FIG. 1

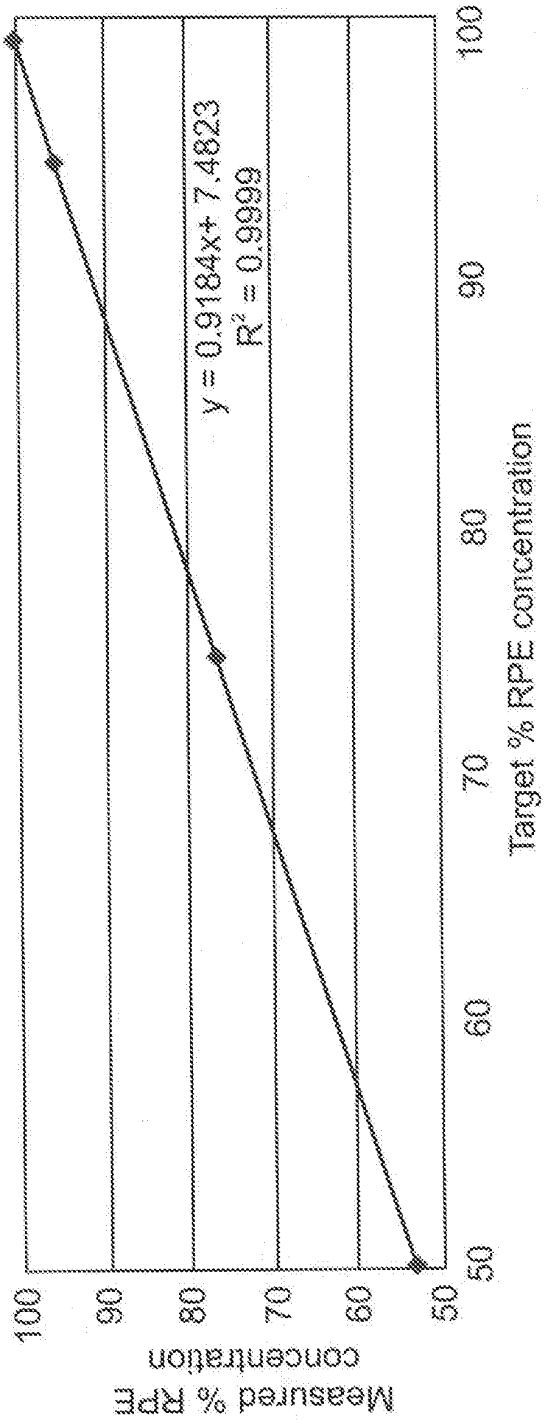

y = 0.9184x + 7.4823
R² = 0.9999
Target % RPE concentration
Measured % RPE concentration
100
90
80
70
60
50

FIG. 2

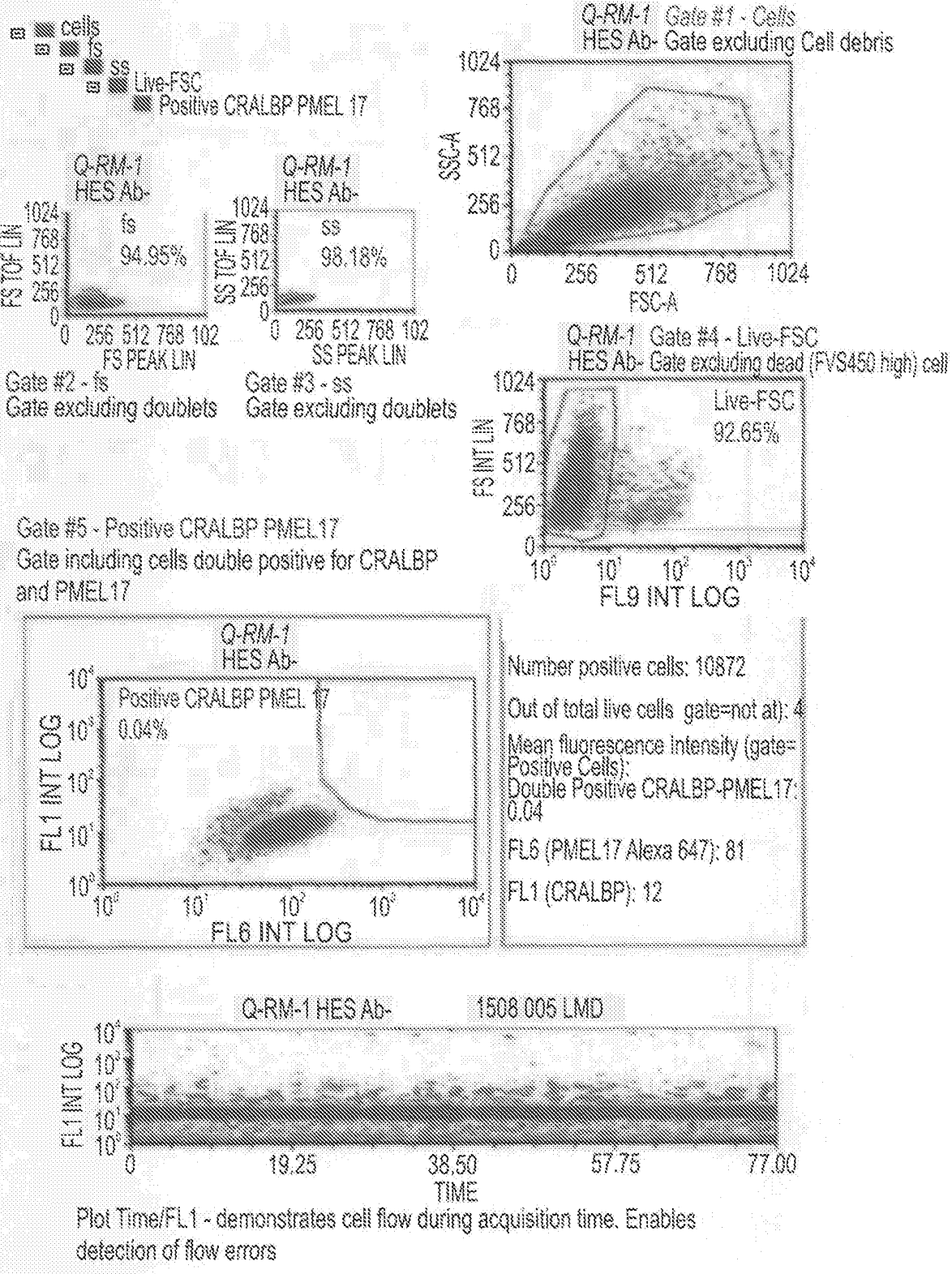

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
HES Ab-
fs
94.95%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
HES Ab-
ss
98.18%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
HES Ab- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
HES Ab- Gate excluding dead (FVS450 high) cell
Live-FSC
92.65%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1
HES Ab-
Positive CRALBP PMEL 17
0.04%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 10872
Out of total live cells gate=not at): 4
Mean fluorescence intensity (gate=Positive Cells):
Double Positive CRALBP-PMEL17: 0.04
FL6 (PMEL17 Alexa 647): 81
FL1 (CRALBP): 12
Q-RM-1 HES Ab-
1508 005 LMD
FL1 INT LOG
TIME
Plot Time/FL1 - demonstrates cell flow during acquisition time. Enables detection of flow errors

FIG. 3

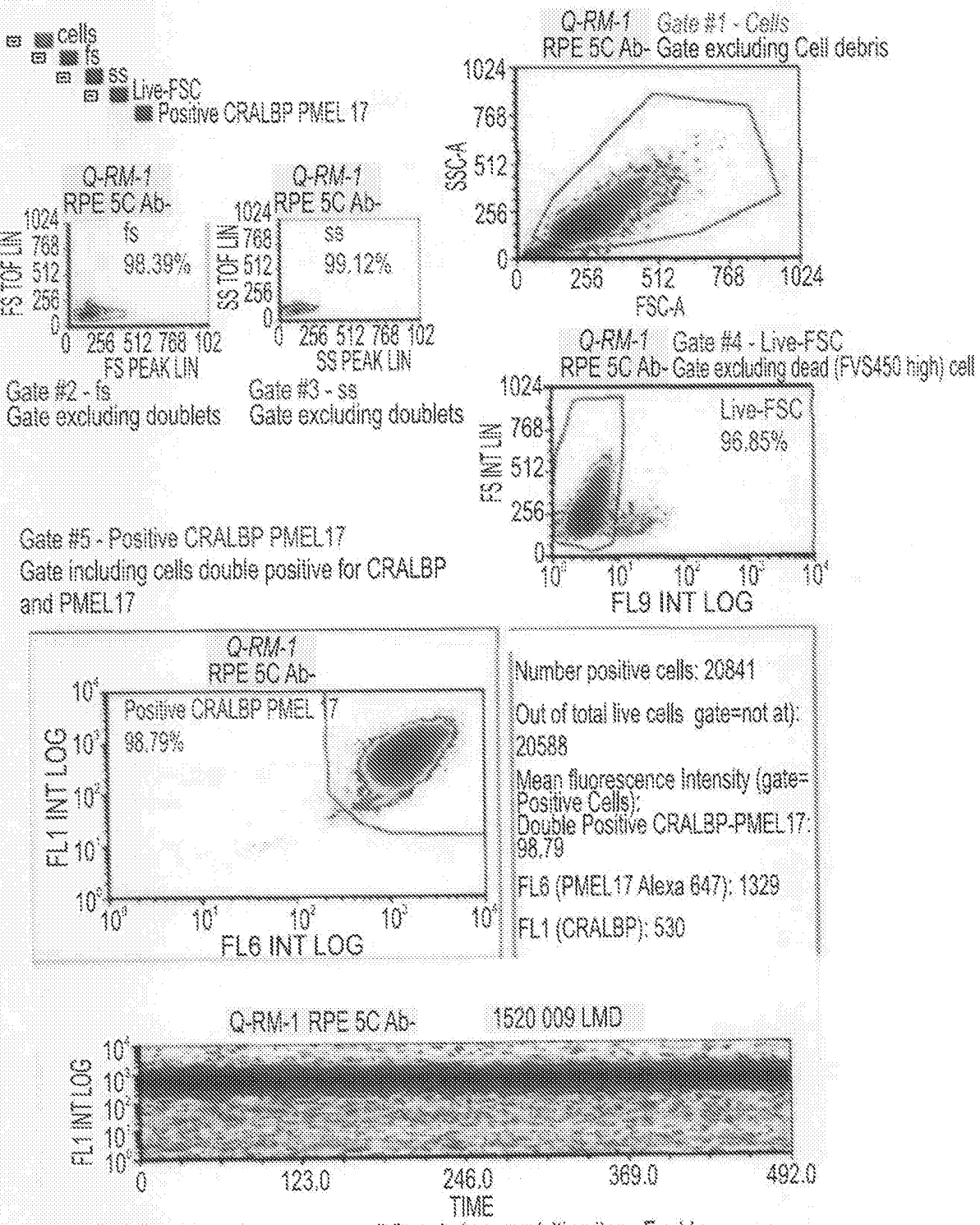

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
RPE 5C Ab-
fs
98.39%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
RPE 5C Ab-
ss
99.12%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
RPE 5C Ab- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
RPE 5C Ab- Gate excluding dead (FVS450 high) cell
Live-FSC
96.85%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1
RPE 5C Ab-
Positive CRALBP PMEL 17
98.79%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 20841
Out of total live cells gate=not at): 20588
Mean fluorescence intensity (gate= Positive Cells):
Double Positive CRALBP-PMEL17: 98.79
FL6 (PMEL17 Alexa 647): 1329
FL1 (CRALBP): 530
Q-RM-1 RPE 5C Ab- 1520 009 LMD
FL1 INT LOG
TIME
Plot Time/FL1 - demonstrates cell flow during acquisition time. Enables detection of flow errors

FIG. 4

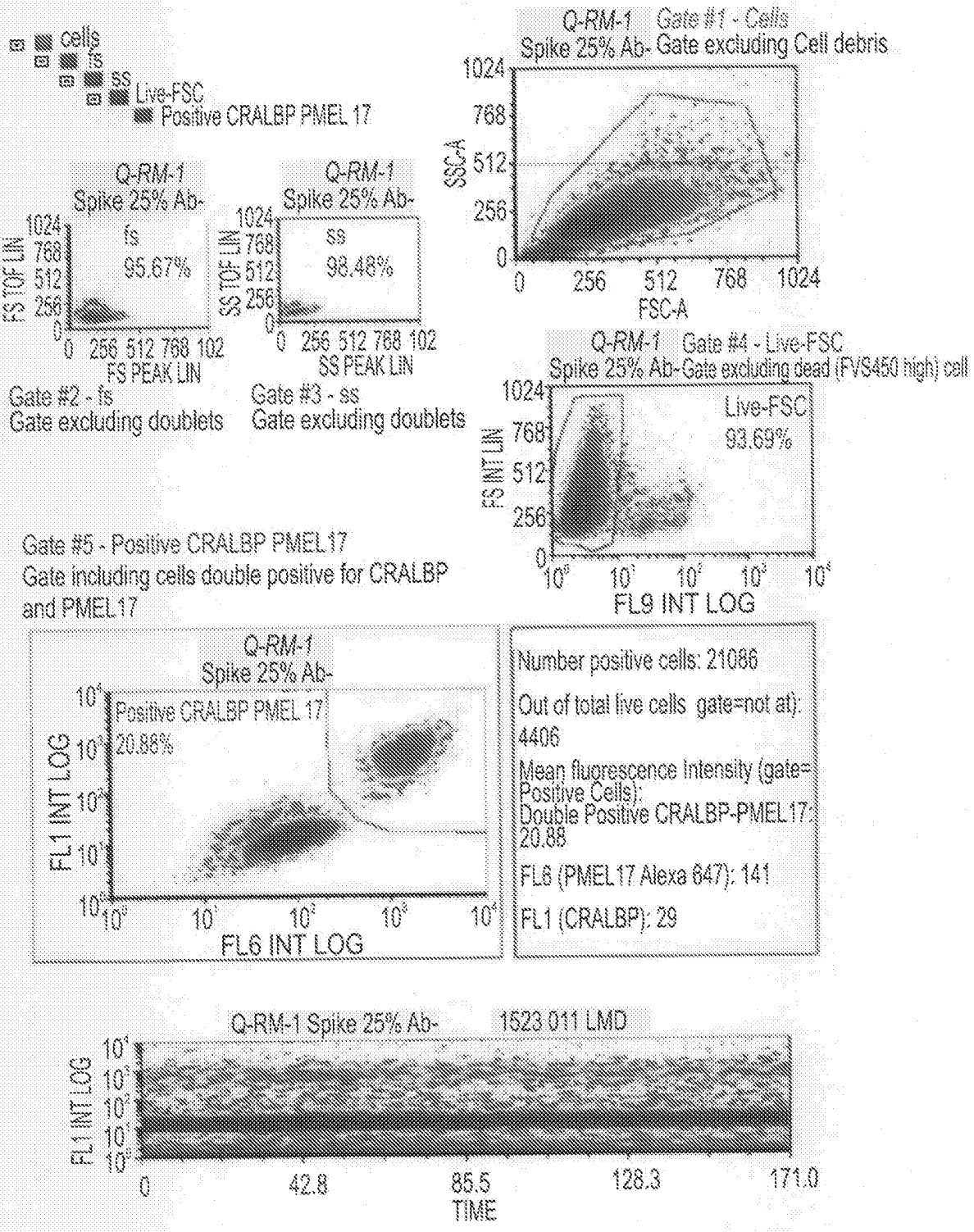

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
Spike 25% Ab-
fs
95.67%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
Spike 25% Ab-
ss
98.48%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
Spike 25% Ab- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
Spike 25% Ab-Gate excluding dead (FVS450 high) cell
Live-FSC
93.69%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1
Spike 25% Ab-
Positive CRALBP PMEL 17
20.86%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 21086
Out of total live cells gate=not at): 4406
Mean fluorescence intensity (gate=Positive Cells):
Double Positive CRALBP-PMEL17: 20.88
FL6 (PMEL17 Alexa 647): 141
FL1 (CRALBP): 29
Q-RM-1 Spike 25% Ab- 1523 011 LMD
FL1 INT LOG
TIME

FIG. 5

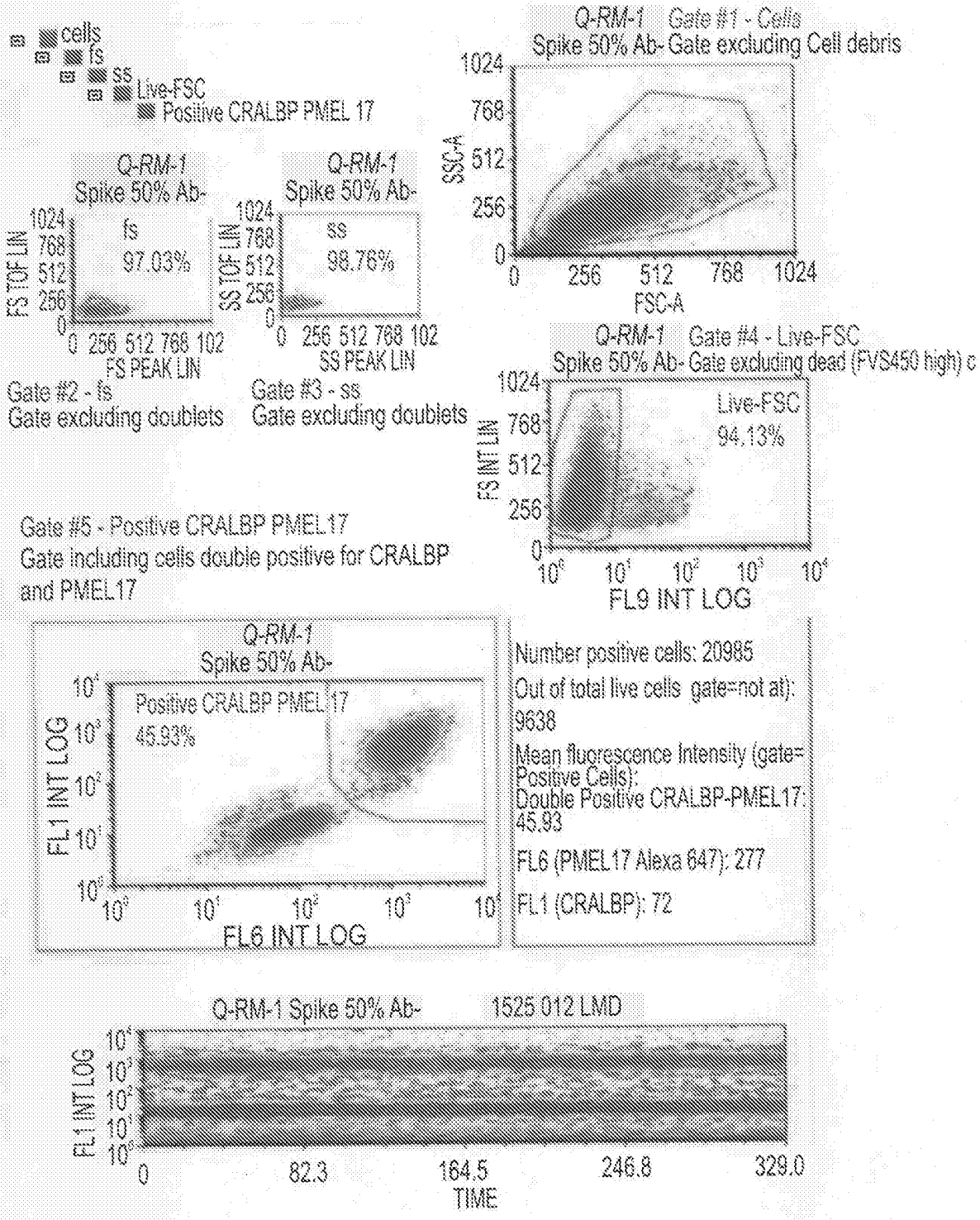

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
Spike 50% Ab-
fs
97.03%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
Spike 50% Ab-
ss
98.76%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
Spike 50% Ab- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
Spike 50% Ab- Gate excluding dead (FVS450 high) c
Live-FSC
94.13%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP
and PMEL17
Q-RM-1
Spike 50% Ab-
Positive CRALBP PMEL 17
45.93%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 20985
Out of total live cells gate=not at):
9638
Mean flugrescence Intensity (gate=
Positive Cells):
Double Positive CRALBP-PMEL17:
45.93
FL6 (PMEL17 Alexa 647): 277
FL1 (CRALBP): 72
Q-RM-1 Spike 50% Ab-
1525 012 LMD
FL1 INT LOG
TIME

