Supporting Information

Supramolecular FRET signal amplification nanoprobe for high contrast and synchronous in-situ imaging of cell surface receptor homodimer/heterodimer

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Experiment

Materials and reagents

The oligonucleotides were synthesized and purified by Sangong Biotechnology Co., Ltd. (Shanghai, China) and the specific sequences are shown in Table S1. DNA Marker, 6×DNA loading buffer and Gelred nucleic acid dye were also purchased from Sangong Biotechnology Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA), phosphate buffer saline (PBS), and Tris (hydroxymethyl) aminomethane-hydrochloric acid and RPMI Medium 1640 were purchased from Solaibao Technology Co., Ltd (Beijing, China). Fetal bovine serum (FBS) was obtained from Gemini (USA). Calcein AM Cell Viability Assay Kit (CCK-F) was obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Hematoxylin Staining Solution and Eosin Staining Solution were purchased from Servicebio Technology Co., Ltd. (Wuhan, China). A549 cells and BEAS-2B cells were obtained from Cell Bank, Chinese Academy of Sciences. All experimental water was Millipore-Q water (resistivity > 18.2 MΩ·cm).

Experimental section

Cell culture. A549 cells (human lung adenocarcinoma cells) and BEAS-2B cells (human normal bronchial epithelial cells) were cultured in RPMI Medium 1640 (containing phenol red indicator and 1% penicillin-streptomycin) with 10% FBS. The conditions of the cell culture incubator were clean and humid, 37 ℃, with 5% CO_2.

Modeling of A549 tumor-bearing mice. The subcutaneous tumor formation in nude mice was used to construct a tumor-bearing mouse model. A suspension containing

1×10^7 A549 cells was injected into the right axilla of nude mice. Following the injection, the mice were monitored every 3 days for tumor formation, during which the tumor size was measured using vernier calipers. 27 days later, mice were euthanized to remove the tumors, with a tumor volume of 1063.02 mm^3, and weight of 0.85 g. The tumor volume was calculated by the formula: $TV=a\times b^2/2$ (a is the long diameter, b is the short diameter). All protocols referring to animals in this study were approved by the Life Science Ethics Committee of Zhengzhou University (ZZUIRB2024-169).

Preparation of FRET signal amplification nanoprobes. The oligonucleotide probe was initially heated at 95 °C for 5 min. Subsequently, it was removed and allowed to cool gradually to room temperature. The concentration of each oligonucleotide probe was adjusted to 4 μmol/L. The supporting chain (e.g., S1) was mixed with the corresponding FRET signal amplification probes (e.g., H1 and H2) in a ratio of 1:4:4 or other ratios, and the final concentration of the supporting chain S1 was controlled at 200 nmol/L. Finally, the mixed solution was incubated at 400 r/min for 30 min in a thermostatic metal shaker at 37 °C.

Fluorescence measurement. Take 50 μL of the prepared probe in a microcuvette. Set the excitation wavelength to 492 nm with the slit width at 1.5 nm. At this point, the collection range of the emission wavelength is from 500 to 750 nm, and measure the fluorescence spectra of FAM and Cy5. Set the excitation wavelength to 540 nm and the slit width to 1.5 nm. At this time, the collection range of the emission wavelength is from 560 to 800 nm, and measure the fluorescence spectra of Cy3 and Cy7.

Flow cytometric analysis. The cell suspension was centrifuged at 1000 r/min for 3 min at room temperature and the supernatant was discarded. After that, the cell precipitate was mixed with the prepared probe, ensuring that the final concentration of the probe in the cell mixture was maintained at 200 nmol/L, resulting in a total reaction volume of 200 μL. The cells were incubated at 400 r/min in a metal oscillator at 37 ℃ for 30 min and then washed with PBS buffer. Finally, the fluorescence intensity of each group of cells was measured by flow cytometry with the corresponding fluorescence channel.

Confocal fluorescence imaging. A total of 5×10^4 cells were seeded in a confocal culture dish and cultured for 24 hours until the cells were completely attached to the wall. After that, the cells were washed with PBS buffer and incubated with the prepared probes for 2 hours. Real-time fluorescence imaging of live cells was performed using a 40× objective lens of a laser confocal microscope. For example, R1 was excited at 488 nm, and the fluorescence signals were collected from 496 nm to 570 nm, with the resulting images assigned a green pseudo-color.

