Figure 1

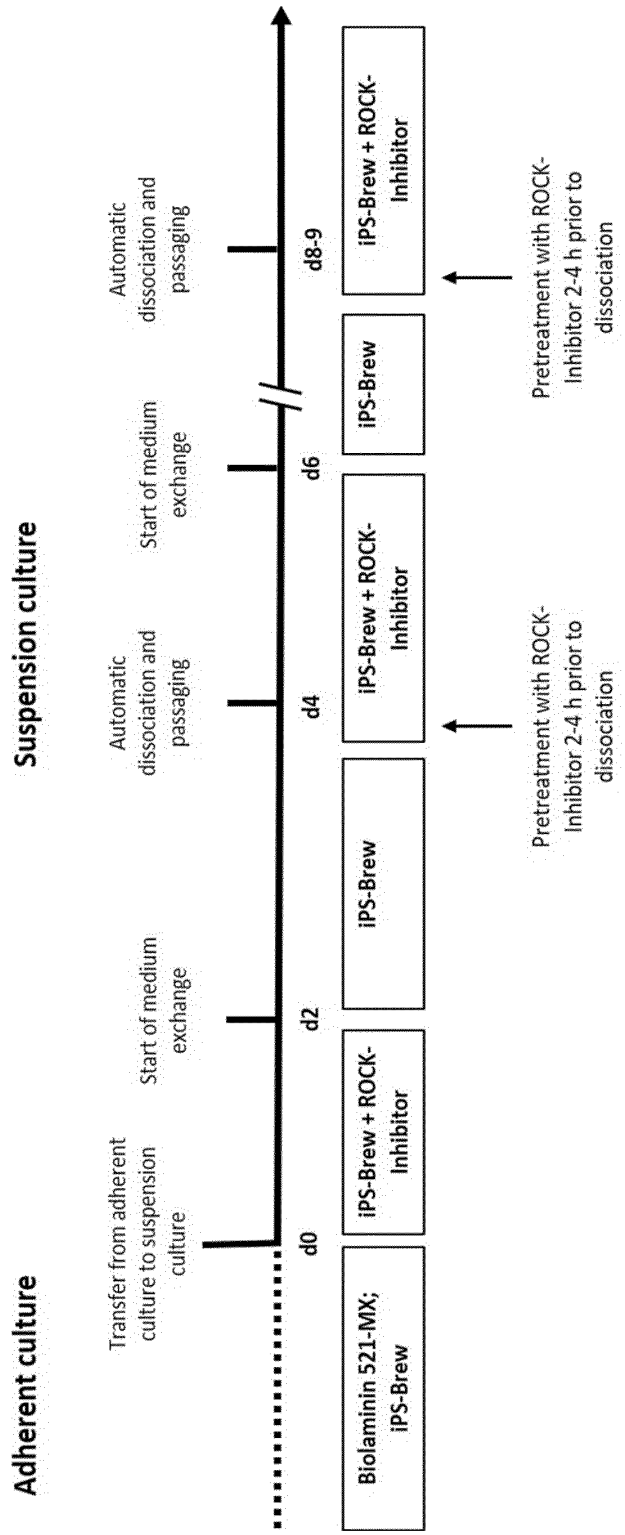

Adherent culture
Suspension culture
Biolaminin 521-MX; iPS-Brew
iPS-Brew + ROCK-Inhibitor
iPS-Brew
iPS-Brew + ROCK-Inhibitor
iPS-Brew
iPS-Brew + ROCK-Inhibitor
d0
d2
d4
d6
d8-9
Transfer from adherent culture to suspension culture
Start of medium exchange
Automatic dissociation and passaging
Start of medium exchange
Automatic dissociation and passaging
Pretreatment with ROCK-Inhibitor 2-4 h prior to dissociation
Pretreatment with ROCK-Inhibitor 2-4 h prior to dissociation

