Fig.1

Induction/Amplification/Differentiation Methods for Human iPS Cell-Derived Primitive Gut Endoderm Cells (PGECs)

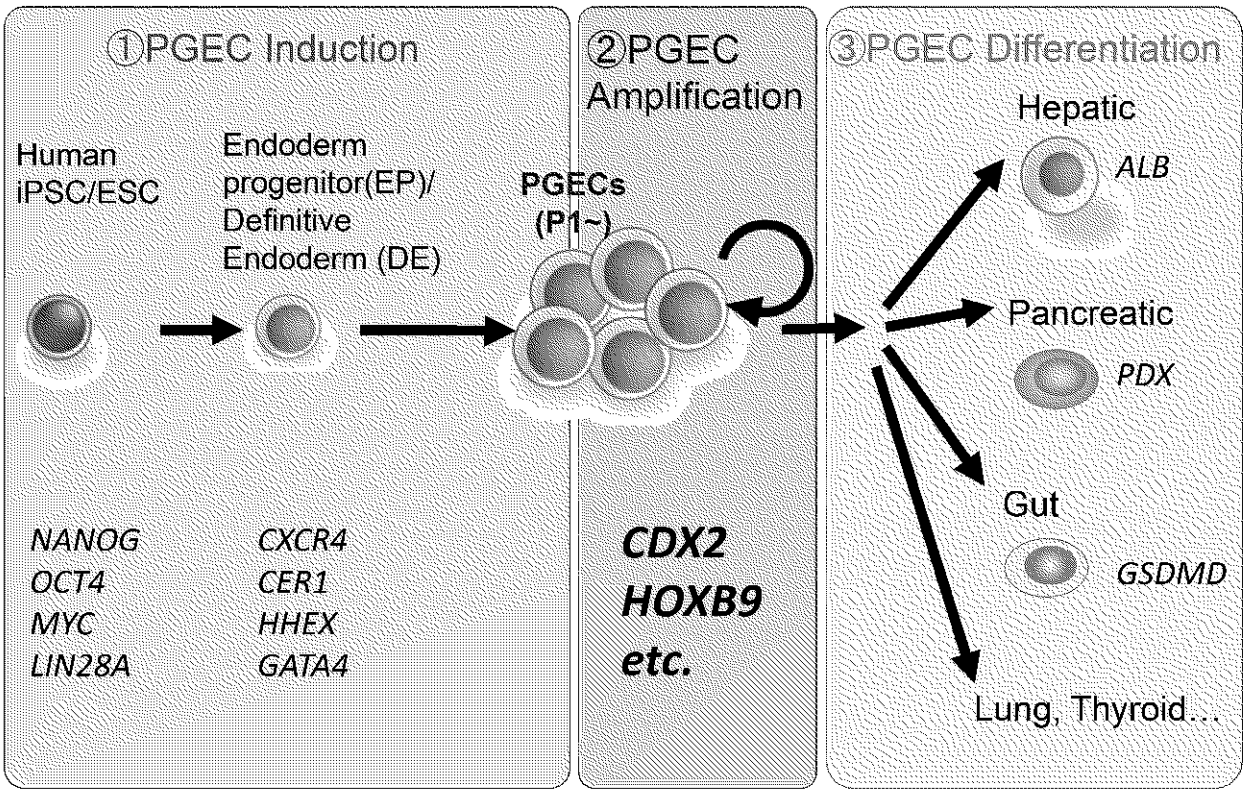


Fig.2

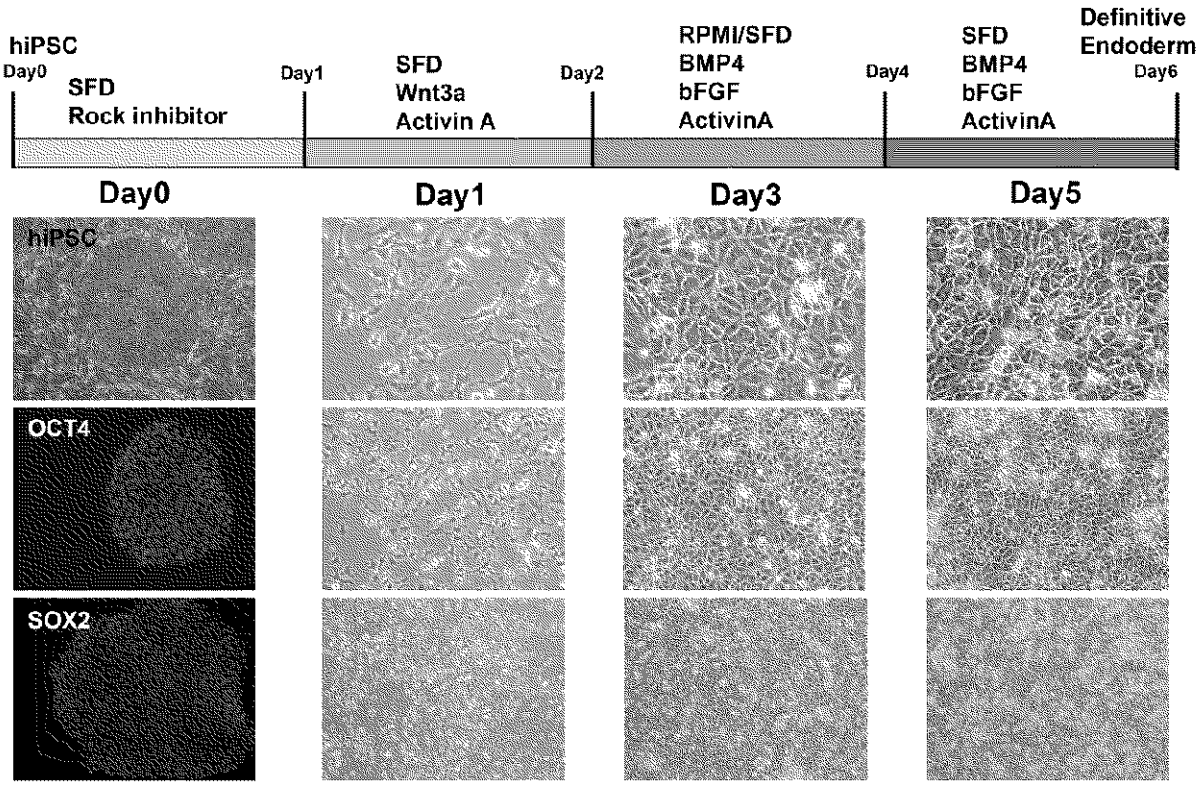

Induction of Human iPS Cell-Derived DE toward PGEC Induction
hiPSC
Day0
SFD
Rock inhibitor
Day1
SFD
Wnt3a
Activin A
Day2
RPMI/SFD
BMP4
bFGF
ActivinA
Day4
SFD
BMP4
bFGF
ActivinA
Definitive Endoderm
Day6
Day0
Day1
Day3
Day5
hiPSC
OCT4
SOX2

Fig.3

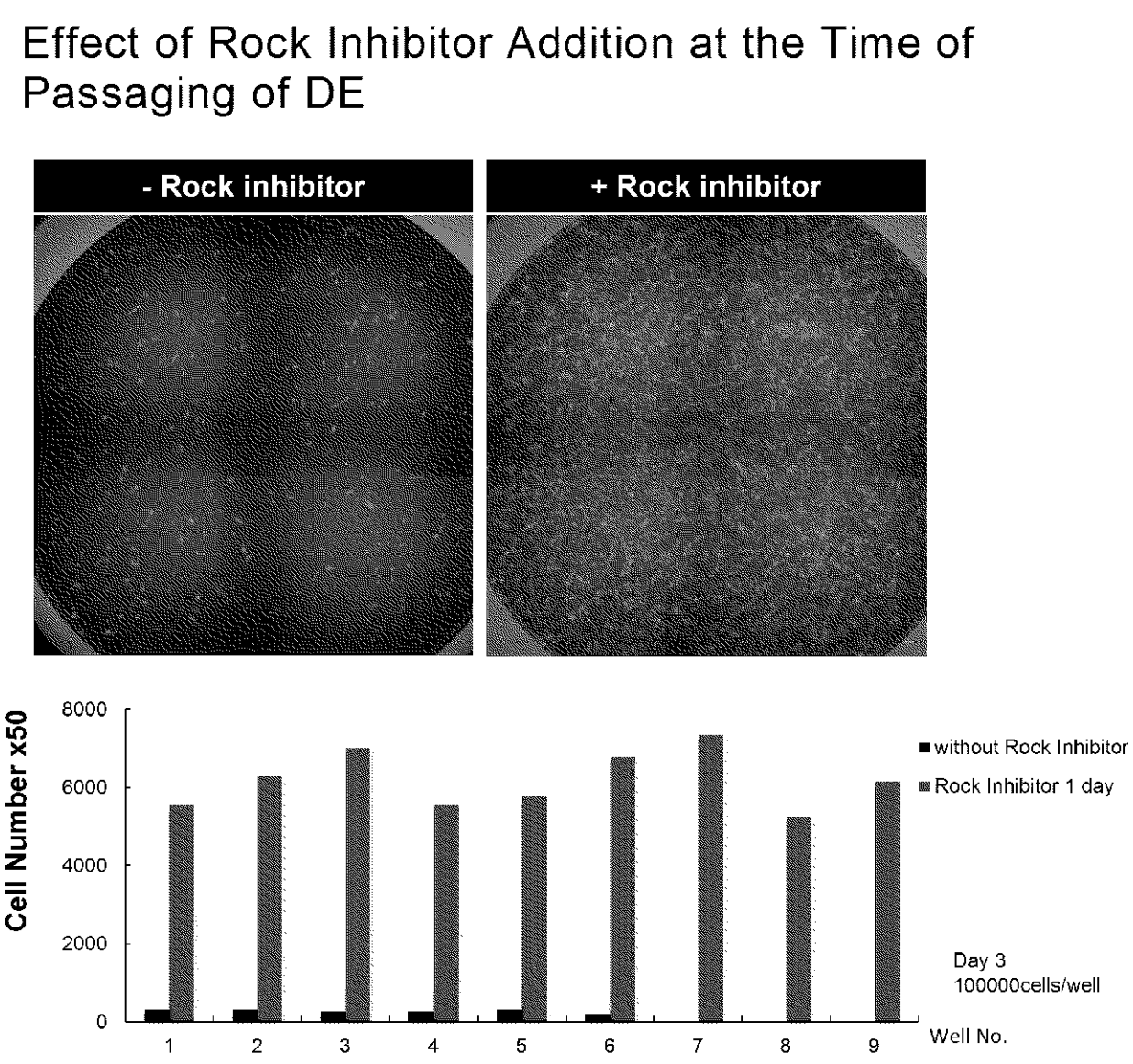

Effect of Rock Inhibitor Addition at the Time of Passaging of DE
- Rock inhibitor
+ Rock inhibitor
Cell Number x50
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
9
Well No.
without Rock Inhibitor
Rock Inhibitor 1 day
Day 3
100000cells/well

Fig.4

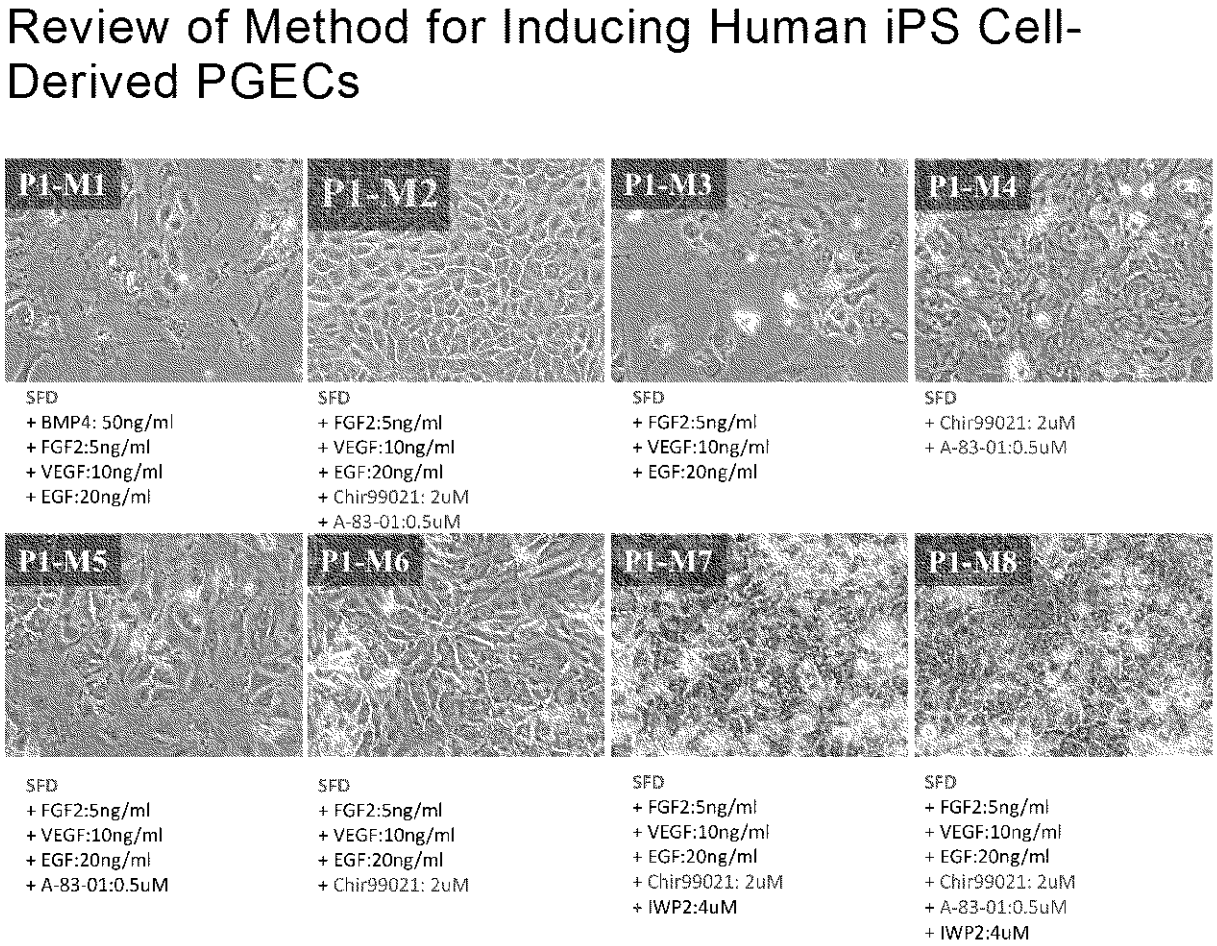

Review of Method for Inducing Human iPS Cell-Derived PGECs
P1-M1
SFD
+ BMP4: 50ng/ml
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
P1-M2
SFD
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
+ Chir99021: 2uM
+ A-83-01:0.5uM
P1-M3
SFD
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
P1-M4
SFD
+ Chir99021: 2uM
+ A-83-01:0.5uM
P1-M5
SFD
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
+ A-83-01:0.5uM
P1-M6
SFD
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
+ Chir99021: 2uM
P1-M7
SFD
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
+ Chir99021: 2uM
+ IWP2:4uM
P1-M8
SFD
+ FGF2:5ng/ml
+ VEGF:10ng/ml
+ EGF:20ng/ml
+ Chir99021: 2uM
+ A-83-01:0.5uM
+ IWP2:4uM