Supporting Figures

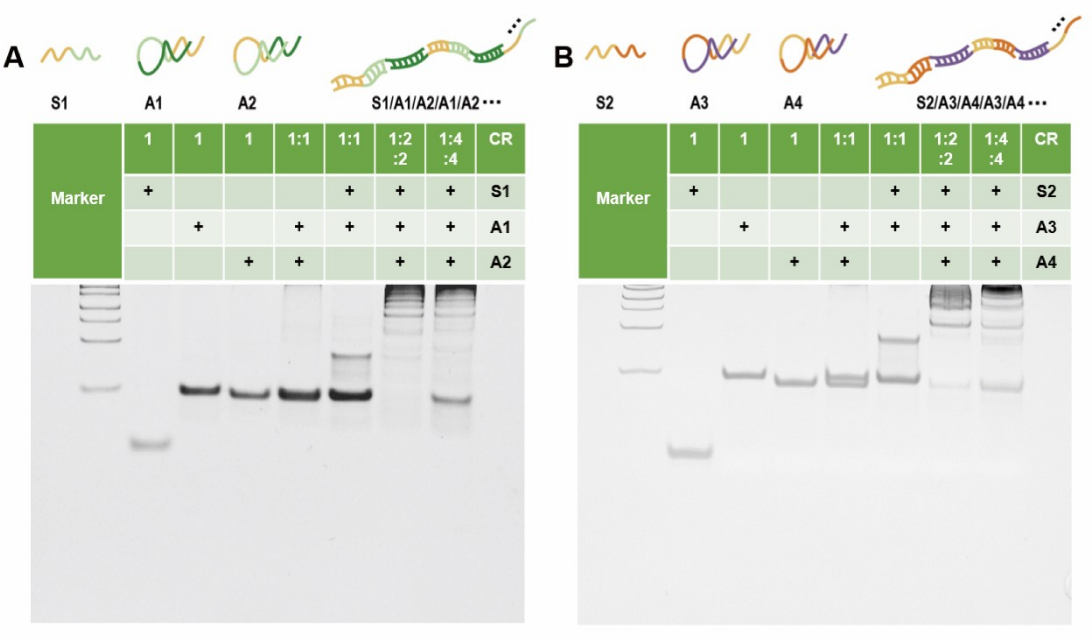

Figure S1. Gel electrophoresis characterization of hybridization chain reaction (HCR) of (A) S1/A1/A2 and (B) S2/A3/A4. CR (Concentration Ratio).

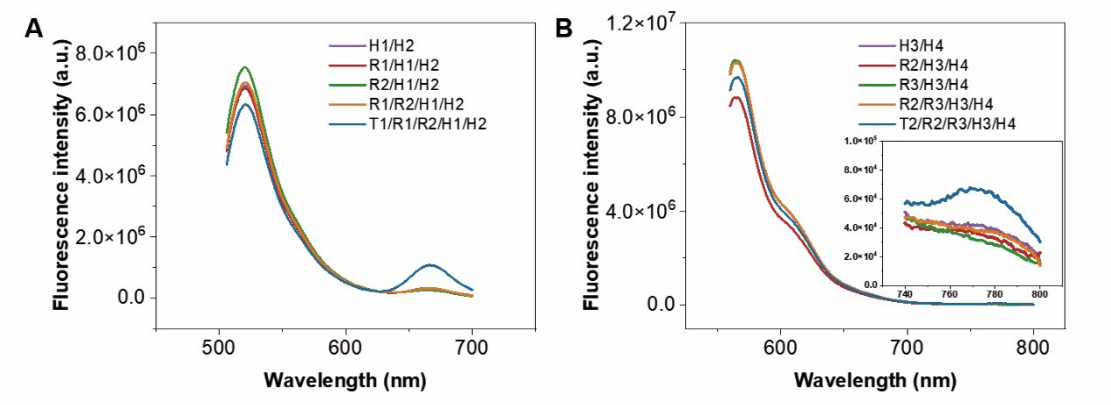

Figure S2. Fluorescence spectra characterization of proximity ligation induced FRET signals of (A) T1/R1/R2/H1/H2 and (B) T2/R2/R3/H3/H4.

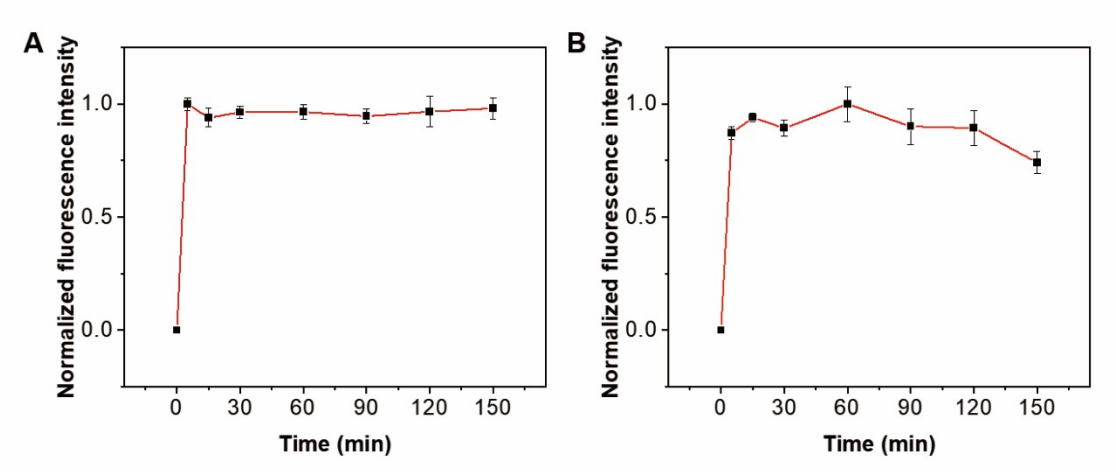

Figure S3. The normalized fluorescence intensity of (A) S1/H1/H2 and (B) S2/H3/H4 FRET signal amplification based on HCR at different reaction times.

