FIG. 1

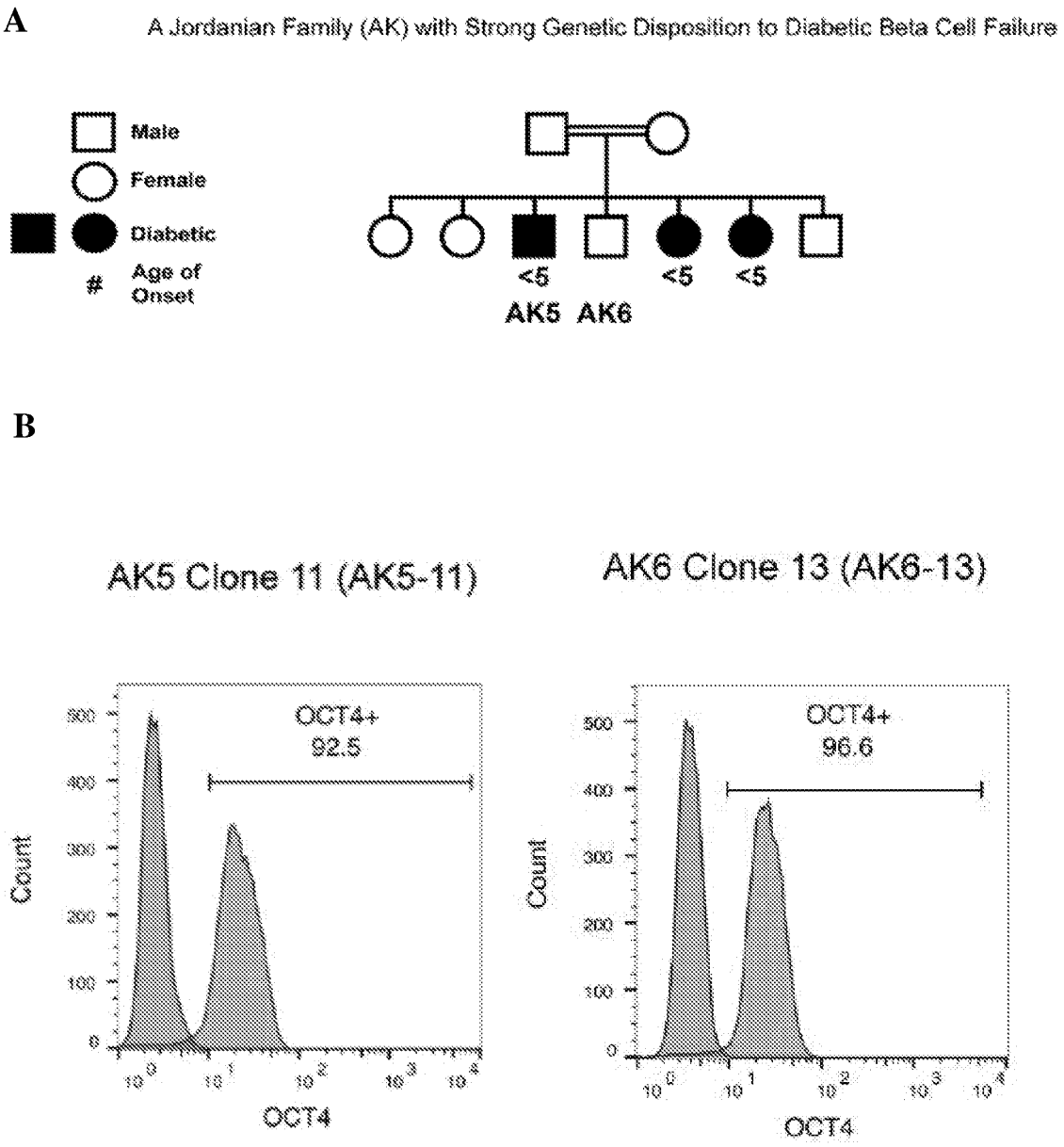

A
A Jordanian Family (AK) with Strong Genetic Disposition to Diabetic Beta Cell Failure
Male
Female
Diabetic
Age of Onset
<5
<5
<5
AK5
AK6
B
AK5 Clone 11 (AK5-11)
AK6 Clone 13 (AK6-13)
OCT4+
92.5
OCT4+
96.6
Count
OCT4

FIG. 1

C

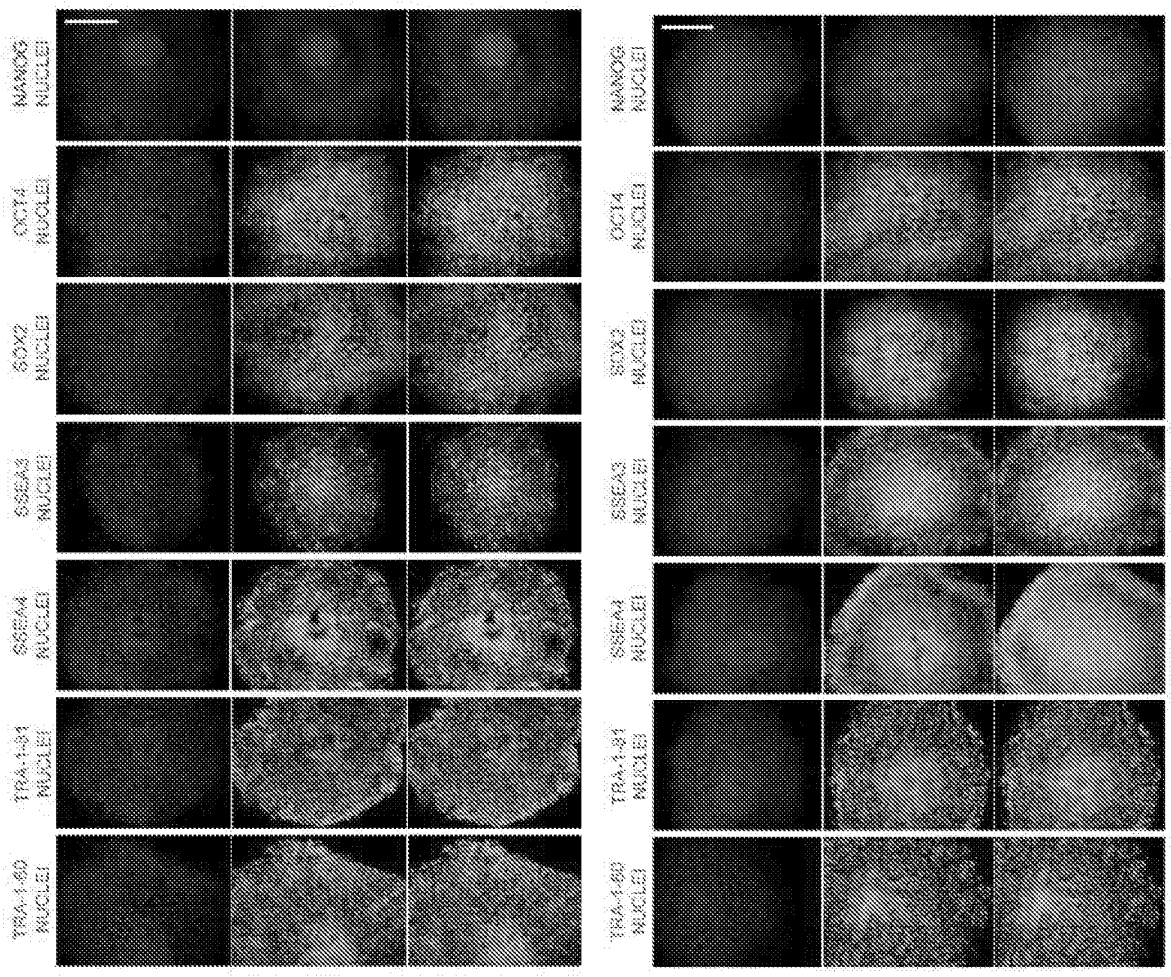
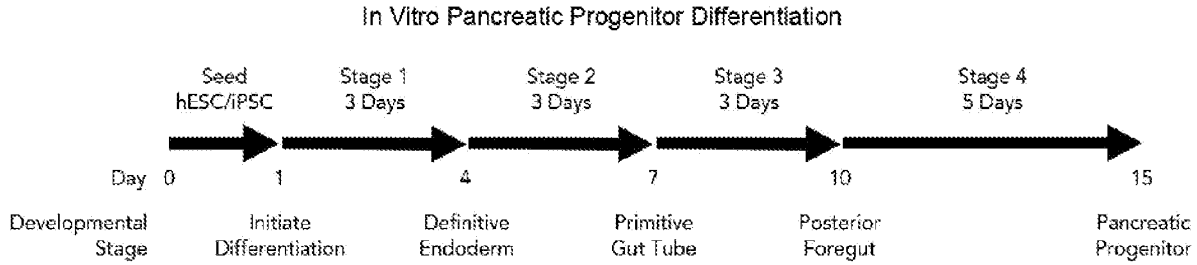

NANOG NUCLEI
OCT4 NUCLEI
SOX2 NUCLEI
SSEA3 NUCLEI
SSEA4 NUCLEI
TRA-1-81 NUCLEI
TRA-1-60 NUCLEI
NANOG NUCLEI
OCT4 NUCLEI
SOX2 NUCLEI
SSEA3 NUCLEI
SSEA4 NUCLEI
TRA-1-81 NUCLEI
TRA-1-60 NUCLEI

FIG. 2

A


In Vitro Pancreatic Progenitor Differentiation
Seed hESC/iPSC
Stage 1 3 Days
Stage 2 3 Days
Stage 3 3 Days
Stage 4 5 Days
Day 0
1
4
7
10
15
Developmental Stage
Initiate Differentiation
Definitive Endoderm
Primitive Gut Tube
Posterior Foregut
Pancreatic Progenitor

B

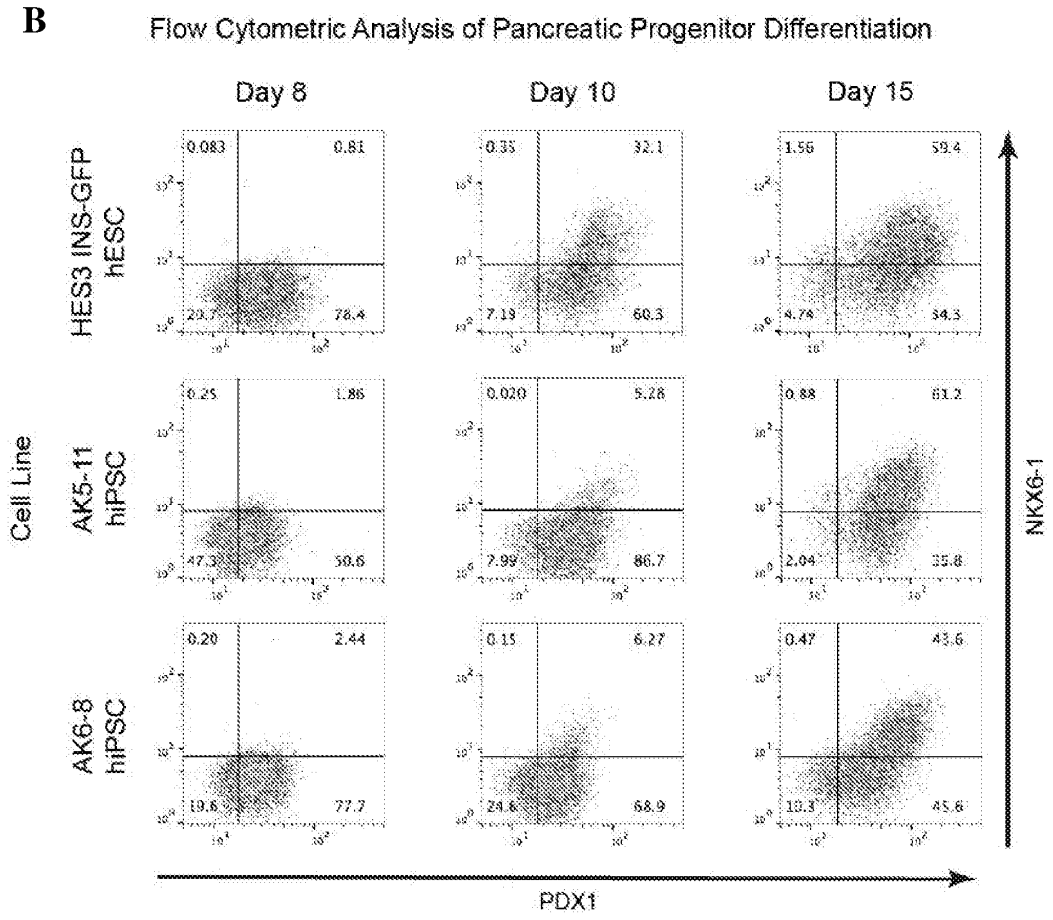

Flow Cytometric Analysis of Pancreatic Progenitor Differentiation
Day 8
Day 10
Day 15
Cell Line
HES3 INS-GFP hESC
AK5-11 hiPSC
AK6-8 hiPSC
0.083
0.81
23.7
78.4
0.35
32.1
7.19
60.3
1.56
59.4
4.78
34.3
0.25
1.86
47.3
50.6
0.020
5.28
7.99
86.7
0.88
61.2
2.04
35.8
0.20
2.44
19.6
77.7
0.15
6.27
24.6
68.9
0.47
43.6
10.3
45.6
NKX6-1
PDX1

FIG. 2

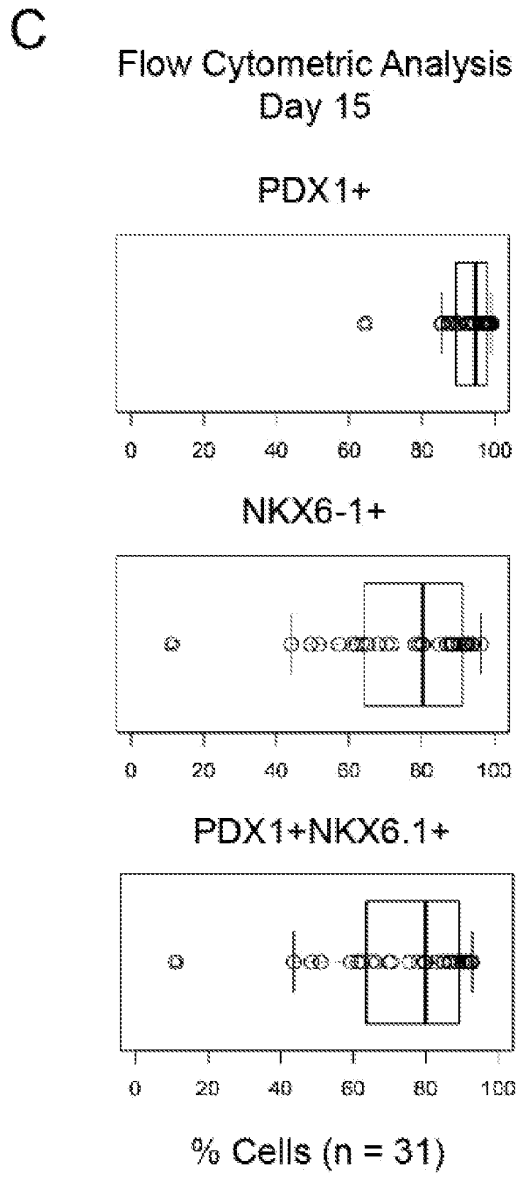

C
Flow Cytometric Analysis
Day 15
PDX1+
0
20
40
60
80
100
NKX6-1+
0
20
40
60
80
100
PDX1+NKX6.1+
0
20
40
60
80
100
% Cells (n = 31)

