
Supplementary information

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Supplementary Information

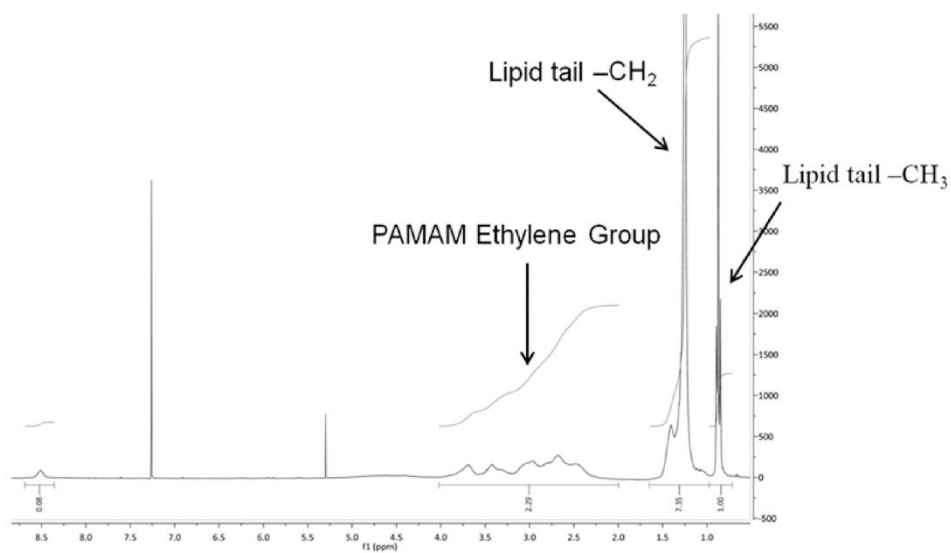
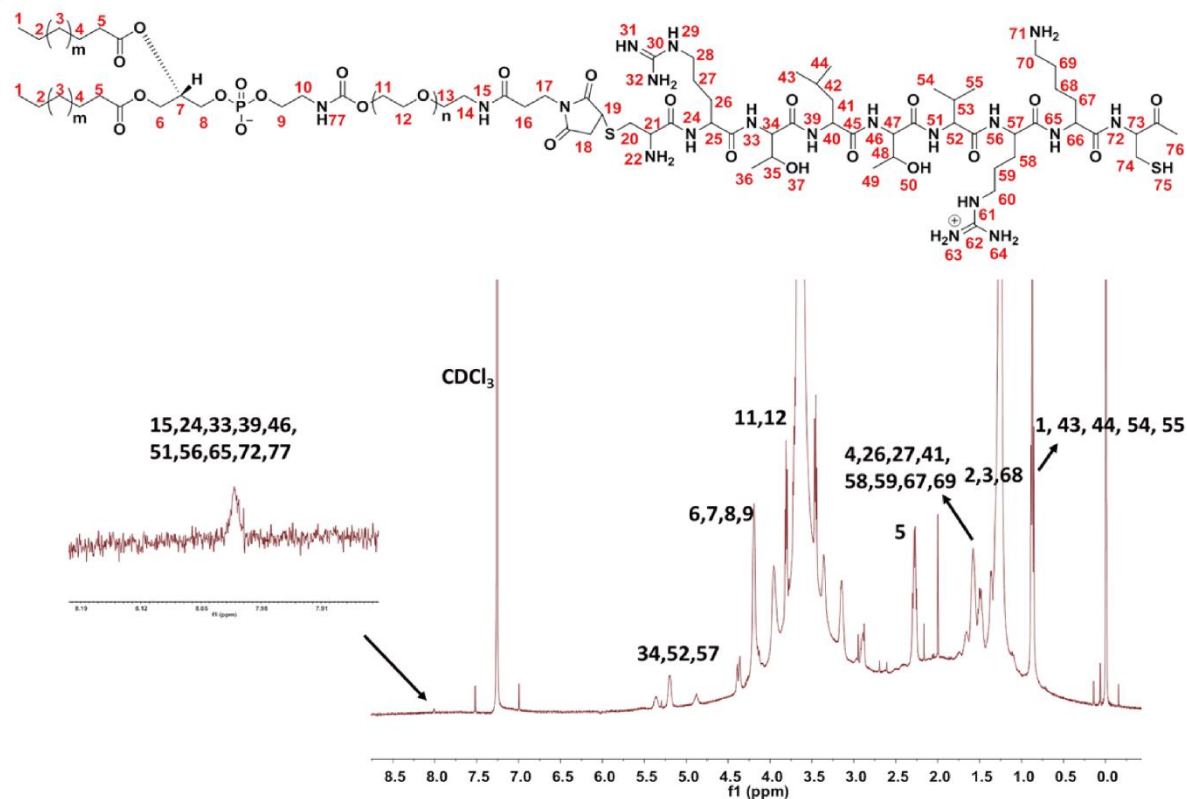
Synthesis of siRNA nanoparticles to silence plaque-destabilizing gene in atherosclerotic lesional macrophages

