Fig. 1

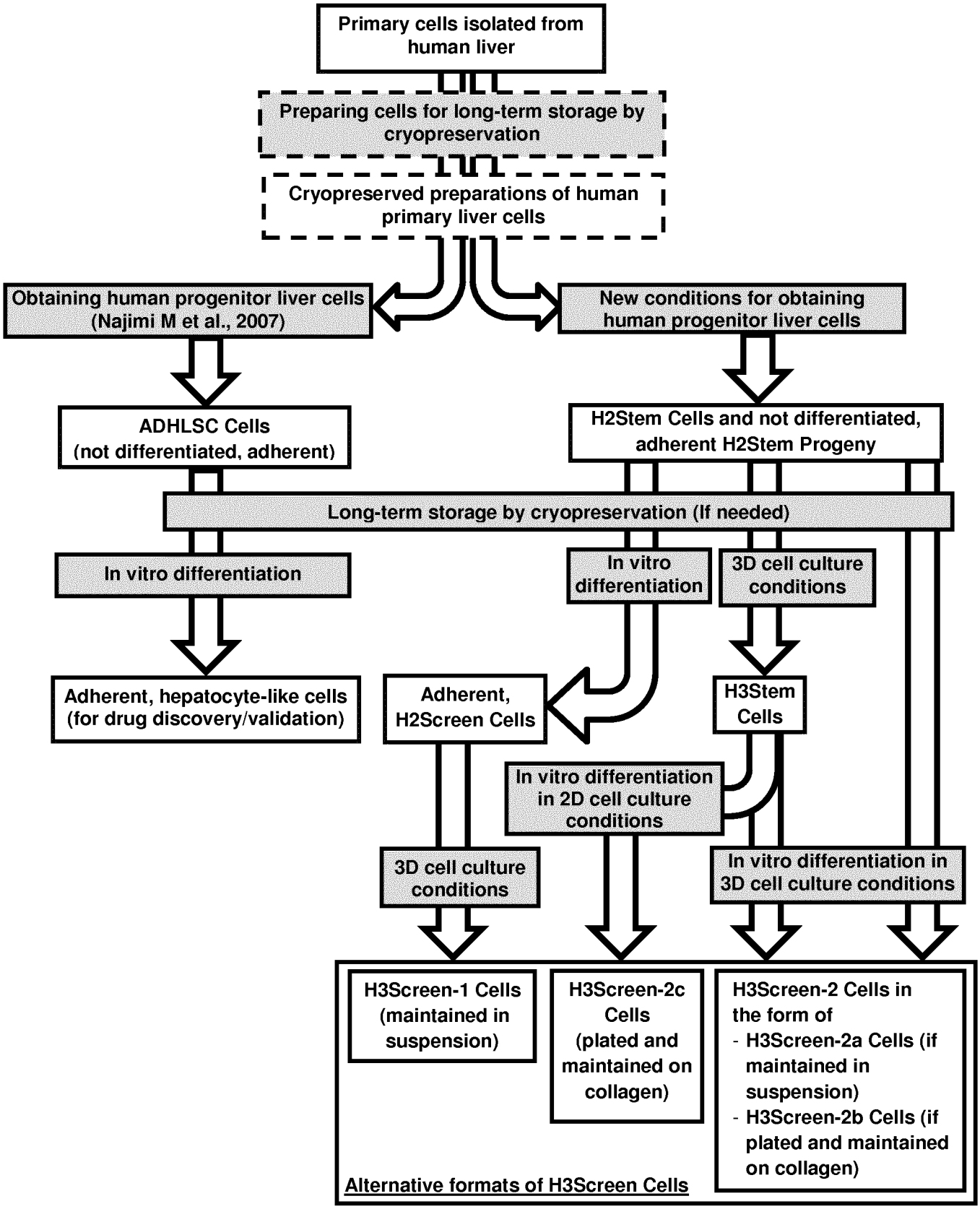
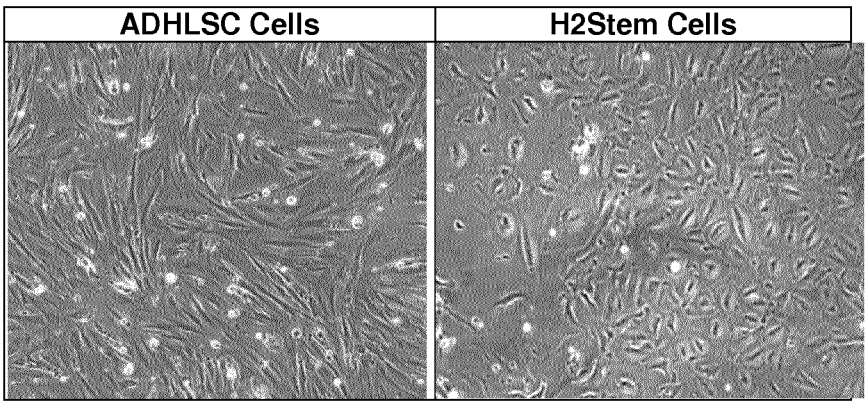

Primary cells isolated from human liver
Preparing cells for long-term storage by cryopreservation
Cryopreserved preparations of human primary liver cells
Obtaining human progenitor liver cells (Najimi M et al., 2007)
New conditions for obtaining human progenitor liver cells
ADHLSC Cells (not differentiated, adherent)
H2Stem Cells and not differentiated, adherent H2Stem Progeny
Long-term storage by cryopreservation (If needed)
In vitro differentiation
In vitro differentiation
3D cell culture conditions
Adherent, hepatocyte-like cells (for drug discovery/validation)
Adherent, H2Screen Cells
H3Stem Cells
In vitro differentiation in 2D cell culture conditions
3D cell culture conditions
In vitro differentiation in 3D cell culture conditions
H3Screen-1 Cells (maintained in suspension)
H3Screen-2c Cells (plated and maintained on collagen)
H3Screen-2 Cells in the form of
- H3Screen-2a Cells (if maintained in suspension)
- H3Screen-2b Cells (if plated and maintained on collagen)
Alternative formats of H3Screen Cells

