Innovative Genomics Institute

Nguyen Lab

Lipid Nanoparticles for DNA Template Delivery to T-cells

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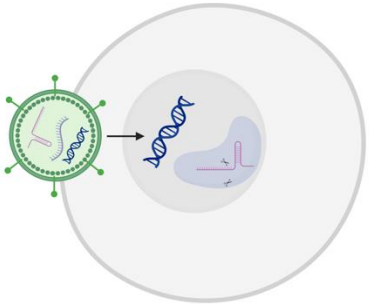
NEXT-GENERATION DELIVERY INNOVATIONS

CRISPR facilitates therapeutic transgene knock-in, however implementation is not clinically scalable

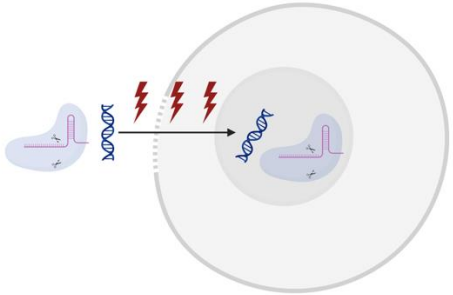
Clinical Standard: Viral Vectors

- Limited packaging capacity
- Risk of insertional mutagenesis
- Immunogenic
- High cost of production

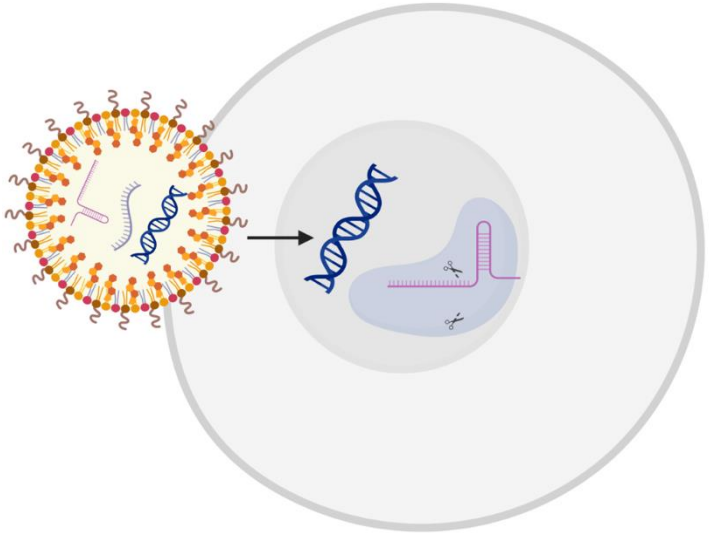


Nonviral Standard: Electroporation

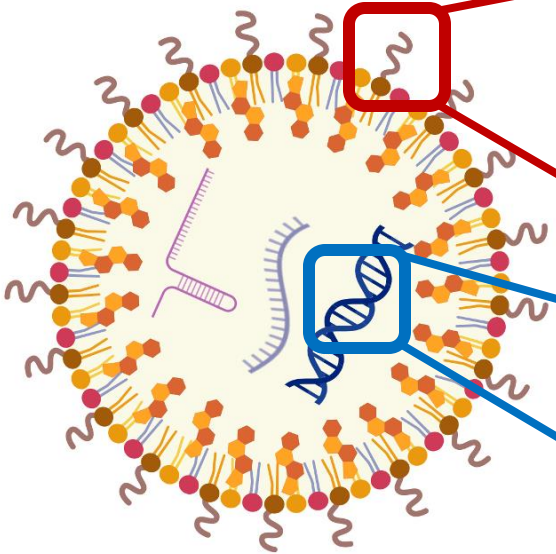
- High toxicity
- Inherently *ex vivo*
- High implementation and equipment cost



Lipid nanoparticles are a scalable non-viral solution, but challenges in nuclear delivery of DNA limit genome editing



Optimizing LNPs for HDR template delivery to T cells is required



LNP Formulation

- Ionizable lipid
- Lipid/cargo composition ratio
- Targeting moiety

Cargo Enhancements

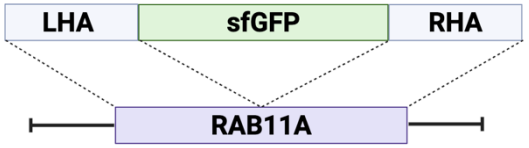
- Active nuclear trafficking
- DNA structure (ssDNA vs dsDNA)

