Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

to

Automated screening of endoderm differentiation with 3D human induced pluripotent stem cell cultures on chip

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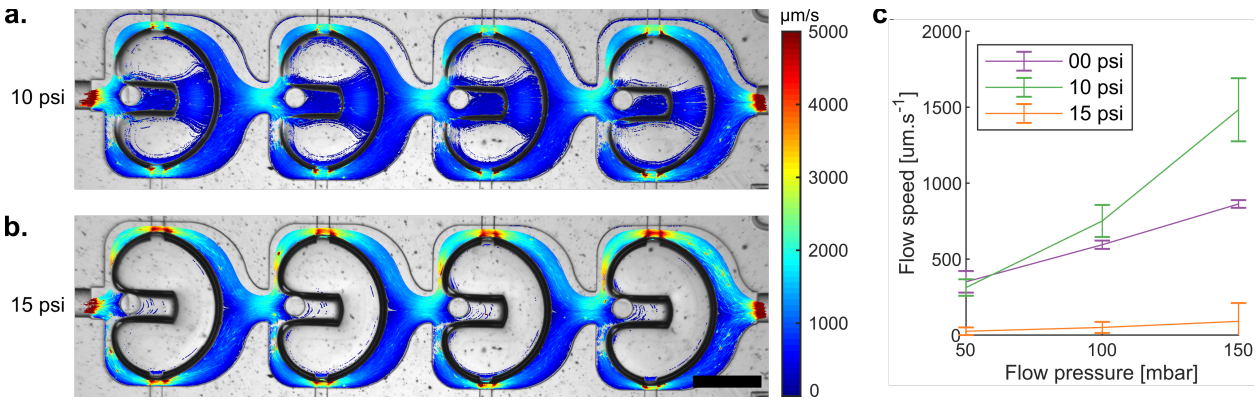
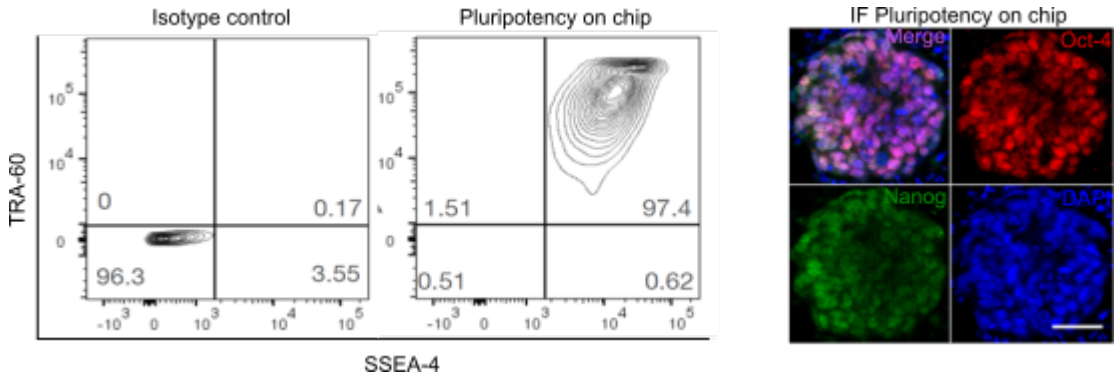
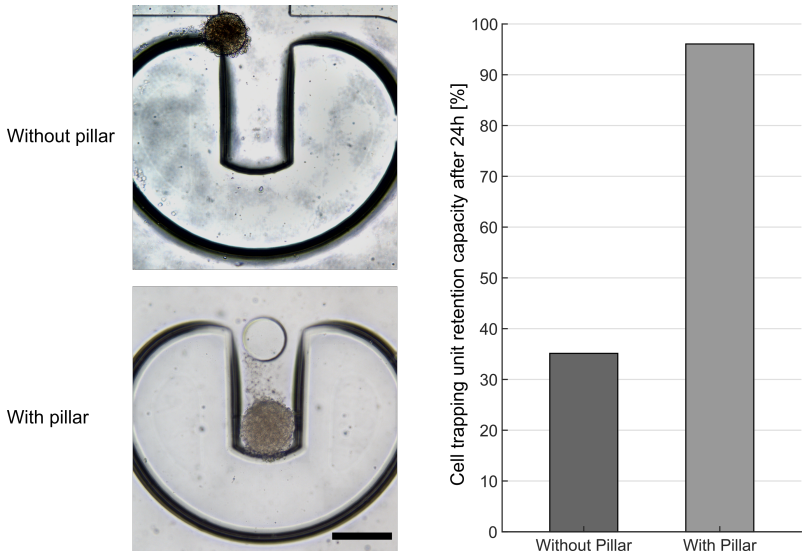
Supplementary Figure 1. Microfluidic large-scale integration chip platform with 128 hiPSC derived 3D cell cultures. Image was taken 24h after seeding. Scale bar denotes for 1000 µm.