Fig. 2

A)


ADHLSC Cells
H2Stem Cells

B)

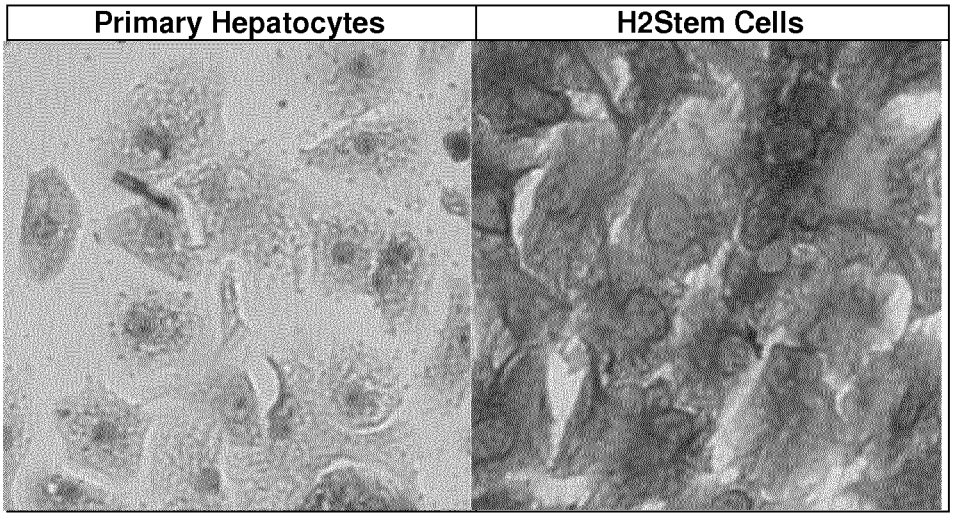

Primary Hepatocytes
H2Stem Cells

Fig. 3

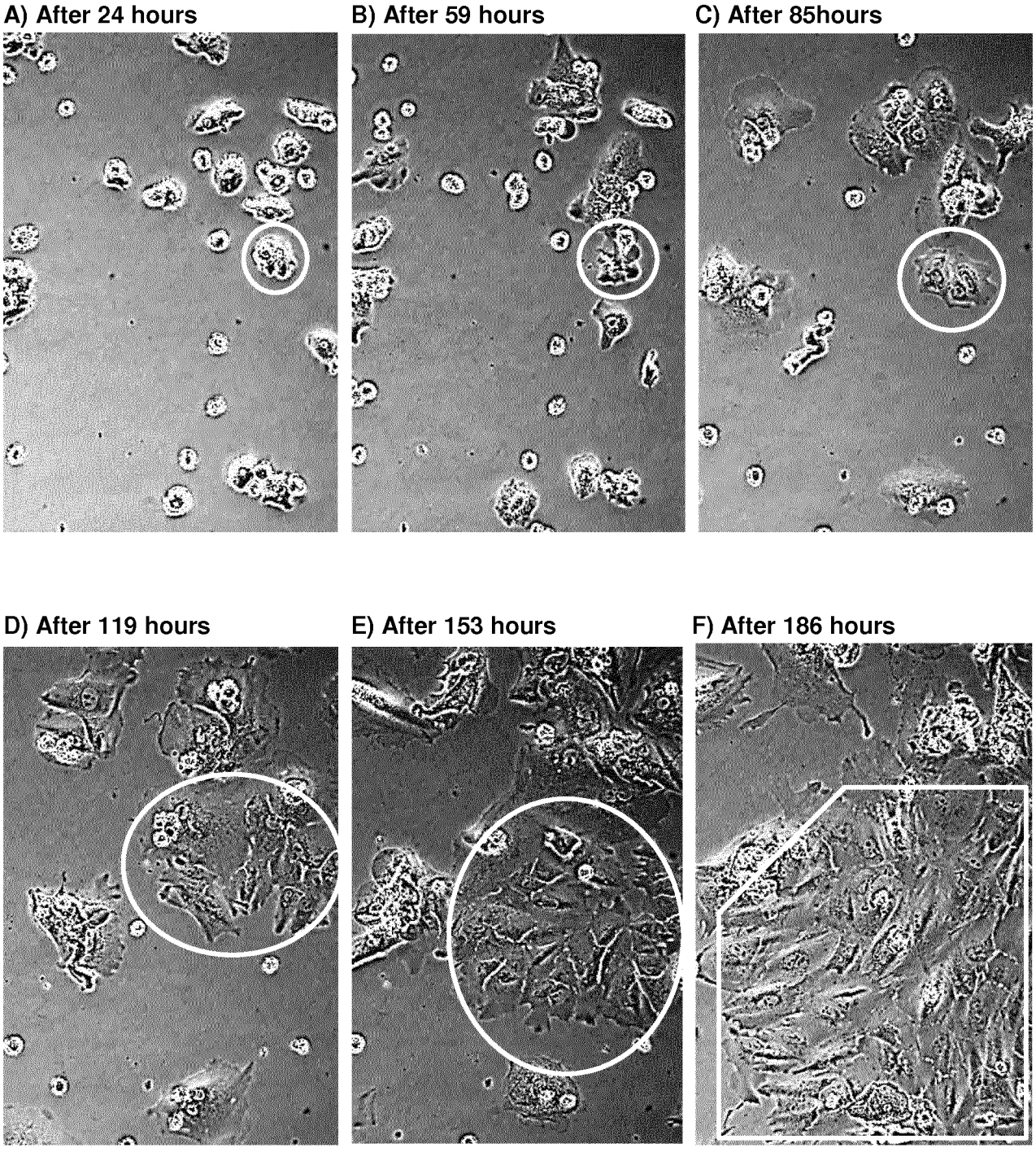
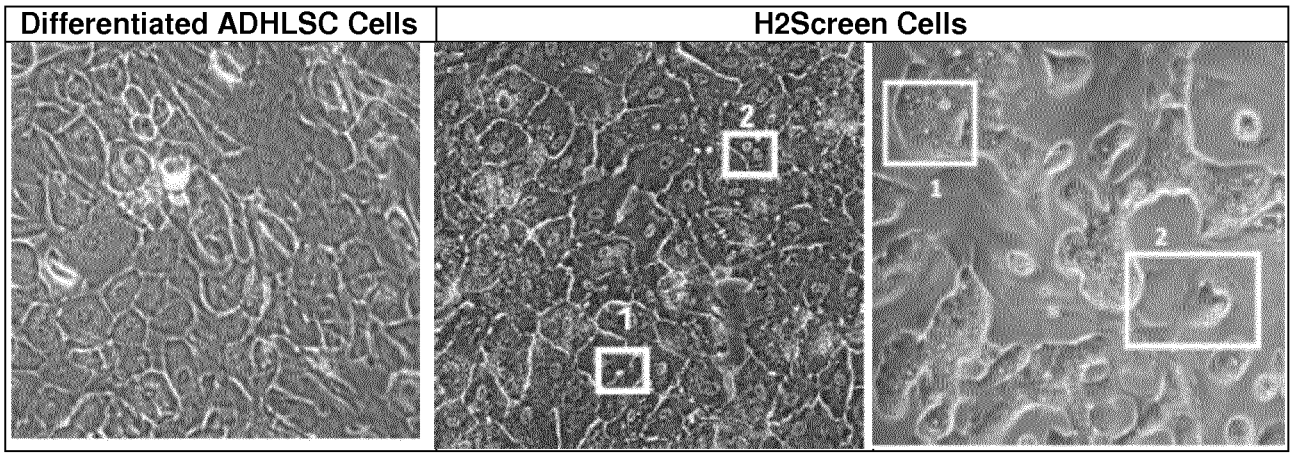
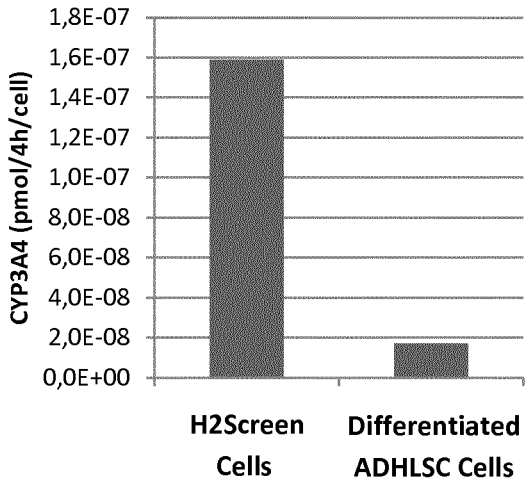

