

Functional modulation of human BEST1 channel by a proline metabolic enzyme, PYCR3

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ABSTRACT

Bestrophin (BEST) Bestrophin (BEST) channels, including human BEST1 (hBEST1), are calcium-activated chloride (Cl⁻) channels that respond to intracellular [Ca²⁺] elevations. Recently, we identified human PYCR3, an enzyme in proline metabolism, as a novel binding partner of hBEST1 through immunoprecipitation-mass spectrometry (IP-MS). The interaction between hBEST1 and hPYCR3 was confirmed via co-immunoprecipitation (Co-IP) and size-exclusion chromatography (SEC), where the two proteins co-migrated, indicating a direct interaction.

To investigate effect of hPYCR3 on hBEST1 channels, we performed whole-cell patch clamp recordings. We found that hPYCR3 overexpression increased hBEST1 current density in HEK293T cells, whereas PYCR3 knockdown significantly reduced it. Furthermore, co-expression of hBEST1 and hPYCR3 in hPYCR3 knockout HEK293T cells augmented hBEST1 current levels, underscoring hPYCR3's critical role in modulating hBEST1 activity. Functional analysis of the enzymatically inactive hPYCR3 G252V mutant revealed no enhancement of hBEST1 current density. This result highlights that hPYCR3 enzymatic activity is essential for hBEST1 modulation. Additionally, treatments with proline and cofactors further supported a mechanistic link between hPYCR3-mediated proline metabolism and hBEST1 activity. These findings identify hPYCR3 as a key enzymatic regulator of hBEST1. We aim to investigate whether the effects of hBEST1 and hPYCR3 observed in vitro are recapitulated in vivo using retinal pigment epithelial (RPE) cells, where hBEST1 is highly expressed, to explore their physiological relevance. Additionally, we aim to resolve the complex structure of hBEST1 and hPYCR3 to elucidate the mechanisms underlying their structural modulation. Ongoing studies aim to elucidate the structural and functional mechanisms underlying hPYCR3-dependent modulation of hBEST1, offering new insights into the interplay between proline metabolism and ion channel regulation.

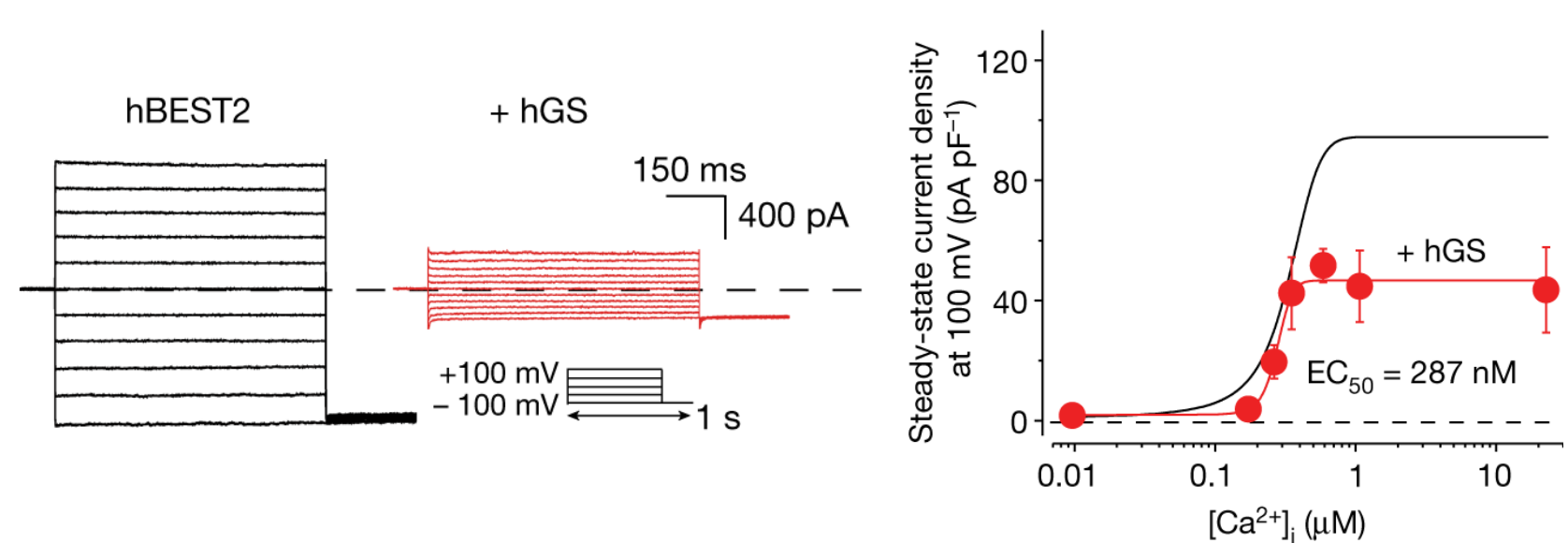
* **Keywords:** BEST1, PYCR3, whole-cell patch-clamp, Co-Immunoprecipitation

INTRODUCTION

Bestrophin (BEST) channel

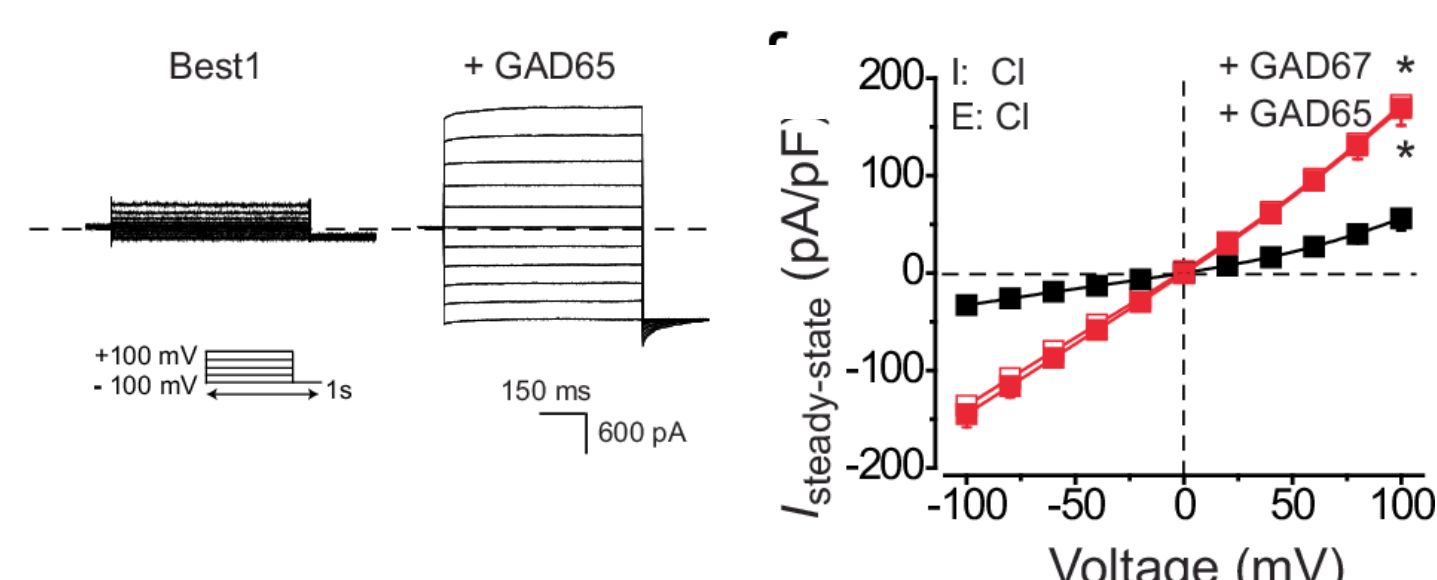
- Bestrophin channels are calcium-activated chloride channels.
- Four paralogs (hBEST1 to hBEST4) are encoded in the human genome.
- Bestrophin channel is encoded by the Vitelliform Macular Dystrophy (VMD) gene.
- BEST1 is primarily expressed in the retinal pigment epithelium (RPE) and is genetically associated with various retinal degenerative disorders (ex. bestrophinopathies).

