Figure 1

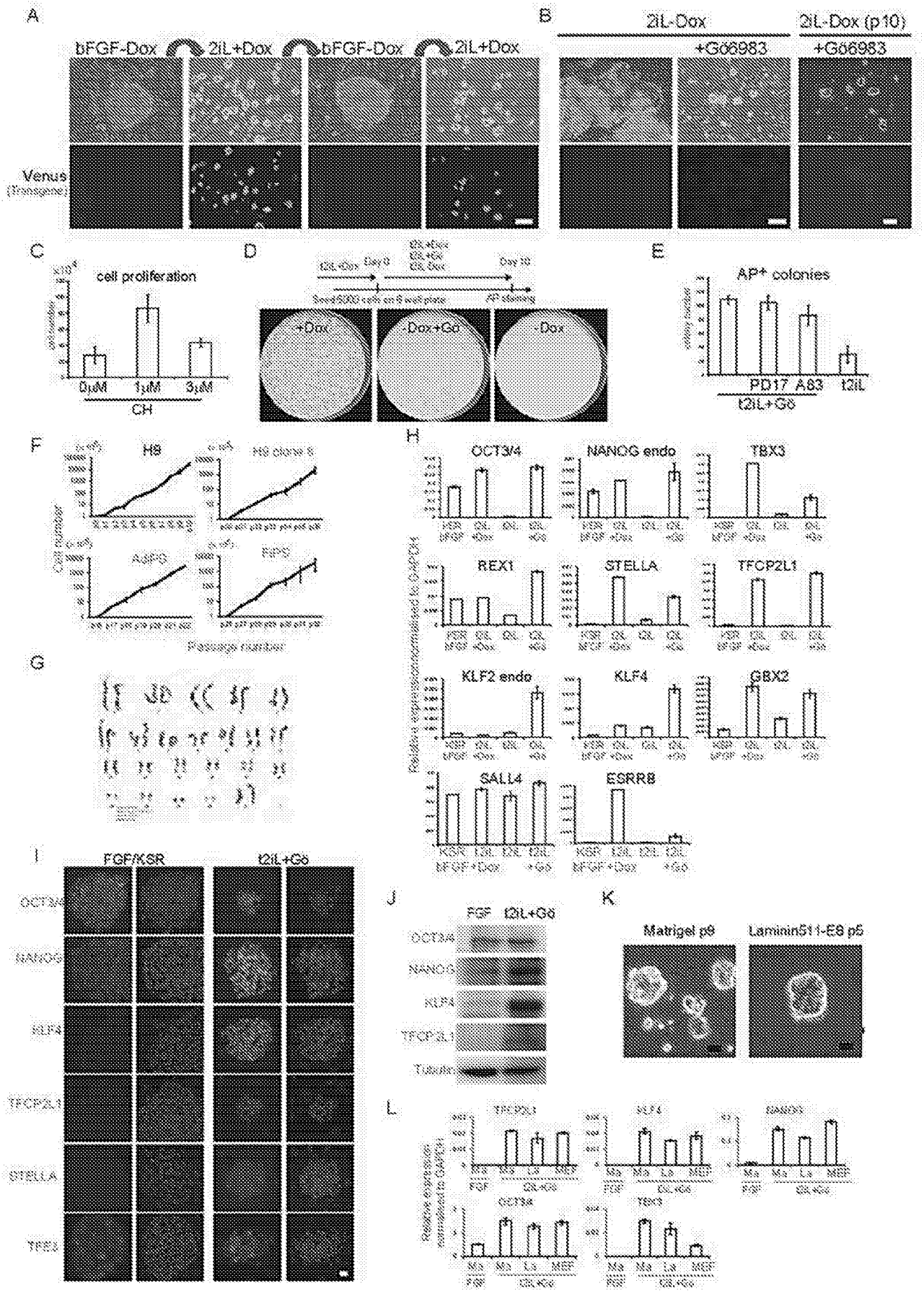

A
bFGF-Dox
2iL+Dox
bFGF-Dox
2iL+Dox
Venus
(Transgene)
B
2iL-Dox
2iL-Dox (p10)
+Gö6983
+Gö6983
C
cell proliferation
0μM
1μM
3μM
CH
D
Day 0
Day 10
AP staining
+Dox
-Dox+Gö
-Dox
E
AP+ colonies
PD17
A83
t2iL
t2iL+Gö
F
H9
H9 clone 6
AdPS
FiPS
Cell number
Passage number
G
H
OCT3/4
NANOG endo
TBX3
REX1
STELLA
TFCP2L1
KLF2 endo
KLF4
GBX2
SALL4
ESRRB
Relative expression normalized to GAPDH
KSR
bFGF+Dox
t2iL
+Gö
I
FGF/KSR
t2iL+Gö
OCT3/4
NANOG
KLF4
TFCP2L1
STELLA
TFEJ
J
FGF
t2iL+Gö
OCT3/4
NANOG
KLF4
TFCP2L1
Tubulin
K
Matrigel p9
Laminin511-E8 p5
L
TFCP2L1
KLF4
GBX3
OCT3/4
Tbx3
Relative expression normalized to GAPDH
Ma
La
MEF
FGF
t2iL+Gö

Figure 2

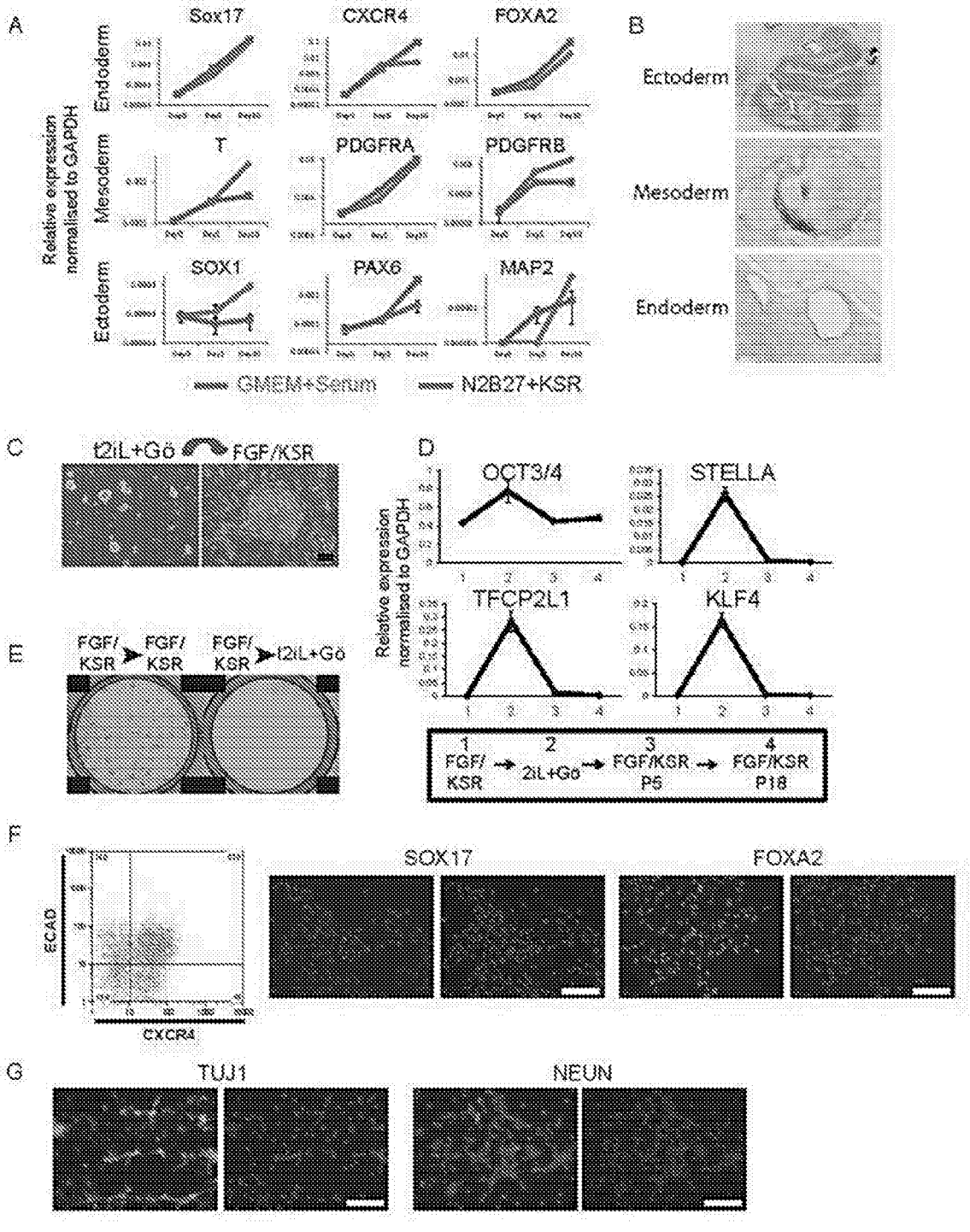
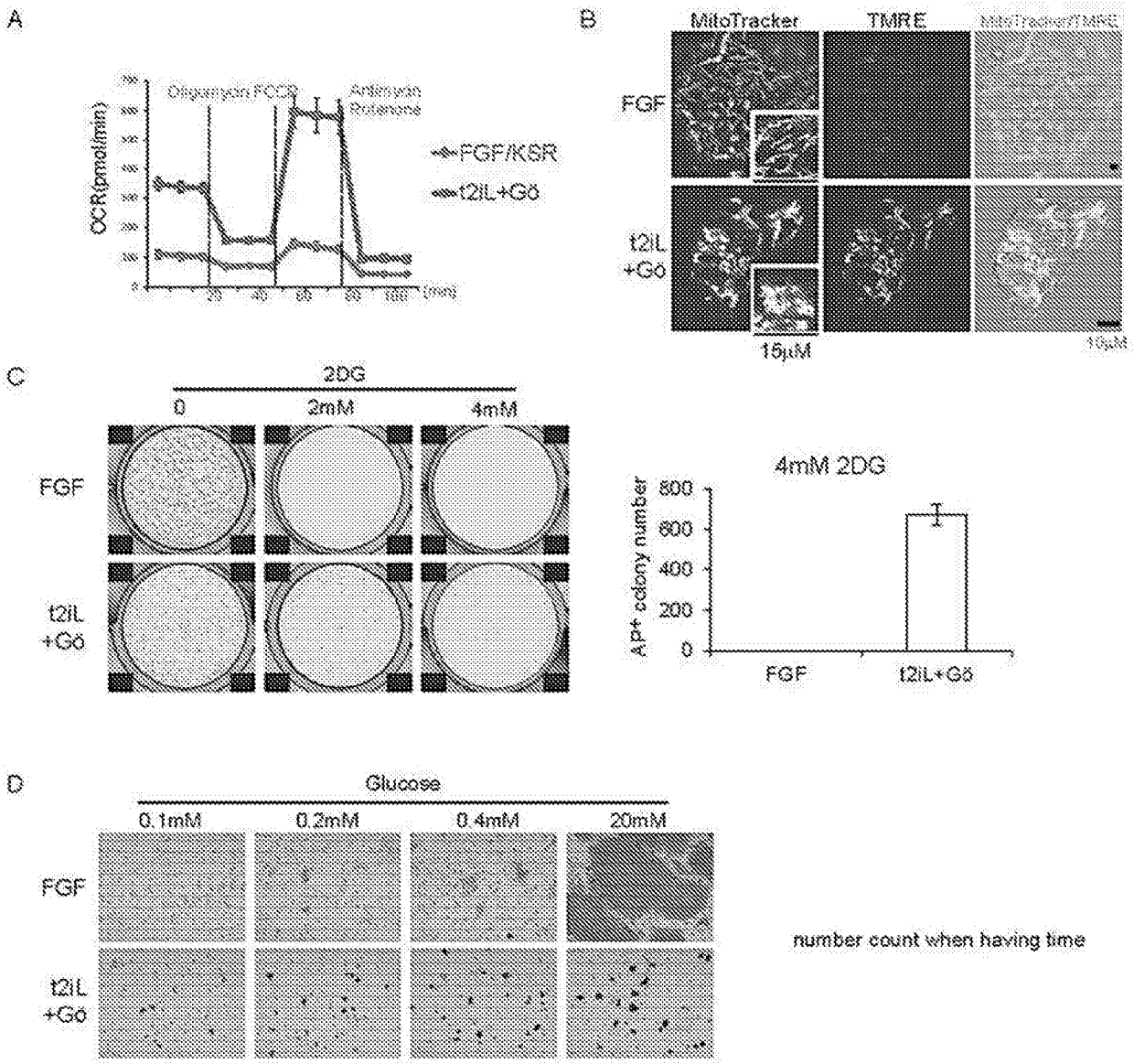

A
Sox17
CXCR4
FOXA2
Endoderm
T
PDGFRA
PDGFRB
Mesoderm
SOX1
PAX6
MAP2
Ectoderm
Relative expression normalised to GAPDH
GMEM+Serum
N2B27+KSR
B
Ectoderm
Mesoderm
Endoderm
C
t2iL+Gö
FGF/KSR
D
OCT3/4
STELLA
TFCP2L1
KLF4
Relative expression normalised to GAPDH
1 FGF/KSR
2 2iL+Gö
3 FGF/KSR P5
4 FGF/KSR P18
E
FGF/KSR
FGF/KSR
FGF/KSR
t2iL+Gö
F
ECAD
CXCR4
SOX17
FOXA2
G
TUJ1
NEUN

Figure 3



C and D

3x10^4 cells are seeded on 12 wells and cultured for 7 days with 2DG or reduced glucose.
(with Rock in KSR and without Rock in t2iL+Go)

Figure 4

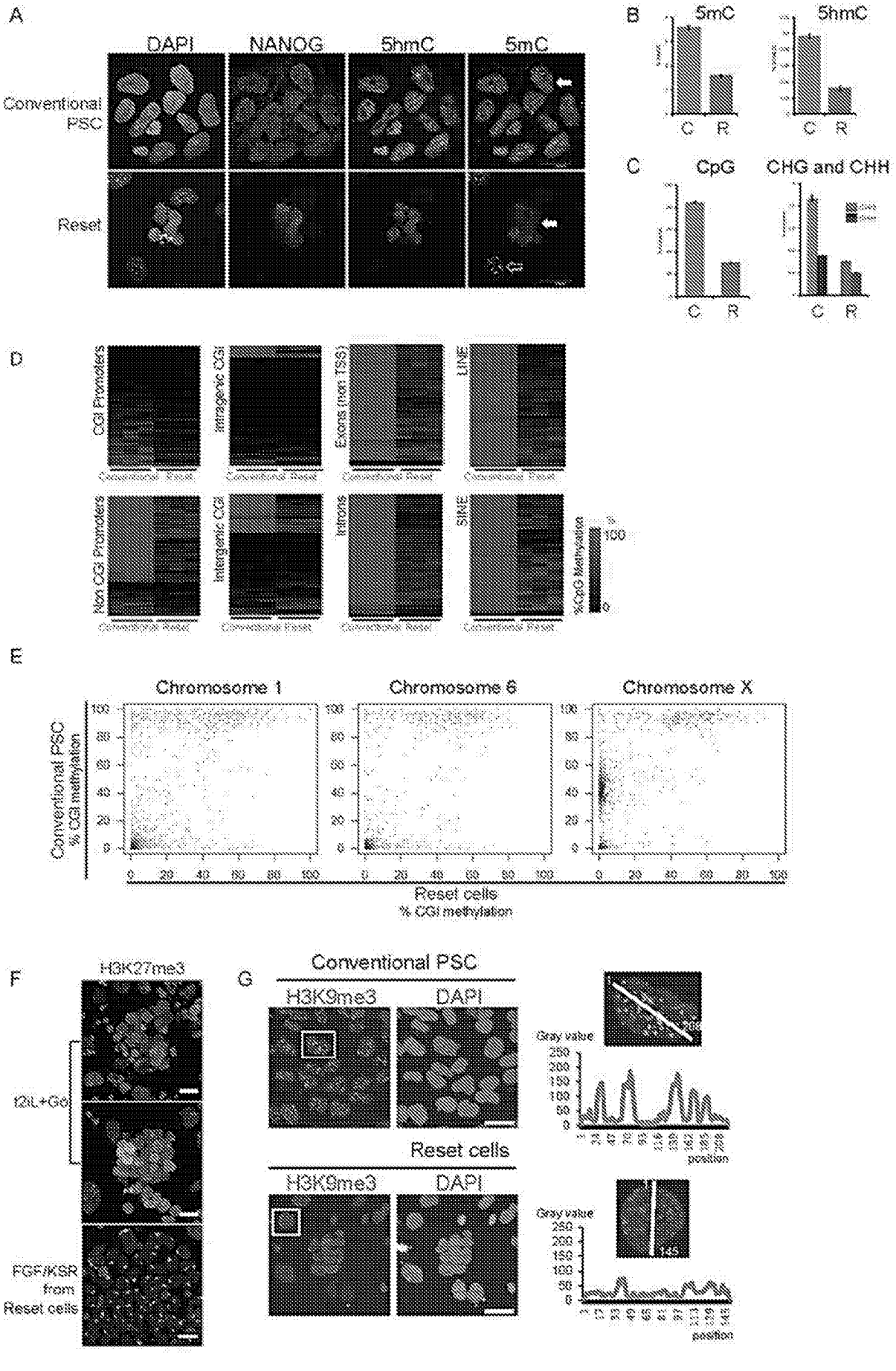

A
DAPI
NANOG
5hmC
5mC
Conventional PSC
Reset
B
5mC
5hmC
C
R
C
CpG
CHG and CHH
D
CGI Promoters
Intragenic CGI
Exons (non TSS)
LINE
Non CGI Promoters
Intergenic CGI
Introns
SINE
%CpG Methylation
100
0
E
Chromosome 1
Chromosome 6
Chromosome X
Conventional PSC
% CGI methylation
Reset cells
% CGI methylation
F
H3K27me3
t2iL+Gö
FGF/KSR
from
Reset cells
G
Conventional PSC
H3K9me3
DAPI
Reset cells
H3K9me3
DAPI
Gray value
position

