

Supplementary material

Title: Development of Caco-2 cells expressing four CYPs via a mammalian artificial chromosome

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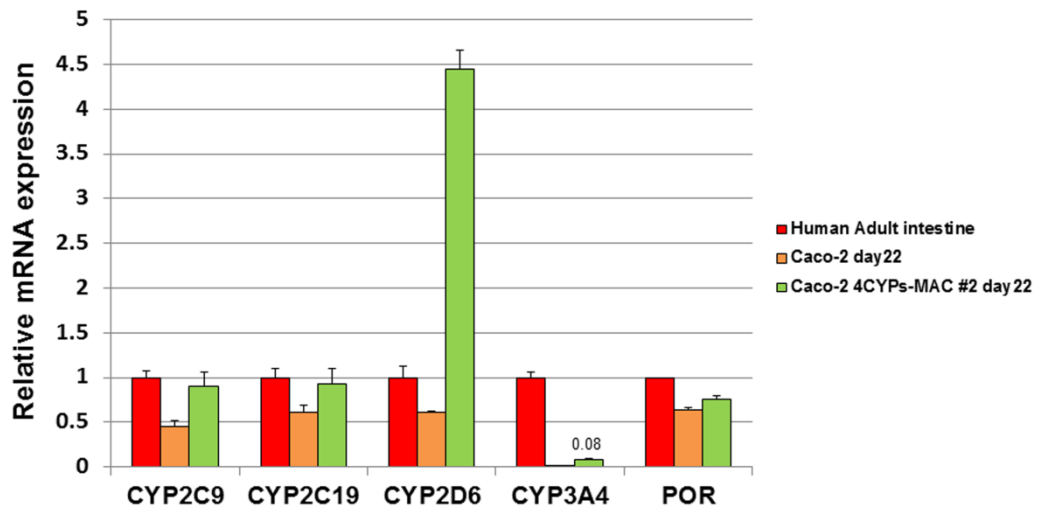
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Supplementary Figure

Figure S1. Comparison of gene expression between human small intestine and day 22 culture of Caco-2 and Caco-2 4CYPs-MAC #2.



Supplementary Tables

Table S1. Primer sequences for genomic PCR and RT-qPCR

Aim	Target gene	Primer name	Sequence (5'-3')
gPCR	CYP2C9	gPCR hCYP2C9_F	ATGGATTCTCTTGTGGTCCT
		gPCR hCYP2C9_R	TCAGACAGGAATGAAGCACA
	CYP2C19	qPCR hCYP2C19_F	ACTTGGAGCTGGGACAGAGA
		CAGpA_rev1764	CAGCCACCACCTTCTGATAGG
	CYP2D6	qPCR hCYP2D6_F	TGATGAGAACCTGCGCATAG
		CAGpA_rev1801	CTCAGTGGTATTTGTGAGCC
	CYP3A4	qPCR hCYP3A4_F	TGTGGGGCTTTTATGATGGT
		CAGpA_rev1801	CTCAGTGGTATTTGTGAGCC
	POR	qPCR hPOR_F	TGGAGGAGGACTTCATCACC
		CAGpA_rev1801	CTCAGTGGTATTTGTGAGCC
RT-qPCR	CYP2C9	qPCR hCYP2C9_F	CCACATGCCCTACACAGATG
		qPCR hCYP2C9_R	TGCCCTTGGGAATGAGATAG
	CYP2C19	qPCR hCYP2C19_F	ACTTGGAGCTGGGACAGAGA
		qPCR hCYP2C19_R	CATCTGTGTAGGGCATGTGG
	CYP2D6	qPCR hCYP2D6_F	TGATGAGAACCTGCGCATAG
		qPCR hCYP2D6_R	CCCTATCACGTCGTCGATCT
	CYP3A4	qPCR hCYP3A4_F	TGTGGGGCTTTTATGATGGT
		qPCR hCYP3A4_R	CCTCCGGTTTGTGAAGACAG
	POR	qPCR hPOR_F	TGGAGGAGGACTTCATCACC
		qPCR hPOR_R	ACAAGCTCGTACTGGCGAAT
	GAPDH	GAPDH real F	AGCCACATCGCTCAGACAC
		GAPDH real R	GCCCAATACGACCAAATCC

Table S2. LC-MS/MS analysis conditions (MDZ, 1'-OH MDZ)

	HPLC condition	MS/MS condition	
column	TSK gel ODS-100V column (50 mm x 0.2 mm, 3 μ m, TOSOH)	Ionization method	ESI+
mobile phase	A: 0.1% formic acid prepared with MQ B:0.1% formic acid prepared with acetonitrile	curtain gas1	30psi
gradient condition	A:B=80:20 (v/v)	curtain gas2	40psi
flow velocity	0.2ml/minute	collision gas	9
column temperature	40°C	Ionspray voltage	5500V
injection amount	10 μ l	Declustering Potential (DP)	100V
		Entrance potential	10V
		Collision energy	40V
		Collision cell exit potential	10V
		Ion source temp	650°C