Non-genotoxic antibody-based conditioning paired with multi-plex base edited HSCs for the potential treatment of sickle cell disease

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FASEB Genome Engineering Conference

June 27th, 2022

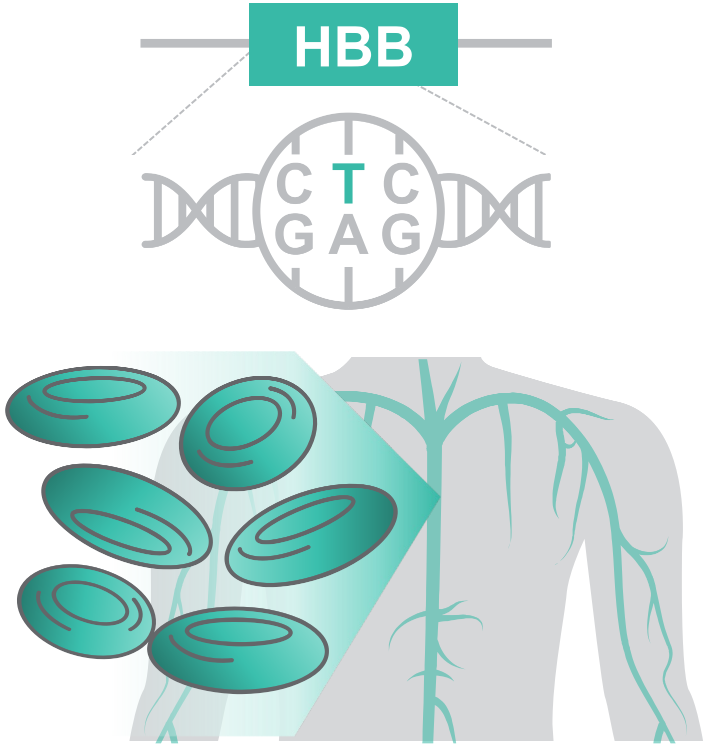
Sickle cell disease (SCD) is a monogenetic disease caused by a single-letter mutation in the *HBB* gene

β-globin gene

Adult β-globin gene

Glutamine at 6th amino acid (HbA)

Normal red blood cells

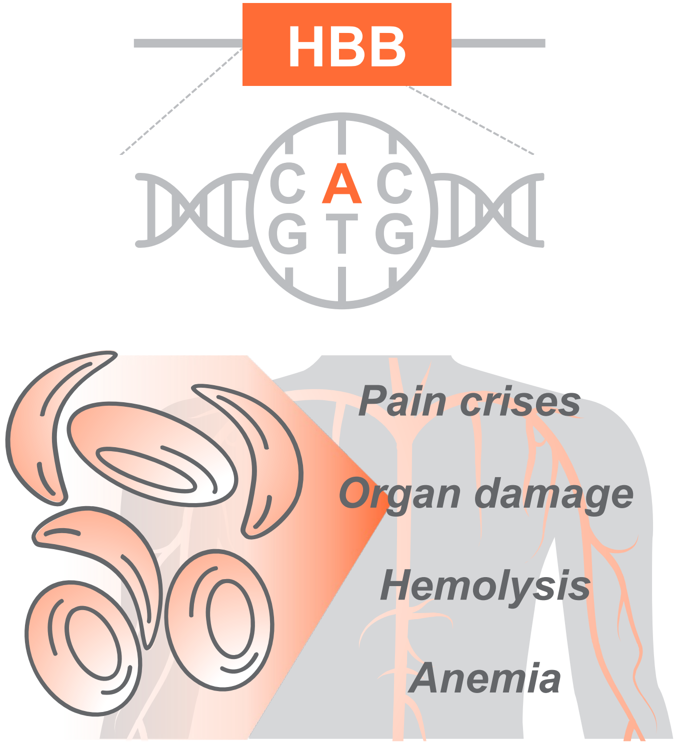


T-to-A mutation causes sickling

Sickle β-globin gene

Valine at 6th amino acid (HbS)

Sickling red blood cells



Pain crises

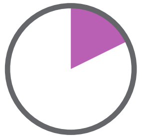
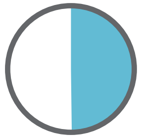
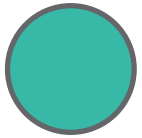
Organ damage

Hemolysis

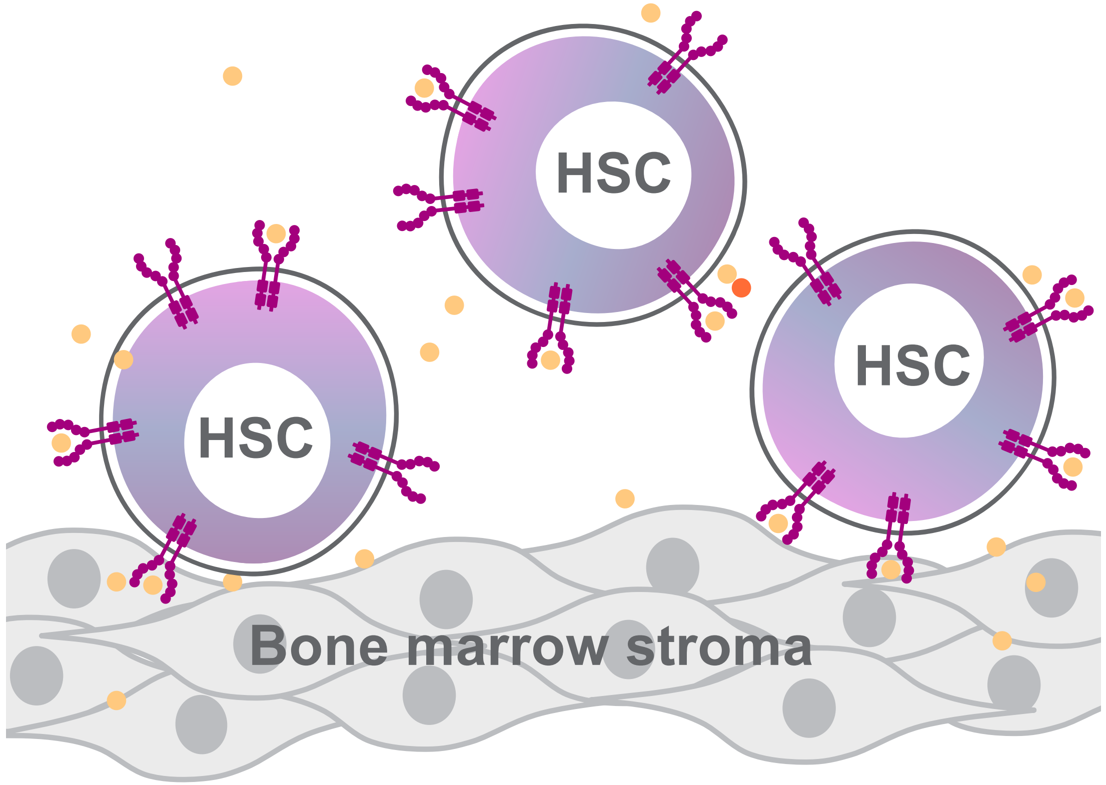
Anemia

There are approximately 100,000 sickle cell disease patients in the US

Long term strategy to potentially cure SCD and build a transformative platform within hematology

	Editing **(HSCT + busulfan)**	**Editing + Conditioning** **(HSCT + novel)**	**Editing + Delivery** **(infusion)**
Goals	• Highly efficient and specific correction of genotype • Non-cutting, non-viral	• Less toxic conditioning to eliminate old cells and make room for edited graft	• Transition from ex vivo editing (transplant) to in vivo editing (infusion)
Potentially addressable patients			
Required technologies	• Base editing (ex vivo)	• Multi-plex base editing • Antibody interference with conditioning target	• HSC-targeted LNP • HSC-targeted viral vectors • Multi-plex base editing • Antibody interference with cell conditioning target
Programs	• BEAM-101 (HPFH) • BEAM-102 (MKSR)	• ESCAPE-1 (HPFH+CD117) • ESCAPE-2 (MKSR+CD117)	• TBD

Bone marrow niche harbors HSCs, the cells that reconstitute the hematopoietic system



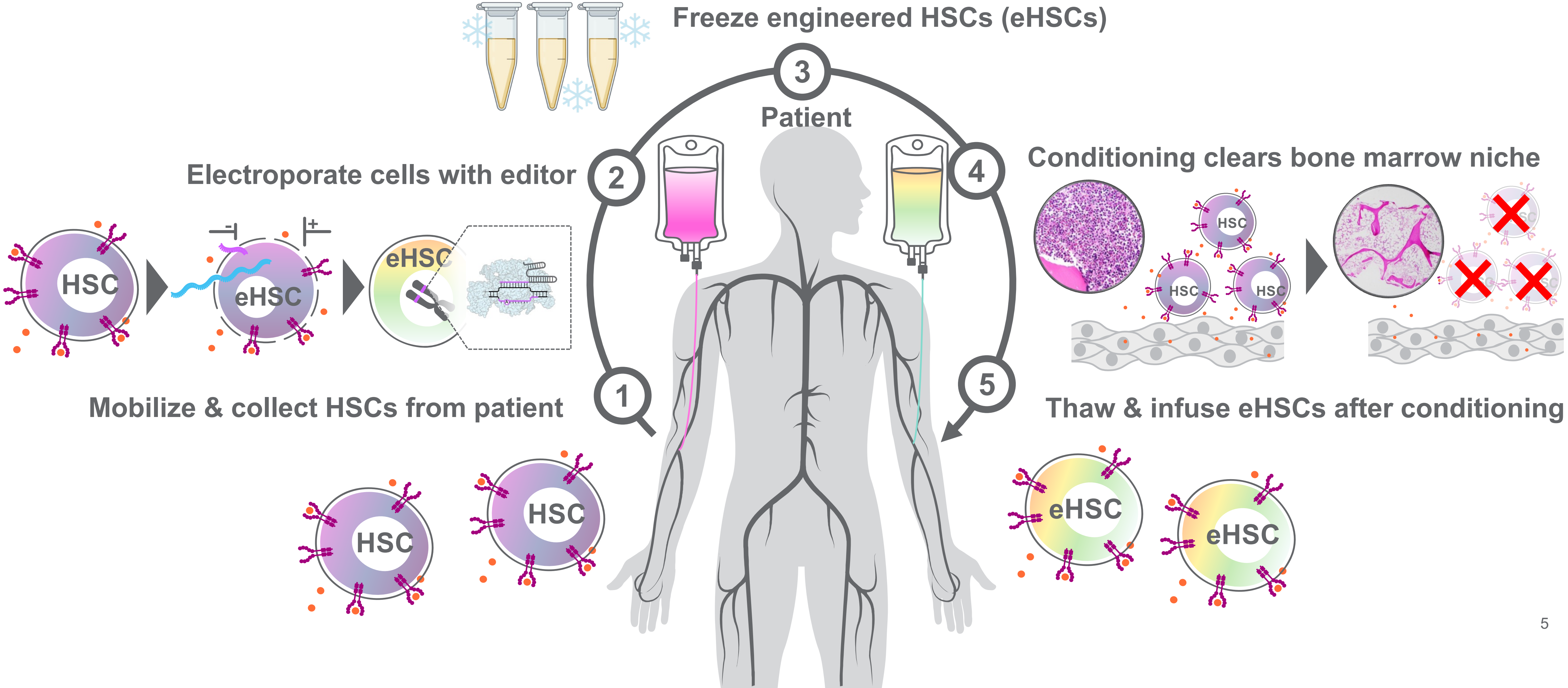
HSC = Hematopoietic stem cells

● = Stem Cell Factor (SCF) the ligand for CD117

Y = CD117 critical for survival & renewal of HSCs

- Hematopoietic stem cells (HSCs) are harbored in the bone marrow microenvironment
- Stromal cells secrete stem cell factor (SCF), that signals through CD117 on HSCs and support survival and renewal of HSCs
- Differentiation of HSCs both in bone marrow and the periphery are responsible for reconstitution of the hematopoietic system
- Genome-edited long-term HSCs are potentially curative for sickle cell disease

