

### Supplementary figures

Figure S1.

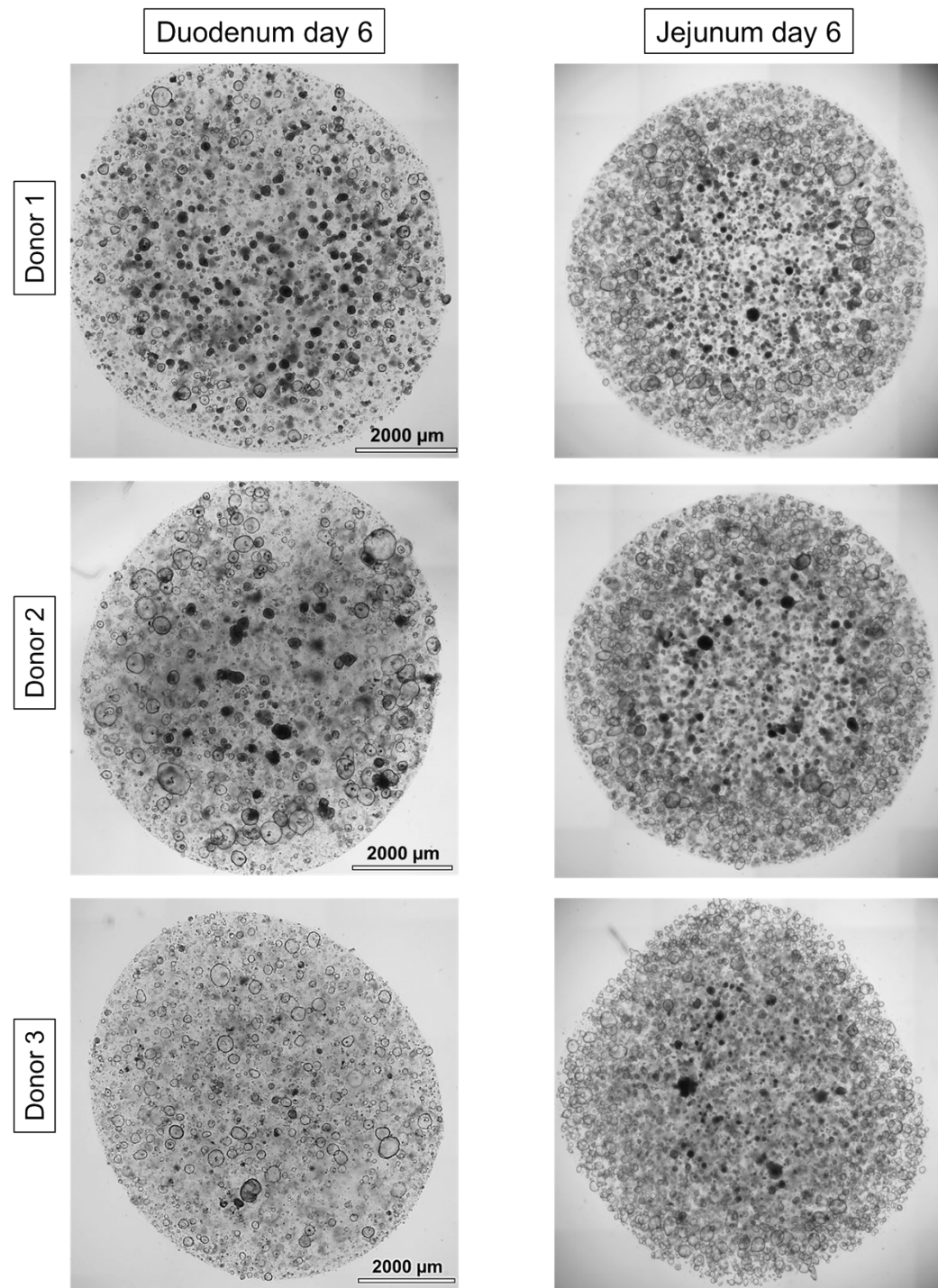


Figure S2.

