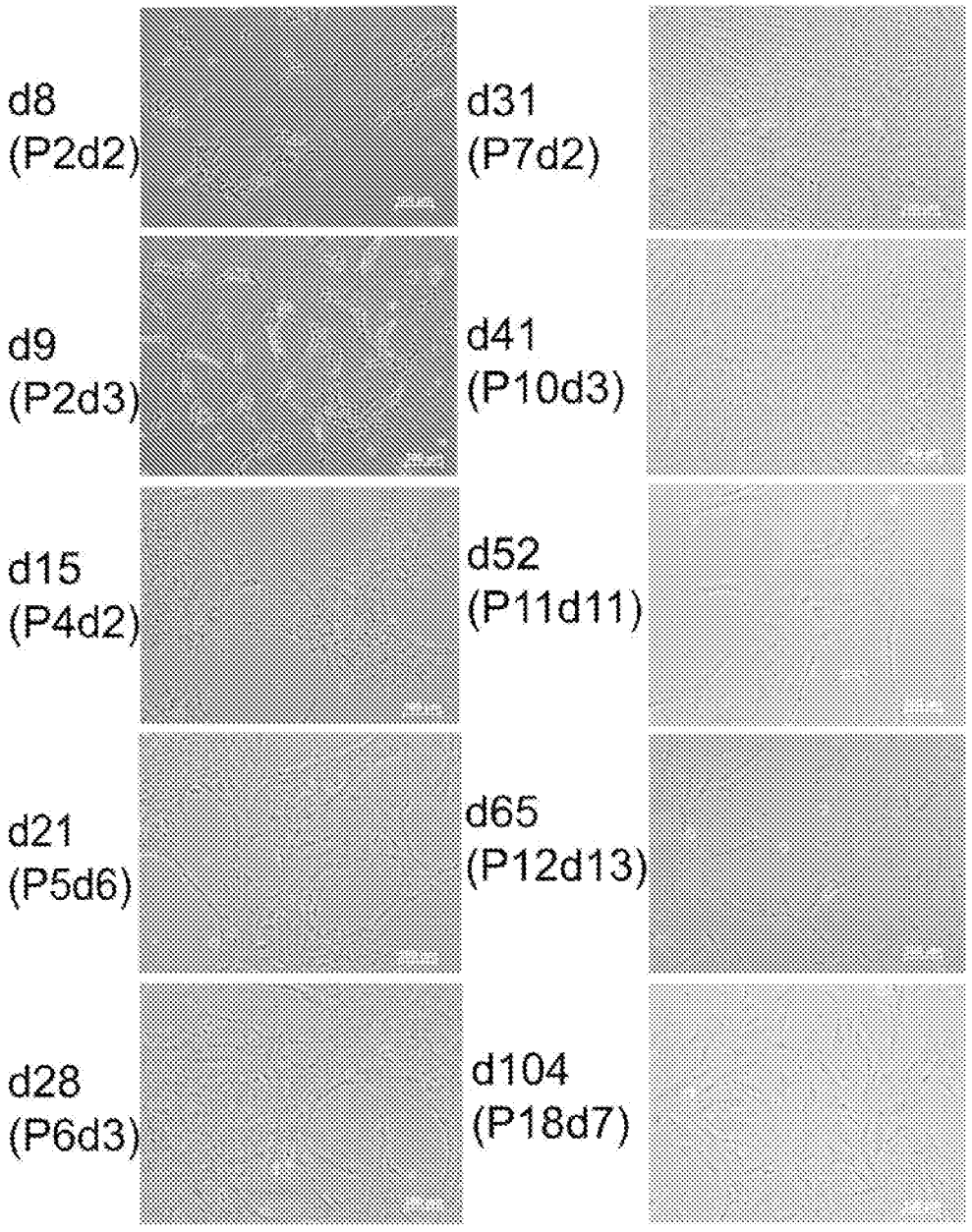



**FIGURE 1**

FIG. 2/55

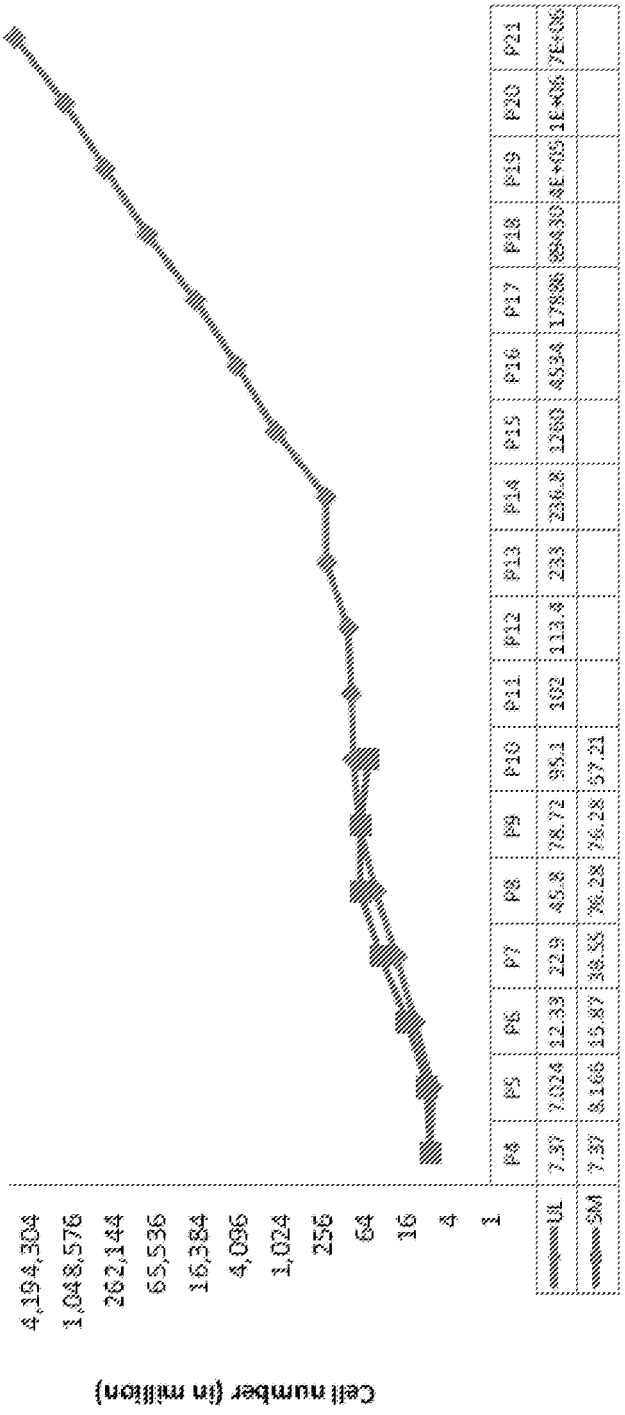



FIGURE 2

FIG. 3/55

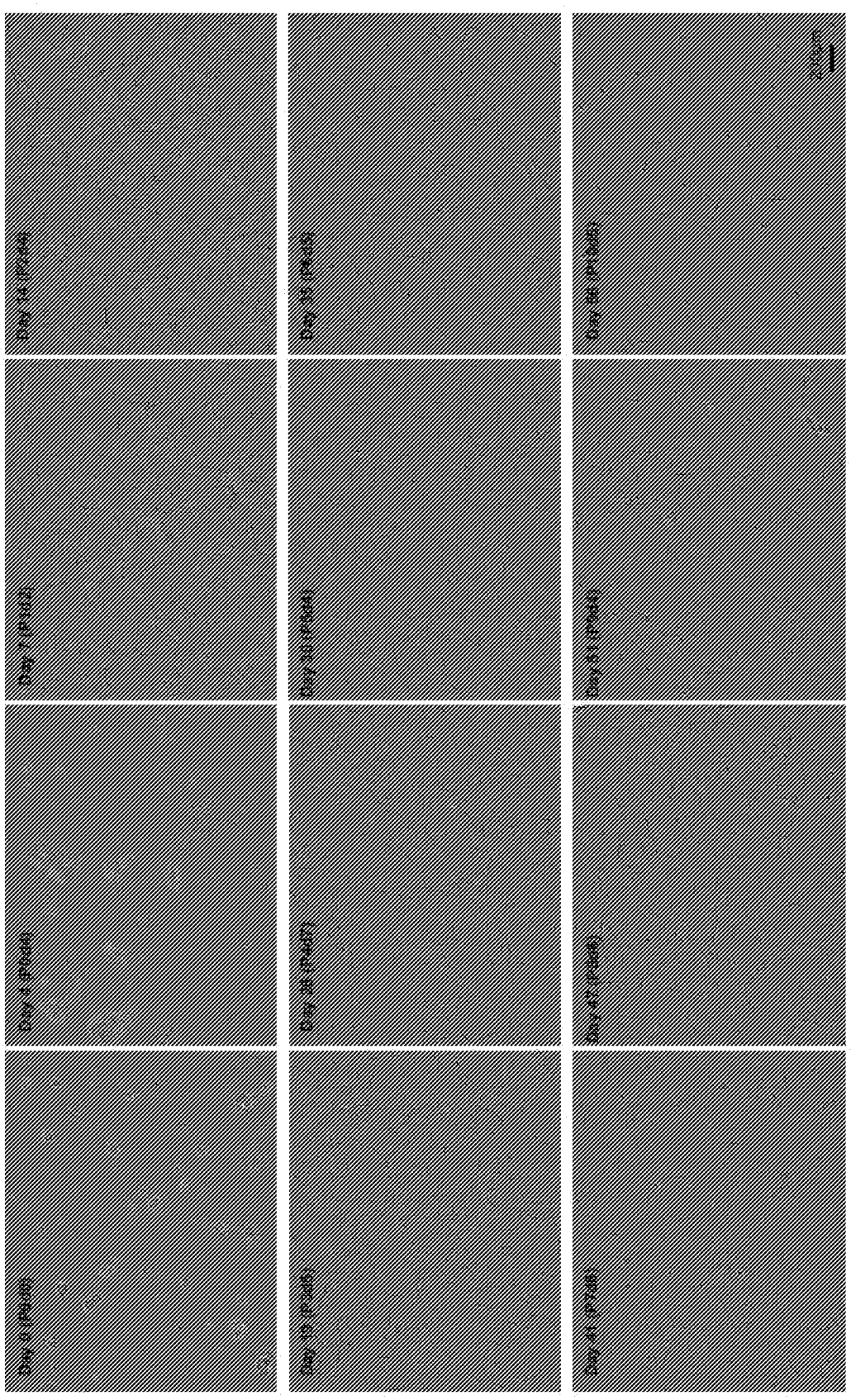
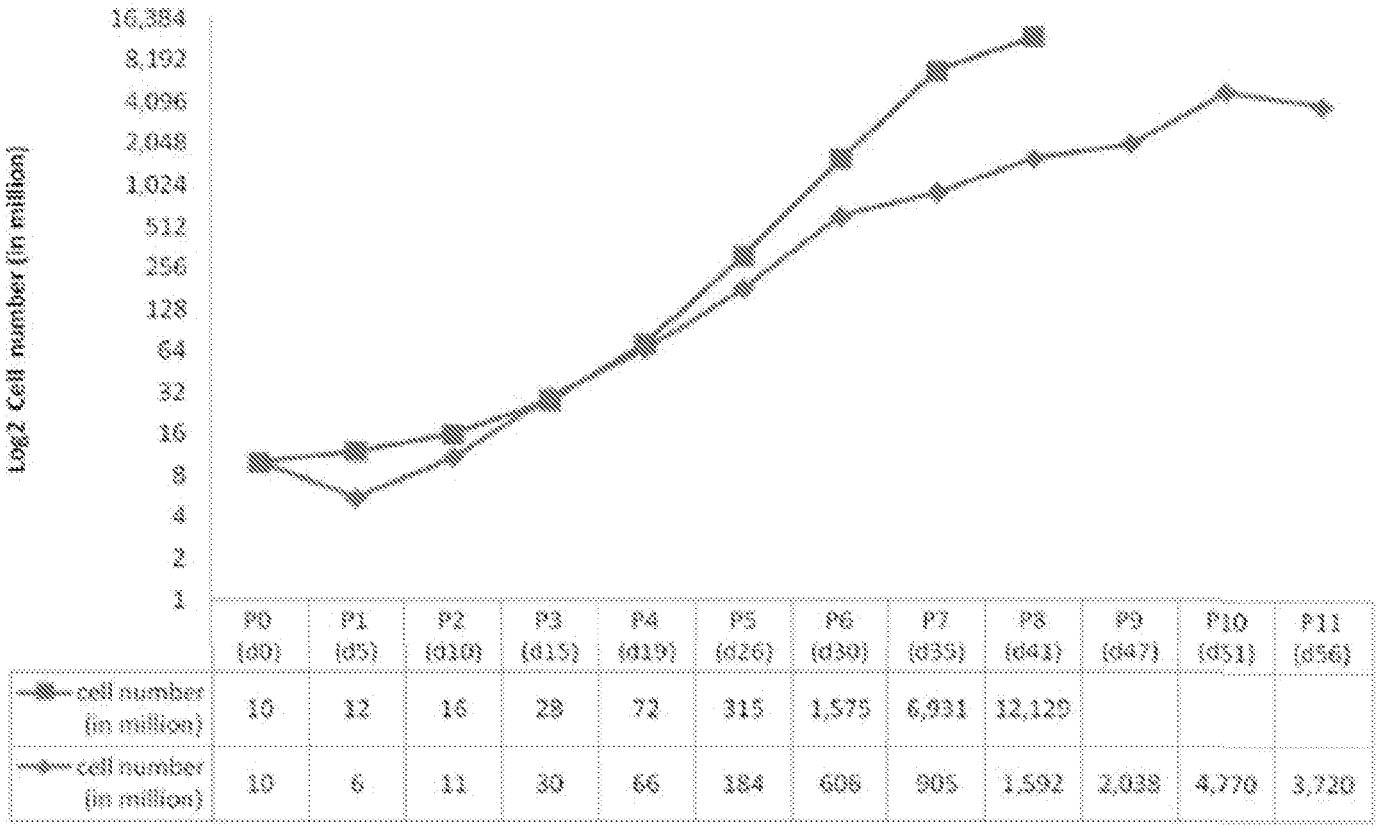



FIGURE 3




| | 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**

WO 2012/158910 PCT/US2012/038342

FIG. 5/55

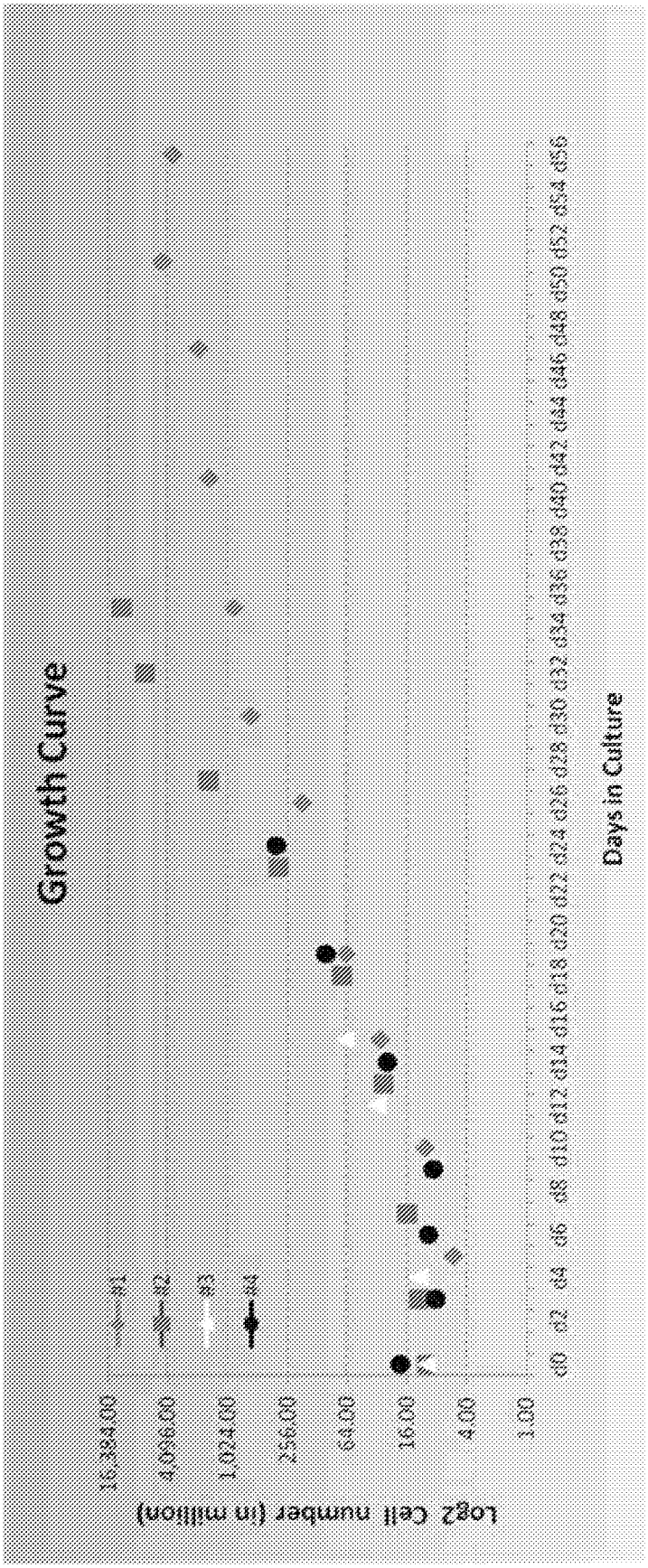
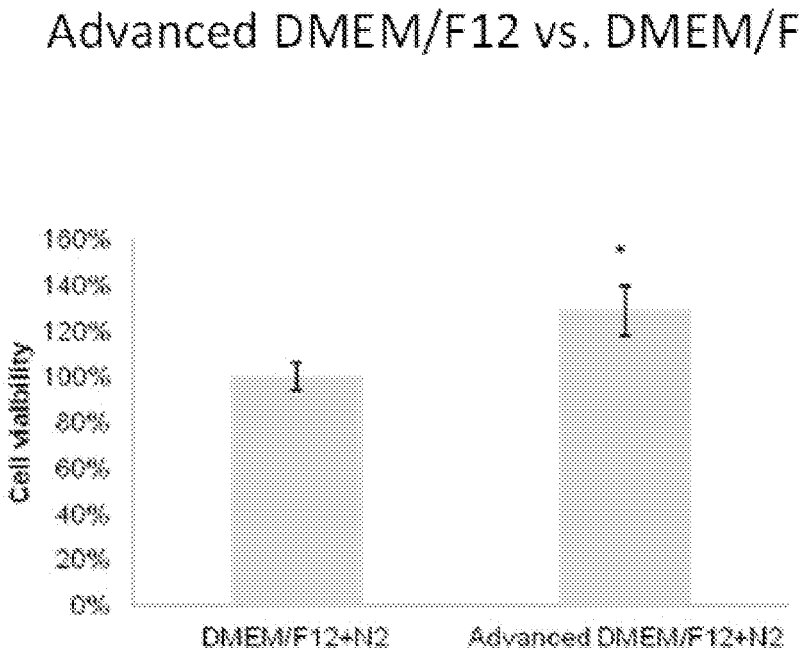



FIGURE 5

FIG. 6/55

A




B

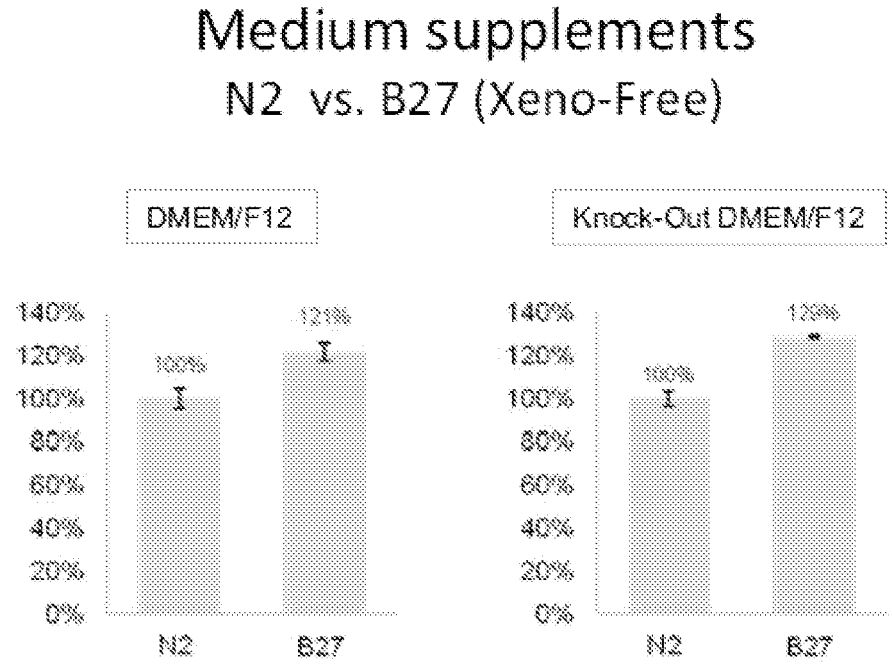
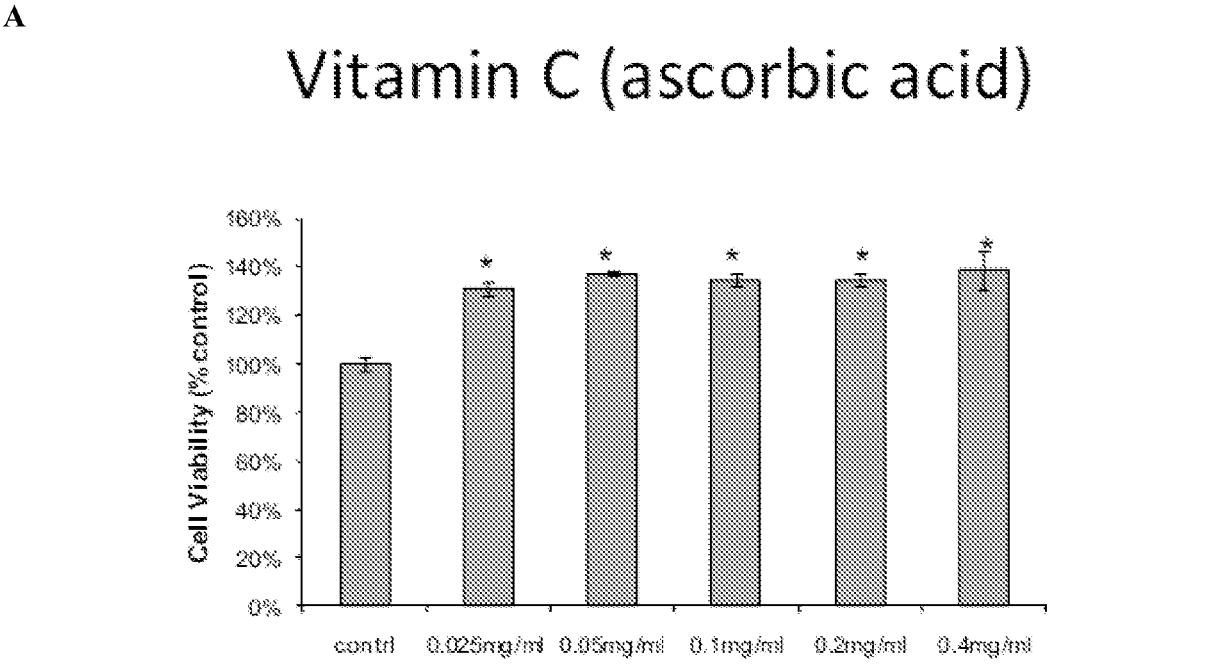


