Supporting Information

A DNA Aptamer That Inhibits the Aberrant Signaling of Fibroblast Growth Factor Receptor in Cancer Cells

Akihiro Eguchi,‡ Ayaka Ueki,† Junya Hoshtyama,† Keiko Kuwata,‡ Yoko Chikaoka,§ Takeshi Kawamura,‡ Satoru Nagatoishi,† Kouhei Tsumoto,‡,^ Ryosuke Ueki,*† and Shinsuke Sando*†,‡

§Department of Chemistry and Biotechnology, †Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
‡Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan
§Proteomics Laboratory, Isotope Science Center, The University of Tokyo, 2-11-16, Yayoi, Bunkyo-Ku, Tokyo 113-0032, Japan
^The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

*e-mail: r.ueki@chembio.t.u-tokyo.ac.jp; ssando@chembio.t.u-tokyo.ac.jp
# Table of Contents

1. General information .......................................................... 2
2. Sequence data ........................................................................ 2
3. Methods .................................................................................. 3
4. Supplementary figures ............................................................ 15
5. References ............................................................................... 25
1. **General information**

Reagents were purchased from standard suppliers and used without further purification. All oligonucleotide samples were purchased from Fasmac or Eurofin. Oligonucleotide samples were folded by heating at 95 °C for 5 min in Dulbecco’s phosphate-buffered saline (DPBS), followed by gradual cooling at 0.1 °C/sec to 25 °C using thermal cycler. A pan-FGFR kinase inhibitor AZD4547 was purchased from Chemscene LLC (#CS-0971) and dissolved in dimethyl sulfoxide (DMSO) at 10 mM. Recombinant human FGF1 and FGF10 was purchased from PeproTech (FGF1: #100-17A, FGF10: #100-26). Ab-10 Rapid HiLyte Fluor 647 Labeling Kit (#LK36, Dojindo) was used for fluorescent labeling of FGF1 and FGF10. Recombinant FGFRs-Fc chimera proteins and human IgG were purchased from R&D systems (FGFR1c: #661-FR-050, FGFR2b: #665-FR-050, FGFR2c: #684-FR-050, FGFR3c: #766-FR-050, FGFR4: #685-FR-050, IgG: #1-001-A).

2. **Sequence data**

**ssDNA pool:** 5’ ATC CAG AGT GAC GCA GCA NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NTG GAC ACG GTG GCT TAG T 3’

**Fw_primer:** 5’ ATC CAG AGT GAC GCA GCA 3’

**Rev_primer:** 5’ ACT AAG CCA CCG TGT CCA 3’

**Apt_76:** 5’ ATC CAG AGT GAC GCA GCA GTT GTT GGG AGG GGA TGG GAC GTA TAG GTA AGG GCG GGG GTG GAC ACG GTG GCT TAG T 3’

**Apt_22:** 5’ ATG GGA CGT ATA GGT AAG GGC G 3’

**Apt_30:** 5’ GGG GAT GGG ACG TAT AGG TAA GGG CGG GGG 3’

**Apt_46:** 5’ TGT TGG GAG GGG ATG GGA CGT ATA GGT AAG GGC GGG GGT GGA CAC G 3’

**Inv_46:** 5’ GCA CAG GTG GGG GCG GGA ATG GAT ATG CAG GGT AGG GGA GGG TTG T 3’

**Control ssDNA:** 5’ TGT TGA GAG AAG ATG AGA CGT ATA GAT AAG AGC GAG AGT GAA CAC G 3’
3. Methods

3-1. Cell culture

SNU16, KATO-III, and KMS11 cells were grown at 37 °C in 5% CO₂ atmosphere in RPMI-1640 supplemented with 10% head-inactivated fetal bovine serum (FBS) and 1% Antibiotic-Antimycotic (#09366-44, Nacalai Tesque). Expi293F cells (#A14635, Thermo Fisher Scientific) were grown at 37°C in 8% CO₂ atmosphere with shaking at 120 rpm in Expi293 Expression Medium (#A1435101, Thermo Fisher Scientific).

3-2. In vitro selection of FGFR2b-binding DNA aptamer

**Protein immobilization:** FGFR2b-Fc was incubated with Dynabeads Protein G (#10004D, Thermo Fisher Scientific) in PBS-T (DPBS supplemented with 0.02% Tween 20 (wt/vol)) for 30 min. A 12.6 µL of slurry (binding capacity = 20 pmol) was used for the immobilization of a 10 pmol protein. After incubation, the beads were washed three times with PBS-T and used for the selection. Human IgG-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 the following table.

