Genome Editing of *HBG1/2* Promoter Leads to Robust HbF Induction *In Vivo* While Editing of *BCL11A* Erythroid Enhancer Shows Erythroid Defect

Kai-Hsin Chang, Minerva Sanchez, Jack Heath, Edouard de Dreuzy, Scott Haskett, Abigail Vogelaar, Kiran Gogi, Jen DaSilva, Tongyao Wang, Andrew Sadowski, Gregory Gotta, Jamaica Siwak, Ramya Viswanathan, Katherine Loveluck, Hoson Chao, Eric Tillotson, Aditi Chalishazar, Abhishek Dass, Frederick Ta, Emily Brennan, Diana Tabbaa, Eugenio Marco, John Zuris, Deepak Reyon, Meltem Isik, Ari Friedland, Terence Ta, Fred Harbinski, Georgia Giannoukos, Sandra Teixeira, Christopher Wilson, Charlie Albright, Haiyan Jiang

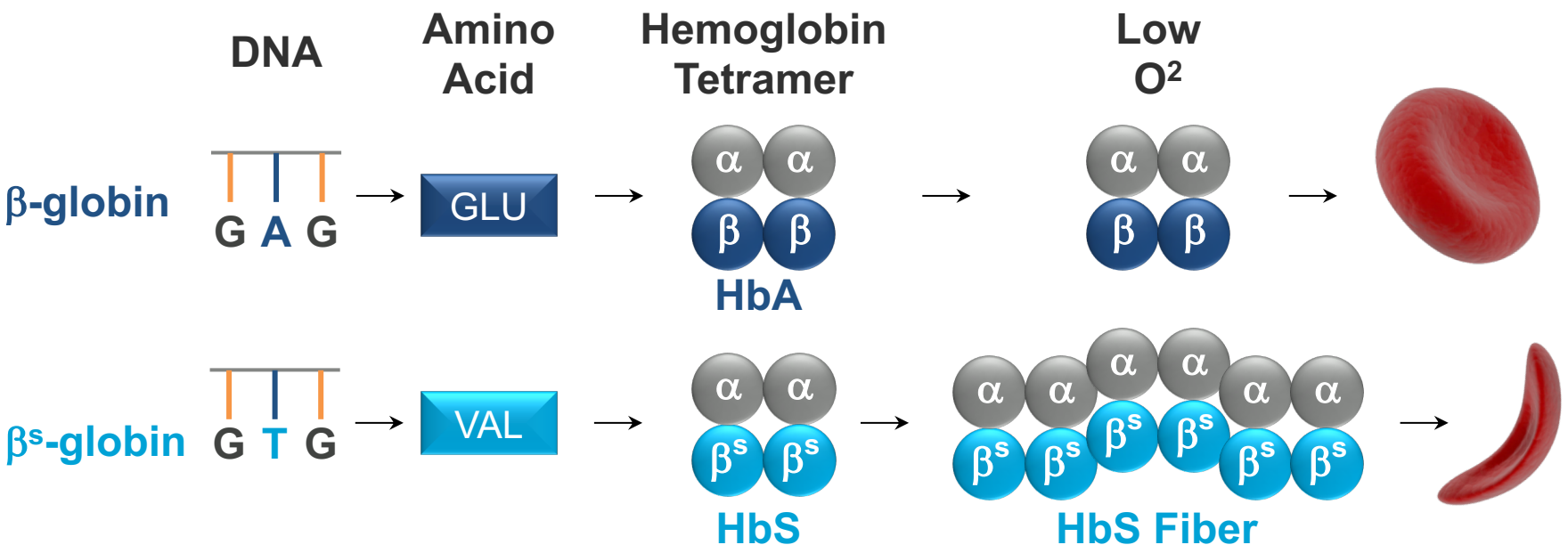
Editas Medicine, Inc., Cambridge, MA

60th Annual Meeting and Exposition of American Society of Hematology
December 2, 2018, San Diego, CA

Overview

- Etiology of Sickle Cell Disease
- *In Vivo* Study Design to Evaluate Two Approaches to Increase Fetal Hemoglobin (HbF) Expression
- Effect of Downregulating BCL11A Expression by Targeting its Erythroid Enhancer
- Editing Cis-regulatory Elements in β-Globin Locus
- Conclusion

