

Supplementary Material

1 Supplementary Tables

Supplementary Table 1. List of Primers used in RT-qPCR analysis		
Gene	Forward primer	Reverse primer
RPL19	ATGAGTATGCTCAGGCTTCAG	GATCAGCCCATCTTTGATGAG
HPRT1	TATTGTAATGACCAGTCAACAG	GGTCCTTTTCACCAGCAAG
LGR5	GCAGTGTTACCTTCCC	GGTCCACACTCCAATTCTG
HNF1B	ATGATCAAGGGTTACATGCAG	GTCTGGTTGAATTGTCGGAG
HES1	ACGTGCGAGGGCGTTAATAC	ATTGATCTGGGTCATGCAGTTG
KRT7	GGACATCGAGATCGCCACCT	ACCGCCACTGCTACTGCCA
KRT19	CTTCCGAACCAAGTTTGAGAC	AGCGTACTGATTTCTCCTC
ABCB1	AATGATGCTGCTCAAGTTAAAGGG	TCAGTAGCGATCTTCCCAGAACC
SLC4A2	ACTACCTGAGTGACTTCCGA	TGCTACAGAACGAGAAGAAGG
SLC10A2	GTCTACCCTGGTACAGGTGC	GCGGGAAGGTGAATACGACA
GPBAR1	CATTGCCCACATTGCCAG	GAGCCAAGTAGACGAGGAG
GGT1	CCTCAAAGGGTACAACCTCTC	TTGTAGTAGGAGATCGGGTG
CFTR	TTCTGGGAGGAGGGATTTGG	GTGAGAAATTACTGAAGAAGAGGC
AQP1	CACCTCCTGGCTATTGACTACAC	ATCCAGTGGTTGCTGAAGTTGTG
SSTR2	GCAGTCCTCACATTCATCT	TGGTCTTCATCTTGGCATAG
SLC51A	TTGTTCGCCTCCCTATTCC	TTGTGGTCTTTCCTTCGGT

SLC51B	TGTGGTGGTCATTATAAGCATGG	TCTTAGGTTGTTTAGGCTGTTGTG
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Supplementary Table 2. List of Antibodies used in immunofluorescent analysis				
Antibody	Species	Supplier	Cat. No	Dilution
K7	Mouse	Dako	M7018	1:50
K19	Rabbit	Abcam	ab76539	1:150
ZO1	Rabbit	Invitrogen	40-2300	1:250
E-cadherin	Mouse	BD Bioscience	610181	1:100
SCTR	Rabbit	Atlas	HPA007269	1:200
GPBAR1	Rabbit	Atlas	HPA007269	1:200
CFTR	Mouse	Abcam	ab2784	1:200
α -tubulin	Mouse	Sigma-Aldrich	T6793	1:200
Phalloidin Alexa 488		Thermo Fisher	A-12379	1:200
Anti-mouse Alexa 488	Goat	Thermo Fisher	A-11029	1:200
Anti-Rabbit Alexa 488	Goat	Thermo Fisher	A-11034	1:200
Anti-mouse Alexa 568	Goat	Thermo Fisher	A-11004	1:200
Anti-Rabbit Alexa 568	Goat	Thermo Fisher	A-11036	1:200

Supplementary Table 3. Statistical analysis of the gene expression comparison between EM and CDM culture conditions				
Gene name	Days	EM (n = 4) Median (Range)	CDM (n = 4) Median (Range)	Two tailed P value
<i>LGR5</i>	Day 4	23.0 (21.7 - 27.6)	0.51 (0.18 - 1.33)	0.0286
	Day 6	18.2 (14.8 - 25.5)	0.10 (0.09 - 0.44)	0.0286
	Day 8	19.1 (14.4 - 28.5)	0.11 (0.08 - 0.51)	0.0286
<i>HNF1B</i>	Day 4	0.51 (0.04 - 1.04)	0.85 (0.07 - 1.60)	0.4857
	Day 6	0.95 (0.79 - 1.00)	1.51 (1.27 - 1.80)	0.0286
	Day 8	1.15 (0.96 - 1.39)	1.61 (1.18 - 2.18)	0.1143
<i>HES1</i>	Day 4	0.81 (0.59 - 0.90)	1.97 (1.20 - 2.55)	0.0286
	Day 6	1.01 (0.66 - 1.09)	2.01 (0.94 - 2.81)	0.2000
	Day 8	1.16 (0.80 - 1.52)	2.18 (1.97 - 2.53)	0.0286
<i>KRT7</i>	Day 4	2.53 (15.25 - 32.79)	67.1 (38.3 - 82.8)	0.0286
	Day 6	21.5 (11.01 - 35.1)	57.3 (39.2 - 88.2)	0.0286
	Day 8	25.3 (7.9 - 37.2)	66.3 (42.2 - 88.0)	0.0286
<i>KRT19</i>	Day 4	4.90 (2.90 - 9.6)	9.6 (6.9 - 17.3)	0.1143
	Day 6	6.18 (5.05 - 8.93)	11.1 (8.7 - 15.6)	0.0571
	Day 8	6.57 (4.43 - 9.23)	10.4 (8.52 - 12.7)	0.0571
<i>ABCB1</i>	Day 4	0.086 (0.008 - 0.424)	0.109 (0.006 - 0.603)	>0.9999
	Day 6	0.11 (0.07 - 0.45)	0.20 (0.15 - 0.54)	0.2000
	Day 8	0.10 (0.04 - 0.45)	0.22 (0.20 - 0.59)	0.2000

