Highly Efficient CRISPR/Cas9 Gene Editing and Long-Term Engraftment of Human Hematopoietic Stem and Progenitor Cells

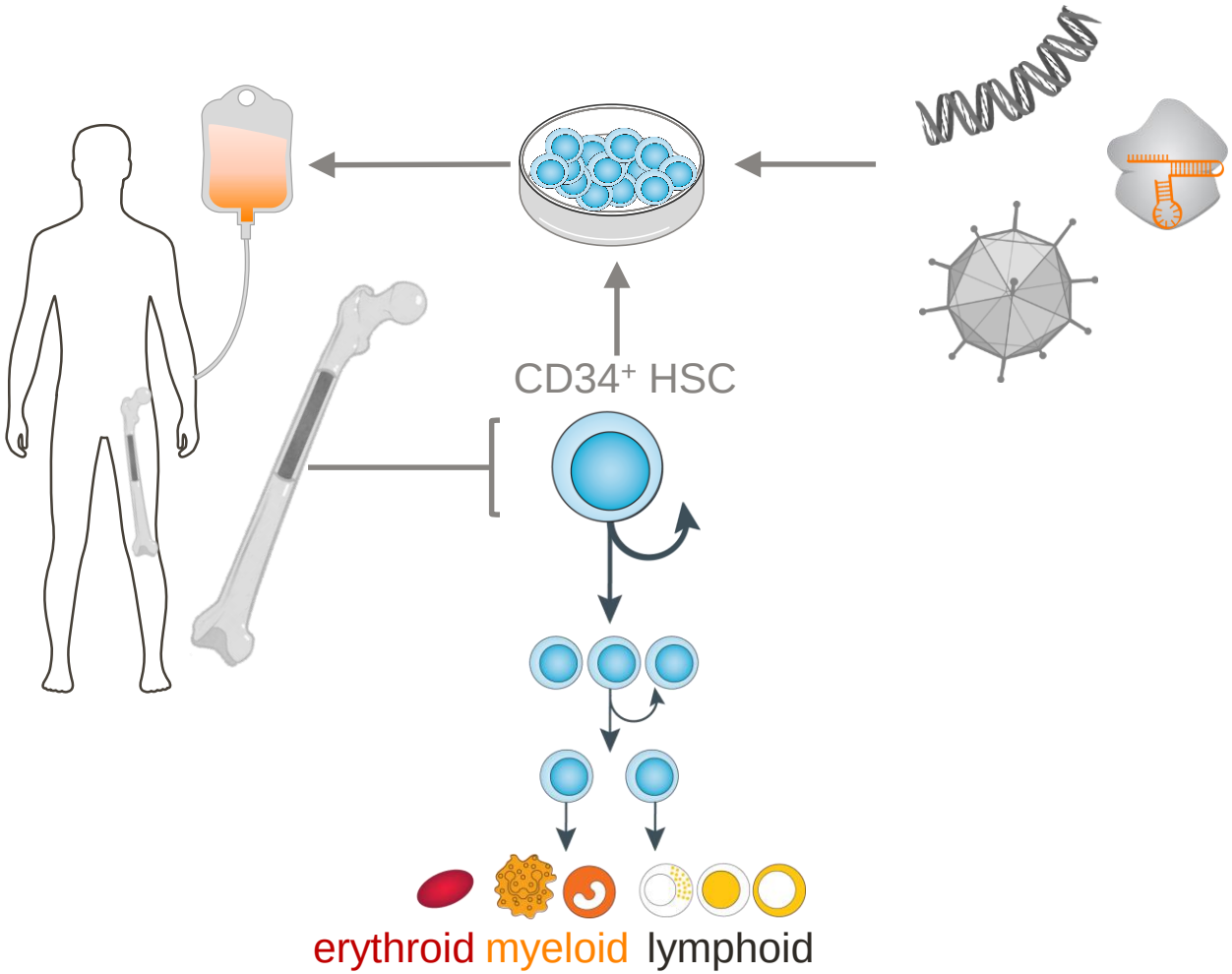
J. M. Heath, A. Chalishazar, C.S. Lee, W. Selleck, C. Cotta-Ramusino, D. Bumcrot, J.L. Gori

