

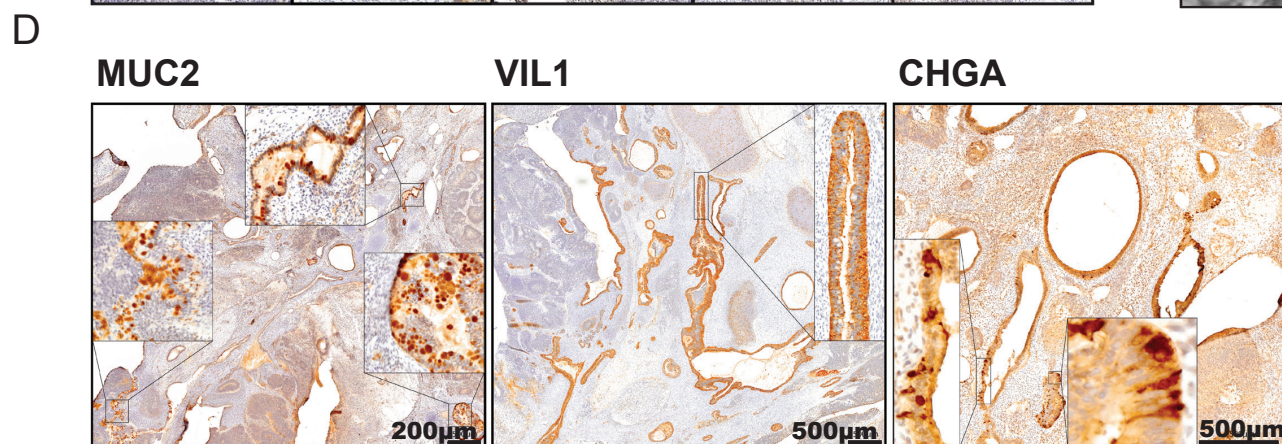
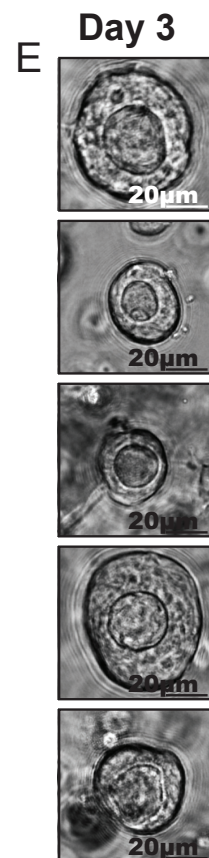
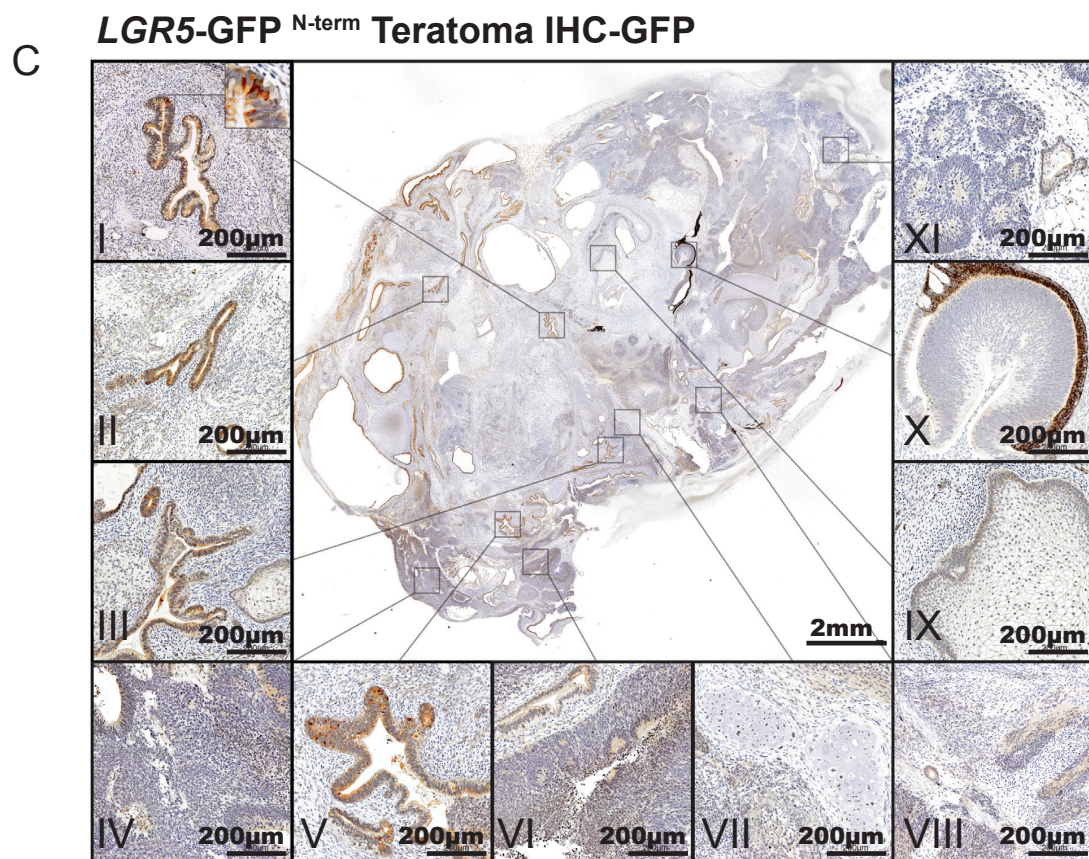
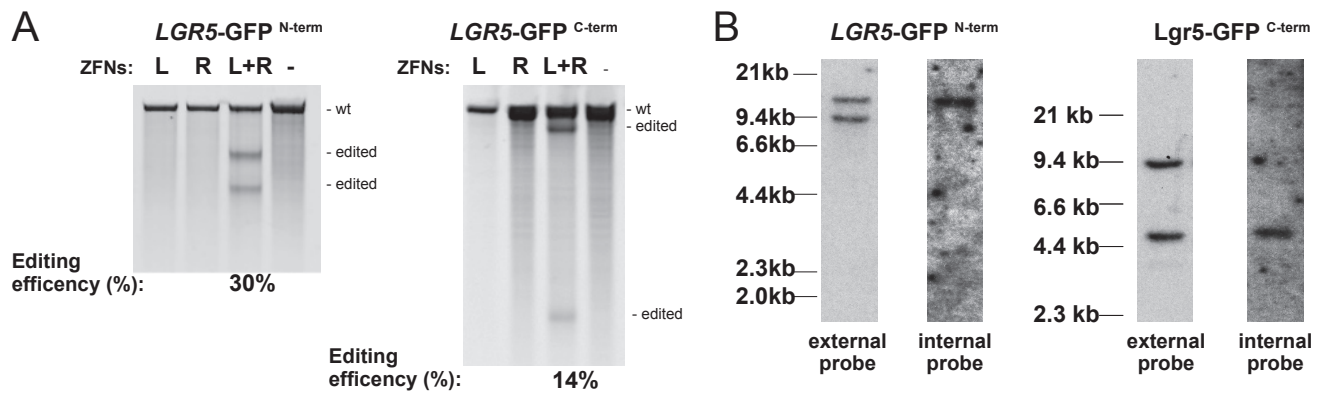
**Stem Cell Reports, Volume 2**