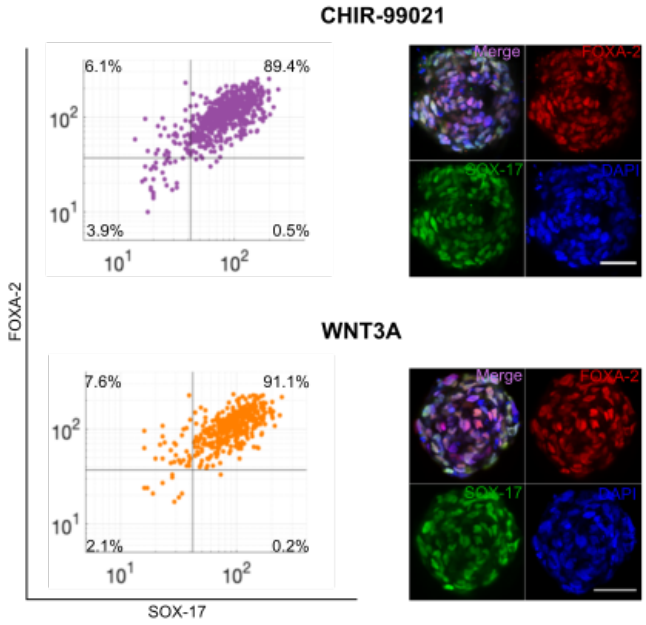
Supplementary Figure 2. Flow characterization of the U-shape valve within a unit cell culture chamber on the mLSI chip. Particle tracking velocity analysis around the U-shape valve within the cell culture chamber actuated with **(a)** Valve restricted state with a control pressure of 10 psi and **(b)** Valve closed state with a control pressure of 15 psi. Particle tracking velocity was achieved using 1:1000 dilution of 2.55µm polystyrene beads (microparticles GmbH), tracked with Trackmate (ImageJ plugin), and analyzed in Matlab. Scale bar denotes for 500 µm. **(c)** Flow speeds in function of flow pressure for the three states of the valves, unrestricted (purple 00psi), restricted (green 10psi), and closed (orange 15psi).