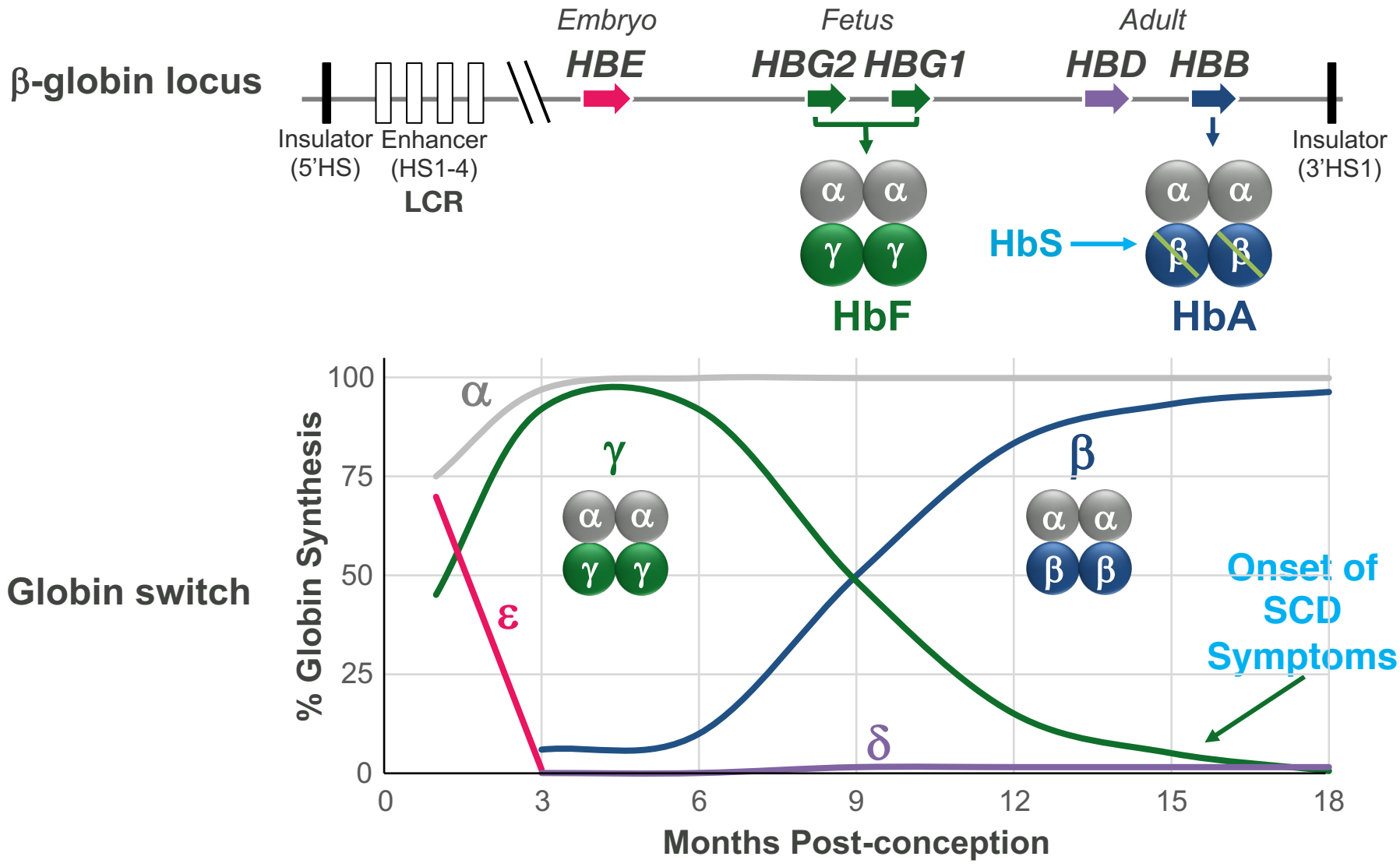
Etiology of Sickle Cell Disease



- Sickle cell disease (SCD) is caused by a single mutation E6V of the β-globin chain, leading to polymerization of hemoglobin (Hb) and formation of sickle hemoglobin (HbS) fibers when deoxygenated.
- Symptoms include anemia, acute chest syndrome, pain crises, and an array of other complications.
- Patients suffer significant morbidity and early mortality.

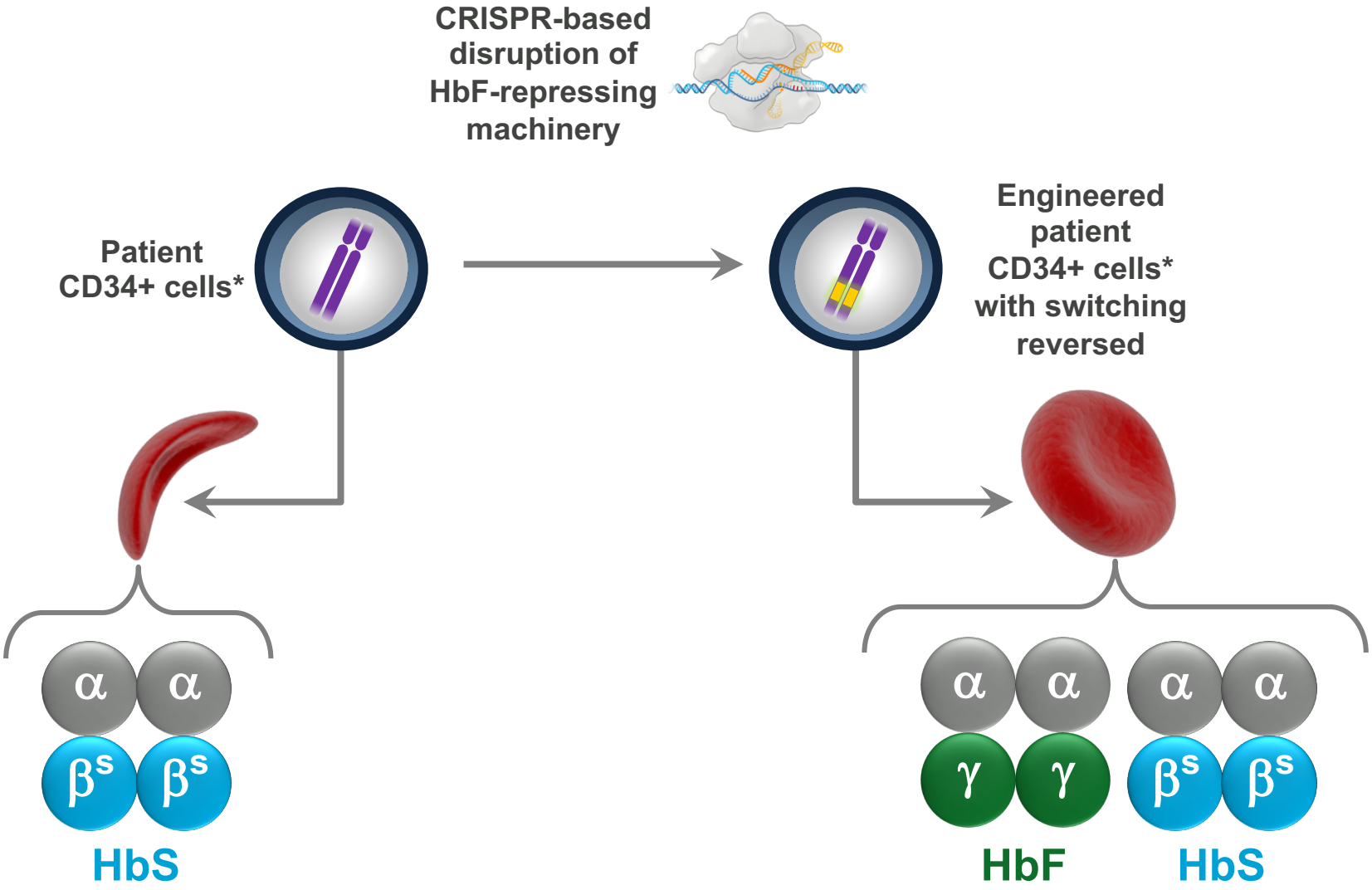
Bender MA. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018.

Harnessing Natural Anti-sickling Hemoglobin to Treat Sickle Cell Disease



Adapted from Canver and Orkin, Blood, 2016

Genome Editing to Reverse Hemoglobin Switching for Treating Sickle Cell Disease



*CD34+ hematopoietic stem and progenitor cells (HSPCs)

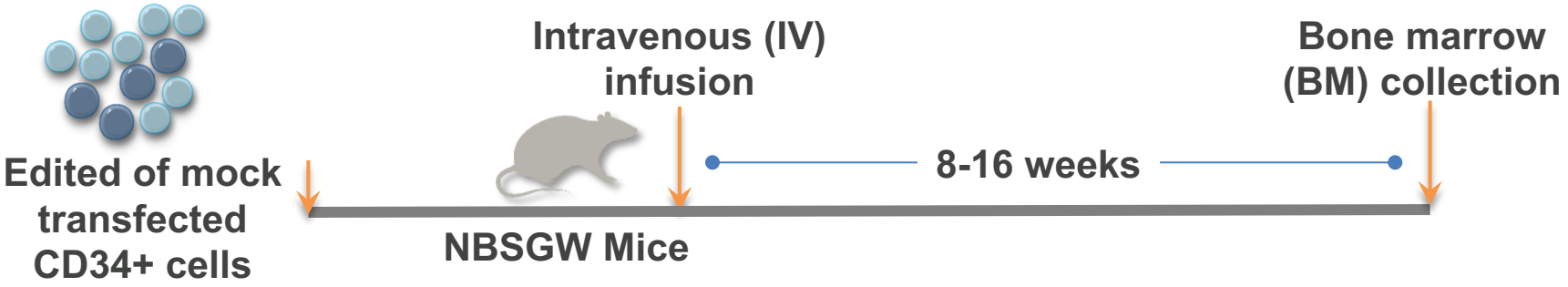
Preclinical Target Criteria

Successful editing of long-term HSCs

Maintenance of normal HSPC functions

Robust long-term induction of HbF

Study Design for Assessment of Multilineage Engraftment Potential of Edited HSPCs