Figure 5

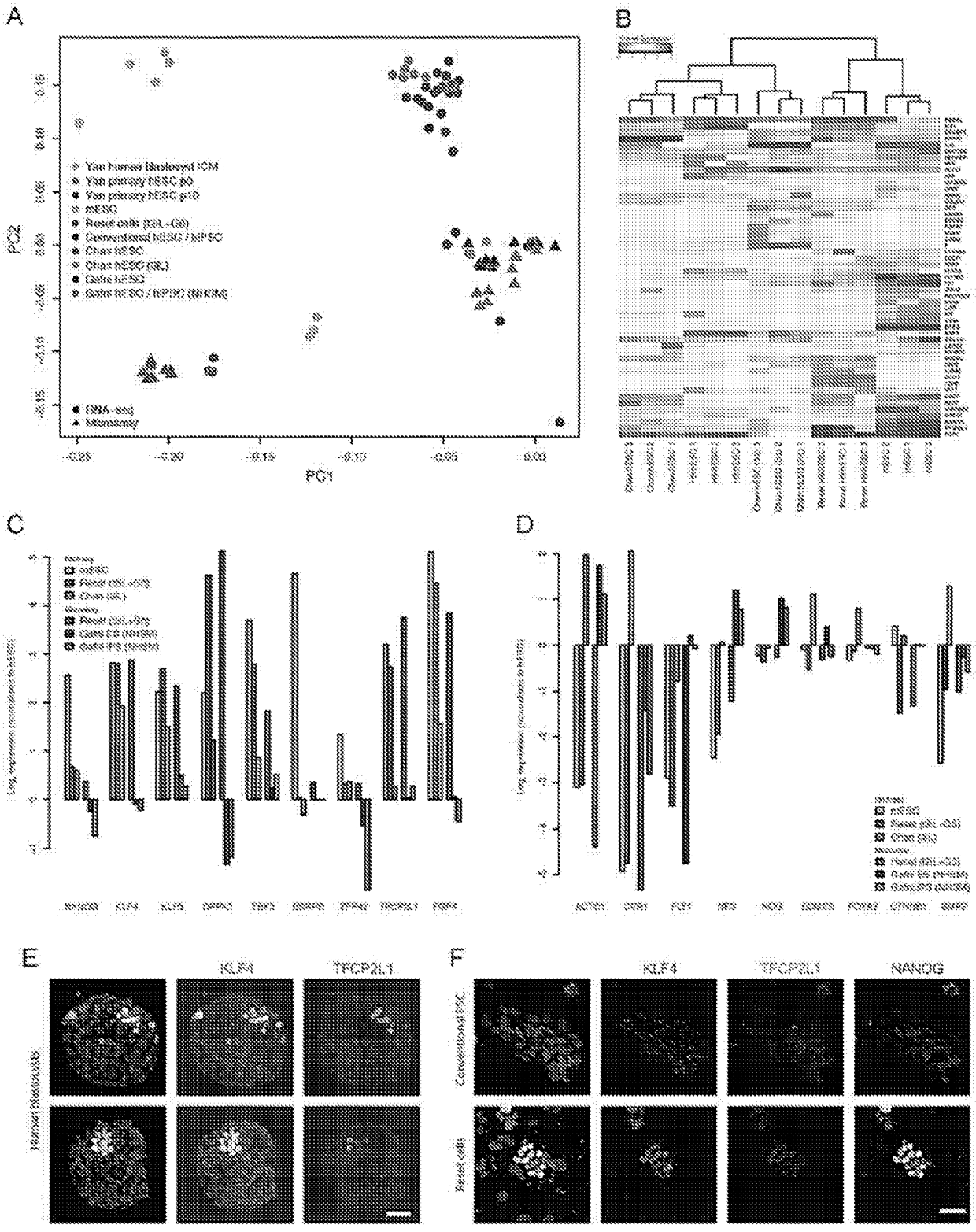


Figure 6

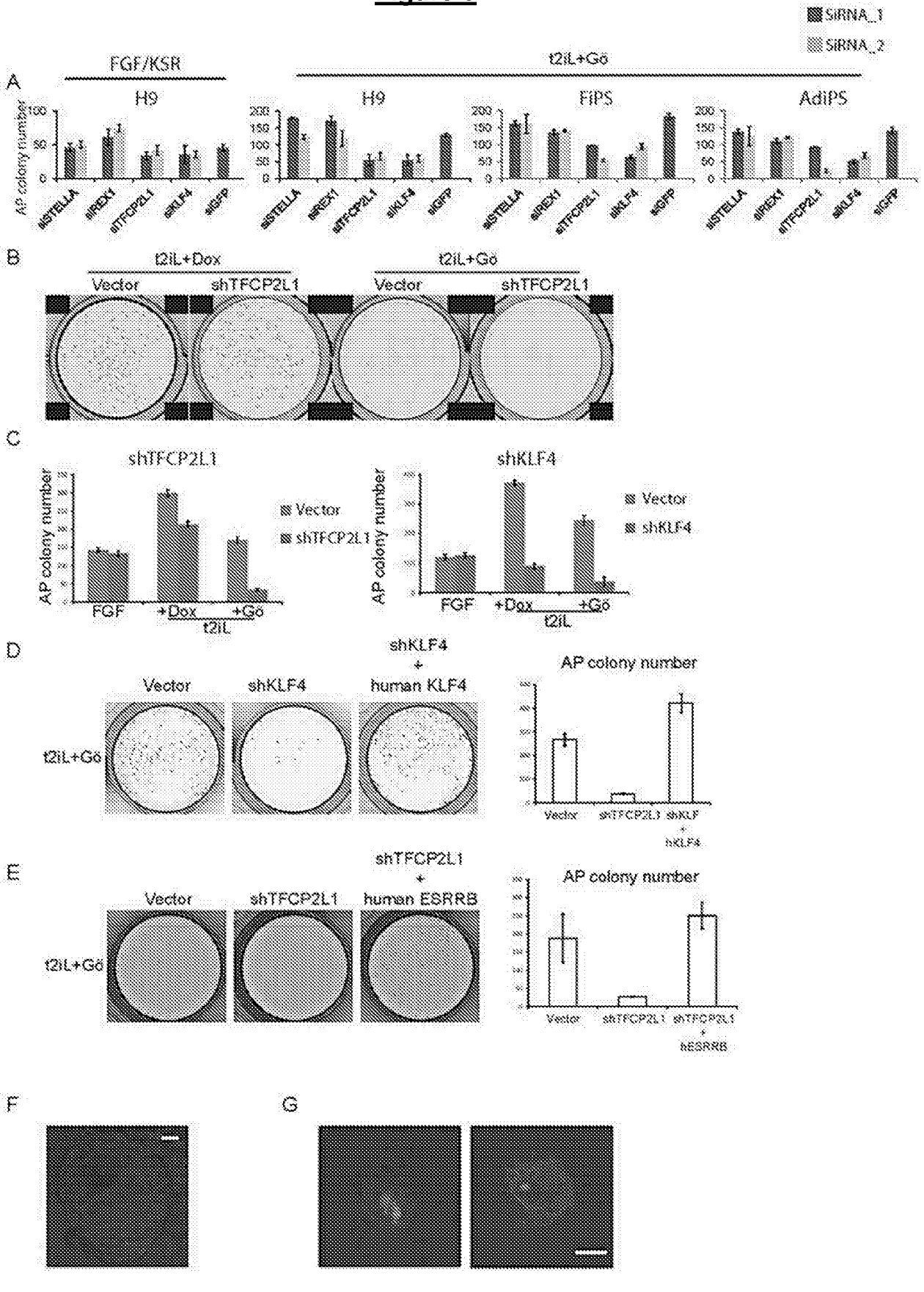

A
FGF/KSR
t2iL+Gö
H9
H9
FiPS
AdiPS
SiRNA_1
SiRNA_2
AP colony number
siSTELLA
siREX1
siTFCP2L1
siKLF4
siGFP
B
t2iL+Dox
t2iL+Gö
Vector
shTFCP2L1
C
shTFCP2L1
shKLF4
AP colony number
Vector
shTFCP2L1
shKLF4
FGF
+Dox
+Gö
t2iL
D
shKLF4
+
human KLF4
Vector
shKLF4
t2iL+Gö
AP colony number
shKLF
+
hKLF4
E
shTFCP2L1
+
human ESRRB
Vector
shTFCP2L1
t2iL+Gö
AP colony number
shTFCP2L1
+
hESRRB
F
G

Figure 7

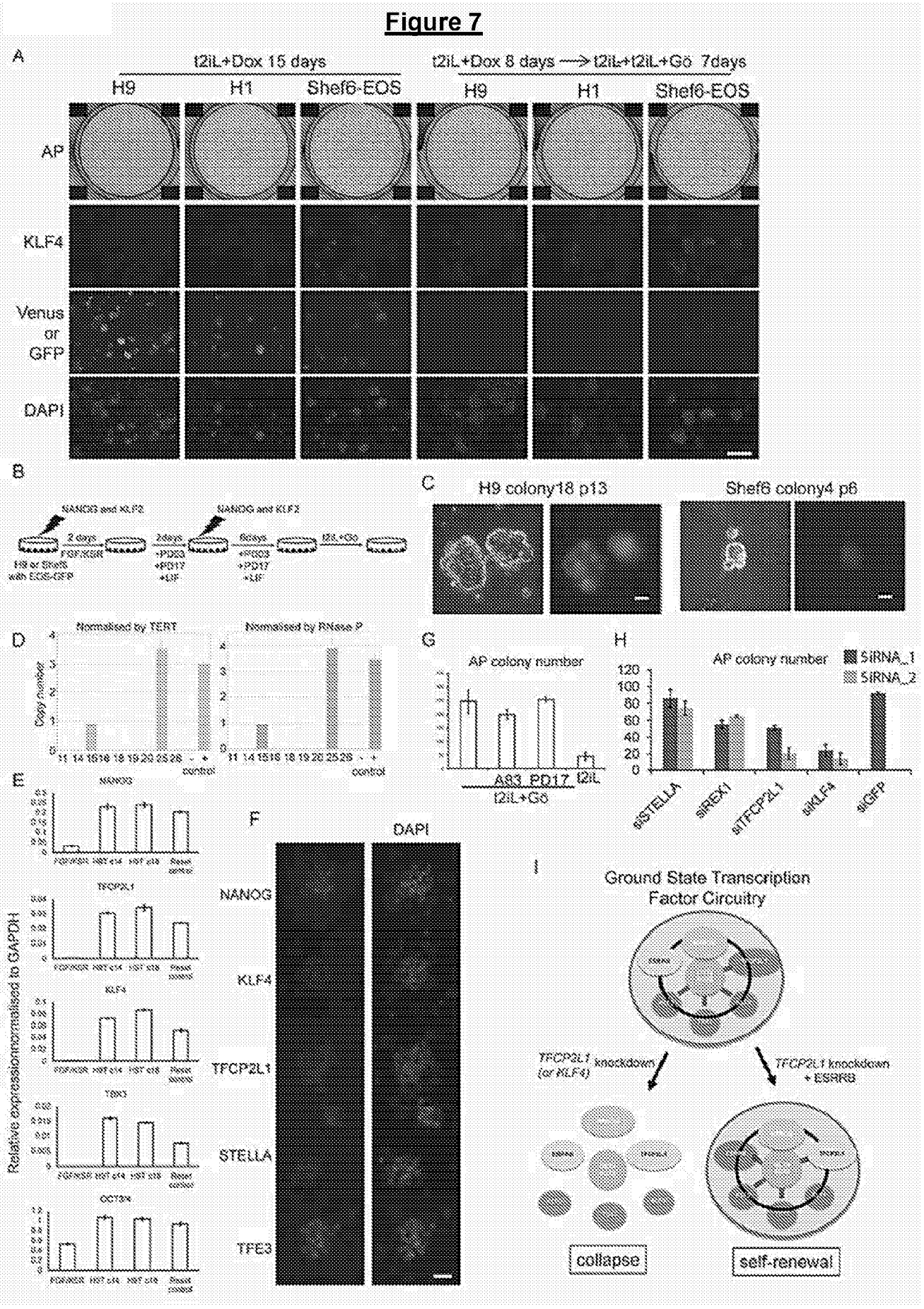

A
t2iL+Dox 15 days
t2iL+Dox 8 days ⟶ t2iL+t2iL+Gö 7days
H9
H1
Shef6-EOS
AP
KLF4
Venus or GFP
DAPI
B
NANOG and KLF2
2 days
FGF/KSR
2days
+PD03
+PD17
+LIF
6days
+PD03
+PD17
+LIF
t2iL+Gö
H9 or Shef6 with ECS-GFP
C
H9 colony18 p13
Shef6 colony4 p6
D
Normalised by TERT
Normalised by RNase P
Copy number
control
E
NANOG
TFCP2L1
KLF4
TBX3
OCT3/4
Relative expressionnormalised to GAPDH
F
DAPI
NANOG
KLF4
TFCP2L1
STELLA
TFE3
G
AP colony number
A83 PD17 t2iL
t2iL+Gö
H
AP colony number
SiRNA_1
SiRNA_2
siSTELLA
siREX1
siTFCP2L1
siKLF4
siGFP
I
Ground State Transcription Factor Circuitry
TFCP2L1 knockdown (or KLF4)
TFCP2L1 knockdown + ESRRB
collapse
self-renewal

Figure 8

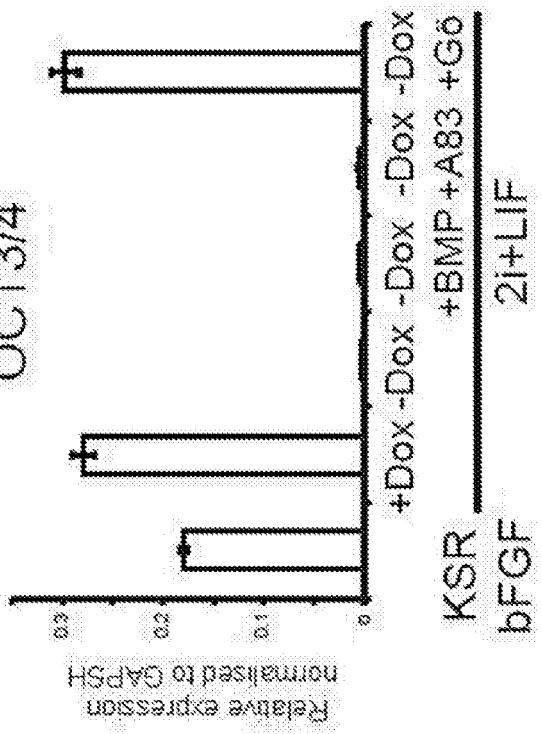

OCT3/4
Relative expression normalised to GAPSH
0
0.1
0.2
0.3
+Dox -Dox -Dox -Dox -Dox
+BMP +A83 +Gö
KSR
bFGF
2i+LIF

