iScience, Volume *25*

Supplemental information

Organoid-derived intestinal epithelial cells are a suitable model for preclinical toxicology and pharmacokinetic studies

Yu Takahashi, Makoto Noguchi, Yu Inoue, Shintaro Sato, Makoto Shimizu, Hirotatsu Kojima, Takayoshi Okabe, Hiroshi Kiyono, Yoshio Yamauchi, and Ryuichiro Sato

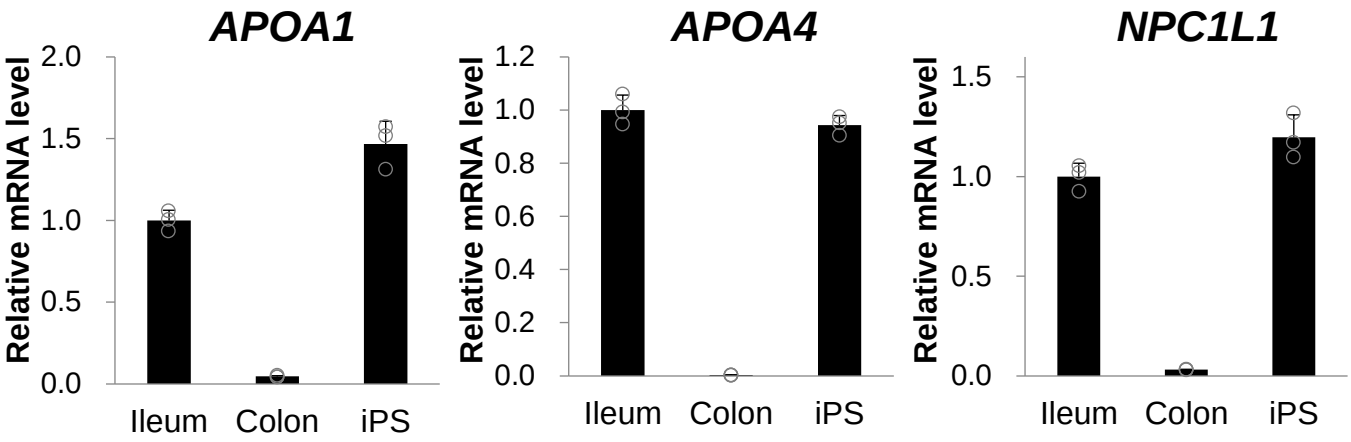

Figure S1. Comparison of small intestinal genes among different types of organoids, Related to Figure 1. Human organoids developed from primary ileum, transverse colon, and iPS cells were cultured in human organoid culture medium for 6 days after passage. After cells were harvested, *APOA1*, *APOA4*, and *NPC1L1* mRNA levels were determined by qRT-PCR and normalized to 18S rRNA levels. Assays were performed in $n = 3$ independent biological replicates (mean ± S.D.).

