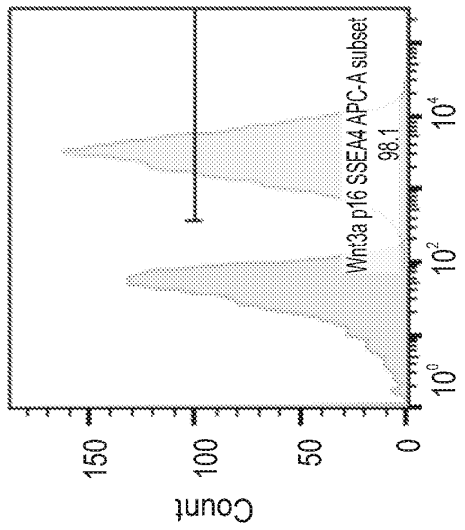


Fig. 1C

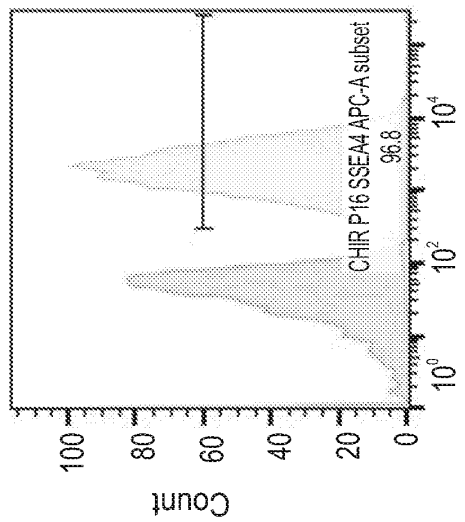


Fig. 1B

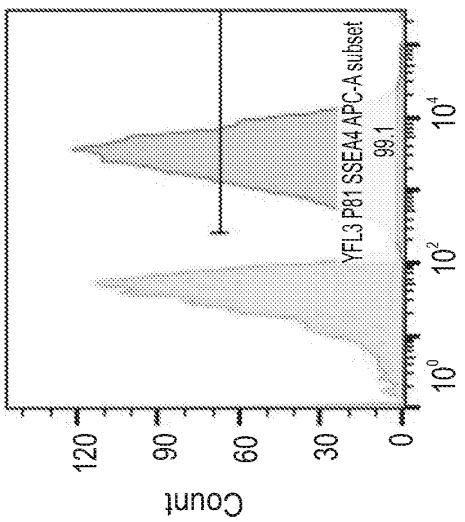


Fig. 1A

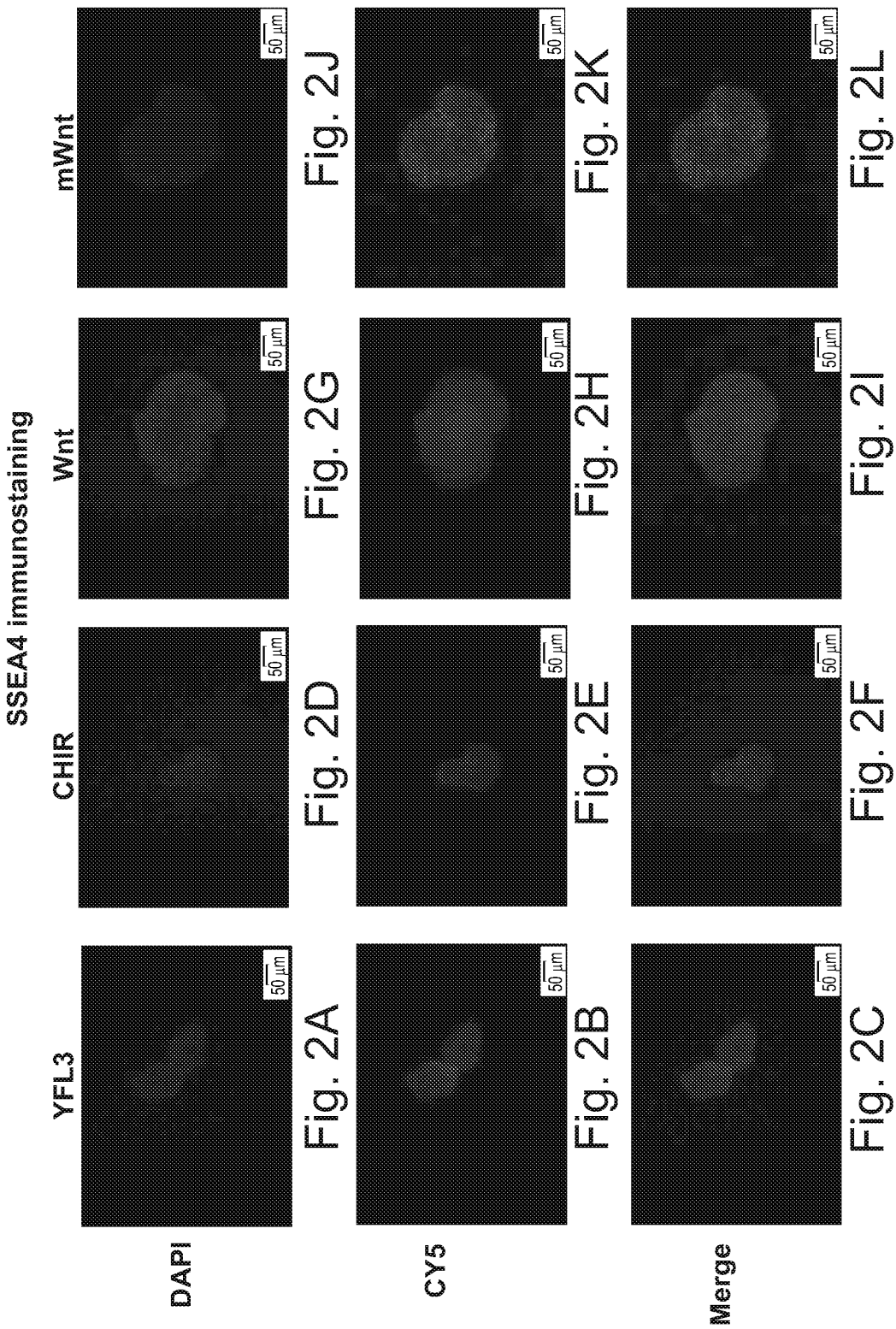
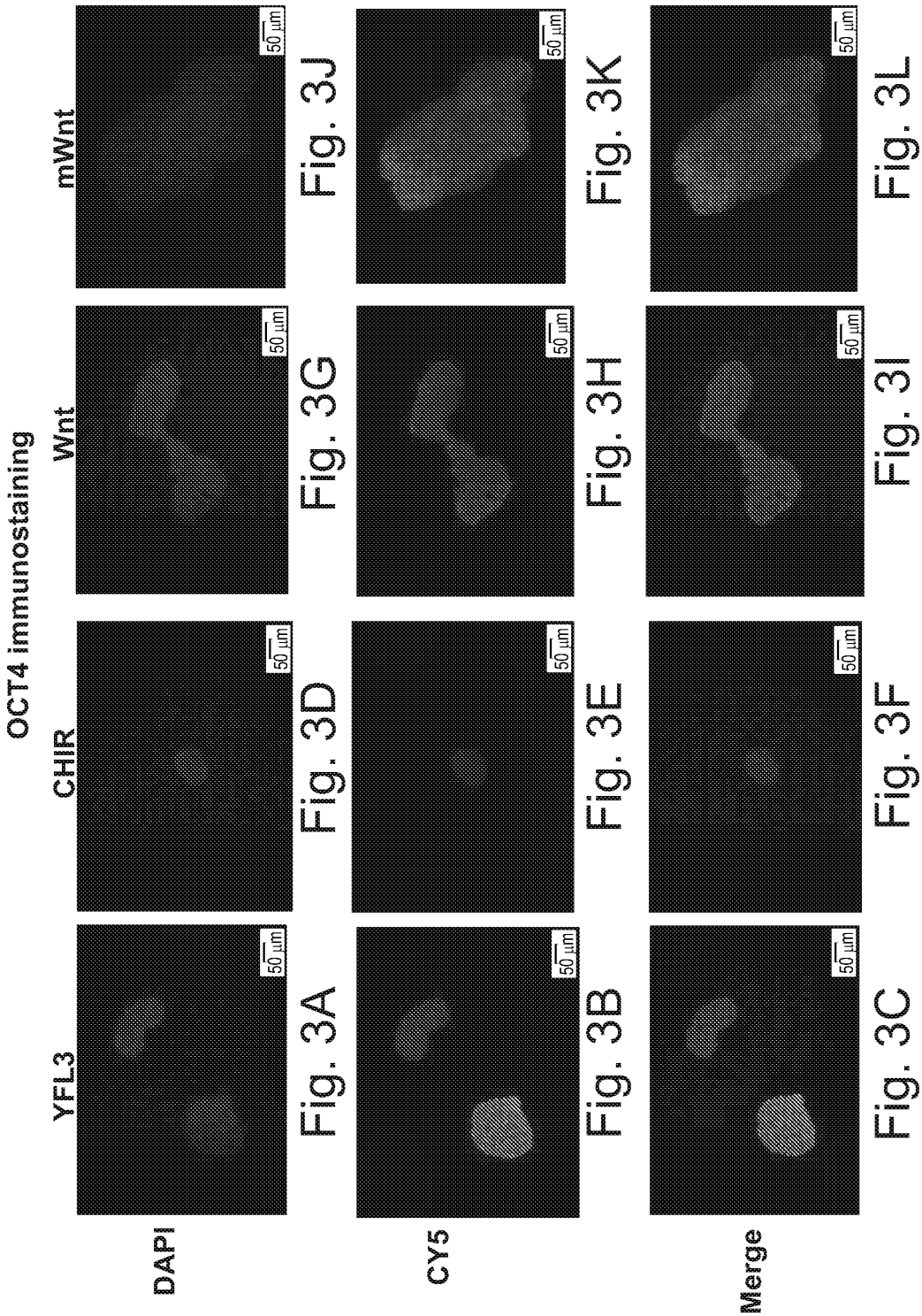


Fig. 2A Fig. 2B Fig. 2C Fig. 2D Fig. 2E Fig. 2F Fig. 2G Fig. 2H Fig. 2I Fig. 2J Fig. 2K Fig. 2L


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

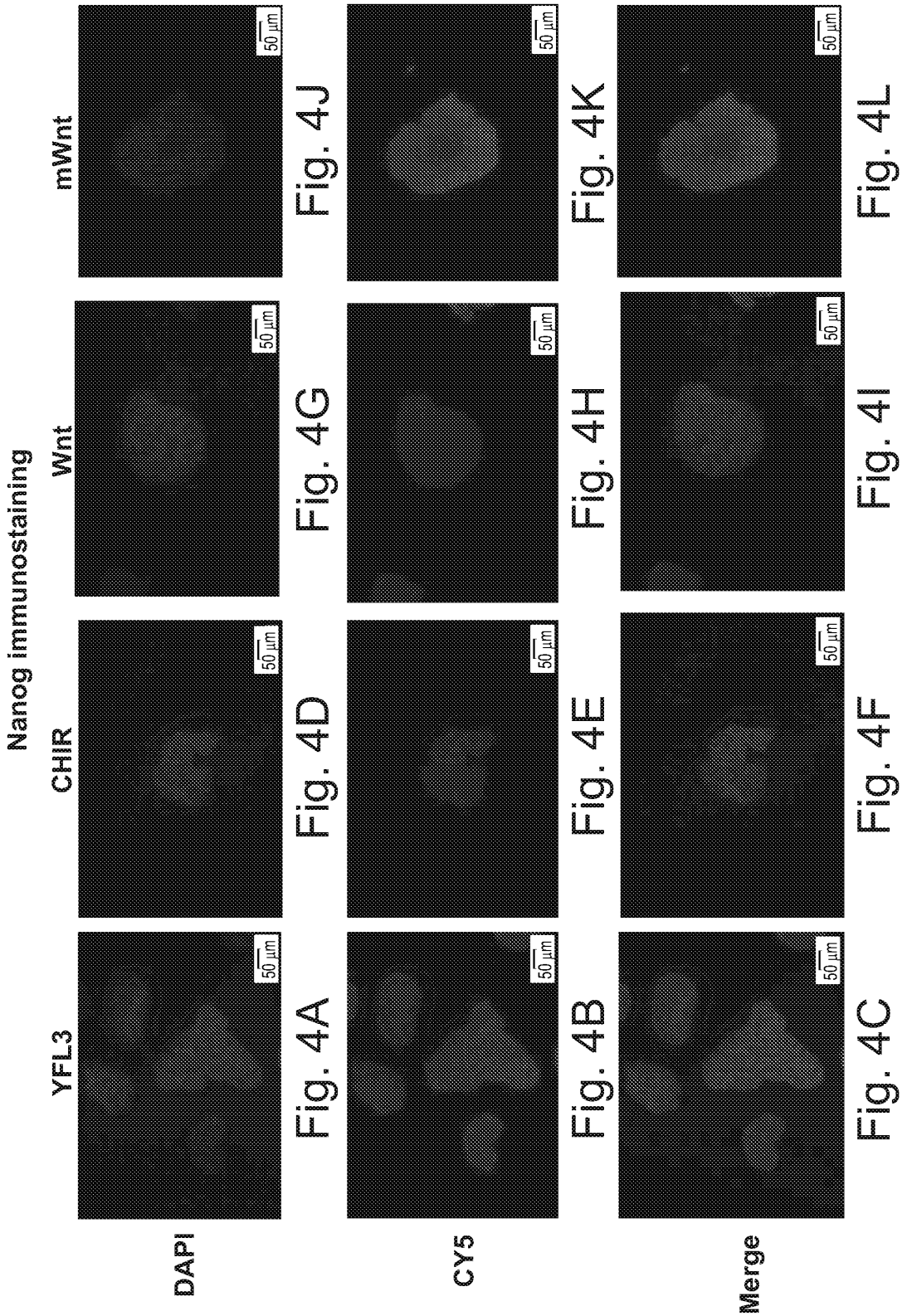


Fig. 4A Fig. 4B Fig. 4C Fig. 4D Fig. 4E Fig. 4F Fig. 4G Fig. 4H Fig. 4I Fig. 4J Fig. 4K Fig. 4L

