Electronic Supplementary Information

For

DNA aptamer to c-Met inhibits cancer cell migration

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1. General information

Reagents were purchased from standard suppliers and used without further purification. DPBS (containing 4.2 mM K^+ ion) was from standard suppliers. All DNAs were purchased from Fasmac Co., Ltd (Japan). Recombinant human c-Met protein was purchased from R&D Systems (USA). Recombinant human HGF was purchased from PeproTech (USA). The primary antibodies for flowcytometry; Human HGF R/c-MET Phycoerythrin (PE) MAb Mouse IgG1 (#FAB3582P) and Mouse IgG1 (PE) Isotype control (#ab81200) were purchased from R&D Systems and Abcam, respectively. The primary antibodies for immunoblotting; c-Met (#8198) and phospho-Met (#3077) were obtained from Cell Signaling Technology (USA). The secondary antibody, Anti-Rabbit Immunoglobulins/HRP (#P0448) was obtained from Dako (Denmark). Blight field and phase contrast images were acquired by inverted microscope (IX-81, Olympus) equipped with CCD cameras (DP72, Olympus).

2. Sequence data

CLN0003_Full: 5’ GGA GGG AAA AGT TAT CAG GCT GGA TGG TAG CTC GGT CGG GGT GGG TGG GTT GGC AAG TCT GAT TAG TTT TGG AGT ACT CGC TCC 3’

CLN0003_SL1: 5’ ATC AGG CTG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA AGT CTG AT 3’

CLN0003_SL1 Reverse: 5’ TAG TCT GAA CGG TTG GGT GGG TGG GGC TGG CTC GAT GGT AGG TCG GAC TA 3’

CLN0003_G-loop: 5’ GGA TGG TAG CTC GGT CGG GGT GGG TGG GTT GG 3’

CLN0003_SL2: 5’ GGA GTA CTC GCT CC 3’

CLN0003_SL1G14A: 5’ ATC AGG CTG GAT GAT AGC TCG GTC GGG GTG GGT GGG TTG GCA AGT CTG AT 3’

CLN0003_SL1G32A: 5’ ATC AGG CTG GAT GGT AGC TCG GTC GGG GTG GAT GGG TTG GCA AGT CTG AT 3’

CLN0003_SL1_del1: 5’ ATC AGG CTG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA AGT CTG A 3’

CLN0003_SL1_del3: 5’ ATC AGG CTG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA AGT CT 3’

CLN0003_SL1_del5: 5’ ATC AGG CTG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA AGT 3’

CLN0003_SL1_del7: 5’ ATC AGG CTG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA A 3’

CLN0003_SL1-CGAT: 5’ CGA TCG ATG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA ATC GAT CG 3’

CLN0003_SL1-GC: 5’ GCG CGC GTG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA ACG CGC GC 3’

3. Methods

3-1 Cell culture

All cell lines were cultured in 5% CO_2 in a humidified incubator at 37 ˚C. A549, SUIT-2, and SNU-1 cells were cultured in RPMI1640 supplemented with 10% FBS and 1% Antibiotic-Antimycotic (Life Technologies). SNU-5 cells were cultured in IMDM supplemented with 20% FBS and 1% Antibiotic-Antimycotic.

3-2 Flow cytometry

The fluorescent signal from each cells were measured by Flow cytometer (guava eacyCyte^{TM}, Merck Millipore). The histograms were drawn by Flowjo (Tree Star). The 4.0×10^5 cells of SNU-1 or SNU-5 cells were incubated with 5’-FITC labelled aptamer in DPBS with 0.5% BSA at 21 ˚C for 15 min. After incubation, the cells were washed with DPBS twice and then were suspended in DPBS before analysis.

In Fig. 3A, biotiylated-HGF and avidin-FITC conjugate from HGF-fluorokine$^{(R)}$ kit (R&D Systems) were used. Briefly, SNU-5 cells (1.0×10^5 cell) were incubated with biotiylated-HGF (1.1 μg/mL) in the absence or presence of SL1 or SL1 Reverse at 21 ˚C. After 15 min, avidin-FITC was added and incubated at 21 ˚C for 30 min. After incubation, the cells were washed with DPBS twice and then were suspended in DPBS before analysis.