Autologous transplantation of engineered HSCs currently requires conditioning with toxic alkylating antineoplastic agents such as busulfan


Freeze engineered HSCs (eHSCs)

Patient

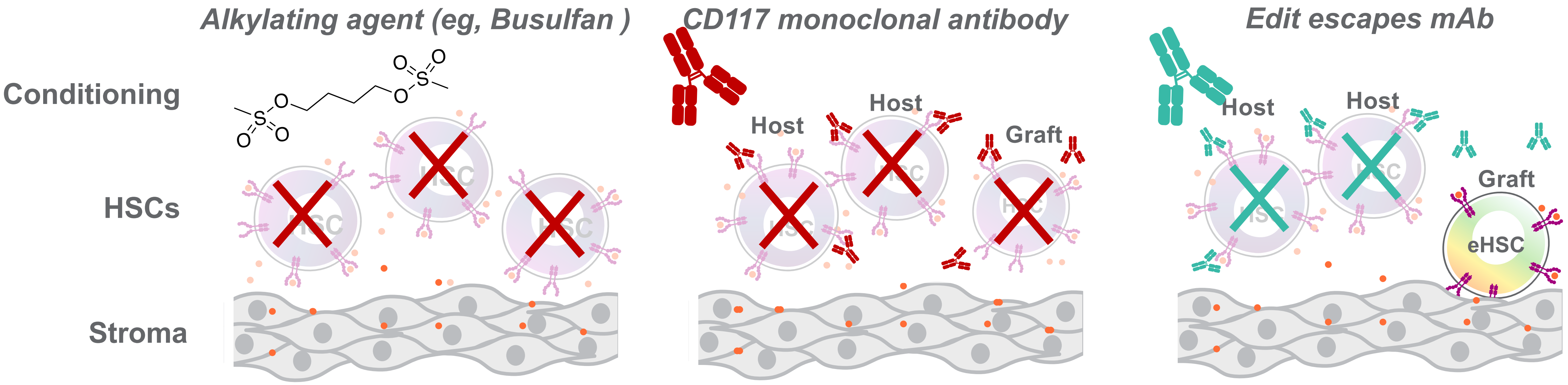
Electroporate cells with editor

Conditioning clears bone marrow niche

Mobilize & collect HSCs from patient

Thaw & infuse eHSCs after conditioning

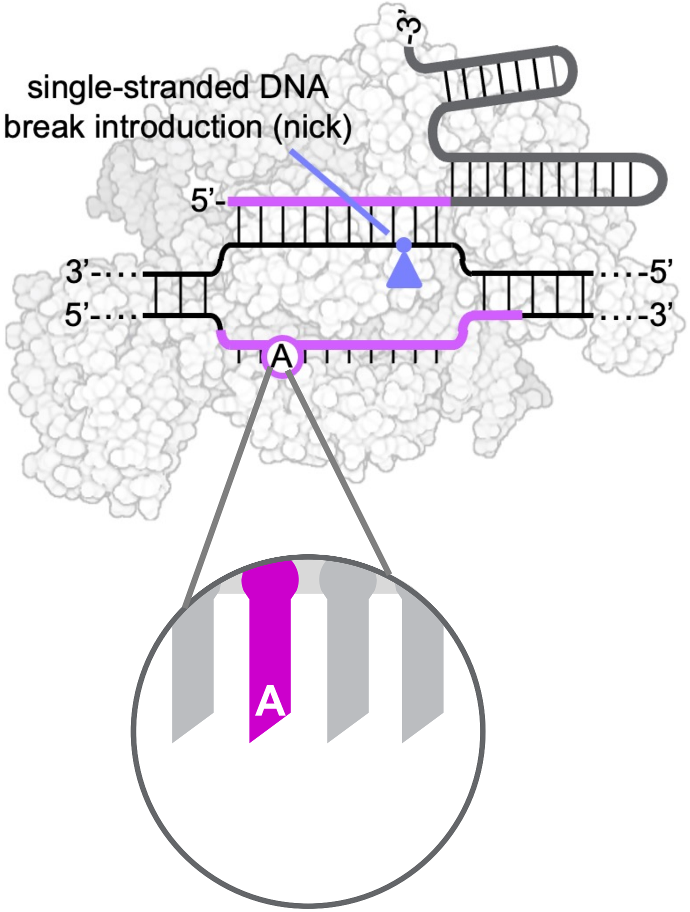
Next generation of conditioning agent are designed to enable graft cells to “ESCAPE” the conditioning agent



Desired attribute:

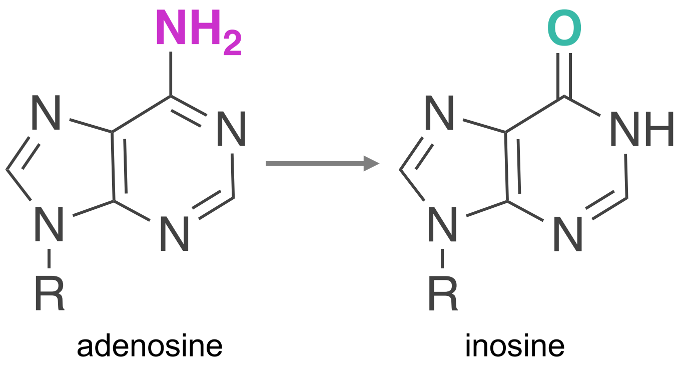
	Alkylating agent (eg, Busulfan)	*CD117 monoclonal antibody*	*Edit escapes mAb*
Myeloablative	✓	?	?
HSC-specific	X	✓	✓
Non-genotoxic	X	✓	✓
Low cancer risk	X	✓	✓
Preserve fertility	X	✓	✓
Long half-life	X	X	✓
Re-dosable	X	X	✓

Adenine base editors (ABEs) chemically modify target bases, permanently and predictably

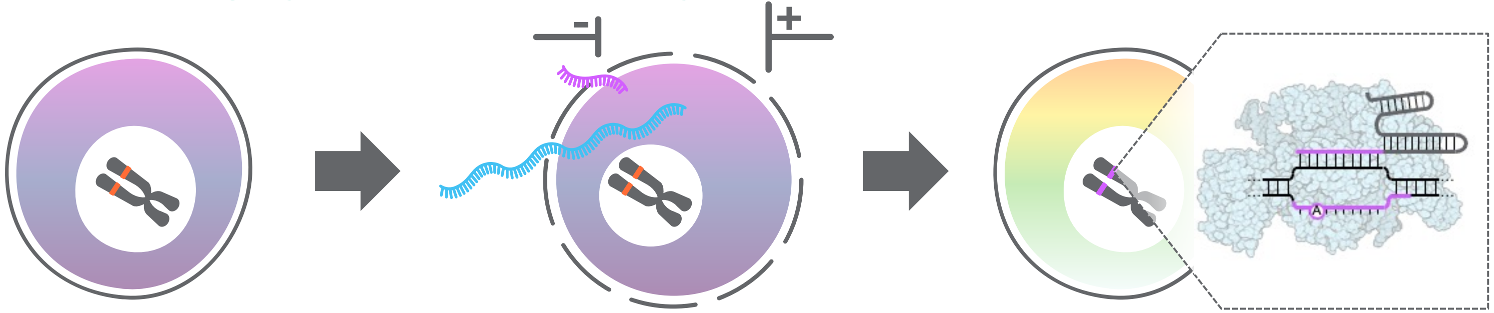


ABEs conduct chemistry on the genome

A-to-G base editor ("ABE")