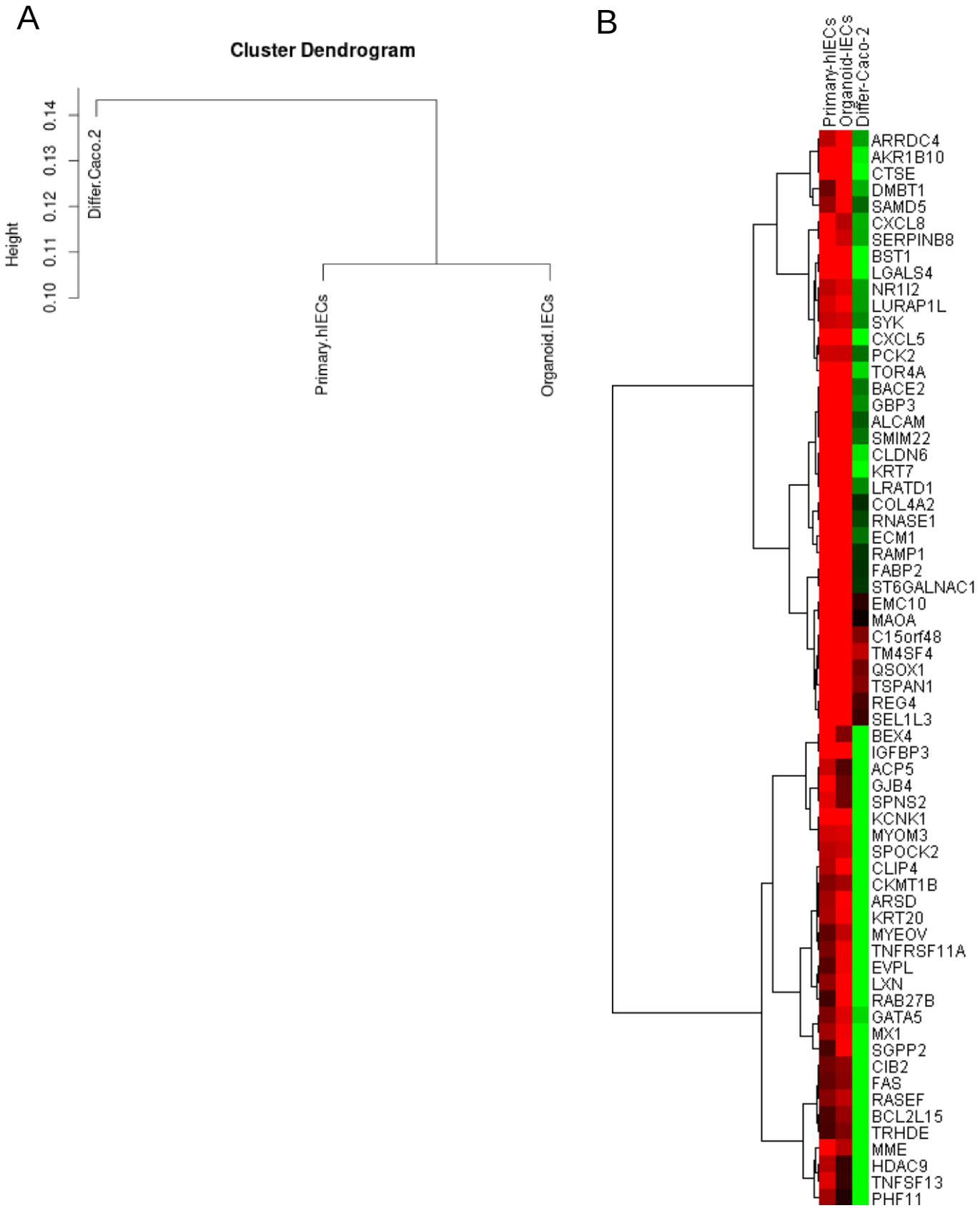

Figure S2. Analysis of RNA sequence data among different types of IECs, Related to Figure 1. RNA was extracted and purified from primary human IECs (Primary hIECs), hiPSO-derived monolayer IECs (Organoid-IECs), and differentiated Caco-2 cells (Differ-Caco-2). (A) A dendrogram was drawn with distances determined by Hierarchical clustering analysis with Spearman correlation of the complete RNA sequence transcriptome data. (B) A heat map of genes with normalized TPM values >1 of Organoid-IECs and more than 20-fold normalized TPM values of Organoid-IECs than those of Differ-Caco-2.

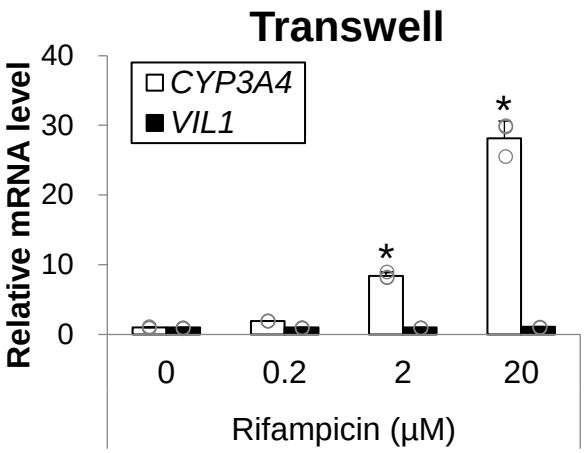

Figure S3. CYP3A4 induction by rifampicin in monolayer organoid-derived IECs cultured in Transwells, Related to Figure 2. hiPSO-derived monolayer IECs in collagen I-coated Transwells were treated with 0, 0.2, 2, 20 μM rifampicin for 48 h. After cells were harvested, *CYP3A4* and *VIL1* mRNA levels were determined by qRT-PCR and normalized to 18S rRNA levels. Assays were performed in $n = 3$ independent biological replicates (mean ± S.D.). Statistical significance was determined by one-way analysis of variance with the Bonferroni test. *$P < 0.05$ (versus no rifampicin).