The influence of GS (glutamine synthetase) on BEST2



(Owji et al. *Nature*, 2022)

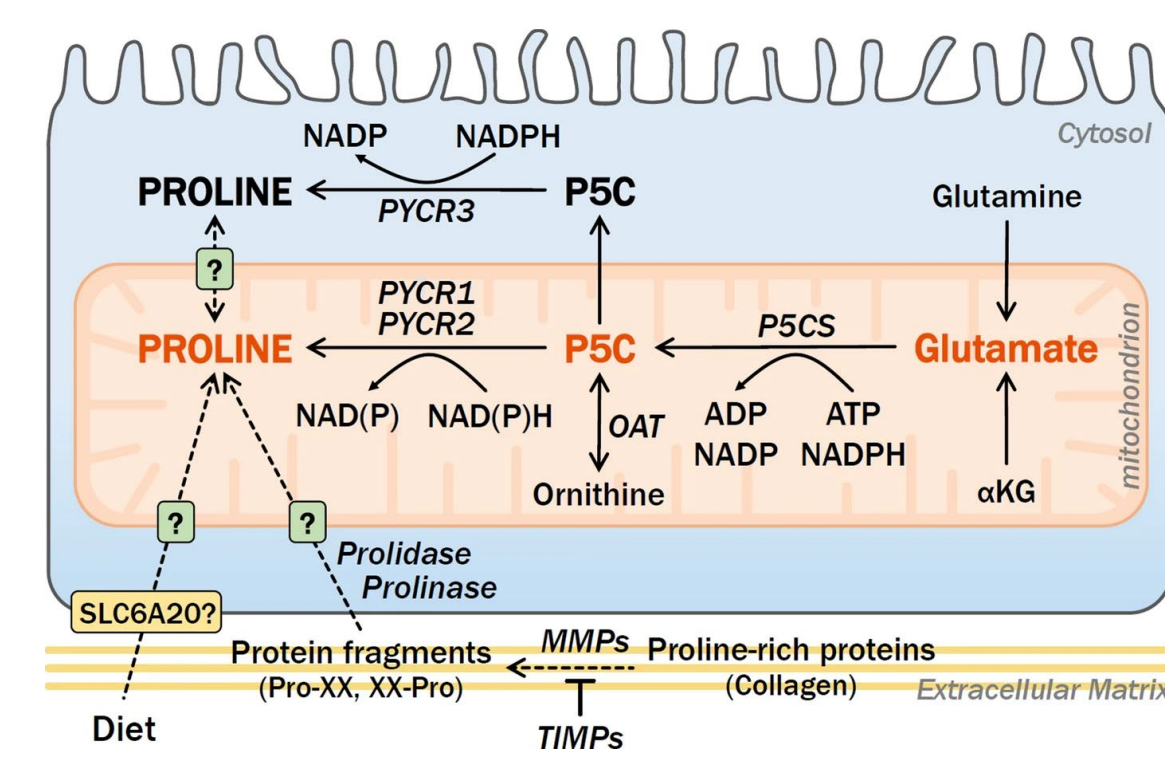
Influence of GADs (glutamic acid decarboxylases) on BEST1



(Wang et al., *Nat. Comm.* 2024)

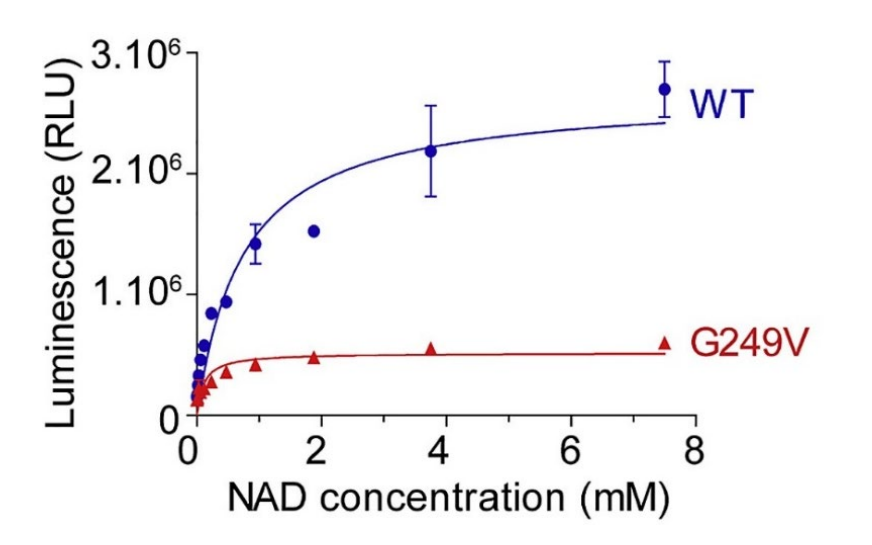
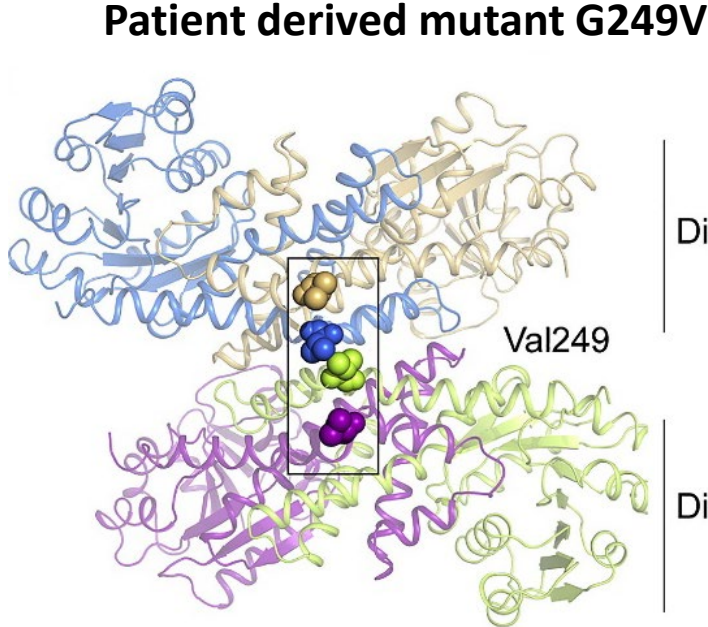
Enzymatic activity of PYCR (Pyrroline-5-carboxylate reductase)

Proline biosynthesis



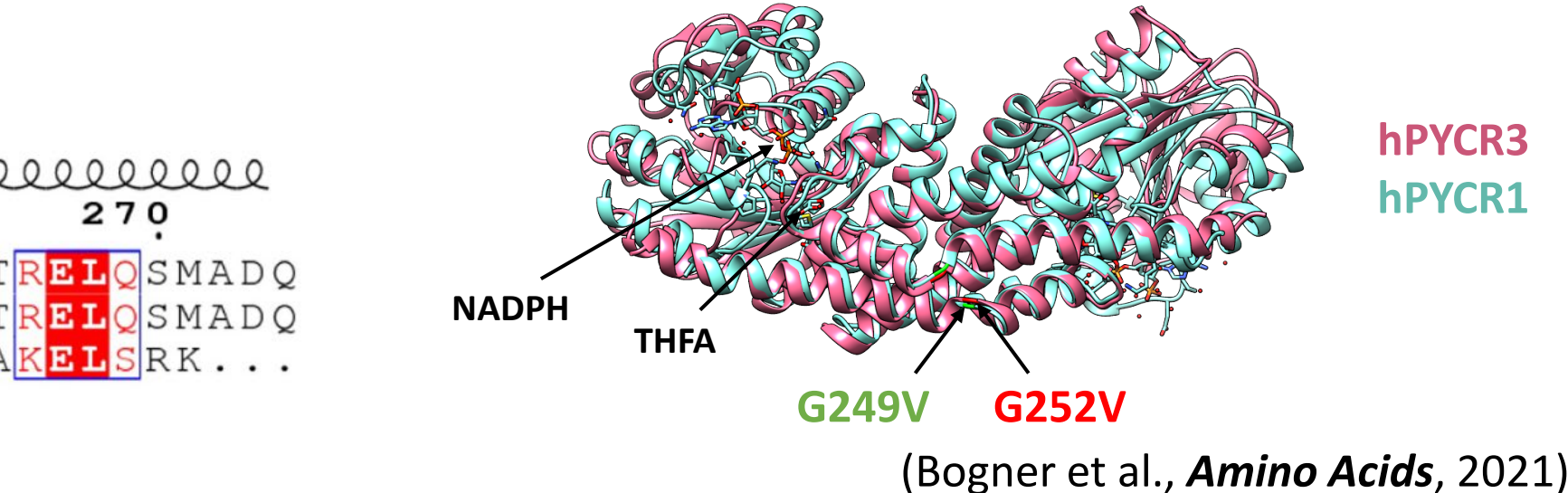
(Du et al., *Amino Acids*, 2021)