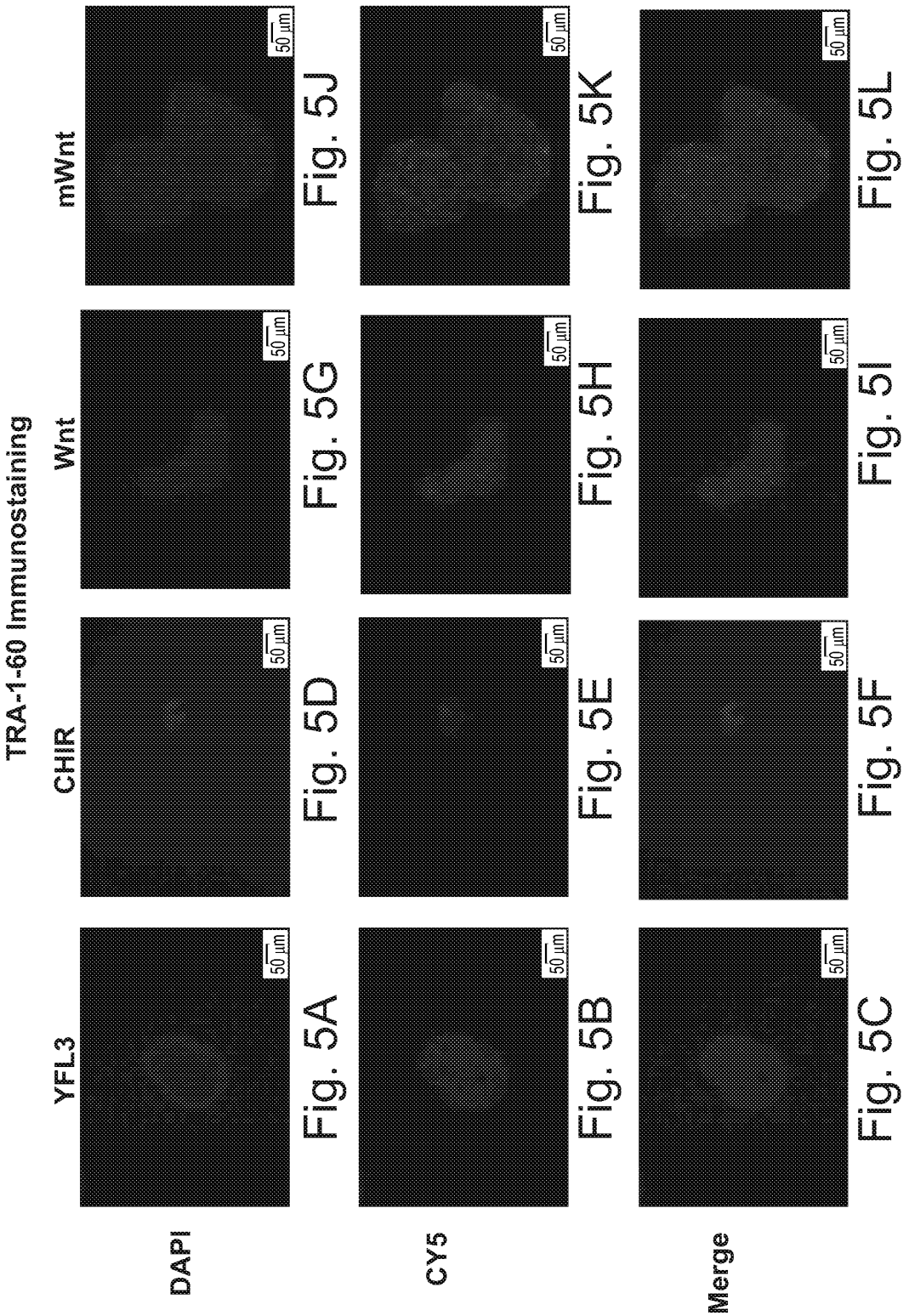


Fig. 5A Fig. 5D Fig. 5G Fig. 5J

Fig. 5B Fig. 5E Fig. 5H Fig. 5K

Fig. 5C Fig. 5F Fig. 5I Fig. 5L

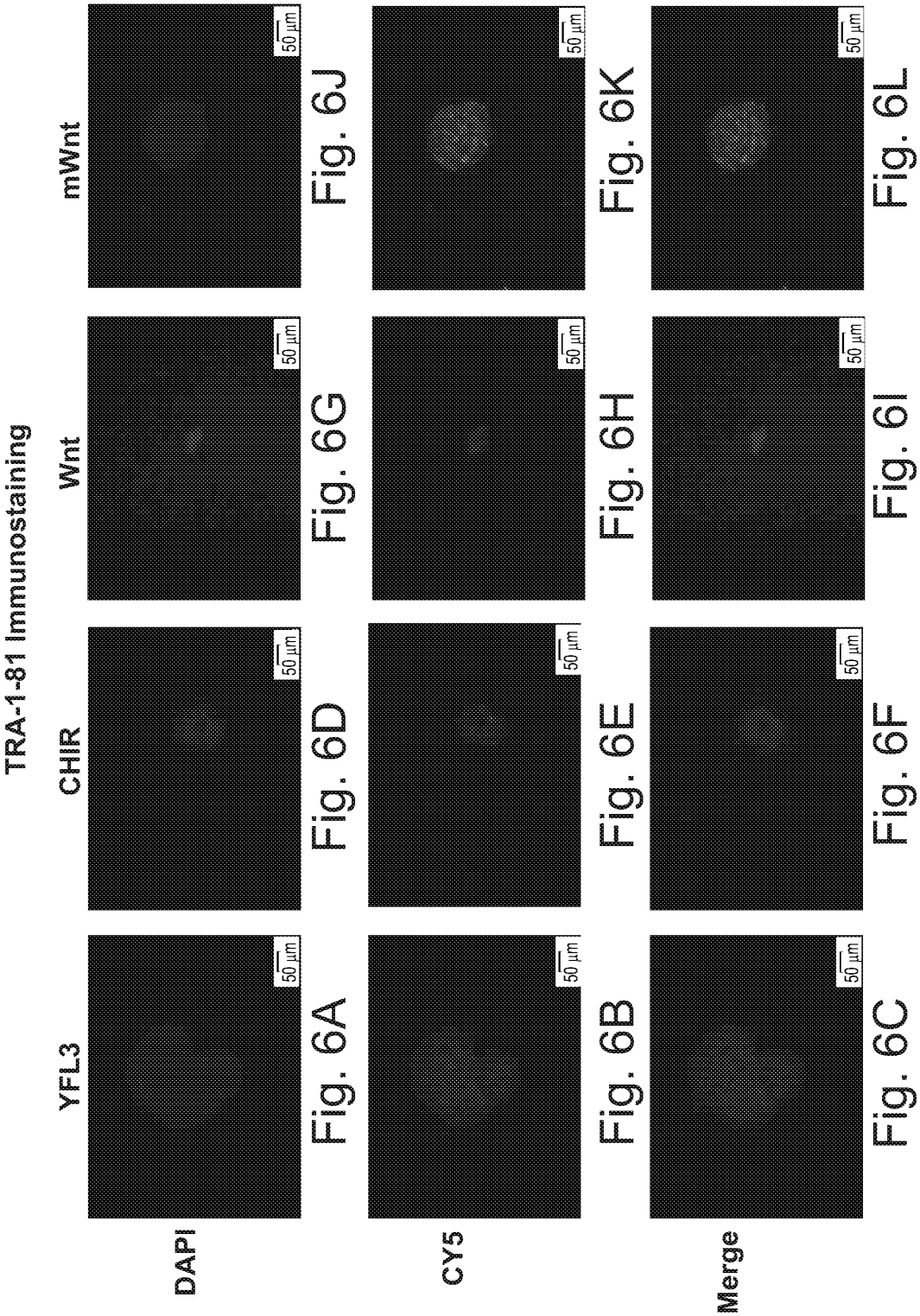


Fig. 6A Fig. 6D Fig. 6G Fig. 6J

Fig. 6B Fig. 6E Fig. 6H Fig. 6K

Fig. 6C Fig. 6F Fig. 6I Fig. 6L

Fig. 7B

Fig. 7D

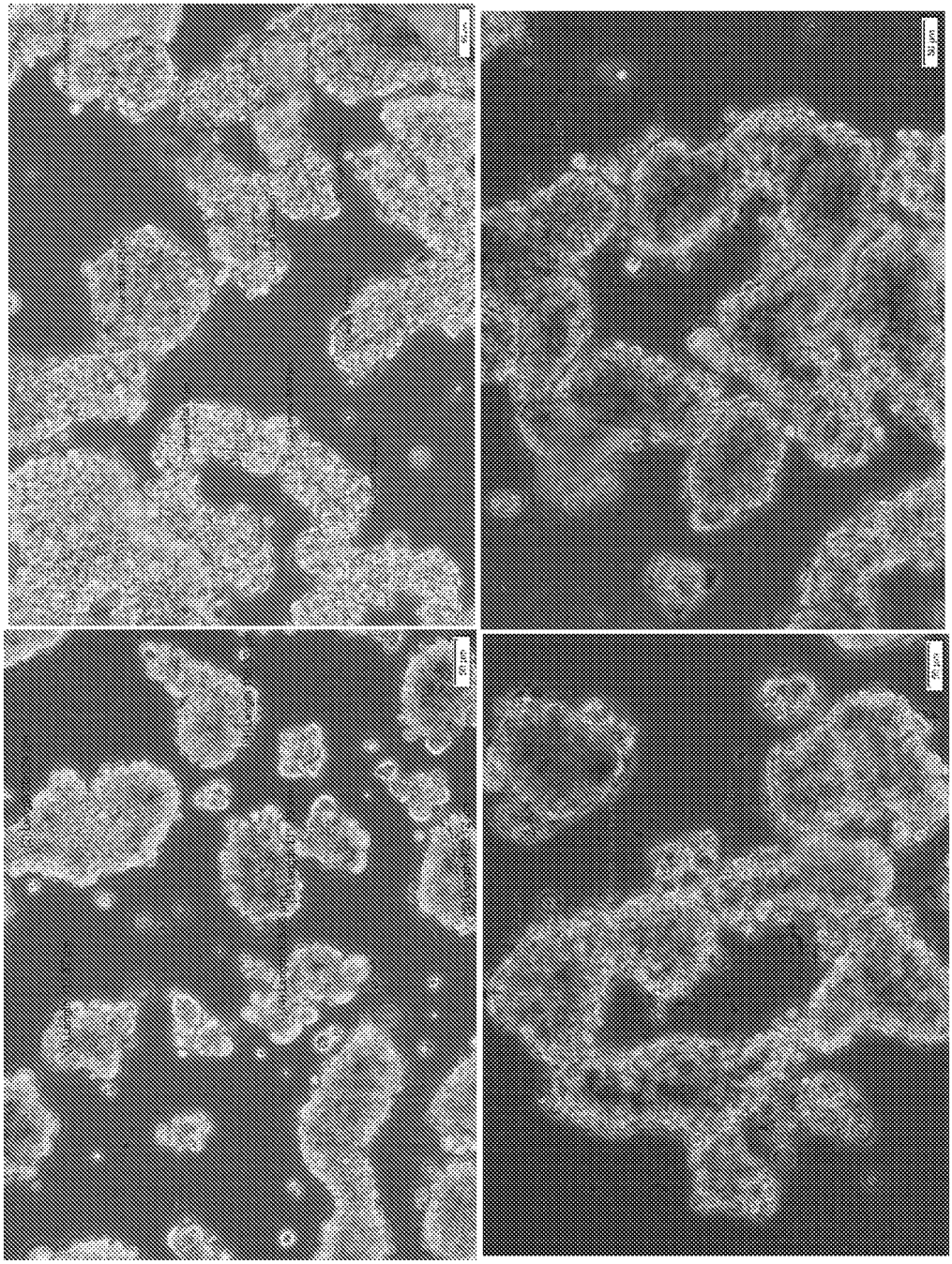
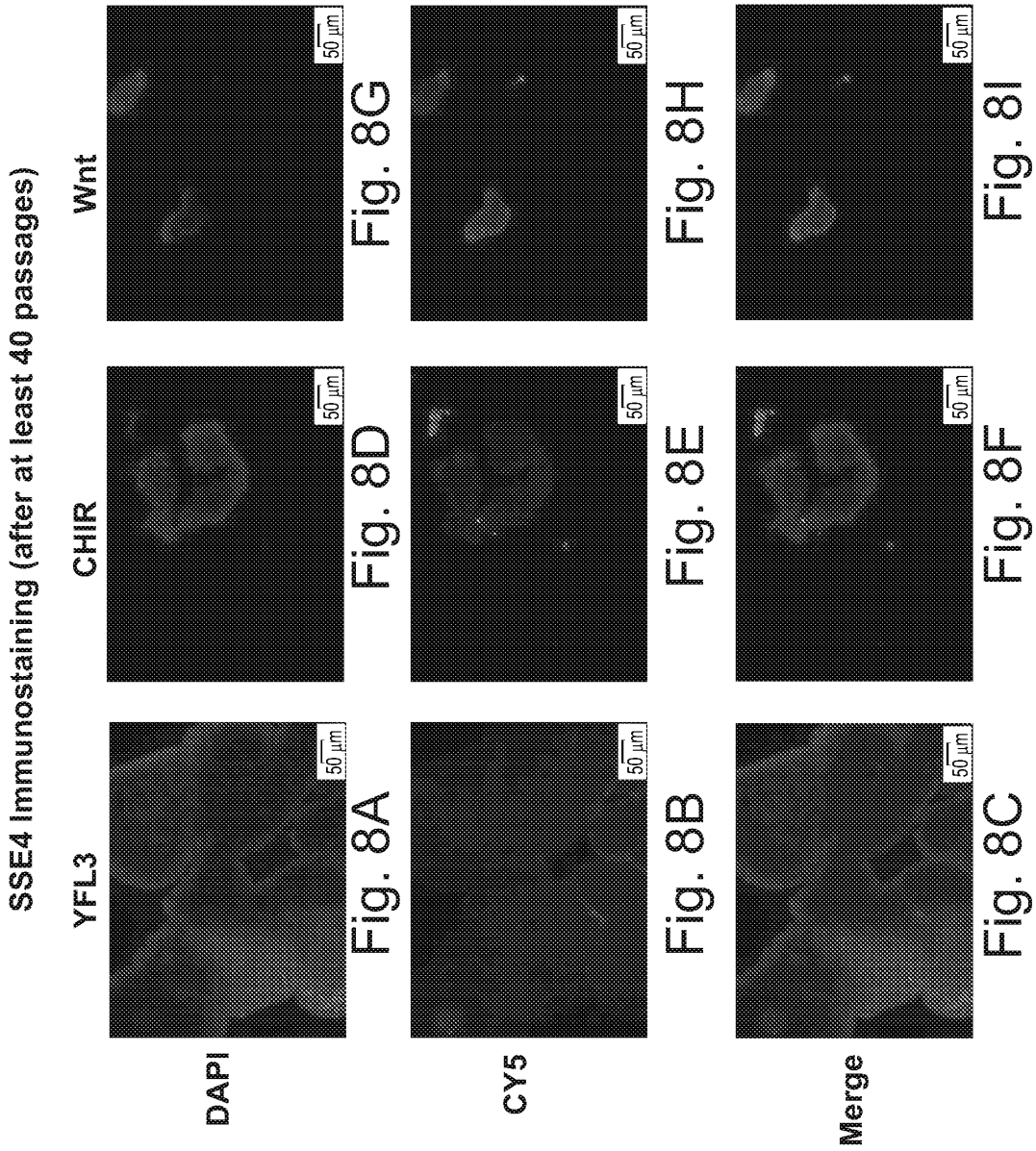


Fig. 7A

Fig. 7C


SSE4 Immunostaining (after at least 40 passages)
YFL3
CHIR
Wnt
DAPI
CY5
Merge
50 µm
Fig. 8A
Fig. 8B
Fig. 8C
Fig. 8D
Fig. 8E
Fig. 8F
Fig. 8G
Fig. 8H
Fig. 8I

