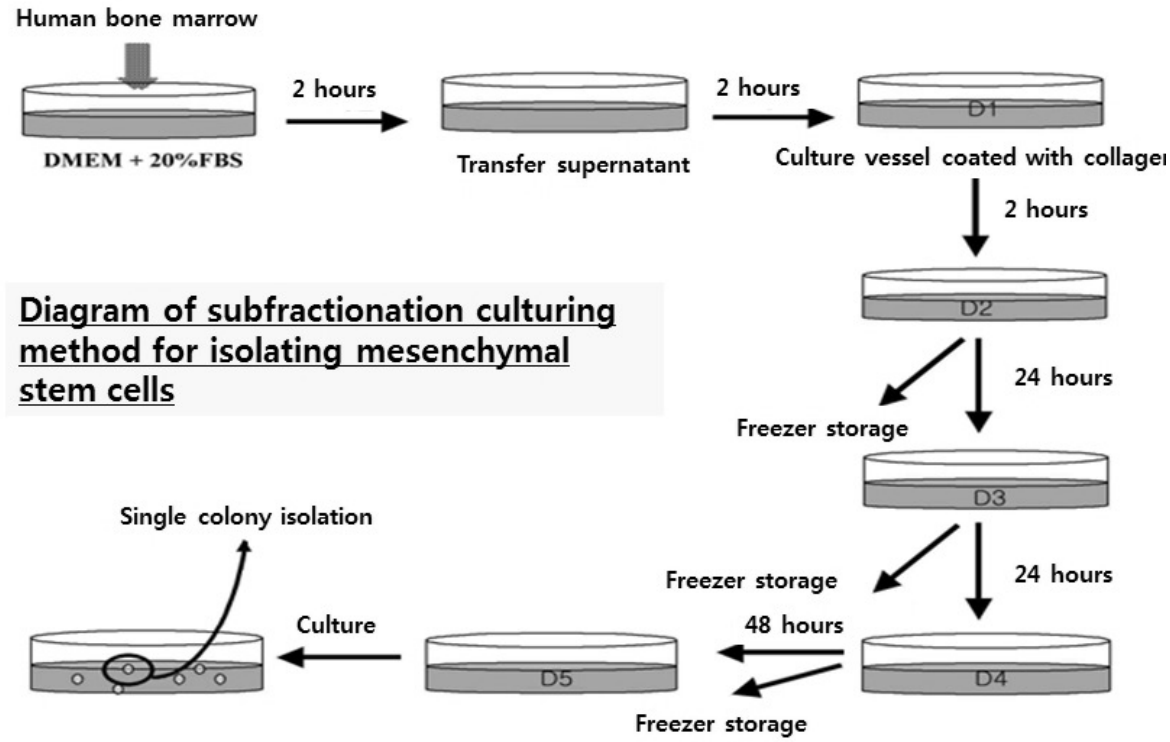

Human bone marrow
DMEM + 20%FBS
2 hours
Transfer supernatant
2 hours
D1
Culture vessel coated with collagen
2 hours
D2
24 hours
Freezer storage
D3
24 hours
Freezer storage
48 hours
D4
Freezer storage
D5
Culture
Single colony isolation
Diagram of subfractionation culturing method for isolating mesenchymal stem cells

FIG. 1

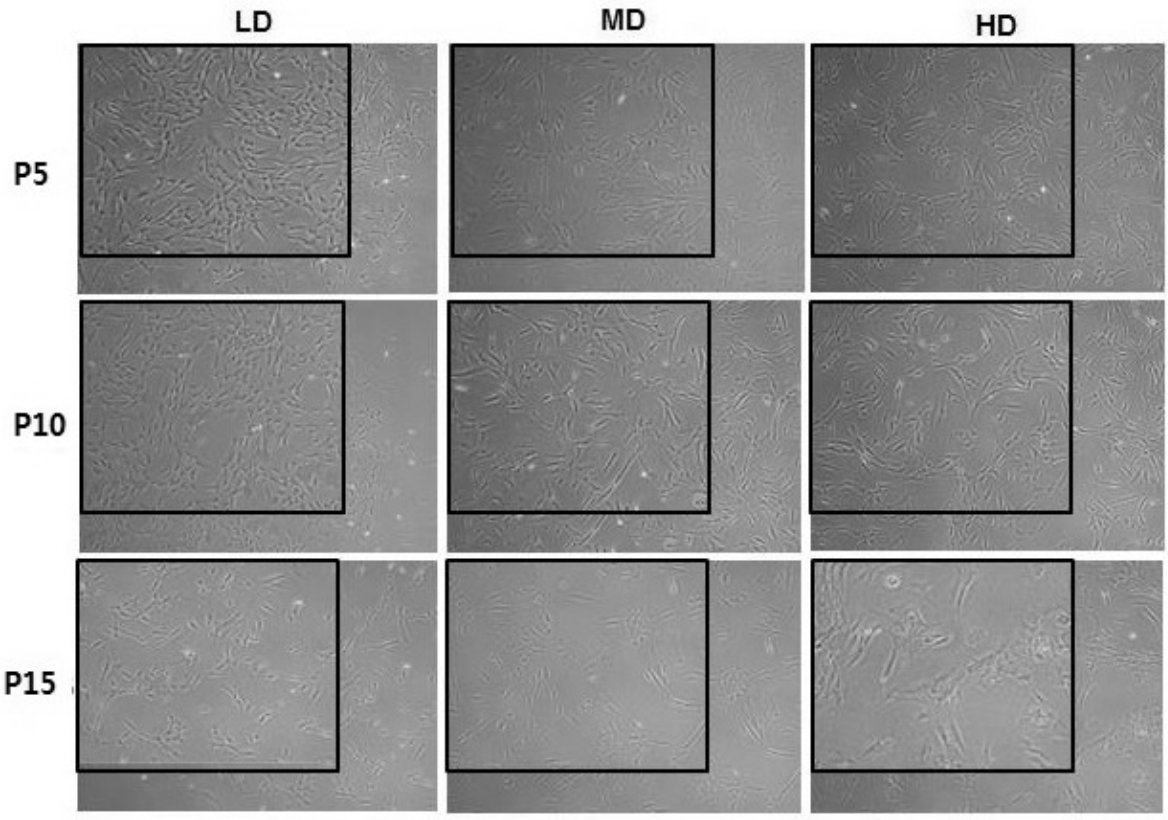

LD
MD
HD
P5
P10
P15

FIG. 2

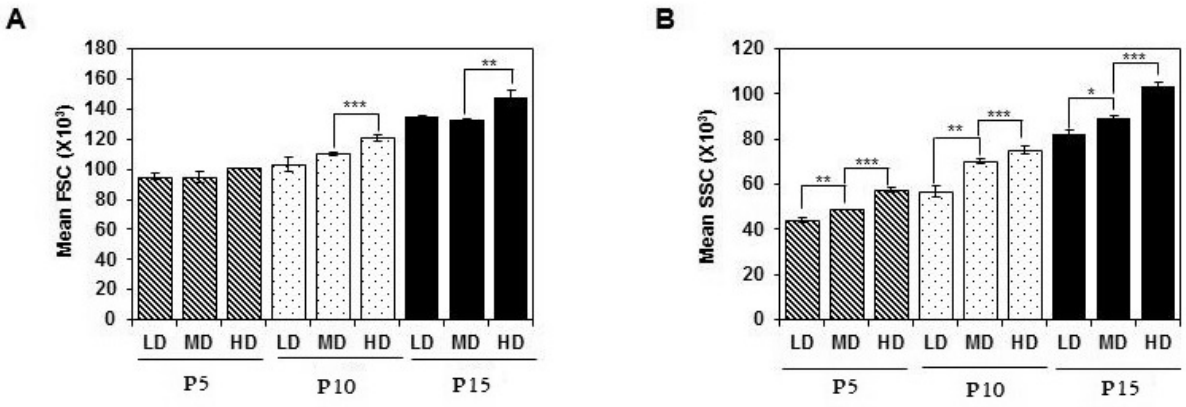

A
Mean FSC (X10³)
180
160
140
120
100
80
60
40
20
0
**
***
LD MD HD LD MD HD LD MD HD
P5
P10
P15
B
Mean SSC (X10³)
120
100
80
60
40
20
0
**
***
**
***
*
***
LD MD HD LD MD HD LD MD HD
P5
P10
P15