<i>GPBAR1</i>	Day 4	0.0037 (0.0034 - 0.0081)	0.020 (0.005 - 0.074)	0.0571
	Day 6	0.0042 (0.0031 - 0.0053)	0.014 (0.005 - 0.018)	0.1143
	Day 8	0.0035 (0.0025 - 0.0046)	0.012 (0.011 - 0.029)	0.0286
<i>SLC4A2</i>	Day 4	1.0085e ⁻¹⁰ (7.149e ⁻¹² - 3.012e ⁻¹⁰)	1.66325e ⁻¹⁰ (1.351e ⁻¹¹ - 5.146e ⁻¹⁰)	0.6857
	Day 6	1.78e ⁻¹⁰ (1.094e ⁻¹⁰ - 2.933e ⁻¹⁰)	3.811e ⁻¹⁰ (1.4e ⁻¹⁰ - 4.123e ⁻¹⁰)	0.1143
	Day 8	2.2785e ⁻¹⁰ (8.888e ⁻¹¹ - 3.492e ⁻¹⁰)	3.832e ⁻¹⁰ (3.335e ⁻¹⁰ - 8.658e ⁻¹⁰)	0.0571
<i>SLC10A2</i>	Day 4	0.0012 (0.0003 - 0.0027)	0.0089 (0.0033 - 0.0258)	0.0571
	Day 6	0.0003 (0.0003 - 0.0009)	0.0034 (0.0008 - 0.00623)	0.1143
	Day 8	0.0004 (0.0002 - 0.0016)	0.0024 (0.0011 - 0.0087)	0.0571
<i>CFTR</i>	Day 4	0.082 (0.024 - 0.166)	0.123 (0.042 - 0.179)	0.4857
	Day 6	0.118 (0.043 - 0.193)	0.184 (0.067 - 0.241)	0.3429
	Day 8	0.128 (0.052 - 0.217)	0.192 (0.113 - 0.256)	0.3429
<i>AQP1</i>	Day 4	0.0030 (0.0028 - 0.0118)	0.0055 (0.0055 - 0.0056)	0.5333
	Day 6	0.0064 (0.0035 - 0.0230)	0.0070 (0.0008 - 0.0151)	0.8857
	Day 8	0.0071 (0.0061 - 0.0261)	0.0094 (0.0019 - 0.0164)	>0.9999
<i>GGT1</i>	Day 4	0.161 (0.008 - 0.409)	0.901 (0.070 - 2.091)	0.3429
	Day 6	0.353 (0.305 - 0.384)	3.97 (1.73 - 4.22)	0.0286
	Day 8	0.38 (0.28 - 0.67)	5.15 (4.40 - 6.13)	0.0286
<i>SSTR2</i>	Day 4	0.0009 (0.0002 - 0.0025)	0.0073 (0.0003 - 0.0005)	0.2000

	Day 6	0.0009 (0.0002 - 0.003)	0.0011 (0.0005 - 0.0021)	>0.9999
	Day 8	0.0007 (0.0005 - 0.0035)	0.0008 (0.0005 - 0.0019)	0.8857

