SUPPORTING INFORMATION

H_2O_2- and Light-Triggered MnO_2-Based Nanoswitches for Microenvironment Reprogramming and Photodynamic Therapy of Hypoxic Atherosclerotic Plaques

Yong Geun Lim[a,1], Jin Hyuk Kim[b,c,1], Ryeong Hyun Kim[b], Hongki Yoo[d], Jin Won Kim[b,c,*], Kyeongsoon Park[a,*]

[a]Department of Systems Biotechnology, Chung-Ang University, Anseong, Gyeonggi 17546, Korea

[b]Multimodal Imaging and Theranostic Laboratory, Cardiovascular Center, Korea University Guro Hospital, Seoul 08308, Korea

[c]BK21 Graduate Program, Department of Biomedical Sciences, Korea University College of Medicine, Seoul, Korea

[d]Department of Mechanical Engineering, KAIST, Daejeon, Korea

[1]These authors contributed equally to this work.

[*]These authors jointly supervised this work.

Correspondence to:

Kyeongsoon Park, Ph.D.

Department of Systems Biotechnology, Chung-Ang University, Anseong, Gyeonggi 17546, Korea; Tel: +82-31-670-3357; Fax: +82-31-675-1381; E-mail: kspark1223@cau.ac.kr

Jin Won Kim, M.D., Ph.D., F.A.C.C., Cardiovascular Center, Korea University Guro Hospital, 148, Gurodong-ro, Guro-gu, Seoul 08308, Korea; Tel: +82-2-2626-3021; Fax: +82-2-863-1109; E-mail: kjwmm@korea.ac.kr

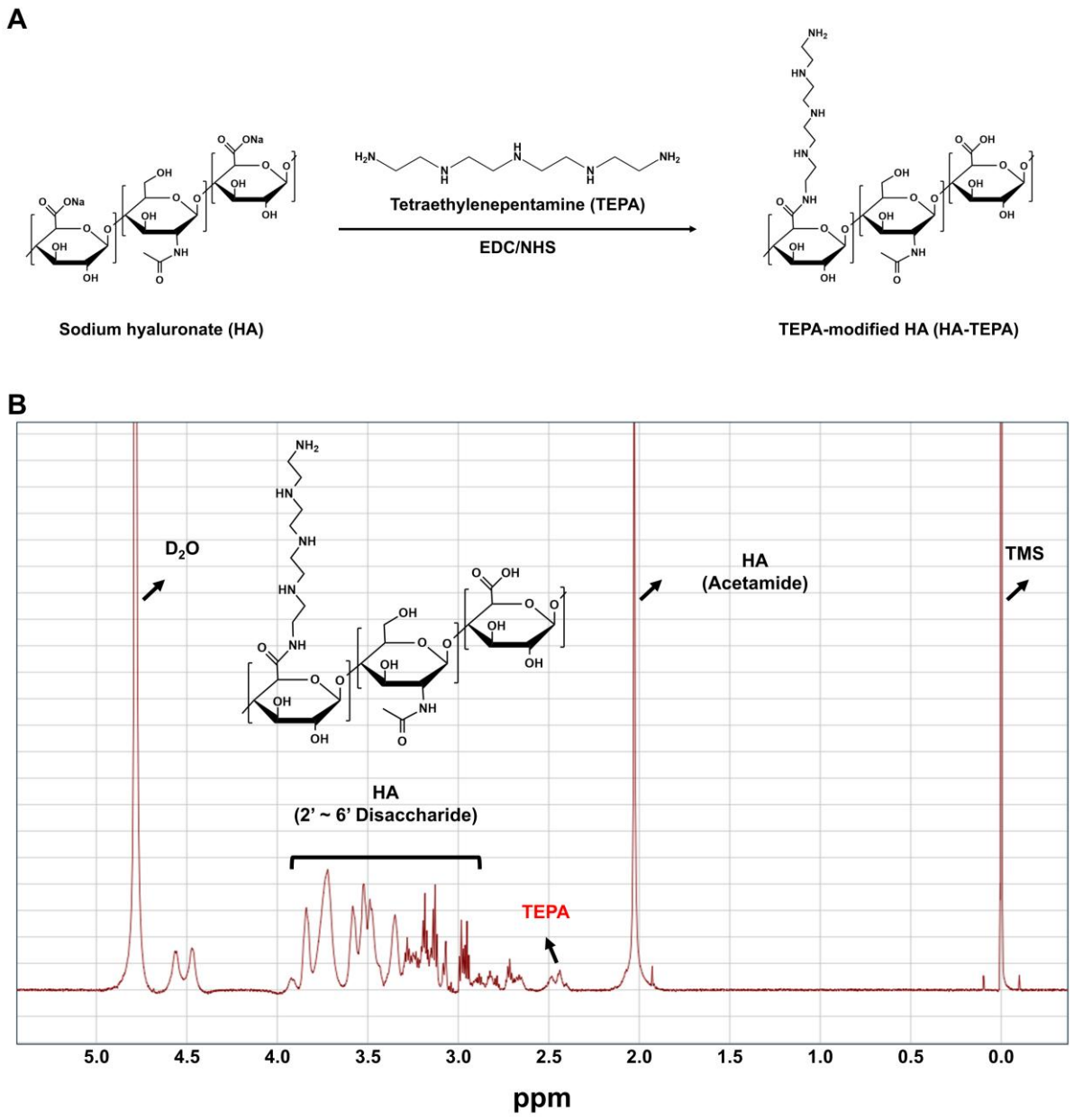

Figure S1. Synthesis and characterization of HA-TEPA. (A) Synthetic mechanism of HA-TEPA via EDC/NHS chemistry. (B) ^{1}H-NMR spectrum of HA-TEPA.

