nature research

https://doi.org/10.1038/s41596-020-00419-8

Supplementary information

In vitro generation of self-renewing human intestinal epithelia over planar and shaped collagen hydrogels

In the format provided by the authors and unedited

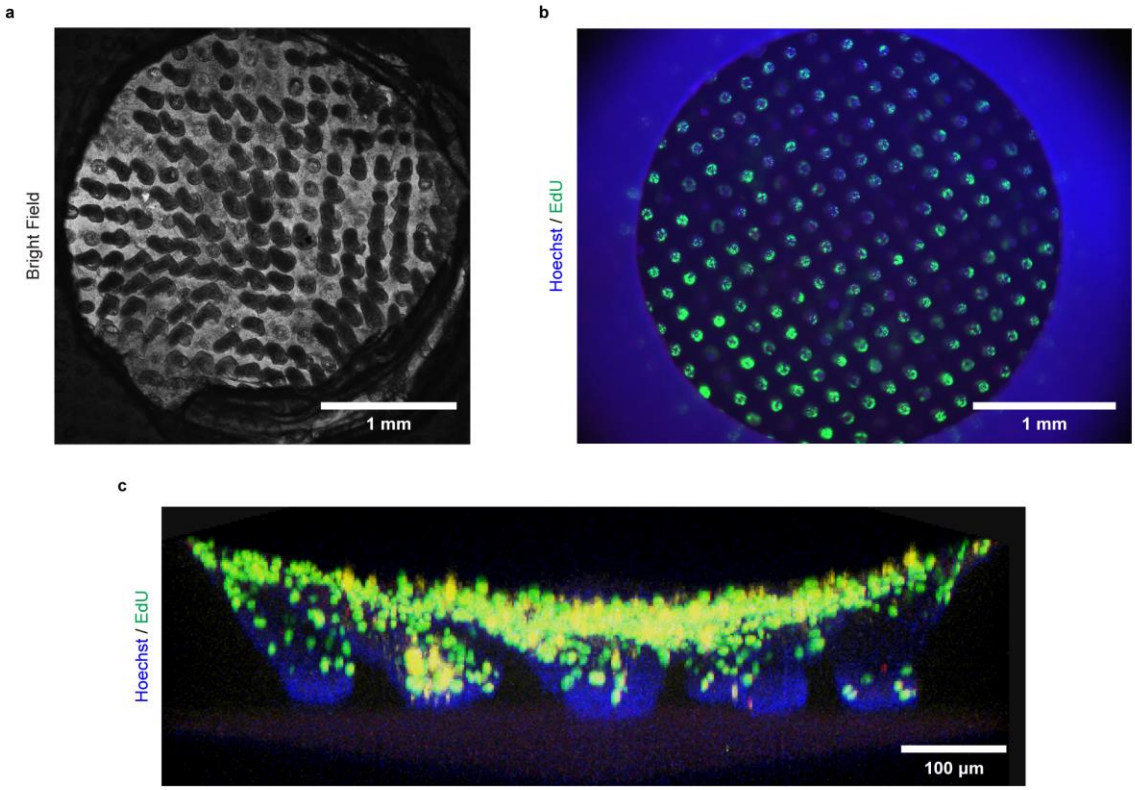
Supplementary Information

In Vitro Generation of Self-Renewing Human Intestinal Epithelia over Planar and Shaped Collagen Hydrogels