In Fig. S1, the 4×10^5 cells of SNU-1 or SNU-5 were incubated with isotype control-PE (mouse IgG_1) or Anti human c-Met antibody. After 30 min incubation on ice, the cells were washed with DPBS twice and then were suspended in DPBS before analysis.

The dissociation constant of aptamer to c-Met protein on cell surface of SNU-5 was determined as follows. Concentration-dependent changes in mean fluorescence intensity were fitted using the equation $F_{max}X/(K_d + X)$, in which F_{max}, X, and K_d represent the maximum change in fluorescent intensity, aptamer concentration, and the dissociation constant between the aptamer and c-Met on cell surface, respectively. The dissociation constant is determined from three individual experiments.

3-3 CD measurement

CD spectra were acquired by CD spectrometer (J-820, JASCO) over a wavelength of 210–320 nm using a quartz cuvette at room temperature. A 5 μM of DNA (strand concentration) in 20 mM Tris-HCl (pH7.6) was supplied to measurements. The scanning speed was set at 50 nm/min, and the response time was 1 s.

3-4 Preparation of cell lysate and immunoblotting

A549 cells were starved in RPMI1640 supplemented with 0.5% BSA for 24 h before assay. After starvation, the medium was replaced and the aptamers were added into the medium. After 15 min, the cells were incubated with HGF (110 pM) and incubated for another 15 min. After incubation, the cells were washed with DPBS twice, and then lysed with lysis buffer (#9803,

CST) containing protease inhibitor cocktail (#25955-11, Nacalai Tesque) and phosphatase inhibitor cocktail (#07575-51, Nacalai Tesque). The lysed cells were placed on ice for 10 min and then centrifuged at 16,000 g for 20 min. The supernatant was recovered and supplied to immunoblotting. After separation in SDS-PAGE, the cell lysates were transferred to PVDF membrane. The membrane reacted with primary antibody at 4 ˚C overnight and then secondary antibody at room temperature for 1 h in appropriate concentration. The membranes were probed by Immobilon Western Chemiluminescent HRP Substrate (WBKLS0500, Merck Millipore) and chemiluminescent images were acquired by Luminescence Detection System (AE-9300H, ATTO).

3-5 Scattering assay

The assay was performed according to the reference 3a in the main text with slight modification. The 2.4×10^3 of SUIT-2 cell were seeded at 6-well plate and cultured in RPMI1640 supplemented with 10% FBS. After 3 days, the cells were incubated with or without HGF (110 pM) in the presence or absence of the aptamer (1 μM) for 18 h. After incubation, the phase contrast images (4× objective) were acquired.

3-6 Migration assay

The assay was performed according to the reference 3a in the main text with slight modification. The 1.5×10^4 of SUIT-2 cell in RPMI1640 supplemented with 10% FBS were seeded at transwell chamber equipped with transparent PET membrane (#353097, Corning) or FluoroBlok insert (#351152, Corning) on 24 well culture plate. The cells were incubated with or without HGF (110 pM) in the presence or absence of the aptamer for 12 h. In Fig. 3D, the SUIT-2 cells that passed through membrane were fixed by methanol and then stained by Hemacolar (#111661, Merck-Millipore). After staining, the bright-field images (4× and 10× objective) were acquired. In Fig. 3E, the cells were labelled with 4 μg/mL Calcein-AM (#C396, Dojindo) in HBSS for 60

min at 37 ˚C. After labelling, the cells were subjected to fluorescent measurement by plate reader (Ex; 485 nm, Em; 530 nm)

3-7 SPR Measurement

SPR measurement was conducted by Biacore 2000 (GE healthcare) using sensor chip SA (GE healthcare) according to the reference[1] in this supporting information with slight modification. The 5'-biotinylated SL1 was immobilized on sensor chip SA. A various concentration (0, 0.5, 0.75, 1, 2, 3, and 5 nM) of recombinant human c-Met Fc chimera protein (#358-MT, R&D systems) was injected (180 sec) to the SL1-immobilized surface at a flow rate of 30 μL/min (25˚C, running buffer: DPBS supplemented with 0.05% Nonidet-P40, regeneration solution: 50 mM NaOH in 1 M NaCl).

