## Supporting Information

## Methods

**Preparation of oligonucleotide-conjugated GNPs**. Rod-shaped GNPs with aspect ratio of 3.5 (Fig. 2 b) are synthesized using a previously reported seed-mediated growth method (33, 34). As shown in Fig. 2 a and e, these GNPs are selected because of the maximum light absorbance at a wavelength in the NIR range of interest (785 nm). Phosphorothioate oligonucleotides sequences directed against the 5' region of the ERBB2 mRNA molecule are purchased from Integrated DNA Technologies (Coralville, IA). The sense sequence is 3'-GTGAGCACCATGGAG-5'-SH and the antisense sequence is 3' -CTCCATGGTGCTCAC-5'. Scrambled sense sequence 5' AAGAUCCAGUGGUAAUCUAC-3'-SH and antisense sequence 5' GUAGAUUACCACUGGAGUCUU-3' are used as control sequences. Unless otherwise specified, all sequences are purchased from Integrated DNA Technologies and all sequences are not labeled with dye. All thiolated oligonucleotides are reduced with dithiothreitol (Fisher Scientific) and are purified using NAP5 purification columns (GE Healthcare). To conjugate sense oligonucleotides with GNPs, 100 μl of 100 μM thiolated, sense oligonucleotides is incubated with 2000 μl of GNPs ($1x10^9$ particles/ml) and 40 μl of phosphate-buffered saline (PBS) on a rocker for 24 hours. The sense oligonucleotides attach to the GNPs through the thiol (-SH) group on the 5' end. To hybridize antisense oligonucleotides to the sense oligonucleotides, 100 μl of 100 μM antisense

oligonucleotides is added to the conjugated GNPs solution. This mixture is then heated for 2 minutes at 80˚C and then heated for 15 minutes at 65˚C. The mixture is finally incubated at room temperature on a rocker for 24 hours to ensure maximum hybridization. For use with cell culture, the conjugated GNPs are concentrated to $1x10^{14}$ particles/μL by centrifugation at 5000 rpm for 30 minutes, removal of the supernatant, and sonication for 1 minute to resuspend the GNPs. The functionalized particles are then mixed with lipid complexes (Metafectene, Biontex) at a 3:1 ratio and allowed to incubate for 20 minutes as suggested by manufacturer's instructions. The complexes are then added to cells and allowed to incubate for 12 hours. After 12 hours, the media is then exchanged back to normal media.

**Surface immobilization of conjugated GNPs**. TAMRA-modified (559 nm excitation/583 nm emission), phosphorothioate antisense oligonucleotides (TAMRA - 3' - CTCCATGGTGCTCAC-5') are used to visually monitor the release of antisense oligonucleotides from the gold GNPs. Conjugated GNPs are electrostatically attached to the glass slide. The nanoparticle solution is firstly dispensed onto the glass slide. Immediately after drying on the slide, the nanoparticles were re-immersed in buffer solutions. A few times of washing steps were also performed to remove free standing nanoparticles. When re-immersed in solution, the conjugated GNPs remain attached to the glass surface.

**Characterizations of laser power, wavelength specificity, and temperature**. A 785 nm laser (model APM50/1557, Power Technologies, Little Rock, AR) is positioned above the sample. The laser spot size is 2 mm. An inverted microscope operating in epi-fluorescence mode is used to visualize ONCOS gene release.

To characterize laser power, the power intensity is measured using an optical power meter (model 1830-C, Newport Corp, Irvine, CA). Immobilized GNPs with TAMRA-labeled antisense oligonucleotides are illuminated at three different power intensities: 4 $mW/mm^2$, 6 $mW/mm^2$, and 9 $mW/mm^2$ for 30 minutes. At 10 minute intervals, the fluorescence intensity of an area away from the conjugated GNPs is captured using a color CCD camera (Qfire, Olympus America, Inc.).

