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*Electronic supplementary information*

# DNA aptamer assemblies as fibroblast growth factor mimics and their application in stem cell culture

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# 1. Supporting figures

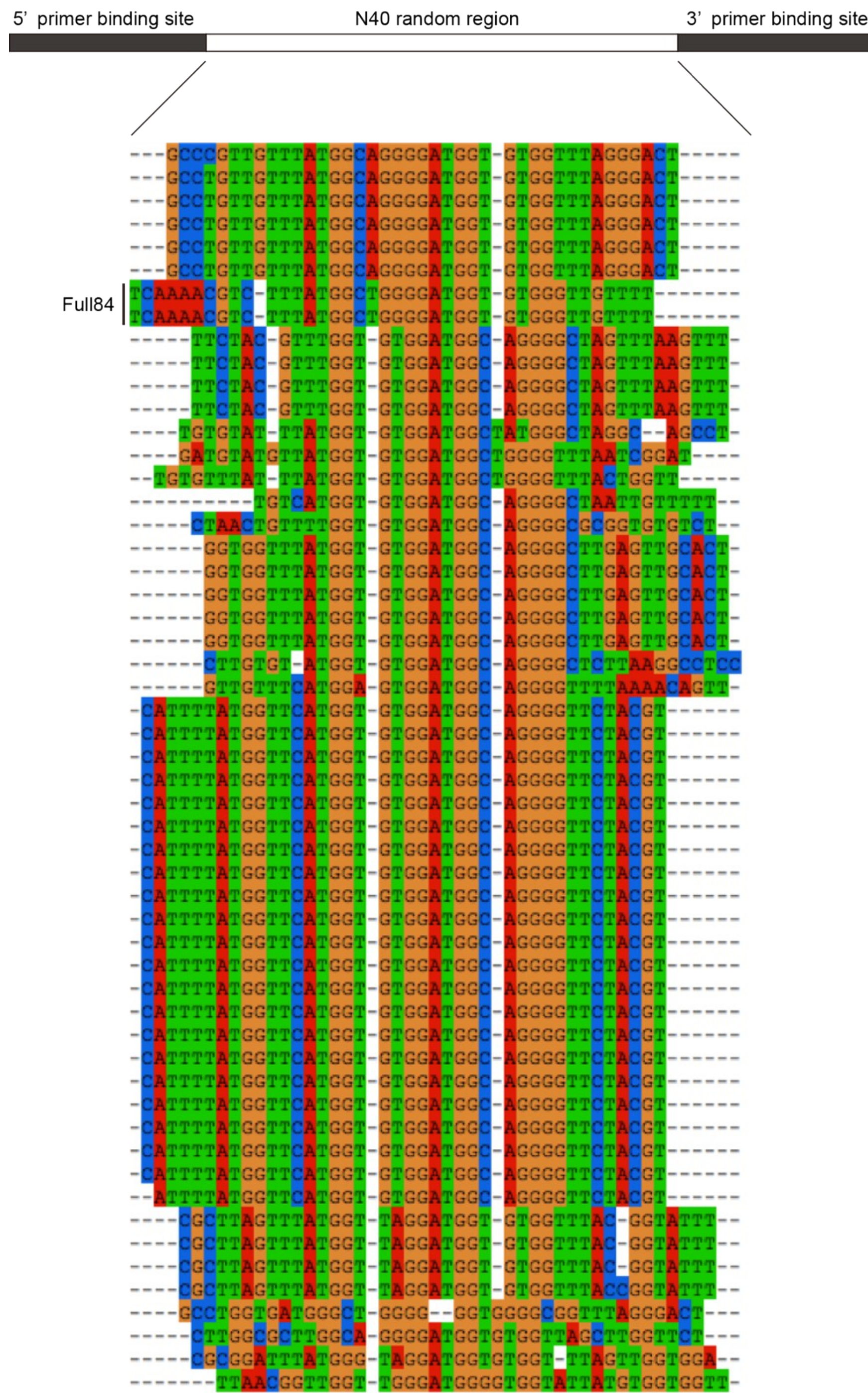



***Fig. S1*** The sequence data of the DNA pool obtained after the 6th round of selection. The sequences of the random regions of $N_{40}$ are shown. Sequence alignment was performed with Clustal X.

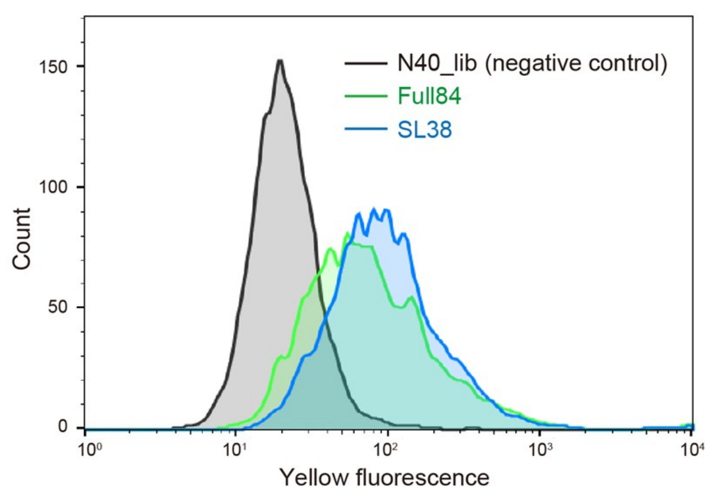



***Fig. S2*** Flow cytometry of aptamer binding to FGFR1-expressing A204 cells. A 5'-biotin-labeled aptamer was incubated with A204 cells for 30 min. After the incubation, aptamer-bound cells were stained with streptavidin-phycoerythrin and analyzed by flow cytometry.

