Fig.1

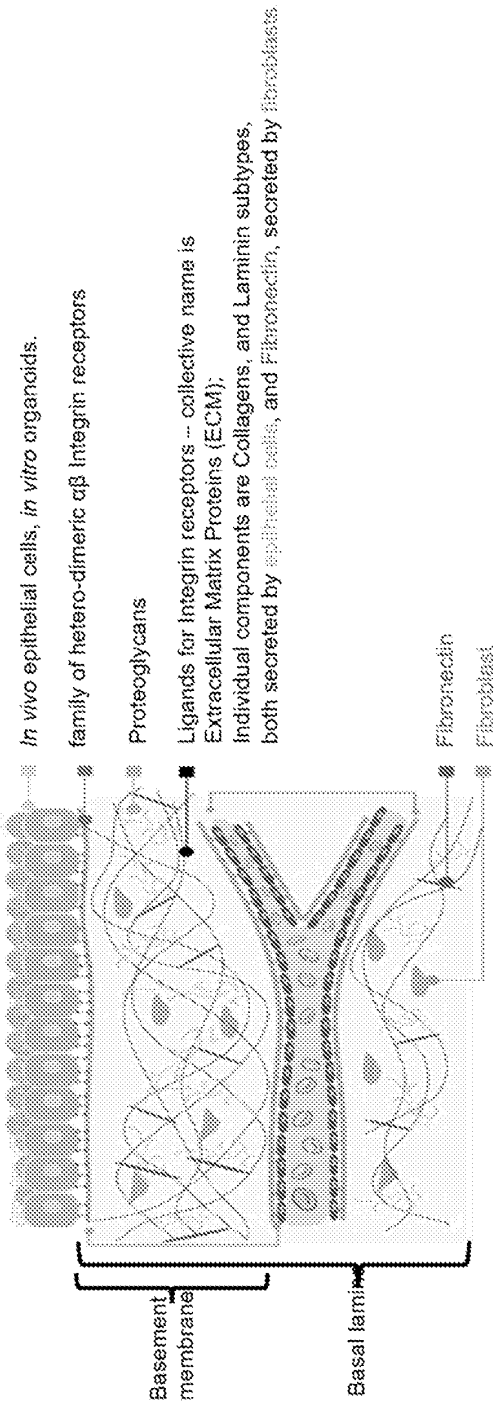

In vivo epithelial cells, in vitro organoids.
family of hetero-dimeric αβ Integrin receptors
Proteoglycans
Ligands for Integrin receptors -- collective name is Extracellular Matrix Proteins (ECM);
Individual components are Collagens, and Laminin subtypes,
both secreted by epithelial cells, and Fibronectin, secreted by fibroblasts
Fibronectin
Fibroblast
Basement membrane
Basal lamina

Fig. 2

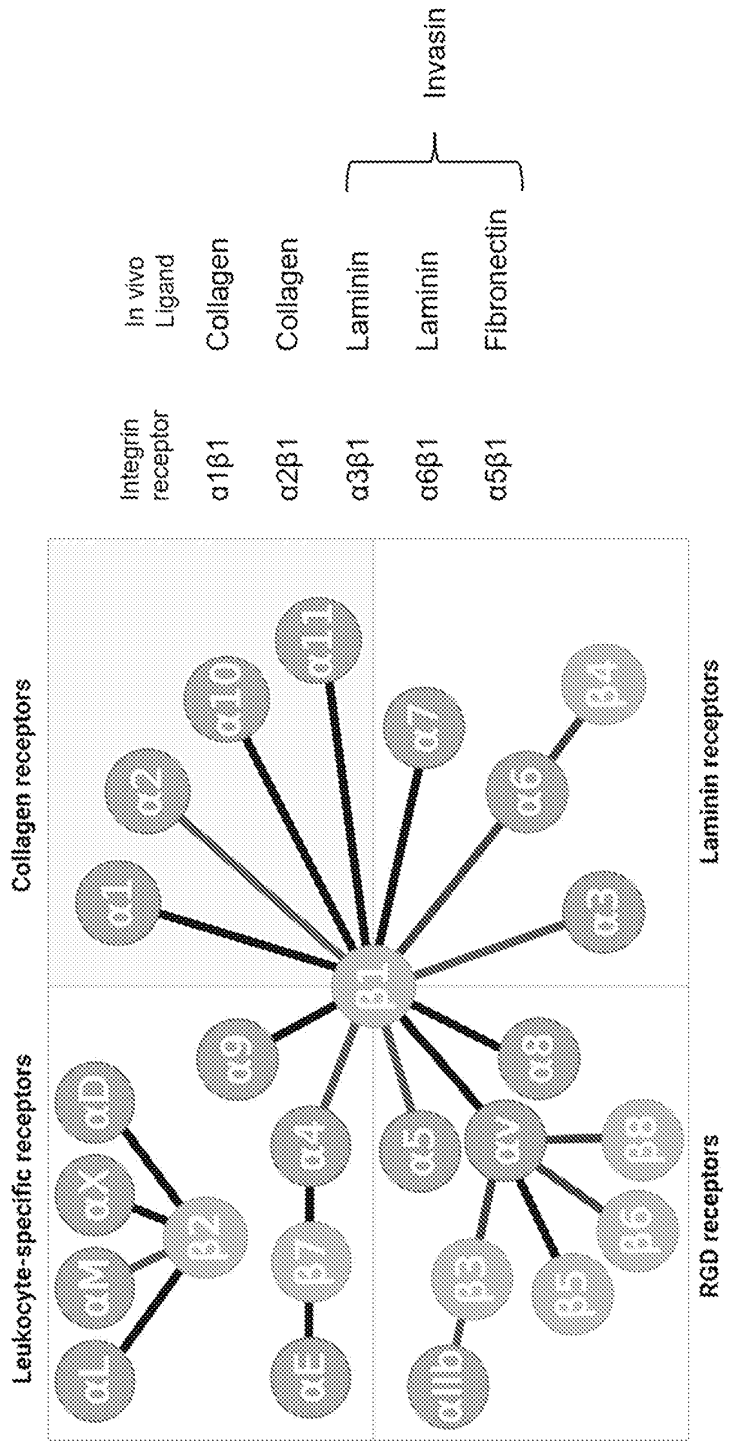
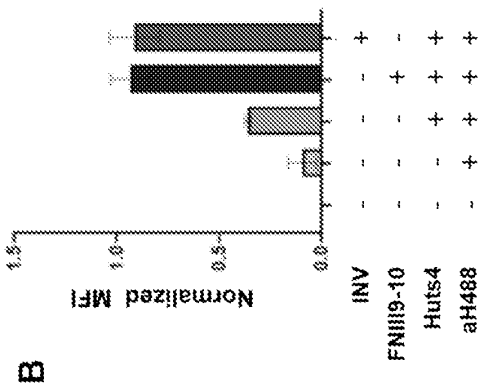

Integrin receptor
In vivo Ligand
α1β1 Collagen
α2β1 Collagen
α3β1 Laminin
α6β1 Laminin
α5β1 Fibronectin
Invasin
Collagen receptors
Laminin receptors
Leukocyte-specific receptors
RGD receptors
β1
α1
α2
α10
α11
α7
α6
β4
α3
α9
α4
β7
α5
αV
α8
β8
β6
β5
α11b
β2
αL
αM
αX
αD

Fig. 3


B
Normalized MFI
1.5
1.0
0.5
0.0
INV
FNIII9-10
Huts4
aH488

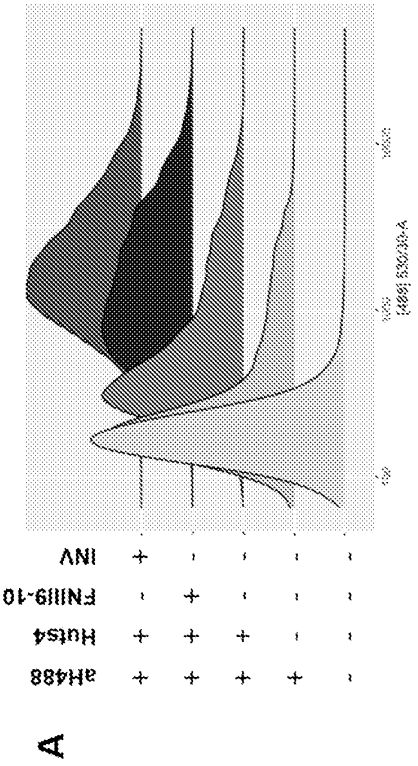

A
INV
FNIII9-10
Huts4
aH488

Fig. 4

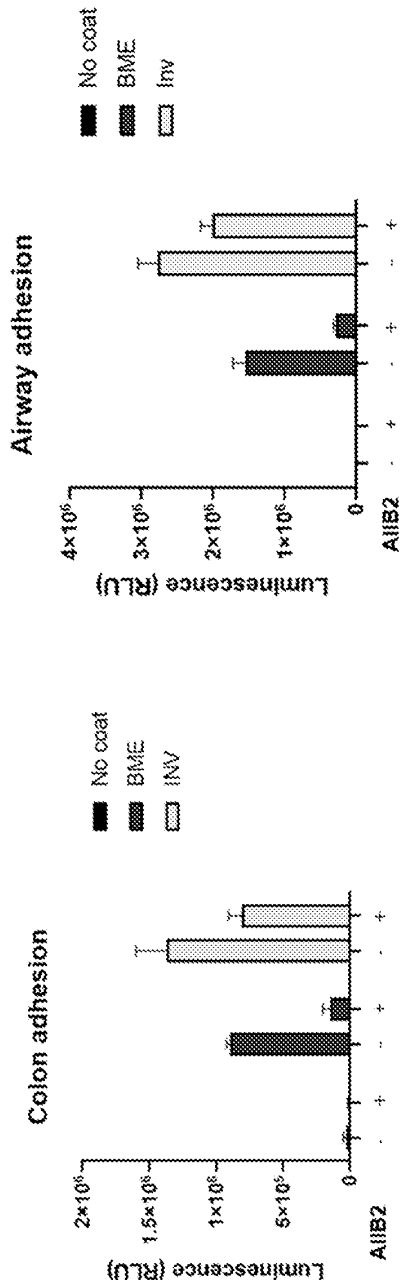

No coat
BME
Inv
Airway adhesion
Luminescence (RLU)
4×10⁶
3×10⁶
2×10⁶
1×10⁶
0
AllB2
No coat
BME
INV
Colon adhesion
Luminescence (RLU)
2×10⁶
1.5×10⁶
1×10⁶
5×10⁵
0
AllB2

Fig. 4 – Cont.

