

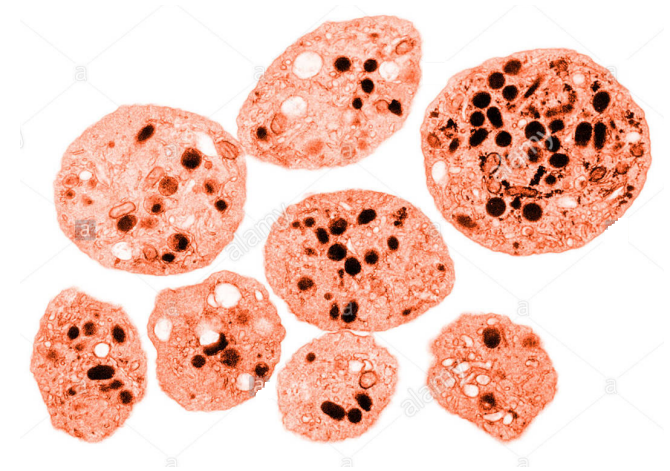
Genetic engineering of megakaryocytes from blood progenitor cells using mRNA lipid nanoparticles

Jerry Leung



The need for better platelet transfusion products

- Bleeding the leading cause of death worldwide
- Platelets transfused to restore hemostasis
 - Not always efficacious
- Enhanced platelets a potential solution



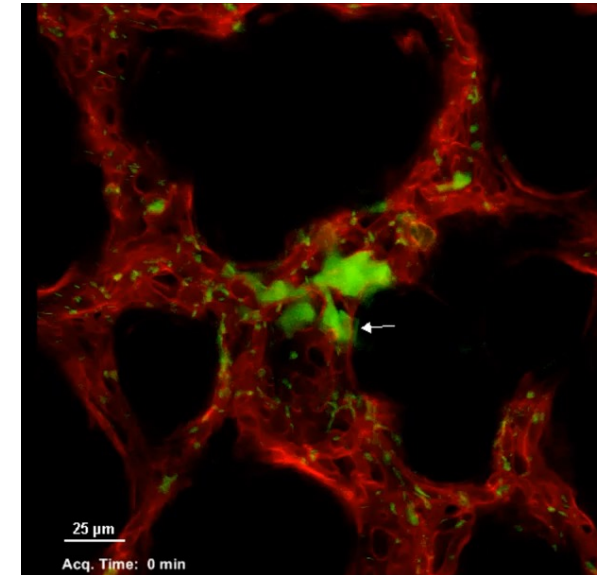
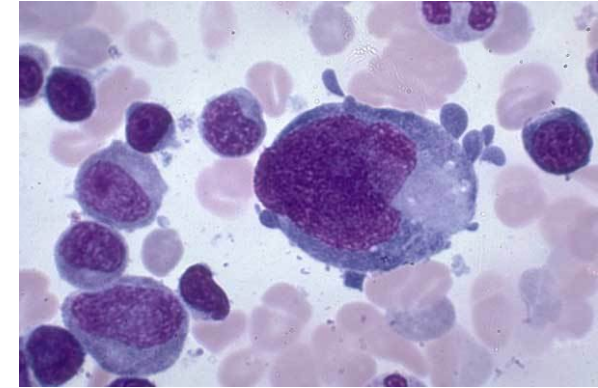
Challenging to modify

<https://www.alamy.com/tem-transmission-electron-microscope-image-of-human-platelets-computer-image5196635.html>

Megakaryocytes – The platelet precursor cells

- Large polyploid cells that produce platelets by cytoplasmic extensions
- Single MK can yield thousands of platelets
 - Exploited for in vitro platelet production

