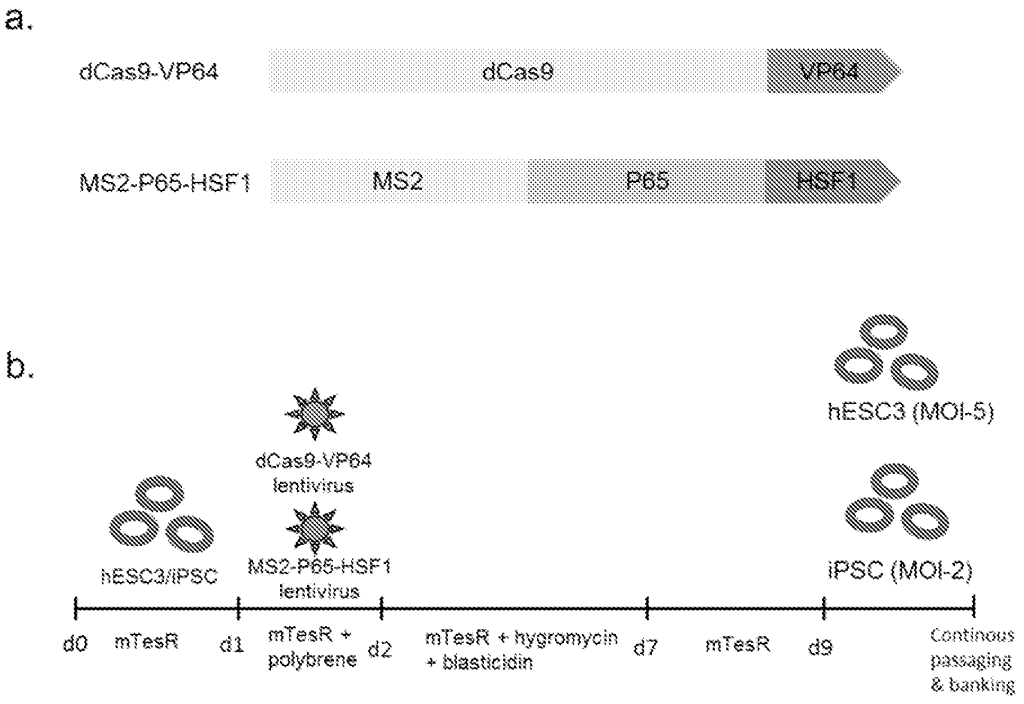

a.
dCas9-VP64
dCas9
VP64
MS2-P65-HSF1
MS2
P65
HSF1
b.
hESC3 (MOI-5)
dCas9-VP64
lentivirus
hESC3/iPSC
MS2-P65-HSF1
lentivirus
iPSC (MOI-2)
d0
mTesR
d1
mTesR +
polybrene
d2
mTesR + hygromycin
+ blasticidin
d7
mTesR
d9
Continous
passaging
& banking

Fig. 1

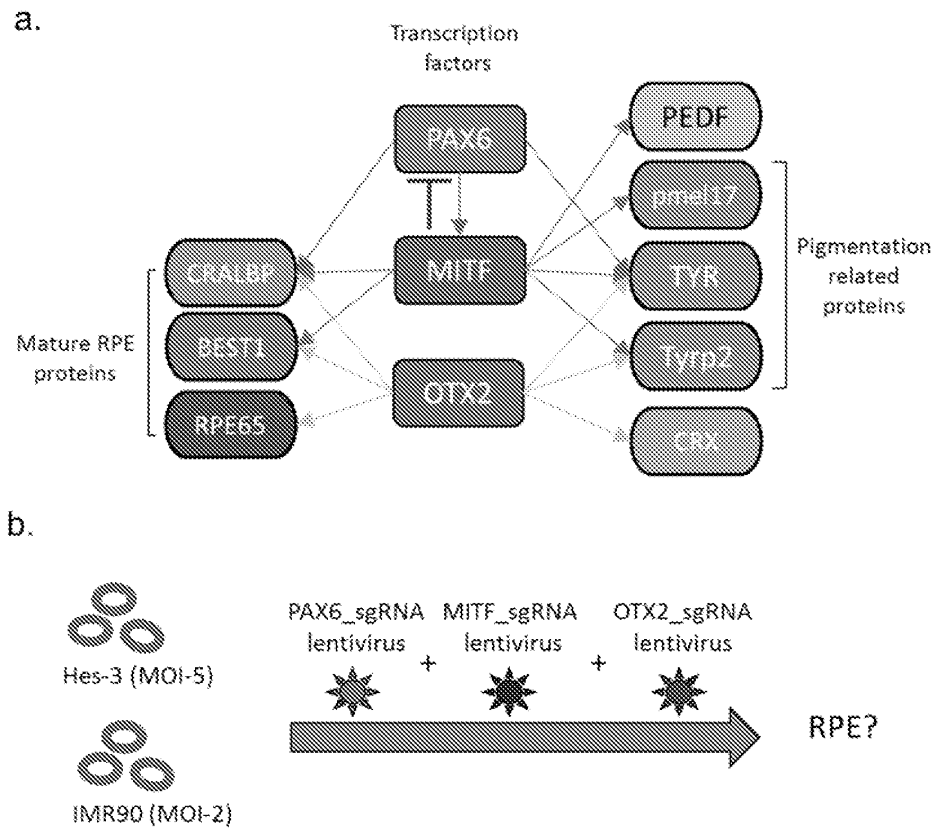

a.
Transcription factors
PAX6
MITF
OTX2
PEDF
pmel17
TYR
Tyrp2
Crx
CRALBP
BEST1
RPE65
Mature RPE proteins
Pigmentation related proteins
b.
Hes-3 (MOI-5)
IMR90 (MOI-2)
PAX6_sgRNA lentivirus
MITF_sgRNA lentivirus
OTX2_sgRNA lentivirus
RPE?

Fig. 2

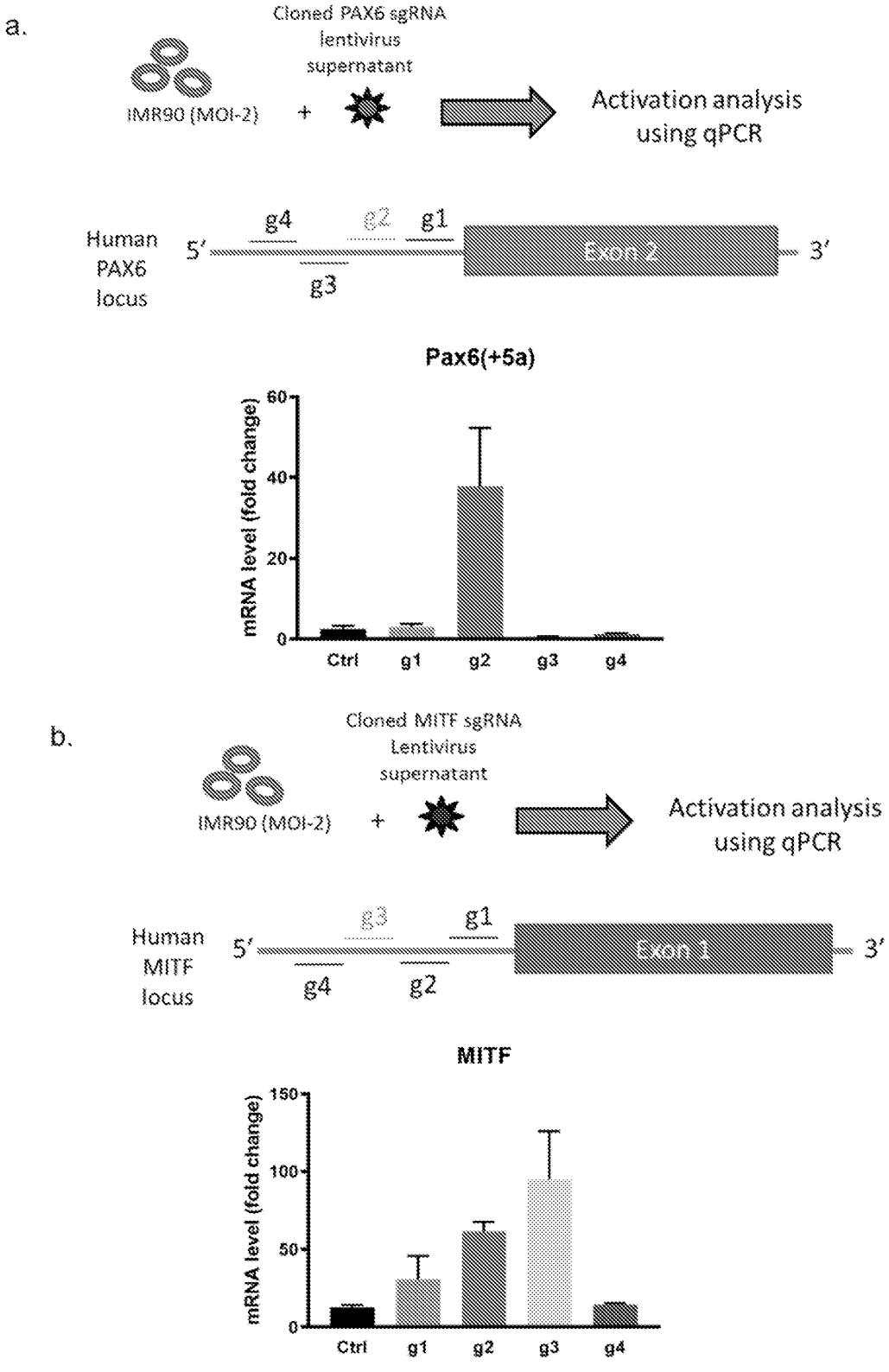


Fig. 3 (continued)

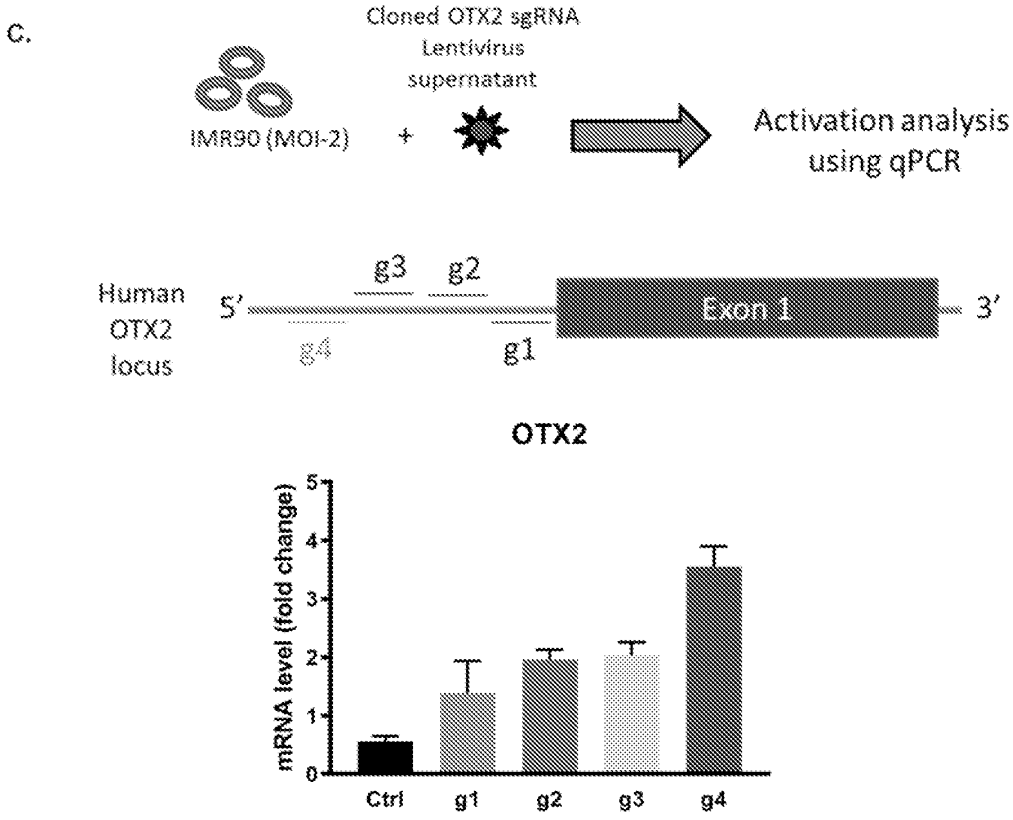
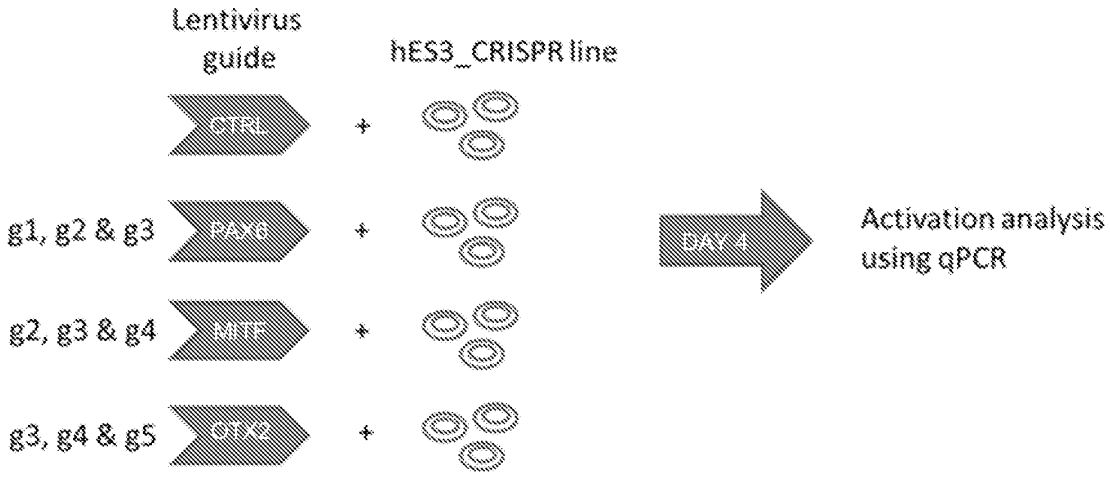

c.
Cloned OTX2 sgRNA
Lentivirus
supernatant
IMR90 (MOI-2)
+
Activation analysis
using qPCR
Human
OTX2
locus
5'
g3
g2
g4
g1
Exon 1
3'
OTX2
mRNA level (fold change)
0
1
2
3
4
5
Ctrl
g1
g2
g3
g4

Fig. 3

a.


Lentivirus guide
hES3_CRISPR line
CTRL
+
g1, g2 & g3
PAX6
+
g2, g3 & g4
MITF
+
g3, g4 & g5
OTX2
+
DAY 4
Activation analysis using qPCR

b.

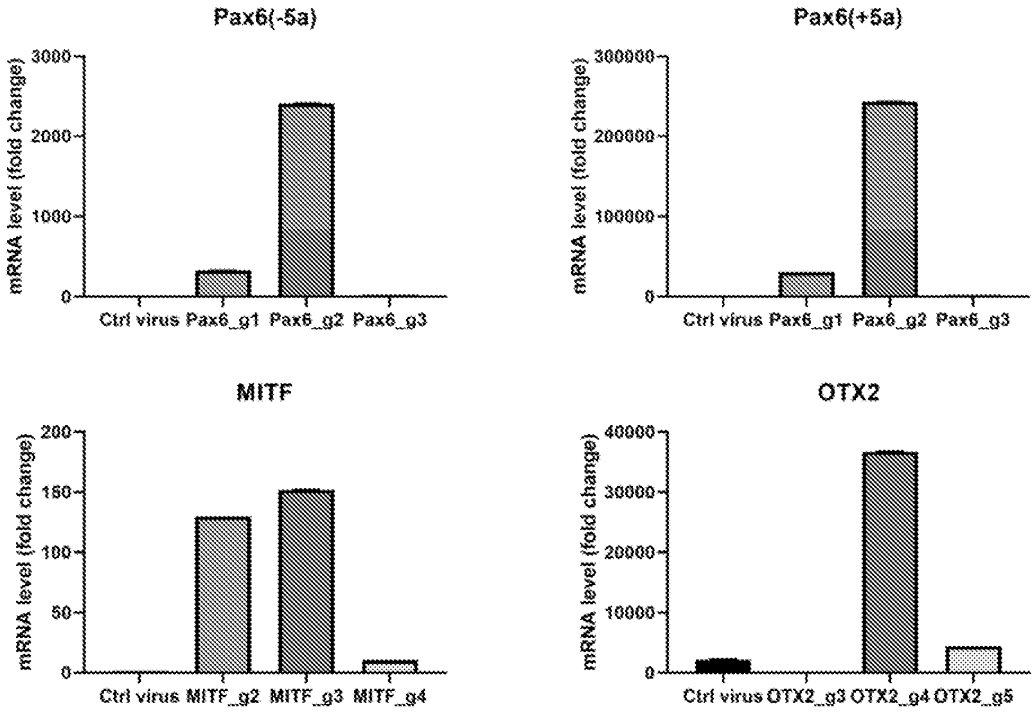
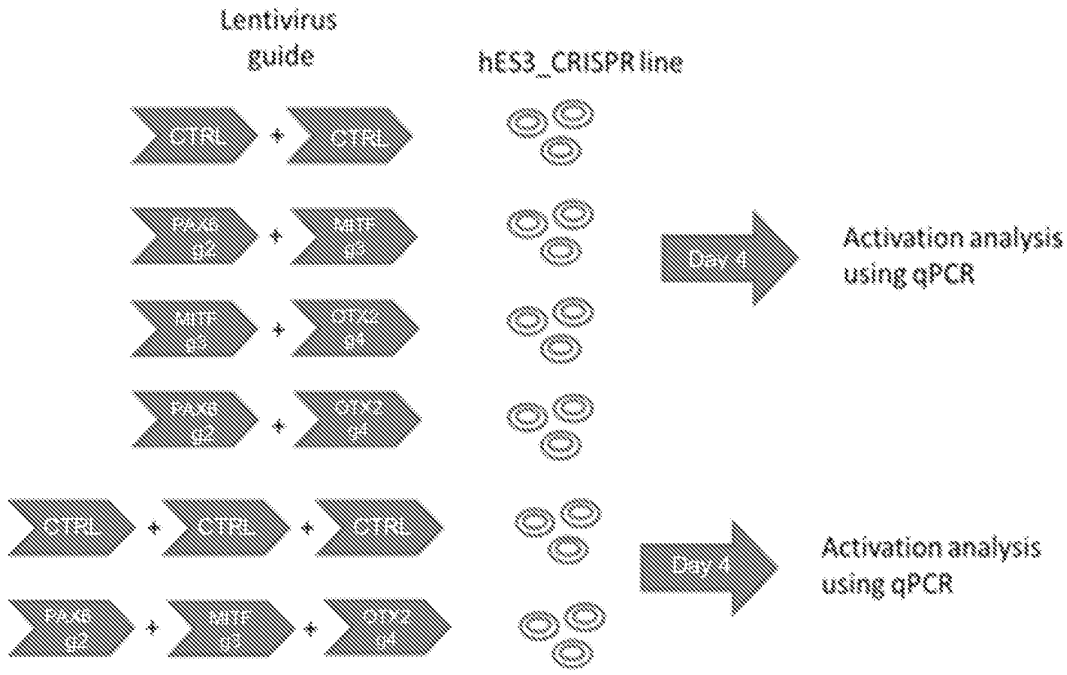

Pax6(-5a)
mRNA level (fold change)
3000
2000
1000
0
Ctrl virus
Pax6_g1
Pax6_g2
Pax6_g3
Pax6(+5a)
mRNA level (fold change)
300000
200000
100000
0
Ctrl virus
Pax6_g1
Pax6_g2
Pax6_g3
MITF
mRNA level (fold change)
200
150
100
50
0
Ctrl virus
MITF_g2
MITF_g3
MITF_g4
OTX2
mRNA level (fold change)
40000
30000
20000
10000
0
Ctrl virus
OTX2_g3
OTX2_g4
OTX2_g5

Fig. 4

a.