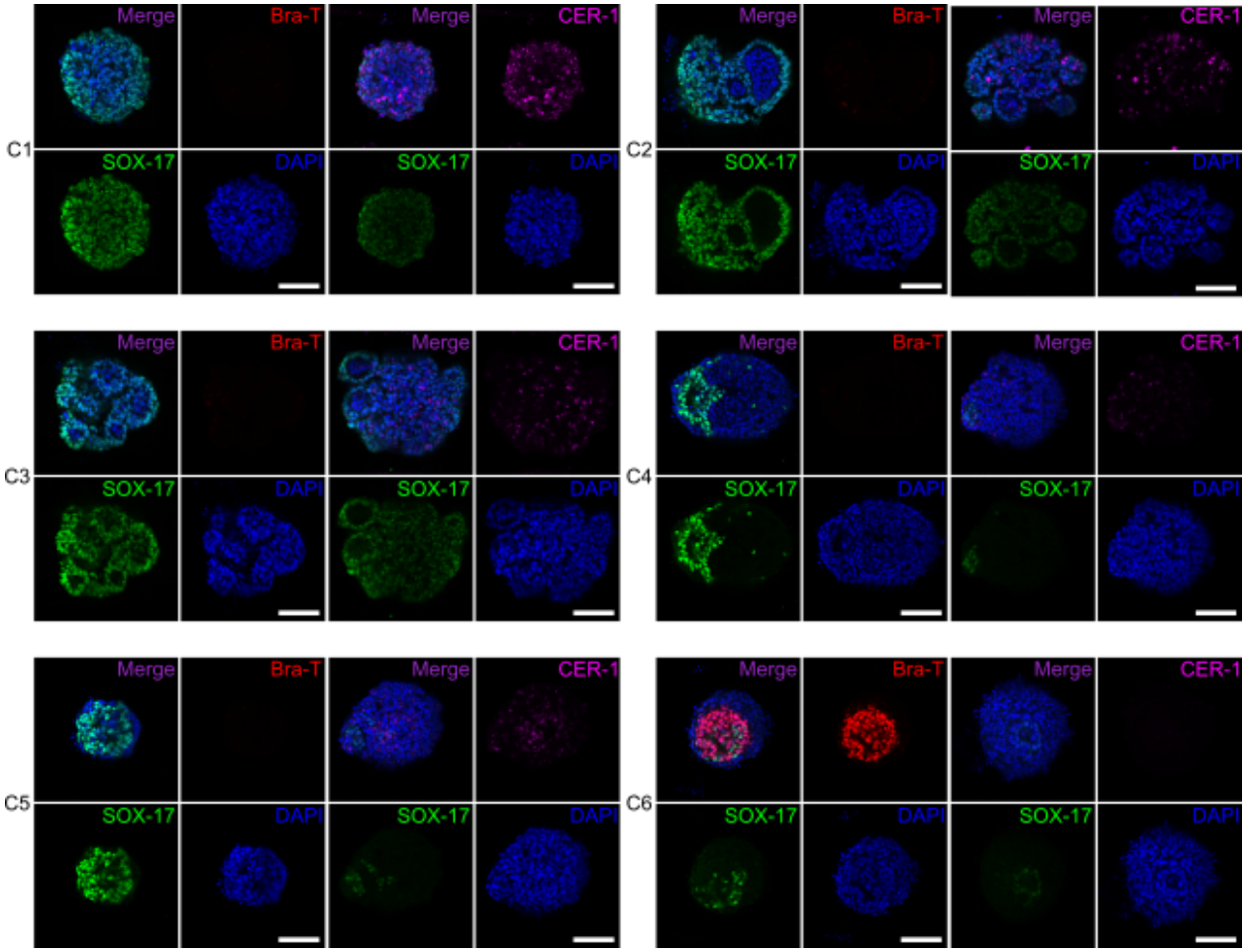
Supplementary figure 3. Evaluation of the hiPSC pluripotency after 3D cell culture formation on-chip. (**left and middle panel**) FACS plot from hiPSCs expressing the pluripotency markers TRA-60 and SSEA-4 after 48 hours of culture inside the mLSi platform. (**right panel**). Representative immunofluorescent image of a 3D hiPSC cell culture expressing the pluripotency transcription factors Oct-4 (red) and Nanog (Green); Nuclei are counterstained with DAPI (blue). Scale bar denotes for 50µm.



Supplementary figure 4: Cell trapping unit retention capacity after 24h. (**left panel**) shows representative brightfield images of 3D cell cultures contained within cell trapping units with and without retention pillar (Scale bar 200µm). (**right panel**) bar plot of the retention capacity of a cell trapping unit expressed as a percentage of the 3D cell cultures still present within the U-shaped traps after 24h of culture.



Supplementary figure 5. On-chip comparison of differentiations induced by either CHIR-99021 or WNT3A. (**left panels**) Distribution of the mean fluorescence intensity of individual nuclei for the transcription factors FOXA-2 and SOX-17 by quantitative confocal image analysis (**right panels**) Representative immunofluorescence images of a DE aggregates induced with CHIR-99021 (top) or WNT3A (bottom) expressing transcription factors FOXA-2 (red) and SOX-17 (green). Nuclei counterstained with DAPI (blue). Scale bar 50µm.



Supplementary Figure 6: Representative IF images of 3D cell aggregates cultured under different conditions (C1-C6), stained for the mesoderm marker Brachyury-T (red), DE marker SOX-17 (green), and anterior DE marker CER-1 (magenta). Nuclei counterstained with DAPI (blue). Scale bar 100 µm.

Supplementary Table 1. Chemical compounds used for definite endoderm differentiations.

Chemicals	**Company, Cat#**
MCDB131	Gibco®, Cat# 10372-019
Glutamax	Life Technologies, Cat# 35050-079
BSA	Sigma, Cat# 10775835001
Sodium bicarbonate	Sigma, MO, Cat# S6297
Glucose	Sigma, Cat# G8769
ITS-X	Life Technologies, Cat# 51500-056
Ascorbic acid	Sigma-Aldrich, Cat# A4544
Penicillin/Streptomycin	Gibco®, Cat# 15140-122

Supplements	**Company, Cat#**
100ng/ml Activin A	Peprotech, Cat# 120-14-300
5μM CHIR-99021	GSK3β inhibitor, Tebu-bio, Cat# 24804-0004
10μM ROCK inhibitor	Y-27632, Santa Cruz Biotechnology, Cat# sc-281642A

Supplementary Table 2. Basal media composition and supplements for conditions P1-P2 in the main text.

