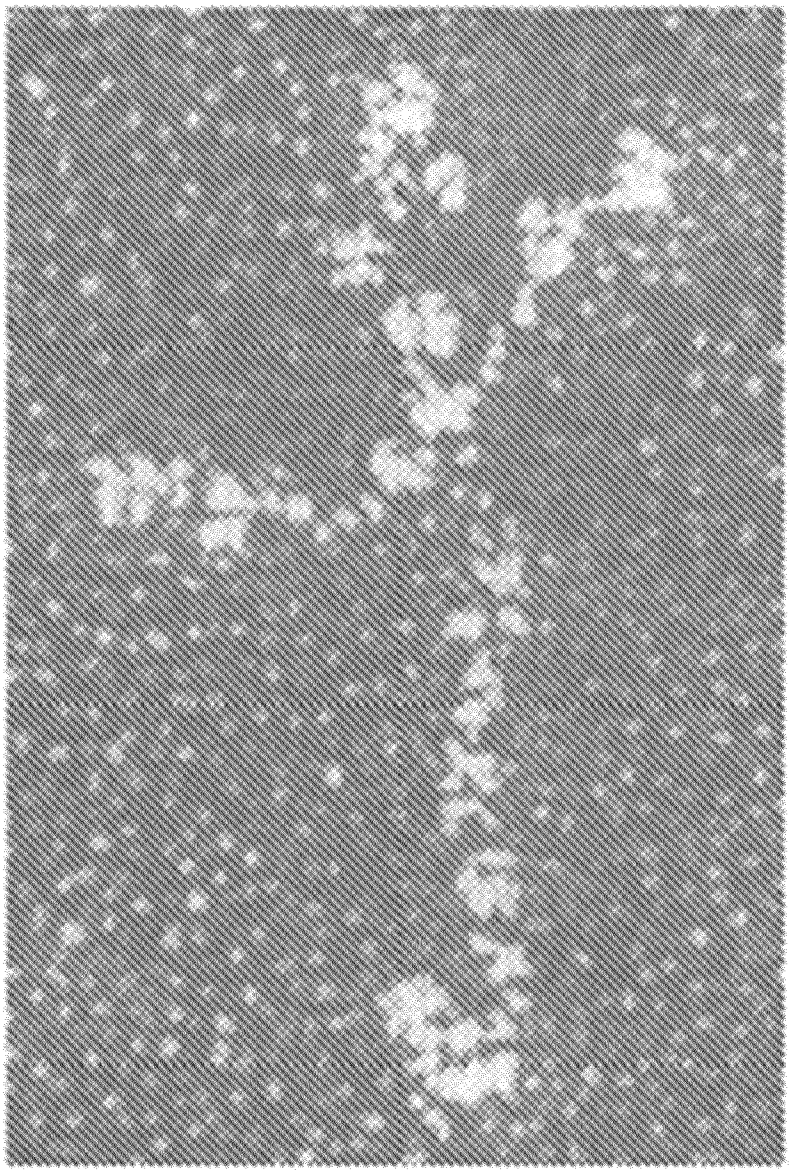


FIG. 1

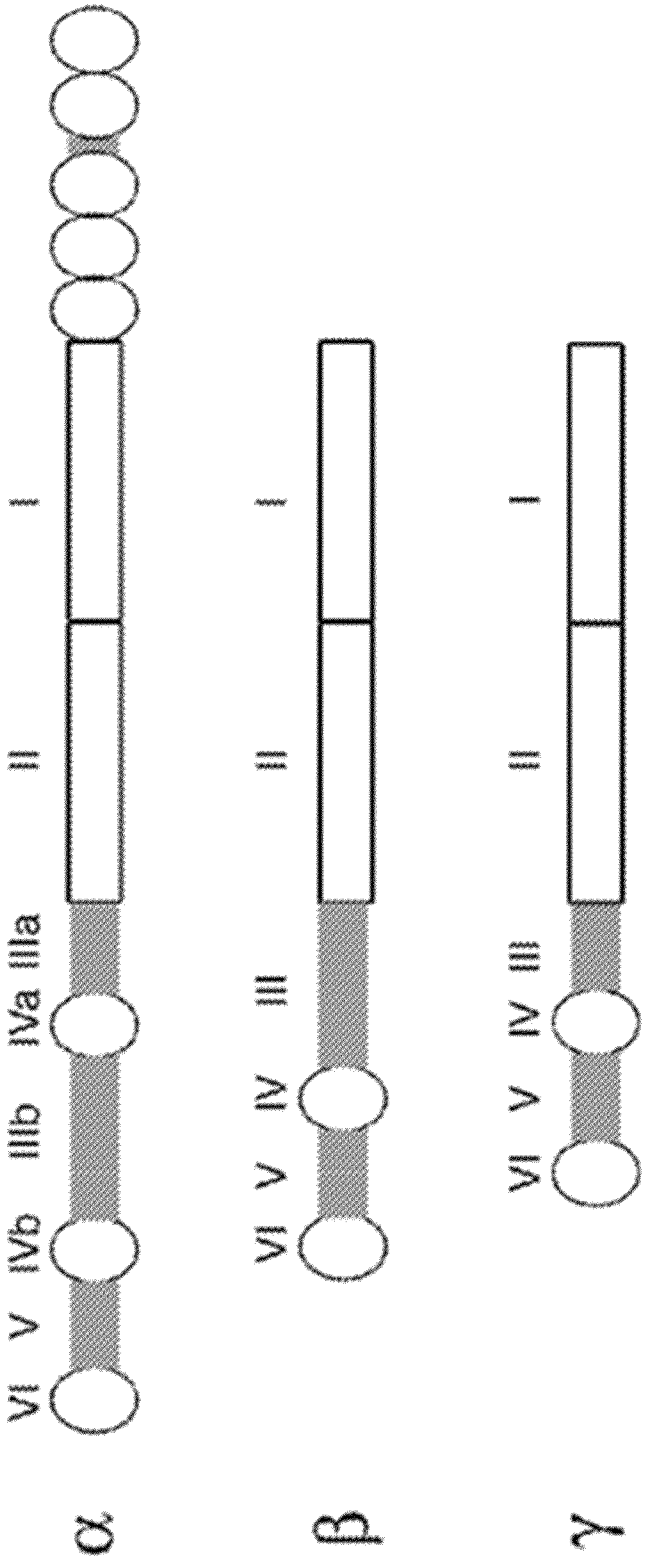


FIG. 2

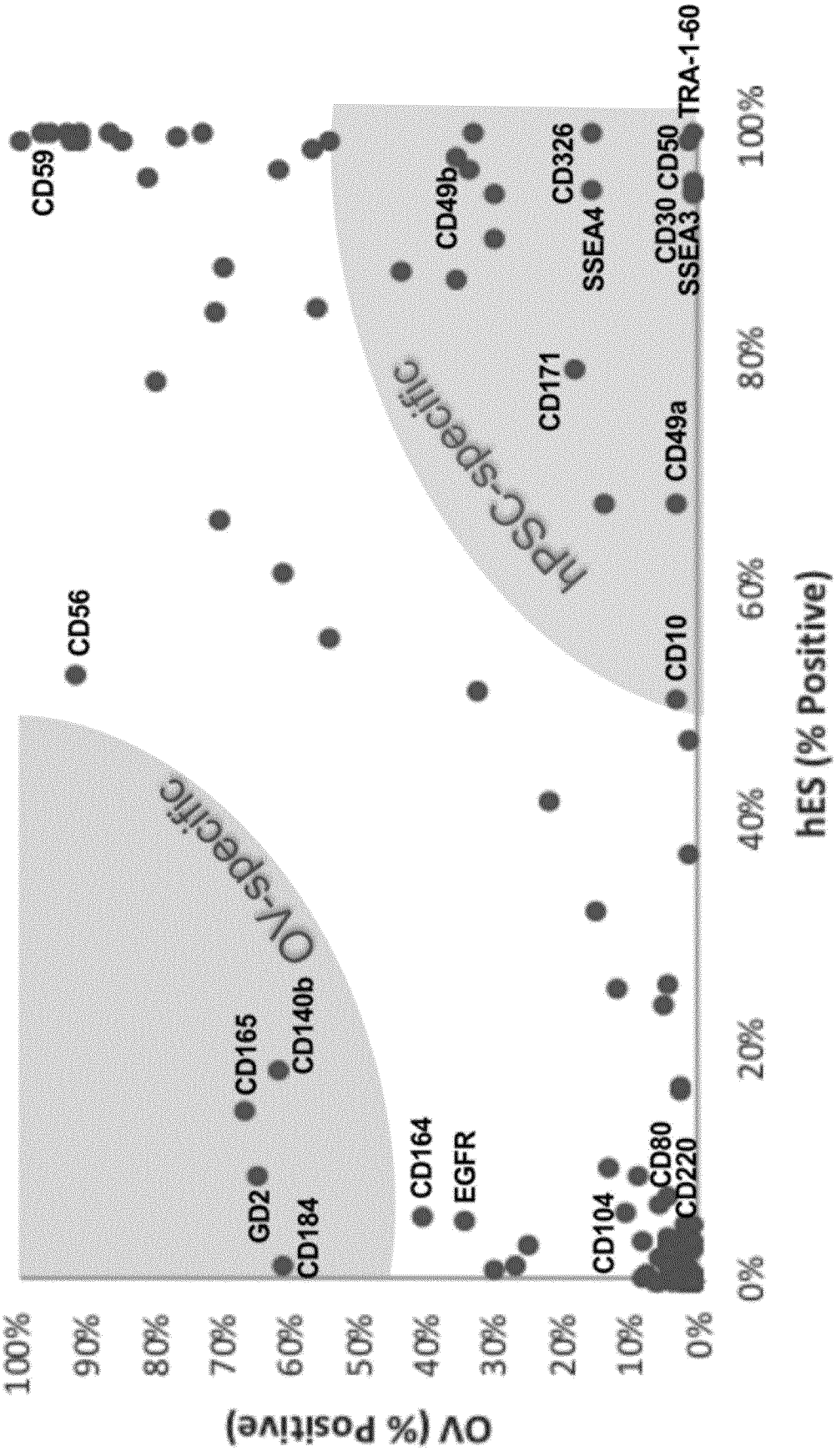

OV (% Positive)
hES (% Positive)
OV-specific
hPSC-specific
CD59
CD56
CD165
CD140b
GD2
CD184
CD164
EGFR
CD104
CD80
CD220
CD10
CD49a
CD171
CD49b
CD326
SSEA4
CD30 CD50
SSEA3
TRA-1-60
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Fig 3

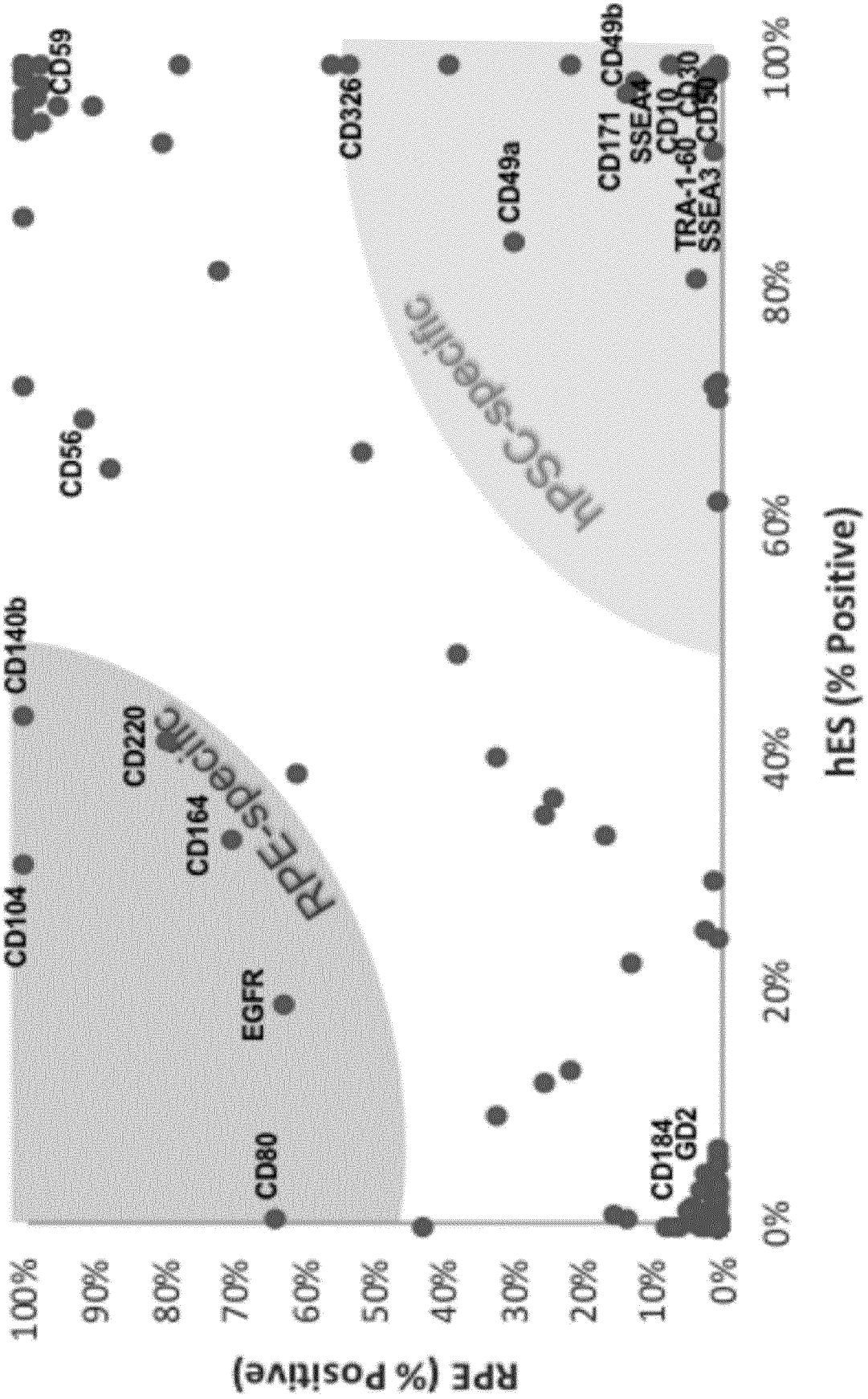

RPE (% Positive)
hES (% Positive)
RPE-specific
hPSC-specific
CD140b
CD104
CD220
CD164
EGFR
CD80
CD184
GD2
CD59
CD56
CD326
CD49a
CD171
CD49b
SSEA4
CD10
CD30
CD50
TRA-1-60
SSEA3
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

