FIG. 1

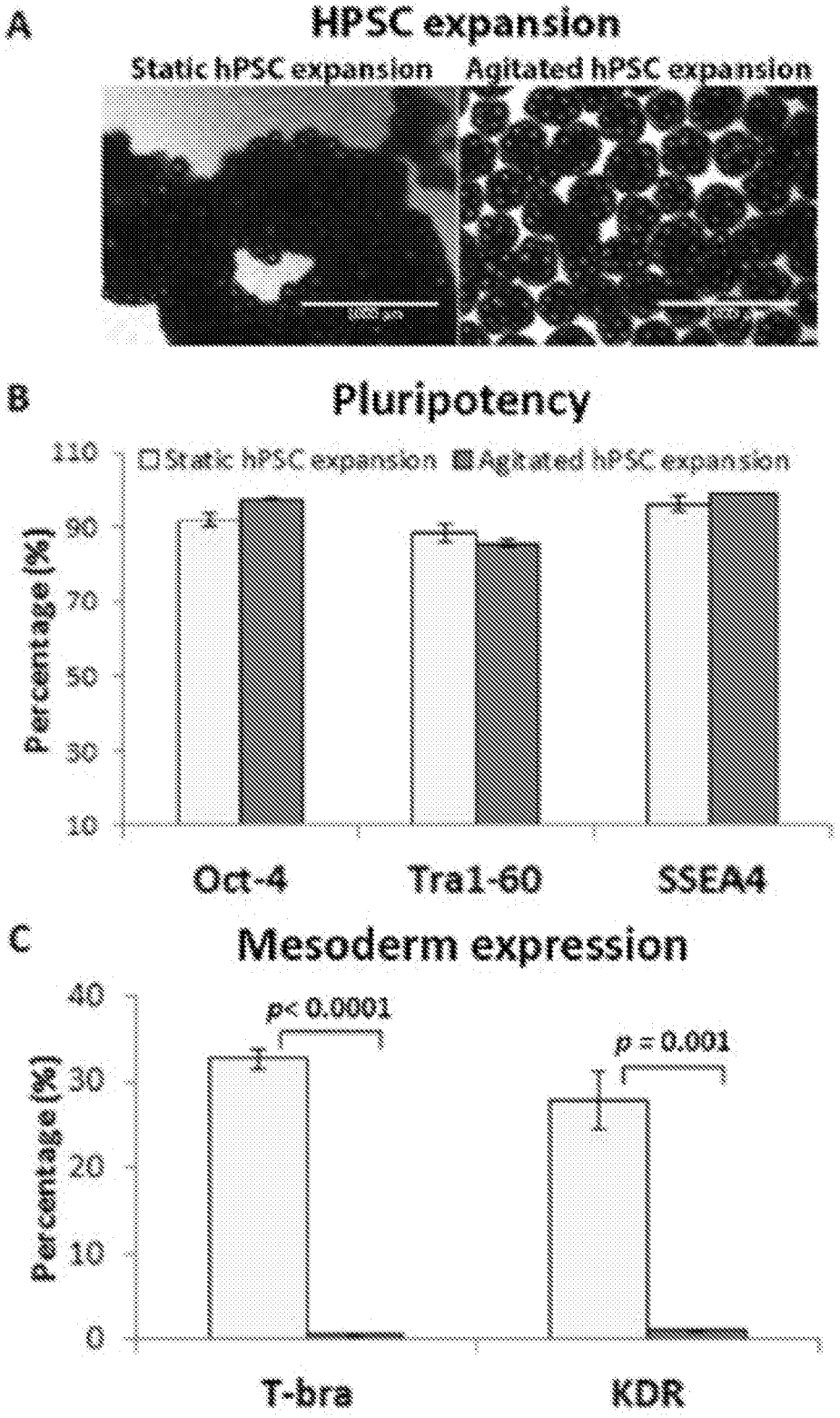
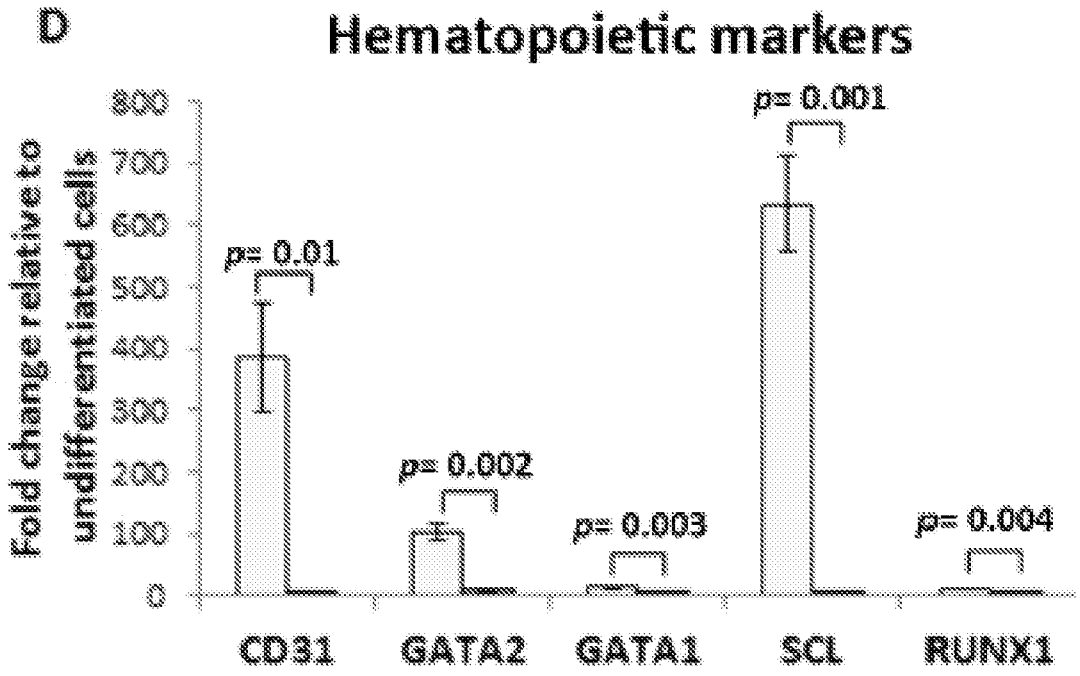

A
HPSC expansion
Static hPSC expansion
Agitated hPSC expansion
B
Pluripotency
Static hPSC expansion
Agitated hPSC expansion
Percentage (%)
110
90
70
50
30
10
Oct-4
Tra1-60
SSEA4
C
Mesoderm expression
Percentage (%)
40
30
20
10
0
p< 0.0001
p = 0.001
T-bra
KDR

FIG. 1 continued



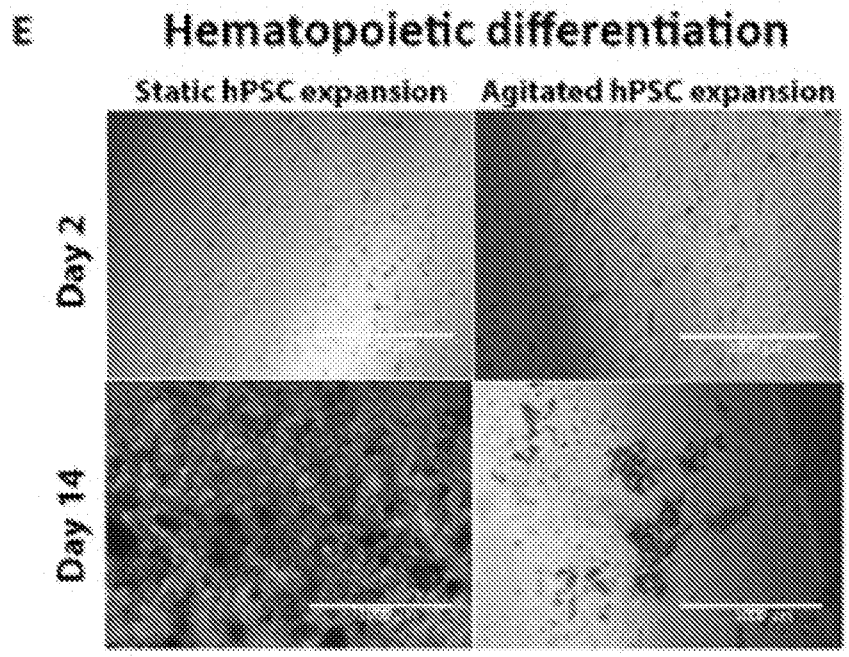


FIG. 1 continued

F

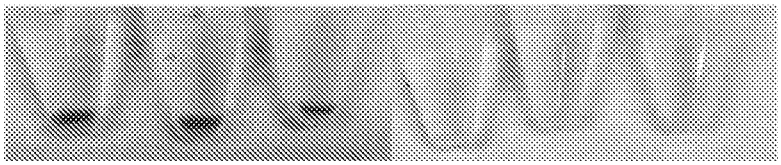
	Static hPSC expansion	Agitated hPSC expansion	*p*-value
Total Hematopoietic precursors per well (Day 14)	$5.77 \times 10^6 \pm 6.30 \times 10^5$	$1.39 \times 10^5 \pm 2.18 \times 10^4$	0.0009
Total Erythroblast per well (Day 28)	$2.62 \times 10^7 \pm 4.72 \times 10^6$	$4.11 \times 10^4 \pm 2.37 \times 10^4$	0.005
CD235a+ cells (%)(Day 28)	51.8 % ± 2.0	0.51 % ± 0.1	<0.0001
HbF+ cells (%)(Day 28)	65.7 % ± 2.3	0.70 % ± 0.05	<0.0001
RBC pellet (Day 28)			

FIG. 2

A

	Dose and duration of factors evaluated				Response outcome	
Condition	Activin A (ng/ml)	$CHIR_{24hr}$ (μM)	$CHIR_{48hr}$ (μM)	BMP4 (ng/ml)	% KDR+ cells	Total number of Hematopoietic precursors
1	0	0	0	50	0.44	136000
2	80	0	0	10	1.07	271500
3	0	15	0	10	9.19	401500
4	80	15	0	50	18.5	469000
5	0	0	15	10	1.18	271000
6	80	0	15	50	2.06	403000
7	0	15	15	50	30.7	185500
8	80	15	15	10	25.6	370500
9	0	0	0	10	0.6	96500
10	80	0	0	50	0.97	92500
11	0	15	0	50	17.7	655000
12	80	15	0	10	16.3	348000
13	0	0	15	50	4.57	75500
14	80	0	15	10	1.38	108500
15	0	15	15	10	38	60000
16	80	15	15	50	38.7	381000
17	0	7.5	7.5	30	5.61	498000
18	80	7.5	7.5	30	19.2	362000
19	40	0	7.5	30	2.42	304500
20	40	15	7.5	30	36.6	469500
21	40	7.5	0	30	9.25	344500
22	40	7.5	15	30	30.8	350500

FIG. 2 continued

Condition	Activin A (ng/ml)	$CHIR_{24hr}$ (μM)	$CHIR_{48hr}$ (μM)	BMP4 (ng/ml)	% KDR+ cells	Total number of Hematopoietic precursors
23	40	7.5	7.5	30	9.24	450500
24	40	7.5	7.5	30	11.4	388000
25	40	7.5	7.5	10	30.1	440500
26	40	7.5	7.5	50	28.3	491000
27	40	7.5	7.5	30	16.9	425000
28	40	7.5	7.5	30	26.7	385500
29	40	7.5	7.5	30	14	368500

B

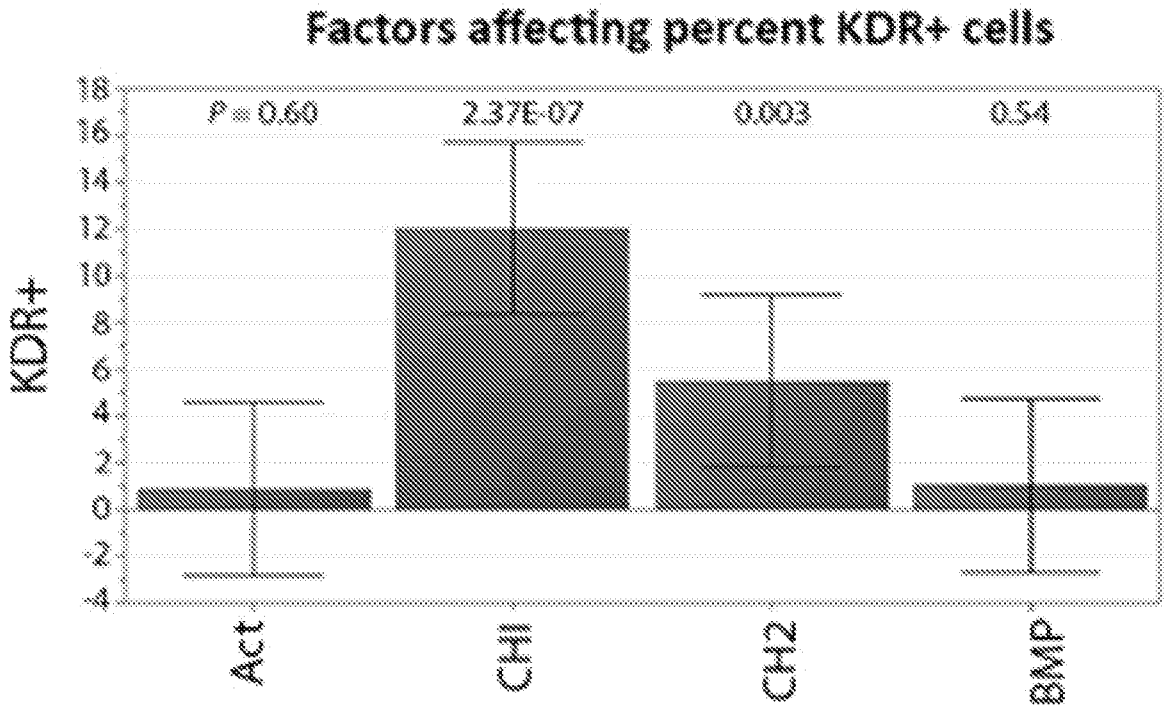


FIG. 2 continued

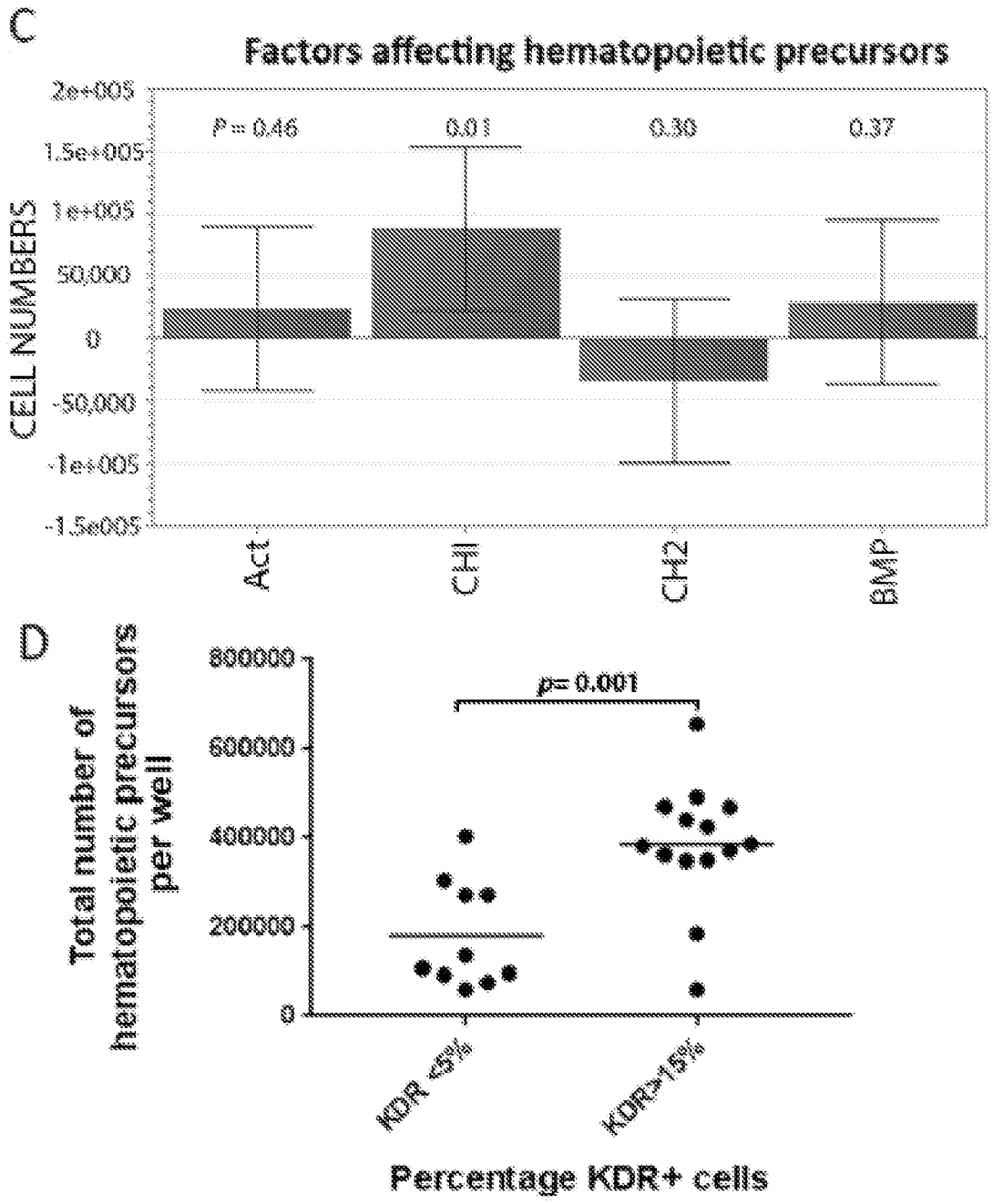
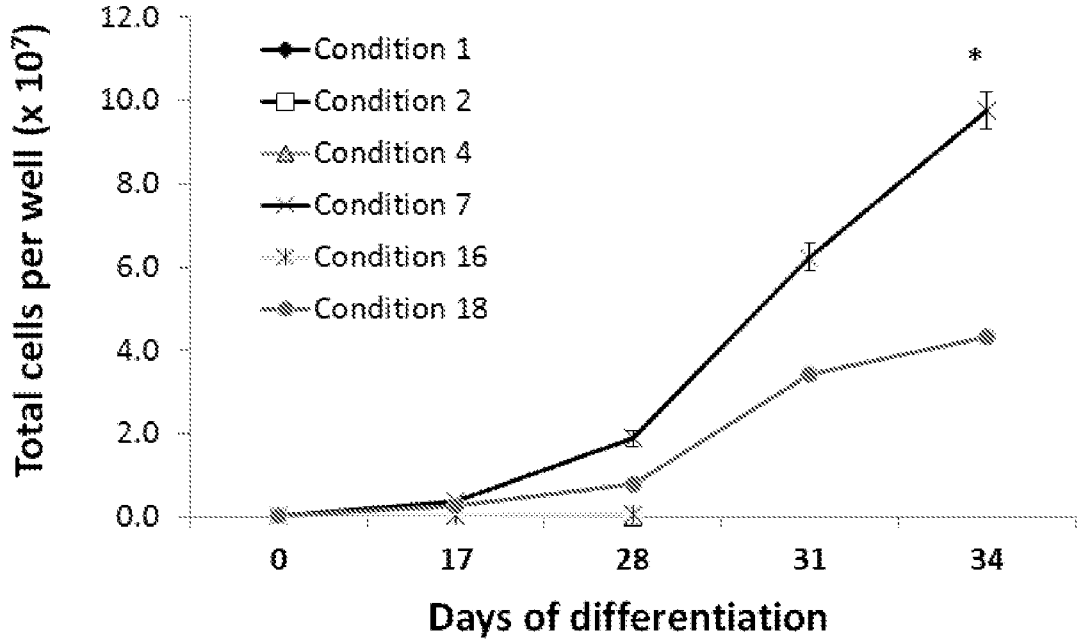


FIG. 3

A



Condition:	1	2	4	7	16	18
BMP4 (ng/ml)	10	50	50	10	50	50
Activin A (ng/ml)	-	-	50	50	50	25
$CHIR_{24hr}$ (μM)	-	-	-	12	12	6
$CHIR_{48hr}$ (μM)	-	-	-	-	12	6

FIG. 3 continued

B

	Condition 7	Condition 18	*p*-value
Total cells per well (Day 34)	$9.77 \times 10^7 \pm 4.6 \times 10^6$	$4.31 \times 10^7 \pm 5.9 \times 10^5$	0.0003
CD235a+ cells (%)	79.2 % ± 2.77	60.07 %± 4.77	0.03
CD235a+ CD71+ cells (%)	63.5 % ± 1.82	34.8 % ± 7.28	0.019
CD235a+ CD71- cells (%)	15.7 % ± 1.30	25.27 %± 2.74	0.03
HbF+ cells (%)	99.43 % ± 0.12	67.10 % ± 0.55	<0.0001

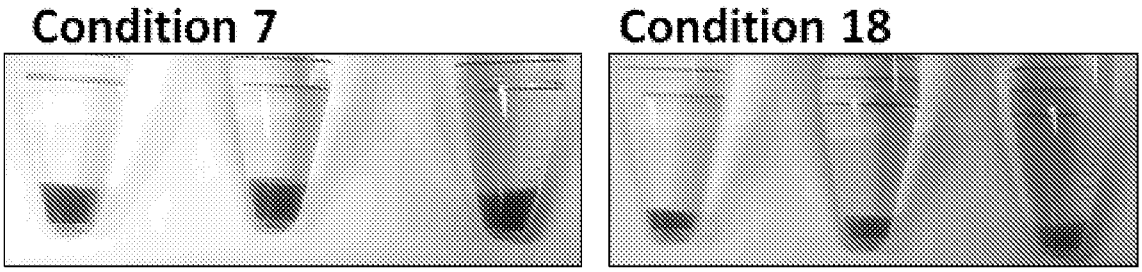


FIG. 4

A

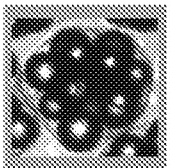
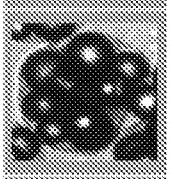
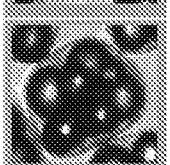
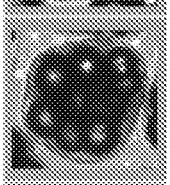
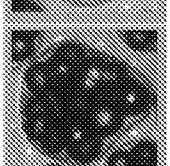
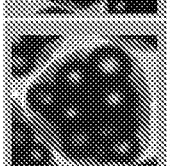
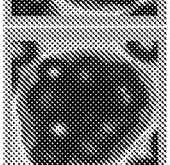
Test	Lines	RES-3	IMR90	BR2	BR7	D12	D11	D5	D9	Y13
Representative microcarrier aggregate images										
Fold-expansion		10.1 ± 1.1	12.5 ± 0.9	6.0 ± 0.7	9.6 ± 0.9	6.0 ± 0.4	7.8 ± 1.4	7.7 ± 0.9	8.0 ± 0.5	5.3 ± 0.8
Pluripotency (%)	Oct-4	94.6 ± 0.7	89.7 ± 0.2	92.3 ± 0.9	72.9 ± 2.1	98.3 ± 0.7	84.9 ± 1.4	83.4 ± 1.9	91.8 ± 1.3	90.6 ± 0.4
	Tra1-60	93.5 ± 1.4	93.1 ± 1.1	90.4 ± 2.0	84.8 ± 1.7	95.7 ± 0.5	89.9 ± 0.8	91.3 ± 0.6	91.5 ± 2.2	91.5 ± 1.1
	SSEA4	99.7 ± 0.1	99.9 ± 0.03	97.2 ± 0.7	99.4 ± 0.1	99.2 ± 0.2	99.1 ± 0.2	98.1 ± 0.2	98.9 ± 0.6	97.2 ± 0.4
Karyotype		46, XX	NA	46, XX	46, XX	46, XX	46, XX	46, XY	46, XY	46, XY
Aggregate diameter (µm)		458 ± 96	510 ± 128	459 ± 112	468 ± 99	255 ± 72	390 ± 148	371 ± 67	377 ± 76	320 ± 104

FIG. 4 continued

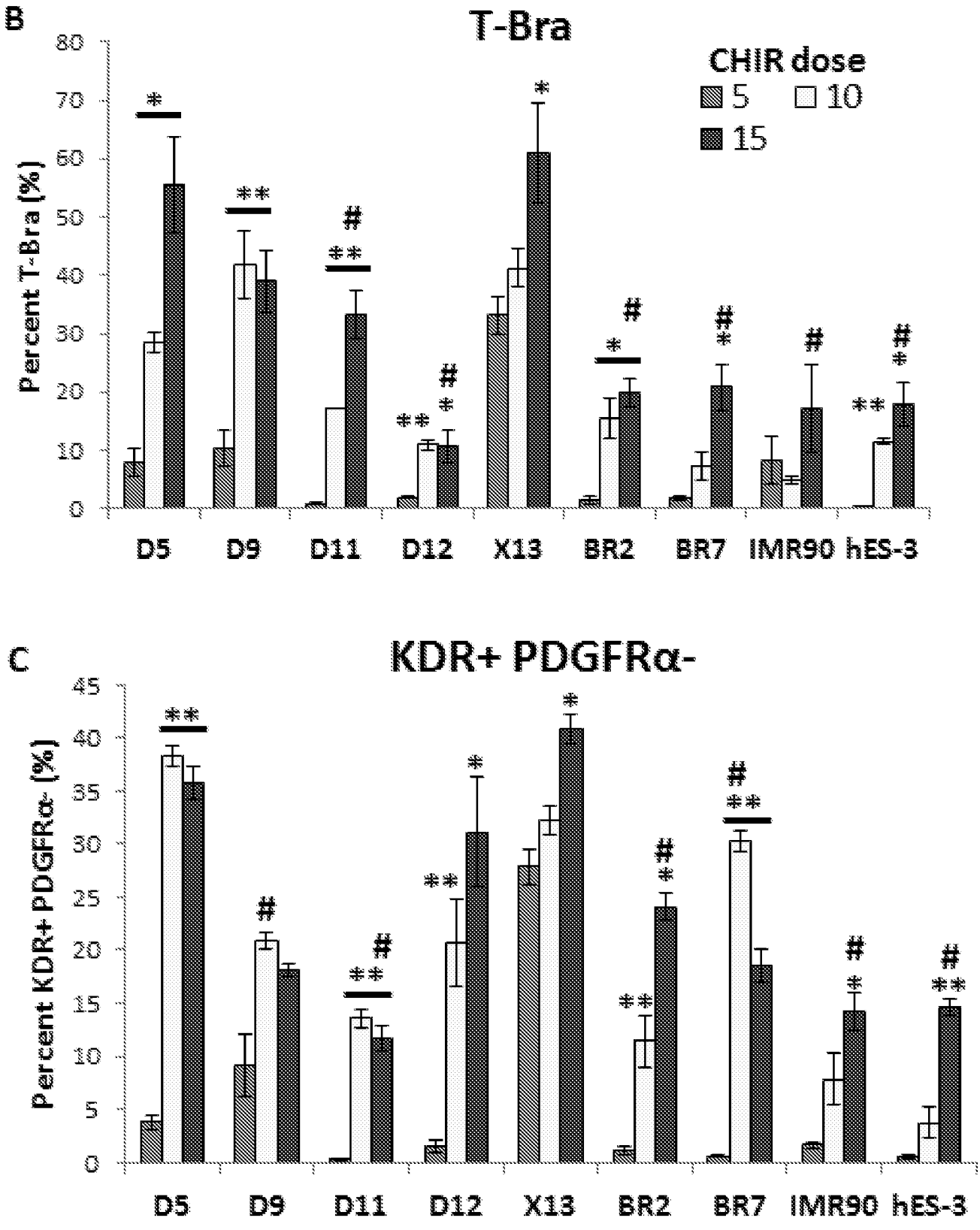
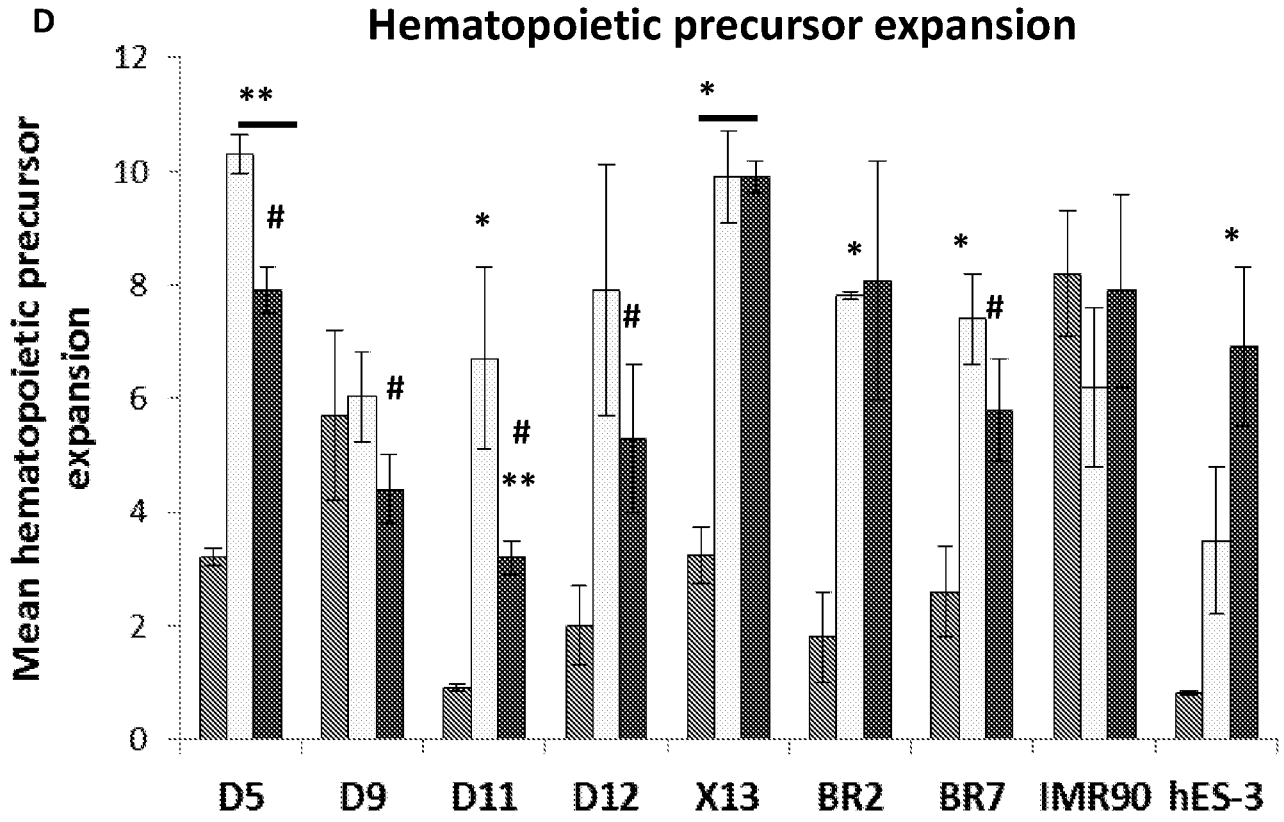


