Supporting Information

For

DNA supersandwich assemblies as artificial receptors to mediate intracellular delivery of catalase for efficient ROS scavenging

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Experimental Section

S1 Chemicals and Materials

All DNA oligonucleotides were synthesized and HPLC purified by Sangon Biotech. Co. Ltd. (China), and dissolved in phosphate buffered saline (PBS) solution (10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4). The sequences of the oligonucleotides are listed in **Table S1**. 3-(4,5-Dimethylthiazol-2-y1)- 2,5-diphenyltetrazolium bromide (MTS) were purchased from Peroeme (USA). Hoechst 33342 (Hoechst), SYBR Green I, Lysotracker Green DND-26, SYBR® Gold and Lactate dehydrogenase (LDH) were purchased from Invitrogen (USA). Bovine serum albumin (BSA) was purchased from Dingguo Biotechnology Co., Ltd. (China). 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA), Propidium iodide (PI) and Calcein-AM were obtained from Sigma-Aldrich (USA). All solutions were prepared and diluted using ultrapure water (≥ 18.2 MΩ cm) from the Millipore Milli-Q system (Barnstead/Thermolyne NANO-pure, Dubuque, IA).

S2 Apparatus and characterization

All fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrometer. Ultraviolet-visible (UV-vis) absorption spectra were recorded on a Shimadzu UV-2600 spectrometer. Fluorescence polarization was measured in a fluorescence spectrometer (Fluoromax-4, HORIBA Scientific, USA) with an excitation wavelength at 643 nm and an emission wavelength at 667 nm. DNA gel electrophoresis was conducted in 2% agarose gel, and the gel was prepared using a 1 × TAE buffer (40 mM Tris AcOH, 2.0 mM Na_2EDTA, pH 8.5). The SYBR® Gold was used as oligonucleotide dye. The gel was run at 100 V for 60 min in 1 × TAE buffer at room temperature, and then photographed in Gel Imaging (Tanon 2500 R, Tianneng Ltd.). Atomic force microscopy (AFM) was used to characterize the morphography of the DNA supersandwich. Diluted DNA supersandwich sample was pipetted on freshly cleaved mica. After 20 min, the mica surface was washed several times by purified water and gently blown dry by nitrogen gas. The prepared sample was scanned by ScanAsyst-air tips in ScanAsyst Imaging Mode on Multimode 8 AFM with a NanoScope V controller.

S3 Assembly of aptamer- supersandwich as artificial receptor

DNA strands were dissolved in certain amount of phosphate buffered saline solution (PBS, 10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4) as stock solution and quantified by the absorbance at 260 nm with UV-vis spectrophotometer. Two steps involved in the assembly of aptamer-supersandwich assemblies. Stoichiometric qualities of ssDNA1 and ssDNA2 were separately added to the Eppendorf tubes containing PBS buffer, yielding a final concentration of 5 μM. Each

tube containing the ssDNA mixture was heated to 95 ℃ for 5 min and cooled to room temperature for 12 h to form the desired dsDNA building block units of DNA supersandwich (Step 1). Then, target aptamer was added to DNA supersandwich solution for further incubation for 3 h, which resulted in the produce of aptamer-supersandwich assemblies (Step 2). Thermal stability of the resultant dsDNA was analysed using the F7000 instrument by mixing the nanowire solution (200 μL) with SYBR Green I dissolved in dimethyl sulfoxide (DMSO, 6 μL, 20×). The fluorescence data for melting curves were acquired in increments of 5 ℃ during the transition from 25 to 90 ℃ (3 min at each temperature). The fluorescence data were then converted to melting peaks using the software and then plotted as the negative derivative of fluorescence intensity as a function of temperature.

Adherent NIH/3T3 cells with density of 1×10^5 cells/mL were treated with aptamer-supersandwich assemblies (1 μM) in 2 mL of DMEM solution containing 5% FBS for 0.5 h, which led to the immobilization of aptamer-supersandwich assemblies on cell membrane as artificial receptor. As a cell-free experiments, the binding ratio of target-aptamer to supersandwich was optimized by agarose gel electrophoresis. Finally, catalase was added into 1 μM aptamer-supersandwich assemblies solution and incubated for 2 h, which resulted in the formation of aptamer-catalase-supersandwich.

