Electronic Supplementary Material (ESI) for Biomaterials Science.

1. Supporting figures

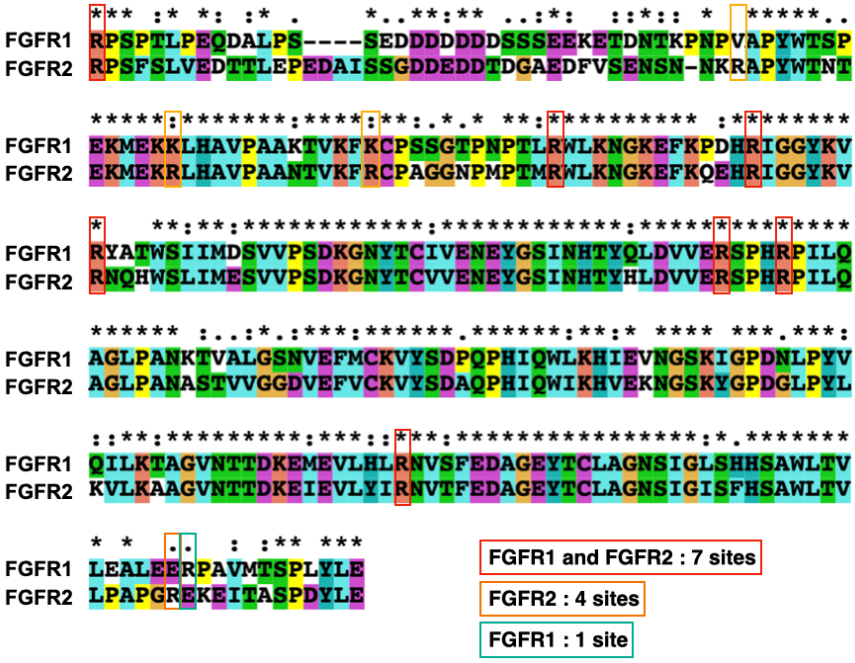

Figure S1. Amino acid sequences of the extracellular domain of human FGFR1 (Accession: NP_075594) and FGFR2 (Accession: NP_001138387). The sequences are aligned using Clustal X2. Arginine residues that exist both in FGFR1 and FGFR2, only in FGFR2, and only in FGFR1 are highlighted by red, orange, and green boxes, respectively.

SL38.2 (FGFR1-binder) HD22 (exosite 2-binder)

Apt-clean 2: CGATCGATGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGGCAATCGATCGAGTCCGTGGTAGGGCAGGTTGGGGTGACT

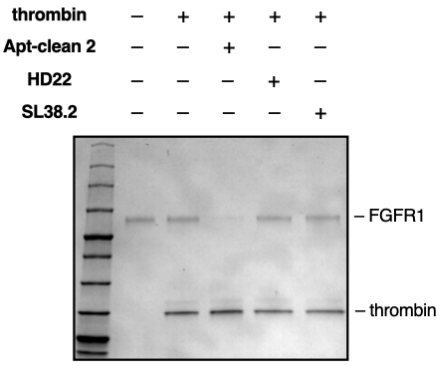

Figure S2. Bispecific aptamer-dependent cleavage of FGFR1-Fc. FGFR1-Fc (0.5 μg) was incubated with thrombin (500 nM) in the presence or absence of aptamers (500 nM) in DPBS for 60 min at 37℃.

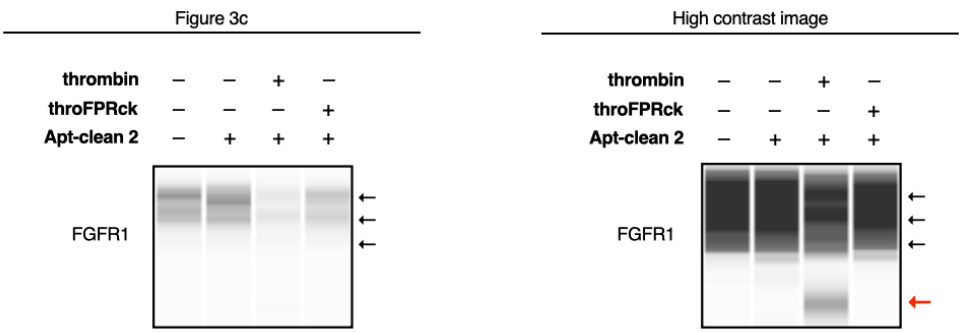

Figure S3. Proteolytic activity-dependent cleavage of FGFR1 (black arrows). 3T3-L1 cells were treated with Apt-clean 2 and thrombin or throFPRck (100 nM) for 2 h. High contrast image of Figure 3c is shown in the right panel. A new band with lower molecular weight is highlighted by a red arrow.

