

Supplementary Information

Bispecific aptamer-decorated and light-triggered nanoparticles targeting tumor and stromal cells in breast cancer derived organoids: implications for precision phototherapies

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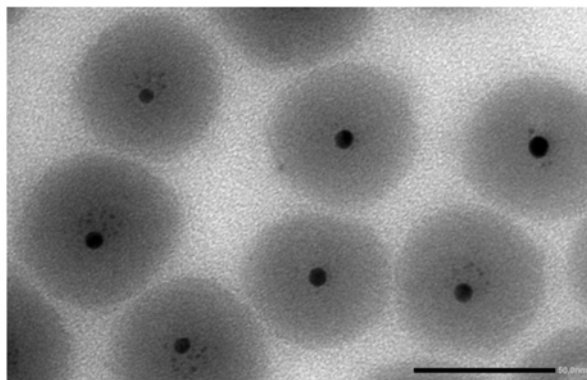
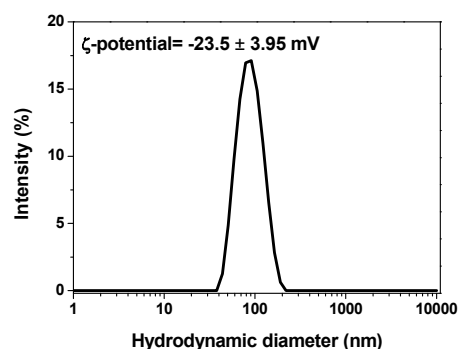
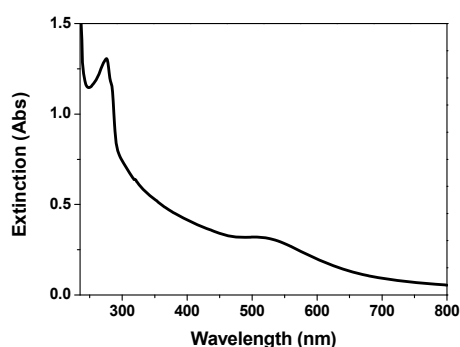
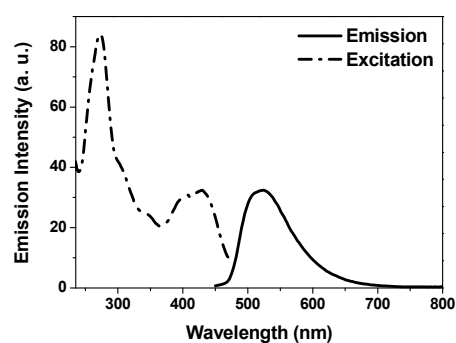
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Supplementary Figure 1. Characterization of Ir_{en}-AuSiO₂-COOH nanoparticles. **A** Representative TEM image. Scale bar: 50 nm. **B** Hydrodynamic diameter and ζ -potential value, **C** extinction spectrum. **D** excitation and emission spectra (λ_{ex} =420 nm; λ_{em} =520 nm) of Ir_{en}-AuSiO₂-COOH dispersed in water.

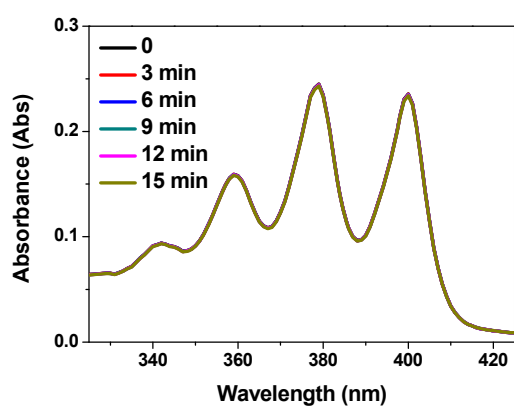
Light-induced singlet oxygen generation and photothermal effects of Ir_{en}-AuSiO₂-COOH

The generation of singlet oxygen by the photosensitizer-doped gold-silica nanoparticles was validated by monitoring spectrophotometrically the chemical oxidation of the molecular probe 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) (Sigma-Aldrich, St. Louis, MO, USA) in

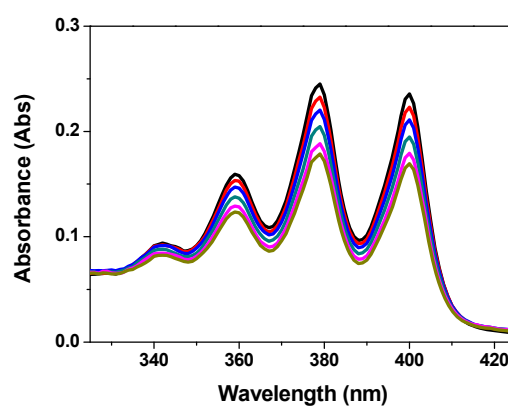
aqueous solution. The ABDA molecule reacts irreversibly with singlet oxygen to form the corresponding endoperoxide, resulting in attenuation of the absorption peak at 378 nm.