FIG. 4 continued



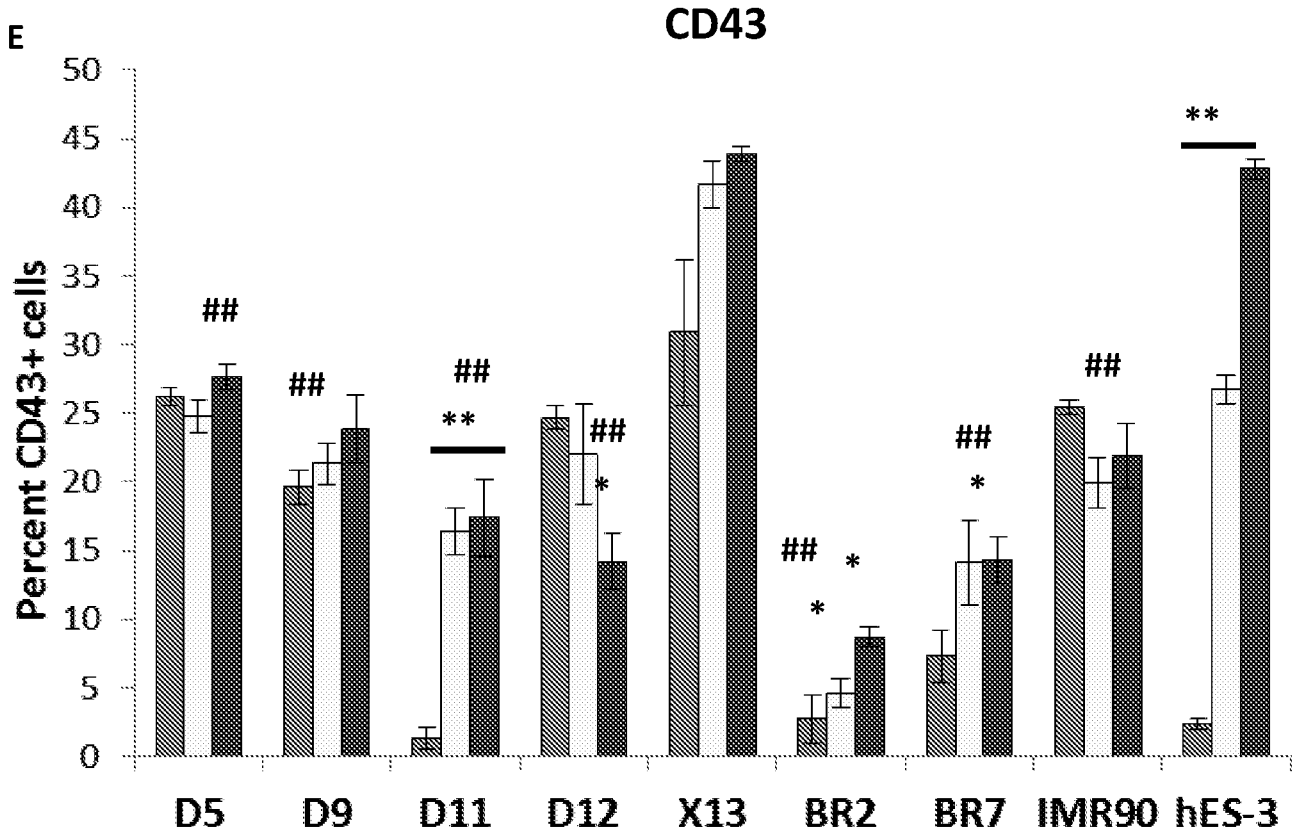


FIG. 4 continued

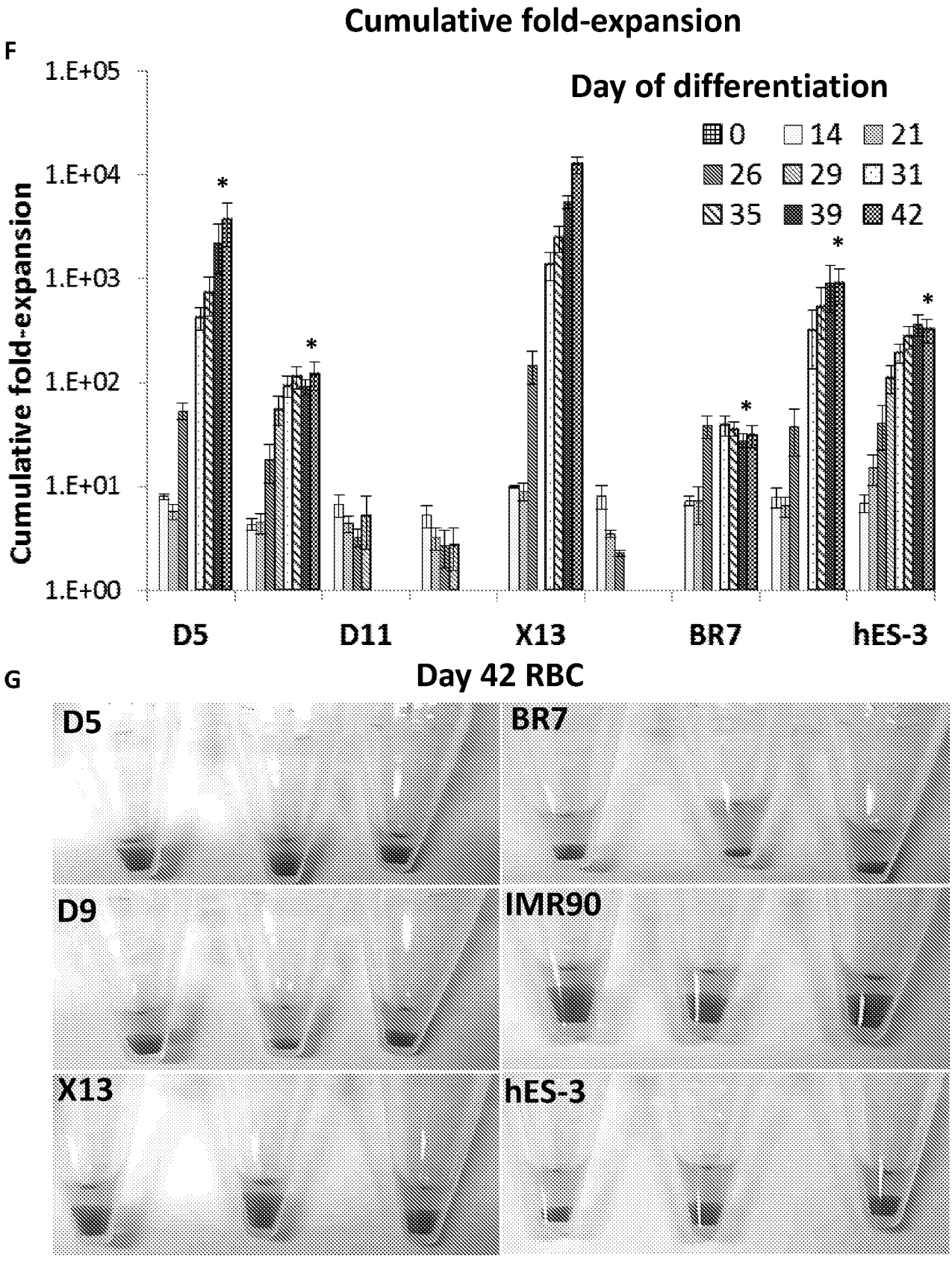


FIG. 5

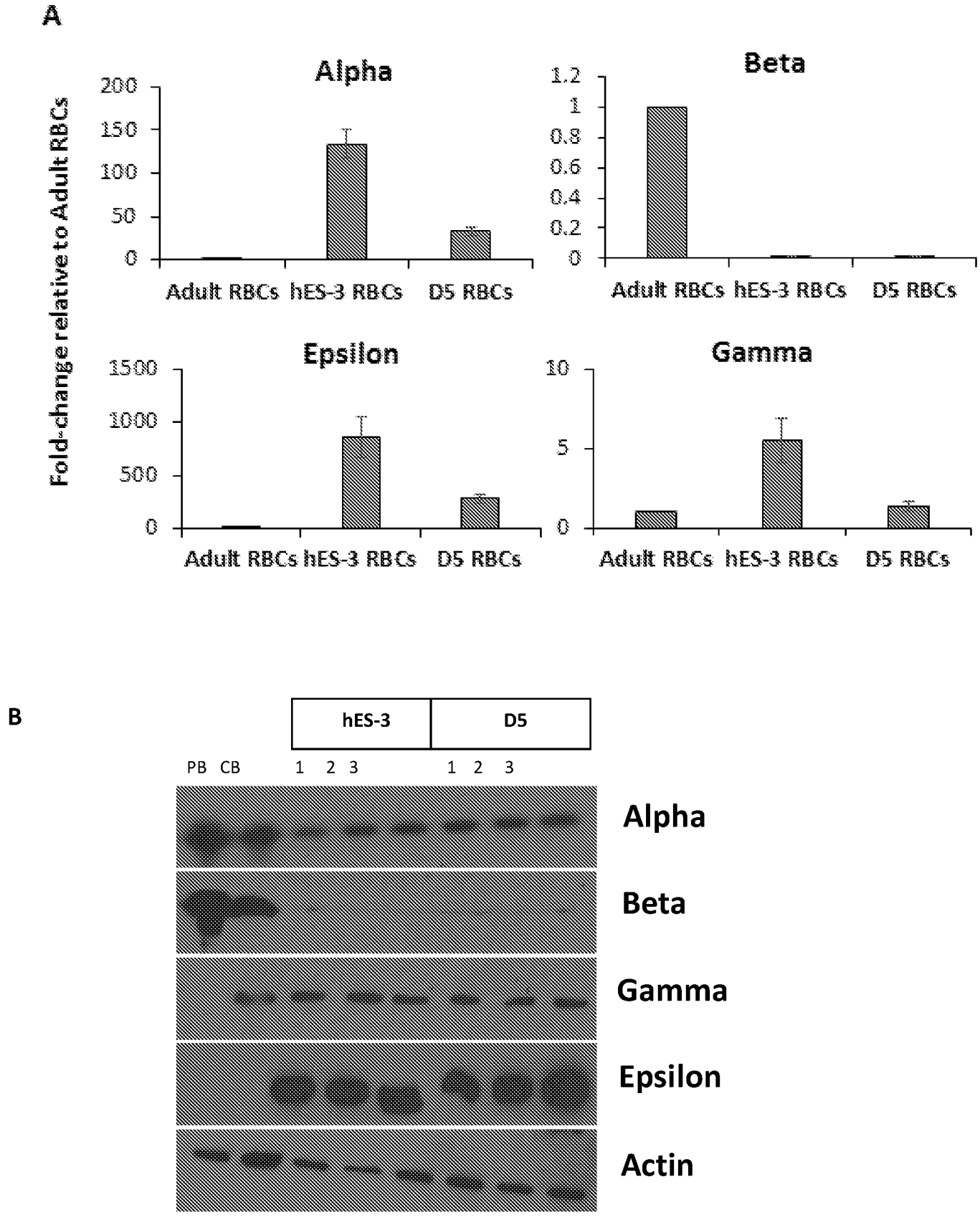
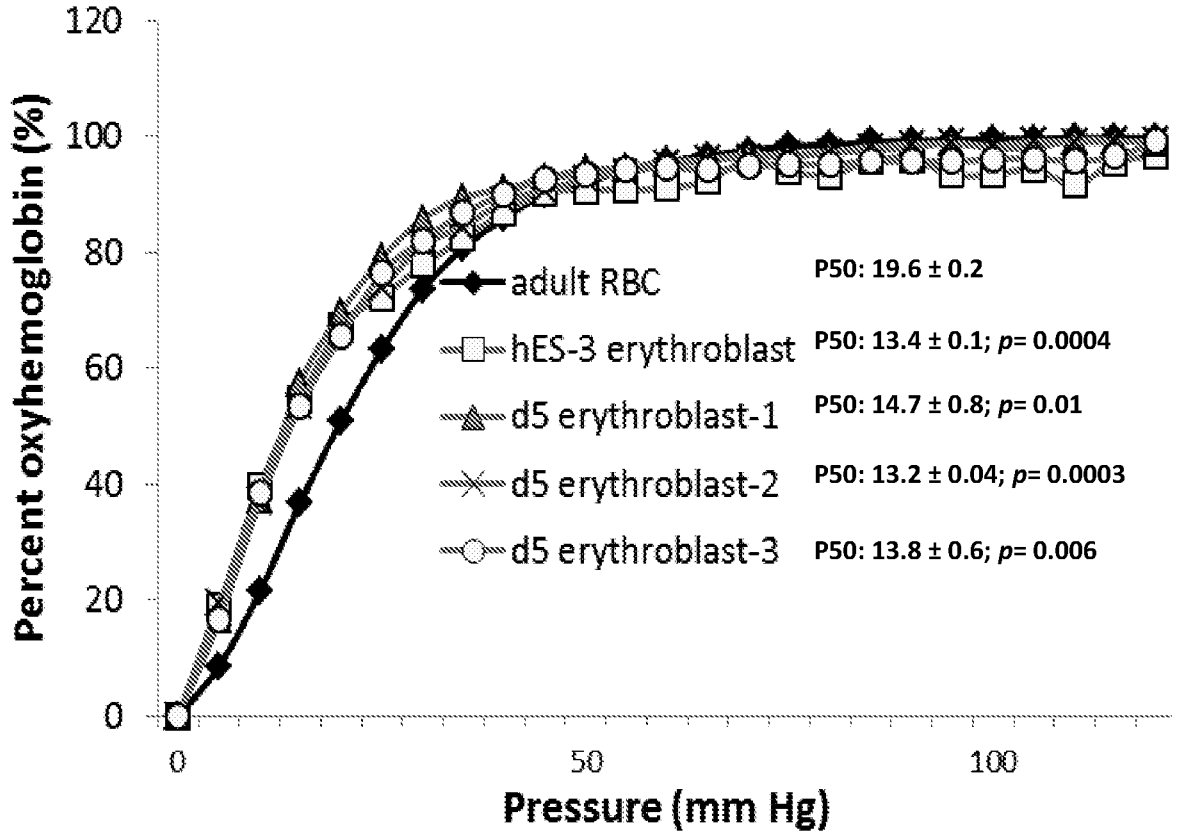

A
Alpha
Beta
Epsilon
Gamma
Fold-change relative to Adult RBCs
200
150
100
50
0
1.2
1
0.8
0.6
0.4
0.2
1500
1000
500
10
5
Adult RBCs
hES-3 RBCs
D5 RBCs
B
hES-3
D5
PB CB
1 2 3
1 2 3
Alpha
Beta
Gamma
Epsilon
Actin

FIG. 5 continued

C



D

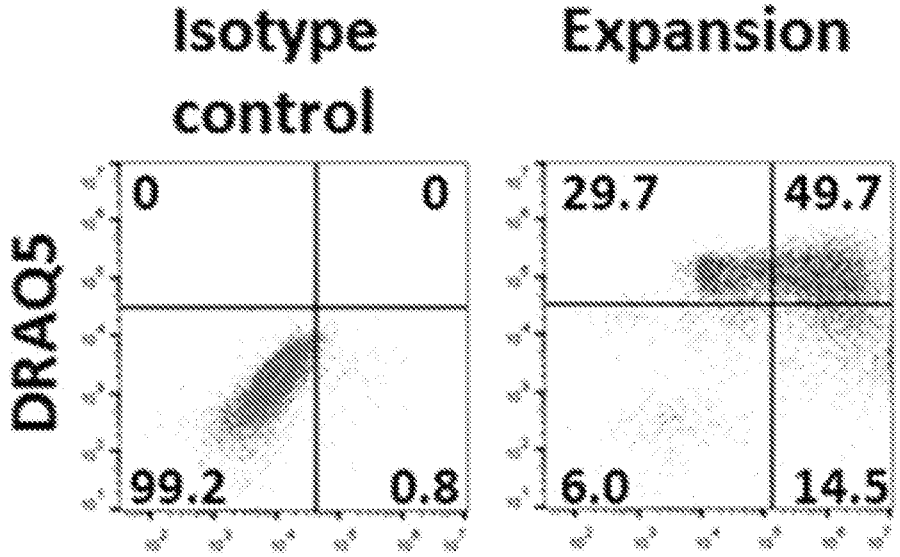
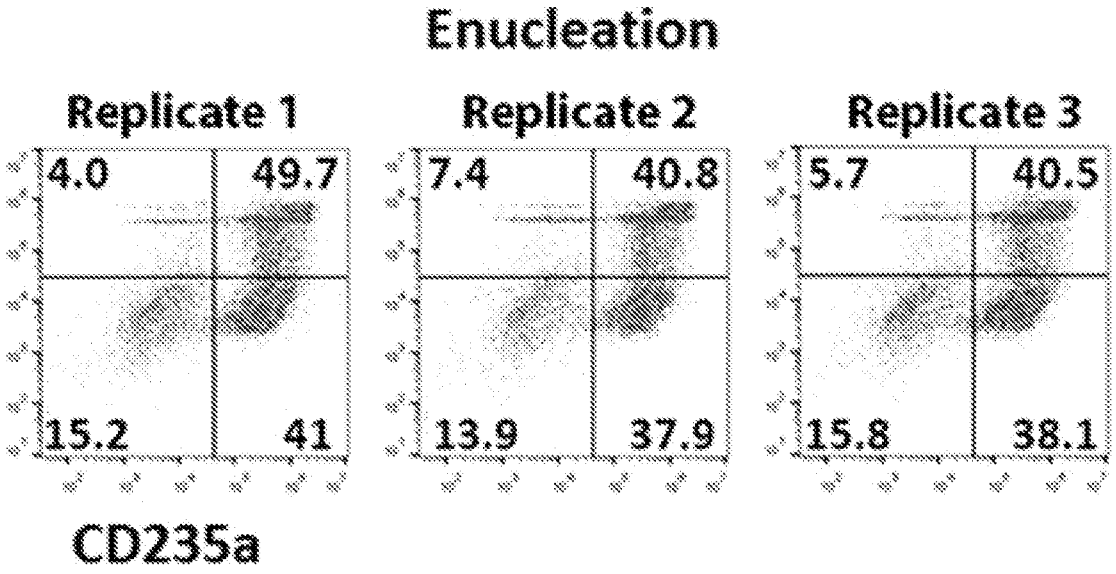


FIG. 5 contiued



E

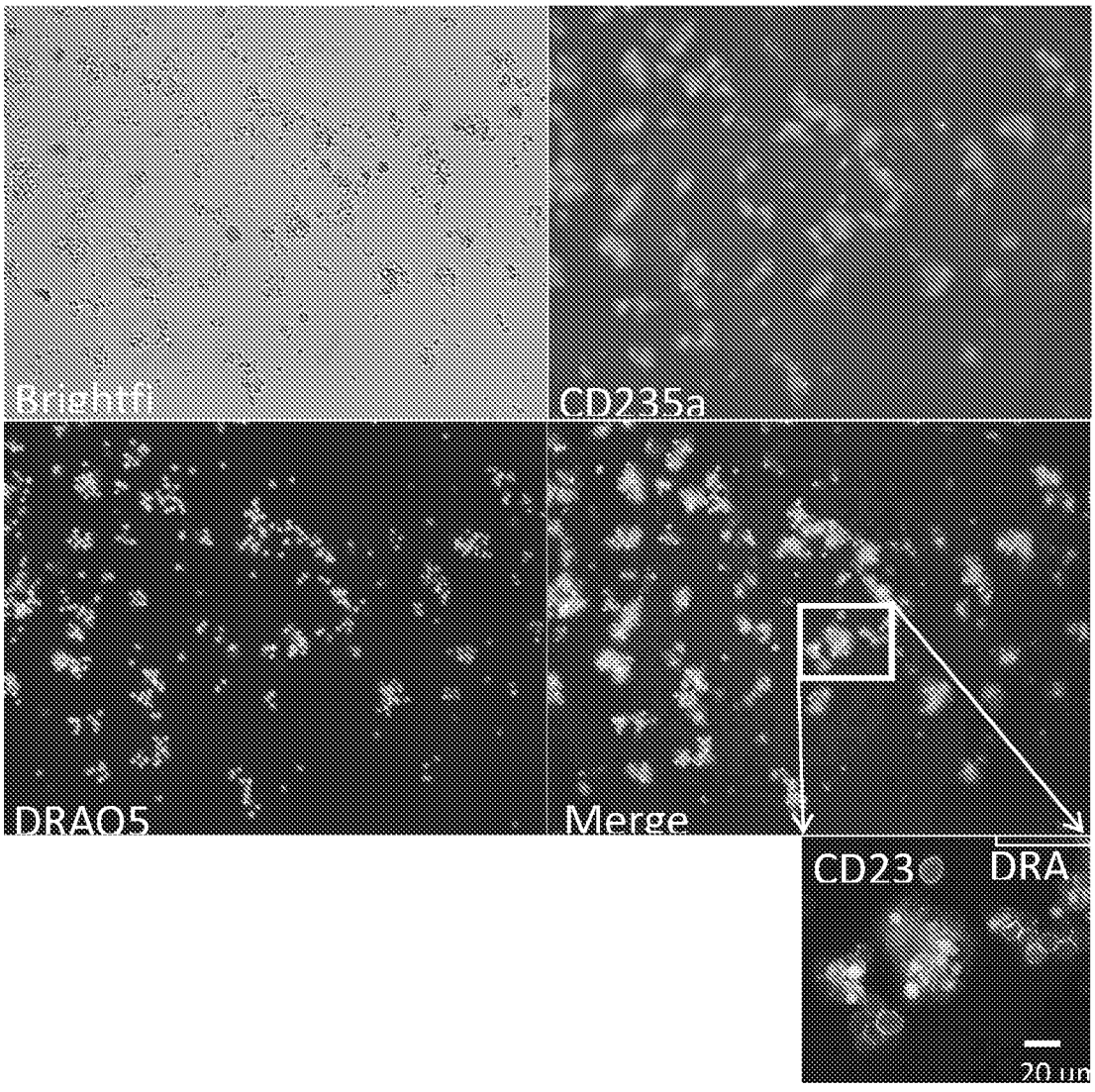


FIG. 5 continued

F

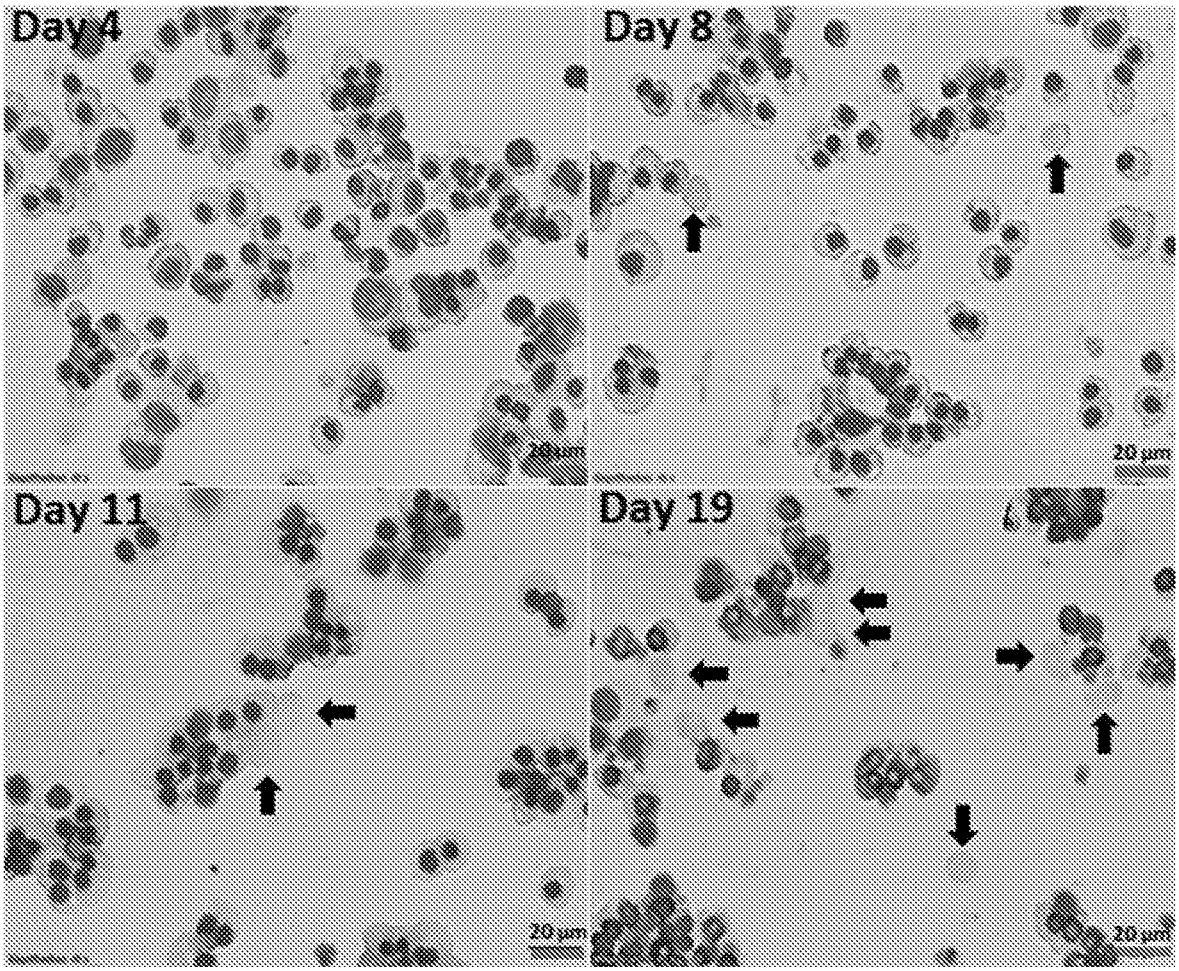


FIG. 6

A

Condition	Dose and duration of factors evaluated				Response outcome
	Activin A (ng/ml)	$CHIR_{24hr}$ (μM)	$CHIR_{48hr}$ (μM)	BMP4 (ng/ml)	% KDR+ cells
1	0	0	0	10	11.4
2	0	0	0	50	12.3
3	50	0	0	10	38.1
4	50	0	0	50	30
5	0	12	0	10	48.5
6	0	12	0	50	53.8
7	50	12	0	10	57.2
8	50	12	0	50	49
9	0	0	12	10	27.2
10	0	0	12	50	33.1
11	50	0	12	10	45.9
12	50	0	12	50	45.4
13	0	12	12	10	53.7
14	0	12	12	50	42.9
15	50	12	12	10	54.3
16	50	12	12	50	69.6
17	25	6	6	10	63.7
18	25	6	6	50	54.6
19	0	6	6	30	49.8
20	50	6	6	30	55.1
21	25	0	6	30	49.4
22	25	12	6	30	47.8
23	25	6	6	30	47

FIG. 6 continued

Condition	Activin A (ng/ml)	$CHIR_{24hr}$ (μM)	$CHIR_{48hr}$ (μM)	BMP4 (ng/ml)	% KDR+ cells
24	25	6	12	30	43.4
25	25	6	6	30	59.9
26	25	6	6	30	69.3
27	25	6	6	30	53.2
28	25	6	6	30	56.6
29	25	6	6	30	59.5

B

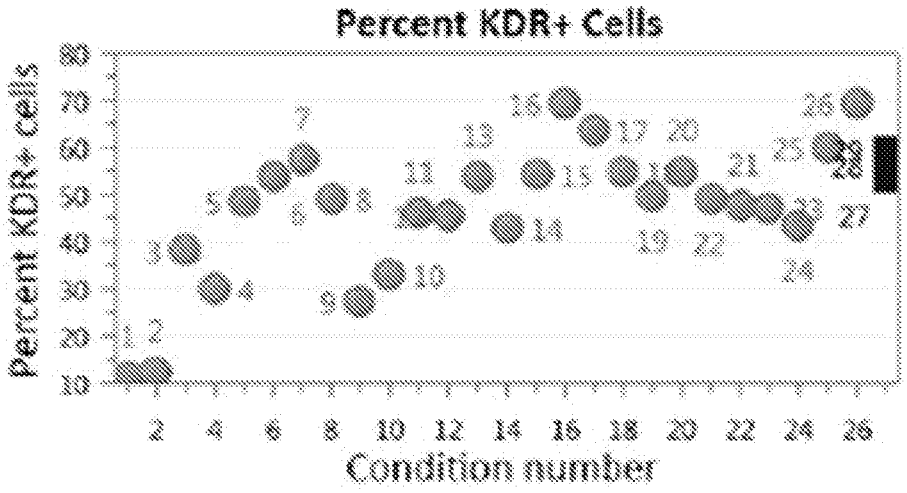
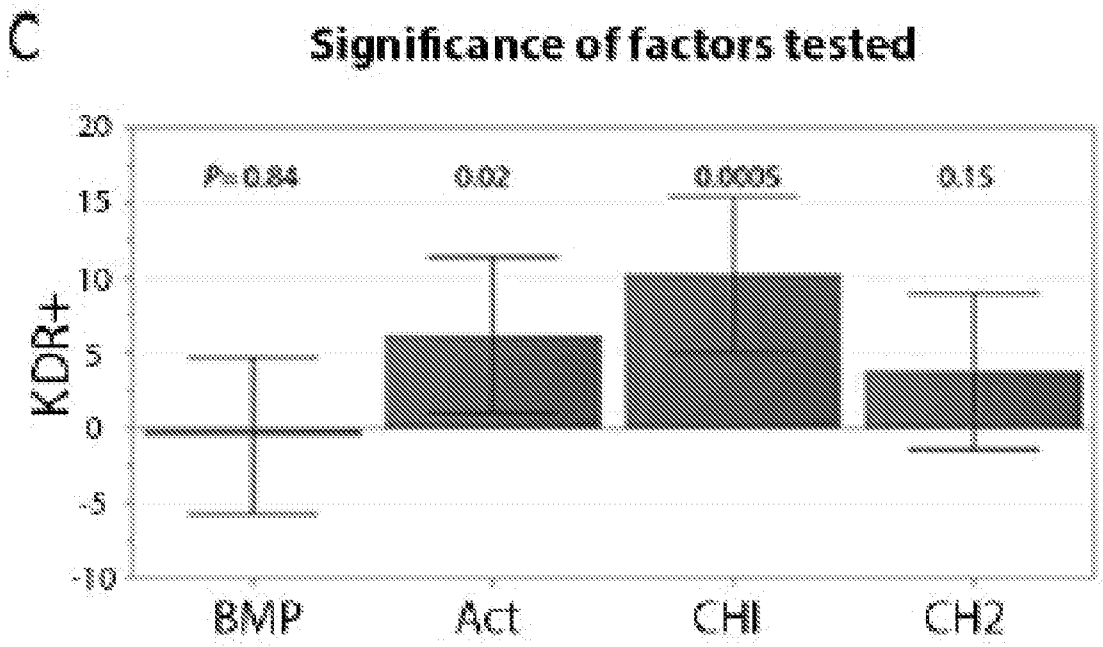