ABEs are highly efficient in primary cells such as HSCs



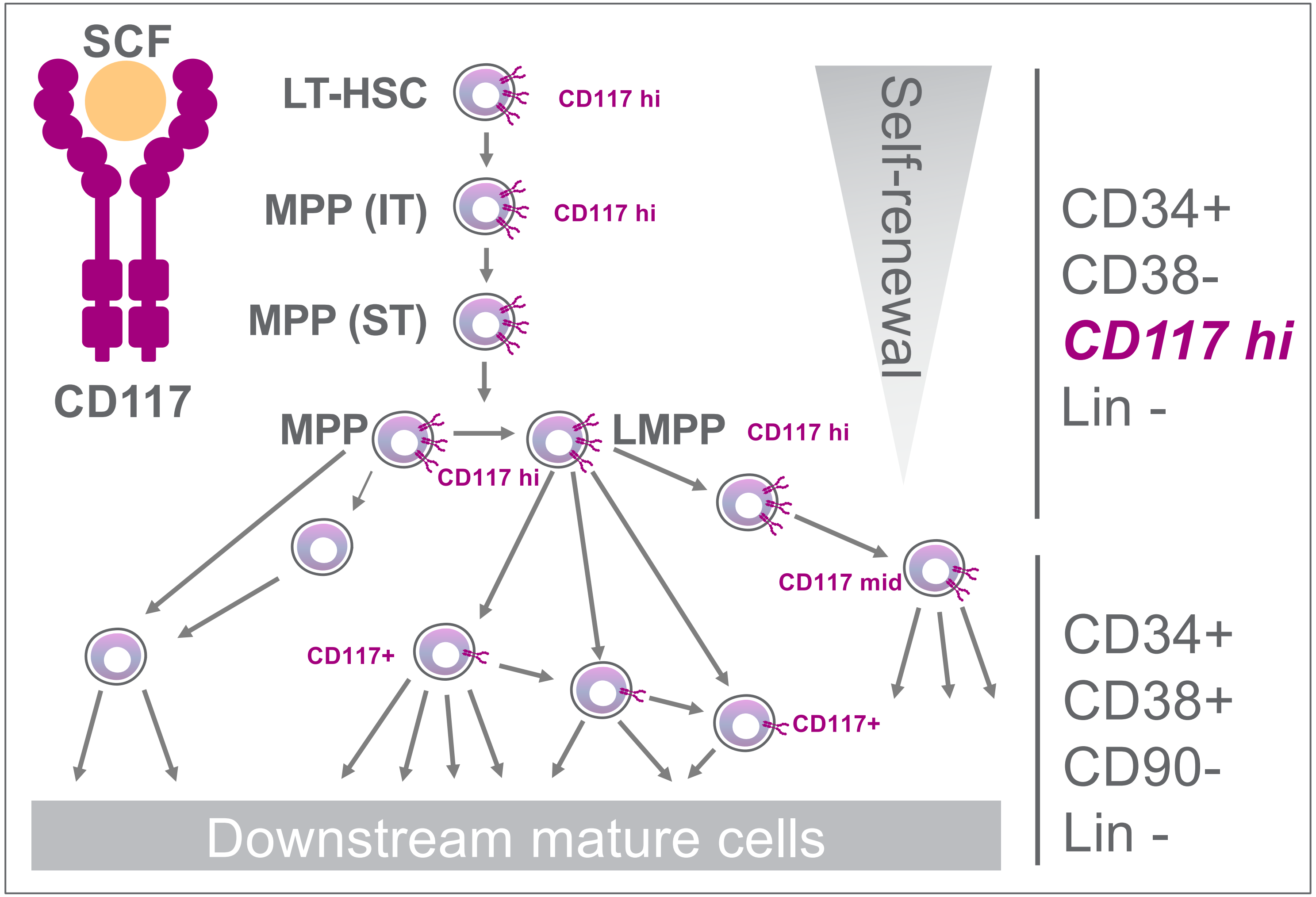
HSCs mobilized from patient or donor

Electroporation of **disease-specific gRNA** and **mRNA encoding ABE**

ABE performs gRNA-dependent base editing to create engineered HSCs

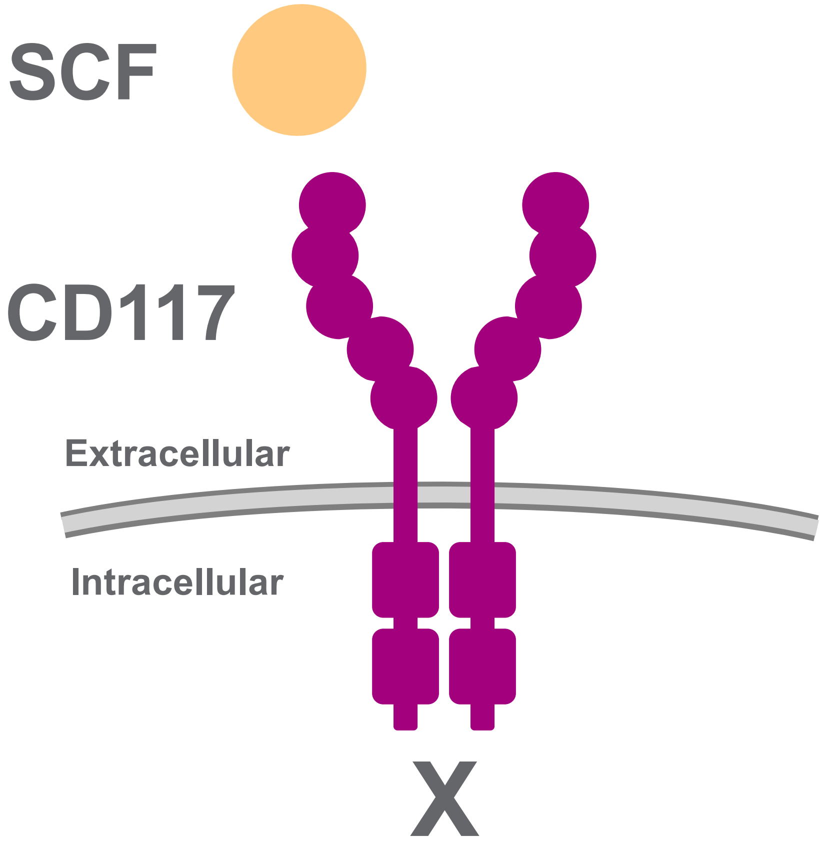
ABE: Gaudelli, N. M. *et al. Nature* **551**, 464-471 (2017)
CBE: Komor, A. *et al. Nature* **533,** 420–424 (2016)

CD117 is an optimal target for conditioning with expression on short- and long-term HSCs



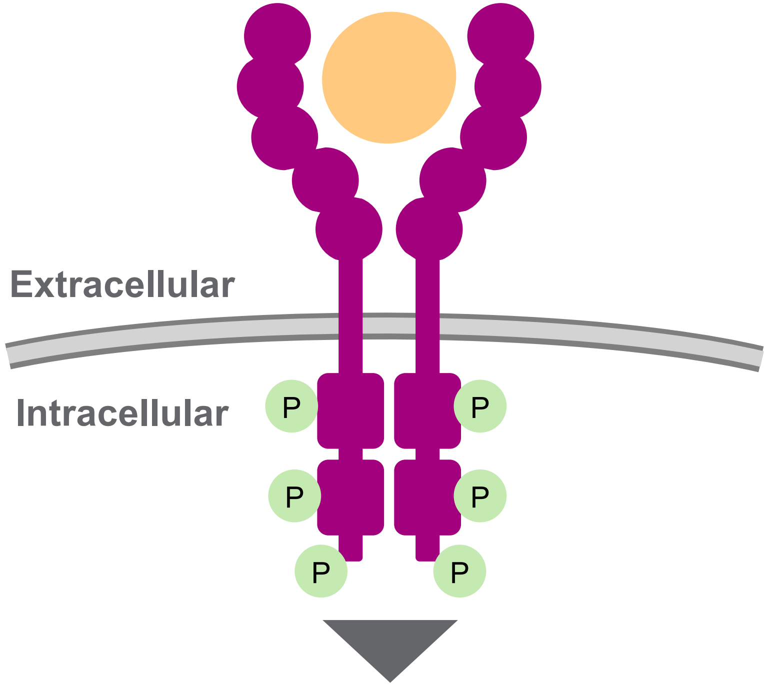
- CD117 is expressed on HSCs and is critical for their self-renewal, survival, & differentiation
- High expression in the long-term and short-term HSCs make CD117 an attractive target for immunologic conditioning

CD117 requires SCF binding and autophosphorylation to enable downstream signaling and HSC cell proliferation



No SCF bound to CD117
No phosphorylation
No downstream signaling

SCF binds CD117

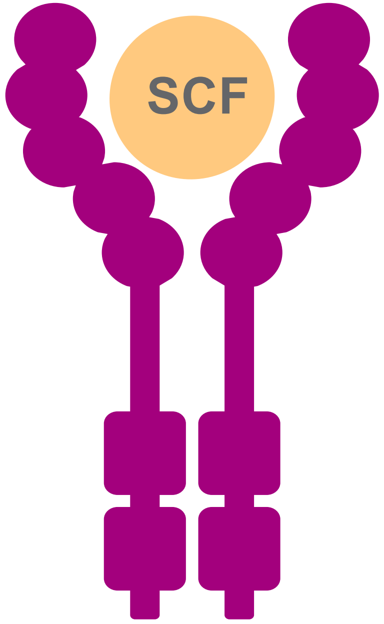


SCF bound to CD117
Intracellular phosphorylation
Downstream signaling

Feng *et al*, *Diabetologia*, 2015

Many CD117 epitopes evaluated and targeted with ABE

Goal: **identify a mutation in CD117 that disallows mAb binding**



CD117

Gene Editing Requirements:

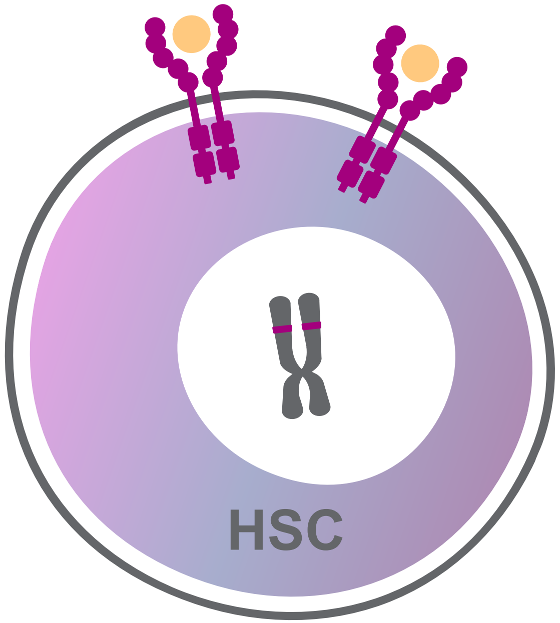
- CD117 gene edit uses an ABE editor previously validated for sickle cell disease correction
  - Enables multi-plex capability

- CD117 DNA targets contain an NGG PAM
  - Adenine nucleobase appropriately placed in the window

- ABE edit causes an amino acid substitution in a residue of CD117 that is solvent exposed for potential orthogonality in mAb binding relative to wild-type.

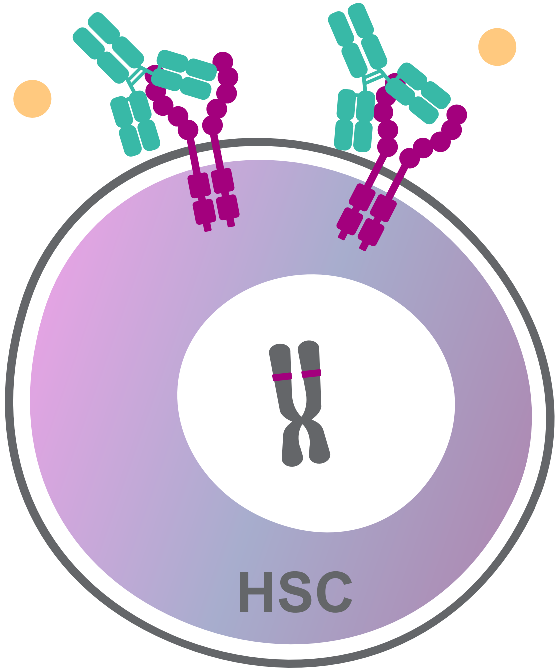
ESCAPE: Engineered Stem Cell Antibody Paired Evasion

Base edited CD117 epitope enables eHSCs to selectively ESCAPE mAb binding



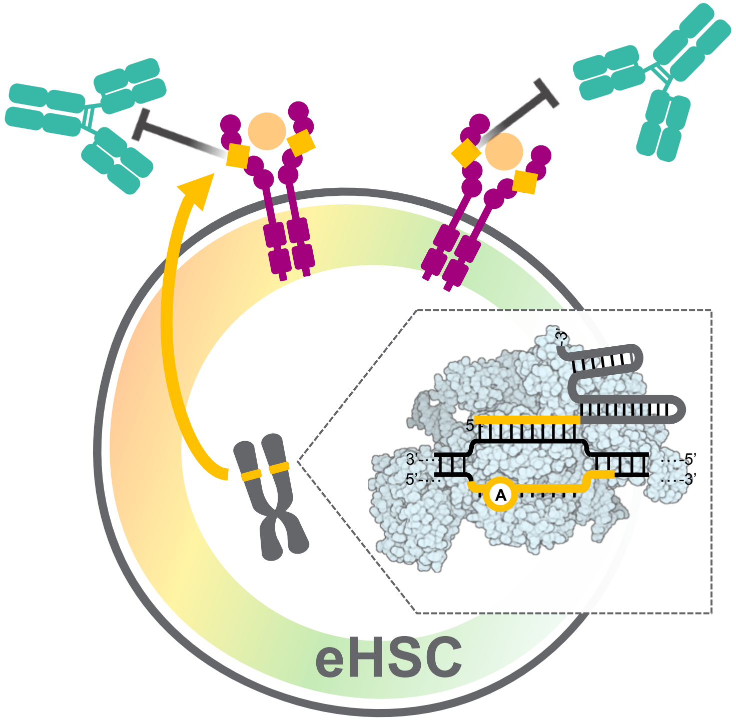
Unedited CD34 cell
No CD117 mAb
Normal signaling