Briefly, 15 μL of ABDA solution (2 mg/mL DMSO) were mixed with 0.75 mL of $\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-COOH}$ solution and placed in a quartz cuvette with an optical path length of 0.2 cm. The generation of singlet oxygen was monitored upon irradiation at 254 nm for 15 min (five cycles of 3 min) by a xenon discharge lamp (equivalent to 20 kW for 8 μs duration). As control solution, 15 μL of ABDA solution were mixed with 0.75 mL of water and irradiated under the same conditions.

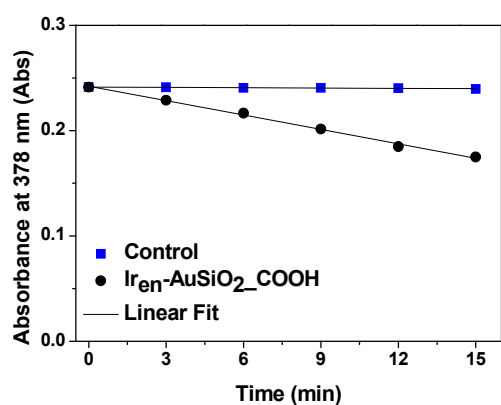
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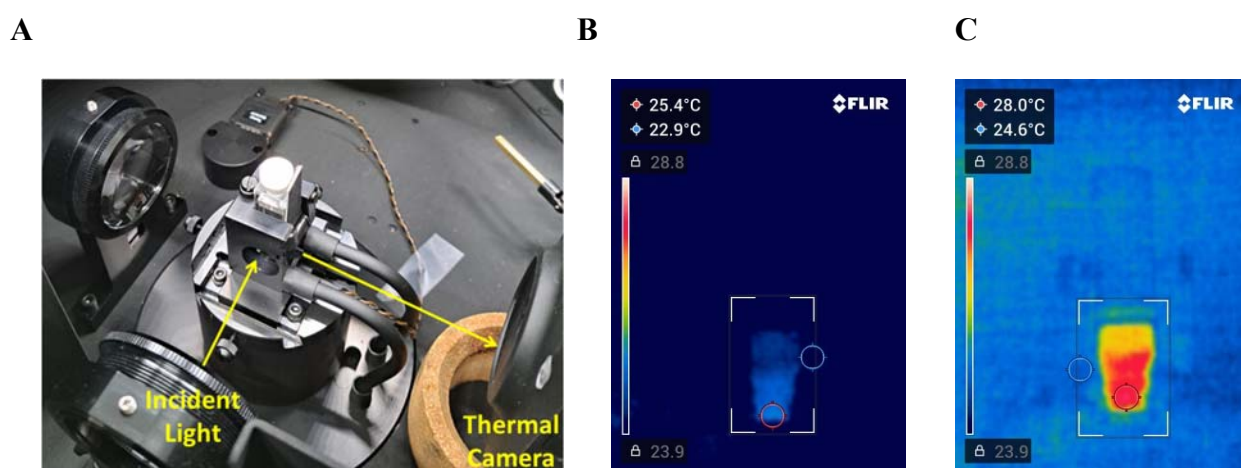


Supplementary Figure 2. Absorbance attenuation of ABDA by singlet oxygen generation. **A** Photobleaching of ABDA at different irradiation times in water (control) and **B** in $\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-COOH}$

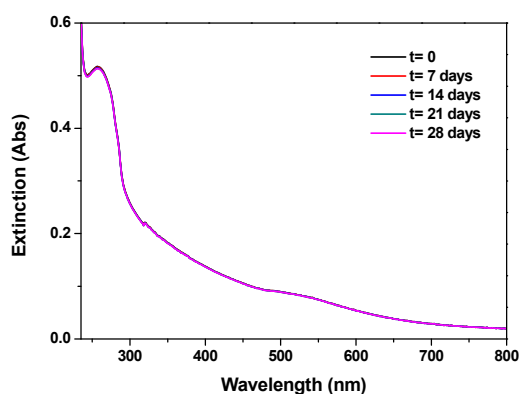
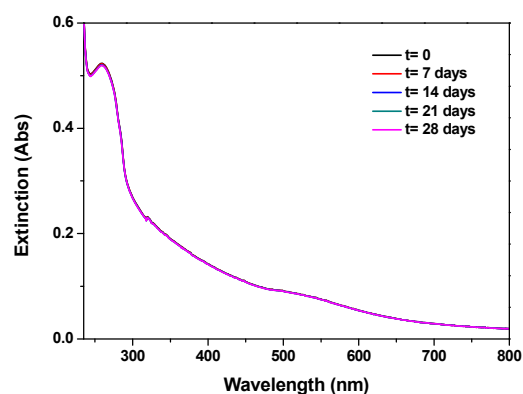
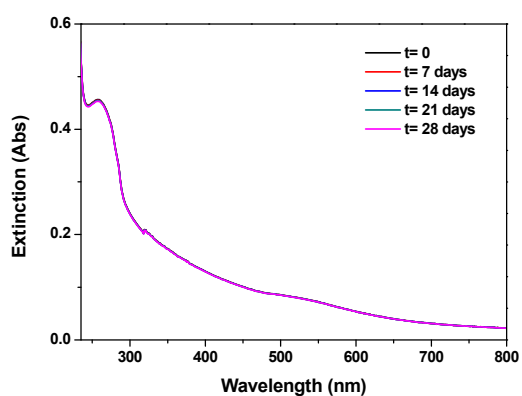
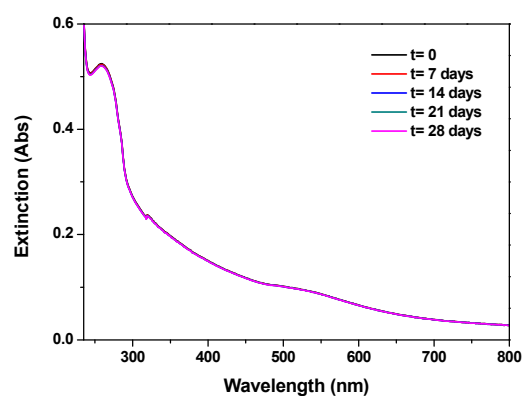
AuSiO₂_COOH aqueous solution. C Plotting of ABDA absorption at 378 nm as a function of illumination time.