FIG. 6 continued



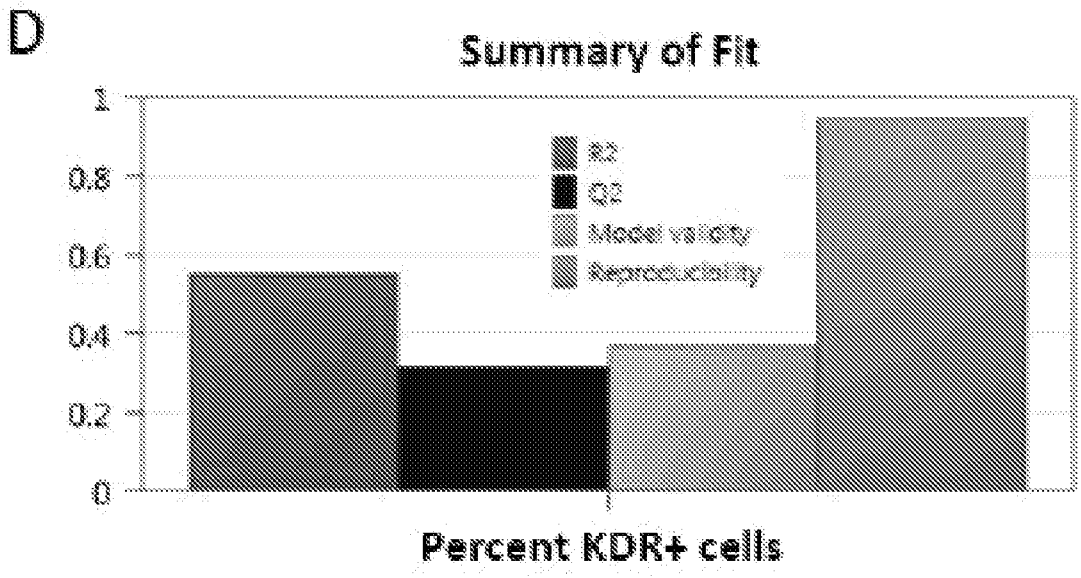


FIG. 7

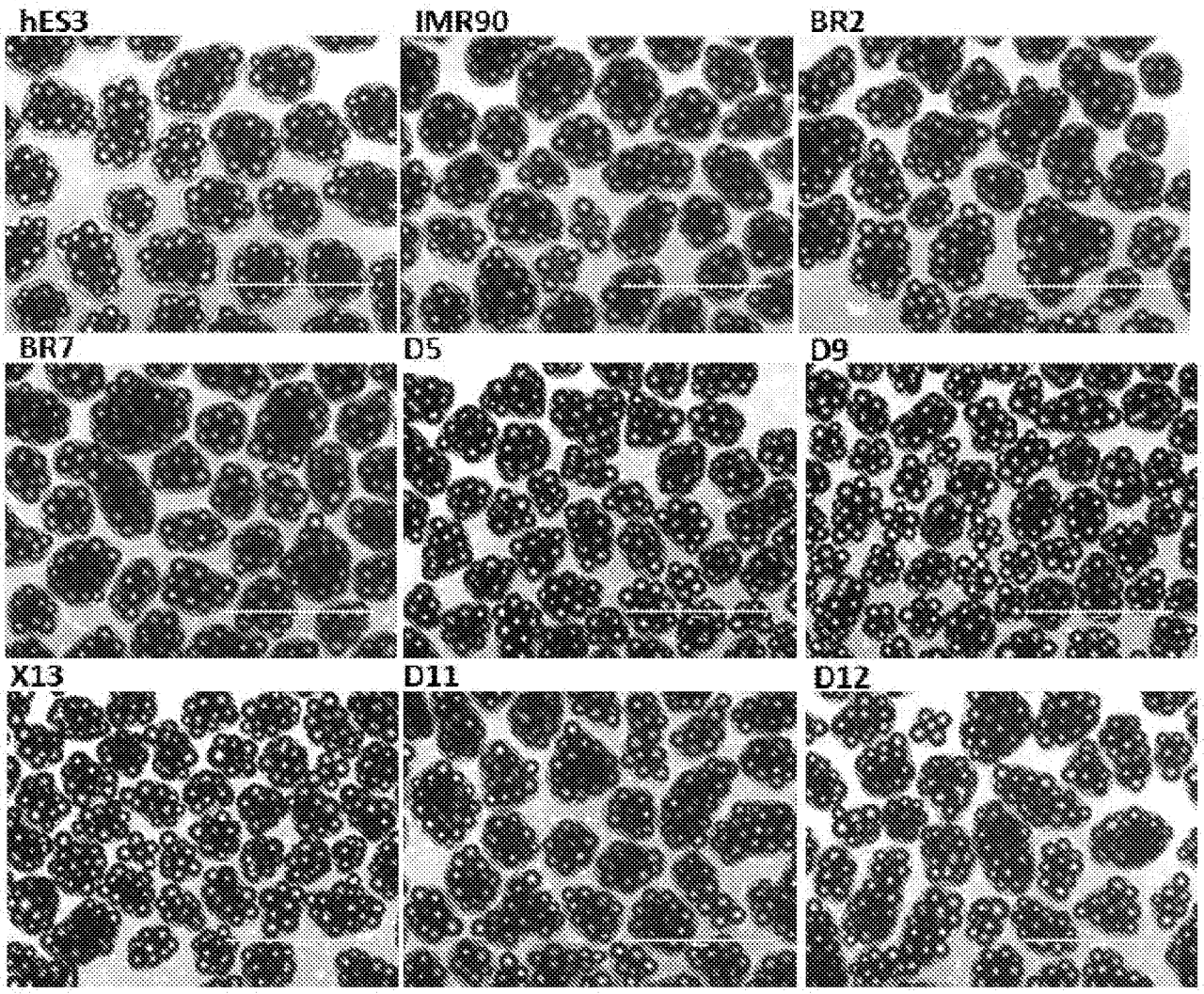


FIG. 8

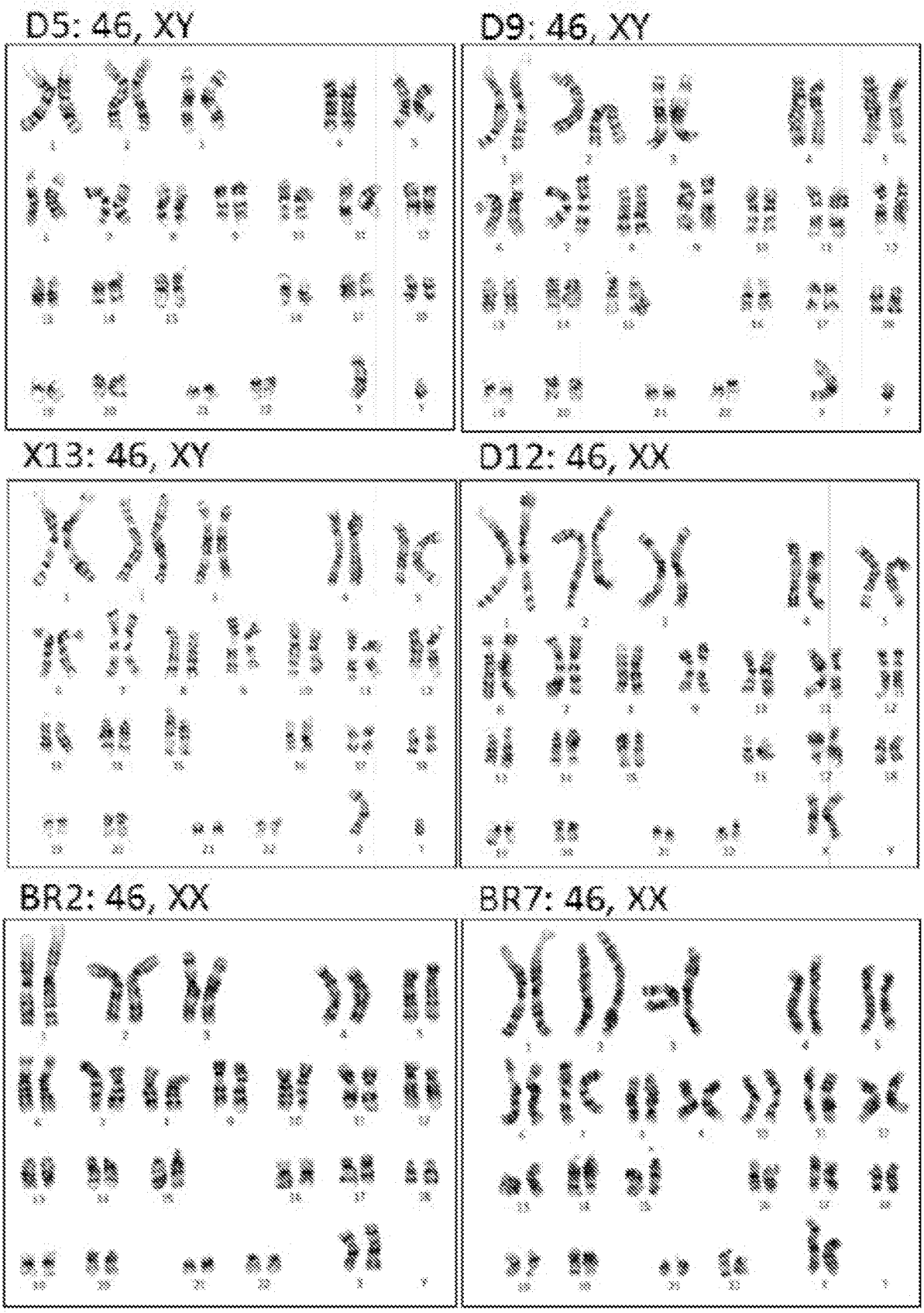


FIG. 8 continued

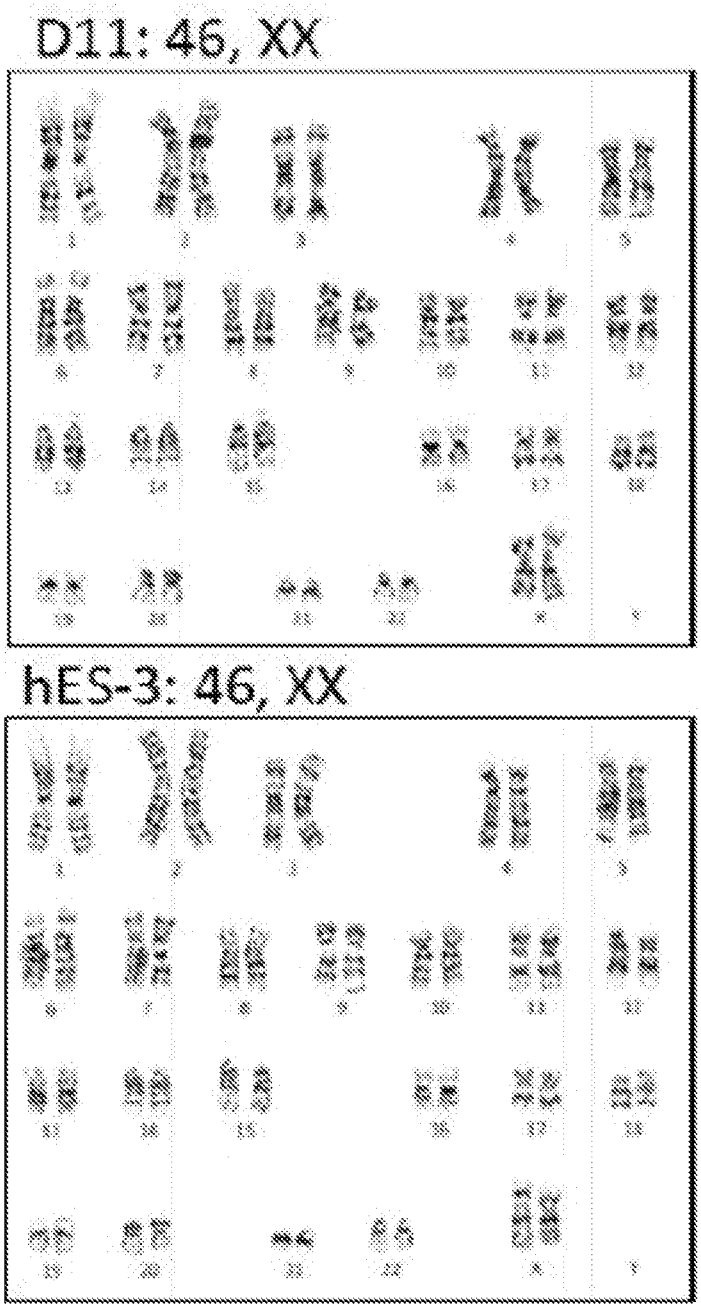


FIG. 9

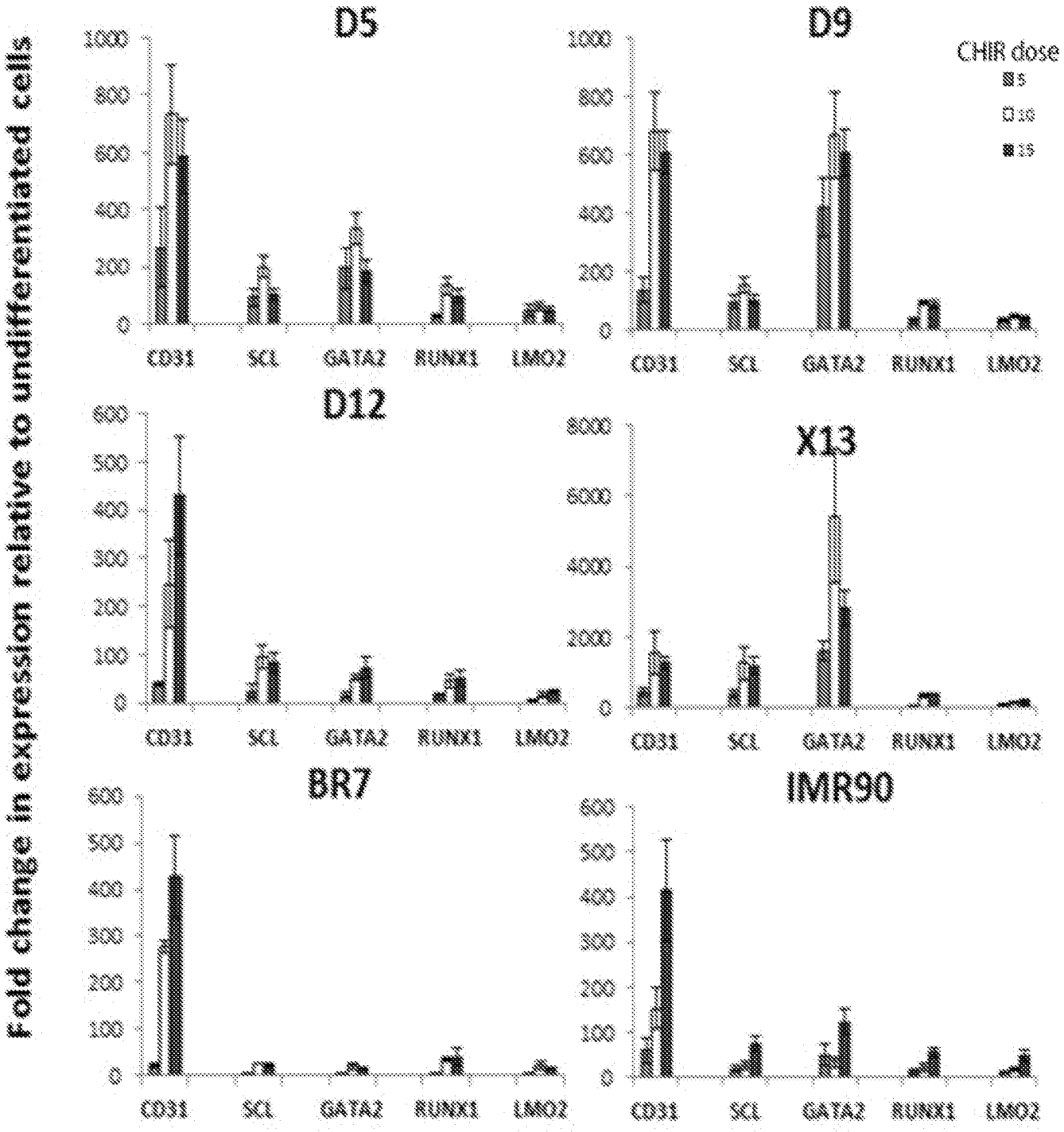

Fold change in expression relative to undifferentiated cells
D5
D9
D12
X13
BR7
IMR90
CHIR dose
5
10
15
CD31
SCL
GATA2
RUNX1
LMO2

FIG. 9 contined

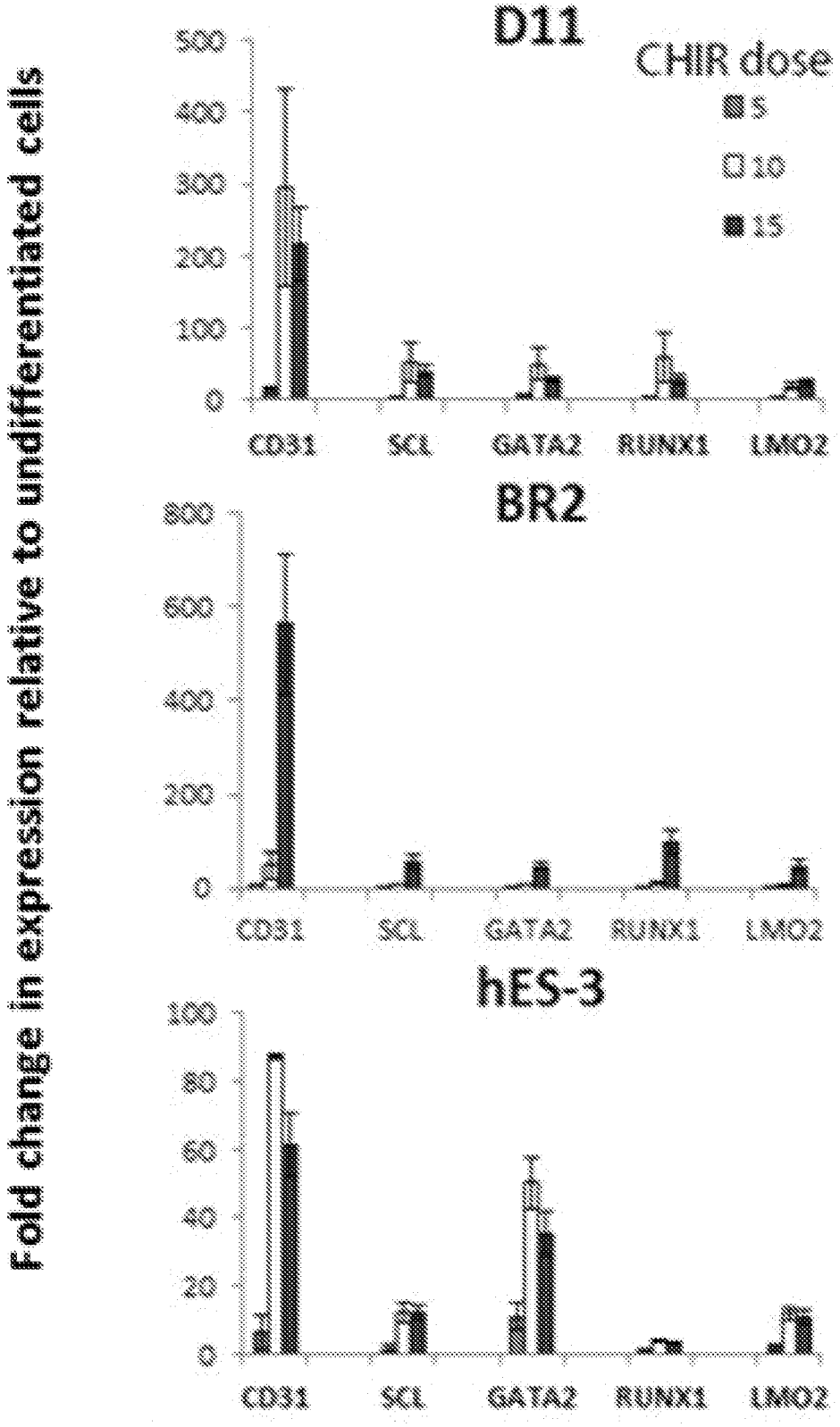


FIG. 10

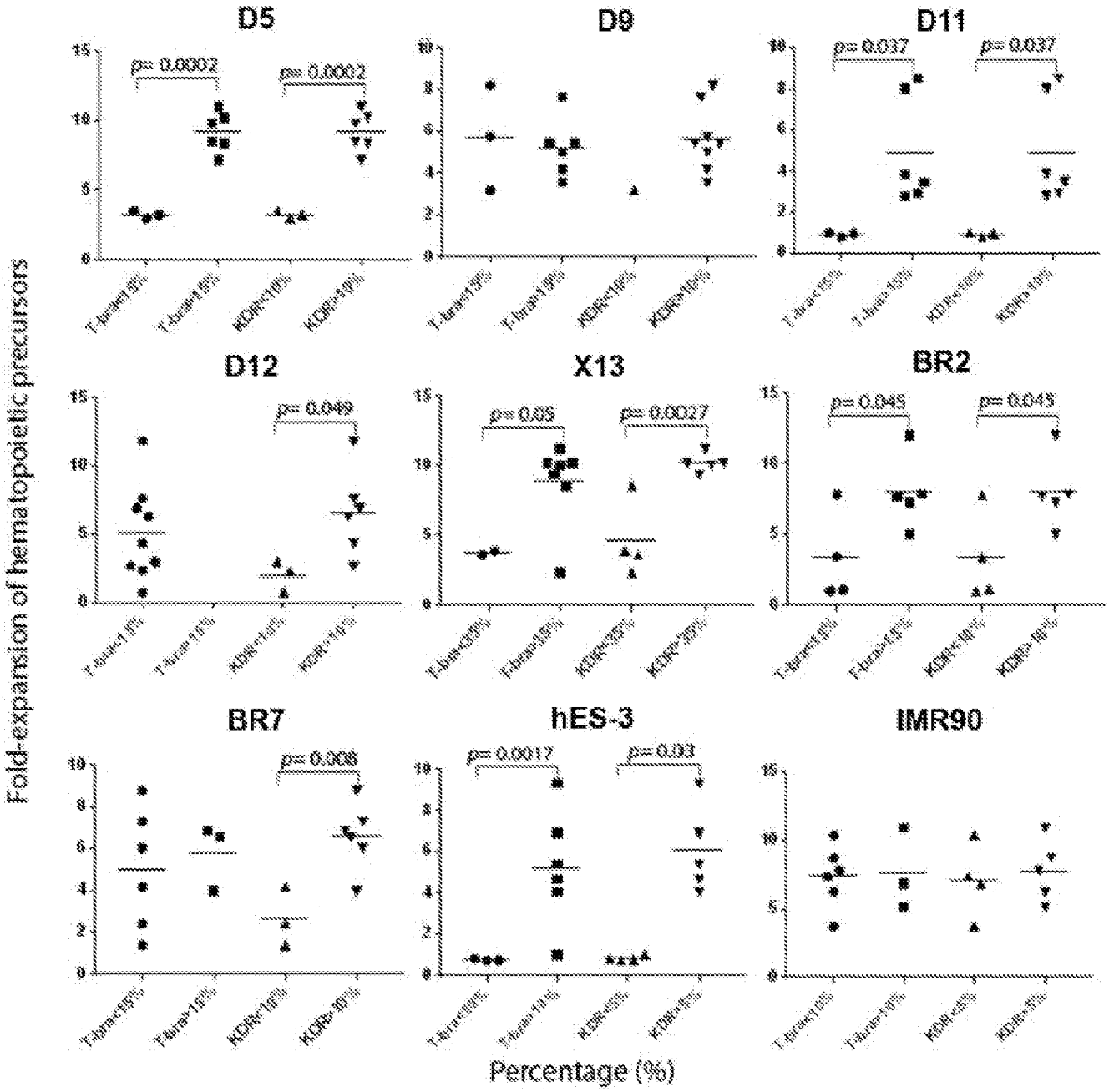

D5
D9
D11
D12
X13
BR2
BR7
hES-3
IMR90
p= 0.0002
p= 0.0002
p= 0.037
p= 0.037
p= 0.049
p= 0.05
p= 0.0027
p= 0.045
p= 0.045
p= 0.008
p= 0.0017
p= 0.03
Fold-expansion of hematopoietic precursors
Percentage (%)

FIG. 11

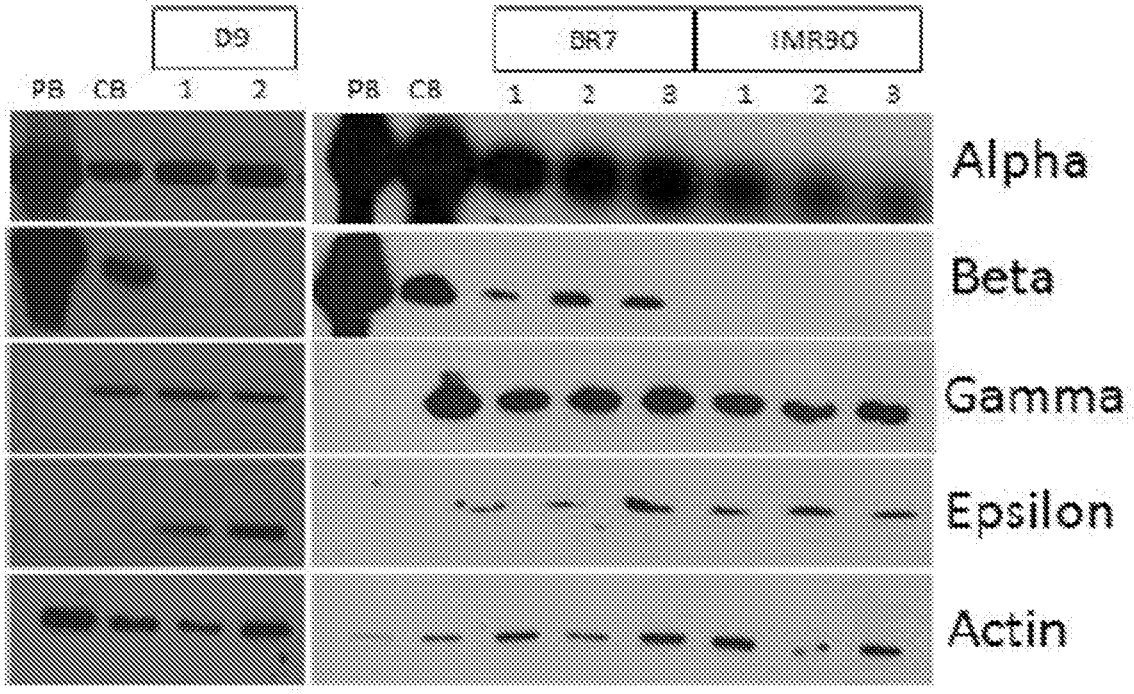

D9
BR7
IMR90
PB
CB
1
2
PB
CB
1
2
3
1
2
3
Alpha
Beta
Gamma
Epsilon
Actin

FIG. 12

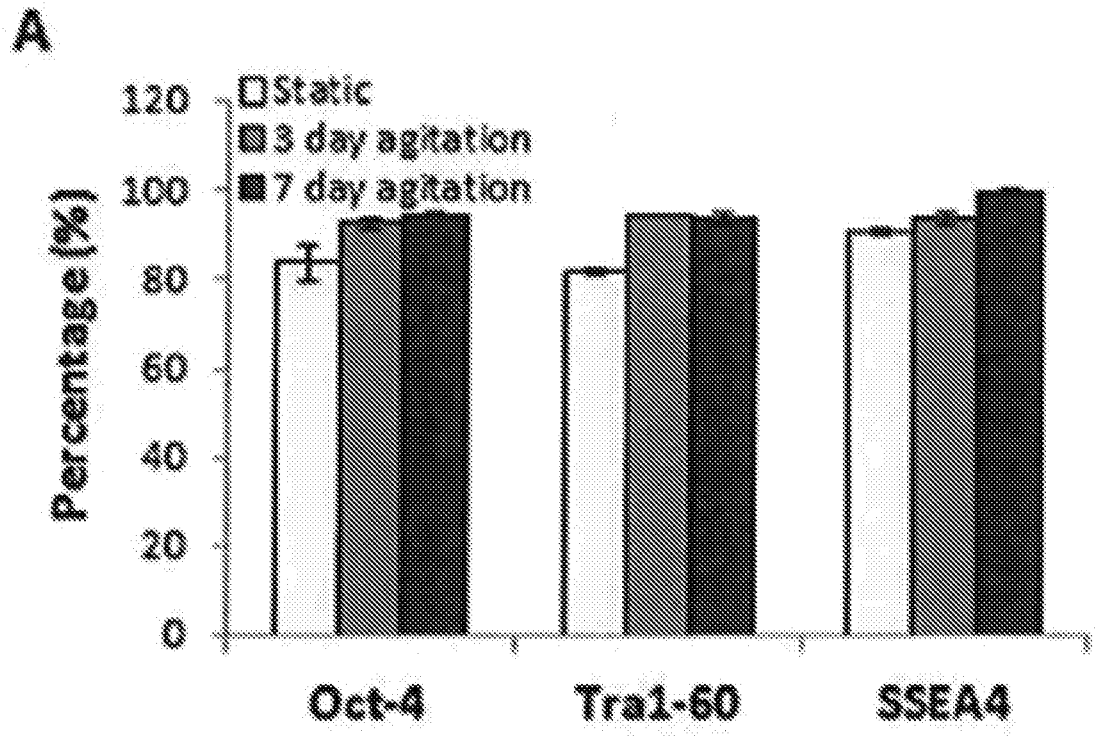

A
Static
3 day agitation
7 day agitation
Percentage (%)
120
100
80
60
40
20
0
Oct-4
Tra1-60
SSEA4

FIG. 12 continued

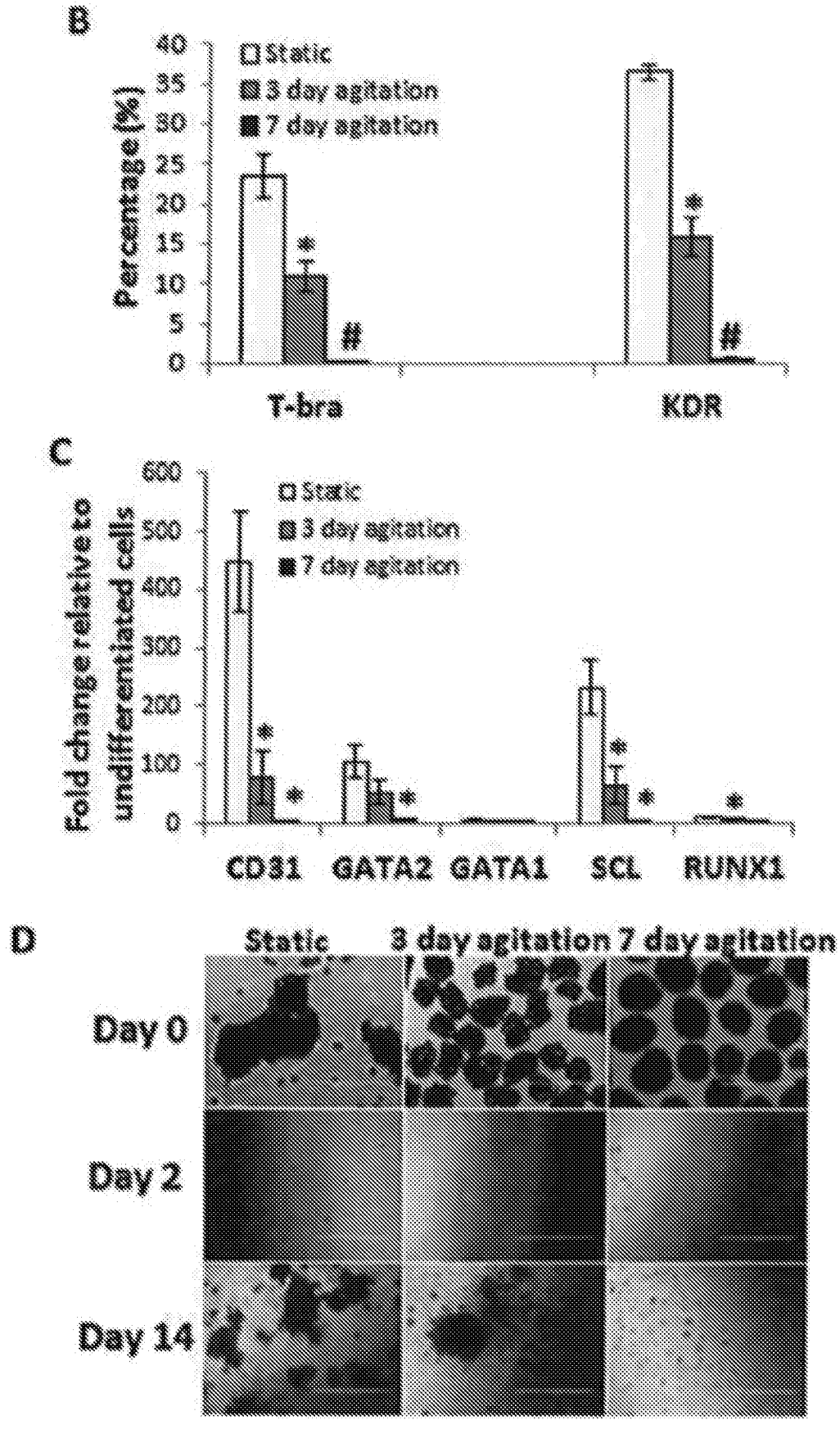


FIG. 12 continued

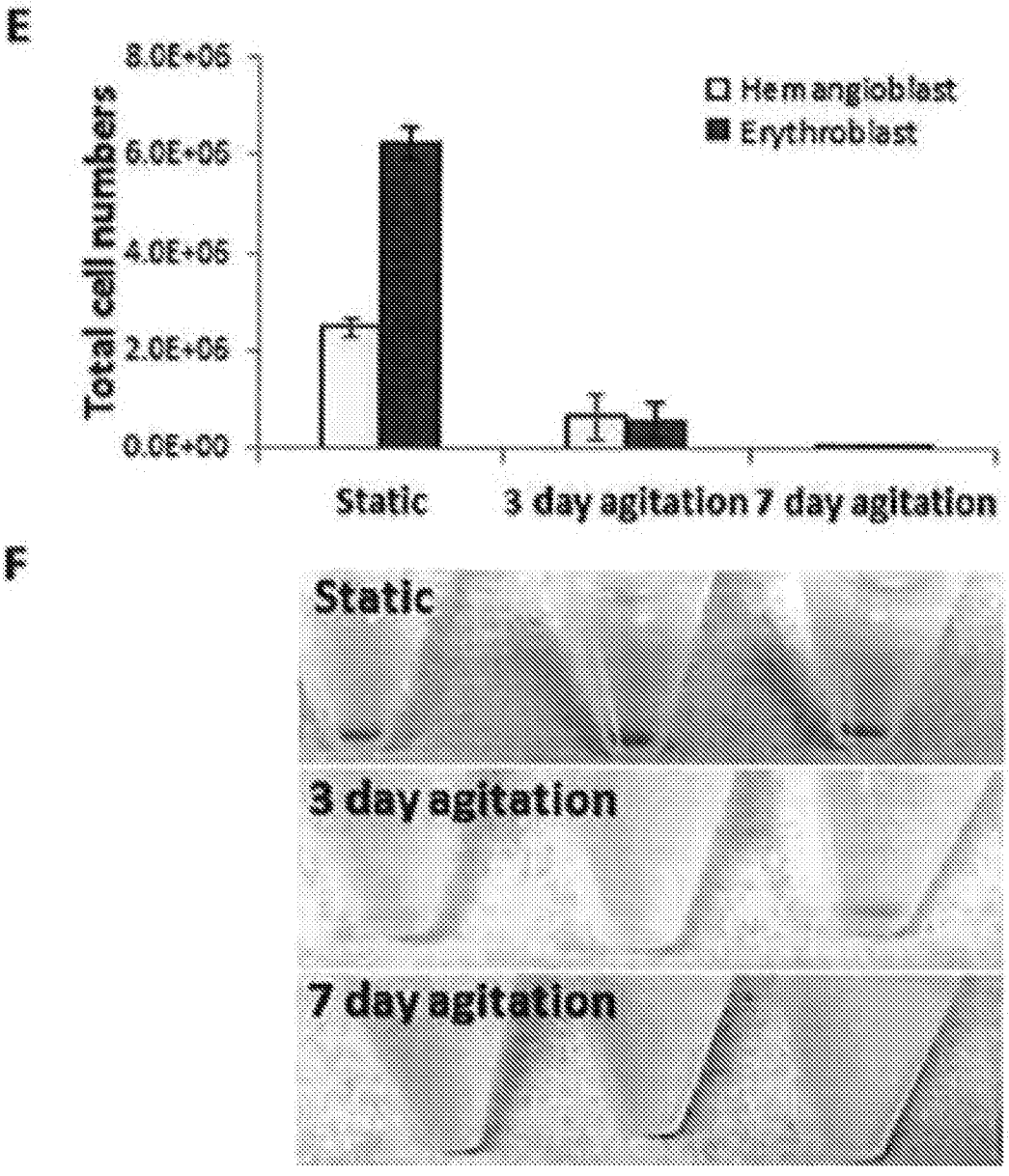


FIG. 13

A

Condition	Activin A (ng/ml)	$CHIR_{12mM}$ (μM)	$CHIR_{6mM}$ (μM)	SB (μM)	BMP4 (ng/ml)	KDR
1	0	0	0	0	50	0.44
2	50	0	0	0	10	1.07
3	0	15	0	0	10	9.19
4	50	15	0	0	50	18.5
5	0	0	15	0	10	1.18
6	50	0	15	0	50	2.05
7	0	15	15	0	50	30.7
8	50	15	15	0	10	25.6
9	0	0	0	30	10	0.6
10	50	0	0	30	50	0.97
11	0	15	0	30	50	17.7
12	50	15	0	30	10	16.3
13	0	0	15	30	50	4.57
14	50	0	15	30	10	1.38
15	0	15	15	30	10	38
16	50	15	15	30	50	33.7
17	0	7.5	7.5	15	30	5.61
18	50	7.5	7.5	15	30	19.2
19	40	0	7.5	15	30	3.42
20	40	15	7.5	15	30	34.6
21	40	7.5	0	15	30	9.25
22	40	7.5	15	15	30	30.8
23	40	7.5	7.5	0	30	8.24
24	40	7.5	7.5	30	30	11.4
25	40	7.5	7.5	15	10	30.1
26	40	7.5	7.5	15	50	28.3
27	40	7.5	7.5	15	30	16.9
28	40	7.5	7.5	15	30	26.7
29	40	7.5	7.5	15	30	14

FIG. 13 continued

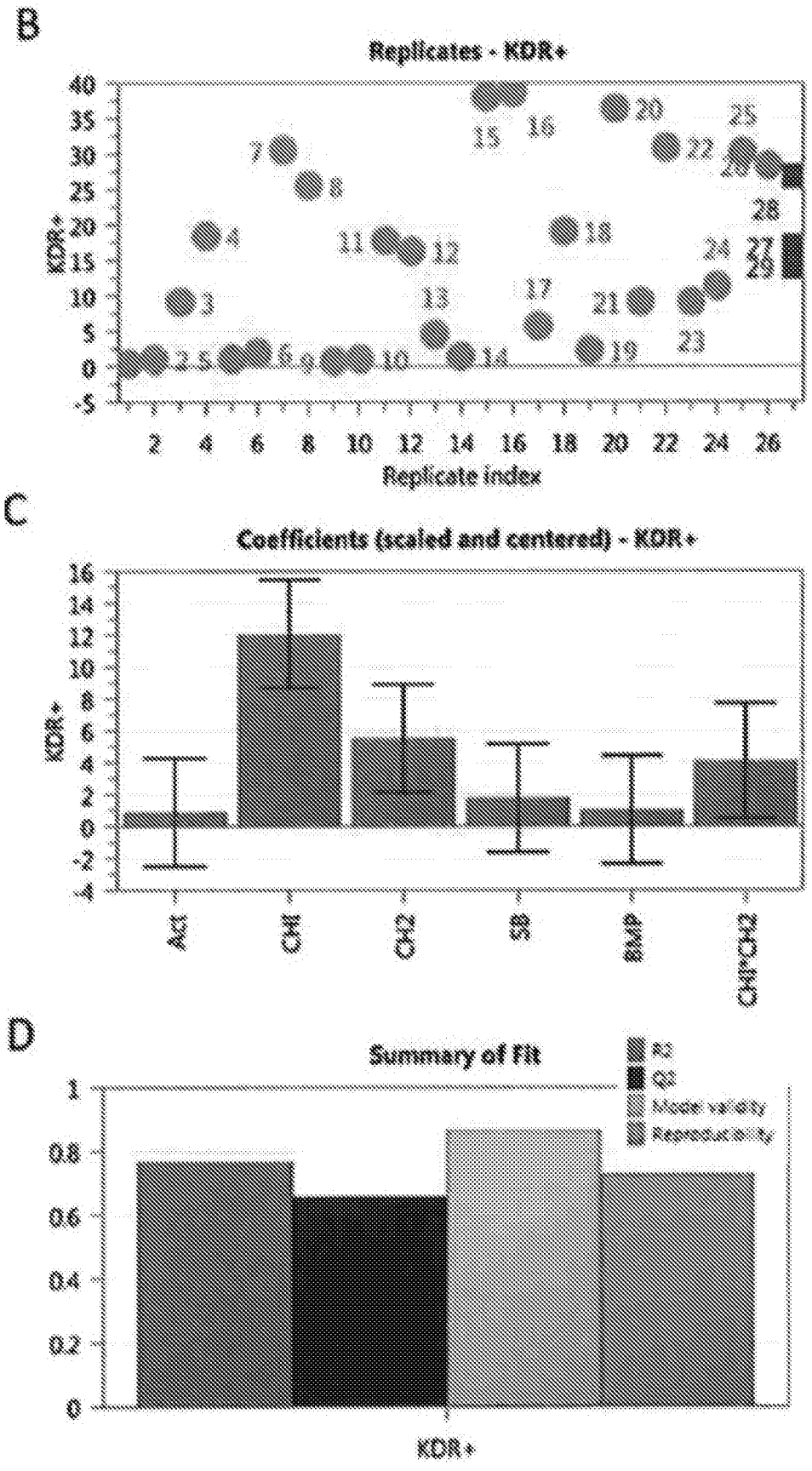


FIG. 14

A

	Dose and duration of factors evaluated				Response outcome
Condition	Activin A (ng/ml)	$CHIR_{24hr}$ (μM)	$CHIR_{48hr}$ (μM)	BMP4 (ng/ml)	% KDR+ cells
1	0	0	0	10	11.4
2	0	0	0	50	12.3
3	50	0	0	10	38.1
4	50	0	0	50	30
5	0	12	0	10	46.3
6	0	12	0	50	53.3
7	50	12	0	10	57.2
8	50	12	0	50	49
9	0	0	12	10	27.2
10	0	0	12	50	33.1
11	50	0	12	10	45.9
12	50	0	12	50	45.4
13	0	12	12	10	53.7
14	0	12	12	50	42.9
15	50	12	12	10	54.3
16	50	12	12	50	49.6
17	25	6	6	30	63.7
18	25	6	6	50	54.6
19	0	6	6	30	49.8
20	50	6	6	30	55.1
21	25	0	6	30	48.4
22	25	12	6	30	47.8
23	25	6	6	30	47
24	25	6	12	30	43.4
25	25	6	6	30	59.9
26	25	6	6	30	60.3
27	25	6	6	30	53.2
28	25	6	6	30	58.6
29	25	6	6	30	58.5