Figure 9

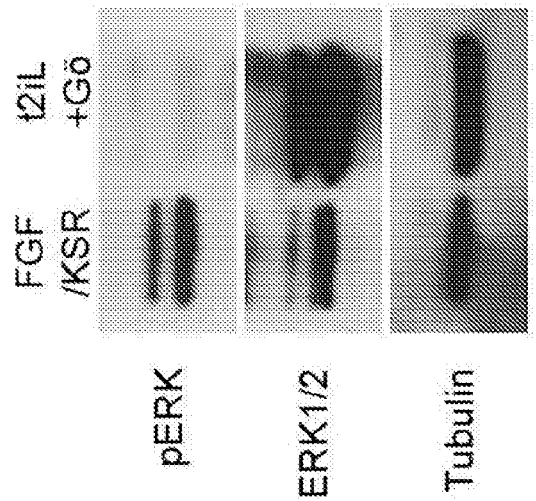


Figure 10

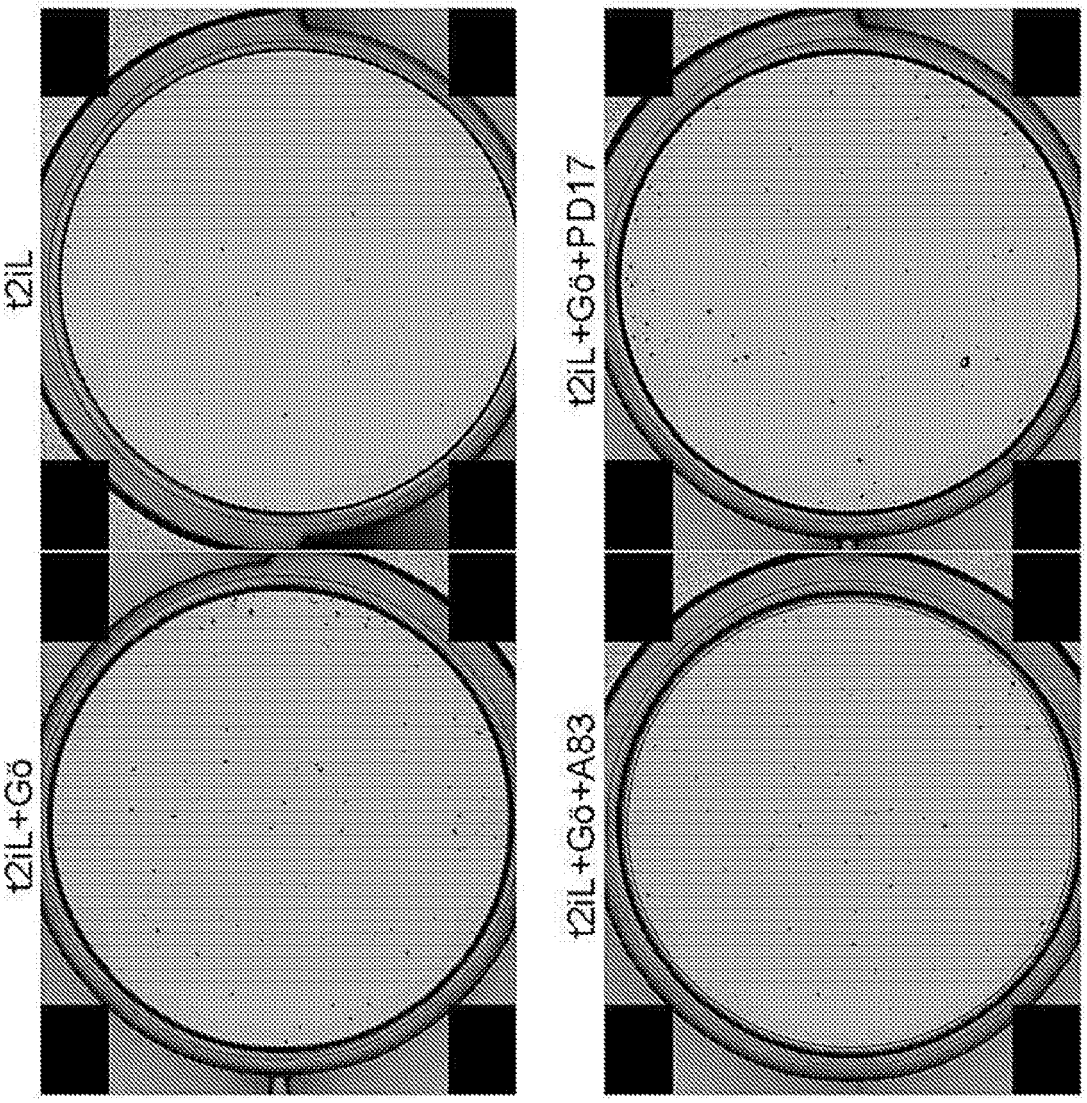


Figure 11

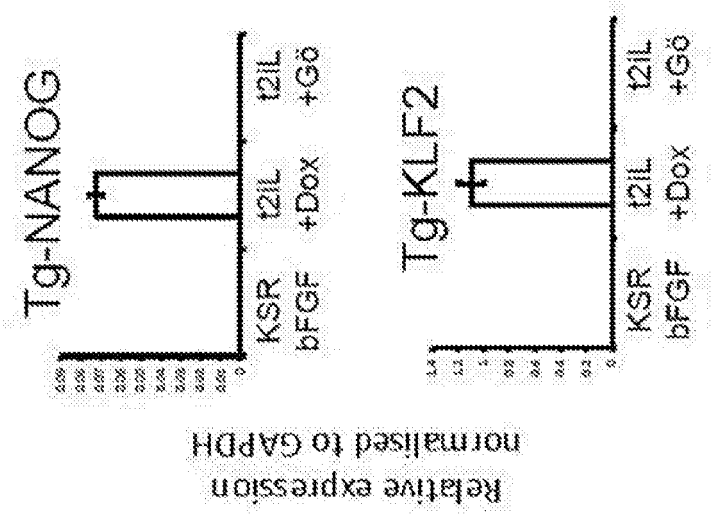
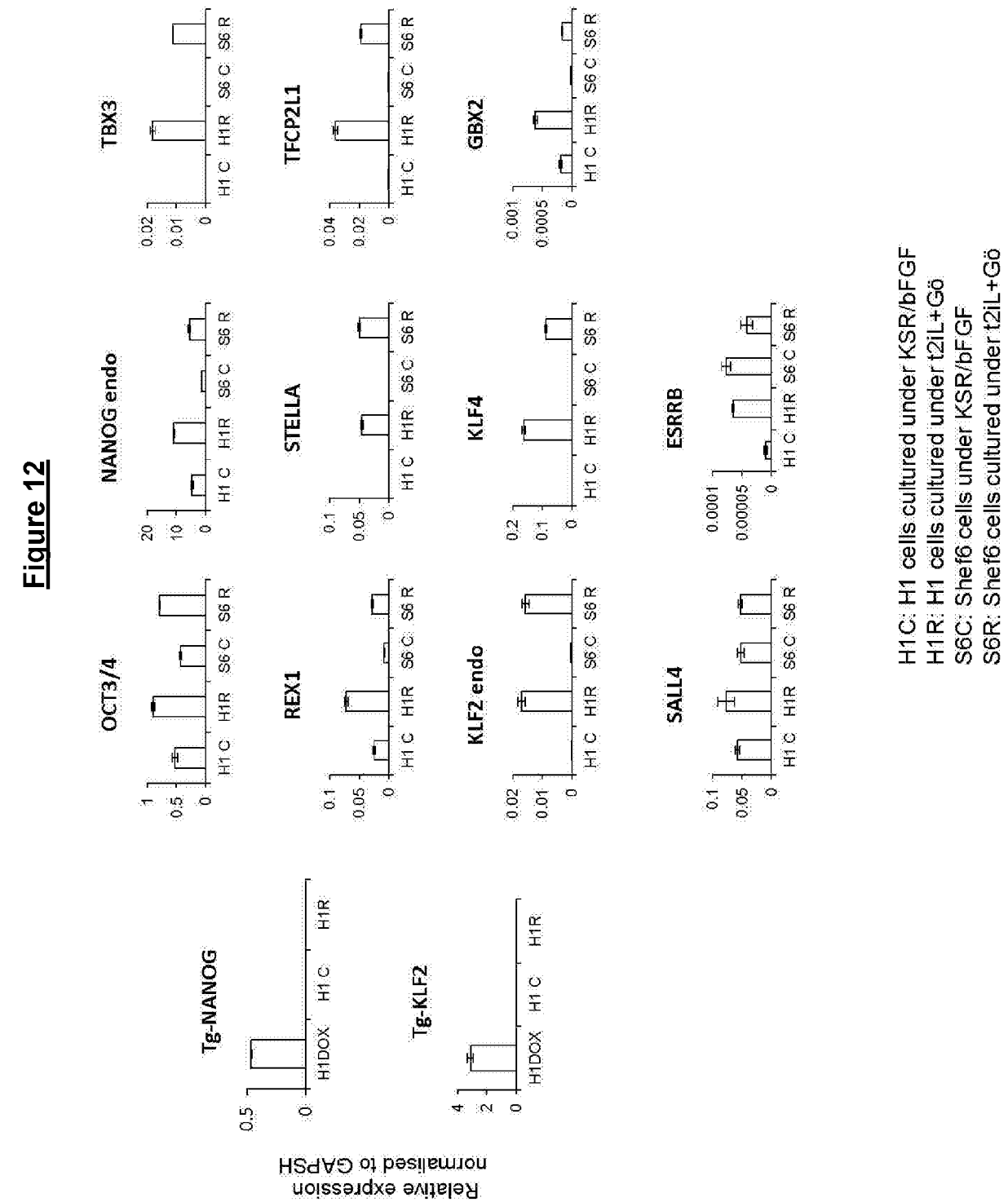

Tg-NANOG
Tg-KLF2
KSR
bFGF
t2iL
+Dox
t2iL
+Gö
Relative expression
normalised to GAPDH

Figure 12



H1C: H1 cells cultured under KSR/bFGF
H1R: H1 cells cultured under t2iL+Gö
S6C: Shef6 cells under KSR/bFGF
S6R: Shef6 cells cultured under t2iL+Gö

Figure 13

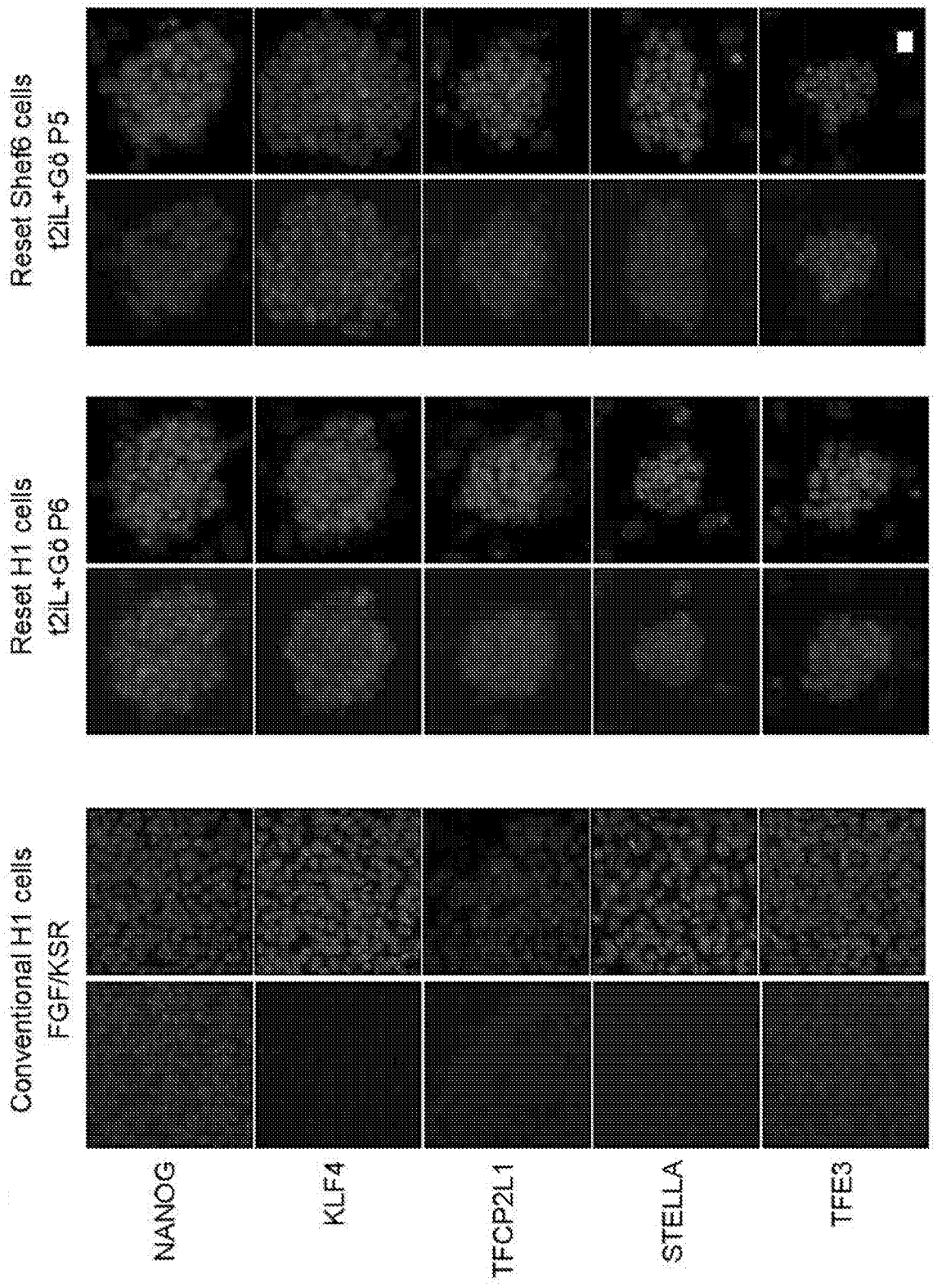

Conventional H1 cells
FGF/KSR
Reset H1 cells
t2iL+Gö P6
Reset Shef6 cells
t2iL+Gö P5
NANOG
KLF4
TFCP2L1
STELLA
TFE3

Figure 14

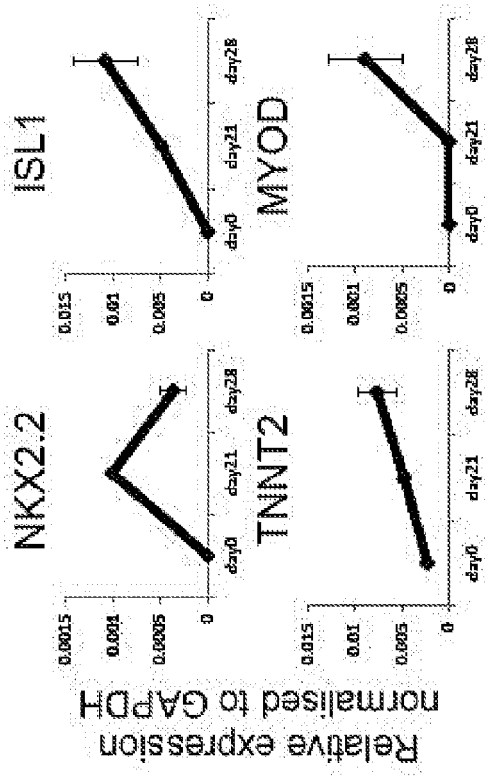

NKX2.2
ISL1
TNT2
MYOD
day0
day21
day28
0
0.0005
0.001
0.0015
0.005
0.01
0.015
0.0025
0.005
0.0075
Relative expression
normalised to GAPDH

Figure 15

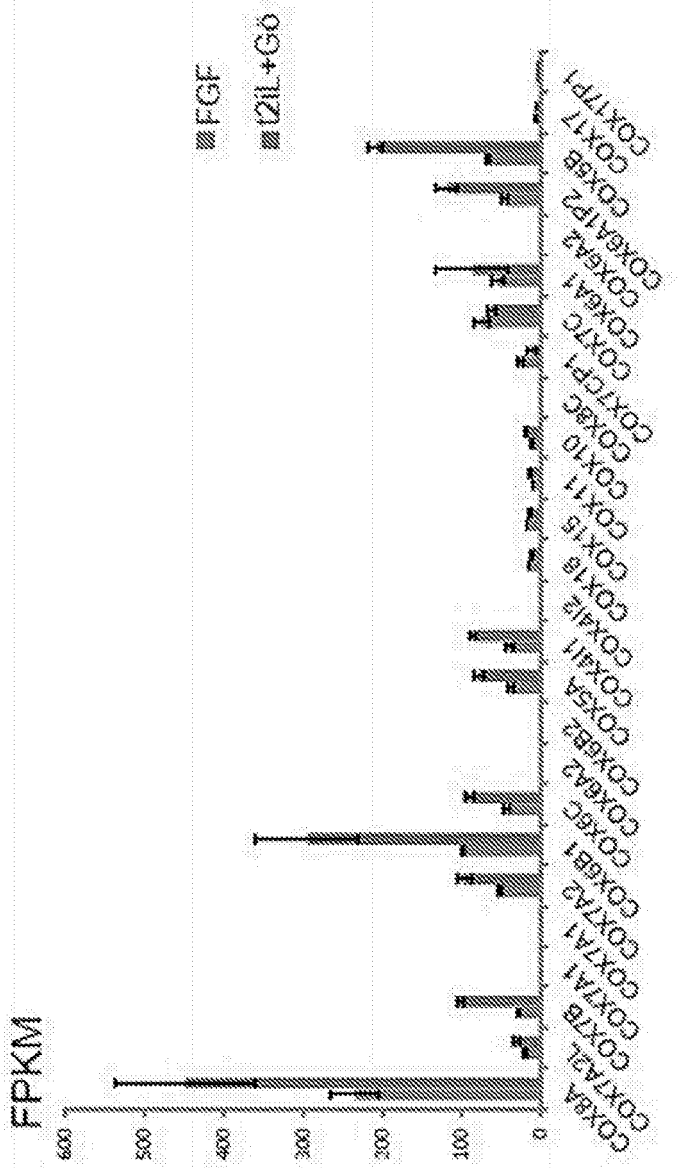

FPKM
600
500
400
300
200
100
0
FGF
I2iL+Gö
COX8A
COX7A2L
COX7B
COX7A1
COX7A2
COX6B1
COX6C
COX6A2
COX6B2
COX5A
COX4I1
COX4I2
COX5B
COX11
COX10
COX6C
COX7CP1
COX7C
COX6A1
COX6A2
COX8A1P2
COX6B
COX1
COX7TP1

Figure 16

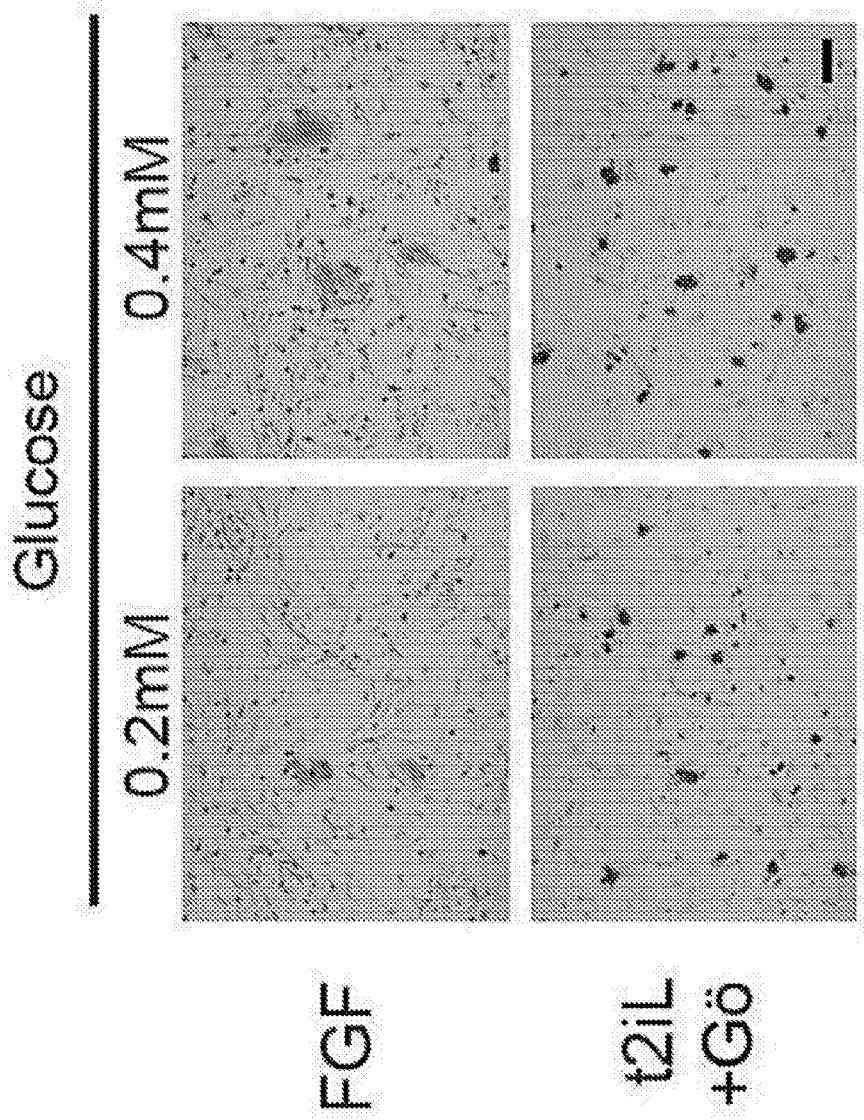


Figure 17

Sequencing Stats

BS-seq Sample	Sequenced reads	Mapping Efficiency (%)	Fold coverage (Genome length GRCh37.p10)
Conventional iPSC19.1	163350819	84.9	4.17
Conventional iPSC19.2	156765441	85.1	4.00
Conventional iPSC19.3	153635899	84.9	3.92
Reset cells 19.1	154802363	67.1	3.12
Reset cells 19.2	155956363	66.0	3.09
Reset cells 19.3	137626880	65.8	2.72