Figure 2

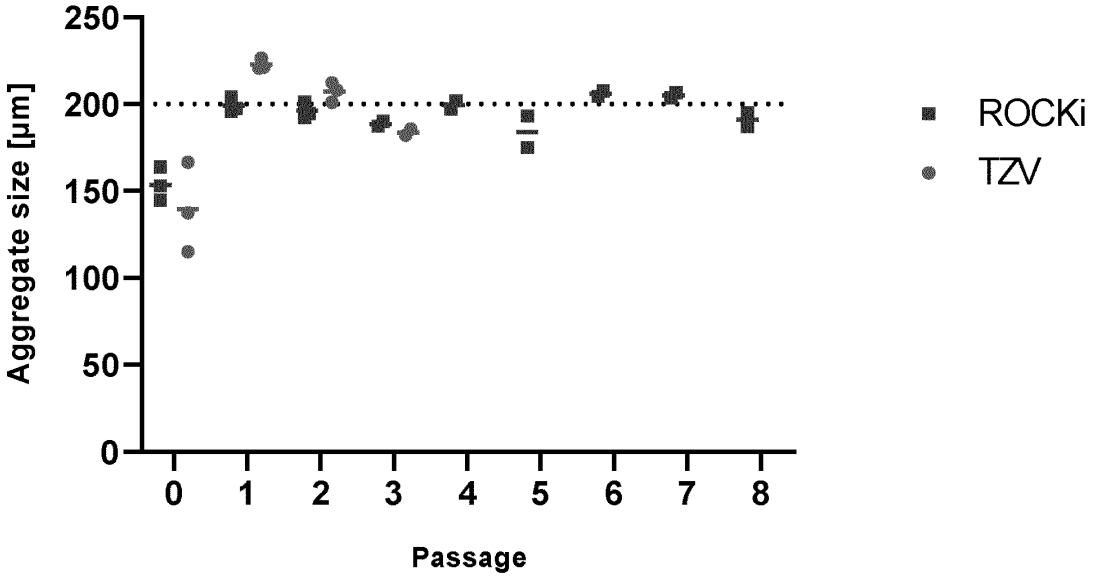


Figure 3

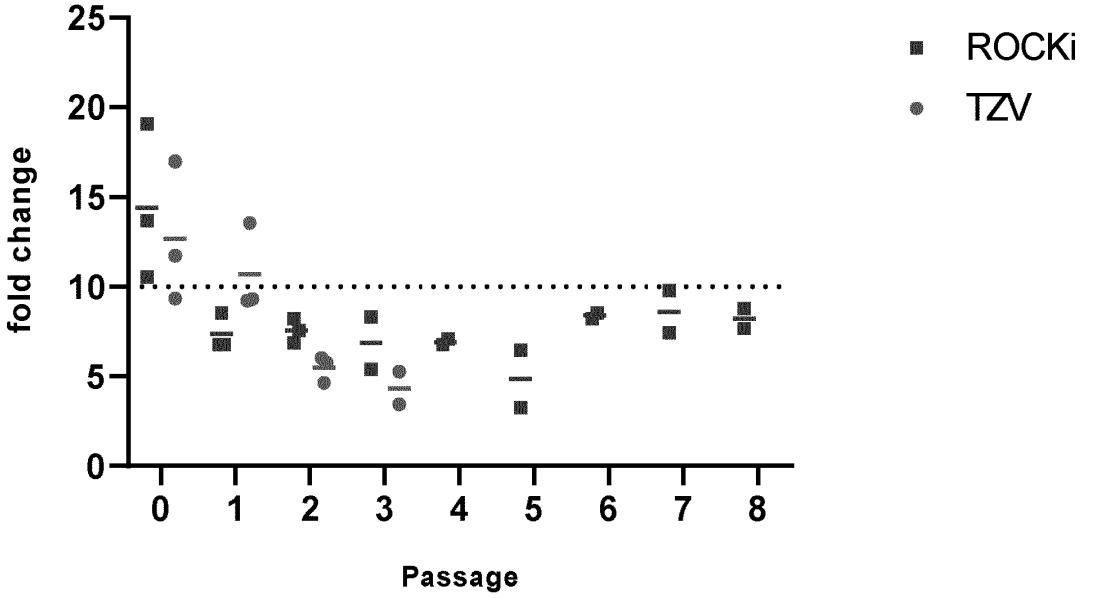


Figure 4

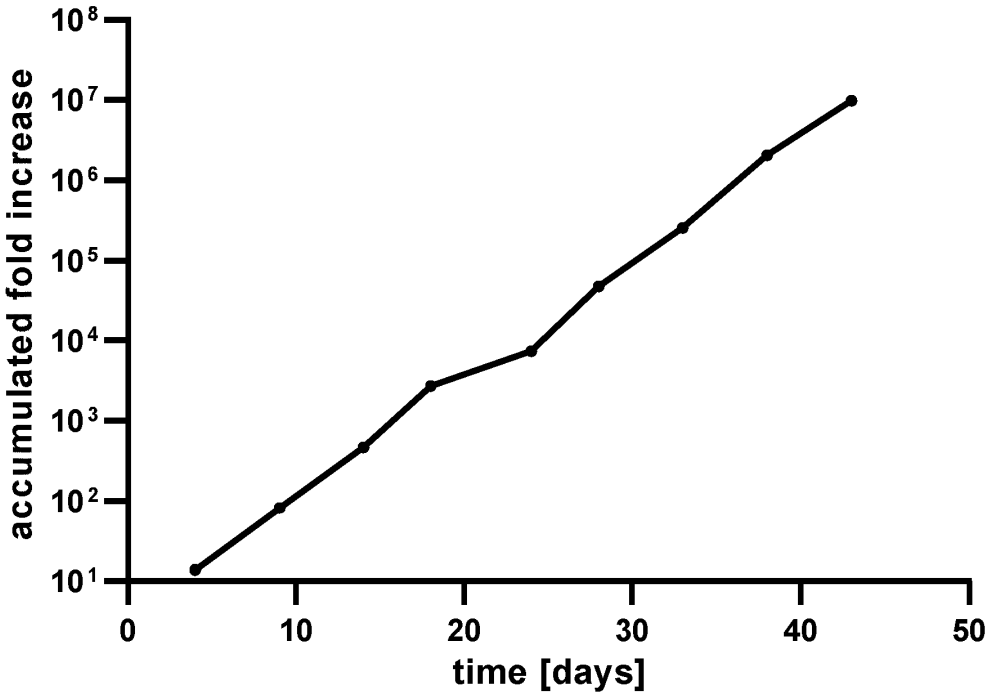

accumulated fold increase
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
10
20
30
40
50
time [days]