FIG. 3

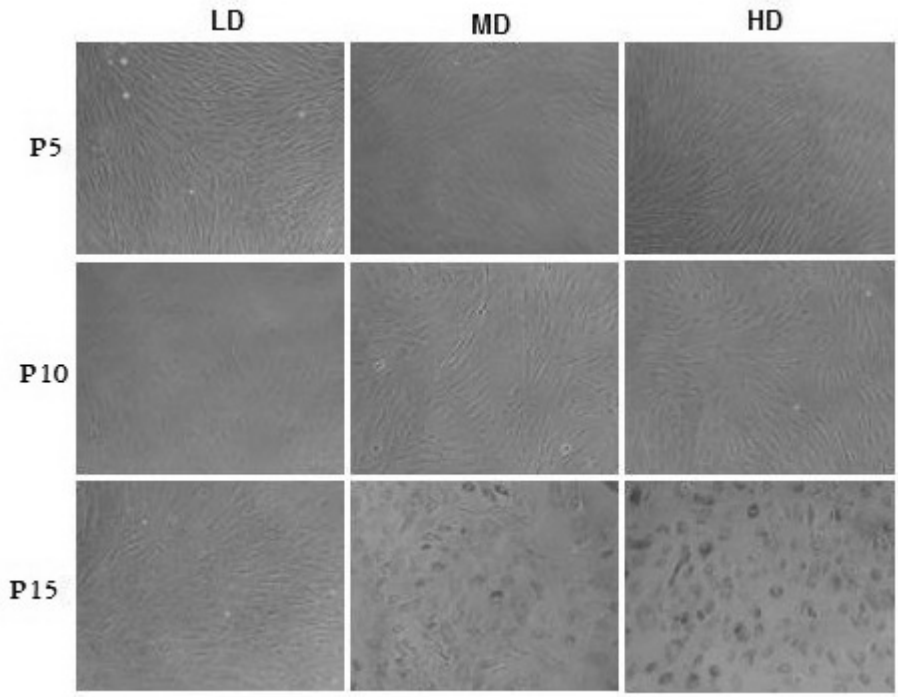

LD
MD
HD
P5
P10
P15

FIG. 4

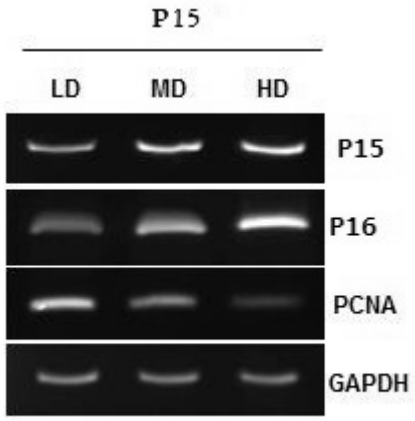

P15
LD
MD
HD
P15
P16
PCNA
GAPDH

FIG. 5

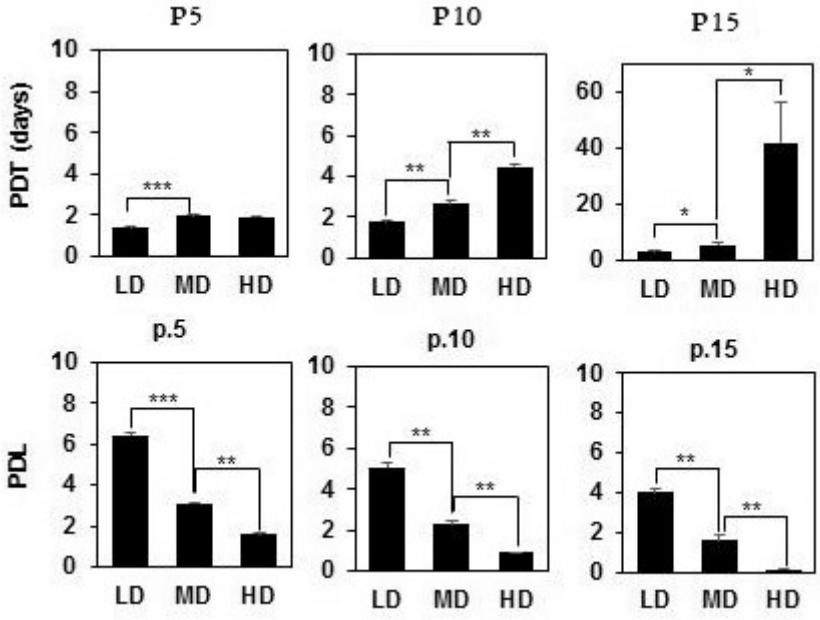

P5
P10
P15
PDT (days)
p.5
p.10
p.15
PDL
LD
MD
HD

FIG. 6

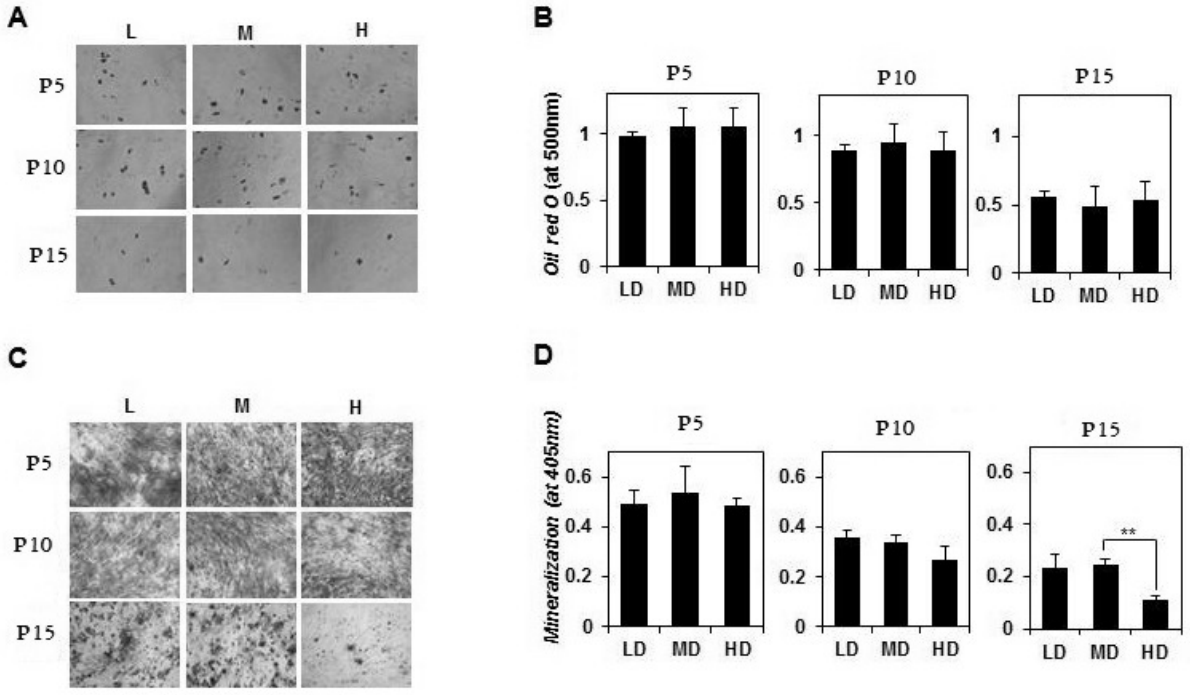

A
L
M
H
P5
P10
P15
B
P5
P10
P15
Oil red O (at 500nm)
1
0.5
0
LD
MD
HD
C
L
M
H
P5
P10
P15
D
P5
P10
P15
Mineralization (at 405nm)
0.6
0.4
0.2
0
**
LD
MD
HD

