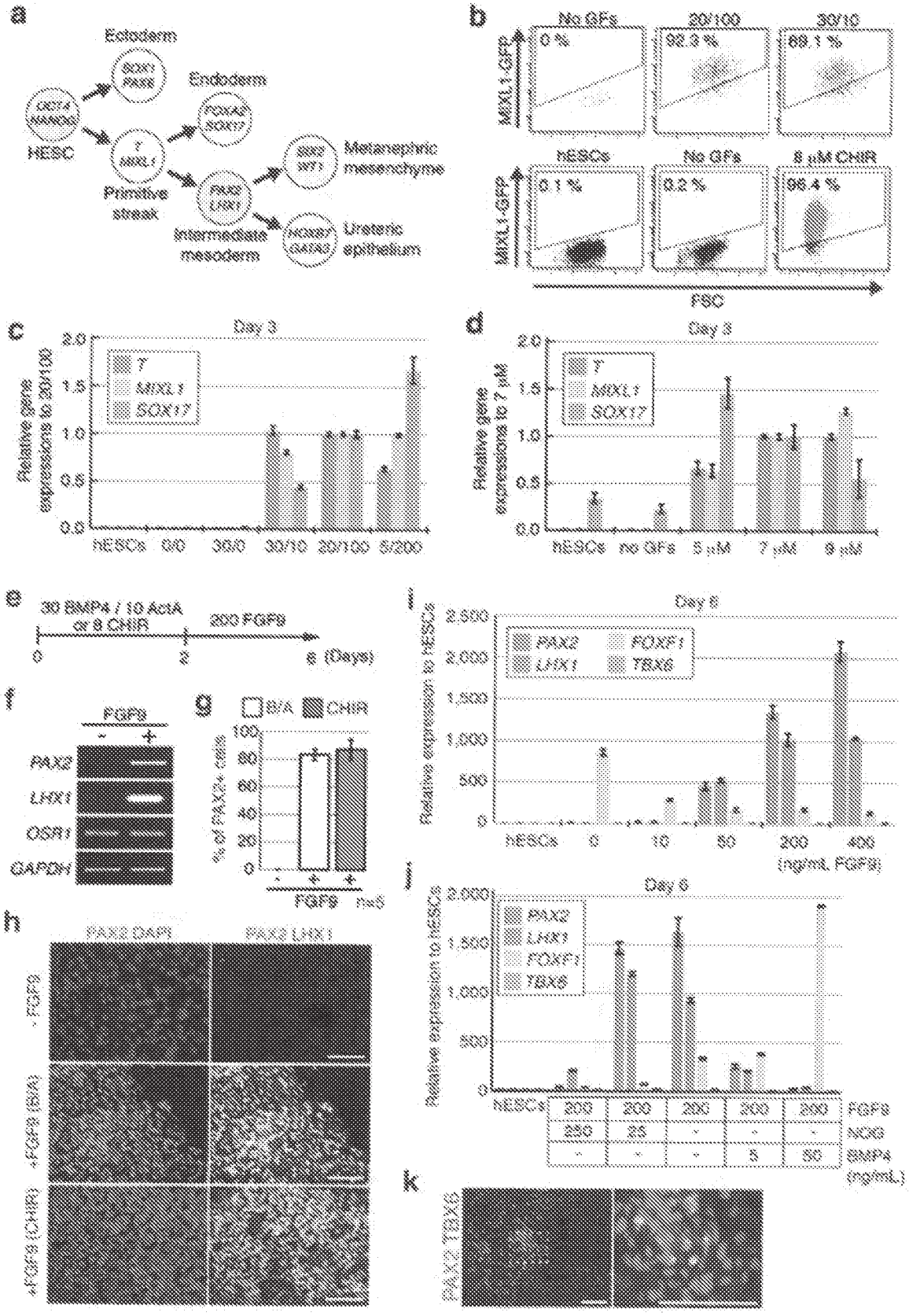

a
Ectoderm
SOX1
PAX6
Endoderm
FOXA2
SOX17
OCT4
NANOG
HESC
T
MIXL1
Primitive streak
PAX2
LHX1
SIX2
WT1
Metanephric mesenchyme
Intermediate mesoderm
HOXB7
GATA3
Ureteric epithelium
b
No GFs
20/100
30/10
0 %
92.3 %
68.1 %
MIXL1-GFP
hESCs
No GFs
8 μM CHIR
0.1 %
0.2 %
96.4 %
FSC
c
Day 3
Relative gene expressions to 30/100
T
MIXL1
SOX17
hESCs 0/0 30/0 30/10 30/100 8/200
d
Day 3
Relative gene expressions to 7 μM
hESCs no GFs 5 μM 7 μM 9 μM
e
30 BMP4 / 10 ActA or 8 CHIR
200 FGF8
0
2
6 (Days)
f
FGF8
PAX2
LHX1
OSR1
GAPDH
g
B/A
CHIR
% of PAX2+ cells
FGF8
h
PAX2 DAPI
PAX2 LHX1
- FGF8
+FGF8 (B/A)
+FGF8 (CHIR)
i
Day 6
Relative expression to hESCs
PAX2
LHX1
FOXF1
TBX6
hESCs 0 10 50 200 400
(ng/mL FGF8)
j
Day 6
Relative expression to hESCs
PAX2
LHX1
FOXF1
TBX6
hESCs
200 200 200 200 200 FGF8
250 25 - - - NOG
- - - 5 50 BMP4
(ng/mL)
k
PAX2 TBX6

