# Therapeutic Platform for BEST1-Associated Blindness: Anticodon-Engineered tRNA and Gene Augmentation

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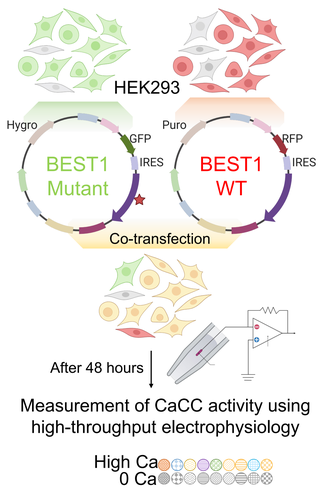
## Introduction

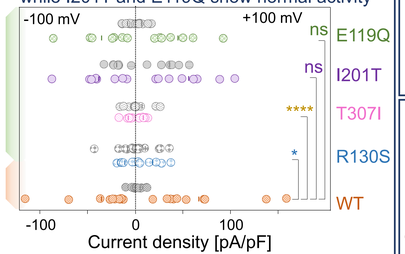
- The ***BESTROPHIN1*** **(*BEST1*)** gene encodes a calcium-activated chloride channel (CaCC), essential for retinal pigment epithelium (RPE) function and photoreceptor health. Mutations (>300 identified) cause dominant and recessive bestrophinopathies (Best disease), leading to progressive central vision loss, with no approved treatments.
- Because BEST1 is small, adeno-associated virus (AAV)-based gene augmentation is feasible for recessive disease. For dominant mutations, prior work showed ~3× increased *BEST1* expression using its native promoter can restore function in some cases. To broaden this approach, ~25 common dominant mutations were prioritized for the development of OPGx-BEST1, an AAV therapy that delivers full-length *BEST1*. The strategy aims to optimize the wild-type-to-mutant subunit ratio, reducing mutant subunit incorporation into channel pentamers and restoring function.
- At the same time, a gene- and position-independent strategy using anticodon-engineered tRNAs (ACE-tRNAs) is being developed to target nonsense mutations, which account for ~24% of inherited variants. ACE-tRNAs promote readthrough of premature stop codons, restoring full-length protein expression and function across experimental models.

## *BEST1* gene augmentation (dominant variants)

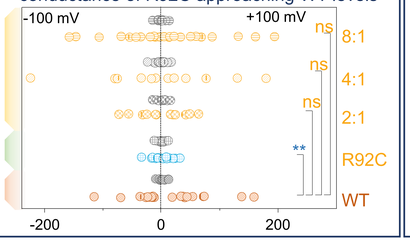
**A** Co-transfection strategy varying WT : mutant ratios (2:1-8:1)




