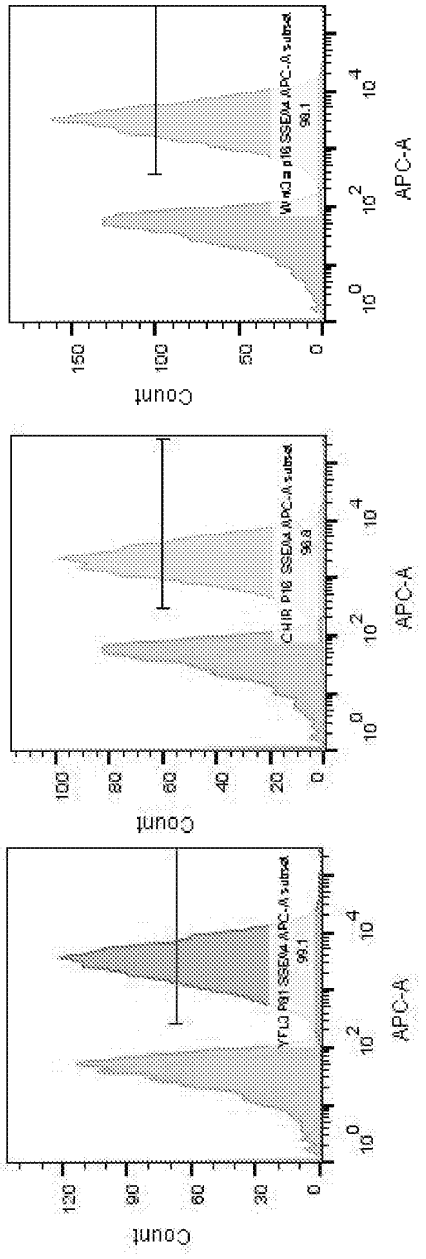


Fig. 1A Fig. 1B Fig. 1C

SSEA4 immunostaining

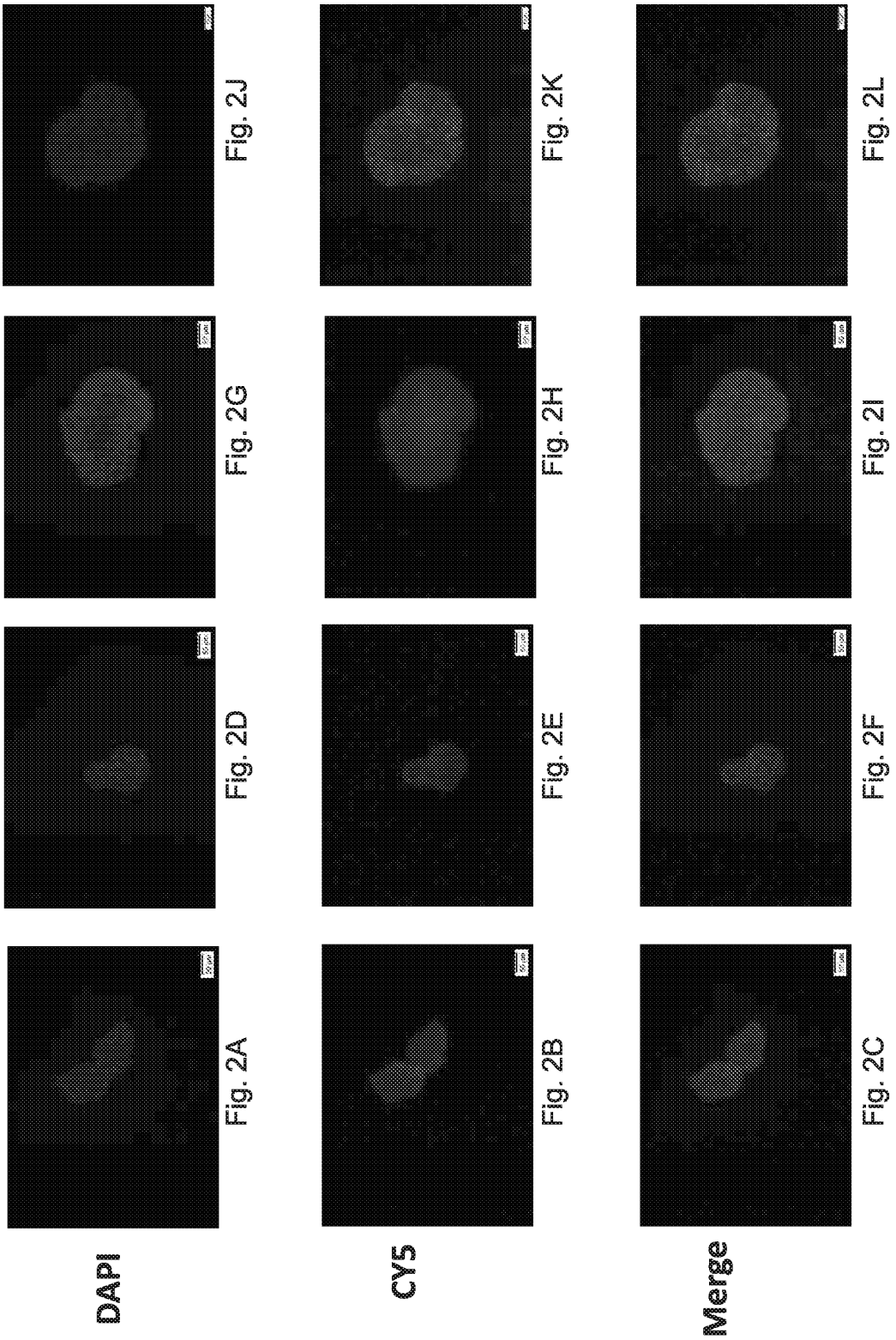
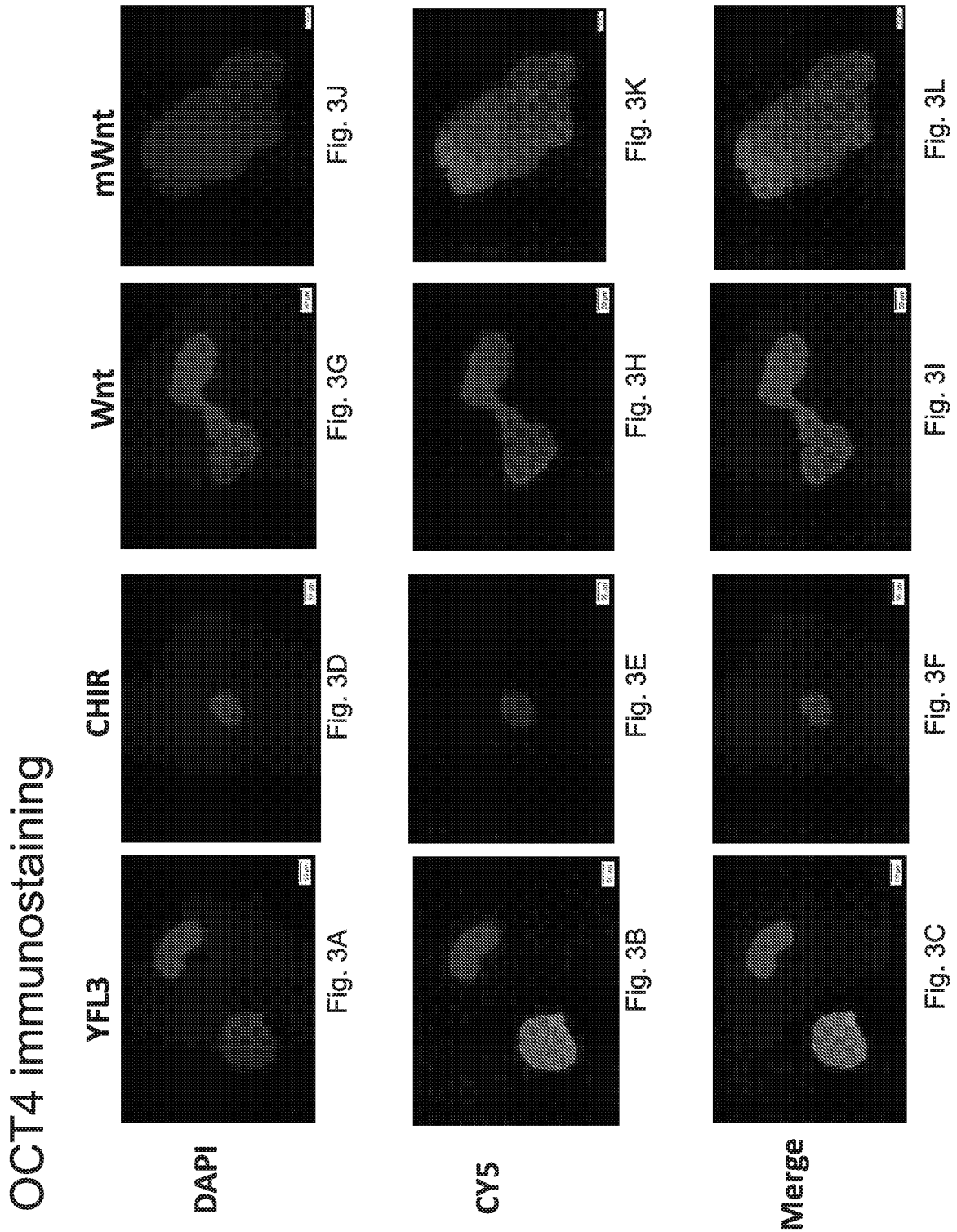


