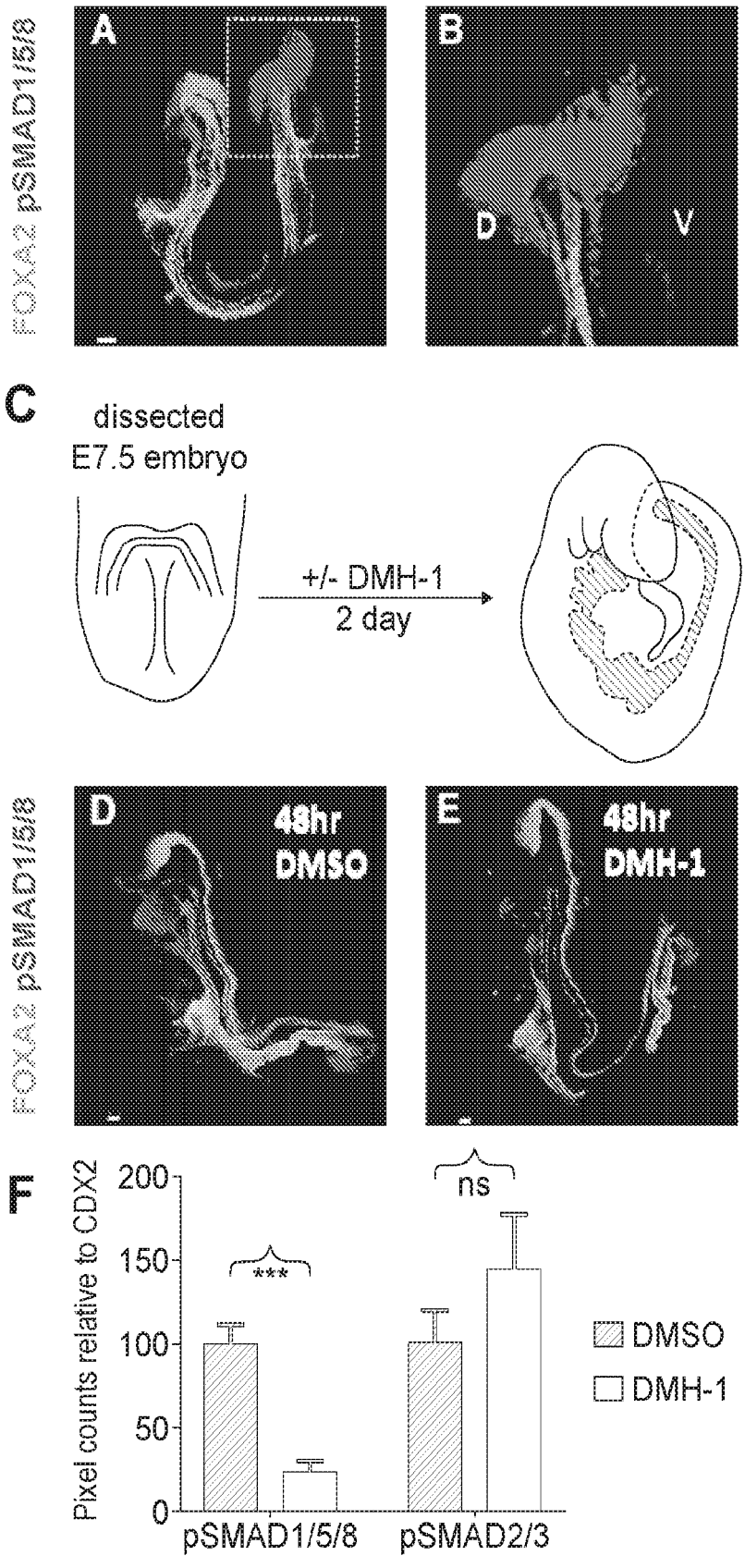

FOXA2 pSMAD1/5/8
A
B
D
V
C
dissected
E7.5 embryo
+/- DMH-1
2 day
FOXA2 pSMAD1/5/8
D
48hr
DMSO
E
48hr
DMH-1
F
Pixel counts relative to CDX2
200
150
100
50
0
***
ns
pSMAD1/5/8
pSMAD2/3
DMSO
DMH-1

FIG. 1A

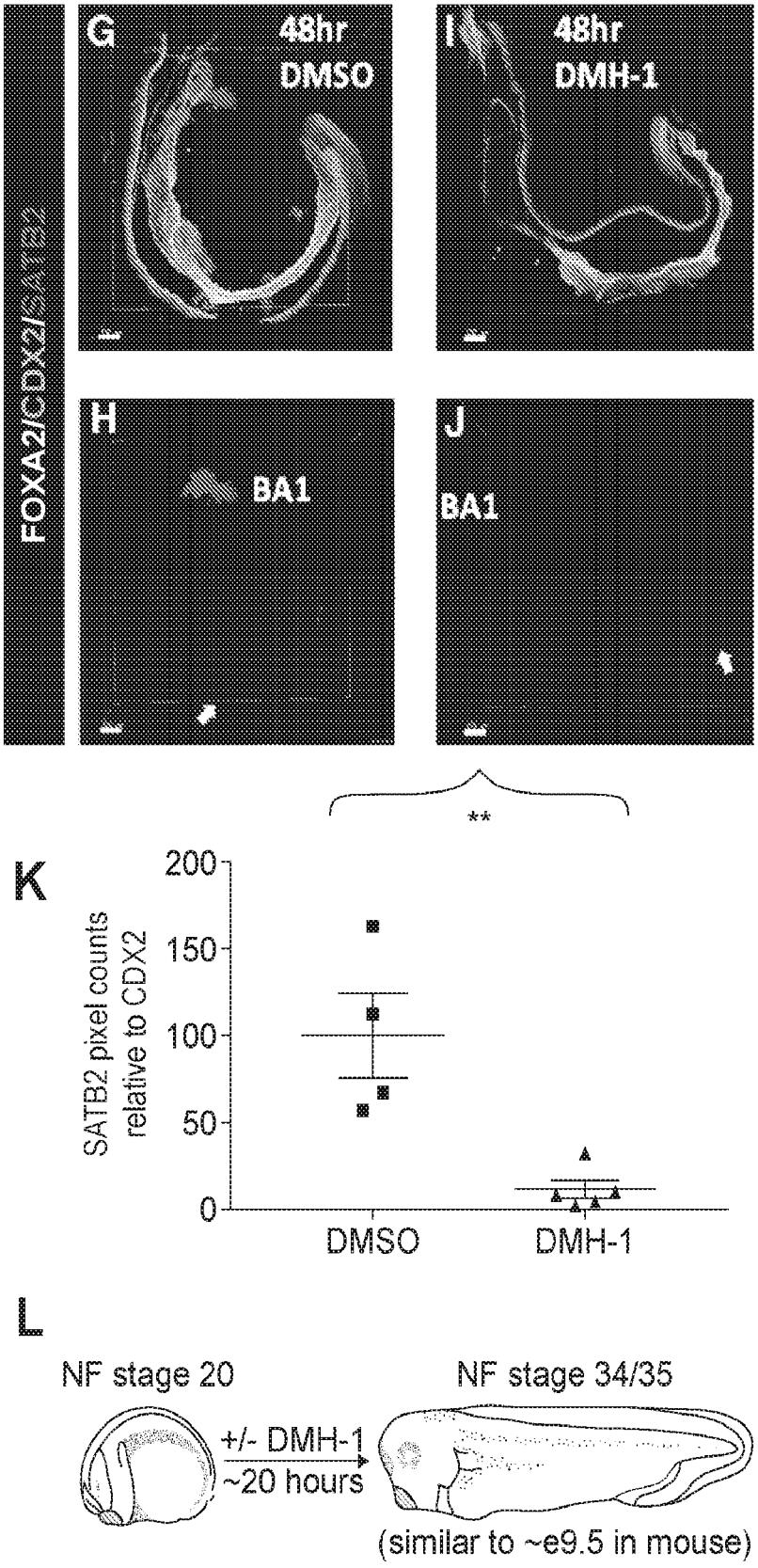

FOXA2/CDX2/SATB2
G
48hr
DMSO
I
48hr
DMH-1
H
BA1
J
BA1
**
K
SATB2 pixel counts relative to CDX2
200
150
100
50
0
DMSO
DMH-1
L
NF stage 20
NF stage 34/35
+/- DMH-1
~20 hours
(similar to ~e9.5 in mouse)

FIG. 1B

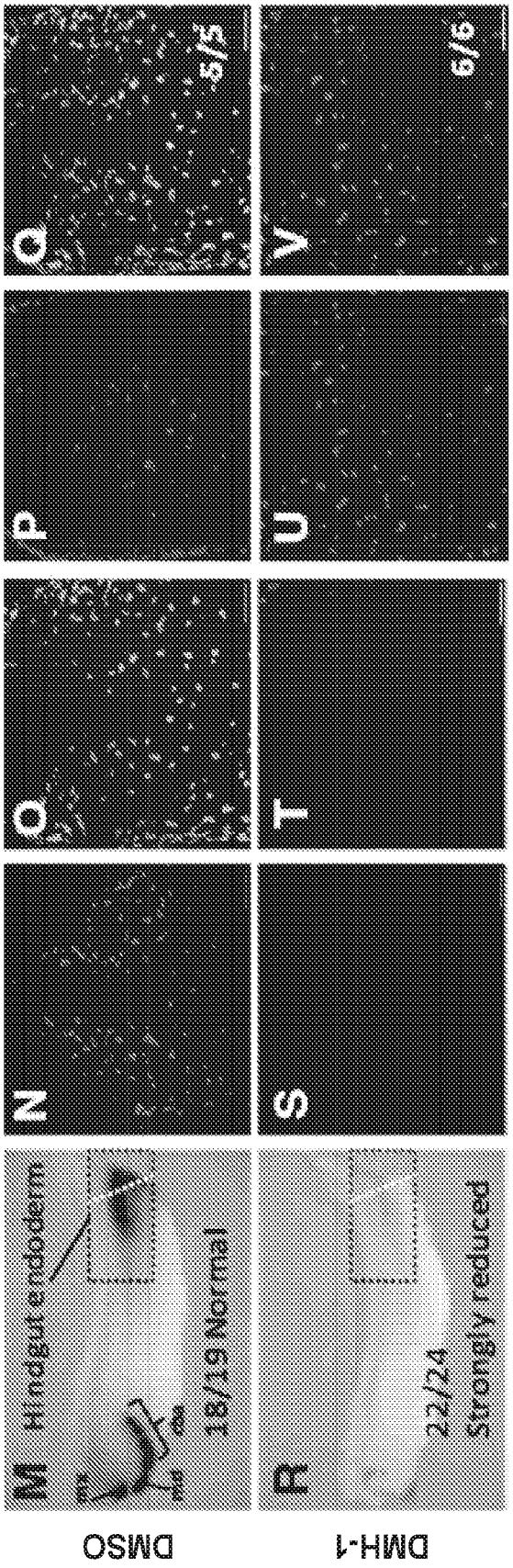

satb2 in situ
Satb2 IF
pSmad1/5/8
DAPI
Merge
DMSO
DMH-1
M
Hindgut endoderm
18/19 Normal
N
O
P
Q
5/5
R
22/24
Strongly reduced
S
T
U
V
6/6

FIG. 1C

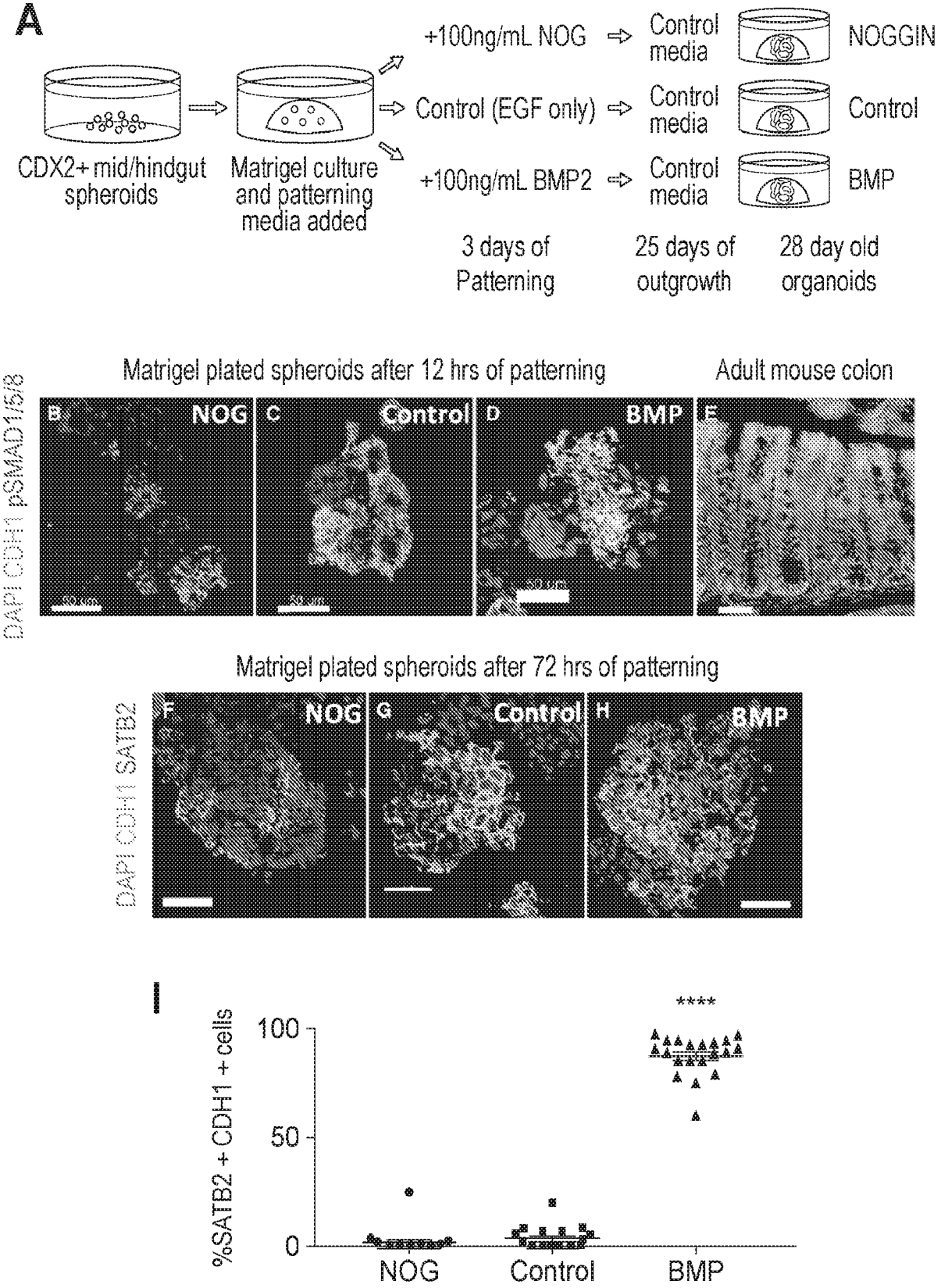
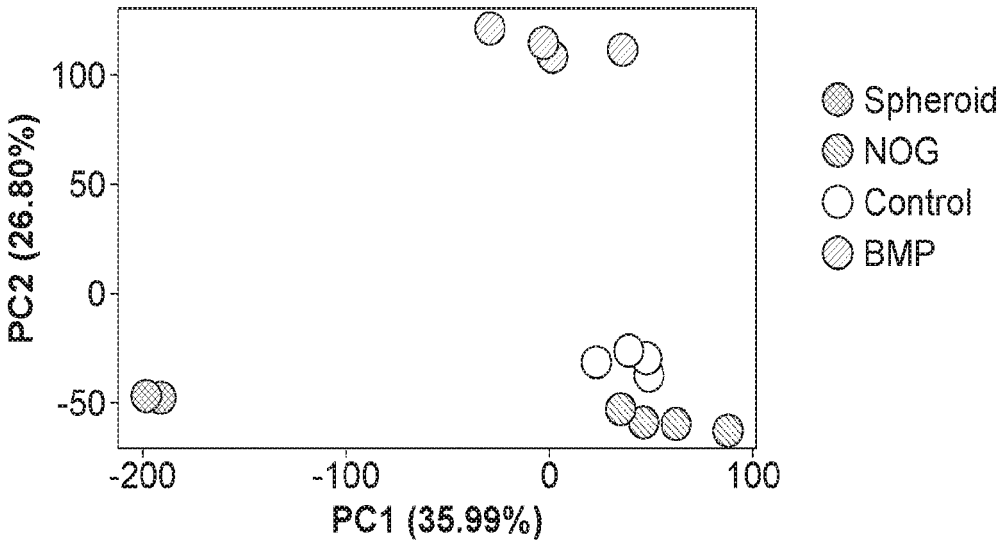

