FIGURE 1

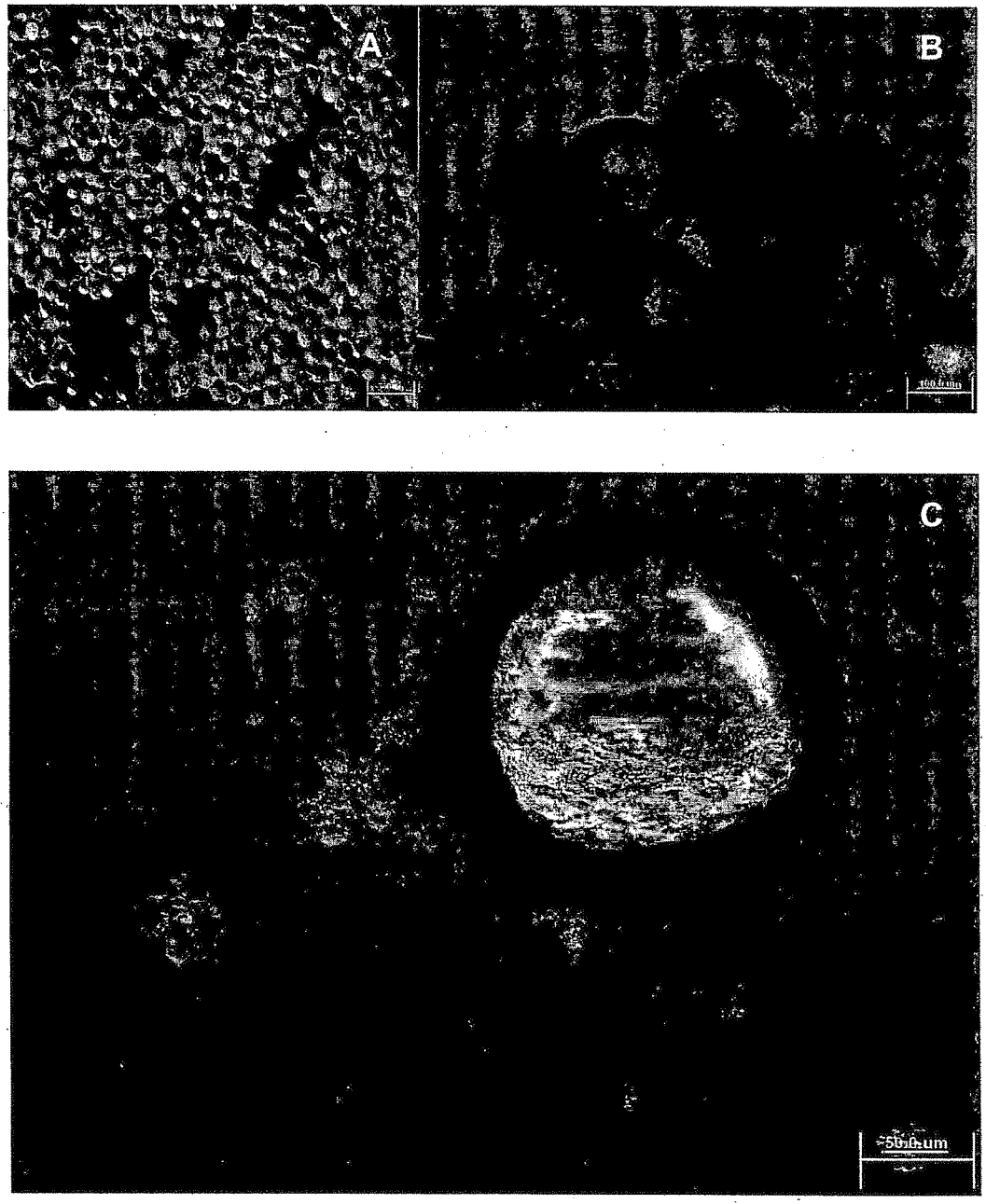


FIGURE 2