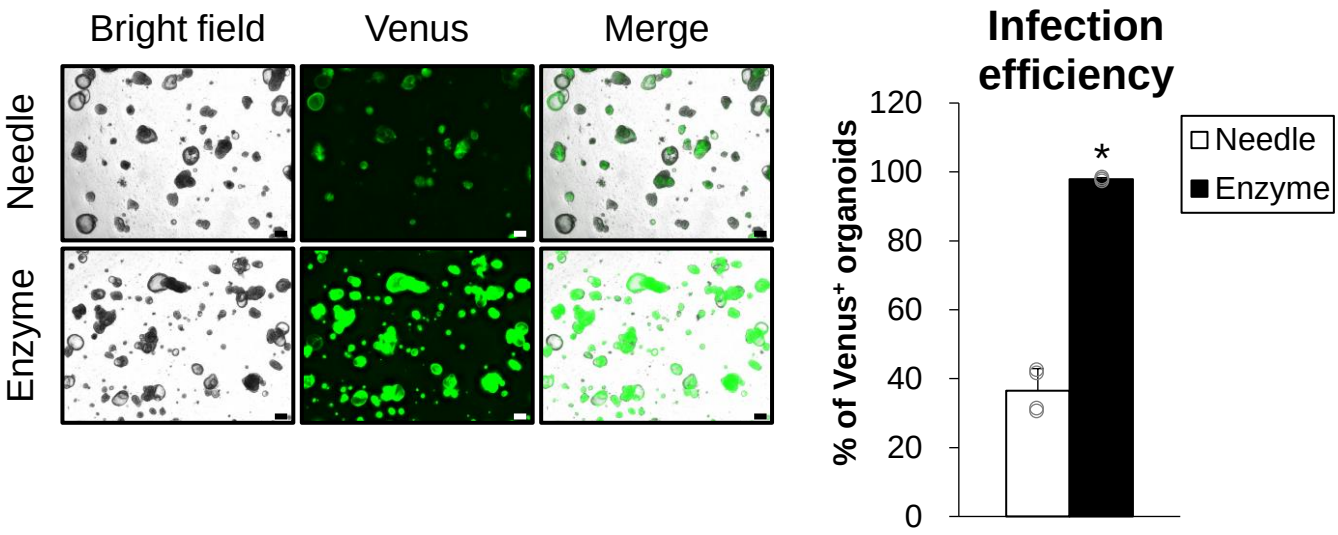

Figure S4. Improved lentiviral infection efficiency into human intestinal organoids, Related to Figure 3. After being disrupted by physical breaking with a 26-gauge needle or dispersed by enzymatic digestion with TrypLE Express solution followed by vigorous pipetting, hiPSOs were seeded on collagen I-coated plates. Cells were cultured for four days (physical breaking, “Needle”) or one day (enzymatic digestion, “Enzyme”), infected with 4-fold diluted internal ribosome entry site-Venus lentiviruses, and embedded in Matrigel to regenerate organoids. (Left) bright-field or fluorescent images of organoids after 9 days of infection were taken. Scale bar, 200 μm. (Right) The proportions of Venus-positive organoids per microscopic bright field after 9 days of infection were calculated. Assays were performed in $n = 4$ independent images (mean ± S.D.). Statistical significance was determined by Student’s t-test. *$P < 0.05$ (versus Needle).

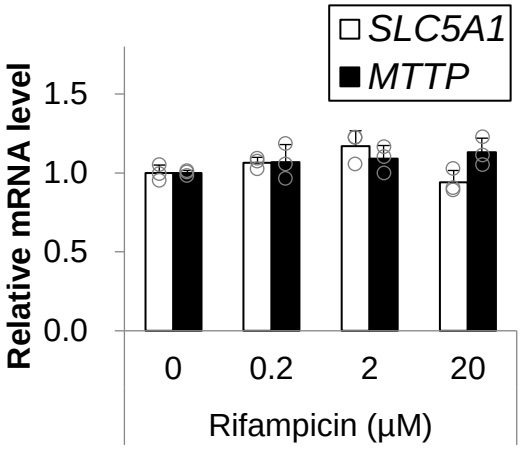

Figure S5. No change in *SLC5A1* and *MTTP* gene expression upon treatment with rifampicin in monolayer organoid-derived IECs, Related to Figure 4. hiPSO-derived monolayer IECs cultured in collagen I-coated 12-well plates were treated with 0, 0.2, 2, 20 μM rifampicin for 48 h. After cells were harvested, *SLC5A1* and *MTTP* mRNA levels were determined by qRT-PCR and normalized to 18S rRNA levels. Assays were performed in $n = 3$ independent biological replicates (mean ± S.D.).