A
CDX2+ mid/hindgut spheroids
Matrigel culture and patterning media added
+100ng/mL NOG
Control (EGF only)
+100ng/mL BMP2
Control media
Control media
Control media
NOGGIN
Control
BMP
3 days of Patterning
25 days of outgrowth
28 day old organoids
Matrigel plated spheroids after 12 hrs of patterning
Adult mouse colon
DAPI CDH1 pSMAD1/5/8
B NOG
C Control
D BMP
E
Matrigel plated spheroids after 72 hrs of patterning
DAPI CDH1 SATB2
F NOG
G Control
H BMP
I
%SATB2 + CDH1 + cells
100
50
0
****
NOG
Control
BMP

FIG. 2A

J




K

**Gene ontology BMP vs NOG**

| Category | Name | p-value |
|---|---|---|
| GO: Cellular Component | intrinsic component of plasma membrane | 4.87E-38 |
| GO: Cellular Component | integral component of plasma membrane | 4.34E-36 |
| GO: Cellular Component | extracellular matrix | 5.08E-36 |
| GO: Biological Process | animal organ morphogenesis | 4.86E-34 |
| GO: Biological Process | cell-cell signaling | 6.46E-33 |
| GO: Biological Process | regulation of multicellular oranismal development | 1.89E-31 |
| GO: Biological Process | *pattern specification process* | 3.43E-30 |
| GO: Biological Process | *cellular response to BMP* | 8.62E-14 |

L

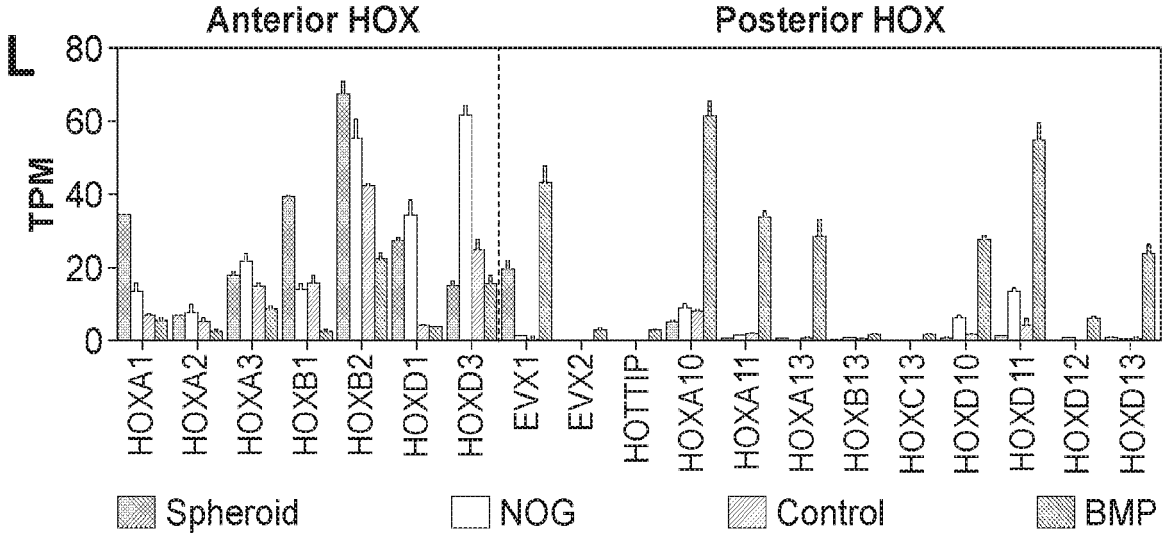



FIG. 2B

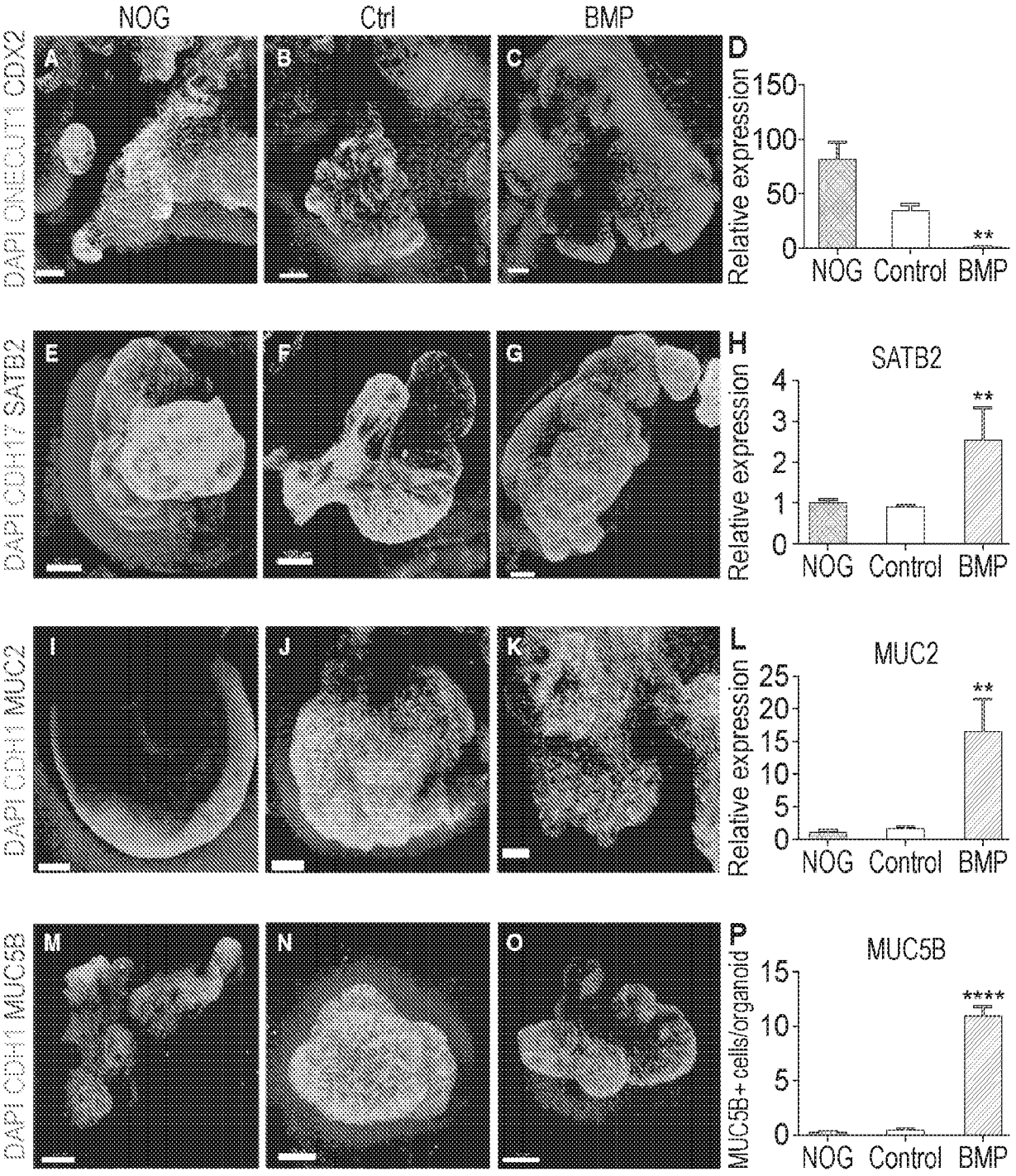

NOG
Ctrl
BMP
DAPI ONECUT1 CDX2
A
B
C
D
Relative expression
150
100
50
0
**
NOG Control BMP
DAPI CDH17 SATB2
E
F
G
H
SATB2
4
3
2
1
0
**
Relative expression
NOG Control BMP
DAPI CDH1 MUC2
I
J
K
L
MUC2
25
20
15
10
5
0
**
Relative expression
NOG Control BMP
DAPI CDH1 MUC5B
M
N
O
P
MUC5B
15
10
5
0
****
MUC5B+ cells/organoid
NOG Control BMP

FIG. 3A

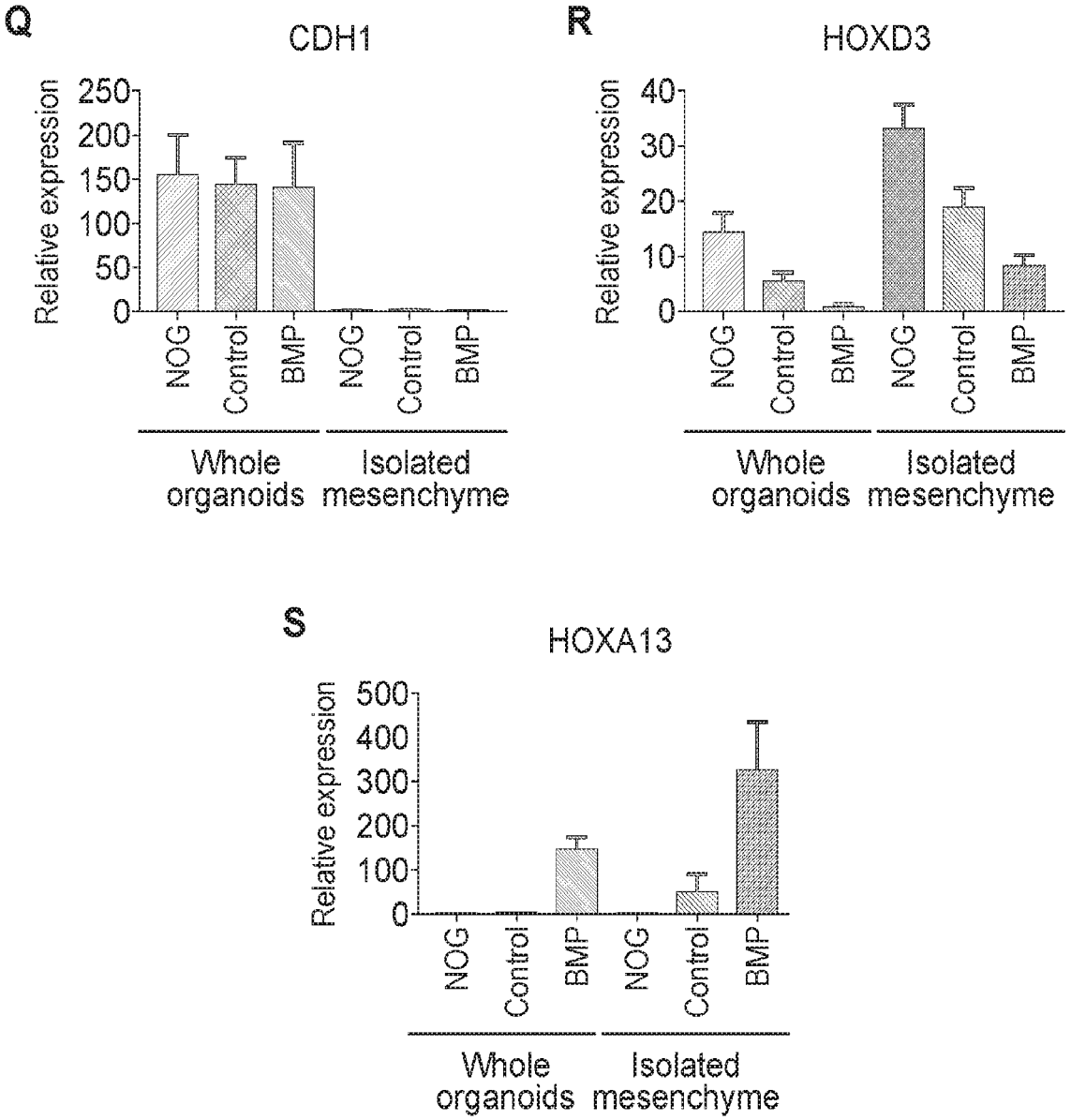

Q
CDH1
Relative expression
250
200
150
100
50
0
NOG
Control
BMP
NOG
Control
BMP
Whole organoids
Isolated mesenchyme
R
HOXD3
Relative expression
40
30
20
10
0
NOG
Control
BMP
NOG
Control
BMP
Whole organoids
Isolated mesenchyme
S
HOXA13
Relative expression
500
400
300
200
100
0
NOG
Control
BMP
NOG
Control
BMP
Whole organoids
Isolated mesenchyme

FIG. 3B

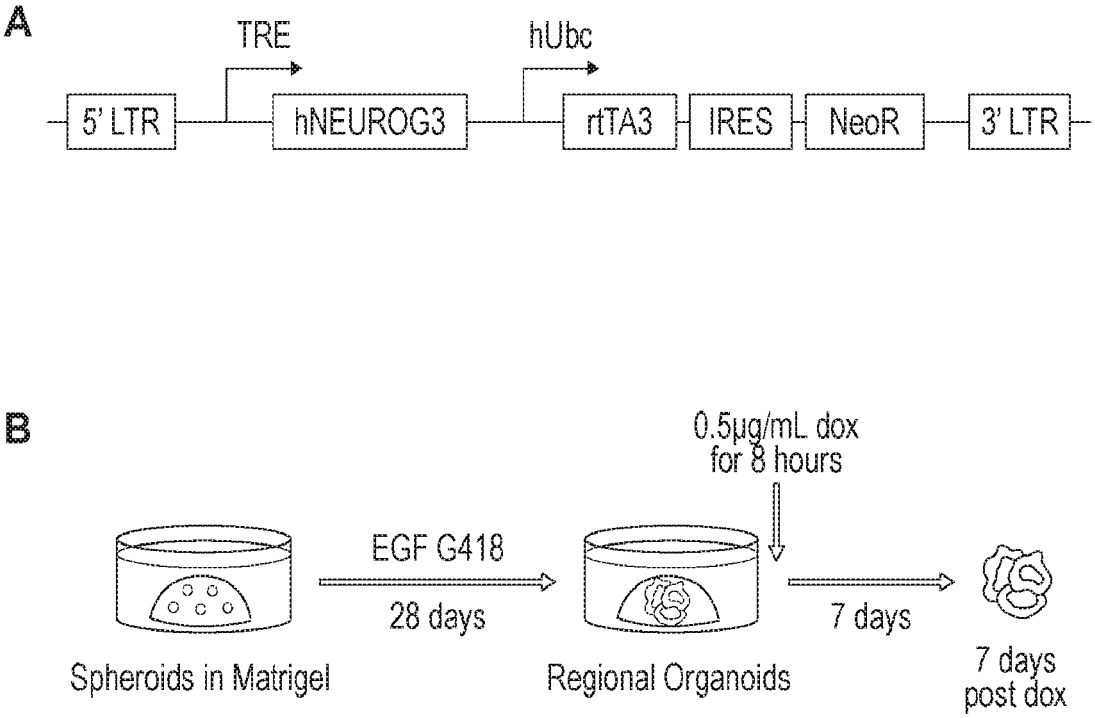

A
TRE
hUbc
5' LTR
hNEUROG3
rtTA3
IRES
NeoR
3' LTR
B
0.5µg/mL dox
for 8 hours
EGF G418
28 days
7 days
Spheroids in Matrigel
Regional Organoids
7 days
post dox
NOG
Control
BMP
DAPI/CHGA/CDX2/INSL5
-DOX
+DOX
C
D
E
F
G
H