FIG. 7

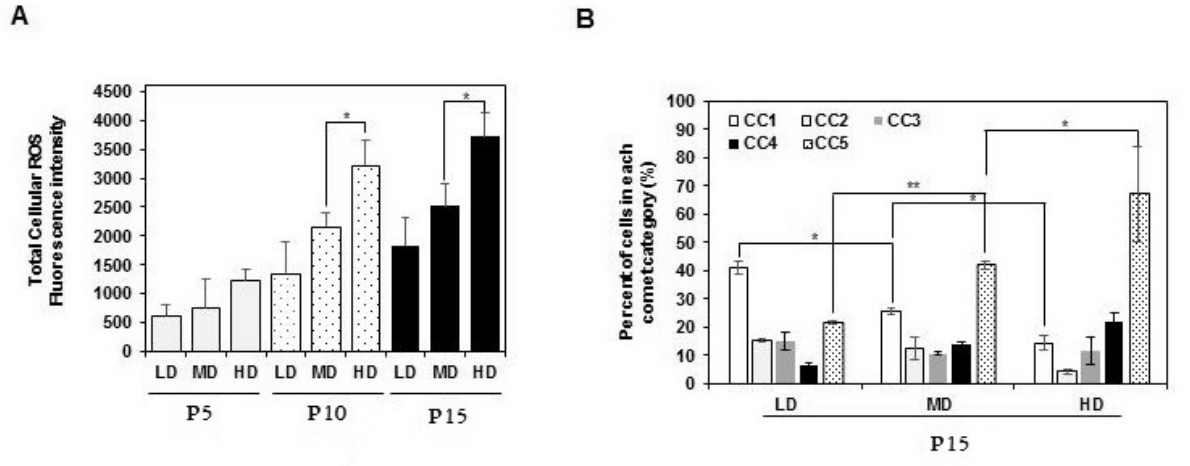

A
Total Cellular ROS Fluorescence intensity
4500
4000
3500
3000
2500
2000
1500
1000
500
0
*
*
LD
MD
HD
P5
P10
P15
B
Percent of cells in each comet category (%)
100
90
80
70
60
50
40
30
20
10
0
CC1
CC2
CC3
CC4
CC5
*
**
*
*
LD
MD
HD
P15

FIG. 8

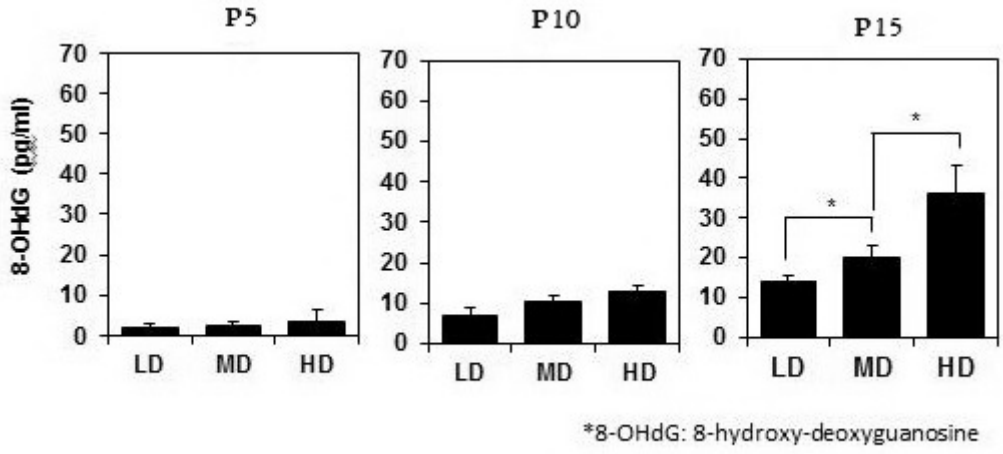


FIG. 9

Fold Increase (F.I)			
cell/cm^2	**P11**	**P13**	**P15**
HD	2.6±0.3	1.9±0.2	1.6±0.1
HD + AA	3.8±0.5	2.9±0.1	2.5±0.2

Relative Proliferation Index			
cell/cm^2	p.11	p.13	p15
HD	1	1	1
HD + AA	1.46	1.52	1.56

FIG. 10

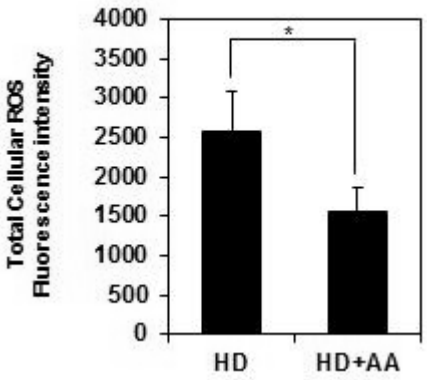

Total Cellular ROS
Fluorescence intensity
4000
3500
3000
2500
2000
1500
1000
500
0
*
HD
HD+AA