**Supplemental Information**

# **Human Intestinal Tissue with Adult Stem Cell Properties Derived from Pluripotent Stem Cells**

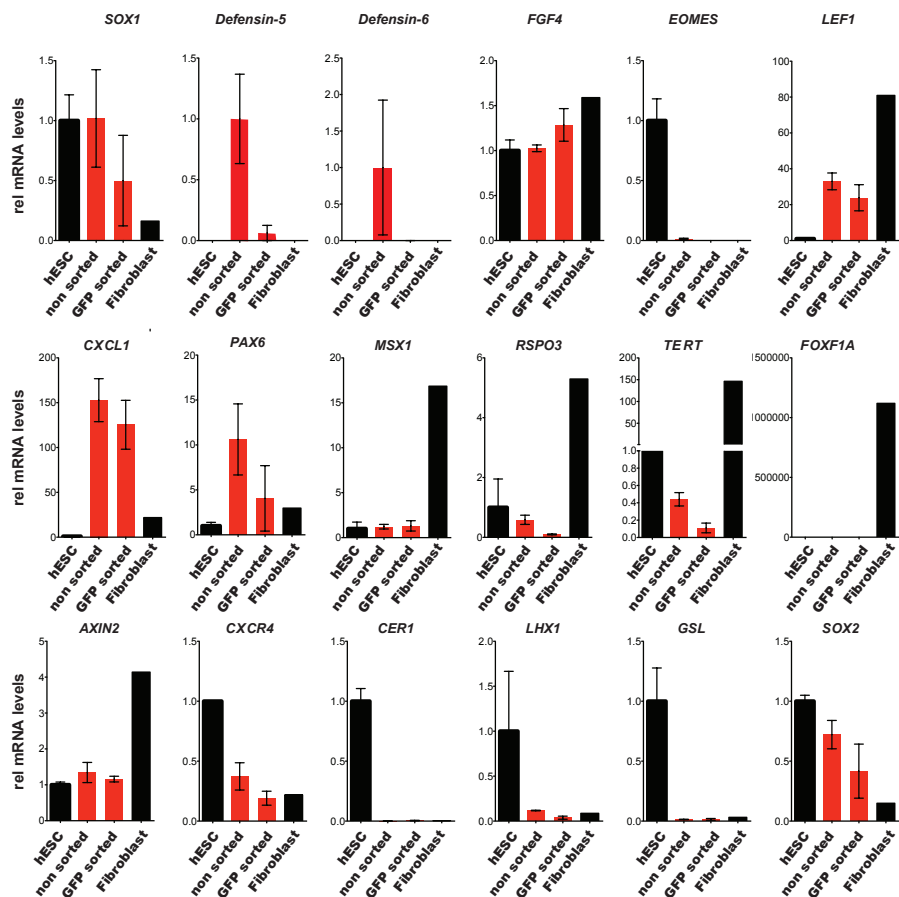
**Ryan Forster, Kunitoshi Chiba, Lorian Schaeffer, Samuel G. Regalado, Christine S. Lai, Qing Gao, Samira Kiani, Henner F. Farin, Hans Clevers, Gregory J. Cost, Andy Chan, Edward J. Rebar, Fyodor D. Urnov, Philip D. Gregory, Lior Pachter, Rudolf Jaenisch, and Dirk Hockemeyer**