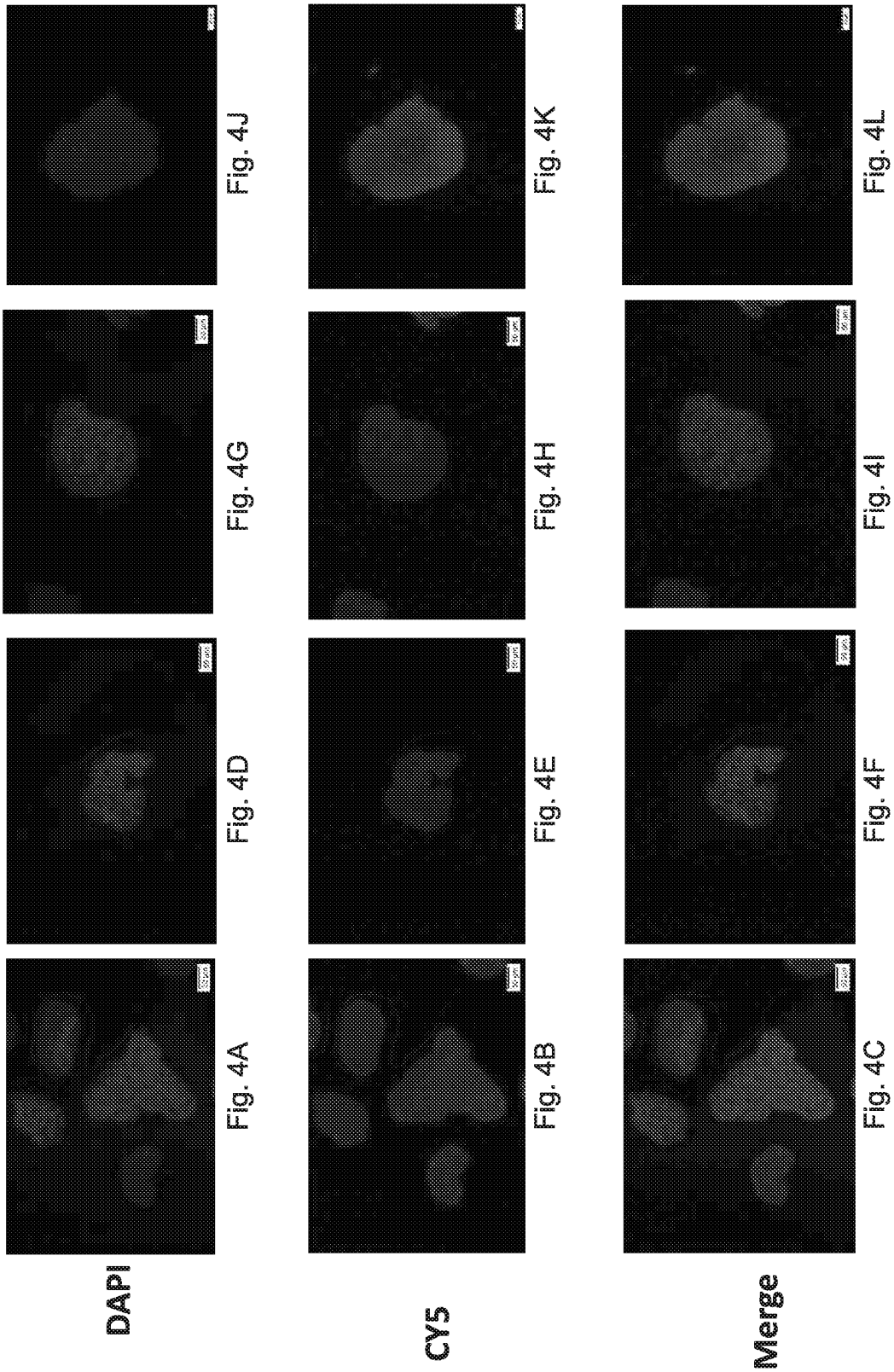
Fig. 2A Fig. 2D Fig. 2G Fig. 2J

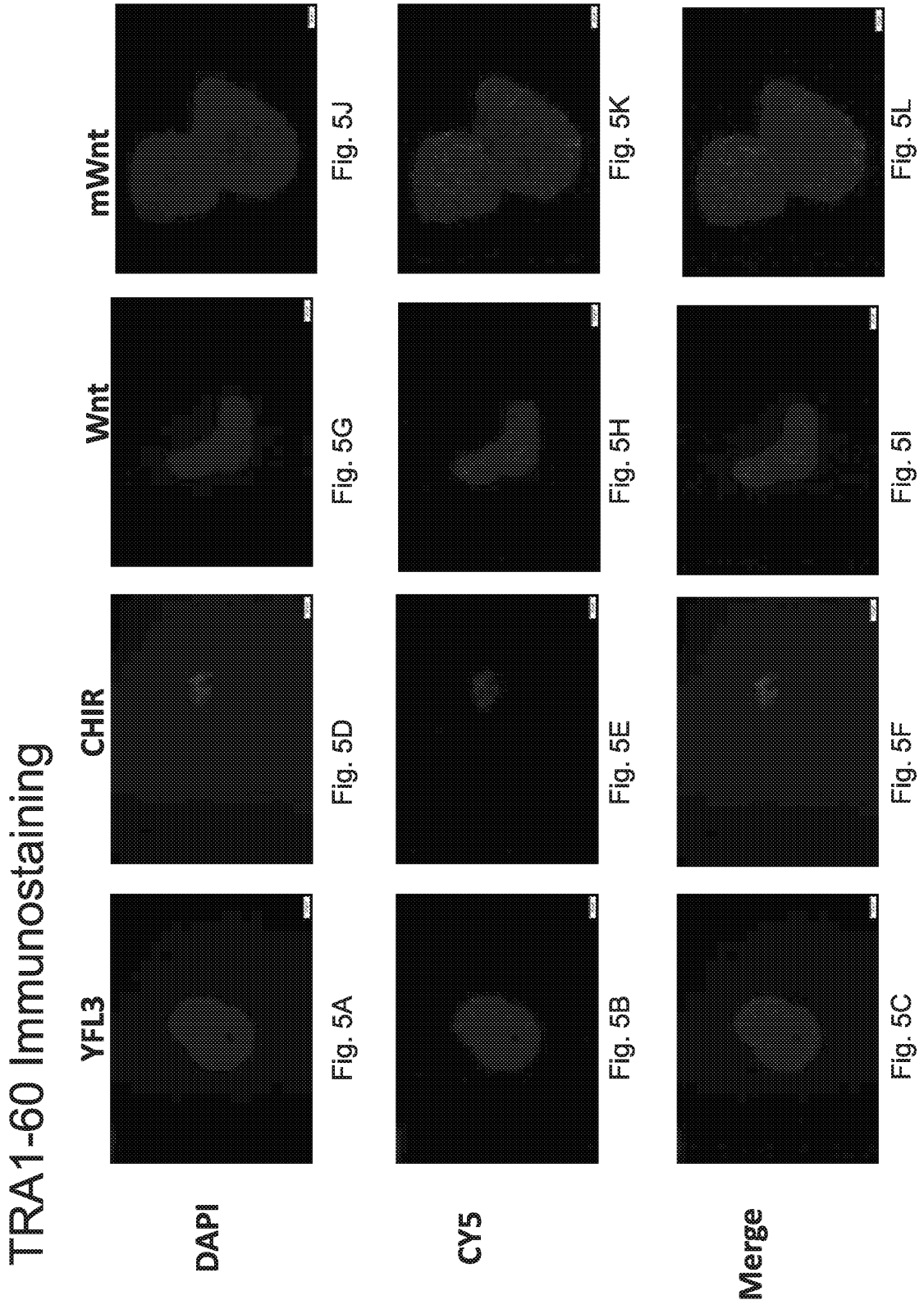
Fig. 2B Fig. 2E Fig. 2H Fig. 2K

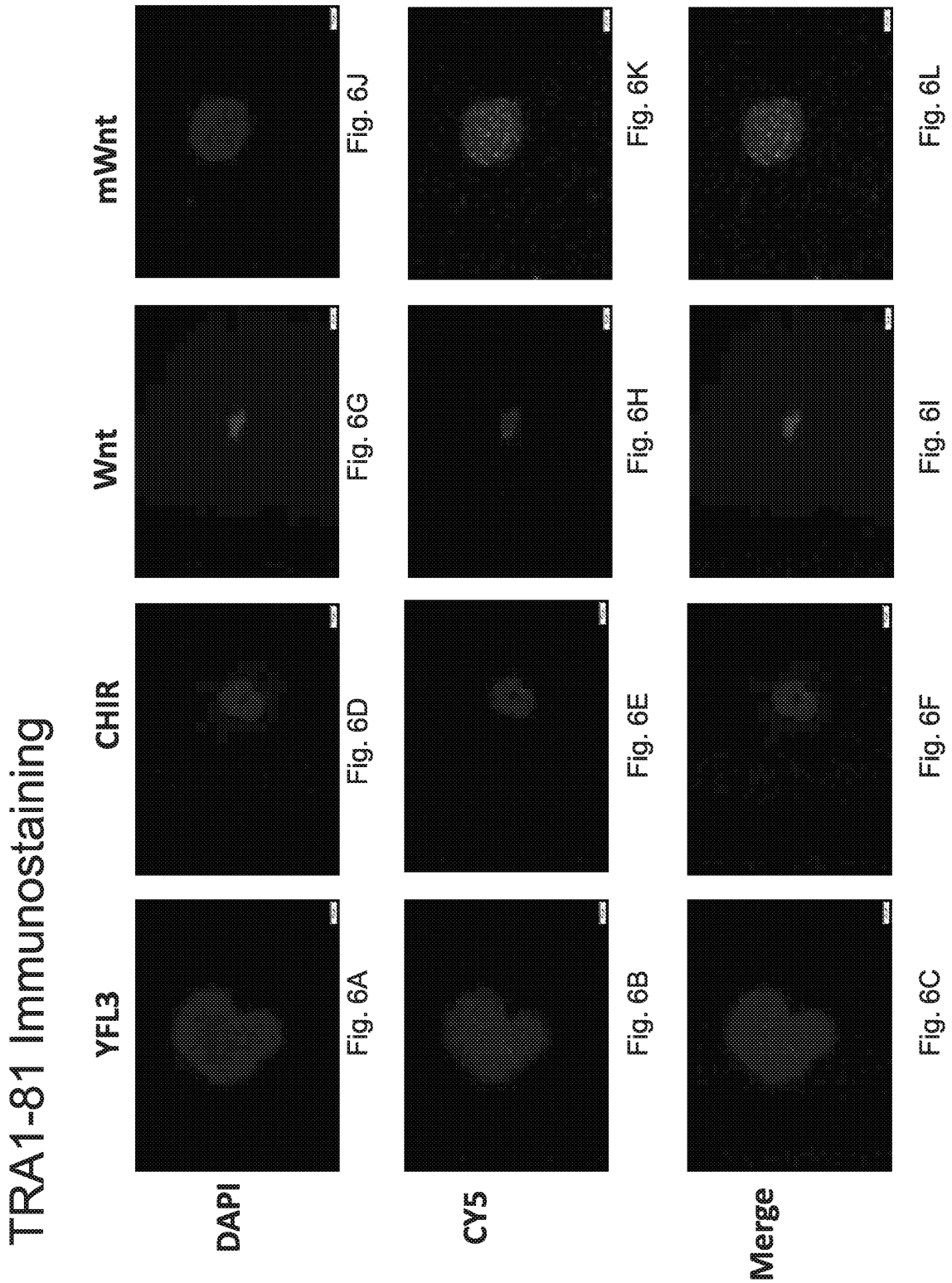
Fig. 2C Fig. 2F Fig. 2I Fig. 2L


OCT4 immunostaining
YFL3
CHIR
Wnt
mWnt
DAPI
CY5
Merge
Fig. 3A
Fig. 3B
Fig. 3C
Fig. 3D
Fig. 3E
Fig. 3F
Fig. 3G
Fig. 3H
Fig. 3I
Fig. 3J
Fig. 3K
Fig. 3L

Nanog immunostaining




TRA1-60 Immunostaining
YFL3
CHIR
Wnt
mWnt
DAPI
CY5
Merge
Fig. 5A
Fig. 5B
Fig. 5C
Fig. 5D
Fig. 5E
Fig. 5F
Fig. 5G
Fig. 5H
Fig. 5I
Fig. 5J
Fig. 5K
Fig. 5L


TRA1-81 Immunostaining
YFL3
CHIR
Wnt
mWnt
DAPI
CY5
Merge
Fig. 6A
Fig. 6B
Fig. 6C
Fig. 6D
Fig. 6E
Fig. 6F
Fig. 6G
Fig. 6H
Fig. 6I
Fig. 6J
Fig. 6K
Fig. 6L

