CRISPR engineering primary human B cells for sustained secretion of therapeutic biologics

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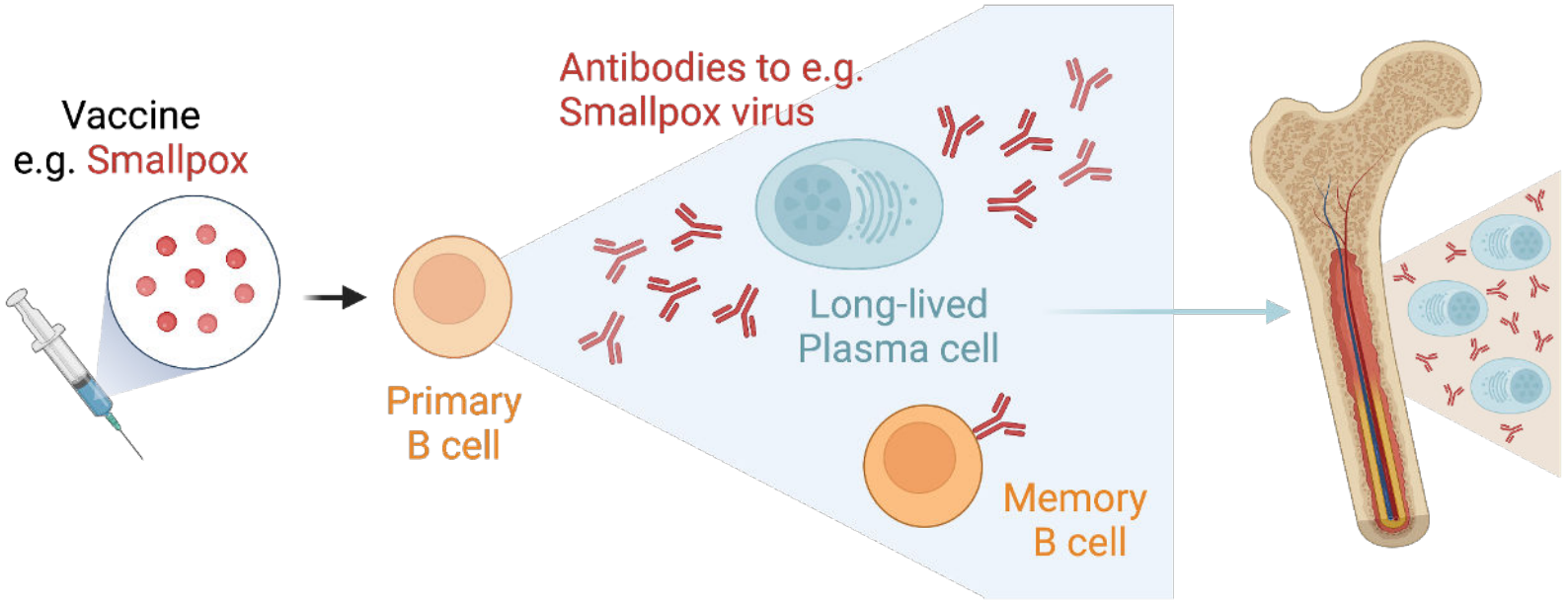
Disclosures

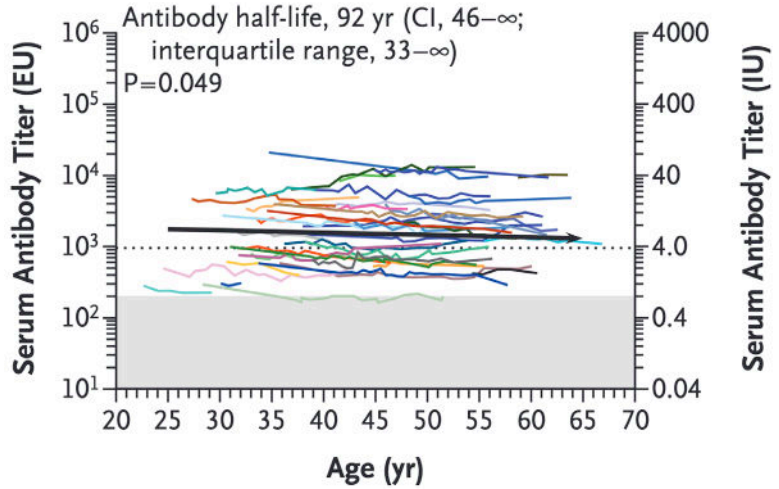
- Employee and stock owner at Be Biopharma

B cell biology enables a new class of cellular medicines

B cell biology



Longitudinal analysis of serum antibody titers in 45 subjects[1]