**Isolation of FGFR2b-binding aptamer:** 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. In the 2nd selection rounds, the ssDNA pool was pre-incubated with Dynabeads Protein G for 30 min × 5 times at ambient temperature to remove bead-binding sequences. During the 3rd and 6th selection rounds, negative selection, using human IgG-immobilized beads, was performed. The supernatant was then incubated with FGFR2b-immobilized beads for 10–30 min at ambient temperature. After incubation, the beads were washed with PBS-T. The FGFR2b-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 ethylenediaminetetraacetic acid (EDTA)) for 3 min at 95 °C. The eluted DNA was isolated by PCI extraction and ethanol precipitation.
The conditions for each selection round are summarized in the following table.

**Generation of the ssDNA pool:** The eluted DNA was amplified by PCR, using the KOD-plus-Neo polymerase (#KOD-401, TOYOBO) according to the manufacturer’s protocols. The primer DNA sequences (Fw_primer and Rev_primer) are shown as “2. Sequence data” in the supplementary information. After the PCR, the reaction mixture was incubated with streptavidin magnetic beads (#21344, Thermo Fisher Scientific) in 10 mM Tris-HCl buffer (pH 7.5) supplemented with 1 M NaCl and 1 mM EDTA for 10 min at ambient temperature. The ssDNA was eluted from the beads by incubation in 150 mM NaOH for 5 min at ambient 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). After standard *E. coli* transformation and cloning procedures, aptamer candidate sequences were identified by Sanger sequencing.

| Round | Input ssDNA (pmol) | FGFR2b-Fc (pmol) | Incubation vol. (mL) | Incubation time (min) | Number of wash | Protein G beads (slurry vol.) | Human IgG (pmol) |
|-------|--------------------|------------------|---------------------|----------------------|----------------|-------------------------------|----------------|
| 1     | 2000               | 100              | 500                 | 30                   | 3              | –                             | –              |
| 2     | 50                 | 20               | 500                 | 30                   | 3              | 25 µL × 3                     | –              |
| 3     | 25                 | 10               | 500                 | 30                   | 3              | –                             | 20 pmol × 3    |
| 4     | 10                 | 5                | 500                 | 30                   | 3              | –                             | 20 pmol × 3    |
| 5     | 5                  | 1                | 1000                | 30                   | 3              | –                             | 20 pmol × 3    |
| 6     | 5                  | 1                | 1000                | 10                   | 5 min × 3      | –                             | 20 pmol × 3    |

**3-3. CD measurements (Figure 2c and S3)**

The formation of G-quadruplex was confirmed using CD measurement. Oligonucleotide samples (5 µM) in 20 mM Tris-HCl (pH 7.6) with or without KCl (100 mM) were used for CD measurement. The samples were folded by heating at 95 °C for 5 min in the buffer followed by gradual cooling at 0.1
˚C/sec to 25 °C using thermal cycler before measurement. CD measurements were performed using CD spectrometer (J-1500, JASCO) over a wavelength of 210–310 nm using a quartz cuvette at 37 °C. The scanning speed was set at 100 nm/min.

3-4. Flow cytometry

The binding of oligonucleotide samples to FGFRs was confirmed using flow cytometry.

Assay using magnetic beads (Figure S2 and 2d): Dynabeads Protein G (1.25 µL of slurry; binding capacity = 2 pmol) was incubated with FGFRs-Fc protein (1 pmol) for 30 min at ambient temperature with rotation. After washing three times with DPBS supplemented with 0.05% Tween 20 (PBS-T), the beads were incubated with 5'-FAM conjugated oligonucleotide samples (100 nM) or HiLyte Fluor 647-labeled FGF1 (100 nM) with 10 µg/mL heparin sodium salt for 15 min at ambient temperature with rotation. After washing three times with PBS-T, the beads were resuspended in PBS-T. The fluorescent signal of FAM or HiLyte Fluor 647 was measured using the Guava easyCyte (Merck Millipore).

Assay using cells (Figure S13): SNU16 or KMS11 cells (2 × 10^5 cells) were incubated with 5'-FAM conjugated oligonucleotide samples (100 nM) in DPBS supplemented with 0.5% BSA and 0.1% tRNA at 21 °C for 15 min. The cells were washed twice with DPBS and resuspended in DPBS. The fluorescent signal of FAM was measured using the Guava easyCyte (Merck Millipore).