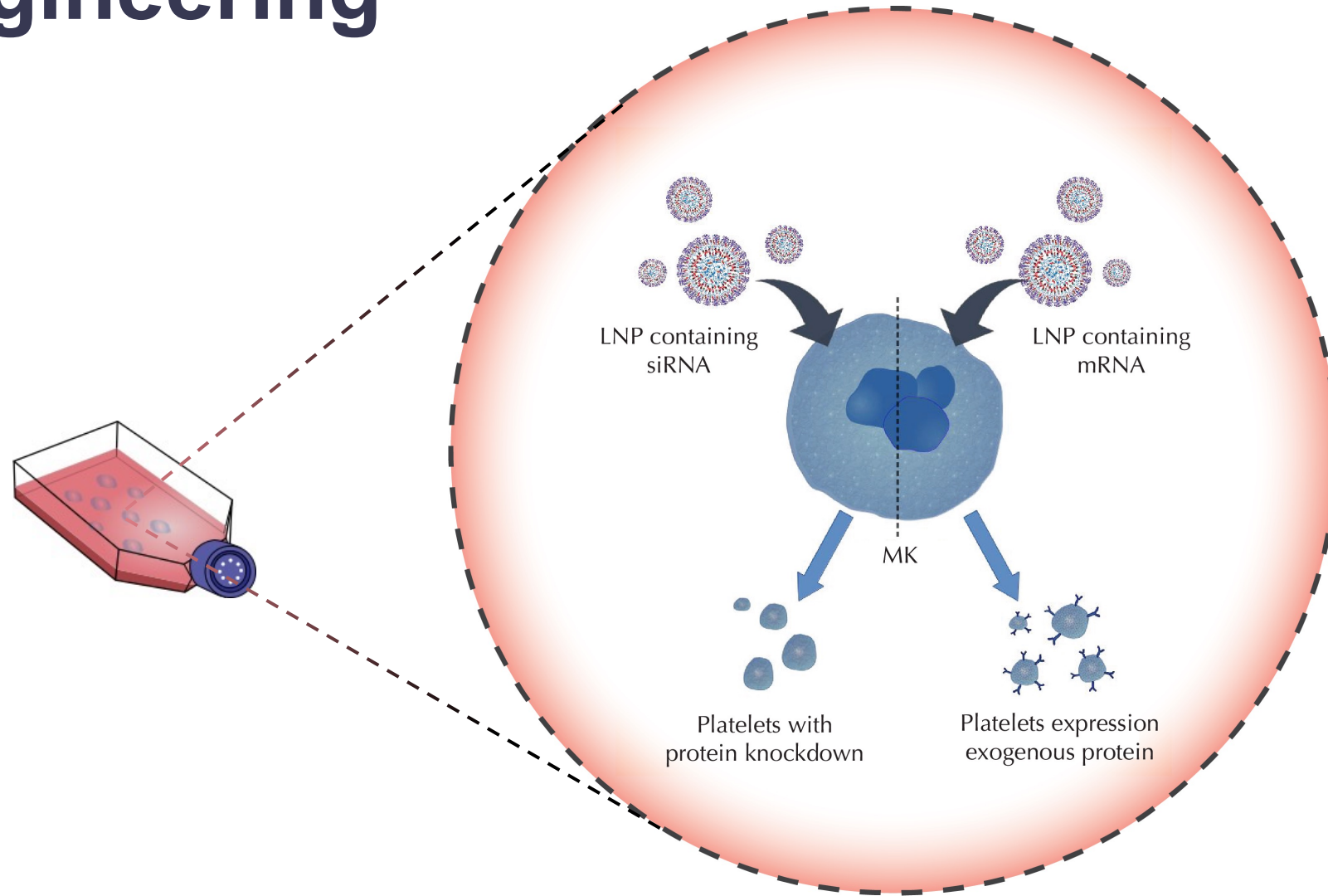
**No non-viral transfection agents
to genetically modify MKs**



<https://www.thrombocyte.com/wp-content/uploads/2015/10/megakaryocytes-definition.jpg>; Lefrançois et al., *Nature* (2017)



