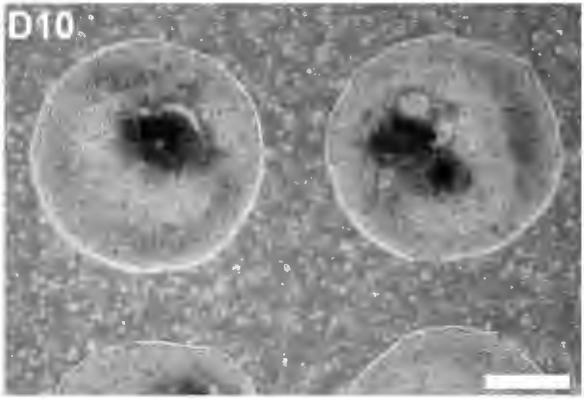
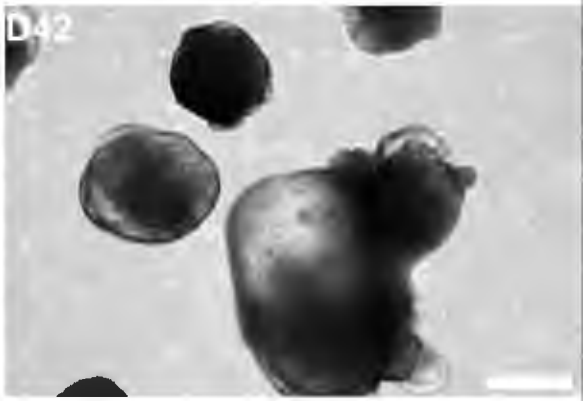
Supplemental Table. List of primers used for quantitative RT-PCR

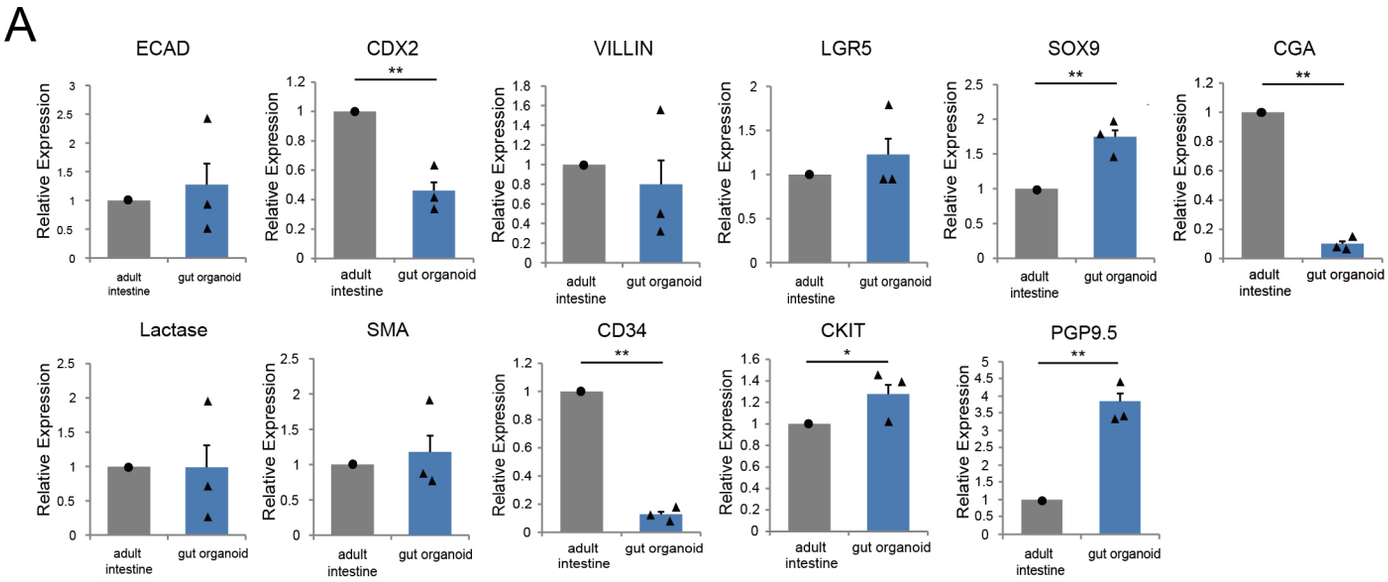
Gene	Forward primer (5′→3′)	Reverse primer (3′→5′)
ABCB1	AAGGCCTAATGCCGAACACA	GTCTGGCCCTTCTTCACCTC
ABCG2	TGTGGCATTAAACAGAGAAGAAGAC	TCACCCCCGGAAAGTTGATG
CD34	AGAAAGGCTGGGCGAAGACCCT	AGTGGGGAAGGGTTGGGCGT
CDX2	GGAACCTGTGCGAGTGGAT	TCGATATTTGTCTTTCGTCCTG
CKIT	TAAAGGTAACAACAAAGAGCAAATCC	AGGTCAGAATCATCACAATAATGCA
CXCR4	GGGCAATGGATTGGTCATCCT	TGCAGCCTGTACTTGTCCG
ECAD	ATTTTTCCCTCGACACCCGAT	TCCCAGGCGTAGACCAAGA
FOXA2	GGAGCAGCTACTATGCAGAGC	CGTGTTCATGCCGTTCATCC
GATA4	GTGTCCCAGACGTTCTCAGTC	GGGAGACGCATAGCCTTGT
GATA6	GTGCCAACTGTCACACCACA	GAGTCCACAAGCATTGCACAC
