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Electronic Supplementary Information

A Dual-Targeted CeO_2-DNA Nanosensor for Real-time Imaging of H_2O_2 to Assess Atherosclerotic Plaque Vulnerability

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Part A: Experimental Methods

Materials. Cerium nitrate hexahydrate (99.5%, $Ce(NO_3)_3 \cdot 6H_2O$) was purchased from Shanghai Civi Chemical Technology Co., Ltd. Sodium hydroxide (NaOH), sodium chloride (NaCl), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 4-hydroxyethylpiperazineethanesulfonic acid (HEPES), dodecyl sulfate (SDS) were purchased from Sinopharm Chemical Reagent Co., Ltd. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), rapamycin and 3-(4,5- Dimethylthiazole-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich(USA). 4,6-biindole-2-phenylindole (DAPI) were purchased from Shanghai Beyotime Biotechnology Co., Ltd. PEGs were purchased from Beijing Keykai Technology Co., Ltd. Anti-CD36 was purchased from Wuhan Boster Biological Technology.,LTD, folic acid (FA) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. All DNA synthesis and purification were completed by Shanghai Sangon Biotechnology Co., Ltd. Cell culture supplies were purchased from Gibco except as otherwise mentioned. Oxidized low density lipoprotein (ox-LDL) was obtained from Guangzhou Yiyuan biotechnology Co., Ltd. Water was purified with a Sartorius Arium 611 VF system (Sartorius AG, Germany) to a resistivity of 18.2 MΩ·cm. All the reagents used were of analytical grade or above.

Characterization. Transmission electron microscopy (TEM) was carried out on a HT7700 electron microscope. Transmission electron microscopy (TEM) was carried out on a HT7700 electron microscope. Dynamic light scattering measurements were performed on a Malvern Zeta Sizer Nano (Malvern Instruments). Absorption spectra were measured on a U-4100 UV-vis-NIR spectrophotometer (HITACHI, Japan). Loading capacity was measured on a 1200 Infinity II high-performance liquid chromatography. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai LeiCi Device Works, Shanghai, China) with a combined glass calomel electrode. Absorbance in MTT assay and pharmacokinetics study was measured in a microplate reader (RT 6000, Rayto, USA). Imaging flow cytometry analysis was performed using an Image-StreamX multispectral imaging flow cytometer (Amnis

Corporation) with 642 nm excitation. In vivo small animal imaging was performed with Caliper IVIS Lumina III (Caliper Co., USA).

DNA loading and release. To demonstrate the ability of FA/CD36-CeO_2 NWs to load DNA, we performed different concentrations of FA/CD36-CeO_2 NWs in a buffer solution containing 100 nM FAM-DNA and measured by fluorescence spectroscopy to determine fluorescence signal intensity. To detect H_2O_2-induced FAM-DNA release, we added different concentrations of H_2O_2 (1, 5, 10, 20, 30, 40, 50 μM) to the buffer solution containing FA/CD36-CeO_2-DNA (40 μg/mL) and detected the recovery of fluorescence.

Cell culture. HUVECs were purchased from Procell Life Science&Technology Co. Ltd. Rat aortic vascular smooth muscle cells (VSMCs) were obtained from Dingguo Changsheng Biotechnology Co. Ltd. The murine macrophage RAW 264.7 cells were purchased from the Committee on Type Culture Collection of the Chinese Academy of Sciences. VSMCs and RAW 264.7 cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% heat-inactivated FBS and 1% antibiotics penicillin/streptomycin. HUVECs were cultured in Ham's F-12K with 100 μg/mL Heparin, 50 μg/mL endothelial cell growth supplement (ECGs), 10% FBS and 1% penicillin/streptomycin. All types of cells were maintained at 37 °C in a 5% CO2/95% air humidified incubator (MCO-15AC, SANYO).

Cytotoxicity assay. RAW 264.7 macrophages were seeded in 96-well plates (10^4 cells/well) and cultured overnight. Then fresh medium containing different concentrations of FA/CD36-CeO_2-DNA（10, 20, 30, 40 and 50 μg/mL） was added to each well and incubated for different time(4 h, 8 h, 12h and 24 h). Finally, the absorbance values of the cells per well were determined with a microplate reader at 490 nm for analyzing the cell viability.

