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Electronic Supplementary Information

Target Induced Reconstruction of DNAzymatic Amplifier Nanomachine in Living Cells for Concurrent Imaging and Gene Silencing

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Experimental Section:

Chemicals and materials. MCF-7 cells (human breast cancer cell line) and MCF-10A cells (normal human mammary epithelial cell line) were obtained from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China). Mammary Epithelia Cell Medium (MEpiCM) was purchased from ScienCell (California, USA). Mcf-7 cell culture media was obtained from Thermo Scientific HyClone (MA, USA). Lipofectmine 3000 and Opti-MEM were obtained from Invitrogen (MA, USA). LysoTracker Green DND-26 was brought from Molecular Probes™ (MA, USA). Oligonucleotides were synthesized and purified through HPLC by Sangon Biotechnology Co., Ltd. (Shanghai, China), except for the hairpins of H1 and H2 unlabeled was purified through ULTRAPAGE. All hairpin probes were heated at 95ºC for 5 min and snap-cooled at 4ºC for at least 20 min before use. All reagents were used without further purification. All solutions were prepared using ultrapure water (≥18.2 MΩ). Sequences of the synthesized oligonucleotides are given in Supporting Information Table S1.

Instruments. The fluorescence measurements of Cy5 were carried out at room temperature in a quartz cuvette on a FS5 spectrometer (FS5, England) and The fluorescence measurements of FAM were carried out using FL-7000 spectrometer (Hitachi, Japan). For the Real-time fluorescence intensity study, Tecan Finite M1000 (Tecan, Switzerland) was used. Agarose gel was visualized via a Tanon 4200SF gel

imaging system (Tanon Science & Technology Co., Ltd., China). All fluorescence images were acquired using an oil dipping objective (60×) on Nikon TI-E+A1 SI confocal laser scanning microscope (Japan).

Gel electrophoresis analysis. All the samples were performed in 20 aliquot of 1×PBS buffer (137 mM NaCl, 2.7 mM KCl, 2.03 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) incubating at 37°C for 2 h. Gel electrophoresis analysis was carried out on 3% (w/w) agarose gels stained by 0.5 μg/mL GoldView and 0.5 μg/mL ethidium bromide running in 0.5× TBE buffer at a constant potential of 101 V for 2 h after loading 10 μL of each sample into the lanes. After electrophoresis, the gel was visualized using Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China).

Fluorescence Measurements. For in vitro fluorescence experiments, the reaction mixture containing 200 nM H1, 200 nM H2, 20 nM target mRNA, 200 nM substrate mRNA and 20 mM Mg^{2+} were incubated in PBS buffer for 2 h at 37ºC, and added 50 μL water when used. Fluorescence spectra of Cy5 were recorded using a Fluorescence spectrometer (FS5 Spectrofluorometer) with excitation at 620 nm. The slit was set to be 5 nm for both the excitation and the emission. FAM fluorescence detection was carried out on an FL-7000 spectrometer. The fluorescence emission spectra was collected from 500 nm to 600 nm at room temperature with a 480 nm excitation wavelength. Target mRNA was added after the mixture containing H1, H2 and substrate mRNA was transfered into 386 wells and real-time monitoring of the fluorescence signal was measured with 625 nm and 480 nm excitation wavelength.

To evaluate its selectivity, the response of DNA amplifier nanomachine to single-mismatched target mRNA, double- mismatched target mRNA, and three-mismatched target mRNA was also detected.

Cell culture and confocal microscopy imaging. MCF-10A cells were cultured using Mammary Epithelia Cell Medium (MEpiCM). MCF-7 cells were incubated in DMEM medium supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin, and 100 U mL^{-1} streptomycin. All cell lines were maintained at 37ºC in a 100% humidified atmosphere containing 5% CO_2. Fluorescence imaging of cells was performed as follows: Cells were plated in 35 mm glass bottomed culture dishes and grown for 48h

to reach ~80% confluency. And the dishes were washed two times with 1× PBS before transfection. Transfection assays were then performed according to the manufacturer's protocol. Briefly, 200 μL of Opti-MEM with 3 μL lipofectamine 3000 and an aliquot of 200 μL Opti-MEM containing 600 nM H1, 600 nM H2 were mixed together, incubating at room temperature for 5 min. Cells were then incubated with 400 μL mixture 37ºC for 2 h. Subsequently, the cells were washed three times with 1× PBS and replaced with normal medium for imaging.