FIG. 1

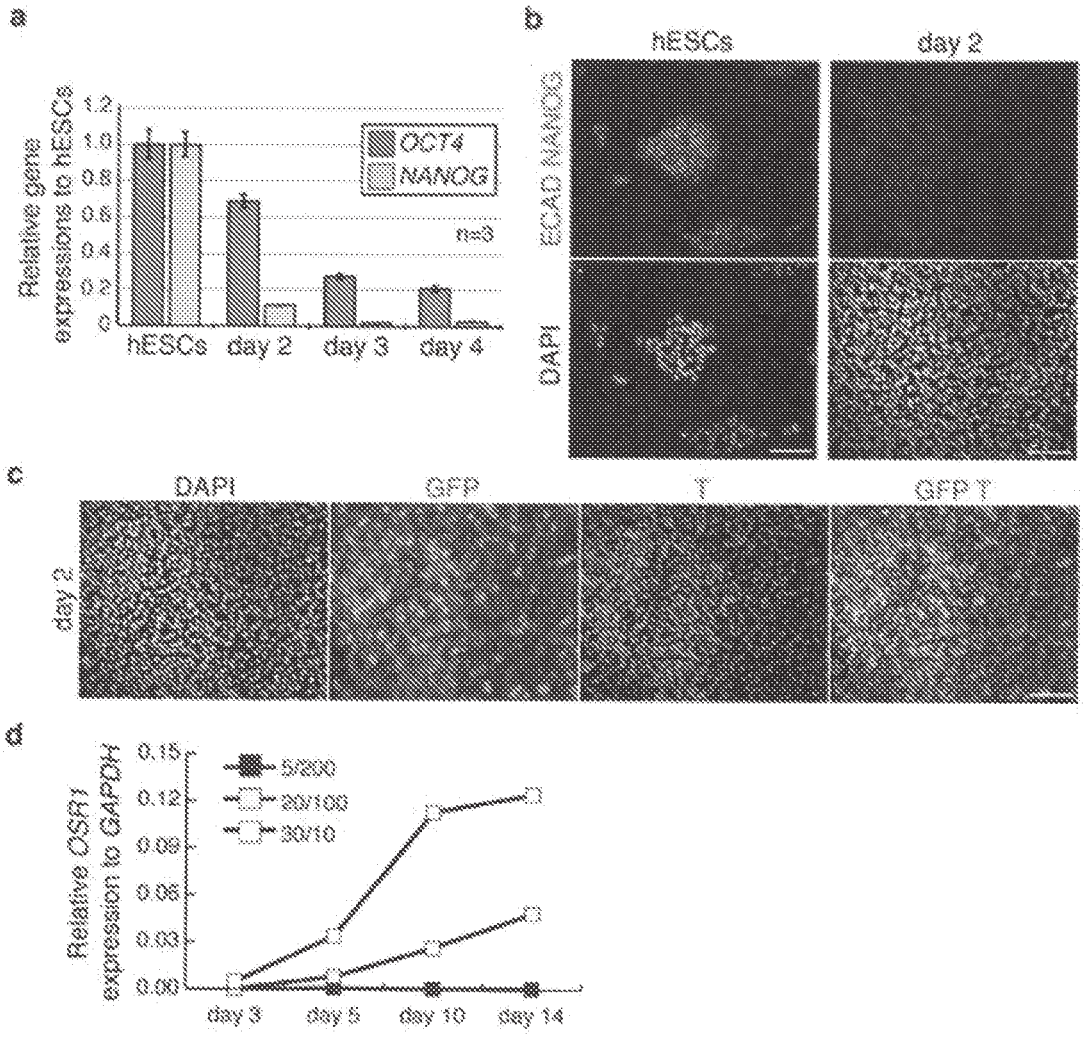
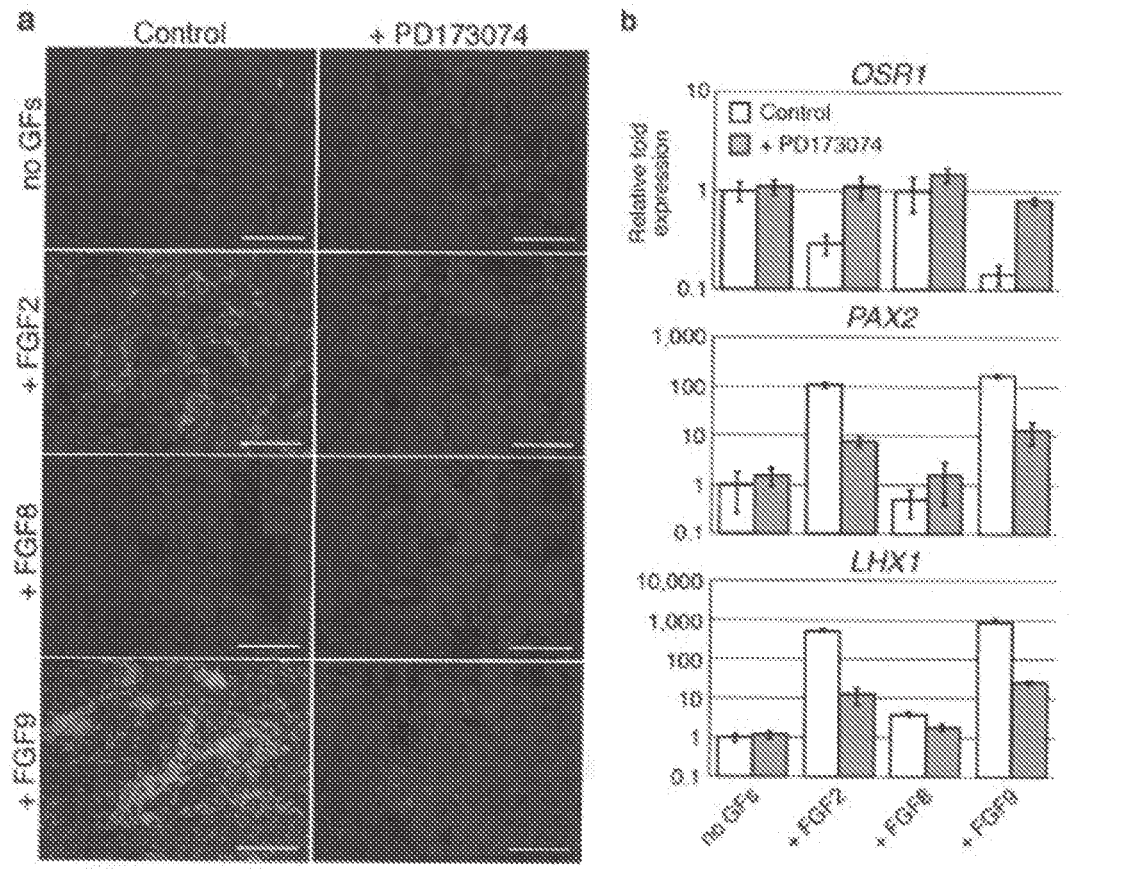

a
Relative gene expressions to hESCs
1.2
1.0
0.8
0.6
0.4
0.2
0
OCT4
NANOG
n=3
hESCs
day 2
day 3
day 4
b
hESCs
day 2
ECAD NANOG
DAPI
c
DAPI
GFP
T
GFP T
day 2
d
Relative OSR1 expression to GAPDH
0.15
0.12
0.09
0.06
0.03
0.00
5/200
20/100
30/10
day 3
day 5
day 10
day 14

FIG. 2



d

	%PAX2⁻	%PAX2⁺	
total	13.6 ± 5.5	86.4 ± 5.5	
%LHX1⁻	13.3 ± 5.2	6.8 ± 2.9	
%LHX1⁺	0.4 ± 0.5	79.5 ± 4.7	(n=5)

FIG. 3

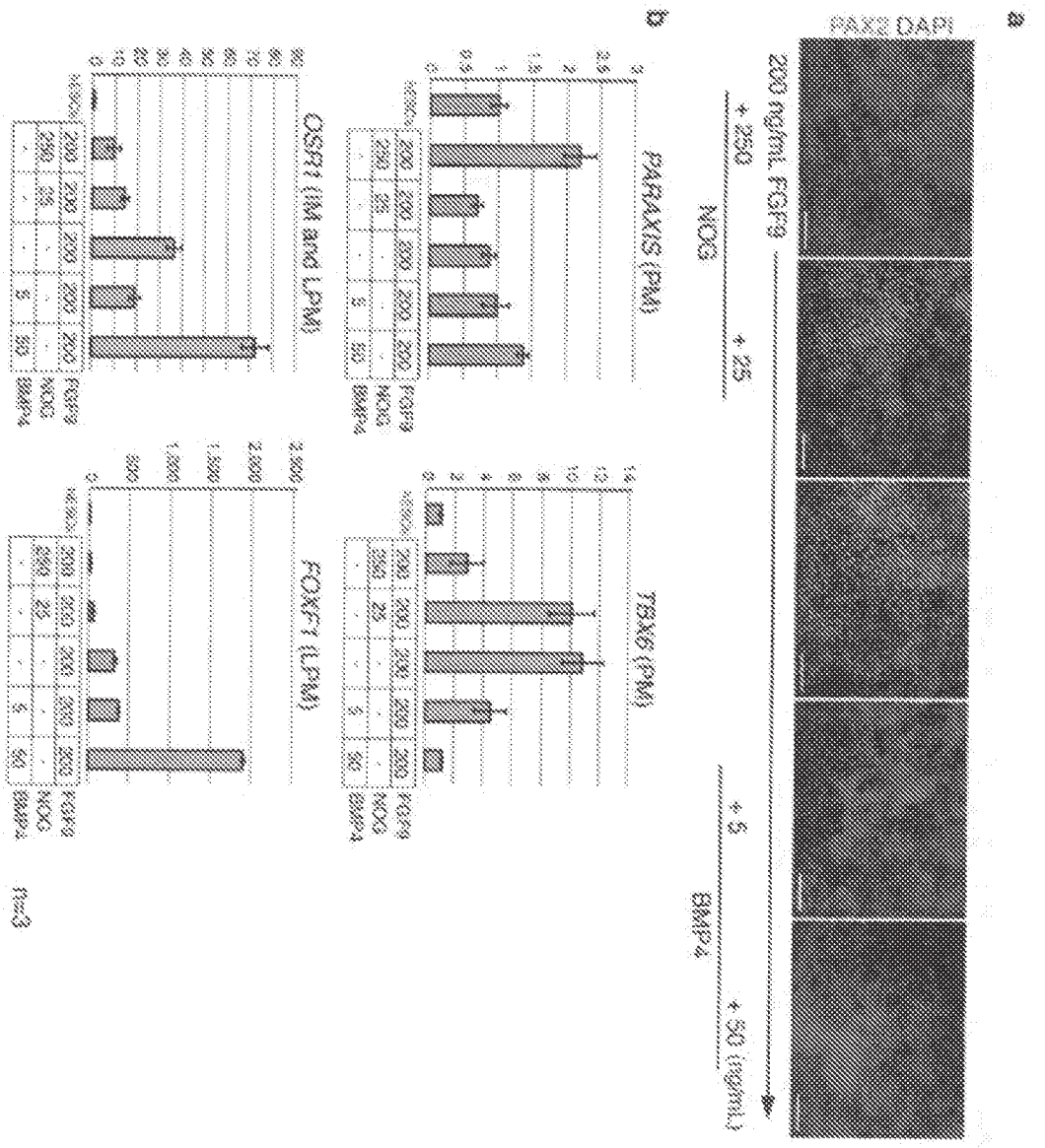

a
PAX3 DAPI
200 ng/mL FGF9
+ 250
NOG
+ 25
BMP4
+ 5
+ 50 (ng/mL)
b
PARAXIS (PM)
TBX6 (PM)
OSR1 (IM and LPM)
FOXF1 (LPM)
n=3

