Development of a Beta-globin Gene Replacement Strategy as a Therapeutic Approach for β-Thalassemia

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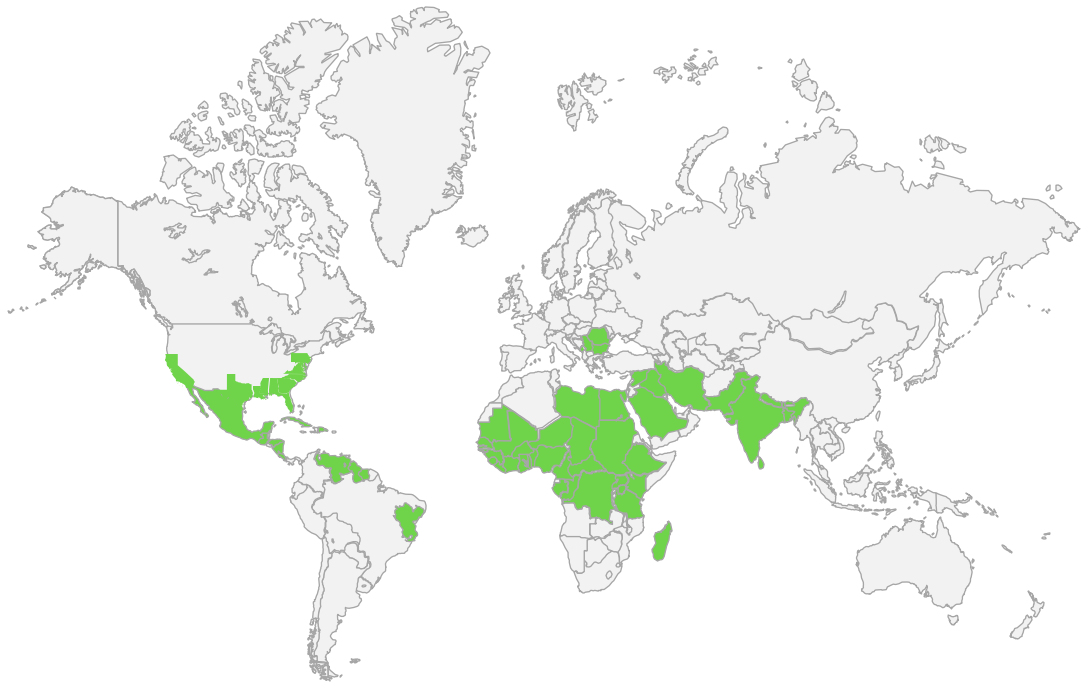
Disclosures

All authors are current employees and equity holders in Graphite Bio. Christopher Bandoro reports equity holdings in Sana Biotechnology and James Partridge reports equity holdings in Global Blood Therapeutics.

Our goal: Restore normal genotype and physiology in people with hemoglobinopathies

Sickle Cell Disease (SCD)[1,2]



β-Thalassemia[2]

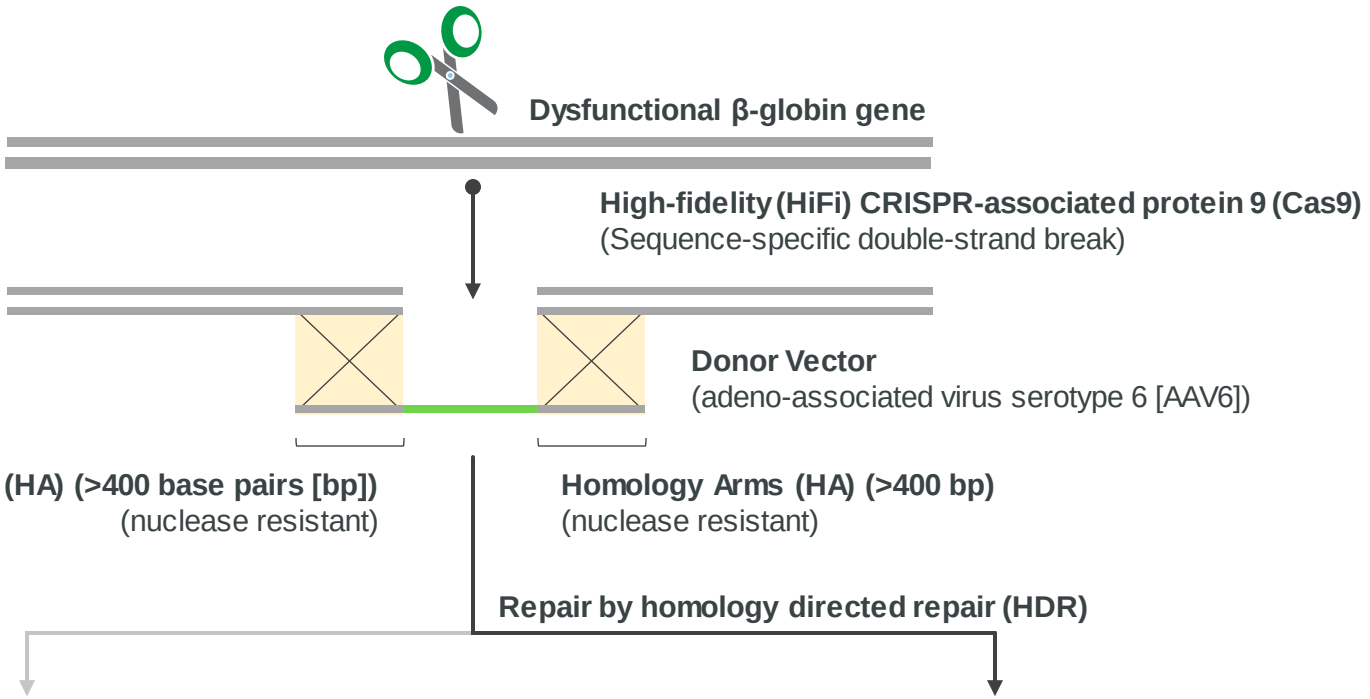
Annually 330,000 infants are born with hemoglobinopathies worldwide, of which sickle cell disease and β-thalassemia are the most common[3]

1. Piel FB, et al. *Nat Commun*. 2010;1:104; 2. Piel FB. *Hematol Oncol Clin North Am*. 2016;30(2):327-341; 3. Modell B and Darlison M. *Bull World Health Organ*. 2008;86(6):480-487.