Disclosure:

Jennifer Gori and Co-Authors are Full-time Employees of Editas Medicine

Gene-Modified Autologous Hematopoietic Stem and Progenitor Cell Therapy

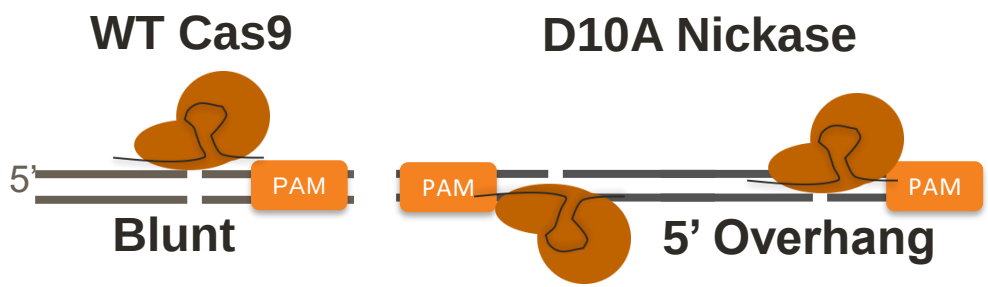
ex vivo approach to gene correction of hematopoietic diseases



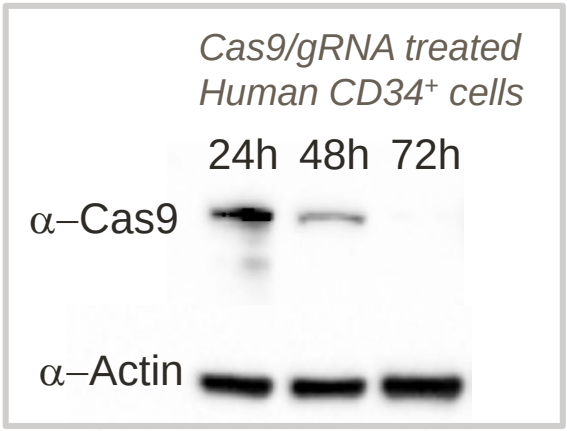
Rationale for Delivery of Cas9 RNP for Gene Editing in HSCs

Effective gene editing and transient nuclease expression

- **Hypothesis**
  - Cas9 RNP would support gene editing in HSCs without impacting viability or functionality *in vivo*
- **Electroporation of Cas9/gRNA ribonucleoprotein (RNP)**
  - High efficiency
  - Limited exposure