Xiangang Huang^{1†}, Chuang Liu^{1†}, Na Kong¹, Yufen Xiao¹, Arif Yurdagul Jr.², Ira Tabas^{3,4,5}, Wei Tao^{1*}

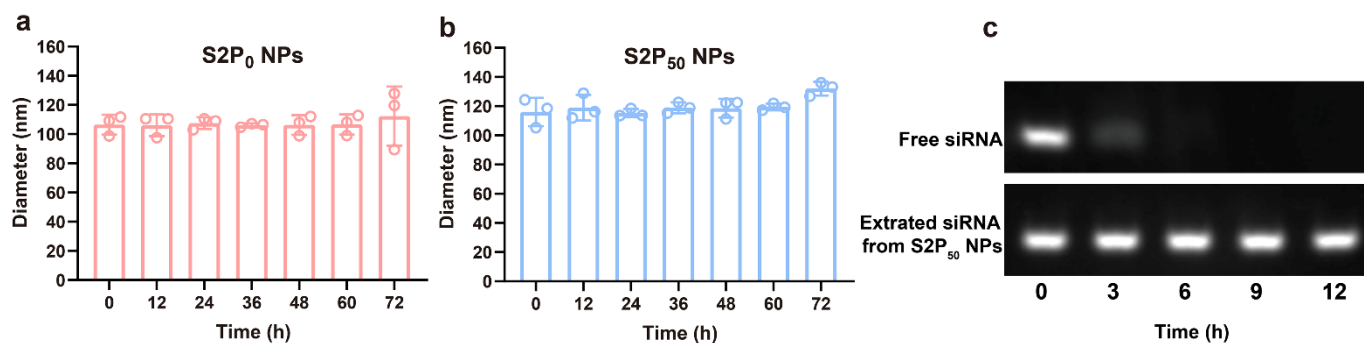
¹Center for Nanomedicine and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. ²Department of Molecular and Cellular Physiology, LSU Health Shreveport, Shreveport, LA, 71130, USA. ³Department of Medicine, Columbia University Irving Medical Center,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

[†]These authors contributed equally to this work.

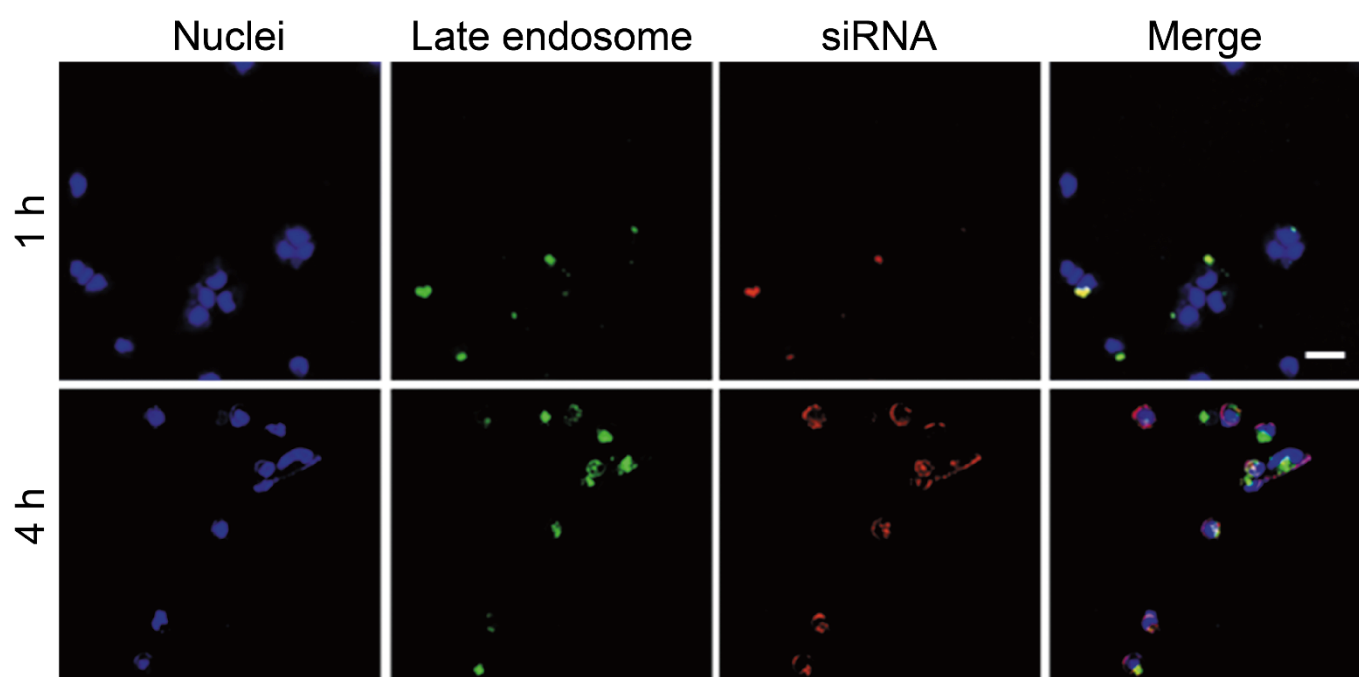
*Corresponding author. Email: wtao@bwh.harvard.edu