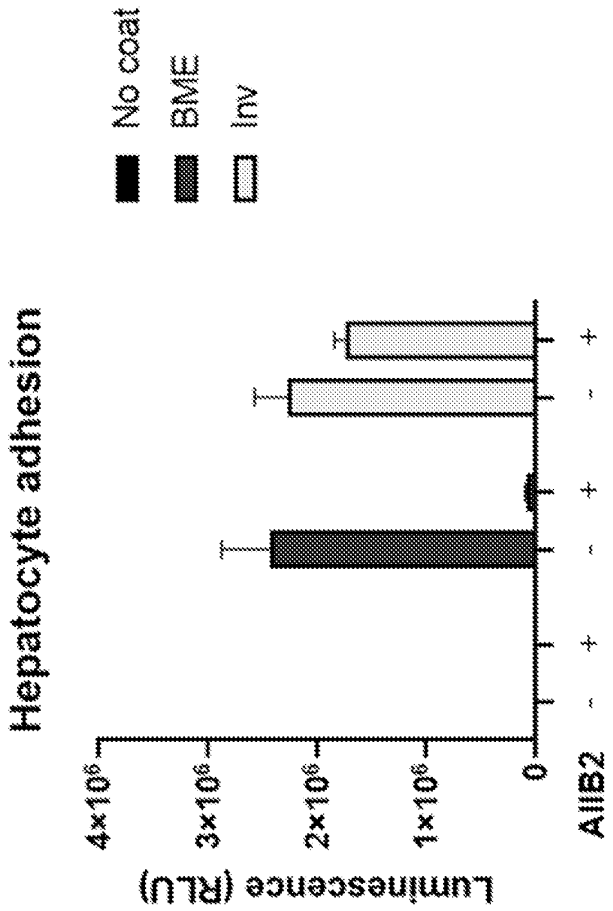

No coat
BME
Inv
Hepatocyte adhesion
Luminescence (RLU)
4×10^6
3×10^6
2×10^6
1×10^6
0
AllB2
+
-
+
-
+
-

Fig. 5

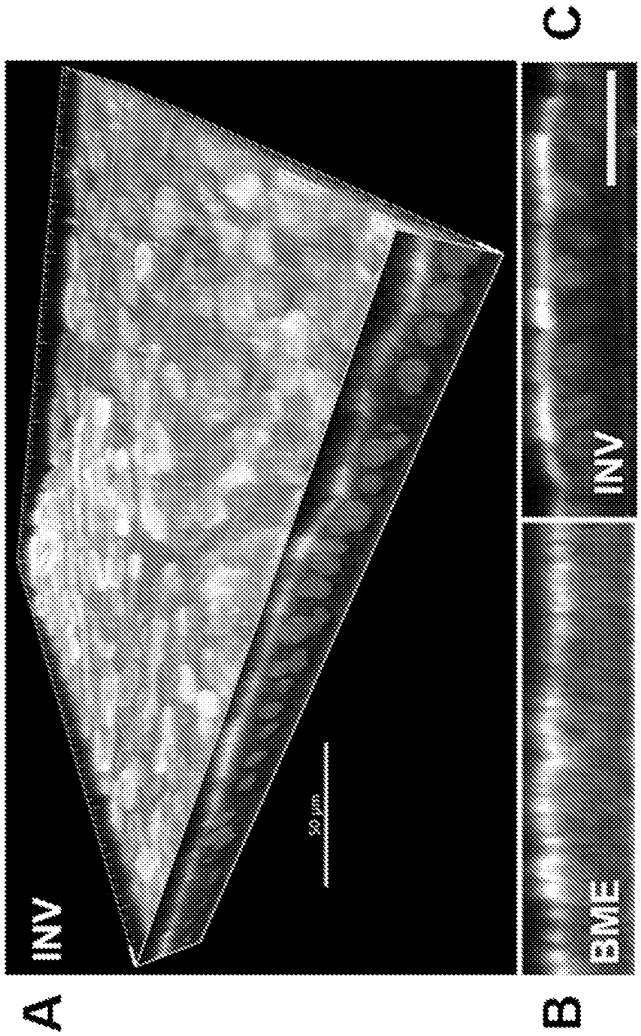


Fig. 6

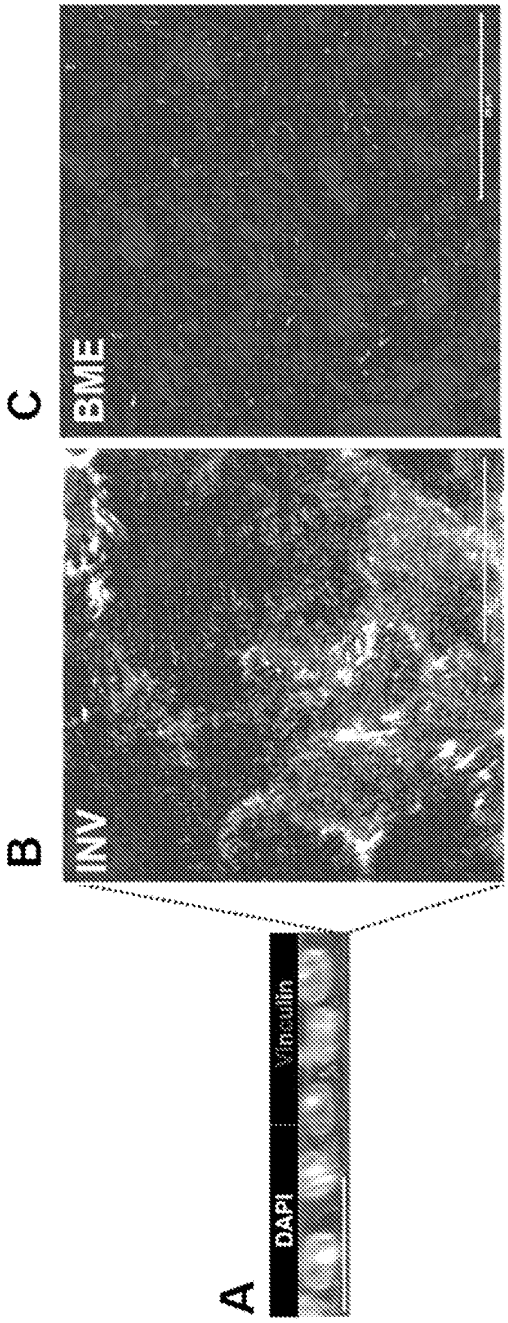


Fig. 7

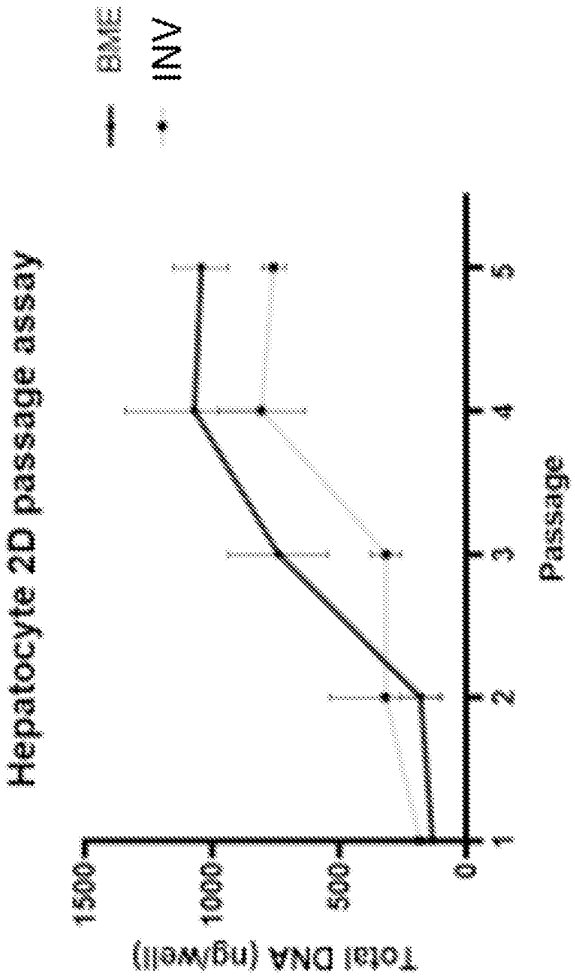

BME
INV
Hepatocyte 2D passage assay
Total DNA (ng/well)
1500
1000
500
0
1
2
3
4
5
Passage

Fig 8.