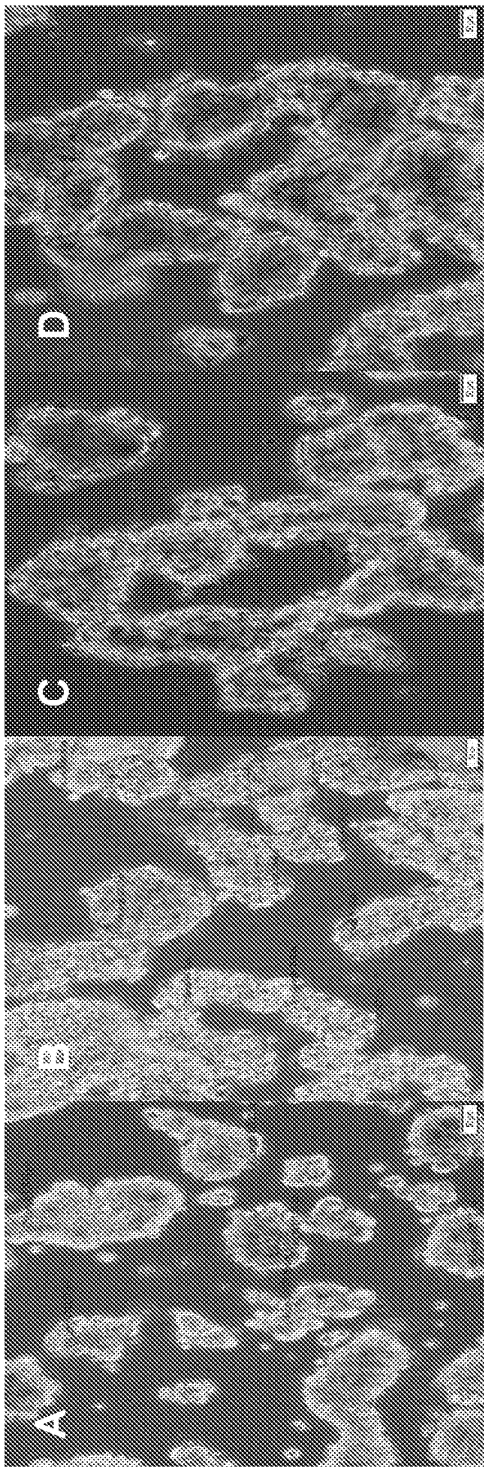


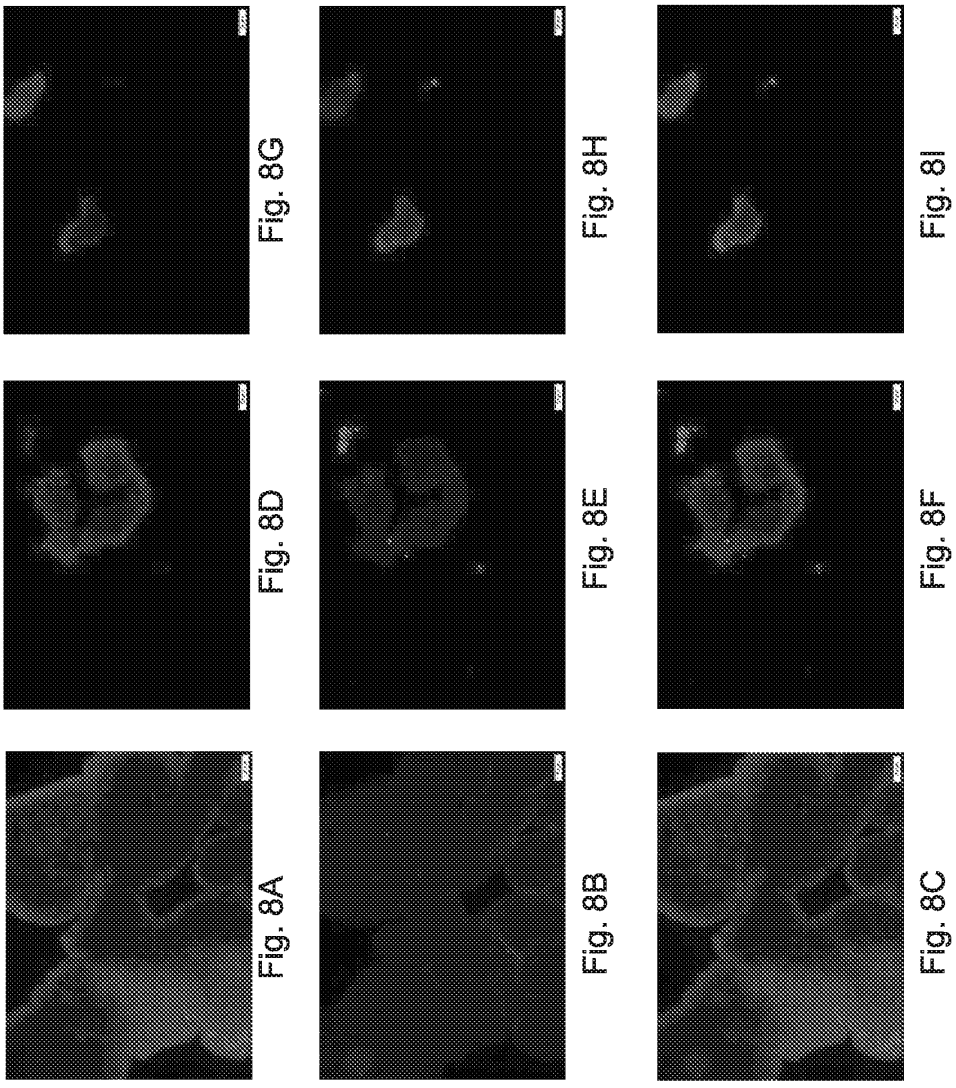
Fig. 7A

Fig. 7B

Fig. 7C

Fig. 7D

SSEA4 Immunostaining (after at least 40 passages)



Nanog Immunostaining (after at least 40 passages)



Fig. 9A

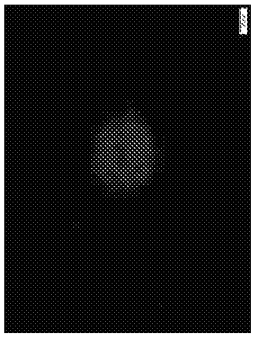


Fig. 9D

Fig. 9G

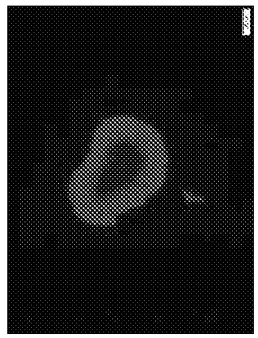


Fig. 9B

Fig. 9E

Fig. 9H

Fig. 9C

Fig. 9F

Fig. 9I

DAPI

CY5

Merge

TRA-1-60 immunostaining (at least 40 passages)

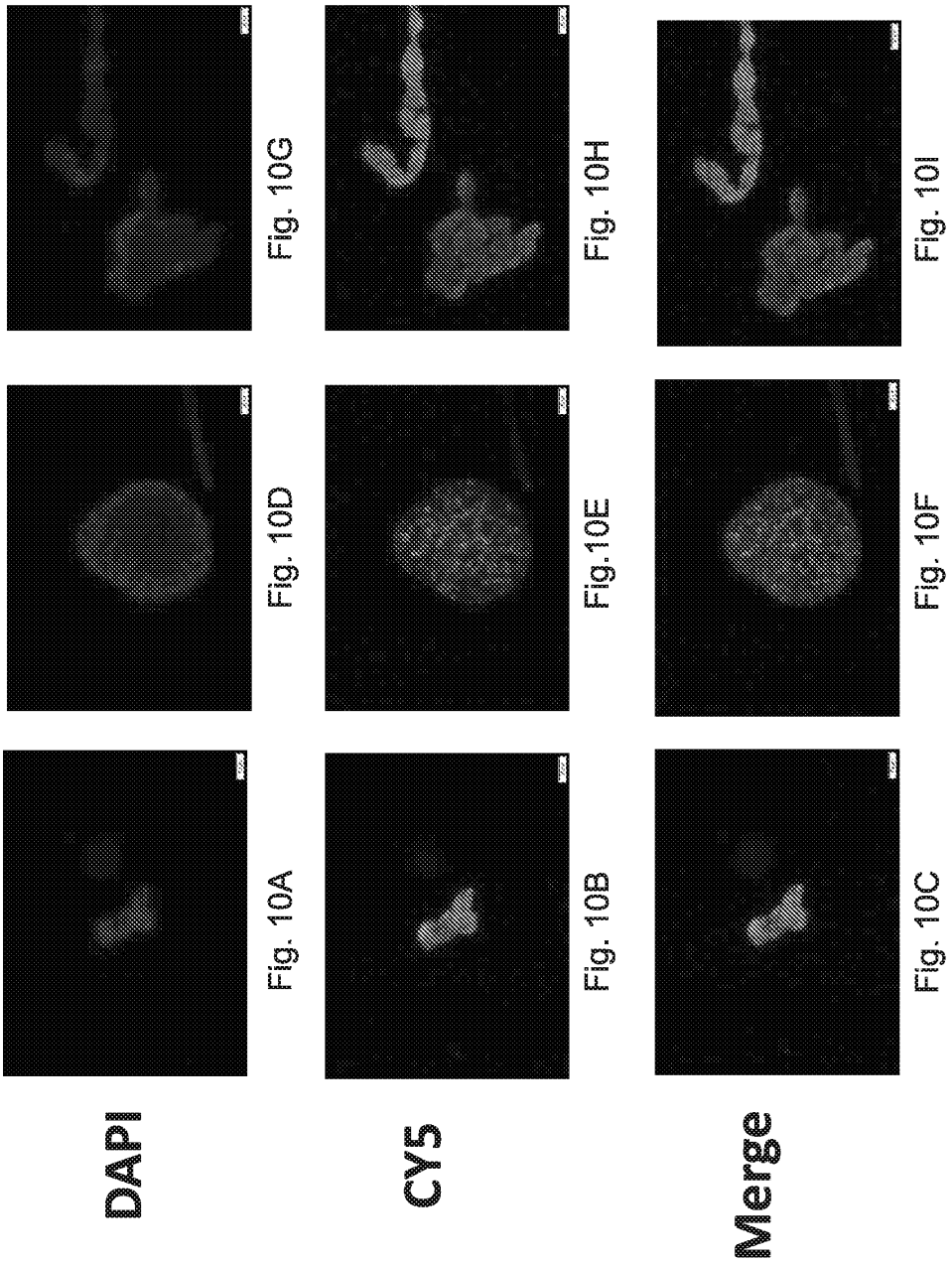
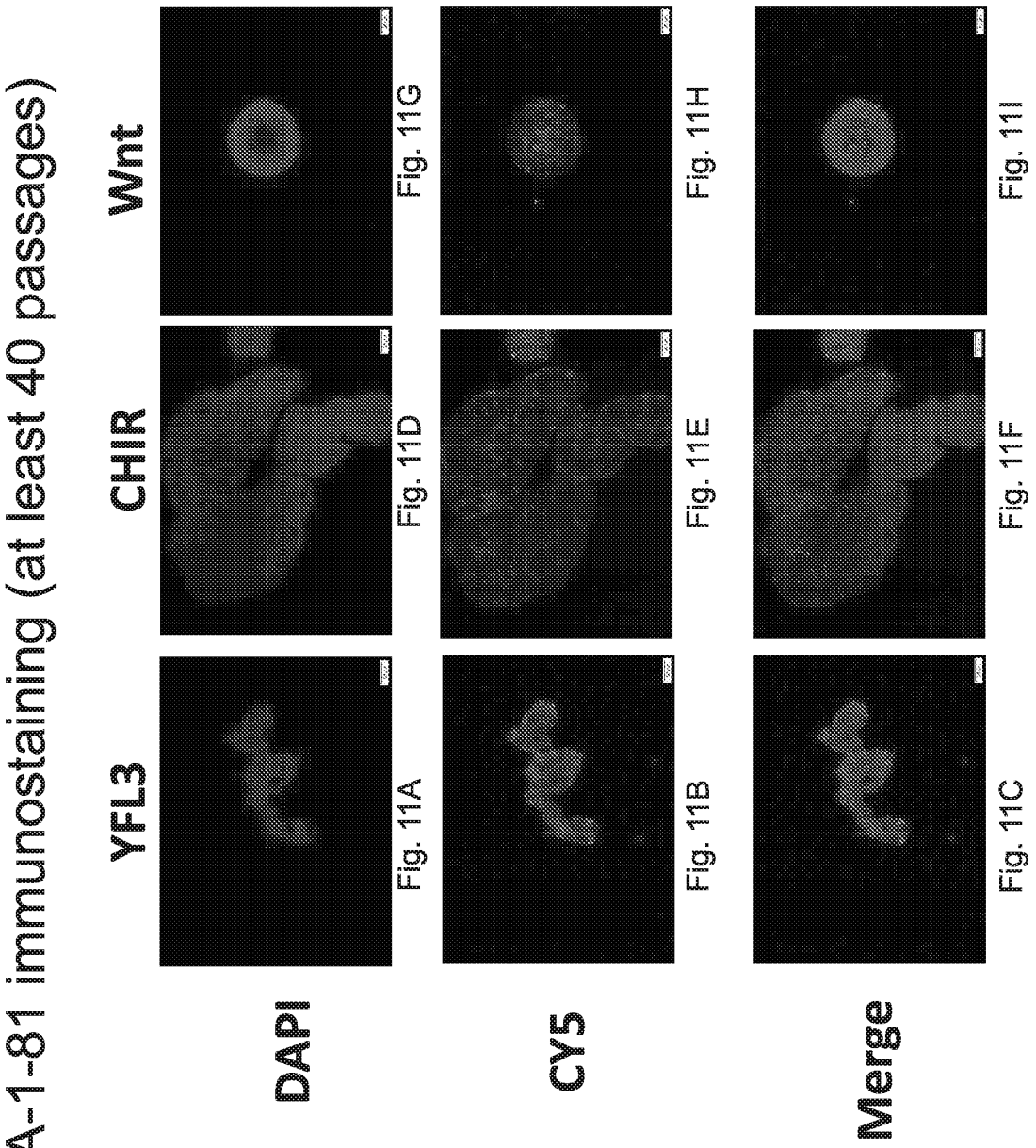