4. Supporting figures

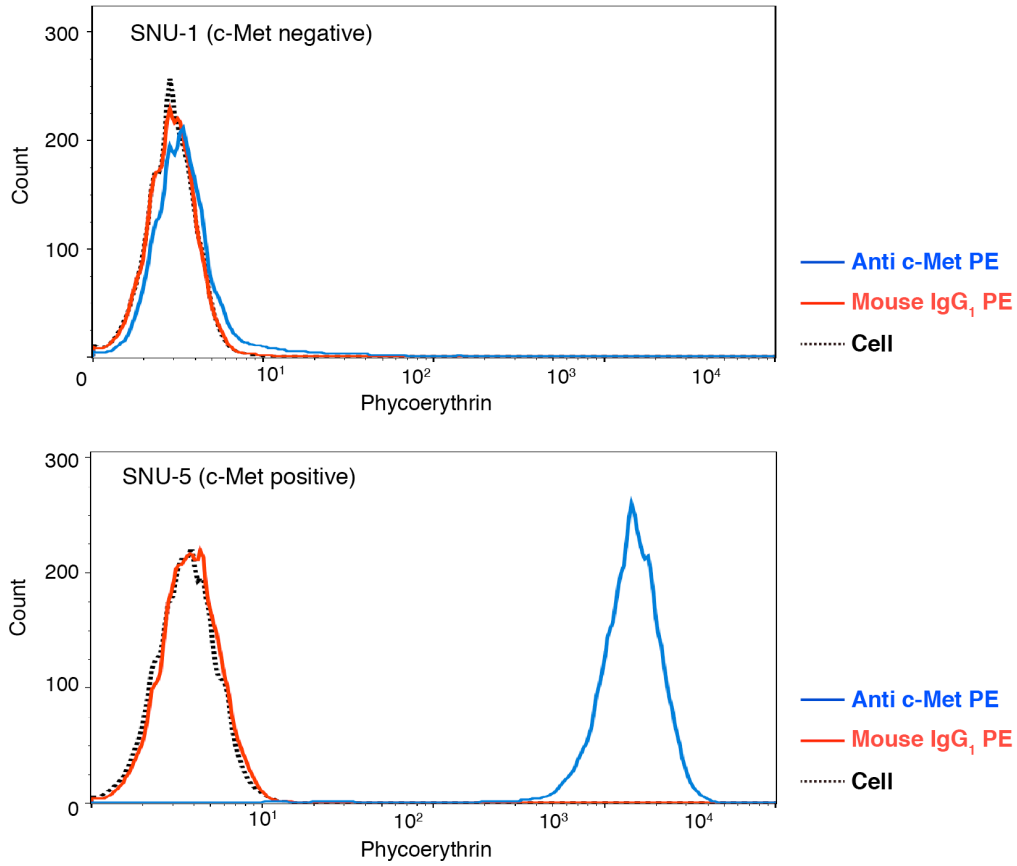

Fig. S1 The expression level of c-Met in SNU-1 (top) or SNU-5 (bottom) cell was evaluated by flowcytometer. The 4×10^5 cells of SNU1 or SNU-5 were incubated with Anti human c-Met-PE (Phycoerythrin) antibody or Mouse IgG_1-PE, as a negative control. After 30 min incubation on ice, the cells were washed by DPBS twice and were suspended in DPBS before analysis.