FIG. 14 continued

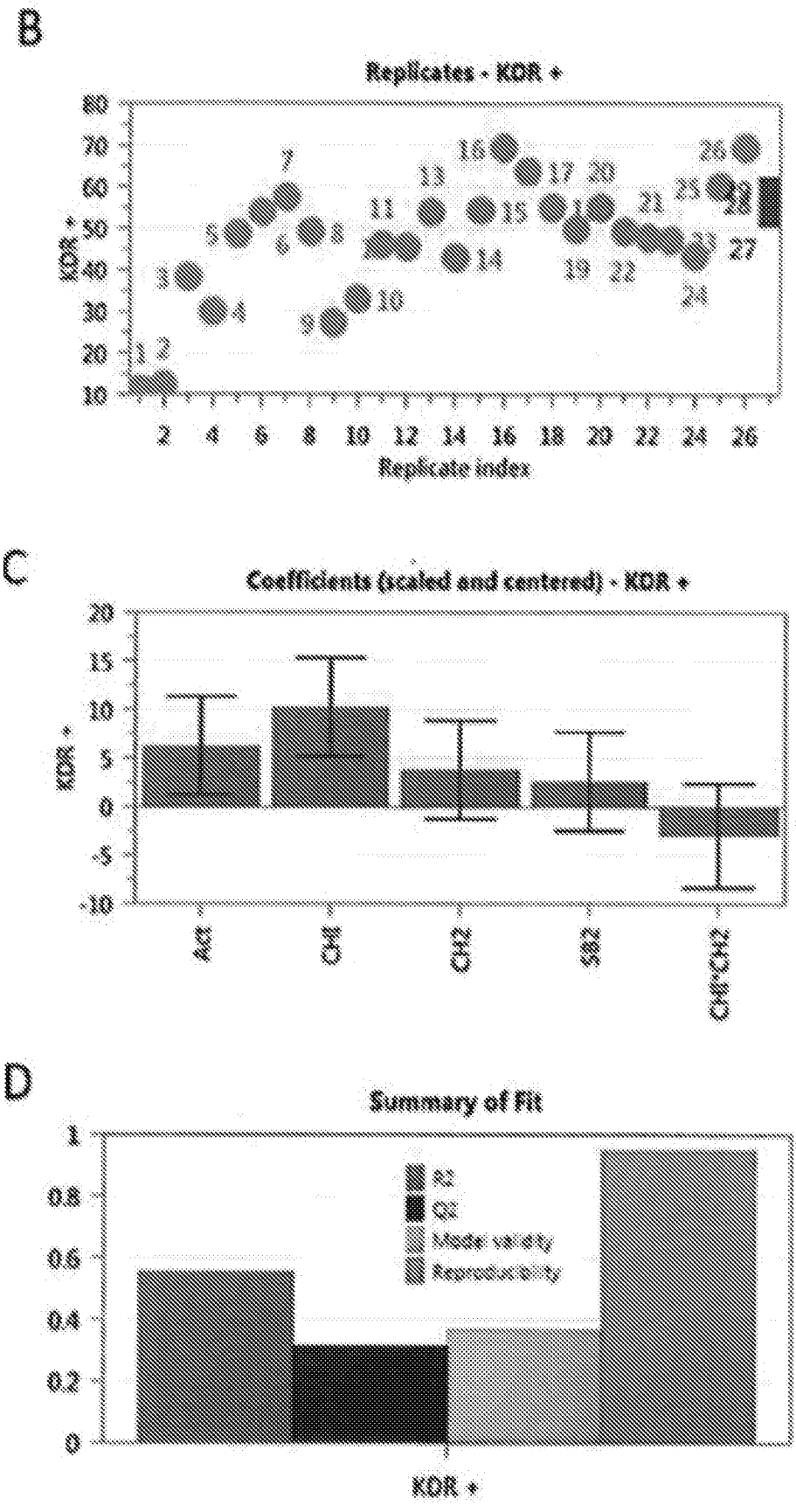


FIG. 15

A

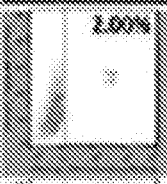
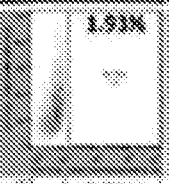
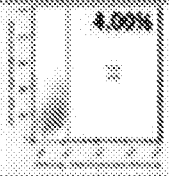
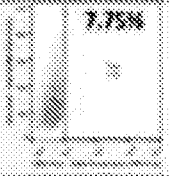
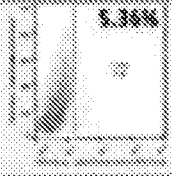
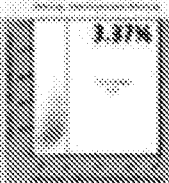
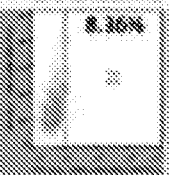
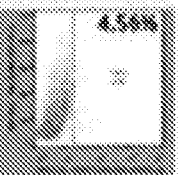
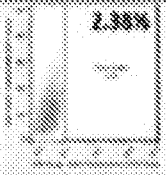
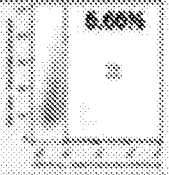
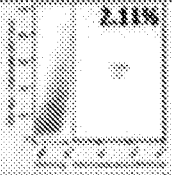
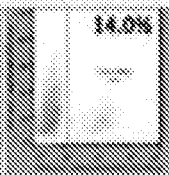
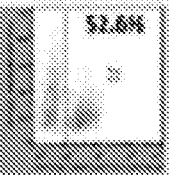
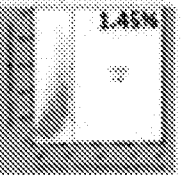
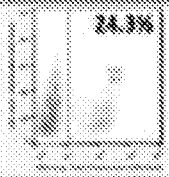
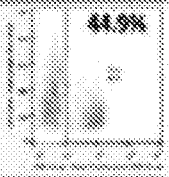
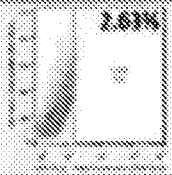
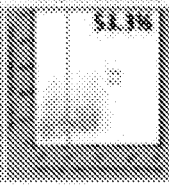
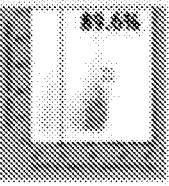
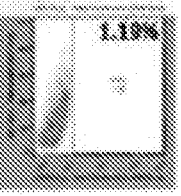
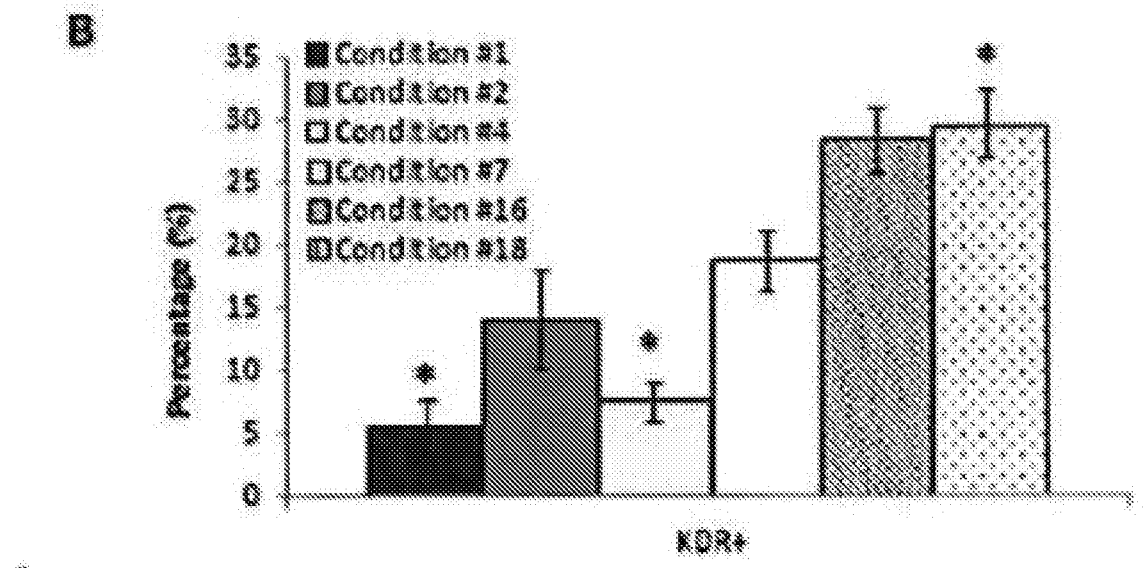
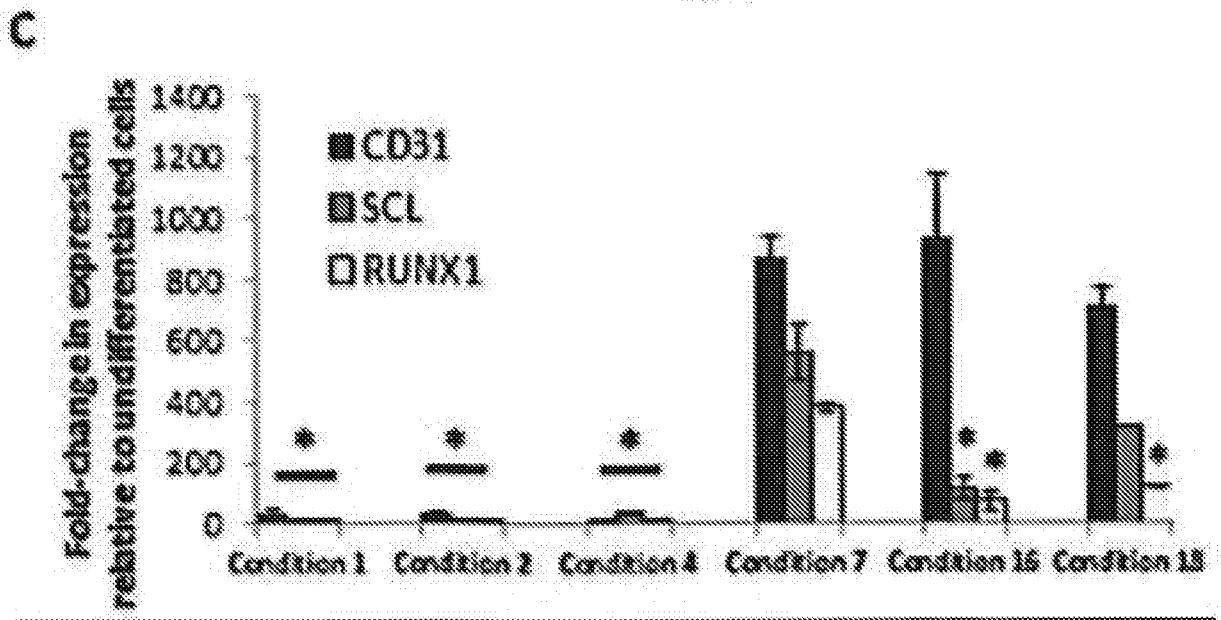
	Condition	T-bra$_{24\ hr}$	T-bra$_{48\ hr}$	T-bra$_{72\ hr}$
	No treatment	 2.60%	 1.91%	
Condition #1	10 ng/ml BMP4	 4.00%	 7.75%	 5.36%
Condition #2	50 ng/ml BMP4	 3.37%	 8.36%	 4.56%
Condition #4	50 ng/ml BMP4; 80 ng/ml Activin A	 2.38%	 8.66%	 2.11%
Condition #7	10 ng/ml BMP4; 80 ng/ml Activin A; 15 uM CHIR$_{24hr}$	 14.0%	 52.6%	 1.45%
Condition #16	50 ng/ml BMP4; 80 ng/ml Activin A; 15 uM CHIR$_{24hr}$, 15 uM CHIR$_{48hr}$	 24.3%	 44.9%	 2.61%
Condition #18	50 ng/ml BMP4,40 ng/ml Activin A; 7.5 uM CHIR$_{24hr}$, 7.5 uM CHIR$_{48hr}$	 51.3%	 85.6%	 1.19%

FIG. 15 continued

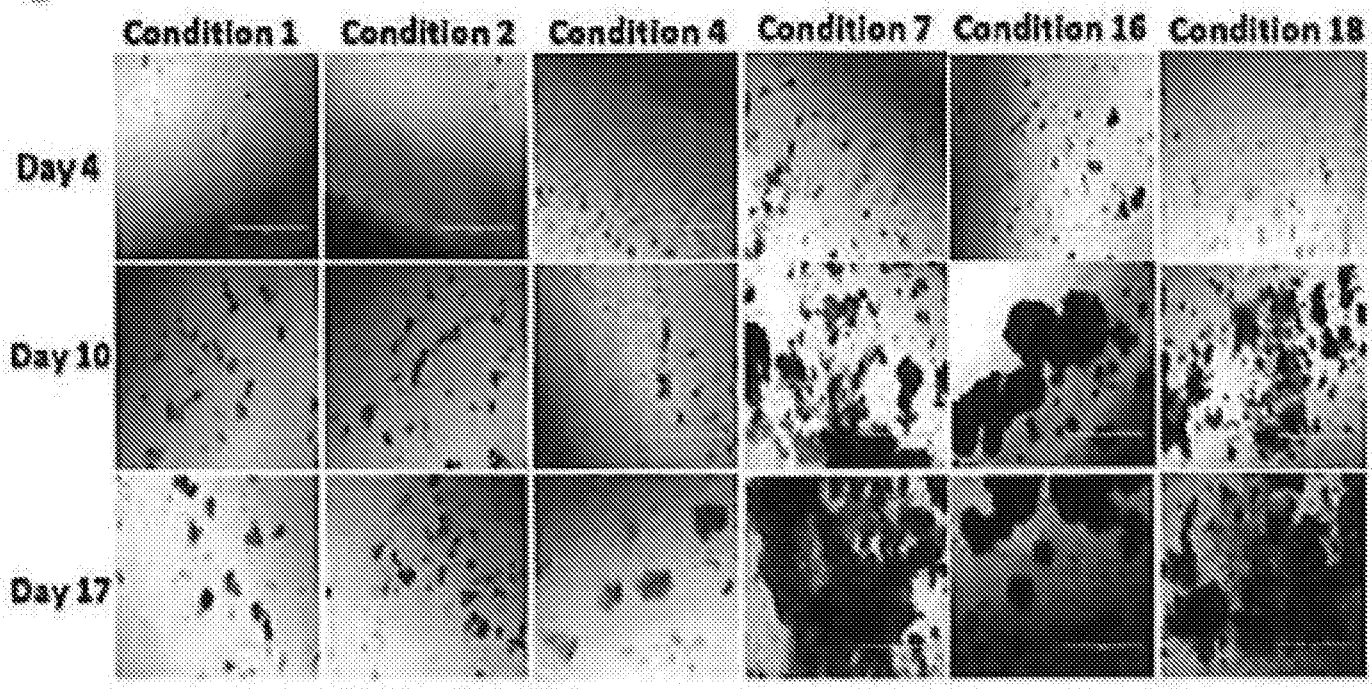




BMP4	low	high	high	low	high	high
Activin A	-	-	high	high	high	low
$CHIR_{24hr}$	-	-	-	15 uM	15 uM	7.5 uM
$CHIR_{48hr}$	-	-	-	-	15 uM	7.5 uM

FIG. 15 continued

D



	Condition 1	Condition 2	Condition 4	Condition 7	Condition 16	Condition 18
Hemangioblast fold-expansion:	0.56 ± 0.1	0.56 ± 0.06	0.60 ± 0.09	7.57 ± 0.28	0.57 ± 0.05	5.23 ± 0.53
Day 2 T-bra expression:	7.75 %	8.36 %	6.56%	52.6%	44.9%	89.6%
D4 CD31 expression:	34 ± 19.3	29.24 ± 6.6	15.35 ± 0.6	863.4 ± 76.9	926.3 ± 218.3	704.4 ± 66
D4 SCL expression:	4.96 ± 2.2	11.34 ± 2.9	35.97 ± 5.6	556.84 ± 94.5	112.5 ± 37.5	321.36 ± 0.1
D4 RunX1 expression:	6.1 ± 4.2	3.84 ± 0.5	5.92 ± 2.9	378.81 ± 14.1	72.42 ± 27.9	122.42 ± 4.9

FIG. 15 continued

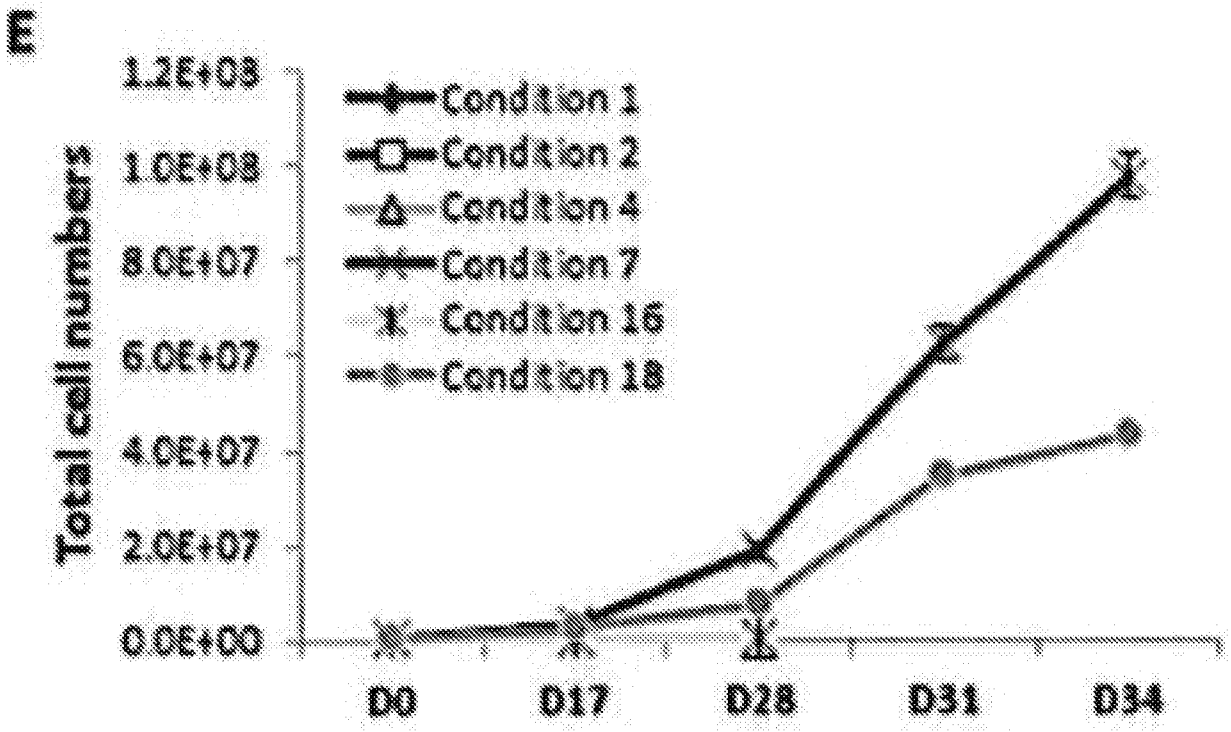
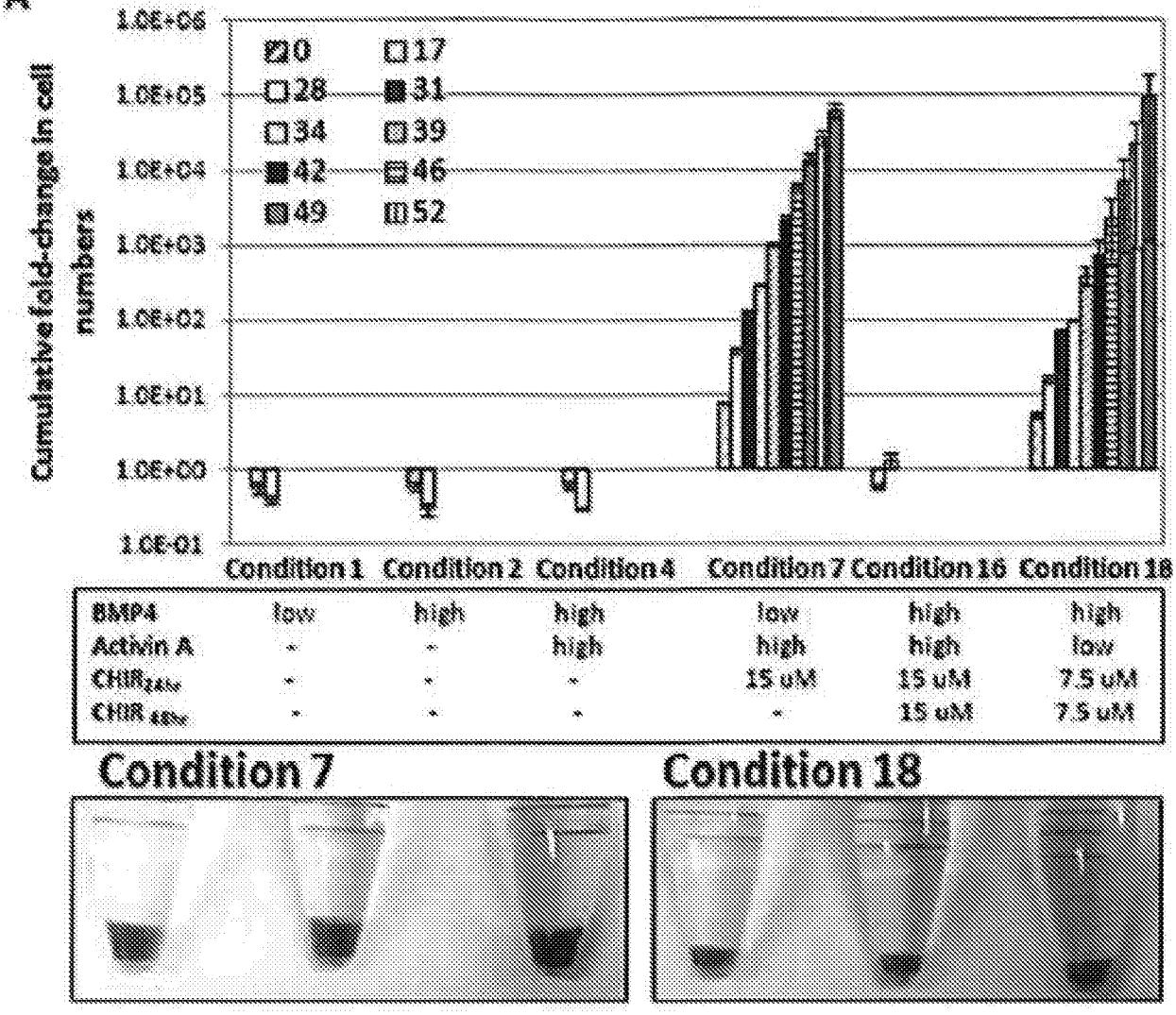


FIG. 16

A



B

	Condition 7	Condition 18	p-value
CD235a+ CD71+	63.5 % ± 1.82	34.8 % ± 7.28	0.019
CD235a+ CD71-	15.7 % ± 1.30	25.27 %± 2.74	0.03
Total CD235a	79.2 % ± 2.77	60.07 %± 4.77	0.03
HbF	99.43 % ± 0.12	67.10 % ± 0.55	<0.0001
Total yield (Day 34)	$9.77 \times 10^7 \pm 4.6 \times 10^6$	$4.31 \times 10^7 \pm 5.9 \times 10^6$	0.0003

FIG. 16 continued

C

	Adult RBCs	hES-3 derived RBCs	iPSC derived O-negative RBCs
Embryonic hemoglobin	-	+++	+
Fetal hemoglobin	-	+++	++
Adult hemoglobin	+++	-	-

D

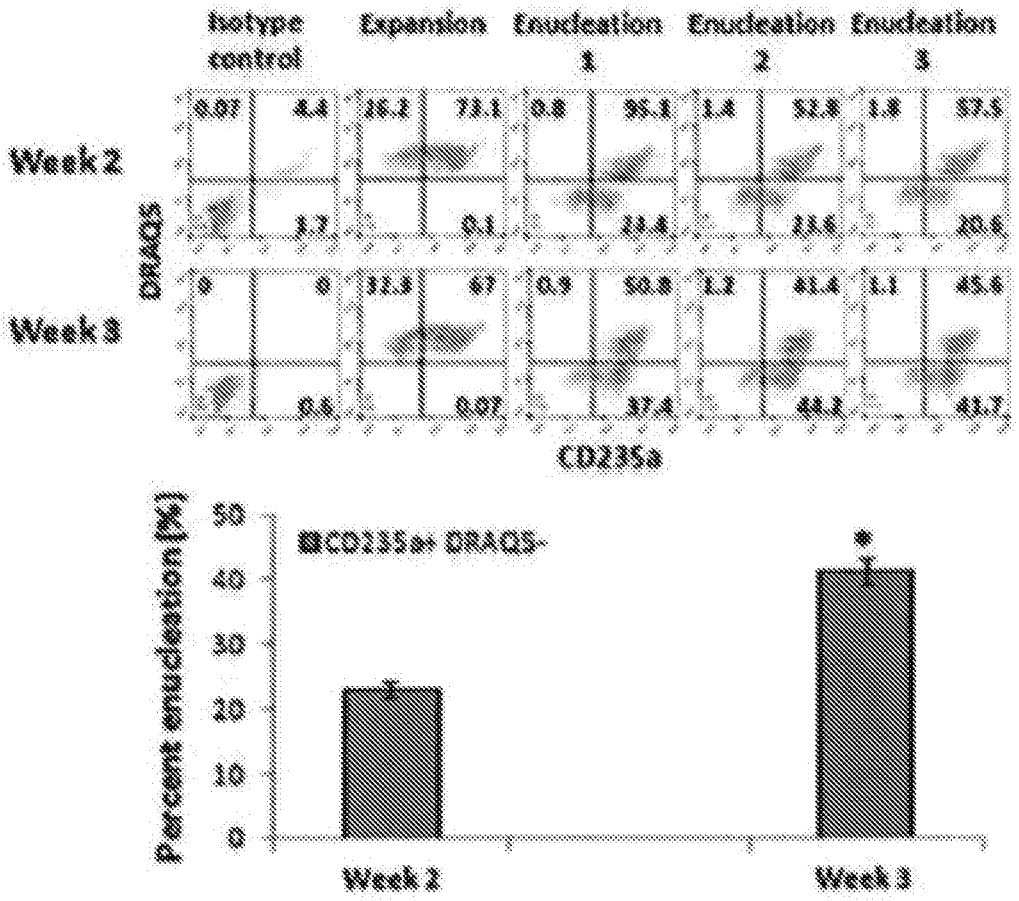


FIG. 16 continued

E

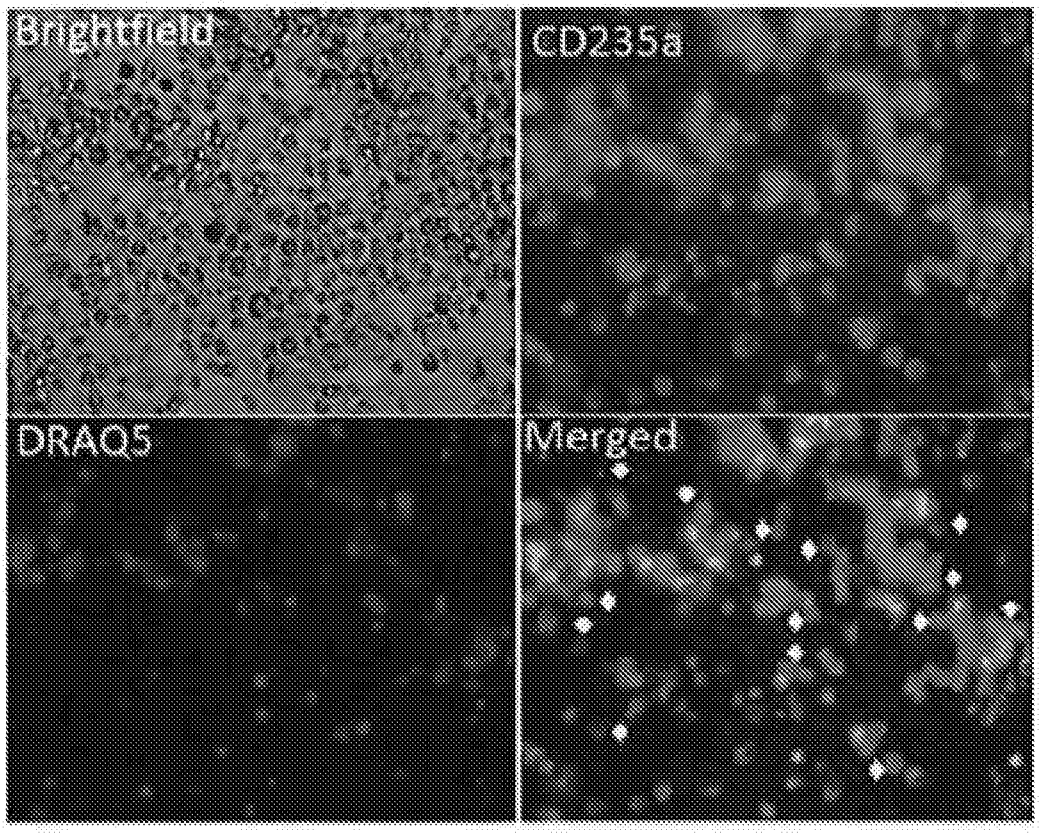


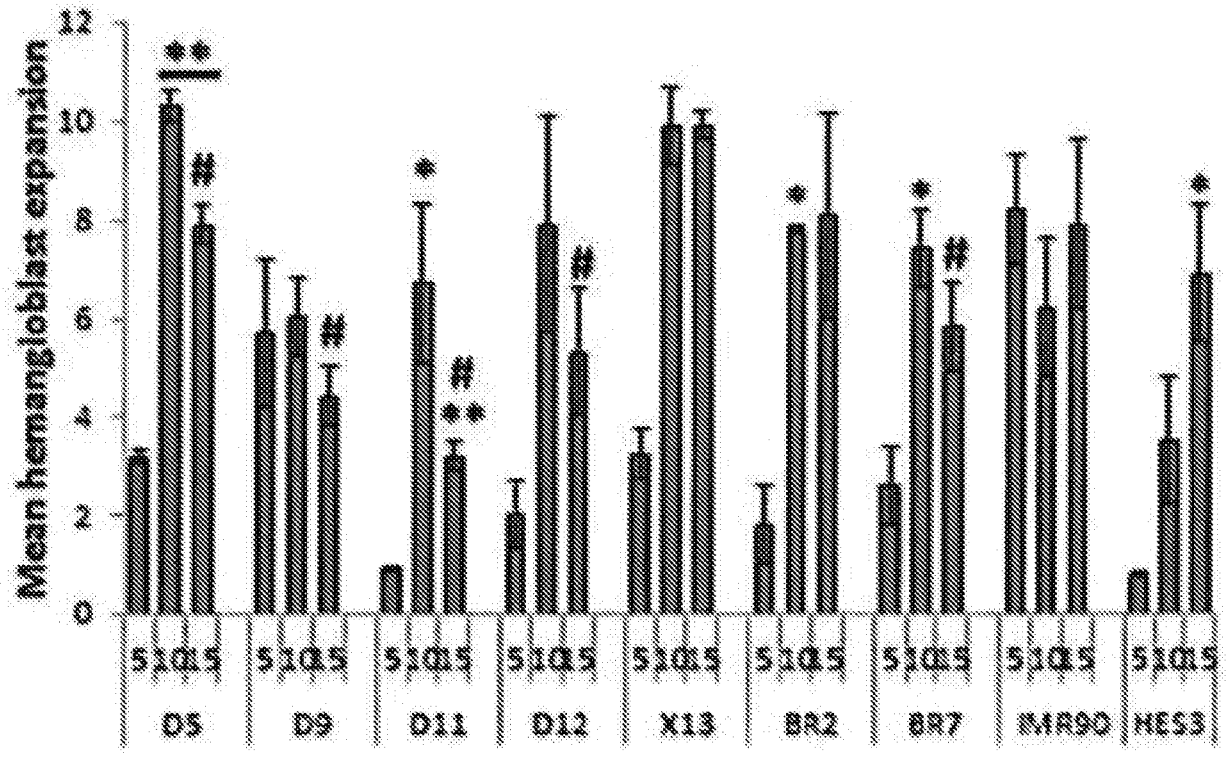
FIG. 17

A

Test	Lines	hES-3	IMR90	BR2	BR7	D12	D11	D5	D9	X13
Representative microscopic images										
Pluripotency (%)	Oct-4	94.6 ± 0.7	83.7 ± 0.2	92.3 ± 0.9	72.9 ± 2.1	94.3 ± 0.7	84.9 ± 1.4	83.4 ± 1.9	91.8 ± 1.1	90.6 ± 0.4
	Tra1-60	95.1 ± 1.8	91.1 ± 1.1	90.4 ± 2.1	84.8 ± 1.7	93.7 ± 0.5	83.1 ± 0.8	90.1 ± 0.6	91.3 ± 2.2	91.3 ± 1.1
	SSEA4	99.7 ± 0.1	99.9 ± 0.03	97.2 ± 0.7	99.4 ± 0.1	99.2 ± 0.2	99.1 ± 0.2	98.1 ± 0.2	98.9 ± 0.6	97.2 ± 0.4
Karyotype		46, XX	NA	46, XX	NA	NA	46, XX	46, XX	46, XX	46, XX
Fold-expansion		10.1 ± 1.1	12.5 ± 0.9	6.0 ± 0.7	9.6 ± 0.9	6.0 ± 0.4	7.8 ± 1.4	7.7 ± 0.9	8.0 ± 0.5	5.3 ± 0.8
Aggregate Size		453 ± 50	510 ± 128	459 ± 111	488 ± 99	455 ± 71	398 ± 148	371 ± 67	377 ± 76	340 ± 104
IGF-2 mRNA level (relative to hES-3)		1	1.3 ± 0.4	0.6 ± 0.2	0.7 ± 0.05	0.6 ± 0.1	0.71 ± 0.08	0.47 ± 0.01	0.36 ± 0.04	0.28 ± 0.07

