FIG. 1

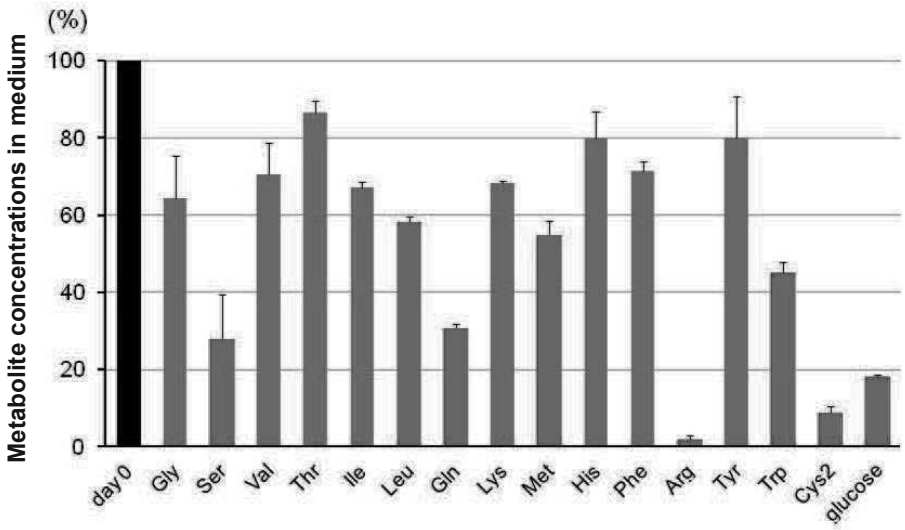

(%)
Metabolite concentrations in medium
100
80
60
40
20
0
day0
Gly
Ser
Val
Thr
Ile
Leu
Gln
Lys
Met
His
Phe
Arg
Tyr
Trp
Cys2
glucose

FIG. 2

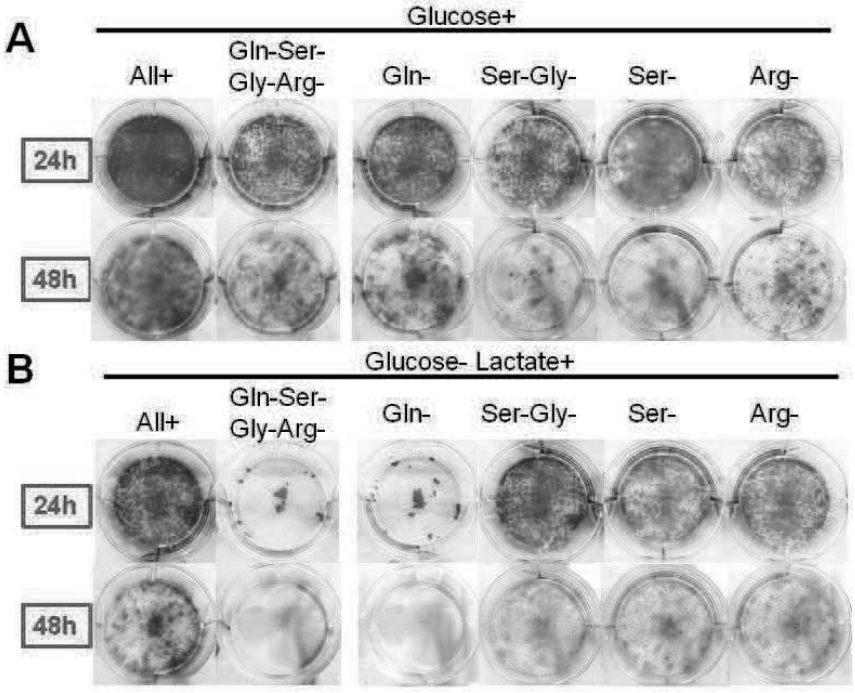

A
Glucose+
All+
Gln-Ser-Gly-Arg-
Gln-
Ser-Gly-
Ser-
Arg-
24h
48h
B
Glucose- Lactate+
All+
Gln-Ser-Gly-Arg-
Gln-
Ser-Gly-
Ser-
Arg-
24h
48h