Fig 4

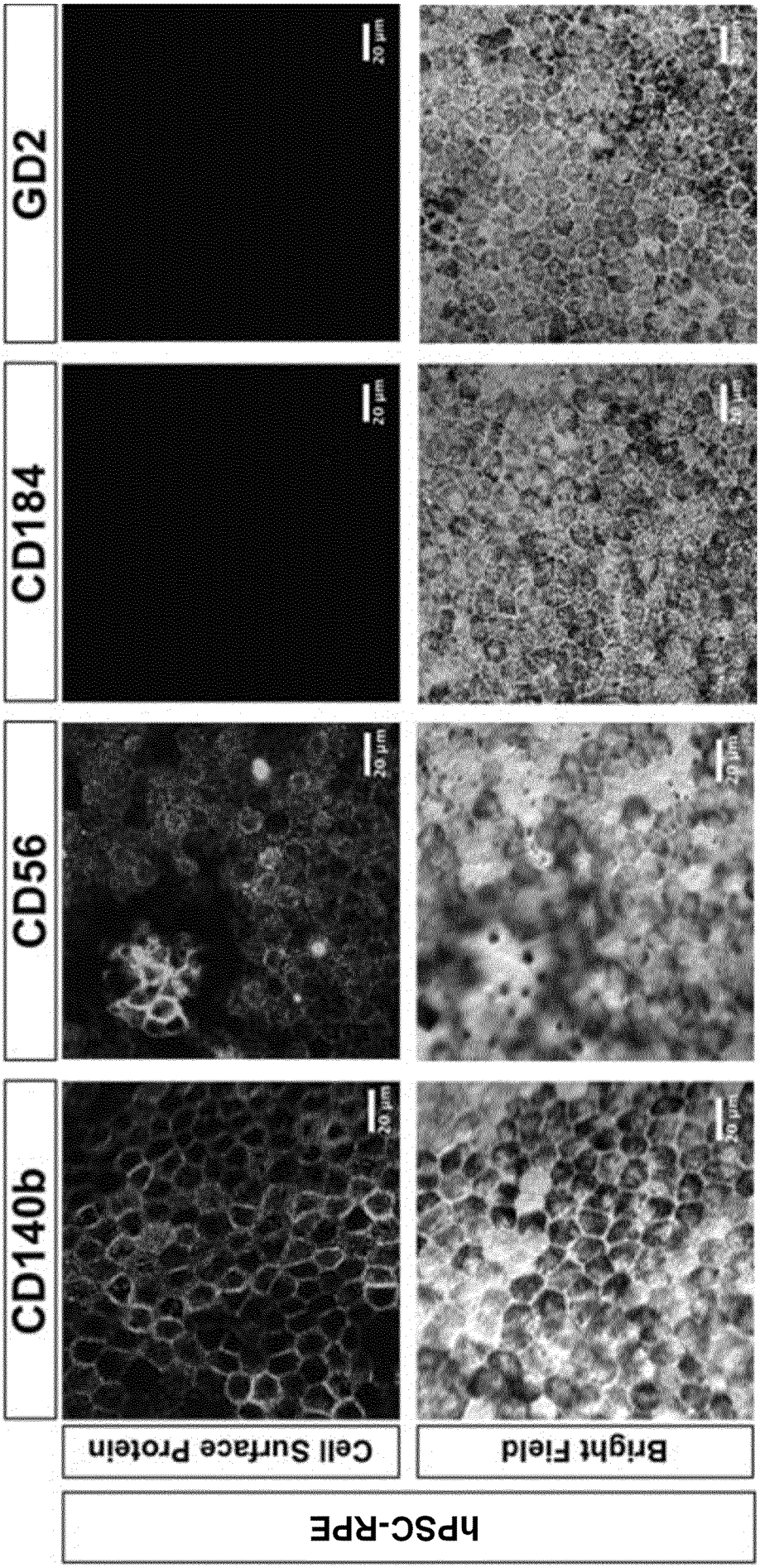

hPSC-RPE
Cell Surface Protein
Bright Field
CD140b
CD56
CD184
GD2
20 µm

FIG. 5

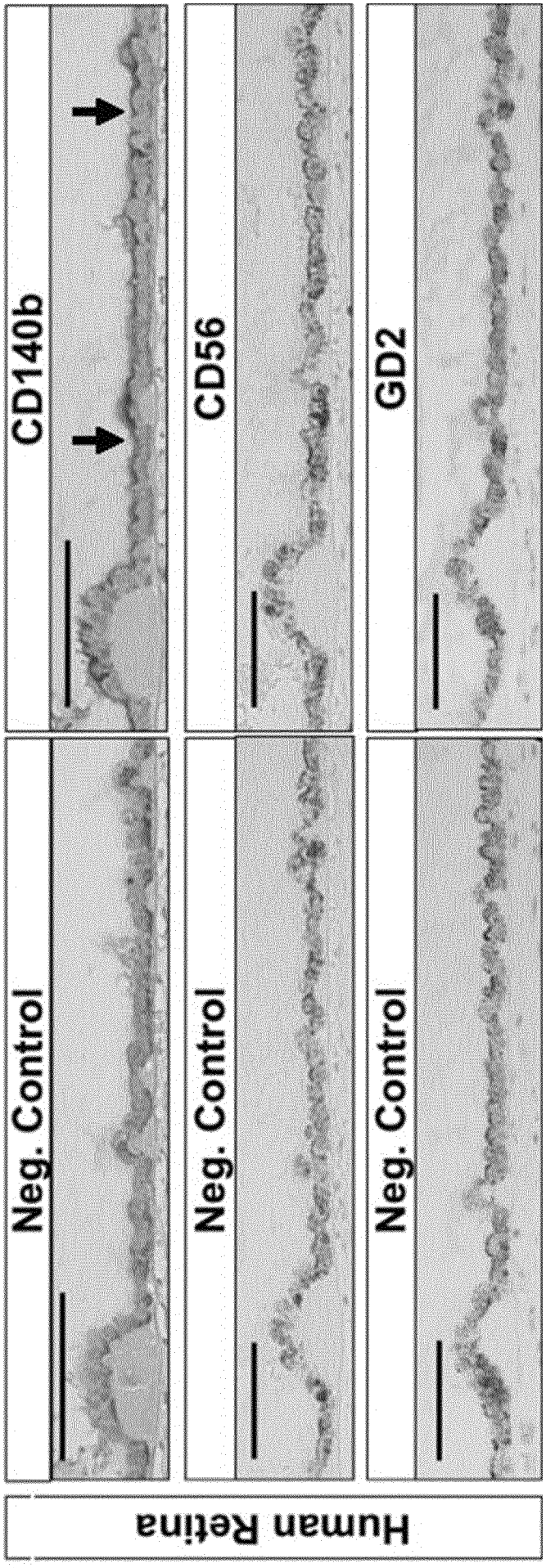

Human Retina
Neg. Control
Neg. Control
Neg. Control
CD140b
CD56
GD2

FIG. 6

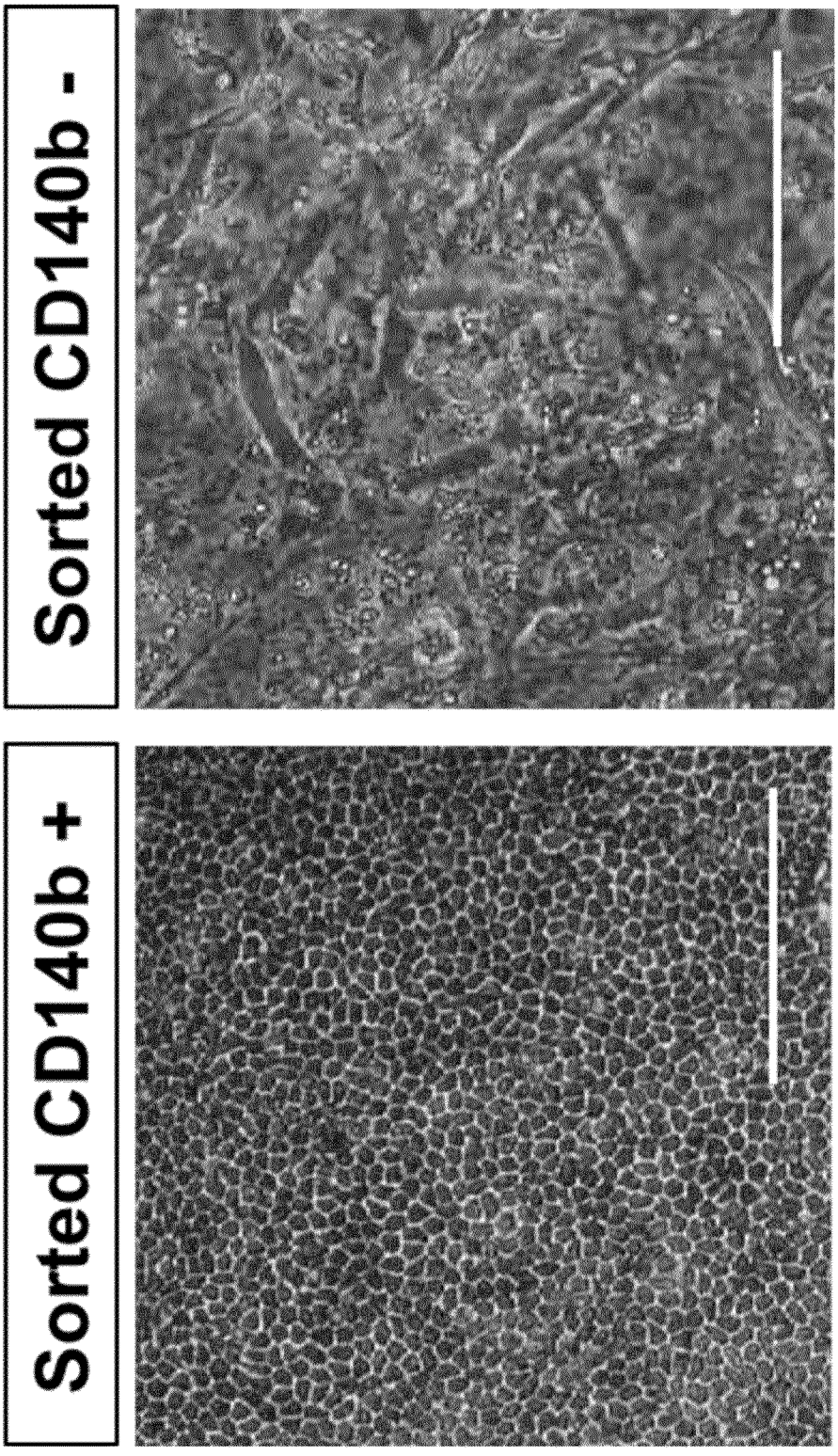


FIG. 7

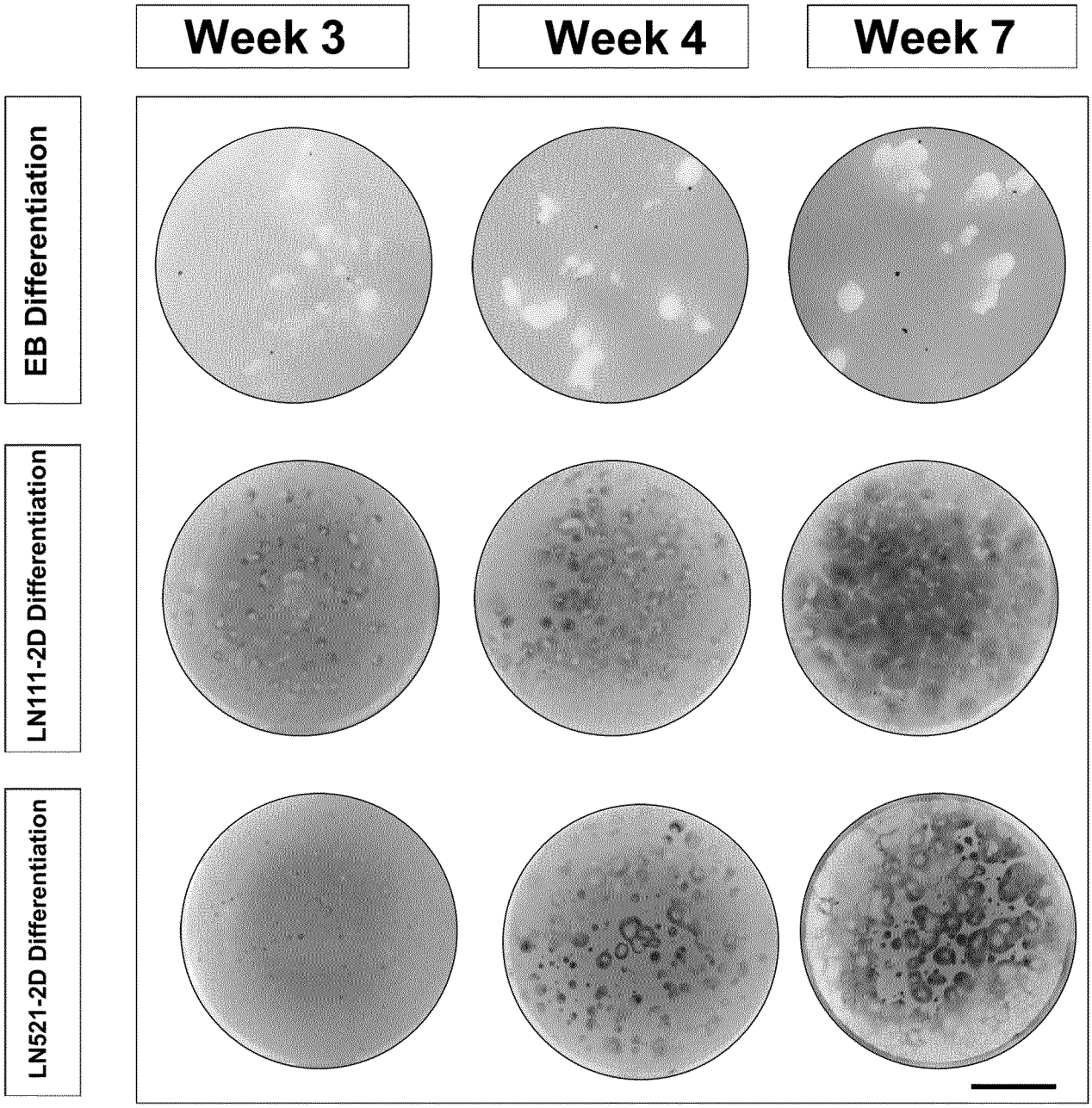

Week 3
Week 4
Week 7
EB Differentiation
LN111-2D Differentiation
LN521-2D Differentiation

FIG. 8

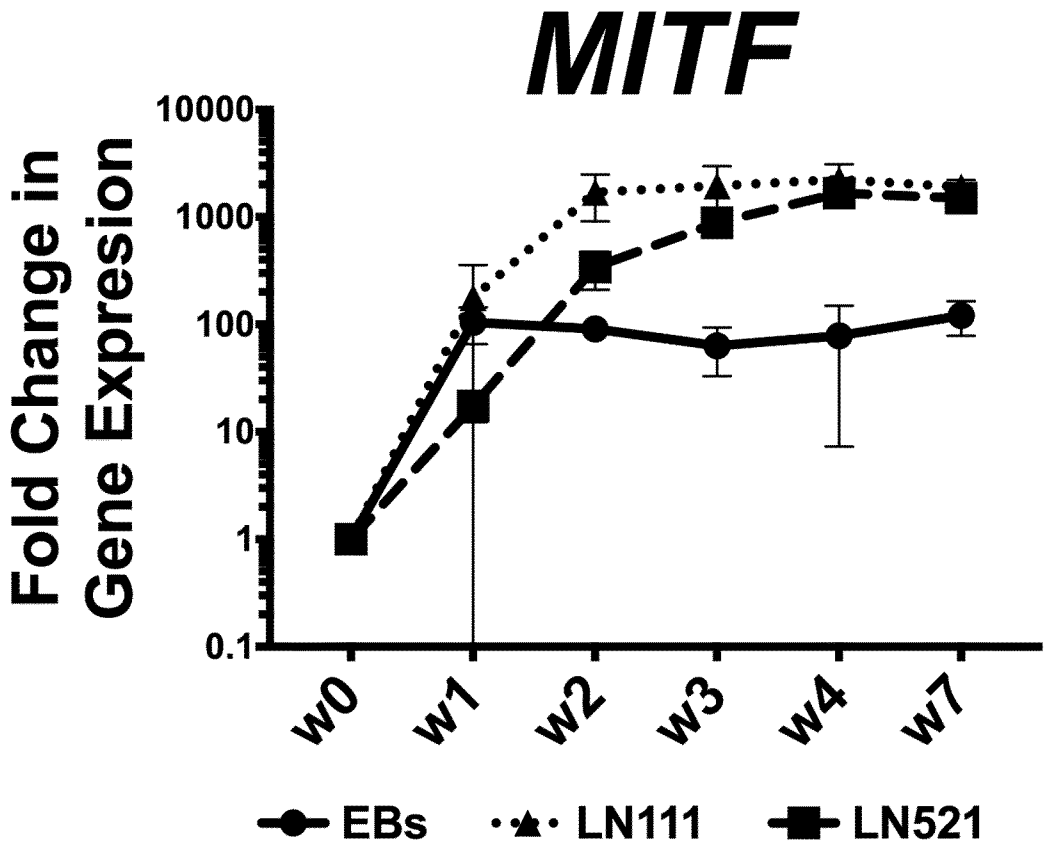

MITF
Fold Change in Gene Expresion
10000
1000
100
10
1
0.1
w0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 9A

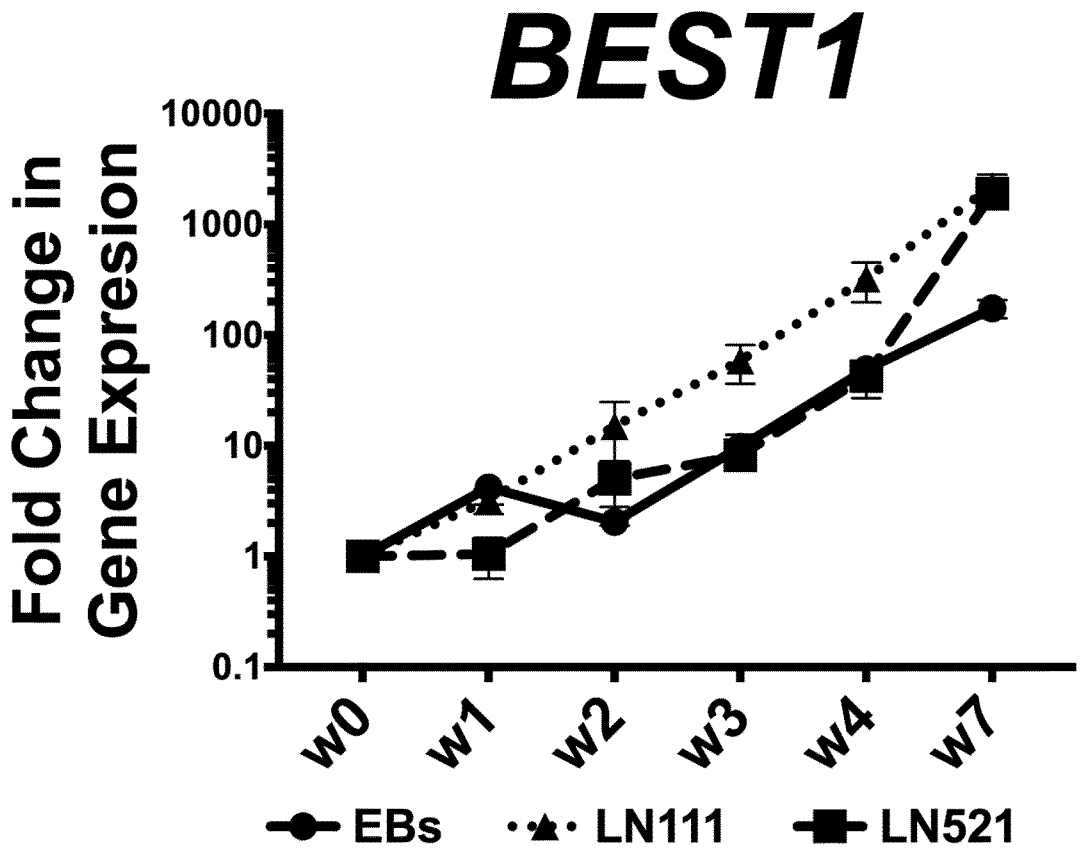

BEST1
Fold Change in Gene Expresion
10000
1000
100
10
1
0.1
w0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 9B

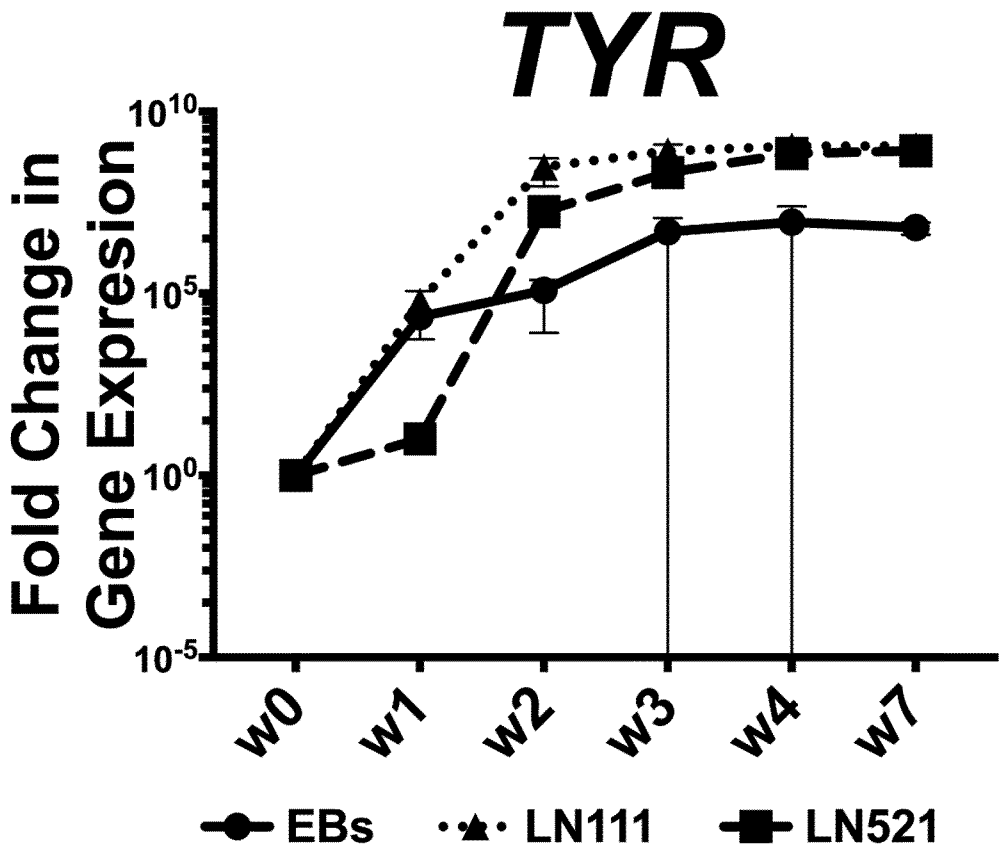

TYR
Fold Change in Gene Expresion
10^{10}
10^{5}
10^{0}
10^{-5}
w0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 9C

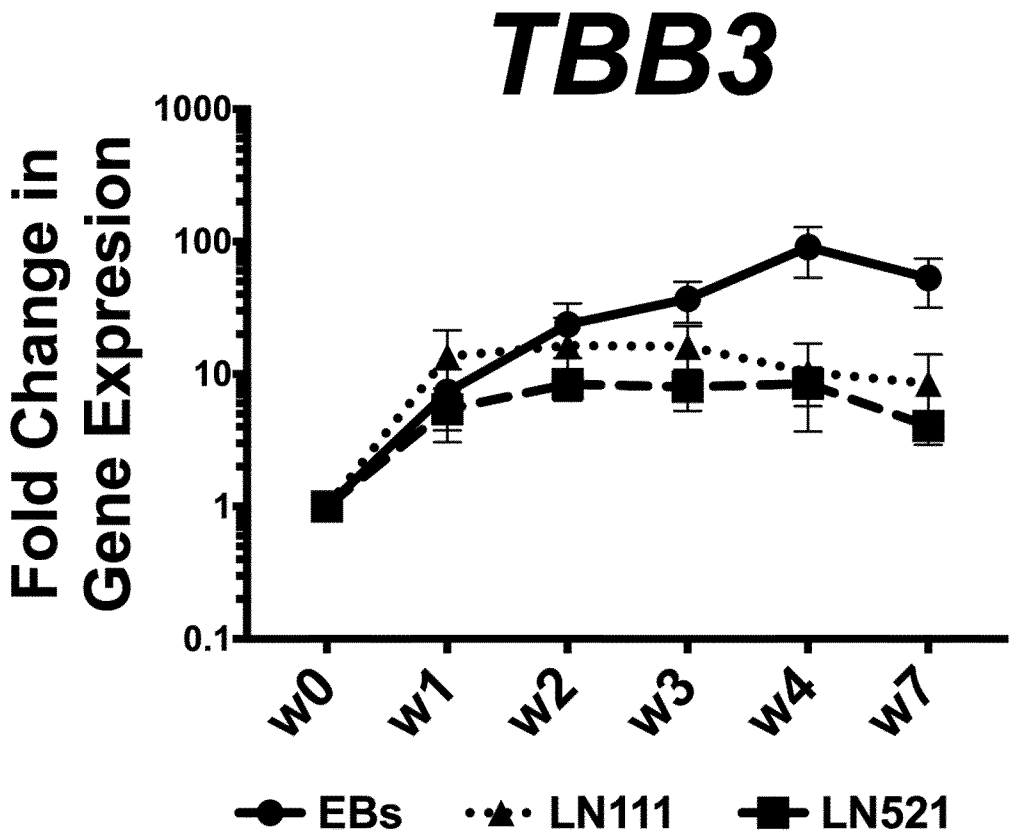

TBB3
Fold Change in Gene Expresion
1000
100
10
1
0.1
w0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 9D

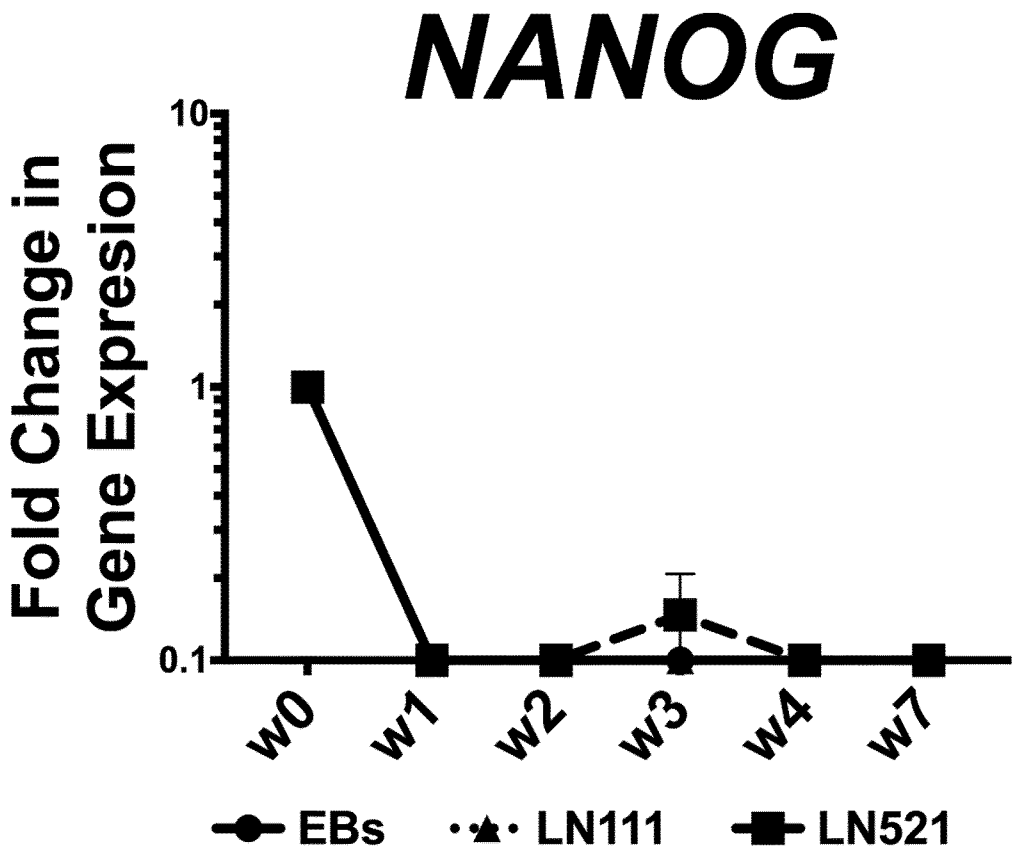

NANOG
Fold Change in Gene Expresion
10
1
0.1
w0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 9E

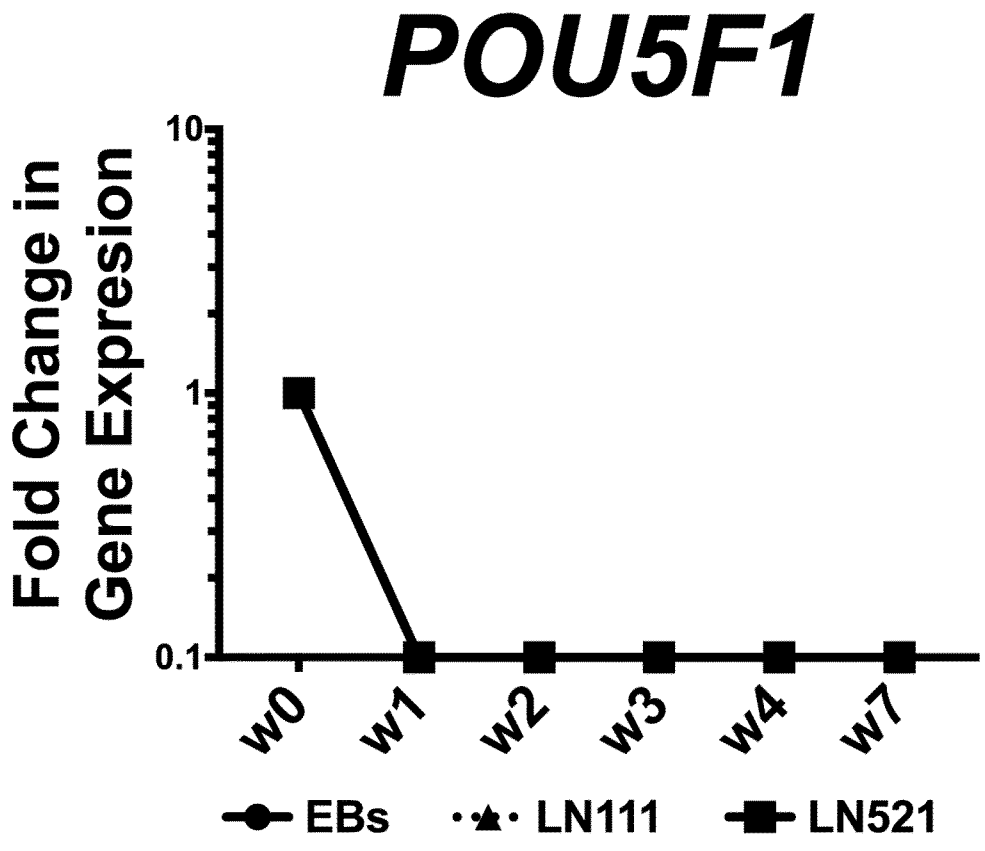

POU5F1
Fold Change in Gene Expresion
10
1
0.1
w0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 9F