The degradation of DNA in MCF-7 cell. MCF-7 cells were plated in 35 mm glass bottomed culture dishes and grown to reach ~80% confluency. After washing twice with 1× PBS, the cells were incubated with 100 nM probe HP2 and 1 μL lipofectamine 3000 and control group were incubated with 100 nM probe HP1 and1 μL lipofectamine 3000 in Opti-MEM at 37 ºC for 4 h. Then, the culture medium was replaced with fresh culture medium for imaging at 4 h, 8 h and 24 h.

Flow cytometry assay. The experiments of transfection was the same as imaging. After transfection cells were washed with 1× PBS for three times and detached by 0.05% trypsin. The suspended cells were centrifuged and dispersed in PBS and analyzted using a CytoFLEX™ flow cytometer.

qRT-PCR quantification of mRNA expression. Total cellular RNA was extracted from MCF-7 cells using the RNeasy Mini Kit (Qiagen, USA) according to its manual. The cDNA samples were prepared with a Revert Aid Premium Reverse Transcriptase kit (Thermo Scientific) according to the indicated protocol. The cDNA samples were diluted 10 times before use. qPCR analysis of cDNA was performed with SybrGreen Fast qPCR Master Mix (ABI, USA) on an ABI Stepone Plus qPCR instrument. Primers used were: EGR-1 forward, 5'-GTTTGCCAGGAGCGATGAA-3'; EGR-1 reverse, 5'- GGGTAGGAAGAGAGAGAGGAGGT -3'.

Western blot analysis. MCF-7 cells (3× 10^5) were seeded in a 30-mm dish and incubated for 24 h. Then cells were incubated with H2, H1 and H2 with Mg^{2+} in culture medium. After 4 h incubation, the culture medium was replaced by another 2 mL fresh culture medium. After 48 h incubation, cells were washed with 1× PBS. All subsequent steps were performed at 4ºC. Cells were lysed for 20 min in 60 μL of lysis

buffer (50 mM HEPES, pH 7.6, 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 1 mM PMSF, 10 mM N-ethylmaleimide, and 2 μg each of aprotinin, bestatin, and leupeptin/mL). The lysates were clarified by microcentrifugation for 20 min. Total cellular proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Protein detection was performed using infrared fluorescent-conjugated secondary antibodies on a ChemiDoc XRS+ with image Lab software (Bio-RAD).

Cell proliferation assays. MCF-7 cells (3×10^3) were seeded in a 96-well plate and incubated for 24 h. After washing twice with 1× PBS, the cells were incubated with different concentrations of H1 and H2 in culture medium supplemented with 5% FBS at 37ºC for 4 h. Then, the culture medium was replaced with 100 μL of fresh culture medium. After 48h incubation, the culture medium was then discarded, and cells were washed with 1× PBS for three times. Finally, MCF-7 cells were cultured in 100 μL of fresh culture medium with 10 μL CCK-8 reagents for the proliferation assay. After 4 h incubation at 37ºC, cell viability was monitored by measuring the absorption at 450 nm using a Synergy 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT).

Table S1. Sequences of synthesized DNA probes

Name	Sequences (5'-3')
Control probe H-C	GTAGAGAAGCGGTGGTCCTTGAGACCGTGTGTAGATCTCA AGGACCACCGCGCCTATGAGGCTAGCT
Single-mismatched mRNA target	GCTTTCACTATCCACATAAGACAC
double-mismatched mRNA target	GCTTTCACTATACACATAAGACAC
three-mismatched mRNA target	GCTTTCACTATACACATAATACAC
EGR-1 mRNA	TCGT(-FAM)CCAGGrArUGGCCGCGG-Dabcyl
Hairpin Probe (HP1)	Cy5-AGATGTGTACCGGCCACCAGGAACTCTCGGTACACAT CTAGAGTTCTTTTTTTTTACGACAGACAGGT
Hairpin Probe (HP2)	Cy5-AGATGTGTACCGGCCACCAGGAACTCTCGGTACACAT C**T**(-BHQ2)AGAGTTCTTTTTTTTTACGACAGACAGGT