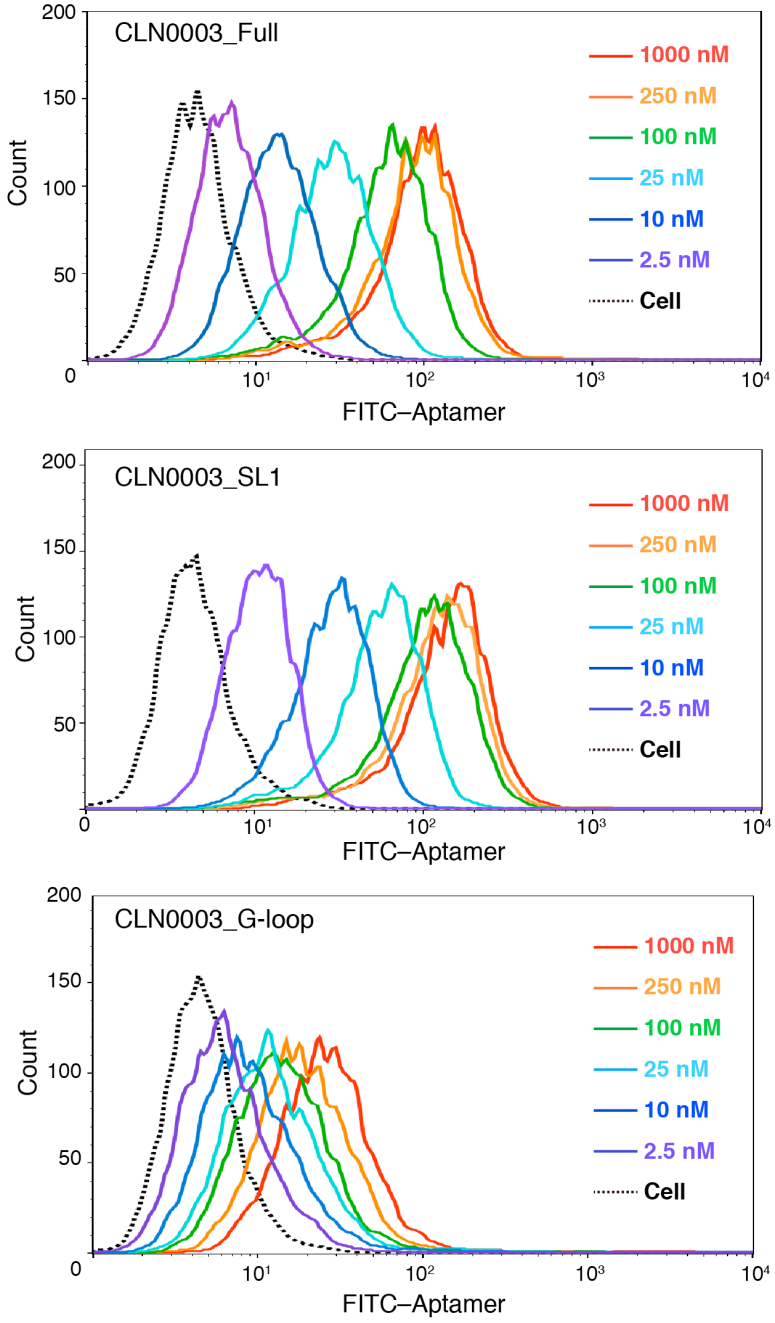

Fig. S2 Flow cytometric analysis of 5'-FITC labelled aptamer binding to SNU-5 cells. Each sequence (2.5-1000 nM) was incubated with 4.0×10^5 cells in DPBS with 0.5% BSA at 21 ˚C for 15 min. After incubation, the cells were washed with DPBS twice and then analyzed by flow cytometry.