Cell Survives



Unedited CD34 cell
CD117 mAb displaces SCF
CD117 signaling blocked

Cell Dies



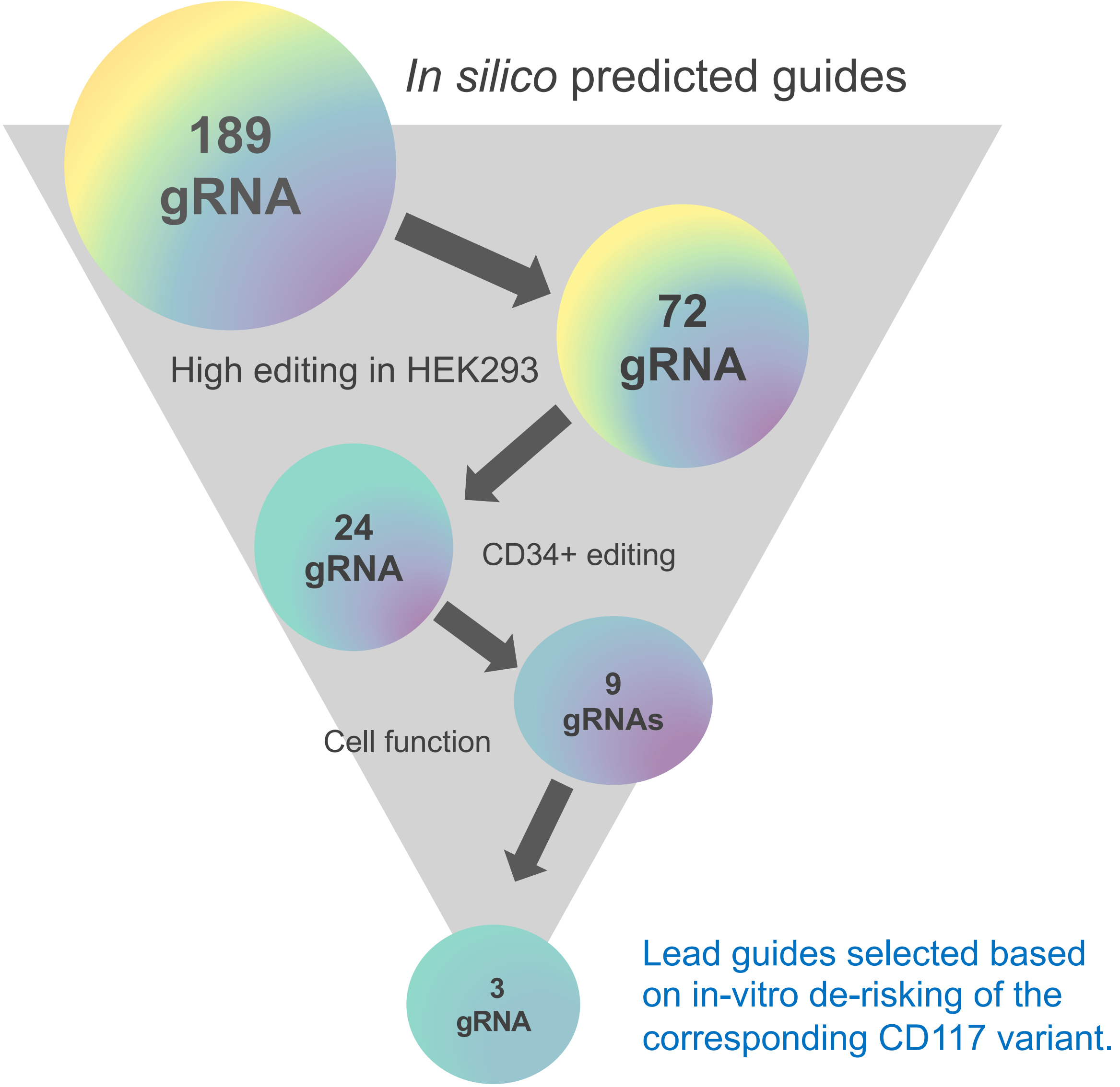
Edited CD34 cell
Escapes CD117 mAb
Normal signaling

Cell survives

ESCAPE: Engineered Stem Cell Antibody Paired Evasion

ABE compatible CD117 antigen engineering and antibody screening summary

Engineered antigen screening funnel:



In silico predicted guides

189 gRNA

High editing in HEK293

72 gRNA

24 gRNA

CD34+ editing

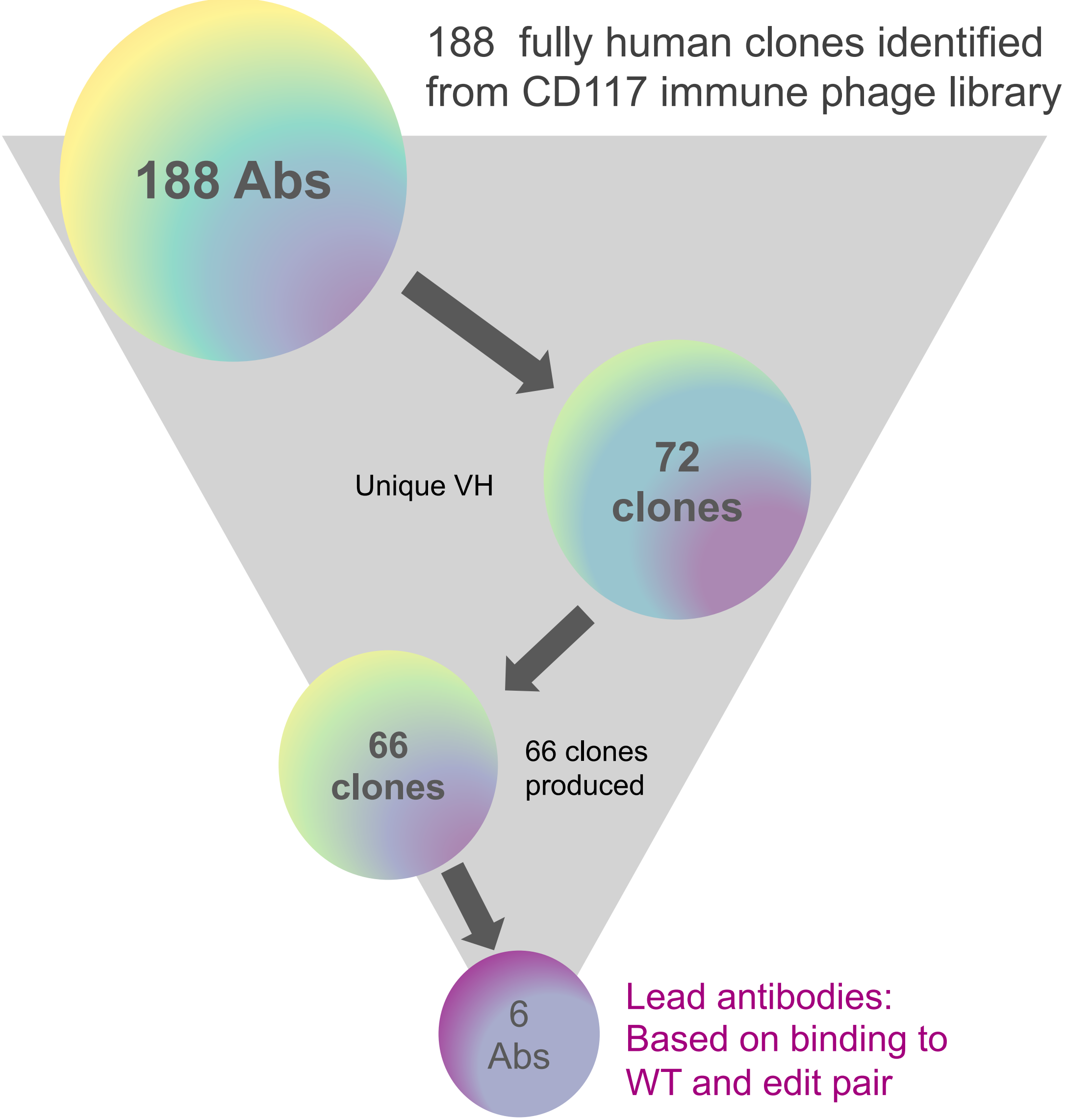
9 gRNAs

Cell function

3 gRNA

Lead guides selected based on in-vitro de-risking of the corresponding CD117 variant.

Antibody screening funnel:



188 fully human clones identified from CD117 immune phage library

188 Abs

Unique VH

72 clones

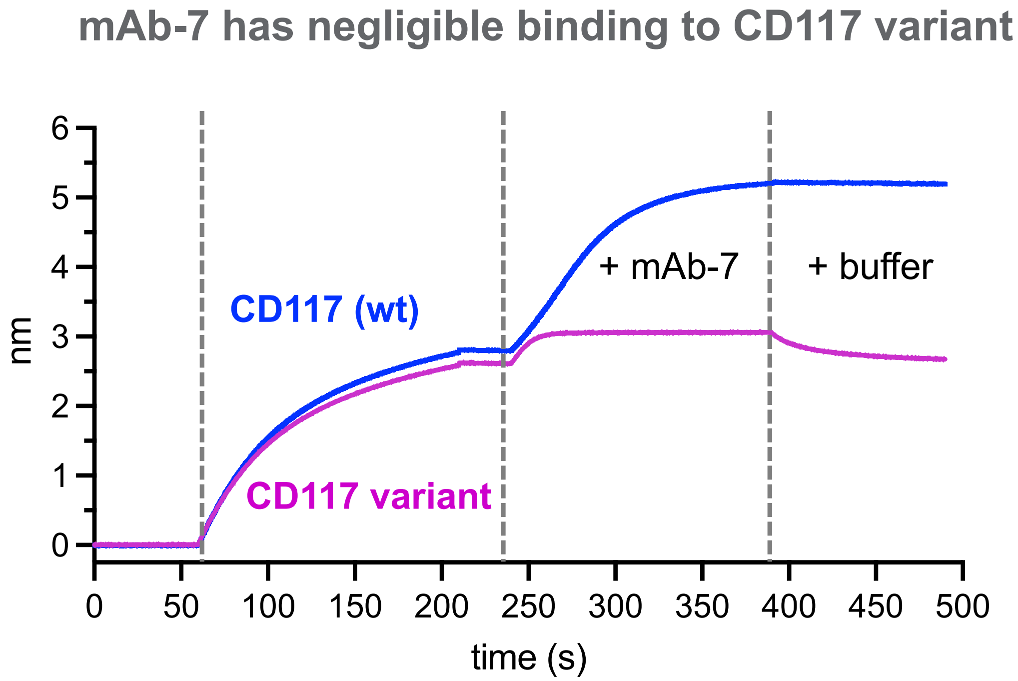
66 clones

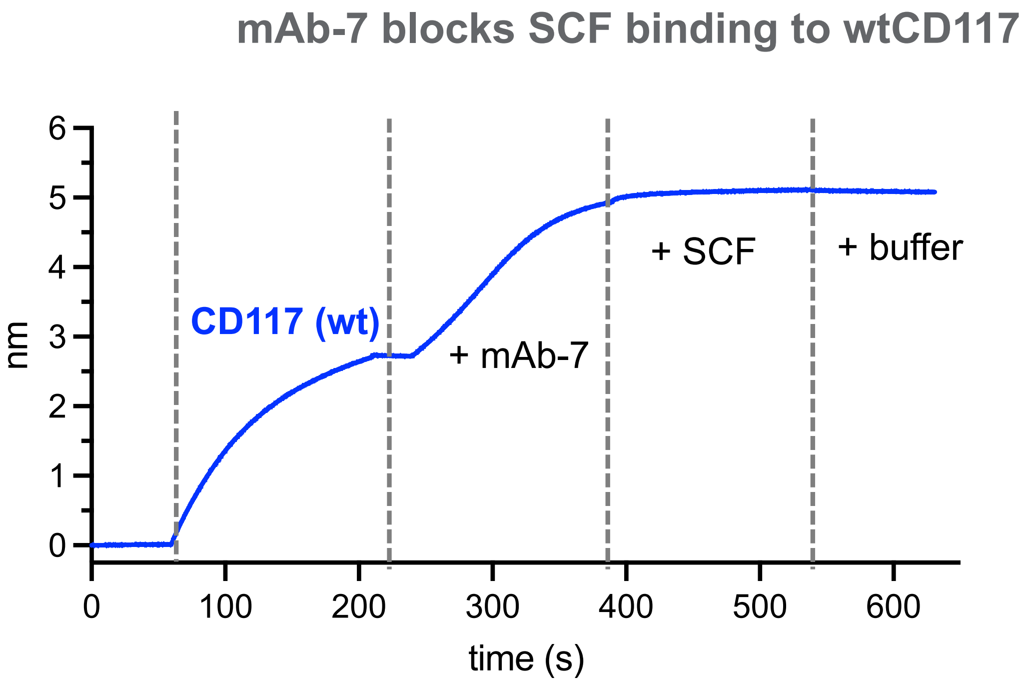
66 clones produced

6 Abs

Lead antibodies: Based on binding to WT and edit pair

mAb-7 binds minimally to CD117 variant (created through base editing) and blocks SCF

