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# Supplementary Materials for

## Functionalized lipid-like nanoparticles for in vivo mRNA delivery and base editing

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Published 21 August 2020, *Sci. Adv.* **6**, eabc2315 (2020)
DOI: 10.1126/sciadv.abc2315

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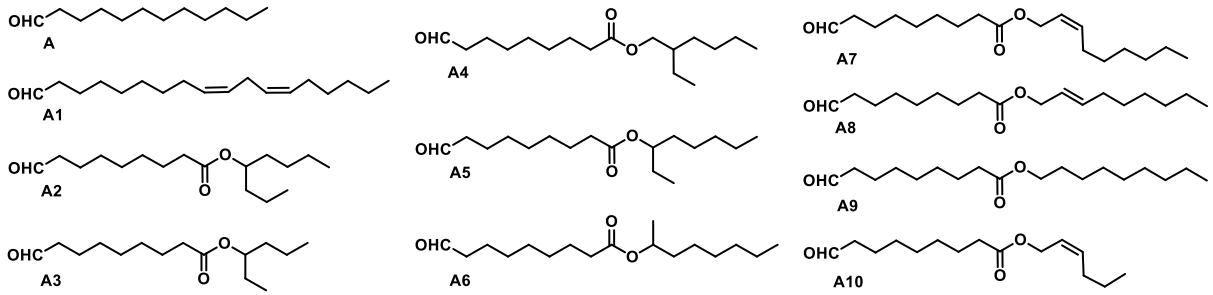
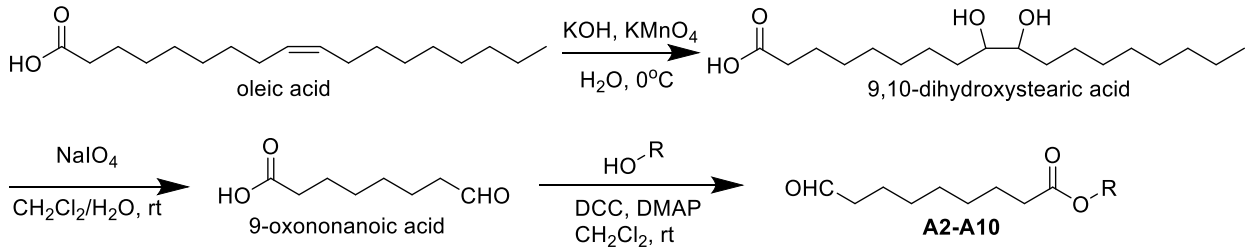
**Synthesis of FTT compounds**

General method for synthesizing aldehydes (**A1-A10**).




**A1** was synthesized according to the reported method.(*24*) 9-oxononanoic acid and 9-Oxononanoic acid esters (**A2-A10**) were synthesized according to the reported method as described below.(*41, 42*)



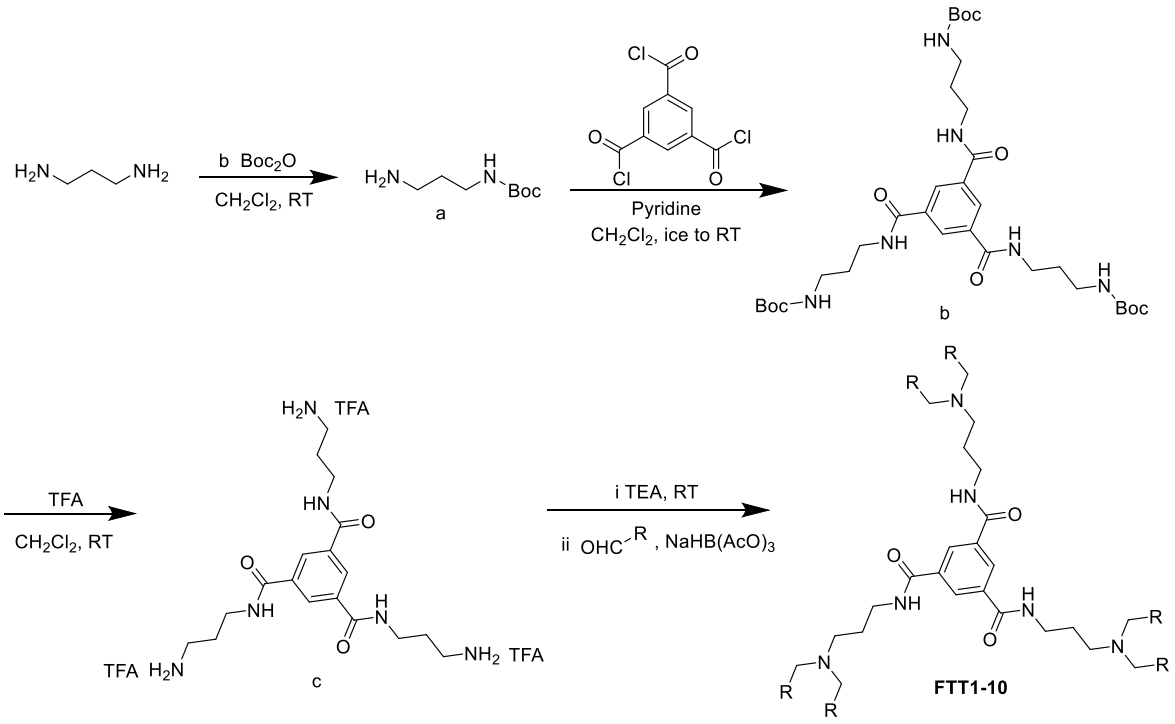

Generally, oleic acid 90% (5.0 g, 17.7 mmol) was added to an aqueous solution of KOH 1.25% (400 mL). The resulting mixture was stirred at 50 °C till a clear solution was formed. The mixture was cooled to 0 °C after another aqueous solution of KOH 1.25% (400 mL) was added. To the resulting mixture powdered $KMnO_4$ (5.0 g, 31.84 mmol) was added and stirred for 15 min at 0 °C. The reacting was quenched by addition of 50 mL of sodium thiosulfate (10% aqueous) and 10 ml of sodium sulfite (20% aqueous). The resulting brown solution was acidified with 50 mL of 37% HCl solution till a white fluffy precipitate was formed and filtered. The white product was crystallized from ethanol to yield 9,10-dihydroxystearic acid.