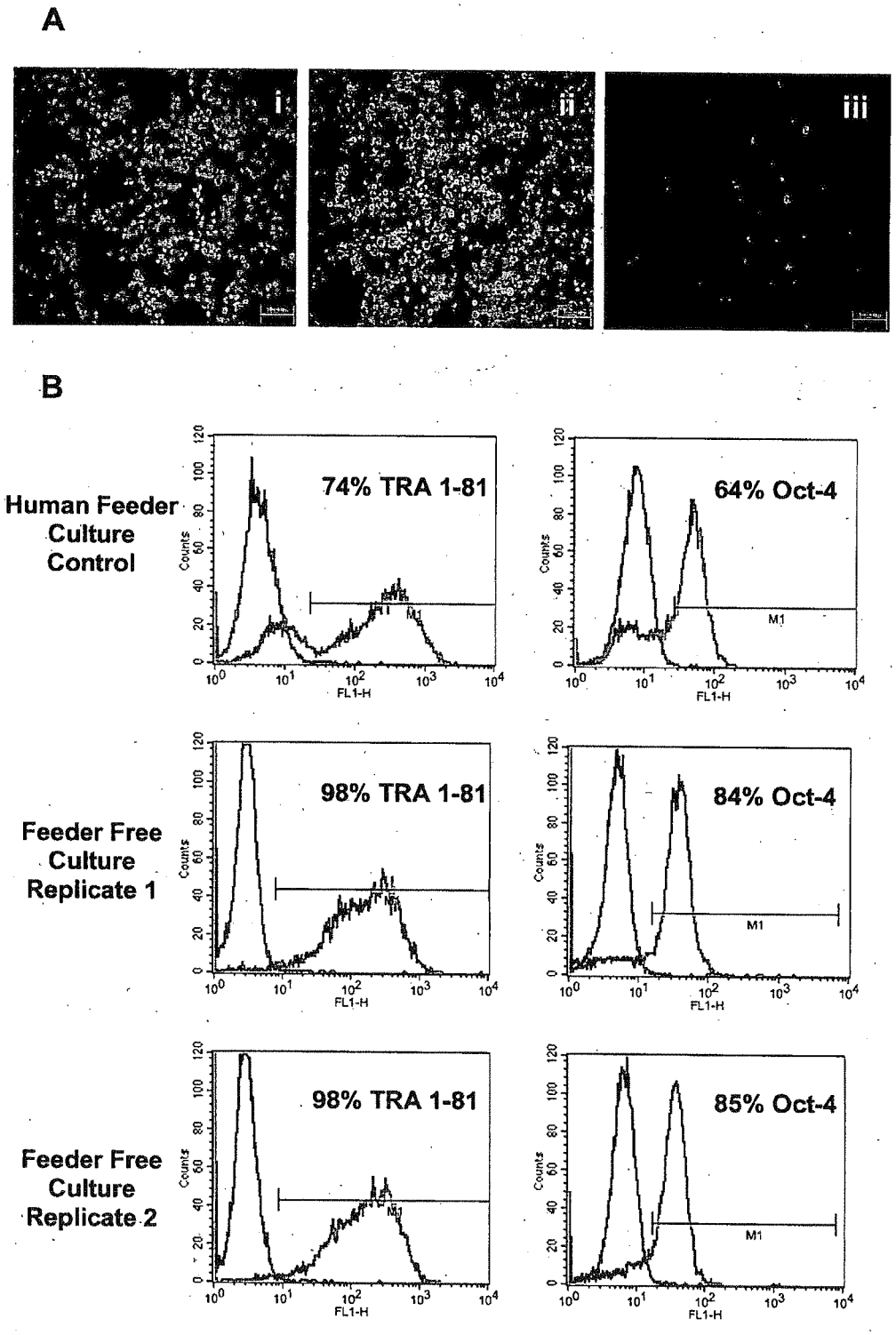
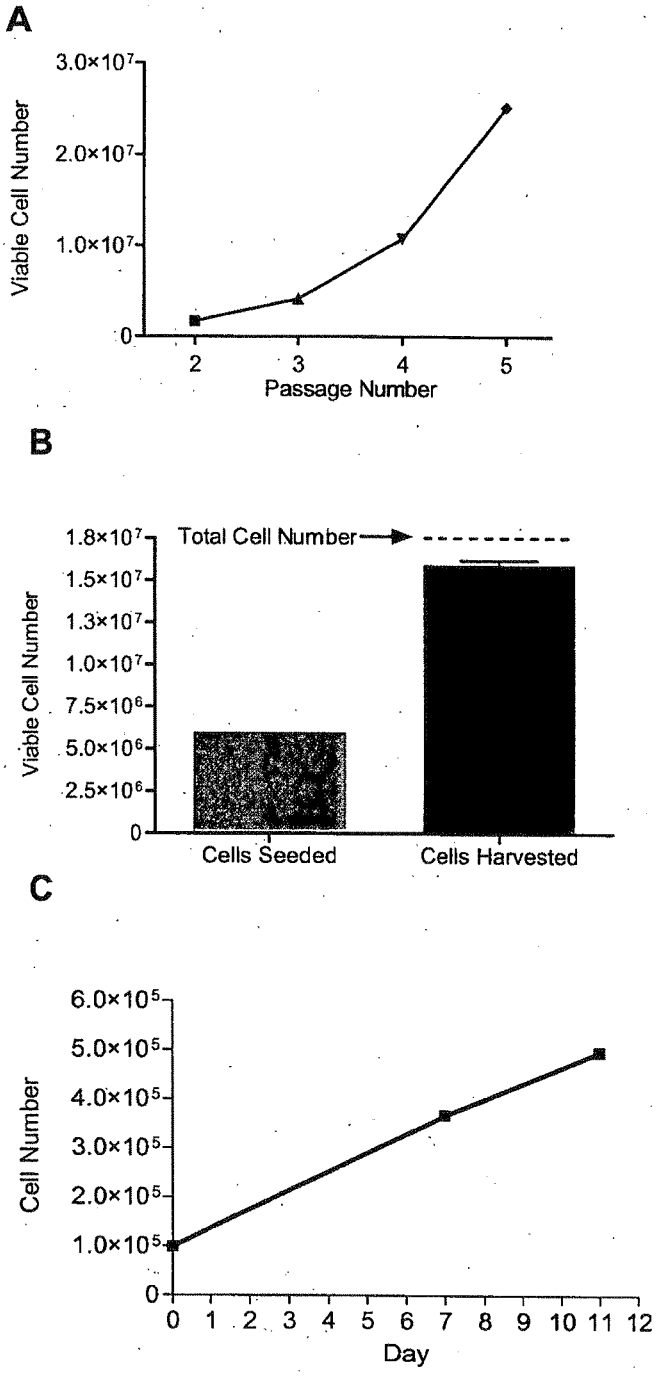