FIG. 4A

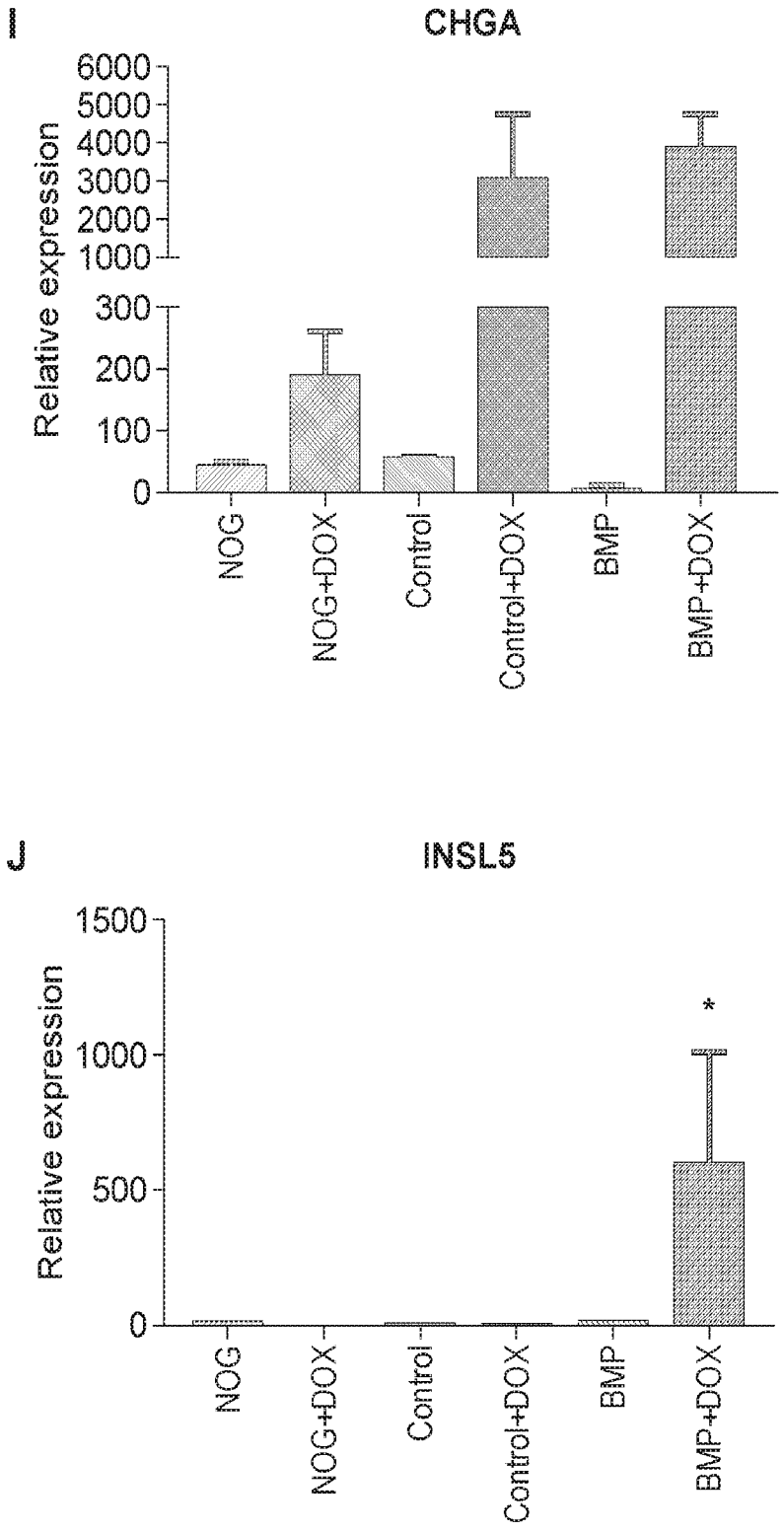

I
CHGA
Relative expression
6000
5000
4000
3000
2000
1000
300
200
100
0
NOG
NOG+DOX
Control
Control+DOX
BMP
BMP+DOX
J
INSL5
Relative expression
1500
1000
500
0
*
NOG
NOG+DOX
Control
Control+DOX
BMP
BMP+DOX

FIG. 4B

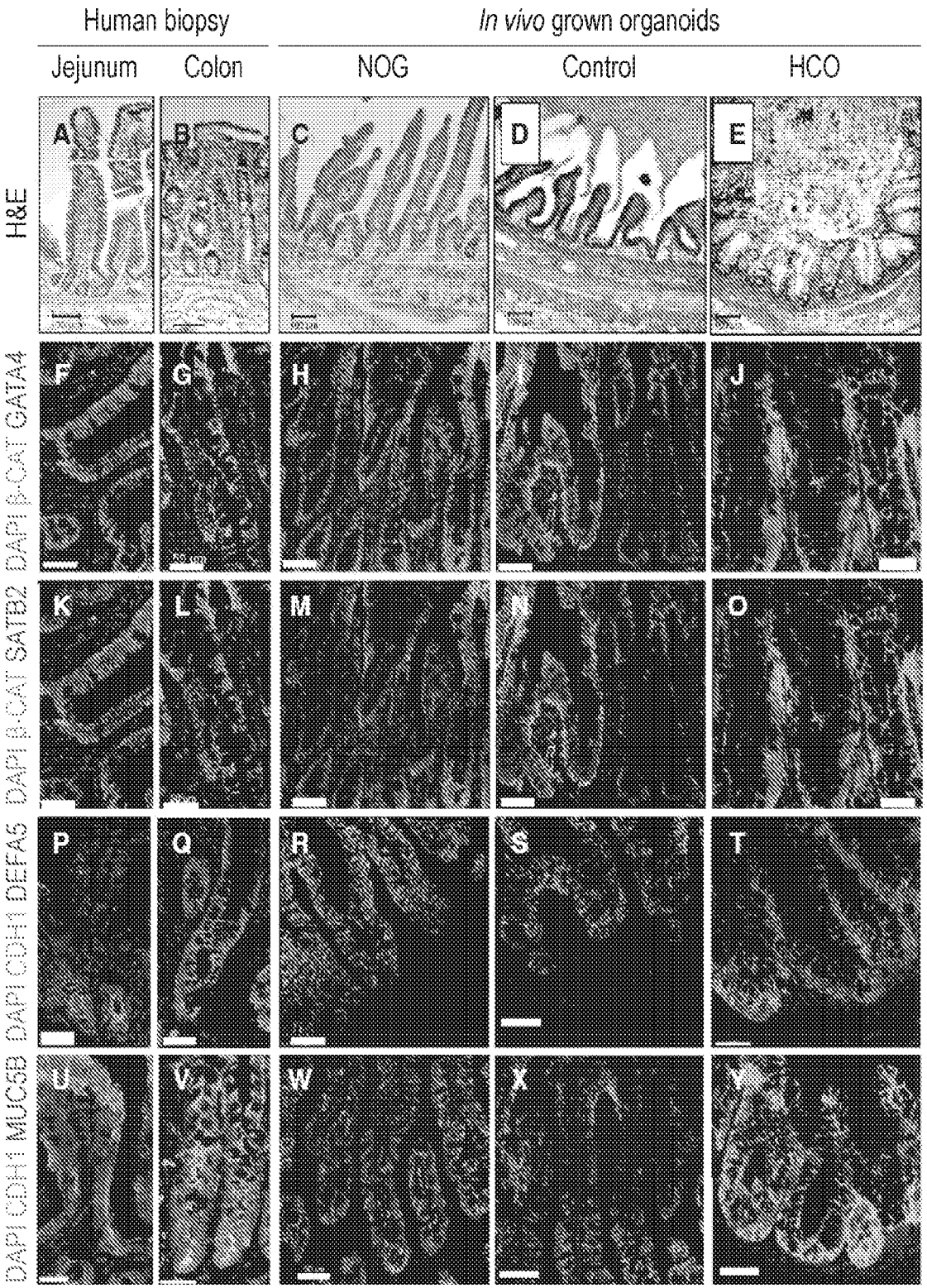

Human biopsy
In vivo grown organoids
Jejunum
Colon
NOG
Control
HCO
H&E
DAPI β-CAT GATA4
DAPI β-CAT SATB2
DAPI CDH1 DEFA5
DAPI CDH1 MUC5B
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y

FIG. 5

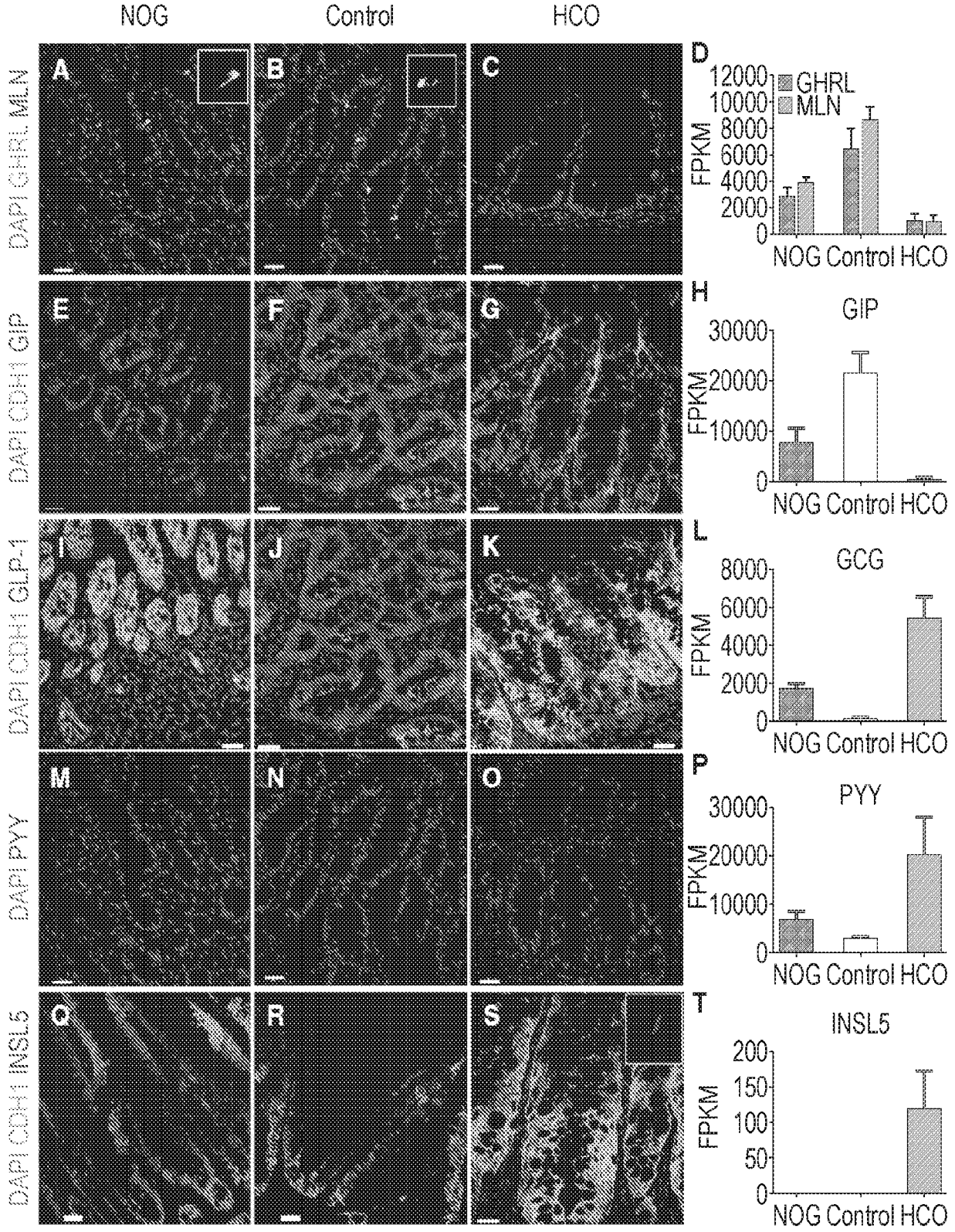
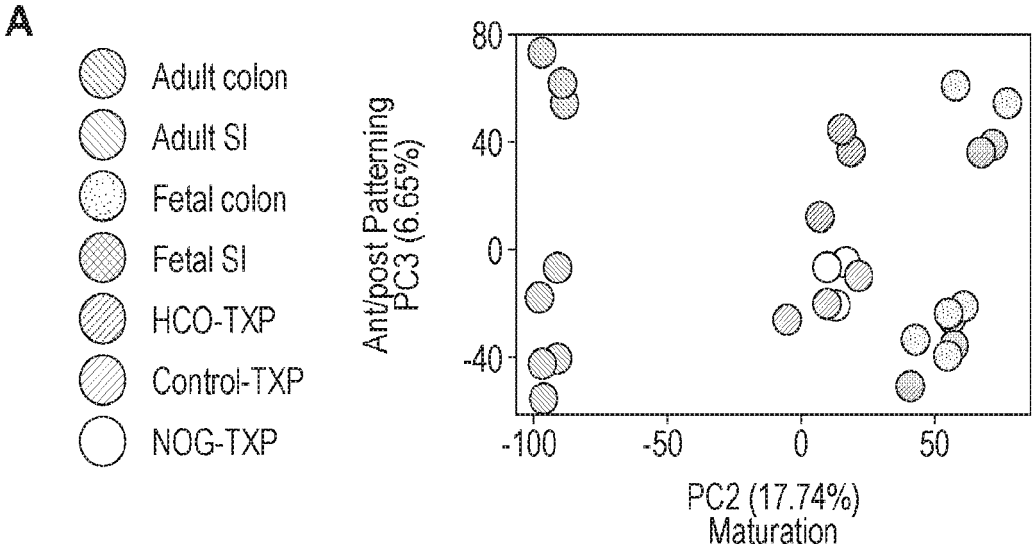

NOG
Control
HCO
DAPI GHRL MLN
DAPI CDH1 GIP
DAPI CDH1 GLP-1
DAPI PYY
DAPI CDH1 INSL5
A
B
C
D
GHRL
MLN
FPKM
12000
10000
8000
6000
4000
2000
0
NOG Control HCO
E
F
G
H
GIP
30000
20000
10000
I
J
K
L
GCG
M
N
O
P
PYY
Q
R
S
T
INSL5
200
150
100
50

FIG. 6


A
Adult colon
Adult SI
Fetal colon
Fetal SI
HCO-TXP
Control-TXP
NOG-TXP
Ant/post Patterning
PC3 (6.65%)
80
40
0
-40
-100
-50
0
50
PC2 (17.74%)
Maturation