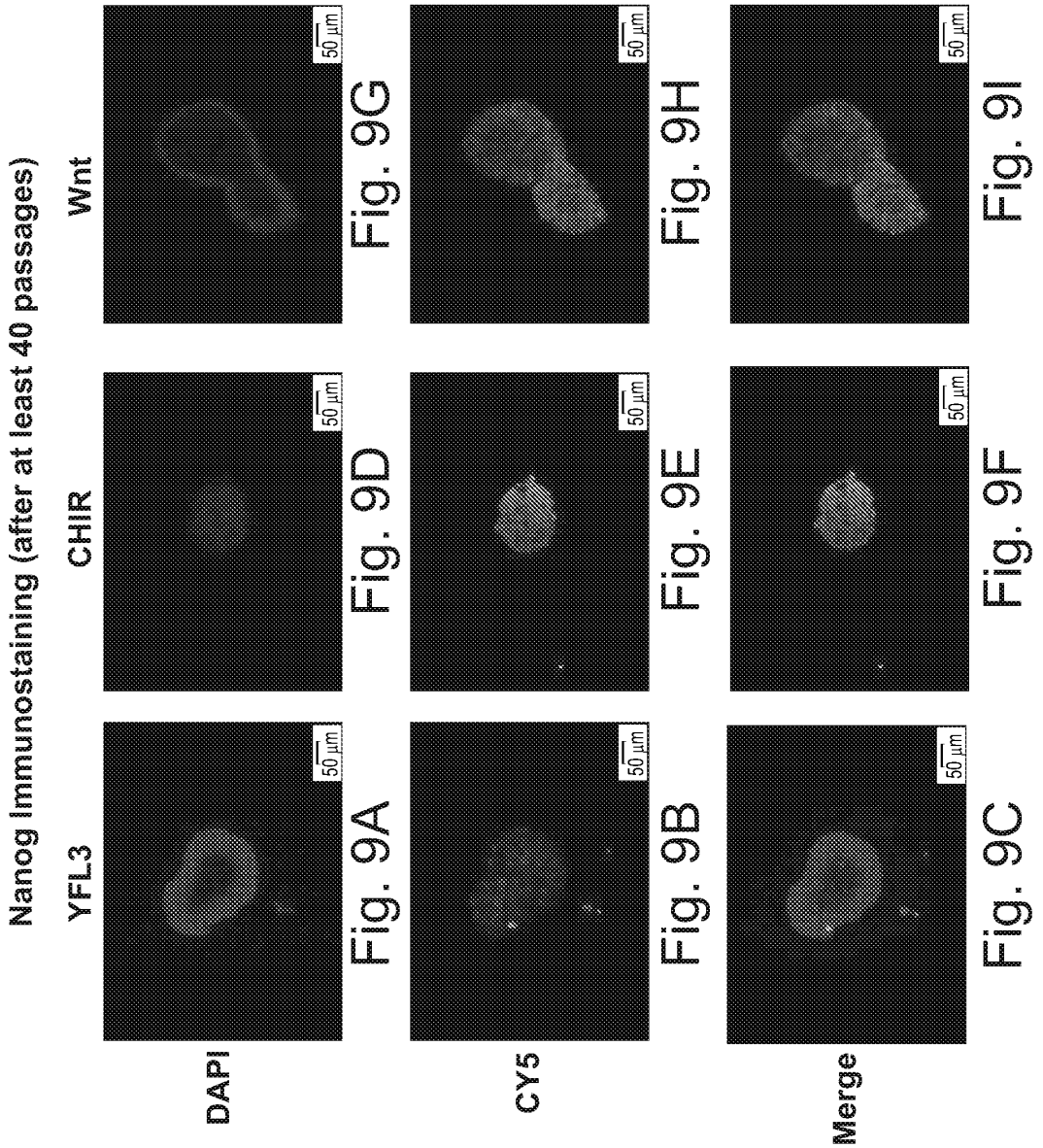


Fig. 9A Fig. 9D Fig. 9G

Fig. 9B Fig. 9E Fig. 9H

Fig. 9C Fig. 9F Fig. 9I

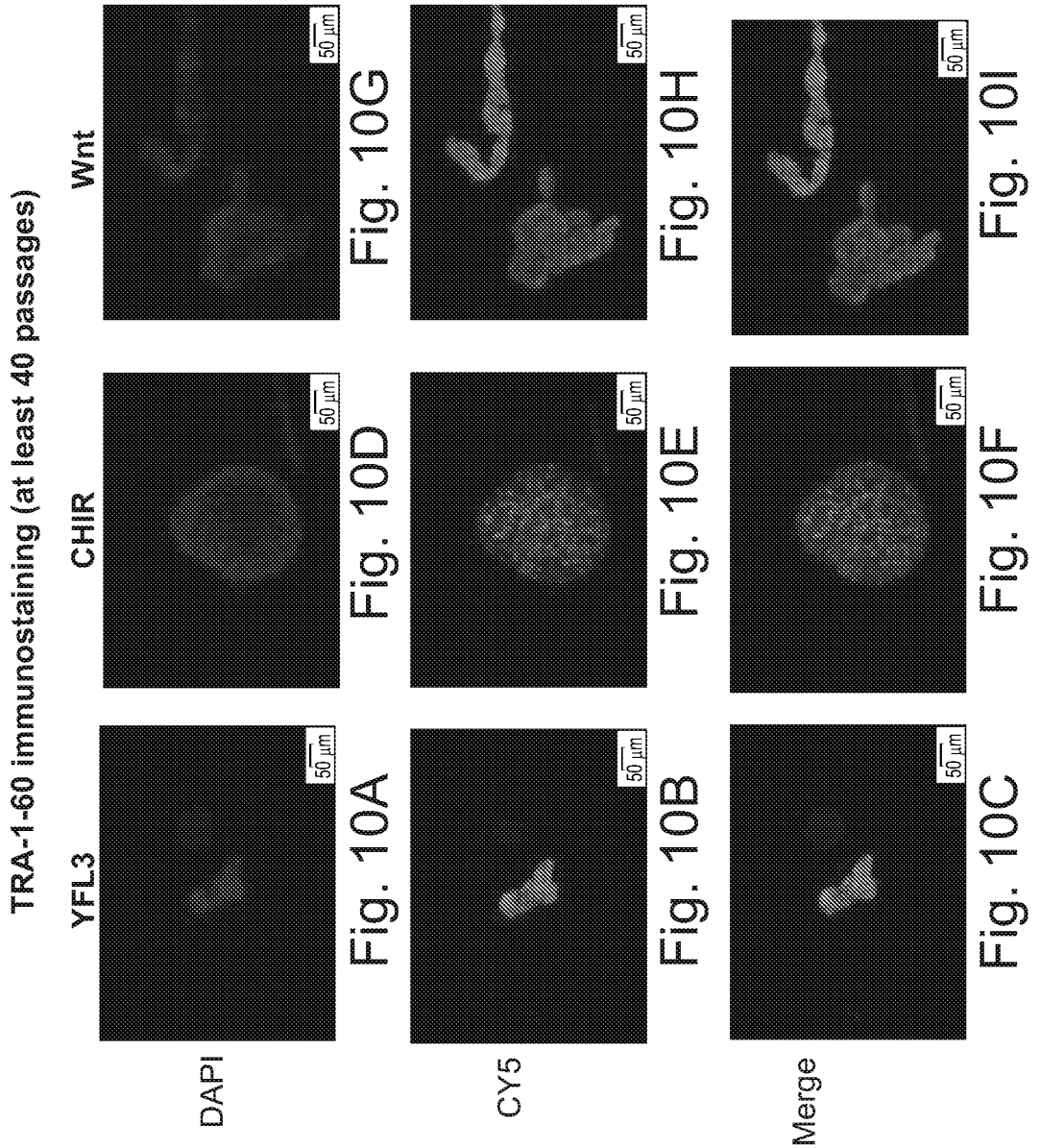


Fig. 10A Fig. 10B Fig. 10C Fig. 10D Fig. 10E Fig. 10F Fig. 10G Fig. 10H Fig. 10I

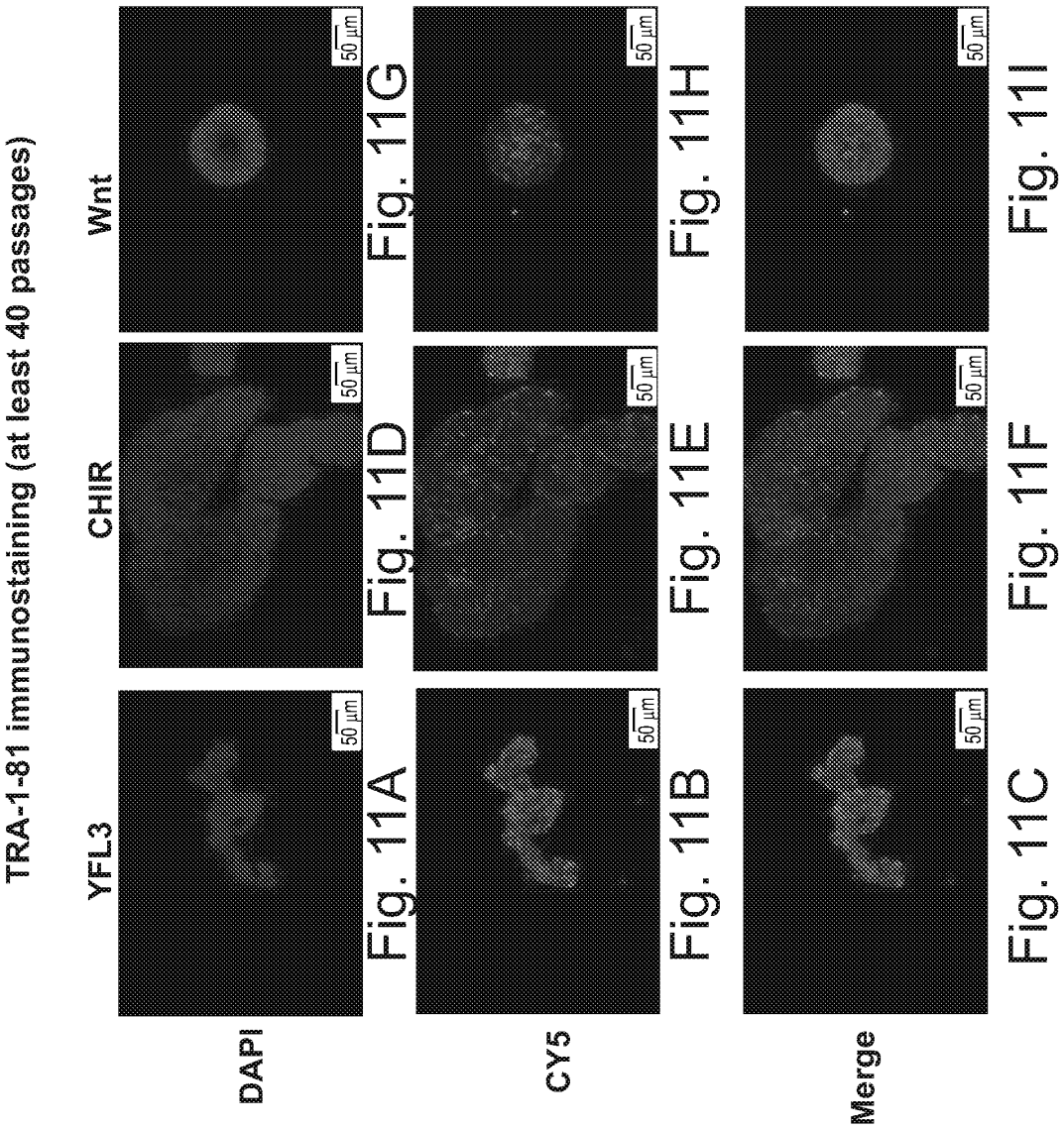


Fig. 11A Fig. 11B Fig. 11C Fig. 11D Fig. 11E Fig. 11F Fig. 11G Fig. 11H Fig. 11I

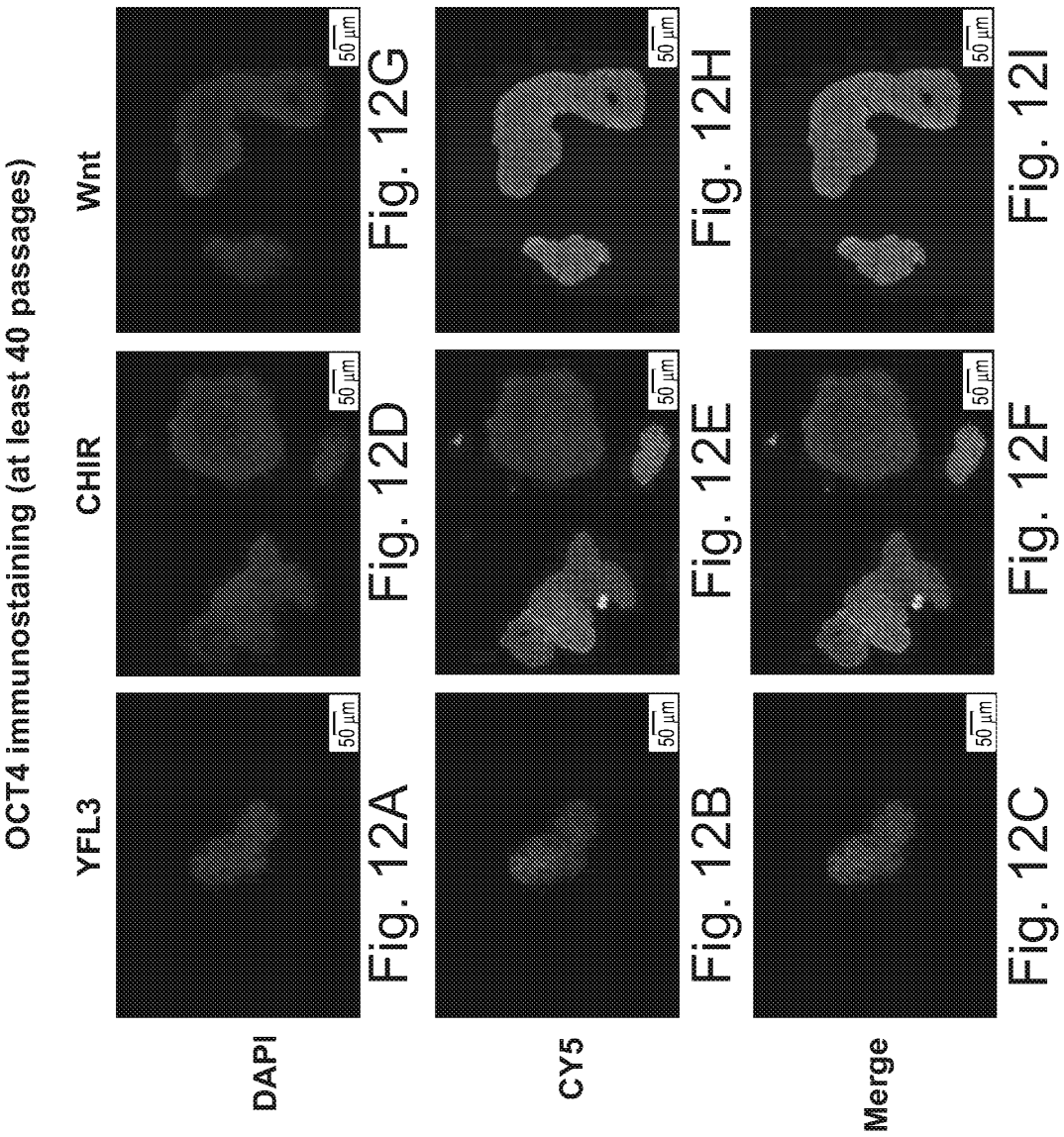


Fig. 12A Fig. 12D Fig. 12G

Fig. 12B Fig. 12E Fig. 12H

Fig. 12C Fig. 12F Fig. 12I