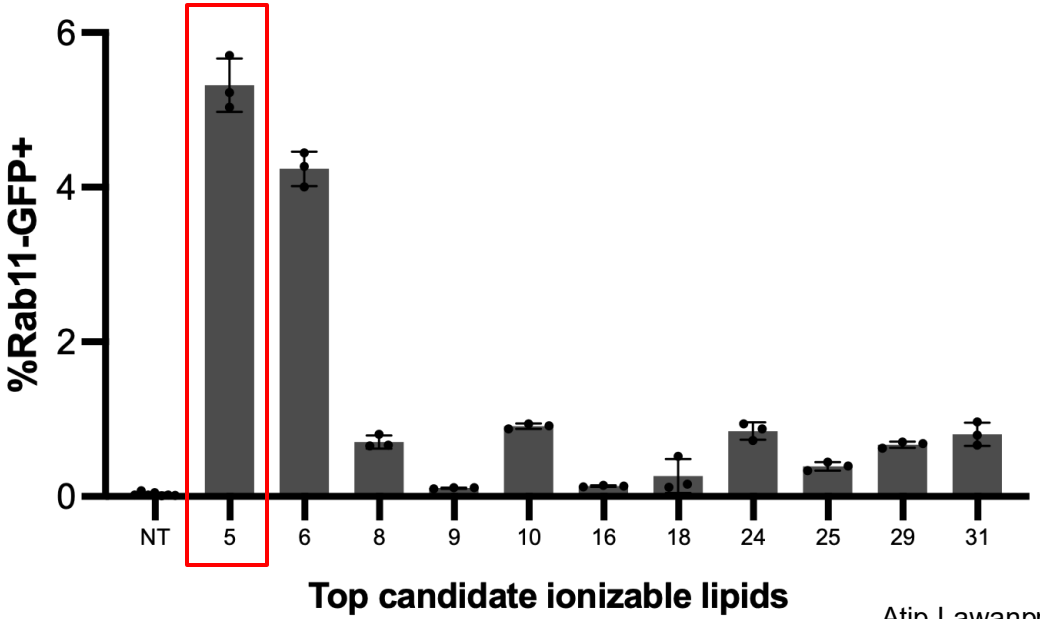
Collaboration with Murthy Lab at UC Berkeley

The optimal ionizable lipid (L5) achieves 5% CRISPR knock-in at Rab11 locus in primary human T cells

LNP encapsulation

- Linear 1.4kB dsDNA HDRT
- Rab11 Exon 1 sgRNA
- Cas9 mRNA





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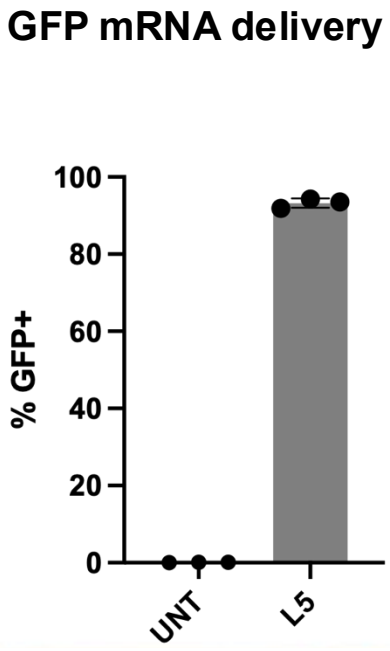
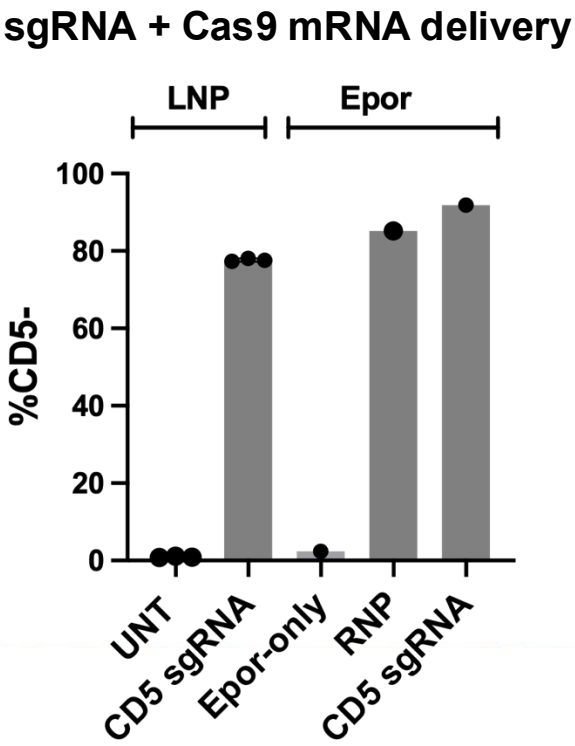
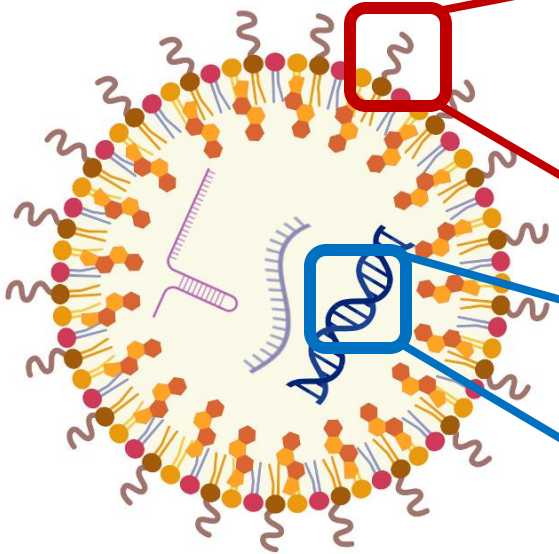
L5 delivers cargoes efficiently to T cells