A) After 24 hours
B) After 59 hours
C) After 85hours
D) After 119 hours
E) After 153 hours
F) After 186 hours

Fig. 4

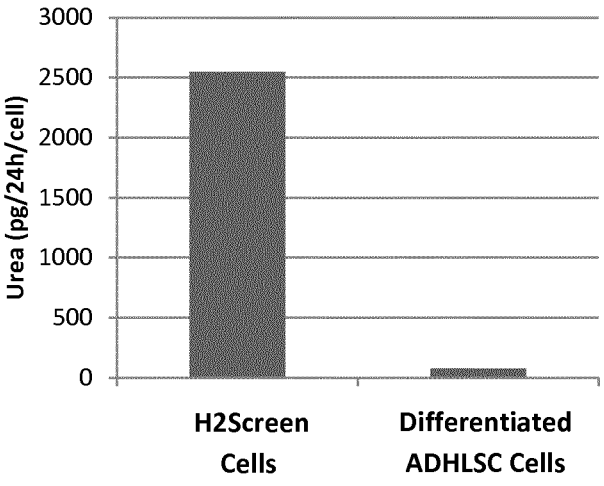
A)


Differentiated ADHLSC Cells
H2Screen Cells
1
2
1
2

B)


CYP3A4 (pmol/4h/cell)
1,8E-07
1,6E-07
1,4E-07
1,2E-07
1,0E-07
8,0E-08
6,0E-08
4,0E-08
2,0E-08
0,0E+00
H2Screen Cells
Differentiated ADHLSC Cells

C)


Urea (pg/24h/cell)
3000
2500
2000
1500
1000
500
0
H2Screen Cells
Differentiated ADHLSC Cells

D)