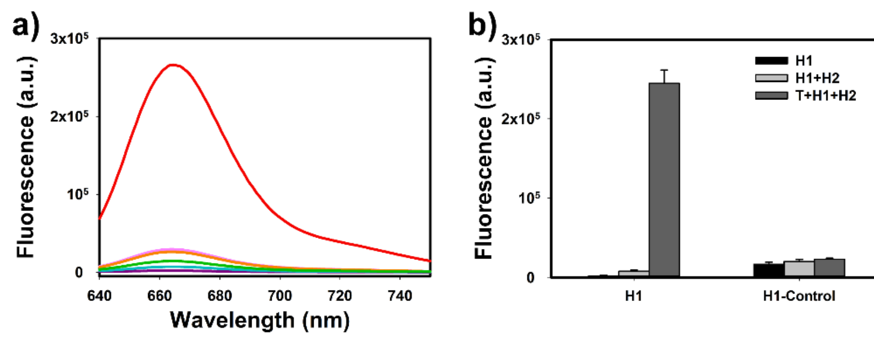

Fig. S1 The fluorescence of DNAzymatic amplifier nanomachine responses to negative control probe H1-Control (H1-C) and probe H1. (a) Red lane: 10 nM target mRNA + 100 nM H1 + 100 nM H2; Pink lane: 10 nM target mRNA + 100 nM H1-C + 100 nM H2; Orange lane: 100 nM H1-C + 100 nM H2; Green lane: 100 nM H1-C; Blue lane: 100 nM H1 + 100 nM H2; Purple lane: 100 nM H1. Histogram (b) corresponding the fluorescence spectral of (a).

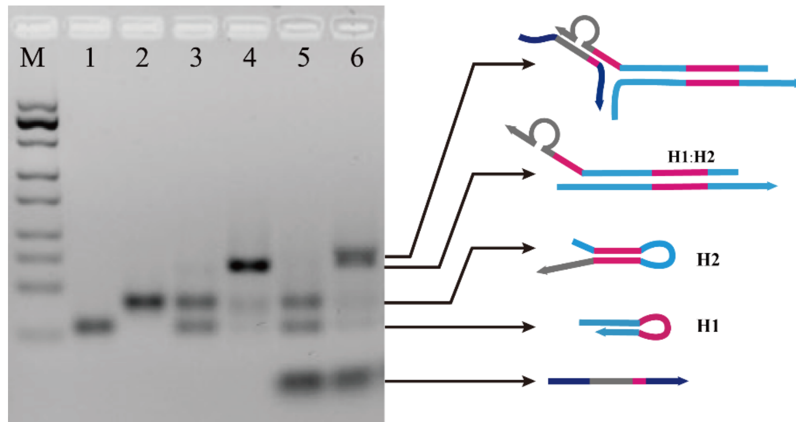

Fig. S2 Agarose gel electrophoresis images. Lane M, DNA marker; lane 1, 500 nM H1; lane 2, 500 nM H2; lane 3, 500 nM H1 and 500 nM H2; lane 4, 50 nM target mRNA, 500 nM H1 and 500 nM H2; lane 5, 500 nM H1, 500 nM H2 and 5μM substrate RNA; lane 6, 50 nM target mRNA, 500 nM H1, 500 nM H2 and 5μM substrate RNA.