Optimizing cargoes for HDR template delivery to T cells is required



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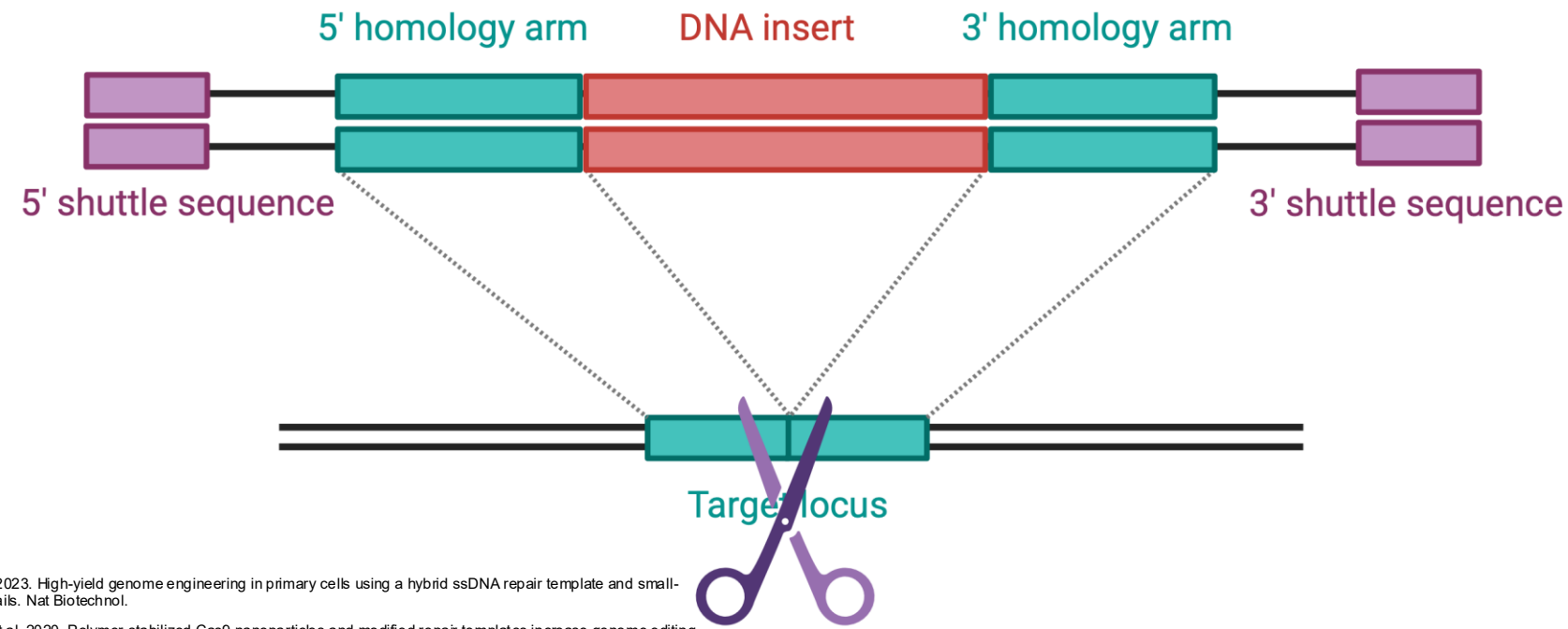
Collaboration with Murthy Lab at UC Berkeley

