

CASEBIA  
THERAPEUTICS

# NHEJ-mediated Targeted Integration in LT-HSCs retain engraftment potential

Gene I Uenishi, PhD – Hematology

Gregory J Cost, PhD

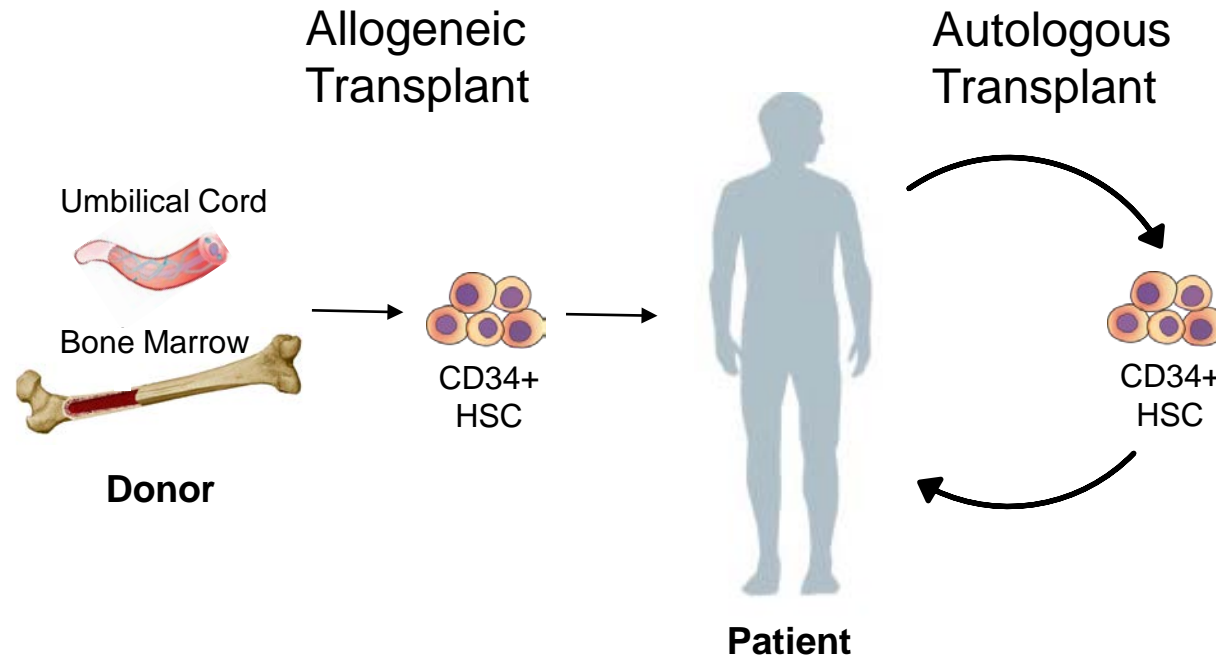
*May 2<sup>nd</sup>, 2019 – 22<sup>nd</sup> ASGCT Annual Meeting*

All authors are employees of Casebia Therapeutics LLC

# Introduction to editing HSCs

## Allogeneic HSCT treatment:

- Aplastic anemia
- Pure red-cell aplasia
- Paroxysmal nocturnal hemoglobinuria
- Fanconi anemia
- Thalassemia major
- Sickle cell anemia
- Severe combined immunodeficiency (SCID)
- Wiskott-Aldrich syndrome
- Hemophagocytic lymphohistiocytosis
- Inborn errors of metabolism
- Epidermolysis bullosa
- Severe congenital neutropenia
- Shwachman-Diamond syndrome
- Diamond-Blackfan anemia
- Leukocyte adhesion deficiency