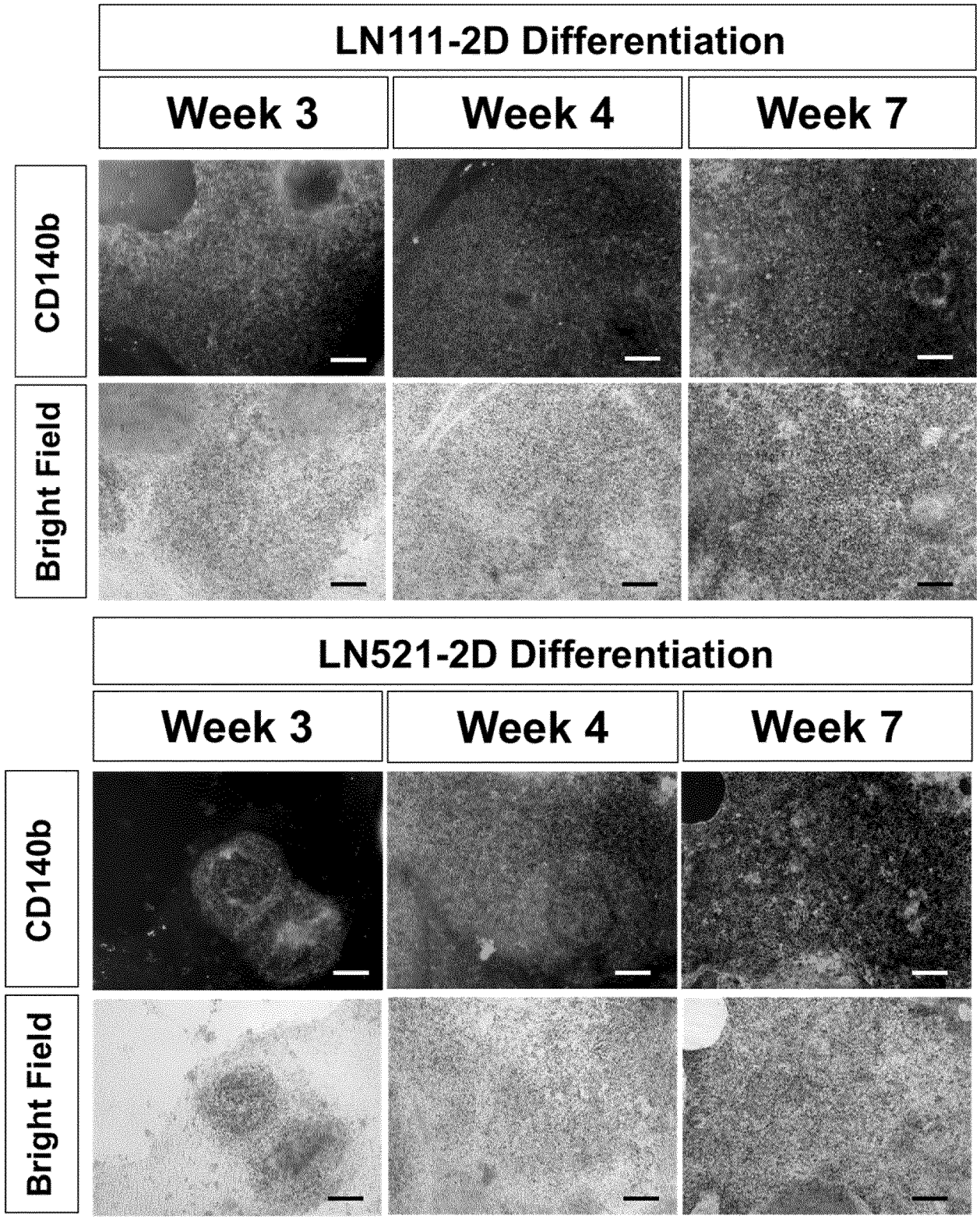

LN111-2D Differentiation
Week 3
Week 4
Week 7
CD140b
Bright Field
LN521-2D Differentiation
Week 3
Week 4
Week 7
CD140b
Bright Field