Fig. 10A Fig. 10B Fig. 10C

Fig. 10D Fig.10E Fig. 10F

Fig. 10G Fig. 10H Fig. 10I


TRA-1-81 immunostaining (at least 40 passages)
YFL3
CHIR
Wnt
DAPI
CY5
Merge
Fig. 11A
Fig. 11B
Fig. 11C
Fig. 11D
Fig. 11E
Fig. 11F
Fig. 11G
Fig. 11H
Fig. 11I

OCT4 immunostaining (at least 40 passages)

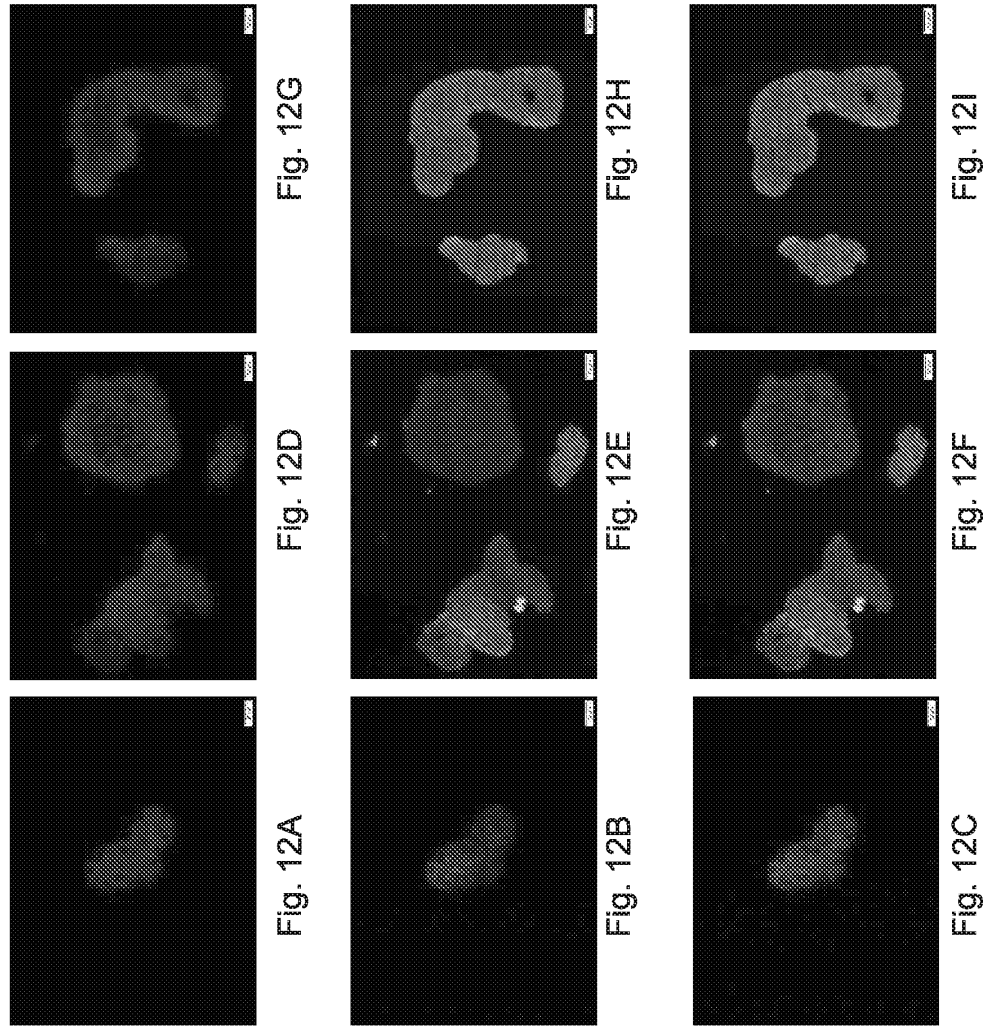


Fig. 12A Fig. 12B Fig. 12C

Fig. 12D Fig. 12E Fig. 12F

Fig. 12G Fig. 12H Fig. 12I

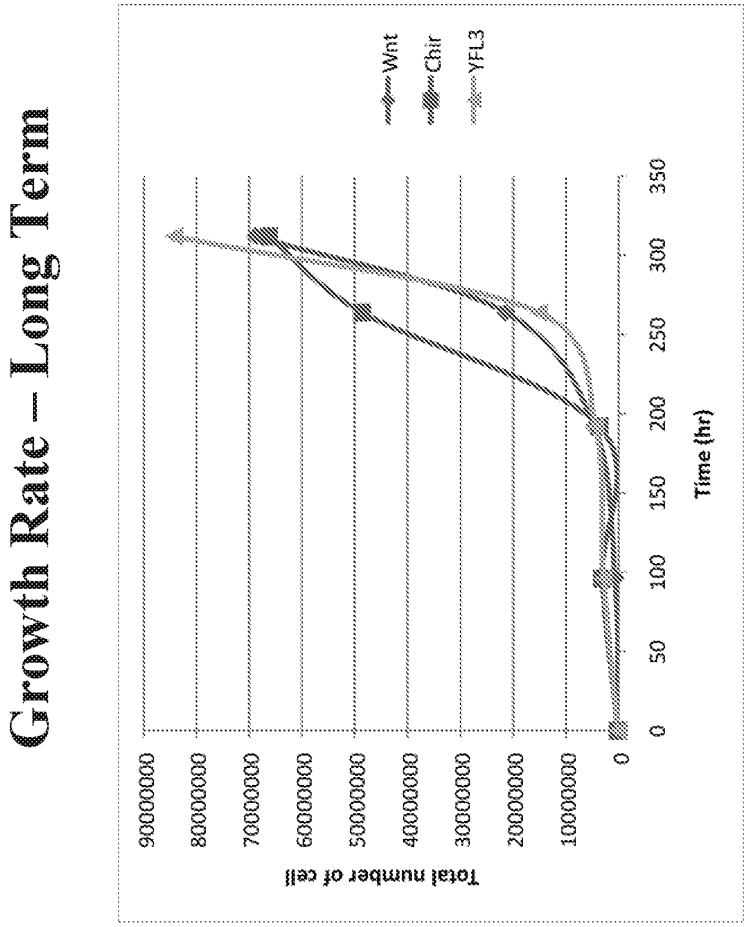

Growth Rate – Long Term
Total number of cell
90000000
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
0
50
100
150
200
250
300
350
Time (hr)
Wnt
Chir
YFL3

Fig. 13A

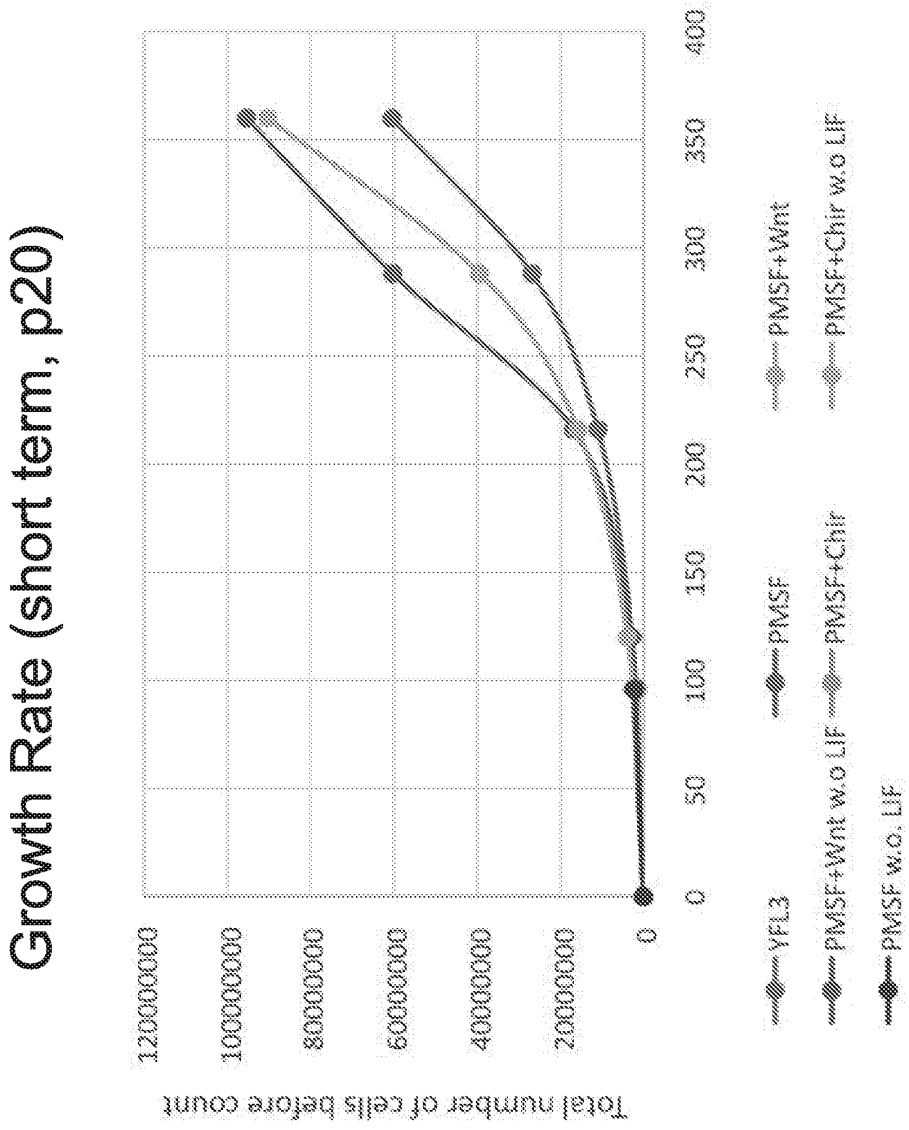

Growth Rate (short term, p20)
Total number of cells before count
120000000
100000000
80000000
60000000
40000000
20000000
0
0
50
100
150
200
250
300
350
400
YFL3
PMSF
PMSF+Wnt
PMSF+Wnt w.o LIF
PMSF+Chir
PMSF+Chir w.o LIF
PMSF w.o. LIF