Supplementary Table 4. Statistical analysis of the gene expression comparison between M/C and HFM			
Gene name	M/C (n = 4) Median (Range)	HFM (n = 4) Median (Range)	Two tailed P value
<i>HNF1B</i>	0.99 (0.19 - 1.58)	1.90 (0.98 - 3.17)	0.3429
<i>HES1</i>	1.71 (0.33 - 3.17)	0.91 (0.65 - 1.17)	0.3429
<i>KRT7</i>	12.3 (8.8 - 27.8)	21.2 (19.4 - 31.6)	0.2000
<i>KRT19</i>	29.0 (6.7 - 36.5)	16.1 (15.6 - 21.5)	0.3429
<i>ABCB1</i>	0.64 (0.22 - 1.12)	2.052 (0.708 - 3.320)	0.3429
<i>GPBAR1</i>	0.0134 (0.0050 - 0.0275)	0.0070 (0.0029 - 0.0109)	0.1143
<i>SLC4A2</i>	5.17 (0.50 - 5.997)	4.147 (2.53 - 5.47)	0.6857
<i>SLC10A2</i>	0.0168 (0.0103 - 0.0585)	0.0341 (0.0034 - 0.0724)	0.6857
<i>CFTR</i>	0.284 (0.170 - 0.491)	1.10 (0.39 - 1.97)	0.0571
<i>AQP1</i>	0.0310 (0.0049 - 0.0573)	0.0315 (0.0026 - 0.1974)	>0.9999
<i>GGT1</i>	24.7 (4.7 - 44.5)	15.0 (5.7 - 18.5)	0.3429
<i>SSTR2</i>	0.0017 (0.0012 - 0.0027)	0.0011 (0.0003 - 0.0015)	0.2286

Supplementary Table 5. Comparison between murine and human ICOs differentiation towards CLCs				
Species	Stage specific differentiation	Culture conditions		
		Medium	3D Plate Scaffold	Bioengineered bile duct
Murine	ICOs Expansion	Advanced DMEM/F12 medium 1% (v/v) Penicillin/Streptomycin 1% (v/v) HEPES (10 Mm) 1% (v/v) GlutaMax 5% (v/v) Rspodin-1 conditioned medium 1% (v/v) B27 supplement without vitamin A 1% (v/v) N2 supplement 10 mM Nicotinamide 1.25 mM n-acetylcysteine 100 ng/mL FGF10 10 nM gastrin 10 µM Forskolin 50 ng/mL EGF 50 ng/mL HGF 5 µM A830	Matrigel	
	ICOs → CP	CEM conditions: Advanced DMEM/F12 medium 1% (v/v) Penicillin/Streptomycin	Matrigel/Collagen 1.2 mg/mL rat-tail type I collagen at a ratio of 2:3	

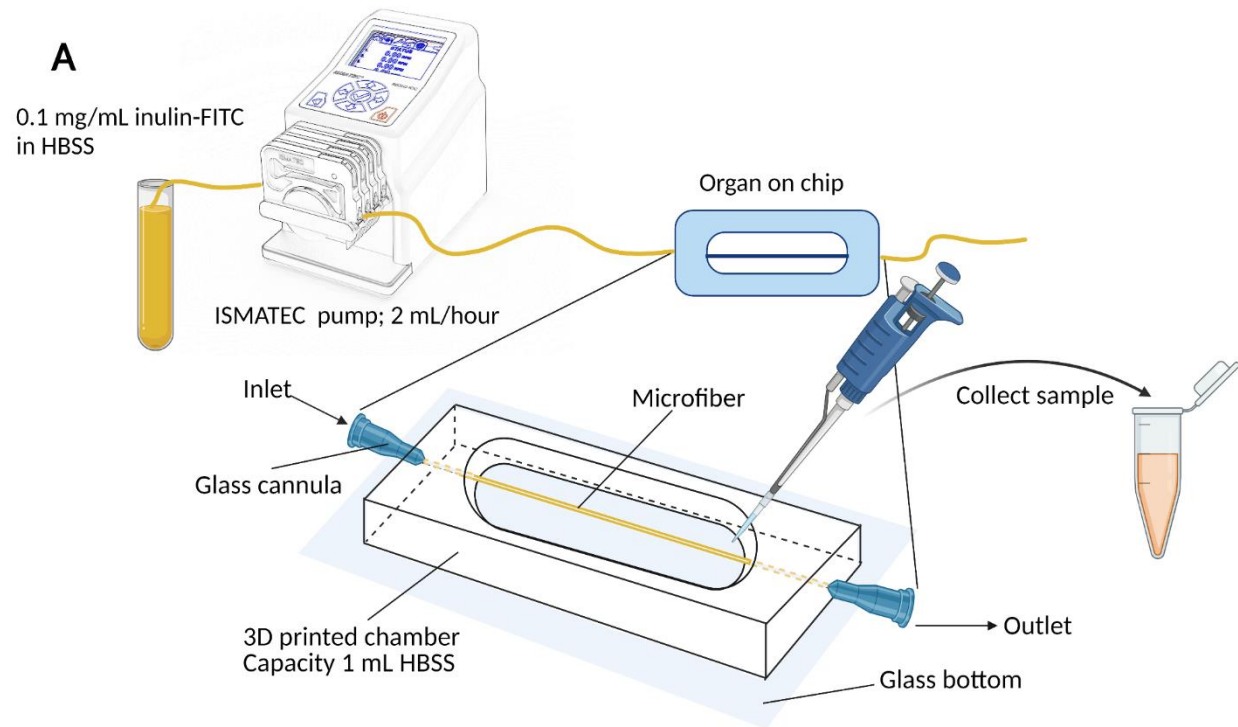
		1% (v/v) HEPES (10 Mm) 1% (v/v) GlutaMax 10% (v/v) FBS 1% (v/v) Non-essential amino acids 50 ng/mL EGH 50 ng/mL HGF 0.1 μ M dexamethasone		
	CP → CLCs	CEM supplemented with 1.25 mM n-acetylcysteine and 10 nM gastrin	Matrigel/Collagen 1.2 mg/mL rat-tail type I collagen at a ratio of 2:3	Double precoated HFM: L-DOPA solution (2 mg/mL Rat-tail type I collagen solution (25 μ g/mL in PBS)
Human	ICOs Expansion	Advanced DMEM/F12 medium 1% (v/v) Penicillin/Streptomycin 1% (v/v) HEPES (10 Mm) 1% (v/v) GlutaMax 10% (v/v) Rspodin-1 conditioned medium 2% (v/v) B27 supplement without vitamin A 1% (v/v) N2 supplement 10 mM Nicotinamide 1.25 mM n-acetylcysteine 100 ng/mL FGF10 10 nM recombinant human (Leu15)-gastrin I 10 μ M Forskolin	Matrigel	Double precoated HFM: L-DOPA solution (2 mg/mL Rat-tail type I collagen solution (25 μ g/mL in PBS)