FIG. 10

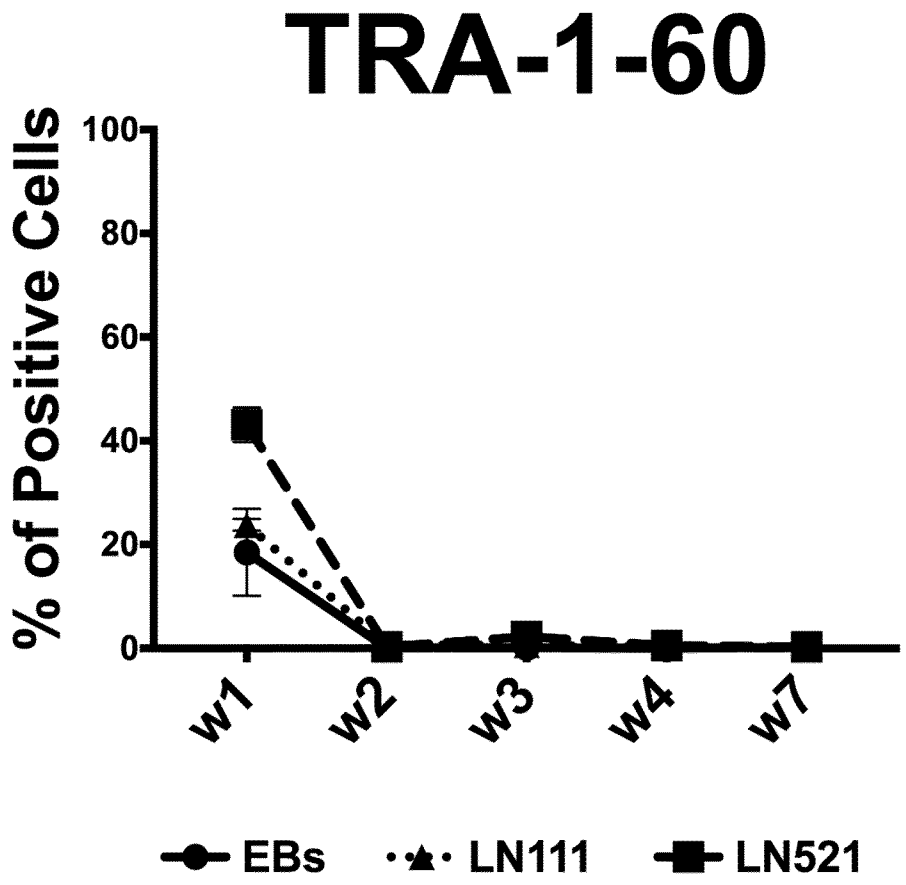


Fig 11A

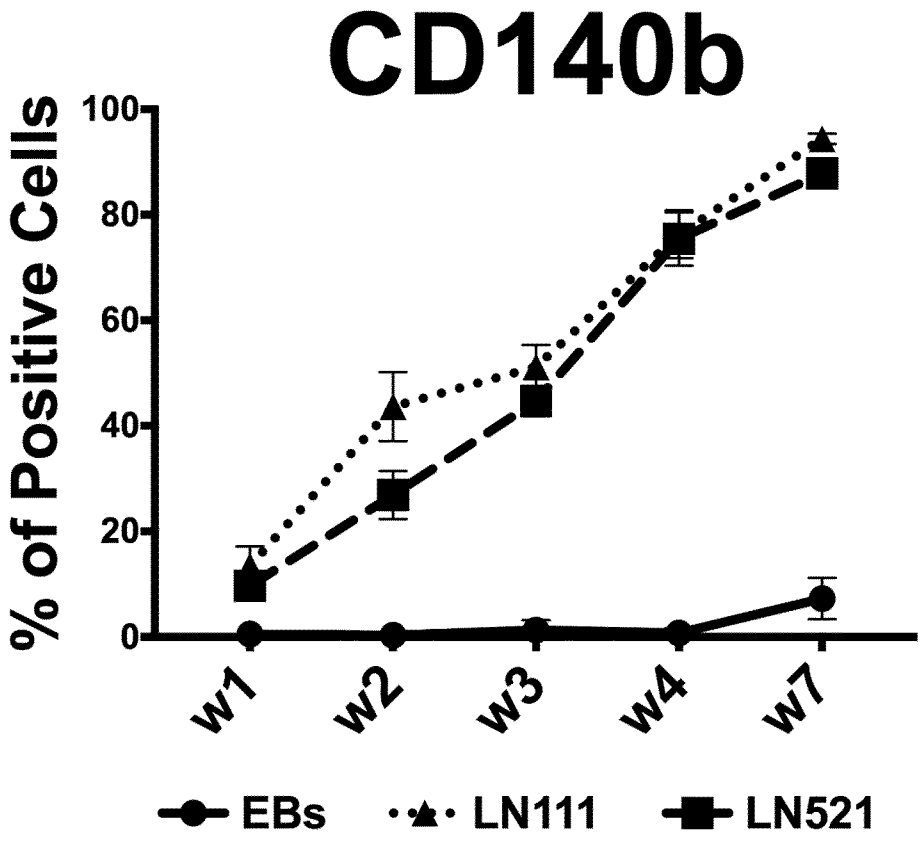


Fig 11B

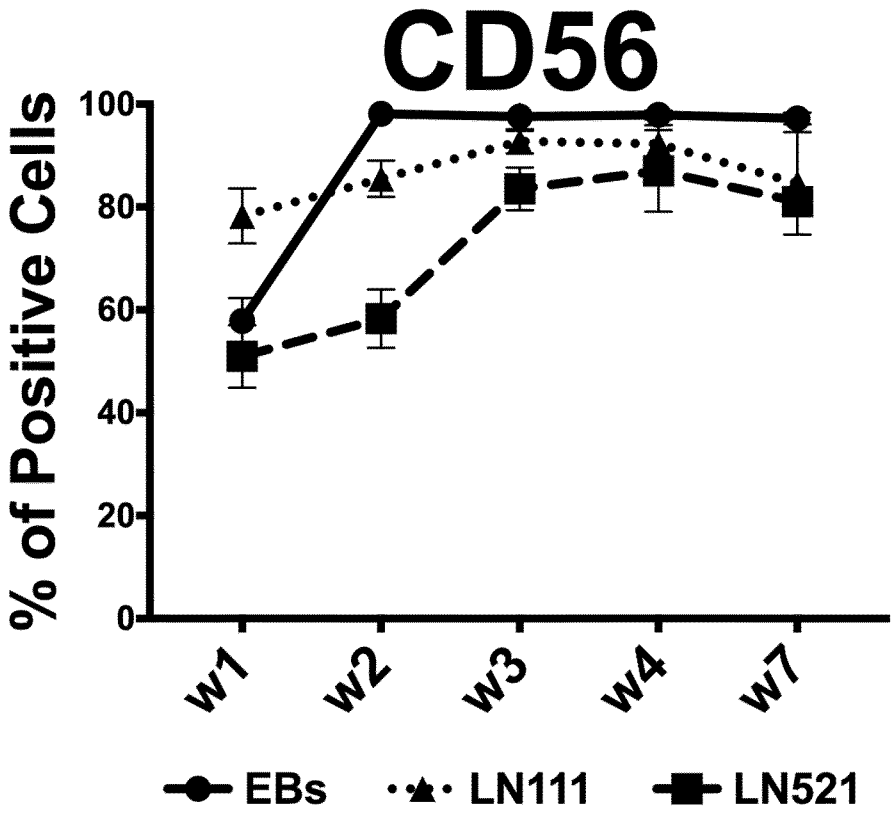

CD56
% of Positive Cells
100
80
60
40
20
0
w1
w2
w3
w4
w7
EBs
LN111
LN521

Fig 11C

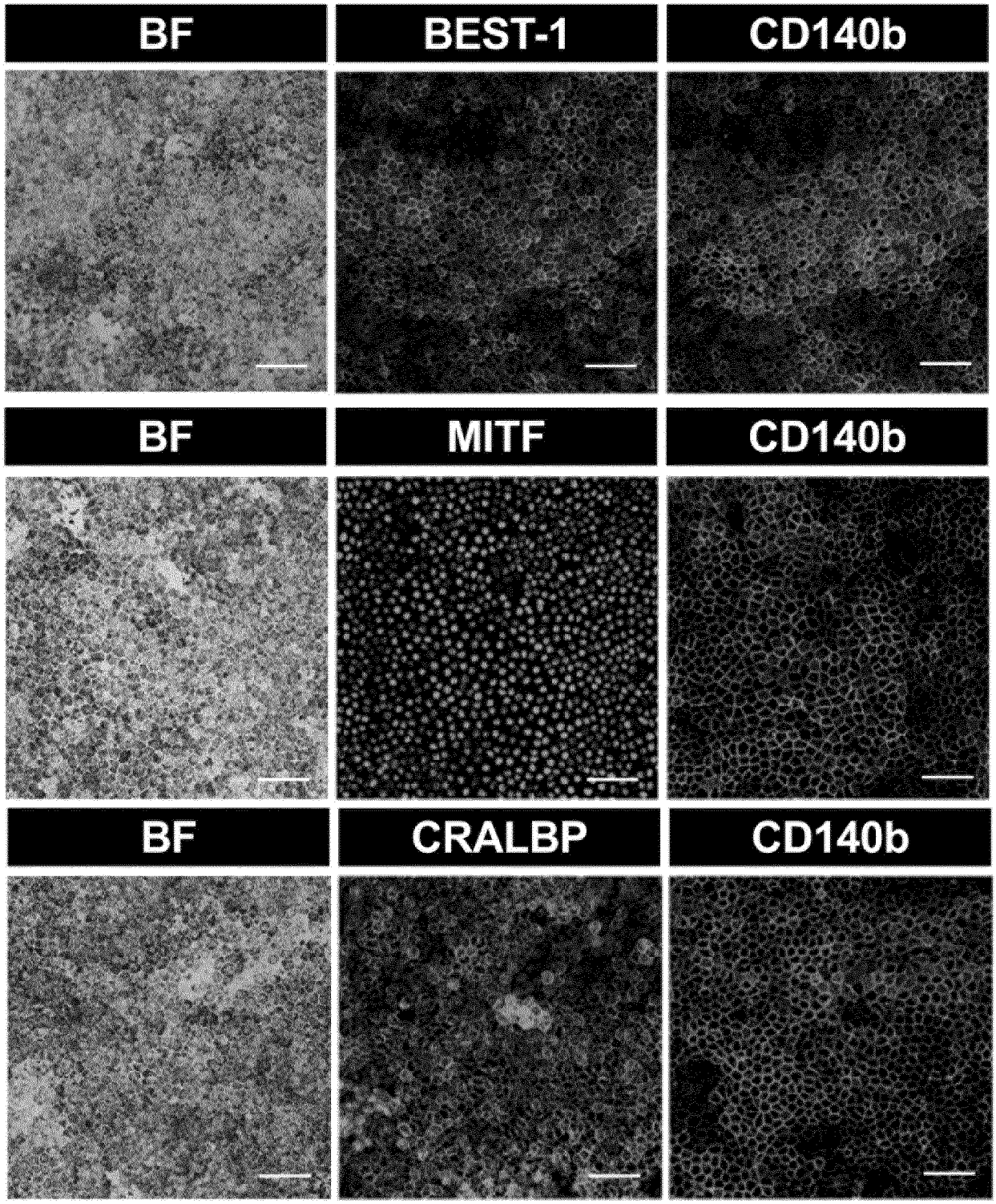


Fig 12

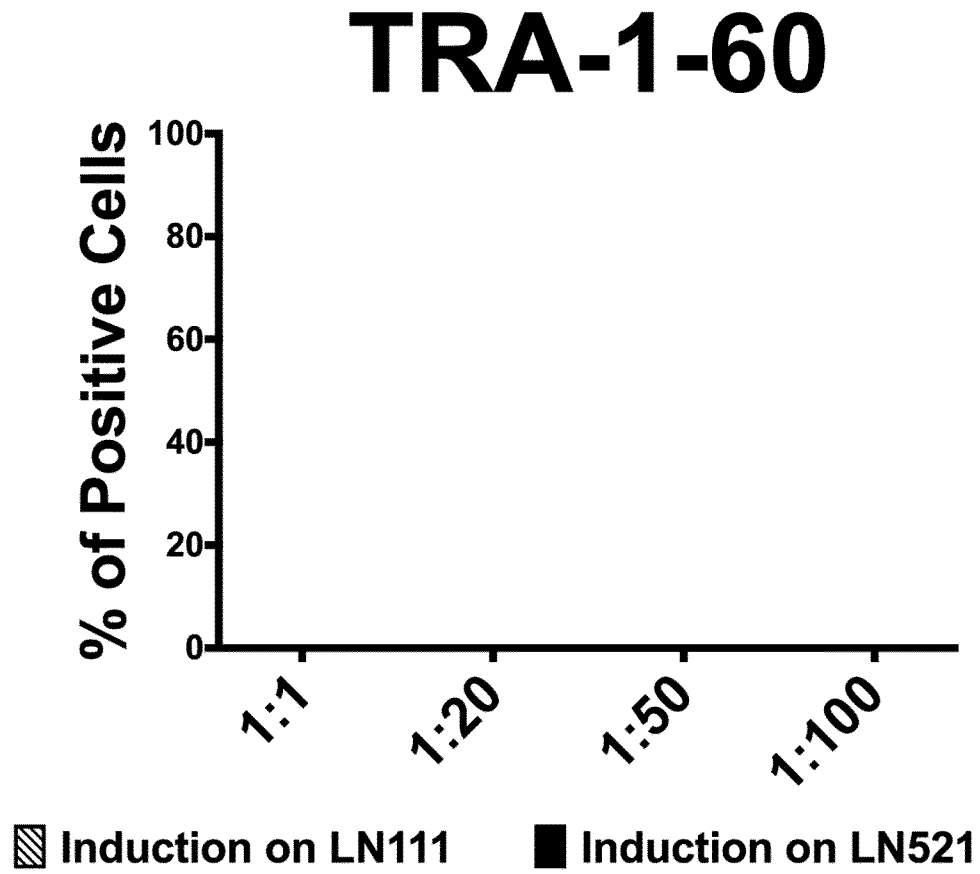


Fig 13A

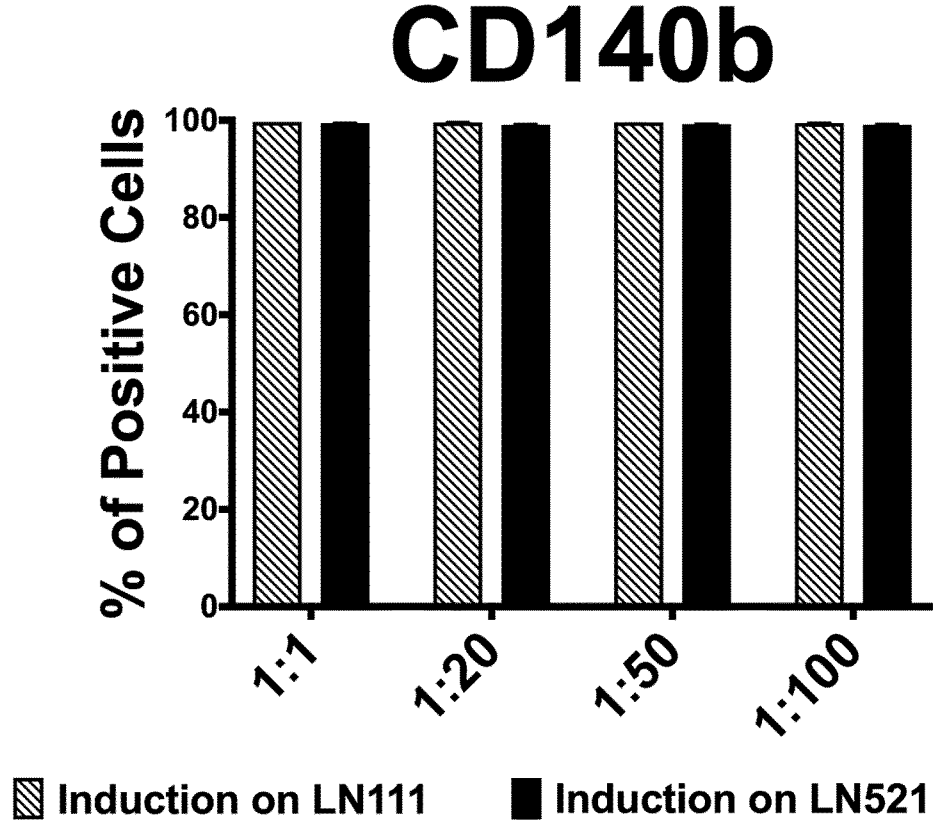


Fig 13B

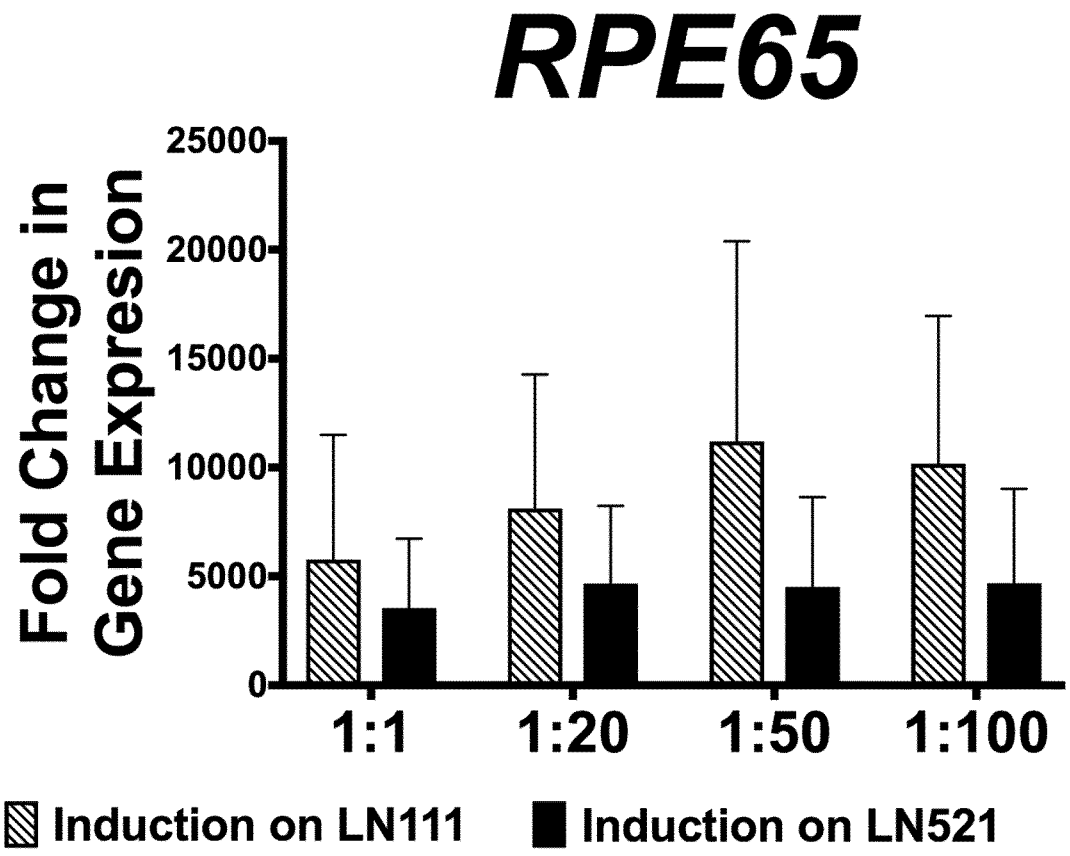


Fig 14A

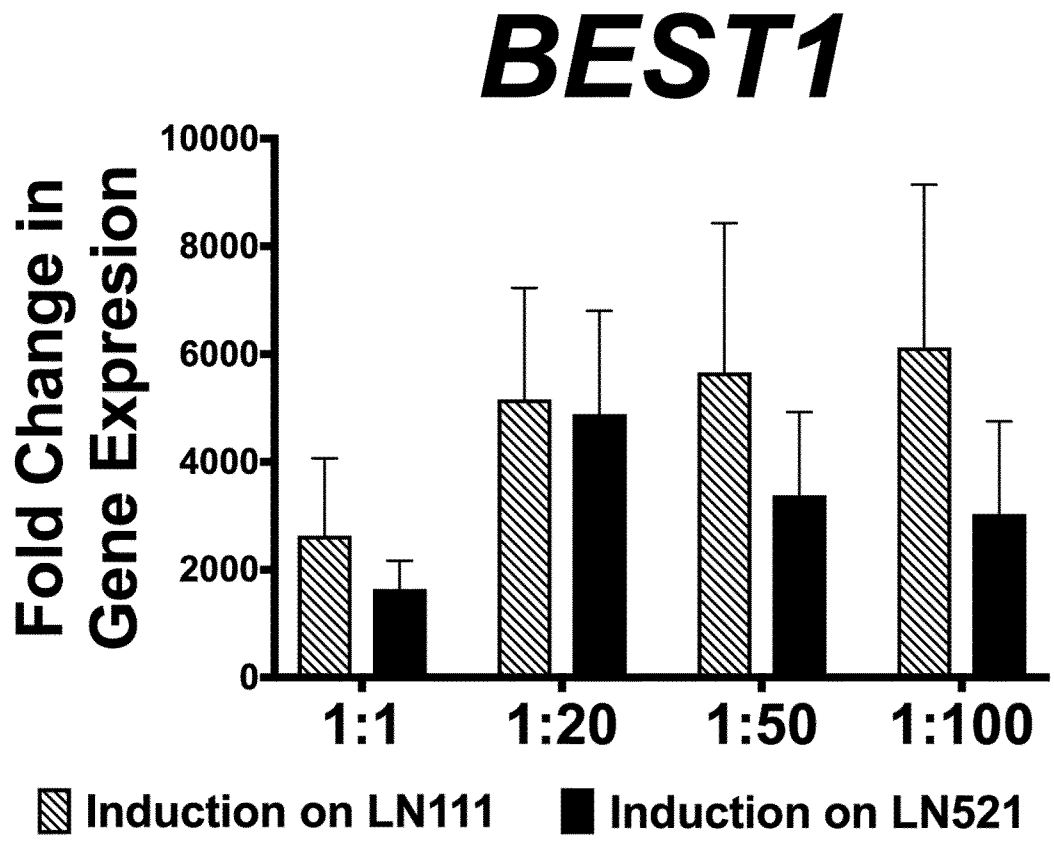


Fig 14B

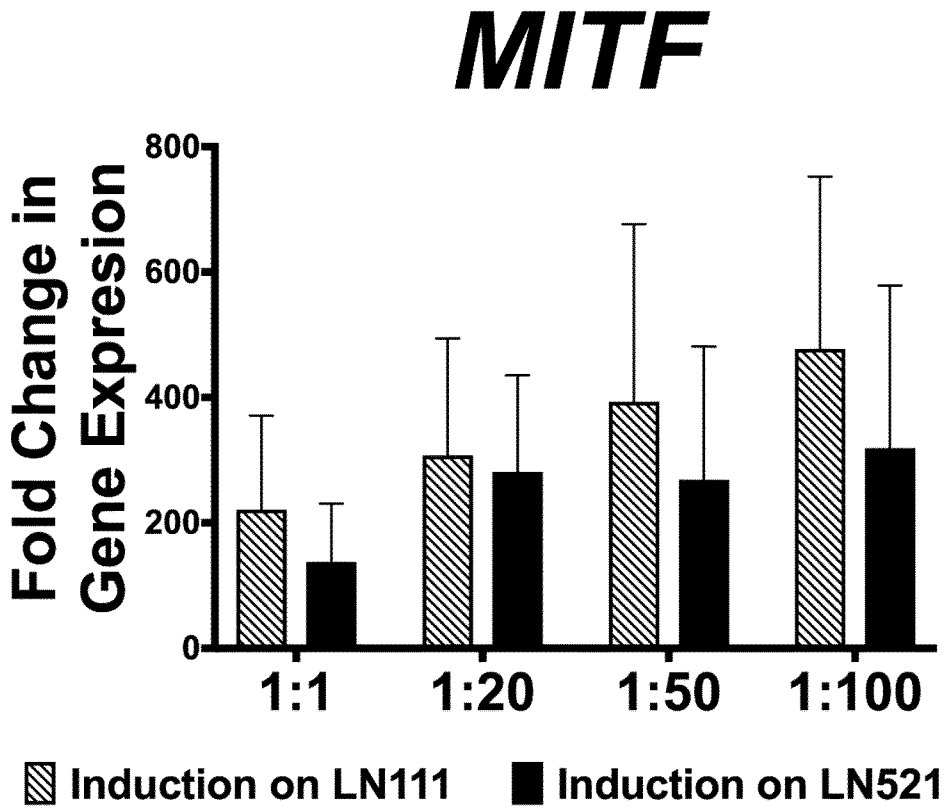


Fig 14C

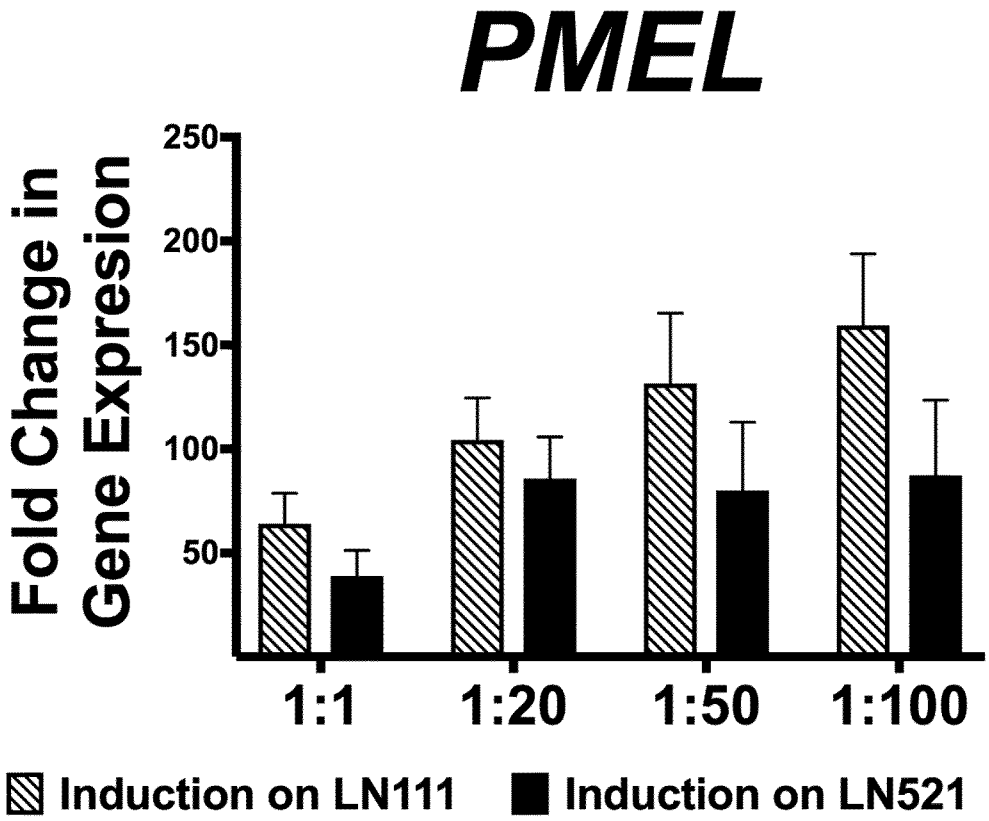


Fig 14D

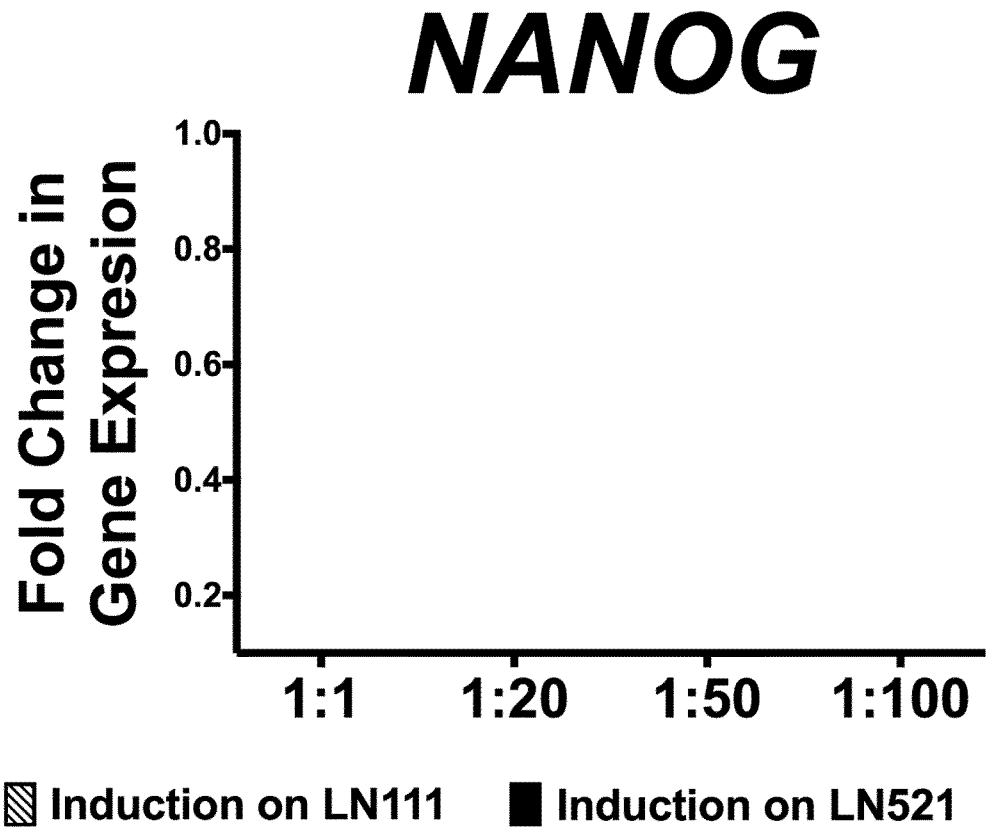


Fig 14E

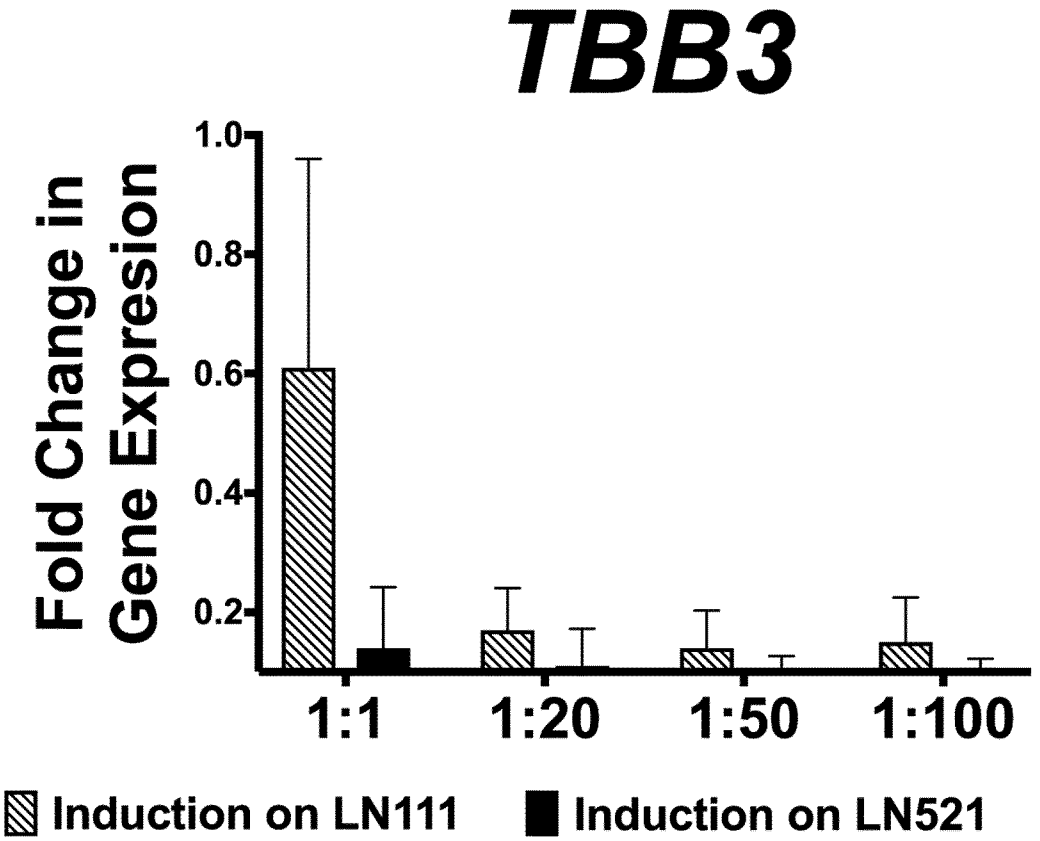


Fig 14F