Loss-of-function mutation



(Escande-Beillard et al., *Neuron*, 2020)

Active site of PYCR

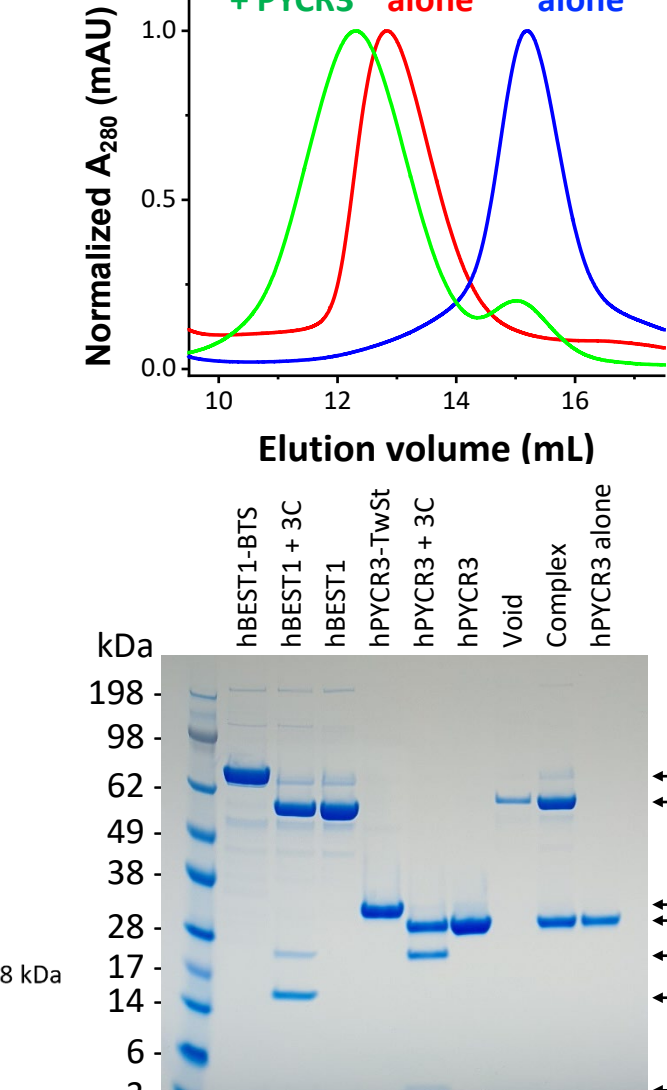
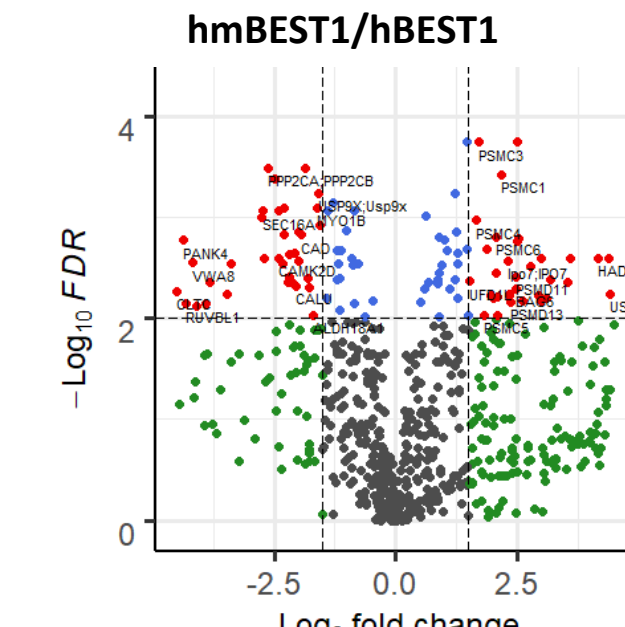
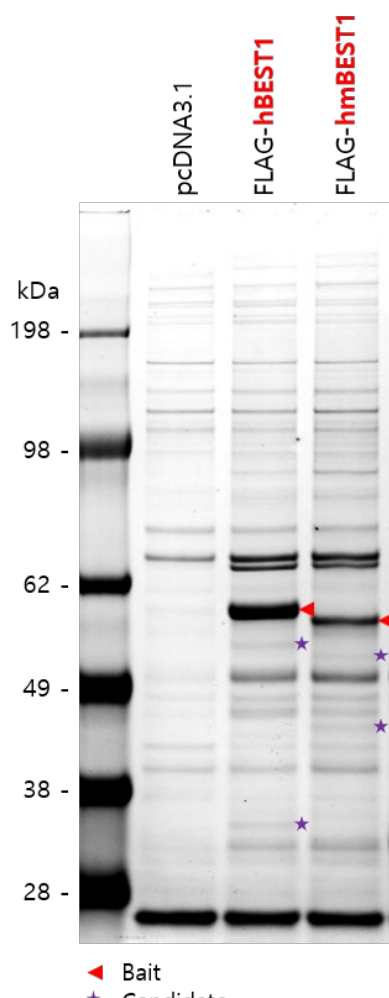
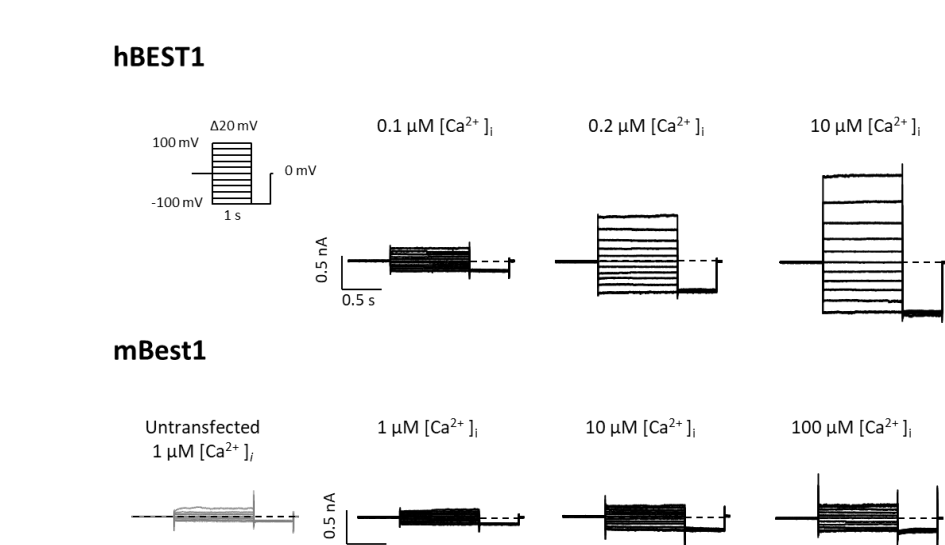


(Bogner et al., *Amino Acids*, 2021)