FIG. 4

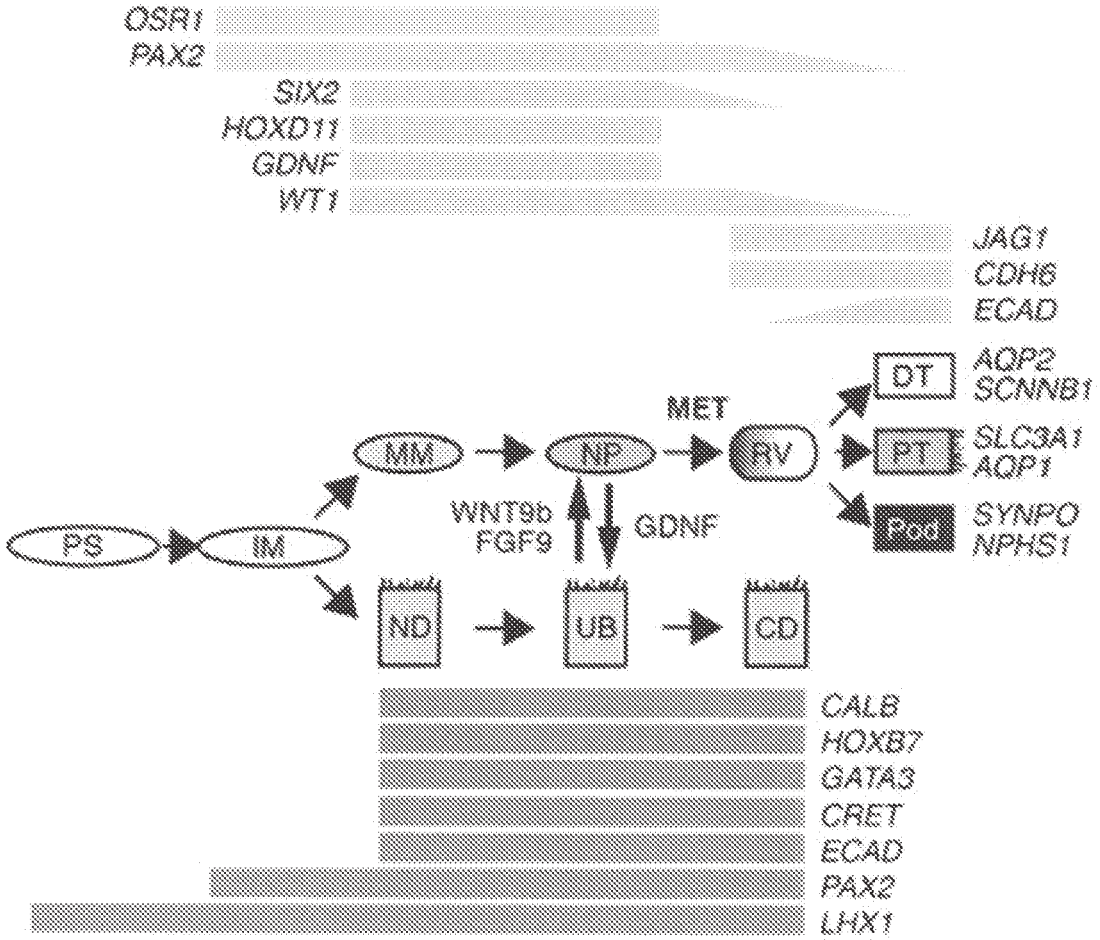

OSR1
PAX2
SIX2
HOXD11
GDNF
WT1
JAG1
CDH6
ECAD
DT
AQP2
SCNN1B
MET
MM
NP
RV
PT
SLC3A1
AQP1
WNT9b
FGF9
GDNF
Pod
SYNPO
NPHS1
PS
IM
ND
UB
CD
CALB
HOXB7
GATA3
CRET
ECAD
PAX2
LHX1

FIG. 5

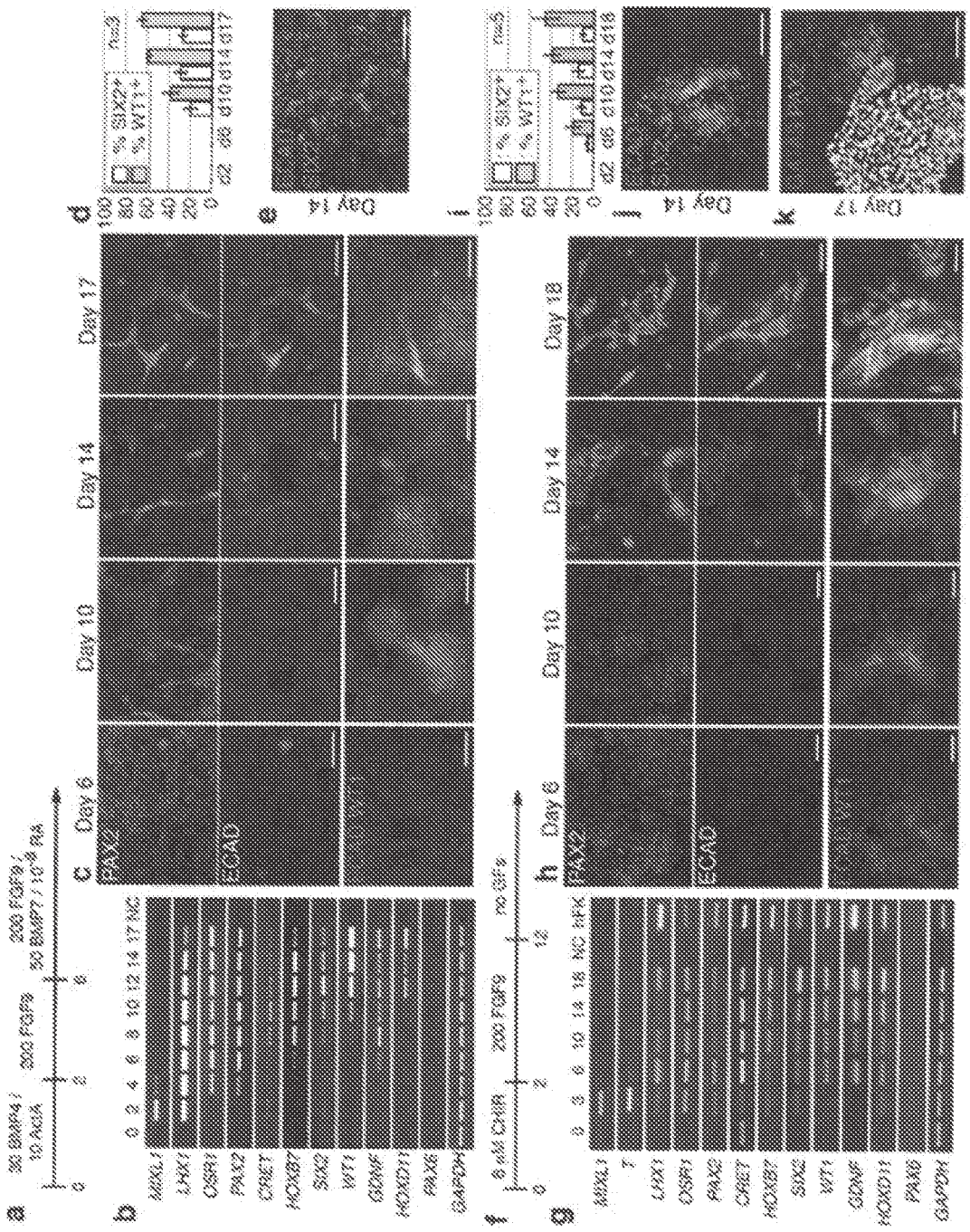

a
30 BMP4 / 10 ActA
200 FGF9
200 FGF9 / 50 BMP7 / 10⁻⁶ RA
0 2 4 6 8 10 12 14 17 NC
b
MIXL1
LHX1
OSR1
PAX2
CRET
HOXB7
SIX2
WT1
GDNF
HOXD11
PAX8
GAPDH
c
Day 6
Day 10
Day 14
Day 17
PAX2
EOAD
d
% SIX2+
% WT1+
n=3
e
Day 14
f
6 uM CHIR
200 FGF9
no GF's
0 2 18
g
0 3 6 10 14 16 NC MPK
T
h
Day 6
Day 10
Day 14
Day 18
i
n=3
j
Day 14
k
Day 17