S4 Enzyme activity assay of catalase

The enzyme activity of catalase was determined by measuring the decomposition rate of H_2O_2. A series of concentration catalase solution (0-200 μM) were mixed with 2.9 mL of 10^{-2} M H_2O_2 in a pH 7.4 PBS buffer solution (50 mM) respectively, and the absorbance of H_2O_2 at 240 nm was monitored. The initial enzyme activities were determined by calculating the differential coefficient of reaction equations

S5 Cell culture and cell internalization

NIH/3T3 cells (mouse fibroblasts cells) and HUVEC (Human umbilical vein endothelial cells) were grown in DMEM media supplemented with 10% inactivated fetal bovine serum, 100 $U \cdot mL^{-1}$ 1% penicillin and streptomycin solution. All cells were cultured in a humid CO_2 incubator containing 5% CO_2 at 37 ^oC.

Confocal imaging was used to evaluate the cell recognition of aptamer-supersandwich. The NIH/3T3 cells (positive cell) and HUVEC cells (negative cell) were plated on 35 mm dishes for 24 h, and the NIH/3T3 cells and HUVEC cells were incubated with Cy5-labled aptamer-supersandwich for 30 min. After the medium was removed, the cells were washed twice with PBS buffer, followed by lysotraker green staining, and visualized under a confocal laser scanning microscope. The lysotraker green was irradiated with 488 nm laser and the Cy5 was irradiated with 633 nm laser. The fluorescence imaging was collected with 40× objective.

FITC labelled catalase was used to evaluate the intracellular distribution of catalase. FITC-labelled catalase was prepared by adding of 0.3 mL 1 $mg \cdot mL^{-1}$ FITC DMSO solution in 4 mL pH 8.0 PBS solution containing 4 mg catalase, and allow it incubation for 2 h at room temperature. Remove unreacted FITC by ultrafilter centrifugation with Millipore amicon ultra-tubes (MWCO100kD), and the FITC-Catalase was collected in 50 mM PBS at -20°C for further use. The NIH/3T3 cells were incubated with aptamer-sandwich for different time intervals. After the medium was removed, the cells were washed twice with PBS buffer, followed by lysotraker green staining, and visualized under a confocal laser scanning microscope. The FITC was irradiated with 488 nm laser.

S6 Intracellular ROS staining

Adherent NIH/3T3 cells with density of 1×10^5 cells/mL immobilized with or without artificial receptor were treated with catalase (1 μM) in 2 mL of DMEM solution containing 5% FBS for 12 h. After wash, the cells were incubated with 200 μM H_2O_2 in serum-free DMEM solution for 30 min. After wash, the cells were incubated with 10 μM DCFH-DA in serum-free DMEM solution and then visualized under a confocal laser scanning microscope under excitation of 488 nm laser. The DCFH-DA stained cells could be digested and analysed using FACS (Beckman Coulter). Data were then analysed using FlowJo SoftwareV10. DCFH-DA, a cell-permeable fluorescent probe for H_2O_2, is deesterified intracellularly and turns to highly fluorescent 2'7'-dichlorofluorescein (DCF) upon oxidation.

S7 LDH cytotoxicity assay

Lactate dehydrogenase (LDH) assay was used to assess cell cytotoxicity and membrane integrity. Cells were seeded at 1×10^4 cells per well into 96-well plates for 24 h and then treated with H_2O_2/LPS and catalase loaded supersandwich and then washed for 3 times to remove the substances on membrane. Subsequently, 100 μL of LDH reaction mixture was added to each well and incubated for 4 h. The absorbance value at 450 nm was recorded using a multi-function plate reader Benchmarks Plus (Bio-Rad). Each concentration was tested at least three times.

S8 MTS cell proliferation assay

MTS assay was used was used to assess in vitro cell proliferation and cytotoxicity. Briefly, cells were firstly treated with H_2O_2/LPS and catalase loaded supersandwich in 96-well plates, and then washed for 3 times to remove the substances on membrane. Then, 10 μL of MTS solution (5 mg/mL) diluted in fresh medium (100 μL) was added to each well and incubated for 4 h. The absorbance value at 490 nm was recorded using a multi-function plate reader Benchmarks Plus (Bio-Rad). Each concentration was tested at least three times.