Binding competition between FGF10 and Apt_46 (Figure 3a): Dynabeads Protein G (1.25 µL of slurry; binding capacity = 2 pmol) was incubated with FGFRs-Fc protein (0.5 pmol) for 30 min at ambient temperature with rotation. After washing three times with DPBS supplemented with PBS-T, the beads were incubated with a mixture of HiLyte Fluor 647-labeled FGF10 (0.1 µM), heparin sodium salt (10 µg/mL), and 5'-FAM-labeled oligonucleotide samples (Apt_46 or Inv_46; 0.01, 0.1, 1, and 10 µM) in DPBS for 15 min at 21 °C. The beads were washed twice with DPBS and resuspended in DPBS. The fluorescent signal of HiLyte Fluor 647 was measured using the Guava easyCyte (Merck Millipore).
3-5. Expression and purification of recombinant FGFR2b

Ig-like domain 2–3 of FGFR2b (A140–I369, Uniprot ID: P21802-3) was subcloned into the expression vector pEFs. The leader sequence of the immunoglobulin kappa chain and hexa-histidine tag were fused at the N-terminus and C-terminus, respectively. The expression and purification of the proteins was conducted as previously described. Expi293F cells were transfected with the expression vector and cultured according to the manufacturer’s protocol. After 4 days of culture, the cells were centrifuged at 3000 × g for 30 min at 4 °C, and the supernatant was dialyzed at 4 °C against nickel-nitrilotriacetic acid (Ni-NTA)-binding buffer (20 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 5 mM imidazole). The dialyzed samples were purified using Ni-NTA agarose beads (#30210, QIAGEN) and Econo-Pac Chromatography Columns (Bio-Rad). The eluted samples were further purified by heparin affinity chromatography (HAC) or size exclusion chromatography (SEC). In the case of HAC, the eluted samples were dialyzed against HAC buffer (20 mM Tris-HCl (pH 7.6), 0.2 M NaCl) and purified using a HiTrap Heparin HP column (GE Healthcare) with a NaCl gradient elution (0.2–1 M). In the case of SEC, the eluted samples were dialyzed against SEC buffer (20 mM Tris-HCl (pH 8.0), and 1 M NaCl) and purified using a Superdex75 column (GE Healthcare). The concentration of the protein was determined by measuring the absorbance at 280 nm using a NanoDrop ND-1000 (Thermo Fisher Scientific). The purity of the final preparation was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie Brilliant Blue (CBB) staining.

3-6. ITC measurements (Figure 3c, S6 and S7)

The purified Ig-like domain 2–3 of FGFR2b was dialyzed against DPBS (Figure 3c and S7) or PBS without potassium (Figure S6; 10 mM sodium hydrogen phosphate, 1.8 mM sodium dihydrogen phosphate, and 137 mM NaCl) twice. Apt_46 (Figure 3c and S6: 30 µM, Figure S7: 80–100 µM) was dissolved in DPBS (Figure 3c and S7) or PBS without potassium (Figure S6). ITC measurements were conducted at 25 °C using iTC200 (GE Healthcare), and the thermal profile was obtained by a single
injection (0.5 µL) and following 19 times sequential titrations (2 µL) of the DNA aptamer to recombinant FGFR2b (Figure 3c and S6: 2 µM, Figure S7: 4 µM). The data was analyzed using Origin 8.0 (Originlab Corp) and the curve fitting was conducted using the OneSites model. The N-value (black), ΔG (blue), and ΔH (red) were obtained from the curve fitting analysis as shown in the figure below. ΔS was calculated from the equation: ΔG = ΔH - TΔS. The parameters were calculated as the mean of the three independent measurements.

3-7. SEC-MALS (Figure S8)

Apt_46 was dissolved in DPBS at 30, 89, or 148 µM and folded in the same buffer. The concentration of the samples was adjusted to 30 µM, and a 100 µL of the sample was applied to SEC-MALS analysis using a Superdex 200 10/300 GL column (GE Healthcare), inline DAWN8+ multi angle light scattering (Wyatt Technology), UV detector (Shimadzu; detection at 280 nm), and RI detector (Shodex). MALS detectors were calibrated using BSA before the analysis of oligonucleotide samples. The molecular weights of the oligonucleotide samples at each elution time point were calculated using a dn/dc value of 0.170 mL/g.
3-8. Chemical crosslinking (Figure 4b, S10, and S11)