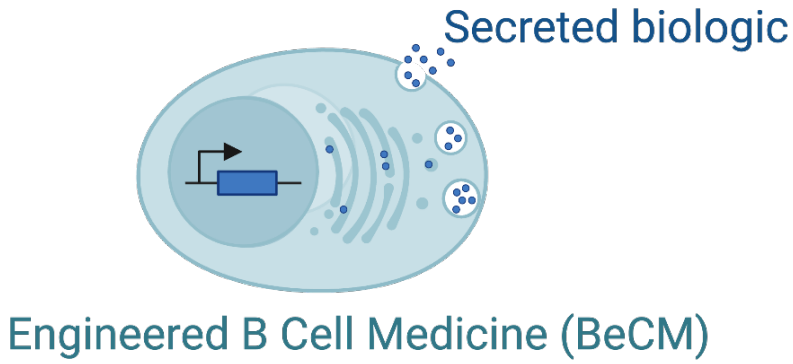


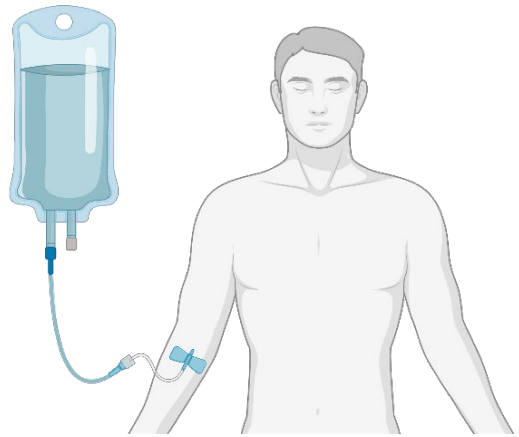
Capacity for protein production: 1000s of molecules/sec/cell[2]
Longevity: Natural human plasma cells can persist for decades[3]

1) Amanna, Carlson, and Slifka (2007) *NEJM*
2) Hibi and Dosch (1986) *Eur J Immunol*; Eyer *et al* (2017) *Nat Biotech*
3) Landsverk *et al* (2017) *J Exp Med*

BeCMs are uniquely suited for sustained supply of biologics

Engineered B Cell Medicines (BeCMs)





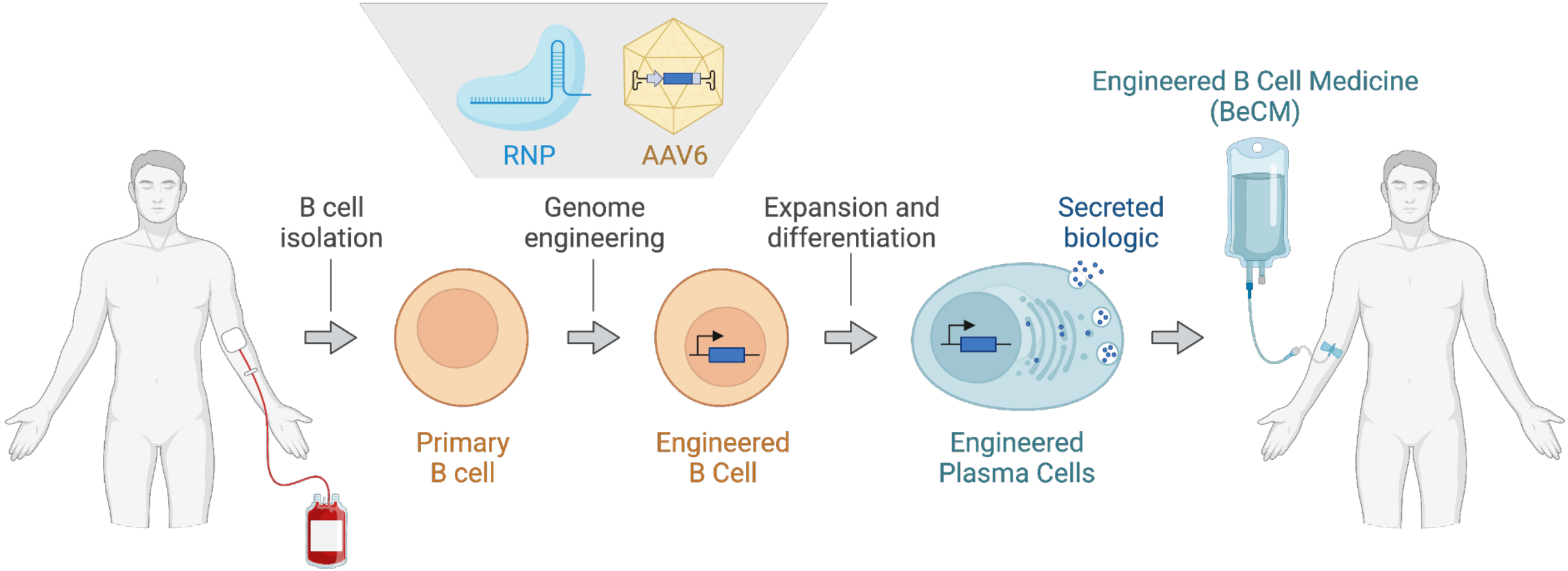
Favorable attributes

Capable of making versatile biologics
Stable protein expression
Continuous secretion
Durable