FIG. 6

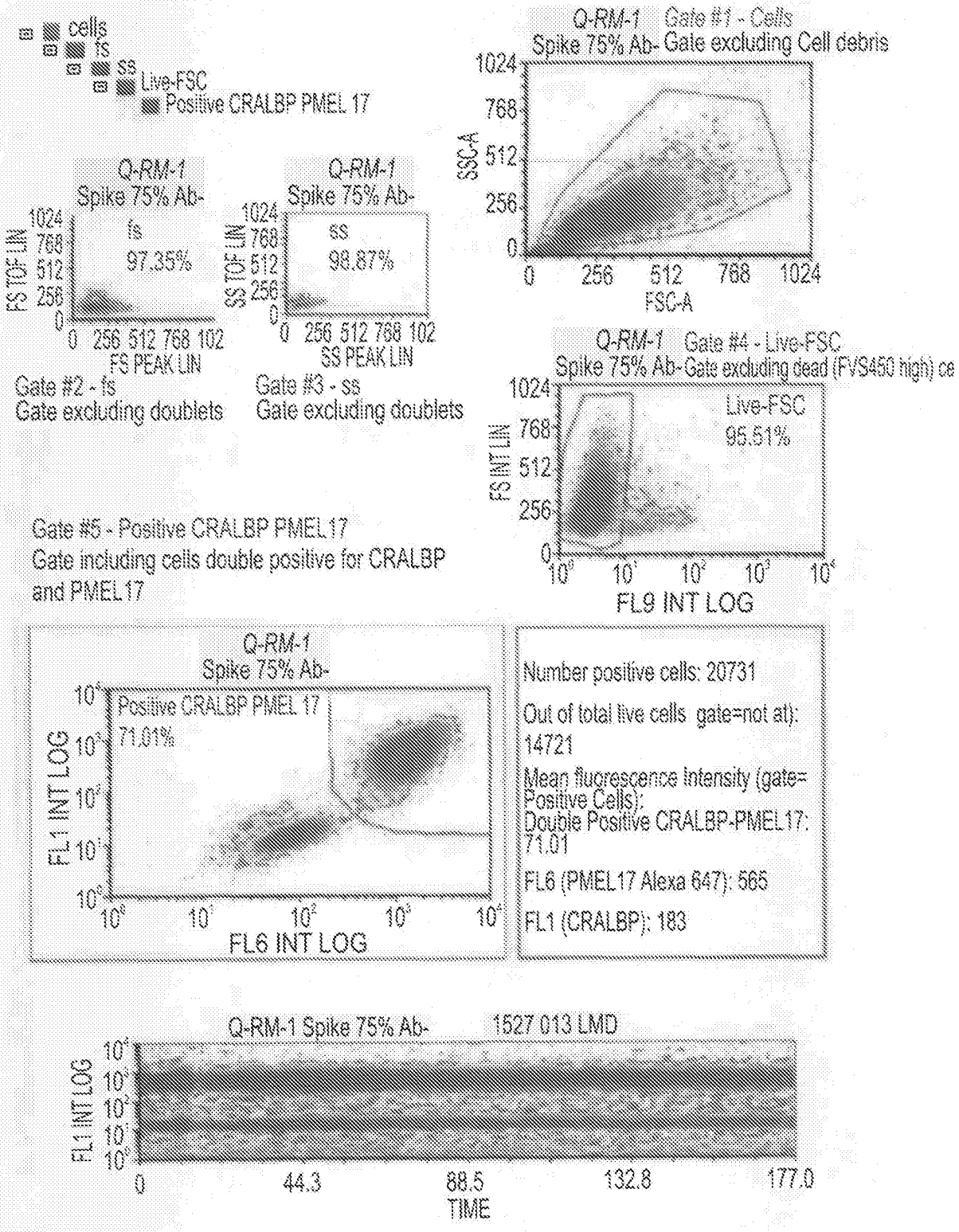

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
Spike 75% Ab-
fs
97.35%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
Spike 75% Ab-
ss
98.87%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
Spike 75% Ab- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
Spike 75% Ab- Gate excluding dead (FVS450 high) ce
Live-FSC
95.51%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1
Spike 75% Ab-
Positive CRALBP PMEL 17
71.01%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 20731
Out of total live cells gate=not at): 14721
Mean fluorescence Intensity (gate= Positive Cells):
Double Positive CRALBP-PMEL17: 71.01
FL6 (PMEL17 Alexa 647): 565
FL1 (CRALBP): 183
Q-RM-1 Spike 75% Ab- 1527 013 LMD
FL1 INT LOG
TIME

FIG. 7

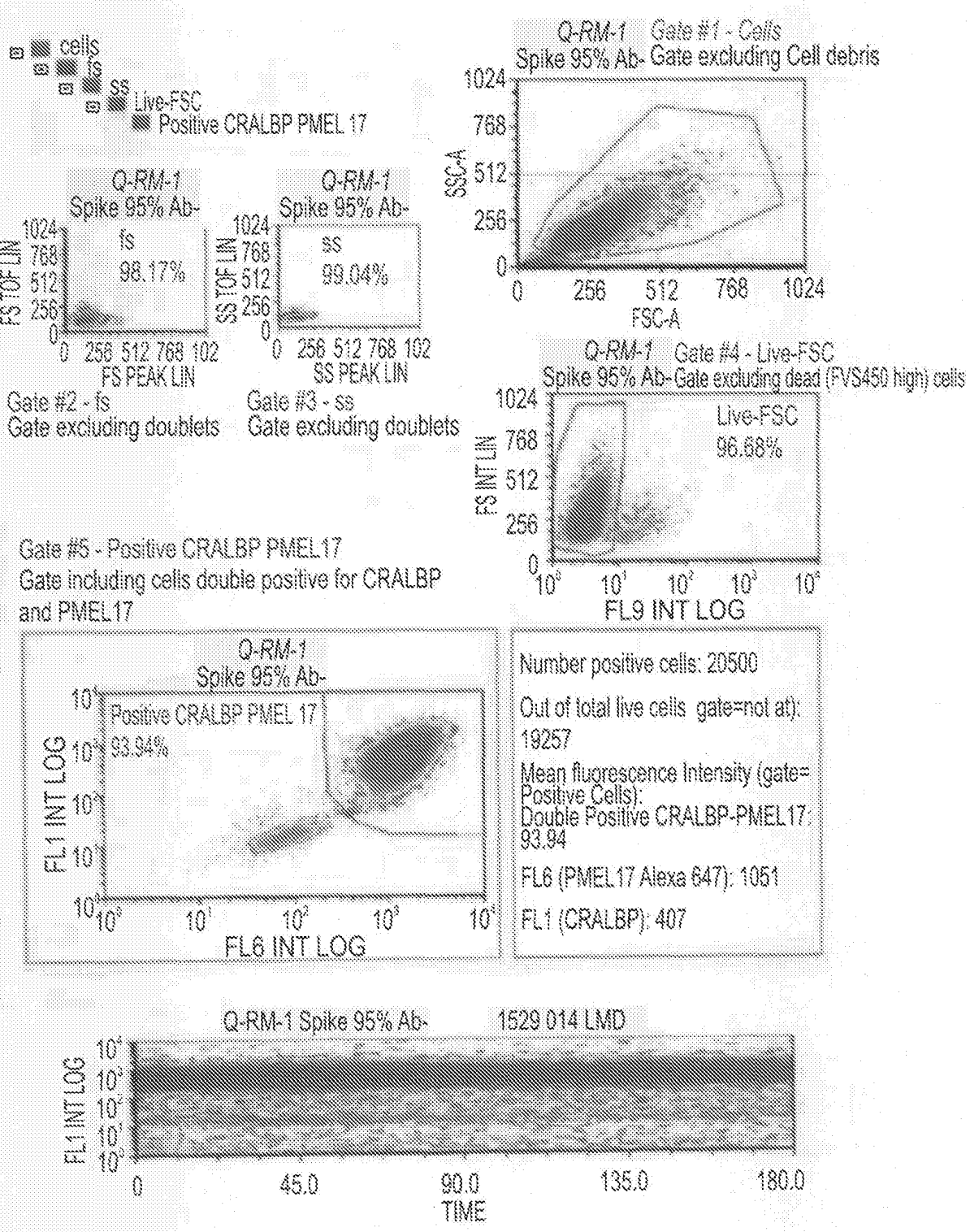

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
Spike 95% Ab-
fs
98.17%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
Spike 95% Ab-
ss
99.04%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
Spike 95% Ab- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
Spike 95% Ab- Gate excluding dead (FVS450 high) cells
Live-FSC
96.68%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1
Spike 95% Ab-
Positive CRALBP PMEL 17
93.94%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 20500
Out of total live cells gate=not at): 19257
Mean fluorescence Intensity (gate= Positive Cells):
Double Positive CRALBP-PMEL17: 93.94
FL6 (PMEL17 Alexa 647): 1051
FL1 (CRALBP): 407
Q-RM-1 Spike 95% Ab- 1529 014 LMD
FL1 INT LOG
TIME

FIG. 8

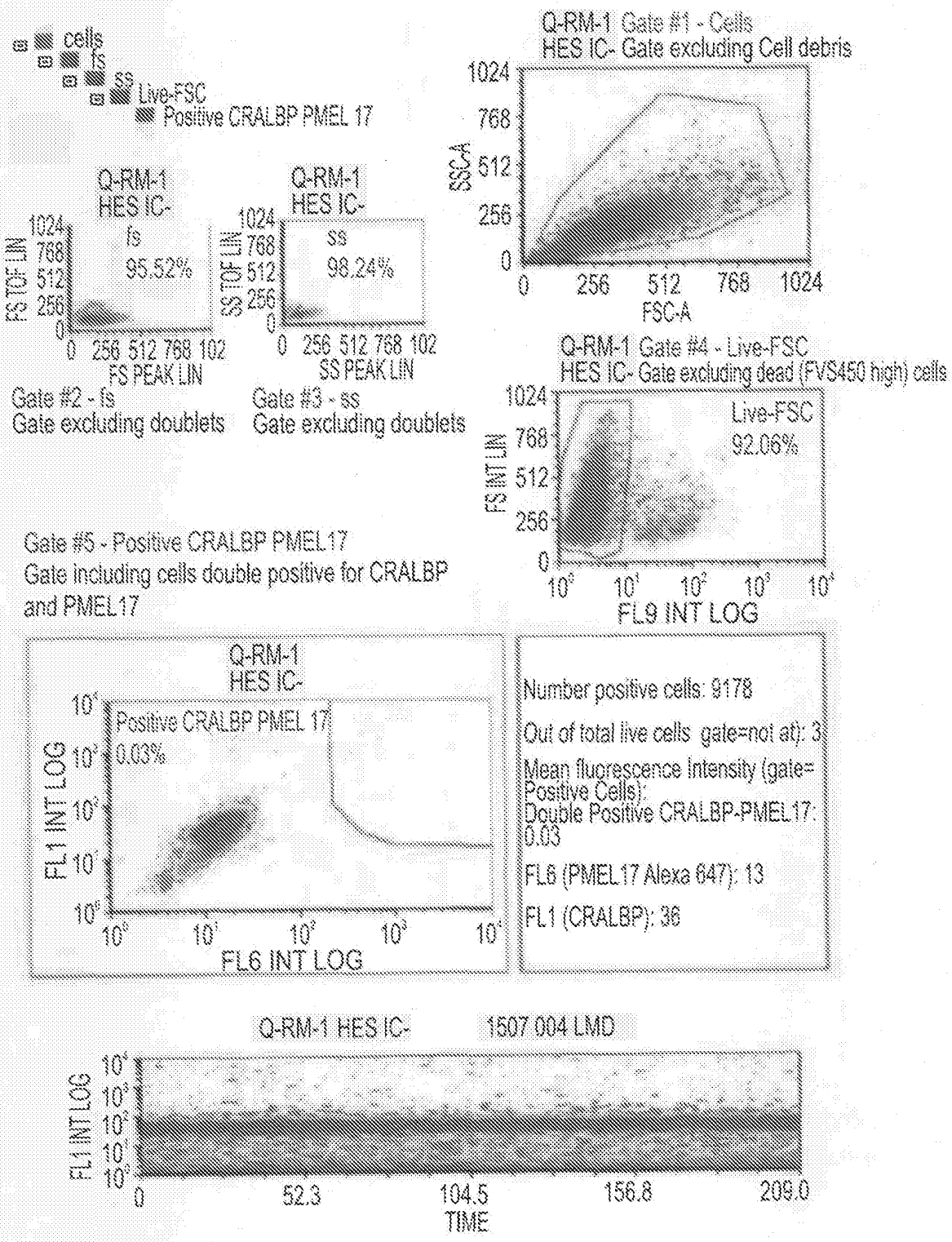

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1 HES IC- fs 95.52%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1 HES IC- ss 98.24%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
HES IC- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1 Gate #4 - Live-FSC
HES IC- Gate excluding dead (FVS450 high) cells
Live-FSC 92.06%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1 HES IC-
Positive CRALBP PMEL 17 0.03%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 9178
Out of total live cells gate=not at): 3
Mean fluorescence Intensity (gate= Positive Cells):
Double Positive CRALBP-PMEL17: 0.03
FL6 (PMEL17 Alexa 647): 13
FL1 (CRALBP): 36
Q-RM-1 HES IC- 1507 004 LMD
FL1 INT LOG
TIME

FIG. 9

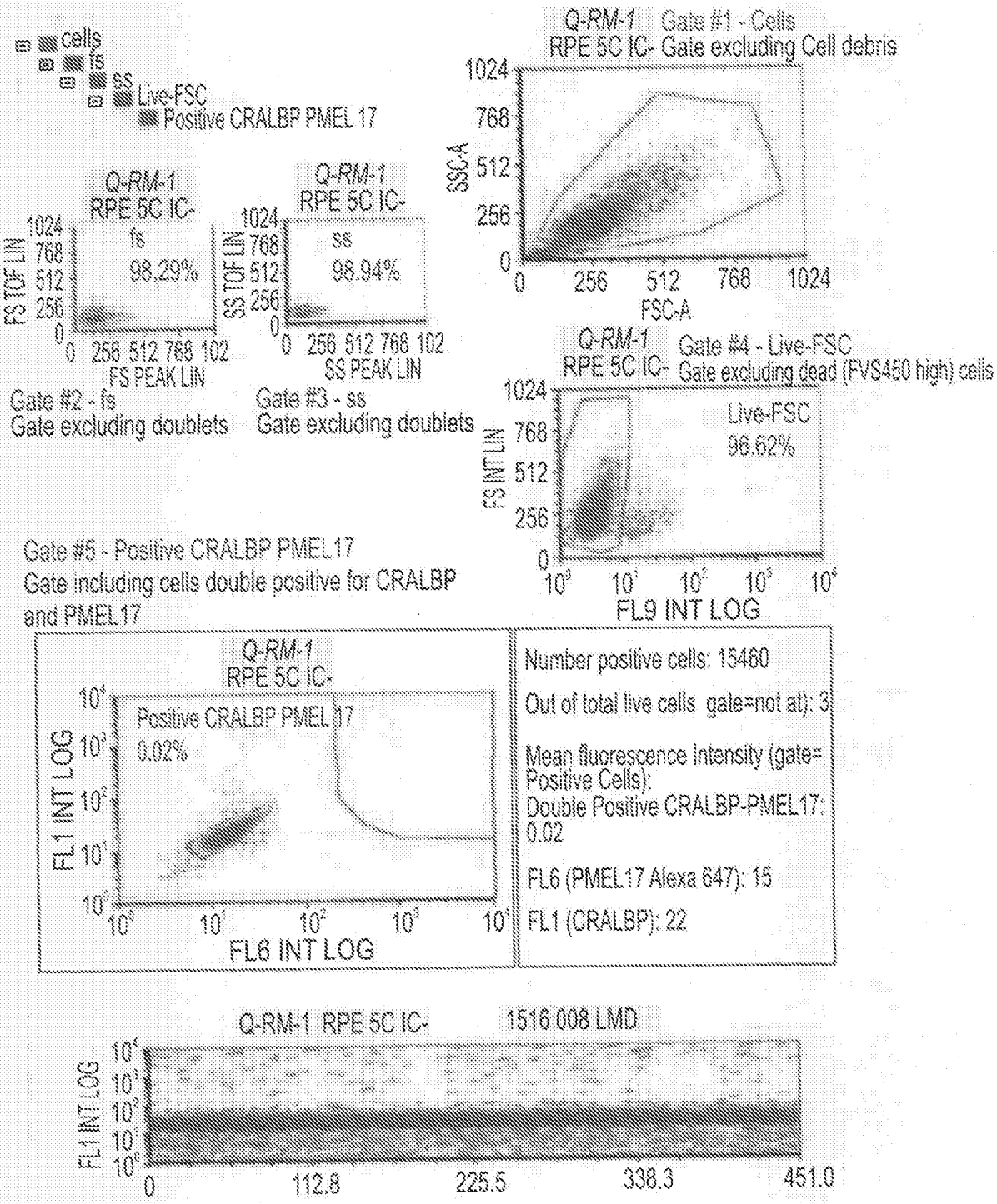

cells
fs
ss
Live-FSC
Positive CRALBP PMEL 17
Q-RM-1
RPE 5C IC-
fs
98.29%
FS TOF LIN
FS PEAK LIN
Gate #2 - fs
Gate excluding doublets
Q-RM-1
RPE 5C IC-
ss
98.94%
SS TOF LIN
SS PEAK LIN
Gate #3 - ss
Gate excluding doublets
Q-RM-1 Gate #1 - Cells
RPE 5C IC- Gate excluding Cell debris
SSC-A
FSC-A
Q-RM-1
RPE 5C IC-
Gate #4 - Live-FSC
Gate excluding dead (FVS450 high) cells
Live-FSC
96.62%
FS INT LIN
FL9 INT LOG
Gate #5 - Positive CRALBP PMEL17
Gate including cells double positive for CRALBP and PMEL17
Q-RM-1
RPE 5C IC-
Positive CRALBP PMEL 17
0.02%
FL1 INT LOG
FL6 INT LOG
Number positive cells: 15460
Out of total live cells gate=not at): 3
Mean fluorescence Intensity (gate= Positive Cells):
Double Positive CRALBP-PMEL17: 0.02
FL6 (PMEL17 Alexa 647): 15
FL1 (CRALBP): 22
Q-RM-1 RPE 5C IC-
1516 008 LMD
FL1 INT LOG
0
112.8
225.5
338.3
451.0