FIG. 6

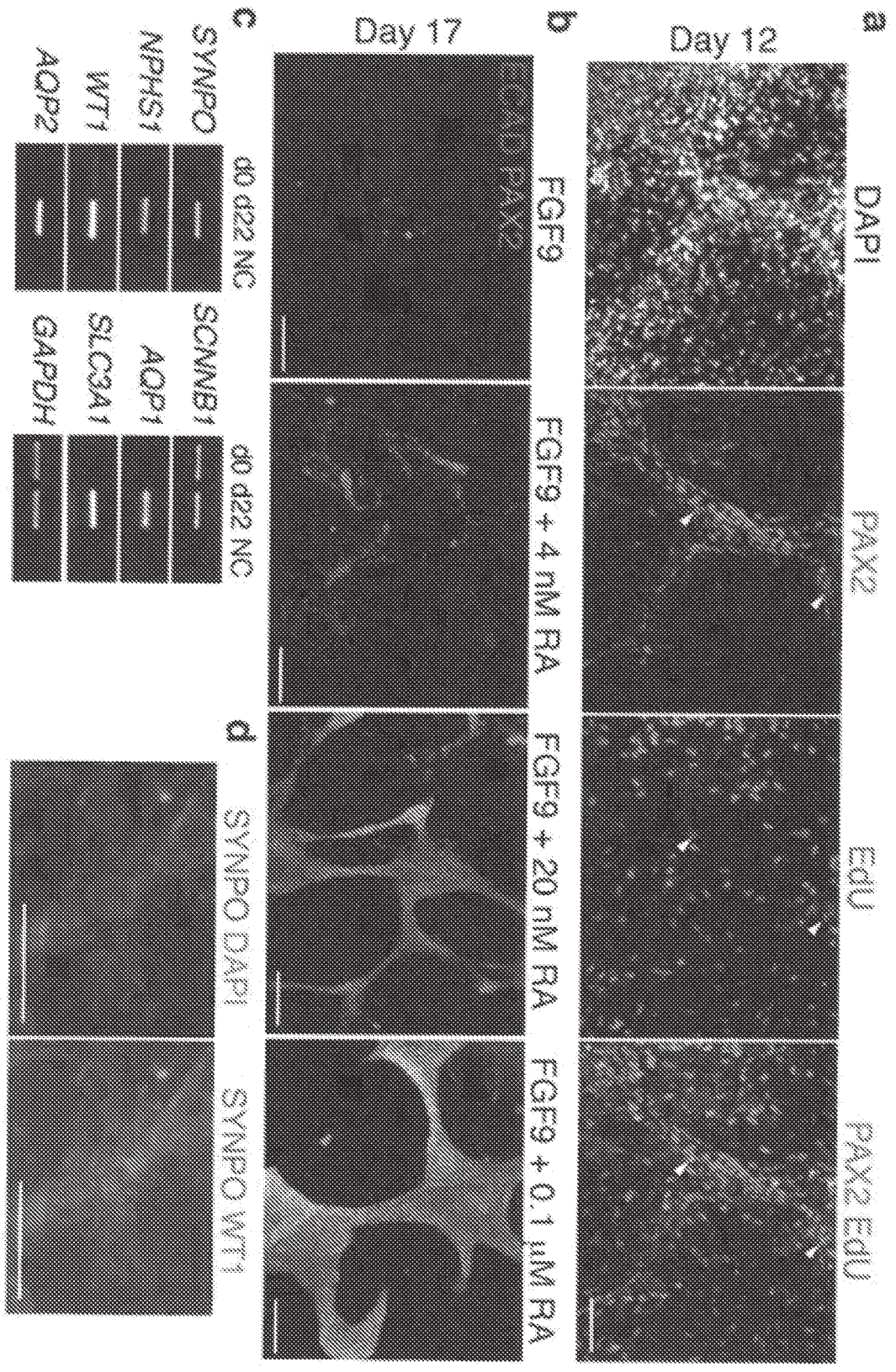

a
Day 12
DAPI
PAX2
EdU
PAX2 EdU
b
Day 17
FGF9
FGF9 + 4 nM RA
FGF9 + 20 nM RA
FGF9 + 0.1 μM RA
c
d0 d22 NC
SYNPO
NPHS1
WT1
AQP2
SCNN1B
AQP1
SLC3A1
GAPDH
d
SYNPO DAPI
SYNPO WT1

FIG. 7

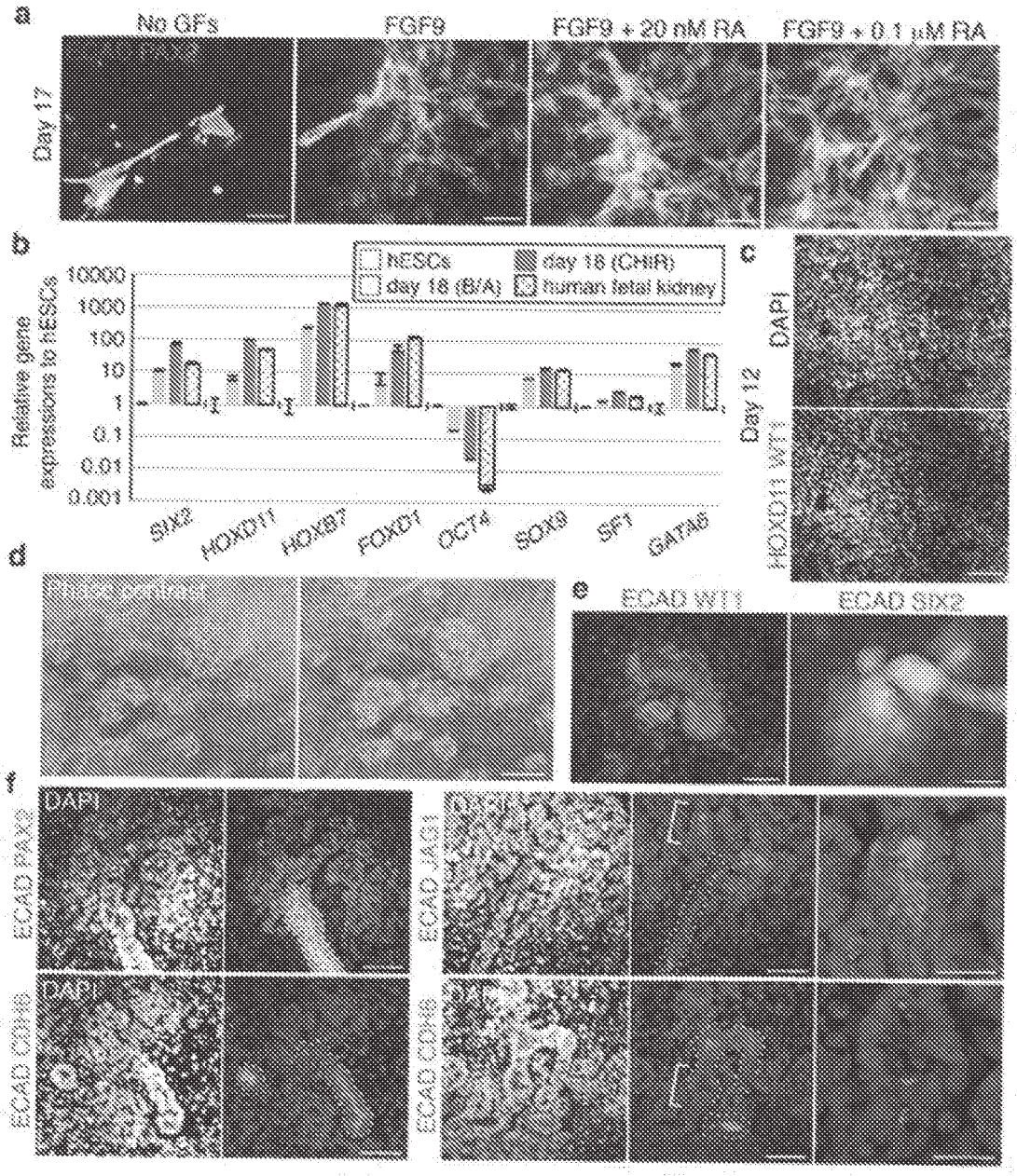

a
No GFs
FGF9
FGF9 + 20 nM RA
FGF9 + 0.1 μM RA
Day 17
b
hESCs
day 18 (B/A)
day 18 (CHIR)
human fetal kidney
Relative gene expressions to hESCs
10000
1000
100
10
1
0.1
0.01
0.001
SIX2
HOXD11
HOXB7
FOXD1
OCT4
SOX9
SF1
GATA6
c
DAPI
Day 12
HOXD11 WT1
d
e
ECAD WT1
ECAD SIX2
f
DAPI
ECAD PAX2
ECAD CDH6
ECAD JAG1
ECAD CDH6