The nanoplateforms heat generation under continuous illumination

To validate the photothermal effects, the thermal behavior of Ir_{en}-AuSiO₂_COOH nanoparticles was investigated under a 450 W continuous Xenon lamp irradiation; a photograph of the experimental setup used to carry out the measurements is shown in Supplementary Figure 3A. The incident light beam (254 nm) impinged on a 1 x 0.4 cm quartz cuvette containing 1 mL of nanoparticles solution. A thermal camera (C-3X model, by FLIR), characterized by a sensitivity of 0.07°C and placed perpendicular to the incident light (forming a 90-degree angle with the surface of the cuvette), was used to monitor the photo-induced temperature variations. The chamber temperature before the start of irradiation, for both control solution (water) and sample, was 22.1°C. After 90 min of irradiation, a thermal image, showing the value of the minimum and maximum temperature recorded in the area of interest, was acquired (Supplementary Fig. 3B,C).



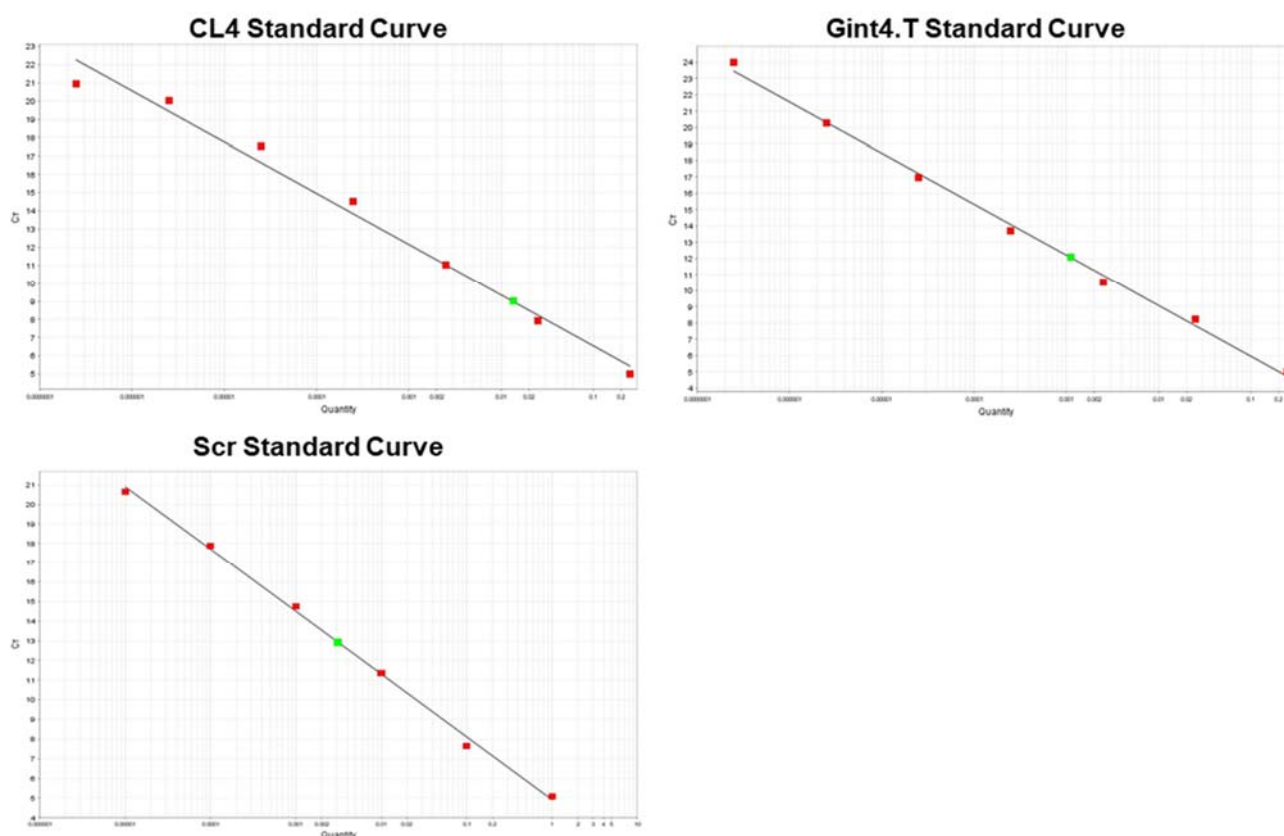
Supplementary Figure 3. Light-induced photothermal effect of Ir_{en}-AuSiO₂_COOH. A Picture of the used measurement setup to investigate the light-induced thermal effect of Ir_{en}-AuSiO₂_COOH. Infrared thermal images of B water (control) and C Ir_{en}-AuSiO₂_COOH nanoparticles dispersed in water (light exposure 254 nm, 90 min).