Animals. All animal experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee (People's Republic of China) and were approved by the Animal Care Committee of Shandong Normal University. $ApoE^{-/-}$ mice (6-8 weeks old, male) were purchased from Changzhou Cavens Laboratory Animal Co. Ltd. (Changzhou, China) and housed under controlled conditions (12/12 h

light/dark cycle; humidity 50-60%; 24-25 °C room temperature), with free access to water and chow. To induce the development of atherosclerotic lesions, mice were fed with the high fat diet (HFD, 20% fat, 20% sugar, and 1.25% cholesterol) for 16 weeks.

Pharmacokinetics study. Healthy ApoE$^{-/-}$ mice were given an intravenous injection of FA/CD36-CeO_2-DNA at 10 mg/kg. At predetermined time intervals, 20 μL of tail vein blood was withdrawn using a tube containing heparin, and the wound was pressed for several seconds to stop the bleeding. Then, fluorescence intensity of FA/CD36-CeO_2-DNA in the blood was determined by an RT 6000 microplate reader. The blood circulation half-life ($t_{1/2}$) was calculated from a first order fit of the data.

Response of FA/CD36-CeO_2-DNA in ox-LDL-activated macrophages. Intracellular response of FA/CD36-CeO_2-DNA was further monitored by imaging flow cytometry. RAW 264.7 macrophages stimulated with ox-LDL were trypsinized and centrifuged (1000 g) to obtain a pellet of about 10^6 cells in 150 μL PBS. Catalase (100 μM) was used as H_2O_2 inhibitor. Cell images were acquired using ImageStreamX multispectral imaging flow cytometer. A 495 nm wavelength laser was used to excite FAM. The fluorescence images were collected using the 450-600 nm spectral detection channels. Unstained cells were used to compensate fluorescence between channel images on a pixel-by-pixel basis. Cell images were analyzed using IDEAS® image-analysis software (Amnis). Laser confocal imaging uses the same conditions.

***In vivo* plaque-response study.** Different stages high fat diet-fed ApoE$^{-/-}$ mice given an intravenous injection of FA/CD36-CeO_2-DNA at 10 mg/kg for 2 h. The mice were sacrificed at predetermined time after injecting, and biodistribution of FA/CD36-CeO_2-DNA was monitored by *ex vivo* fluorescence imaging. The fluorescence signals in each tissue were normalized to the corresponding pre-injection baseline values.

Biodistribution study. To study the biodistribution of nanoparticles, plaque-bearing ApoE$^{-/-}$ mice (n = 3) were intravenously injected with FA/CD36-CeO_2-DNA, Catalase + FA/CD36-CeO_2-DNA, CeO_2-DNA, anti-CD36 + FA/CD36-CeO_2-DNA, FA + FA/CD36-CeO_2-DNA, anti-CD36 + FA + FA/CD36-CeO_2-DNA and PBS (10 mg/kg) via the tail vein. At 2 h post-injection, distribution of nanoparticles was detected by *ex vivo* fluorescence imaging and histology study. For ex vivo fluorescence imaging,

aortas, heart, liver, spleen, lung and kidney from ApoE$^{-/-}$ mice were isolated and imaged by Caliper IVIS Lumina III *in vivo* small animal fluorescence imaging system (Caliper Co., USA). The fluorescence signals in each tissue were normalized to the corresponding pre- injection baseline values. For histology study, 5-μm frozen sections of aortic roots from ApoE$^{-/-}$ mice were blocked with goat serum. Sections were incubated by primary anti-F4/80 (1:250) overnight at 4°C and subsequently incubated by TRITC-tagged secondary antibody for 1 h at 37 °C. After washing 3 times with PBS, the nucleus was stained with DAPI and observed by TCS SP8 confocal laser scanning microscopy.

Statistical Analysis. Each experiment was repeated three times in duplicate if not stated otherwise. Data were presented as mean ± S.D.