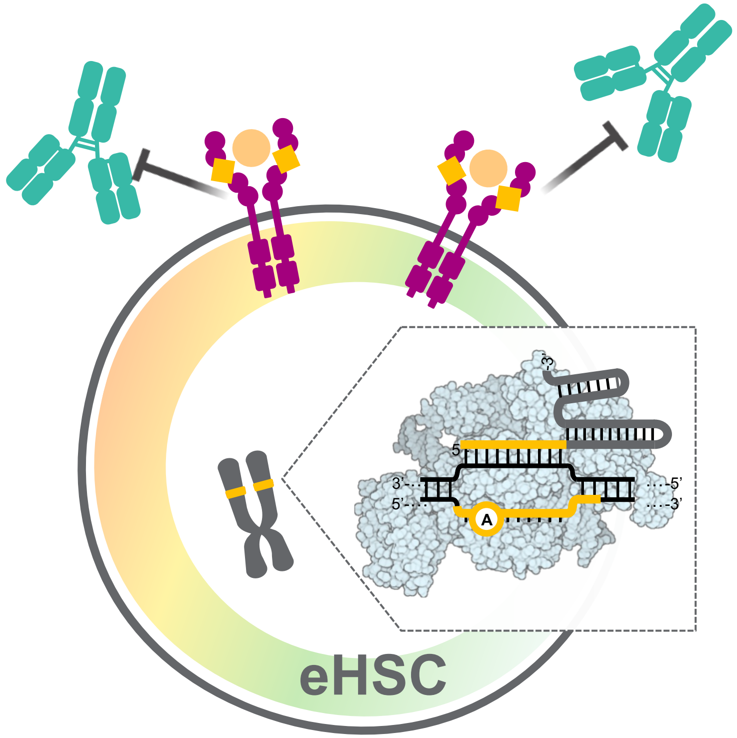

mAb-7 has negligible binding to CD117 variant

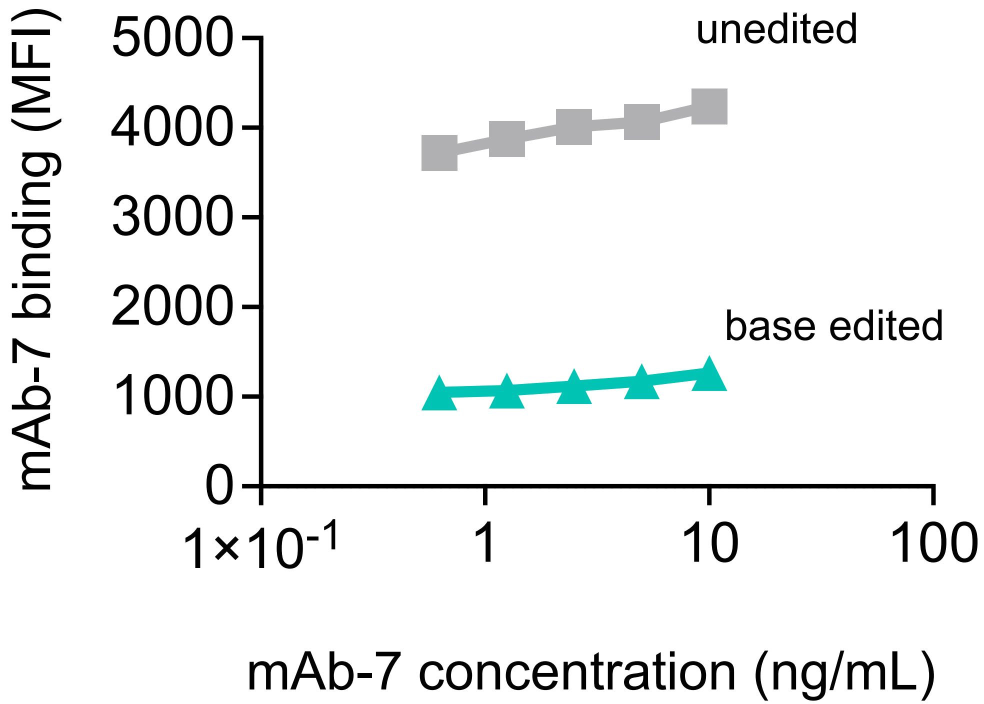

mAb-7 blocks SCF binding to wtCD117

	M07e cells expressing wt CD117	M07e cells expressing CD117 ESCAPE variant	SCF blocking	KD(M) Human CD117	KD(M) Cyno CD117
mAb7	Yes	L/N	Yes	<1.0E-12	<1.0E-12

- mAb-7 binds CD117 with high affinity (pM)
- mAb-7 binds minimally to CD117 variant as purified protein with rapid dissociation
- mAb-7 blocks SCF binding to CD117

CD34+ cells edited with ABE8 + CD117-targeting sgRNA escape recognition by antibodies that bind to wild type CD117

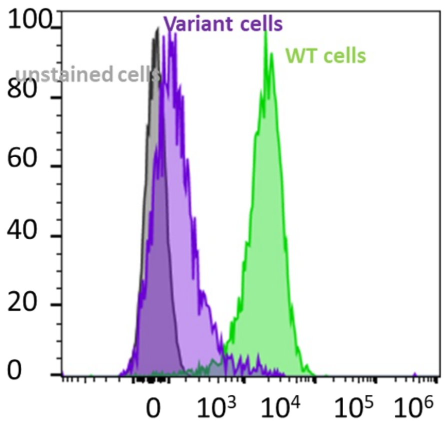




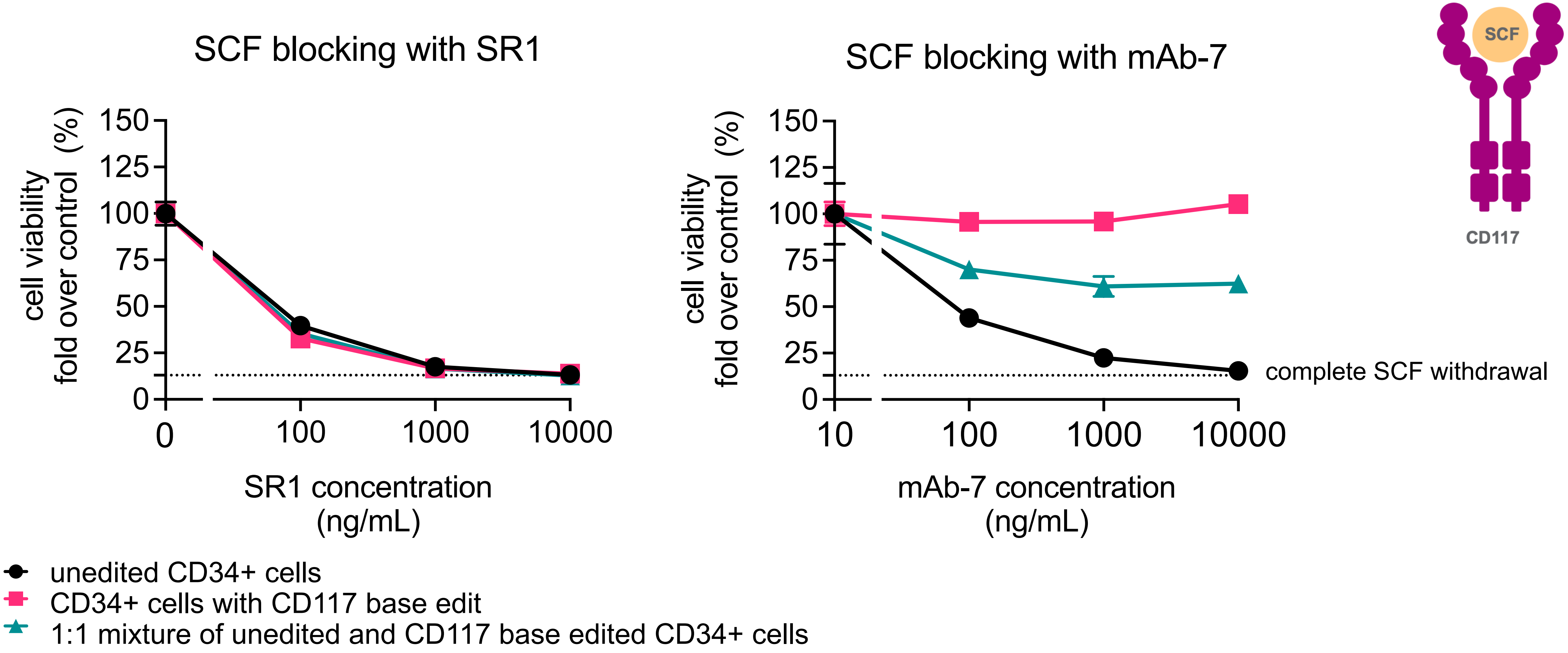
■ unedited CD34+ cells

▲ CD34+ cells expressing CD117 ESCAPE variant

mAb-7 cell Binding



Exposure of mAb-7 to wild-type CD34+ cells mimics complete SCF withdrawal

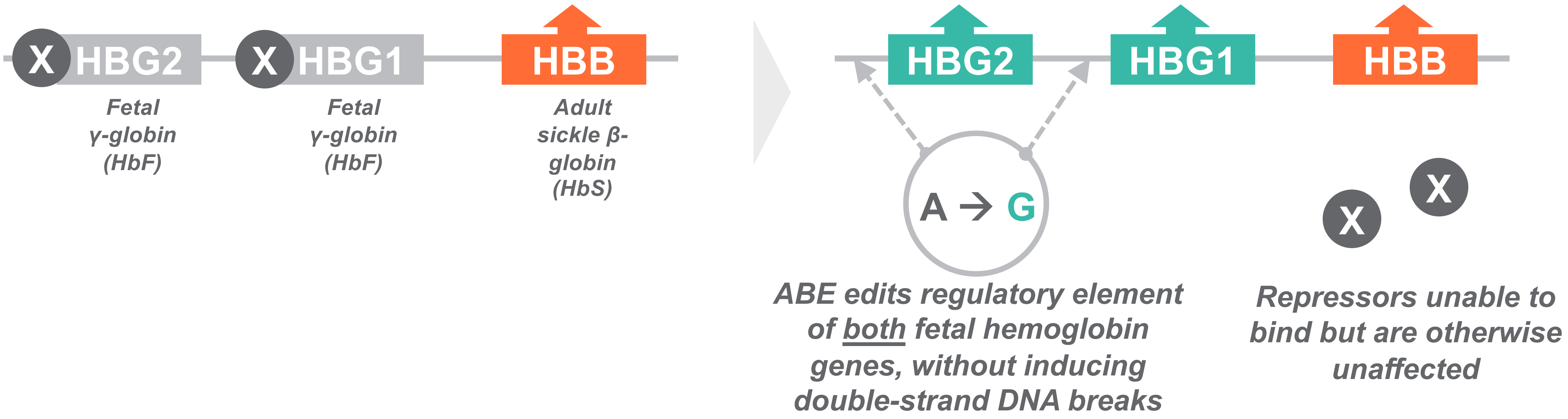


CD34+ cells edited with CD117 sgRNA maintain viability upon exposure to mAb-7

BEAM-101: Recreating hereditary persistence of fetal hemoglobin (HPFH) with base editing

Sickle cell disease patient

Reactivating fetal hemoglobin genes



Fetal γ-globin (HbF)

Fetal γ-globin (HbF)

Adult sickle β-globin (HbS)