FIG. 17 continued

B



C

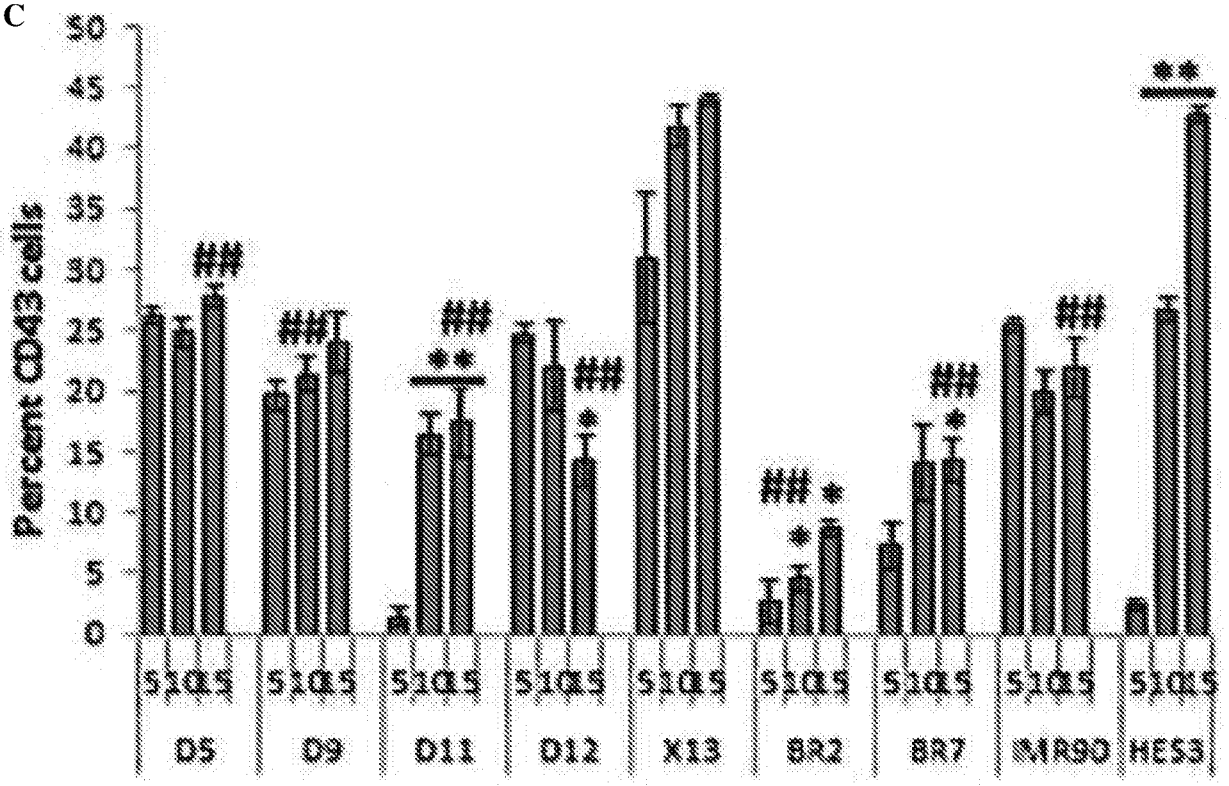


FIG. 18

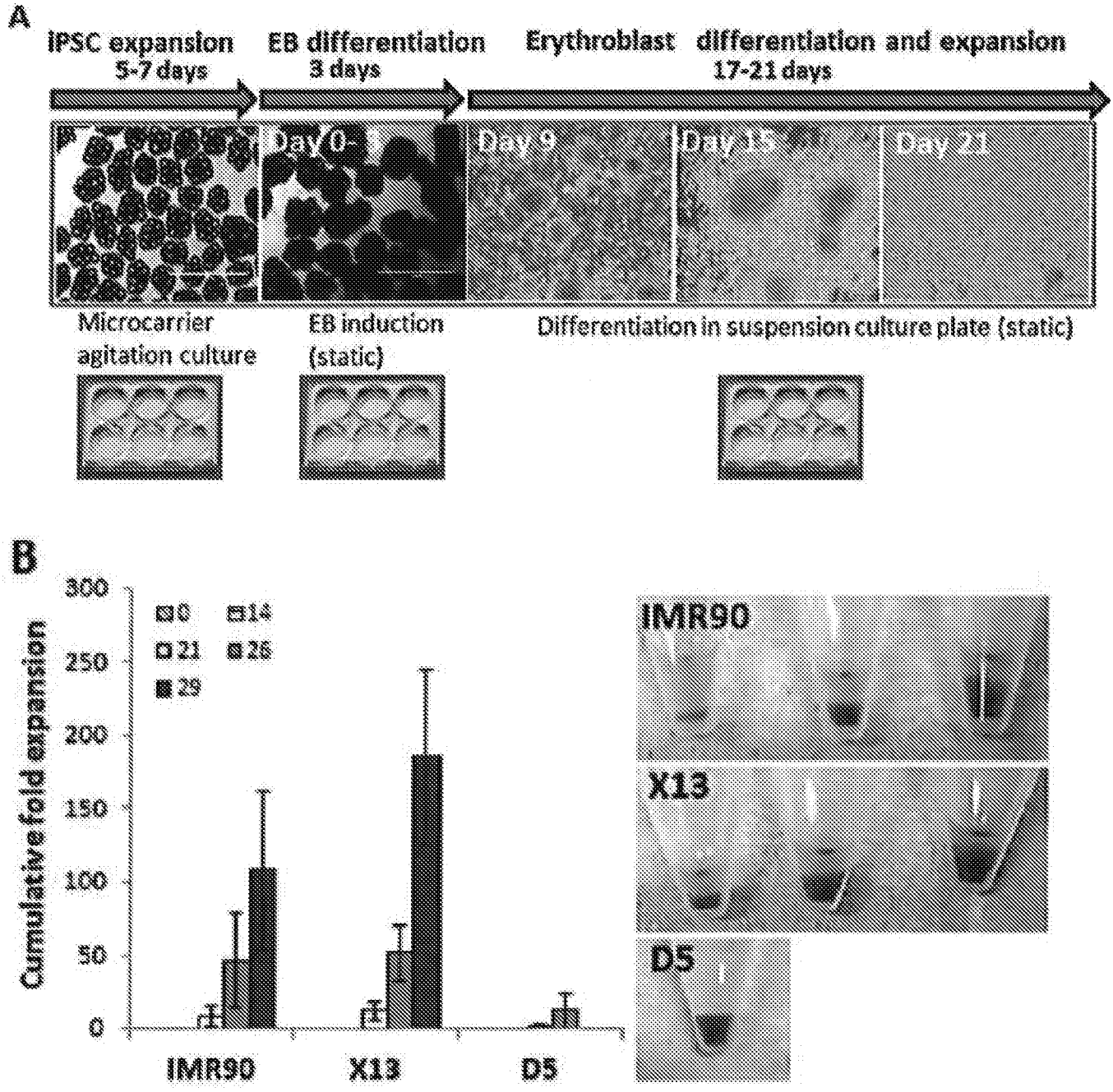

A
IPSC expansion
5-7 days
EB differentiation
3 days
Erythroblast differentiation and expansion
17-21 days
Day 0-3
Day 9
Day 15
Day 21
Microcarrier agitation culture
EB induction (static)
Differentiation in suspension culture plate (static)
B
Cumulative fold expansion
300
250
200
150
100
50
0
0
14
21
26
29
IMR90
X13
D5
IMR90
X13
D5

FIG. 18 continued

C

	IMR90	X13
Percent erythroid cells (CD235a)	86.8 % ± 1.8	78.3 % ± 2.4
HbF	98.9 % ± 0.5	98.2 % ± 0.9
Cumulative fold expansion (Day 29)	108 ± 54	185 ± 60

FIG. 19

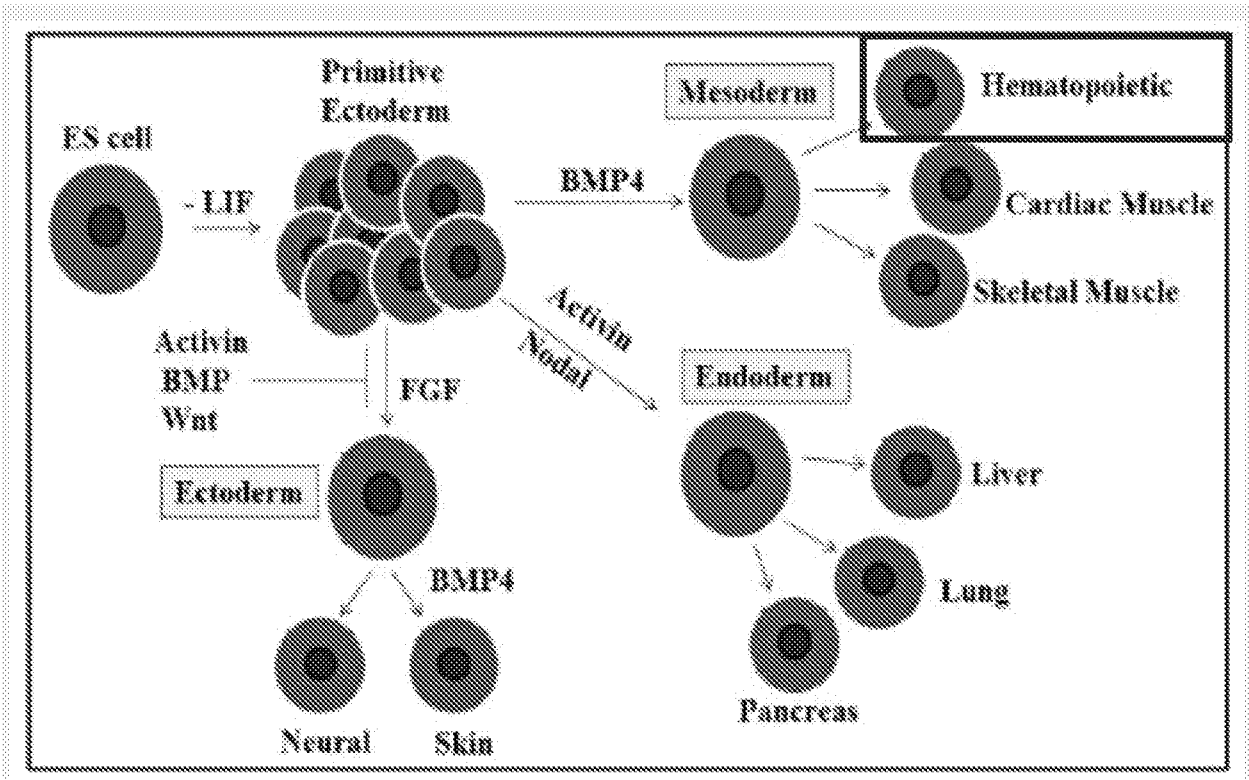
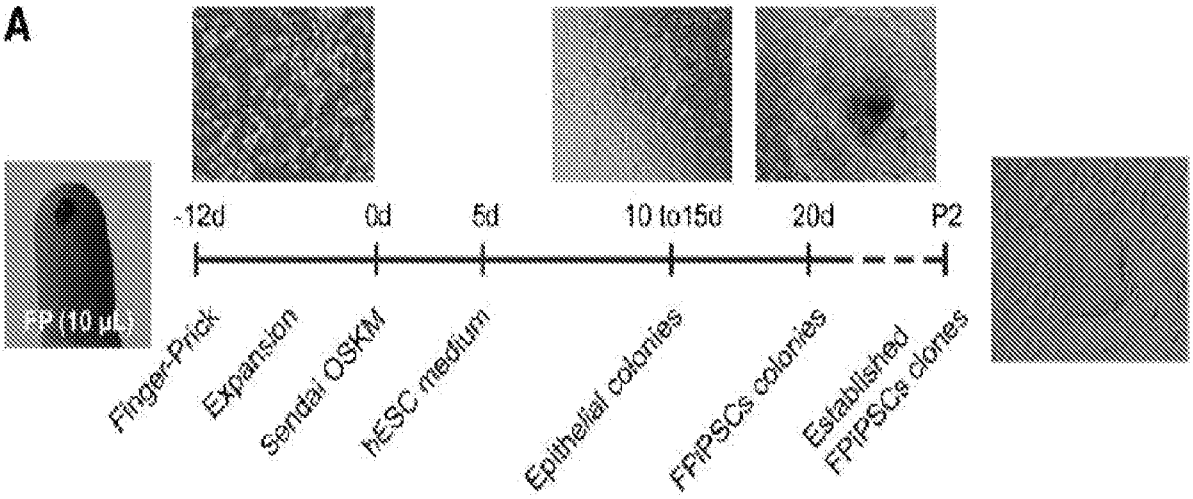


FIG. 20

A



B

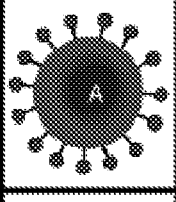
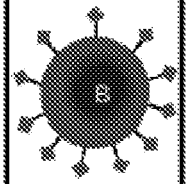
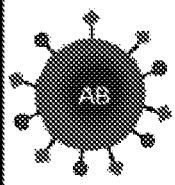
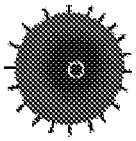
	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	 A and B antigens	None

FIG. 21

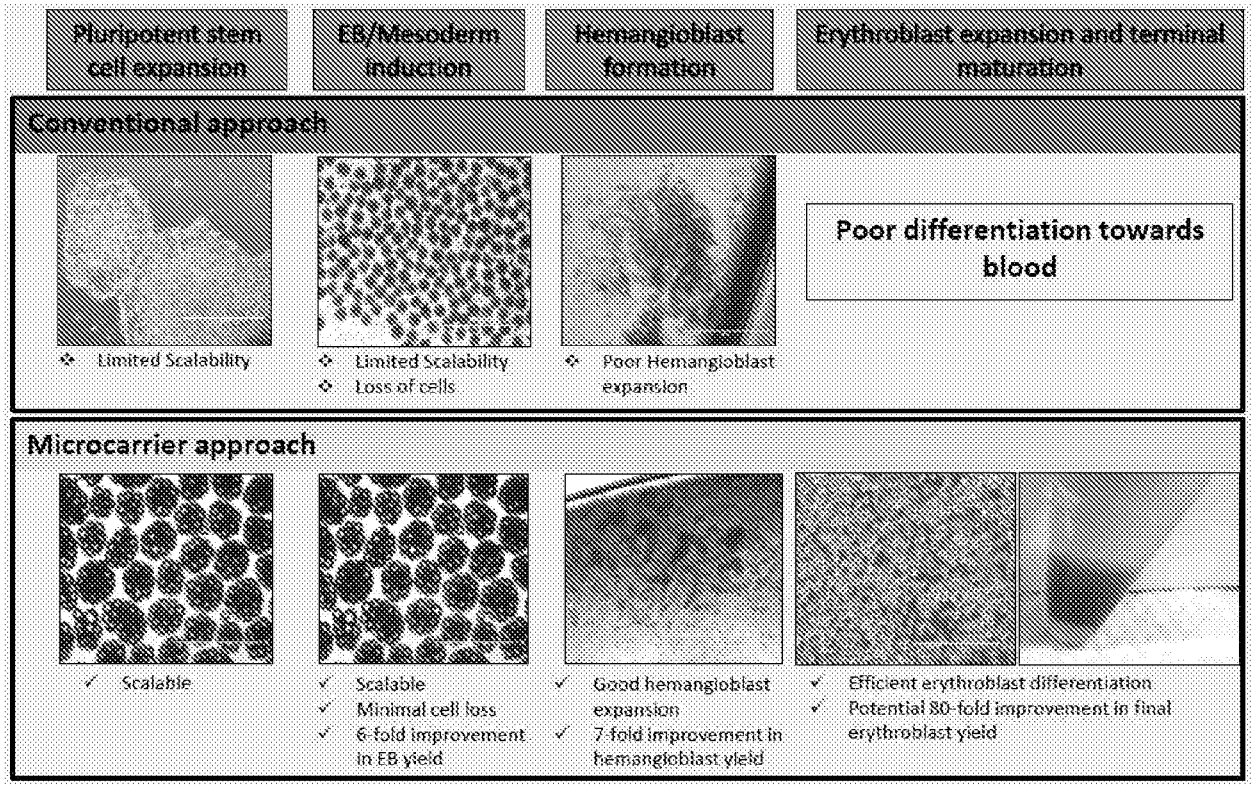

Pluripotent stem cell expansion
EB/Mesoderm induction
Hemangioblast formation
Erythroblast expansion and terminal maturation
Conventional approach
Poor differentiation towards blood
Limited Scalability
Limited Scalability
Loss of cells
Poor Hemangioblast expansion
Microcarrier approach
Scalable
Scalable
Minimal cell loss
6-fold improvement in EB yield
Good hemangioblast expansion
7-fold improvement in hemangioblast yield
Efficient erythroblast differentiation
Potential 80-fold improvement in final erythroblast yield

FIG. 22

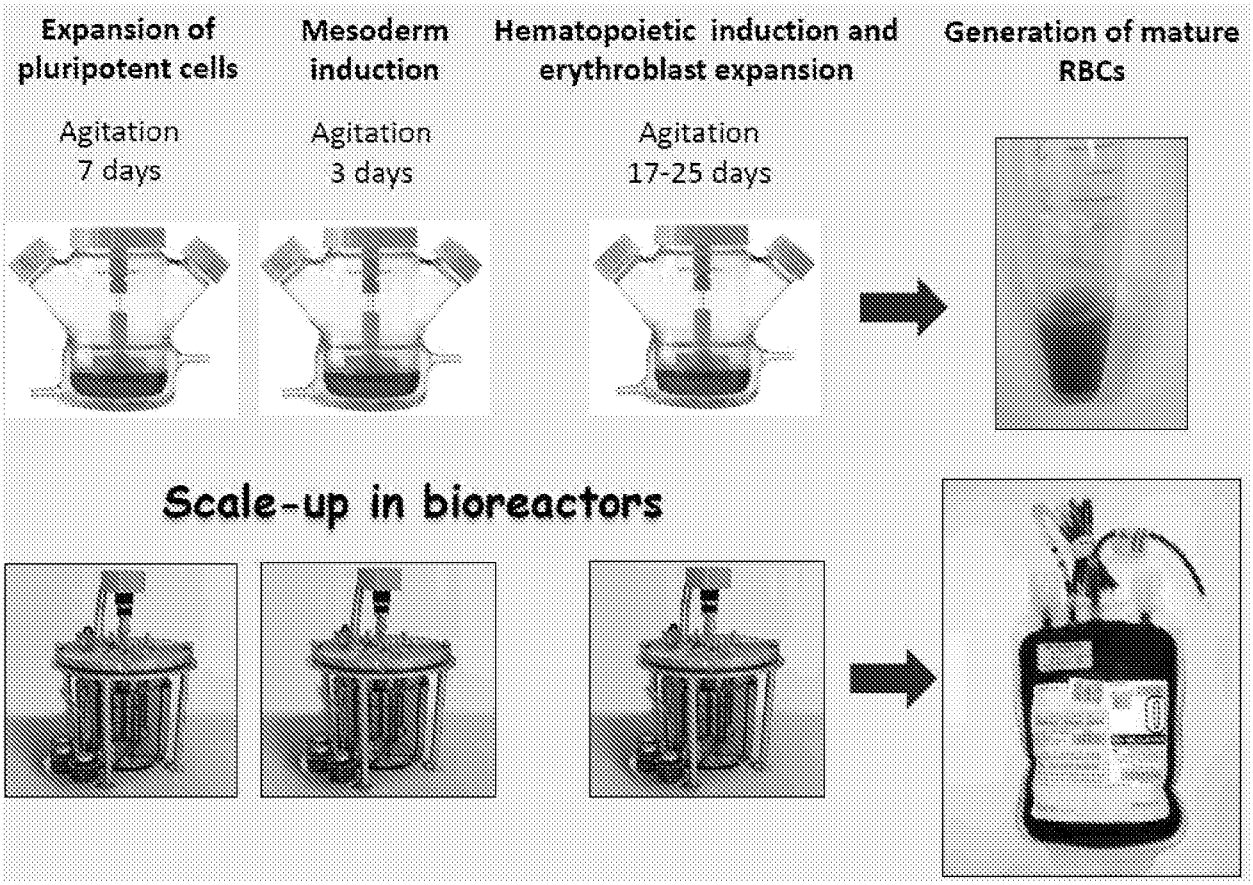

Expansion of pluripotent cells
Agitation 7 days
Mesoderm induction
Agitation 3 days
Hematopoietic induction and erythroblast expansion
Agitation 17-25 days
Generation of mature RBCs
Scale-up in bioreactors

FIG. 23

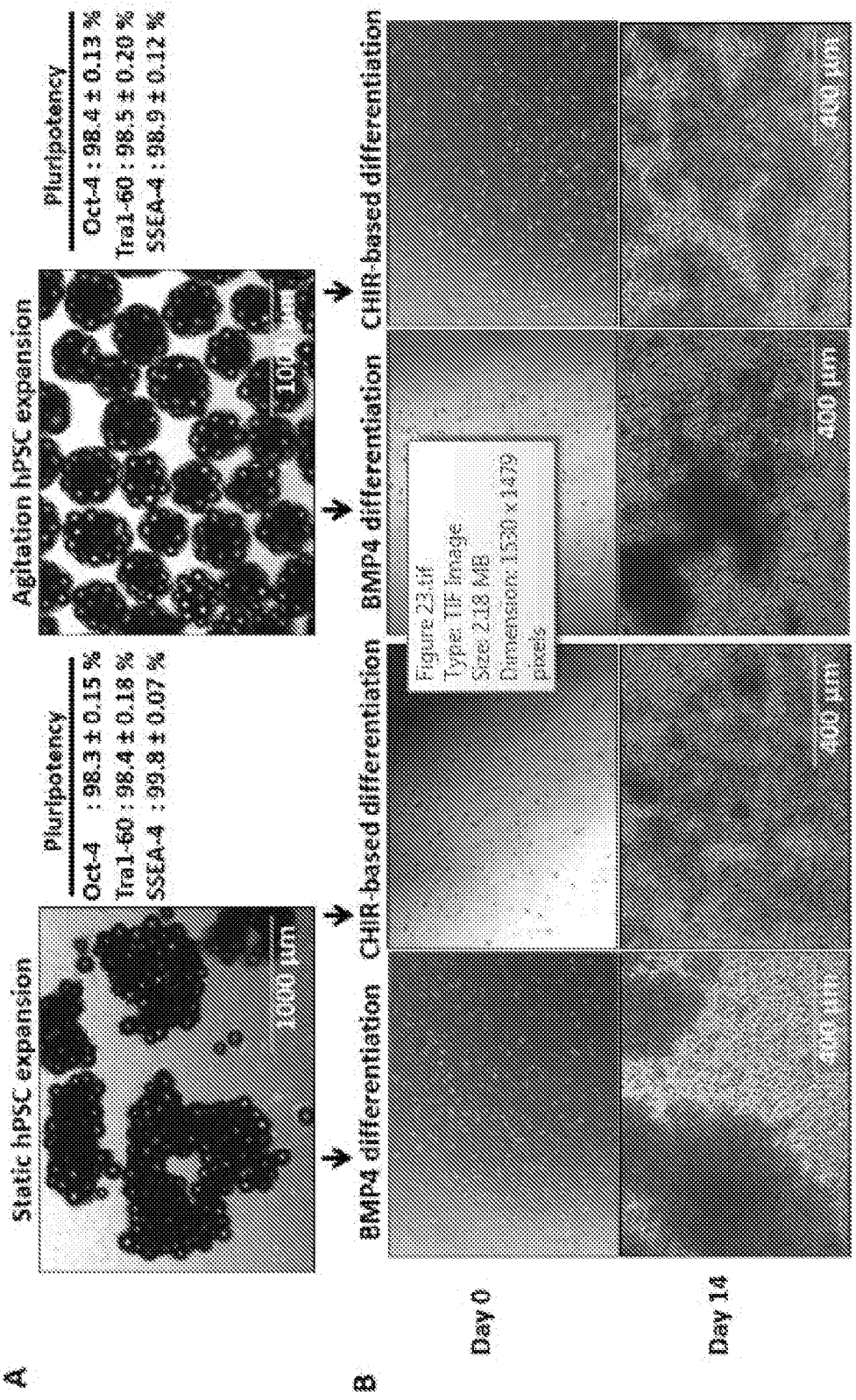
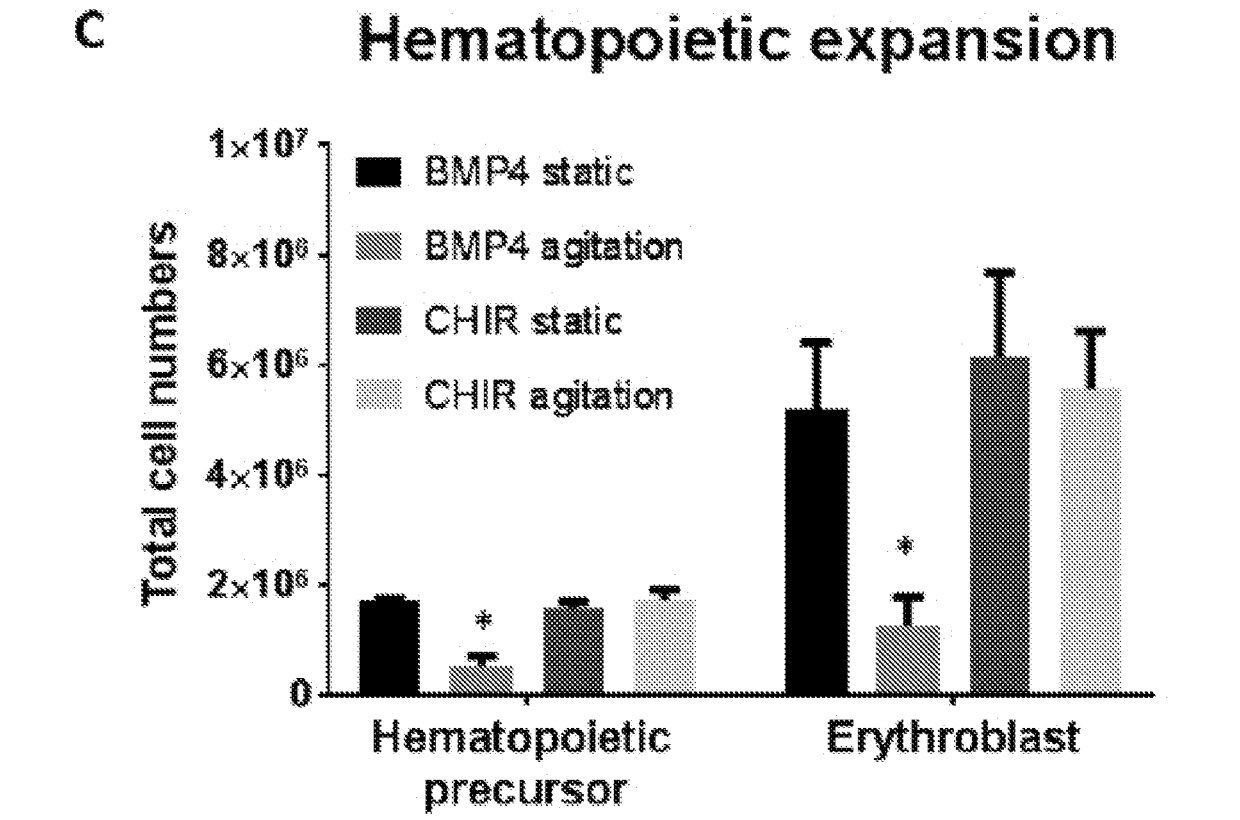

A
Static hPSC expansion
Pluripotency
Oct-4 : 98.3 ± 0.15 %
Tra1-60 : 98.4 ± 0.18 %
SSEA-4 : 99.8 ± 0.07 %
1000 µm
Agitation hPSC expansion
Pluripotency
Oct-4 : 98.4 ± 0.13 %
Tra1-60 : 98.5 ± 0.20 %
SSEA-4 : 98.9 ± 0.12 %
1000 µm
B
BMP4 differentiation
CHIR-based differentiation
BMP4 differentiation
CHIR-based differentiation
Figure 23.tif
Type: TIF Image
Size: 2.18 MB
Dimension: 1530 x 1479 pixels
Day 0
Day 14
400 µm
400 µm
400 µm
400 µm

FIG. 23 continued



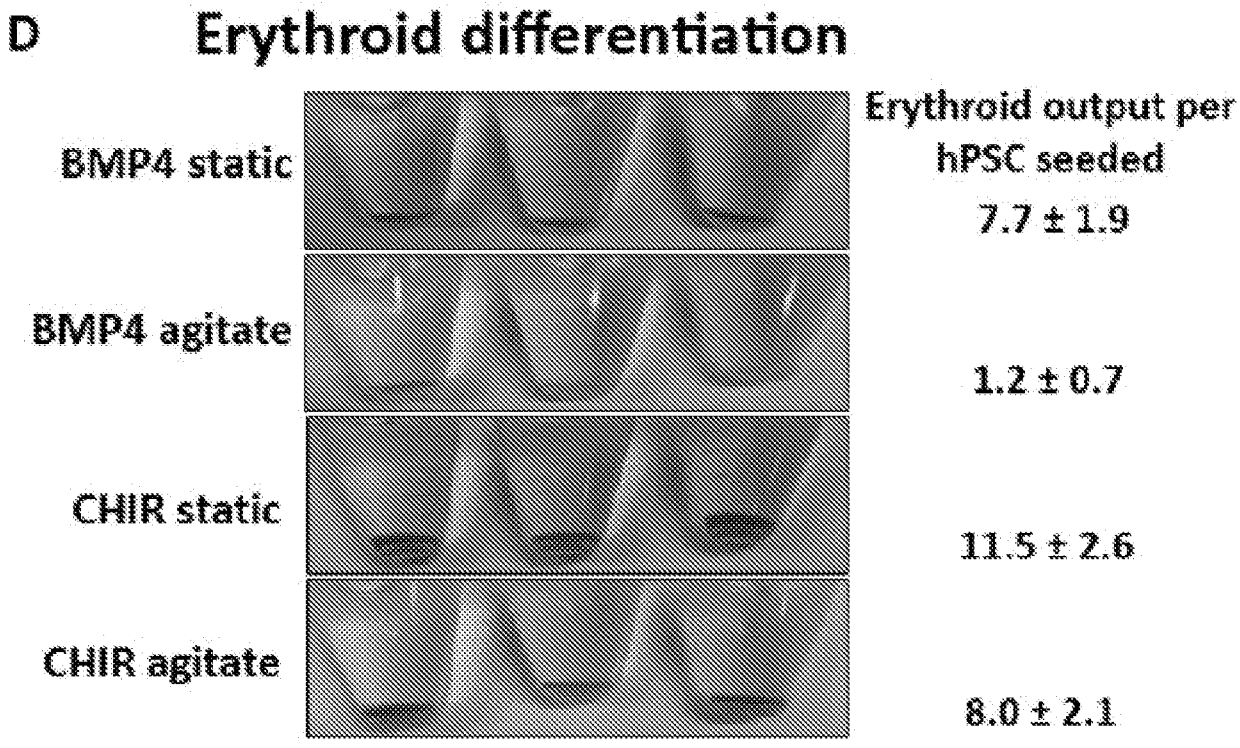


FIG. 24

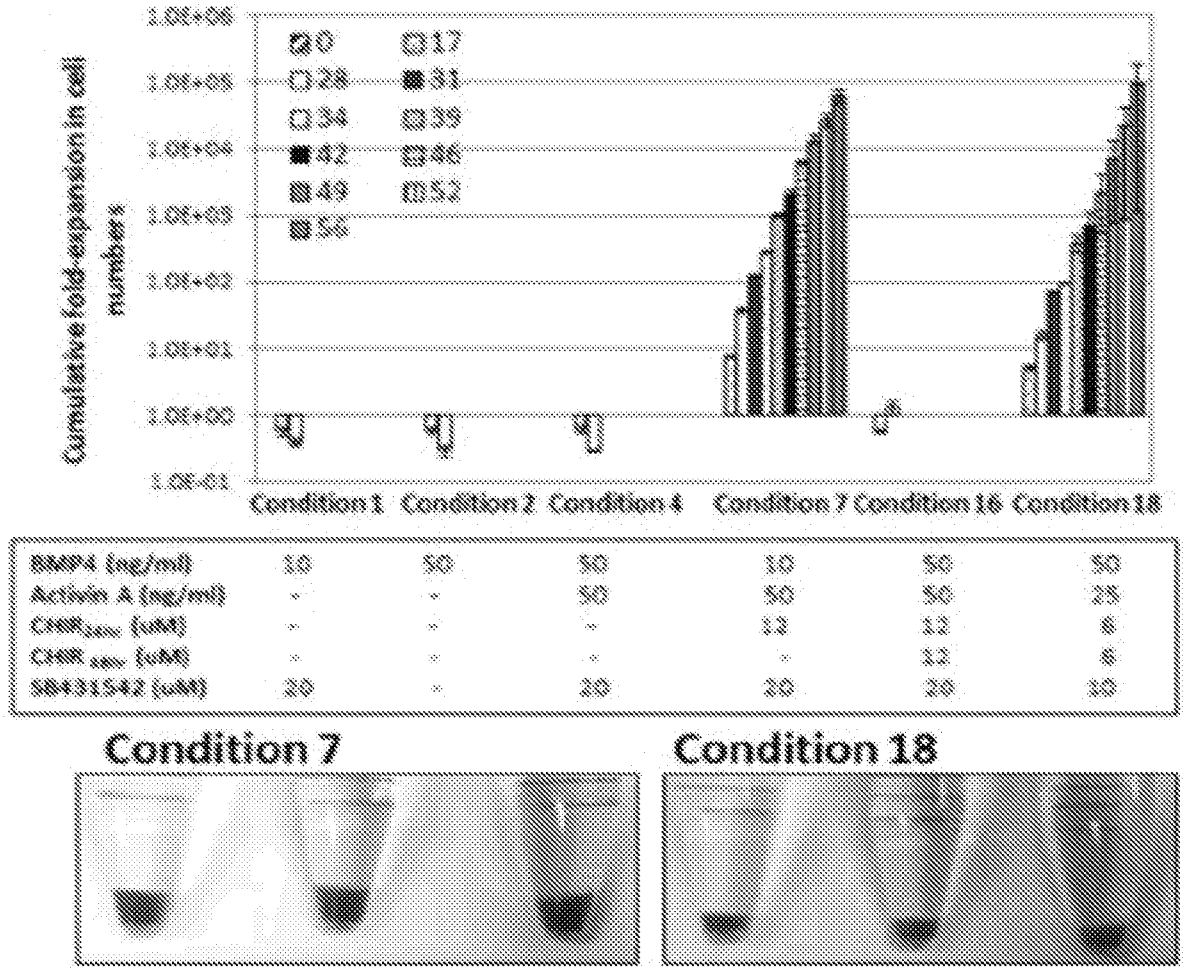


FIG. 25

A

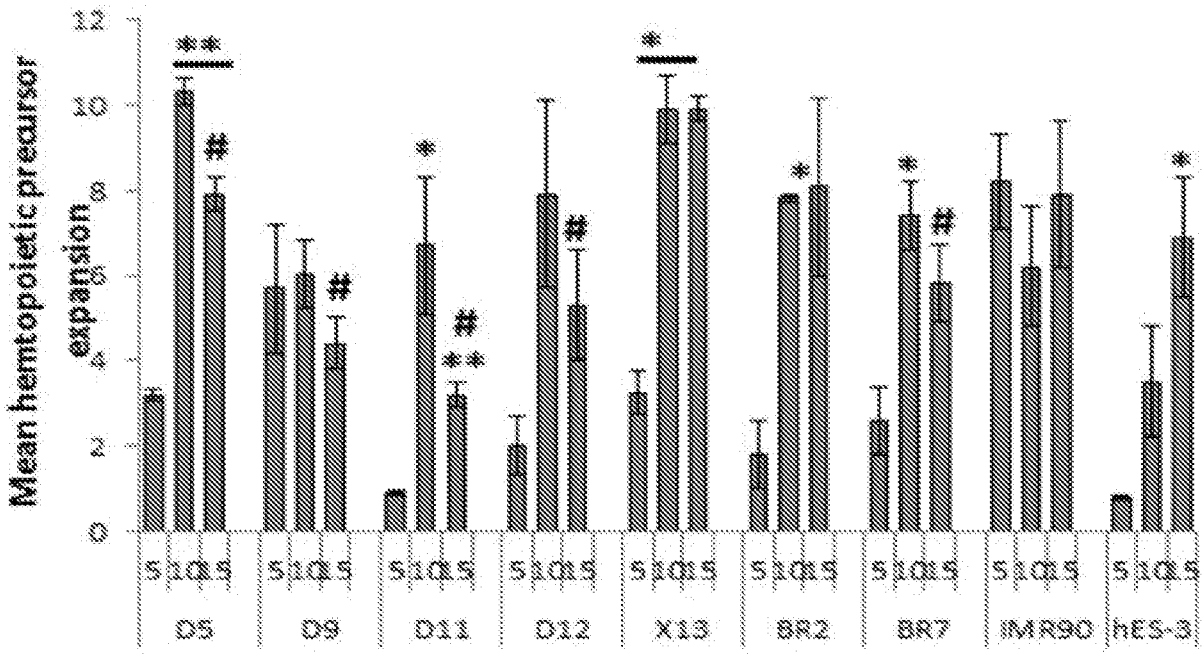


FIG. 25 continued

B

	Erythroid cells (% CD235a)	HbF (%)	Cumulative fold expansion (Day 42)
D5	74.6 ± 6.8	99.2 ± 0.1	3712 ± 1651
D9	57.5 ± 4.6	97.8 ± 0.3	121 ± 37
D11^	33.9 ± 13.6	53.6 ± 14.4	N.A
D12^	36.1 ± 4.2	58.5 ± 11.3	N.A
X13	67.4 ± 3.8	96.9 ± 1.4	12605 ± 2125
BR2^	37.1 ± 3.5	48.9 ± 8.0	N.A
BR7	33.8 ± 3.6	95.5 ± 1.5	31 ± 7.7
IMR90	75.9 ± 1.8	98.1 ± 0.5	913 ± 342
hES-3	78.5 ± 3.0	99.3 ± 0.1	324 ± 84