Part B: Supplementary figures

DNA loaded FA/CD36-PEGylated CeO_2 NW	Mean Size (nM)	Zeta potential (mV)	Encapsulation efficiency (%)	DNA molecules /NW
FA/CD36-CeO_2-DNA	159.1 × 13.3	-9.6 ± 0.4	59.5	353
FA/CD36-CeO_2 NW	158.6 × 13.2	6.9 ± 0.2	—	—
PEGy-CeO_2 NW	153.3 × 11.3	5.3 ± 0.4	—	—
CeO_2 NW	136.2 × 9.5	-13.8 ± 0.5	—	—

Table S1. Mean size, zeta potential, encapsulation efficiency and DNA molecules quantity of different nanomaterials.

Name	Sequence (5' to 3')
A30-FAM	FAM-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Table S2. DNA sequences employed in this work.

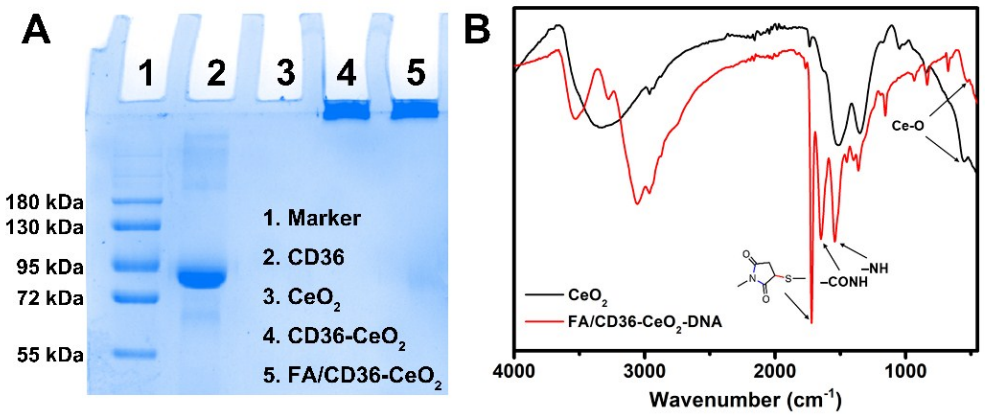

Fig. S1. (A) Gel electrophoresis assay for CD36, CeO_2, CD36-CeO_2 and FA/CD36-CeO_2 were incubated with a loading buffer (5x) at 100℃ for 5 min and loaded onto the 8% SDS-PAGE gel. (B) FT-IR spectra of CeO_2 and FA/CD36- CeO_2. CeO_2 displayed feature absorption band at 412 cm^{-1}, which were attributed to Ce-O groups. Peak intensities at 1635 cm^{-1} and 1554 cm^{-1} were due to the introduction of –CONH amide band and –NH amide band derived from folic acid. After the Michael addition reaction of the thiol group (from anti-CD36 antibody) and the maleimide (from Mal-PEG-NH_2), a strong peak at 1695 cm^{-1} was observed.

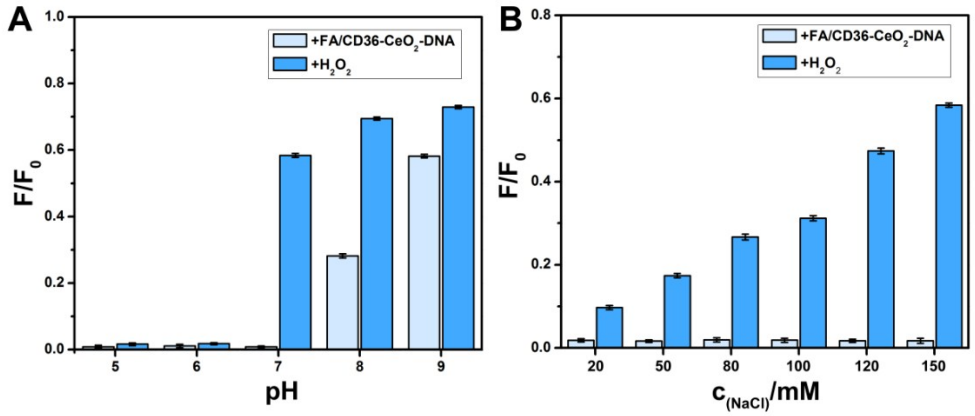

Fig. S2. Hydrogen peroxide responsiveness of FA/CD36-CeO_2-DNA in different buffer. (A) Fluorescence intensity of FA/CD36-CeO_2-DNA incubate with or without H_2O_2 in different acidity (pH =5, 6, 7, 8, and 9). (B) Fluorescence intensity of FA/CD36-CeO_2-DNA incubate with or without H_2O_2 in different concentration of NaCl (20, 50, 80, 100, 120 and 150 mM).