Fig. 13B

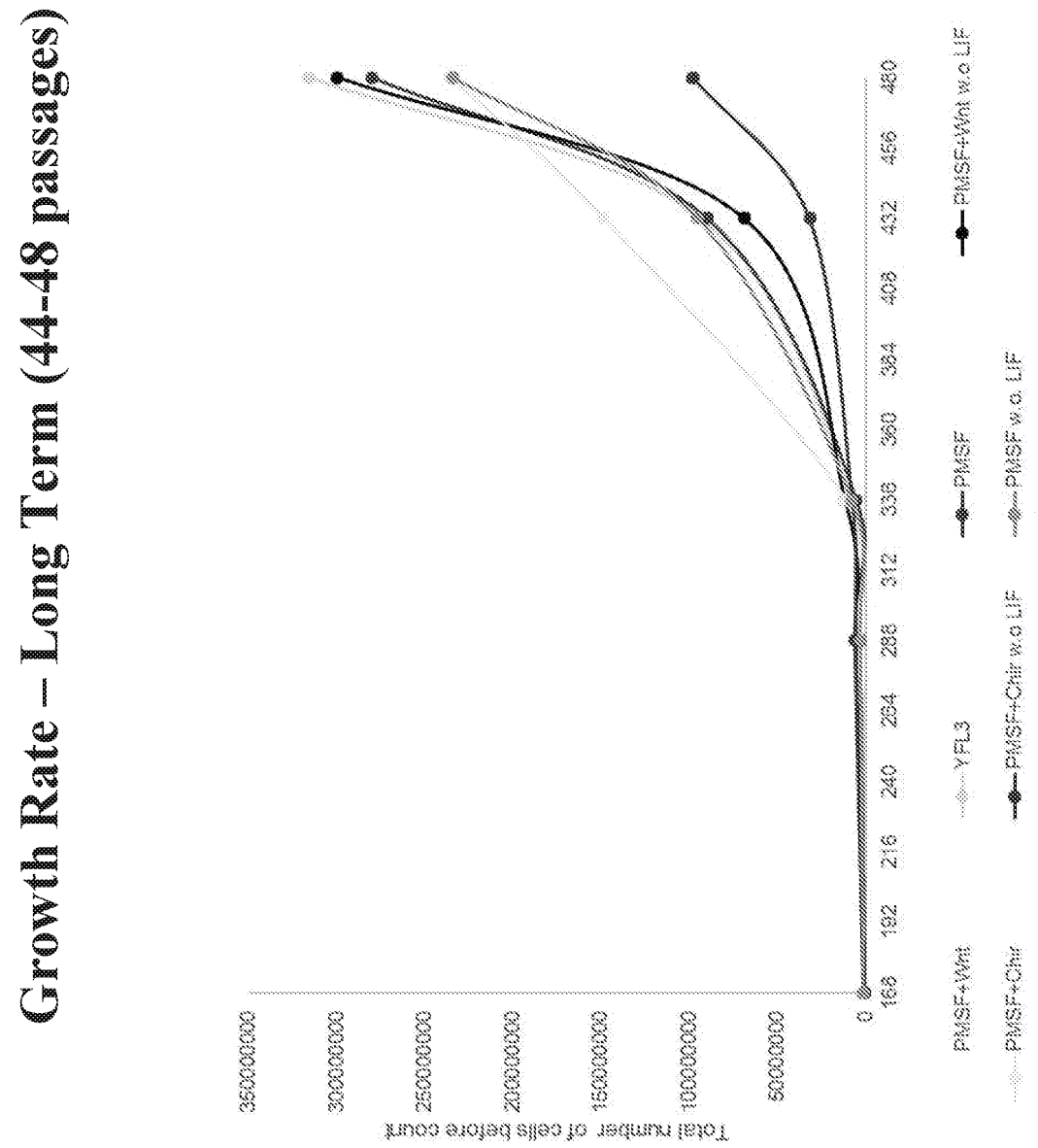

Growth Rate – Long Term (44-48 passages)
Total number of cells before count
360000000
300000000
250000000
200000000
150000000
100000000
50000000
0
168
192
216
240
264
288
312
336
360
384
408
432
456
480
PMSF+Wnt
YFL3
PMSF
PMSF+Wnt w.o LIF
PMSF+Chir
PMSF+Chir w.o LIF
PMSF w.o. LIF

Fig. 13C

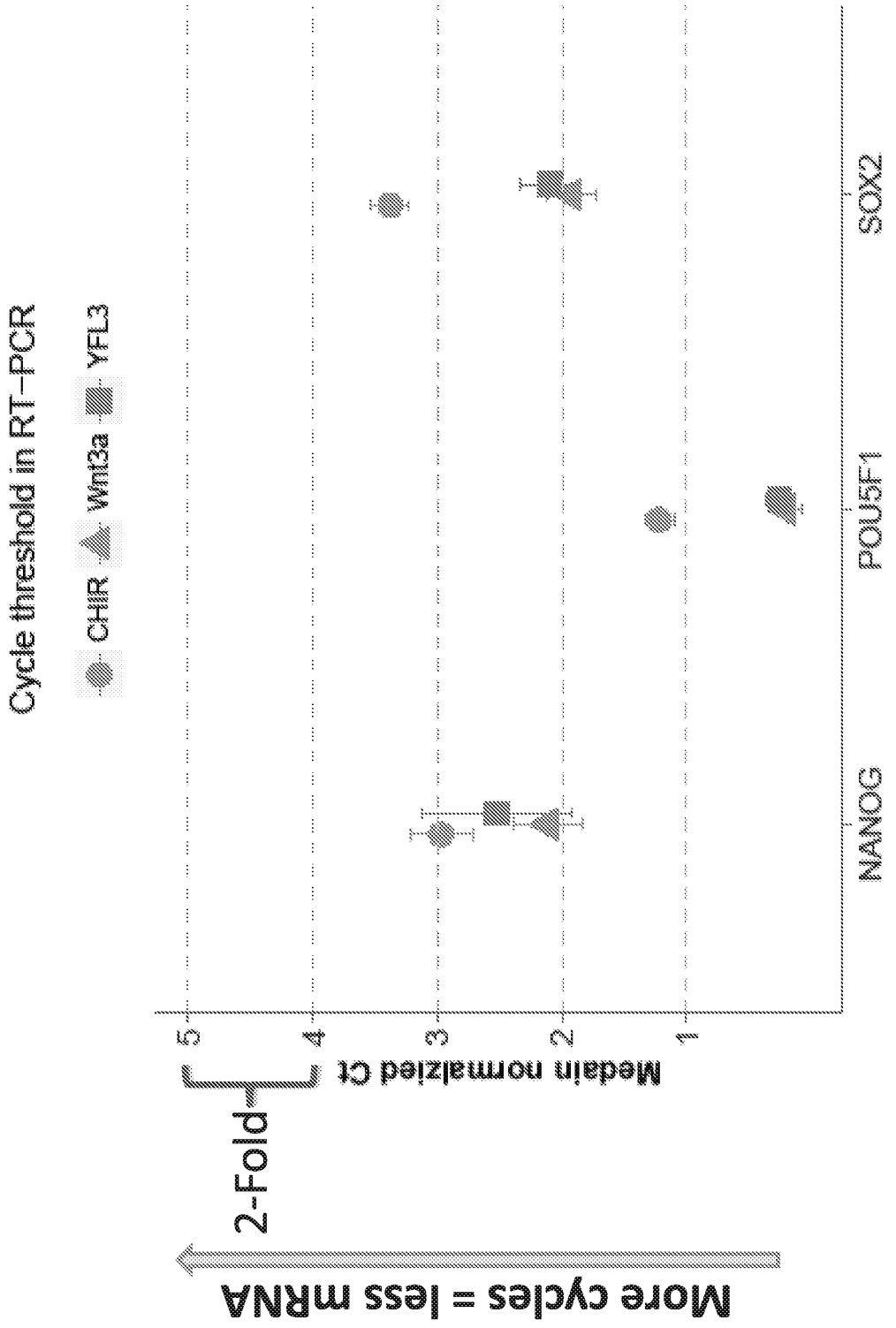

More cycles = less mRNA
2-Fold
Median normalized ct
5
4
3
2
1
NANOG
POU5F1
SOX2
Gene name
Cycle threshold in RT–PCR
CHIR
Wnt3a
YFL3

