Supporting Information

for

DNAsome with self-boosting ROS generation via tumour acidosis for enhanced and targeted chemodynamic cancer therapy

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Experimental methods

Chemicals

Tamoxifen was obtained from Sigma Aldrich. Hoechst, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), acridine orange (AO), propidium iodide (PI), Annexin V-FITC, Fetal bovine serum (FBS), Tetramethylrhodamine Methyl Ester Perchlorate (TMRM), penicillin-streptomycin and 0.25% trypsin-EDTA were purchased from Thermo Fischer Scientific. All the these chemicals were used without further purification. Deionized (DI) water was used for all the studies.

Instruments

AFM imaging was done on Multimode SPM (Veeco Nanoscope V). The probe used for imaging was an antimony-doped silicon cantilever with a resonance frequency of 300 kHz and a spring constant of 40 Nm^{-1}. TEM analyses were carried out on an FEI Tecnai 30 G2 (120 kV). Absorption spectra were recorded on a Peltier-attached Shimadzu UV-3600 Vis-NIR spectrophotometer in a quartz cuvette of 10 mm path length. DLS analyses were done on a Malvern Zetasizer Nano Zs equipped with a 655 nm laser. Experiments were performed at 25°C at a backscattering angle of 173°. Flow cytometry analysis was carried out using the FACS LSR Fortessa Flow Cytometer (BD, USA). Confocal images have been carried out using Nikon Eclipse Ti with a 100 X oil immersion objective. MTT assay has been carried on Tecan Infinite 200 PRO microplate reader.

Sample preparation for microscopic analyses

For the TEM analyses, an annealed solution of **DNA1some/TAM** (5/10 μM) was drop cast onto a 400-mesh carbon-coated copper grid (Ted Pella, Inc.) and kept for 2 minutes, and the excess sample was wiped off using tissue paper. The process was repeated 2-3 times, and samples were kept for drying. The samples were further kept in a high vacuum before taking the analyses. The samples were then analysed using TEM. For the AFM analyses, **DNA1some/TAM** (5/10 μM) was drop cast onto a freshly cleaved mica surface and allowed to dry in air, and the images were taken using AFM.

Synthesis of DNA1some/TAM

Self-assembly of **DNA1** was achieved by annealing **DNA1** (5 μM) in water (pH 7) at 90 °C for 10 min followed by slow cooling (1 h) to room temperature. Further, Tamoxifen (**TAM**) (10 μM in MeOH) was added dropwise at 55 °C during the annealing process and allowed to cool to room

temperature. Further performed centrifugal cut-off filtration using a molecular weight cut-off filter to remove unbound **TAM**. The solution obtained where used for further studies.

Measurement of peroxidase-like activity of DNA1some/TAM

The peroxidase-like activity of **DNA1some/TAM** was studied using 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate. The **DNA1some/TAM** (**DNA1**:10 μM, **TAM**: 20 μM), TMB (500 μM) using H_2O_2 (200 μM) and GSH (20 μM) at pH 5.0. in NaAc buffer in the presence of GSH was studied. The absorbance of oxidized TMB at 650 nm was monitored to assess the catalytic performance of the CDT agents.

Cellular uptake and subcellular localization studies

MDA-MB-231 cells were seeded in μ-Slide 8 well slides at a seeding density of 1000 cells per well and cultured with DMEM containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 for 24 h. After confluency has reached up to 70 %, cell culture media was replaced with replacing the medium containing **DNA1some/TAM/DNA3** (**DNA1**:20 μM, **TAM**:20 μM **DNA3**:1 μM) and the cells were further incubated for 12 h. After 12 h of incubation, the medium was replaced with fresh DMEM containing 10 μL Hoechst 33342 (0.35 mg/mL), 10 μL Lysotracker deep red (0.35 mg/mL), 10 μL Mitotracker deep red (0.35 mg/mL). After 15 minutes of incubation, the medium was removed and the cells were washed three times with PBS and imaged using CLSM.