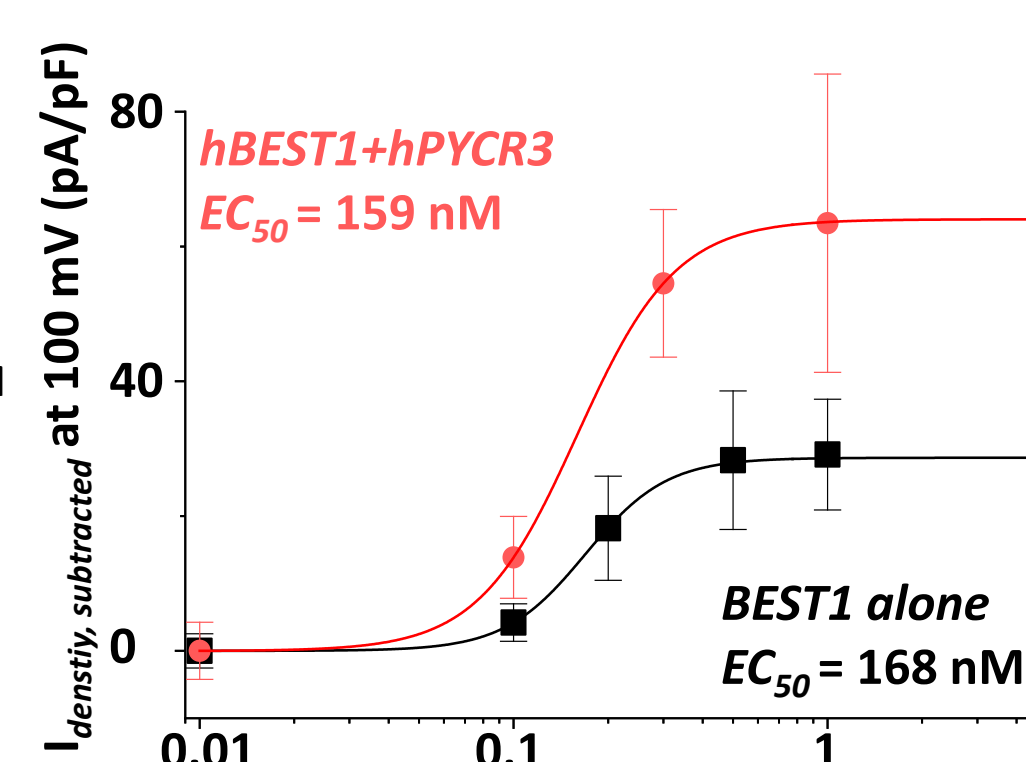
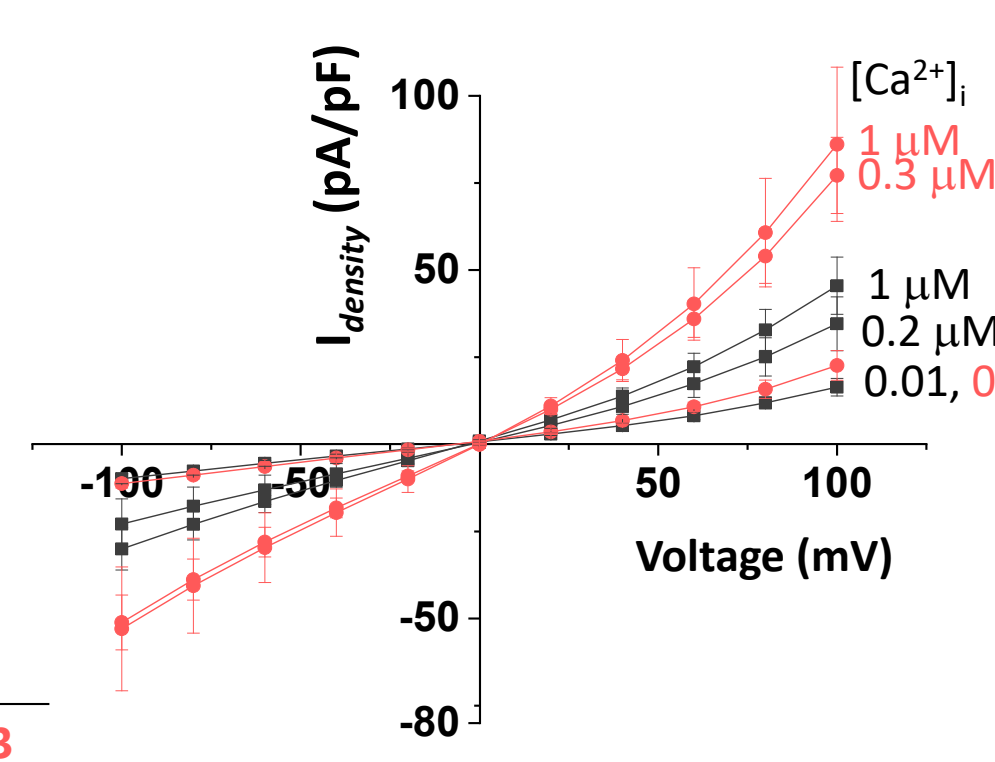
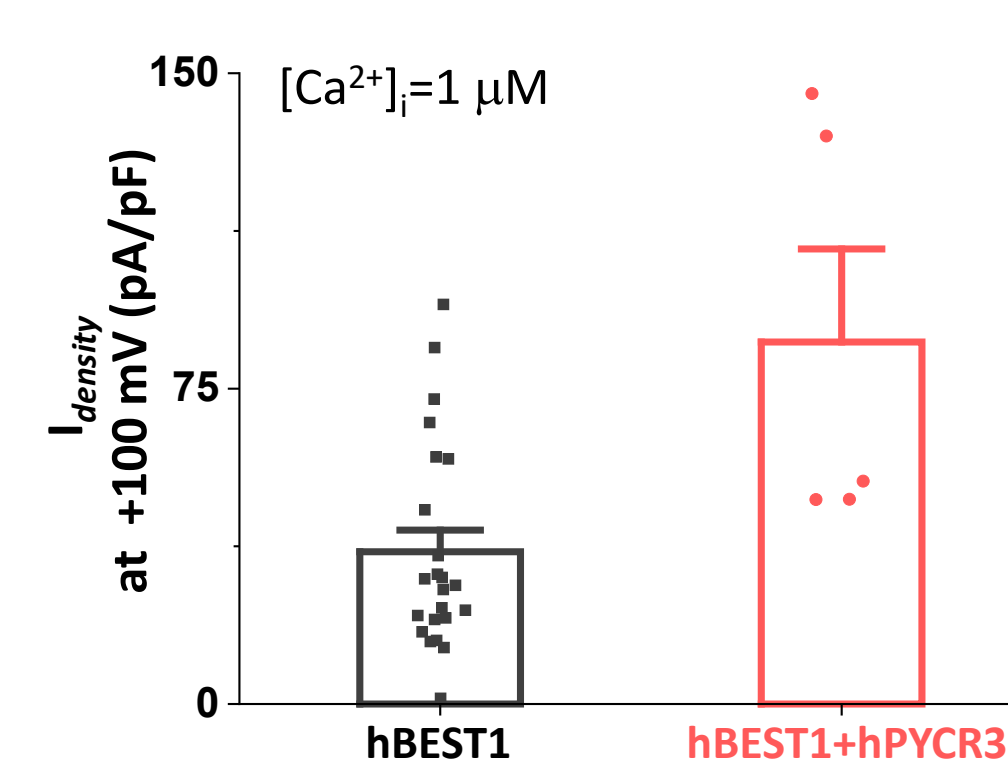
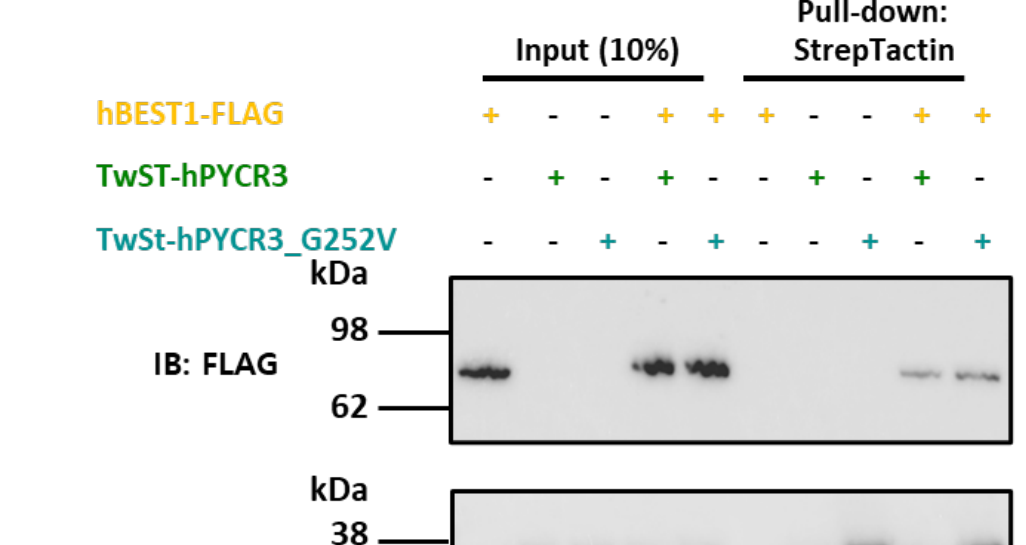
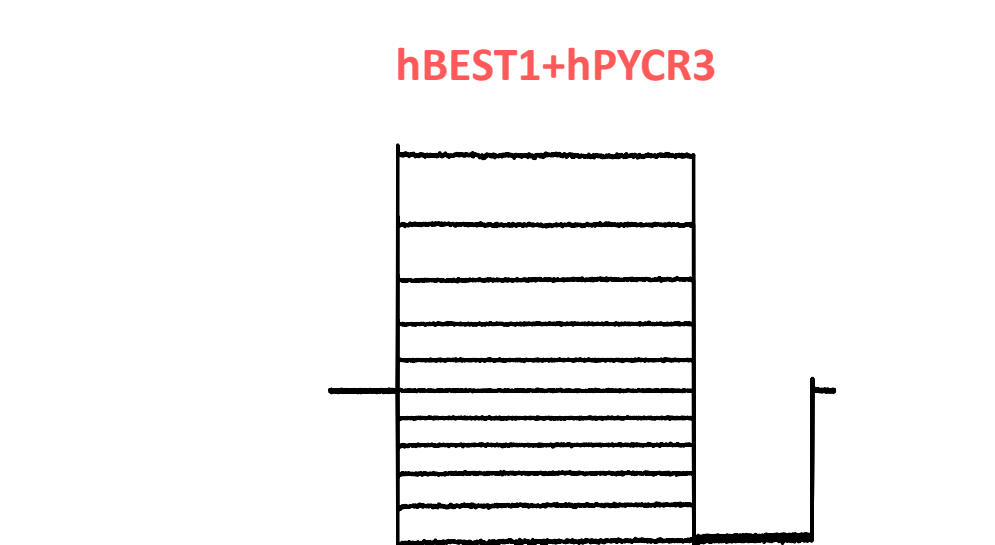
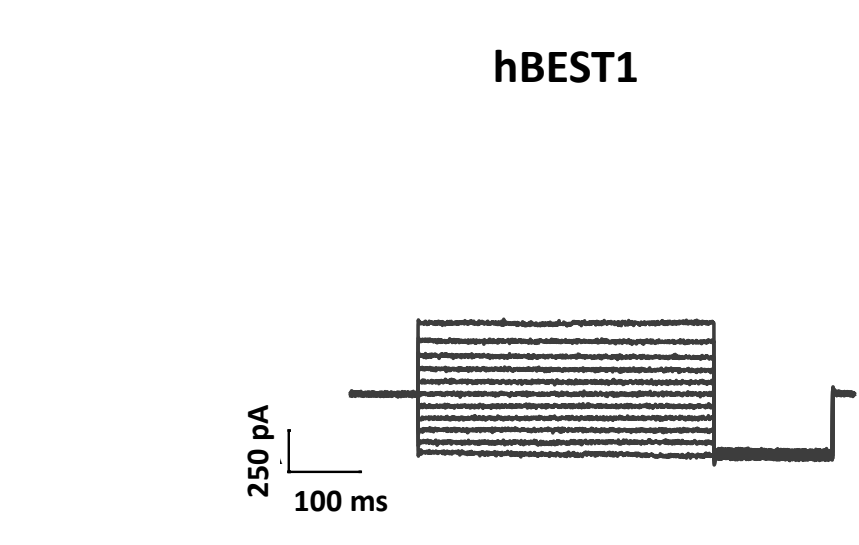
RESULTS

