Preclinical development of a TALEN®-based genome editing therapy for RAG1 deficiency

ESGCT Collaborative Virtual Congress 2021

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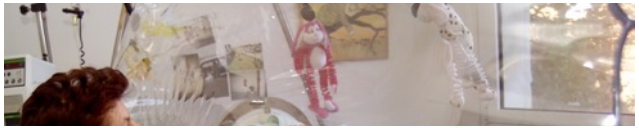
21.10.2021

Introduction

RAG1-SCID

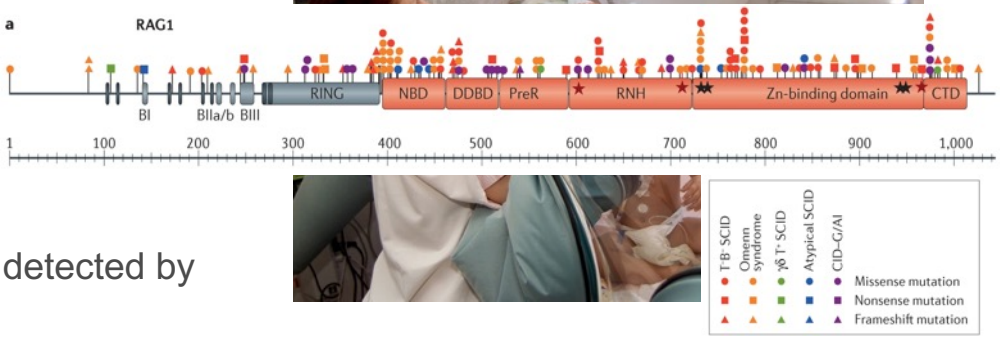
RAG1/2 are…

- **…essential for V(D)J recombination**
- **…required for maturation of B and T cells**



Characteristics:

- Absence of **T and B cells**
- Diminished/absent Ig levels
- Patients suffer from **infections**
- Lethal during infancy if untreated
- **RAG1-SCID** accounted for **8/49 SCID** cases detected by newborn screening in California (2010-2017)
- Only curative option to date HSCT



Challenges for gene therapy:

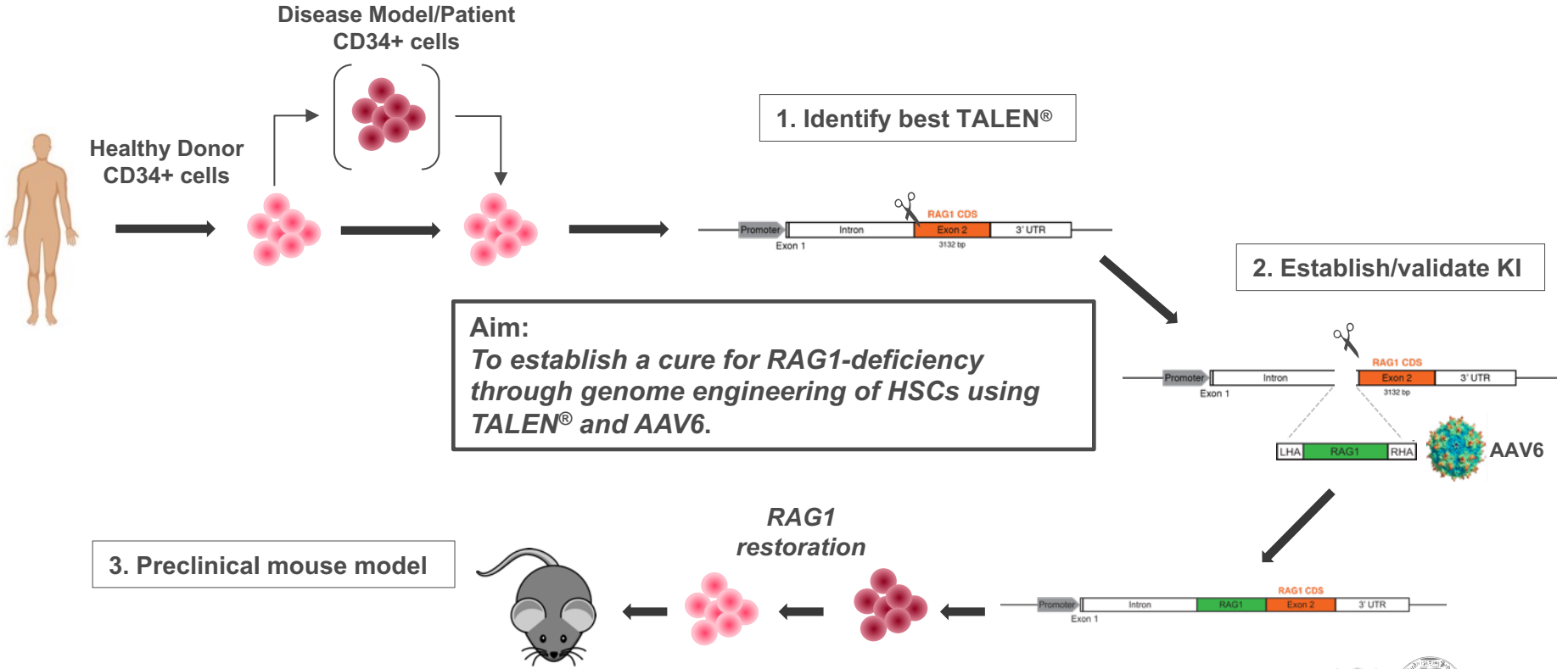
→ Spatiotemporal control of RAG1 expression