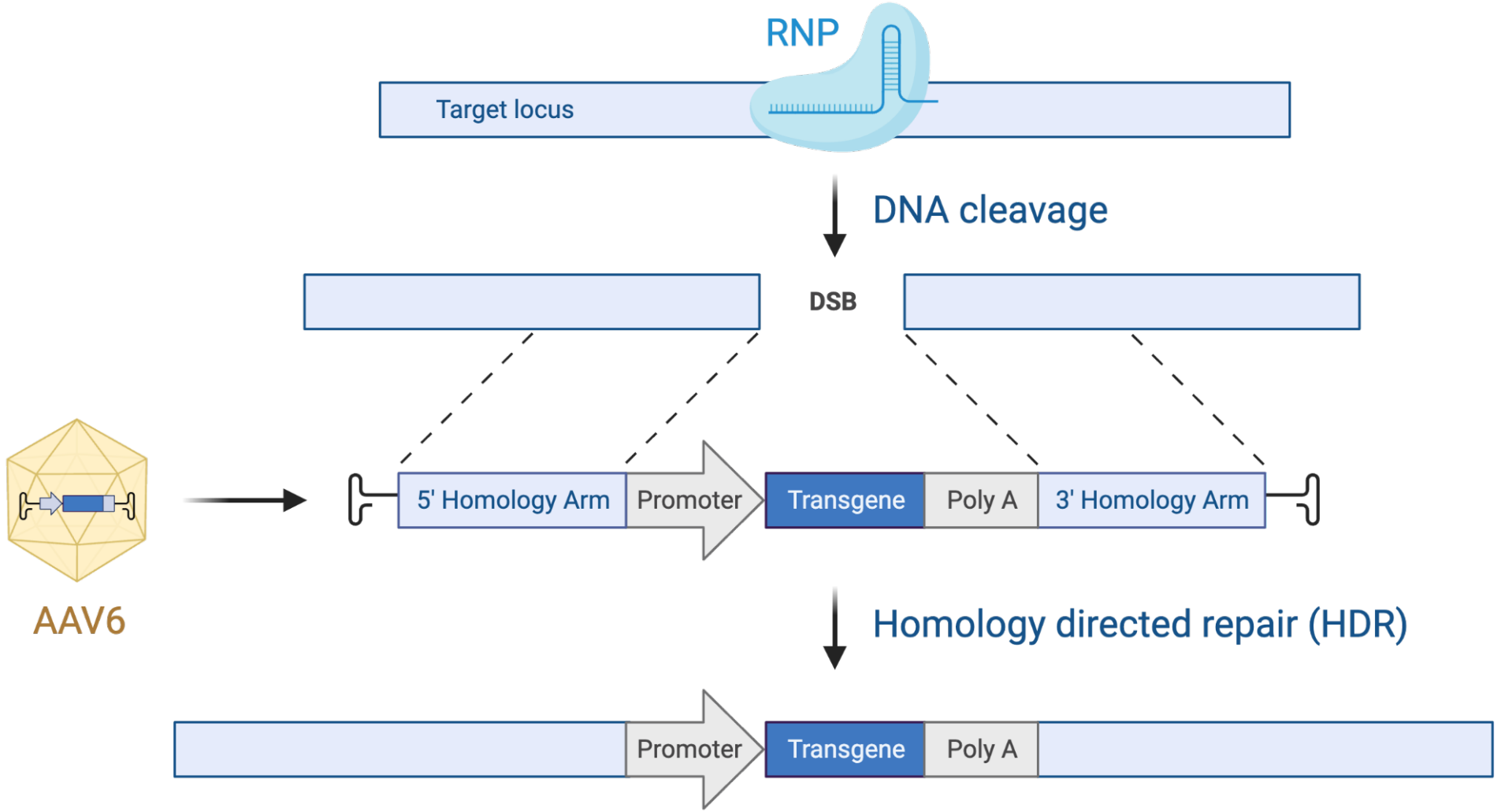
Engraftment without pre-conditioning
Long-term persistence
Redosable
Inherently immunologically stealthy

The challenge: culturing, engineering, and differentiating B cells.

BeCMs are engineered and differentiated *ex vivo* to serve as protein factories in the body upon infusion