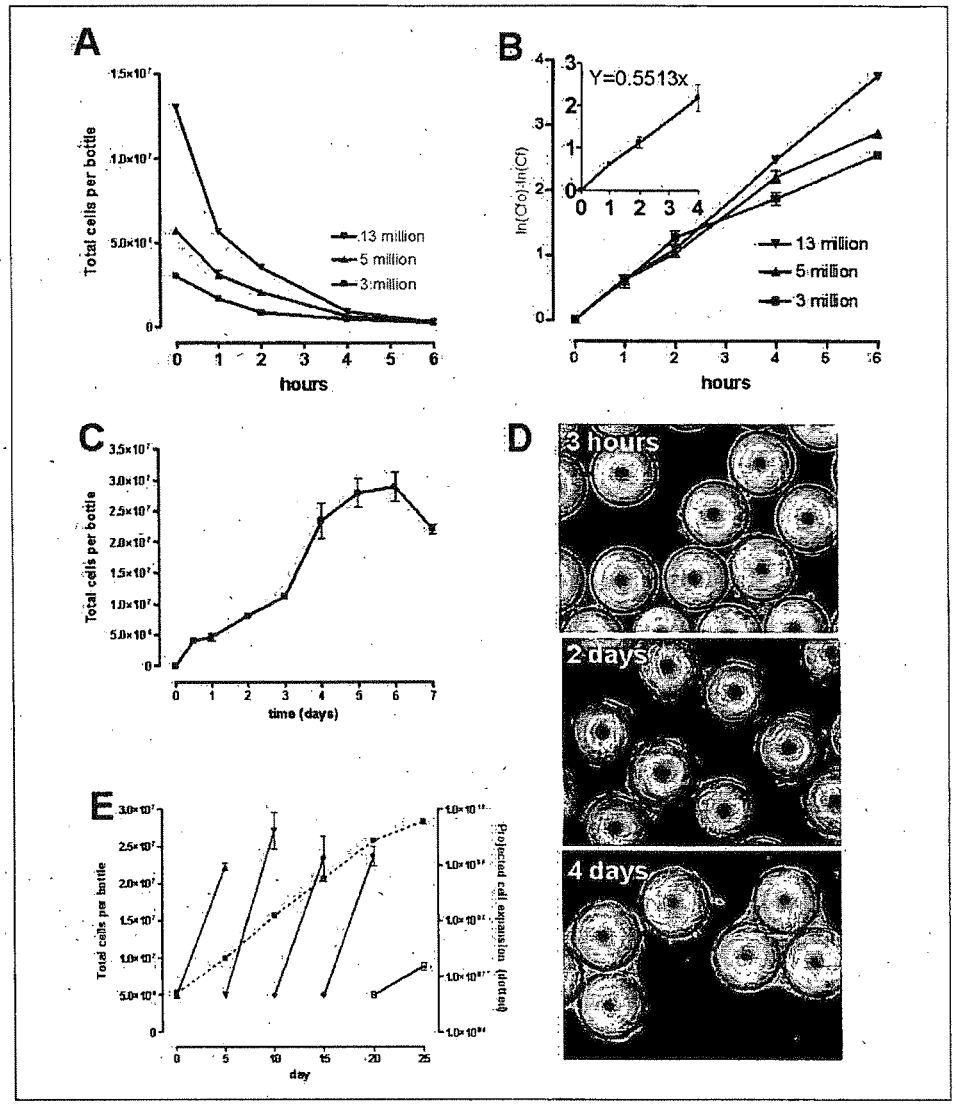