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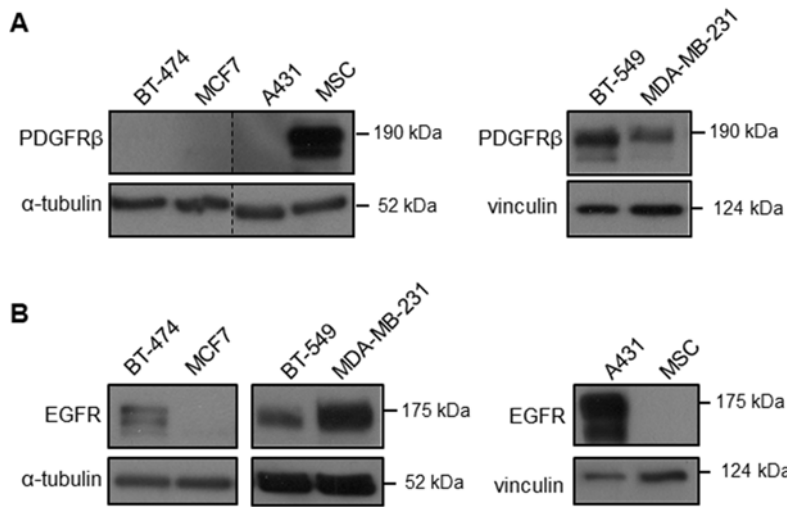
Supplementary Figure 4. Extinction spectra acquired in the time range 0-28 days of aptamers-conjugated nanoparticles dispersed in water. Extinction spectra of **A** Ir_{en}-AuSiO₂-CL4, **B** Ir_{en}-AuSiO₂-Scr, **C** Ir_{en}-AuSiO₂-Gint4.T and **D** Ir_{en}-AuSiO₂-CL4-Gint4.T dispersed in water over 7, 14, 21 and 28 days.