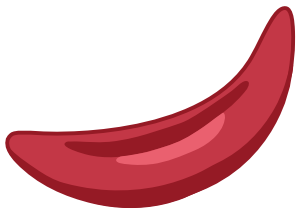
The UltraHDR™ platform allows for gene correction and gene replacement in sickle cell disease and β-thalassemia

Trial in Progress – Poster 806

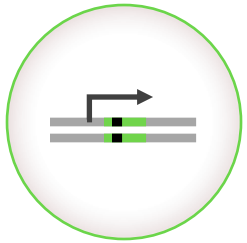
CEDAR



Sickle Cell Disease (GPH101)



GENE CORRECTION

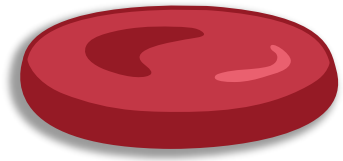


Correct point mutations or short DNA stretches in endogenous locus

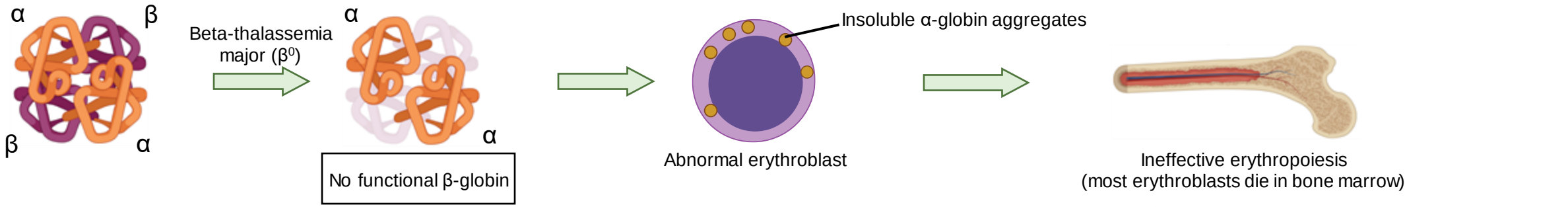
GENE REPLACEMENT

Gene replacement driven by endogenous promoter

β-thalassemia (GPH102)



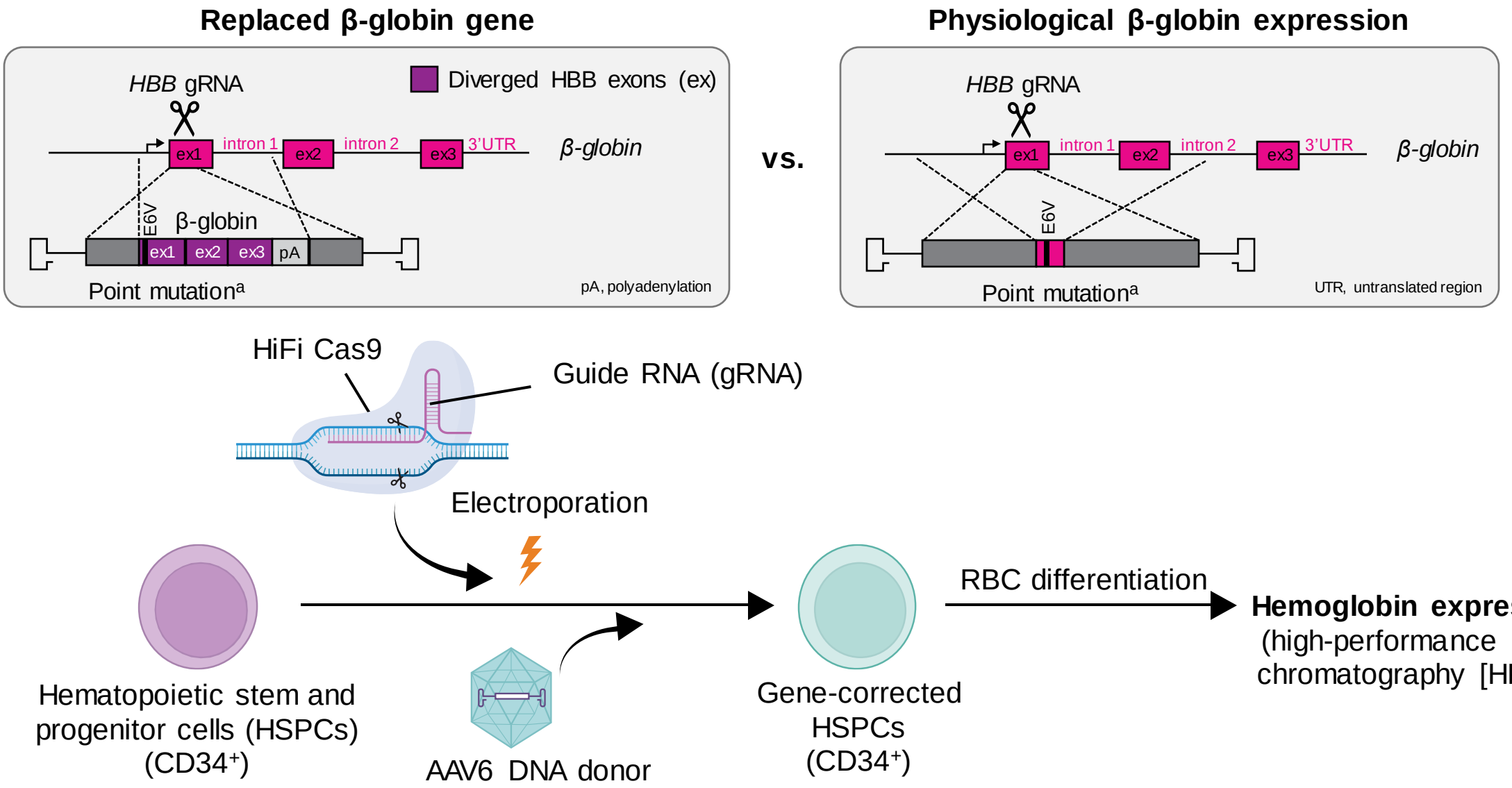
β-thalassemia is a genetic disorder with high unmet need characterized by reduced production of β-globin[1]



- More than 300 mutations are known to cause β-thalassemia[2]
- Globally, 1.5% of the population are carriers of β-thalassemia mutations[3]
- Approximately 68,000 symptomatic individuals are born annually[4]
- β-globin (HBB) gene replacement would be an ideal therapeutic strategy with the potential to reproduce a normal genotype and restore adult hemoglobin (HbA) expression

1. Beta thalassemia. National Organization for Rare Diseases (NORD). https://rarediseases.org/rare-diseases/thalassemia-major/; 2. Taher AT, et al. *N Engl J Med*. 2021;384(8):727-743; 3. Galanello R and Origa R. *Orphanet J Rare Dis*. 2010;5:11; 4. Origa R. *Genet Med.* 2017;19(6):609-619.
Figures created with BioRender.com

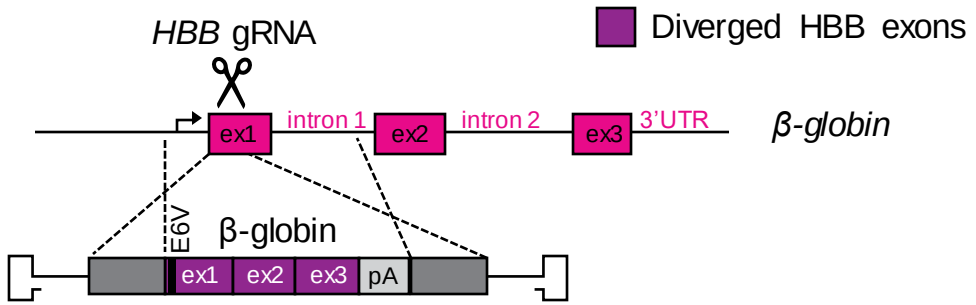
How do β-globin expression levels from a replaced β-globin coding sequence compare to physiological β-globin levels?