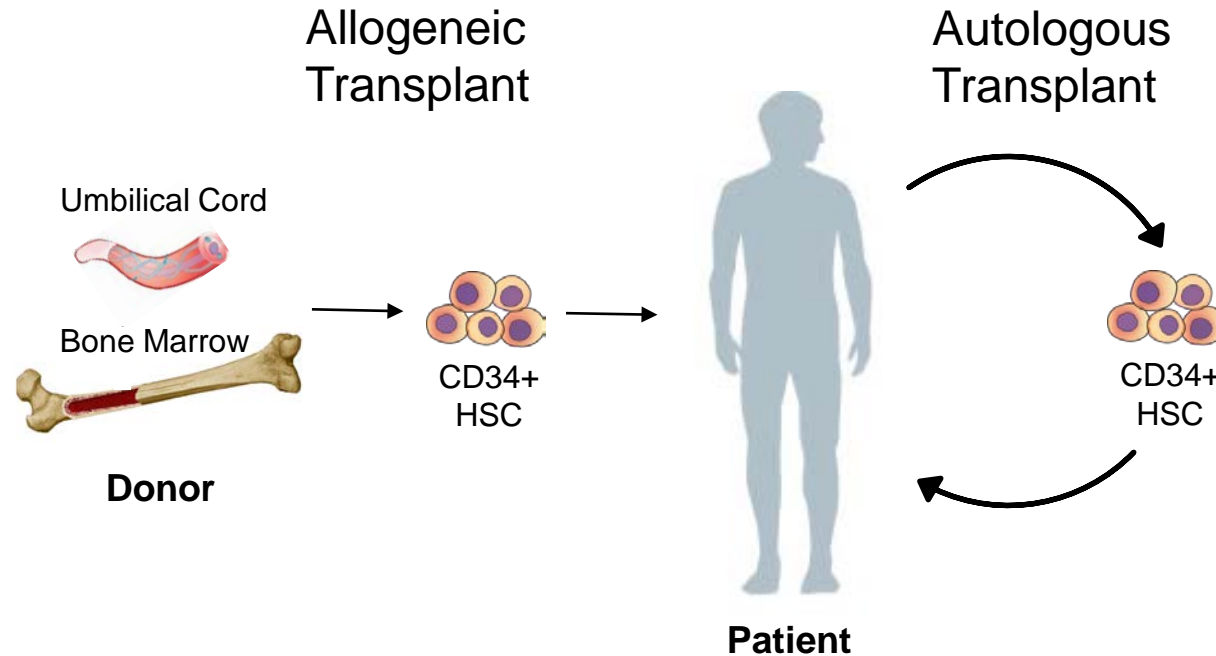


## Autologous HSCT treatment:

- Autoimmune disorders
- Amyloidosis

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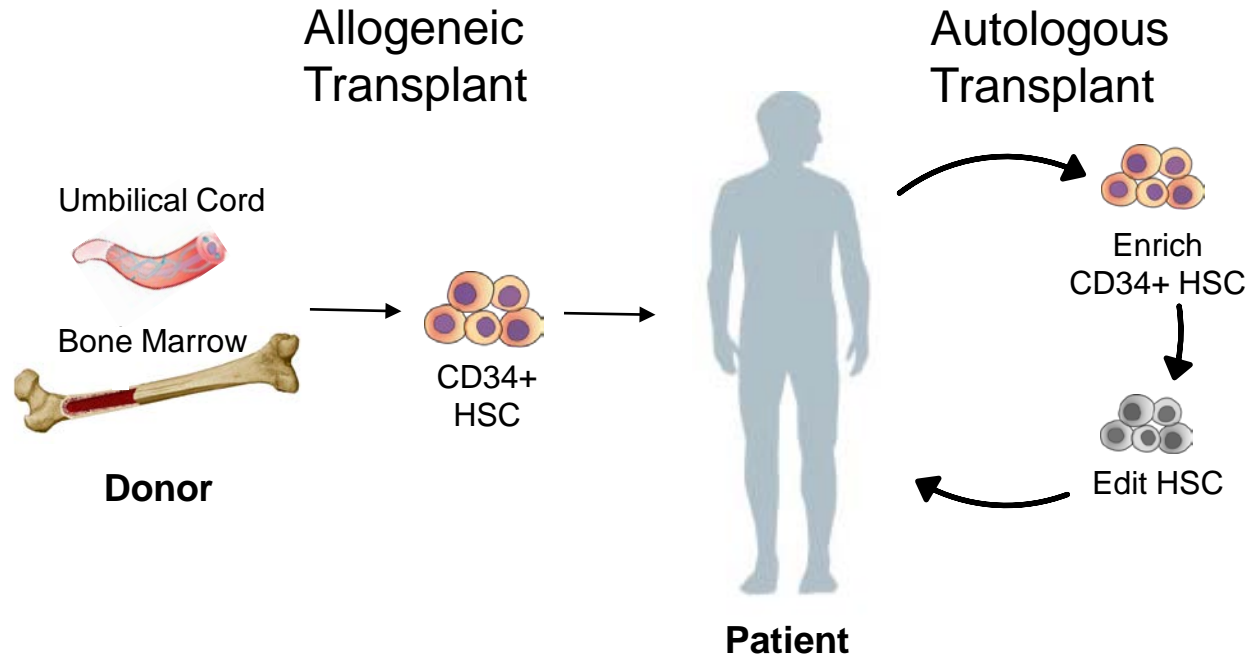


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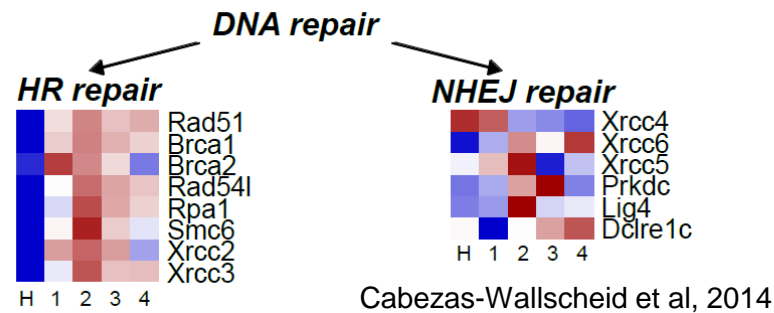
## Autologous HSCT treatment:

- Autoimmune disorders
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LT-HSCs edited for gene KO via NHEJ-mediated INDEL formation maintain engraftment potential

Mobilized PB-derived HSCs edited by HDR have extremely low engraftment potential

Mouse and human LT-HSCs do not express machinery required for HDR; NHEJ more likely