FIG. 10

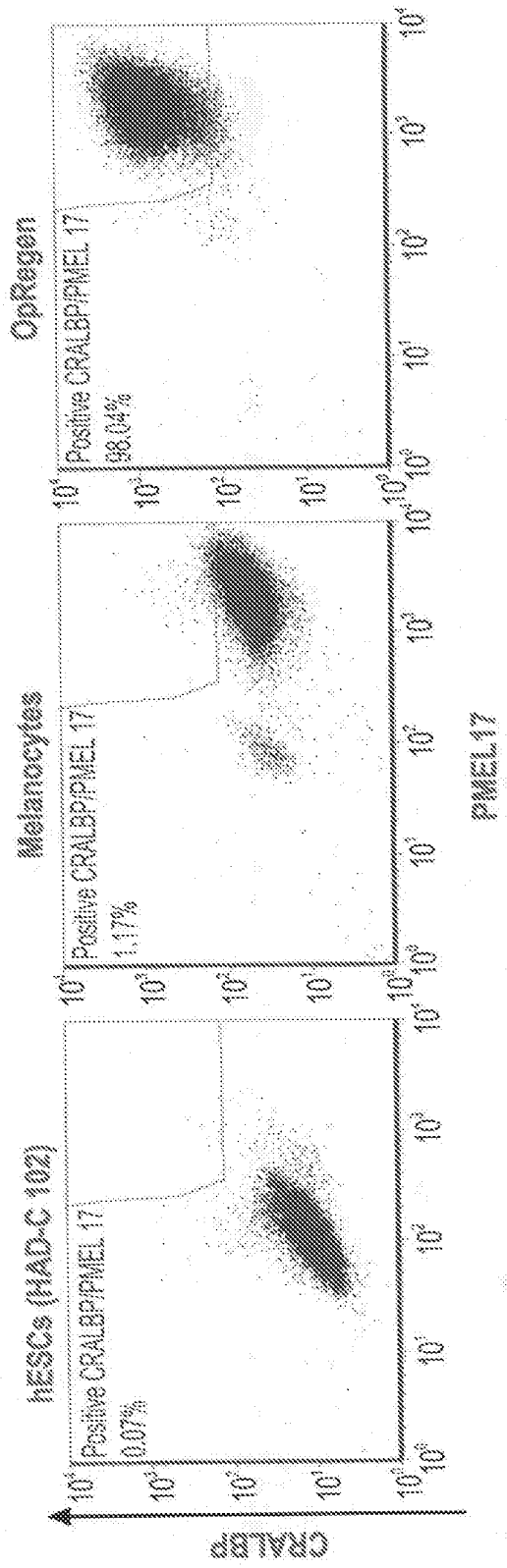

hESCs (HAD-C 102)
Melanocytes
OpRegen
Positive CRALBP/PMEL 17
0.07%
1.17%
98.04%
CRALBP
PMEL17

FIG. 11

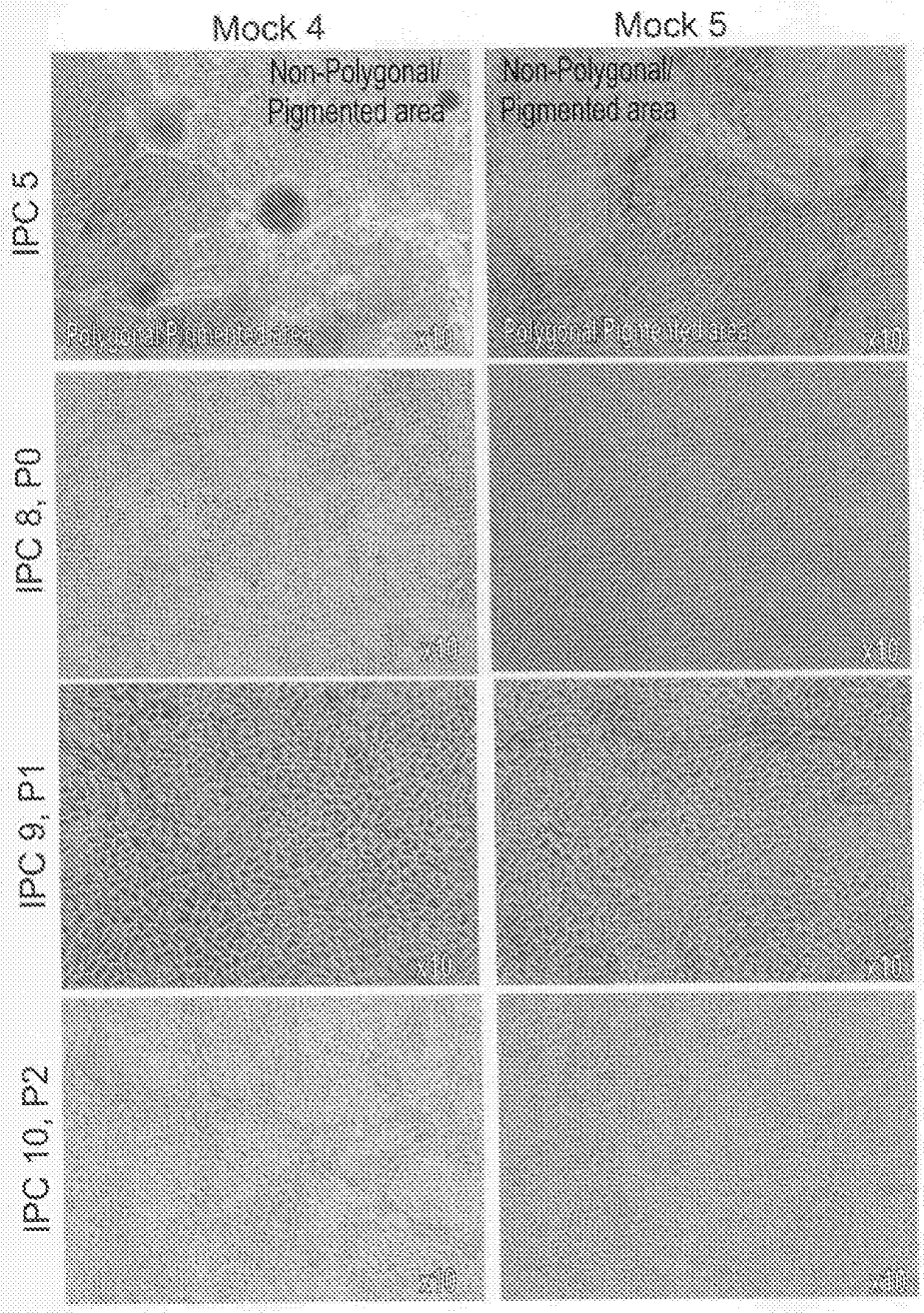

Mock 4
Mock 5
IPC 5
IPC 8, P0
IPC 9, P1
IPC 10, P2
Non-Polygonal Pigmented area
Non-Polygonal Pigmented area
Polygonal Pigmented area
Polygonal Pigmented area
x10
x10
x10
x10
x10
x10
x10
x10

FIG. 12

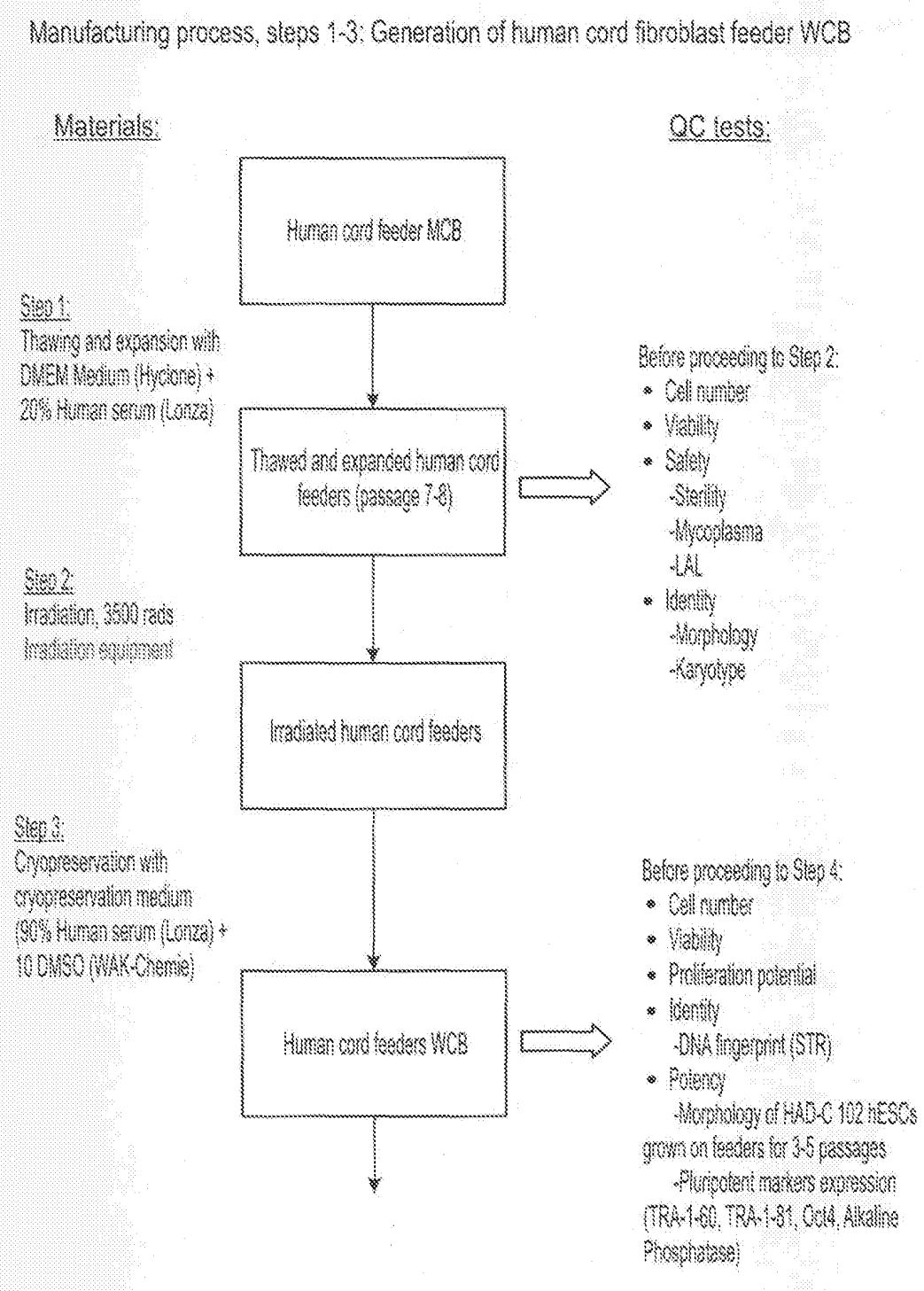
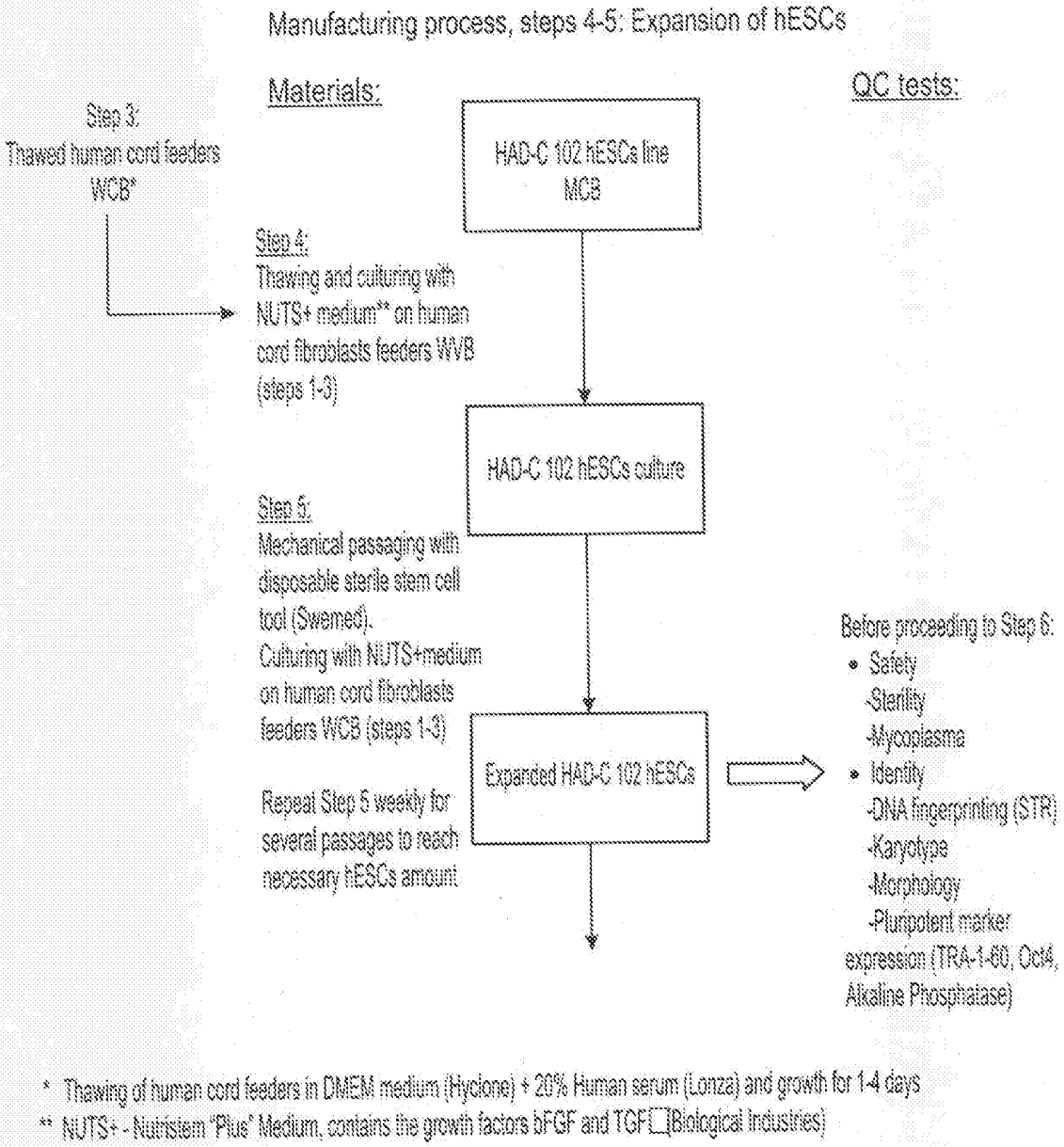

Manufacturing process, steps 1-3: Generation of human cord fibroblast feeder WCB
Materials:
QC tests:
Human cord feeder MCB
Step 1:
Thawing and expansion with DMEM Medium (Hyclone) + 20% Human serum (Lonza)
Thawed and expanded human cord feeders (passage 7-8)
Before proceeding to Step 2:
Cell number
Viability
Safety
-Sterility
-Mycoplasma
-LAL
Identity
-Morphology
-Karyotype
Step 2:
Irradiation, 3500 rads
Irradiation equipment
Irradiated human cord feeders
Step 3:
Cryopreservation with cryopreservation medium (90% Human serum (Lonza) + 10 DMSO (WAK-Chemie)
Human cord feeders WCB
Before proceeding to Step 4:
Cell number
Viability
Proliferation potential
Identity
-DNA fingerprint (STR)
Potency
-Morphology of HAD-C 102 hESCs grown on feeders for 3-5 passages
-Pluripotent markers expression (TRA-1-60, TRA-1-81, Oct4, Alkaline Phosphatase)

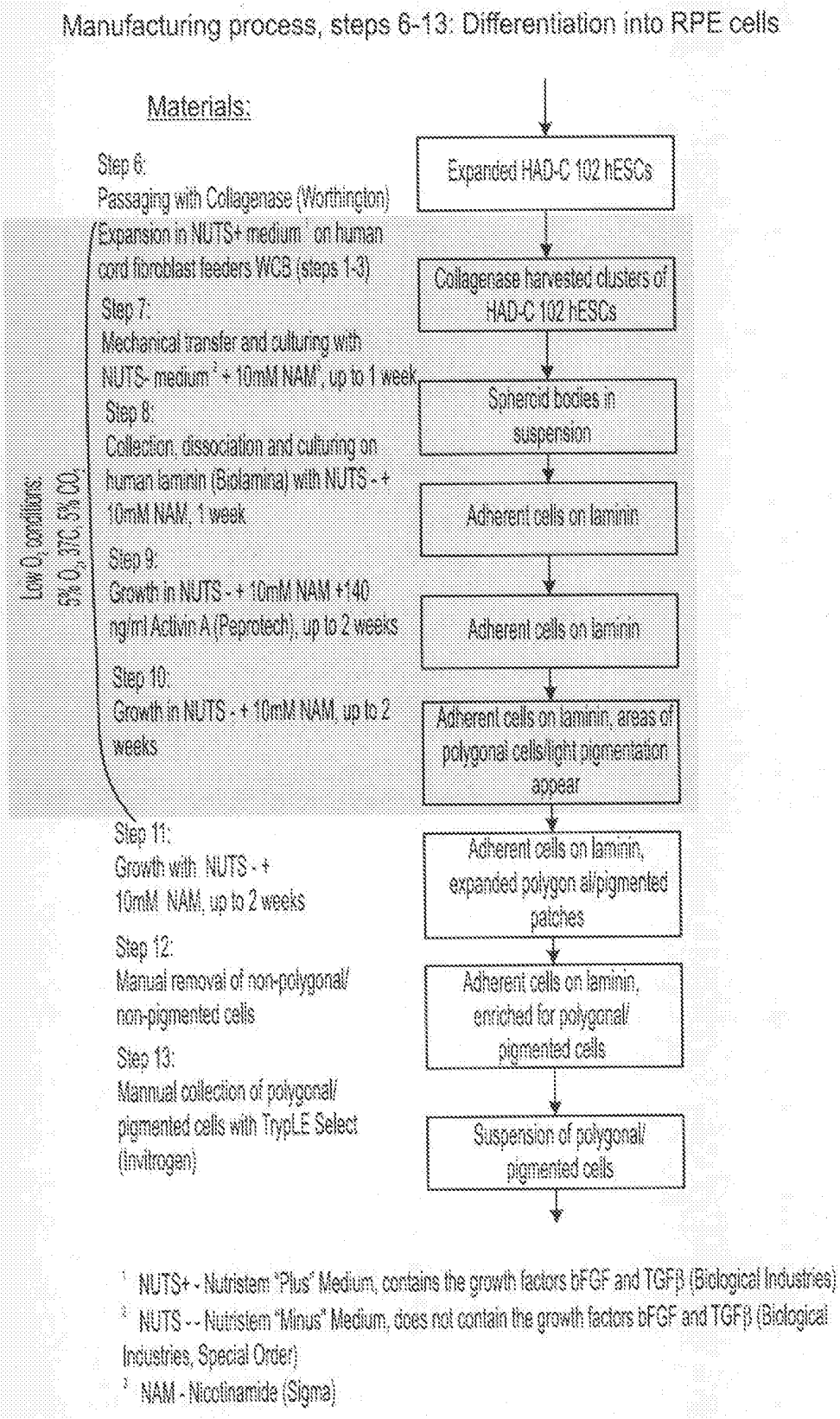
FIG. 13



* Thawing of human cord feeders in DMEM medium (Hyclone) + 20% Human serum (Lonza) and growth for 1-4 days

** NUTS+ - Nutristem "Plus" Medium, contains the growth factors bFGF and TGF□(Biological Industries)

FIG. 14

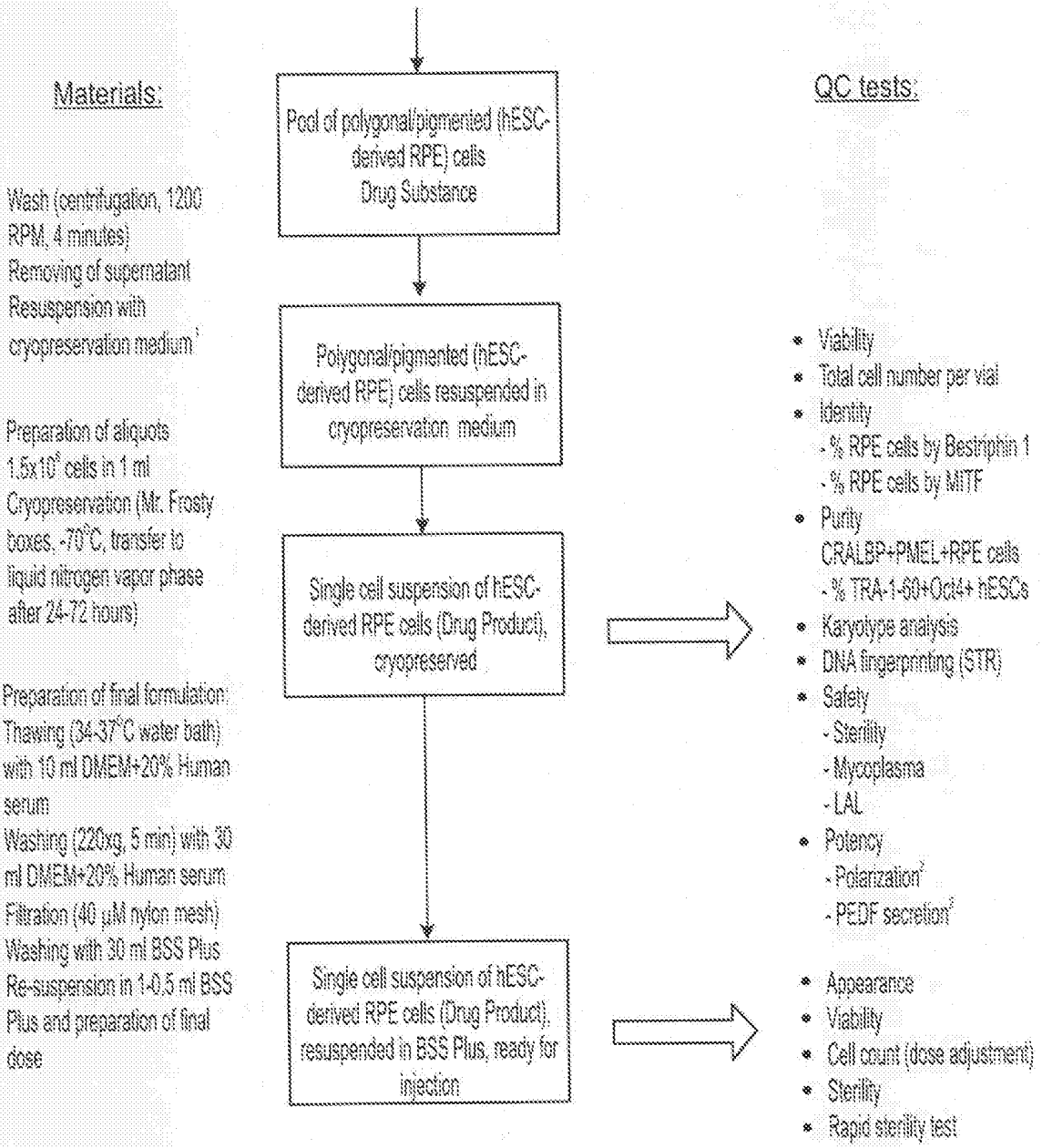


[1] NUTS+ - Nutristem "Plus" Medium, contains the growth factors bFGF and TGFβ (Biological Industries)