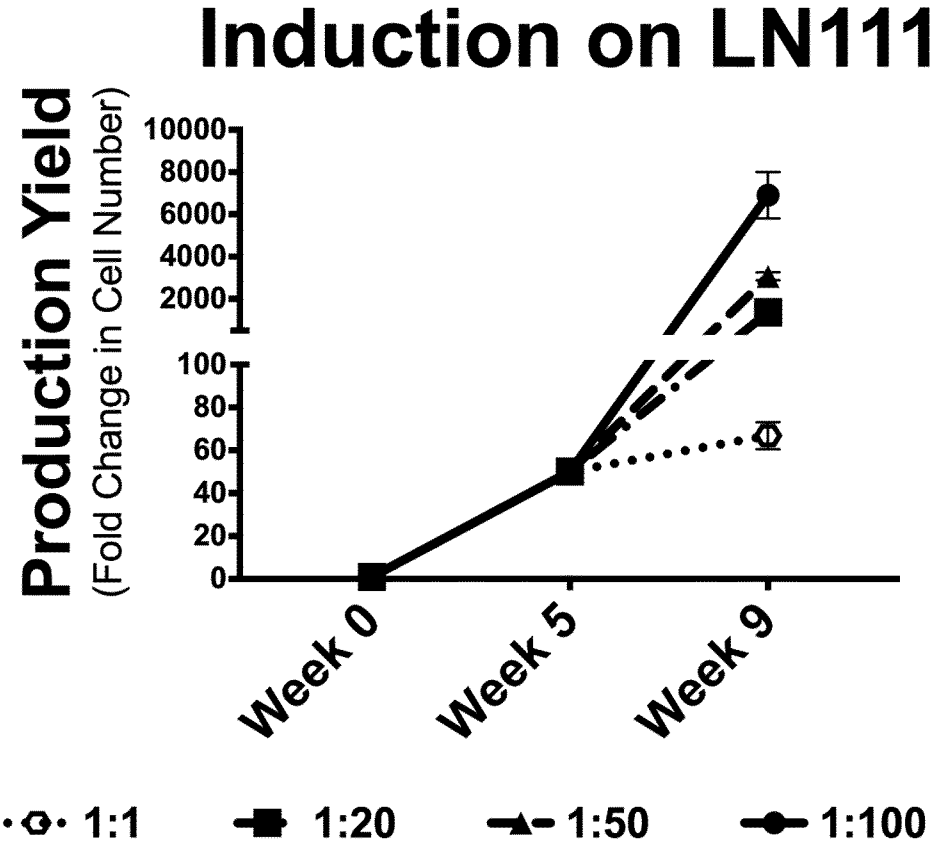


Fig 15A

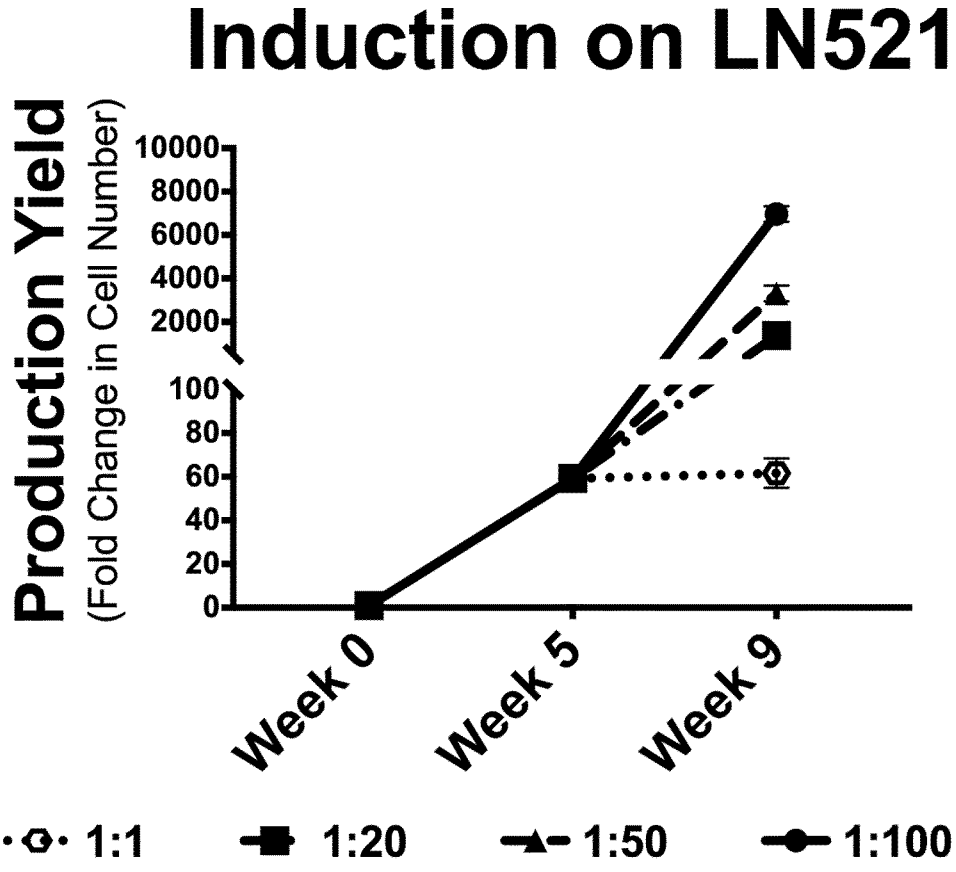


Fig 15B

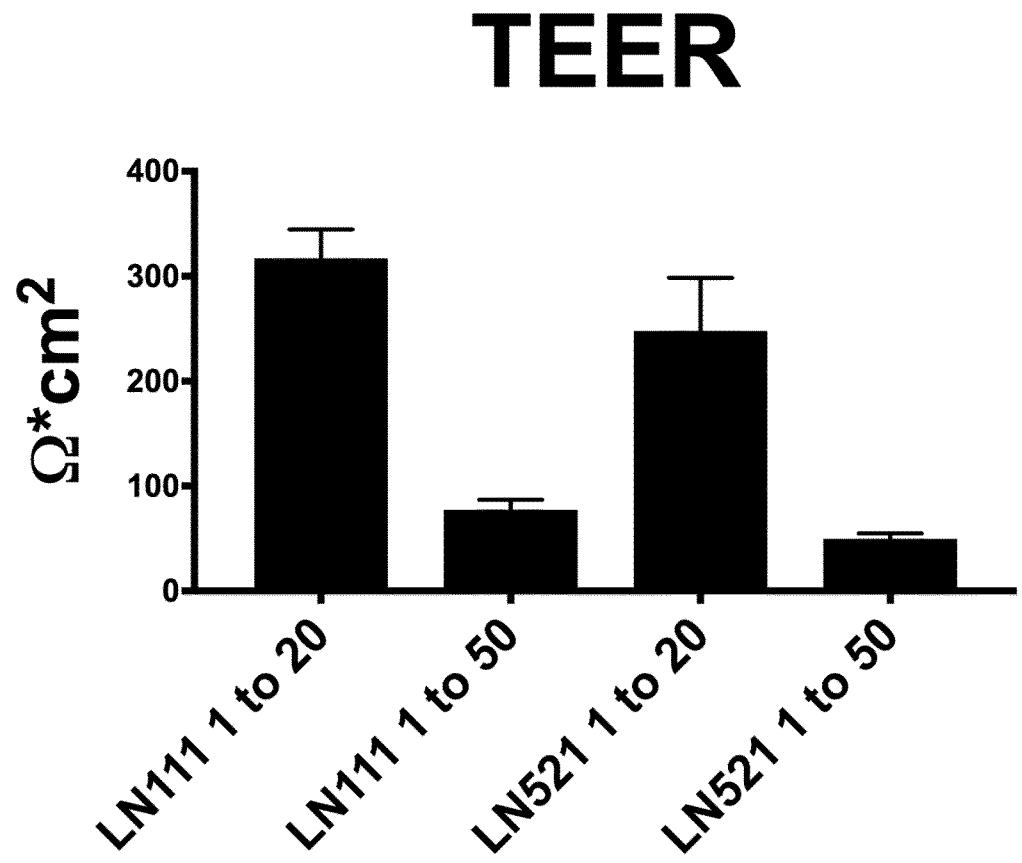


Fig 16A

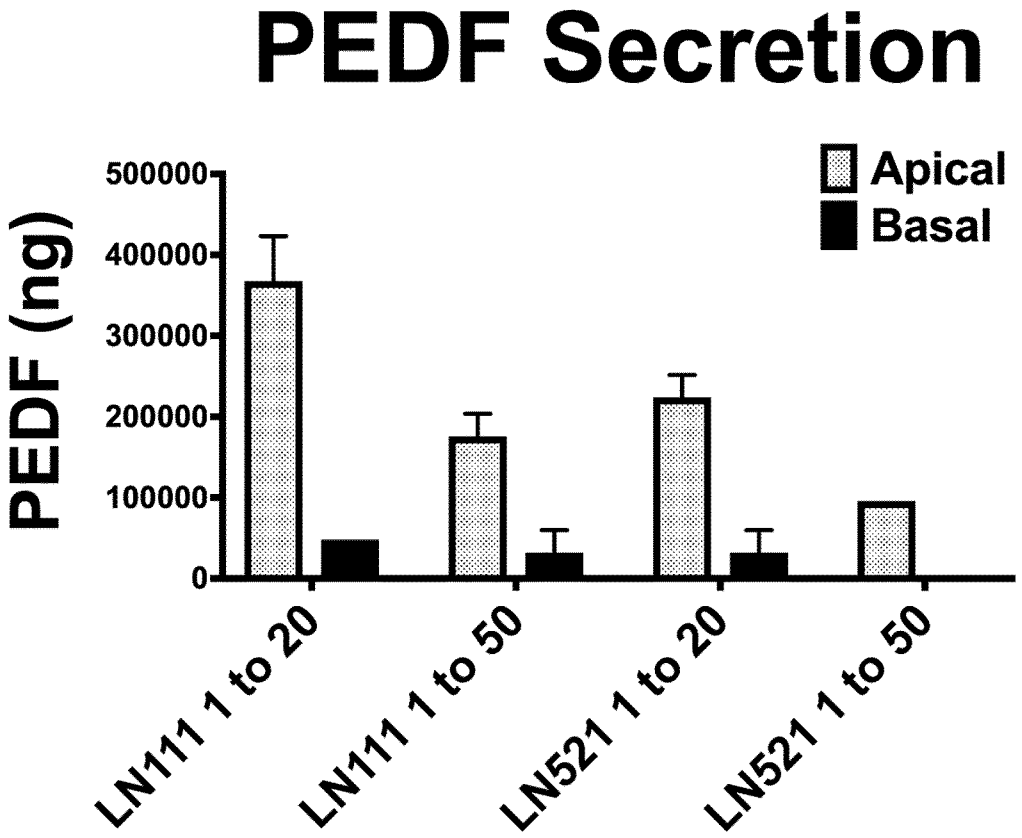


Fig 16B

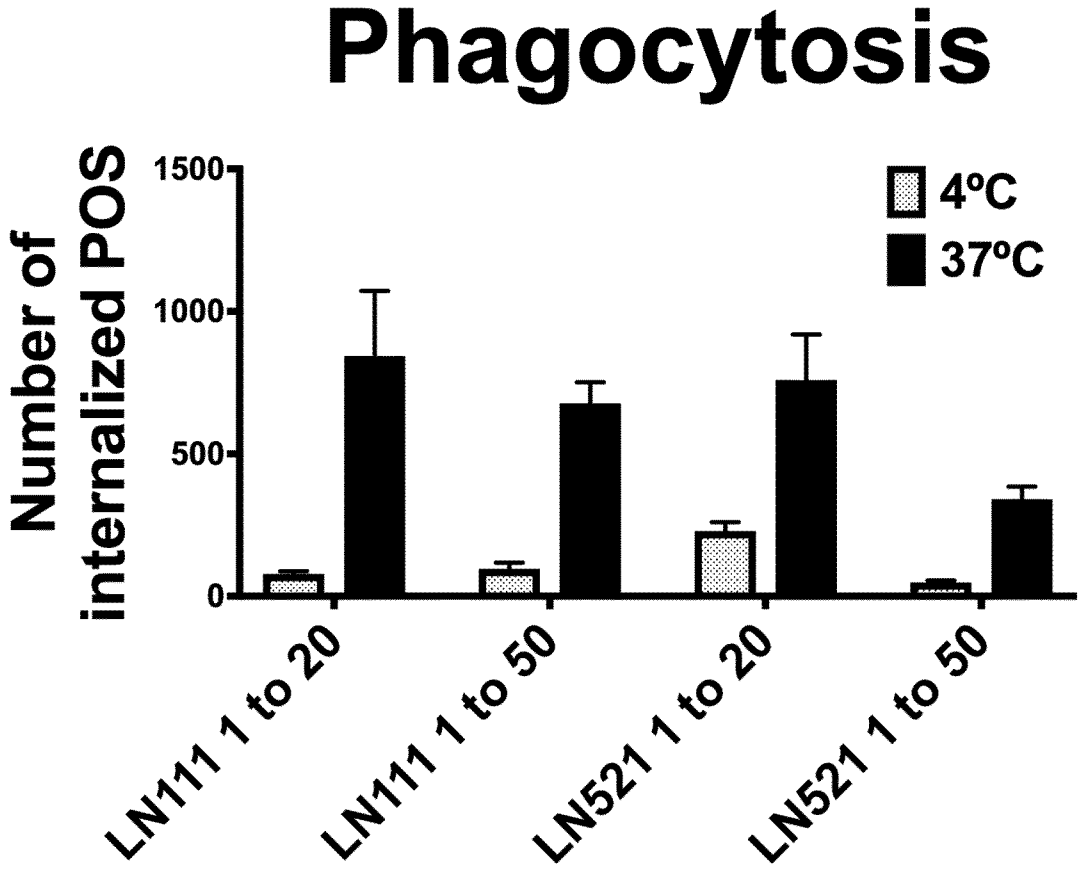


Fig 16C

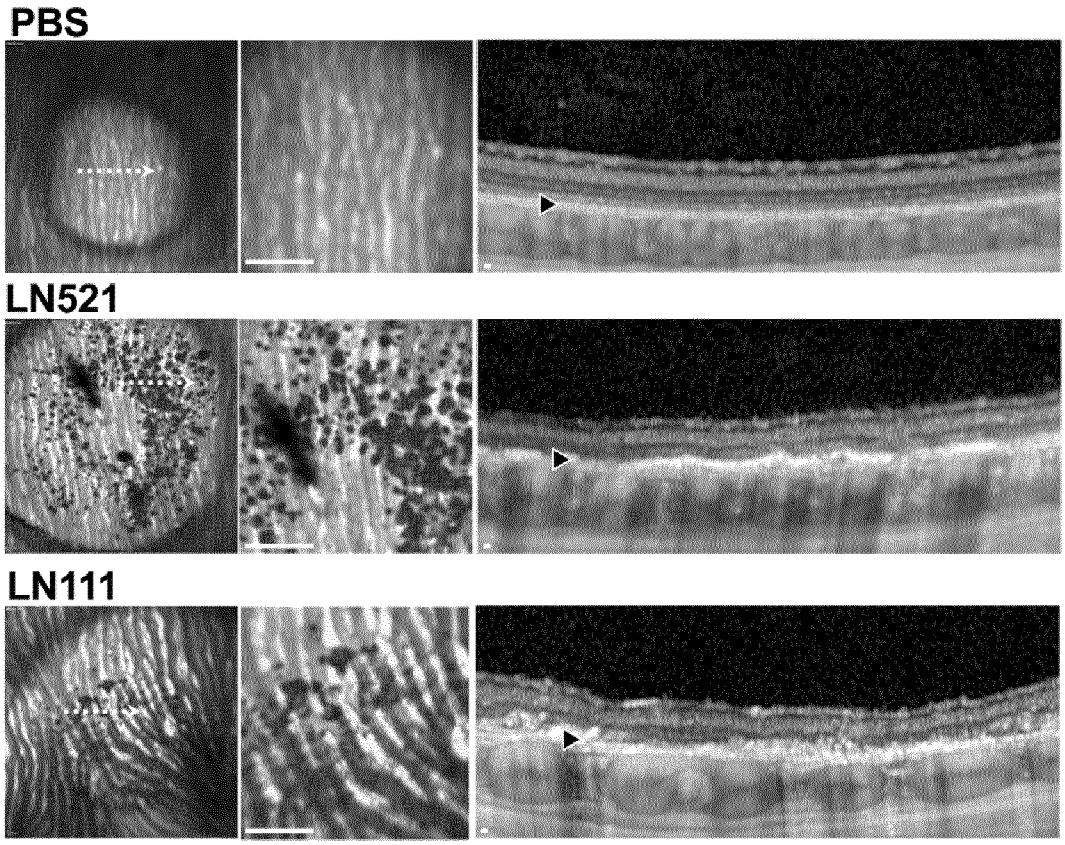
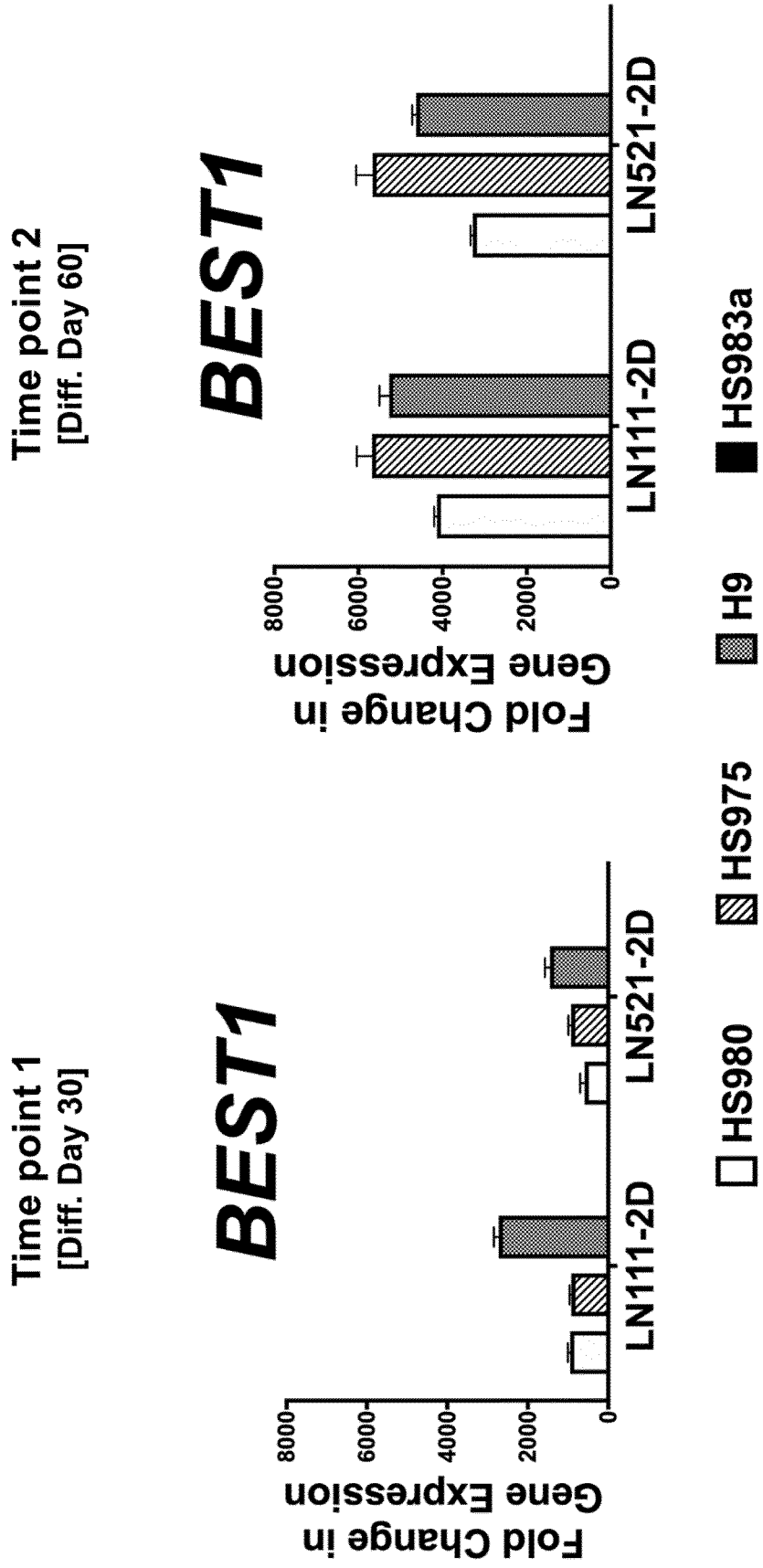


Fig 16D


Time point 2
[Diff. Day 60]
BEST1
Fold Change in
Gene Expression
8000
6000
4000
2000
0
LN111-2D
LN521-2D
Time point 1
[Diff. Day 30]
BEST1
Fold Change in
Gene Expression
8000
6000
4000
2000
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a

Figs 17A,17B

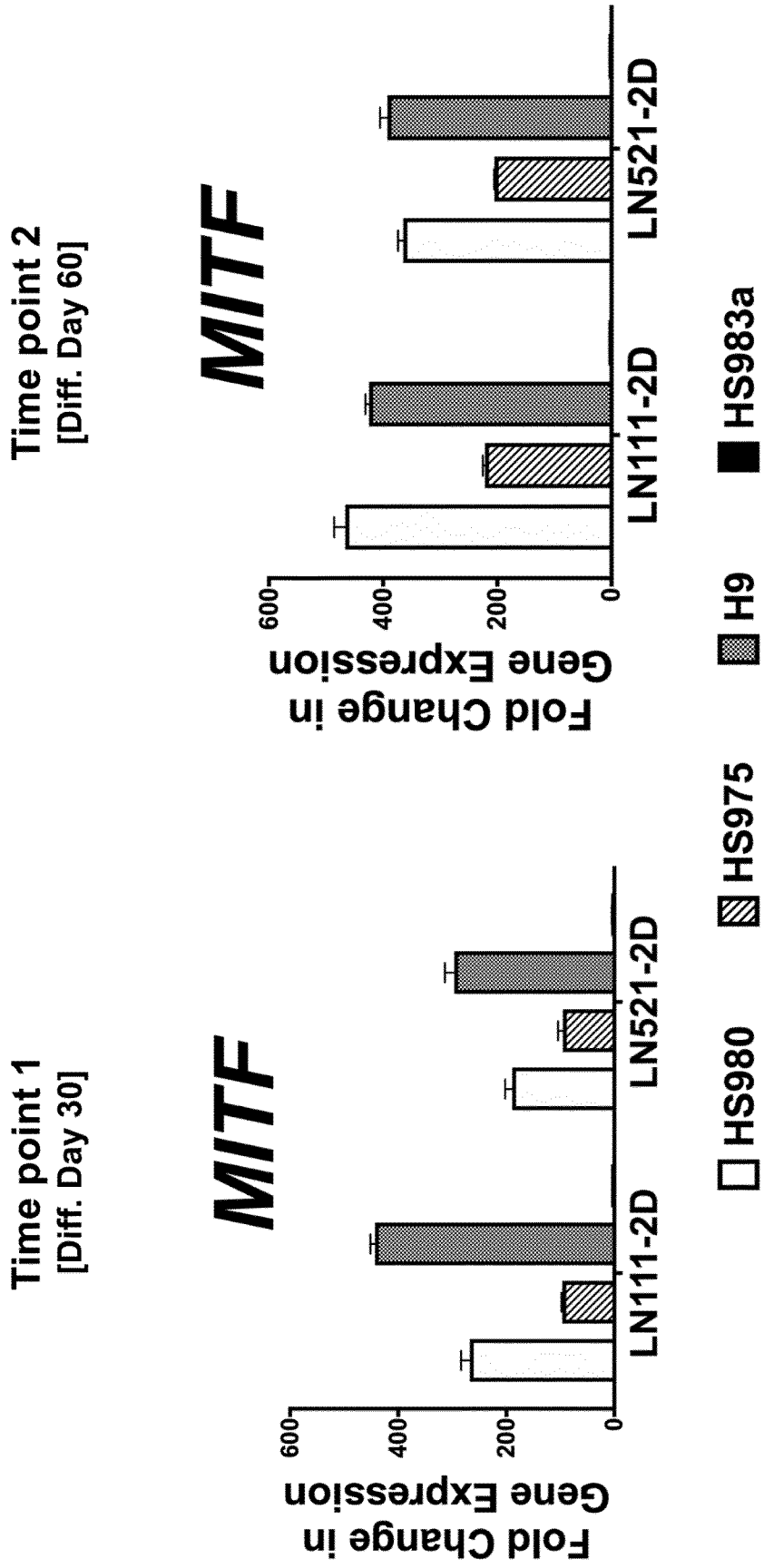
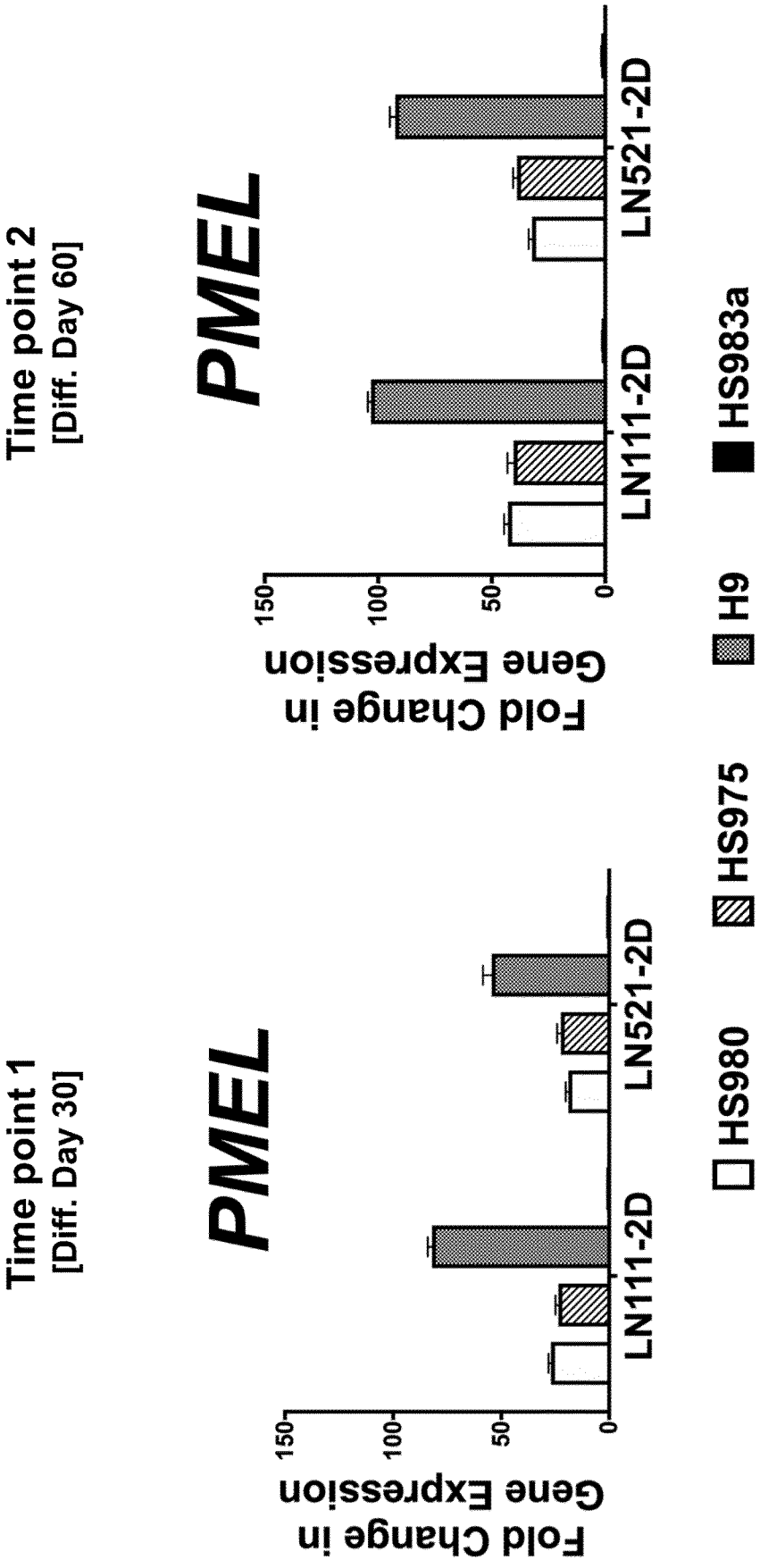
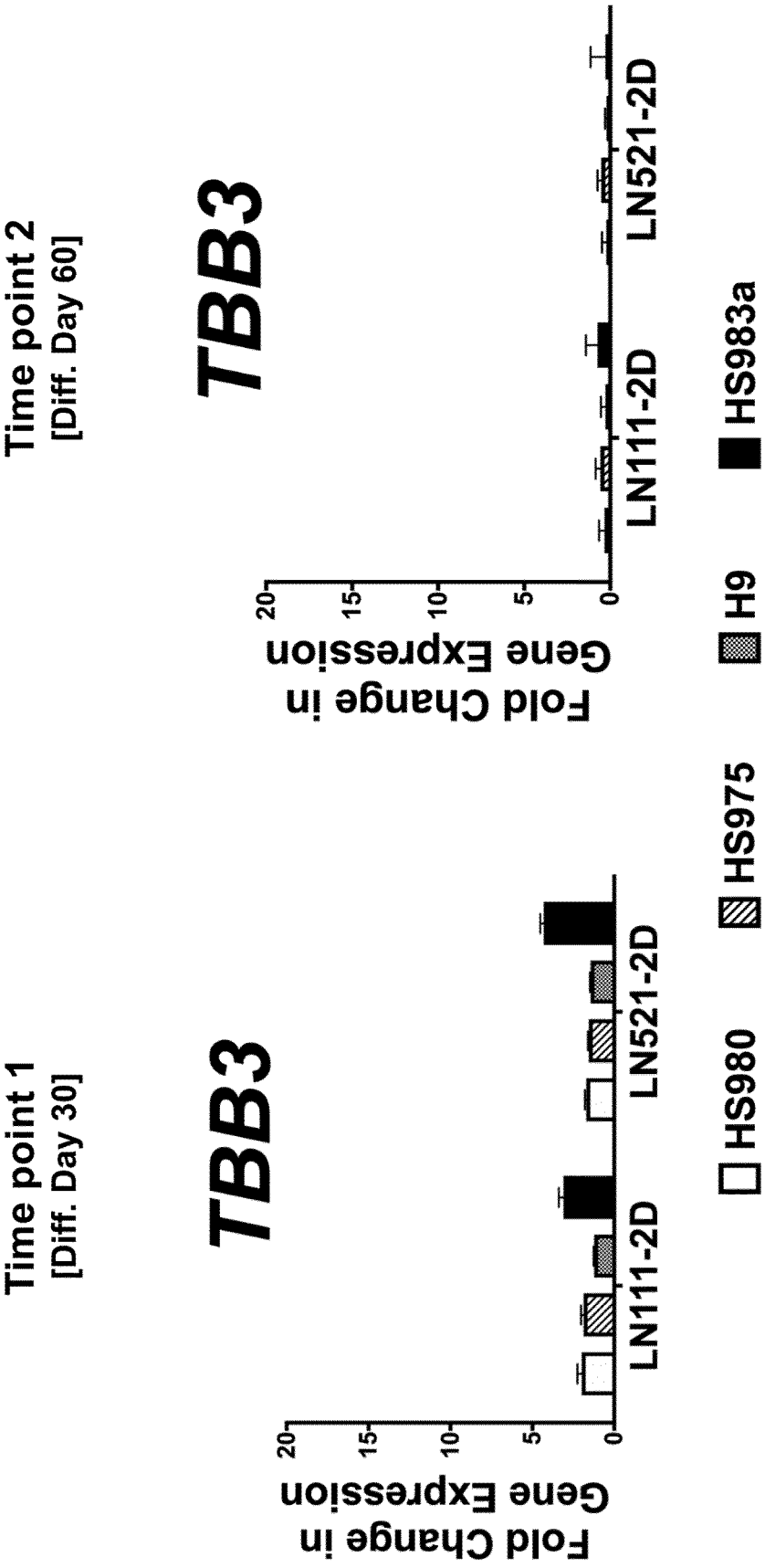