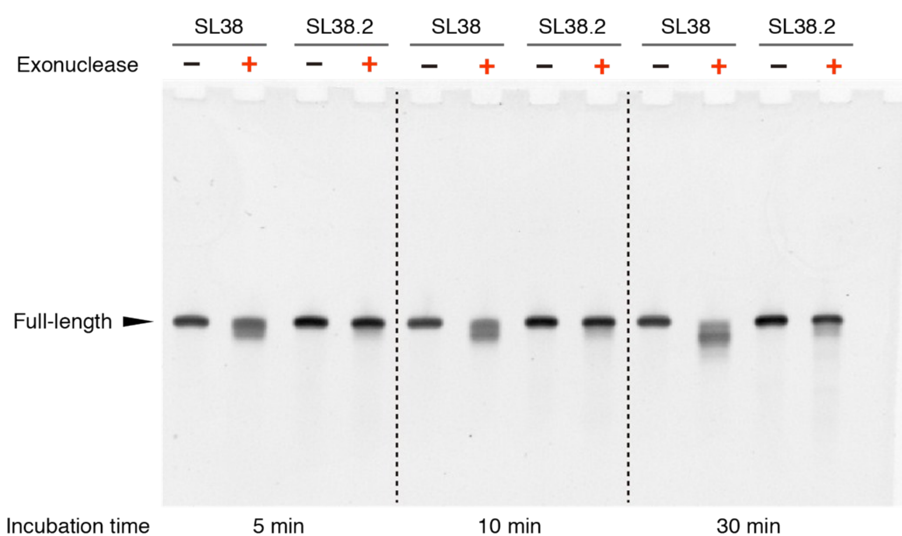



***Fig. S3*** Exonuclease stability of SL38 and SL38.2. Each aptamer (2 μM) was incubated in DPBS containing exonuclease T (25 unit/mL) and 1 mM $MgCl_2$ for the indicated time at 37 °C. After incubation, the samples were immediately analyzed using denaturing 15% polyacrylamide gel electrophoresis.

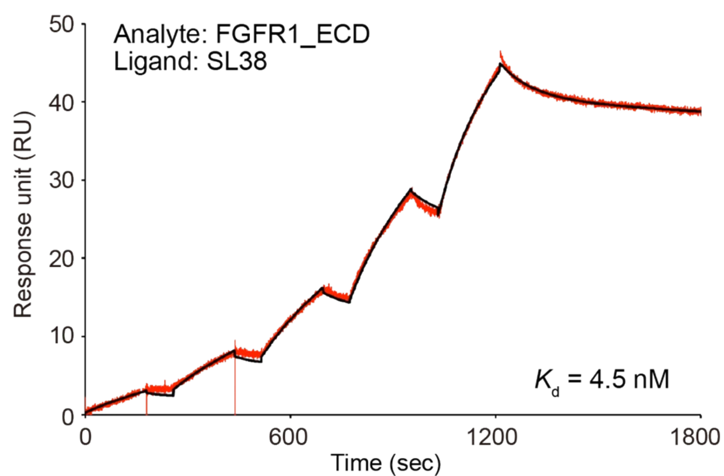



***Fig. S4*** Surface plasmon resonance measurement of binding kinetics of SL38 against the extracellular domain of FGFR1 (FGFR1_ECD). Various concentrations (2.5, 10, 25, 50, 100 nM) of FGFR1_ECD were injected onto the immobilized-DNA surface at a flow rate of 30 μL/min (25˚C, running buffer: DPBS supplemented with 0.2% Nonidet-P40, contact time: 3 min, dissociation time: 1 and 3 min). The sensorgram was fitted to a two-state binding model.