Fig. 2

D

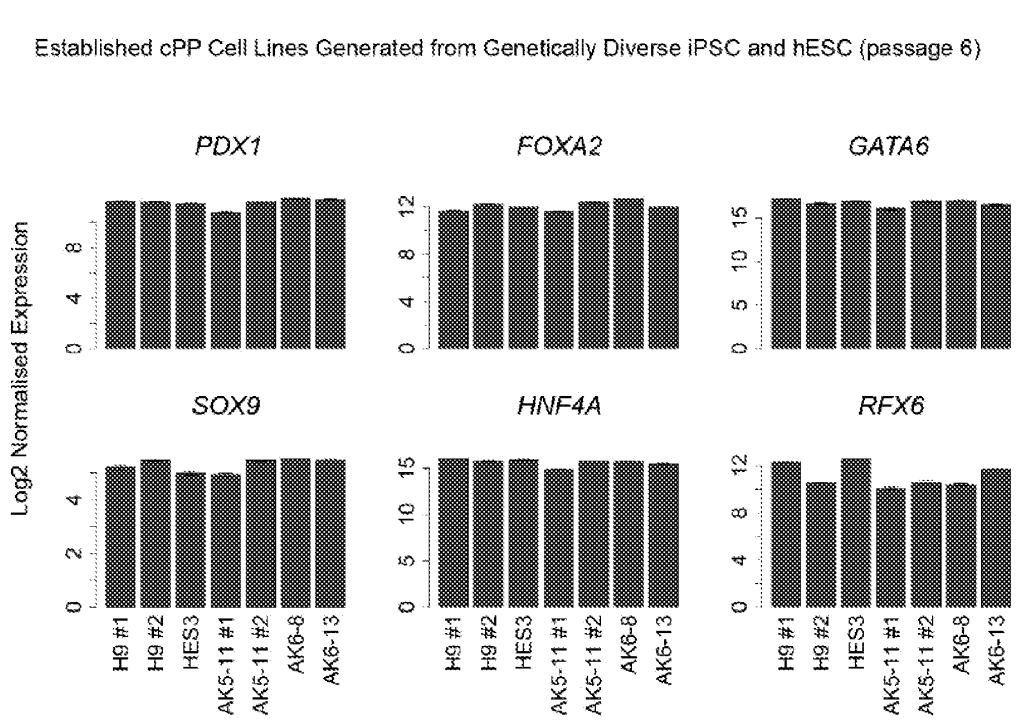

Established cPP Cell Lines Generated from Genetically Diverse iPSC and hESC (passage 6)
PDX1
FOXA2
GATA6
SOX9
HNF4A
RFX6
Log2 Normalised Expression
H9 #1
H9 #2
HES3
AK5-11 #1
AK5-11 #2
AK6-8
AK6-13

Fig. 3

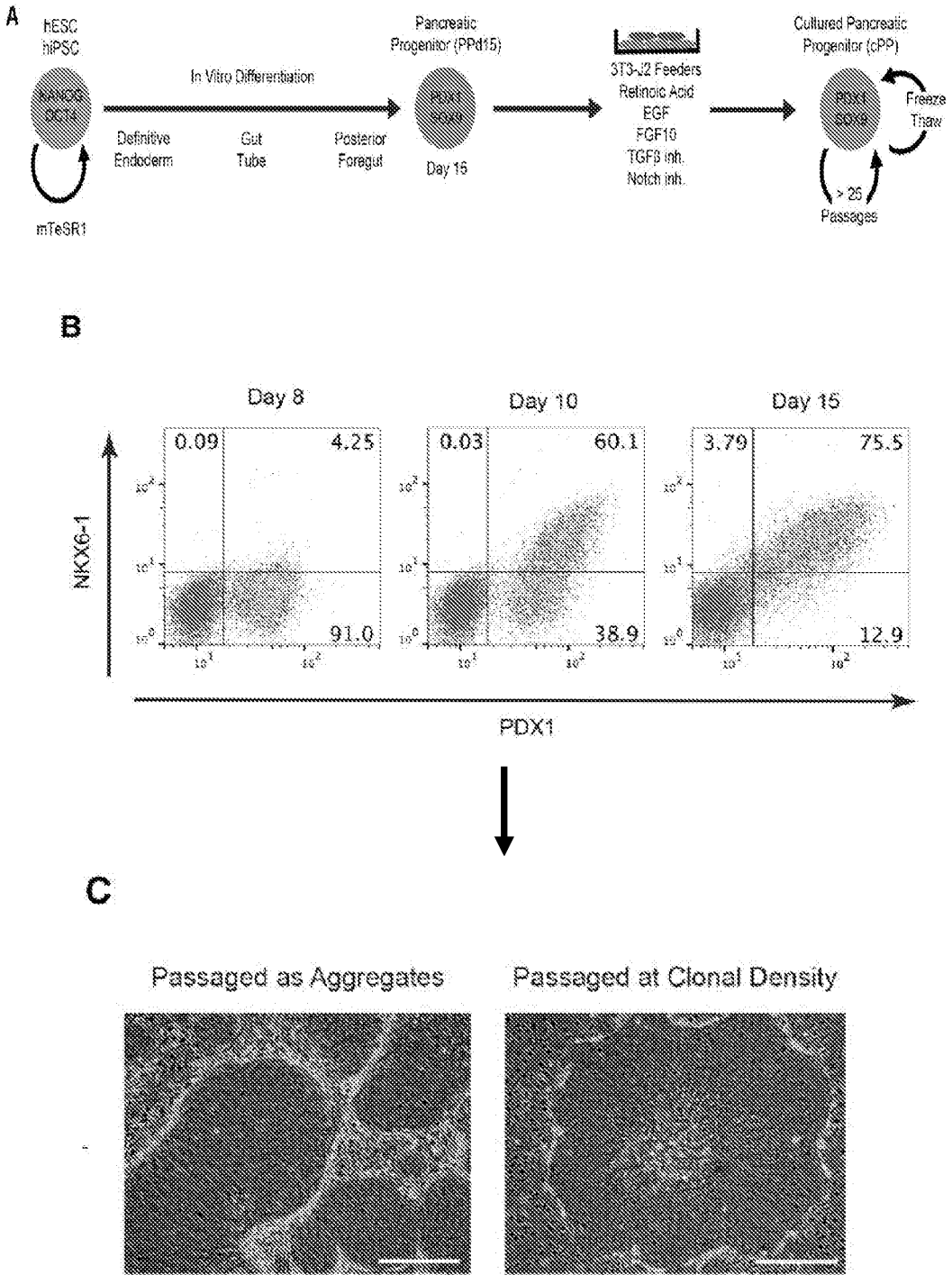

A
hESC
hiPSC
In Vitro Differentiation
Definitive Endoderm
Gut Tube
Posterior Foregut
Pancreatic Progenitor (PPd15)
Day 15
3T3-J2 Feeders
Retinoic Acid
EGF
FGF10
TGFβ inh.
Notch inh.
Cultured Pancreatic Progenitor (cPP)
Freeze
Thaw
> 25 Passages
mTeSR1
B
Day 8
Day 10
Day 15
0.09
4.25
91.0
0.03
60.1
38.9
3.79
75.5
12.9
NKX6-1
PDX1
C
Passaged as Aggregates
Passaged at Clonal Density

FIG. 3

D

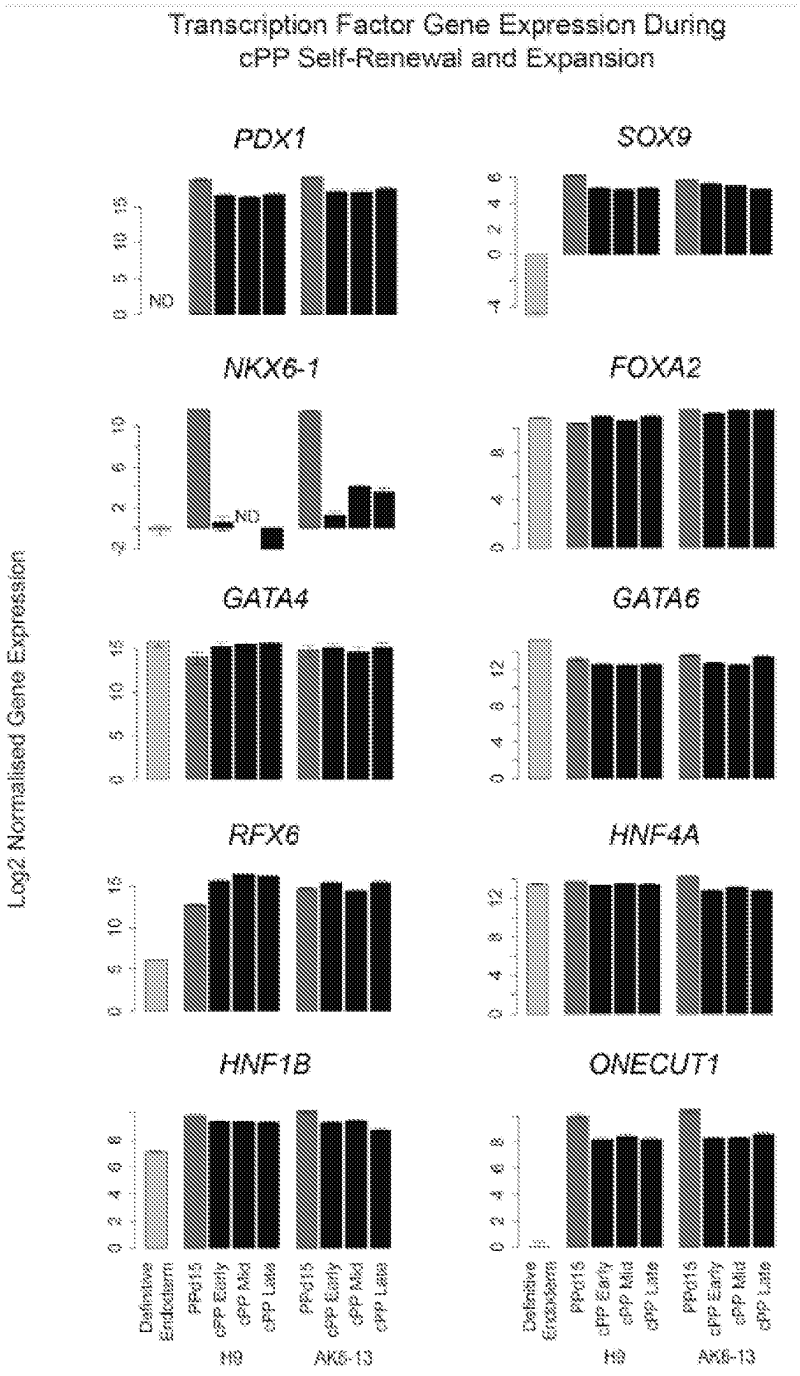

Transcription Factor Gene Expression During
cPP Self-Renewal and Expansion
Log2 Normalised Gene Expression
PDX1
SOX9
NKX6-1
FOXA2
GATA4
GATA6
RFX6
HNF4A
HNF1B
ONECUT1
ND
Definitive Endoderm
PPoS15
cPP Early
cPP Mid
cPP Late
H9
AK6-13

FIG. 3

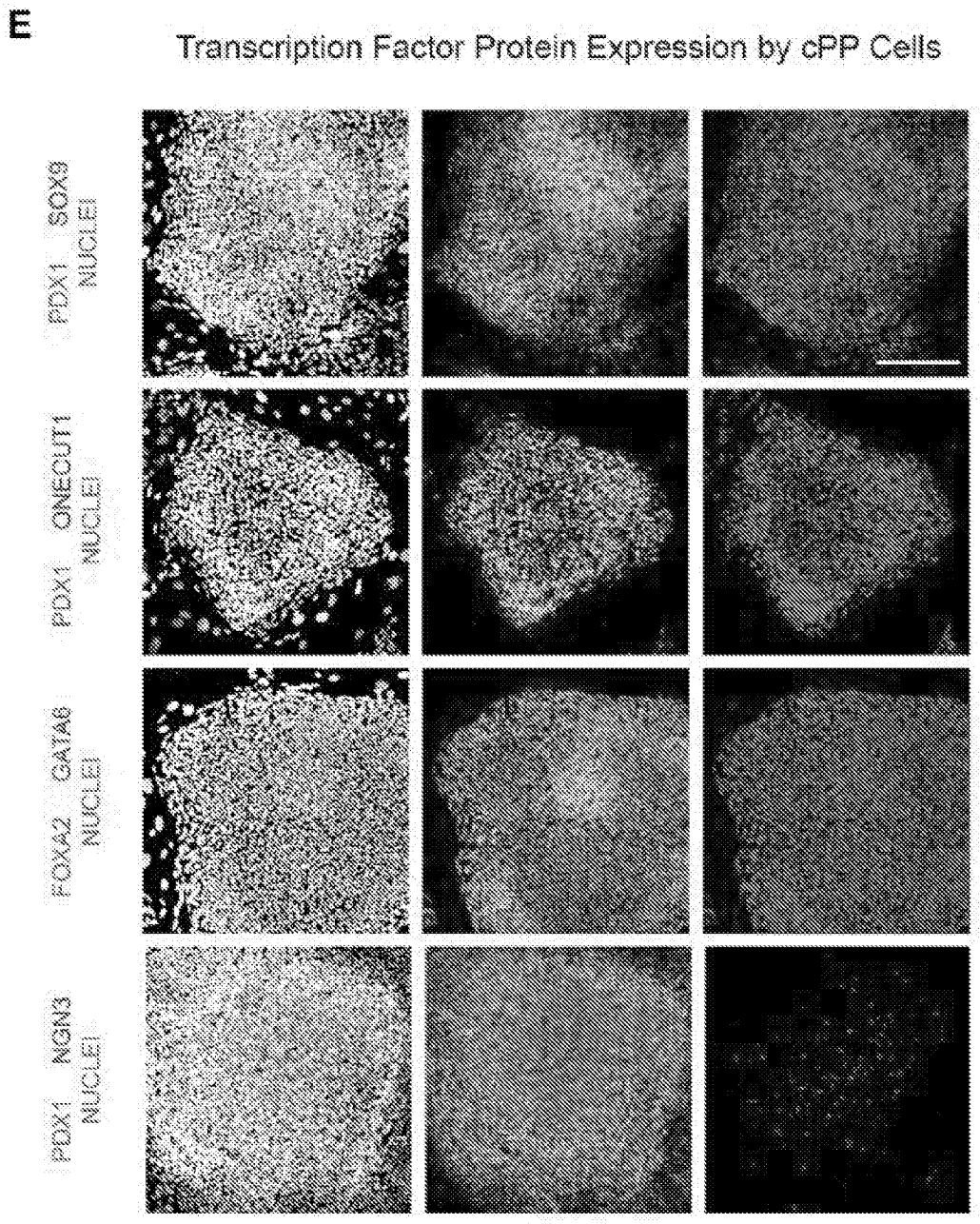

E
Transcription Factor Protein Expression by cPP Cells
PDX1 SOX9 NUCLEI
PDX1 ONECUT1 NUCLEI
FOXA2 GATA6 NUCLEI
PDX1 NGN3 NUCLEI

