Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting information for

Quantifying genetically inserted fluorescent protein in single iPS cells to monitor Nanog expression using electroactive microchamber arrays

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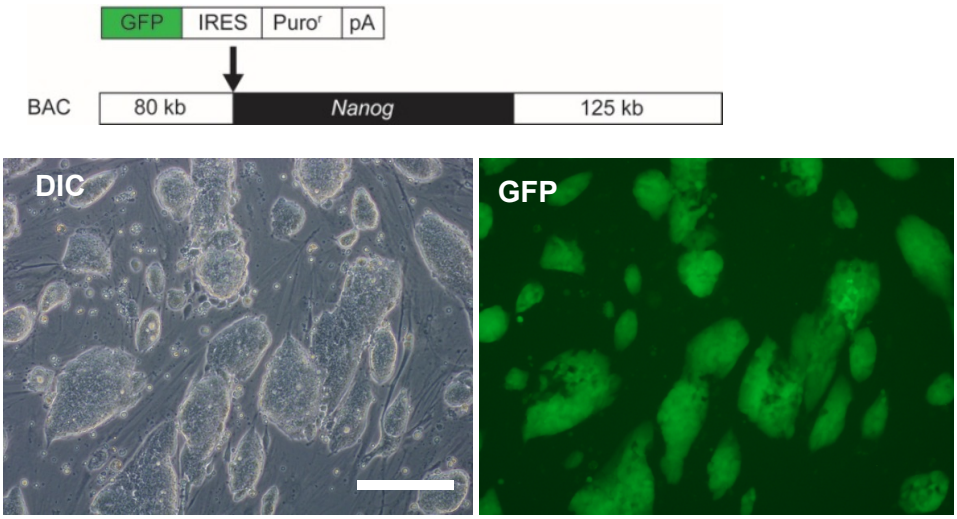

Figure S1. Mouse iPS cells (miPSCs; iPS-MEF-Ng-20D-17 cell line).

A green fluorescent protein (GFP)-internal ribosome entry site (IRES)-puromycin resistance gene (Puror) cassette was inserted into the untranslated region of a bacterial artificial chromosome (BAC) containing the mouse Nanog gene at its center[1]. The scale bar is 500 μm.

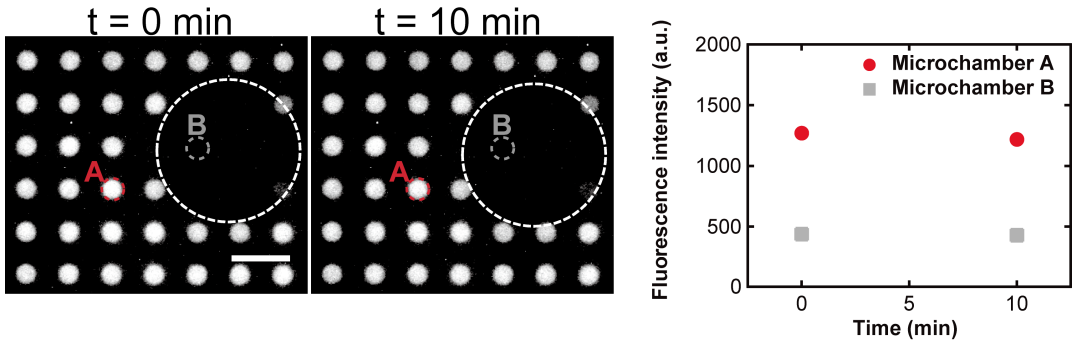

Figure S2. Photobleaching experiment to check for leakage from the microchambers.

The closing of the chamber was evaluated by checking the diffusion of fluorescence dye from one microchamber to the other microchambers. A 1-μM solution of fluorescein dye (Wako Pure Chemical Industries Ltd.) in PBS buffer (Sigma Chemical Co.) was introduced into the device and the PDMS membrane was pressed with a rounded plastic tip to close the microchambers. Then, a randomly selected local area was photobleached for several seconds with strong UV-illumination. The images were taken just after the photobleaching and 10 minutes later, where the white dotted circle indicates the bleached area (left). We plotted a time course of the fluorescence intensity of microchambers (right). Diffusion of the dye across the microchambers, which would result in an increase in fluorescence intensity in the bleached area, was not observed for 10 minutes. The scale bar is 50 μm.