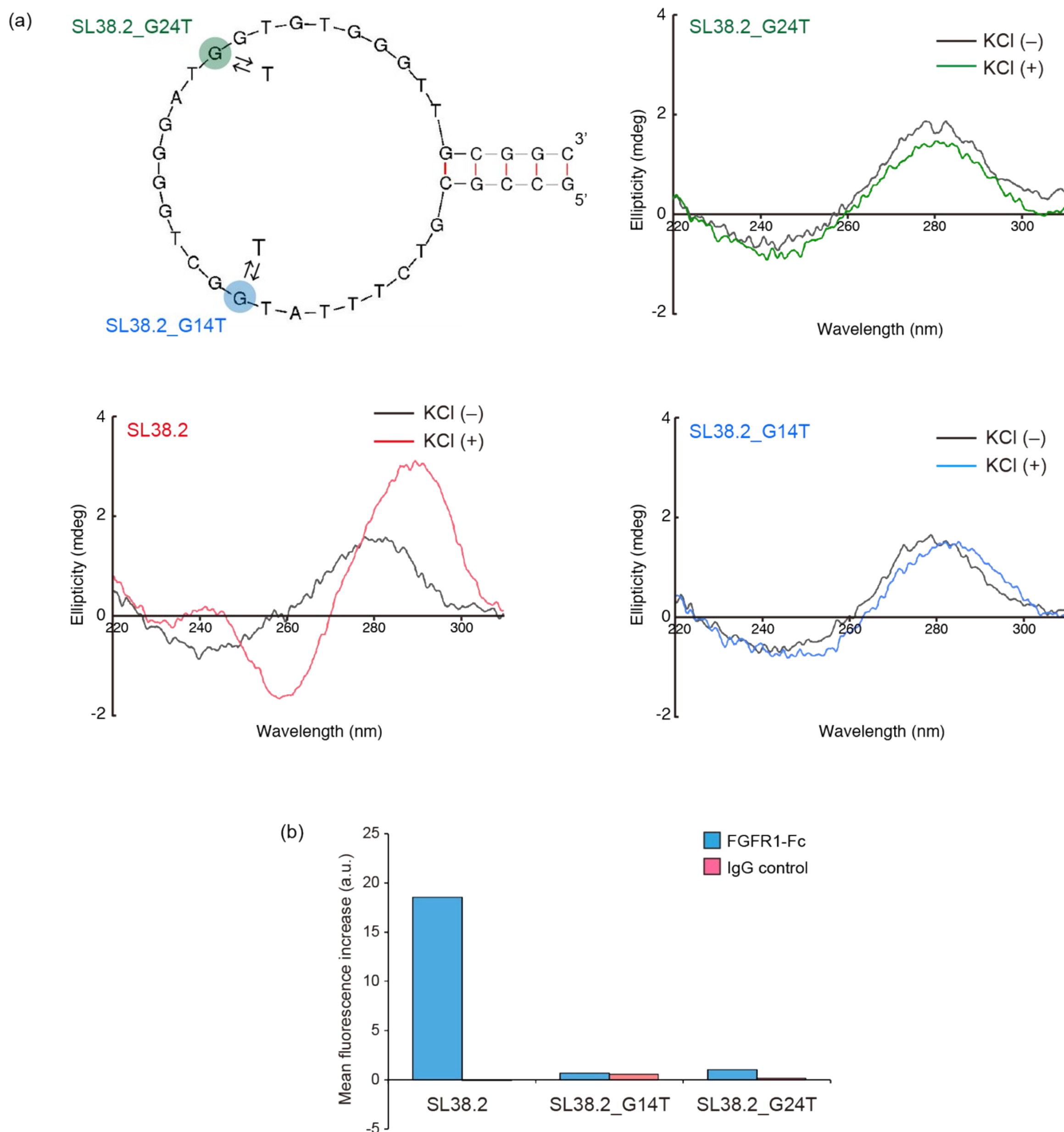



***Fig. S5*** **(a)** CD spectra of SL38.2 and its mutants. DNA samples (5 μM) were prepared in 20 mM Tris-HCl buffer (pH 7.6) with or without KCl (100 mM). **(b)** Flow cytometry of aptamer binding to protein-immobilized beads. A 5'-FAM-labeled aptamer (400 nM) was incubated with FGFR1-Fc (1 pmol) or human IgG (1 pmol)-immobilized beads for 30 min at 37°C. The increase of fluorescence from the beads incubated with the aptamer relative to that from the untreated beads was expressed as mean fluorescence increase.

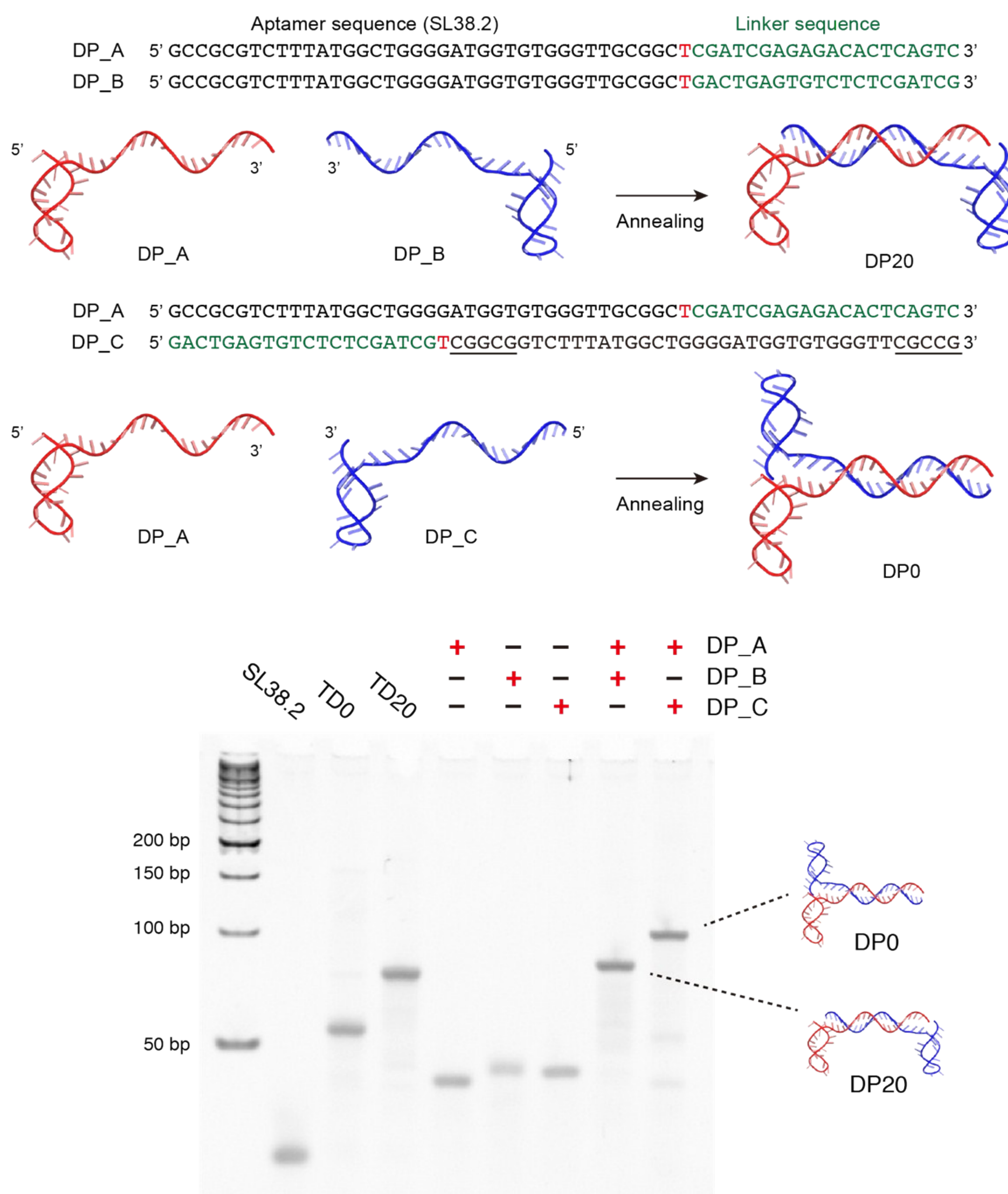



***Fig. S6*** **(Top)** Schematic representation of DP0 and DP20. One thymidine (red) was inserted between the linker sequence (green) and the aptamer sequence (black). The hybridization of DP_A and DP_B yielded DP20. The hybridization of DP_A and DP_C yielded DP0. As shown in the figure, the stem sequence of SL38.2 in DP_C (underlined) was replaced with a different duplex-forming sequence to prevent hybridization to the stem sequence of SL38.2 in DP_A. **(Bottom)** Native PAGE analysis of aptamer assembly. Each DNA (0.5 µM in DPBS) was refolded or annealed with a thermal cycler (95 °C for 5 min, and then cooled at 0.1 °C/sec to 25 °C) and analyzed with 8% Native PAGE.