MK Engineering



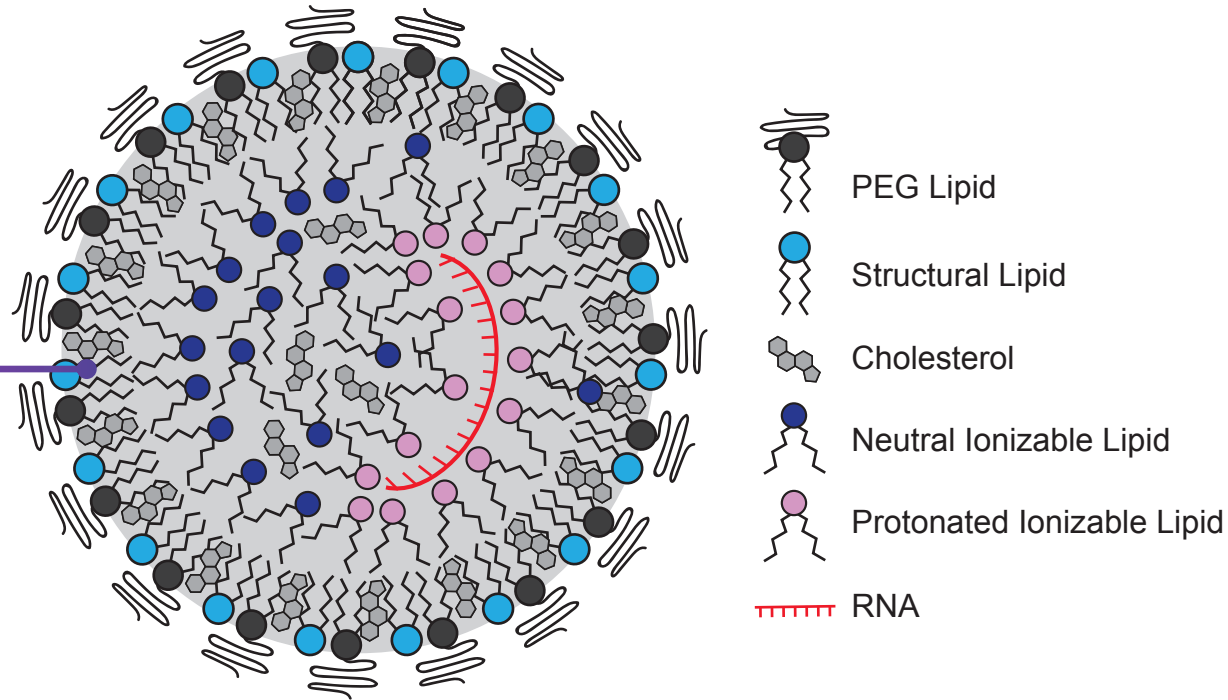
LNP Formulations

Ethanol phase

- Ionizable Lipid
- DSPC
- Cholesterol
- PEG Lipid

Aqueous phase

- mRNA
 - EGFP
 - Firefly luciferase
 - Factor VII



Patisiran

- **DLin-MC3-DMA**
- DSPC
- Cholesterol
- PEG-c-DMG

Tozinameran

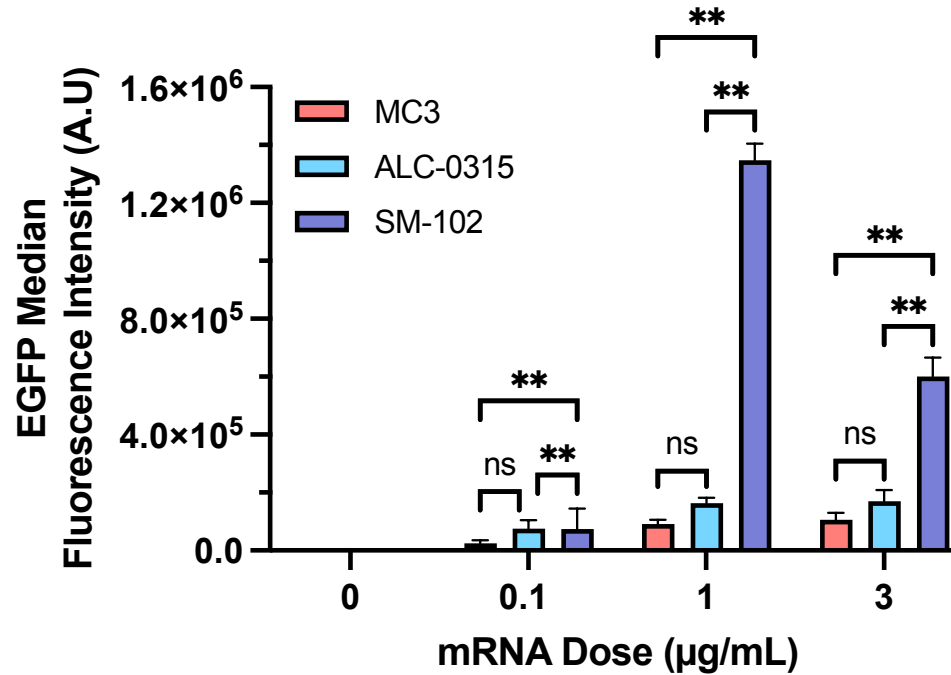
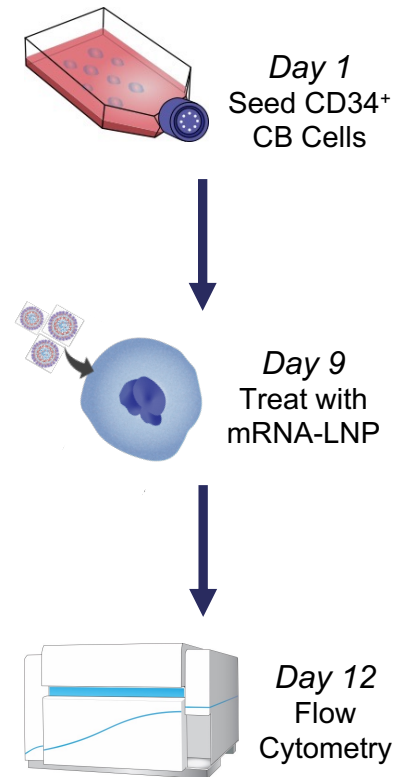
- **ALC-0315**
- DSPC
- Cholesterol
- ALC-0159