S9 Calcein-AM/PI cell viability assay

Calcein-AM/PI staining, simultaneous fluorescence staining of viable and dead cells, was used to assess the cell viability. Briefly, The NIH/3T3 cells were seeded in 35 mm confocal laser culture dishes at a density of 1 $\times 10^5$ cells per mL for 24 h. cells were firstly treated with H_2O_2/LPS and catalase loaded supersandwich, and then washed for 3 times to remove the substances on membrane. Subsequently, calcein-AM solution (10 μM) and PI solution (10 μM) were added into the cell suspension and incubated for 30 min and washed before imaging. The stained cells were imaged on a confocal laser scanning microscope with 20 × objectives. Calcein-AM fluorescence was collected in the range of 505–525 nm with excitation at 488 nm, and PI fluorescence was collected above 610 nm with excitation at 543 nm.

Table and Figures

Table S1 Oligonucleotide sequences used in this work. Poly$(T)_5$ in ssDNA1 was designed to decrease steric hindrance. The blue sequences in ssDNA1 and orange sequence in ssDNA2 constructed the double chains of the supersandwich structure. The underlined sequence in the anti-TfR aptamer was a terminal binding site, which was complementary to the underlined sequence in ssDNA1, and poly $(T)_{10}$ was designed to decrease steric hindrance.

DNA names	sequences (5′-3′)
ssDNA1	CTTCTGCCCGCCTCCTTCCGACCTAGCAGTGGACATGTGGC AGGGTGAAGTGGCATCGTCGGAGACGAGATAGGCGGACAC $(T)_5$TCGTCCTACACTCCTGGCAGTCTCGTTCTAGTCTCGCGTT G
ssDNA2	ACTGCCAGGAGTGTAGGACGCAACGCGAGACTAGAACGAG
anti-TfR aptamer	ACTGCCAGGAGTGTAGGACG$(T)_{10}$GAATTCCGCGTGTGCACA CGGTCACAGTTAGTATCGCTACGTTCTTTGGTAGTCCGTTCG GGAT
Cy5-labelled ssDNA1	Cy5ACTGCCAGGAGTGTAGGACGCAACGCGAGACTAGAACG AG

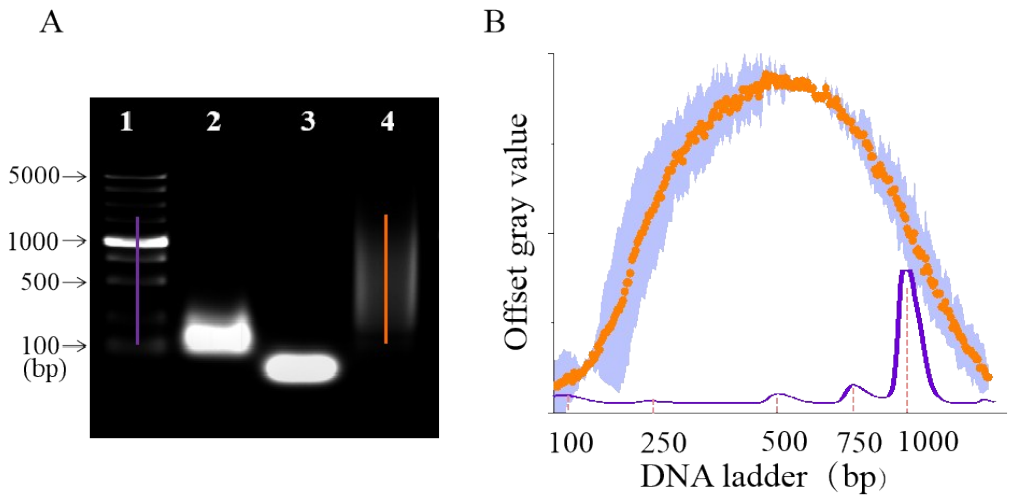

Figure S1 (A) Agarose gel electrophoresis of the DNA supersandwich: lane 1, DNA ladder; lane 2, ssDNA1; lane 3, ssDNA2; lane 4, DNA supersandwich. (B) Gray value analysis of DNA ladder and supersandwich in (A). Compared to DNA ladder, most of the DNA supersandwich were around 500 bp. The orange dots represent the gray value of supersandwich; The purple line represent the the gray value of DNA ladder; And the blue lines represent the standard deviations.

