# Genetic Engineering of Megakaryocytes from Blood Progenitor Cells using mRNA-Lipid Nanoparticles

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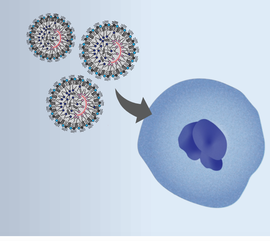


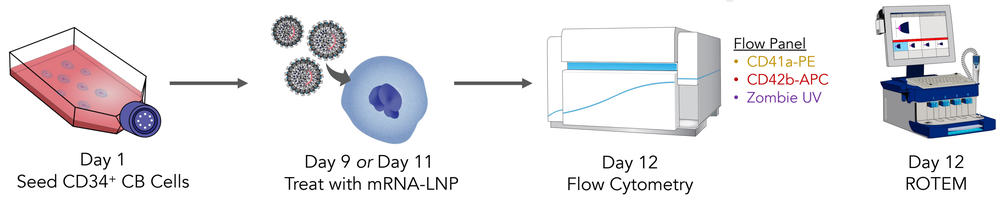
## Introduction

Platelets are an essential component of hemorrhage control and management, and engineering platelets to express therapeutic proteins could expand their use as a cell therapy. Genetically engineered platelets can be achieved by modifying the platelet precursor cells, megakaryocytes (MKs). Current strategies include transfecting MK progenitors ex vivo with viral vectors harbouring lineage-driven transgenes and inducing the production of "in vitro" modified platelets. The use of viruses, however, poses challenges in clinical implementation, and **no methods currently exist to genetically modify MKs with non-viral techniques.** Lipid nanoparticles (LNP) are a non-viral delivery system that could enable a facile strategy to modify MKs with a variety of nucleic acid payloads.

GOAL: To investigate whether (1) LNP resembling the clinically approved LNP formulations can transfect cultured hematopoietic stem/progenitor cell (HSPC)-derived MKs to express exogenous proteins and (2) elicit functional changes when expressing a physiologically relevant protein.

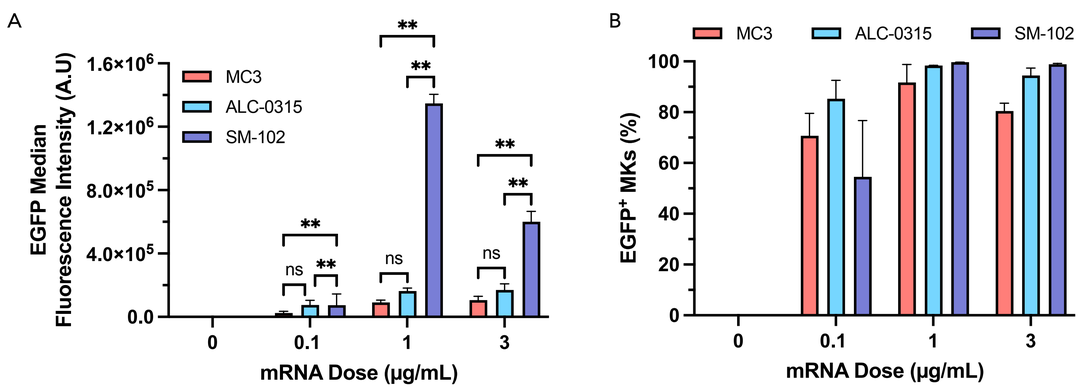
## Experimental Design

- mRNA encoding enhanced GFP (EGFP), firefly luciferase (FLuc) or factor VII (FVII) formulated into LNP resembling the clinically approved RNA LNP
- Cells treated on either Day 9 or 11 of culture, and assayed on Day 12 by flow cytometry, and rotational thromboelastometry (ROTEM)