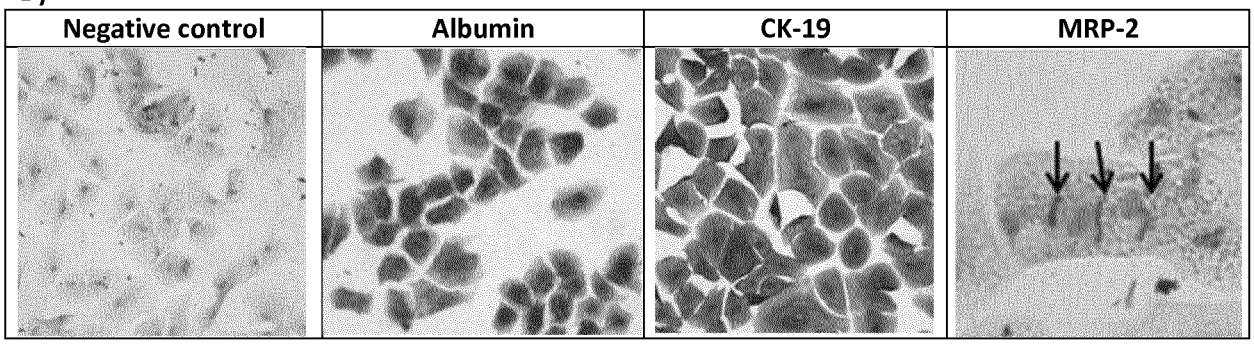
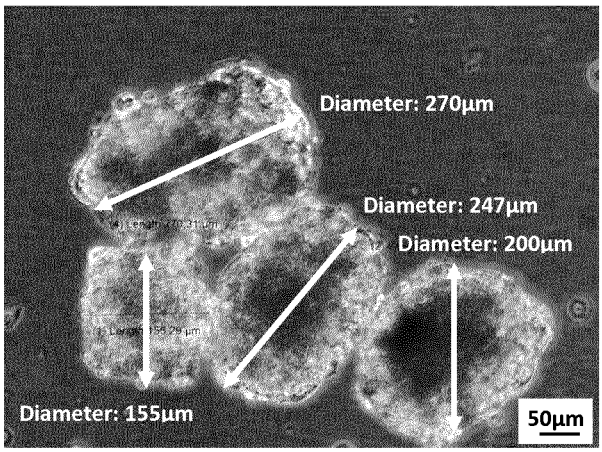
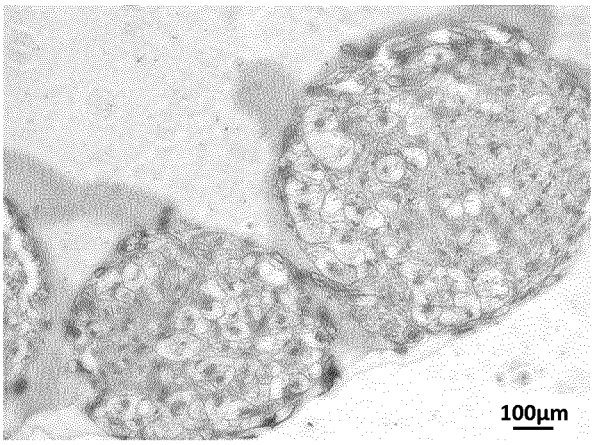

Negative control
Albumin
CK-19
MRP-2

Fig. 5

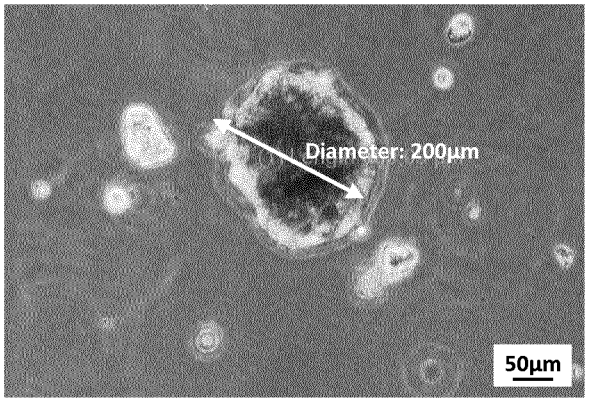
A) H3Stem Cells



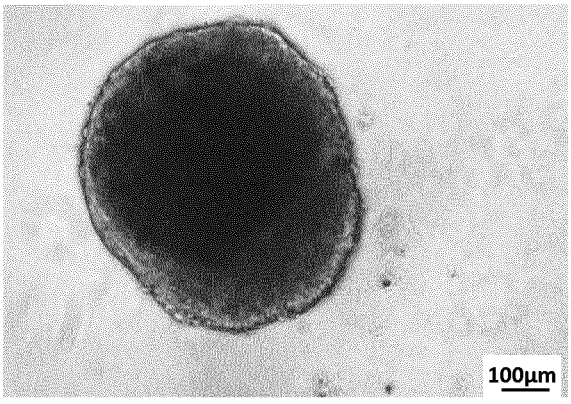
B) H3Screen-1 Cells



C) H3Screen-2a Cells (low binding plates)



D) H3Screen-2a Cells (in U-shaped wells)