The existence of receptor dimer was confirmed using the chemical crosslinking agent bis(sulfosuccinimidy)suberate (BS3) (#21580, Thermo Fisher Scientific). SNU16 cells (4 × 10^5 cells) or KATO-III cells (4 × 10^5 cells) were washed twice with a starvation medium (RPMI-1640 supplemented with 0.5% BSA) and seeded in 6-well plates and cultured for 24 h. After the starvation, medium was replaced, and the cells were stimulated with FGF10 (2 nM) and heparin sodium salt (10 µg/mL), Apt_46 (1 µM), or Inv_46 (1 µM) for 1 h on ice (Figure 4b and S10) or for 5–15 min at 37 °C (Figure S11). After the stimulation, the cells were washed with ice-cold DPBS three times, followed by incubation with BS3 (0.5 mM) in ice-cold DPBS for 1 h on ice. After the incubation, quenching buffer (1 M Tris-HCl (pH 7.6)) was added and incubated for 15 min on ice. The cells were washed twice with DPBS and lysed with 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 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF). The lysates were incubated on ice for more than 30 min followed by centrifugation at 10,000 × g for 20 min at 4 °C. The supernatants were recovered and used for Western blotting. Total protein concentration of each cell lysate was determined by using the Protein Assay BCA kit (#06385-00, Nacalai Tesque), and it was adjusted to the same value. The absorbance was detected using Infinite M200 pro (Tecan). The cell lysates were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked using Blocking One (#03953-95, Nacalai Tesque). Immunoblotting was performed with anti-FGFR2 primary antibody (#11835, Cell Signaling Technology, 1:2000) at 4 °C overnight and horseradish peroxidase (HRP) conjugated anti-rabbit secondary antibody (#P0447, Dako, 1:3000) for 1 h at ambient temperature. Membranes were probed by ImmunoStar LD (#296-69901, Fujifilm Wako Pure Chemical Corporation) and luminescence was detected using Ez-Capture MG AE9300H-CSP (ATTO).
SNU16 cells (2 × 10⁶ cells) were seeded in 10-cm dishes in RPMI-1640 supplemented with 10% FBS. After 2 days of culture, SNU16 cells were collected and resuspended in a starvation medium followed by stimulation with Apt_46 at 37 °C for 15 min. The cells were washed twice with DPBS and then lysed with lysis buffer (#9803, Cell Signaling Technology) supplemented with 1 mM AEBSF. After sonication on ice for 2 min, the lysates were centrifuged at 10,000 × g for 20 min at 4 °C. The supernatants were recovered and used for immunoprecipitation. Dynabeads Protein G (50 µL) was incubated with anti-FGFR2 antibody (#11835, Cell Signaling Technology, 1:50) in PBS-T for 15 min at ambient temperature with rotation. After washing three times with PBS-T, the beads were incubated with each lysate sample for 25 min at ambient temperature with rotation. After washing three times with PBS-T, the beads were suspended in gel loading buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and 100 mM 2-mercaptoethanol), boiled at 68 °C for 10 min, and the supernatants were resolved by SDS-PAGE using 4–15% precast polyacrylamide gels (#4561085, Bio-Rad). The gels were stained with CBB in 10% acetic acid/40% methanol in water, and the stained bands were cut from the gel. The obtained gel pieces were subjected to in-gel tryptic digestion and subsequent MS analysis. The digested peptides were analyzed by nano-flow reverse-phase LC followed by tandem MS, using a Q-Exactive hybrid mass spectrometer (Thermo Fisher Scientific). The system of capillary reverse-phase HPLC–MS/MS was composed of a Dionex U3000 gradient pump equipped with a VICI CHEMINERT valve. Q-Exactive was equipped with a nano-electrospray ionization (NSI) source (AMR). The desalted peptides were loaded into a separation capillary C18 reverse-phase column (NTCC-360/100–3–125, 125 × 0.1 mm, Nikkyo Technos). The record of peptide spectra over the mass range of m/z 350–1,800 was conducted using the Xcalibur 3.0.63 system (Thermo Fisher Scientific). Repeatedly, MS spectra were recorded followed by 10 data-dependent high energy collisional dissociation (HCD) MS/MS spectra generated from the 10 highest intensity precursor ions. Multiple charged peptides were chosen for MS/MS experiments because they showed good fragmentation.
characteristics. MS/MS spectra were interpreted and peak lists were generated using Proteome Discoverer 2.4.1.15 (Thermo Fisher Scientific). Searches were performed by using SEQUEST (Thermo Fisher Scientific) against a Homo sapiens (SwissProt TaxID = 9606) peptide sequence. Peptide identification was based on a significant Xcorr (high-confidence filter). The information of peptide identification and modification returned from SEQUEST was manually inspected and filtered to obtain confirmed peptide identification and modification lists of HCD MS/MS. The cell lysates from three experiments were used for the analysis and each sample was measured twice. The abundance of peptides was estimated from the peak area of mass chromatogram of the peptides. The abundance of phosphopeptides was normalized by total peptide amount of FGFR2 (Uniprot ID: #P21802-16). The average of the normalized abundance of phosphopeptides (RPPGMEYSYDINR for pY586/Y588 (pY = 1), DINNIDYYK and DINNIDYYKK for pY656/Y657 (pY = 1 or 2), and ILTTLTNEELDSQPLEYSPSYDPDR for pS780 (pS = 1)) of the three measurements is shown in Figure 5a. For each phosphopeptide in each lysate, the average of the two measurements was used for the calculation of the average of the normalized abundance. When the phosphopeptides were detected only in one measurement, the normalized abundance of the measurement was used for the calculation.