FIG. 26

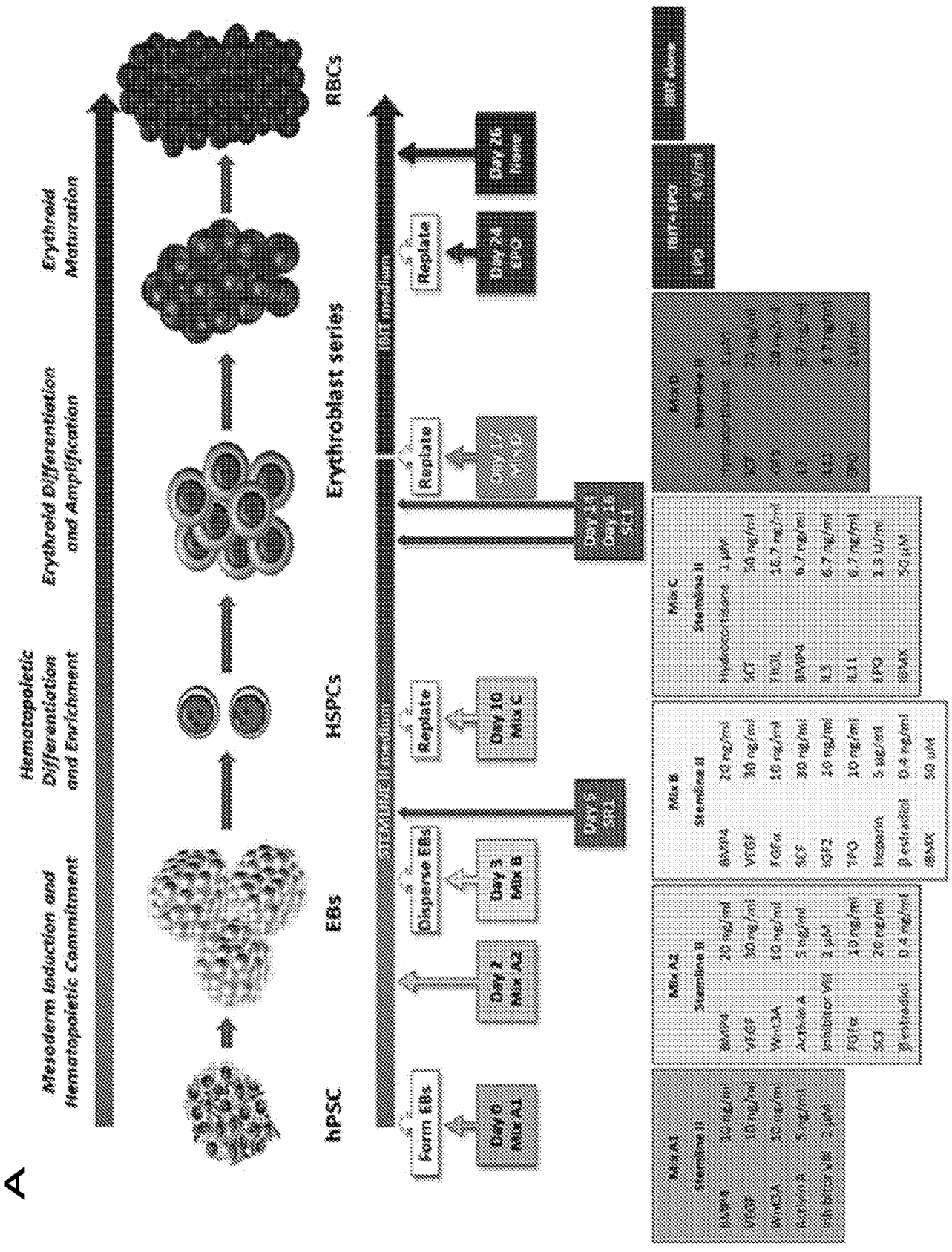

A
Mesoderm Induction and Hematopoietic Commitment
Hematopoietic Differentiation and Enrichment
Erythroid Differentiation and Amplification
Erythroid Maturation
hPSC
EBs
HSPCs
Erythroblast series
RBCs
Form EBs
Day 0 Mix A1
Day 2 Mix A2
Disperse EBs
Day 3 Mix B
Replate
Day 10 Mix C
Day 17 Mix D
Day 21 EPO
Day 24 Hemo
Mix A1
Mix A2
Mix B
Mix C
Mix D
Stemline II
BMP4
VEGF
Wnt3A
Activin A
Inhibitor VIII
FGFa
SCF
β-estradiol
TPO
Heparin
IGF2
IL3
IL11
EPO
IBMX
Hydrocortisone
SCF
FASL
BMP4
Transferrin
Insulin
Dexamethasone
Rifampicin
Doxycycline
Puromycin
Blasticidin
Hygromycin
Ganciclovir
Cell culture

FIG. 26 continued

B

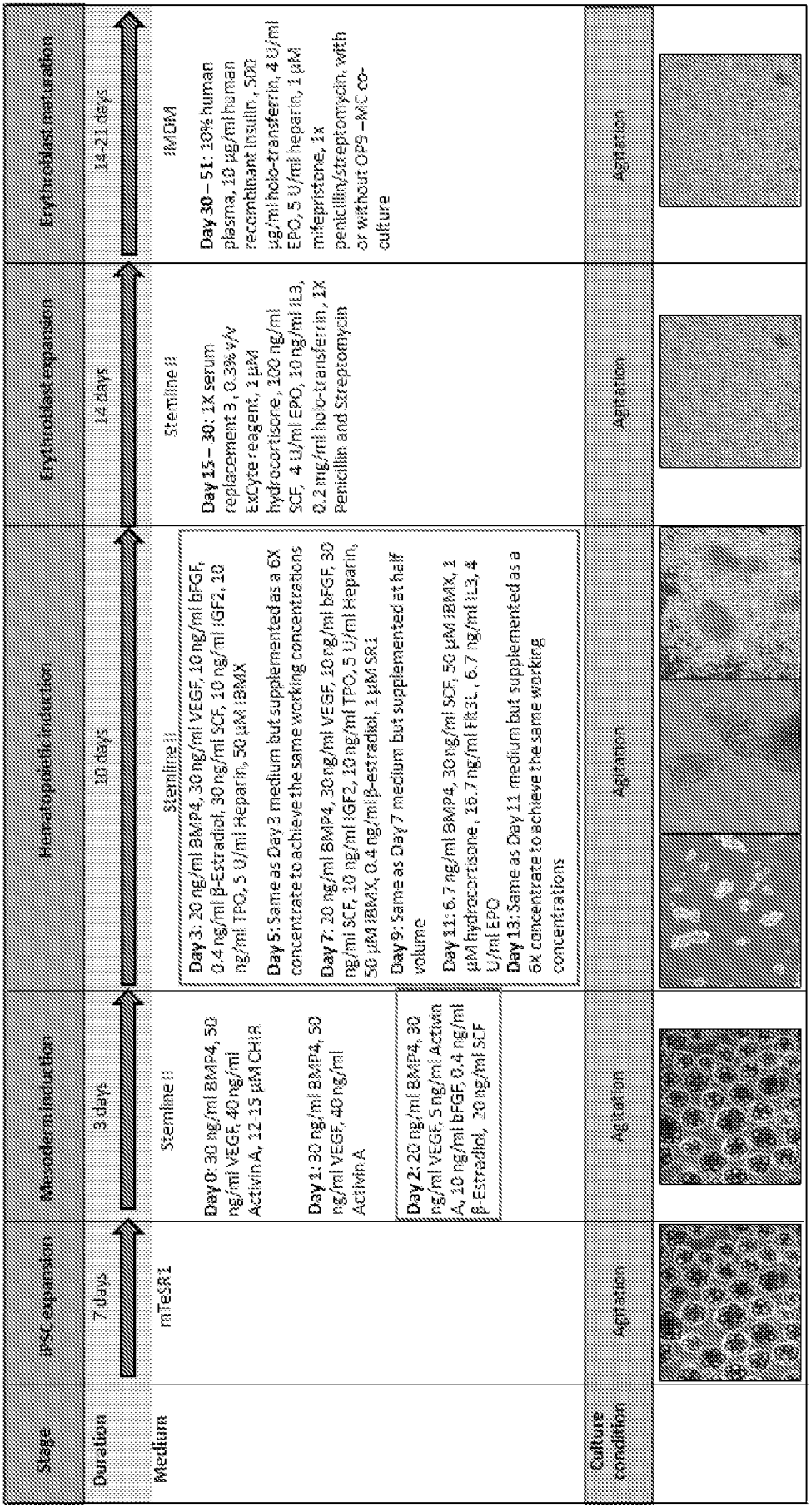


FIG. 27

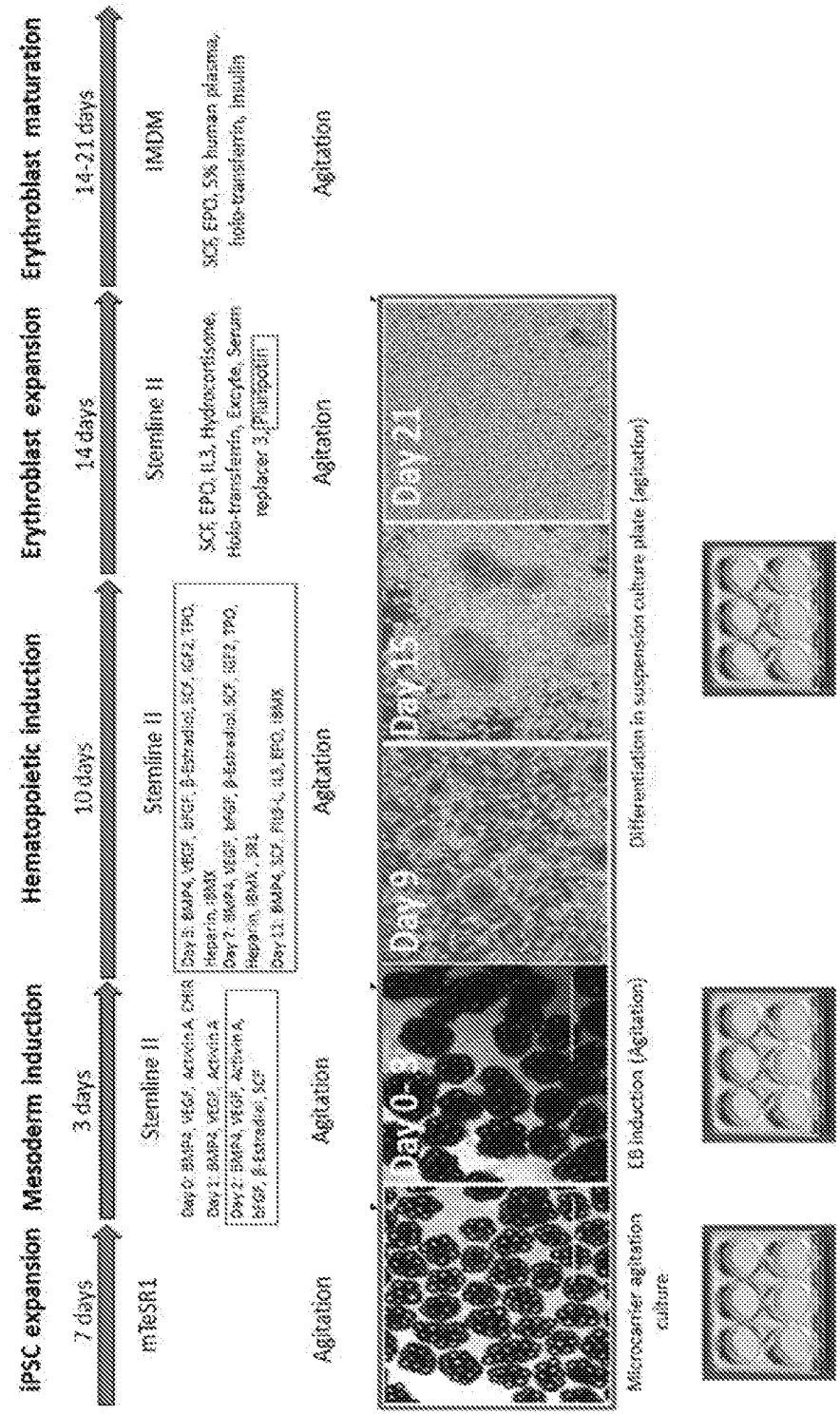
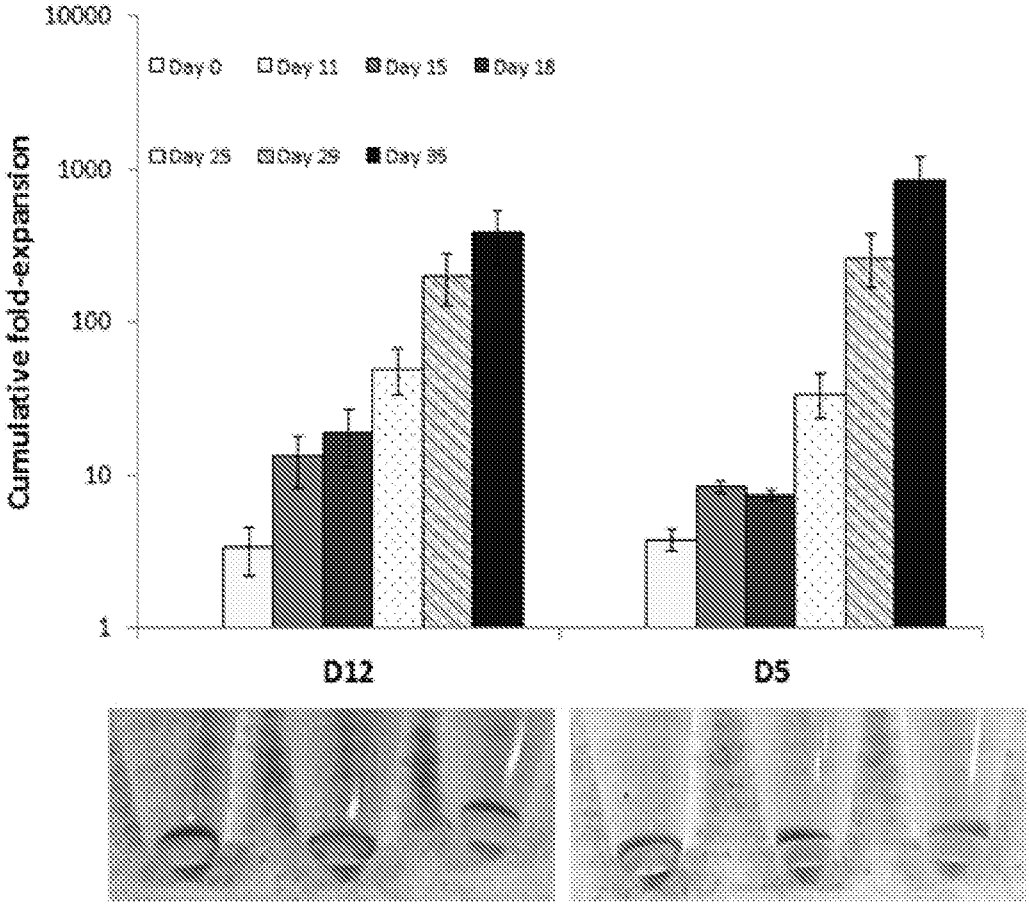

A
Stage
iPSC expansion
Mesoderm induction
Hematopoietic induction
Erythroblast expansion
Erythroblast maturation
Duration
7 days
3 days
10 days
14 days
14-21 days
Medium
mTeSR1
Stemline II
Stemline II
Stemline II
IMDM
Day0: BMP4, VEGF, Activin A, CHIR
Day1: BMP4, VEGF, Activin A
Day2: BMP4, VEGF, Activin A, bFGF, β-Estradiol, SCF
Day3: BMP4, VEGF, bFGF, β-Estradiol, SCF, IGF2, TPO, Heparin, IBMX
Day7: BMP4, VEGF, bFGF, β-Estradiol, SCF, IGF2, TPO, Heparin, IBMX, 3I4
Day11: BMP4, SCF, FGF-1, IL3, EPO, IBMX
SCF, EPO, IL3, Hydrocortisone, Holo-transferrin, Excyte, Serum replacer 3, Pluronic
SCF, EPO, 5% human plasma, holo-transferrin, insulin
Culture condition
Agitation
Agitation
Agitation
Agitation
Agitation
Day0
Day3
Day11
Day21
Microcarrier agitation culture
EB induction (Agitation)
Differentiation in suspension culture plate (agitation)

FIG. 27 continued

B



C

	D12 agitate	D5 agitate
CD235a+ CD71+	55.2 % ± 2.56	48.9 % ± 3.13
CD235a+ CD71-	8.18 % ± 0.63	6.39 % ± 0.30
CD235a+ CD36+	57.1 % ± 2.33	50.1 % ± 3.27
CD235a+CD36-	9.86 % ± 0.86	4.81 % ± 0.07
Total CD235a	63.4 % ± 2.22	55.3 % ± 2.84
HbF	93.6 % ± 0.67	76.3 % ± 4.63
HbA	56.8 % ± 1.14	11.78 % ± 1.81
CD14	0.02 % ± 0.004	0.007 % ± 0.017
CD15	0.05 % ± 0.02	0.02 % ± 0.05
CD133	0.06 % ± 0.02	0.89 % ± 0.007
CXCR4	0.14 % ± 0.058	1.10 % ± 0.20
Cumulative fold-expansion (Day 35)	385 ± 152	835 ± 373

FIG. 28

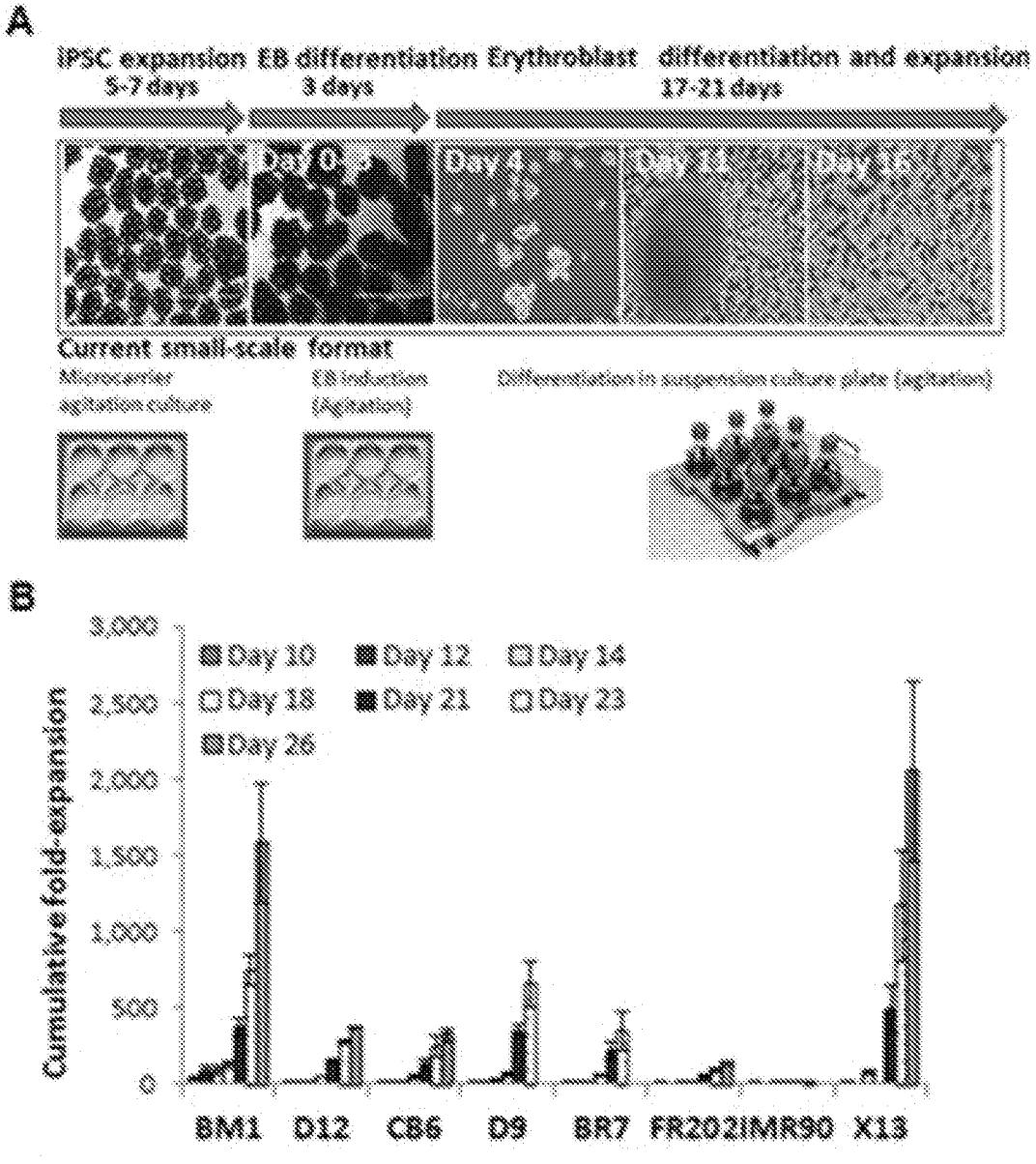
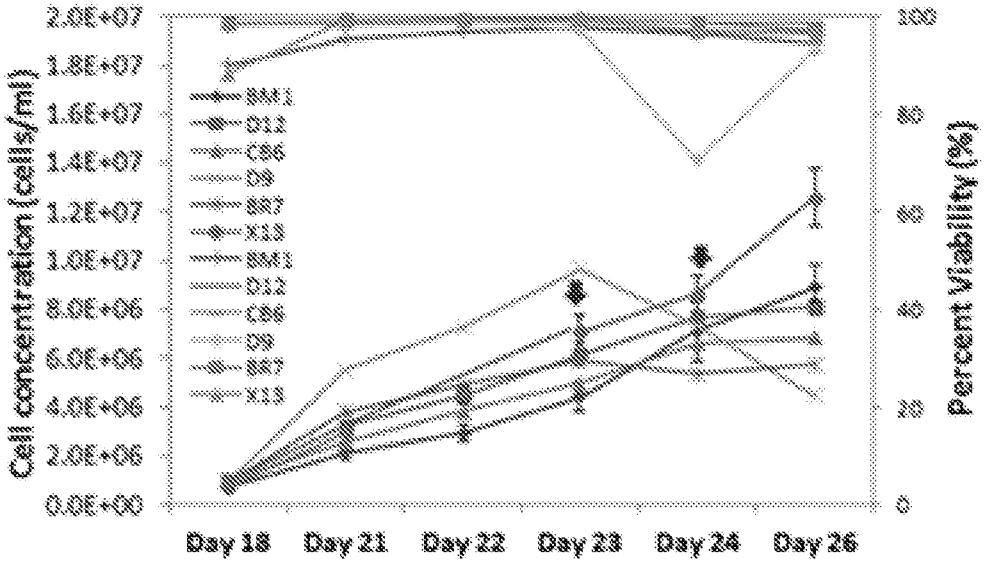

A
iPSC expansion
5-7 days
EB differentiation
3 days
Erythroblast differentiation and expansion
17-21 days
Day 0
Day 4
Day 11
Day 16
Current small-scale format
Microcarrier agitation culture
EB induction (Agitation)
Differentiation in suspension culture plate (agitation)
B
Cumulative fold-expansion
3,000
2,500
2,000
1,500
1,000
500
0
Day 10
Day 12
Day 14
Day 18
Day 21
Day 23
Day 26
BM1
D12
CB6
D9
BR7
FR202
IMR90
X13
C
BM1
D12
IMR90
D9
BR7
X13
CB6
FR202

FIG. 28 continued

D



E

	Glucose (g/L)	Lactate (g/L)	Ammonia (mM)
Media	3.61 ± 0.03	0.41 ± 0.02	0
D9	1.85 ± 0.03	2.49 ± 0.02	4.65 ± 0.10
D12	3.92 ± 0.01	0.97 ± 0.13	3.92 ± 0.01
BM1	3.94 ± 0.19	1.18 ± 0.06	2.47 ± 0.15
C86	3.6 ± 0.25	1.34 ± 0.14	3.28 ± 0.30
BR7	3.98 ± 0.20	1.02 ± 0.09	2.97 ± 0.25
X13	4.09 ± 0.10	0.96 ± 0.10	1.05 ± 0.12

FIG. 29

A

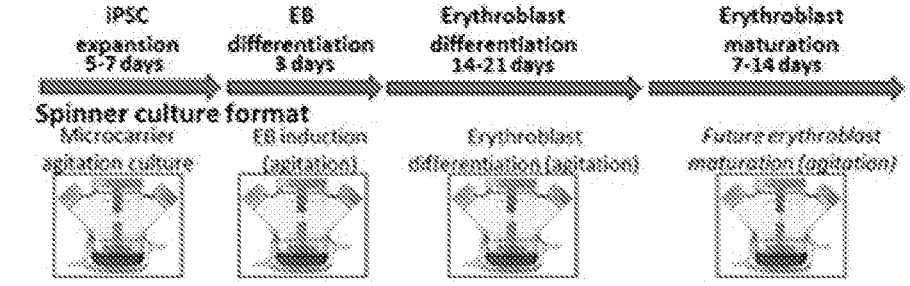
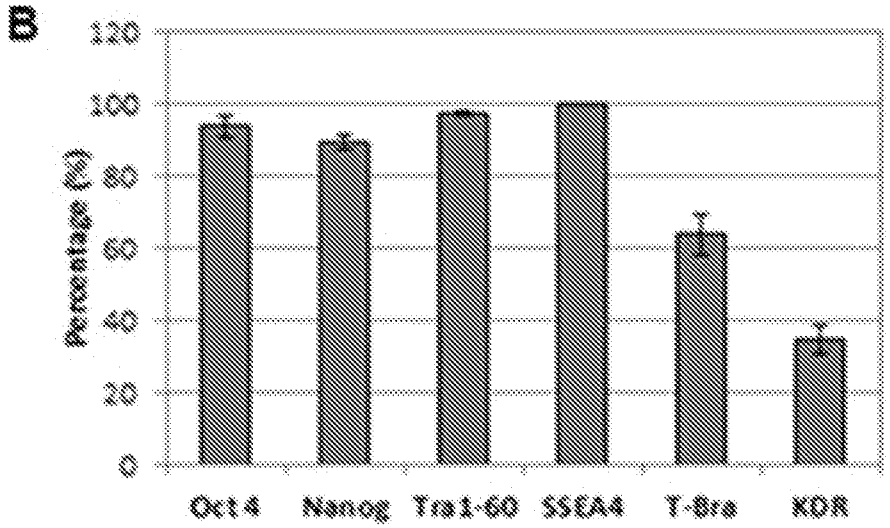
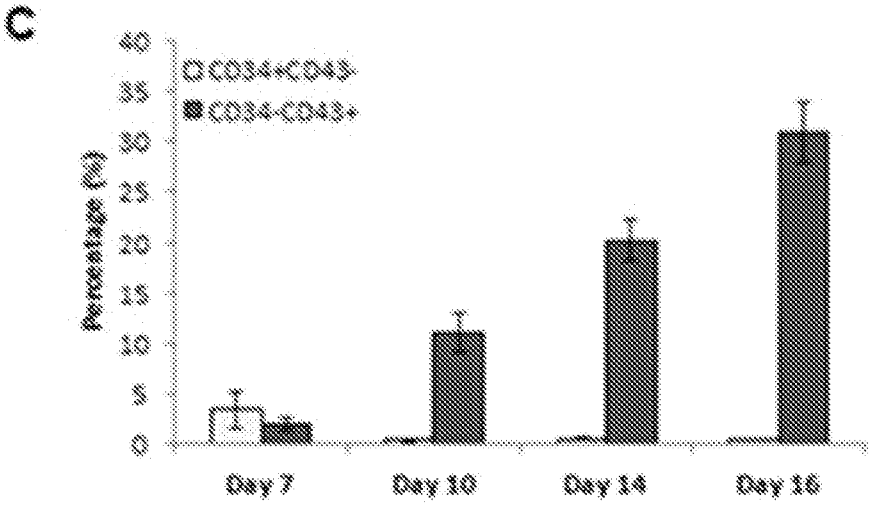


FIG. 29 continued





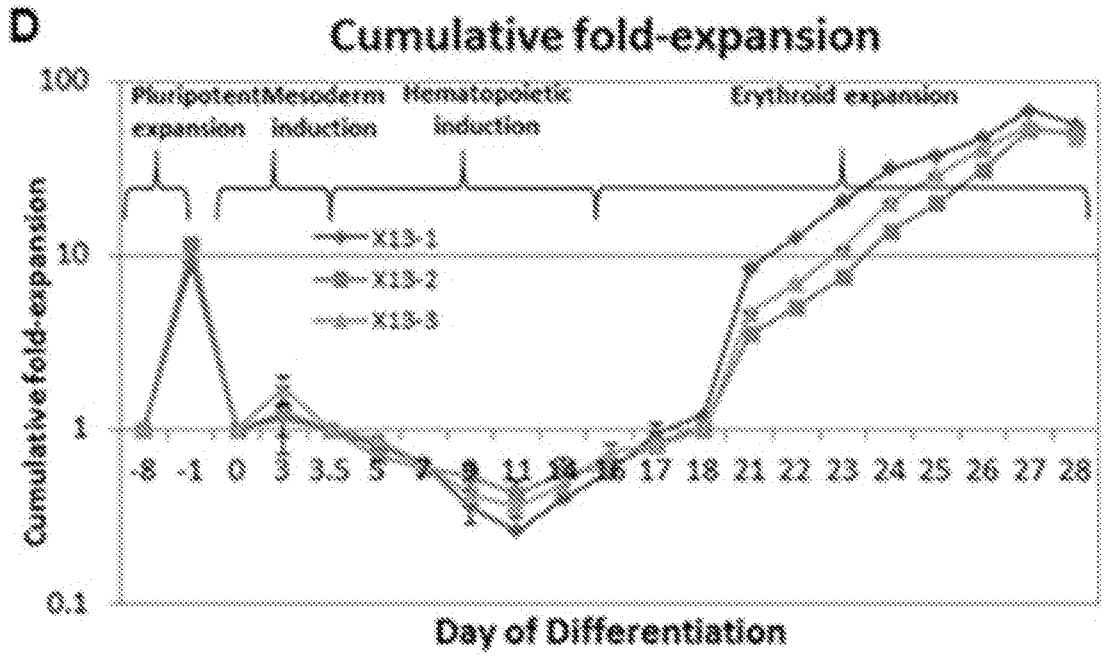
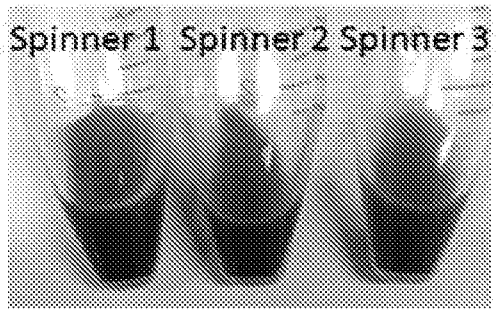


FIG. 29 continued

E



F

Day 27	Spinner 1	Spinner 2	Spinner 3
Concentration (cells/ml)	1.7×10^7 $\pm 5.2 \times 10^6$	1.32×10^7 $\pm 3.2 \times 10^6$	1.38×10^7 $\pm 3.2 \times 10^6$
Total cells/ 50 ml	8.5×10^8 $\pm 1.5 \times 10^7$	6.6×10^8 $\pm 1.6 \times 10^7$	6.9×10^8 $\pm 1.6 \times 10^7$
Total media used (ml)	575	575	575
Lactate production (g/L/per 24hr/8.5e8 cells)	1.5	1.46	1.53
Ammonia production (mM/per 24 hr/ 8.5e8 cells)	1.32	1.17	1.55
Glucose consumption (g/L/per 24hr/8.5e8 cells)	1.47	0.94	1.072