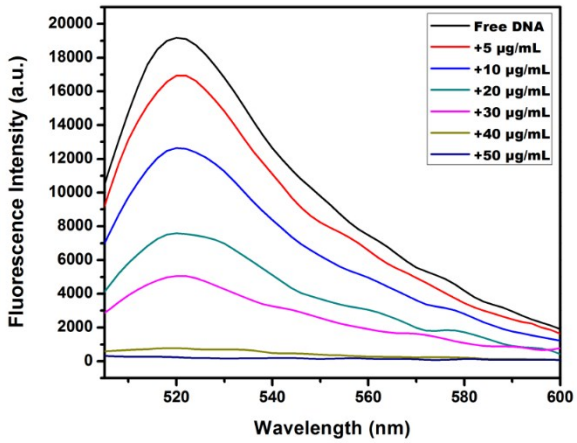

Fig. S3. Fluorescence Spectroscopy of different concentration of FA/CD36-CeO_2 (5, 10, 20, 30, 40, 50 μL) incubate with 100nM FAM-DNA or free FAM-DNA.

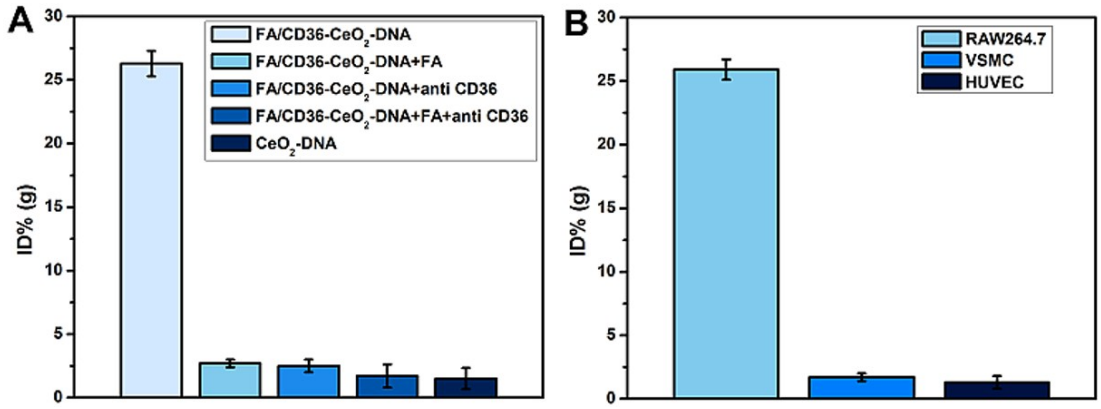

Fig. S4. (A) ICP-AES of Ce content in RAW 264.7 cells after incubated with FA/CD36-CeO_2-DNA, FA + FA/CD36-CeO_2-DNA, anti-CD36+ FA/CD36-CeO_2-DNA, FA + anti-CD36 + FA/CD36-CeO_2-DNA and CeO_2-DNA for 6 h. (B) ICP-AES of Ce content in RAW 264.7, VSMCs and HUVECs after incubated with FA/CD36-CeO_2-DNA for 6 h.