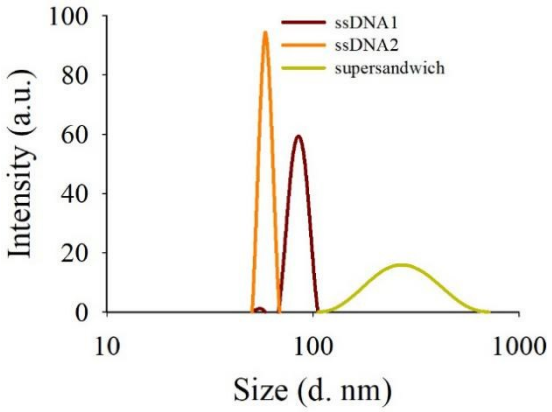

Figure S2 Dynamic light scattering analysis of the formation of DNA supersandwich. The hydrodynamic sizes of ssDNA1, ssDNA2 and DNA supersandwich were determined to be 85.0 ± 2.6 nm, 62.4 ± 1.8 nm and 255.0 ± 133.8 nm, respectively. Compared with ssDNA, the size increase of DNA supersandwich also confirmed the formation of hybridization nanostructure.

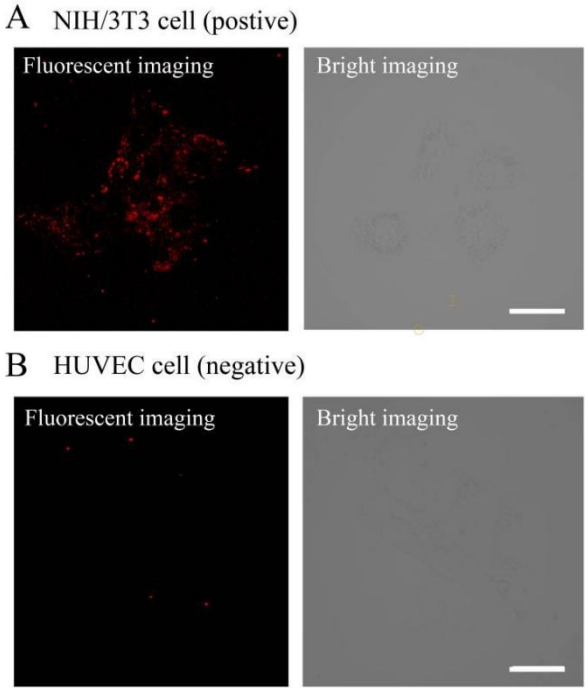

Figure S3 Aptamer-supersandwich assemblies as artificial receptors. (A, B) confocal images comparing Cy5-labelled aptamer-supersandwich selective binding with TfR-expressed cells. (A) NIH/3T3 cells (positive cells) and (B) HUVEC cells (negative cells) were incubated with aptamer-supersandwich assemblies at 37 °C for 30 min. Scale bar, 10 μm.

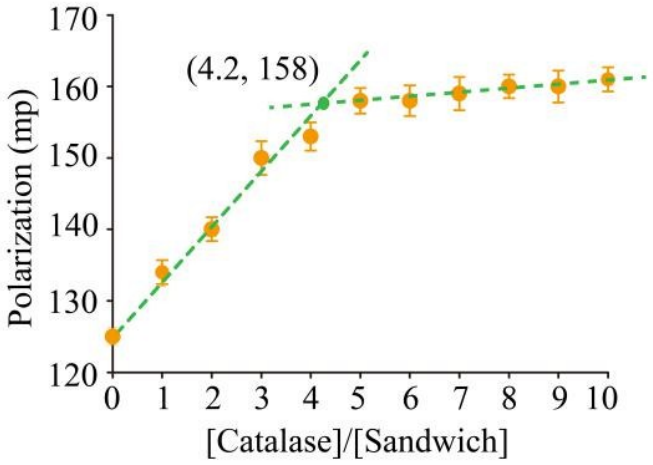

Figure S4 Fluorescence polarisation change upon the aptamer-sandwich (100 nM) challenged with target catalase at different concentrations (0–1.0 μM).