[a] To distinguish β-globin produced from the HDR allele from endogenously produced β-globin, a sickle point mutation was introduced in the DNA donor.

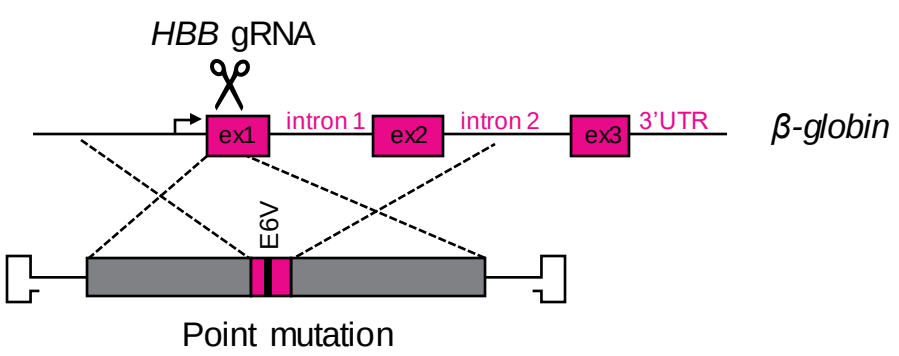
The β-globin gene requires introns for physiological protein expression

Replaced β-globin gene



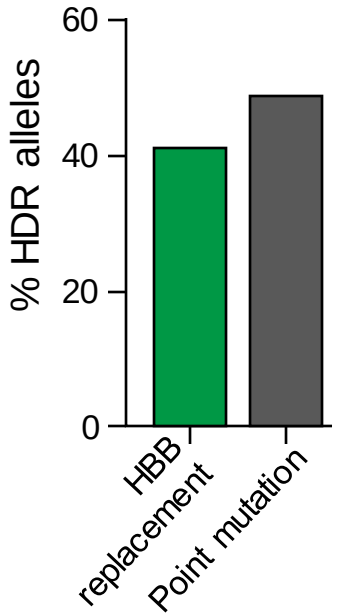
vs.

Physiological β-globin expression

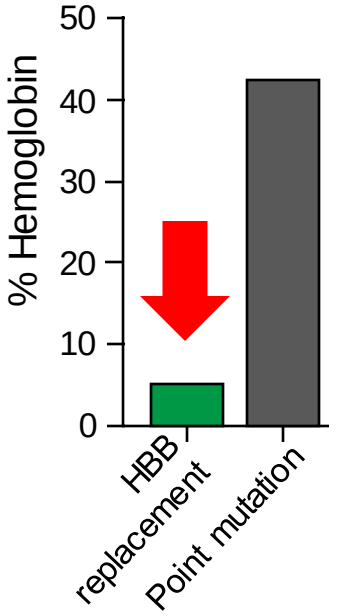


- Replacement with a diverged β-globin coding sequence reduces hemoglobin expression
- Introns are necessary for physiological expression of β-globin
- Requirement for non-homologous DNA donor prevents use of β-globin introns
- **We sought to develop a non-homologous AAV6 DNA donor that results in physiological β-globin expression levels**