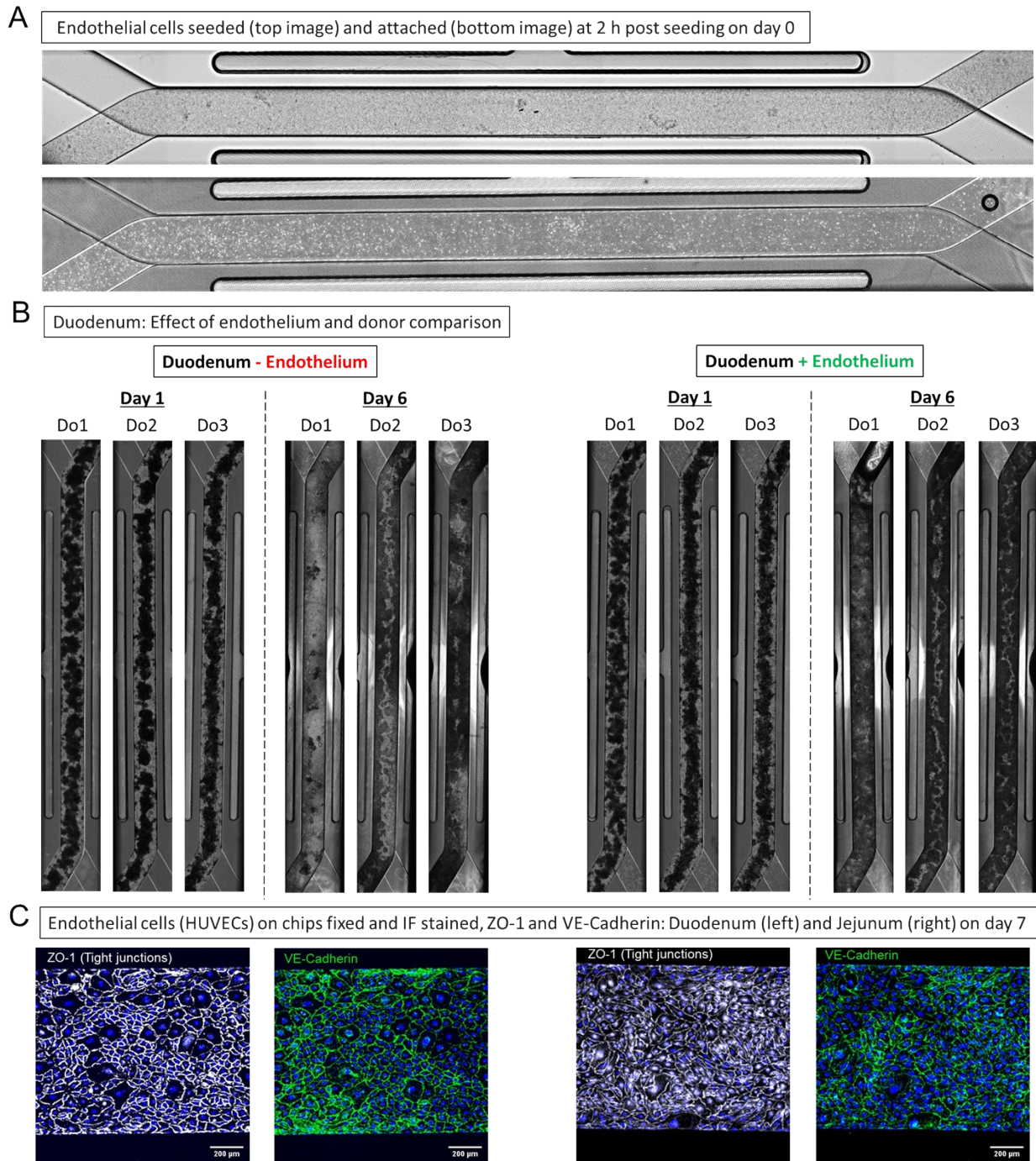


Figure S3.

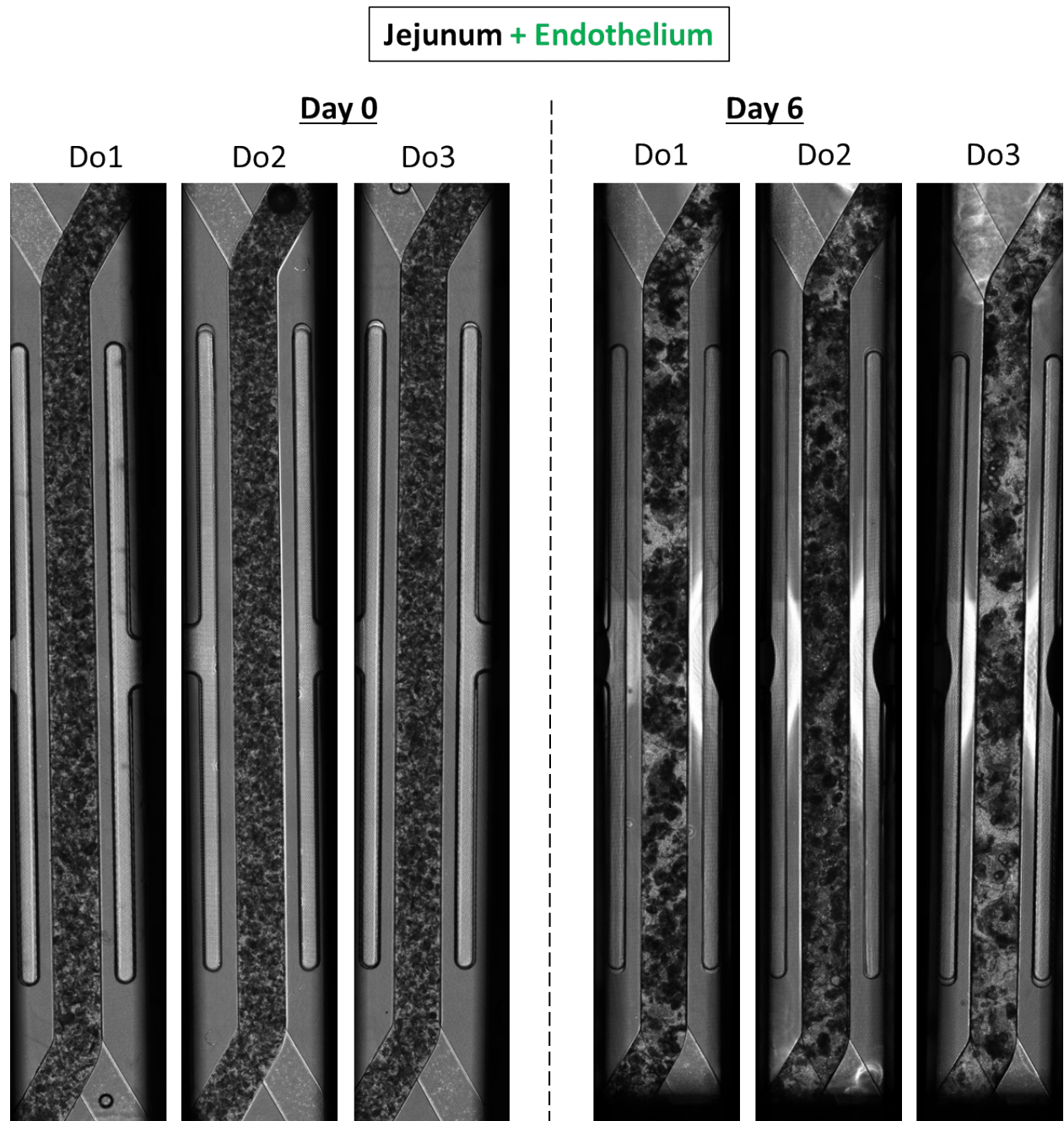
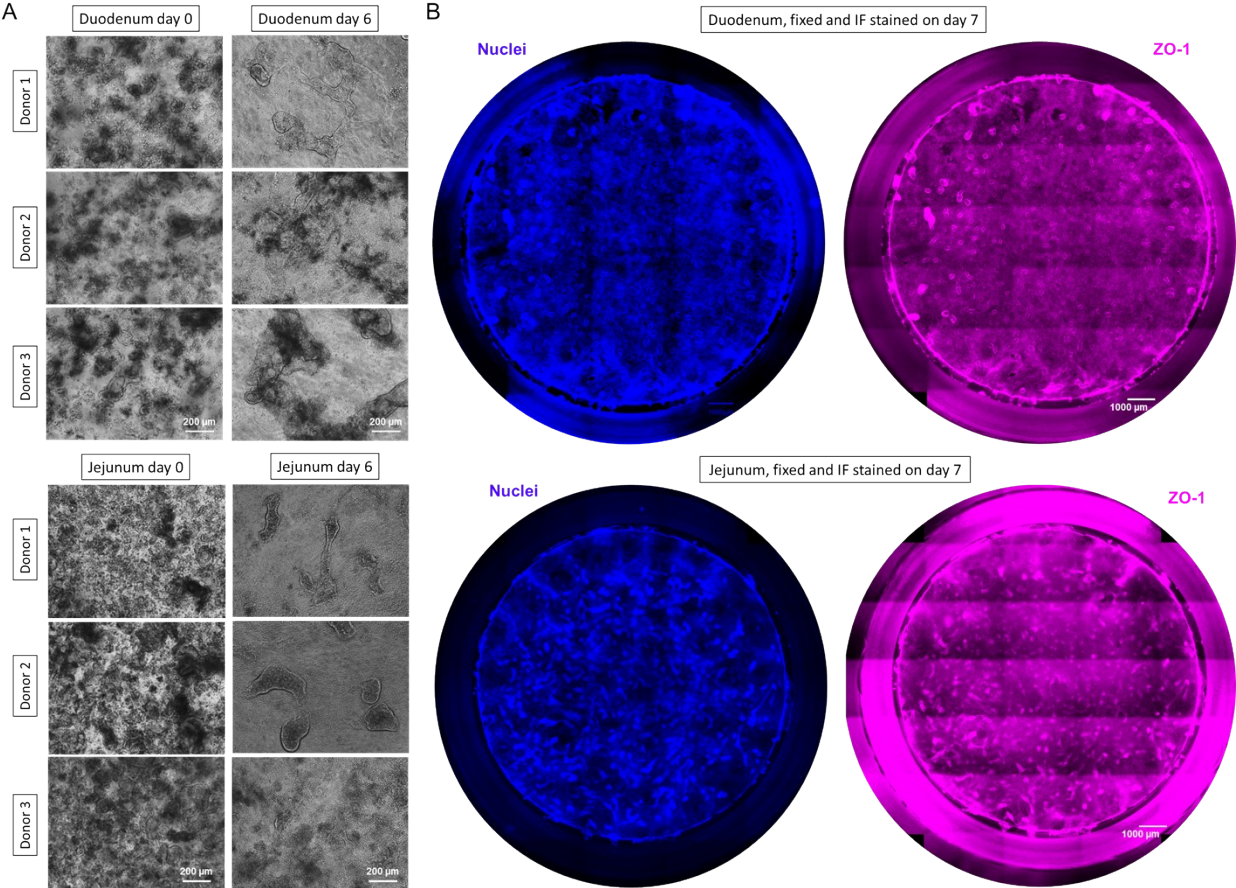