FIG. 4

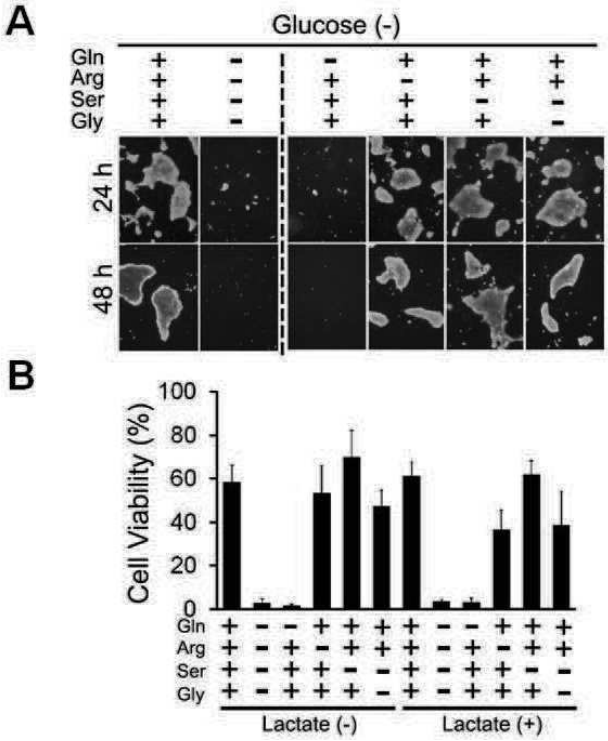
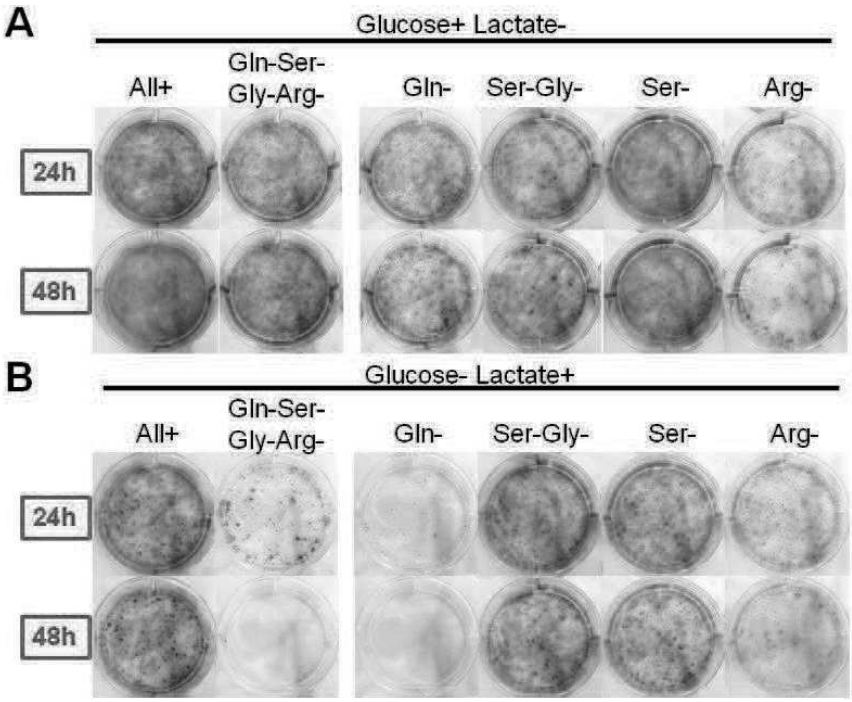
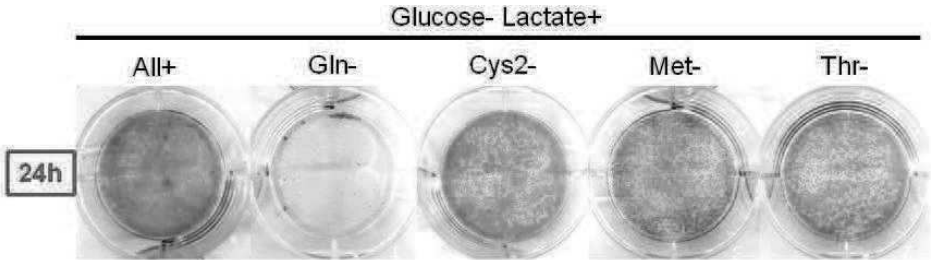