FIG. 11

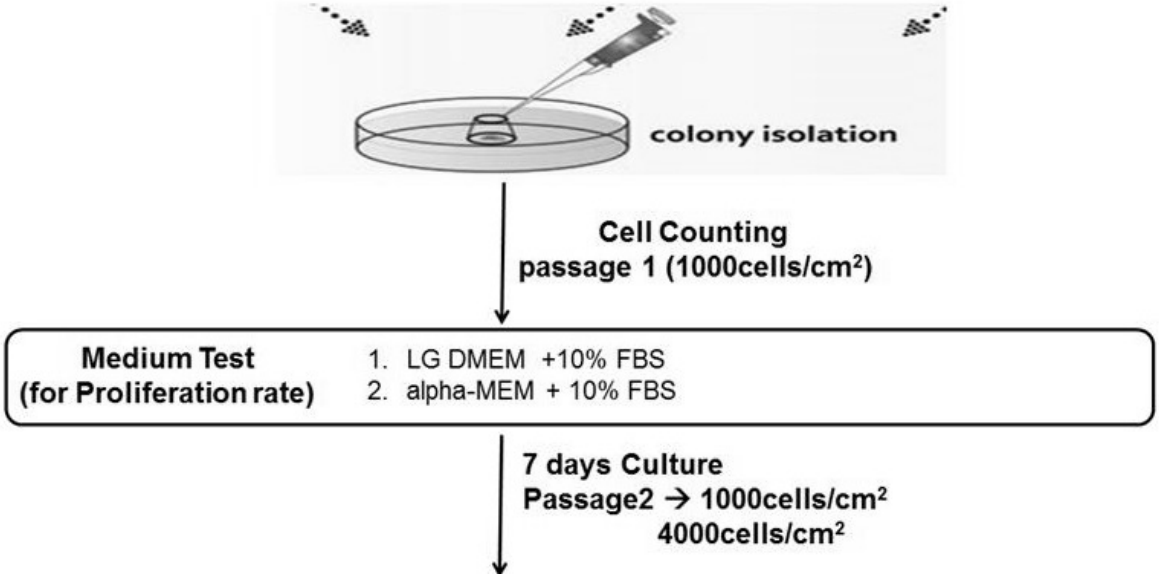
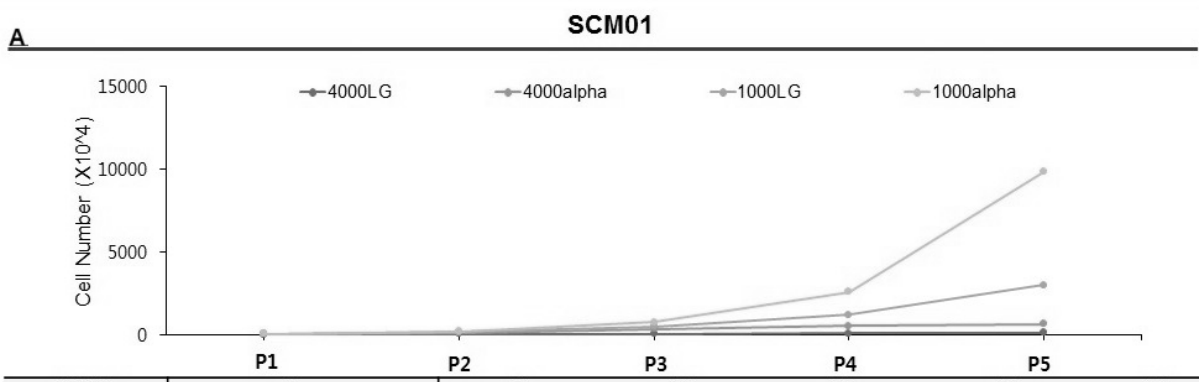

colony isolation
Cell Counting
passage 1 (1000cells/cm²)
Medium Test
(for Proliferation rate)
1. LG DMEM +10% FBS
2. alpha-MEM + 10% FBS
7 days Culture
Passage2 → 1000cells/cm²
4000cells/cm²

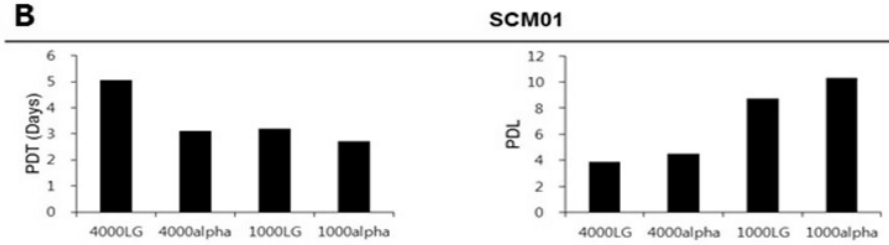
FIG. 12

A



SCM01	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	2.10	1.66	1.46	1.34
	Cell Numbers (x10^4)	22.000	46.20	76.69	111.97	150.04
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	3.49	3.43	1.66	1.14
	Cell Numbers (x10^4)	22.000	76.78	263.36	437.17	498.37
1000LG	1000N		7	7	7	7
	Fold Increase	0	6.86	4.80	3.60	3.60
	Cell Numbers (x10^4)	5.500	37.73	181.10	651.97	2347.11
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	7.19	6.34	5.57	4.91
	Cell Numbers (x10^4)	5.500	39.55	250.72	1396.48	6856.74

B

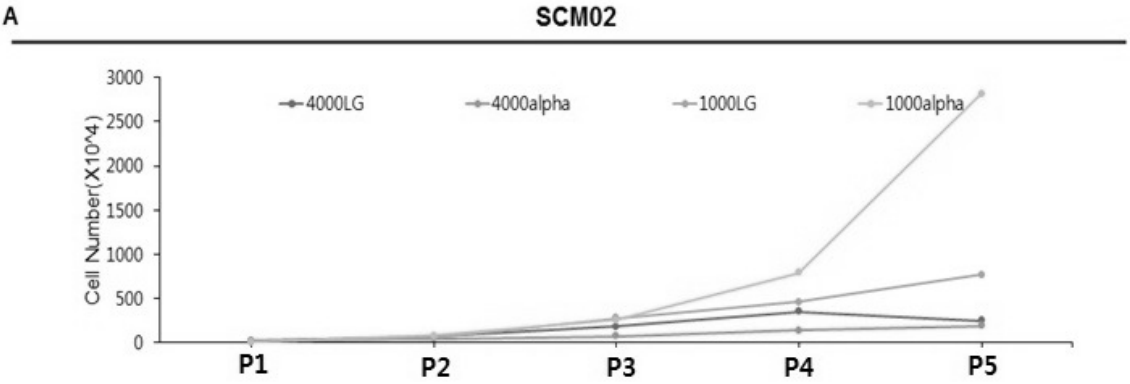


Population Doubling Time (PDT)	
4000LG	5.05
4000alpha	3.11
1000LG	3.20
1000alpha	2.72

Population Doubling Level (PDL)	
4000LG	3.84
4000alpha	4.50
1000LG	8.74
1000alpha	10.28

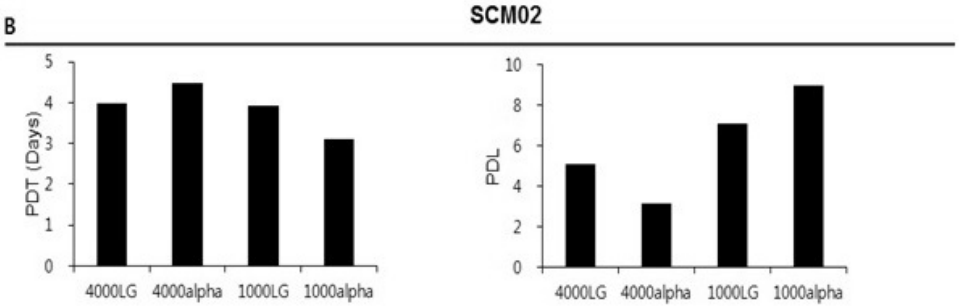
FIG. 13

A



SCM02	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	3.06	2.67	1.96	0.71
	Cell Numbers ($x10^4$)	22.000	67.32	179.74	352.30	250.13
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	1.94	1.77	1.86	1.37
	Cell Numbers ($x10^4$)	22.000	42.68	75.54	140.51	192.50
1000LG	1000N		7	7	7	7
	Fold Increase	0	10.56	4.76	1.67	1.67
	Cell Numbers ($x10^4$)	5.500	58.08	276.46	461.69	771.02
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	13.89	3.33	3.10	3.57
	Cell Numbers ($x10^4$)	5.500	76.40	254.40	788.63	2815.39