LCT	TGACCAATCCGAACACGGAG	CAGAGACCAGGCGACATACC
LGR5	CACCTCCTACCTAGACCTCAGT	CGCAAGACGTAACTCCTCCAG
OCT4	CGAGGAATTTGCCAAGCTCTGA	TTCGGGCACTGCAGGAACAAATTC
SLC15A1/PEPT1	CACCTCCTTGAAGAAGATGGCA	GGGAAGACTGGAAGAGTTTTATCG
PGP9.5	GTCCCCTGAAGACAGAGCAAA	TTCACCGGAAAAGGCATTCG
SMA	TGGCTTGGCTTGTCAGGGCTT	CCCGGGGGCTGTTAGGACCTT
SOX9	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
SOX17	GTGGACCGCACGGAATTTG	GGAGATTCACACCGGAGTCA
VIL1	CGGAAAGCACCCGTATGGAG	CGTCCACCACGCCTACATAG
Lysozyme	AAATACTGGGGCCAGCTCAC	GCCCTGGACCGTAACAGAAA
INS	CCAGCATCTGCTCCCTCTAC	TGCTGGTTCAAGGGCTTTAT
ALBUMIN	CGCTATTAGTTCGTTACACCA	TTTACAACATTTGCTGCCCA
T	TATGAGCCTCGAATCCACATAGT	CCTCGTTCTGATAAGCAGTCAC
SOX1	ACCAGGCCATGGATGAAG	CTTAATTGCTGGGGAATTGG
GAPDH	CTCACCGGATGCACCAATGTT	CGCGTTGCTCACAATGTTCAT

