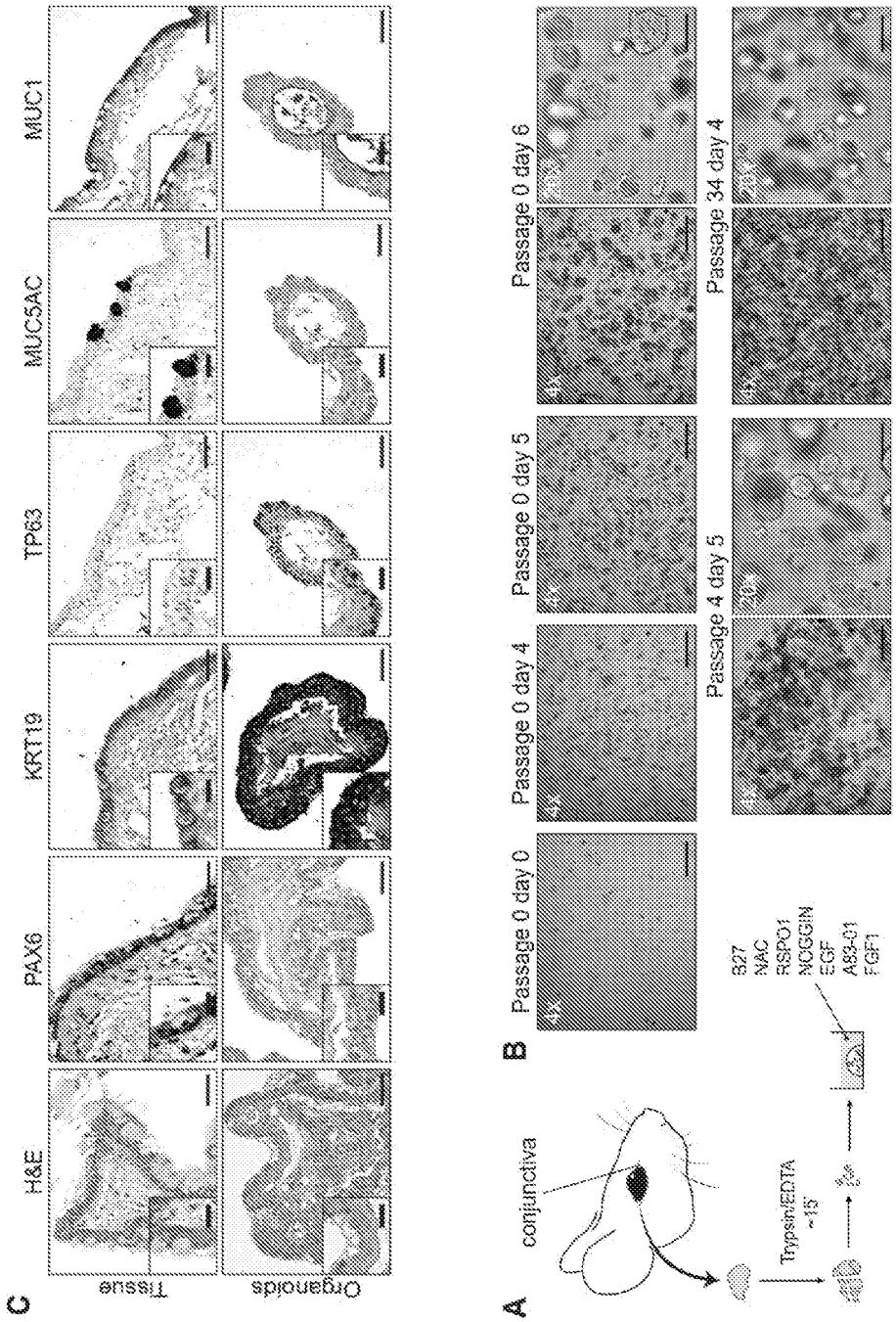
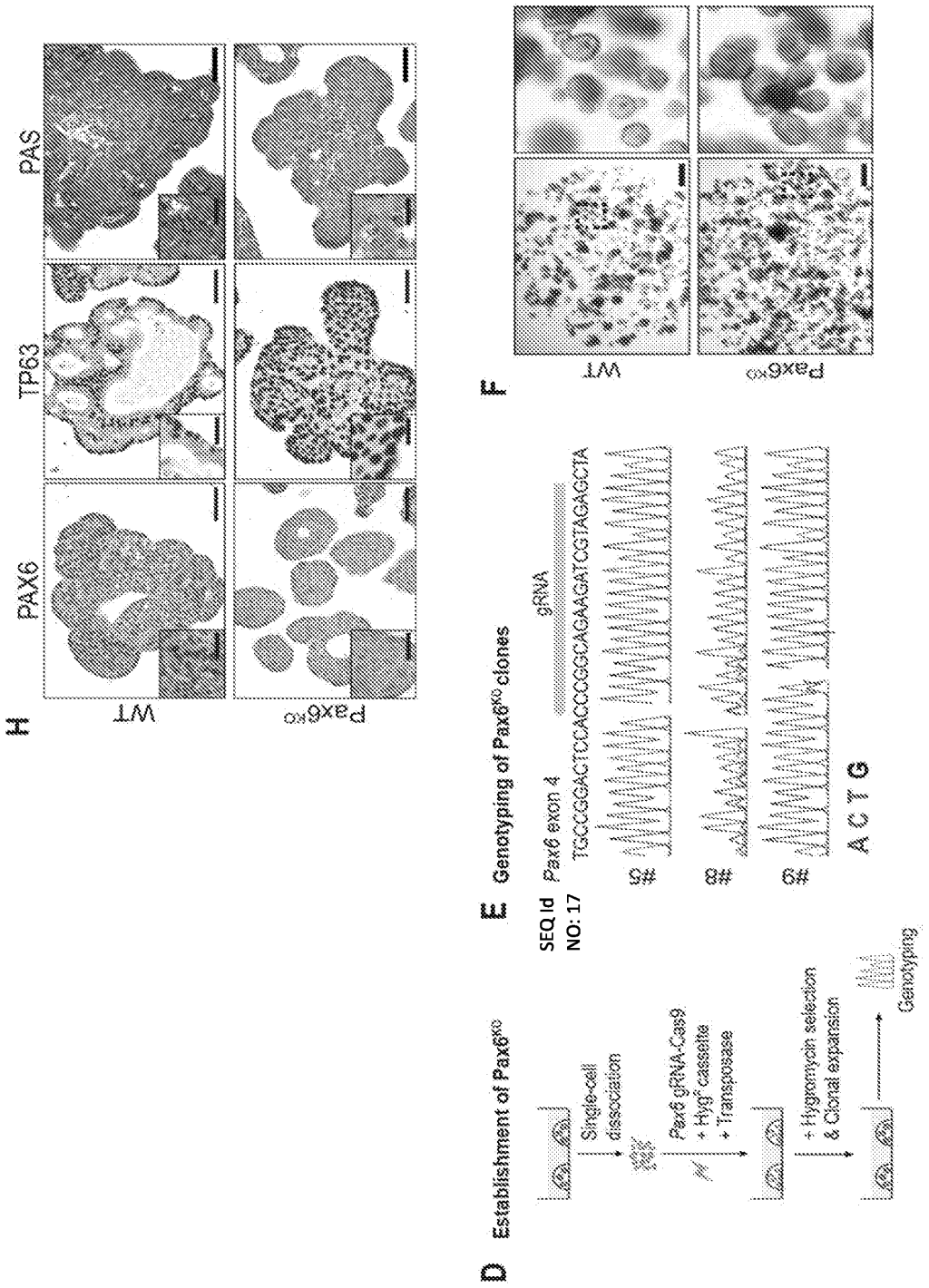
FIGURE 1