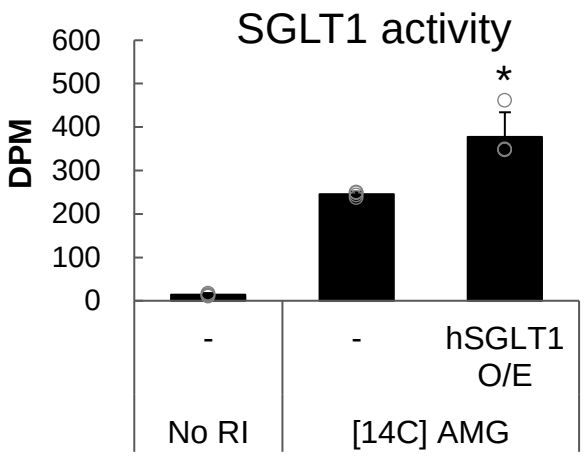

Figure S6. An increase in SGLT1 activity of Caco-2 cells exogenously overexpressing human SGLT1, Related to Figure 4. Caco-2 cells were transiently transfected with either empty (mock) or human SGLT1 expression plasmid using Lipofectamine 3000 reagent. After 72 h, cells were treated with 100 μM AMG and 1 μM [14C]-AMG for 1 h and lysed. A scintillation counter determined radioactivity. Assays were performed in $n = 4$ independent biological replicates (mean ± S.D.). Statistical significance was determined by Student's t-test. *$P < 0.05$ (versus mock).

Table S1. IC_{50} values and Hill coefficients of 4-HPR in hiPSO-derived IECs in the presence or absence of 20 μM rifampicin, Related to Figure 6.

	IC_{50} (μM) (95% confidence interval)		**Hill coefficient** (95% confidence interval)	
	−Rifampicin	+Rifampicin	−Rifampicin	+Rifampicin
−Pretreatment	**0.98** (0.89 - 1.1)	**2.9** (2.2 - 3.7)	**1.2** (1.1 - 1.3)	**1.5** (1.0 - 2.9)
+Pretreatment (24 h)	**0.87** (0.83 - 0.90)	**4.8** (4.6 - 4.9)	**1.9** (1.8 - 2.0)	**4.9** (4.0 - 8.0)

Table S2. A list of qPCR probes and primers used in this study, Related to STAR Methods.

Probe		
Gene	Supplier	ID
CDH17	Integrated DNA Technologies	Hs.PT.58.38621861
CDX2	Integrated DNA Technologies	Hs.PT.58.20039761
CHGA	Integrated DNA Technologies	Hs.PT.58.26803667
CYP3A4	Integrated DNA Technologies	Hs.PT.58.1272782
DGAT1	Thermo Fisher Scientific	Hs00201385_m1
LYZ	Integrated DNA Technologies	Hs.PT.58.24761205
MTTP	Integrated DNA Technologies	Hs.PT.58.94887
NPC1L1	Integrated DNA Technologies	Hs.PT.58.2402286
NR1I3	Integrated DNA Technologies	Hs.PT.58.1610919
NR1I2	Integrated DNA Technologies	Hs.PT.58.417352
PPARA	Integrated DNA Technologies	Hs.PT.58.45310483
VIL1	Integrated DNA Technologies	Hs.PT.58.4630053
18S	Integrated DNA Technologies	Hs.PT.39a.22214856.g

Primer		
Gene	Forward	Reverse
APOA1	5'-AGAGACTATGTGTCCCAGTTTGAAG-3'	5'-CAGTTGTCAAGGAGCTTTAGGTTT-3'
APOA4	5'-CGTGGAACATCTCCAGAAATCT-3'	5'-CTTCCCAATCTCCTCCTTCAGT-3'
APOE	5'-TGCGTTGCTGGTCACATTC-3'	5'-TCTGTCTCCACCGCTTGCT-3'
MUC2	5'-ACTCTCCACACCCAGCATCATC-3'	5'-GTGTCTCCGTATGTGCCGTTGT-3'
SLC5A1	5'-CTACACCATGACCACCAAGTTC-3'	5'-CGGGCCTTTTAAGCAGTATCAA-3'