Fig. 3

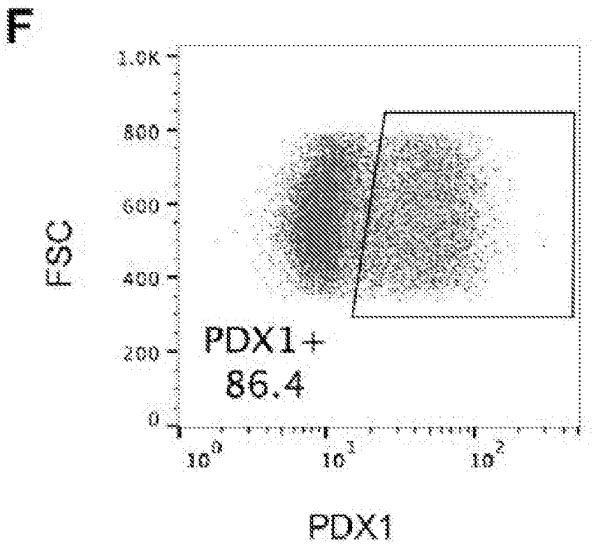


Fig. 4

A

Chromosome Counting

cPP Cell Line	H9 Pedigree #1	H9 Pedigree #1	H9 Pedigree #1	H9 Pedigree #2	H9 Pedigree #3	AK6_13	AK6_13	AK6-8
Pluripotent Cells	hESC	hESC	hESC	hESC	hESC	iPSC	iPSC	iPSC
cPP Passage	8	13	19	12	15	6	11	12
Spread Count	50	50	51	50	50	50	50	50
<40	0	2	0	0	0	0	0	0
40	0	0	0	2	0	2	0	0
41	0	4	0	0	0	2	0	0
42	0	0	0	0	0	0	2	0
43	0	0	0	0	0	4	4	2
44	0	6	0	2	2	0	0	0
45	4	4	0	6	0	0	6	4
46	80	28	5.9	86	96	84	80	88
47	10	52	94.1	2	2	8	6	6
48	4	0	0	2	0	0	0	0
49	2	2	0	0	0	0	0	0
50	0	2	0	0	0	0	0	0
>50	0	0	0	0	0	0	2	0

Fig. 4

B

M-FISH Analysis of Chromosomal Translocations
AK6-13 cPP Passage 20

46, XY - No structural abnormalities detected [19/20 Spreads]

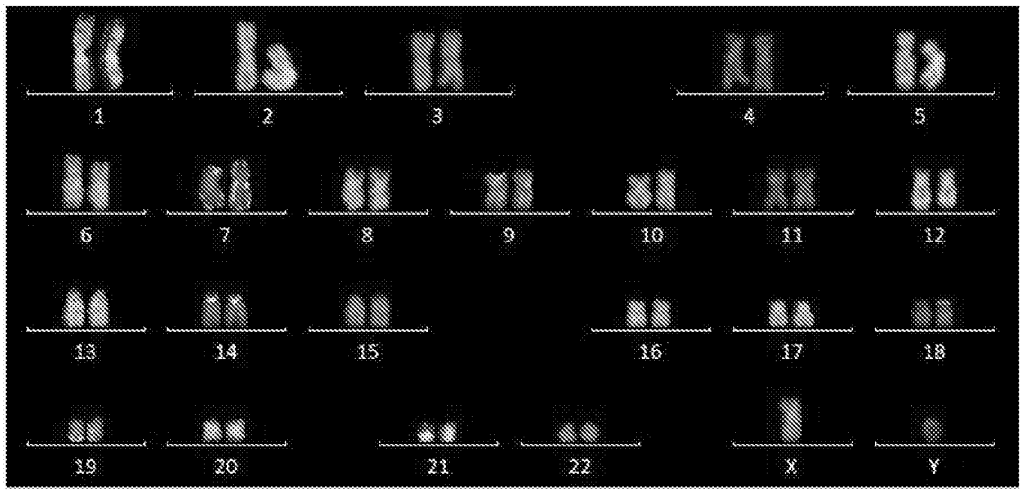


Fig. 5

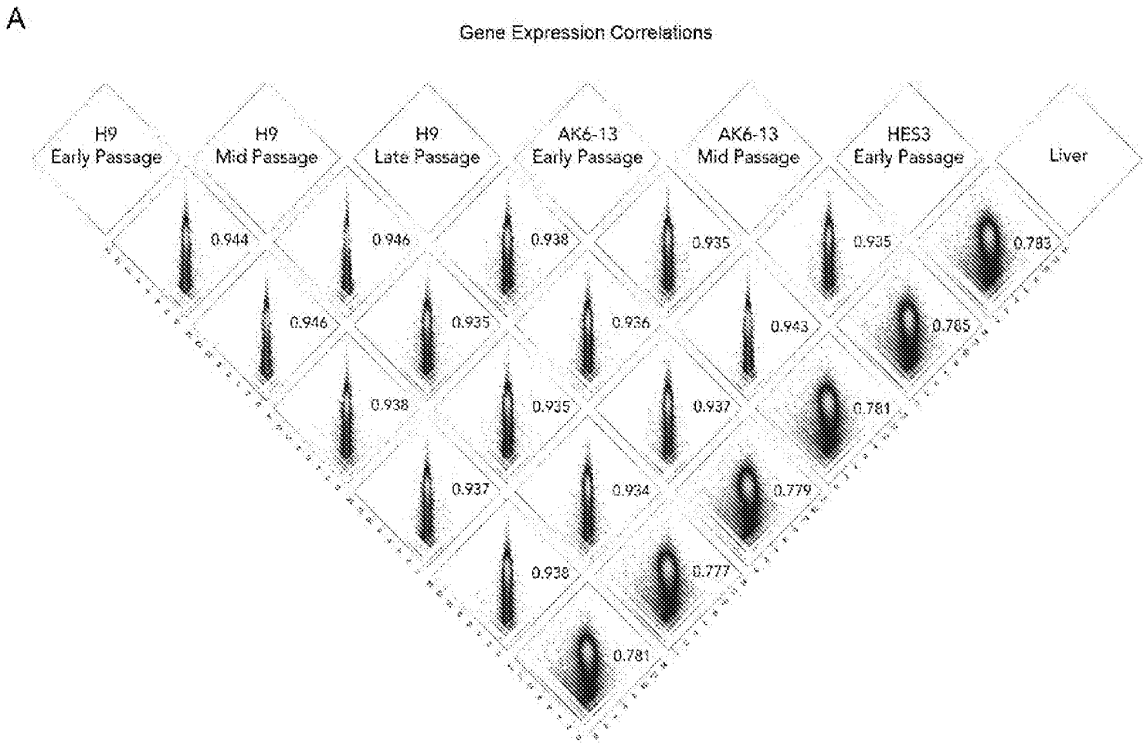

A
Gene Expression Correlations
H9 Early Passage
H9 Mid Passage
H9 Late Passage
AK6-13 Early Passage
AK6-13 Mid Passage
HES3 Early Passage
Liver
0.944
0.946
0.938
0.935
0.935
0.783
0.946
0.935
0.936
0.943
0.785
0.938
0.935
0.937
0.781
0.937
0.934
0.779
0.938
0.777
0.781

Fig. 5

B

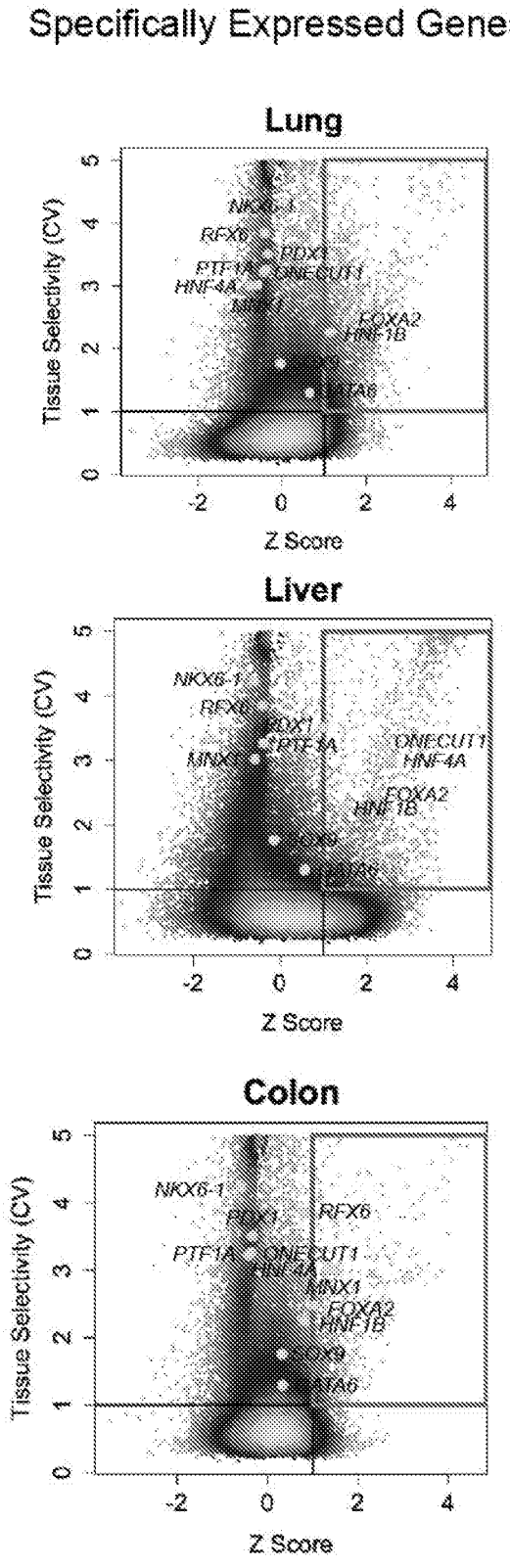

Specifically Expressed Genes
Lung
Tissue Selectivity (CV)
Z Score
NKX6-1
RFX6
PDX1
PTF1A
ONECUT1
HNF4A
MNX1
FOXA2
HNF1B
CDX2
ATA6
Liver
Tissue Selectivity (CV)
Z Score
NKX6-1
RFX6
PDX1
PTF1A
MNX1
ONECUT1
HNF4A
FOXA2
HNF1B
CDX2
ATA6
Colon
Tissue Selectivity (CV)
Z Score
NKX6-1
PDX1
RFX6
PTF1A
ONECUT1
HNF4A
MNX1
FOXA2
HNF1B
CDX2
ATA6

