Figure S1 Related to Figure 1

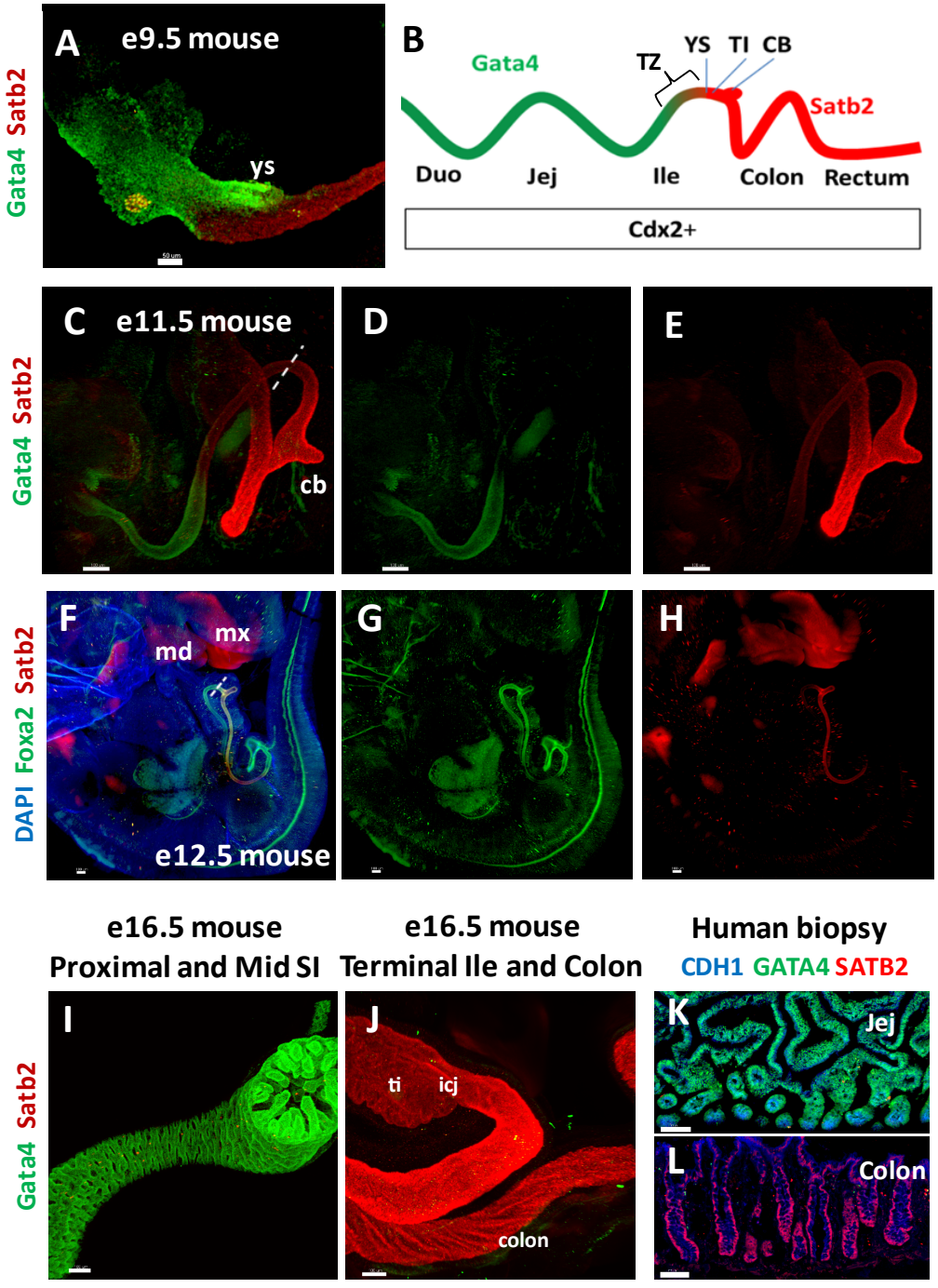
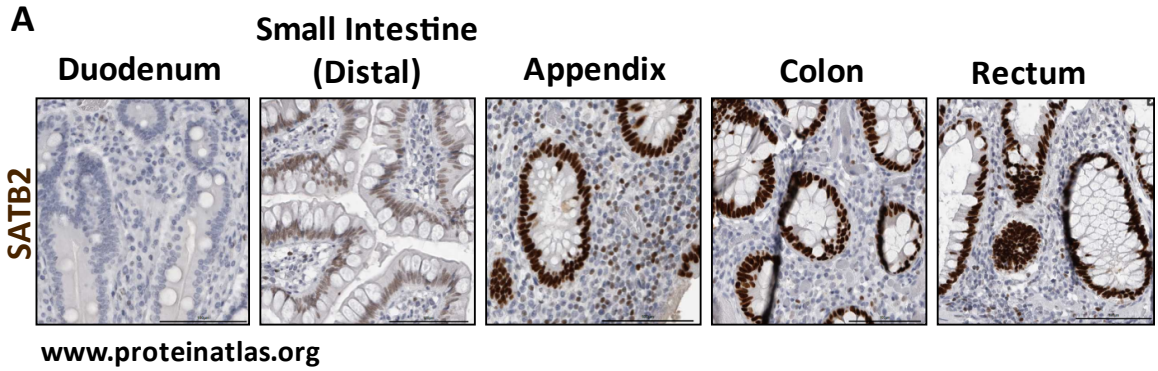
