Supporting Information

*Margaret M. Billingsley[1], Nathan Singh[2,3], Pranali Ravikumar[2], Rui Zhang[1], Carl H. June[4,5], and Michael J. Mitchell[1,4,6,7,8#]*

[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

[2]Division of Oncology, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

[3]Division of Oncology, Section of Stem Cell Biology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, United States

[4]Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

[5] Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

[6] Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[7] Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[8] Institute for Regenerative Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[#]To whom correspondence should be addressed: mjmitch@seas.upenn.edu

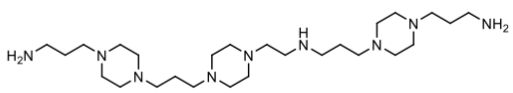
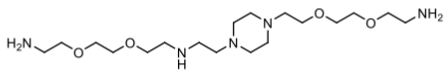
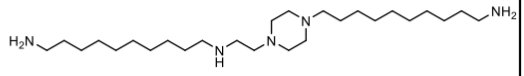
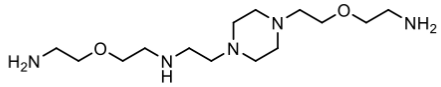
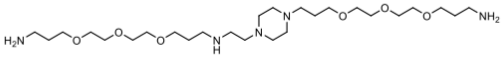
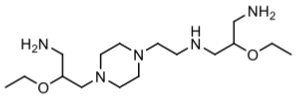
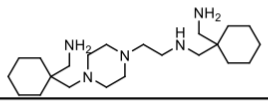
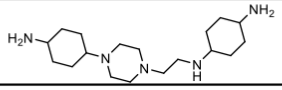
| Amine Core (#) | Name | Structure |
|---|---|---|
| 1 | 3-[4-(3-{[2-(4-{3-[4-(3-aminopropyl)piperazin-1-yl]propyl}piperazin-1-yl)ethyl]amino}propyl)piperazin-1-yl]propan-1-amine |  |
| 2 | {2-[2-(2-aminoethoxy)ethoxy]ethyl}[2-(4-{2-[2-(2-aminoethoxy)ethoxy]ethyl}piperazin-1-yl)ethyl]amine |  |
| 3 | 10-(4-{2-[(10-aminodecyl)amino]ethyl}piperazin-1-yl)decan-1-amine |  |
| 4 | 2-{2-[4-(2-{[2-(2-aminoethoxy)ethyl]amino}ethyl)piperazin-1-yl]ethoxy}ethan-1-amine |  |
| 5 | 1-[4-(3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propyl)piperazin-1-yl]-7,10,13-trioxa-3-azahexadecan-16-amine |  |
| 6 | 3-(4-{2-[(3-amino-2-ethoxypropyl)amino]ethyl}piperazin-1-yl)-2-ethoxypropan-1-amine |  |
| 7 | [1-({[2-(4-{[1 (aminomethyl) cyclohexyl]methyl}piperazin-1- yl) ethyl] amino}methyl)cyclohexyl]methanamine |  |
| 8 | 1-N-{2-[4-(4-aminocyclohexyl)piperazin-1-yl]ethyl}cyclohexane-1,4-diamine |  |

Table S1. Amine Cores. The named structures of the amine cores used to generate the ionizable lipid library.

| Formulation | Diameter (nm) | PDI | Luciferase mRNA (ng/uL) |
|---|---|---|---|
| **C12-1** | 68.85 ± 1.07 | .094 ± .007 | 40.25 ± 0.07 |
| **C12-2** | 82.92 ± 1.21 | .238 ± .006 | 38.65 ± 0.78 |
| **C12-3** | 51.22 ± 0.66 | .158 ± .013 | 45.8 ± 0.28 |
| **C12-4** | 51.05 ± 2.75 | .219 ± .015 | 36.25 ± 1.20 |
| **C12-5** | 65.6 ± 1.13 | .203 ± .015 | 37.05 ± 0.21 |
| **C12-6** | 91.79 ± 1.17 | .115 ± .018 | 39.8 ± 1.13 |
| **C12-7** | 64.73 ± 2.88 | .219 ± .021 | 37.55 ± 0.07 |
| **C12-8** | 70.25 ± 0.75 | .086 ± .003 | 47.55 ± 0.49 |
| **C14-1** | 73.15 ± 2.23 | .176 ± .052 | 34.3 ± 0.85 |
| **C14-2** | 75.61 ± 1.21 | .179 ± .136 | 35.25 ± 0.35 |
| **C14-3** | 74.09 ± 0.35 | .238 ± .009 | 40.4 ± 1.13 |
| **C14-4** | 70.17 ± 0.41 | .176 ± .006 | 35.6 ± 2.12 |
| **C14-5** | 64.57 ± 3.10 | .232 ± .007 | 34.25 ± 0.49 |
| **C14-6** | 64.71 ± 1.73 | .129 ± .020 | 36.8 ± 2.12 |
| **C14-7** | 67.14 ± 1.81 | .175 ± .027 | 39.7 ± 1.56 |
| **C14-8** | 65.44 ± 0.51 | .091 ± .004 | 40.3 ± 2.40 |
| **C16-1** | 83.82 ± 3.16 | .029 ± .014 | 46.85 ± 0.50 |
| **C16-2** | 97.01 ± 2.57 | .14 ± .023 | 36.1 ± 0.99 |
| **C16-3** | 82.72 ± 2.32 | .113 ± .010 | 40.3 ± 1.84 |
| **C16-4** | 68.12 ± 1.41 | .211 ± .009 | 38 ± 1.27 |
| **C16-5** | 75.82 ± 0.91 | .284 ± .029 | 35.95 ± 0.64 |
| **C16-6** | 96.03 ± 1.95 | .053 ± .031 | 37.35 ± 0.21 |
| **C16-7** | 89.46 ± 0.59 | .188 ± .009 | 33.3 ± 4.67 |
| **C16-8** | 87.7 ± 0.33 | .101 ± .004 | 48.3 ± 0.85 |