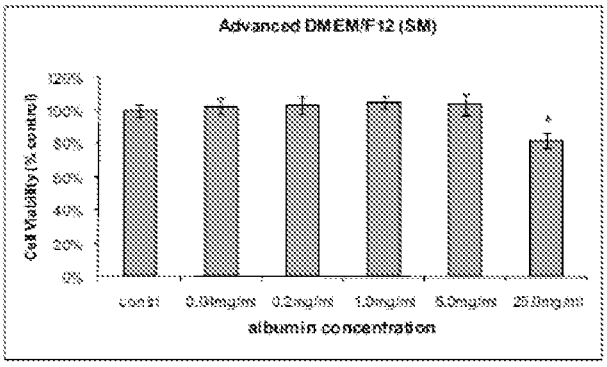

FIGURE 6

FIG. 7/55

A




B




C

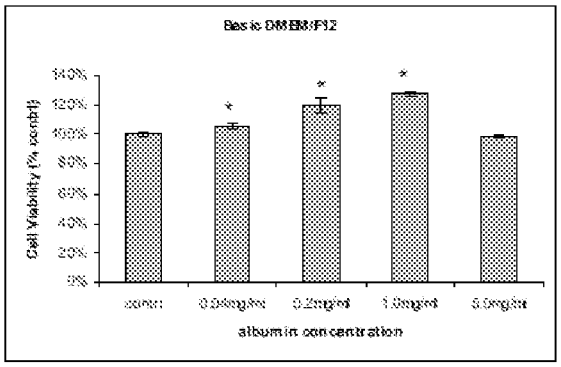



**FIGURE 7**

FIG. 8/55

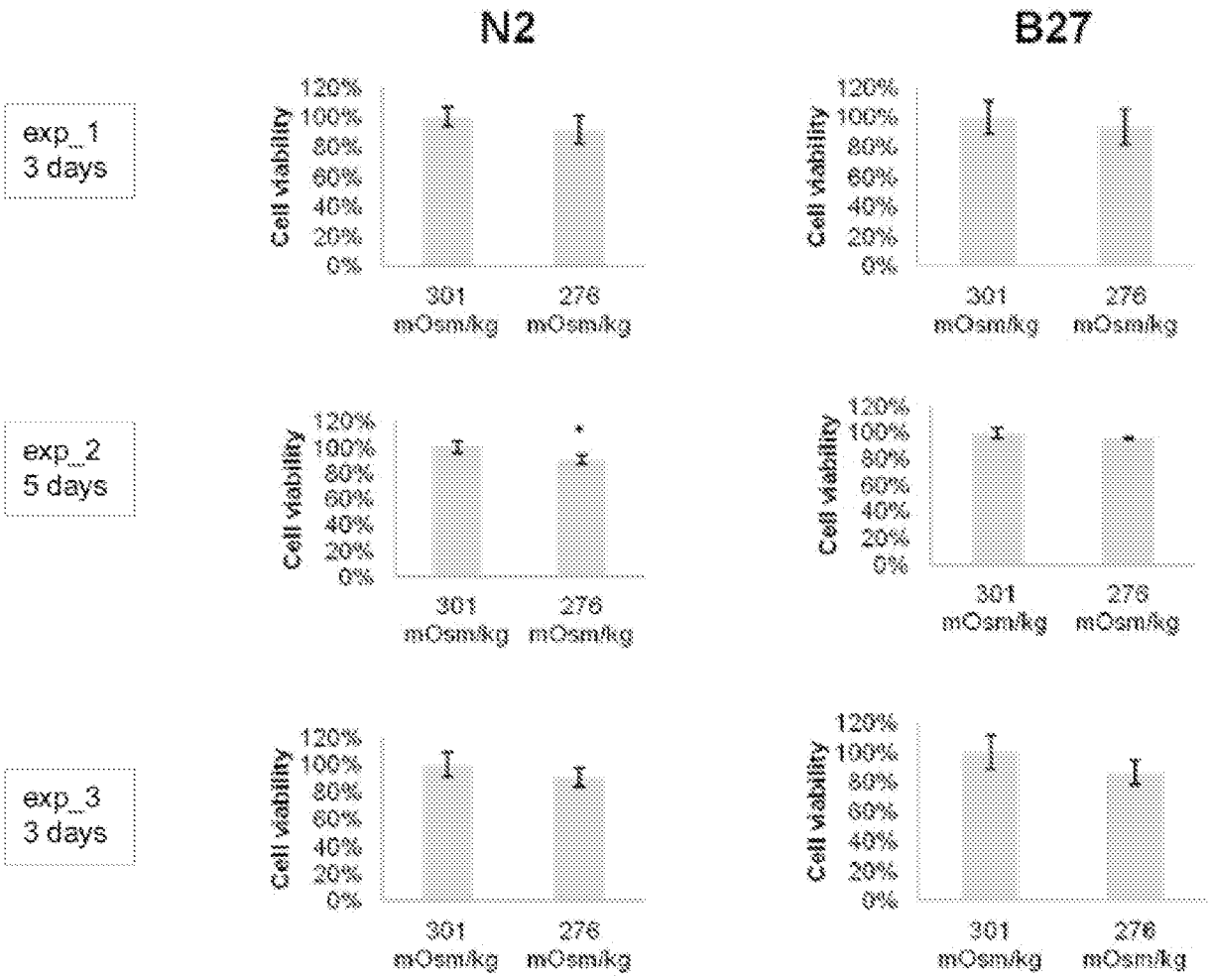



**FIGURE 8**

FIG. 9/55

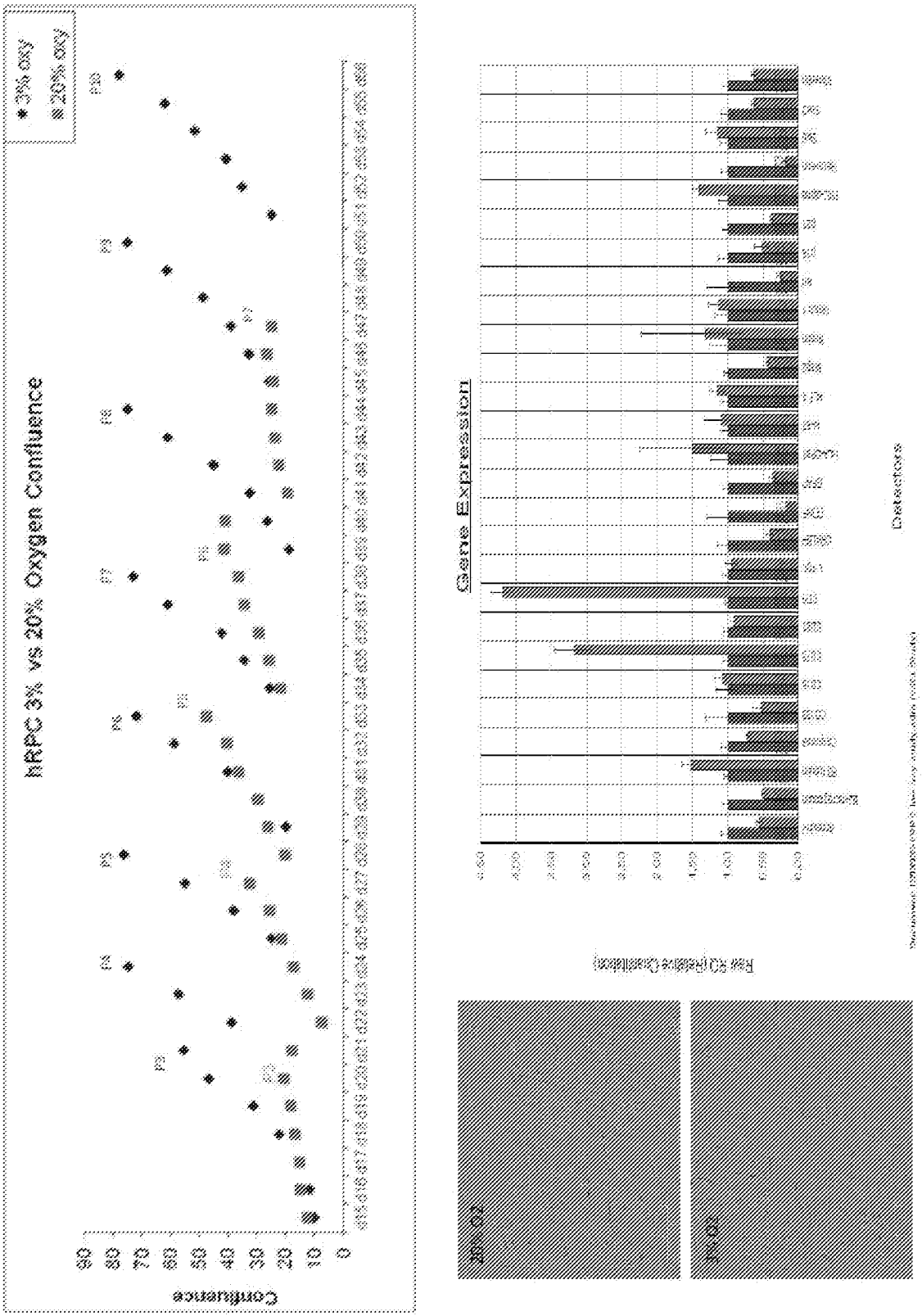



FIGURE 9

FIG. 10/55

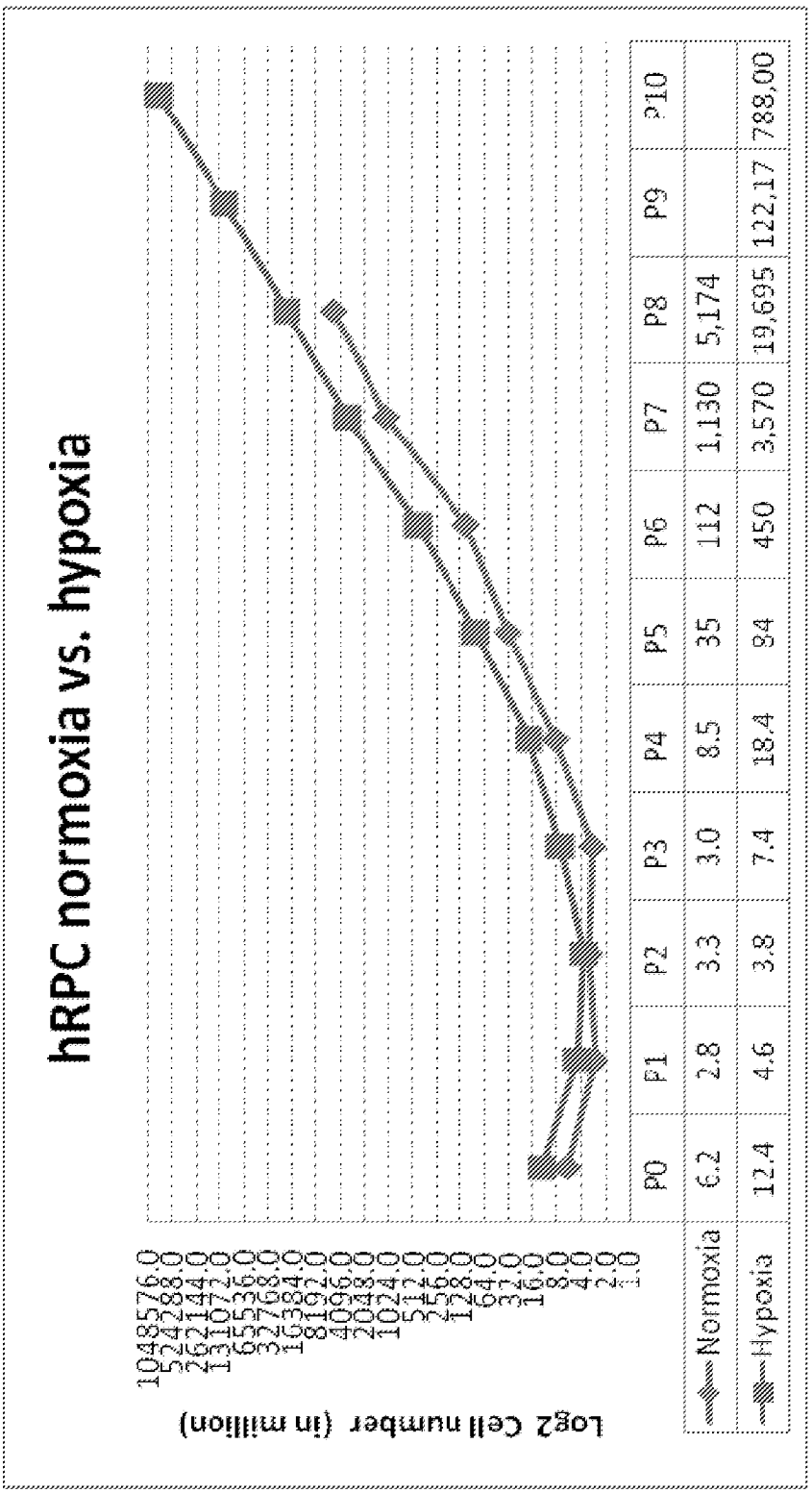



FIGURE 10

FIG. 11/55

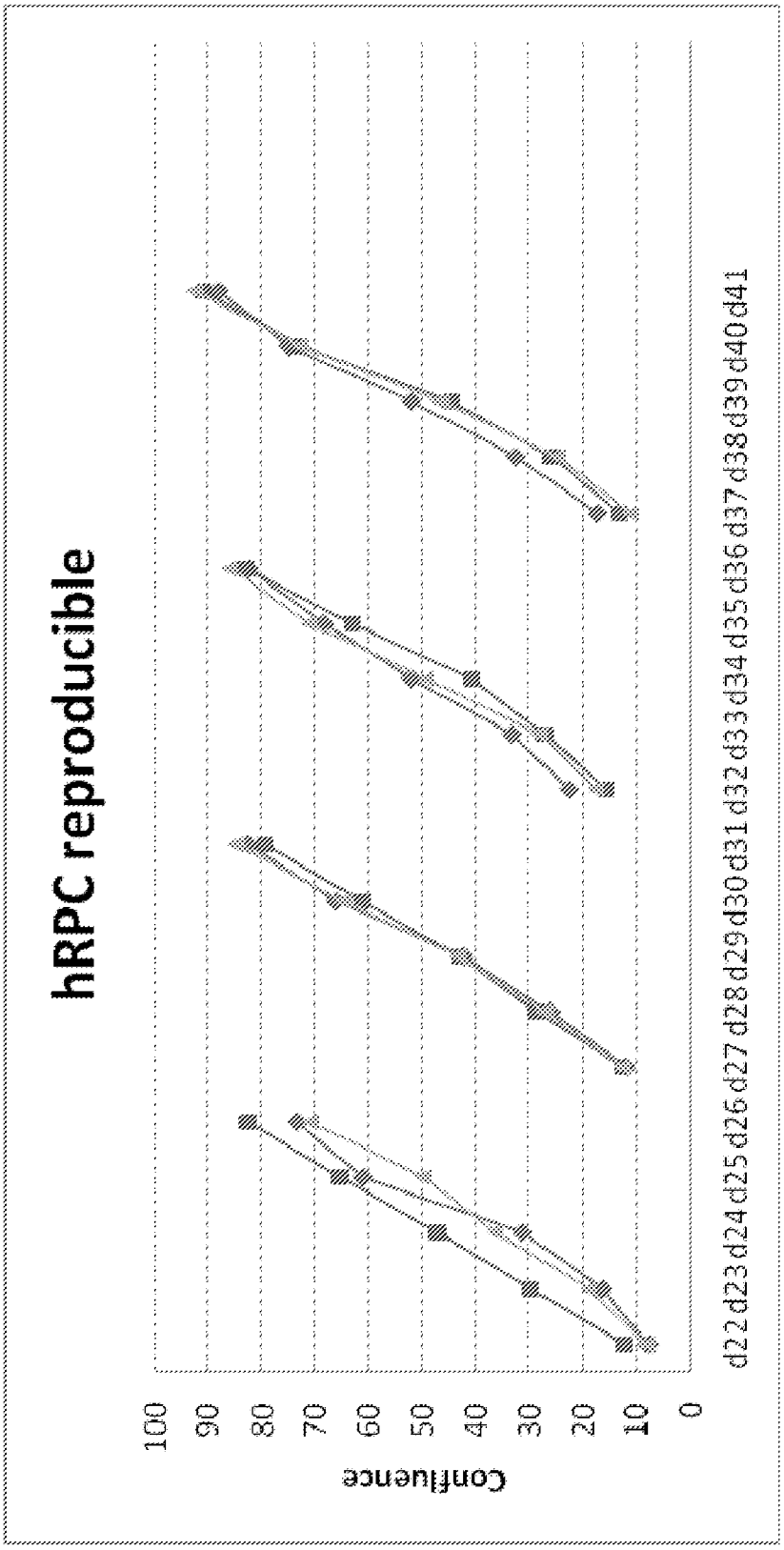



FIGURE 11

FIG. 12/55

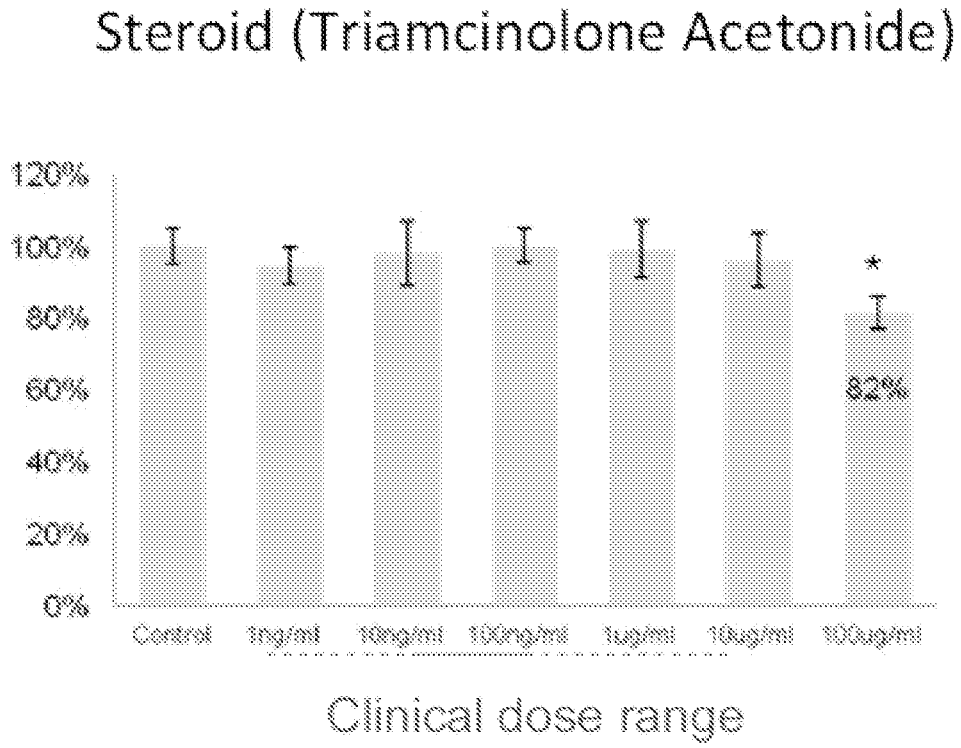
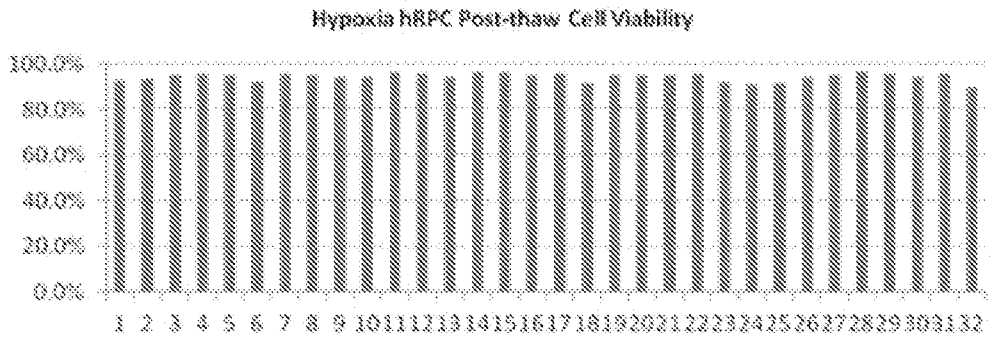
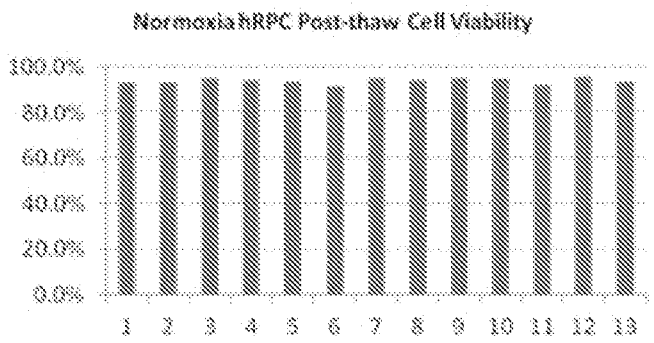