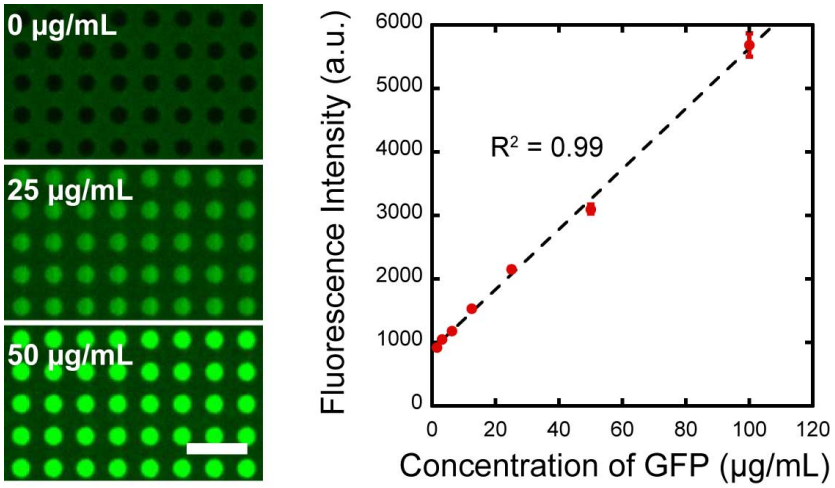

Figure S3. Calibration curve.

Various concentrations of recombinant GFP molecules were introduced into the device, and tightly enclosed microchambers were formed. The averaged fluorescence intensities for the 100 randomly selected microchambers are plotted versus the concentration of GFP molecules. The limit of detection (LOD) of GFP was determined to be 8×10^4 molecules per chamber, where the LOD was determined from the extrapolation of concentration of GFP at the fluorescence intensity equal to the background plus 3 times standard deviation of the background. The scale bar is 100 μm.

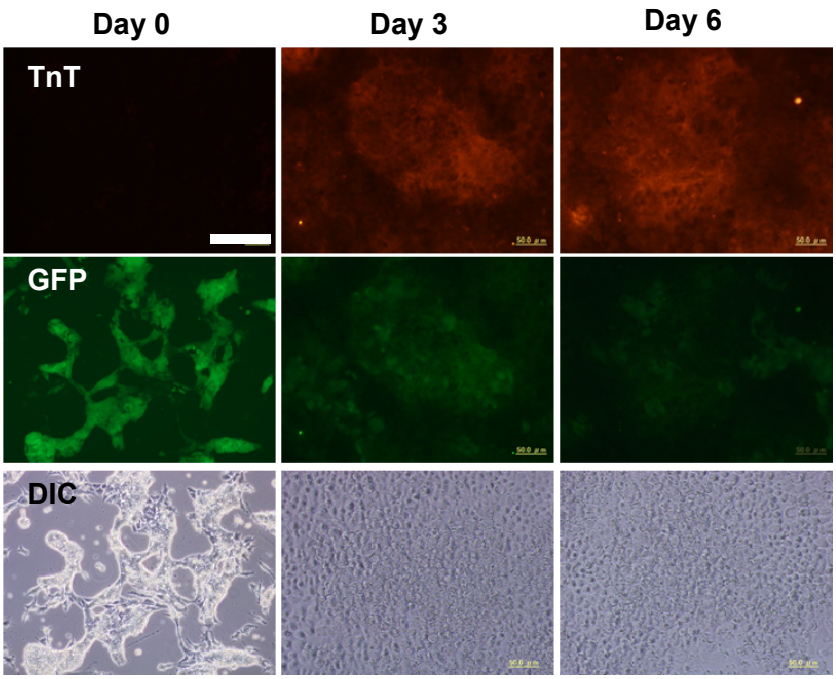

Figure S4. Cardiac differentiation of miPSCs.

In order to induce cardiac differentiation, miPSCs were cultured in medium containing 20% fetal bovine serum (FBS). The cells cultured in each dish were immunostained with anti-Troponin T (TnT) antibodies (red) at days 0, 3, and 6 to verify cardiac differentiation, where TnT is a marker protein for cardiac cells. The expression of the cardiac marker TnT increased, and that of GFP decreased with cardiac differentiation. The scale bar is 500 μm.

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Supplementary movie legends

Movie S1. miPSC trapping using dielectrophoresis.

miPSCs were trapped into the microwell array by inducing the DEP force. A suspension of miPSCs was introduced by laminar flow at flow rate of 1 μL min^{-1}. Because the cells were cultured to maintain their pluripotency, they emitted strong GFP fluorescence. No cells were trapped into the microwell array in the absence of DEP force. When the DEP force of 3.5 Vpp at 5 MHz electric potential was started, cells were gradually and stably trapped into the microwell array.

Movie S2. miPSC lysis using electroporation.

After closing the microchamber, trapped miPSCs were lysed by applying a series of 50 Vpp, 10-μs square pulses at 1 KHz to the electrodes for 10 seconds. Following membrane disruption, the fluorescence intensity of the cell decreased and diffused out, and inside of the microchamber was filled with the intracellular GFP molecules.

Supplementary references

1. K. Okita, T. Ichisaka and S. Yamanaka, *Nature*, 2007, **448**, 313-317.