[2] NUTS - - Nutristem "Minus" Medium, does not contain the growth factors bFGF and TGFβ (Biological Industries, Special Order)

[3] NAM - Nicotinamide (Sigma)

FIG. 15



[1] Cryopreservation medium - 90% human serum + 10% DMSO (WAK-DMSO-10, WAK-Chemie
[2] Assay is performed for information only, no release criteria is set

FIG. 16

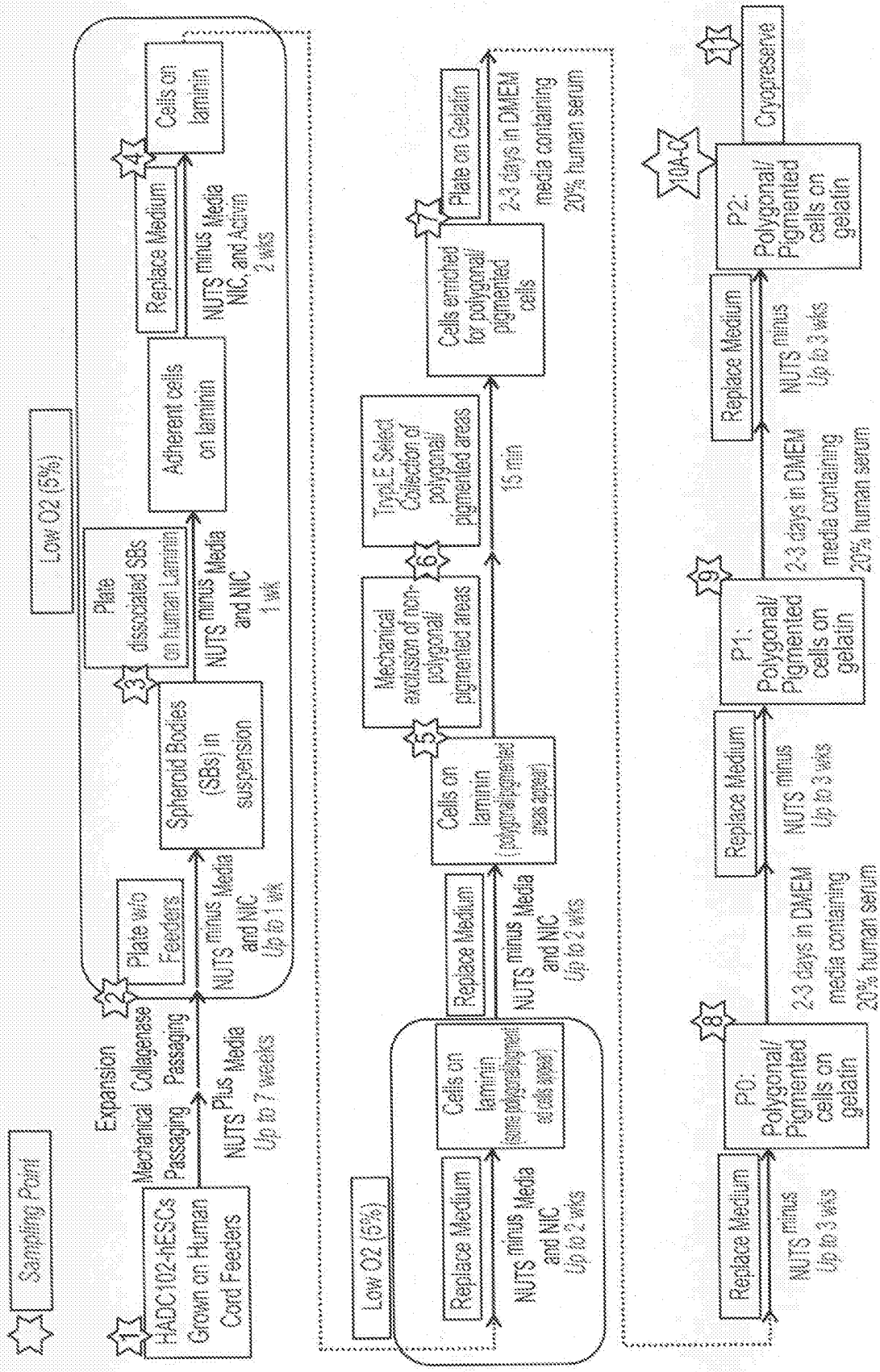

Sampling Point
1
HADC102-hESCs Grown on Human Cord Feeders
Replace Medium
NUTS minus Media and NIC Up to 2 wks
Cells on laminin (some polygonal/pigmented areas open)
Low O2 (6%)
Expansion
Mechanical Passaging
Collagenase Passaging
2
Plate w/o Feeders
NUTS Plus Media Up to 7 weeks
NUTS minus Media and NIC Up to 1 wk
Low O2 (6%)
3
Spheroid Bodies (SBs) in suspension
Plate dissociated SBs on human Laminin
NUTS minus Media and NIC 1 wk
4
Adherent cells on laminin
Replace Medium
Cells on laminin
NUTS minus Media NIC, and Activin 2 wks
5
Cells on laminin (polygonal/pigmented areas open)
Replace Medium
NUTS minus Media and NIC Up to 2 wks
6
Mechanical excision of non-polygonal/ pigmented areas
TrypLE Select Collection of polygonal/ pigmented areas
16 min
7
Cells enriched for polygonal/ pigmented cells
Plate on Gelatin
2-3 days in DMEM media containing 20% human serum
8
P0: Polygonal/ Pigmented cells on gelatin
Replace Medium
NUTS minus Up to 3 wks
2-3 days in DMEM media containing 20% human serum
9
P1: Polygonal/ Pigmented cells on gelatin
Replace Medium
NUTS minus Up to 3 wks
2-3 days in DMEM media containing 20% human serum
10A-C
P2: Polygonal/ Pigmented cells on gelatin
Cryopreserve
11

FIG. 17

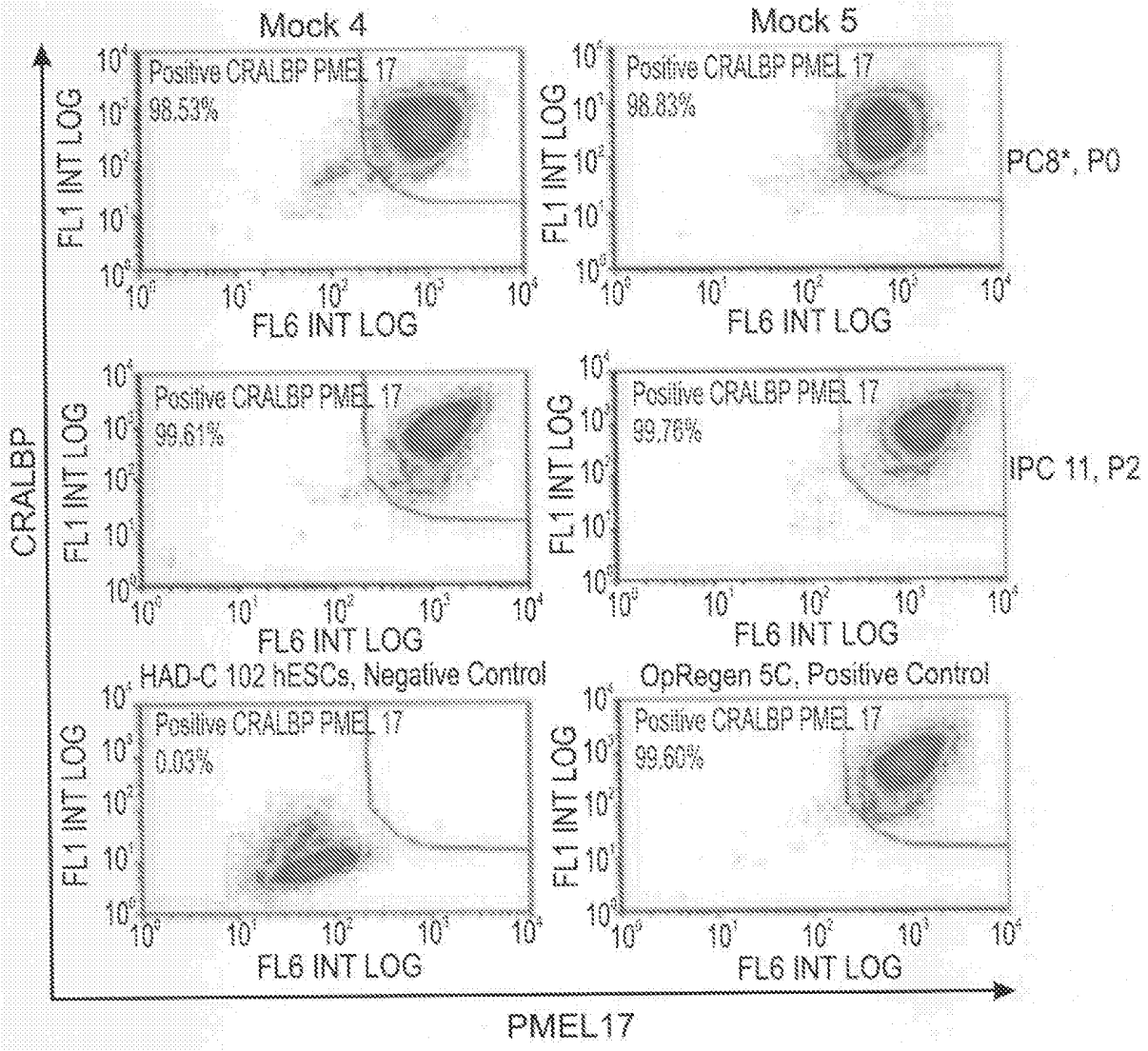
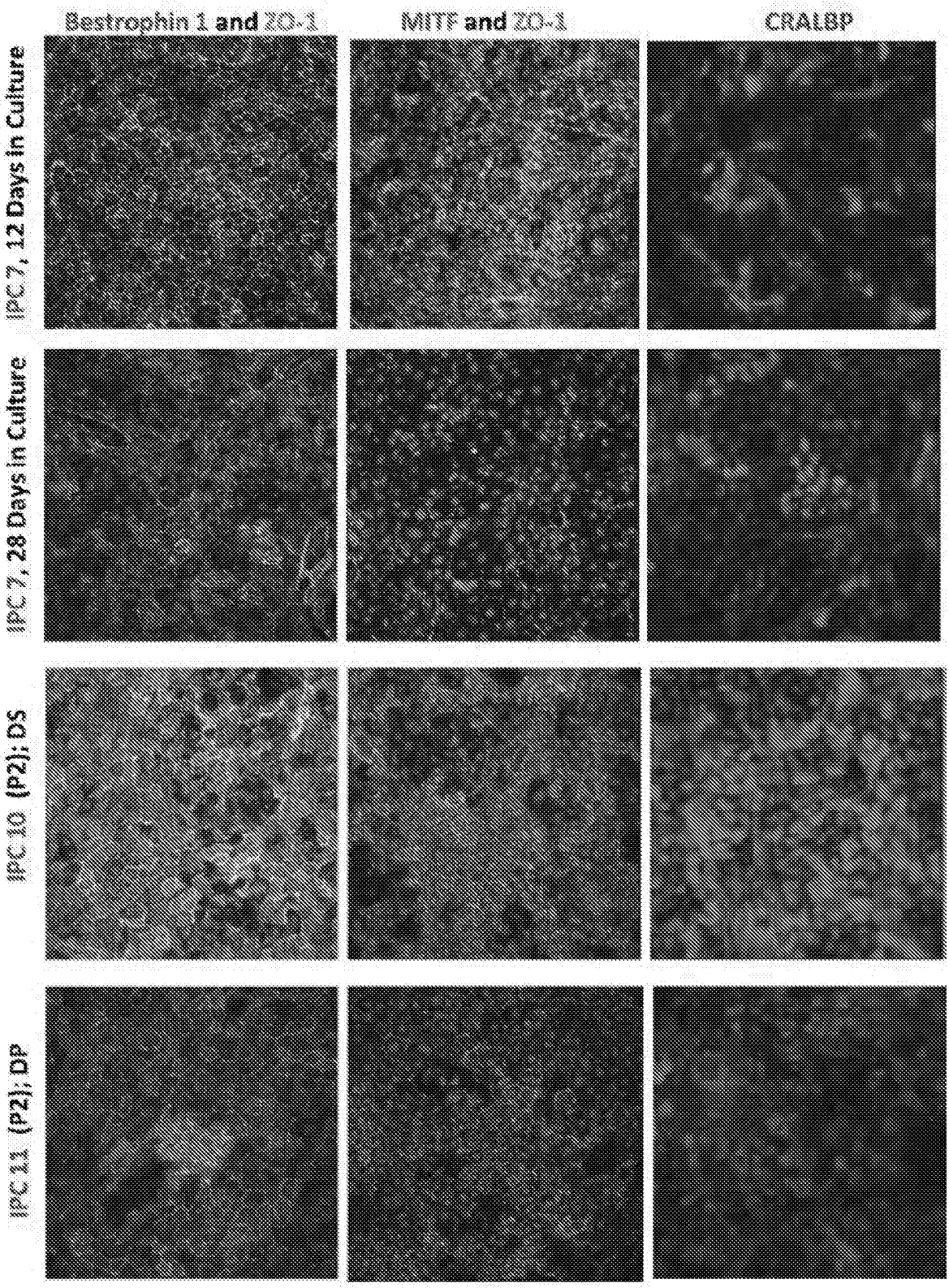

Mock 4
Positive CRALBP PMEL 17
98.53%
Mock 5
Positive CRALBP PMEL 17
98.83%
PC8*, P0
Positive CRALBP PMEL 17
99.61%
Positive CRALBP PMEL 17
99.76%
IPC 11, P2
HAD-C 102 hESCs, Negative Control
Positive CRALBP PMEL 17
0.03%
OpRegen 5C, Positive Control
Positive CRALBP PMEL 17
99.60%
FL1 INT LOG
FL6 INT LOG
CRALBP
PMEL17

FIG. 18


Bestrophin 1 and ZO-1
MITF and ZO-1
CRALBP
IPC 7, 12 Days in Culture
IPC 7, 28 Days in Culture
IPC 10 (P2); DS
IPC 11 (P2); DP

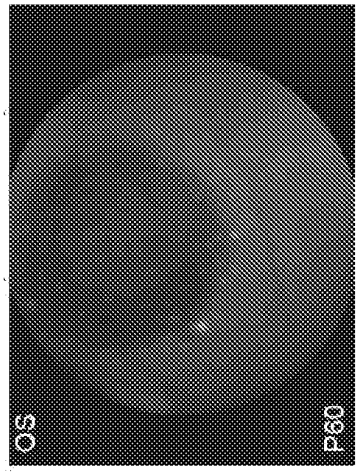


FIG. 19C

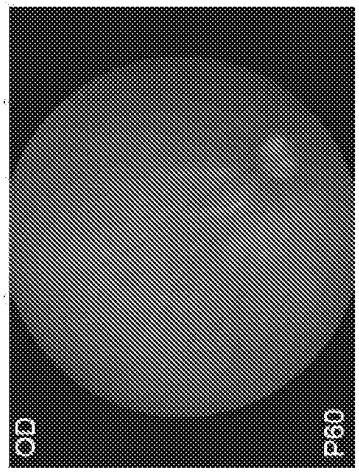


FIG. 19B

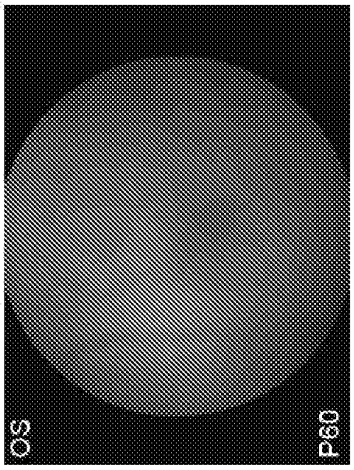
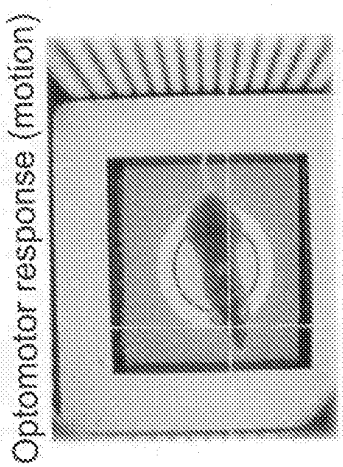


FIG. 19A

FIG. 20


Optomotor response (motion)

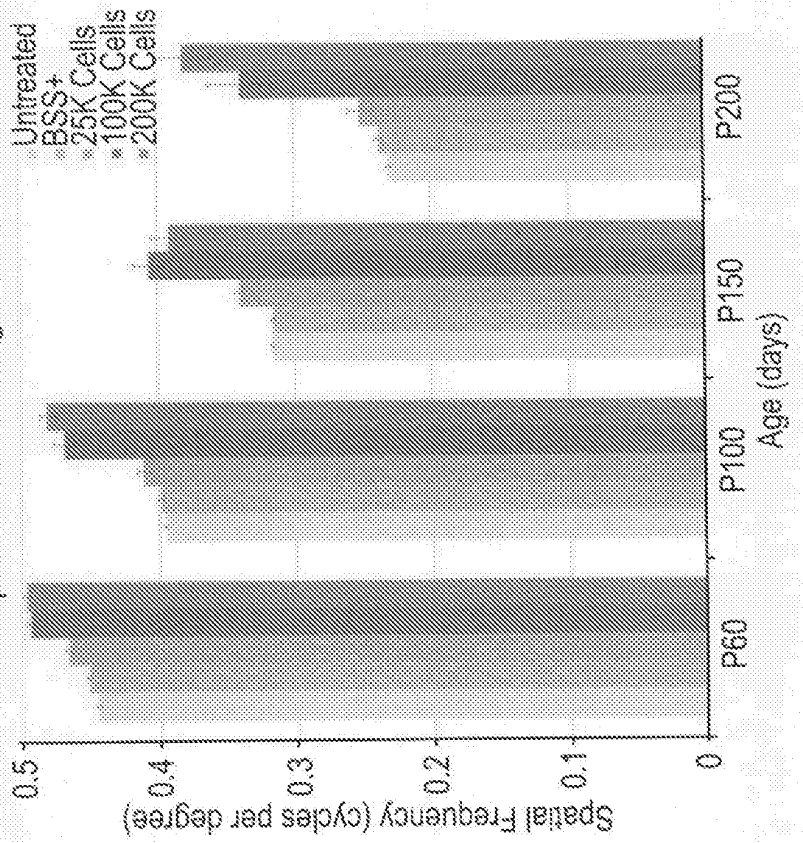

Optomotor Tracking Thresholds
Untreated
BSS+
25K Cells
100K Cells
200K Cells
0.5
0.4
0.3
0.2
0.1
0
Spatial Frequency (cycles per degree)
P60
P100
P150
P200
Age (days)