Table S2: Library Characterization. The diameter (z-average), PDI, and mRNA concentration of each LNP formulation showing a narrow range in LNP size, monodispersity, and similar mRNA loading across LNP formulations. (± standard deviation)

| **C14-4 LNP (luciferase mRNA)** | **Crude** | **Purified** |
|---|---|---|
| mRNA (ng/uL) ± St. Dev. | 35.6 ± 2.12 | 29.8 ± 2.31 |
| Encapsulation Efficiency (%) | 92.53 | 86.3 |
| Diameter (nm) ± St. Dev. | 70.17 ± 0.41 | 65.19 ± 0.83 |
| Polydispersity ± St. Dev. | .176 ± .006 | .189 ± .014 |
| pKa | 6.143 | 6.505 |

Table S3: Crude and Purified C14-4 LNPs. Comparison of characteristics of crude and purified C14-4 LNPs encapsulating luciferase mRNA.

A

| Lipid | pka |
|---|---|
| C12-1 | 7.054 |
| C12-2 | 6.589 |
| C12-3 | 6.52 |
| C12-4 | 6.655 |
| C12-5 | 5.771 |
| C12-6 | 6.026 |
| C12-7 | 6.758 |
| C12-8 | 5.706 |
| C14-1 | 7.049 |
| C14-2 | 5.543 |
| C14-3 | 5.782 |
| C14-4 | 5.889 |
| C14-5 | 6.596 |
| C14-6 | 5.993 |
| C14-7 | 7.317 |
| C14-8 | 7.406 |
| C16-1 | 6.261 |
| C16-2 | 4.597 |
| C16-3 | 6.2228 |
| C16-4 | 5.61 |
| C16-5 | 6.103 |
| C16-6 | 5.671 |
| C16-7 | 6.457 |
| C16-8 | 4.622 |