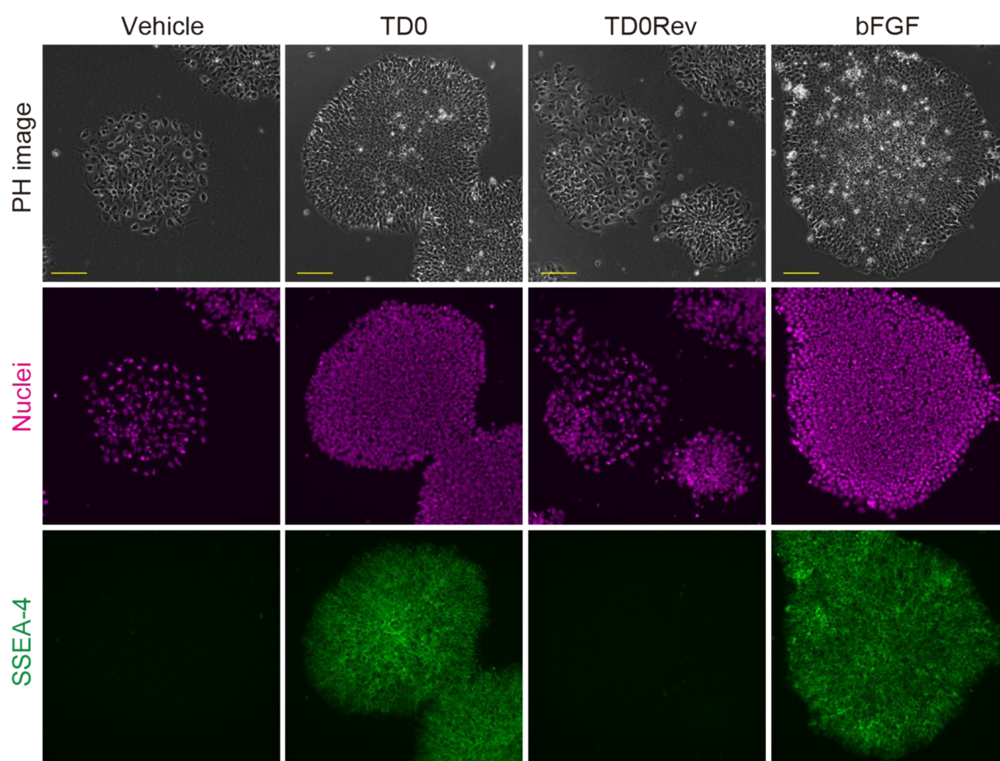



***Fig. S7*** Immunofluorescence staining of SSEA-4 (green) and nuclei (magenta) in the cultured hiPSCs 7 days after seeding. 409B2 hiPSCs were maintained using the same culture protocol as described in Fig. 3e. Scale bars: 100 µm.

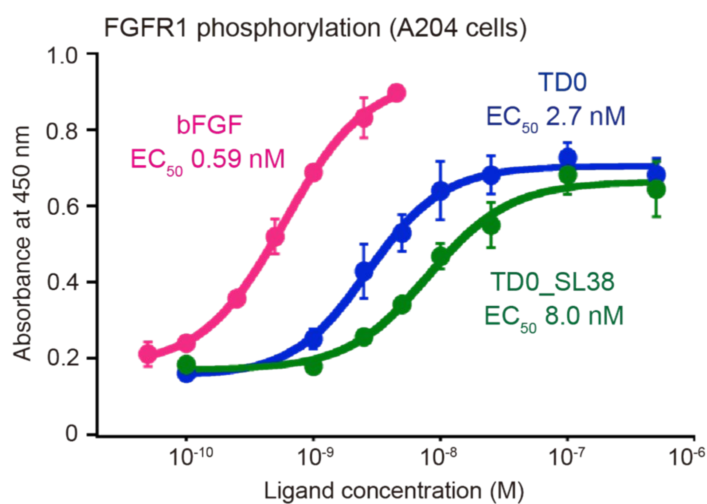
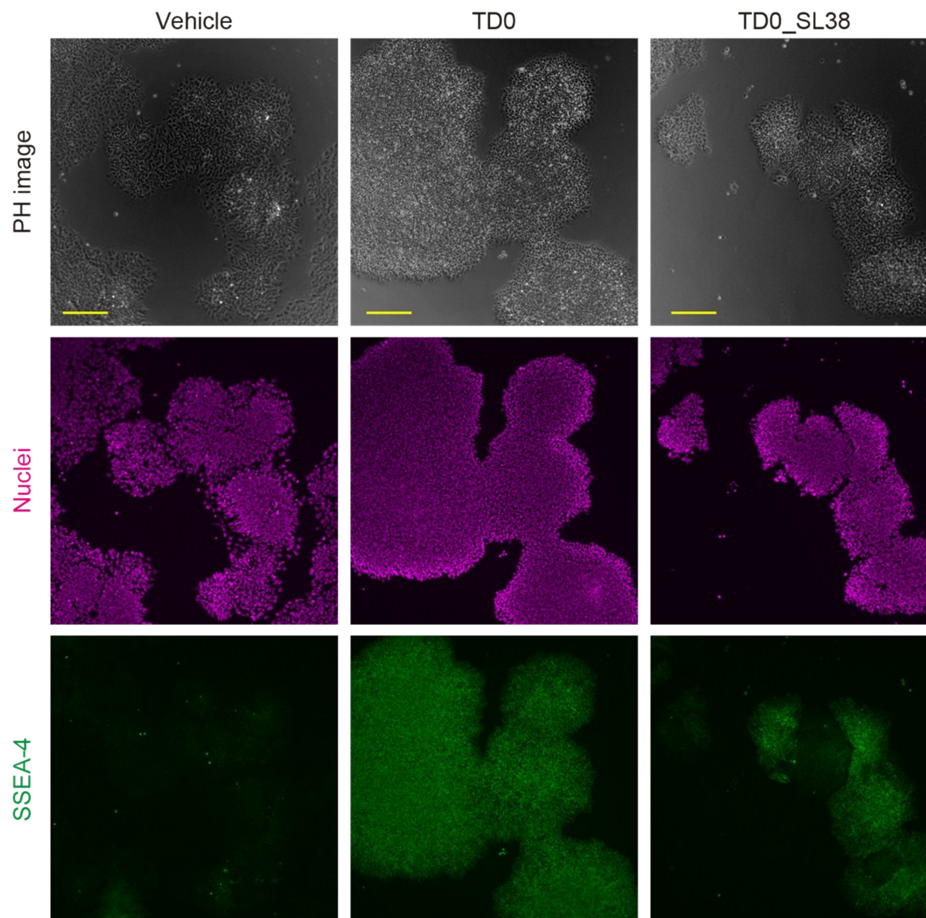