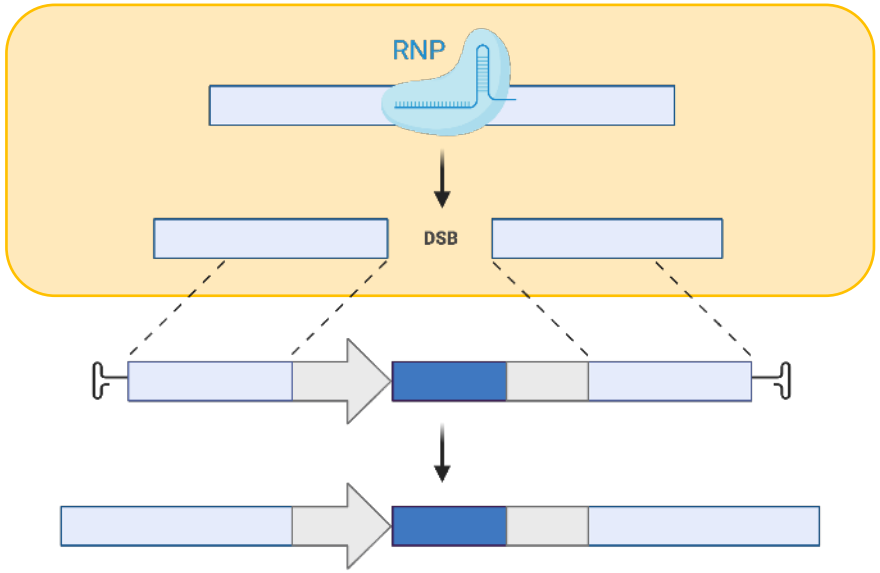


Our engineering strategy stably inserts transgenes at defined genomic sites

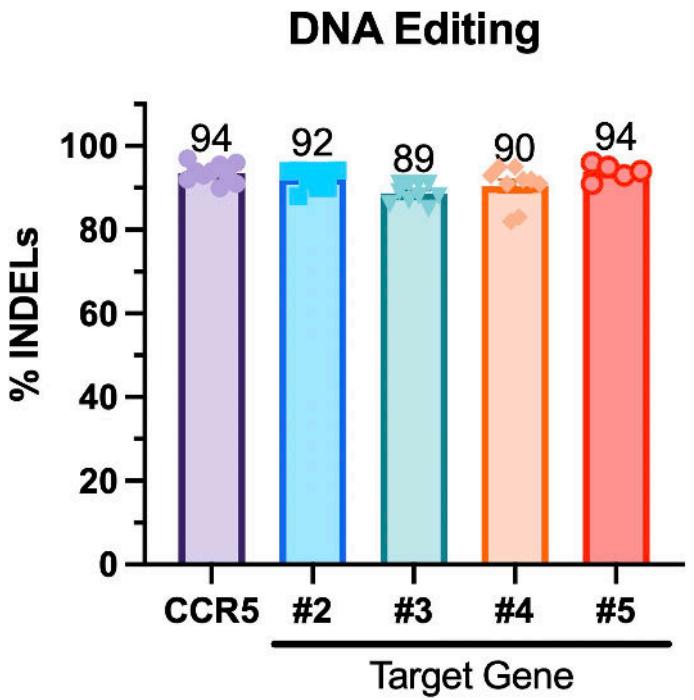


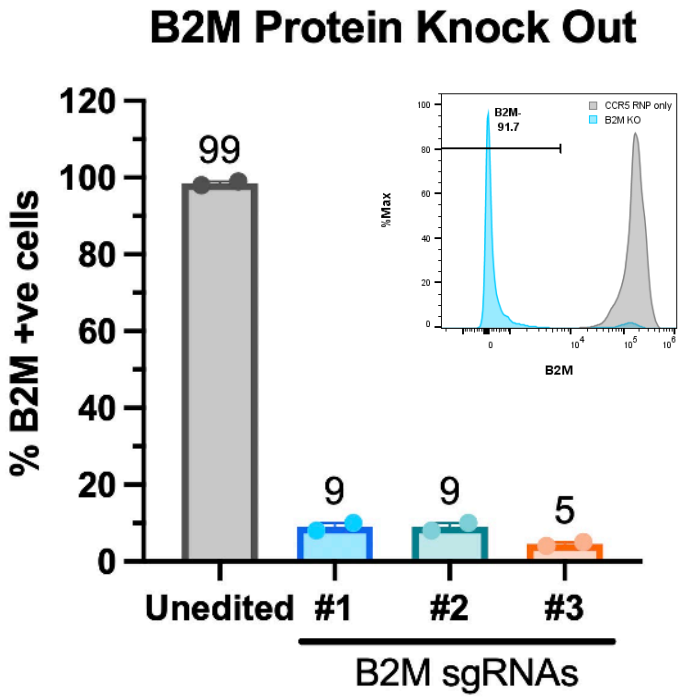
>90% gene editing efficiency in primary B cells

Optimization of DNA editing and gene **KNOCK OUT**



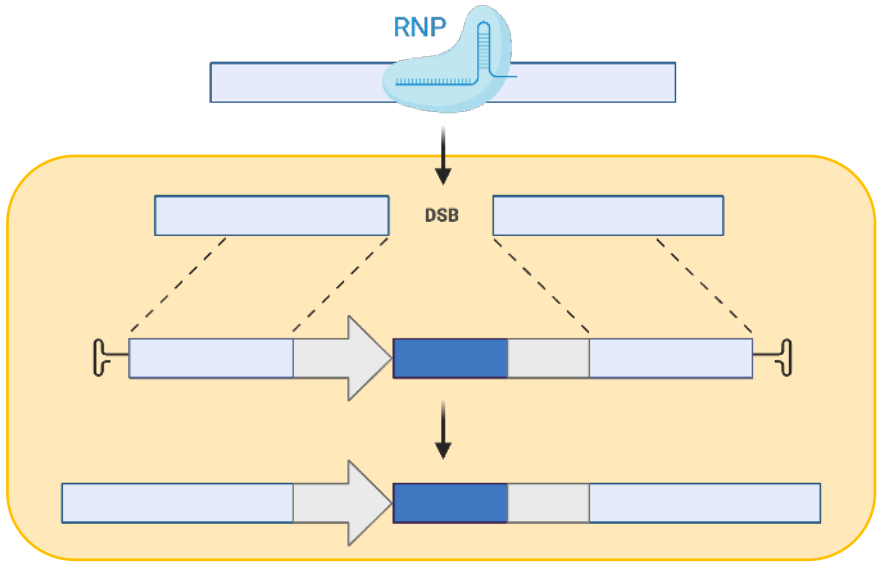
Optimization parameters
Electroporator & electroporation code
Guide:Cas9 ratio
RNP concentration
Cell concentration during electroporation
Day of electroporation