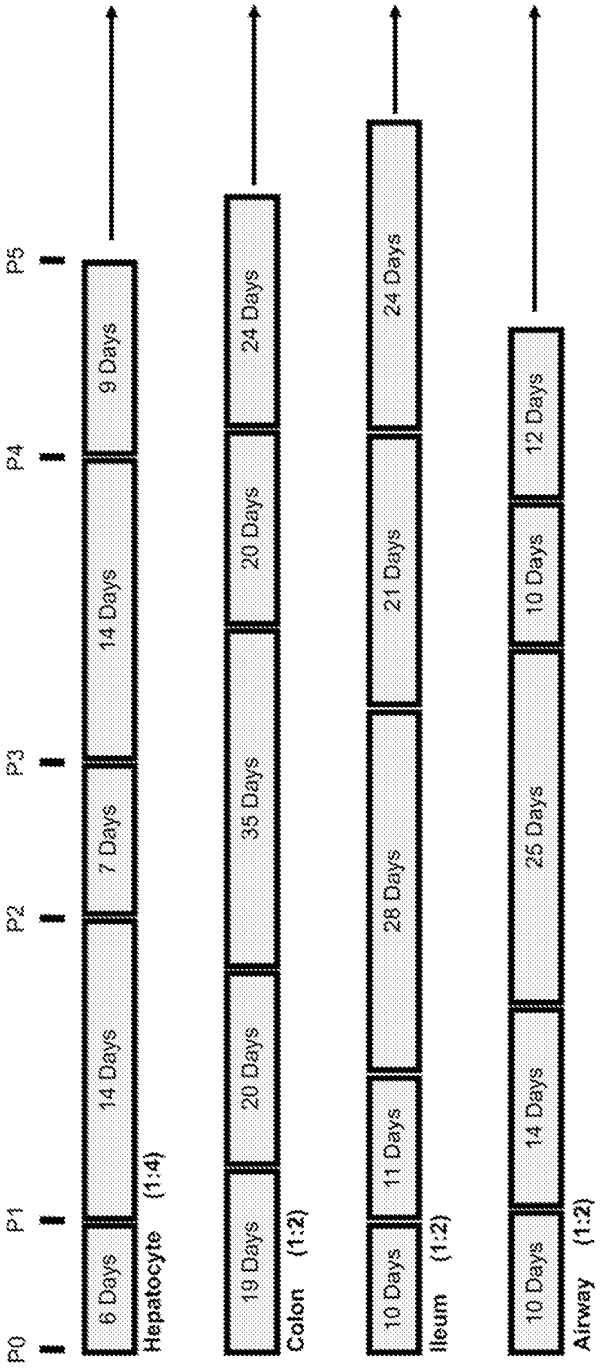

P0
P1
P2
P3
P4
P5
6 Days
14 Days
7 Days
14 Days
9 Days
Hepatocyte (1:4)
19 Days
20 Days
35 Days
20 Days
24 Days
Colon (1:2)
10 Days
11 Days
28 Days
21 Days
24 Days
Ileum (1:2)
10 Days
14 Days
25 Days
10 Days
12 Days
Airway (1:2)

Fig. 9

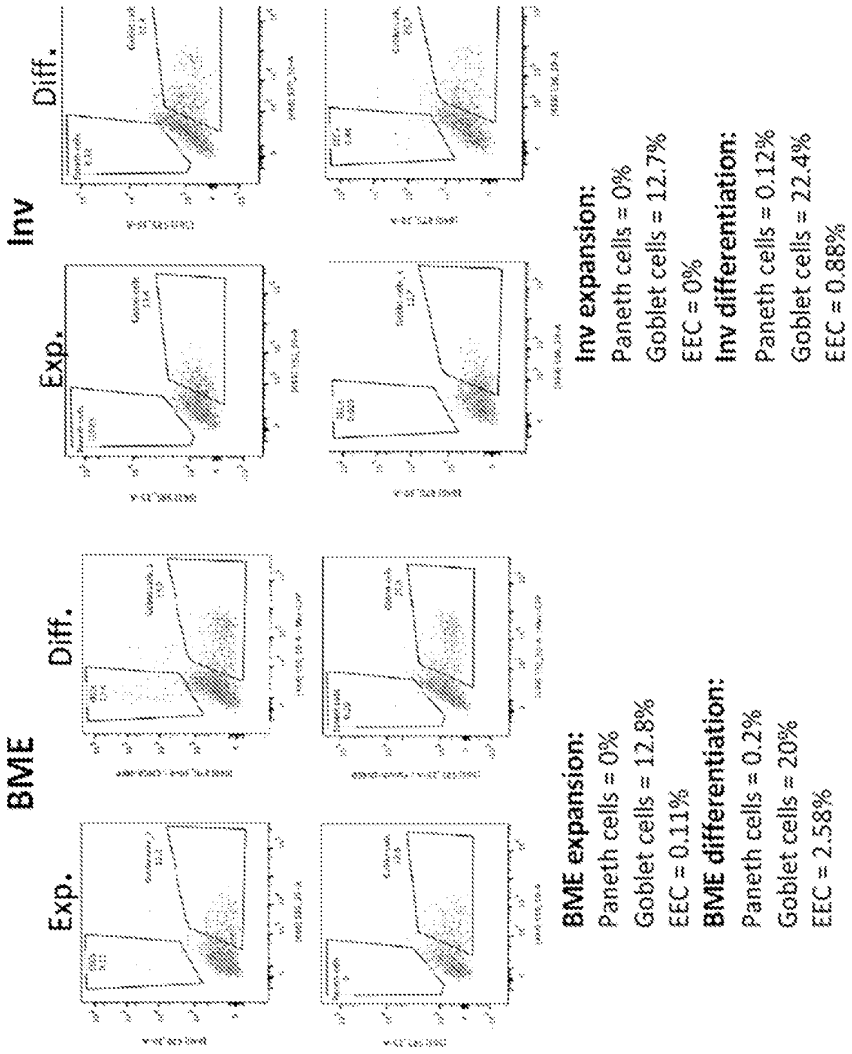

BME
Exp.
Diff.
Inv
Exp.
Diff.
BME expansion:
Paneth cells = 0%
Goblet cells = 12.8%
EEC = 0.11%
BME differentiation:
Paneth cells = 0.2%
Goblet cells = 20%
EEC = 2.58%
Inv expansion:
Paneth cells = 0%
Goblet cells = 12.7%
EEC = 0%
Inv differentiation:
Paneth cells = 0.12%
Goblet cells = 22.4%
EEC = 0.88%

Fig. 10

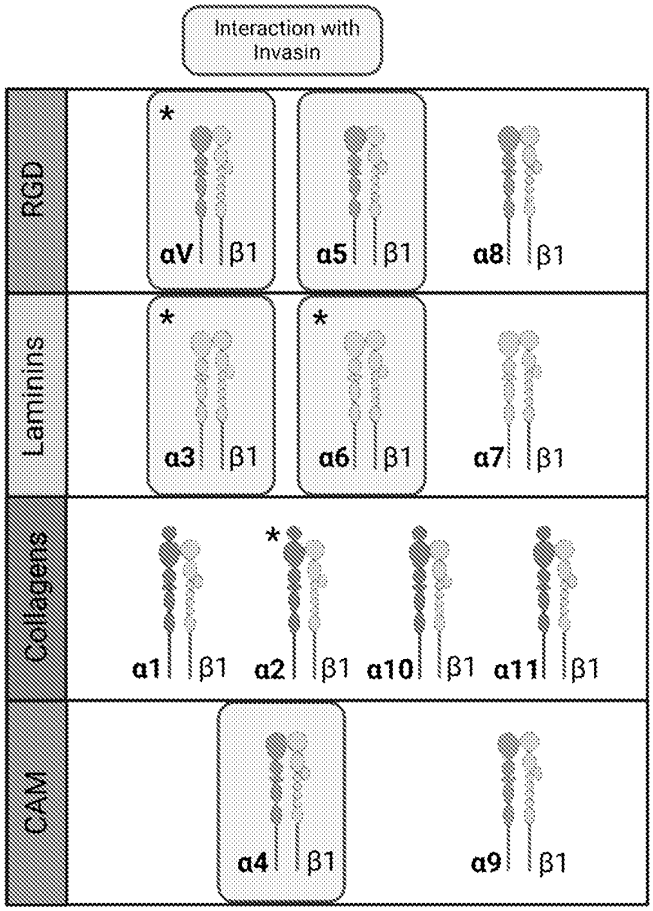

Interaction with Invasin
RGD
Laminins
Collagens
CAM
αV β1
α5 β1
α8 β1
α3 β1
α6 β1
α7 β1
α1 β1
α2 β1
α10 β1
α11 β1
α4 β1
α9 β1
*