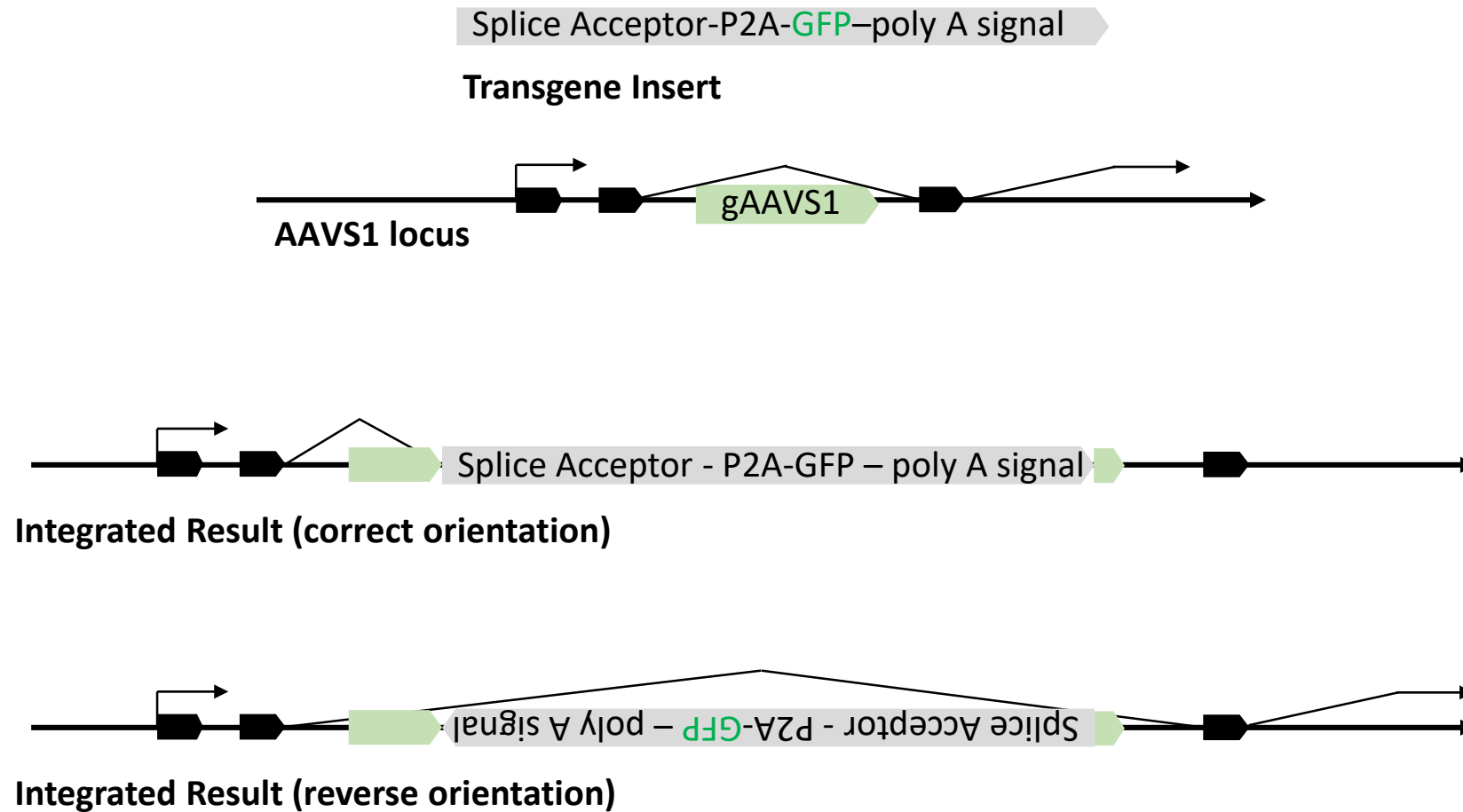


**Can we utilize LT-HSC's endogenous ability to do NHEJ for targeted integration?**

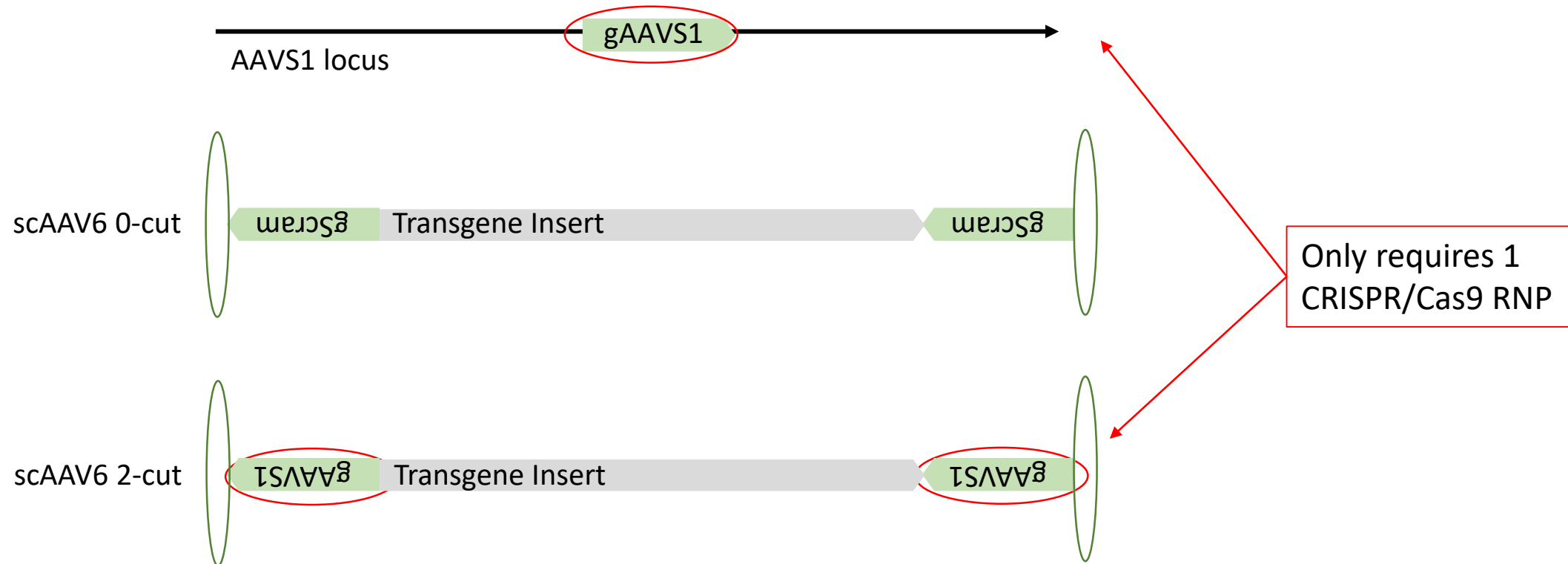
**Will LT-HSCs edited via NHEJ-mediated TI retain engraftment potential?**