Detection of ROS inside the cells

MDA-MB-231 cells were seeded in μ-Slide 8 well slides at a seeding density of 1000 cells per well and cultured with DMEM containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 for 24 h. After confluency had reached up to 70 %, cell culture media was replaced with the medium containing **DNA1some/TAM/DNA2** (**DNA1**:20 μM, **TAM**:20 μM **DNA2**:1 μM) for 12 hr and costained with 2,7-dichlorofluorescein-diacetate (DCFH-DA) as trapping agent. After incubation time was over,washed off three times with PBS and imaged using CLSM.

Evaluation of the mitochondrial membrane potential

TMRM is a fluorescence probe that localizes at mitochondria, and emits red fluorescence from healthy mitochondria. However, the fluorescence intensity get significantly diminished upon the mitochondrial disruption. To investigate the mitochondrial membrane potential, MDA-MB-231 were seeded in μ-Slide 8 well slides at a seeding density of 1000 cells per well and cultured with DMEM containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 for 24 h. After confluency has reached up to 70 %, cell culture media was replaced with the medium containing

DNA1some/TAM/DNA2 (**DNA1**:20 μM, **TAM**:20 μM **DNA2**:1 μM) and the cells were further incubated for 12 h. After the incubation period was over, cells were then stained with TMRM (10 μM) in PBS for 45 min and washed off three times with PBS band imaged using CLSM.

Evaluation of the intracellular pH using BCECF-AM

To measure intracellular pH, confocal laser scanning microscope and flow cytometry analysis were carried out by using BCECF-AM pH fluorescent probe. Briefly, MDA-MB-231 were pre-seeded in 6-well plate for 12 h, and then incubated with **DNA1some**, **DNA1some/TAM/DNA2** (**DNA1**:20 μM, **TAM**:20 μM **DNA2**:1 μM). Next, those cells were stained with BCECF-AM (2.5 μM) for 40 min, at 37 °C in dark. After washing three times with PBS, the intracellular fluorescent emission of BCECF were observed by confocal laser scanning microscope (Ex = 488 nm, Em = 530 nm). Moreover, the intracellular fluorescent intensity was also quantified by flow cytometry analysis (BD FACS). Specifically, MDA-MB-231 cells were incubated with **DNA1**, **DNA2/TAM/DNA1some** (**DNA2:**1μM, **DNA1:**20 μM, **TAM**: 20 μM). Subsequently, those cells were stained with BCECF-AM (2.5 μM) for 40 min. Samples were detected with the flow cytometer (Ex = 488 nm, Em = 530 nm). The data were analyzed using FlowJo V10.

Cytotoxicity assessments

Cell-viability was studied by methyl thiazolyl tetrazolium (MTT) assay. For this, MDA-MB-231 were seeded in 96-well plates at 100 cells per well and cultured for 24 h until it reached confluency. The cells were then treated with **TAM**, **DNA1some**, **DNA2/TAM/DNA1some** (**DNA2:**1μM, **DNA1:**20 μM, **TAM**: 20 μM) for 24 hr. After incubating for 24 h, the medium was replaced with fresh culture medium and 20 μL of 5 mg/mL MTT solution was added. The cells were incubated for another 4 h and 100 μL DMSO was added to solubilize the formazan crystals and the absorbance at 565 nm was measured using a microplate reader to evaluate the cytotoxicity.

Live/Dead cell assay

The cytotoxicity of **TAM**, **DNA1some**, **DNA2/TAM/DNA1some** (**DNA2:**1μM, **DNA1:**20 μM, **TAM**: 20 μM) was analyzed using live/dead cell assay. For this, MDA-MB-231 cells were seeded in μ-Slide 8 well slides at a seeding density of 1000 cells per well and cultured with DMEM containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 for 24 h. After confluency had reached up to 70 %, cell culture media was replaced with the medium containing **TAM**, **DNA1some**, **DNA2/TAM/DNA1some** and the cells were further incubated for 24 h. After the incubation period was over, cells were washed with PBS and calcein AM (1 μM) and propidium

iodide (10 μL from 10 μg/mL) in fresh medium were added and incubated for 30 min. The plates were further washed with 1 X PBS and then imaged using CLSM.

