Supplementary Information

Supplementary Table 1. List of primers

Genes		Primer Sequence (5'- 3')
GAPDH	F	GAA GGT GAA GGT CGG AGT C
	R	GAA GAT GGT GAT GGG ATT TC
OCT4 (POU5F1)	F	CCT GAA GCA GAA GAG GAT CAC C
	R	TCT TGA AGC TAA GCT GCA GA
SOX2	F	GCT ACA GCA TGA TGC AGG ACC A
	R	TCT GCG AGC TGG TCA TGG AGT T
CXCR4	F	CTC CTC TTT GTC ATC ACG CTT CC
	R	GGA TGA GGA CAC TGC TGT AGA G
FOXA2	F	GGA ACA CCA CTA CGC CTT CAA C
	R	GGA TGA GGA CAC TGC TGT AGA G
HNF4A	F	GGT GTC CAT ACG CAT CCT TGA C
	R	AGC CGC TTG ATC TTC CCT GGA T
AFP	F	GCA GAG GAG ATG TGC TGG ATT G
	R	ACG TTC AGC GTG GTC AGT T
ALB	F	GAT GAG ATG CCT GCT GAC TTG C
	R	CAC GAC AGA GTA ATC AGG ATG C
ASGR1	F	GAG AGA GAC GTT CAG CAA CTT C
	R	GGG ACT CTA GCG ACT TCA TCT T
SOX9	F	AGC GAA CGC ACA TCA AGA C
	R	CTG TAG GCG ATC TGT TGG GG
VM	F	CAG GCA AAG CAG GAG TCC AC
	R	AGT GTC TTG GTA GTT AGC AGC
CD68	F	GGA AAT GCC ACG GTT CAT CCA
	R	TGG GGT TCA GTA CAG AGA TGC
HNF4A	F	GGT GTC CAT ACG CAT CCT TGA C
	R	AGC CGC TTG ATC TTC CCT GGA T
CYP3A4	F	GGC AAG CCT GTC ACC TTG AA
	R	CGA GGC GAC TTT CTT TCA TCC TT
UGT1A1	F	AAC AAG GAG CTC ATG GCC TCC
	R	CCA CAA TTC CAT GTT CTC CAG
SULT2A1	F	CGT GAT GAG TTC GTG ATA AGG G
	R	GAC TTG GGG AAT AAC TGG ATG G
F7	F	CCT CAA GTC CAT GCC AGA ATG
	R	CAC AGA TCA GCT GGT CAT CCT

Supplementary Table 2. List of primary antibodies

Primary antibodies	Host/Isotype	Vendor	Dilution/ working concentration
HNF3β/FOXA2	Goat/polyclonal IgG	AF2400; R&D Systems	5 µg/mL
SOX2	Mouse/monoclonal (IgG1)	sc-365823; SantaCruz	1:40
HNF4a	Mouse/monoclonal (IgG1)	sc-374229; SantaCruz	1:40
ALB	Rabbit/polyclonal (IgG)	ab2406; Abcam	5 µg/mL
E-cad	Mouse/monoclonal (IgG1)	sc-21791; SantaCruz	1:40
VM	Rabbit/polyclonal (IgG)	SAB1305445; Sigma-Aldrich	1:40