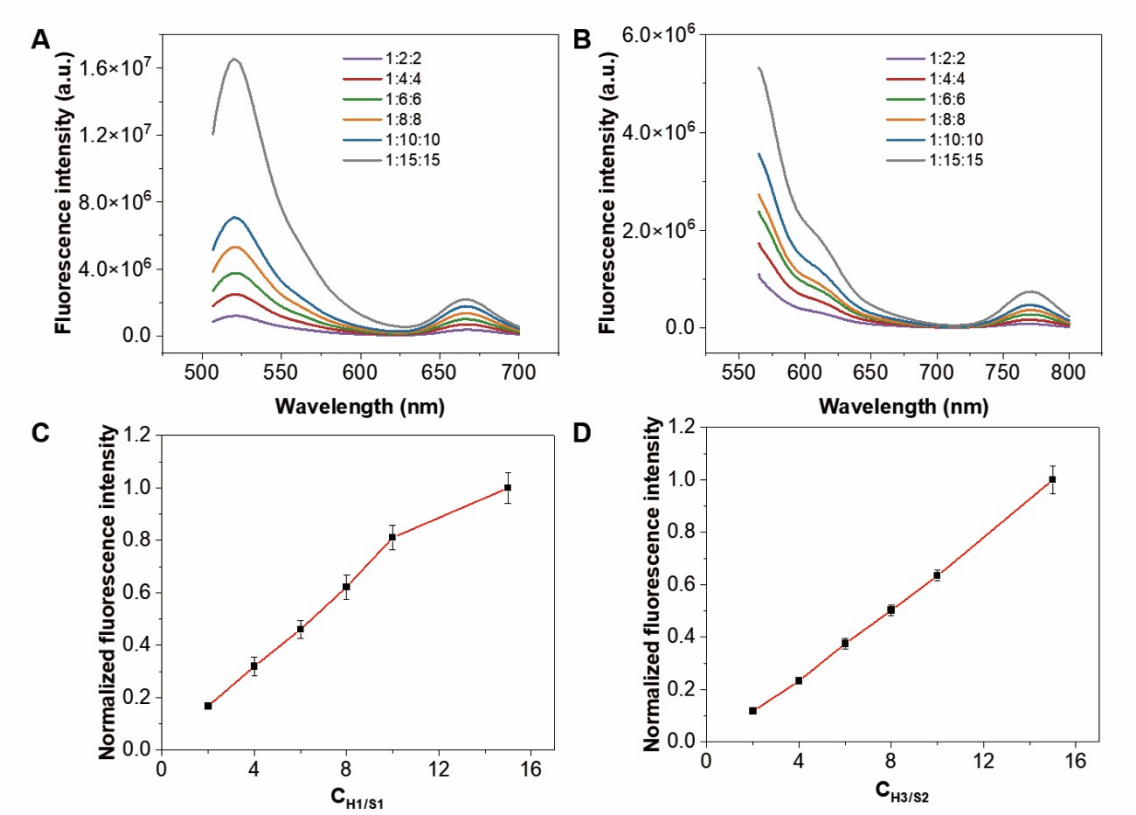

Figure S4. Fluorescence spectra (A) and normalized fluorescence intensity (C) of S1/H1/H2 FRET signal amplification based on HCR at different concentration ratios (S:H:H). Fluorescence spectra (B) and normalized fluorescence intensity (D) were shown for S2/H3/H4. $C_{H1/S1}$ represents the concentration ratio of H1/S1; $C_{H3/S2}$ represents the concentration ratio of H3/S2.