1 Lineage reconstitution by flow cytometry

Human Chimerism	Erythroid	B Cell	Monocyte	Neutrophils	HSPCs
hCD45/WBC	CD235a/total	hCD19/hCD45	hCD14/hCD45	hCD15/hCD45	hCD34/hCD45

2 Editing analysis by Next-Gen Sequencing (NGS)

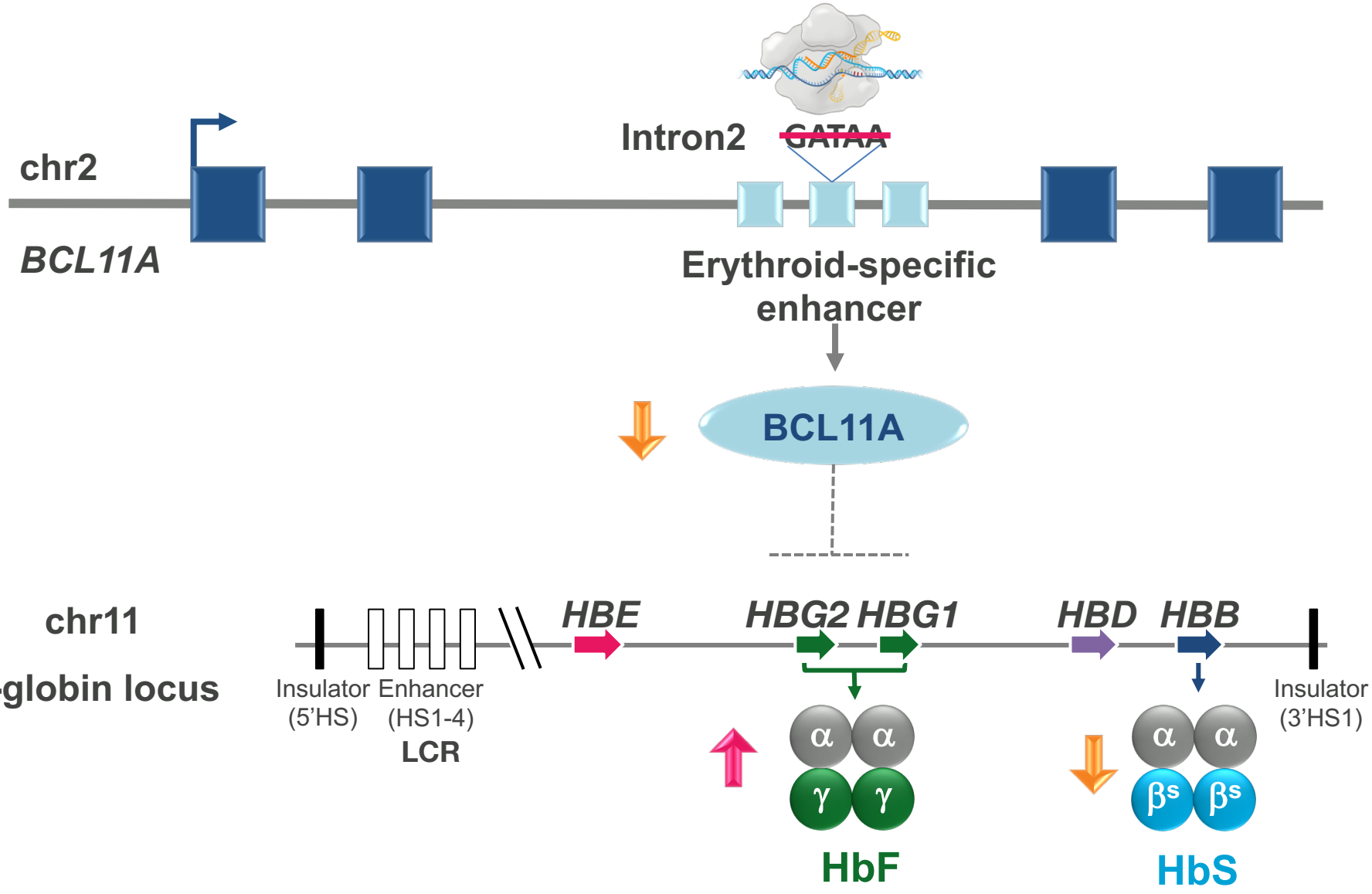
- Unfractionated BM
- Flow sorted erythroids, B cells, neutrophils, and Lin-HSPCs