FIG. 8

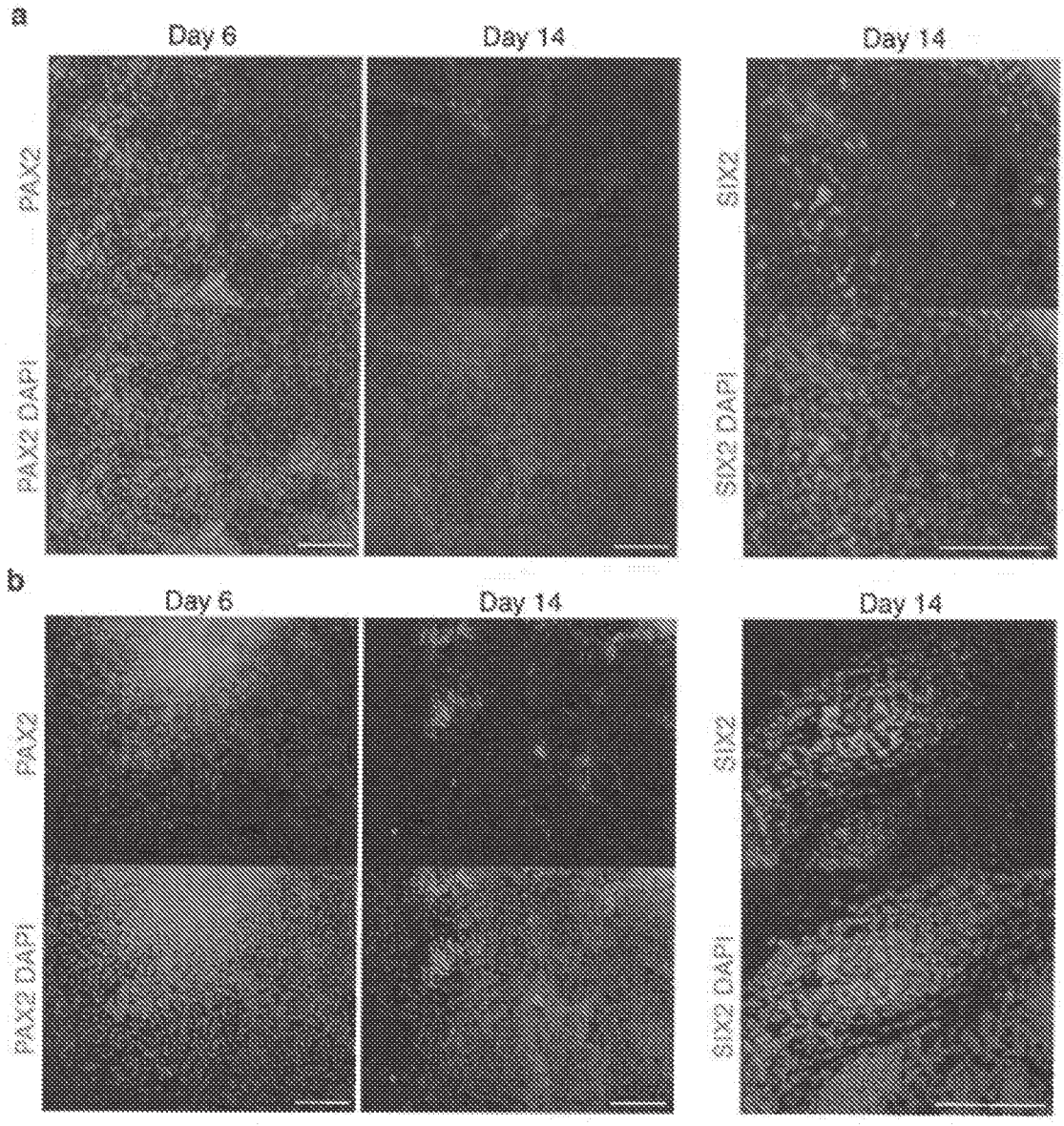

a
Day 6
Day 14
Day 14
PAX2
PAX2 DAPI
SIX2
SIX2 DAPI
b
Day 6
Day 14
Day 14
PAX2
PAX2 DAPI
SIX2
SIX2 DAPI

FIG. 9

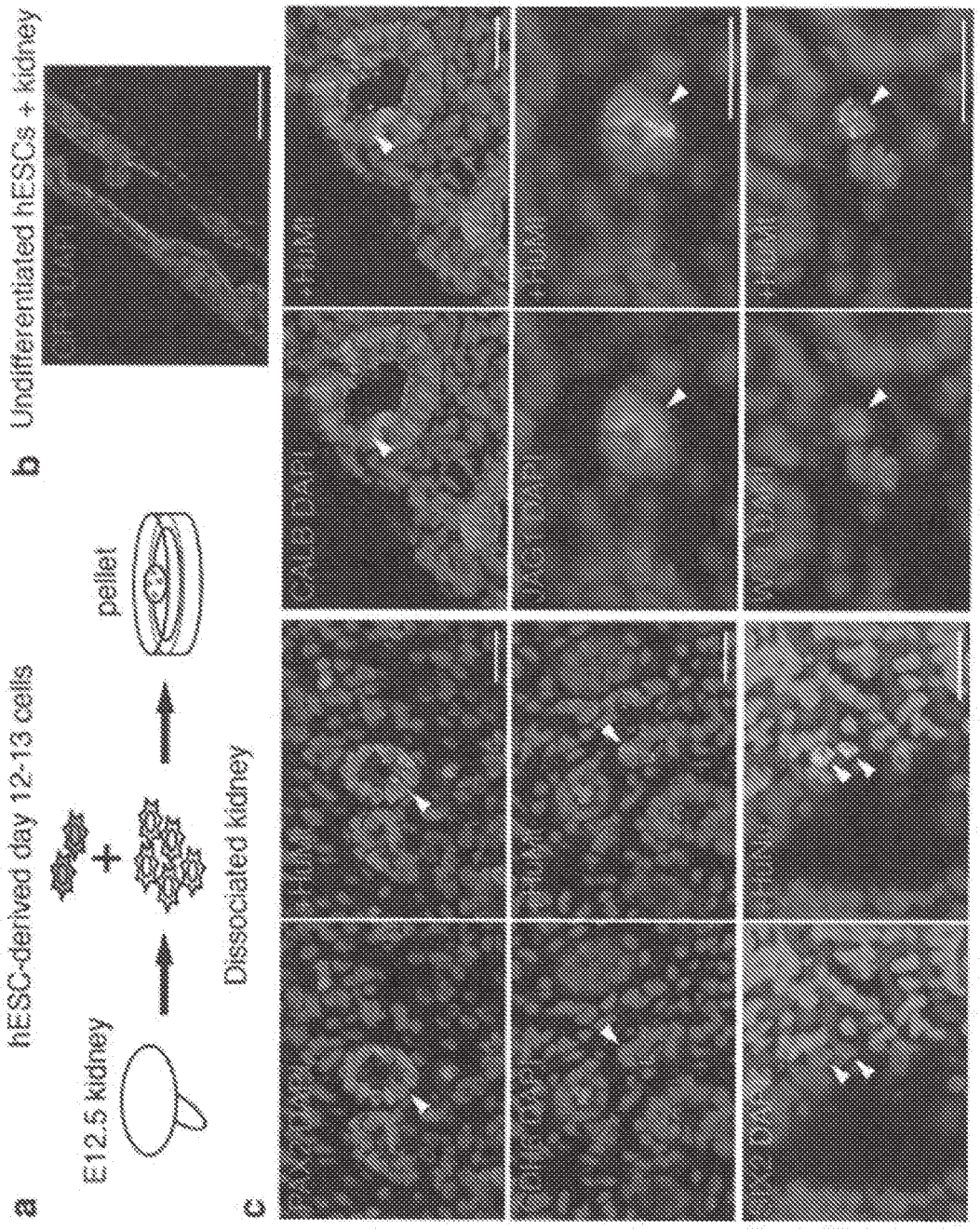

a
hESC-derived day 12-13 cells
E12.5 kidney
pellet
Dissociated kidney
b
Undifferentiated hESCs + kidney
c