To characterize wavelength specificity, a 658 nm laser (model LM658-65C, Newport Corporation, Irvine, CA), outside the peak optical absorption, is also used in addition to the 785 nm laser. Immobilized GNPs with TAMRA-labeled antisense oligonucleotides are illuminated at the same power density of 9 $mW/mm^2$ for 30 minutes using each laser. At 10 minute intervals, the fluorescence intensity of an area away from the conjugated GNPs is captured using a color CCD camera (Qfire, Olympus America, Inc.). Matlab$^{TM}$ software is used to analyze the fluorescence intensity by integrating the signal over the area of the image. Various areas located at the same radial distance from the immobilized GNPs are measured to obtain statistical information (mean, standard deviation).

To characterize the temperature on the GNPs, three different lengths of oligonucleotides (15 bp, 25 bp, and 50 bp) with known melting temperatures (50ºC,

63ºC, and 70ºC respectively) are purchased: (15 bp sense) 5' – GTG AGC ACC ATG GAG - 3' SH, (15 bp antisense) 5'- CTC CAT GGT GCT CAC - 3' FAM, (25 bp sense) 5' - AAC CTC CTC GAA CCT CCT CGA ACC T - 3' SH, (25 bp antisense) 5' - AGG TTC GAG GAG GTT CGA GGA GGT T - 3' FAM, (50 bp sense) 5' - AAC CTC CTC GAA CCT CCT CGA ACC T AAC CTC CTC GAA CCT CCT CGA ACC T- 3' SH, and (50 bp antisense) 5' - AGG TTC GAG GAG GTT CGA GGA GGT TAGG TTC GAG GAG GTT CGA GGA GGT T- 3' FAM. Here, all the antisense strands are labeled with FAM dye (495 nm excitation/520 nm emission) on the 3' end to visually monitor the release of antisense oligonucleotides from the GNPs. To characterize the temperature, immobilized GNPs with different lengths of oligonucleotides are illuminated with 9 $mW/mm^2$ for 15 minutes. The fluorescence intensity of the conjugated GNPs is captured at 3 frames/sec using a color CCD camera (Qfire, Olympus America, Inc.).

**Cell preparation**. The human breast carcinoma line BT474 was purchased from the American Type Culture Collection (Rockville, MD). Cells are cultured in DMEM media supplemented with 10% heat-inactivated fetal bovine serum and are maintained in a 37˚C incubator with 5% $CO_2$ humidified air. Cells are initially seeded in either 6-well plate at a cell density of 400,000 cells/well (9.6 $cm^2$) or 96-well plates at 20,000 cells/well. The cells are allowed to plate for 24 hours prior to treatment with conjugated GNPs.

**Experimental setup for localized activation of photothermal gene release**. To show highly localized activation of photothermal gene release, 785 nm laser is positioned above a sample using a micro-manipulated xyz stage (MP-285, Sutter Instrument Company). The laser is then focused to a 5 μm diameter spot size using a 4X objective lens located above the sample. An inverted microscope operating in epi-fluorescence mode is used to visualize ONCOS gene release. Conjugated GNPs are illuminated using the focused laser at 9 mW/mm$^2$ for 2 minutes without illuminating other neighboring GNPs. Images were captured using a color CCD camera (Qfire, Olympus America, Inc.) before and after activation.

For highly localized intracellular activation, 10 μL of conjugated GNPs (1x10$^{14}$ particles/μL) are internalized inside BT474 breast carcinoma cells (which are plated on glass coverslips in the 6-well plates) for 8 hours. The antisense strand is labeled with FAM fluorescent dye. After 8 hours, the coverslips containing cells are removed from the 6-well plate and are washed three times with 1X PBS. The coverslip containing cells is then placed on a microscope slide. Cured PDMS is used to form a well around the coverslip on the microscope slide. This well is then filled with media. Conjugated GNPs are illuminated using the focused laser at 9 mW/mm$^2$ for 2 minutes without illuminating other neighboring GNPs within a cell. Images were captured using a highly sensitive monochrome CCD camera (Cascade 512B, Photometrics) before and after activation.