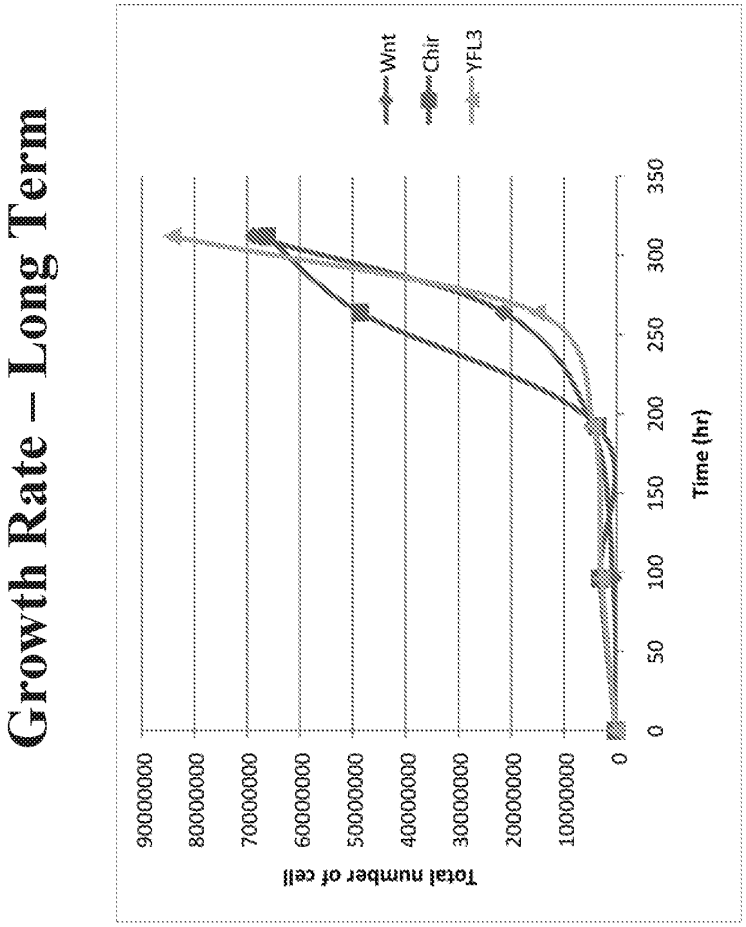

Growth Rate – Long Term
Total number of cell
90000000
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
0
50
100
150
200
250
300
350
Time (hr)
Wnt
Chir
YFL3

Fig. 13A

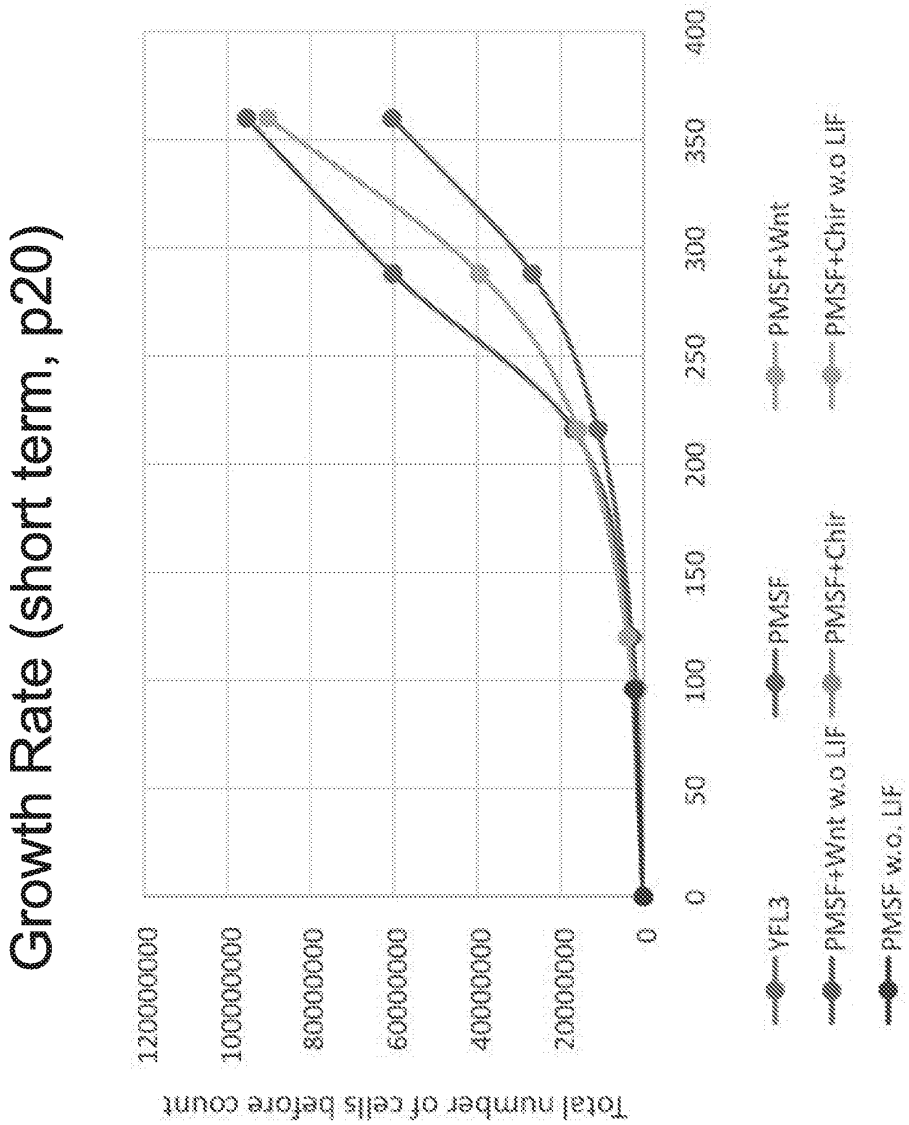

Growth Rate (short term, p20)
Total number of cells before count
12000000
10000000
8000000
6000000
4000000
2000000
0
0
50
100
150
200
250
300
350
400
YFL3
PMSF
PMSF+Wnt
PMSF+Wnt w.o LIF
PMSF+Chir
PMSF+Chir w.o LIF
PMSF w.o. LIF