FIG. 21A

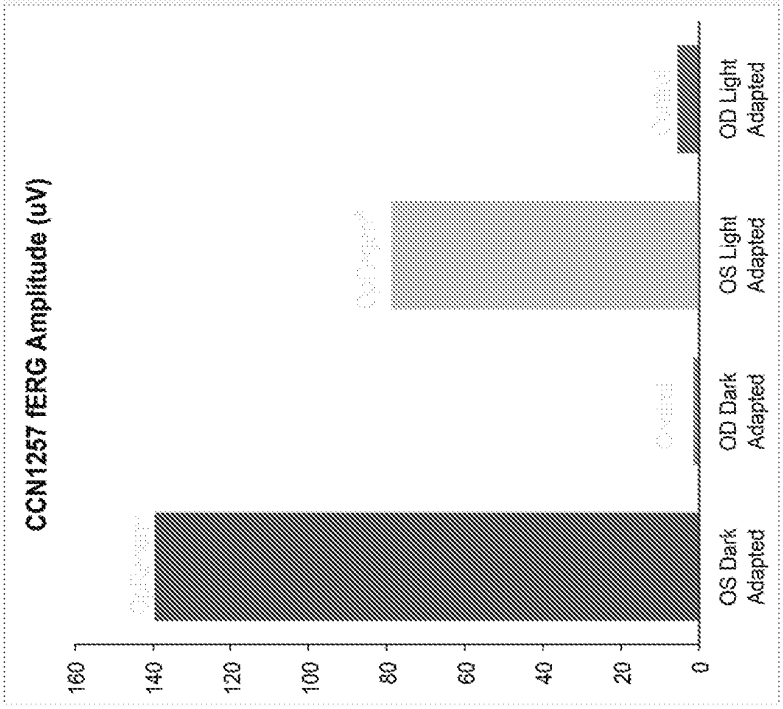
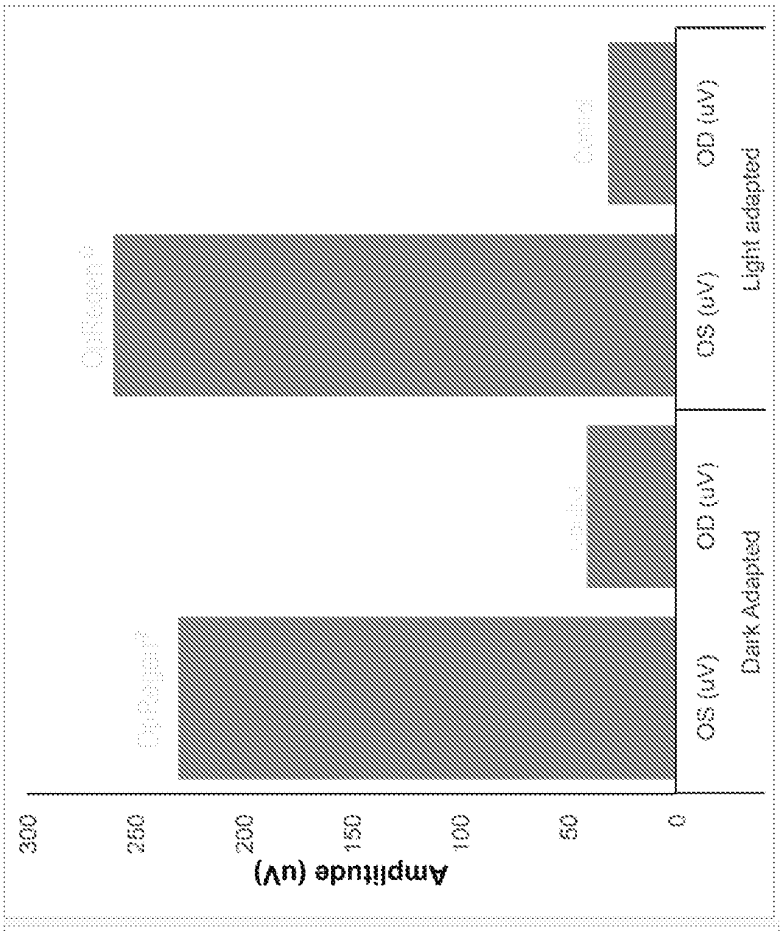


FIG. 21B



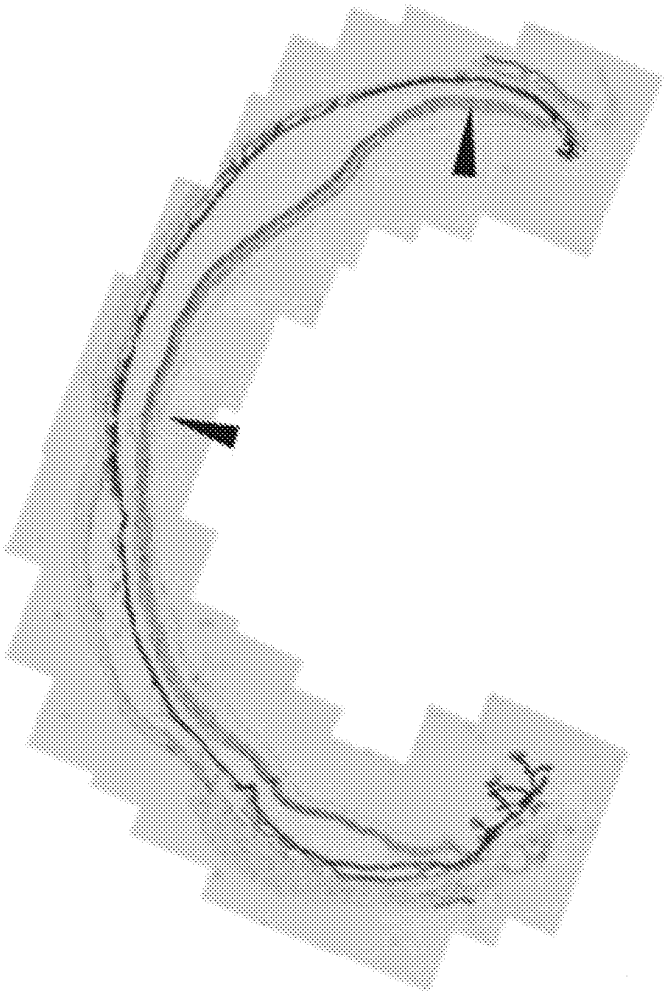


FIG. 22A

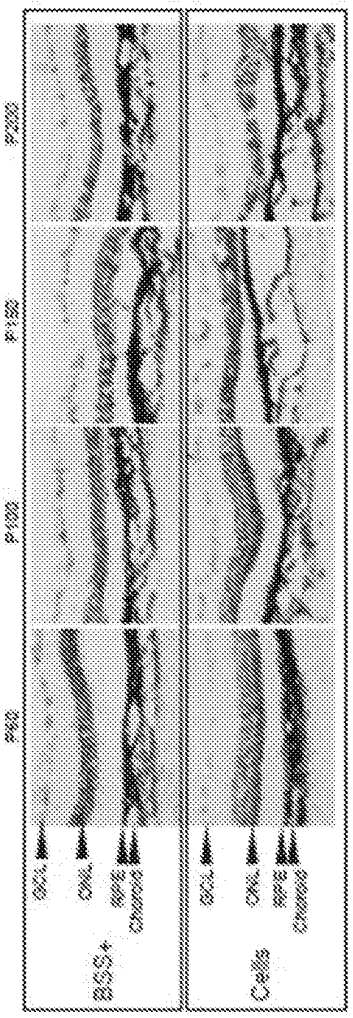


FIG. 22B

FIG. 23

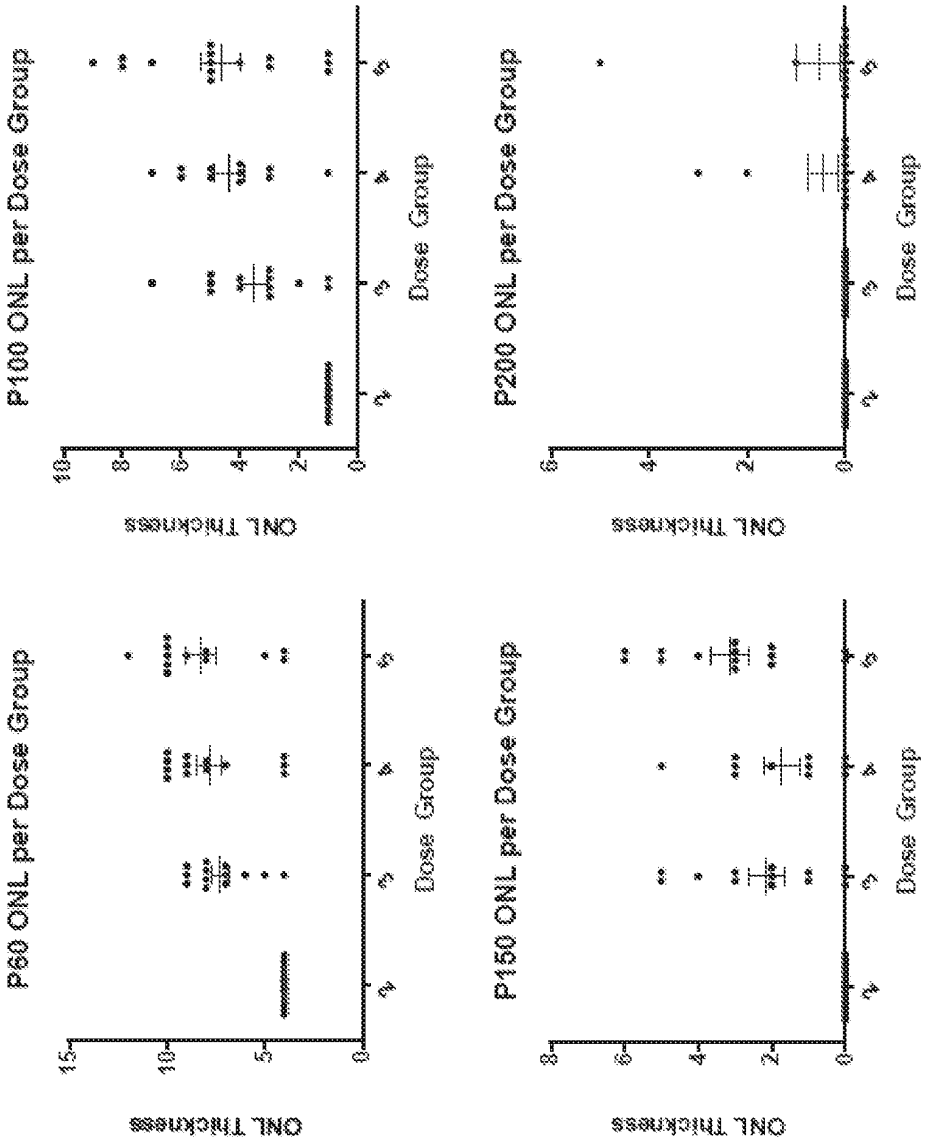

P60 ONL per Dose Group
P100 ONL per Dose Group
P150 ONL per Dose Group
P200 ONL per Dose Group
ONL Thickness
Dose Group

FIG. 24

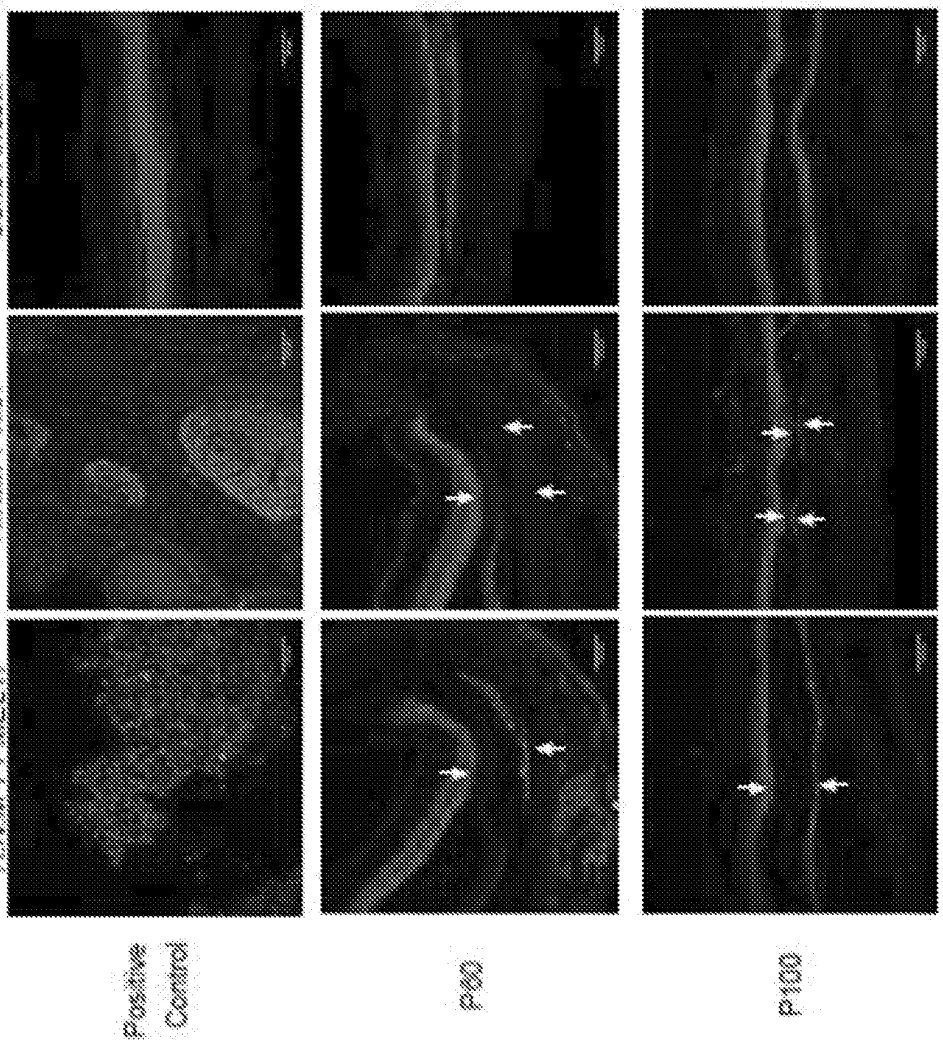

H.N.M + PMEL17
H.N.M + Ki67
Cone Arrestin
Positive Control
P60
P180

FIG. 24 – continued

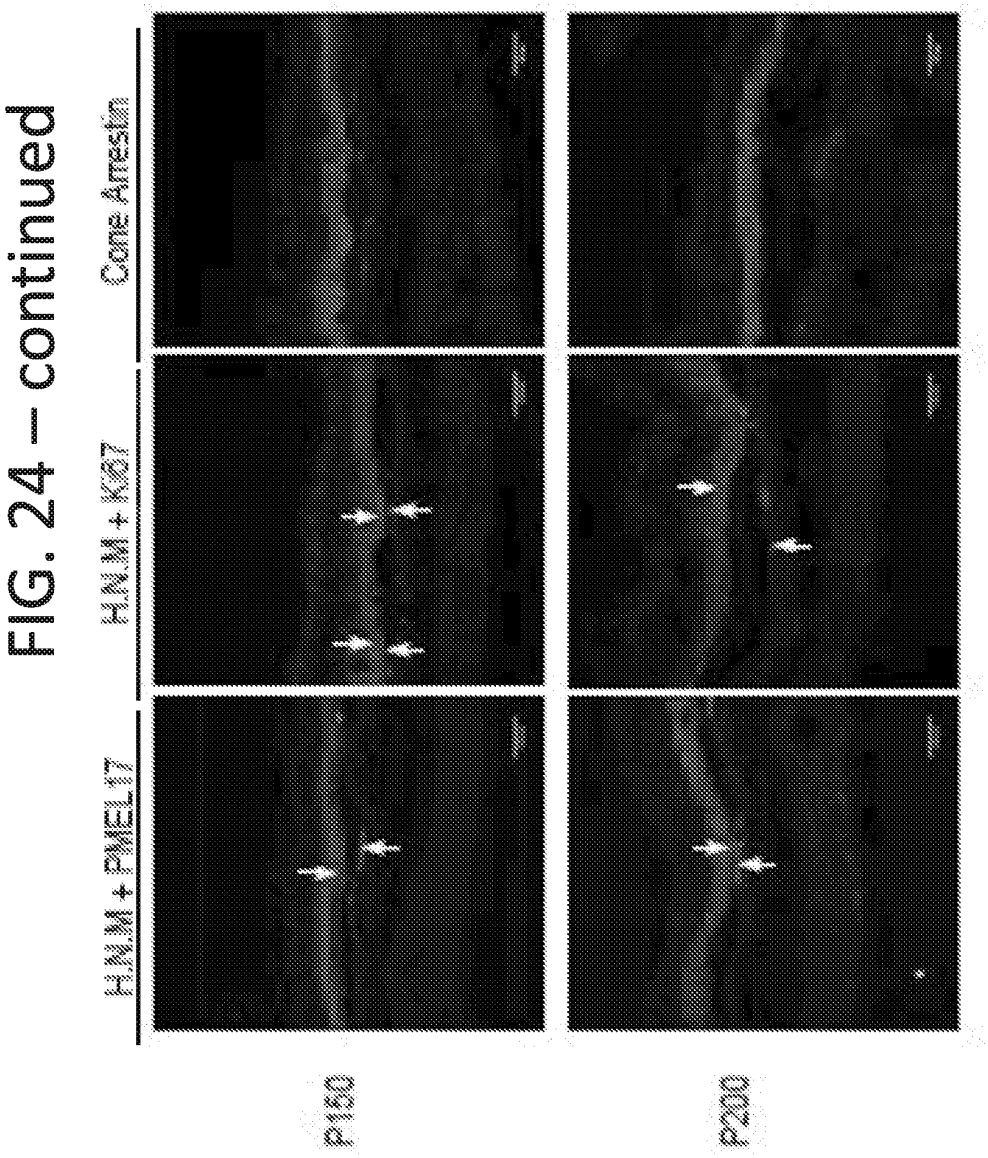
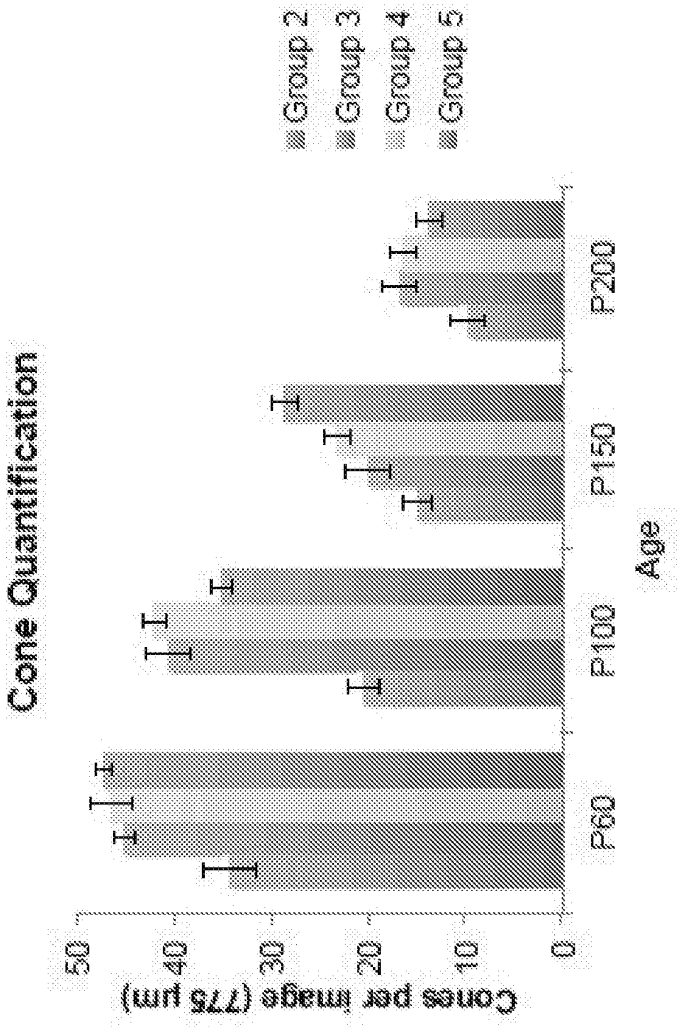