**FIGURE 12**

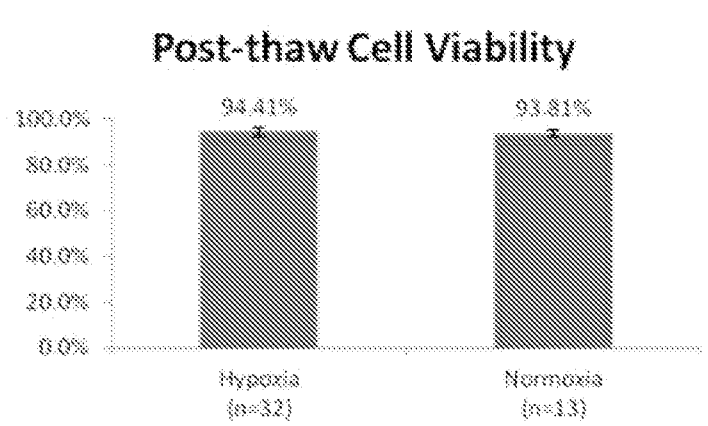
A




B




C




D

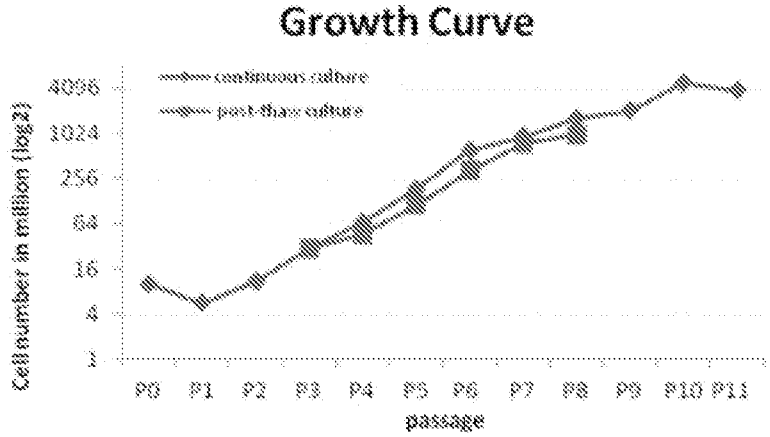



**FIGURE 13**

FIG. 14/55

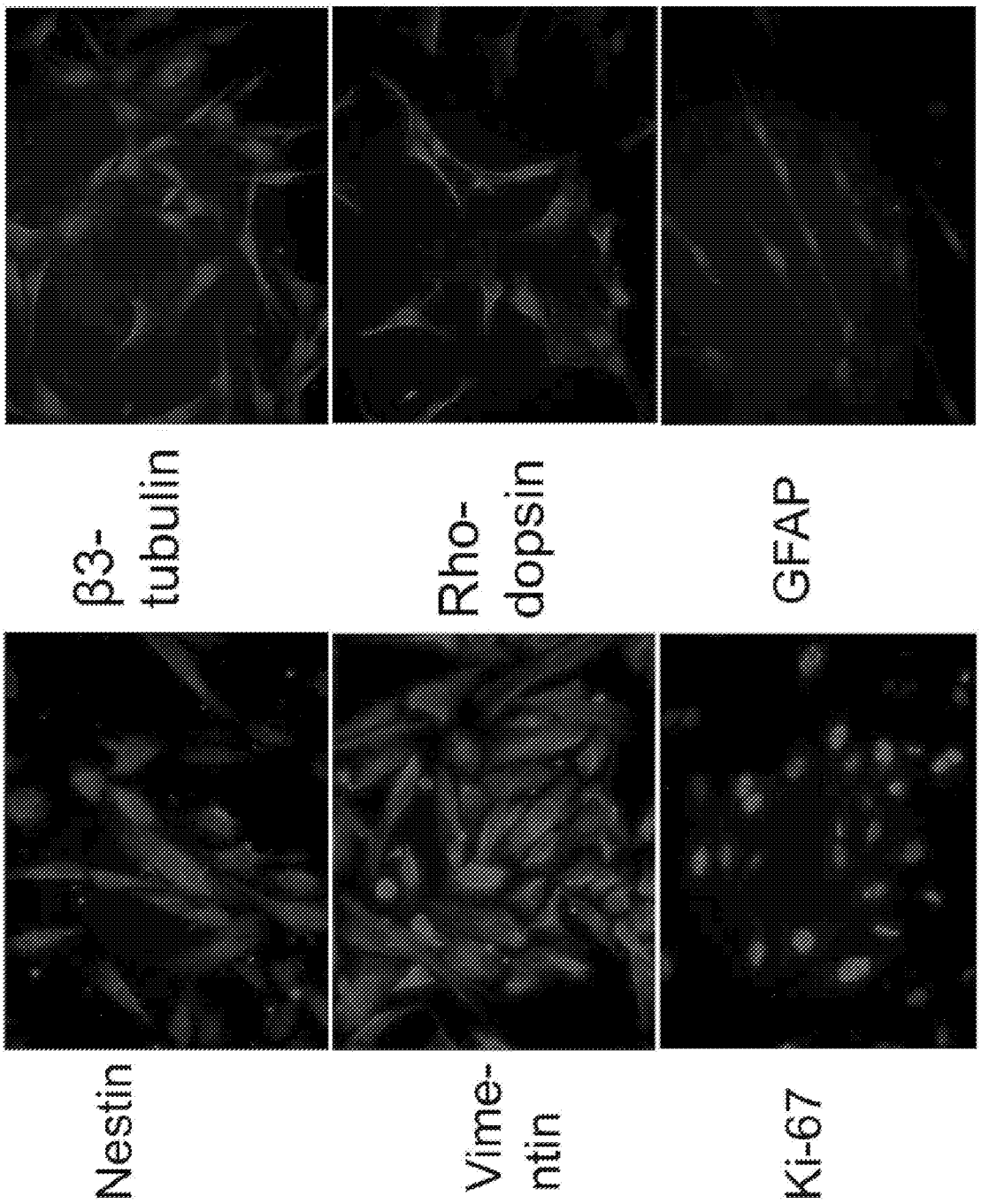



FIGURE 14

# 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)

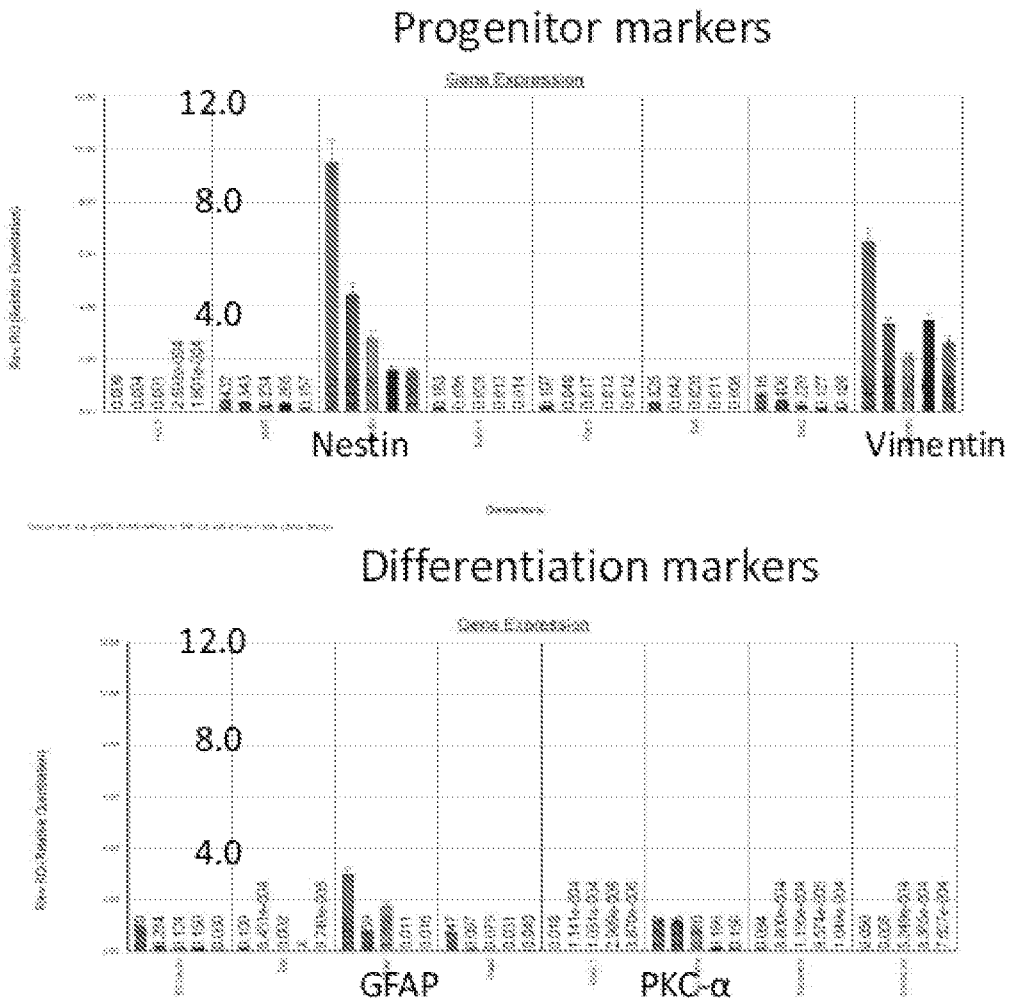
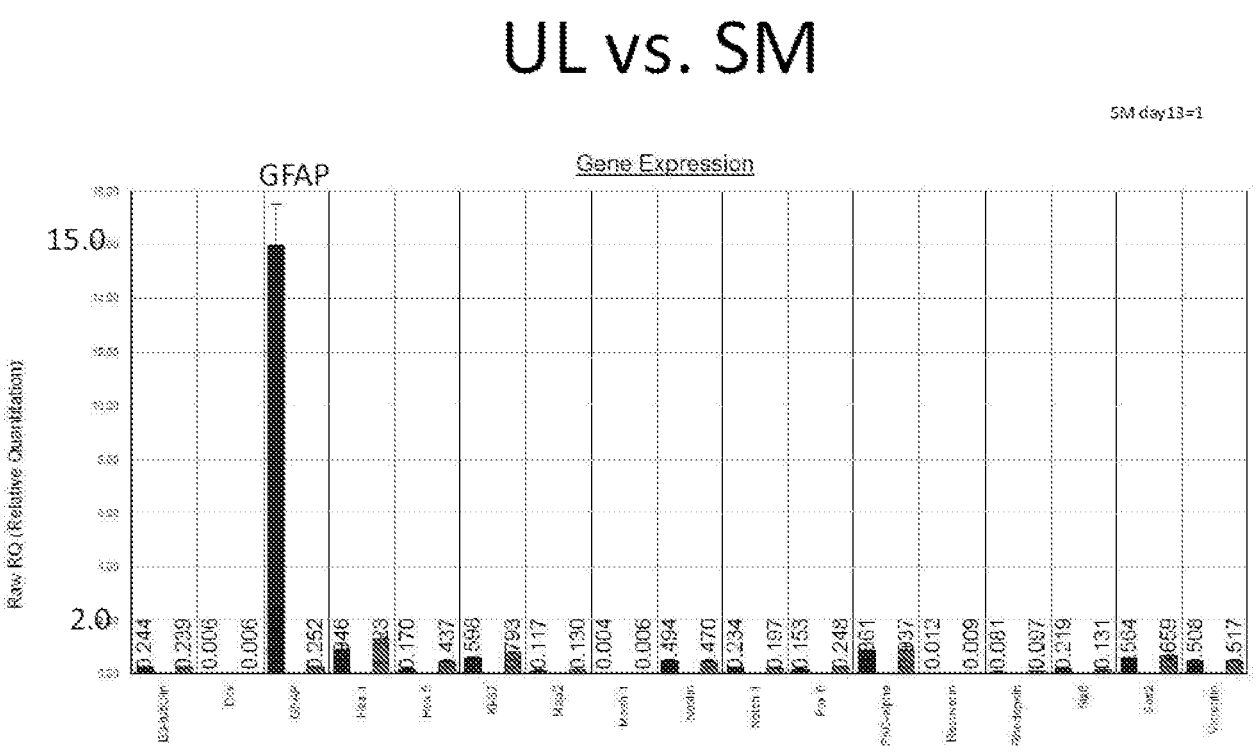



**FIGURE 15**

WO 2012/158910 PCT/US2012/038342

FIG. 16/55

A




B

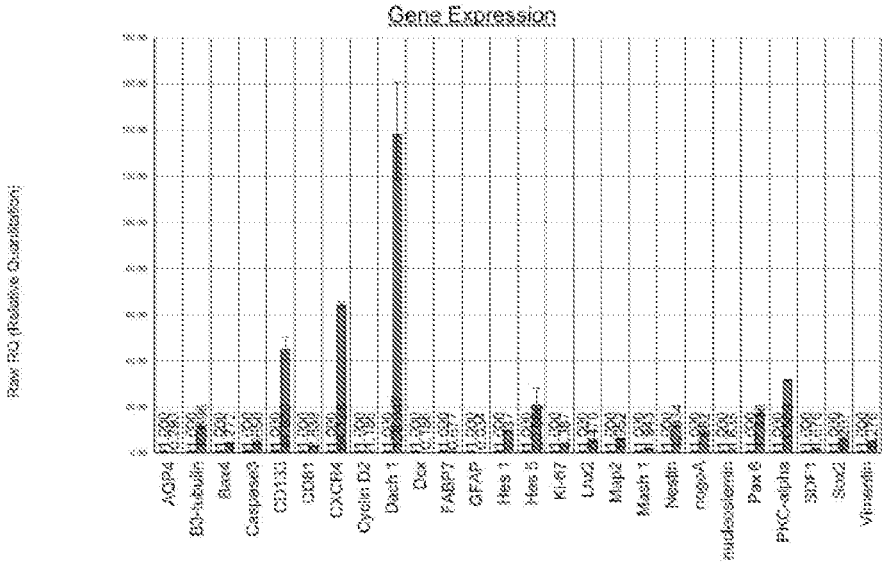



FIGURE 16

WO 2012/158910

ANTIBODIES USED Labeling

Anti-RFP injected cells

Anti-vimentin Müller cells

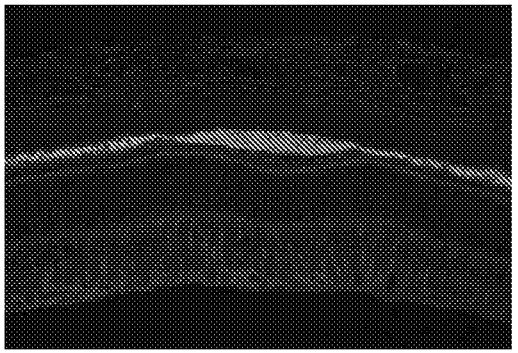
Anti-rod opsin rod outer segments and some rod cell bodies as the protein redistributes due to the degeneration

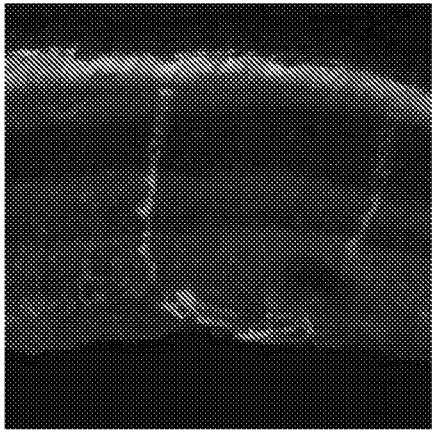
OR

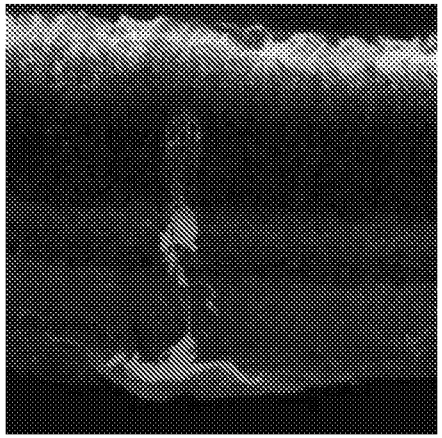
Anti-Ezrin RPE microvilli and Müller cell microvilli at the OLM

