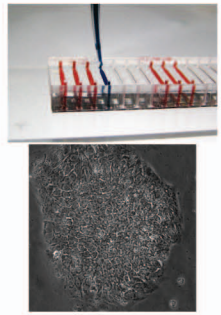
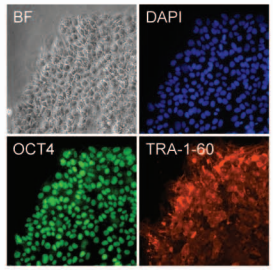
1. Performing hPSC culture on-chip

1-1. Sterlize a microfluidic hPSC array
1-2. Coat the ECM on the cell culture chambers using an electrical pipette (4 μL of each solution, 6 μL sec^{-1} of dispencing rate) at 4˚C for overnight.
1-3. Passage hPSCs cultured in a 6-well plate mechanically or enzymatically with collagenase type IV.
1-4. Load the solution containing hPSCs into the cell culture chambers.
1-5. A few hours after hPSC loading, change medium for every 12 hours using an electrical pipette.



2. On-chip immunocytochemistry

2-1. Fix hPSC colonies with 4% paraformaldehyde in a microfluidic hPSC array.
2-2. Permeabilize hPSCs with PBS containing 0.5% Triton X100 at room temperature for 30 min.
2-3. Incubate the blocking solution.
2-4. Incubate the solution containing primary antibodies.
2-5. Incubate the solution containing secondary antibodies.
2-6. Incubate 300 nM of DAPI solution.
2-7 Obtain image sets of BF, DAPI, OCT4 and SSEA1 (MetaMorph).



3. Segmentaion & Quantification (CellProfiler)

3-1. Identify cells in each image using DAPI channel.
3-2. Quantify OCT4/SSEA1 fluorescent intensity in each cell.
3-3. Export quantified data to Excel.

4. Histogram (Excel & Origin)

4-1. Transform the value of fluorescent intensity (Mean intensity units) of individual cells from linear scale to log scale.
4-2. Draw a histogram of transformed fluorescent intensity in individual cells.

5. Hierarchical clustering & Heat map (TIRG MeV)

5-1. Make average of fluorecent intensity of OCT4/SSEA1 in individual cells for each sample.
5-2. Construct a hierarchy of sample groups represented by a dendgram.
5-3. Display the values of averaged fluoscent intensity of each sample in a heat map.

Fig. S2 Kamei et. al.