Elasomeran

- **SM-102**
- DSPC
- Cholesterol
- PEG-DMG

MKs robustly express exogenous EGFP reporter

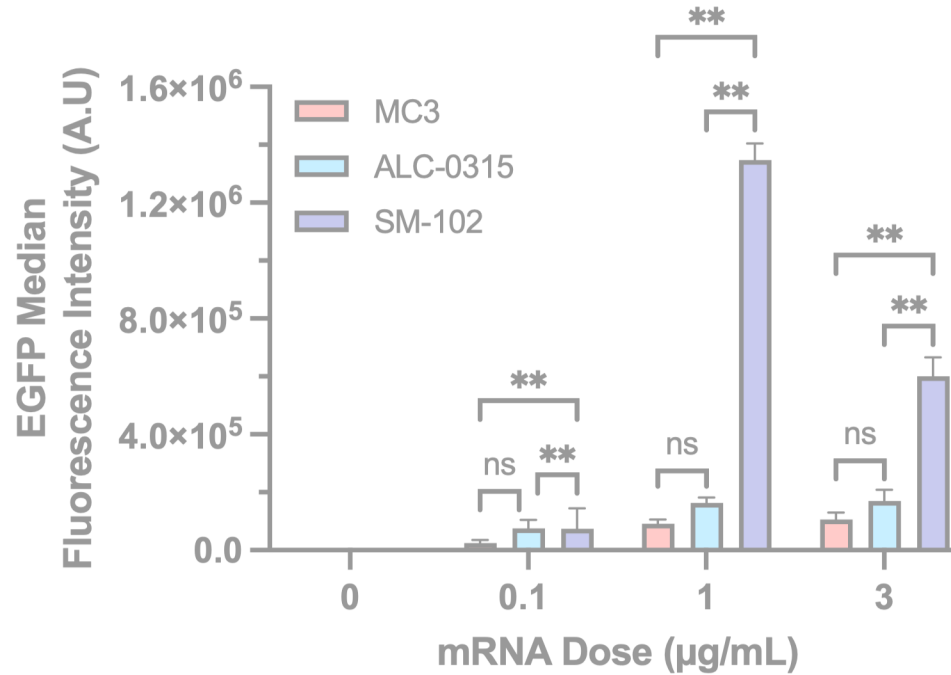
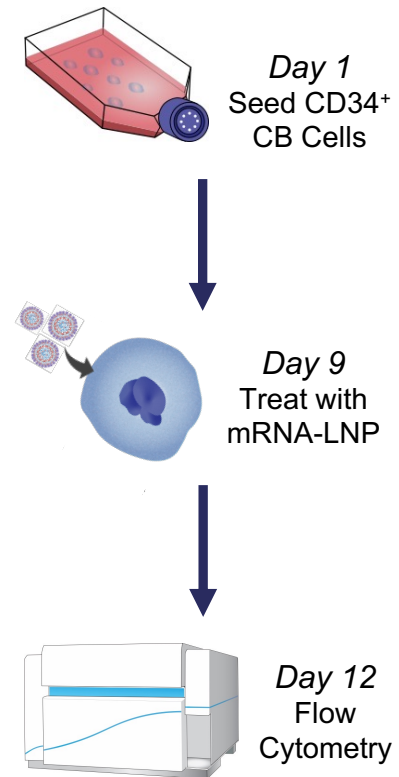
Method