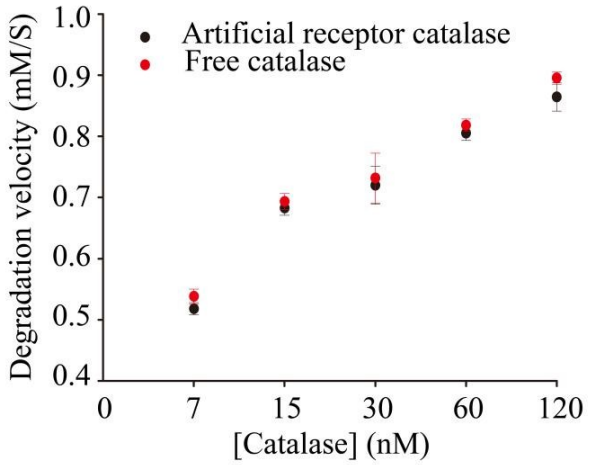

Figure S5 Enzyme activity of catalase before and after binding with the aptamer-supersandwich, as determined by measuring the decomposition rate of H_2O_2 via UV–Vis spectroscopy.

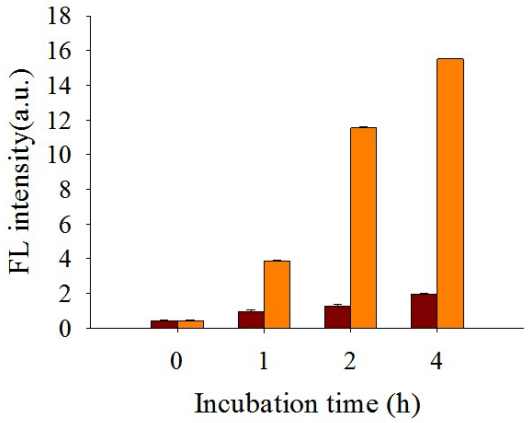

Figure S6 Flow cytometry qualification of the FITC-catalase (1 μM catalase) internalised into the cell through artificial receptor mediated delivery or nonspecific delivery. The mean fluorescent intensity was determined from flow cytometry at different time intervals. Fluorescence from the FITC-labelled catalase was used to qualify the intracellular catalase.

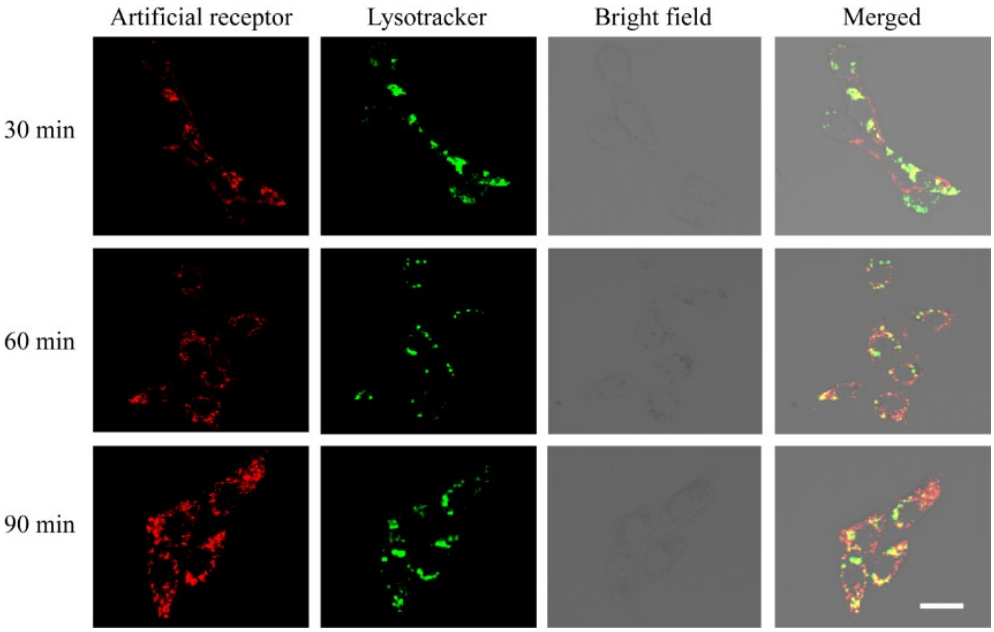

Figure S7 Time-dependent confocal imaging of the cell endocytosis of Cy5-labelled aptamer-supersandwich (1 μM DNA monomer) at 37 °C. The cells were incubated with the aptamer-sandwich for different times, then co-stained with Lysotracker green. The green fluorescence originated from Lysotracker, whereas the red fluorescence originated from the aptamer-supersandwich. Cy5-labelled ssDNA1 was used to construct the aptamer-supersandwich and real-time fluorescent imaging. Scale bar: 20 μm.