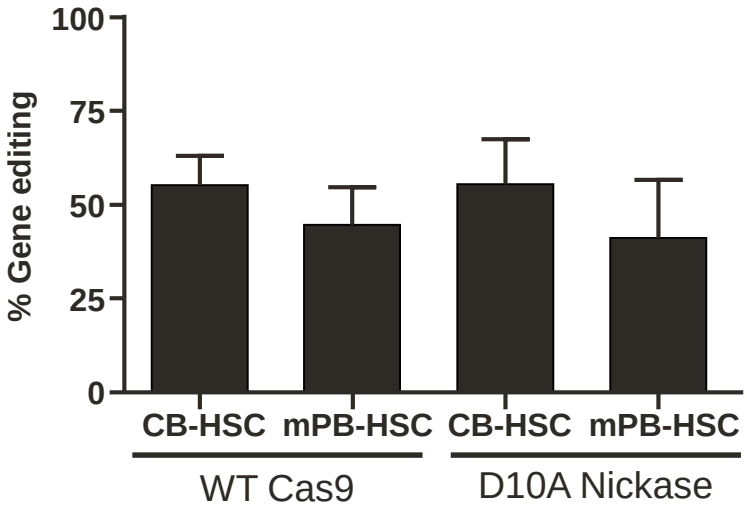


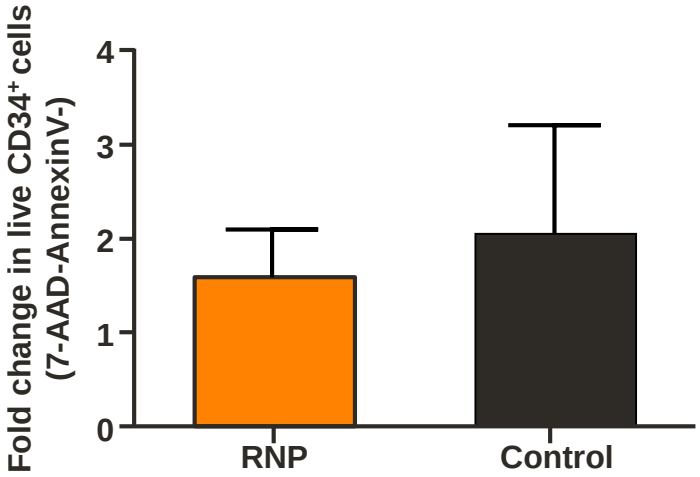
Effective for NHEJ ***Effective for HDR***



Efficient and Reproducible Editing in HSCs

Comparison of Wild-Type and D10A SpCas9 at β-hemoglobin locus (*HBB)*

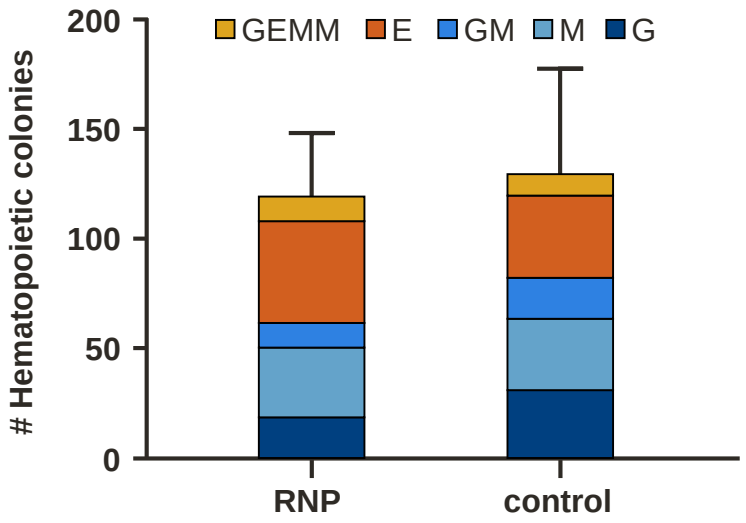


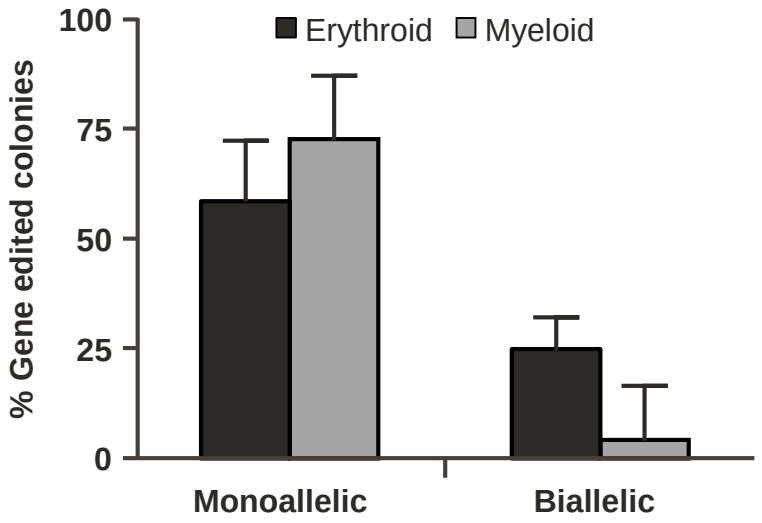


- Reproducible gene editing across 20 donors
- Maintenance of viability of RNP treated HSCs