3 Analysis of HbF (γ/β-like) expression by reverse phase UPLC

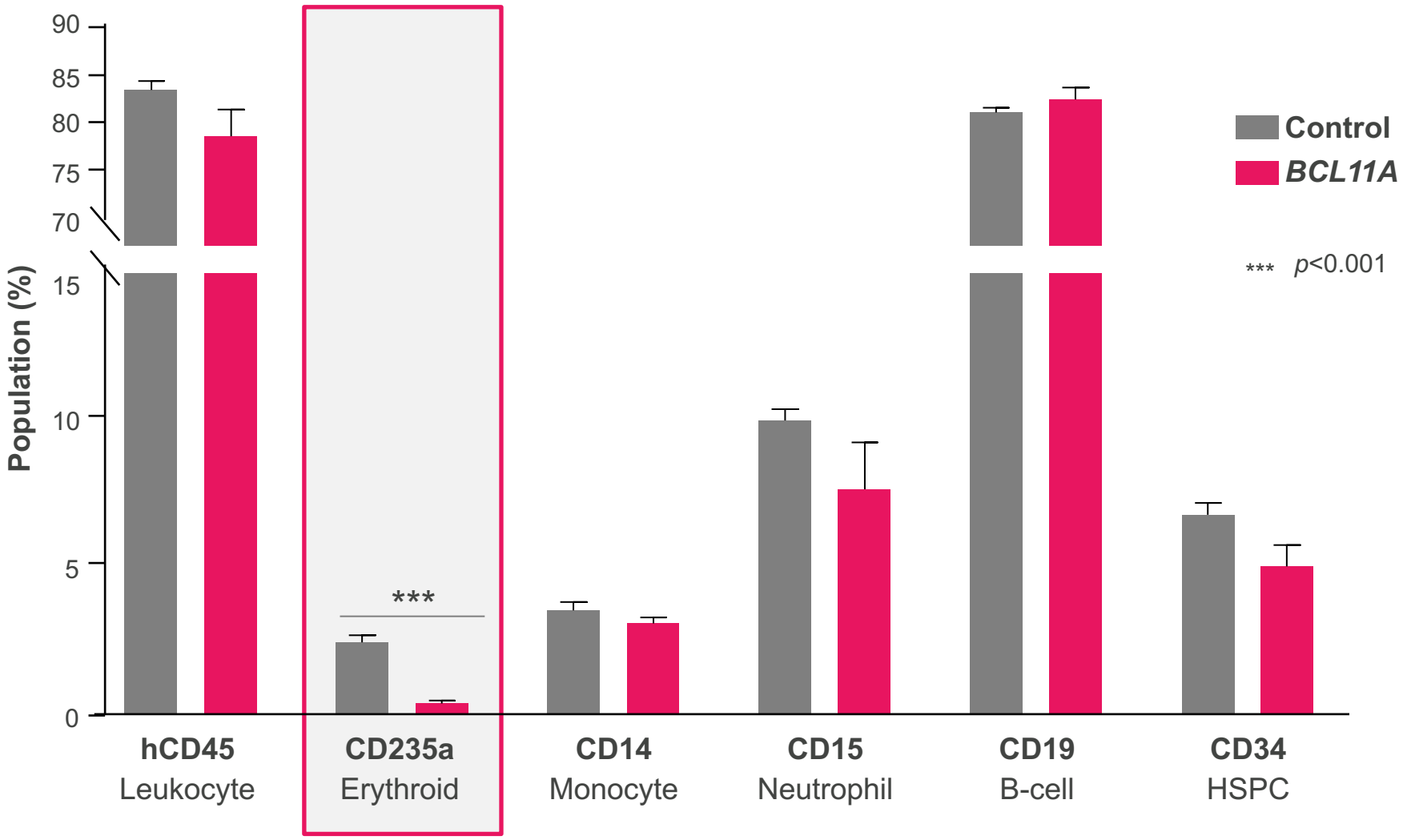
- Flow sorted CD235a+ erythroid cells
- *Ex vivo* cultured erythroid cells from chimeric BM

4 Apoptosis assessment of cultured erythroid cells by flow cytometry

Approach 1: Downregulation of BCL11A Expression by Targeting Its Erythroid Enhancer



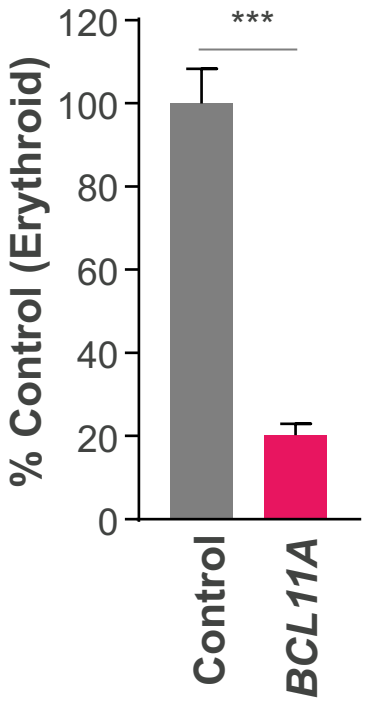
BCL11A Erythroid Enhancer-editing Displayed Reduced Erythroid Output in BM of NBSGW Mice



Reduced *BCL11A* Erythroid Output Coincided with Increased Non-productive Indels and Increased Apoptosis