Annexin V-FITC/PI assay

Cell apoptosis was studied using Annexin V-FITC/PI assay. For this, MDA-MB-231 were seeded in μ-Slide 8 well slides at a seeding density of 1000 cells per well and cultured with DMEM containing 10 % fetal bovine serum (FBS) at 37 °C in 5% CO_2 for 24 h. After confluency had reached up to 70 %, cell culture media was replaced with the medium containing **DNA2/TAM/DNA1some** (**DNA2:**1μM, **DNA1:**20 μM, **TAM**: 20 μM) and the cells were further incubated for 24 h. After the incubation period was over, cells were washed with PBS. The Annexin V-FITC (5 μL) in Annexin V-FITC binding buffer (200 μL), and PI solution (10 μL from 10 μg/mL) were added and incubated for 20 min. Washed with 1 X PBS and then imaged using CLSM. For the FACS analysis, MDA-MB-231 cells were seeded at 24 well plates and grown in a 5 % CO_2 incubator at 37 °C in DMEM cell culture media for 24 h. Once the cells has reached 70 % confluency, **DNA2/TAM/DNA1some** was diluted with DMEM and kept for 24 h. Once the incubation time was over, the media was changed with fresh media, and cells were collected by trypsinization and washed three times with 1 X PBS. Cells were then collected in Annexin V-FITC binding buffer and treated with Annexin V-FITC (5 μL) in Annexin V-FITC binding buffer and PI solution (10 μL from 10 μg/mL) and centrifuged the cell suspension at 150 RCF for 3 minutes and supernatant were removed. Cells were then washed with Annexin binding buffer and resuspended in Annexin binding buffer to 300 μL volume. The cell suspension was then analysed using a flow cytometer.

Spheroid culture

MDA-MB-231 cells were cultured to prepare the 3D spheroids, and the method adopted for the growth was the hanging drop method. The cells were grown on a T75 flask, and once it reached more than 90% confluency, cells were trypsinized and made cell stock solution with 5×10^3 cells and mounted over the lid of a petri dish and kept upside down with base filled with 15 mL of PBS to provide humidity for the spheroid. Followed by keeping a CO_2 incubator for 24 h, spheroid formation was confirmed using an optical microscope. After confirming the formation of spheroids, the spheroids were transferred to a 24-well plate mounted with 10 mm coverslip having 3:1 collagen to media, and it was incubated at 37 °C for 1 h. The spheroids were then incubated with **DNA2/TAM/DNA1some** (**DNA2:**1μM, **DNA1:**20 μM, **TAM**: 20 μM) for 24 h at 37 °C.

Once the incubation was over, the spheroids were fixed using 4% PFA for 15 min at 37 °C. Further the spheroids were permeabilized using 0.1% TritonX and incubated for 10 min at room temperature. Subsequently, 0.1 × TritonX + Phalloidin solution was added and incubated at 37 °C for 30 min. Spheroids were washed gently twice with 1 × PBS and mounted using Mowiol on a glass slide and kept overnight for drying and the spheroids were imaged using CLSM.

