Fig.1

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

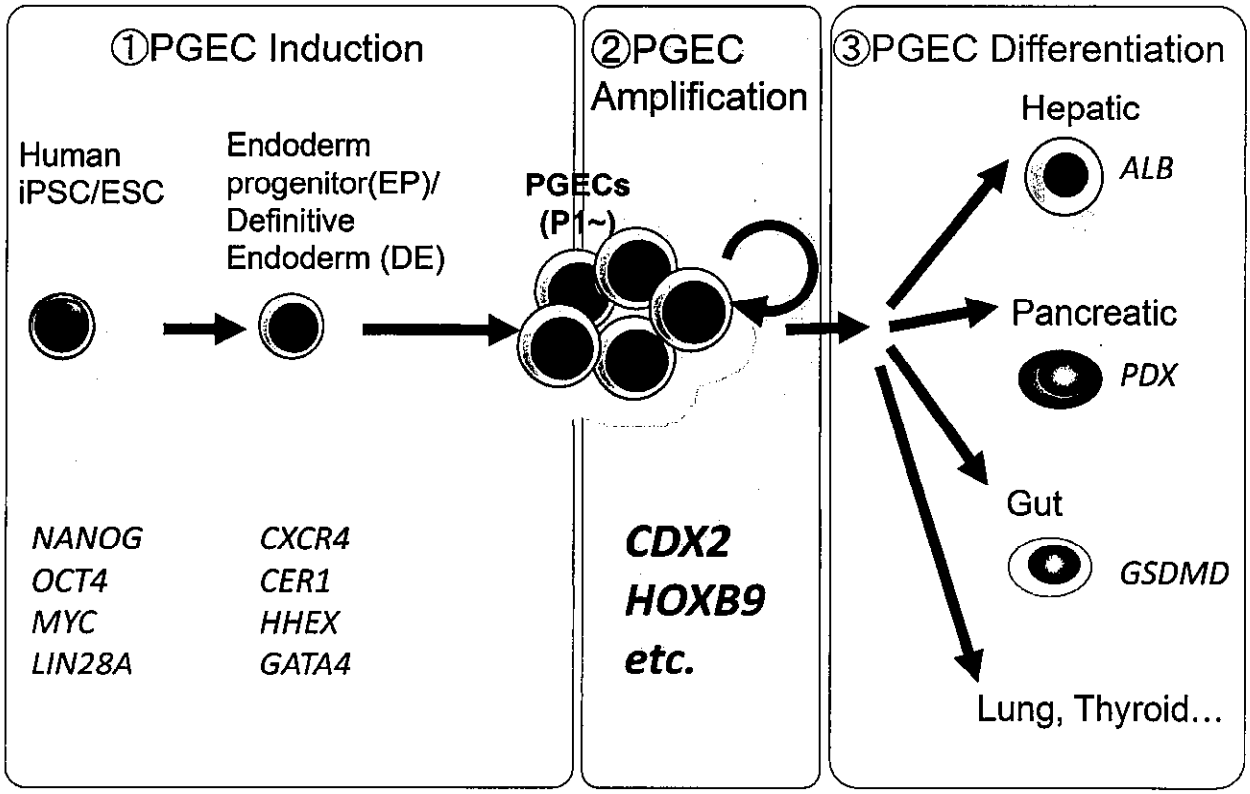


Fig.2

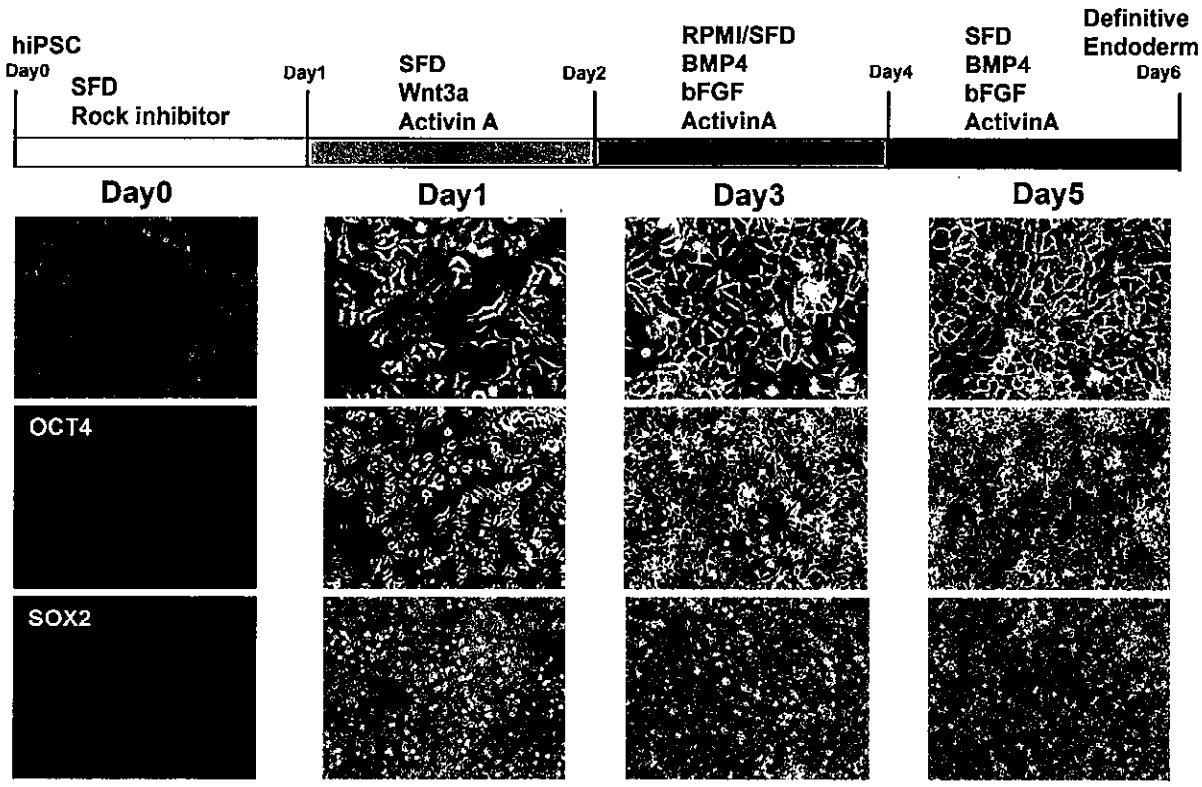

Induction of Human iPS Cell-Derived DE toward PGEC Induction
hiPSC
Day0
SFD
Rock inhibitor
Day1
SFD
Wnt3a
Activin A
Day2
RPMI/SFD
BMP4
bFGF
ActivinA
Day4
SFD
BMP4
bFGF
ActivinA
Definitive Endoderm
Day6
Day0
Day1
Day3
Day5
OCT4
SOX2

Fig.3

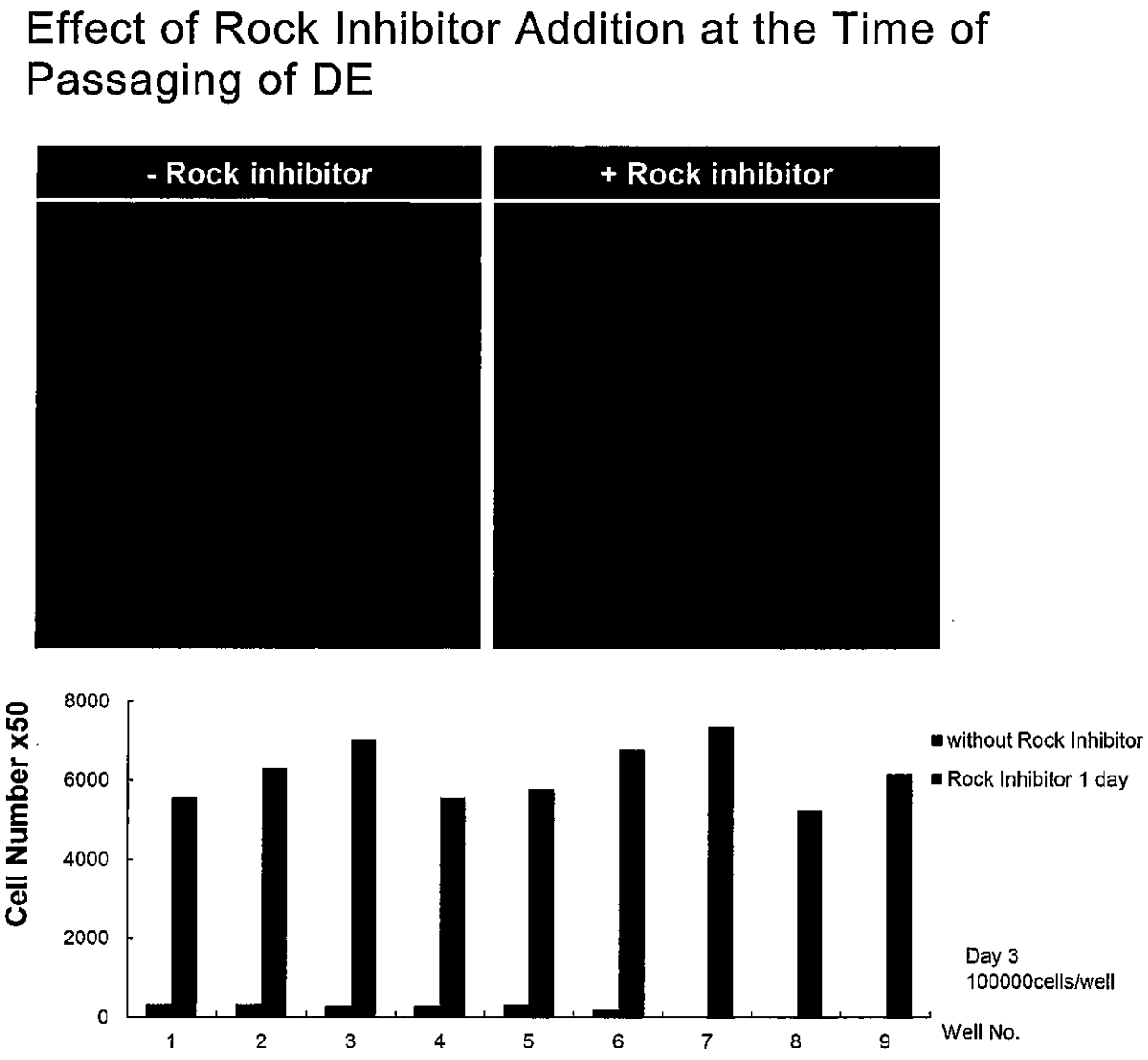

Effect of Rock Inhibitor Addition at the Time of Passaging of DE
- Rock inhibitor
+ Rock inhibitor
Cell Number x50
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
9
Well No.
without Rock Inhibitor
Rock Inhibitor 1 day
Day 3
100000cells/well