Figure S1



# Figure S2

## A



\*All q PCR data are biological replicates n=3, bars represent the SEM

## B

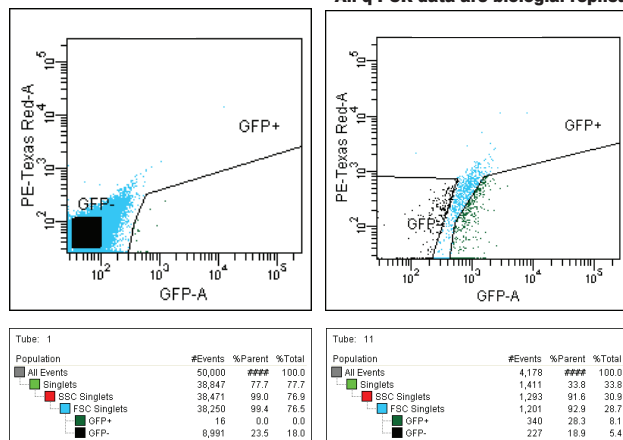


Figure S3

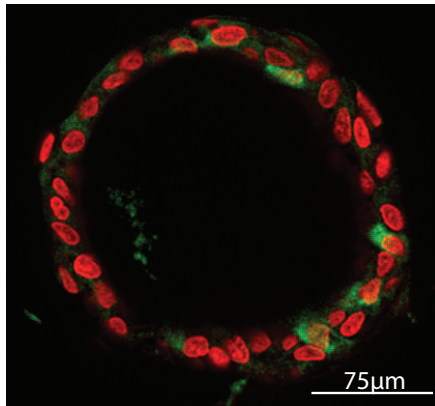




Figure S4

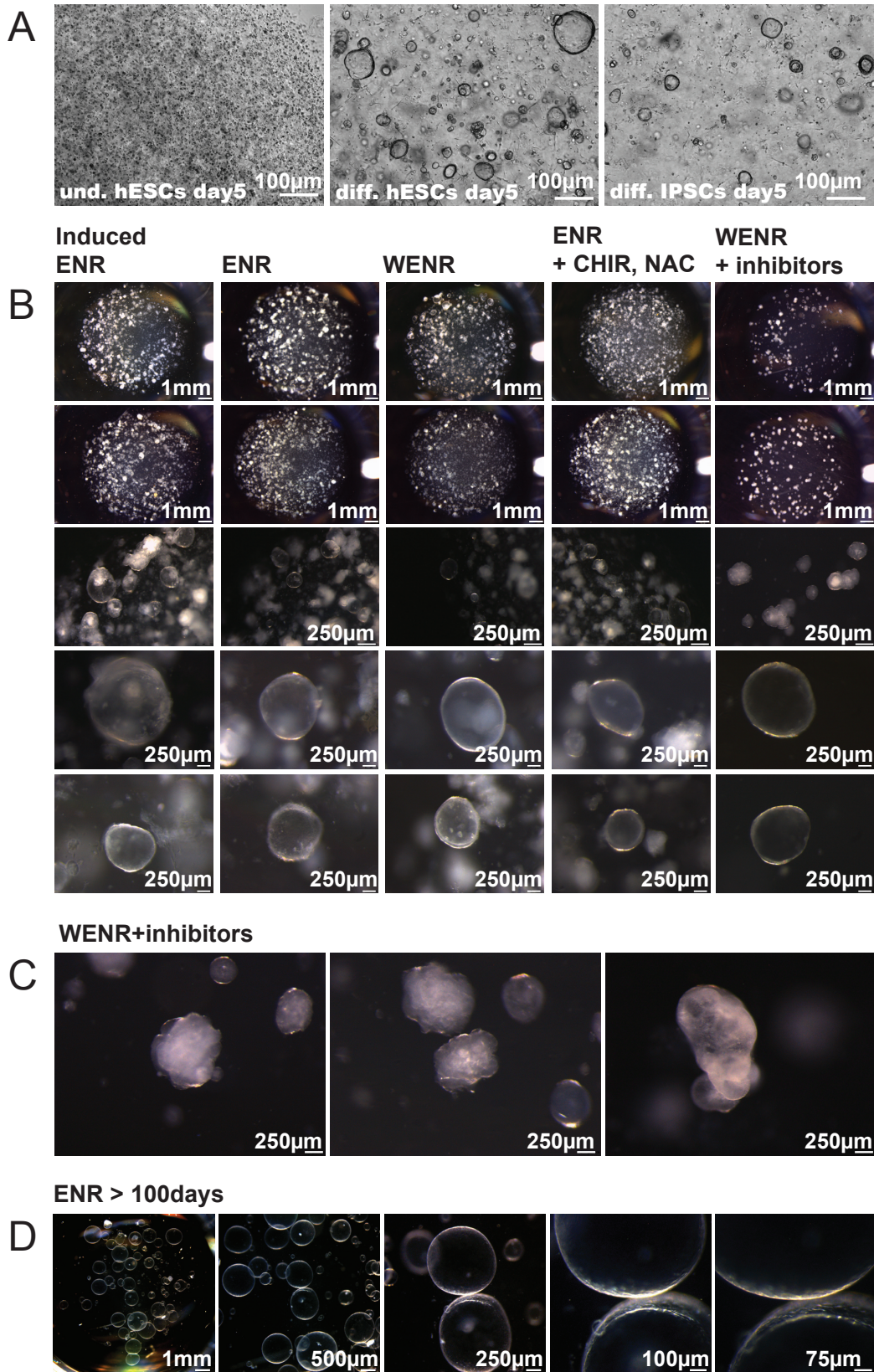
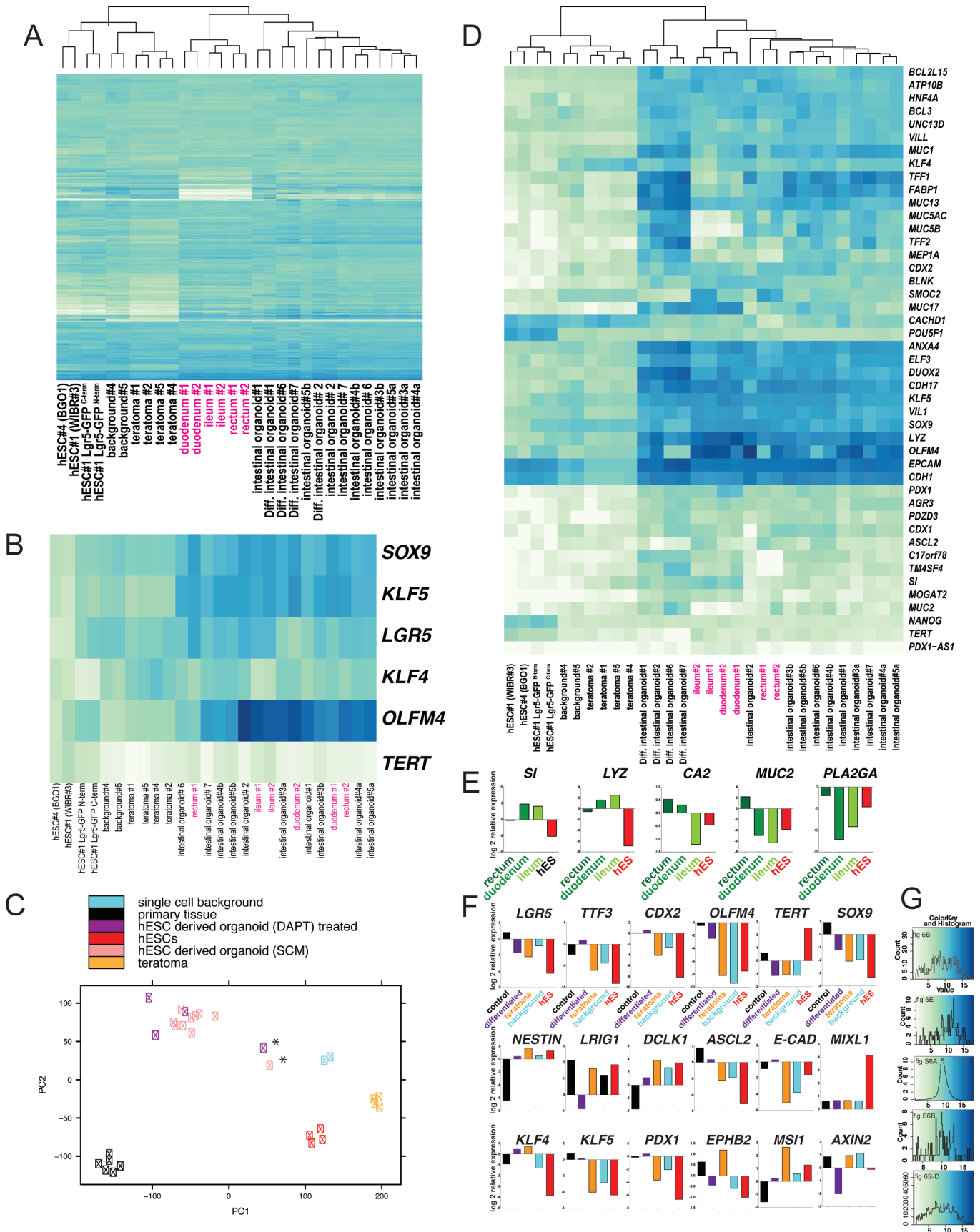