Figure 5

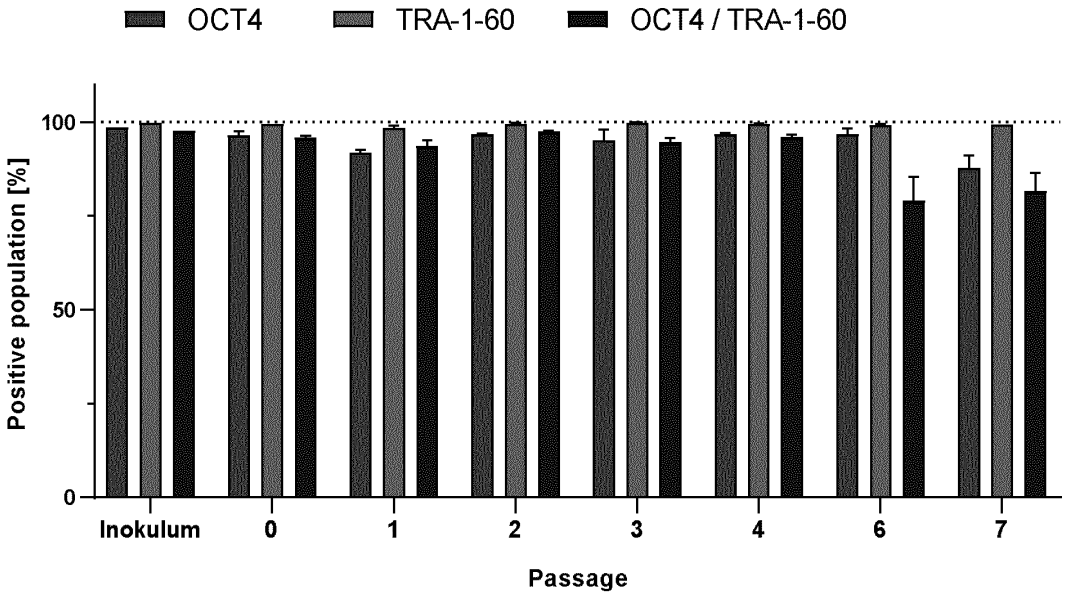
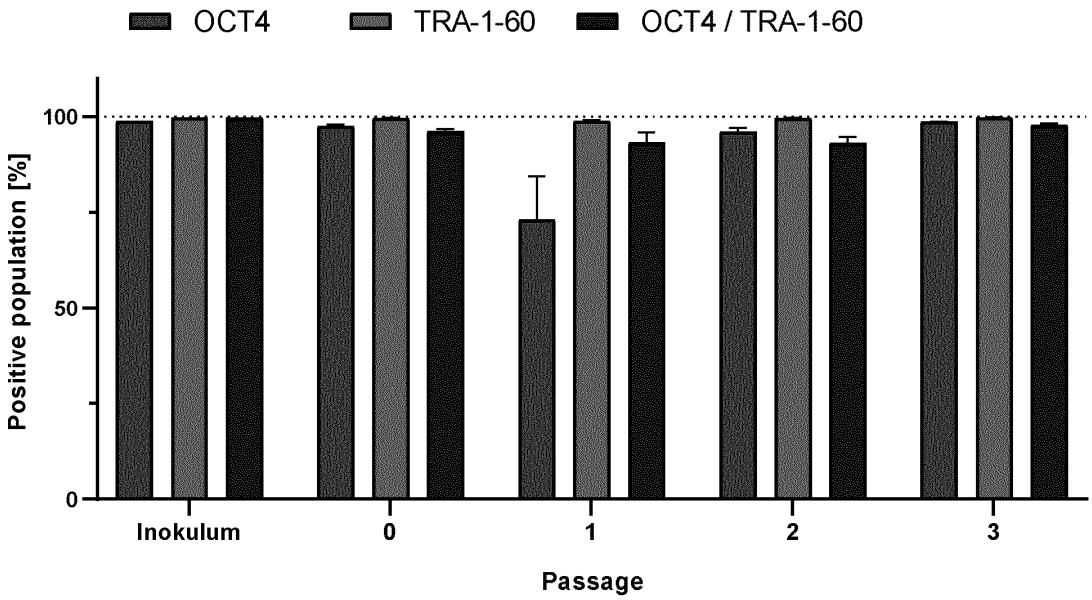