A
i
ii
iii
B
Human Feeder Culture Control
74% TRA 1-81
64% Oct-4
Feeder Free Culture Replicate 1
98% TRA 1-81
84% Oct-4
Feeder Free Culture Replicate 2
98% TRA 1-81
85% Oct-4
Counts
FL1-H
M1

FIGURE 3


A
3.0×10⁷
2.0×10⁷
1.0×10⁷
0
Viable Cell Number
2
3
4
5
Passage Number
B
1.8×10⁷
1.5×10⁷
1.3×10⁷
1.0×10⁷
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
Viable Cell Number
Total Cell Number
Cells Seeded
Cells Harvested
C
6.0×10⁵
5.0×10⁵
4.0×10⁵
3.0×10⁵
2.0×10⁵
1.0×10⁵
0
Cell Number
0 1 2 3 4 5 6 7 8 9 10 11 12
Day

4/12

FIGURE 4


A
Total cells per bottle
13 million
5 million
3 million
hours
B
Y=0.5513x
ln(Co)-ln(Cf)
13 million
5 million
3 million
hours
C
Total cells per bottle
time (days)
D
3 hours
2 days
4 days
E
Total cells per bottle
day

5/12

FIGURE 5

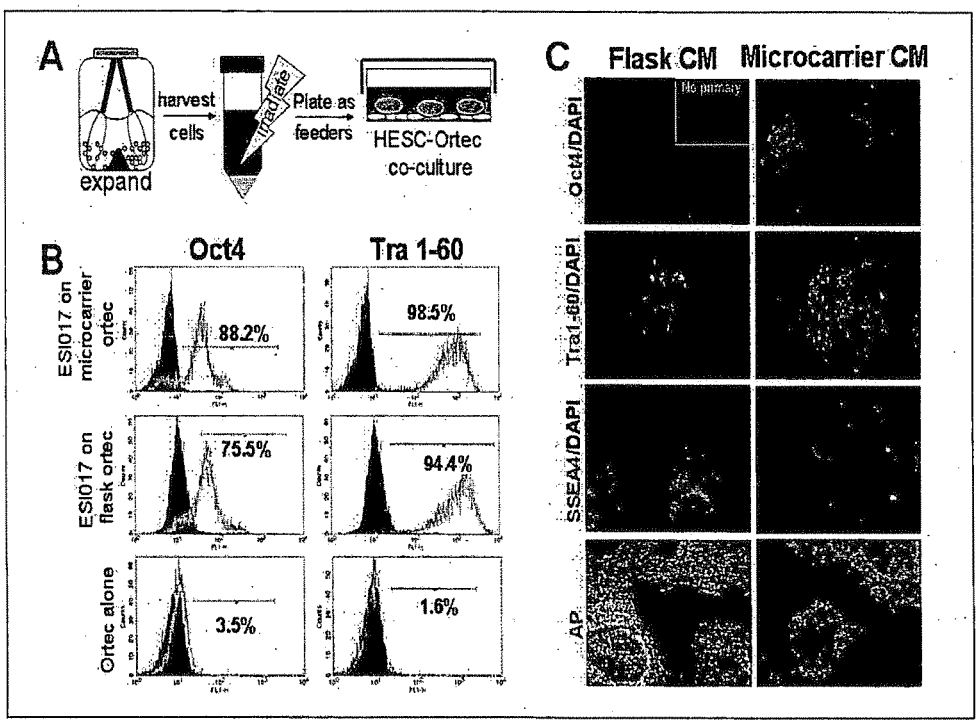

A
expand
harvest cells
irradiate
Plate as feeders
HESC-Ortec co-culture
B
Oct4
Tra 1-60
ESI017 on microcarrier ortec
88.2%
98.5%
ESI017 on flask ortec
75.5%
94.4%
Ortec alone
3.5%
1.6%
C
Flask CM
Microcarrier CM
No primary
Oct4/DAPI
Tra1-60/DAPI
SSEA4/DAPI
AP

FIGURE 6

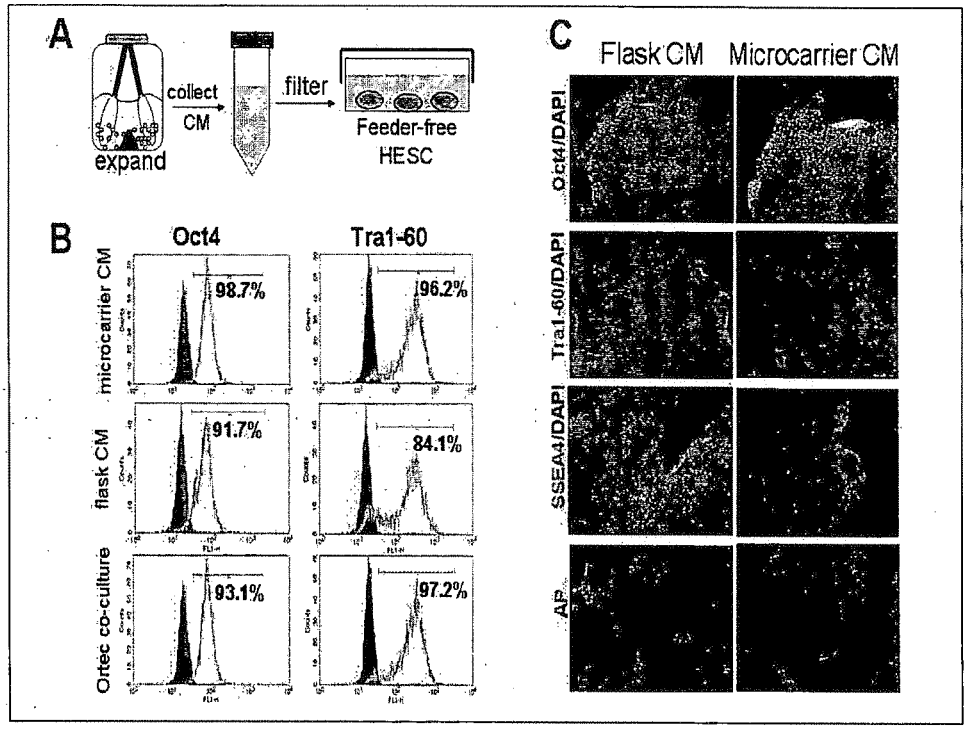

A
collect
CM
filter
expand
Feeder-free
HESC
B
Oct4
Tra1-60
microcarrier CM
flask CM
Ortec co-culture
98.7%
96.2%
91.7%
84.1%
93.1%
97.2%
C
Flask CM
Microcarrier CM
Oct4/DAPI
Tra1-60/DAPI
SSEA4/DAPI
AP