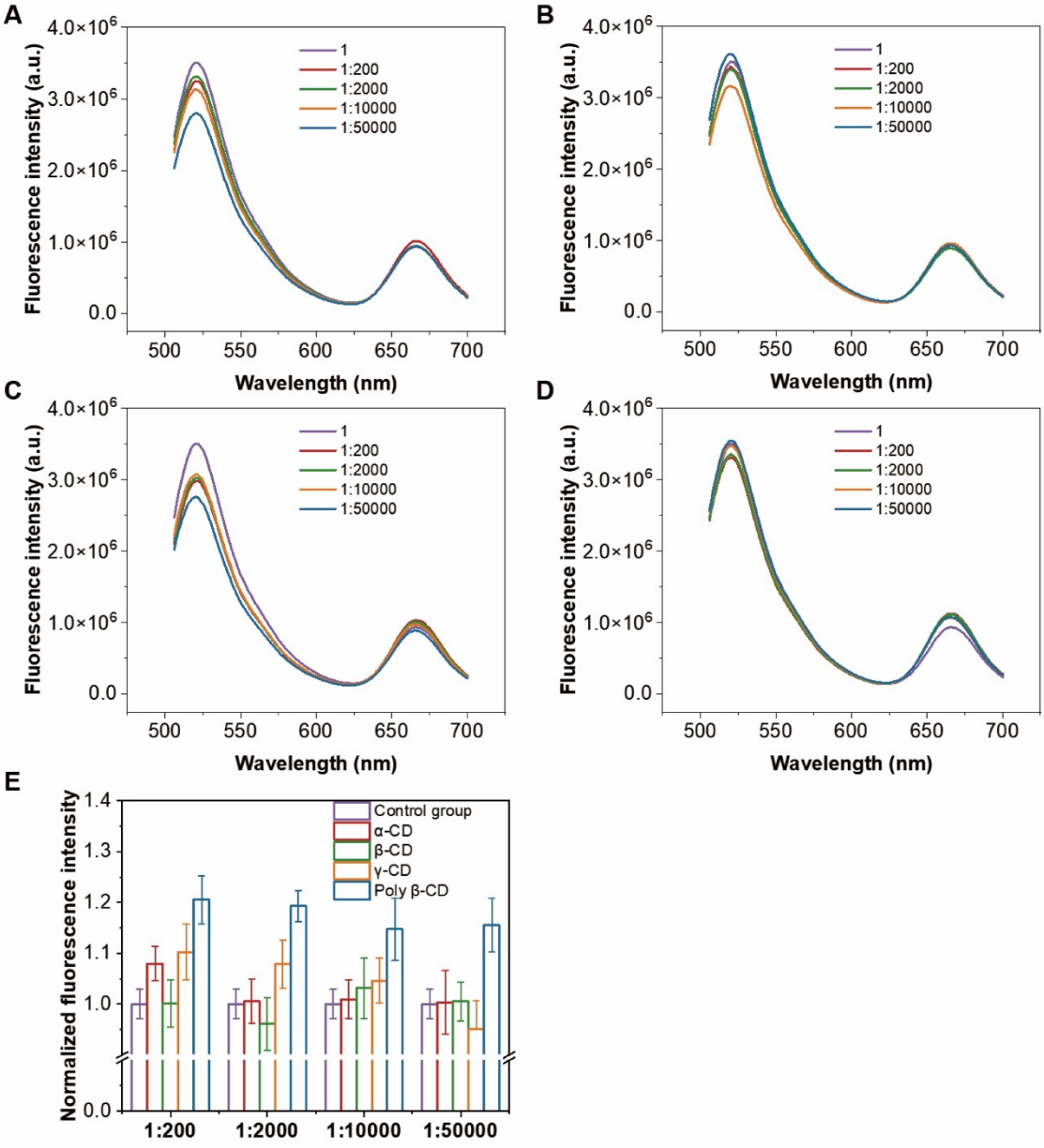

Figure S5. Fluorescence spectra of (A) α-CD, (B) β-CD, (C) γ-CD and (D) poly β-CD enhancing FRET signal amplification with different concentration ratios (considering T1/R1/R2/H1/H2 (1:1:1:4:4) as one, the ratio of its concentration to that of different cyclodextrins was 1:200, 1:2000, 1:10000, and 1:50000, respectively). (E) Normalized fluorescence intensity of α-CD, β-CD, γ-CD and Poly β-CD enhancing FRET signal amplification with different concentration ratios.

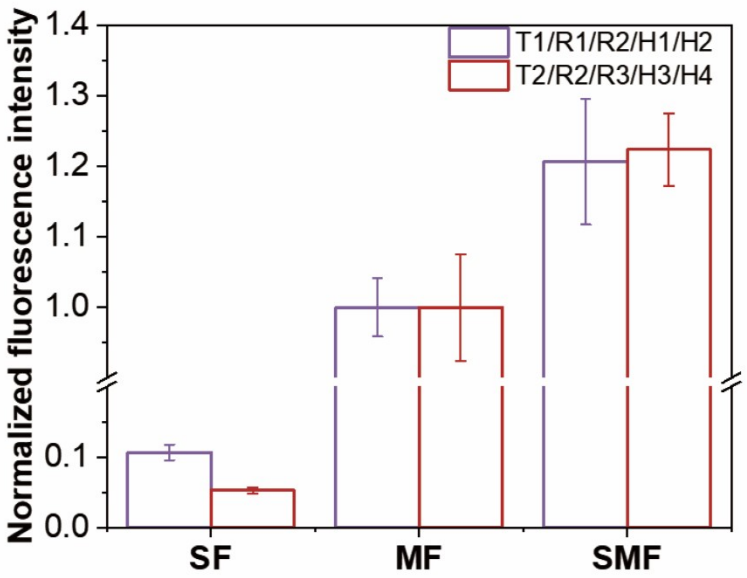

Figure S6. Normalized fluorescence intensity of Poly β-CD enhancing FRET signal amplification under optimal conditions. SF (Single-FRET group): T/R/R/A/H; MF (Multi-FRET group): T/R/R/H/H (1:1:1:10:10); SMF (Supramolecular FRET group). Considering T/R/R/H/H (1:1:1:10:10) as one, the ratio of its concentration to that of Poly β-CD was 1:200.

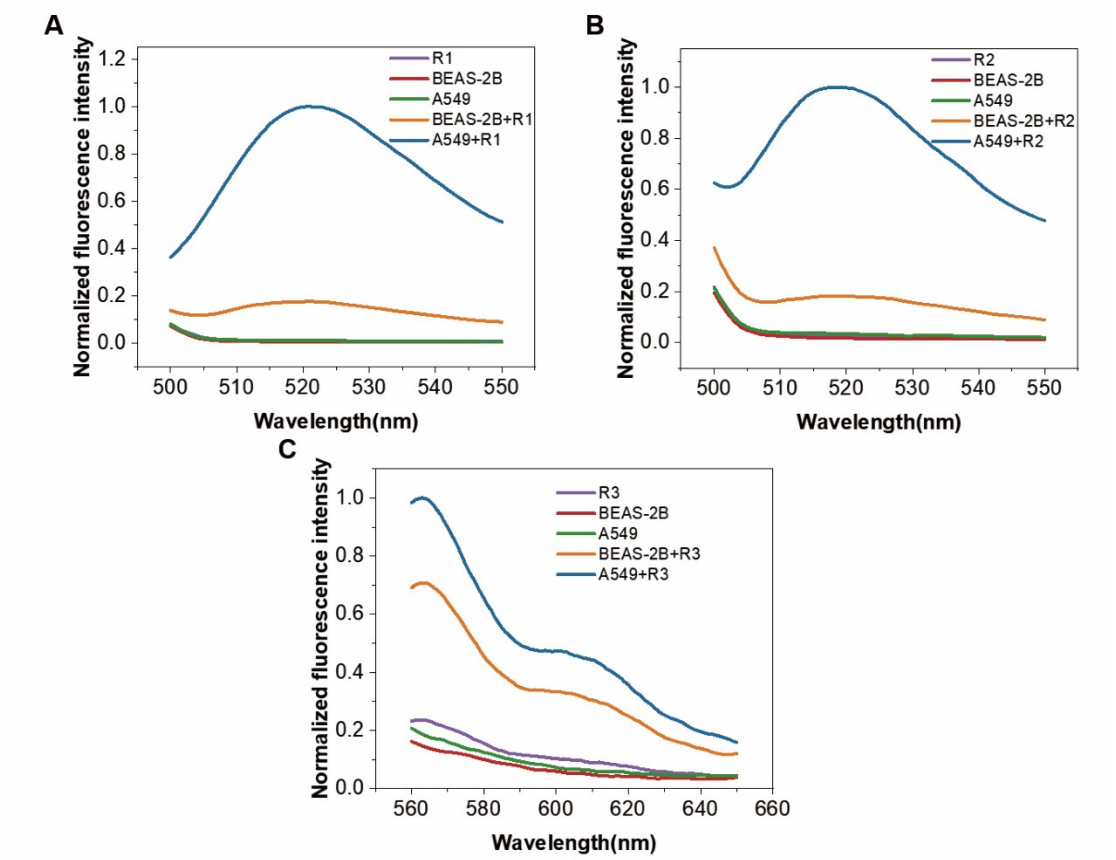

Figure S7. Fluorescence spectra of activatable aptamer fluorescent probes (A) R1, (B) R2 and (C) R3 incubation with cells.