Time point 2
[Diff. Day 60]
MITF
Fold Change in
Gene Expression
600
400
200
0
LN111-2D
LN521-2D
Time point 1
[Diff. Day 30]
MITF
Fold Change in
Gene Expression
600
400
200
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a

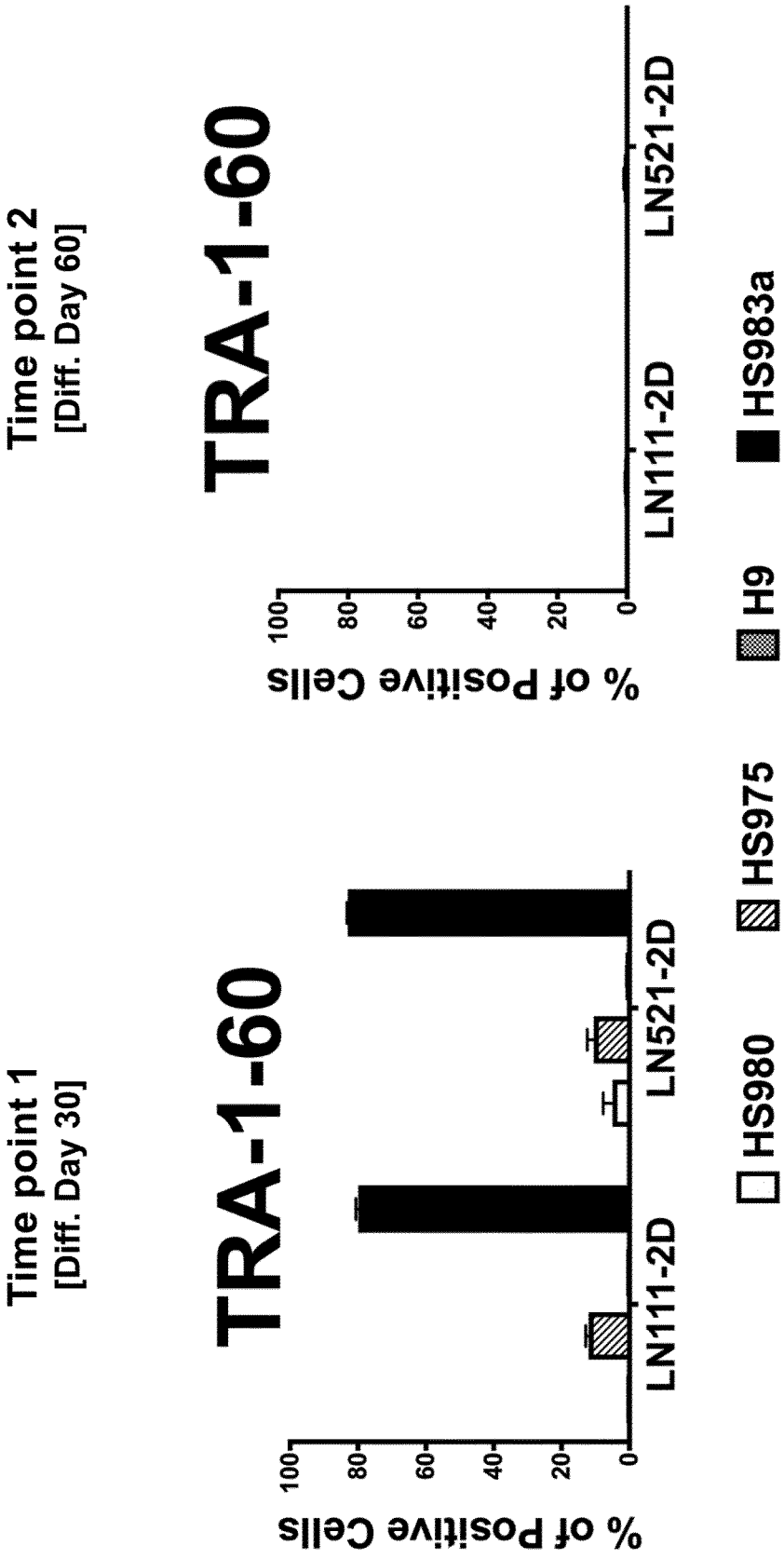
Fig 18A, 18B


Time point 1
[Diff. Day 30]
PMEL
Fold Change in
Gene Expression
150
100
50
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a
Time point 2
[Diff. Day 60]
PMEL
Fold Change in
Gene Expression
150
100
50
0
LN111-2D
LN521-2D

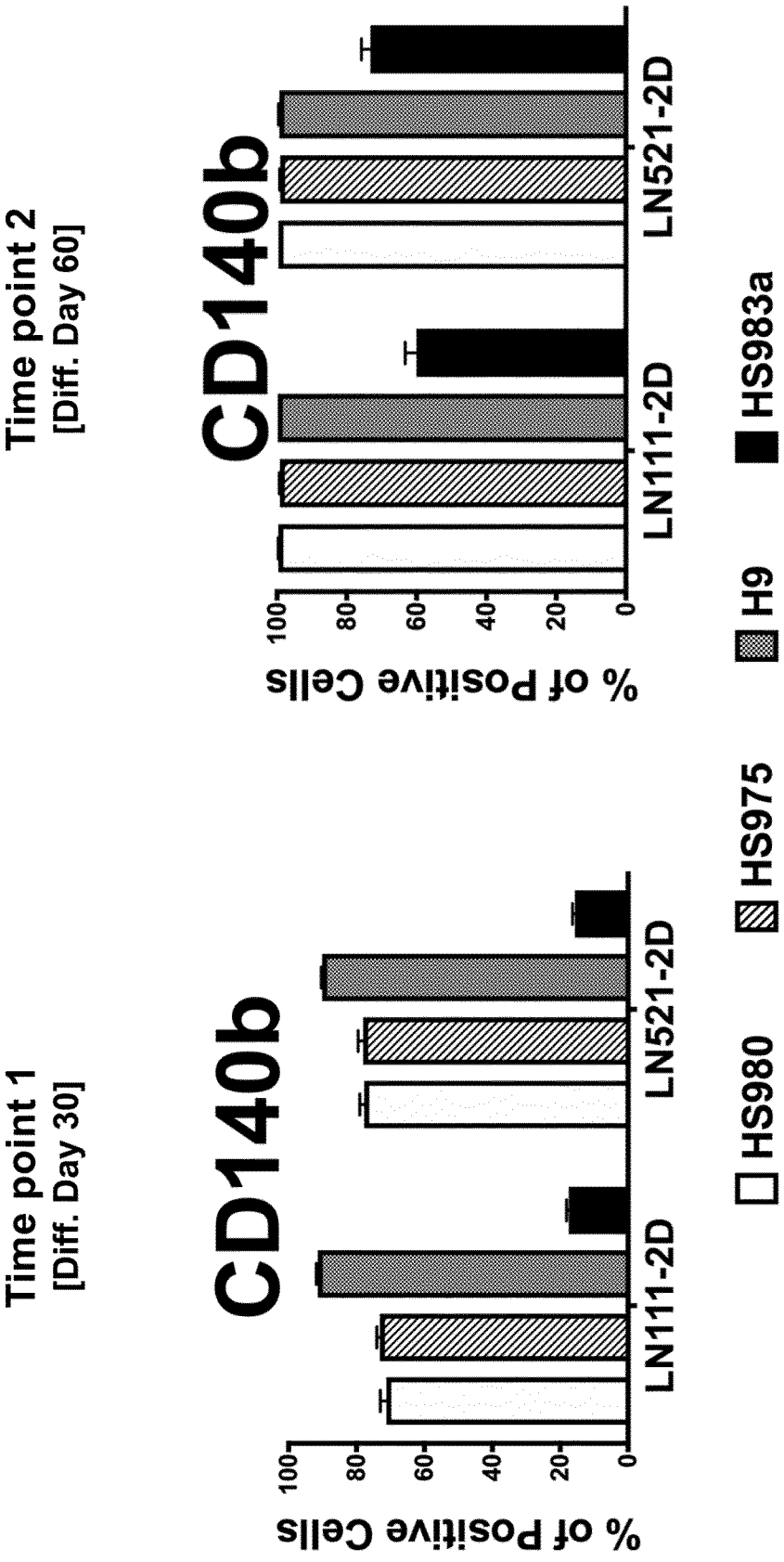
Figs 19A, 19B


Time point 1
[Diff. Day 30]
TBB3
Fold Change in
Gene Expression
0
5
10
15
20
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a
Time point 2
[Diff. Day 60]
TBB3
Fold Change in
Gene Expression
0
5
10
15
20
LN111-2D
LN521-2D

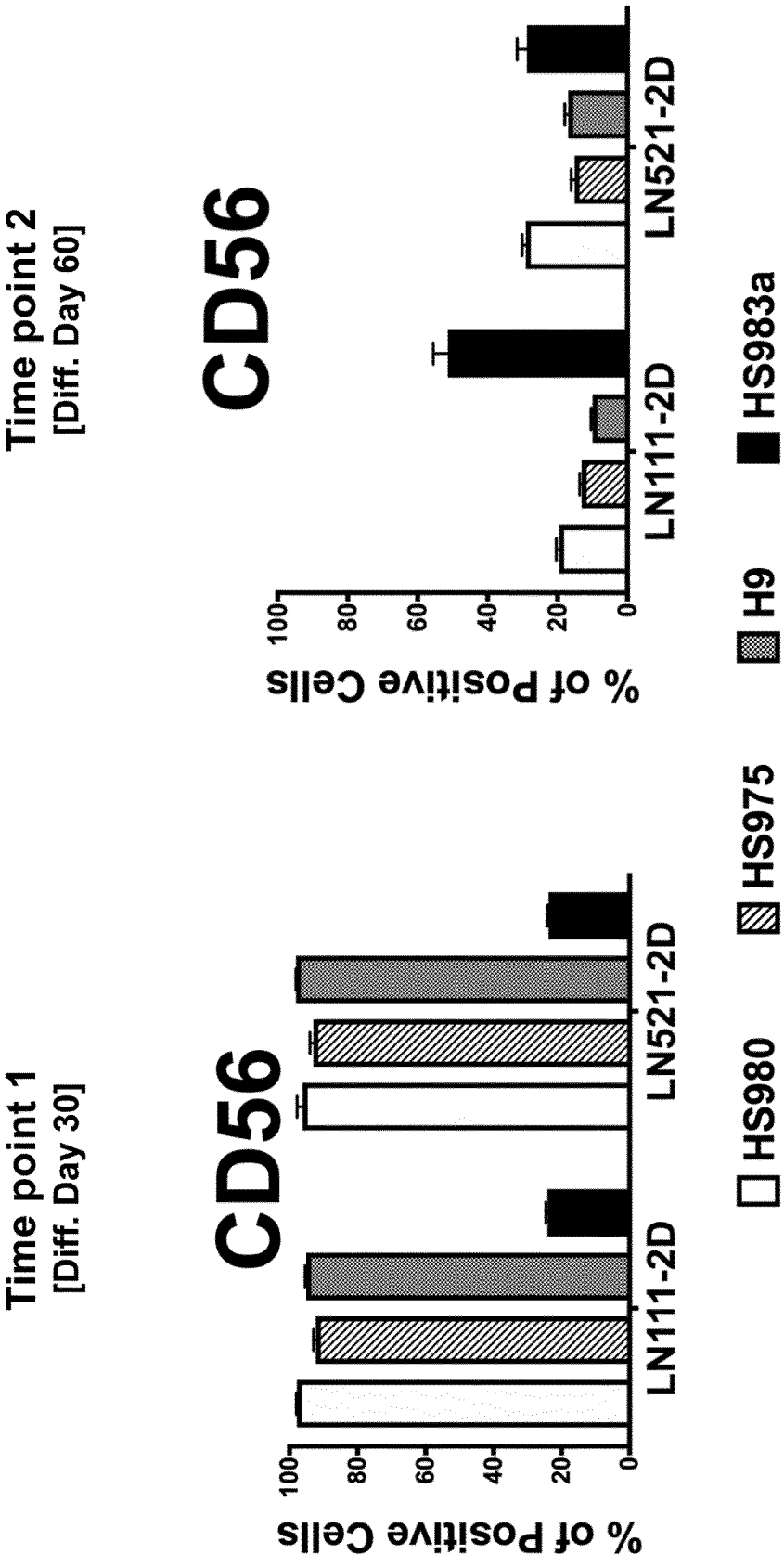
Figs 20A, 20B


Time point 1
[Diff. Day 30]
TRA-1-60
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a
Time point 2
[Diff. Day 60]
TRA-1-60
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D

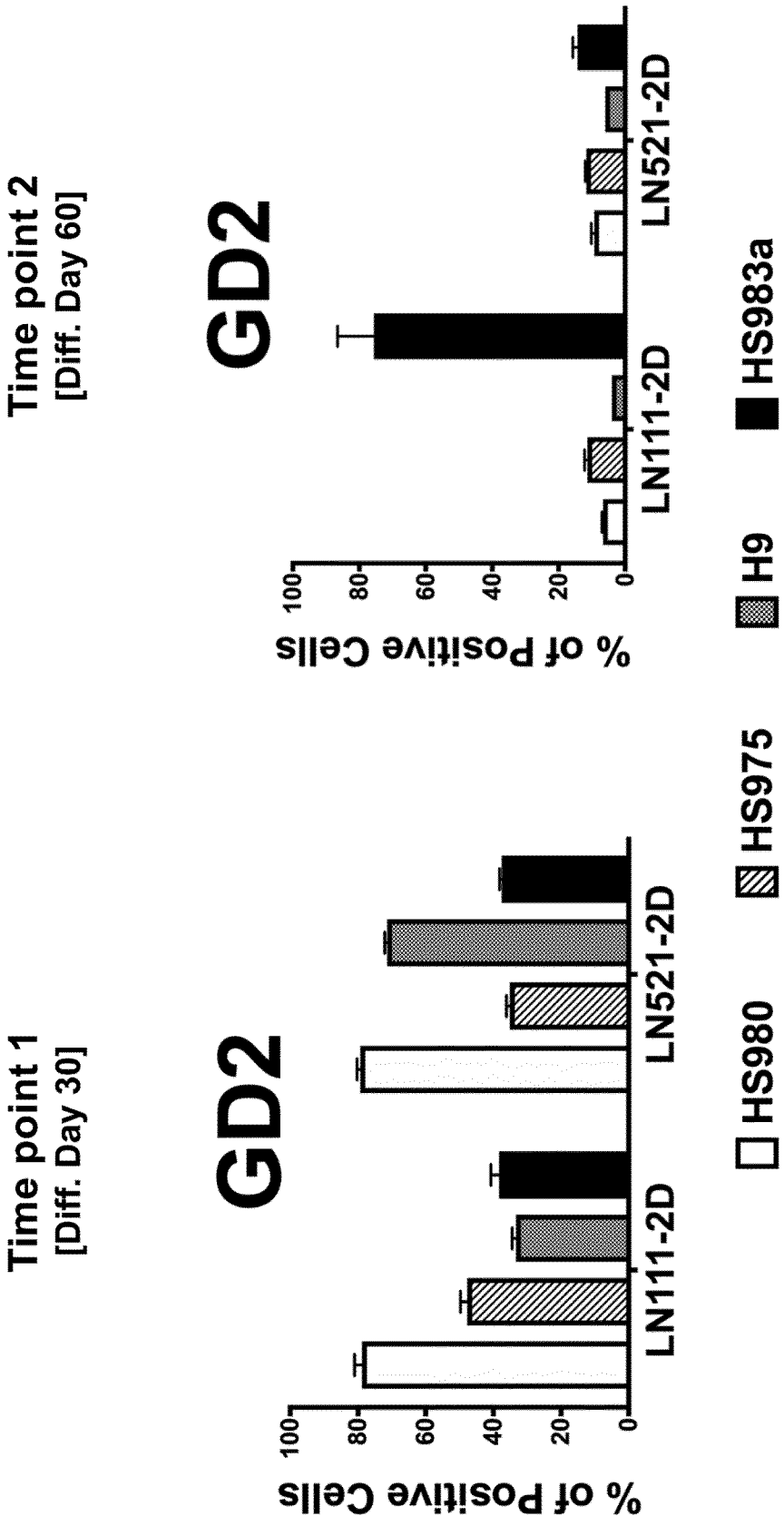
Figs 21A, 21B


Time point 2
[Diff. Day 60]
CD140b
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a
Time point 1
[Diff. Day 30]
CD140b
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a

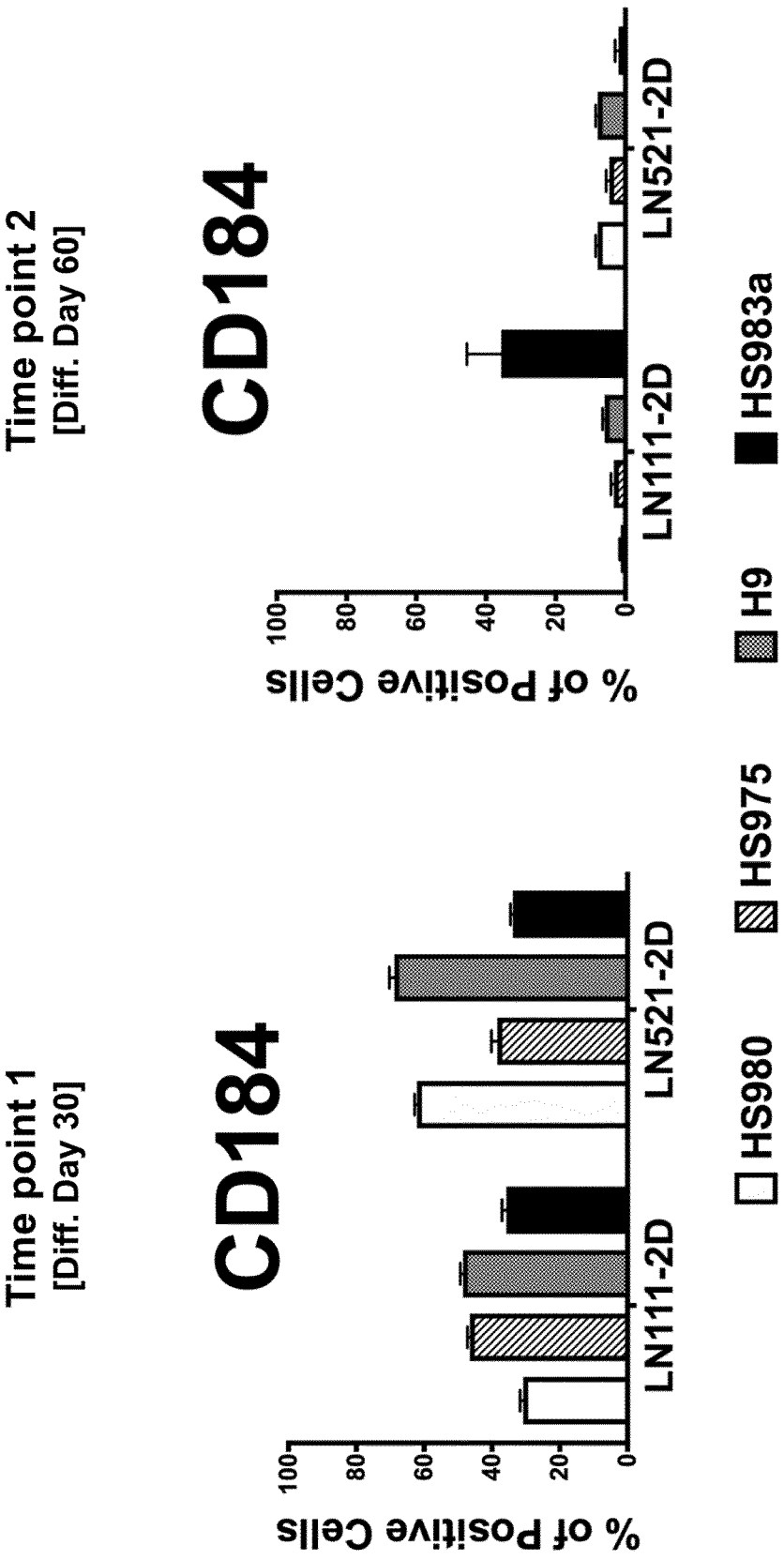
Figs 22A, 22B


Time point 1
[Diff. Day 30]
CD56
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
HS980
HS975
Time point 2
[Diff. Day 60]
CD56
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
H9
HS983a