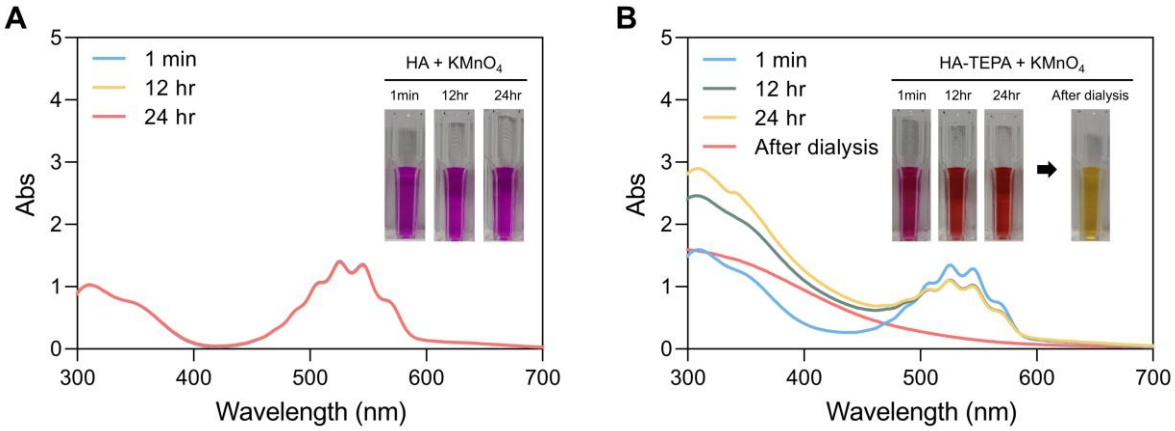

Figure S2. Comparison of the reducing reactivity of (A) HA and (B) HA-TEPA towards $KMnO_4$. Reducing activity was confirmed by time-dependent changes in solution color and UV/Vis spectra.

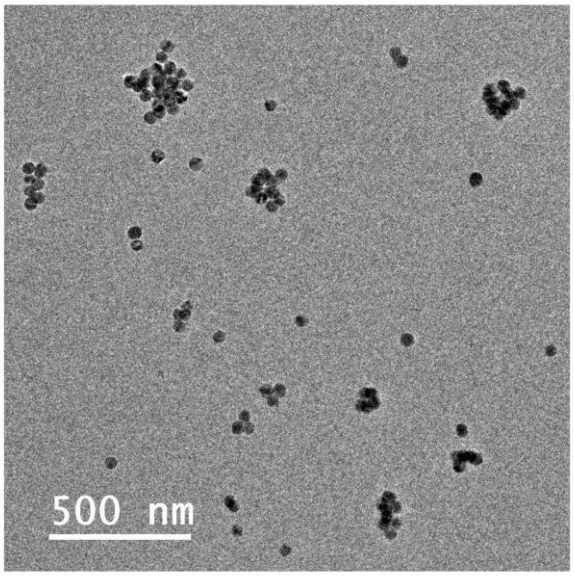

Figure S3. Wide-view TEM image of the HA-TEPA/MnO_2/Ce6 nanoswitch. Scale bar: 500 nm.

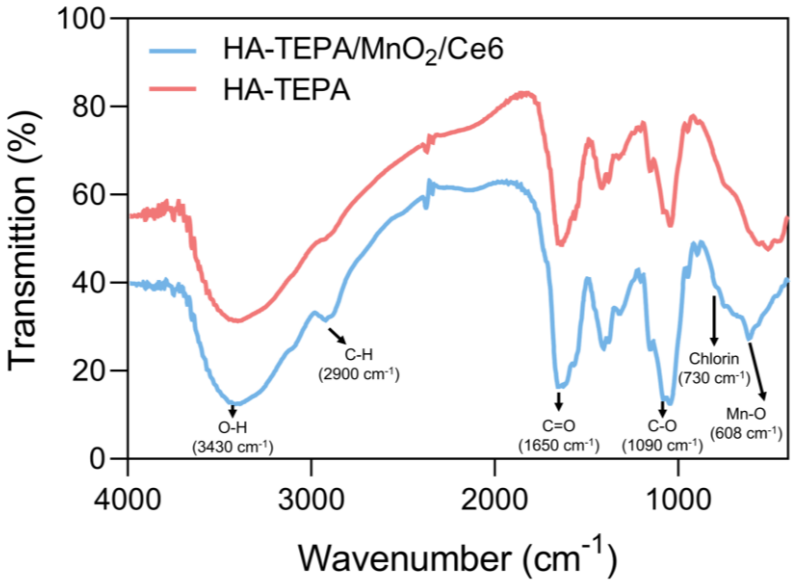

Figure S4. FT-IR spectra of HA-TEPA and the HA-TEPA/MnO_2/Ce6 nanoswitch.