Fig. 13B

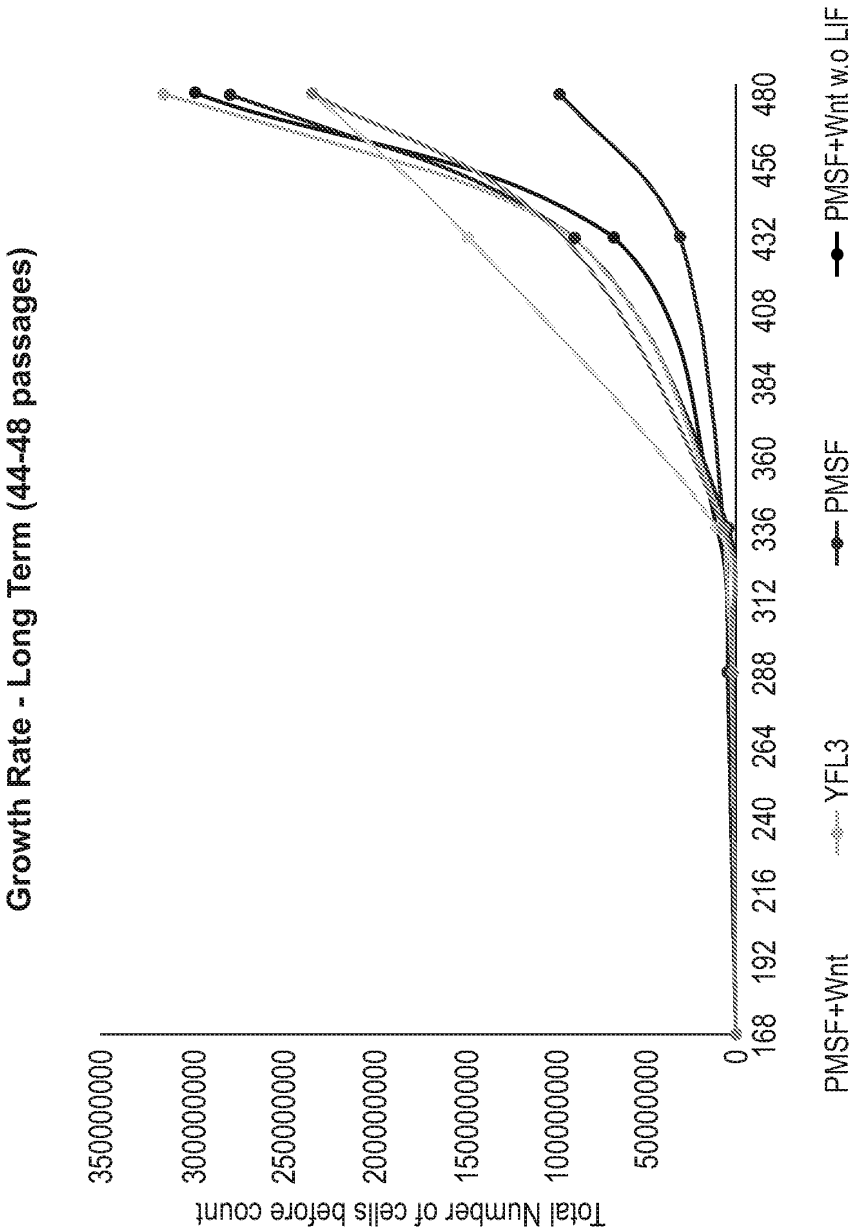

Growth Rate - Long Term (44-48 passages)
Total Number of cells before count
350000000
300000000
250000000
200000000
150000000
100000000
50000000
0
168
192
216
240
264
288
312
336
360
384
408
432
456
480
PMSF+Wnt
YFL3
PMSF
PMSF+Wnt w.o LIF
PMSF+Chir
PMSF+Chir w.o LIF
PMSF w.o. LIF

Fig. 13C

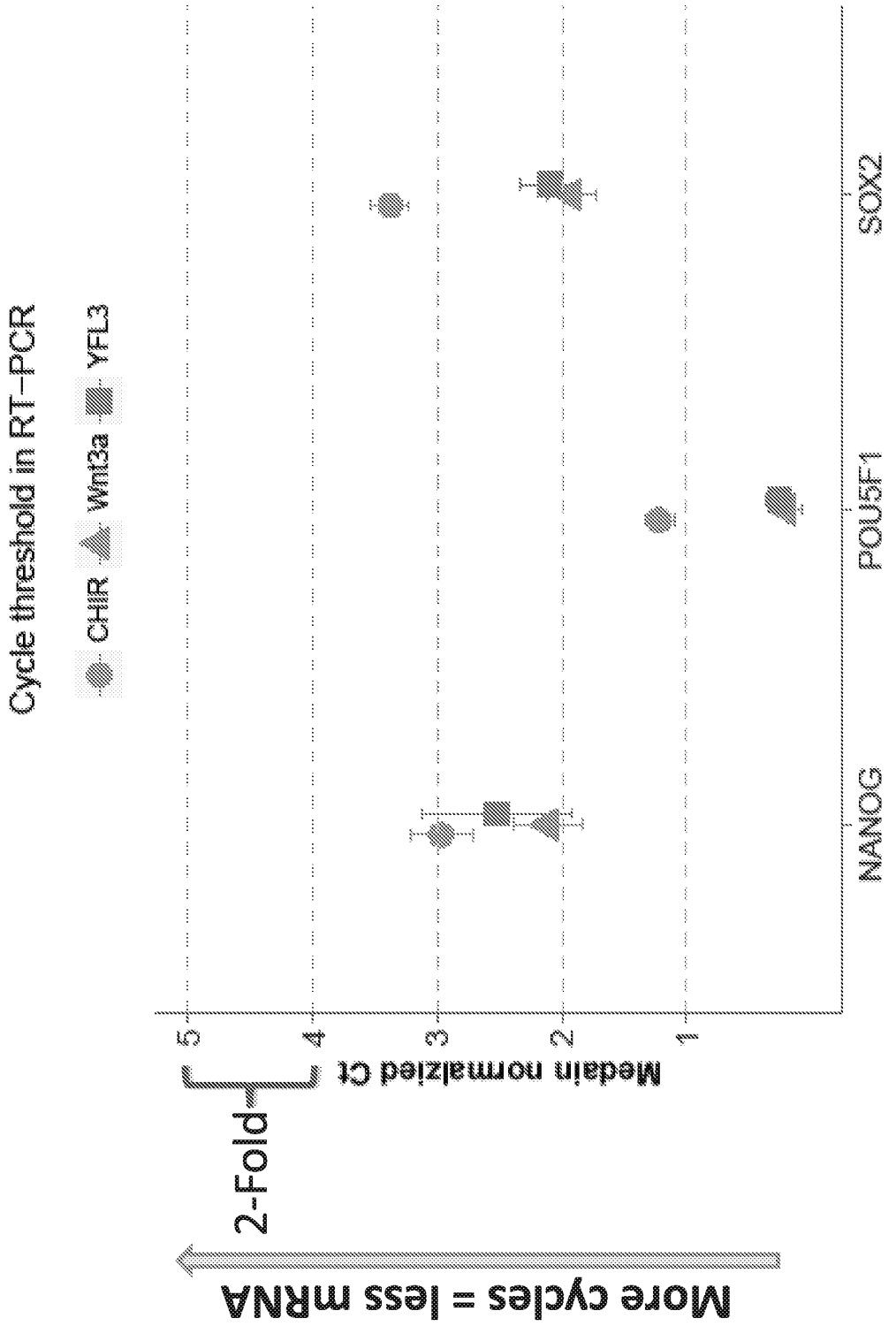
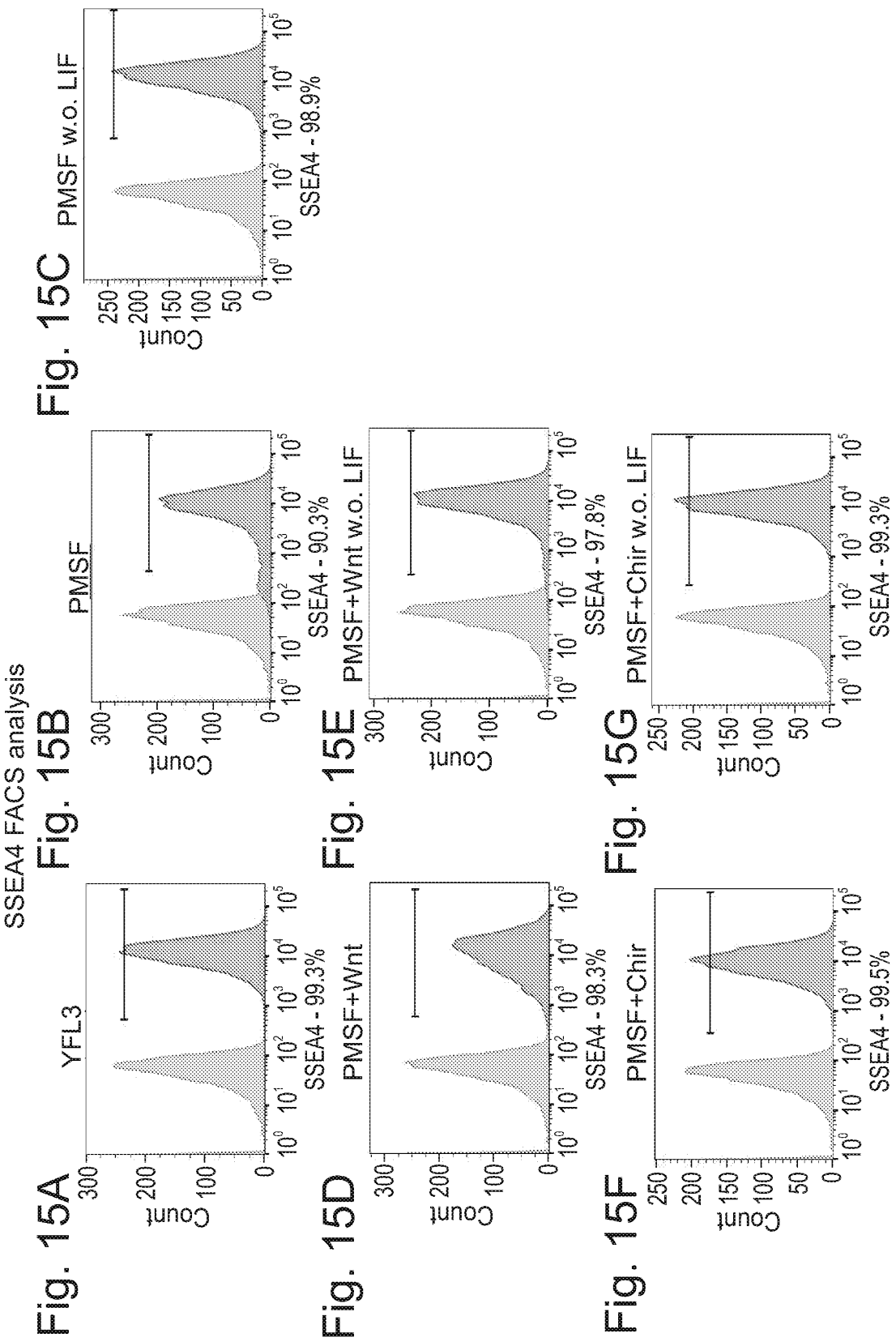
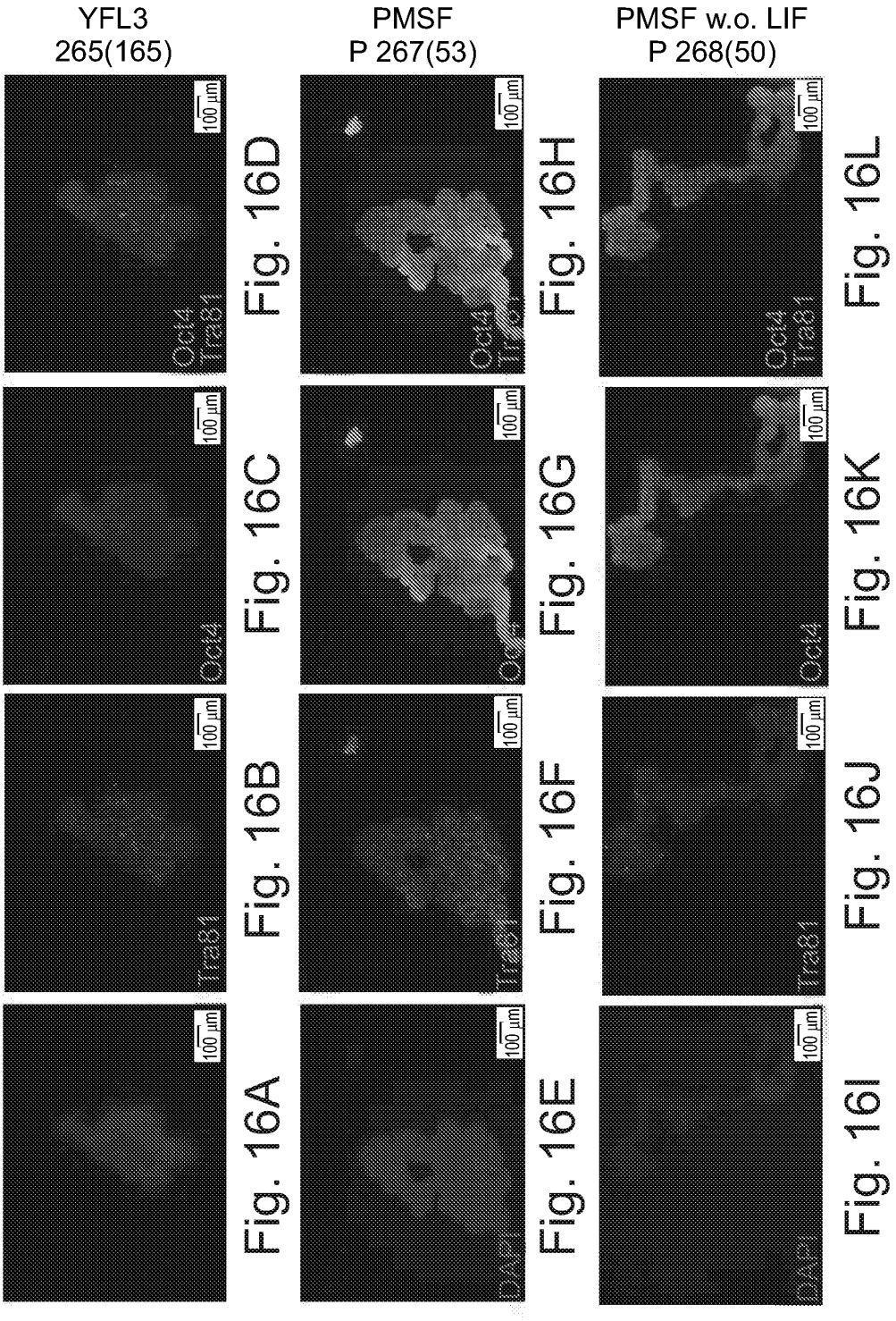