3-10. Analysis of cellular signaling by Western blotting (Figure 4a, 5b, S9, S14, and S17)

SNU16 cells (4 × 10⁵ cells) or KATO-III cells (3–4 × 10⁵ cells) were washed with a starvation medium (RPMI-1640 supplemented with 0.5% BSA) and seeded in 35-mm dishes and cultured for 24 h. KMS11 cells (1 × 10⁵ cells) were seeded in 35-mm dishes and culture in RPMI-1640 supplemented with 10% FBS overnight. Then the medium was replaced by a starvation medium and cultured for 24 h. After the starvation, medium was replaced, and the cells were stimulated with the indicated concentration of oligonucleotide samples or AZD4547 for the indicated time at 37 °C. The cells were washed twice with DPBS and lysed with 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 20 min at 4 °C. The supernatants were recovered and used for Western blotting as described in section 3-7. The PVDF membrane was blocked with Blocking One (#03953-95, Nacalai Tesque) or Blocking One P (#05999-84, Nacalai Tesque). Immunoblotting was performed with the following primary antibodies at 4 °C overnight and horseradish peroxidase (HRP) conjugated anti-rabbit secondary antibody (#P0447, Dako, 1:3000) for 1 h at ambient temperature: anti-phospho-FGFRs (#AF3285, R&D Systems, 1:2000), anti-FGFR2 (#11835, Cell Signaling Technology, 1:2000), anti-FGFR3 (#4574, Cell Signaling Technology, 1:2000), anti-phospho-Akt (#9271, Cell Signaling Technology, 1:2000), anti-Akt (#9272, Cell Signaling Technology, 1:2000), anti-phospho-Erk1/2 (#9101, Cell Signaling Technology, 1:2000), anti-Erk1/2 (#9102, Cell Signaling Technology, 1:2000).

3-11. Expression of FGFR 2 and 3 in SNU16 and KMS11 cells (Figure S12)

SNU16 and KMS11 cells were washed twice with DPBS and lysed with lysis buffer supplemented with 1 mM AEBSF. The lysates were incubated on ice for more than 30 min followed by centrifugation at 10,000 × g for 20 min at 4 °C. The supernatants were recovered and used for Western blotting as described in section 3-7. Immunoblotting was performed with the following primary antibodies at 4 °C overnight and horseradish peroxidase (HRP) conjugated anti-rabbit secondary antibody (#P0447, Dako, 1:3000) for 1 h at ambient temperature: anti-FGFR2 (#11835, Cell Signaling Technology, 1:2000), anti-FGFR3 (#4574, Cell Signaling Technology, 1:2000).

3-12. Serum nuclease stability of the oligonucleotide samples (Figure S15)

Control ssDNA (see “2. Sequence data”), Apt_46, and 3´-inverted dT-modified Apt_46 (2 µM) were incubated in DPBS containing 10% non-heat-inactivated FBS at 37 °C for indicated times. After the
incubation, the samples were immediately mixed with the same volume of sample buffer (20 mM Tris-HCl (pH 8.0), 8 M urea, and 1 mM EDTA) and analyzed by denaturing PAGE.

