FIG. 1

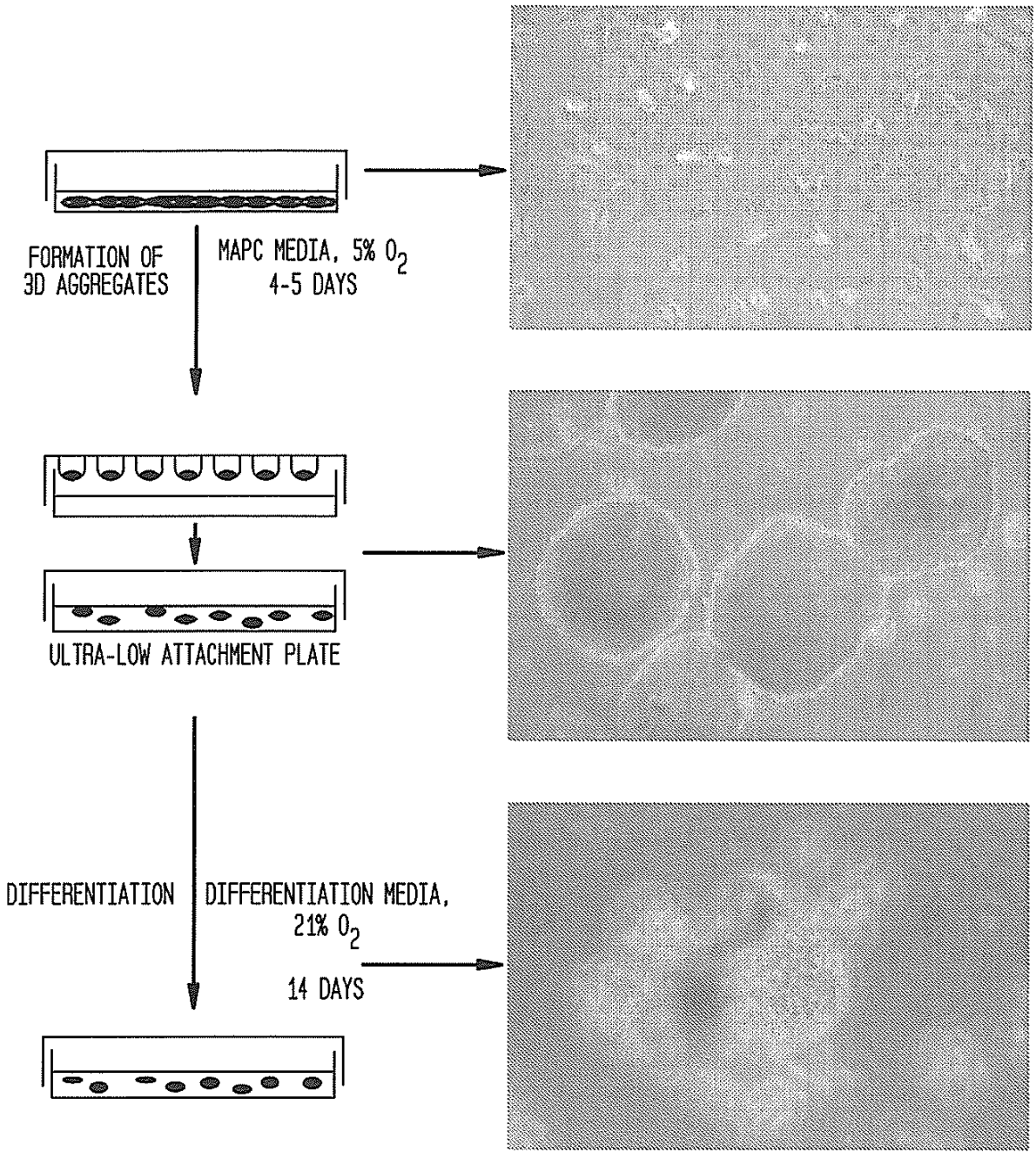

FORMATION OF
3D AGGREGATES
MAPC MEDIA, 5% O_2
4-5 DAYS
ULTRA-LOW ATTACHMENT PLATE
DIFFERENTIATION
DIFFERENTIATION MEDIA,
21% O_2
14 DAYS