Supplementary Table 1. Properties of aptamers-nanoplatforms conjugates

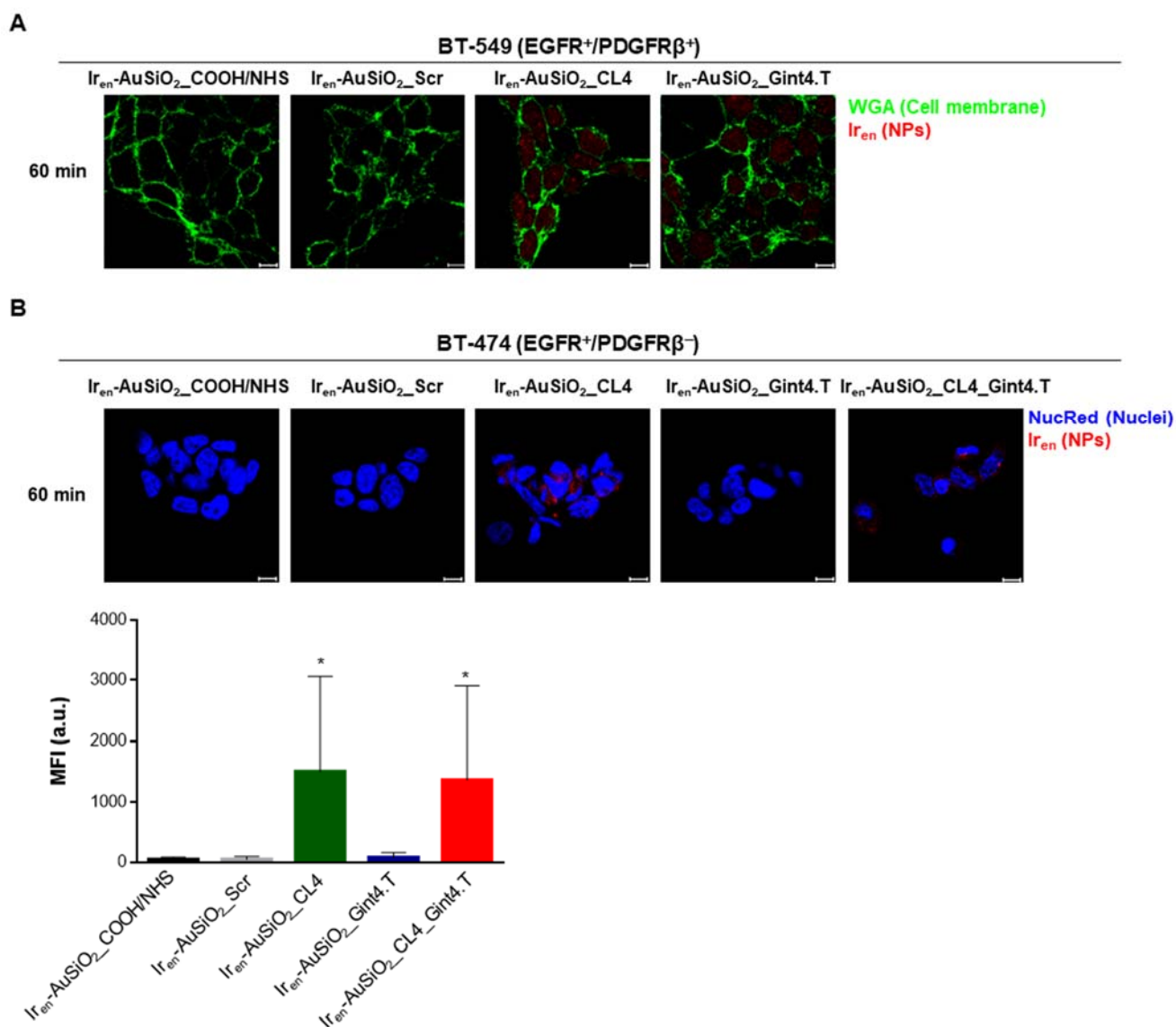
Nanoparticles formulation	Hydrodynamic diameter (nm)	PDI	ζ -potential (mV)
$\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-COOH/NHS}$	102.0 ± 0.15	0.172	-26.0 ± 1.01
$\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-CL4}$	102.1 ± 0.93	0.163	-22.3 ± 2.12
$\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-Scr}$	101.4 ± 0.20	0.162	-22.9 ± 2.25
$\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-Gint4.T}$	104.4 ± 0.47	0.168	-28.3 ± 3.12
$\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-CL4_Gint4.T}$	103.7 ± 0.82	0.162	-26.4 ± 8.12



Supplementary Figure 5. Quantification of the amount of aptamer conjugated to $\text{Ir}_{\text{en}}\text{-AuSiO}_2\text{-COOH/NHS}$. PCR calibration curve with unconjugated aptamer (red); sample, green.