Fig.4

Review of Method for Inducing Human iPS Cell-Derived PGECs

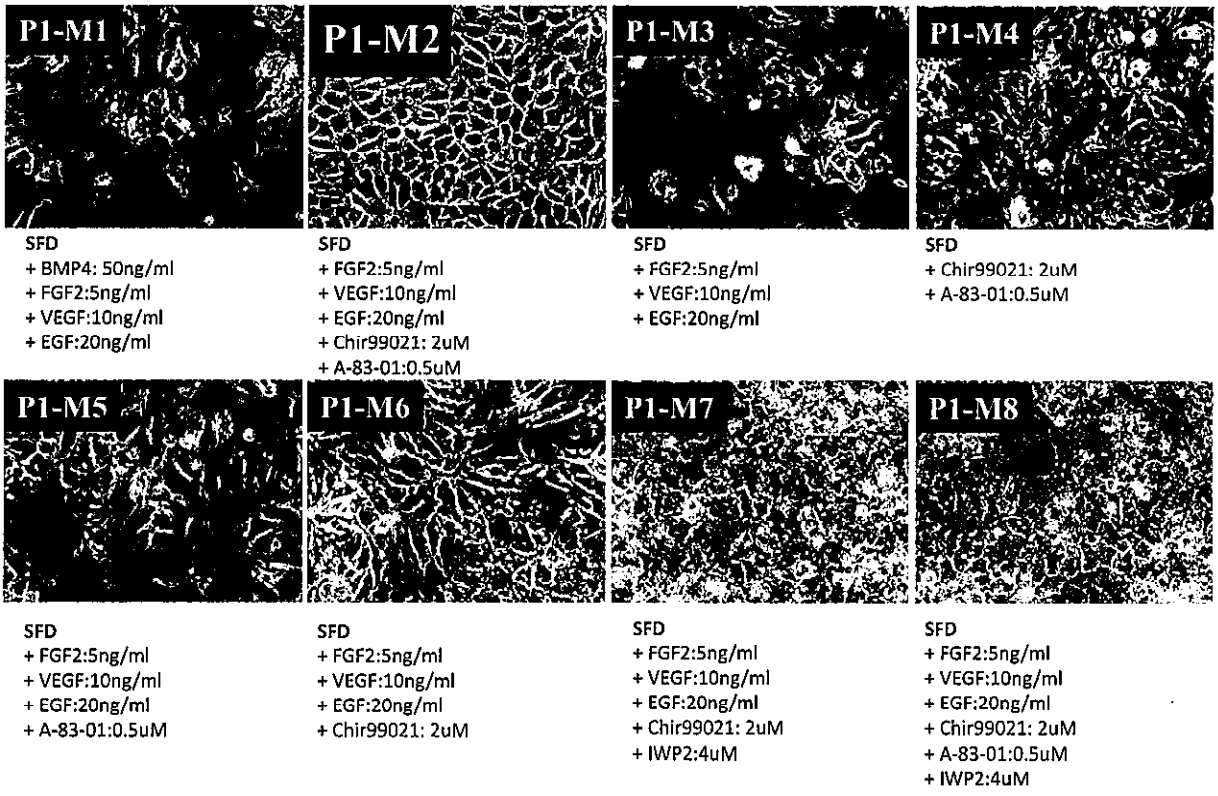


Fig.5

Review of Method for Amplifying Human iPS Cell-Derived PGECs

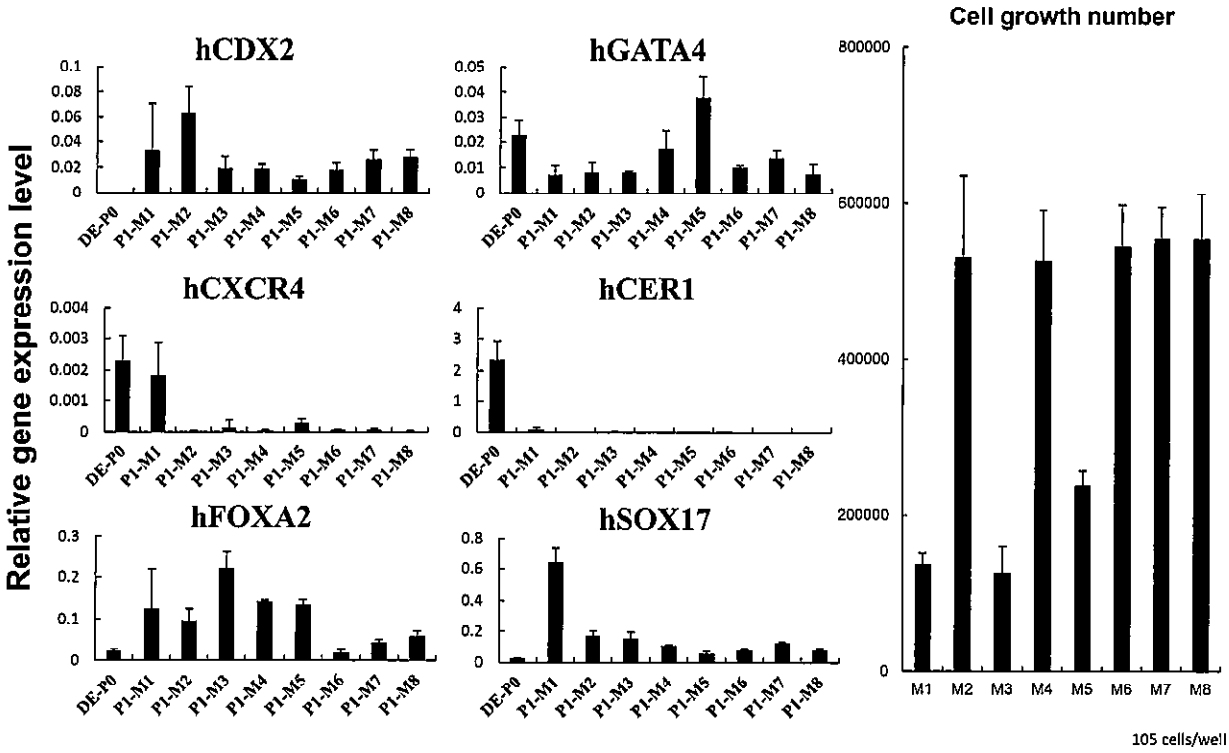


Fig.6

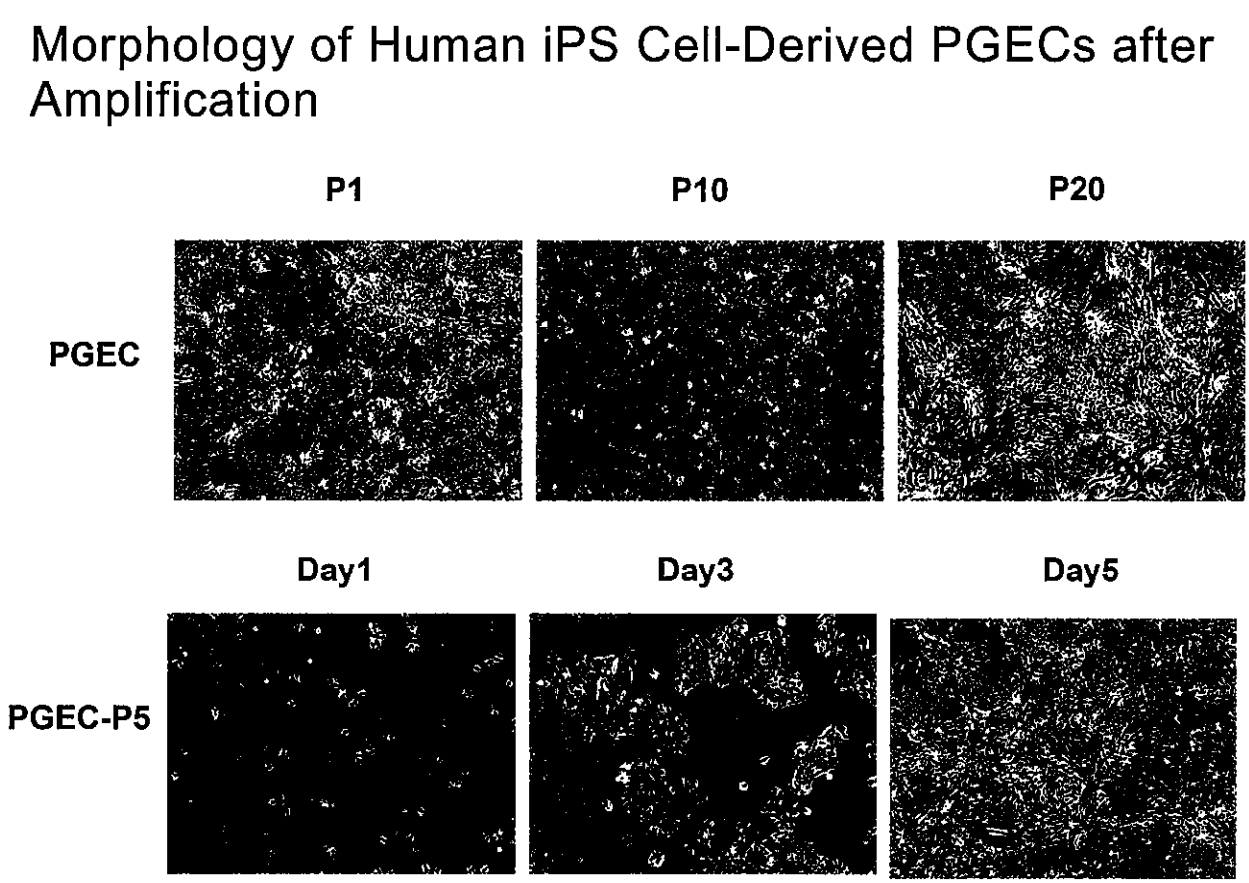

Morphology of Human iPS Cell-Derived PGECs after Amplification
P1
P10
P20
PGEC
Day1
Day3
Day5
PGEC-P5

Fig.7

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

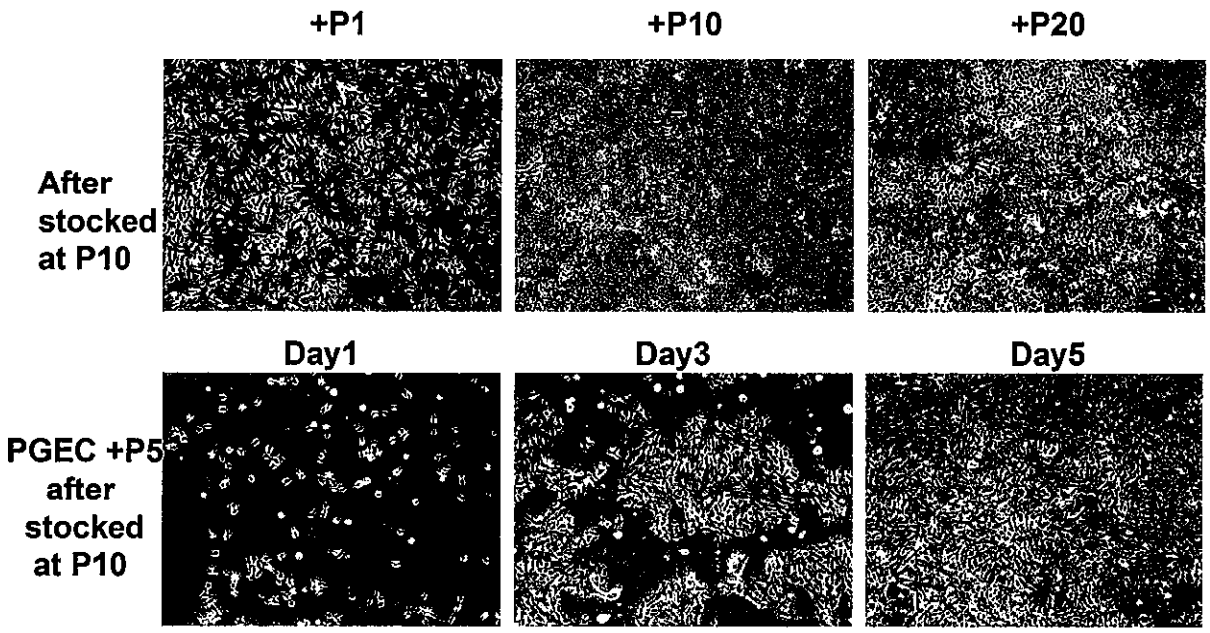


Fig.8

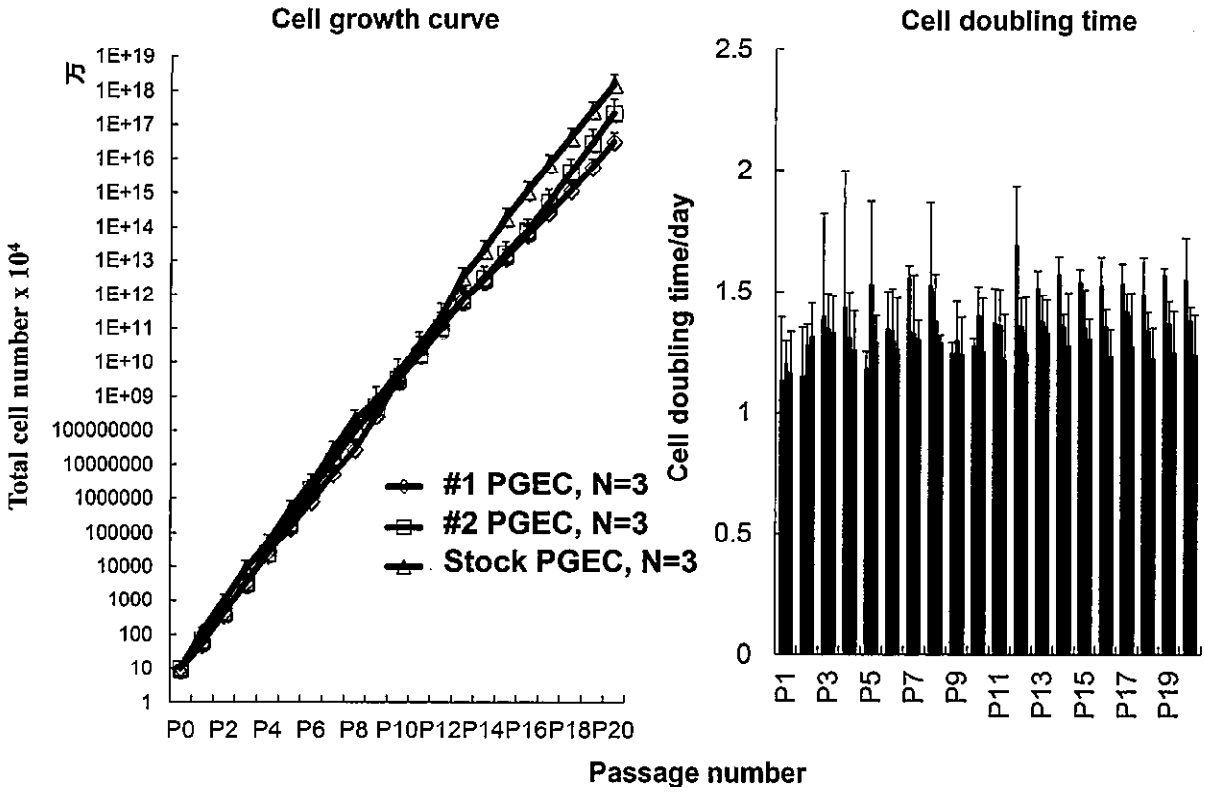

Growth Curve of Human iPS Cell-Derived PGECs
Cell growth curve
Total cell number x 10⁴
1E+19
1E+18
1E+17
1E+16
1E+15
1E+14
1E+13
1E+12
1E+11
1E+10
1E+09
100000000
10000000
1000000
100000
10000
1000
100
10
1
P0 P2 P4 P6 P8 P10P12P14P16P18P20
#1 PGEC, N=3
#2 PGEC, N=3
Stock PGEC, N=3
Cell doubling time
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