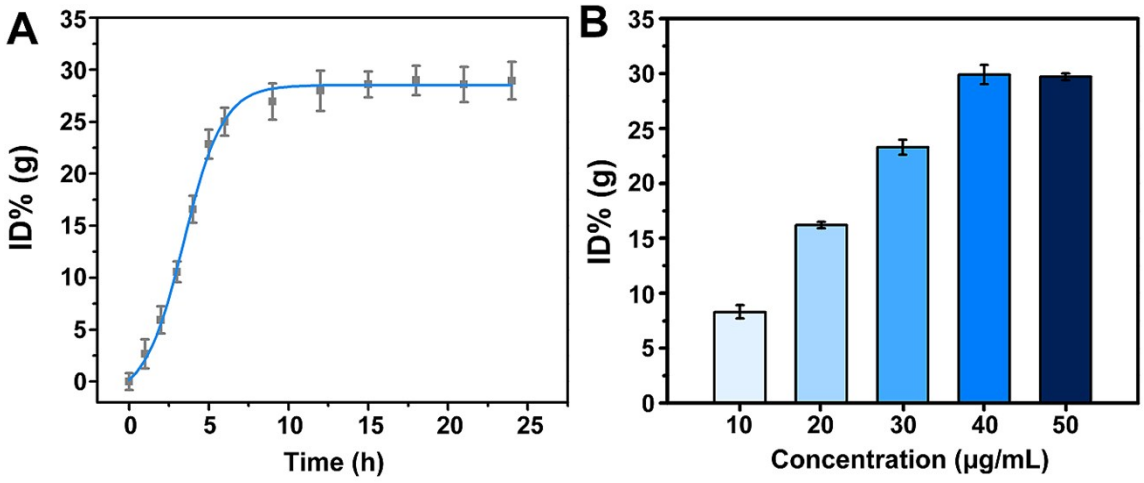

Fig. S5. ICP-AES of Ce content in RAW 264.7 cells at different times (A) or concentrations (B) after incubated with FA/CD36-CeO_2-DNA. Data are shown as mean ± S.D. of three independent experiments.

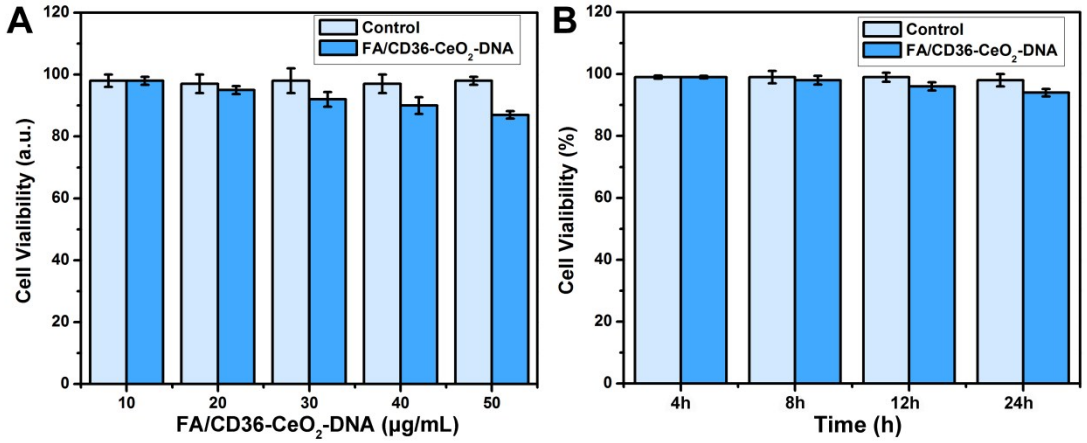

Fig. S6. Cell viabilities of the murine macrophage RAW 264.7 cells after incubated with FA/CD36-CeO_2-DNA in different concentrations (A) and different time (B).