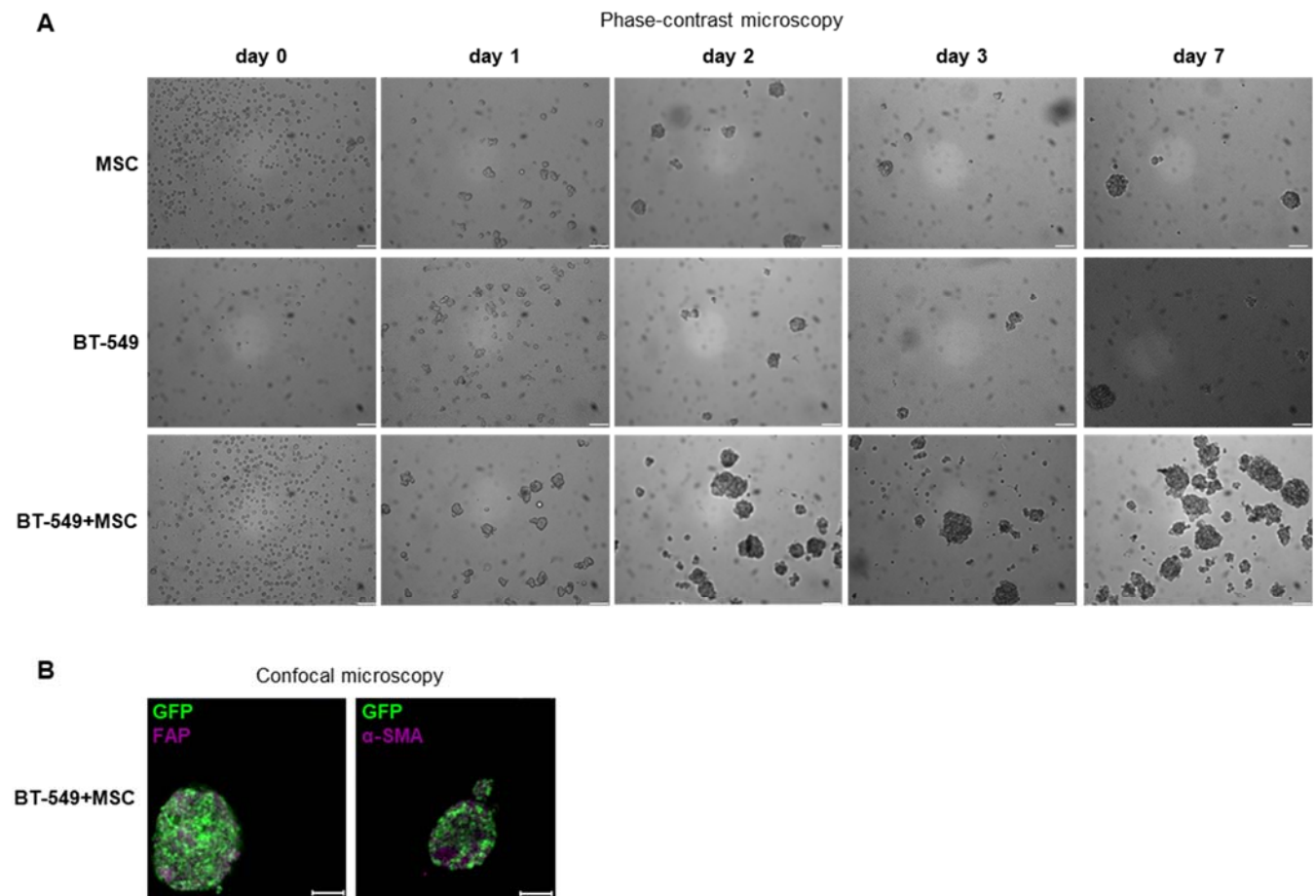


Supplementary Figure 6. Expression of PDGFR β and EGFR in different human cell lines. Lysates from cancer BT-474, MCF7, MDA-MB-231, BT-549, and A431 cell lines and stromal MSCs were immunoblotted with **A** anti-PDGFR β or **B** anti-EGFR antibodies, as indicated. Equal loading was confirmed by immunoblot with anti-vinculin or anti- α -tubulin antibody, as indicated. The molecular weights of the indicated proteins are reported. In **A**, black dashed lines delineate the boundary between non-contiguous lanes of the same gel.