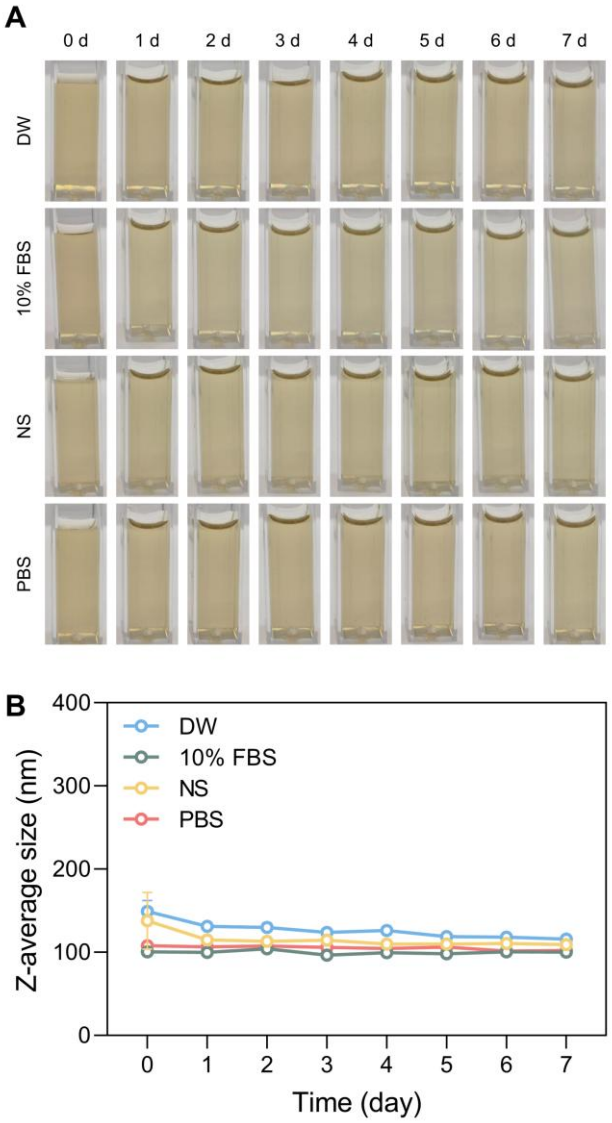

Figure S5. Particle stability study. (A) Photographs of the HA-TEPA/MnO_2/Ce6 nanoswitch in DW, 10% FBS, 0.9% NS, and PBS (pH 7.4) over 7 days. (B) Z-average sizes of the HA-TEPA/MnO_2/Ce6 nanoswitch stored in various solutions (DW, 10% FBS, 0.9% NS, and PBS (pH 7.4)) measured at different time points.

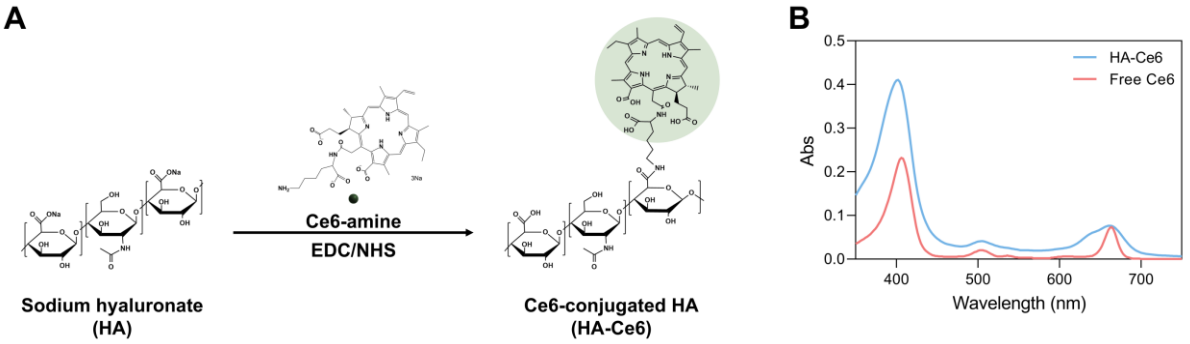

Figure S6. Synthesis and characterization of HA-Ce6. (A) Synthetic mechanism of HA-Ce6. HA-Ce6 was synthesized via EDC/NHS chemistry between the carboxylic acid of HA and the primary amine of Ce6-NH_2. (B) UV/Vis spectra of free Ce6 (red) and HA-Ce6 (blue).