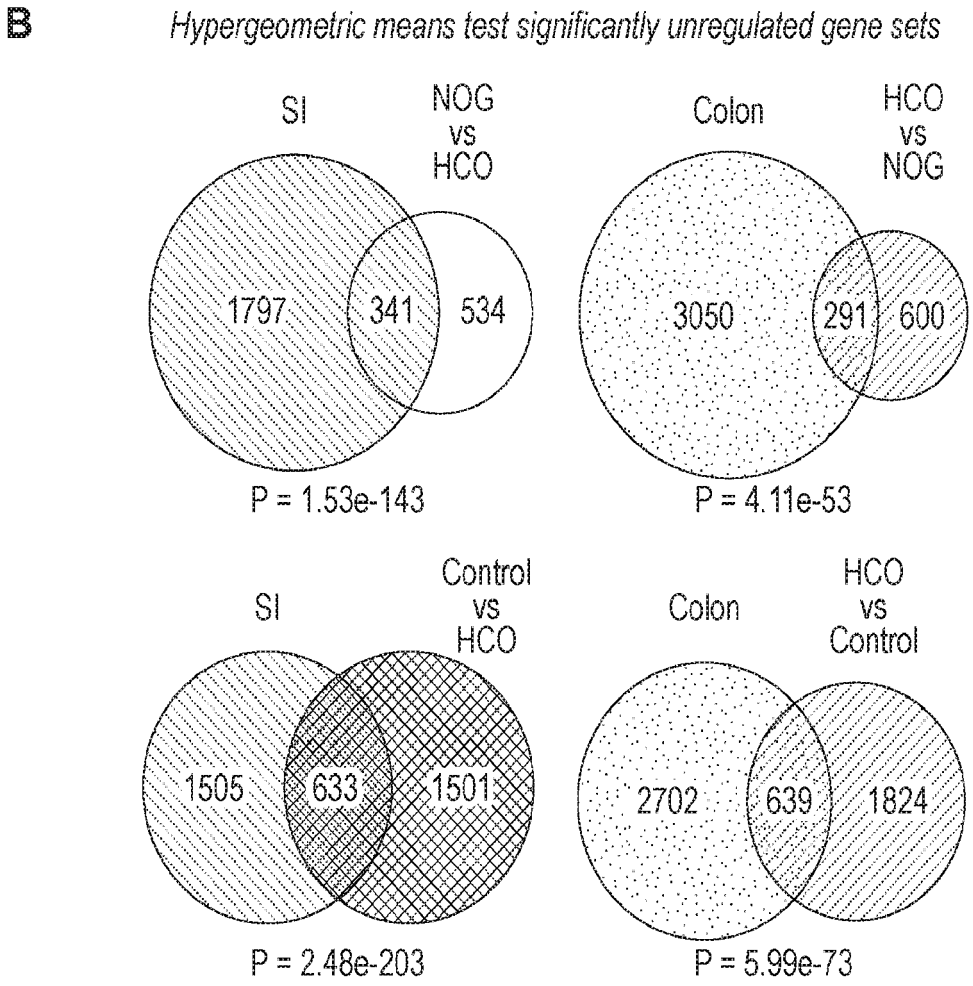

B
Hypergeometric means test significantly unregulated gene sets
SI
NOG
vs
HCO
1797
341
534
P = 1.53e-143
Colon
HCO
vs
NOG
3050
291
600
P = 4.11e-53
SI
Control
vs
HCO
1505
633
1501
P = 2.48e-203
Colon
HCO
vs
Control
2702
639
1824
P = 5.99e-73

FIG. 7A

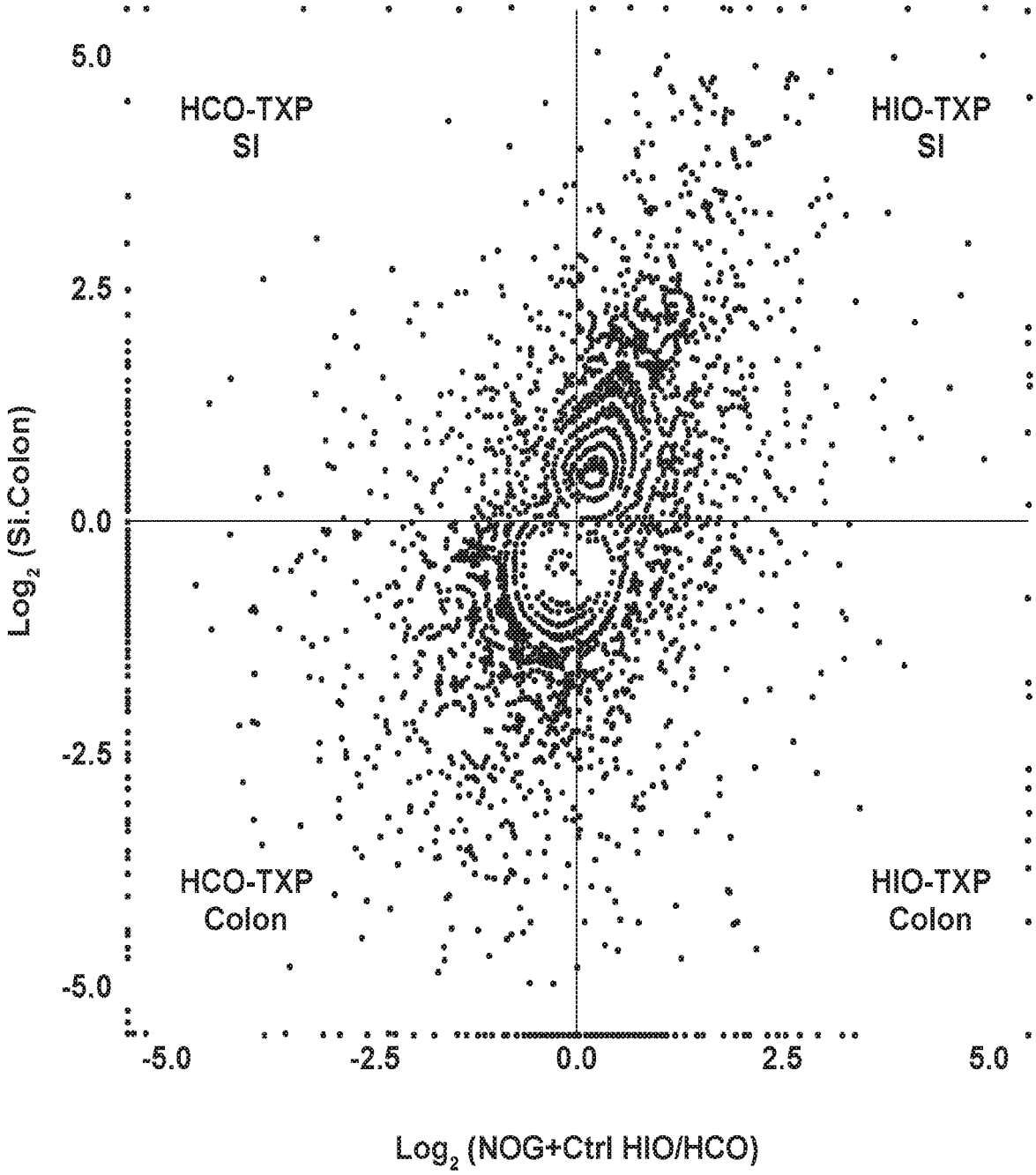

HCO-TXP
SI
HIO-TXP
SI
HCO-TXP
Colon
HIO-TXP
Colon
5.0
2.5
0.0
-2.5
-5.0
-5.0
-2.5
0.0
2.5
5.0
Log2 (Si.Colon)
Log2 (NOG+Ctrl HIO/HCO)

FIG. 7B

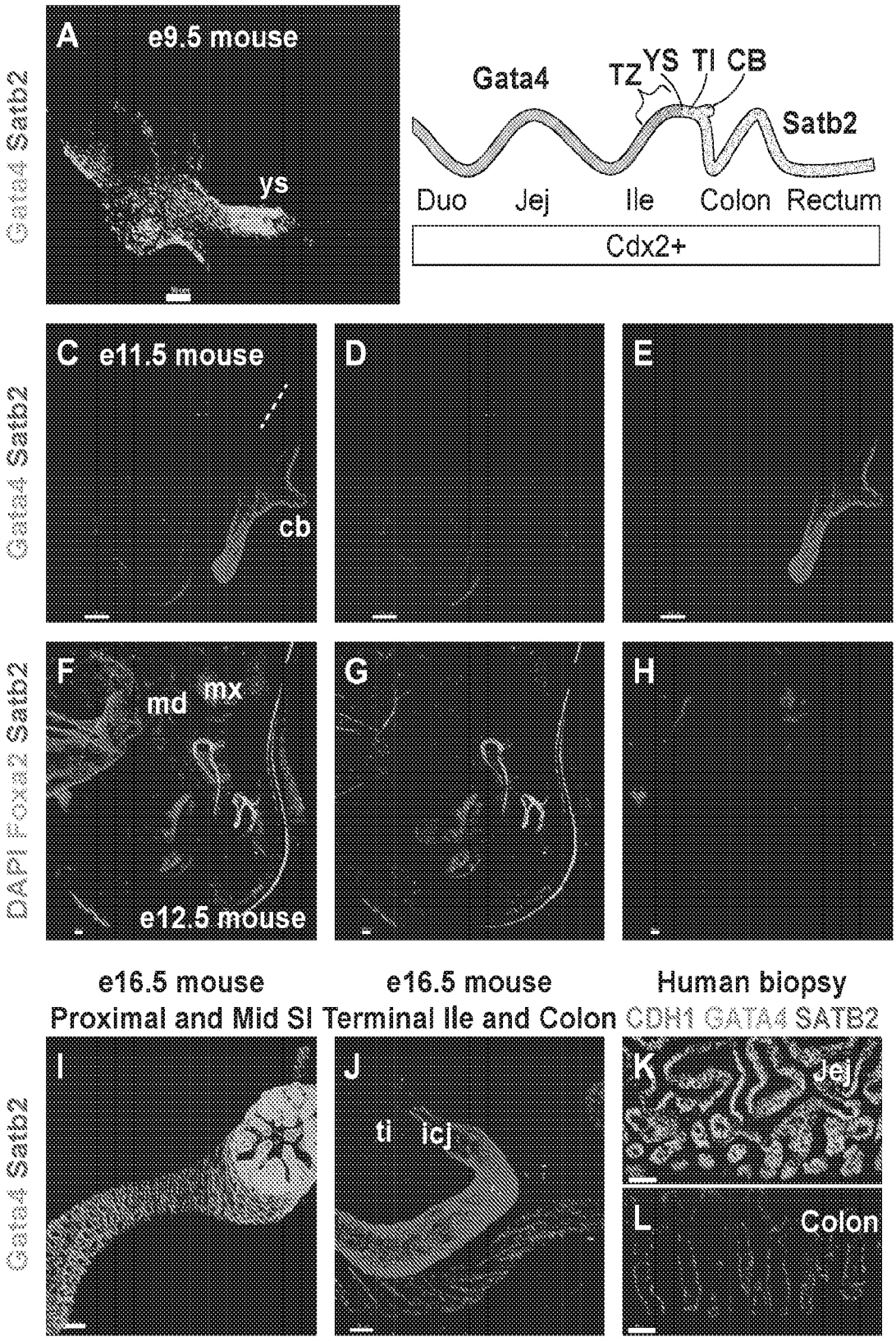

A
e9.5 mouse
ys
Gata4 Satb2
Gata4
TZ
YS
TI
CB
Satb2
Duo
Jej
Ile
Colon
Rectum
Cdx2+
C
e11.5 mouse
cb
D
E
Gata4 Satb2
F
md
mx
e12.5 mouse
G
H
DAPI Foxa2 Satb2
e16.5 mouse
Proximal and Mid SI
e16.5 mouse
Terminal Ile and Colon
Human biopsy
CDH1 GATA4 SATB2
I
J
ti
icj
K
Jej
L
Colon
Gata4 Satb2

FIG. 8

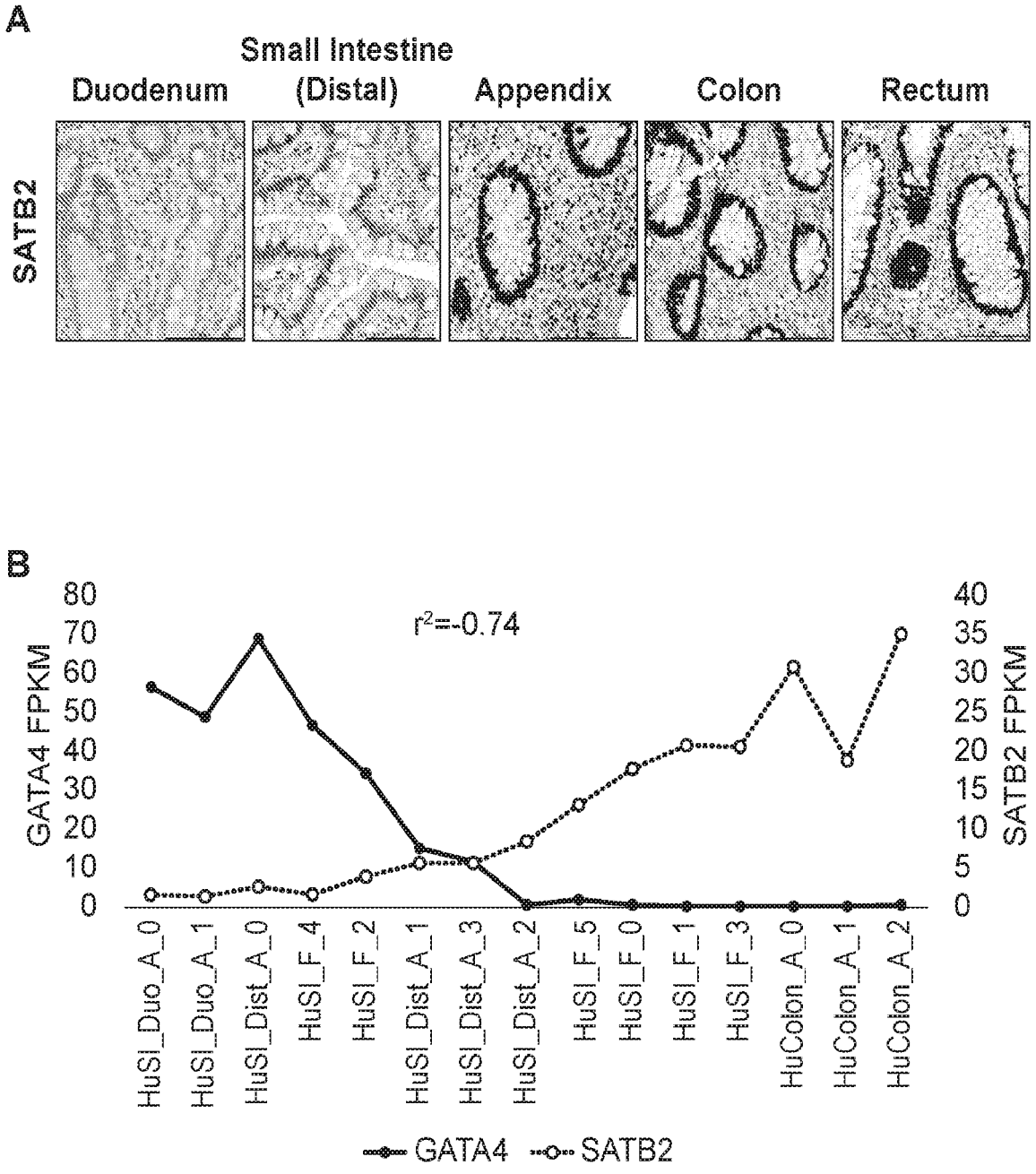
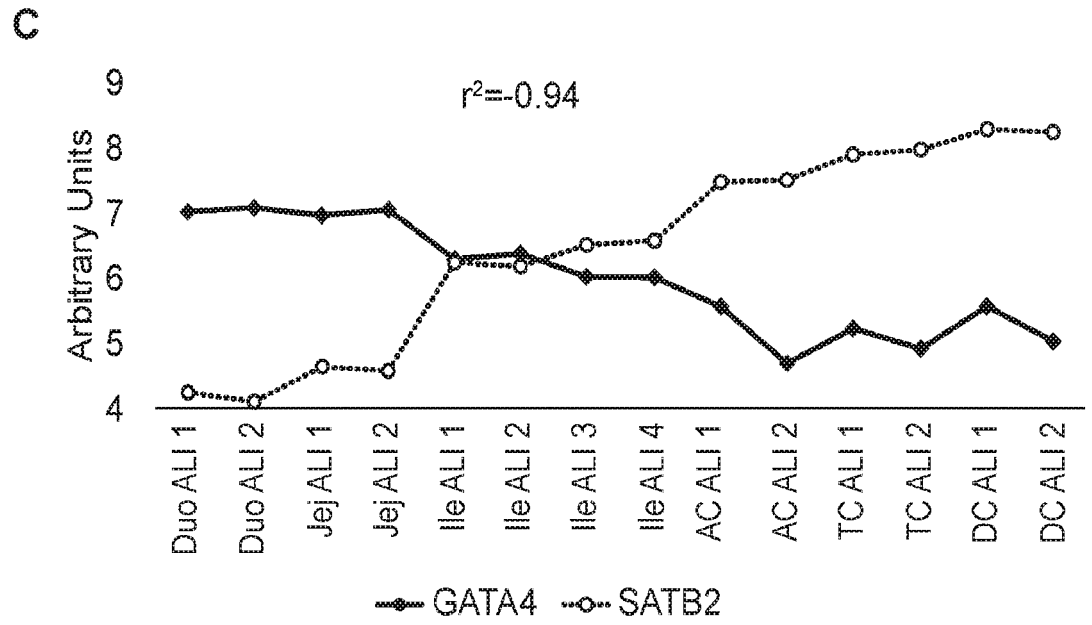