Fig. 14

SSEA4 FACS analysis

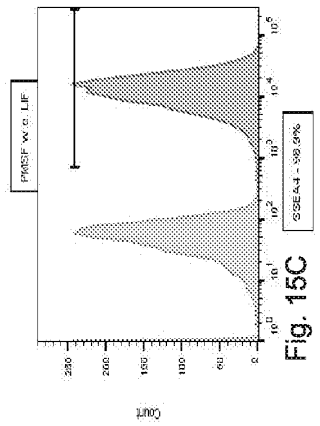


Fig. 15C

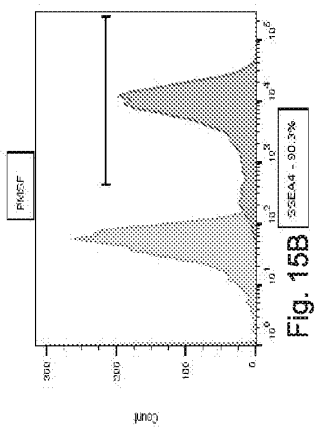


Fig. 15B

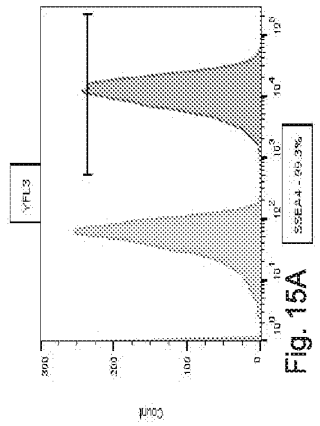


Fig. 15A

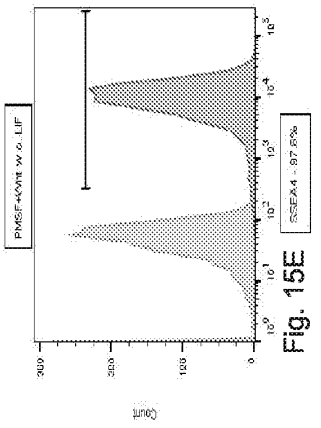


Fig. 15E

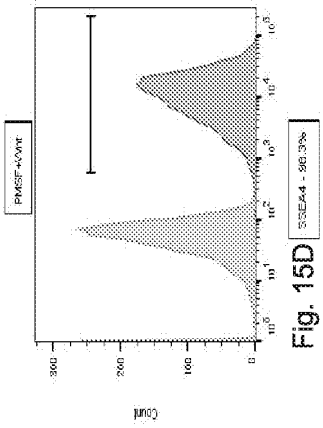


Fig. 15D

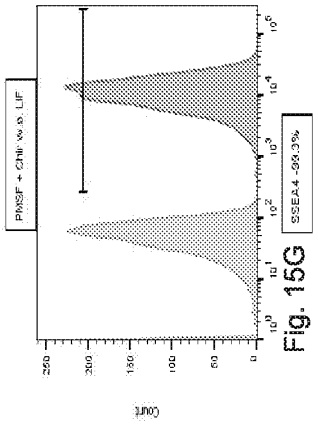


Fig. 15G

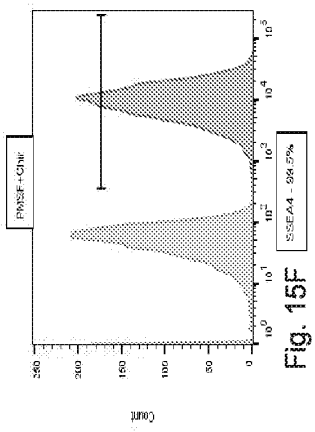
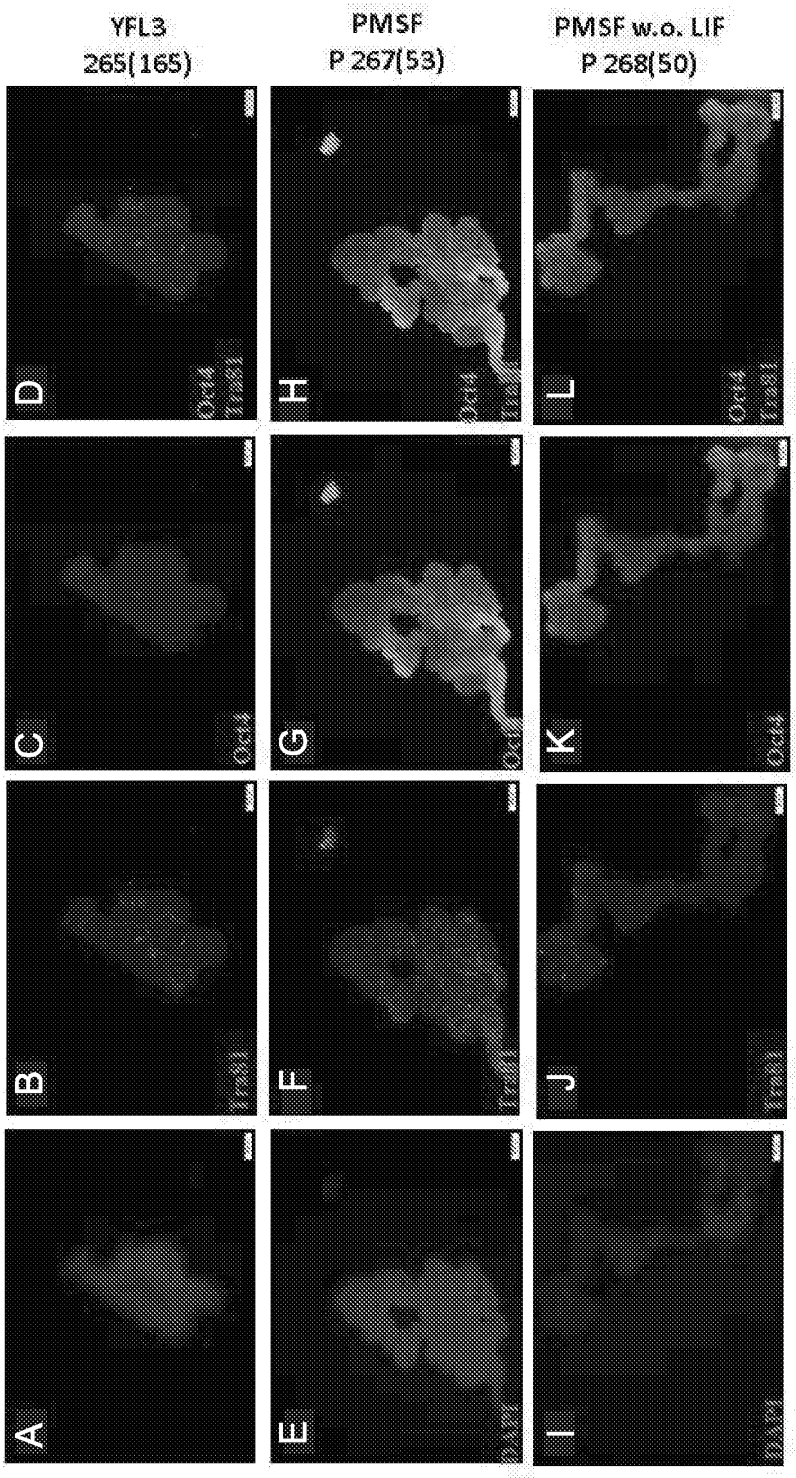
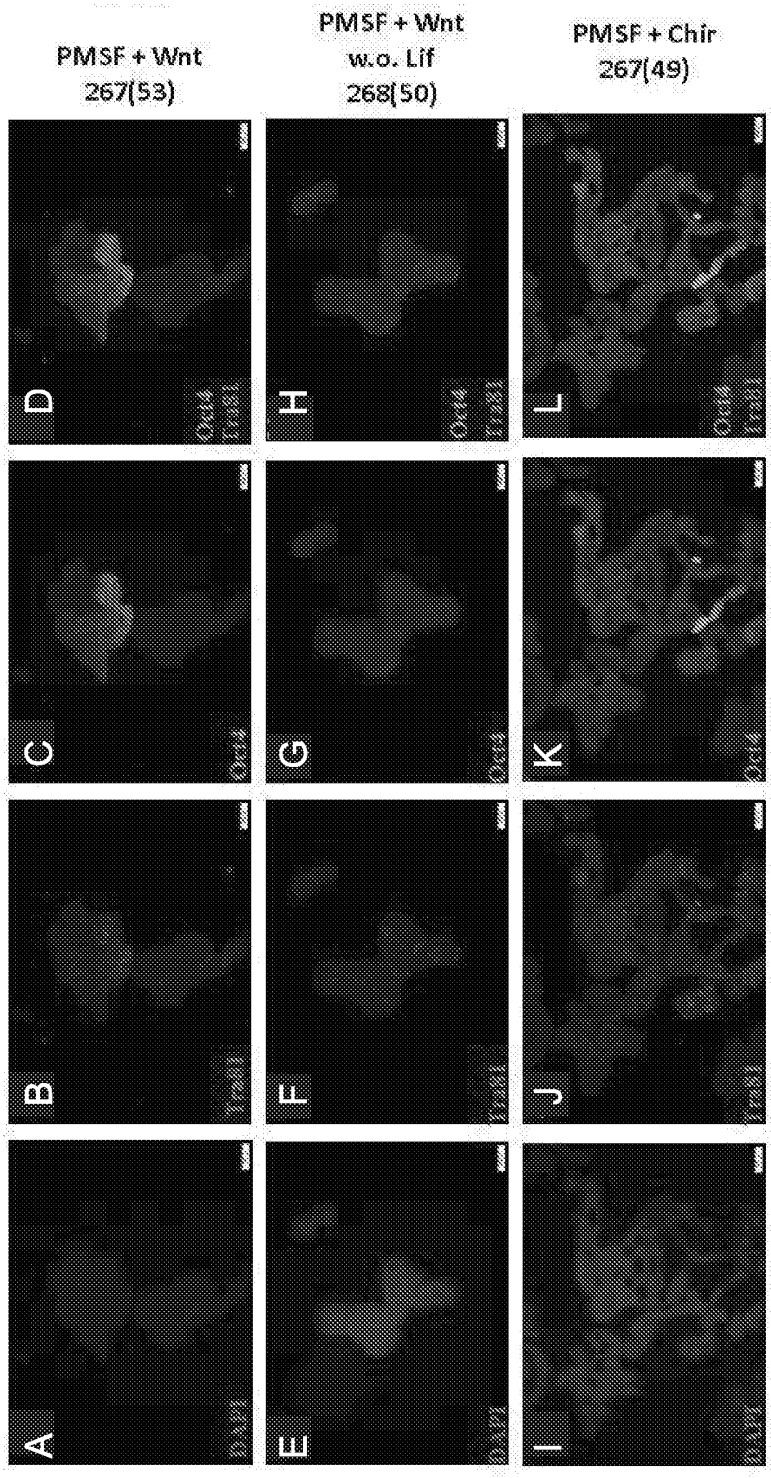


Fig. 15F


YFL3
265(165)
PMSF
P 267(53)
PMSF w.o. LIF
P 268(50)
A
B
C
D
E
F
G
H
I
J
K
L
Oct4
Tra-1
DAPI

Figs. 16A-L


PMSF + Wnt
267(53)
PMSF + Wnt
w.o. Lif
268(50)
PMSF + Chir
267(49)
A
B
C
D
E
F
G
H
I
J
K
L
DAPI
Tra81
Oct4
Oct4
Tra81