Figure 6



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Figure 7

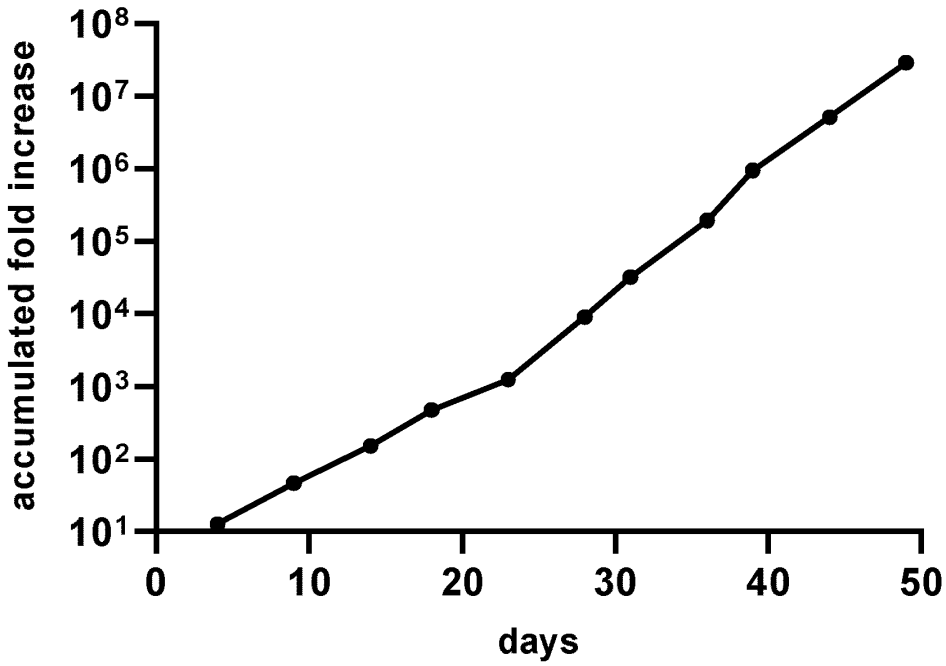

accumulated fold increase
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
10
20
30
40
50
days