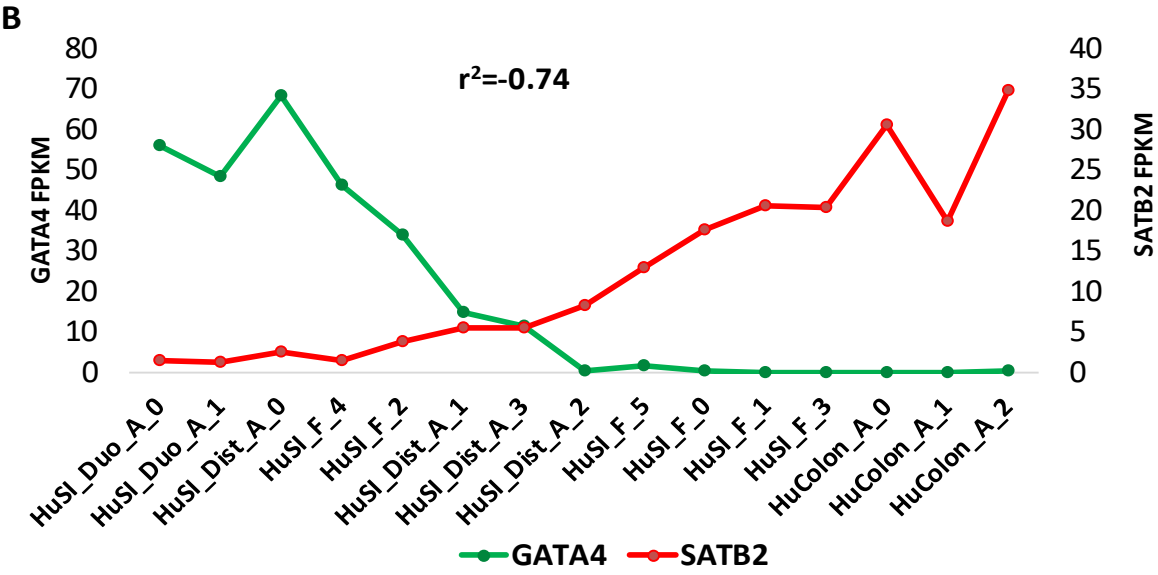
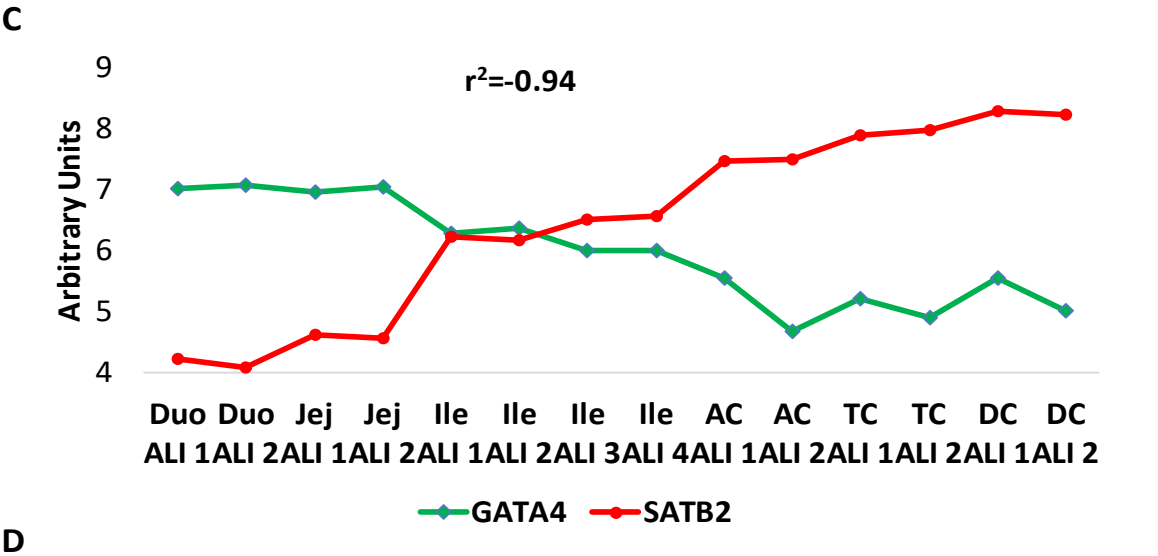