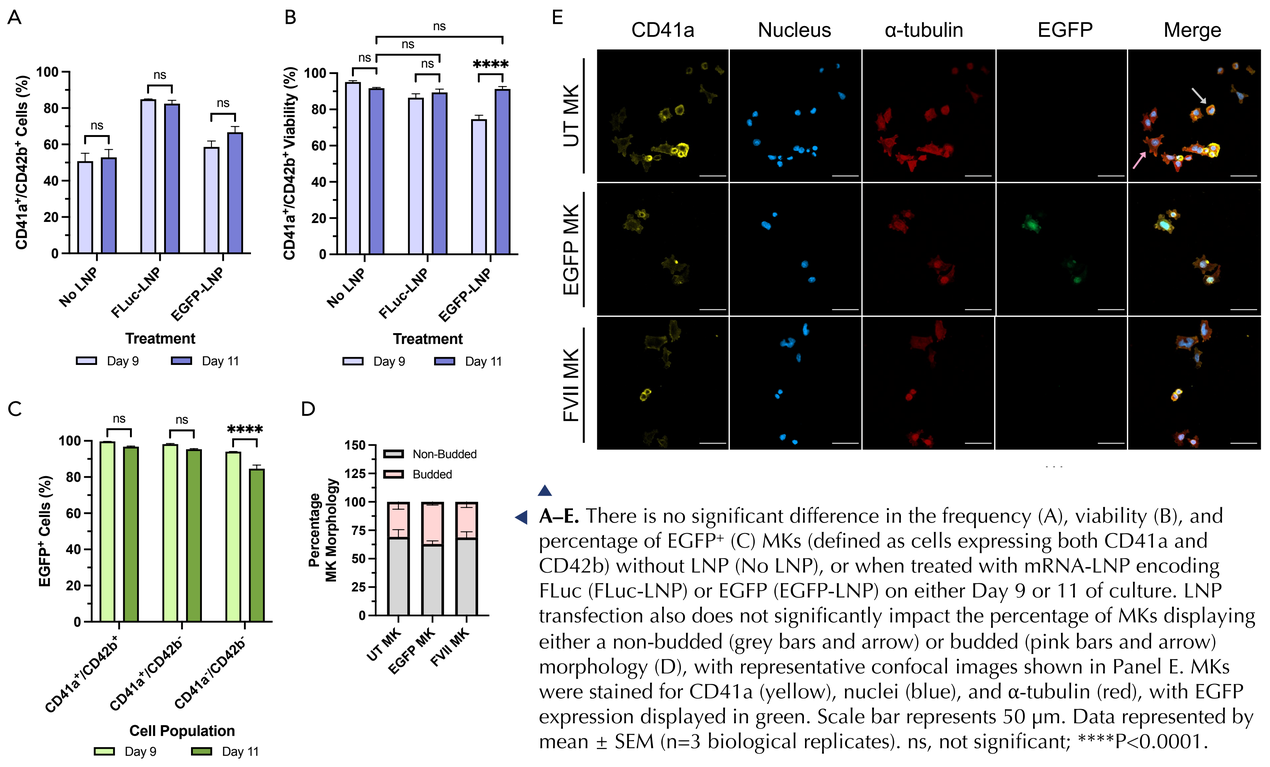

## Results

### 1. Exogenous-mRNA delivered via LNP can be translated with high transfection efficiency



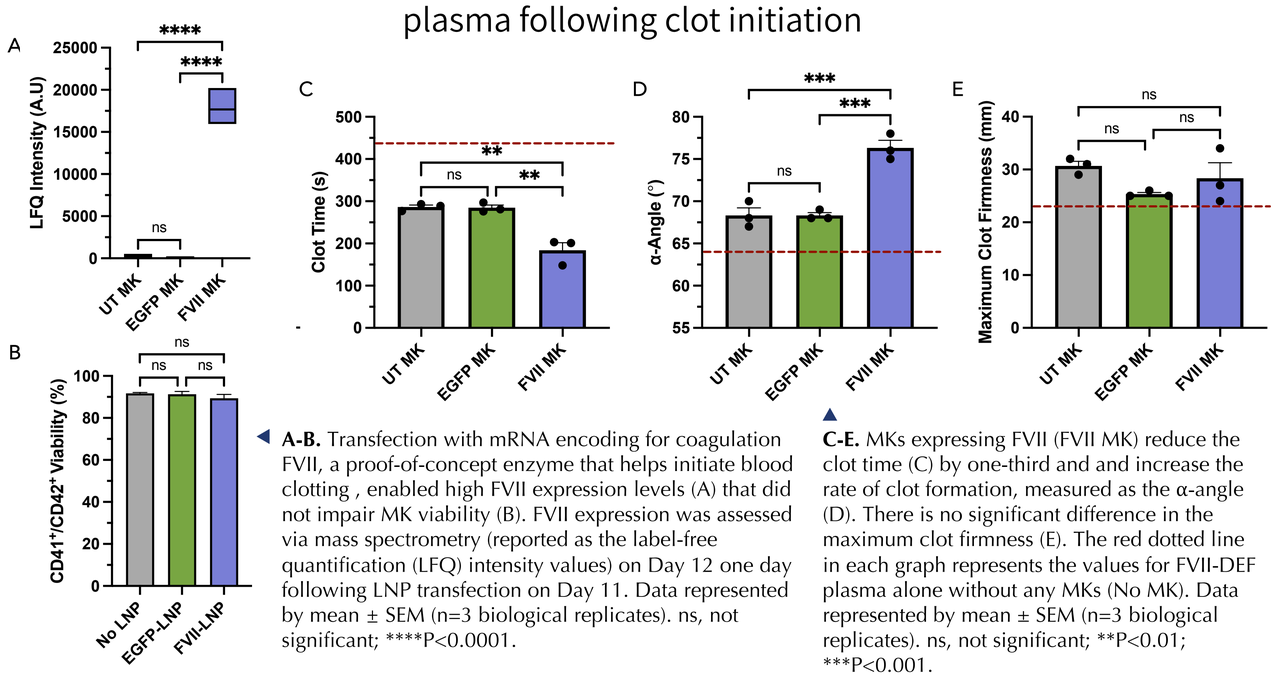

**A–B.** Quantification of the median fluorescence intensity (A) and percentage of MKs positive for EGFP (B) 72-hours post-treatment with mRNA-LNP encoding for EGFP and containing the ionizable lipids MC3, ALC-0315, or SM-102. A dose of 1 µg/mL in transfecting with LNP containing SM-102 yield the highest expression levels, and up to 99% of MKs being positive for EGFP. Data represented by mean ± SEM (n=3 biological replicates). ns, not significant; **P<0.01.

### 2. LNP transfection does not impact MK maturation, viability, or morphology




**A–E.** There is no significant difference in the frequency (A), viability (B), and percentage of EGFP+ (C) MKs (defined as cells expressing both CD41a and CD42b) without LNP (No LNP), or when treated with mRNA-LNP encoding FLuc (FLuc-LNP) or EGFP (EGFP-LNP) on either Day 9 or 11 of culture. LNP transfection also does not significantly impact the percentage of MKs displaying either a non-budded (grey bars and arrow) or budded (pink bars and arrow) morphology (D), with representative confocal images shown in Panel E. MKs were stained for CD41a (yellow), nuclei (blue), and α-tubulin (red), with EGFP expression displayed in green. Scale bar represents 50 µm. Data represented by mean ± SEM (n=3 biological replicates). ns, not significant; ****P<0.0001.

### 3. MKs engineered to express exogenous FVII decreased clotting time in FVII-deficient plasma following clot initiation




**A-B.