Electronic Supplementary Material (ESI) for Integrative Biology

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Gut-on-a-Chip Microenvironment Induces Human Intestinal Cells to Undergo Villus Differentiation

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ESI Method

Microfluidic HT-29/MTX cell culture

The mucus-secreting HT-29/MTX cells (kindly provided by Dr. T. Lesuffleur, INSERM)[1] were routinely maintained in DMEM (Gibco) containing 4.5 g L^{-1} glucose and 25 mM HEPES supplemented with 20% (v/v) FBS (Gibco), 100 units mL^{-1} penicillin, and 100 µg mL^{-1} streptomycin (Gibco) at 37^{o}C, humidified condition with 5% CO_2 under air. HT-29/MTX cells were cultured under the same microfluidic conditions as described for Caco-2 cells (see main Materials and Methods).

ESI FIGURES

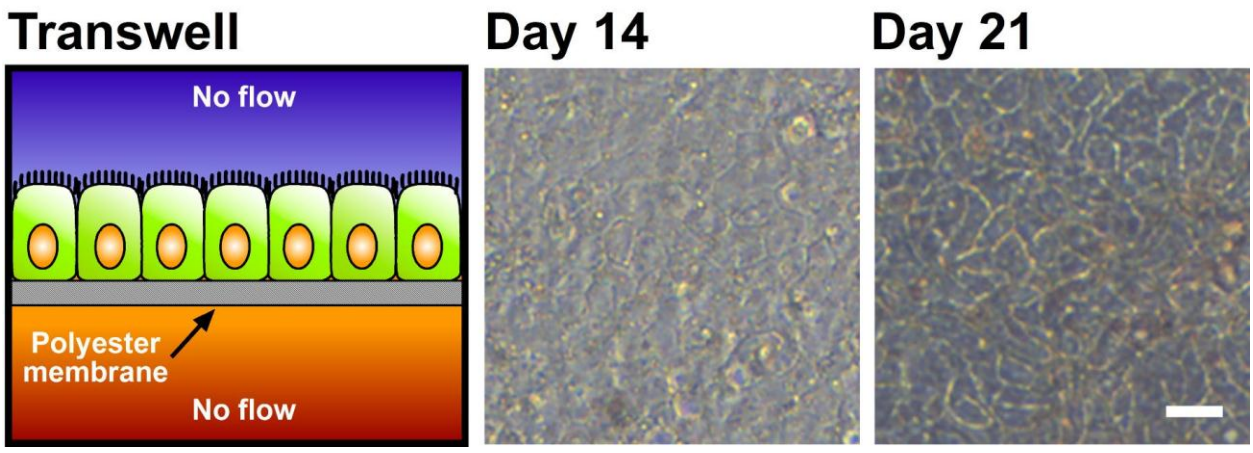

Fig. S1. Caco-2 cell morphology when cultured in the static Transwell system. Left schematic showing how the cells are cultured on the porous membrane insert in the absence of fluid flow in the Transwell insert. Phase contrast microscopic views show that Caco-2 cells form a planar cell monolayer even after 14 and 21 days of static culture (bar, 20 µm).