A
conjunctiva
Trypsin/EDTA
~15'
B27
NAC
RSPO1
NCGGIN
EGF
A83-01
FGF1
B
Passage 0 day 0
Passage 0 day 4
Passage 0 day 5
Passage 0 day 6
Passage 4 day 5
Passage 34 day 4
C
H&E
PAX6
KRT19
TP63
MUC5AC
MUC1
Tissue
Organoids

Cont. FIGURE 1



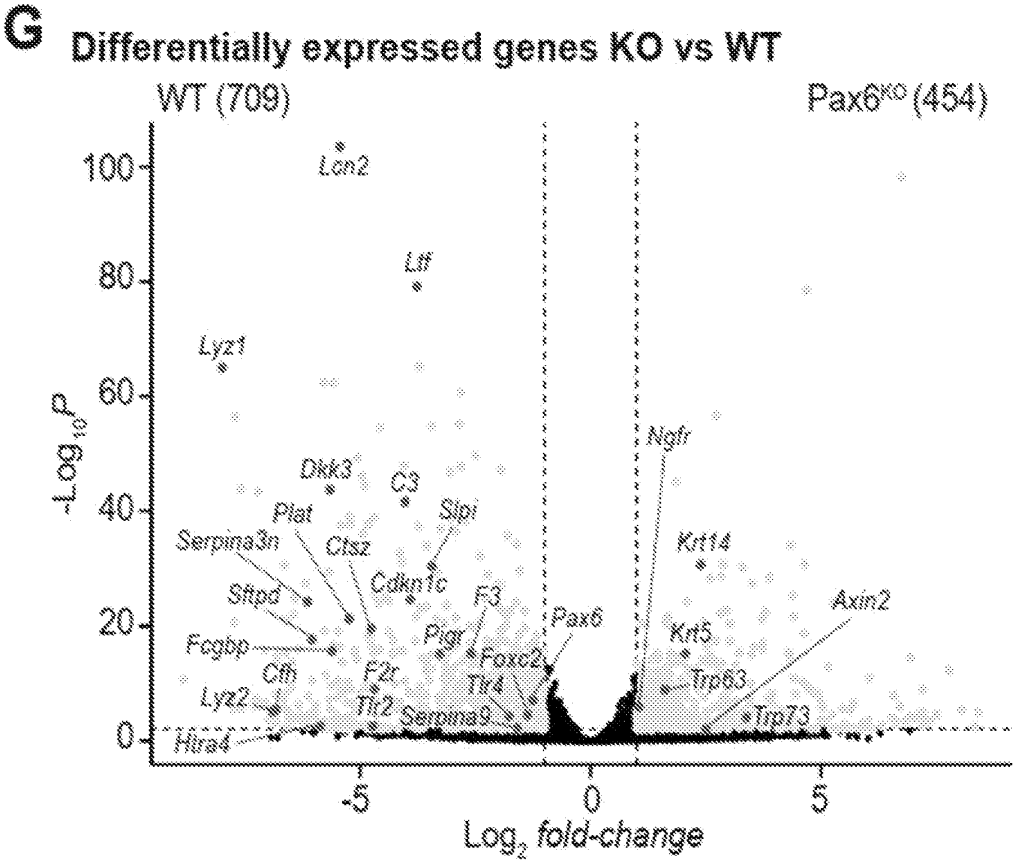
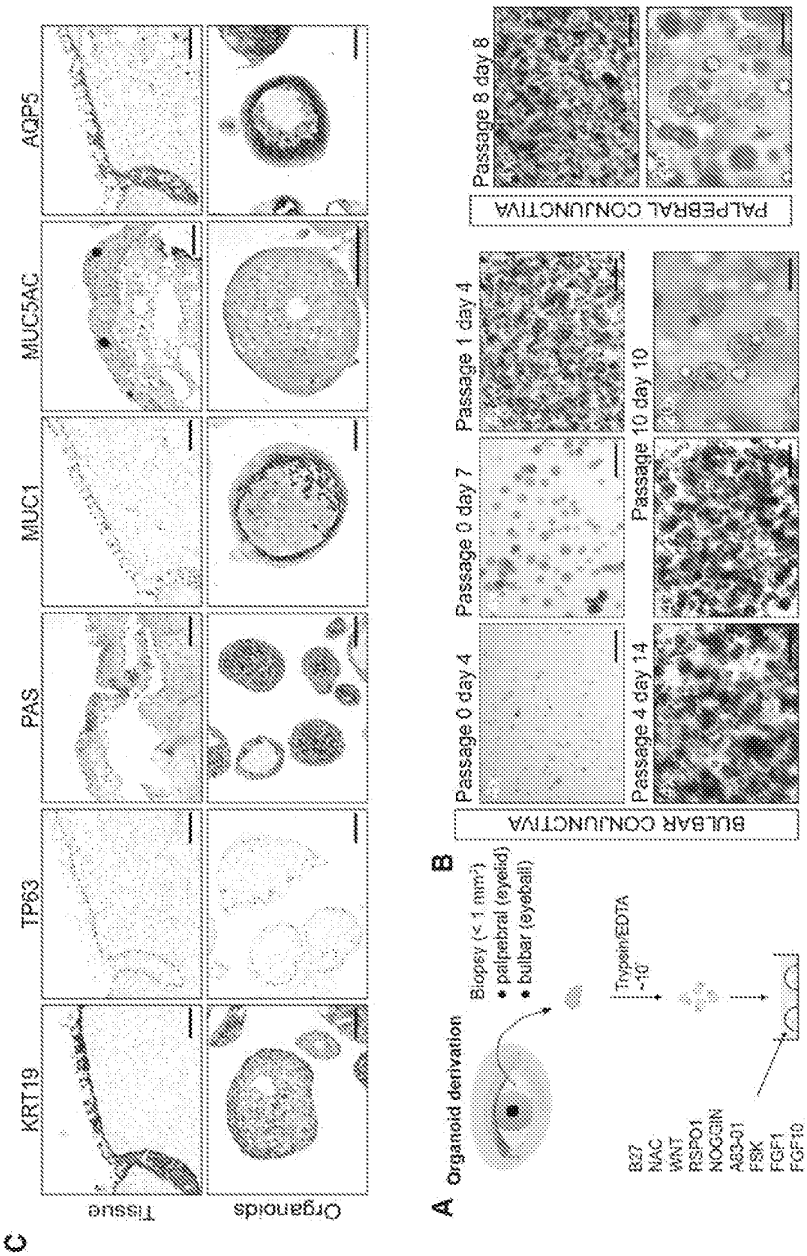