Fig.5

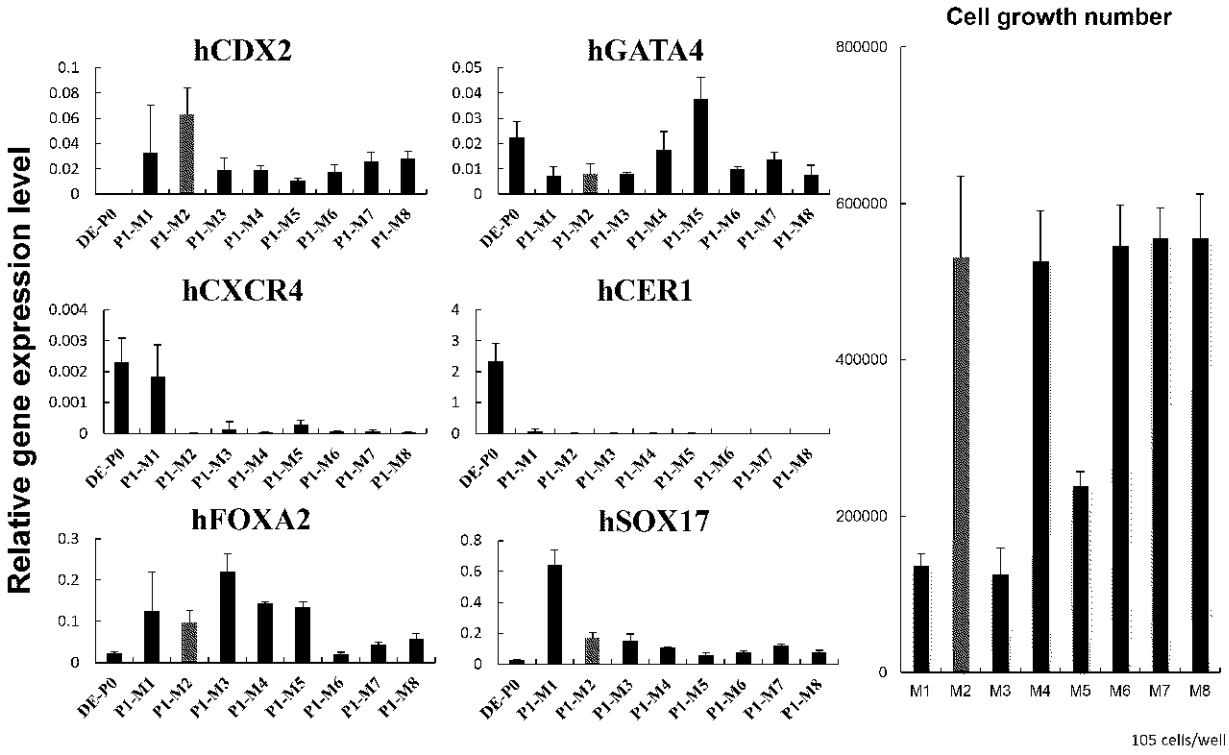

Review of Method for Amplifying Human iPS Cell-Derived PGECs
Relative gene expression level
hCDX2
hGATA4
hCXCR4
hCER1
hFOXA2
hSOX17
DE-P0
P1-M1
P1-M2
P1-M3
P1-M4
P1-M5
P1-M6
P1-M7
P1-M8
Cell growth number
M1
M2
M3
M4
M5
M6
M7
M8
105 cells/well

Fig.6

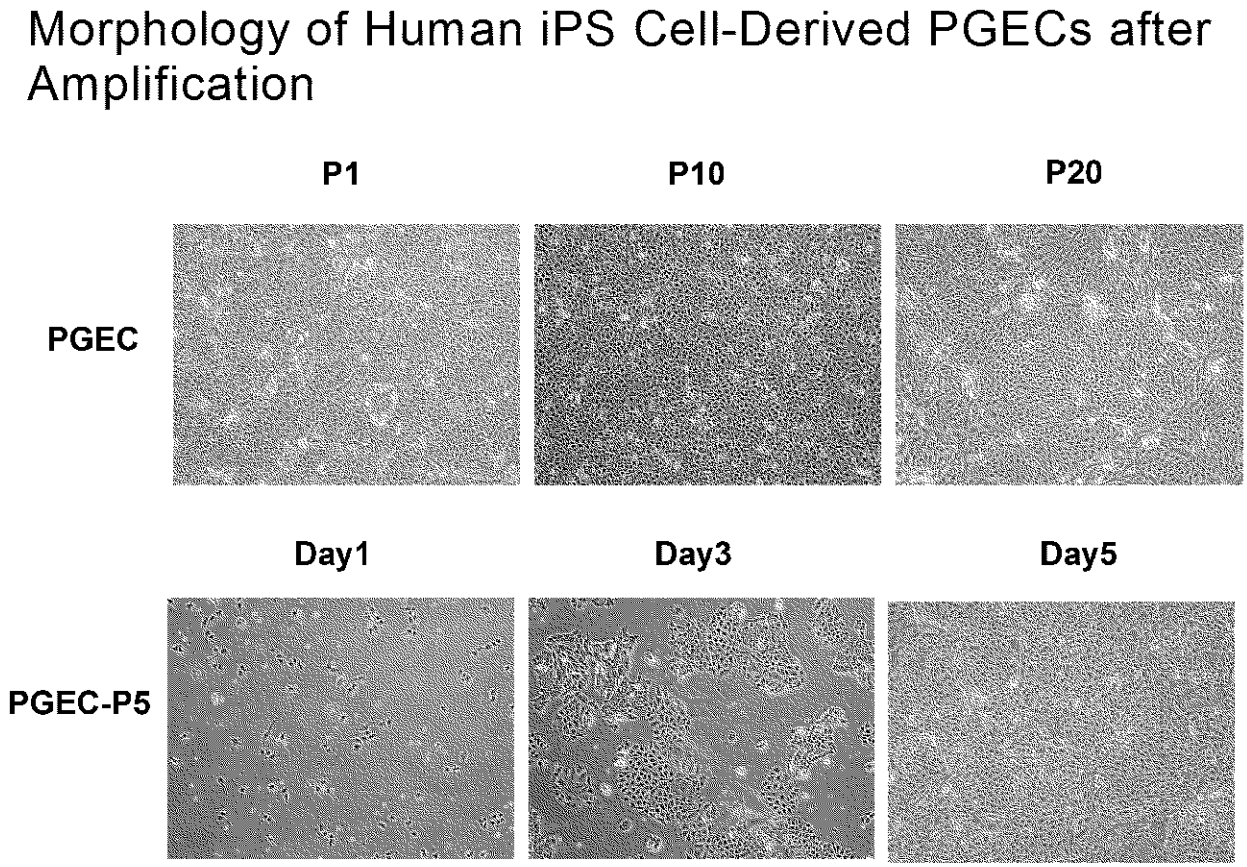

Morphology of Human iPS Cell-Derived PGECs after Amplification
P1
P10
P20
PGEC
Day1
Day3
Day5
PGEC-P5