Figure S5



Supplemental Table 1

Sample	Tissue	Genetic Background	Group	Growth Cond.
duodenum #1	primary organoid	wt human biopsy	NA	*WENR+ inhibitors
duodenum #2	primary organoid	wt human biopsy	NA	*WENR+ inhibitors
ileum #1	primary organoid	wt human biopsy	NA	*WENR+ inhibitors
ileum #2	primary organoid	wt human biopsy	NA	*WENR+ inhibitors
rectum #1	primary organoid	wt human biopsy	NA	*WENR+ inhibitors
rectum #2	primary organoid	wt human biopsy	NA	*WENR+ inhibitors
intestinal organoid#1	hESC-Organoid	WIBR3- N-term	1	GF-Media
Diff. intestinal organoid#1	hESC-Organoid	WIBR3- N-term	1	**GF -Media+DAPT
hESC#1 LGR5-GFP N-term	hES	WIBR3- N-term	1	hES MEDIA
intestinal organoid# 2	hESC-Organoid	WIBR3- C-term	2	GF-Media
Diff. intestinal organoid# 2	hESC-Organoid	WIBR3- C-term	2	**GF -Media+DAPT
hESC#1 LGR5-GFP C-term	hES	WIBR3- C-term	2	hES MEDIA
hESC#1 (WIBR3)	hES	WIBR3	3	hES MEDIA
intestinal organoid#3a	hESC-Organoid	WIBR3	3a	GF-Media
teratoma #4	Teratoma	WIBR3-AI9	4	Mouse
intestinal organoid#4a	hESC-Organoid	WIBR3-AI9	4a	GF-Media
teratoma #5	Teratoma	WIBR3-AI9	5	Mouse
intestinal organoid#5a	hESC-Organoid	WIBR3-AI9	5a	GF-Media
teratoma #1	Teratoma	WIBR3	1	Mouse
teratoma #2	Teratoma	WIBR3	2	Mouse
intestinal organoid#3b	hESC-Organoid	WIBR3	3b	GF-Media
intestinal organoid#4b	hESC-Organoid	WIBR3-AI9	4b	GF-Media
intestinal organoid#5b	hESC-Organoid	WIBR3-AI9	5b	GF-Media
intestinal organoid# 6	hESC-Organoid	BGO1	6	GF-Media
Diff. intestinal organoid#6	hESC-Organoid	BGO1	6	**GF -Media+DAPT
intestinal organoid# 7	hESC-Organoid	BGO1	7	GF-Media
Diff. intestinal organoid#7	hESC-Organoid	BGO1	7	*GF -Media+DAPT
background#4	Non-Organoid	WIBR3-AI9	4	GF-Media
background#5	Non-Organoid	WIBR3-AI9	5	GF-Media
hESC#6-7 (BGO1)	hES	BGO1	6,7	hES MEDIA

Footnotes: RNA samples that were analyzed by RNA-seq (column 1). The second column (tissue) indicates the tissue type of each RNA sample. RNA from primary organoid samples was isolated from organoid lines that were cultured for 1-6 months and derived from duodenum, ileum, or rectum biopsies of human subjects.

hESC-derived organoids, hESC and teratoma indicates RNA samples that were isolated from these steps in the differentiation protocol. Non-organoid material indicates single-cell contaminants that were removed by the enrichment protocol described as “single cell background” in the text. The third column indicates the “*Genetic Background*” of the samples in respect to the parent tissue. hESC-derived organoids were isolated from 5 genetically distinct hESC lines: WIBR3, WIBR3 Lgr5-GFP<sup>N-term</sup>, WIBR3 Lgr5-GFP<sup>C-term</sup>, WIBR3-AI9, and BGO1. Column 4 shows the correspondence of independently derived teratomas (1-7) to the parental hESCs and derived organoid samples and shows biological replicates (indicated a or b).

The last column shows the specific growth conditions as described in the methods section with GF equivalent to the stem cell growth factors except when differentiation required WNT3a withdrawal. Media called \*WENR+inhibitors elsewhere was prepared as described previously (Sato et al., 2011).

\*\*GF-Media+DAPT was GF-Media supplemented with 10 $\mu$ M DAPT and absent Wnt3a for five days.