G
Differentially expressed genes KO vs WT
WT (709)
Pax6KO (454)
-Log10 p
Log2 fold-change
Lcn2
Ltf
Lyz1
Dkk3
C3
Slpi
Plat
Serpina3n
Ctsz
Cdkn1c
F3
Sftpd
Pax6
Pigr
Foxc2
Fcgbp
Cfh
F2r
Tlr4
Lyz2
Tlr2
Serpina9
Htra4
Ngfr
Krt14
Krt5
Axin2
Trp63
Trp73

FIGURE 2


A
Organoid derivation
Biopsy (< 1 mm³)
palpebral (eyelid)
bulbar (eyeball)
Trypsin/EDTA
~10
B27
NAC
WNT
RSPO1
NOGGIN
A83-01
FSK
FGF1
FGF10
B
BULBAR CONJUNCTIVA
PALPEBRAL CONJUNCTIVA
Passage 0 day 4
Passage 0 day 7
Passage 1 day 4
Passage 4 day 14
Passage 10 day 10
Passage 8 day 8
C
Tissue
Organoids
KRT19
TP63
PAS
MUC1
MUC5AC
AQP5

Cont. FIGURE 2

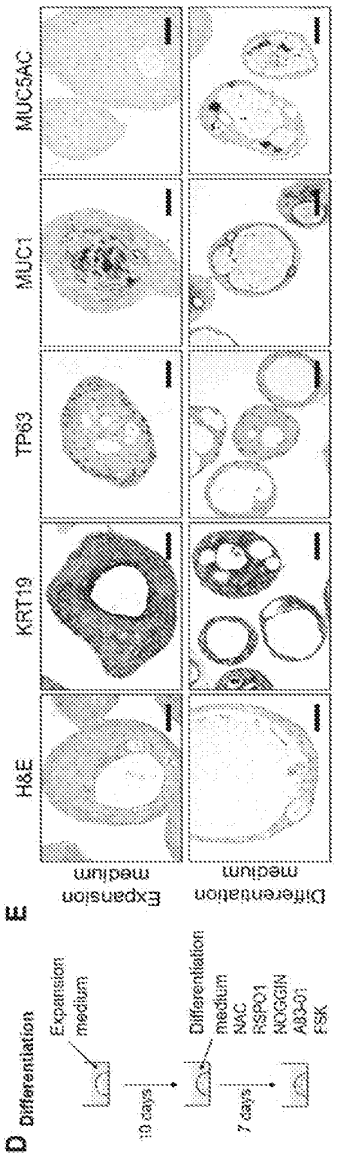


FIGURE 3

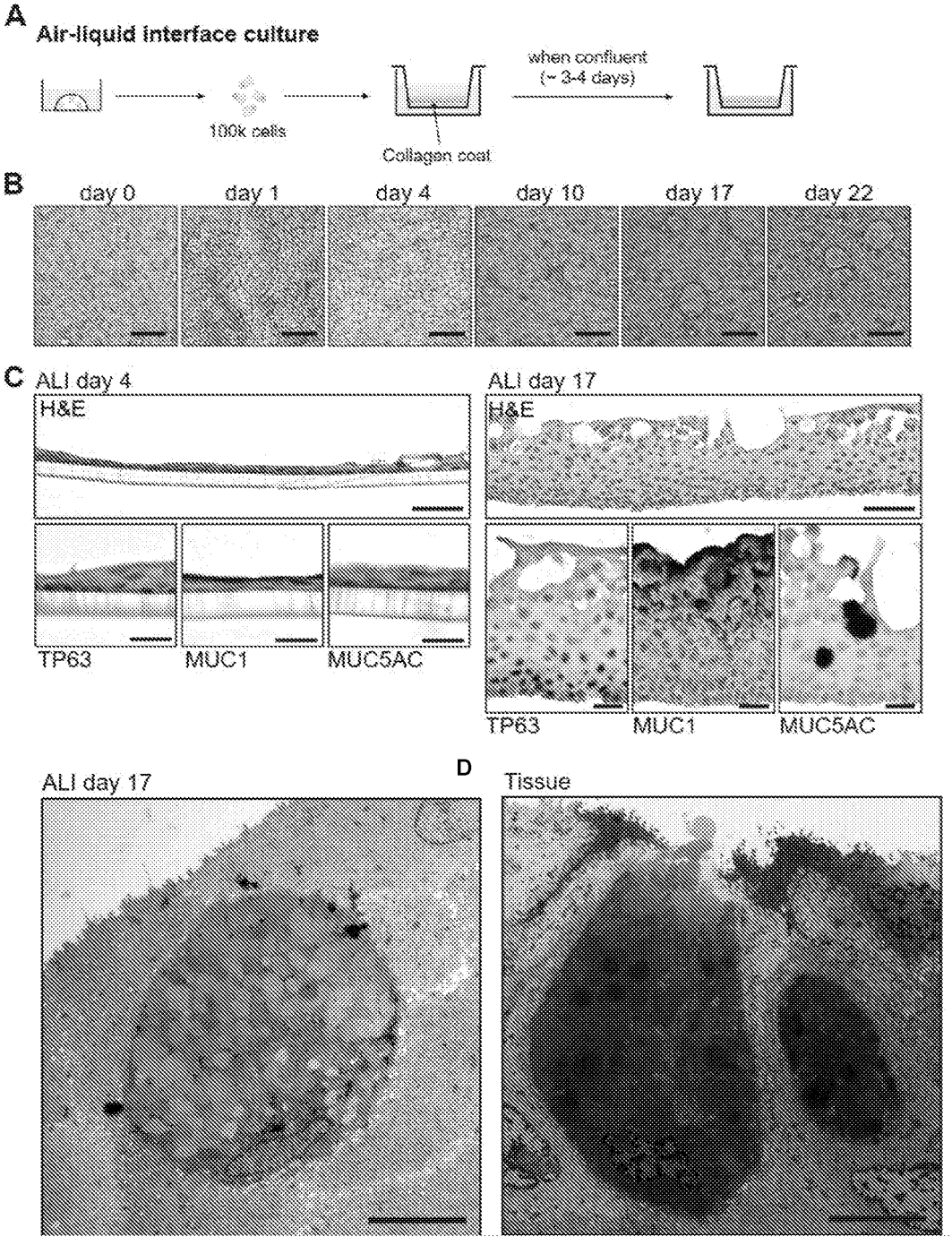

A
Air-liquid interface culture
100k cells
Collagen coat
when confluent
(~ 3-4 days)
B
day 0
day 1
day 4
day 10
day 17
day 22
C
ALI day 4
H&E
TP63
MUC1
MUC5AC
ALI day 17
H&E
TP63
MUC1
MUC5AC
D
ALI day 17
Tissue

FIGURE 4

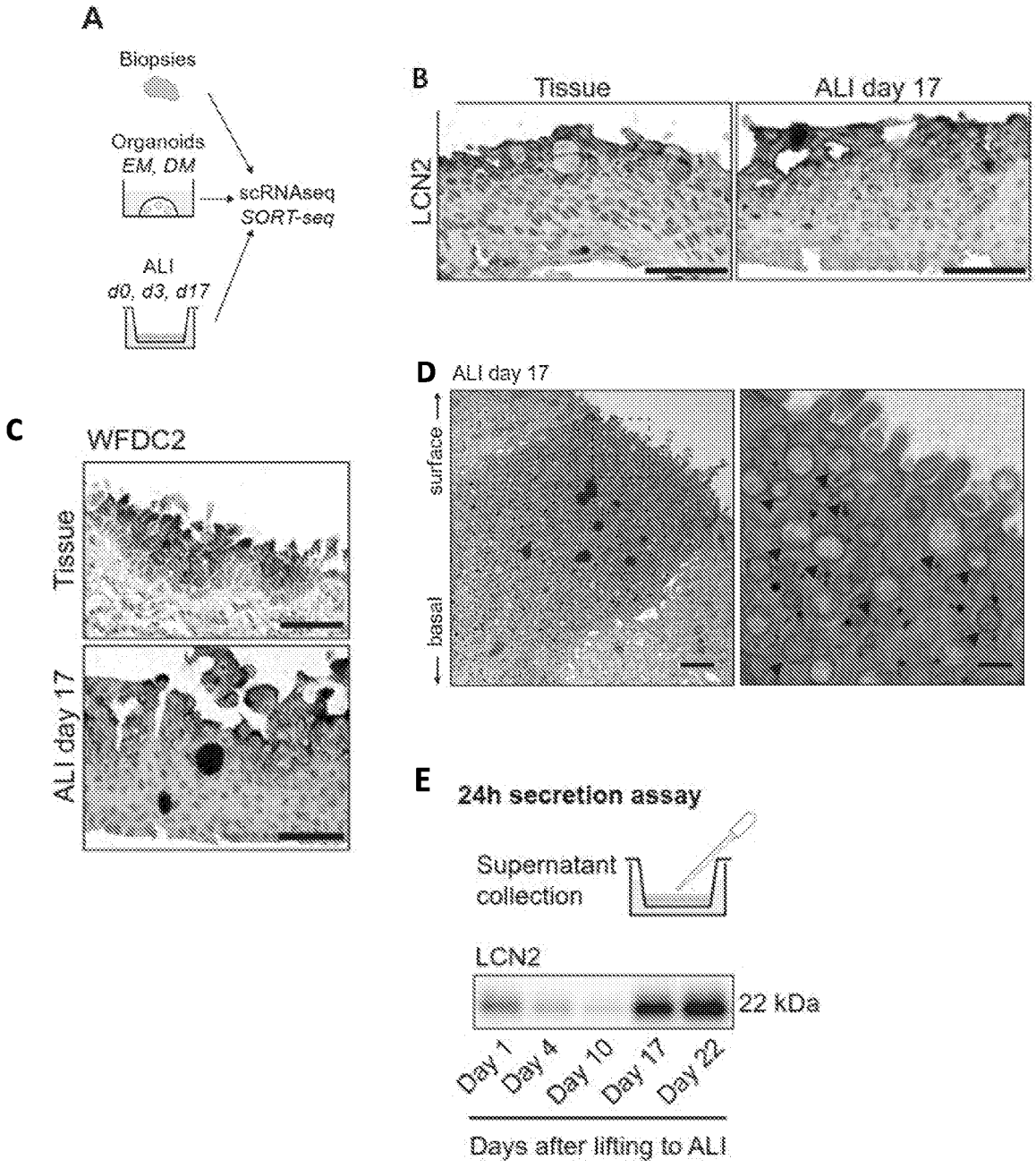

A
Biopsies
Organoids
EM, DM
scRNAseq
SORT-seq
ALI
d0, d3, d17
B
Tissue
ALI day 17
LCN2
C
WFDC2
Tissue
ALI day 17
D
ALI day 17
surface
basal
E
24h secretion assay
Supernatant collection
LCN2
22 kDa
Day 1
Day 4
Day 10
Day 17
Day 22
Days after lifting to ALI