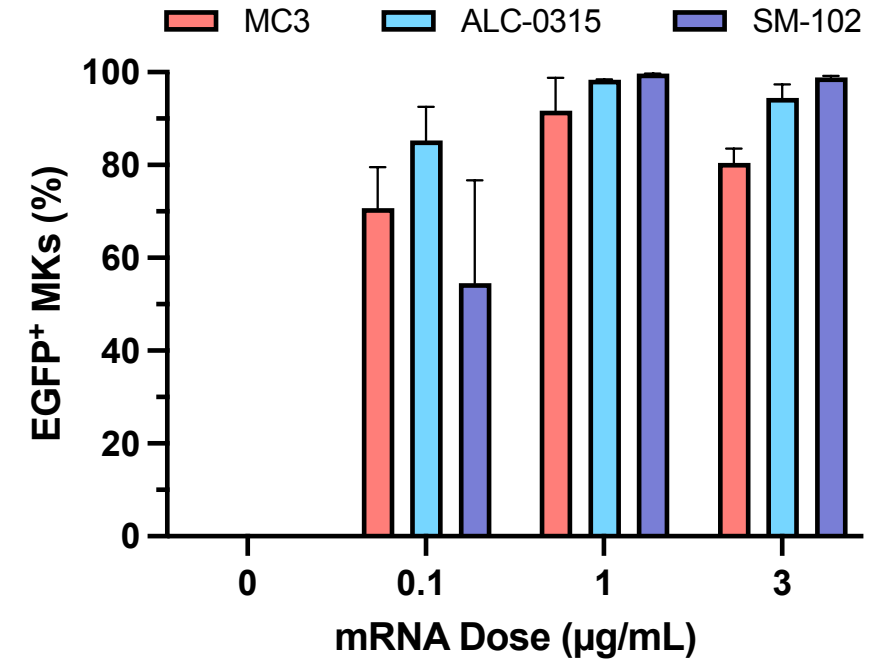
SM-102 optimal for MK
transfection

MKs robustly express exogenous EGFP reporter

Method



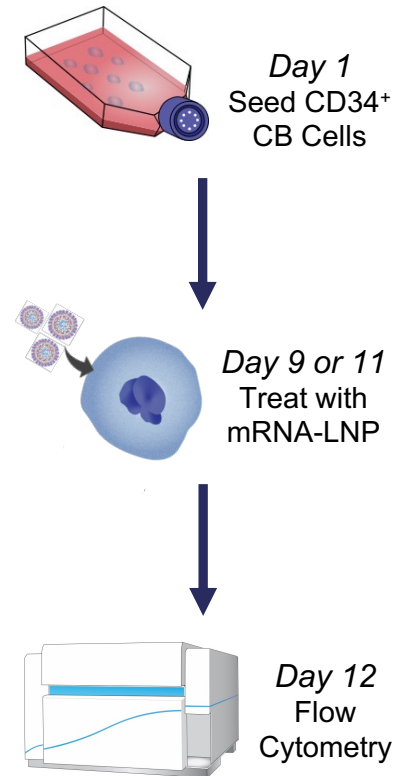
SM-102 optimal for MK transfection