FIG. 2

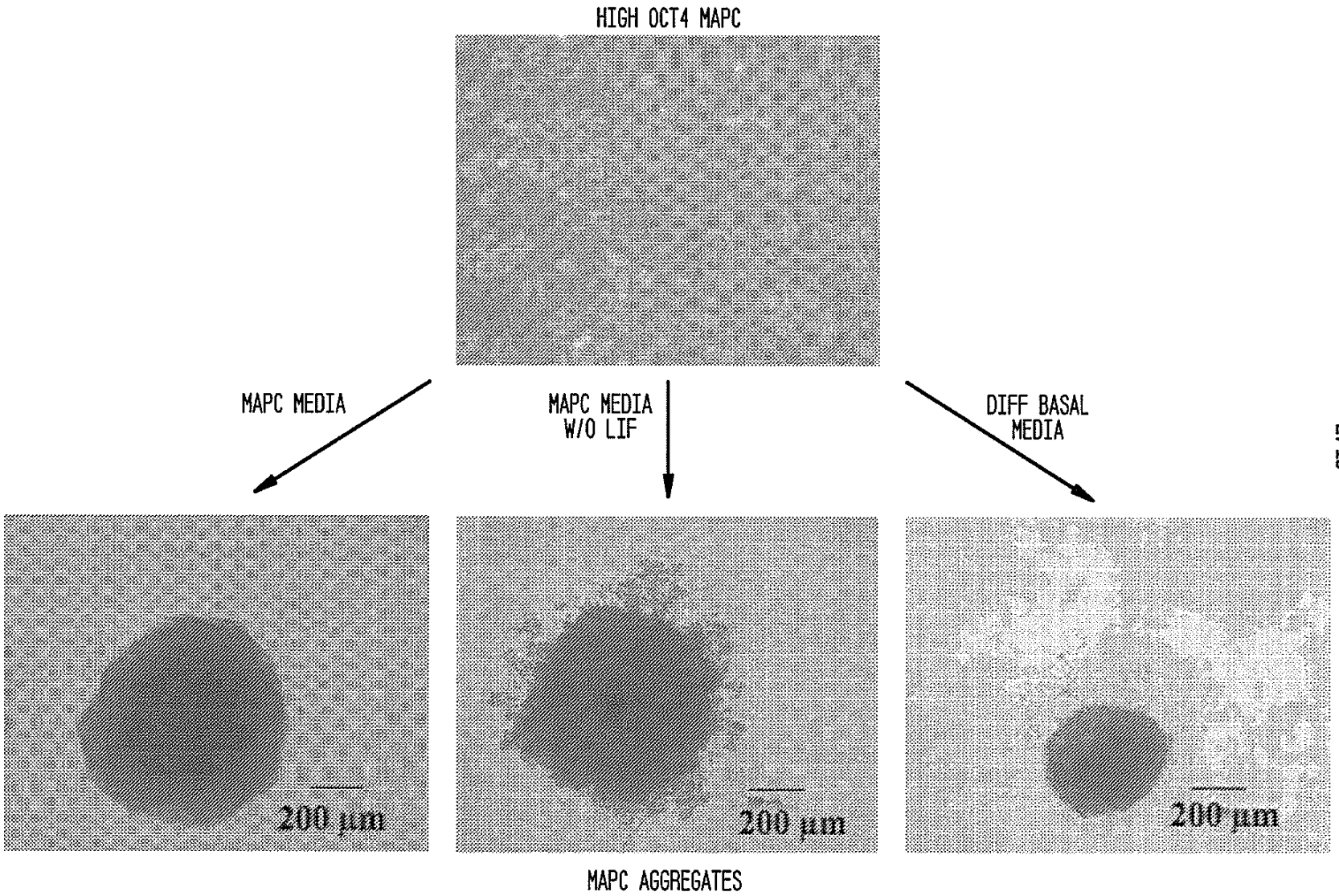

HIGH OCT4 MAPC
MAPC MEDIA
MAPC MEDIA W/O LIF
DIFF BASAL MEDIA
200 µm
200 µm
200 µm
MAPC AGGREGATES

FIG. 3

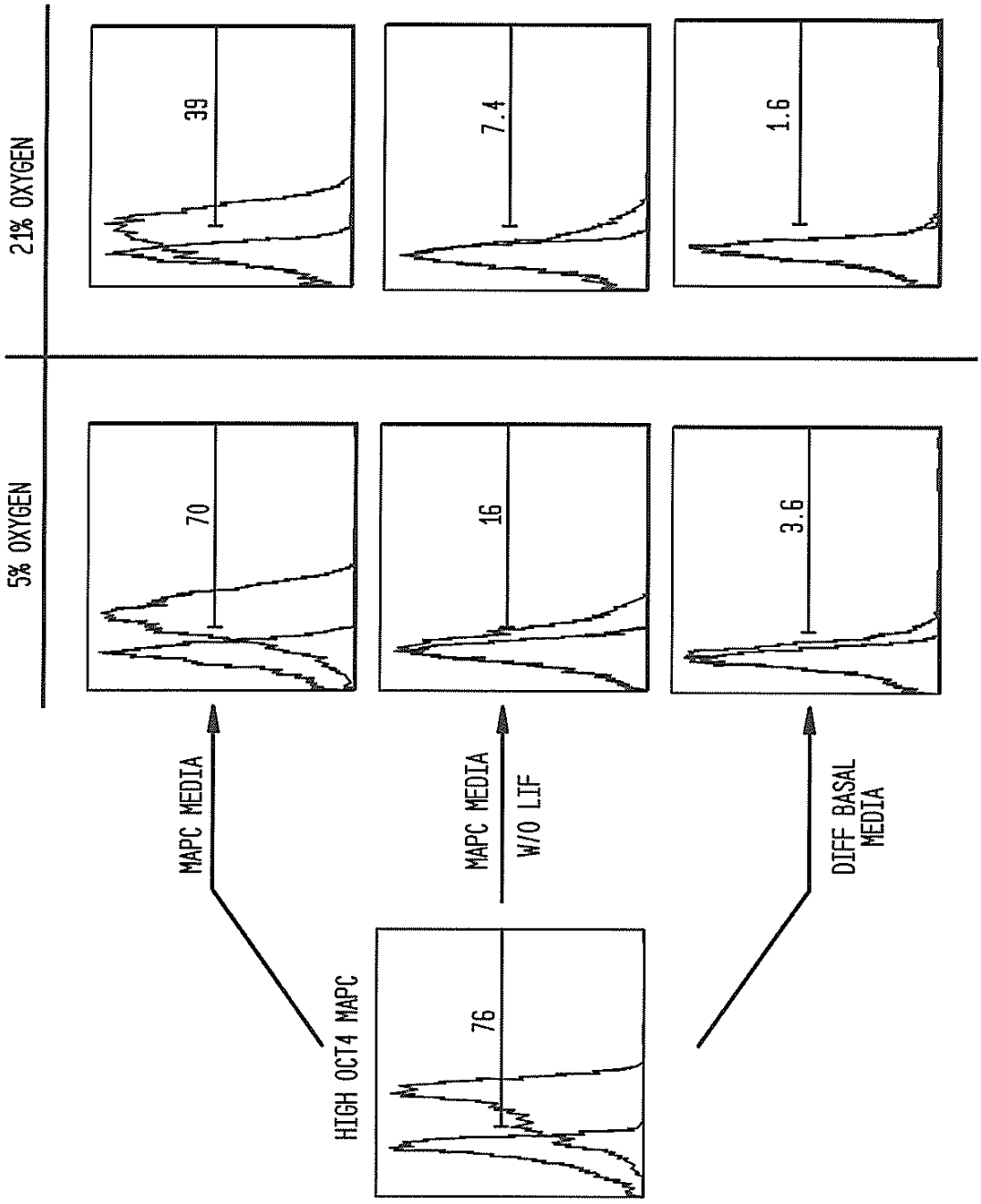

5% OXYGEN
21% OXYGEN
70
16
3.6
39
7.4
1.6
76
HIGH OCT4 MAPC
MAPC MEDIA
MAPC MEDIA
W/O LIF
DIFF BASAL
MEDIA

FIG. 4

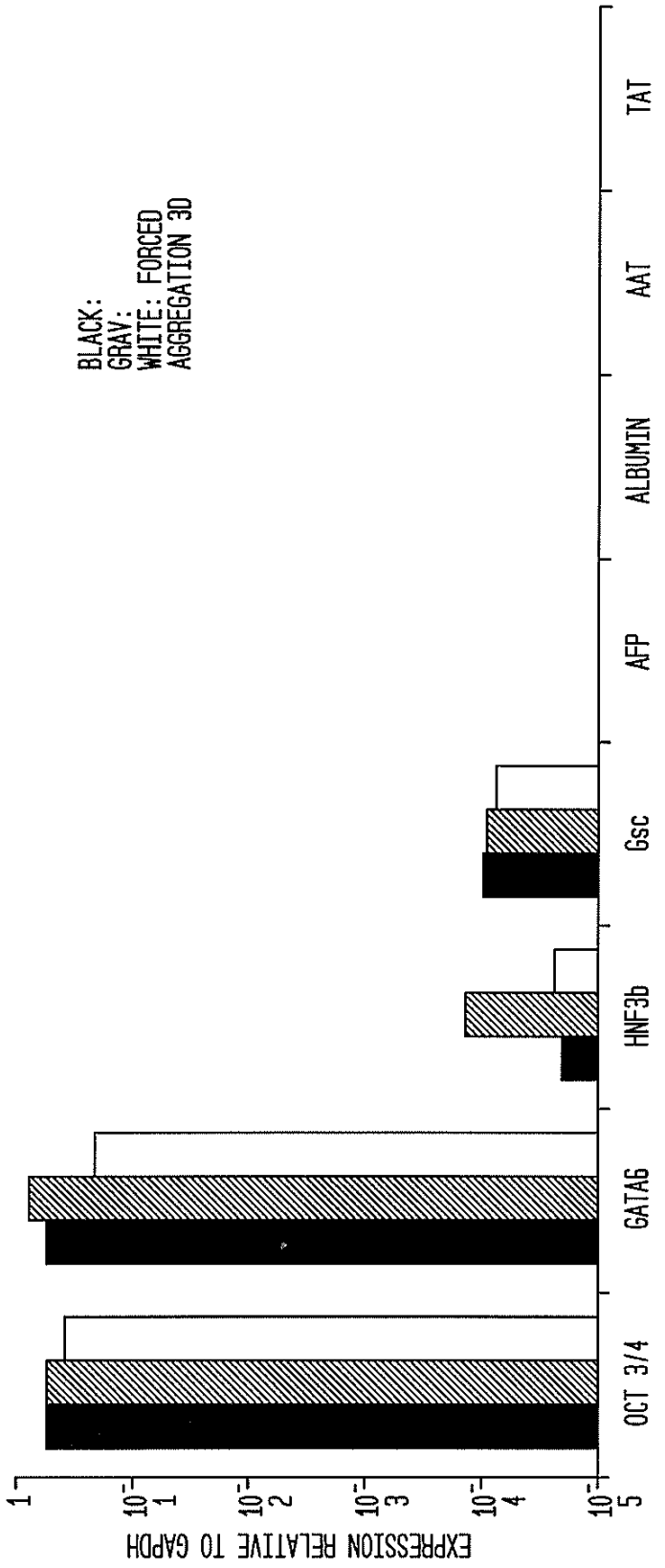

BLACK:
GRAV:
WHITE: FORCED
AGGREGATION 3D