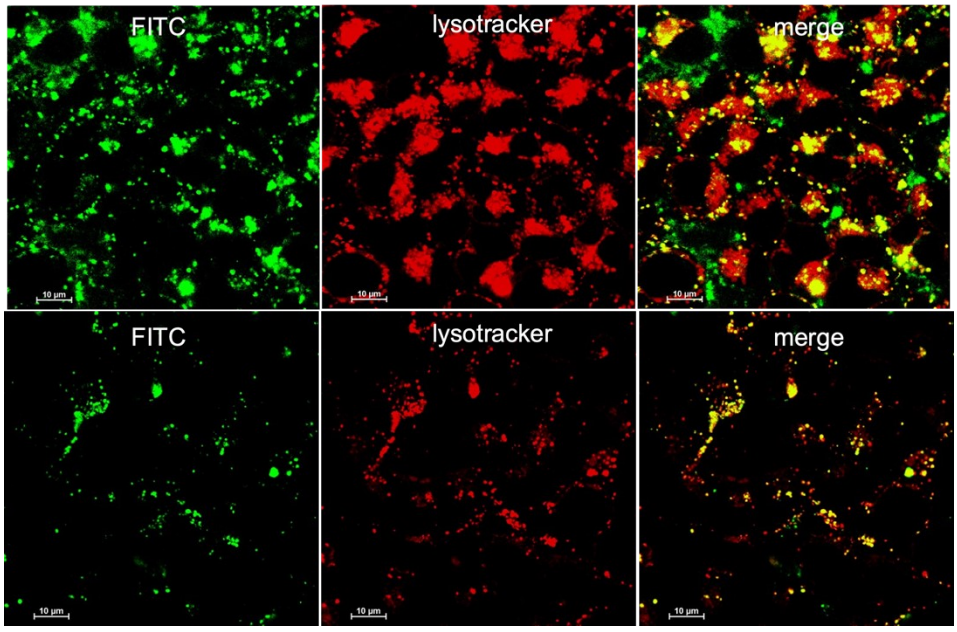

Figure S1. Additional CLSM images for the lysosomal localization of **DNA1some/TAM/DNA3**.

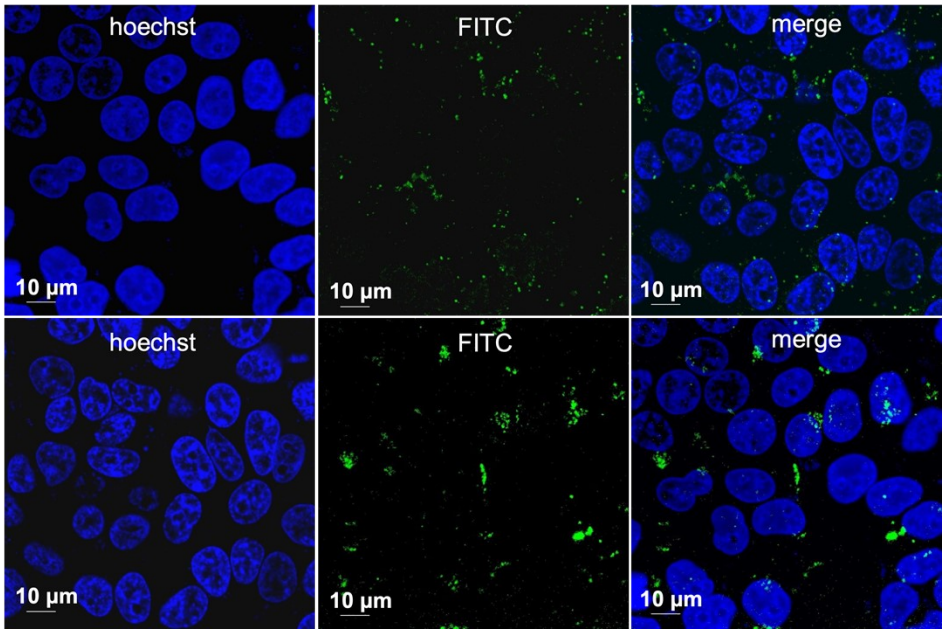

Figure S2. Additional CLSM images for the cellular internalization of **DNA1some/TAM/DNA3**.

