Supplementary Information

1. Preparation of Wnt3A and R-Spondin 2-conditioned media

L-cells and HEK-293 cells producing and secreting Wnt-3A protein and R-spondin-1 protein, respectively, were kind gift from Dr. Jeffery Whitsett (Cincinati Childrens hospital). The cells were cultured in advanced DMEM/F12 medium supplemented with 10% FBS, GlutaMAX (1×), penicillin (100 unit/mL), streptomycin (100 μg/mL). Selective antibiotic G418 sulfate (400 μg/mL) was added in the medium for the passage of the cells, but it was not added for the preparation of the conditioned media.

To prepare the Wnt-3A-conditioned medium, 5×10^5 L-cells suspended in 10 mL culture medium (*note: no addition of G418 sulfate*) were plated in a 10-cm, tissue-culture dish. The cells were allowed to grow for 3-4 days until they reached to 80-90% confluency. The medium was removed and centrifuged at 3,000Xg for 10 min. The supernatant constituted the first batch of medium and was stored at 4°C. 15 mL fresh medium was added to the dish and the cells were cultured for an additional 3 days. The medium was taken off and centrifuged at 3,000Xg for 10 min. This supernatant was the second batch of medium. The first batch and second batch of media were mixed, filtered through a 0.22 μm membrane, and stored at -80°C.

R-spondin-1 conditioned medium was prepared in the same manner as the Wnt-3A conditioned medium using the HEK-293 cell line.

2. Preparation of the PDMS microstrainer array

A PDMS membrane containing an array of microstrainers was prepared in three microfabrication steps as illustrated in Fig. S1.

(1) Fabrication of the master mold on a glass substrate (Fig. S1A).[1] A glass slide (75 mm x 50 mm x 1 mm, Corning) was cleaned using a plasma cleaner (Harrick Plasma) for 5 min. A 10-μm thick layer of 1002F photoresist was spin-coated onto the glass slide using 1002F photoresist (formulation 10)[2] on a spin coater (Laurell Technologies) at 500 rpm, acceleration setting of 1 (108 rpm/second), 10 s followed by 1500 rpm, A=4, 30 s. The photoresist was baked in a 95 °C oven for 15 min to evaporate the solvent. The photoresist was then exposed to UV light (800 mJ/cm^2) through a photomask to pattern the resist. The post-exposure baking was performed in a 95 °C oven for 5 min followed by incubation on a 120 °C hotplate for 5 min. The sample was then developed for 1 min, rinsed with propylene glycol monomethyl ether acetate (PGMEA) and isopropyl alcohol (IPA), and dried by purging with a stream of nitrogen. The sample was hard baked on a 120 °C hotplate for 60 min to harden the film.

A second layer of photoresist was then placed onto the first photoresist layer. To prevent the trapping of air bubbles during placement of this second layer, the sample (with first layer) was treated with plasma for 5 min to make its surface hydrophilic. A second 1002F layer of 150-μm thickness was spin-coated at 500 rpm, A=1, 10 s followed by 1500 rpm, A=4, 30 s using 1002F photoresist formulation 100. The layer was baked at 95 °C for 60 min and exposed to UV light (800 mJ/cm^2) through a photomask to place the patterns into the photoresist. The film was then post-baked at 95 °C for 10 min and developed for 15 min. The film was solidified by baking on a 120 °C hotplate for 60 min. Finally, this 1002F master mold was treated with octyltrichlorosilane in a vapor-phase silanization process in a polycarbonate desiccator (Fisher Scientific). 50 μL octyltrichlorosilane was added in a small vial and placed in the desiccator. The dessicator was degassed using an oil-free pump for 2 min and then sealed for 16 h.

The master mold contained an array of microstrainers firmly adhered to the glass substrate. The base layer of the epoxy microstrainer was composed of a 10-μm thick grid within 30-μm square or circle openings. The top layer was a 150-μm thick epoxy film with holes of 150 μm in diameter.

(2) Fabrication of a PDMS mold by replicate molding (Fig. S1B). Two PDMS spacers (thickness, 200 μm) were placed at the ends of the master mold. PDMS pre-polymer was spread on the master mold, and trapped air bubbles were removed by degasing under house vacuum. A glass slide (75 mm × 50 mm × 1 mm) was treated with air plasma for 5 min and placed on the master mold. Two paper clips were applied at the ends to ensure the flatness of PDMS sandwiched between the master mold and the glass slide. The assembly was then baked in a 95 °C oven for 10 min. After separating the glass slide from the master mold, PDMS remained on the glass slide (since PDMS has very good adhesion to plasma cleaned glass, but it has very poor adhesion to a octyltrichlorosilane treated surface). The PDMS mold was baked on a 120 °C hotplate for 60 min to fully polymerize the elastomer. Finally, the PDMS mold was treated with plasma for 2 min, and reacted with octyltrichlorosilane in a vapor-phase silanization process for 16 h.