Figure S2 Realted to Figure 1 and Figure 2







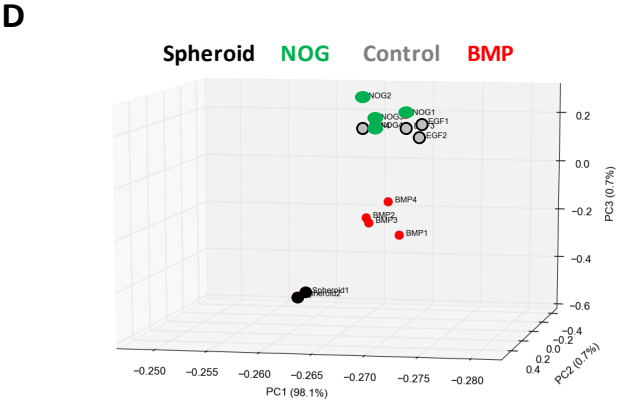


Figure S3 Related to Figure 2

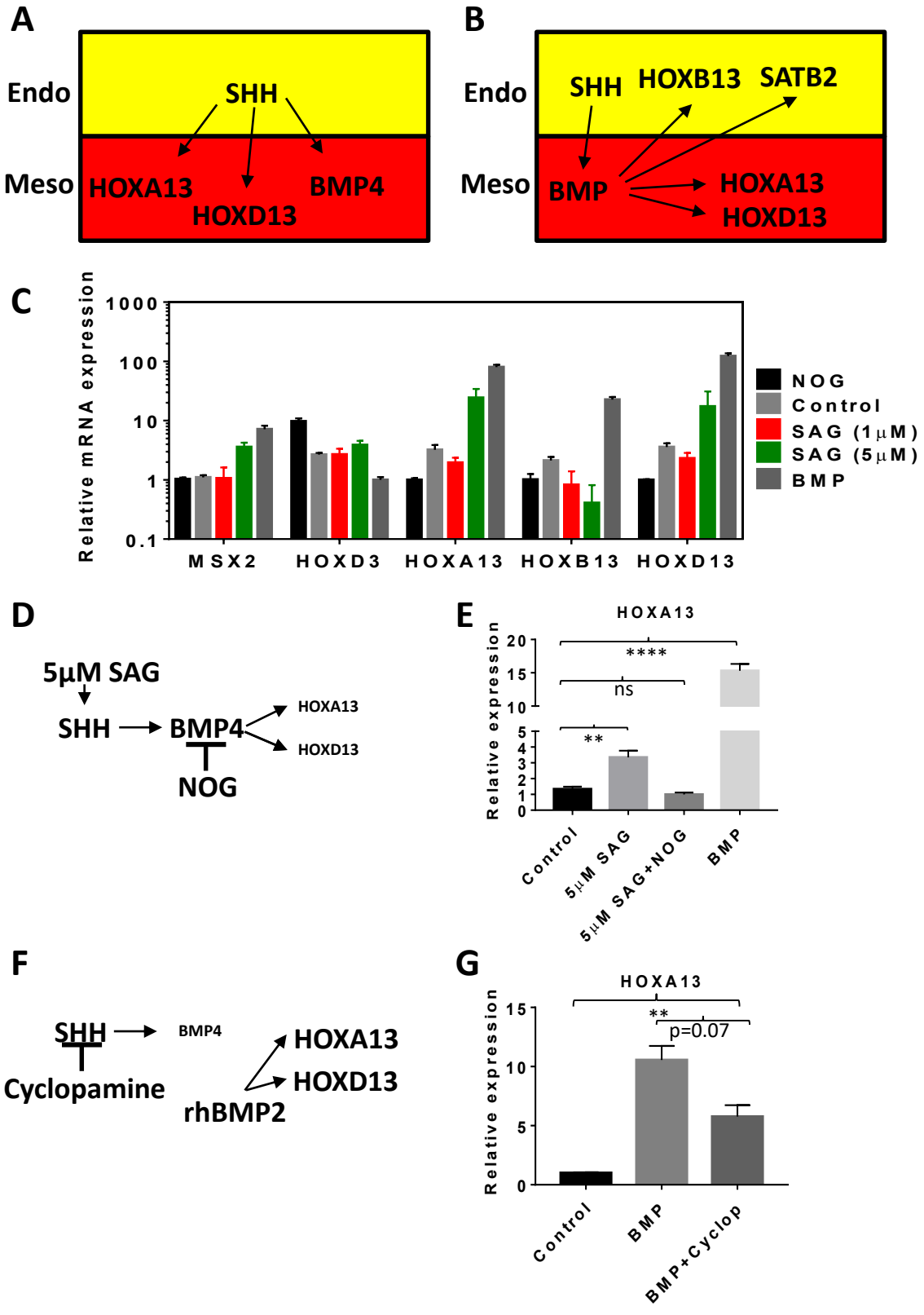


Figure S4 Related to Figure 3

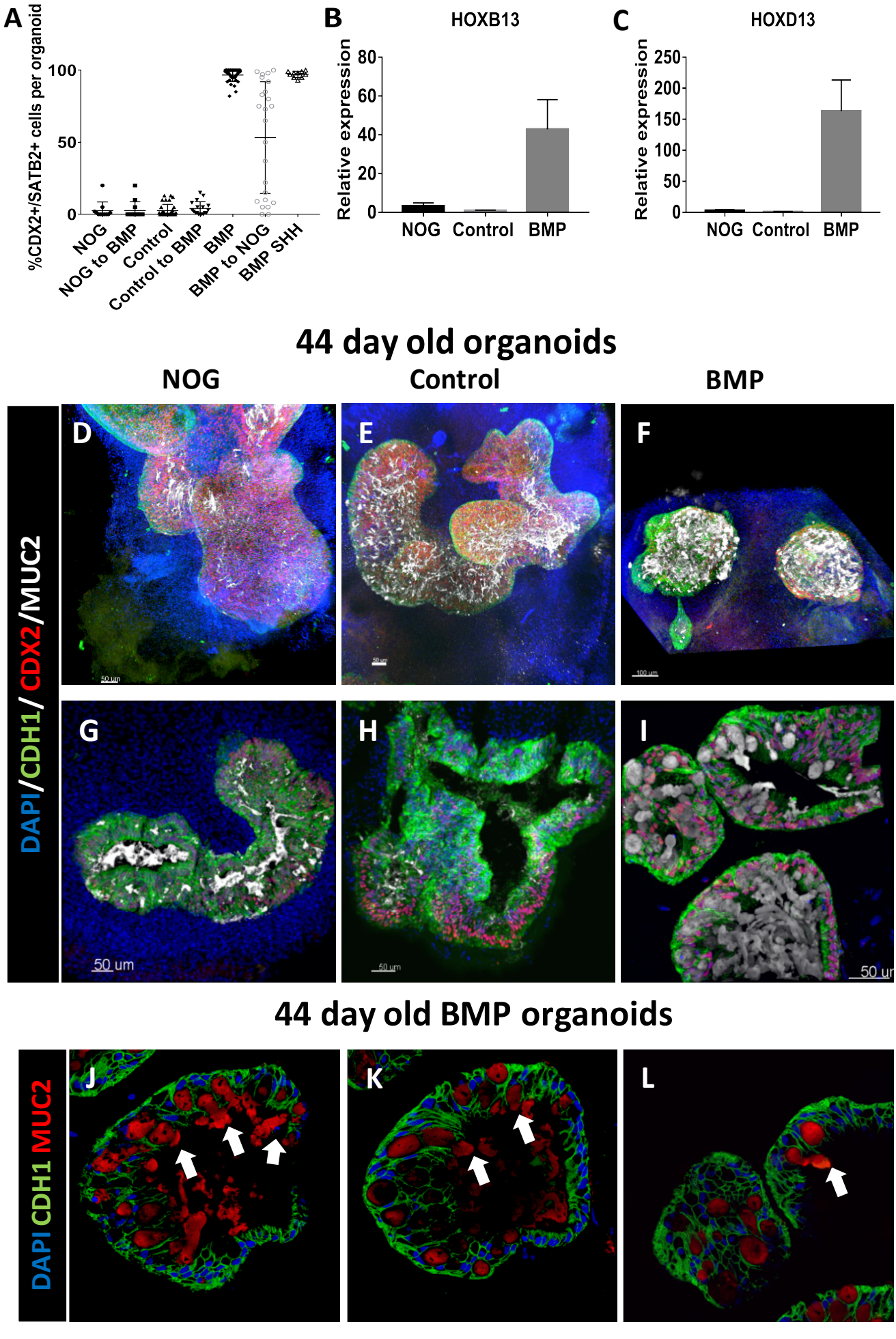


Figure S5 Related to Figure 5