Fig. 11



Fig. 12

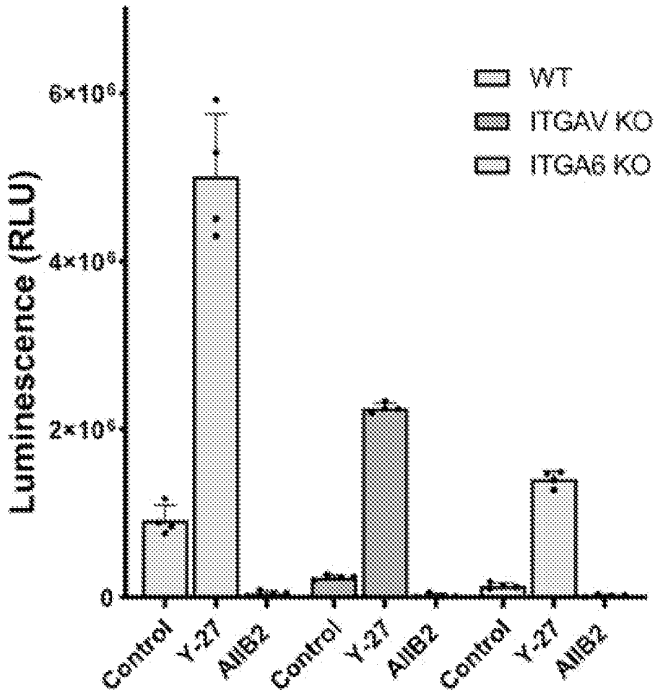

WT
ITGAV KO
ITGA6 KO
Luminescence (RLU)
6×10⁵
4×10⁵
2×10⁵
0
Control
Y-27
AIIB2
Control
Y-27
AIIB2
Control
Y-27
AIIB2

Fig. 13

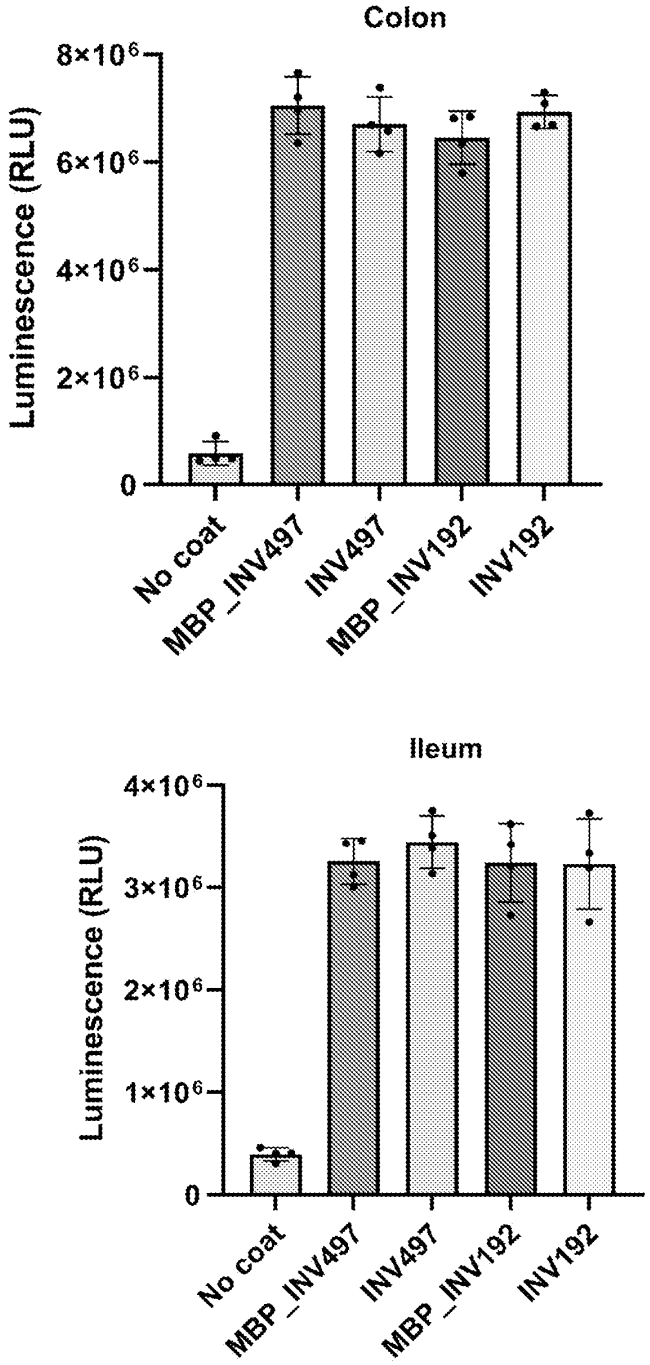
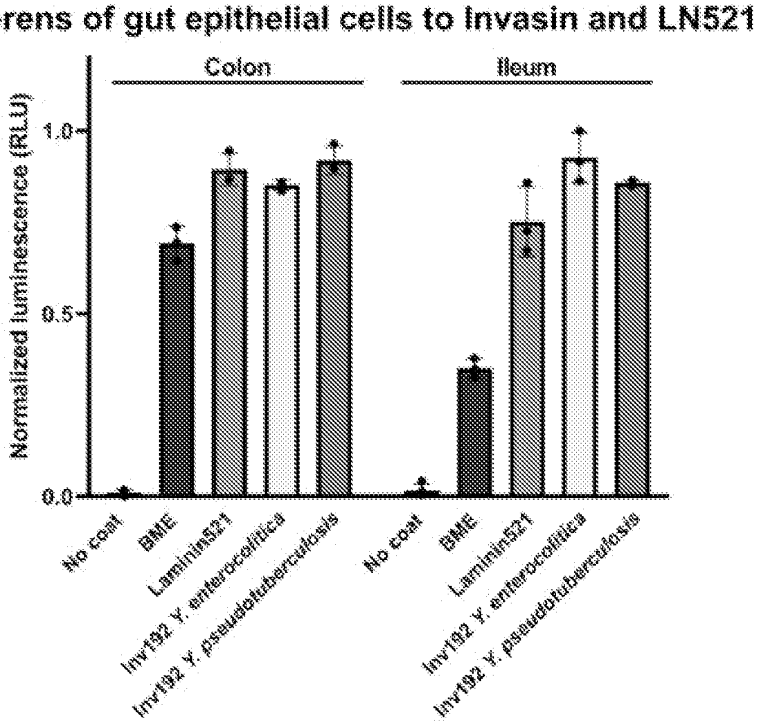
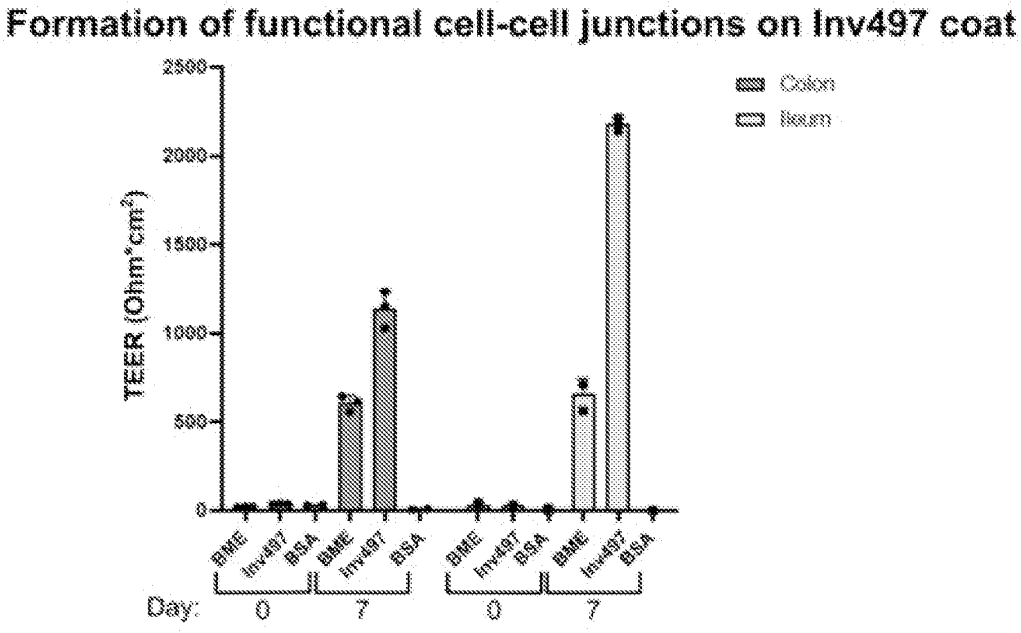

Colon
Luminescence (RLU)
8×10^6
6×10^6
4×10^6
2×10^6
0
No coat
MBP_INV497
INV497
MBP_INV192
INV192
Ileum
Luminescence (RLU)
4×10^6
3×10^6
2×10^6
1×10^6
0
No coat
MBP_INV497
INV497
MBP_INV192
INV192

Fig. 14

A


Adherens of gut epithelial cells to Invasin and LN521
Colon
Ileum
Normalized luminescence (RLU)
1.0
0.5
0.0
No coat
BME
Laminin521
Inv192 Y. enterocolitica
Inv192 Y. pseudotuberculosis
No coat
BME
Laminin521
Inv192 Y. enterocolitica
Inv192 Y. pseudotuberculosis

C


Formation of functional cell-cell junctions on Inv497 coat
Colon
Ileum
TEER (Ohm*cm²)
2500
2000
1500
1000
500
0
BME
Inv497
BSA
BME
Inv497
BSA
BME
Inv497
BSA
BME
Inv497
BSA
Day:
0
7
0
7

Fig, 14 – cont.