Cycle threshold in RT–PCR
CHIR
Wnt3a
YFL3
More cycles = less mRNA
2-Fold
Medain nommalzied ct
5
4
3
2
1
NANOG
POU5F1
SOX2
Gene name

Fig. 14


SSEA4 FACS analysis
Fig. 15A
YFL3
SSEA4 - 99.3%
Fig. 15B
PMSF
SSEA4 - 90.3%
Fig. 15C
PMSF w.o. LIF
SSEA4 - 98.9%
Fig. 15D
PMSF+Wnt
SSEA4 - 98.3%
Fig. 15E
PMSF+Wnt w.o. LIF
SSEA4 - 97.8%
Fig. 15F
PMSF+Chir
SSEA4 - 99.5%
Fig. 15G
PMSF+Chir w.o. LIF
SSEA4 - 99.3%
Count


YFL3
265(165)
PMSF
P 267(53)
PMSF w.o. LIF
P 268(50)
100 µm
Oct4
Tra81
DAPI
Fig. 16A
Fig. 16B
Fig. 16C
Fig. 16D
Fig. 16E
Fig. 16F
Fig. 16G
Fig. 16H
Fig. 16I
Fig. 16J
Fig. 16K
Fig. 16L

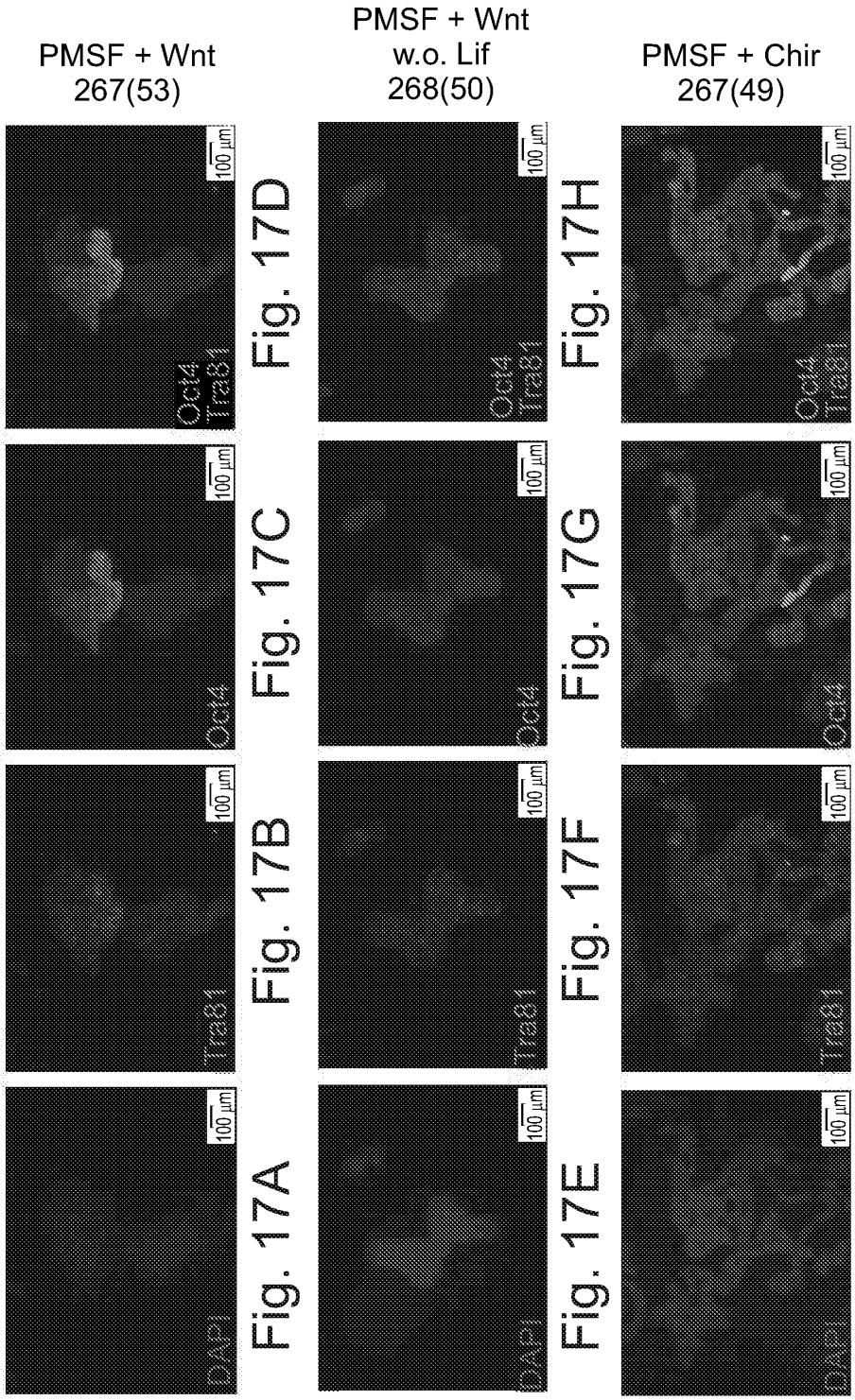


Fig. 17A Fig. 17B Fig. 17C Fig. 17D

Fig. 17E Fig. 17F Fig. 17G Fig. 17H

Fig. 17I Fig. 17J Fig. 17K Fig. 17L

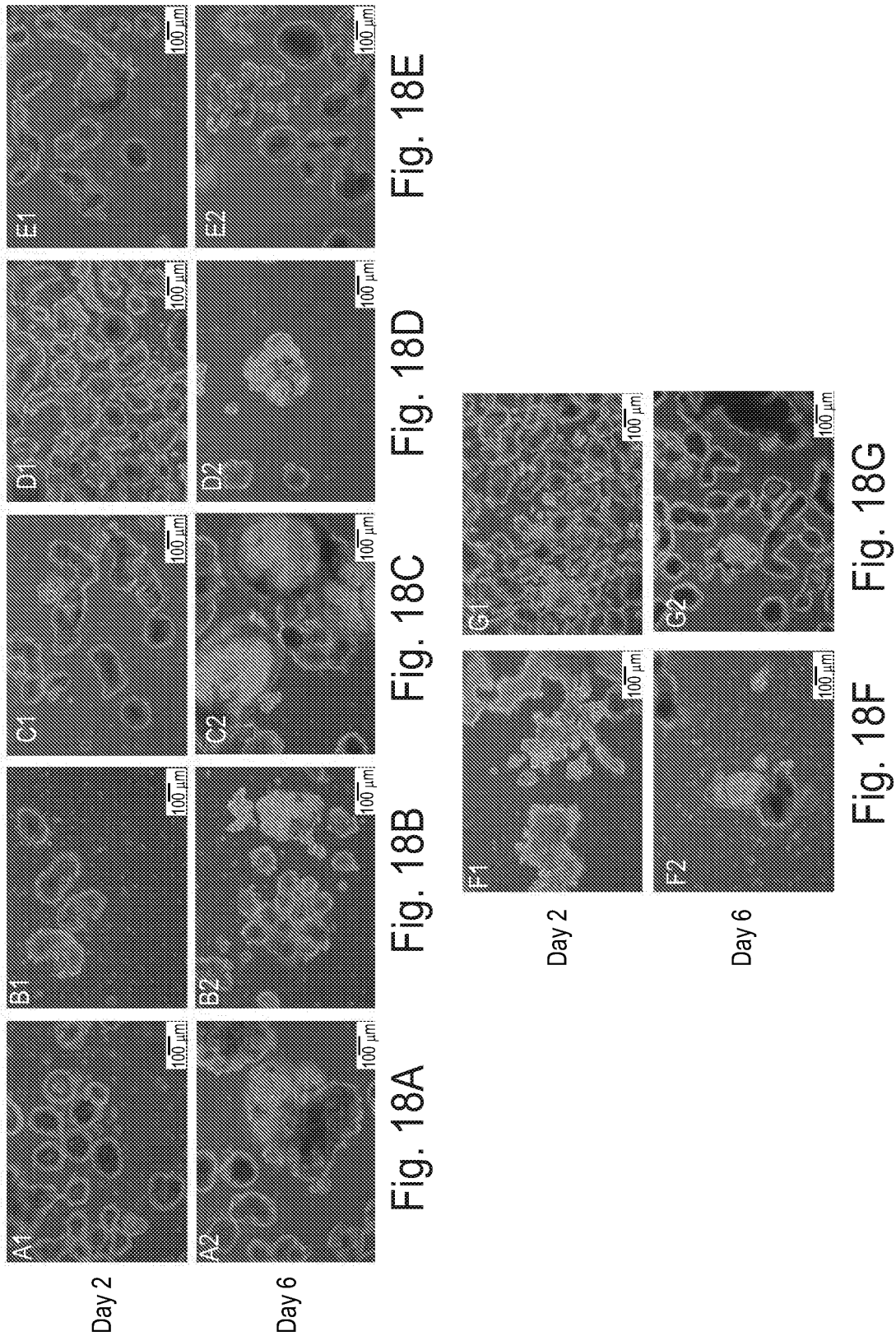


Fig. 18A Fig. 18B Fig. 18C Fig. 18D Fig. 18E

Fig. 18F Fig. 18G

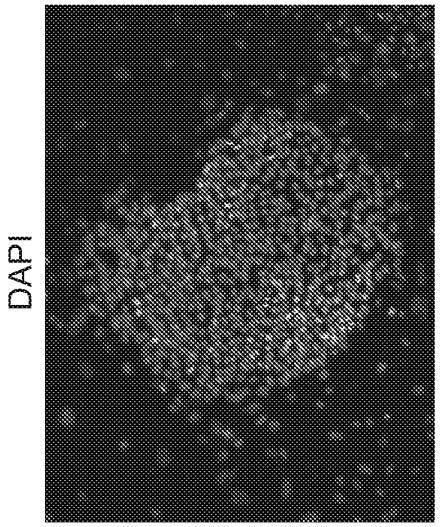


Fig. 19B

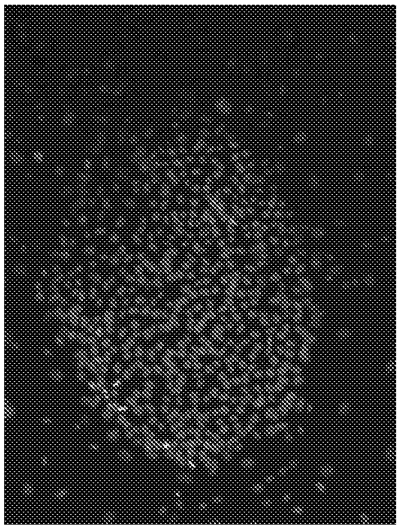


Fig. 19D

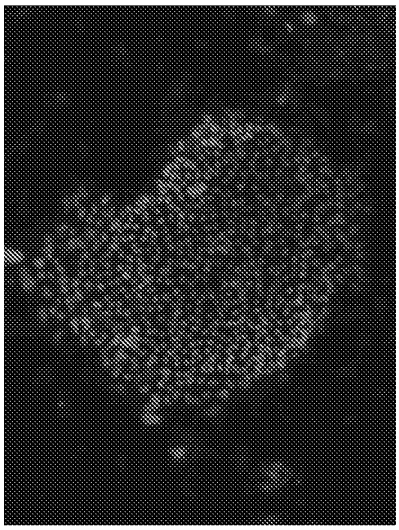


Fig. 19A

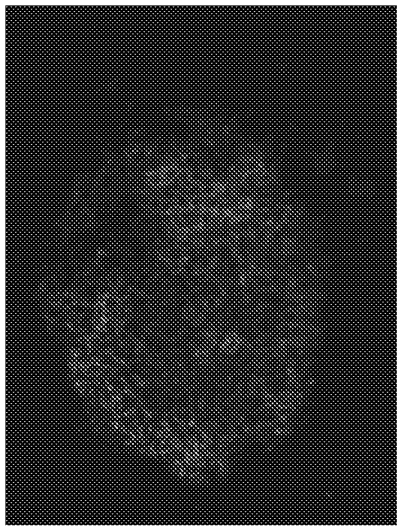
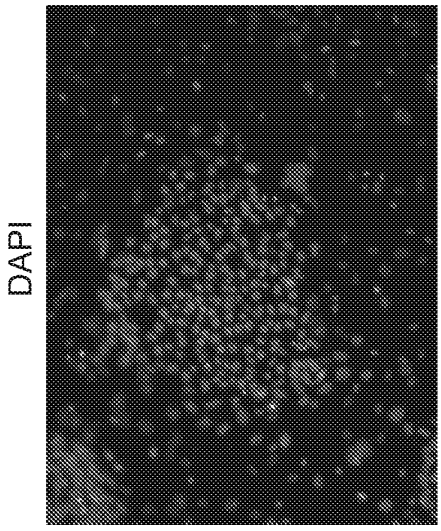


Fig. 19C



DAPI

Fig. 20B

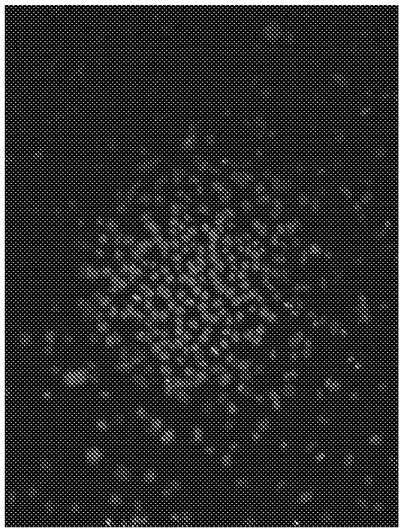
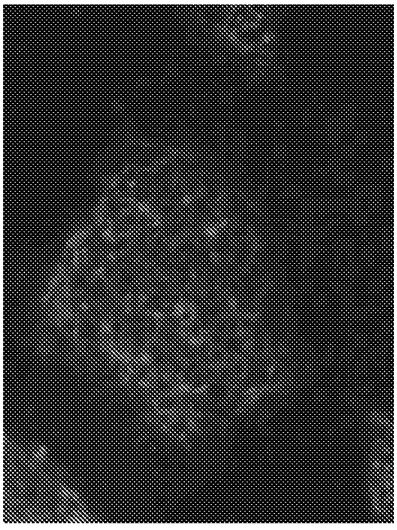