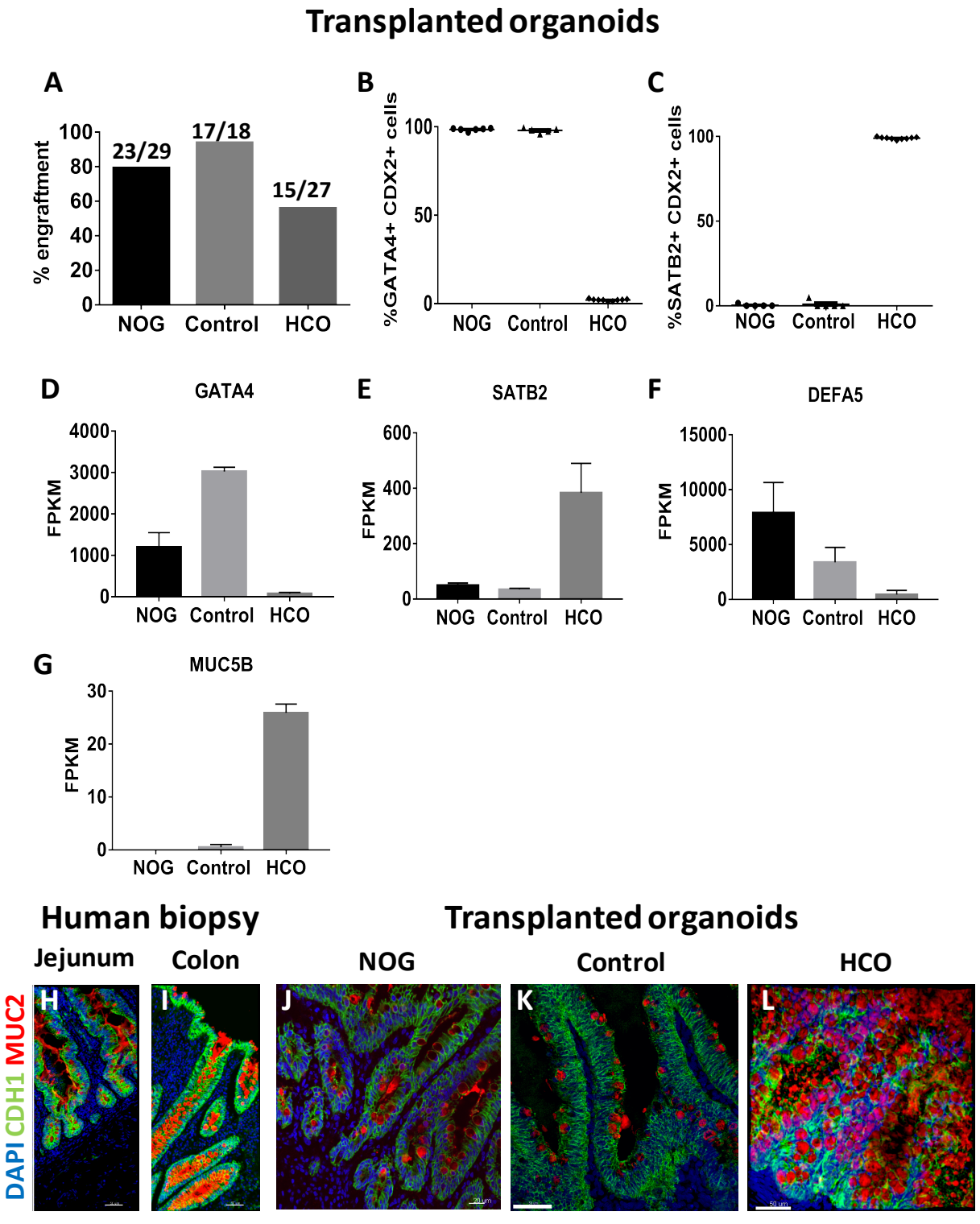


Figure S6 Related to Figure 6

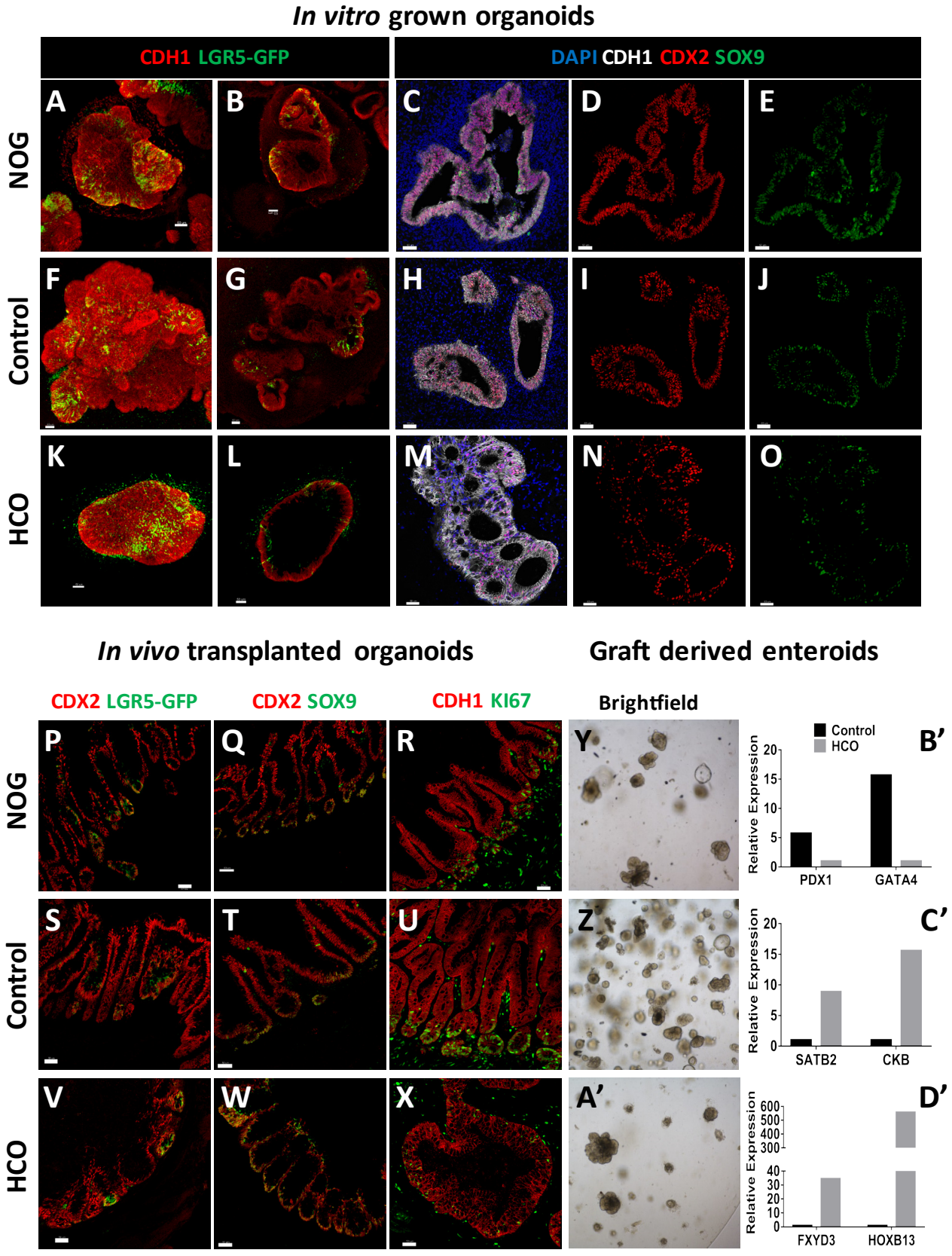
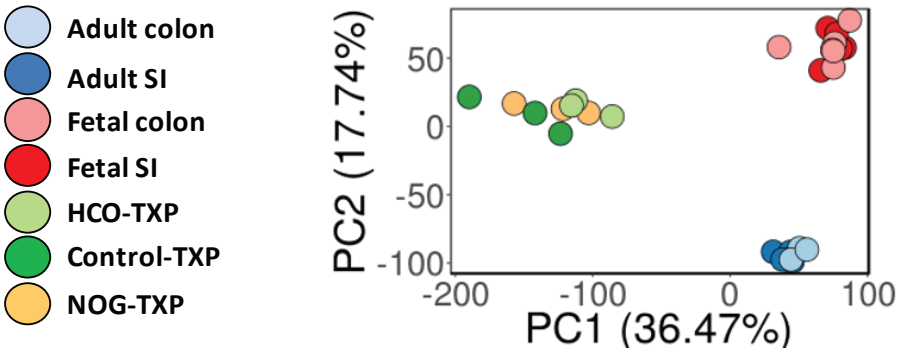