3-13. Cell growth assay (Figure 5c)

SNU16 (5.0 × 10^3 cells), KATO-III (5.0 × 10^3 cells), and KMS11 cells (1.0 × 10^4 cells) were seeded in 96-well plate in a 100 μL of RPMI-1640 supplemented with 10% FBS and 3′-dT conjugated oligonucleotide samples (final concentration: 31.3–2000 nM). A 1 μL of the oligonucleotide samples (3.13–200 μM) were additionally supplemented every 24 h. After 72 h incubation, cell viability was evaluated using a Cell Counting Kit-8 (#347-07621, Dojindo) according to the manufacturer’s instruction. The data were relative values standardized by the absorbance (450 nm) of vehicle control.

3-14. Quantitative analysis of phosphoproteome based on stable isotope labeling by amino acids in cell culture (SILAC)^4 (Figure 6 and Table S1)

SNU16 cells were grown in RPMI for SILAC (#88365, Thermo Fisher Scientific) supplemented with 10% dialyzed FBS (#A3382001, Thermo Fisher Scientific) and 1% Antibiotic-Antimycotic containing “light” or “heavy” amino acids. The “light” SILAC media were supplemented with L-lysine and L-arginine (#123-06081 and #010-24041, Thermo Fisher Scientific) and “heavy” SILAC media were supplemented with L-lysine-U-^{13}C_6-^{15}N_2 and L-arginine-U-^{13}C_6-^{15}N_4 (#L8662-25G and A6969-25G, Fujifilm Wako Pure Chemical Corporation). The cells were cultured in these SILAC media for 1 week before the analysis. The “heavy” labeled cells were treated with AZD4547 (1 μM) or Apt_46 (1 μM) for 15 min at 37 °C in the “heavy” SILAC media, and the “light” labeled cells were treated with vehicle (0.01% DMSO/DPBS) in the “light” SILAC media. The cells were twice with ice-cold DPBS and treated with ice-cold 10% trichloroacetic acid for 30 min on ice, followed by scraping. The cell lysates were centrifuged at 3,000 × g for 10 min at 4 °C. The cell pellets were resuspended in ice-cold DPBS and centrifuged again. The supernatants were discarded, and the cell pellets were dissolved with
protein solubilization buffer (50 mM bicine (pH 8.5) and 8 M guanidine-HCl), and incubated for 30–120 min on ice. The suspension was centrifuged at 20,000 × g for 30 min, and the supernatant was collected. The total protein concentration was determined using the Protein Assay BCA kit, and equal amounts of protein from “heavy” and “light” samples were mixed. Proteins (0.3 mg/150 µL) were reduced with 25 mM tris(2-carboxyethyl)phosphine (TCEP) at 37 °C for 15 min and alkylated using 25 mM iodoacetamide at 37 °C for 30 min in the dark, both with gentle shaking (1,000 rpm) following the standard protocol. Proteins were digested with Lys-C (Fujifilm Wako Pure Chemical Corporation) at an enzyme-protein ratio of 1:100 at 37 °C for 3 h. This Lys-C digest was diluted to 2 M urea with 100 mM Tris-HCl, pH 8.5, followed by digestion with trypsin (Promega) at an enzyme-protein ratio of 1:100 at 37 °C overnight. After digestion was stopped by the addition of a 1/20 volume of 20% TFA, digested peptides were desalted using a MonoSpin C18 spin column (GL Science) according to the manufacturer's instructions. Eluates were evaporated in vacuo to dryness, reconstituted in 200 µL of 80% acetonitrile (0.1% TFA) and subjected to phosphopeptide enrichment using Fe(III)-IMAC cartridges (5 µL) on an AssayMAP Bravo platform (Agilent) at a flow rate of 5 µL/min according to the manufacturer’s protocol. Phosphopeptides were eluted three times with 20 µL of 20% acetonitrile (1% TFA), 20 µL of 25% acetonitrile (5% ammonia), and 25% acetonitrile (5% pyrrolidine), respectively. The 2nd and 3rd eluted fractions were mixed and acidified with 20% TFA. The eluted fraction was concentrated and desalted using GL-Tip SDB (GL Science) according to the manufacturer's instructions. The desalted sample was dried down and stored at −80 °C until LC-MS/MS analysis. The 1st eluent and the 2nd/3rd eluents were separately analyzed by LC-MS/MS. MS/MS spectra were interpreted and peak lists were generated by Proteome Discoverer 2.4.1.15 (Thermo Fisher Scientific) using SILAC 2plex (Arg10, Lys8) as a quantification method. Searches were performed by using SEQUEST (Thermo Fisher Scientific) against a Homo sapiens (SwissProt TaxID = 9606) peptide sequence. Peptide identification was based on a significant Xcorr (high-confidence filter). Peptide identification and modification information returned from SEQUEST was
manually inspected and filtered to obtain confirmed peptide identification and modification lists of HCD MS/MS. Three SILAC samples were prepared and each sample was measured twice. Detected phosphopeptides whose phosphorylated sites are confidently identified (Site Probability ≥ 75%) were used for the following quantitative analysis. SILAC ratios calculated using Proteome Discoverer 2.4.1.15 were used for quantification. Phosphopeptides that was quantified for a minimum of two of three samples in both conditions are listed. Phosphopeptides that were only detected in either the “light” or “heavy” peptides were excluded upon quantitative analysis. By the above steps, we identified and quantified 2,386 peptides (grey plots in Figure 6). The phosphopeptides whose SILAC ratio is more than 2 or less than 0.5 in either or both of Apt_46-treated condition and AZD4547-treated condition are defined as “Regulated” phosphopeptides (orange plots in Figure 6). “Regulated” phosphopeptides whose SILAC ratio differs more than twice between Apt_46-treated and AZD4547-treated conditions are defined as “Changed (Apt vs AZD)” phosphopeptides (red plots in Figure 6).
4. Supplementary figures