FIG. 10

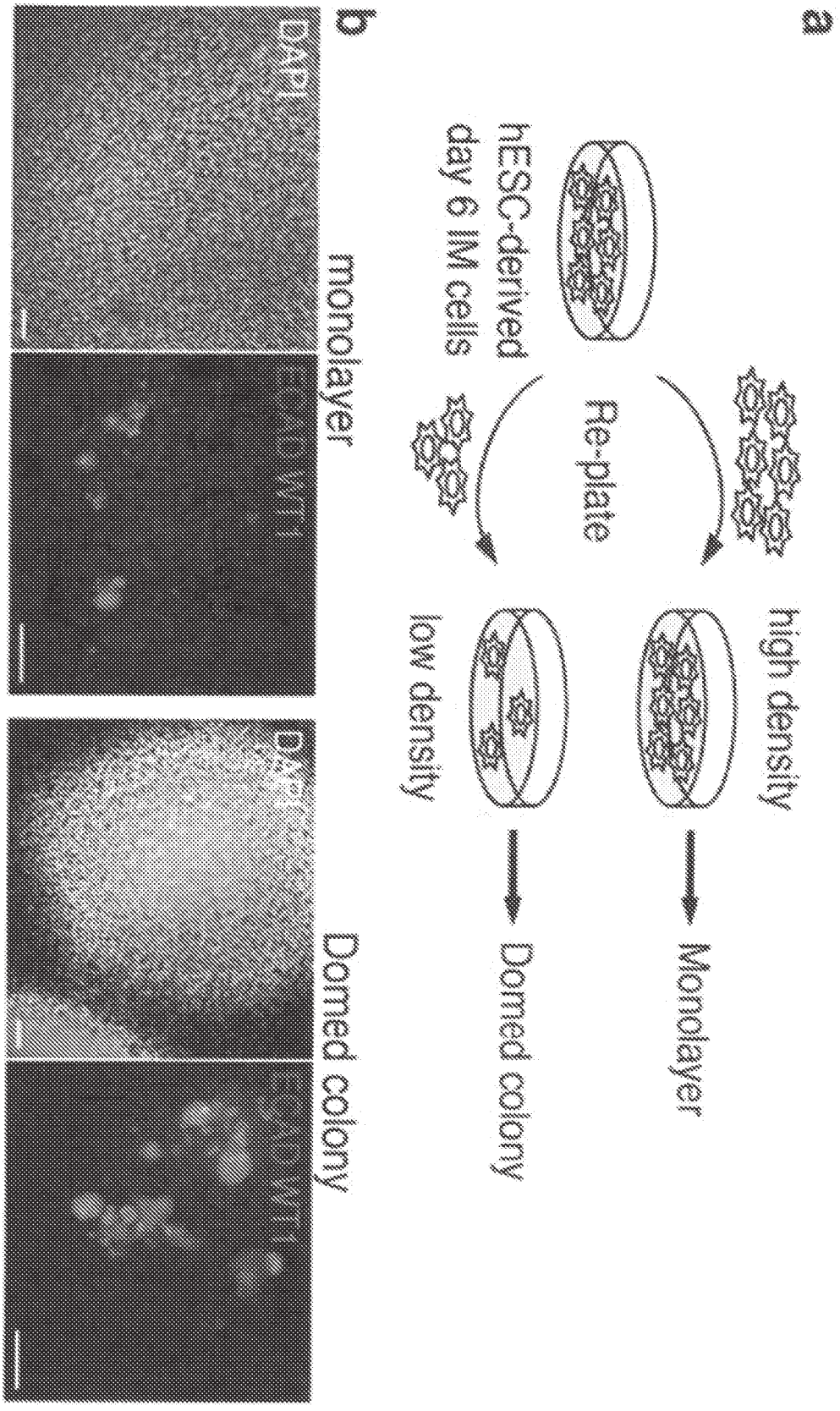

a
hESC-derived day 6 IM cells
Re-plate
high density
low density
Monolayer
Domed colony
b
DAPI
WT1
monolayer
Domed colony

FIG. 11

Figure 12

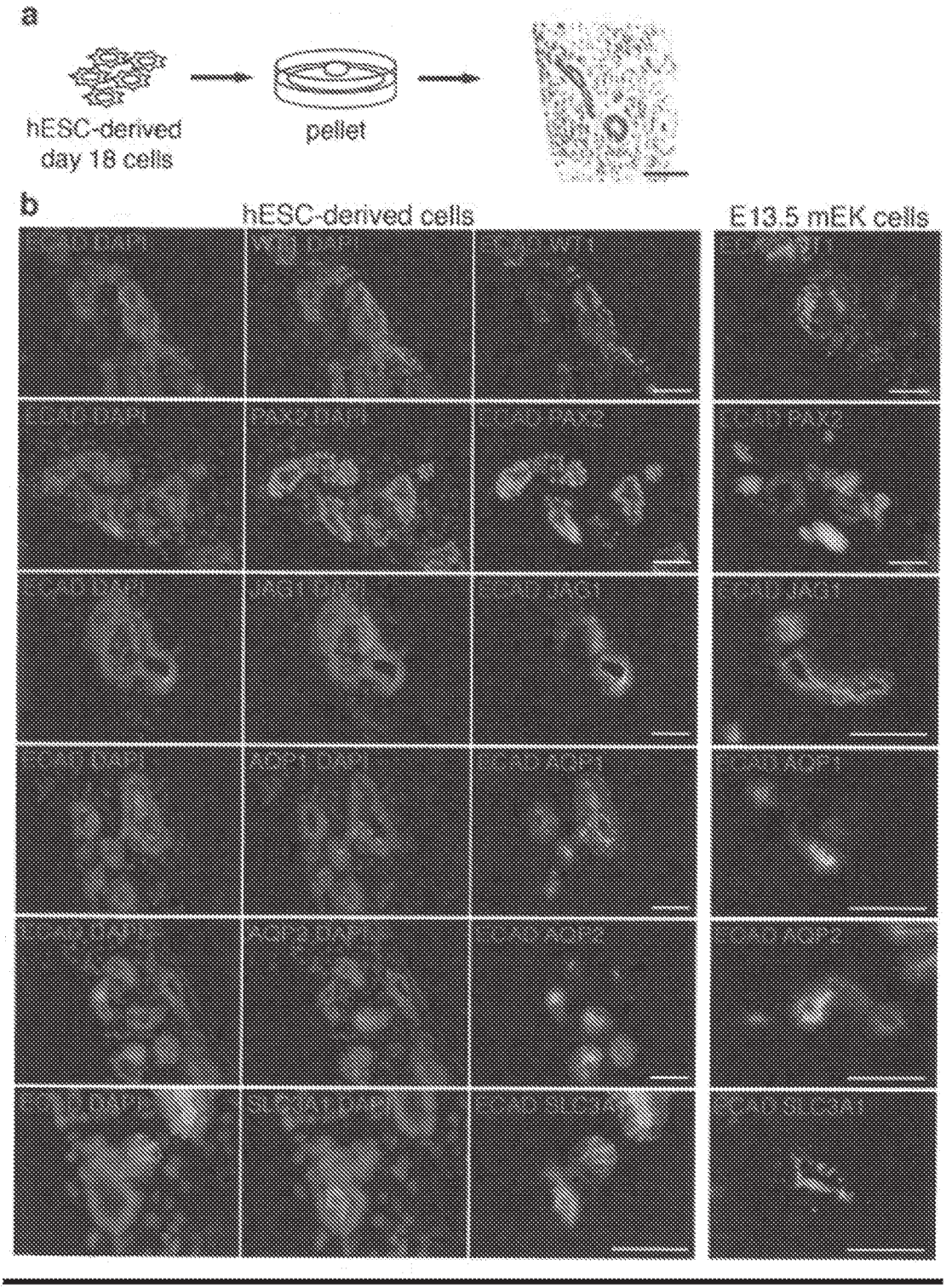


FIG. 12

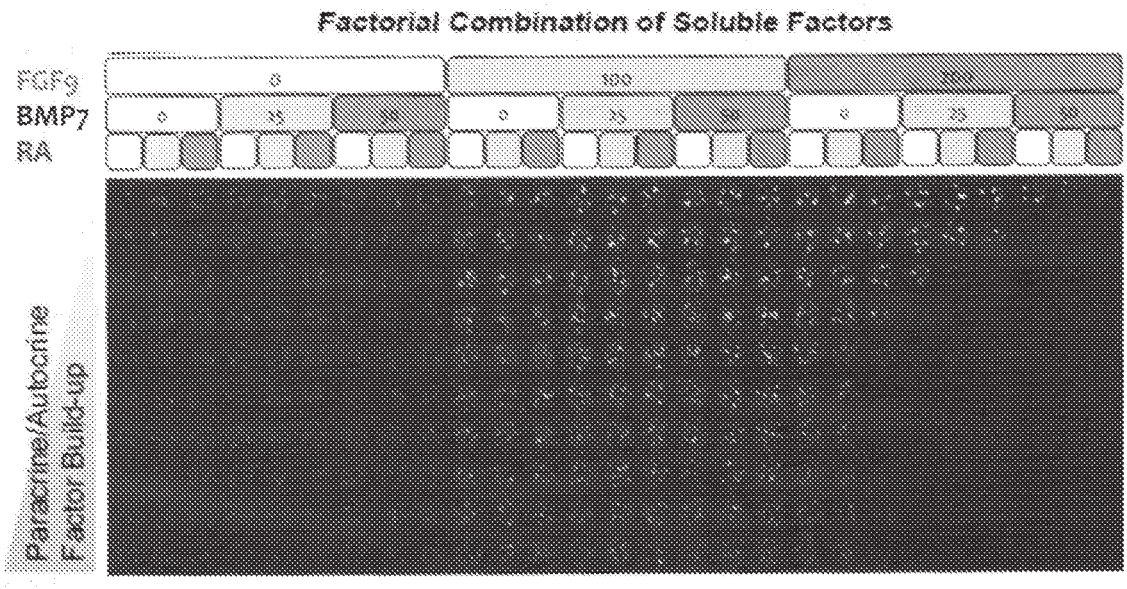

Factorial Combination of Soluble Factors
FGF9
BMP7
RA
Paracrine/Autocrine
Factor Build-up

