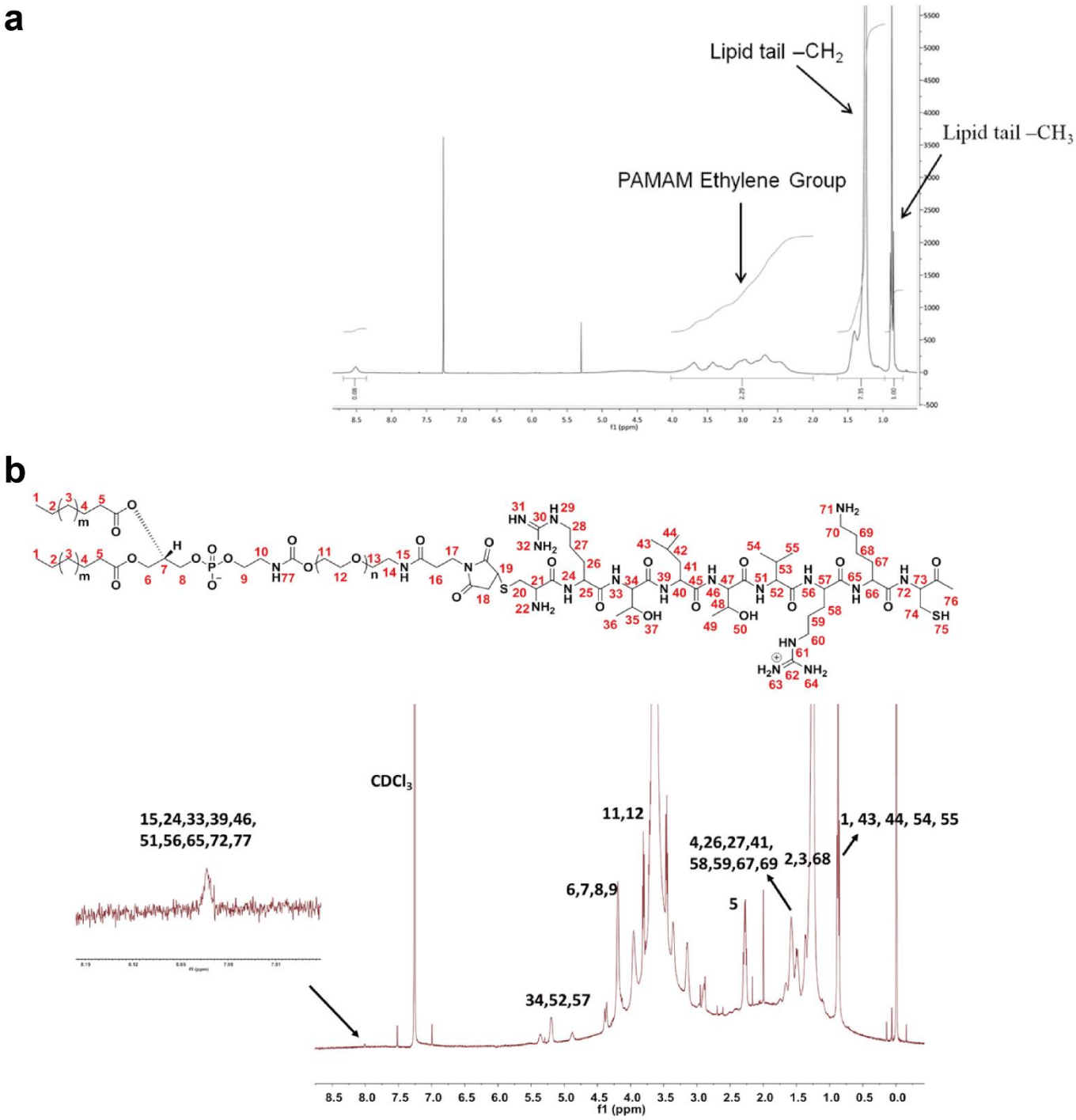
Supplementary Information

Engineering targeted siRNA nanoparticles to silence plaque-destabilizing gene in atherosclerotic lesional macrophages