$NaIO_4$ (3 eq) in 15 mL was added to 9,10-dihydroxystearic acid (400 mg) in 15 mL DCM at room temperature. The resulting mixture was sitrred at room temperature overnight. The two layers were separated in a separating funnel. The aqueous layer was extracted with DCM twice. The organic phases were combined and dried over $Na_2SO_4$. After removing the solvent, the resulting product was purified by silica column using hexanes and ethyl acetate (1:1) to yield 9,10-dihydroxystearic acid as colorless oil.

9-oxononanoic acid (500 mg, 3.2 mmol) and DCC (700 mg, 3.4 mmol) were dissolved in 15 mL $CH_2Cl_2$ and cooled to 0 °C. After adding DMAP (10 mg, 0.1 mmol), the reacting mixture was stirred for 30 min at 0 °C. 1.5 eq vary alcohol was added and stirred for 2 h at 0 °C. The reacting mixture was warmed to RT and stirred overnight. The reacting mixture was then washed with water twice. The organic phase was dried with sodium sulfate and $CH_2Cl_2$ was evaporated. The crude product was purified by silica column using hexane and ethyl acetate (95:5) to give the corresponding product as a colorless oil.

General method for synthesizing **FTT1-10**.

**FTT1-10** were synthesized according to the reported method as described below.




Generally, a solution of $Boc_2O$ (14.4 mmol) in $CHCl_3$ (30 mL) was added to 1,3-diaminopropane (75 mL) in $CHCl_3$ (70 mL) dropwise over 3 h. 100 mL of $NaHCO_3$ (1N) was slowly added to the above reacting mixture under stirring. The organic layer was washed with 100 mL of $NaHCO_3$ (1N) and 20 mL of brine, then dried over $MgSO_4$. The solvent was removed to yield compound a.

10 mL of pyridine was added to a solution of Benzene-1,3,5-tricarbonyl trichloride (1.9 mmol) in $CH_2Cl_2$ (30 mL) at 0 °C. A solution of compound a (7.5 mmol) in $CH_2Cl_2$ (30 mL) was added dropwise to the above reacting mixture with stirring. The reaction mixture was allowed to warm to RT, then washed with 50 mL of $NaHCO_3$, and 50 mL of brine. The organic layer dried over $MgSO_4$. After removing the solvent, the residue was purified by silica column using $CH_2Cl_2$ and ultra (85:15, ultra is a mixture of $CH_2Cl_2$/MeOH/$NH_4OH$=75/22/3) to yield compound b.

Trifluoroacetic acid (TFA, 14 mmol) was added to compound b (1.4 mmol) in DCM and stirred at RT for 1 h. The solvent was removed under reduced pressure to yield compound c, which was used for the next step without further purification.

Triethylamine (0.4 mmol) was added to compound c in 10 mL tetrahydrofuran and stirred for 30 min at room temperature. Aldehyde (1.2 mmol) and $NaBH(OAc)_3$ were added and stirred at RT for 24 h. After the solvent was removed, the residue was purified by silica column using $CH_2Cl_2$ and ultra (85:15, ultra is a mixture of $CH_2Cl_2$/MeOH/$NH_4OH$=75/22/3) to yield **FTT1-10**.

**FTT1**: yield 36%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.64 (3H, s), 8.39 (3H, s), 5.71 (6H, m), 5.37-5.31 (27H, m), 3.56-3.55 (5H, m), 3.00-2.91 (8H, m), 2.79-2.75 (19H, m), 2.07-2.0 (31H, m), 1.58 (11H, m), 1.37-1.25 (90H, m), 0.92-0.88 (18H, m). MS (*m/z*): $[M + H]^+$ calcd. for $C_{126}H_{223}N_6O_3$, 1868.7482; found, 1868.7478.

**FTT2**: yield 30%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.42 (4H, m), 4.92-4.86 (6H, m), 3.68-3.53 (5H, m), 2.65-2.56 (5H, m), 2.28-2.25 (12H, m), 1.80 (5H, m), 1.69-1.50 (48H, m), 1.30-1.26 (84H, m), 0.92-0.87 (36H, m). MS (*m/z*): $[M + H]^+$ calcd. for $C_{120}H_{223}N_6O_{15}$, 1988.6871; found, 1988.6903.

**FTT3**: yield 45%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.43-8.39 (5H, m), 4.85-4.81 (6H, m), 3.57-3.53 (5H, m), 2.63-2.60 (6H, m), 2.53-2.49 (10H, m), 2.29-2.25 (12H, m), 1.61-1.58

(6H, m), 1.57-1.47 (48H, m), 1.25 (60H, m), 0.92-0.86 (36H, m). MS (*m/z*): $[M + H]^+$ calcd. for $C_{108}H_{199}N_6O_{15}$, 1820.4993; found, 1820.5090.

**FTT4**: yield 27%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.42 (7H, s), 4.00-3.98 (12H, m), 3.71-3.68 (17H, m), 3.55-3.54 (6H, m), 2.64-2.50 (29H, m), 2.31-2.27 (12H, t, *J* = 8), 1.81-1.78 (6H, m), 1.71-1.68 (19H, m), 1.61-1.56 (8421H, m), 1.47 (14H, s), 1.38-1.26 (101H, m), 0.92-0.88 (36H, m). MS (*m/z*): $[M + H]^+$ calcd. for $C_{120}H_{223}N_6O_{15}$, 1988.6871; found, 1988.6834.

**FTT5**: yield 27%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.45-8.43 (3H, t, *J* = 4), 8.38 (3H, s), 4.86-4.79 (6H, m), 3.59-3.54 (6H, m), 2.63-2.60 (6H, t, *J* = 4), 2.53-2.49 (11H, t, *J* = 8), 2.30-2.26 (12H, t, *J* = 8), 1.78-1.75 (6H, m), 1.64-1.47 (49H, m), 1.29-1.26 (84H, m), 0.90-0.87 (36H, m). MS (*m/z*): $[M + H]^+$ calcd. for $C_{120}H_{223}N_6O_{15}$, 1988.6871; found, 1988.6841.