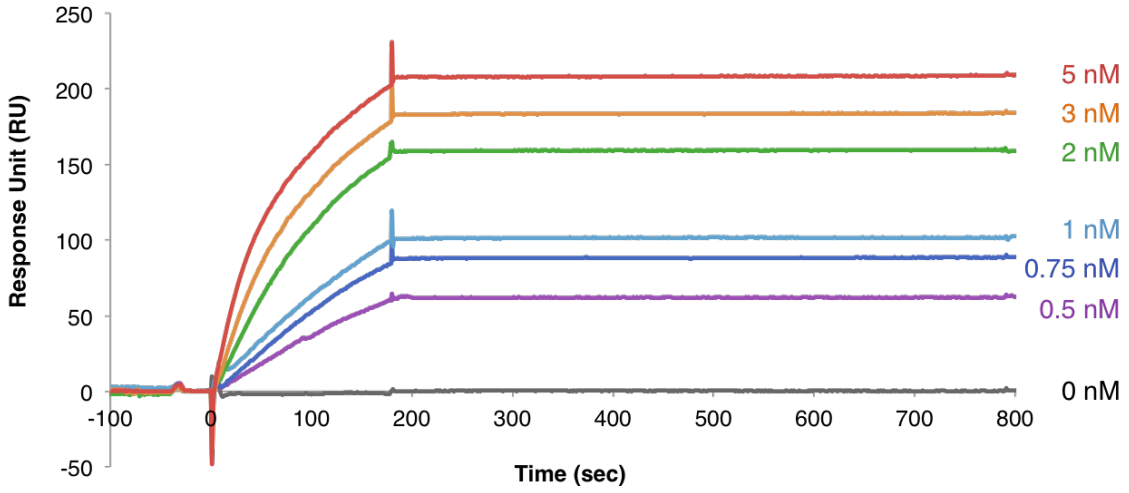

Fig. S3 Kinetic analysis of 5'-biotinylated SL1 on SA-chip using Biacore 2000. The recombinant human c-Met (0, 0.5, 0.75, 1, 2, 3, and 5 nM) was injected onto the SL1-immobilized SA-chip (25°C, running buffer: DPBS supplemented with 0.05% Nonidet-P40, flow rate = 30 μL/min, injection time = 180 sec).