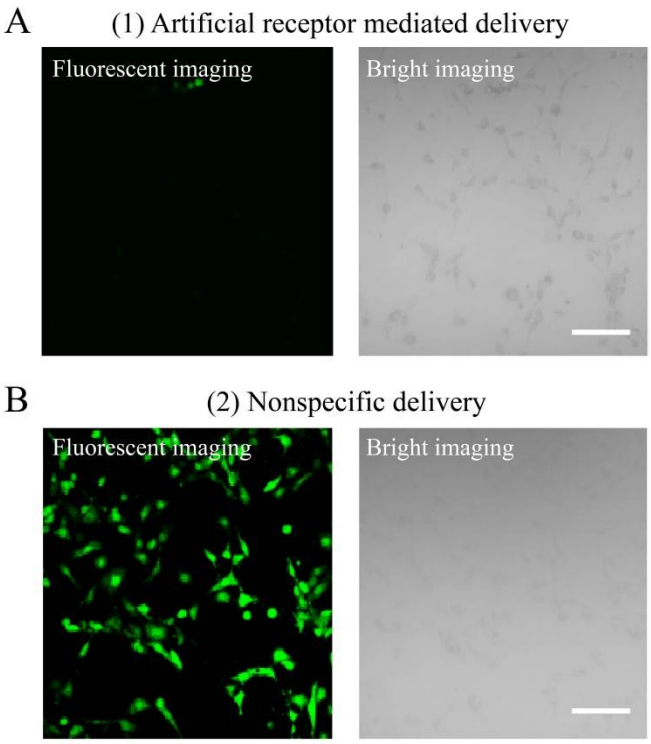

Figure S8 Comparison of the ROS scavenging capability between artificial receptor-mediated catalase delivery and nonspecific catalase delivery. Fluorescence microscopy images of the DCFH-DA stained cell exposed to H_2O_2 (exogenous ROS). NIH/3T3 cells modified with artificial receptor (A) or without artificial receptor as the control (B) were incubated with catalase for 12 h; Then, H_2O_2 (200 μM) was added in each sample for further incubation. Scale bar: 50 μm.

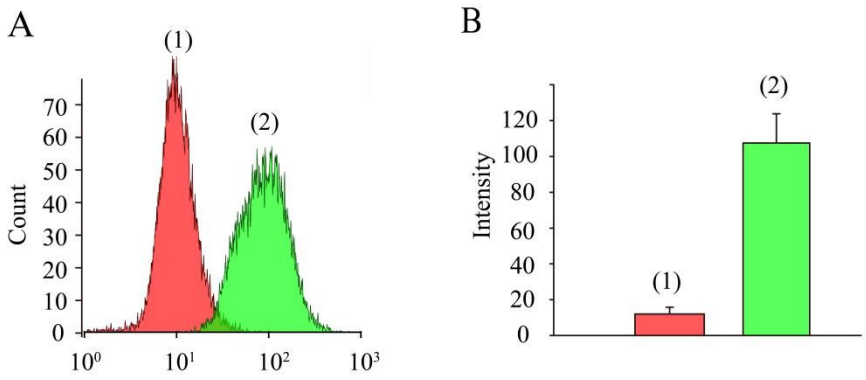

Figure S9 FCS analysis of cell tolerance against a high level of H_2O_2. The fluorescence intensity represents the ROS level inside the cells. (B) The corresponding fluorescence intensity obtained from the histograms of (A).

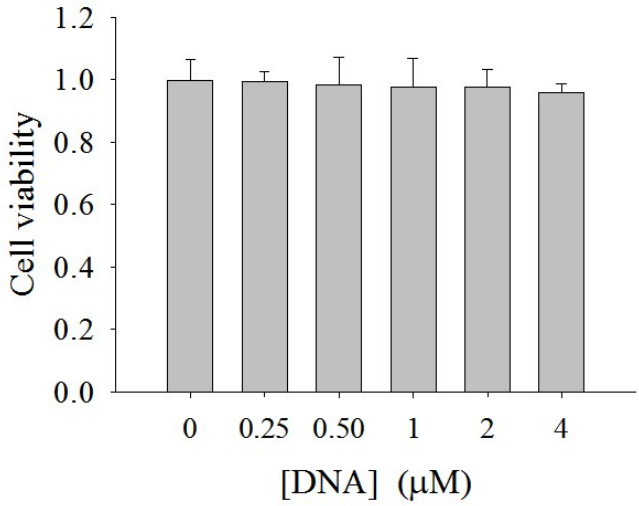

Figure S10 Determination of the biocompatibility of the DNA supersandwich. The NIH/3T3 cells were incubated with different concentrations of the DNA supersandwich at 37 °C for 12 h, and the cell viability was measured using an MTS assay.