**FTT6**: yield 27%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.43-8.39 (5H, t, *J* = 4), 4.92-4.88 (6H, m), 3.57-3.55 (6H, m), 2.63-2.61 (6H, t, *J* = 4), 2.53-2.50 (11H, t, *J* = 8), 2.27-2.36 (12H, t, *J* = 8), 1.79-1.77 (6H, m), 1.60-1.55 (19H, m), 1.48-1.46 (19H, m), 1.28-1.19 (84H, m), 0.91-0.87 (18H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{120}H_{223}N_6O_{15}$, 1988.6871; found, 1988.6809.

**FTT7**: yield 78%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.45-8.40 (5H, m), 5.68-5.61 (6H, m), 5.56-5.49 (6H, m), 4.64-4.62 (12H, d, *J* = 8), 3.58-3.54 (6H, m), 2.64-2.62 (5H, t, *J* = 4), 2.54-2.50 (11H, t, *J* = 8), 2.31-2.27 (12H, t, *J* = 8), 2.13-2.08 (12H, m), 1.78 (5H, m), 1.60-1.55 (14H, m), 1.55-1.25 (114H, m), 0.91-0.88 (18H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{126}H_{223}N_6O_{15}$, 2060.6871; found, 2060.6816.

**FTT8**: yield 45%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.46-8.39 (6H, m), 5.80-5.73 (6H, m), 5.60-5.52 (6H, m), 4.52-4.50 (11H, m), 3.77-3.74 (2H, t, *J* = 4), 3.58-3.54 (6H, m), 2.63-2.60 (6H, t, *J* = 4), 2.53-2.49 (12H, t, *J* = 8), 2.30-2.27 (12H, t, *J* = 8), 2.08-2.03 (12H, m), 1.88-1.76 (6H, m), 1.62-1.55 (13H, m), 1.46-1.24 (109H, m), 0.91-0.87 (18H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{126}H_{223}N_6O_{15}$, 2060.6871; found, 2060.6870.

**FTT9**: yield 30%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.44-8.40 (5H, m), 4.07-4.04 (11H, m), 3.56-3.55 (4H, m), 3.63 (5H, m), 2.54-2.50 (10H, t, *J* = 8), 2.29-2.25 (11H, t, *J* = 8), 1.78 (5H, m), 1.63-1.56 (23H, m), 1.46 (11H, m), 1.31-1.24 (116H, m), 0.91-0.87 (18H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{126}H_{235}N_6O_{15}$, 2072.7810; found, 2072.7869.

**FTT10**: yield 50%, $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.47 (3H, s), 8.40 (3H, s), 5.67-5.60 (6H, m), 5.57-5.50 (6H, m), 4.63-4.61 (11H, m), 3.55-3.54 (6H, m), 2.69-2.66 (6H, m), 2.58-2.54 (12H, m), 2.30-2.26 (12H, t, *J* = 8), 2.11-2.03 (14H, m), 1.83-1.80 (6H, t, *J* = 4), 1.61-1.57 (13H, m), 1.48-1.37 (25H, m), 1.25 (52H, s), 0.93-0.89 (18H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{108}H_{187}N_6O_{15}$, 1808.4054; found, 1808.4028.

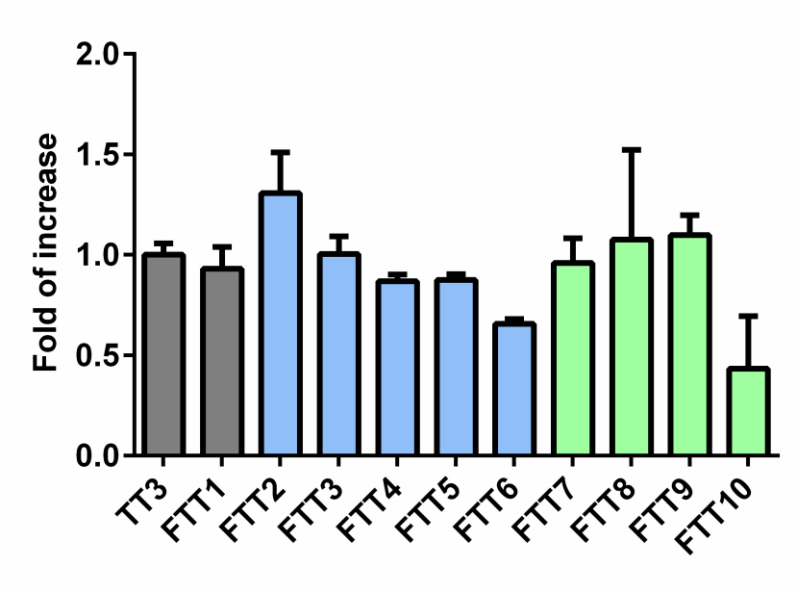



**fig. S1. *In vitro* screening of FTT1-10 LLNs.** *In vitro* delivery efficiency of FLuc mRNA encapsulated FTT1-10 LLNs as compared to TT3 LLNs in Hep3B cells. FTT1-10 possess three different types of lipid side chains, which are presented as grey (carbon chain), blue (branched ester chain), and green (linear ester chain), respectively.