A
Small Intestine
Duodenum
(Distal)
Appendix
Colon
Rectum
SATB2
B
GATA4 FPKM
SATB2 FPKM
r²=-0.74
HuSI_Duo_A_0
HuSI_Duo_A_1
HuSI_Dist_A_0
HuSI_F_4
HuSI_F_2
HuSI_Dist_A_1
HuSI_Dist_A_3
HuSI_Dist_A_2
HuSI_F_5
HuSI_F_0
HuSI_F_1
HuSI_F_3
HuColon_A_0
HuColon_A_1
HuColon_A_2
GATA4
SATB2

FIG. 9A


C
r²=-0.94
Arbitrary Units
9
8
7
6
5
4
Duo ALI 1
Duo ALI 2
Jej ALI 1
Jej ALI 2
Ile ALI 1
Ile ALI 2
Ile ALI 3
Ile ALI 4
AC ALI 1
AC ALI 2
TC ALI 1
TC ALI 2
DC ALI 1
DC ALI 2
GATA4
SATB2

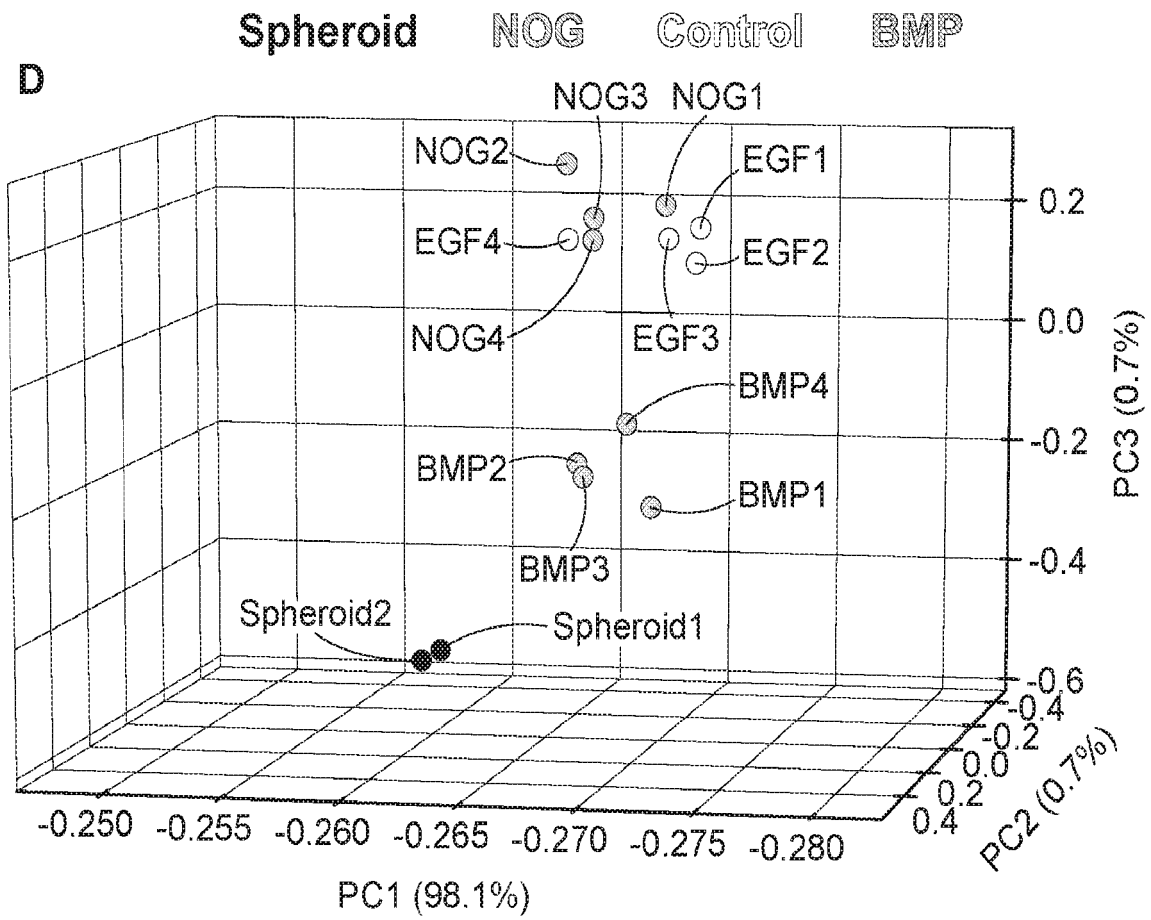

Spheroid
NOG
Control
BMP
D
NOG3
NOG1
NOG2
EGF1
EGF4
EGF2
NOG4
EGF3
BMP4
BMP2
BMP1
BMP3
Spheroid2
Spheroid1
0.2
0.0
-0.2
-0.4
-0.6
PC3 (0.7%)
-0.250
-0.255
-0.260
-0.265
-0.270
-0.275
-0.280
PC1 (98.1%)
-0.4
-0.2
0.0
0.2
0.4
PC2 (0.7%)

FIG. 9B

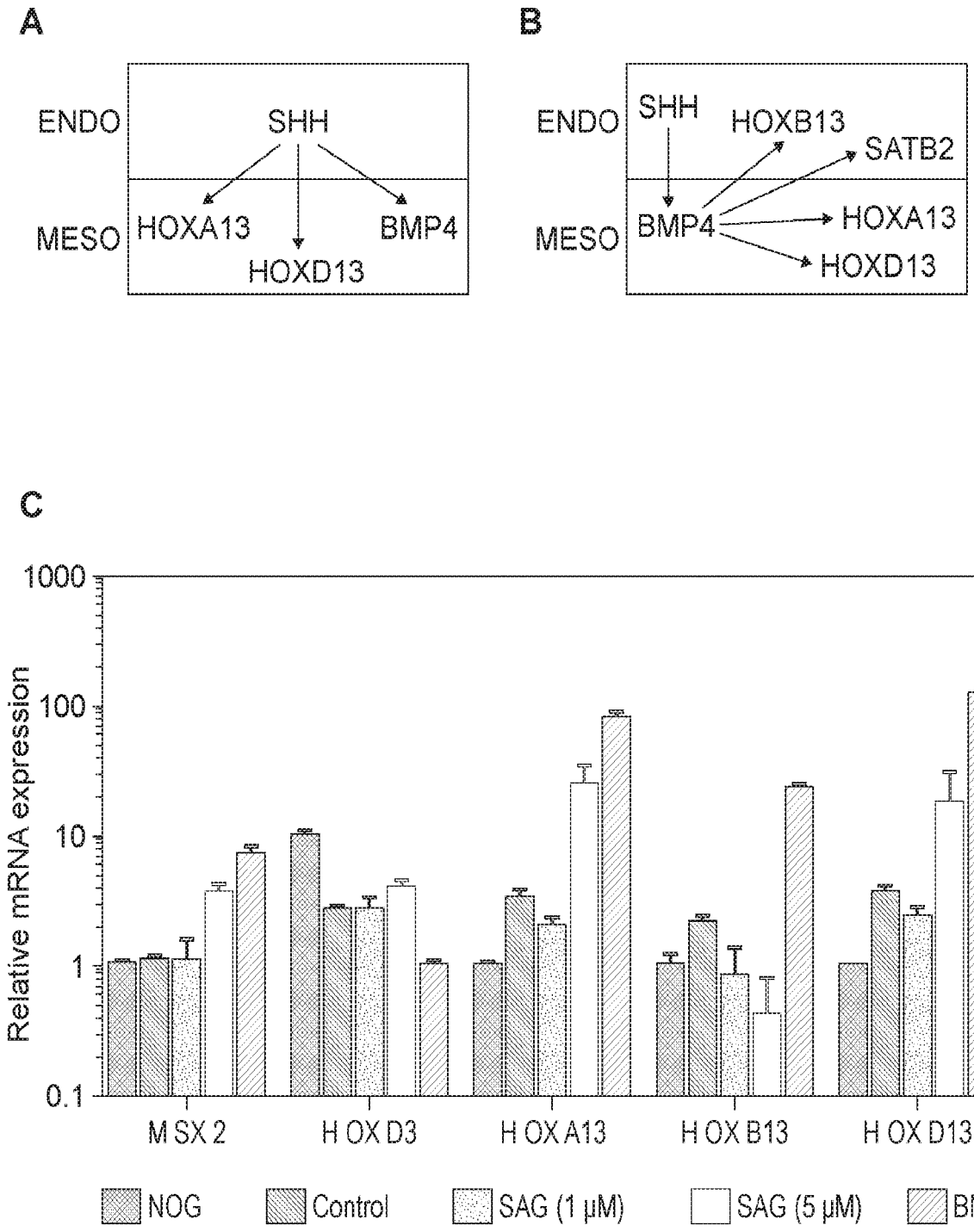

A
ENDO
SHH
MESO
HOXA13
BMP4
HOXD13
B
ENDO
SHH
HOXB13
SATB2
MESO
BMP4
HOXA13
HOXD13
C
Relative mRNA expression
1000
100
10
1
0.1
MSX2
HOXD3
HOXA13
HOXB13
HOXD13
NOG
Control
SAG (1 μM)
SAG (5 μM)
BMP

FIG. 10A

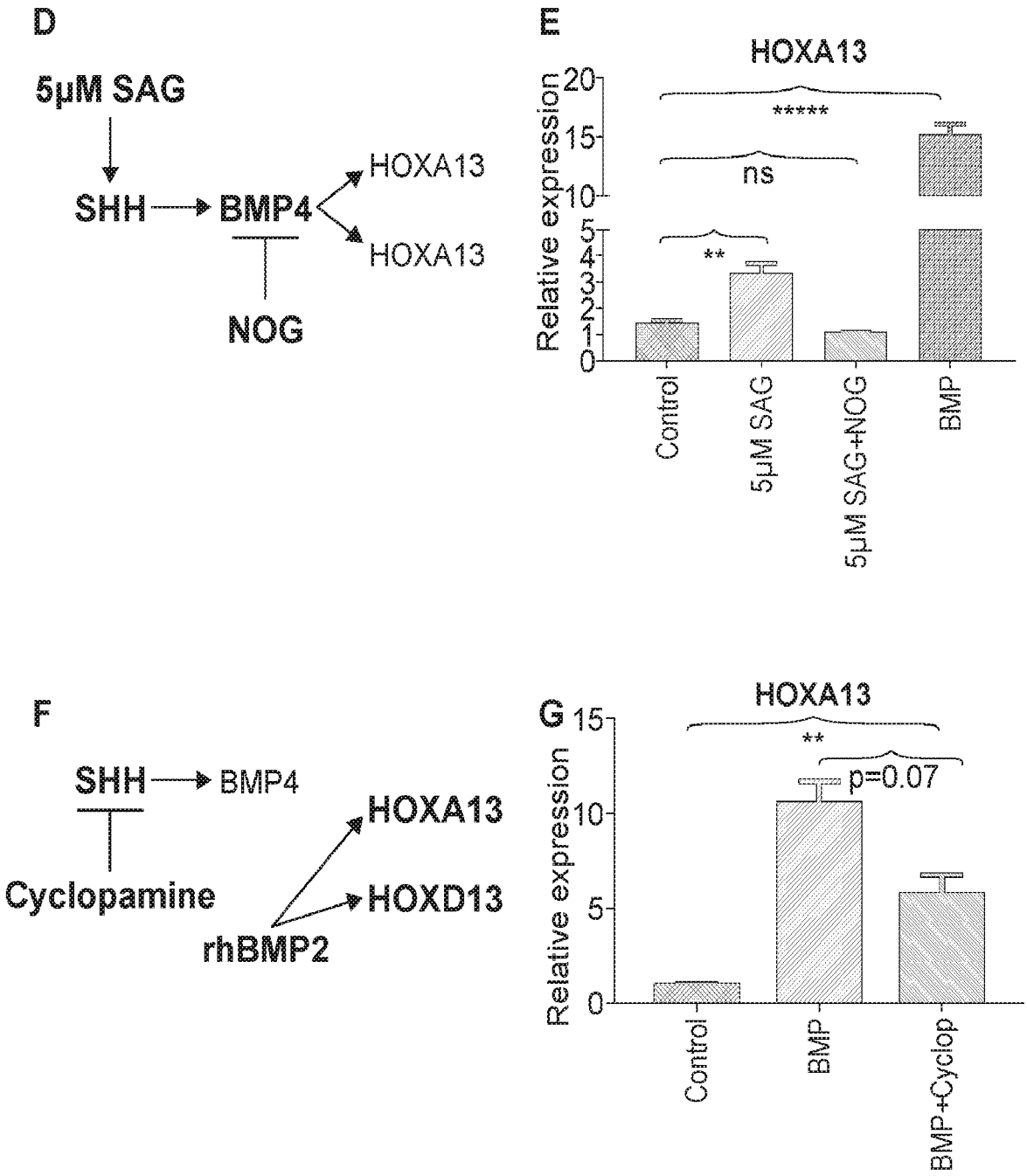

D
5μM SAG
SHH
BMP4
HOXA13
HOXA13
NOG
E
HOXA13
Relative expression
20
15
10
5
0
*****
ns
**
Control
5μM SAG
5μM SAG+NOG
BMP
F
SHH
BMP4
Cyclopamine
HOXA13
HOXD13
rhBMP2
G
HOXA13
Relative expression
15
10
5
0
**
p=0.07
Control
BMP
BMP+Cyclop