B

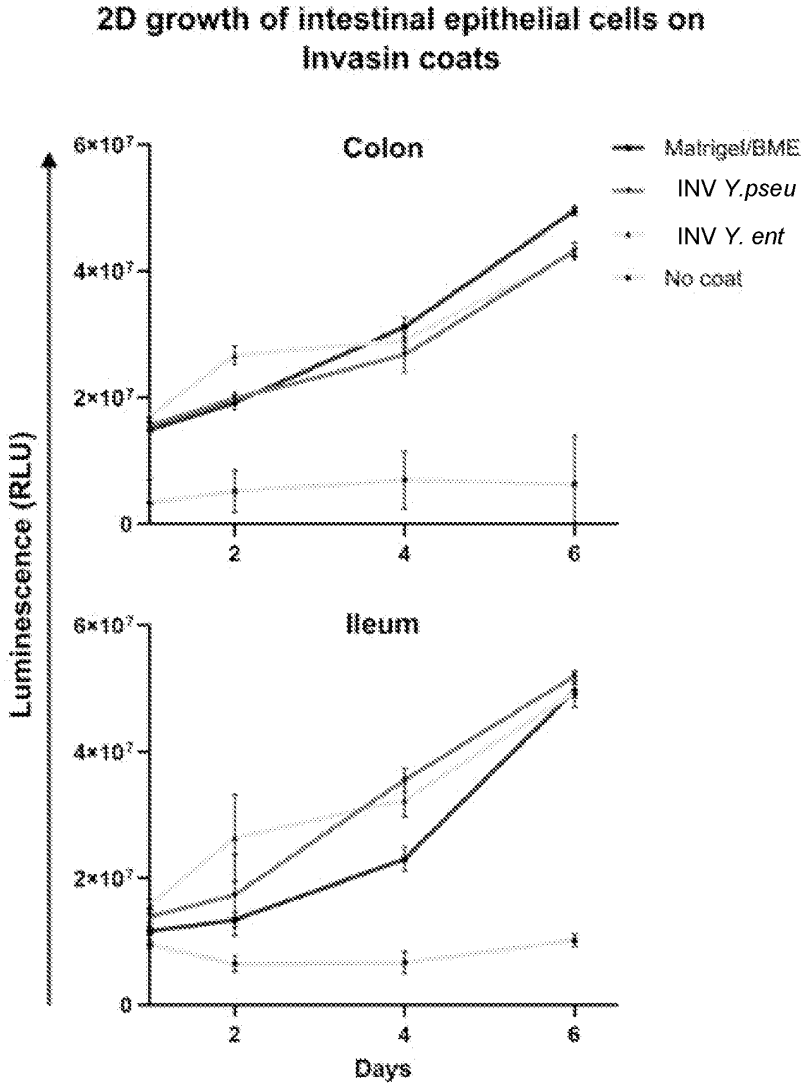
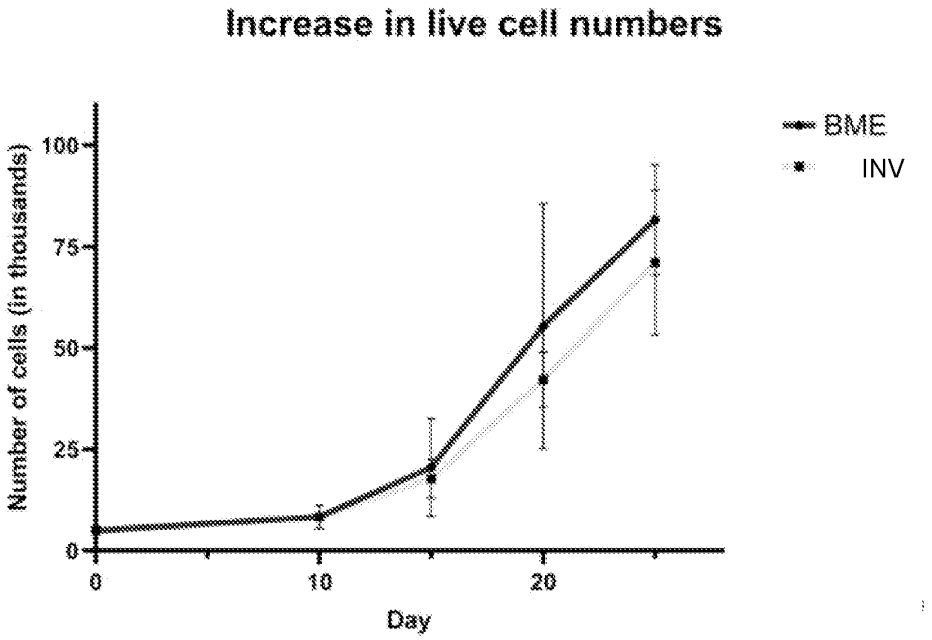

2D growth of intestinal epithelial cells on Invasin coats
Colon
Ileum
Matrigel/BME
INV Y.pseu
INV Y. ent
No coat
Luminescence (RLU)
6×10⁷
4×10⁷
2×10⁷
0
2
4
6
Days

Fig. 15

A


Increase in live cell numbers
Number of cells (in thousands)
100
75
50
25
0
0
10
20
Day
BME
INV

B

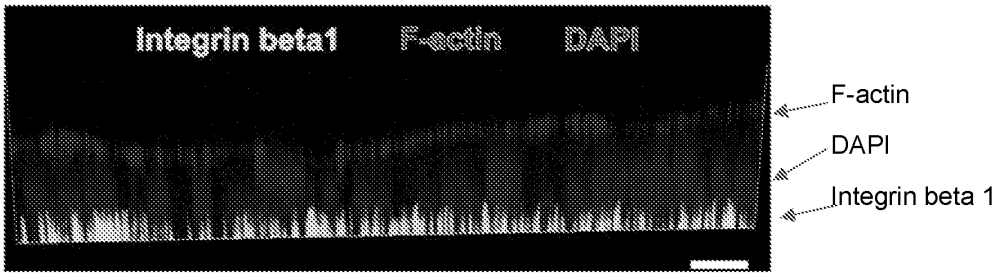
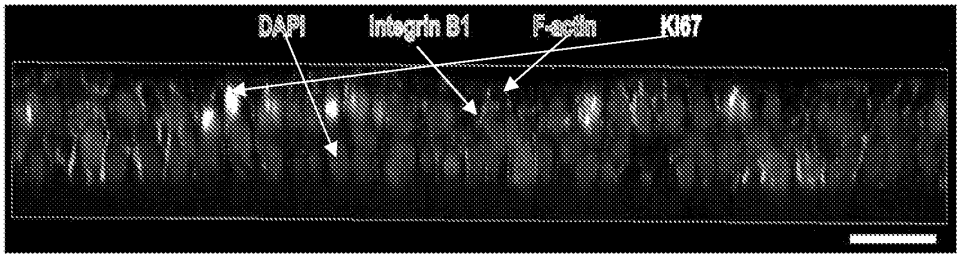
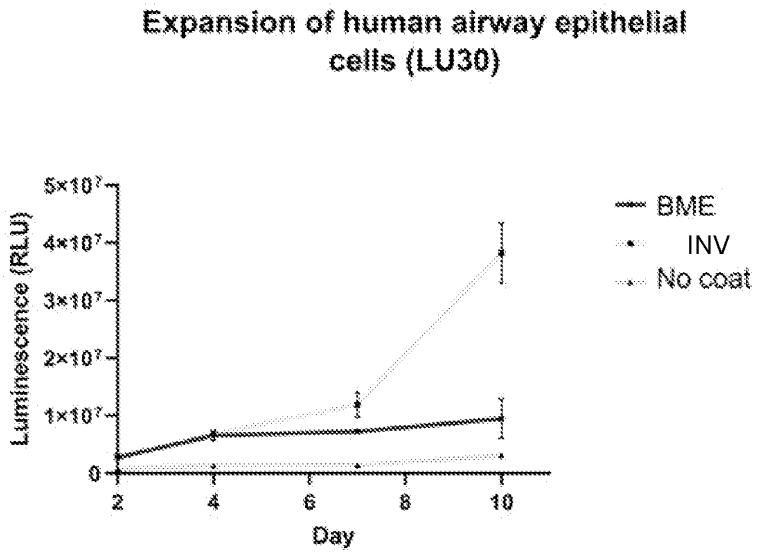

Integrin beta1
F-actin
DAPI
F-actin
DAPI
Integrin beta 1

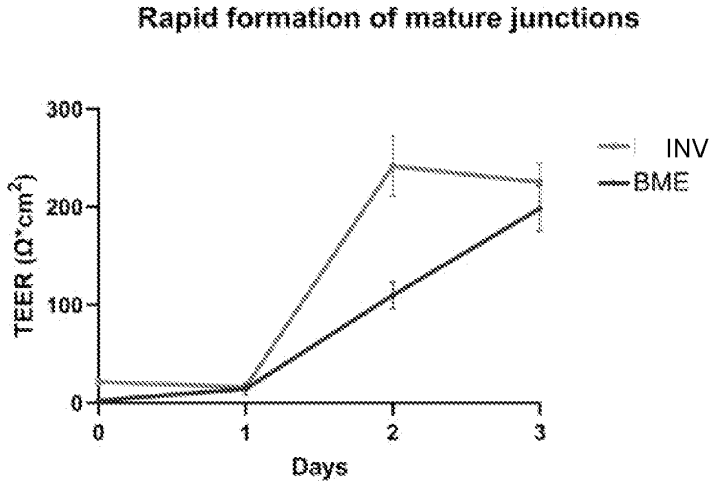
Fig. 16

A


DAPI
Integrin B1
F-actin
KI67

B


Expansion of human airway epithelial cells (LU30)
Luminescence (RLU)
5×10⁷
4×10⁷
3×10⁷
2×10⁷
1×10⁷
0
2
4
6
8
10
Day
BME
INV
No coat