E) Collagen-plated H3Screen-2b Cells

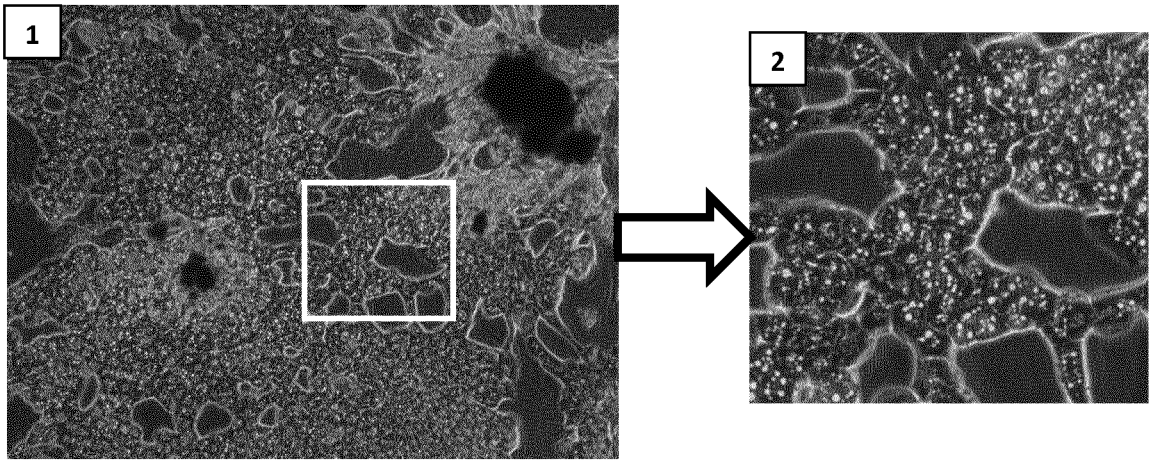


Fig. 6

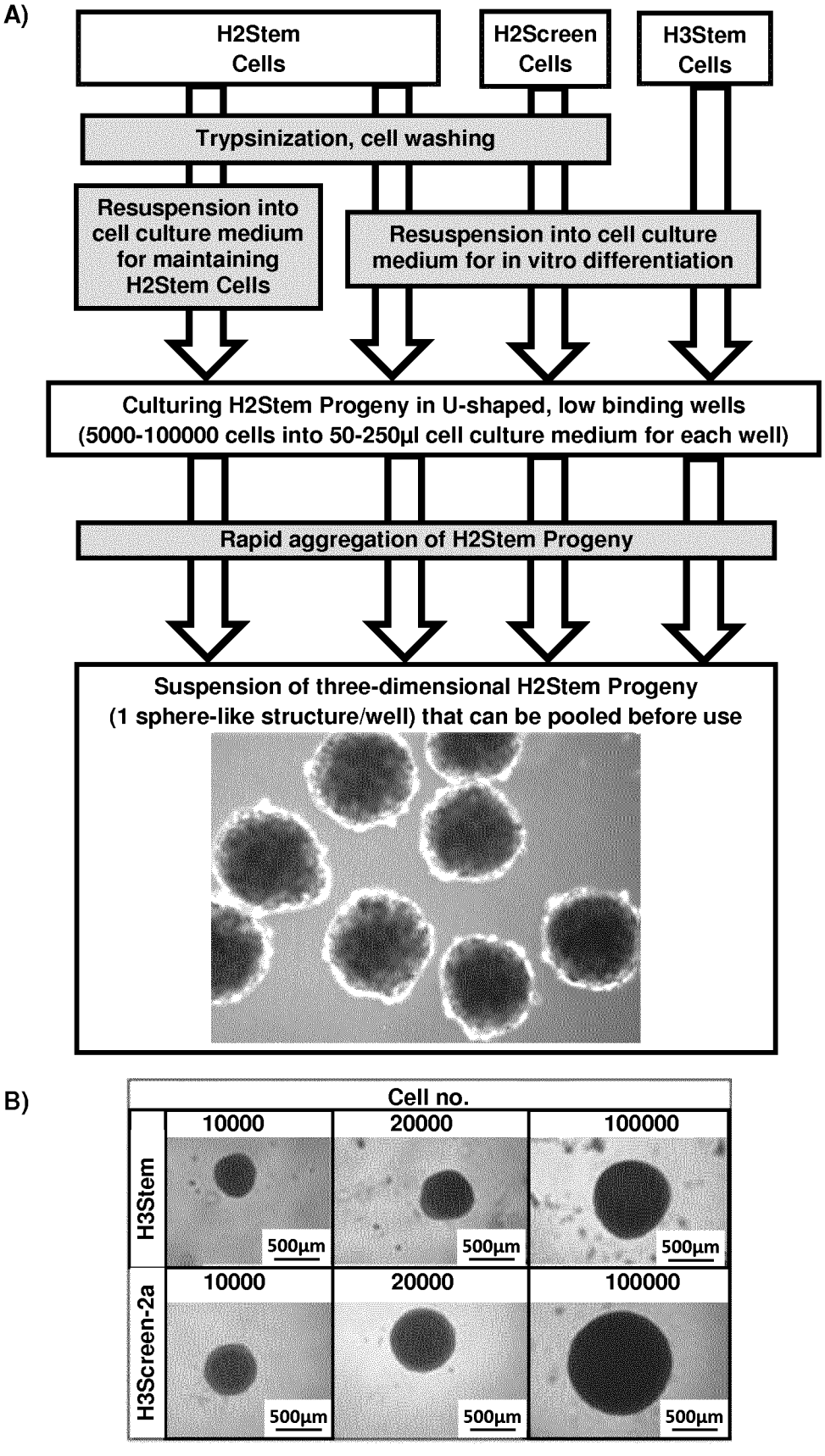
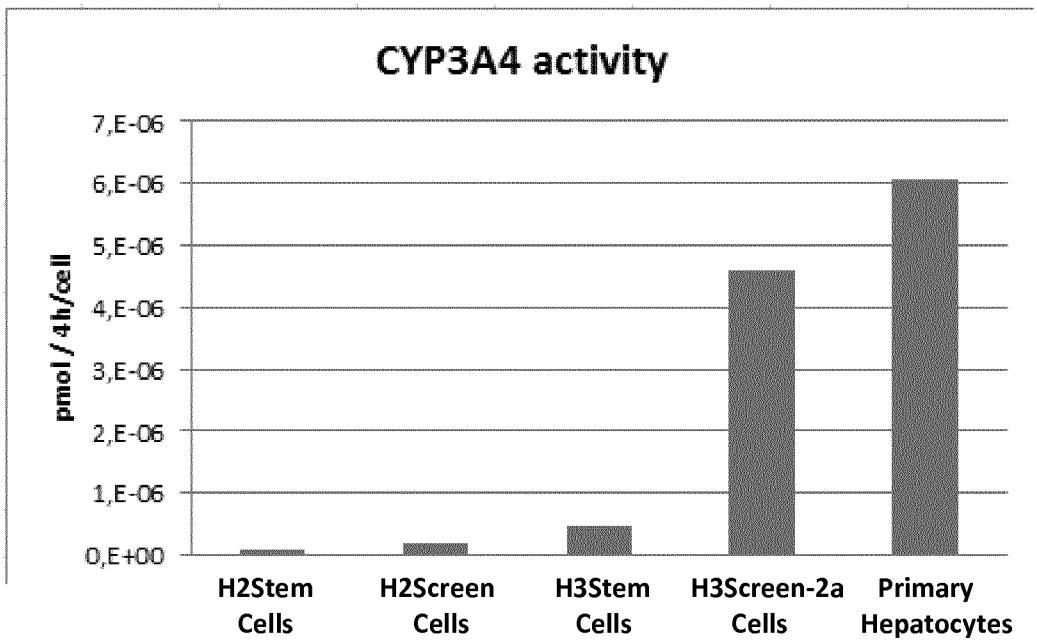
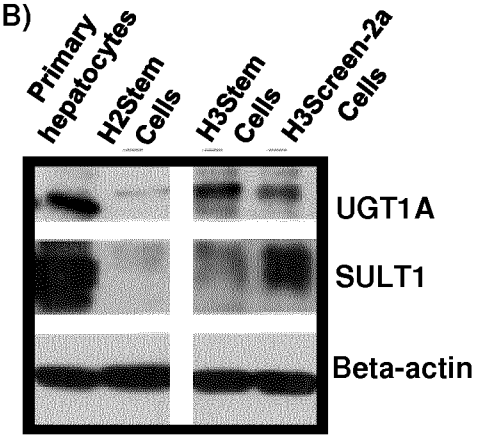