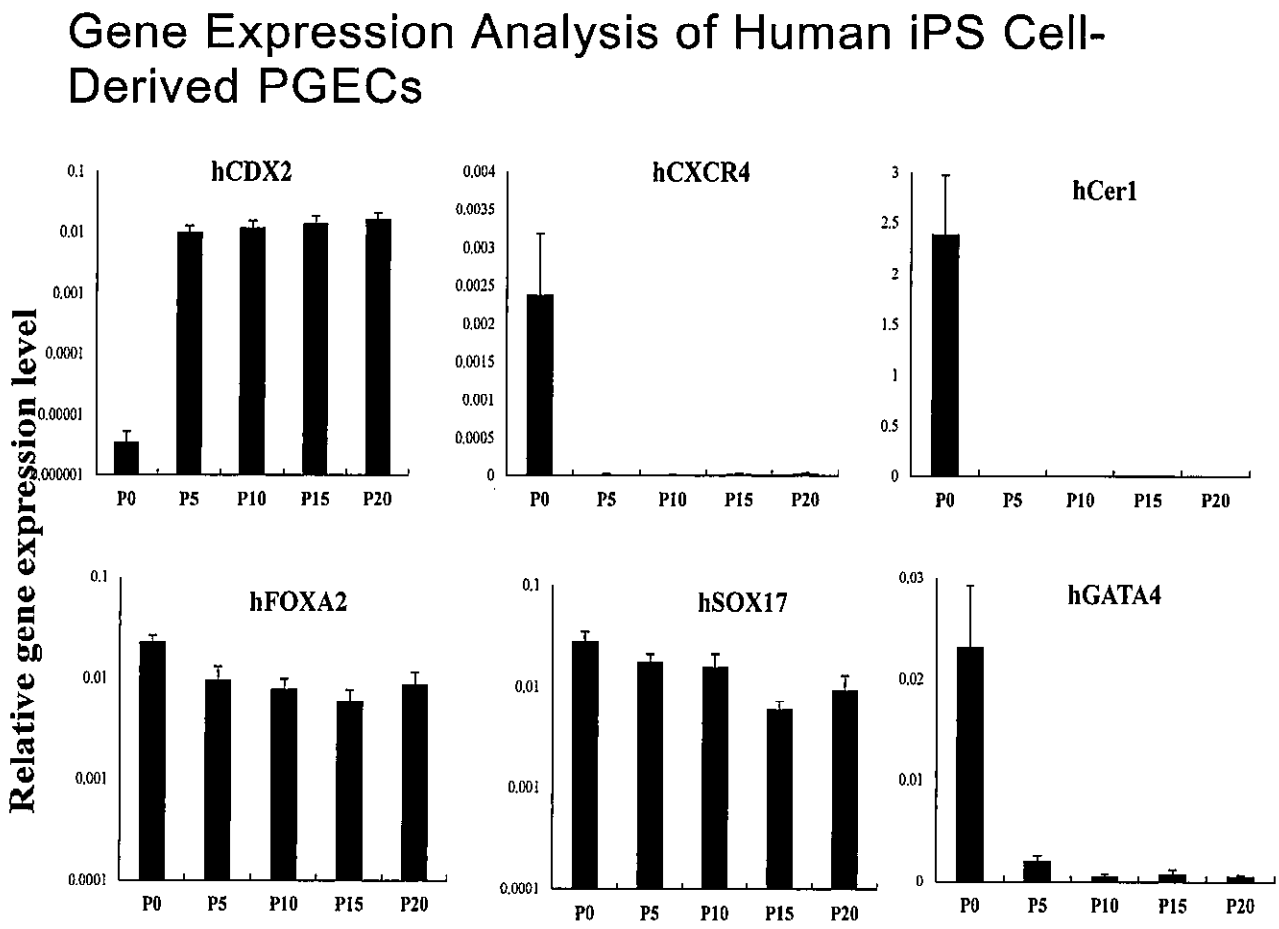

Gene Expression Analysis of Human iPS Cell-Derived PGECs
Relative gene expression level
hCDX2
hCXCR4
hCer1
hFOXA2
hSOX17
hGATA4
P0
P5
P10
P15
P20