Rapid formation of mature junctions
TEER (Ω*cm²)
300
200
100
0
0
1
2
3
Days
INV
BME

C

Fig. 17

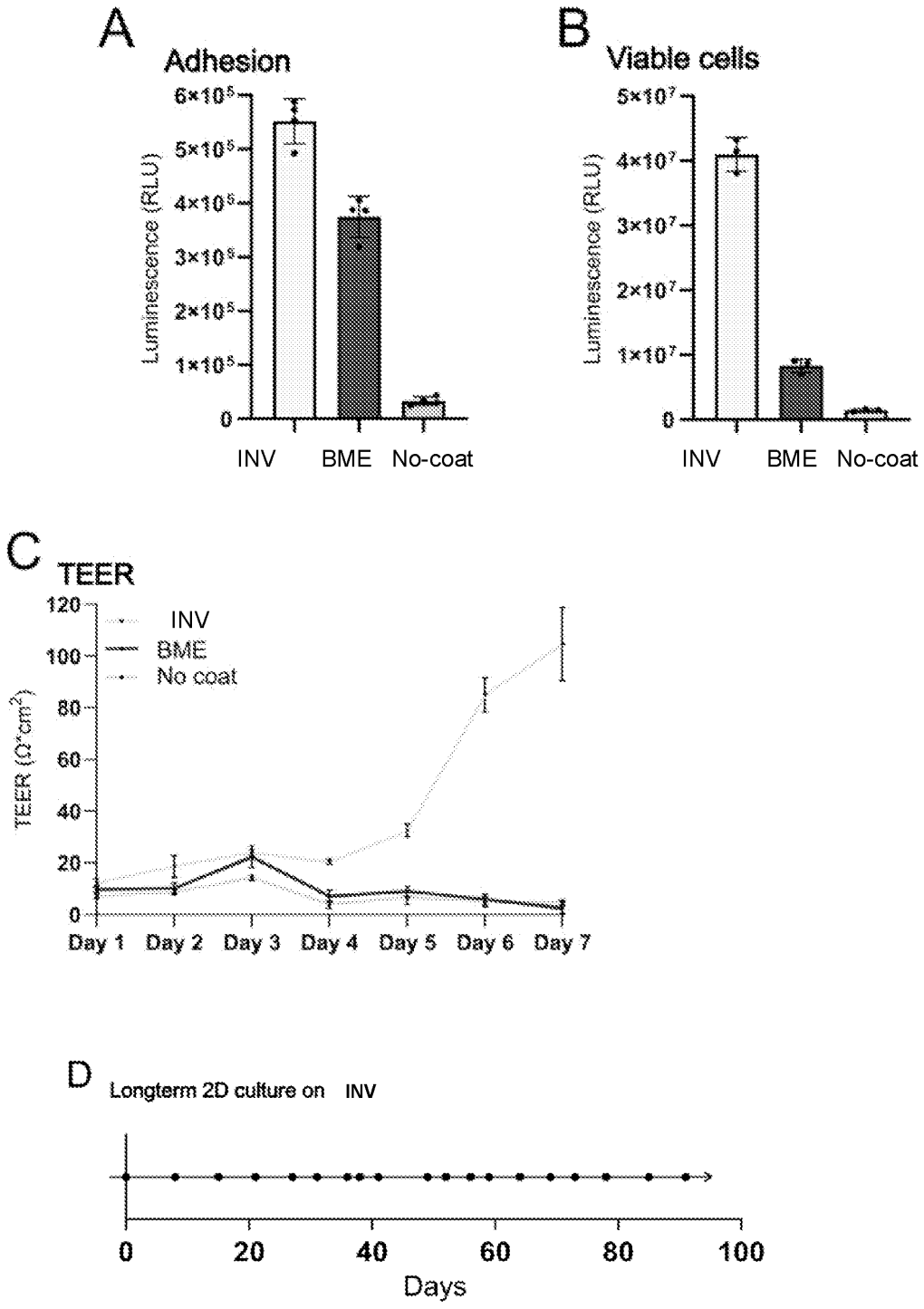

A
Adhesion
Luminescence (RLU)
6×10^5
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
INV
BME
No-coat
B
Viable cells
Luminescence (RLU)
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
INV
BME
No-coat
C
TEER
TEER (Ω*cm^2)
120
100
80
60
40
20
0
INV
BME
No coat
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
D
Longterm 2D culture on INV
0
20
40
60
80
100
Days

Fig. 18

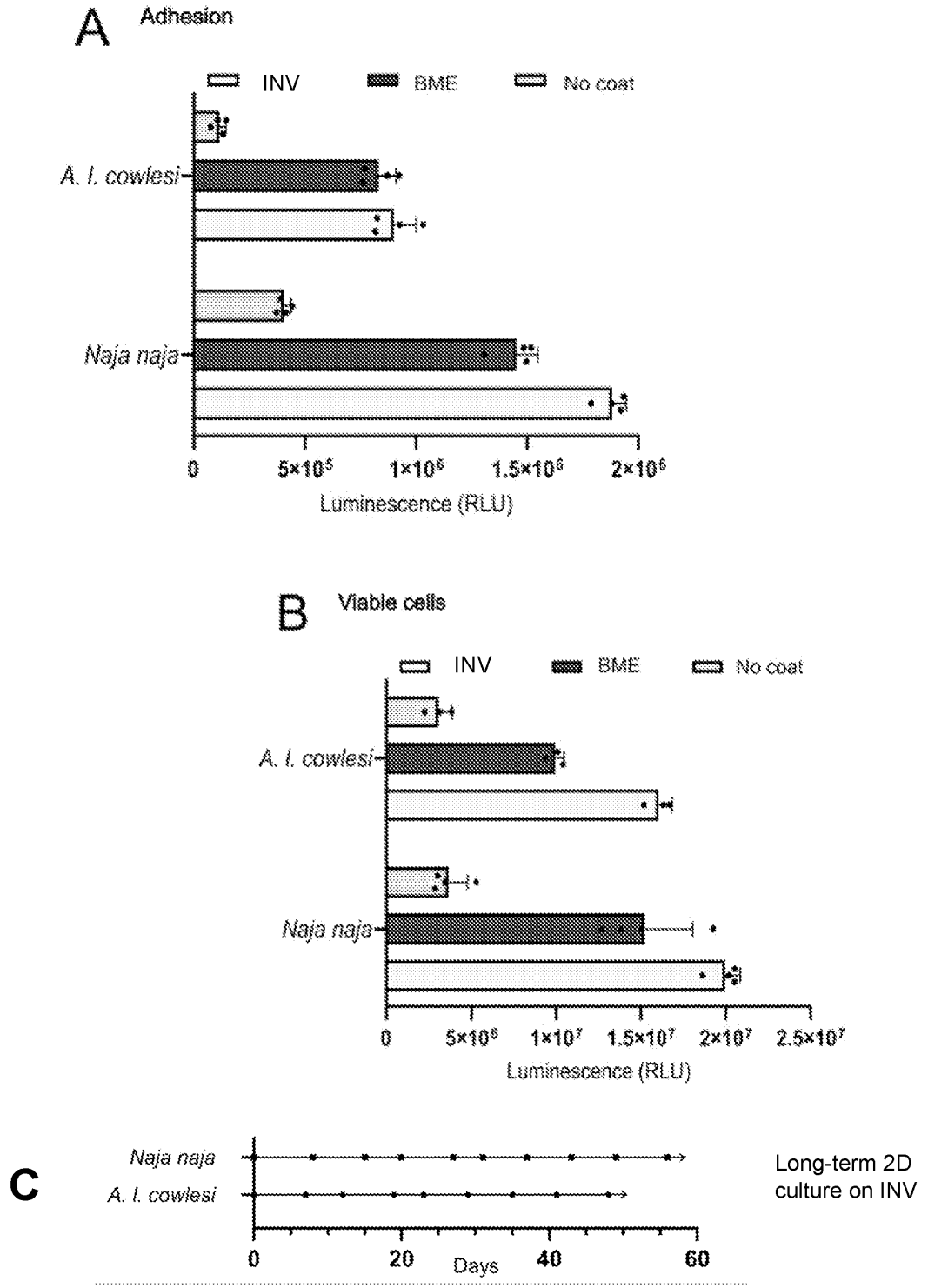
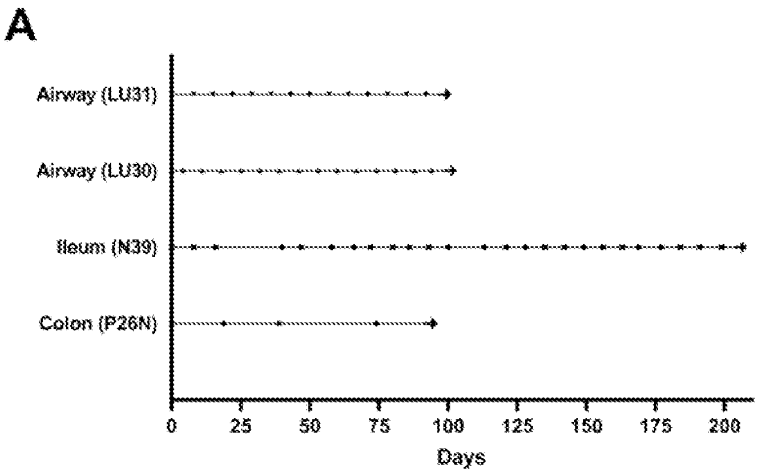
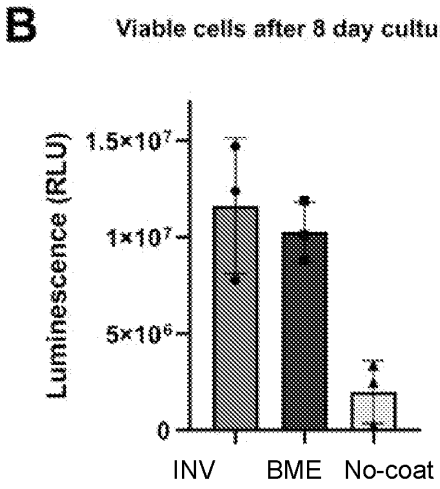