# Test NHEJ-TI in AAVS1 locus of LT-HSCs

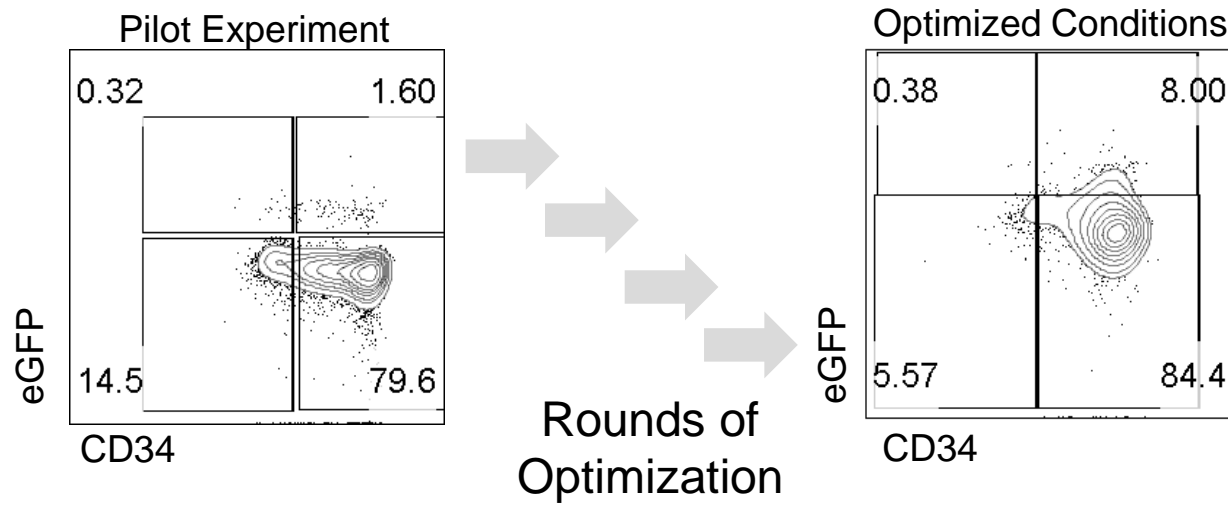




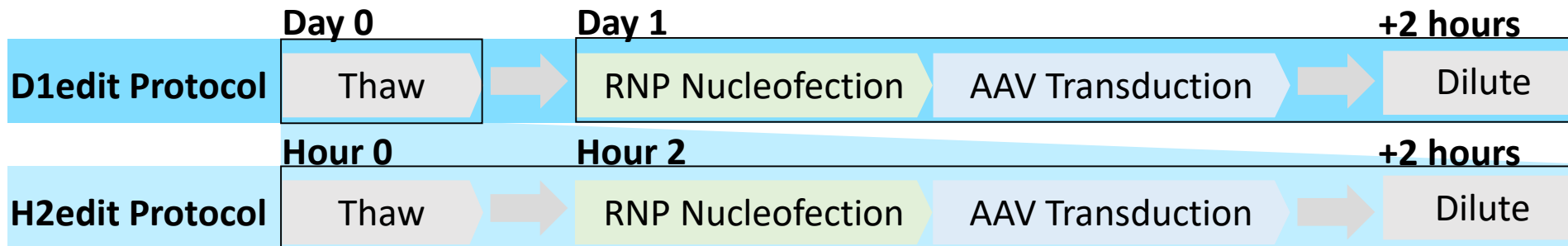
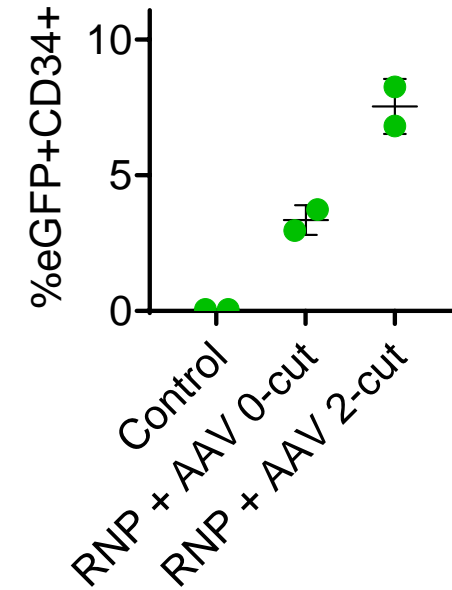
- eGFP reporter expression dependent on AAVS1 endogenous promoter