Figs. 17A-L

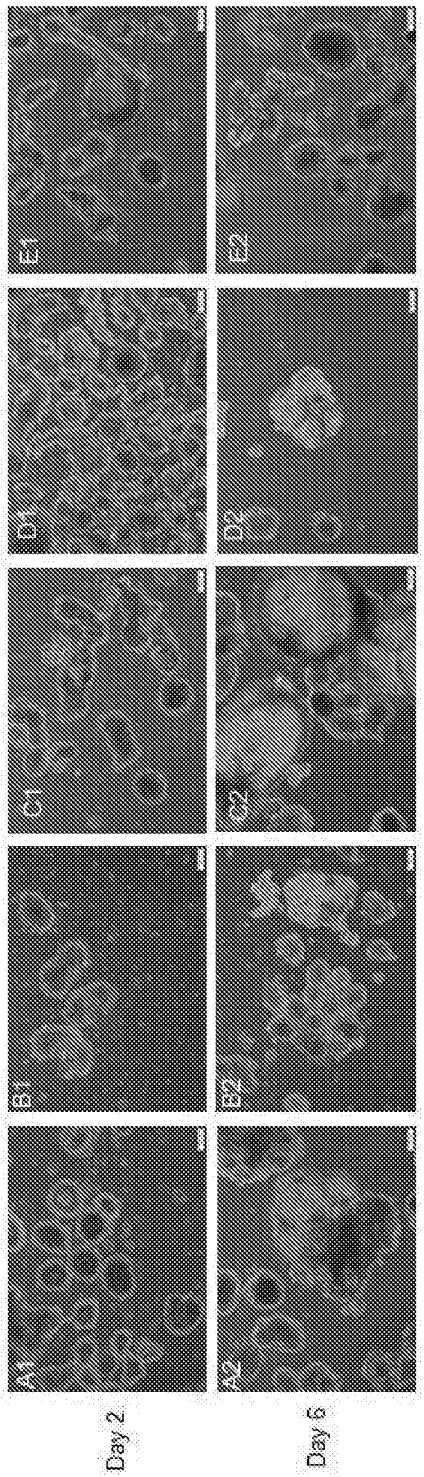


Fig. 18A Fig. 18B Fig. C Fig. 18D Fig. 18E

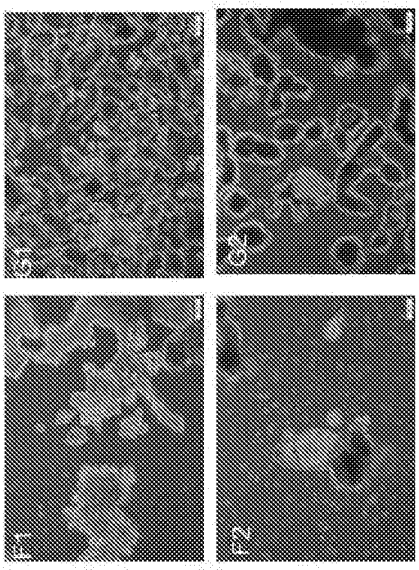
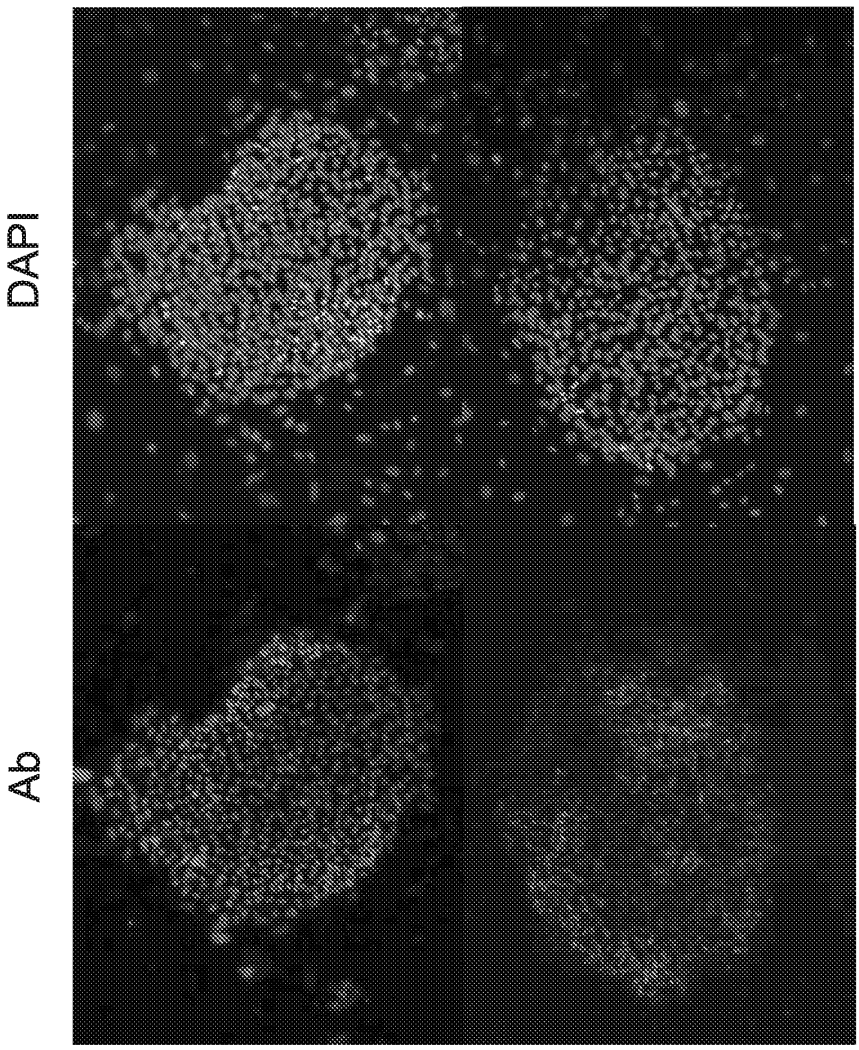
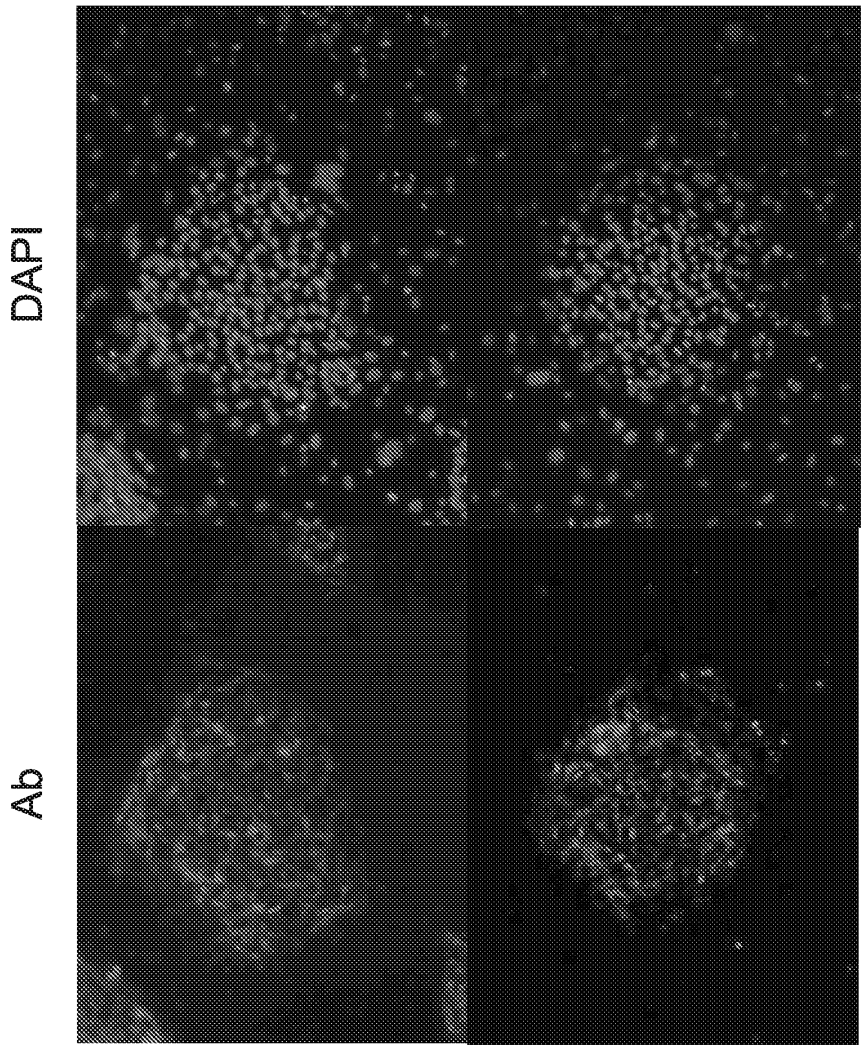


Fig. 18F Fig. 18G


Fig. 19B
Fig. 19D
DAPI
Ab
Fig. 19A
Oct 4
Fig. 19C
SSEA4


Fig. 20B
Fig. 20D
DAPI
Ab
Fig. 20A
TRA-1-60
Fig. 20C
TRA-1-81

HFF



Fig. 21A

Matrigel

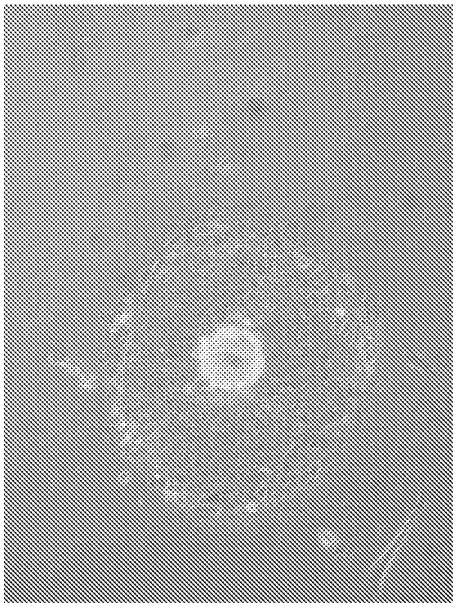
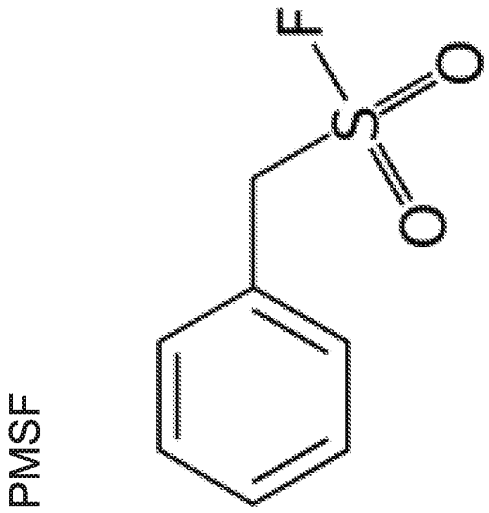


