Electronic Supporting Information

Formation of arrays of planar intestinal crypts possessing a stem/proliferative cell compartment and differentiated cell zone

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Table S1. The composition of expansion and differentiation media used in this study

	Expansion Media (EM)	Differentiation Media (DM)
Component	**Concentration**	**Concentration**
Advanced DMEM/F12 (Thermo Fisher)	50% (v/v)	50% (v/v)
WRN	50% (v/v)	--
FBS	--	10% (v/v)
GlutaMAX (Thermo Fisher)	1X	1X
HEPES (Thermo Fisher)	10 mM	10 mM
Murine EGF (Peprotech)	50 ng/mL	50 ng/mL
A83-01 (Sigma Aldrich)	500 nM	500 nM
N-Acetyl cysteine (MP Bio)	1 mM	1 mM
Y-27632 (ApexBio)	10 μM	--
Primocin (InvivoGen)	50 μg/mL	50 μg/mL

Figure S1

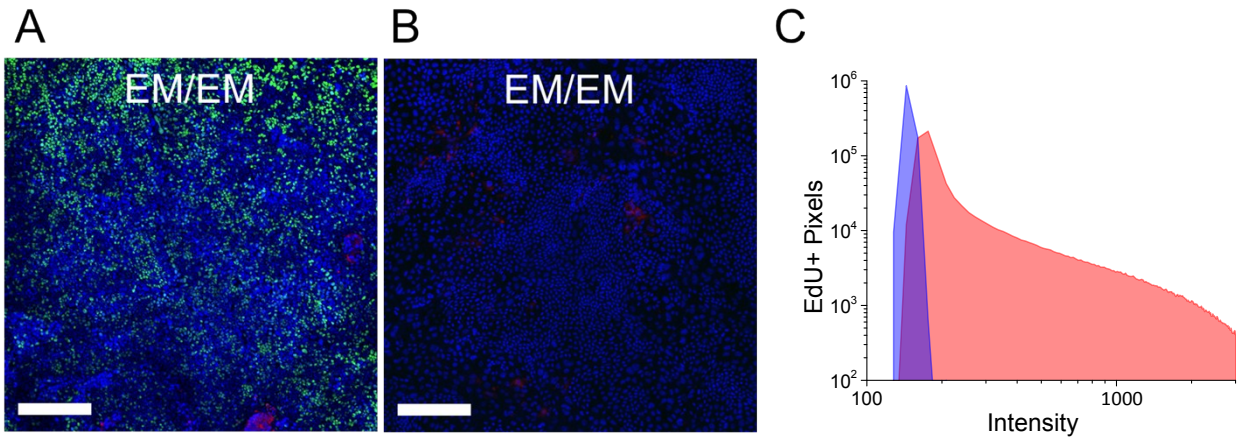

Figure S1. Confocal microscopy images (plane) of the intestinal epithelial cell growth on: A) a suspended collagen layer without the underlying 1002F film and B) a collagen-coated 1002F film without microholes. Cells were grown for 4 days in EM/EM. Green, red, blue represent EdU-incorporation, ALP activity and DNA-Hoechst 33342, respectively. The white scale bar represents 200 μm. C) The histogram of the EdU fluorescence intensity versus the number of pixels in A (red) and B (blue).

Figure S2

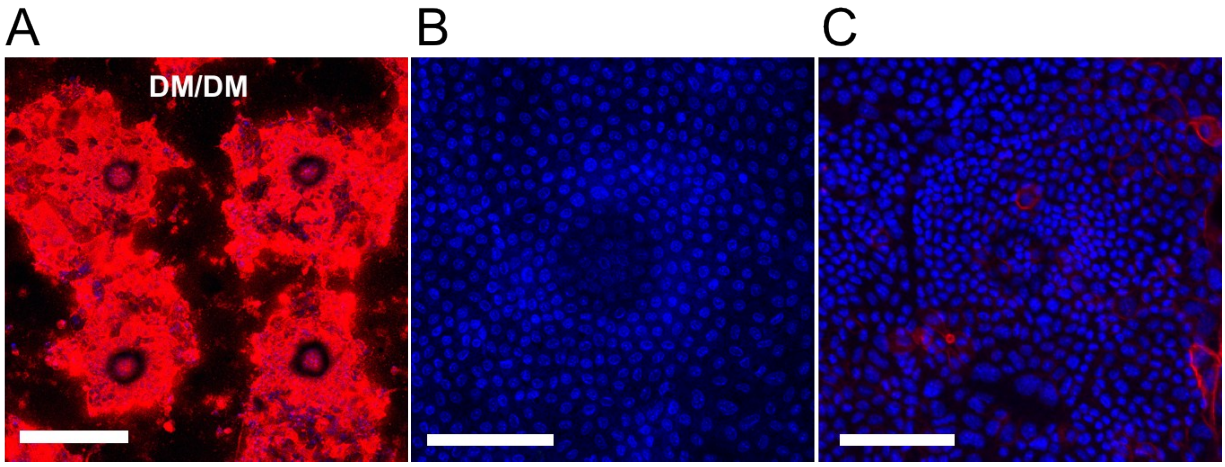

Figure S2. Representative confocal microscopy images of the cells on the array after culture for 2 days in EM/EM and then under DM/DM for an additional 2 days prior to imaging. For all images, blue represents Hoechst 33342. A) Measurement of ALP activity (red) Green and red represent EdU and ALP respectively and EdU incorporation (green). EdU is not visible due to its lack of incorporation into the cell's DNA. The white bar indicates 200 μm. B) EdU incorporation (green) in a single planar crypt showing low incorporation. The white bar indicates 100 μm. C) Z-projected image of β-catenin immunofluorescence (red) in a single planar crypt. The white bar indicates 100 μm.