FIG. 25


Cone Quantification
Cones per image (775 µm)
0
10
20
30
40
50
P60
P100
P150
P200
Age
Group 2
Group 3
Group 4
Group 5

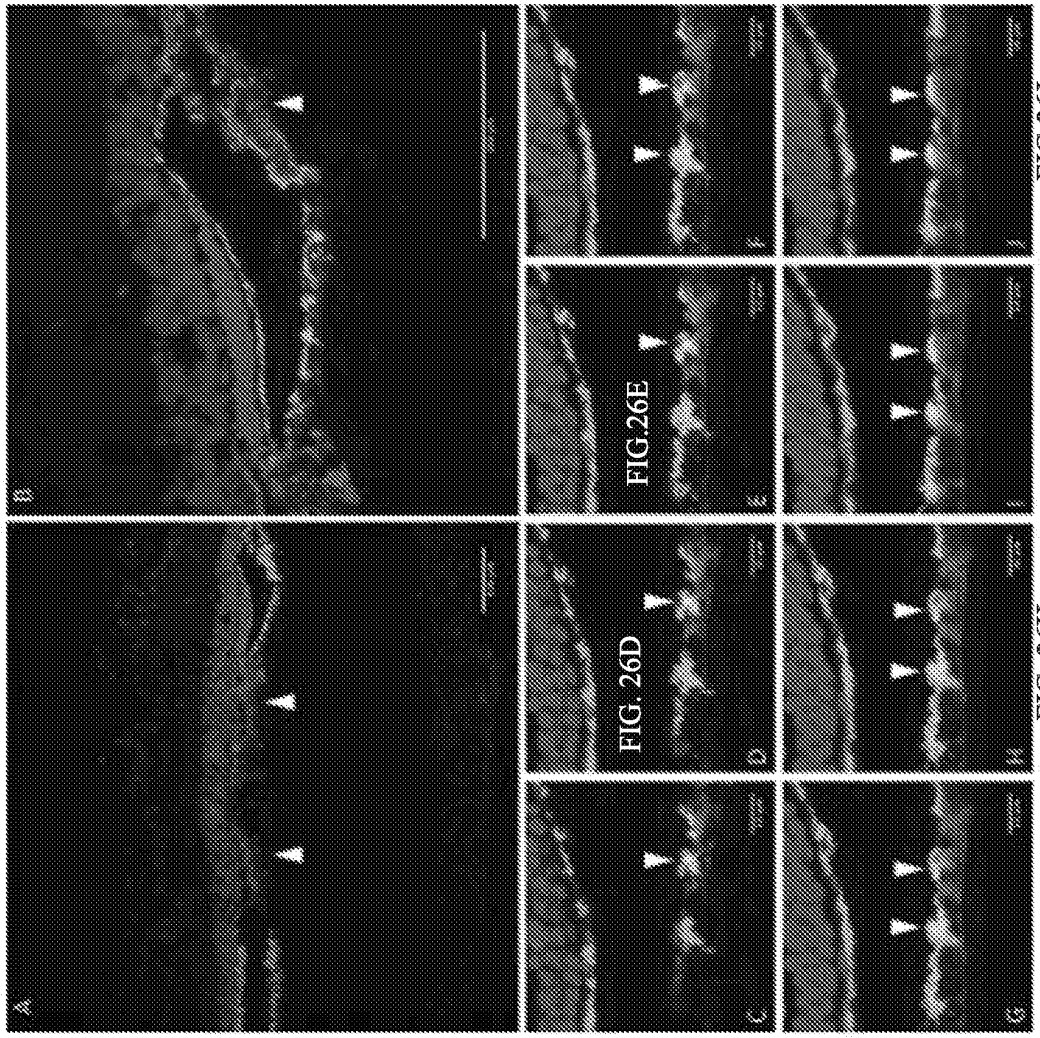

FIG. 26B
FIG. 26F
FIG.26A
FIG.26C
FIG. 26D
FIG.26E
FIG. 26H
FIG. 26I
FIG.26G
FIG.26J

FIG. 27A

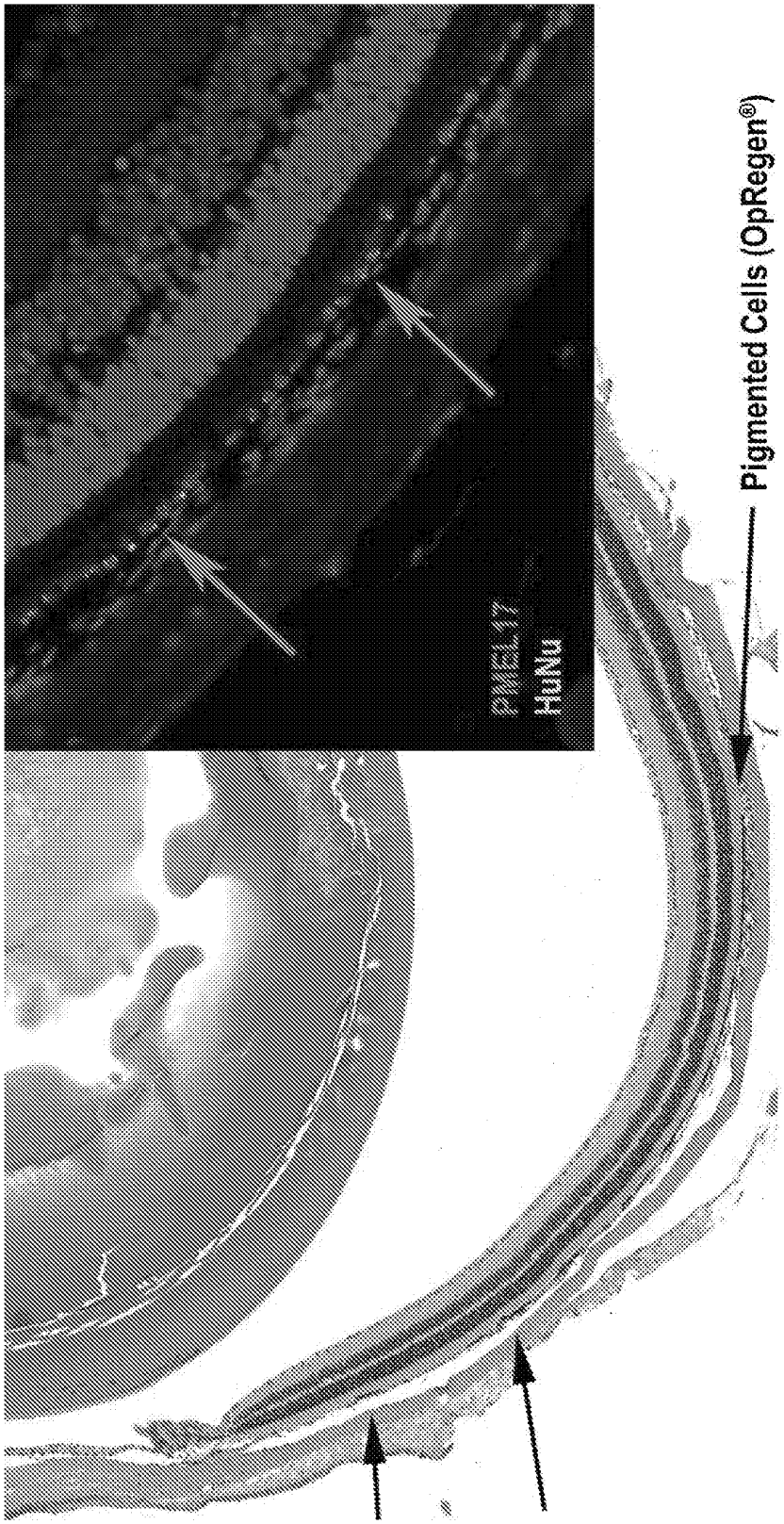


FIG. 27C

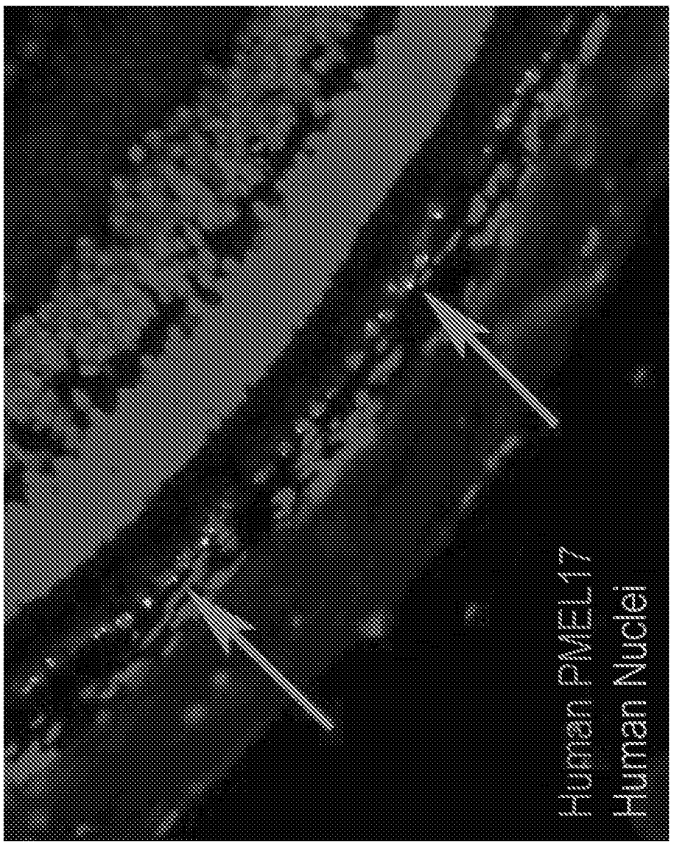


FIG. 27B

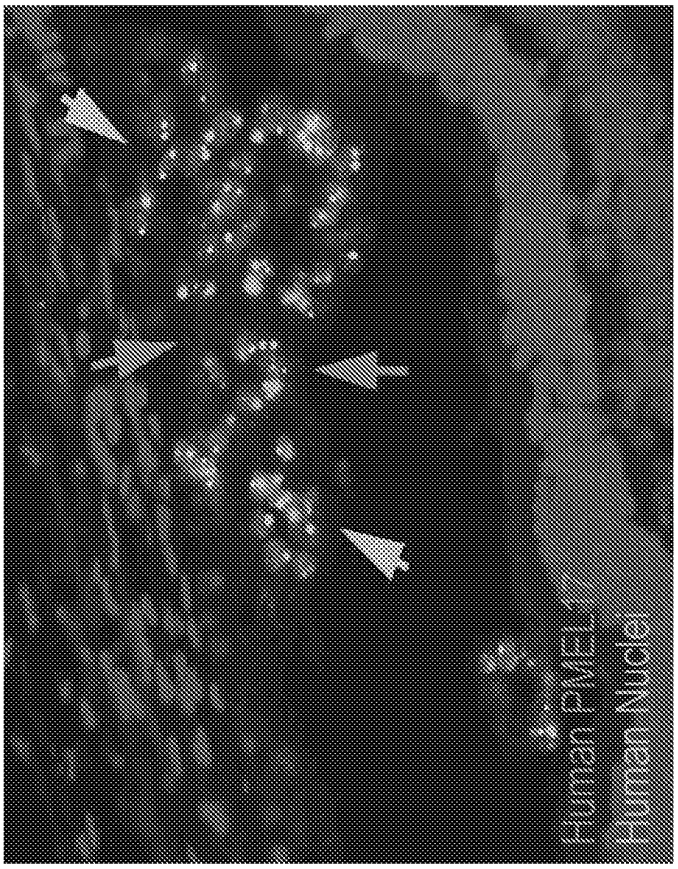


FIG. 28

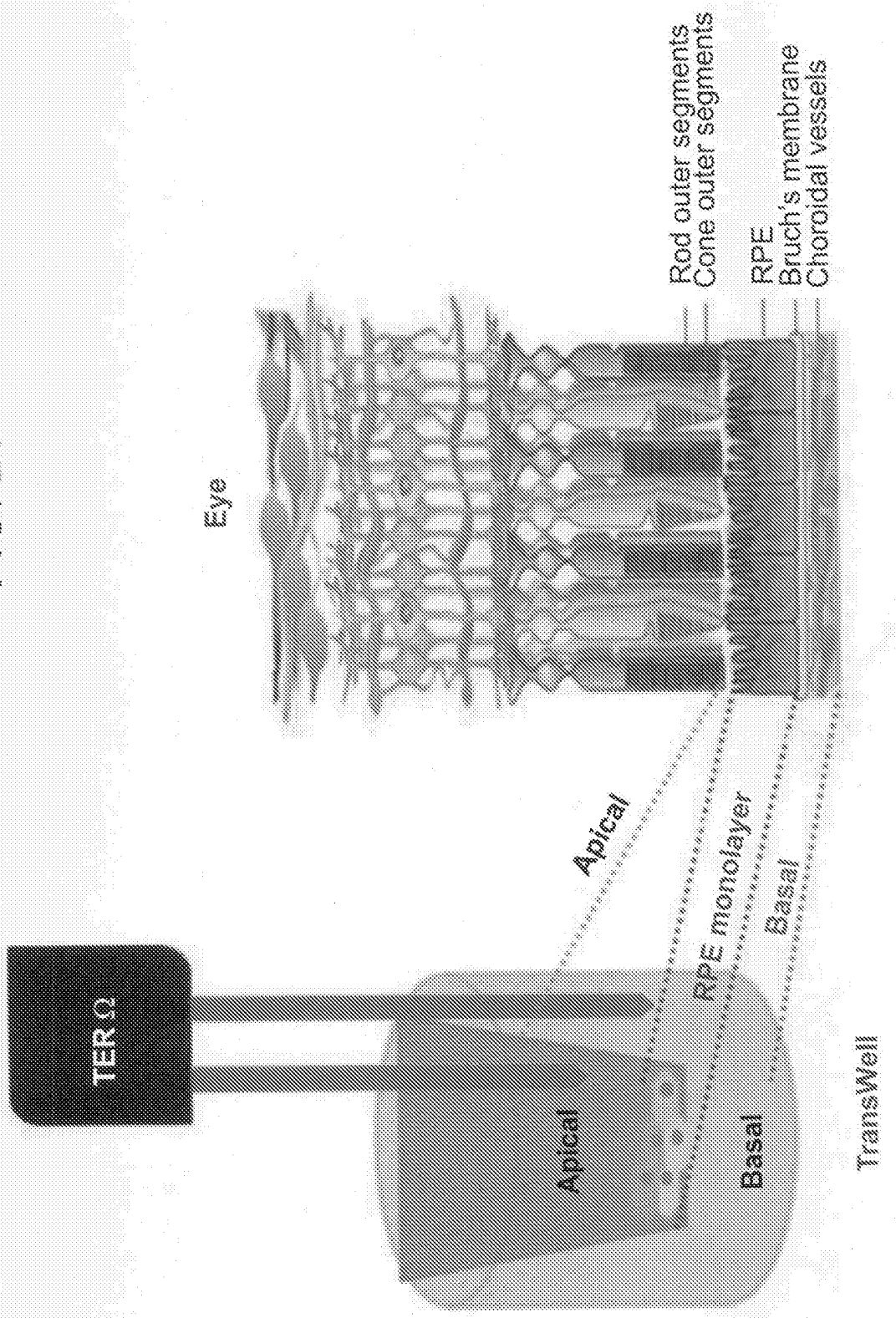

Eye
Rod outer segments
Cone outer segments
RPE
Bruch's membrane
Choroidal vessels
Apical
RPE monolayer
Basal
TER Ω
Apical
Basal
TransWell

FIG. 29

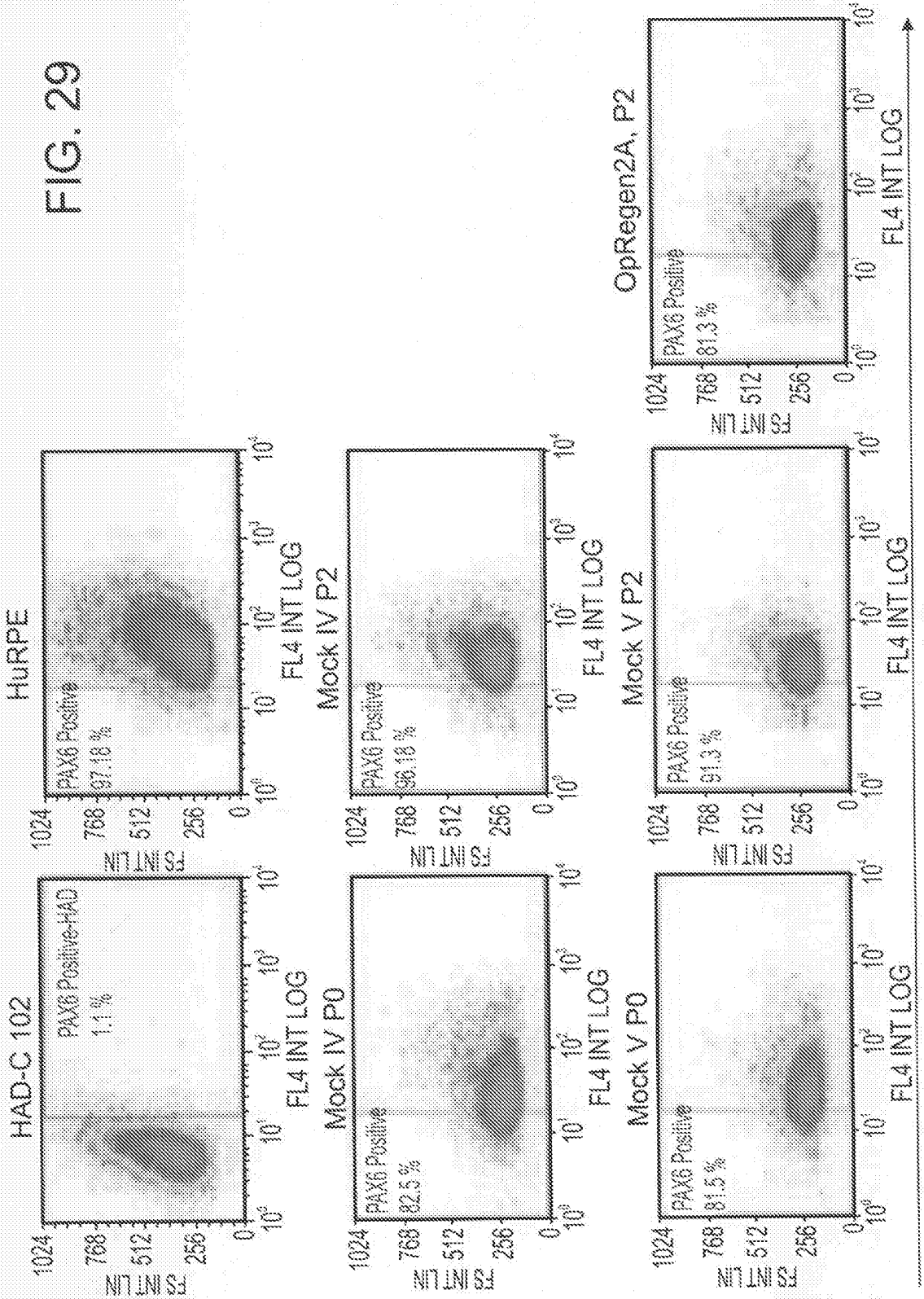

HuRPE
HAD-C 102
Mock IV P2
Mock V P2
OpRegen2A, P2
Mock IV P0
Mock V P0
PAX6 Positive
PAX6 Positive-HAD
97.18 %
1.1 %
96.18 %
82.5 %
91.3 %
81.5 %
81.3 %
FS INT LIN
FL4 INT LOG
PAX6 - Alexa647