### **Supplemental Figure 1: Genome editing of LGR5 locus in WIBR3 hESCs, Teratoma histology and organoid formation**

- (A) Validating genome-editing activity of engineered ZFNs targeting LGR5 (LGR5<sup>N-term</sup> and LGR5<sup>C-term</sup>) in K562 cells. Following transient transfection of the ZFN expression construct, the sequence of the LGR5 locus surrounding the respective ZFN cut sites was PCR-amplified, and genome editing efficiency was measured using the Surveyor nuclease assay. The degree of target locus disruption was quantified and is shown below the lane.
- (B) Southern blot analysis of WIBR3 hESCs targeted in the LGR5 locus with the respective donor plasmid shown in Figure 1A. Genomic DNA was digested with HindIII for LGR5-GFP<sup>N-term</sup> targeted cells and with EcoRV for LGR5-GFP<sup>C-term</sup> targeted cells. DNA was hybridized with the <sup>32</sup>P-labeled external 3'-probe or with the internal eGFP probe.
- (C) Immunohistochemical staining for indicated proteins in teratoma sections generated from LGR5-GFP<sup>N-term</sup> hESC reporter cells (GFP= enhanced green fluorescent protein, large panel shows whole teratoma section with regions of interest showing GFP positive cells in intestinal regions (I-III and V) or other cell types where interpretable [IV: not specified (NS), VI: NS, VII: cartilage, VIII: NS, IX: NS, X: ectoderm/neuroepithelium, XI: neural rosettes]).
- (D) Immunohistochemical staining for MUC2= mucin2, VIL1= villin1, and CHGA= Chromogranin A in the context of lower magnification images of teratoma sections.
- (E) Higher magnification images of independently derived organoids (n=5) on the third day after embedding single-cell dissociated teratoma samples into matrigel. Image shows representative pictures of organoids that form a central lumen at day three.

### **Supplemental Figure 2: Isolation of intestinal organoids independent of the LGR5-GFP reporter system**

- (A) Expression profiling of organoids from GFP positive and unsorted LGR5-GFP teratoma cells compared to hESCs and fibroblast-like cells. Quantitative RT-PCR for the indicated genes in hESCs, organoids derived from eGFP-positive cells (n=3) or unsorted (n=3) cells of LGR5-GFP<sup>N-term</sup>, hESCs and fibroblast-like cells [derived from hESCs, as described previously (Hockemeyer et al., 2008), expressing telomerase from the AAVS1 locus]. Relative expression levels were normalized to expression levels of these genes in hESCs. Data are biological replicates from independent experiments, bars represent the SEM.
- (B) Left: Representative FACS analysis (top) and sorting statistics of LGR5-GFP<sup>N-term</sup> hESCs isolated from teratoma explants. Gates were chosen based on undifferentiated hESC cells. Right: Representative FACS analysis (top) and sorting statistics of LGR5-GFP<sup>N-term</sup> hESCs isolated from teratoma explants not sorted for GFP, but cultured for 5 days prior to this analysis in organoid culture conditions. FACS settings were identical in both plots.

### **Supplemental Figure 3: hESC-derived organoids contain Lysozyme positive cells indicating the presence of Paneth cells.**

Immunofluorescence staining of a hESC-derived human intestinal organoid stained for Lysozyme (green) and DAPI (red) by confocal microscopy (see also Supplemental movie 1).

### **Supplemental Figure 4: hESCs and iPSCs can form intestinal organoid cultures when differentiated into a teratoma but not when directly embedded into matrigel.**

- (A) Left- Undifferentiated hESCs after 5 days grown under intestinal stem cell conditions and embedded into matrigel. Middle panel and right panel show teratoma cells isolated from hESCs and iPSCs after 5 days— grown in parallel under identical conditions.
- (B) Increasing magnification top to bottom shows LGR5-GFP N- and C- term derived teratoma cells assayed for the ability to form organoids. Conditions were adapted as described by

- (Spence et al., 2011). Cells labeled “Induced” ENR were cultured for 4 days in 500ng/ml FGF4 and 500ng/ml WNT3a (as described in (Spence et al., 2011) and then continuously cultured in ENR. Cells labeled ENR and WENR were cultures as described in methods; ENR/CHIR/NAC as described by (Wang et al., 2013); or WENR + inhibitors as described by (Sato et al., 2011).
- (C) A fraction of organoids in “WENR + inhibitors” media showed morphological changes not observed in other conditions, but did not explicitly bud into crypt-like structures after >30 days in culture.
  - (D) Long-term cultures (>100 days shown) of organoids grown in WENR (not shown) or ENR persist and exhibit increasingly uniform morphology over time.

### **Supplemental Figure 5: hESC-derived organoids share a transcriptional profile with primary intestinal tissue derived organoids and display the characteristic responses to differentiation stimuli**

- (A) Heat map of hESCs (n=4), teratoma samples (n=4), bulk hESC-derived organoid cultures grown in WENR (n=10), bulk cultures of hESC-derived organoids grown in differentiation medium (n=4), and primary- duodenum (n=2), rectum (n=2) or ileum (n=2) derived organoids. Shown are the Euclidian distances calculated from the expression of the top 5000 differentially expressed transcripts.
- (B) Heat map as in S5A showing selected intestinal stem cell marker genes.
- (C) First two components of principal component analysis of expression data from all samples, labeled by sample type. Samples indicated with \* were contaminated with non-organoid teratoma cells “single cell background cells” (organoids #1 and organoids #1-differentiated). Early analysis of sample #1 showed unexpected expression; however, subsequent organoids were cultured more stringently as described in methods. “Single cell background” is the RNA isolated from the non-organoid teratoma cells that can be removed partially (please see Supplemental methods and additional discussion of the RNA-seq data) but might represent some residual contamination.
- (D) Heat map displaying unbiased cluster analysis of the samples analyzed in Figure 6B, but for an extended subset of genes with intestine specific expression and function. Shown are the Euclidian distances calculated from the relative expression of the genes as determined by RNA-seq.
- (E) Intermediate DESeq calculation of RNA-seq results. Shown is the log2 fold change of three types of human-derived intestinal samples and parental hESCs relative to organoids cultured in WENR media (please see supplemental methods).
- (F) Intermediate DESeq calculation of RNA-seq results. Shown is the log2 fold change of intestinal stem cell marker genes shown in Figures 2 and S2 for control (all human primary derived samples), differentiated (ENR+DAPT), parental hES, teratoma, and the background (non-organoid cells removed from the cultures) relative to organoids cultured in WENR.
- (G) Histograms for heat maps generated for Figures 6B, 6E, S5A, S5B, and S5D.