Figure S4.



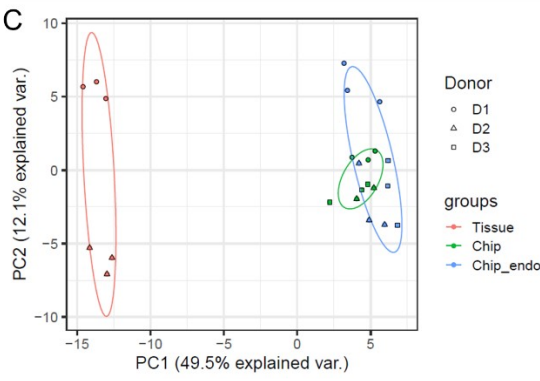
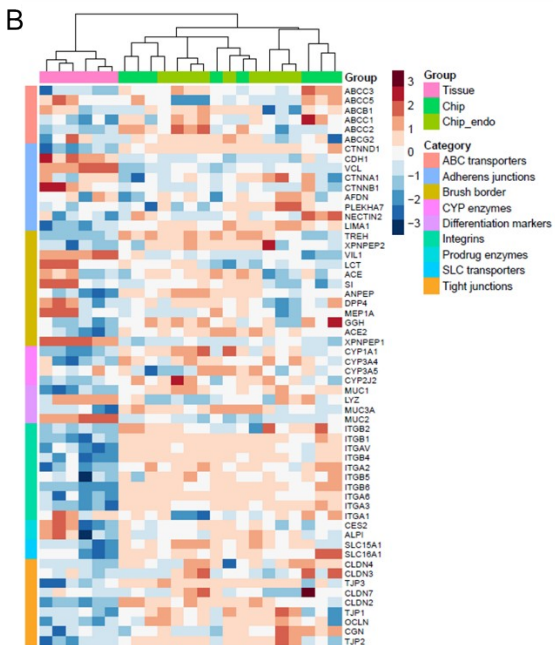
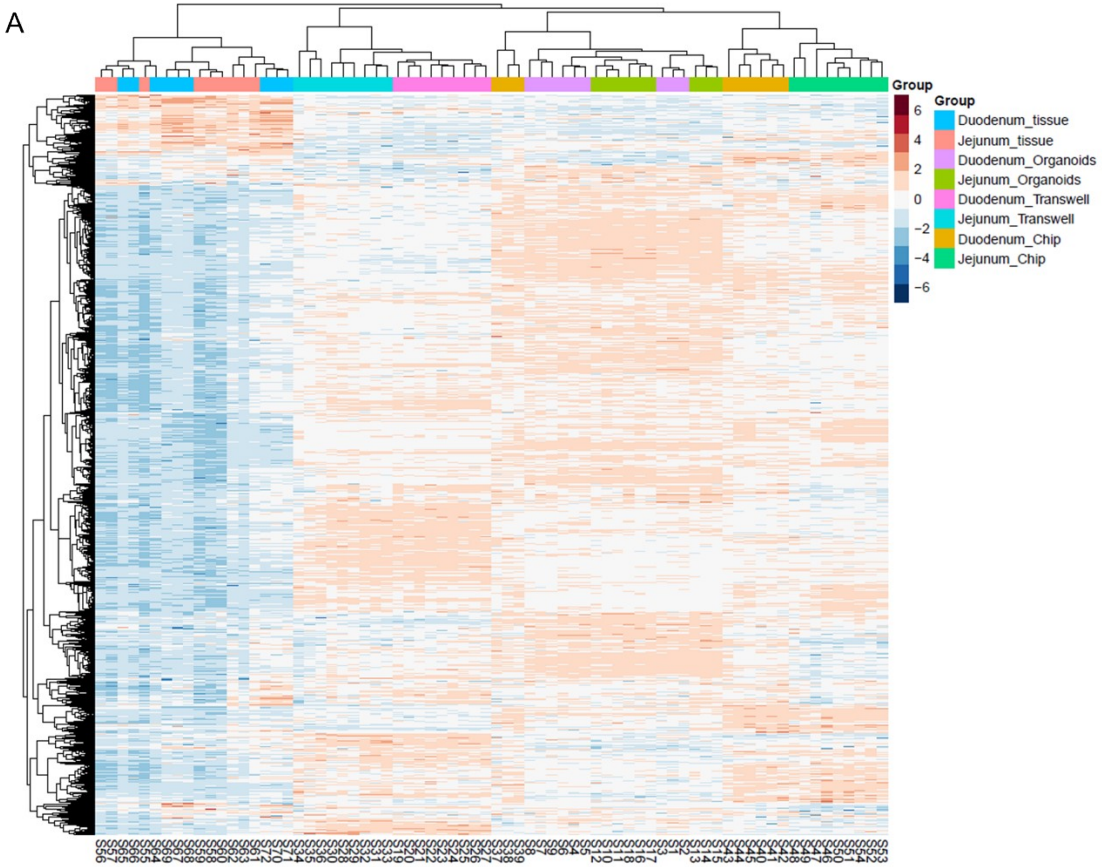