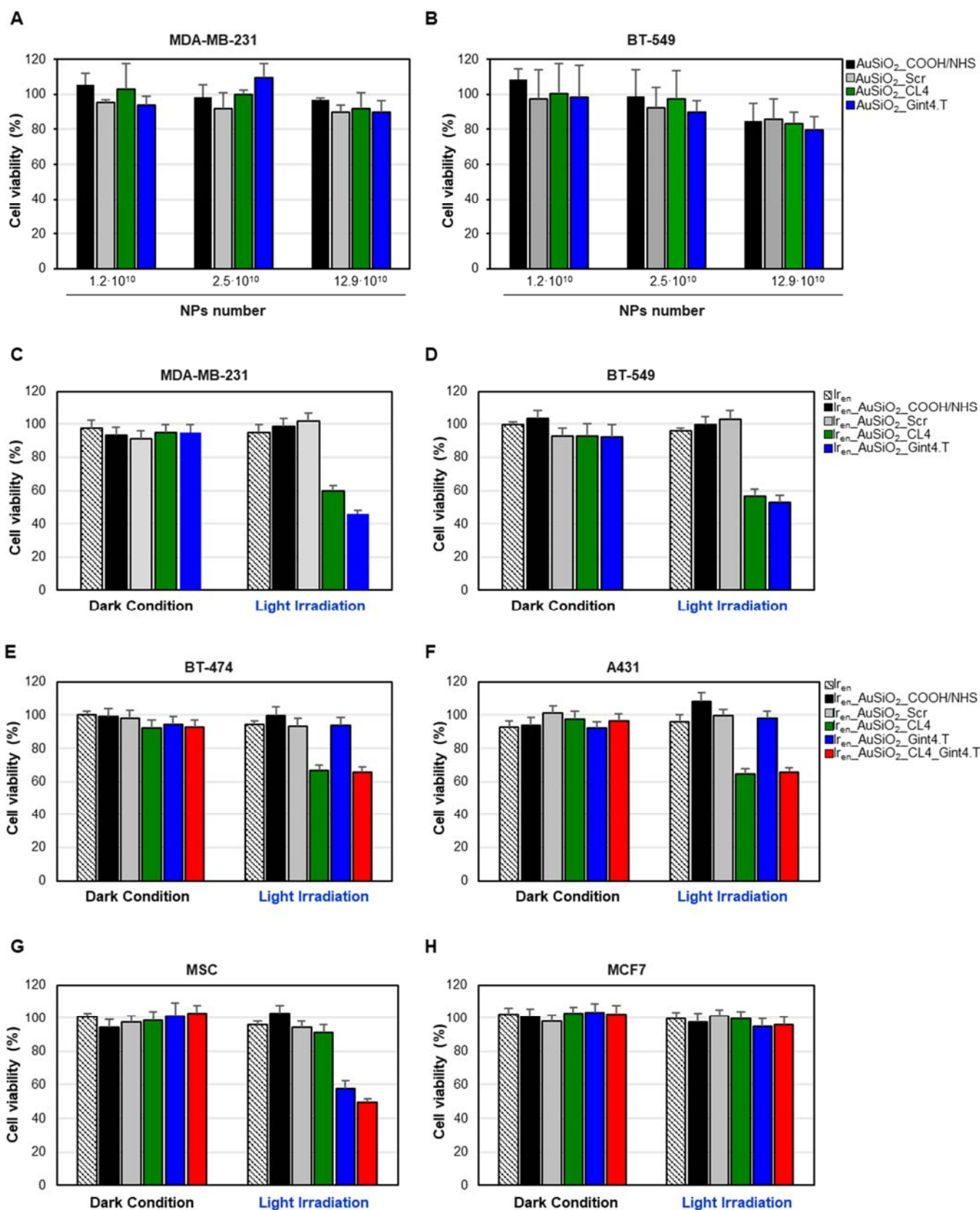


Supplementary Figure 7. Selective cell uptake of CL4 and/or Gint4.T-decorated Ir_{en}-AuSiO₂_Aptamer nanoplatforms in 2D BT-549 or BT-474 cultures. **A** Representative confocal images of BT-549 (EGFR⁺/PDGFR β ⁺) cells incubated for 60 min at 37°C with Ir_{en}-AuSiO₂_COOH/NHS or Ir_{en}-AuSiO₂_Aptamer nanoparticles. After washing and fixation, cells were labeled with WGA-488 (green) to visualize the cell membrane. **B** Representative confocal images of BT-474 (EGFR⁺/PDGFR β ⁻) cells incubated for 60 min at 37°C with the indicated nanoparticles. After washing and fixation, cells were labeled with NucRed (blue) to stain nuclei. Mean fluorescence intensity (MFI) was evaluated by Zeiss software on 10 separate images for each condition. Bars depict means $\pm$ SD (n = 3). * p < 0.05 relative to Ir_{en}-AuSiO₂_Scr. **A,B** Nanoparticles are displayed in red. All digital images were captured at the same

setting to allow direct comparison of staining patterns. Magnification 63 $\times$, 1.0 $\times$ digital zoom, scale bar = 10 μ m.