Figure 18

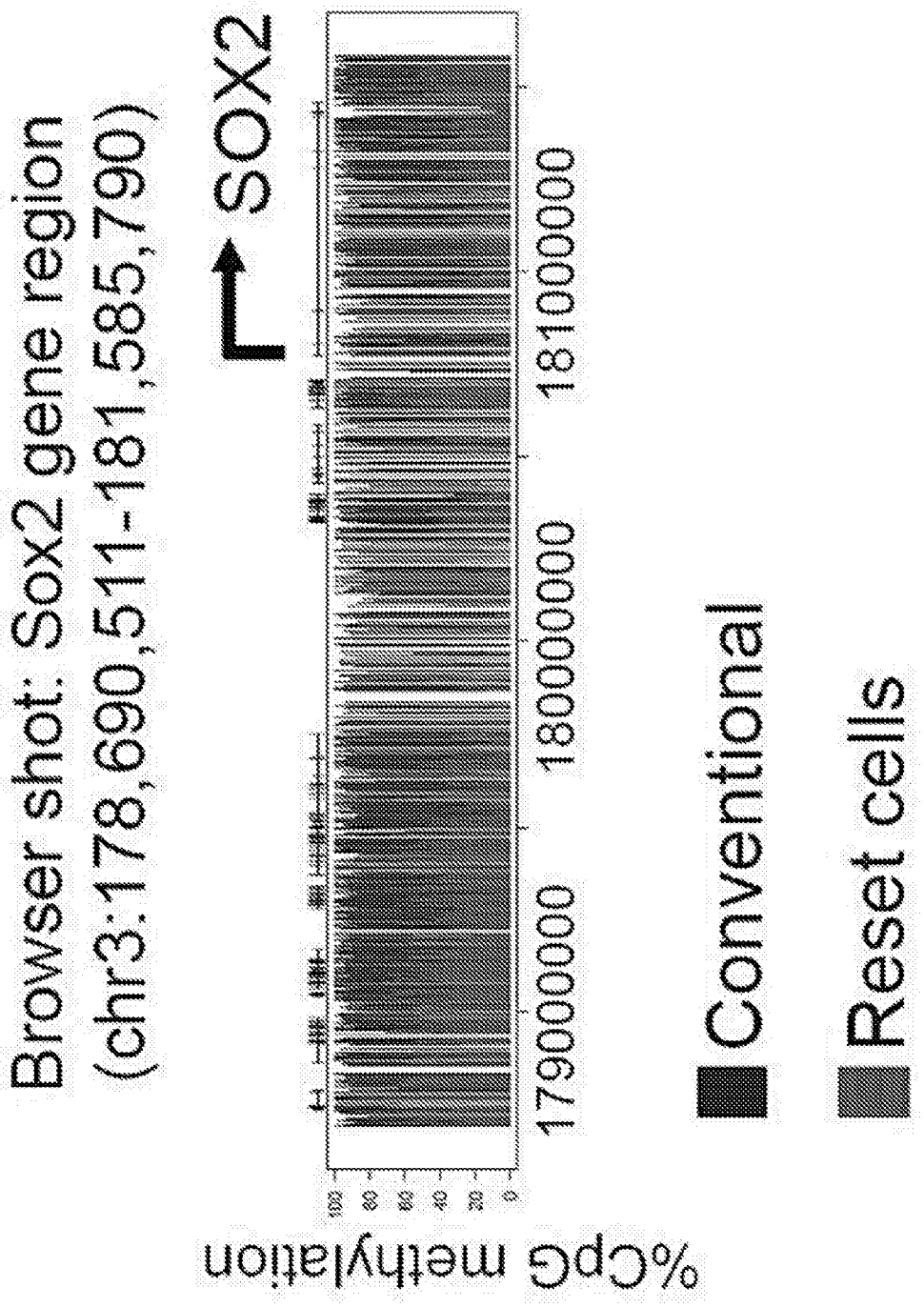

Browser shot: Sox2 gene region
(chr3:178,690,511-181,585,790)
SOX2
%CpG methylation
100
80
60
40
20
0
179000000
180000000
181000000
Conventional
Reset cells