### **Supplemental Table 2: RNA-seq Data**

Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values for all RNA-seq samples generated as described in materials and methods. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar, Domrachev, & Lash, 2002) and are accessible through GEO Series accession number GSE56930  
<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56930>

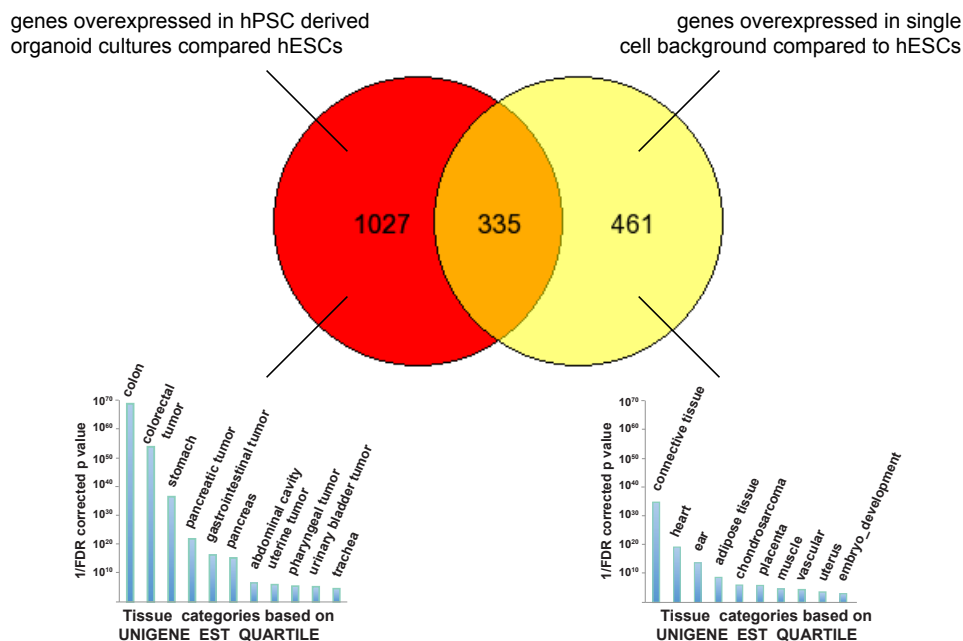
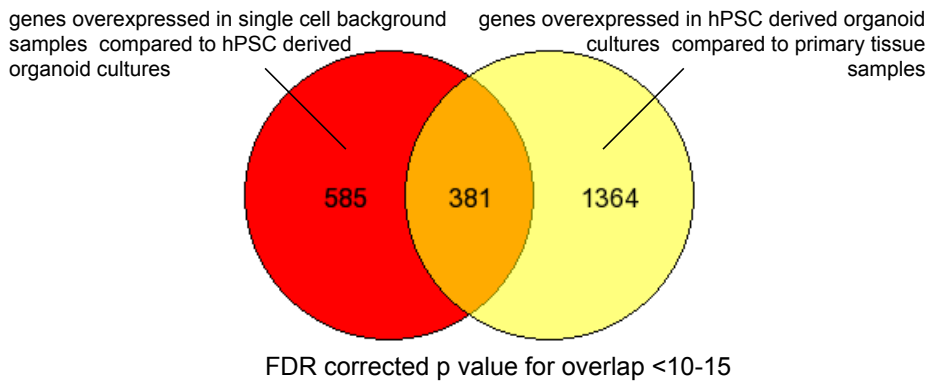
**Supplemental Movie1: hESC-derived organoids contain lysozyme positive cells indicating the presence of Paneth cells**

Movie of sequential Z-stack of a hESC-derived human intestinal organoid stained for lysozyme (green) and DAPI (red). Please see S3.

## Additional discussion of the transcriptional profiling data:

### Single cell contaminants can account largely for the expression differences between the hESC derived organoids and primary tissues samples

We also analyzed the genes that were differentially expressed between the human primary-derived samples and the hESC-derived organoids (Supplemental material). Unexpectedly we found a significant enrichment of genes overexpressed in hESC-derived organoid samples, particularly organoid #1 (please see S5C, organoid #1 is indicated with an asterisks). We hypothesized that this difference in gene expression was the result of small amounts of non-organoid differentiated teratoma cells contaminating the hESC-derived organoids. To test this we separated organoids from these single cells and performed transcriptional profiling of both hESC-derived organoids and these single-cell contaminants ("single cell background") that are normally discarded. Indeed, transcriptional profiling of these contaminating cells showed elevated expression of genes with non-intestinal ontology that can largely explain the transcriptional differences between hPSC-derived organoids and primary tissue cultures. Cultures subsequent to the derivation organoid sample #1 were passaged more stringently against the non-enteric teratoma cells as described in the methods and aligned much closer to the profiles of human-derived samples.





**Top:** Venn diagram depicting the overlap of genes significantly overexpressed ( $p < 0.05$  and  $2^{2.5}$ -fold upregulation) in single cell background samples compared to hPSC-derived organoid cultures and genes overexpressed in hPSC-derived organoid cultures compared to primary tissue samples. Red circle indicates total number of genes overexpressed in background samples (non-organoid outgrowth of teratoma cells) compared to hPSC-derived organoid cultures. Yellow circle indicates total number of genes overexpressed in hPSC-derived organoid cultures compared to primary-derived tissue samples. The high amount of overlap between both gene lists suggests that a significant fraction of the difference between hPSC-derived organoid cultures and primary-derived intestine tissue comes from contamination by background material. P-value for overlap was calculated using a hypergeometric distribution and found to be less than  $10^{-15}$ .

**Bottom:** Venn diagram depicting the overlap of genes significantly overexpressed ( $p < 0.05$  and  $2^{2.5}$ -fold upregulation) in hPSC-derived organoid samples compared to hPSCs as well as genes overexpressed in single-cell background samples compared to hPSC samples. Red circle indicates total number of genes overexpressed hPSC-derived organoid samples compared to hPSCs. Yellow circle indicates total number of genes overexpressed in single-cell background samples compared to hPSC samples. Shown below is the tissue specific expression gene ontology analysis for these genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.abcc.ncifcrf.gov/home.jsp>) and the "UNIGENE\_EST\_QUARTILE" expression profile databases. Analyzed were genes significantly (FDR corrected p value  $< 0.05$ ) unregulated ( $> 2^{2.5}$  fold) in hESC-derived intestinal organoids ( $n=10$ ) compared to hESCs ( $n=4$ ).