EXPRESSION RELATIVE TO GAPDH
1
10-1
10-2
10-3
10-4
10-5
OCT 3/4
GATA6
HNF3b
Gsc
AFP
ALBUMIN
AAT
TAT

FIG. 5

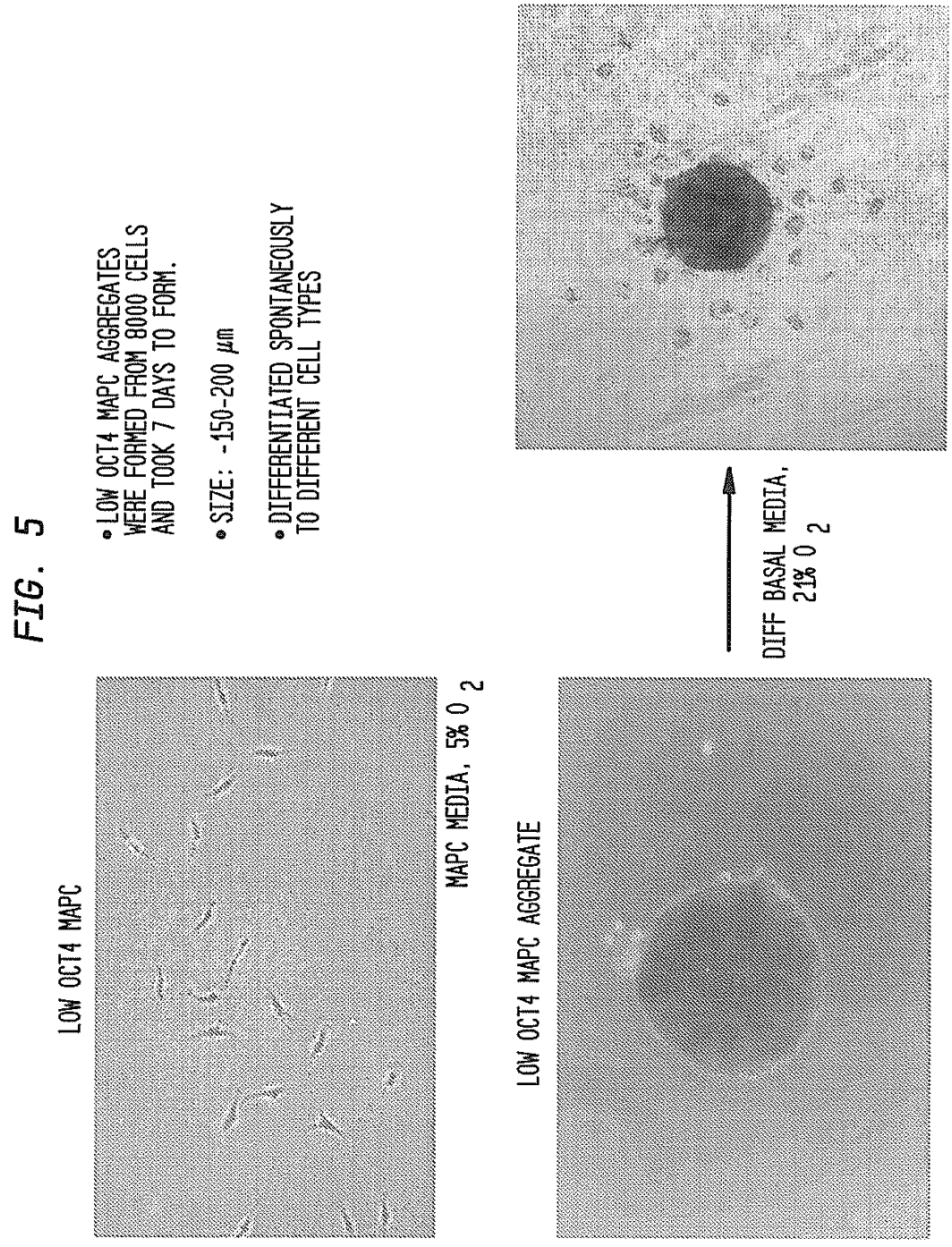

• LOW OCT4 MAPC AGGREGATES WERE FORMED FROM 8000 CELLS AND TOOK 7 DAYS TO FORM.
• SIZE: ~150-200 μm
• DIFFERENTIATED SPONTANEOUSLY TO DIFFERENT CELL TYPES
LOW OCT4 MAPC
MAPC MEDIA, 5% O 2
LOW OCT4 MAPC AGGREGATE
DIFF BASAL MEDIA, 21% O 2