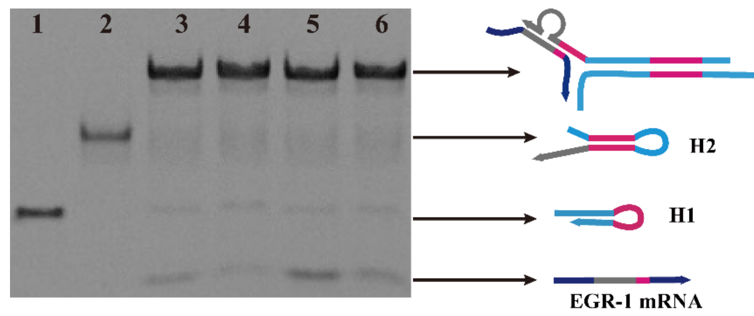

Fig. S3 10% native PAGE gel for the activity assay of reconstructed DNAzyme. lane 1, 500 nM H1; lane 2, 500 nM H2; lane 3, 50 nM target mRNA, 500 nM H1, 500 nM H2 and 500 nM substrate RNA; lane 4, 50 nM target mRNA, 500 nM H1, 500 nM H2, 500 nM substrate RNA and 20 mM Mg^{2+}; lane 5, 50 nM target mRNA, 500 nM H1, 500 nM H2 and 1μM substrate RNA; lane 6, 50 nM target mRNA, 500 nM H1, 500 nM H2, 1 μM substrate RNA and 20 mM Mg^{2+}.

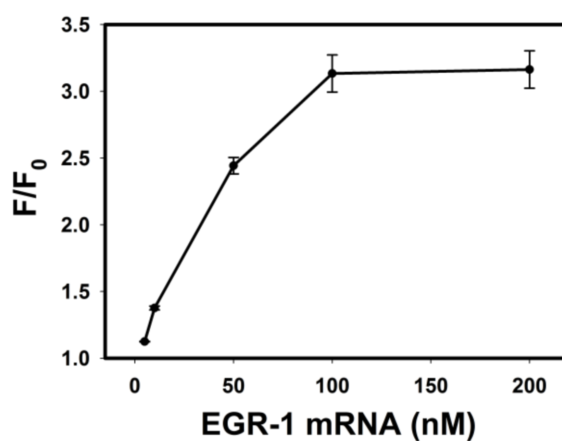

Fig. S4 Fluorescence responses of DNAzyme cleavage to different EGR-1 mRNA concentrations. The fluorescence was obtained by incubating 20 nM target mRNA, 200 nM H1, 200 nM H2, 20 mM $Mg^{2+}$and different concentrations of EGR-1 mRNA (5 nM, 10 nM, 50 nM, 100 nM and 200 nM) in 1× PBS for 2 h. F_0 and F represent the fluorescence signals of nanomachine in the absence and in the presence of Mg^{2+}, respectively. Error bars are standard deviations of three repetitive experiments.

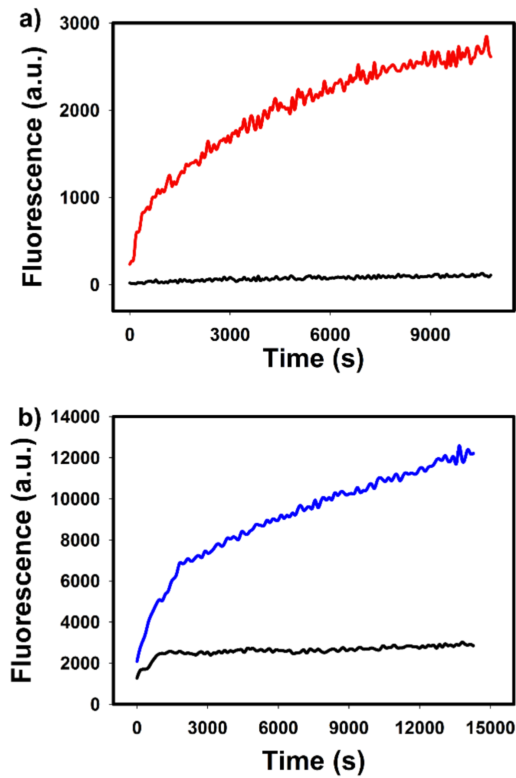

Fig. S5 (a) Time dependent fluorescence measurement of DNAzymatic amplifier nanomachine to target mRNA. (b) Time dependent fluorescence measurement of EGR-1 mRNA cleavage by DNAzyme. Black lane in (a) and (b): 200 nM H1, 200 nM H2, 200 nM substrate mRNA and 20 mM Mg^{2+} were incubated in 1× PBS buffer at 37ºC. Red lane in (a) and Blue lane in (b): 20 nM target mRNA, 200 nM H1, 200 nM H2, 200 nM substrate mRNA and 20 mM Mg^{2+} were incubated in 1× PBS buffer at 37ºC.