FIGURE 5

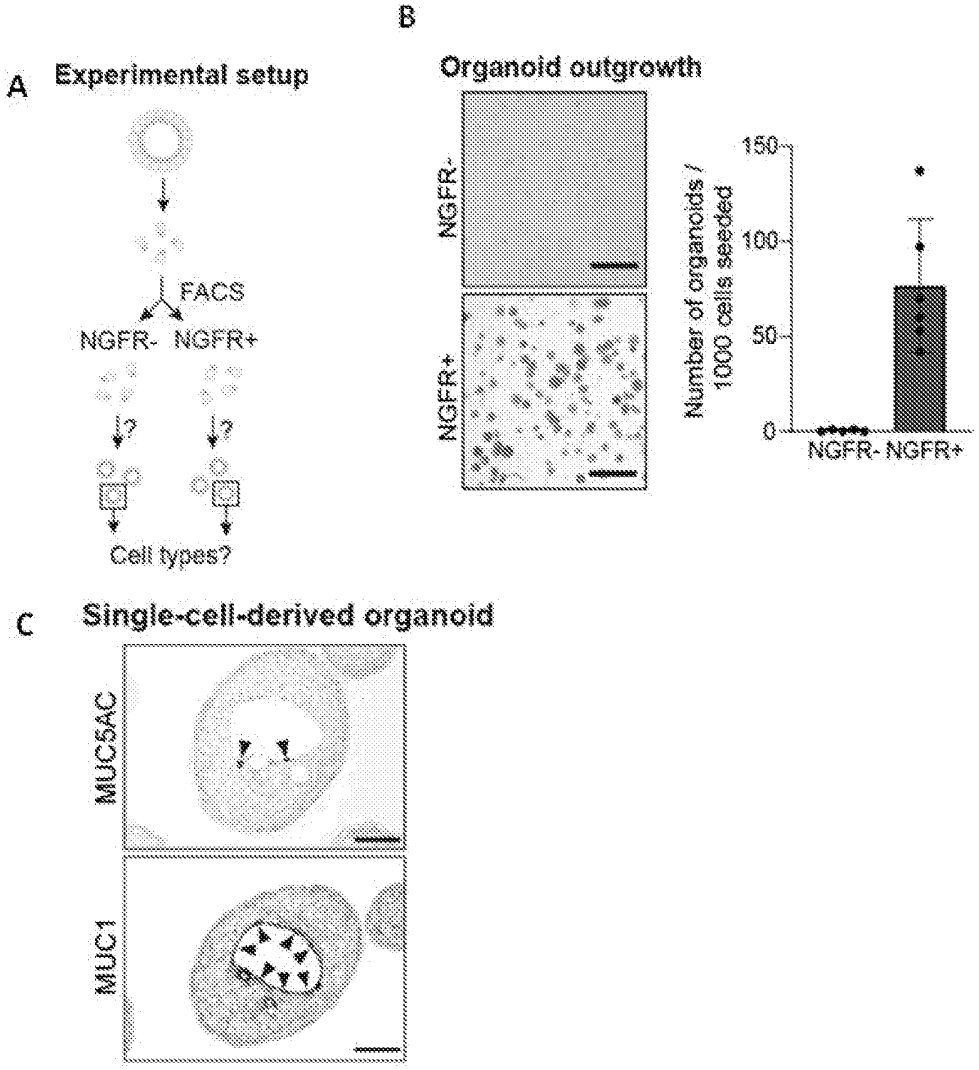
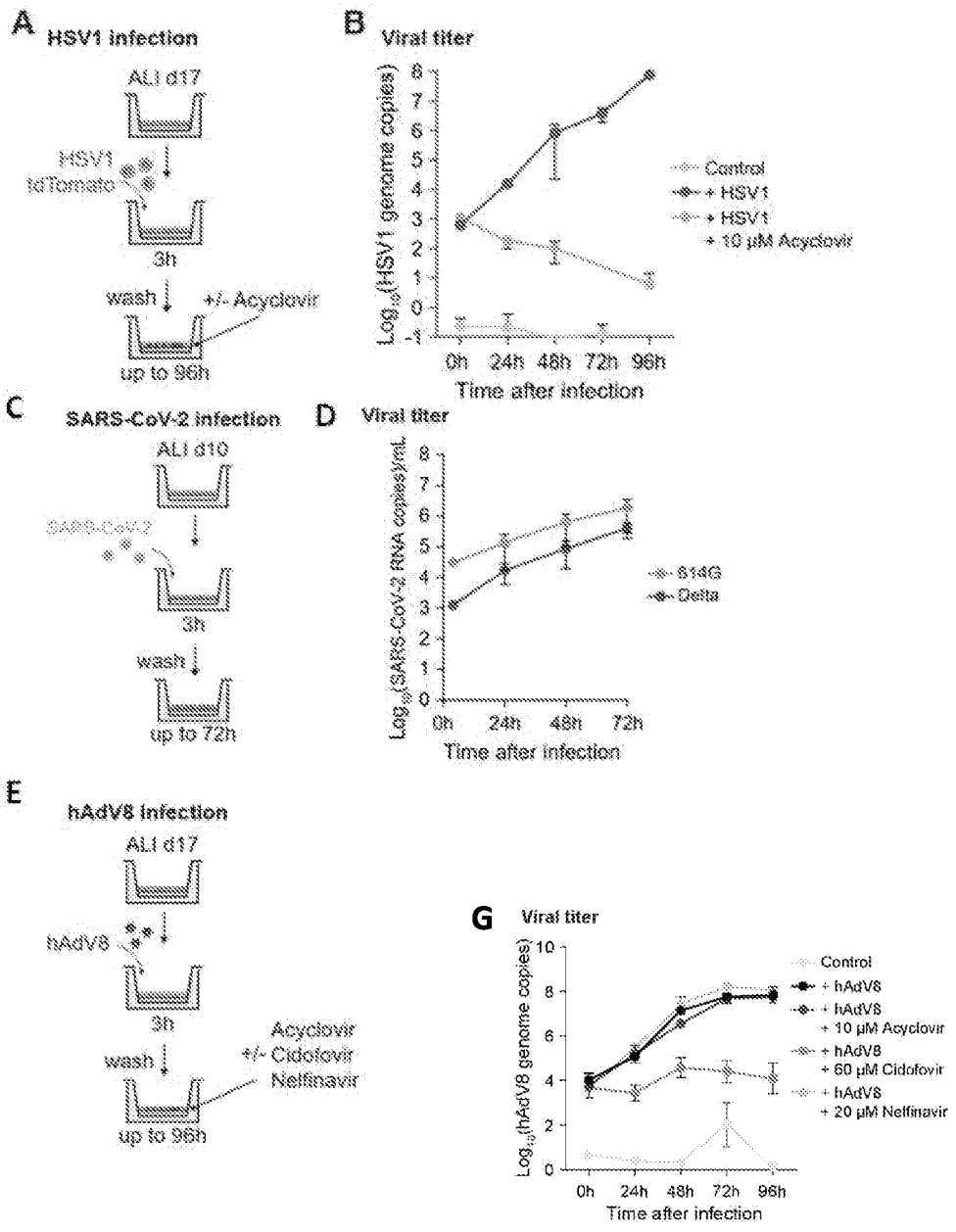

A
Experimental setup
FACS
NGFR- NGFR+
?
?
Cell types?
B
Organoid outgrowth
NGFR-
NGFR+
Number of organoids / 1000 cells seeded
150
100
50
0
NGFR- NGFR+
C
Single-cell-derived organoid
MUC5AC
MUC1