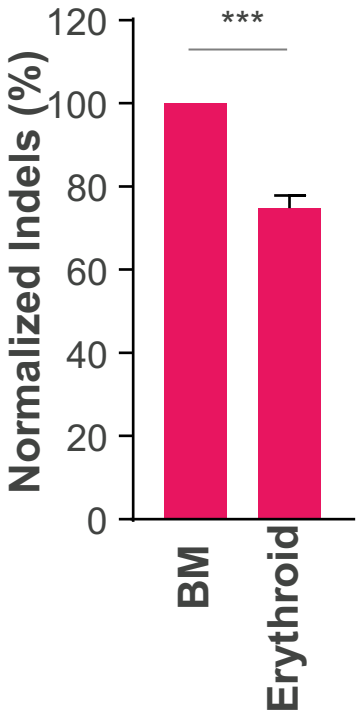
BM

Reduced erythroid output

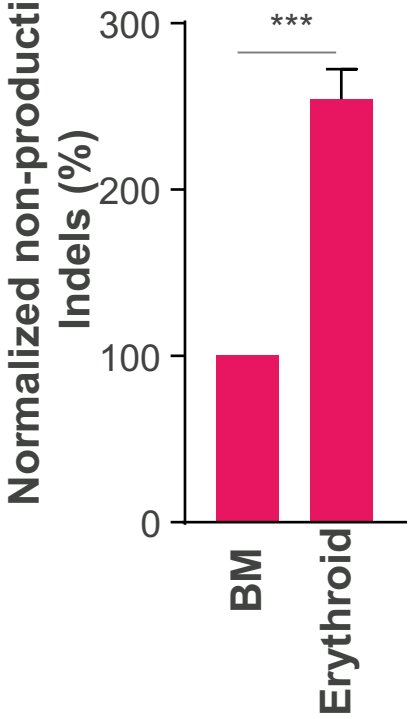


Sorted erythroid cells

Less editing

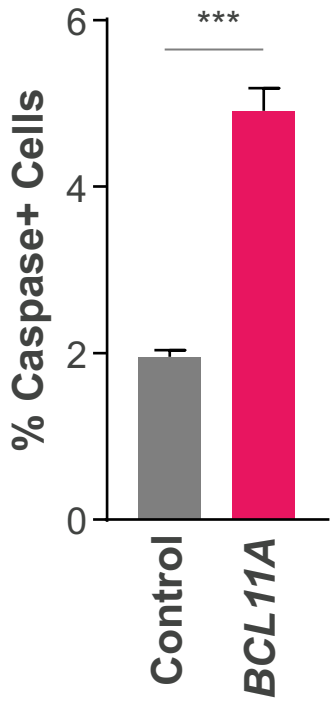


Enrichment Of non-productive edits



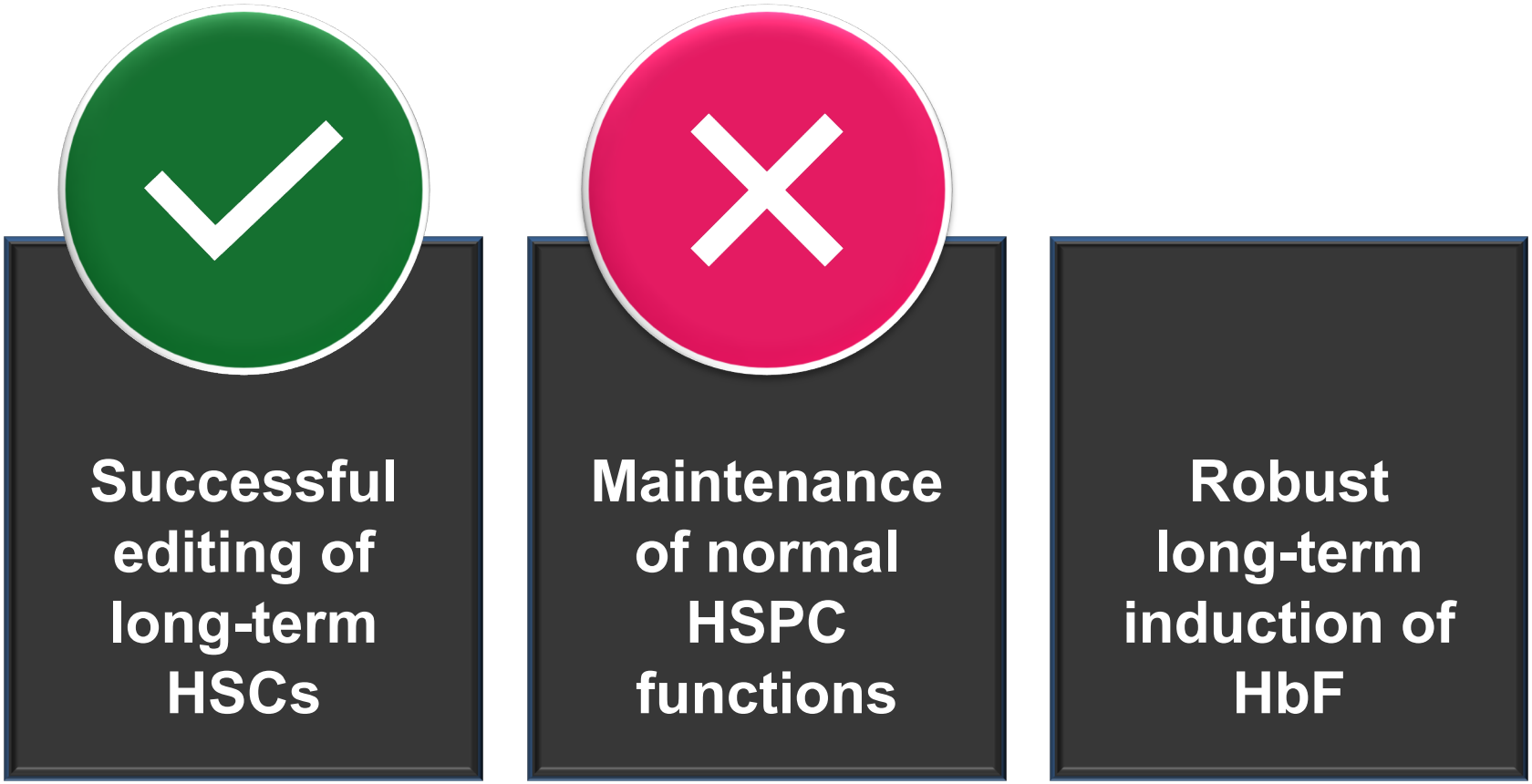
Cultured erythroids

Increased apoptosis

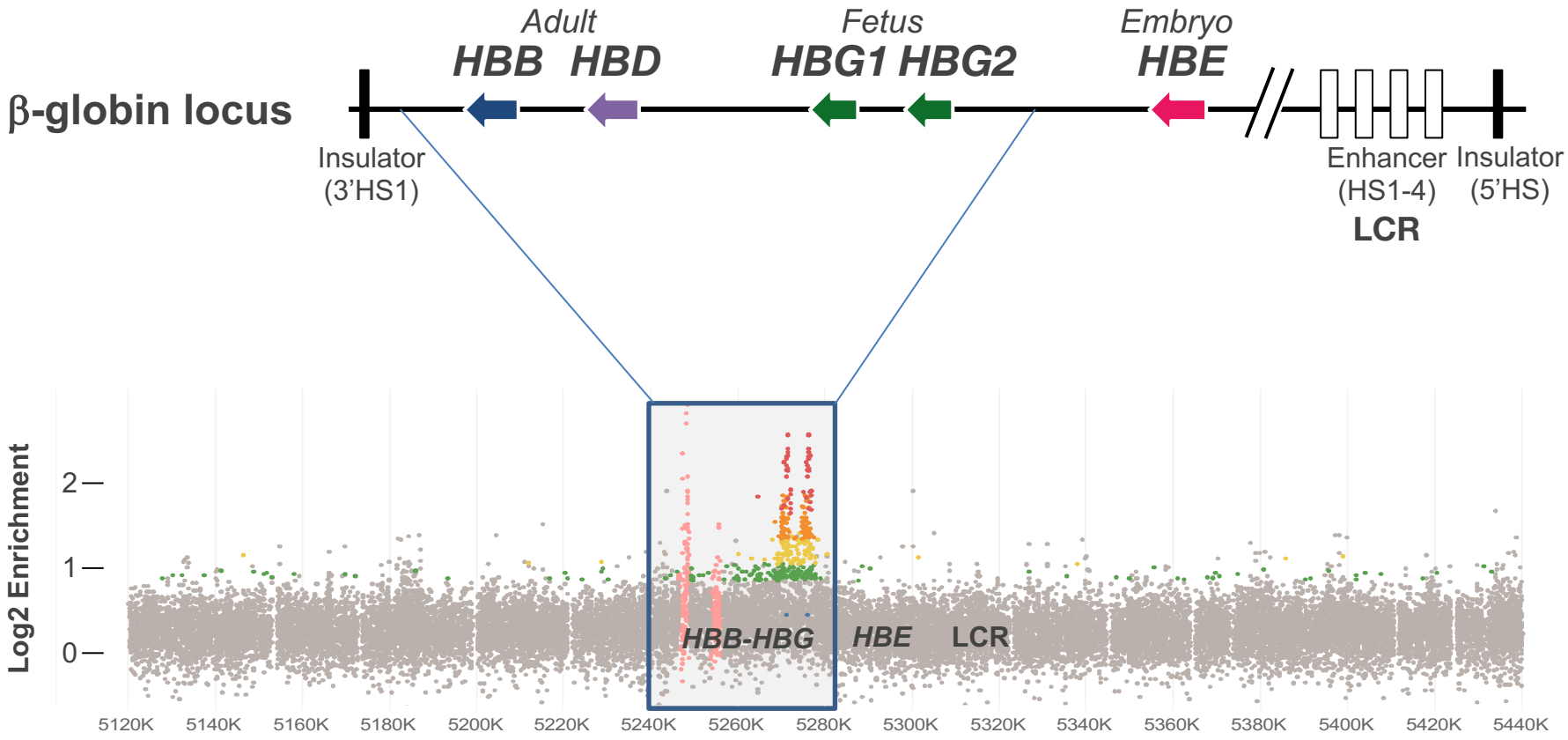


p<0.001