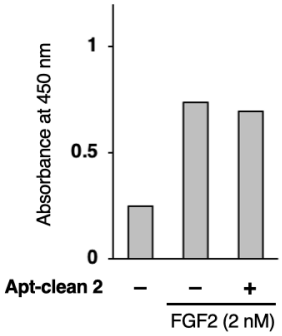

Figure S4. Effect of Apt-clean 2 on FGF2-induced FGFR1 phosphorylation in the absence of thrombin. 3T3-L1 cells were treated with FGF2 (2 nM) for 15 min without the replacement of medium after the incubation in the presence or absence of Apt-clean 2 (300 nM) for 2 h. The absorbance at 450 nm was measured and the mean values are indicated (N = 2).

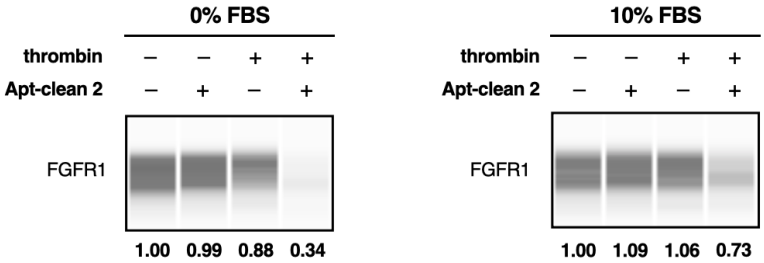

Figure S5. Apt-clean-induced FGFR1 degradation in 3T3-L1 cells in the presence or absence of 10% FBS. The cells were incubated in the presence or absence of Apt-clean 2 (300 nM) and thrombin (300 nM) for 2 h.

SL38.2 (FGFR1-binder) HD22 (exosite 2-binder)

Apt-clean 2: CGATCGATGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGGCAATCGATCGAGTCCGTGGTAGGGCAGGTTGGGGTGACT

$T_{16}N_{38}$: TTTTTTTTTTTTTTTTTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

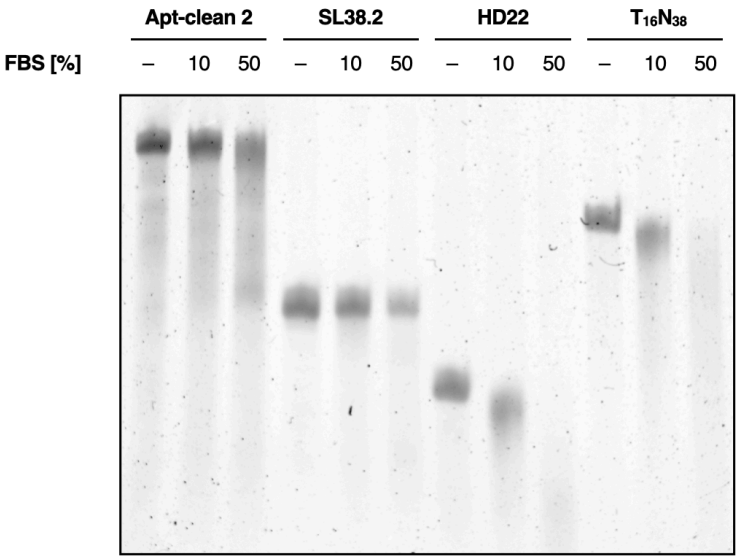

Figure S6. Serum stability of aptamers. Oligonucleotides (2 μM) in DPBS were mixed with fetal bovine serum (final FBS concentrations; 10 or 50%) and incubated for 2 h at 37 °C. After the incubation, the samples were analyzed with 10% denaturing PAGE.

2. Methods

General information

Reagents were purchased from standard suppliers and used without further purification. All DNA samples were purchased from Fasmac or Eurofins Genomics. 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). Recombinant FGFR1β (IIIc)-Fc Chimera (#661-FR) or FGFR2β (IIIc)-Fc Chimera (#684-FR) were purchased from R&D Systems. Dulbecco's phosphate buffered saline (DPBS) was obtained from Wako chemicals (Japan). Recombinant human FGF1 (#100-17A) or FGF2 (#100-18B) were purchased from PeproTech. Human alpha-thrombin (#HCT-0020) or Human alpha-thrombin-FPRCK (#HCT-FPRCK) were purchased from Haematologic Technologies.