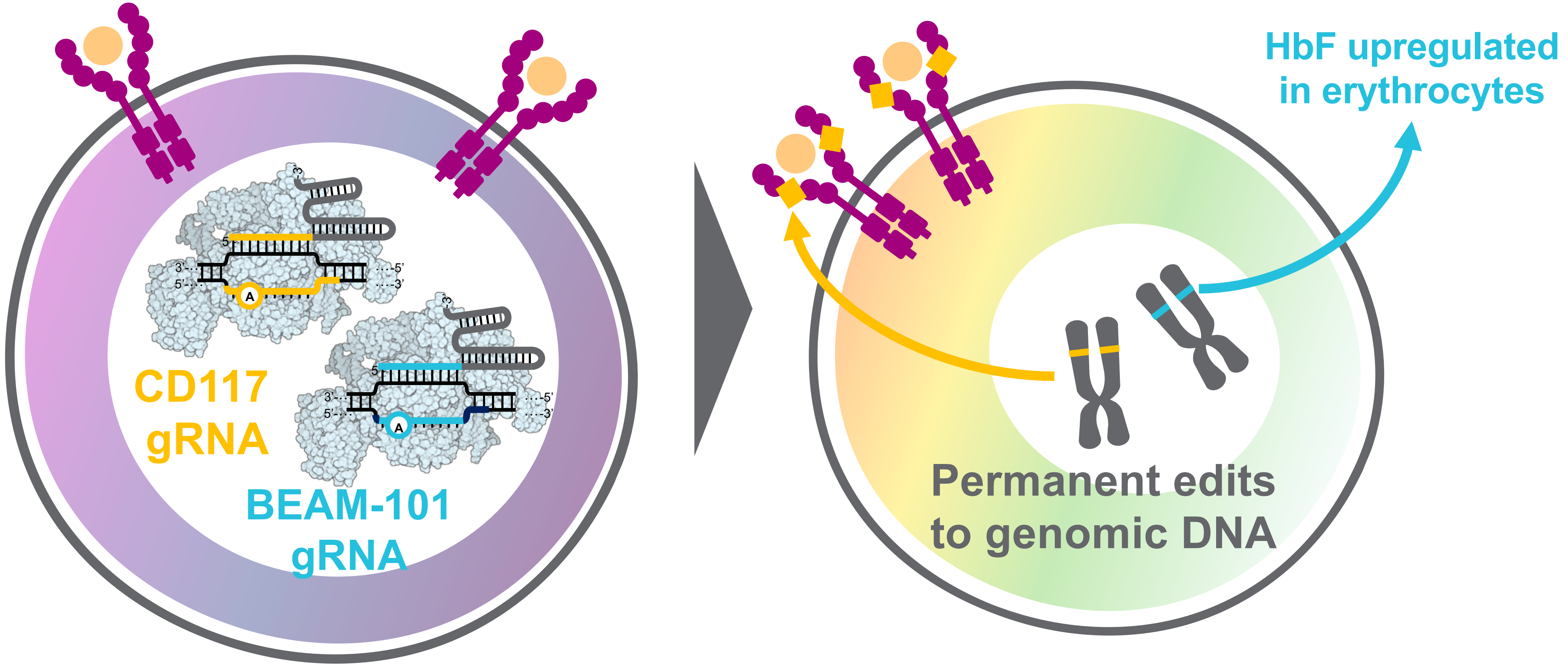
ABE edits regulatory element of both fetal hemoglobin genes, without inducing double-strand DNA breaks

Repressors unable to bind but are otherwise unaffected

- Naturally-occurring base changes cause Hereditary Persistence of Fetal Hemoglobin (HPFH), which protects patients from SCD/B-Thal
- Base editors can reproduce these changes, leading to high, consistent levels of fetal hemoglobin
- Higher fetal hemoglobin likely to correlate with further reductions in disease symptoms

1. Mussallam, et al. 2012. *Blood.*

ESCAPE-1: Base editing enables efficient multiplex editing of both ESCAPE CD117 edit and HbF induction



CD117 gRNA

BEAM-101 gRNA

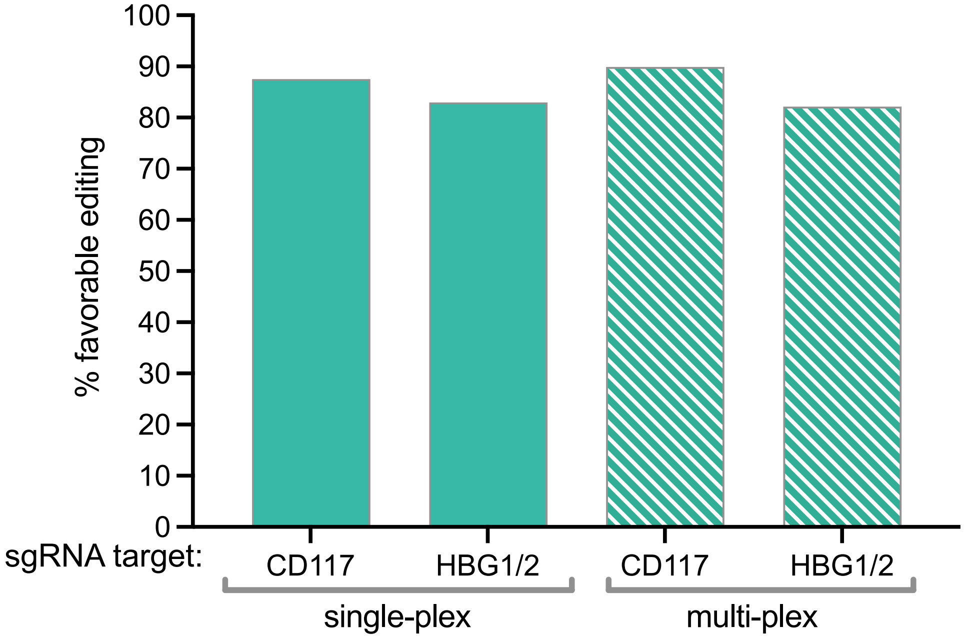
HbF upregulated in erythrocytes

Permanent edits to genomic DNA

- gRNA for therapeutic edit such as for BEAM-101 or BEAM-102 (Makassar)
- gRNA for surface antigen epitope change that does not impact HSC biology but protects from mAb
- Single **base editor** capable of efficient multiplex edit of both therapeutic edit and antigen modification

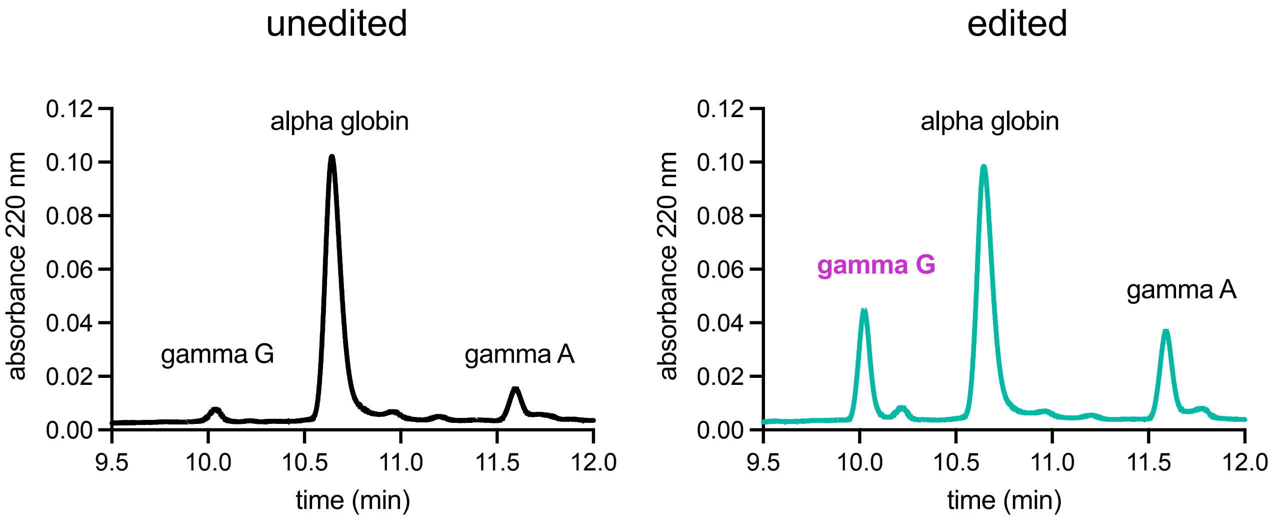
Highly efficient multiplex editing of CD117 and HBG1/2 can be achieved in CD34+ HSPCs

multiplex base editing efficiency is equivalent to single-plex



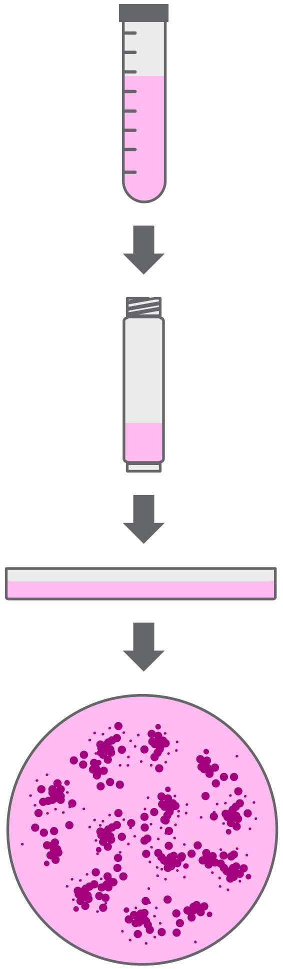
- Multiplexing of HBG1/2a and CD117 sgRNAs with ABE leads to efficient base editing of both targets (>85%)
- Single clonal analysis reveals that all cells have HBG1/2 editing in the multiplex editing condition.
- CD117 multiplex editing outcomes are: >97% bi-allelic, 1.5% mono-allelic, and 1.5% unedited

multiplex base editing of CD117 target with HBG1/2 site does not hinder ɣ-globin induction



- ~60% gamma globin induction detected in IVED cells differentiated from multiplex edited CD34+ cells
- multi-plex editing (CD117 site + HBG1/2) leads to similar levels of gamma G in IVED cells as base editing of HBG1/2 alone.