Lentivirus guide
hES3_CRISPR line
CTRL + CTRL
PAX6 g2 + MITF g3
MITF g3 + OTX2 g4
PAX6 g2 + OTX2 g4
Day 4
Activation analysis using qPCR
CTRL + CTRL + CTRL
PAX6 g2 + MITF g3 + OTX2 g4
Day 4
Activation analysis using qPCR

b.

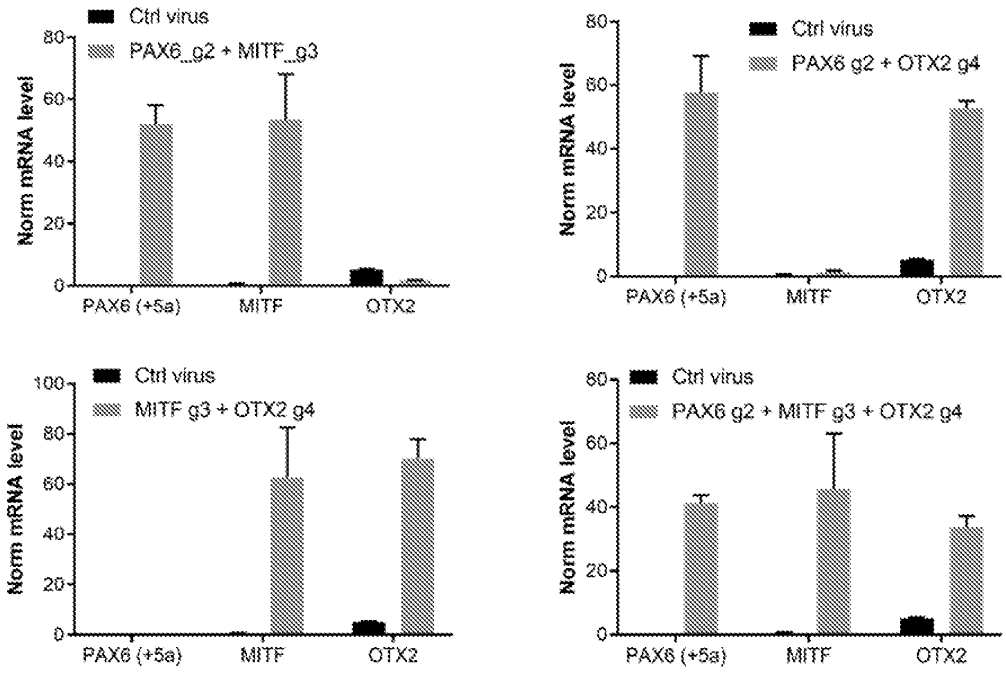
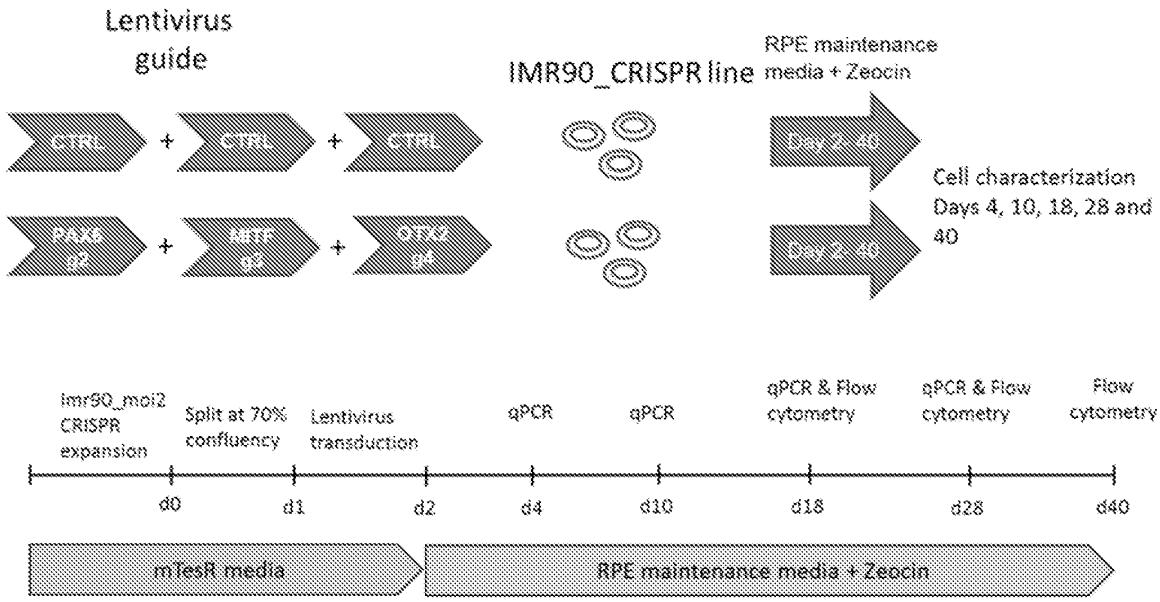

Ctrl virus
PAX6_g2 + MITF_g3
Norm mRNA level
PAX6 (+5a)
MITF
OTX2
Ctrl virus
PAX6 g2 + OTX2 g4
Norm mRNA level
PAX6 (+5a)
MITF
OTX2
Ctrl virus
MITF g3 + OTX2 g4
Norm mRNA level
PAX6 (+5a)
MITF
OTX2
Ctrl virus
PAX6 g2 + MITF g3 + OTX2 g4
Norm mRNA level
PAX6 (+5a)
MITF
OTX2

Fig. 5

a.



b.

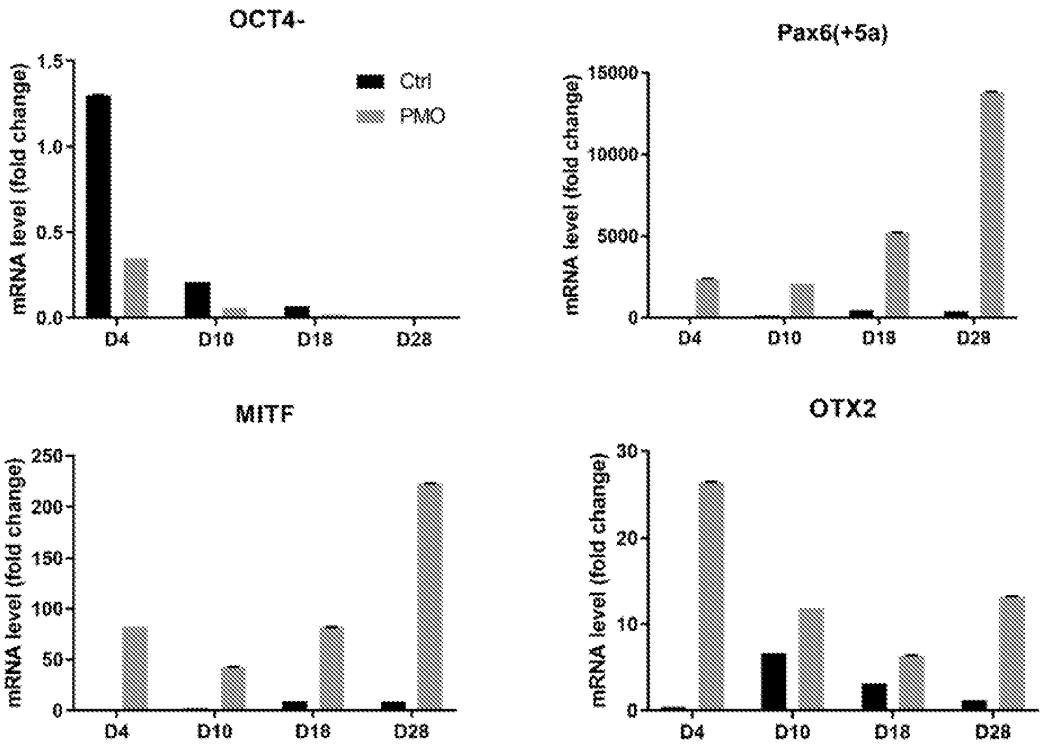


Fig. 6 (continued)

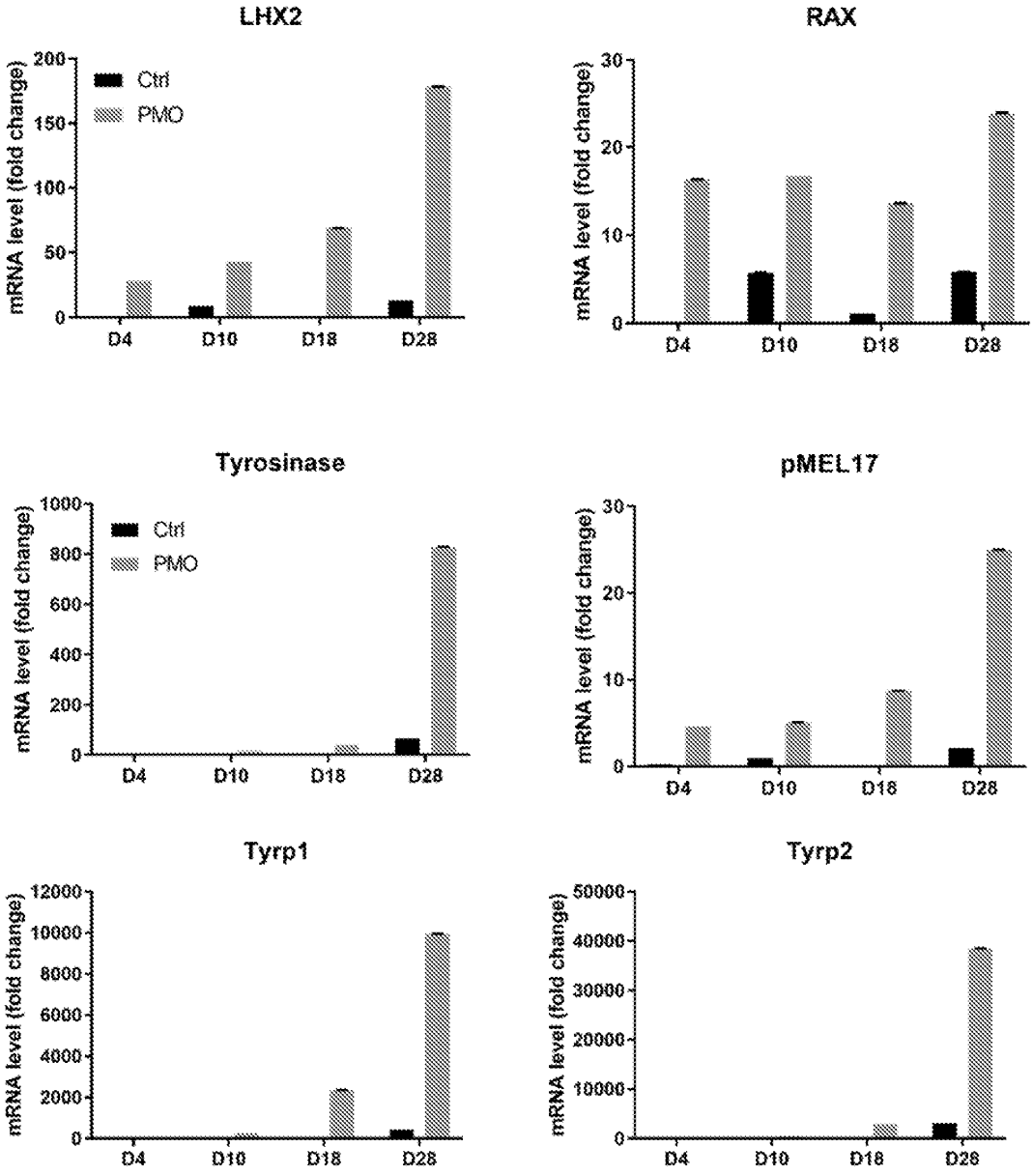


Fig. 6 (continued)

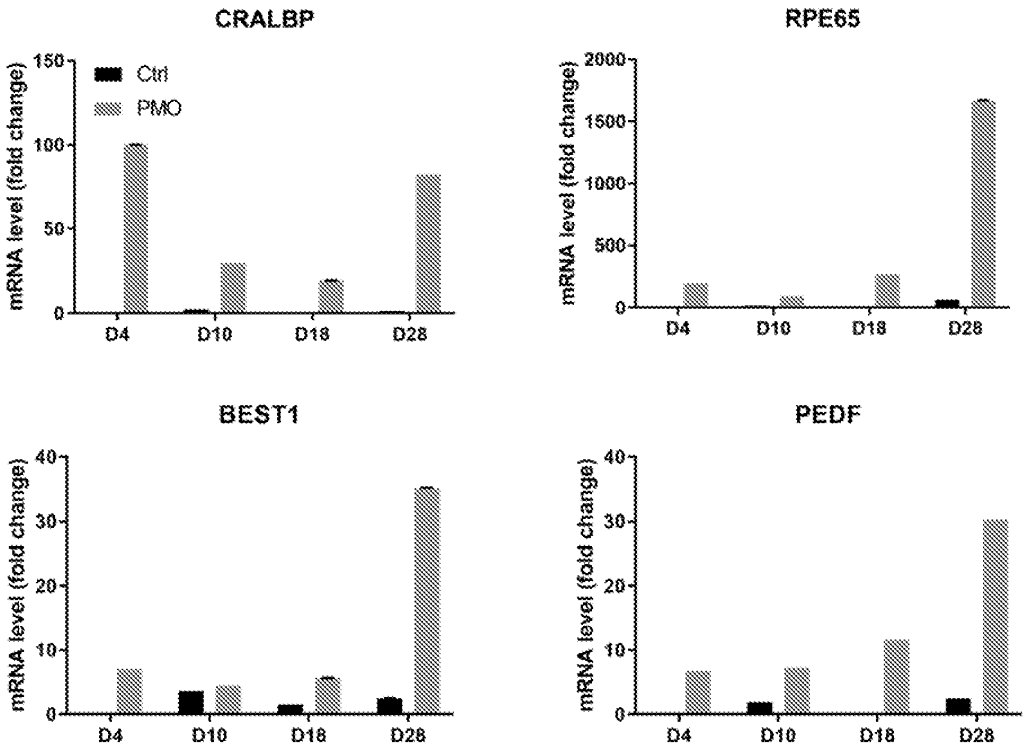
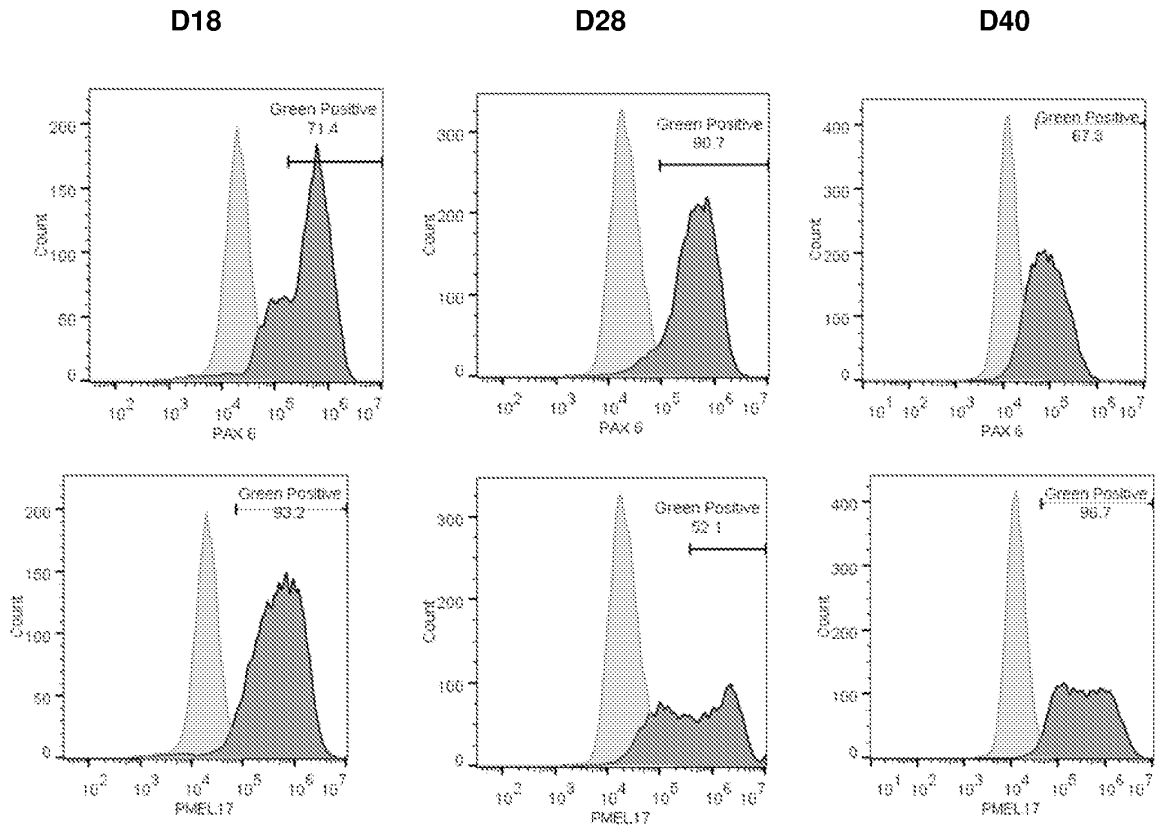
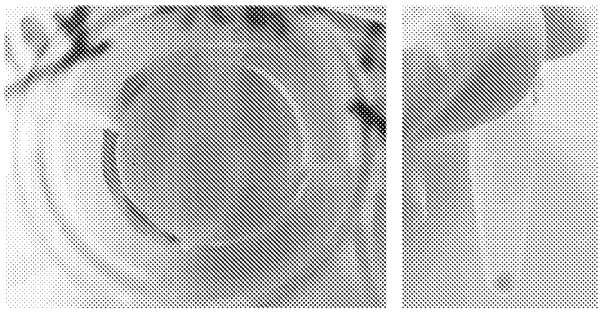


Fig. 6 (continued)

c.


D18
D28
D40
Green Positive
71.4
Green Positive
80.7
Green Positive
67.3
Count
PAX 6
Green Positive
93.2
Green Positive
52.1
Green Positive
96.7
PMEL17

d.



e.