**Supplementary Table 3.** List of secondary antibodies

Secondary antibodies	Host	Vendor	Dilution
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Donkey	A-21202; Invitrogen	1:400
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647	Donkey	A-31571; Invitrogen	1:400
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Donkey	A-21206; Invitrogen	1:400
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Donkey	A-31573; Invitrogen	1:400
Donkey anti-Goat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Donkey	A-21447; Invitrogen	1:400
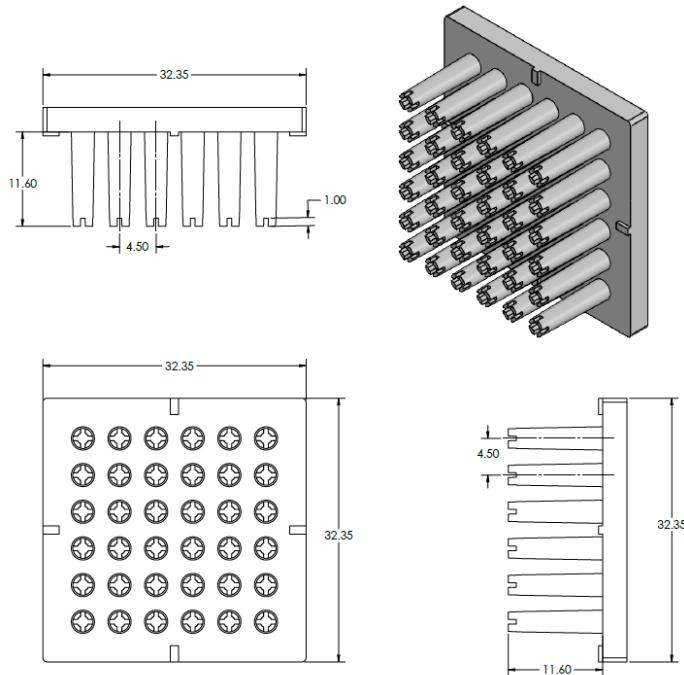
Supplementary Figure 1. SolidWorks design of the 36PillarPlate with a 6 x 6 array of pillars. The unit of the dimension is millimeter (mm).
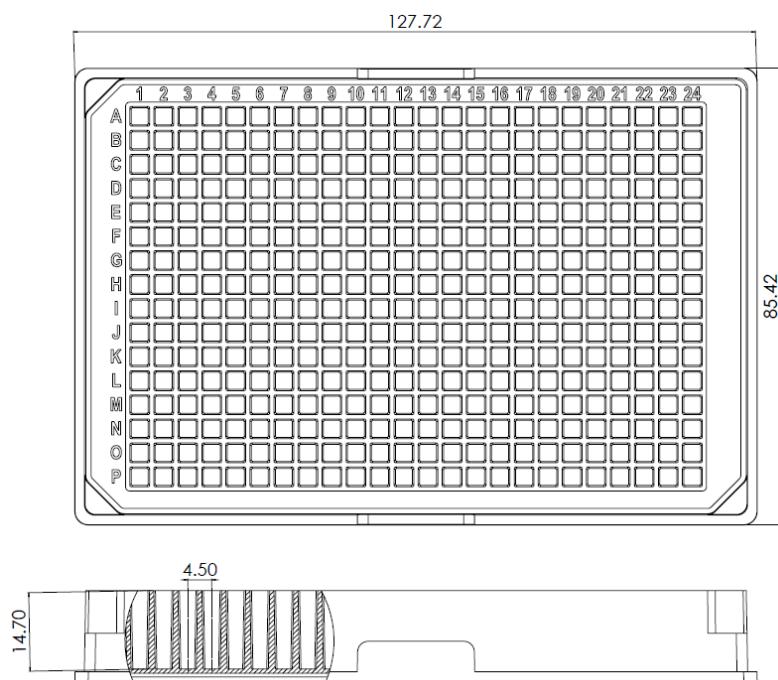
Supplementary Figure 2. SolidWorks design of the 384DeepWellPlate with a 16 x 24 array of deep wells. The unit of the dimension is millimeter (mm).
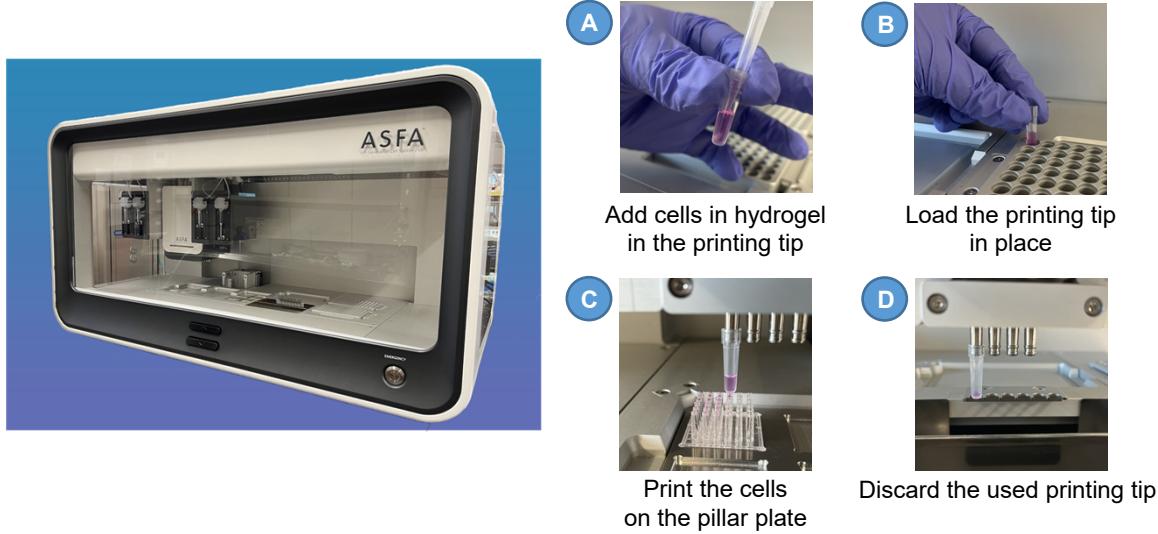
Supplementary Figure 3. The 3D bioprinter used for cell printing on the pillar plate. The 3D bioprinter is operated by microsolenoid valves and pneumatic pressure for sample printing. The pictures A – D illustrate crucial steps necessary for printing cells in hydrogel on the pillar plate.