Fig. 21C

MEF



Fig. 21B



PMSF

Fig. 22

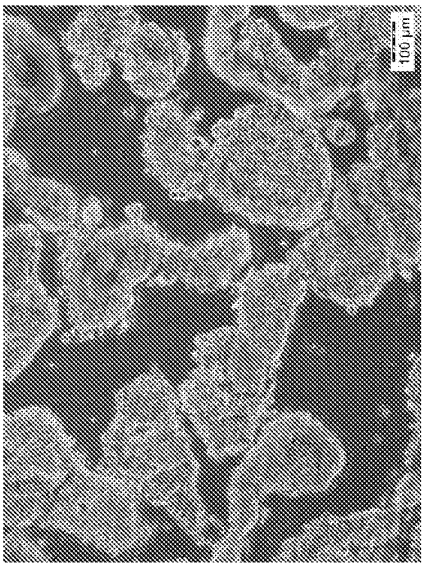


Fig. 23A

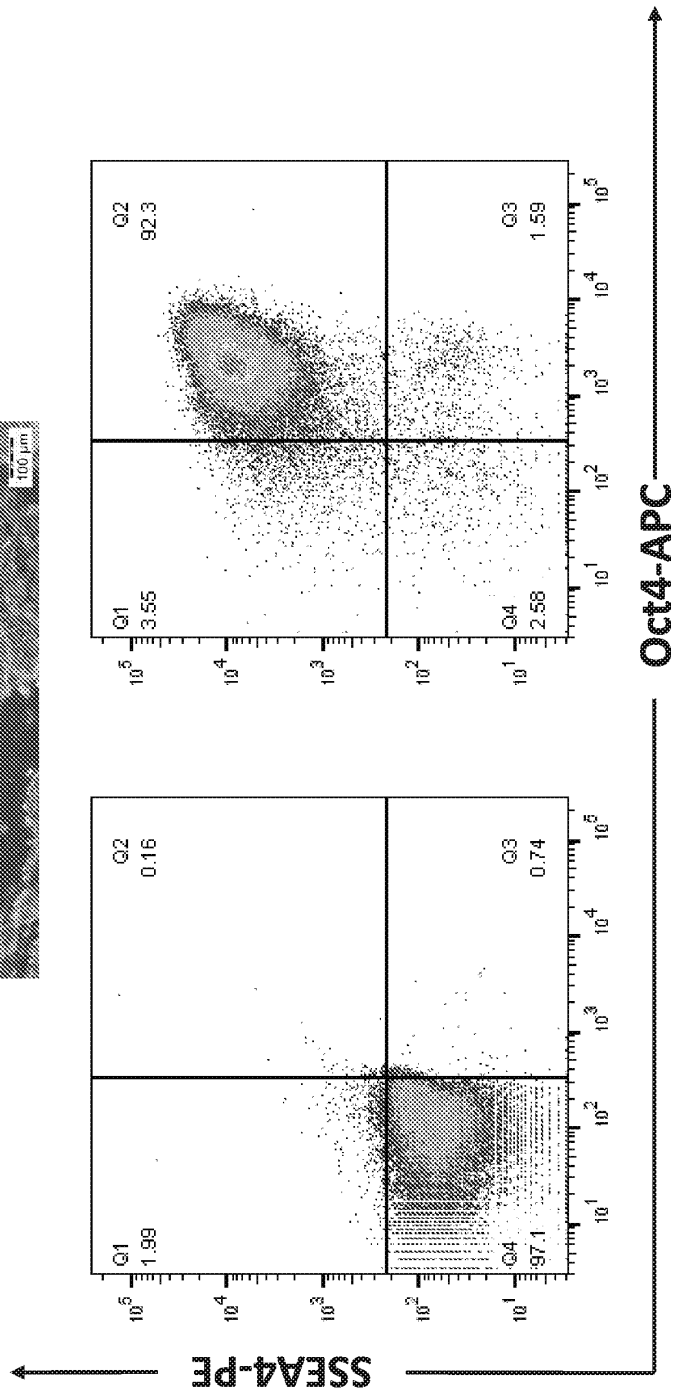


Fig. 23B

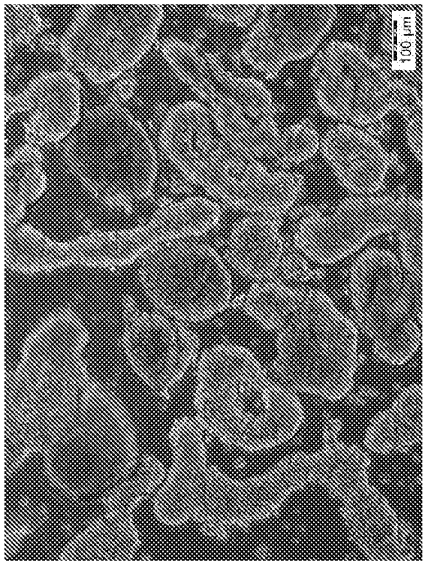


Fig. 24A

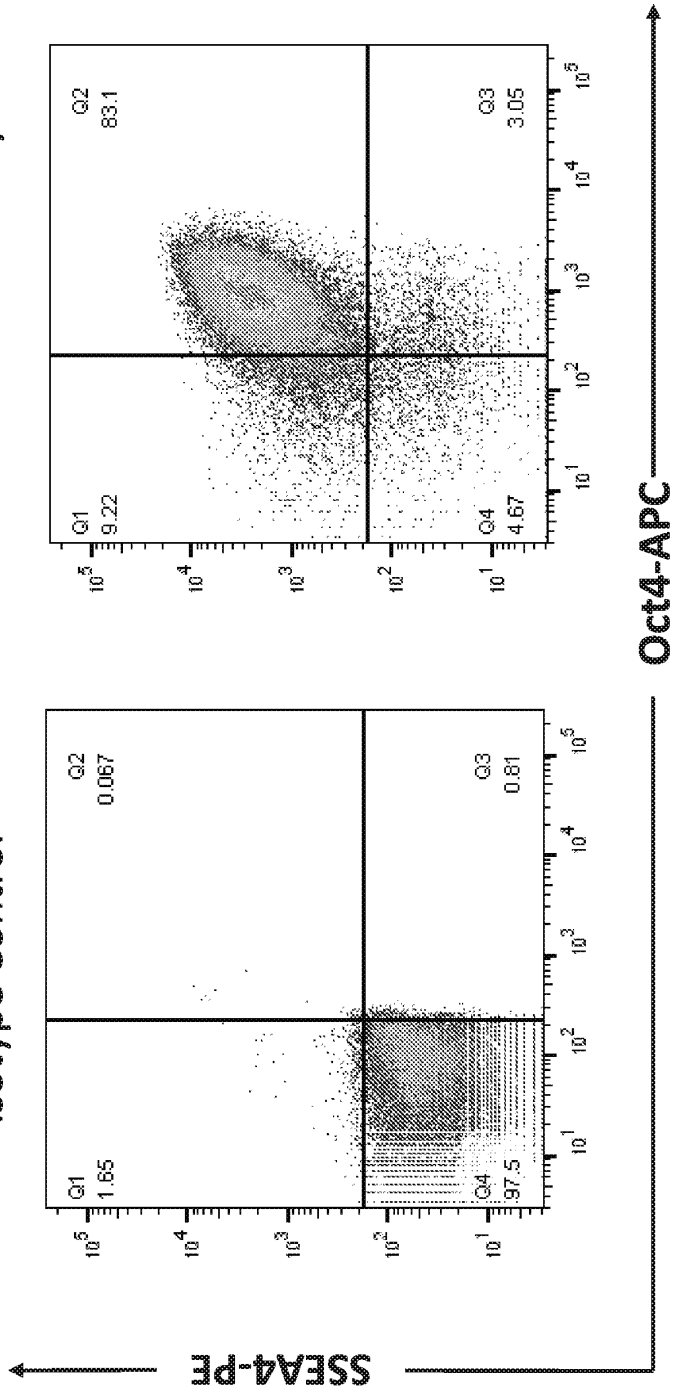


Fig. 24B

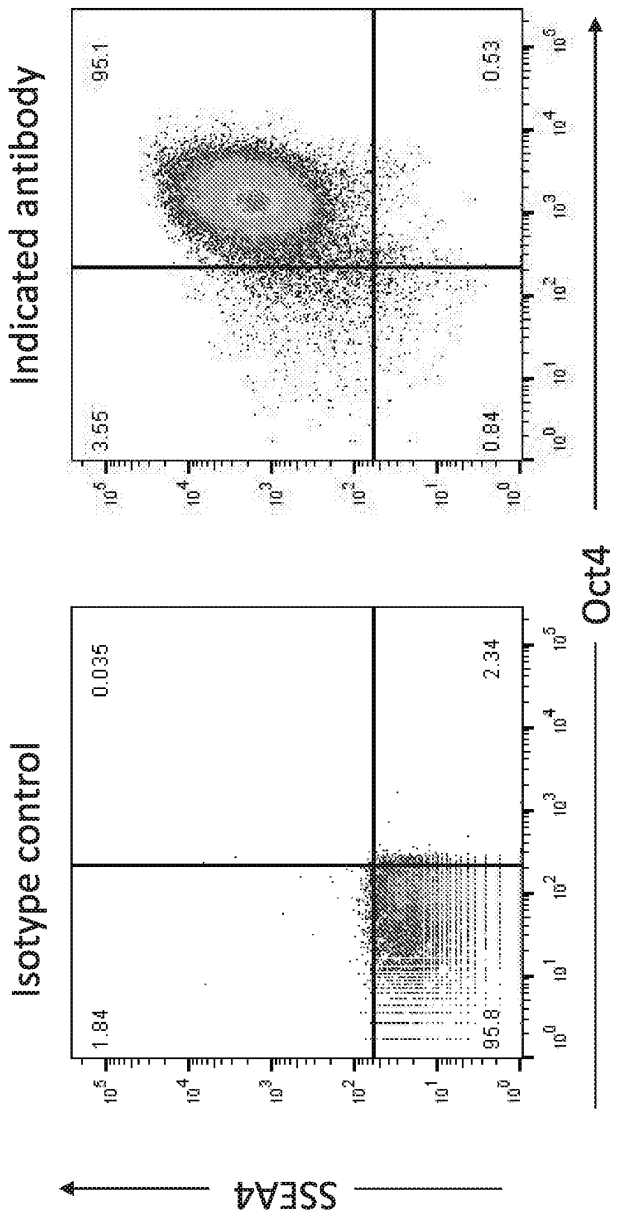

Indicated antibody
Isotype control
95.1
0.53
3.55
0.84
0.035
2.34
1.84
95.8
SSEA4
Oct4

Fig. 25

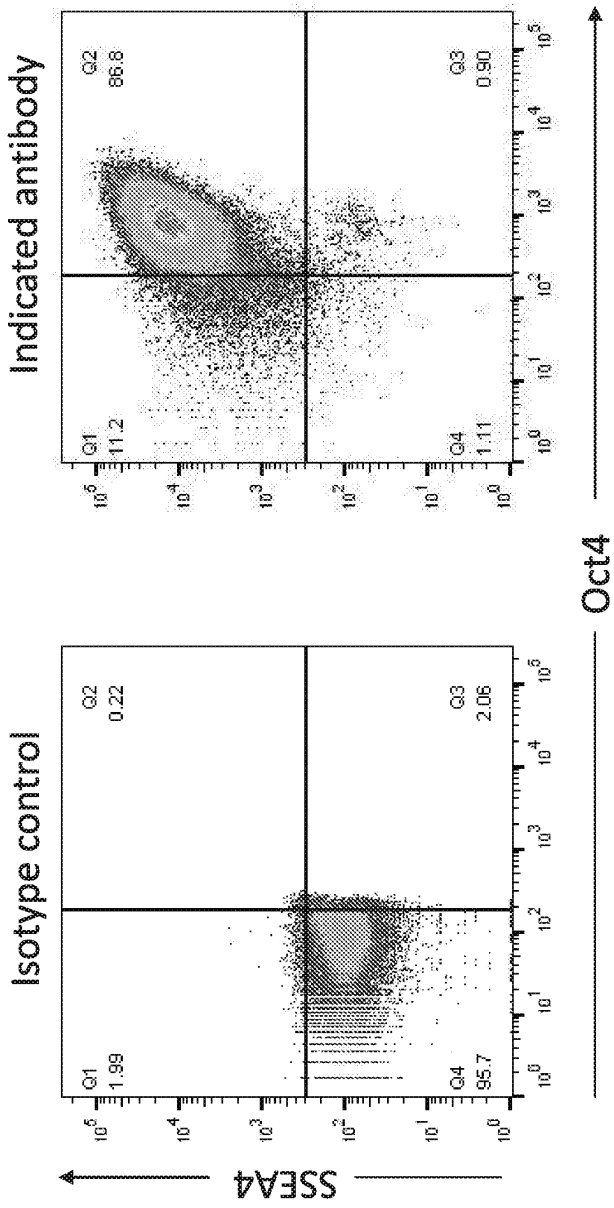

Indicated antibody
Isotype control
Q1
11.2
Q2
86.8
Q3
0.90
Q4
1.11
Q1
1.99
Q2
0.22
Q3
2.06
Q4
95.7
SSEA4
Oct4

Fig. 26

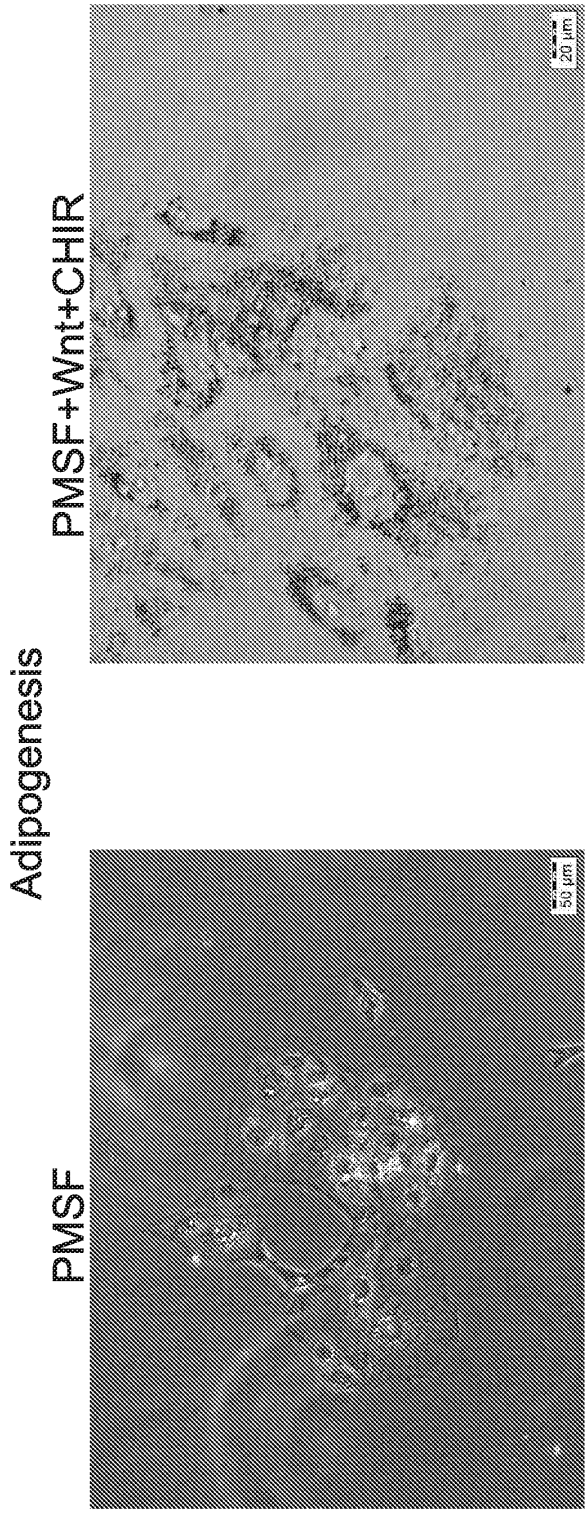


Fig. 27A

Fig. 27B

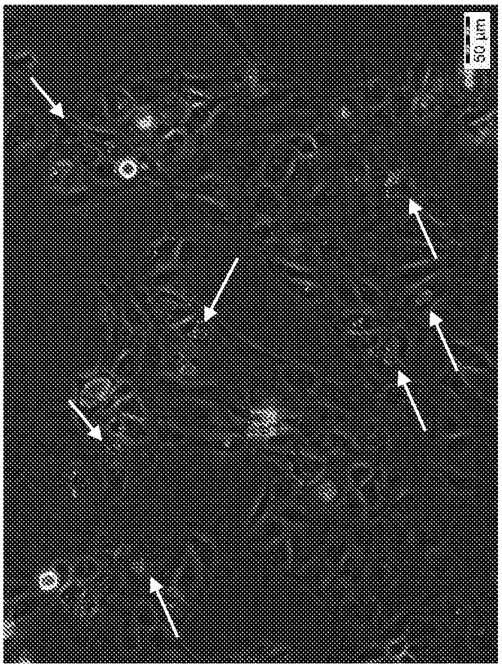


Fig. 27C