Figure 19A

H3K9me3 staining for Mouse ES cells and EpiSC

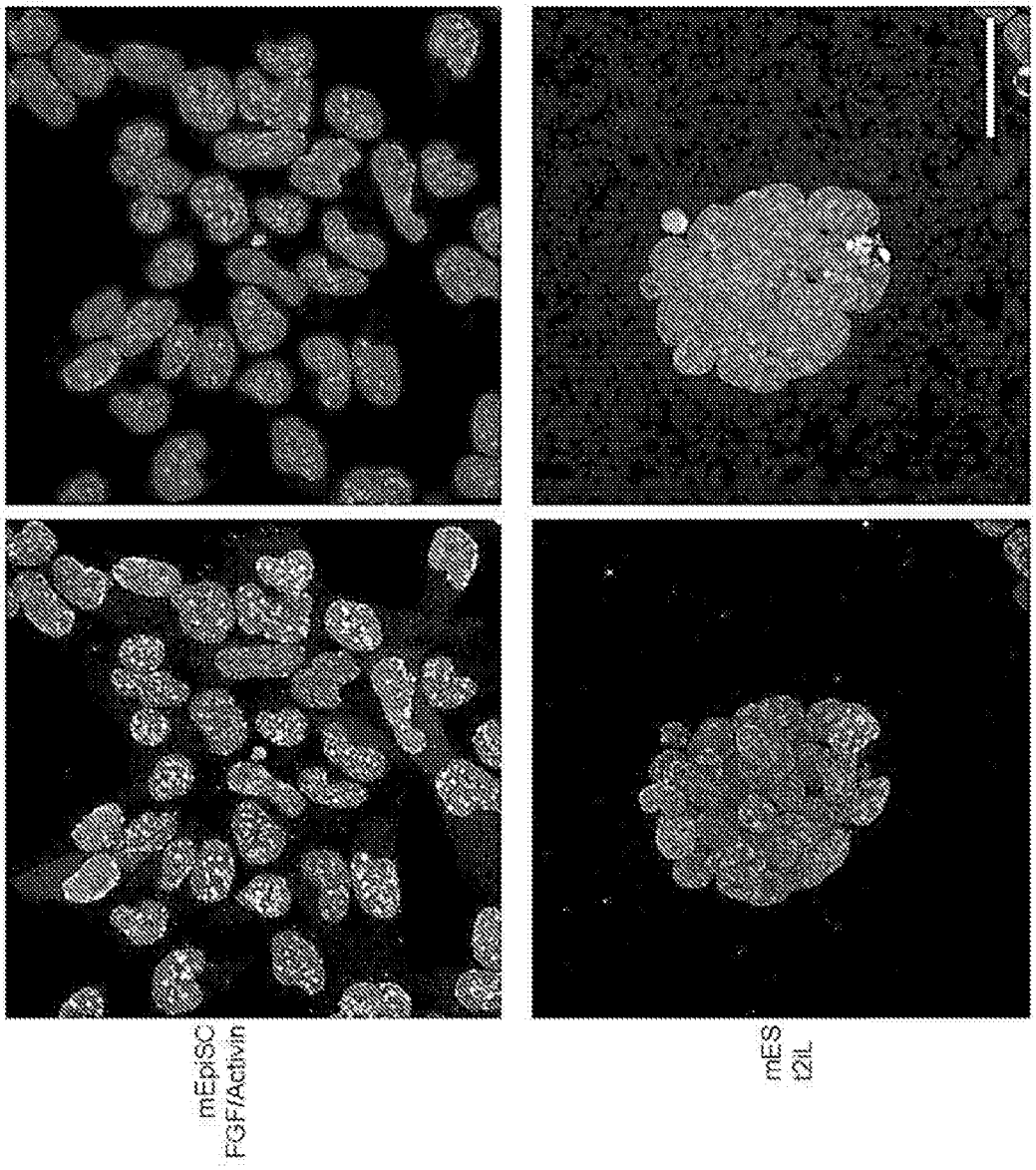


Figure 19B

H3K9me3 staining for Human conventional ES cells in KSR/FGF

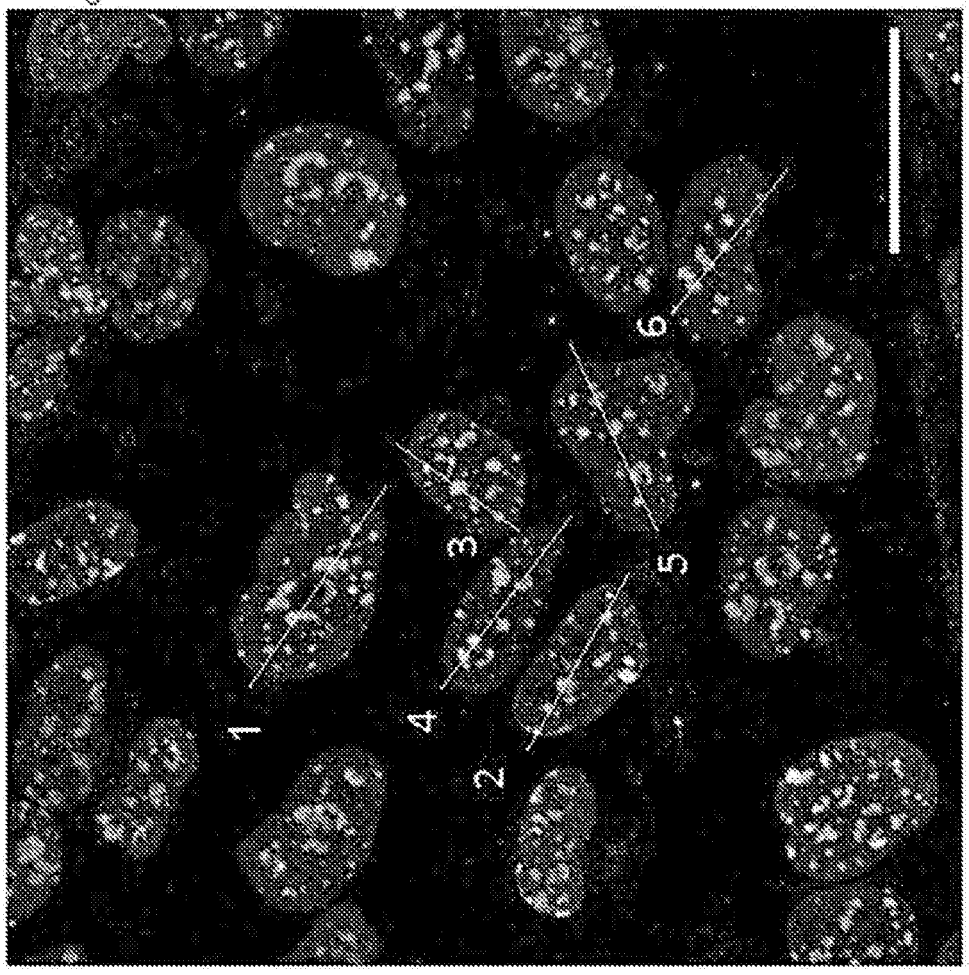


Figure 19C

H3K9me3 staining for Human reset cells in t2iL+Gö

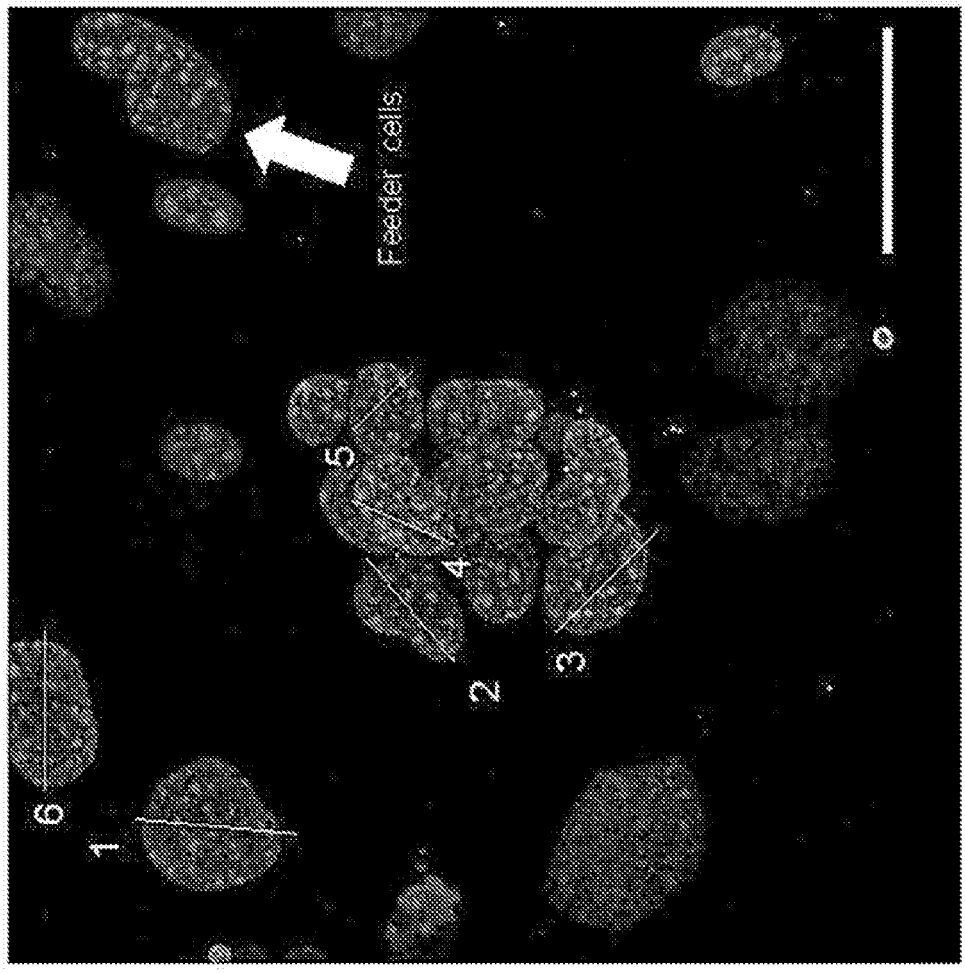


Figure 19D

H3K9me3 staining for mEpiSC bFGF/Activin

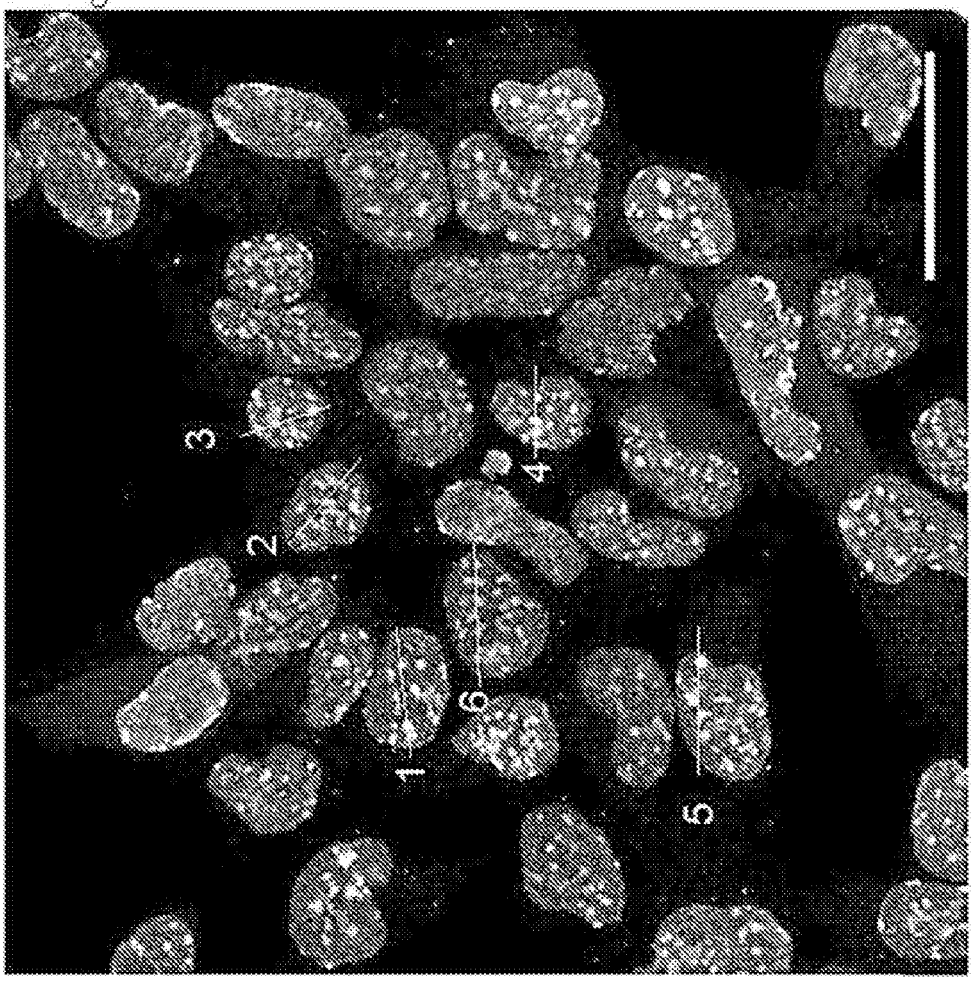


Figure 19E

H3K9me staining for mES cells 2iL

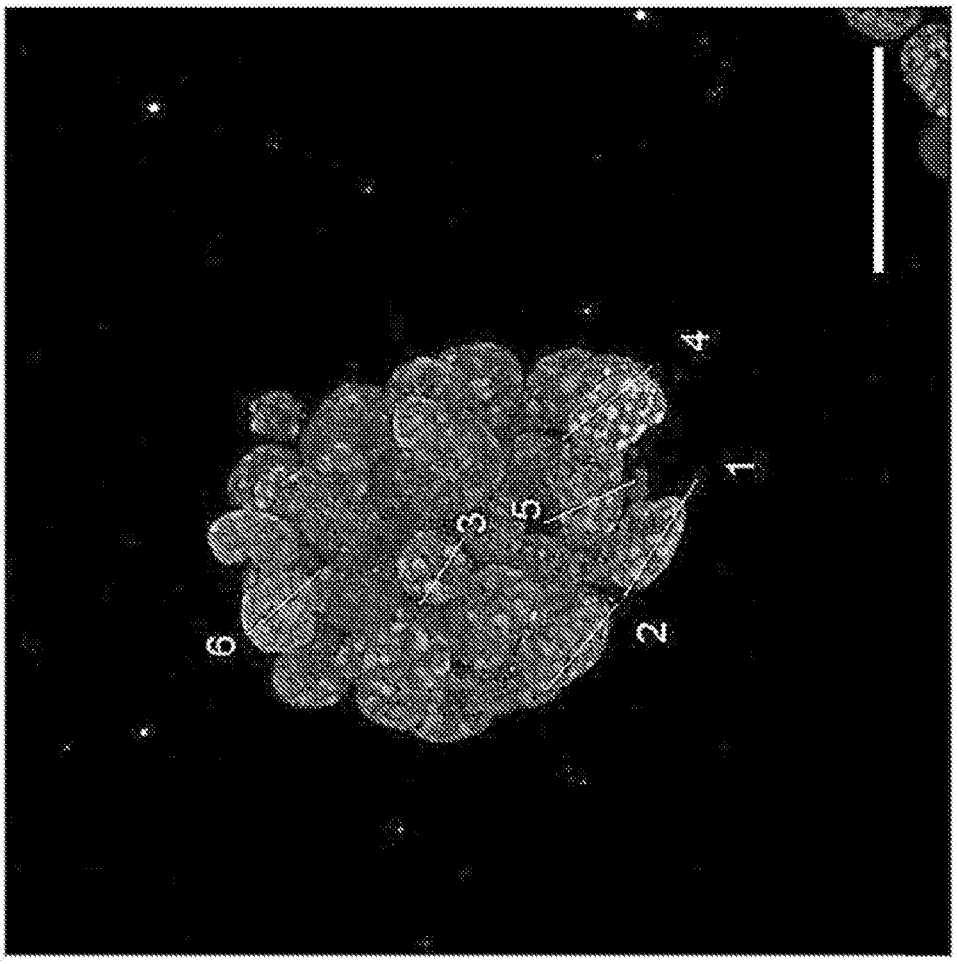


Figure 20

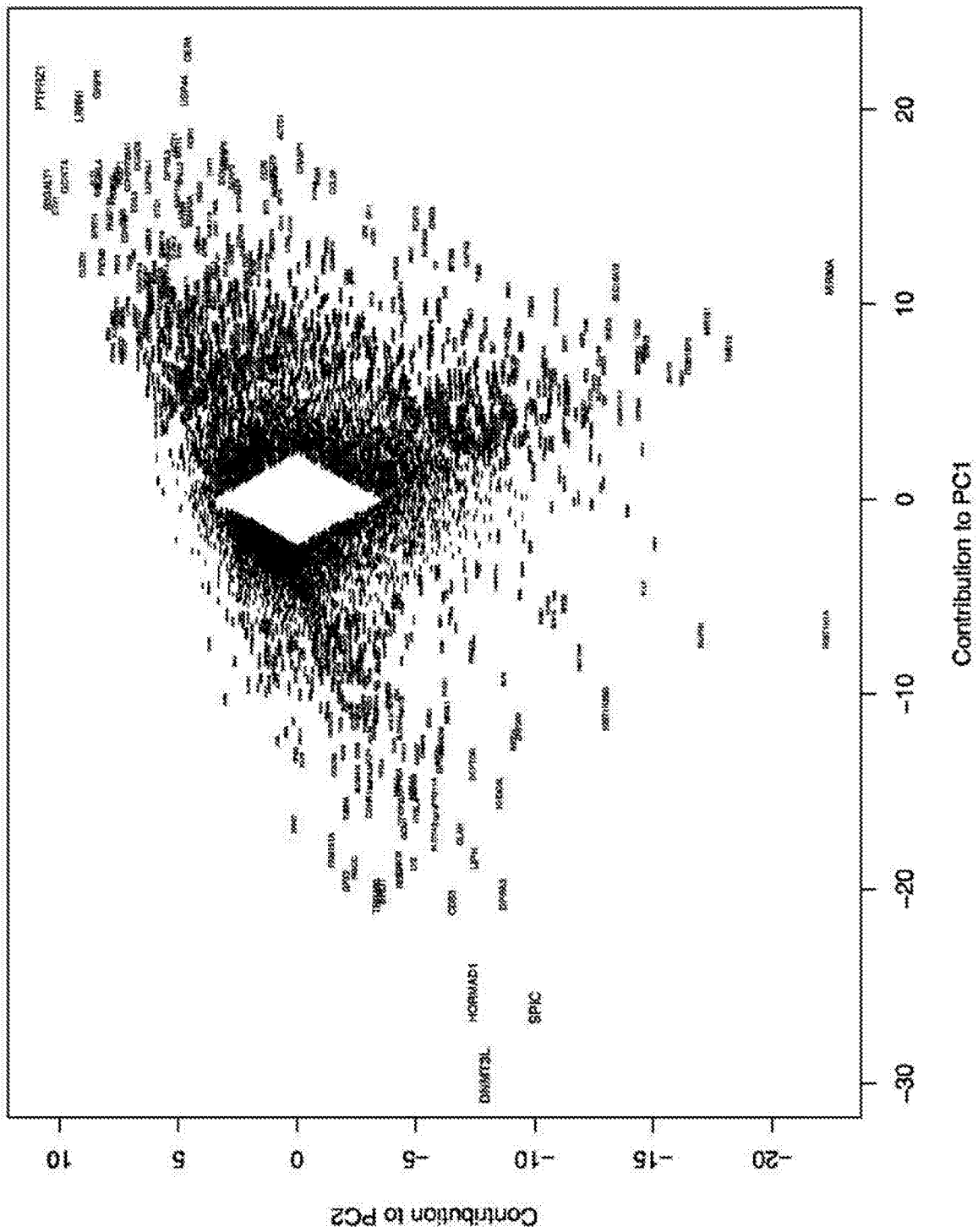

Contribution to PC2
10
5
0
−5
−10
−15
−20
Contribution to PC1
20
10
0
−10
−20
−30
SPIC
HOMMAD1
DMMT3L

Figure 21

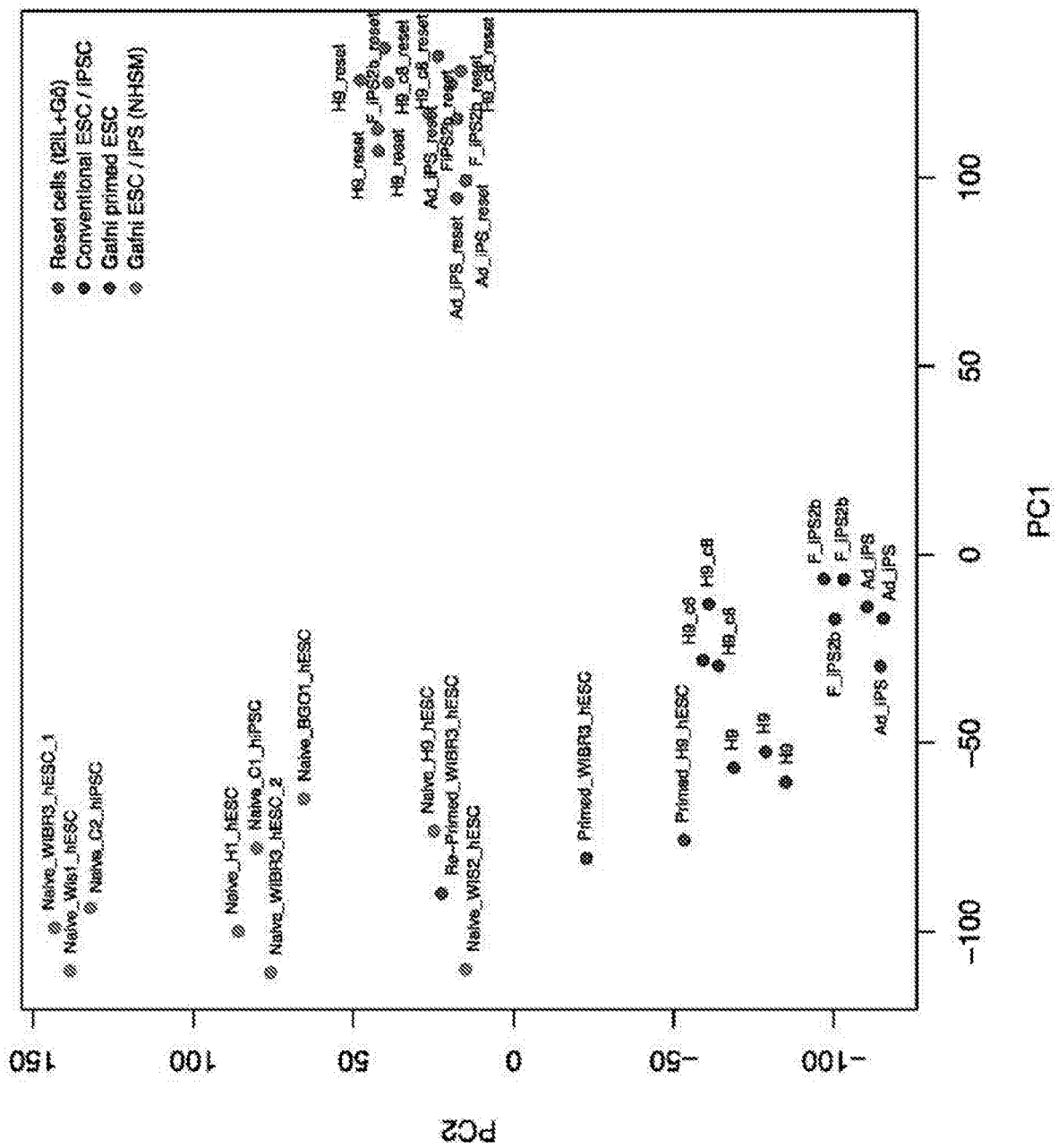

Reset cells (t2iL+Gö)
Conventional ESC / iPSC
Gafni primed ESC
Gafni ESC / iPS (NHSM)
Naive_WIBR3_hESC_1
Naive_Wis1_hESC
Naive_C2_hiPSC
Naive_H1_hESC
Naive_C1_hiPSC
Naive_WIBR3_hESC_2
Naive_BG01_hESC
Naive_H9_hESC
Re-Primed_WIBR3_hESC
Naive_WIS2_hESC
Primed_WIBR3_hESC
Primed_H9_hESC
H9
H9
H9
H9_c8
H9_c8
H9_c8
F_iPS2b
F_iPS2b
F_iPS2b
Ad_iPS
Ad_iPS
Ad_iPS
H9_reset
H9_reset
H9_reset
H9_c8_reset
H9_c8_reset
H9_c8_reset
Ad_iPS_reset
Ad_iPS_reset
Ad_iPS_reset
PC2
150
100
50
0
−50
−100
PC1
−100
−50
0
50
100

Figure 22

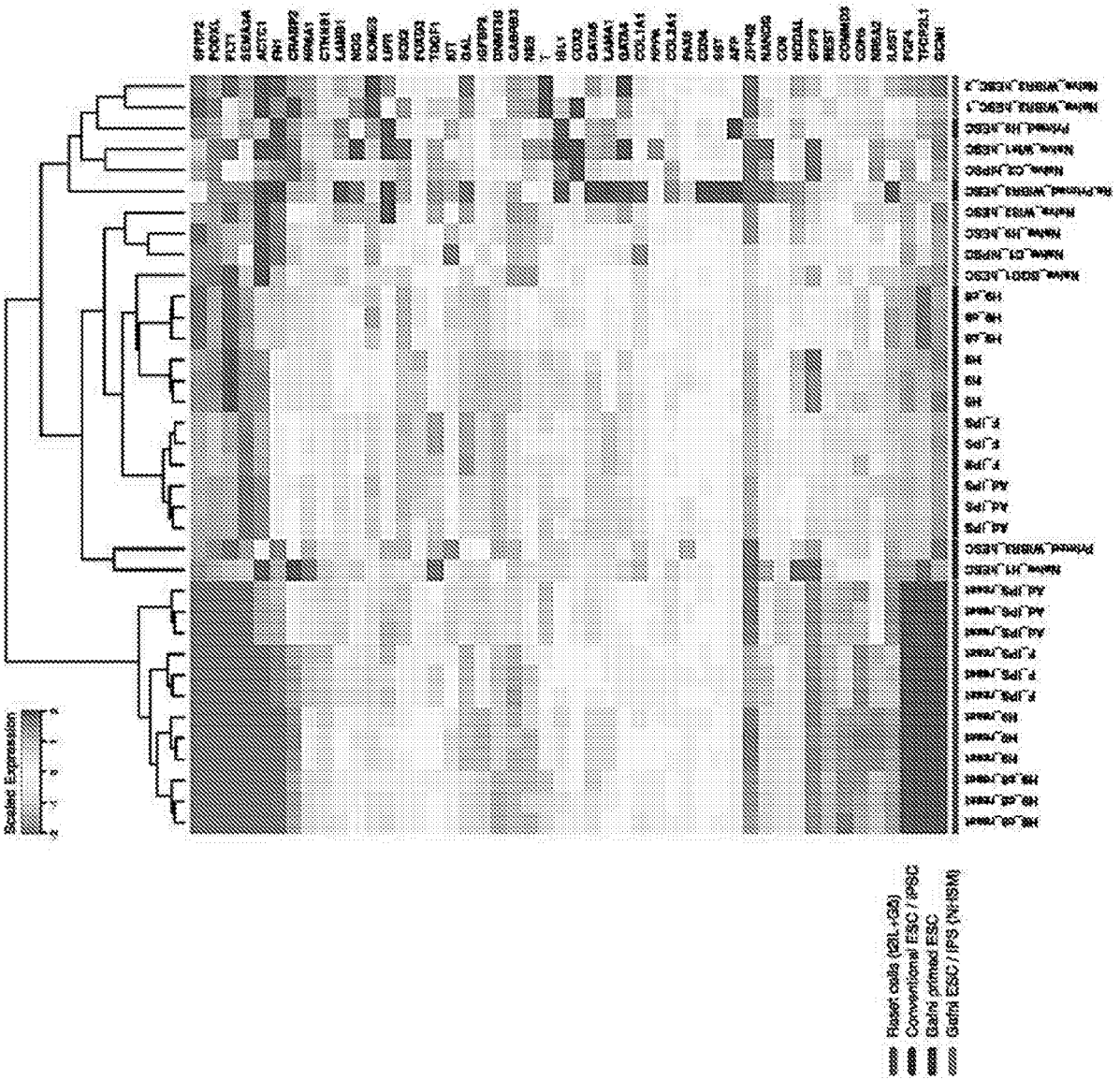

Scaled Expression
Reset cells (iZIL +2i)
Conventional ESC / iPSC
Gsk3i primed ESC
Gsk3i ESC / iFS (N+SM)