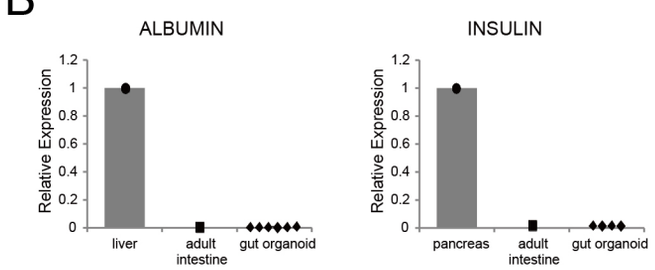
Supplemental Figure 1

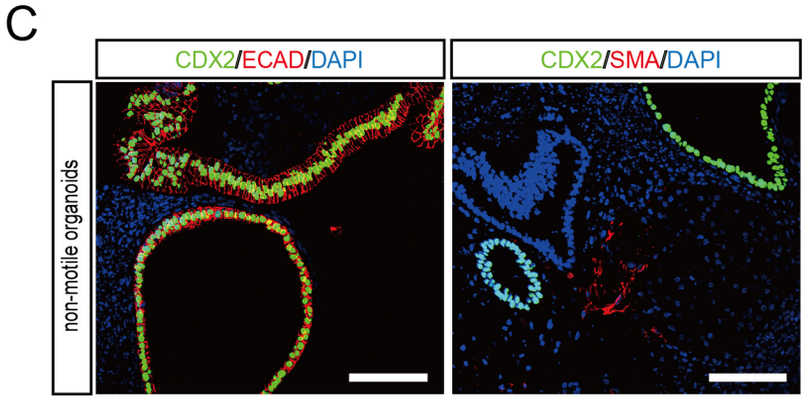


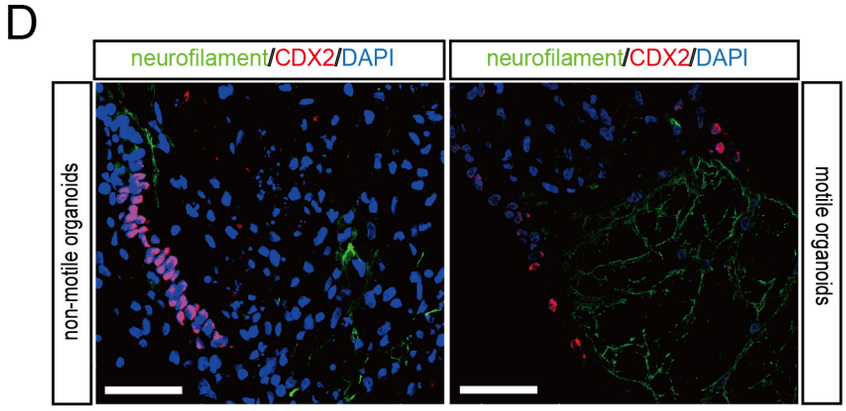


Supplementary Figure 2

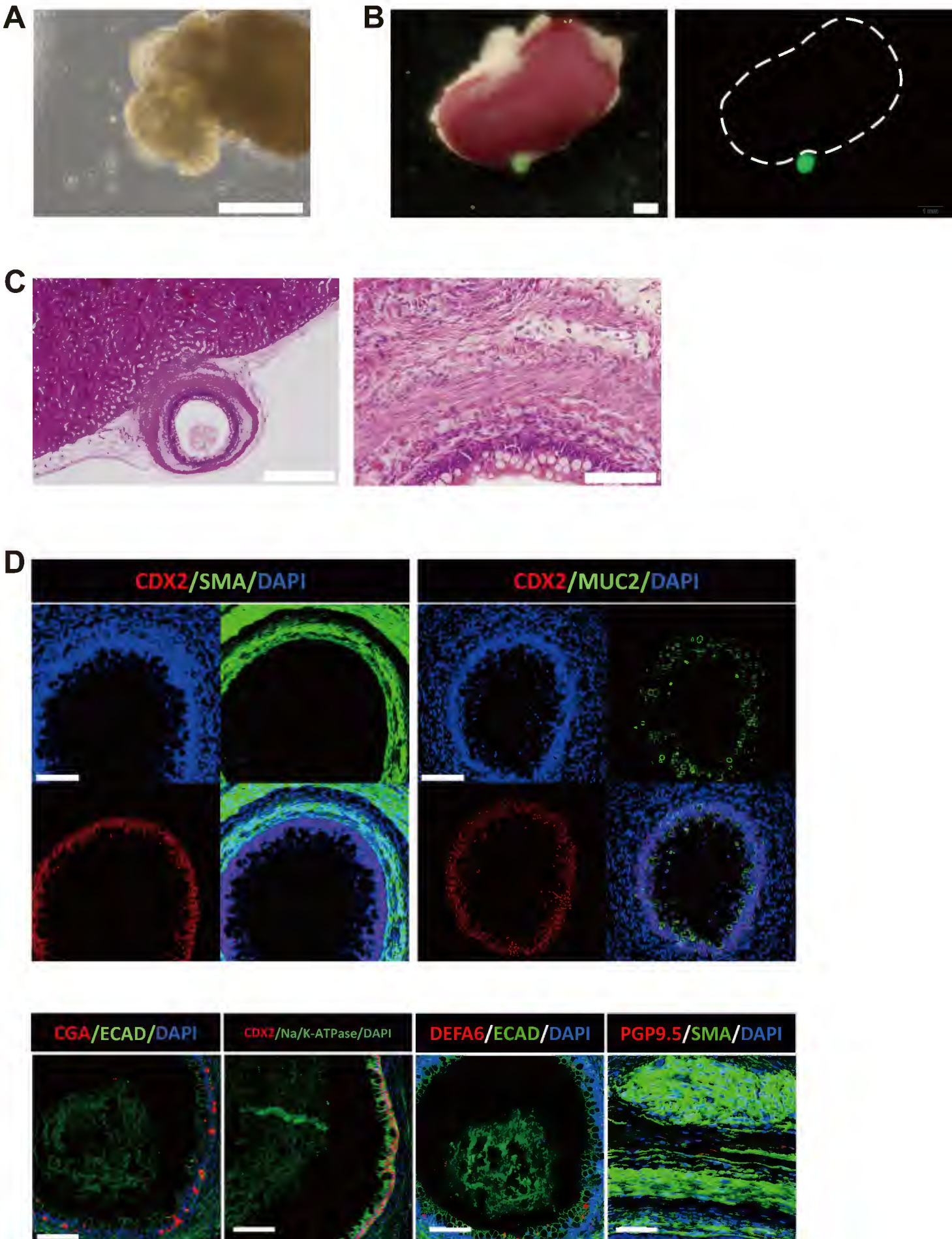








Supplemental Figure 3



Supplemental Figure 1. Time course of organoid growth from human iPSCs in culture. Human induced stem cells (hiPSCs) attached and grew only within each zone at D10. Scale bar: 500 μm. (B) The organoids from hiPSCs at D42 are self-organized cystic spheroids. Similarly to hESCs-gut organoids, these belong to simple cystic forms with thin cellular walls; and bi-component spheroids with solid and partially cystic protrusions. Scale bar: 200 μm.

Supplemental Figure 2. Characterization of non-motile gut organoids. (A) Analysis of expression of cell biomarkers genes in differentiated non-motile organoids (D52). Values were normalized against GAPDH. Relative expression of cell biomarker genes in the non-motile organoids and human adult small intestine. Statistical analysis was performed using a t-test or a Mann-Whitney rank sum test (* $P < 0.05$, ** $P < 0.01$). The data are reported as means (%) ± SEM from three independent experiments. ($n = 3$). (B) Non-motile organoids were examined for ALBUMIN and INSULIN expression levels. Gut organoids showed almost no detectable expression. Single organoids provided cDNA and three independent samples were prepared. The data were obtained from three independent experiments. (n=3-6). (C) Non-motile organoids immunostained with markers for CDX2, E-Cadherin (ECAD), and a-smooth muscle actin (SMA). Cell

nuclei were counterstained with DAPI. Non-motile organoids showed CDX2 and ECAD positive epithelial layers, but a very low level of SMA in the mesenchymal area. Scales bars: 100 μm. (D) Non-motile and motile organoids immunostained for neurofilaments and CDX2. Cell nuclei were counterstained with DAPI. Motile organoids showed neuron networks with neurofilaments in the mesenchymal area; however, little evidence of neurofilaments was found in non-motile organoids. Scale bar: 50 μm.