FIG. 4



Survival rates (ALP staining) of hiPSCs under different conditions

FIG. 5



Survival rates (ALP staining) of hESCs under different conditions

Gln: Glutamine Cys2: Cystine Met: Methionine Thr: Threonine

FIG. 6

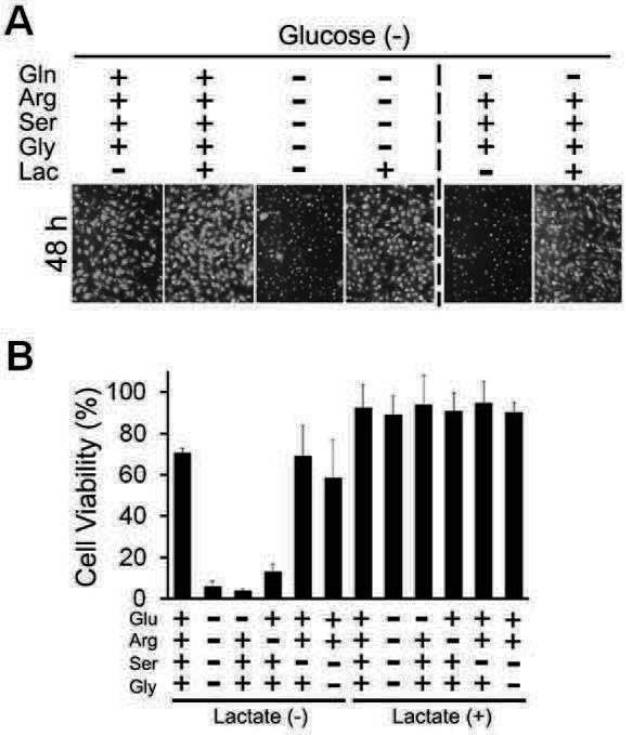


FIG. 7

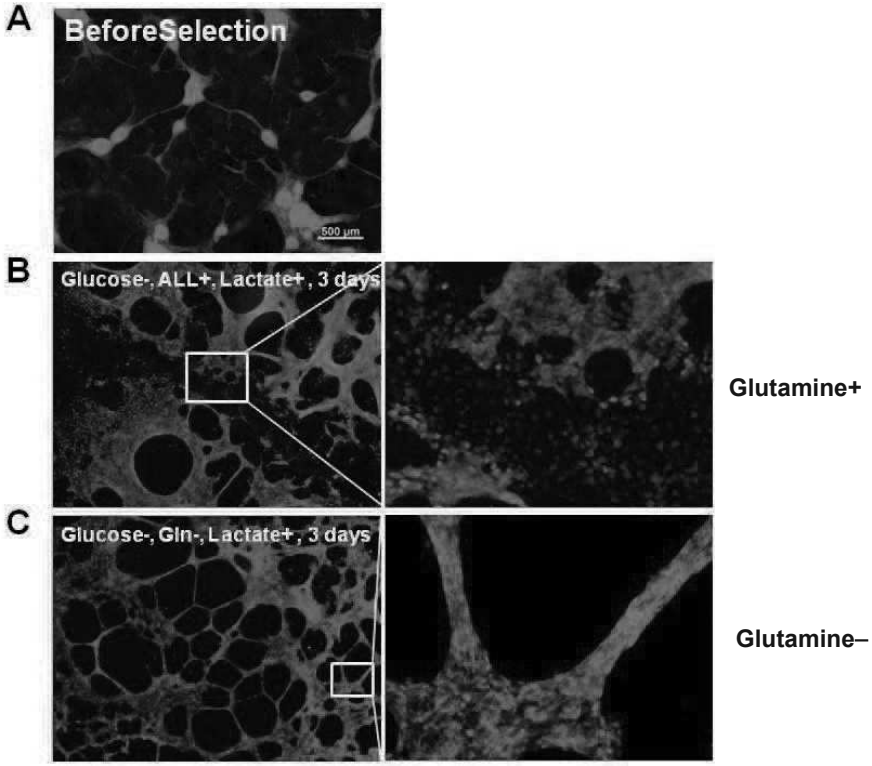

A
BeforeSelection
500 μm
B
Glucose-, ALL+, Lactate+, 3 days
Glutamine+
C
Glucose-, Gln-, Lactate+, 3 days
Glutamine–