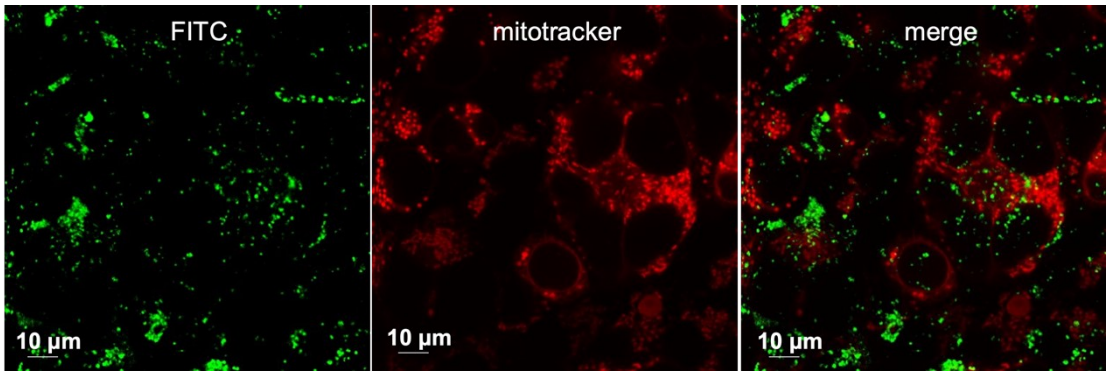

Figure S3. Additional CLSM images for the mitochondrial colocalization of **DNA1some/TAM/DNA3**.

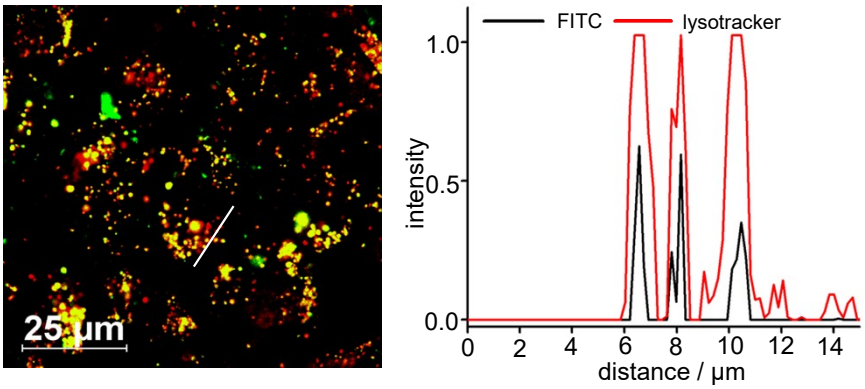

Figure S4. CLSM images of lysosomal colocalization of **DNA1some/TAM/DNA3** (left) and the corresponding line analysis representing the colocalization (right).

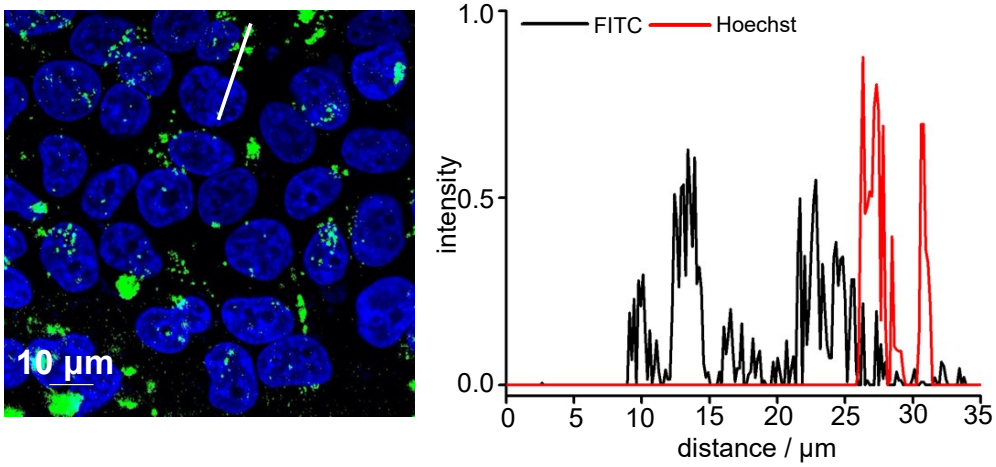

Figure S5. CLSM images of nuclear colocalization of **DNA1some/TAM/DNA3** (left) and the corresponding line analysis representing the colocalization (right).