***Fig. S8*** Dose-response curve of FGFR1 phosphorylation induced by bFGF or an aptamer assembly. A204 cells were stimulated by bFGF (2 nM) in the presence of heparin (10 µg/mL) or each aptamer assembly (500 nM) in the absence of heparin for 5 min. The data were expressed as mean values (n = 3) of absorbance at 450 nm.



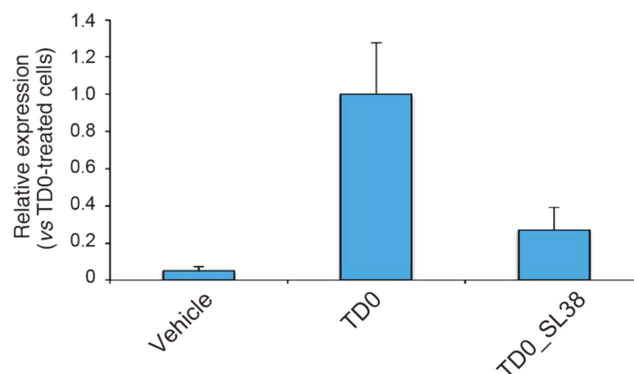




***Fig. S9*** **(a)** Immunofluorescence staining of SSEA-4 (green) and nuclei (magenta) in the cultured hiPSCs 7 days after seeding. 409B2 iPSCs were seeded at day 0 and cultured for 7 days with medium changes on days 1, 3, and 5. The cells were maintained in the presence of bFGF (3 nM) or an aptamer assembly (500 nM). Scale bars: 200 μm. **(b)** RT-qPCR analysis of the gene expression level of OCT4 in the cultured hiPSCs 7 days after seeding. The relative mRNA expression was normalized to the control gene RPLP0, and fold changes were calculated with reference to the TD0-treated sample. Error bars indicate standard deviation (n = 3).