B

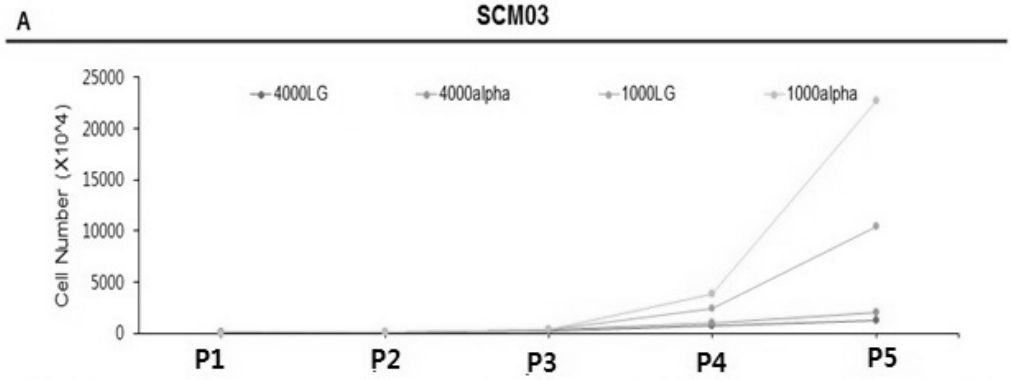


Population Doubling Time (PDT)	
4000LG	3.99
4000alpha	4.47
1000LG	3.93
1000alpha	3.11

Population Doubling Level (PDL)	
4000LG	5.12
4000alpha	3.13
1000LG	7.13
1000alpha	9.00

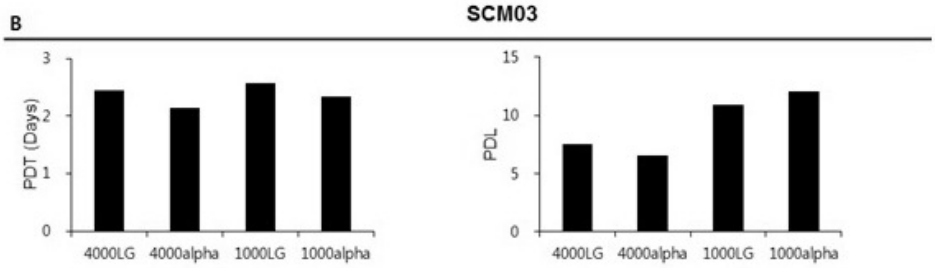
FIG. 14

A



SCM03	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	3.50	3.39	2.70	1.67
	Cell Numbers (x10^4)	22.000	77.00	261.03	704.78	1176.98
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	4.10	3.79	2.77	2.16
	Cell Numbers (x10^4)	22.000	90.20	341.86	946.95	2045.40
1000LG	1000N		7	7	7	7
	Fold Increase	0	8.46	6.17	8.40	4.34
	Cell Numbers (x10^4)	5.500	46.53	287.09	2411.56	10466.16
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	8.40	7.66	10.80	5.94
	Cell Numbers (x10^4)	5.500	46.20	353.89	3822.03	22702.88

B

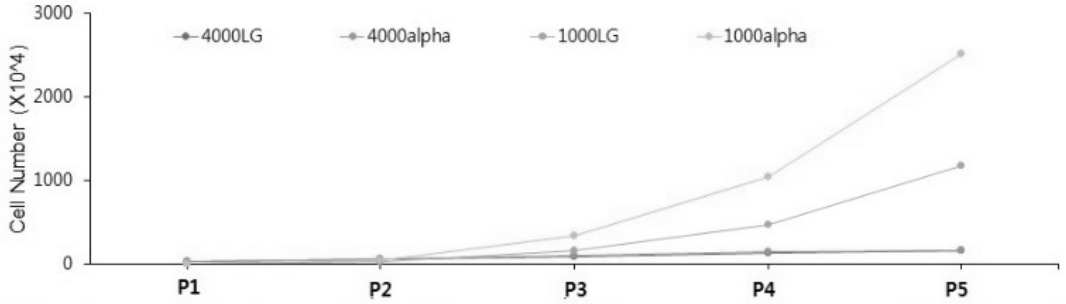


Population Doubling Time (PDT)	
4000LG	2.44
4000alpha	2.14
1000LG	2.57
1000alpha	2.33

Population Doubling Level (PDL)	
4000LG	7.55
4000alpha	6.54
1000LG	10.89
1000alpha	12.01

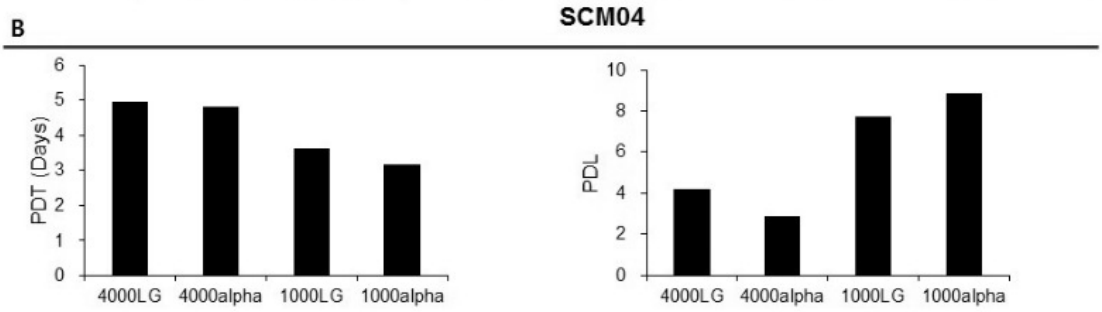
FIG. 15

A



SCM04	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	2.57	1.57	1.51	1.16
	Cell Numbers (x10^4)	22.000	56.54	88.77	134.04	155.49
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	2.70	1.82	1.33	1.14
	Cell Numbers (x10^4)	22.000	59.40	108.11	143.78	163.91
1000LG	1000N		7	7	7	7
	Fold Increase	0	6.29	4.80	2.86	2.49
	Cell Numbers (x10^4)	5.500	34.60	166.06	474.92	1182.55
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	9.00	6.69	3.17	2.40
	Cell Numbers (x10^4)	5.500	49.50	331.16	1049.76	2519.43

B

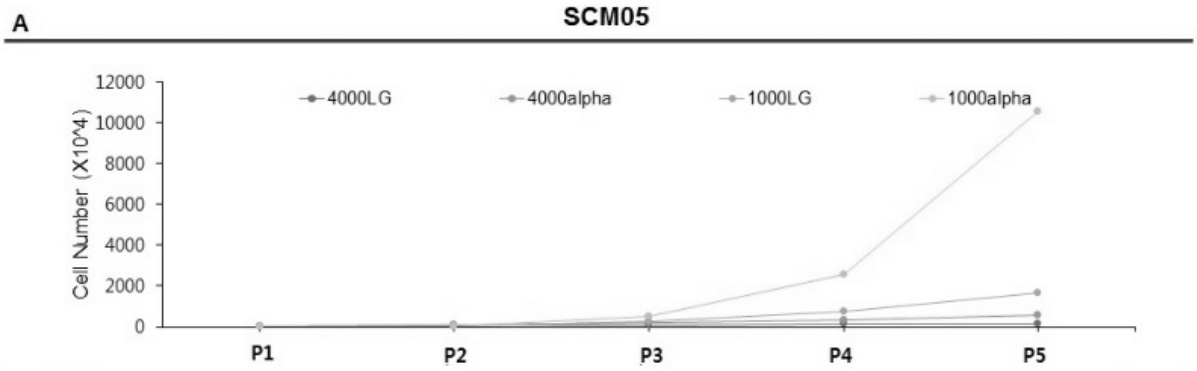


Population Doubling Time (PDT)	
4000LG	4.96
4000alpha	4.83
1000LG	3.61
1000alpha	3.17

Population Doubling Level (PDL)	
4000LG	4.18
4000alpha	2.90
1000LG	7.75
1000alpha	8.84