IP-MS fished out hPYCR3 as an interacting protein to hBEST1.

BEST1 orthologs	Sequence identity to hBEST1 (%)		
	Overall (1 - end)	N-domain (1 - 367)	C-domain (368 - end)
rat	68.1	81.2	39.2
mouse	67.8	82.8	36.4
chicken	60.0	74.9	35.2



hPYCR3 Enhances BEST1 channel function in HEK 293T.



Validation of hPYCR3 Knock out cell line.

1. Sequencing

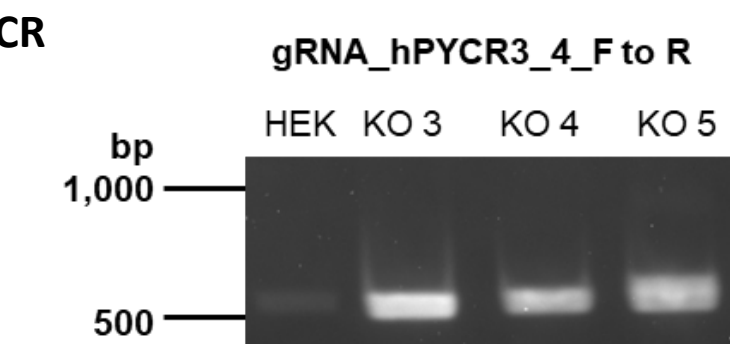
HEK293T WT 5' CCT GAA GCC TAC GTC GAC ATC CAC 3' P E A Y V D I H

HEK hPYCR3 KO 3 (10 bp deletion) CCT GAA - - - - - AC ATC CAC

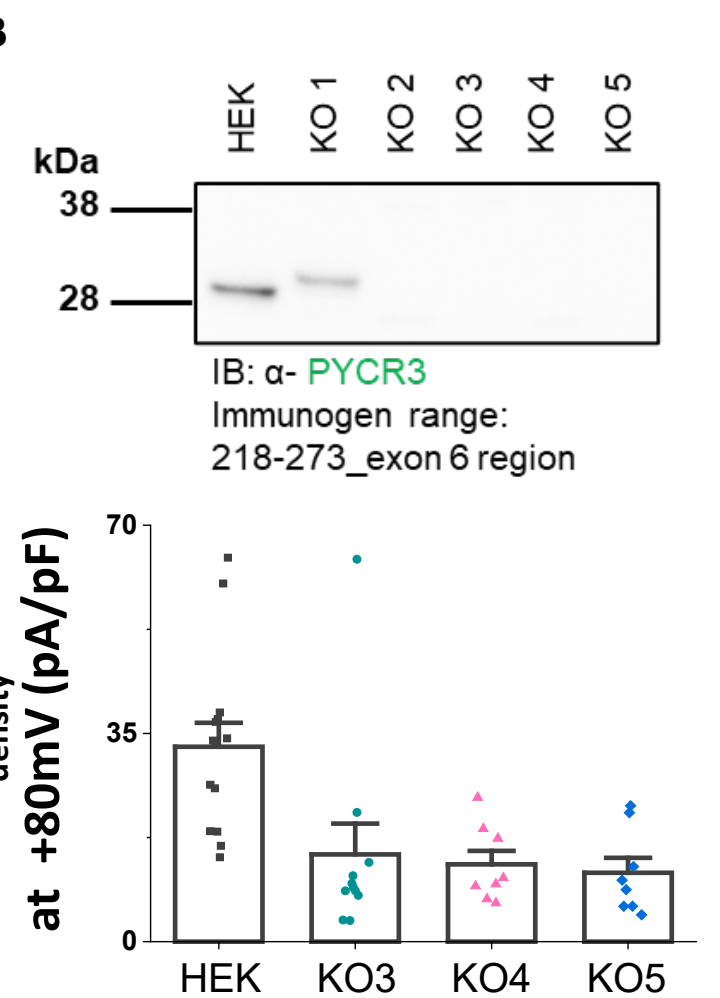
HEK hPYCR3 KO 4 (2 bp deletion) CCT GAA - - C TAC GTC GAC ATC CAC

