

## Supplementary

### Age specific ADME gene expression in infant intestinal enteroids

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Supplementary Table 1: composition of solutions used in enteroid isolation and culturing.

Solution	Components	End concentration	Supplier
Storage Buffer	Williams' E		Gibco, Thermo Fisher Scientific, Walthman, MA
	Glucose	2.8 mg/mL	Sigma-Aldrich, Burlington, MA
	Amphotericin B	2.5 µg/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	Gentamicin	500 µg/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	GlutaMAX	2mM	Gibco, Thermo Fisher Scientific, Walthman, MA
	HEPES	10mM	Gibco, Thermo Fisher Scientific, Walthman, MA
Wash Buffer	PBS		Gibco, Thermo Fisher Scientific, Walthman, MA
	Pen/Strep	100 U/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	Gentamicin	500 µg/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	Amphotericin B	2,5 µg/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	Primocin	50 µg/mL	Invitrogen, Carlsbad, CA
Crypt releasing solution	Wash buffer		See above
	EDTA	4 mM	Sigma-Aldrich, Burlington, MA
	DTT	2 mM	Roche, Basel, Switzerland
Advance DMEM +++	Advanced DMEM		Gibco, Thermo Fisher Scientific, Walthman, MA
	Pen/strep	100 U/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	GlutaMAX 100x	2 mM	Gibco, Thermo Fisher Scientific, Walthman, MA
	HEPES	10 mM	Gibco, Thermo Fisher Scientific, Walthman, MA
Organoid Growth Medium	IntestiCult™ Organoid Growth Medium (Human)		STEMCELL™ Technologies, Vancouver, BC, Canada
	IntestiCult™ Organoid Supplement		STEMCELL™ Technologies, Vancouver, BC, Canada
	Pen/Strep	100 U/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	Rock-inhibitor (Y-27632)*	10 µM	STEMCELL™ Technologies, Vancouver, BC, Canada
Organoid Differentiation Medium	IntestiCult™ Organoid Differentiation Medium (Human)		STEMCELL™ Technologies, Vancouver, BC, Canada
	IntestiCult™ Organoid Supplement		STEMCELL™ Technologies, Vancouver, BC, Canada
	Pen/Strep	100 U/mL	Gibco, Thermo Fisher Scientific, Walthman, MA
	DAPT	5 µM	STEMCELL™ Technologies, Vancouver, BC, Canada

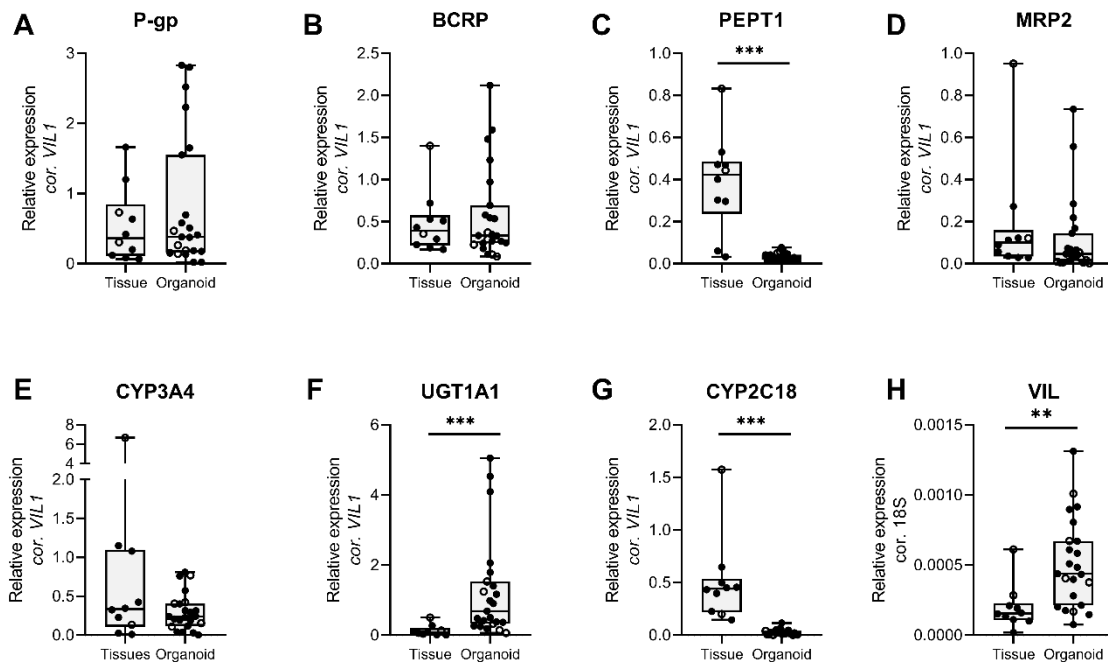
\*Only added during the first 3 days after enteroid isolation.