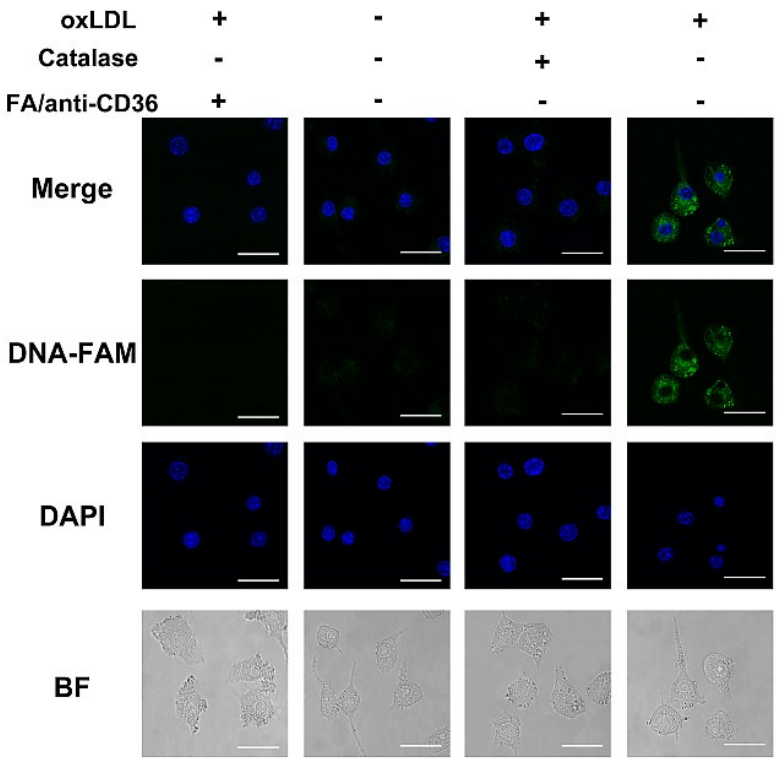

Fig. S7. Laser confocal imaging of RAW 264.7 cells treatment with or without ox-LDL, catalase and FA/anti-CD36. From top to bottom, Merge, FAM, DAPI and BF. Scale bar = 25 μm

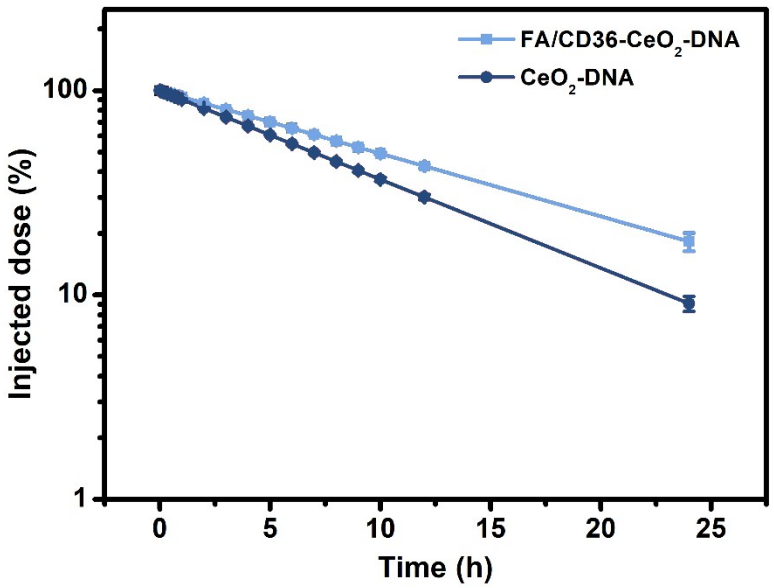

Fig. S8. Pharmacokinetics of FA/CD36-CeO_2-DNA and CeO_2-DNA. Data are shown as mean ± SD (n = 3).