Fig.10

FACS Time Lapse Analysis of Human iPS Cell-Derived PGECs

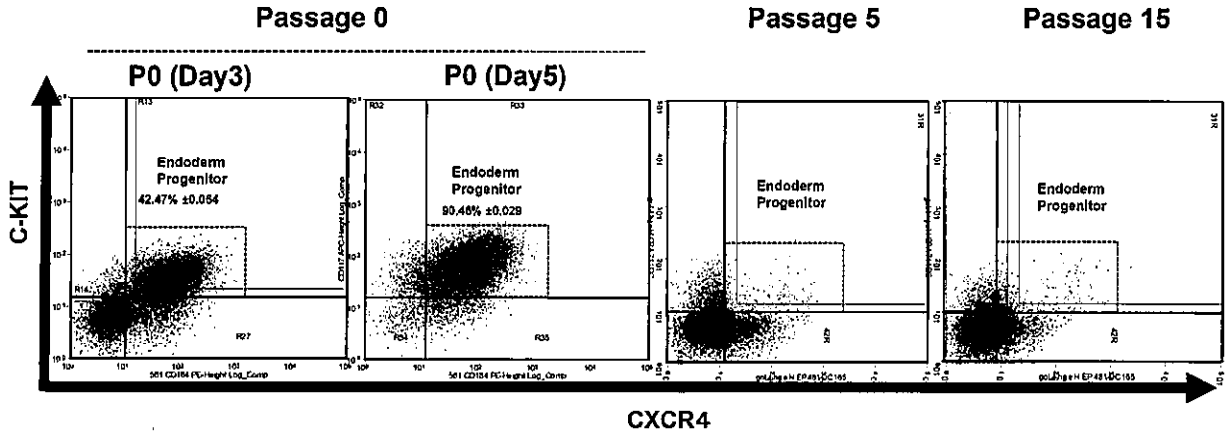


Fig.11

Immunological Analysis of Human iPS Cell-Derived PGECs

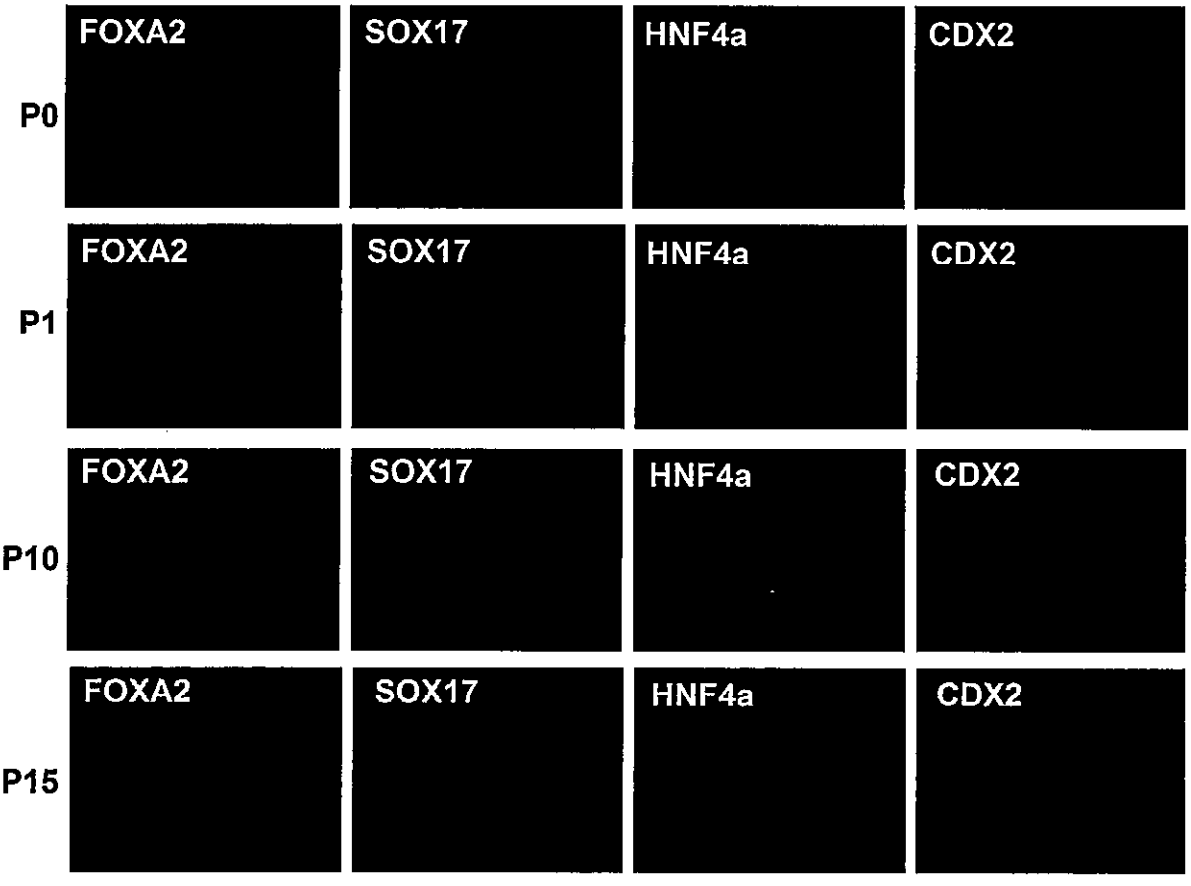


Fig.12

Immunological Analysis of Human iPS Cell-Derived PGECs

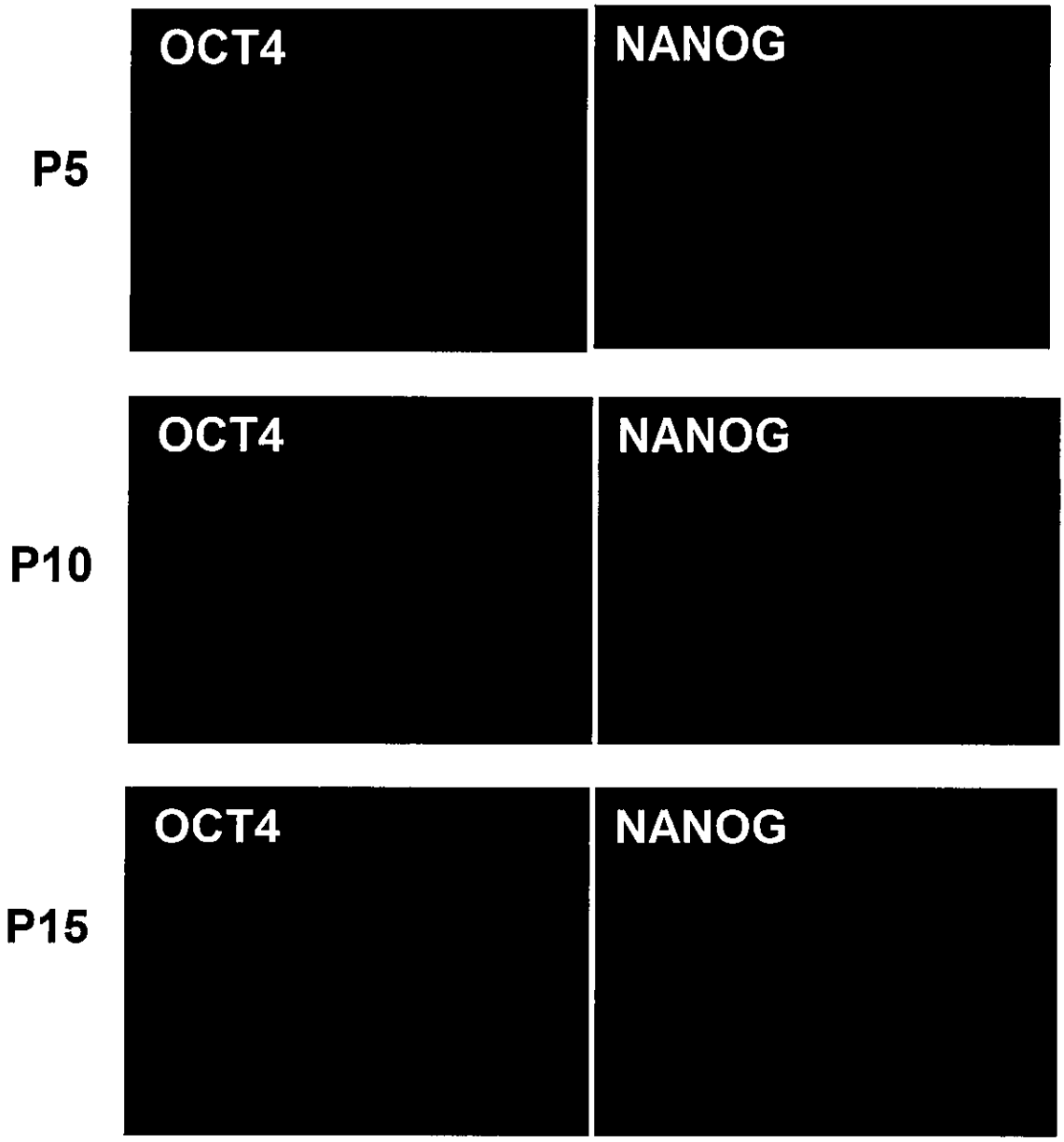


Fig.13

Principal Component Analysis of Human iPS Cell-Derived PGECs

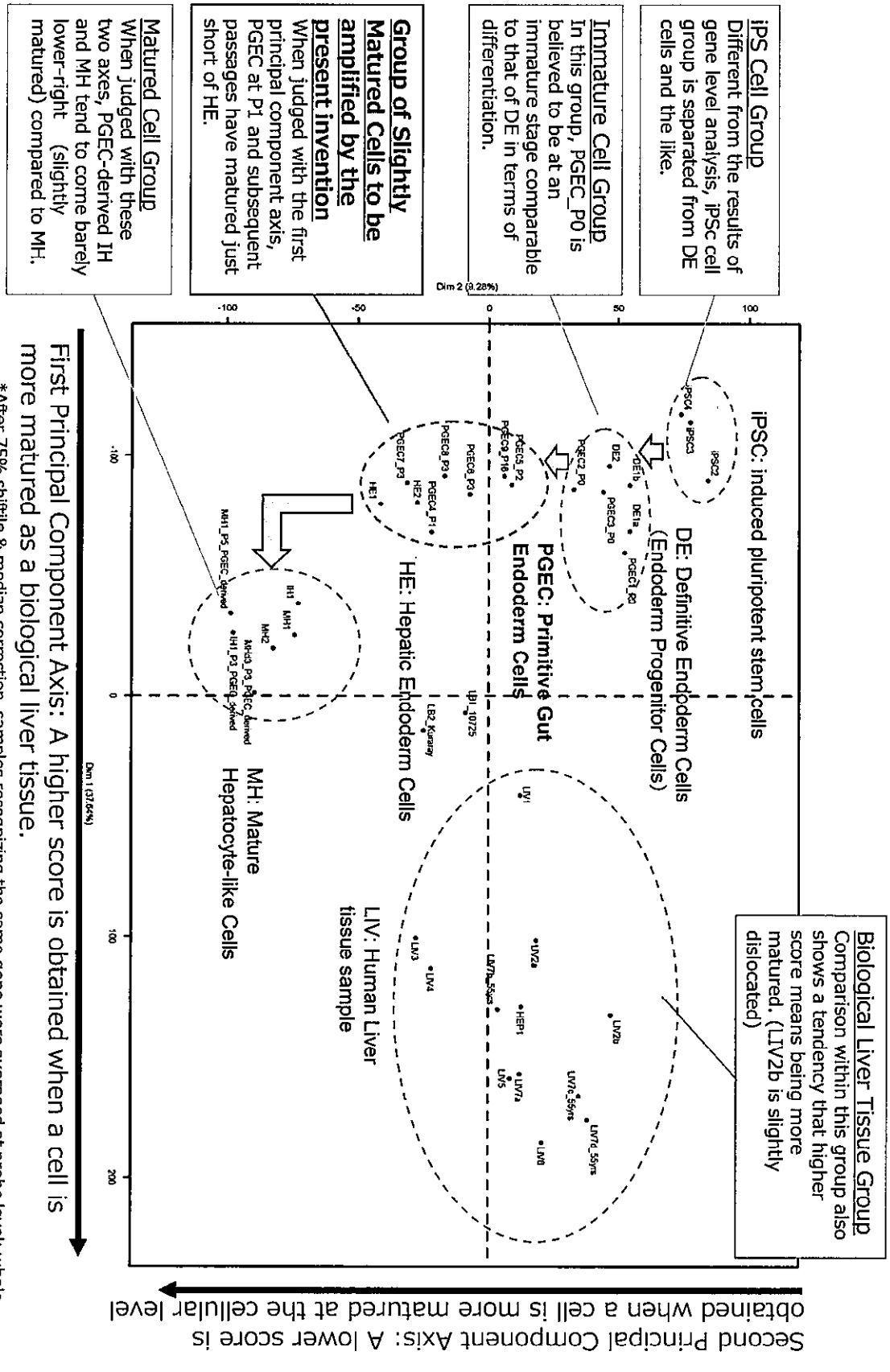
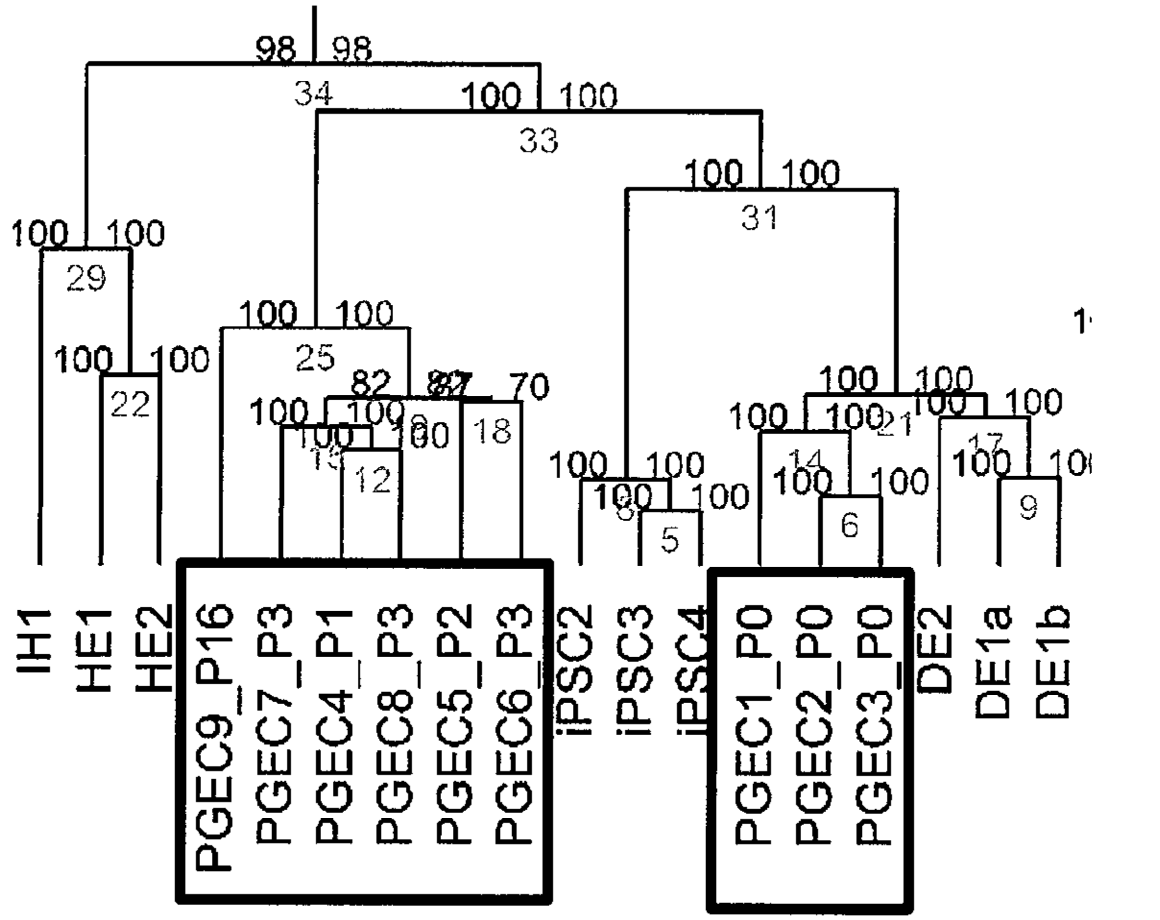


Fig.14

Hierarchical Clustering of Human iPS Cell-Derived PGECs



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

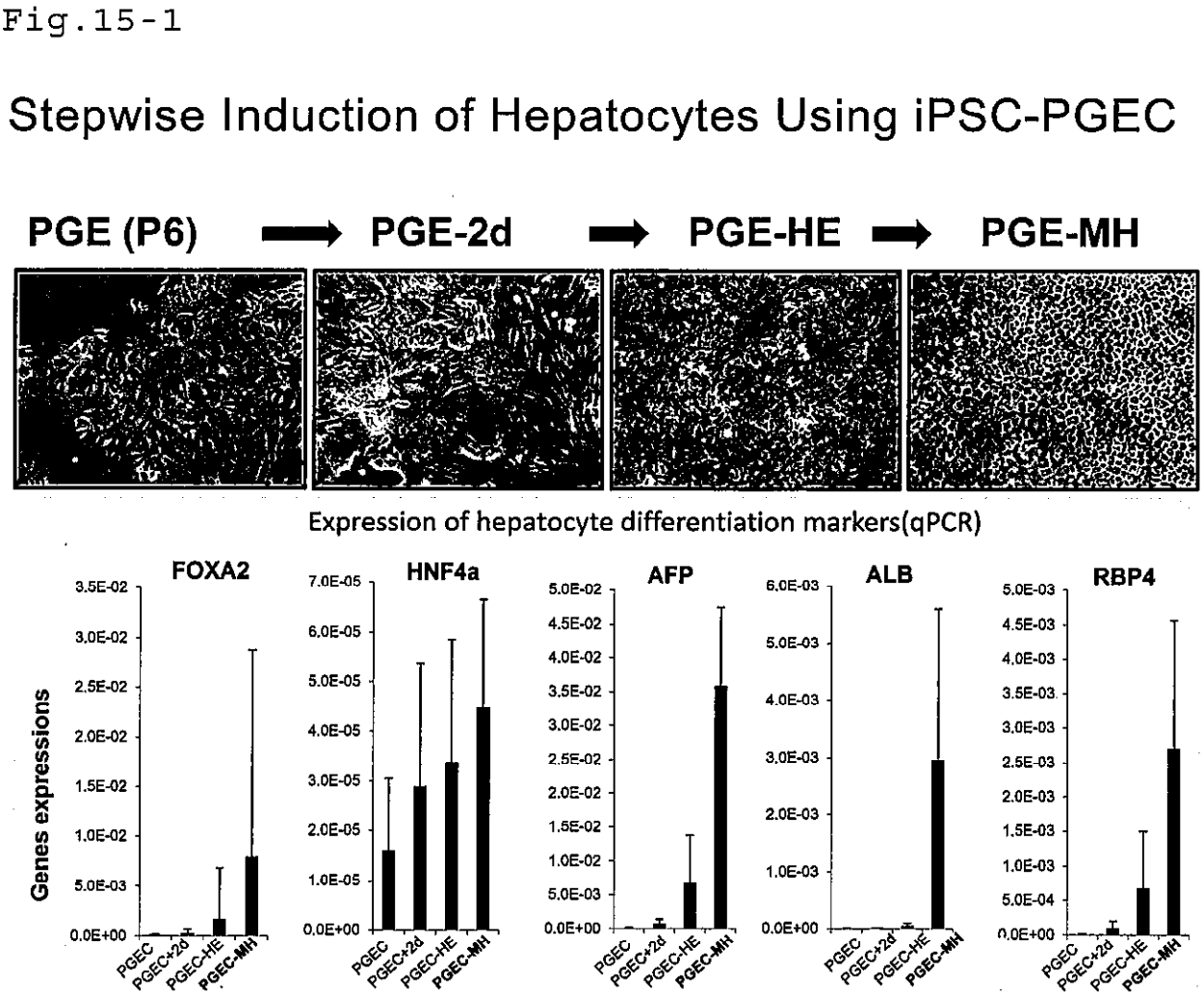

Stepwise Induction of Hepatocytes Using iPSC-PGEC
PGE (P6)
PGE-2d
PGE-HE
PGE-MH
Expression of hepatocyte differentiation markers(qPCR)
Genes expressions
FOXA2
HNF4a
AFP
ALB
RBP4
PGEC
PGEC+2d
PGEC-HE
PGEC-MH