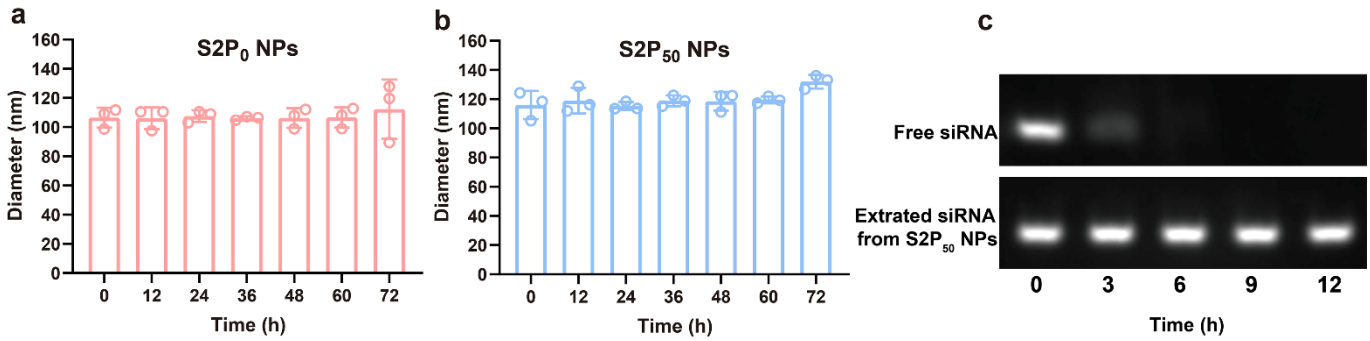
Xiangang Huang[1†], Chuang Liu[1†], Na Kong[1], Yufen Xiao[1], Arif Yurdagul Jr.[2], Ira Tabas[3,4,5], Wei Tao[1*]

[1]Center for Nanomedicine and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. [2]Department of Molecular and Cellular Physiology, LSU Health Shreveport, Shreveport, LA, 71130, USA. [3]Department of Medicine, Columbia University Irving Medical Center, New York, NY 10032, USA. [4]Department of Physiology and Cellular Biophysics, Columbia University Irving Medical Center, New York, NY 10032, USA. [5]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY 10032, USA.

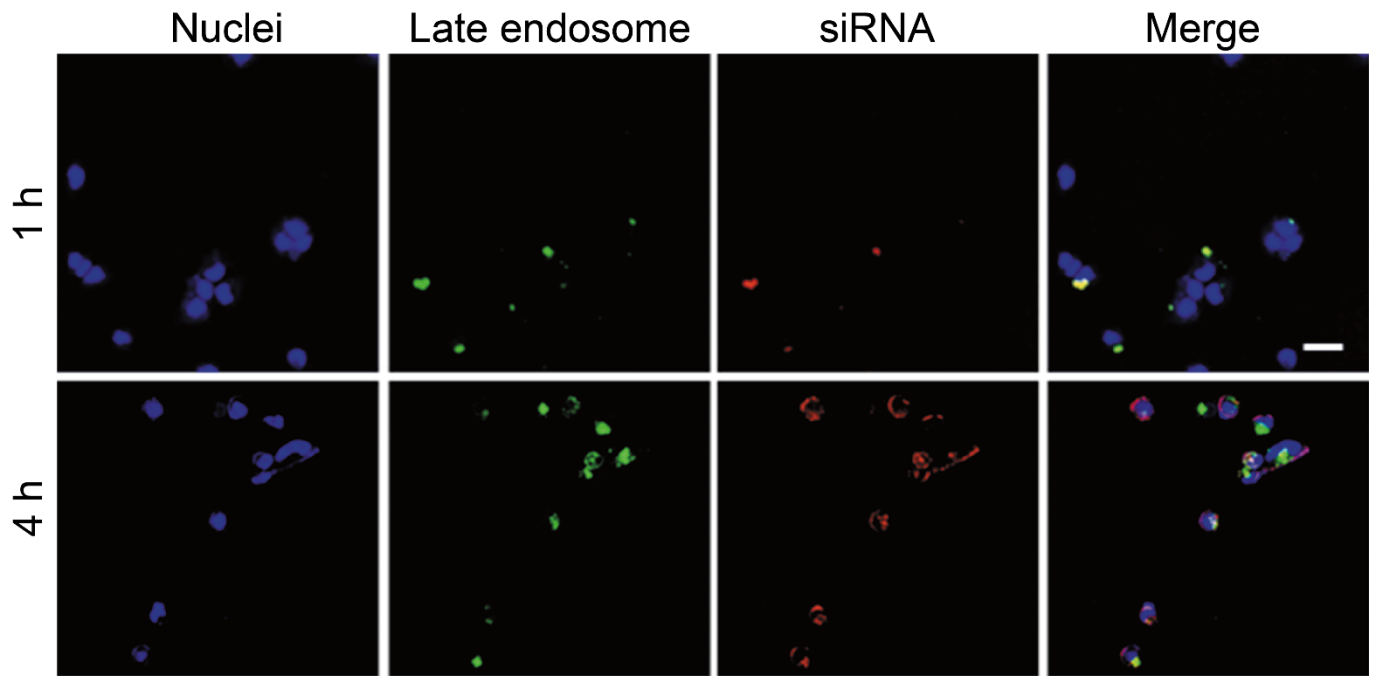
[†]These authors contributed equally to this work.
*Corresponding author. Email: wtao@bwh.harvard.edu



