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         |

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

| Basal media P1-P2 | Component | Day | P1    | P2    |
|-------------------|-----------|-----|-------|-------|
| MCDB131           | ActA(ng/ml) | D1  | 100   | 100   |
|                   | CHIR (µM)  |     | 5     | 3     |
|                   | ROCK inhibitor (µM) |     | 0     | 10    |
| 1x Glutamax       | ActA(ng/ml) | D2  | 100   | 100   |
| 0.5% BSA          | CHIR (µM)  |     | 0.3   | 0.3   |
| 1.5g/l Sodium Bicarbonate | ActA(ng/ml) | D3  | 100   | 100   |
| 1.8g/l Glucose    | CHIR (µM)  |     | 0     | 0     |
| 1% penicillin/streptomycin |             |     |       |       |
**Supplementary Table 3.** Basal media composition and supplements for conditions P3-P4 in the main text.

| Basal media P3-P4 | Component     | Day  | P3  | P4  |
|-------------------|---------------|------|-----|-----|
| MCDB131           | ActA(ng/ml)   | D1   | 100 | 100 |
|                   | CHIR (μM)     | D1   | 5   | 3   |
| 1x Glutamax       |               |      |     |     |
| 2% BSA            |               |      |     |     |
| 2.46 g/l Sodium Bicarbonate | | | | |
| 0.44 g/l Glucose |               |      |     |     |
| ITS-X 20 μl/l    | rocky inhibitor (μM) | | | |
| 250 μM ascorbic acid | | |     |     |
| 1% penicillin/streptomycin | | | | |

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

| Basal media C1-C6 | Component     | Day  | C1  | C2  | C3  | C4  | C5  | C6  |
|-------------------|---------------|------|-----|-----|-----|-----|-----|-----|
| MCDB131           | ActA(ng/ml)   | D1   | 100 | 50  | 50  | 25  | 25  | 0   |
|                   | CHIR (μM)     | D1   | 5   | 5   | 7   | 7   | 10  | 10  |
| 1x Glutamax       |               |      |     |     |     |     |     |     |
| 0.5% BSA          |               |      |     |     |     |     |     |     |
| 1.5g/l Sodium Bicarbonate | | | | |
| 1.8g/l Glucose    |               |      |     |     |     |     |     |     |
| 1% penicillin/streptomycin | | | | |

**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                  |
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       |