Figure S3

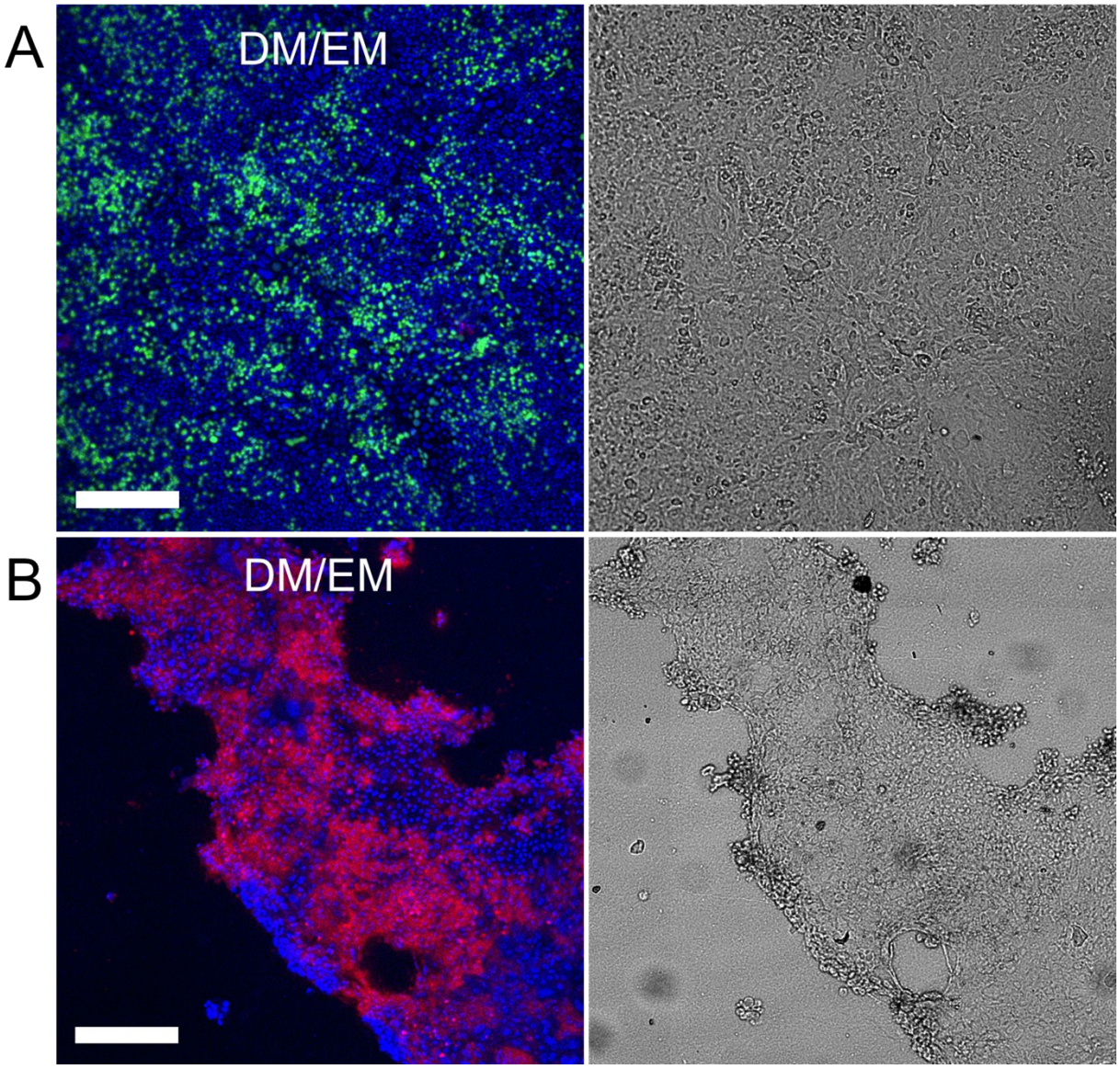

Figure S4. Representative confocal microscopy images of the cells on the array after culture for 2 days in EM/EM and then under DM/EM for an additional 2 days prior to imaging. Cells were cultured on a suspended collagen layer without the underlying 1002F film (A) or a collagen-coated 1002F film without microholes (B). Left images are fluorescence overlays with green, red, and blue representing EdU-incorporation, ALP activity and DNA-Hoechst 33342, respectively. Right panels are differential interference contrast images of the same area. The white scale bars represent 200 μm.