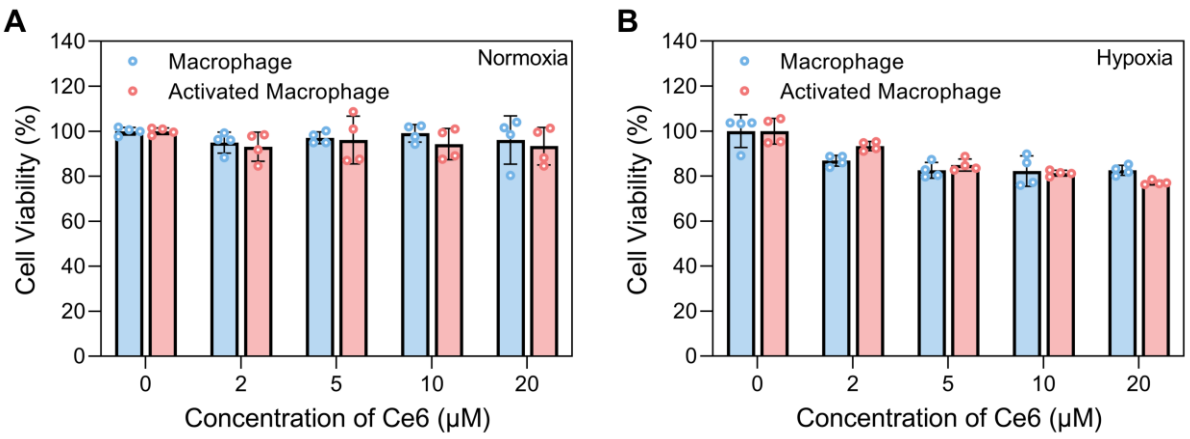

Figure S7. In vitro cytotoxicity of HA-TEPA/MnO_2/Ce6 nanoswitches in macrophages and activated macrophages under (A) normoxic and (B) hypoxic conditions.