→ SCID-causing mutations spread across entire protein-sequence

→ Need to deliver full length copy for “one-size-fits-all” treatment option

Objective

Strategy and project overview

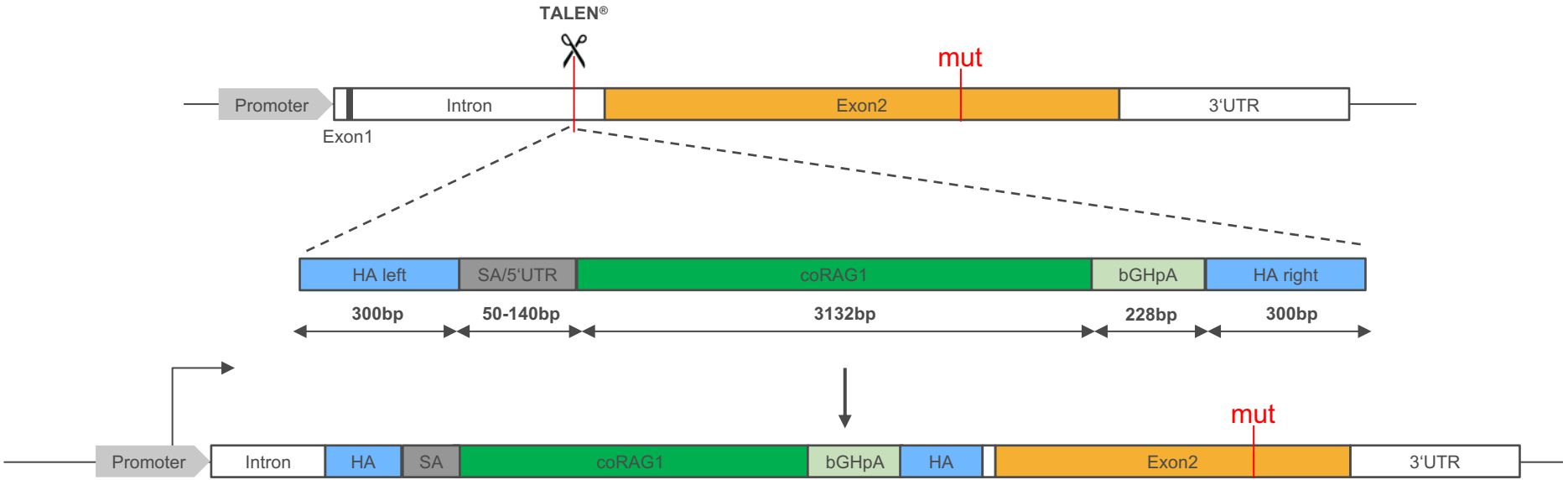


Introduction

Strategy

RAG1 deficiency $T^-B^-NK^+$SCID – gene editing Strategy

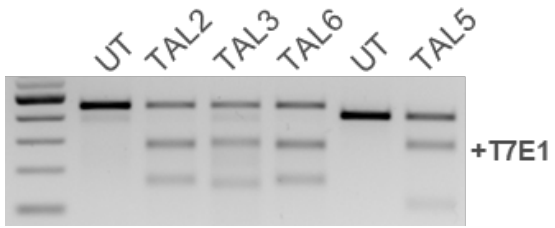
-Insertion of full length coRAG1 in Intron1→Expression under endogenous promoter

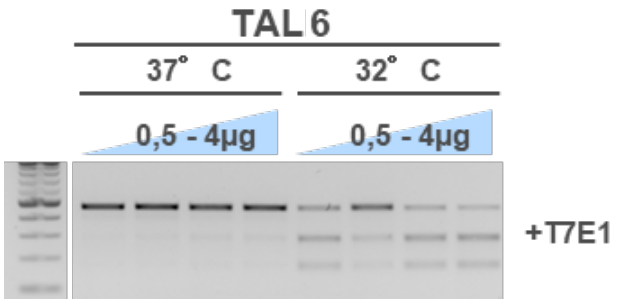


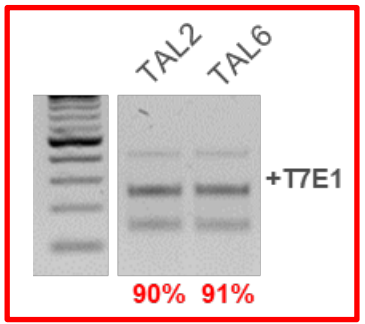
Identifying the best TALEN®

TALEN®-cleavage optimization in CD34+ cells

Initial screen → Optimization: Concentration/Temperature → High cleavage efficiency