Fig.7

Morphology of Human iPS Cell-Derived PGECs after Cryopreservation/Thawing

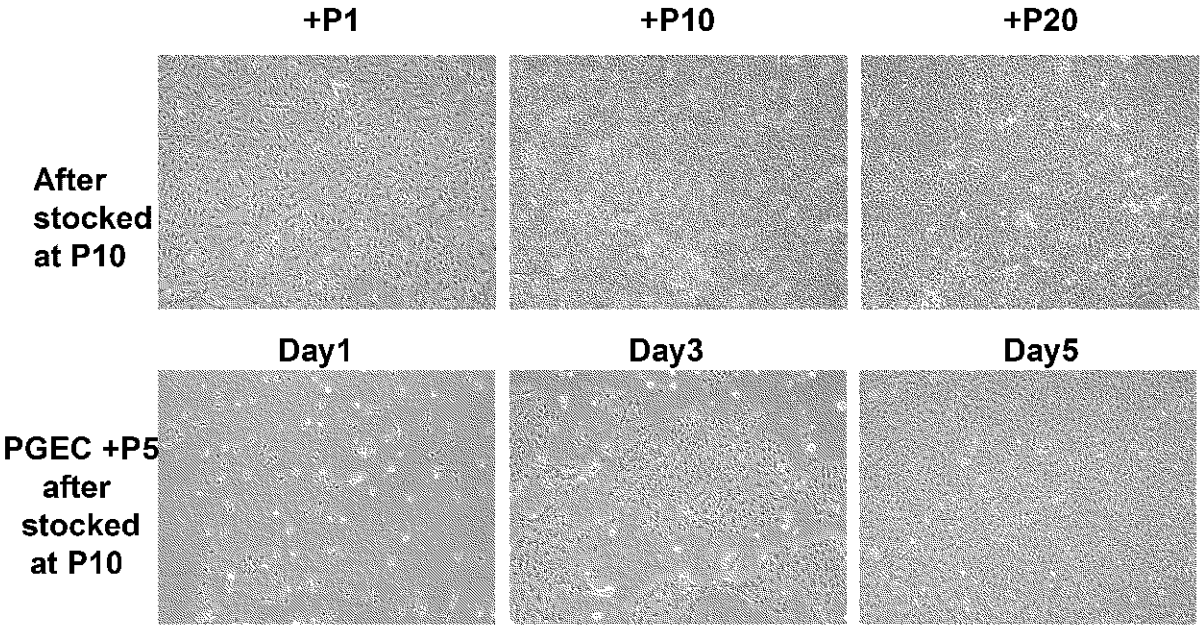


Fig.8

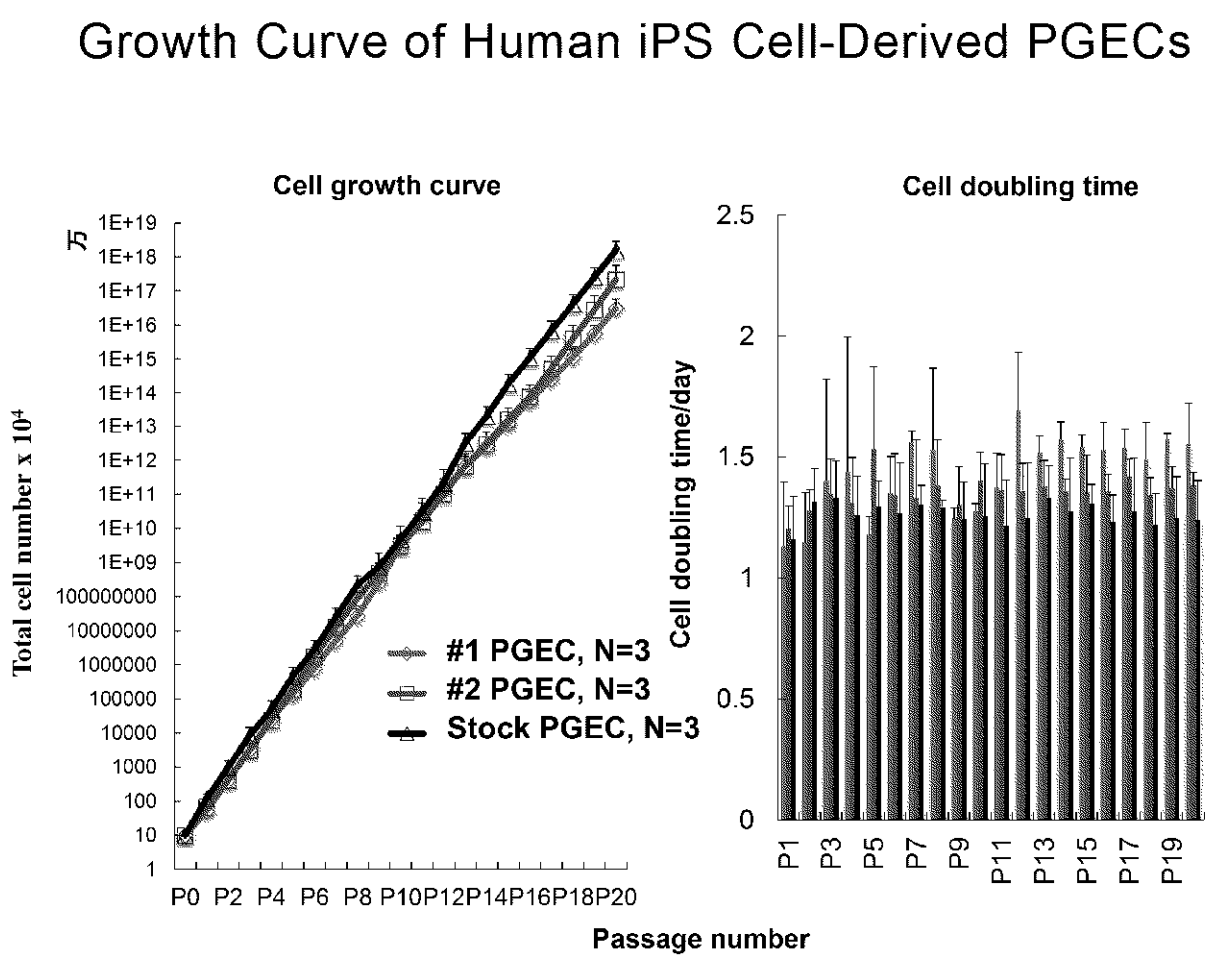

Growth Curve of Human iPS Cell-Derived PGECs
Cell growth curve
Total cell number x 10⁴
1E+19
1E+18
1E+17
1E+16
1E+15
1E+14
1E+13
1E+12
1E+11
1E+10
1E+09
100000000
10000000
1000000
100000
10000
1000
100
10
1
P0 P2 P4 P6 P8 P10 P12 P14 P16 P18 P20
#1 PGEC, N=3
#2 PGEC, N=3
Stock PGEC, N=3
Cell doubling time
Cell doubling time/day
2.5
2
1.5
1
0.5
0
P1 P3 P5 P7 P9 P11 P13 P15 P17 P19
Passage number

Fig.9

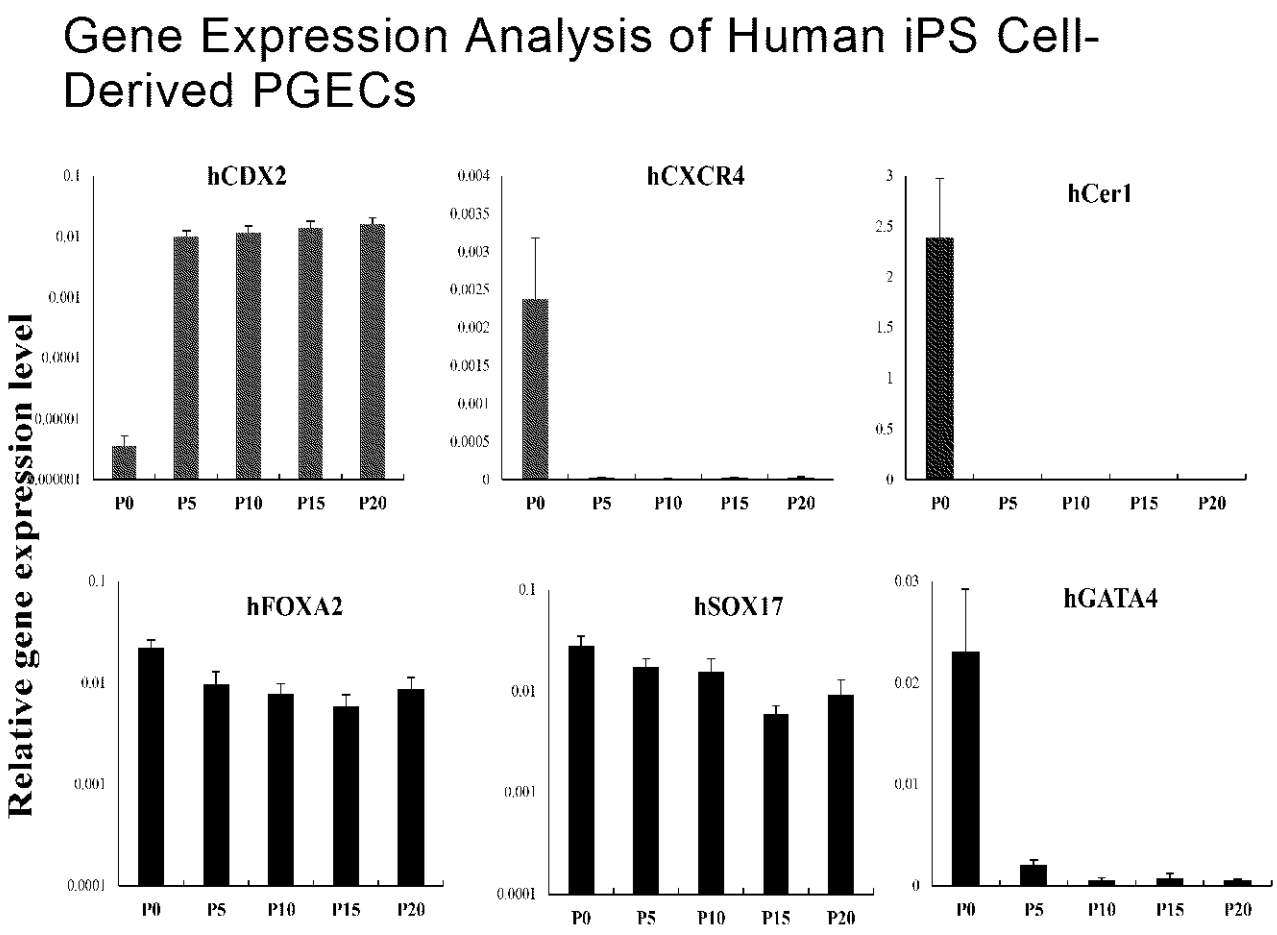

Gene Expression Analysis of Human iPS Cell-Derived PGECs
Relative gene expression level
hCDX2
hCXCR4
hCer1
hFOXA2
hSOX17
hGATA4
P0
P5
P10
P15
P20