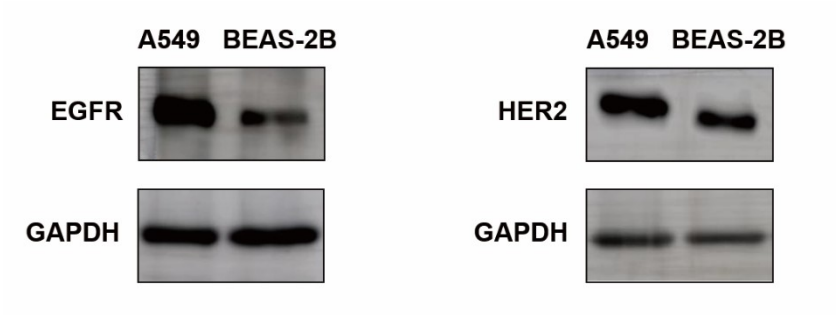

Figure S8. Western blot analysis of EGFR and HER2 expression in A549 cells and BEAS-2B cells.

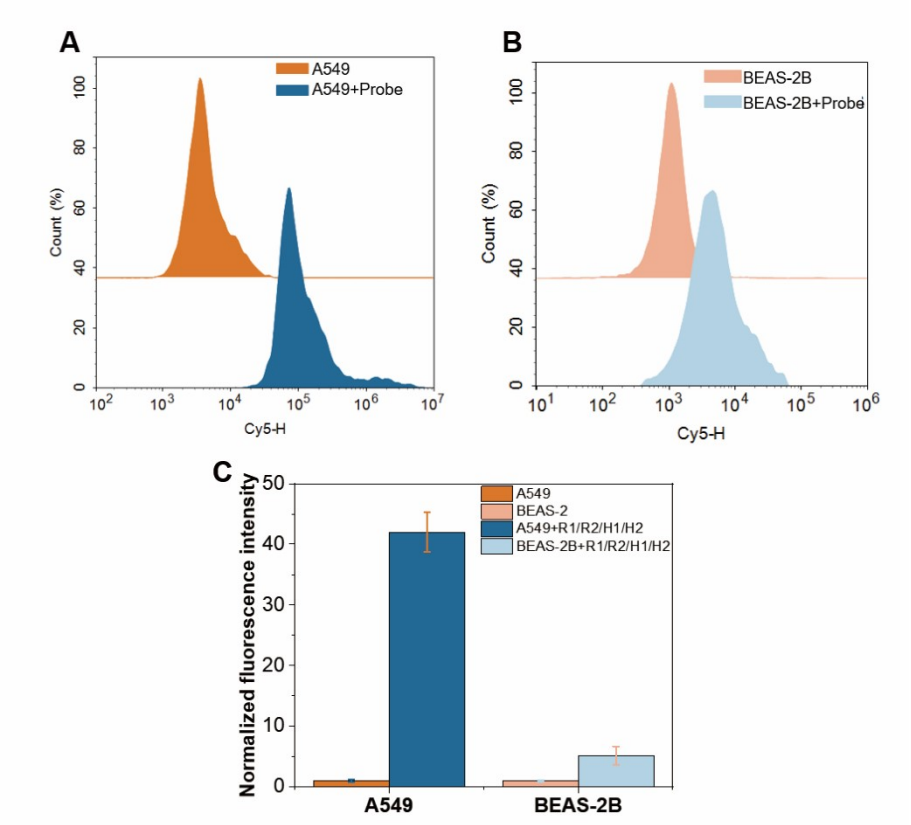

Figure S9. Flow cytometry assay of EGFR/EGFR homodimer of A549 cells (A) and BEAS-2B cells (B) and their quantitative fluorescence analysis (C).