Fig. 5

Z-Scores Correlations

C

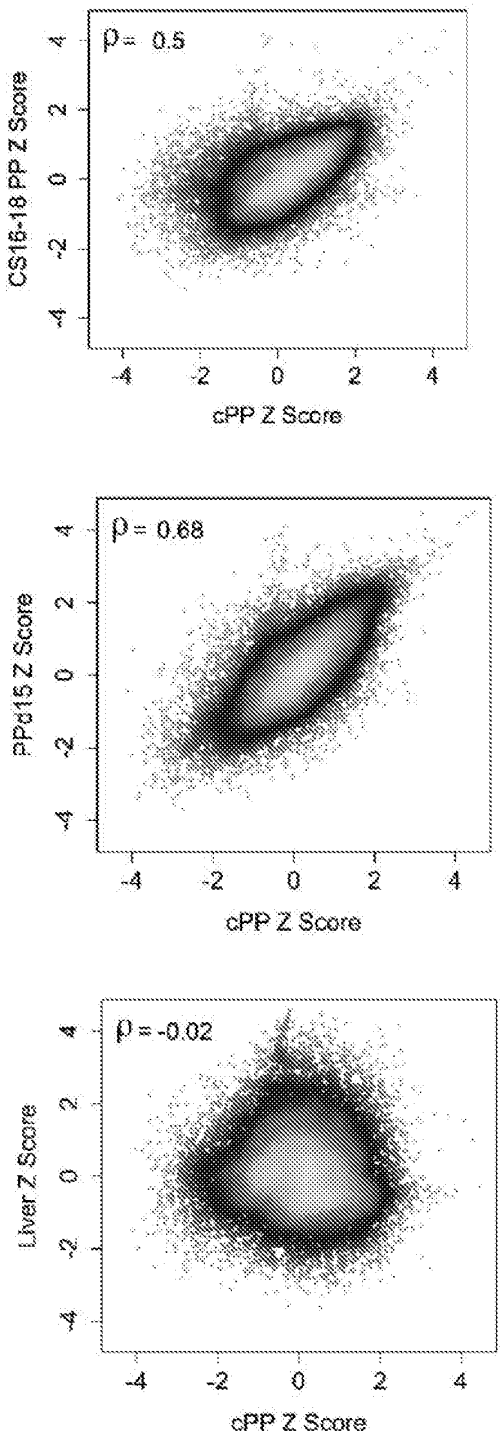


Fig. 6

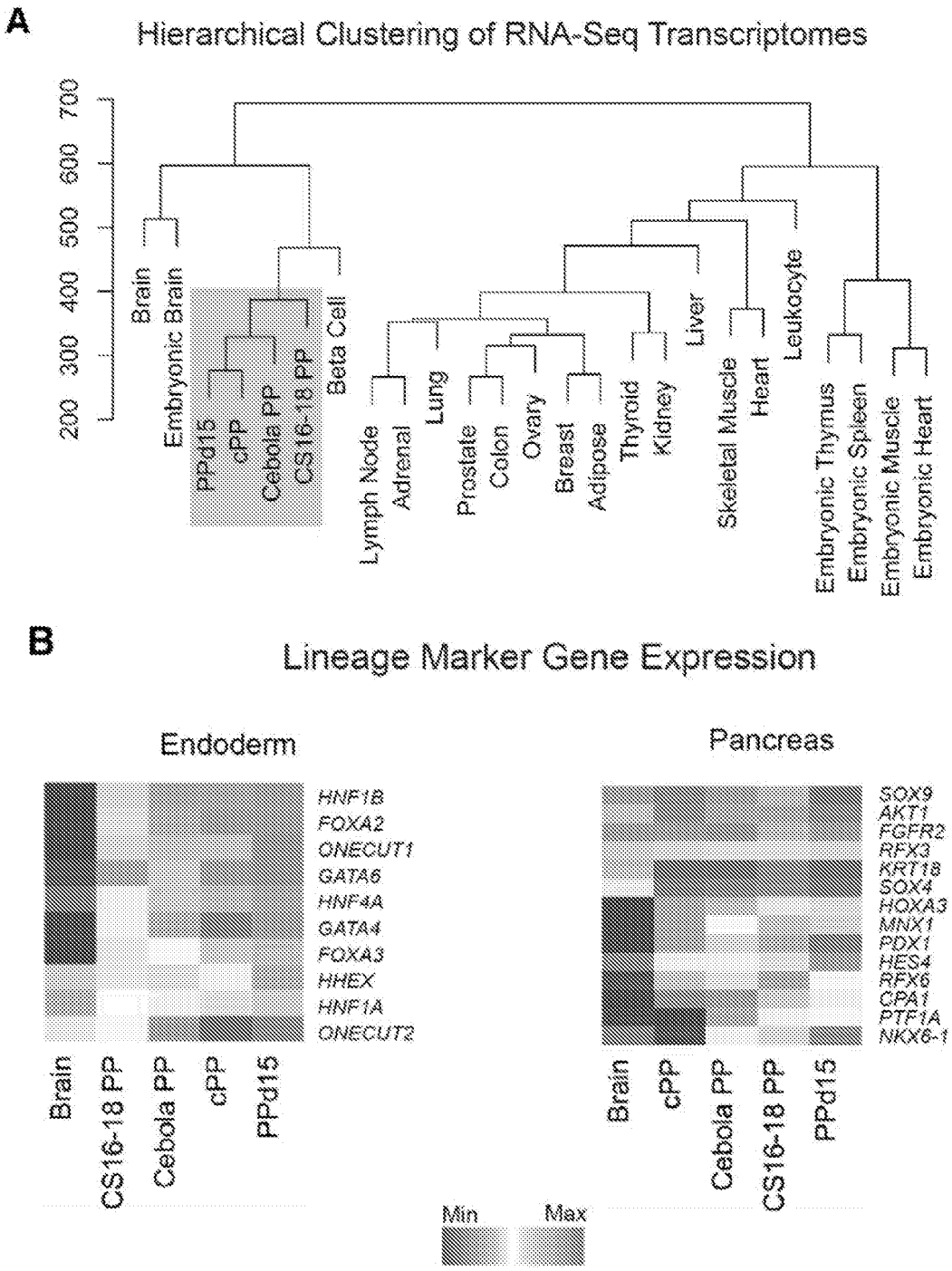

A
Hierarchical Clustering of RNA-Seq Transcriptomes
700
600
500
400
300
200
Brain
Embryonic Brain
PPd15
cPP
Cebola PP
CS16-18 PP
Beta Cell
Lymph Node
Adrenal
Lung
Prostate
Colon
Ovary
Breast
Adipose
Thyroid
Kidney
Liver
Skeletal Muscle
Heart
Leukocyte
Embryonic Thymus
Embryonic Spleen
Embryonic Muscle
Embryonic Heart
B
Lineage Marker Gene Expression
Endoderm
HNF1B
FOXA2
ONECUT1
GATA6
HNF4A
GATA4
FOXA3
HHEX
HNF1A
ONECUT2
Brain
CS16-18 PP
Cebola PP
cPP
PPd15
Pancreas
SOX9
AKT1
FGFR2
RFX3
KRT18
SOX4
HOXA3
MNX1
PDX1
HES4
RFX6
CPA1
PTF1A
NKX6-1
Brain
cPP
Cebola PP
CS16-18 PP
PPd15
Min
Max

Fig. 6

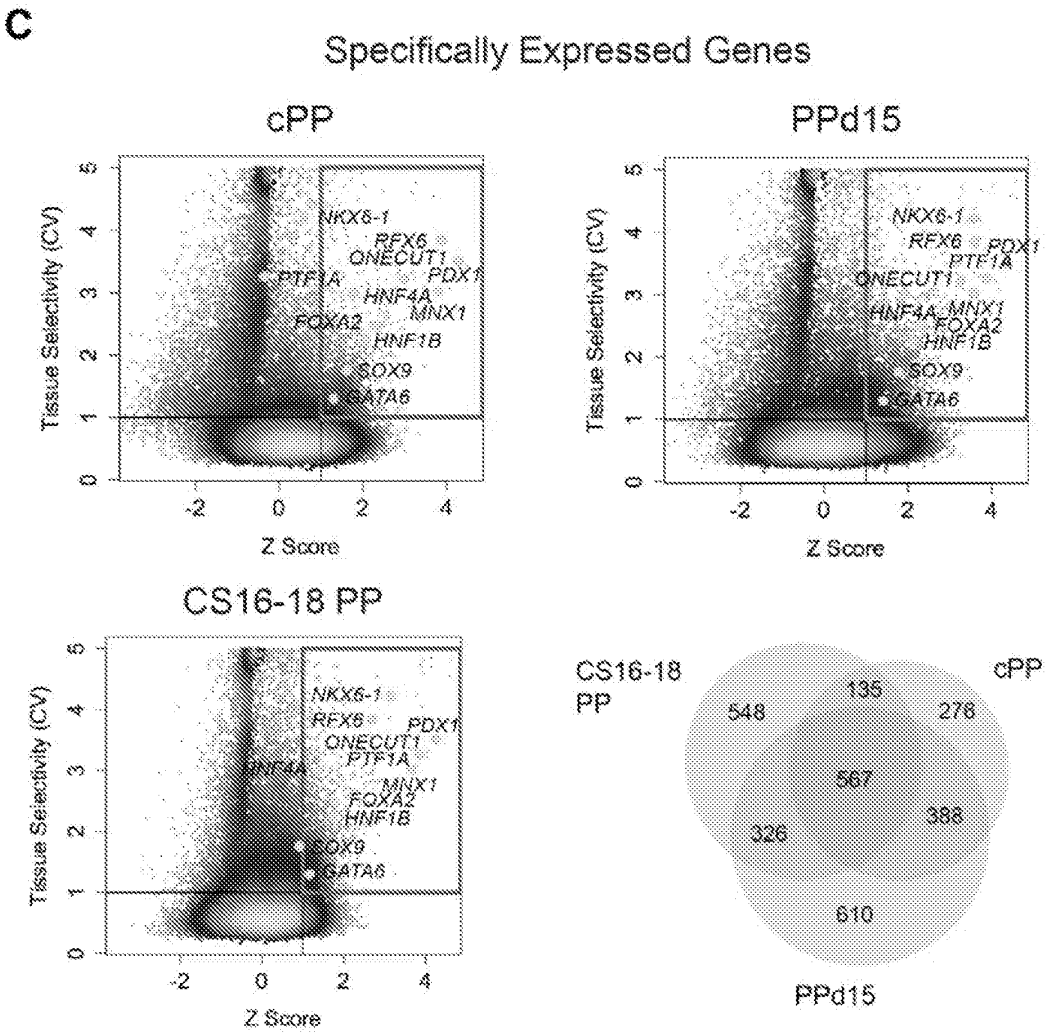

C
Specifically Expressed Genes
cPP
PPd15
CS16-18 PP
Tissue Selectivity (CV)
Z Score
NKX6-1
RFX6
ONECUT1
PTF1A
PDX1
HNF4A
MNX1
FOXA2
HNF1B
SOX9
GATA6
CS16-18 PP
cPP
PPd15
135
548
276
567
388
326
610

Fig. 6

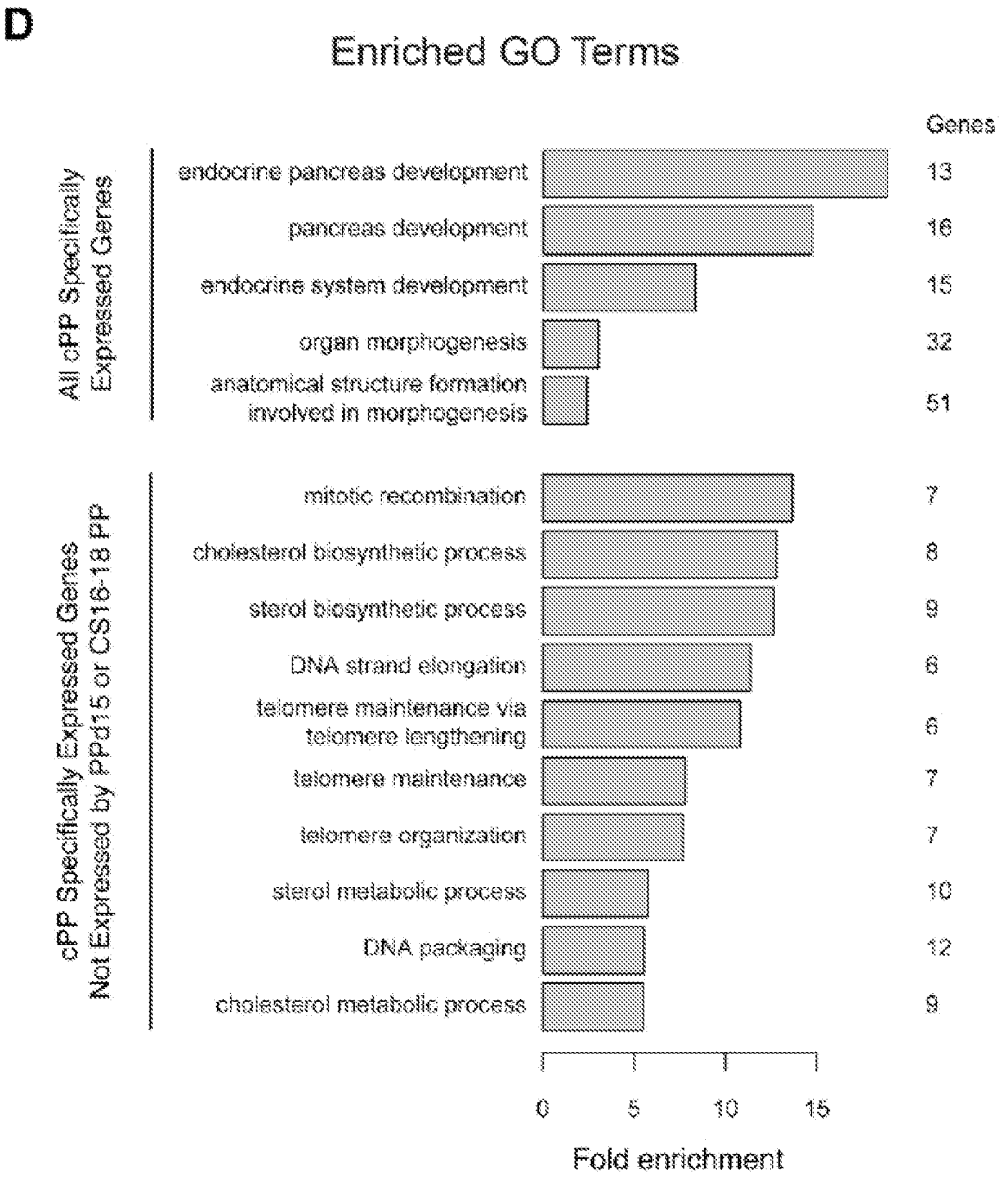

D
Enriched GO Terms
Genes
All cPP Specifically Expressed Genes
endocrine pancreas development
13
pancreas development
16
endocrine system development
15
organ morphogenesis
32
anatomical structure formation involved in morphogenesis
51
cPP Specifically Expressed Genes Not Expressed by PPd15 or CS16-18 PP
mitotic recombination
7
cholesterol biosynthetic process
8
sterol biosynthetic process
9
DNA strand elongation
6
telomere maintenance via telomere lengthening
6
telomere maintenance
7
telomere organization
7
sterol metabolic process
10
DNA packaging
12
cholesterol metabolic process
9
0
5
10
15
Fold enrichment

Fig. 6

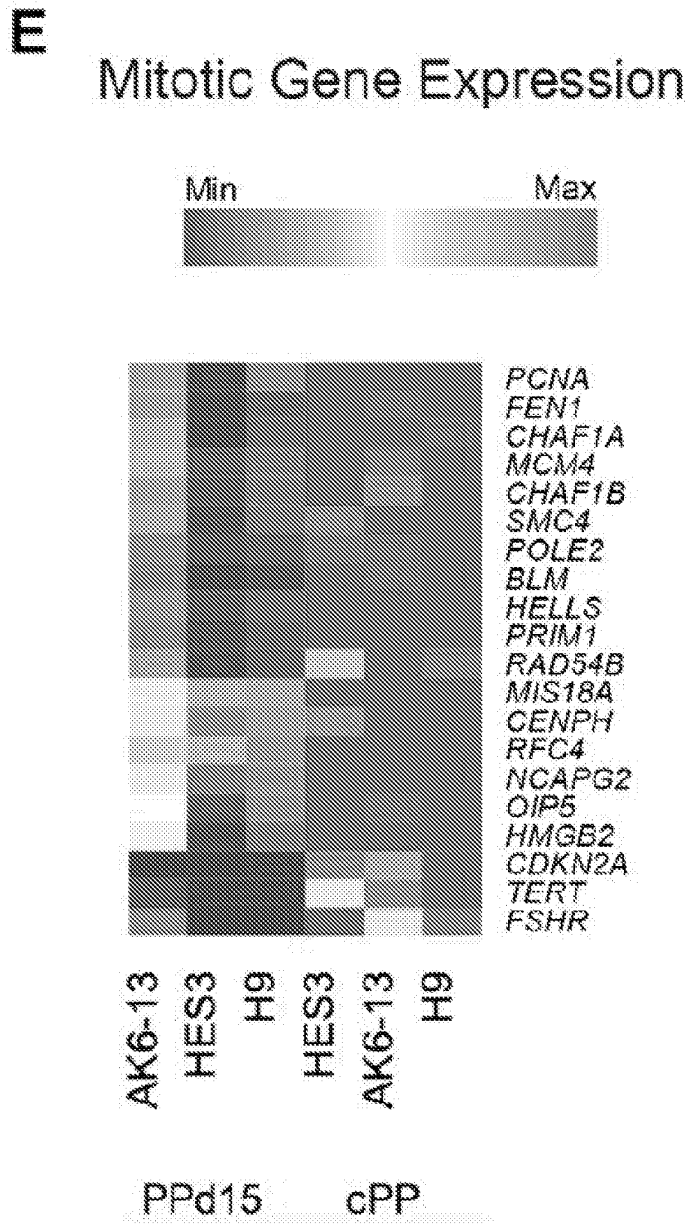

E
Mitotic Gene Expression
Min
Max
PCNA
FEN1
CHAF1A
MCM4
CHAF1B
SMC4
POLE2
BLM
HELLS
PRIM1
RAD54B
MIS18A
CENPH
RFC4
NCAPG2
OIP5
HMGB2
CDKN2A
TERT
FSHR
AK6-13
HES3
H9
HES3
AK6-13
H9
PPd15
cPP