FIG. 30

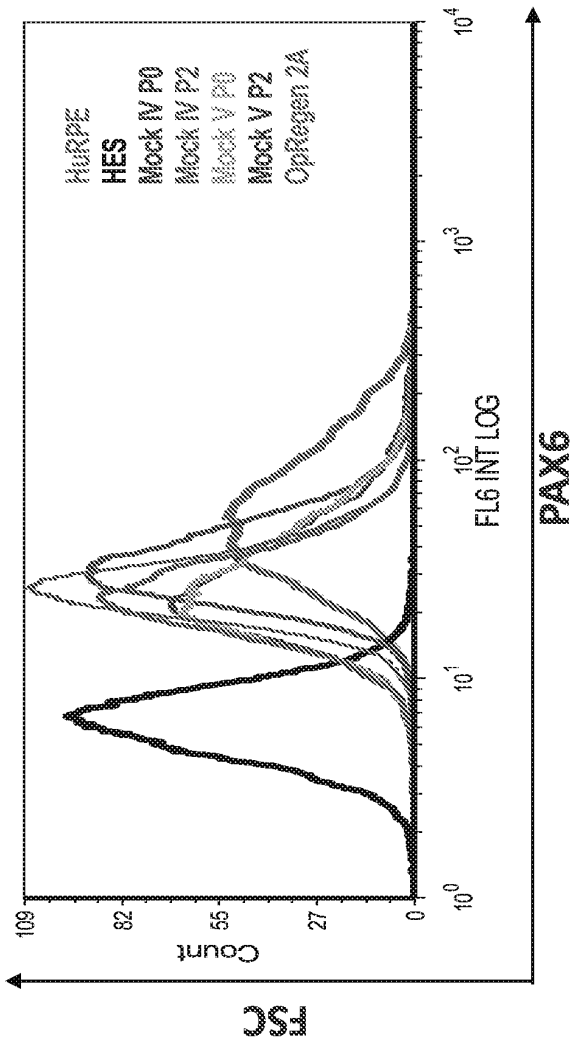

FSC
Count
109
82
55
27
0
10^0
10^1
10^2
10^3
10^4
FL6 INT LOG
PAX6
huRPE
HES
Mock IV P0
Mock IV P2
Mock V P0
Mock V P2
Optiregen 2A

FIG. 31

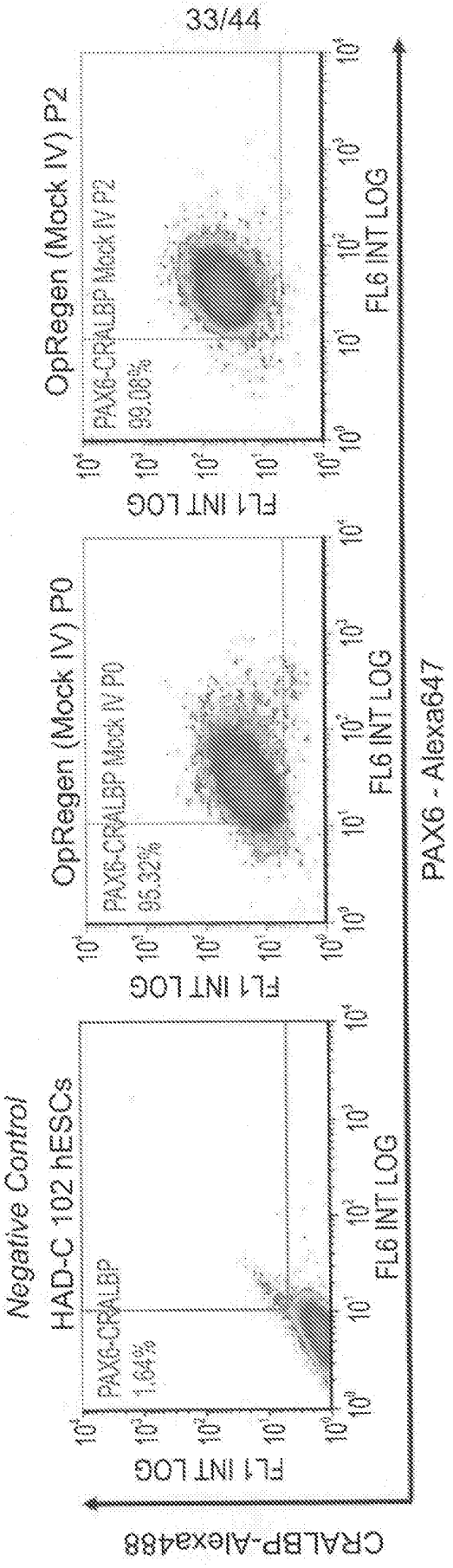

Negative Control
HAD-C 102 hESCs
PAX6-CRALBP
1.64%
OpRegen (Mock IV) P0
PAX6-CRALBP Mock IV P0
95.32%
OpRegen (Mock IV) P2
PAX6-CRALBP Mock IV P2
99.06%
FL1 INT LOG
FL6 INT LOG
CRALBP-Alexa488
PAX6 - Alexa647

FIG. 32A

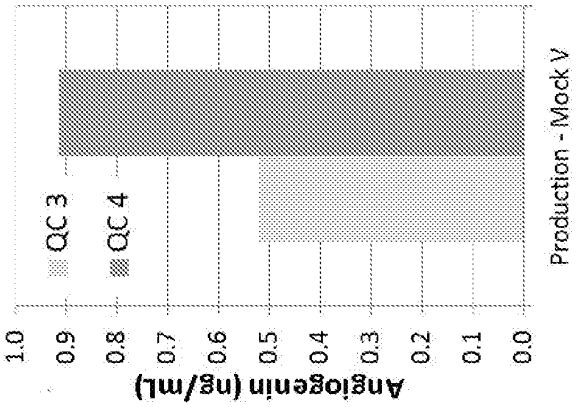


FIG. 32B

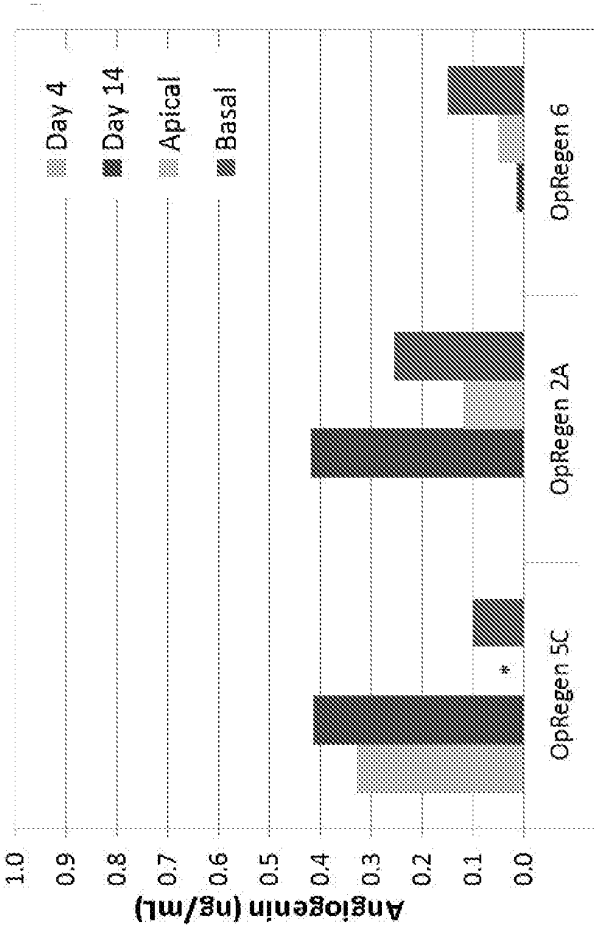


FIG. 32C

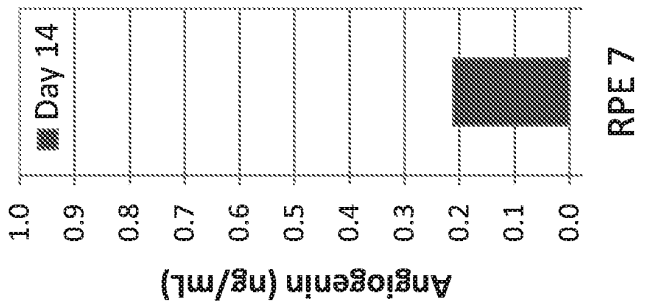
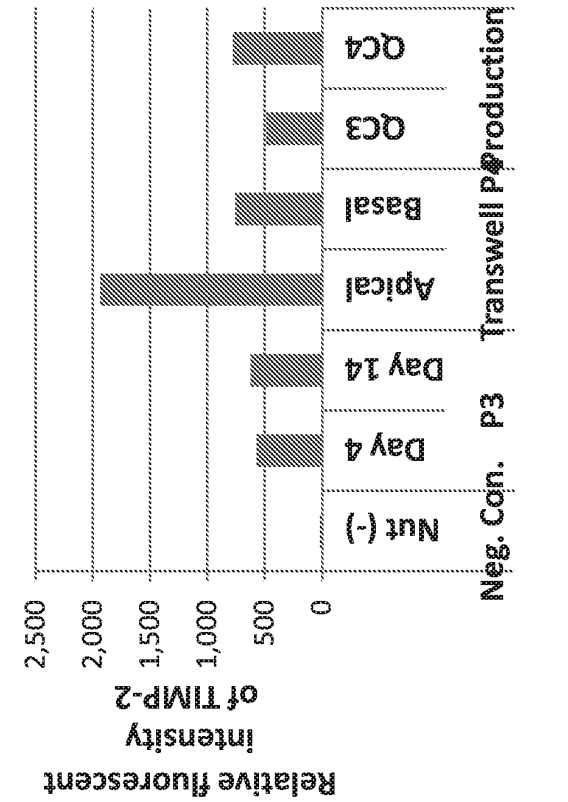


FIG. 33A


Relative fluorescent intensity of TIMP-2
2,500
2,000
1,500
1,000
500
0
Neg. Con.
P3
Transwell P4
Production
Nut (-)
Day 4
Day 14
Apical
Basal
QC3
QC4

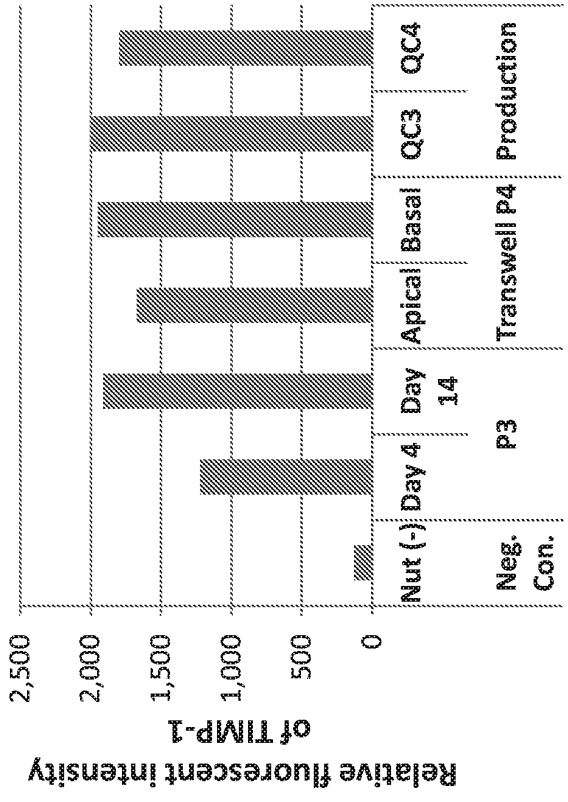

Relative fluorescent intensity of TIMP-1
2,500
2,000
1,500
1,000
500
0
Neg. Con.
P3
Transwell P4
Production
Nut (-)
Day 4
Day 14
Apical
Basal
QC3
QC4