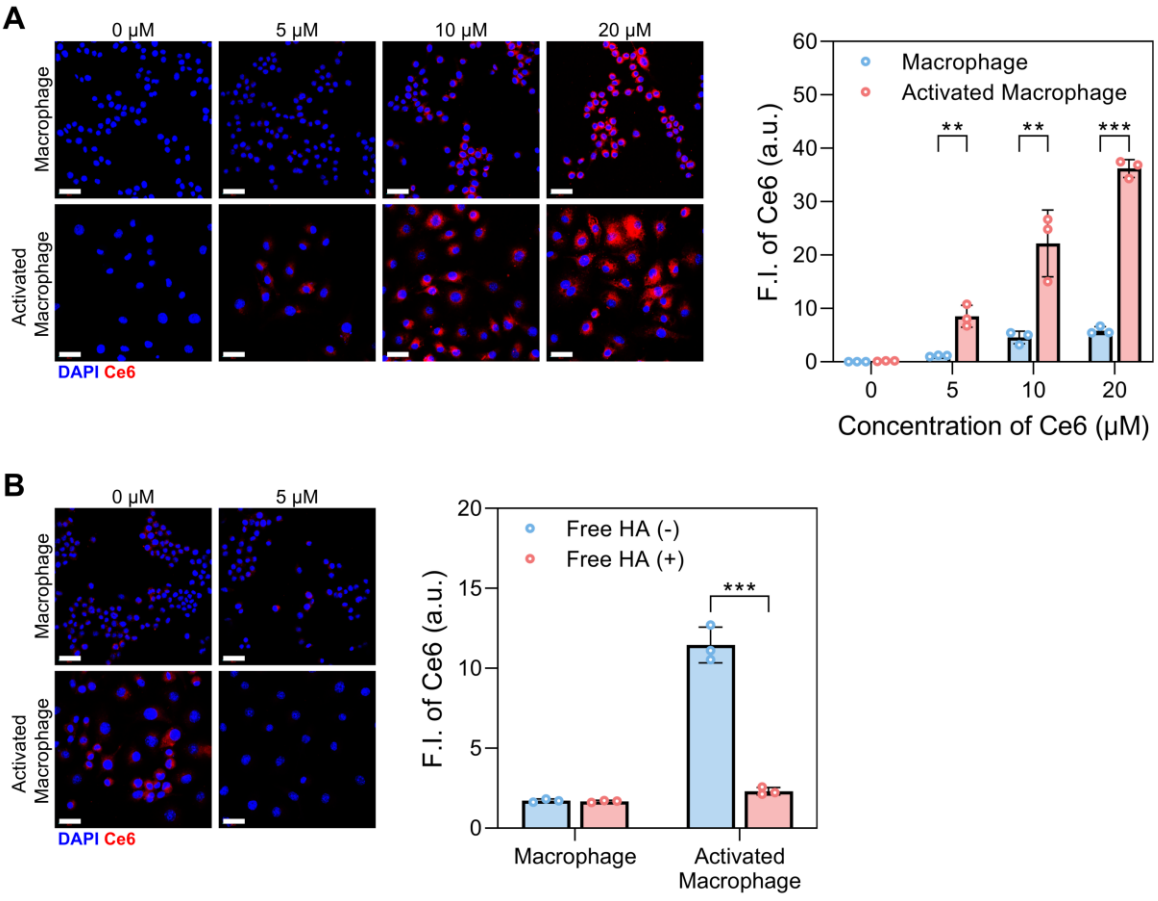

Figure S8. Intracellular CD44-mediated endocytosis of HA-TEPA/MnO_2/Ce6 nanoswitches. (A) Dose-dependent intracellular uptake and quantitative comparison of HA-TEPA/MnO_2/Ce6 nanoswitches in macrophages and activated macrophages under normoxic conditions. Scale bar: 30 μm. $^{**}P < 0.01$, $^{***}P < 0.001$. (B) Blocking experiments and quantitative fluorescence intensities of HA-TEPA/MnO_2/Ce6 nanoswitches (equivalent to 5 μM Ce6) in macrophages and activated macrophages with or without pretreatment with free HA. Scale bar: 30 μm. $^{***}P < 0.001$.

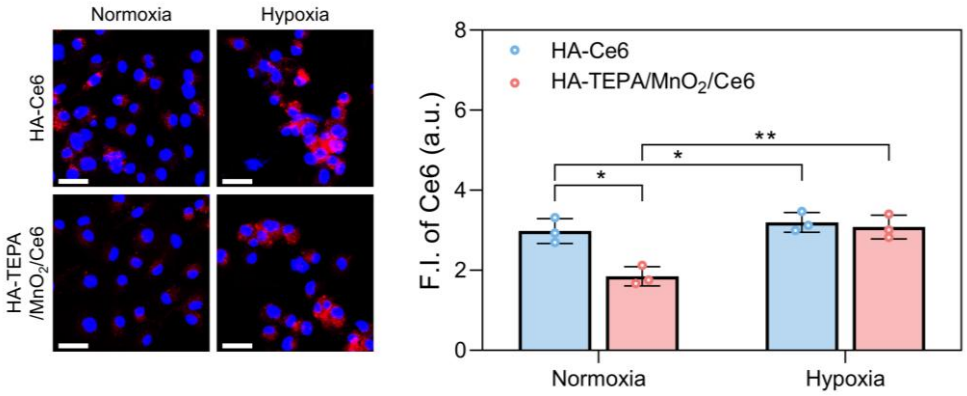

Figure S9. Comparison of intracellular uptake of HA-Ce6 (equivalent to 5 μM Ce6) and HA-TEPA/MnO_2/Ce6 nanoswitches (equivalent to 5 μM Ce6) in activated macrophages under normoxic or hypoxic conditions. Scale bar: 30 μm. *$P < 0.05$, **$P < 0.01$.

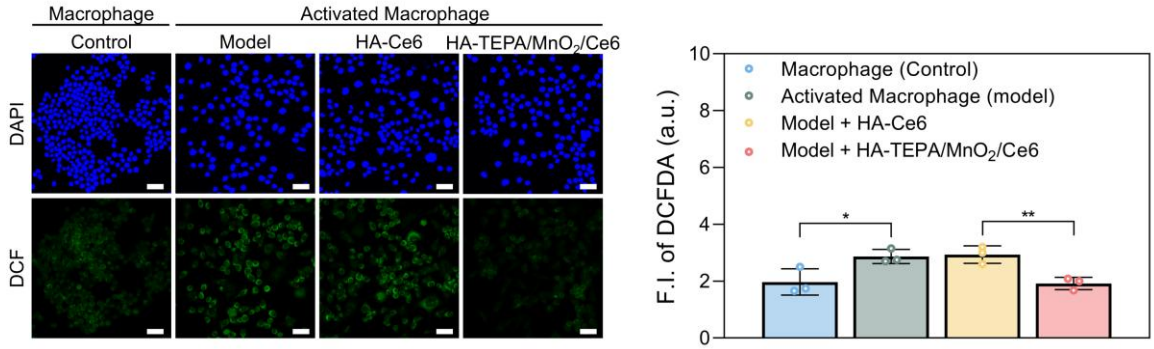

Figure S10. In vitro intracellular ROS-scavenging capacity of HA-TEPA/MnO_2/Ce6 nanoswitches in hypoxically activated macrophages. Fluorescence images and quantitative analysis of hypoxically activated macrophages treated with HA-Ce6 (equivalent to 5 μM Ce6) or HA-TEPA/MnO_2/Ce6 nanoswitches (equivalent to 5 μM Ce6). Scale bar: 30 μm. *$P < 0.05$, **$P < 0.01$.