Gene-Edited HSCs Maintain Erythroid and Myeloid Multipotency *ex vivo*

Analysis of gene editing in clonal derivatives of edited HSCs

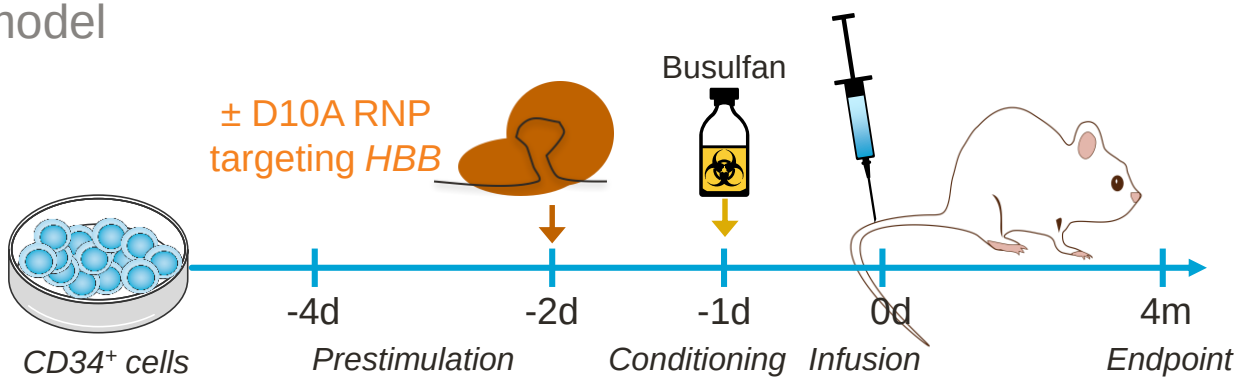




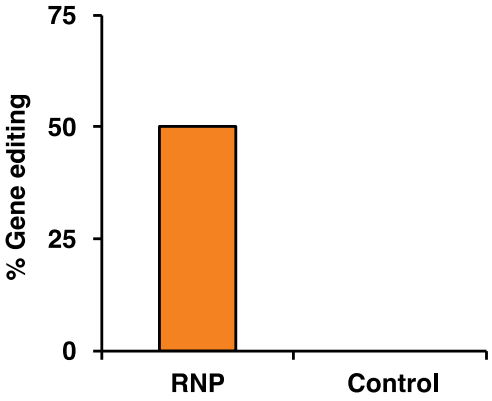
- RNP treated HSCs
  - Differentiate into erythroid and myeloid colonies
  - Monoallelic and biallelic gene disruption detected in HSC clones

Long-Term Engraftment of Cas9/gRNA RNP Treated Human HSCs

Compare engraftment of RNP treated and control human CD34$^+$ cells in mouse xenograft model



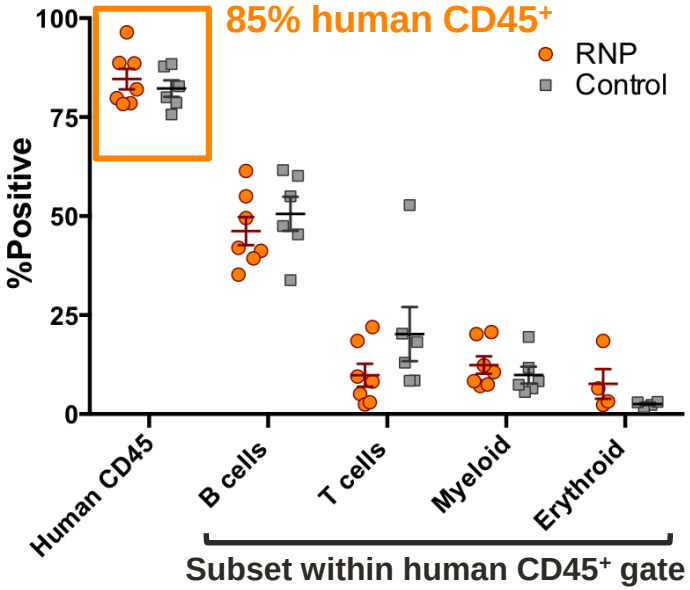
Parameter	n
Busulfan (mg/kg)	25
Control HSC mice	6
RNP HSC mice	7
CD34$^+$ cell dose	570,000