Basal media P1-P2
MCDB131
1x Glutamax
0.5% BSA
1.5g/l Sodium Bicarbonate
1.8g/l Glucose
1% penicillin/streptomycin

Component	**Day**	**P1**	**P2**
ActA(ng/ml)	D1	100	100
CHIR (μM)		5	3
ROCK inhibitor (μM)		0	10
ActA(ng/ml)	D2	100	100
CHIR (μM)		0.3	0.3
ActA(ng/ml)	D3	100	100
CHIR (μM)		0	0

Supplementary Table 3. Basal media composition and supplements for conditions P3-P4 in the main text.

Basal media P3-P4
MCDB131
1x Glutamax
2% BSA
2.46 g/l Sodium Bicarbonate
0.44 g/l Glucose
ITS-X 20 µl/l
250 µM ascorbic acid
1% penicillin/streptomycin

Component	Day	P3	P4
ActA(ng/ml)	D1	100	100
CHIR (µM)		5	3
ROCK inhibitor (µM)		0	10
ActA(ng/ml)	D2	100	100
CHIR (µM)		0	0
ActA(ng/ml)	D3	100	100
CHIR (µM)		0	0

Supplementary Table 4. Basal media composition and supplements for the DE differentiation conditions C1-C6 in the main text.

Basal media C1-C6
MCDB131
1x Glutamax
0.5% BSA
1.5g/l Sodium Bicarbonate
1.8g/l Glucose
1% penicillin/streptomycin

Component	Day	C1	C2	C3	C4	C5	C6
ActA(ng/ml)	D1	100	50	50	25	25	0
CHIR (µM)		5	5	7	7	10	10
ActA(ng/ml)	D2	100	50	50	25	25	0
CHIR (µM)		0.3	0.3	0.3	0.3	0.3	0.3
ActA(ng/ml)	D3	100	50	50	25	25	0
CHIR (µM)		0	0	0	0	0	0

Supplementary Table 5. Antibody vendors information.

Target	Antibody	Dilution	Company, Cat#
Pluripotency Markers	Mouse anti-OCT3/4	1:11	Cell signaling Technology, Cat# 75463S
	Human anti-SSEA4-FITC	1:11	Miltenyi Biotec, Cat# 130-098-371
	Human anti-TRA-1-60-PE	1:11	Miltenyi Biotec, Cat# 130-100-347
DE Markers	Rabbit anti-FOXA2	1:400	Cell signaling Technology, Cat# 8186S
	Goat anti-SOX17	1:400	Neuromics, Cat# GT15094
	Mouse anti-human FOXA2-PE	1:20	BD Pharmingen™, Cat# 561589
	Mouse anti-human SOX17-A647	1:20	BD Pharmingen™, Cat# 562594
	Rabbit anti-T	1:300	Abcam, Cat# ab209665

Germ layer markers	Goat anti-CER-1	1:300	R&D systems, Cat #AF1075
Membrane marker	Rat anti-Cadherin-E	1:400	TaKaRa, Cat# M108
Isotype controls	REA Control (S)-PE-Vio615	1:11	Miltenyi Biotec, Cat# 130-107-146
	REA Control (S)-FITC	1:11	Miltenyi Biotec, Cat# 130-104-610
	PE Mouse IgG1	1:160	BD Pharmingen™, Cat# 561589
	AlexaFluor® 647 Mouse IgG1	1:333	BD Pharmingen™, Cat# 561589
Secondary antibodies	Donkey anti-rabbit Alexa Fluor 555 IgG	1:600	Invitrogen, Cat# A31572#
	Donkey anti-goat Alexa Fluor 488 IgG	1:600	Invitrogen, Cat# A11055
	Donkey anti-mouse 647	1:600	Dianova, Cat# 706-545-148
	Donkey anti-rat IgG (H+L)-Cy3	1:600	Dianova, Cat# 712-165-153

Supplementary Table 6. Summary of geometric parameters evaluated for the catching valve design and operation.

Wb [µm]	Wm [µm]	Repeats
220	250	6
	300	6
233	250	6
	300	6
250	150	8
	175	8
	200	8
	250	6
	275	8
	300	6
270	250	6
	300	6
280	150	8
	175	8
	200	8
	275	8
290	250	6
	300	6
320	300	4