Homology directed repair

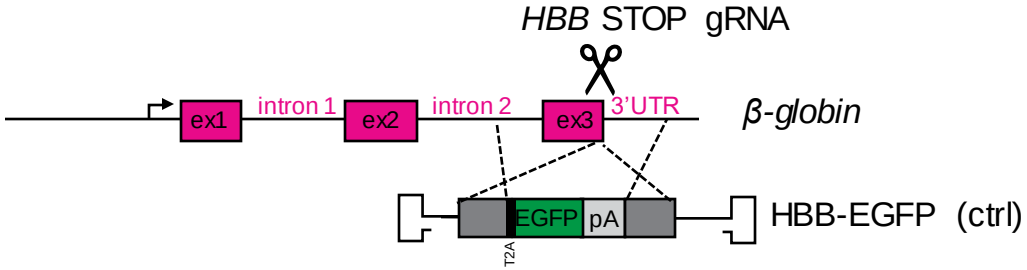


Hemoglobin

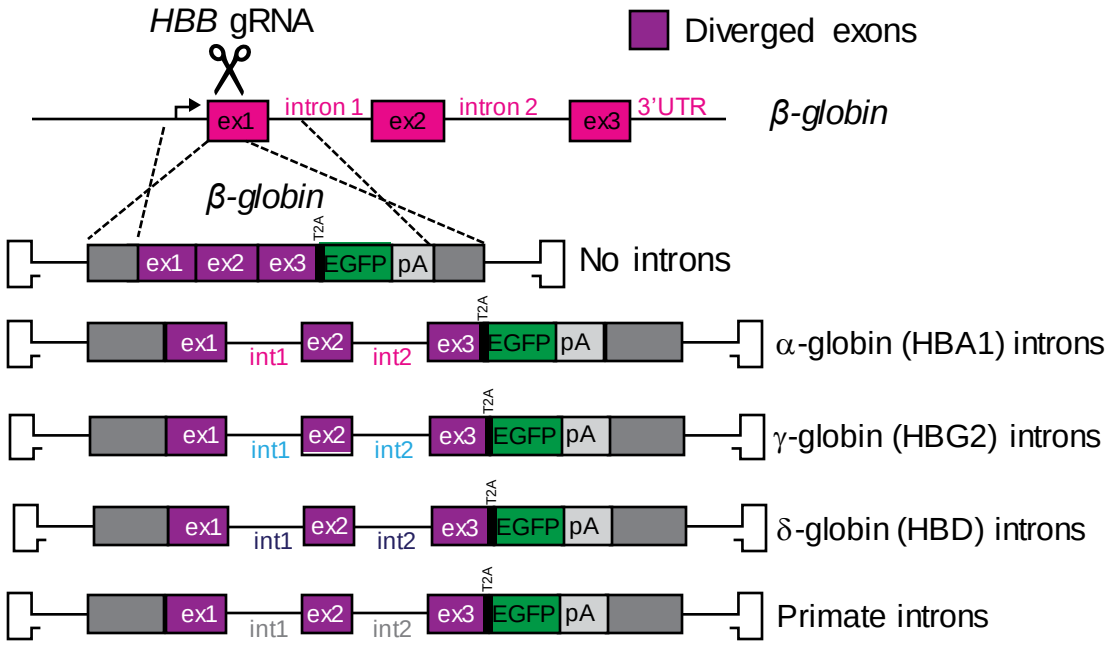


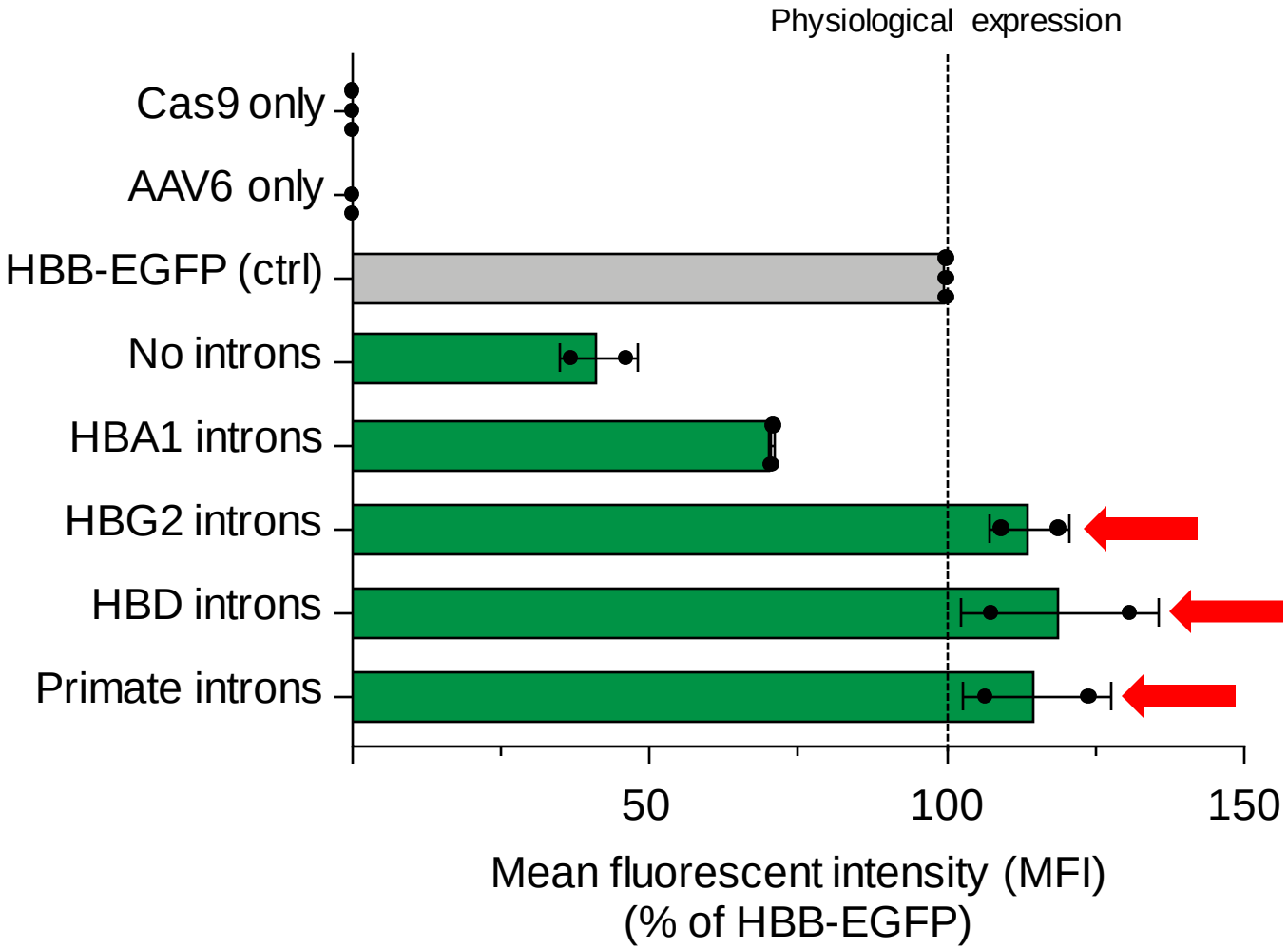
A T2A-EGFP reporter system allows screening for high-expressing DNA donors

Physiological β-globin control:



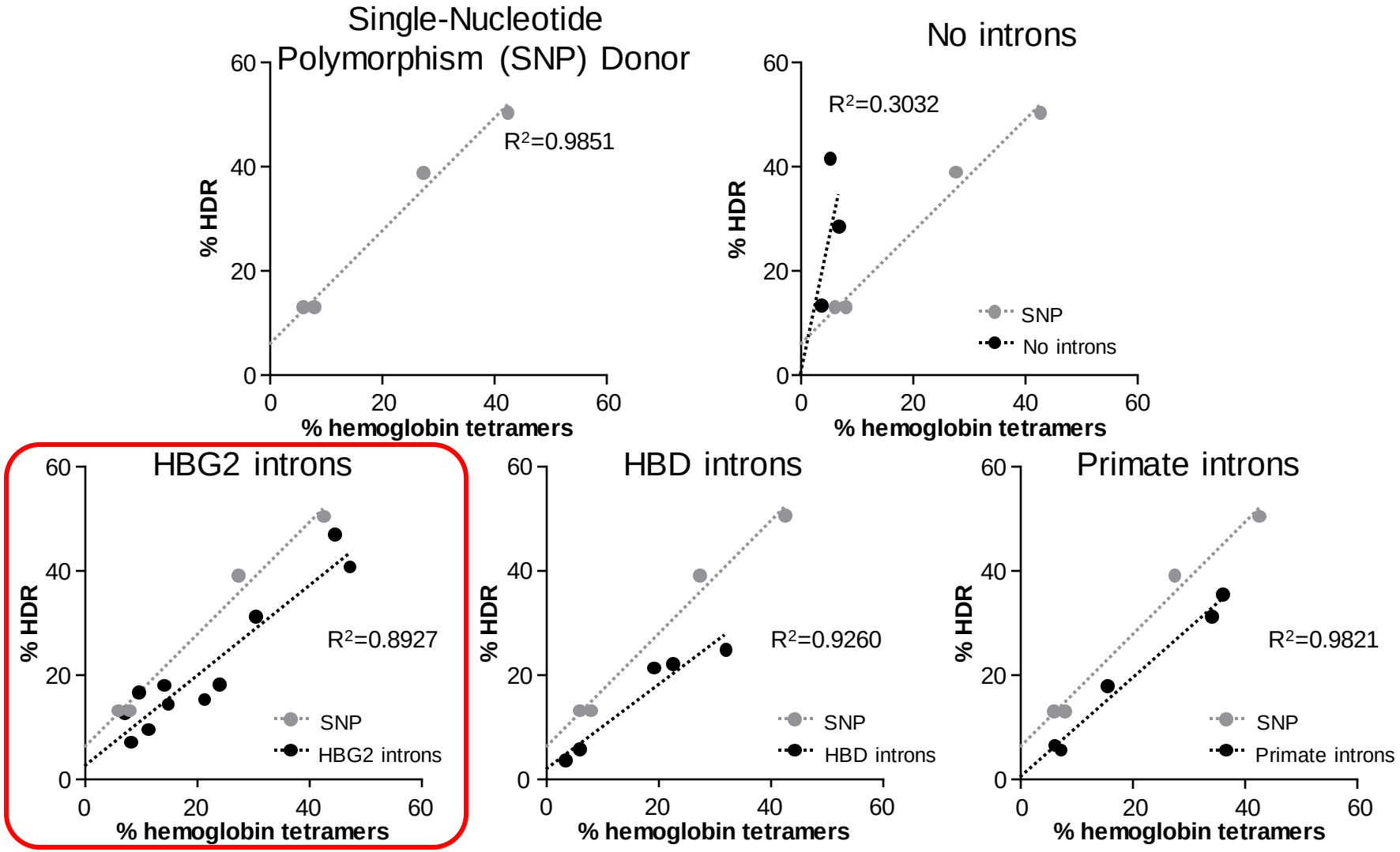
β-globin gene replacement:





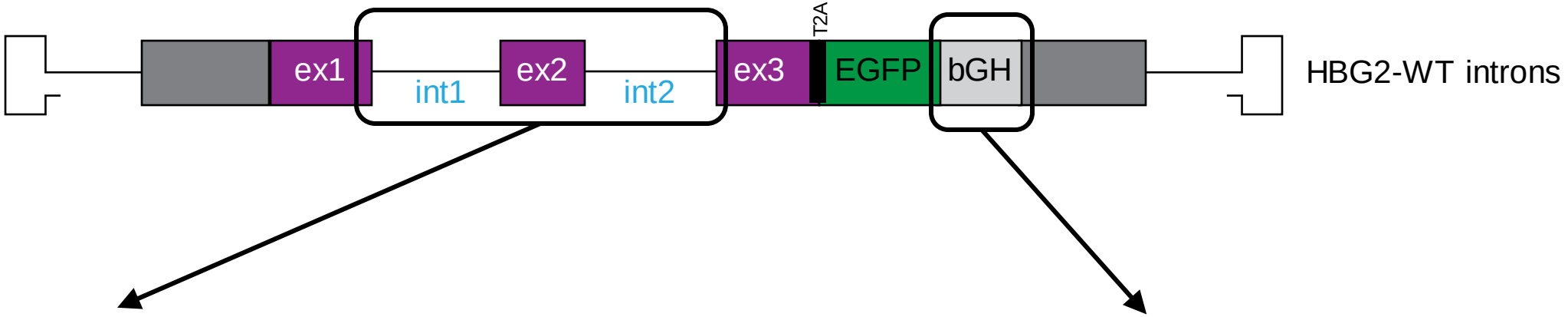
Incorporating heterologous introns restores β-globin expression to physiological levels

Heterologous intron donors produce hemoglobin tetramers



HBB coding sequence with HBG2 introns results in up to 40% HDR and physiological hemoglobin protein levels

Can the HBG2 β-globin DNA donor be further optimized?



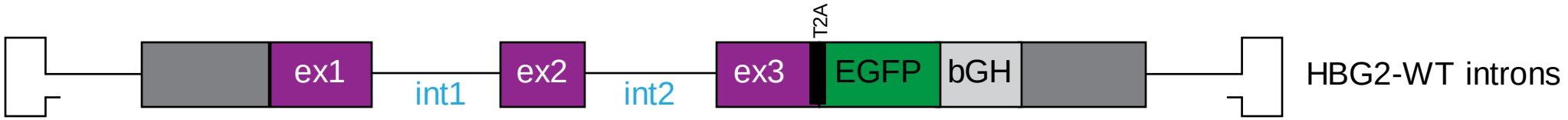
Intron length:

- Shorter intron sequences, and thereby a shorter gene cassette, may result in higher rates of HDR
- Shorter intron sequences reduce the potential for homologous recombination with γ-globin gene

Polyadenylation (pA) sequences:

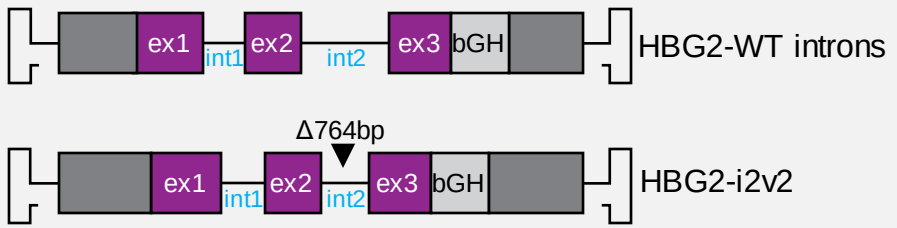
- We assessed if different pA sequences could increase protein expression
- Screened a total of 11 different pA tails
- The original bovine growth hormone (bGH) pA performed best