- Self-complementary AAV genome allows for CRISPR/Cas9 cleavage



## Integration frequency of No-cut vs two-cut donors

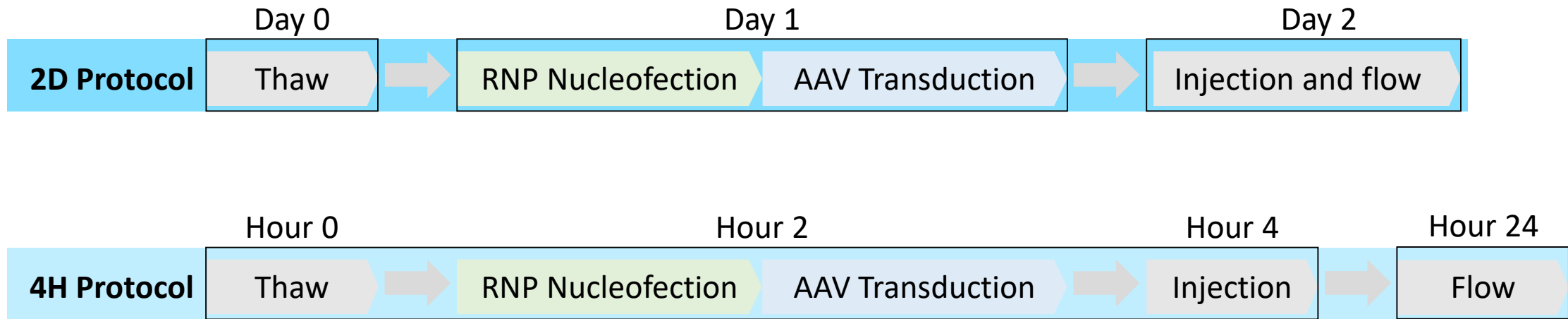


### Analysis

- 0-cut: 1-3% eGFP+
- 2-cut: 4-8% eGFP+

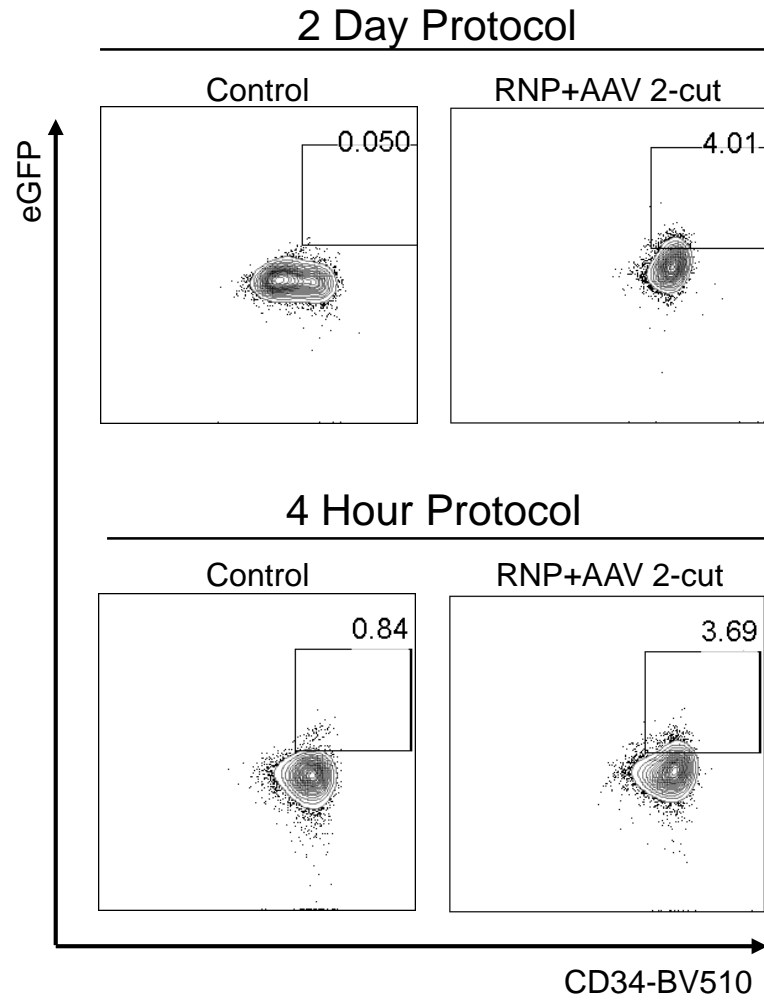