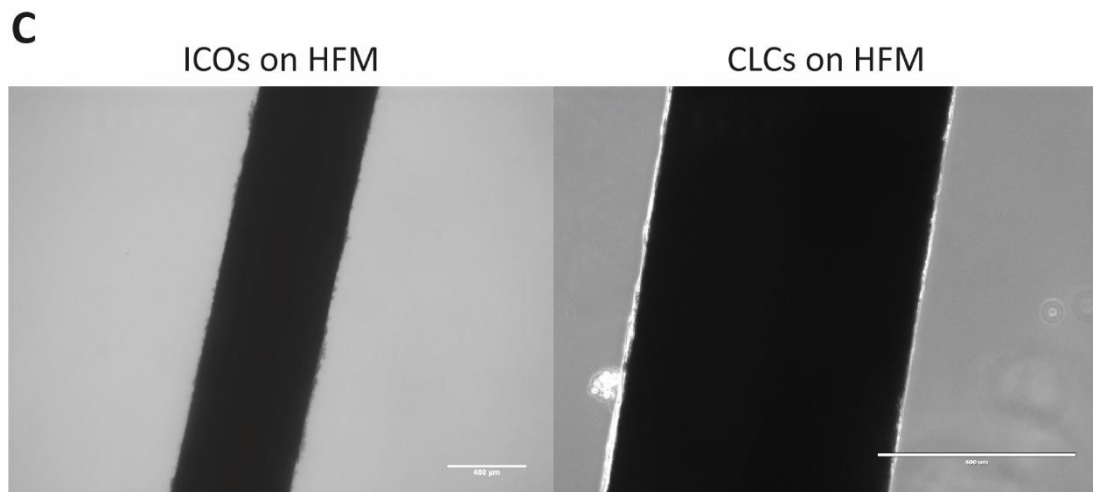
		50 ng/mL EGF 25 ng/mL HGF 5 μ M A830		
	ICOs → CLCs	CDM conditions: Advanced DMEM/F12 medium 1% (v/v) Penicillin/Streptomycin 1% (v/v) HEPES (10 Mm) 1% (v/v) GlutaMax 2% (v/v) B27 supplement without vitamin A 1% (v/v) ITS Premix (contains insulin, human transferrin and selenous acid) 1.25 mM n-acetylcysteine 100 ng/mL FGF10 10 nM gastrin 50 ng/mL EGF 25 ng/mL HGF 5 μ M A830	Matrigel/Collagen 1.2 mg/mL rat-tail type I collagen at a ratio of 2:3	Double precoated HFM: L-DOPA solution (2 mg/mL Rat-tail type I collagen solution (25 μ g/mL in PBS)
FBS: Fetal Bovine Serum; EGF: Epidermal Growth Factor; HGF: Hepatocyte Growth Factor; FGF 10: Fibroblast Growth Factor 10.				

2 Supplementary Figures

2.1 Supplementary Figures

Supplementary Figure 1.





Supplementary Figure 2.