FIG. 6

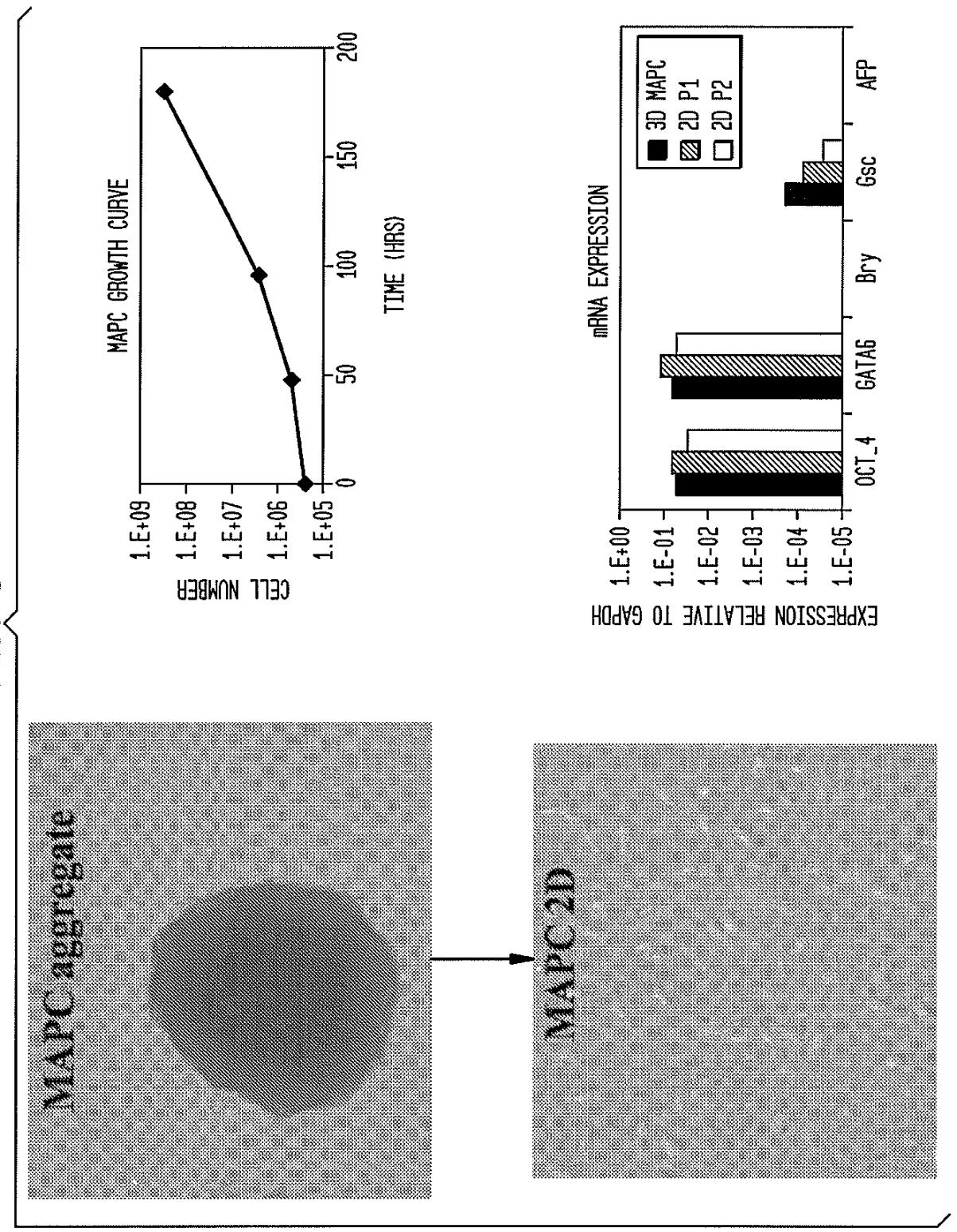

MAPC aggregate
MAPC 2D
MAPC GROWTH CURVE
CELL NUMBER
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
0
50
100
150
200
TIME (HRS)
mRNA EXPRESSION
3D MAPC
2D P1
2D P2
EXPRESSION RELATIVE TO GAPDH
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
OCT_4
GATA6
Bry
Gsc
AFP