Supplemental Figure 3. Engrafted gut organoids (D35) displayed highly structured intestinal tract morphology. (A) Single organoids were transplanted under the kidney capsules of adult immunodeficient mice. Scale bar: 200 μm. (B) Six weeks after engraftment, the organoid formed a compact EGFP-expressing structure; the size of the engraftment was compared to that of the mouse kidney (dotted white line in the right panel). Scale bars: 1 mm. (C) Hematoxylin and eosin (HE) staining of the engrafted gut organoid; note the lumen and laminated appearance. Scale bars on the left panel: 500 μm and on the right panel 100 μm.(D) The engrafted organoid developed into a laminated myenteric gut (α-SMA staining); MUC2 and CDX2 were expressed in the epithelial layer. The distribution of E-cadherin (ECAD) and Na+/K+-ATPase in highly

differentiated enteroendocrine cells positive for chromogranin A (CGA) and Paneth cells positive for DEFA6 is shown; the expression of enteric neuronal marker PGP9.5 was also observed in the α-SMA-positive laminated mesenchyme. Scale bars on the upper panel: 100 μm and on the lower panel 50 μm.

Supplemental Video 1. Peristalsis-like movements of a D54 hESC-derived organoid in culture. Organoid contractions were recorded using an inverted microscope. Playback speed, 10 times actual.

Supplemental Video 2. Modulation of gut organoid contractile activity in culture by pharmacological agents. hESC-derived contracting gut organoids (D80–90) were treated with peristalsis stimulator histamine (0.2 μM) and cholinergic antagonist atropine sulfate (0.2 μM), and gut contractility response was recorded using an inverted microscope. Playback speed, 20 times actual.

Supplemental Video 3. Non-motile gut organoid in culture by histamine administration. hESC-derived non-motile gut organoids (D80–90) were treated with peristalsis stimulator histamine (0.2 μM) and gut contractility response was recorded using an inverted microscope. Playback speed, 16 times actual.

Supplemental Video 4. Forskolin-induced gut organoid swelling. Organoids derived from EGFP-SEES1 cells were used to visualize volume changes. Organoid morphology was monitored by time-lapse fluorescence laser confocal microscopy (Keyence) following forskolin treatment. This movie shows time-lapse images of swelling organoid. The time-lapse covers a period of 19 min. Selected frames from this movie are shown in Figure 6B.