FIGURE 7

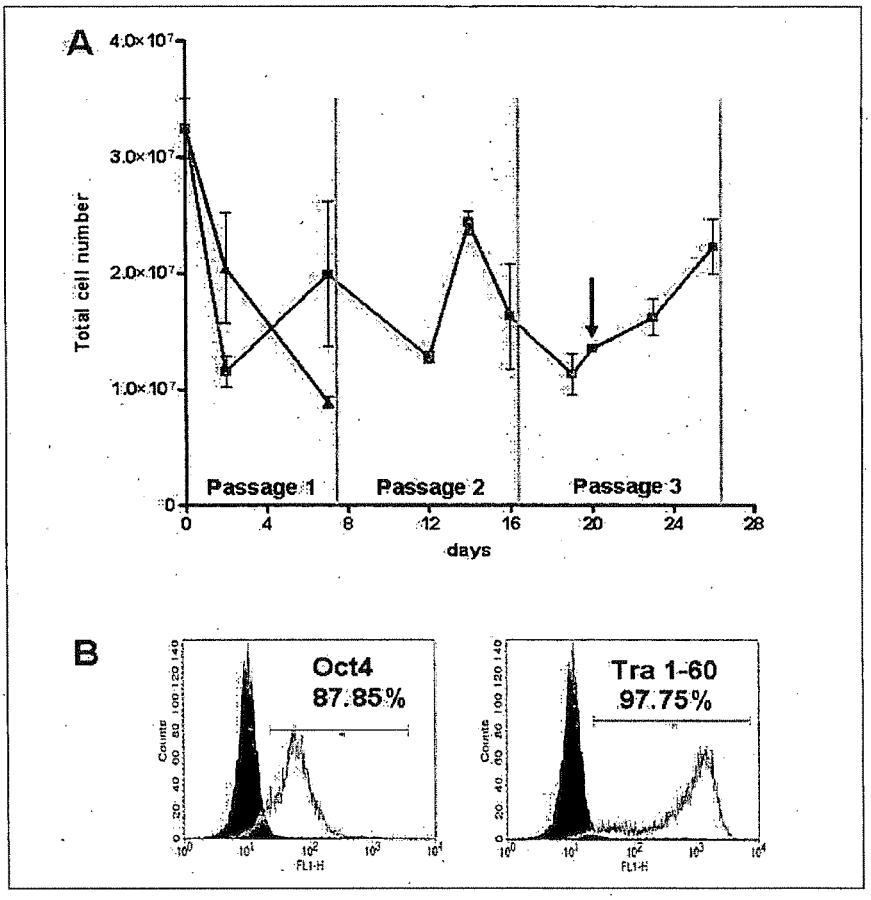

A
4.0×10⁷
3.0×10⁷
2.0×10⁷
1.0×10⁷
0
Total cell number
Passage 1
Passage 2
Passage 3
0
4
8
12
16
20
24
28
days
B
Oct4
87.85%
Tra 1-60
97.75%
Counts
FL1-H

FIGURE 8

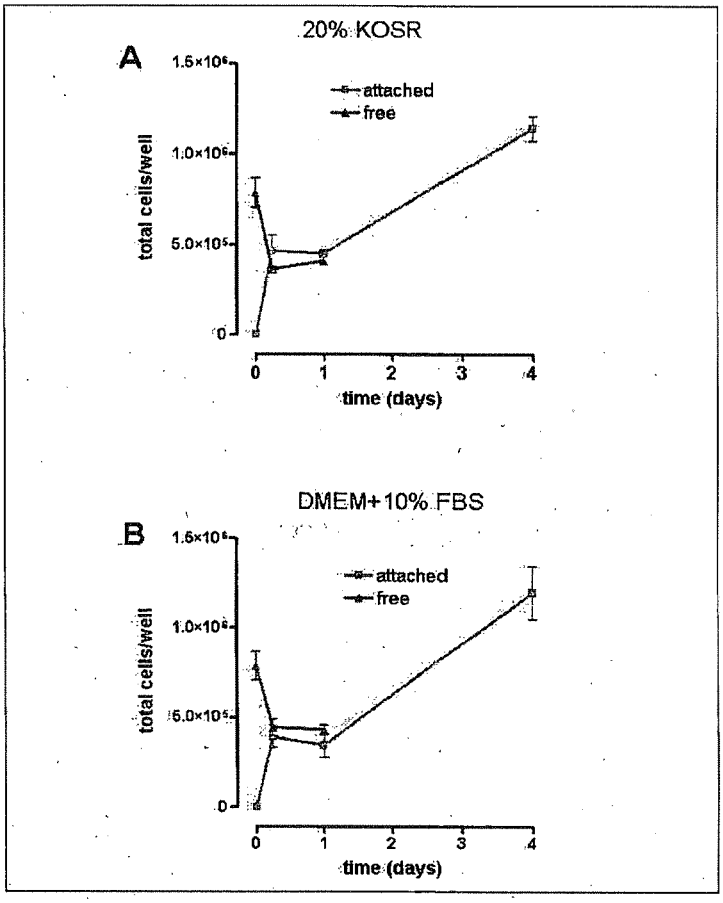

20% KOSR
A
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
total cells/well
attached
free
0
1
2
3
4
time (days)
DMEM+10% FBS
B
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
total cells/well
attached
free
0
1
2
3
4
time (days)