Fig.15-2

Stepwise Induction of Hepatocytes Using iPSC-PGEC

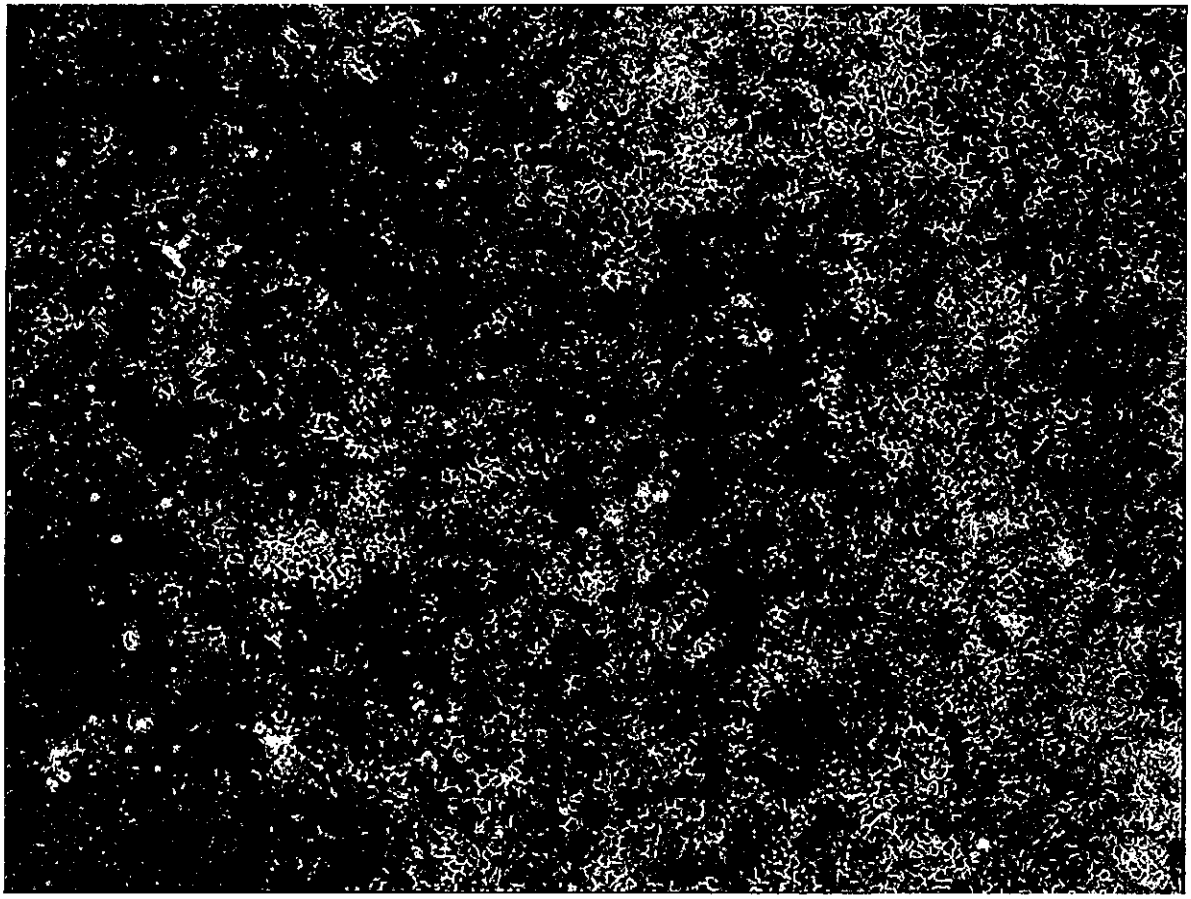


Fig.16

Functional Analysis of iPSC-PGEC-Derived Hepatocytes

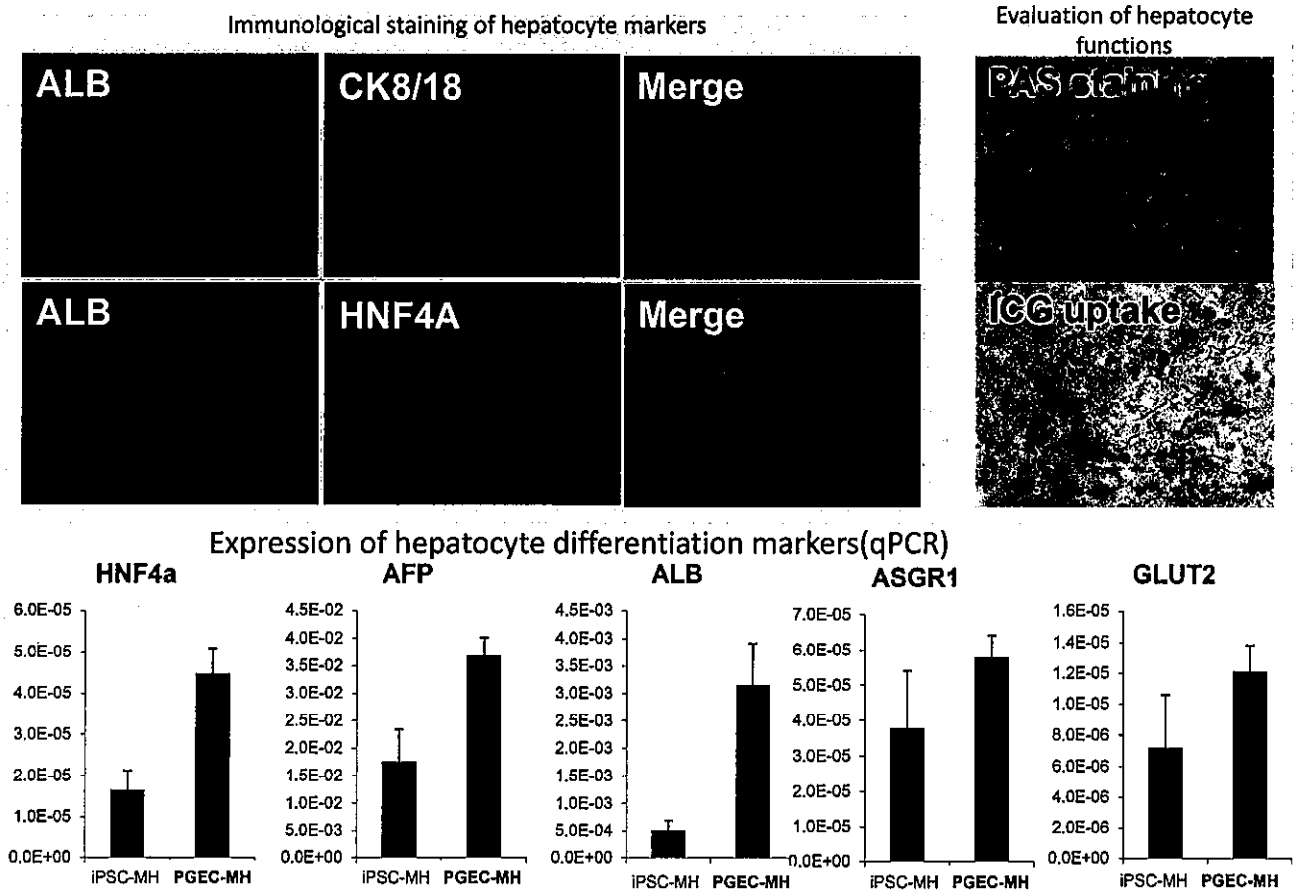


Fig.17-1

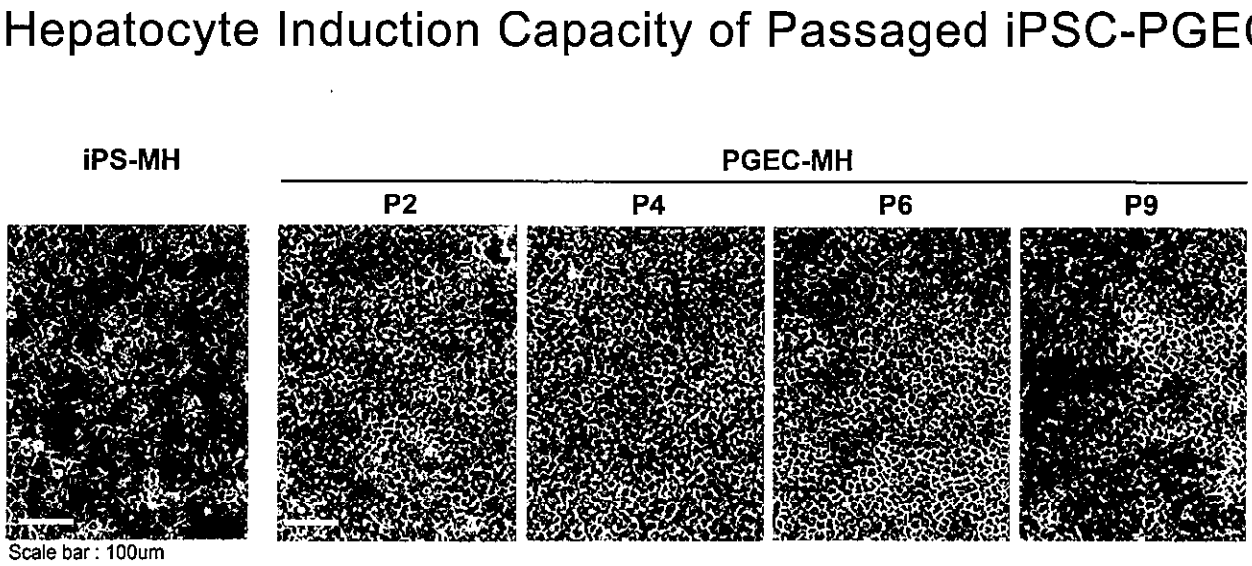


Fig.17-2

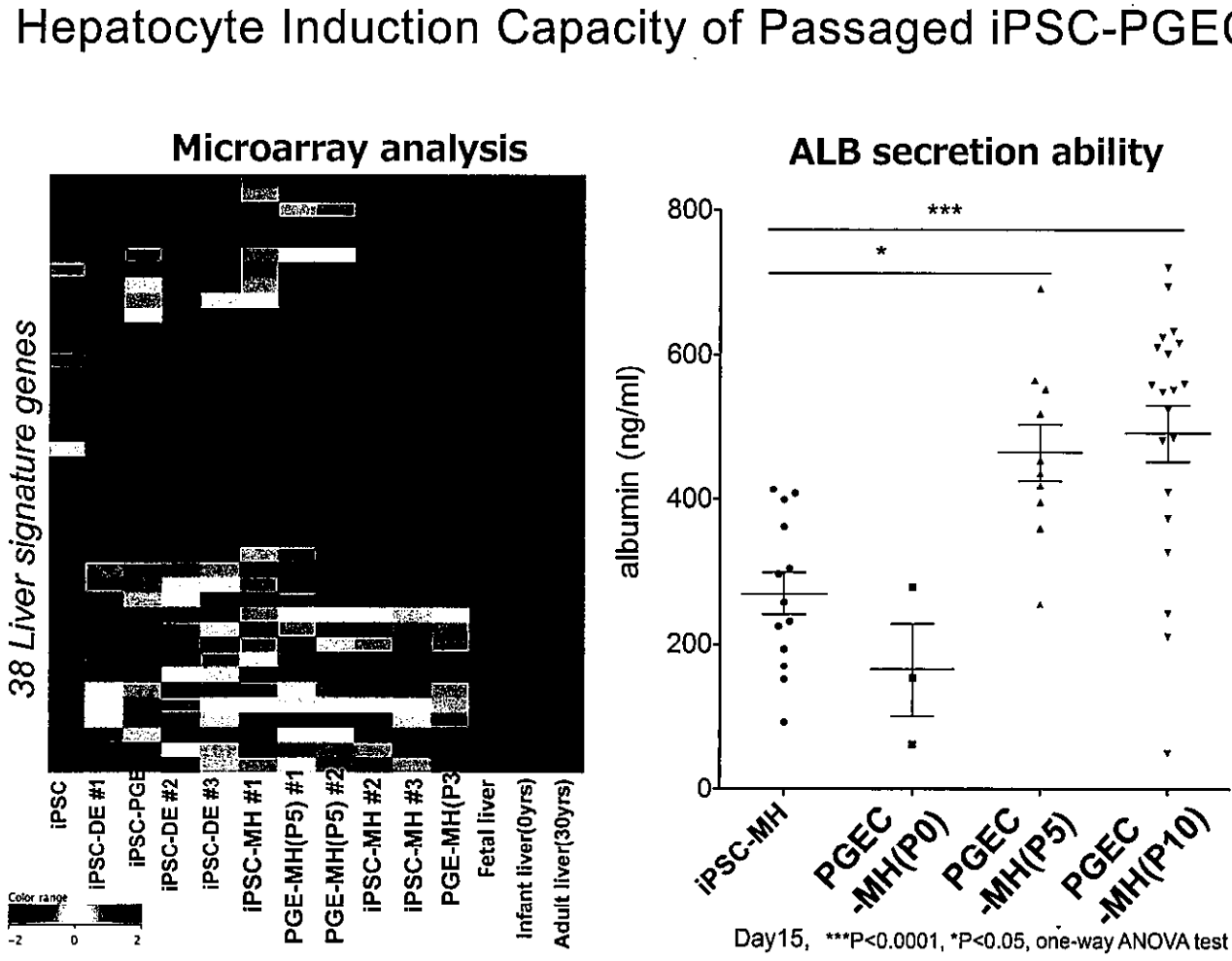


Fig.18

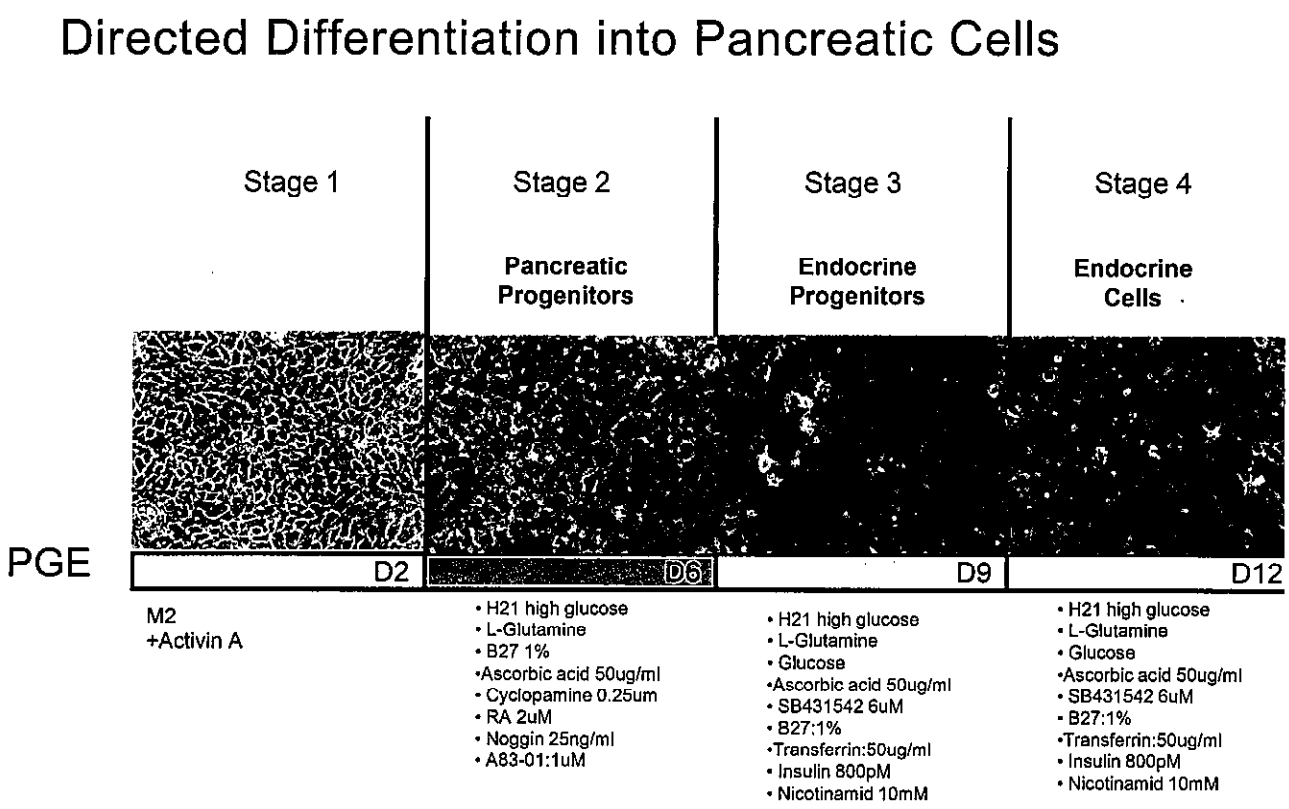
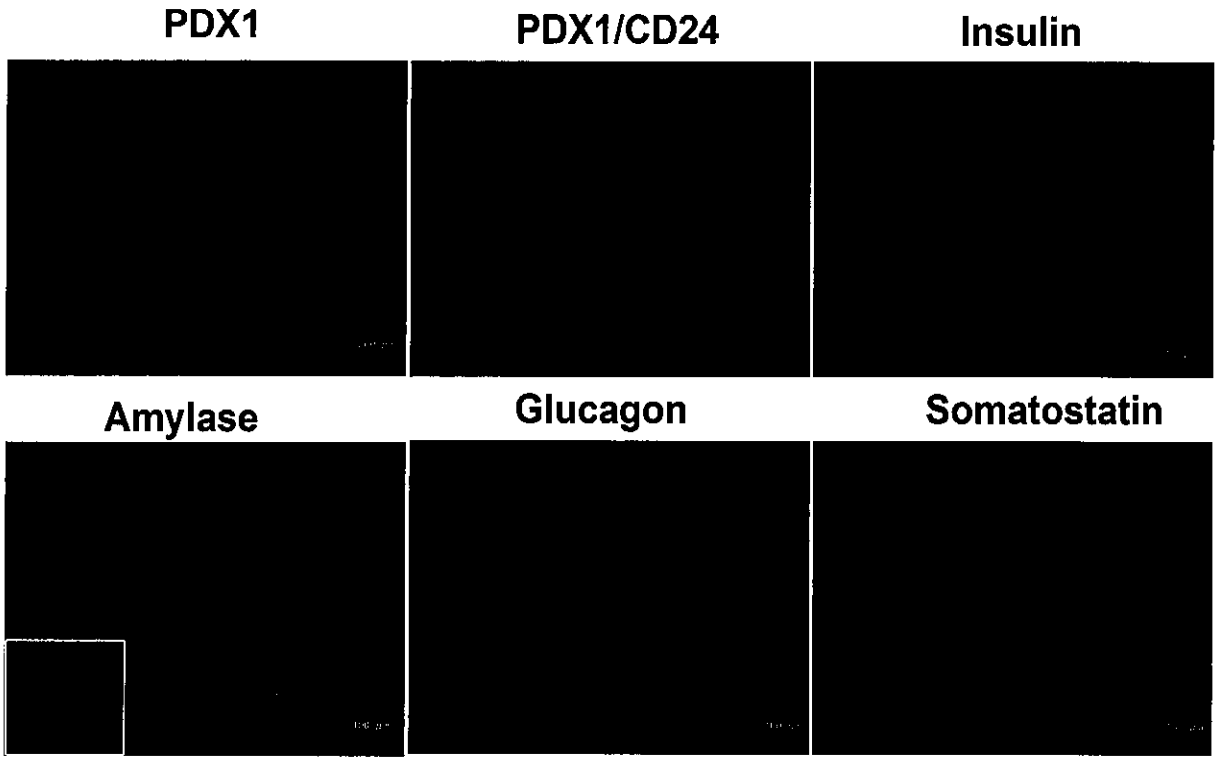

Directed Differentiation into Pancreatic Cells
Stage 1
Stage 2
Pancreatic Progenitors
Stage 3
Endocrine Progenitors