FIG. 13

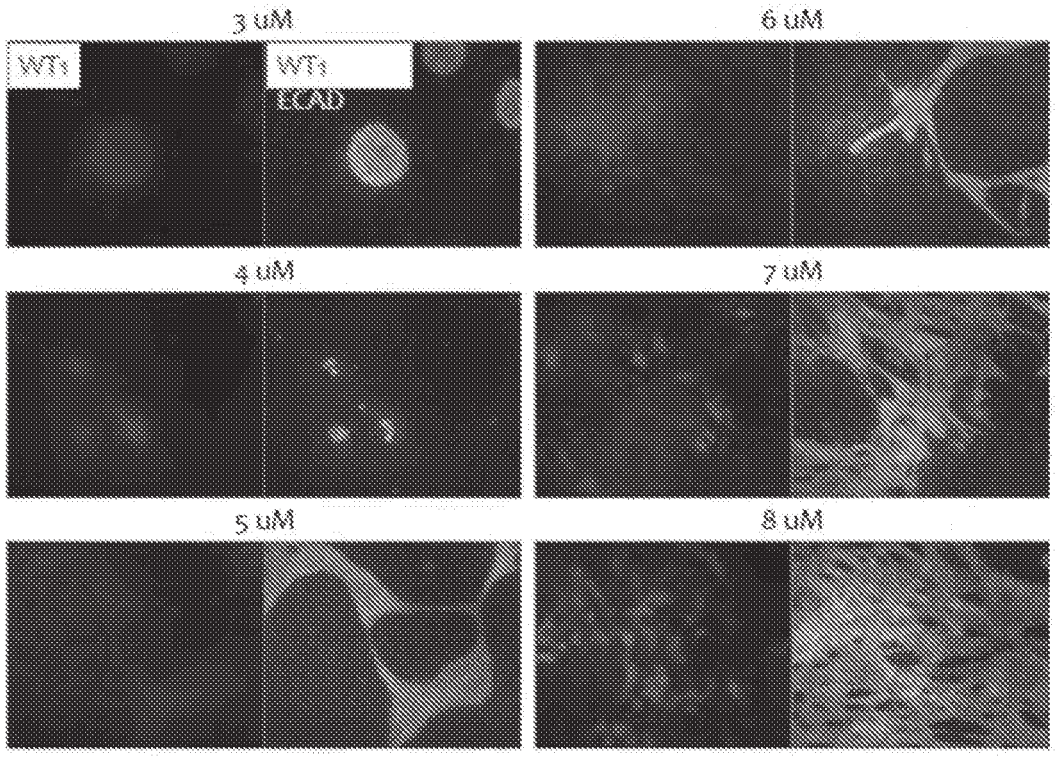


FIG. 14

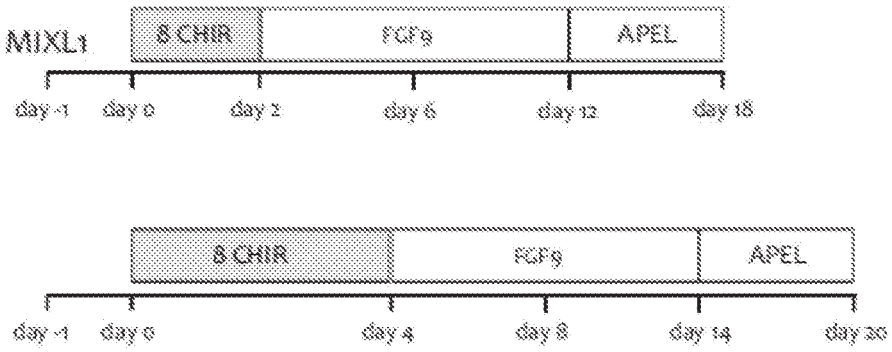

MIXL1
8 CHIR
FGF9
APEL
day -1
day 0
day 2
day 6
day 12
day 18
8 CHIR
FGF9
APEL
day -1
day 0
day 4
day 8
day 14
day 20

FIG. 15

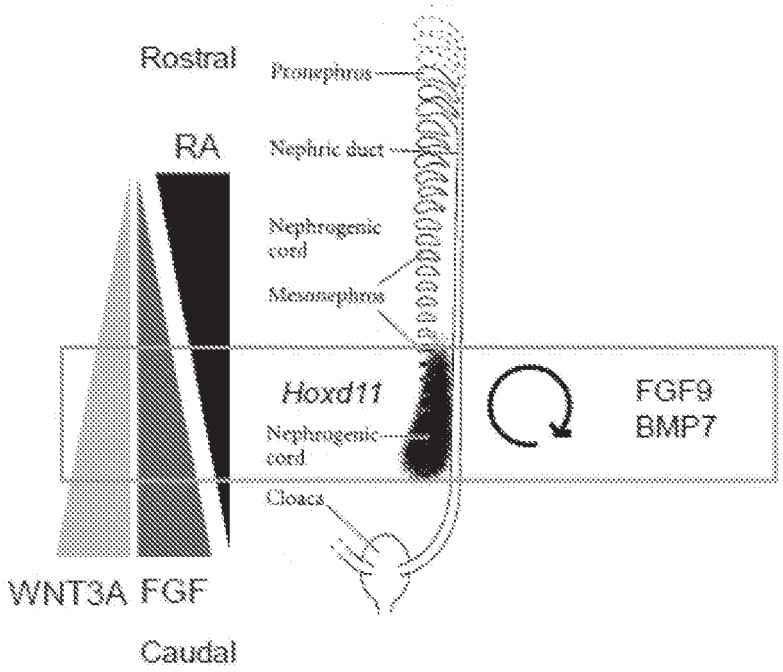

Rostral
Pronephros
RA
Nephric duct
Nephrogenic cord
Mesonephros
Hoxd11
Nephrogenic cord
Cloaca
FGF9
BMP7
WNT3A FGF
Caudal

FIG. 16

*AGN193109: a highly active antagonist of retinoid action, which binds to RARα, RARβ, and RARγ.