FIG. 10B

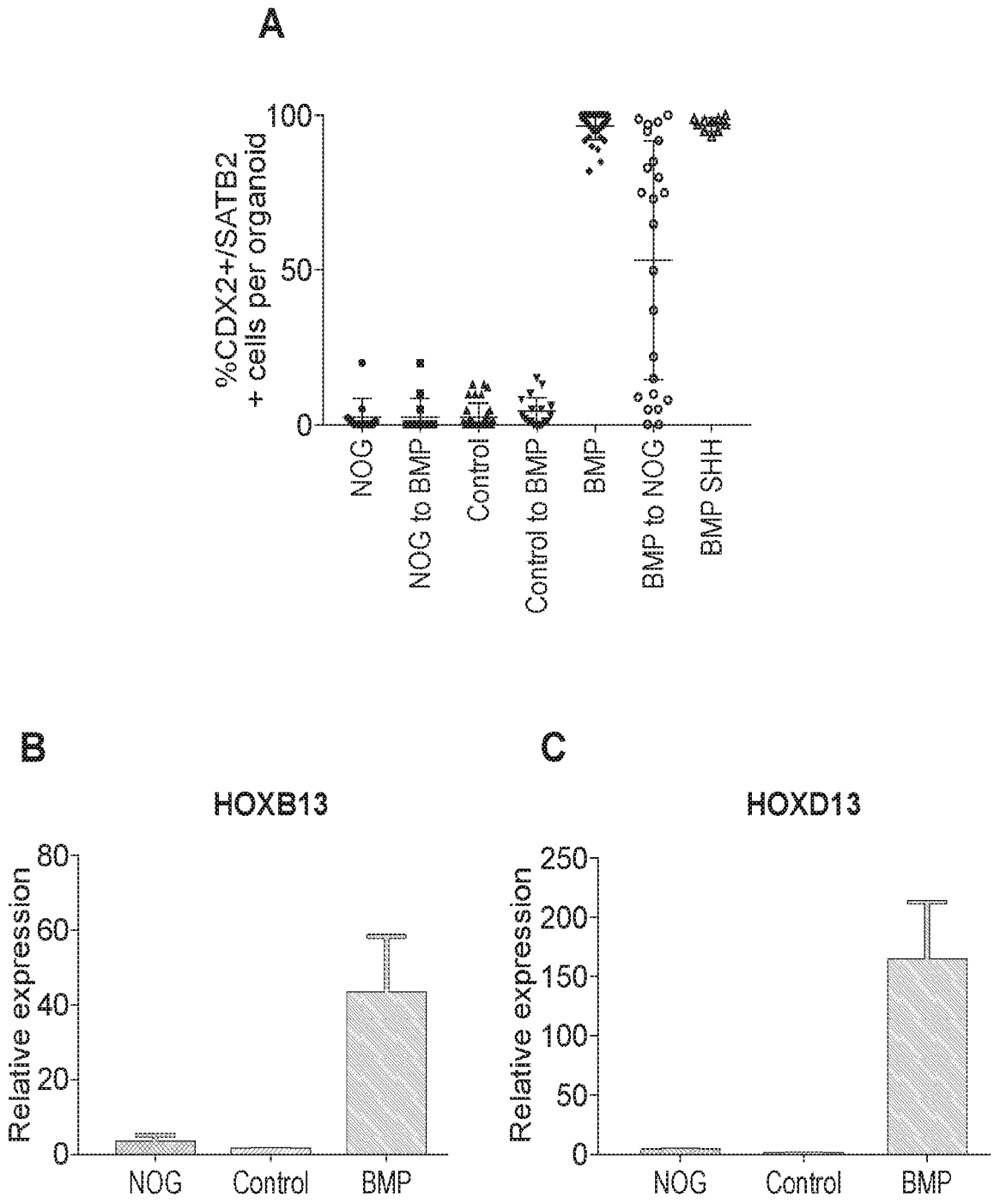

A
%CDX2+/SATB2 + cells per organoid
100
50
0
NOG
NOG to BMP
Control
Control to BMP
BMP
BMP to NOG
BMP SHH
B
HOXB13
Relative expression
80
60
40
20
0
NOG
Control
BMP
C
HOXD13
Relative expression
250
200
150
100
50
0
NOG
Control
BMP

FIG. 11A

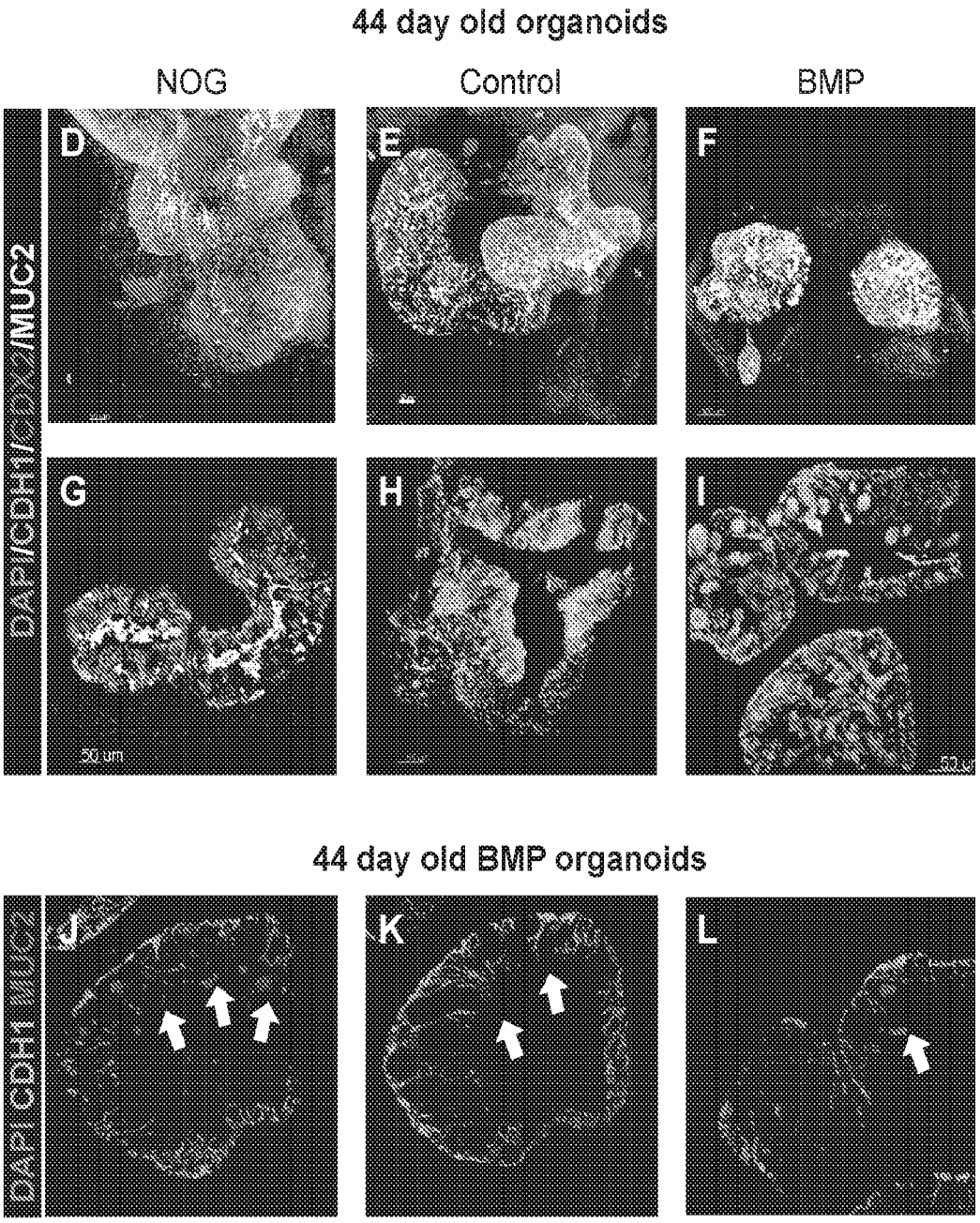

44 day old organoids
NOG
Control
BMP
DAPI/CDH1/CDX2/MUC2
D
E
F
G
H
I
50 um
44 day old BMP organoids
DAPI CDH1 MUC2
J
K
L

FIG. 11B

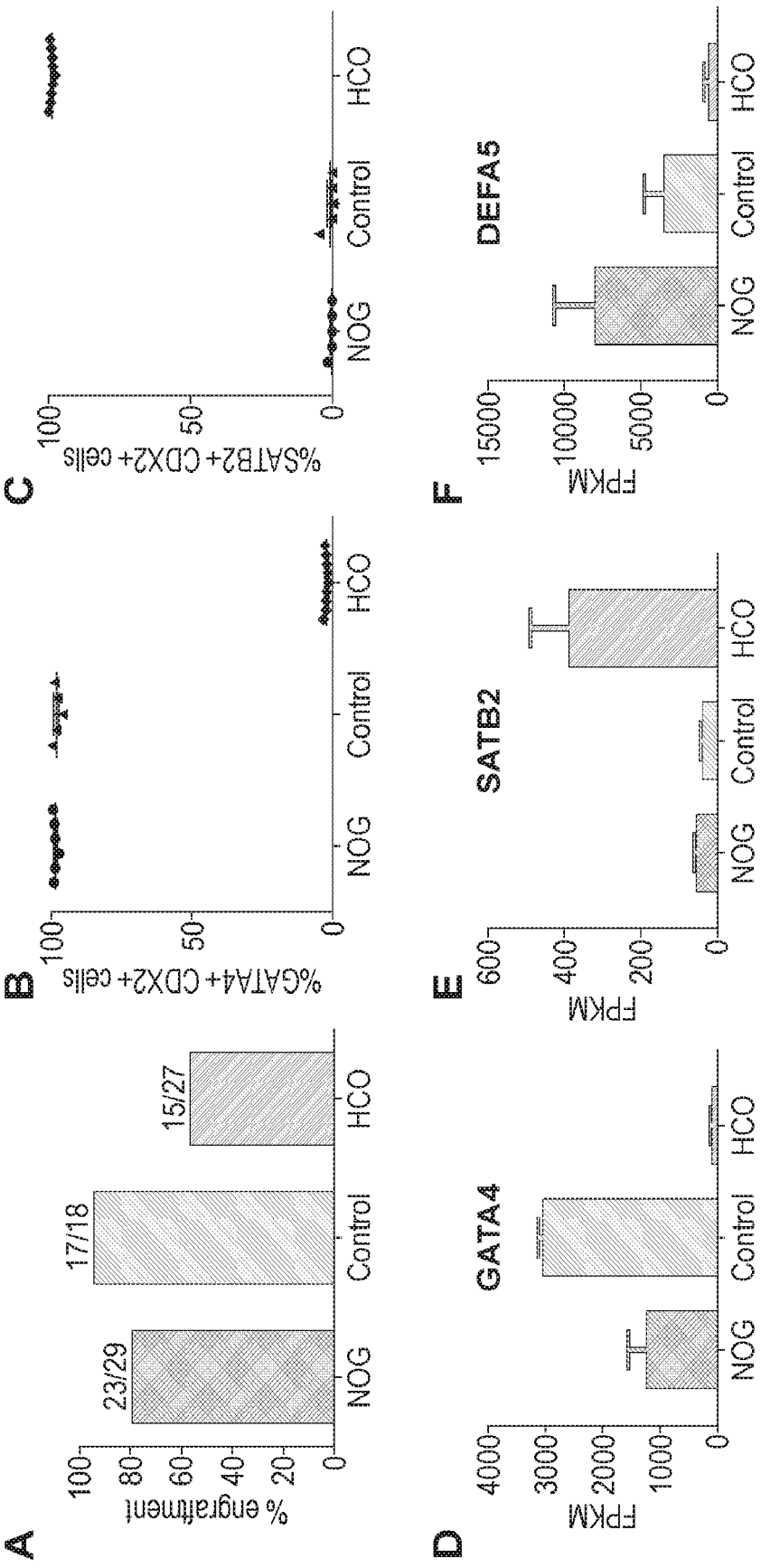

A
% engraftment
100
80
60
40
20
0
23/29
17/18
15/27
NOG
Control
HCO
B
%GATA4+ CDX2+ cells
100
50
0
NOG
Control
HCO
C
%SATB2+ CDX2+ cells
100
50
0
NOG
Control
HCO
D
GATA4
FPKM
4000
3000
2000
1000
0
NOG
Control
HCO
E
SATB2
FPKM
600
400
200
0
NOG
Control
HCO
F
DEFA5
FPKM
15000
10000
5000
0
NOG
Control
HCO

FIG. 12A

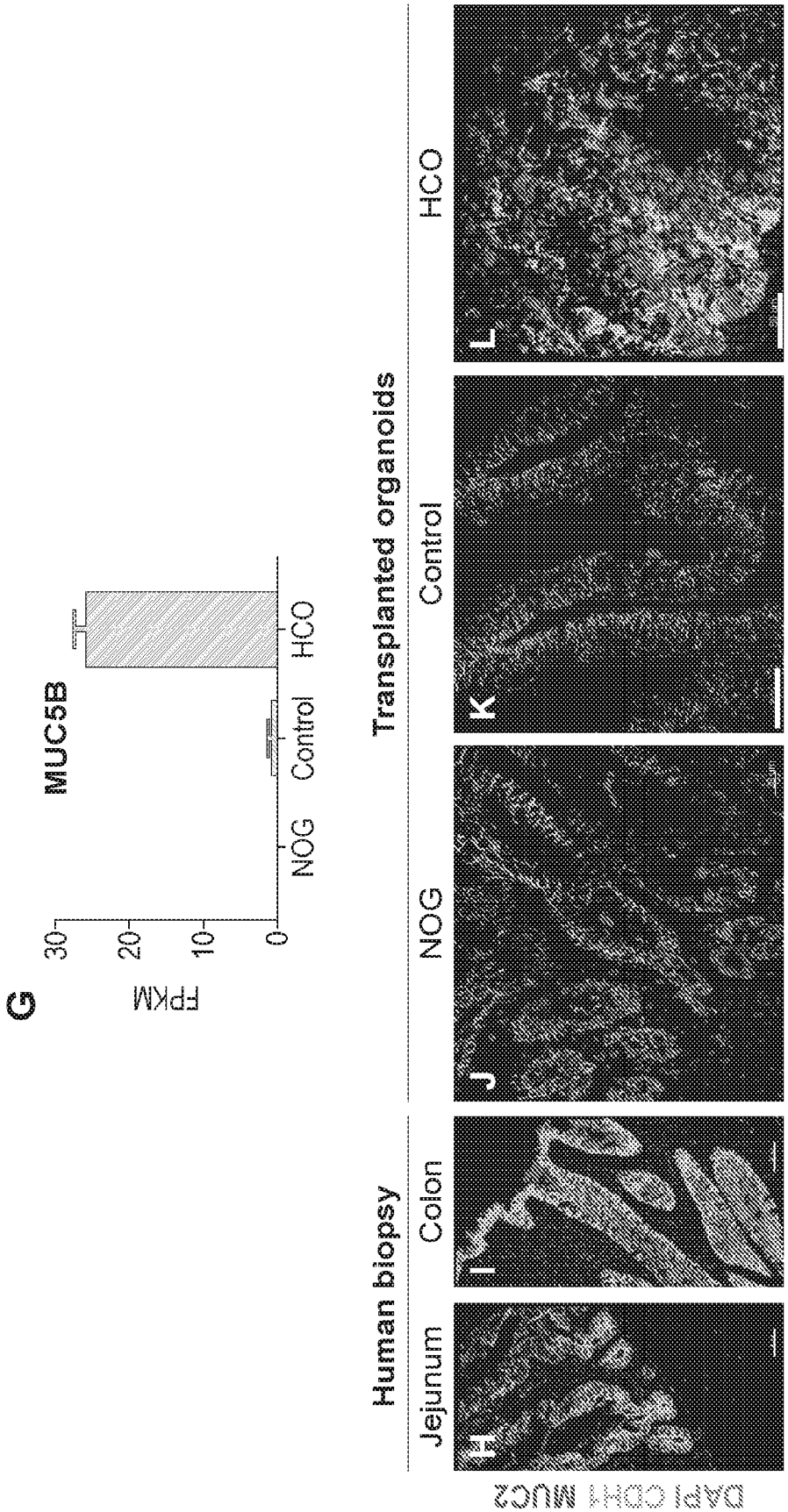

G
MUC5B
FPKM
30
20
10
0
NOG
Control
HCO
Transplanted organoids
Human biopsy
Jejunum
Colon
NOG
Control
HCO
H
I
J
K
L
DAPI CDH1 MUC2