Figure 23

Epigenetic Modifiers

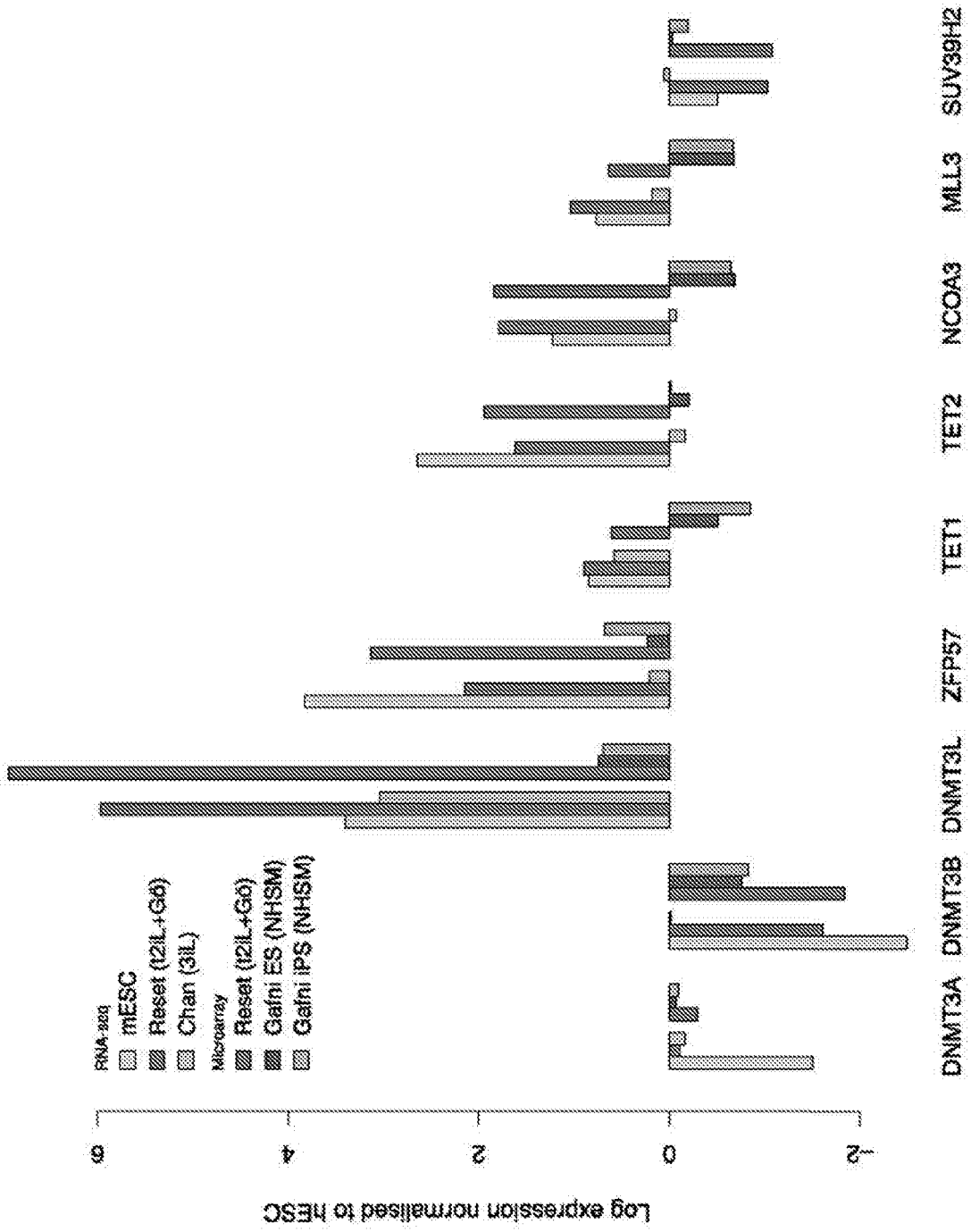


Figure 24

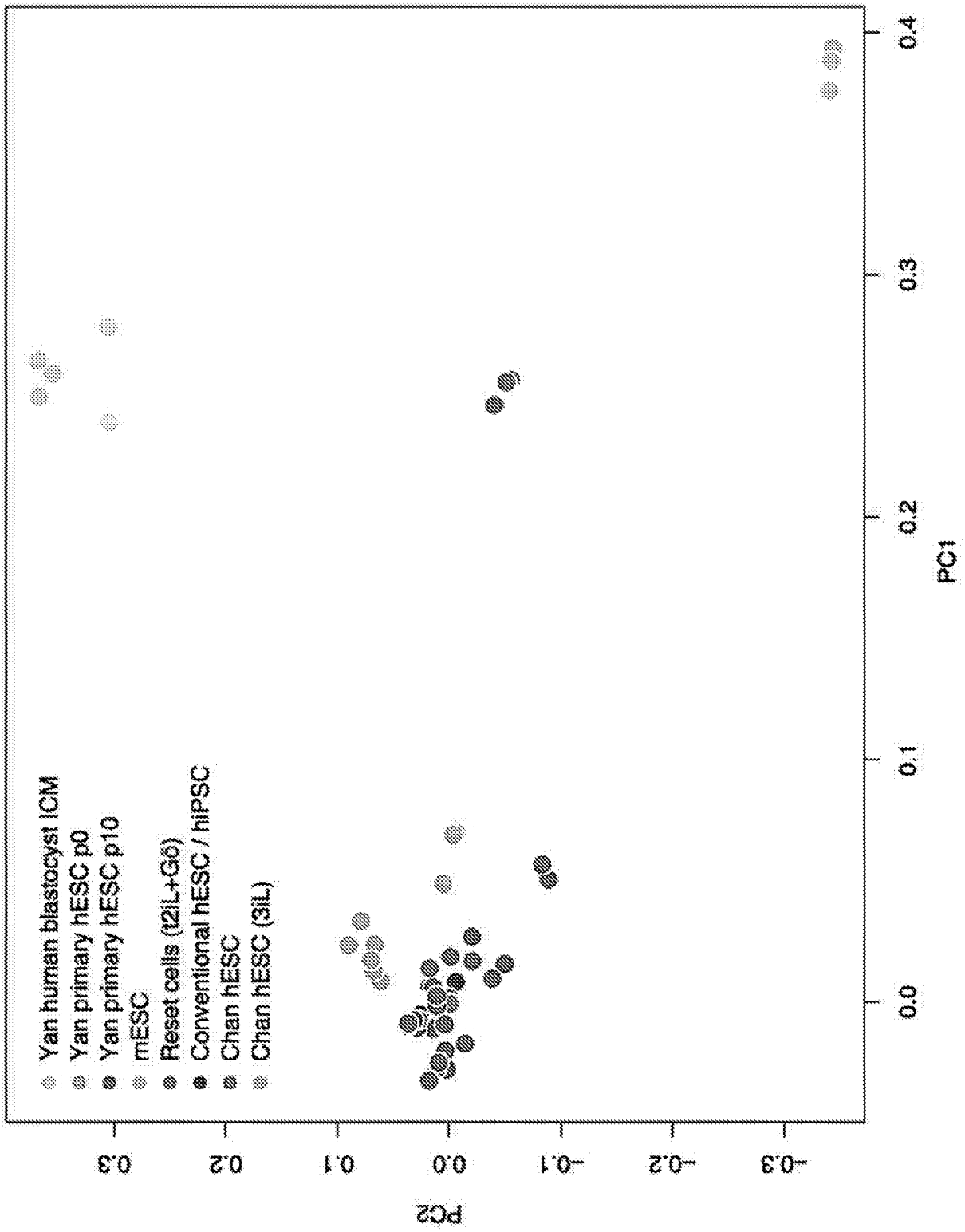

Yan human blastocyst ICM
Yan primary hESC p0
Yan primary hESC p10
mESC
Reset cells (t2iL+Gö)
Conventional hESC / hiPSC
Chan hESC
Chan hESC (3iL)
PC1
PC2
0.0
0.1
0.2
0.3
0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3

Figure 25

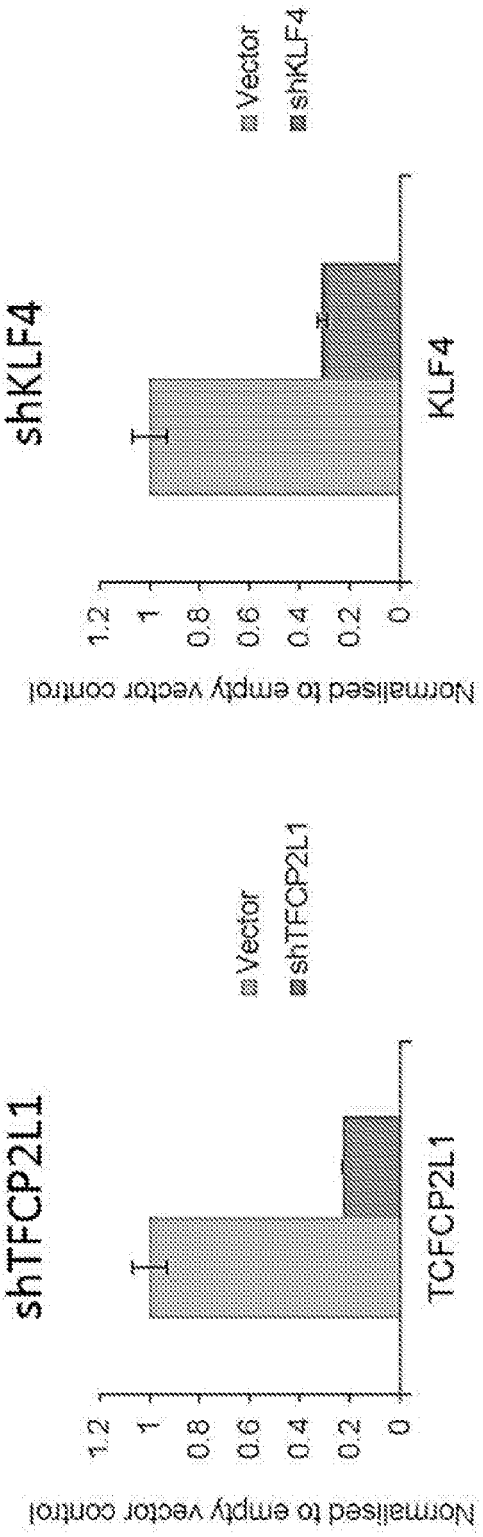

shKLF4
Vector
shKLF4
1.2
1
0.8
0.6
0.4
0.2
0
KLF4
Normalised to empty vector control
shTFCP2L1
Vector
shTFCP2L1
1.2
1
0.8
0.6
0.4
0.2
0
TCFCP2L1
Normalised to empty vector control

Figure 26

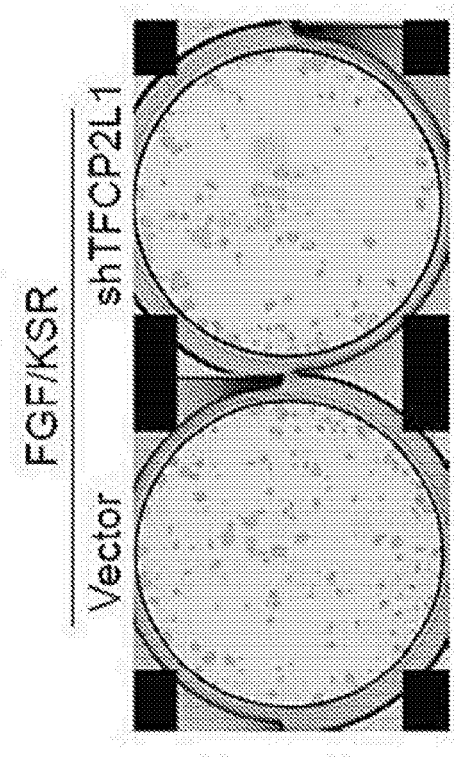


Figure 27

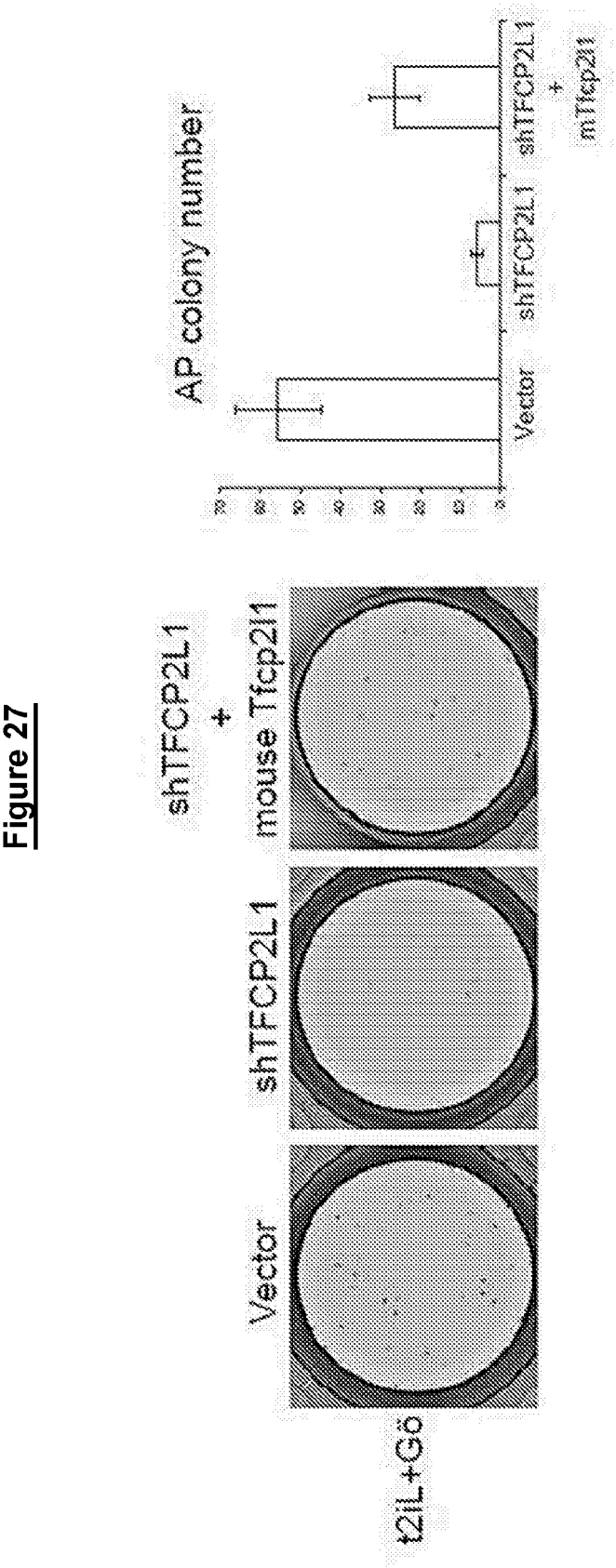

t2iL+Gö
Vector
shTFCP2L1
shTFCP2L1
+
mouse Tfcp2l1
AP colony number
Vector
shTFCP2L1
shTFCP2L1
+
mTfcp2l1

Figure 28

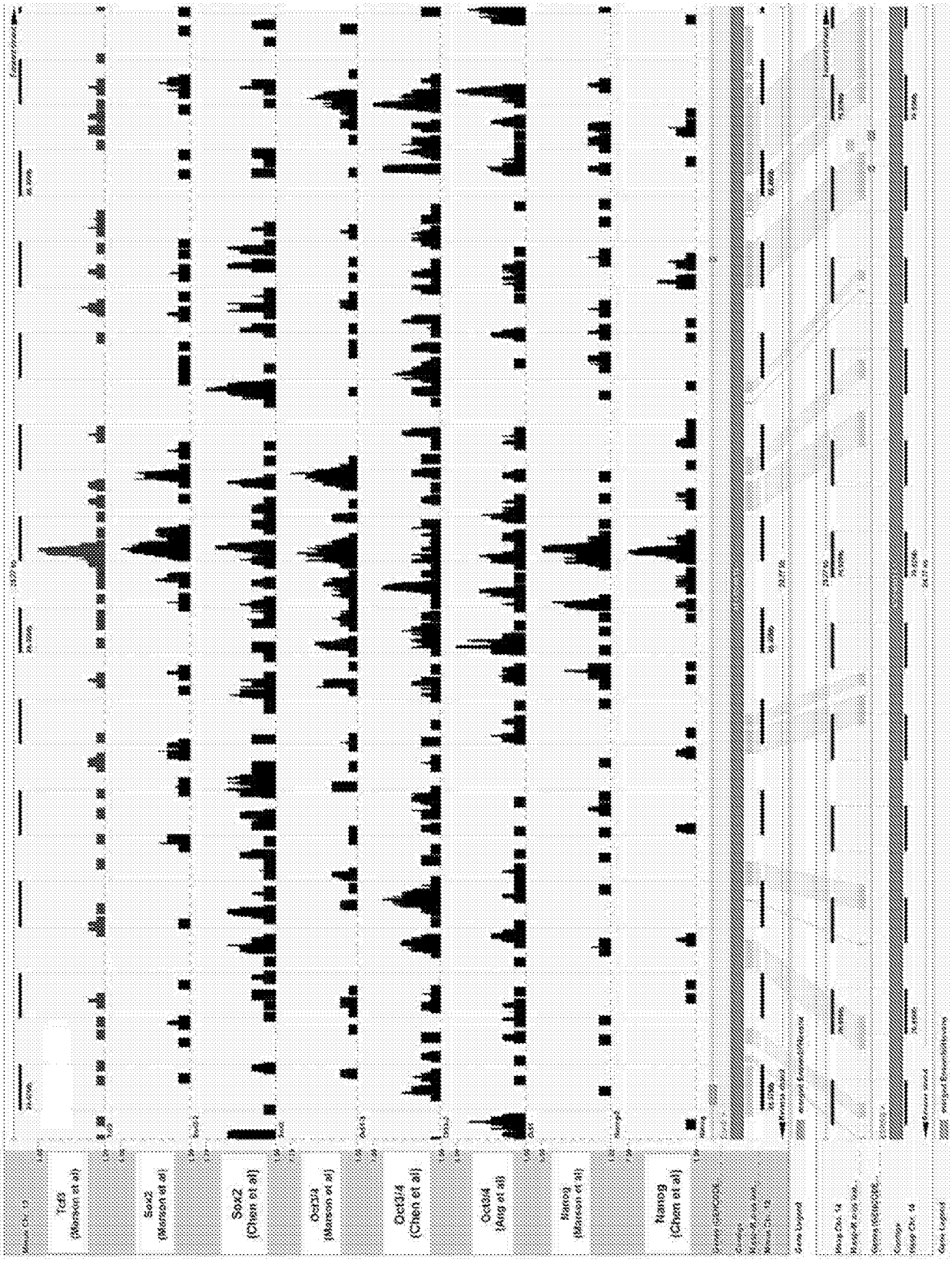

Tcf3
(Marson et al)
Sox2
(Marson et al)
Sox2
(Chen et al)
Oct3/4
(Marson et al)
Oct3/4
(Chen et al)
Oct3/4
(Aug et al)
Nanog
(Marson et al)
Nanog
(Chen et al)

Figure 29

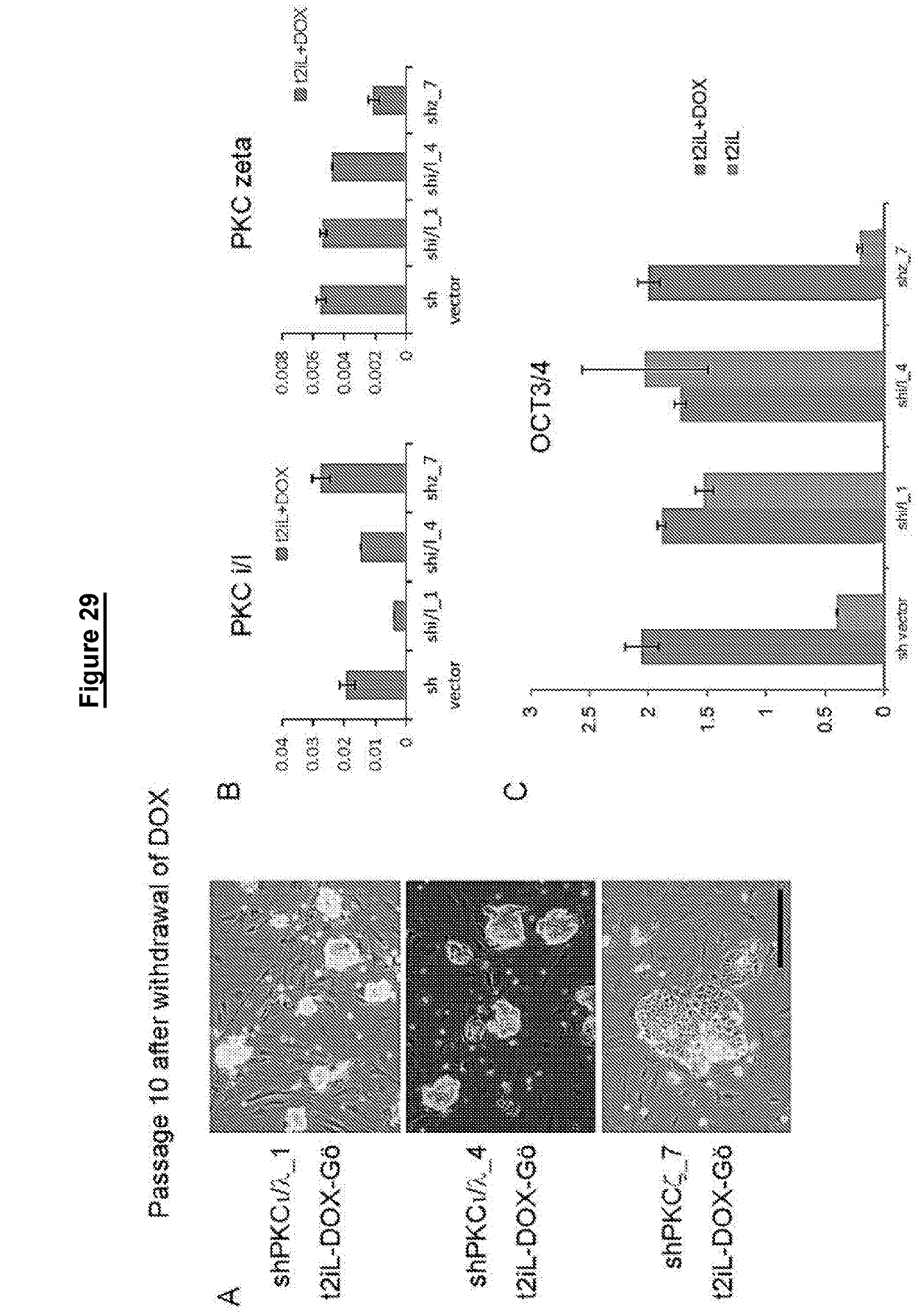

Passage 10 after withdrawal of DOX
A
shPKCι/λ_1
t2iL-DOX-Gö
shPKCι/λ_4
t2iL-DOX-Gö
shPKCζ_7
t2iL-DOX-Gö
B
PKC i/l
t2iL+DOX
0.04
0.03
0.02
0.01
0
sh vector
shi/l_1
shi/l_4
shz_7
PKC zeta
t2iL+DOX
0.008
0.006
0.004
0.002
0
sh vector
shi/l_1
shi/l_4
shz_7
C
OCT3/4
t2iL+DOX
t2iL
3
2.5
2
1.5
1
0.5
0
sh vector
shi/l_1
shi/l_4
shz_7