BCL11A Erythroid Enhancer-editing Failed to Meet Preclinical Target Criteria

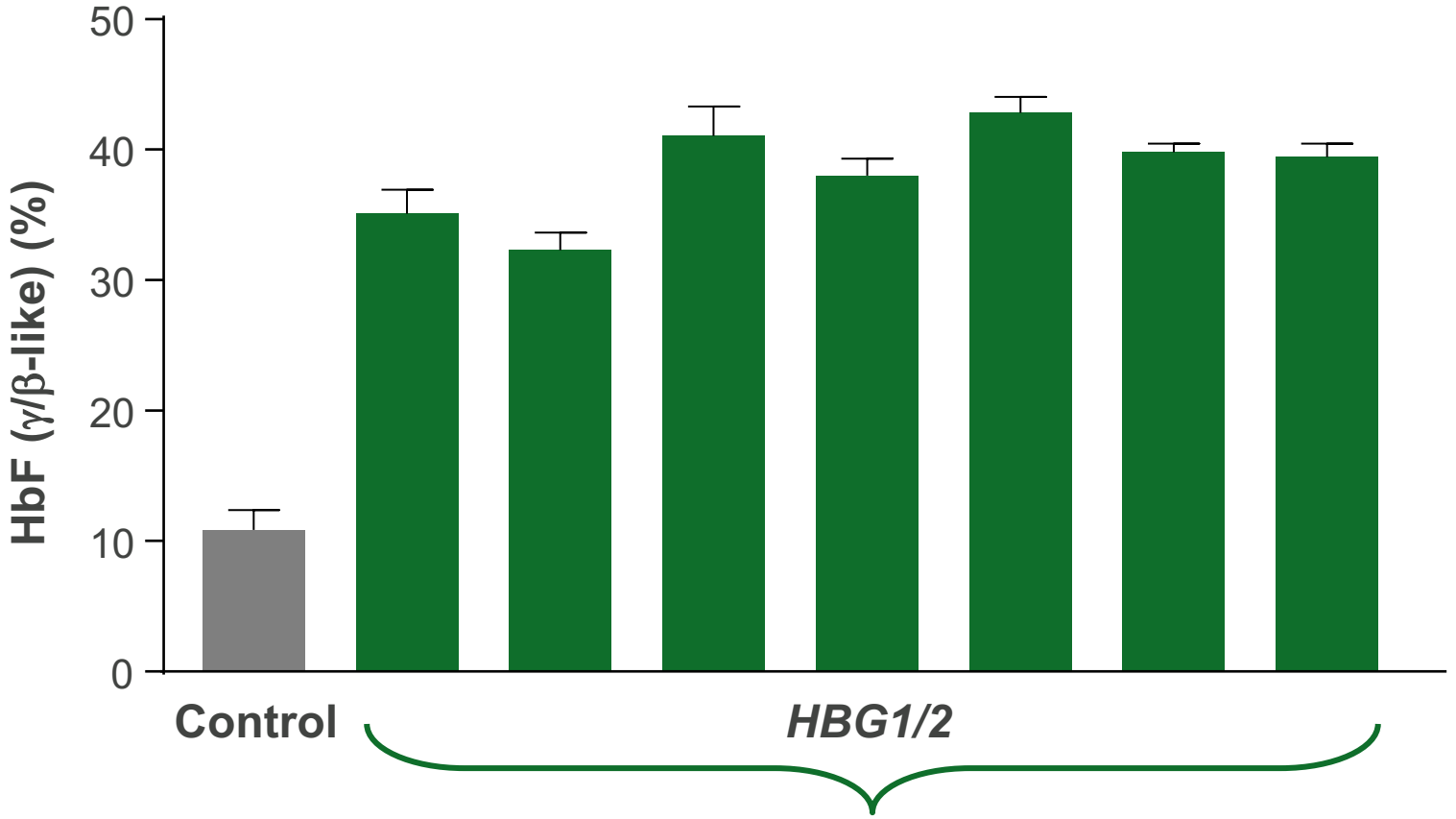


Approach 2: Editing Cis-regulatory Elements in β-Globin Locus



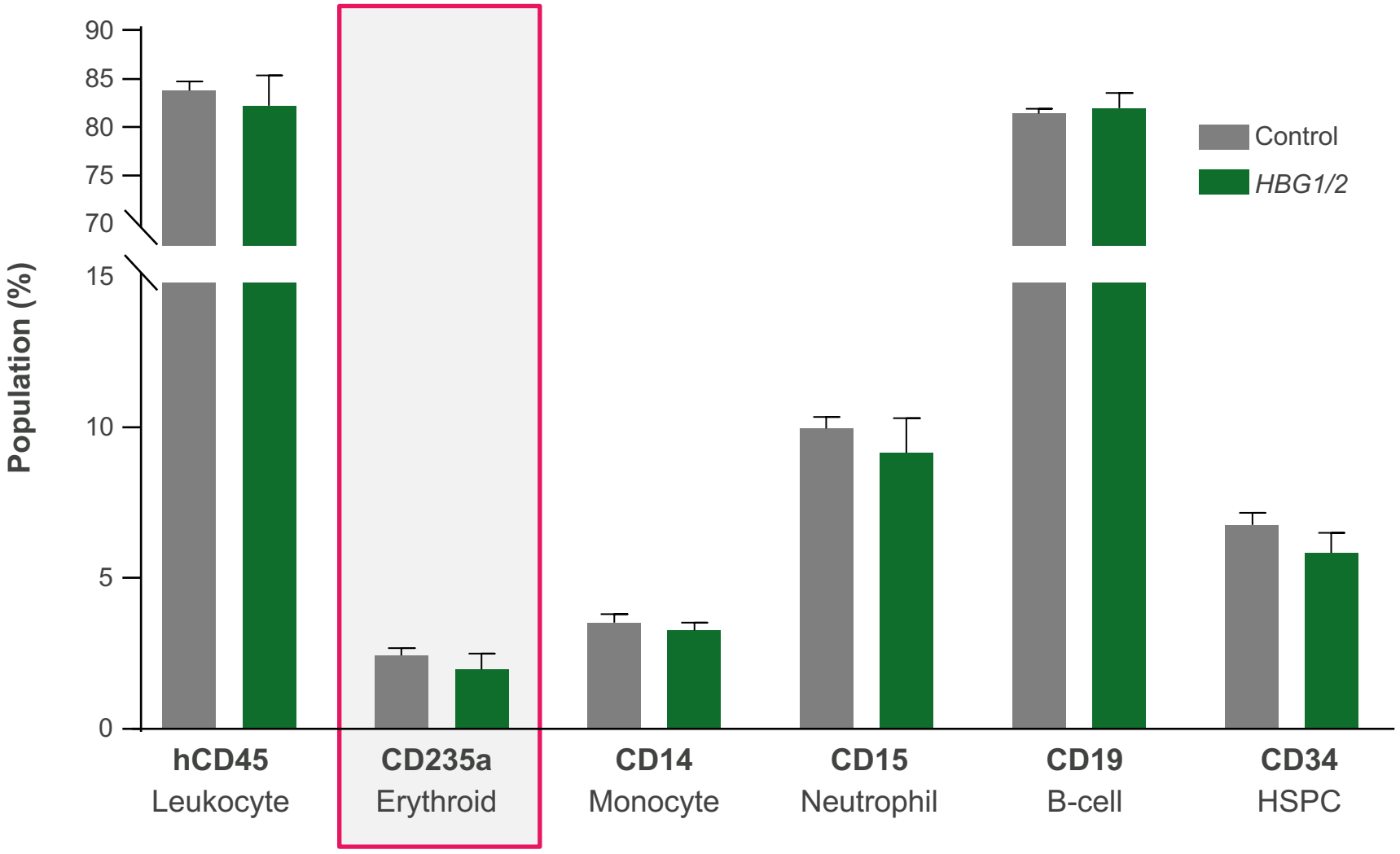
- ~26,000 gRNAs were tested covering 320kb genomic region
- ~300 HbF-inducing gRNA were identified
- Most were mapped to β-globin locus including *HBG*, *HBD*, and *HBB* genes