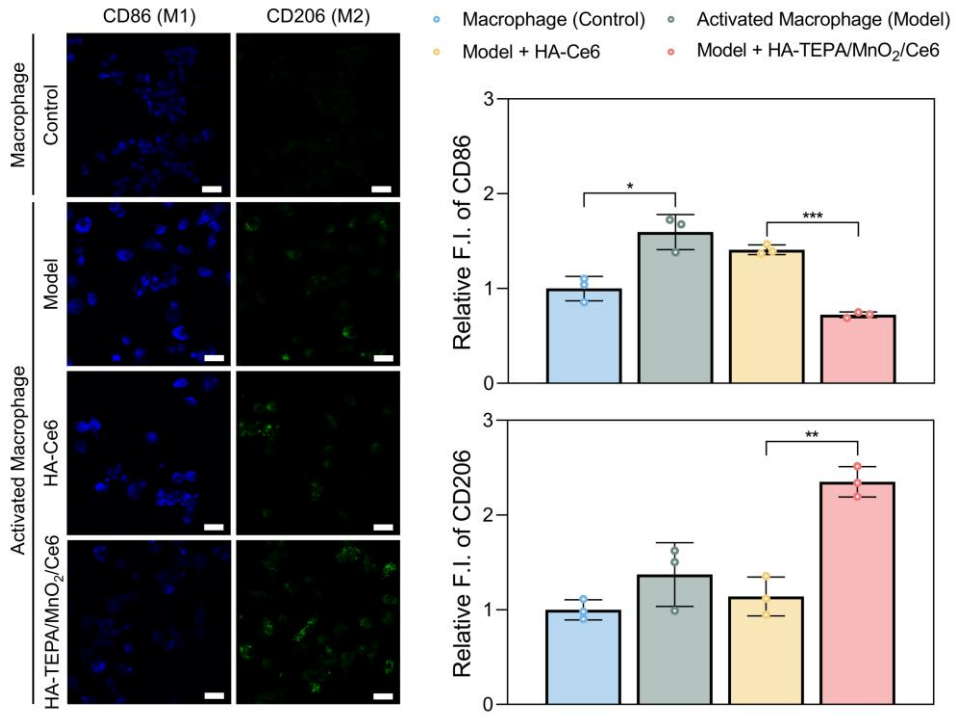

Figure S11. Comparison of M1-to-M2 macrophage reprogramming induced by HA-Ce6 and HA-TEPA/MnO_2/Ce6 nanoswitches in hypoxically activated macrophages. Fluorescence images and quantitative fluorescence analysis of hypoxically activated macrophages treated with HA-Ce6 (equivalent to 5 μM Ce6) or HA-TEPA/MnO_2/Ce6 nanoswitches (equivalent to 5 μM Ce6). Scale bar: 30 μm. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

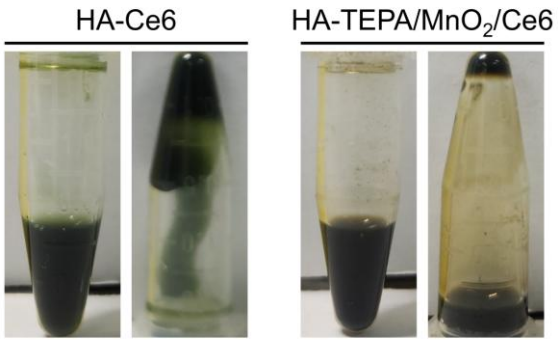

Figure S12. Photographs of HA-Ce6 (left) and HA-TEPA/MnO_2/Ce6 (right) solutions at the injection dose (equivalent to 3 mg/kg Ce6). Photographs were also acquired after 180° rotation. In contrast to the high viscous HA-Ce6 solution, the HA-TEPA/MnO_2/Ce6 solution exhibited lower viscosity suitable for intravenous injection.

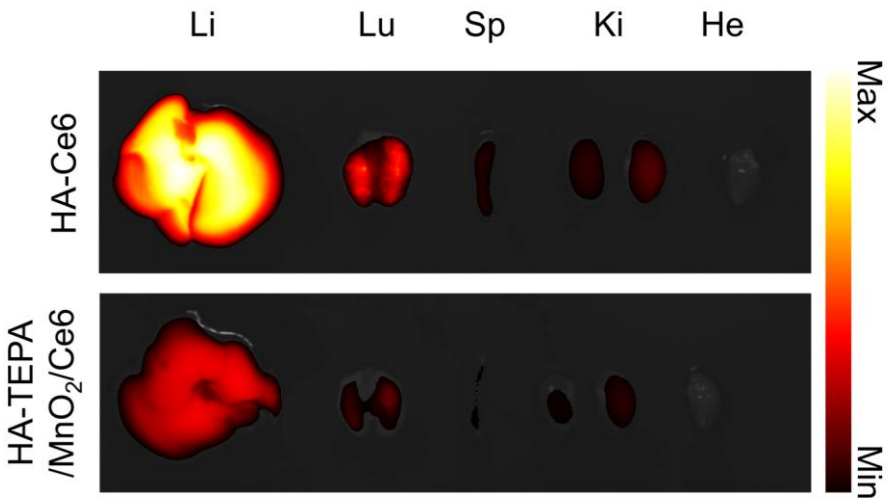

Figure S13. Ex vivo fluorescence images of harvested liver (Li), lung (Lu), spleen (Sp), kidney (Ki), and heart (He).

