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SUPPLEMENTARY INFORMATION

A simple three-dimensional gut model constructed in a restricted ductal microspace induces intestinal epithelial cell integrity and facilitates absorption assays

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1. Supplementary Figures

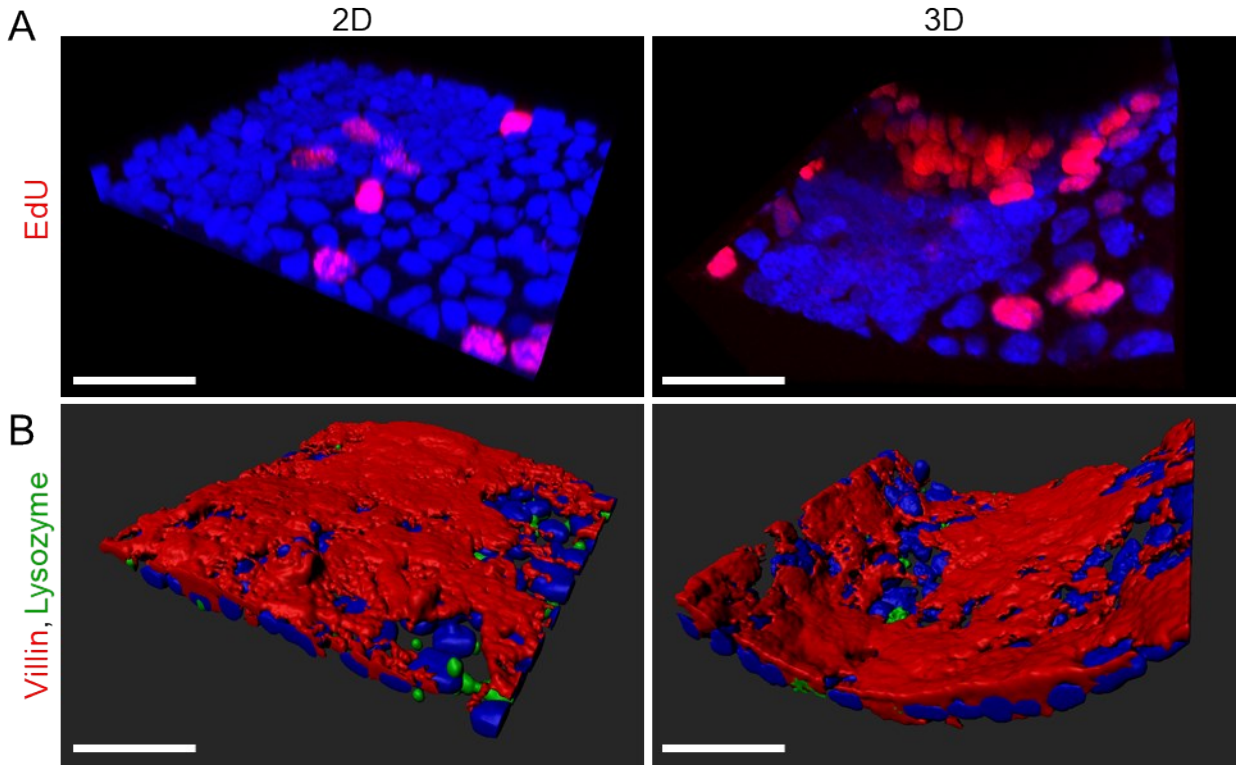

Figure S1. Confocal images for EdU assay and immunofluorescence for differentiation marker proteins in 2D or 3D gut model cultured for 16 days. (A) Confocal microscopies of EdU assay. EdU-positive nuclei (red) and nuclei (blue) were visualized. (B) Binarized immunofluorescent images for villin (red) and lysozyme (green). Scale bar: 50 μm.

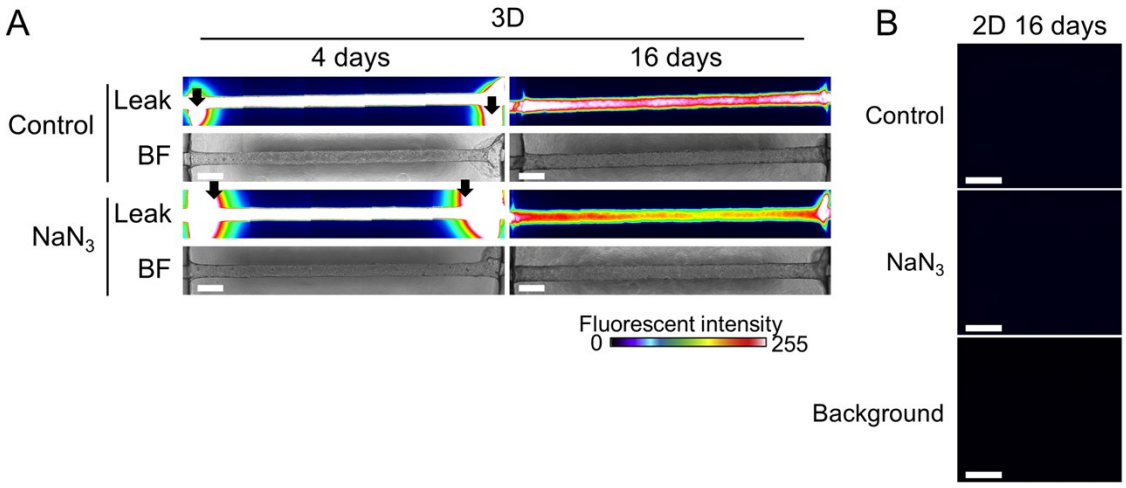

Figure S2. Fluorescent images for leak check at absorption assay in 2D and 3D gut models. (A) Fluorescent and bright-field (BF) images at 20 min after FITC-4 kDa dextran treatment in the 3D gut model cultured for 4 or 16 days with or without 10 mM NaN_3 treatment. Scale bar: 500 μm. (B) Fluorescent images at 20 min after FITC-4 kDa dextran treatment in the bottom well of the 2D gut model cultured for 16 days with or without 10 mM NaN_3 treatment to check for absence of leakage. Background: without FITC-dextran treatment. In the region on the filter, fluorescent intensity was saturated in all samples. Scale bar: 200 μm.

2. Supplementary Table

	Forward	Reverse
ACTB	GGGCATGGGTCAGAAGGATT	AGGTCTCAAACATGATCTGGGT
LGR5	AGCCTTCAATCCCTGCGTCT	TAACGCATTGTCATCCAGCCA
LYZ	ATCAGCCTAGCAAACTGGATG	TGACAGGCATTAACTGCTCCT
MUC2	ACGTGGCTGTTCAGGACTAC	TCCGTCCTCCCCATGAAGAT
VIL1	GATATGGAGGATCGAGGCCAT	GTCCTGGCCAATCCAGTAGT