Robust HbF Induction Achieved with Editing of *HBG1/2* Promoters *Ex Vivo*



Multiple enzymes including SpCas9, SaCas9, Cpf1 (Cas12a) and gRNA combinations were tested

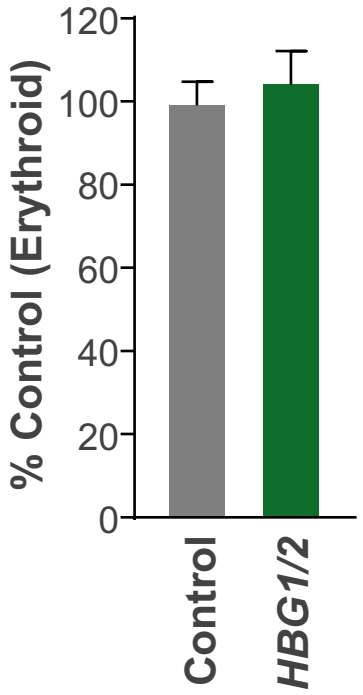
HBG1/2 Promoter-editing Displayed Normal Erythroid Output in BM of NBSGW Mice



HBG1/2 Promoter Editing Demonstrated No Erythroid Defect

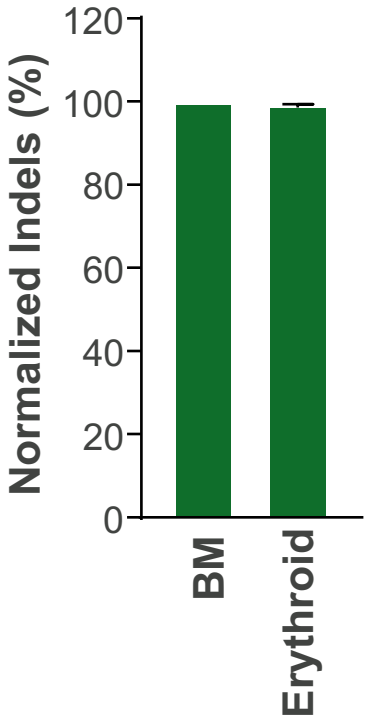
BM

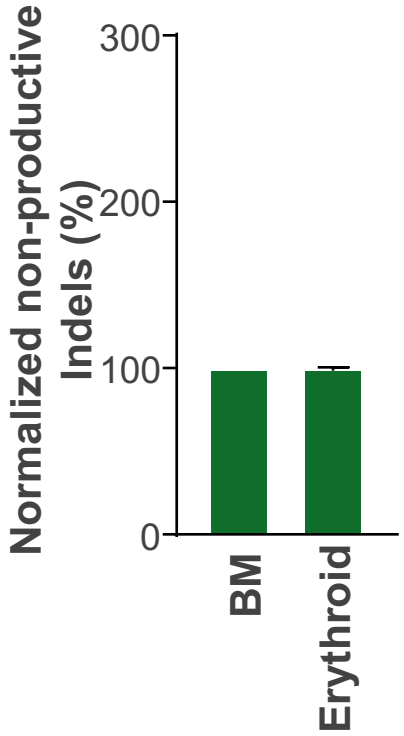
Similar levels of erythroid output



Sorted erythroid cells

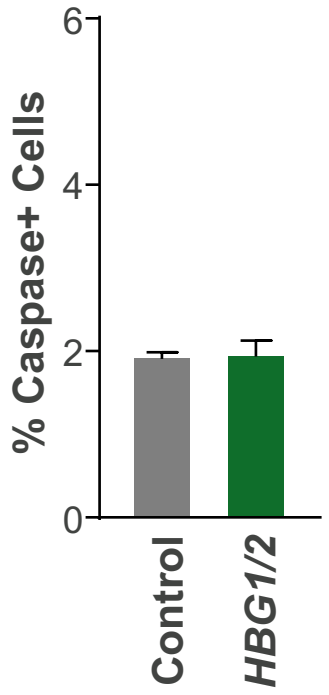
Similar levels of indels in erythroid compared to BM