(A) Dispensing condition

V(nl)	O.T.(µs)	P(kPa)	Vel	I.T.(ms)	Ps
5000	13800	6	40000	10	0

(B) Plate position

Name	Type		X(µm)	Y(µm)	Z(µm)	W2W(µm)
Target_plate_38	384	▼	8030	78600	38000	4500
Source_96	96	▼	334300	15900	49000	9000
Target_well_384	384	▼	7950	78500	40000	4500
Target_plate_36	384	▼	8030	78600	38000	4500

Supplementary Figure 4. (A) Example of a sample dispensing condition. To dispense 5 µL of samples, 13,800 µs of open time (O.T.) and 6 kPa of pressure (P) were used. **(B)** The X, Y, and Z position and the well-to-well (W2W) or pillar-to-pillar distance of sample plates and pillar plates in µm
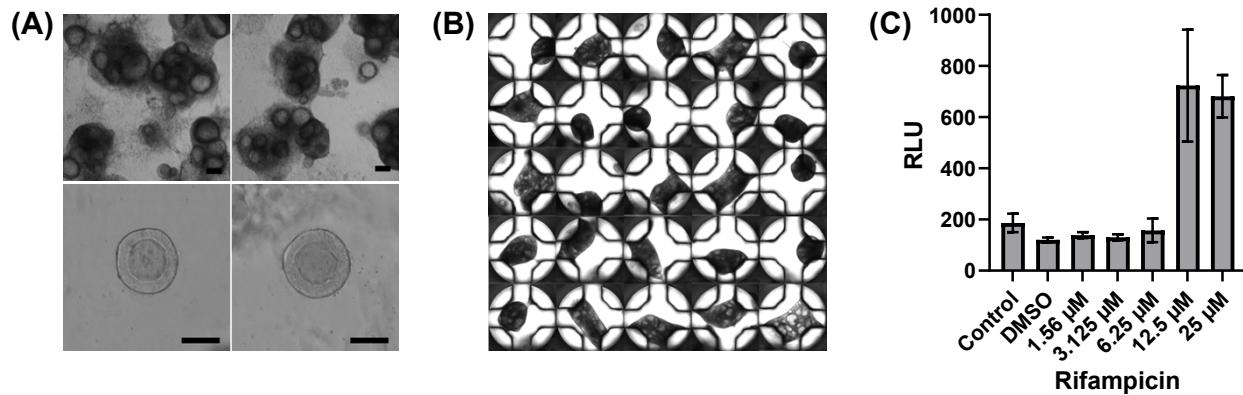
Supplementary Figure 5. Generation of HLOs by differentiation of 72-3 iPSCs. (A) Representative images of HLOs generated in 50 µL Matrigel domes in a 24-well plate (Upper row, scale bars: 200 µm) and magnified images of individual HLOs (Bottom row, scale bars: 50 µm). **(B)** Stitched image of day 25 HLOs on the 384PillarPlate. Day 7 foregut cells were suspended in 2-fold diluted Matrigel and printed on the pillar plate at the seeding density of 3,000 cells/pillar. **(C)** Induction of CYP3A4 by treatment of HLOs with rifampicin at the concentration range of 1.6 µM - 25 µM. The control condition contains no DMSO and no rifampicin whereas the DMSO condition contains 0.5% DMSO alone in the culture medium. All rifampicin treatment conditions contain 0.5% DMSO.