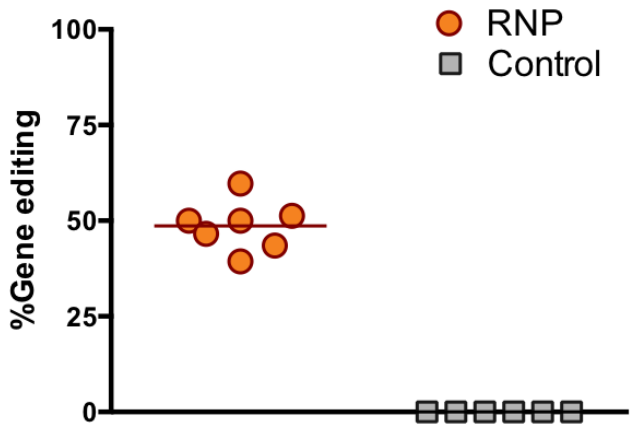


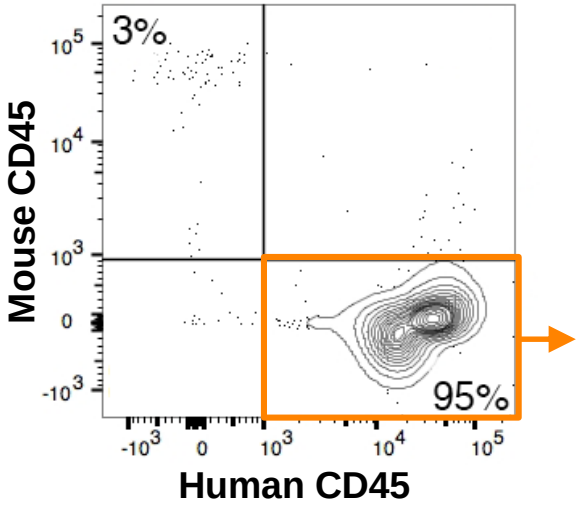
- **Reconstitution** of human hematopoiesis *in vivo* (4+ months)
- **Gene editing** in marrow, spleen, blood (human subsets)

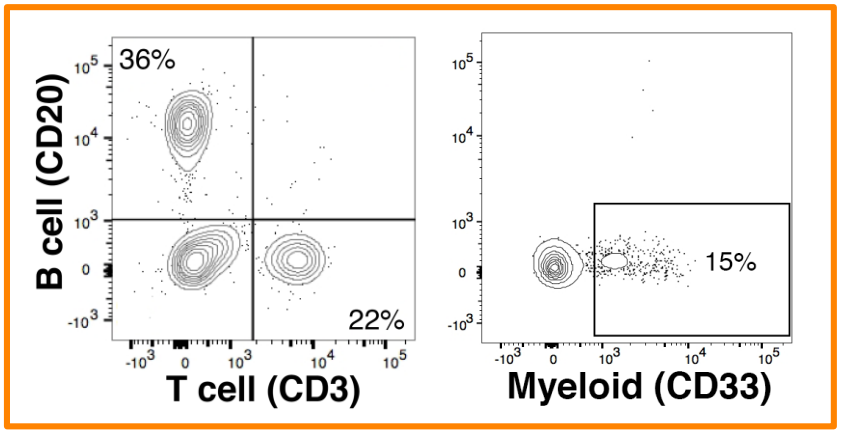
Gene Edited Cells Reconstitute Peripheral Blood