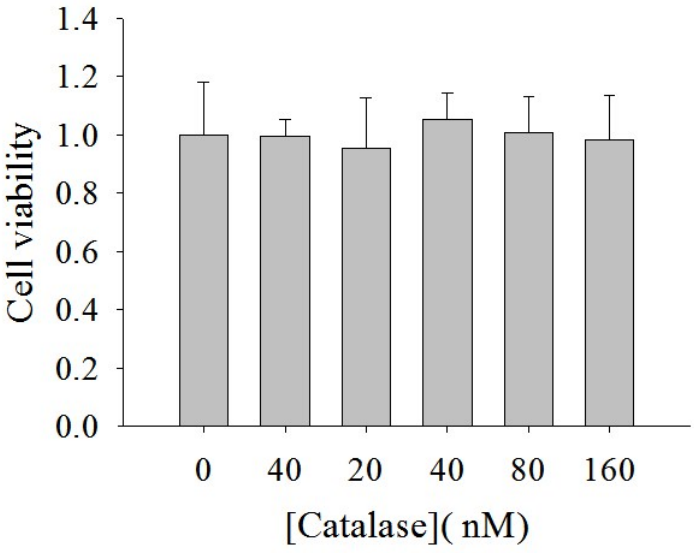

Figure S11 Determination of cell viability after incubation with different concentrations of catalase. The NIH/3T3 cells were incubated with different concentrations of catalase at 37 °C for 12 h, and the cell viability was measured using an MTS assay.

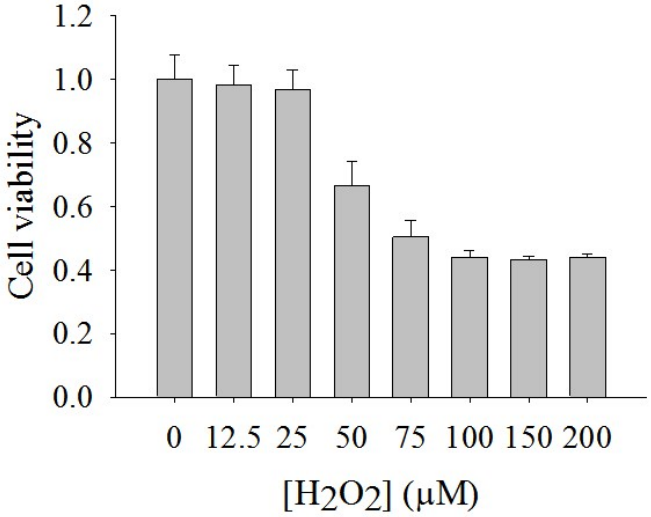

Figure S12 Determination of cell viability after incubation with different concentrations of H_2O_2. The NIH/3T3 cells were incubated with different concentrations of H_2O_2 at 37 °C for 12 h, and the cell viability was measured using an MTS assay.

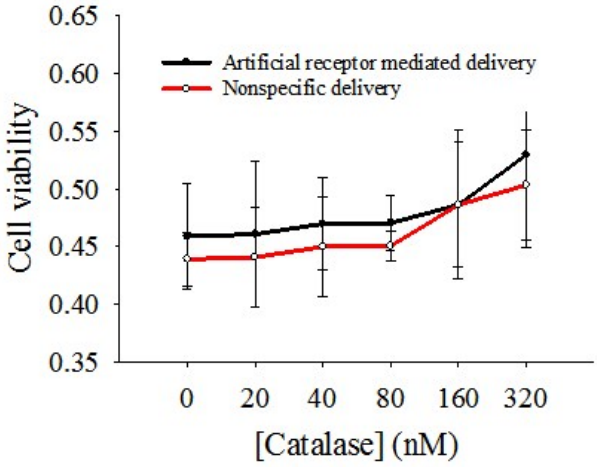

Figure S13 Determination of cell viability of HUVEC cells pre-treated with or without the artificial receptor and catalase and then incubated with different concentrations of H_2O_2. The HUVEC cells were incubated with different concentrations of catalase at 37 °C for 12 h, and then the cell viability was measured using an MTS assay.