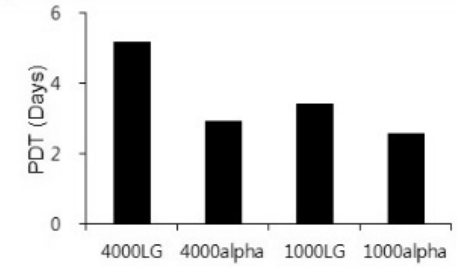
FIG. 16

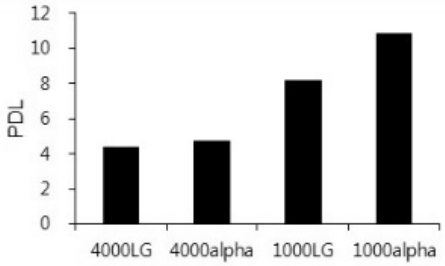
A



SCM05	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	3.27	1.49	1.19	1.13
	Cell Numbers ($x10^4$)	22.000	71.94	107.19	127.56	144.14
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	4.64	2.14	1.67	1.63
	Cell Numbers ($x10^4$)	22.000	102.08	218.45	364.81	594.65
1000LG	1000N		7	7	7	7
	Fold Increase	0	8.57	5.71	2.86	2.14
	Cell Numbers ($x10^4$)	5.500	47.14	269.14	769.74	1647.25
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	10.95	8.24	5.24	4.05
	Cell Numbers ($x10^4$)	5.500	60.23	496.25	2600.37	10531.50

B



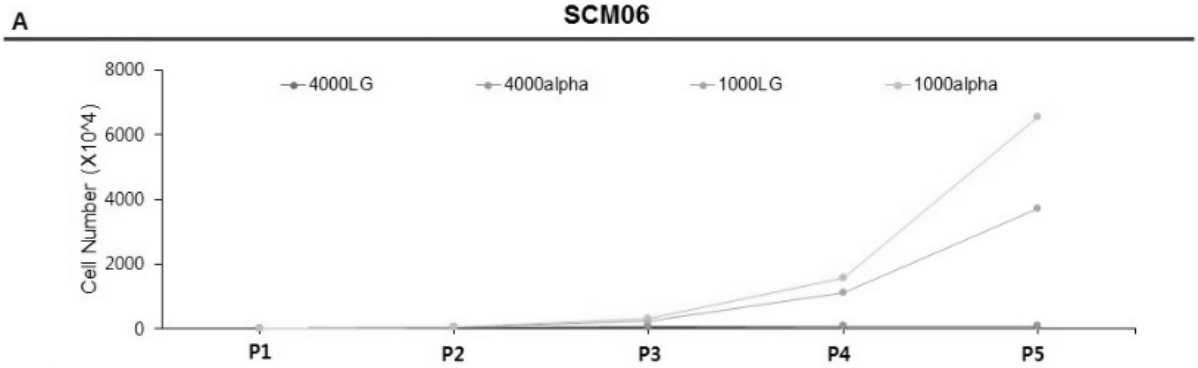


Population Doubling Time (PDT)	
4000LG	5.16
4000alpha	2.94
1000LG	3.40
1000alpha	2.57

Population Doubling Level (PDL)	
4000LG	4.42
4000alpha	4.76
1000LG	8.23
1000alpha	10.90

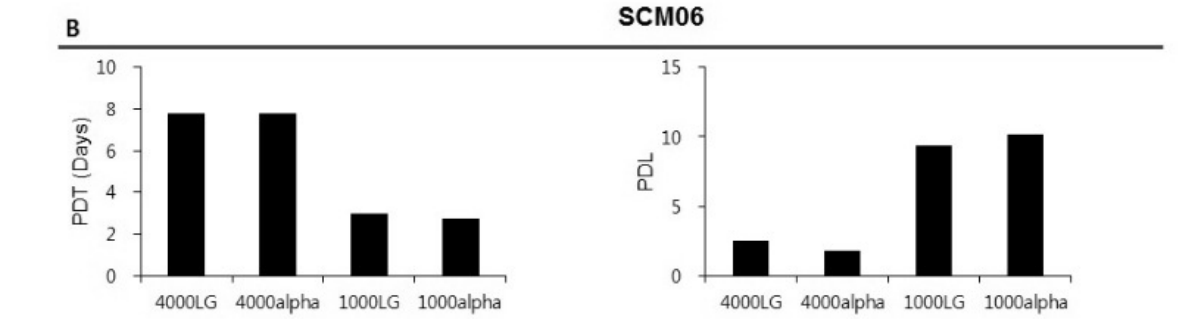
FIG. 17

A



SCM06	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	1.67	1.67	1.31	0.95
	Cell Numbers (x10^4)	22.000	36.74	61.36	80.38	76.36
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	2.14	1.83	1.25	0.71
	Cell Numbers (x10^4)	22.000	47.08	86.16	107.70	76.46
1000LG	1000N		7	7	7	7
	Fold Increase	0	7.14	5.95	4.76	3.33
	Cell Numbers (x10^4)	5.500	39.27	233.66	1112.20	3703.64
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	10.00	5.71	5.00	4.17
	Cell Numbers (x10^4)	5.500	55.00	314.05	1570.25	6547.94

B

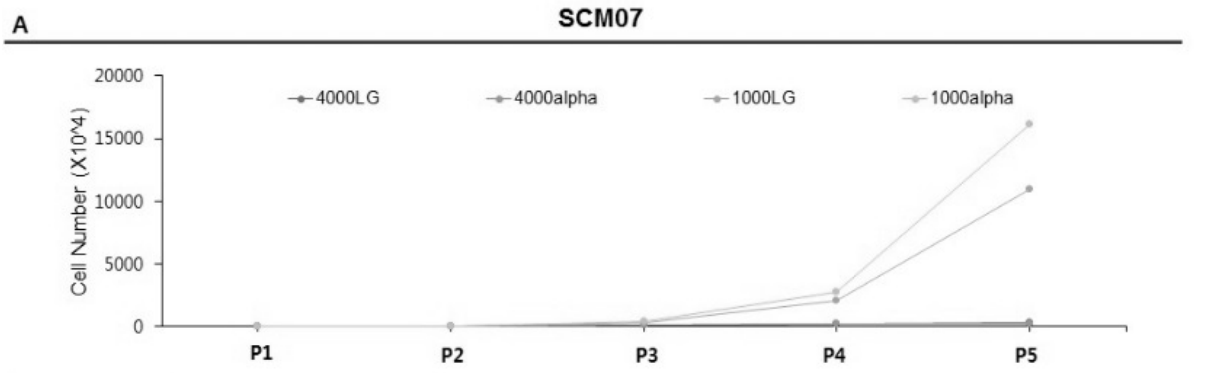


Population Doubling Time (PDT)	
4000LG	7.80
4000alpha	7.79
1000LG	2.98
1000alpha	2.74

Population Doubling Level (PDL)	
4000LG	2.54
4000alpha	1.80
1000LG	9.40
1000alpha	10.22