Figure 8

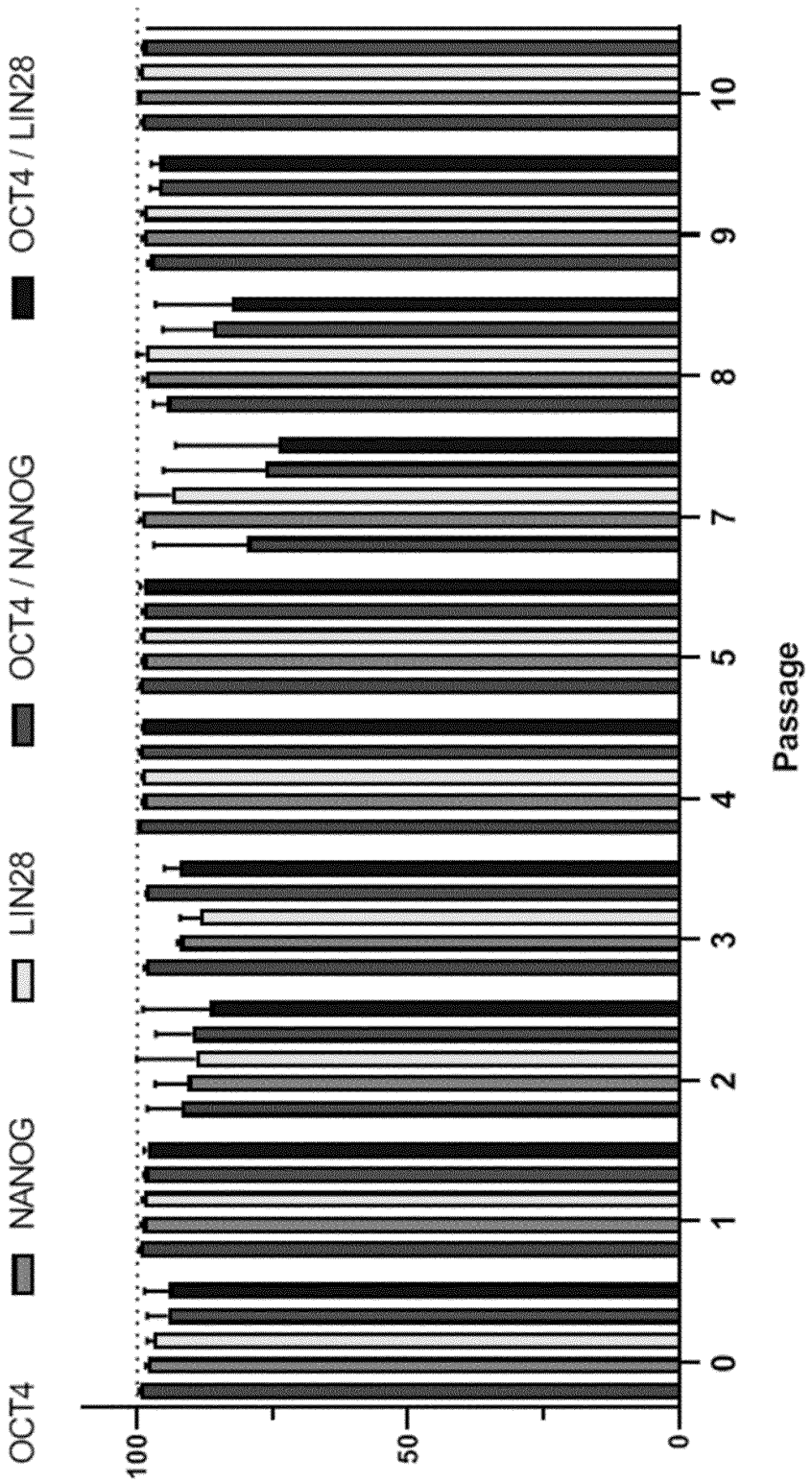

OCT4
NANOG
LIN28
OCT4 / NANOG
OCT4 / LIN28
100
50
0
0
1
2
3
4
5
7
8
9
10
Passage

Figure 9

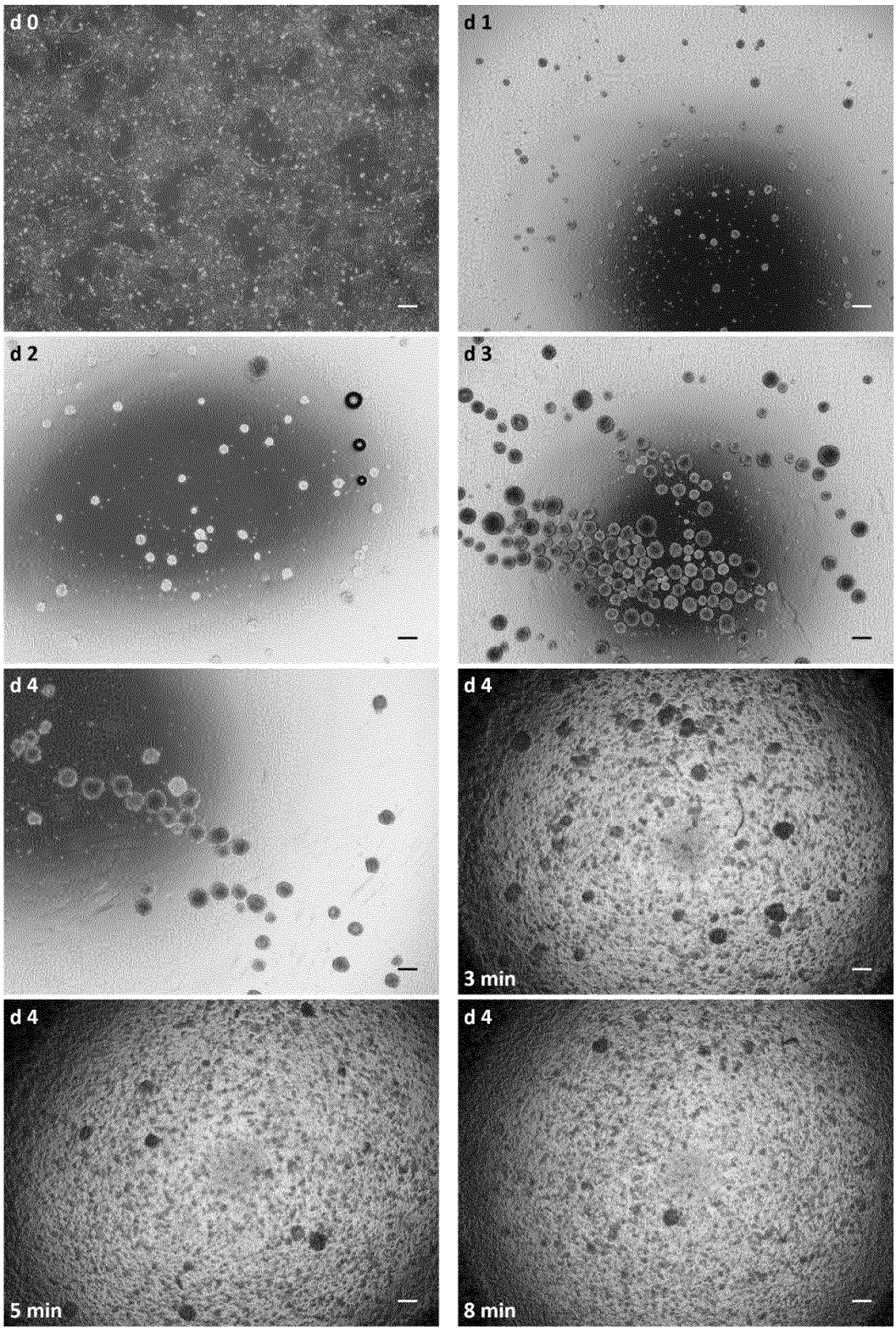


Figure 10

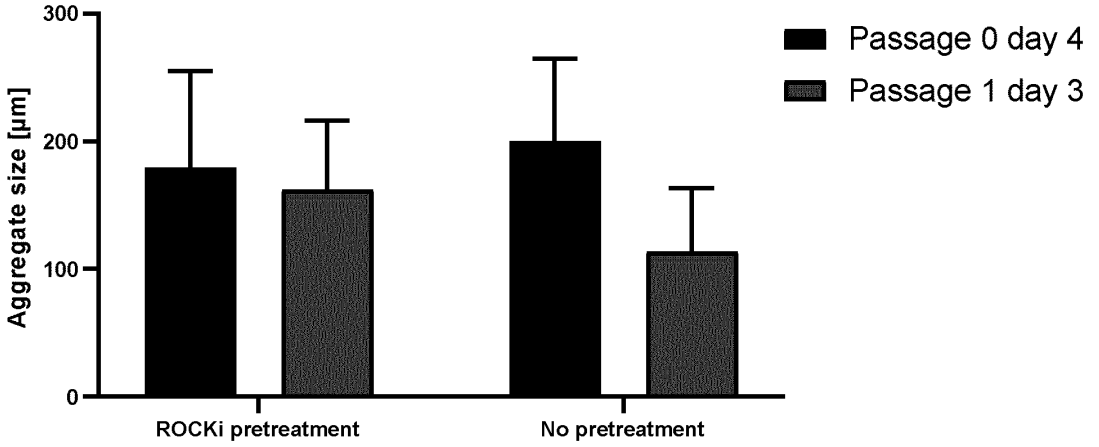