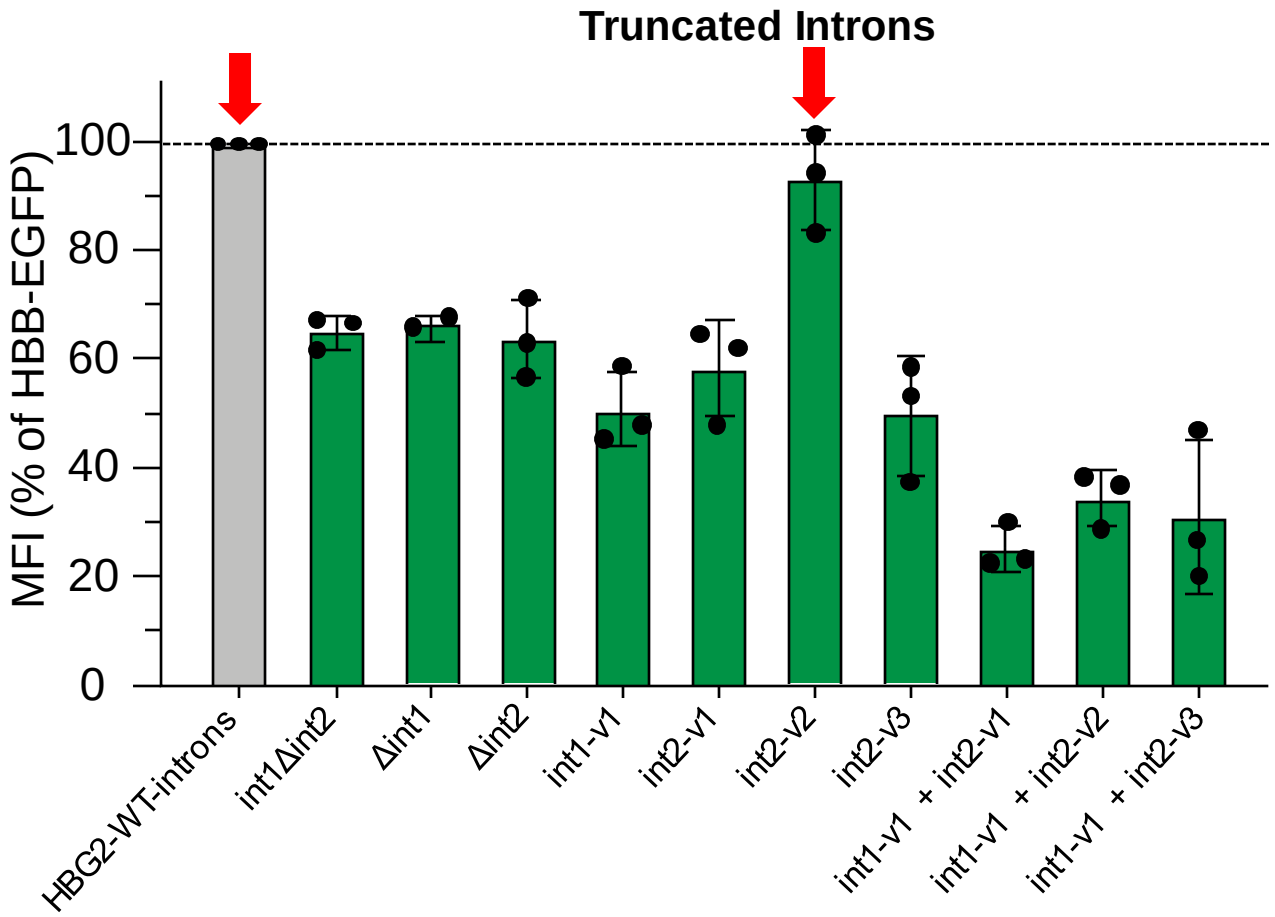
Optimization of the HBG2 β-globin DNA donor identified an additional β-globin DNA donor with truncated introns



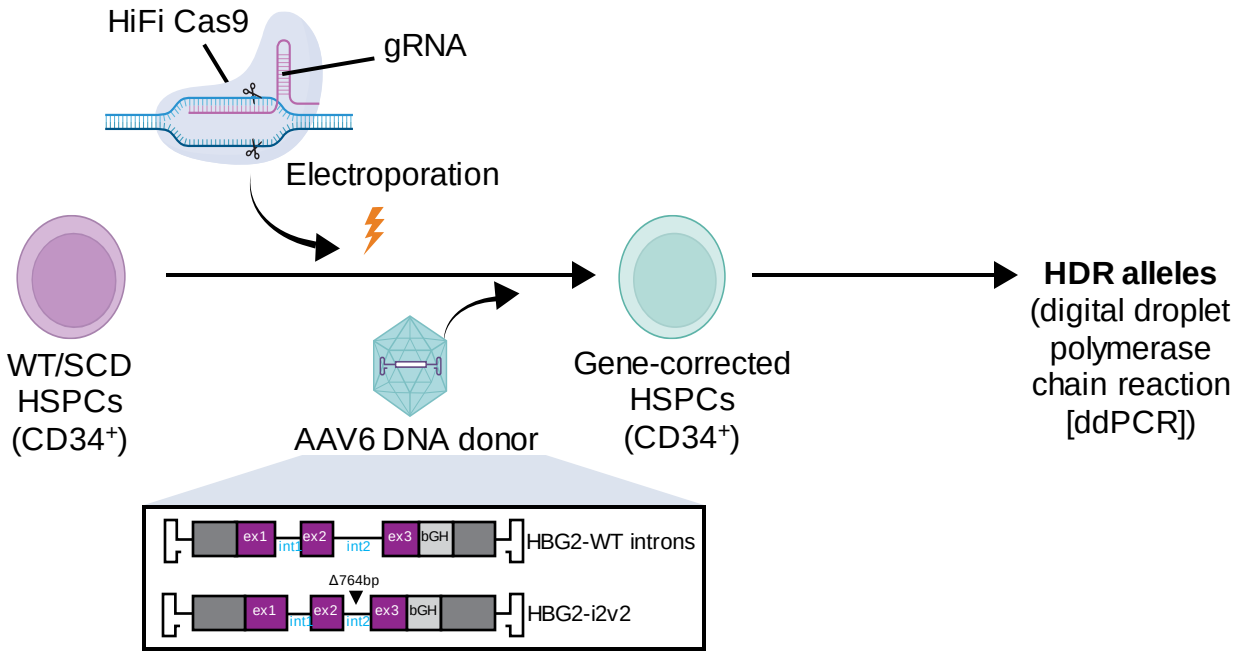
- Most intron deletions resulted in a relative loss of expression
- We identified one truncated intron 2 DNA donor that resulted in sustained HBB-EGFP expression