Figure S7 Related to Figure 7

A



B

Up in TXPs 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-46
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

C

Up primary tissues vs in TXPs

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 organismal cell-cell adhesion	8.69E-18
GO: Biological Process	immune response	8.81E-18
GO: Biological Process	intracellular signal transduction	2.82E-17

Table S2. List of QPCR primers used in this study. Related to Figures 3 and 4.

GENE	Sequence
CDH1 FWD	GACCGGTGCAATCTTCAAA
CDH1 REV	TTGACGCCGAGAGCTACAC
CHGA FWD	TGTGTCGGAGATGACCTCAA
CHGA REV	GTCCTGGCTCTTCTGCTCTG
CKB FWD	CCCACACCAGGAAGGTCTTA
CKB REV	CCTCTTCGACAAGCCCGT
FXYD3 FWD	AGGGTCACCTTCTGCATGTC
FXYD3 REV	CTTCGGATAAACGCAGGACT
GATA4 FWD	TAGCCCCACAGTTGACACAC
GATA4 REV	GTCCTGCACAGCCTGCC
HOXA13 FWD	GCACCTTGGTATAAGGCACG
HOXA13 REV	CCTCTGGAAGTCCACTCTGC
HOXB13 FWD	GCTGTACGGAATGCGTTTCT
HOXB13 REV	AACCCACCAGGTCCCTTTT
HOXD13 FWD	CCTCTTCGGTAGACGCACAT
HOXD13 REV	CAGGTGTACTGCACCAAGGA
HOXD3 FWD	CACCTCCAATGTCTGCTGAA
HOXD3 REV	CAAAATTCAAGAAAACACACACA
INSL5 FWD	GAAGGTTTTGCGCTGGATT
INSL5 REV	GATCCCTCAAGCTCAGCAAG
MSX2 FWD	GGTCTTGTGTTTCCTCAGGG
MSX2 REV	AAATTCAGAAGATGGAGCGG
MUC2 FWD	TGTAGGCATCGCTCTTCTCA
MUC2 REV	GACACCATCTACCTCACCCG
ONECUT1 Fwd	TTTTTGGGTGTGTTGCCTCT
ONECUT1 Rev	AGACCTTCCGGAGGATGTG
PDX1 FWD	CGTCCGCTTGTTCTCCTC
PDX1 REV	CCTTTCCCATGGATGAAGTC
PPIA (CPHA) FWD	CCCACCGTGTTCTTCGACATT
PPIA (CPHA) REV	GGACCCGTATGCTTTAGGATGA
SATB2 FWD	CCACCTTCCCAGCTTGATT
SATB2 REV	TTAGCCAGCTGGTGGAGACT

Table S3. List of antibodies used in this study. Related to Figures 1-6.