Human CD45+ lymphoid and myeloid cells at 4 months





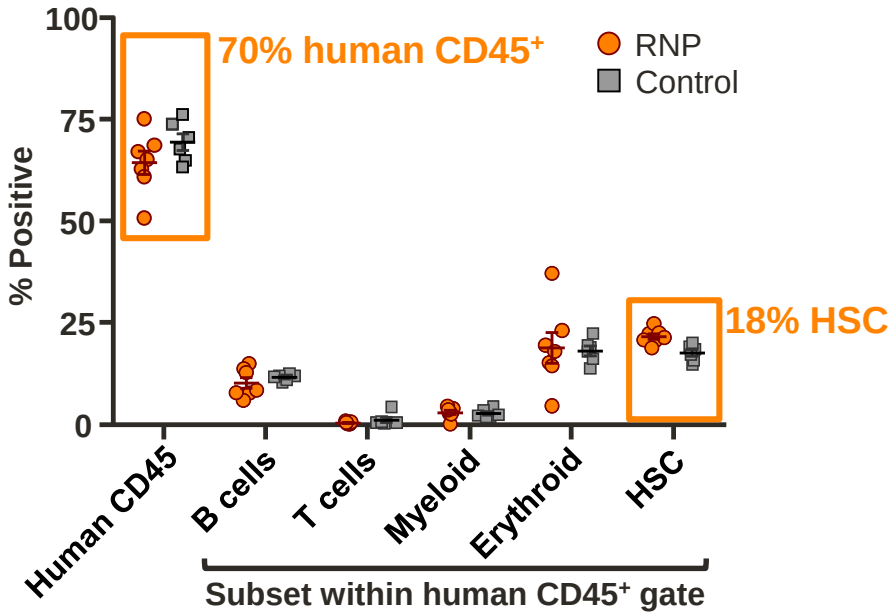




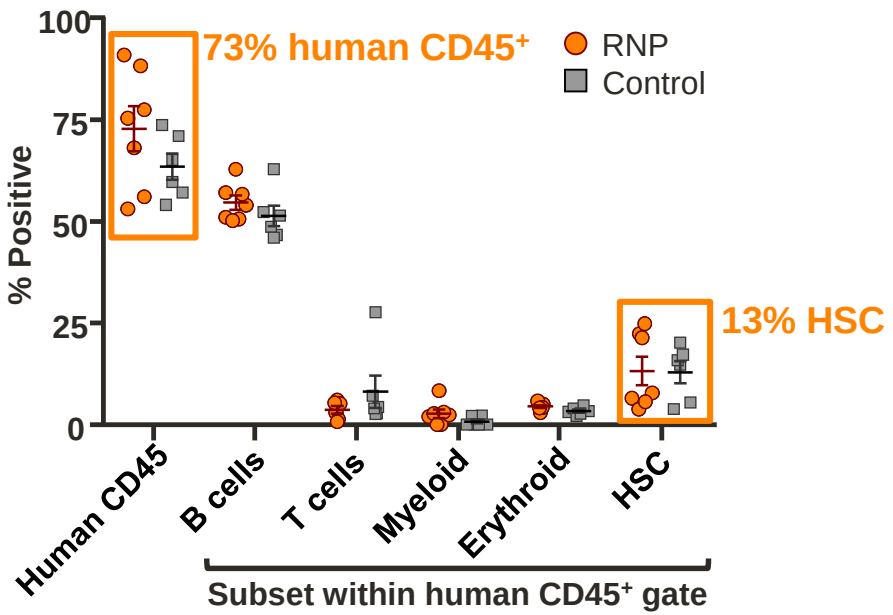
Gene Edited Human Blood Cells and HSCs Repopulate the Bone Marrow and Spleen