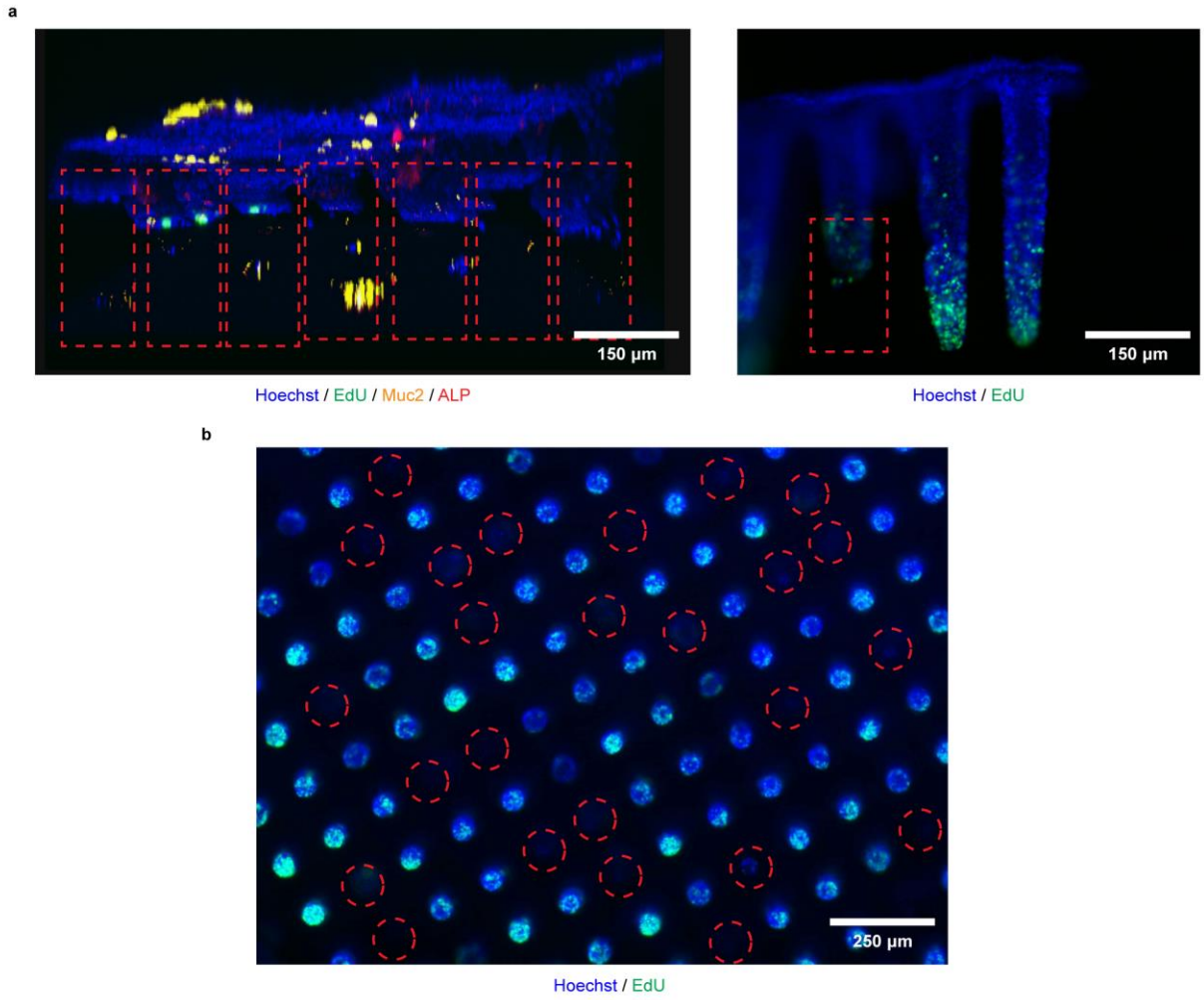
Samuel S. Hinman, Yuli Wang, Raehyun Kim, Nancy L. Allbritton*

Department of Bioengineering, University of Washington, Seattle, WA 98195, USA

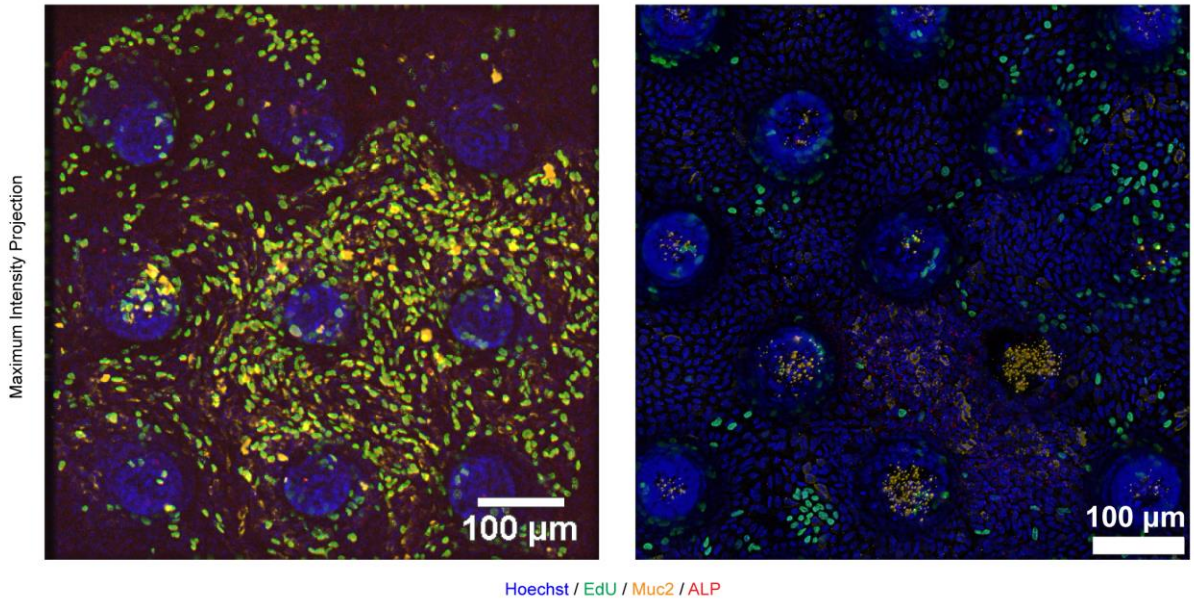
*Corresponding Author: nlallbr@uw.edu



Supplementary Figure 1 | Effects of improper micromolding. **a,** PDMS stamp was not lifted vertically from the gel, causing its deformation. **b,** PDMS stamps was not perfectly horizontal during micromolding. The collagen hydrogel (underneath the microwells) is therefore titled, generating an uneven gradient of growth factors across the array. The lower half has more stem/proliferative cells (EdU^+) than the upper half. **c,** Pressure was not applied during micromolding, and the collagen hydrogel did not extend up the full length of the PDMS micropillars. The human colonic biopsy specimen (male, 52 y) utilized to generate the data in this figure was obtained during a routine screening colonoscopy performed at the University of North Carolina (UNC) Hospitals Meadowmount Endoscopy Center under UNC IRB #14-2013.