A
Adhesion
INV
BME
No coat
A. l. cowlesi
Naja naja
0
5×10⁵
1×10⁶
1.5×10⁶
2×10⁶
Luminescence (RLU)
B
Viable cells
INV
BME
No coat
A. l. cowlesi
Naja naja
0
5×10⁶
1×10⁷
1.5×10⁷
2×10⁷
2.5×10⁷
Luminescence (RLU)
C
Naja naja
A. l. cowlesi
0
20
Days
40
60
Long-term 2D culture on INV

Fig. 19


A
Airway (LU31)
Airway (LU30)
Ileum (N39)
Colon (P26N)
0
25
50
75
100
125
150
175
200
Days


B
Viable cells after 8 day culture
Luminescence (RLU)
1.5×10⁷
1×10⁷
5×10⁶
0
INV
BME
No-coat

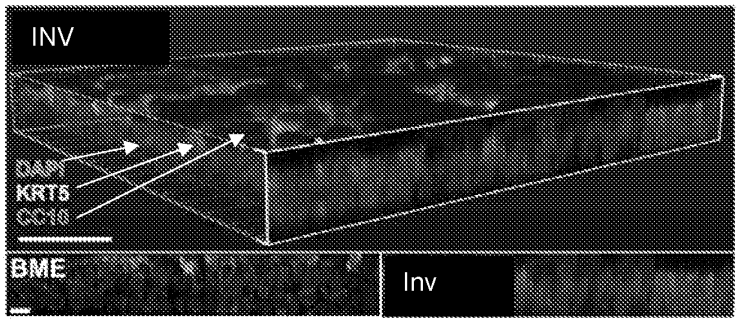
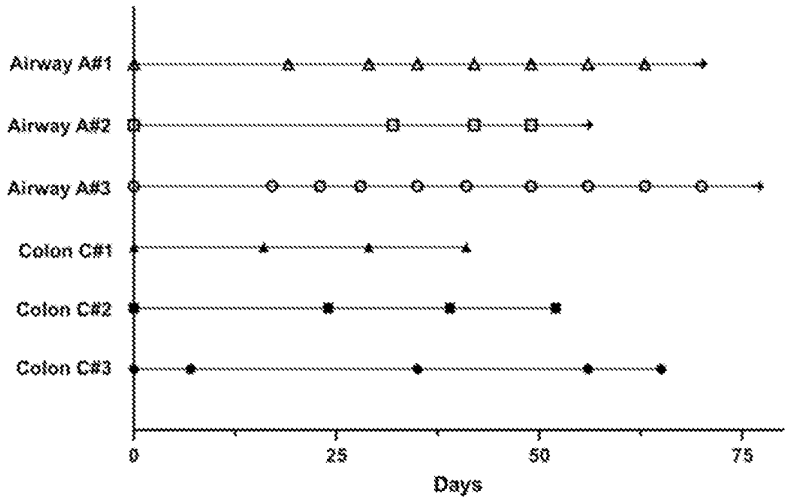

C
Freshly isolated, priary airway epithelial cells grown on INV or BME
INV
DAPI
KRT5
CC10
BME
Inv

Fig. 19 – cont.

D

2D cultures on INV of airway and colon epithelial cells started from freshly isolated tissue biopsies



¶

Fig. 20

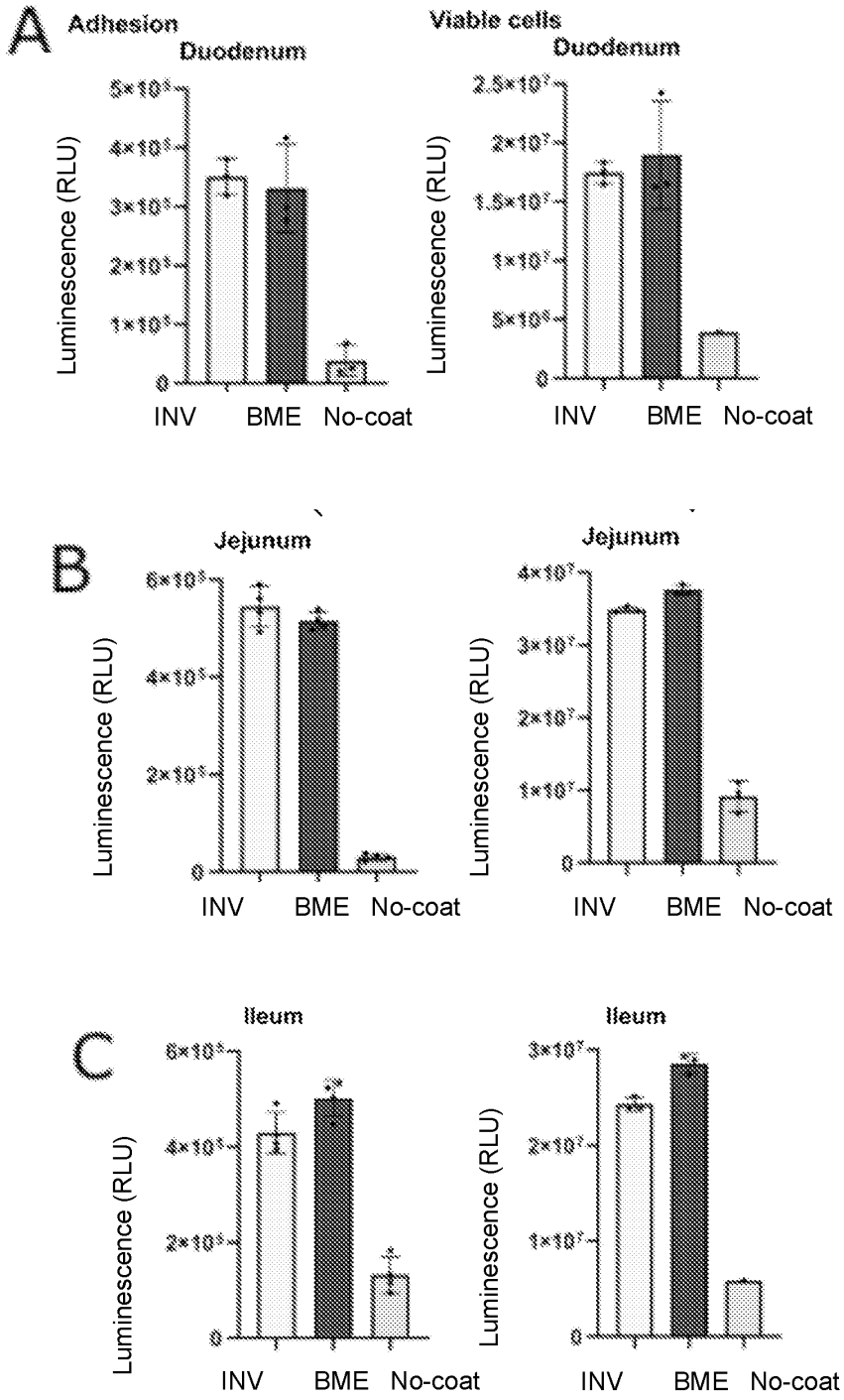

A
Adhesion
Duodenum
Luminescence (RLU)
INV
BME
No-coat
Viable cells
Duodenum
Luminescence (RLU)
INV
BME
No-coat
B
Jejunum
Luminescence (RLU)
INV
BME
No-coat
Jejunum
Luminescence (RLU)
INV
BME
No-coat
C
Ileum
Luminescence (RLU)
INV
BME
No-coat
Ileum
Luminescence (RLU)
INV
BME
No-coat