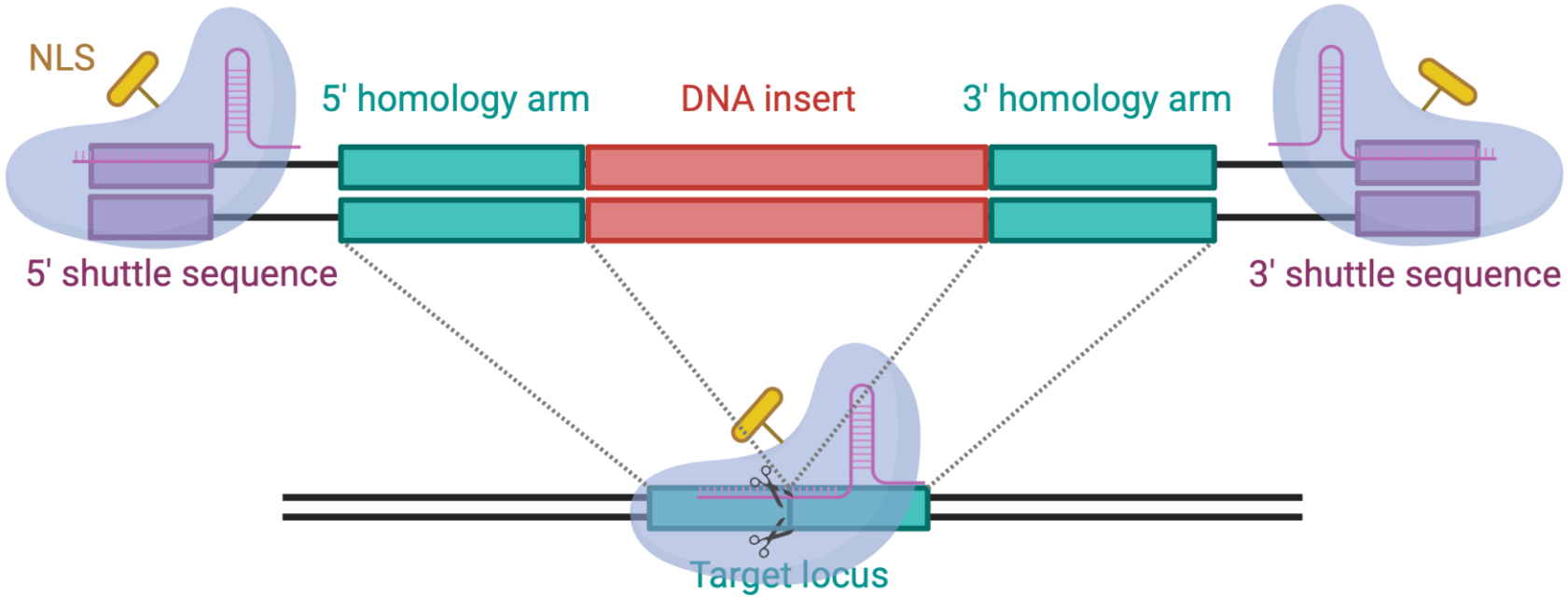
Truncated Cas9 target sequences (tCTSs) flank the 5’ and 3’ ends



Shy, B.R et al. 2023. High-yield genome engineering in primary cells using a hybrid ssDNA repair template and small-molecule cocktails. Nat Biotechnol.