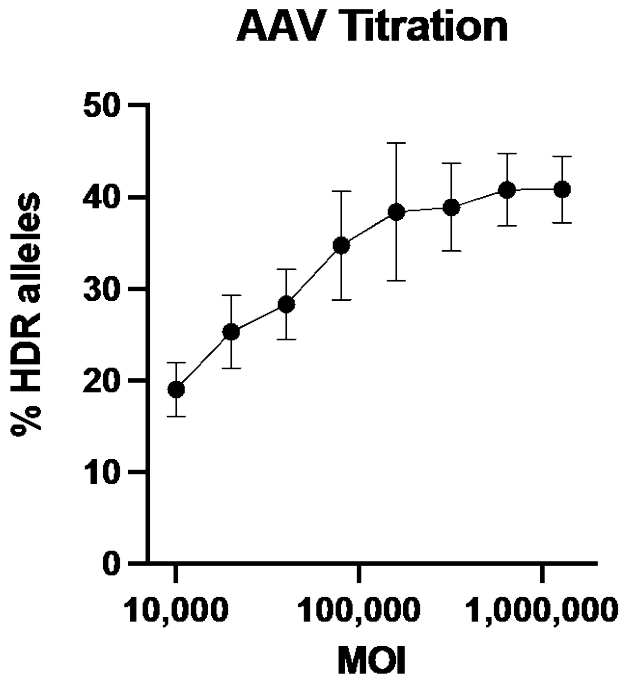


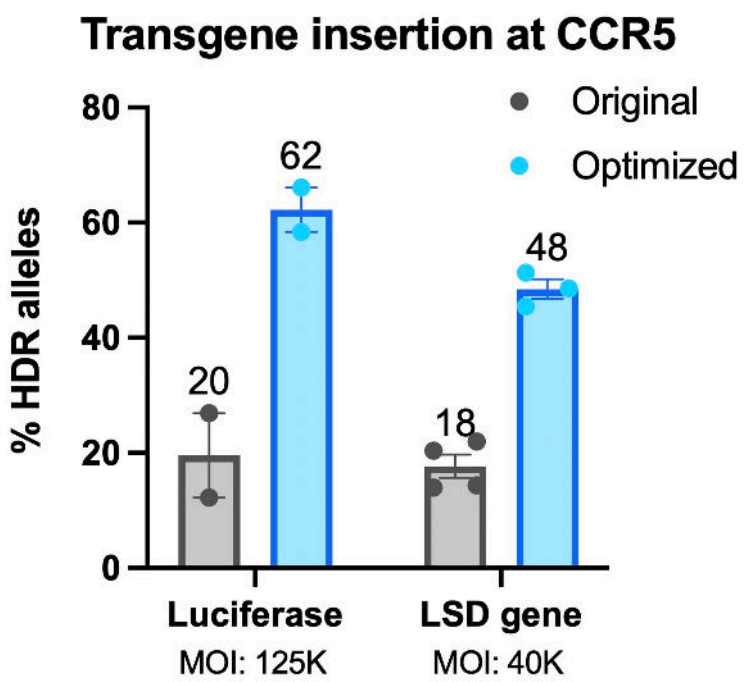
Up to 60% transgene insertion in primary B cells

Optimization of transgene **KNOCK IN**

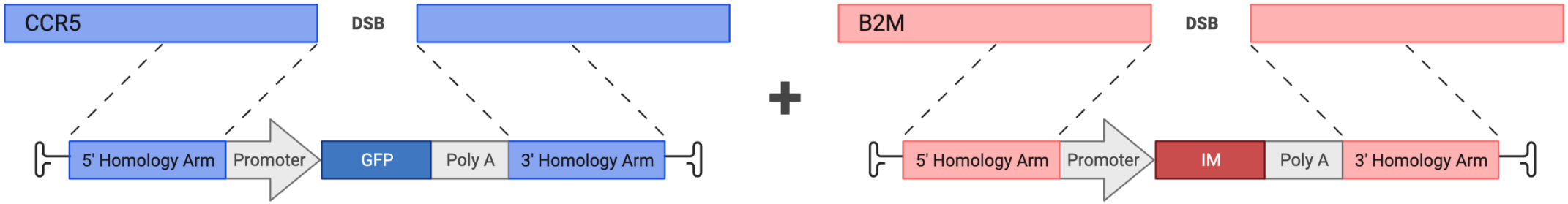


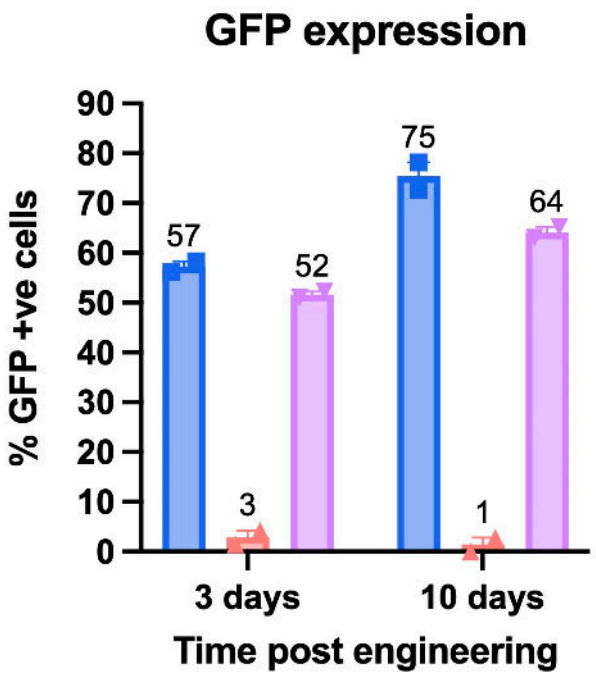
Optimization parameters
AAV MOI
Timing and duration of AAV transduction
Transduction volume
Media composition