HEK hPYCR3 KO 5 (1 bp insertion+3 bp change) CCG AAA AGC CTA CGT CGA CAT CCA C

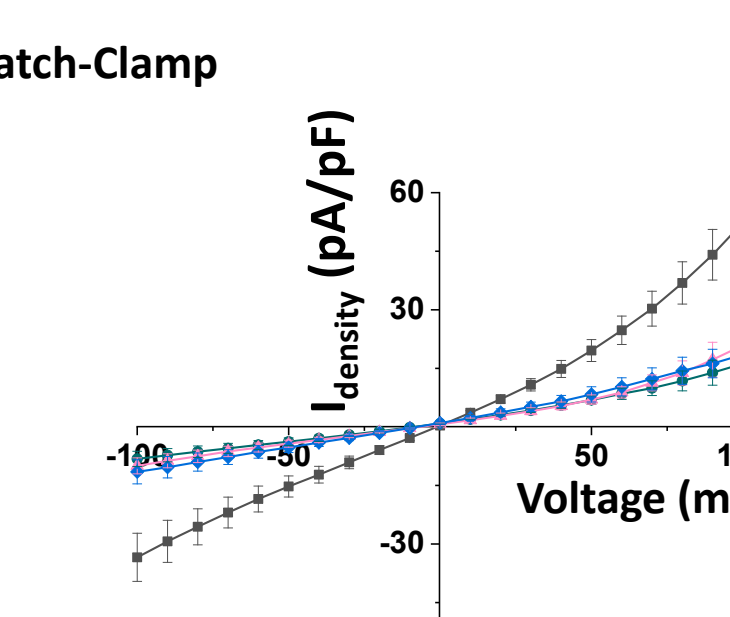
2. PCR



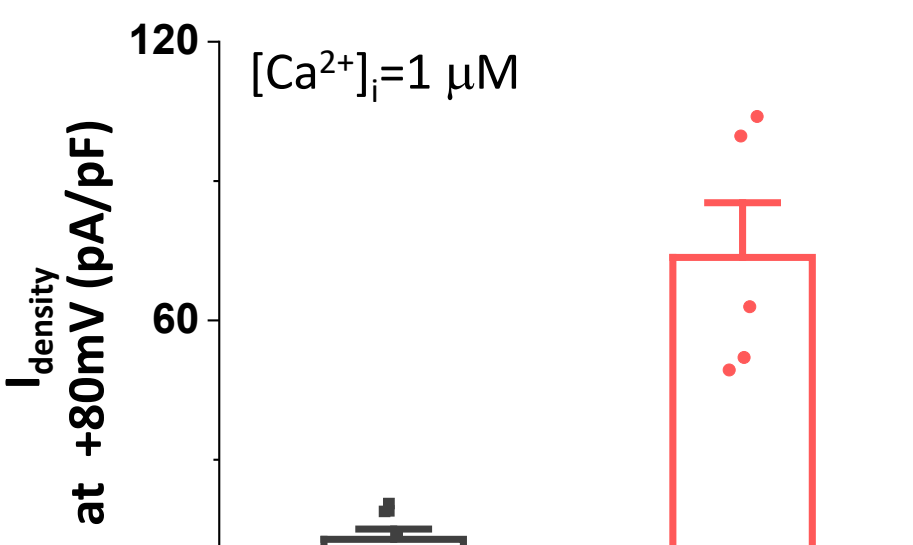
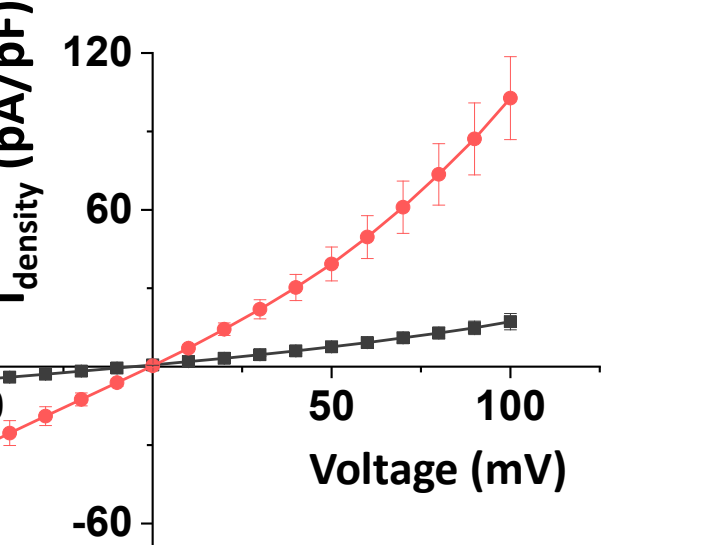
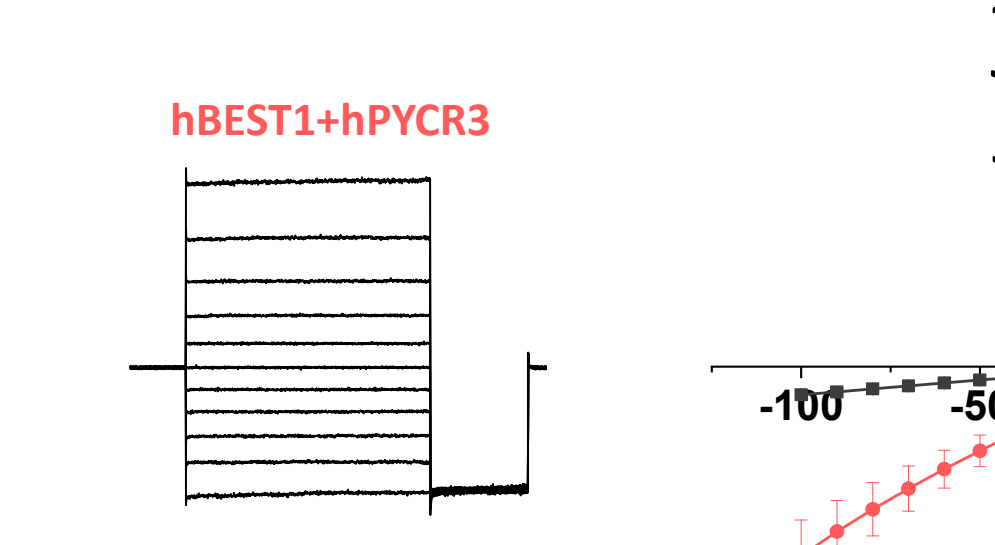
3. WB