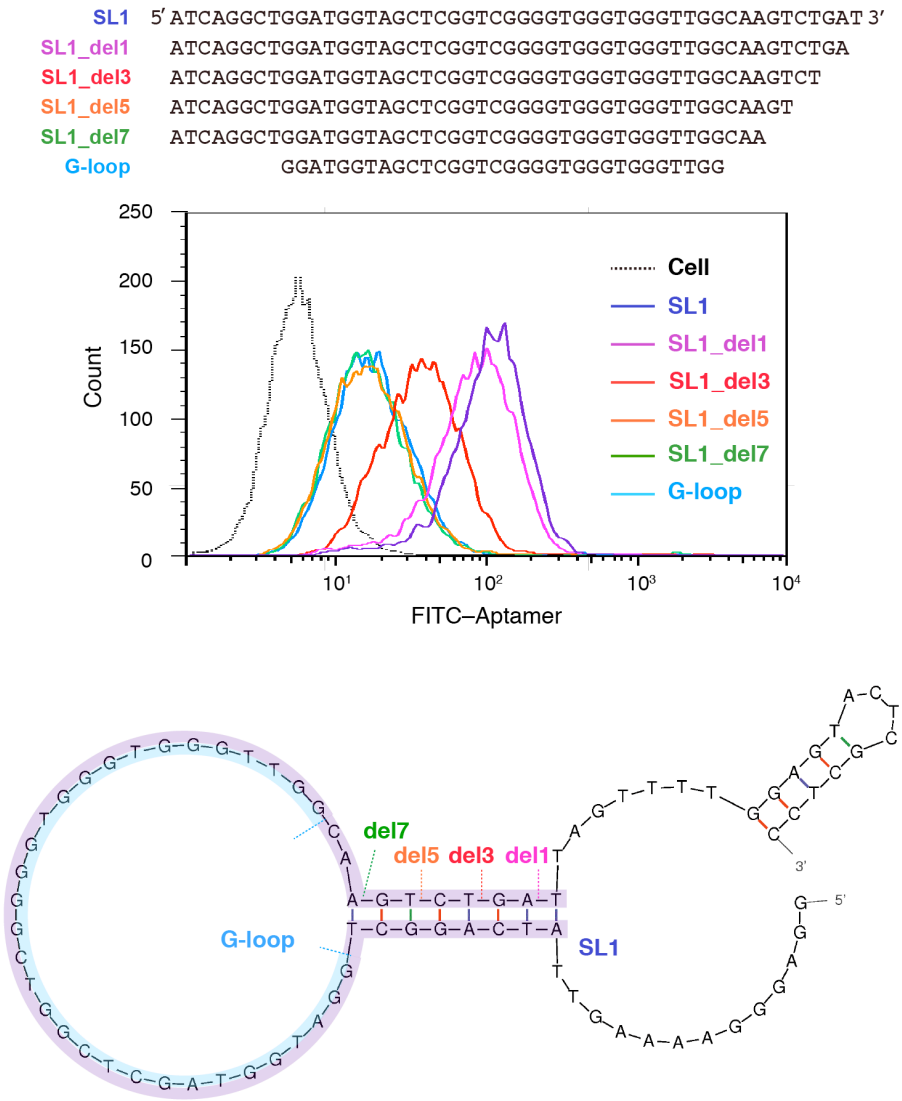

Fig. S4 Flow cytometric analysis of 5'-FITC labelled aptamers binding to SNU-5 cells. Each sequence (100 nM) was incubated with 4.0×10^5 cells in DPBS with 0.5% BSA at 21 ˚C for 15 min. After incubation, the cells were washed with DPBS twice and then analyzed by flow cytometry.

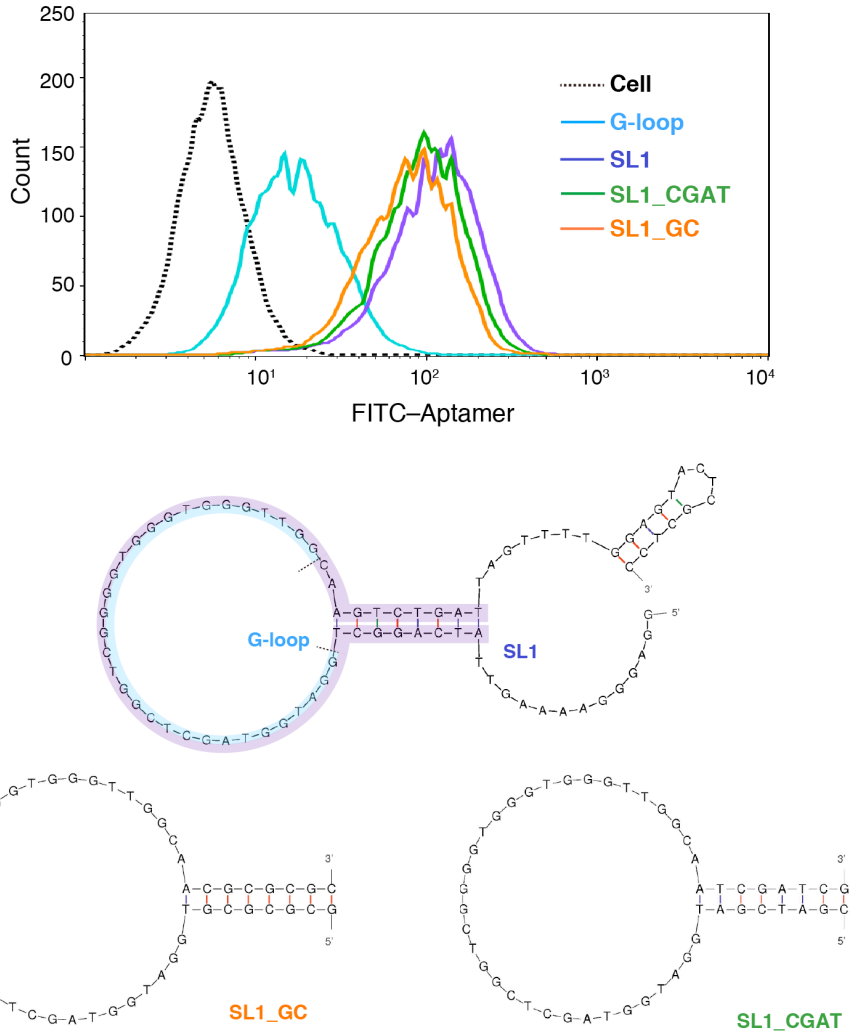

Fig. S5 Flow cytometric analysis of 5'-FITC labelled aptamer binding to SNU-5 cells. Each sequence (100 nM) was incubated with 4.0×10^5 cells in DPBS with 0.5% BSA at 21 ˚C for 15 min. After incubation, the cells were washed with DPBS twice and then analyzed by flow cytometry.