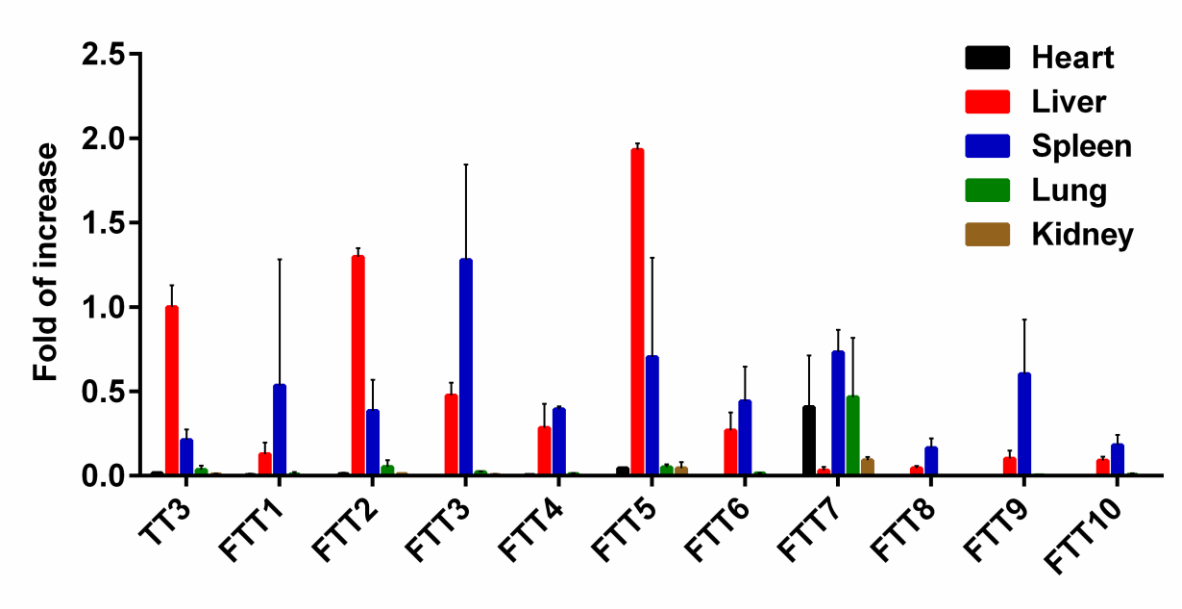



**fig. S2. *In vivo* biodistributions of FTT1-10 LLNs as compared to TT3 LLNs.** *In vivo* biodistributions of FLuc mRNA encapsulated FTT1-10 LLNs in the heart, liver, spleen, lung and kidneys of mice as compared to TT3 LLNs. ($n$ = 2) All the bioluminescence intensity data were normalized to that in the livers of TT3 LLNs treated mice.

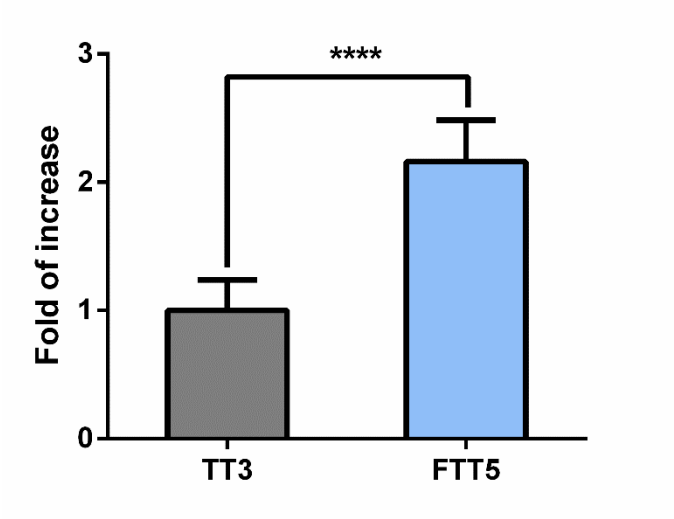



**fig. S3. *In vivo* luciferase expression mediated by TT3 and FTT5 LLNs in the livers of mice.** *In vivo* luciferase expression of FLuc mRNA encapsulated FTT5 LLNs in the livers of mice as compared to TT3 LLNs. ($n$ = 6; two-tailed student $t$-test; ****, $P < 0.0001$)

A

| | FTT5 | DOPE | Cholesterol | DMG-PEG$_{2000}$ |
|---|---|---|---|---|
| 1 | 10 | 20 | 25 | 0.5 |
| 2 | 10 | 30 | 30 | 0.75 |
| 3 | 10 | 40 | 35 | 1 |
| 4 | 10 | 50 | 40 | 1.25 |
| 5 | 20 | 20 | 30 | 1 |
| 6 | 20 | 30 | 25 | 1.25 |
| 7 | 20 | 40 | 40 | 0.5 |
| 8 | 20 | 50 | 35 | 0.75 |
| 9 | 30 | 20 | 35 | 1.25 |
| 10 | 30 | 30 | 40 | 1 |
| 11 | 30 | 40 | 25 | 0.75 |
| 12 | 30 | 50 | 30 | 0.5 |
| 13 | 40 | 20 | 40 | 0.75 |
| 14 | 40 | 30 | 35 | 0.5 |
| 15 | 40 | 40 | 30 | 1.25 |
| 16 | 40 | 50 | 25 | 1 |