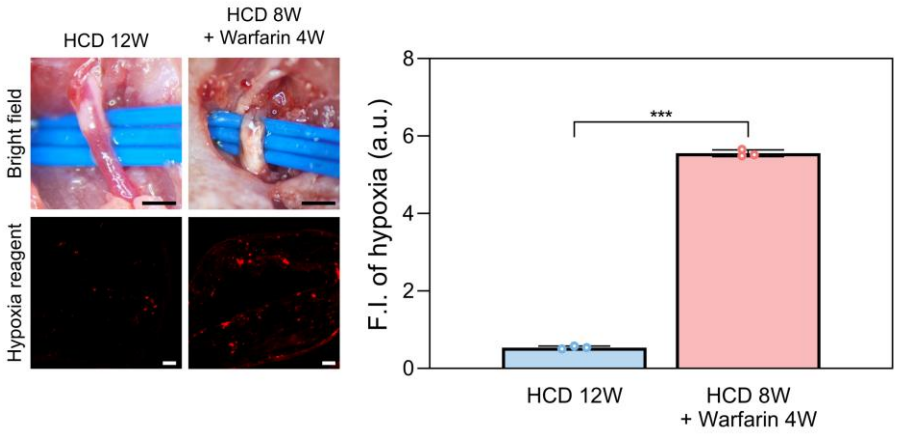

Figure S14. In vivo modeling of hypoxic atherosclerotic plaques in ApoE$^{-/-}$ mice. Representative bright-field (scale bar: 500 μm) and hypoxia fluorescence images (scale bar: 30 μm) of carotid plaques from mice fed a standard atherogenic diet (high-cholesterol diet (HCD) for 12 weeks) or a hypoxia-enhanced atherogenic diet (HCD for 8 weeks followed by a warfarin-containing diet (WD) for an additional 4 weeks). ***$P < 0.001$.

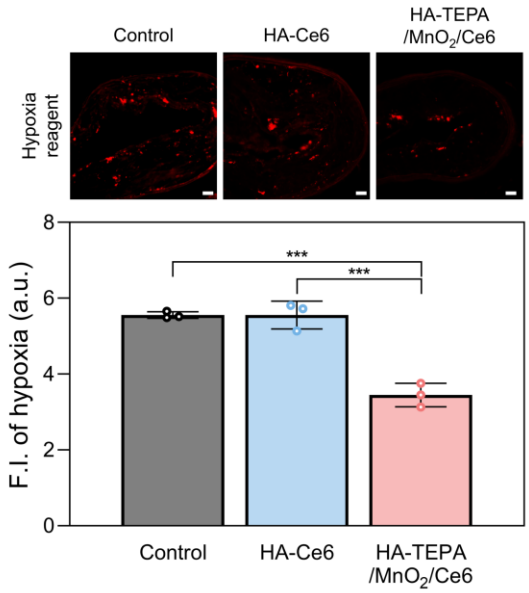

Figure S15. In vivo hypoxia alleviation by HA-TEPA/MnO_2/Ce6 nanoswitches. CLSM images and quantitative analysis of hypoxia signals in sectioned carotid atheroma. Consistent with the immunofluorescence results for HIF-1α, the fluorescence of the hypoxia probe decreased in the HA-TEPA/MnO_2/Ce6 nanoswitches-treated groups. Scale bar: 60 μm. ***$P < 0.001$.

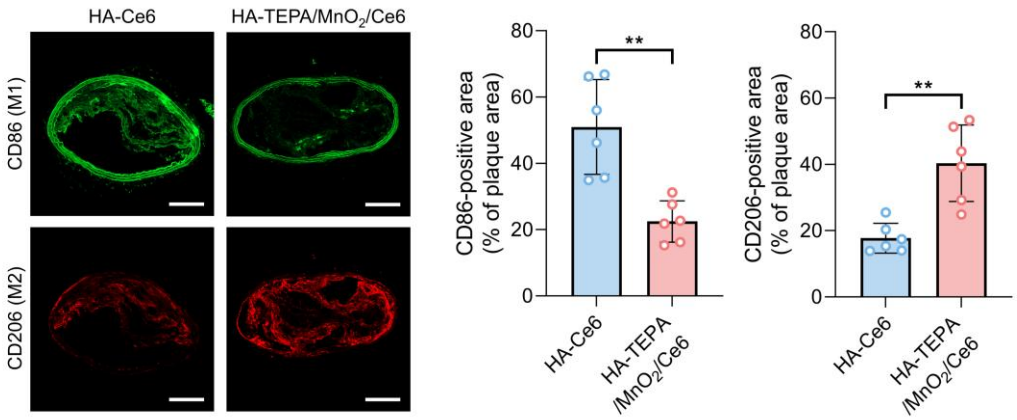

Figure S16. In vivo M1-to-M2 macrophage reprogramming induced by HA-TEPA/MnO_2/Ce6 nanoswitches. CLSM images and immunofluorescence analysis of sectioned carotid atheroma after treatment with HA-Ce6 or HA-TEPA/MnO_2/Ce6 nanoswitches. Immunofluorescence staining reveals CD86 (green) and CD206 (red) expression within plaque regions. Scale bar: 200 μm. Quantitative immunofluorescence analysis of sectioned carotid atheroma. $^{**}P < 0.01$.