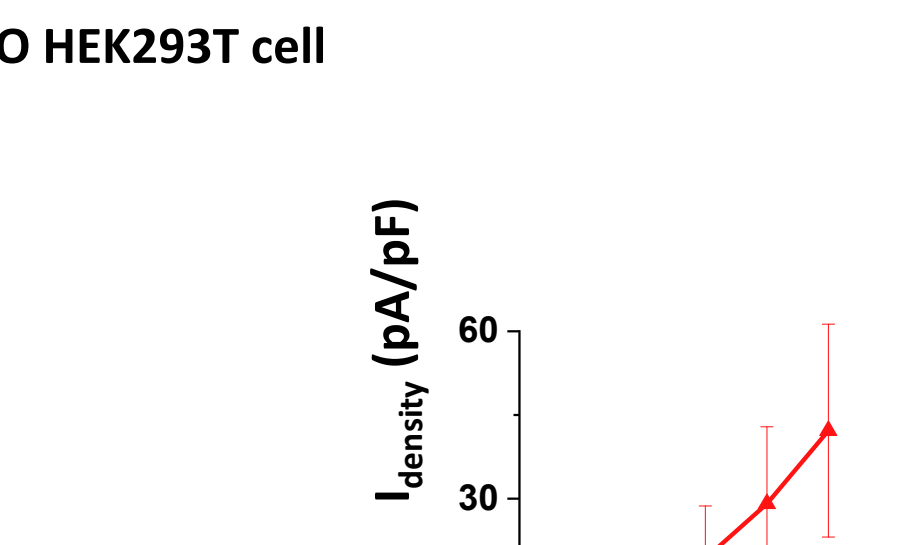
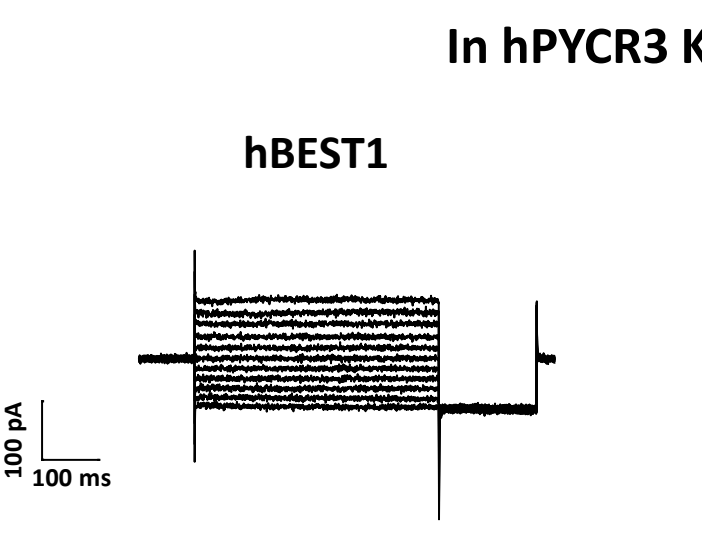
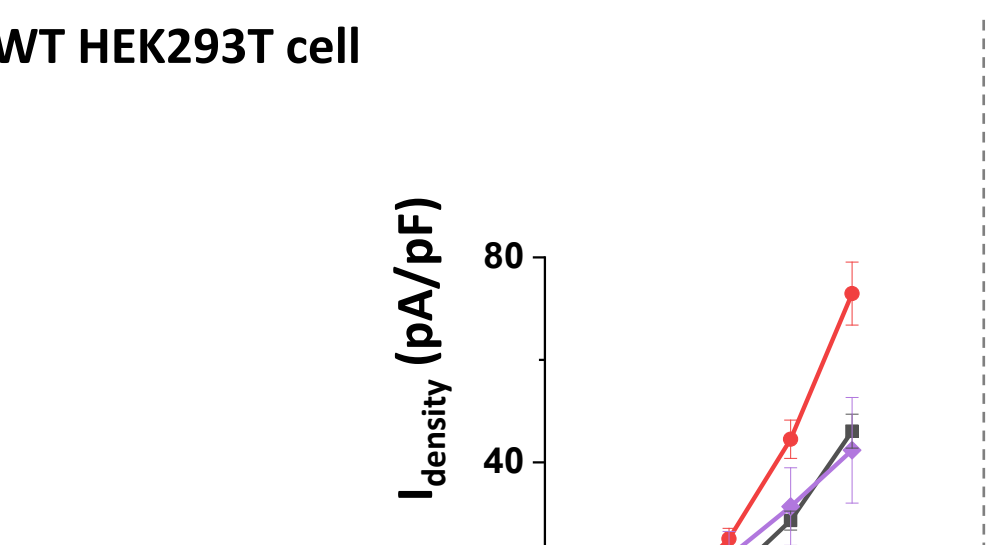
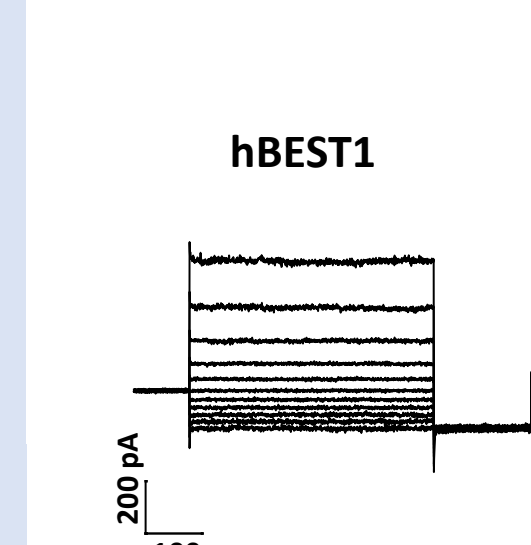
4. Patch-Clamp



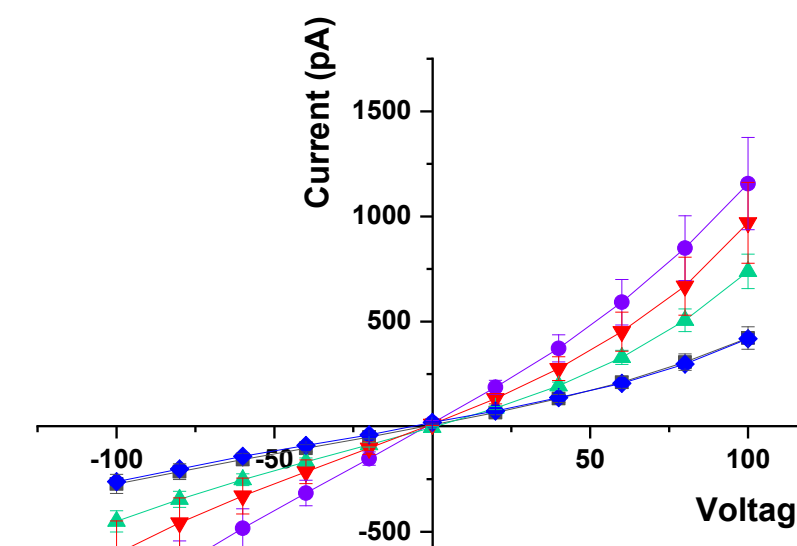
hPYCR3 Enhances BEST1 channel function in hPYCR3 KO cell line.