Figure S5.

Figure S6.

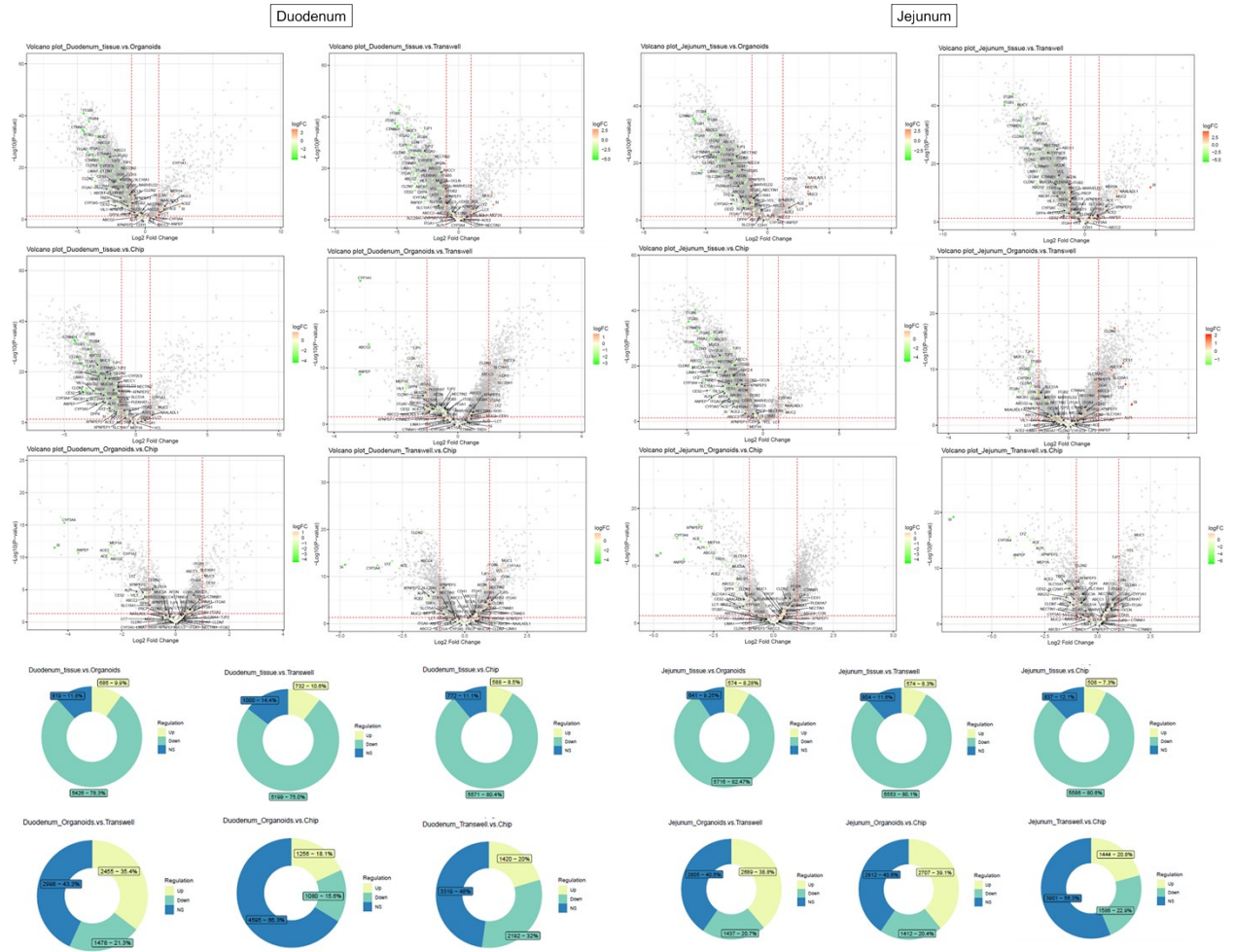


Figure S7.