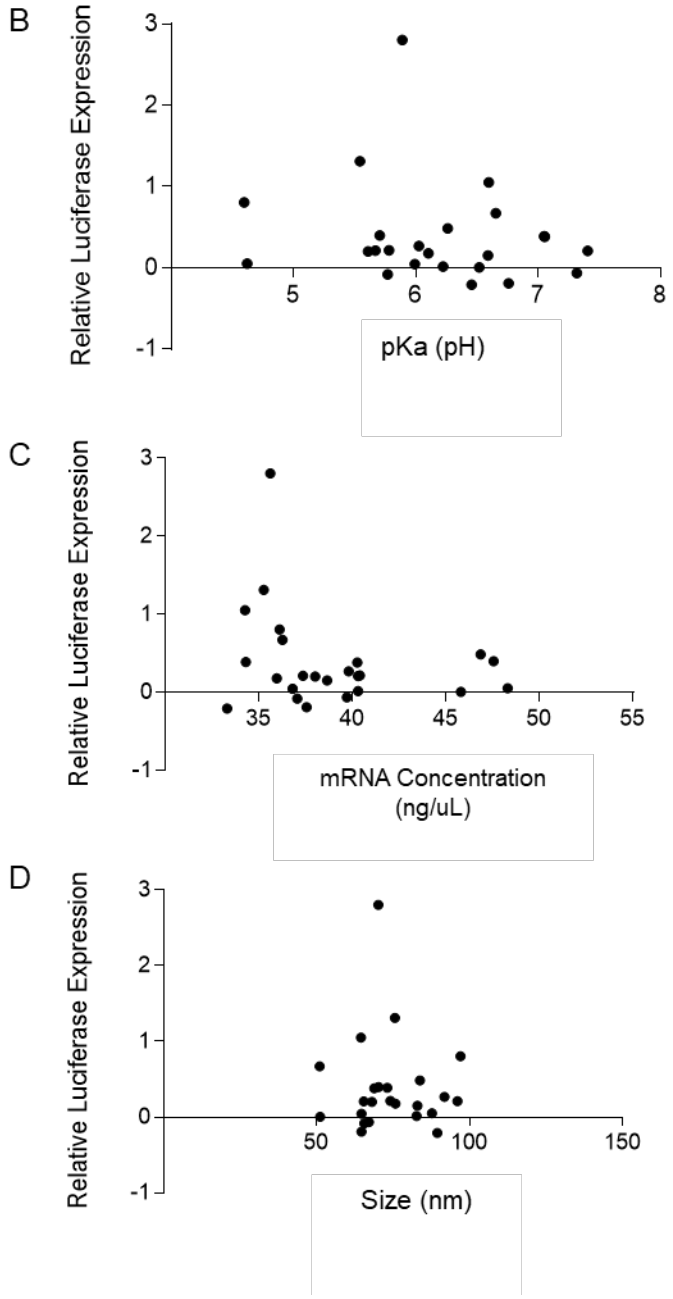



**Figure S1.** (A) Table of the polyamine cores and their pKa values as determined via TNS assay. (B-D) The relative luciferase expression produced by each LNP formulation (at 30 ng/ 60,000 Jurkat cells) graphed against the polyamine core pKa, LNP mRNA concentration, and LNP size, respectively. No correlations between these factors were found.