OR

Protein kinase C rod bipolar cells







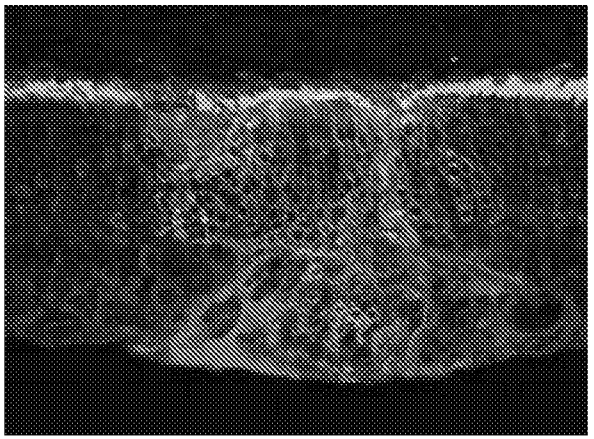


**FIGURE 17**

PCT/US2012/038342

FIG. 18/55

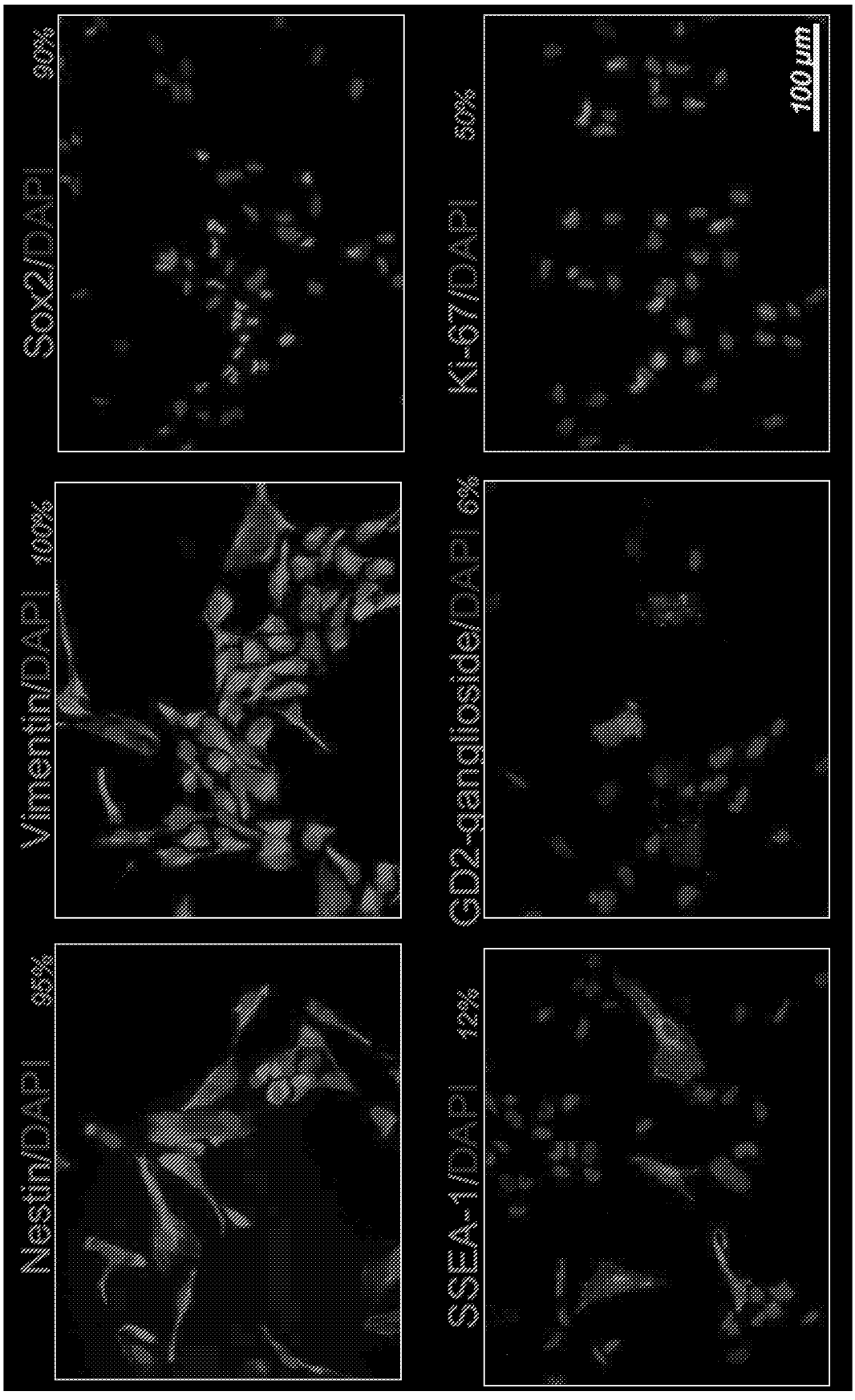



FIGURE 18

FIG. 19/55

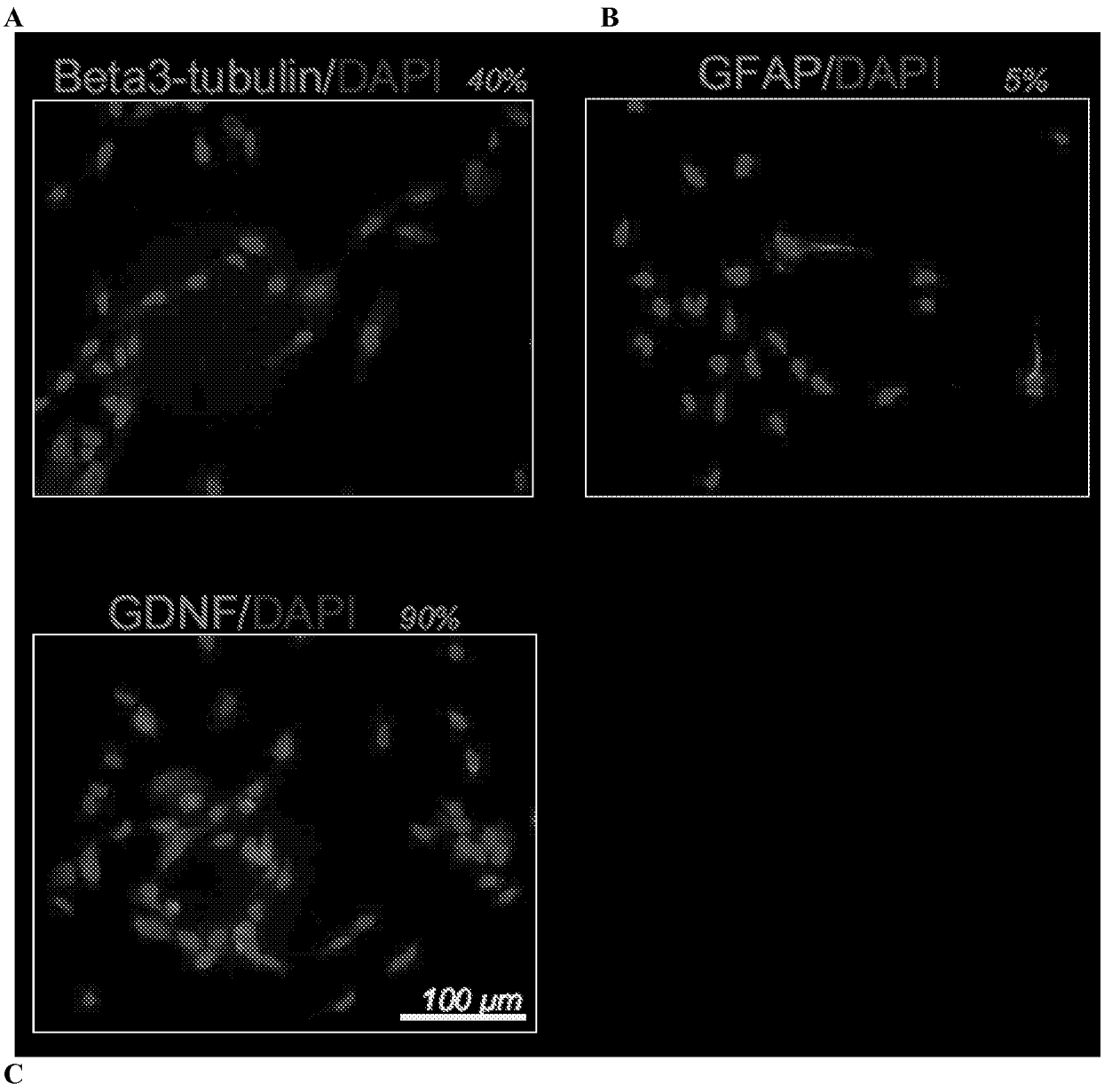



**FIGURE 19**

FIG. 20/55

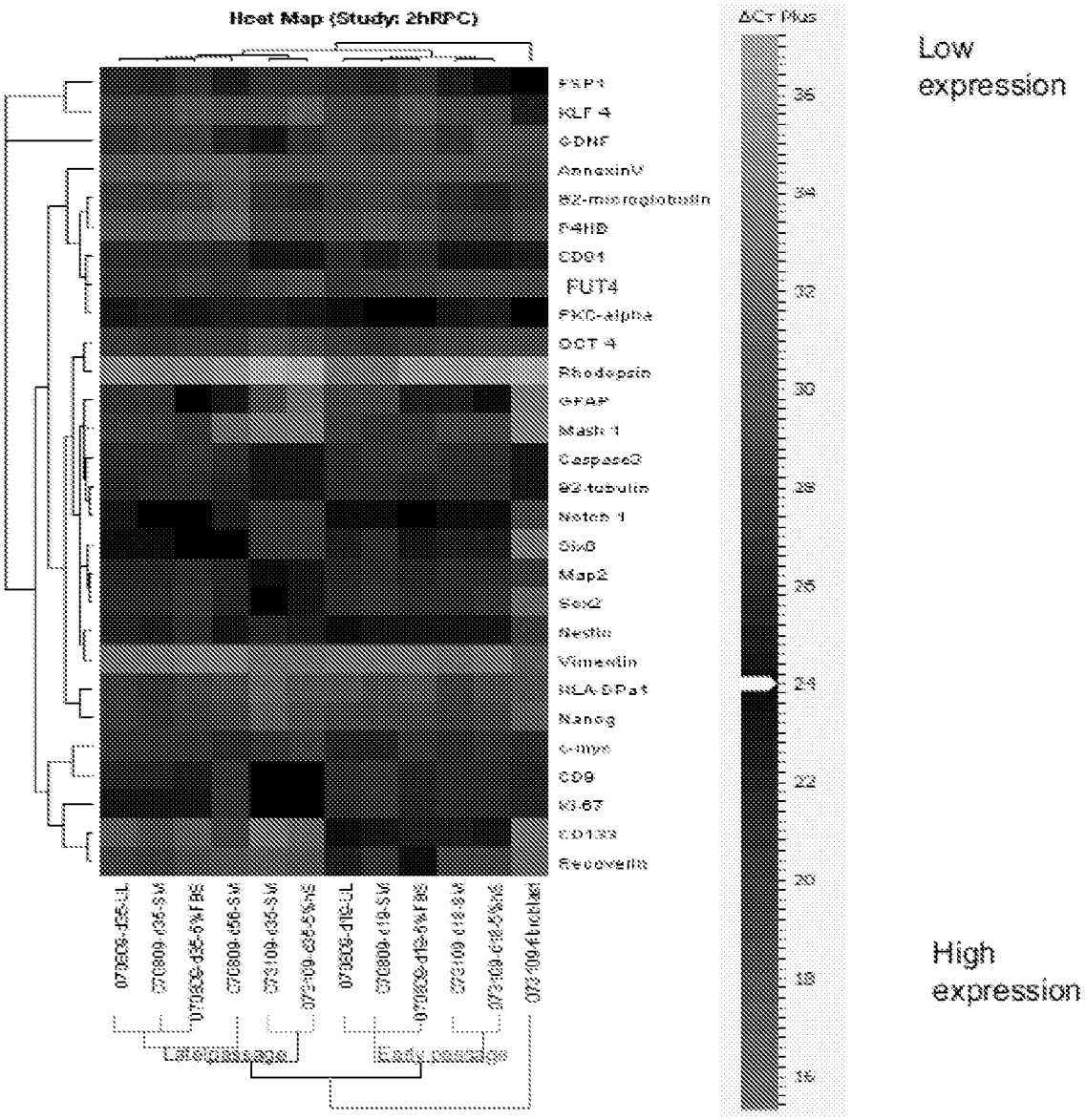
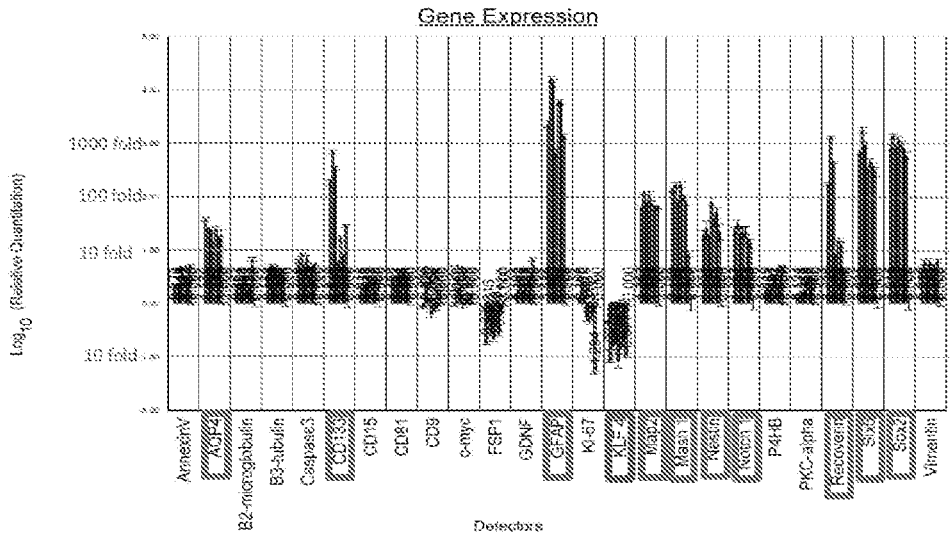