# In Vivo Data

NHEJ-TI Engraftment Study

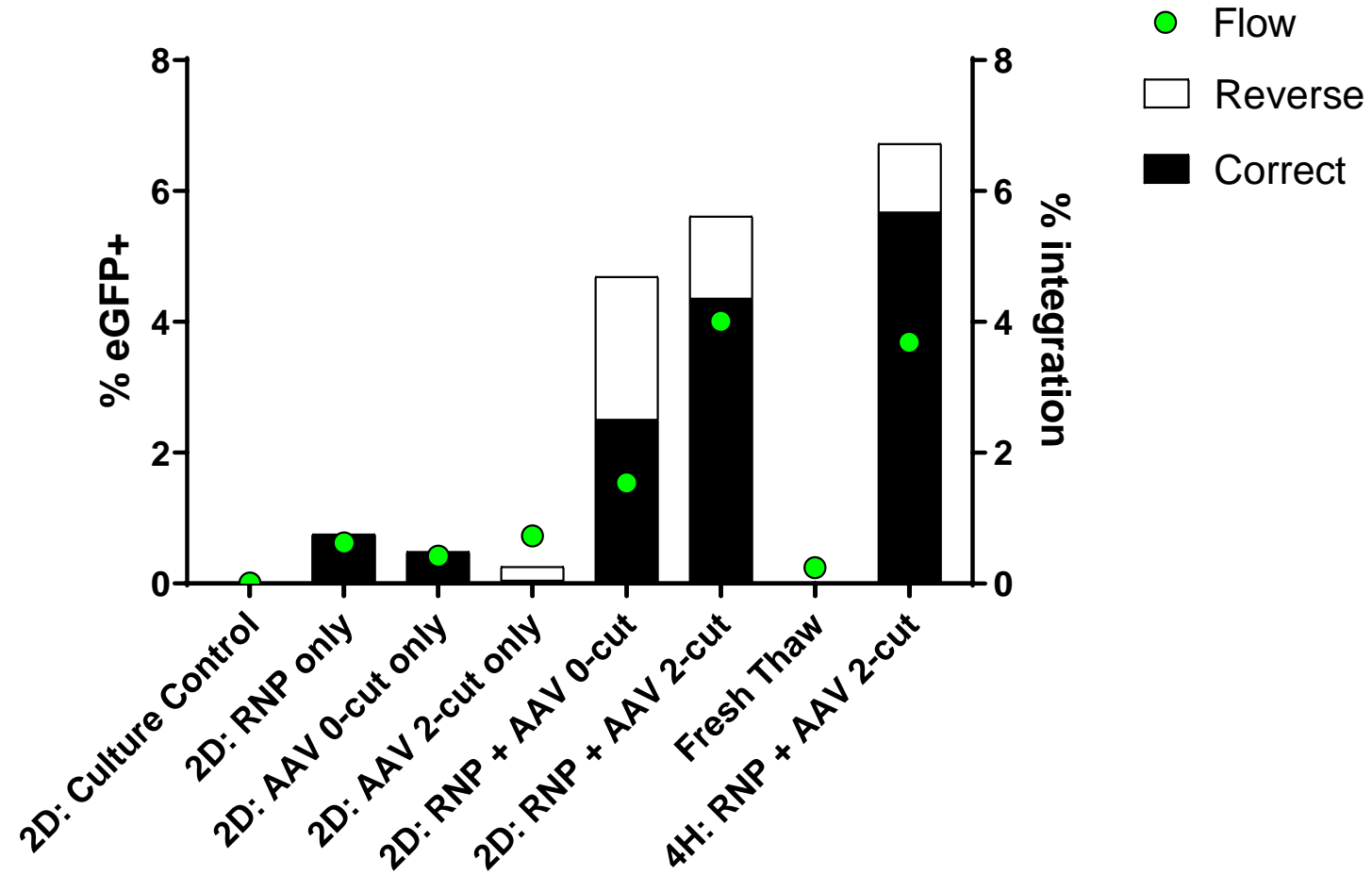


## Conditions

- Long and short editing protocol

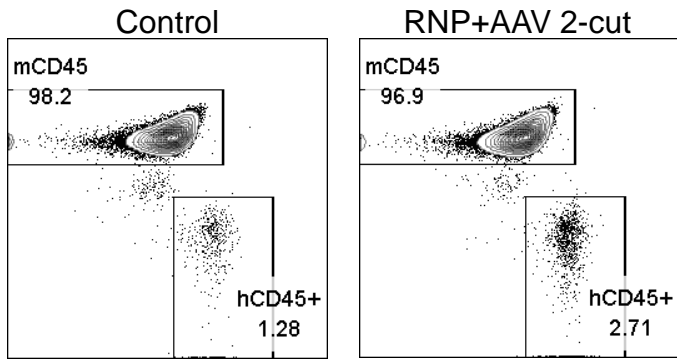


Flow Cytometry vs ddPCR results

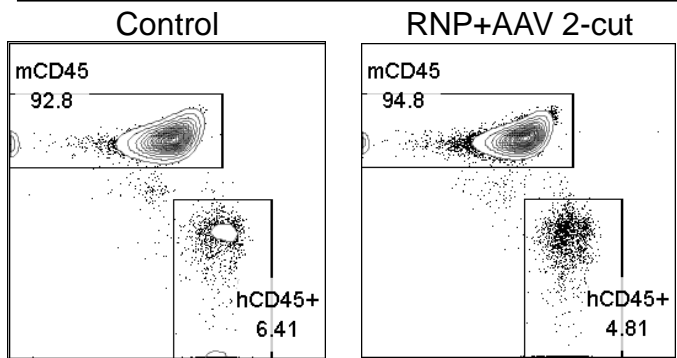