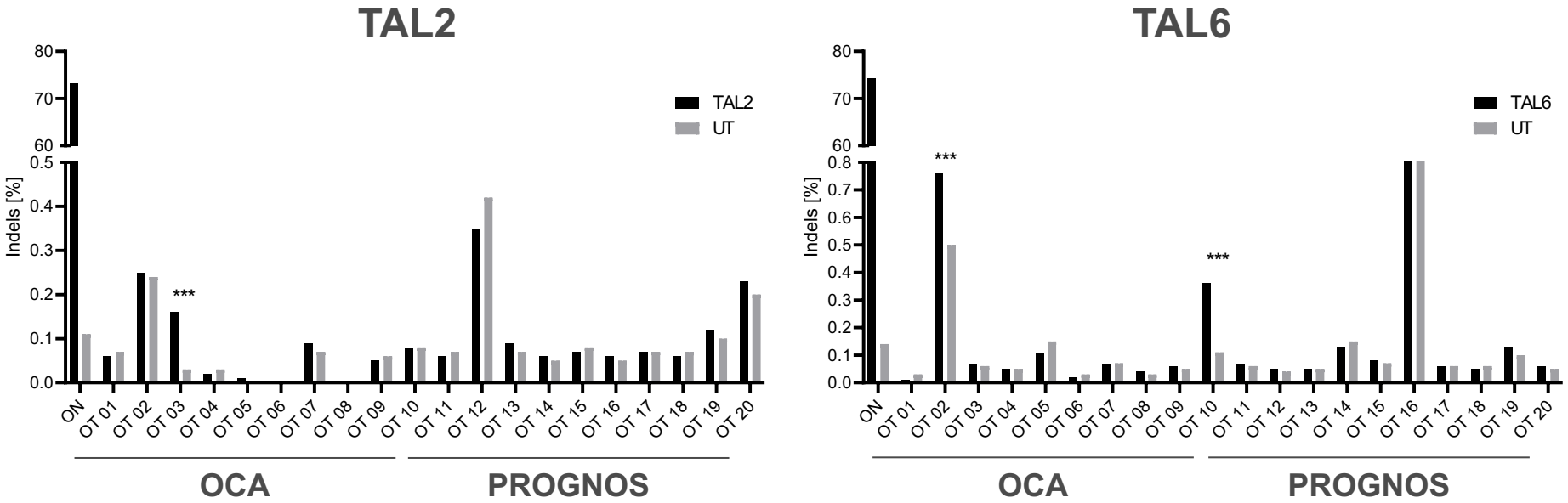




→Identification of two highly active TALEN® lead candidates

Off-target profile of TALEN® lead candidates

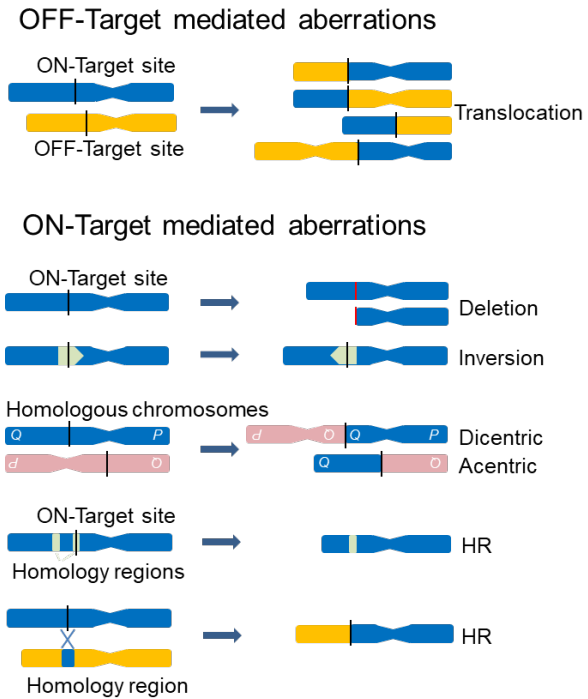
Oligo-capture (OCA) and *In silico* prediction hits



- Amplicon NGS confirms high on-target activity (~74%) for both TALEN®
- Minor off-target activity in one (TAL2) or two (TAL6) sites
- No off-target activity at *in silico*-predicted (PROGNOS) sites