**Experimental setup for conventional transfection of antisense oligonucleotides**. Antisense oligonucleotides that are complementary to ERBB2 mRNA (FAM - 3' - CTCCATGGTGCTCAC-5') are transfected into BT474 breast carcinoma cells using Fugene 6 (Roche Diagnostics) transfection reagent. Briefly, 10 μl of Fugene is mixed with 90 μl of Optimem media (Gibco) and incubated for 5 minutes at room temperature. Then 0.5 μg of antisense oligonucleotides is added to the Fugene/Optimem mixture and allowed to incubate for 20 minutes at room temperature. Finally, this solution is added to one wells of the 6-well plate containing BT474 cells and media. After 24 hours and 48 hours, the samples are prepared for flow cytometry analysis (described below).

**Experimental setup for ERBB2 interference using ONCOS**. 10 μL of conjugated GNPs ($1x10^{14}$ particles/μL) to each well of the 96-well plate. The BT474 breast carcinoma cells are incubated for 24 hours at 37°C with the conjugated GNPs. After 24 hours, A 785 nm laser is positioned above a sample (spot size of the laser is 2 mm in diameter). The laser is used to illuminate the cells at 9 $mW/mm^2$ for 2 minutes. The cells are then incubated at 37°C for 48 hours.

**Indirect immuno-fluorescence staining of ERBB2**. Cells are washed three times with 1X PBS, fixed using 2% paraformaldehyde (15712-S, Electron Microscope Sciences, Hatfield, PA) for 10 minutes, and blocked with bovine serum albumin (15260-037, Invitrogen Corporation) for 30 minutes. The cells are then stained for

ERBB2 using mouse anti-ERBB2 (OP15T, Calbiochem) primary antibody (1:100 dilution) for 90 minutes and anti-mouse IgG (F5262-1ML, Sigma-Aldrich) secondary antibody conjugated with (1:128 dilution) FITC dye (488 nm excitation/518 nm emission) for 30 minutes.

**Viability analysis**. Cells are seeded in 6-well plates as explained in the cell preparation method above. After 24 hours, cells are incubated with 10 μl of concentrated GNPs ($1x10^{14}$ particles/μL) in 2 ml of Optimem media per well for 24 hours. After 24 hours, the cells are washed with 1X PBS and placed back in media. The laser is used to illuminate cells at 9 $mW/mm^2$ for 2 minutes. The cells are then incubated at 37°C for 2 days and 5 days.

Two days and five days, the cells are prepared for analysis with flow cytometry. The cells are not fixed when they are analyzed. The cells are detached from the 6 well plates using 0.25% trypsin for 2 minutes, collected using centrifugation (1800 rpm, 4 minutes), and re-suspended in 1X PBS which contains 2 μM Calcein AM. As a control, un-adhered dead cells are collected from the media in the 6 well plates and sonnicated for 10 seconds. These dead cells are then collected using centrifugation (1800 rpm, 4 minutes), and re-suspended in 1X PBS which contains 2 μM Calcein AM (Invitrogen) for 20 minutes. These samples are then immediately transferred to FACS tubes for flow cytometry analysis. Their fluorescence is analyzed by flow cytometry using the Coulter EPICS XL flow

cytometer (Beckman Coulter Inc., Fullerton, CA) at an average flow rate of 500 cells/second. Flow cytometry profiles were analyzed using WinMDI software.

To qualitatively view viability, cells are adhered onto glass-coverslips and prepared for ONCOS activation in the same way as explained the above paragraph. Five days after activation, the cells are washed in 1X PBS and incubated in 1X PBS containing 2 μM Calcein AM and 4 μM Ethidium homodimer (Invitrogen). The cells are imaged using DIC and fluorescence microscopy.