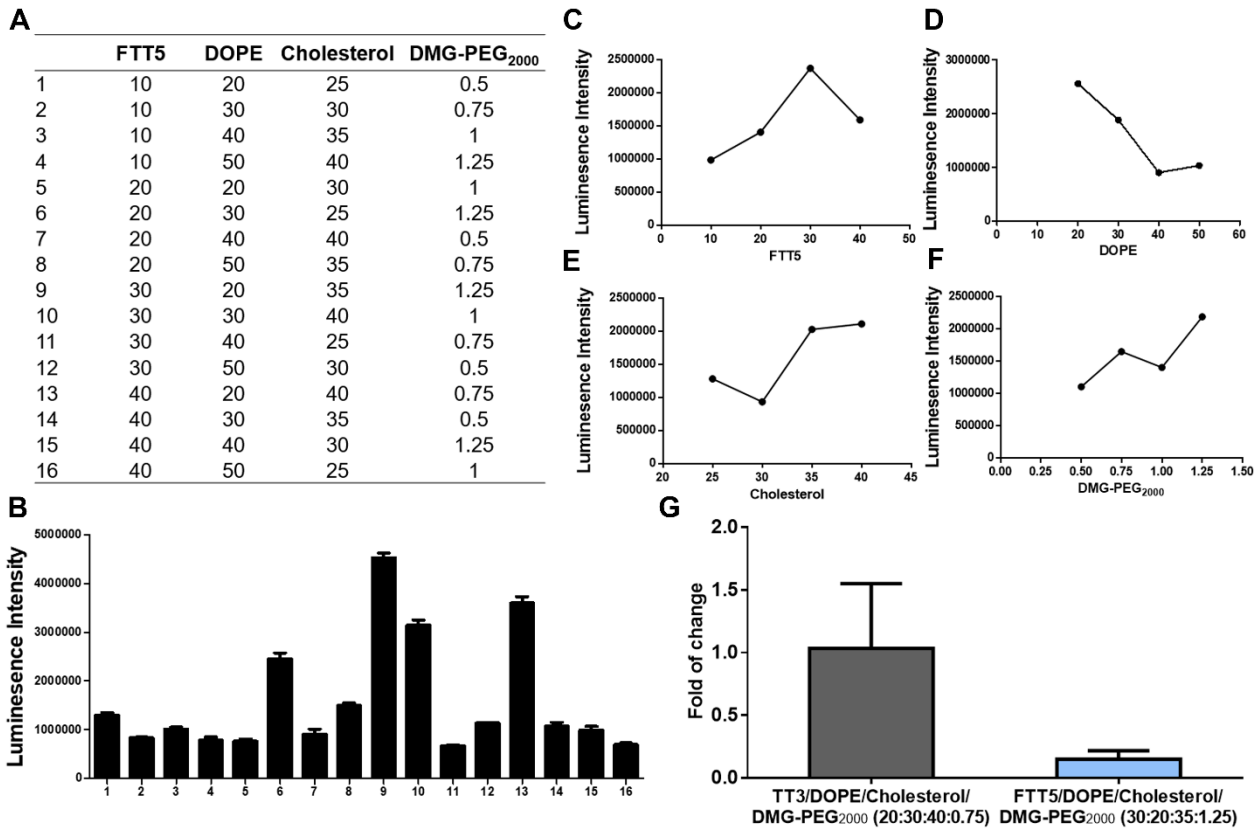
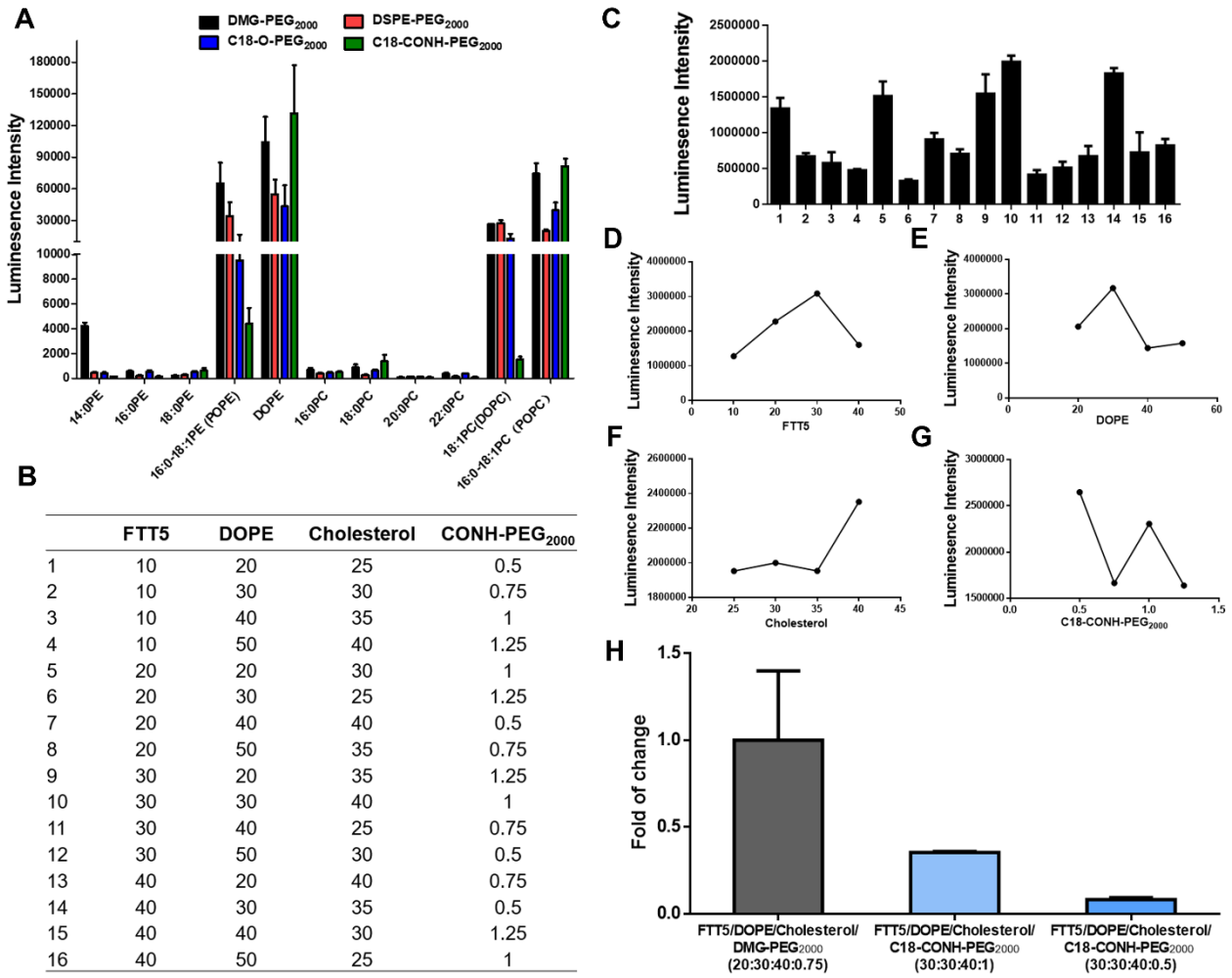



**fig. S4. An *in vitro* optimization of FTT5 LLNs on formulation components ratio through orthogonal array analysis. (A)** A four-parameter and four-level orthogonal array was adopted for the optimization of FTT5 LLNs on formulation components ratio. 16 FTT5 formulations generated from the orthogonal array design were listed in the table. **(B)** Luciferase expression levels of 16 different FTT5 LLNs in the orthogonal array chart. **(C), (D)**, **(E)**, and **(F)** The impact trend of FTT5 lipids, DOPE, cholesterol and DMG-PEG$_{2000}$, respectively, on the *in vitro* mRNA delivery efficiency of FTT5 LLNs. **(G)** Evaluation on *in vivo* delivery efficiency of the optimal FTT5 LLNs (FTT5/DOPE/Cholesterol/DMG-PEG$_{2000}$=30:20:35:1.25) identified through the orthogonal design as compared with the previously reported TT3 LLNs in the livers of mice. ($n$ = 3)




| | FTT5 | DOPE | Cholesterol | CONH-PEG$_{2000}$ |
|---|---|---|---|---|
| 1 | 10 | 20 | 25 | 0.5 |
| 2 | 10 | 30 | 30 | 0.75 |
| 3 | 10 | 40 | 35 | 1 |
| 4 | 10 | 50 | 40 | 1.25 |
| 5 | 20 | 20 | 30 | 1 |
| 6 | 20 | 30 | 25 | 1.25 |
| 7 | 20 | 40 | 40 | 0.5 |
| 8 | 20 | 50 | 35 | 0.75 |
| 9 | 30 | 20 | 35 | 1.25 |
| 10 | 30 | 30 | 40 | 1 |
| 11 | 30 | 40 | 25 | 0.75 |
| 12 | 30 | 50 | 30 | 0.5 |
| 13 | 40 | 20 | 40 | 0.75 |
| 14 | 40 | 30 | 35 | 0.5 |
| 15 | 40 | 40 | 30 | 1.25 |
| 16 | 40 | 50 | 25 | 1 |