A)
H2Stem Cells
H2Screen Cells
H3Stem Cells
Trypsinization, cell washing
Resuspension into cell culture medium for maintaining H2Stem Cells
Resuspension into cell culture medium for in vitro differentiation
Culturing H2Stem Progeny in U-shaped, low binding wells (5000-100000 cells into 50-250µl cell culture medium for each well)
Rapid aggregation of H2Stem Progeny
Suspension of three-dimensional H2Stem Progeny (1 sphere-like structure/well) that can be pooled before use
B)
Cell no.
10000
20000
100000
H3Stem
H3Screen-2a
500µm

Fig. 7

A)



B)



C)

	Primary hepatocytes	H2Stem Cells	H3Stem Cells	H3Screen-2a Cells
UGT1A activity	+++	-	++	++
SULT activity	+++	+	+	++

D)

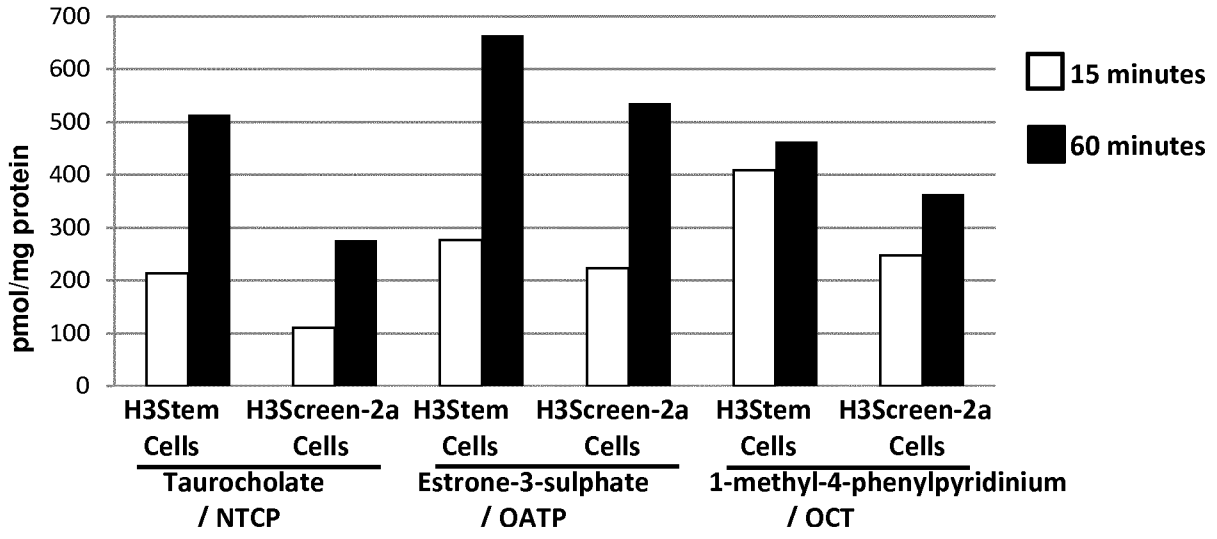
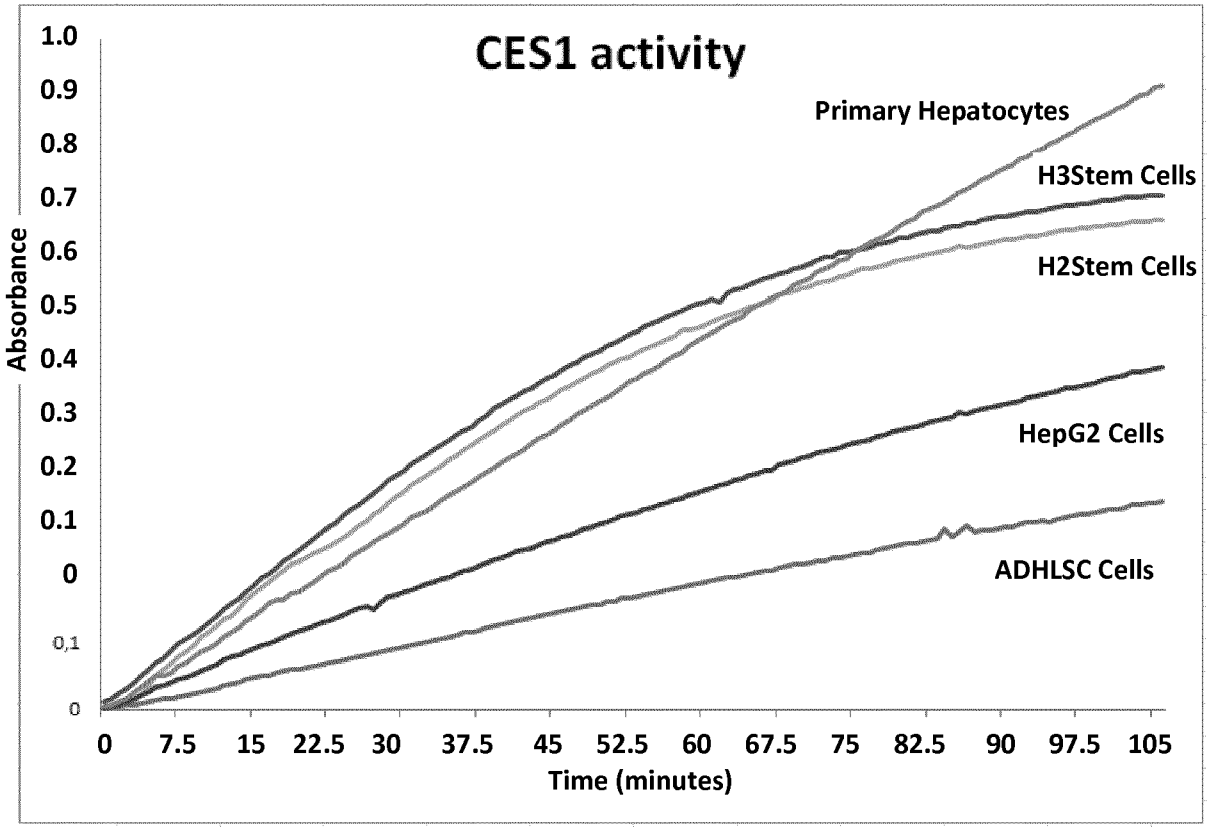