Stage 4
Endocrine Cells
PGE
D2
D6
D9
D12
M2
+Activin A
• H21 high glucose
• L-Glutamine
• B27 1%
•Ascorbic acid 50ug/ml
• Cyclopamine 0.25um
• RA 2uM
• Noggin 25ng/ml
• A83-01:1uM
• H21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

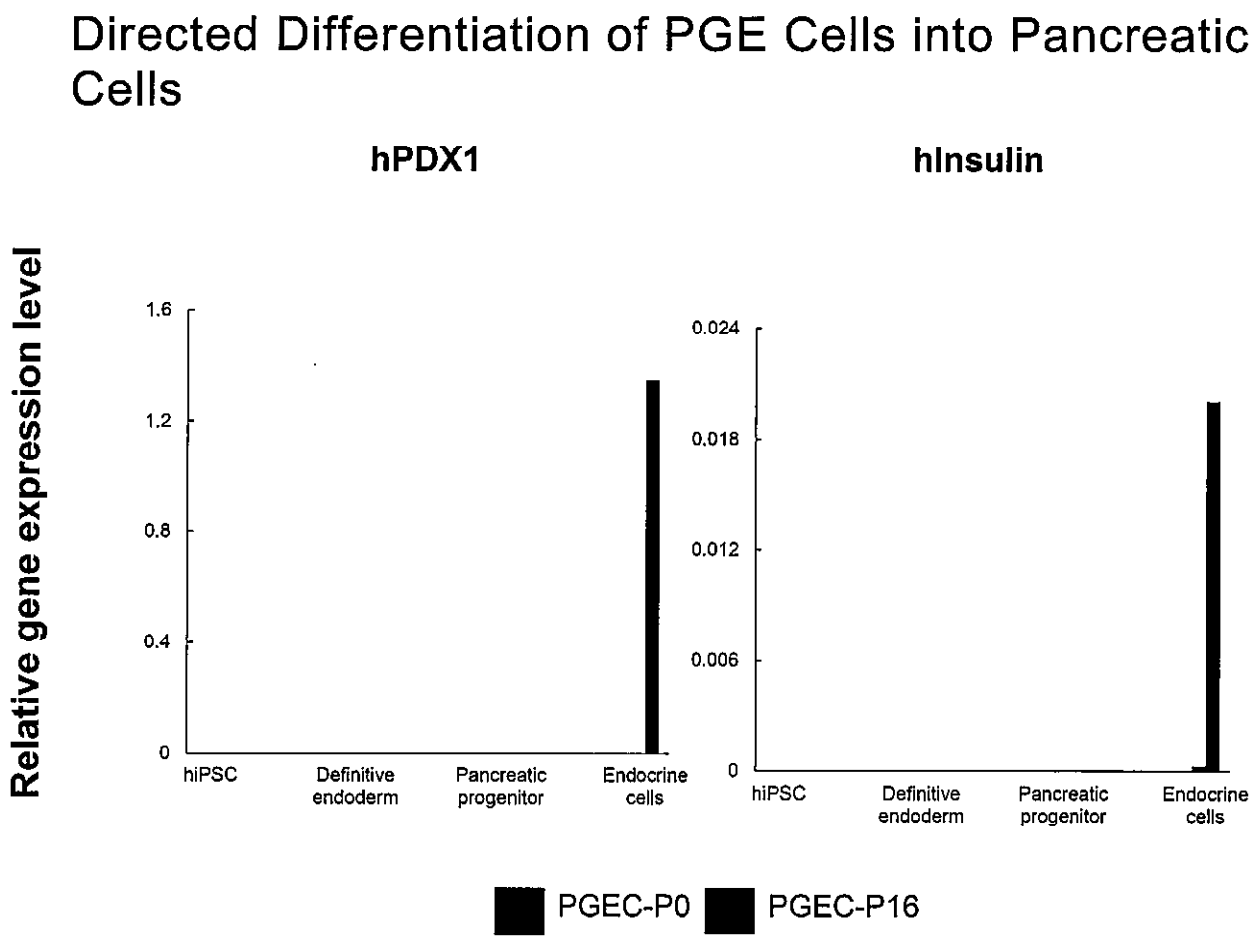

Directed Differentiation of PGE Cells into Pancreatic Cells
hPDX1
hInsulin
Relative gene expression level
1.6
1.2
0.8
0.4
0
0.024
0.018
0.012
0.006
0
hiPSC
Definitive endoderm
Pancreatic progenitor
Endocrine cells
PGEC-P0
PGEC-P16