Nguyen, D.N. et al. 2020. Polymer-stabilized Cas9 nanoparticles and modified repair templates increase genome editing efficiency. Nat Biotechnol.

tCTSs bind RNP, which has engineered nuclear localization sequences (NLS) that “shuttle” template to the nucleus



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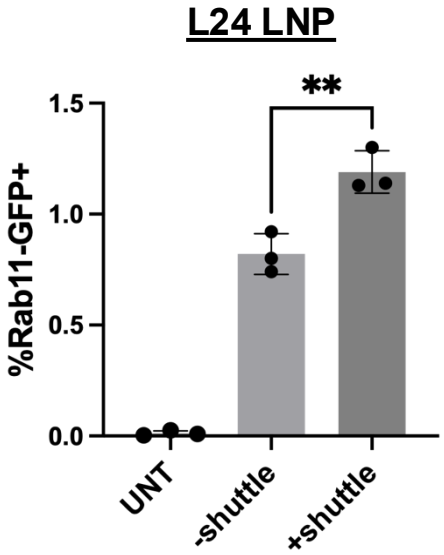
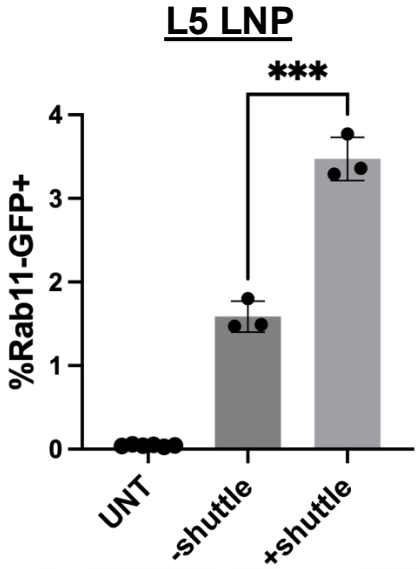
Shuttle enables more efficient HDR delivery across multiple LNP at the Rab11 locus





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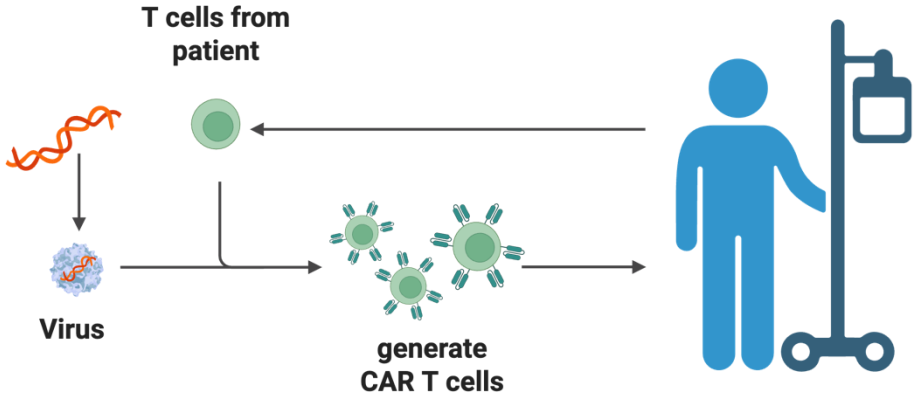
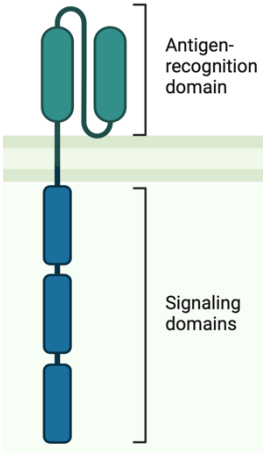
Clinical Applications: Generating CD19 CAR-T