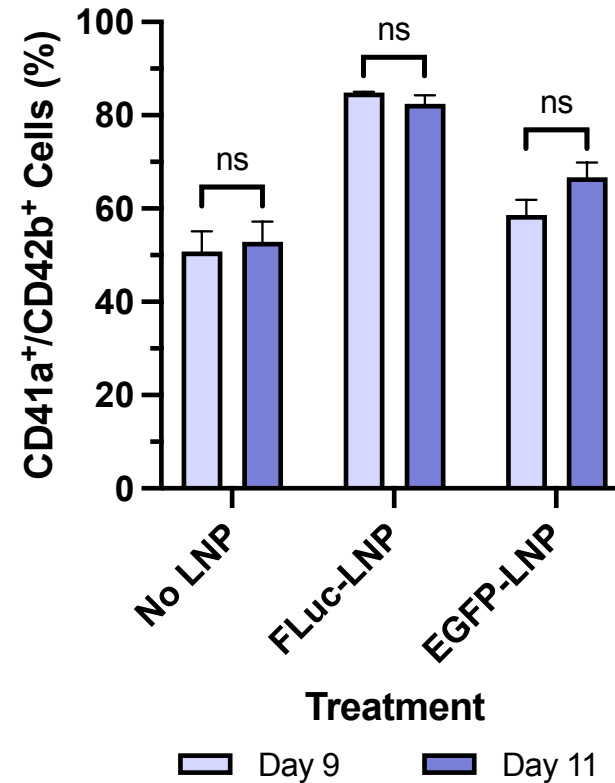
>95% transfection efficiency

LNP transfection does not impair MK maturation or viability

Method

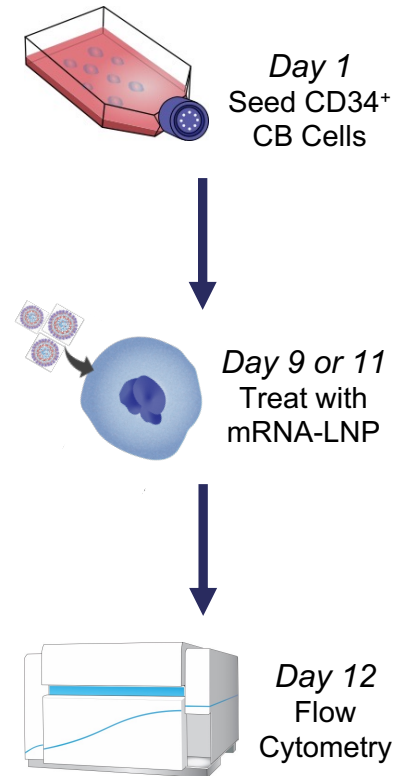


Maturation

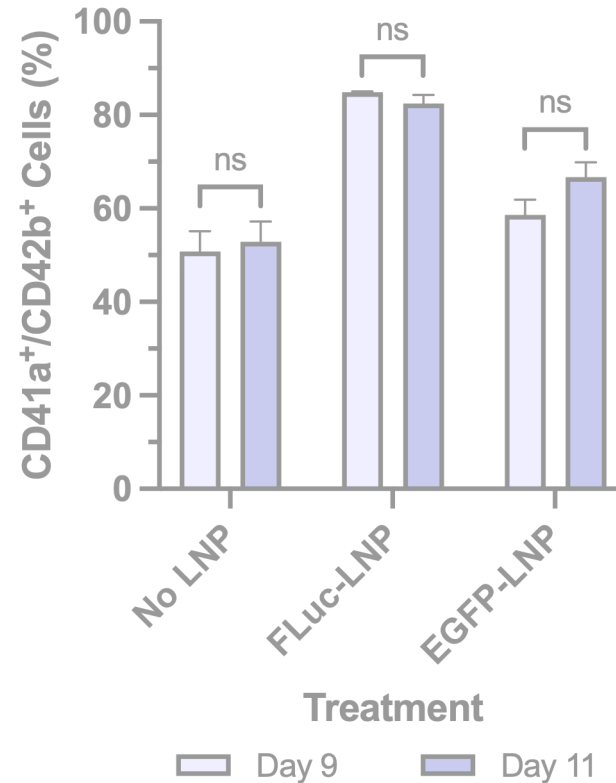