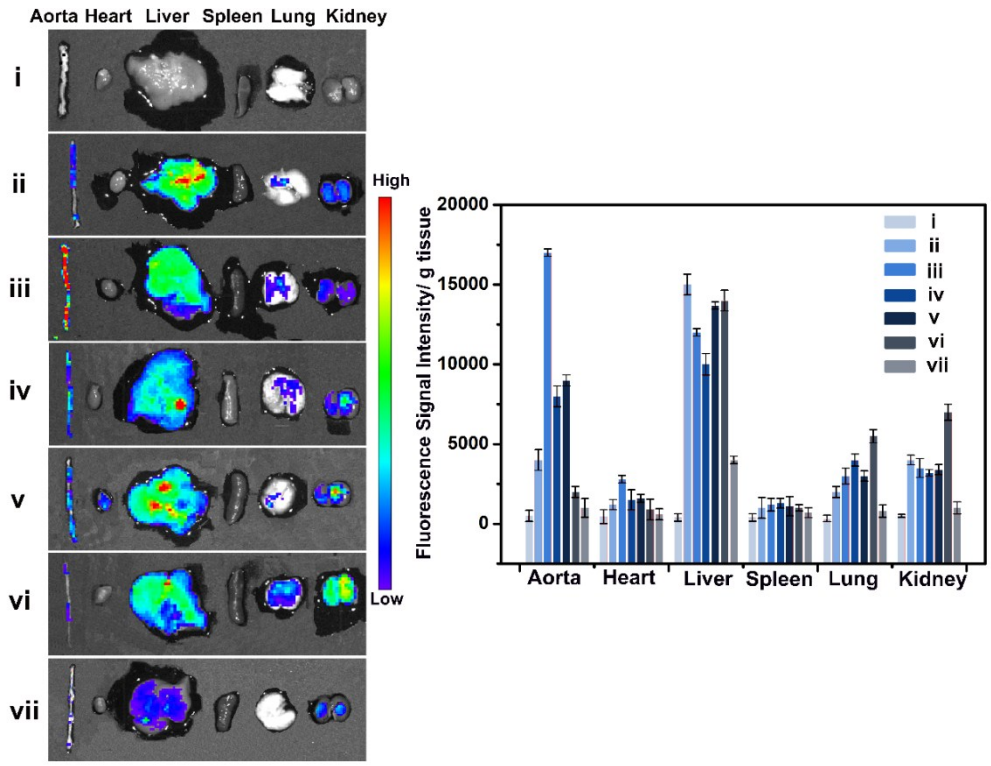

Fig. S9. Quantification of the fluorescence intensity in the aortas, heart, liver, spleen, lung and kidney of the plaque-bearing ApoE$^{-/-}$ mice sacrificed 2 h post-injection of (i) PBS, (ii) CeO_2-DNA, (iii) FA/CD36-CeO_2-DNA, (iv) FR-β blocking + FA/CD36-CeO_2-DNA, (v) CD36 blocking + FA/CD36-CeO_2-DNA, (vi) FR-β/CD36 blocking + FA/CD36-CeO_2-DNA, (vii) Catalase + FA/CD36-CeO_2-DNA. Data are shown as mean ± SD (n = 3).

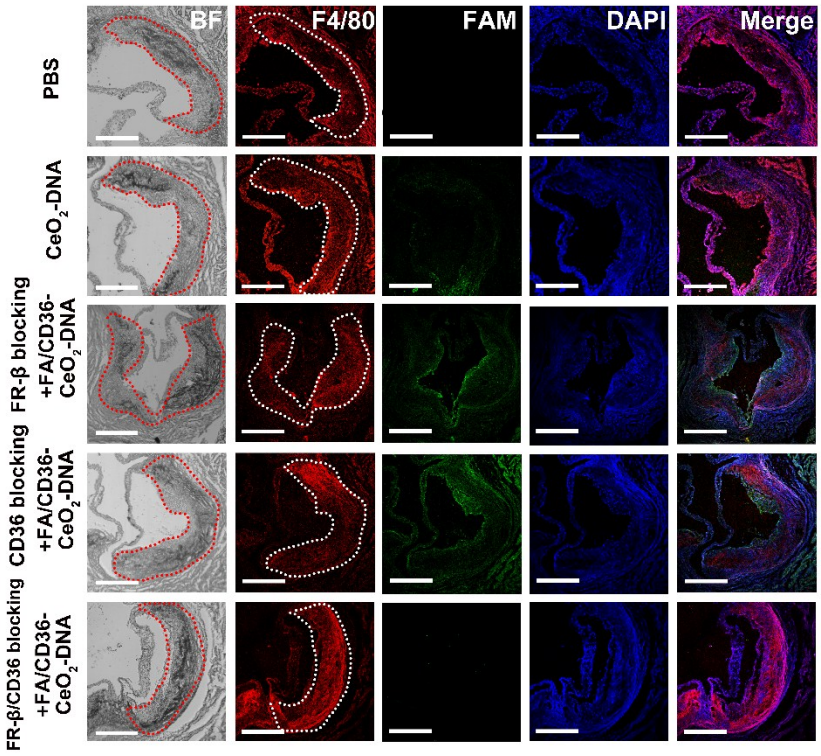

Fig. S10. Immunofluorescence micrographs of aortic plaque region cross sections of mice with 16 weeks on high-fat diet. From top to bottom were intravenously injected with PBS, CeO_2-DNA, FA + FA/CD36-CeO_2-DNA, anti-CD36+ FA/CD36-CeO_2-DNA, FA + anti-CD36+ FA/CD36-CeO_2-DNA (10 mg/kg) via the tail vein. From left to right, BF, red (macrophages marker F4/80), green (FAM), blue (DAPI) and merge. Scale bar =75 μm.