## Week 16: Gated on Live

### 2 Day Protocol

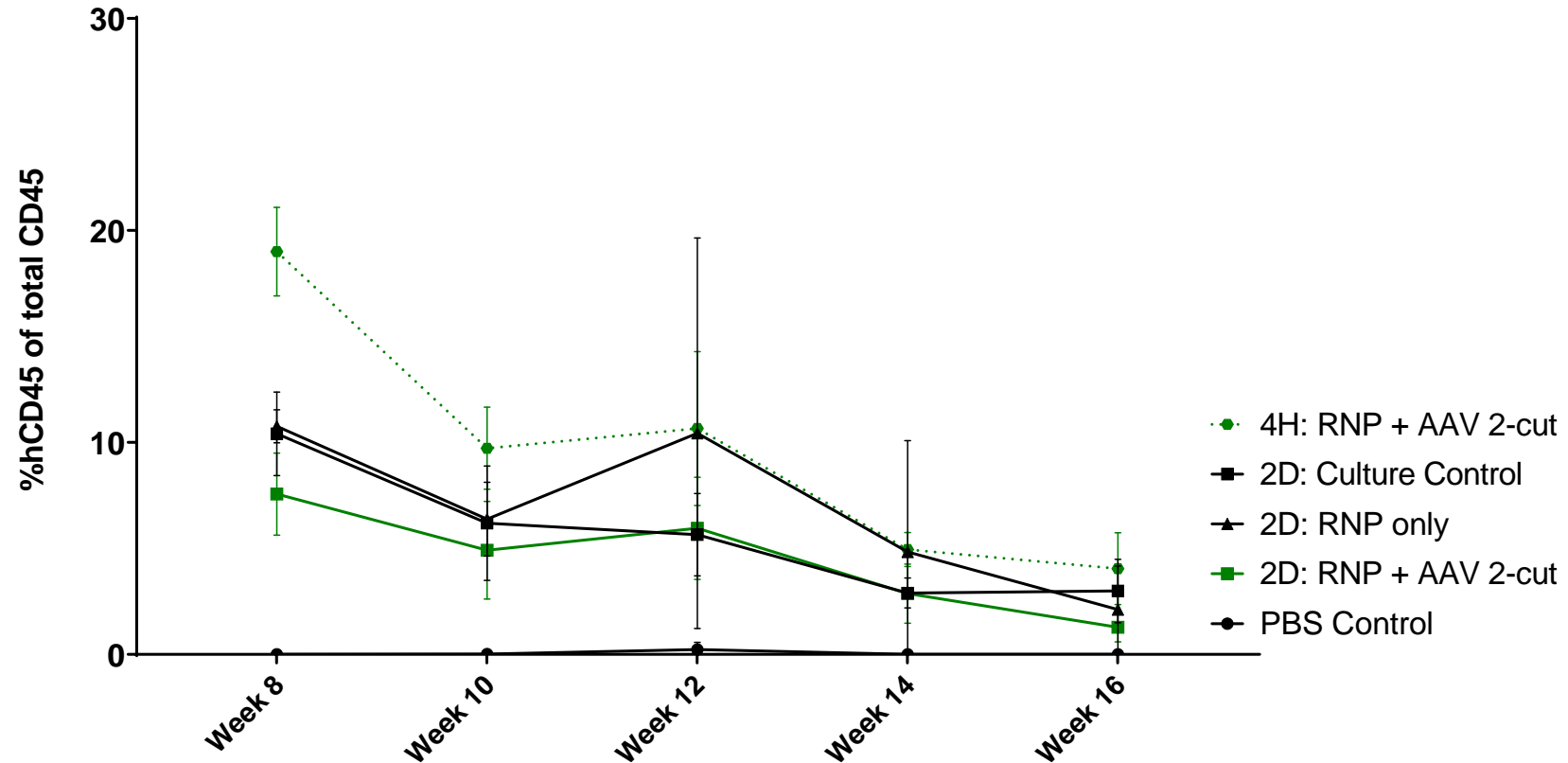


### 4 Hour Protocol

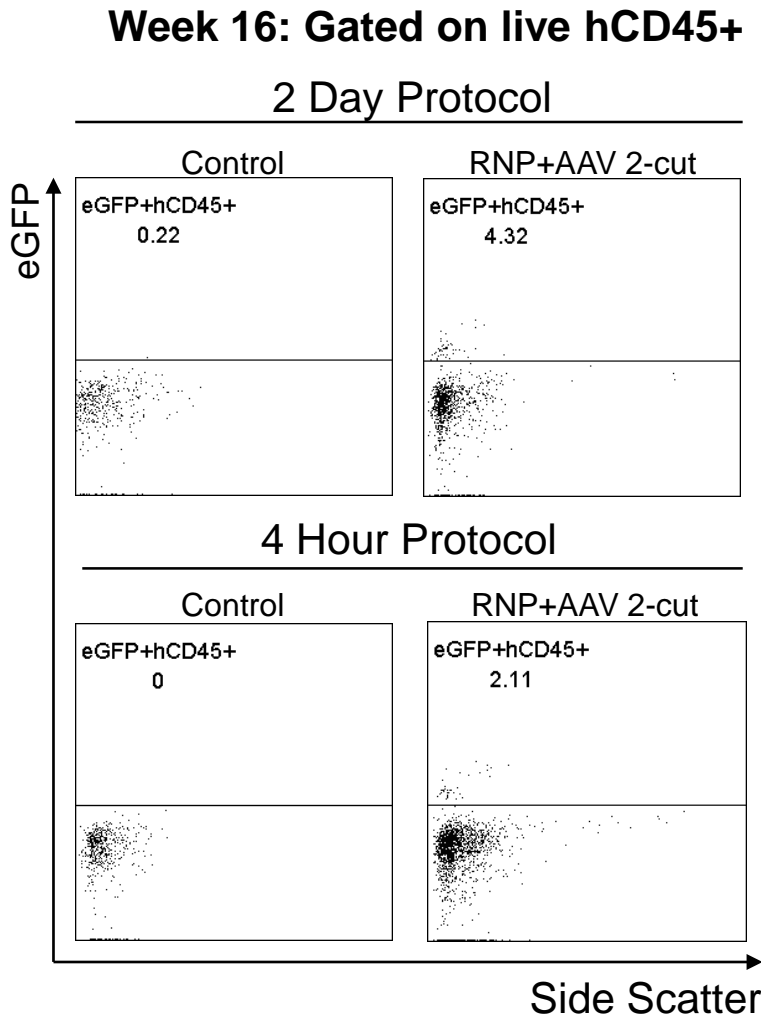


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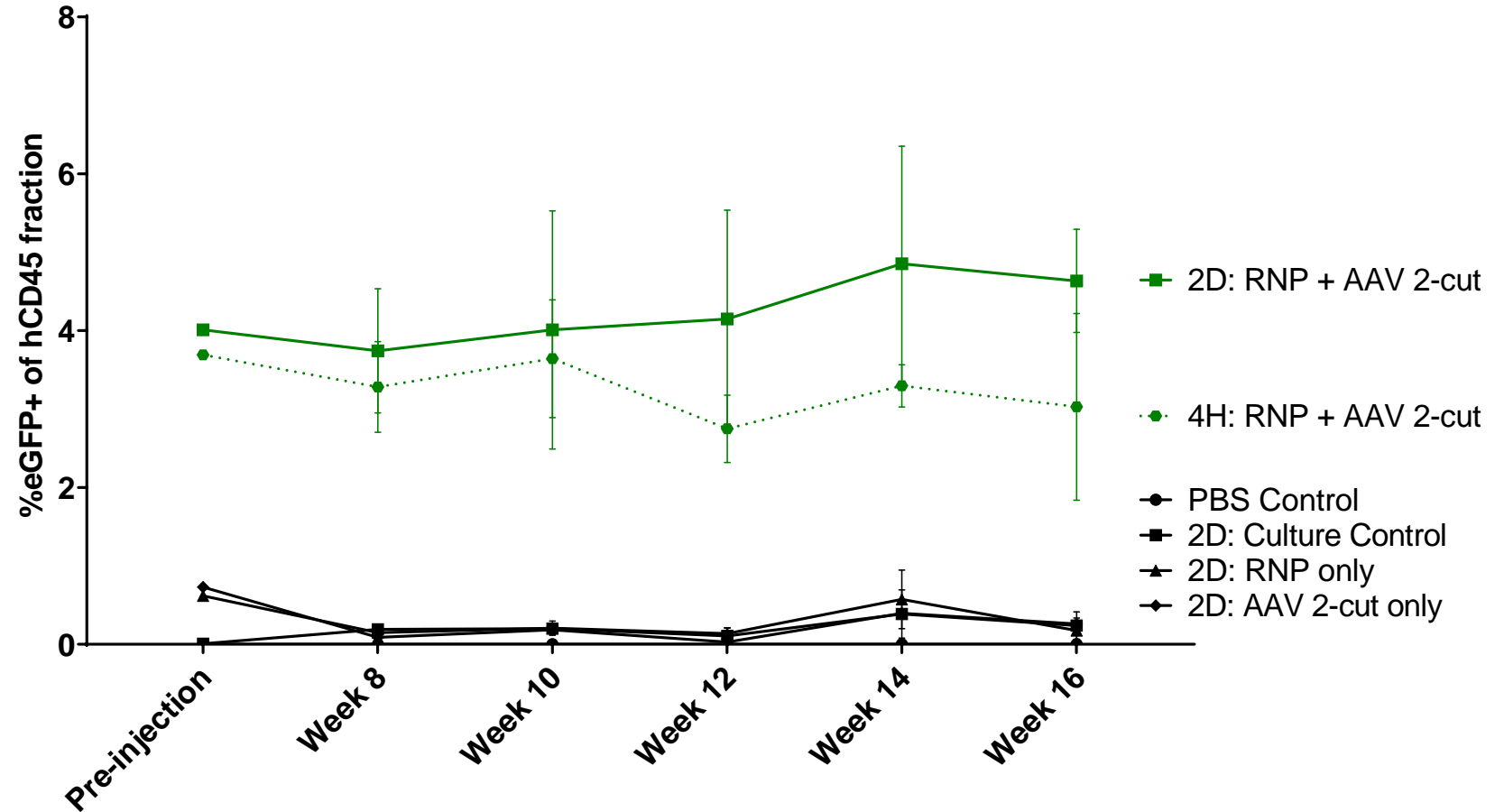
## Time Course of Engraftment