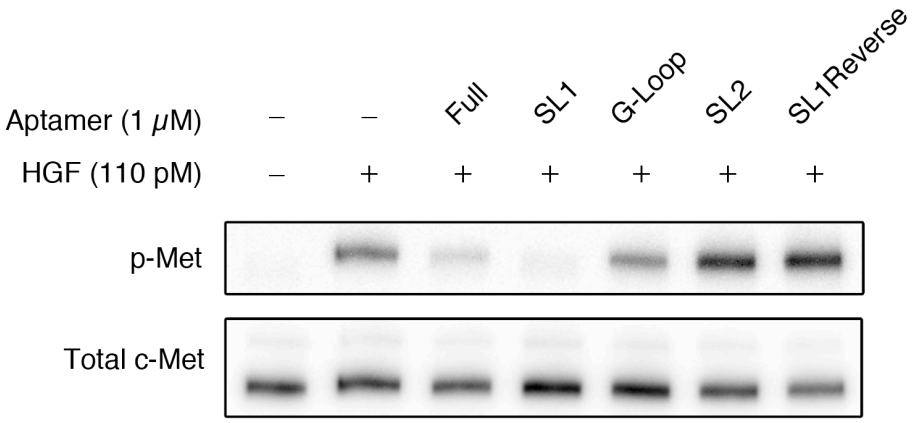

Fig. S6 The immunoblotting of HGF-treated A549 cells in the presence or absence of the aptamers designed from CLN0003. After A549 cells were incubated with the aptamers (1 μM) for 15 min, HGF (110 pM) was added to the medium. After 15 min incubation, the cells were lysed with lysis buffer. The cell lysates were separated by SDS-PAGE, and then transferred to PVDF membrane. The membrane was reacted with primary antibody overnight at 4˚C and HRP-conjugated secondary antibody for 1 h at room temperature.