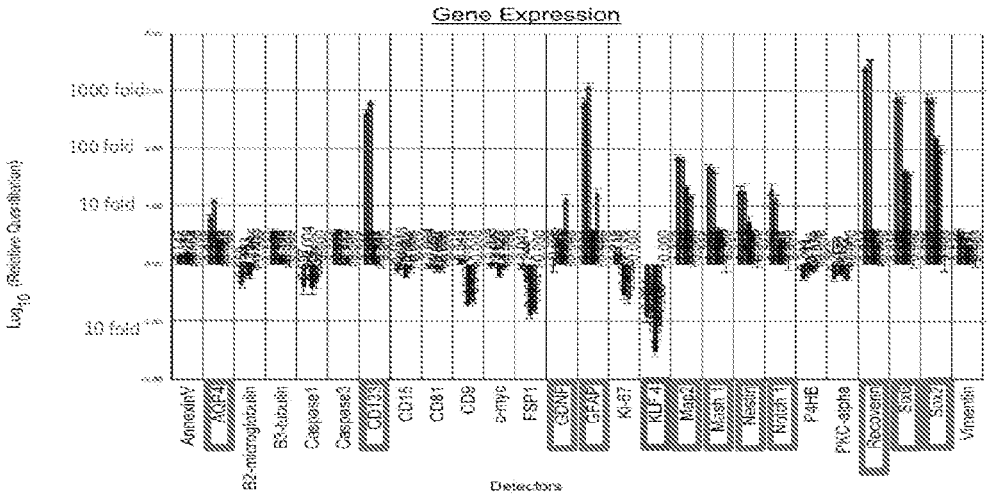

**FIGURE 20**

FIG. 21/55

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**

WO 2012/158910 PCT/US2012/038342

FIG. 23/55

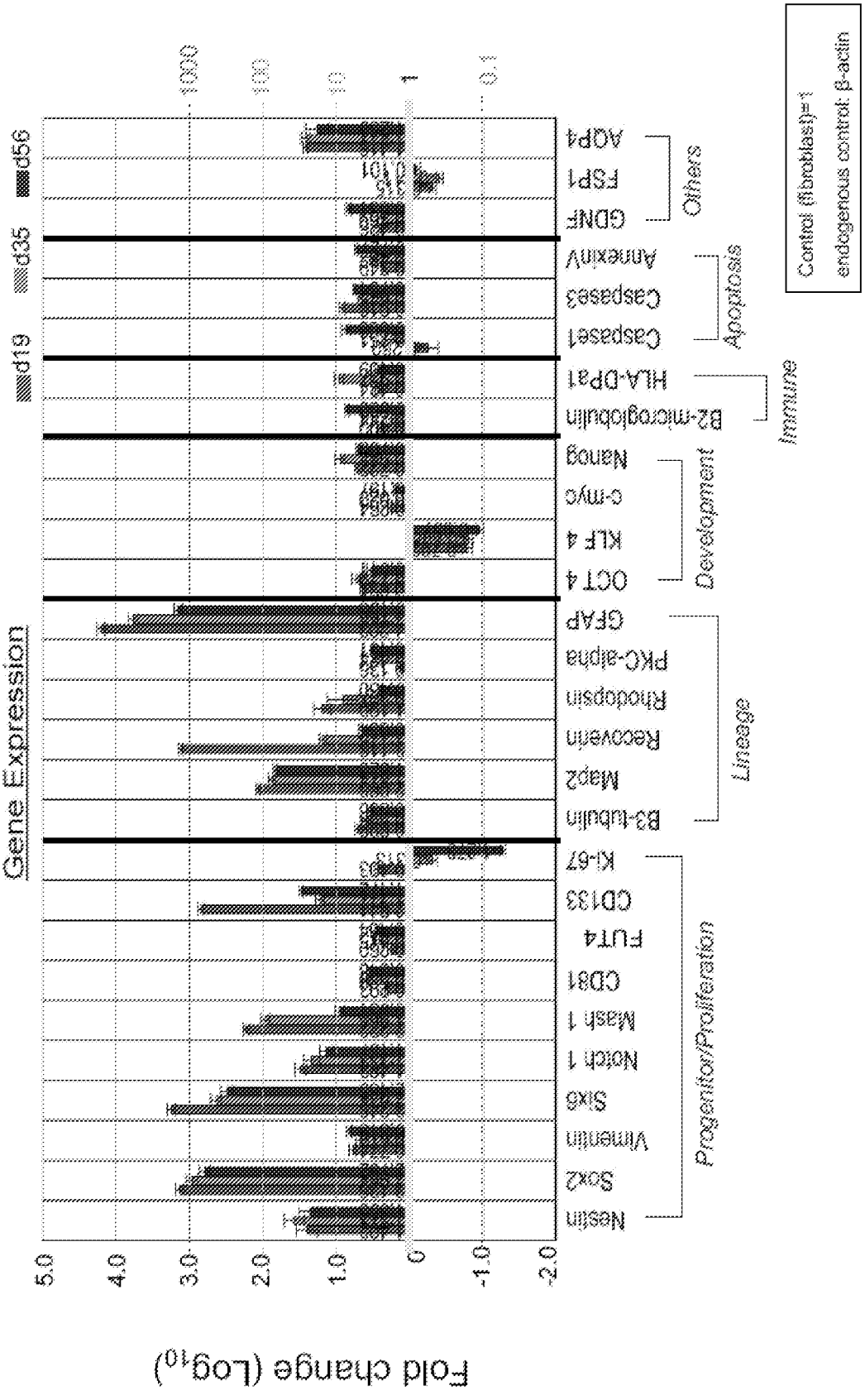



FIGURE 23

FIG. 24/55

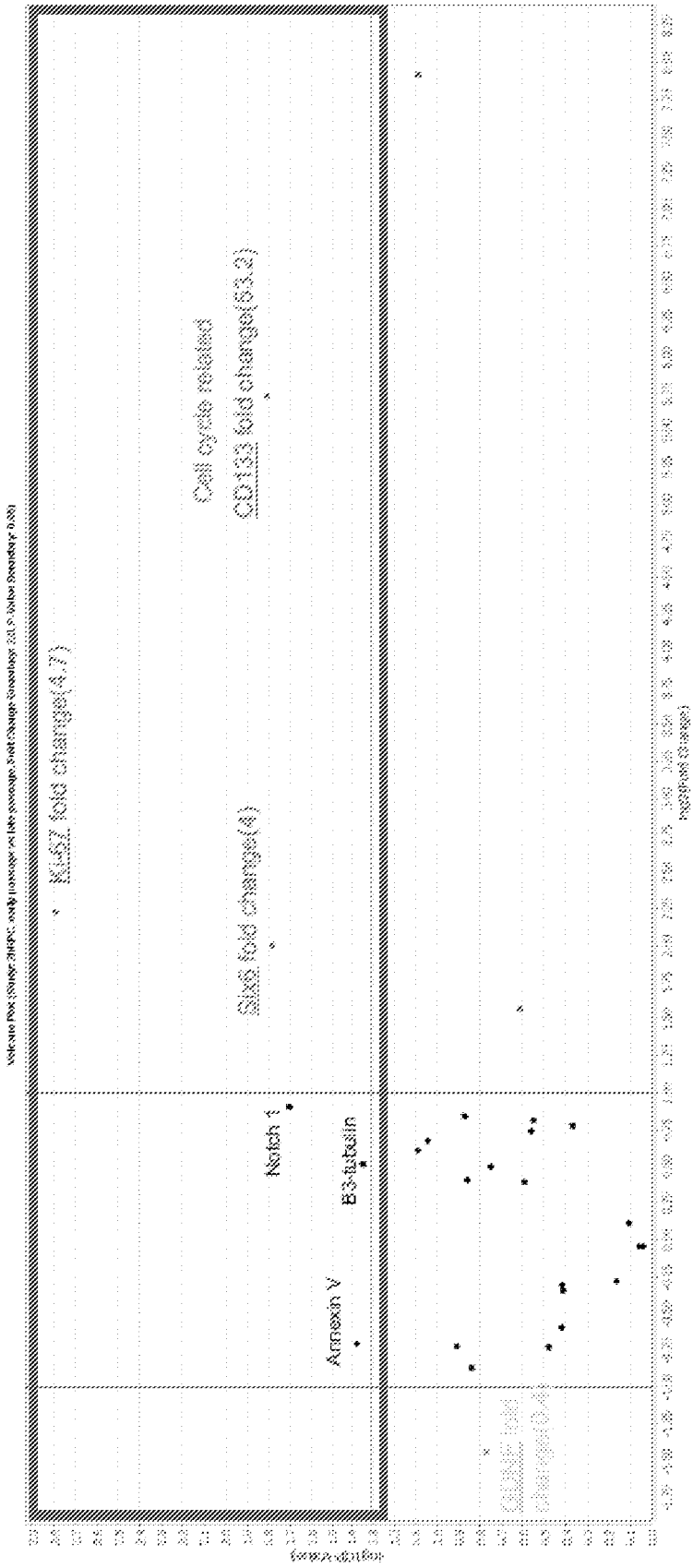



FIGURE 24

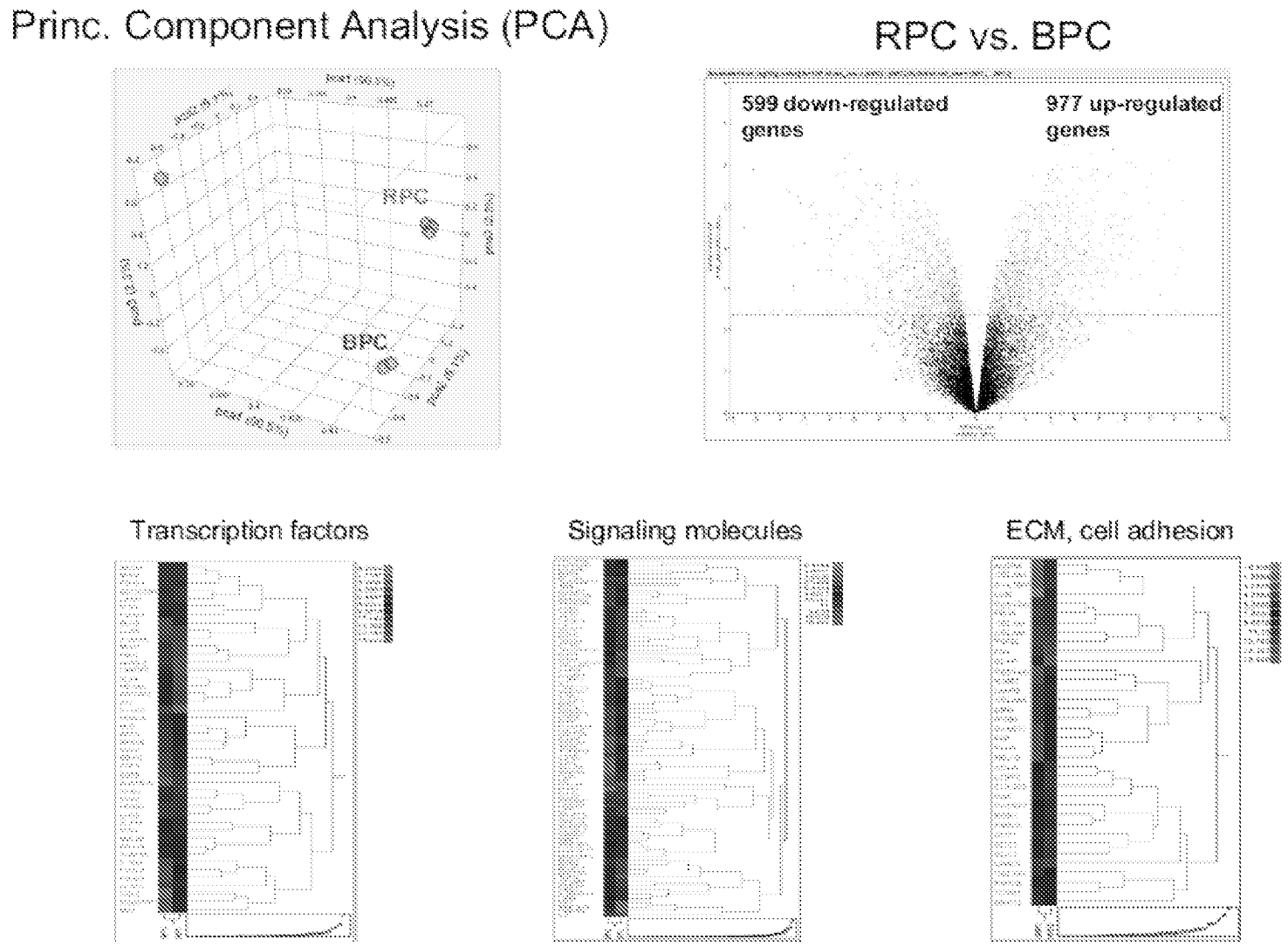



**FIGURE 25**

WO 2012/158910 PCT/US2012/038342

FIG. 26/55

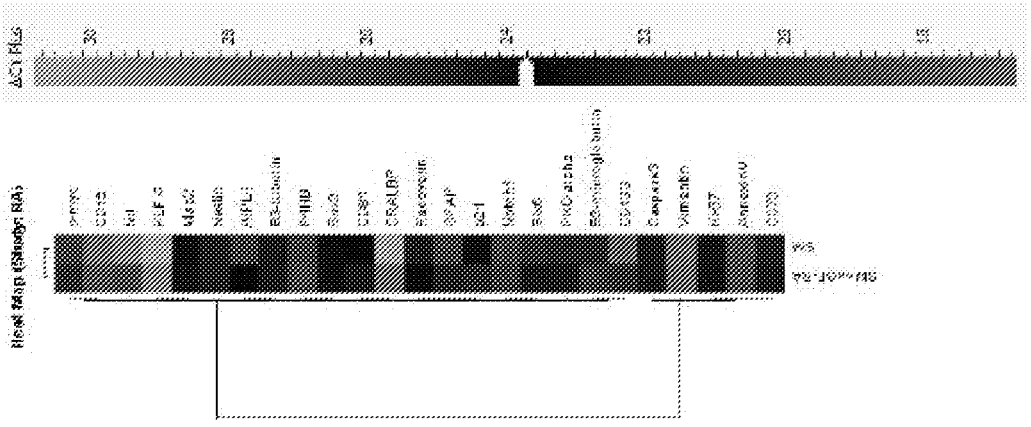


FIGURE 26

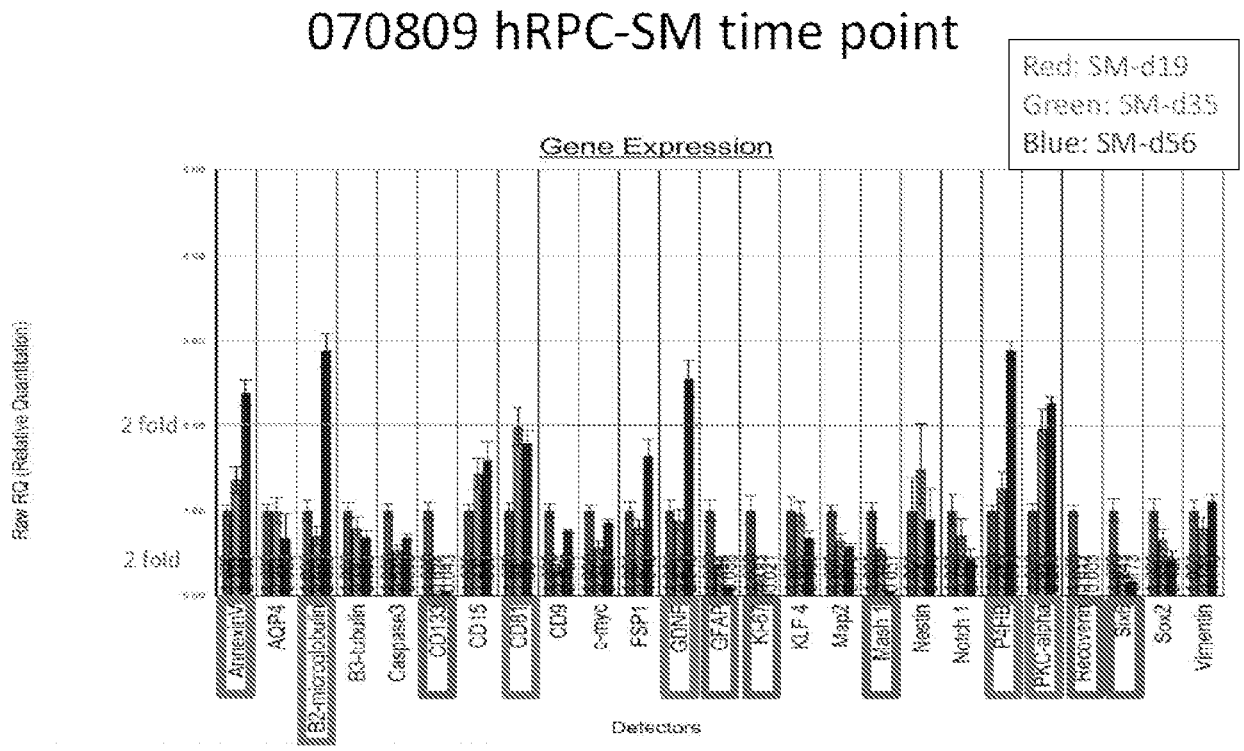
# hRPC time point design

| 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**

FIG. 28/55

A




B

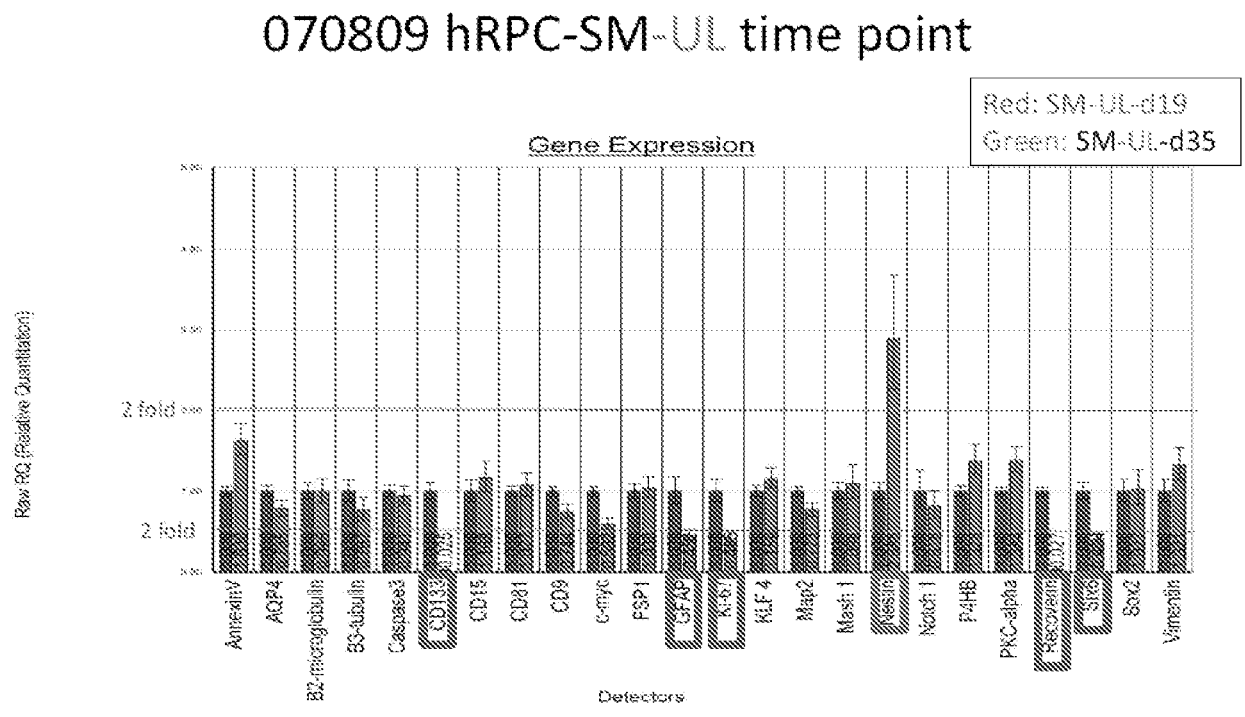
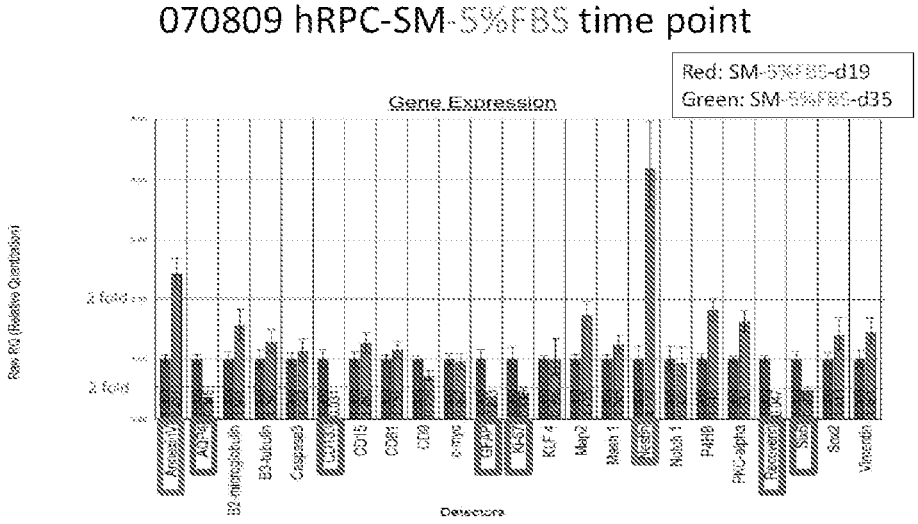


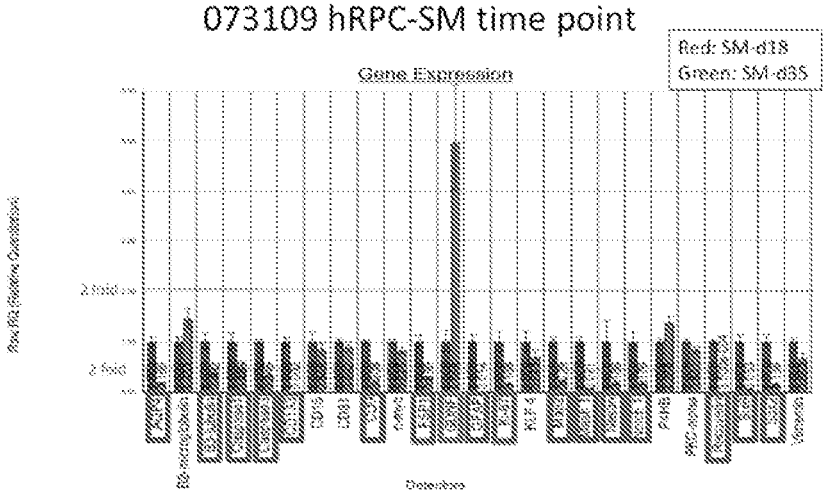

**FIGURE 28**

FIG. 29/55

A




B




C

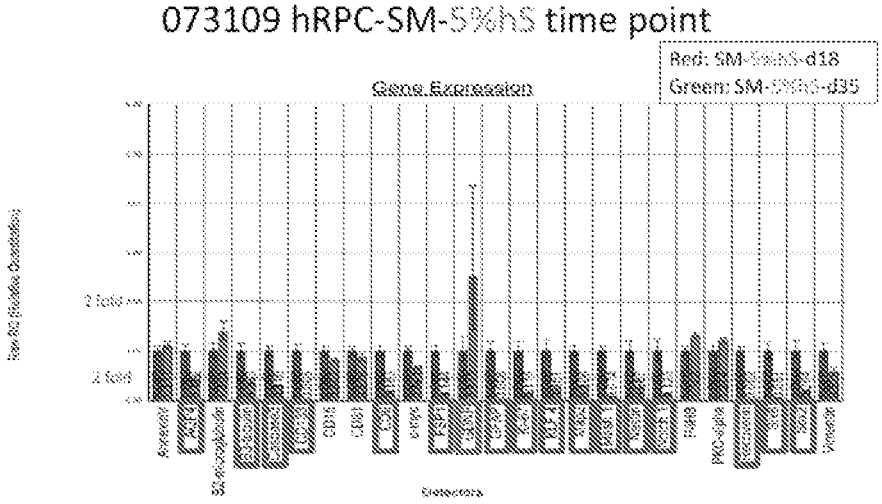



**FIGURE 29**

hRPC time point comparison

Red ↓ green ↑

| 01220thRPC | | 07dep9hRPC | | | 07310thRPC | |
|---|---|---|---|---|---|---|
| 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 | | | | |
| PKCa? | | PKCa | | | | |
| 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 | | | | | |
| AnexinV | AnexinV | AnexinV | | AnexinV | | |

**FIGURE 30**

WO 2012/158910 PCT/US2012/038342

FIG. 31/55

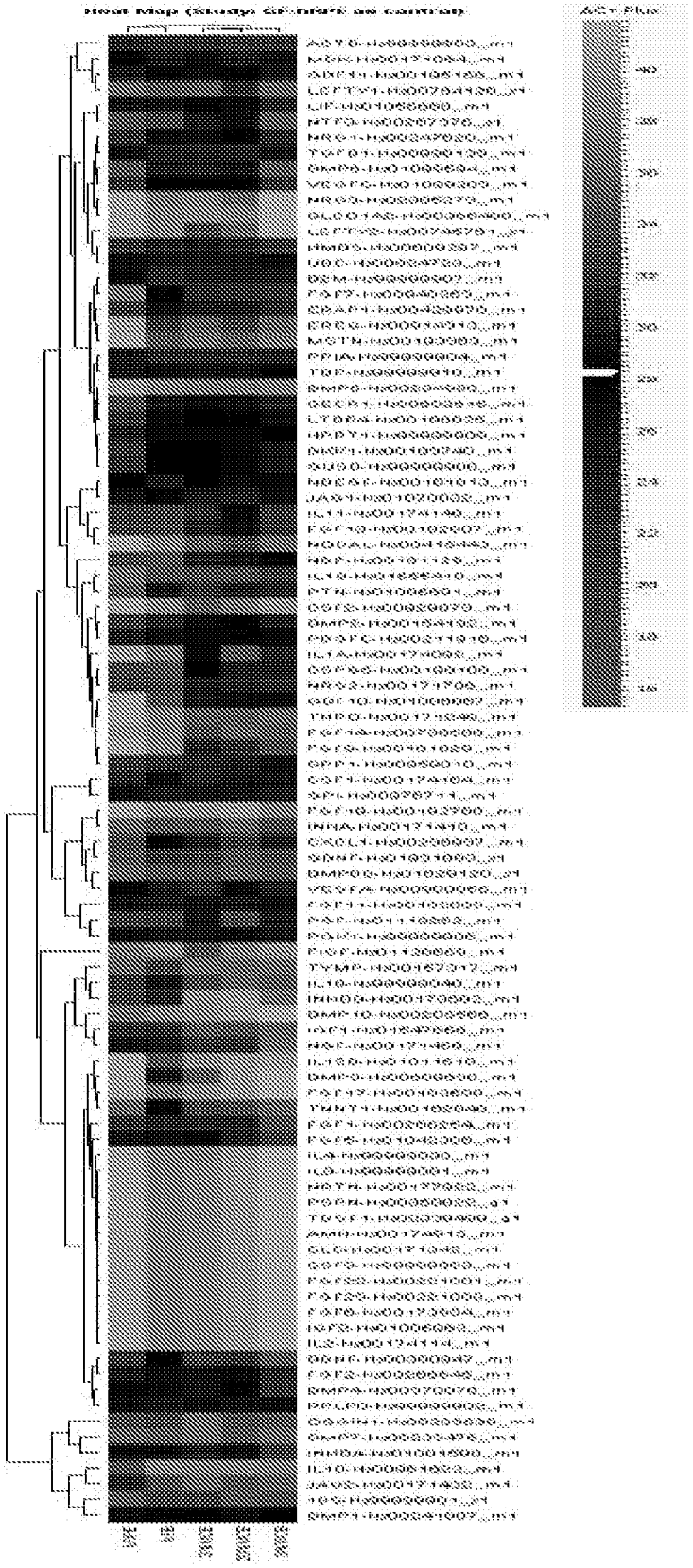


FIGURE 31

FIG. 32/55

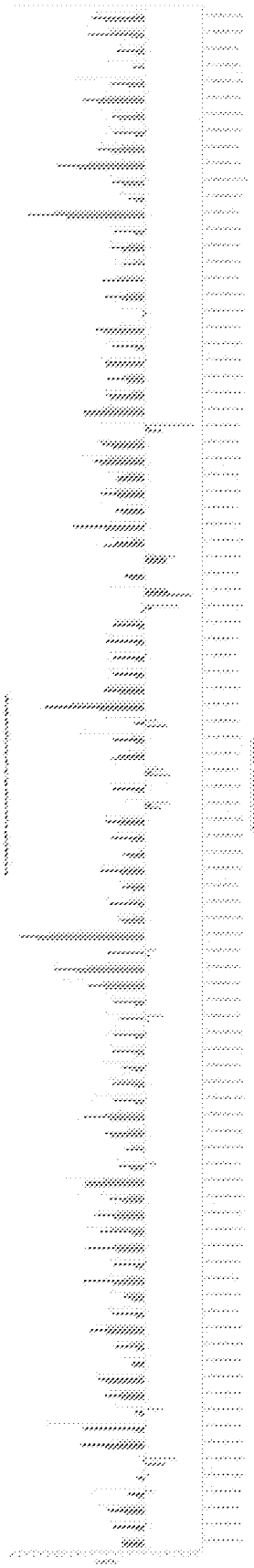


FIGURE 32

FIG. 33/55

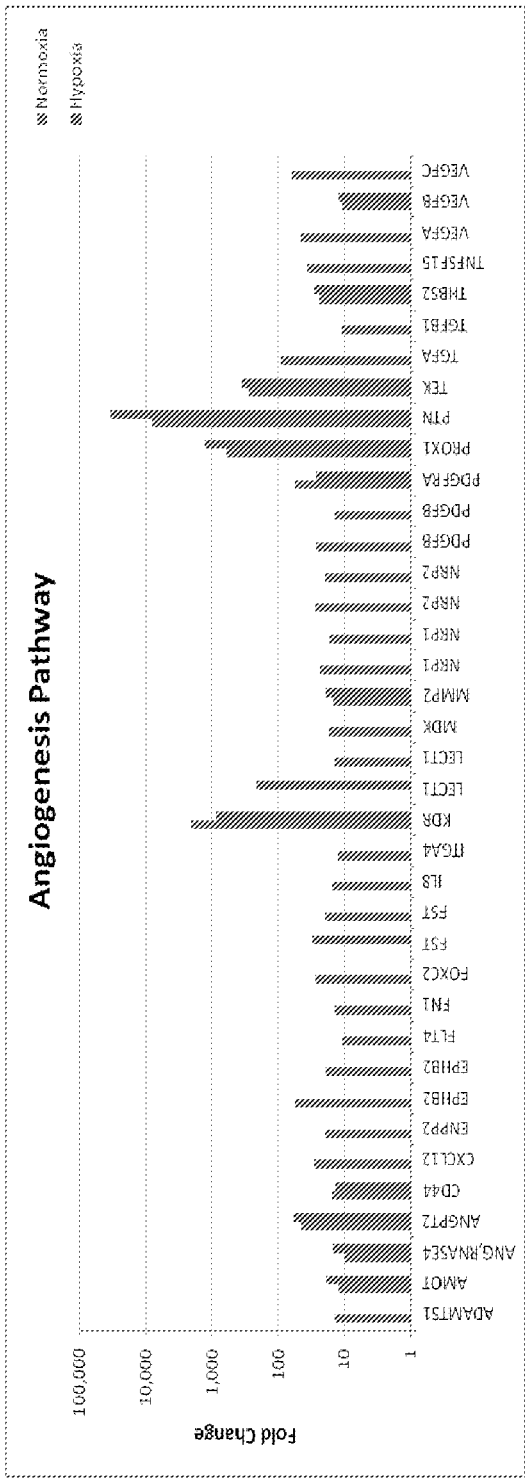
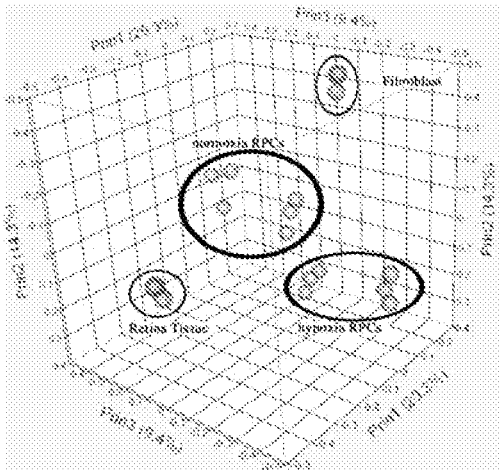