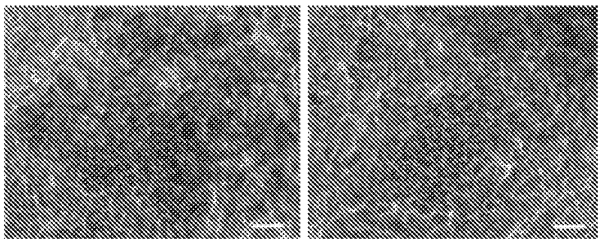
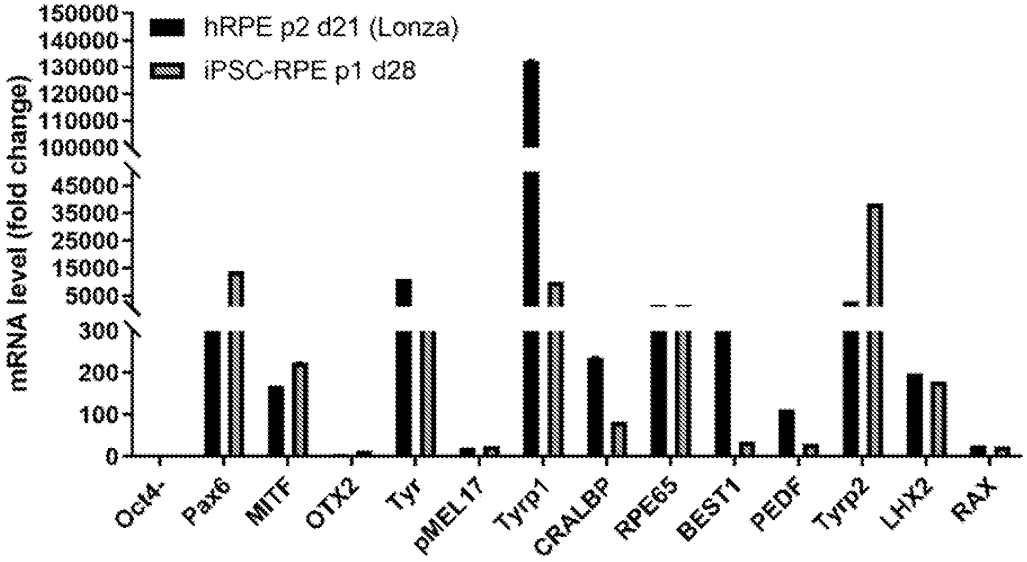


Fig. 6

(a)



(b)

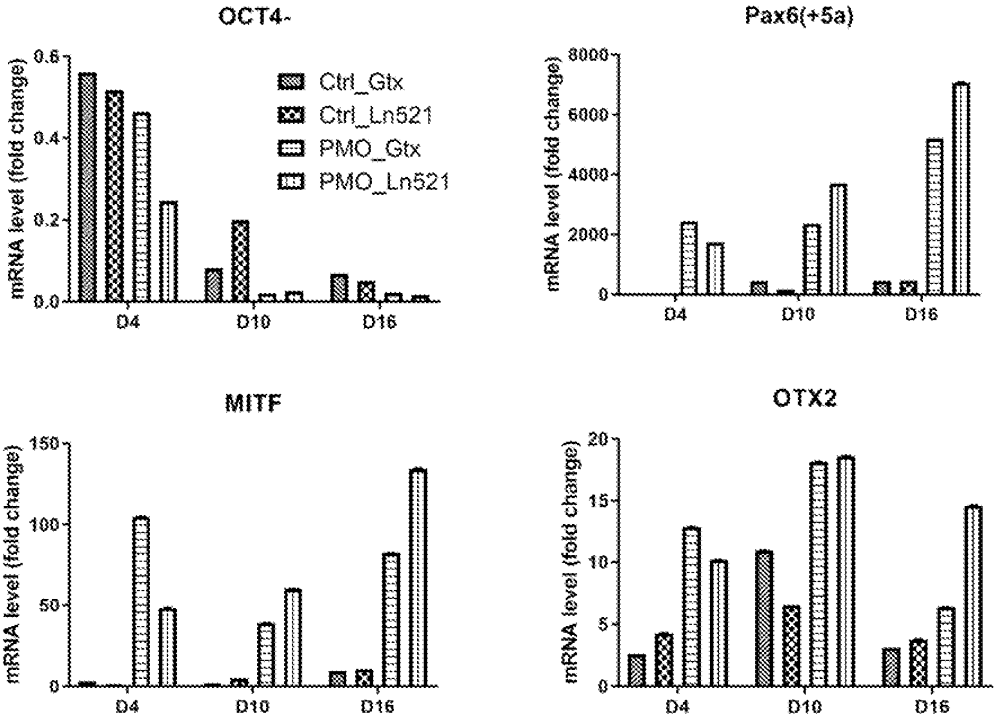


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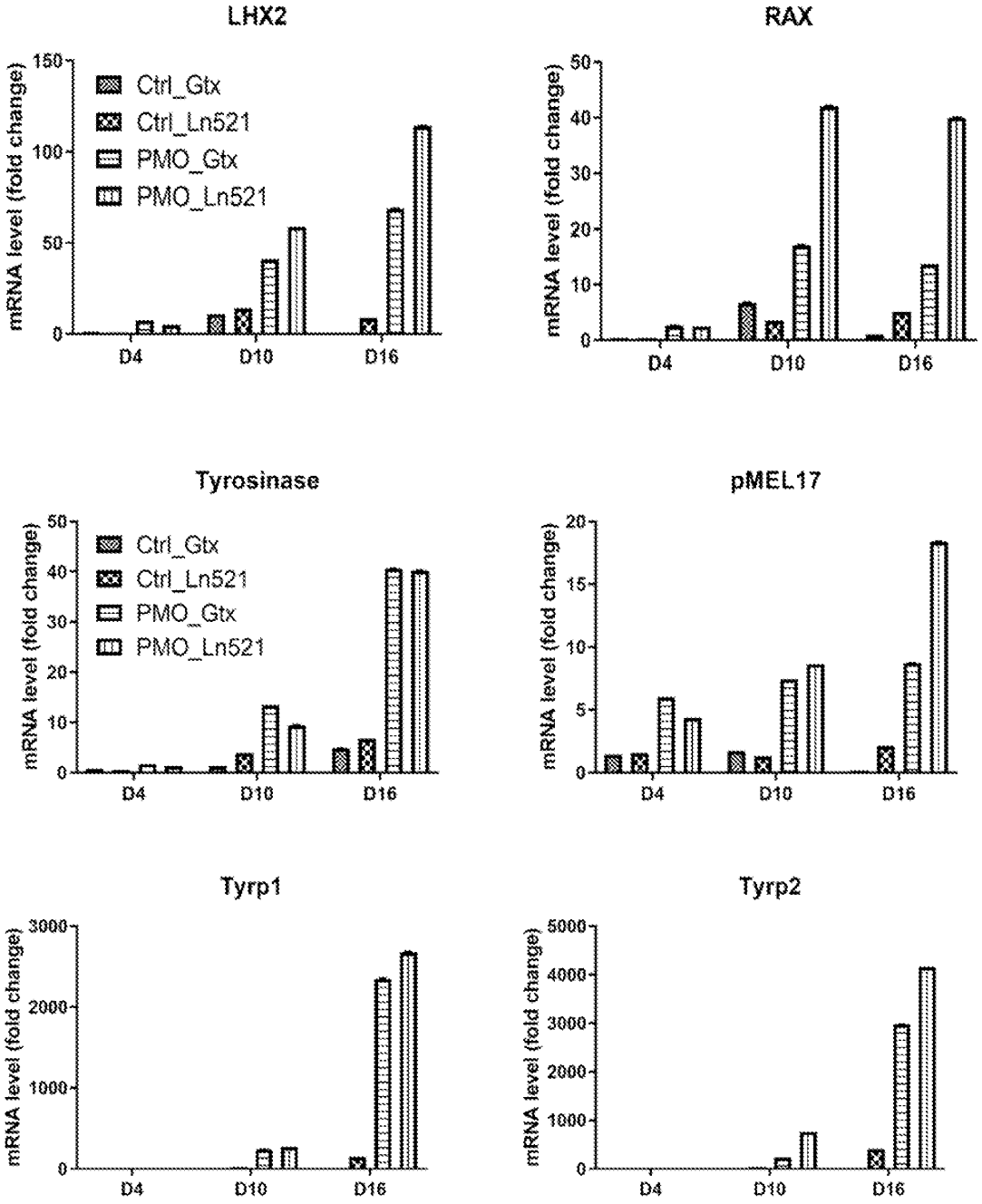


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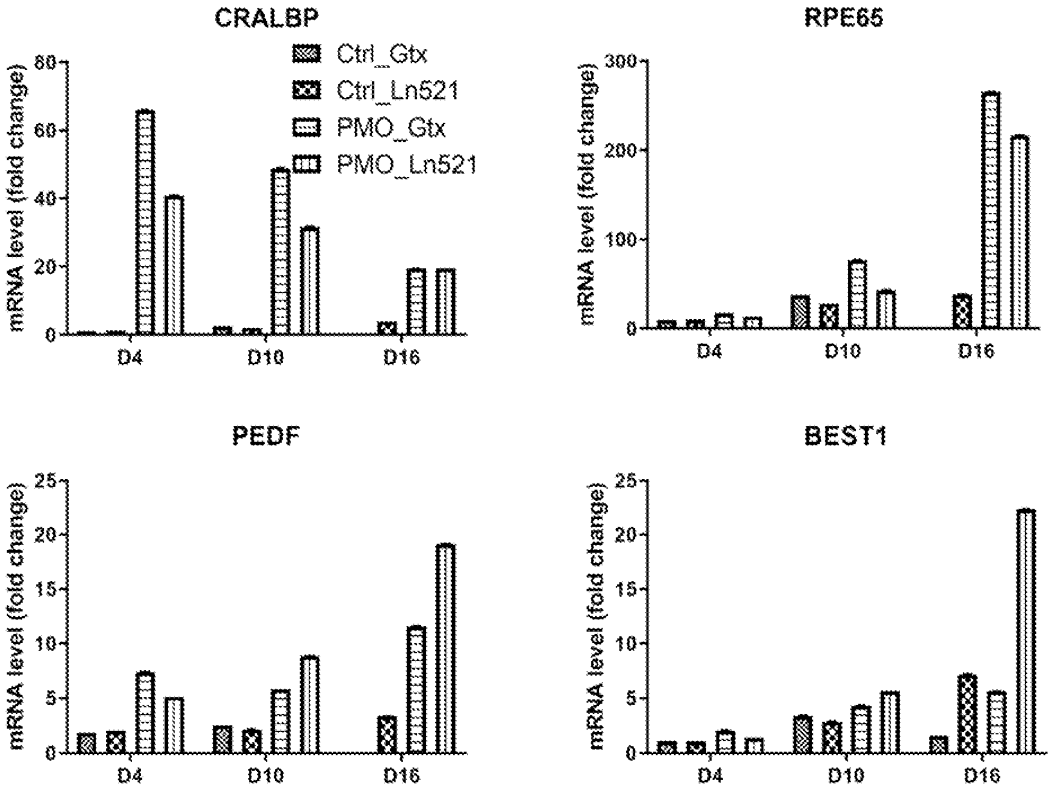

CRALBP
Ctrl_Gtx
Ctrl_Ln521
PMO_Gtx
PMO_Ln521
mRNA level (fold change)
0
20
40
60
80
D4
D10
D16
RPE65
mRNA level (fold change)
0
100
200
300
D4
D10
D16
PEDF
mRNA level (fold change)
0
5
10
15
20
25
D4
D10
D16
BEST1
mRNA level (fold change)
0
5
10
15
20
25
D4
D10
D16

Fig. 7

(a)

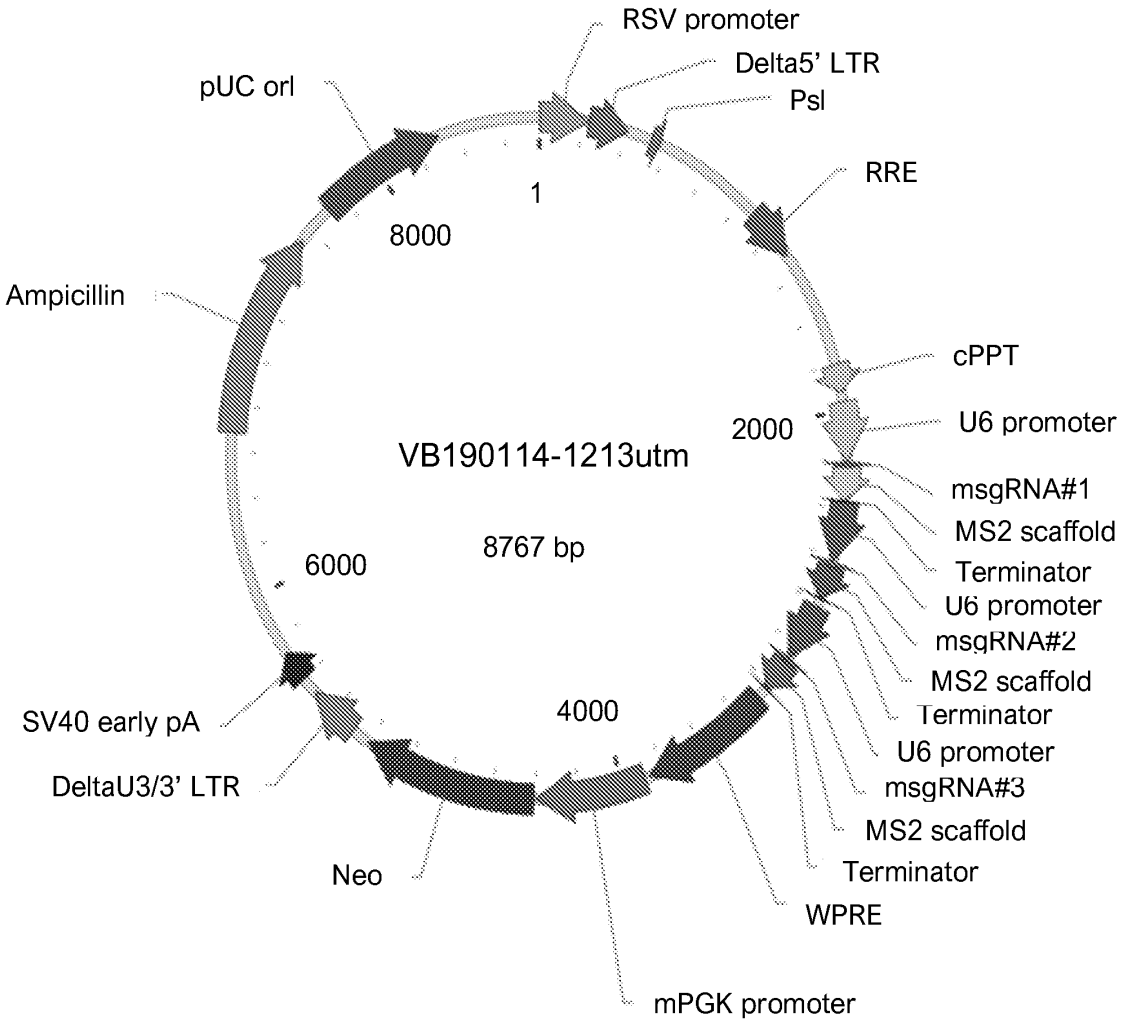


Fig. 8 (continued)

(b)

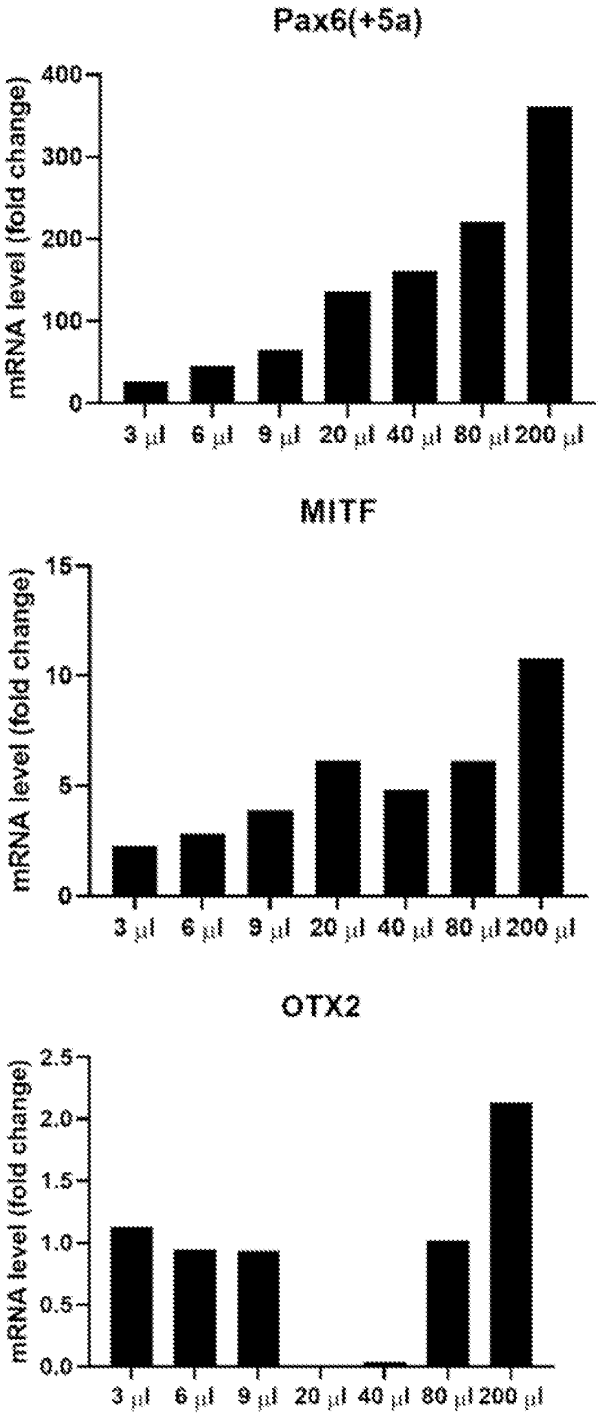
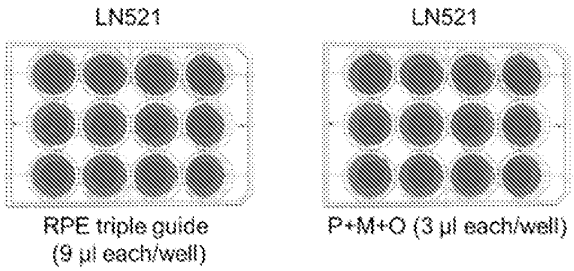

Pax6(+5a)
mRNA level (fold change)
400
300
200
100
0
3 µl
6 µl
9 µl
20 µl
40 µl
80 µl
200 µl
MITF
mRNA level (fold change)
15
10
5
0
3 µl
6 µl
9 µl
20 µl
40 µl
80 µl
200 µl
OTX2
mRNA level (fold change)
2.5
2.0
1.5
1.0
0.5
0.0
3 µl
6 µl
9 µl
20 µl
40 µl
80 µl
200 µl

Fig. 8

(a)



(b)