Figs 23A, 23B


Time point 2
[Diff. Day 60]
GD2
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
Time point 1
[Diff. Day 30]
GD2
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a

Figs 24A, 24B


Time point 2
[Diff. Day 60]
CD184
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
Time point 1
[Diff. Day 30]
CD184
% of Positive Cells
100
80
60
40
20
0
LN111-2D
LN521-2D
HS980
HS975
H9
HS983a

Figs 25A, B

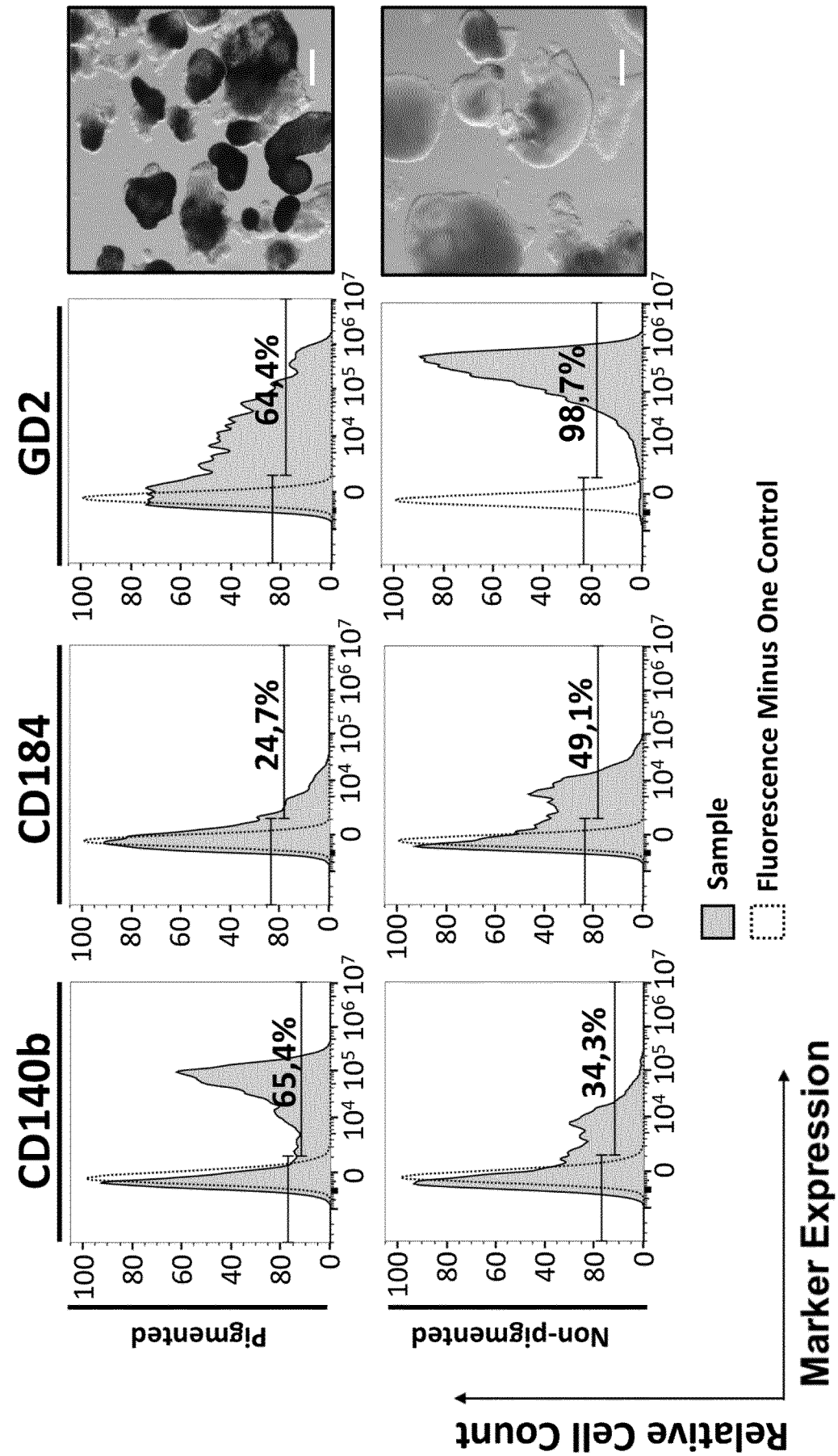

CD140b
CD184
GD2
Pigmented
Non-pigmented
65,4%
24,7%
64,4%
34,3%
49,1%
98,7%
Relative Cell Count
Marker Expression
Sample
Fluorescence Minus One Control

Fig 26

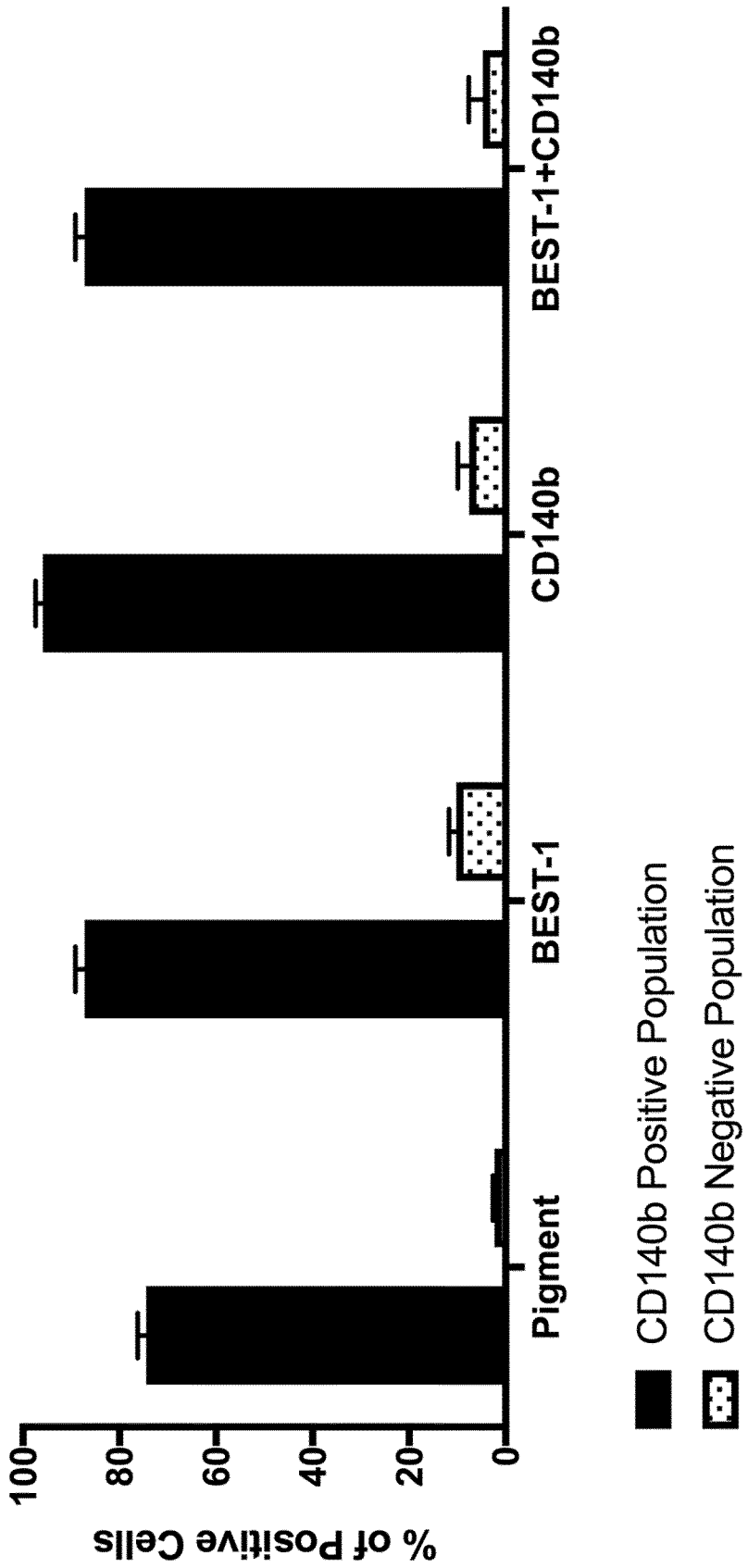

% of Positive Cells
100
80
60
40
20
0
Pigment
BEST-1
CD140b
BEST-1+CD140b
CD140b Positive Population
CD140b Negative Population

Fig 27

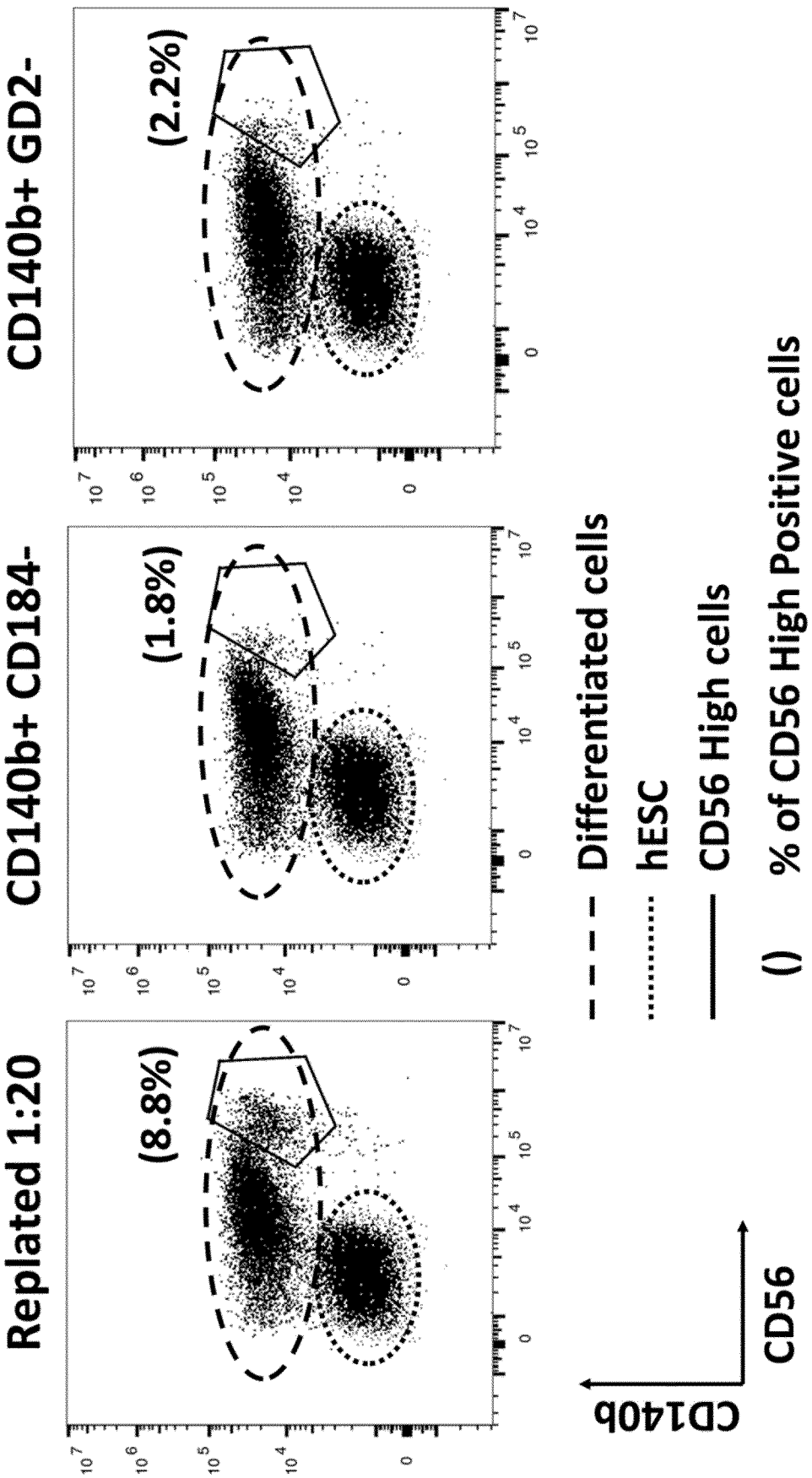

Replated 1:20
CD140b+ CD184-
CD140b+ GD2-
(8.8%)
(1.8%)
(2.2%)
Differentiated cells
hESC
CD56 High cells
() % of CD56 High Positive cells
CD140b
CD56

Fig 28

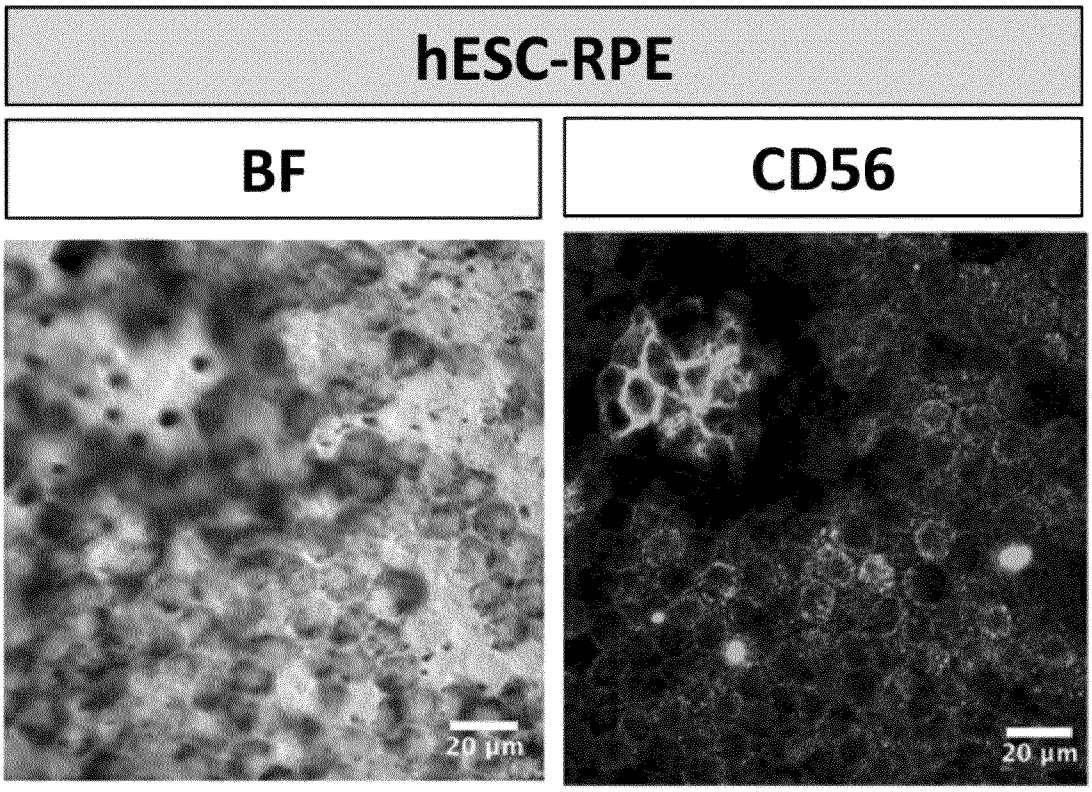

hESC-RPE
BF
CD56
20 µm
20 µm

Fig 29

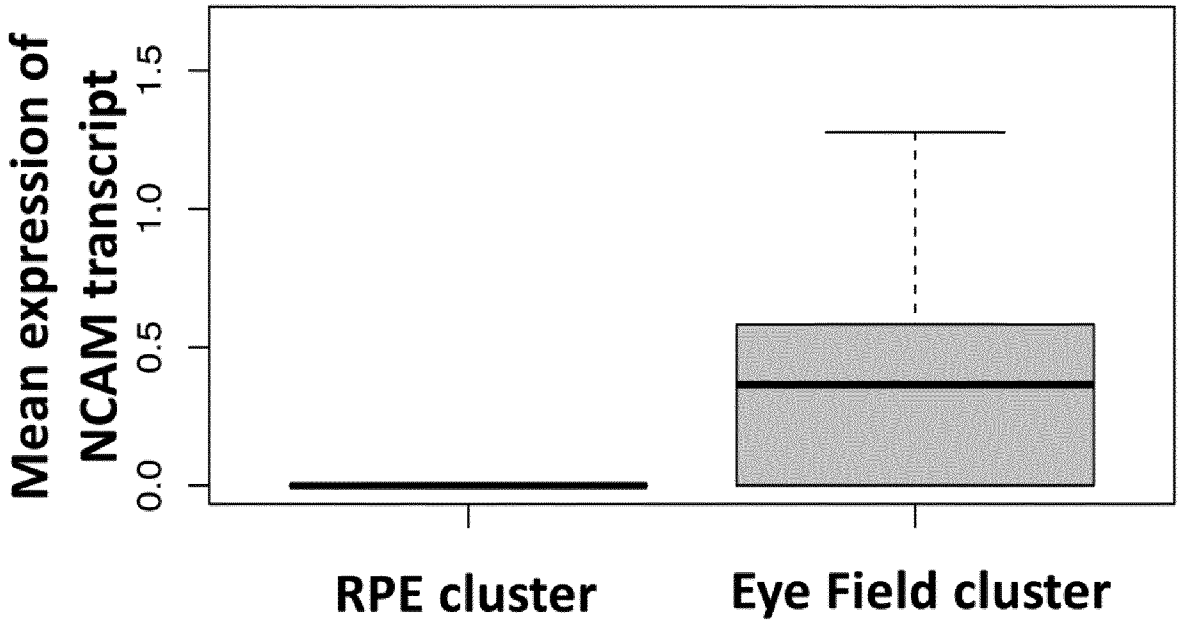

Mean expression of NCAM transcript
0.0
0.5
1.0
1.5
RPE cluster
Eye Field cluster

Fig 30

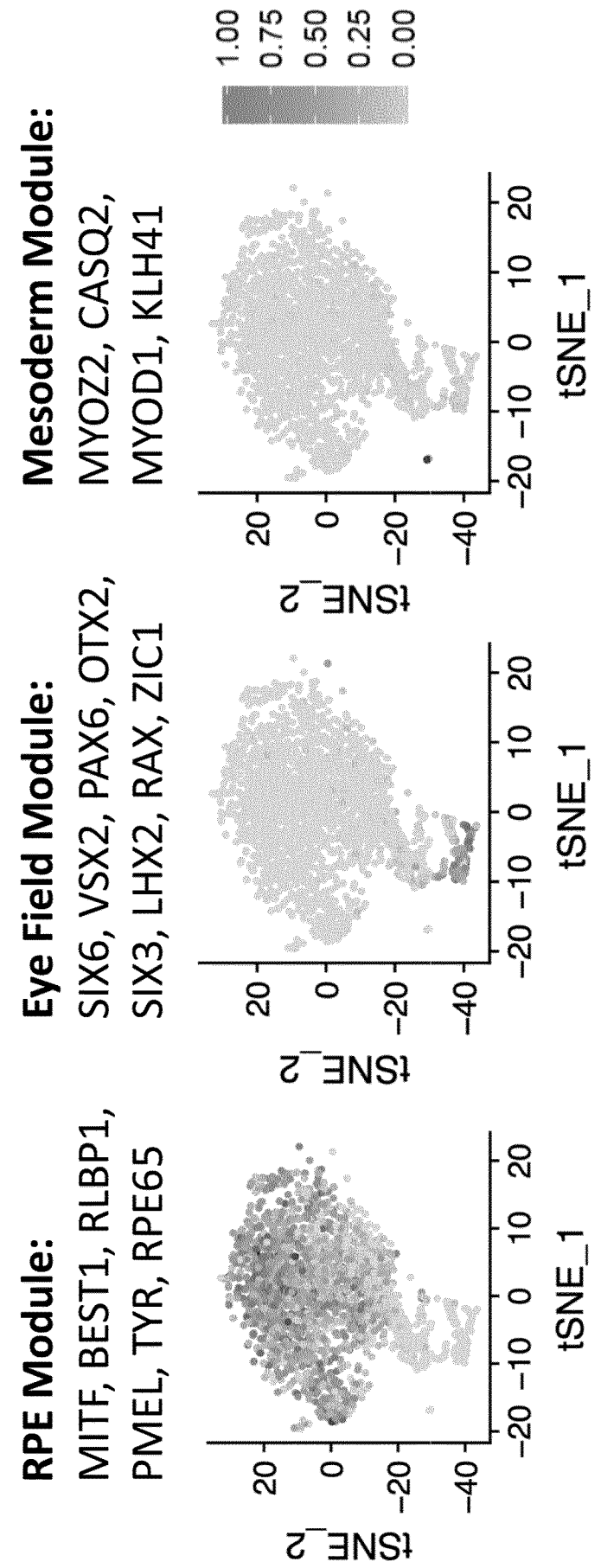
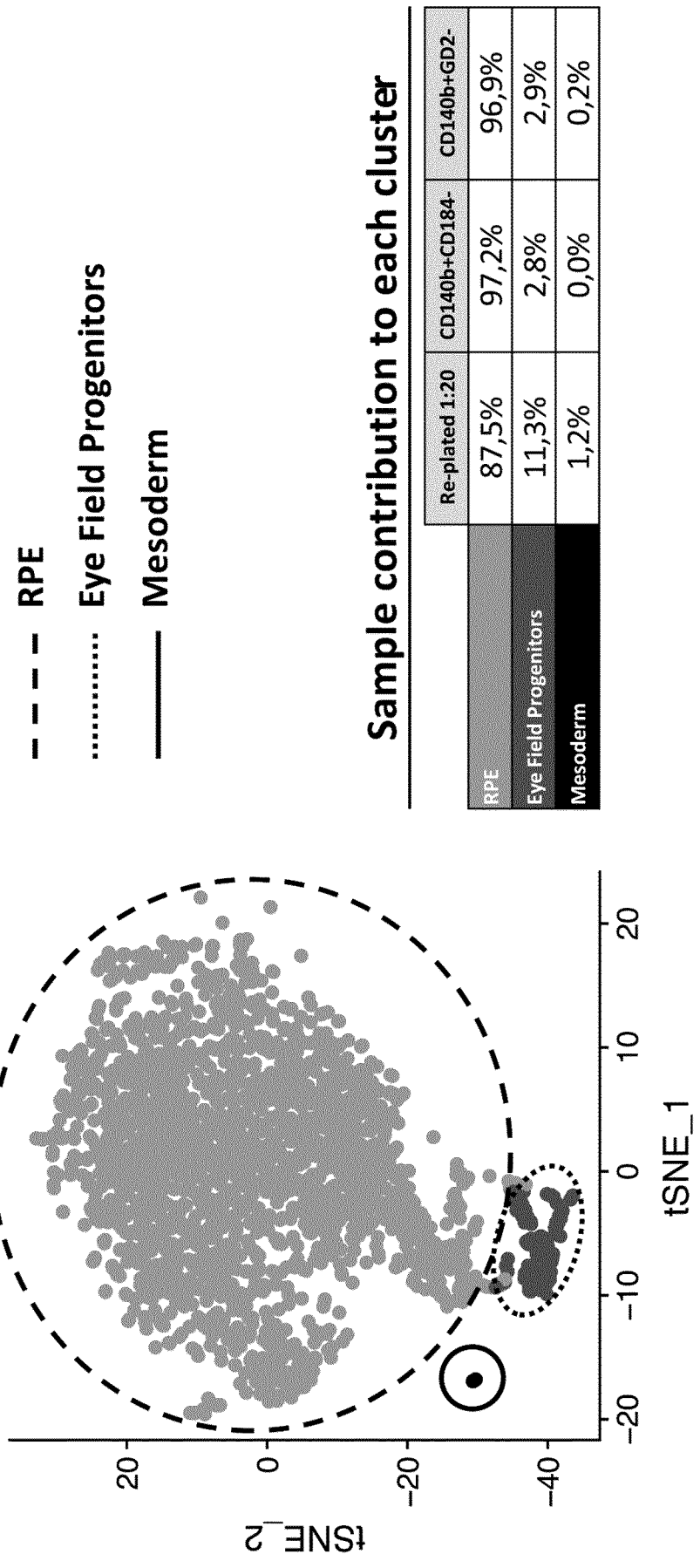