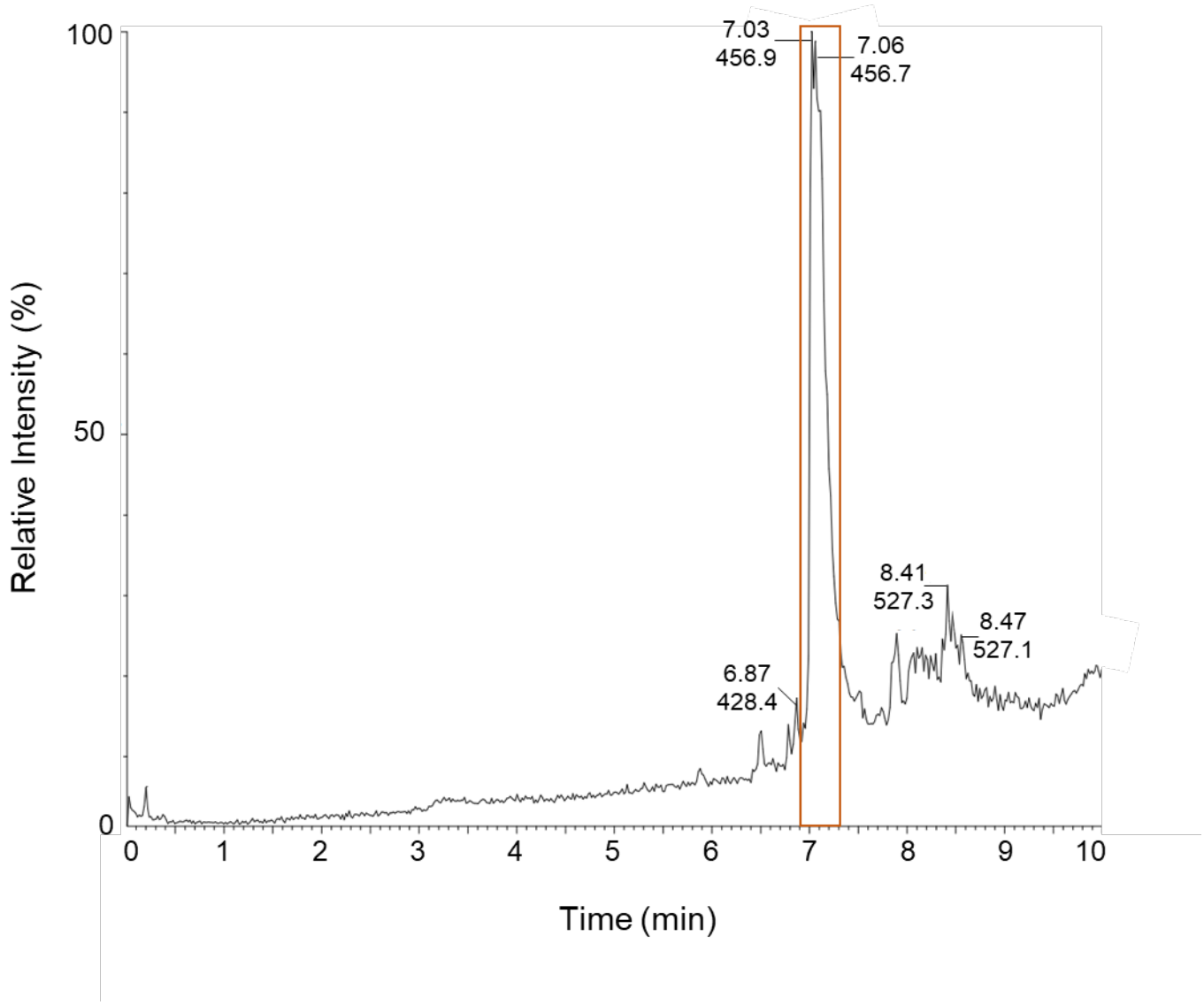



**Figure S2**. LCMS spectra of the crude C14-4 product with the fully saturated amine core highlighted with in the orange box.

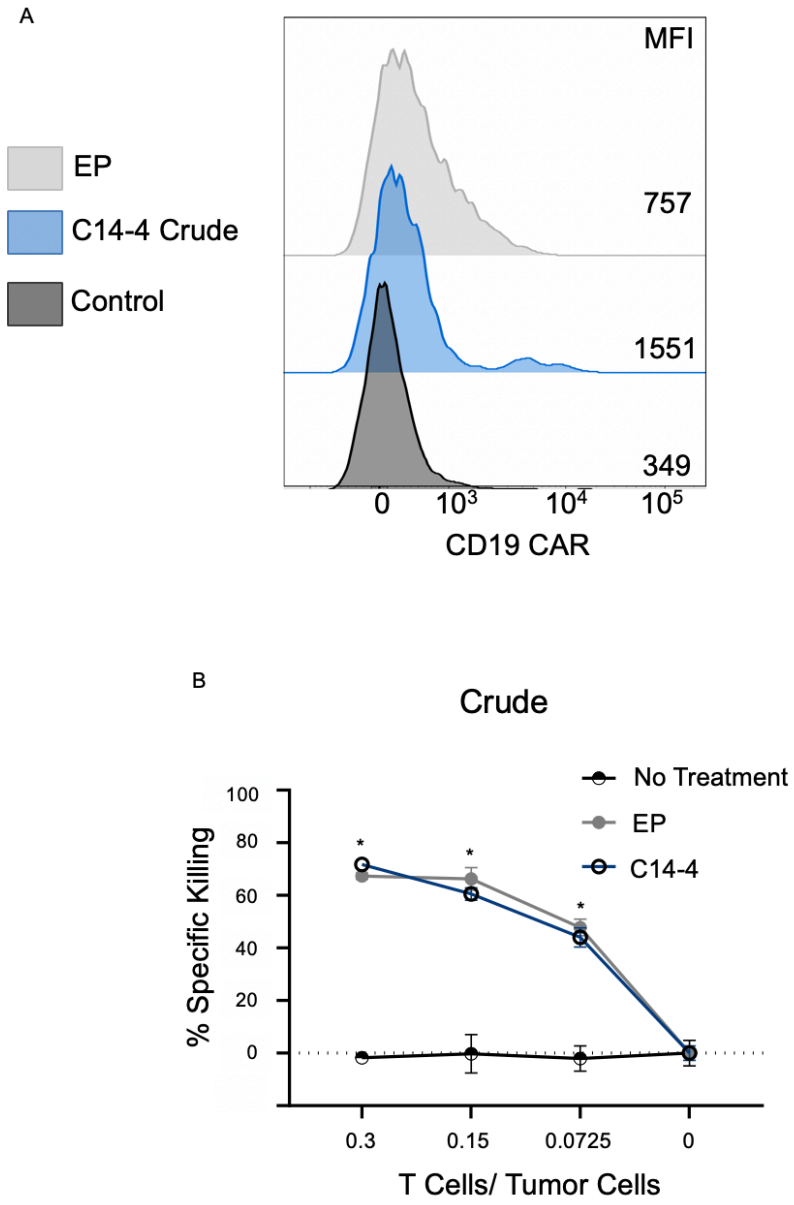



**Figure S3.** (A) Surface expression of CAR on primary T cells assessed using flow cytometry (B) Results of Nalm6 and CAR T cell co-plating at different effector-to-target ratios for 48 hrs. n=3 wells. * = p<.0001 in paired t test to No Treatment (for both EP and C14-4 LNPs)