Fig.10

FACS Time Lapse Analysis of Human iPS Cell-Derived PGECs

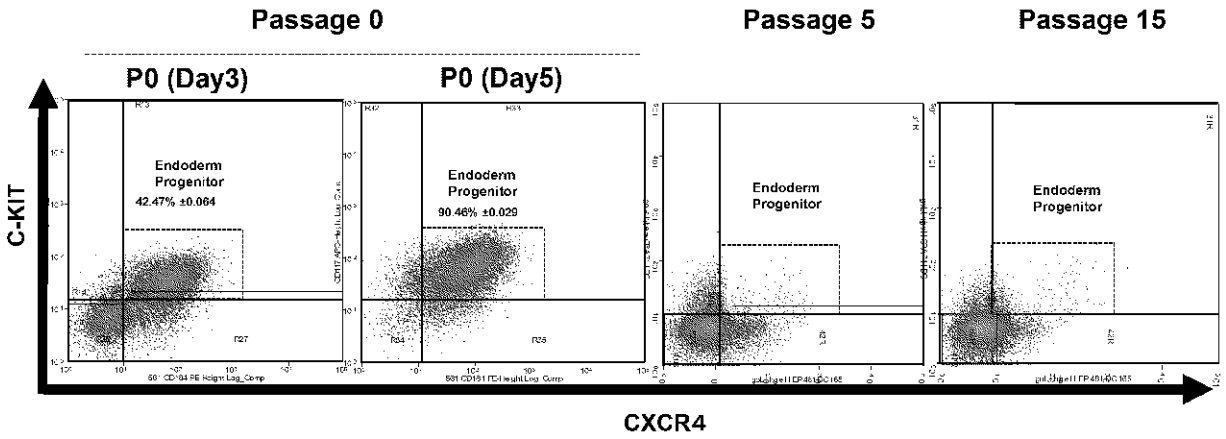


Fig.11

Immunological Analysis of Human iPS Cell-Derived PGECs

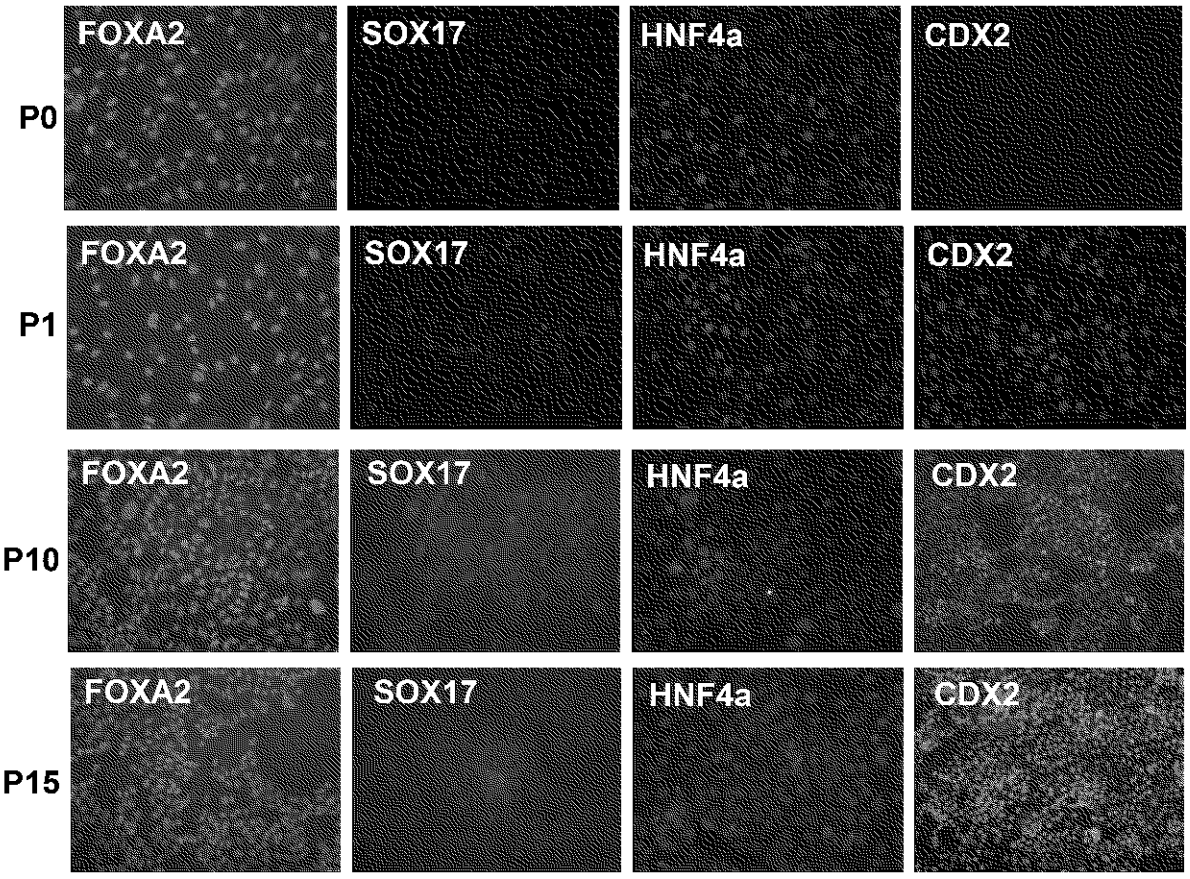


Fig.12

Immunological Analysis of Human iPS Cell-Derived PGECs

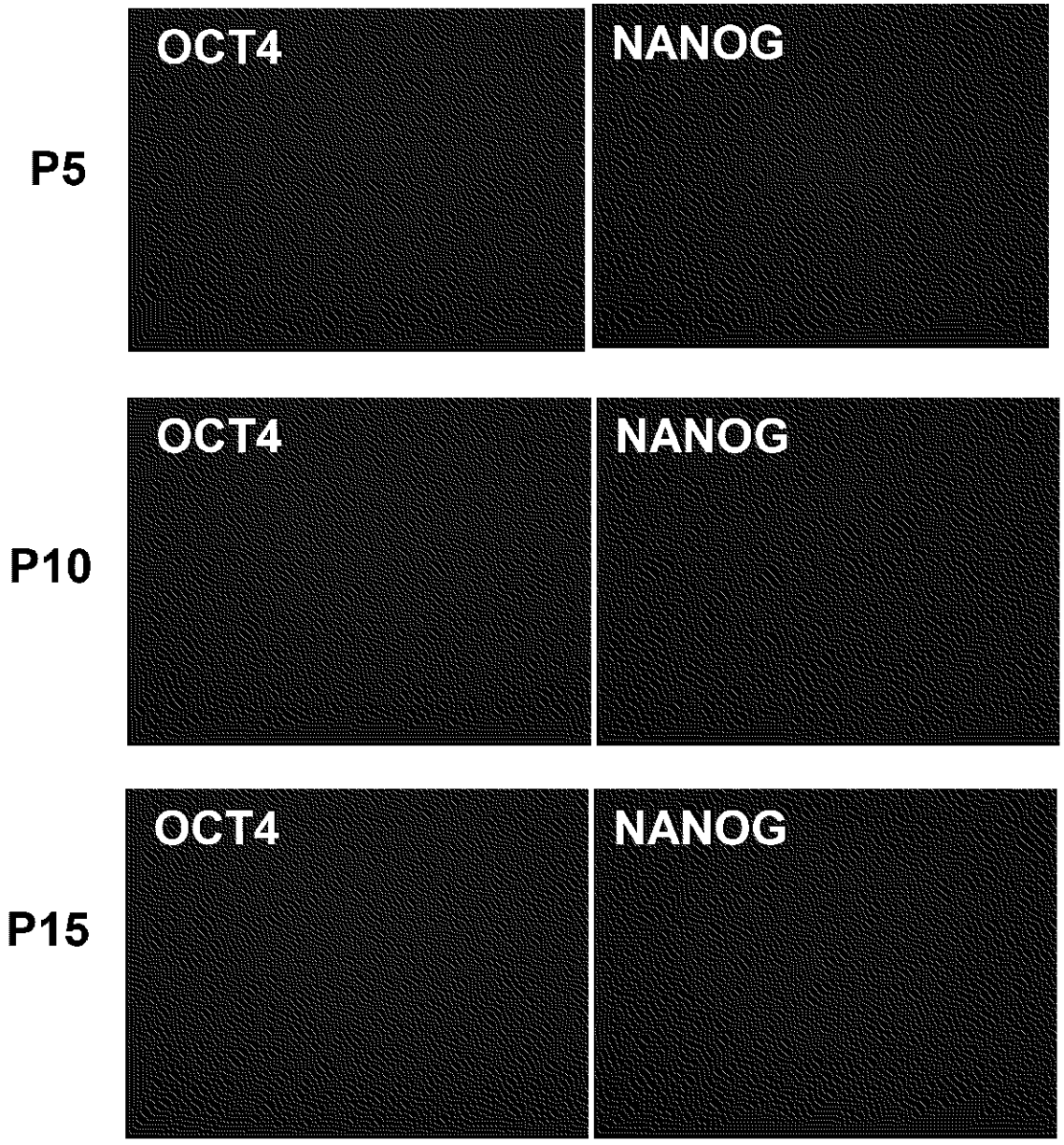


Fig.13

Principal Component Analysis of Human iPS Cell-Derived PGECs

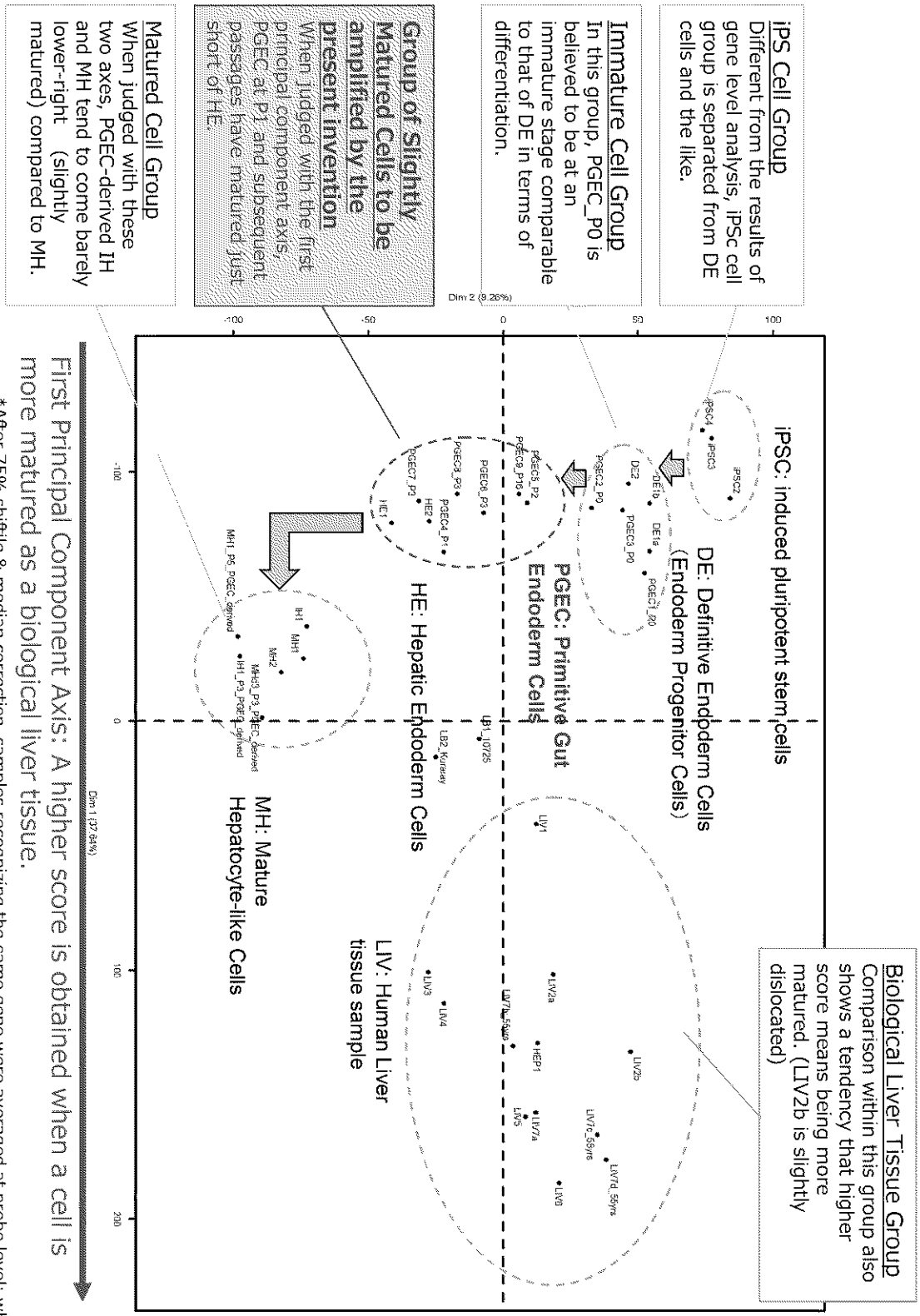


Fig.14

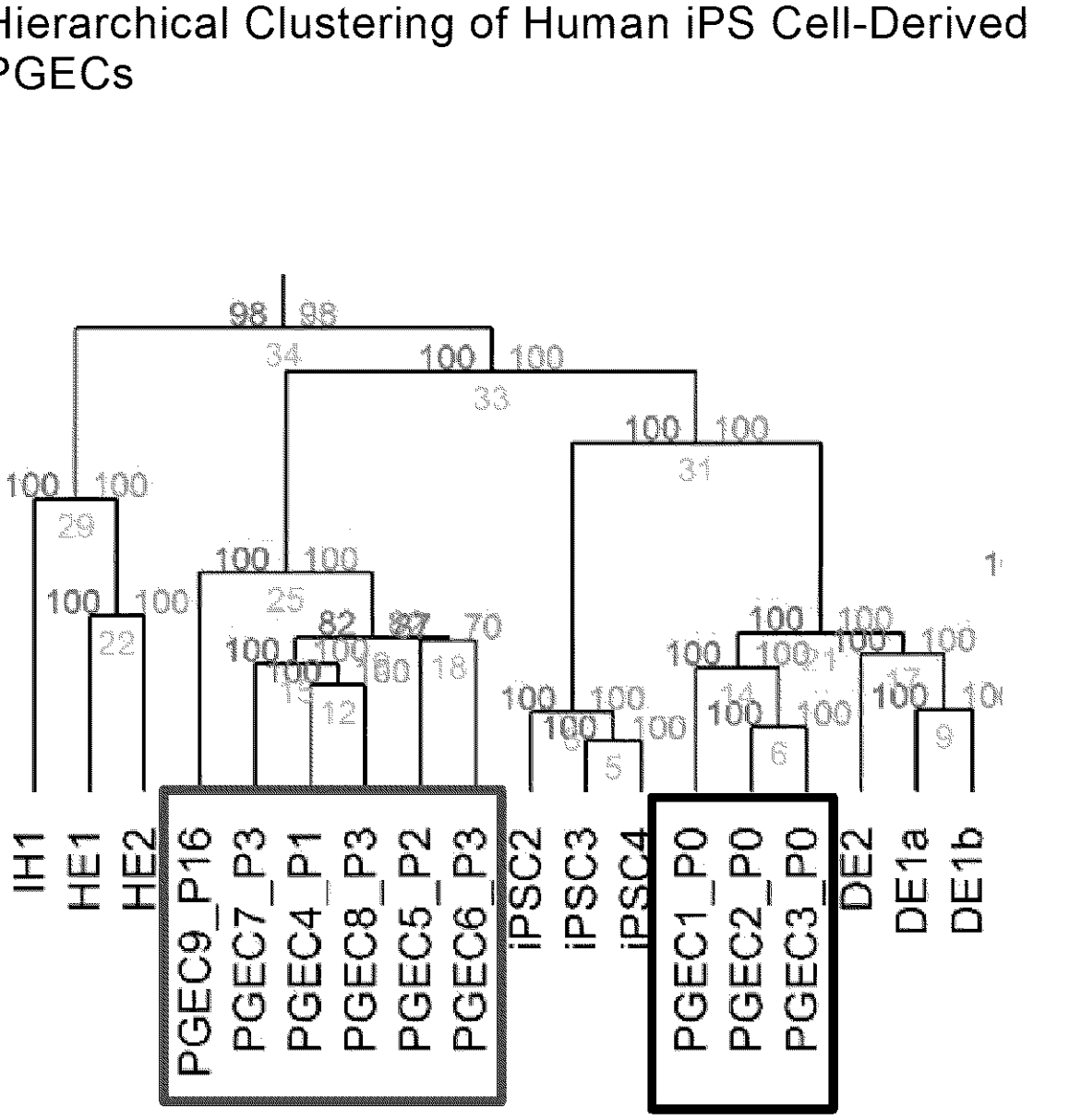

Hierarchical Clustering of Human iPS Cell-Derived PGECs
IH1
HE1
HE2
PGEC9_P16
PGEC7_P3
PGEC4_P1
PGEC8_P3
PGEC5_P2
PGEC6_P3
iPSC2
iPSC3
iPSC4
PGEC1_P0
PGEC2_P0
PGEC3_P0
DE2
DE1a
DE1b
It is suggested that samples at P1 and thereafter are located at a ifferentiation stage between DE and HE.
It is suggested that samples at P0 are located at a differentiation stage corresponding to DE (EP).

Fig.15-1

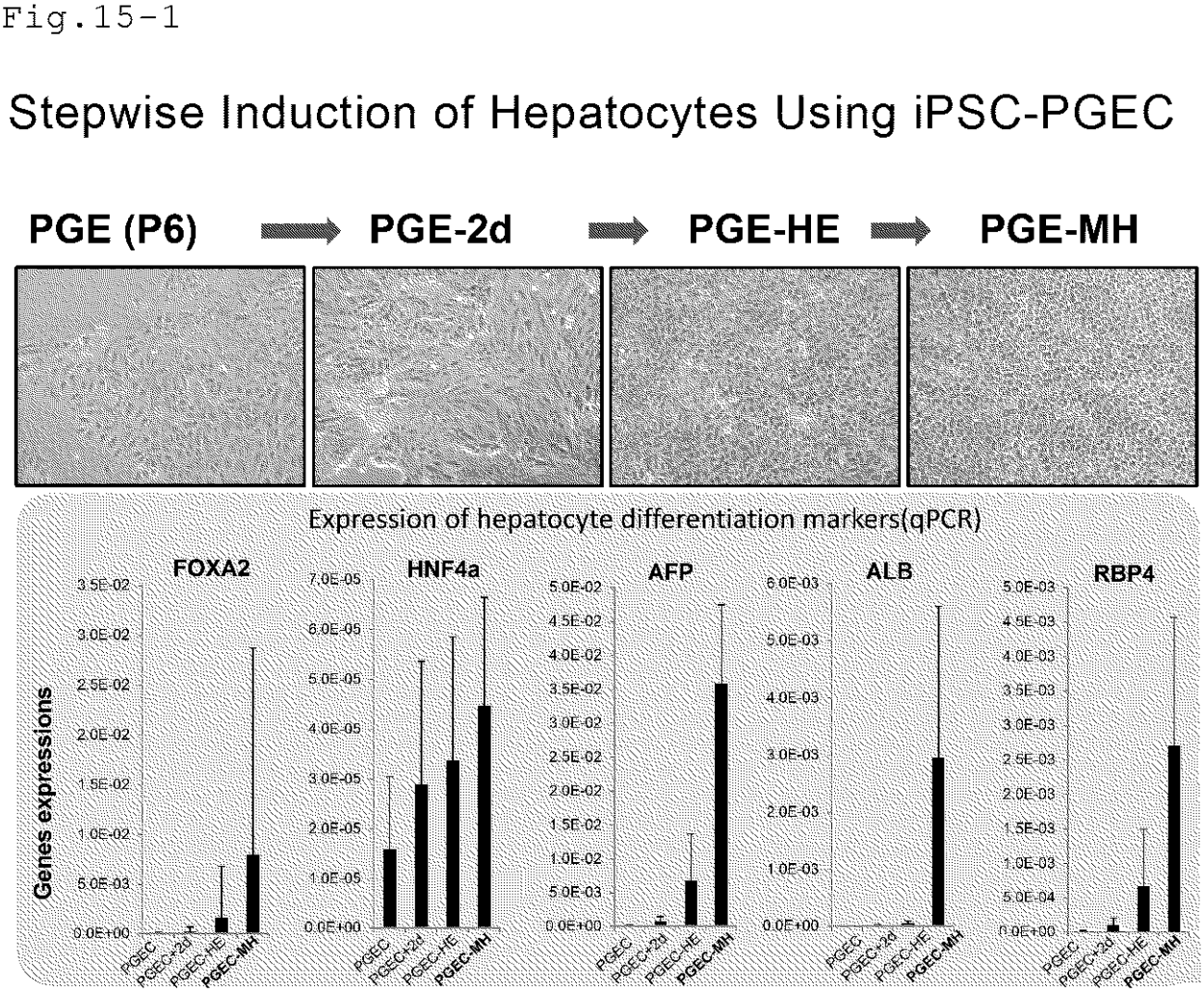

Stepwise Induction of Hepatocytes Using iPSC-PGEC
PGE (P6)
PGE-2d
PGE-HE
PGE-MH
Expression of hepatocyte differentiation markers(qPCR)
Genes expressions
FOXA2
HNF4a
AFP
ALB
RBP4
PGEC
PGEC+2d
PGEC-HE
PGEC-MH