Figure 30

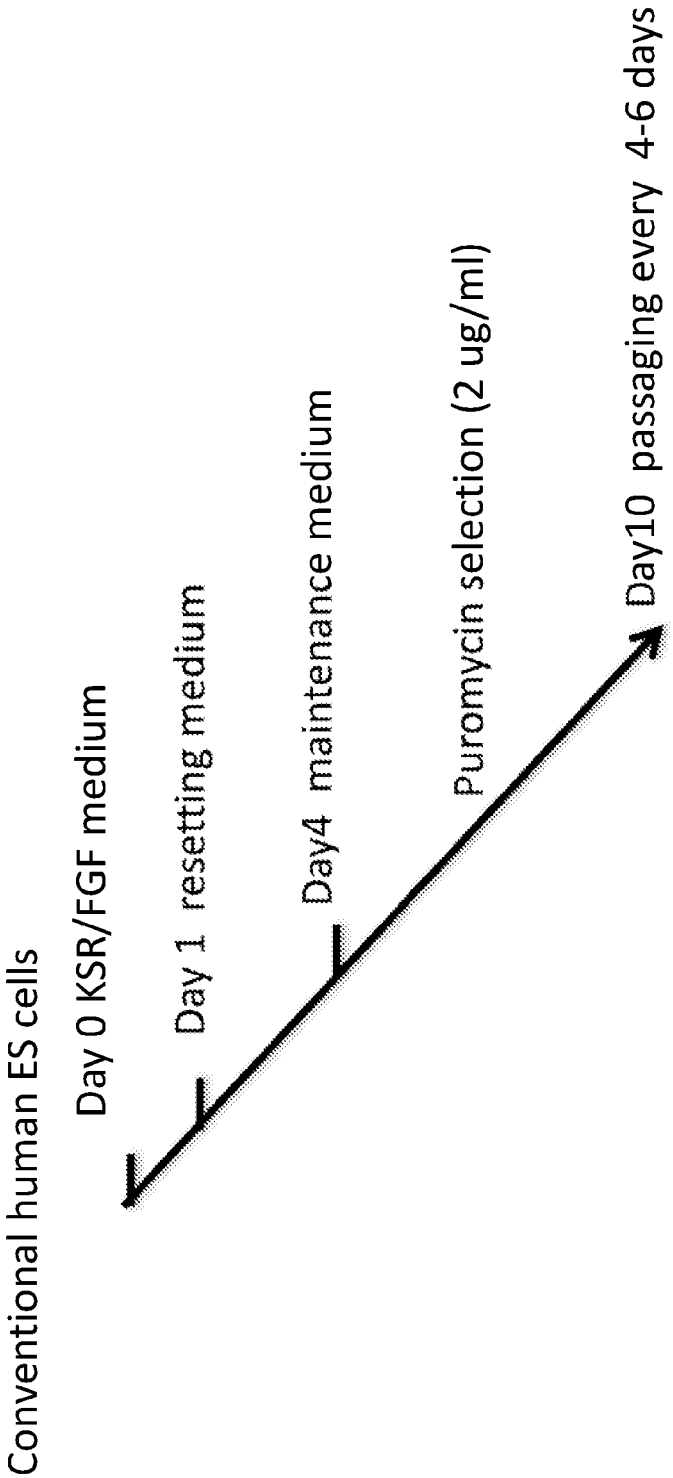

Conventional human ES cells
Day 0 KSR/FGF medium
Day 1 resetting medium
Day4 maintenance medium
Puromycin selection (2 ug/ml)
Day10 passaging every 4-6 days

Figure 31

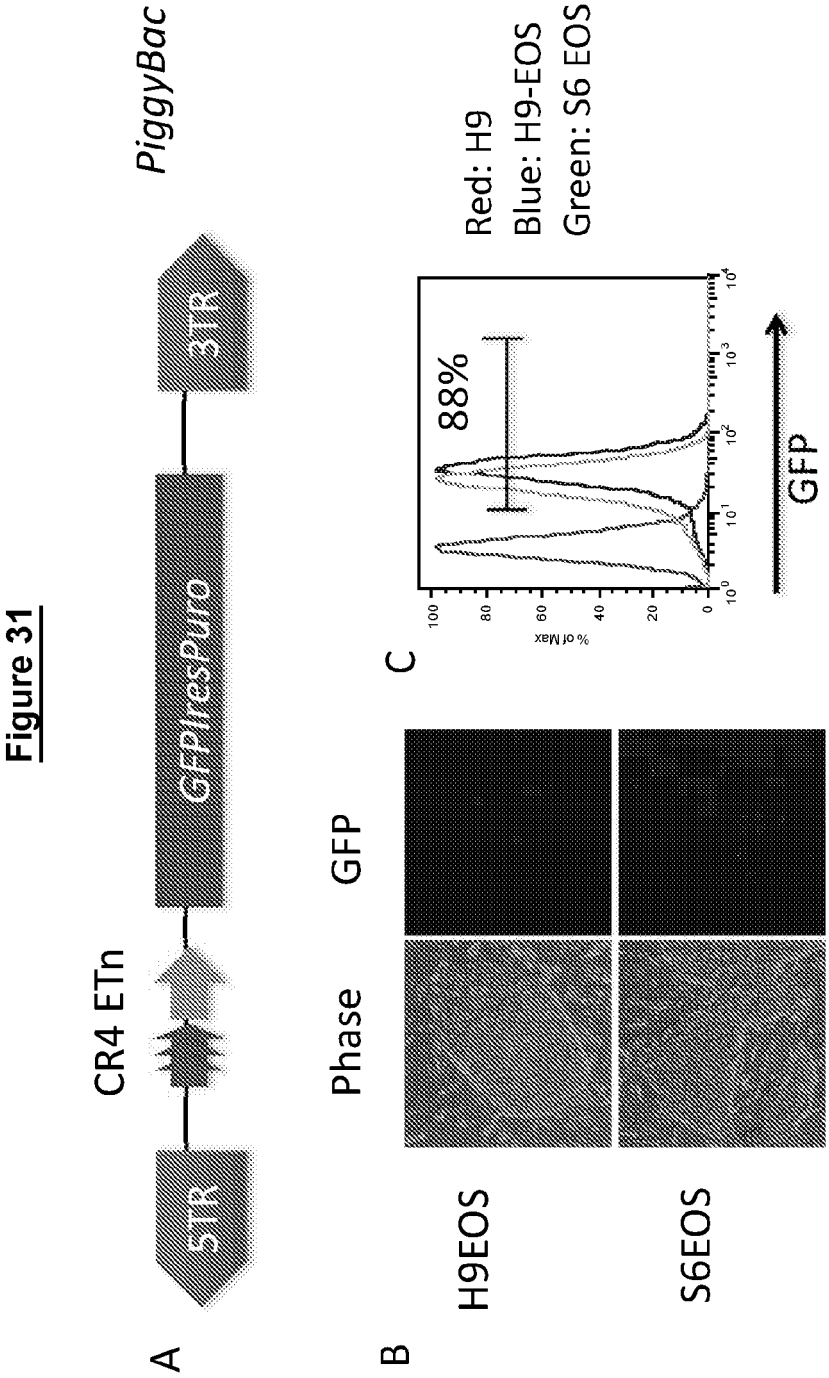

A
PiggyBac
5TR
CR4 ETn
GFPIresPuro
3TR
B
Phase
GFP
H9EOS
S6EOS
C
Red: H9
Blue: H9-EOS
Green: S6 EOS
88%
% of Max
GFP

Figure 32

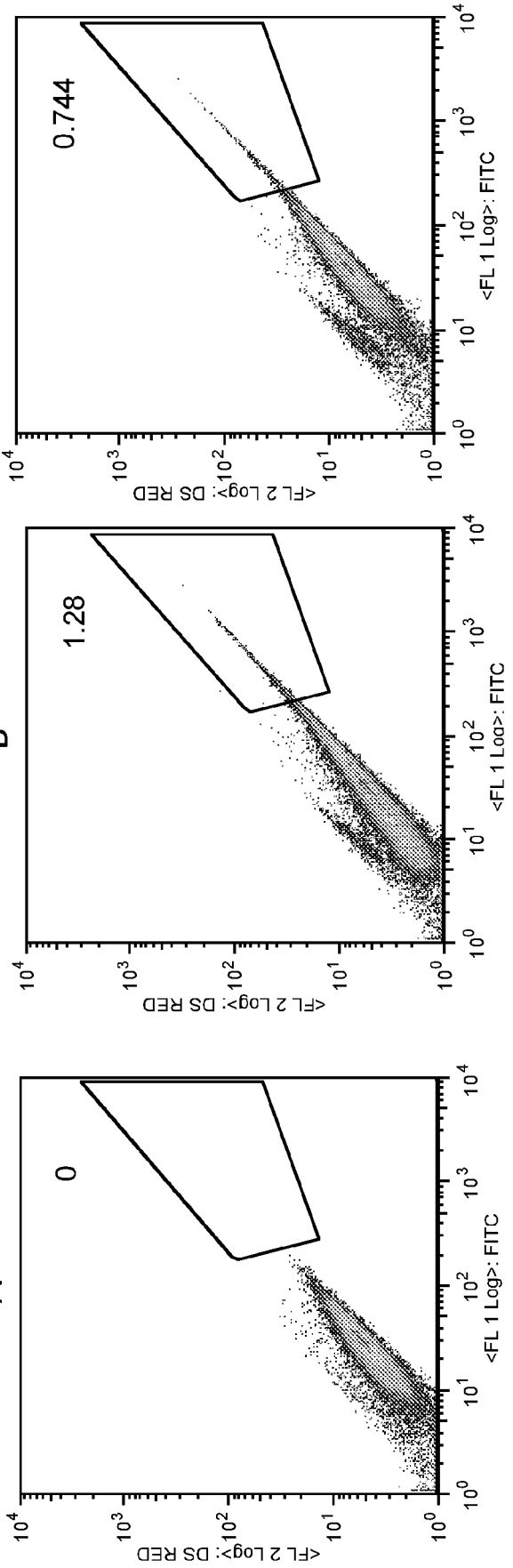


Figure 33

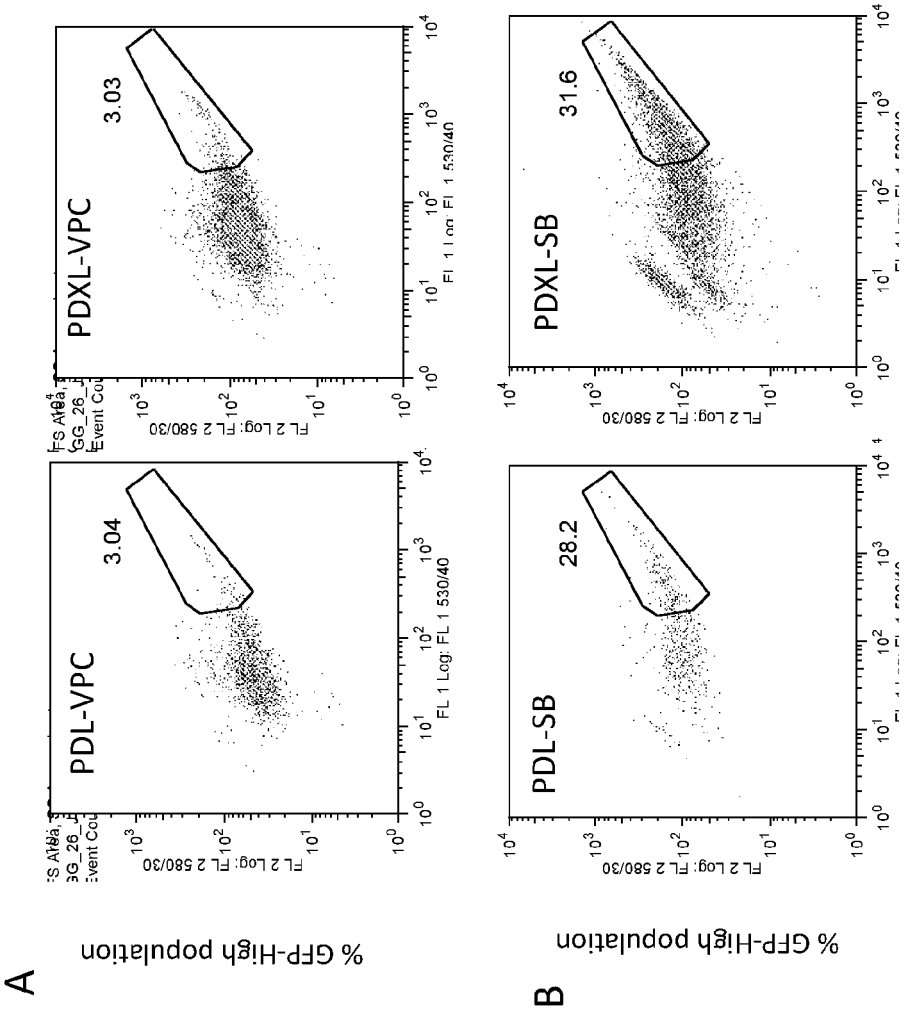

A
% GFP-High population
PDL-VPC
3.04
PDXL-VPC
3.03
B
% GFP-High population
PDL-SB
28.2
PDXL-SB
31.6
FL 2 Log: FL 2 580/30
FL 1 Log: FL 1 530/40

Figure 34

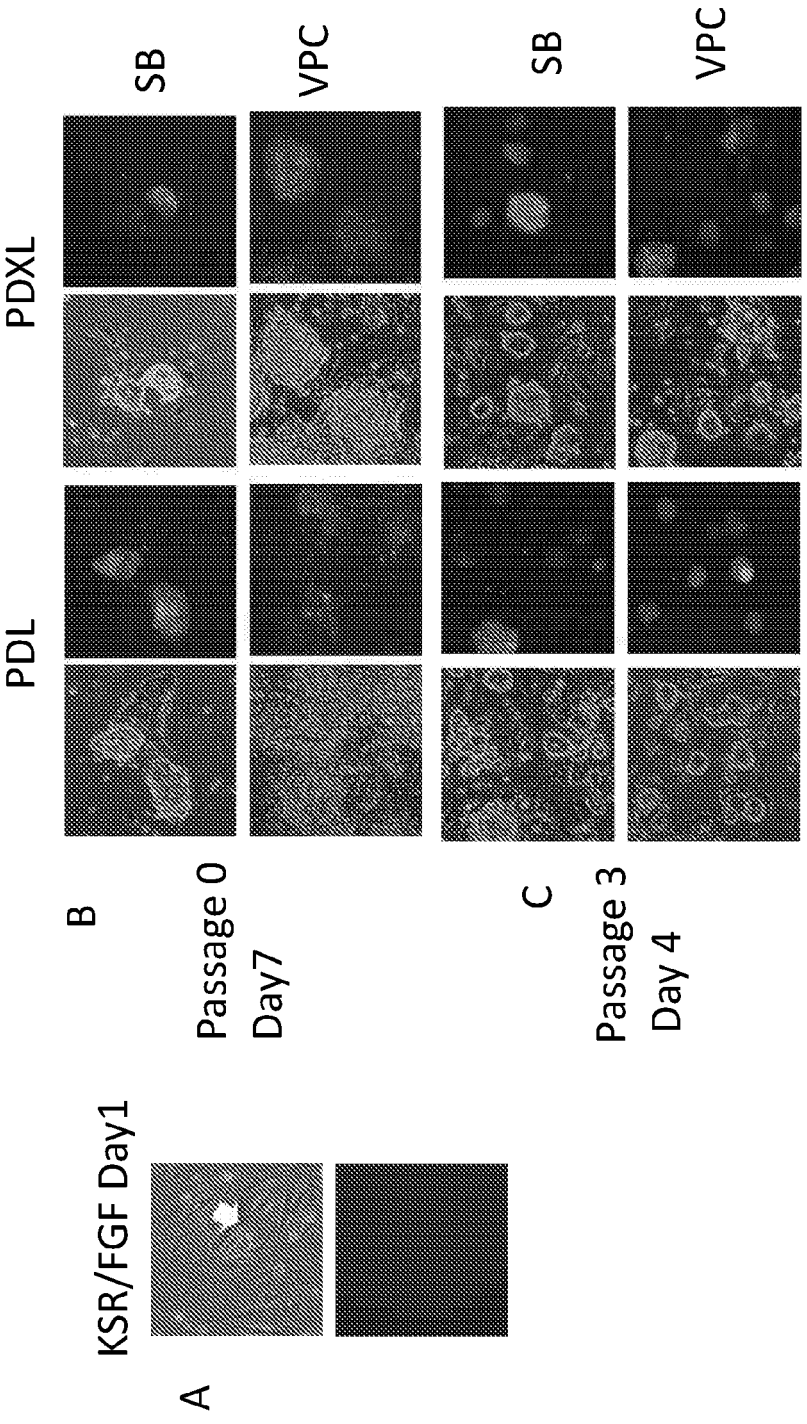

A
KSR/FGF Day1
B
Passage 0
Day7
PDL
PDXL
SB
VPC
C
Passage 3
Day 4
SB
VPC

Figure 35

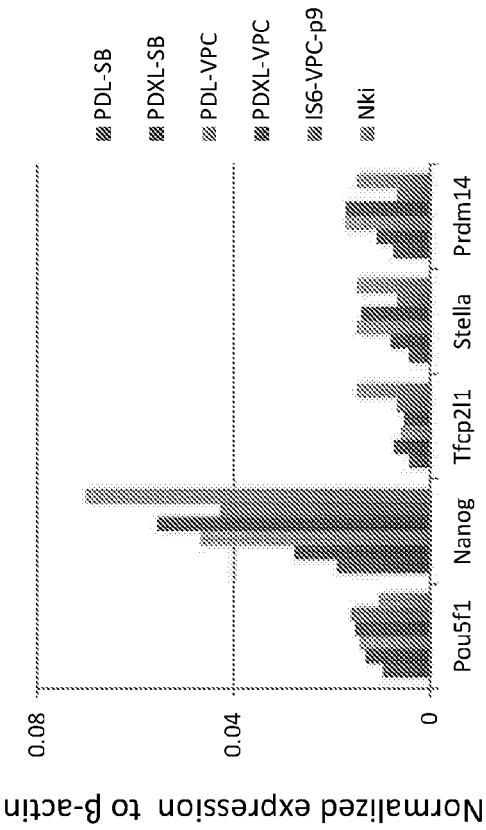
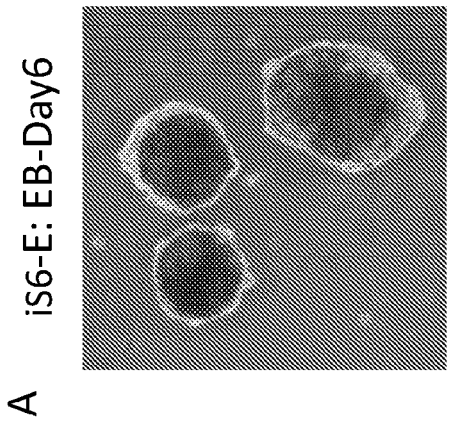