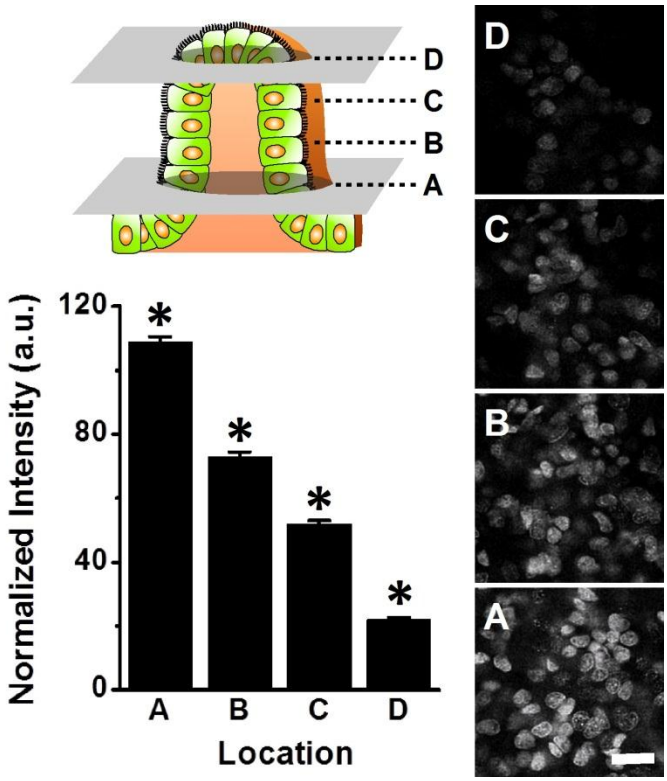

Fig. S2. Gradient of fluorescence intensity in EdU-positive cells at four different Z-positions in Caco-2 villi. A schematic (left top) displays four different Z-positions from the crypt (A) to the villus tip (D) along the villus axis, which corresponds to the bar chart (left bottom) and the horizontal cross-section confocal views of EdU-positive cells (right). Bar, 20 µm. (* $p < 0.001$). Note that staining intensity decreases progressively as the cells move from their site of EdU labeling in the crypt (A) and undergo cell divisions as they progressively move up towards the tip of the villus (B-D).

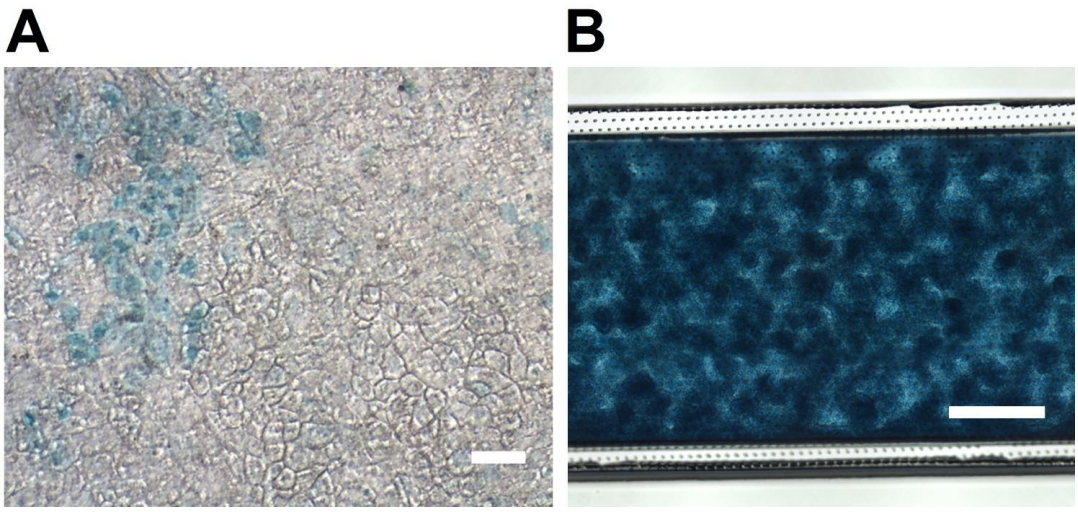

Fig. S3. Evaluation of intestinal mucus production by alcian blue staining. (A) Caco-2 cells cultured in the static Transwell for 3 weeks display virtually no mucus production as indicated by the lack of positive alcian blue staining, confirming past work that claimed Caco-2 cells do not have this capacity[2] (bar, 20 µm). (B) HT-29/MTX cells that have been previously shown to produce mucus at high levels in culture[1] produce strong, dark alcian blue staining when cultured in Gut-on-a-chip for only 5 days. Note these data are shown as a positive control to confirm the effectiveness of using alcian blue to detect mucus production *in vitro* (bar, 300 µm).

ESI References

1. T. Lesuffleur, A. Barbat, C. Luccioni, J. Beaumatin, M. Clair, A. Kornowski, E. Dussaulx, B. Dutrillaux and A. Zweibaum, *J. Cell Biol.*, 1991, **115**, 1409-1418.

2. P. Artursson and R. T. Borchardt, *Pharm. Res.*, 1997, **14**, 1655-1658.