FIG. 8

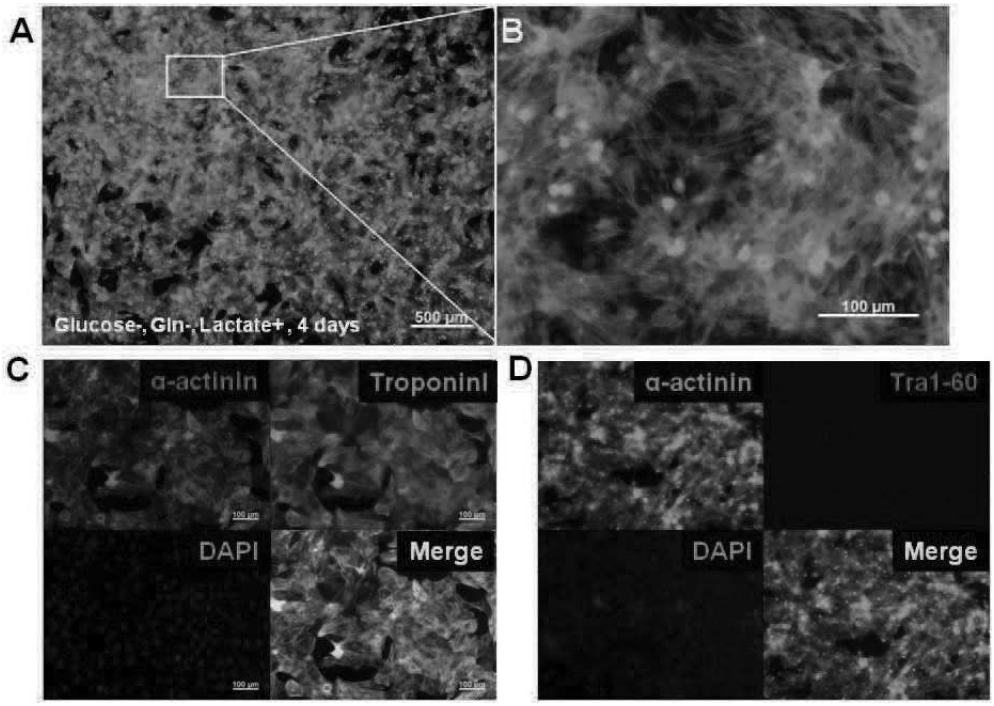

A
Glucose-, Gln-, Lactate+, 4 days
500 μm
B
100 μm
C
α-actinin
Troponin I
DAPI
Merge
D
α-actinin
Tra1-60
DAPI
Merge

FIG. 9

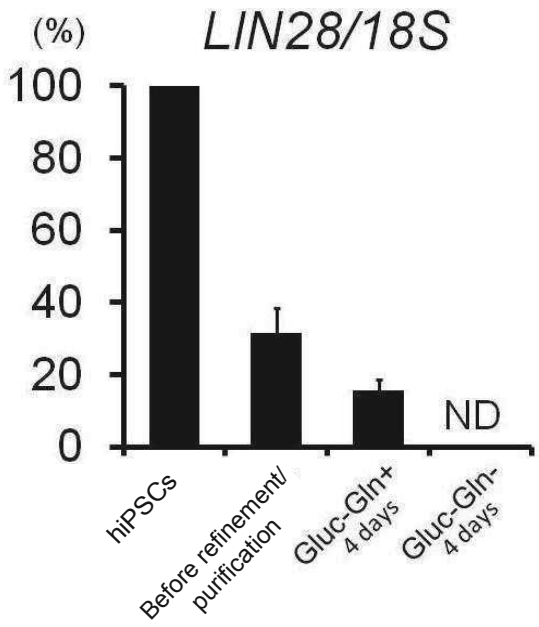

(%)
LIN28/18S
100
80
60
40
20
0
ND
hiPSCs
Before refinement/ purification
Gluc-Gln+ 4 days
Gluc-Gln- 4 days