** Transfection with mRNA encoding for coagulation FVII, a proof-of-concept enzyme that helps initiate blood clotting , enabled high FVII expression levels (A) that did not impair MK viability (B). FVII expression was assessed via mass spectrometry (reported as the label-free quantification (LFQ) intensity values) on Day 12 one day following LNP transfection on Day 11. Data represented by mean ± SEM (n=3 biological replicates). ns, not significant; ****P<0.0001.

**C-E.** MKs expressing FVII (FVII MK) reduce the clot time (C) by one-third and and increase the rate of clot formation, measured as the α-angle (D). There is no significant difference in the maximum clot firmness (E). The red dotted line in each graph represents the values for FVII-DEF plasma alone without any MKs (No MK). Data represented by mean ± SEM (n=3 biological replicates). ns, not significant; **P<0.01; ***P<0.001.

## Conclusions

- LNP enabled transfection efficiencies of 99% in MKs resulting in functional changes without impairing MK maturation, viability, and morphology.
- Future studies will focus on validating the production of engineered platelets from transfected MKs.
- **Leveraging LNP with in vitro platelet production can ultimately provide an easy-to-use modular platform to genetically modify MKs, potentially extending to modified platelets.**

## Acknowledgements

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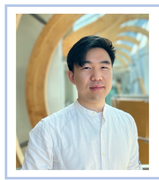

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