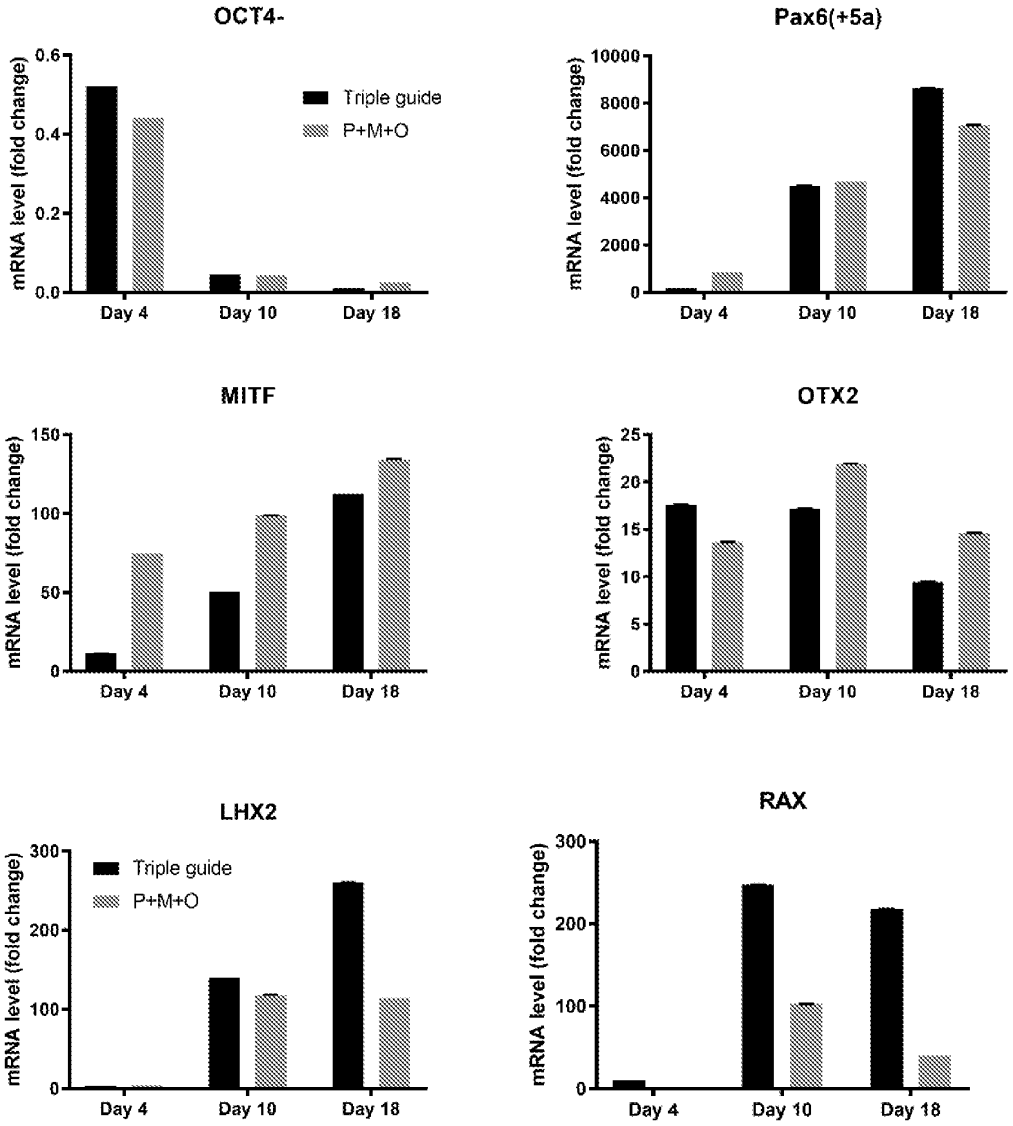


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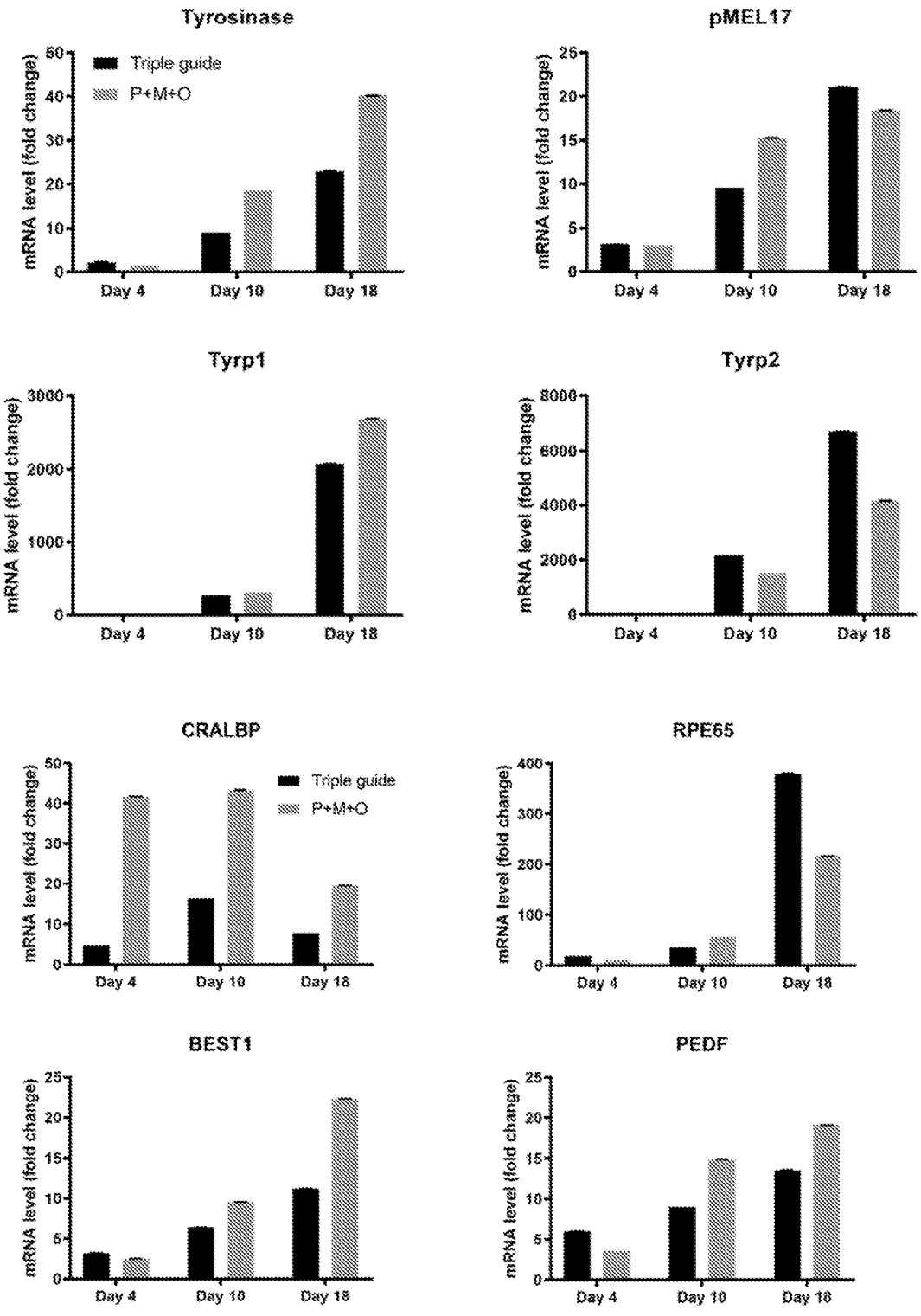

Tyrosinase
pMEL17
Tyrp1
Tyrp2
CRALBP
RPE65
BEST1
PEDF
Triple guide
P+M+O
mRNA level (fold change)
Day 4
Day 10
Day 18

Fig. 9

(a)

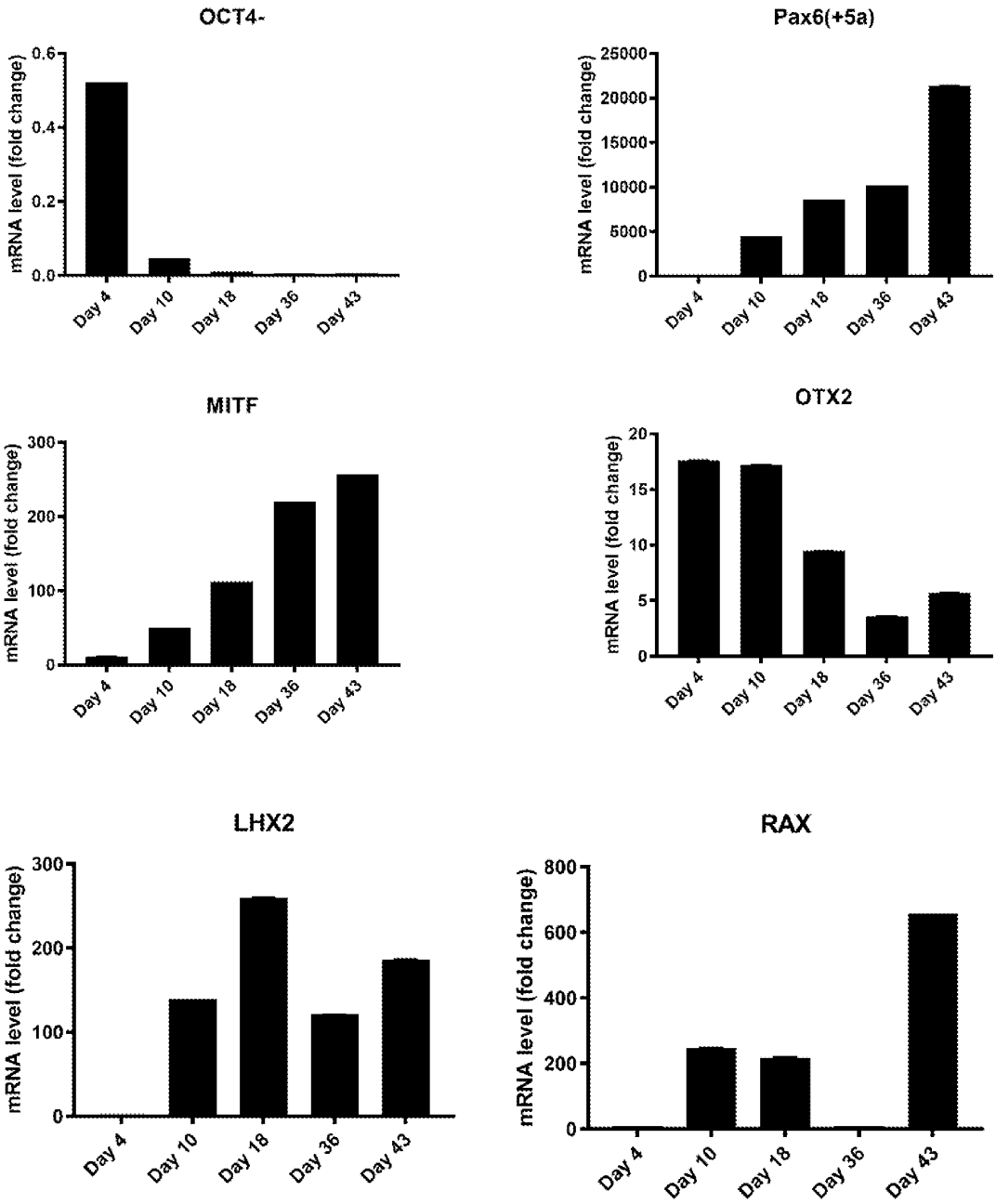


Fig. 10 (continued)

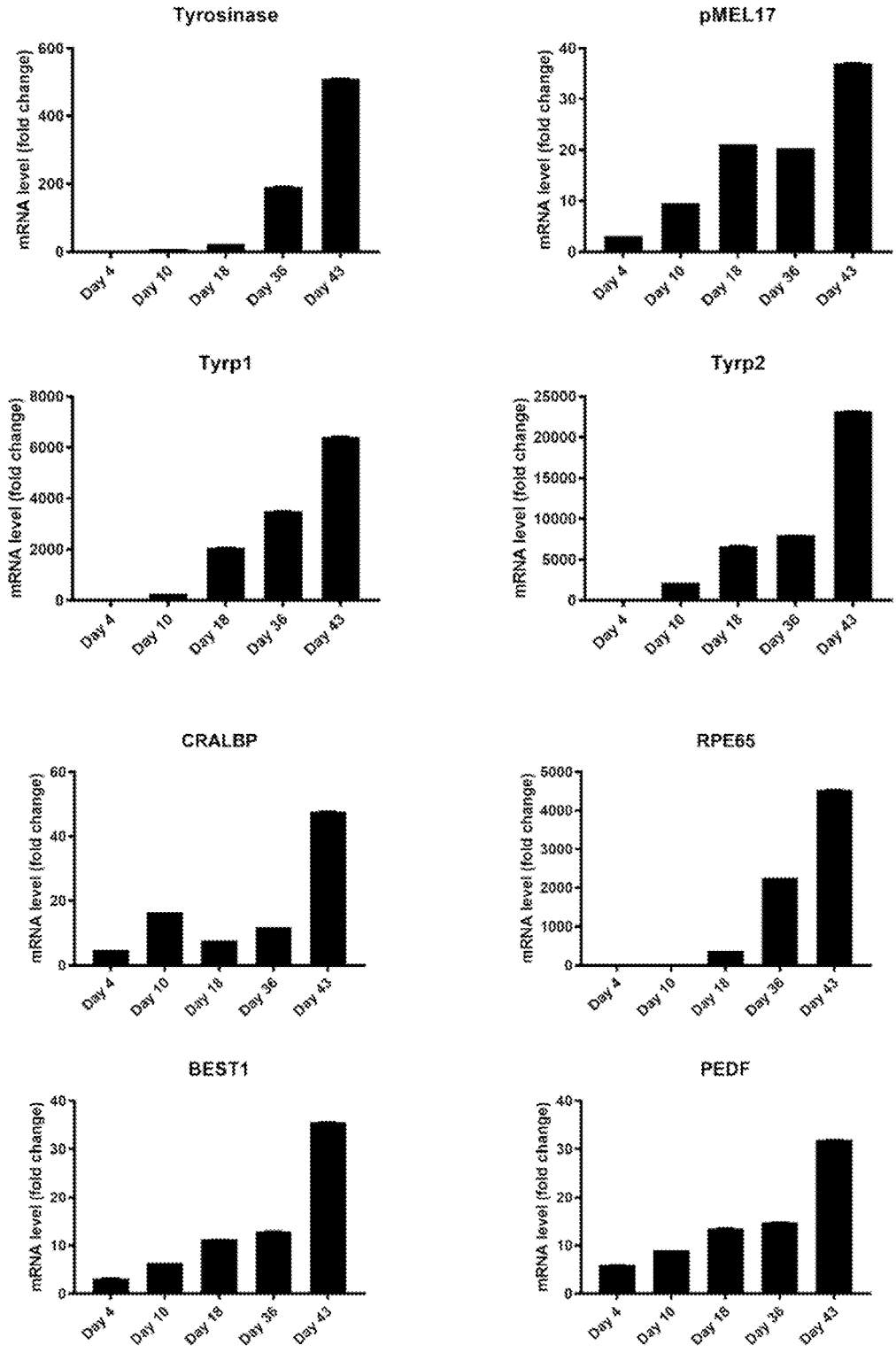


Fig. 10 (continued)

(b)

Days	PMEL17 (%)	PAX6 (%)	OTX2 (%)	MITF (%)
D22	99.3	95.8	79.0	91.6
D36	99.9	99.9	86.5	97.0
D43	99.5	98.2	76.9	93.7

(c)

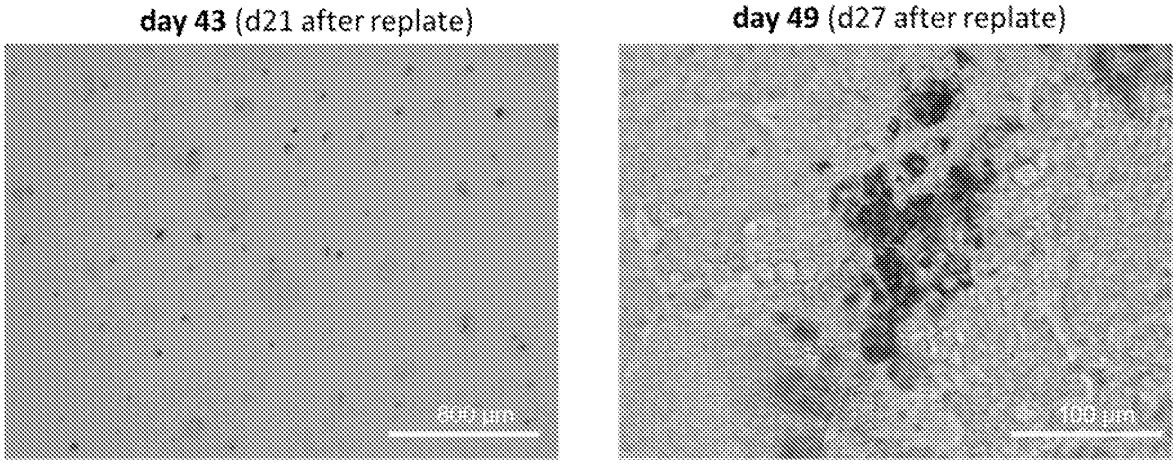
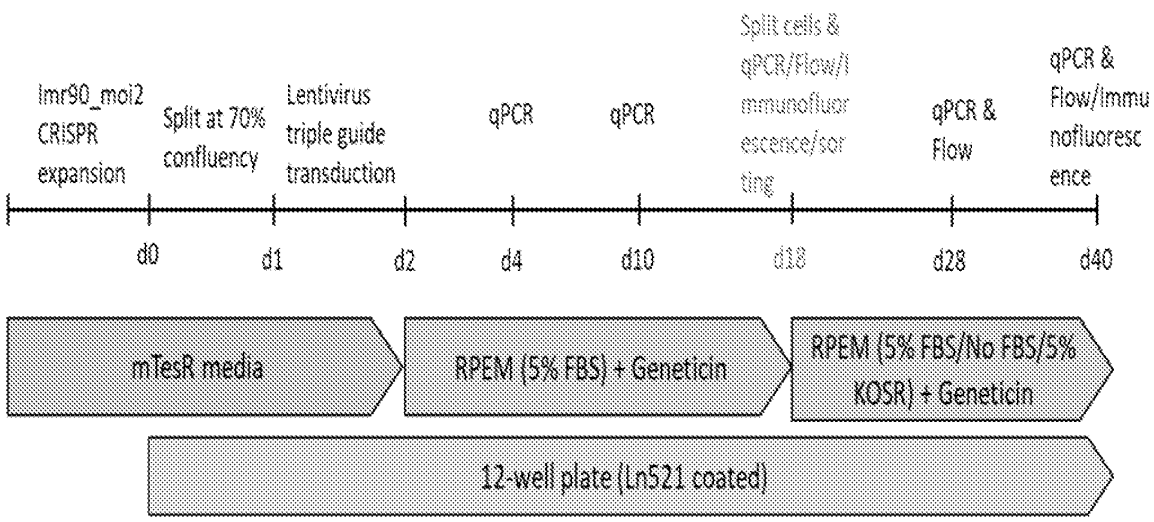


Fig. 10

(a)



(b)

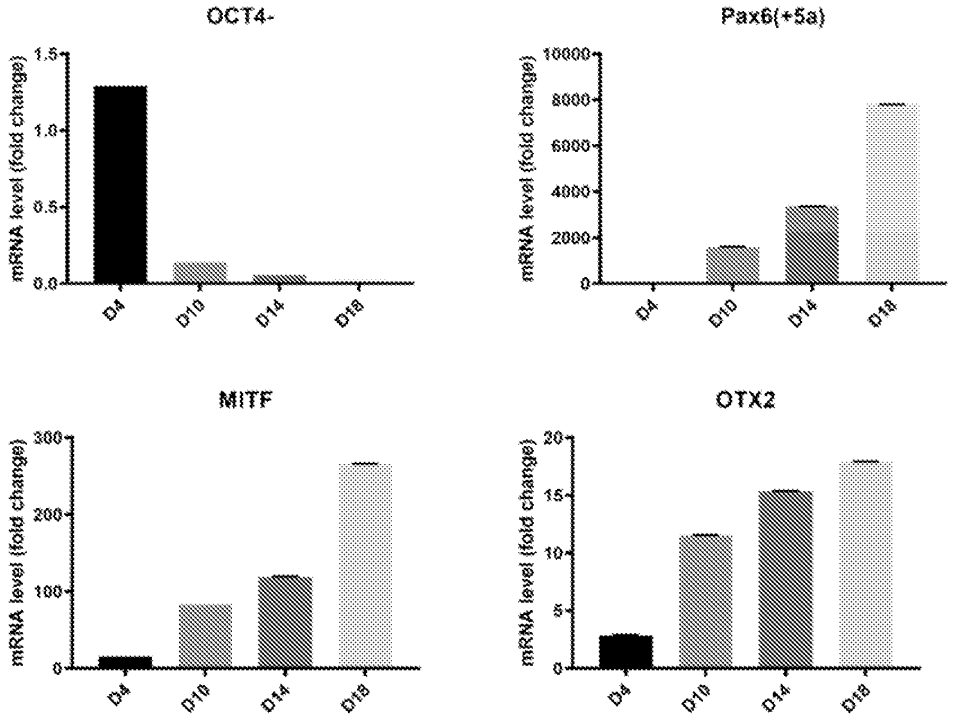


Fig. 11 (continued)