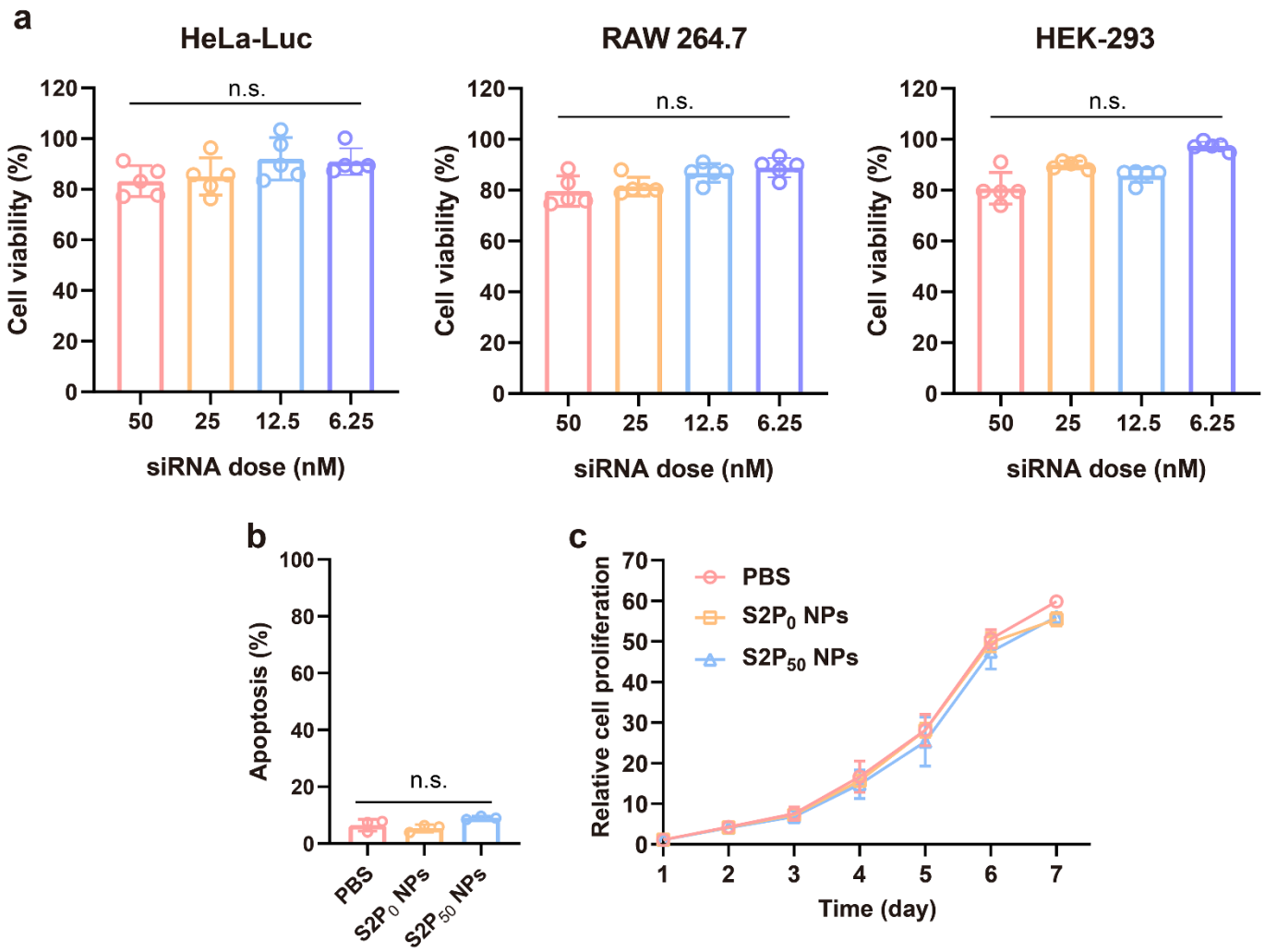
Supplementary Figure 1. ^{1}H NMR analysis. **a**, G0-C14 ($CDCl_3$). Adapted with permission from ref.[1], National Academy of Sciences; **b**, DSPE-PEG-S2P ($CDCl_3$). Adapted with permission from ref.[2], American Association for the Advancement of Science.