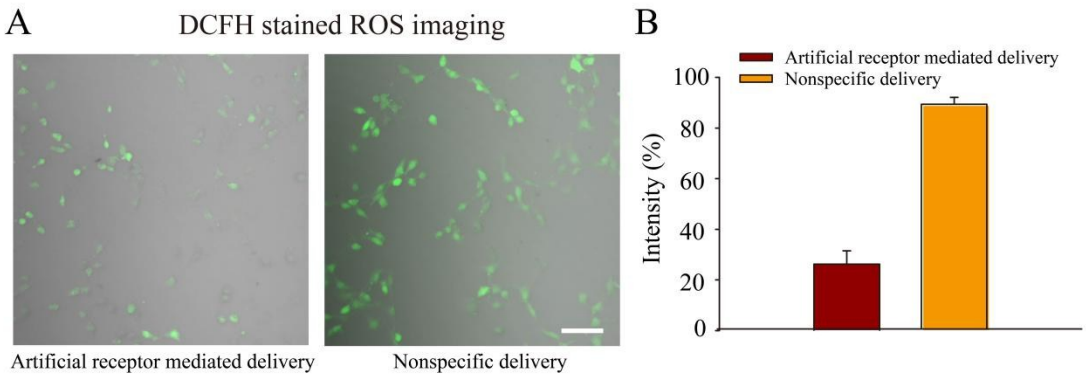

Figure S14 Comparison of the endogenous ROS scavenging and toxicity alleviation capability between artificial receptor-mediated catalase delivery and nonspecific catalase delivery. (A) DCFH-AM fluorescence images of intracellular ROS stimulated from LPS treatment. (B) Corresponding percentage of fluorescence intensity histograms obtained from A. Scale bars: 50 μm.

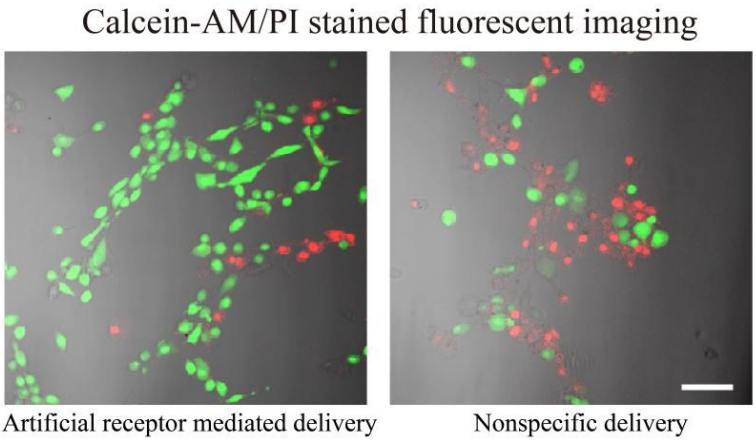

Figure S15 Fluorescence images of calcein-AM and PI-stained live/dead cells. Green fluorescence originated from calcein-AM stained live cells, whereas red fluorescence originated from PI-stained dead cells. Scale bars: 50 μm.

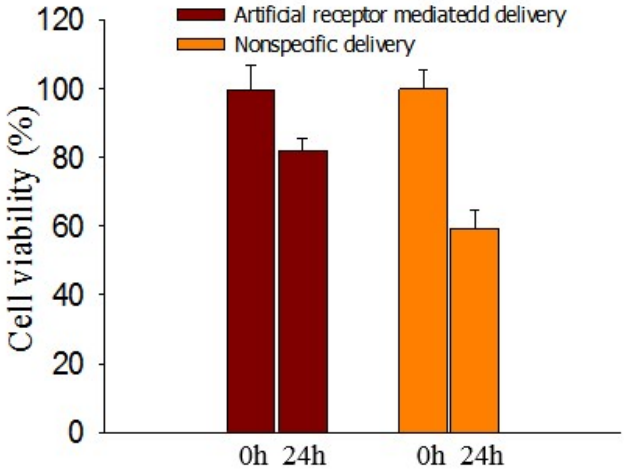

Figure S16 MTS cell viability assay for assessing the toxicity alleviation capability between these two approaches. The NIH/3T3 cells were incubated with 1 μM catalase at 37 °C for 0 h or 24h, and then the cell viability was measured using an MTS assay.