Supplementary Figure 2 | Images of stunted and dead *in vitro* crypts. **a,** In vitro crypts only grow halfway down the microwells (outlined within red dashed line), caused by insufficient fragmentation of cell colonies while plating over the shaped scaffolds. The crypts effectively become "blocked" by these larger cell colonies. The images in both subpanels depict the same issue in separate experiments. **b,** Many microwells will not obtain stem/proliferative cells during plating, and therefore may not grow to full length crypts. If greater than 30% of the microwells do not grow to full length *in vitro* crypts, time of passage must be optimized. However, proper polarization of full length *in vitro* crypts will not be affected by surrounding microwells. The human colonic biopsy specimen (male, 52 y) utilized to generate the data in this figure was obtained during a routine screening colonoscopy performed at the University of North Carolina (UNC) Hospitals Meadowmount Endoscopy Center under UNC IRB #14-2013.



Supplementary Figure 3 | Unpolarized *in vitro* crypts. Both of the above examples demonstrate crypts that did not polarize into stem/proliferative and differentiated cell zones, with proliferative cells (EdU^+) along the entire crypt length. This may be caused by a leak of growth factors between basal and luminal compartments, growth factor concentrations (*i.e.*, Wnt3a, R-spondin 3, and noggin) with the basal medium being too high, or utilization of non-optimized scaffold dimensions (*e.g.*, shorter height). The human colonic biopsy specimen (male, 52 y) utilized to generate the data in this figure was obtained during a routine screening colonoscopy performed at the University of North Carolina (UNC) Hospitals Meadowmount Endoscopy Center under UNC IRB #14-2013.

Supplementary Table 1 | Routine solutions validated for labeling primary human intestinal epithelial cells cultured over collagen hydrogel scaffolds. PFA = 4% paraformaldehyde, RT = room temperature, MeOH = 100% methanol.

Target	Antibody (with RRID) or Labeling Kit Information	Fixation Strategy	Concentration/Dilution Ratio
Nuclei	B2261, Millipore-Sigma	15 min PFA (RT) or 60 min MeOH (-20 °C)	2 μg mL^{-1}
Olfactomedin 4 (Olfm4)	14369, Cell Signaling Tech. (RRID:AB_2798465)	15 min PFA (RT)	1:200
SRY-box transcription factor 9 (Sox9)	AB5535, Millipore-Sigma (RRID:AB_2239761)	15 min PFA	1:100
Nuclei of proliferative cells	C10340, ThermoFisher	15 min PFA (RT) or 60 min MeOH (-20 °C)	Manufacturer-recommended
Cytokeratin-20 (KRT20)	13063, Cell Signaling Tech. (RRID:AB_2798106), 60183-1, ProteinTech (RRID:AB10858399)	15 min PFA (RT)	1:500
Mucin-2 (Muc2)	sc-15334, Santa Cruz (RRID:AB_2146667), sc-515032, Santa Cruz (RRID:AB_2815005), ab76774, Abcam (RRID:AB_1523987)	15 min PFA (RT) or 30 min Carnoy's solution (RT)	1:200
Alkaline phosphatase	SK5100, Vector Laboratories (RRID:AB_2336847)	15 min PFA (RT)	Manufacturer-recommended
Chromogranin A	ab15160, Abcam (RRID:AB_301704)	15 min PFA (RT)	1:1000
Villin	sc-2823, Santa Cruz	15 min PFA (RT)	1:200
Ezrin	PA5-17518, ThermoFisher (RRID:AB_10977904)	15 min PFA (RT)	1:200
Integrin-β4	sc-9090, Santa Cruz (RRID:AB_2129021)	15 min PFA (RT)	1:200
Actin	A12379, ThermoFisher R37110, ThermoFisher	15 min PFA	Manufacturer-recommended
Zonula-occludens-1 (ZO-1)	21773-1-AP, ProteinTech (RRID:AB_10733242)	60 min MeOH (-20 °C)	1:500
E-cadherin	20874-1-AP, ProteinTech (RRID:AB_10697811), sc-7870, Santa Cruz (RRID:AB_2076666)	60 min MeOH (-20 °C)	1:200
β-catenin	sc-7963, Santa Cruz (RRID:AB_626807)	60 min MeOH (-20 °C)	1:200
Occludin	13409-1-AP, ProteinTech (RRID:AB_2156308)	60 min MeOH (-20 °C)	1:100