Fig.21

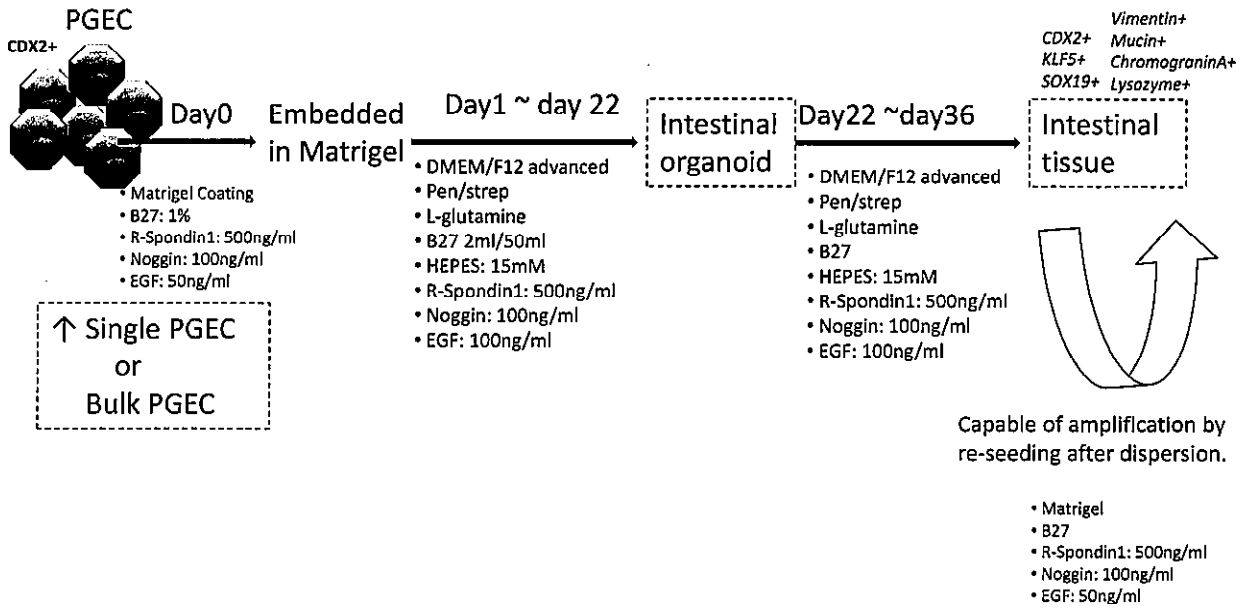

Induction of Intestinal Tissues Using PGEC
PGEC
CDX2+
Day0
Embedded in Matrigel
Day1 ~ day 22
Intestinal organoid
Day22 ~day36
Intestinal tissue
CDX2+ KLF5+ SOX19+
Vimentin+ Mucin+ ChromograninA+ Lysozyme+
• Matrigel Coating
• B27: 1%
• R-Spondin1: 500ng/ml
• Noggin: 100ng/ml
• EGF: 50ng/ml
↑ Single PGEC or Bulk PGEC
• DMEM/F12 advanced
• Pen/strep
• L-glutamine
• B27 2ml/50ml
• HEPES: 15mM
• R-Spondin1: 500ng/ml
• Noggin: 100ng/ml
• EGF: 100ng/ml
• DMEM/F12 advanced
• Pen/strep
• L-glutamine
• B27
• HEPES: 15mM
• R-Spondin1: 500ng/ml
• Noggin: 100ng/ml
• EGF: 100ng/ml
Capable of amplification by re-seeding after dispersion.
• Matrigel
• B27
• R-Spondin1: 500ng/ml
• Noggin: 100ng/ml
• EGF: 50ng/ml

Fig.22

Intestinal Tissues Induced from PGEC

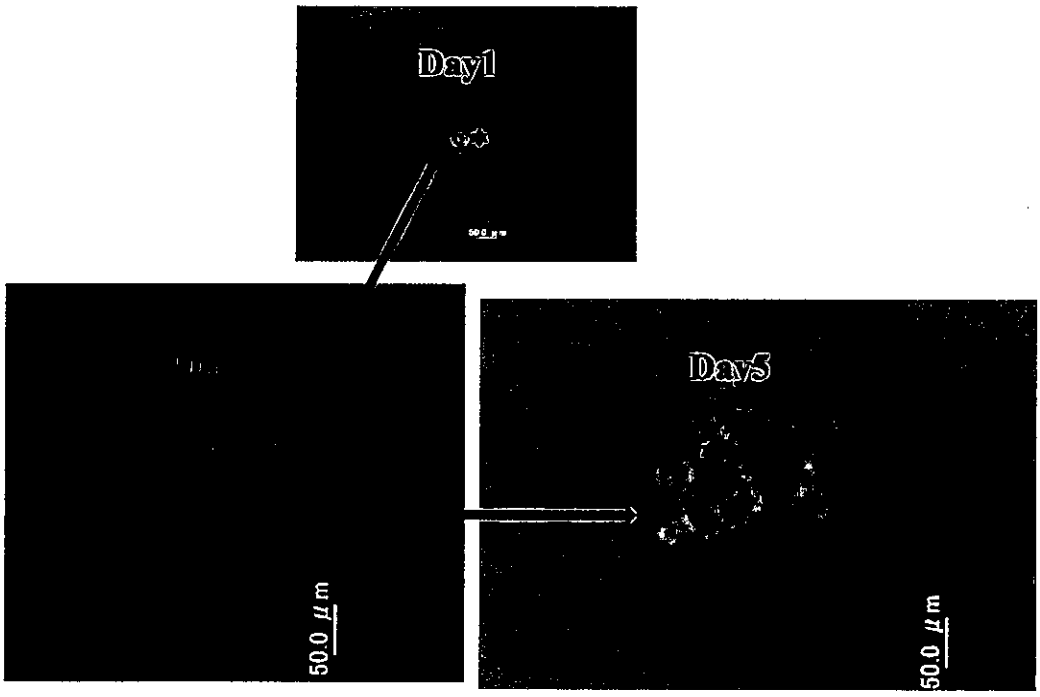


Fig.23

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

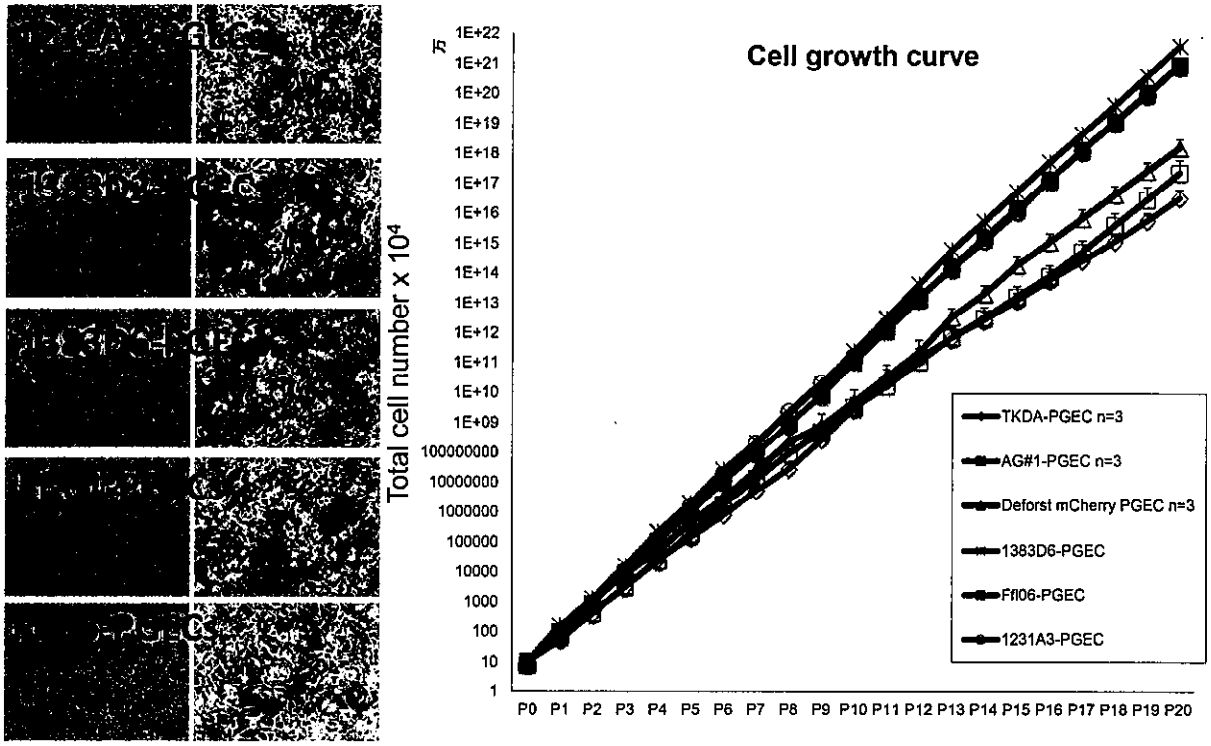


Fig.25

Generating multiple tissues of endodermal origin from transplanted PGEC

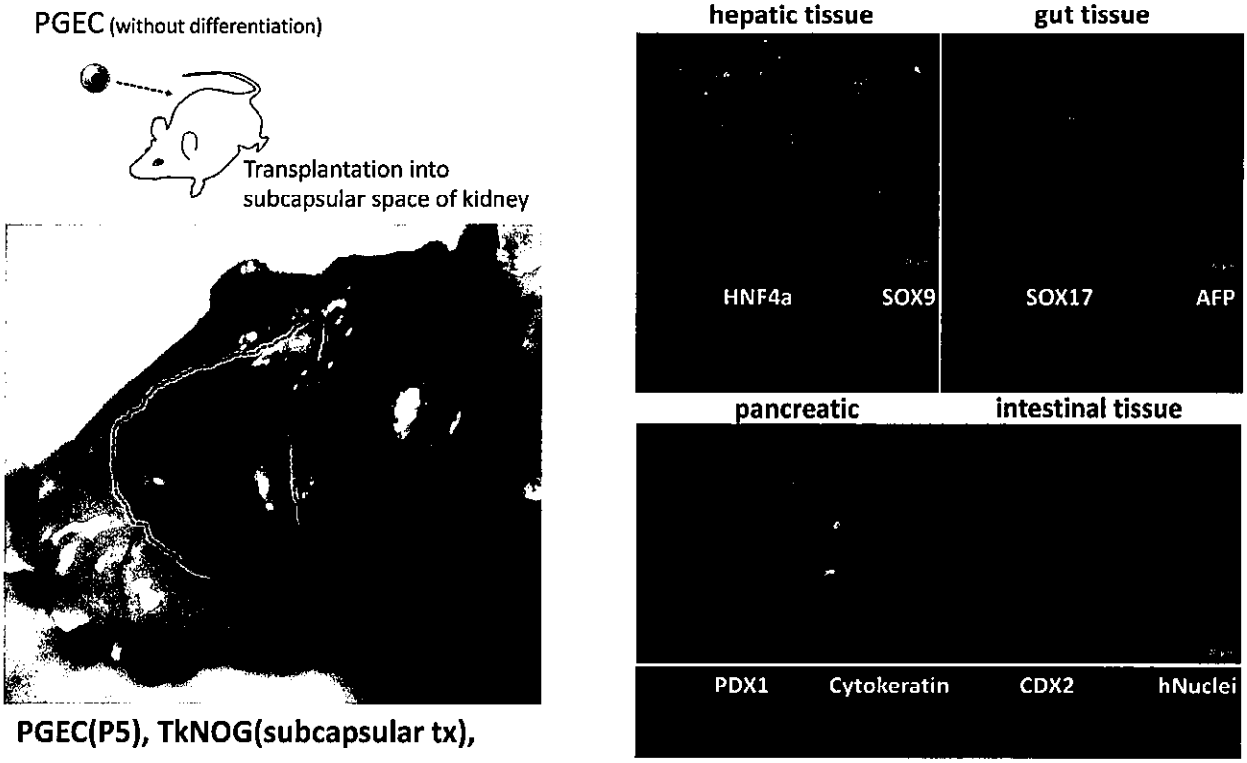
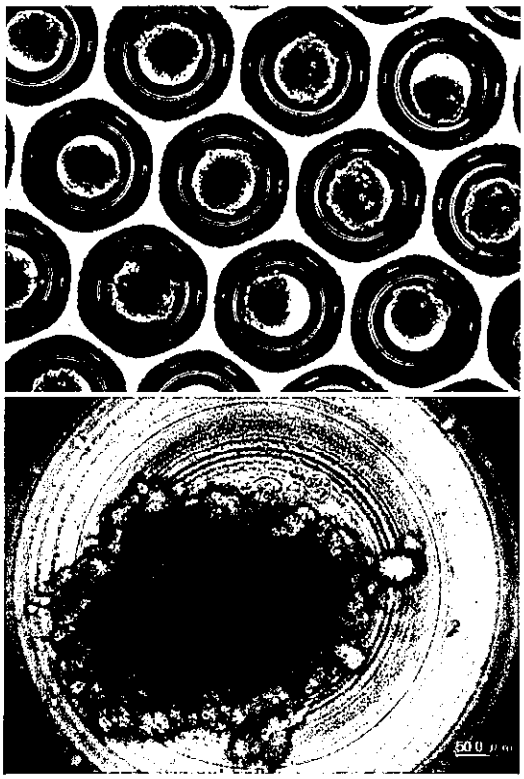