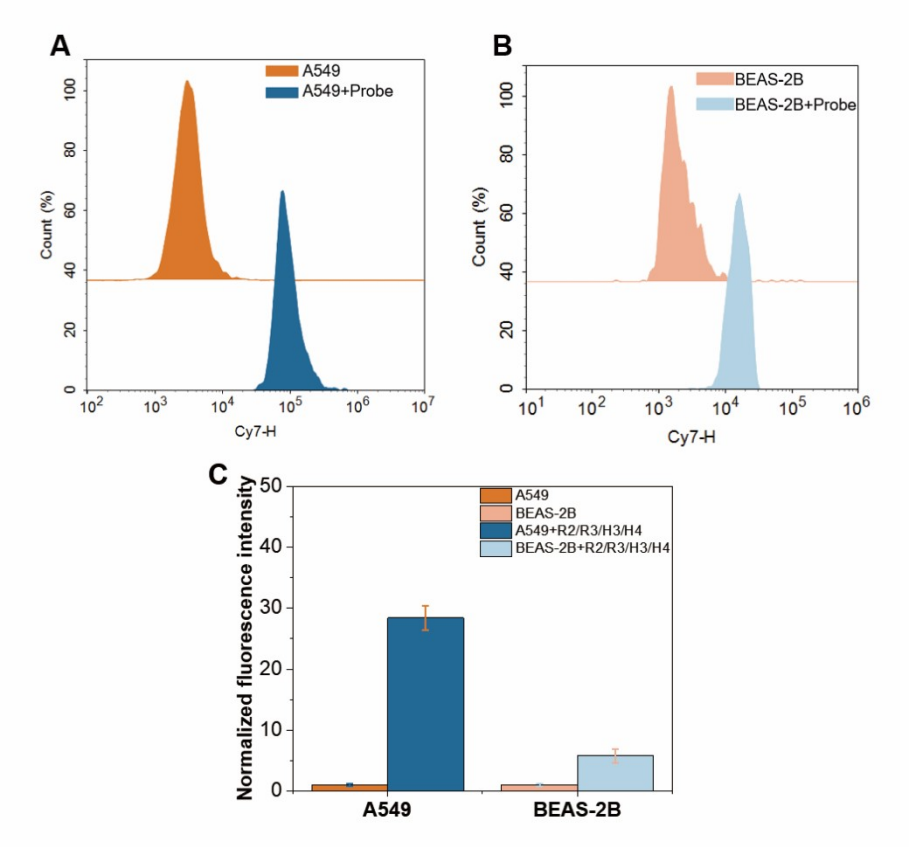

Figure S10. Flow cytometry assay of EGFR/HER2 heterodimer of A549 cells (A) and BEAS-2B cells (B) and their quantitative fluorescence analysis (C).

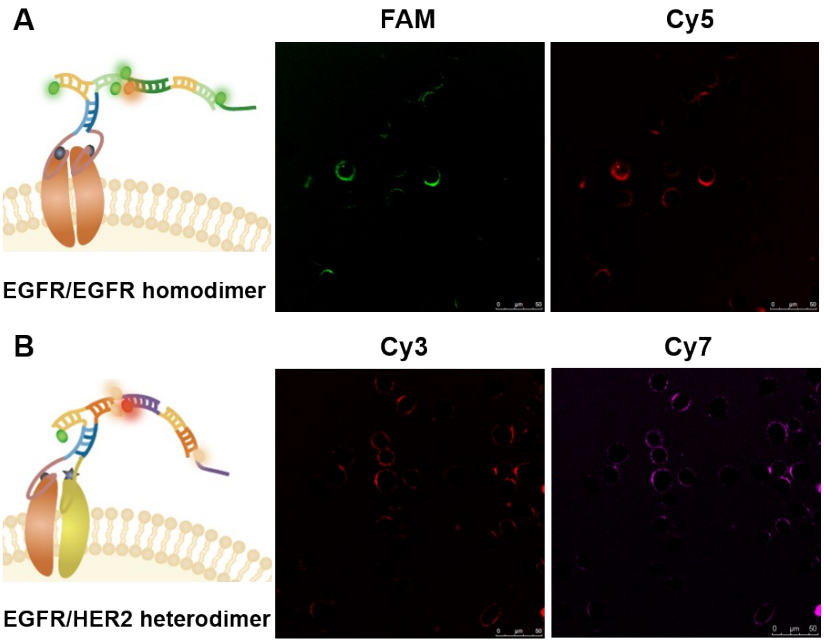

Figure S11. Confocal fluorescence imaging of EGFR/EGFR homodimer (A) and EGFR/HER2 heterodimer (B) of A549 cells by activatable FRET signal amplification nanoprobe. FAM channel: λex = 488 nm, λem = 496-570 nm; Cy5 channel: λex = 638 nm, λem = 650-700 nm; Cy3 channel: λex = 552 nm, λem = 560-600 nm; Cy7 channel: λex = 638 nm, λem = 700-800 nm. Scale bar: 50 μm.

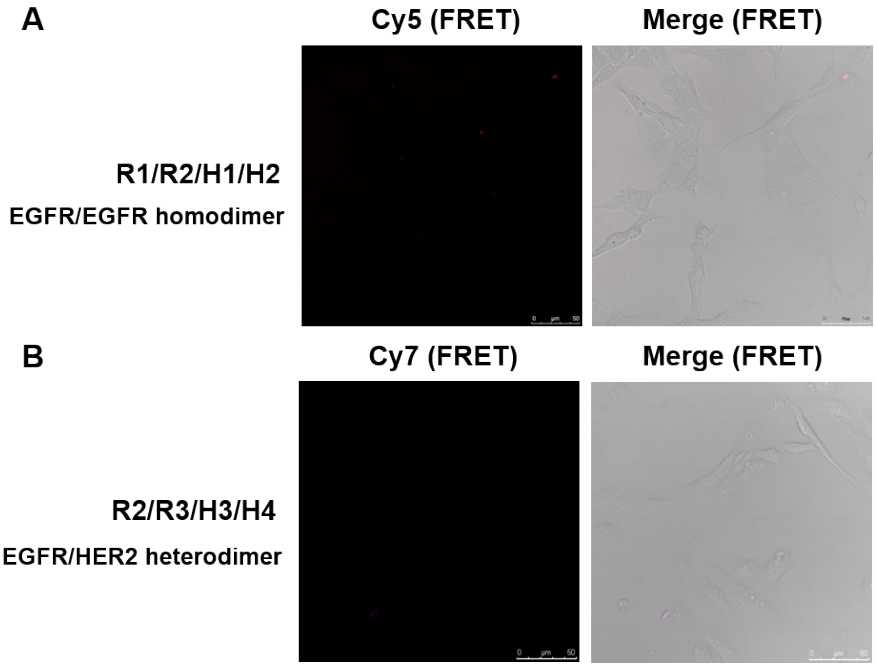

Figure S12. Confocal fluorescence imaging of EGFR/EGFR homodimer (A) and EGFR/HER2 heterodimer (B) of BEAS-2B cells by activatable FRET signal amplification nanoprobe. Cy5 channel (FRET): λex = 488 nm, λem = 650-700 nm; Cy7 channel (FRET): λex = 552 nm, λem = 700-800 nm. Scale bar: 50 μm.