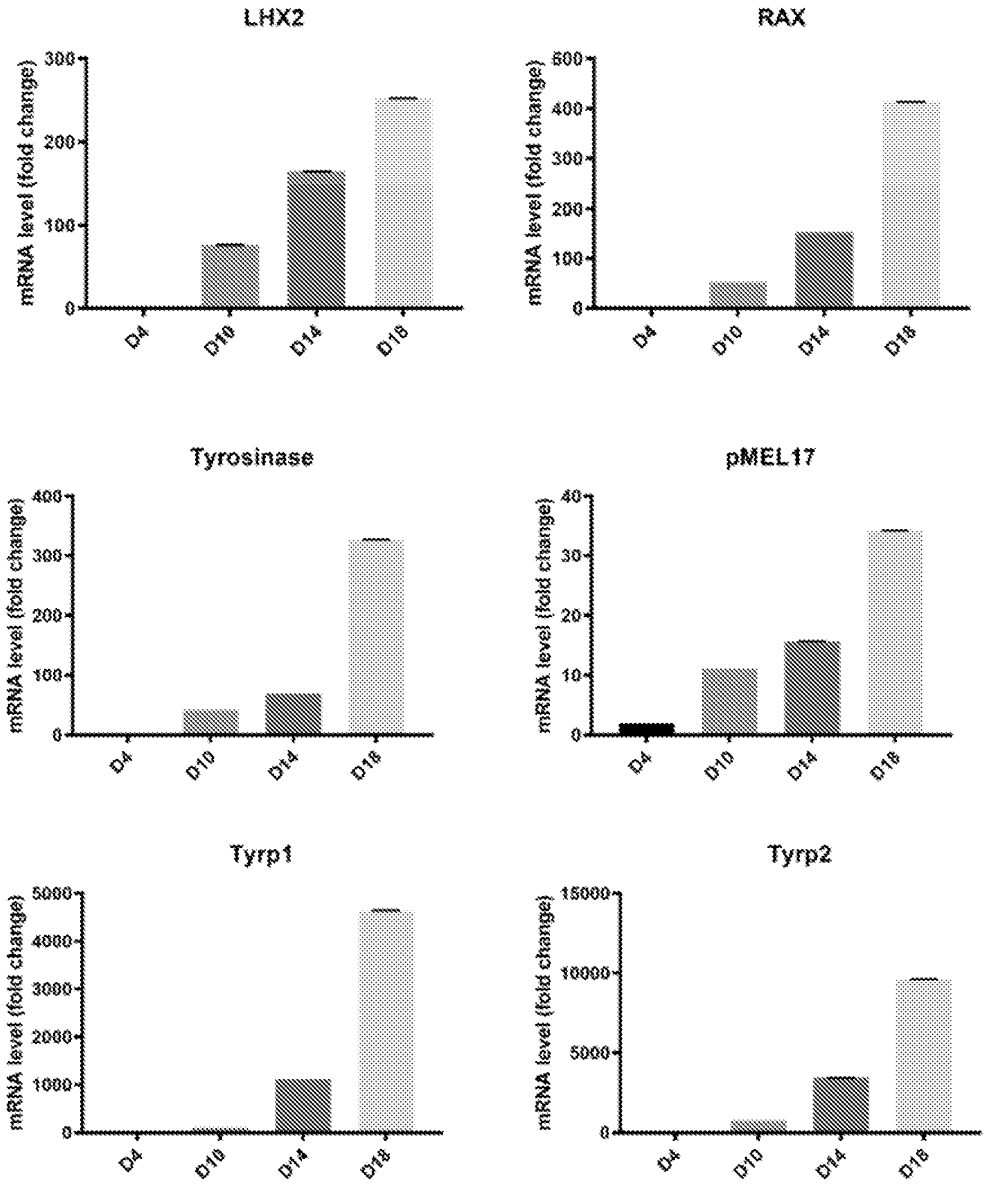


Fig. 11 (continued)

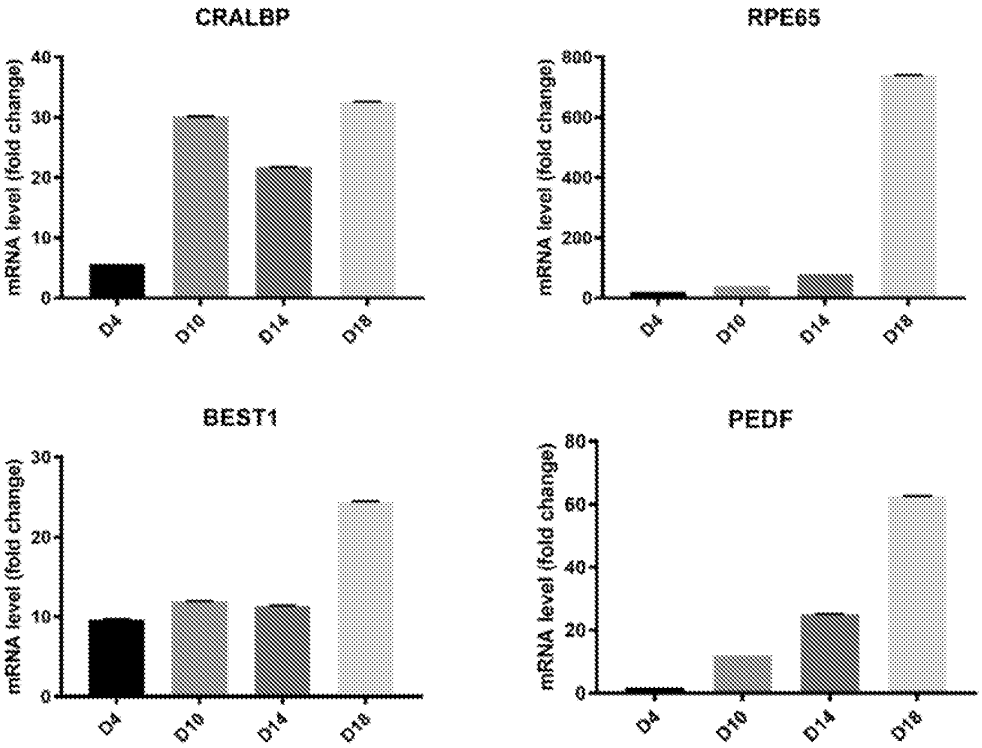


Fig. 11 (continued)

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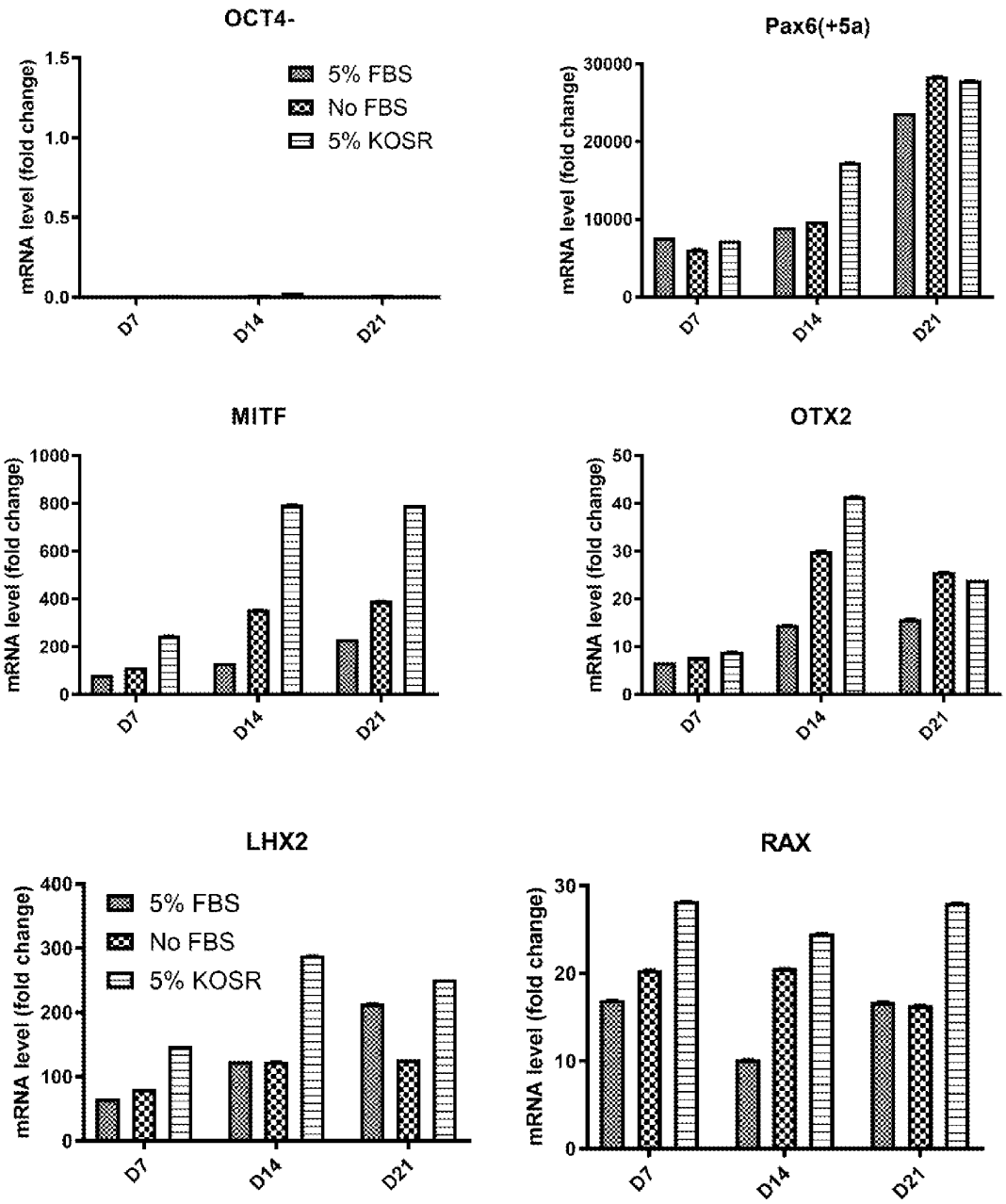


Fig. 11 (continued)

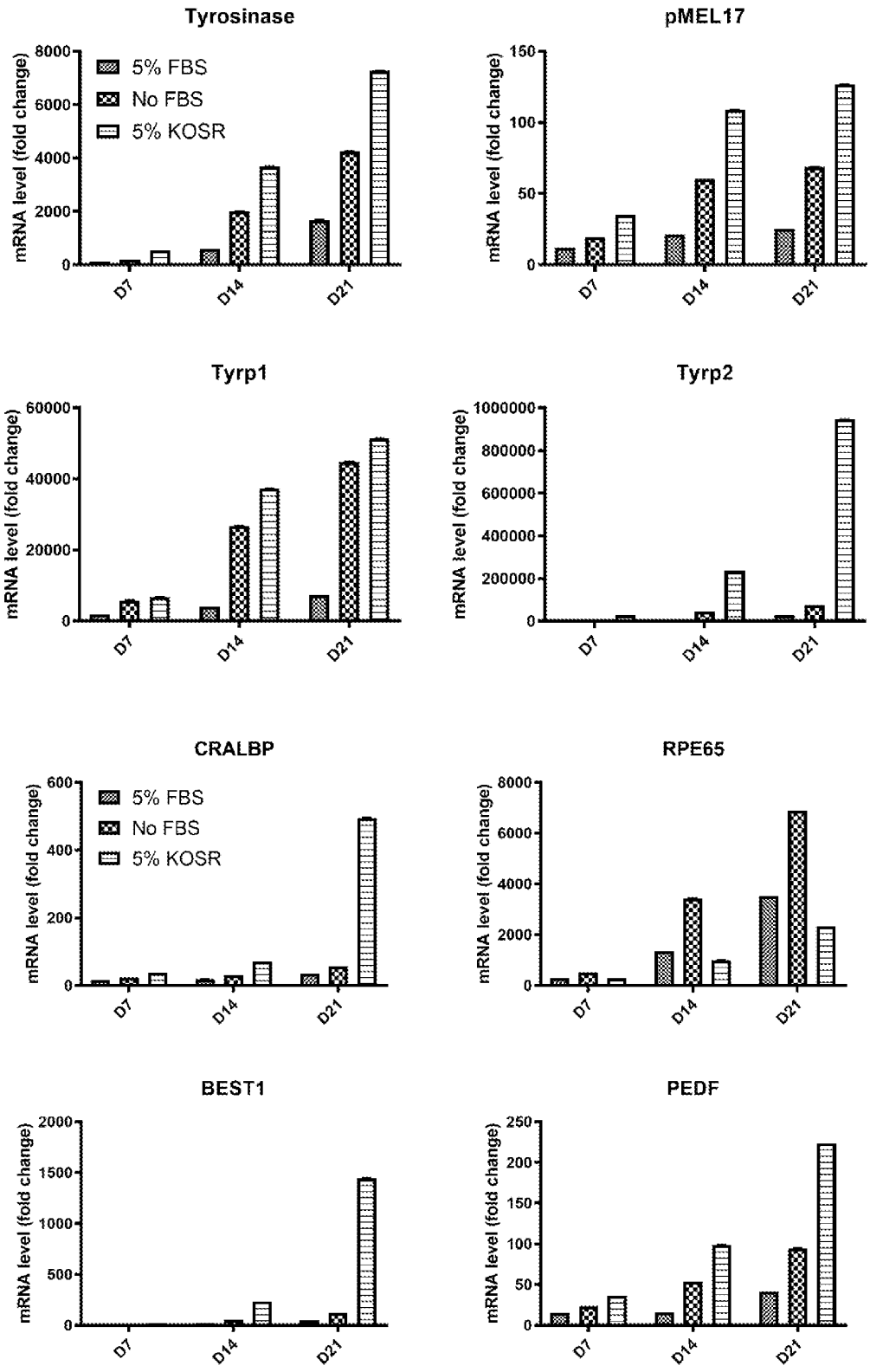


Fig. 11 (continued)

(d)

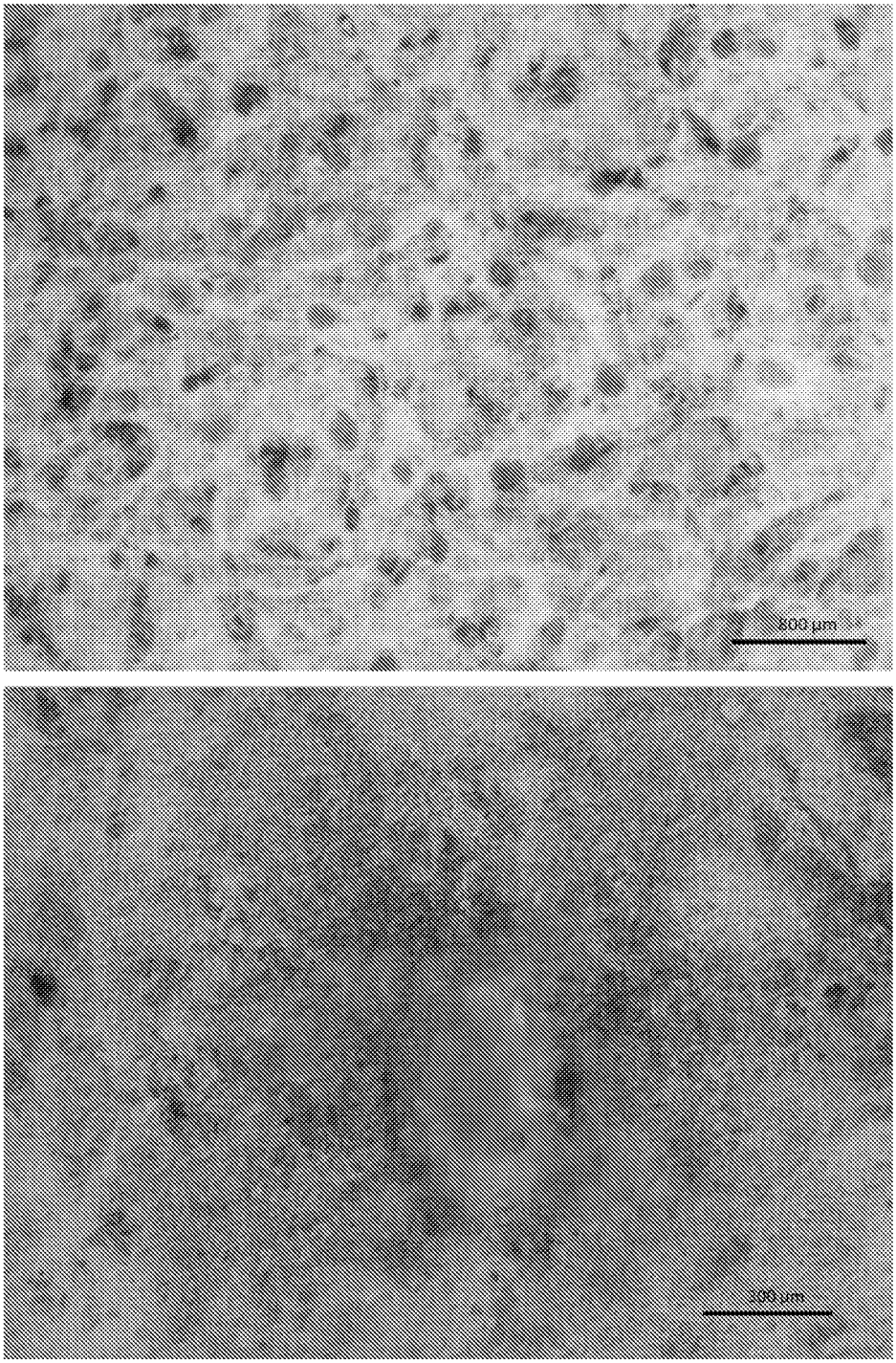


Fig. 11 (continued)

(e)