Supplementary Table 2: statistical values of Figure 2, Wilcoxon matched-paired signed rank test. IQR: interquartile range.

	Median ± IQR early passage	Median ± IQR late passage	p-value paired donors early vs late passage
P-gp	0.47 ± 1.36	0.26 ± 0.46	0.3594
BCRP	0.37 ± 0.549	0.27 ± 0.44	0.0547
PEPT1	0.028 ± 0.02	0.03 ± 0.03	0.6523
MRP2	0.04 ± 0.25	0.05 ± 0.12	0.2500
CYP3A4	0.31 ± 0.34	0.21 ± 0.23	0.4258
UGT1A1	0.99 ± 1.71	0.69 ± 1.32	0.9102
CYP2C18	0.02 ± 0.02	0.02 ± 0.05	0.2500
VIL-1	0.0006 ± 0.0008	0.0004 ± 0.0003	0.2500

Supplementary Table 3: statistical values of Figure 3, Wilcoxon matched-paired signed rank test. IQR: interquartile range.

	Median ± IQR tissue	Median ± IQR enteroid	p-value paired donors tissue vs enteroid
P-gp	0.36 ± 0.74	0.27 ± 0.72	>0.9999
BCRP	0.39 ± 0.36	0.27 ± 0.39	0.7695
PEPT1	0.42 ± 0.25	0.03 ± 0.03	0.0020
MRP2	0.10 ± 0.12	0.04 ± 0.13	0.1934
CYP3A4	0.34 ± 0.99	0.27 ± 0.16	0.2754
UGT1A1	0.07 ± 0.16	0.54 ± 1.10	0.0039
CYP2C18	0.44 ± 0.32	0.024 ± 0.03	0.0020
VIL-1	0.0002 ± 0.00012	0.0005 ± 0.0005	0.0039



Supplementary Figure 1: relative gene expression in all tissues and enteroid samples corrected for Villin-1 (VIL1), Villin-1 corrected for 18S. Shown in Median ± Min. to Max. Shows similar expression in tissue and enteroids for P-gp, BCRP, MRP2, CYP3A4. PEPT1, UGT1A1, CYP2C18 and VIL1 show deviating expressions between tissue and enteroids. Individual dots represent individual donors for tissue and individual passages of all donors for enteroids. Open circles indicate adult donors, closed circles pediatric donors. A: P-gp (ABCB1), B: BCRP (ABCG2), C: PEPT1 (SLC15A1), D: MRP2 (ABCC2), E: CYP3A4, F: UGT1A1, G: CYP2C18, H: Villin-1. \*\*: p < 0.01, \*\*\*: p < 0.001, exact p-values are presented in Supplementary table 4.

Supplementary Table 4: statistical p-values of Supplementary Figure 1, Mann-Whitney test

<b>Early vs late passage</b>	
<b>P-gp</b>	0.7727
<b>BCRP</b>	>0.9999
<b>PEPT1</b>	<0.0001
<b>MRP2</b>	0.1928
<b>CYP3A4</b>	0.3633
<b>UGT1A1</b>	0.0001
<b>CYP2C18</b>	<0.0001
<b>VIL-1</b>	0.0021