## **Supplementary Methods**

### **ZFN-driven targeted genetic engineering of the LGR5 locus in hESCs and hiPSCs using ZFN-mediated homologous recombination**

hESCs and hiPSCs were cultured in Rho Kinase (ROCK)-inhibitor (Calbiochem; Y-27632) 24 hours prior to electroporation. Cells were harvested using 0.25% trypsin/EDTA solution (Invitrogen), and  $1 \times 10^7$  cells were resuspended in phosphate buffered saline (PBS) and electroporated with 40  $\mu$ g of donor plasmids (previously described in (Hockemeyer et al., 2009) or designed and assembled by D.H.) and 5  $\mu$ g of each ZFN or TALEN encoding plasmid (Gene Pulser Xcell System, Bio-Rad: 250 V, 500 $\mu$  F, 0.4 cm cuvettes). Cells were subsequently plated on MEF feeder layers (DR4 MEFs for puromycin selection) in hESC medium supplemented with ROCK-inhibitor for the first 24 hours. Individual colonies were picked and expanded after puromycin selection (0.5  $\mu$ g/ml) 10 to 14 days after electroporation. hESC-derived fibroblast-like cells used as negative controls for the expression analysis shown in Figure 2 and Supplemental Figure 2 were generated from hESCs overexpressing hTERT from the AAVS1 locus (unpublished data) by differentiation as previously described in (Hockemeyer et al., 2008).

### **ZFN design and ZFN expression plasmids**

ZFNs against the human LGR5 locus were designed using an archive of validated two-finger modules (Perez et al., 2008; Urnov et al., 2005); complete sequences of the ZFNs, which carried obligate heterodimer forms of the FokI endonuclease (Miller et al., 2007).

### **ZFN target sites**

ZFN Target site and corresponding recognition alpha helices (NH<sub>2</sub> to COOH) engineered against the LGR5 locus were as follows: N-term L ZFN target– AATGACAGTGTGTGGGGC, DRSHLTR, RSDHLTT, RSDSLLR, LQHHLTD, DRSNLSR, LRQNLIM; N-term R ZFN target– CCGACGGCAGGA<sub>t</sub>GTTGCT, QSSDLSR, YKWTLRN, QSGHLAR, QSGDLTR, RSDTLSQ, RSDDRKK; C-term L ZFN target –ACAGTTTAATGGGGG, RSAHLSR, RSDHLST, QSANRTK, TSGSLSR, QSSVRNS; C-term R ZFN target– GGGGTCATCGCAGCAGTG, RSDSLSV, QSGDLTR, QSGDLTR, DTGARLK, DRSAISR, RSDHLTR. The ZFNs were tested by transient transfection into K562 cells followed by Surveyor (Cel-1) endonuclease based measurement of NHEJ at the target locus exactly as described (Miller et al., 2007; Perez et al., 2008).

## **hESC culture**

Cell culture was carried out as described previously (Soldner et al 2009). All hESC lines were maintained on a layer inactivated mouse embryonic fibroblasts (MEFs) in hESC medium (DMEM/F12 [Invitrogen] supplemented with 15% fetal bovine serum [Hyclone], 5% KnockOut™ Serum Replacement [Invitrogen], 1mM glutamine [Invitrogen], 1% nonessential amino acids [Invitrogen], 0.1 mM β-mercaptoethanol [Sigma], penicillin/streptomycin [Gibco], and 4 ng/ml FGF2 [R&D systems]). Cultures were passaged every 5-7 days either manually or enzymatically with collagenase typeIV (Invitrogen; 1.5 mg/ml).

## **Teratoma Isolation**

Subcutaneous hESC-derived teratomas were excised from NOD-SCID mice (Taconic) after 4-8 weeks, briefly washed in 70% EtOH, then PBS supplemented with penicillin/streptomycin. Tissues were minced and disaggregated in 10ml trypsin for 10-15 minutes, treated with 750,000U DNaseI (Roche), and suspended in 30ml of FBS-containing hES media followed by isolation of single cells through a 70µm nylon mesh filter (Falcon) for subsequent FACS or direct matrigel embedding for 3D cultures.

## **Organoid Reagents and Growth Factors**

Matrigel (BD Biosciences), Dispase (Stemcell Technologies), JAG-1 (AnaSpec; no. 61298), N2 (R&D Systems), B27 (Invitrogen), L-glutamine, NEAA (Gibco), WNT3A (R&D Systems), RSPO1 (R&D Systems), EGF (R&D Systems), Noggin (R&D Systems) and 10µM ROCK inhibitor (Stemgent; Stemolecule™ Y27632)

## **Immunocytochemistry**

Immunostaining of paraffin section was performed with standard techniques using the following antibodies: anti-GFP (Abcam 290), Sox9 (AB5535 Millipore), Mucin2 (H-300 Santa Cruz), Villin (C-19, sc-7672 Santa Cruz), chromogranin A (SP-1 ImmunoStar), EphB2 (AF467 R&D System), Epcam AF960 R&D systems, FABP1 (HPA028275 Sigma), E-cadherin 610181 BD), Lysozyme (Dako), CDX2 (CDX2-88 BioGenex), PDX1 (abcam® ab47267) and appropriate Molecular Probes Alexa Fluor® dye conjugated secondary antibodies (Invitrogen). Phalloidin was Alexa Fluor® 488 Phalloidin or Rhodamine Phalloidin (Molecular Probes – Life Technologies).

## **Electron microscopy analysis**

The material was fixed in 2.5% gluteraldehyde, 3% paraformaldehyde with 5% sucrose in 0.1M sodium cacodylate buffer (pH 7.4). When possible, the organoids were pelleted. Larger organoids



were carried through processing in mesh bottom baskets. Material was post-fixed in 1% OsO<sub>4</sub> in veronal-acetate buffer, stained en bloc overnight with 0.5% uranyl acetate in veronal-acetate buffer, dehydrated and embedded in Embed-812 resin. Ultra-thin sections were cut on a Reichert Ultracut E microtome with a Diatome diamond knife, stained with uranyl acetate, and lead citrate. The sections were examined using a FEI Tecnai spirit at 80KV and photographed with an AMT ccd camera.