Fig. 8

A)


CES1 activity
Primary Hepatocytes
H3Stem Cells
H2Stem Cells
HepG2 Cells
ADHLSC Cells
Absorbance
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0,1
0
0 7.5 15 22.5 30 37.5 45 52.5 60 67.5 75 82.5 90 97.5 105
Time (minutes)

B)

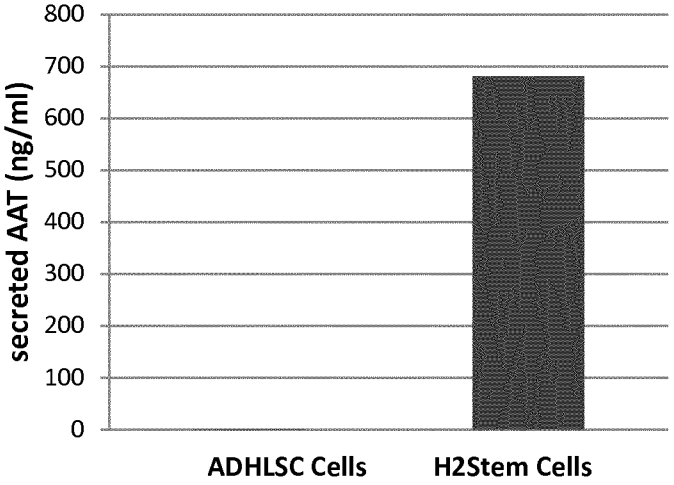

secreted AAT (ng/ml)
800
700
600
500
400
300
200
100
0
ADHLSC Cells
H2Stem Cells

Fig. 9

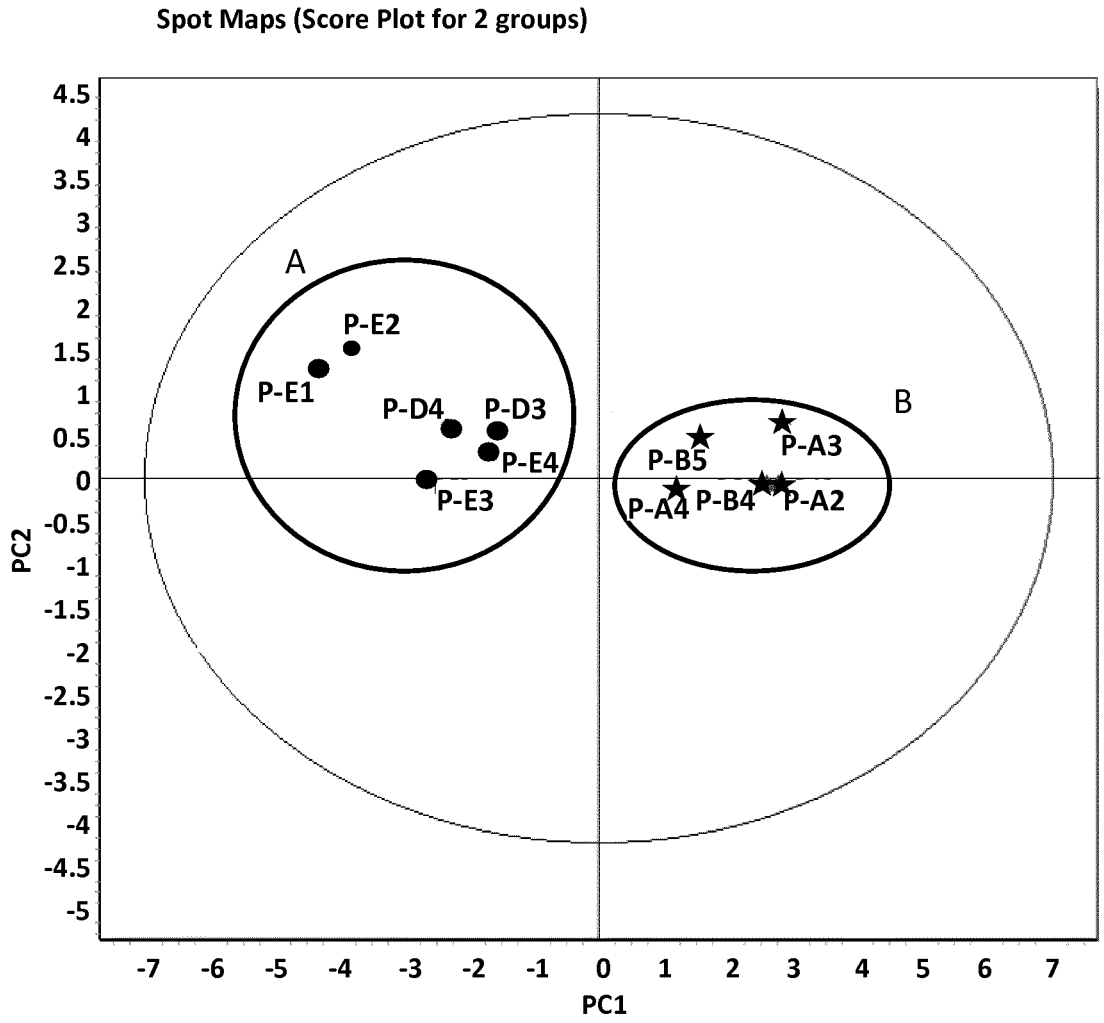
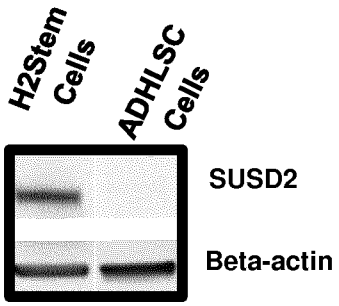
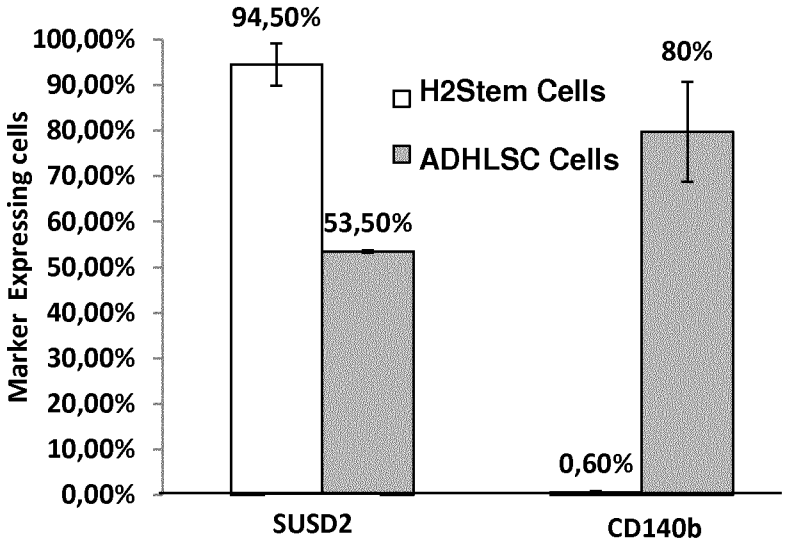