In vitro cleavage of recombinant FGFR

Recombinant FGFR1c-Fc or FGFR2c-Fc chimera (0.5 μg) was incubated with 500 nM thrombin and/or 500 nM aptamer in 10 uL of DPBS for 60 min at 37 °C. After incubation, 2.5 μL of 5 × SDS loading buffer (250 mM Tris-HCl (pH 6.8), 10% SDS, 50% glycerol, 0.5 M 2-mercaptoethanol) was added to each sample and incubated for 5 min at 95 °C. Samples were run in Mini-PROTEAN TGX gel (#4561084, BIO-RAD) for 30 min at 200 V and stained by Coomassie Brilliant Blue. The gel images were captured by GelDoc Go (BIO-RAD).

Cell culture

3T3-L1 cells were obtained from Japanese collection of research bioresources (JCRB) cell bank (#JCRB9014) and cultured in DMEM (#08456-65, Nacalai Tesque) supplemented with 10% calf serum (#C8056-500ML, Sigma-Aldrich) and 1% antibiotic-antimycotic mixed solution (#09366-44, Nacalai Tesque), and maintained in 5% CO_2 in a humidified incubator at 37 °C.

Preparation of cell lysates

3T3-L1 cells were seeded in 35 mm dishes and cultured in DMEM supplemented with 10% CS and 1% antibiotic-antimycotic. After overnight incubation, the medium was replaced with a starvation medium (DMEM supplemented with 1% antibiotic-antimycotic) and cultured for 24 h. After the starvation, the medium was replaced with the fresh starvation medium, and the cells were incubated in the presence or absence of thrombin and DNA aptamer for 2 h at 37 °C. In figure S5, FBS (#CCP-FBS-BR-500) was supplemented to the medium (final FBS concentration: 10%) during Apt-clean treatment. For FGF stimulation after the digestion, the medium was replaced with the fresh starvation medium and FGF was added and incubated for 15 min at 37 °C. The cells were washed twice with DPBS and lysed with a lysis buffer (20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% TritonX-100, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 μg/mL leupeptin, and 10% glycerol) supplemented with 1 mM AEBSF. The lysates were incubated on ice for more than 30 min followed by centrifugation at 10,000 × *g* for 15 min at 4 °C. The supernatants were recovered and used for capillary electrophoresis immunoassay or ELISA.

Capillary electrophoresis immunoassay

The Jess™ Simple Western system (ProteinSimple) was used to quantify protein signals, following the manufacturer's standard method for separation module (#SM-W004), anti-rabbit detection module (#DM-001), and total protein detection module (#DM-TP01). Cell lysates was mixed with 10× Sample buffer and Fluorescent 5× Master mix (ProteinSimple), then incubated at 95 °C for 5 min. Antibodies were prepared in antibody diluent by

indicated ratio: anti-phospho-FGFR1 Tyr653/654 (#52928S, Cell Signaling Technology, 1:100), anti-phospho-ERK (#4370S, Cell Signaling Technology, 1:250), anti-FGFR1 (#9740S, Cell Signaling Technology, 1:100), anti-ERK (#4695S, Cell Signaling Technology, 1:100), anti-PDGFRβ (#3169S, Cell Signaling Technology, 1:100), anti-pan-Cadherin (#4068S, Cell Signaling Technology, 1:100), and anti-Insulin Receptor β (#3025, Cell Signaling Technology, 1:100). Compass Simple Western software (ProteinSimple) was used to produce digital image of chemiluminescence and calculate corrected peak area which is normalized by total protein detection. The peaks corresponding to FGFR1 signal was fitted by "Dropped Lines" method and those from other proteins were fitted by "Gaussian" method.

ELISA

The detection of phosphorylated FGFR1 were performed using the PathScan® Phospho-FGF Receptor 1 (panTyr) Sandwich ELISA Kit (#12909C, CST signaling). The assay was performed according to the manufacturer's instructions. The absorbance at 450 nm was measured with Infinite M200 pro (Tecan).

3. Sequence data

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

HD1: 5′ GGT TGG TGT GGT TGG 3′

HD22: 5′ AGT CCG TGG TAG GGC AGG TTG GGG TGA CT 3′

Apt-clean 1: 5′ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCG GTT GGT GTG GTT GG 3′

Apt-clean 2: 5′ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCA GTC CGT GGT AGG GCA GGT TGG GGT GAC T 3′

Apt-clean 3: 5′ GGT TGG TGT GGT TGG GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GC 3′

Apt-clean 4: 5′ AGT CCG TGG TAG GGC AGG TTG GGG TGA CTG CCG CGT CTT TAT GGC TGG GGA TGG TGT GGG TTG CGG C 3′

Apt-clean 5: 5′ GCC GCG TCT TTA TGG CTG GGG ATG GTG TGG GTT GCG GCT TTT TTT TTT AGT CCG TGG TAG GGC AGG TTG GGG TGA CT 3′

$T_{16}N_{38}$: 5′ TTT TTT TTT TTT TTT TNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN 3′