LNP transfection does not impair MK maturation or viability

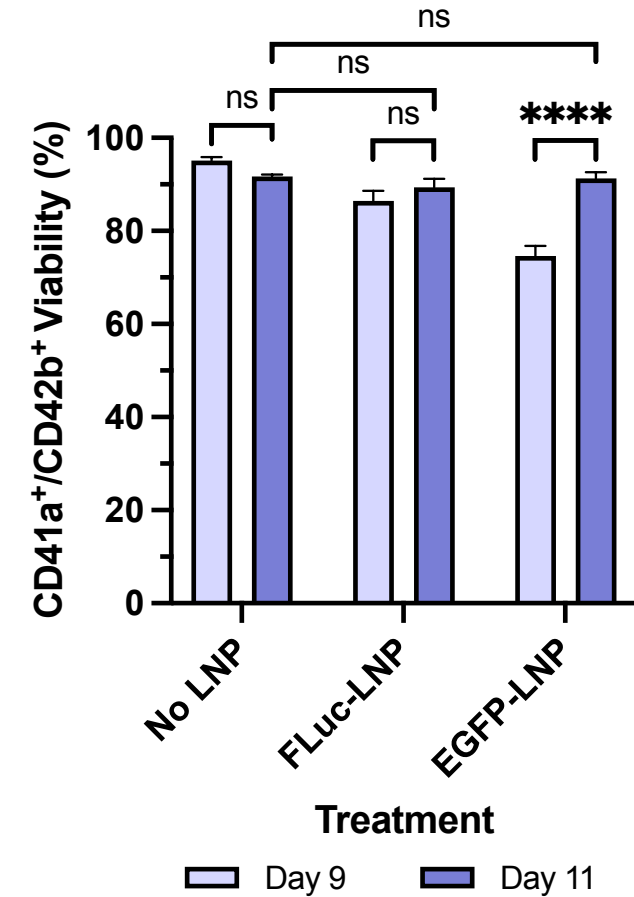
Method



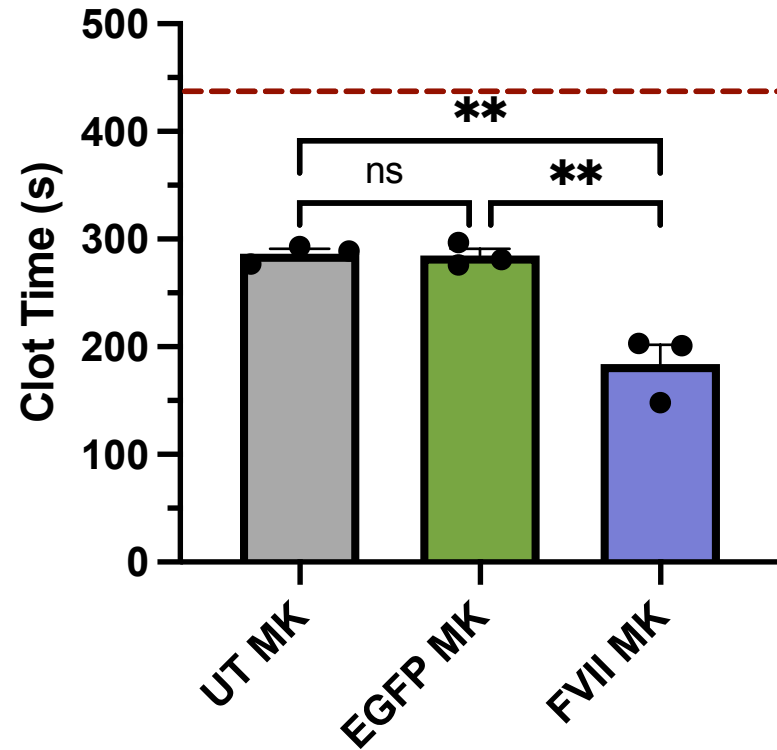
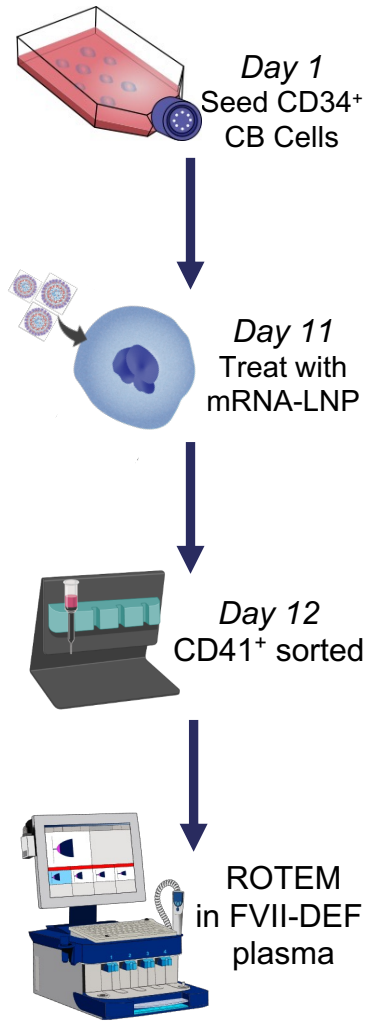
Maturation