Fig.15-2

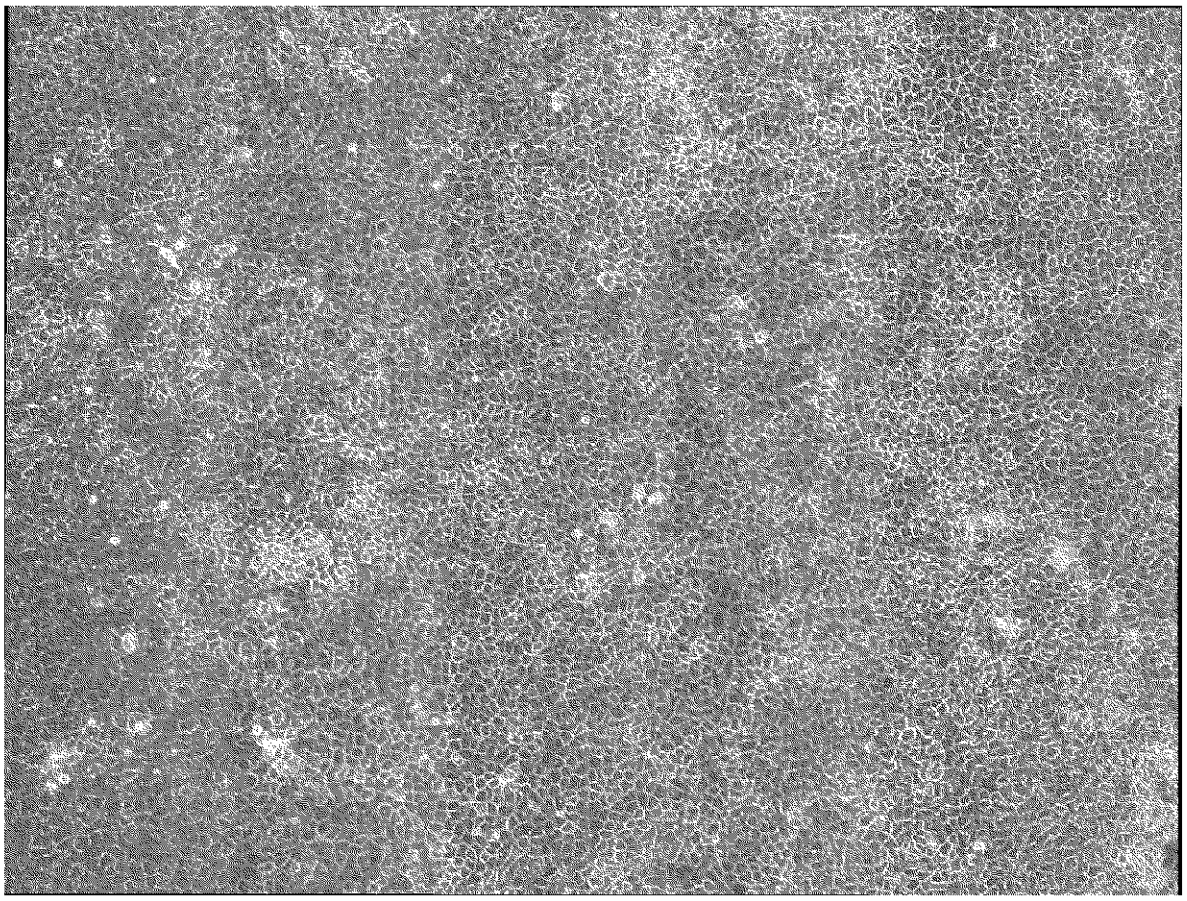


Fig.16

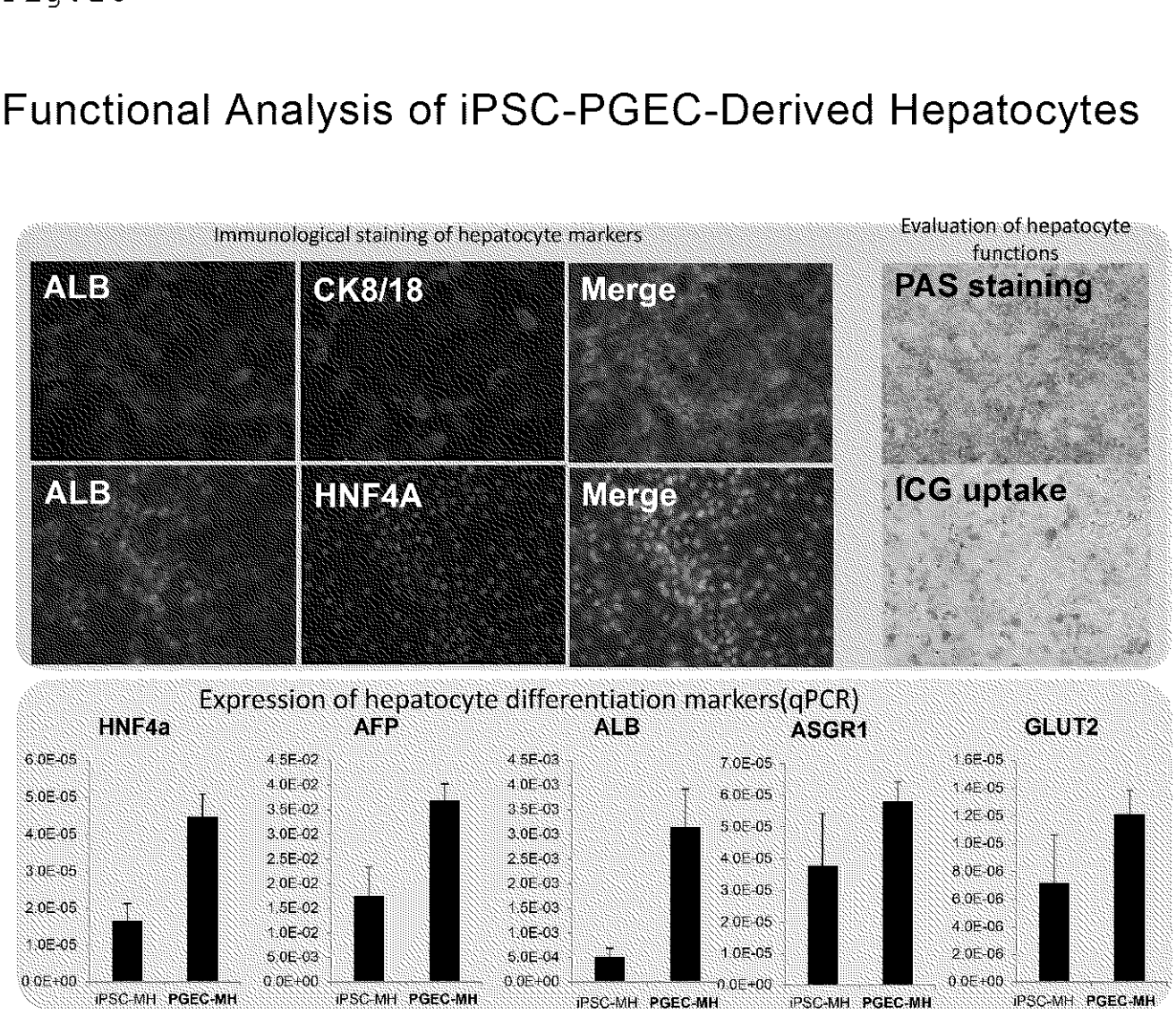

Functional Analysis of iPSC-PGEC-Derived Hepatocytes
Immunological staining of hepatocyte markers
ALB
CK8/18
Merge
ALB
HNF4A
Merge
Evaluation of hepatocyte functions
PAS staining
ICG uptake
Expression of hepatocyte differentiation markers(qPCR)
HNF4a
AFP
ALB
ASGR1
GLUT2
iPSC-MH
PGEC-MH