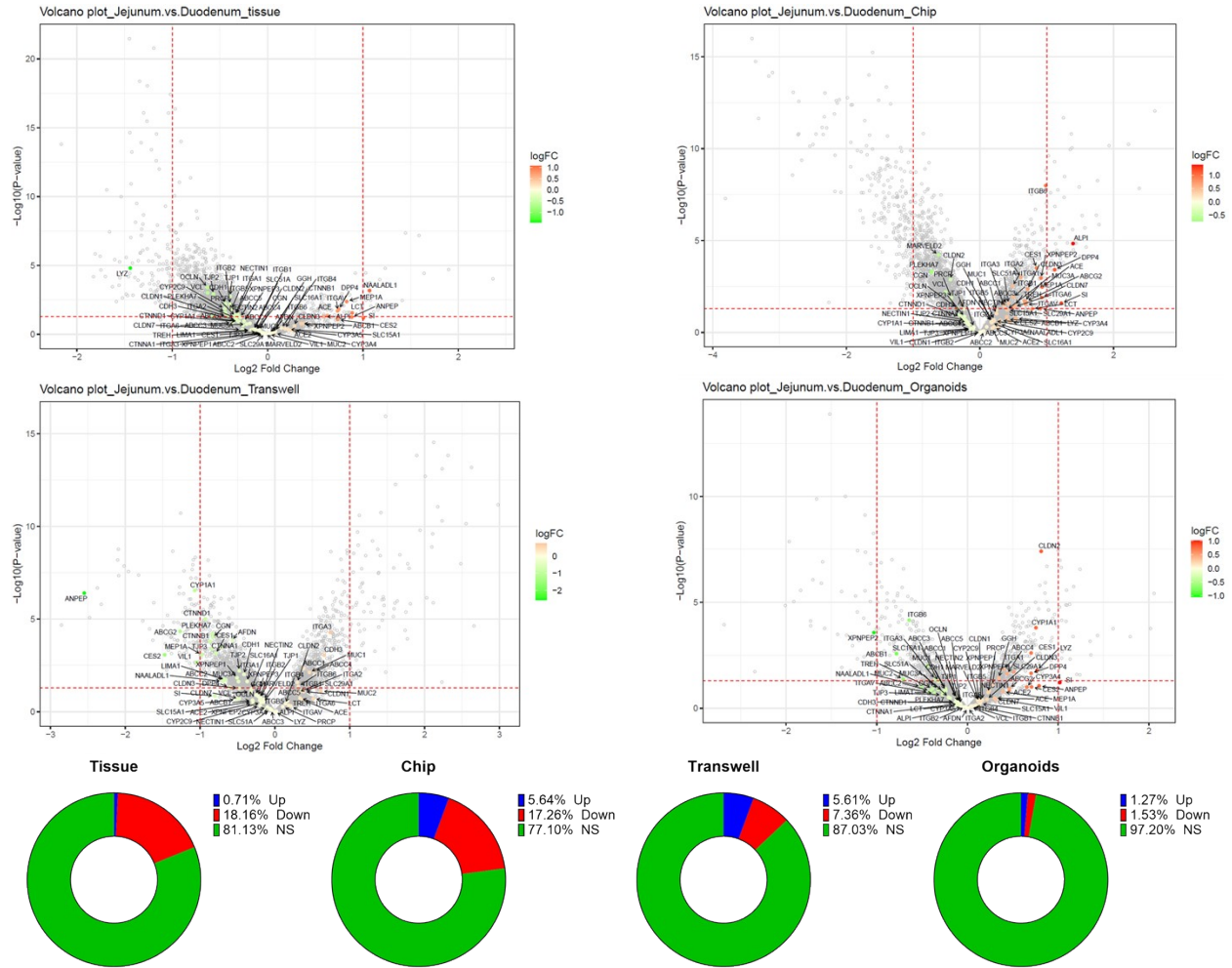


Figure S8.

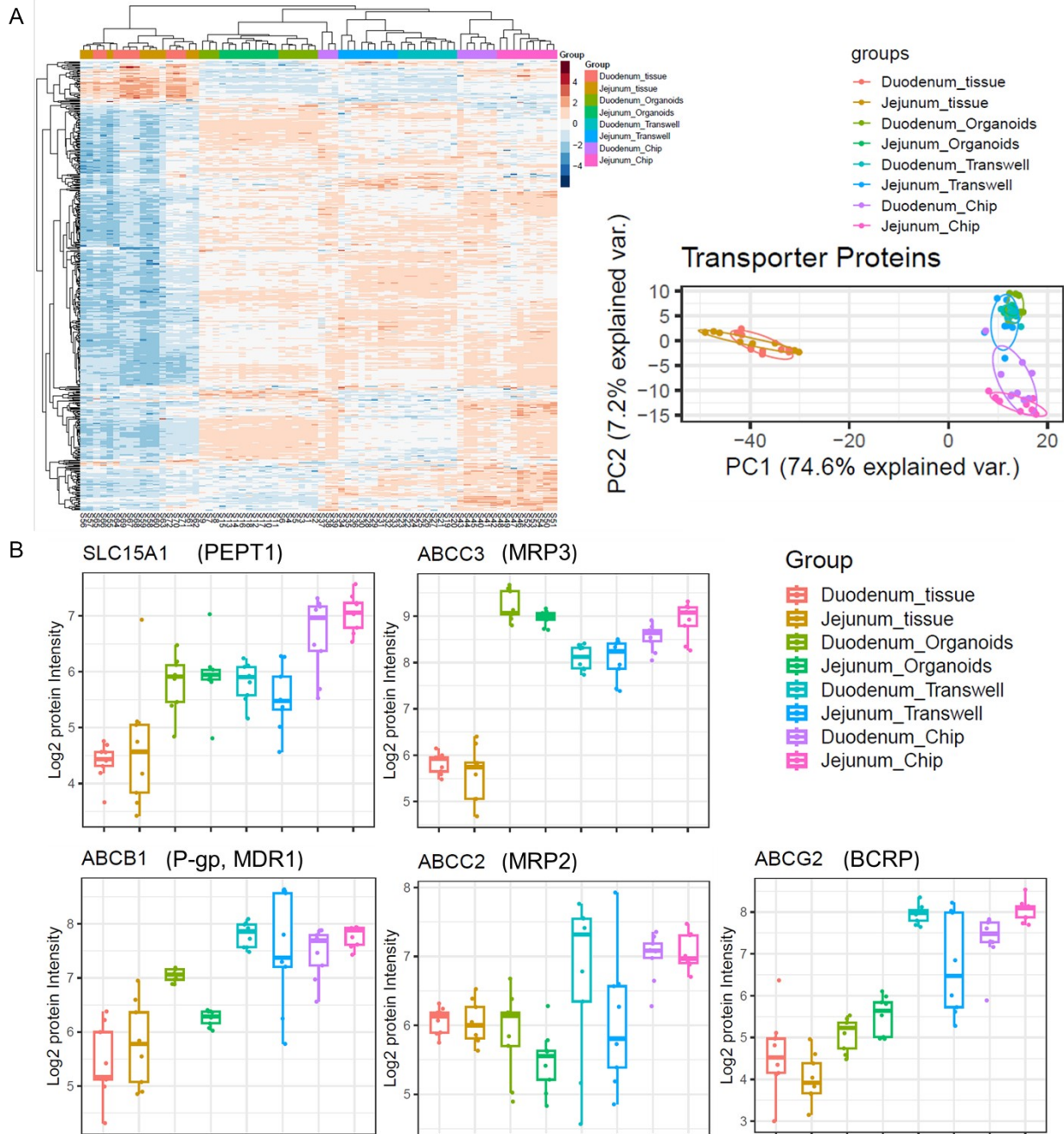




Figure S9.