SCF blocking by mAb-7 significantly inhibits erythroid colony formation



❶ Prepare cells (1:1 mixture of CD117 base edited and unedited CD34+ cells) and adjust concentration of mAb-7

❷ Add cells to methylcellulose

❸ Plate and incubate

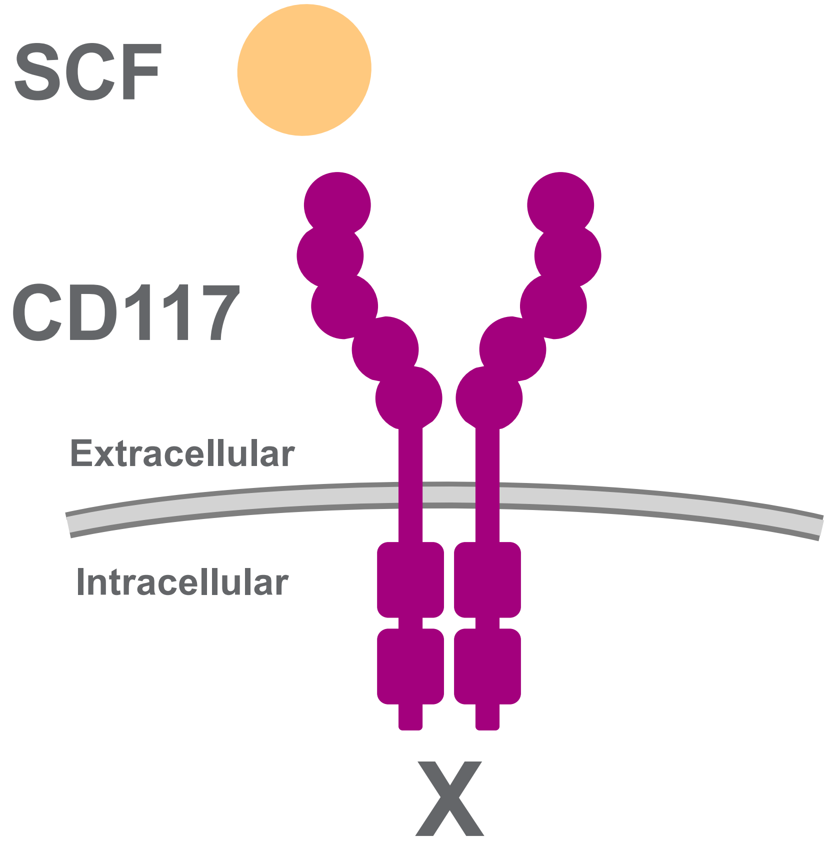
❹ Count colonies

BFU-E colonies (n=24) were sequenced in each group

without mAb-7, there is an even distribution of edited to non-edited cells

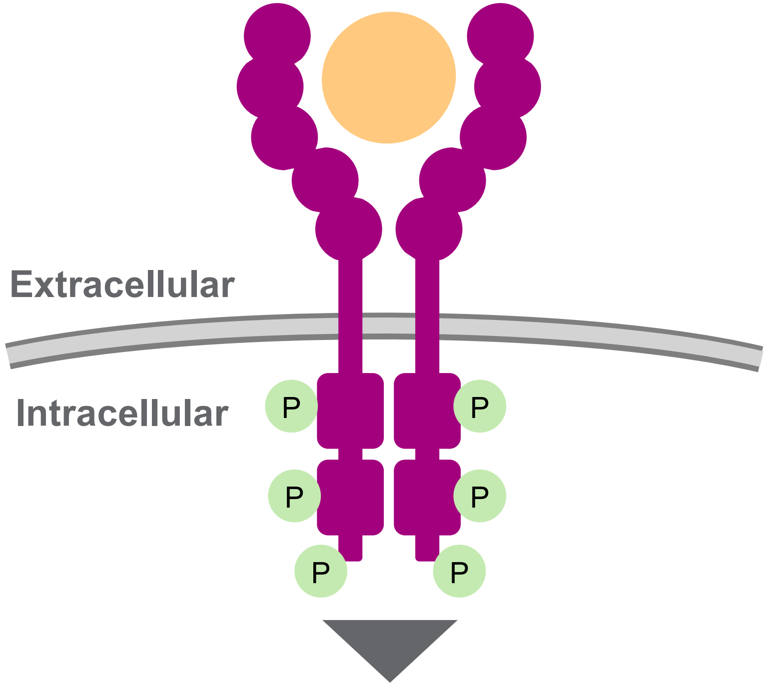
increasing [conc.] of mAb-7 yielded higher proportion of CD117 edited colonies

CD117 requires SCF binding and autophosphorylation to enable downstream signaling and HSC cell proliferation



No SCF bound to CD117
No phosphorylation
No downstream signaling

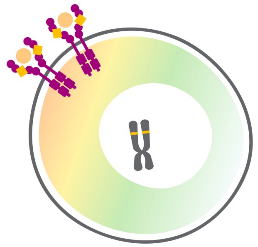
SCF binds CD117

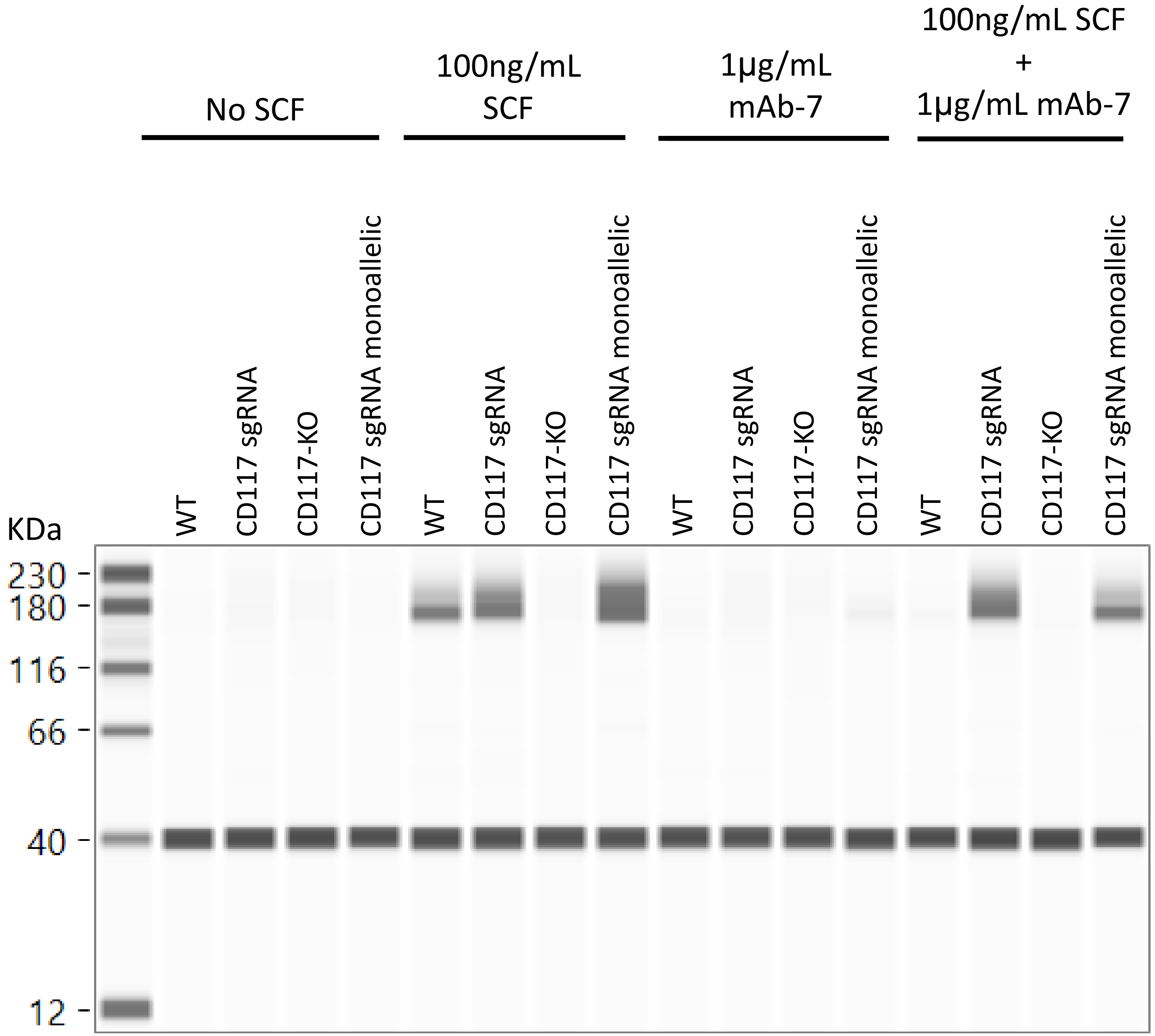


SCF bound to CD117
Intracellular phosphorylation
Downstream signaling

Feng *et al*, *Diabetologia*, 2015

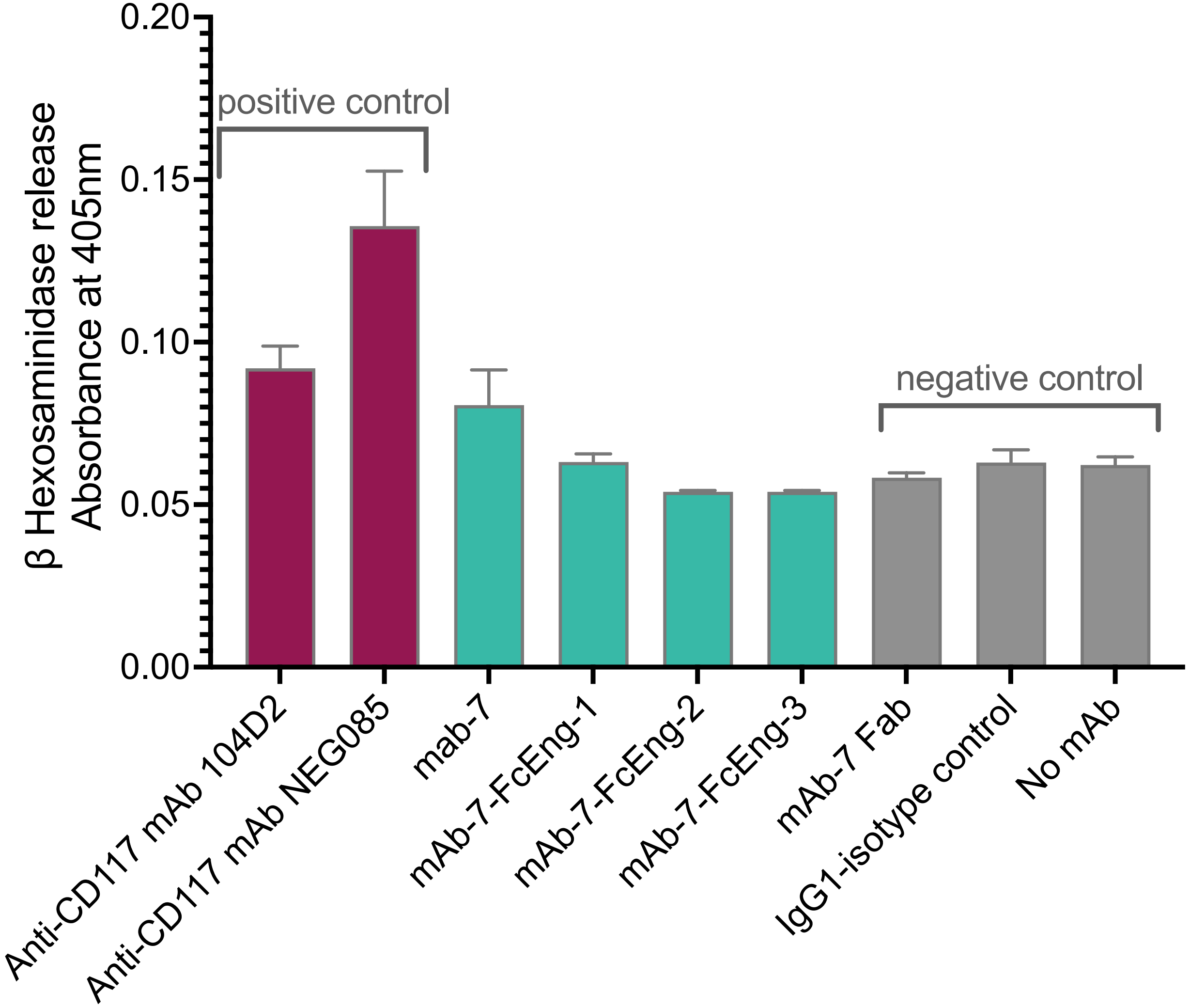
Base edited CD117 cells can bind to ligand SCF and induce phosphorylation *in vitro*





- Cells edited with CD117 sgRNA retain the ability to bind SCF
- Upon SCF binding, edited CD117 undergoes phosphorylation similar to WT protein
- mAb-7 blocks SCF binding as well as CD117 phosphorylation in unedited cells
- mAb-7 fails to block SCF binding to edited protein
- Edited protein undergoes phosphorylation even in the presence of mAb-7