Fig.17-1

Hepatocyte Induction Capacity of Passaged iPSC-PGEC

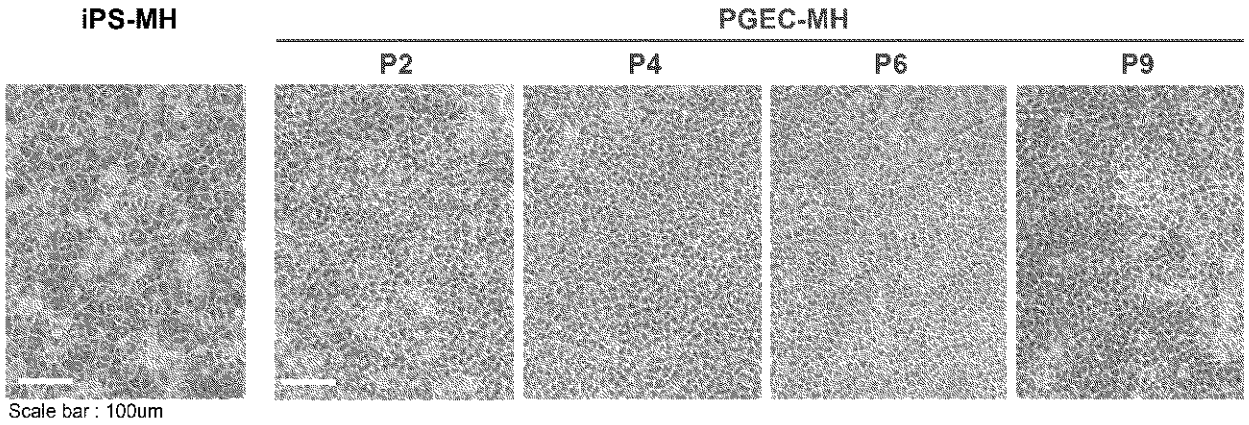


Fig.17-2

Hepatocyte Induction Capacity of Passaged iPSC-PGEC

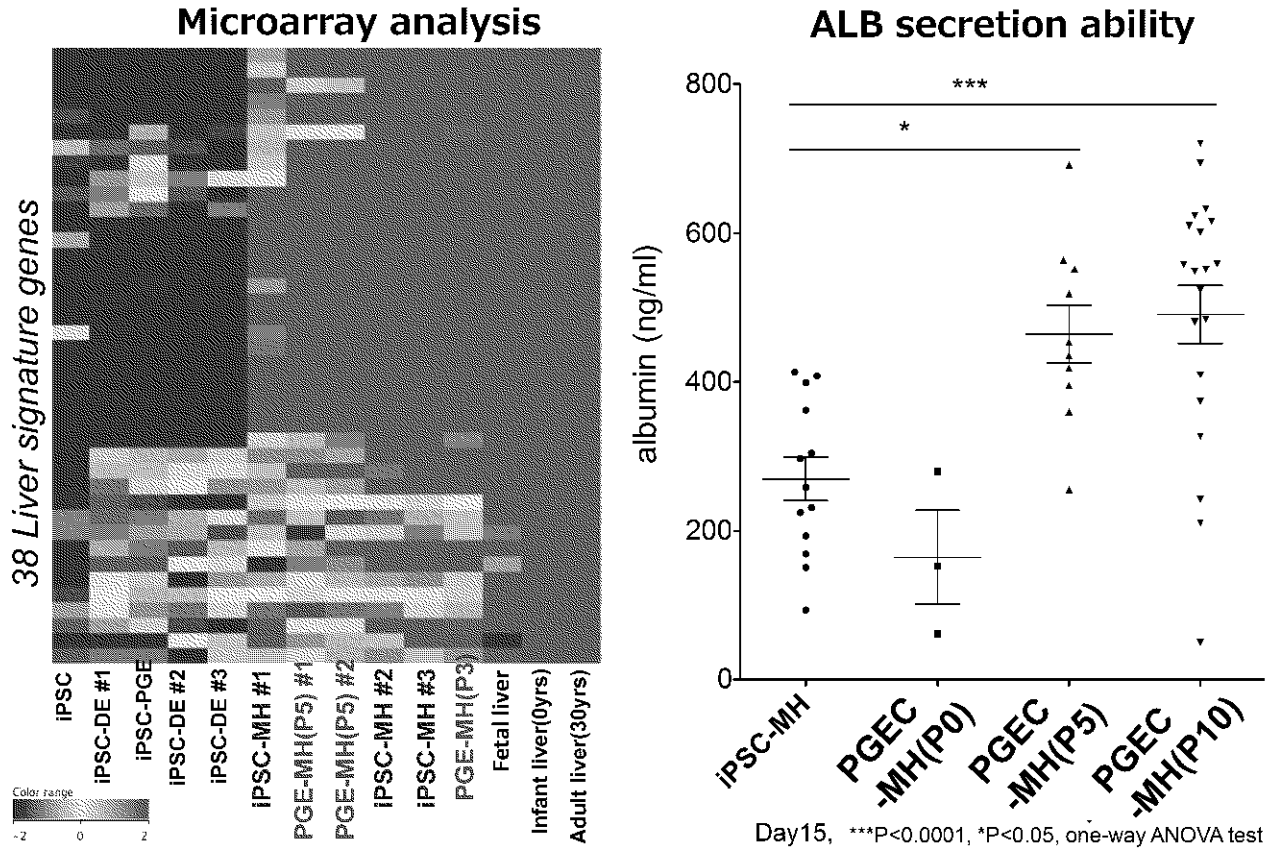


Fig.18

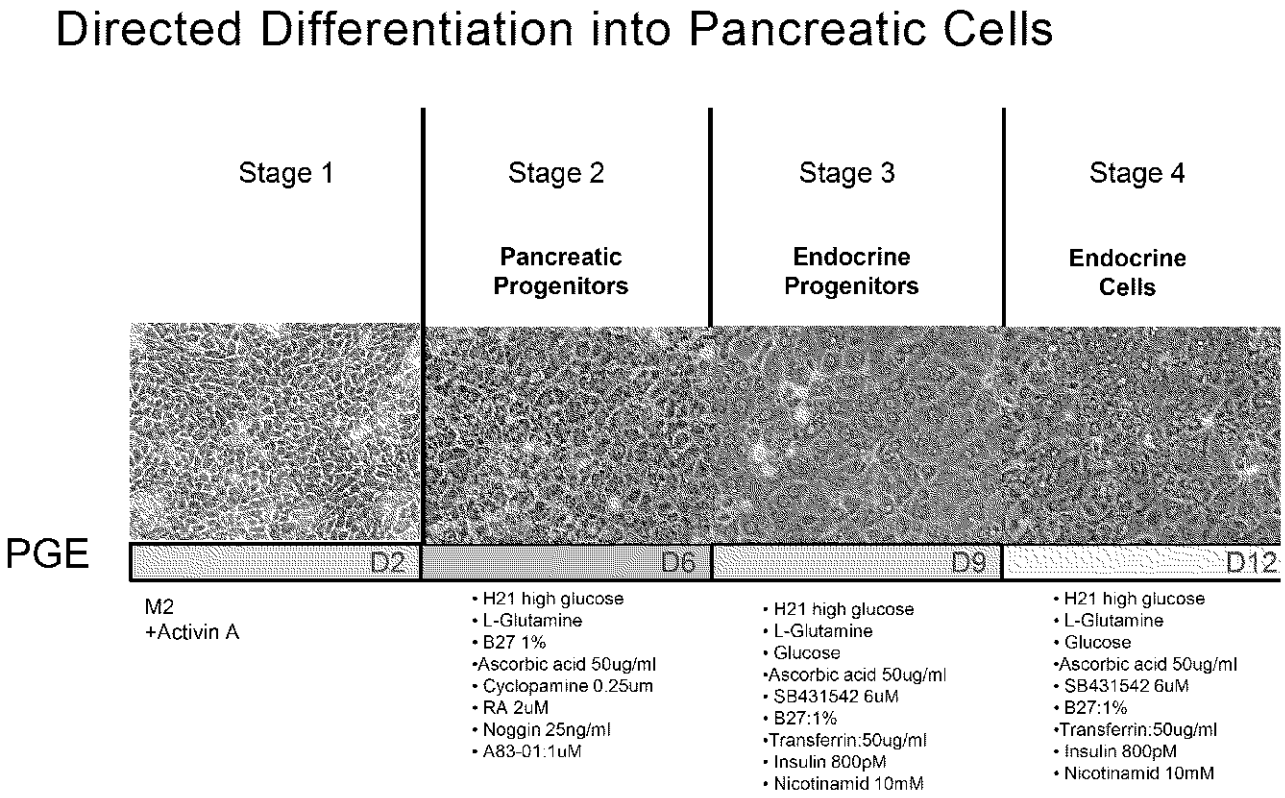
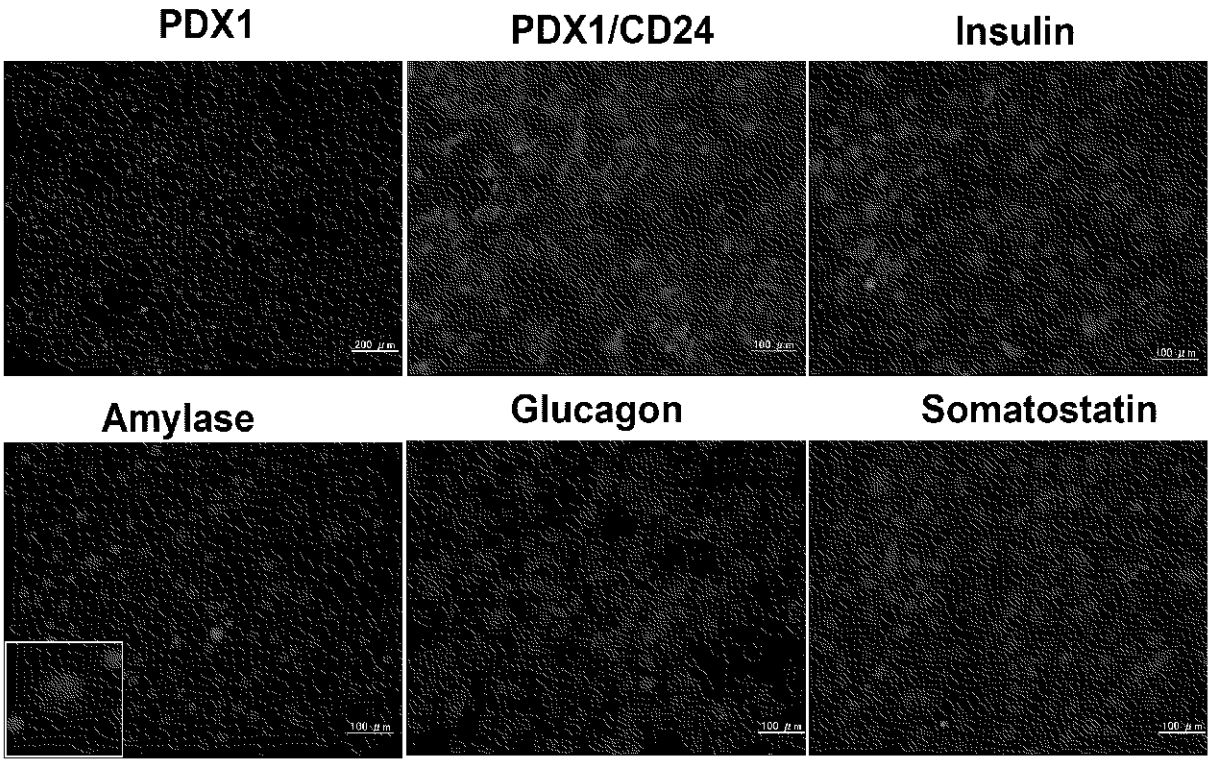

Directed Differentiation into Pancreatic Cells
Stage 1
Stage 2
Pancreatic Progenitors
Stage 3
Endocrine Progenitors
Stage 4
Endocrine Cells
PGE
D2
D6
D9
D12
M2
+Activin A
• H21 high glucose
• L-Glutamine
• B27 1%
•Ascorbic acid 50ug/ml
• Cyclopamine 0.25um
• RA 2uM
• Noggin 25ng/ml
• A83-01:1uM
• H21 high glucose
• L-Glutamine
• Glucose
•Ascorbic acid 50ug/ml
• SB431542 6uM
• B27:1%
•Transferrin:50ug/ml
• Insulin 800pM
• Nicotinamid 10mM
• H21 high glucose
• L-Glutamine
• Glucose
•Ascorbic acid 50ug/ml
• SB431542 6uM
• B27:1%
•Transferrin:50ug/ml
• Insulin 800pM
• Nicotinamid 10mM

Fig.19

Directed Differentiation of PGEC into Pancreatic Cells



Insulin: beta cells
Somatostatin: δ-cells or D cells
Glucagon: alpha cells of the pancreas
Amylase: pancreatic acinar cells
CD24-positive cells could differentiate into insulin-producing cells P16

Fig.20

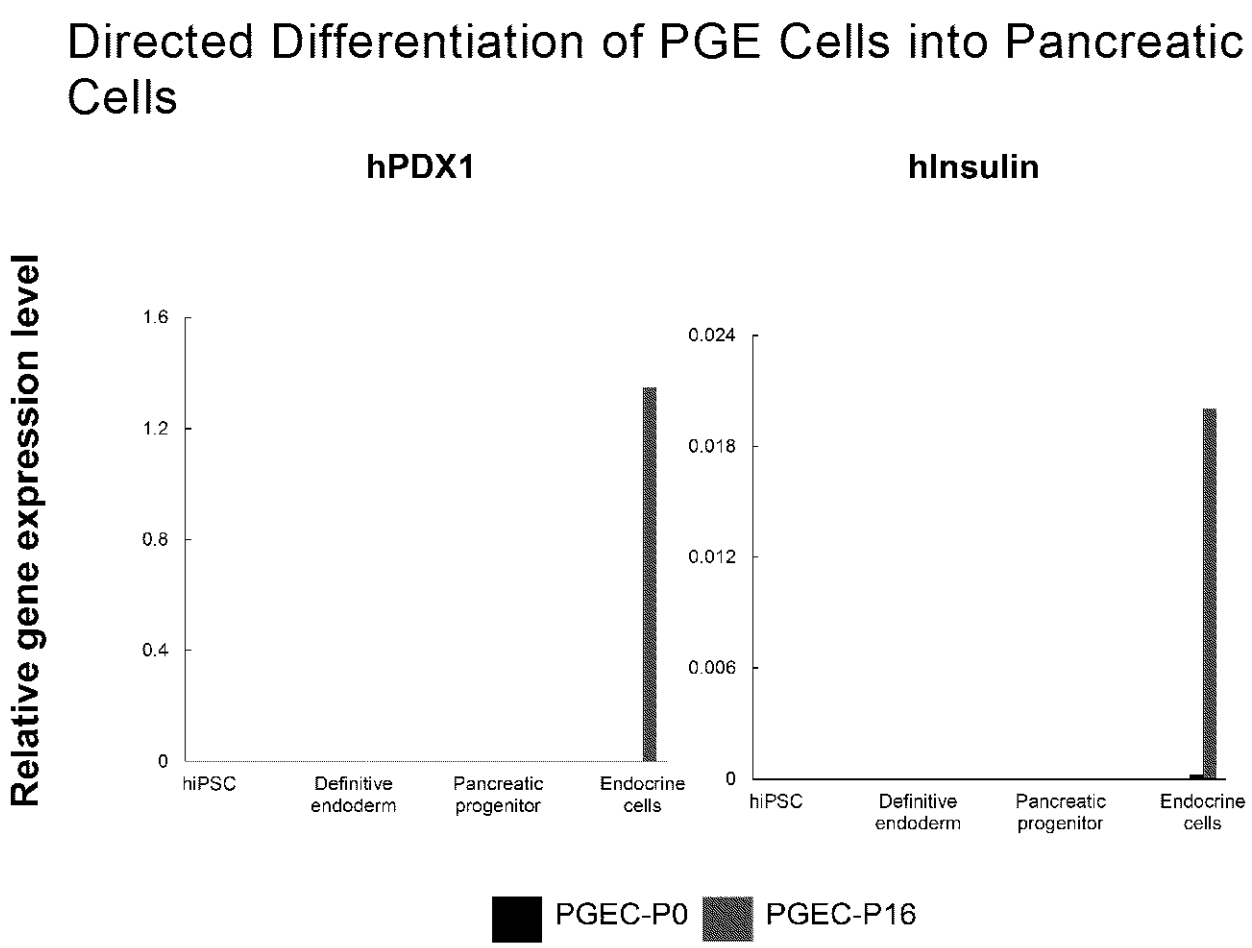

Directed Differentiation of PGE Cells into Pancreatic Cells
hPDX1
hInsulin
Relative gene expression level
1.6
1.2
0.8
0.4
0
0.024
0.018
0.012
0.006
0
hiPSC
Definitive endoderm
Pancreatic progenitor
Endocrine cells
PGEC-P0
PGEC-P16