FIG. 7

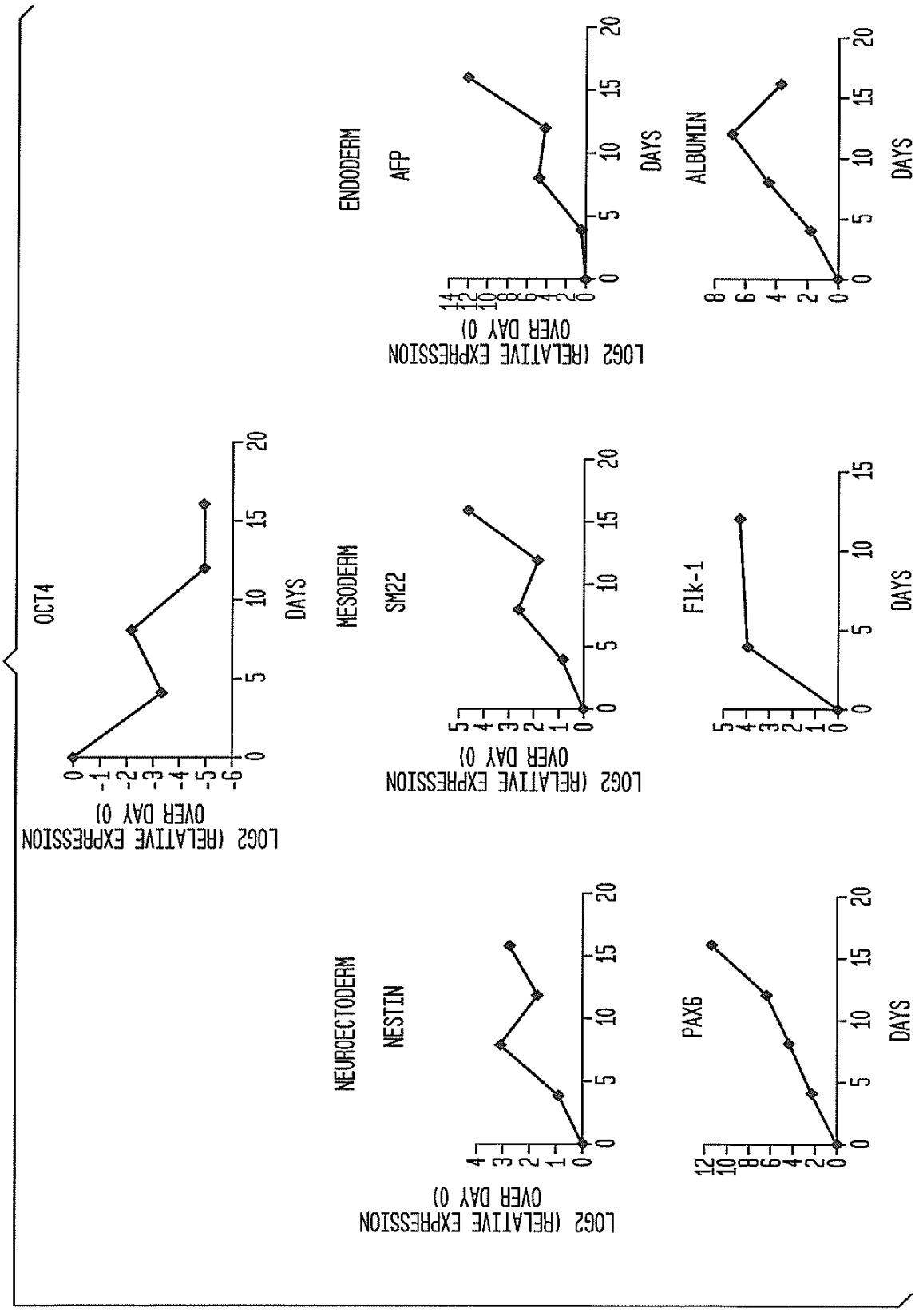

OCT4
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
DAYS
MESODERM
SM22
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
DAYS
ENDODERM
AFP
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
DAYS
ALBUMIN
DAYS
NEUROECTODERM
NESTIN
LOG2 (RELATIVE EXPRESSION OVER DAY 0)
DAYS
Flk-1
DAYS
PAX6
DAYS

FIG. 8

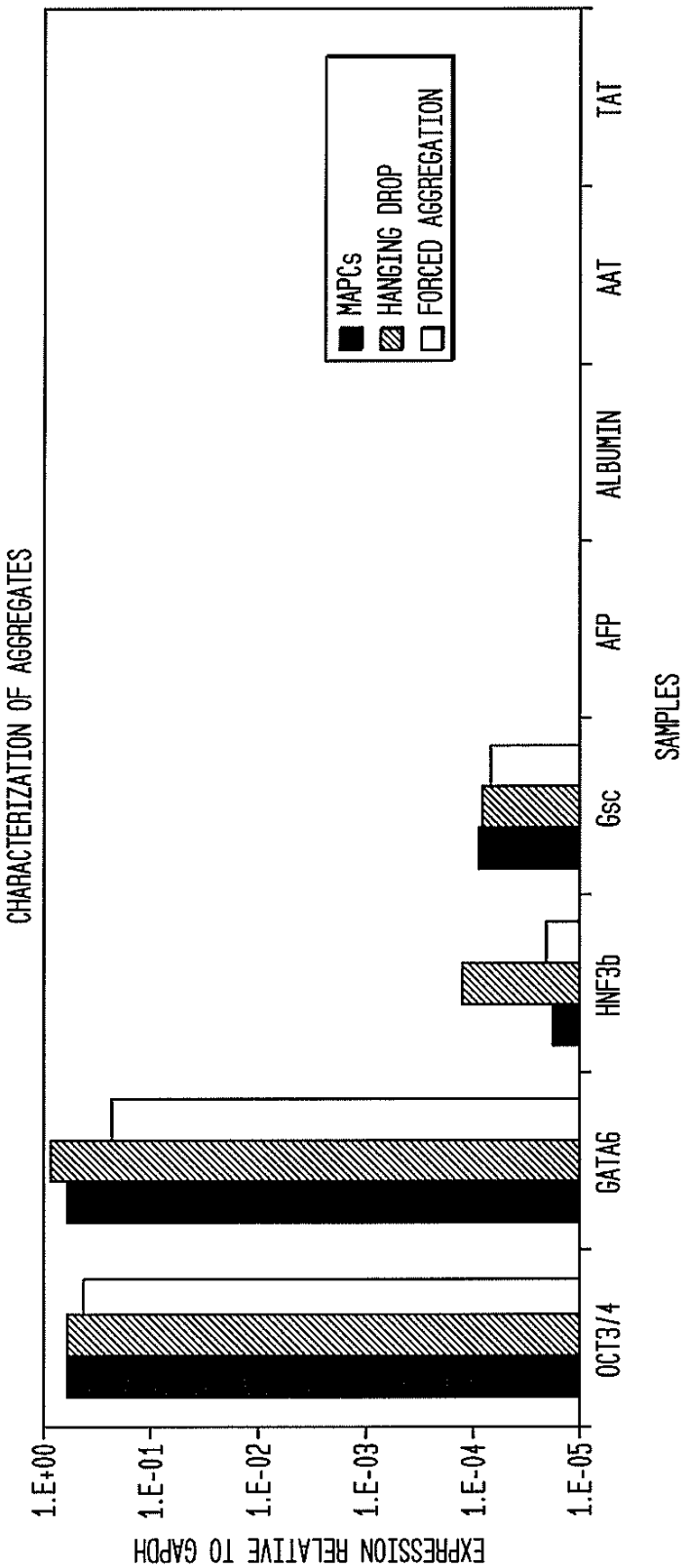

CHARACTERIZATION OF AGGREGATES
MAPCs
HANGING DROP
FORCED AGGREGATION
EXPRESSION RELATIVE TO GAPDH
1.E+00
1.E-01
1.E-02
1.E-03
1.E-04
1.E-05
OCT3/4
GATA6
HNF3b
Gsc
AFP
ALBUMIN
AAT
TAT
SAMPLES