FIGURE 9

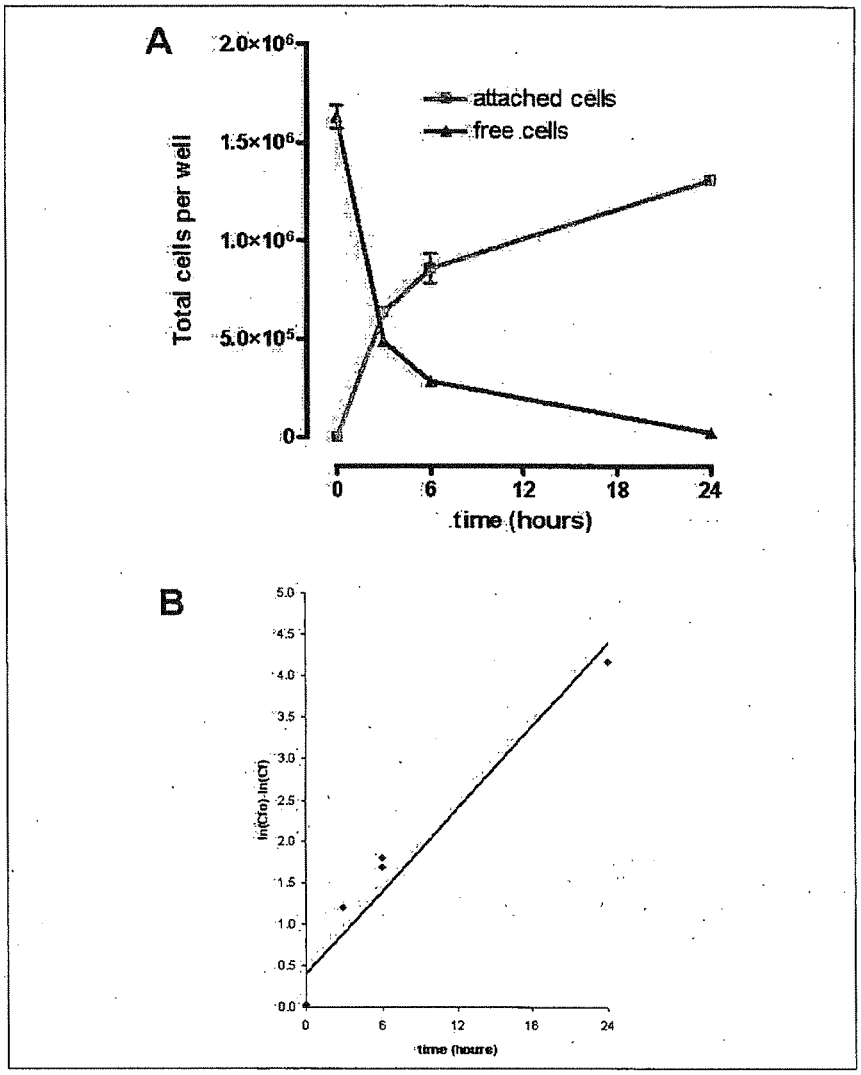

A
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
Total cells per well
attached cells
free cells
0
6
12
18
24
time (hours)
B
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ln(C0)-ln(Ct)
0
6
12
18
24
time (hours)