CAST-Seq

Principles



CAST-Seq is capable of

Nominating off-target sites

OFF-target mediated gross chromosomal aberrations:

-chromosomal translocations

-chromosomal rearrangements

ON-target mediated gross chromosomal aberrations:

-large deletions

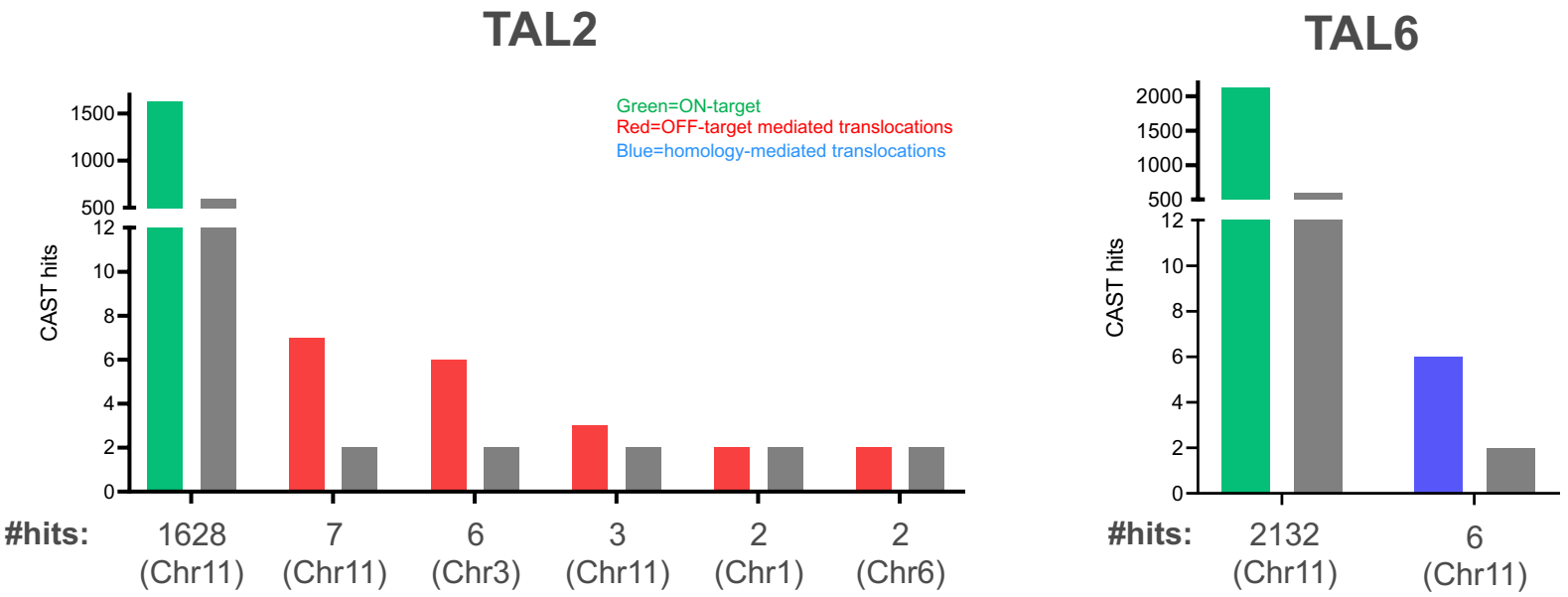
-large inversions

-homology-mediated translocations

Turchiano et al. (2021) Cell Stem Cell

CAST-Seq

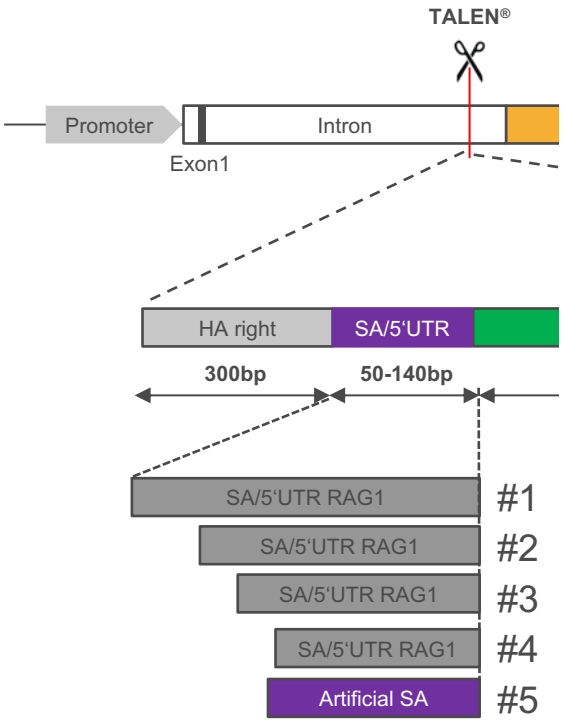
Hits

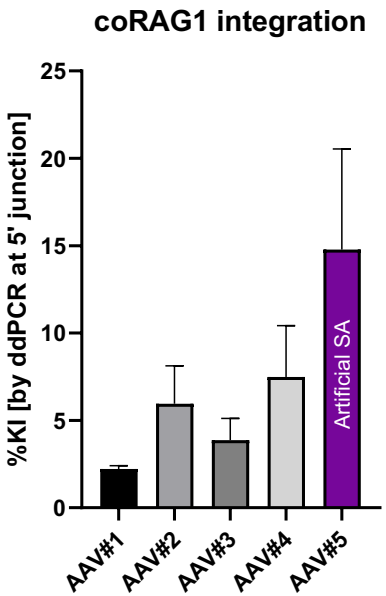


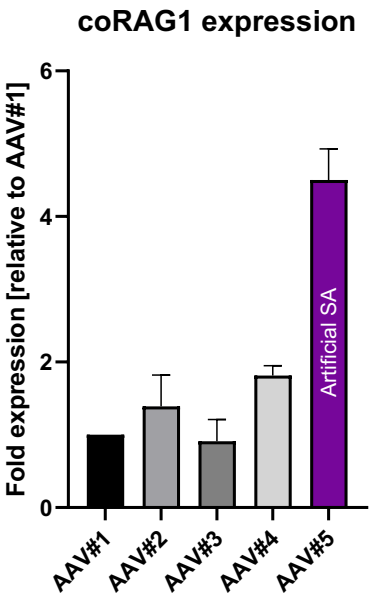
- All identified OMTs/HMT have few hits (≤0.05% of alleles)