**Figure S1** The sequence data of the DNA pool obtained after 6th round of the selection. The sequences of N40 random region and the primer binding sites are shown. The alignment was performed using Clustal X.

**Figure S2** Binding analysis of truncated aptamers to FGFR2b. FAM-labeled Apt_46, Apt_30, or Apt_22 was incubated with FGFR2b-Fc-immobilized magnet beads for 15 min at ambient temperature. The fluorescence from FGFR2b-bound DNA was detected using flow cytometry.
**Figure S3** CD spectra of Apt_22 (left) and Apt_30 (right). Oligonucleotide samples (5 µM) were refolded in 20 mM Tris-HCl (pH 7.6) with (red) or without (blue) KCl (100 mM) and subjected to CD measurement at 37 °C.

**Figure S4** Crystal structures of FGFR2b–FGF10 complex (left) and FGFR1c–FGF2 complex (right). The binding fashion of FGFs to FGFRs are generally preserved in all FGF and FGFR family members. FGFs bind around Ig-like domain 2 to 3.
The comparison of amino acid sequence between extracellular domains of FGFR2b (Uniprot ID: P21802-3) and FGFR2c (Uniprot ID: P21802-1) isoforms. The differences are mainly located in Ig-like domain 3. Sequence alignment was performed with IdentityX 13.0.0.

**Figure S5**

**Figure S6** Isothermal titration calorimetry measurement in PBS without potassium at 25 °C. The profile was obtained by the sequential titrations of Apt_46 (30 µM) to recombinant FGFR2b extracellular domain (2 µM) in the sample cell.
Figure S7 Isothermal titration calorimetry measurements were conducted in Dulbecco’s phosphate-buffered saline at 25 °C. The profile was obtained by the sequential titrations of Apt_46 (80–100 µM) to a solution of recombinant FGFR2b extracellular domain (4 µM). The parameters are shown as the mean of three measurements ± SD. The representative titration curve (Apt_46 concentration: 80 µM) is shown.

Figure S8 SEC-MALS chromatograms of Apt_46. Apt_46 was folded in DPBS at various concentrations (30, 89, and 148 µM) and diluted to 30 µM immediately before the sample injection. The chromatography was conducted in DPBS using a Superdex 200 10/300 GL column at ambient temperature.
**Figure S9** Western blotting of the cell lysates of SNU16 and KATO-III cells incubated with Apt_46 (16, 40, 80, 200, 400, 1000, 2000, and 5000 nM) for 15 min. The intensity of each band was calculated using ImageJ.
**Figure S10** Western blotting analysis of crosslinked FGFR2b dimer. KATO-III cells were incubated with FGF10 (2 nM) and heparin sodium salt (10 µg/mL), Apt_46 (1 µM), or Inv_46 (1 µM) for 1 h on ice, followed by crosslinking reaction with BS3 (500 µM) for 1 h on ice. The cell lysates were used for Western blotting and FGFR2b was detected.