The PDMS mold was replicated from the master mold so that it contained an array of large posts (150 μm in height, 150 μm in diameter) with small posts (10 μm in height, 30 μm in diameter or length) at the top of each large post.

(3) Fabrication of a PDMS microstrainer array by replica molding under pressure (Fig. S1C). PDMS pre-polymer was spread on the PDMS mold, and trapped air bubbles were removed by degasing under house vacuum. An octyltrichlorosilane-treated glass slide (75 mm × 50 mm × 1 mm) was placed on the PDMS mold with its silanized side facing the PDMS. A stainless steel rectangular block (weight = 0.75 Kg) was placed above the glass slide to generate even pressure on the assembly. Excess PDMS pre-polymer was expelled by the weight of the block. The assembly (with stainless steel block) was placed on a 120 °C hotplate to cure the PDMS for 30 min. After cooling to room temperature, the glass slide was slowly separated from the PDMS mold. A thin membrane of PDMS with the imprinted strainer array remained on the glass slide (even though both the glass slide and the PDMS mold were treated with octyltrichlorosilane). The PDMS strainer array was easily detached from the glass slide to form a freestanding film.

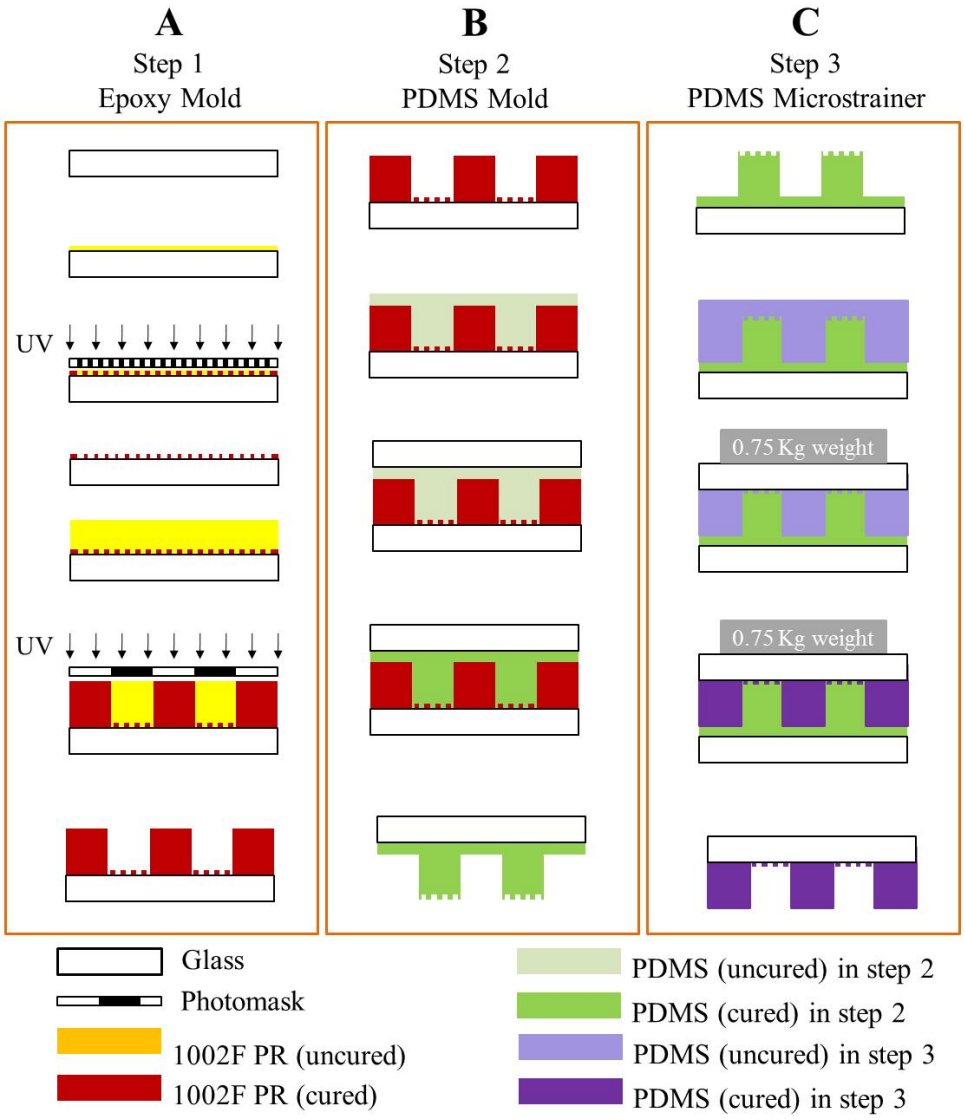

Fig. S1. Three fabrication steps for PDMS microstrainer array. (A) Step 1: making an epoxy master mold. (B) Step 2: making a PDMS mold by replicate molding. (3) Step 3: making a PDMS strainer array by replica molding under pressure.