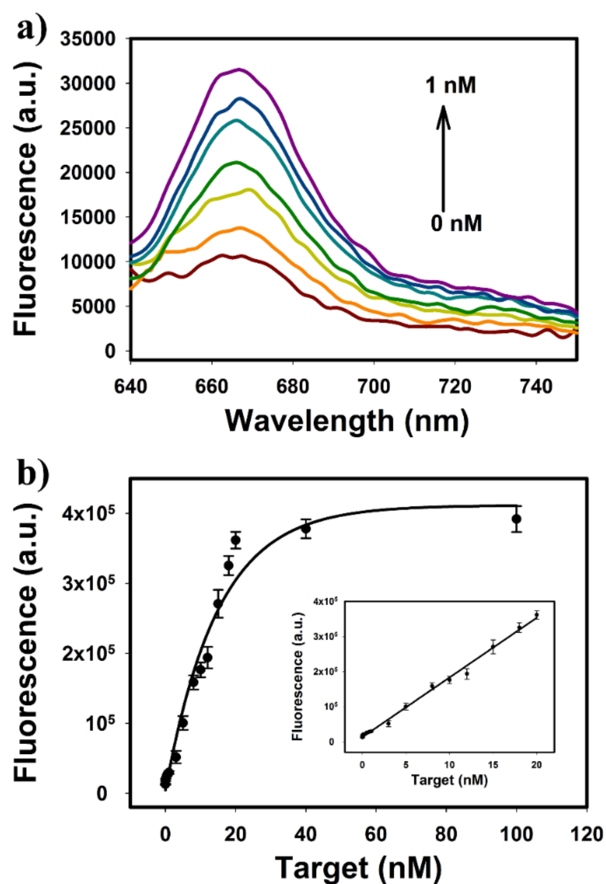

Fig. S6 (a) The fluorescence spectra of DNAzymatic amplifier nanomachine to different concentration of target mRNA (0 pM, 10 pM, 50 pM, 80 pM, 100 pM, 500 pM, 1 nM). (b) Linear relationship of fluorescence intensity to the concentrations of target mRNA. Error bars are standard deviations of three repetitive experiments.

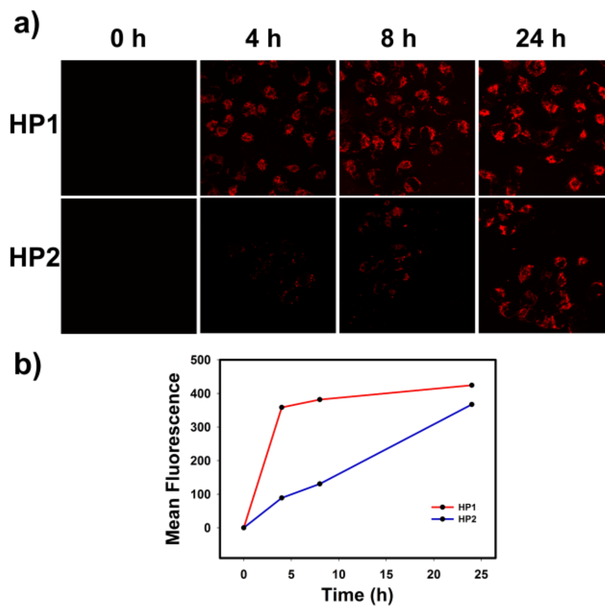

Fig. S7 Degradation of DNA probes in MCF-7 cell. (a) Confocal microscopy imaging of MCF-7 cells incubated with HP1 and HP2 for 0 h, 4 h, 8 h and 24 h. (b) Mean fluorescence of confocal microscopy images from (a).

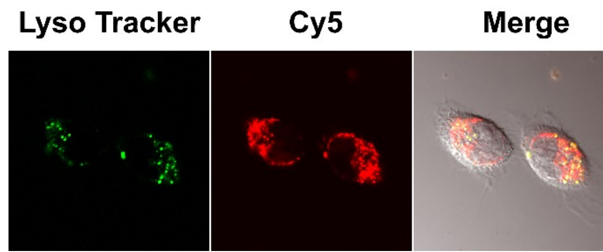

Fig. S8 Confocal microscopy imaging of subcellular localization of probes.