FIGURE 6


A
HSV1 infection
ALI d17
HSV1
tdTomato
3h
wash
+/- Acyclovir
up to 96h
B
Viral titer
Log10(HSV1 genome copies)
Control
+ HSV1
+ HSV1
+ 10 µM Acyclovir
0h 24h 48h 72h 96h
Time after infection
C
SARS-CoV-2 infection
ALI d10
SARS-CoV-2
3h
wash
up to 72h
D
Viral titer
Log10(SARS-CoV-2 RNA copies/mL
614G
Delta
0h 24h 48h 72h
Time after infection
E
hAdV8 infection
ALI d17
hAdV8
3h
wash
Acyclovir
+/- Cidofovir
Nelfinavir
up to 96h
G
Viral titer
Log10(hAdV8 genome copies)
Control
+ hAdV8
+ hAdV8
+ 10 µM Acyclovir
+ hAdV8
+ 60 µM Cidofovir
+ hAdV8
+ 20 µM Nelfinavir
0h 24h 48h 72h 96h
Time after infection

Cont. FIGURE 6

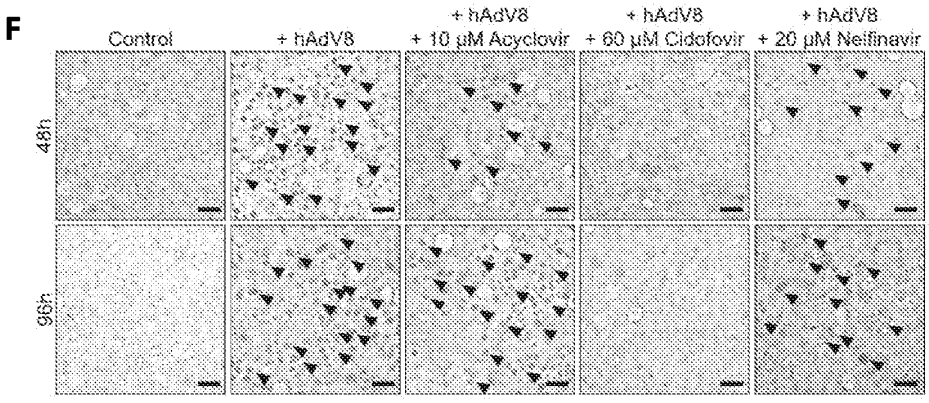
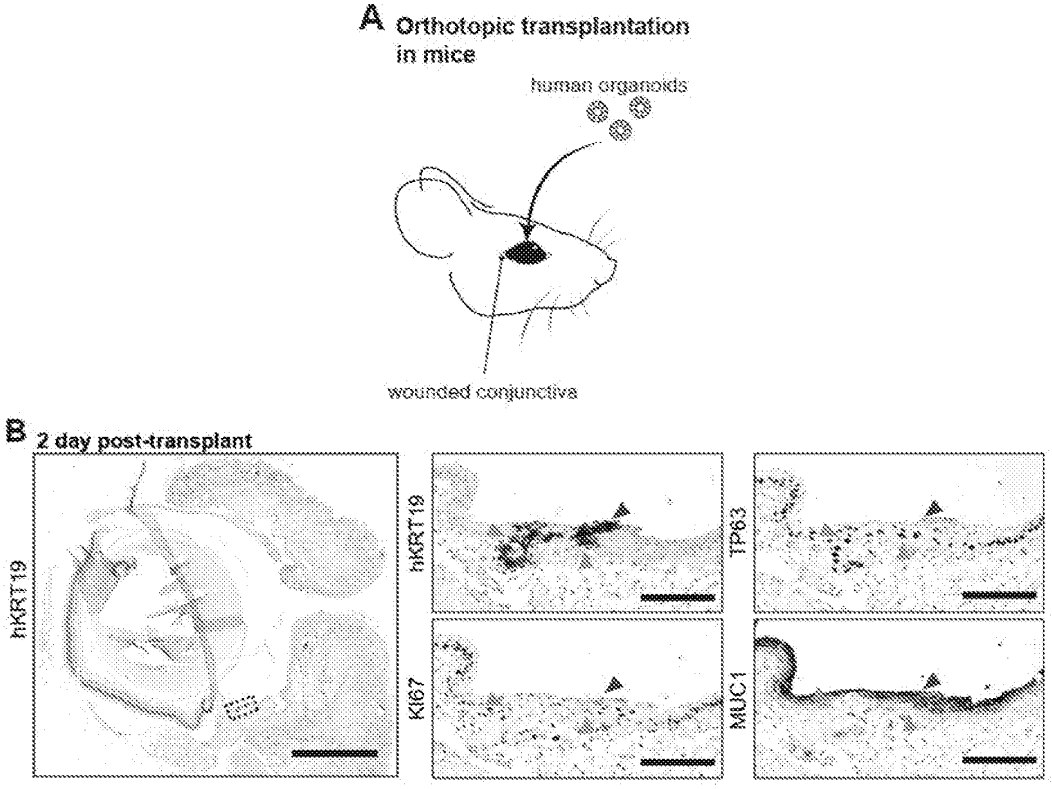
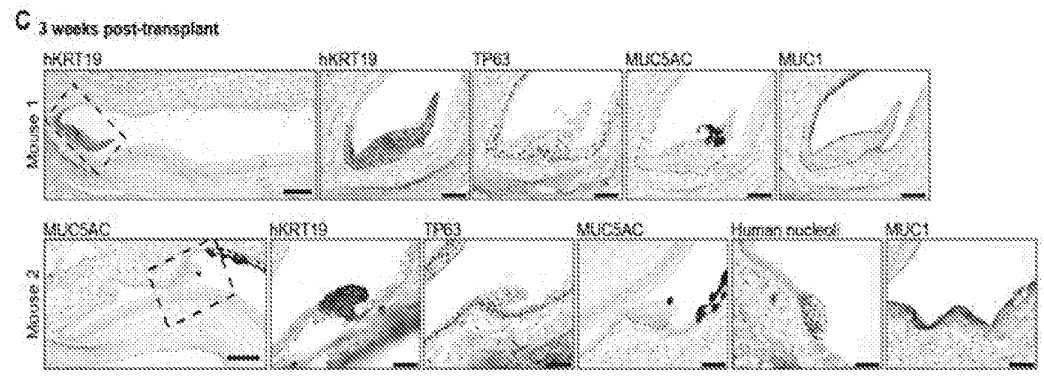


FIGURE 7


A
Orthotopic transplantation in mice
human organoids
wounded conjunctiva
B
2 day post-transplant
hKRT19
hKRT19
TP63
KI67
MUC1