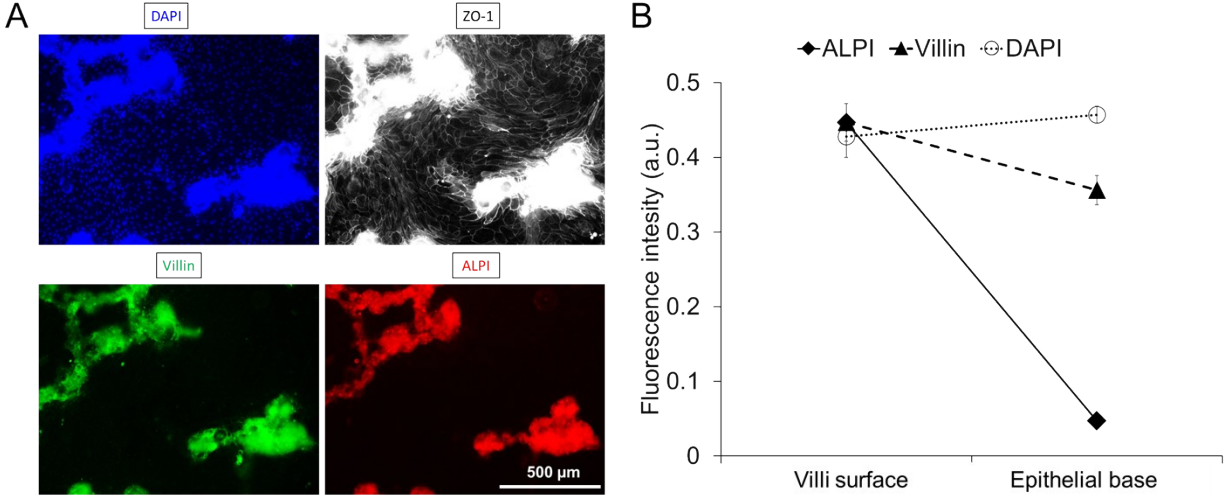


Figure S10.

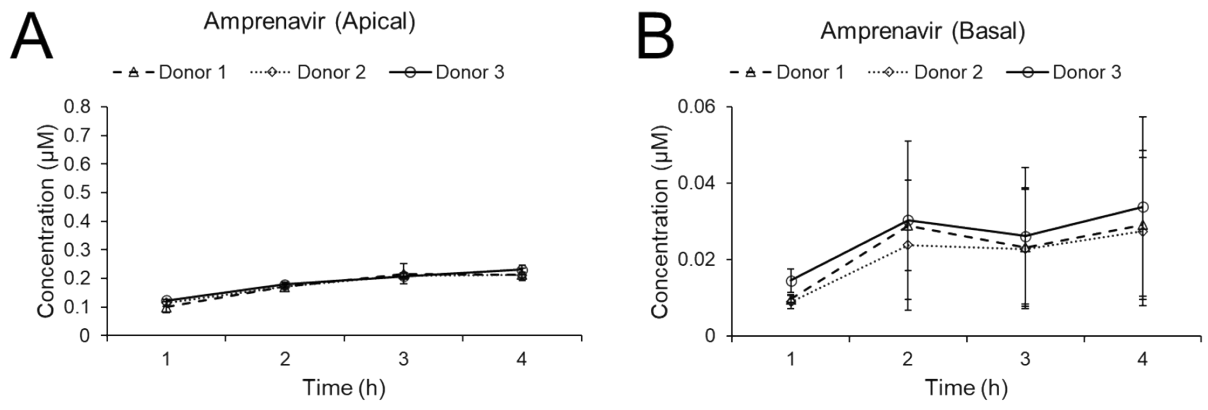


Figure S11.

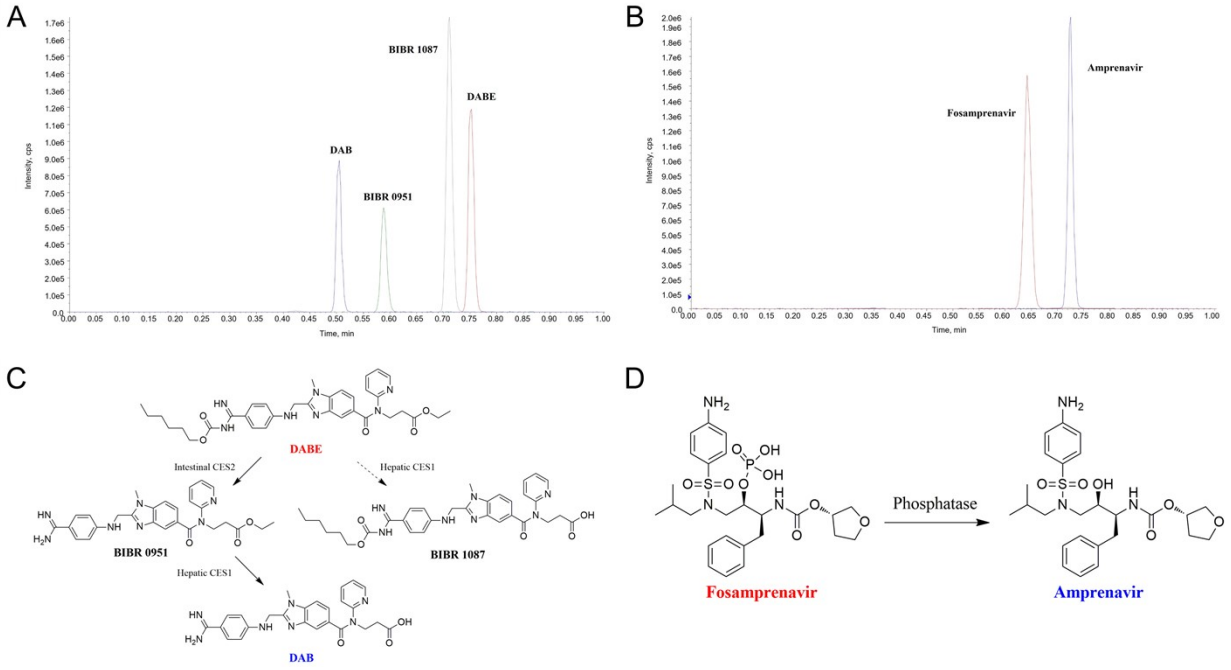
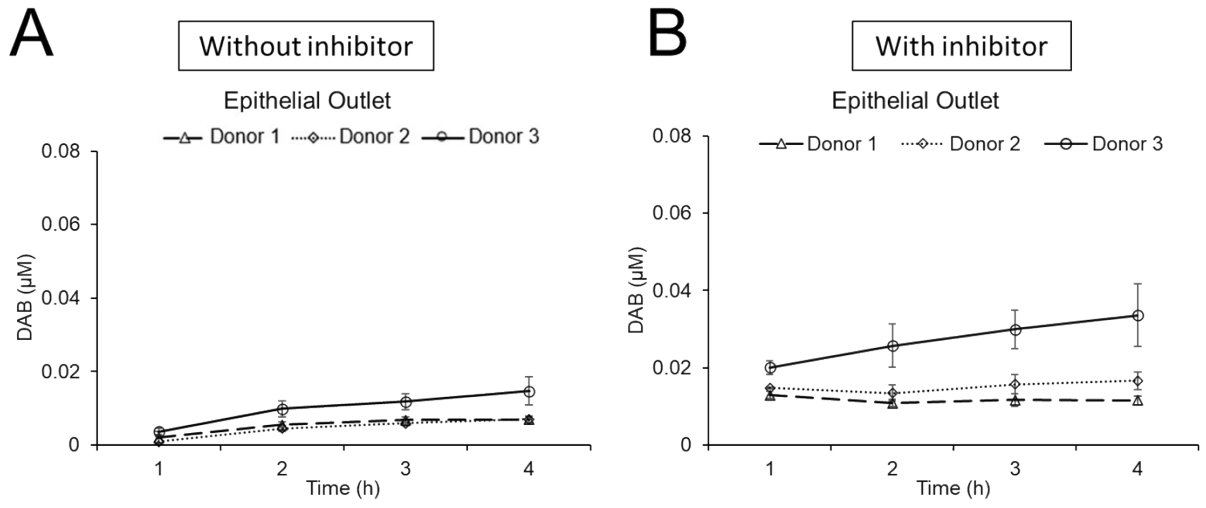