FIG. 33B

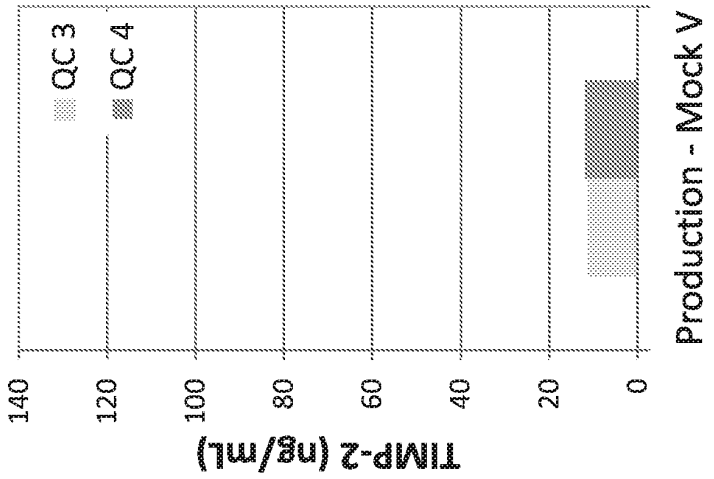


FIG. 33C

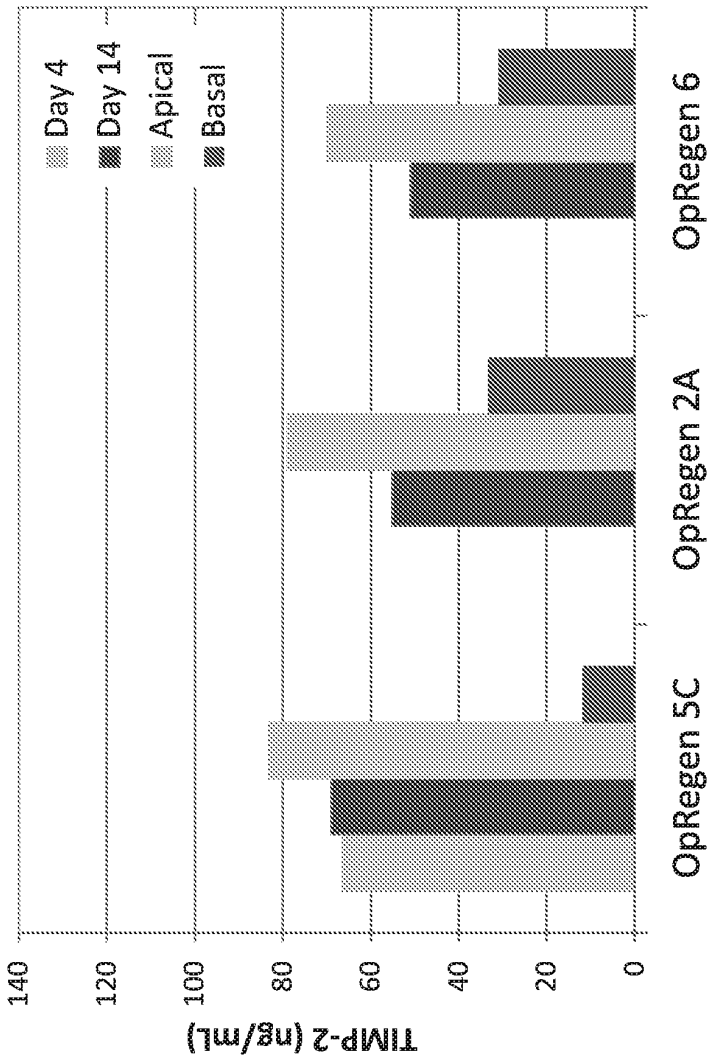


FIG. 33E

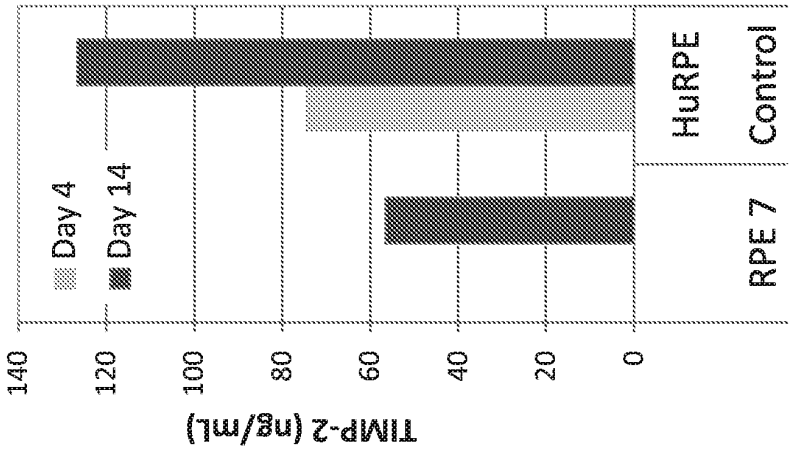


FIG. 33D

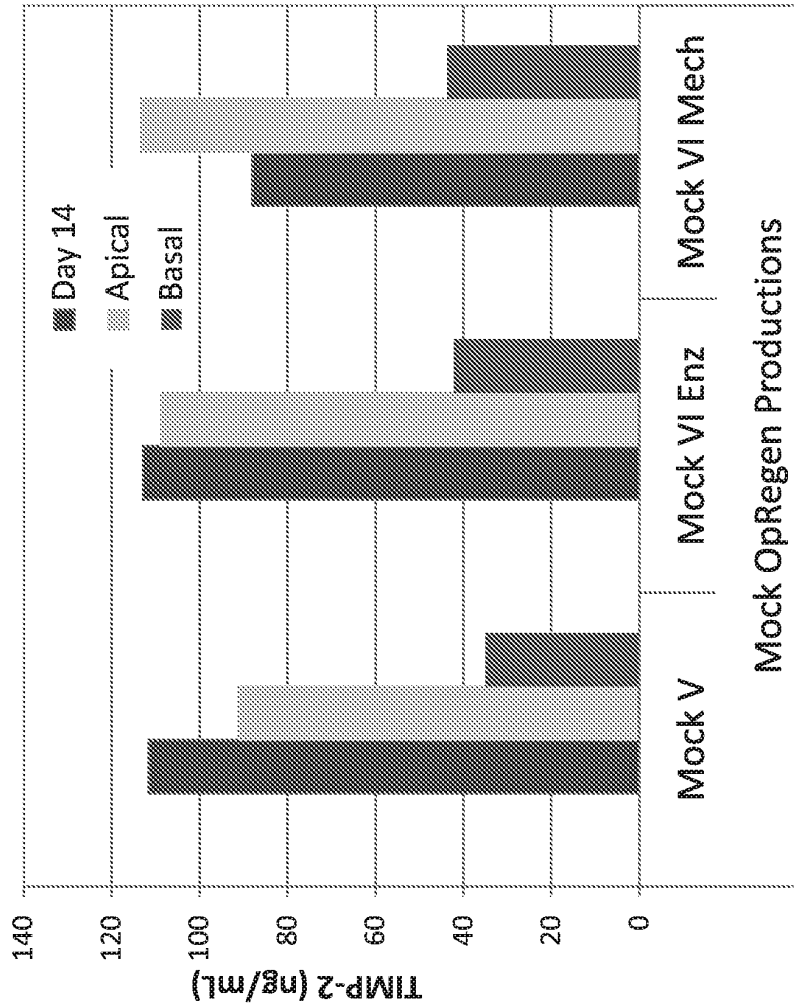


FIG. 34A

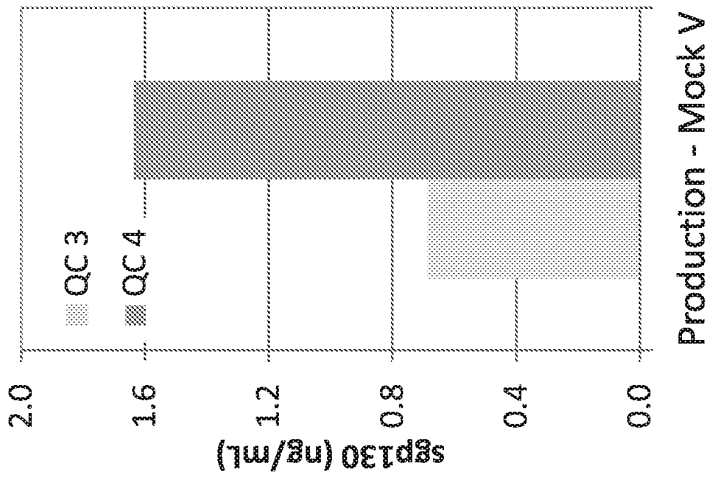


FIG. 34B

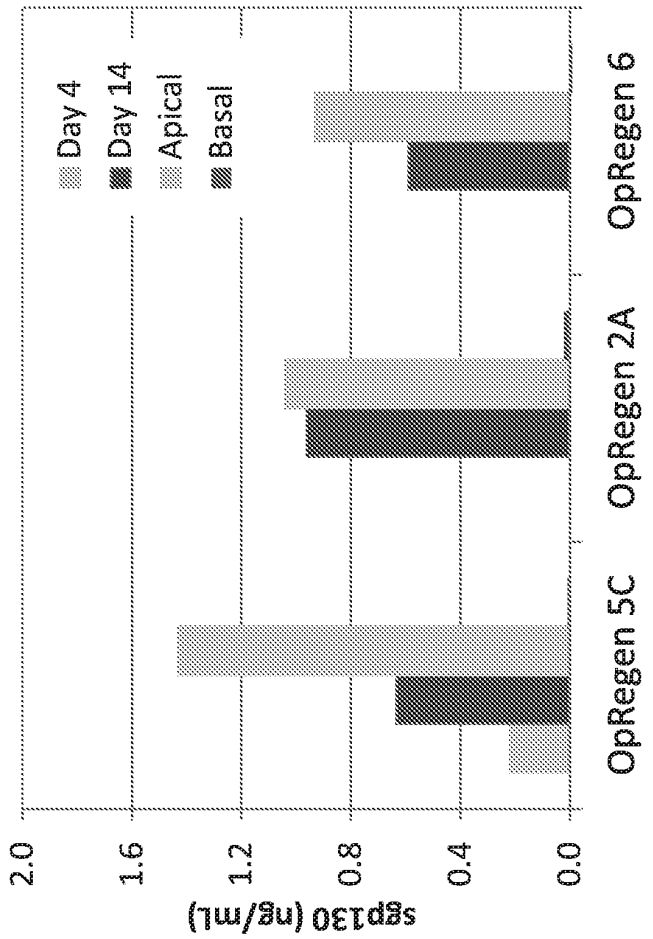


FIG. 34D

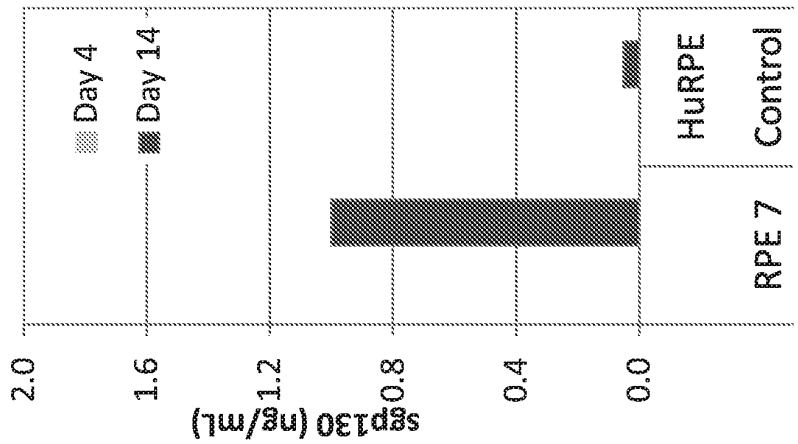


FIG. 34C

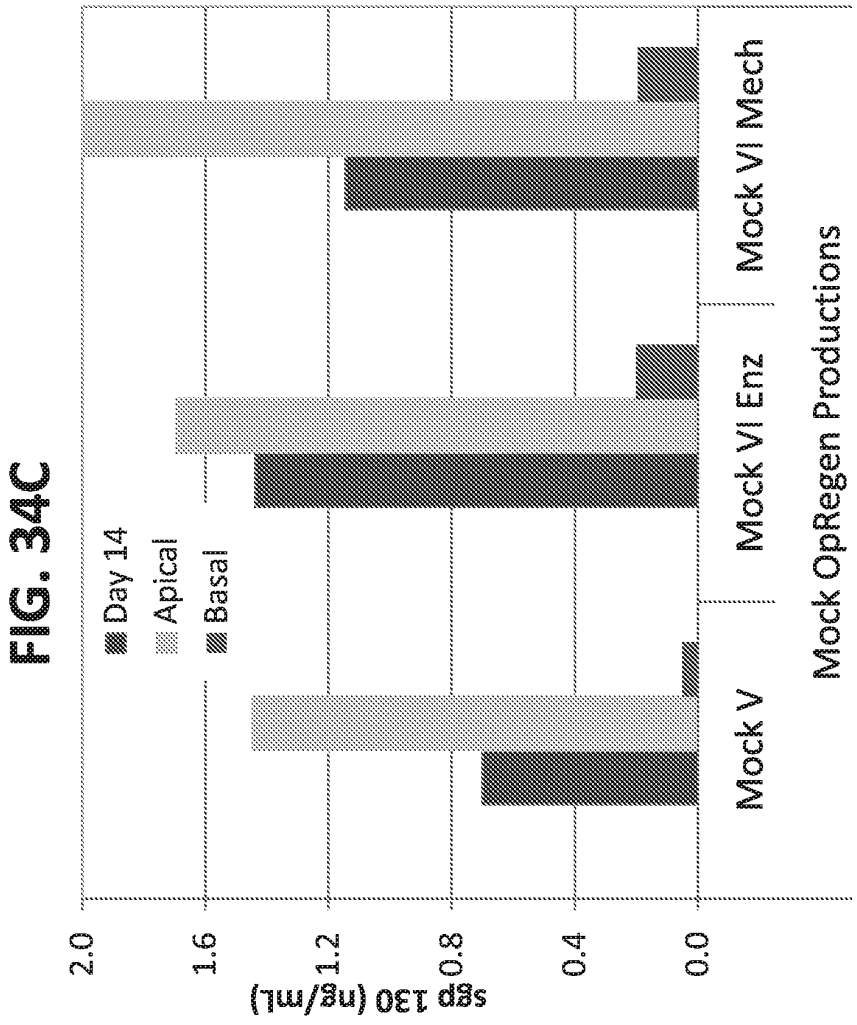


FIG. 35A

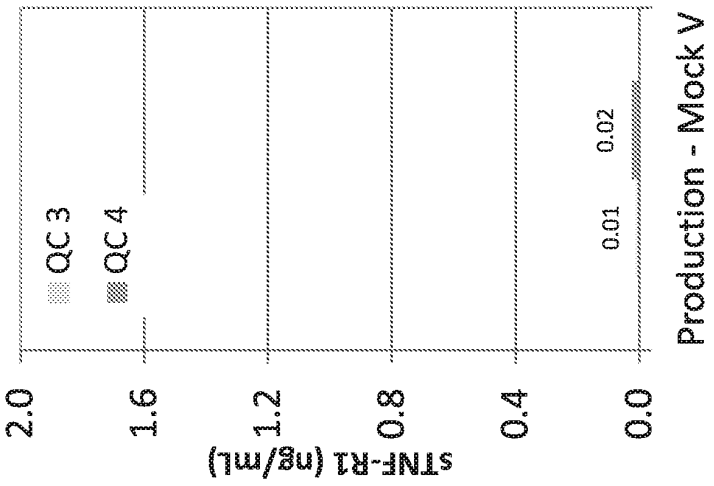


FIG. 35B

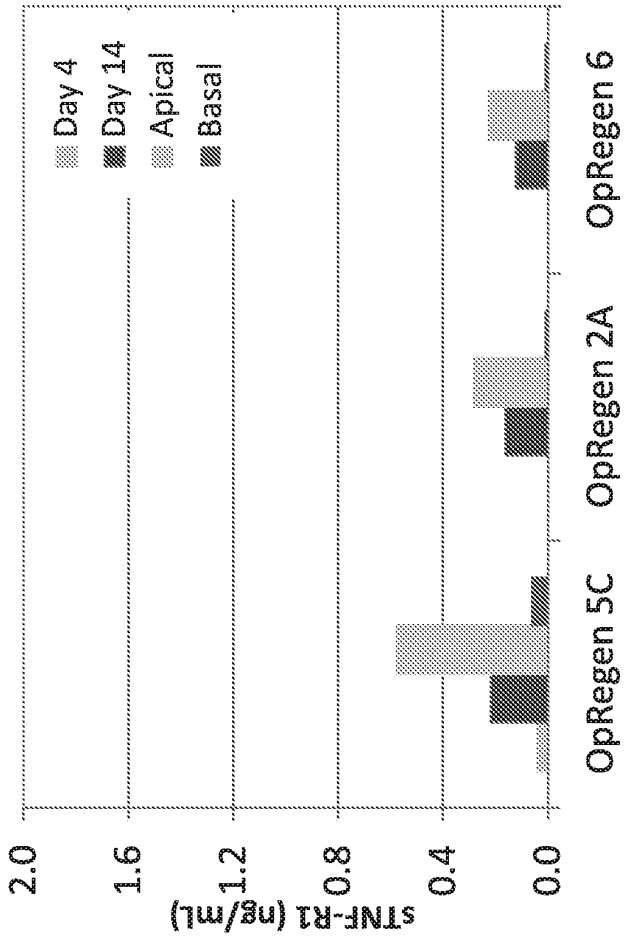


FIG. 35C

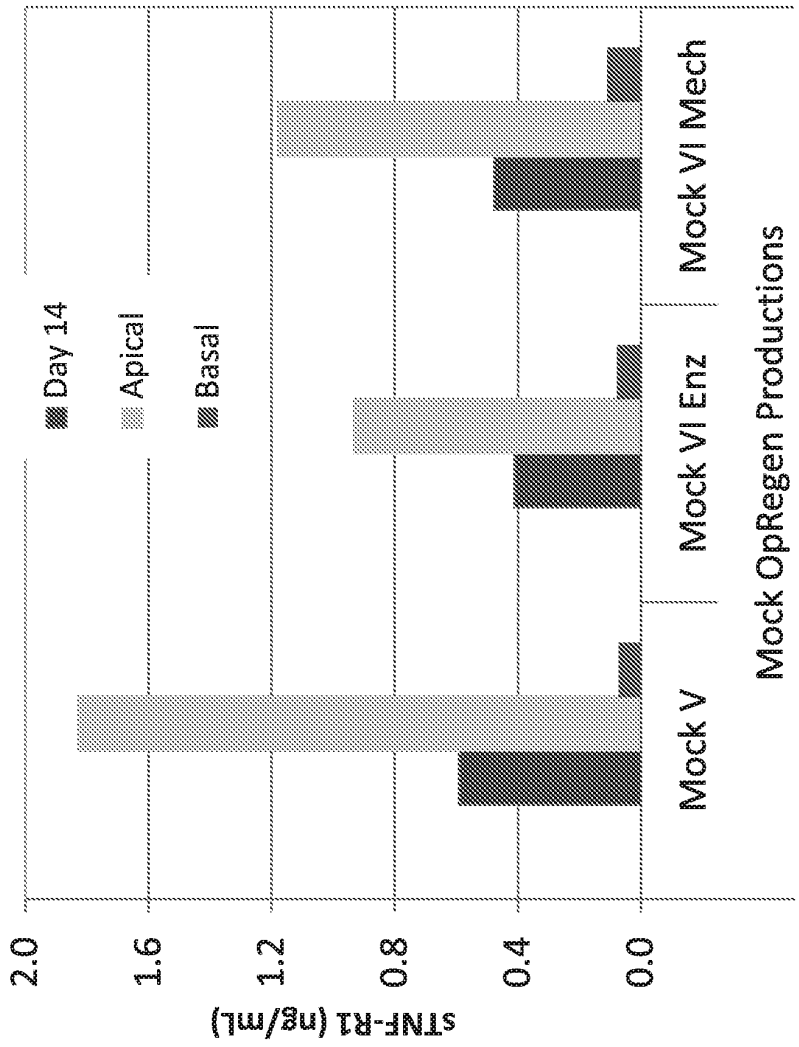


FIG. 35D

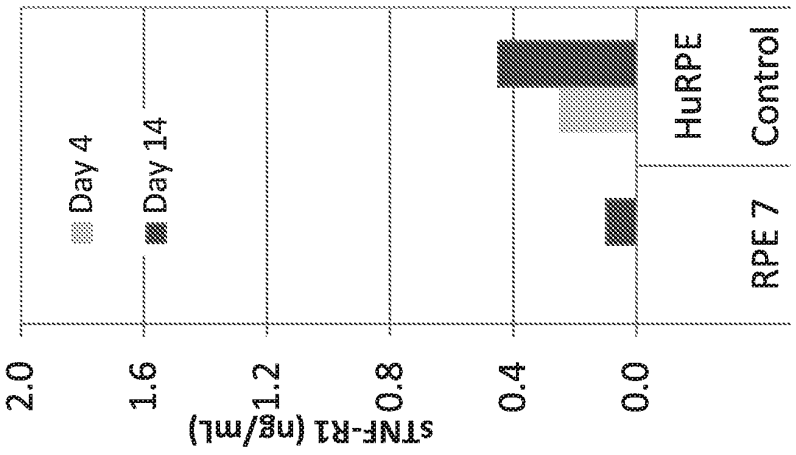
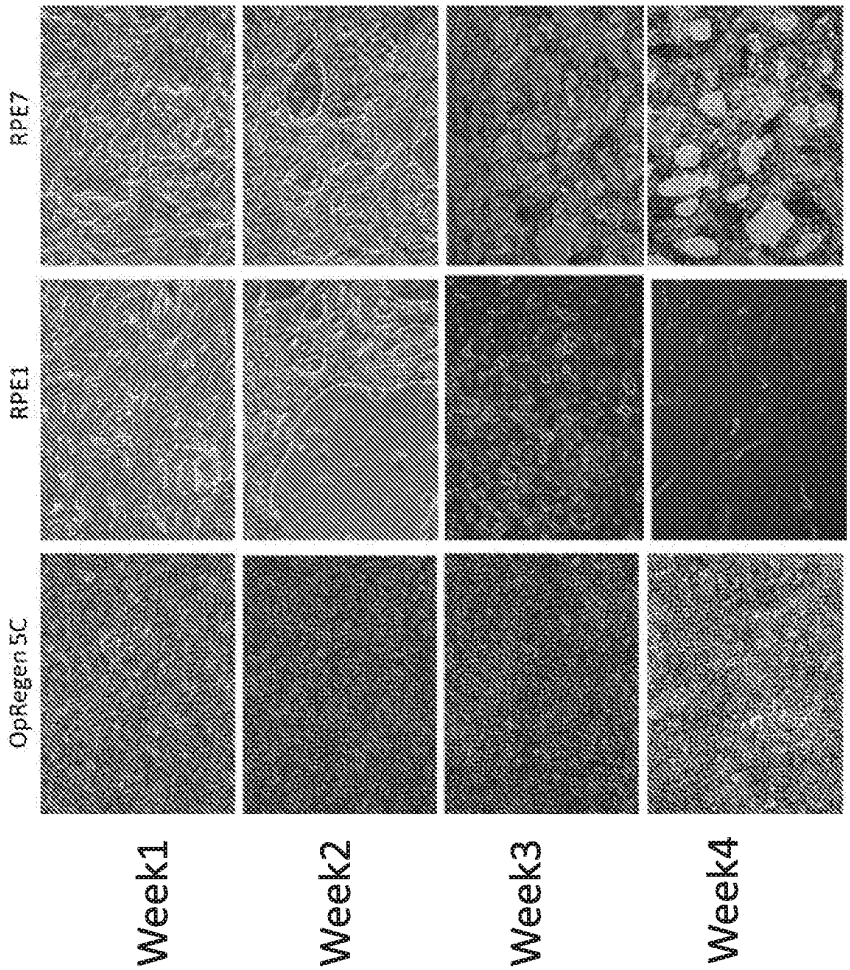


FIG. 36



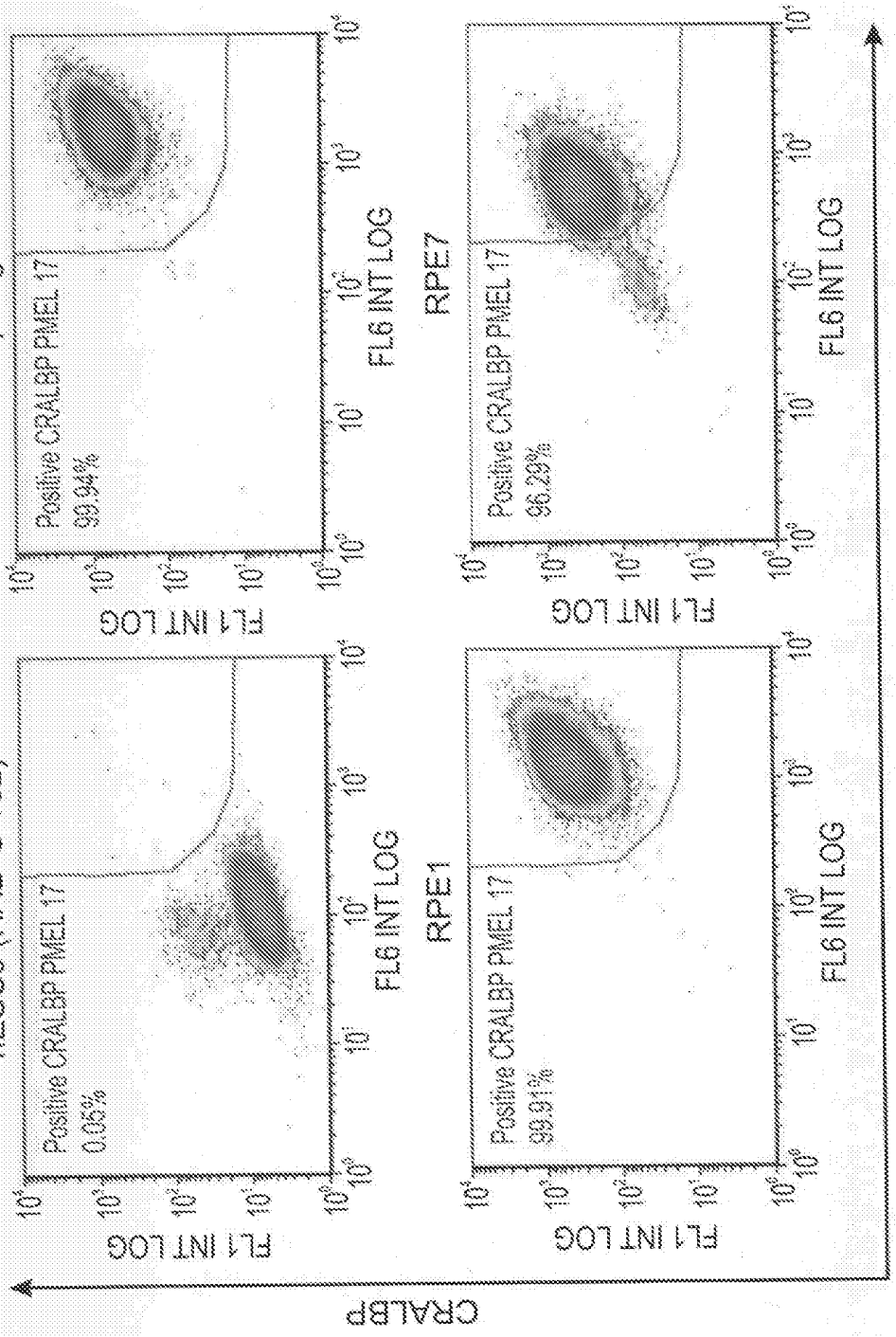

OpRegen®
hESCs (HAD-C 102)
RPE7
RPE1
Positive CRALBP PMEL 17
99.94%
0.05%
96.29%
99.91%
FL1 INT LOG
FL6 INT LOG
CRALBP
PMEL-17

FIG. 37