**Flow cytometry analysis of ERBB2**. For flow cytometry analysis, duplicate samples are made in the 96-well plates so that the total cell number per sample is approximately 500,000 cells. After treatment, the cells are washed three times with 1X PBS, detached from the cell culture dish using 1m M EDTA for 10 minutes, collected using centrifugation (1800 rpm, 4 minutes), and re-suspended in PBS containing 2% fetal bovine serum (16000-036, Invitrogen Corporation) and 0.1% sodium azide. The suspended cells are then stained for ERBB2 using 10 μl of mouse anti-ERBB2 conjugated with PE dye (340552, BD Biosciences) for 60 minutes in the dark. Cells in the isotype control are stained with mouse IgG1 conjugated with phycoerythrin (PE) dye (340761, BD Biosciences) to ensure no non-specific antibody binding takes place. The suspended cells are finally fixed in 2% paraformaldehyde for 10 minutes and transferred to FACS tubes for flow cytometry analysis. Their fluorescence is analyzed by flow cytometry using the Coulter EPICS XL flow cytometer (Beckman Coulter Inc., Fullerton, CA) at an

average flow rate of 500 cells/second. Flow cytometry profiles were analyzed using WinMDI software.

**Supplementary Figures**

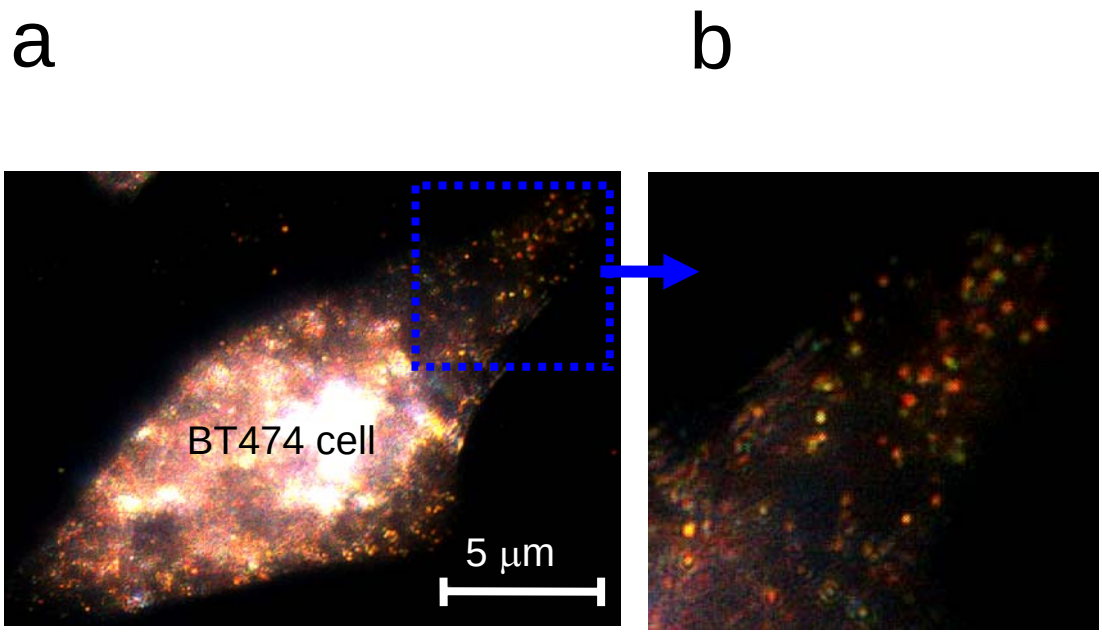