AAV6 donor design

Integration in Jurkat cells

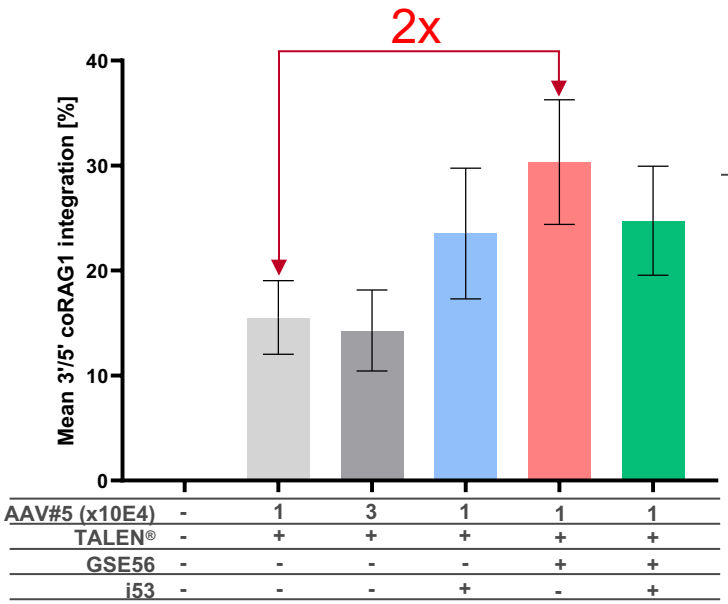


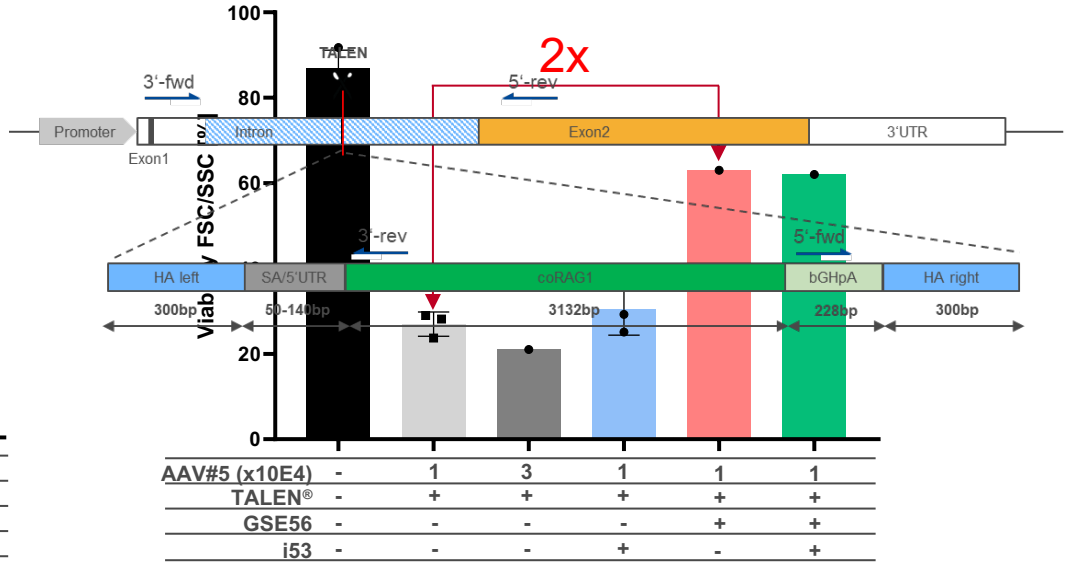




Targeted knock-in in CD34+ cells

Leveraging integration of corrective cDNA



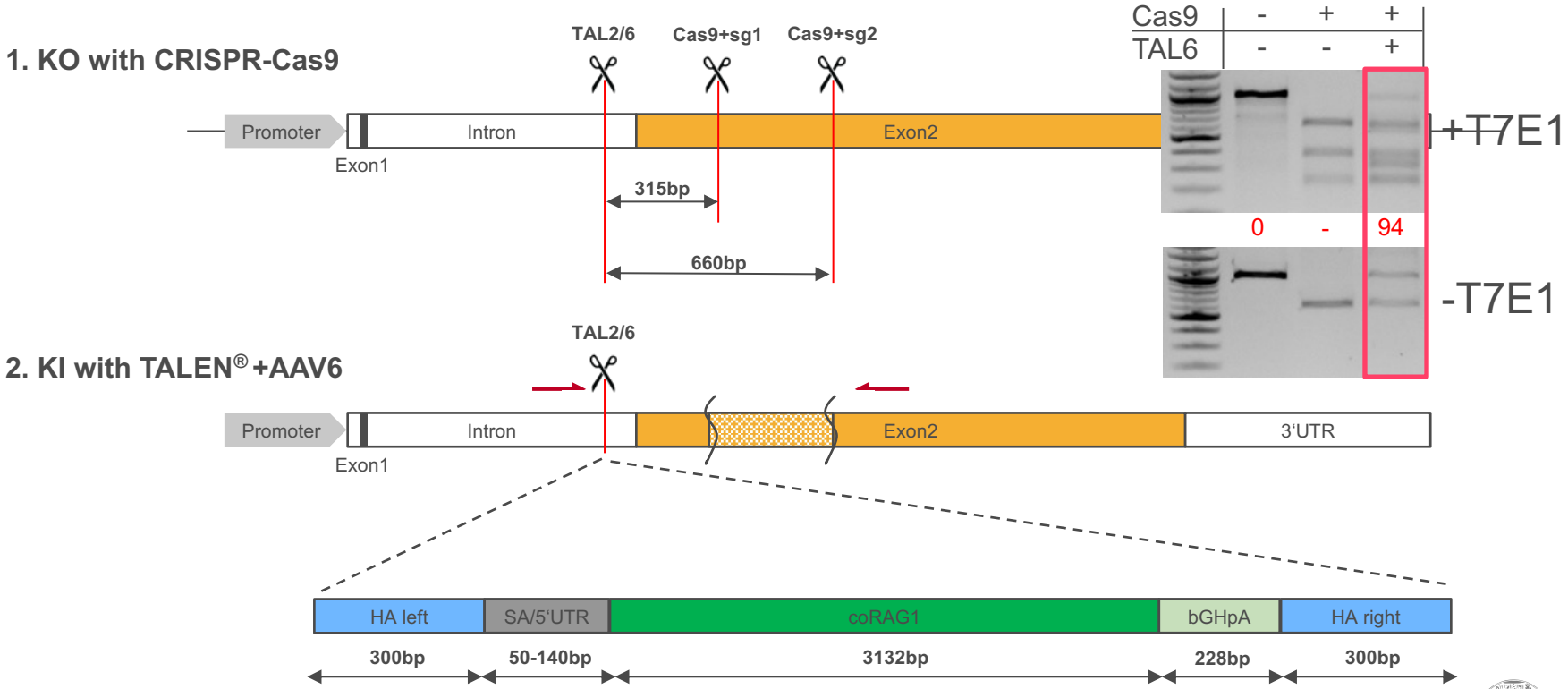


➢ 2-fold increase in knock-in and viability by GSE56

GSE56: Schiroli et al. (2019) Cell Stem Cell; i53: Canny et al. (2018) Nature Biotechnology