**fig. S5. An *in vitro* optimization of FTT5 LLNs with C18-CONH-PEG$_{2000}$ on formulation components ratio through an orthogonal array analysis. (A)** The DOPE and DMG-PEG$_{2000}$ in FTT5 LLNs were replaced by several different helper lipids or PEG with different lipid chains, respectively. **(B)** A four-parameter and four-level orthogonal array was adopted for the optimization of FTT5 LLNs with C18-CONH-PEG$_{2000}$ on formulation components ratio. 16 FTT5 formulations generated from the orthogonal array design were listed in the table. **(C)** Luciferase expression levels of 16 different FTT5 LLNs with C18-CONH-PEG$_{2000}$ in the orthogonal array. **(D)**, **(E)**, **(F)**, and **(G)** The impact trend of FTT5 lipids, DOPE, cholesterol and C18-CONH-PEG$_{2000}$, respectively, on the *in vitro* mRNA delivery efficiency of FTT5 LLNs with C18-CONH-PEG$_{2000}$. **(H)** Evaluation on *in vivo* mRNA delivery efficiency of two optimal FTT5 LLNs with C18-CONH-PEG$_{2000}$ (FTT5/DOPE/Cholesterol/ C18-CONH-PEG$_{2000}$=30:30:40:1 or 30:30:40:0.5) identified through the orthogonal design as compared with original FTT5 LLNs in the livers of mice. ($n$ = 2 or 3)

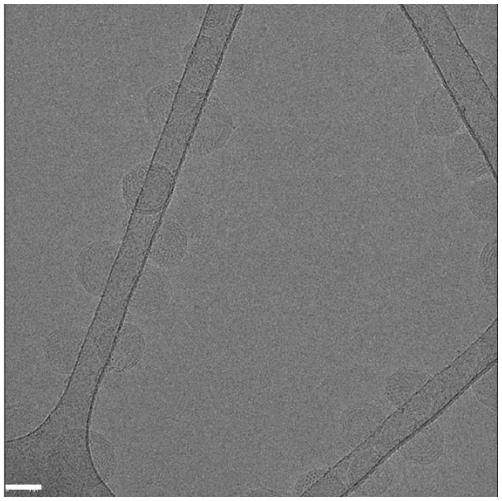


**fig. S6. Cryo-TEM image of FTT5 LLNs.** Scale bar = 50 nm.

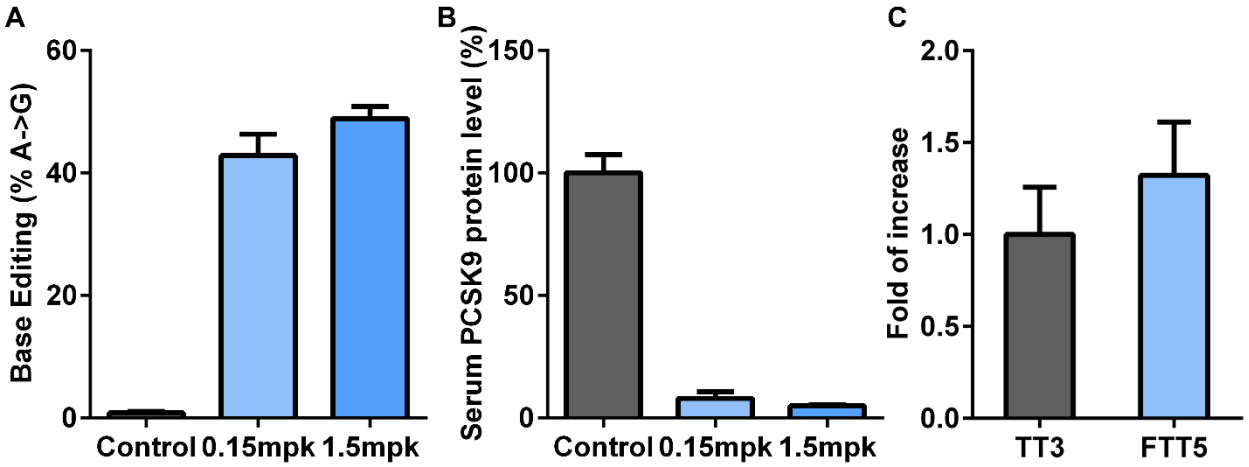



**fig. S7. *In vivo* base editing using TT3 and FTT5 LLNs. (A)** *In vivo* base editing efficiency on the PCSK9 gene using TT3 LLNs at 0.15 and 1.5 mg/kg doses. PBS was used as a control. ($n$ = 3) **(B)** Serum PCSK9 protein level after base editing of the PCSK9 gene using TT3 LLNs at 0.15 and 1.5 mg/kg doses. PBS was used as a control. ($n$ = 3) **(C)** A comparison of *in vivo* base editing efficiency between TT3 and FTT5 LLNs at a dose of 0.5 mg/kg. ($n$ = 3 for TT3 group, $n$ = 6 for FTT5 group)

**table. S1. Size and PDI of FTT5 LLNs used in *in vitro* bioassays.**

| | Size (nm) | PDI |
|---|---|---|
| **FLuc mRNA encapsulated FTT5 LLNs** | 160.53 ± 2.87 | 0.16 ± 0.03 |
| **Alexa-Fluor 647-labelled RNA containing FTT5 LLNs** | 148.87 ± 2.94 | 0.19 ± 0.03 |