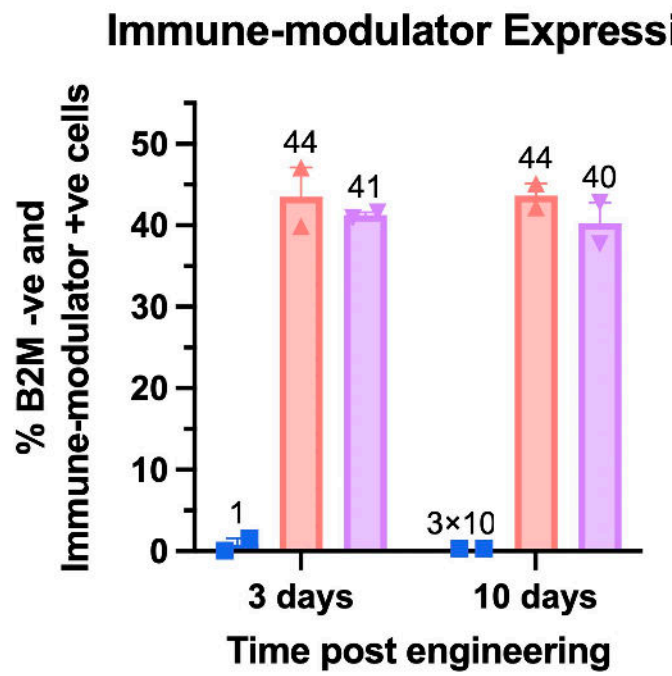


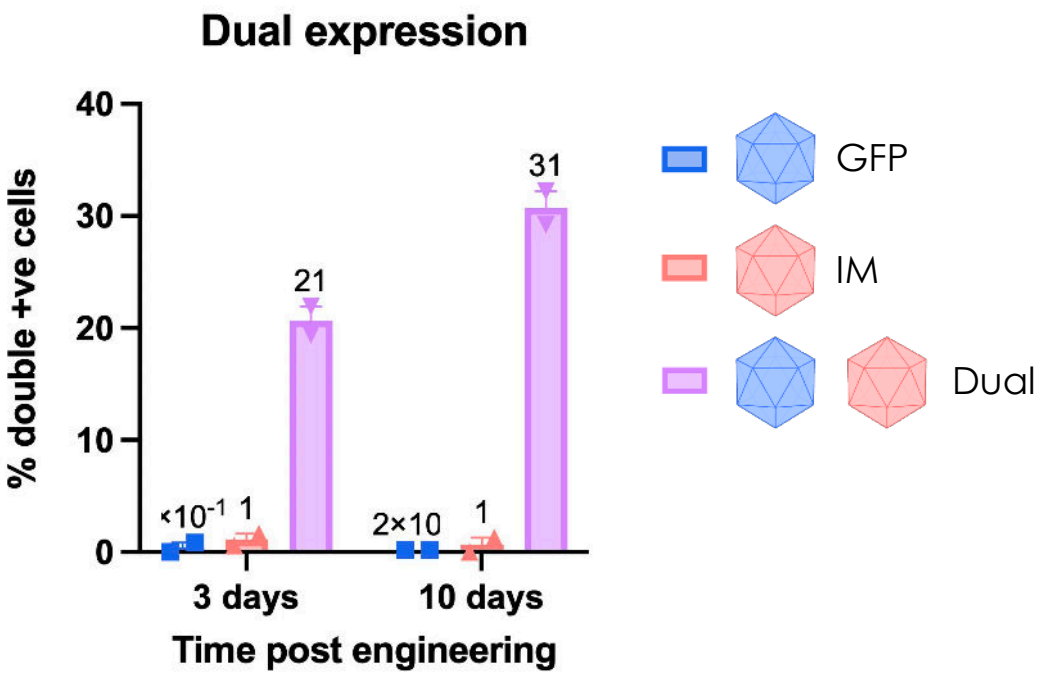


>20% multiplexed insertion of two genes in single reaction









A clinical guide selection pipeline in primary B cells

Potency testing

Computational guide design
Ranking by predicted on-/off-target activity

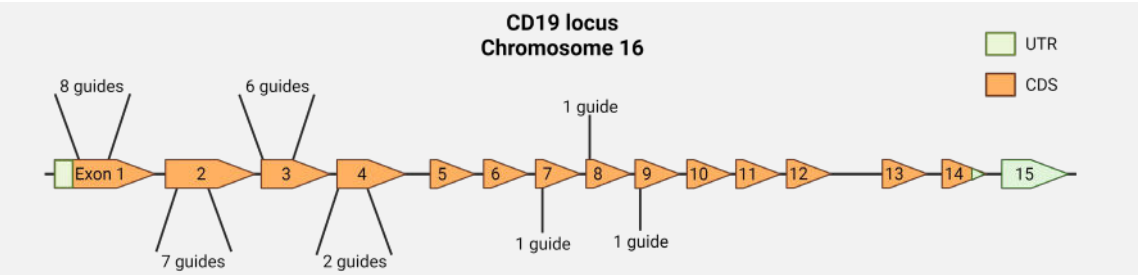
Knock-out
DNA editing
% editing, INDEL pattern; protein knock down

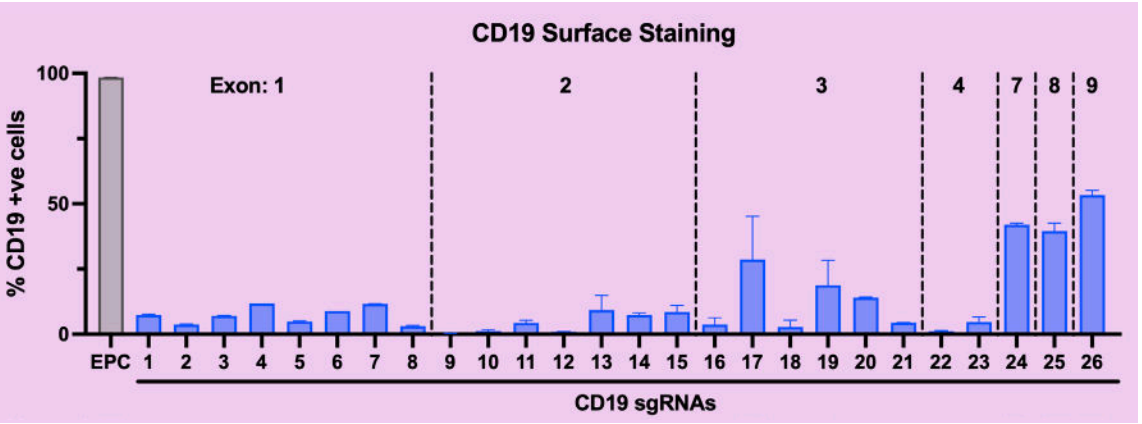
Knock-in
Transgene expression
% Reporter expression, MFI