**Figure S11** Western blotting analysis of crosslinked FGFR2b dimer. SNU16 cells were incubated with Apt_46 (1 µM) at 37 °C for 5 or 15 min, followed by crosslinking reaction with BS3 (500 µM) for 1 h on ice. The cell lysates were used for Western blotting and FGFR2b was detected.
**Figure S12** The expression of FGFR2 and 3 in SNU16 and KMS11 cells. The volume of cell lysates was adjusted by total protein concentration and the lysates were used for Western blotting.

**Figure S13** Binding specificity of Apt_46 to FGFR2b on the cell surface. SNU16 (FGFR2b +) and KMS11 (FGFR3 +) were treated with FAM-labeled Apt_46 (100 nM) or Inv_46 (100 nM) in DPBS supplemented with 0.5% BSA and 0.1% tRNA at 21 °C for 15 min. The fluorescence from FAM-labeled DNA bound to cells was detected using flow cytometry.
**Figure S14** Western blotting analysis of cell lysates of SNU16 or KMS11 incubated with Apt_46 (16, 80, 400, and 2000 nM), Inv_46 (2000 nM), or AZD4547 (3.2, 16, 80, 400, and 2000 nM) for 15 min.

**Figure S15** Evaluation of stability of Apt_46 (2 µM) in DPBS supplemented with 10% non-heat-inactivated fetal bovine serum. After incubation for the indicated time, the samples were immediately applied to denaturing PAGE and the gel was stained using GelRed.
**Figure S16** Growth assay of SNU16 cells. Cells were cultured in the presence of 3’-inverted dT-modified Inv_46 or Apt_46 for 72 h. The initial concentration of the oligonucleotide samples was 31.3–2000 nM and the samples were newly supplemented every 24 h. Cell viability was measured using cell counting kit-8. The bar graph shows mean ± SD (N = 3).

**Figure S17** Western blotting analysis of cell lysate of SNU16 incubated with Apt_46 (1 µM) or Inv_46 (1 µM) for indicated times. SNU16 was cultured in RPMI supplemented with 10% heat-inactivated FBS.
Table S1 The summery of phosphopeptides defined as “Changed” in Figure 6. SNU16 cells grown in heavy SILAC medium were lysed using 8 M guanidium-HCl (pH 8.5) after 15 min incubation with Apt_46 (1 µM) or AZD4547 (1 µM). The cells grown in light SILAC medium were lysed after 15 min incubation with vehicle. The lysates were mixed at 1:1 mass ratio based on the extracted protein and subjected to quantitative LC-MS/MS measurements. Three SILAC samples were prepared. Phosphopeptides that was quantified for a minimum of two of three samples in both conditions are used for quantification. The phosphopeptides whose SILAC ratio is more than 2 or less than 0.5 in either or both of Apt_46-treated condition and AZD4547-treated condition are defined as “Regulated” phosphopeptides. “Regulated” phosphopeptides whose SILAC ratio differs more than twice between Apt_46-treated and AZD4547-treated conditions are defined as “Changed (Apt vs AZD)”.
The Log2 ratios of SILAC ratios (Apt_46/AZD4547) are summarized in the table. The phosphopeptides used for quantification are summarized in Supplementary_table.xlsx.

5. References

[1] A. López-Perrote, R. Castaño, R. Melero, T. Zamarro, H. Kurosawa, T. Ohnishi, A. Uchiyama, K. Aoyagi, G. Buchwald, N. Kataoka, A. Yamashita, O. Llorca, *Nucleic Acids. Res.* **2016**, *44*, 1909–1923.

[2] A. Eguchi, M. Nakakido, S. Nagatoishi, D. Kuroda, K. Tsumoto, T. Nagamune, M. Kawahara, *Biotechnol. Bioeng.* **2019**, *116*, 1742–1751.

[3] N. Shindo, H. Fuchida, M. Sato, K. Watari, T. Shibata, K. Kuwata, C. Miura, K. Okamoto, Y. Hatsuyama, K. Tokunaga, S. Sakamoto, S. Morimoto, Y. Abe, M. Shiroishi, J. M. M. Caaveiro, T. Ueda, T. Tamura, N. Matsunaga, T. Nakao, S. Koyanagi, S. Ohdo, Y. Yamaguchi, I. Hamachi, M. Ono, A. Ojida, *Nat. Chem. Biol.* **2019**, *15*, 250–258.

[4] S. E. Ong and M. Mann, *Nat. Protoc.* **2006**, *1*, 2650–2660.

[5] D. M. Ornitz, N. Itoh, *WIREs Dev. Biol.* **2015**, *4*, 215–266.