G

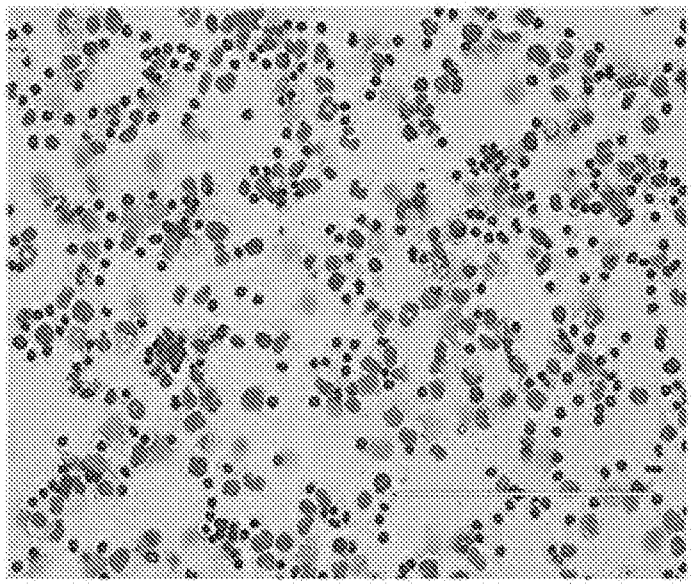


FIG. 30

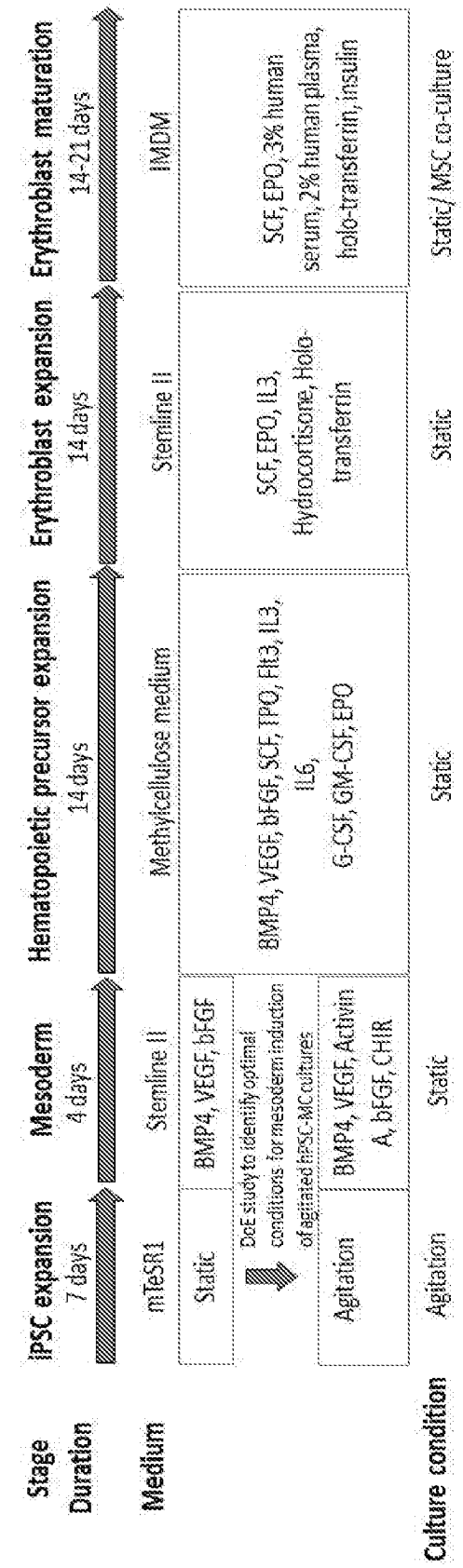

Stage
iPSC expansion
Mesoderm
Hematopoietic precursor expansion
Erythroblast expansion
Erythroblast maturation
Duration
7 days
4 days
14 days
14 days
14-21 days
Medium
mTeSR1
Stemline II
Methylcellulose medium
Stemline II
IMDM
Culture condition
Static
BMP4, VEGF, bFGF
DoE study to identify optimal conditions for mesoderm induction of agitated hPSC-MC cultures
BMP4, VEGF, bFGF, SCF, TPO, Flt3, IL3, IL6, G-CSF, GM-CSF, EPO
SCF, EPO, IL3, Hydrocortisone, Holo-transferrin
SCF, EPO, 3% human serum, 2% human plasma, holo-transferrin, insulin
Agitation
BMP4, VEGF, Activin A, bFGF, CHIR
Agitation
Static
Static
Static
Static/ MSC co-culture

FIG. 31

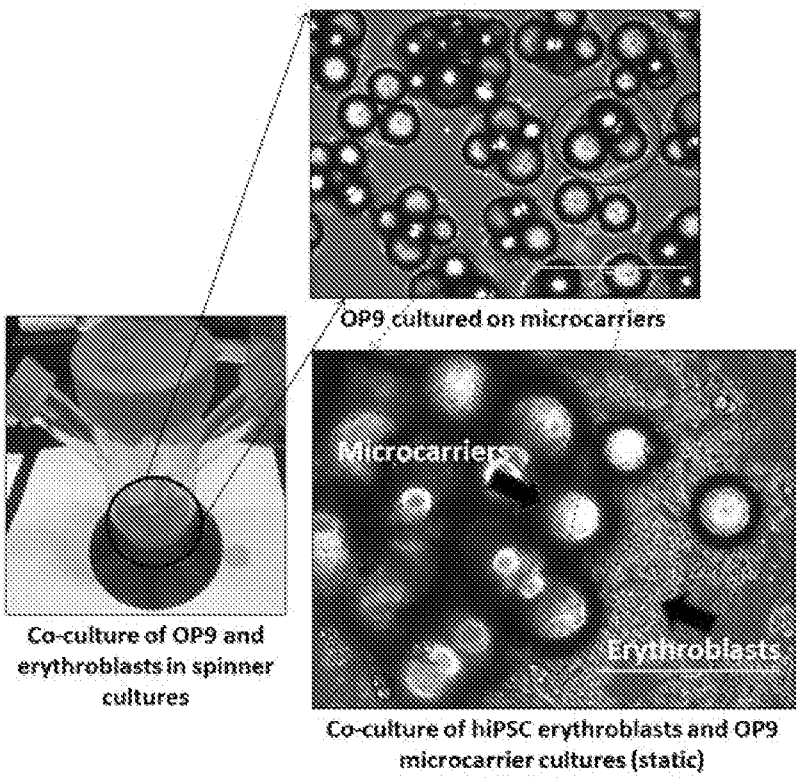

OP9 cultured on microcarriers
Microcarriers
Erythroblasts
Co-culture of OP9 and erythroblasts in spinner cultures
Co-culture of hiPSC erythroblasts and OP9 microcarrier cultures (static)

FIG. 32

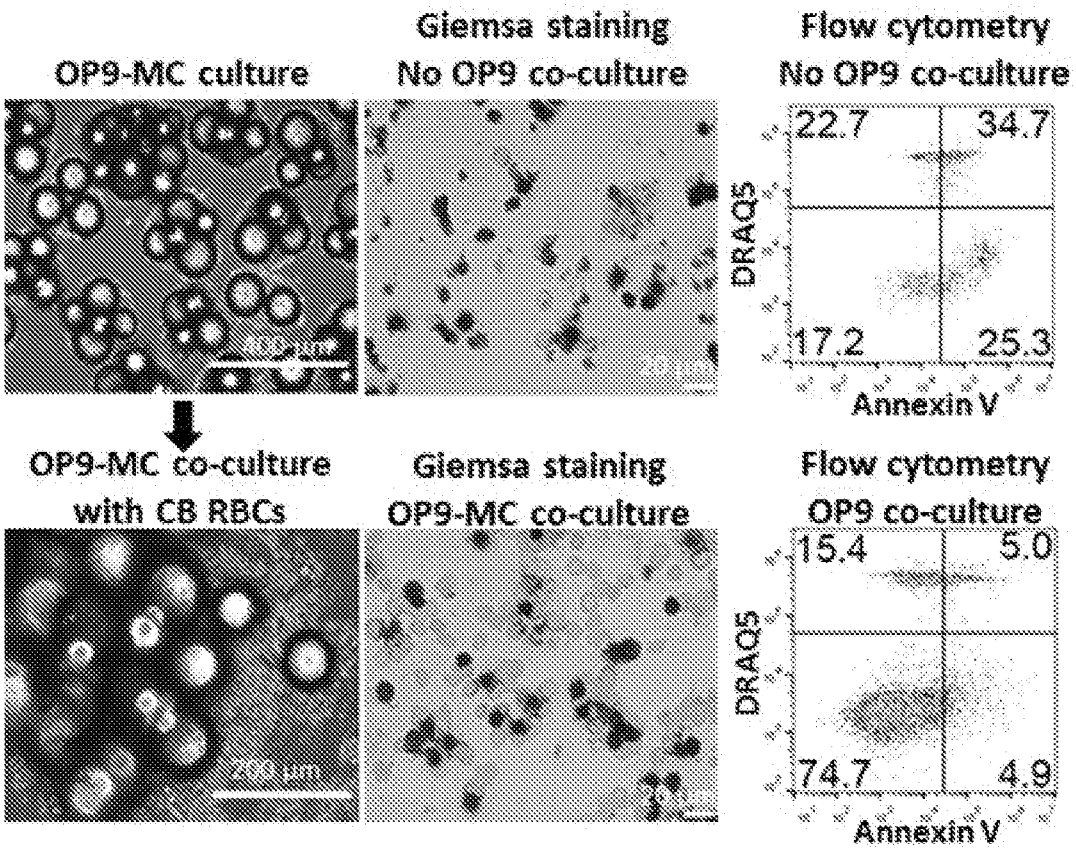

OP9-MC culture
Giemsa staining
No OP9 co-culture
Flow cytometry
No OP9 co-culture
22.7
34.7
17.2
25.3
DRAQ5
Annexin V
OP9-MC co-culture with CB RBCs
Giemsa staining
OP9-MC co-culture
Flow cytometry
OP9 co-culture
15.4
5.0
74.7
4.9
DRAQ5
Annexin V

FIG. 33

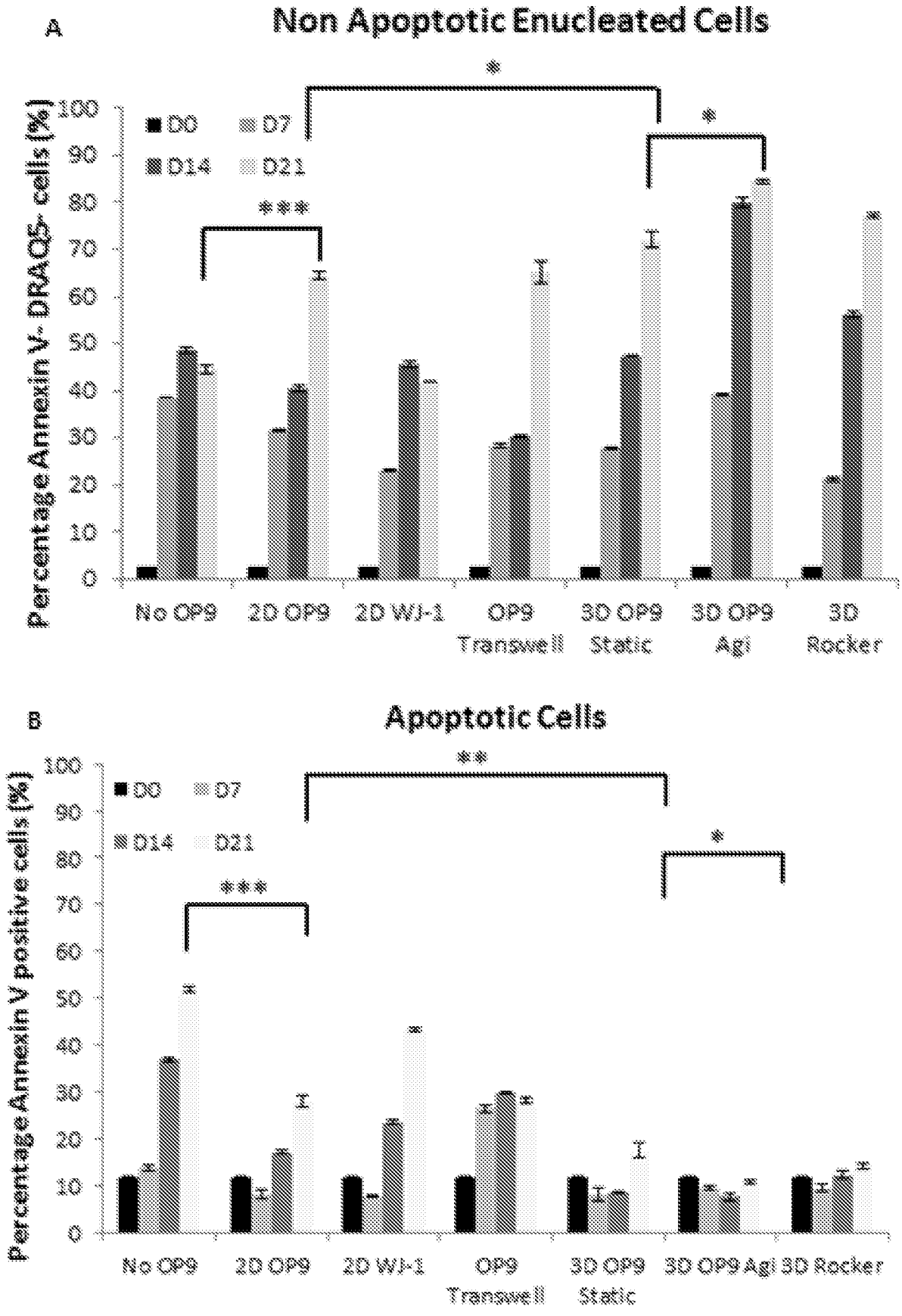

A
Non Apoptotic Enucleated Cells
Percentage Annexin V- DRAQ5- cells (%)
D0
D7
D14
D21
*
*
***
No OP9
2D OP9
2D WJ-1
OP9 Transwell
3D OP9 Static
3D OP9 Agi
3D Rocker
B
Apoptotic Cells
Percentage Annexin V positive cells (%)
**
*
***
No OP9
2D OP9
2D WJ-1
OP9 Transwell
3D OP9 Static
3D OP9 Agi
3D Rocker

FIG. 34

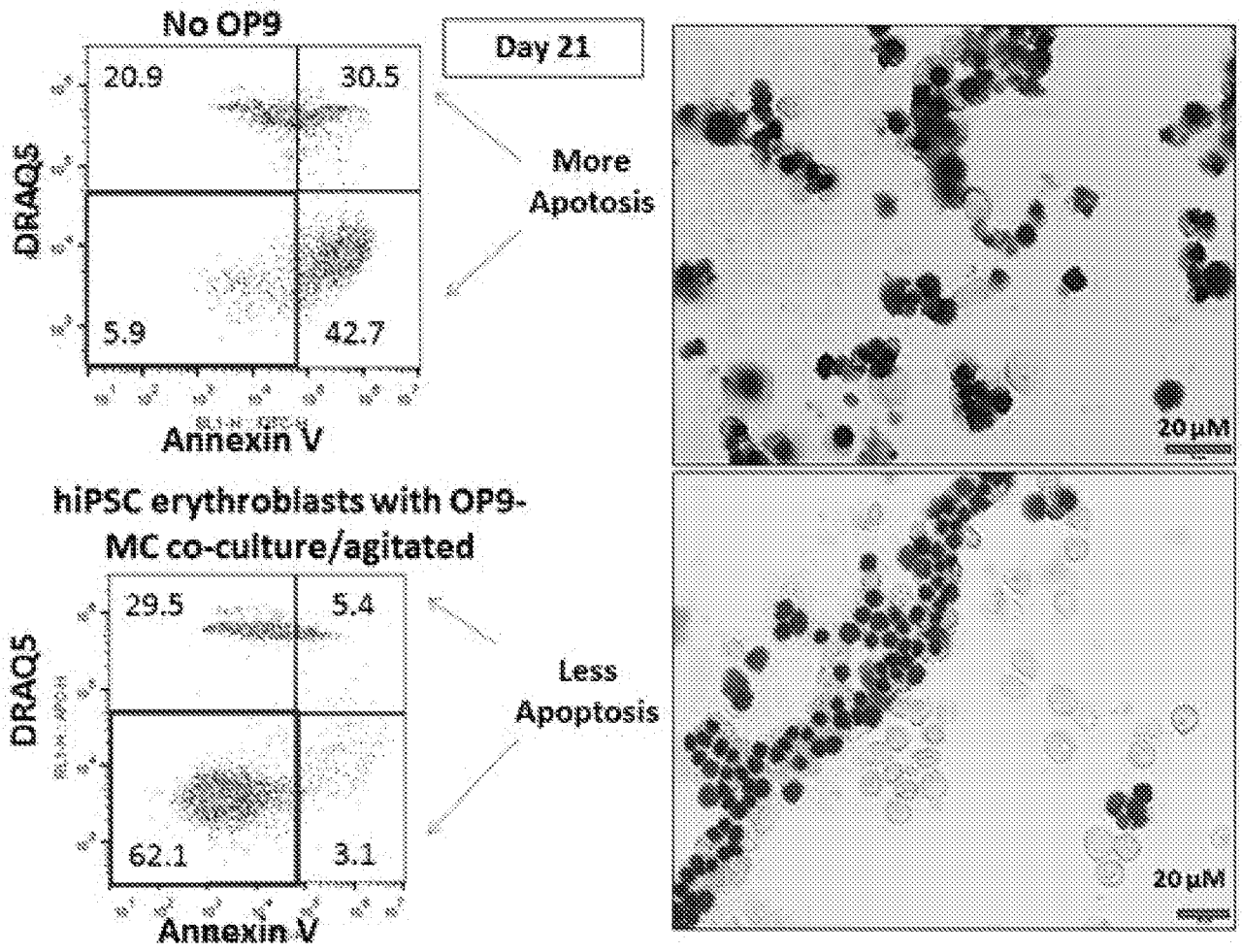

No OP9
Day 21
20.9
30.5
5.9
42.7
DRAQ5
Annexin V
More Apotosis
20 µM
hiPSC erythroblasts with OP9-MC co-culture/agitated
29.5
5.4
62.1
3.1
DRAQ5
Annexin V
Less Apoptosis
20 µM

FIG. 35

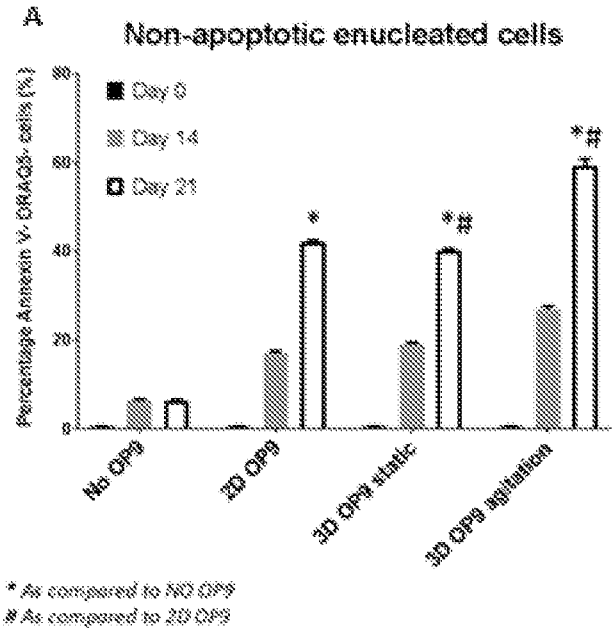

A
Non-apoptotic enucleated cells
Day 0
Day 14
Day 21
Percentage Annexin V- DRAQ5- cells (%)
No OP9
2D OP9
3D OP9 static
3D OP9 agitation
* As compared to NO OP9
# As compared to 2D OP9

FIG. 35 continued

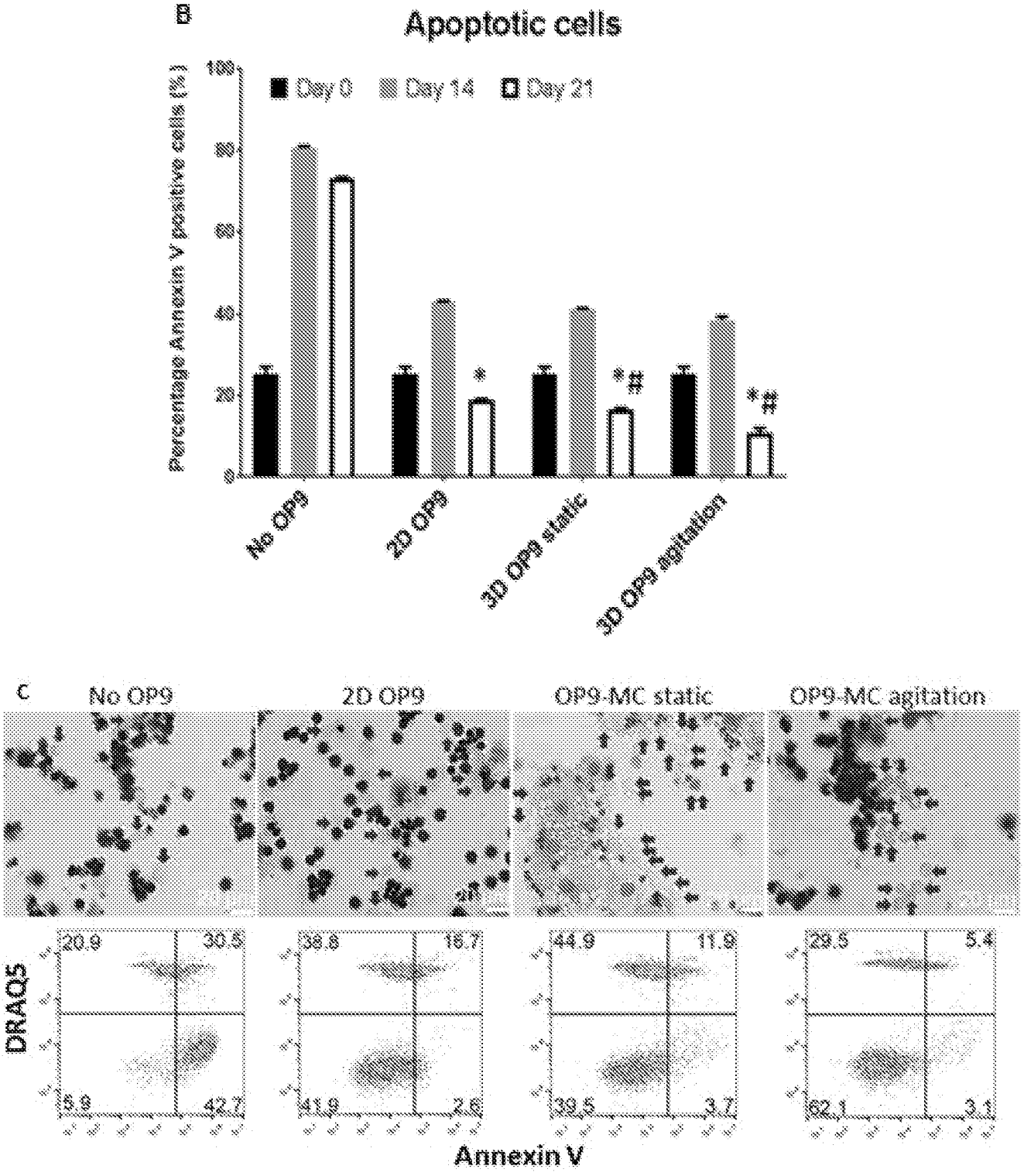


FIG. 36

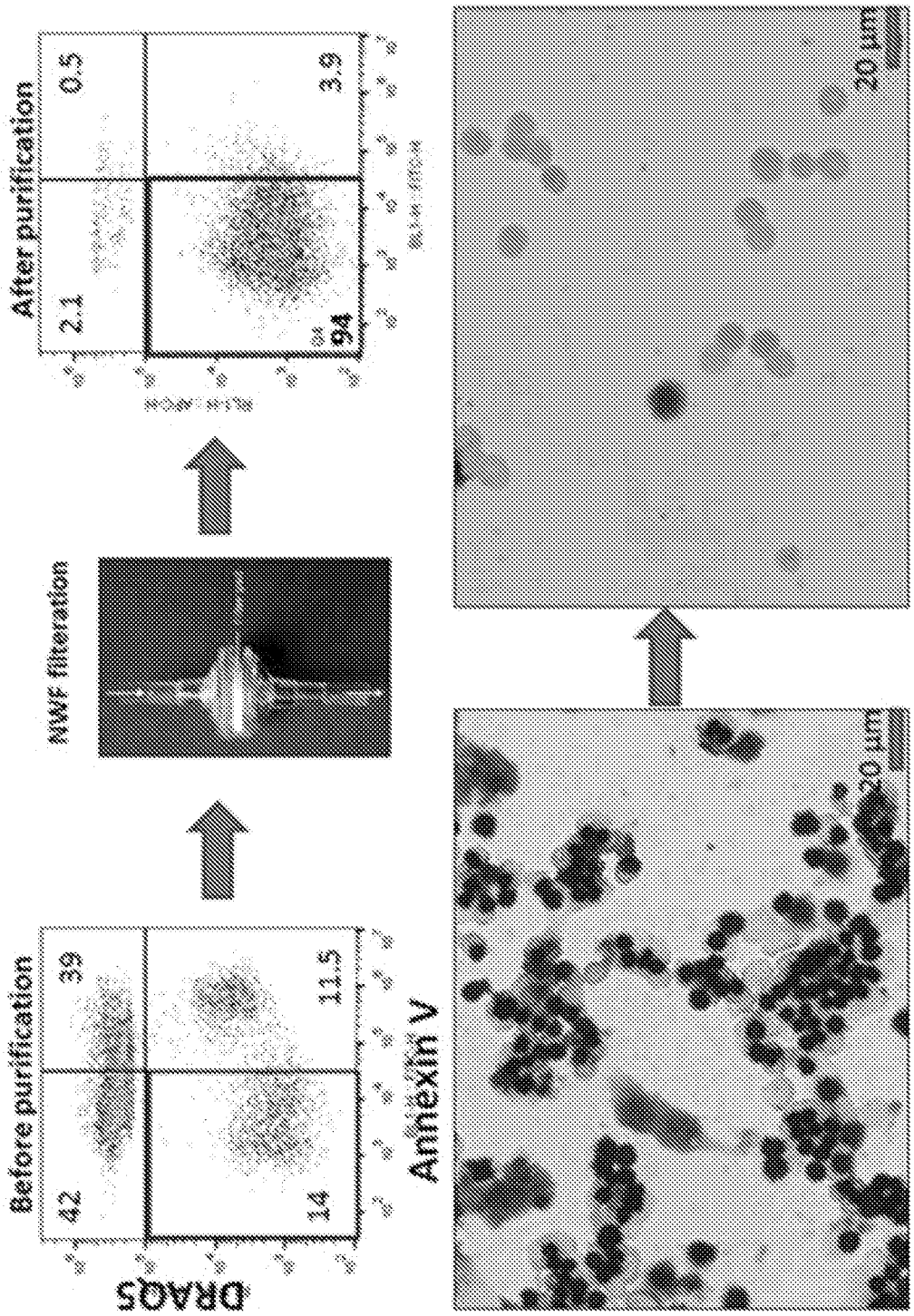

Before purification
42
39
14
11.5
DRAQ5
Annexin V
NWF filteration
After purification
2.1
0.5
94
3.9
20 μm
20 μm

FIG. 37

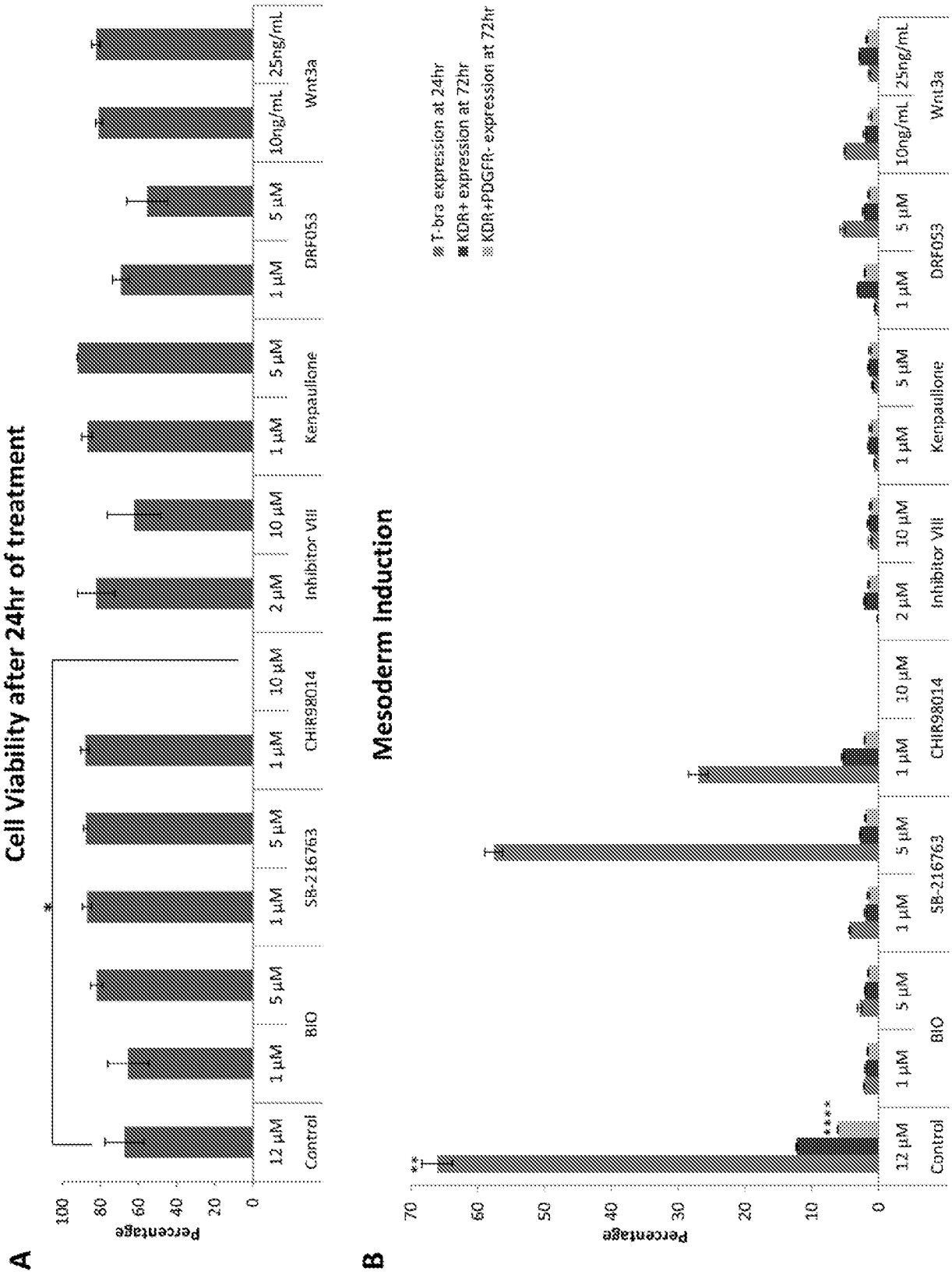

A
Cell Viability after 24hr of treatment
Percentage
100
80
60
40
20
0
12 µM
Control
1 µM
5 µM
BIO
1 µM
5 µM
SB-216763
1 µM
10 µM
CHIR98014
2 µM
10 µM
Inhibitor VIII
1 µM
5 µM
Kenpaullone
1 µM
5 µM
DRF053
10ng/mL
25ng/mL
Wnt3a
B
Mesoderm Induction
T-bra expression at 24hr
KDR+ expression at 72hr
KDR+PDGFR- expression at 72hr
Percentage
70
60
50
40
30
20
10
0
**
****
12 µM
Control
1 µM
BIO
5 µM
BIO
1 µM
SB-216763
5 µM
SB-216763
1 µM
CHIR98014
10 µM
CHIR98014
2 µM
Inhibitor VIII
10 µM
Inhibitor VIII
1 µM
Kenpaullone
5 µM
Kenpaullone
1 µM
DRF053
5 µM
DRF053
10ng/mL
Wnt3a
25ng/mL
Wnt3a

FIG. 38

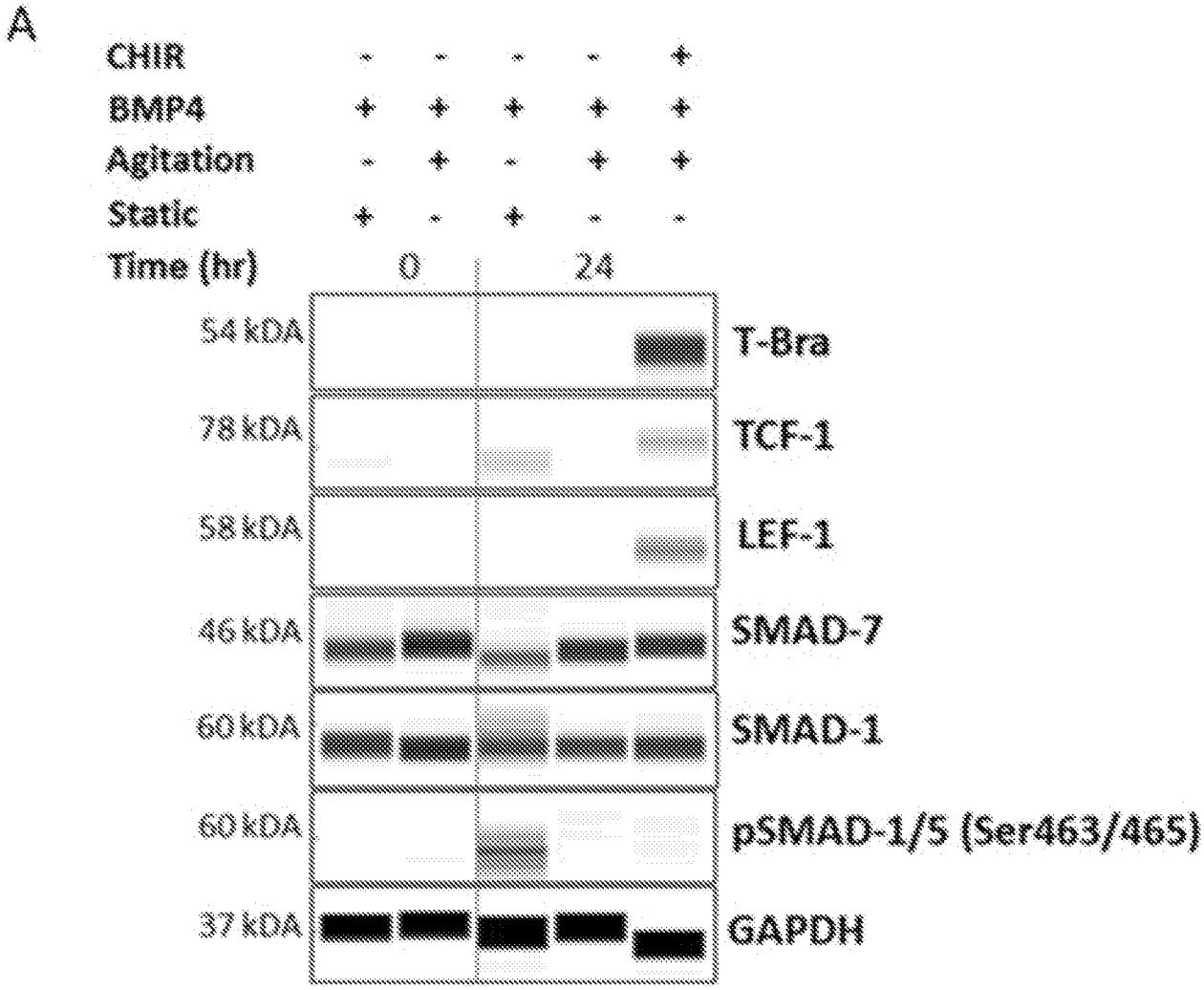

A
CHIR - - - - +
BMP4 + + + + +
Agitation - + - + +
Static + - + - -
Time (hr) 0 24
54 kDA
T-Bra
78 kDA
TCF-1
58 kDA
LEF-1
46 kDA
SMAD-7
60 kDA
SMAD-1
60 kDA
pSMAD-1/5 (Ser463/465)
37 kDA
GAPDH