- **Short editing protocol gives higher overall engraftment**

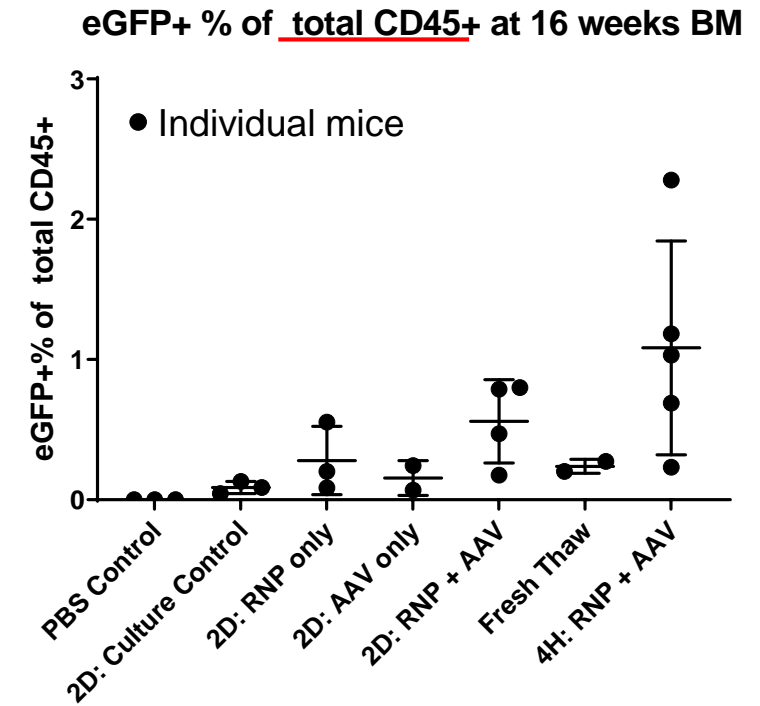
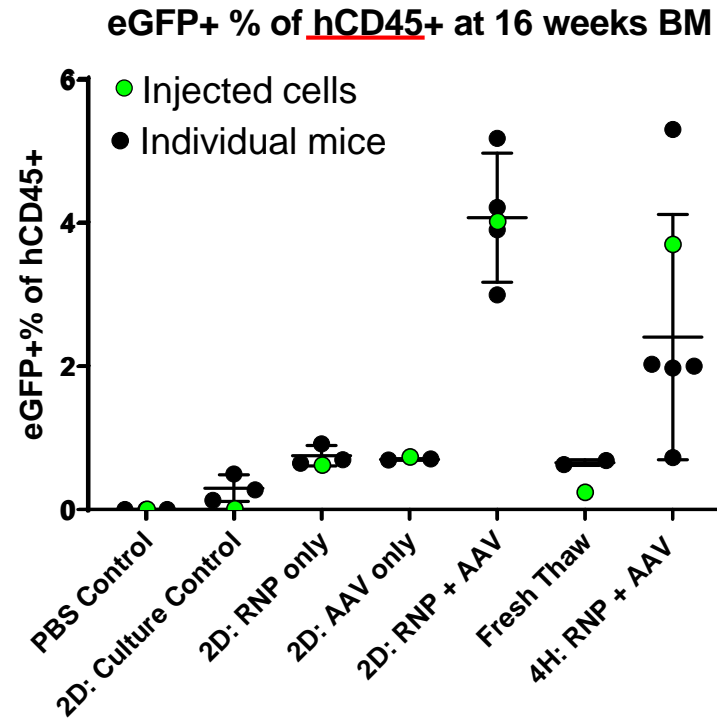
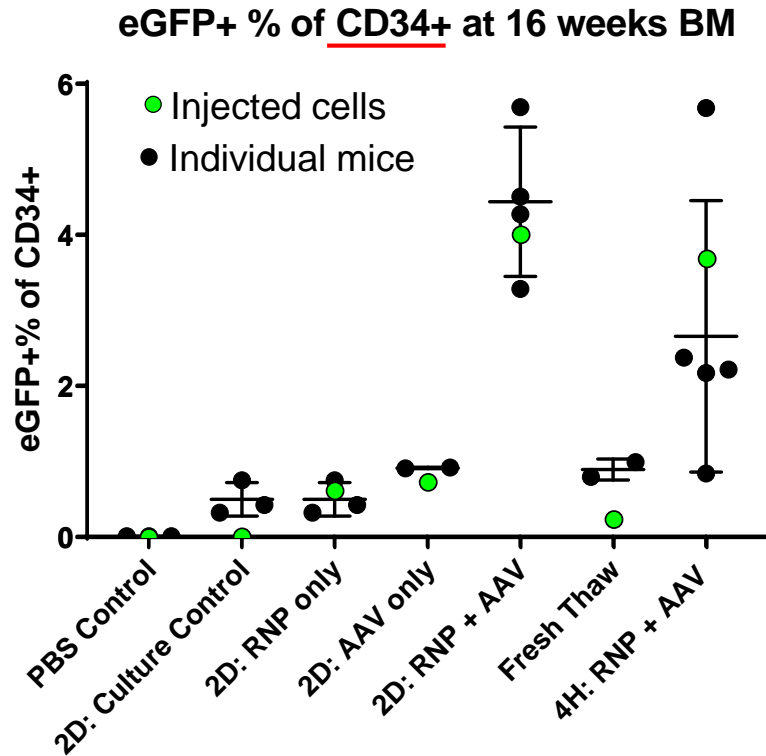


**Time Course of eGFP+**



**• Cells edited with NHEJ-TI are completely stable during engraftment**





- **Cells edited with short culture period have increased engraftment potential**

## **NHEJ can be used for targeted integration in mPB-derived LT-HSCs**

### **LT-HSCs edited by NHEJ-TI retain engraftment potential and persist in NSG mice**

- Allows targeting of non-dividing cells
- Short culture duration maintains stemness



**Gregory J. Cost, PhD: Principal Investigator**

**Andy Scharenberg, MD: Chief Scientific Officer**

**Thank you, ASGCT 2019!**

## Casebia Therapeutics

- Cade Ito
- Basha Stankovich, PhD: Senior Scientist
- Mamle Quarmyne, PhD
- Faye Wu
- Greg Boucher
- Samantha Chin
- Keith Abe: Senior Scientist
- Val Guerrero
- Cornell Mallari

## CRISPR Therapeutics

- Sanjay D'Souza, PhD
- Jason West, PhD
- Ying Zhang, PhD