FIG. 9

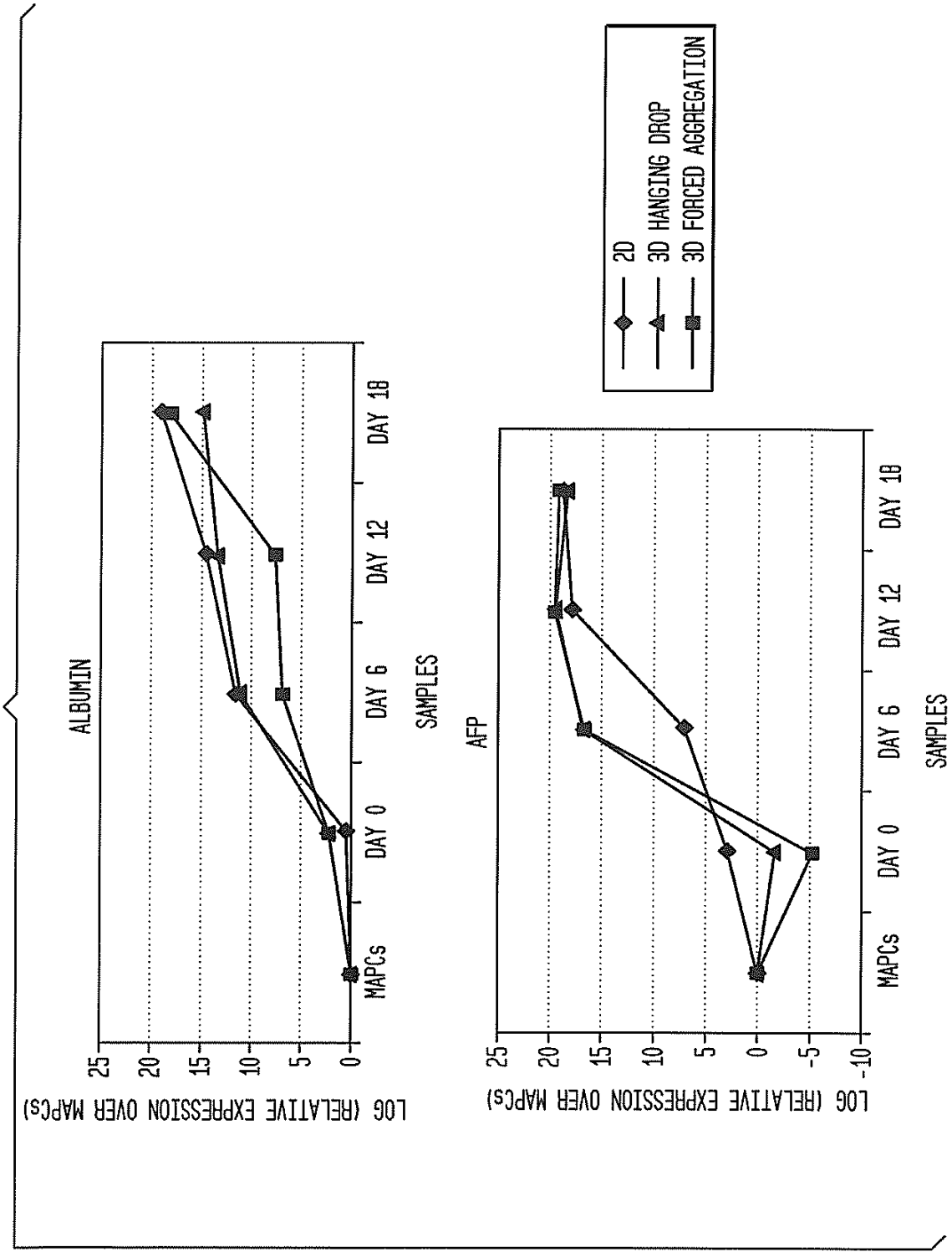

ALBUMIN
LOG (RELATIVE EXPRESSION OVER MAPCs)
25
20
15
10
5
0
MAPCs
DAY 0
DAY 6
DAY 12
DAY 18
SAMPLES
AFP
LOG (RELATIVE EXPRESSION OVER MAPCs)
25
20
15
10
5
0
-5
-10
MAPCs
DAY 0
DAY 6
DAY 12
DAY 18
SAMPLES
2D
3D HANGING DROP
3D FORCED AGGREGATION