Figure 11

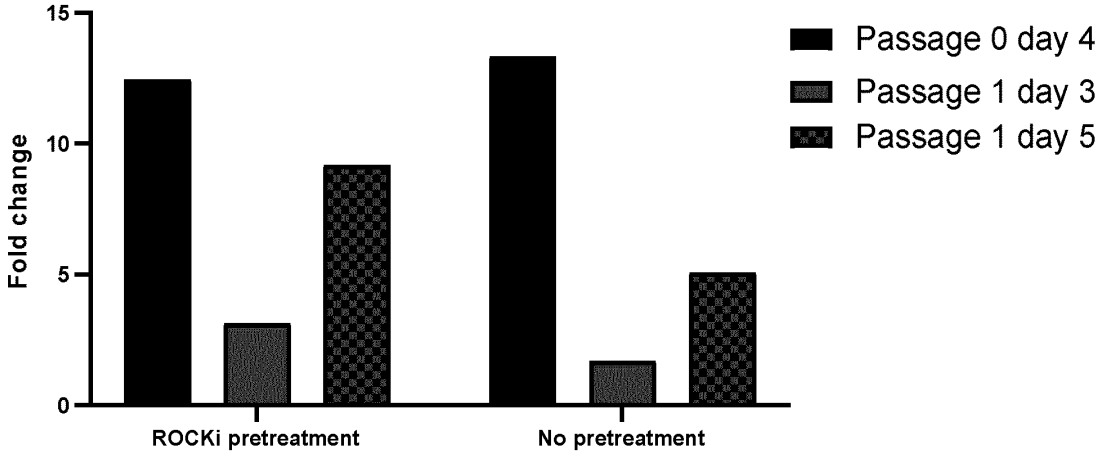


Figure 12

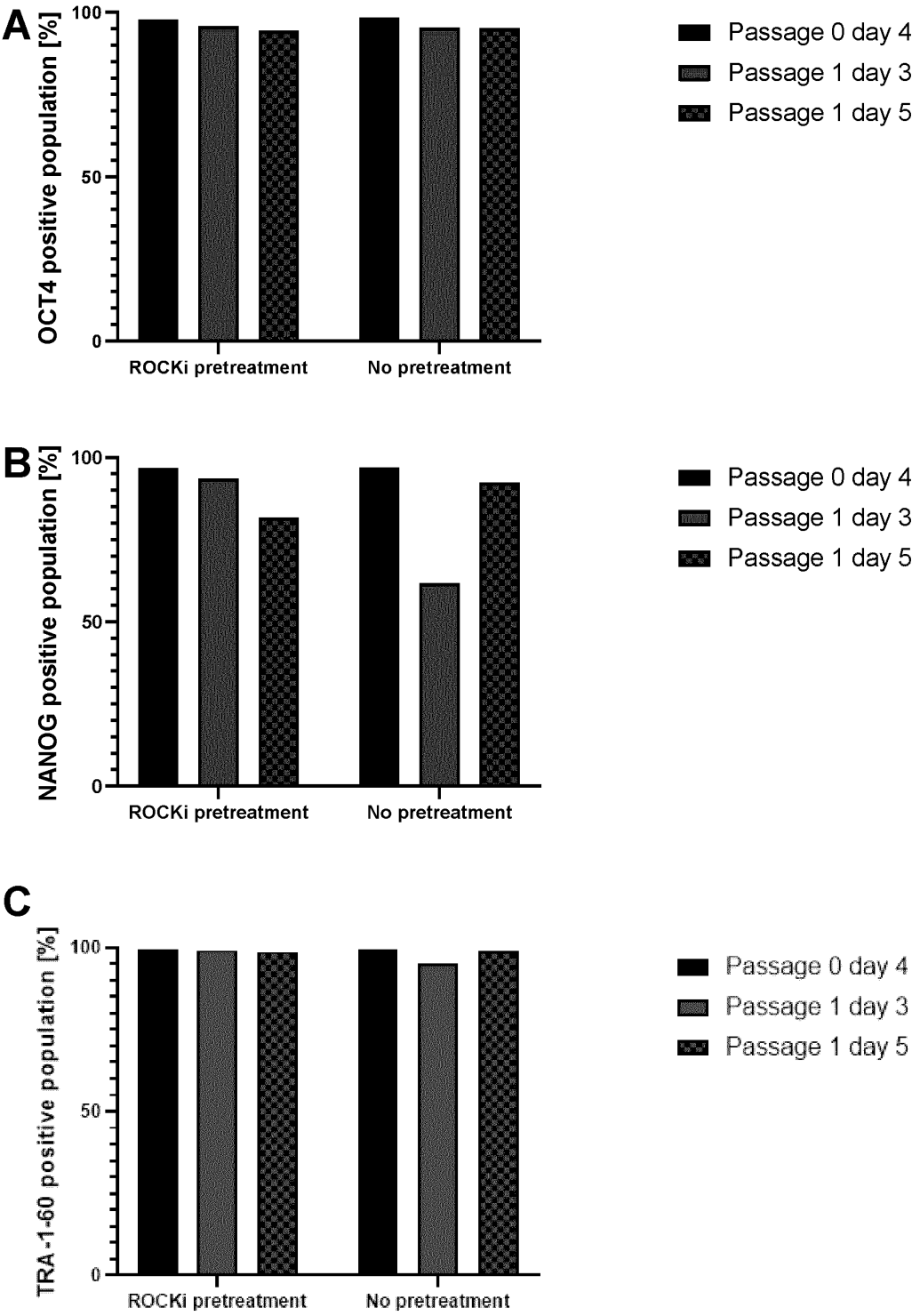

A
OCT4 positive population [%]
100
50
0
ROCKi pretreatment
No pretreatment
Passage 0 day 4
Passage 1 day 3
Passage 1 day 5
B
NANOG positive population [%]
100
50
0
ROCKi pretreatment
No pretreatment
Passage 0 day 4
Passage 1 day 3
Passage 1 day 5
C
TRA-1-60 positive population [%]
100
50
0
ROCKi pretreatment
No pretreatment
Passage 0 day 4
Passage 1 day 3
Passage 1 day 5