**REFERENCES AND NOTES**


1. K. A. Hajj, K. A. Whitehead, Tools for translation: Non-viral materials for therapeutic mRNA delivery. *Nat. Rev. Mater.* **2**, 17056 (2017).
2. U. Sahin, K. Karikó, Ö. Türeci, mRNA-based therapeutics—developing a new class of drugs. *Nat. Rev. Drug Discov.* **13**, 759–780 (2014).
3. Q. Xiong, G. Y. Lee, J. Ding, W. Li, J. Shi, Biomedical applications of mRNA nanomedicine. *Nano Res.* **11**, 5281–5309 (2018).
4. N. Pardi, M. J. Hogan, F. W. Porter, D. Weissman, mRNA vaccines – a new era in vaccinology. *Nat. Rev. Drug Discov.* **17**, 261–279 (2018).
5. S. Patel, A. Athirasala, P. P. Menezes, N. Ashwanikumar, T. Zou, G. Sahay, L. E. Bertassoni, Messenger RNA delivery for tissue engineering and regenerative medicine applications. *Tissue Eng. Part A* **25**, 91–112 (2019).
6. P. S. Kowalski, A. Rudra, L. Miao, D. G. Anderson, Delivering the messenger: Advances in technologies for therapeutic mRNA delivery. *Mol. Ther.* **27**, 710–728 (2019).
7. W. Zhao, X. Hou, O. G. Vick, Y. Dong, RNA delivery biomaterials for the treatment of genetic and rare diseases. *Biomaterials* **217**, 119291 (2019).
8. M. A. Islam, E. K. G. Reesor, Y. Xu, H. R. Zope, B. R. Zetter, J. Shi, Biomaterials for mRNA delivery. *Biomater. Sci.* **3**, 1519–1533 (2015).
9. B. Li, X. Zhang, Y. Dong, Nanoscale platforms for messenger RNA delivery. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **11**, e1530 (2019).
10. H.-X. Wang, M. Li, C. M. Lee, S. Chakraborty, H.-W. Kim, G. Bao, K. W. Leong, CRISPR/Cas9-based genome editing for disease modeling and therapy: Challenges and opportunities for nonviral delivery. *Chem. Rev.* **117**, 9874–9906 (2017).
11. L. Li, S. Hu, X. Chen, Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. *Biomaterials* **171**, 207–218 (2018).
12. C. Liu, L. Zhang, H. Liu, K. Cheng, Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *J. Control. Release* **266**, 17–26 (2017).
13. J. B. Miller, D. J. Siegwart, Design of synthetic materials for intracellular delivery of RNAs: From siRNA-mediated gene silencing to CRISPR/Cas gene editing. *Nano Res.* **11**, 5310–5337 (2018).
14. J. Liu, J. Chang, Y. Jiang, X. Meng, T. Sun, L. Mao, Q. Xu, M. Wang, Fast and efficient CRISPR/Cas9 genome editing in vivo enabled by bioreducible lipid and messenger RNA nanoparticles. *Adv. Mater.* **31**, e1902575 (2019).

15. Y. B. Kim, A. C. Komor, J. M. Levy, M. S. Packer, K. T. Zhao, D. R. Liu, Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nat. Biotechnol.* **35**, 371–376 (2017).
16. H. A. Rees, D. R. Liu, Base editing: Precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* **19**, 770–788 (2018).
17. A. C. Rossidis, J. D. Stratigis, A. C. Chadwick, H. A. Hartman, N. J. Ahn, H. Li, K. Singh, B. E. Coons, L. Li, W. Lv, P. W. Zoltick, D. Alapati, W. Zacharias, R. Jain, E. E. Morrisey, K. Musunuru, W. H. Peranteau, In utero CRISPR-mediated therapeutic editing of metabolic genes. *Nat. Med.* **24**, 1513–1518 (2018).
18. N. M. Gaudelli, A. C. Komor, H. A. Rees, M. S. Packer, A. H. Badran, D. I. Bryson, D. R. Liu, Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 (2017).
19. T. Jiang, J. M. Henderson, K. Coote, Y. Cheng, H. C. Valley, X.-O. Zhang, Q. Wang, L. H. Rhym, Y. Cao, G. A. Newby, H. Bihler, M. Mense, Z. Weng, D. G. Anderson, A. P. McCaffrey, D. R. Liu, W. Xue, Chemical modifications of adenine base editor mRNA and guide RNA expand its application scope. *Nat. Commun.* **11**, 1979 (2020).
20. C.-Q. Song, T. Jiang, M. Richter, L. H. Rhym, L. W. Koblan, M. P. Zafra, E. M. Schatoff, J. L. Doman, Y. Cao, L. E. Dow, L. J. Zhu, D. G. Anderson, D. R. Liu, H. Yin, W. Xue, Adenine base editing in an adult mouse model of tyrosinaemia. *Nat. Biomed. Eng.* **4**, 125–130 (2020).
21. J. M. Levy, W.-H. Yeh, N. Pendse, J. R. Davis, E. Hennessey, R. Butcher, L. W. Koblan, J. Comander, Q. Liu, D. R. Liu, Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses. *Nat. Biomed. Eng.* **4**, 97–110 (2020).
22. M. P. Lokugamage, Z. Gan, C. Zurla, J. Levin, F. Z. Islam, S. Kalathoor, M. Sato, C. D. Sago, P. J. Santangelo, J. E. Dahlman, Mild innate immune activation overrides efficient nanoparticle-mediated RNA delivery. *Adv. Mater.* **32**, e1904905 (2020).
23. M. M. Billingsley, N. Singh, P. Ravikumar, R. Zhang, C. H. June, M. J. Mitchell, Ionizable lipid nanoparticle-mediated mRNA delivery for human CAR T cell engineering. *Nano Lett.* **20**, 1578–1589 (2020).
24. O. S. Fenton, K. J. Kauffman, R. L. McClellan, E. A. Appel, J. R. Dorkin, M. W. Tibbitt, M. W. Heartlein, F. DeRosa, R. Langer, D. G. Anderson, Bioinspired alkenyl amino alcohol ionizable lipid materials for highly potent in vivo mRNA delivery. *Adv. Mater.* **28**, 2939–2943 (2016).