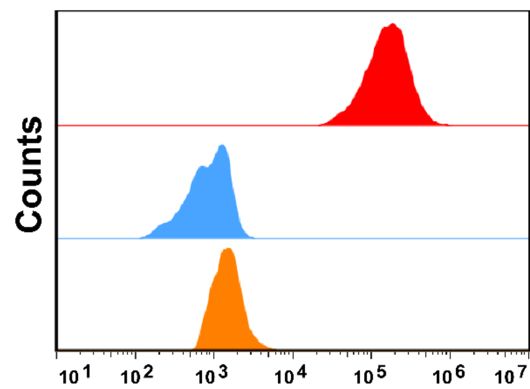

Fig. S9 Flow cytometric assay of MCF-10A and MCF-7. MCF-7 cells were transfected with H1 and H2 (red); MCF-10A cells were transfected with H1 and H2 (blue); MCF-7 cells were transfected with H1-C and H2 (orange).

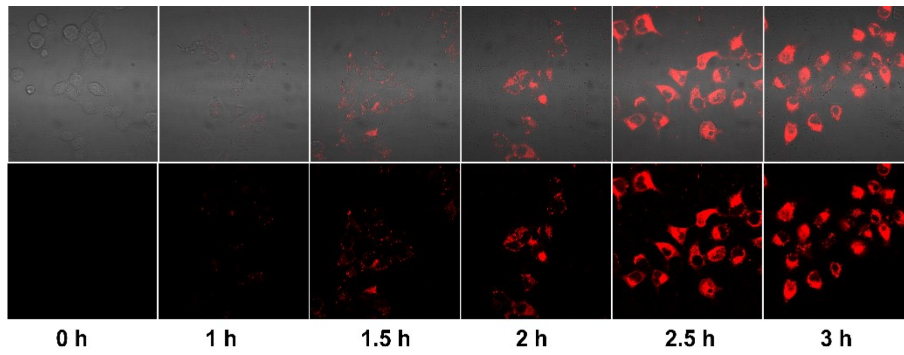

Fig. S10 Confocal microscopy imaging of MCF-7 cells incubated with H1 and H2 for 0 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h.

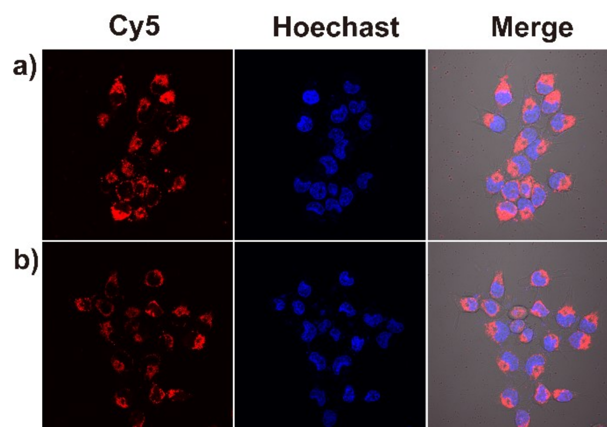

Fig. S11 Confocal microscopy imaging of MCF-7 cells transfected with H1 and H2 (a), H1 (b).

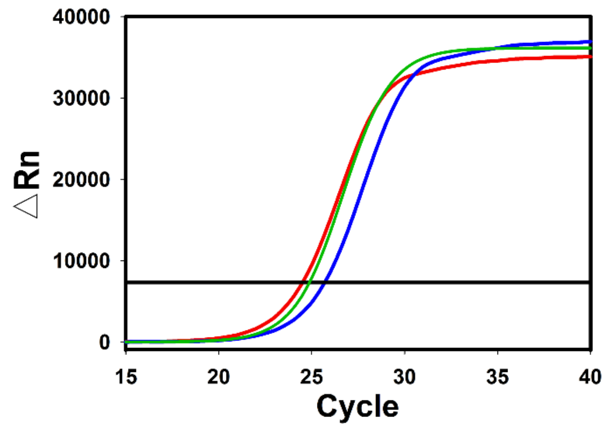

Fig. S12 Reverse transcriptase quantitative PCR analysis of EGR-1 mRNA expression in MCF-7 cell. Red lane, untreated; green lane, treated with probe H2; blue lane, treated with probes H1 and H2.