FIG. 10

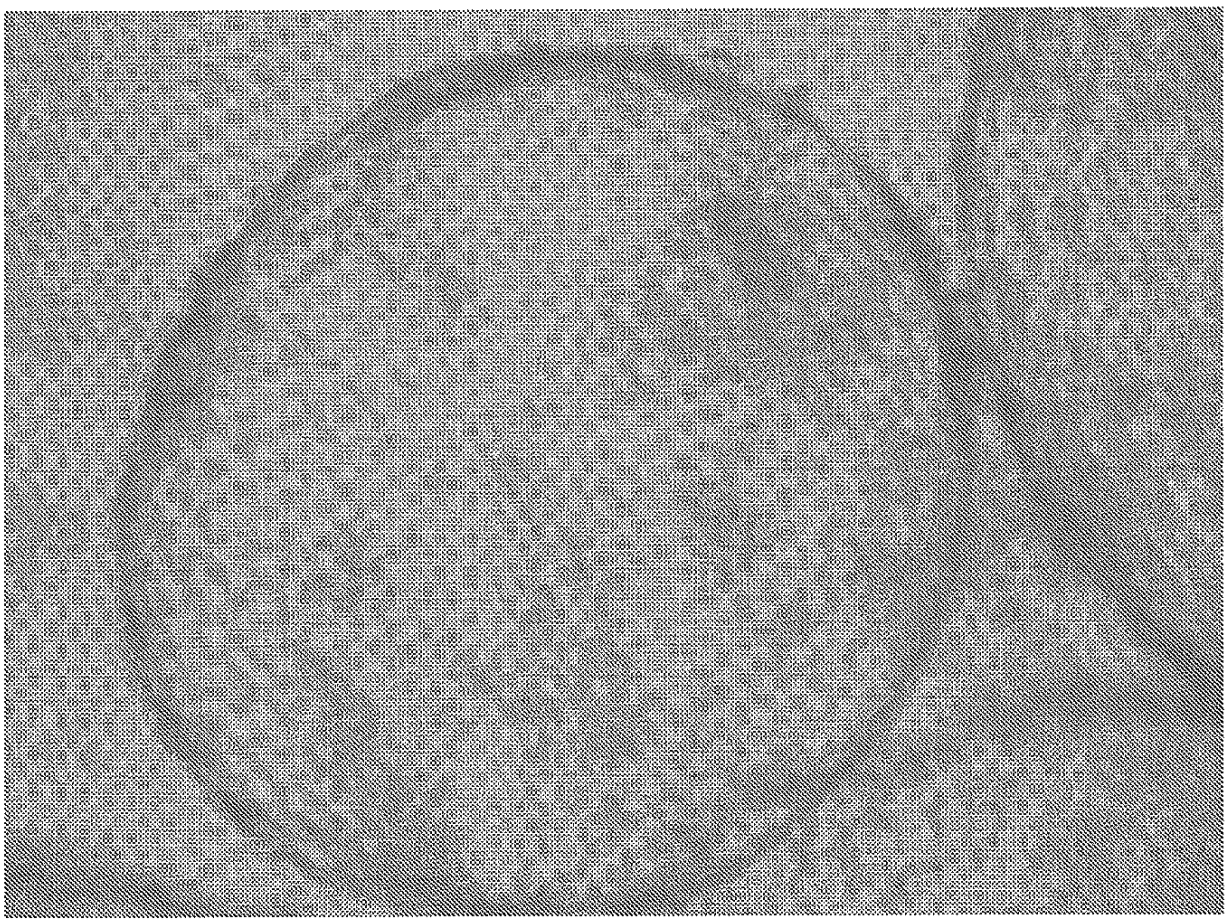


FIG. 11A

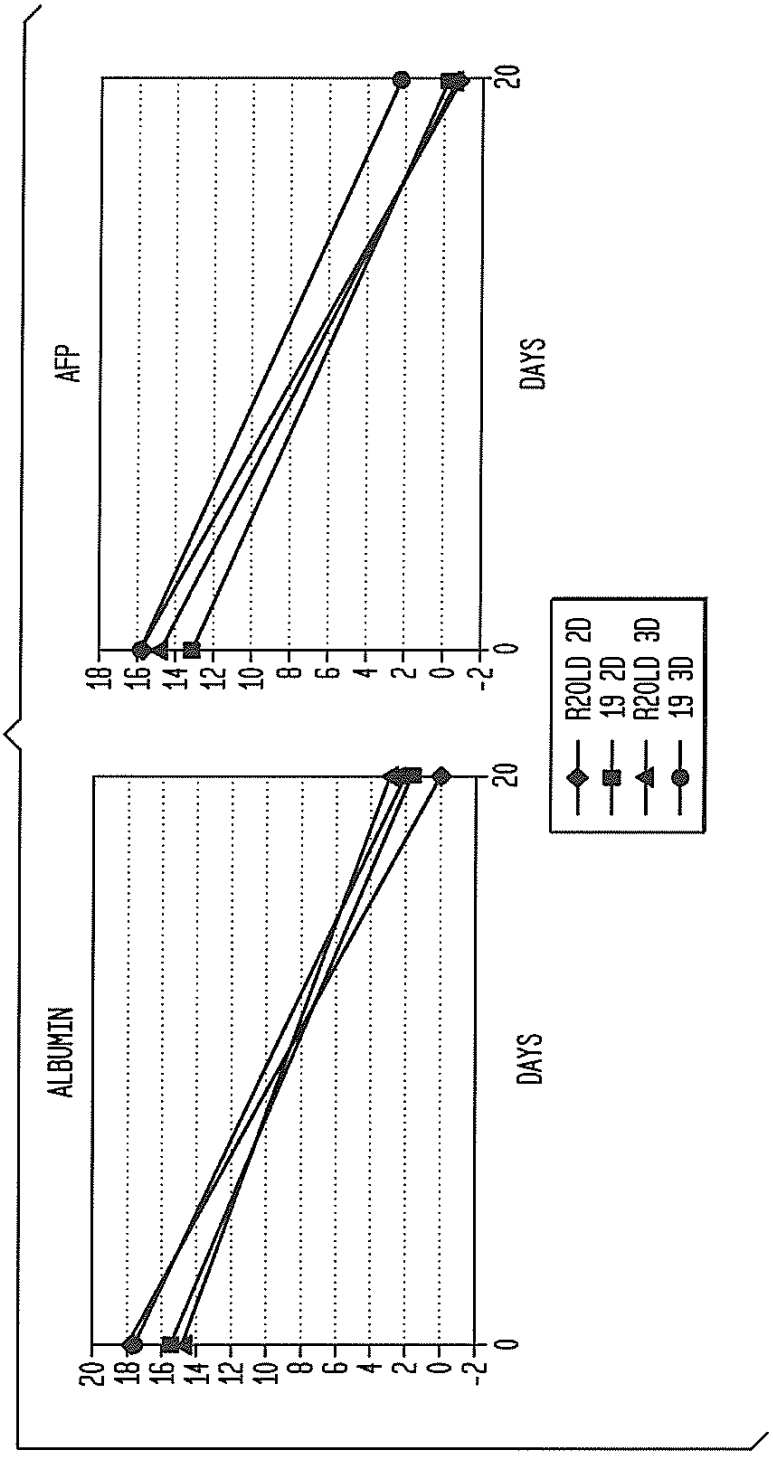


FIG. 11B

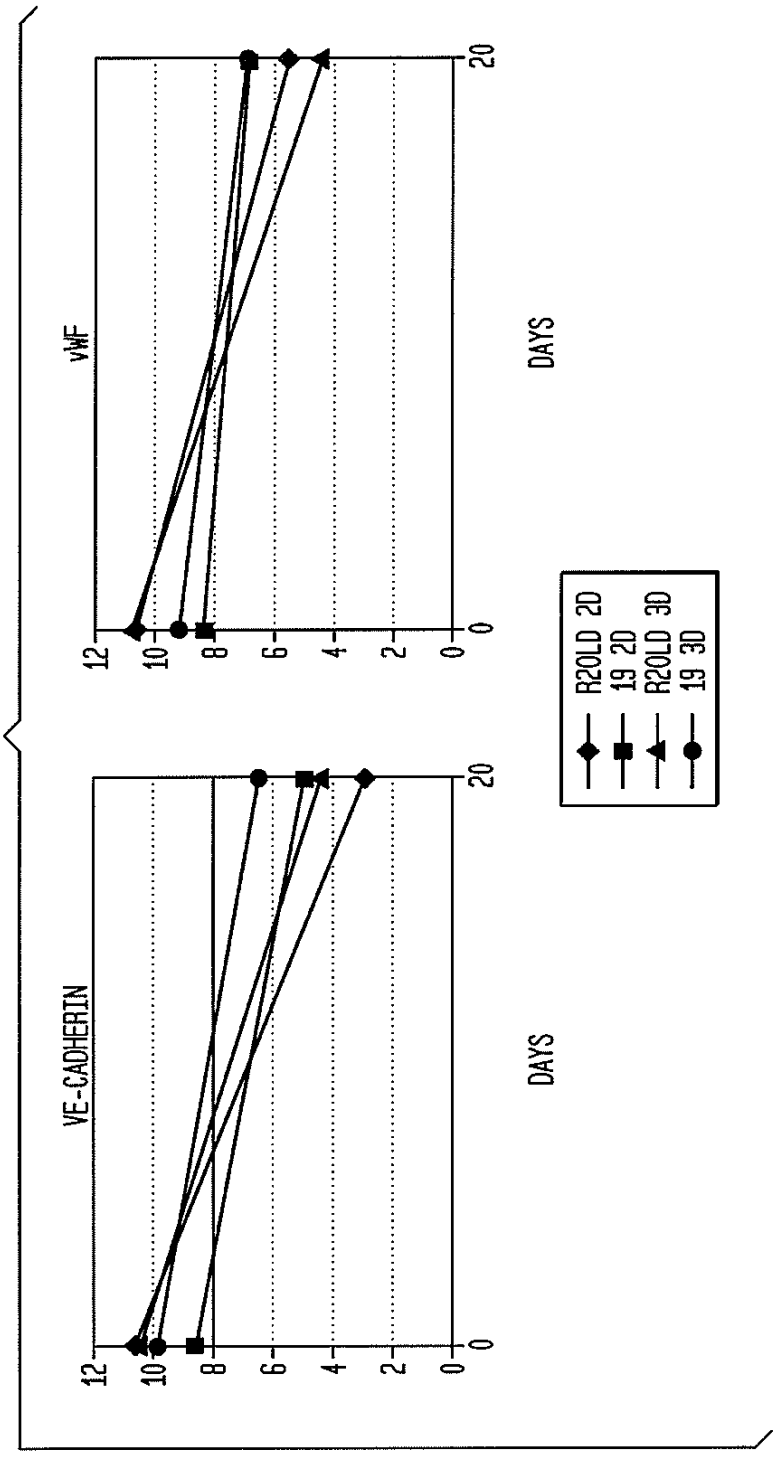
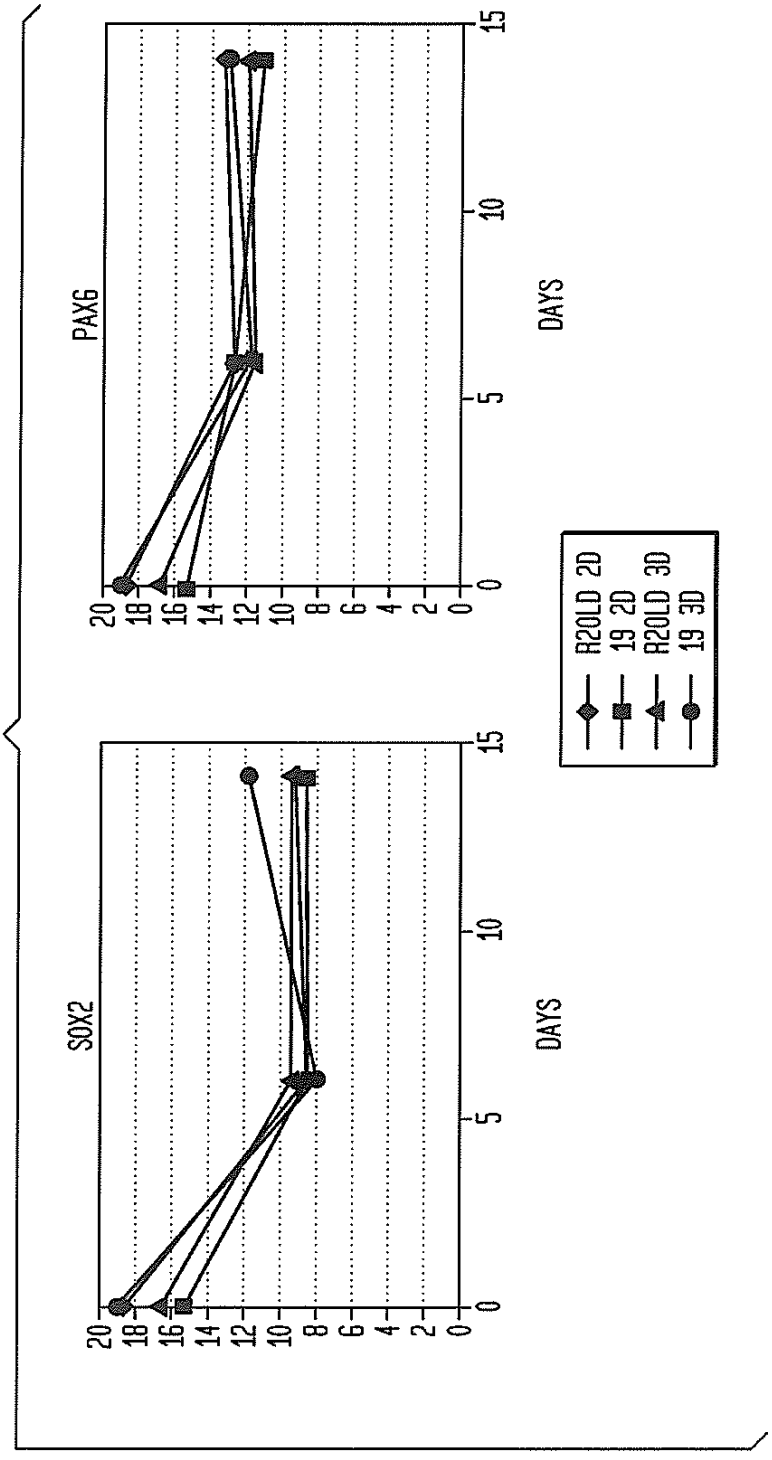

vWF
VE-CADHERIN
0
2
4
6
8
10
12
0
20
DAYS
R20LD 2D
19 2D
R20LD 3D
19 3D

FIG. 11C


SOX2
PAX6
DAYS
DAYS
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
R20LD 2D
19 2D
R20LD 3D
19 3D