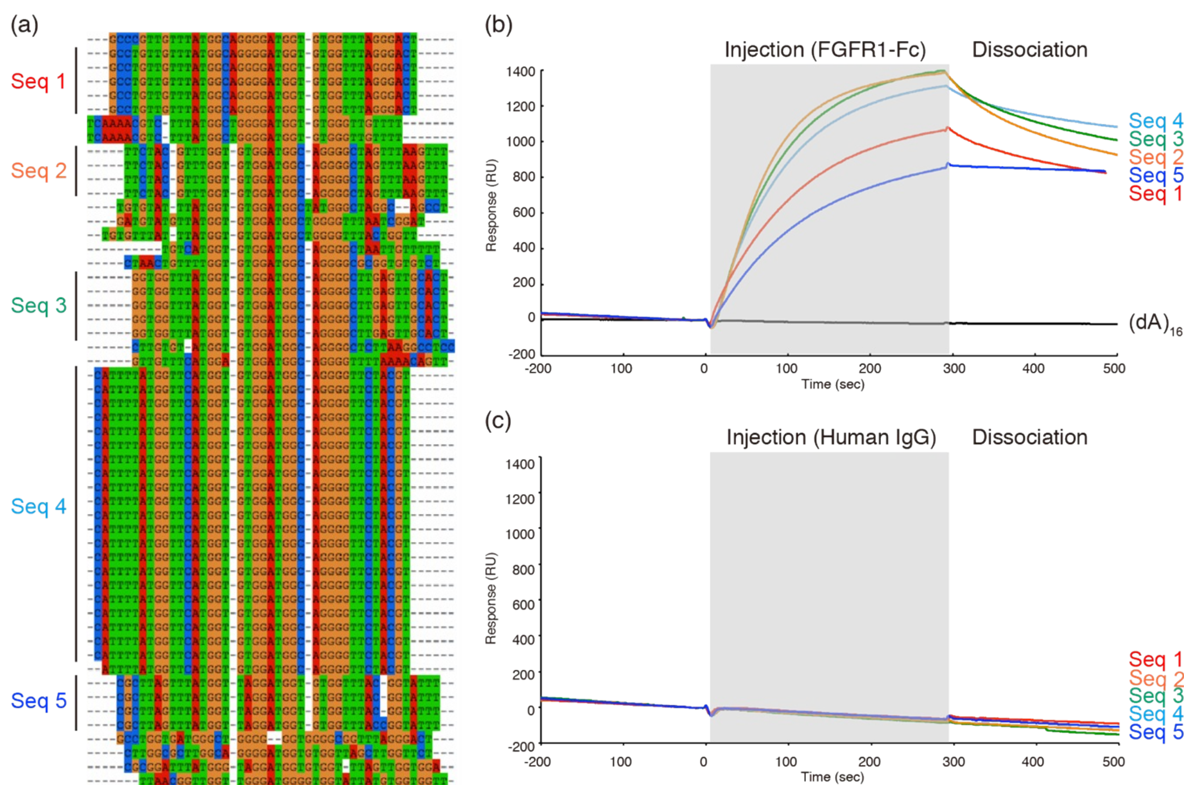



***Fig. S10*** SPR analysis of aptamer candidates. (a) The sequence data of the aptamer candidates used in the experiments. (b) and (c) Sensorgram of SPR analysis. The aptamer candidates were immobilized on sendor chip by DNA hybridization according to the literatures (*e.g.* Y. J. Yuan *et al. Optical Engineering,* 2011, *50,* 034402) with some modifications. Briefly, the 3'-biotinylated poly(dA) 16-nucleotides was immobilized on the streptavidin sensor chip. The candidate aptamers were synthesized with poly(dT) 16-nucleotides linker at the 5' end for the annealing to the biotinylated poly(dA) on the sensor chip. The aptamer candidates were dissolved in DPBS supplemented with 500 mM NaCl and injected at a flow rate of 5 µL/min. After immobilization, the recombinant human FGFR1 protein (100 nM) or human IgG (100 nM) were injected onto the immobilized-DNA surface at a flow rate of 5 µL/min (25˚C, running buffer: DPBS supplemented with 0.05% Nonidet-P40, contact time: 5 min, dissociation time: > 3 min). After injection of the protein, the immobilized candidate DNA was removed with regeneration solution (50 mM NaOH in 1 M NaCl) and the same analysis procedure was repeated for the next sample.

## 2. Sequence data

**N40_lib**: 5’ GGA GGG AAA AGT TAT CAG GCN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN GAT TAG TTT TGG AGT ACT CGC TCC 3’

**Fw_primer**: 5’ (FITC) GGA GGG AAA AGT TAT CAG GC 3’

**Rev_primer**: 5’ (biotin) GGA GCG AGT ACT CCA AAA CTA ATC 3’

**Full84**: 5’ GGA GGG AAA AGT TAT CAG GCT CAA AAC GTC TTT ATG GCT GGG GAT GGT GTG GGT TGT TTT GAT TAG TTT TGG AGT ACT CGC TCC 3’

**SL38**: 5’ AAA ACG TCT TTA TGG CTG GGG ATG GTG TGG GTT GTT TT 3’

**SL38Rev**: 5’ TTT TGT TGG GTG TGG TAG GGG TCG GTA TTT CTG CAA AA 3’

**L30**: 5’ CGT CTT TAT GGC TGG GGA TGG TGT GGG TTG 3’

**SL38.2**: 5’ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GC 3’

**SL38.2_G14T**: 5’ GCC GCG TCT TTA TTG CTG GGG ATG GTG TGG GTT GCG GC 3’

**SL38.2_G24T**: 5’ GCC GCG TCT TTA TGG CTG GGG ATT GTG TGG GTT GCG GC 3’

**TD0**: 5’ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCG CCG CGT CTT TAT GGC TGG GGA TGG TGT GGG TTG CGG C 3’

**TD0_SL38**: 5’ AAA ACG TCT TTA TGG CTG GGG ATG GTG TGG GTT GTT TTA AA ACG TCT TTA TGG CTG GGG ATG GTG TGG GTT GTT TT 3’

**TD0Rev**: 5’ CGG CGT TGG GTG TGG TAG GGG TCG GTA TTT CTG CGC CGC GGC GTT GGG TGT GGT AGG GGT CGG TAT TTC TGC GCC G 3’

**TD20**: 5’ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCT TTT TTT TTT TTT TTT TTT TGC CGC GTC TTT ATG GCT GGG GAT GGT GTG GGT TGC GGC 3’

**DP_A**: 5’ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCT CGA TCG AGA GAC ACT CAG TC 3’

**DP_B**: 5’ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCT GAC TGA GTG TCT CTC GAT CG 3’

**DP_C**: 5’ GAC TGA GTG TCT CTC GAT CGT CGG CGG TCT TTA TGG CTG GGG ATG GTG TGG GTT CGC CG 3’

# 3. Methods

### 3-1 General information

Reagents were purchased from standard suppliers and used without further purification. All DNA samples were purchased from Fasmac (Japan) and Eurofins Genomics Tokyo (Japan). Refolding and annealing of DNA aptamer was performed with a thermal cycler (95 °C for 5 min, and then cooled at 0.1 °C/sec to 25 °C or cooled to room temperature). Dulbecco's phosphate buffered saline (DPBS) was obtained from Wako chemicals (Japan). Recombinant human basic FGF (#100-18B) and recombinant human TGF-β (#100-21) were purchased from PeproTech (USA). Recombinant human FGFR1-Fc chimera protein (#661-FR), recombinant human Met-Fc chimera protein (#358-MT) and human IgG (#1-001-A) were purchased from R&D systems (USA). Recombinant human FGFR1 extracellular domain (#7421-20) was purchased from BioVision (USA). Exonuclease T (M0265) was obtained from New England Biolabs (USA). Fluorescent images were obtained with an inverted microscope (IX-81, Olympus, Japan) equipped with a sCMOS camera (Zyla4.2, Andor, United Kingdom). Absorbance measurements and fluorescence measurements were performed with Infinite M200 pro (Tecan, Switzerland).

### 3-2 *In vitro* selection of FGFR1-binding DNA aptamer

Protein immobilization: FGFR1-Fc was incubated with Dynabeads Protein G (#10004D, Thermo Fisher Scientific, USA) in PBS-T buffer (DPBS supplemented with 0.02% Tween 20 (wt/vol)) for 30 min. Slurry (12.6 μL; binding capacity = 20 pmol) was used for the immobilization of a 10 pmol protein. After incubation, the beads were washed with PBS-T buffer three times and used for the selection. Human Met-Fc-immobilized beads were prepared by the same procedure and used for the negative selection. The amount of recombinant proteins used for each selection round is summarized in Table S1.