FIG. 38 continued

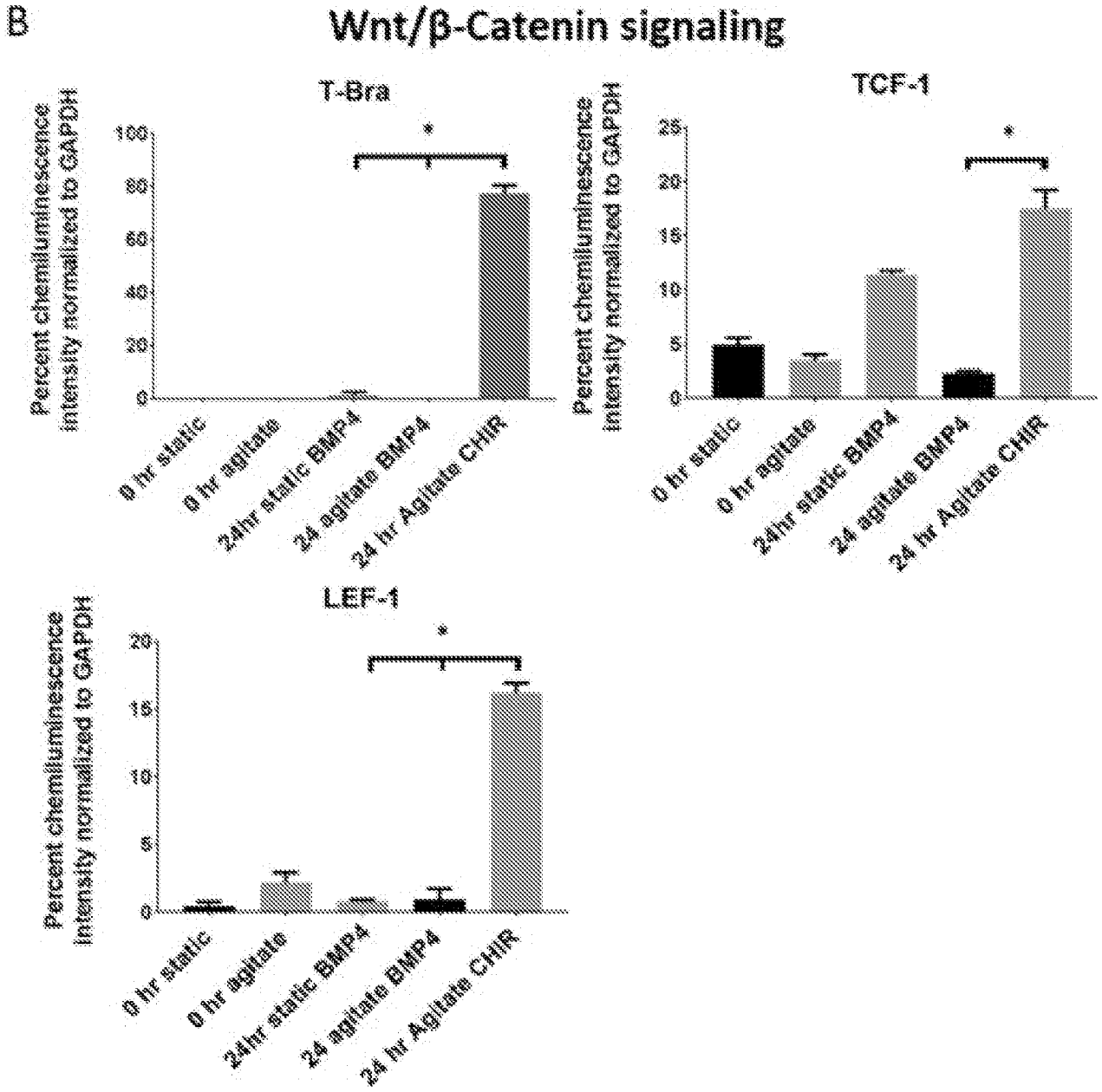


FIG. 38 continued

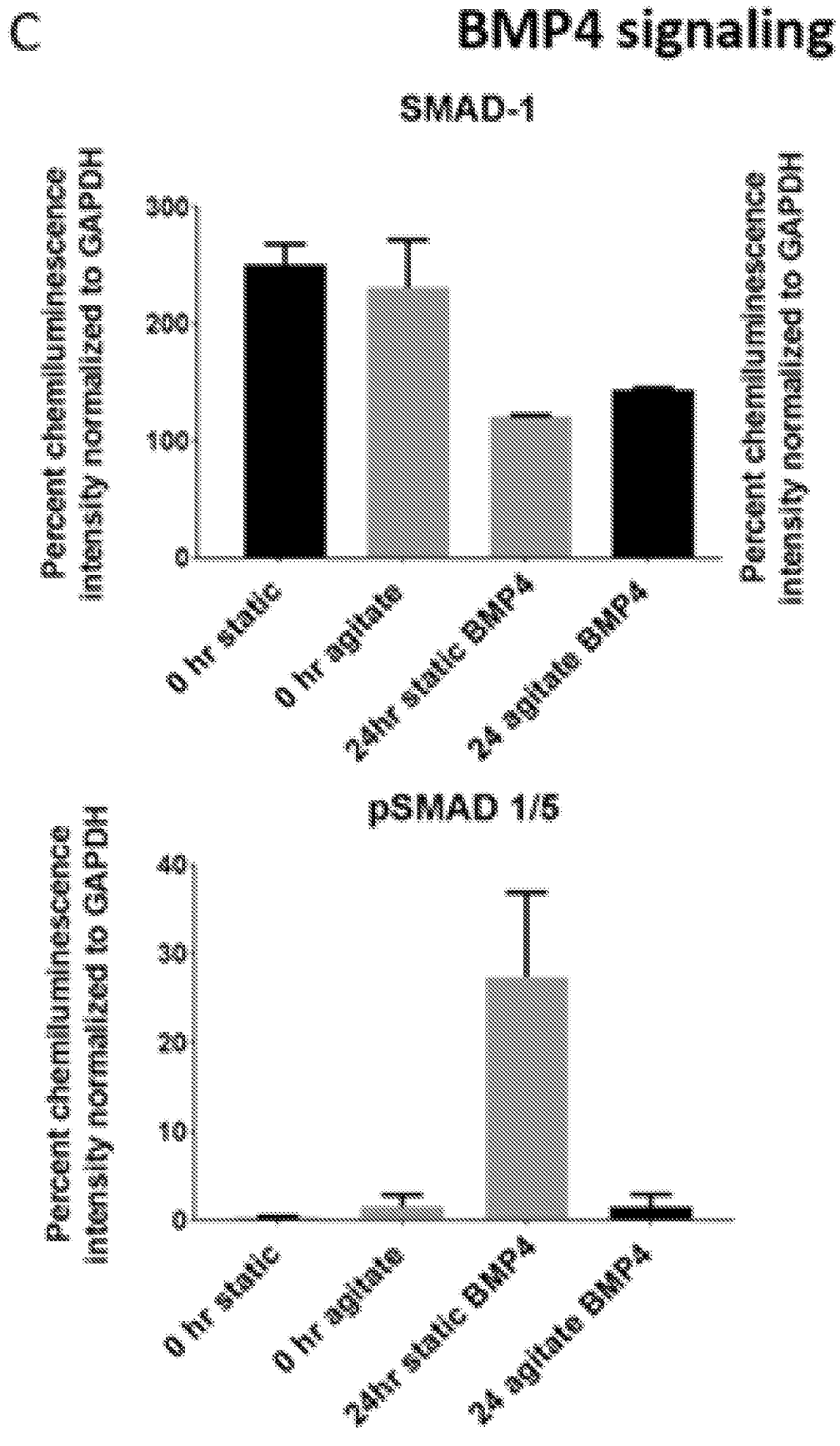


FIG. 38C continued

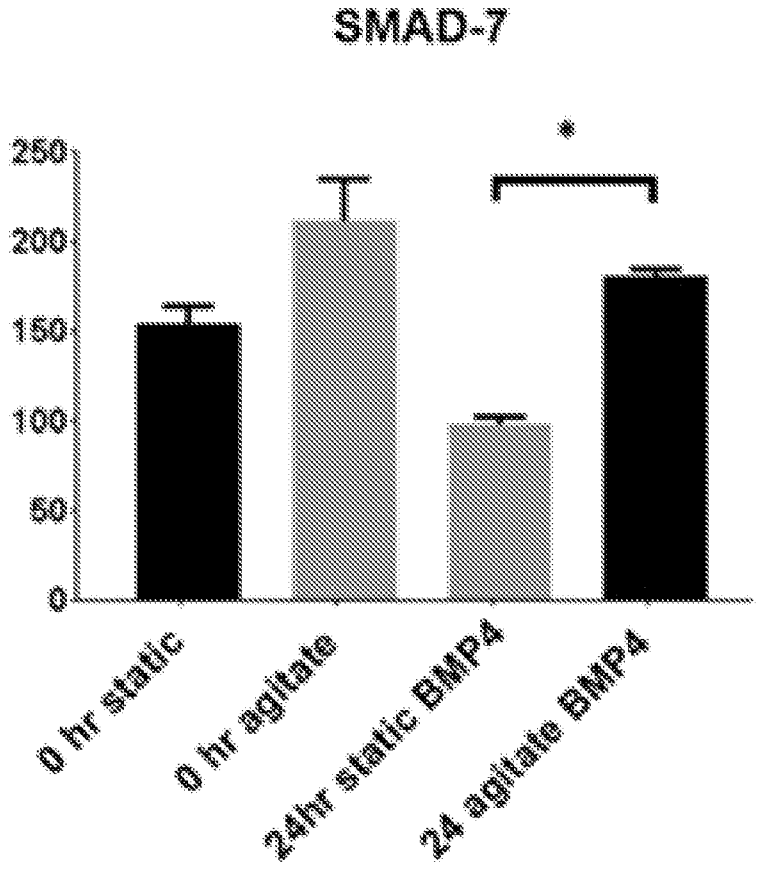


FIG. 39

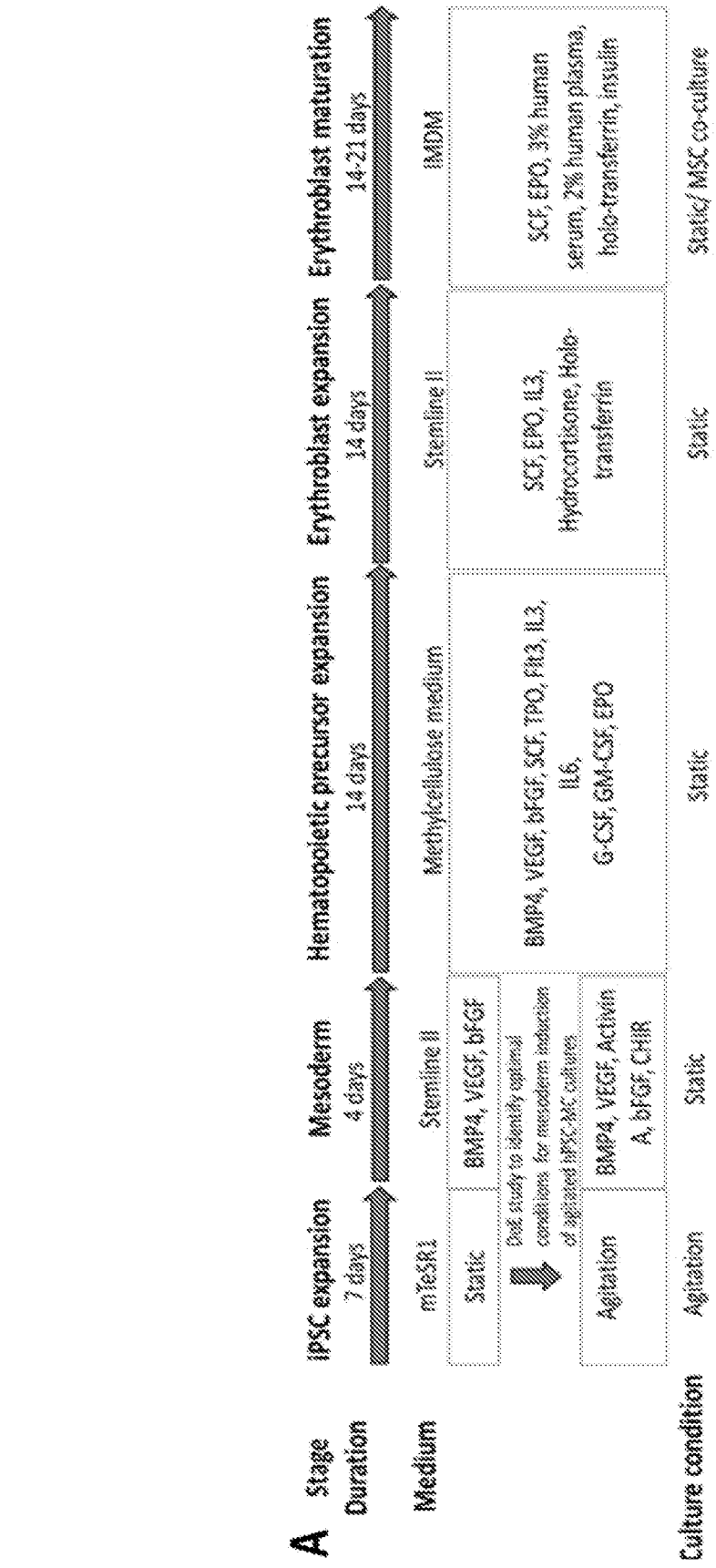
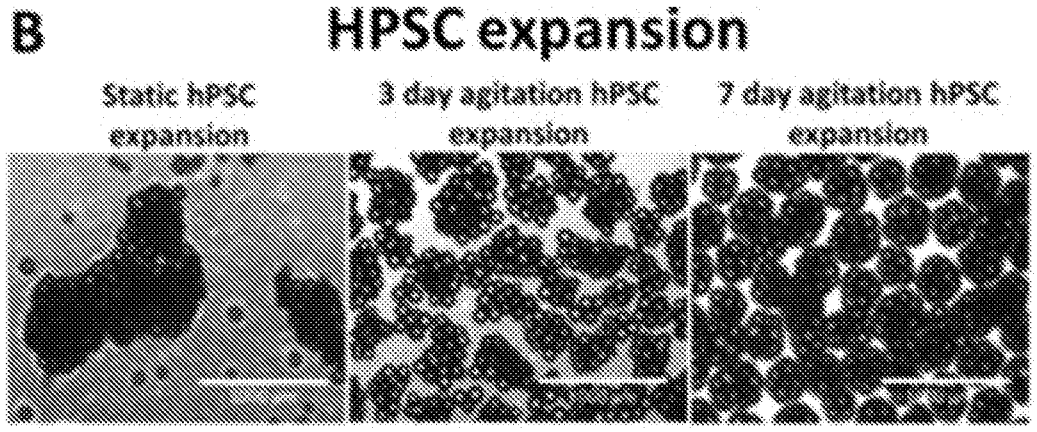
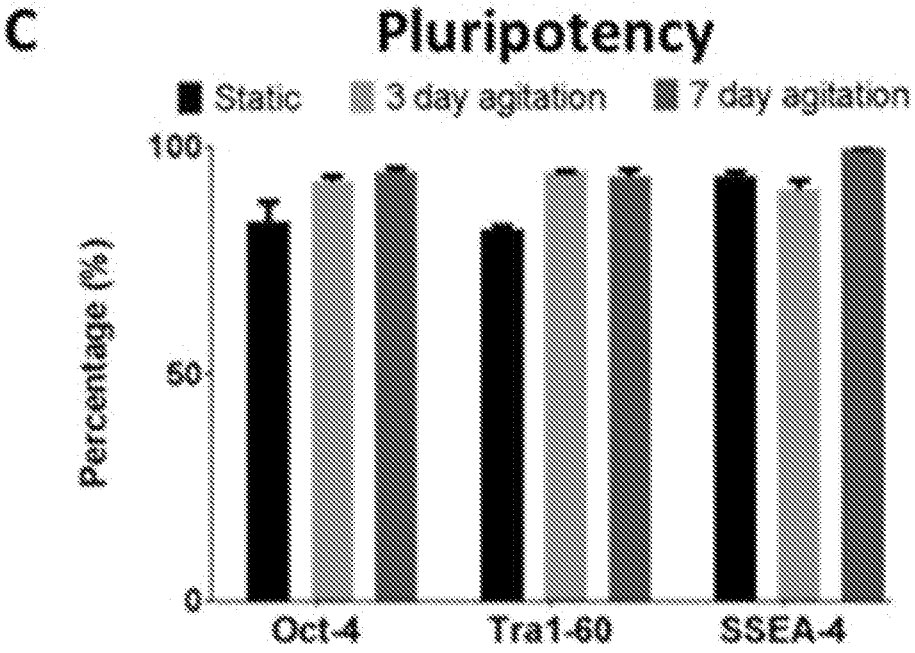

A
Stage
Duration
Medium
Culture condition
iPSC expansion
7 days
mTeSR1
Static
Agitation
Mesoderm
4 days
Stemline II
BMP4, VEGF, bFGF
BMP4, VEGF, Activin A, bFGF, CHIR
Static
Agitation
Dot study to identify optimal conditions for mesoderm induction of agitated hPSC-MC culture
Hematopoietic precursor expansion
14 days
Methylcellulose medium
BMP4, VEGF, bFGF, SCF, TPO, Flt3, IL3, IL6, G-CSF, GM-CSF, EPO
Static
Erythroblast expansion
14 days
Stemline II
SCF, EPO, IL3, Hydrocortisone, Holo-transferrin
Static
Erythroblast maturation
14-21 days
IMDM
SCF, EPO, 3% human serum, 2% human plasma, holo-transferrin, insulin
Static/ MSC co-culture

FIG. 39 contined





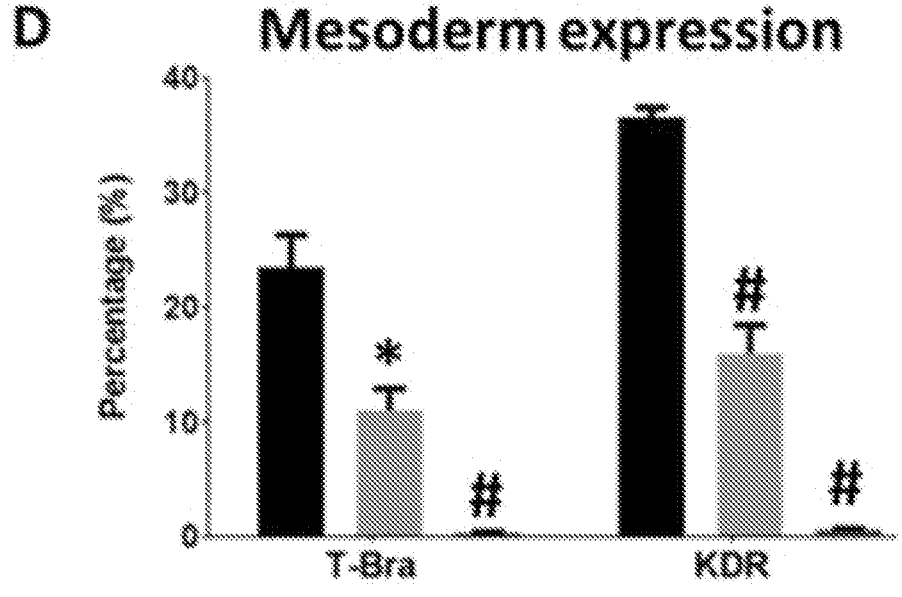


FIG. 39 continued

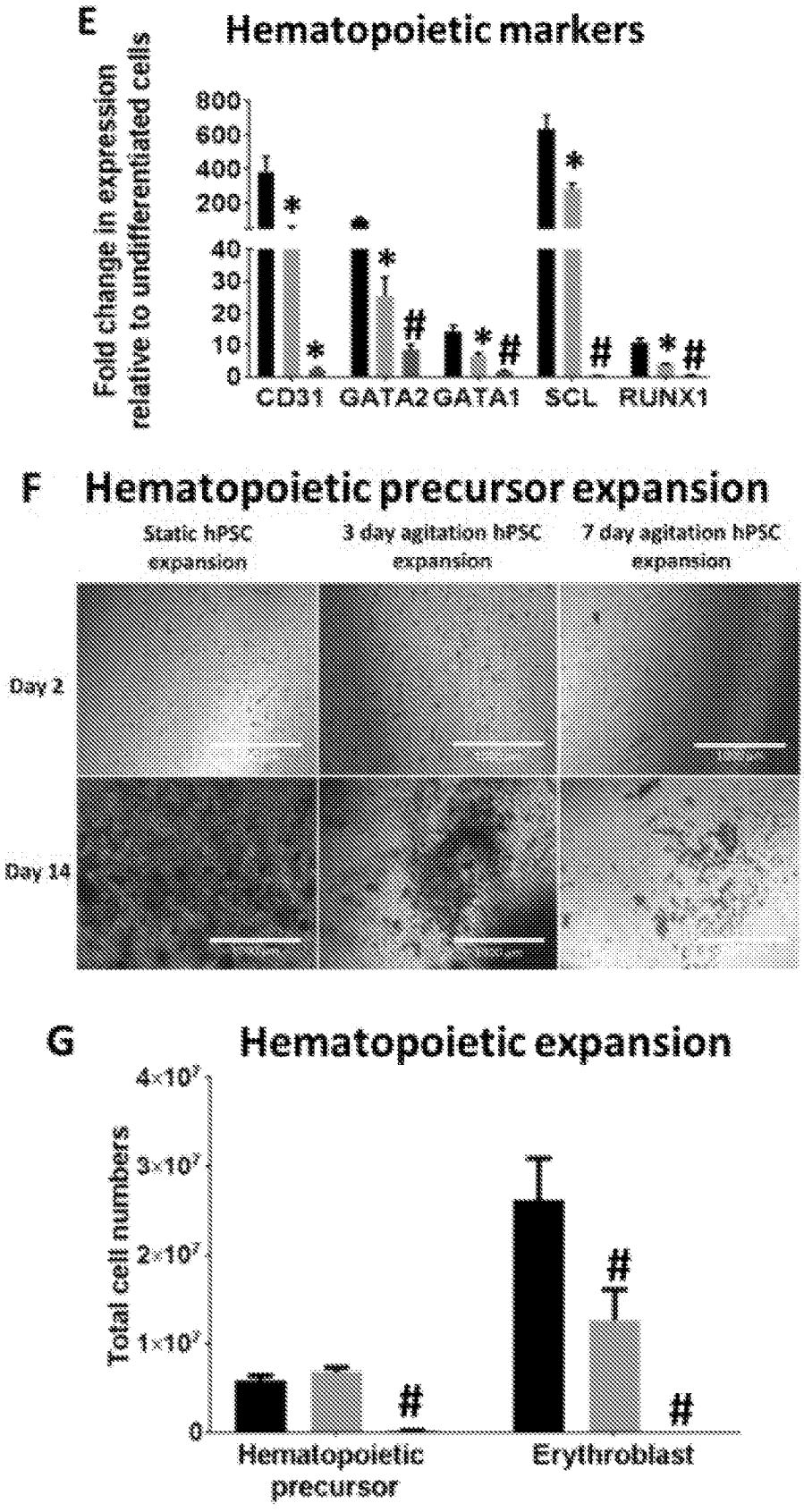


FIG. 39 continued

H

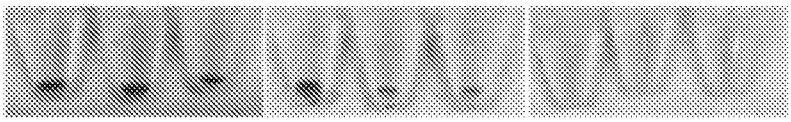
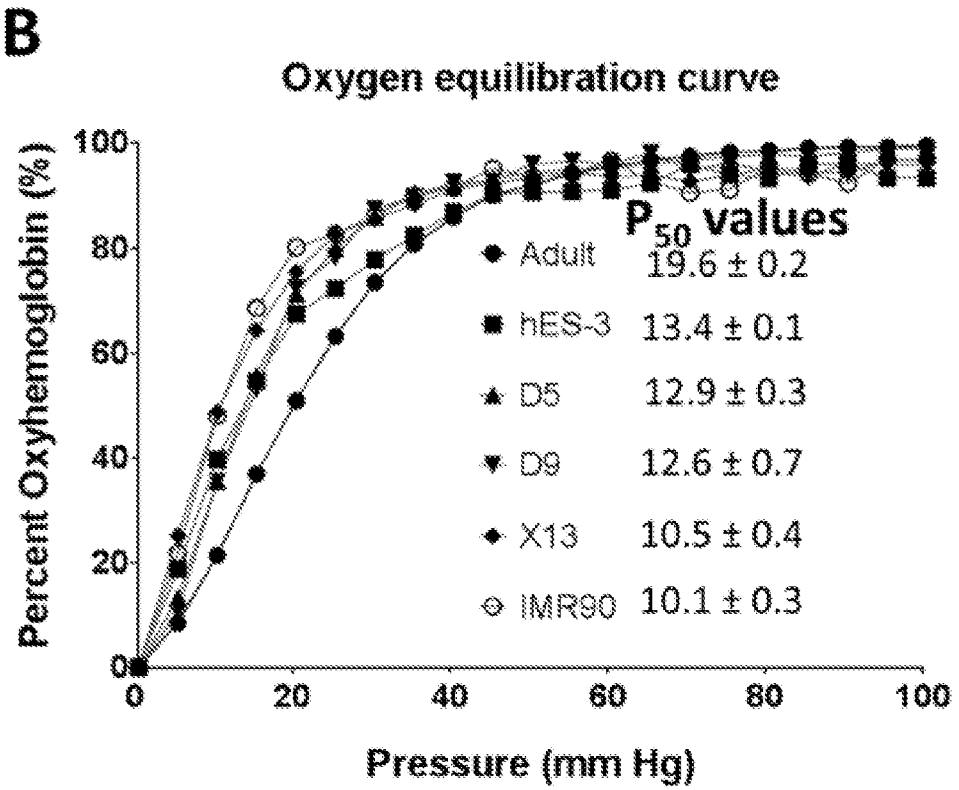
	Static	3 day agitation	7 day agitation	*Static vs 7 day p-value*	*Static vs 3 day p-value*
CD235a+ CD71+ (%)	27.5 % ± 1.2	21.6 % ± 1.8	0.23 % ± 0.04	<0.0001	0.053
Total CD235a+ (%)	51.8 % ± 2.0	40.4 % ± 3.4	0.51 % ± 0.1	<0.0001	0.045
HbF (%)	65.7 % ± 2.3	60.2 % ± 3.8	0.70 % ± 0.05	<0.0001	0.28
Total yield (Day 28)	$2.62 \times 10^7 \pm 4.7 \times 10^6$	$1.26 \times 10^7 \pm 3.5 \times 10^6$	$4.11 \times 10^6 \pm 2.4 \times 10^6$	0.0052	0.08
RBC pellet (Day 28)					

FIG. 40

A

		D5	X13	D9	BR7	IMR90	hES-3
Hemox analysis (P_{50} value)		12.9 ± 0.3	10.5 ± 0.4	12.6 ± 0.7	NA	10.1 ± 0.3	13.4 ± 0.1
Hemoglobin (% of total hemoglobin)	Alpha	21.8 ± 2.2	37.2 ± 1.0	54 ± 0.2	71.1 ± 1.3	74.7 ± 2.6	20.9 ± 1.5
	Beta	2.7 ± 0.7	10.6 ± 0.4	NIL	5.5 ± 1.0	NIL	1.3 ± 0.3
	Gamma	13.1 ± 0.7	15.7 ± 0.2	25.9 ± 2.9	19.6 ± 0.7	19.7 ± 2.1	15.3 ± 1.4
	Epsilon	66.7 ± 5.4	36.5 ± 0.8	20.1 ± 2.7	3.4 ± 1.1	5.6 ± 1.0	62.5 ± 2.4
Enucleation (Percent CD235a+ DRAQ5-)		39 ± 1.0	32 ± 1.6	28 ± 1.2	NA	40.6 ± 1.4	NA

FIG. 40 continued



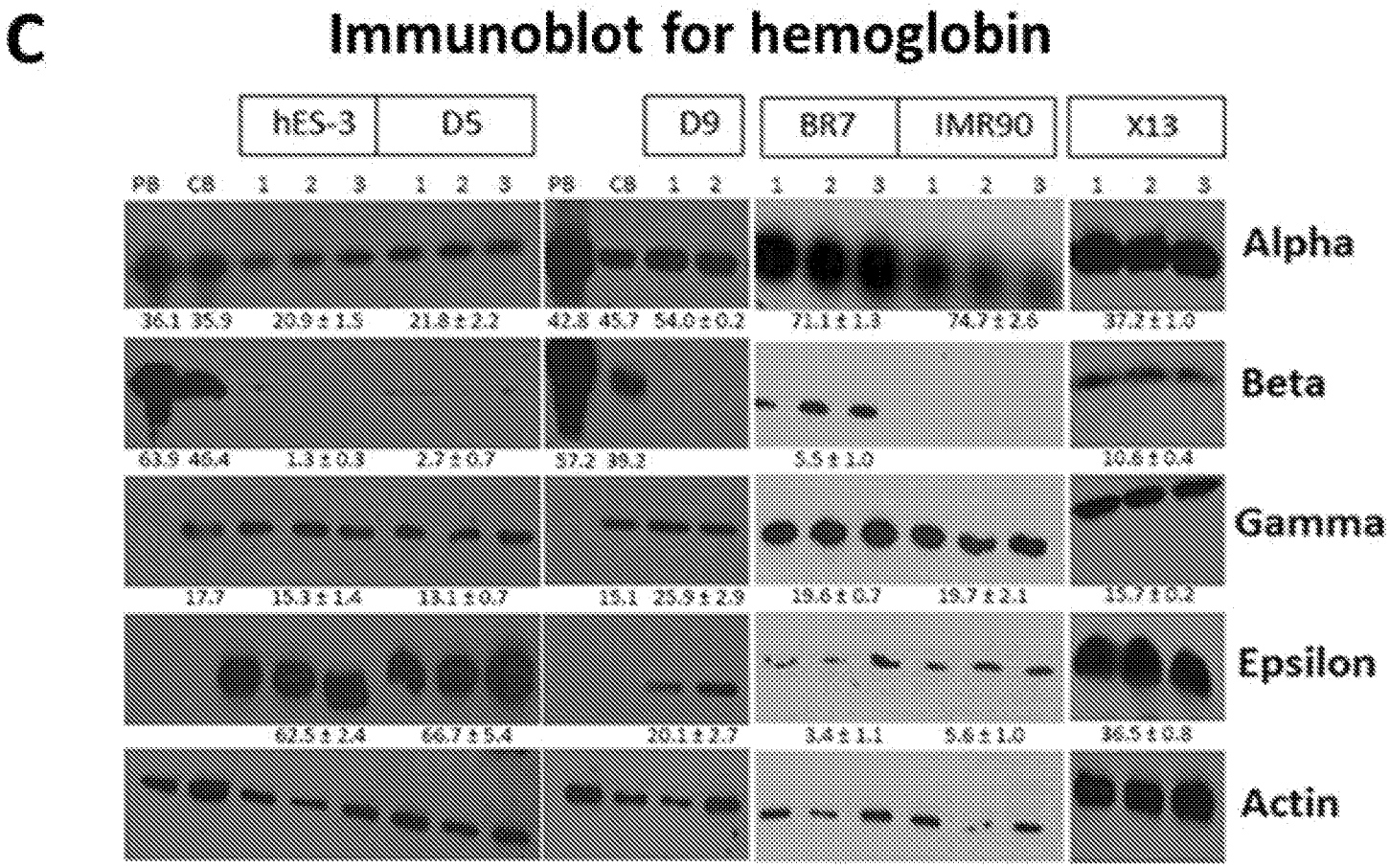


FIG. 40 continued

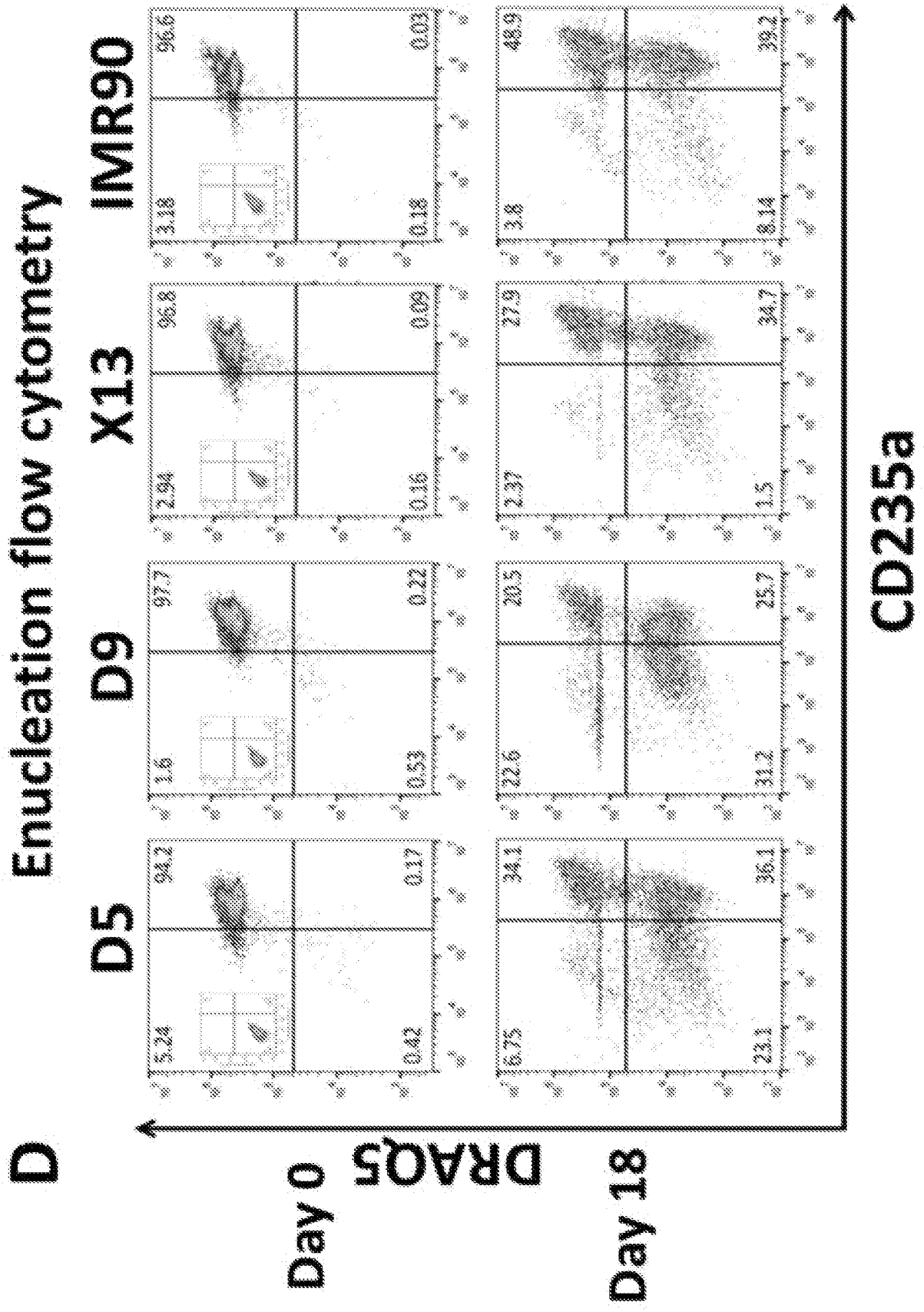


FIG. 40 continued