Spot Maps (Score Plot for 2 groups)
A
B
P-E2
P-E1
P-D4
P-D3
P-E4
P-E3
P-B5
P-A3
P-A4
P-B4
P-A2
PC2
PC1
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7

Fig. 10

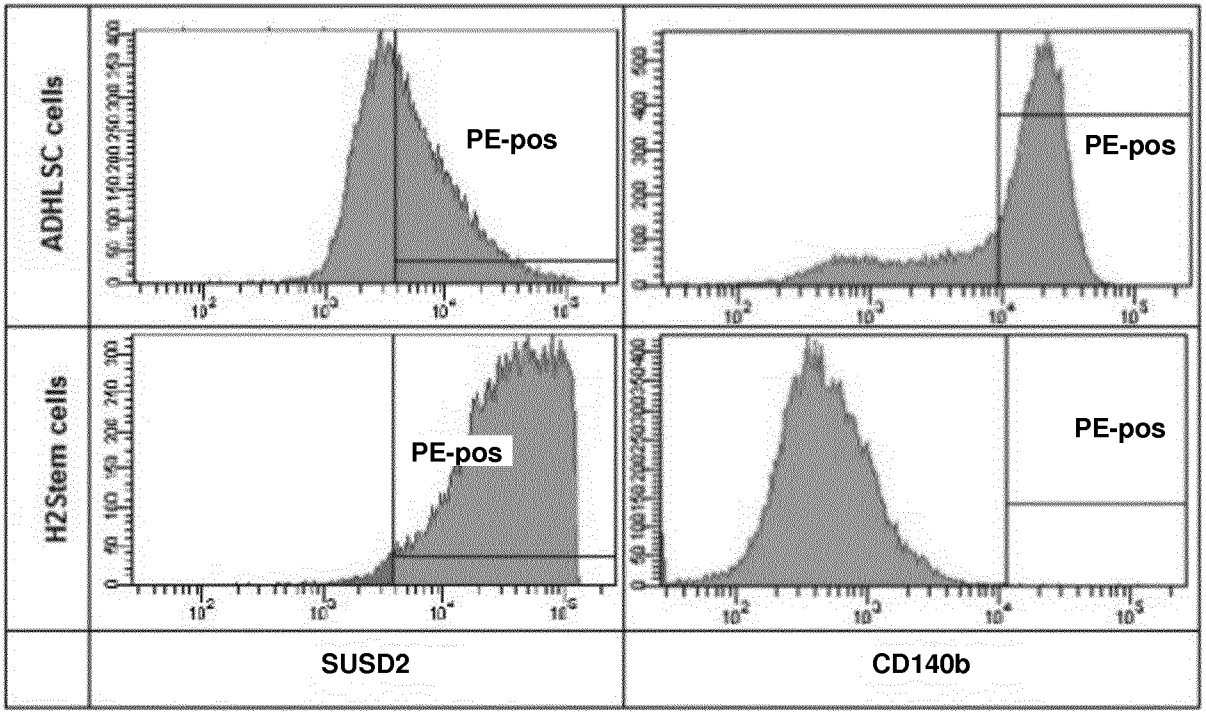
A)


H2Stem Cells
ADHLSC Cells
SUSD2
Beta-actin

B)


94,50%
80%
53,50%
0,60%
100,00%
90,00%
80,00%
70,00%
60,00%
50,00%
40,00%
30,00%
20,00%
10,00%
0,00%
Marker Expressing cells
H2Stem Cells
ADHLSC Cells
SUSD2
CD140b

C)


ADHLSC cells
H2Stem cells
PE-pos
PE-pos
PE-pos
PE-pos
SUSD2
CD140b