FIG. 12B

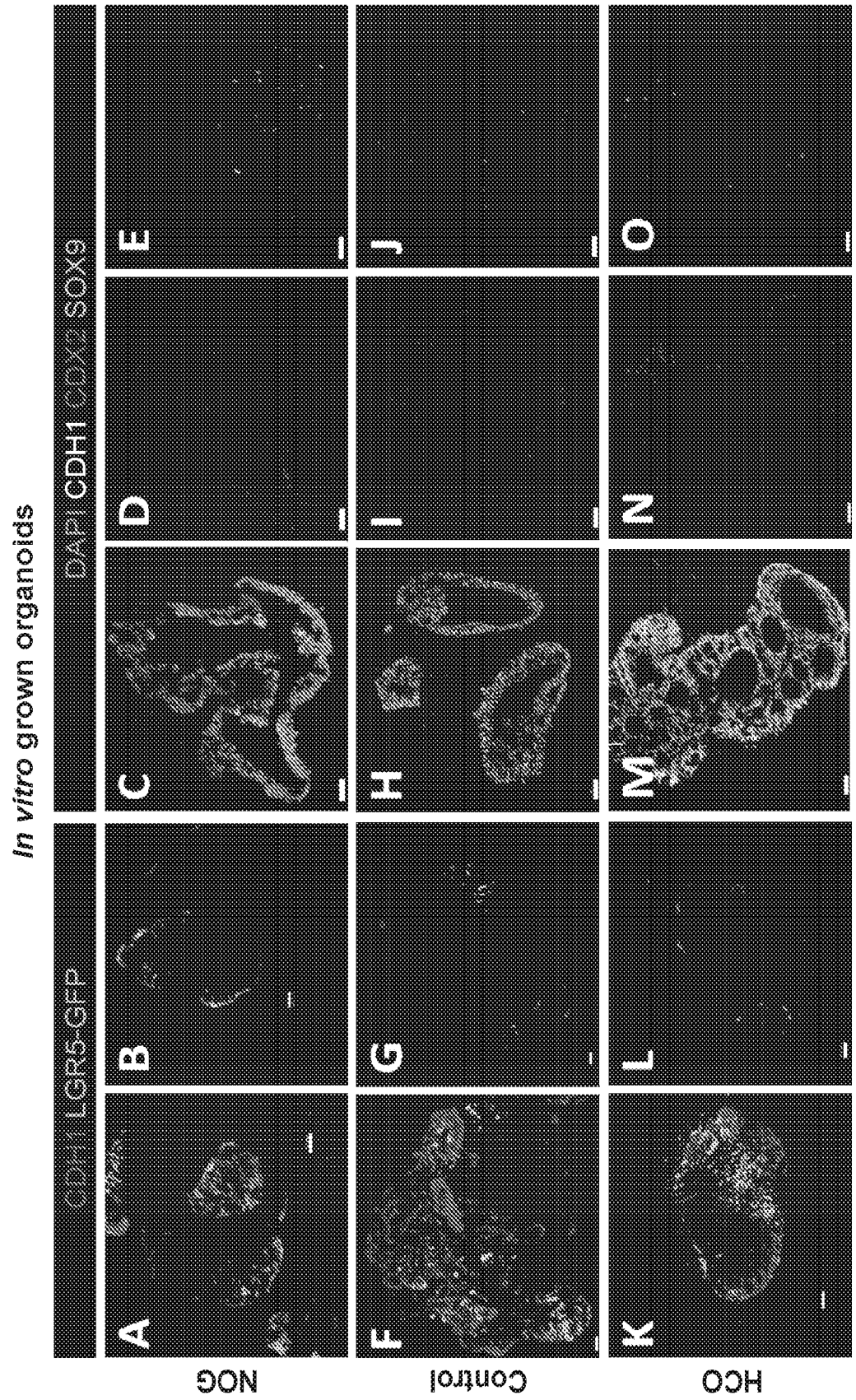

In vitro grown organoids
CDH1 LGR5-GFP
DAPI CDH1 CDX2 SOX9
NOG
Control
HCO
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O

FIG. 13A

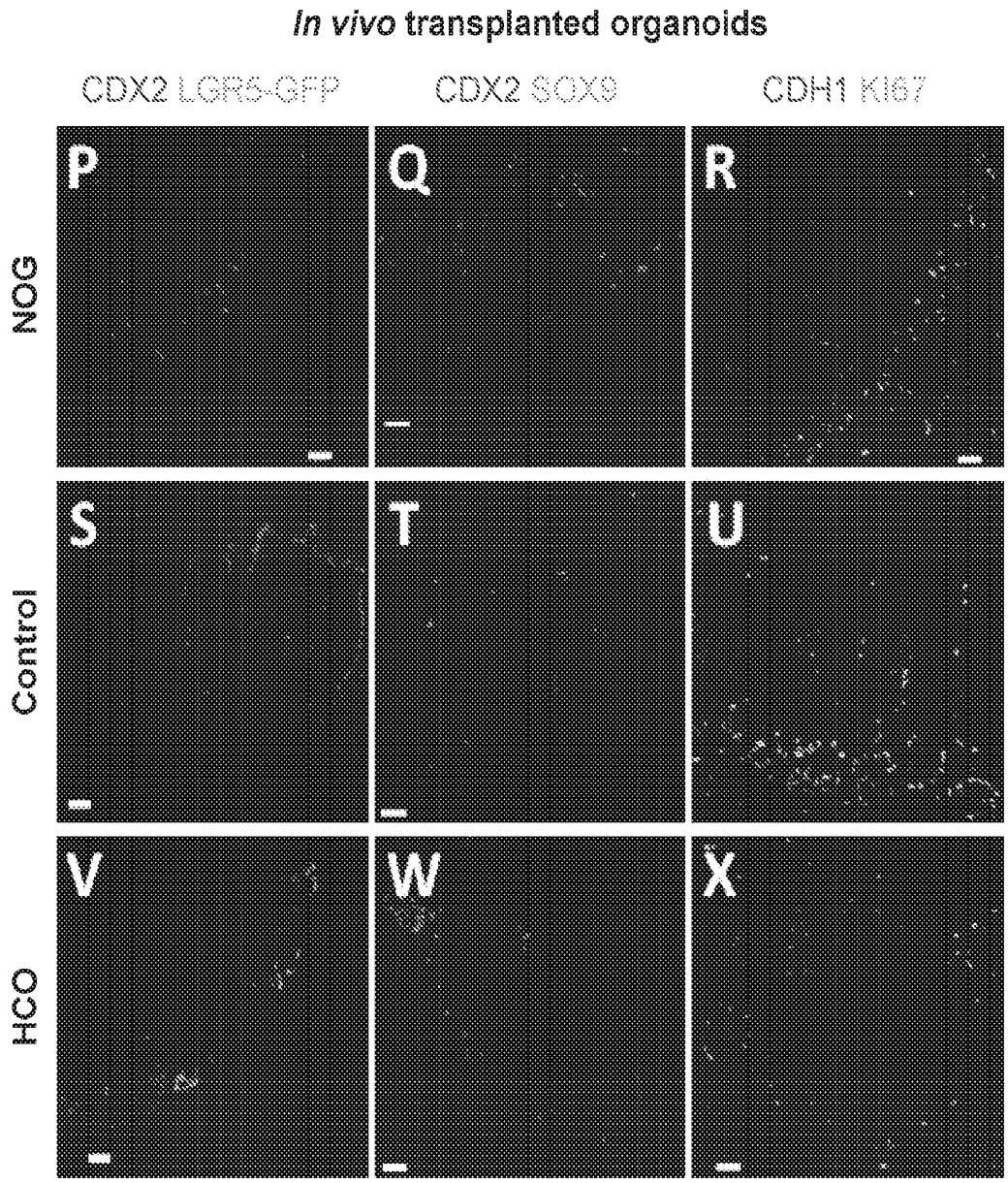

*In vivo* transplanted organoids
CDX2 LGR5-GFP
CDX2 SOX9
CDH1 KI67
NOG
Control
HCO
P
Q
R
S
T
U
V
W
X

FIG. 13B

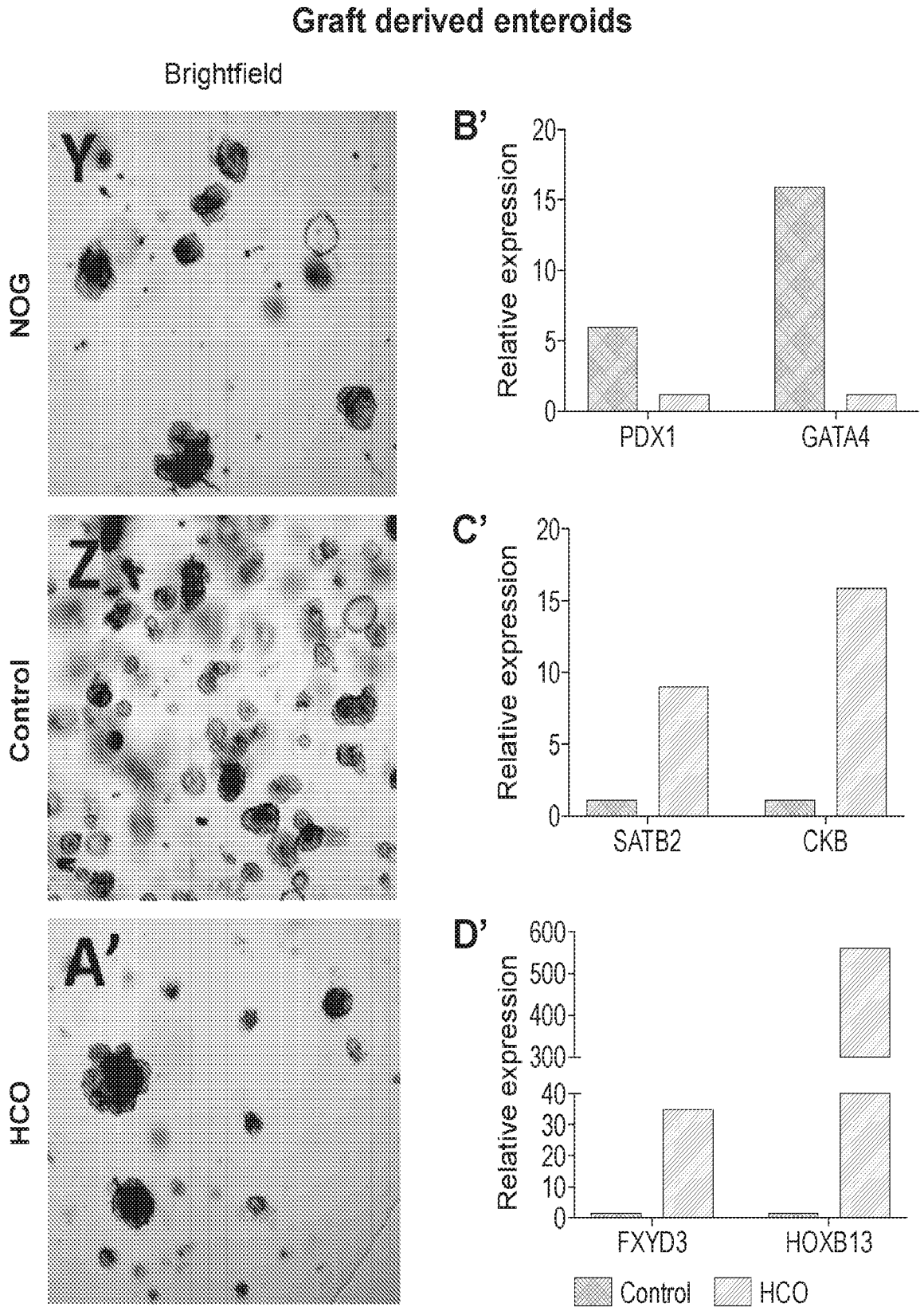
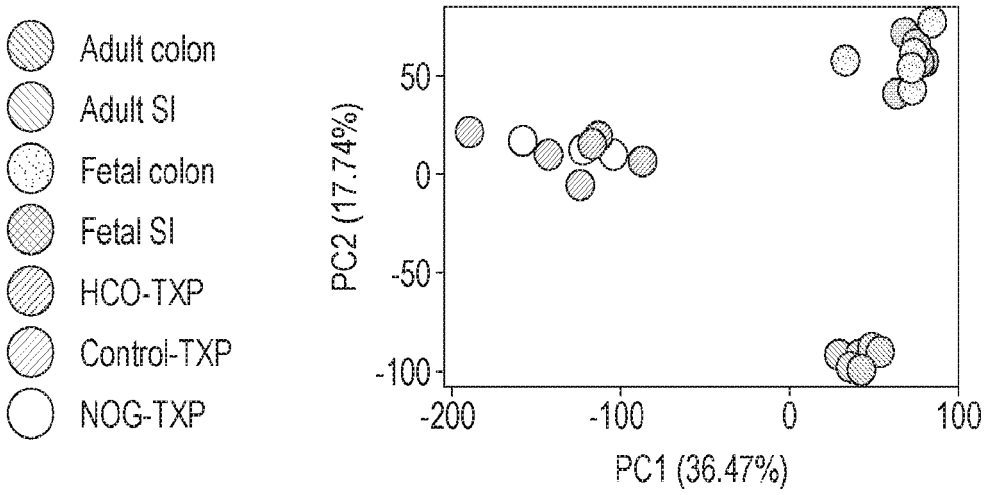

Graft derived enteroids
Brightfield
NOG
Control
HCO
Y
Z
A'
B'
C'
D'
Relative expression
PDX1
GATA4
SATB2
CKB
FXYD3
HOXB13
Control
HCO

FIG. 13C

A




B

**Up in TXP's vs primary tissues**

| Category | Name | p-value |
|---|---|---|
| Pathway | Ribosome | 2.20E-57 |
| GO: Cellular Component | ribosomal subunit | 3.20E-55 |
| GO: Cellular Component | ribosome | 2.61E-48 |
| GO: Molecular Function | structural constituent of ribosome | 5.07E-26 |
| GO: Cellular Component | cytosolic ribosome | 1.66E-41 |
| GO: Cellular Component | large ribosomal subunit | 2.43E-39 |
| GO: Biological Process | SRP dependent cotranslational protein targeting to membrane | 8.02E-39 |
| GO: Biological Process | protein targeting to ER | 1.14E-36 |
| GO: Cellular Component | mitochondrion | 7.71E-36 |
| GO: Biological Process | cotranslational protein targeting to membrane | 1.57E-35 |

FIG. 14A

C

**Up primary tissues vs in TXP's**

| Category | Name | p-value |
|---|---|---|
| GO: Biological Process | leukocyte activation | 2.26E-22 |
| GO: Biological Process | immune system process | 3.25E-22 |
| GO: Biological Process | cell activation | 1.46E-21 |
| GO: Biological Process | positive regulation of immune system process | 2.72E-19 |
| GO: Cellular Component | plasma membrane part | 1.89E-18 |
| GO: Biological Process | lymphocyte activation | 3.14E-18 |
| GO: Biological Process | leukocyte cell-cell adhesion | 6.71E-18 |
| GO: Biological Process | single organism cell-cell adhesion | 8.69E-18 |
| GO: Biological Process | immune response | 8.81E-18 |
| GO: Biological Process | intracellular signal transduction | 2.82E-17 |