Fig. 6

F

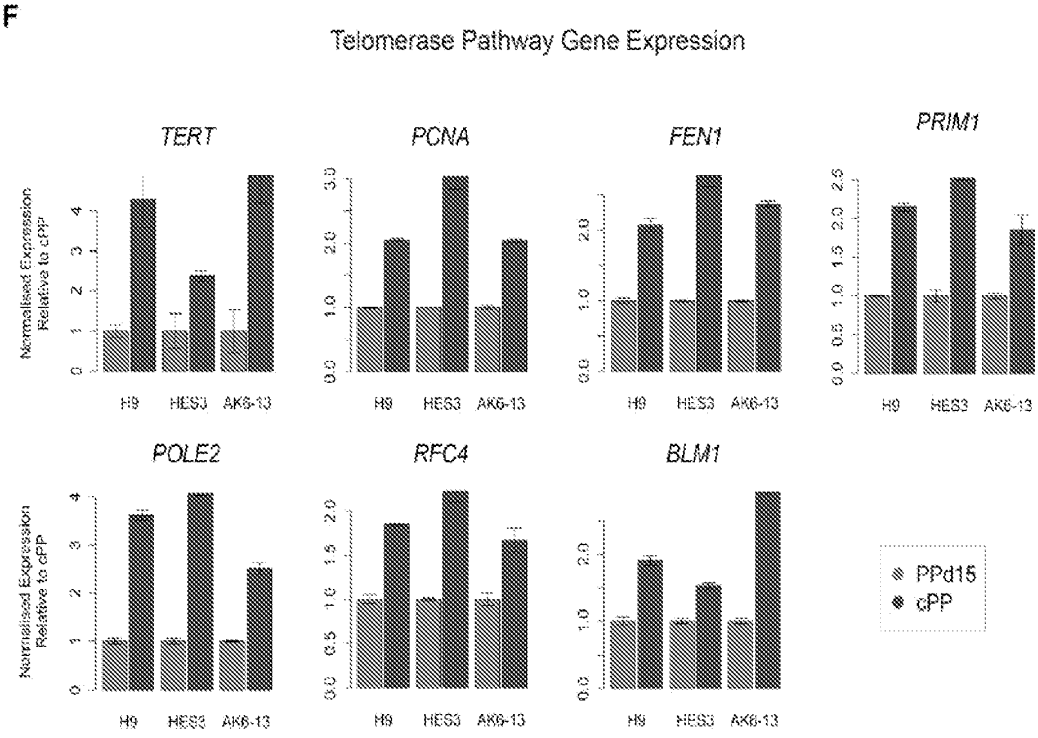

Telomerase Pathway Gene Expression
TERT
PCNA
FEN1
PRIM1
POLE2
RFC4
BLM1
Normalised Expression Relative to cPP
H9
HES3
AK6-13
PPd15
cPP

Fig. 7

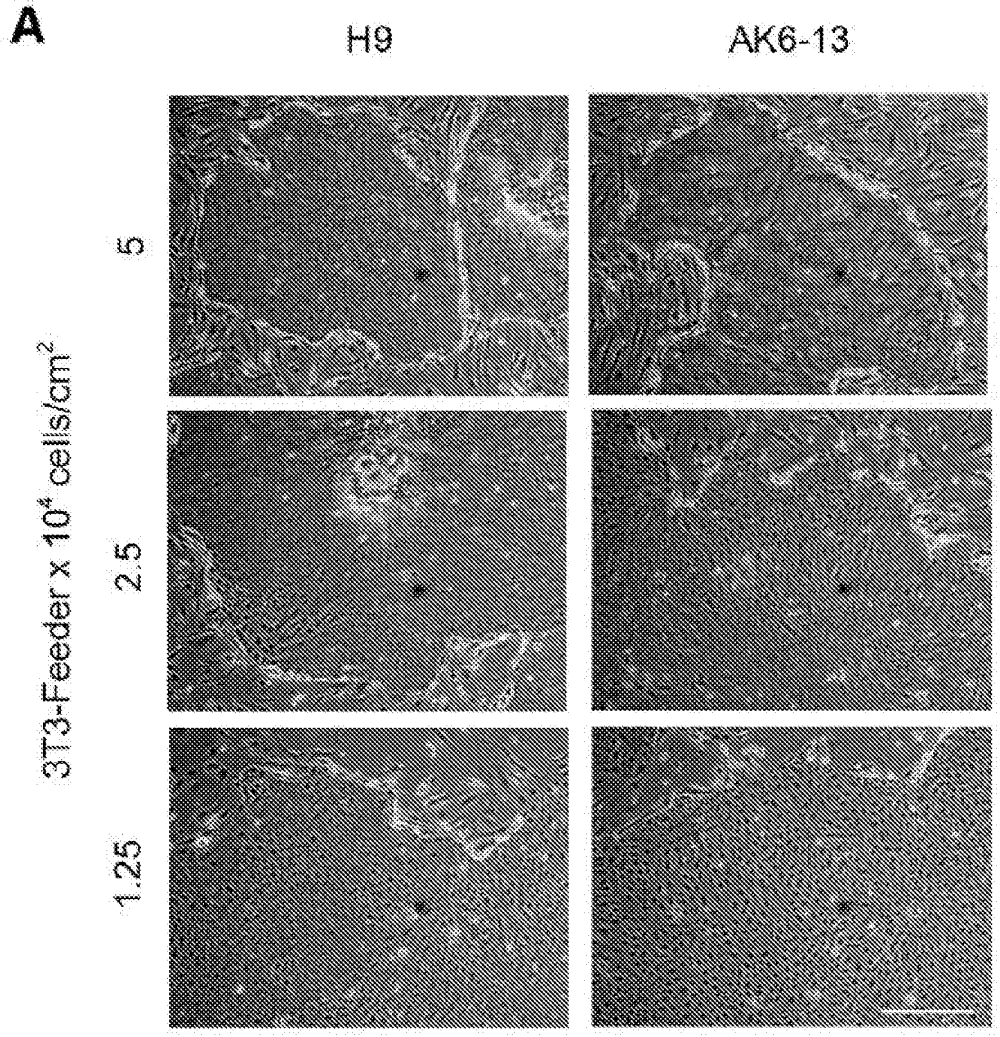


Fig. 7

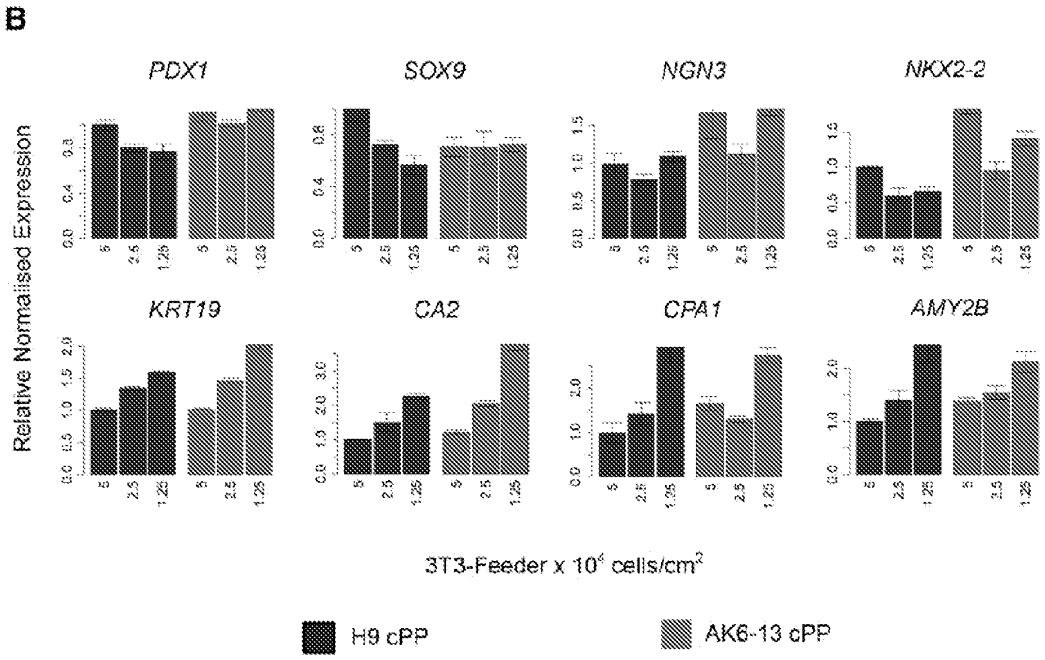
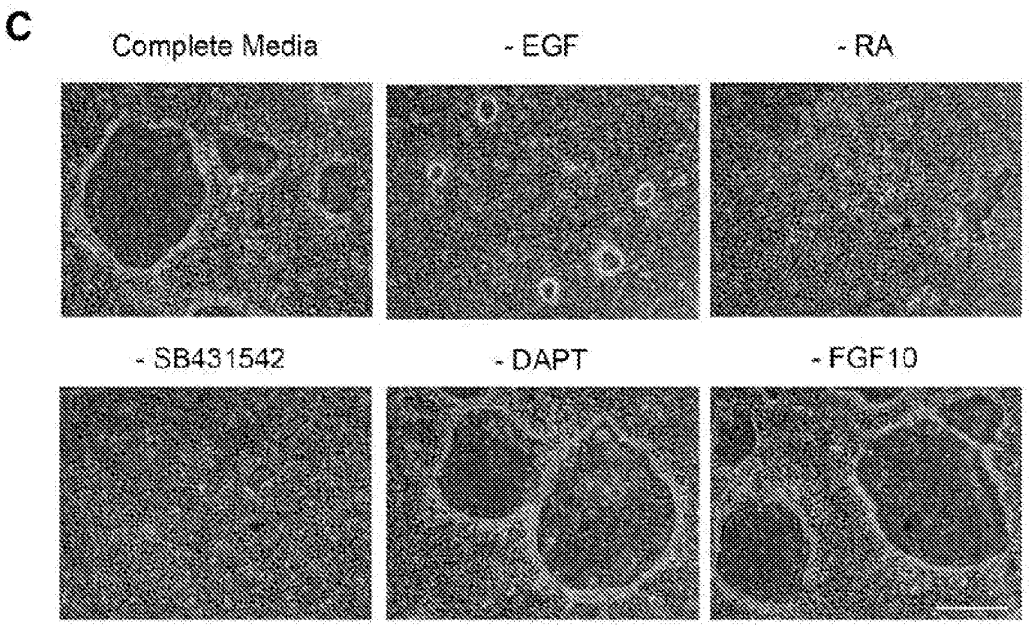

B
PDX1
SOX9
NGN3
NKX2-2
KRT19
CA2
CPA1
AMY2B
Relative Normalised Expression
3T3-Feeder x 10⁴ cells/cm²
H9 cPP
AK6-13 cPP

Fig. 7


C
Complete Media
- EGF
- RA
- SB431542
- DAPT
- FGF10

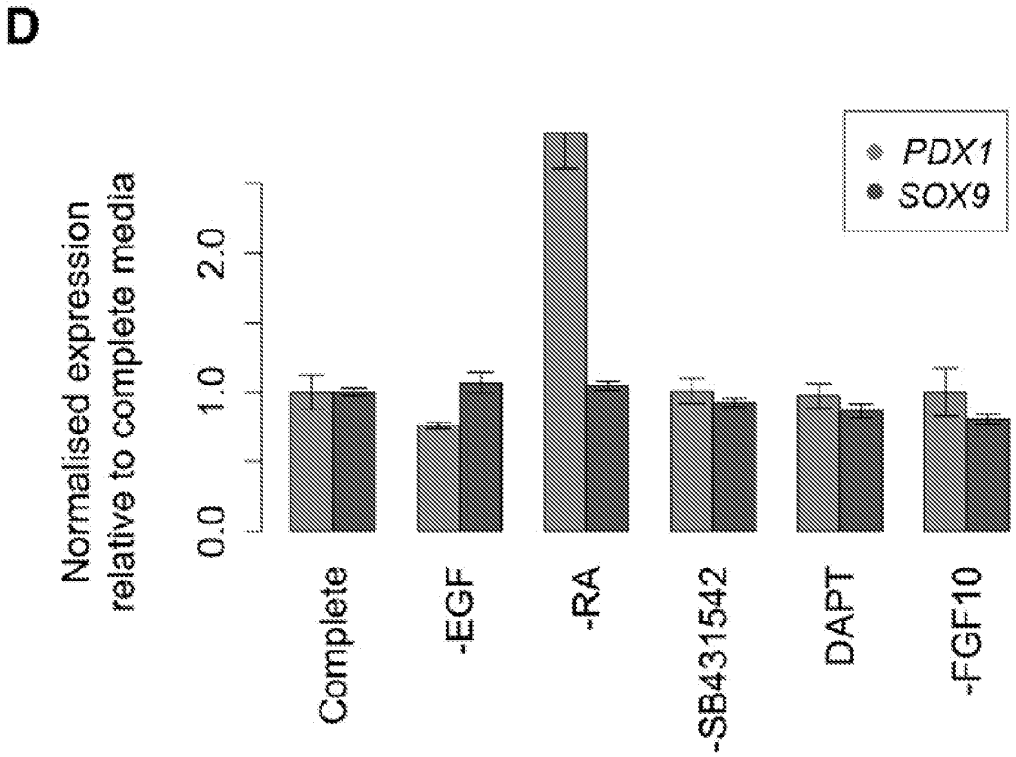

D
Normalised expression relative to complete media
0.0
1.0
2.0
PDX1
SOX9
Complete
-EGF
-RA
-SB431542
DAPT
-FGF10

Fig. 7

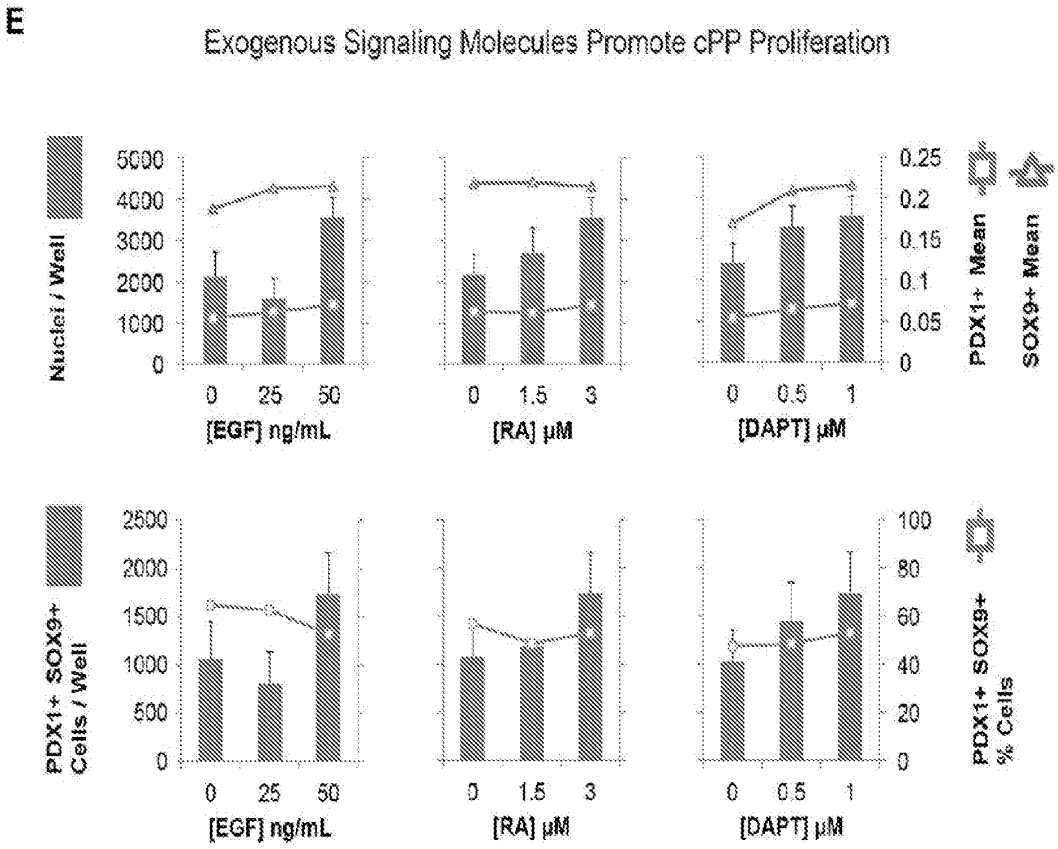

E
Exogenous Signaling Molecules Promote cPP Proliferation
Nuclei / Well
5000
4000
3000
2000
1000
0
0 25 50
[EGF] ng/mL
0 1.5 3
[RA] µM
0 0.5 1
[DAPT] µM
0.25
0.2
0.15
0.1
0.05
0
PDX1+ Mean
SOX9+ Mean
PDX1+ SOX9+ Cells / Well
2500
2000
1500
1000
500
0
PDX1+ SOX9+ % Cells
100
80
60
40
20
0