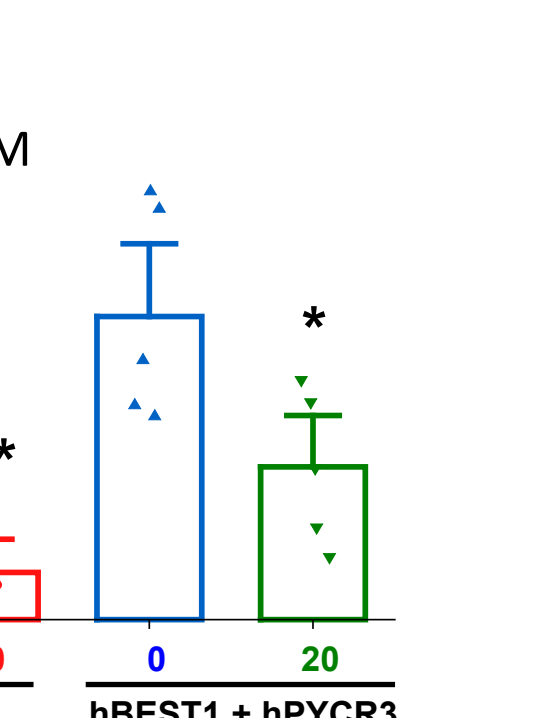
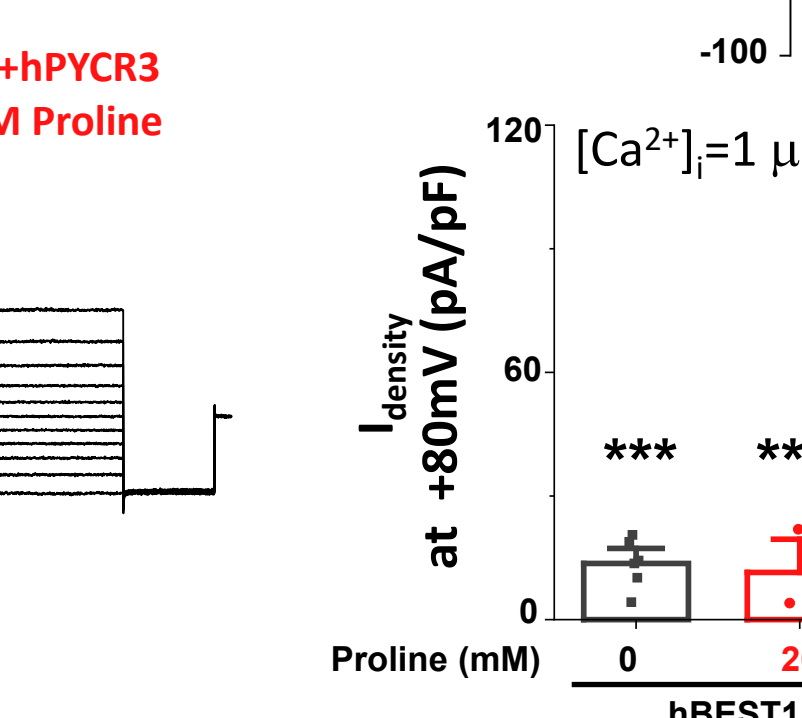
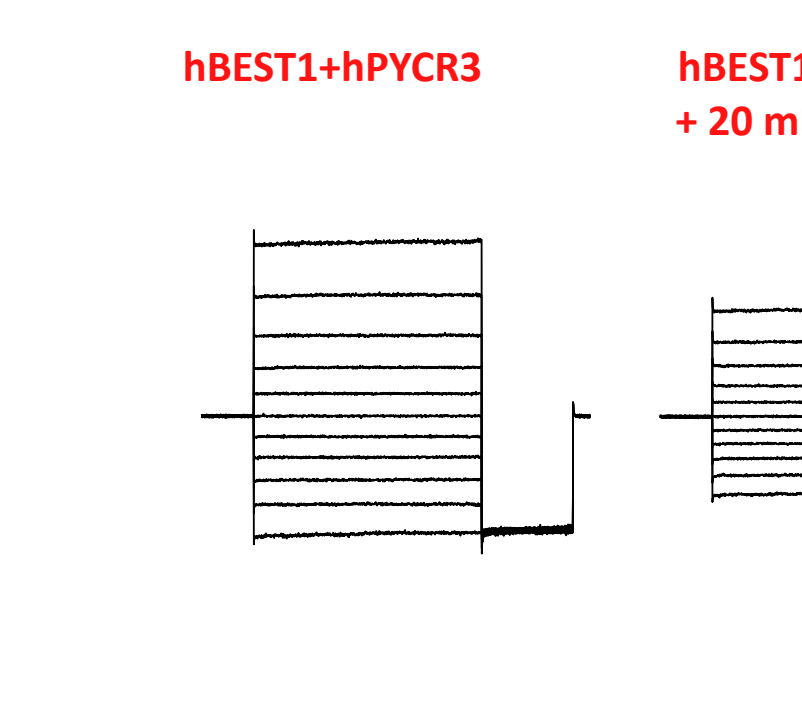
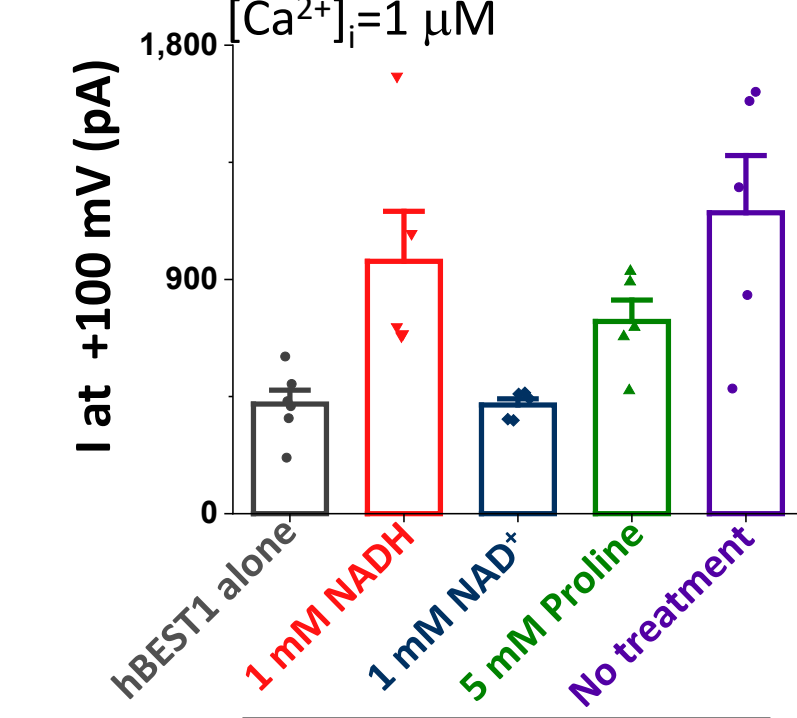
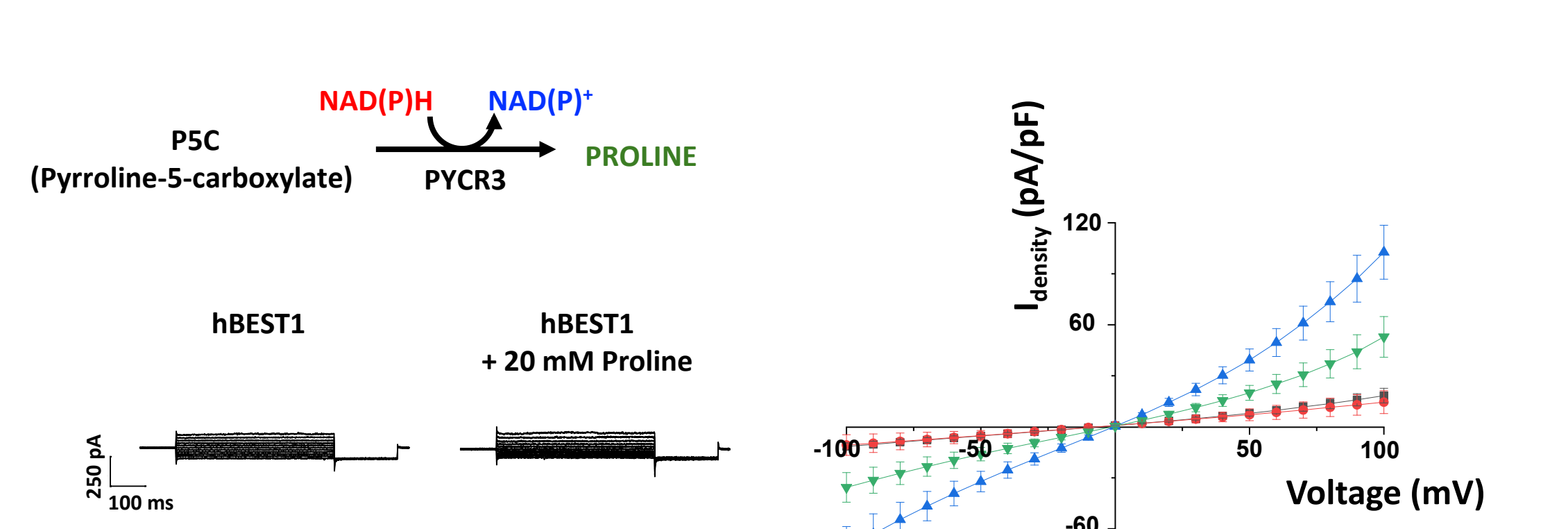
Regulation of hBEST1 Activity by WT hPYCR3 and Its Enzymatic Activity-Deficient GV Mutant.



Enzymatic activity of hPYCR3 may modulate hBEST1 channel in HEK293T.



The correlation between hBEST1 and hPYCR3 enzymatic activity in hPYCR3 KO.



CONCLUSION & ON-GOING DIRCTION

- PYCR3, a metabolic enzyme for proline synthesis was identified as a binding partner of hBEST1 channel.
- hBEST1 activity is significantly increased by co-expression of hPYCR3.
- hPYCR3 enzymatic activity is essential for hBEST1 modulation.
- Understanding the mechanism of functional augmentation of hBEST1 by hPYCR3.
- Investigating activity-dependent mutual regulation of BEST1 channel and PYCR3.

ACKNOWLEDGEMENT

The Basic Science Research Program (NRF, 2021R1A2C1004884) and the KBRI basic research program (24-BR-01-02).