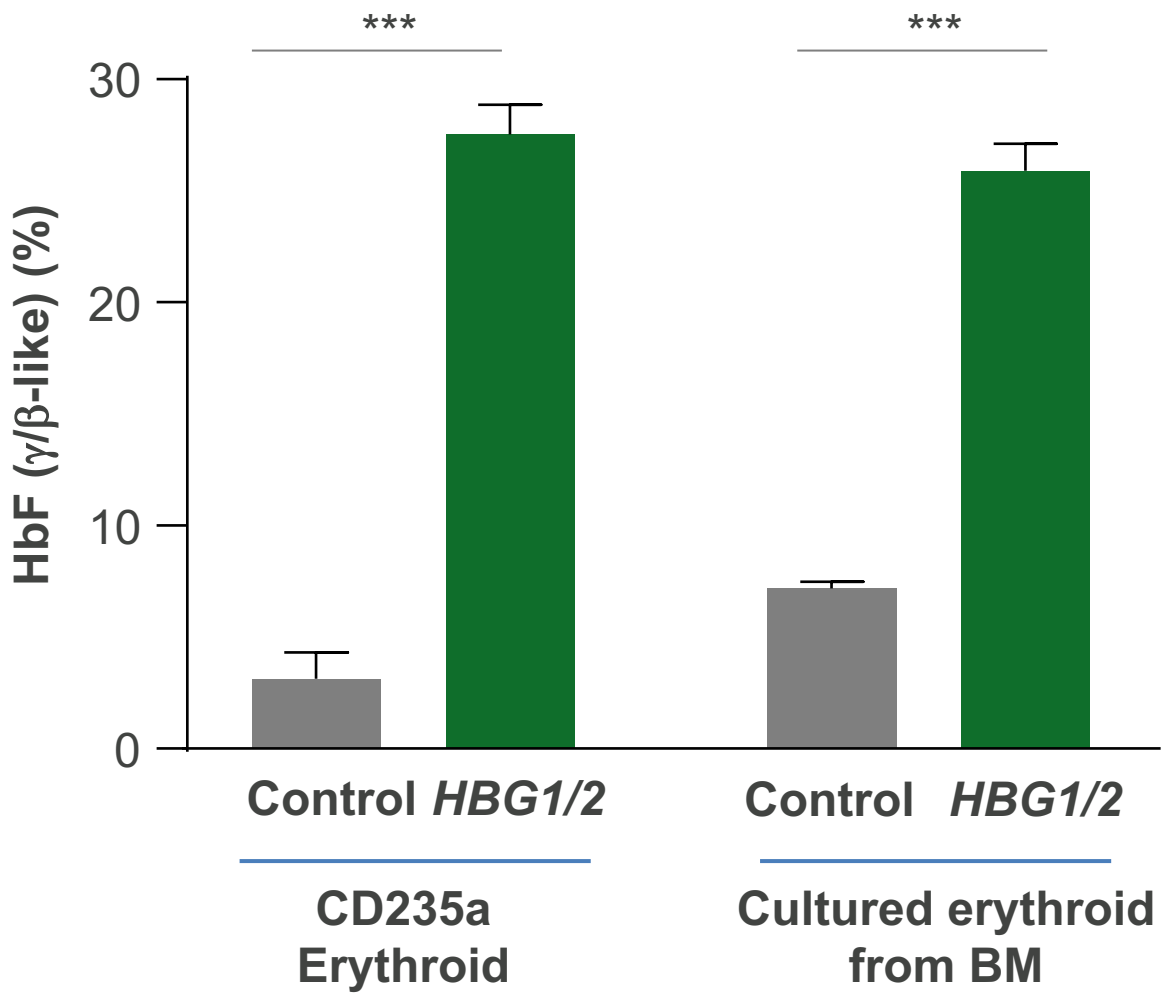


Cultured erythroids

Similar levels of apoptosis

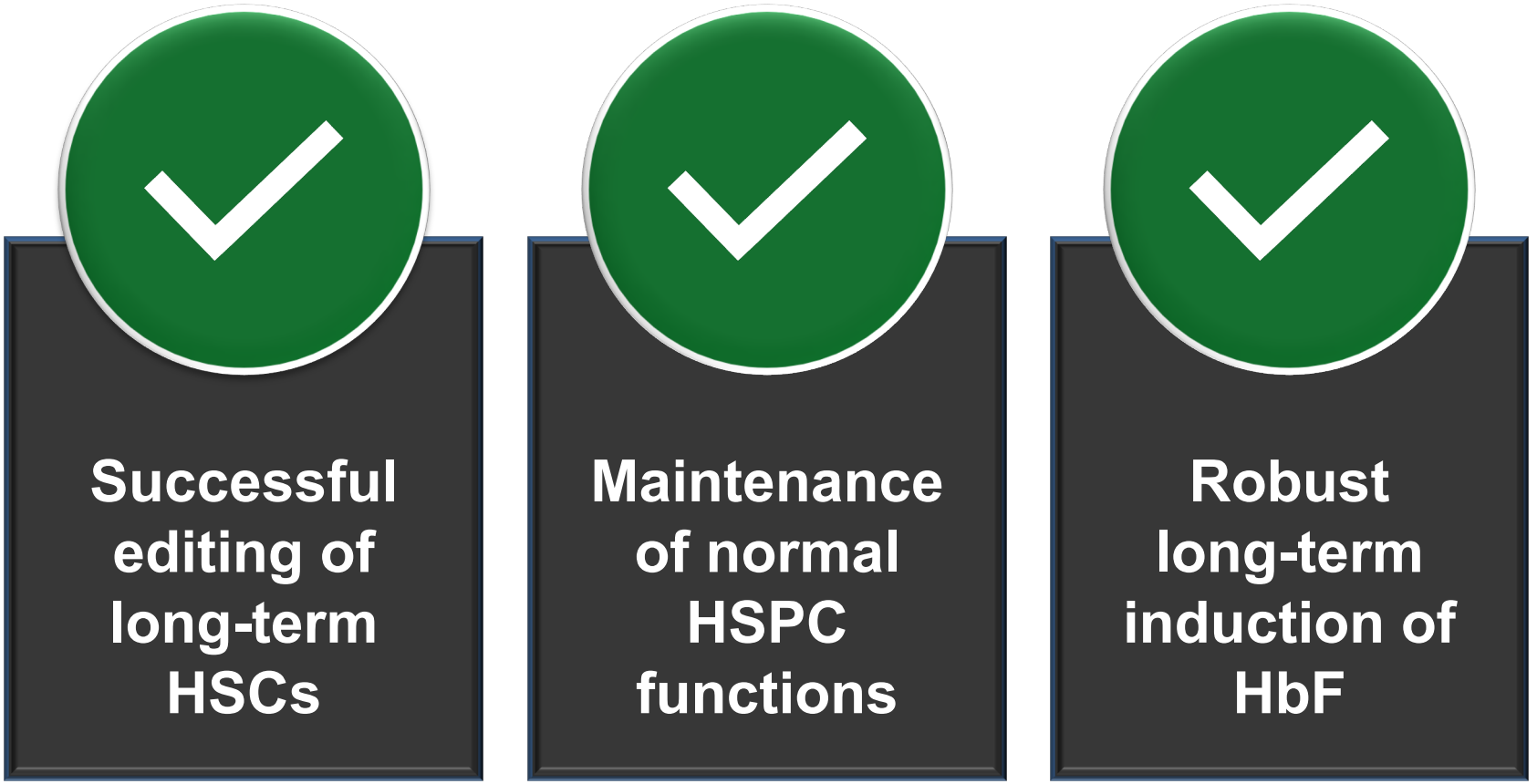


HBG1/2 Promoter Editing Demonstrated Long-term HbF Induction



p<0.001

Editing *HBG1/2* Promoters Met Critical Preclinical Target Criteria



Conclusions

Long-term engraftment observed in immunocompromised NBSGW mice with both *BCL11A* erythroid enhancer-edited and *HBG1/2* promoter-edited CD34+ HSPCs

In this study, *BCL11A*-edited CD34+ HSPCs had an erythroid differentiation defect in the NBSGW mouse model that was not observed in *HBG1/2*-edited CD34+ HSPCs

Robust induction of HbF in *HBG1/2* promoter-edited erythroid cells from long-term *in vivo* study

Acknowledgements

Pipeline

Platform

Program Management

CMC

Clinical

Lab Operations