Fig. 7

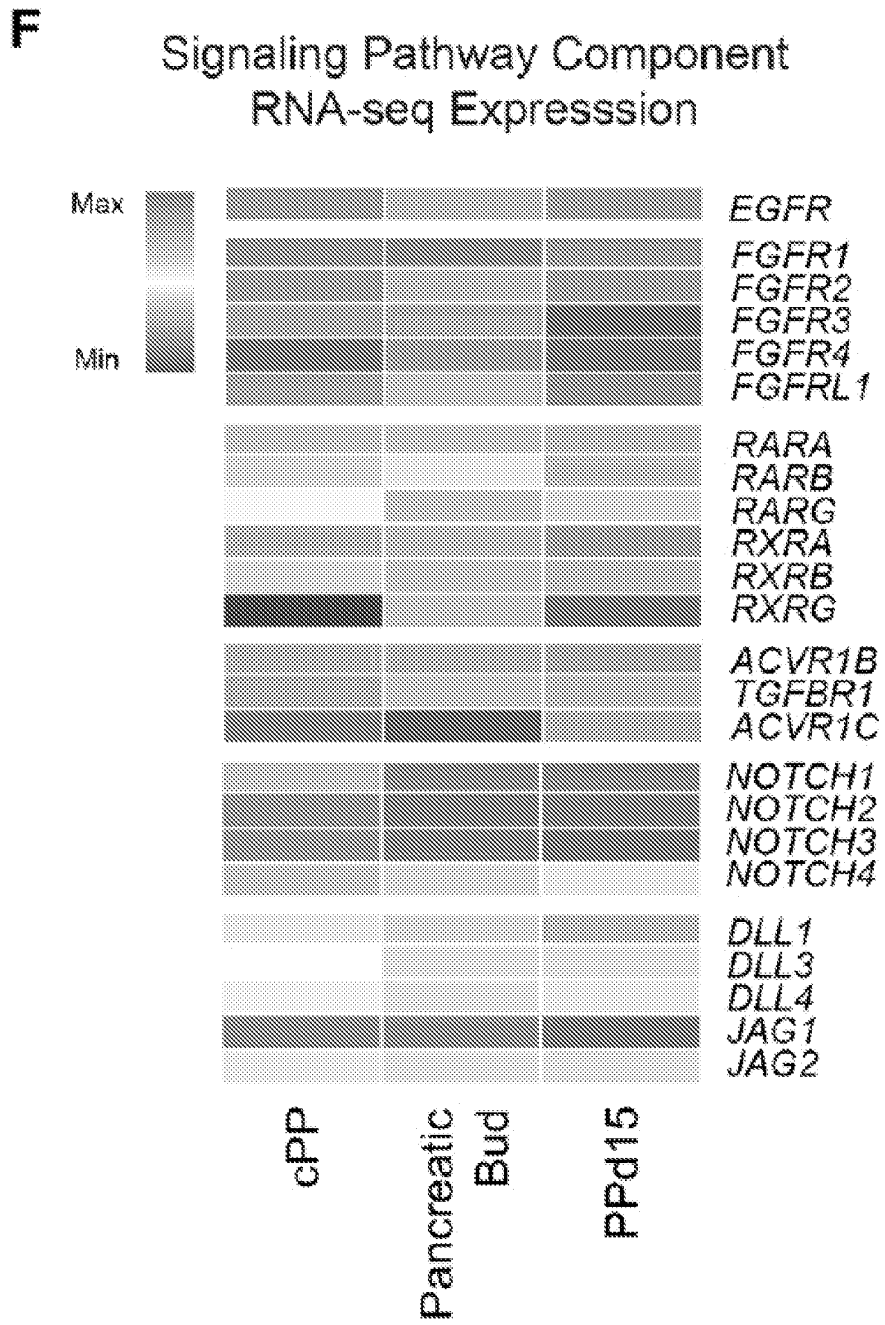

F
Signaling Pathway Component
RNA-seq Expresssion
Max
Min
EGFR
FGFR1
FGFR2
FGFR3
FGFR4
FGFRL1
RARA
RARB
RARG
RXRA
RXRB
RXRG
ACVR1B
TGFBR1
ACVR1C
NOTCH1
NOTCH2
NOTCH3
NOTCH4
DLL1
DLL3
DLL4
JAG1
JAG2
cPP
Pancreatic Bud
PPd15

Fig. 8

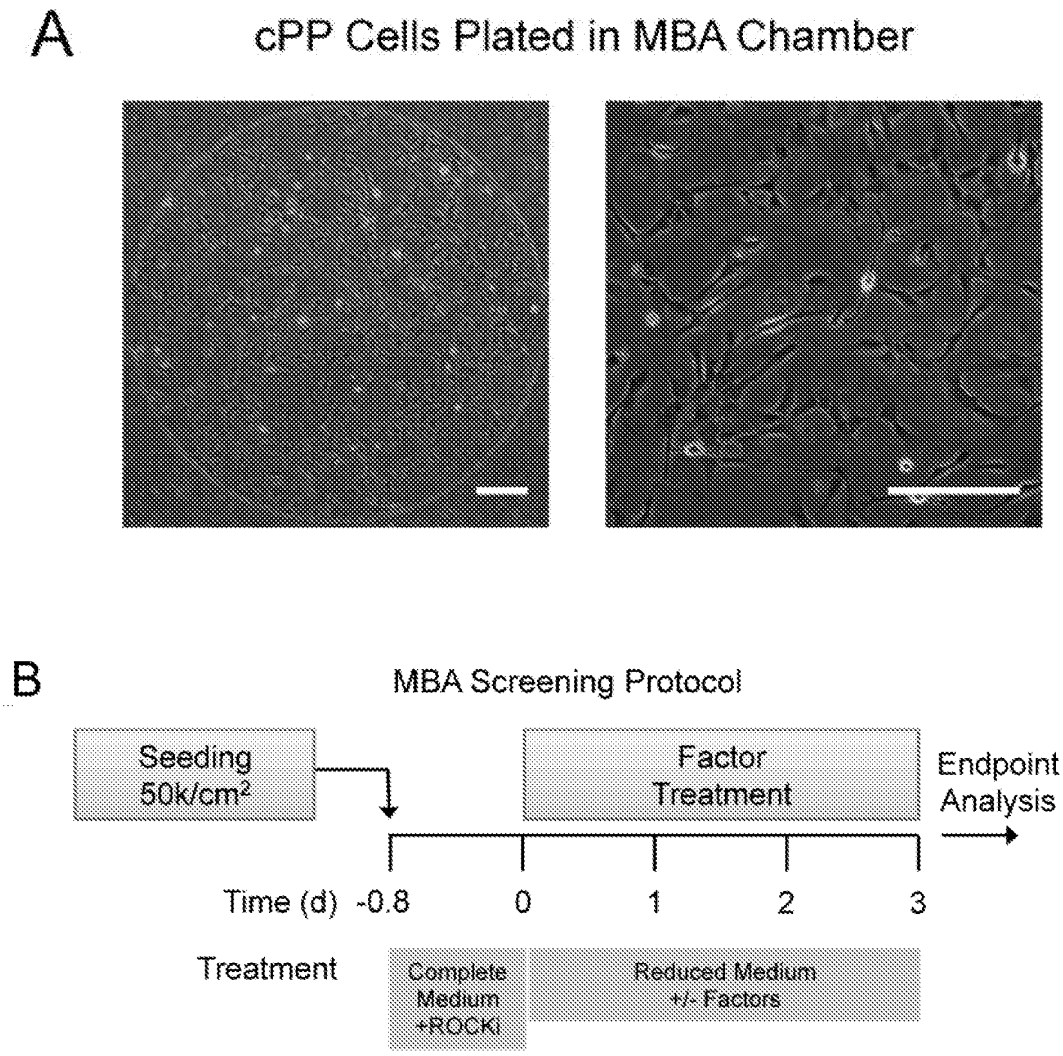

A
cPP Cells Plated in MBA Chamber
B
MBA Screening Protocol
Seeding
50k/cm2
Factor
Treatment
Endpoint
Analysis
Time (d) -0.8 0 1 2 3
Treatment
Complete
Medium
+ROCKi
Reduced Medium
+/- Factors

Fig. 8

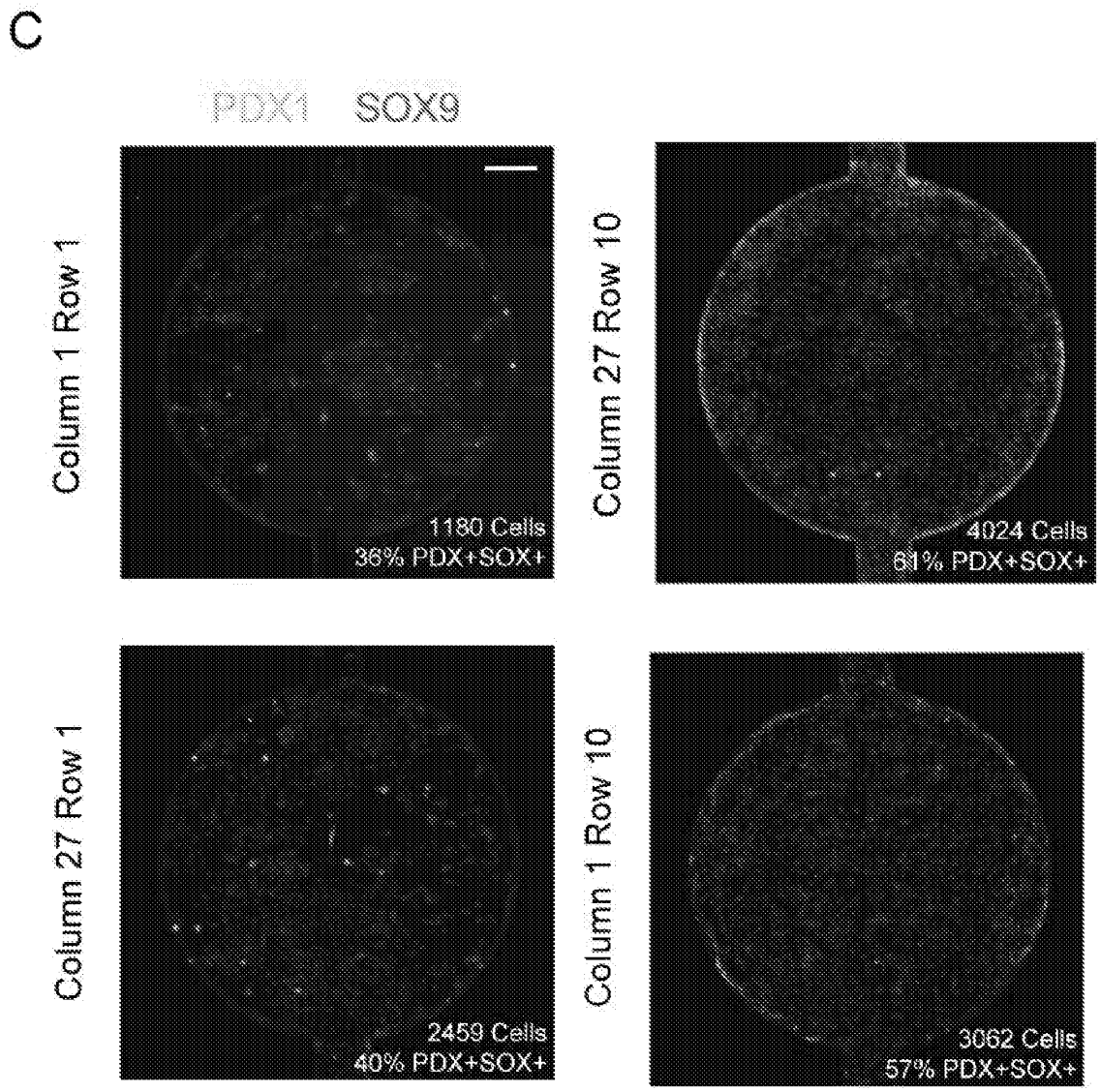

C
PDX1 SOX9
Column 1 Row 1
1180 Cells
36% PDX+SOX+
Column 27 Row 10
4024 Cells
81% PDX+SOX+
Column 27 Row 1
2459 Cells
40% PDX+SOX+
Column 1 Row 10
3062 Cells
57% PDX+SOX+

Fig. 8

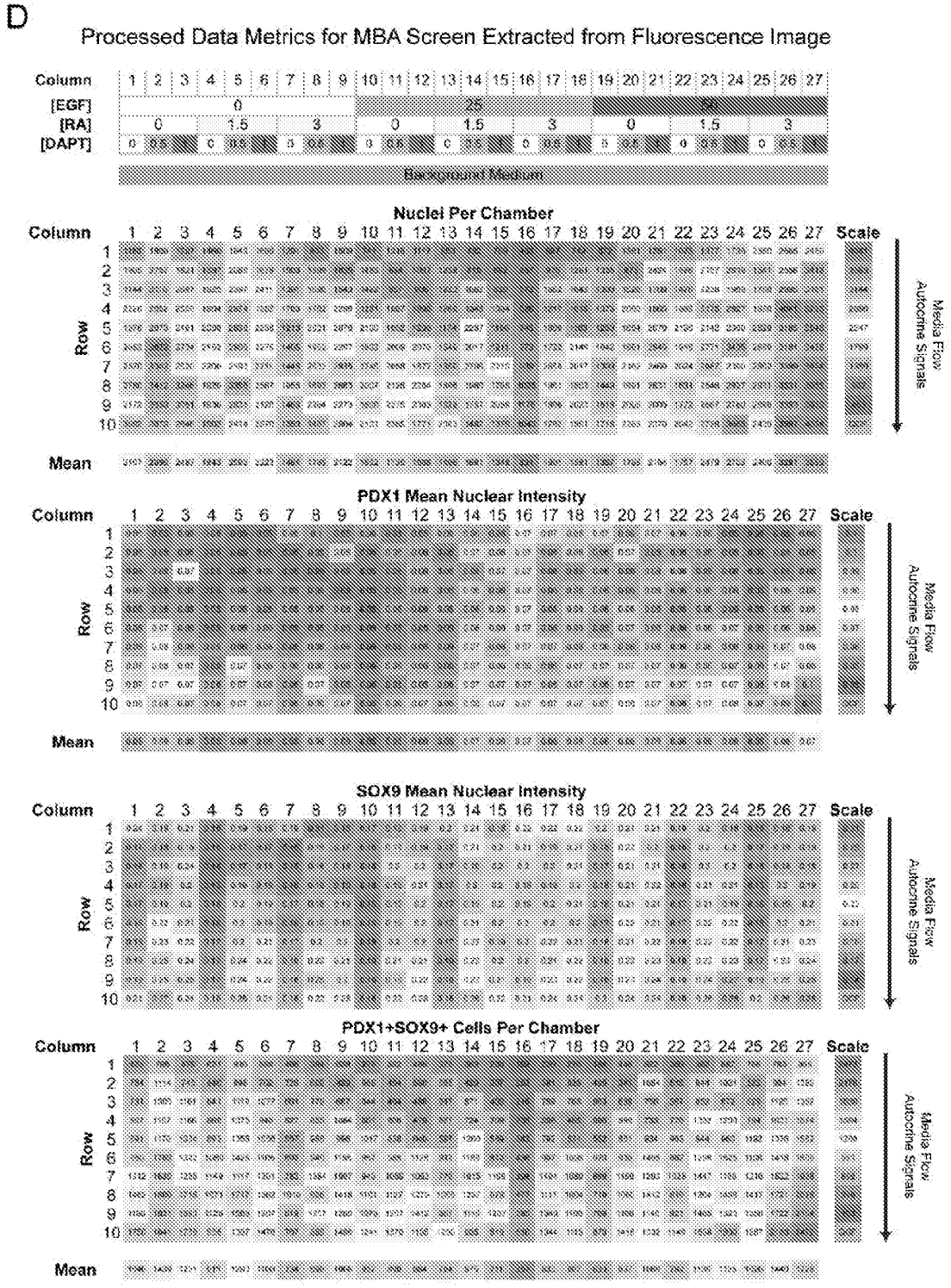

D
Processed Data Metrics for MBA Screen Extracted from Fluorescence Image
Column
[EGF]
[RA]
[DAPT]
Background Medium
Nuclei Per Chamber
PDX1 Mean Nuclear Intensity
SOX9 Mean Nuclear Intensity
PDX1+SOX9+ Cells Per Chamber
Row
Mean
Scale
Media Flow
Autocrine Signals