ANTIBODY	HOST	Catalog number	Dilution	Notes
B-Catenin	rabbit	Santa Cruz #sc-7199	1:200	
CDH17*	rabbit	Sigma #HPA023616	1:1,500	
Cdx2	mouse	BioGenex cdx2-88	1:300	
Cdx2	rabbit monoclonal	Cell Marque EPR2764Y	1:100	
Chr-A (C20)	goat	Santa Cruz #sc-1488	1:100	
DEFA5*	mouse monoclonal	Novus BiologicalsNB110-60002	1:60,000	
E-Cadherin	goat	R&D #AF648	1:400	
E-Cadherin (mouse-specific)	rat	R&D #MAB7481	1:500	
E-Cadherin	mouse	R&D #AF648	1:500	
FoxA2	goat	Santa Cruz #sc-6554	1:500	
GATA4	goat	Santa Cruz #sc-1237	1:100	
GATA4	rabbit	Santa Cruz #sc-9053	1:100	
GFP (green fluorescent protein)	rabbit	Invitrogen #A11122	1:1,000	
Ghrelin	goat	Santa Cruz #sc-10368	1:500	
GIP (Gastric Inhibitory Polypeptide)	goat	Santa Cruz #sc-23554	1:500	
GLP-1	mouse	BioVision #3104-100	1:200	
HNF-6 (ONECUT1)	rabbit	Santa Cruz #sc-13050	1:100	
INSL5 (H-110)*	rabbit	Santa Curz #sc-67190	1:100	
KI67	rabbit monoclonal	Cell Marque SP6	1:100	
Motilin	mouse monoclonal	Santa Cruz #sc-376605	1:100	
Mucin 5B*	rabbit	Santa Cruz #sc-20119	1:100	
Mucin2 (MUC2)	rabbit	Santa Cruz #sc-15334	1:200	
Peptide YY	rabbit	Abcam #ab22663	1:1000	

Table S3. List of antibodies used in this study. Related to Figures 1-6.

pSmad 1/5/8	rabbit	Cell Signaling 9511S	1:100	Discontinued and replaced with 13820S
pSmad 2/3	rabbit	Cell Signaling 9510S	1:100	
SATB2	rabbit monoclonal	Cell Marque EP281	1:100	
SATB2 (SATBA4610)*	mouse monoclonal	Santa Cruz #sc-81376	1:100	
Sox9	rabbit	Millipore #AB5535	1:10,000	
Alexafluor ® Donkey anti-goat 488	donkey	Life Technologies A-11055	1:500	
Alexafluor ® Donkey anti-goat 568	donkey	Life Technologies A-11057	1:500	
Alexafluor ® Donkey anti-mouse 568	donkey	Life Technologies A-10037	1:500	
Alexafluor ® Donkey anti-rabbit 647	donkey	Life Technologies A-31573	1:500	
Alexafluor ® Donkey anti-rat 488	donkey	Life Technologies A-21208	1:500	

Figure S1 Related to Figure 1. Gata4 and Satb2 mark discreet regional boundaries during development of the small and large intestines.

(A) Whole-mount staining of Gata4 (green) and Satb2 (red) in an e9.5 mouse embryo showing expression boundary at the yolk stalk (n=9). **(B)** Model depicting Gata4 and Satb2 expression domains e11.5 intestine showing a transitional zone of low Gata4 and low Satb2 expression. **(C-E)** Whole-mount staining of Gata4 and Satb2 in an e11.5 mouse embryo showing posterior boundary of Gata4 and anterior boundary of Satb2 at the yolk stalk (n=3). **(F-H)** Whole-mount staining of Satb2 and Foxa2 in an e12.5 mouse embryo showing that the anterior boundary of Satb2 expression is maintained (n=3). **(I)** Whole-mount staining of Gata4 and Satb2 in proximal intestine isolated from an e16.5 mouse embryo (n=6). **(J)** Whole-mount staining of Gata4 and Satb2 in distal small intestine and large intestine isolated from an e16.5 mouse embryo (n=6). Staining of GATA4 and SATB2 in section of **(K**) human jejunum (n=2) and **(L)** colon (n=2). Scale bars = 50 μm **(B-D)** and 100 μm **(E-M)**. Dotted lines in **(C)** and **(F)** mark the approximate location of the umbilicus. Abbreviations: ys, yolk stalk; cb, cecal bud; tz, transition zone; mx, maxilliary; and md, mandibular portion of first brachial arch; ti, terminal ileum; icj, ileocecal junction.

Figure S2 Related to Figure 1. SATB2 is expressed in GATA4 negative human small and large intestine.