Fig.26

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



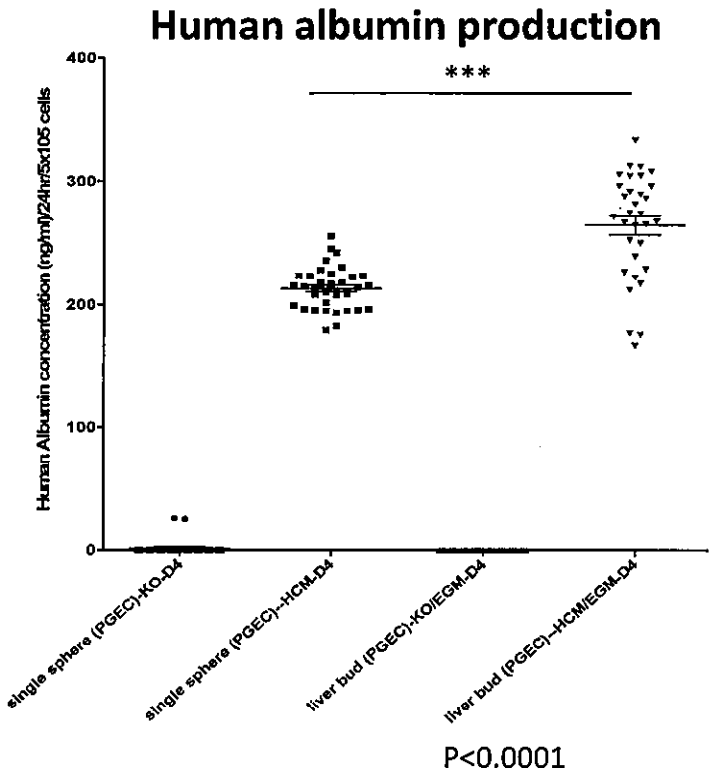


Fig.27

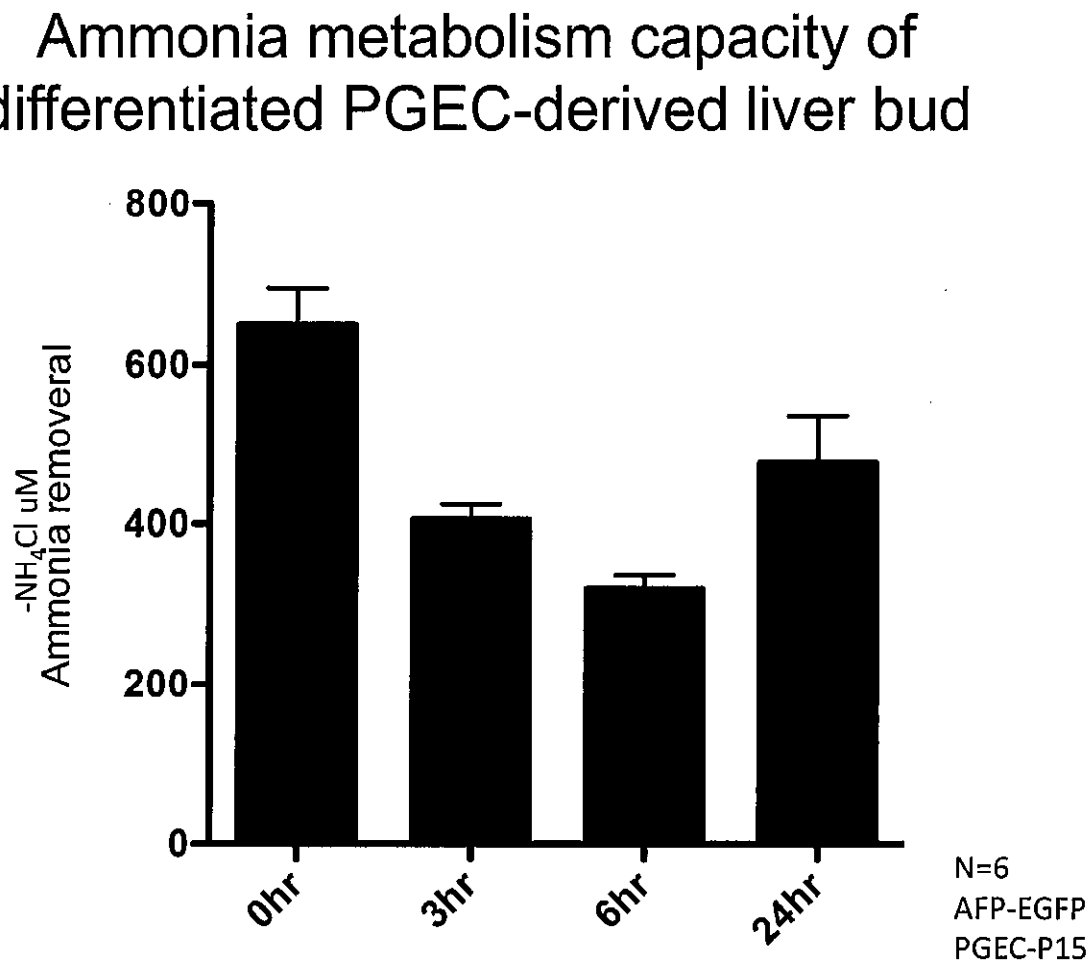

Ammonia metabolism capacity of differentiated PGEC-derived liver bud
-NH4Cl uM
Ammonia removeral
800
600
400
200
0
0hr
3hr
6hr
24hr
N=6
AFP-EGFP
PGEC-P15

Fig.28

Survival improvements following fulminant liver failure by way of PGEC derived liver bud transplantation

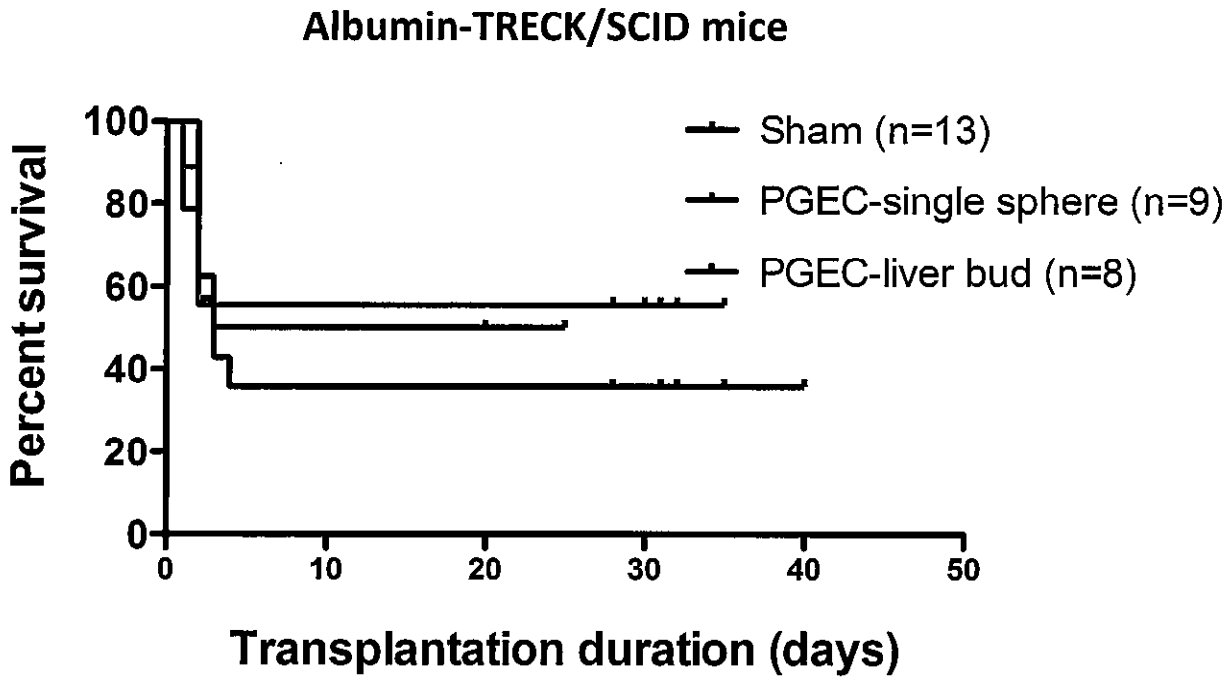
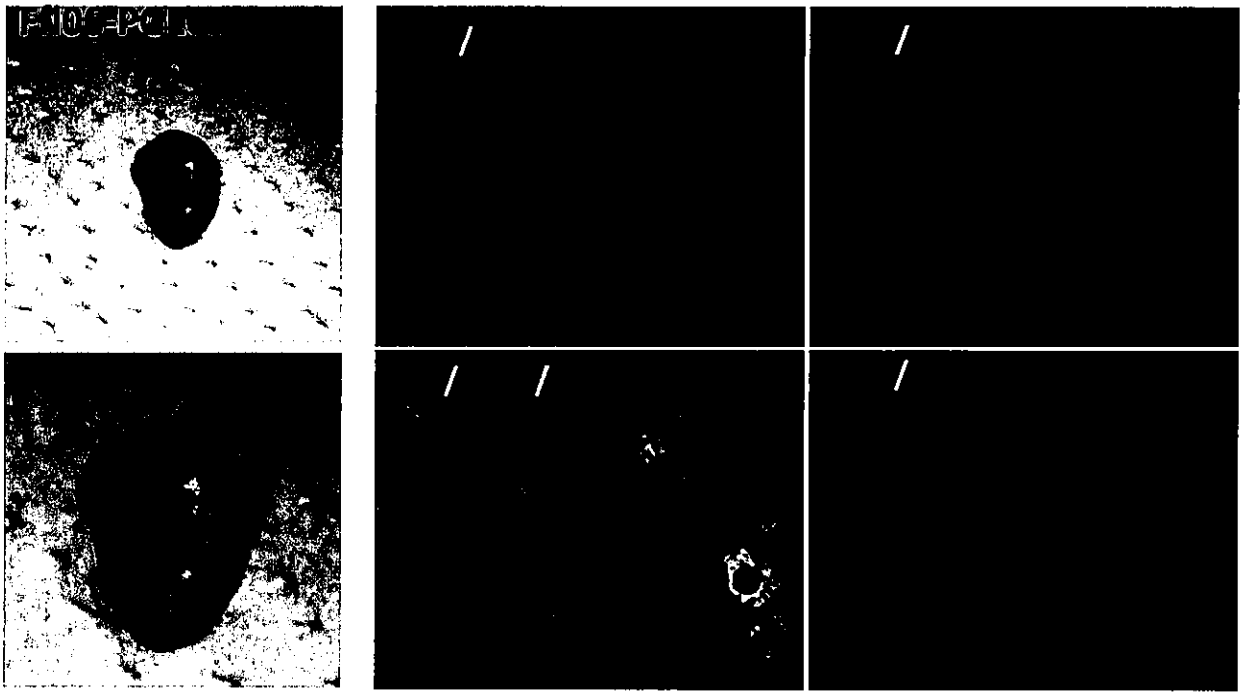


Fig.29

Immunostaining confirmed the bi-directional differentiation into hepatocytes and cholangiocytes *in vivo*



Recipients: Alb-TRECK/SCID mice
DT dose: 1.5 ug/kg

Donor: PGEC-MH-Sphere
Plate: kuraray plate

Duration: 1 month
Kidney capsule