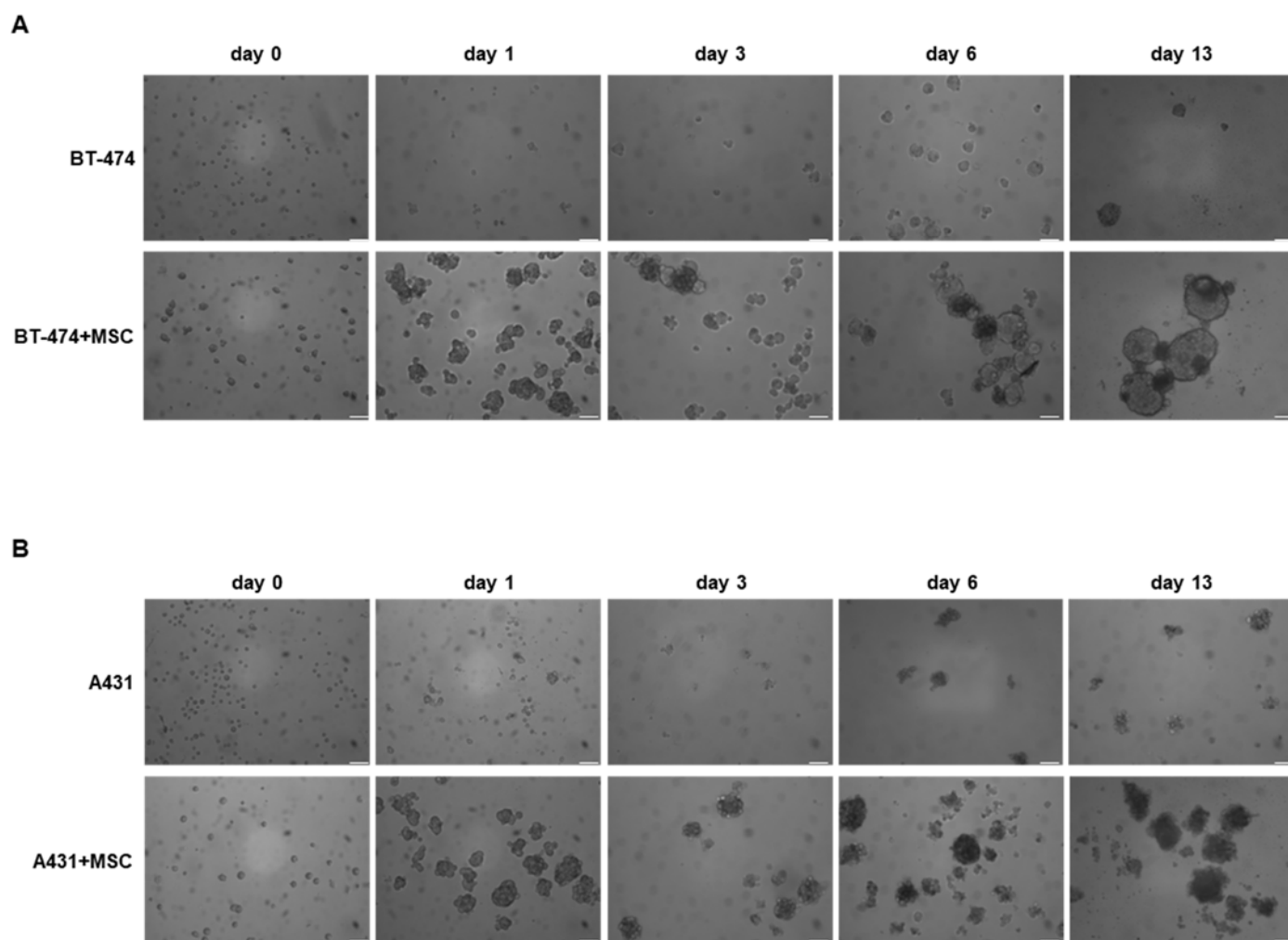


Supplementary Figure 8. Formation of 3D spheroids of BT-549 cells and MSC. **A** Representative phase-contrast microscopy images showing the formation of homotypic (MSC; BT-549) and heterotypic (BT-549+MSC) spheroids over seven days. Magnification: 10 $\times$, scale bar = 100 μ m. **B** Representative confocal images of the heterotypic spheroids grown at day 13 and then stained with α -SMA and FAP stromal markers. BT-549-GFP cells and stromal markers are displayed in green and magenta, respectively. Magnification: 10 $\times$, 1.0 $\times$ digital zoom, scale bar = 100 μ m.



Supplementary Figure 9. Photodynamic effect of nanoplatfroms in 2D cell cultures. **A,B**, AuSiO₂_COOH/NHS, AuSiO₂_Scr, AuSiO₂_CL4 or AuSiO₂_Gint4.T, without the photosensitizing

and luminescent molecule Ir_{en}, were incubated onto MDA-MB-231 or BT-549 cells for 24 h and cell viability was assessed to exclude basal toxicity. **C,D** MDA-MB-231 and BT-549 (EGFR⁺/PDGFRβ⁺) cells, **E,F** BT-474 and A431 (EGFR⁺/PDGFRβ⁻) cells, **G** MSCs (EGFR⁻/PDGFRβ⁺), and **H** MCF7 (EGFR⁻/PDGFRβ⁻) cells, were treated with free Ir_{en}, Ir_{en}-AuSiO₂_Aptamer or unconjugated Ir_{en}-AuSiO₂_COOH/NHS nanoparticles for 60 min at 37°C, washed and kept in the dark or exposed to 254 nm light irradiation for 1 h. After 24 h, cell viability was assessed. Treatment with specific aptamer-decorated nanoplateforms, but not with free Ir_{en}, Ir_{en}-AuSiO₂_COOH/NHS or Ir_{en}-AuSiO₂_Scr, inhibits cell viability, expressed as percentage of viable treated cells with respect to untreated cells. **A-H** Bars depict mean ± SD (n = 3). No statistically significant variations among Ir_{en}-AuSiO₂_COOH/NHS or Ir_{en}-AuSiO₂_Scr and untreated were obtained.



Supplementary Figure 10. Formation of 3D spheroids of EGFR⁺/PDGFR β ⁻ cancer cells and MSC.

A,B Representative phase-contrast microscopy images showing the formation of homotypic spheroids of BT-474 or A431 cells, and heterotypic spheroids of BT-474 or A431+MSCs over thirteen days. Magnification: 10 $\times$, scale bar = 100 μ m.

Supplementary Table 2. Clinicopathological features of three selected tumor samples.

Tumor sample	Age	Histological diagnosis	TNM stage	ER	PgR	HER2	Ki67
M23	63	Ductal carcinoma in situ (DCIS)	pTis pN0	90%	80%	NA	10%
M41	47	Invasive carcinoma no special type (NST)	pT2G 2N2a	90%	90%	Score 1+	28%
M43	71	Invasive carcinoma no special type (NST)	pT2G 3Nx	90%	80%	Score 1+	40%