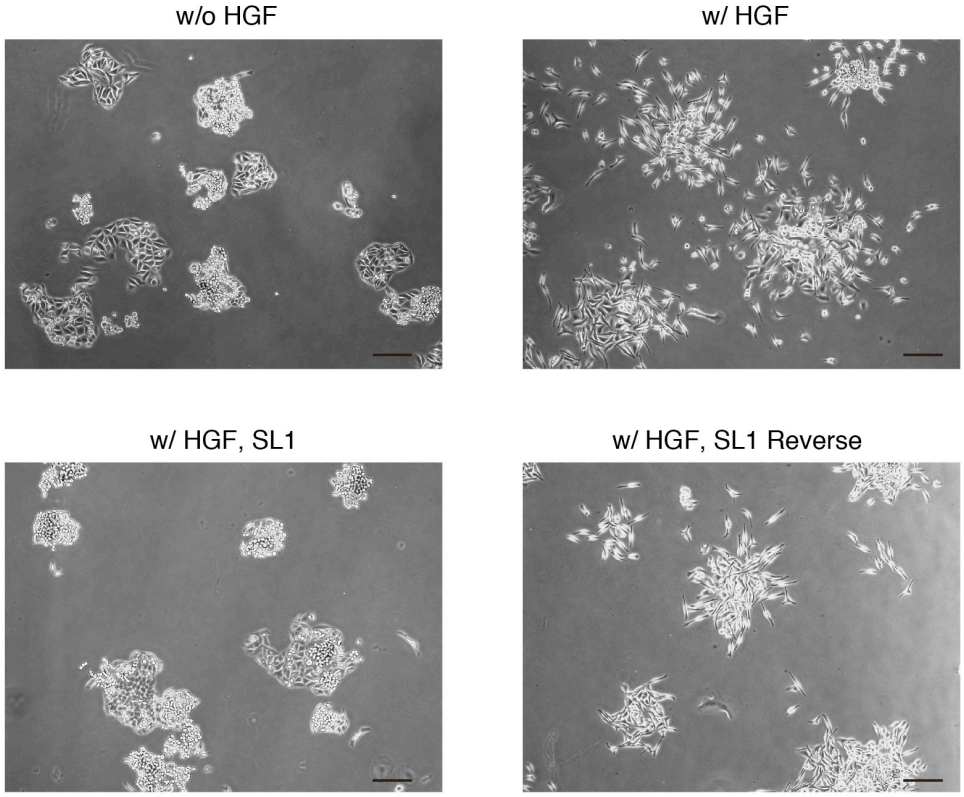

Fig. S7 Cell scattering assay. SUIT-2 cells (2.4×10^3 cells) were seeded at 6-well culture plate and incubated for 3 days. After the medium was replaced, the aptamers (1 μM) and HGF (110 pM) were added. After incubation for 18 h, the phase-contrast images were obtained (4× objective). Parts of these images are shown as Figure 3C. All scale bars indicate 200 μm.

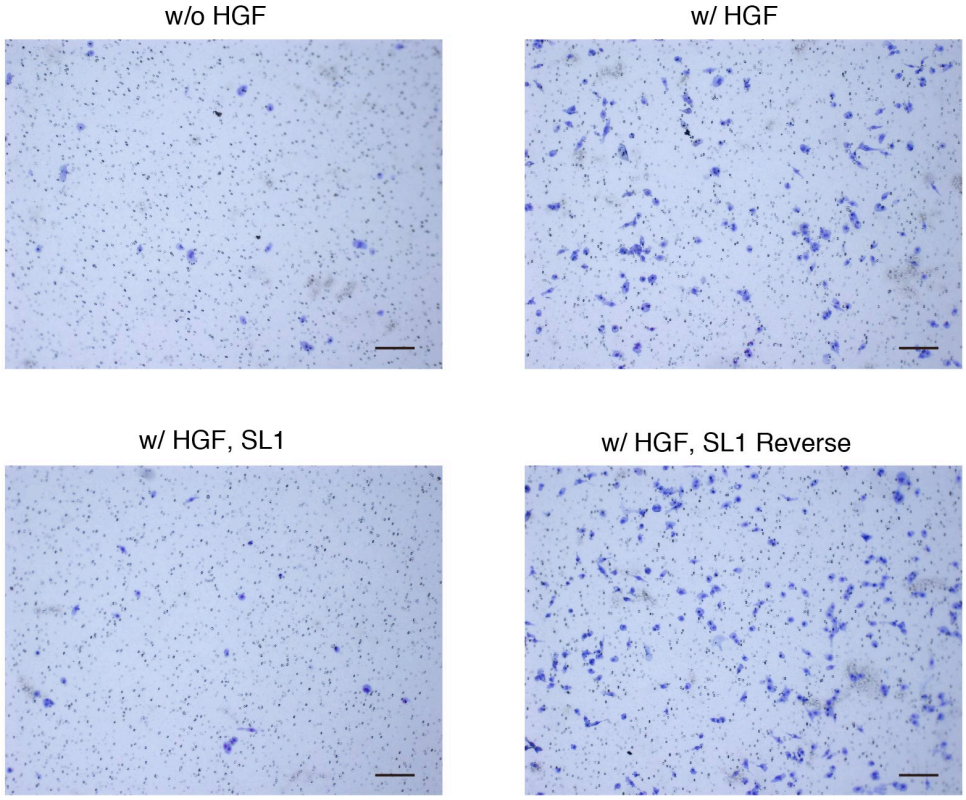

Fig. S8 Cell migration assay. SUIT-2 cells (1.5×10^4 cells) were cultured on PET membrane with 8 μm pore with or without HGF (110 pM) in the absence or presence of aptamer (500 nM) for 12 h. After the cells were fixed and stained, the bright-field images (×4 objective) were obtained. All scale bars indicate 200 μm.

5. Reference

(1) M. Kimoto, R. Yamashige, K. Matsunaga, S. Yokoyama and I. Hirao, *Nat. Biotechnol.*, 2013, **31**, 453.