Figure S12.



**Figure S1.** Tiled brightfield images of the ASC organoids embedded in Matrigel domes from duodenum and jejunum of three human donors.

**Figure S2.** (A) Tiled brightfield images of HUVECs as a single cell suspension (top) and attached after 2 hours (bottom) in the microfluidic chips. (B) Tiled brightfield images of duodenum organoid fractions from 3 donors in the epithelial channel of the microfluidic chips without (left) and with (right) endothelial cells in the bottom channel. (C) IF images showing junctional protein expression (ZO-1 and VE-cadherin) of the endothelial cells in duodenum (left) and jejunum (right) chips.

**Figure S3.** Tiled brightfield images of jejunum organoid fractions (Day 0) and formed an epithelial barrier (Day 6) from 3 donors (indicated as Do1, Do2 and Do3) in the apical channel of the microfluidic chips with endothelial cells in the bottom channel.

**Figure S4.** (A) Brightfield images of organoid fractions seeded in the apical side of the transwell inserts on day 0 (Duodenum on the top and Jejunum on the bottom; 3 donors) and epithelial layer with 3D morphological structures on day 6. (B) Representative tiled fluorescent images showing full coverage of the transwell membrane (nuclei, DAPI) and expressing junctional protein (ZO-1) on day 7 (Duodenum on the top and Jejunum on the bottom).

**Figure S5.** (A) Quantitative proteomic comparison of tissue, ASC organoids in matrix, transwells, and microfluidic chips for all detected proteins (global dataset) using heatmap and unsupervised hierarchical clustering. Values presented from 3 donors (duodenum and jejunum) and chip experiments repeated 3 times. Quantitative proteomic comparison of tissue, ASC organoids in microfluidic chips with, and without endothelial cells for all detected proteins using (A) heatmap and unsupervised hierarchical clustering and (B) principal component analysis (PCA) for a defined epithelial specific dataset. Values presented from 3 donors (duodenum only) and chip experiments repeated 3 times.

**Figure S6.** Up and down expression level of proteins and comparison for (Tissue, Organoids, Transwells and Microfluidic chips).

**Figure S7.** Sectional differences (Duodenum vs Jejunum) in protein expression for (Tissue, Organoids, Transwells and Microfluidic chips).

**Figure S8.** (A) Heatmap and unsupervised hierarchical clustering of all the detected transporters in duodenum and jejunum tissue vs in vitro models. Principal component analysis (PCA) of the selected proteins comparing the clusters from tissue and the in vitro cultures for duodenum and jejunum. (B) Bar graphs showing the comparison of expression across the samples for key drug transporters (Cytochrome P450; CYP3A4: intestinal alkaline phosphatase; ALPI: carboxylesterases 1 and 2; CES1 and CES2). Values represented as average ( $n = 3$  donors)  $\pm$  standard deviation, chip experiments repeated 3 times and data generated from all samples in a single **proteomics** workflow.

**Figure S9.** (A) Immunofluorescence confocal microscopy images showing the nuclei (DAPI, blue), expression of tight junction protein-1 (ZO-1, white), intestinal alkaline phosphatase (ALPI, red), and epithelial brush border membrane protein Villin (green) at the villi surface, epithelial base, and the endothelial base. (B) Quantification of the fluorescence signal intensity of the enzyme CES2 compared to DAPI at the villi surface and the epithelial base. Values represented as average of transwell (duodenum and

jejunum) experiments repeated 3 times (n = 1 donor)  $\pm$  standard deviation, and data generated by uniform normalization across all acquired images.

**Figure S10.** Prodrug enzyme kinetics for fosamprenavir to amprenavir bioconversion on static transwells.

**Figure S11.** Representative extracted ion chromatograms of target analytes: (A) the ester prodrug dabigatran etexilate (DABE) and its metabolites (BIBR 1087, BIBR 0951, and DAB); (B) the phosphate prodrug fosamprenavir and its active form amprenavir. Conversion of (C) ester prodrug DABE to DAB in the presence of intestinal and hepatic CES and (D) phosphate prodrug fosamprenavir to amprenavir in the presence of phosphatase.

**Figure S12.** Prodrug enzyme kinetics for DABE to DAB bioconversion on jejunum organoid chips (A) without BNPP and (B) with BNPP.