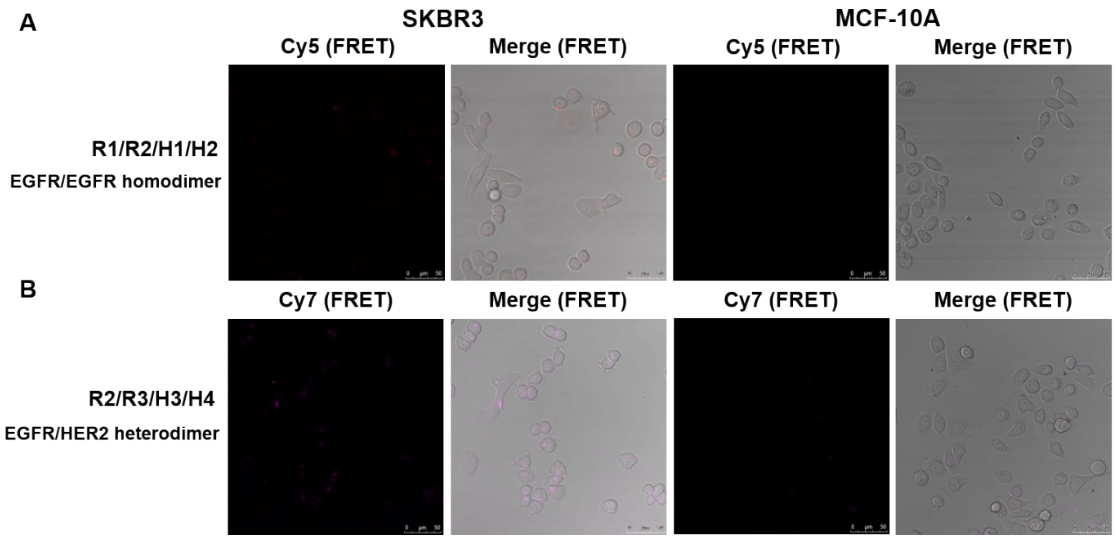

Figure S13. Confocal fluorescence imaging of EGFR/EGFR homodimer (A) and EGFR/HER2 heterodimer (B) of human breast cancer cells (SKBR3) and human normal breast cells (MCF-10A) by activatable FRET signal amplification nanoprobe. Cy5 channel (FRET): λex = 488 nm, λem = 600-700 nm; Cy7 channel (FRET): λex = 552 nm, λem = 700-800 nm. Scale bar: 50 μm.

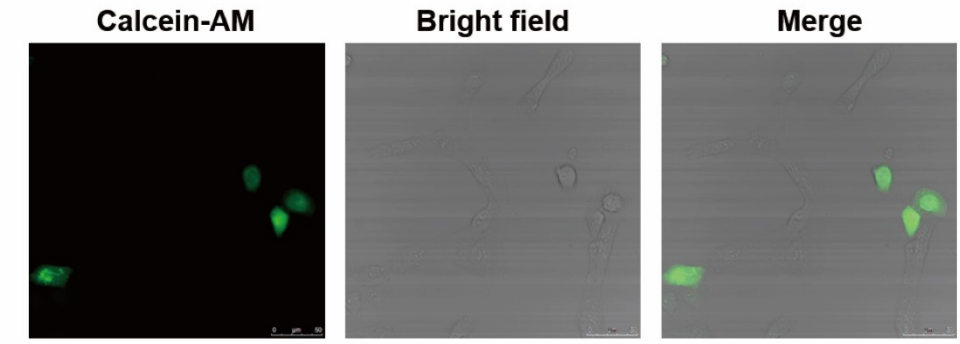

Figure S14. Confocal fluorescence imaging of Calcein-AM dye-labeled A549 after mixed culture with unlabeled BEAS-2B. Calcein-AM channel: λex = 488 nm, λem = 496-550 nm. Scale bar: 50 μm.

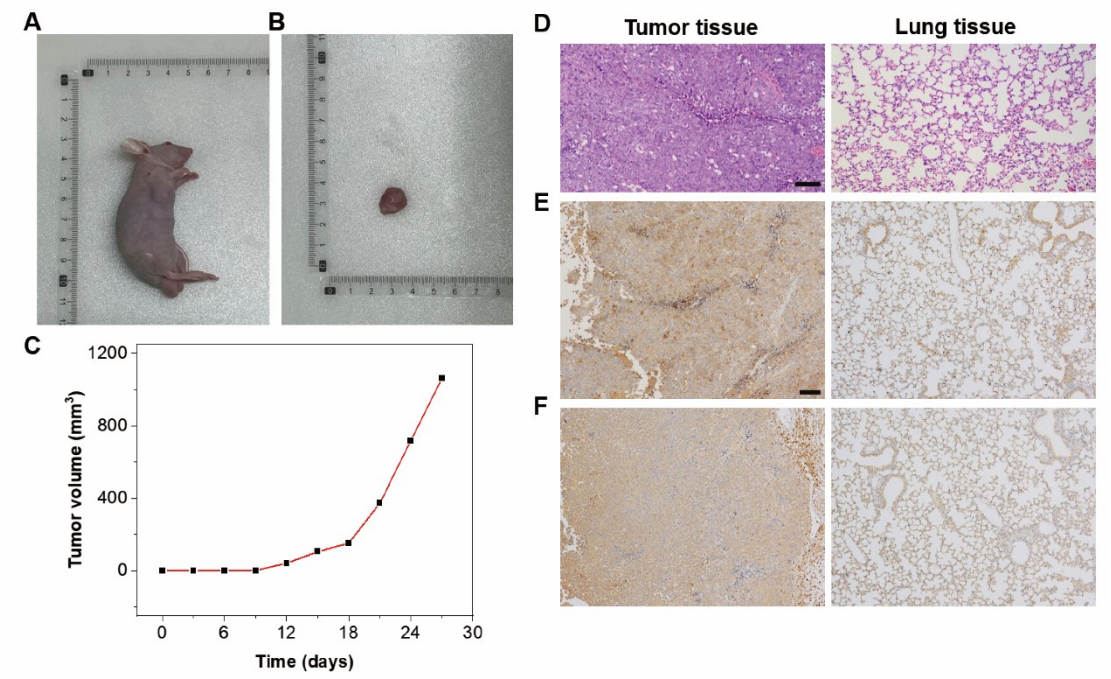

Figure S15. Images of (A) A549 tumor-bearing mouse model and (B) transplanted tumor. (C) Growth curve of tumor tissue during the observation period. (D) H&E staining of tissue section (the blue-purple color was the nucleus, and the red or pink color was the cytoplasm and extracellular matrix). IHC staining for detection of EGFR expression (E) and HER2 expression (F) (the positive area was brown). Scale bar: 100 μm.