FIGURE 10

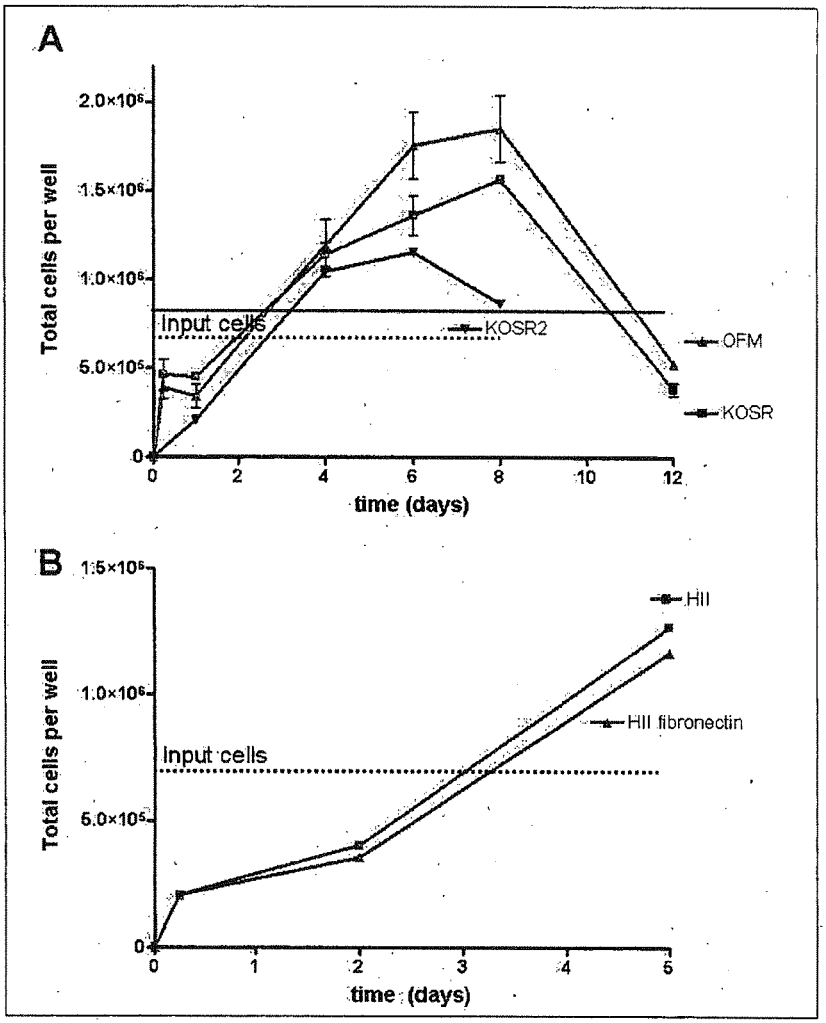
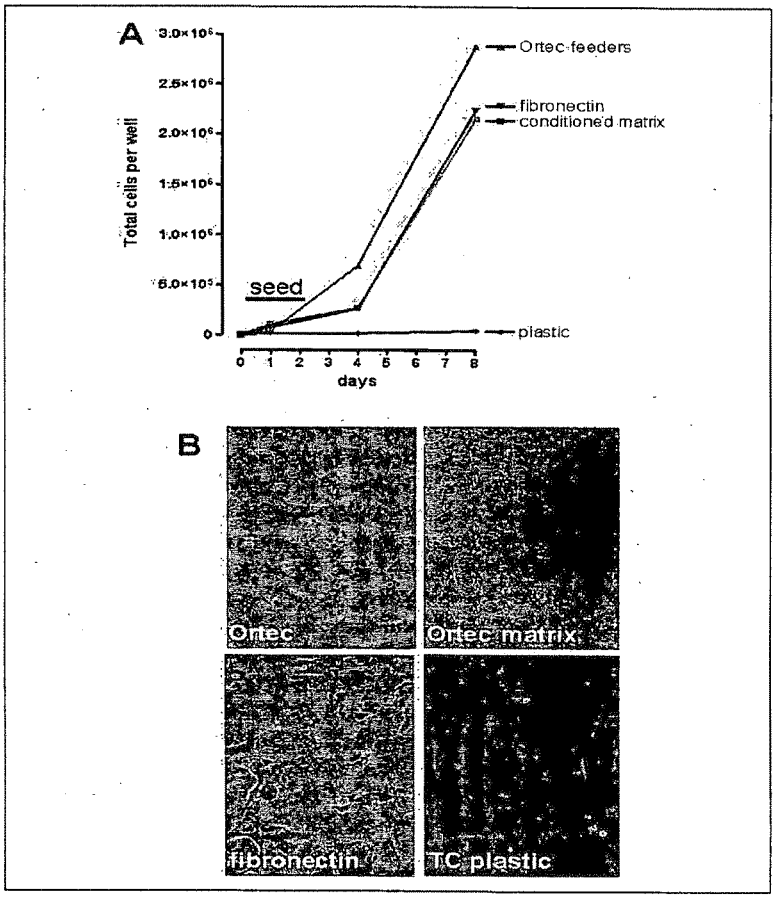

A
Total cells per well
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
Input cells
KOSR2
OFM
KOSR
0
2
4
6
8
10
12
time (days)
B
Total cells per well
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
Input cells
HII
HII fibronectin
0
1
2
3
4
5
time (days)

FIGURE 11


A
Total cells per well
3.0×10⁶
2.5×10⁶
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
seed
Ortec feeders
fibronectin
conditioned matrix
plastic
0 1 2 3 4 5 6 7 8
days
B
Ortec
Ortec matrix
fibronectin
TC plastic