Fig.21

Induction of Intestinal Tissues Using PGEC

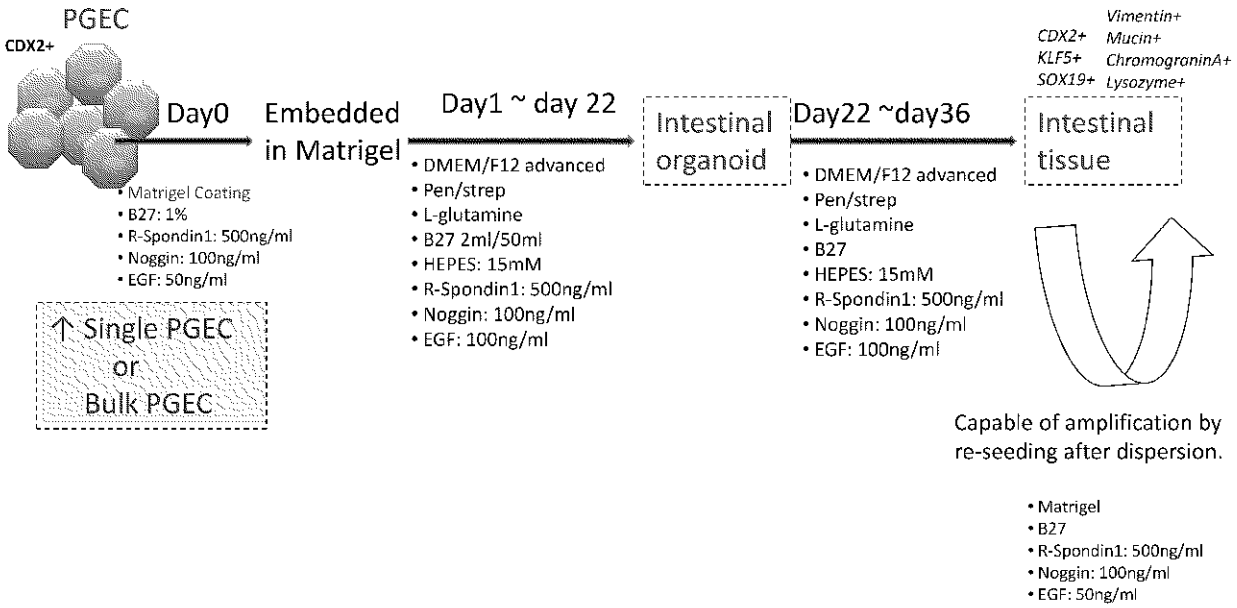


Fig.22

Intestinal Tissues Induced from PGEC

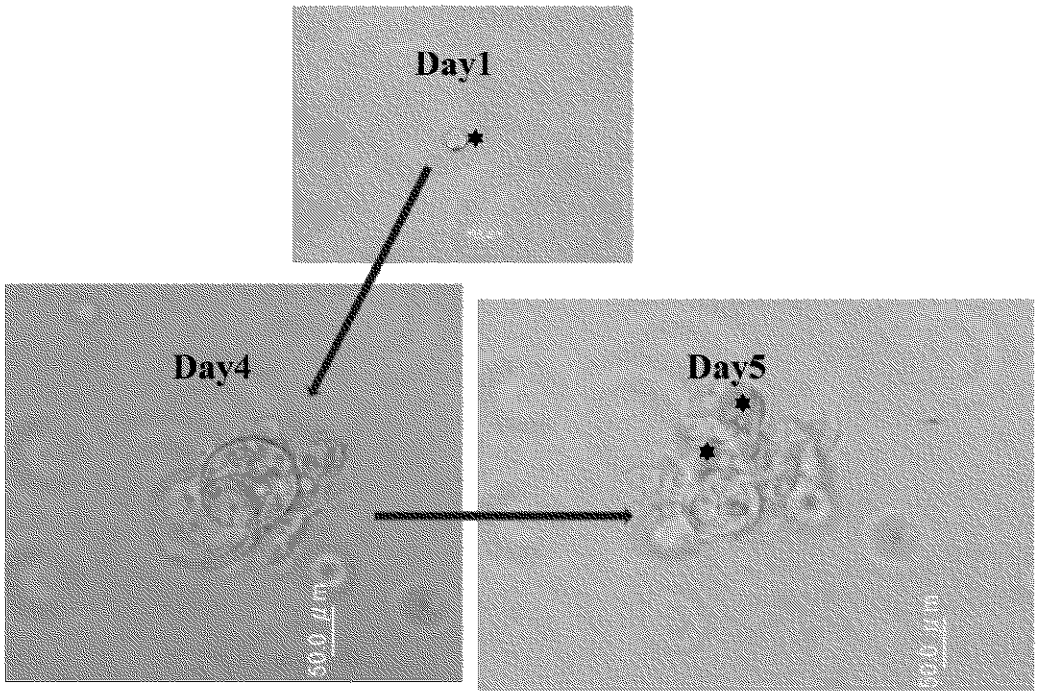


Fig.23

Extraction of Positive/Negative Marker Genes Specific to PGEC (P1 and thereafter)

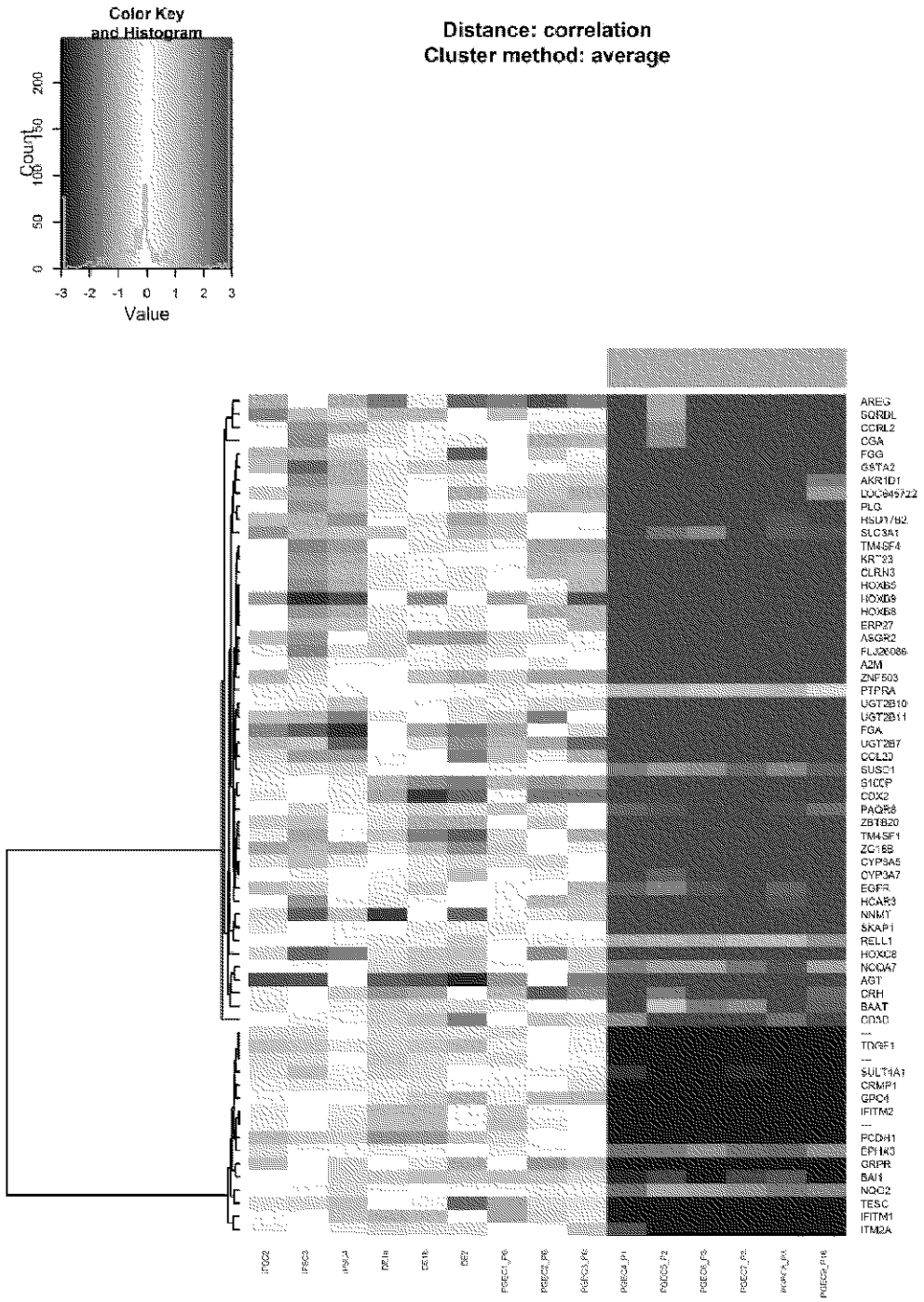


Fig.24

Reproducible generation of primitive gut endoderm cells from different patient-derived iPSC lines

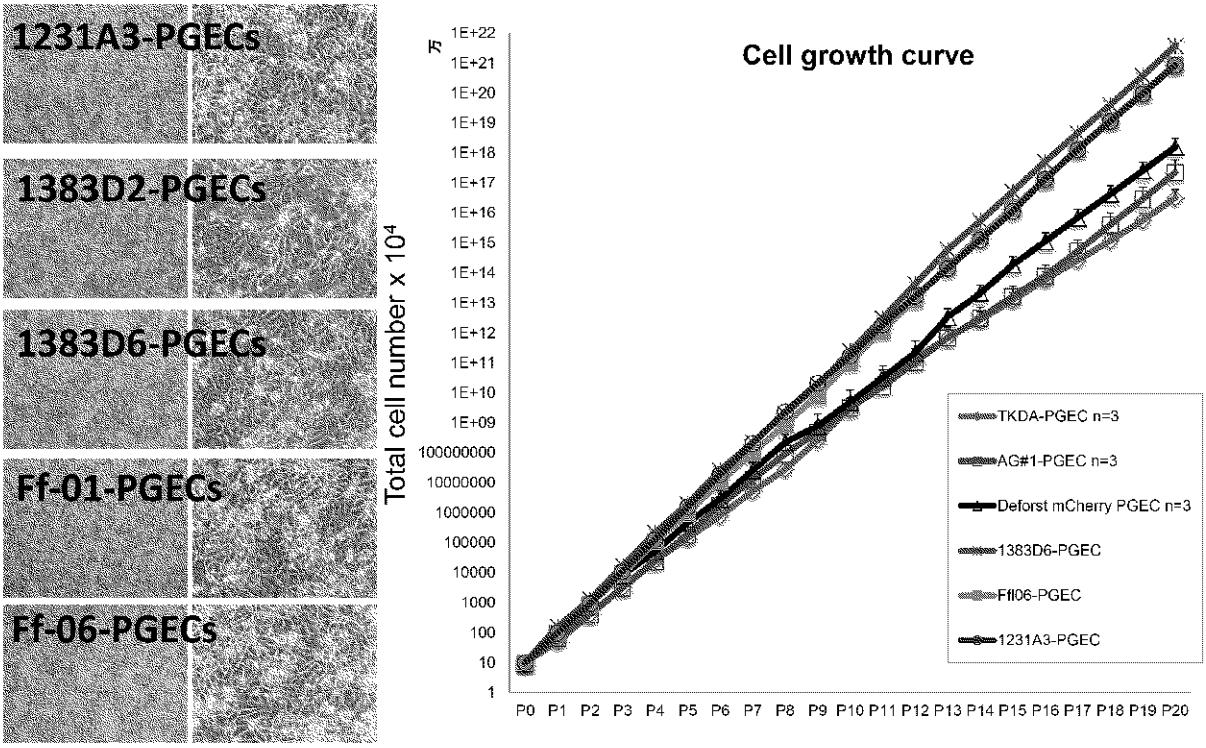
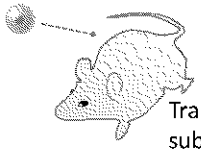


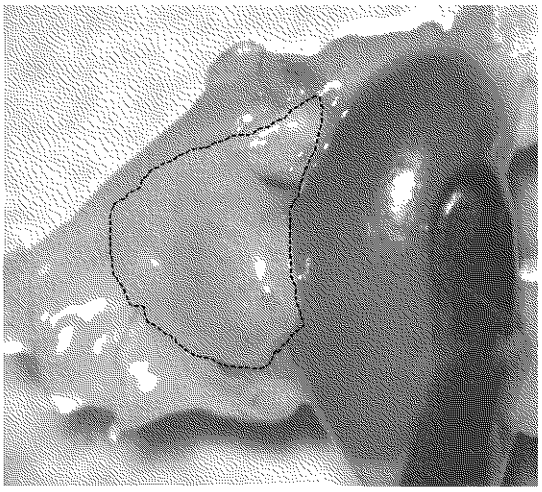
Fig.25

Generating multiple tissues of endodermal origin from transplanted PGEC

PGEC (without differentiation)



Transplantation into subcapsular space of kidney



PGEC(P5), TkNOG(subcapsular tx),

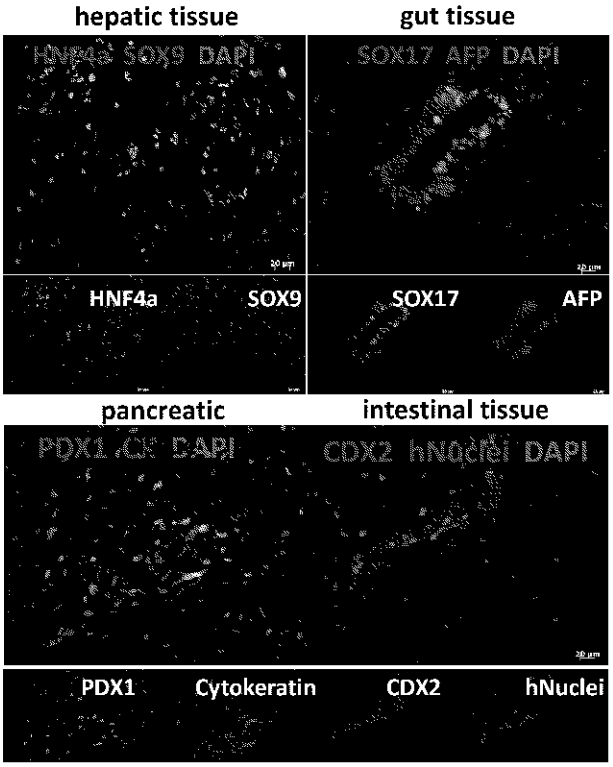
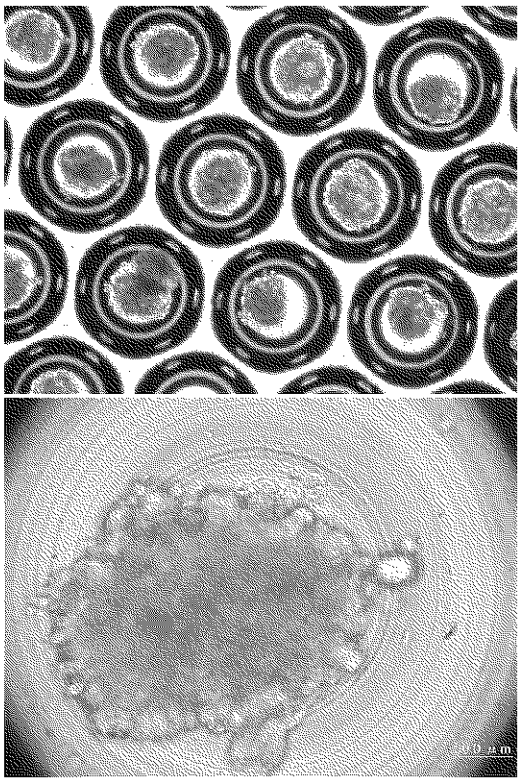