5 million HSCs recovered from bone marrow of each recipient

Bone Marrow



Spleen



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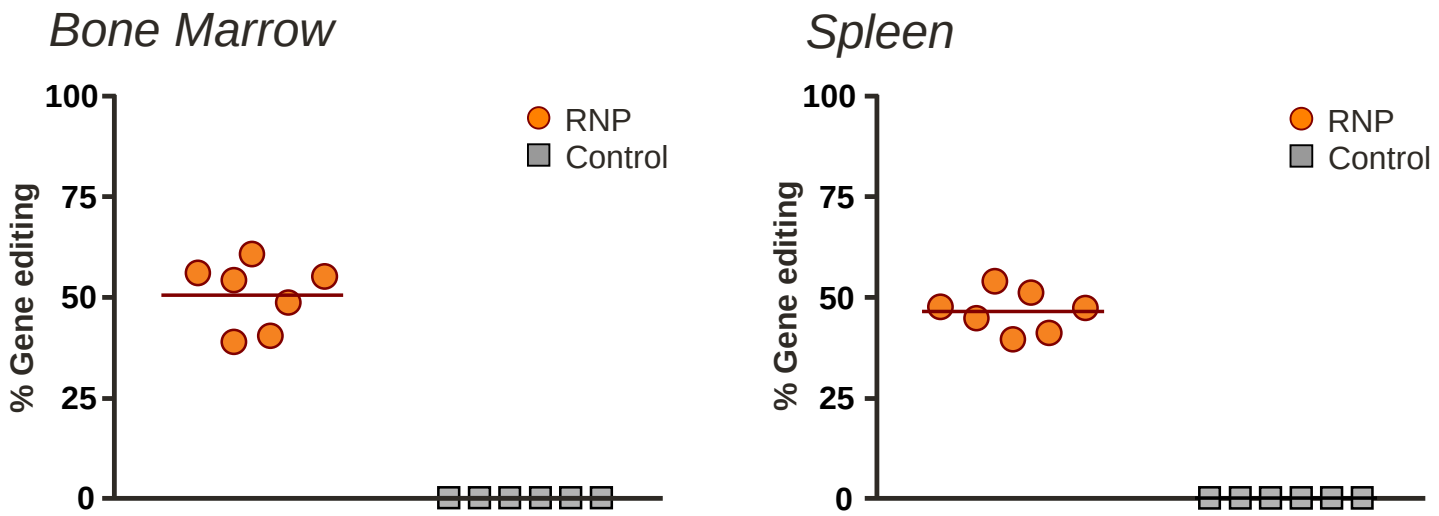
Efficient Gene Editing Detected in Human Blood Cells in the Bone Marrow and Spleen

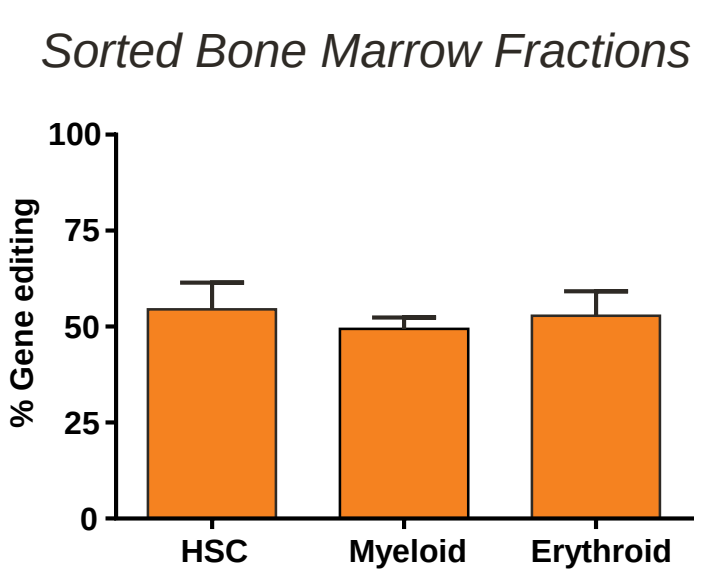
Gene editing *in vivo* equal to editing in pre-infusion product