Isolation of FGFR1-binding sequences: The ssDNA pool was dissolved in DPBS, denatured at 95 °C for 5 min, and slowly cooled at 0.1 °C/sec to 25 °C. After refolding, an equivalent volume of 0.04% Tween 20 (wt/vol) in DPBS was added to the ssDNA pool. The ssDNA pool was incubated with Dynabeads Protein

G for 30 min at room temperature to remove bead-binding sequences. During the 5th and 6th selection rounds, negative selection, using human Met-Fc-immobilized beads, was also performed. The supernatant was then incubated with FGFR1-immobilized beads for 10–30 min at room temperature. After incubation, the beads were washed with PBS-T buffer. The FGFR1-binding sequences were eluted from the beads by incubating the beads two times with elution buffer (7 M urea in 100 mM sodium acetate, 3 mM EDTA) for 3 min at 95 °C. The eluted DNA was isolated by PCI extraction and ultrafiltration with Amicon Ultra (#UFC501096). The conditions for each selection round are summarized in Table S1.

Generation of the ssDNA pool: The eluted DNA was amplified by PCR, using the KOD polymerase according to the manufacturer's protocols. The primer DNA sequences (Fw_primer and Rev_primer) are shown as "sequence data" in the supplementary information. After the PCR, the reaction mixture was incubated with streptavidin magnetic beads (#21344, Thermo Fisher Scientific, USA) in 10 mM Tris-HCl, 1M NaCl, and 1 mM EDTA for 10 min at room temperature. The ssDNA was eluted from the beads by incubation in 150 mM NaOH for 5 min at room temperature. The supernatant was neutralized with 150 mM HCl and desalted with a G-25 spin column. The ssDNA was eluted from the column with nuclease-free water and used for the next round of selection.

Sequencing: After the 6th selection round, the recovered DNA was amplified by PCR using the non-modified primers. The PCR product was inserted into a cloning vector using a TArget Clone™-Plus kit. (#TAK-201, TOYOBO, Japan). After standard *E. coli* transformation and cloning procedures, aptamer candidate sequences were identified by DNA sequencing. The whole sequence data is shown Fig. S1.

| | | Positive selection | | | | Negative selection | |
|---|---|---|---|---|---|---|---|
| Round | input ssDNA (pmol) | FGFR1-Fc (pmol) | Incubation volume (mL) | Incubation time (min) | Number of wash | Protein G beads (slurry vol.) | Met-Fc (pmol) |
| 1 | 2000 | 100 | 1 | 30 | 3 | 20 uL × 1 | - |
| 2 | 100 | 40 | 0.5 | 30 | 3 | 20 uL × 5 | - |
| 3 | 100 | 40 | 0.5 | 30 | 3 | 20 uL × 5 | - |
| 4 | 10 | 10 | 0.5 | 30 | 3 | 20 uL × 5 | - |
| 5 | 5 | 1 | 1 | 10 | 5 min × 3 | 20 uL × 3 | 5 pmol × 3 |
| 6 | 5 | 1 | 1 | 10 | 5 min × 3 | 20 uL × 3 | 5 pmol × 3 |

***Table. S1*** Experimental conditions for *in vitro* selection of FGFR1-binding DNA aptamer.

**3-3 Flow cytometry**

The fluorescent signal from each bead (Fig. 1c and Fig. S5) or cell (Fig. 2e and Fig. S2) was measured by flow cytometry (Guava eacyCyteTM, Merck Millipore, USA).

Aptamer binding to FGFR1-immobilized beads (Fig. 1c and Fig. S5): Human FGFR1-Fc or human IgG (1 pmol for each sample) was incubated with Dynabeads Protein G in PBS-T for 30 min. After incubation, the beads were washed with the PBS-T for three times. A 20 pmol of 5'-FITC-modified DNA was incubated with FGFR1-immobilized beads in PBS-T (50 μL) for 30 min at 37 °C. After incubation, the beads were washed with PBS-T and analyzed by flow cytometry. The increase of fluorescence from the beads incubated with the aptamer relative to that from the untreated beads was expressed as mean fluorescence increase.

Expression of SSEA-4 in hiPSCs (Fig. 2e): 409B2 cells were detached from the dish and washed with the DPBS. The cells were incubated with anti-SSEA-4 Alexa 488 conjugate (1:100 dilution, #53-8843, Thermo Scientific Pierce, USA) in FCM buffer (DPBS with 1% BSA) for 30 min on ice. After incubation, the cells were washed with the FCM buffer two times and analyzed by flow cytometry. The increase of fluorescence from the cells stained with the antibody relative to that from the unstained cells was expressed as mean fluorescence increase.

Aptamer binding to FGFR1-expressing cells (Fig. S2): A 25 pmol of 5'-biotin-modified DNA was incubated with FGFR1-expressing A204 cells in PBS containing 0.5% BSA (50 μL at 21 °C. After 30 min, streptavidin-phycoerythrin (19.3 pmol, #405203, BioLegend, USA) was added to the solution. After 30 min incubation, the cells were washed with PBS two times and analyzed by flow cytometry.

**3-4 SPR analysis**

Kinetic analysis of FGFR1-binding aptamers (Fig. 1e and Fig. S4): SPR measurement (single cycle kinetics) was performed with the Biacore T100 (GE Healthcare) using the Series S Sensor Chip SA (#29104992, GE healthcare). The 5'-biotinylated DNA was immobilized on the sensor chip. Various

concentrations (2.5, 10, 25, 50, 100 nM) of recombinant human FGFR1 extracellular domain (#7421-20, BioVision) were injected onto the immobilized-DNA surface at a flow rate of 30 μL/min (25˚C, running buffer: DPBS supplemented with 0.2% Nonidet-P40, contact time: 3 min, dissociation time: 1 and 3 min). The sensorgrams were fitted to a two-state binding model.