Fig. 20D

Ab

Fig. 20A



TRA-1-60

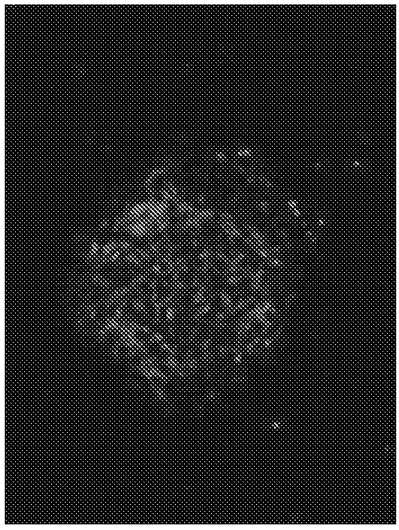


Fig. 20C

TRA-1-81

HFF



Fig. 21A

Matrigel

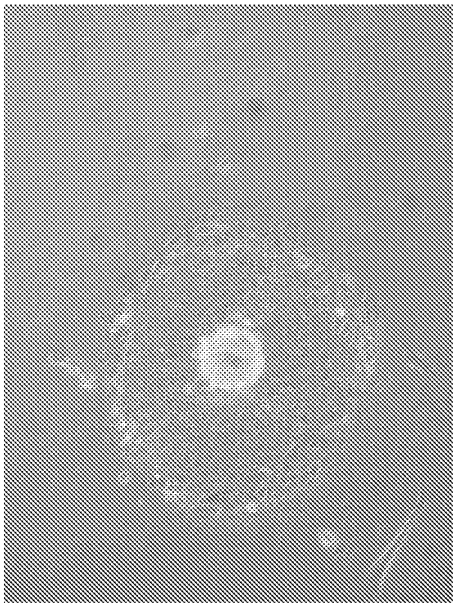


Fig. 21C

MEF



Fig. 21B

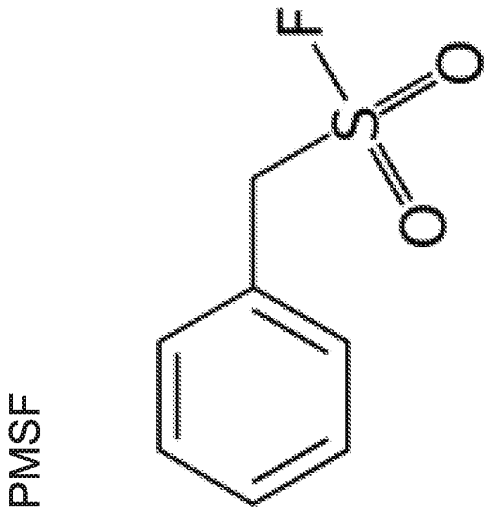


Fig. 22

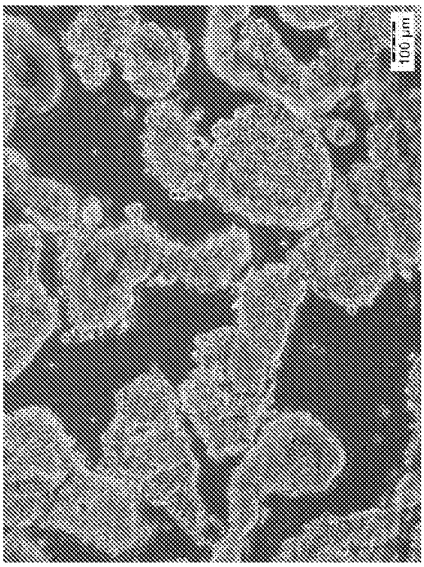


Fig. 23A

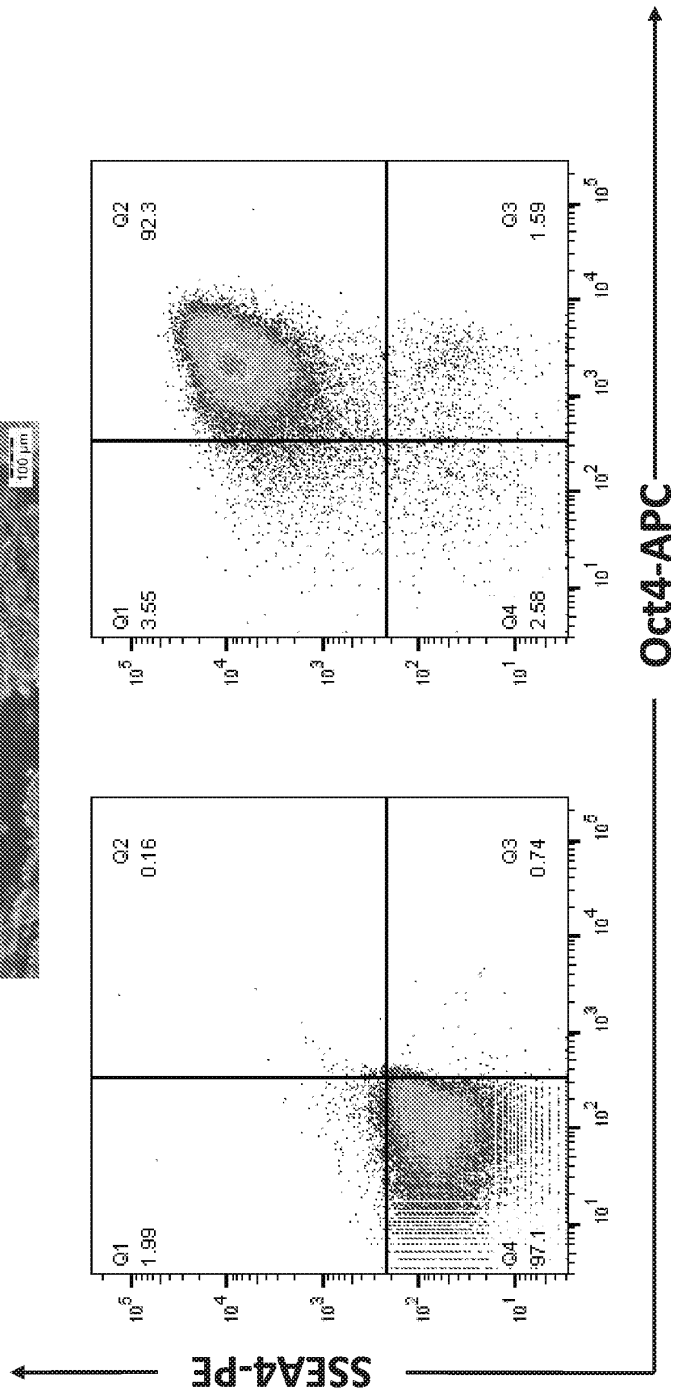


Fig. 23B

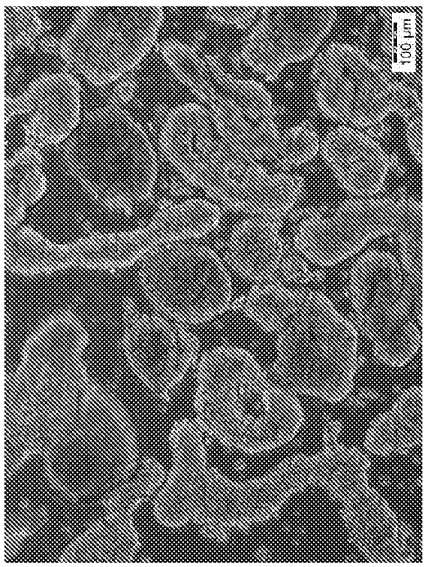


Fig. 24A

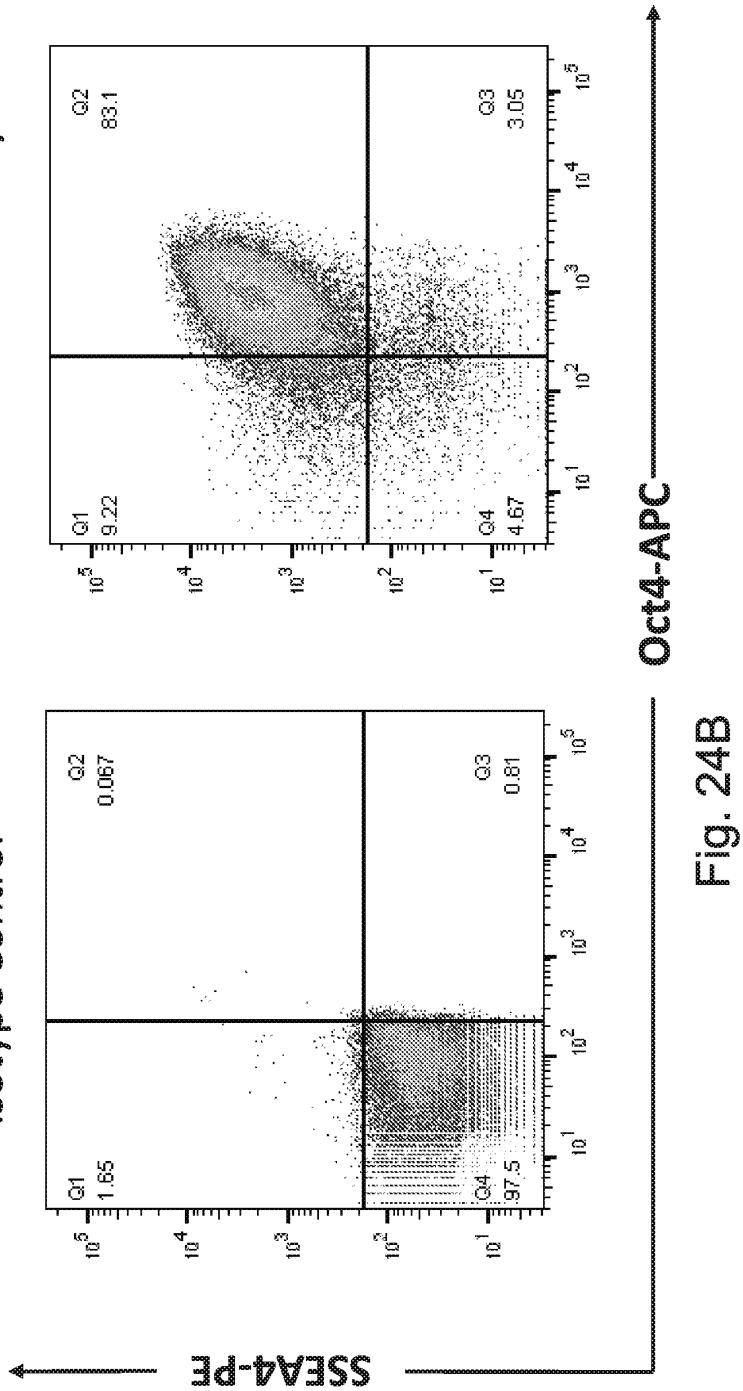