Fig. 9

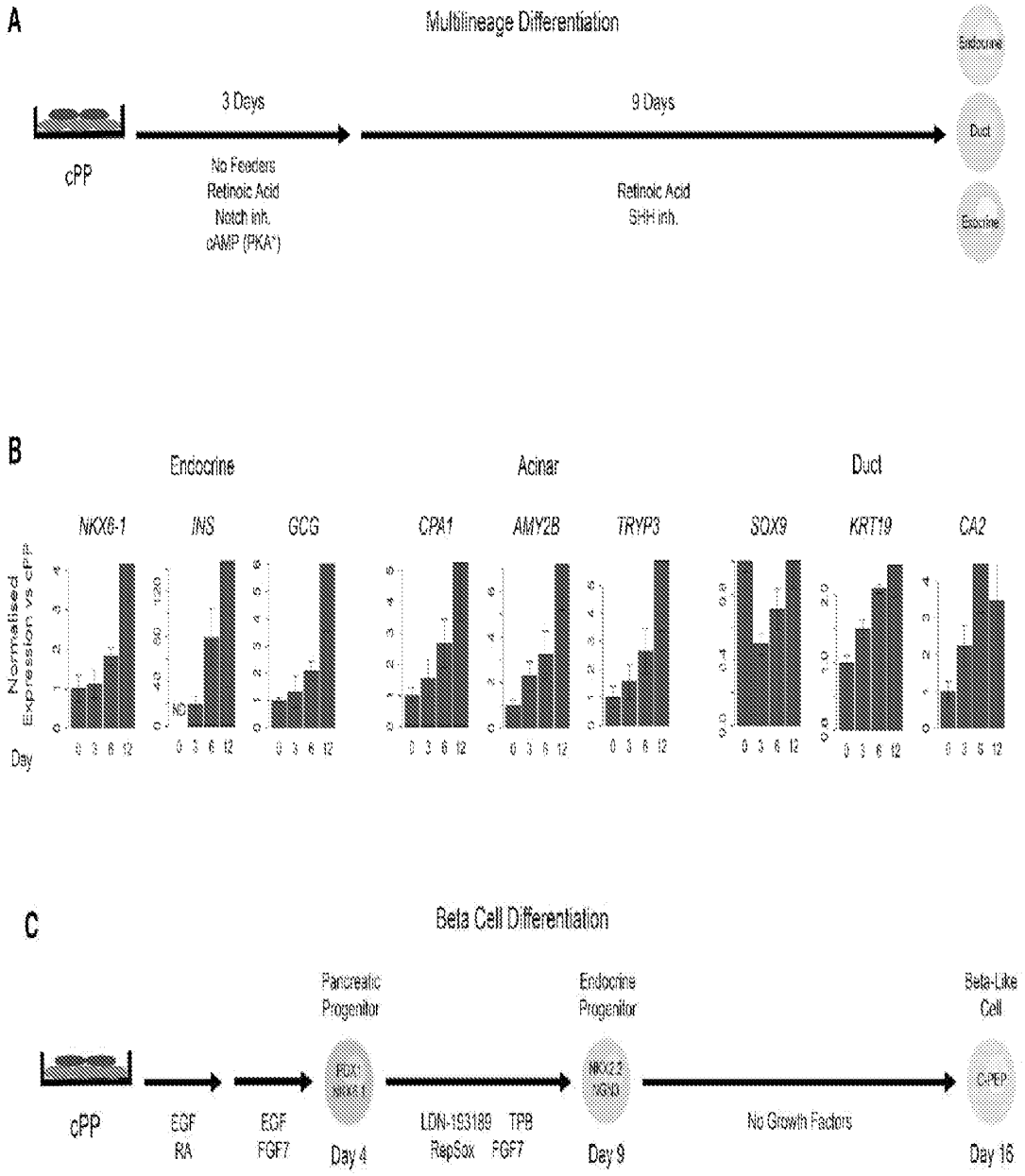

A
Multilineage Differentiation
3 Days
9 Days
cPP
No Feeders
Retinoic Acid
Notch inh.
cAMP (PKA*)
Retinoic Acid
SHH inh.
Endocrine
Duct
Endocrine
B
Endocrine
Acinar
Duct
NKX6-1
INS
GCG
CPA1
AMY2B
TRYP3
SOX9
KRT19
CA2
Normalised Expression vs cPP
Day
C
Beta Cell Differentiation
Pancreatic Progenitor
Endocrine Progenitor
Beta-Like Cell
cPP
EGF
RA
EGF
FGF7
Day 4
LDN-193189
RepSox
TPB
FGF7
Day 9
No Growth Factors
C-PEP
Day 16

Fig. 9

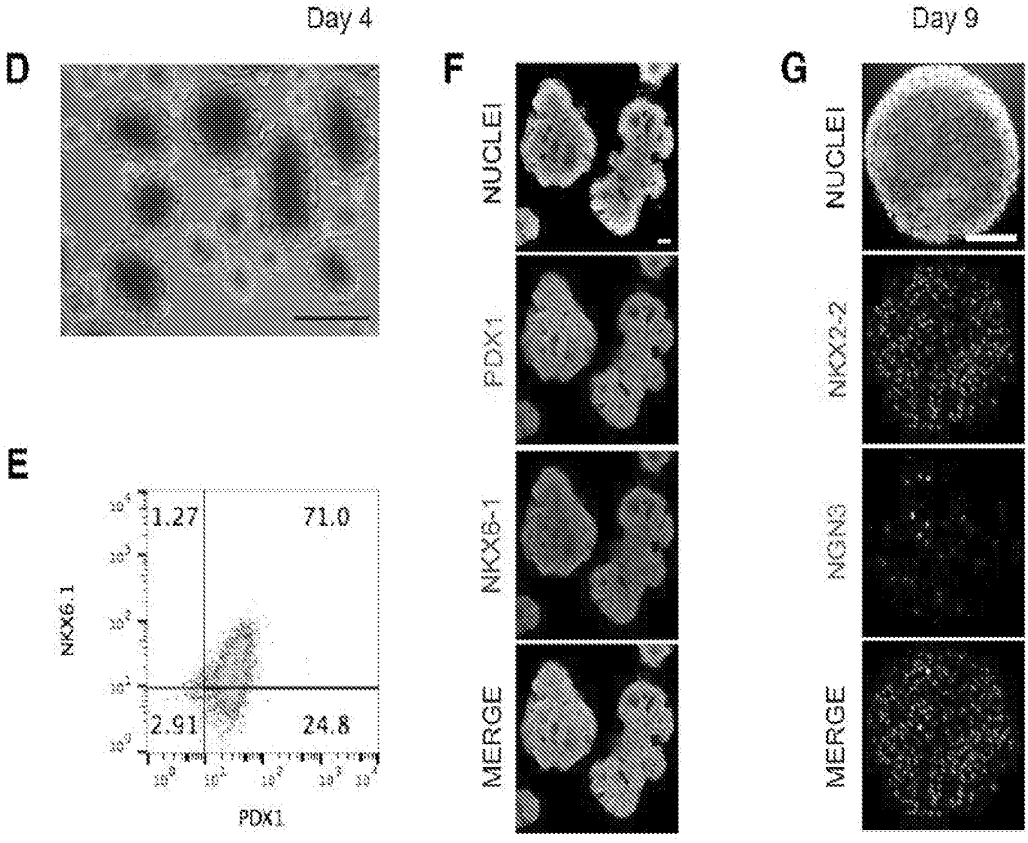

Day 4
D
E
1.27
71.0
2.91
24.8
NKX6.1
PDX1
F
NUCLEI
PDX1
NKX6-1
MERGE
Day 9
G
NUCLEI
NKX2-2
MGN3
MERGE

Fig. 9

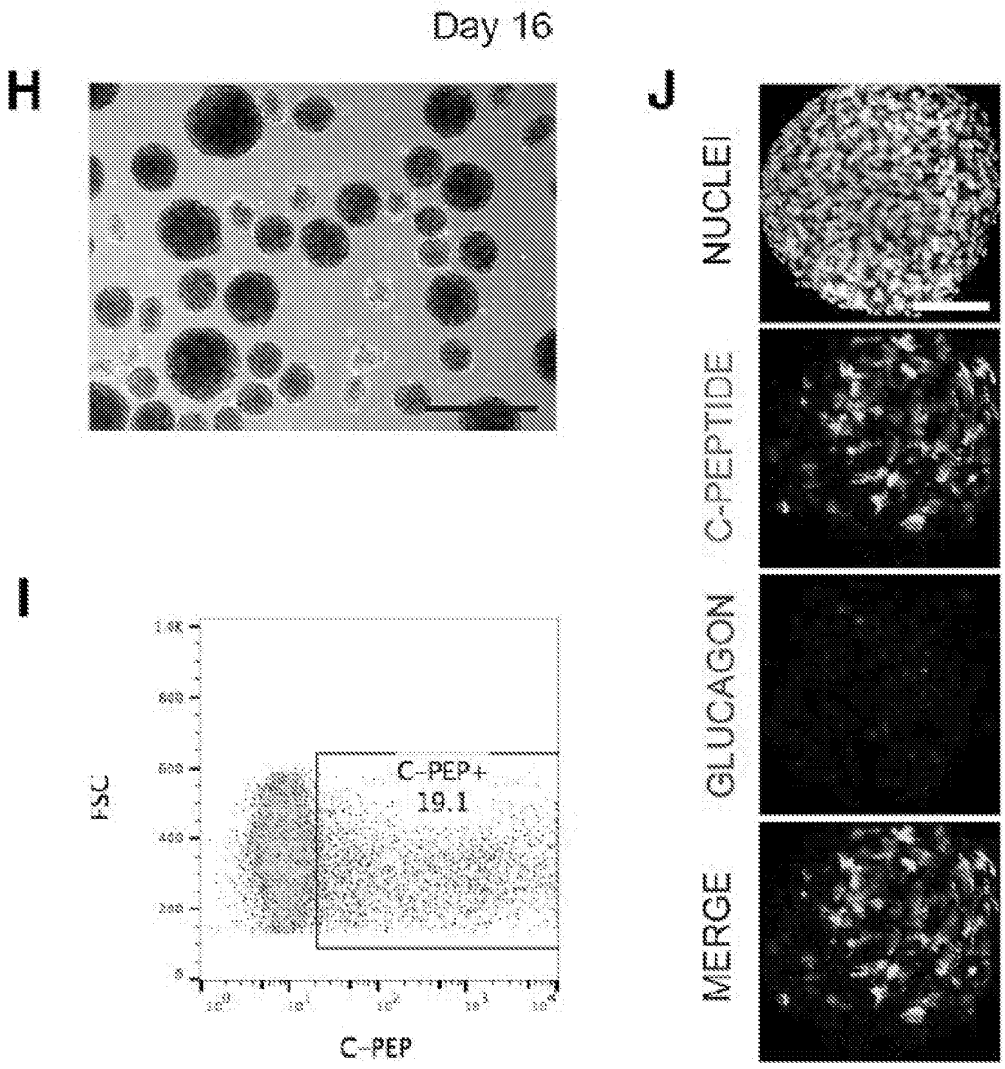


Fig. 9

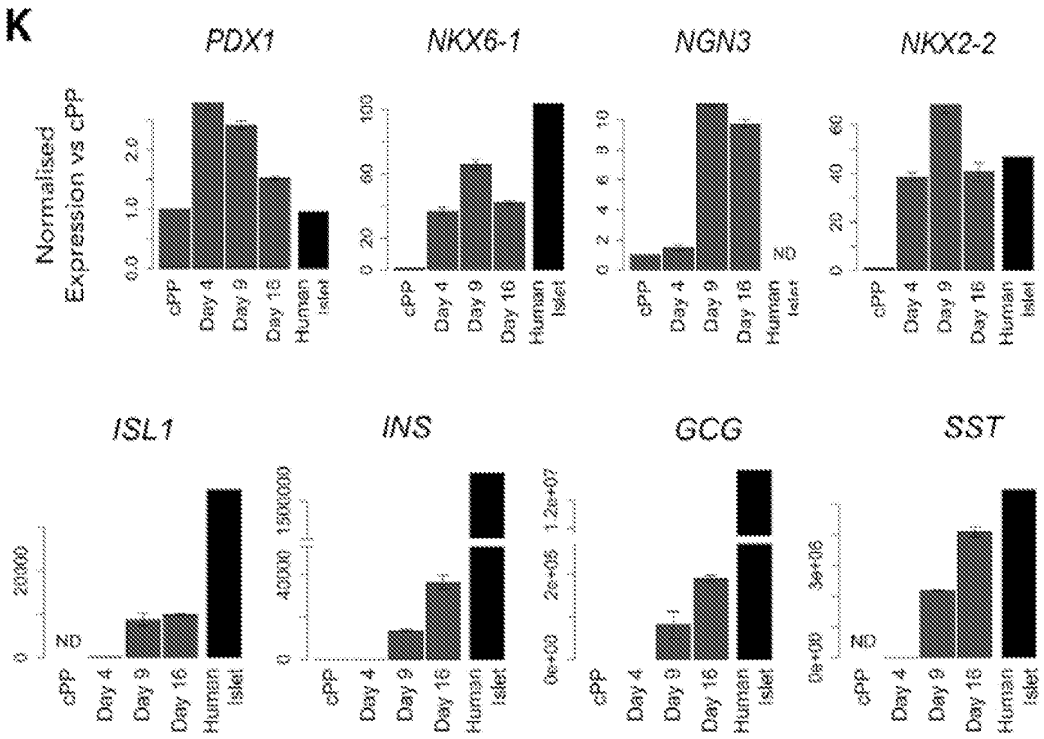

K
PDX1
NKX6-1
NGN3
NKX2-2
Normalised Expression vs cPP
ISL1
INS
GCG
SST
ND
cPP
Day 4
Day 9
Day 16
Human Islet