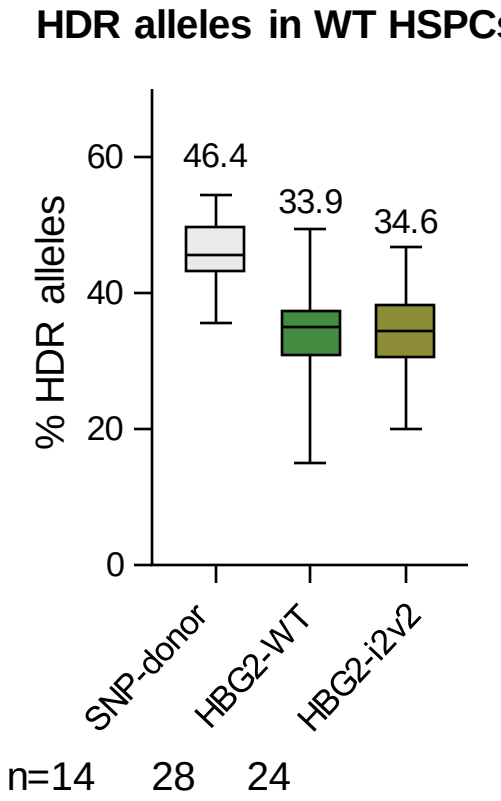
Two high-expressing DNA donors

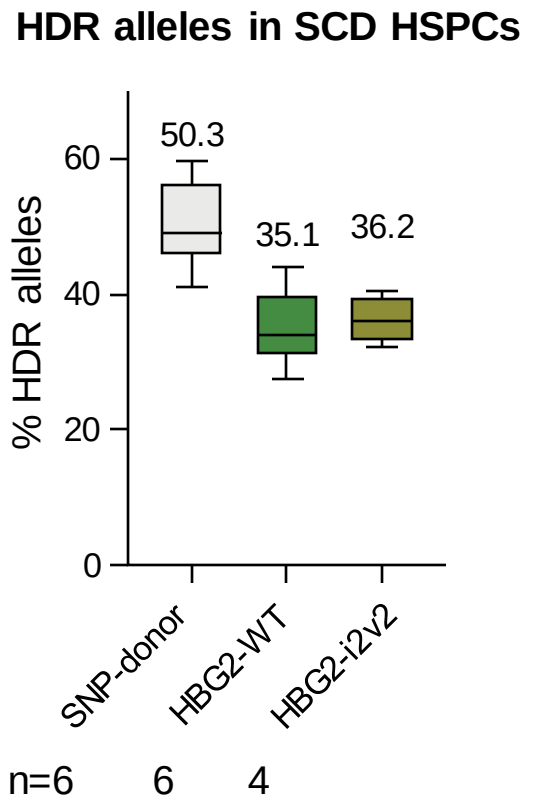




β-globin gene replacement is effective in both healthy and SCD patient HSPCs



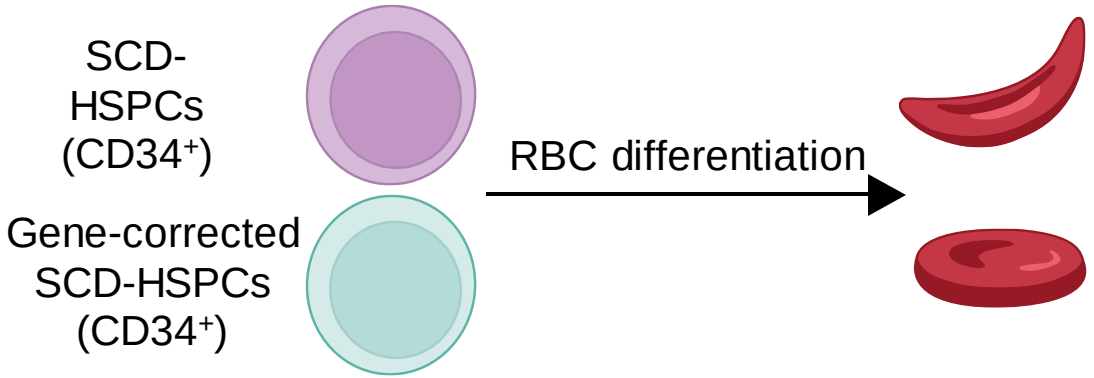




HSPCs from sickle-cell patients serve as a surrogate for β-thalassemia patient HSPCs

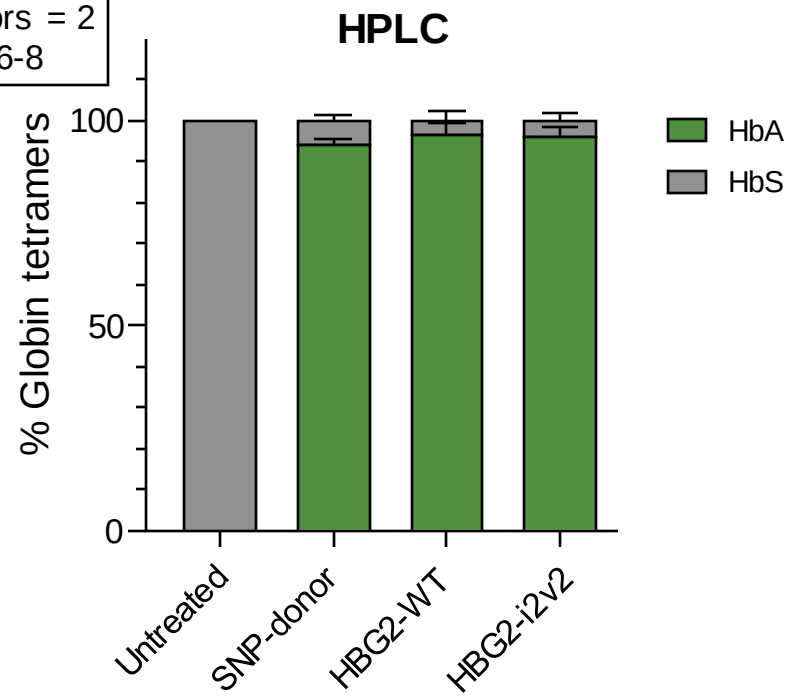
Similar frequencies of HDR are achieved in HSPCs derived from healthy volunteers and from patients with sickle cell disease

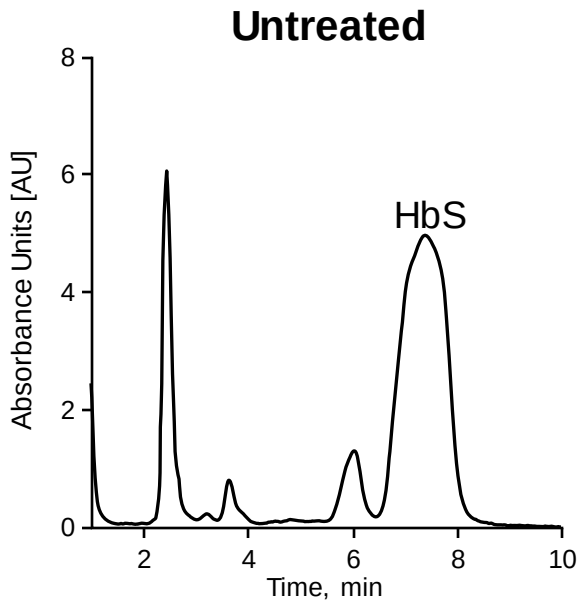
β-globin gene replacement restores adult hemoglobin (HbA) expression in patient SCD-HSPCs