Figure 13

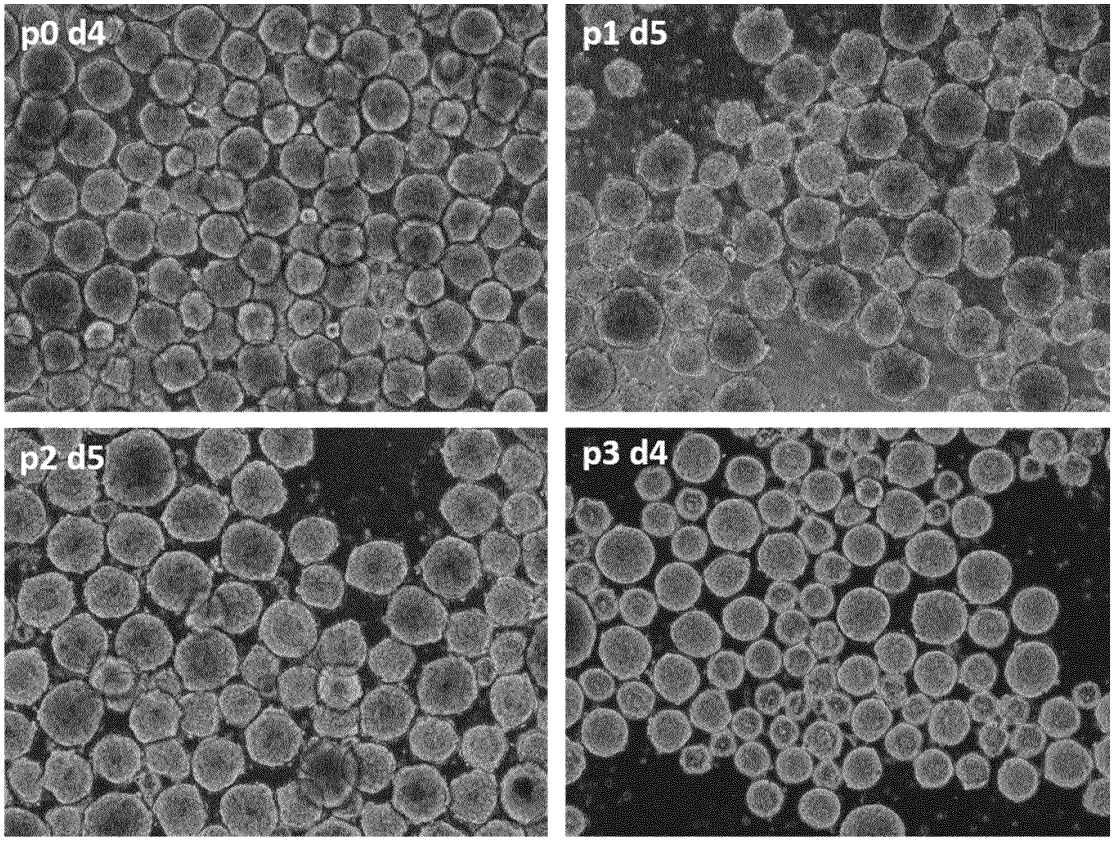


Figure 14

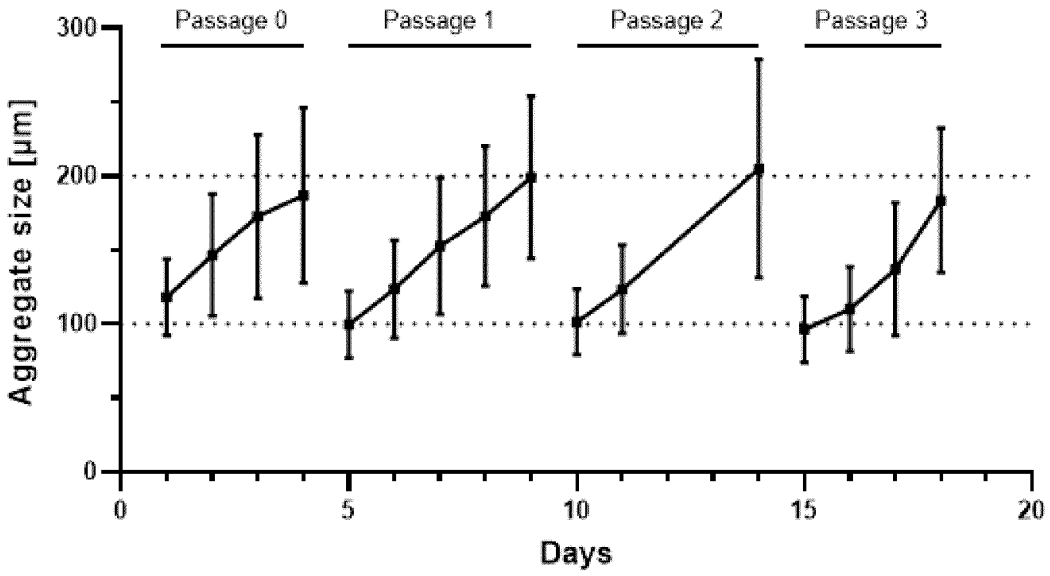


Figure 15

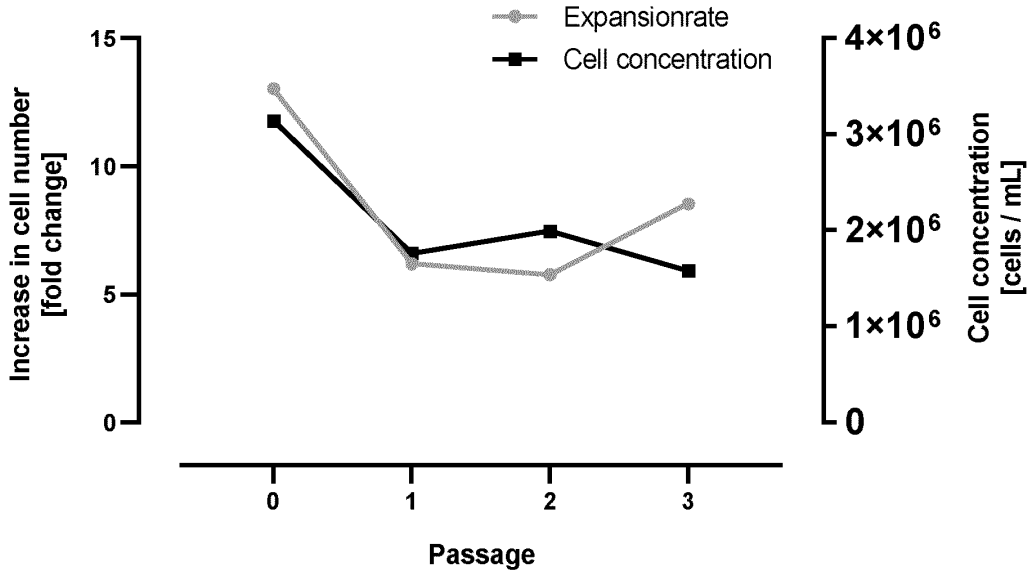
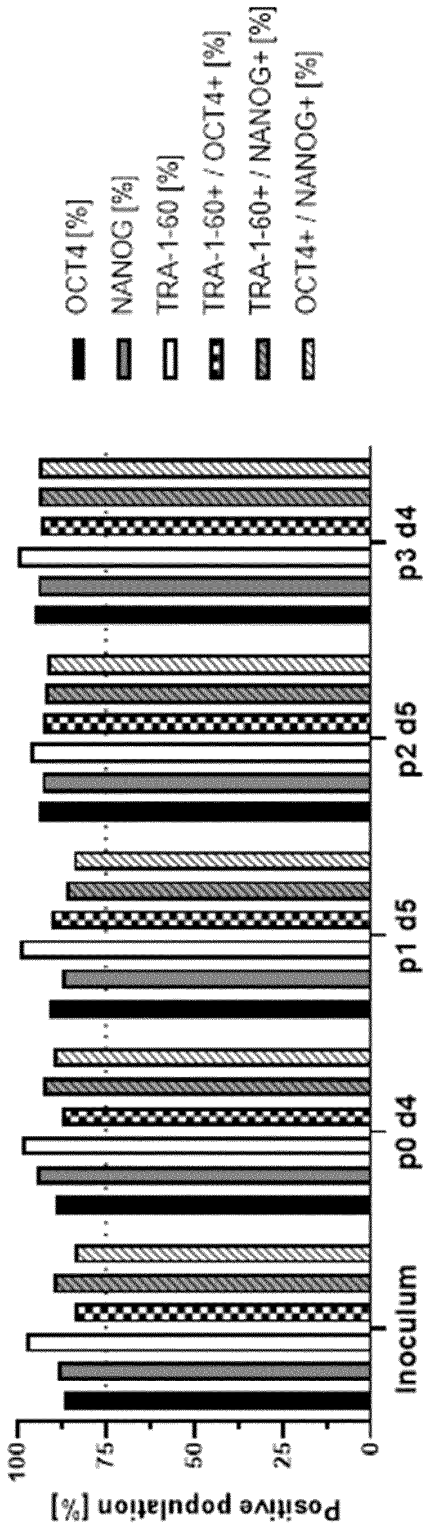

Expansionrate
Cell concentration
Increase in cell number [fold change]
15
10
5
0
4×10^6
3×10^6
2×10^6
1×10^6
0
Cell concentration [cells / mL]
0
1
2
3
Passage

Figure 16


OCT4 [%]
NANOG [%]
TRA-1-60 [%]
TRA-1-60+ / OCT4+ [%]
TRA-1-60+ / NANOG+ [%]
OCT4+ / NANOG+ [%]
Positive population [%]
100
75
50
25
0
Inoculum
p0 d4
p1 d5
p2 d5
p3 d4