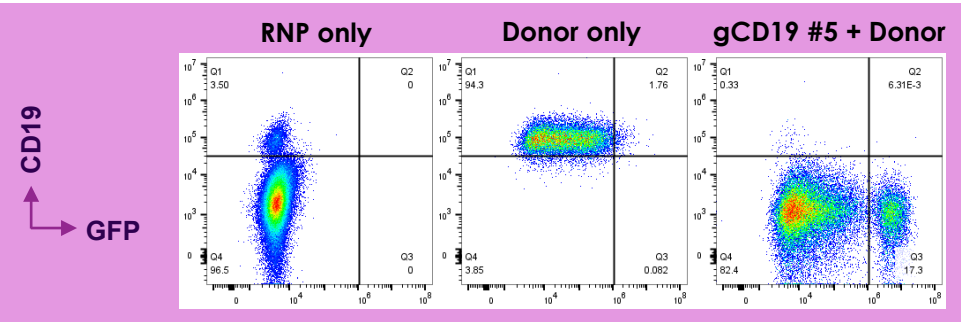
Safety testing

Off-target discovery
In silico, cellular, biochemical

Off-target validation
rhAmpSeq, Amplicon Seq

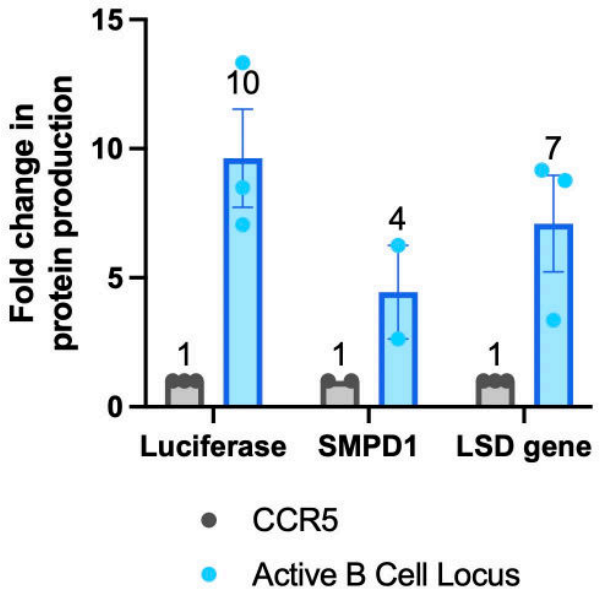






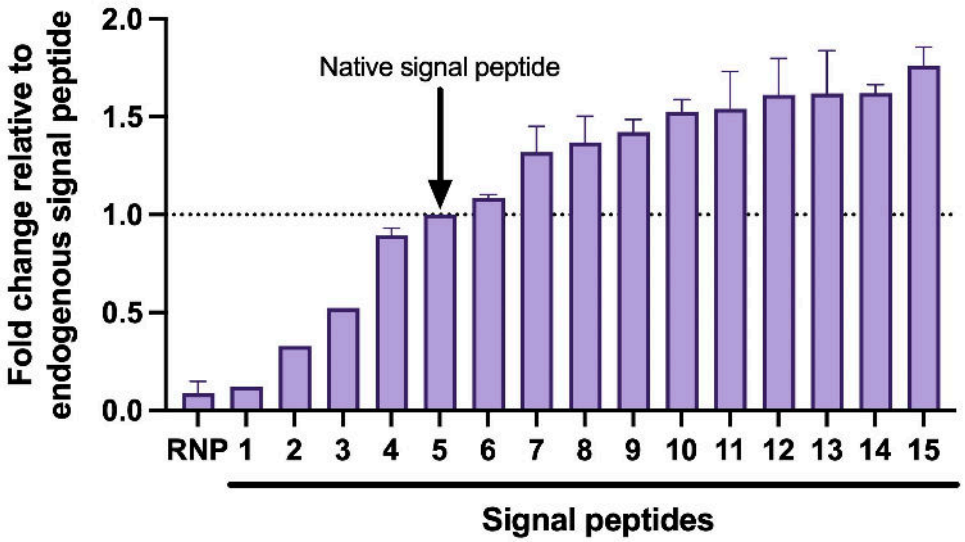
Enhanced transgene expression via construct optimization

Target locus selection



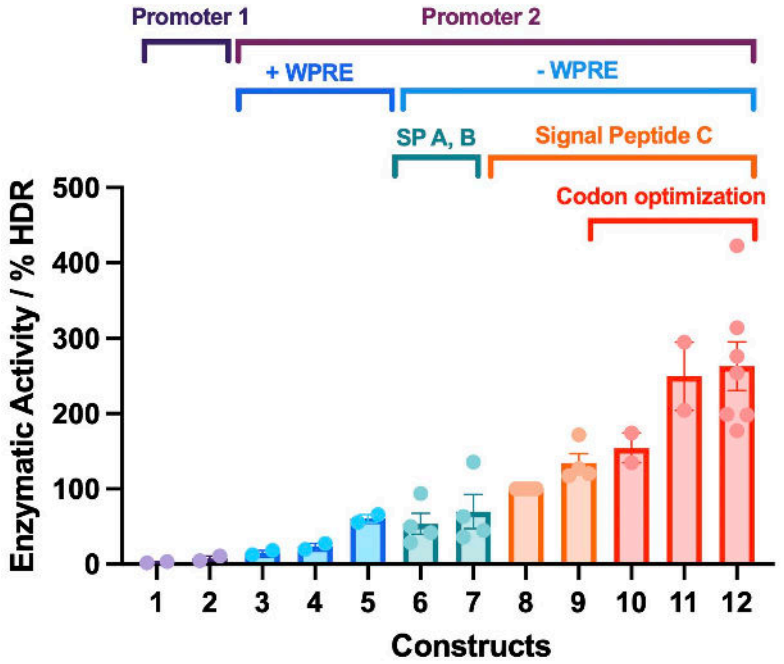
Insertion at active B cell locus enhances protein production