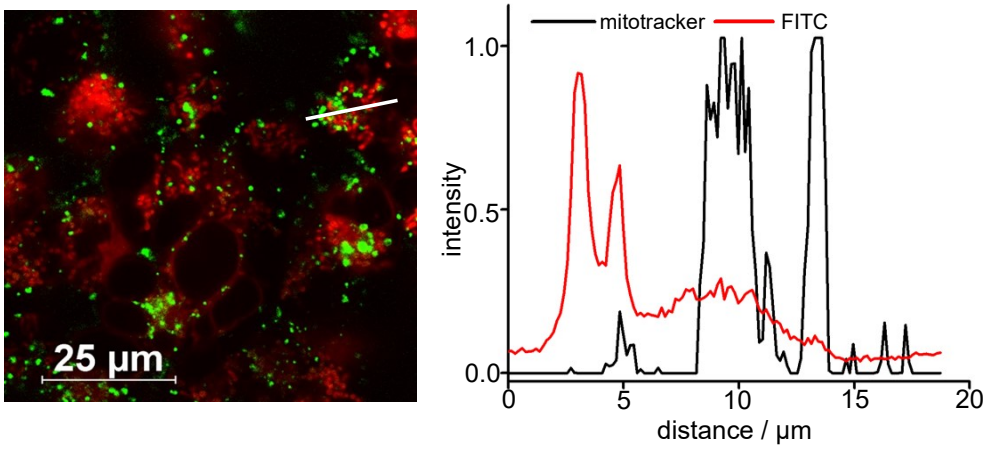

Figure S6. CLSM images of mitochondrial colocalization of **DNA1some/TAM/DNA3** (left) and the corresponding line analysis representing the colocalization (right).

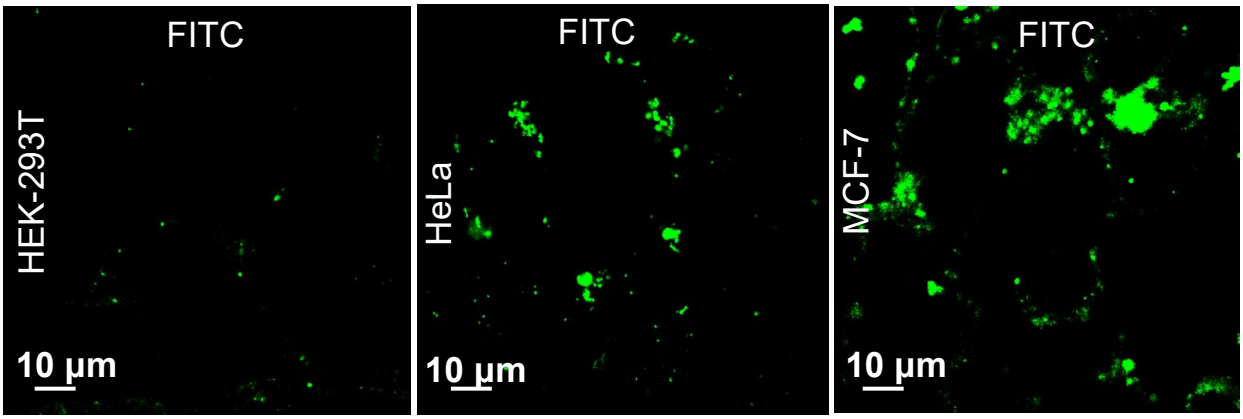

Figure S7. CLSM images of **DNA1some/TAM/DNA3**-treated HEK-293T, HeLa, and MCF-7 cells.

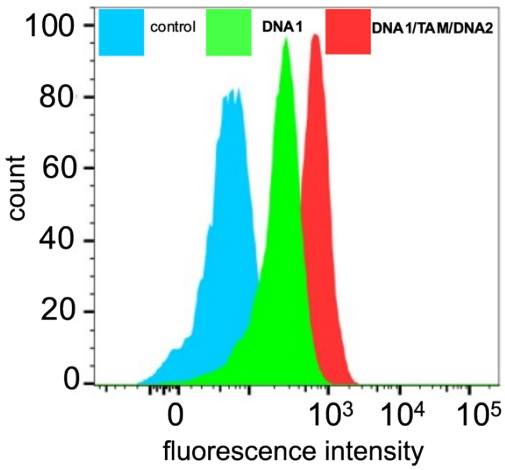

Figure S8. FACS analysis for ROS generation for **DNA1some/TAM/DNA2**-treated MDA-MB-231 cells.