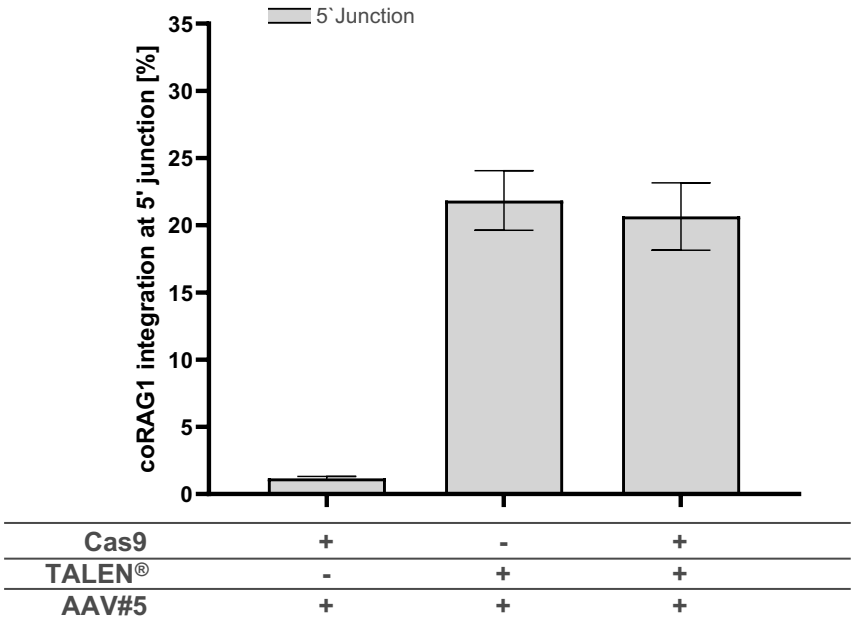
Establishing disease model

Disease model for functional rescue



Establishing disease model

Successive KO/KI in HSPCs

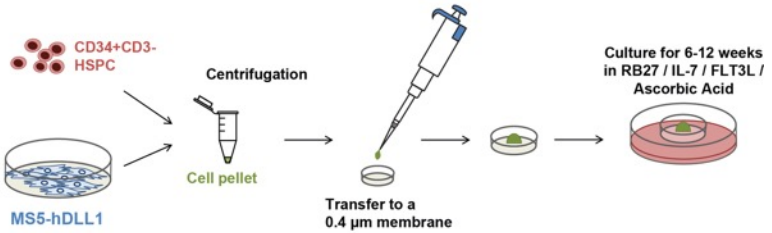


- Identical integration frequency (~20%) in Cas9 pre-treated samples
- **Successful establishment of potential disease model**