Cont. FIGURE 7



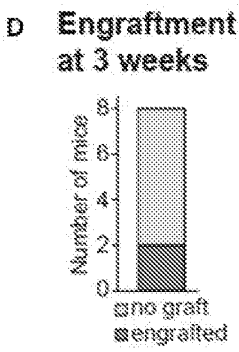


FIGURE 8

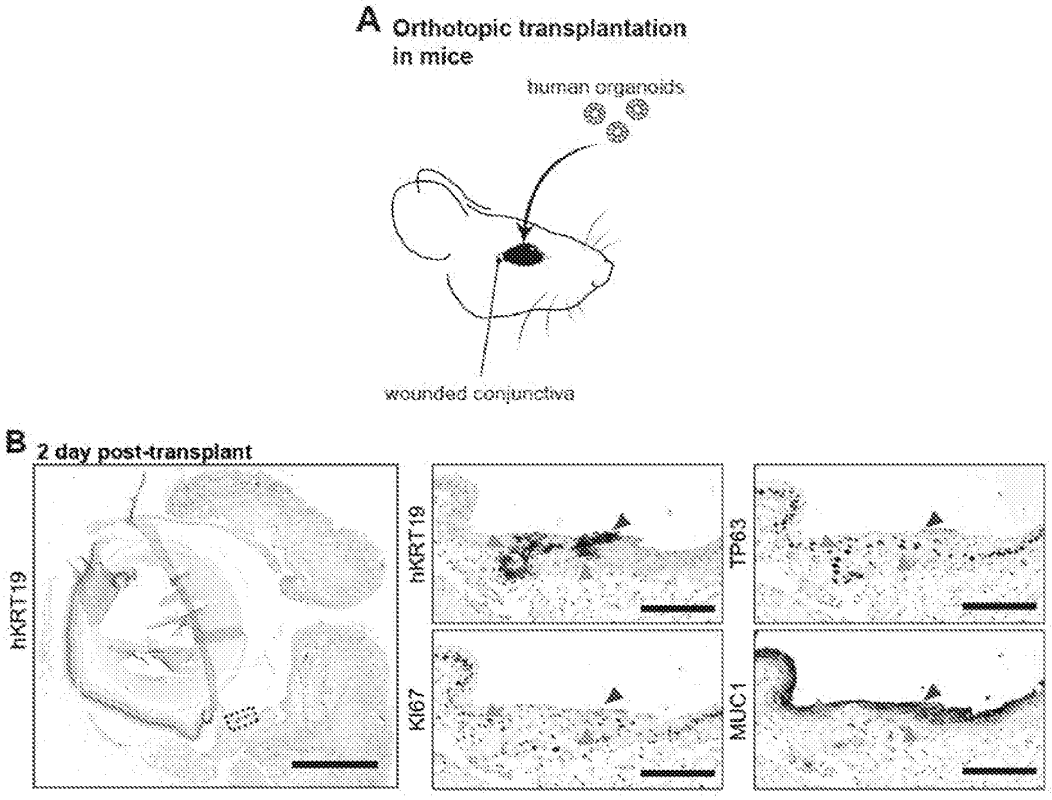

A Orthotopic transplantation in mice
human organoids
wounded conjunctiva
B 2 day post-transplant
hKRT19
hKRT19
TP63
KI67
MUC1