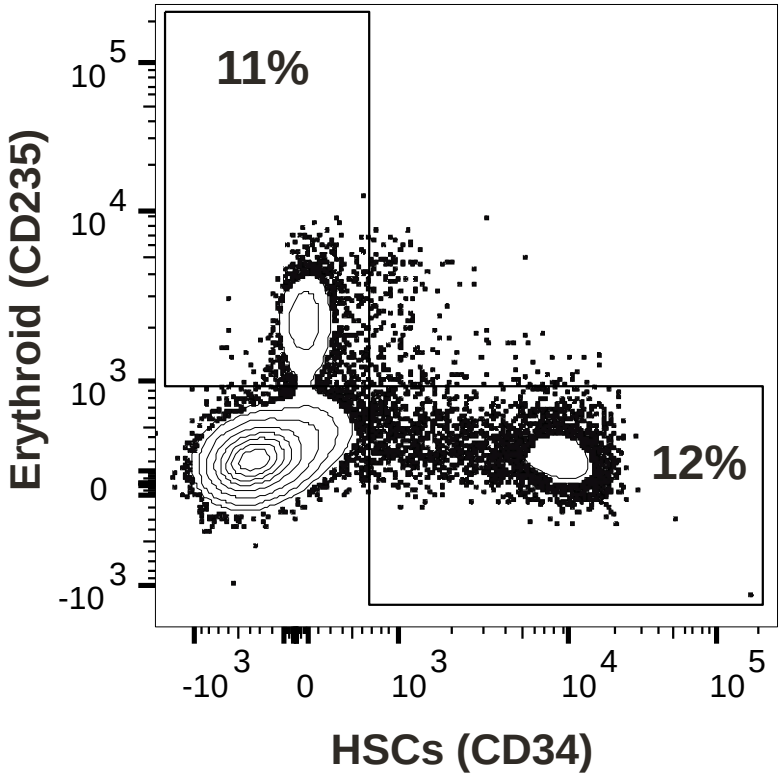


- **50% gene editing HSC before transplantation**
- **50% gene editing in engrafted cells in the blood, marrow, and spleen** 4 months after transplantation

Gene Editing is Maintained in HSC Progeny Differentiated *in vivo*

Gene editing in engrafted HSCs is maintained in progeny *in vivo*





Summary and Conclusions

- Cas9/gRNA RNP supports efficient and reproducible gene editing in human HSCs across donors (57% ± 8)
- Gene edited HSCs retain phenotype, viability, and differentiation potential *ex vivo*
- Gene edited human HSCs retain long-term engraftment and multipotency *in vivo* (50% editing and 85% human blood reconstitution)

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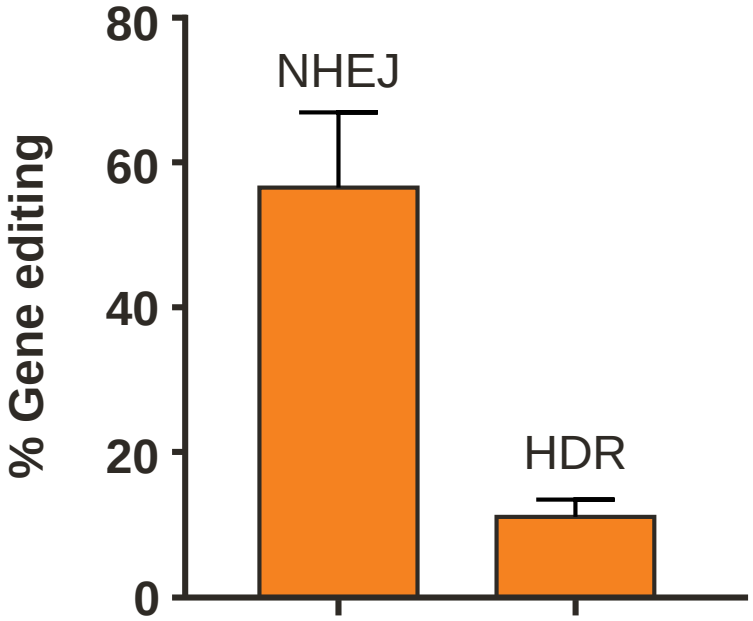
Electroporation of D10A RNP with Donor Supports Homology Directed Repair in HSCs



- 12% homology directed repair achieved after co-delivery of D10A RNP and single strand oligonucleotide donor

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Jack Heath
Aditi Chalishazar
Christina Lee
Will Selleck
Tanushree Phadke
Erik Corcoran
Cecilia Cotta-Ramusino
David Bumcrot