**B** R130S, and T307I show reduced CaCC activity, while I201T and E119Q show normal activity



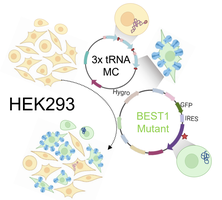

**C** Increasing WT dose (4:1 and 8:1) restores CaCC conductance of R92C approaching WT levels



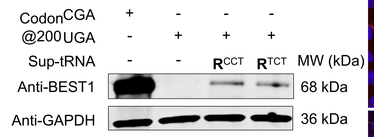

## Methods and Results

### Restoration of BEST1 function using 3x ACE-t$^{UGA.R}$ minicircle (MC) in HEK293 cells

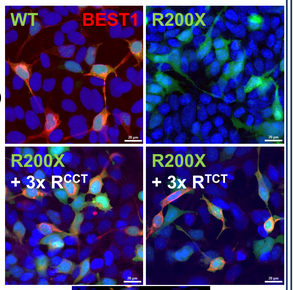
**A** Overall strategy



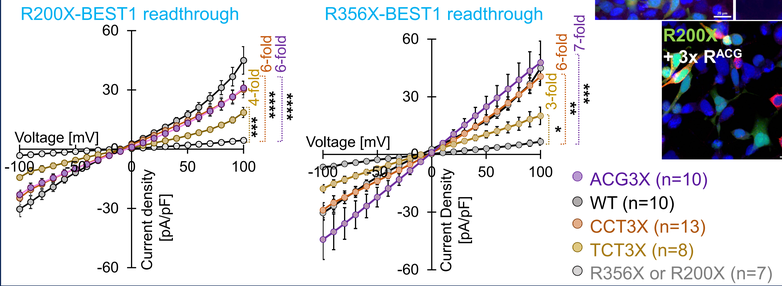

**B** ACE-t$^{UGA.R}$ MC treatment restores BEST1 full-length protein expression




**C** Immunofluorescence confirms recovered membraned localization

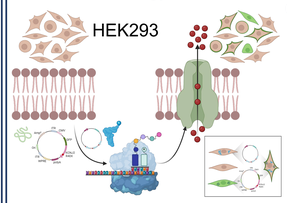



**D** ACE-t$^{UGA.R}$ MC treatment restores CaCC activity in R200X and R356X HEK293 cells

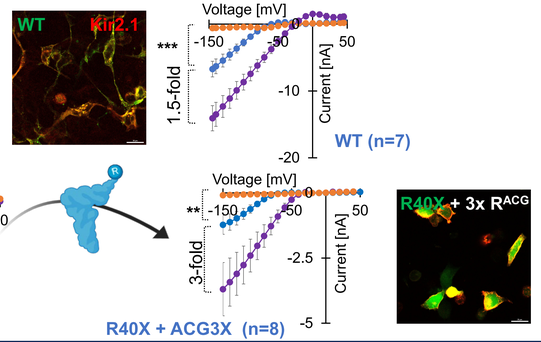



### Gene agnostic restoration of Kir2.1 function using 3x ACE-t$^{UGA.R}$ MC in HEK293 cells

**A** Overall strategy

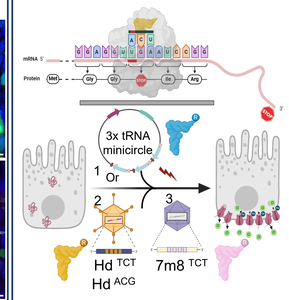



**B** Immunofluorescence confirms recovered membraned localization and Current-voltage (I-V) plots demonstrate inward rectifying Kir2.1 current

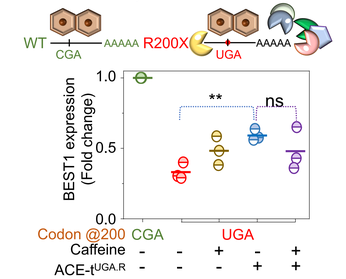



### Restoration of CaCC channel activity in R200X-BEST1 hiPSC RPE using ACE-t$^{UGA.R}$

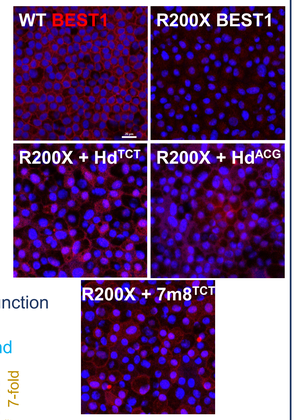
**A** Overall strategy



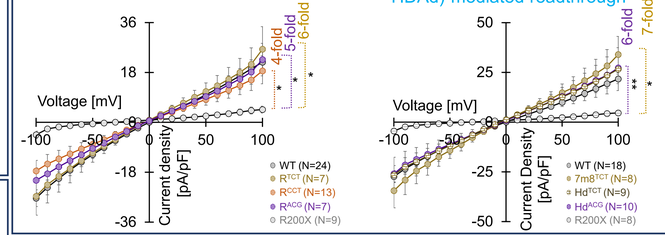

**B** ACE-t$^{UGA.R}$ treatment rescues nonsense-mediated decay in R200X-hiPSC RPE




**C** Immunofluorescence confirms recovered membraned localization




**D** Both non-viral and viral delivery of ACE-t$^{UGA.R}$ show restoration of CaCC function comparable to WT




*$p$ < 0.05, **$p$ < 0.01, ***$p$ < 0.001, ****$p$ < 0.0001, ns; non-significant.

## Conclusions

- *BEST1* augmentation and gene-agnostic ACE-tRNA therapies can restore BEST1 channel function, depending on mutation class.
- These findings outline initial dosing for *BEST1* augmentation and support developing a broad therapeutic approach for prevalent autosomal dominant *BEST1* mutations, advancing the path toward clinical translation.
- In parallel, ACE-tRNA-mediated nonsense suppression offers a precise, personalized strategy by enabling insertion of WT amino acids at premature termination codons.
- Although further development is needed before clinical evaluation, ACE-tRNA offers a scalable, versatile, and clinically relevant alternative to gene-specific therapies for nonsense-mutation-driven diseases.

## Recent publication

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## Acknowledgement

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