FIGURE 9

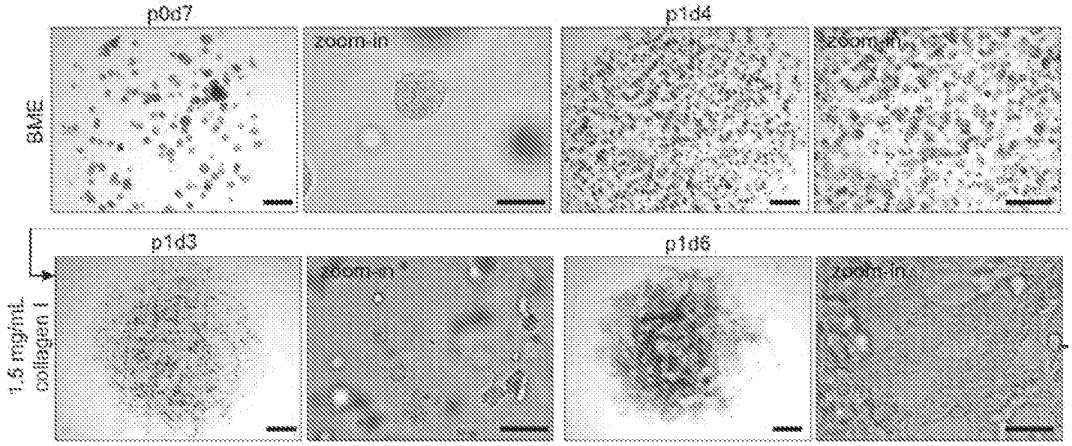

p0d7
zoom-in
p1d4
zoom-in
BME
p1d3
zoom-in
p1d6
zoom-in
1.5 mg/mL collagen I

FIGURE 10

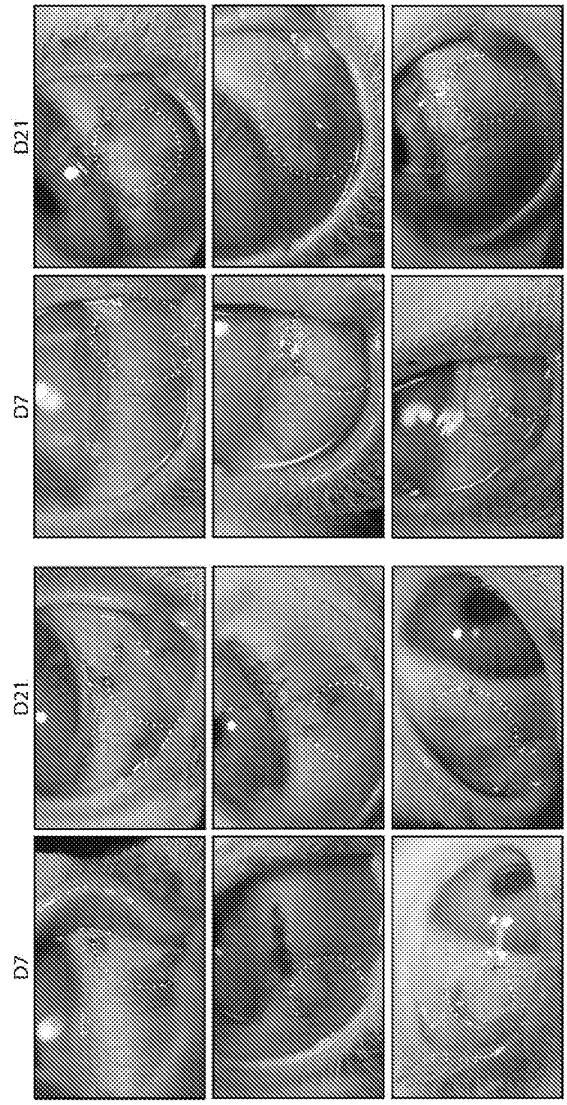


FIGURE 11

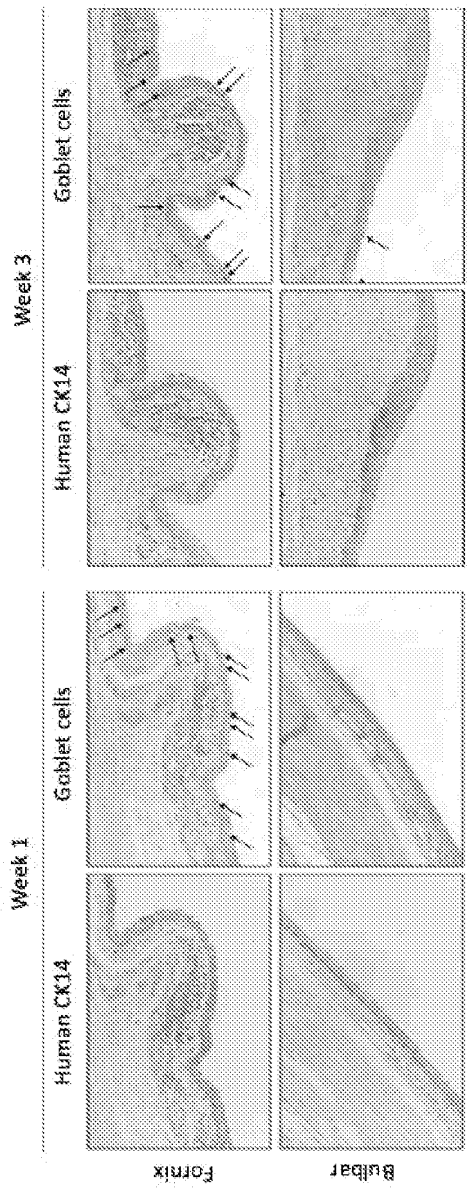

Week 3
Goblet cells
Human CK14
Week 1
Goblet cells
Human CK14
Fornix
Bulbar

FIGURE 12

A:

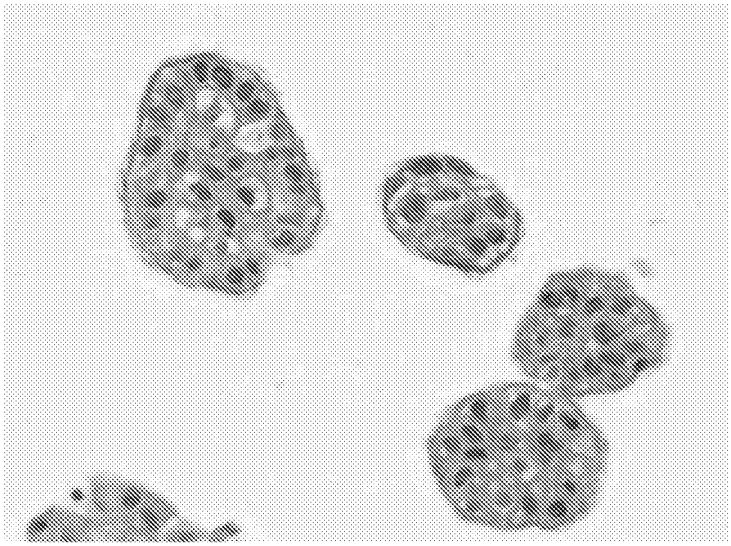


FIGURE 12 – Cont.

B:

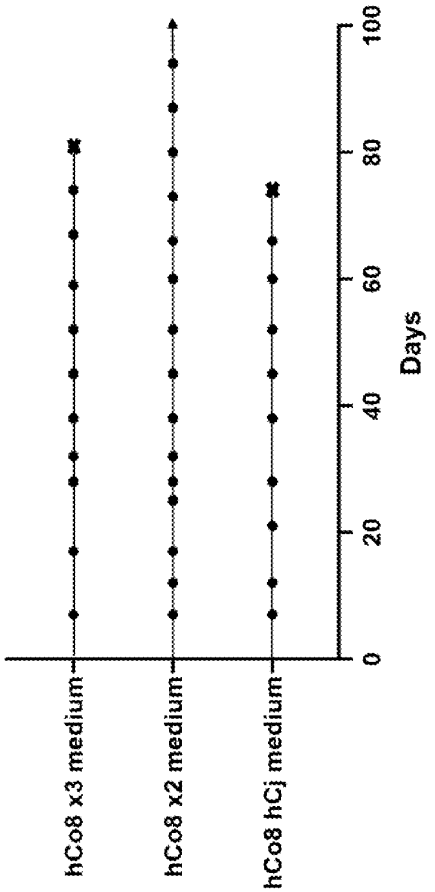


FIGURE 13

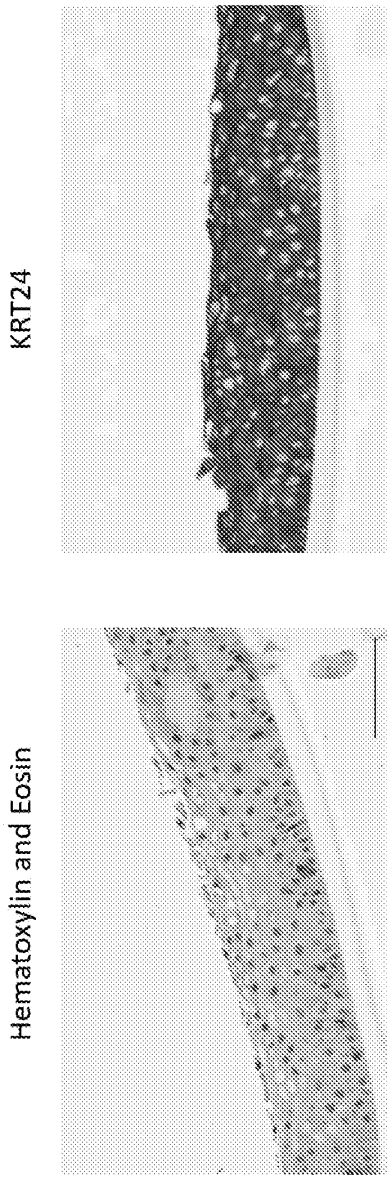


FIGURE 13 – Cont.