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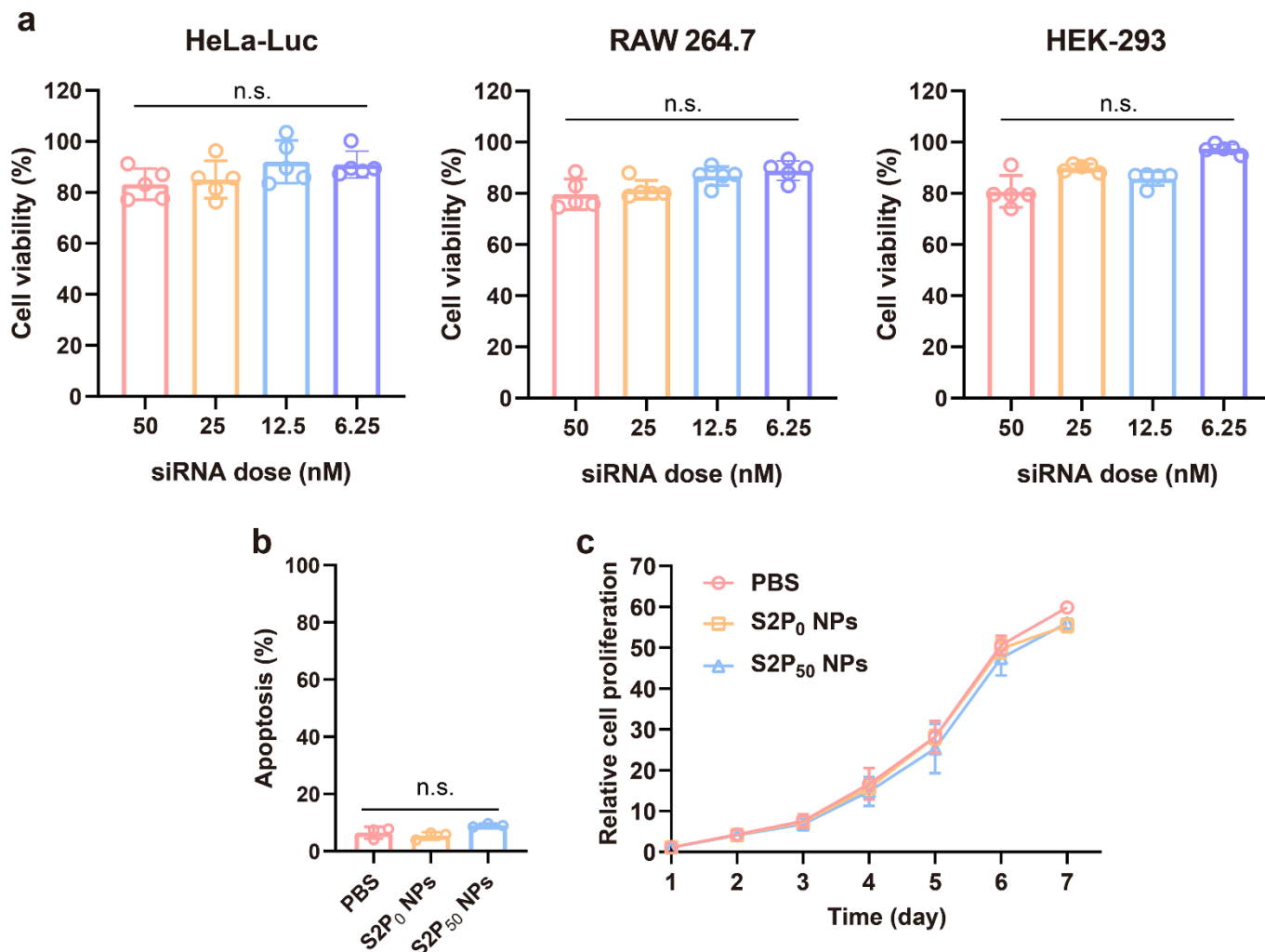
Supplementary Figure 1. ^1H NMR analysis. **a**, G0-C14 (CDCl_3). Adapted with permission from ref.¹, National Academy of Sciences; **b**, DSPE-PEG-S2P (CDCl_3). Adapted with permission from ref.², American Association for the Advancement of Science.



Supplementary Figure 2. Serum stability and protective effect of siRNA-loaded NPs for siRNA. **a-b**, Stability of S2P₀ and S2P₅₀ NPs in PBS solution containing 10% serum at 37 °C 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 extrated siRNA from S2P₅₀ 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 S2P₅₀ 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 S2P₅₀ 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 \pm SEM. **b**, RAW 264.7 cells were treated with PBS, S2P₀ NPs and S2P₅₀ 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, S2P₀ NPs and S2P₅₀ 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.

References

- 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 CaMKII γ in lesional macrophages improve atherosclerotic plaque stability in mice. *Sci. Transl. Med.* **12**, eaay1063 (2020).