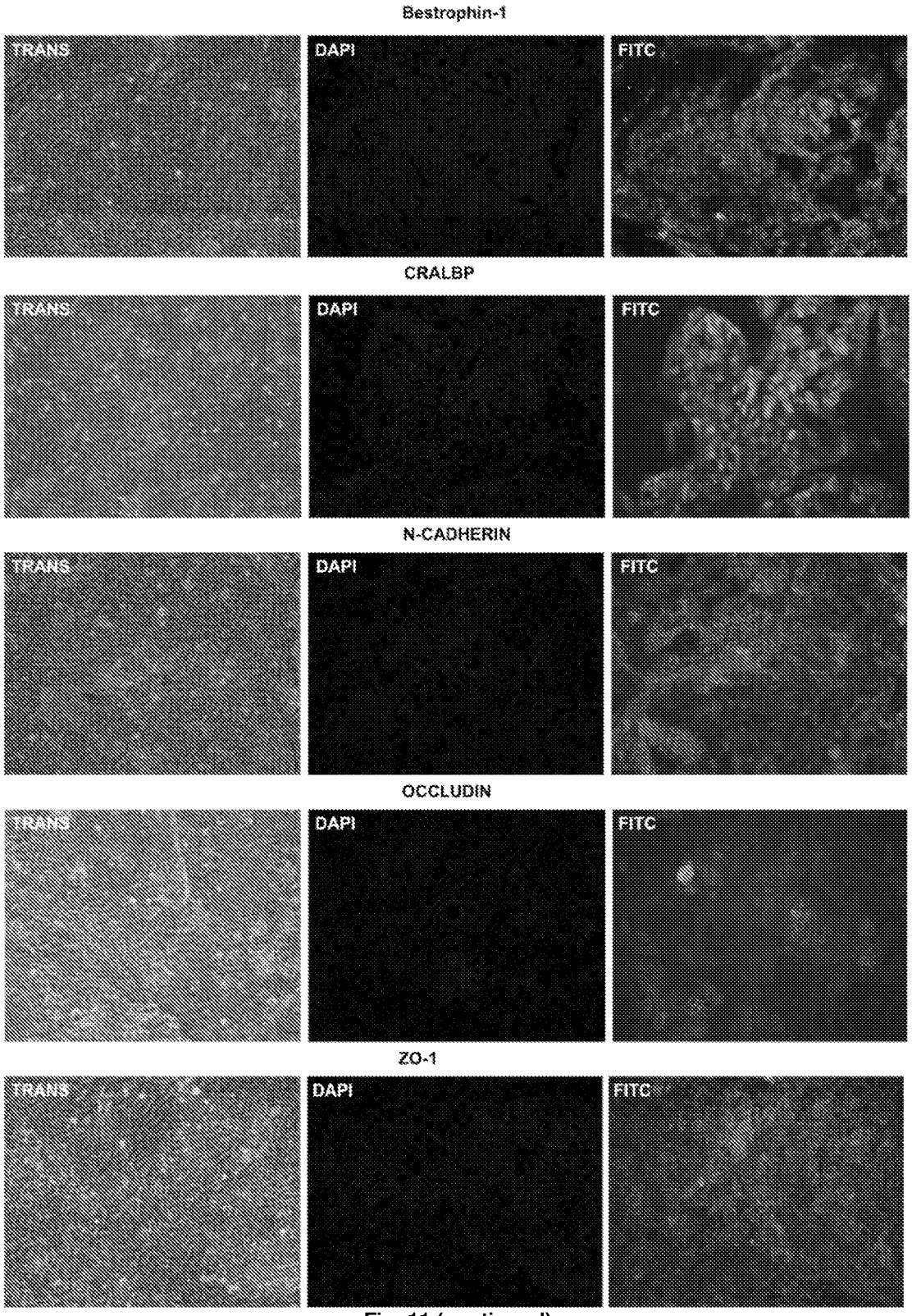


Fig. 11 (continued)

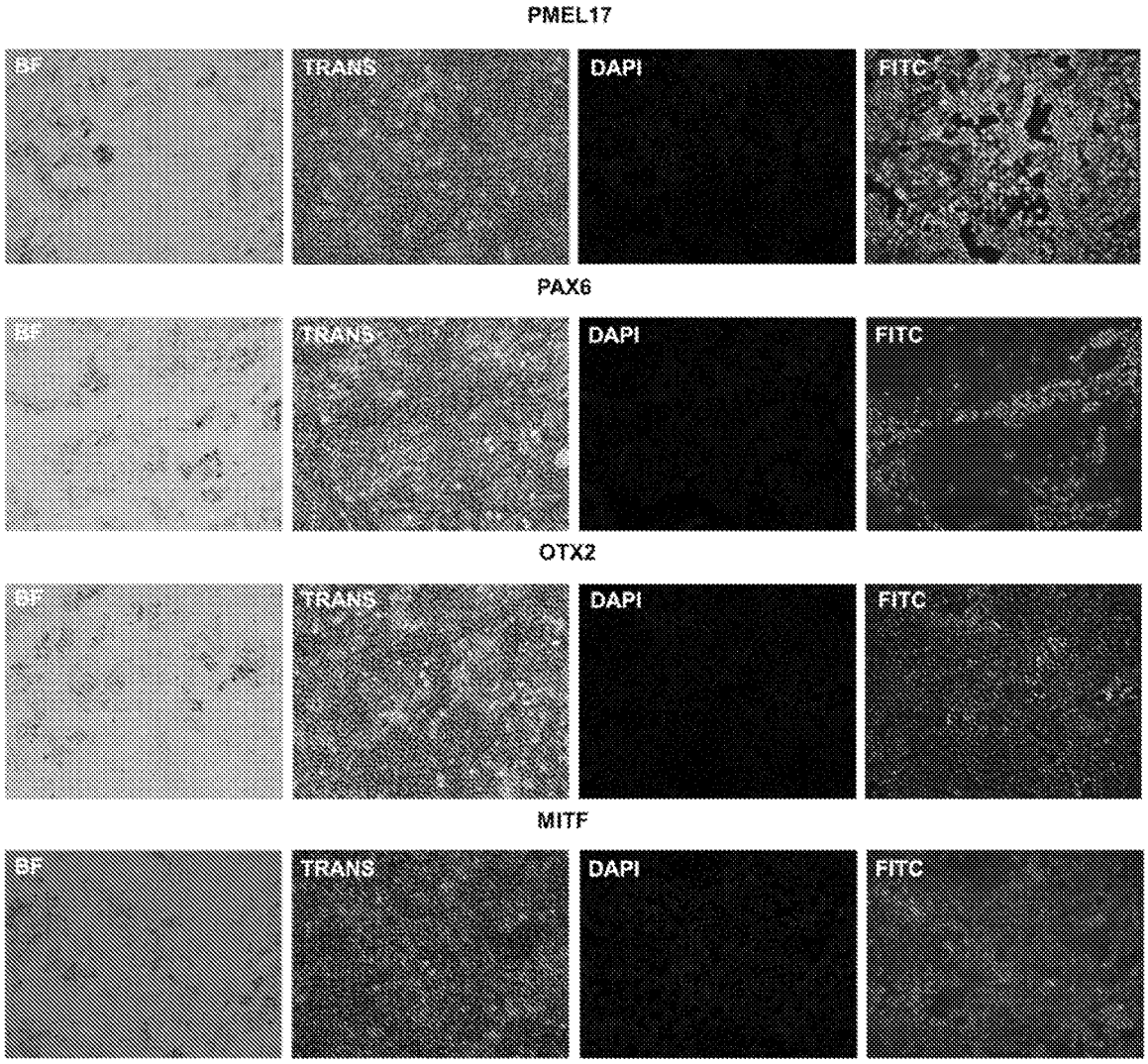


Fig. 11 (continued)

(f)

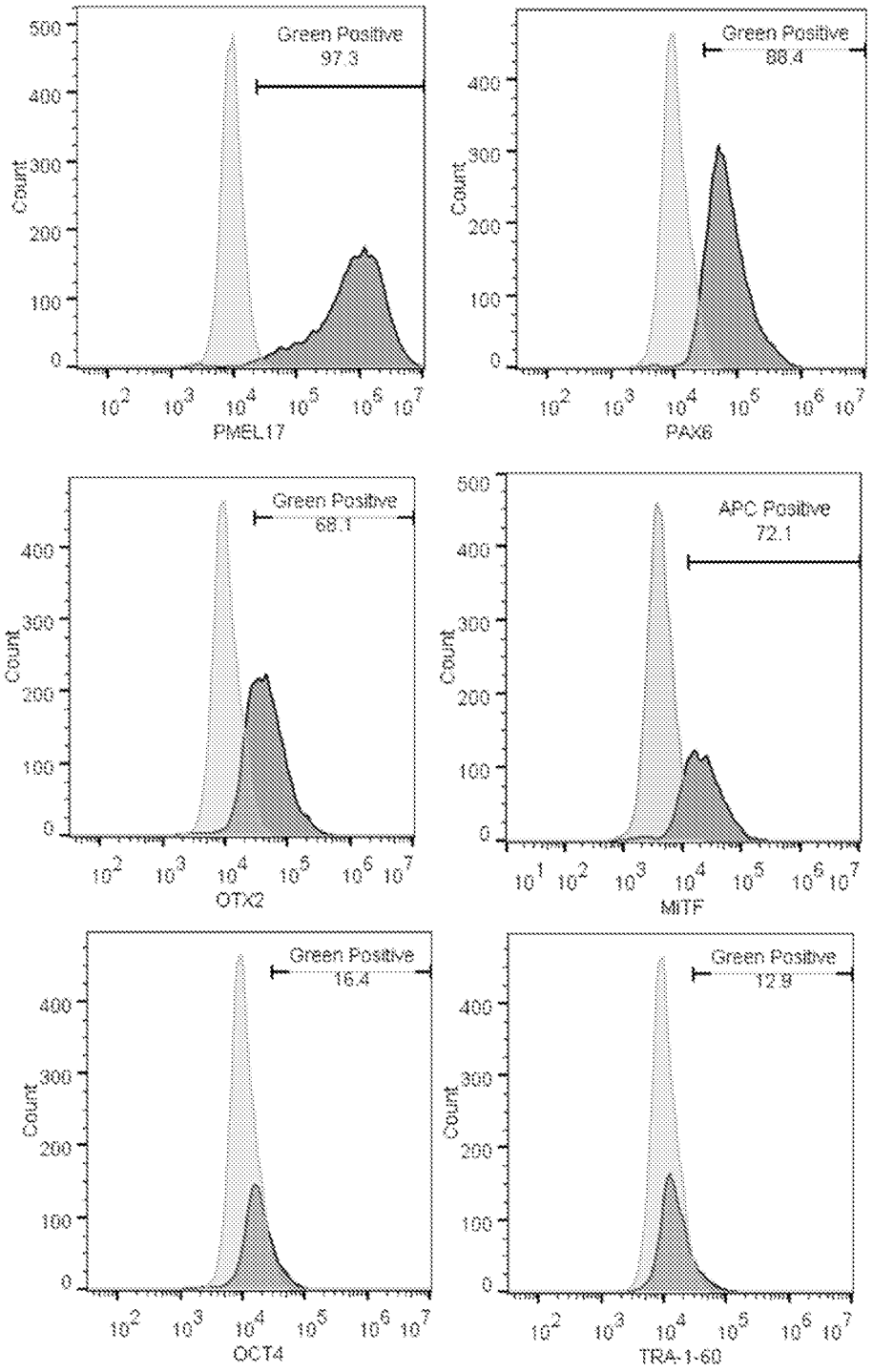

Green Positive
97.3
Count
PMEL17
Green Positive
86.4
PAX6
Green Positive
68.1
OTX2
APC Positive
72.1
MITF
Green Positive
16.4
OCT4
Green Positive
12.9
TRA-1-60

Fig. 11

(a)

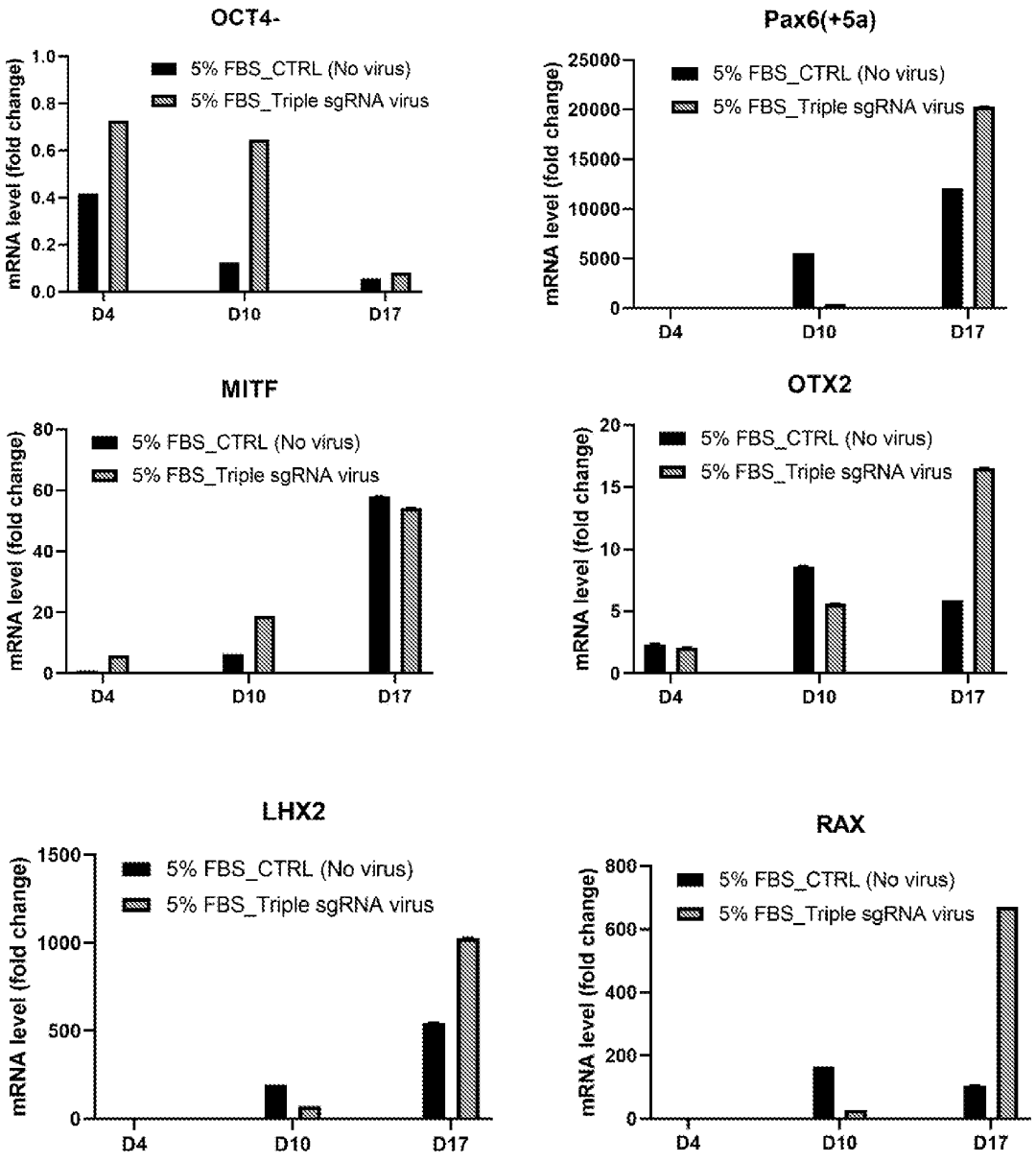


Fig. 12 (continued)

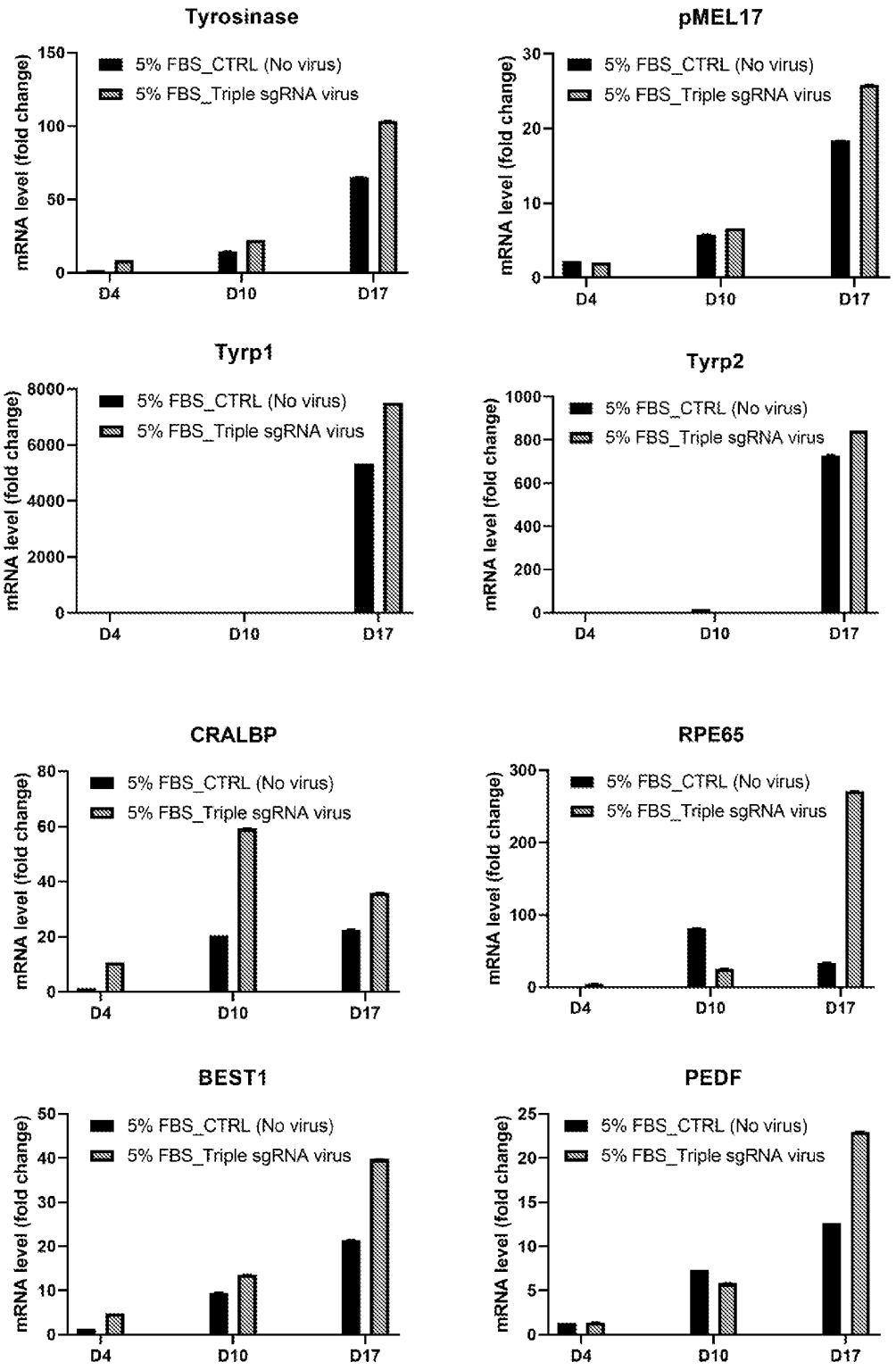


Fig. 12 (continued)

(b)