<u>CAR KI to TRAC locus</u>
Uniform CAR expression

Enhanced CAR T cell potency

Delayed effector T cell differentiation/exhaustion

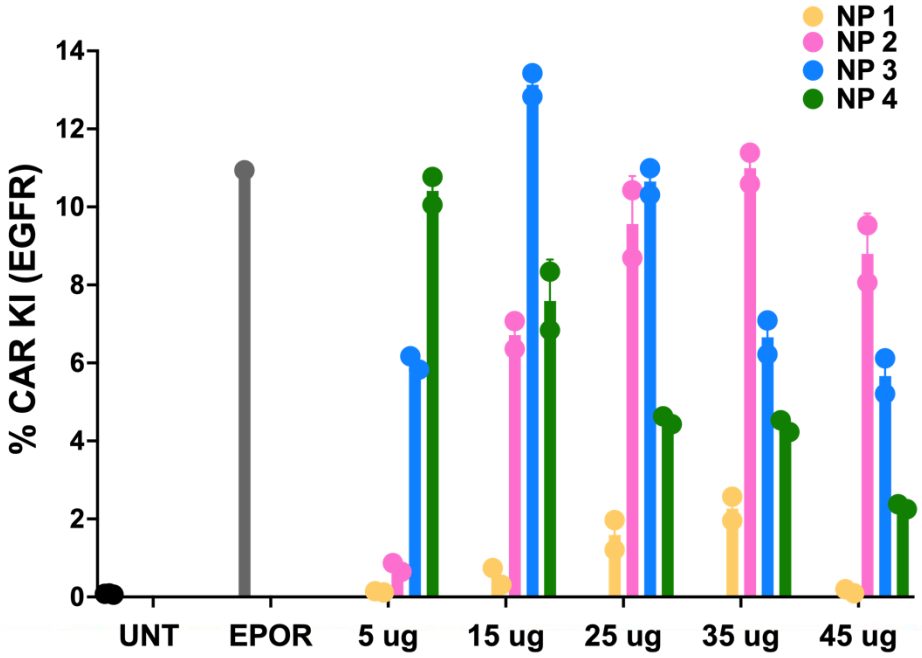
Collaboration with Eyquem Lab at UCSF

L5 delivery achieves 12% CAR HDR knock-in, matching electroporation

LNP encapsulation

- EGFR-CAR HDRT
- TRAC sgRNA
- Cas9 mRNA

All components at a 1:1:1 mass ratio



4kB CAR DNA payload:

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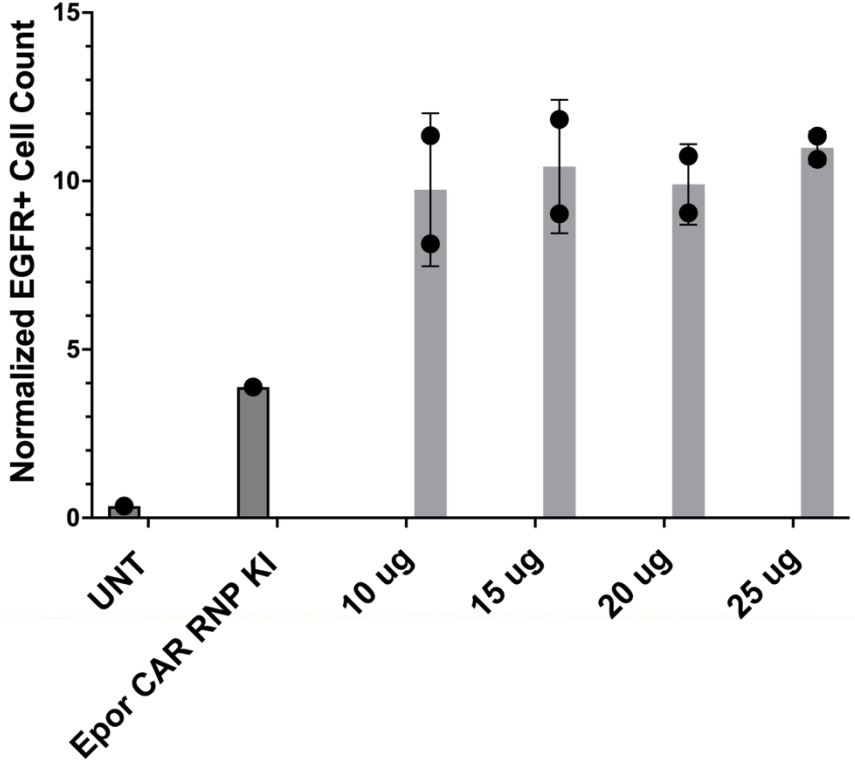
L5 achieves equivalent knock-in as electroporation but with less toxicity



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Takeaways

- The ionizable lipid, L5, efficiently transfects T-cells
- tCTS ”shuttle" HDRT modifications increase HDR rates in the context of LNPs
- L5 LNPs deliver large CRISPR-targeted HDR constructs (1.4kB Rab11 and 4 kB CAR)
- L5 LNPs achieve 12% CAR knock-in, matching the efficiency of electroporation with lower toxicity

Acknowledgements

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