25. K. J. Kauffman, J. R. Dorkin, J. H. Yang, M. W. Heartlein, F. DeRosa, F. F. Mir, O. S. Fenton, D. G. Anderson, Optimization of lipid nanoparticle formulations for mRNA delivery in vivo with fractional factorial and definitive screening designs. *Nano Lett.* **15**, 7300–7306 (2015).
26. P. Midoux, C. Pichon, Lipid-based mRNA vaccine delivery systems. *Expert Rev. Vaccines* **14**, 221–234 (2015).
27. O. S. Fenton, K. J. Kauffman, J. C. Kaczmarek, R. L. McClellan, S. Jhunjhunwala, M. W. Tibbitt, M. D. Zeng, E. A. Appel, J. R. Dorkin, F. F. Mir, J. H. Yang, M. A. Oberli, M. W. Heartlein, F. DeRosa, R. Langer, D. G. Anderson, Synthesis and biological evaluation of ionizable lipid materials for the in vivo delivery of messenger RNA to B lymphocytes. *Adv. Mater.* **29**, 1606944 (2017).
28. B. Li, X. Luo, B. Deng, J. Wang, D. W. McComb, Y. Shi, K. M. L. Gaensler, X. Tan, A. L. Dunn, B. A. Kerlin, Y. Dong, An orthogonal array optimization of lipid-like nanoparticles for mRNA delivery in vivo. *Nano Lett.* **15**, 8099–8107 (2015).
29. E. Robinson, K. D. MacDonald, K. Slaughter, M. McKinney, S. Patel, C. Sun, G. Sahay, Lipid nanoparticle-delivered chemically modified mRNA restores chloride secretion in cystic fibrosis. *Mol. Ther.* **26**, 2034–2046 (2018).
30. L. Jiang, P. Berraondo, D. Jericó, L. T. Guey, A. Sampedro, A. Frassetto, K. E. Benenato, K. Burke, E. Santamaría, M. Alegre, Á. Pejenaute, M. Kalariya, W. Butcher, J.-S. Park, X. Zhu, S. Sabnis, E. S. Kumarasinghe, T. Salerno, M. Kenney, C. M. Lukacs, M. A. Ávila, P. G. V. Martini, A. Fontanellas, Systemic messenger RNA as an etiological treatment for acute intermittent porphyria. *Nat. Med.* **24**, 1899–1909 (2018).
31. Q. Cheng, T. Wei, Y. Jia, L. Farbiak, K. Zhou, S. Zhang, Y. Wei, H. Zhu, D. J. Siegwart, Dendrimer-based lipid nanoparticles deliver therapeutic FAH mRNA to normalize liver function and extend survival in a mouse model of hepatorenal tyrosinemia type I. *Adv. Mater.* **30**, e1805308 (2018).
32. L. Miao, L. Li, Y. Huang, D. Delcassian, J. Chahal, J. Han, Y. Shi, K. Sadtler, W. Gao, J. Lin, J. C. Doloff, R. Langer, D. G. Anderson, Delivery of mRNA vaccines with heterocyclic lipids increases anti-tumor efficacy by STING-mediated immune cell activation. **37**, 1174–1185 (2019).
33. J. D. Finn, A. R. Smith, M. C. Patel, L. Shaw, M. R. Youniss, J. van Heteren, T. Dirstine, C. Ciullo, R. Lescarbeau, J. Seitzer, R. R. Shah, A. Shah, D. Ling, J. Growe, M. Pink, E. Rohde, K. M. Wood, W. E. Salomon, W. F. Harrington, C. Dombrowski, W. R. Strapps, Y. Chang, D. V. Morrissey, A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Rep.* **22**, 2227–2235 (2018).

34. C. Jiang, M. Mei, B. Li, X. Zhu, W. Zu, Y. Tian, Q. Wang, Y. Guo, Y. Dong, X. Tan, A non-viral CRISPR/Cas9 delivery system for therapeutically targeting HBV DNA and *pcsk9* in vivo. *Cell Res.* **27**, 440–443 (2017).
35. D. E. Sabatino, T. C. Nichols, E. Merricks, D. A. Bellinger, R. W. Herzog, P. E. Monahan, Animal models of hemophilia. *Prog. Mol. Biol. Transl. Sci.* **105**, 151–209 (2012).
36. A. M. Lange, E. S. Altynova, G. N. Nguyen, D. E. Sabatino, Overexpression of factor VIII after AAV delivery is transiently associated with cellular stress in hemophilia A mice. *Mol. Ther. Methods Clin. Dev.* **3**, 16064 (2016).
37. J. I. Siner, N. P. Iacobelli, D. E. Sabatino, L. Ivanciu, S. Zhou, M. Poncz, R. M. Camire, V. R. Arruda, Minimal modification in the factor VIII B-domain sequence ameliorates the murine hemophilia A phenotype. *Blood* **121**, 4396–4403 (2013).
38. X. Hou, X. Zhang, W. Zhao, C. Zeng, B. Deng, D. W. McComb, S. Du, C. Zhang, W. Li, Y. Dong, Vitamin lipid nanoparticles enable adoptive macrophage transfer for the treatment of multidrug-resistant bacterial sepsis. *Nat. Nanotechnol.* **15**, 41–46 (2020).
39. G. N. Nguyen, L. A. George, J. I. Siner, R. J. Davidson, C. B. Zander, X. L. Zheng, V. R. Arruda, R. M. Camire, D. E. Sabatino, Novel factor VIII variants with a modified furin cleavage site improve the efficacy of gene therapy for hemophilia A. *J. Thromb. Haemost.* **15**, 110–121 (2017).
40. S. I. Chavin, Factor VIII: Structure and function in blood clotting. *Am. J. Hematol.* **16**, 297–306 (1984).
41. M. Rajabi, M. Lanfranchi, F. Campo, L. Panza, Synthesis of a series of hydroxycarboxylic acids as standards for oxidation of nonanoic acid. *Synth. Commun.* **44**, 1149–1154 (2014).
42. X. Zhang, B. Li, X. Luo, W. Zhao, J. Jiang, C. Zhang, M. Gao, X. Chen, Y. Dong, Biodegradable amino-ester nanomaterials for Cas9 mRNA delivery in vitro and in vivo. *ACS Appl. Mater. Interfaces* **9**, 25481–25487 (2017).