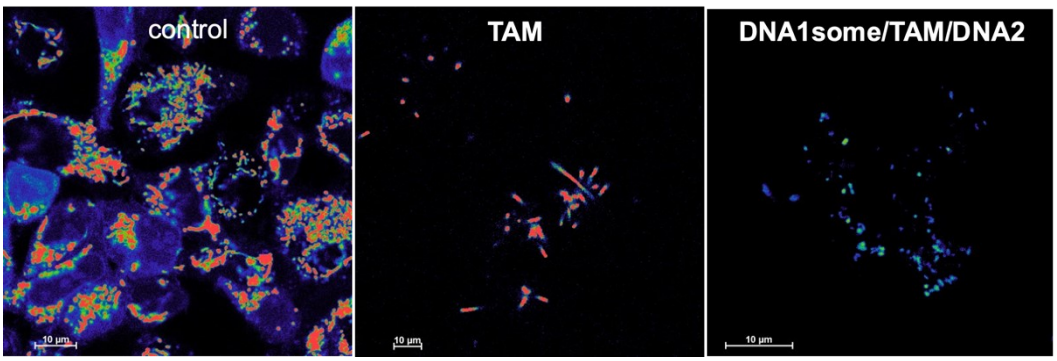

Figure S9. CLSM images for **TAM** and **DNA1some/TAM/DNA2**-treated MDA-MB-23 cells stained with TMRM.

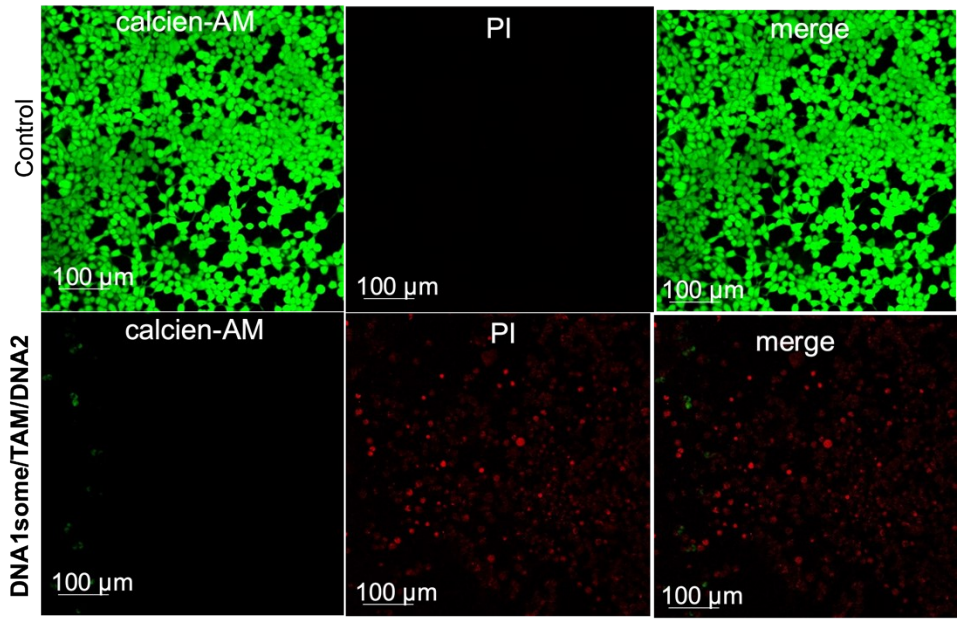

Figure S10. Additional images of live/dead cell assay of **DNA1some/TAM/DNA2**.