Fig. 21

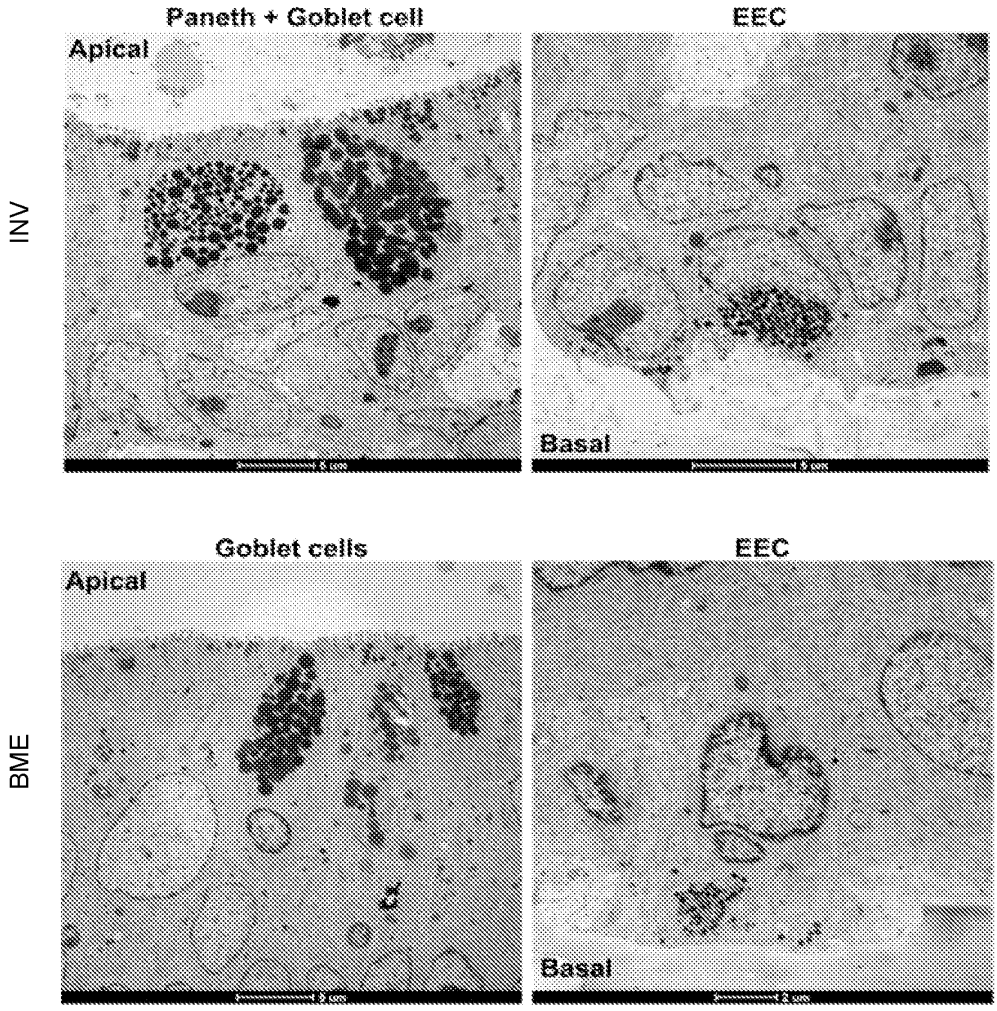

Paneth + Goblet cell
EEC
Apical
INV
Basal
Goblet cells
EEC
Apical
BME
Basal

Fig. 22

INV BME

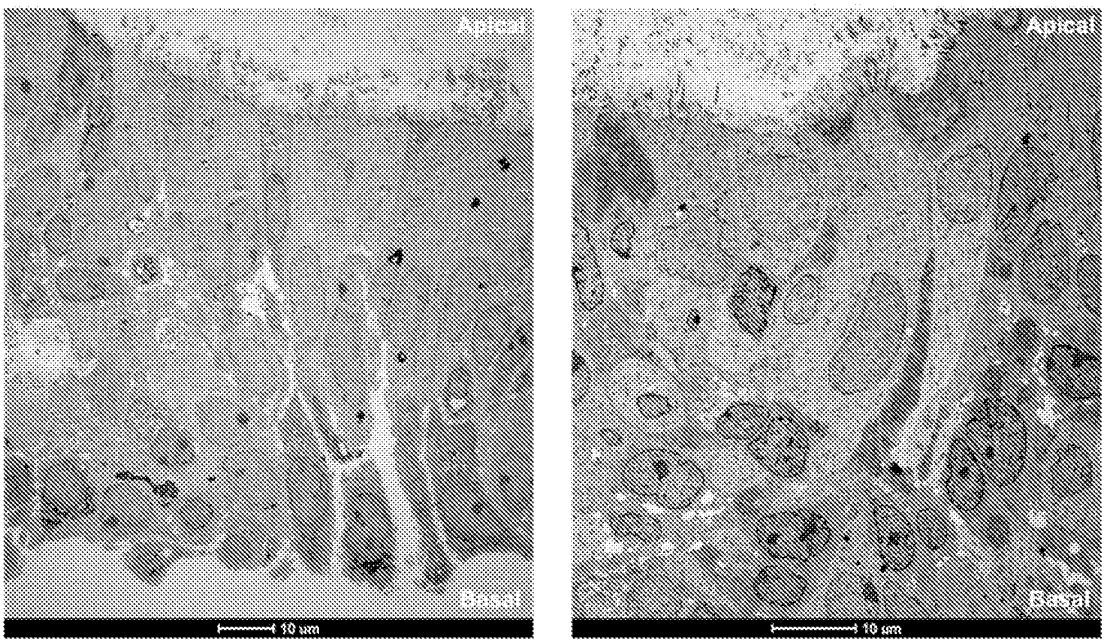
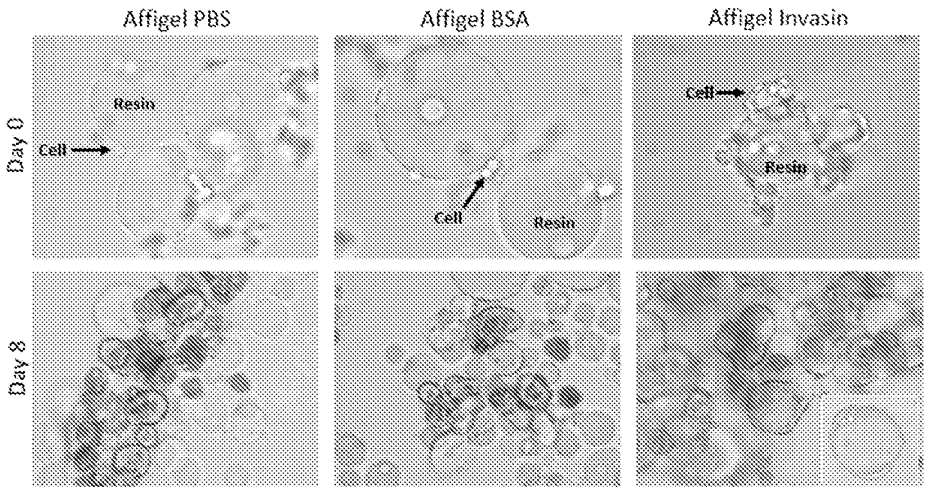


Fig. 23

A


Affigel PBS
Affigel BSA
Affigel Invasin
Day 0
Day 8
Resin
Cell
Cell
Resin
Cell
Resin

B

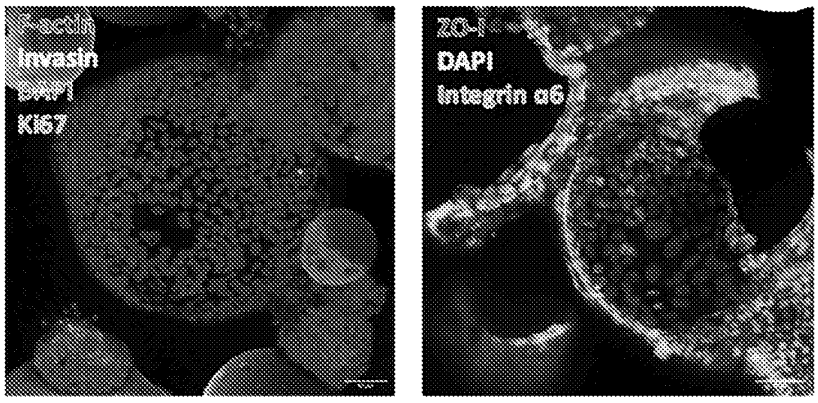

Invasin
DAPI
Ki67
DAPI
Integrin α6

Fig. 24

A

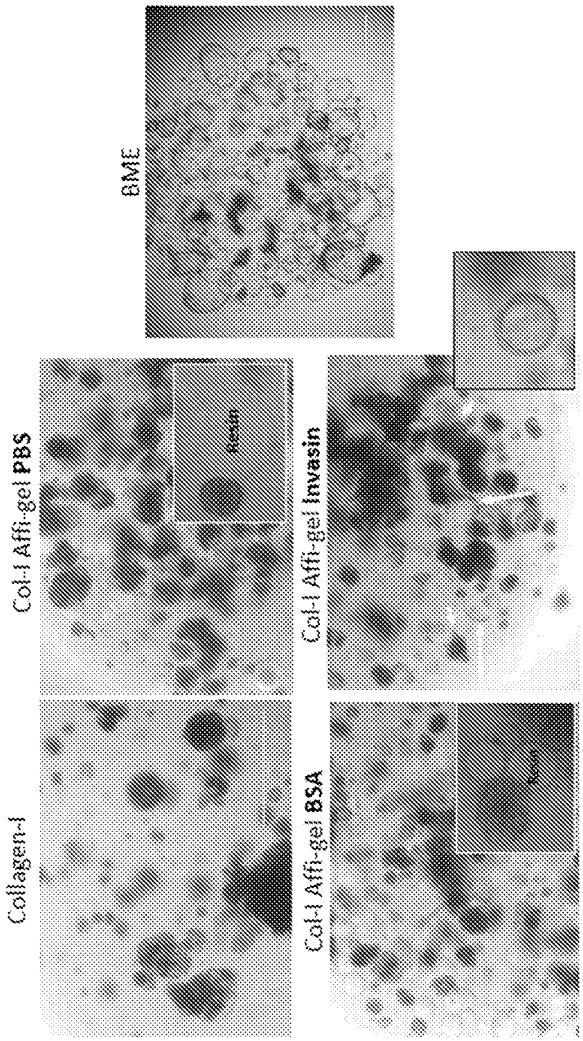

BME
Col-I Affi-gel PBS
Col-I Affi-gel Invasin
Collagen-I
Col-I Affi-gel BSA

Fig. 24 – cont.

B