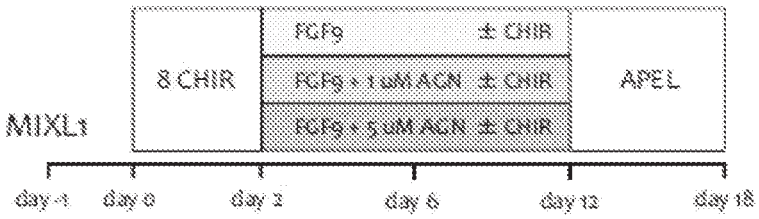


FIG. 17

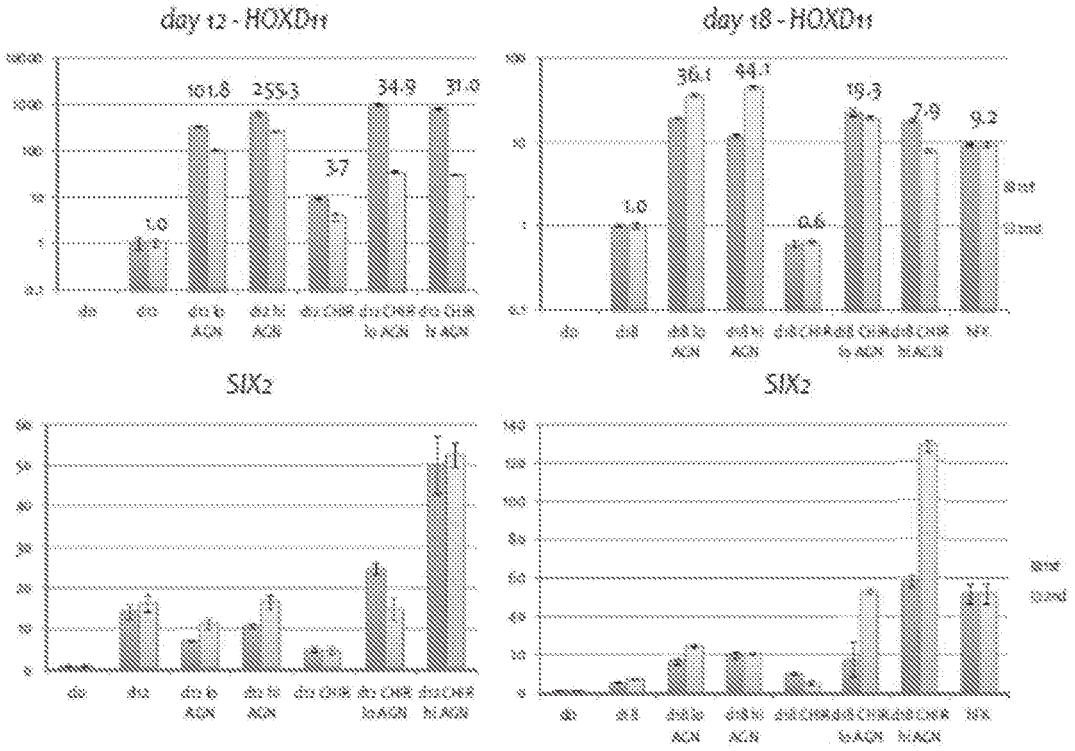


FIG. 18

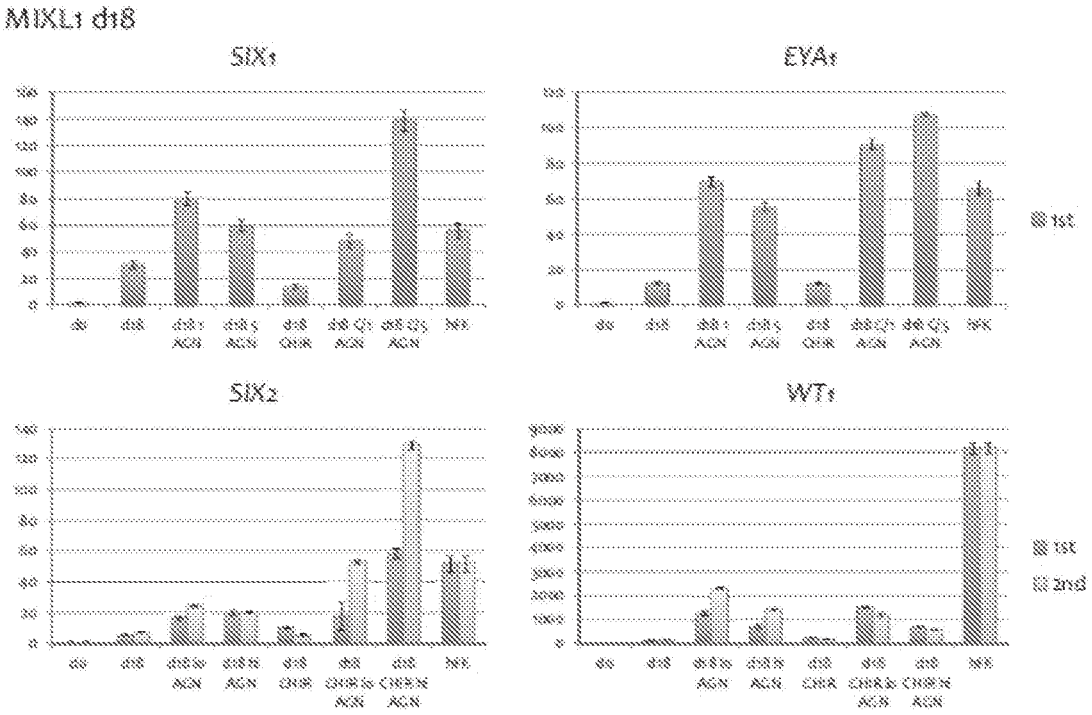


FIG. 19

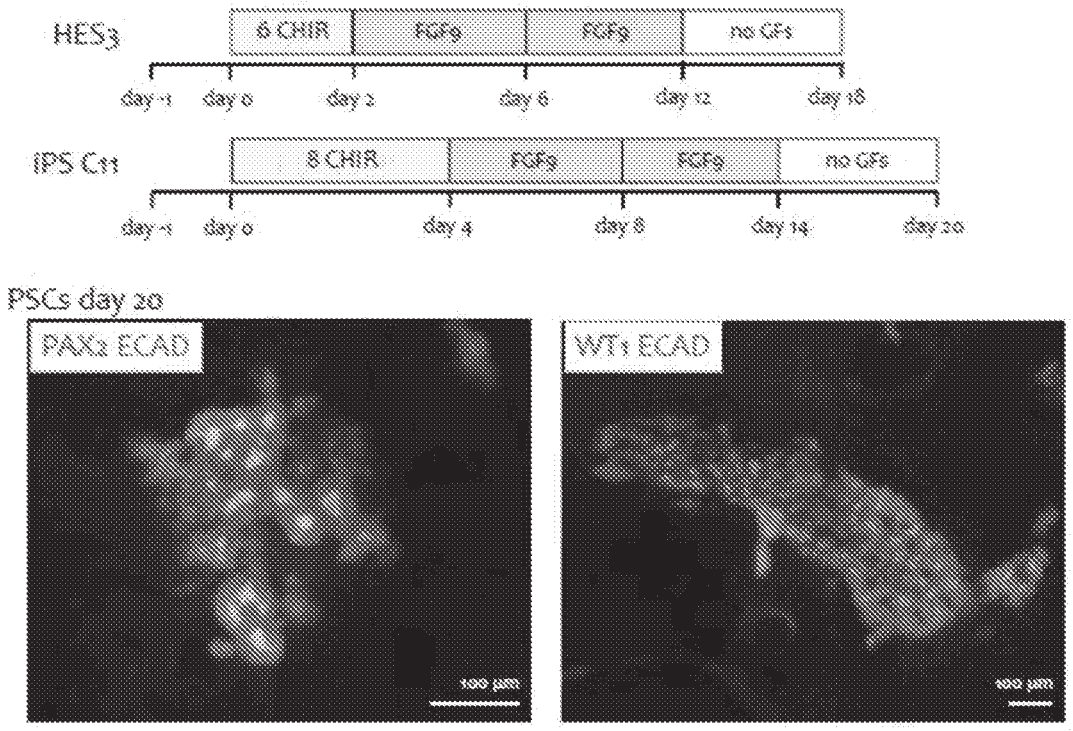

HES3
8 CHIR
FGF9
FGF9
no GFs
day -1
day 0
day 2
day 6
day 12
day 18
iPS C11
8 CHIR
FGF9
FGF9
no GFs
day -1
day 0
day 4
day 8
day 14
day 20
PSCs day 20
PAX2 ECAD
100 µm
WT1 ECAD
100 µm

FIG. 20

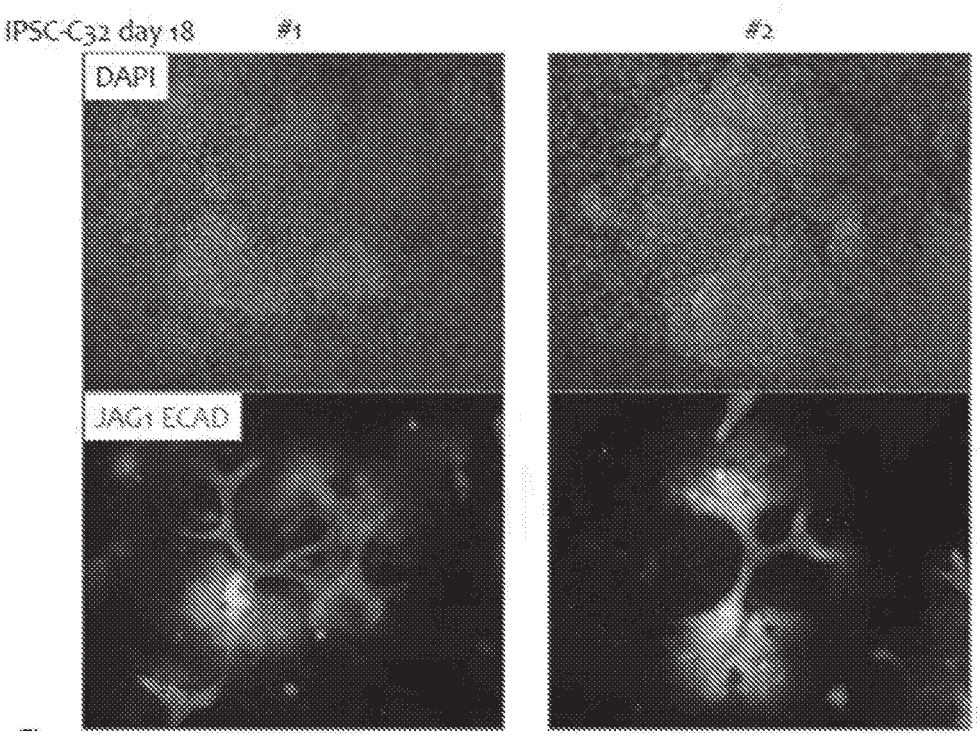


FIG. 21