Evaluation of candidate aptamers (Fig. S10): SPR measurement was performed with the Biacore 2000 (GE Healthcare) using the Sensor Chip SA (#BR100398, GE healthcare). The 3'-biotinylated $(dA)_{16}$ was dissolved in DPBS supplemented with 500 mM NaCl and immobilized on the sensor chip. The candidate aptamers were synthesized with $(dT)_{16}$ nucleotides linker at the 5' end for the annealing to the biotinylated $(dA)_{16}$ on the sensor chip. The candidate DNA sequences (500 nM) were dissolved in DPBS supplemented with 500 mM NaCl and injected at a flow rate of 5 μL/min. After immobilization of DNA, the recombinant human FGFR1-Fc protein (100 nM; #661-FR, R&D systems) or human IgG (100 nM; #1-001-A, R&D systems) were injected onto the immobilized-DNA surface at a flow rate of 5 μL/min (25˚C, running buffer: DPBS supplemented with 0.05% Nonidet-P40, contact time: 5 min sec). After injection of the protein, the immobilized candidate DNA was removed by regeneration solution (50 mM NaOH in 1 M NaCl) and the same analysis procedure was repeated for the next sample.

### 3-5 CD measurement

CD measurement was performed with CD spectrometer (J-1500, JASCO) over a wavelength of 210–320 nm using a quartz cuvette at 37˚C. DNA samples were prepared in 20 mM Tris-HCl buffer (pH 7.6) supplemented with various concentrations of KCl. DNA refolding was performed with a thermal cycler (95 °C for 5 min, and then cooled at 0.1 °C/sec to 25 °C) before measurements. The scanning speed was set at 50 nm/min.

### 3-6 Cell culture

A204 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic (#15240, Thermo Scientific Pierce, USA). 409B2 cells were cultured in StemFit® AK02N (#AK02N, Ajinomoto, Japan) supplemented with 1% penicillin-streptomycin mixed solution (#26253-84,

Nacalai Tesque, Japan). iMatrix-511 (0.25 μg/cm$^2$, #892011, nippi, Japan) was used as a culture substrate. All cells were maintained in 5% $CO_2$ in a humidified incubator at 37 °C.

**3-7 ELISA assay**

The detection of phosphorylated FGFR1 and phosphorylated Erk1/2 were performed using the PathScan® Phospho-FGF Receptor 1 (panTyr) Sandwich ELISA Kit (#12909C, CST signaling) and Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) DuoSet IC ELISA (#DYC1018B, R&D systems), respectively. The assay was performed according to the manufacturer's instructions. Three independent experiments were carried out and the mean values were indicated with error bars (SD). The dose-response curve of FGFR1 phosphorylation was fitted to four-parameter logistic models.

**3-8 Maintenance of pluripotency of hiPSC in the presence of FGFR agonist**

Formulation of culture media: To evaluate the potential effects of FGFR agonists on the maintenance of the pluripotency of iPS cells, we formulated an "ABT medium" as a basal medium for the experiments. The ABT medium was composed of solution A (400 mL) and solution B (100 mL) of StemFit® AK02N and recombinant TGF-β (2 ng/mL, final concentration), whereas the complete StemFit® AK02N medium was composed of solution A (400 mL), solution B (100 mL), and solution C (2 mL). We confirmed that the ABT medium could not maintain the pluripotency of the 409B2 iPS cells, under the culture conditions described in "**3-5 Cell culture**", in the absence of the recombinant bFGF.

Culture protocol: The 409B2 cells were maintained in standard feeder-free culture conditions as described in "**3-5 Cell culture**". The cells ($2 \times 10^3$ cells/cm$^2$) were seeded onto a culture dish in an ABT medium containing an appropriate FGFR agonist, Y-27632 (10 μM), and iMatrix-511 (0.3 μg/cm$^2$). The medium was replaced with an ABT medium containing an appropriate FGFR agonist on the following day. The cells were then maintained by changing the medium every other day.

**3-9 Immuno-fluorescence imaging**

The 409B2 cells were washed twice with DPBS and fixed with 4% paraformaldehyde phosphate buffer solution for 30 min at room temperature. After fixation, the cells were washed with DPBS three times and

then incubated in a permeabilization buffer (DPBS with 0.5% Triton X-100) for 15 min at room temperature. After permeabilization, the cells were washed with DPBS three times and incubated in blocking buffer (DPBS with 3% BSA) for 15 min at room temperature. Next, the cells were reacted with anti-SSEA-4 Alexa 488 conjugate (1:100, #53-8843, Thermo Scientific Pierce) overnight at 4 °C. The cells were then washed with DPBS three times and stained with Hoechst 33258 (1:1000, #H341, DOJINDO, Japan) before the fluorescence microscopy imaging.

### 3-10 RT-qPCR analysis

Total RNA was purified with FastGene™ RNA Premium Kit (#FG-81050, NIPPON Genetics, Japan). The purified RNA was used for the reverse transcription reaction with iScript cDNA Synthesis Kit (#1708890, Bio-Rad, USA) according to the manufacturer's instructions. KOD SYBR qPCR Mix (#QKD-201, TOYOBO, Japan) and appropriate primers were used to measure gene expression levels. The relative mRNA expression was normalized to the control gene RPLP0, and fold changes were calculated with reference to the vehicle-treated sample (Fig. 2f) or TD0-treated sample (Fig. S9). The primer sequences are summarized in Table S2.

| | | |
|---|---|---|
| RPLP0 | Forward | 5'-AATCTCCAGGGGCACCATT-3' |
| | Reverse | 5'-CGCTGGCTCCCACTTTGT-3' |
| OCT4 | Forward | 5'-ACATCAAAGCTCTGCAGAAAGAACT-3' |
| | Reverse | 5'-CTGAATACCTTCCCAAATAGAACCC-3' |

***Table. S2*** Sequences of the primers used in qPCR analysis.

### 3-11 Cell growth assay

The 409B2 cells were washed twice with DPBS and dethatched from the dish with 0.5 mM EDTA in DPBS. The cells were then stained with 0.4% trypan blue solution (#207-17081, Wako chemical, Japan) and counted with a cell counter.