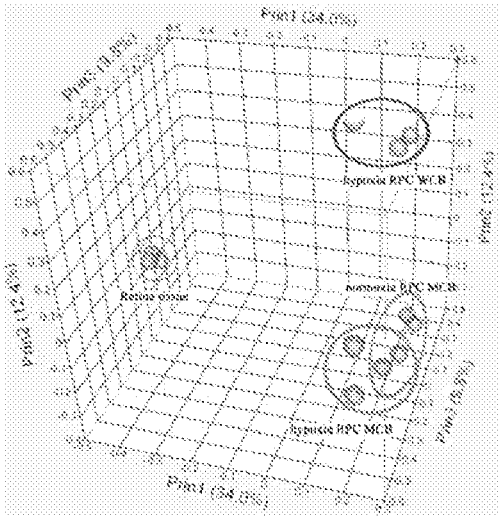

FIGURE 33

FIG. 34/55

A




B




C

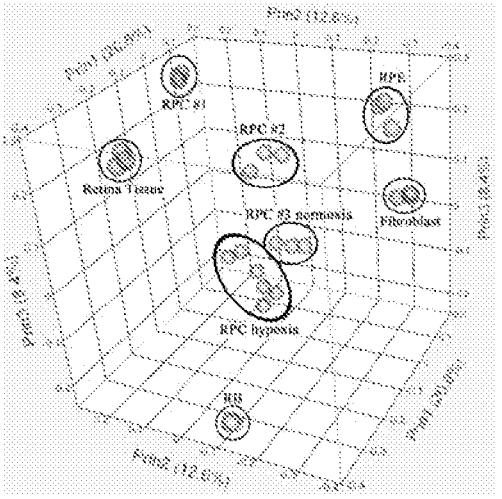



**FIGURE 34**

FIG. 35/55

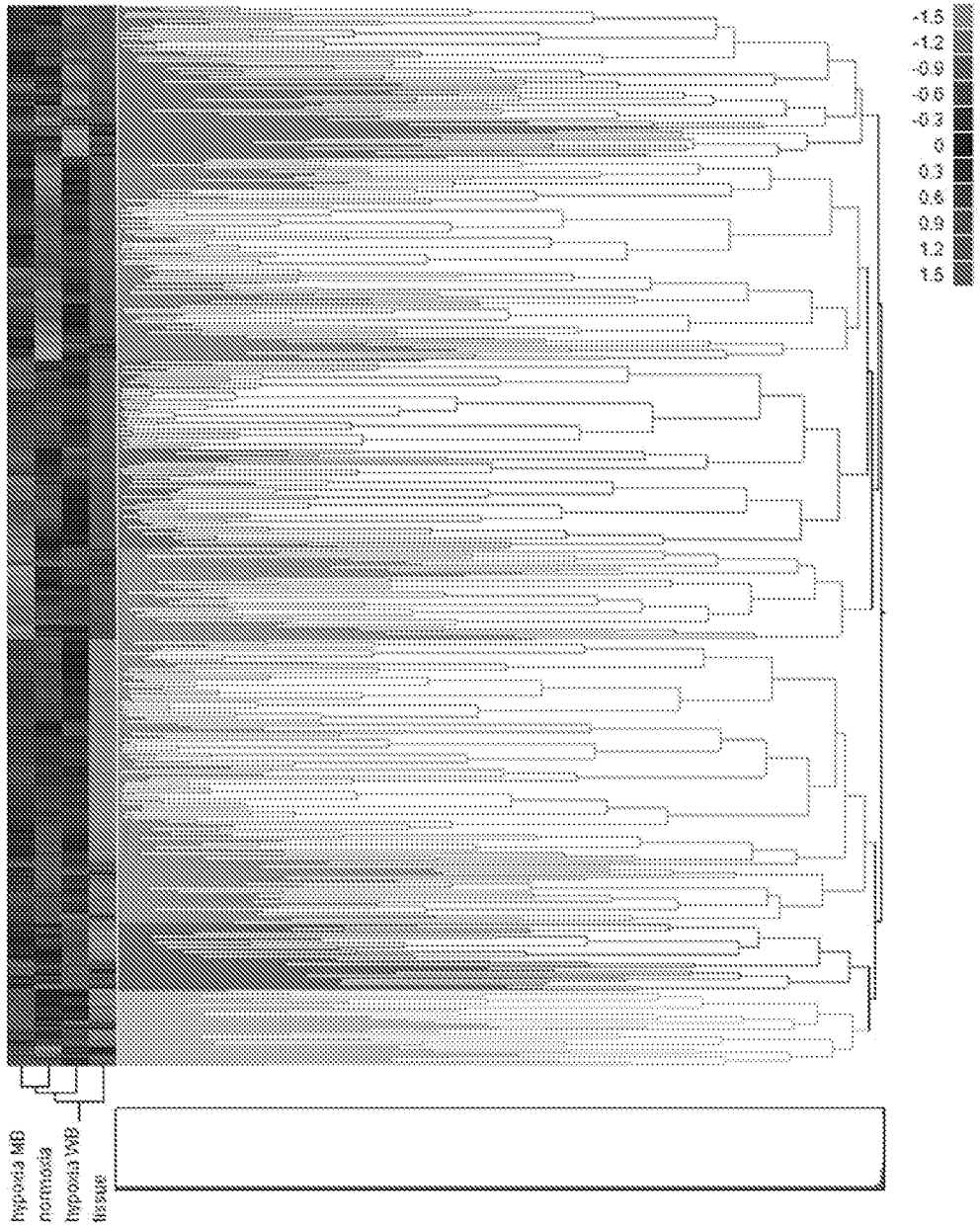



**FIGURE 35**

FIG. 36/55

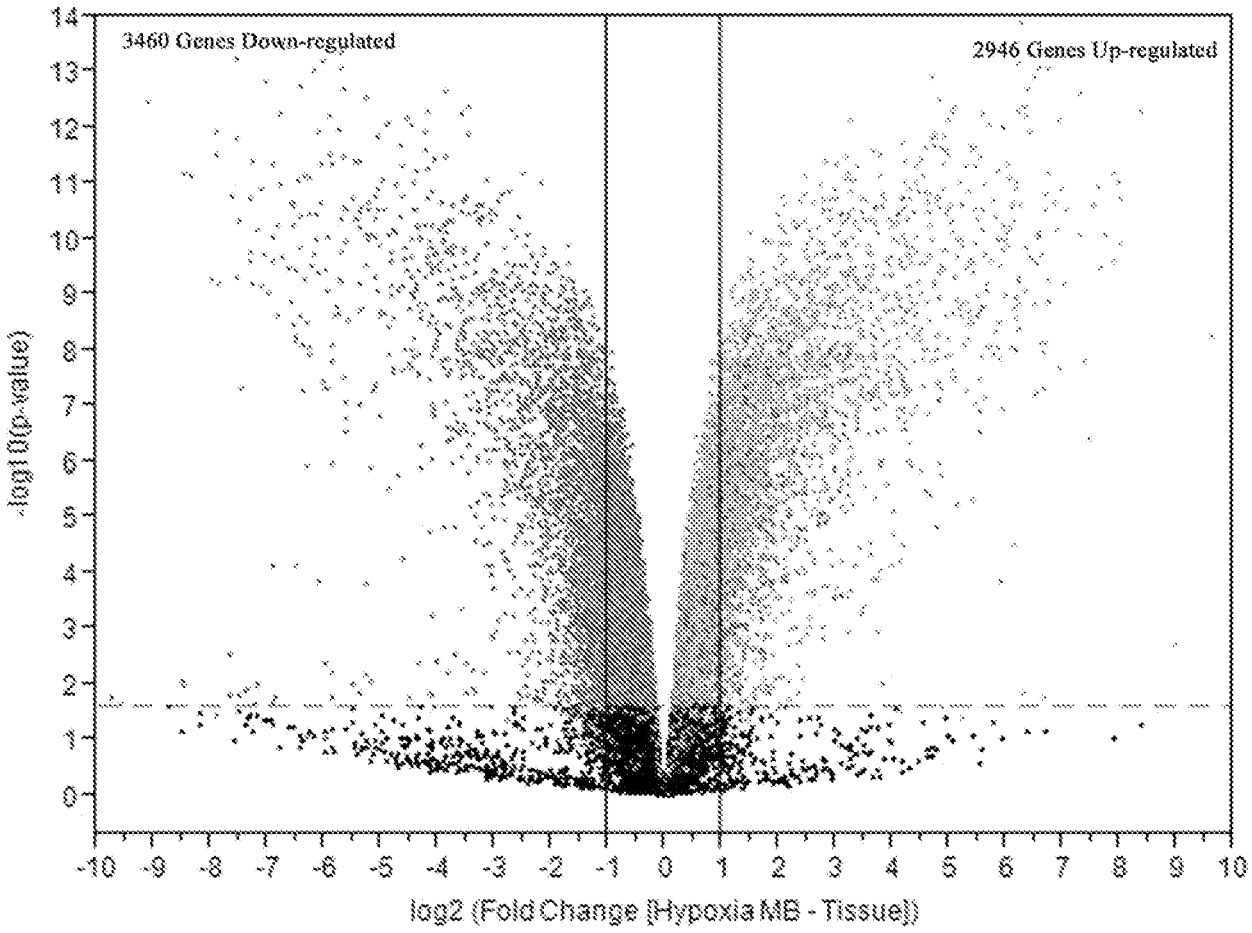
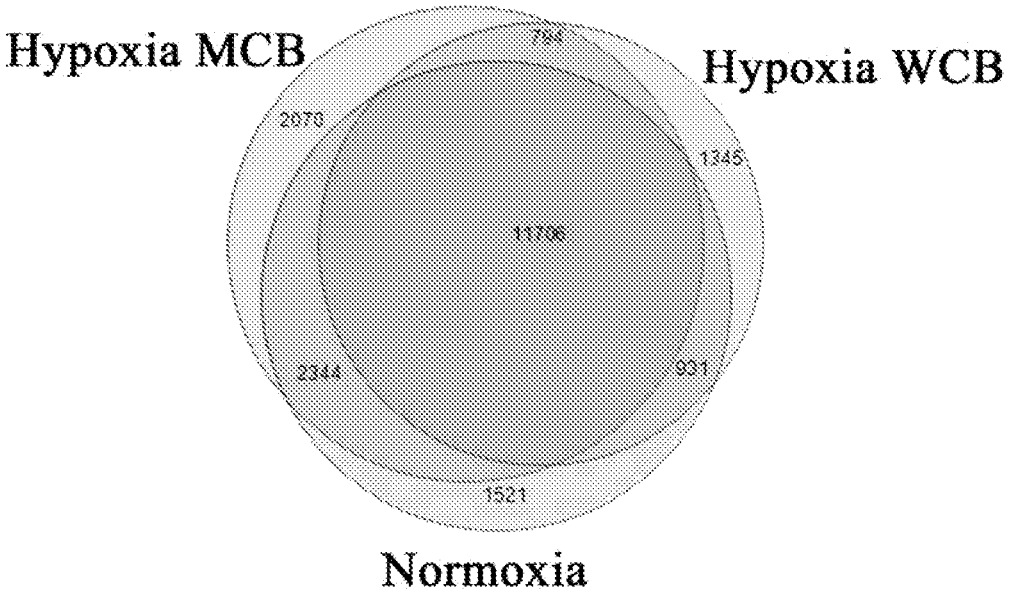



**FIGURE 36**

FIG. 37/55

A




B

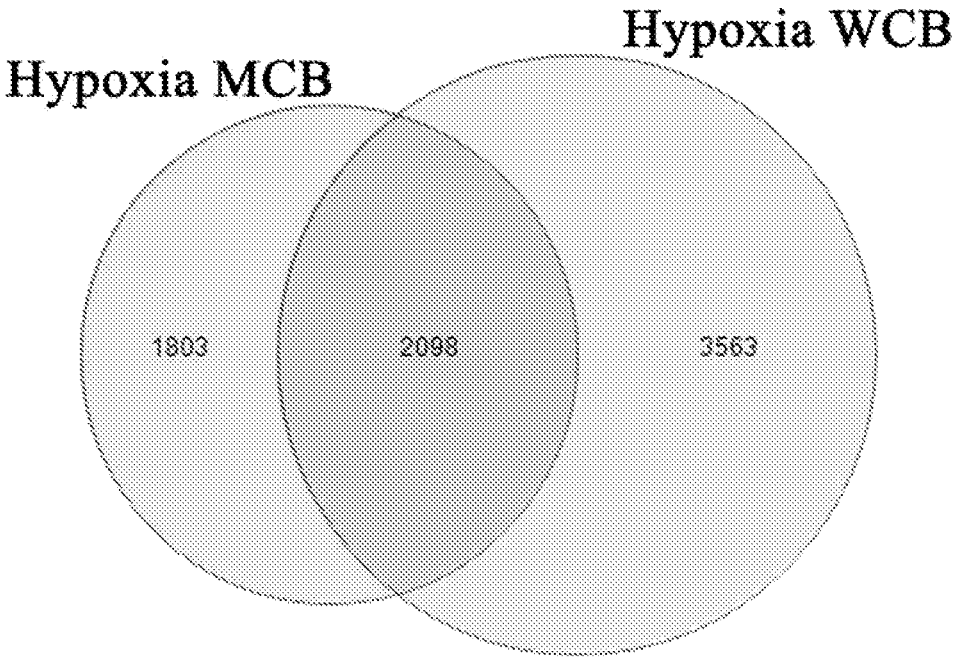
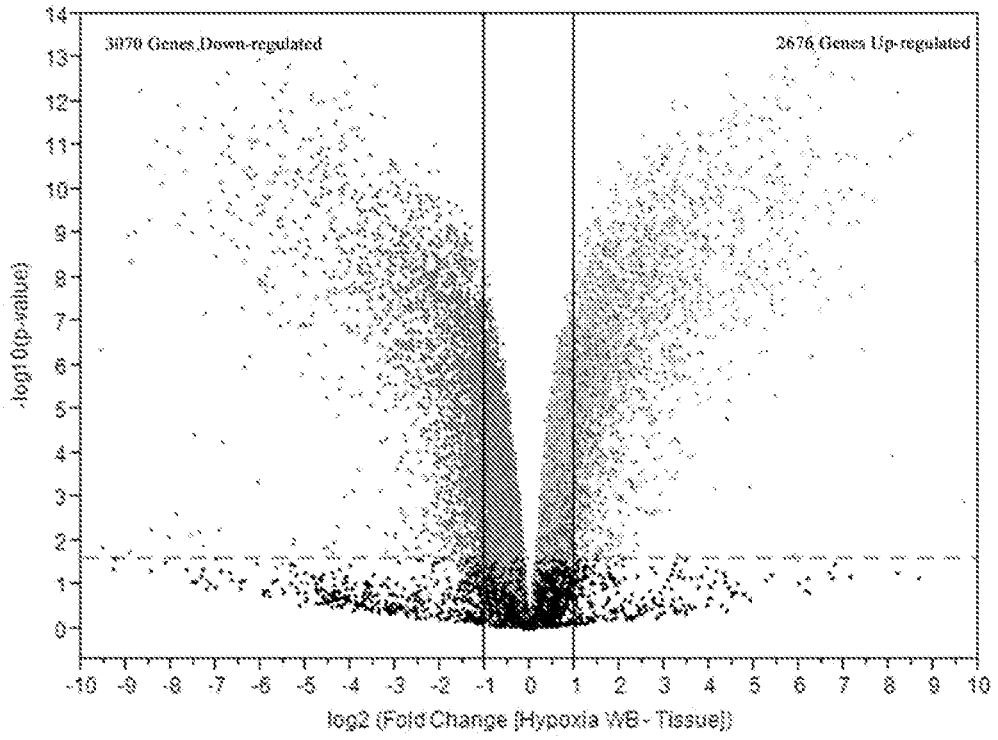



FIGURE 37

FIG. 38/55

A




B

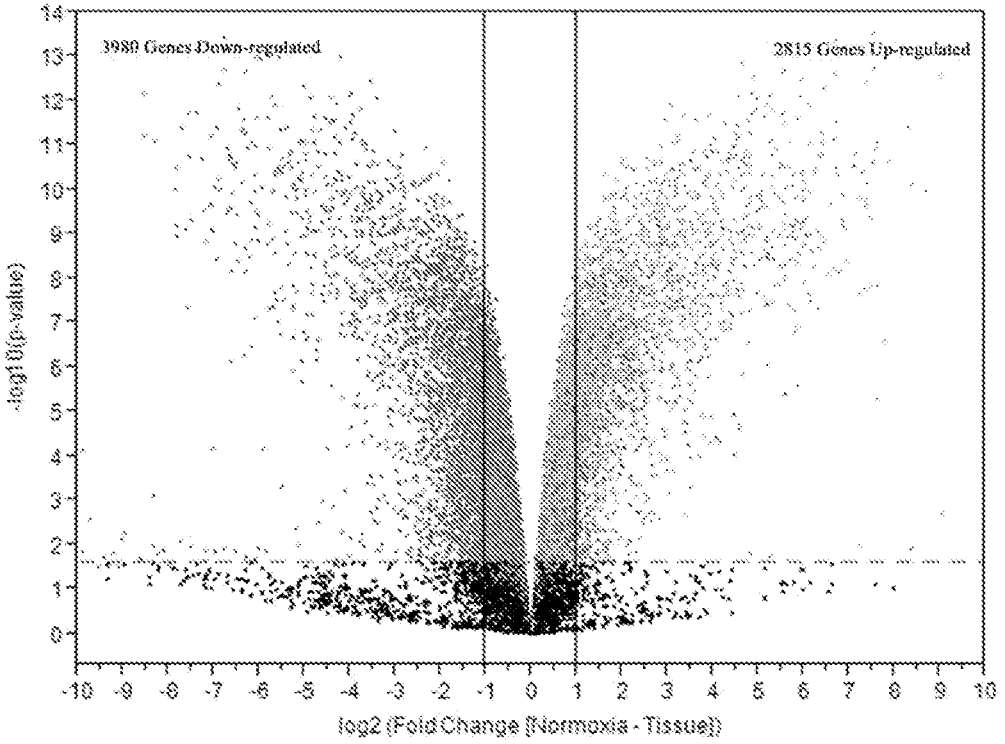
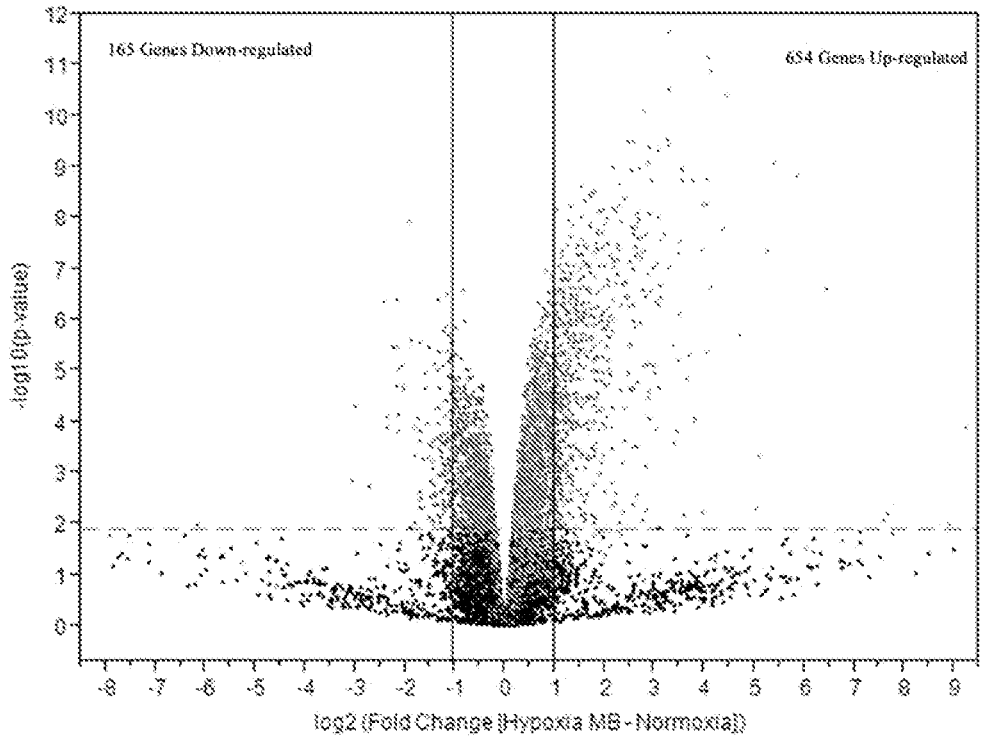



**FIGURE 38**

FIG. 39/55

A




B

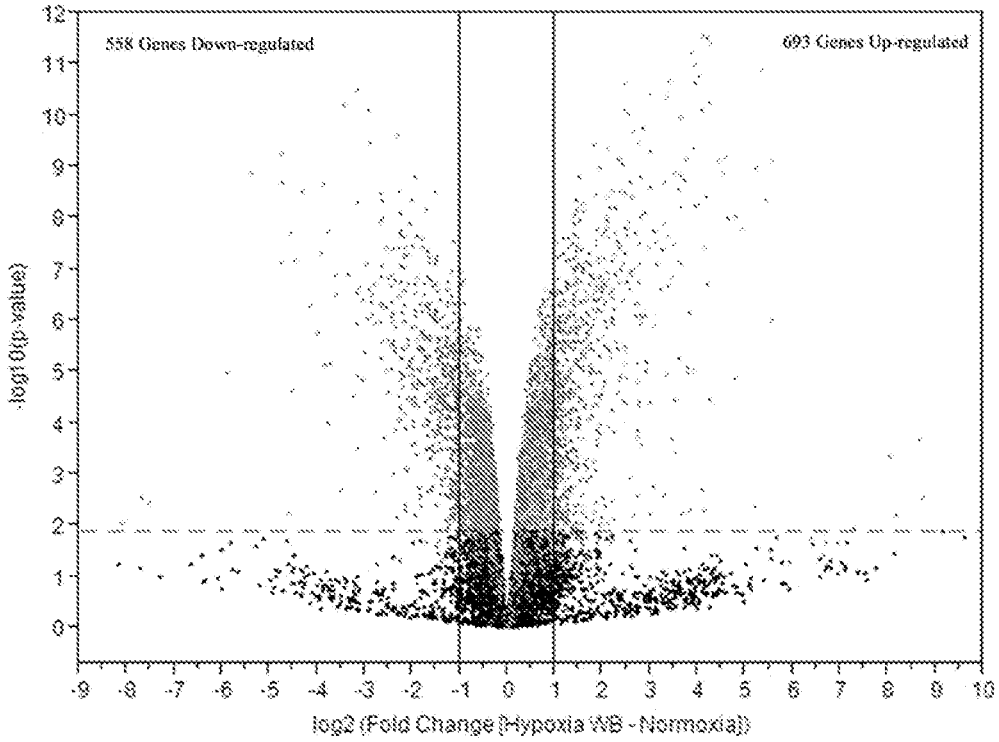



**FIGURE 39**

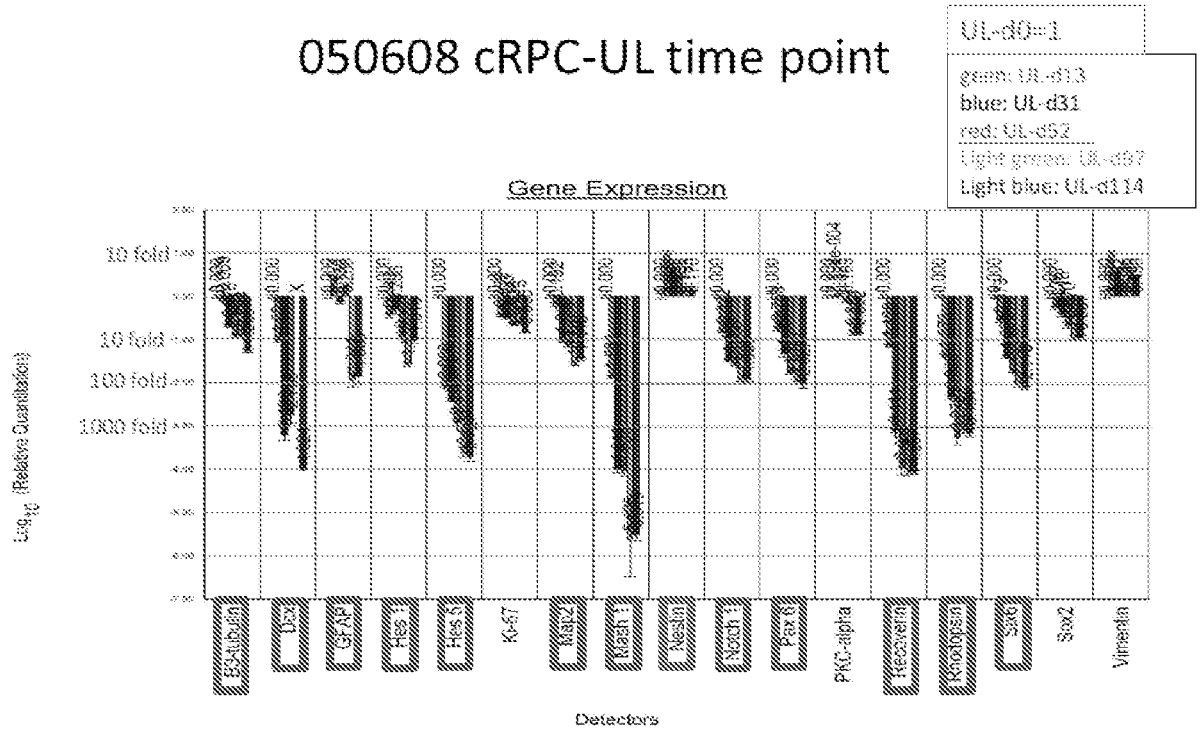
# cRPC time point design

| 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**

FIG. 41/55

A




B

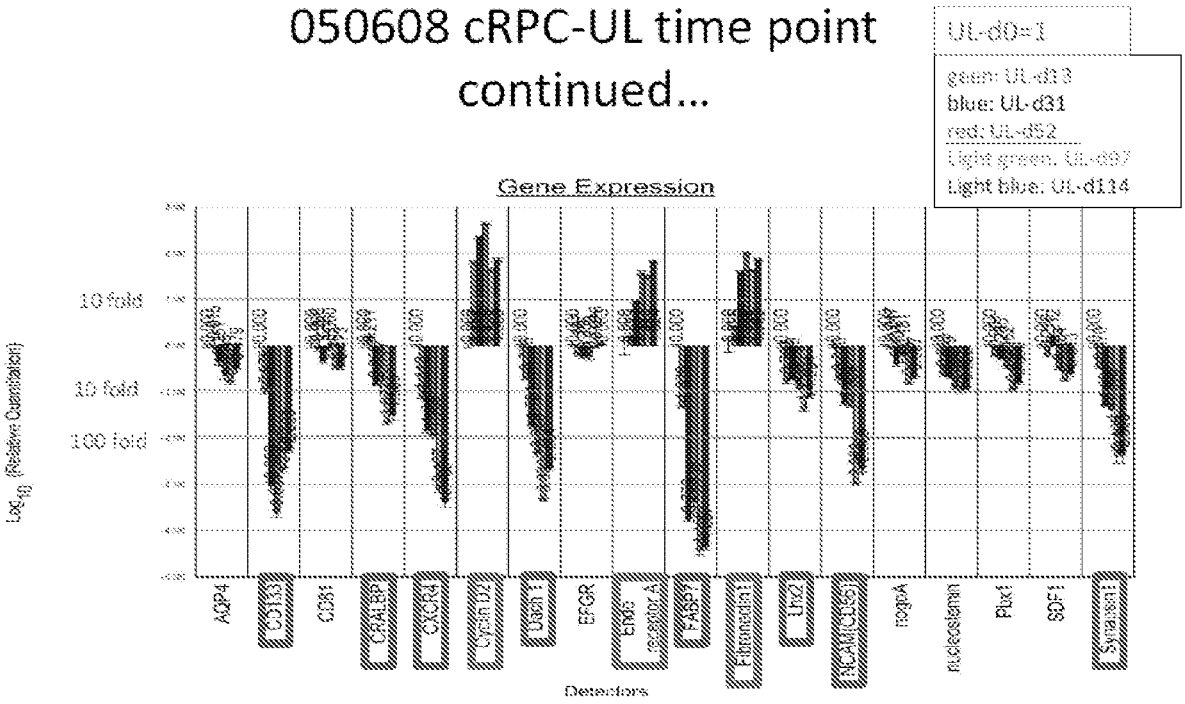
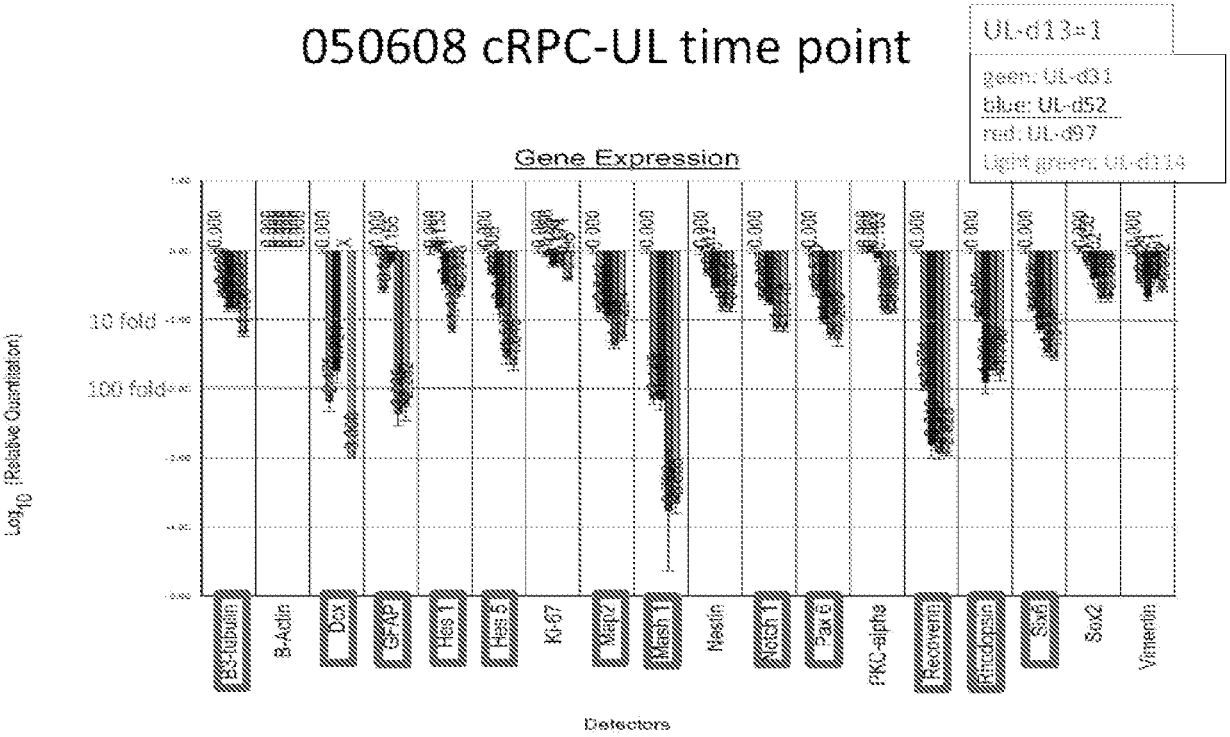