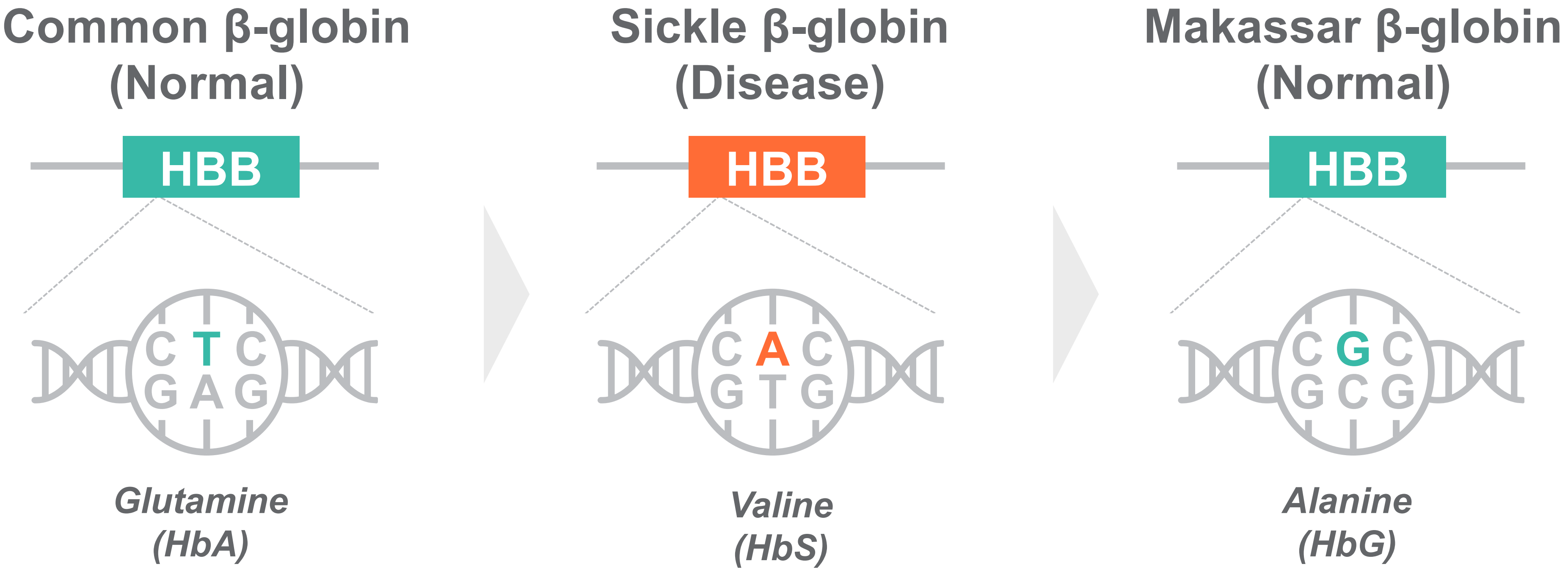
Fc Engineered versions of anti CD117 mAb-7 do not produce mast cell degranulation *in vitro*



104D2 & NEG085 mAb controls: L'Italien L, *Clin Cancer Res*. **14** 3465-3474, (2018)

ESCAPE-2: Makassar Editing for SCD + CD117 edit paired with mAb-7 for conditioning

BEAM-102: Direct correction of the sickle causing mutation



Common β-globin (Normal)

Glutamine (HbA)

Sickle β-globin (Disease)

Valine (HbS)

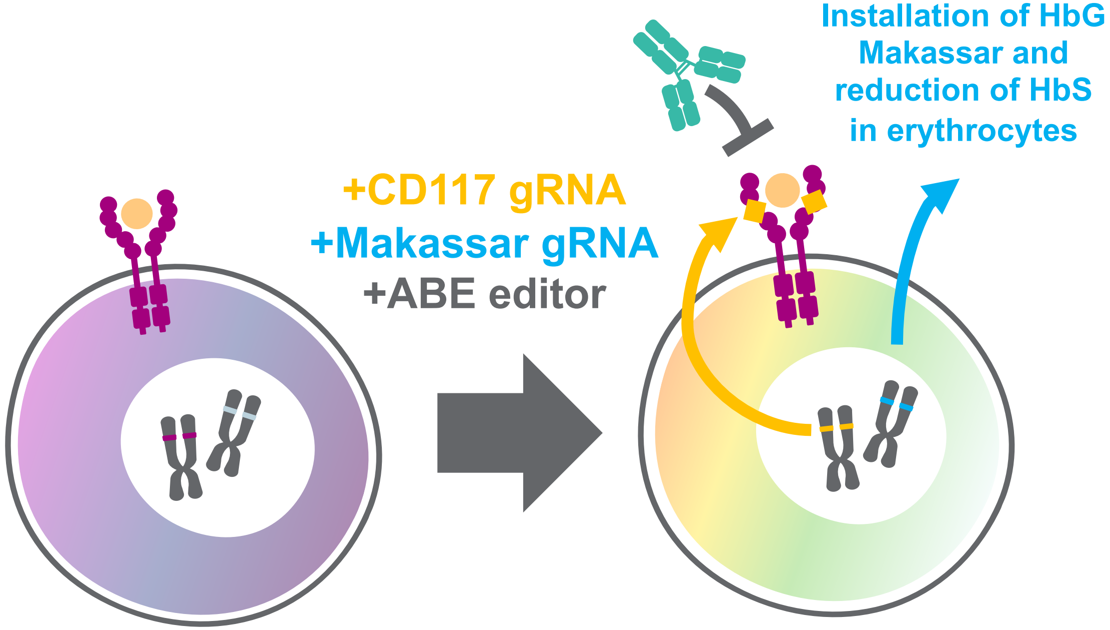
Makassar β-globin (Normal)

Alanine (HbG)

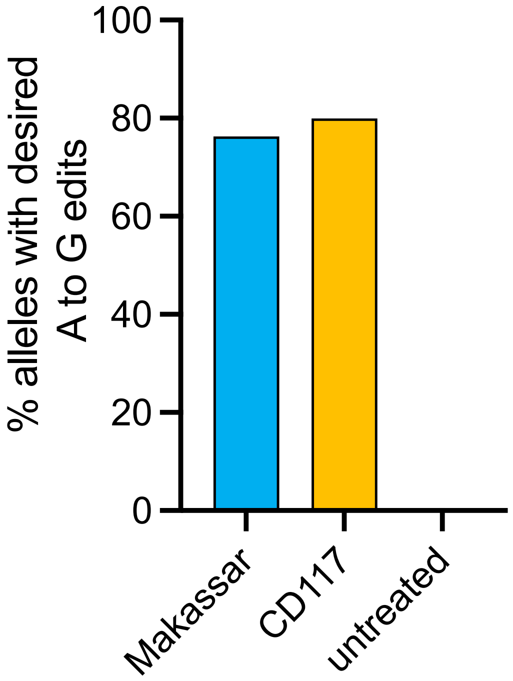
- Base editing recreates naturally-occurring human variant Hb-G Makassar which has alanine (E6A) instead of sickle-causing valine (E6V)[1]
- Hb-G Makassar is a normal β-globin variant and does not cause sickle disease, e.g., blood smear shows negative for sickle cells[2]

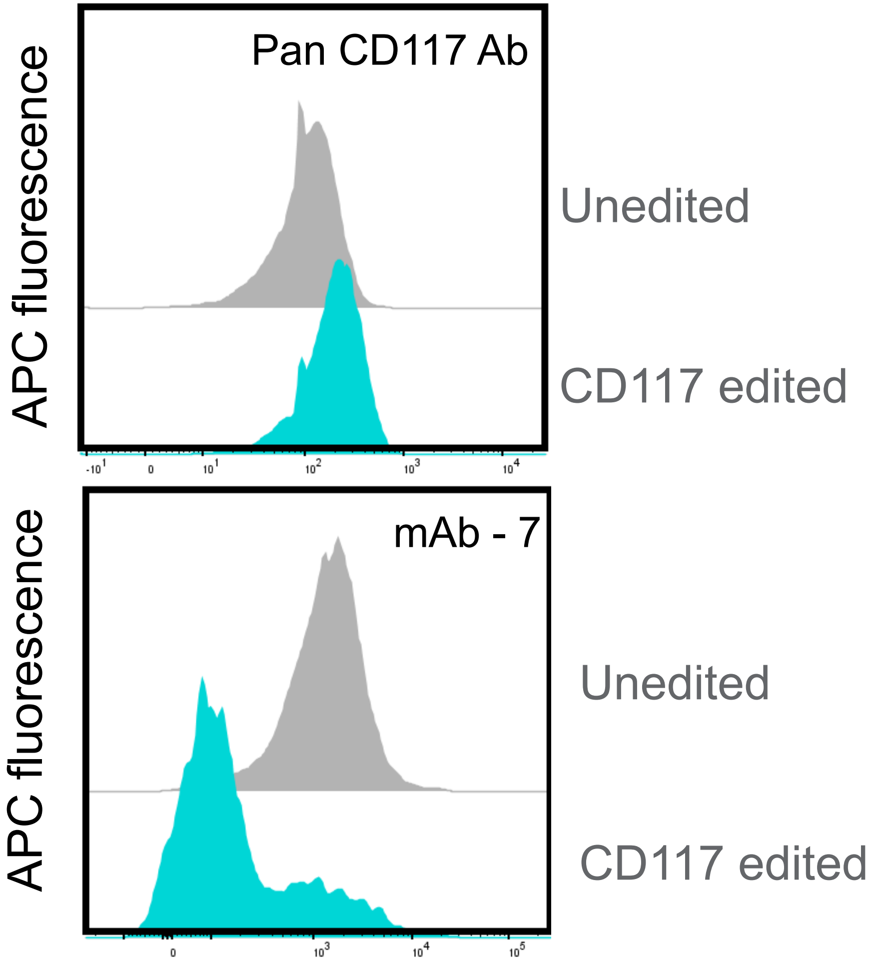
1. Cummings, MR. 2006. Human Heredity: Principles and Issues, Updated Edition.; 2. Mohamad, et al. 2018. Hematology Reports.

Identification of guide that can be multiplexed with Makassar installation that evades mAb-7 binding


+CD117 gRNA
+Makassar gRNA
+ABE editor

Installation of HbG Makassar and reduction of HbS in erythrocytes





- High levels of base editing at the HBB gene locus for sickle to Makassar gene correction can be achieved with an ABE
- A second CD117 variant identified that escapes mAb-7 binding and can be accessed through ABE editing

Summary

- Genotoxic conditioning continues to be a major barrier to the adoption of HSC transplant
- New conditioning agents show promise but cannot discriminate between disease and transplanted cells, and therefore are designed with short half-life or given at low doses well before transplant
- To solve this, we developed a concept we call Engineered Stem Cell Antibody Paired Evasion (ESCAPE), where a base-edited antigen: antibody pair enables edited cells to be resistant to mAb binding. Contrastingly, this conditioning mAb, binds to and interferes with CD117 :
    - mAb-7 binds wild-type CD117 with high affinity but minimally to base edited CD117 variant protein
    - Edited CD117 behaves normally *in vitro* vs wild-type in proliferation, differentiation, viability, and phosphorylation assays
    - Fc engineered mAb-7 does not induce mast-cell degranulation *in vitro*
- (ESCAPE-1) Multiplexing CD117 sgRNA with therapeutic sgRNAs (e.g. *HBG1/2*) with a single ABE8 editor achieved >85% CD117 base editing in CD34+ cells also containing therapeutic edit
    - Multiplex base edited CD34+ cells evade mAb-mediated effects and SCF-ligand blocking, allowing for escape *in vitro*
- (ESCAPE-2) mAb-7:CD117 edit pair, that can be installed by an ABE, is also compatible with efficient base editing to convert the causative SCD mutation into a variant encoding the naturally-occurring, non-polymerizing hemoglobin Makassar

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Lisa Hardy
High Kromer
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AND many more!!

Beam
THERAPEUTICS