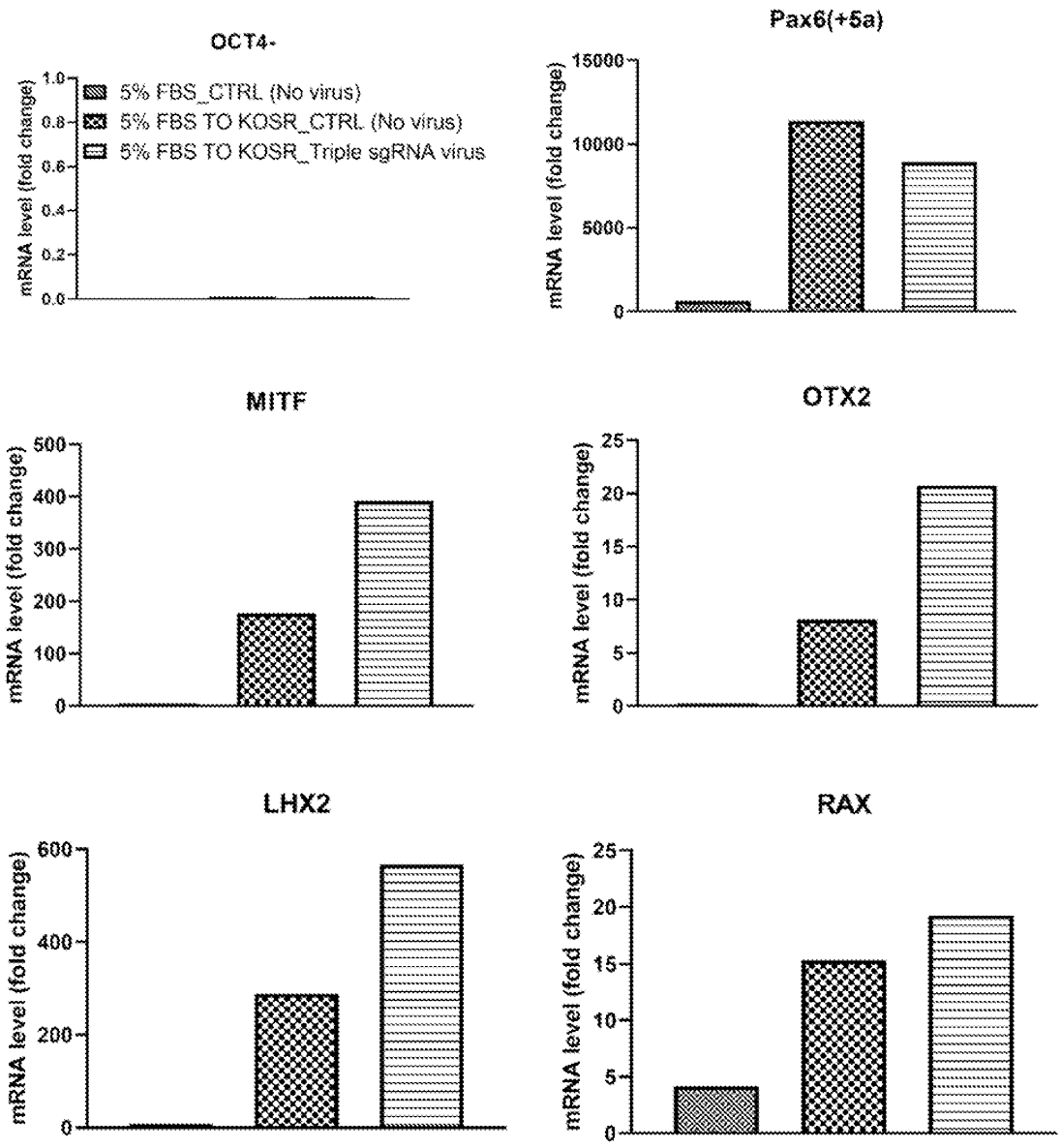


Fig. 12 (continued)

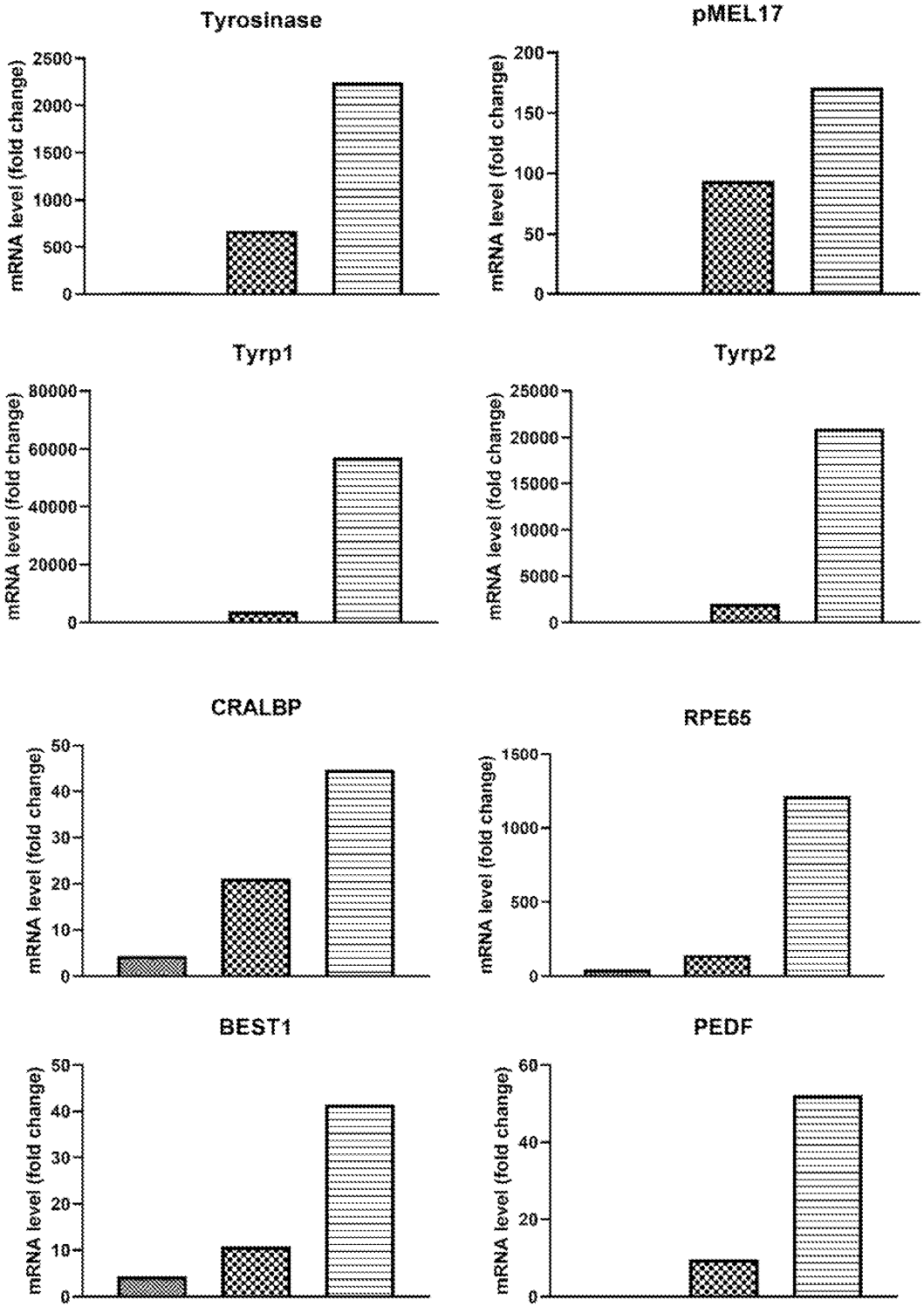


Fig. 12 (continued)

(c)

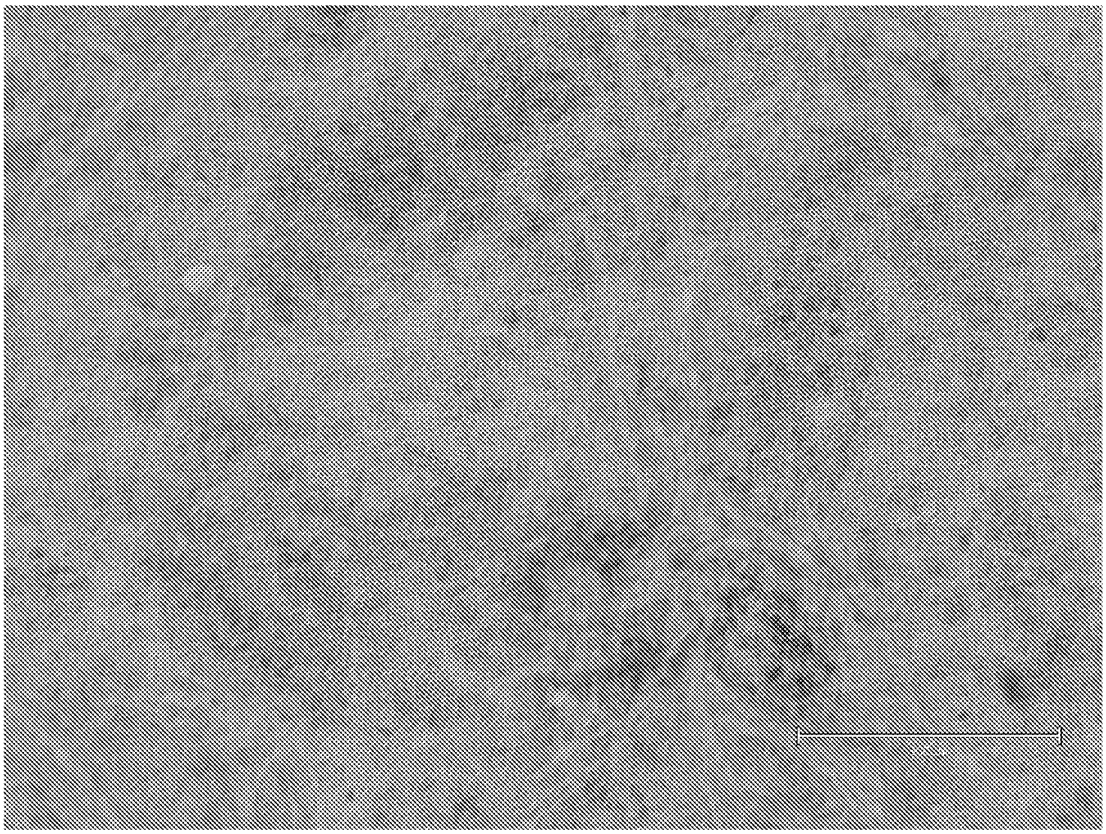


Fig. 12

(a)

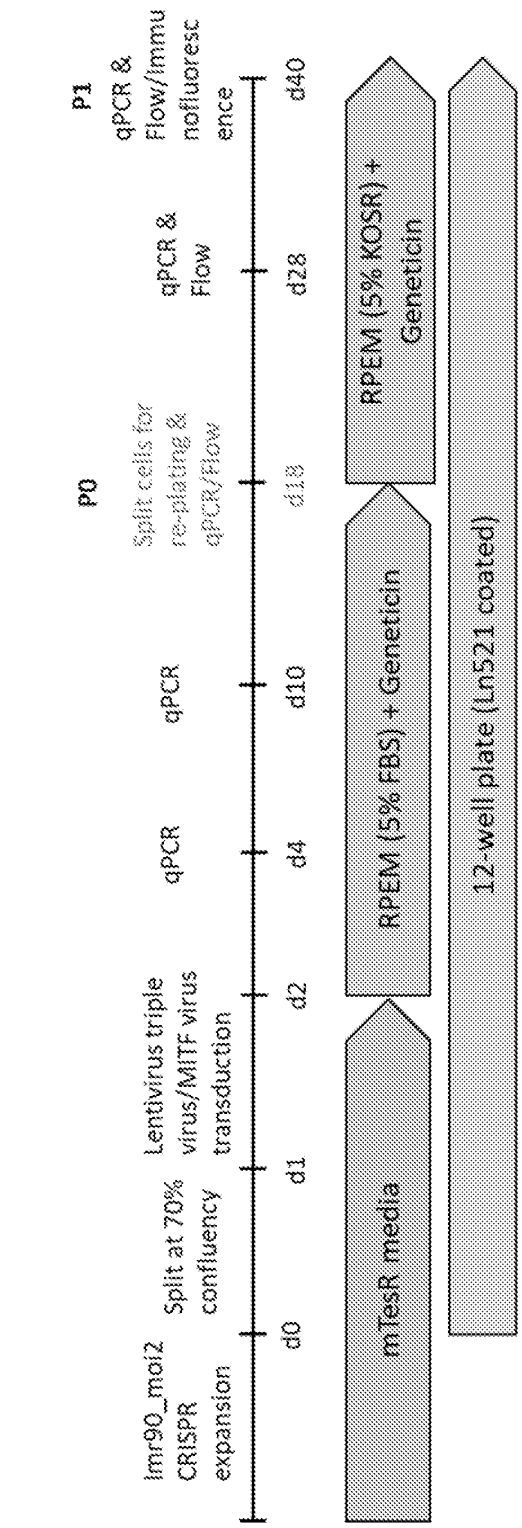


Fig. 13 (continued)

(b)

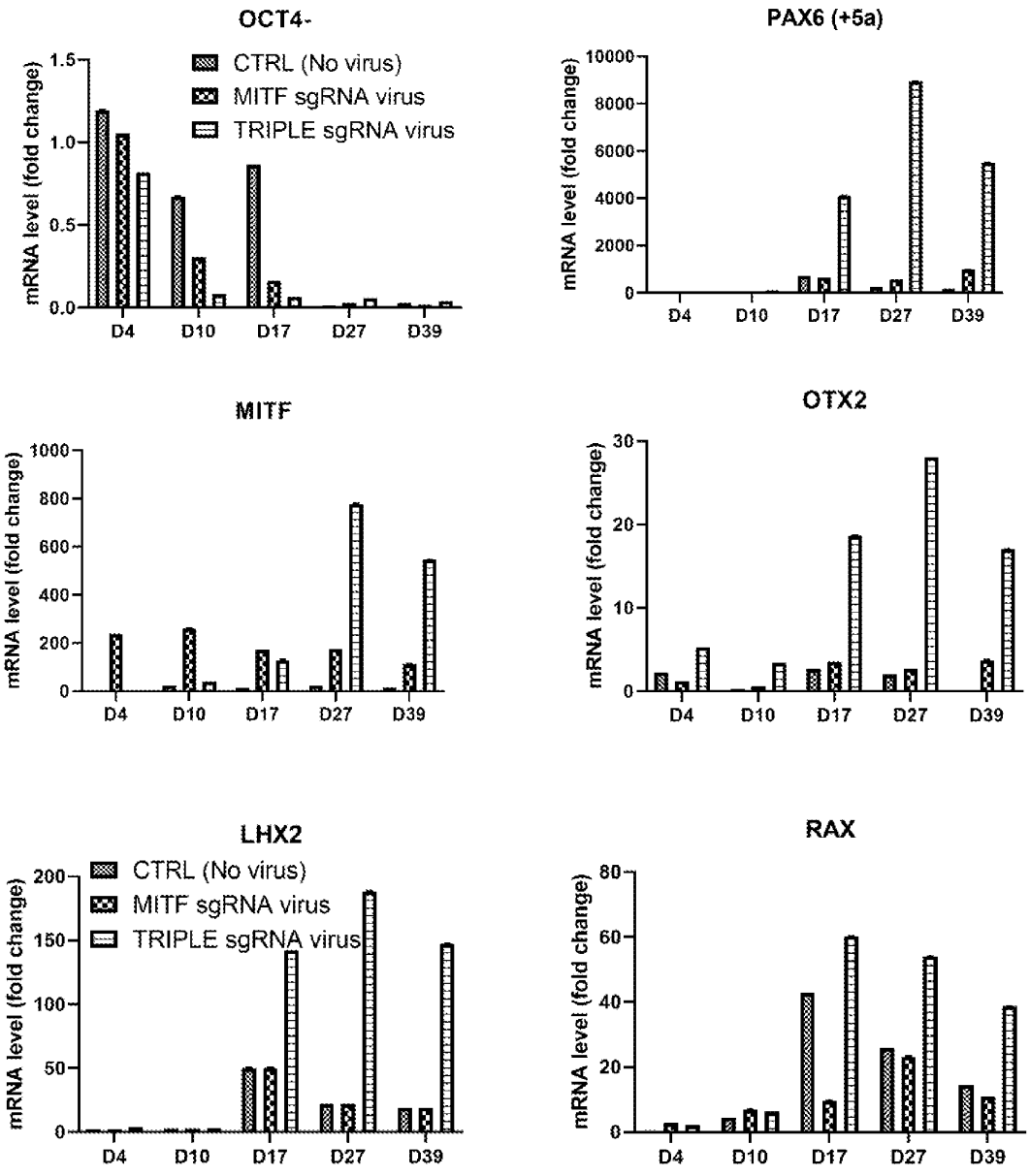


Fig. 13 (continued)

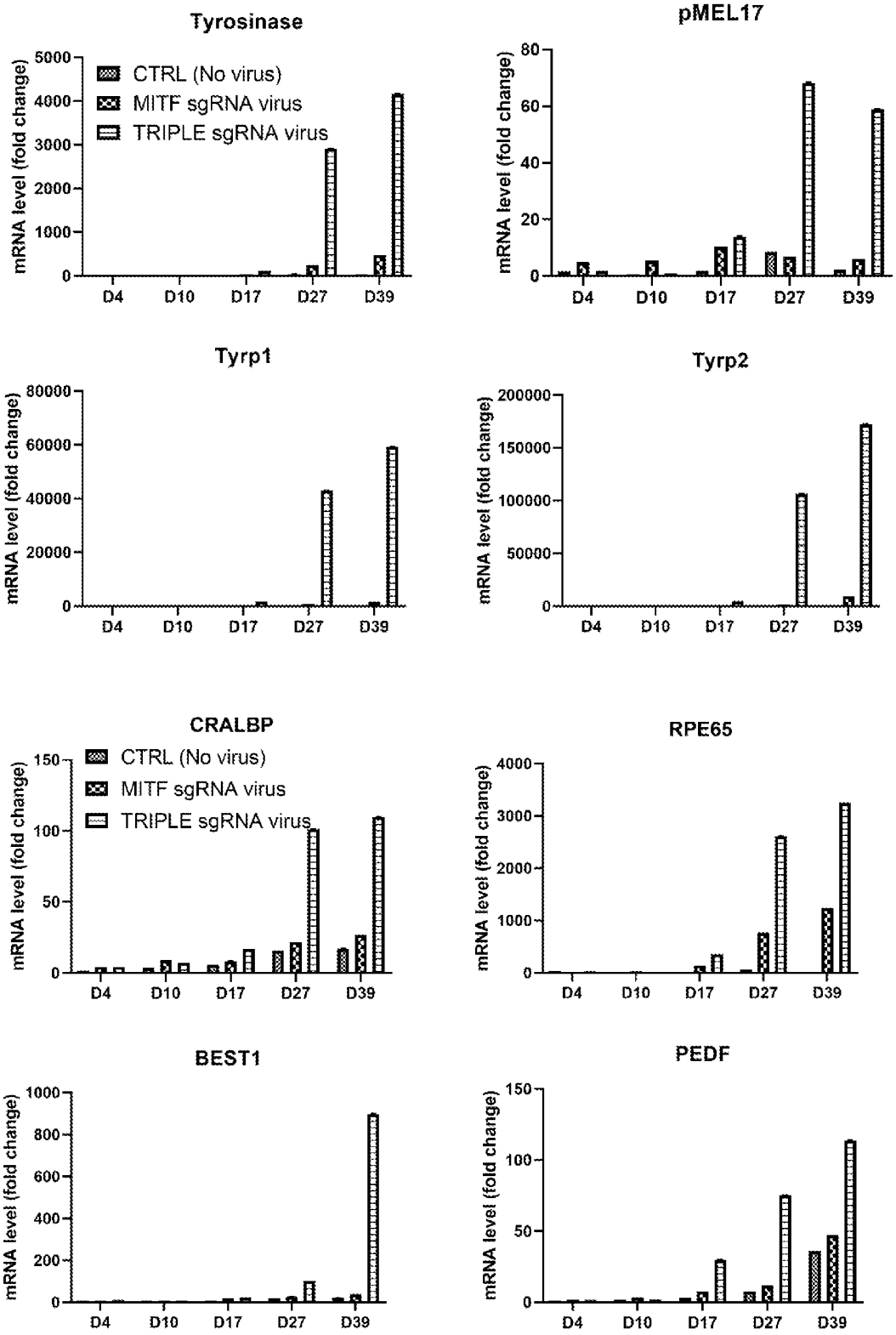
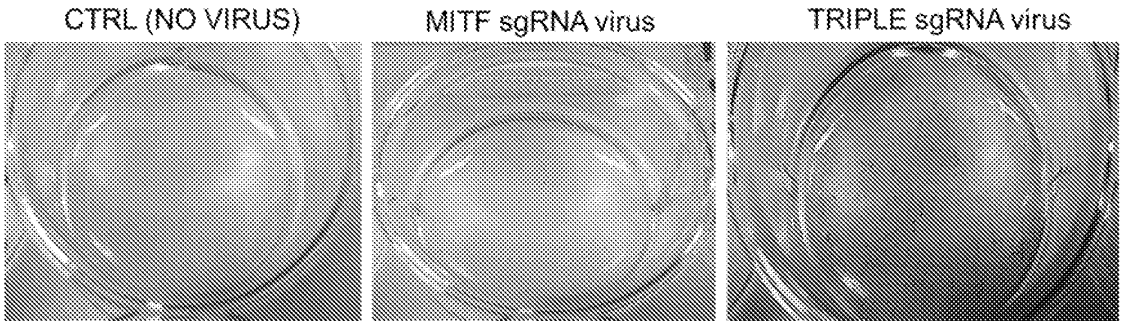
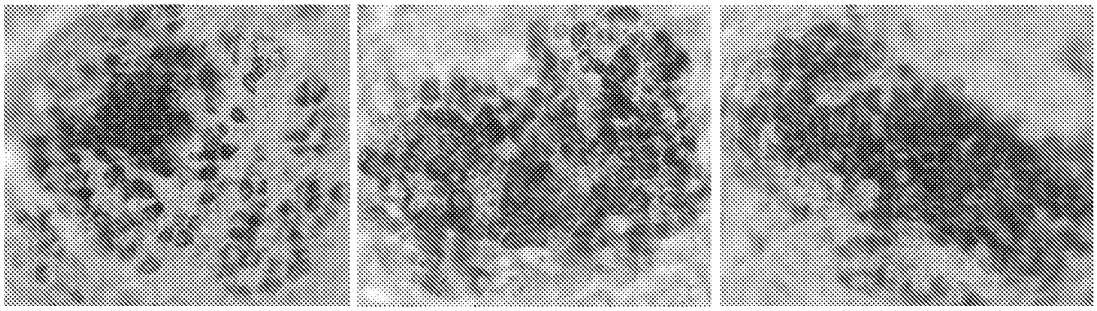