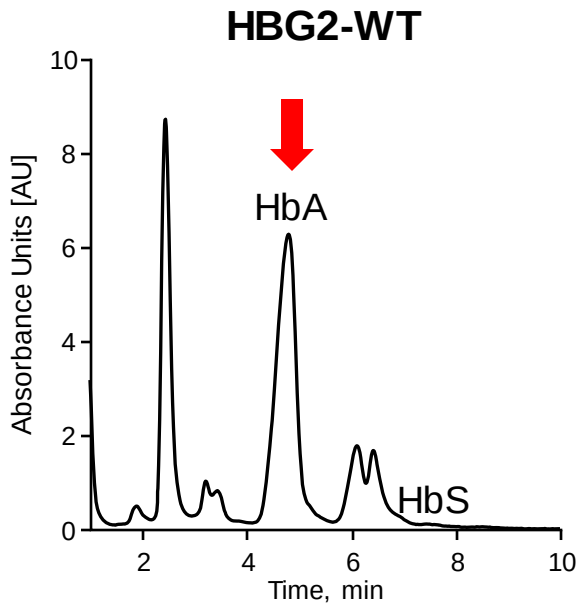


HbA/Sickle hemoglobin (HbS) levels (HPLC)

SCD-HSPC donors = 2
Replicates = 6-8



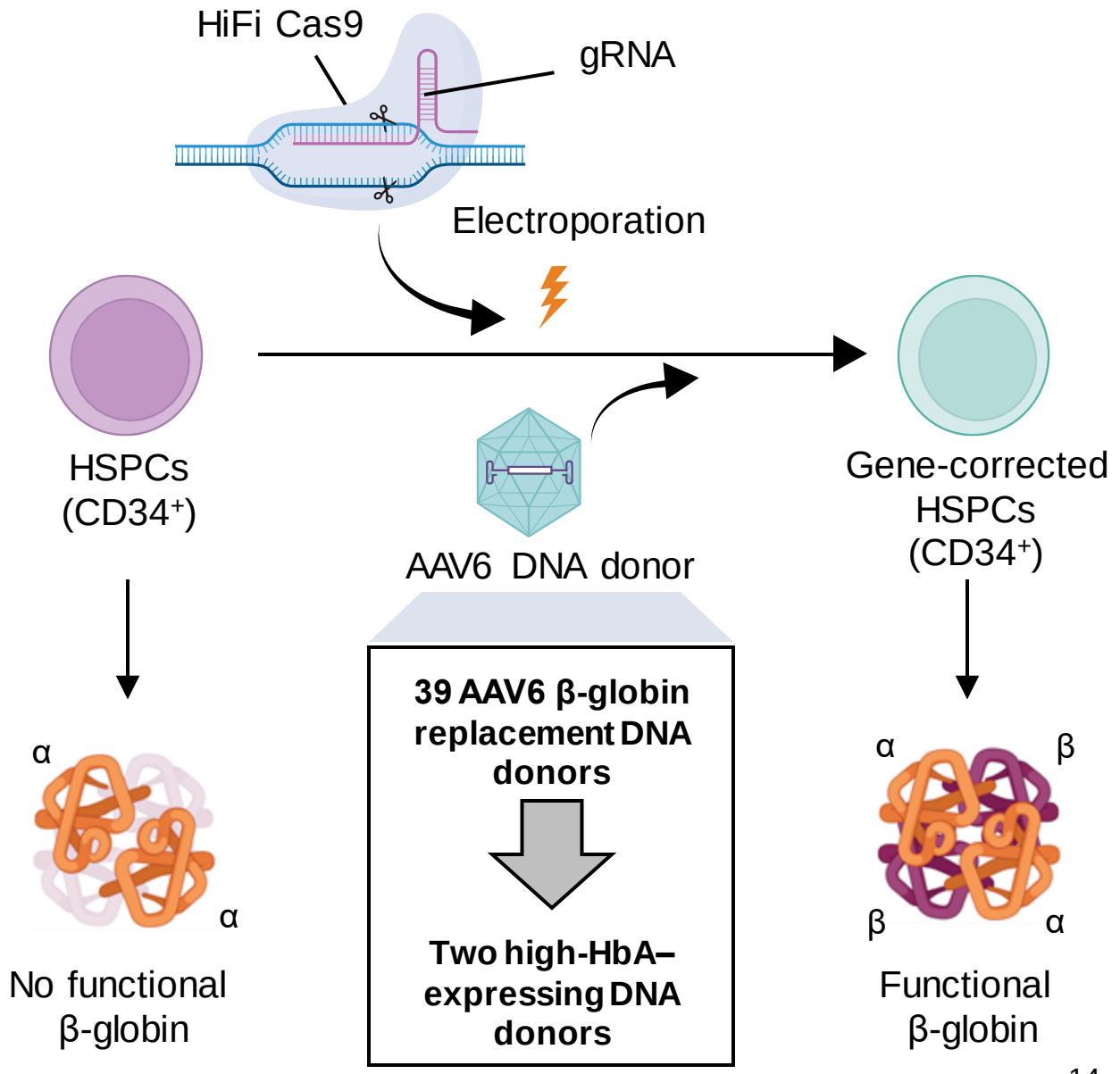




HBB gene replacement using the optimized DNA donors restored HbA expression to a level comparable to a SCD point mutation-correction strategy

Conclusions

- β-thalassemia is a genetic disorder with high unmet need
- Using our UltraHDR™ platform, we developed a precise β-globin gene replacement strategy that restores HbA expression, offering a differentiated approach for treating β-thalassemia
- Additional preclinical studies are planned to further study this approach and advance the GPH102 program



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List of Abbreviations

AAV6, adeno-associated virus serotype 6
AU, absorbance unit
bGH, bovine growth hormone
bp, base pair
Cas9, CRISPR-associated protein 9
CD34, cluster of differentiation 34
CRISPR, clustered regularly interspaced short palindromic repeats
ddPCR, digital droplet polymerase chain reaction
EGFP, enhanced green fluorescent protein
ex, exon
gRNA, guide RNA
HA, homology arms
HbA, adult hemoglobin
HBA1, hemoglobin subunit alpha 1
HBB, β-globin
HBD, δ-globin
HBG2, hemoglobin subunit gamma 2
HbS, sickle hemoglobin
HDR, homology directed repair
HiFi, high-fidelity
HPLC, high-performance liquid chromatography
HSPC, hematopoietic stem and progenitor cell
int, intron
MFI, mean fluorescent intensity
pA, polyadenylation
RBC, red blood cell
SCD, sickle cell disease
SNP, single-nucleotide polymorphism
UTR, untranslated region
WT, wild type