Fig.26

In vitro generation and differentiation of liver bud into mature hepatic tissues grown from PGEC



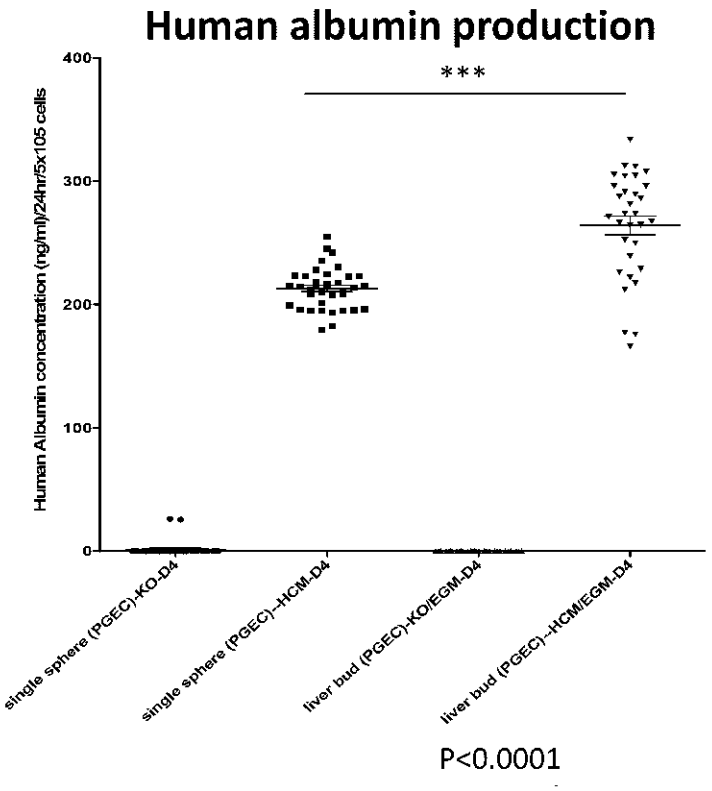


Fig.27

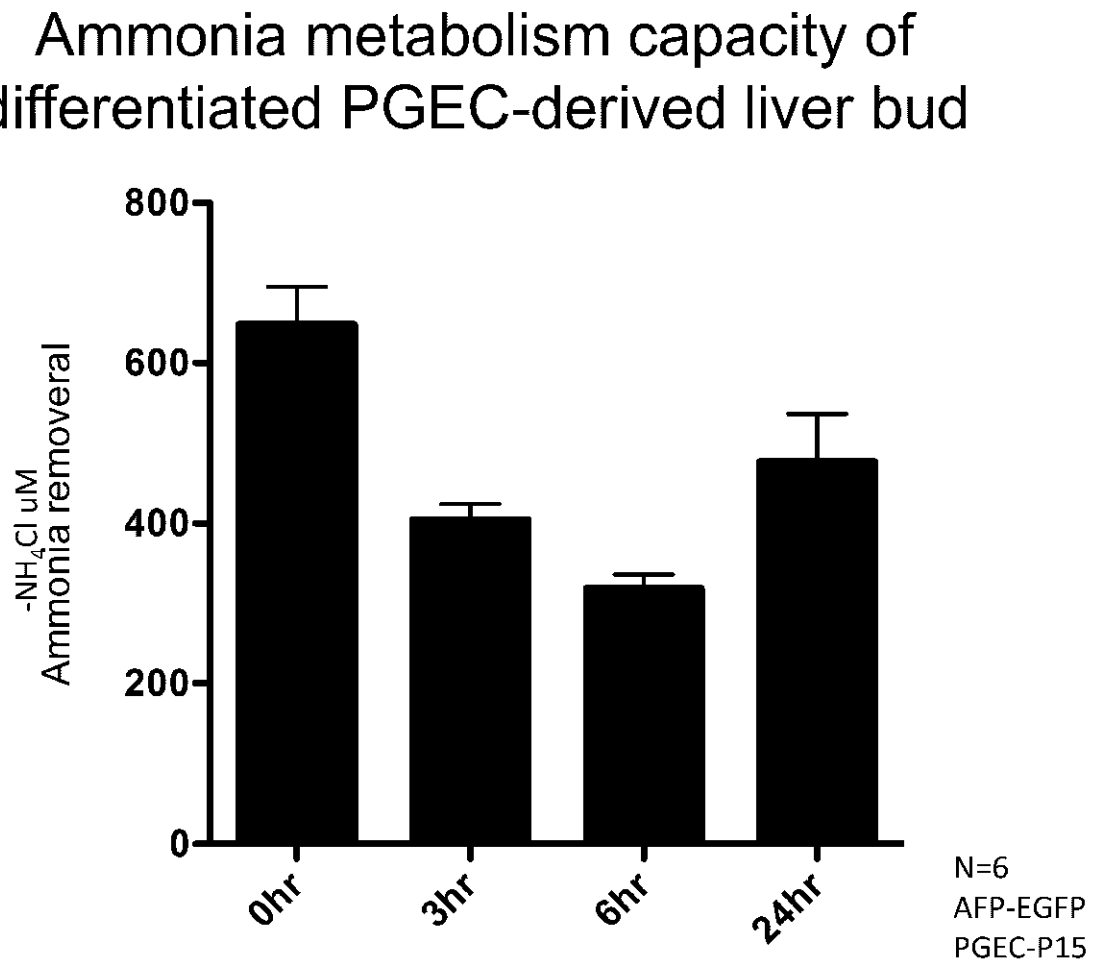

Ammonia metabolism capacity of differentiated PGEC-derived liver bud
-NH4Cl uM
Ammonia removeral
800
600
400
200
0
0hr
3hr
6hr
24hr
N=6
AFP-EGFP
PGEC-P15

Fig.28

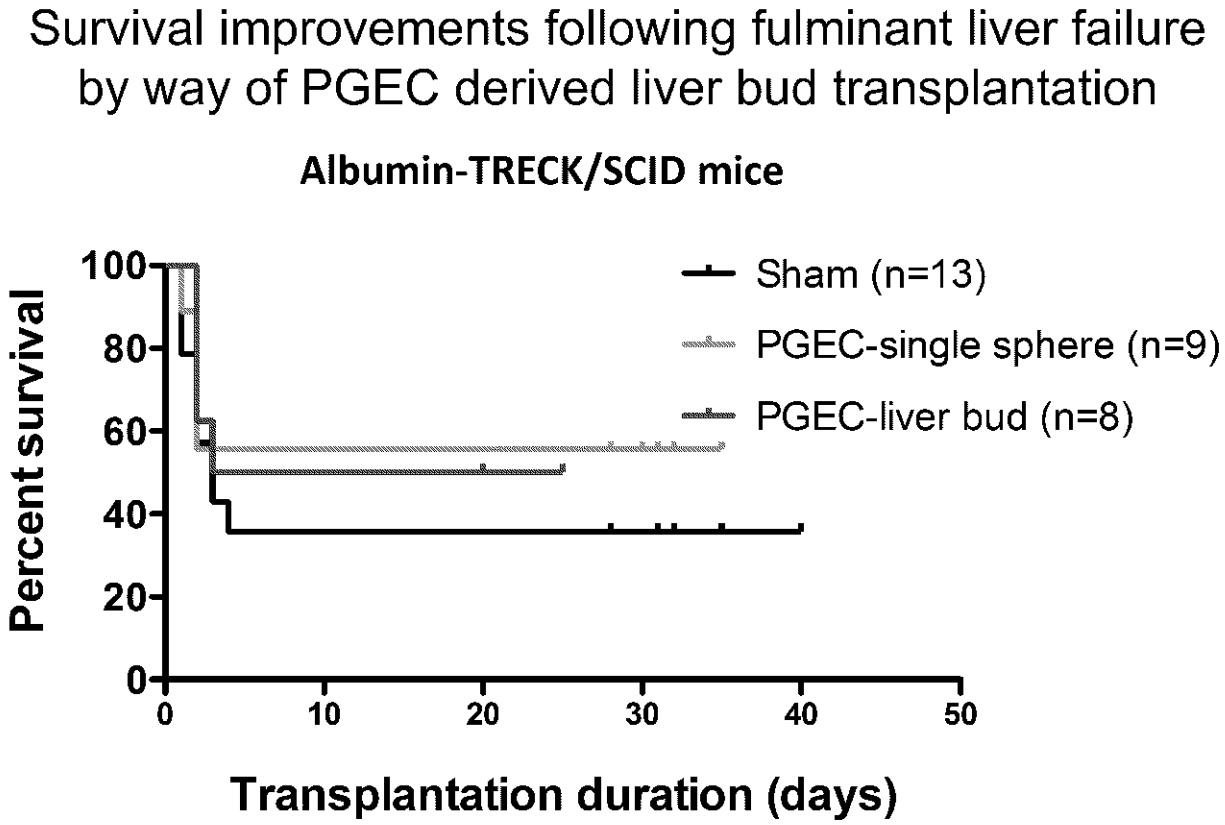
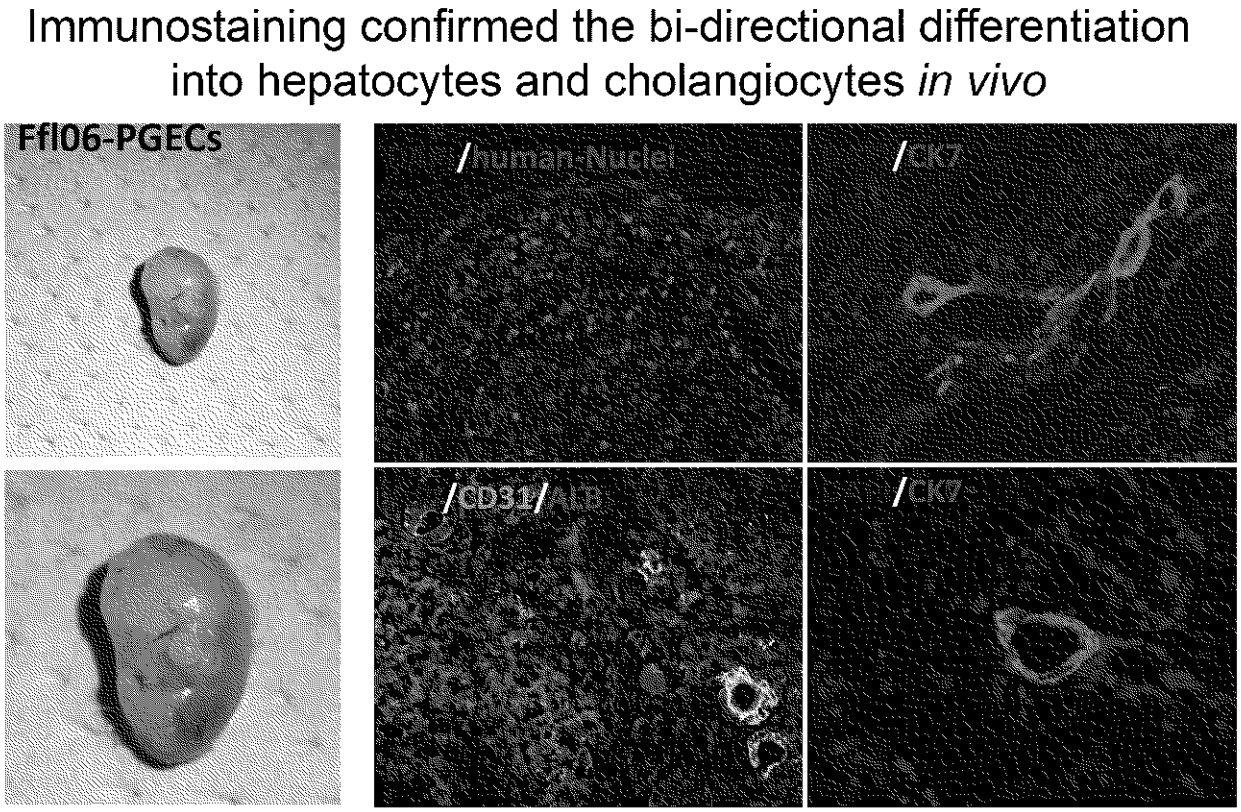

Survival improvements following fulminant liver failure by way of PGEC derived liver bud transplantation
Albumin-TRECK/SCID mice
Percent survival
100
80
60
40
20
0
0
10
20
30
40
50
Transplantation duration (days)
Sham (n=13)
PGEC-single sphere (n=9)
PGEC-liver bud (n=8)

Fig.29


Immunostaining confirmed the bi-directional differentiation into hepatocytes and cholangiocytes *in vivo*
Ffl06-PGECs
/human-Nuclei
/CK7
/CD31/ALB
/CK7
Recipients: Alb-TRECK/SCID mice
DT dose: 1.5 ug/kg
Donor: PGEC-MH-Sphere
Plate: kuraray plate
Duration: 1 month
Kidney capsule