FIG. 10

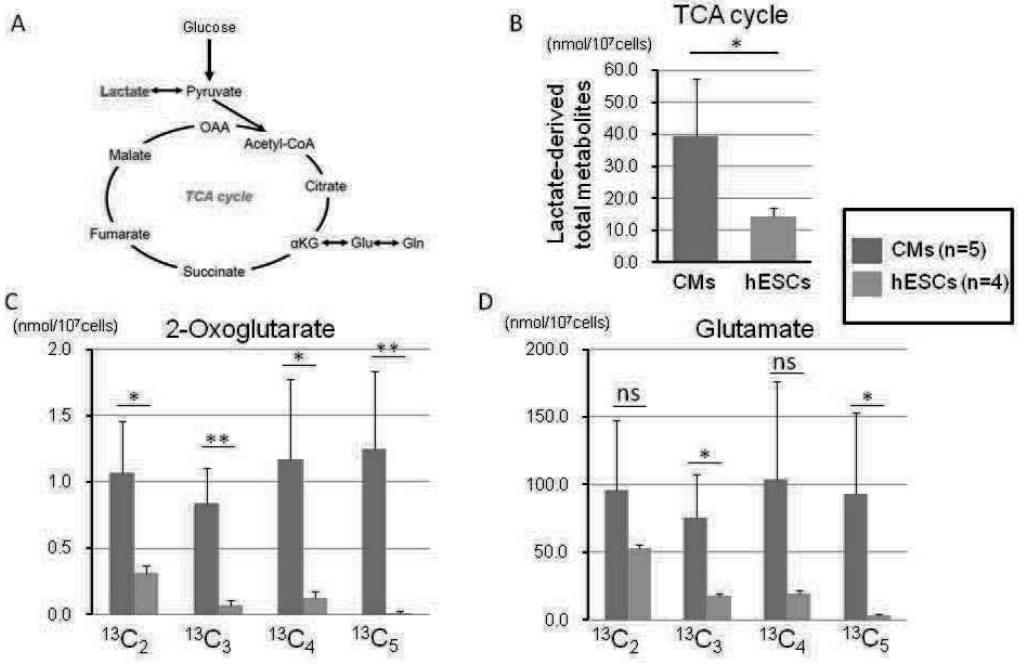

A
Glucose
Lactate
Pyruvate
OAA
Acetyl-CoA
Malate
Citrate
TCA cycle
Fumarate
αKG
Glu
Gln
Succinate
B
TCA cycle
(nmol/10⁷cells)
Lactate-derived total metabolites
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CMs
hESCs
CMs (n=5)
hESCs (n=4)
C
(nmol/10⁷cells)
2-Oxoglutarate
2.0
1.5
1.0
0.5
0.0
13C2
13C3
13C4
13C5
D
(nmol/10⁷cells)
Glutamate
200.0
150.0
100.0
50.0
0.0
ns
13C2
13C3
13C4
13C5

FIG. 11

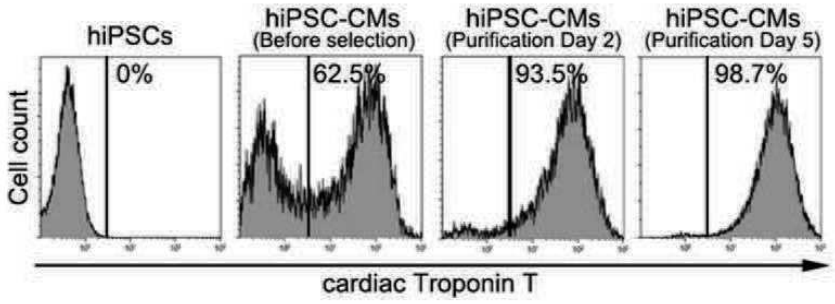

hiPSCs
0%
hiPSC-CMs
(Before selection)
62.5%
hiPSC-CMs
(Purification Day 2)
93.5%
hiPSC-CMs
(Purification Day 5)
98.7%
Cell count
cardiac Troponin T