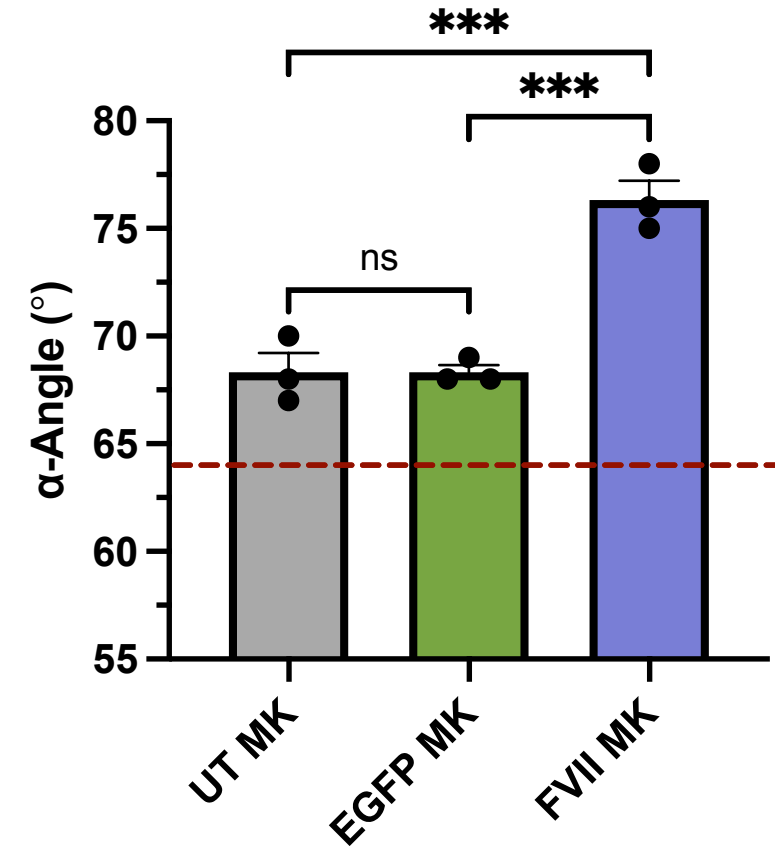
Viability



MKs can express FVII to elicit a functional change



Decreased clot time



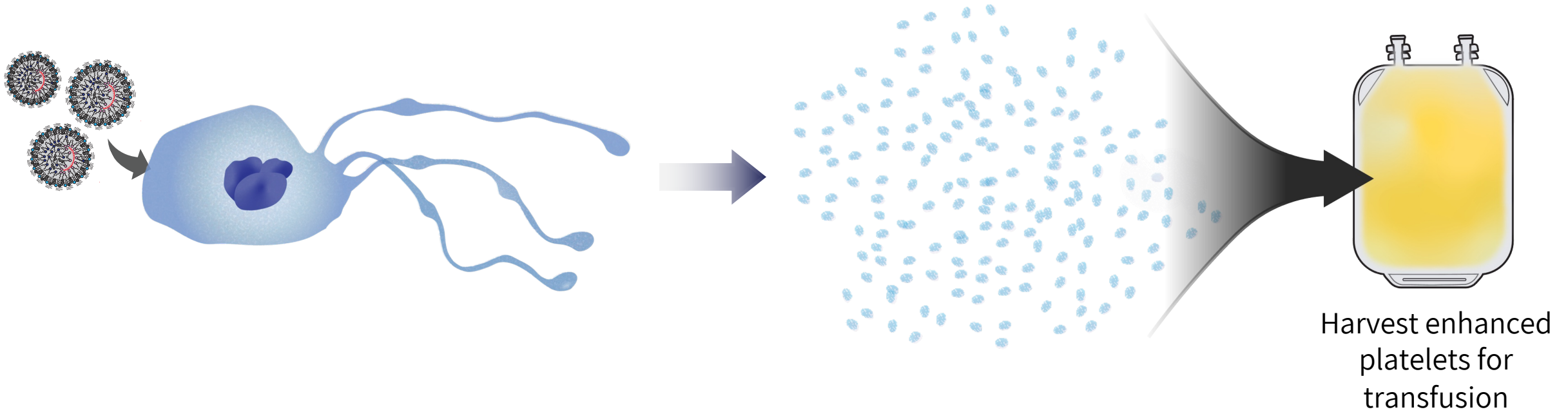
Increased rate of clot formation

Summary

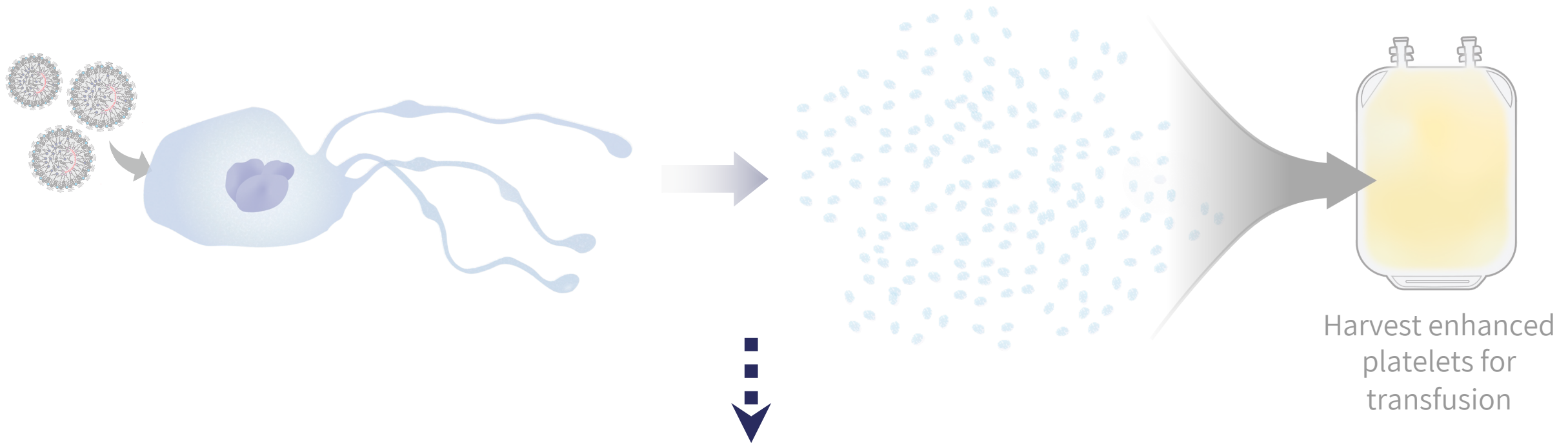
- ① LNP readily transfect MKs without impacting maturation
- ② Transfection yields functional changes



Summary



Summary



Platform Technology



Effective
Transfusions



Targeted
Therapeutics



Research
Tool

Acknowledgements

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