Supplementary Figure 2. Serum stability and protective effect of siRNA-loaded NPs for siRNA. **a**-**b**, Stability of $S2P_0$ and $S2P_{50}$ NPs in PBS solution containing 10% serum at 37 ℃ was determined by monitoring the particle size using DLS over 3 days. n = 3. Data are presented as mean ± SEM. **c**, Protective effect of siRNA-loaded NPs for siRNA in 10% (vol/vol) serum medium was characterized by assessing the extracted siRNA from $S2P_{50}$ NPs using agarose gel electrophoresis. **a**-**c**, Adapted with permission from ref.[2], American Association for the Advancement of Science.



Supplementary Figure 3. Endosomal escape of siRNA-loaded NPs. Confocal microscopy images of HeLa-Luc cells treated with Dy647-siRNA-loaded $S2P_{50}$ NPs (red) for 1 and 4 h. The late endosomes were stained with LysoTracker Green (green) and the nuclei were stained with Hoechst 33342 (Blue). Adapted with permission from ref.[2], American Association for the Advancement of Science.



Supplementary Figure 4. In vitro biocompatibility of siRNA-loaded NPs. **a**, HeLa-luc, RAW 264.7 and HEK-293 cells were treated with $S2P_{50}$ NPs (siLuc 6.25-50 nM) for 24 h. After another 48 h incubation with fresh medium, the viability of the cells was assessed using alamarBlue reagent (n.s., not significant). n = 5. Data are presented as mean ± SEM. **b**, RAW 264.7 cells were treated with PBS, $S2P_0$ NPs and $S2P_{50}$ NPs (siLuc 50 nM) for 24 h. After another 48 h incubation with fresh medium, the apoptosis of the cells was assessed using Annexin V-FITC and propidium iodide (PI) (Annexin V-FITC Apoptosis Detection kit). n = 3. **c**, RAW 264.7 cells were treated with PBS, $S2P_0$ NPs and $S2P_{50}$ NPs (siLuc 50 nM) for 24 h. After another 48 h incubation with fresh medium, the proliferation of the cells was assessed using alamarBlue reagent. n = 3. **a**-**c**, Adapted with permission from ref.[2], American Association for the Advancement of Science.

Supplementary Table 1. siRNA sequences used in this protocol

siRNA	Strands	Sequences
siLuc	Sense	5'-CUU ACG CUG AGU ACU UCG AdTdT-3'
	Antisense siLuc	5'-UCG AAG UAC UCA GCG UAA GdTdT-3'
Dy647-siLuc	Sense	5'-Dy647-CUU ACG CUG AGU ACU UCG AdTdT-3'
	Antisense	5'-UCG AAG UAC UCA GCG UAA GdTdT-3'
siCamK2g	Sense	5'-AAC GUG GUA CAU AAU GCU ACA-3'
	Antisense	5'-UGU AGC AUU AUG UAC CAC GUU-3'

Reference

1 Xu, X. *et al.* Enhancing tumor cell response to chemotherapy through nanoparticle-mediated codelivery of siRNA and cisplatin prodrug. *Proc. Natl. Acad. Sci.* **110**, 18638-18643 (2013).

2 Tao, W. *et al.* siRNA nanoparticles targeting CaMKIIγ in lesional macrophages improve atherosclerotic plaque stability in mice. *Sci. Transl. Med.* **12**, eaay1063 (2020).