Fig. 24B

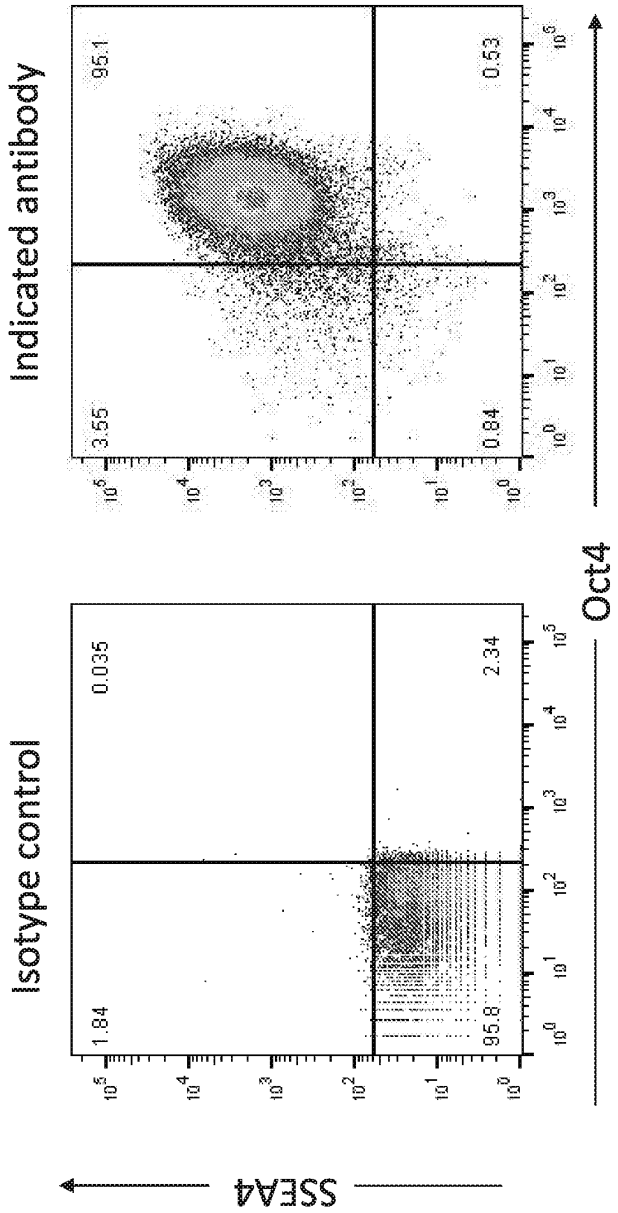

Indicated antibody
Isotype control
95.1
0.53
3.55
0.84
0.035
2.34
1.84
95.8
SSEA4
Oct4

Fig. 25

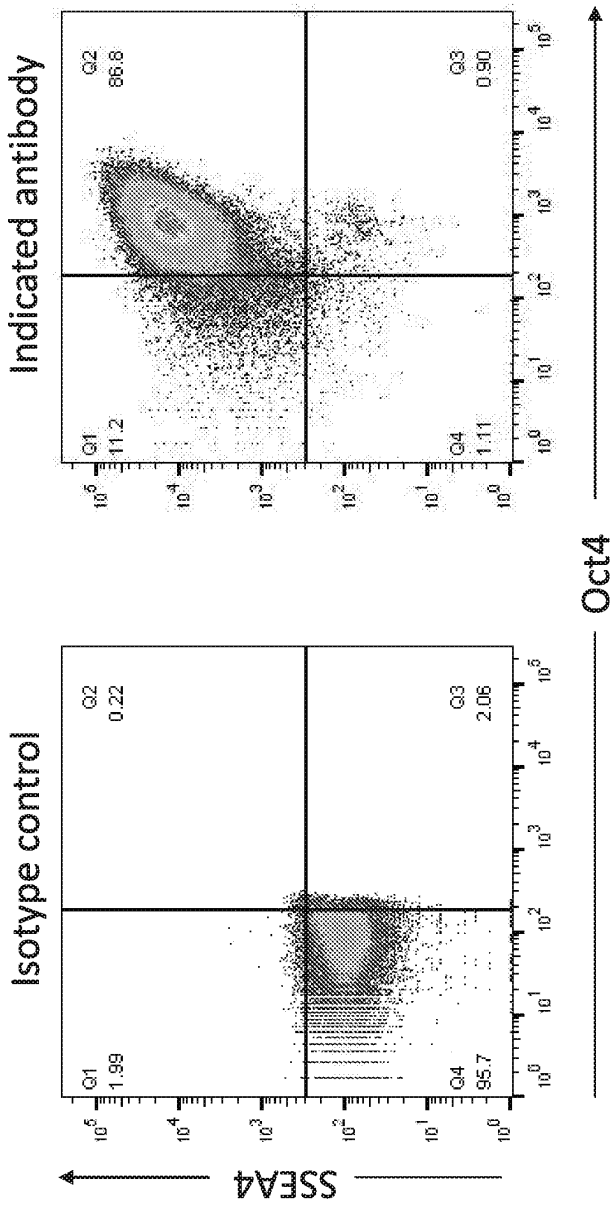

Indicated antibody
Isotype control
Q1
11.2
Q2
86.8
Q3
0.90
Q4
1.11
Q1
1.99
Q2
0.22
Q3
2.06
Q4
95.7
SSEA4
Oct4

Fig. 26

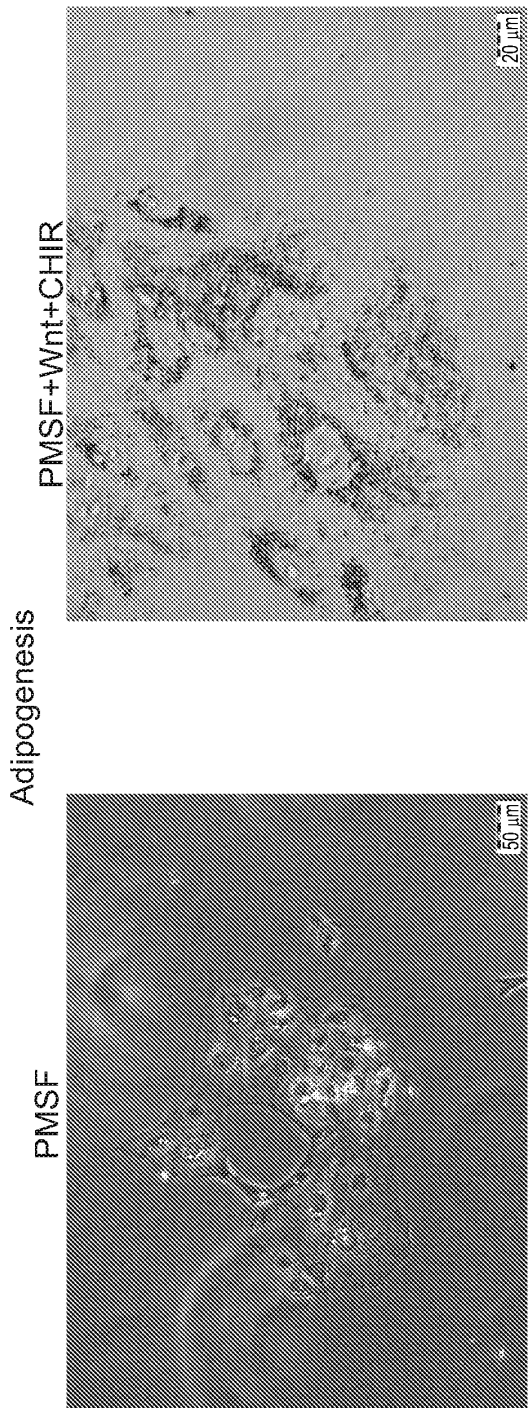


Fig. 27A

Fig. 27B

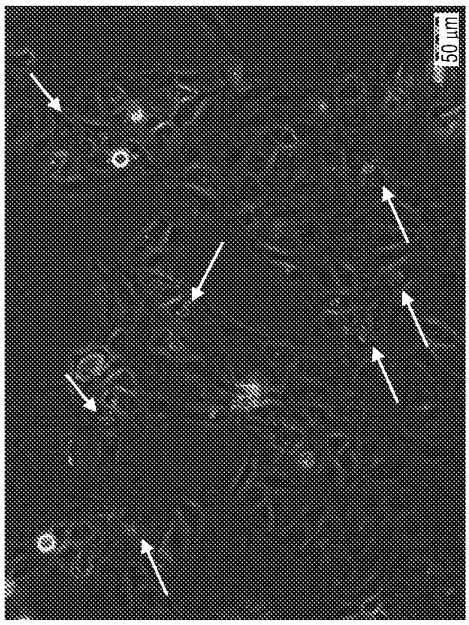


Fig. 27C