### **RT-PCR analysis**

RNA was isolated using either the RNeasy Mini Kit (Qiagen) or Trizol extraction followed by ethanol precipitation. Reverse transcription was performed on 150ng of total RNA using oligo dT priming and Thermoscript reverse transcriptase at 50°C (Invitrogen). qRT-PCR was performed in an ABI Prism 7000 (Applied Biosystems) with Platinum SYBR green pPCR SuperMIX-UDG with ROX (Invitrogen) using primers that were in part previously described (Soldner et al., 2009).

#### qRT-PCR Primer sequences

SOX9F CTGAGCAGCGACGTCATCTC

SOX9R GTTGGGCGGCAGGTACTG

KLF4F ACCAGGCACTACCGTAAACACA

KLF4R GGTCCGACCTGGAAAATGCT

KLF5F CCCTTGACACATACACAATGC

KLF5R GGATGGAGGTGGGGTTAAAT

EOMESF AAGGCATGGGAGGGTATTAT

EOMESR AAACACCACCAAGTCCATCT

VillinF ACACAGGTGGAGGTGCAGAAT

VillinR GGTTGGTCGCTGTCCACTTC

TTF3F CTTGCTGTCCTCCAGCTCT

TTF3R CCGGTTGTTGCACTCCTT

FOXF1F CACTCCCTGGAGCAGCCGTAT C

FOXF1R AAG GCTTGATGTCTTGGTAGGTGA

CDX1F AGCCGTTACATCACAATC

CDX1R GAGACTCGGACCAGACCT

CDX2 B F GAGCTGGAGAAGGAGTTT

CDX2 B R GGTGACGGTGGGGTTTAG

MUC2F TGGGTGTCCTCGTCTCCTACA

MUC2R TGTTGCCAAACCGGTGGTA

ALPF GCAACCCTGCAACCCACCCAAGGAG

ALPR CCAGCATCCAGATGTCCCGGGAG  
Sucrase (SI) F TGGCAAGAAAGAAATTTAGTGGA  
Sucrase (SI) R TTATTCTCACATTGACAGGATC  
Defensin-5 F GACAACCAGGACCTTGCTATCT  
Defensin-5 R ACGGGTAGCACAACGGC  
Defensin-6F GACAACCAGGACCTTGCTATCT  
Defensin-6R ACGGGTAGCACAACGGC

### **Telomeric repeat amplification protocol (TRAP)**

Organoids were isolated from matrigel using dispase, washed with PBS and lysed with HLB buffer (20mM HEPES, 2mM MgCl<sub>2</sub>, 0.2mM EGTA) and 10% glycerol supplemented with 0.5% CHAPS, 1mM DTT and 0.1mM PMSF. The lysate was rocked at 4° C for 30min and cellular debris was removed by centrifugation. Dilution of the supernatant (0.04 to 1µg of total protein) was incubated with 0.1µg of the TS primer (AATCCGTCGAGCAGAGTT) for primer elongation in TRAP PCR buffer (20mM Tris-HCl pH8.0, 2.5mM MgCl<sub>2</sub>, 68mM KCl, 0.05% Tween20, 1mM EGTA) including 1mM dNTPs at 30 C° for 1hr. The following PCR reaction was performed supplied with PCR master mix including 0.04µg of the reverse ACX primer (GCGCGGCTTACCCTTACCCTAACC), [ $\alpha$ -<sup>32</sup>P]dGTP (3000 Ci/mmol; PerkinElmer), 1.25 unit of Taq DNA polymerase and 0.04µg of semi-competitive primer sets [TSNT forward primer; ATTCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT, NT reverse primer; ATCGCTTCTCGGCCTTTT] as previously reported (Kim and Wu, 1997)]. The PCR products were analyzed by non-denatured polyacrylamide gel electrophoresis.

### **Transcriptional profiling**

RNA-seq library was prepared at the Functional Genomics Laboratory at UC Berkeley following the standard Illumina library preparation protocol. Total RNA isolation was performed on a pooled culture of organoids from a single 50µl matrigel matrix in the respective condition, colonies of hES cells, or a triturated pellet of minced teratoma. cDNA libraries for high throughput sequencing were prepared from 50~100ng of total RNA from each sample. Ribosomal RNAs were depleted using a oligo(dT) 25 magnetic bead kit (Life Technologies), and the libraries were generated using the PrepX™ SPIA® RNA-Seq and PrepX Library kit (IntegenX) according to the manufacturer's protocol. Then, samples were quantified using the Qubit and PCR amplified for 18 cycles to incorporate indexes and flow cell-binding regions. Final libraries were quantified using the Qubit, Bioanalyzer and qPCR before being sequenced on the HiSeq2000 for 50bps Single-End reads with multiplexing using V3 SBS chemistry reagents.

Reads were mapped to the Ensembl cDNA release 72 (Flicek et al., 2013) with Bowtie2 version 2.1.0

(Langmead and Salzberg, 2012) using the parameters `-rdg 6,5`, `-rfg 6,5`, and `-score-min L,-.6,-.4`. Transcript abundances were calculated with eXpress version 1.4.0 (Roberts and Pachter, 2013) and the resulting effective counts for each transcript were used to calculate fold changes. Effective counts were also processed with DESeq version 1.12.0 (Anders and Huber, 2010) to identify statistically significant differentially expressed genes and transcripts. For bar graphs of gene expression, transcripts were grouped by their originating gene, based on their Ensembl annotation. The expression level of a given gene was calculated as the sum of the estimated abundances of all of its transcripts. The resulting intermediate log<sub>2</sub> fold analysis shown as relative expression bar graphs used DESeq normalized expression results to generate pair wise analysis. Experiments were clustered using complete linkage clustering applied to abundance estimates and projection of the transcript abundance matrix onto the first two principal components was used to visualize similarity between experiments. IGV version 2.3.13 (Thorvaldsdóttir et al., 2013) was used to create read coverage tracks. FPKM available in supplement and raw data for RNA-seq is available at <http://www.ncbi.nlm.nih.gov/geo/> (GSE56930).

## **Supplementary References:**

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