T cell differentiation in ATOs

Artificial thymic organoid (ATO) formation

a



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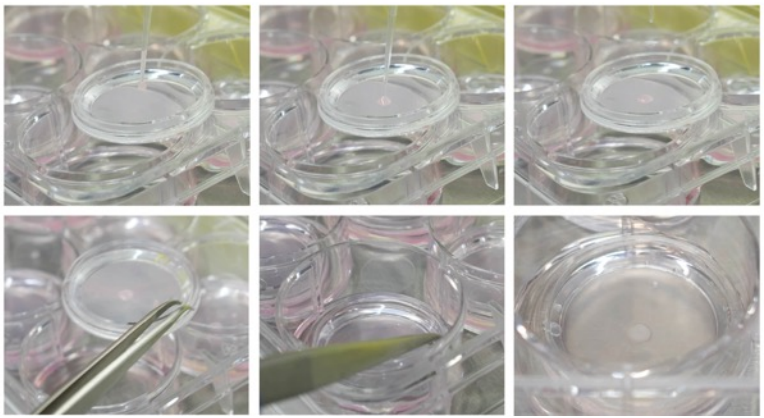
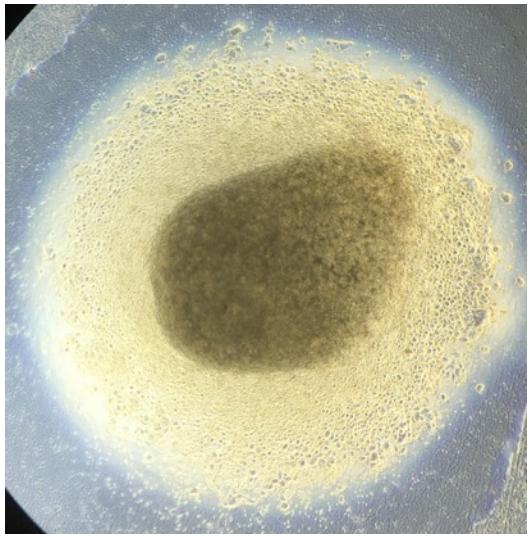
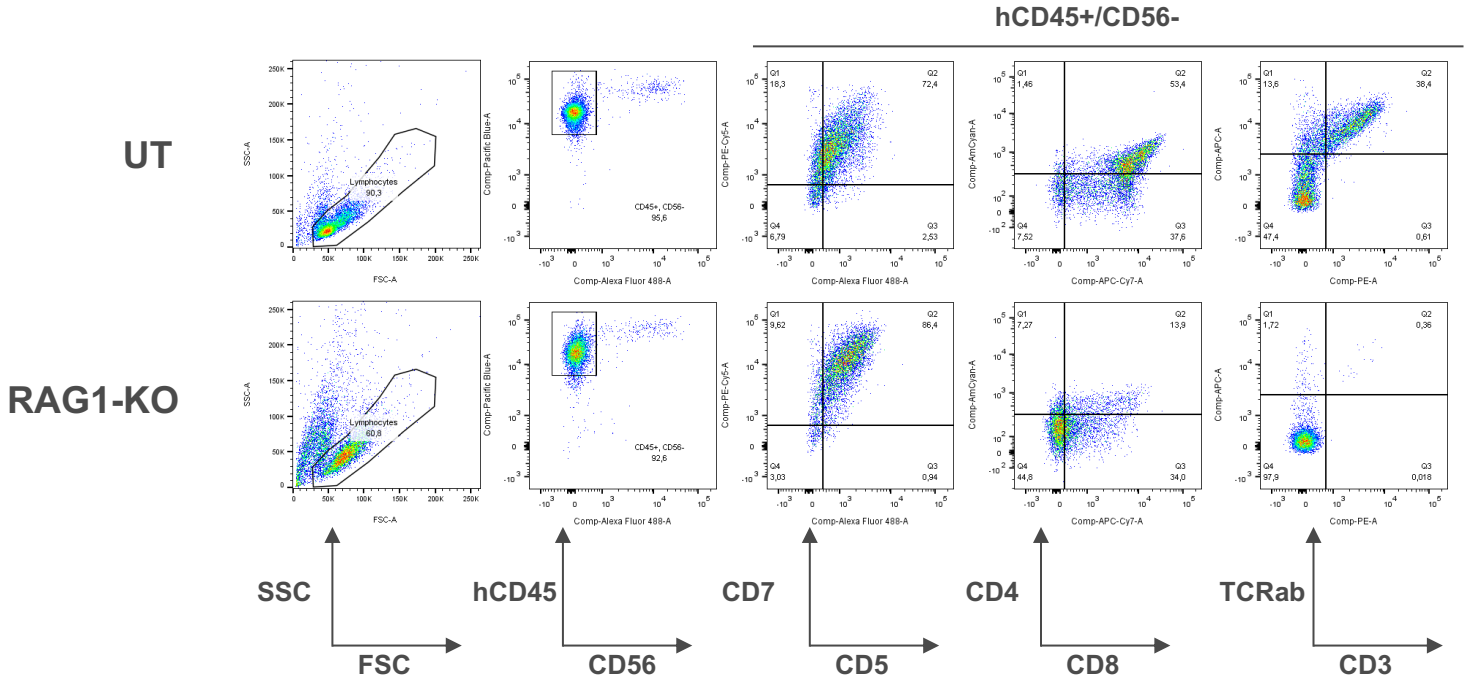




Seet et al. (2017) Nature Methods

T cell differentiation in ATOs

Role of *RAG1* in T cell development *in vitro* (7 weeks)



Successful establishment of RAG1 deficiency *in vitro* model

Summary & Outlook

Summary

1) Identification of two highly active TALEN® with good safety record
2) Leveraging knock-in of *coRAG1* (rAAV donor) in CD34+ cells through inclusion of additional RNAs (i53, GSE56): up to ~30% in bulk HSCs and ~20% in LT-HSCs
3) Increasing the viability of edited HSCs 2-fold by transient p53 inhibition
4) Establishing ATOs as *in vitro* model system to evaluate functional restoration of RAG1 deficiency (~40% CD3/TCRab-DP cells)

Outlook

1) Functional rescue experiment (RAG1-KO followed by coRAG1-KI → seeding into ATOs)
2) Sequencing of T cell receptor repertoire
3) Transplantation of rescued HSCs into NSG mice to show functional restoration of T/ B cell development

Acknowledgments

Toni Cathomen
Jamal Al-Zu'bi
Kay Ole Chmielewski
Tobias Deller
Ala'a Dibas
Melina el Gaz
Carla Fuster
Simone Haas
Ruba Hammad
Marcel Heugel
Beate vom Hövel
Masako Kaufmann
Julia Klermund
Lei Lei
Richard Schäfer
Michalis Papacharalampous
Christine Reichenbach-Braun
Anika Schmith
Francisco Villena
Alessio Conci

Tatjana Cornu
Viviane Dettmer-Monaco
Manuel Rhiel
Julia Rositzka
Kerstin Geiger

Claudio Mussolino
Antonio Carusillo
Klara Penkert
Silvia Roman Azcona
Daniel Türk
Sibtain Haider
Nitish Sabharwal

cellectis
Philippe Duchateau
Alexandre Jullierat

Gay M. Crooks
Shawn Lopez

Melanie Börries
Geoffroy Andrieux