Signal peptide screen



Signal peptide optimization can enhance protein secretion

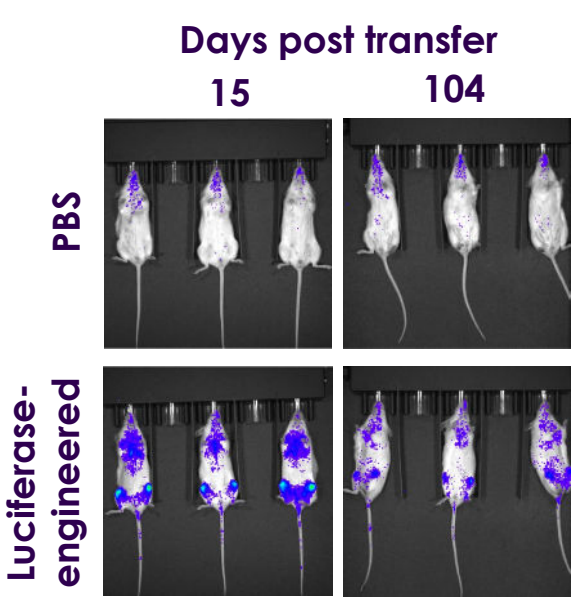
Expression cassette optimization

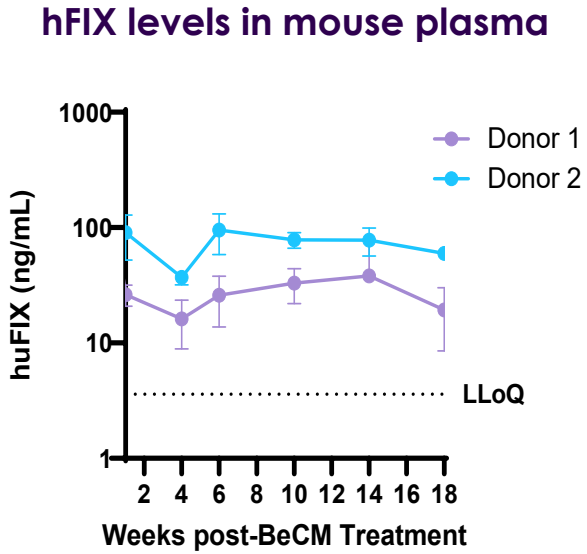


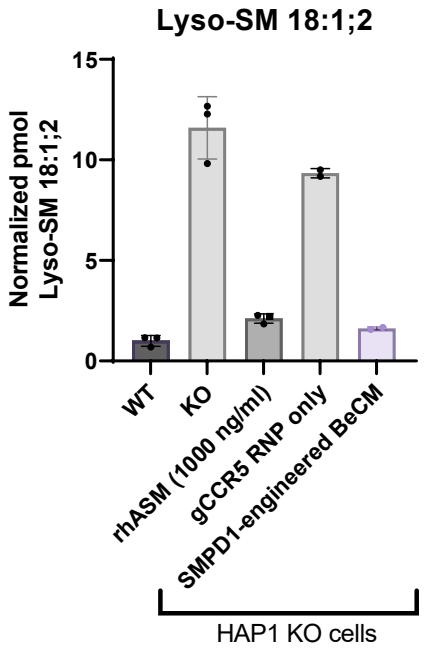
Optimization of regulatory and coding sequences can increase transgene expression

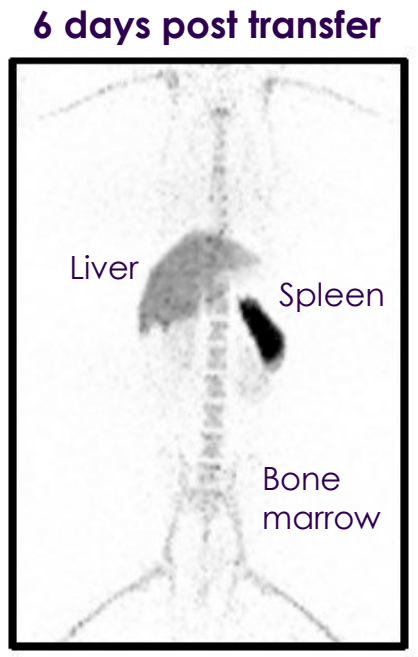
BeCMs durably produce functional biologics

Persistence	Lasting protein production	Secretion of active protein	Engraftment without pre-conditioning









BeCMs engraft in NOG-IL6 mice and persist for >100 days	hFIX detected in plasma for 18 weeks post BeCM transfer	BeCM-secreted Acid Sphingomyelinase corrects disease phenotype in KO cells	*Ex vivo* cultured Rhesus plasma cells engraft in immuno-competent host

A versatile CRISPR-based B cell engineering platform…

- CRISPR-mediated gene knockouts above 90%
- Targeted gene insertions up to 60% (multiplexed above 20%)
- Rapid screening of guides/constructs directly in primary B cells
- Optimized construct design

…enables a new class of cellular medicines designed for sustained delivery of therapeutic biologics.

Acknowledgements

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