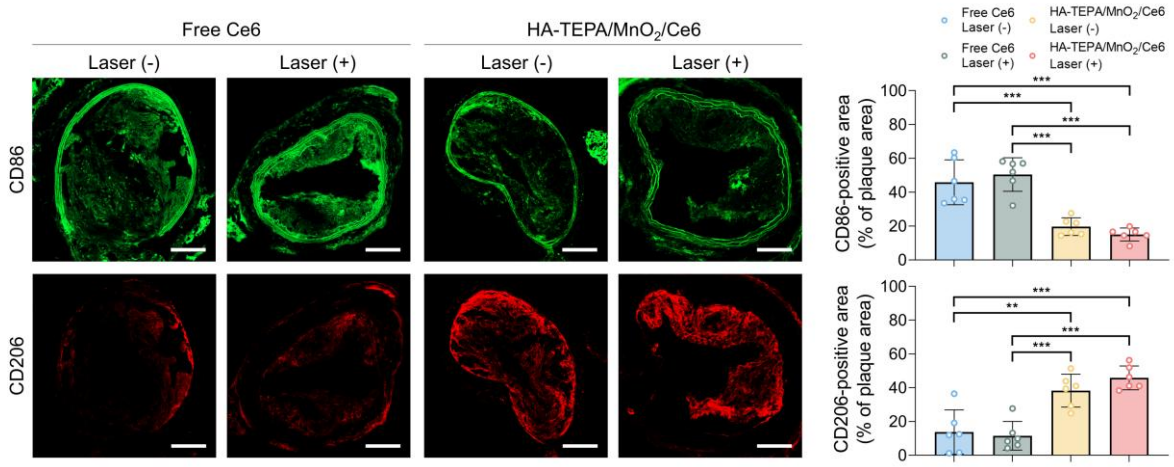

Figure S17. In vivo M1-to-M2 macrophage reprogramming following photoactivation of HA-TEPA/MnO_2/Ce6 nanoswitches. CLSM images and immunofluorescence analysis of sectioned carotid atheroma after treatment with free Ce6 or HA-TEPA/MnO_2/Ce6 nanoswitches, with or without laser irradiation. Immunofluorescence staining reveals CD86 (green) and CD206 (red) expression within plaque regions. Scale bar: 200 μm. Quantitative immunofluorescence analysis of sectioned carotid atheroma. $^{**}P < 0.01$, $^{***}P < 0.001$.

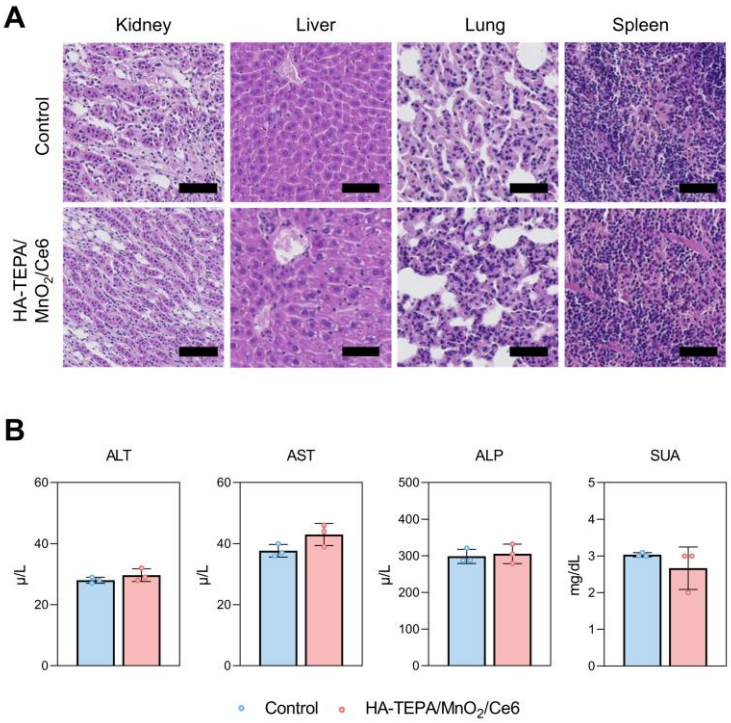

Figure S18. Biosafety of HA-TEPA/MnO_2/Ce6 nanoswitches. (A) Hematoxylin and eosin (H&E)-stained sections of the kidney, liver, lung, and spleen showed no evidence of tissue damage following treatment, indicating no apparent histopathological toxicity. Scale bar: 100 μm. (B) Blood biochemistry showed no evidence of hepatic or renal dysfunction. ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; SUA: serum uric acid.