RPE Module:
MITF, BEST1, RLBP1,
PMEL, TYR, RPE65
Eye Field Module:
SIX6, VSX2, PAX6, OTX2,
SIX3, LHX2, RAX, ZIC1
Mesoderm Module:
MYOZ2, CASQ2,
MYOD1, KLH41
1.00
0.75
0.50
0.25
0.00
tSNE_2
tSNE_1
20
0
−20
−40
−20 −10 0 10 20

Fig 31



Sample contribution to each cluster

	Re-plated 1:20	CD140b+CD184-	CD140b+GD2-
RPE	87,5%	97,2%	96,9%
Eye Field Progenitors	11,3%	2,8%	2,9%
Mesoderm	1,2%	0,0%	0,2%

Fig 32

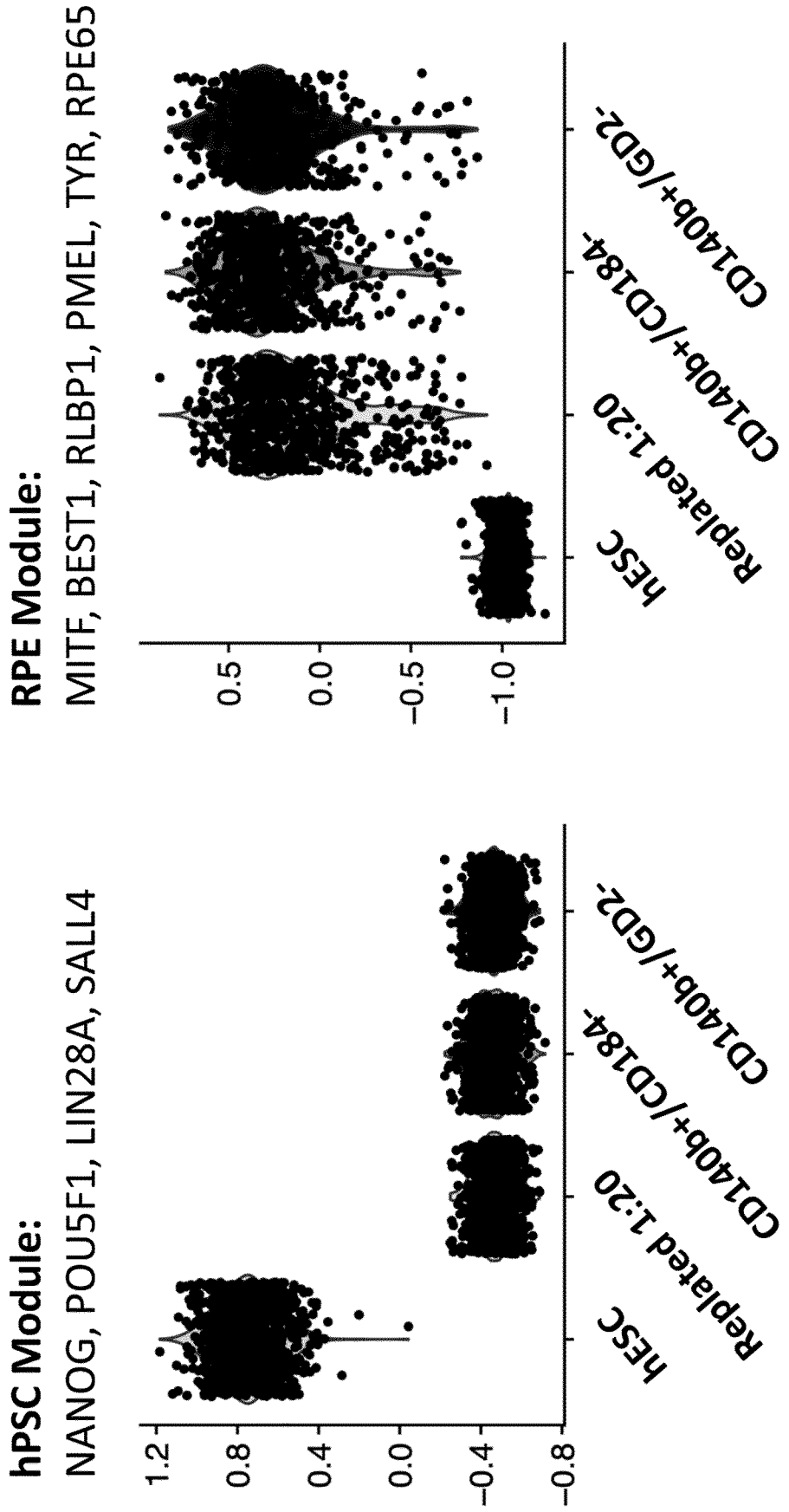

RPE Module:
MITF, BEST1, RLBP1, PMEL, TYR, RPE65
0.5
0.0
−0.5
−1.0
hESC
Replated 1:20
CD140b+/CD184-
CD140b+/GD2-
hPSC Module:
NANOG, POU5F1, LIN28A, SALL4
1.2
0.8
0.4
0.0
−0.4
−0.8
hESC
Replated 1:20
CD140b+/CD184-
CD140b+/GD2-

Fig 33

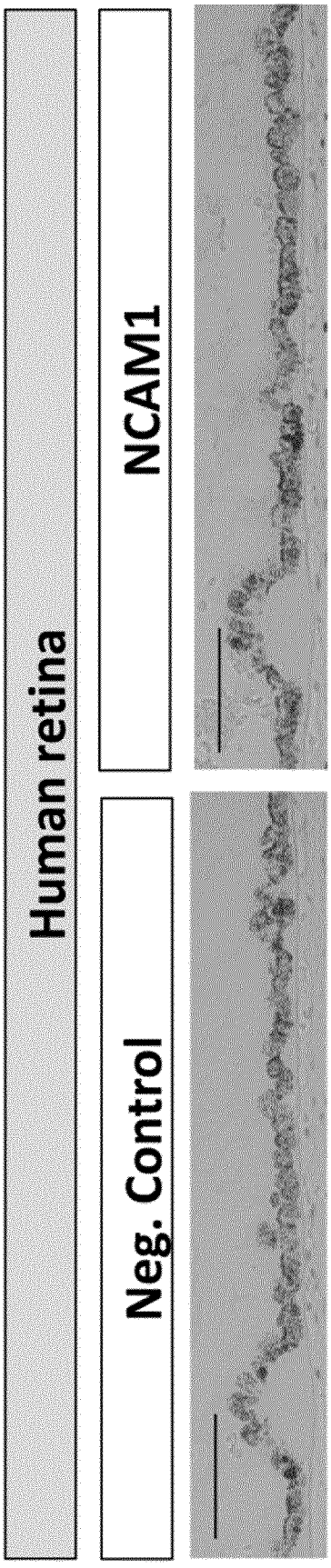


Fig 34

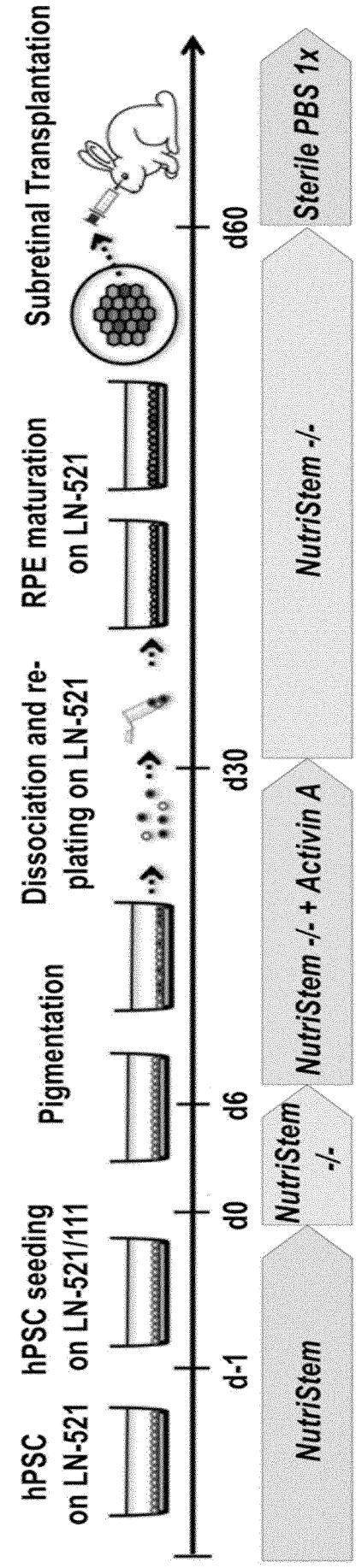

hPSC on LN-521
hPSC seeding on LN-521/111
Pigmentation
Dissociation and re-plating on LN-521
RPE maturation on LN-521
Subretinal Transplantation
d-1
d0
d6
d30
d60
NutriStem
NutriStem -/-
NutriStem -/- + Activin A
NutriStem -/-
Sterile PBS 1x

Fig 35

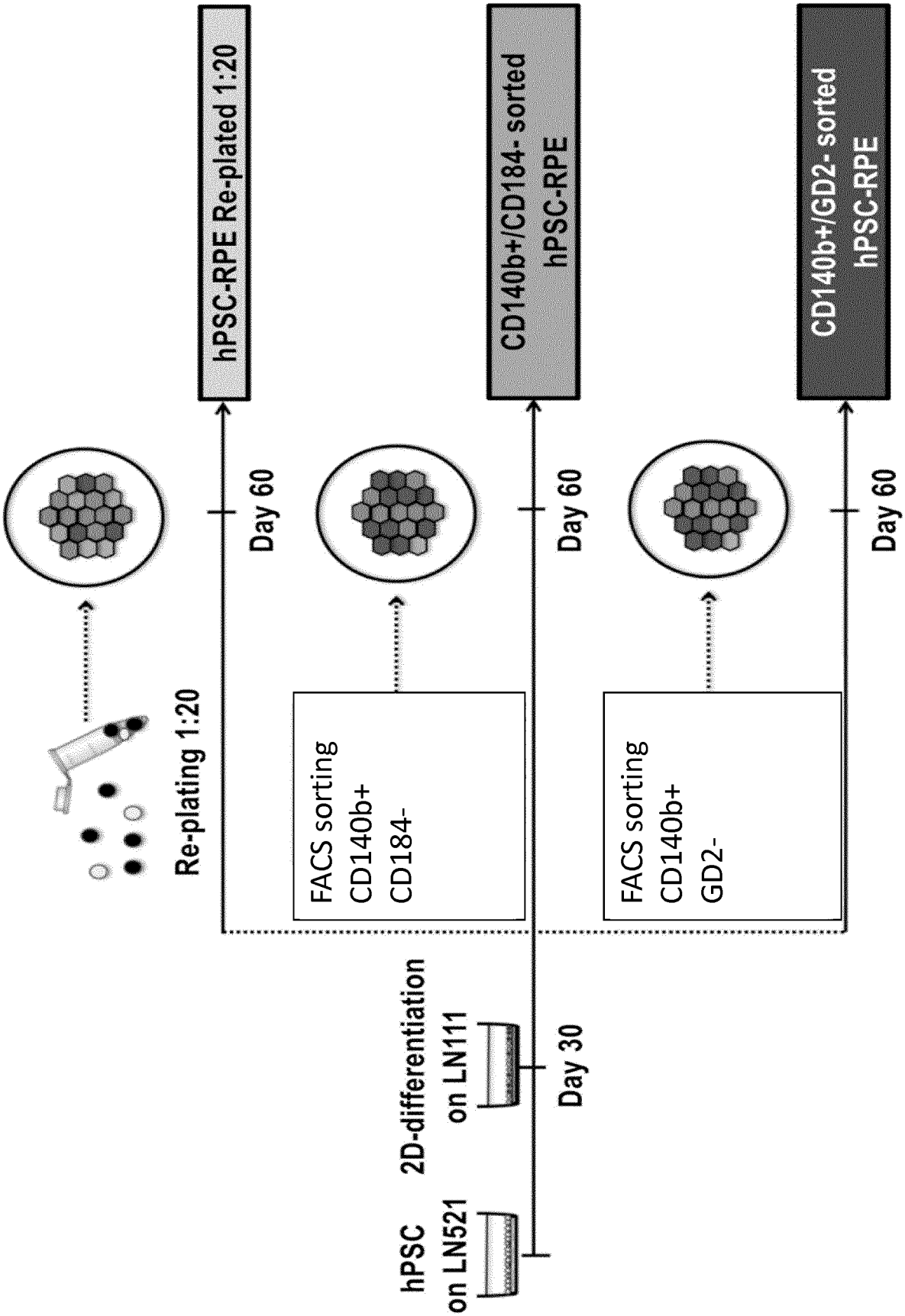

hPSC on LN521
2D-differentiation on LN111
Day 30
Re-plating 1:20
Day 60
hPSC-RPE Re-plated 1:20
FACS sorting CD140b+ CD184-
Day 60
CD140b+/CD184- sorted hPSC-RPE
FACS sorting CD140b+ GD2-
Day 60
CD140b+/GD2- sorted hPSC-RPE

Fig 36

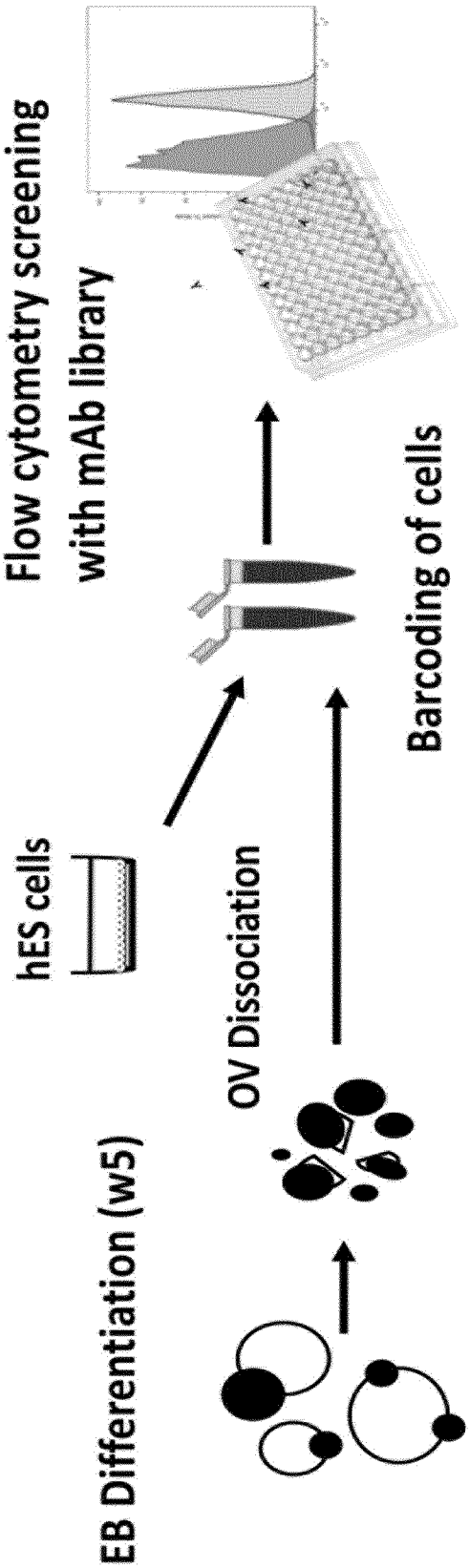

Flow cytometry screening
with mAb library
Barcoding of cells
hES cells
OV Dissociation
EB Differentiation (w5)

Fig 37A

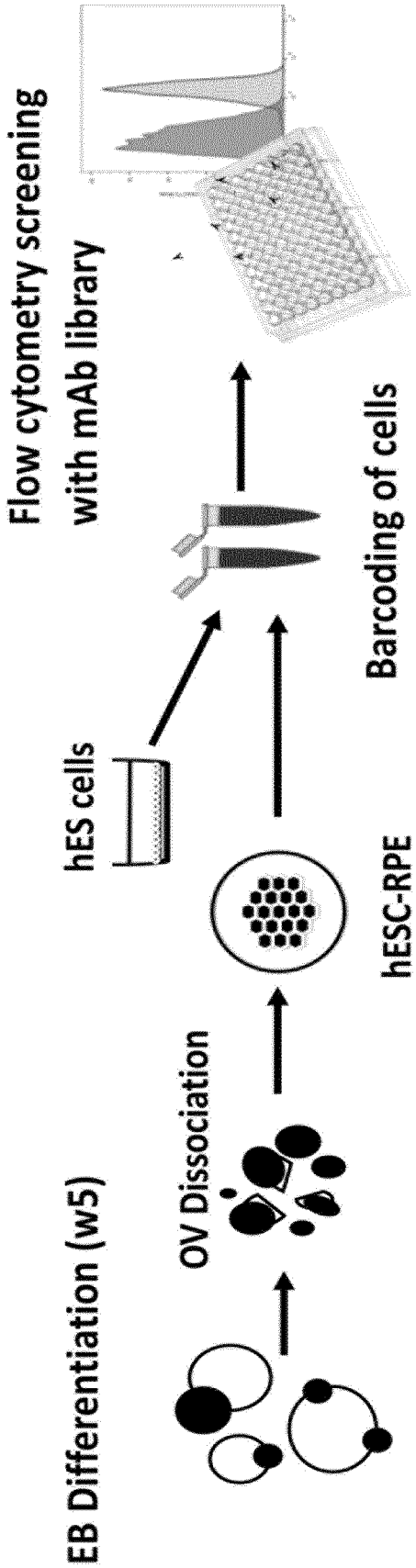

EB Differentiation (w5)
OV Dissociation
hESC-RPE
hES cells
Barcoding of cells
Flow cytometry screening
with mAb library

Fig 37B

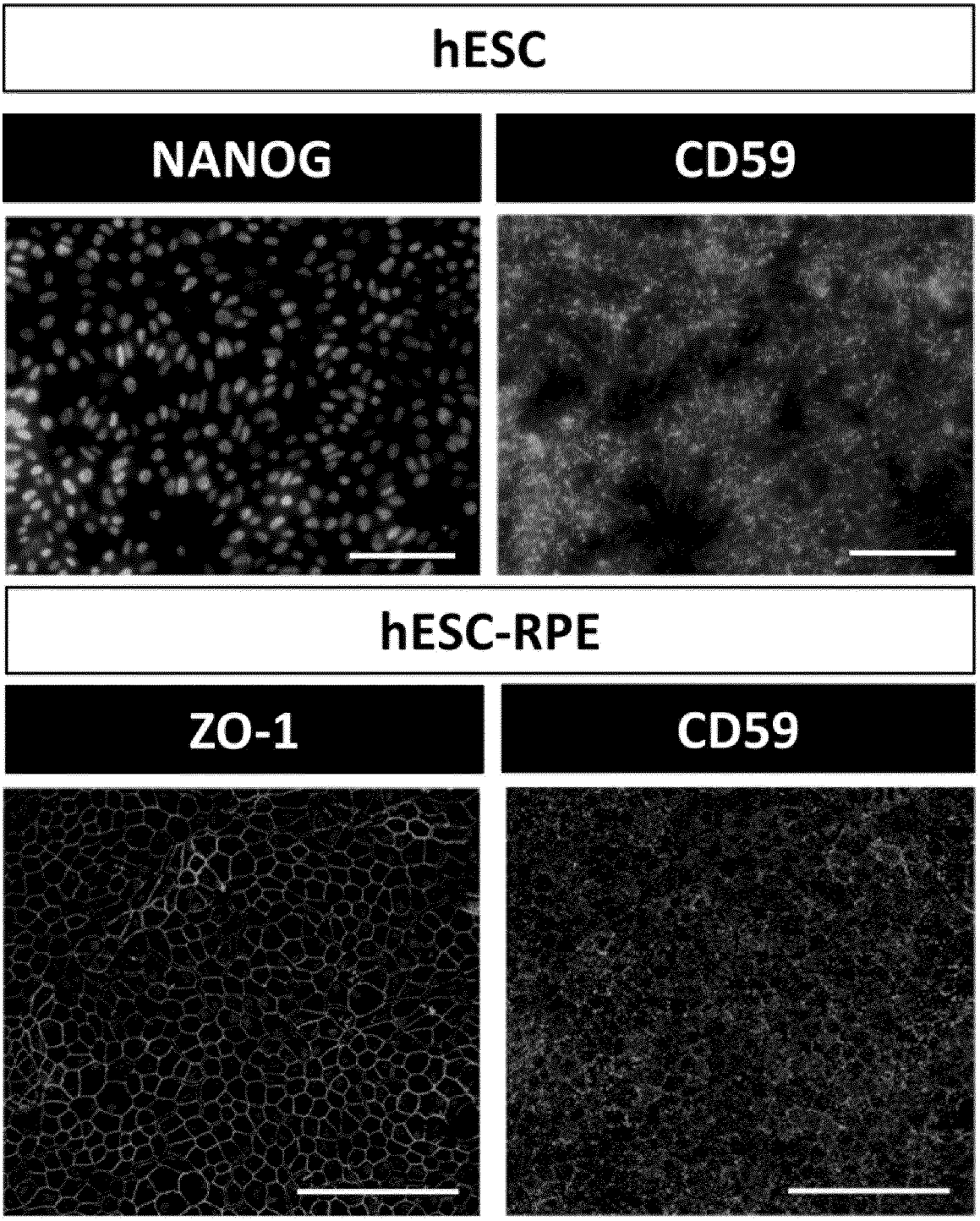


Fig 38