**FIGURE 41**

FIG. 42/55

A




B

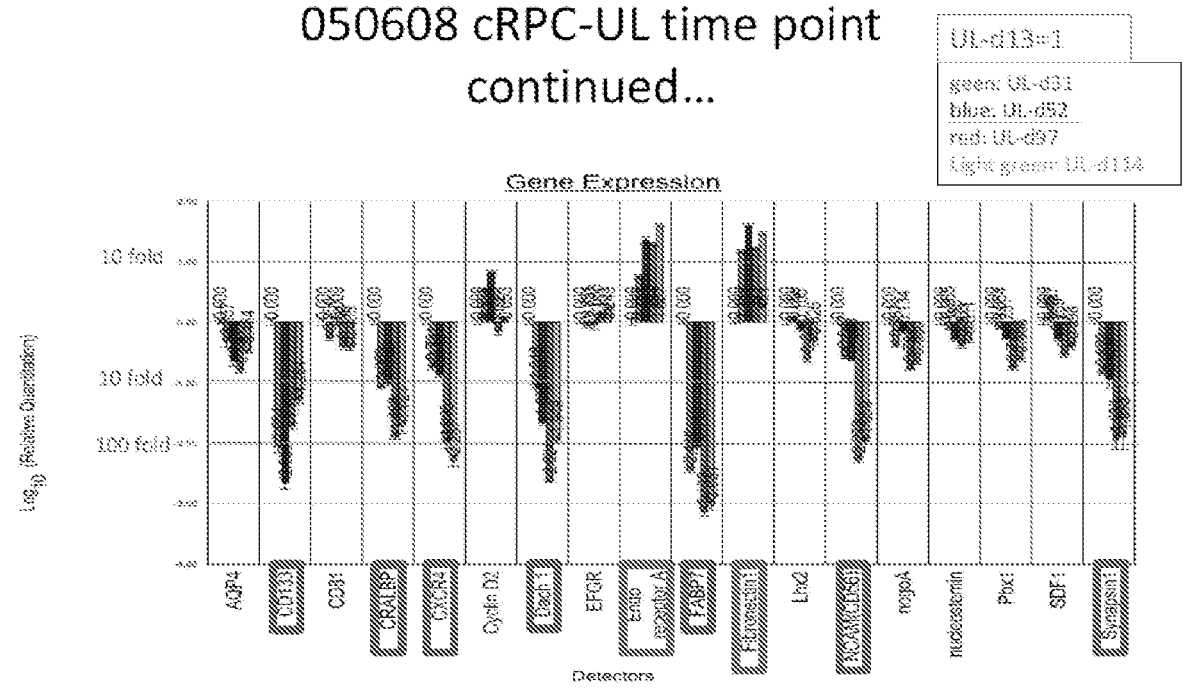
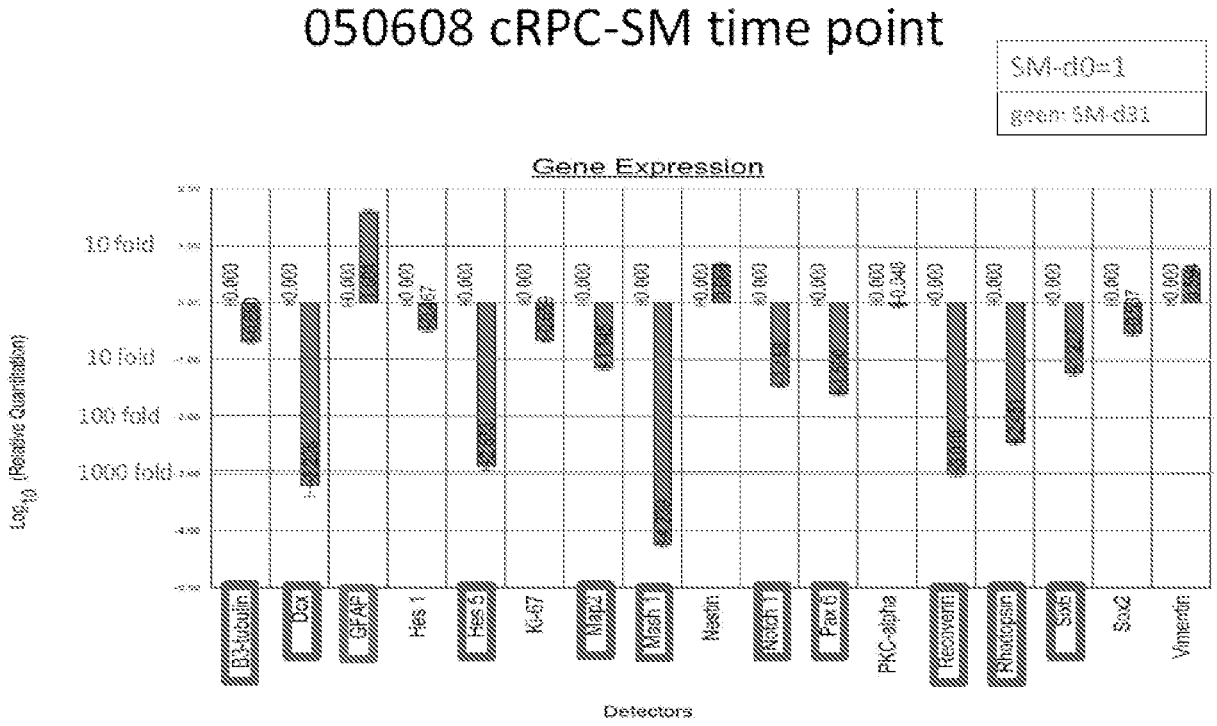



FIGURE 42

FIG. 43/55

A




B

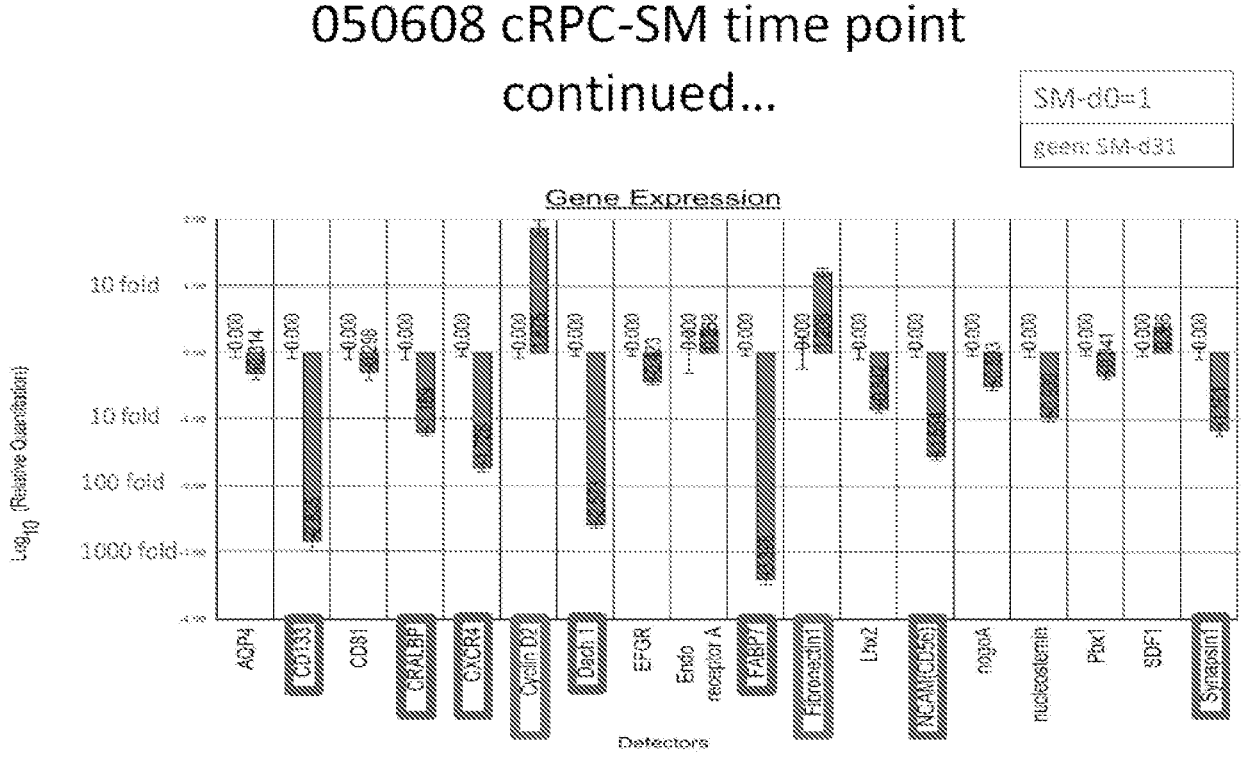



**FIGURE 43**

FIG. 44/55

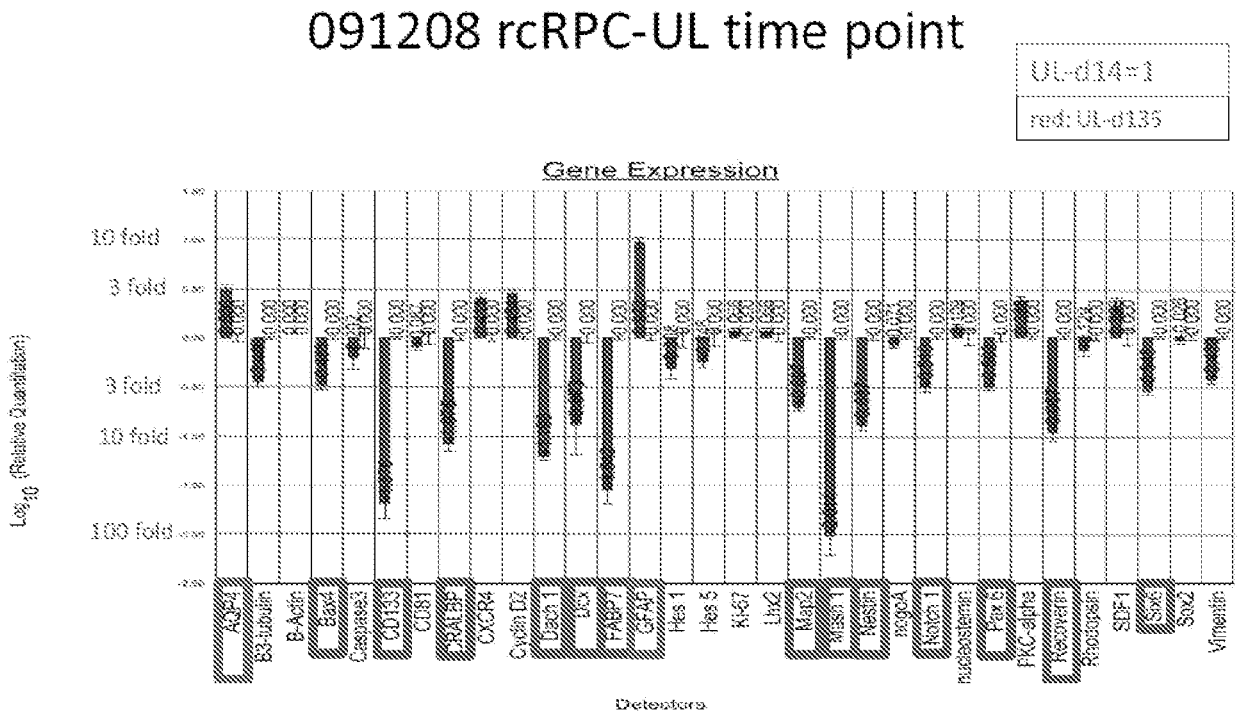
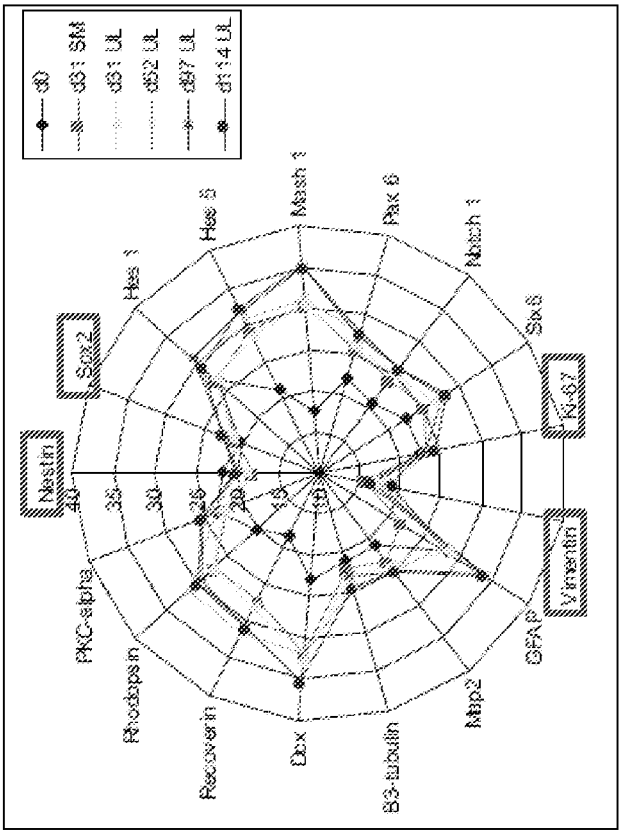



**FIGURE 44**

FIG. 45/55

cRPC time point



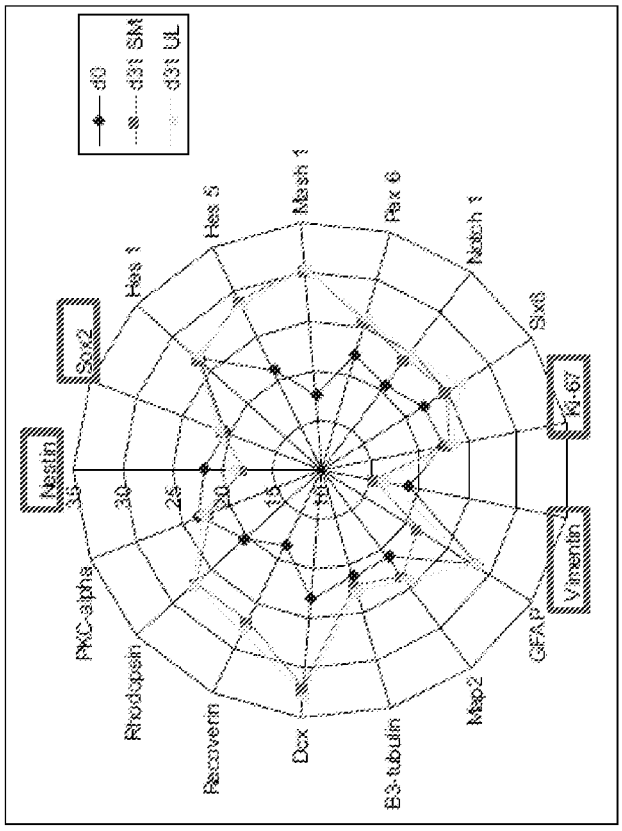




**FIGURE 45**

| Undifferentiated | Neural Induction | Differentiated | Comments with HEPES |
|---|---|---|---|
| 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 |
| | | | Rax6 |

**FIGURE 46**

WO 2012/158910 PCT/US2012/038342

FIG. 47/55

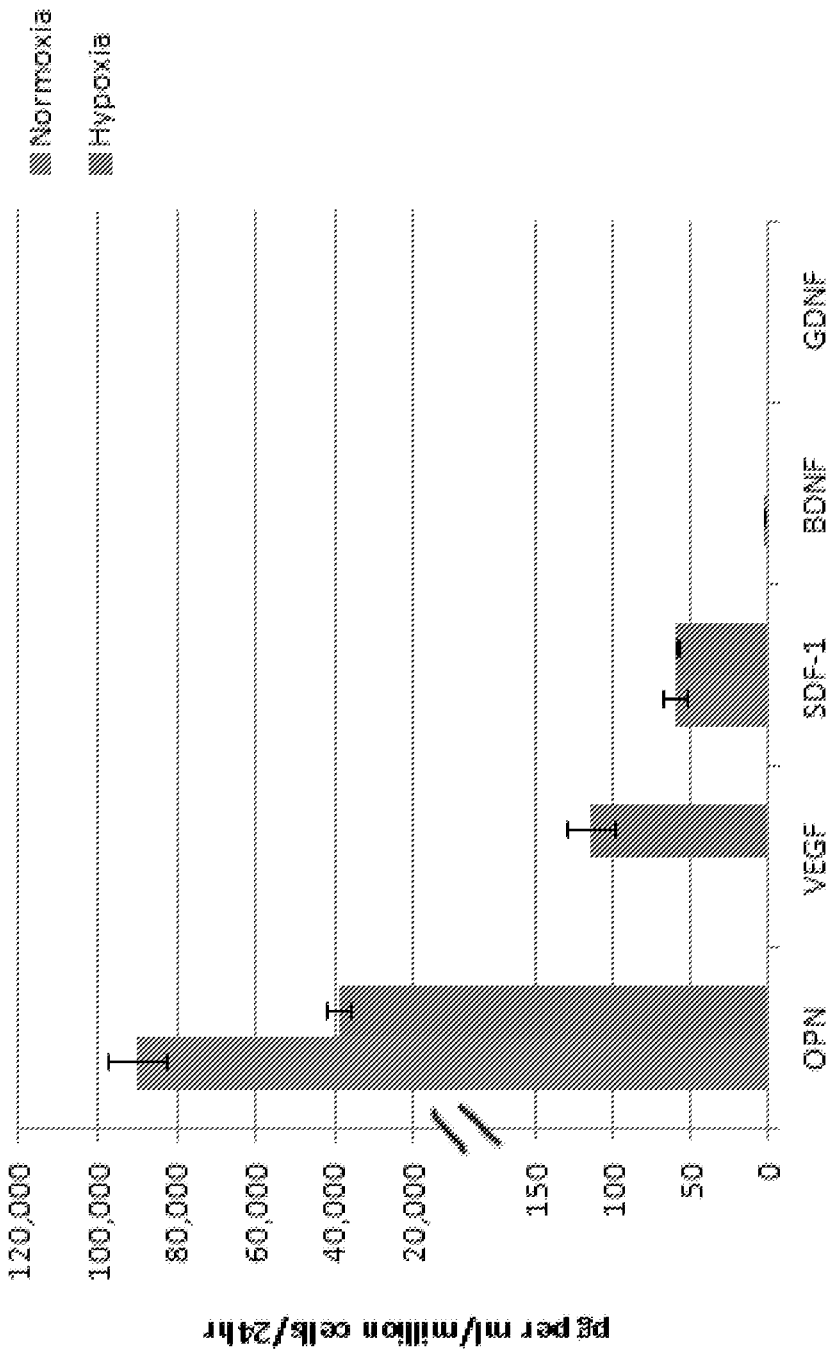



**FIGURE 47**

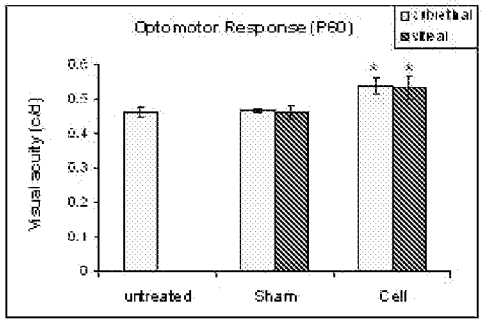
FIG. 48/55

| | 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% |

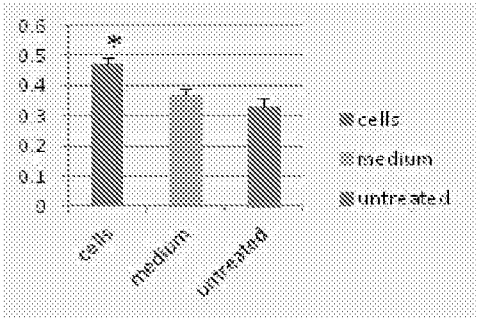
**FIGURE 48**

WO 2012/158910

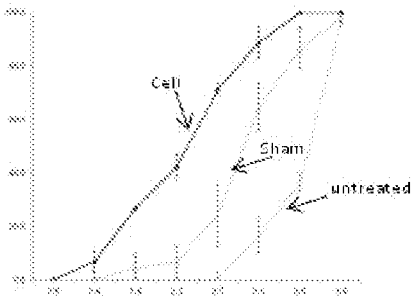
A Optomotor response P60



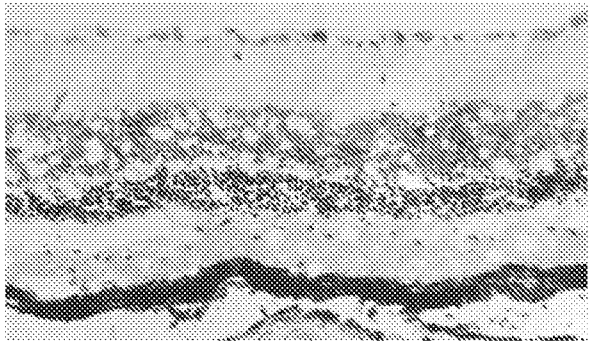

B Optomotor response P90




C Luminance threshold response P90




D Histology P60 (intravitreal injection)