**Supplementary tables**

**Table S1.** Donor information for organoids and tissues

<b>Organoids</b>						
<b>Duodenum</b>			<b>Jejunum</b>			
<b>Donor 1</b>	<b>Donor 2</b>	<b>Donor 3</b>	<b>Donor 1</b>	<b>Donor 2</b>	<b>Donor 3</b>	
M, 39	M, 50	F, 51	M, 23	M, 50	F, 51	<b>Donor ID</b>
Emulate	Altis Biosystems	Altis Biosystems	Altis Biosystems	Altis Biosystems	Altis Biosystems	<b>Vendor</b>
DP41N2	HISC-DUO/5	HISC-DUO/4	HISC-JEJ/1	HISC-JEJ/5	HISC-JEJ/4	<b>Cat #</b>
<b>Tissue</b>						
<b>Duodenum</b>			<b>Jejunum</b>			
<b>Donor 1</b>	<b>Donor 2</b>	<b>Donor 3</b>	<b>Donor 1</b>	<b>Donor 2</b>	<b>Donor 3</b>	
M, 27	M, 38	M, 49	M, 27	M, 38	M, 49	<b>Donor ID</b>
BioIVT	BioIVT	BioIVT	BioIVT	BioIVT	BioIVT	<b>Vendor</b>
S03006	S03006	S03006	S03006	S03006	S03006	<b>Cat #</b>
MHU-I-022822	MHU-I-111721	MHU-I-120621	MHU-I-022822	MHU-I-111721	MHU-I-120621	<b>Lot#</b>

**Table S2.** Reagents and materials

<b>Catalog No</b>	<b>Description</b>	<b>Supplier</b>
MSPP-06010	MEDIUM INTESTICULT ORGANOID GROWTH MEDIUM KIT	Stemcell Technologies
CC-3162	EGM-2 Endothelial Cell Growth Medium-2 BulletKit	LONZA
MSPP-ANTPM1	PRIMOCIN 500MG	INVIVOGEN
47743-722	BD MATRIGEL MATRIX GROWTH FACTOR REDUCED PHENOL RED-FREE	CORNING
C5533-5MG	COLLAGEN IV FROM FROM HUMAN PLACENTA	SIGMA ALDRICH
47743-654	FIBRONECTIN HUMAN NATURAL 5MG	CORNING
ALX270333-MO05	ROCK INHIBITOR Y27632, 5/MG	ENZO
SML1046-25MG	CHIR99021 POWDER	SIGMA ALDRICH
47743-696	SOLUTION CELL RECOVERY 100ML	CORNING
12634010	ADVANCED D-MEM/F-12	ThermoFisher Scientific
SE1M003M00	FILTER UNIT MILLIPORE STERIFLIP-HV 0.45UM 50ML 25/CS	EMD Millipore
21-031-CV	Dulbecco's PBS (DPBS -/-) (without Ca <sup>2+</sup> , Mg <sup>2+</sup> )	CORNING
ER-1	5 mg POWDER	EMULATE
ER-2	25 mL BOTTLE	EMULATE
TrypLE Express	1X, NO PHENOL RED	ThermoFisher Scientific
A9576	BSA 30%, STERILE	SIGMA ALDRICH
D2650	DMSO STERILE	SIGMA ALDRICH
MT25055CV	Cell Culture Grade Water	CORNING
C2519A	HUVEC - Human Umbilical Vein Endothelial Cells, Pooled	LONZA
C02-micro-10	S-Trap™ micro columns (≤ 100 µg)	PROTIFI
V5280	Promega Trypsin Gold, Mass Spectrometry Grade	PROMEGA
4465938	Beta-Galactosidase Digest	SCIEX
	Acetonitrile, HPLC Grade	SIGMA ALDRICH
	Methanol, HPLC Grade	SIGMA ALDRICH
	Milli-Q water, ultra pure	Purified by Millipore Milli-Q lab water system
	Trifluoroacetic acid, 99%	ThermoFisher Scientific
	Formic acid	ThermoFisher Scientific



**Table S3.** Equipment and instrumentation

<b>Equipment</b>	<b>Vendor</b>
Zoë CM-1® Culture Module	EMULATE
Orb-HM1® Hub Module	EMULATE
Chip-S1™	EMULATE
Pod™ Portable Modules	EMULATE
UV Light Box	EMULATE
Chip Cradle	EMULATE
ECM coated transwells	ALTIS Biosystems
TEER measurement device	EVOM3 - World Precision Instruments
FV1000 MPE microscope	Olympus
EVOS M7000	ThermoFisher Scientific
Dionex Ultimate 3000 HPLC	ThermoFisher
Triple TOF 6600 mass spectrometer	Sciex
Bioruptor® Pico sonication device	Bioruptor
5500 Triple quadrupole mass spectrometer	SCIEX
6500+ Triple quadrupole mass spectrometer	SCIEX
Agilent 1290 UPLC	Agilent
CTC PAL Autosampler	CTC Analytics AG

**Table S4.** Antibodies and IF reagents

<b>Cat#</b>	<b>Antibody</b>	<b>Vendor</b>
MA339100A647	ZO-1, ALEXA FLUOR 647	ThermoFisher Scientific
NB100-2638AF594	Alkaline Phosphatase, Intestinal Antibody (AAP1) [Alexa Fluor 594]	NOVUS BIOLOGICALS
NBP3-08929AF488	Villin 1 Antibody (VIL1/2310R) [Alexa Fluor 488]	NOVUS BIOLOGICALS
PA582645	CES2 Polyclonal Antibody	ThermoFisher Scientific
NBP2-48854-25UL	CES1 ANTIBODY	NOVUS BIOLOGICALS
PA514896	ANTI-CYP3A4	
53-1449-42	CD144 (VE-cadherin) Monoclonal Antibody (16B1), Alexa Fluor 488	ThermoFisher Scientific
A32731	GOAT ANTI-RABBIT IGG (H+L) HIGHLY CROSS-ADSORBED SECONDARY ANTIBODY, ALEXA FLUOR PLUS 488	ThermoFisher Scientific
5087410001-MIL	DAPI	EMD Millipore
A30106	AF+ 555-PHALLOIDIN	ThermoFisher Scientific
D7132	DEXTRAN, CASCADE BLUE	ThermoFisher Scientific
J19943-K2	PARAFORMALDEHYDE SOLUTION, 4% IN PBS, 1L, AFFYMETRIX	ThermoFisher Scientific
J63209AK	SAPONIN PERMEATING SOLUTION	ThermoFisher Scientific