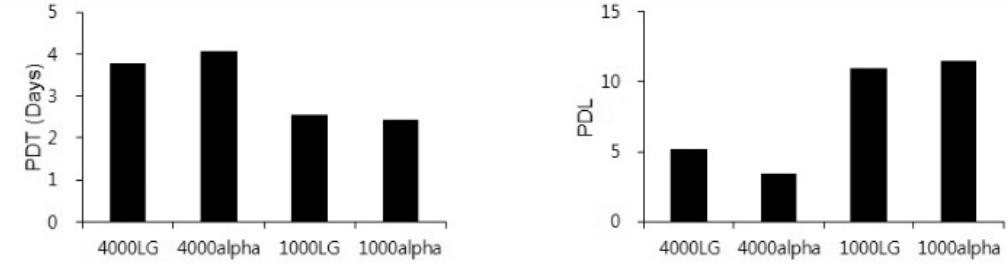
FIG. 18

A



SCM07	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	2.83	2.06	1.94	1.16
	Cell Numbers ($x10^4$)	22.000	62.26	128.26	248.82	288.63
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	3.19	2.23	1.33	1.14
	Cell Numbers ($x10^4$)	22.000	70.18	156.50	208.15	237.29
1000LG	1000N		7	7	7	7
	Fold Increase	0	7.89	7.37	6.43	5.31
	Cell Numbers ($x10^4$)	5.500	43.40	319.82	2056.45	10919.75
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	9.77	7.34	6.97	5.86
	Cell Numbers ($x10^4$)	5.500	53.74	394.41	2749.07	16109.56

B

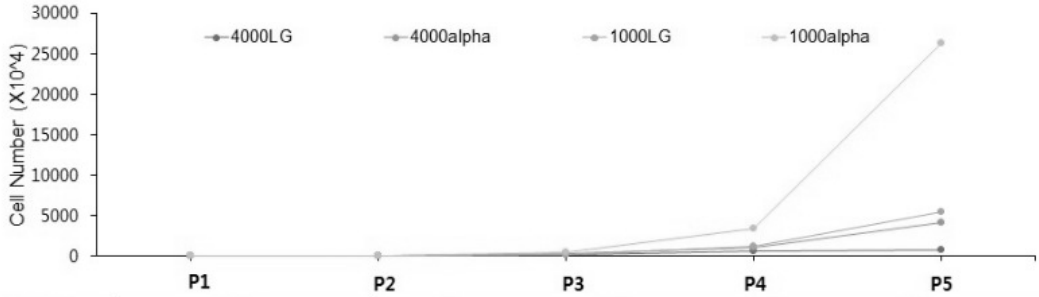


Population Doubling Time (PDT)	
4000LG	3.77
4000alpha	4.08
1000LG	2.56
1000alpha	2.43

Population Doubling Level (PDL)	
4000LG	5.21
4000alpha	3.43
1000LG	10.96
1000alpha	11.52

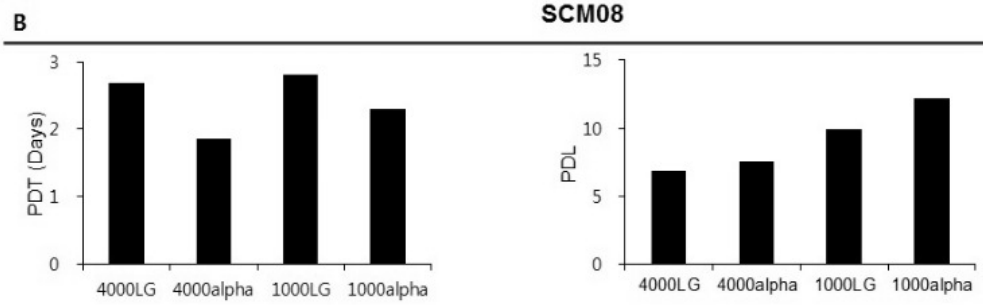
FIG. 19

A



SCM08	Item	P1	P2	P3	P4	P5
4000LG	4000N		4	3	4	3
	Fold Increase	0	3.14	3.00	2.82	1.41
	Cell Numbers ($x10^4$)	22.000	69.08	207.24	584.42	824.03
4000alpha	4000alpha		4	3	4	3
	Fold Increase	0	4.00	3.87	3.36	3.64
	Cell Numbers ($x10^4$)	22.000	88.00	340.56	1144.28	4165.19
1000LG	1000N		7	7	7	7
	Fold Increase	0	6.18	6.36	5.55	4.55
	Cell Numbers ($x10^4$)	5.500	33.99	216.18	1199.78	5458.99
1000alpha	1000alpha		7	7	7	7
	Fold Increase	0	9.27	8.45	8.00	7.64
	Cell Numbers ($x10^4$)	5.500	50.99	430.82	3446.59	26331.92

B



Population Doubling Time (PDT)	
4000LG	2.68
4000alpha	1.85
1000LG	2.81
1000alpha	2.29

Population Doubling Level (PDL)	
4000LG	6.88
4000alpha	7.56
1000LG	9.96
1000alpha	12.23

FIG. 20

Cell Name		A0108							
		SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08
4000LG	**P2** (X10^4)	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
	P5 (X10^4)	150.04	250.13	1176.98	155.49	144.14	76.36	288.63	824.03
	P5/P2 proliferation rate	**6.82**	**11.37**	**53.50**	**7.07**	**6.55**	**3.47**	**13.12**	**37.46**
1000LG	**P2** (X10^4)	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
	P5 (X10^4)	2347.11	771.02	10466.16	1182.55	1647.25	3703.64	10919.75	5458.99
	P5/P2 proliferation rate	**426.75**	**140.19**	**1902.94**	**215.01**	**299.50**	**673.39**	**1985.41**	**992.54**
1000 cells/cm2 / 4000 cells/cm2 proliferation rate (P5)		**15.64**	**3.08**	**8.89**	**7.61**	**11.43**	**48.50**	**37.83**	**6.62**

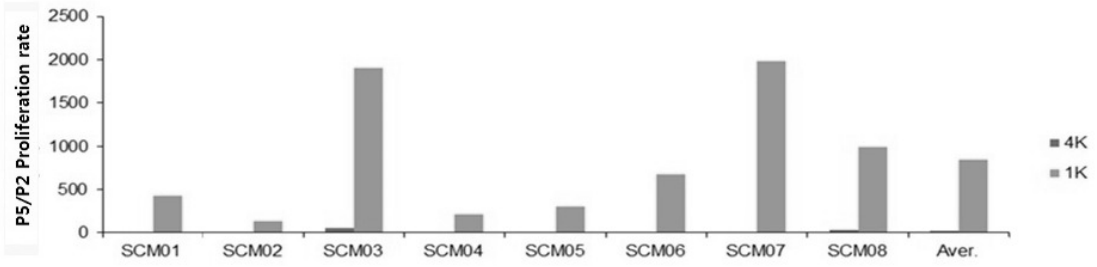


FIG. 21

Cell Name		A0108							
		SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08
PDT	4000LG	5.05	3.99	2.44	4.96	5.16	7.80	3.77	2.68
	1000LG	3.20	3.93	2.57	3.61	3.40	2.98	2.56	2.81
PDL	4000LG	3.84	5.12	7.55	4.18	4.42	2.54	5.21	6.88
	1000LG	8.74	7.13	10.89	7.75	8.23	9.40	10.96	9.96