PDL-SB
PDXL-SB
PDL-VPC
PDXL-VPC
IS6-VPC-p9
Nki
0.08
0.04
0
Pou5f1
Nanog
Tfcp2l1
Stella
Prdm14
Normalized expression to β-actin

Figure 36


A
iS6-E: EB-Day6

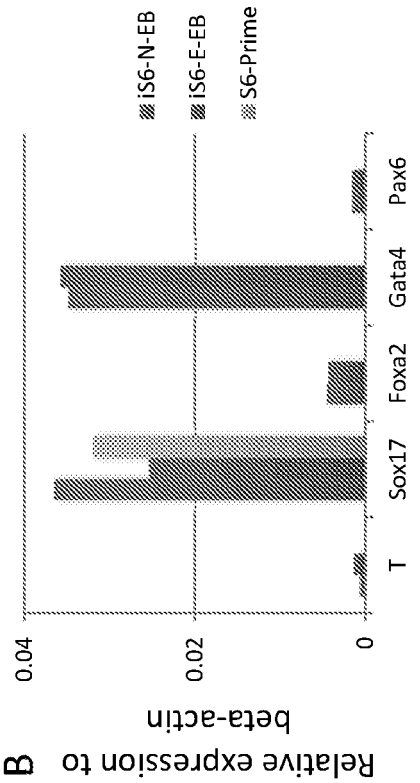

B
Relative expression to beta-actin
0.04
0.02
0
T
Sox17
Foxa2
Gata4
Pax6
iS6-N-EB
iS6-E-EB
S6-Prime

Figure 37

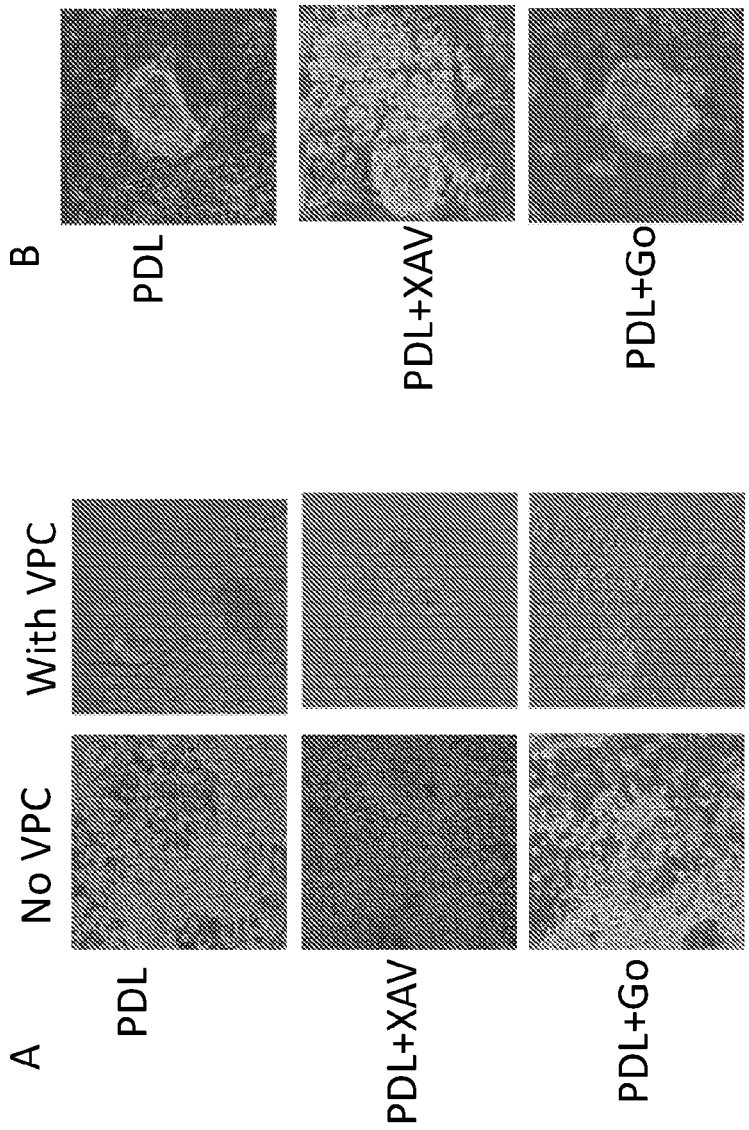


Figure 38

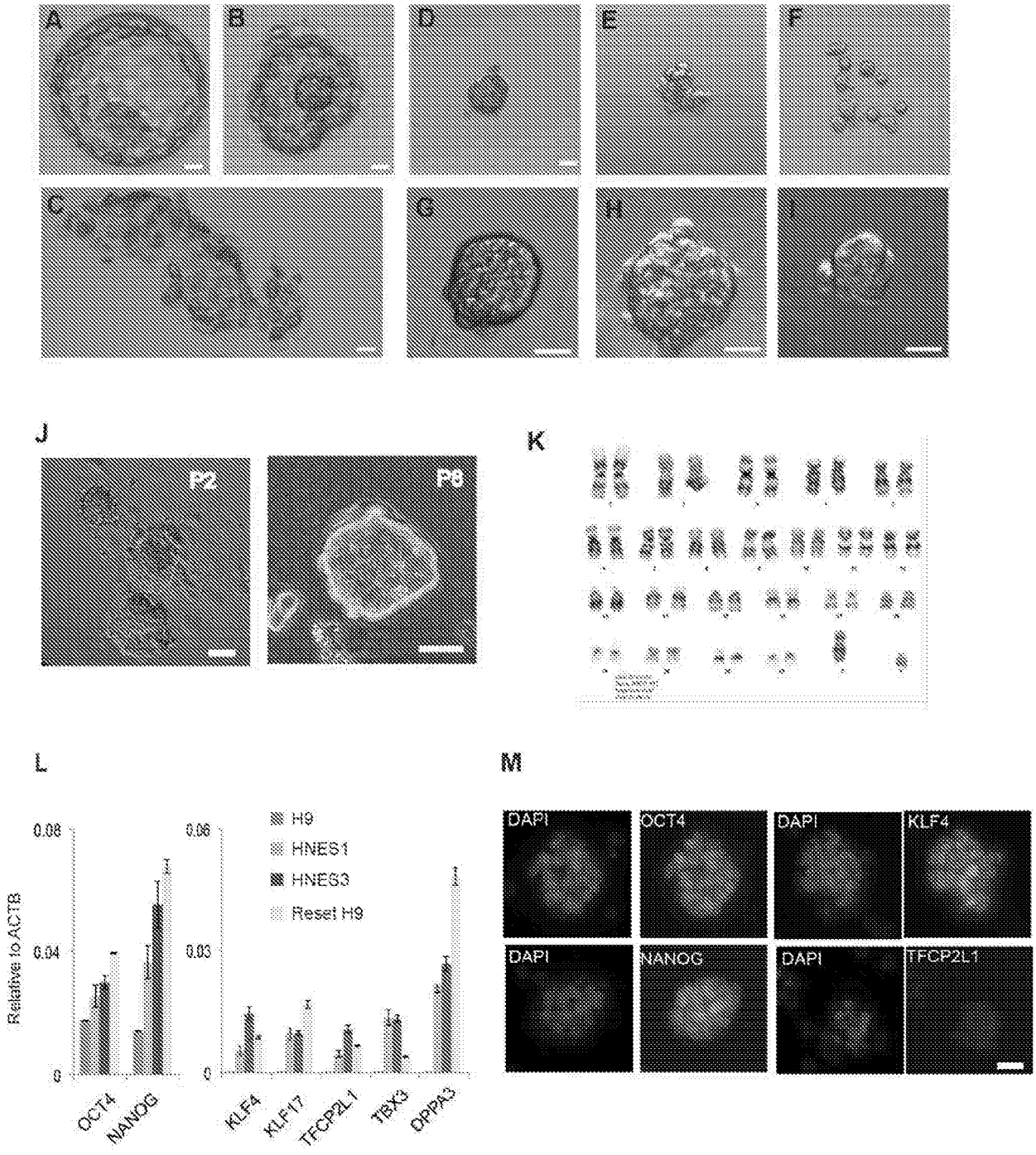

A
B
D
E
F
C
G
H
I
J
P2
P8
K
L
Relative to ACTB
0.08
0.04
0
0.06
0.03
0
H9
HNES1
HNES3
Reset H9
OCT4
NANOG
KLF4
KLF17
TFCP2L1
TBX3
DPPA3
M
DAPI
OCT4
DAPI
KLF4
DAPI
NANOG
DAPI
TFCP2L1

Figure 39

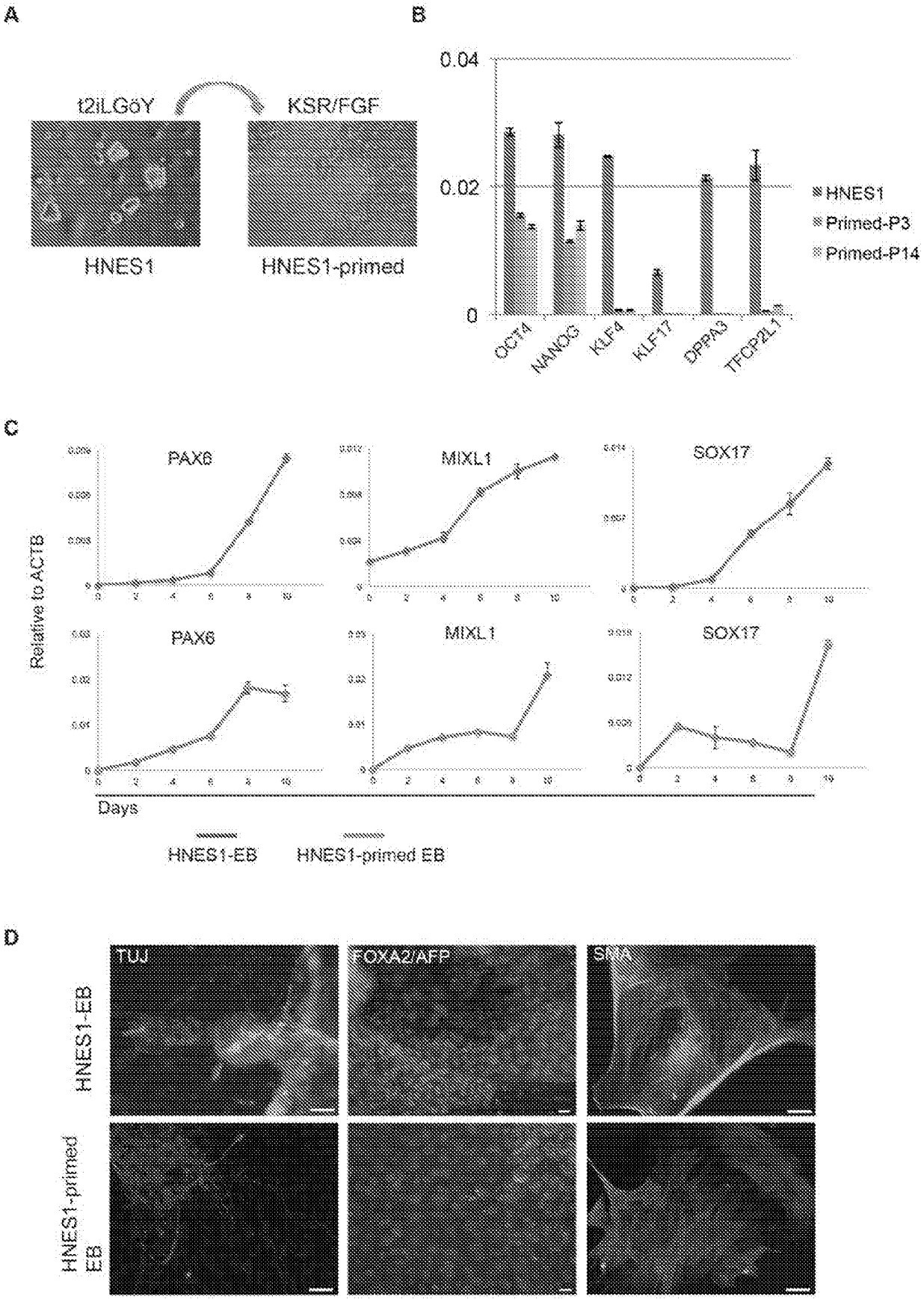

A
t2iLGöY
KSR/FGF
HNES1
HNES1-primed
B
0.04
0.02
0
HNES1
Primed-P3
Primed-P14
OCT4
NANOG
KLF4
KLF17
DPPA3
TFCP2L1
C
PAX6
MIXL1
SOX17
Relative to ACTB
Days
HNES1-EB
HNES1-primed EB
D
TUJ
FOXA2/AFP
SMA
HNES1-EB
HNES1-primed EB

Figure 40

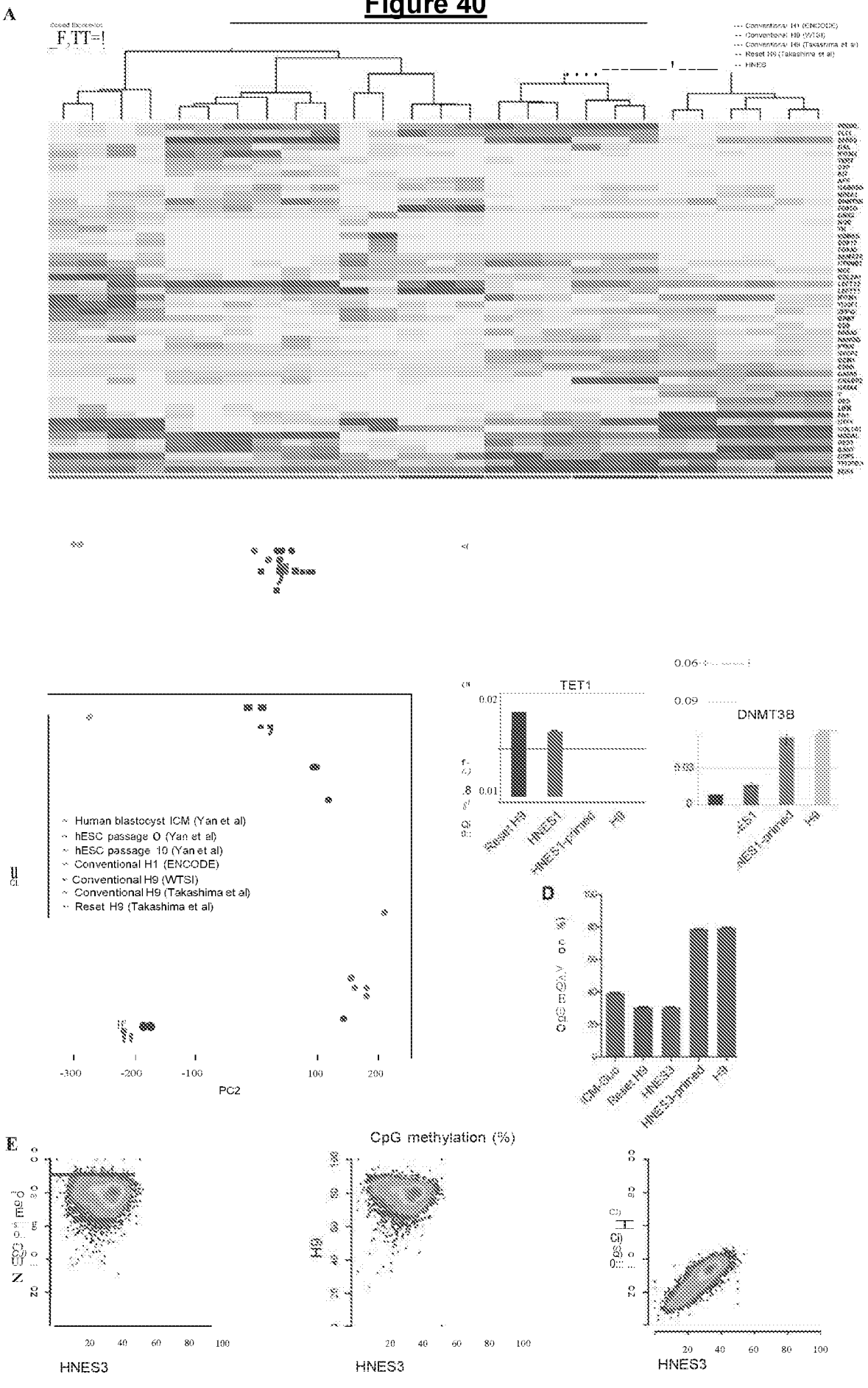

A
Conventional H1 (ENCODE)
Conventional H9 (WTSI)
Conventional H9 (Takashima et al)
Reset H9 (Takashima et al)
HNES3
Human blastocyst ICM (Yan et al)
hESC passage 0 (Yan et al)
hESC passage 10 (Yan et al)
Conventional H1 (ENCODE)
Conventional H9 (WTSI)
Conventional H9 (Takashima et al)
Reset H9 (Takashima et al)
PC2
-300
-200
-100
100
200
TET1
DNMT3B
Reset H9
HNES1
HNES1-primed
H9
D
ICM-Blue
Reset H9
HNES3
HNES1-primed
H9
E
CpG methylation (%)
HNES3
H9
HNES3
HNES3

Figure 41