FIG. 12


Gluc- All+
Gluc- Gln-
Gluc- Gln-
DM-αKG+
Gluc- Gln- Pyr+

FIG. 13

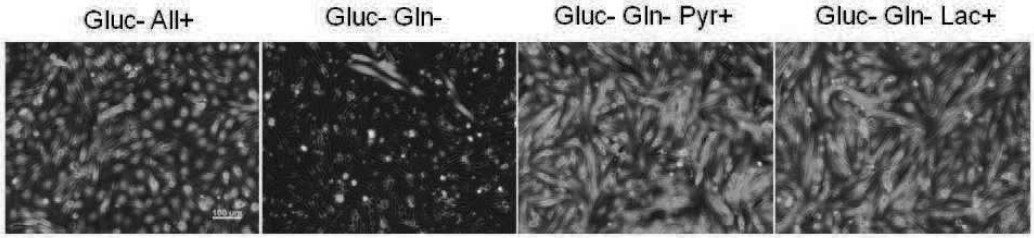

Gluc- All+
Gluc- Gln-
Gluc- Gln- Pyr+
Gluc- Gln- Lac+

FIG. 14

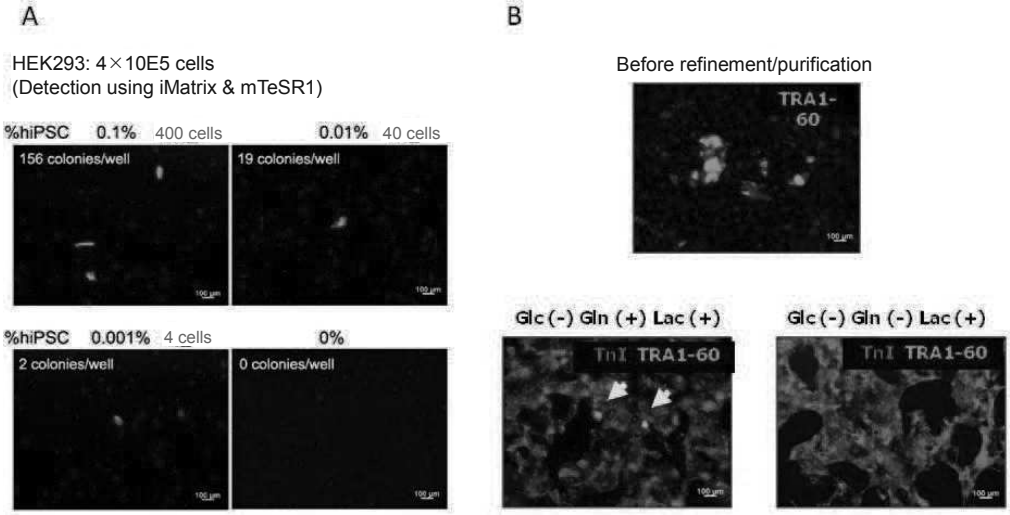

A
HEK293: 4×10E5 cells
(Detection using iMatrix & mTeSR1)
%hiPSC 0.1% 400 cells
156 colonies/well
0.01% 40 cells
19 colonies/well
%hiPSC 0.001% 4 cells
2 colonies/well
0%
0 colonies/well
B
Before refinement/purification
TRA1-60
Glc(-) Gln (+) Lac (+)
TnI TRA1-60
Glc(-) Gln (-) Lac (+)
TnI TRA1-60