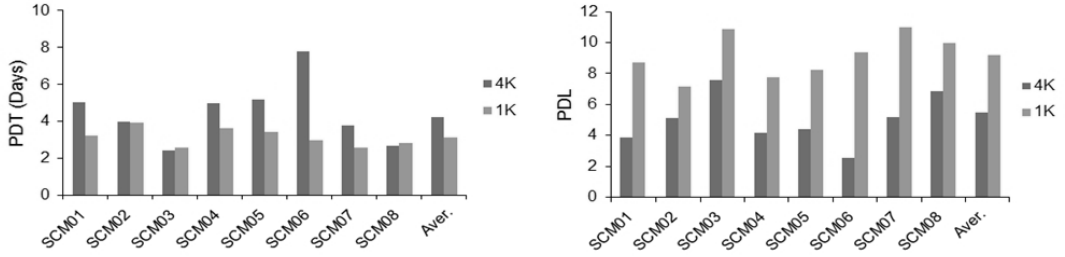


FIG. 22

Cell Name		A0108							
		SCM01	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08	SCM09
4000alpha	**P2** (X10^4)	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
	P5 (X10^4)	498.37	192.50	2045.40	163.91	594.65	76.46	237.29	4165.19
	P5/P2 proliferation rate	**22.65**	**8.75**	**92.97**	**7.45**	**27.03**	**3.48**	**10.79**	**189.33**
1000alpha	**P2**(X10^4)	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
	P5 (X10^4)	6856.74	2815.39	22702.88	2519.43	10531.50	6547.94	16109.56	26331.92
	P5/P2 proliferation rate	**1246.68**	**511.89**	**4127.80**	**458.08**	**1914.82**	**1190.54**	**2929.01**	**4787.62**
1000 cells/cm2 / 4000 cells/cm2 proliferation rate (P5)		**13.76**	**14.63**	**11.10**	**15.37**	**17.71**	**85.63**	**67.89**	**6.32**

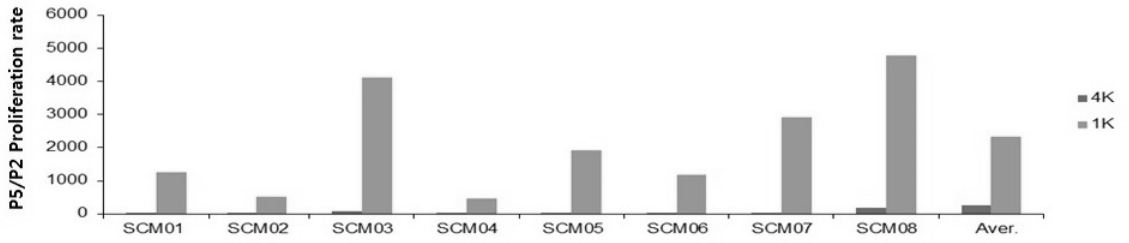


FIG. 23

Cell Name		A0108							
		SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08
PDT	4000alpha	3.11	4.47	2.14	4.83	2.94	7.79	4.08	1.85
	1000alpha	2.72	3.11	2.33	3.17	2.57	2.74	2.43	2.29
PDL	4000alpha	4.50	3.13	6.54	2.90	4.76	1.80	3.43	7.56
	1000alpha	10.28	9.00	12.01	8.84	10.90	10.22	11.52	12.23

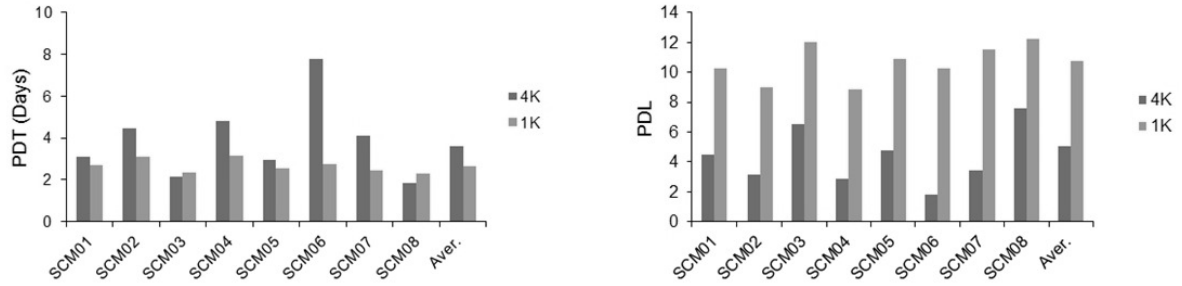


FIG. 24

Cell Name		A0108							
		SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08
1000 LG	**P2** (X10^4)	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
	P5 (X10^4)	2347.11	771.02	10466.16	1182.55	1647.25	3703.64	10919.75	5458.99
	P5/P2 proliferation rate	**426.75**	**140.19**	**1902.94**	**215.01**	**299.50**	**673.39**	**1985.41**	**992.54**
1000 alpha	**P2** (X10^4)	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
	P5 (X10^4)	6856.74	2815.39	22702.88	2519.43	10531.50	6547.94	16109.56	26331.92
	P5/P2 proliferation rate	**1246.68**	**511.89**	**4127.80**	**458.08**	**1914.82**	**1190.54**	**2929.01**	**4787.62**
α-MEM/LG DMEM proliferation rate (P5)		**2.92**	**3.65**	**2.17**	**2.13**	**6.39**	**1.77**	**1.48**	**4.82**

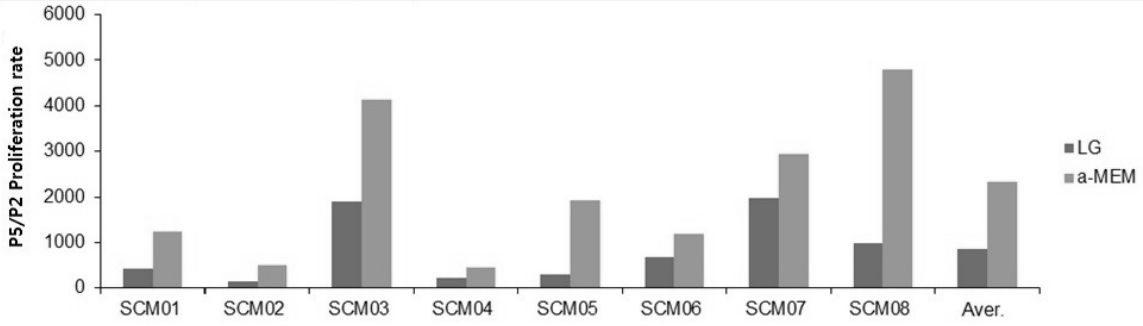


FIG. 25

Cell Name		A0108							
		SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08
PDT	1000LG	3.20	3.93	2.57	3.61	3.40	2.98	2.56	2.81
	1000alpha	2.72	3.11	2.33	3.17	2.57	2.74	2.43	2.29
PDL	1000LG	8.74	7.13	10.89	7.75	8.23	9.40	10.96	9.96
	1000alpha	10.28	9.00	12.01	8.84	10.90	10.22	11.52	12.23

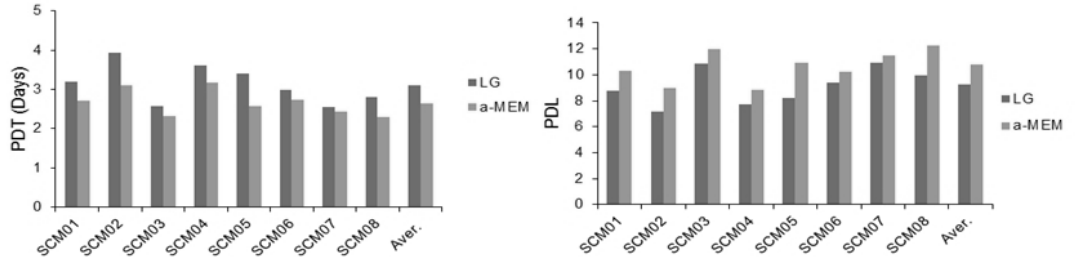


FIG. 26