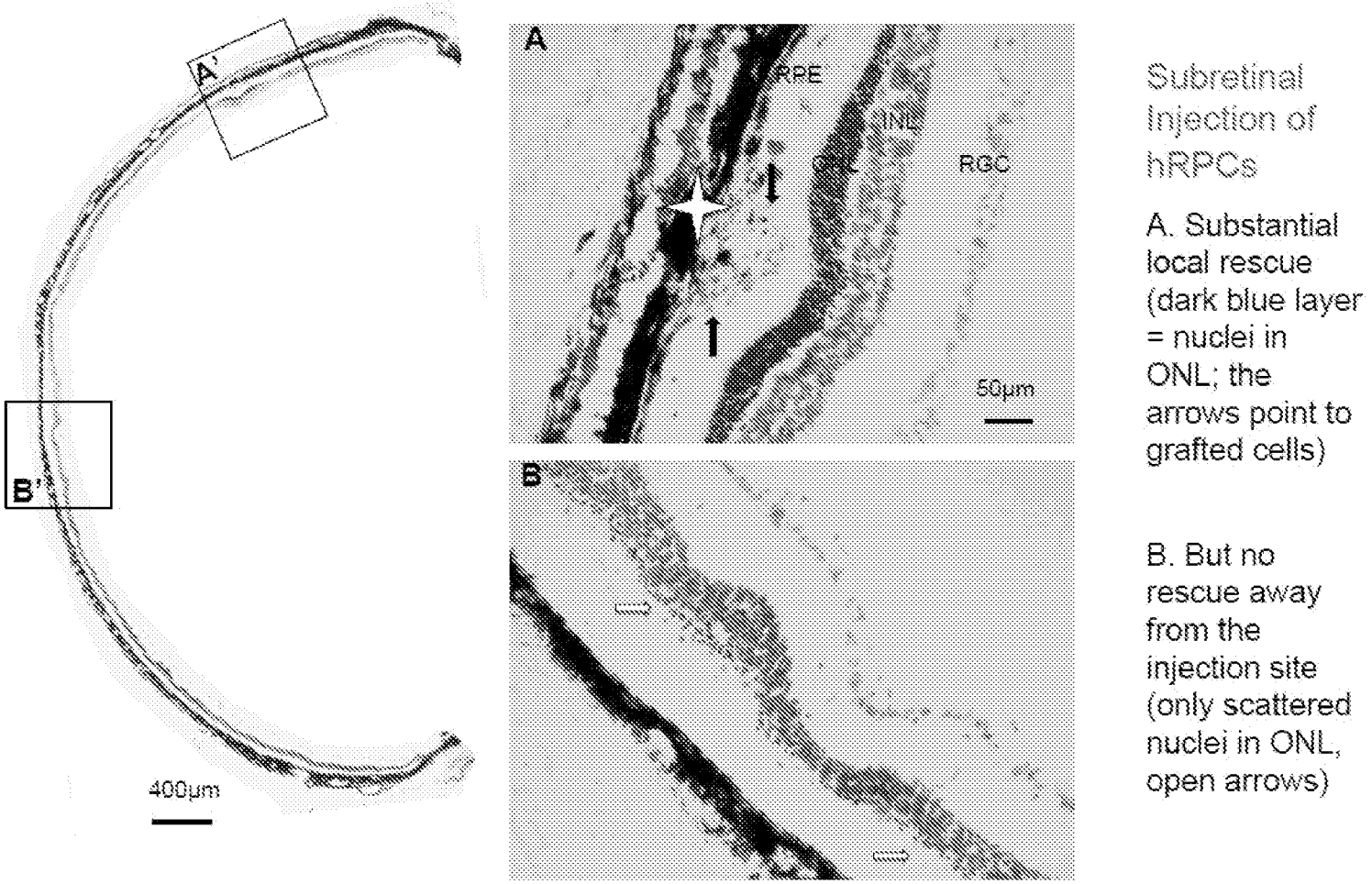
E Histology P90 (intravitreal injection)



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

**FIGURE 49**

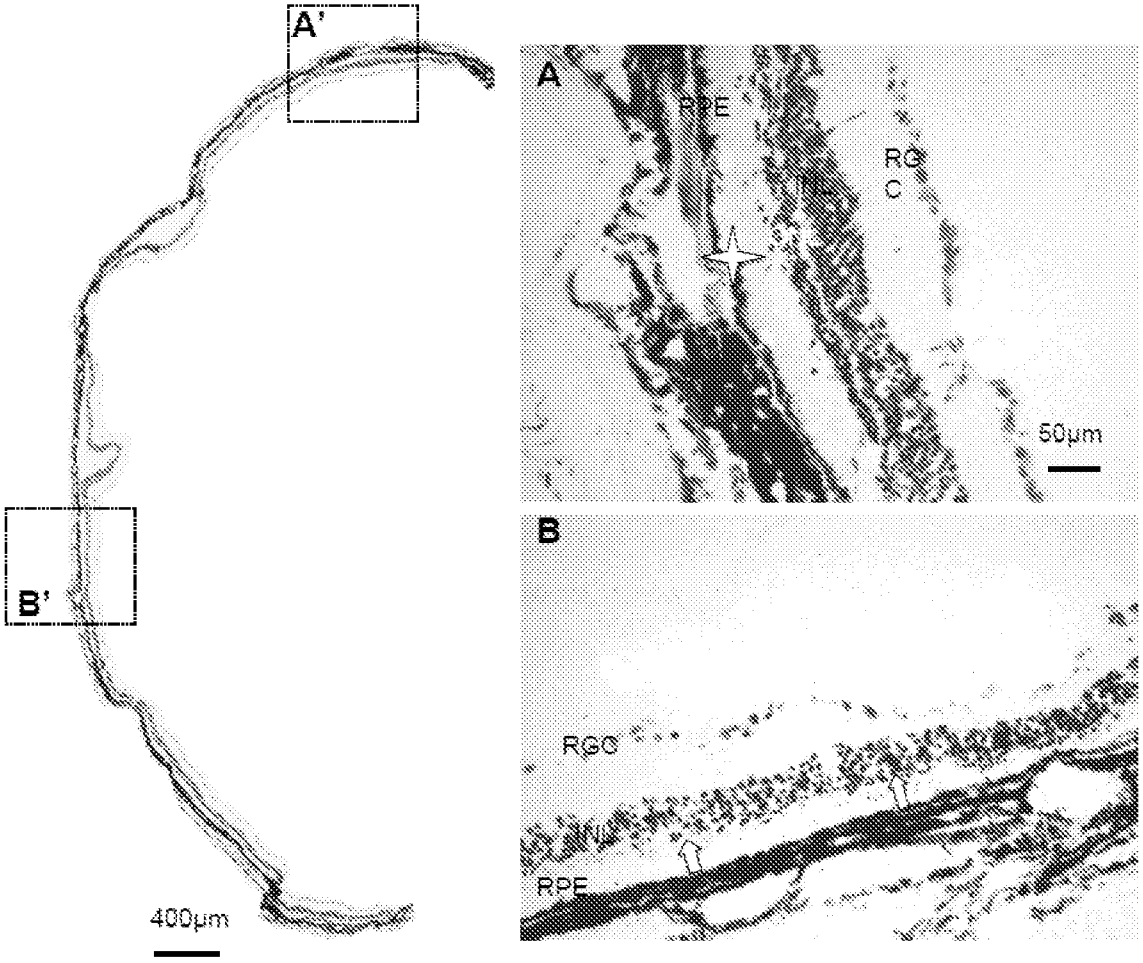
PCT/US2012/038342




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**

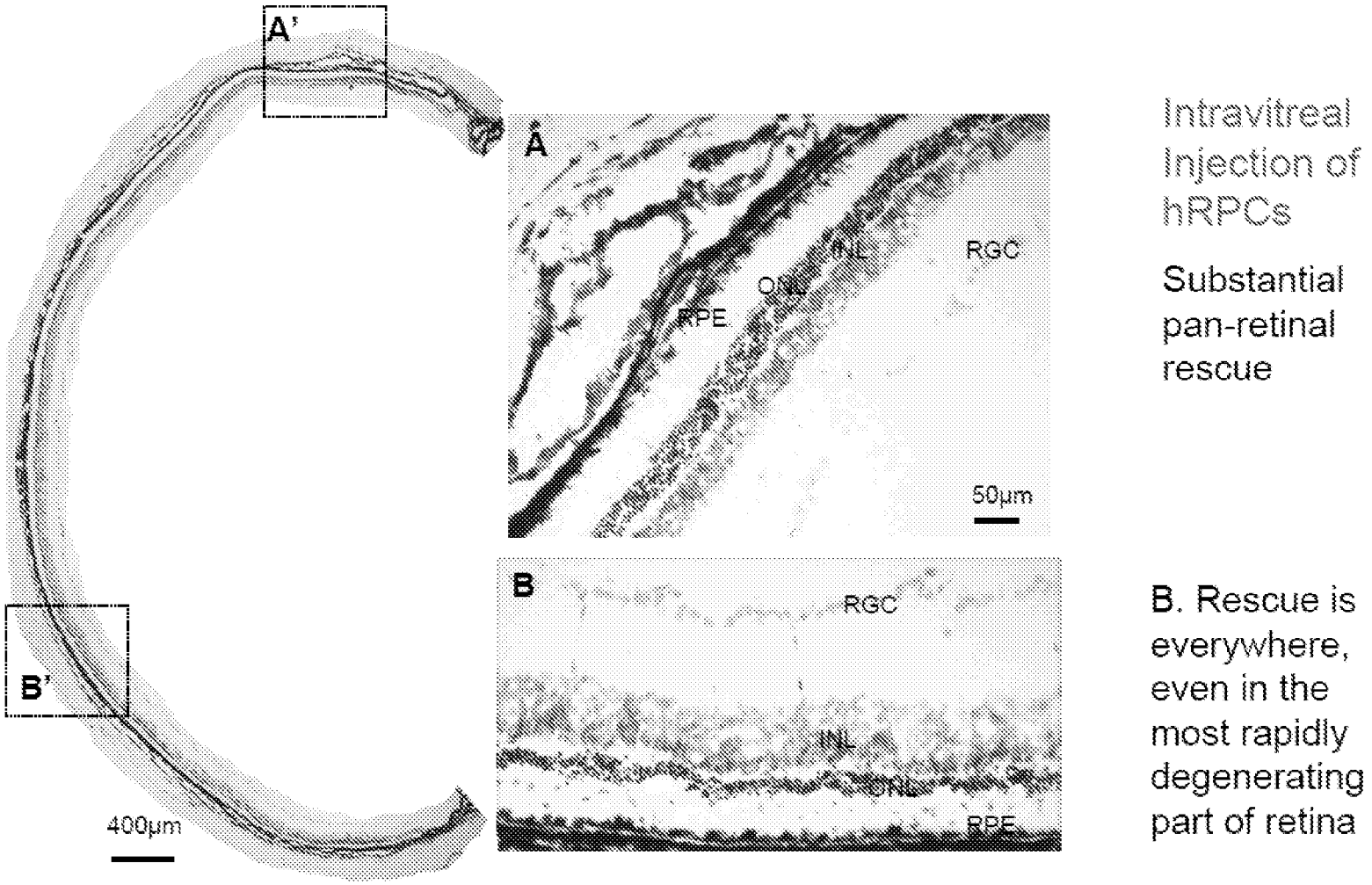
WO 2012/158910 PCT/US2012/038342




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**

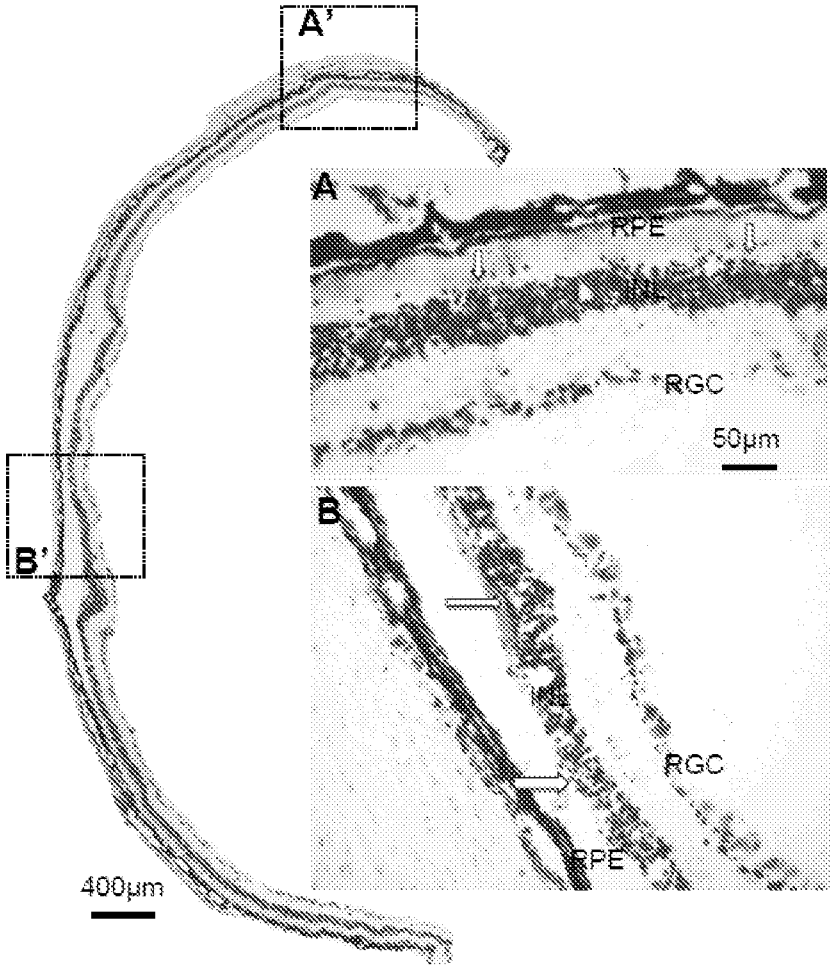
WO 2012/158910 PCT/US2012/038342




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**

WO 2012/158910 PCT/US2012/038342




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)

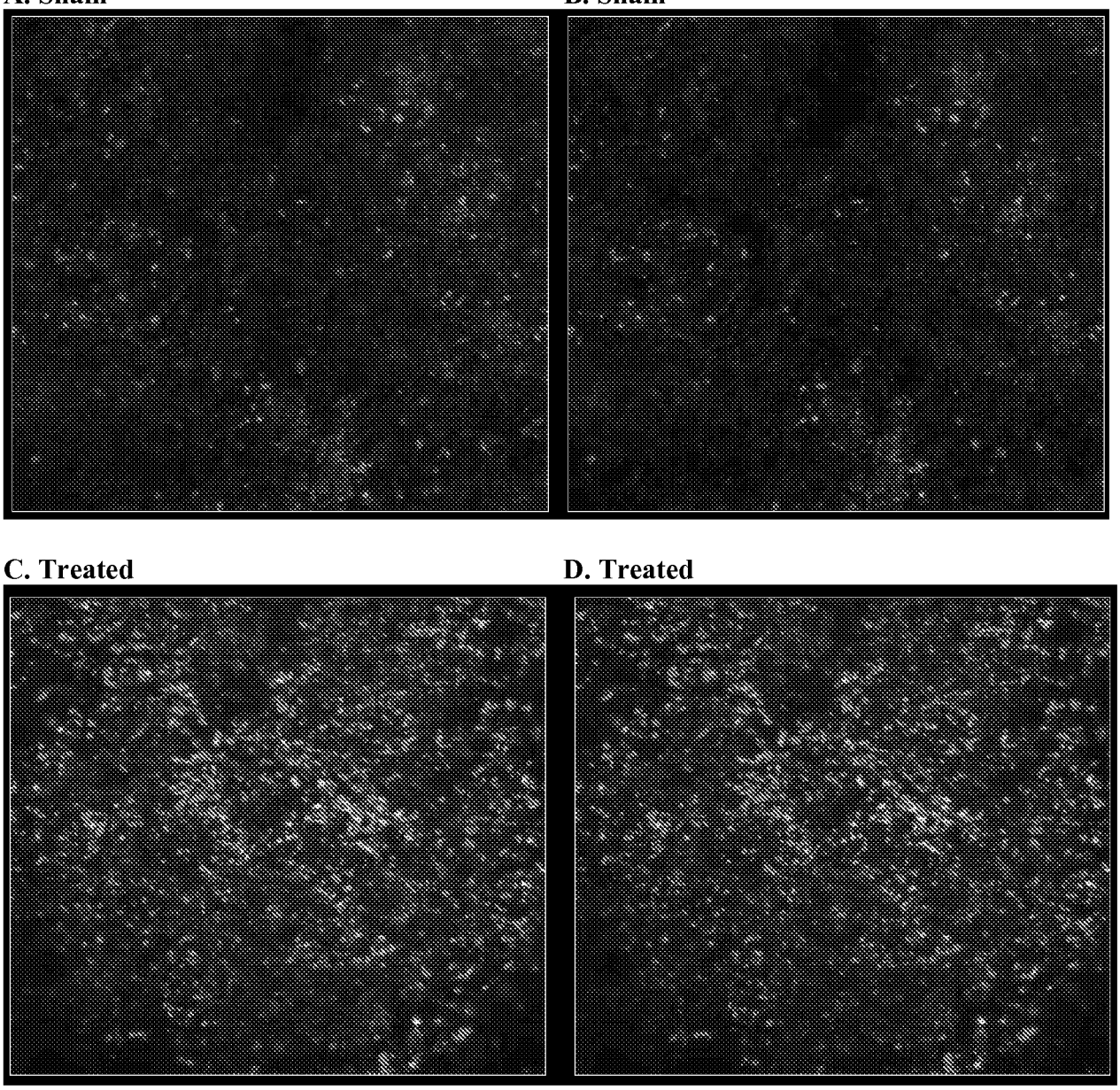
**FIGURE 53**

WO 2012/158910 PCT/US2012/038342

FIG. 54/55

**A. Sham** **B. Sham**

**C. Treated** **D. Treated**



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

**FIGURE 54**

FIG. 55/55

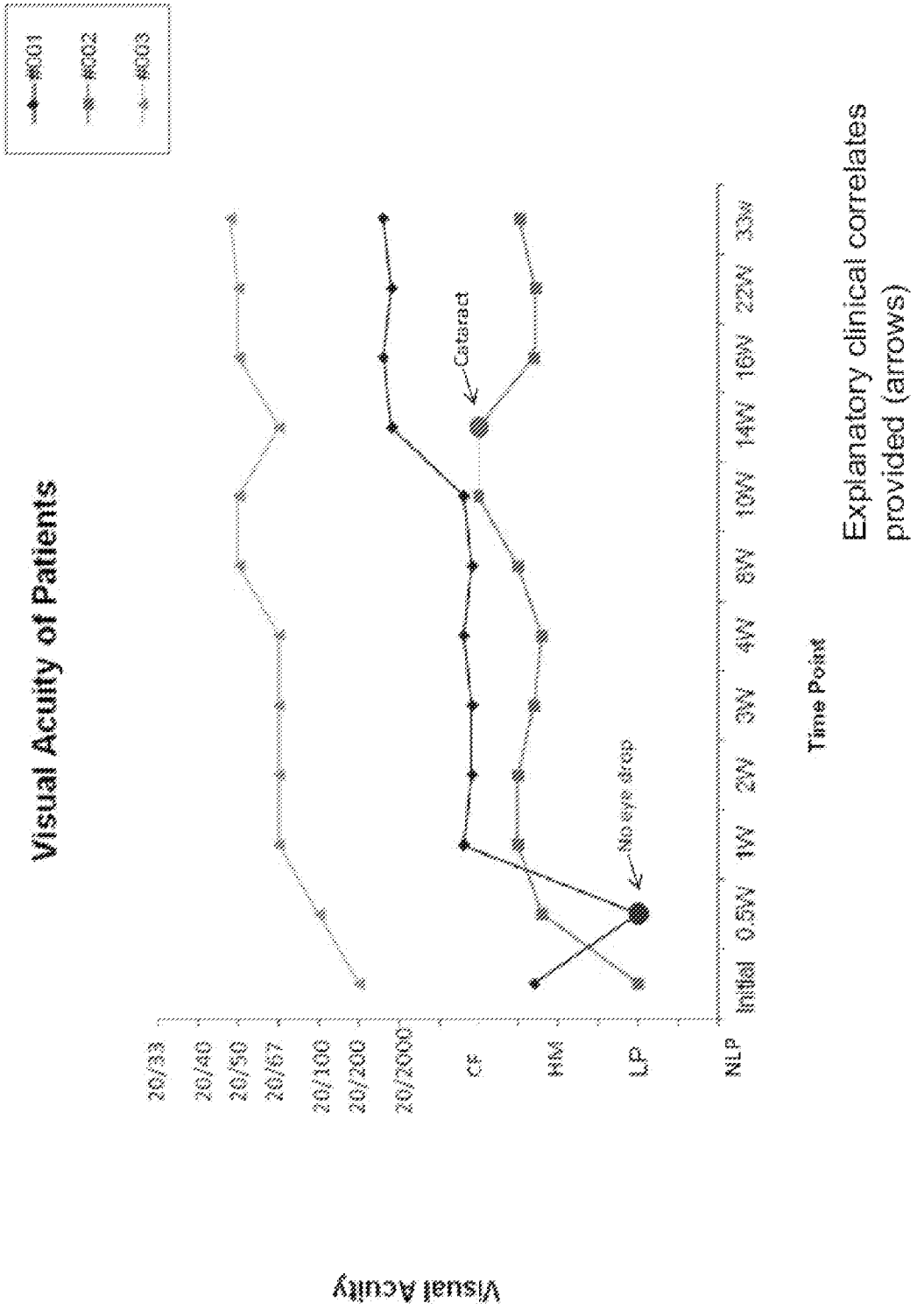



**FIGURE 55**