FIG. 15

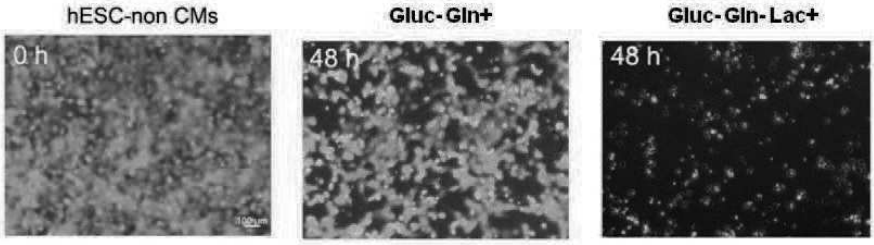

hESC-non CMs
Gluc-Gln+
Gluc-Gln-Lac+
0 h
48 h
48 h

FIG. 16

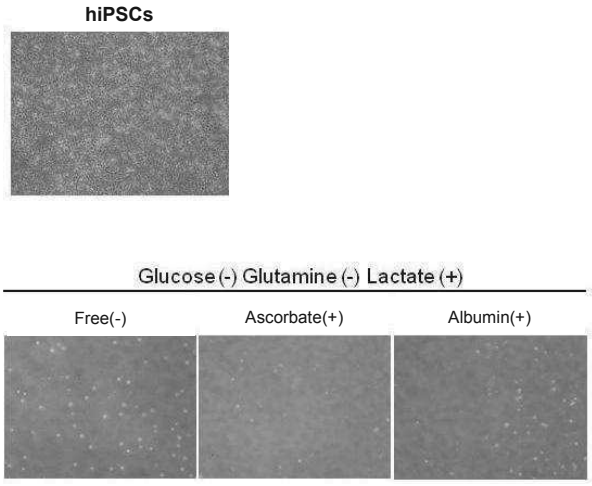

hiPSCs
Glucose (-) Glutamine (-) Lactate (+)
Free(-)
Ascorbate(+)
Albumin(+)

FIG. 17

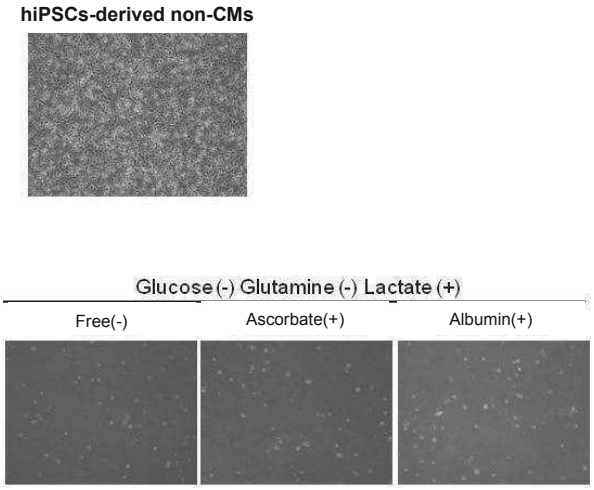

hiPSCs-derived non-CMs
Glucose (-) Glutamine (-) Lactate (+)
Free(-)
Ascorbate(+)
Albumin(+)

FIG. 18

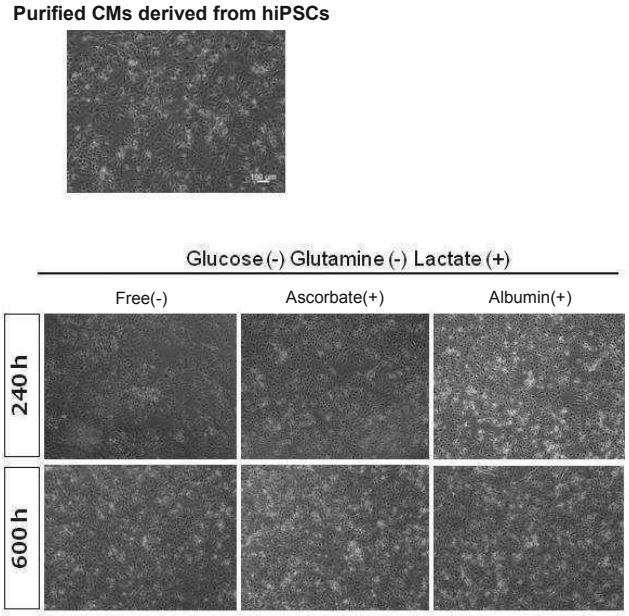

Purified CMs derived from hiPSCs
Glucose (-) Glutamine (-) Lactate (+)
Free(-)
Ascorbate(+)
Albumin(+)
240 h
600 h

FIG. 19