3. Capture of crypts/colonoids on the microstrainer array

Crypts were in vitro cultured in collagen for 48 h to form colonoids. The collagen was then digested by collagenase to release the colonoids. The suspension of colonoids was added to the microstrianer in the same manner as freshly isolated crypts. The colonoids can be captured on the array at >90% capture efficiency. Fig. S2 shows 59 out of 63 microstrainers (93%) were filled with colonoids. Thus the colonoid tissue was arrayed.

The distribution of crypts/colonoids on the array depends on the ratio of crypt/well. A high capture efficiency can be achieved by using a loading ratio of crypt/well = 2, but many microstrainers accommodated more than one crypt ((Fig. S2-B, left). If we don't consider the effect of fluid flow (which is the case when crypt/well ratio is <<1), Poisson distribution can be used to estimate the distribution of crypts on the array. If crypt/well = 0.2, Poisson distribution predicts that 82% wells are empty, 16% wells capture single crypts, and 2% wells capture more than more crypts. Therefore crypt/well ratio can be reduced to 0.2 to achieve individual capturing of crypts. Experimental data verifies this prediction (Fig. S2-B, right).

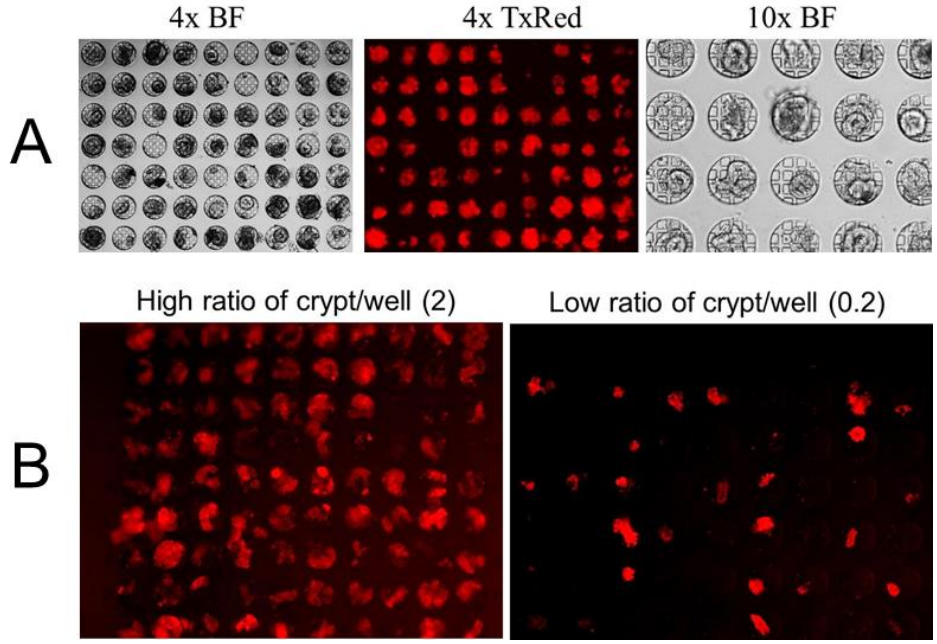

Fig. S2. (A) Capture of colonoids by microstrainer array. (B) Distribution of crypts on the array depends on the crypt/well ratio: left: crypt/well ratio =2, right: crypt/well ratio =0.2.

4. Colonoids on the microstrainer array possessed the full repertoire of differentiated lineages.

Differentiated cells were found in virtually all wells imaged. For example, Fig. S3-A shows all of colonoids in 56 imaged wells contained goblet cells ($Muc2^+$). Only a few enteroendocrine cells (CGA^+) can be found in each colonoid, but they are also present in the vast majority of wells imaged (Fig. S3-B). Enterocytes ($CA\text{-}II^+$) can also be found in the majority of wells (Fig. S3-C).