Fig. 9

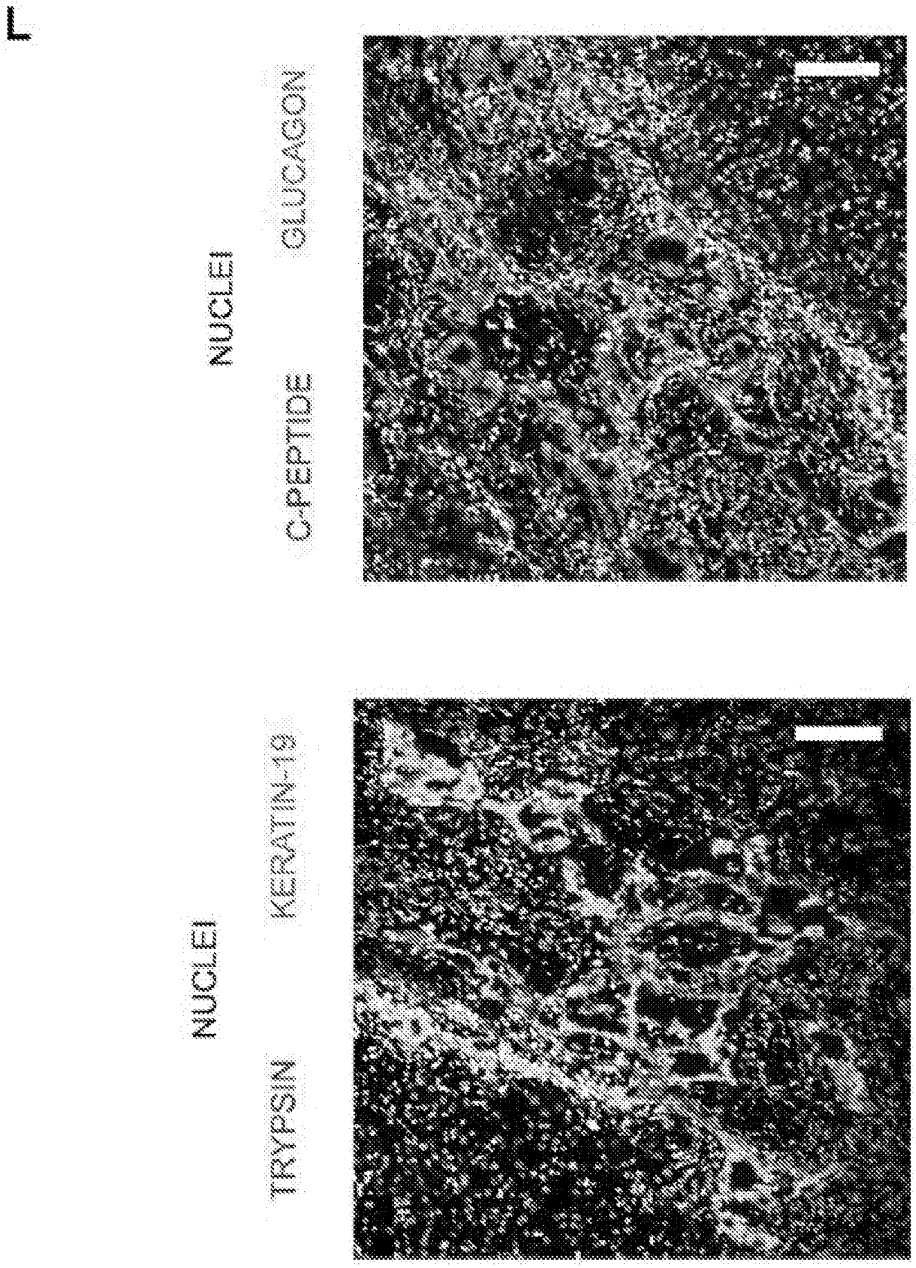


Fig. 10

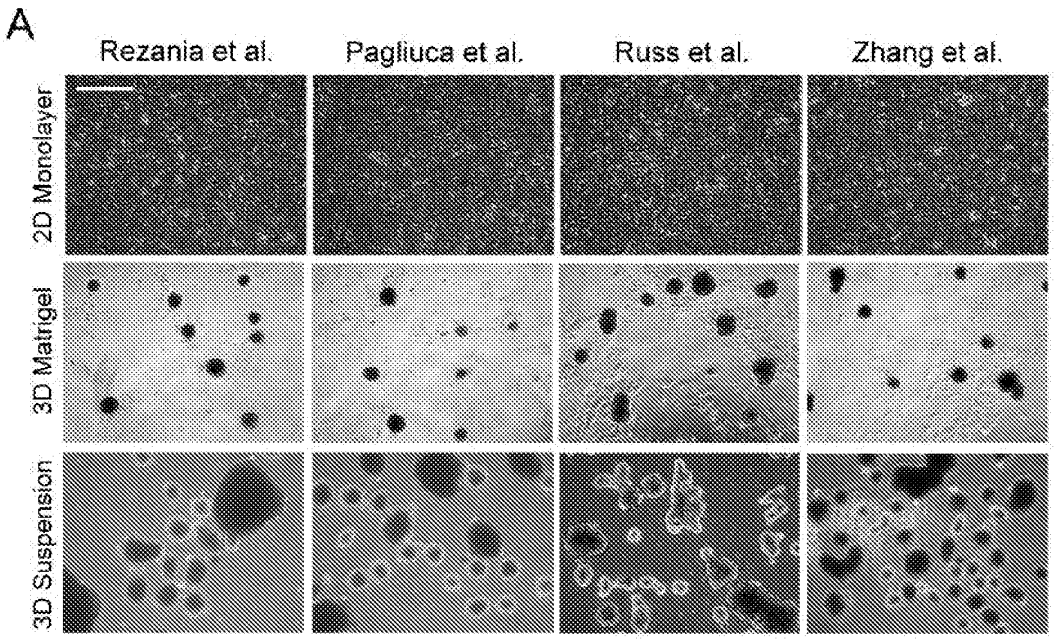

A
Rezania et al.
Pagliuca et al.
Russ et al.
Zhang et al.
2D Monolayer
3D Matrigel
3D Suspension

Fig. 10

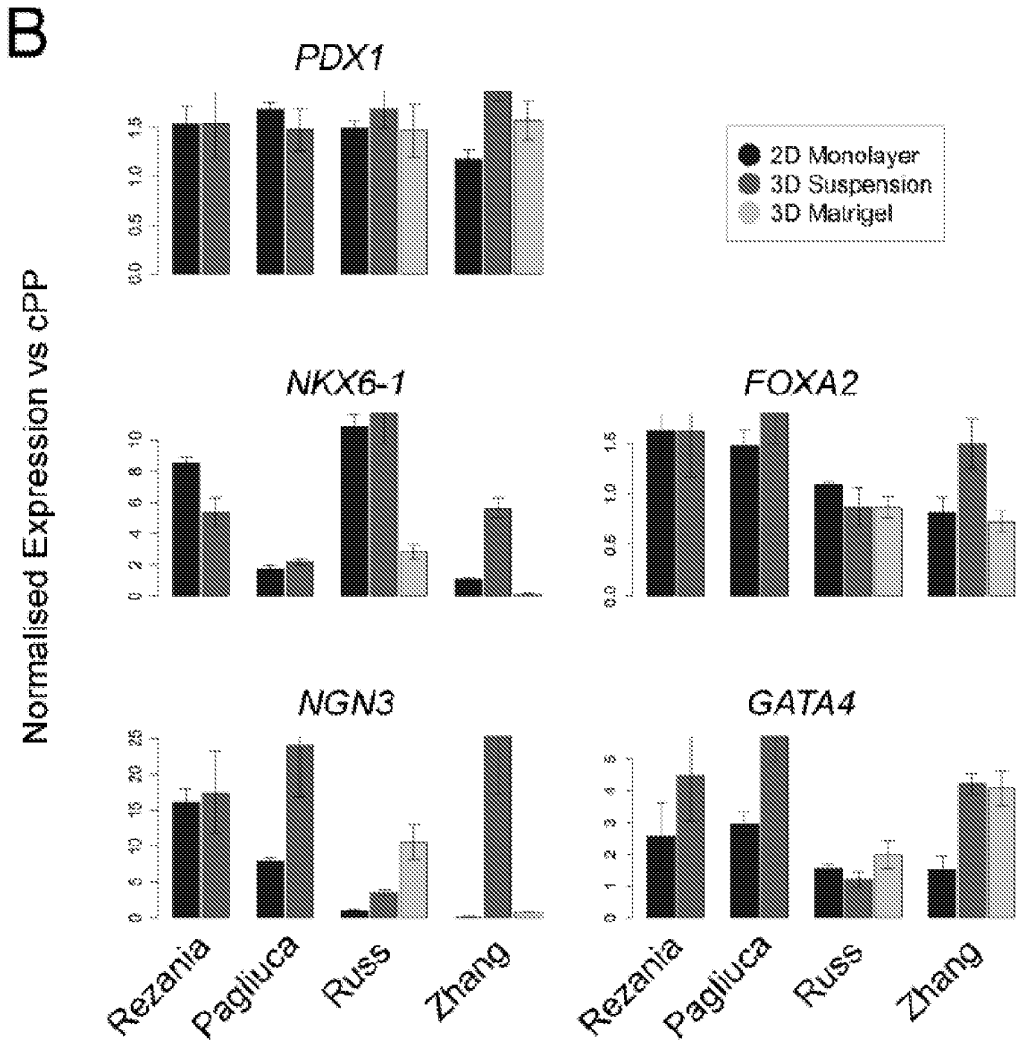

B
PDX1
NKX6-1
FOXA2
NGN3
GATA4
2D Monolayer
3D Suspension
3D Matrigel
Normalised Expression vs cPP
Rezania
Pagliuca
Russ
Zhang

Fig. 10

C

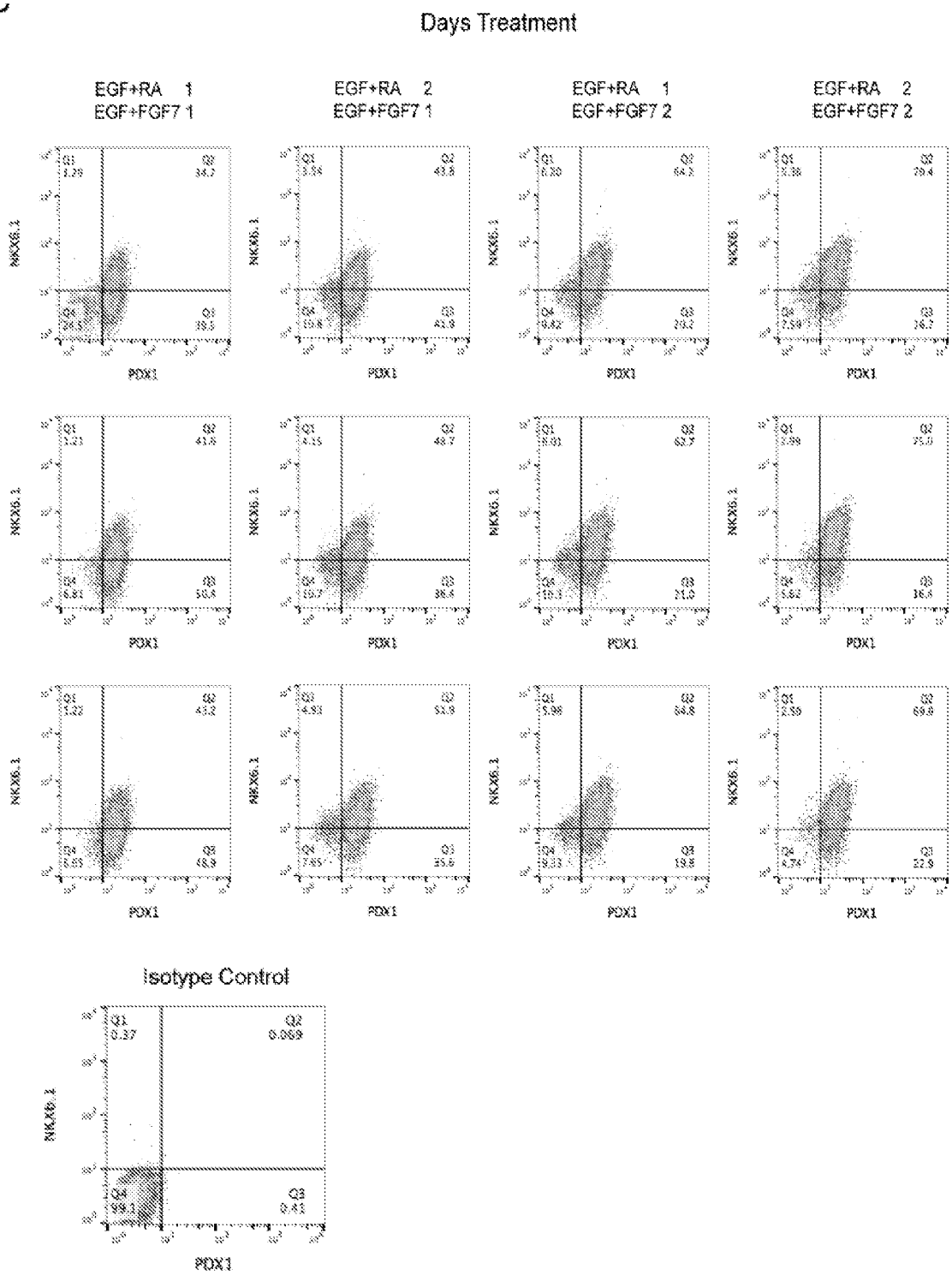
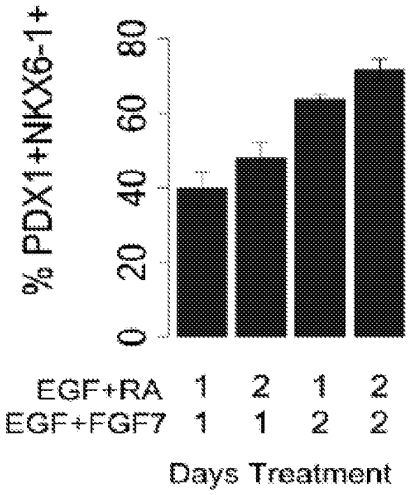

Days Treatment
EGF+RA 1
EGF+FGF7 1
EGF+RA 2
EGF+FGF7 1
EGF+RA 1
EGF+FGF7 2
EGF+RA 2
EGF+FGF7 2
NKX6.1
PDX1
Isotype Control
Q1 0.37
Q2 0.069
Q4 99.1
Q3 0.41

Fig. 10

D


% PDX1+NKX6-1+
0
20
40
60
80
EGF+RA 1 2 1 2
EGF+FGF7 1 1 2 2
Days Treatment