FIG. 14B

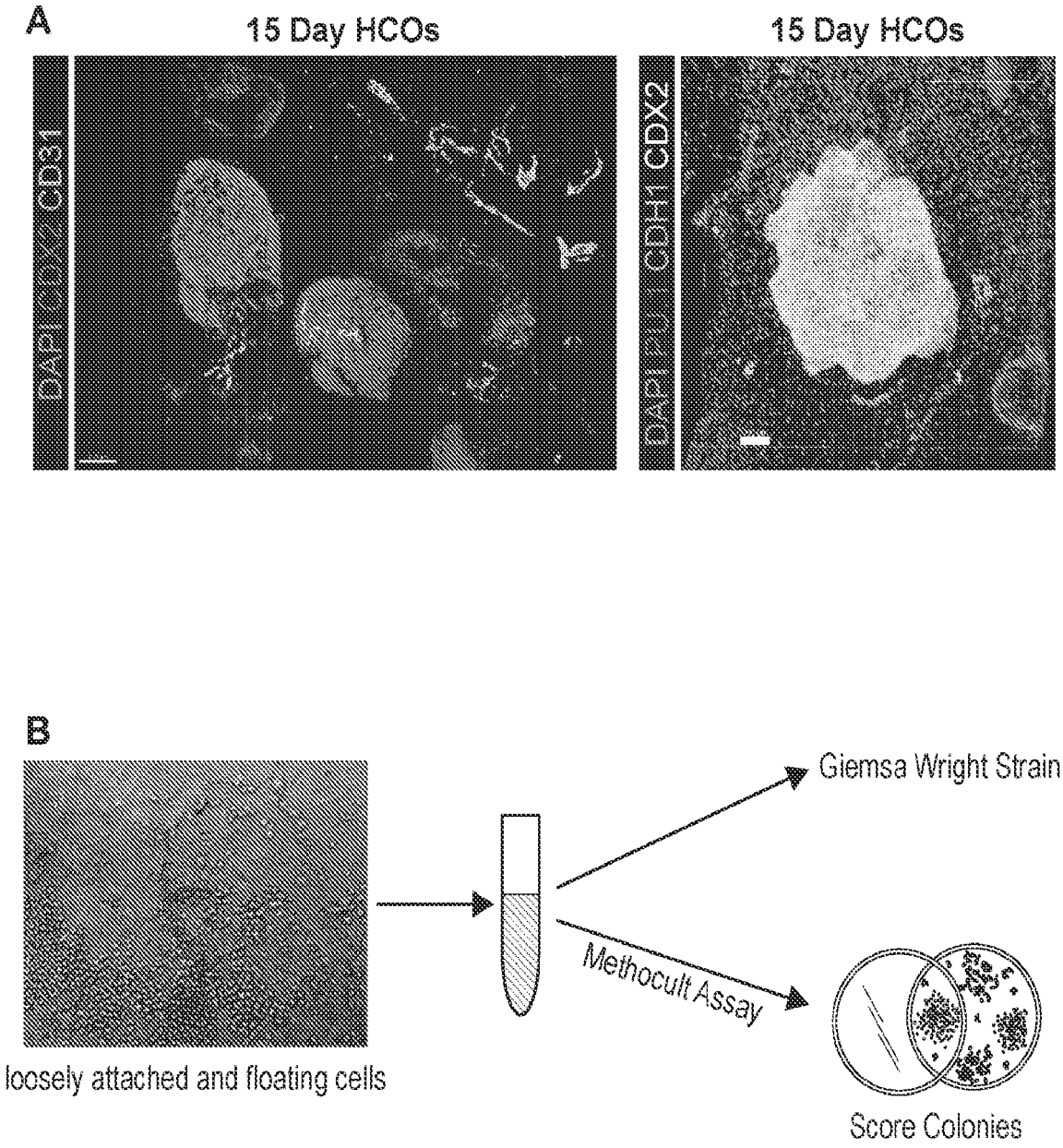

A
15 Day HCOs
DAPI CDX2 CD31
15 Day HCOs
DAPI CDH1 CDX2
B
Giemsa Wright Strain
Methocult Assay
loosely attached and floating cells
Score Colonies

FIG. 15A

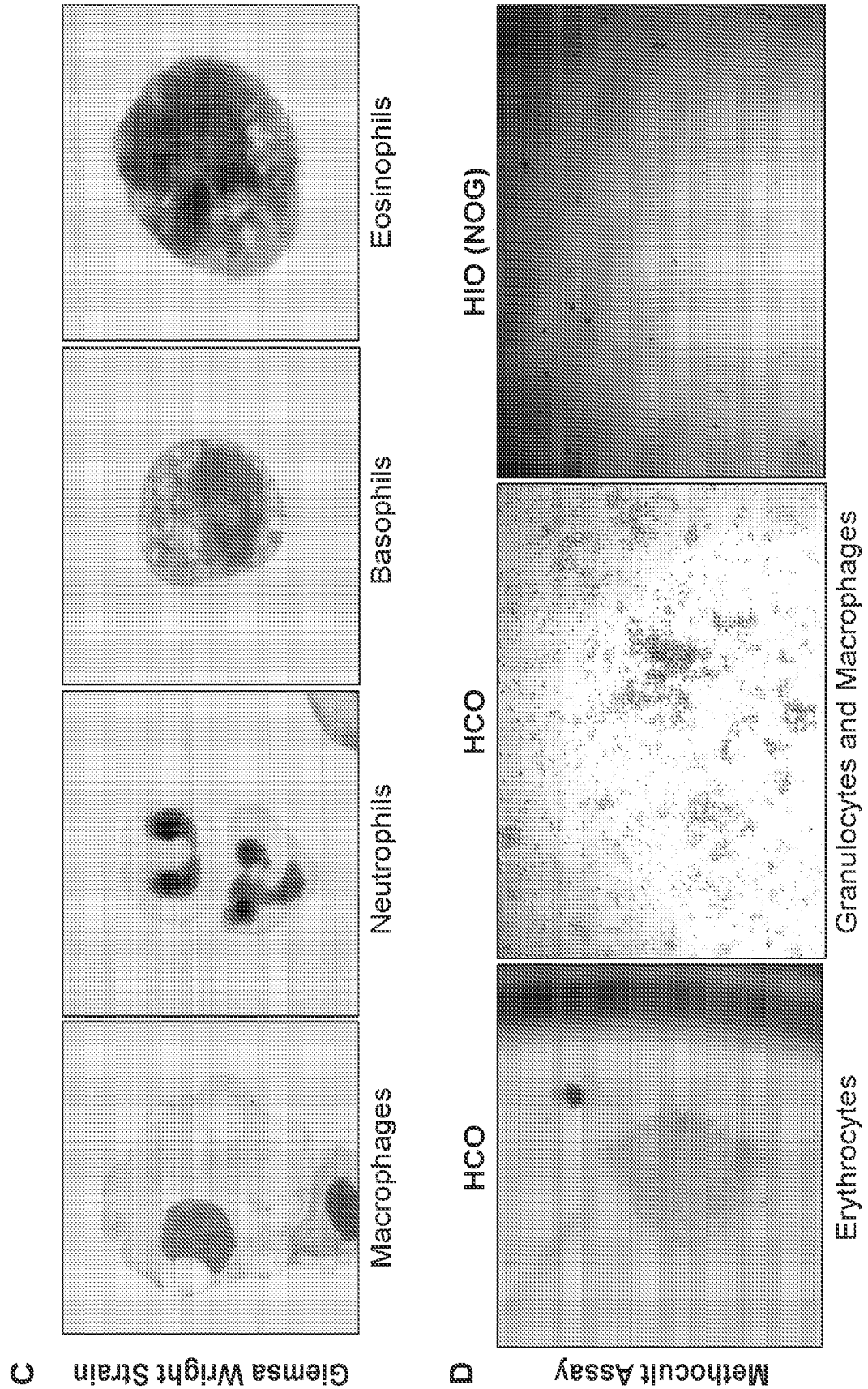

C
Giemsa Wright Stain
Macrophages
Neutrophils
Basophils
Eosinophils
D
Methocult Assay
HCO
HCO
HIO (NOG)
Erythrocytes
Granulocytes and Macrophages

FIG. 15B

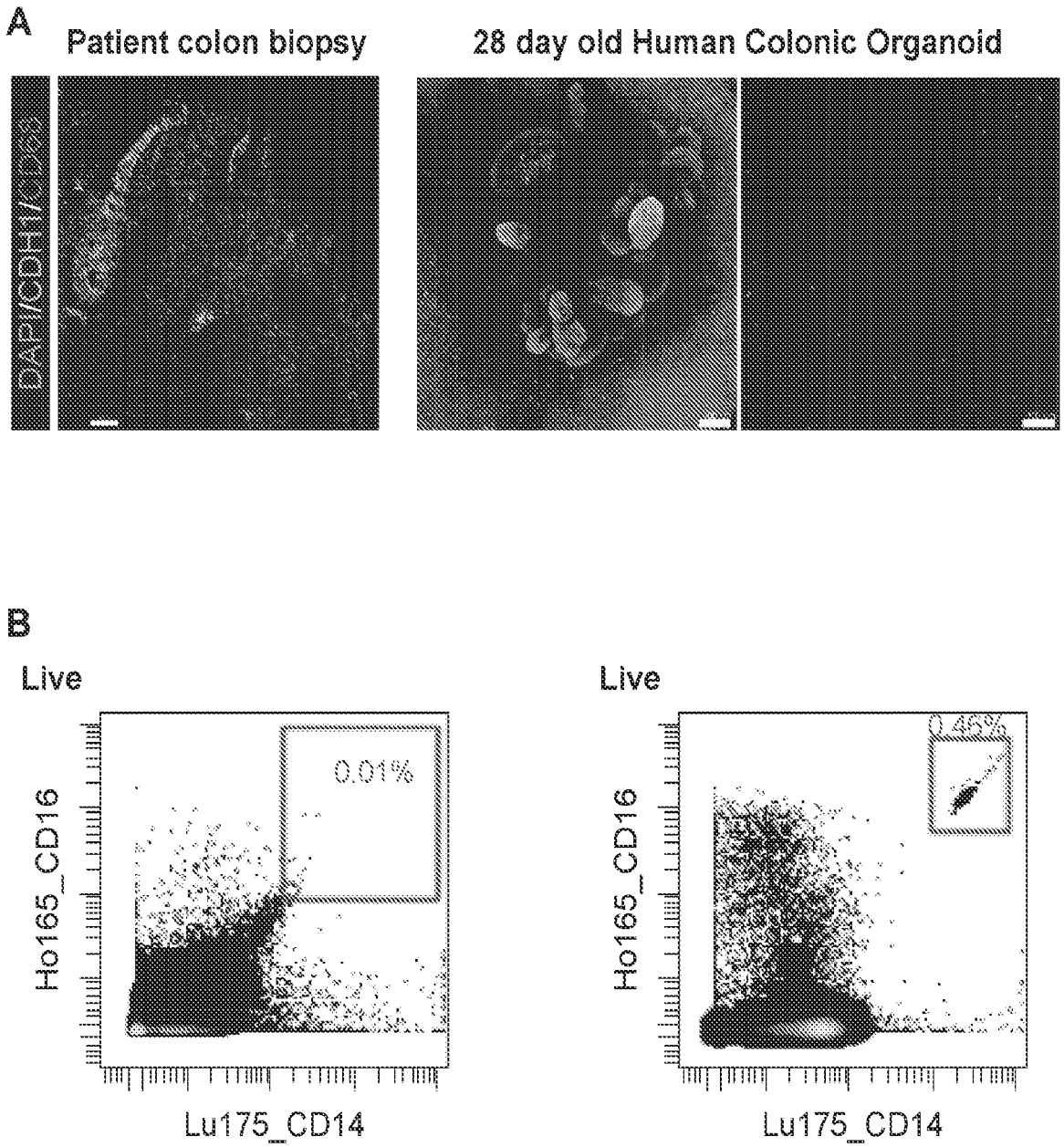

A
Patient colon biopsy
28 day old Human Colonic Organoid
DAPI/CDH1/CD326
B
Live
Ho165_CD16
0.01%
Lu175_CD14
Live
Ho165_CD16
0.45%
Lu175_CD14

FIG. 16A

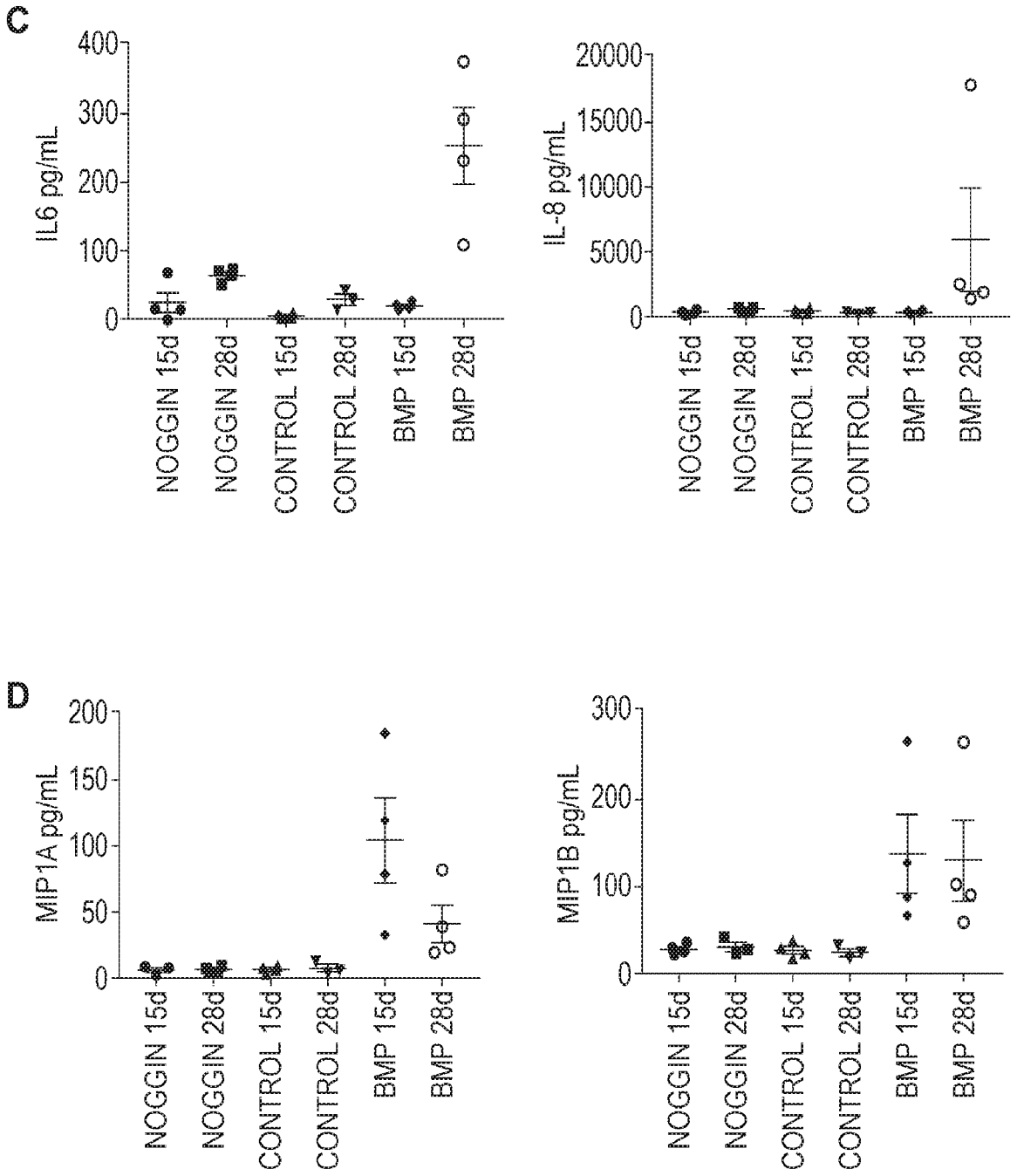

C
IL6 pg/mL
400
300
200
100
0
NOGGIN 15d
NOGGIN 28d
CONTROL 15d
CONTROL 28d
BMP 15d
BMP 28d
IL-8 pg/mL
20000
15000
10000
5000
0
NOGGIN 15d
NOGGIN 28d
CONTROL 15d
CONTROL 28d
BMP 15d
BMP 28d
D
MIP1A pg/mL
200
150
100
50
0
NOGGIN 15d
NOGGIN 28d
CONTROL 15d
CONTROL 28d
BMP 15d
BMP 28d
MIP1B pg/mL
300
200
100
0
NOGGIN 15d
NOGGIN 28d
CONTROL 15d
CONTROL 28d
BMP 15d
BMP 28d

FIG. 16B