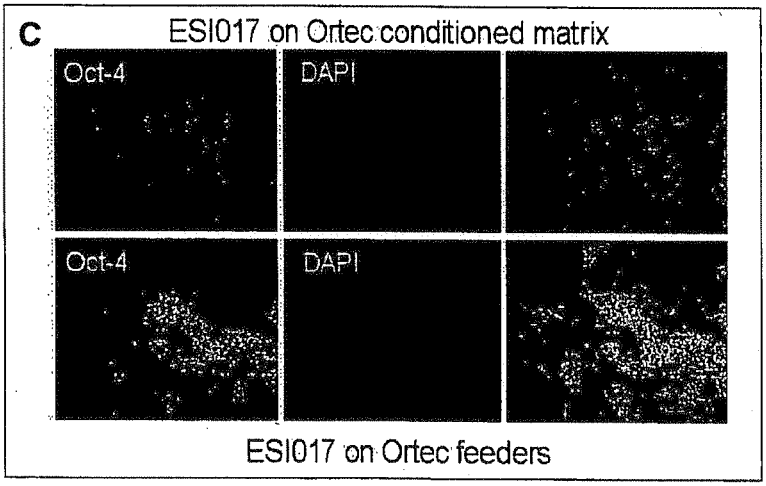
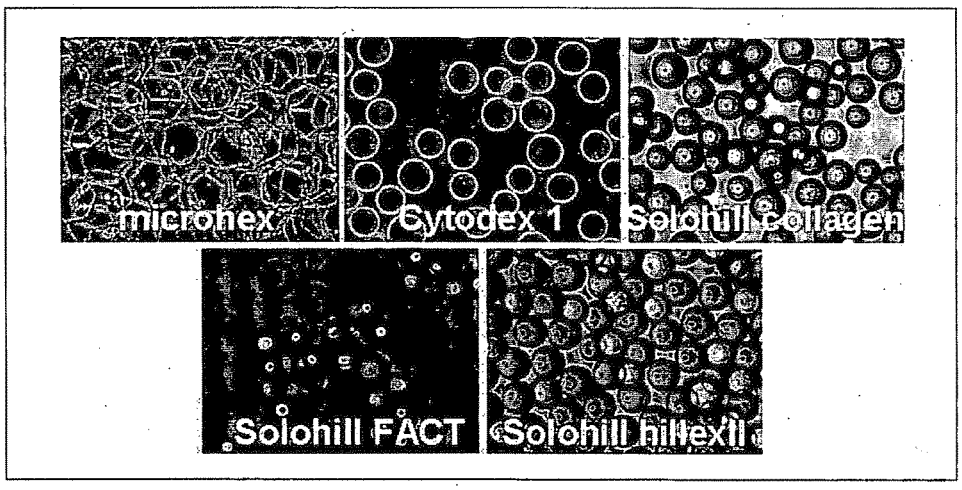
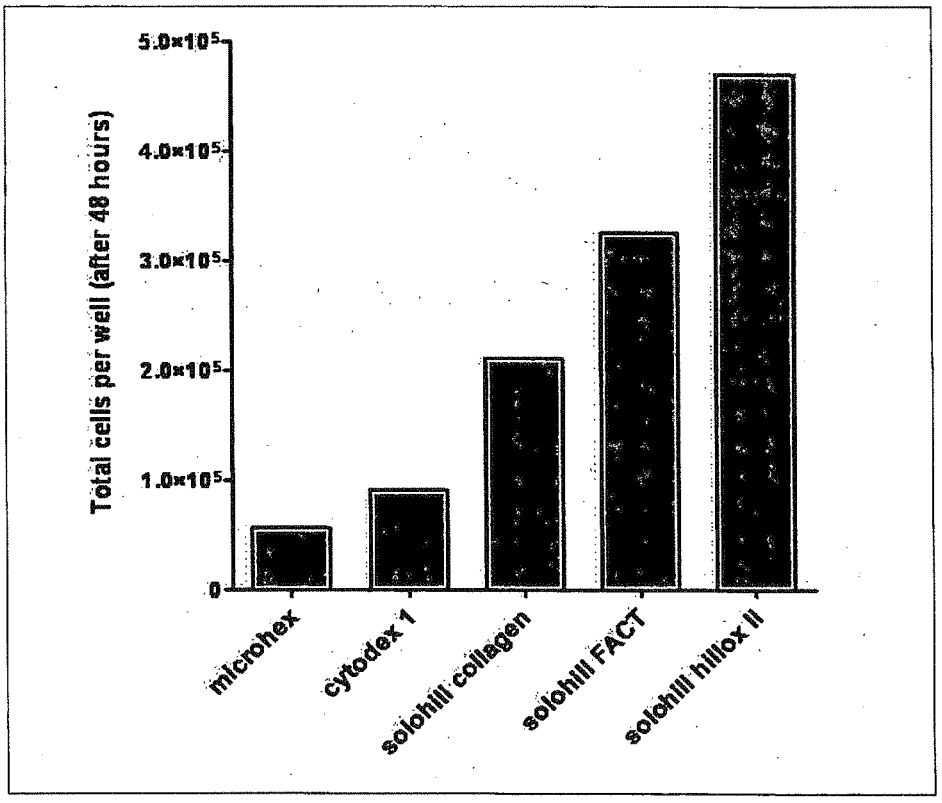

C
ESI017 on Ortec conditioned matrix
Oct-4
DAPI
Oct-4
DAPI
ESI017 on Ortec feeders

FIGURE 12

A


microhex
Cytodex 1
Solohill collagen
Solohill FACT
Solohill hillexII

B


Total cells per well (after 48 hours)
5.0x10^5
4.0x10^5
3.0x10^5
2.0x10^5
1.0x10^5
0
microhex
cytodex 1
solohill collagen
solohill FACT
solohill hillox II