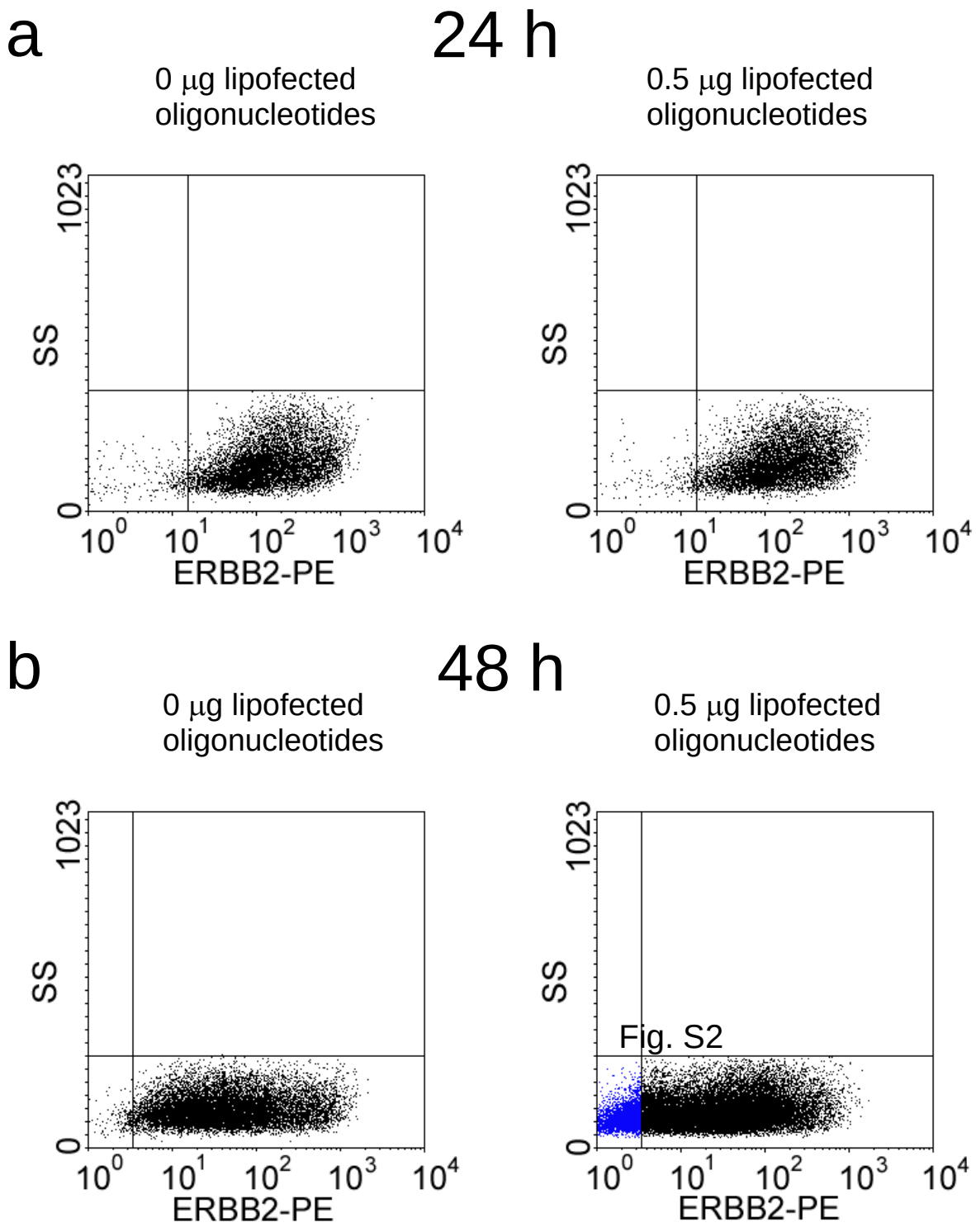



Fig. S1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ERBB2-PE
Fig. S2

Fig. S2

**Figure Captions**

**Fig. S1. Optically-active GNPs in living cells (a)** Darkfield scattering image of nanoparticles internalized within a BT474 breast cancer cell. Nanoparticles are illuminated with unpolarized white light and the light scattered by individual nanoparticles is collected using a darkfield microscope in transmission mode. **(b)** Inset shows a magnified image of the scatter from individual nanoparticles.

**Fig. S2. ERBB2 interference by conventional transfection.** Antisense oligonucleotides are transfected into BT474 breast carcinoma cells. Untreated (control cells which are not incubated with oligonucleotides) and treated cells (cell incubated with oligonucleotides) are analyzed for ERBB2 using flow cytometry **(a)** 24 hours after transfection. No significant change in expression is seen after 24 hours (lower left hand box shows - 0 μg oligonucleotides: 2.3%, 0.5 μg oligonucleotides: 1.8%). **(b)** 48 hours after transfection. A 9.0% of cells show ERBB2 levels below threshold after 48 hours (lower left hand box shows - 0 μg oligonucleotides: 2.1%, 0.5 μg oligonucleotides: 11.1%).