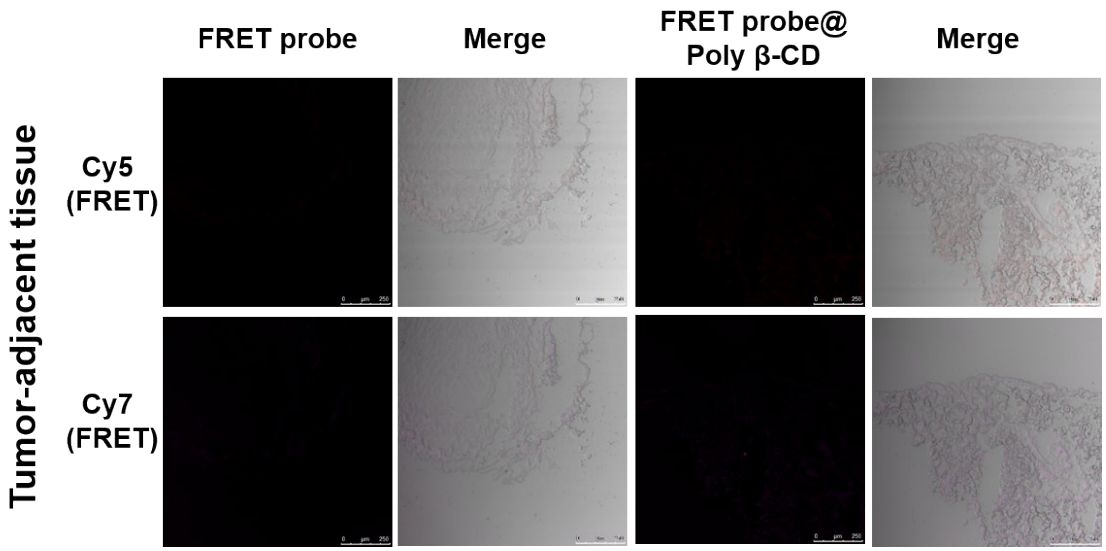

Figure S16. Confocal fluorescence imaging of EGFR/EGFR homodimer (Cy5) and EGFR/HER2 heterodimer (Cy7) of tumor-adjacent tissue sections from A549 tumor-bearing mice with supramolecular FRET signal amplification nanoprobe. Cy5 channel (FRET): λex = 488 nm, λem = 600-700 nm; Cy7 channel (FRET): λex = 552 nm, λem = 700-800 nm. Scale bar: 250 μm.

Supporting Table

Table S1. Oligonucleotide sequences were used in this study.

Name	Sequence (from 5′ to 3′)
R1	Dabcyl-TGCCGTTTCTTCTCTTTCGCTTTTTTTGCTTTTGAGCA TGTTTTTTTTTTCAATTATATTTCCTAAACGGCA-6-FAM
R2	6-FAM-CGGCAAAGTCCTTTTATAATTGTTTTTTTTTTGTACGA GTTTTCGTTTTTTTCGCTTTCTCTTCTTTGCCGT-Dabcyl
R3	BHQ2-CCGCAACCACGACCGAAAGACAACGCAATCTGACAC GTGGTTTTTTTTTTCAATTATATTCTTCGGTTGCGG-Cy3
R3-FAM	Dabcyl-CCGCAACCACGACCGAAAGACAACGCAATCTGACA CGTGGTTTTTTTTTTCAATTATATTCTTCGGTTGCGG-6-FAM
T1	ACGGCAAAGAAGAGAAAGCGAAAAAAACGAAAACTCGTACCA TGCTCAAAAGCAAAAAAAGCGAAAGAGAAGAAACGGCA
T2	ACGGCAAAGAAGAGAAAGCGAAAAAAACGAAAACTCGTACCC ACGTGTCAGATTGCGTTGTCTTTCGGTCGTGGTTGCGG
A1 (H1 without FAM)	GTCCTTCCTAAACGGCATTGGTTTGCCGTTTAGGAAGGACTTTG CCG
A2 (H2 without Cy5)	AACCAATGCCGTTTAGGAAGGACCGGCAAAGTCCTTCCTAAAC GGCA
A3 (H3 without Cy3)	GTCCTCTTCGGTTGCGGTTGGTTCCGCAACCGAAGAGGACTTTG CCG
A4 (H4 without Cy7)	AACCAACCGCAACCGAAGAGGACCGGCAAAGTCCTCTTCGGTT GCGG
S1	CGGCAAAGTCCTTCCTAAACGGCA
S2	CGGCAAAGTCCTCTTCGGTTGCGG
H1	GTCCTTCCTAAACGGCATTGGTT/i6FAMdT/TGCCGTTTAGGAAG GACTTTGCCG
H2	Cy5-AACCAATGCCGTTTAGGAAGGACCGGCAAAGTCCTTCC TAAACGGCA
H3	GTCCTCTTCGGTTGCGGTTGGTT/iCy3dT/CCGCAACCGAAGAGG ACTTTGCCG
H4	Cy7-AACCAACCGCAACCGAAGAGGACCGGCAAAGTCCTCT TCGGTTGCGG