(A) SATB2 staining in human adult duodenum, small intestine, appendix, colon and rectum showing that SATB2 expression is present in distal small intestine and the entire large intestine. **(B)** Analysis of GATA4 and SATB2 from published RNA-seq data from human adult and fetal intestinal samples. Samples plotted include human adult duodenum (HuSI_Duo_A), human adult small intestine distal to duodenum (HuSI_Dist_A), human adult colon (HuColon_A) and human fetal small intestine (HuSI_F). **(C)** Analysis of GATA4 and SATB2 expression from microarray data generated by Wang et al. 2015 on fetal intestinal stem cells from duodenum (Duo), jejunum (Jej), ileum (Ile), ascending colon (AC), transverse colon (TC) and Descending colon grown in Air Liquid Interface (ALI). r^2 values were determined using CORREL function in Excel. **(D)** 3D principal component (without Z-correction) of nascent spheroids and spheroids after 3 days of patterning.

Figure S3 Related to Figure 2. BMP mediates SHH activation of posterior HOX genes.

(A) Previous model of SHH-mediated activation of posterior HOX genes. **(B)** New model of SHH mediated activation of posterior HOX genes and BMP-mediated activation of endoderm HOX genes. **(C)** QPCR analysis of HOX factors following treatment with NOGGIN, control, Smoothened agonist (SAG), or BMP2 (n=3 per condition). **(D)** Model of BMP4 dependent activation of HOX13 genes induced by SAG. **(E)** QPCR analysis of HOXA13 in control, 5 μM SAG, 5 μM SAG + NOG and BMP2 treated organoids after 3 days. **(F)** Model of SHH independent activation of HOX13 genes induced by exogenous recombinant human BMP2. **(G)** QPCR analysis of HOXA13 in control, BMP, and BMP + Cyclopamine treated organoids after 3 days (n=6 per condition).

Figure S4 Related to Figure 3. Extended *in vitro* culture allows maturation of goblet cells.

(A) Quantitation of the percentage of CDX2+ SATB2+ cells in organoids which were patterned and were then re-patterned. QPCR analysis of HOXB13 **(B)** and HOXD13 **(C)** in 28 day old NOGGIN (n=6), Control (n=8) and BMP2 (n=5) organoids. **(D-F)** Whole-mount and **(G-I)** cross section staining with CDH1 (green), CDX2 (red), and MUC2 (white) from 44 day old NOGGIN, Control, and BMP treated organoids. **(J-L)** Staining of sections from 44 day old BMP2 treated organoids. White arrows points to goblet cells which were in the process of secreting Mucin 2. For IF a minimum of 10 organoids per condition were examined. Scale bars= 50 μm.

Figure S5 Related to Figure 5. BMP patterning of organoids is stable *in vitro* and *in vivo*.

(A) Efficiency of organoid engraftment of NOGGIN, Control, and BMP patterned organoids. Quantitation of the percentage of GATA4+ CDX2+ cells **(B)** and SATB2+ CDX2+ cells **(C)** in transplanted patterned organoids. FPKM values from RNA-seq data for GATA4 **(D)** SATB2 **(E)** DEFA5 **(F)** and MUC5B **(G)** in transplanted organoids (n=3 per condition). MUC2 (red) staining of **(H-I)** human jejunum and colon biopsies (n=2 per region) and **(J-L)** transplanted organoids (n=5 per condition). Scale bars= 50 μm.

Figure S6 Related to Figure 6. *In vitro* and *in vivo* grown organoids contain intestinal progenitors.

Representative whole-mount **(A,F,K)** and slice section **(B,G,L)** images of CDH1 and GFP from H9-LGR5-GFP derived organoids treated with NOGGIN, control, or BMP. CDX2 (red) and SOX9 (green) staining on sections from **(C-E)** NOGGIN, **(H-J)** control, or **(M-O)** BMP2 treated organoids (n=5 organoids per condition for each staining combination). Representative images of CDX2 and LGR5-GFP **(P,S,V)**, CDX2 and SOX9 **(Q,T, W)**, and CDH1 and KI67 **(R,U,X)** stained *in vivo* organoids derived from H9-LGR5-GFP organoids treated with NOGGIN, control, or BMP (n=3 organoids per condition). **(Y-A')** Stereomicrographs showing enteroids derived from NOGGIN, control or BMP transplants respectively. **(B'-D')** QPCR analysis of proximal and distal genes in control enteroids (>100 pooled enteroids from 2 transplants) and BMP2 treated colonoids (>50 colonoids from 1 transplant). Scale bars= 50 μm.

Figure S7 Related to Figure 7. Ribosome and immune cell signatures are differentially expressed between transplanted organoids and primary human tissues.

(A) Principal component analysis of patterned transplanted organoids and human adult and fetal small intestine and colon. **(B)** Gene ontology analysis of genes upregulated in transplants versus human primary tissues. **(C)** Gene ontology analysis of genes upregulated in human primary tissues versus transplants.

Table S1. Genes upregulated in adult small intestine and colon which are also upregulated in HIOs and HCOs respectively. Related to Figure 7.

Table S2. List of QPCR primers used in this study. Related to Figure 3 and 4.

Table S3. List of antibodies used in this study. Related to Figure 1-6.