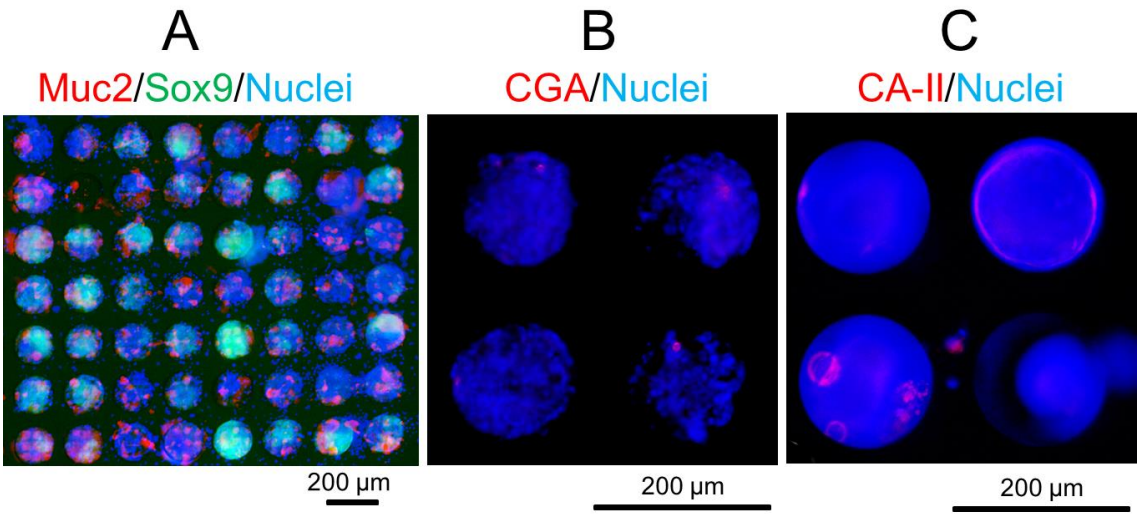

Fig. S3. Immunofluorescence staining of colonoids on the array. (A) Sox9 (green, stem/progenitor cells) and Muc2 (red, goblet cells), (B) chromogranin A (red, enteroendocrine cells), and (C) carbonic anhydrase II (red, enterocytes). Hoechst 33342 was used as a counter stain to mark the nuclei (blue) in all images. The crypts were cultured on the array for 72 h.

4. Immunofluorescence staining of crypts and colonoids.

Fig S4 shows the immunofluorescence staining of the three tissues: fresh crypts, colonoids cultured from crypts on the device for 72 h, and colonoids cultured from off-chip colonoids on the device for 48 h. Immunofluorescence staining of colonoids on the array demonstrated that the colonoid properties were independent of the source of tissue (fresh crypts or off-chip cultured colonoids) Stem /progenitor cells ($Sox9^{+}$) and differentiated cells were present.

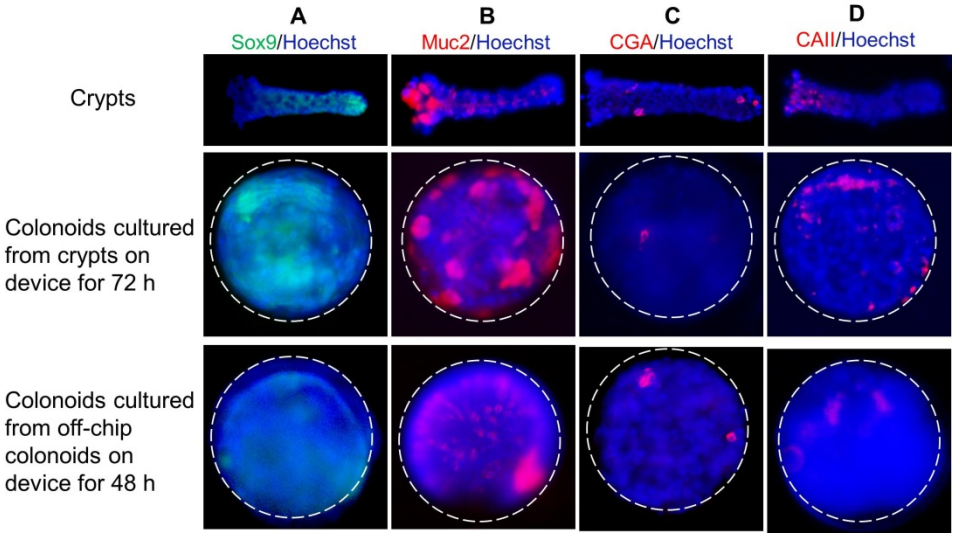

Fig. S4. Immunofluorescence staining of three tissues: fresh crypts, colonoids cultured from crypts on the device for 72 h, and colonoids cultured from off-chip colonoids on the device for 48 h. (A) Sox9 (green, stem/progenitor cells), (B) Muc2 (red, goblet cells), (C) chromogranin A (red, enteroendocrine cells), and (D) carbonic anhydrase II (red, enterocytes). Hoechst 33342 was used as a counter stain to mark the nuclei (blue) in all images.

References

1. Y. L. Wang, R. Dhopeshwarkar, R. Najdi, M. L. Waterman, C. E. Sims and N. Allbritton, *Lab Chip*, 2010, **10**, 1596-1603.
2. J. H. Pai, Y. Wang, G. T. Salazar, C. E. Sims, M. Bachman, G. P. Li and N. L. Allbritton, *Analytical Chemistry*, 2007, **79**, 8774-8780.