Fig. 13 (continued)

(c)



(d)



e)

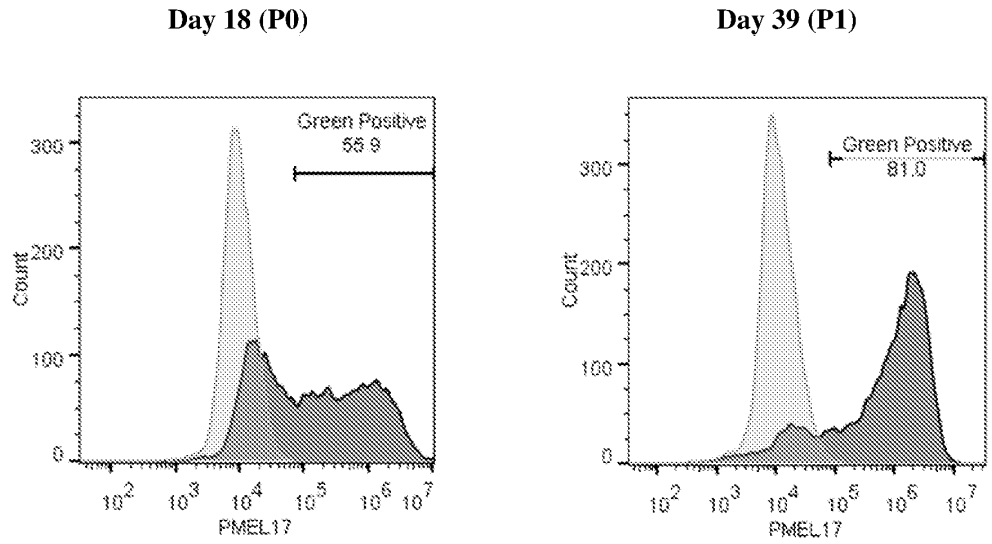


Fig. 13 (continued)

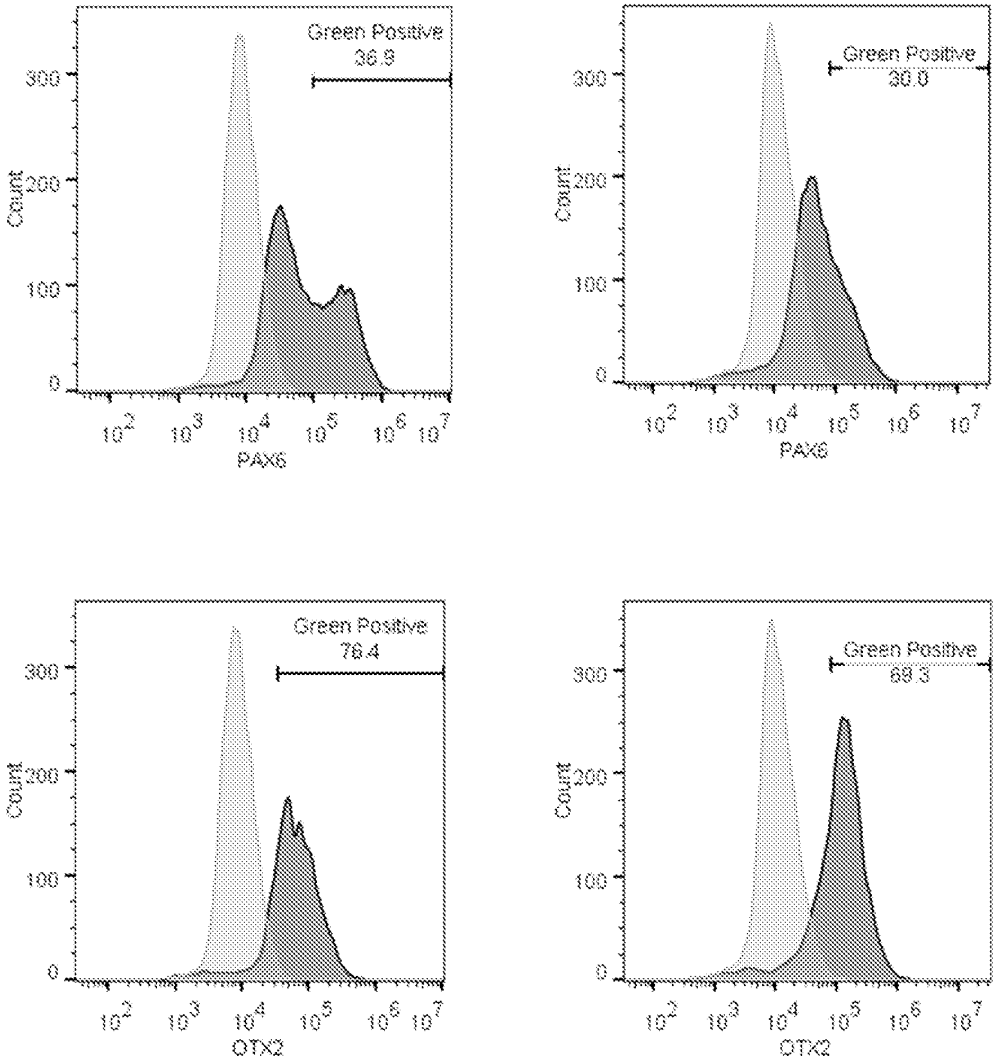


Fig. 13 (continued)

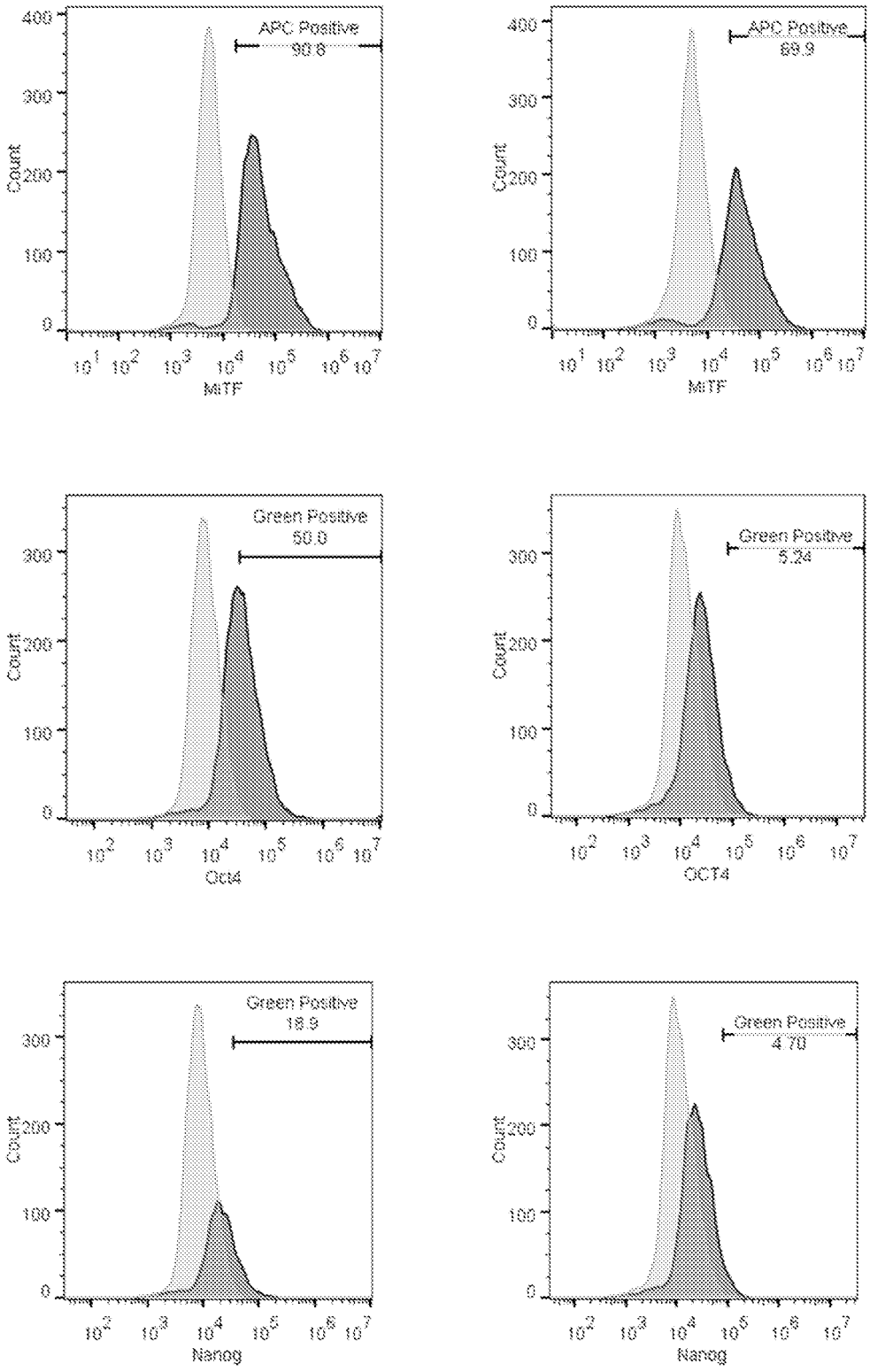
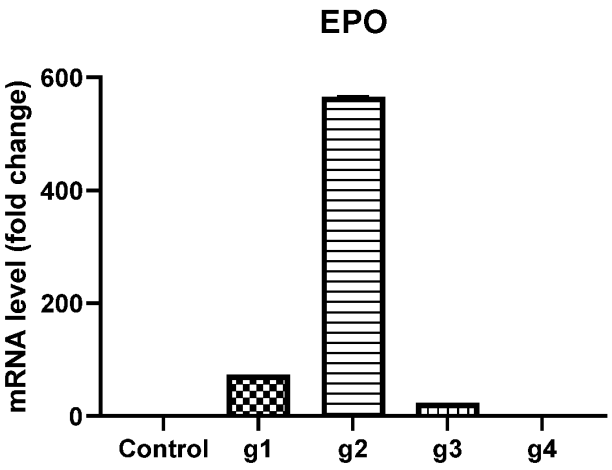

APC Positive
90.8
Count
MiTF
APC Positive
89.9
Count
MiTF
Green Positive
50.0
Count
Oct4
Green Positive
5.24
Count
OCT4
Green Positive
18.9
Count
Nanog
Green Positive
4.70
Count
Nanog

Fig. 13

(a)


EPO
mRNA level (fold change)
600
400
200
0
Control
g1
g2
g3
g4

(b)

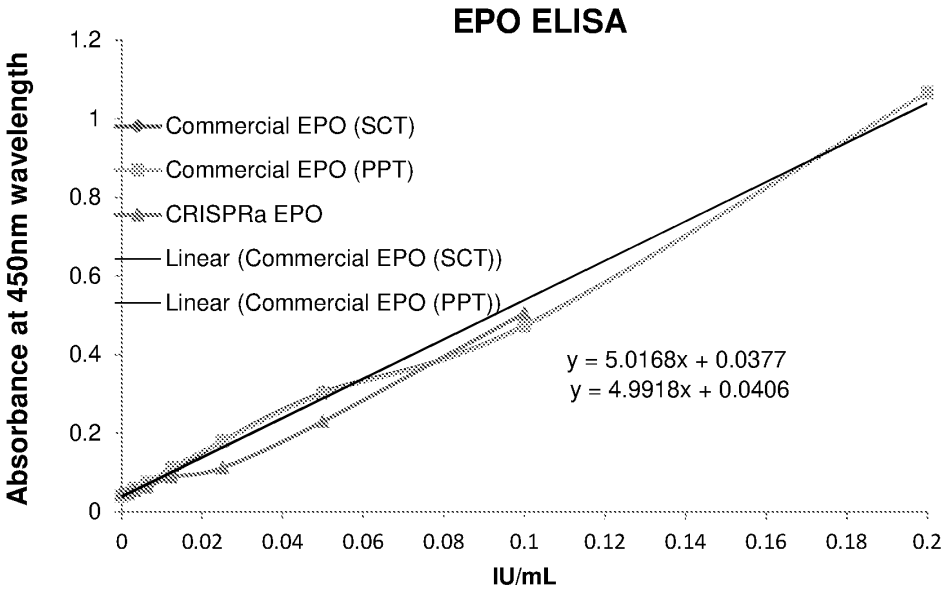

EPO ELISA
Absorbance at 450nm wavelength
1.2
1
0.8
0.6
0.4
0.2
0
Commercial EPO (SCT)
Commercial EPO (PPT)
CRISPRa EPO
Linear (Commercial EPO (SCT))
Linear (Commercial EPO (PPT))
y = 5.0168x + 0.0377
y = 4.9918x + 0.0406
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
IU/mL

Fig. 14

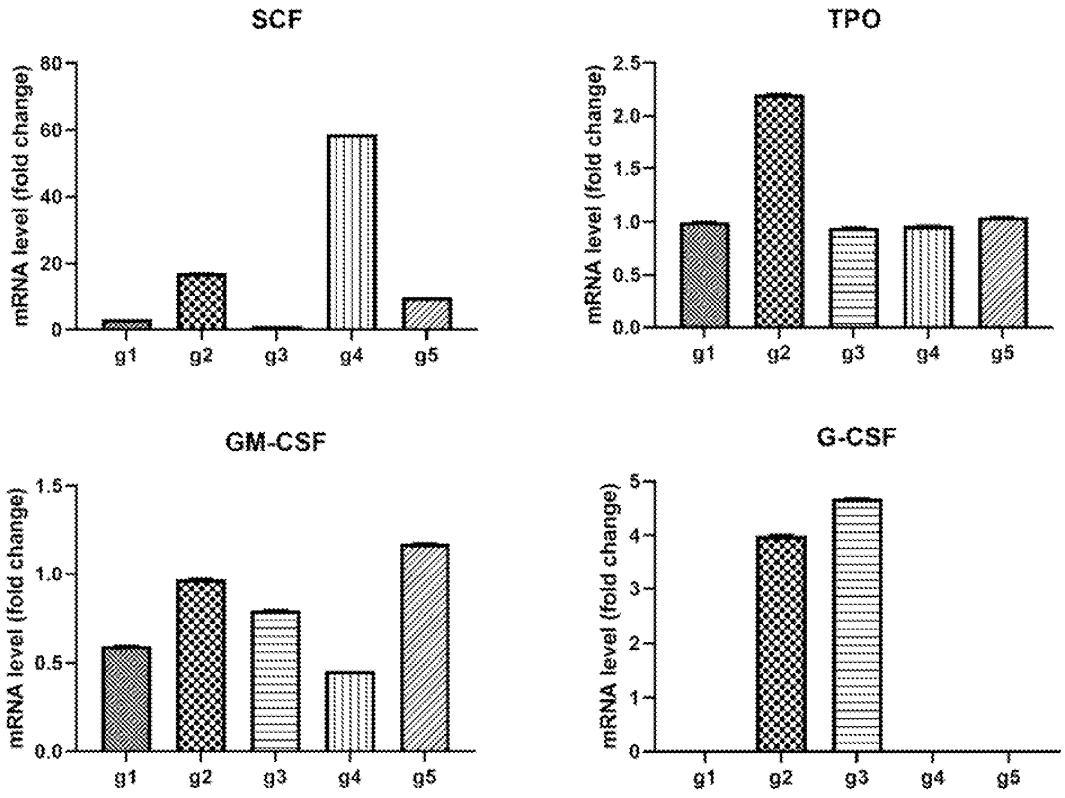

SCF
TPO
GM-CSF
G-CSF
mRNA level (fold change)
g1
g2
g3
g4
g5

Fig. 15

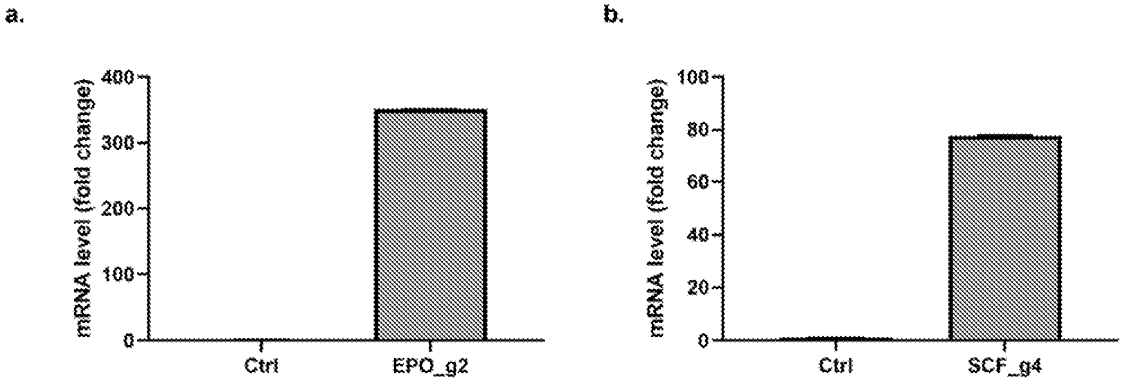

a.
b.
mRNA level (fold change)
Ctrl
EPO_g2
SCF_g4

Fig. 16