

ABSTRACT

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 IP-MS. The interaction between hBEST1 and hPYCR3 was confirmed via Co-IP and SEC, where the two proteins co-migrated, indicating a direct interaction.

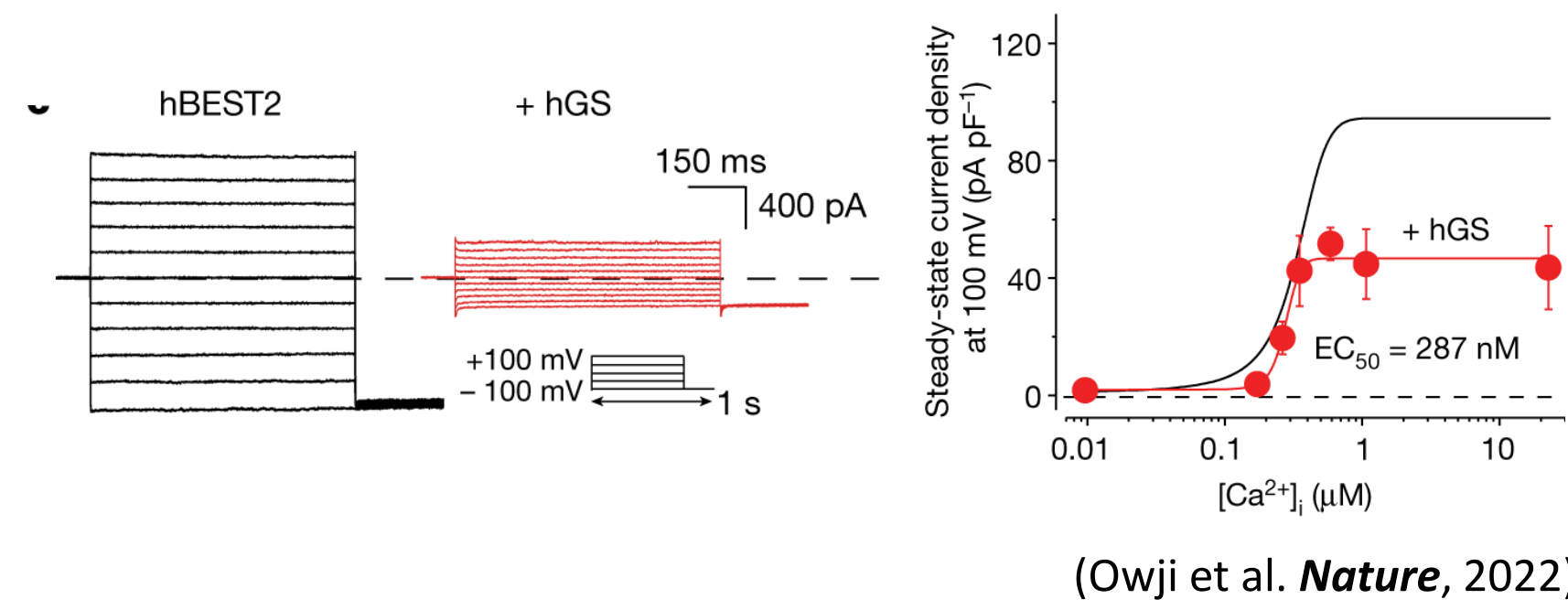
To investigate effect of hPYCR3 on hBEST1 channels, we performed whole-cell recordings. We found that hPYCR3 overexpression increased hBEST1 current density in HEK293T cells, whereas PYCR3 knockdown significantly reduced it. Furthermore, co-expression of hBEST1 and hPYCR3 in hPYCR3 knockout HEK293T cells 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

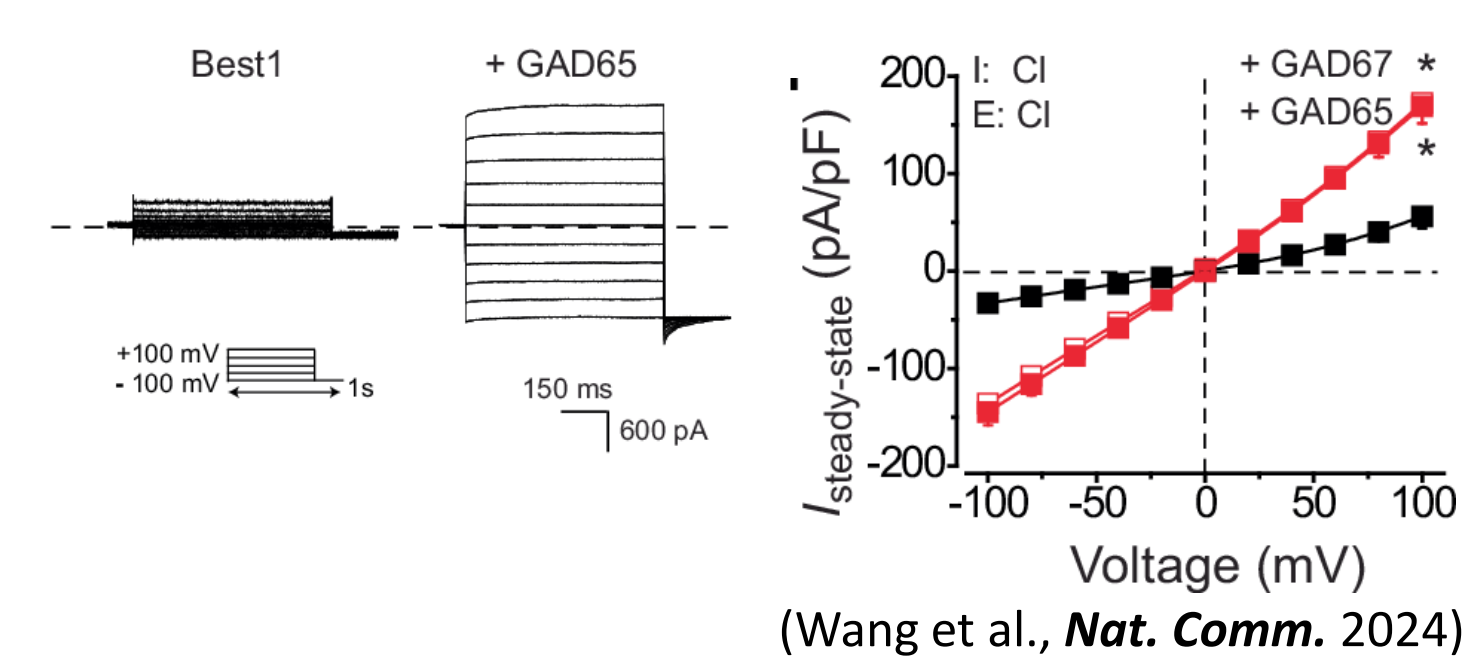
INTRODUCTION

- Bestrophin 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 BEST1 channel in mouse astrocytes has been reported to mediate the release of gliotransmitters such as Glu, GABA, and D-ser.
- Functional regulation of BEST channels is mediated through complexes with metabolic enzymes, such as BEST2-GS and BEST1-GAD65.

• The influence of GS (glutamine synthetase) on BEST2

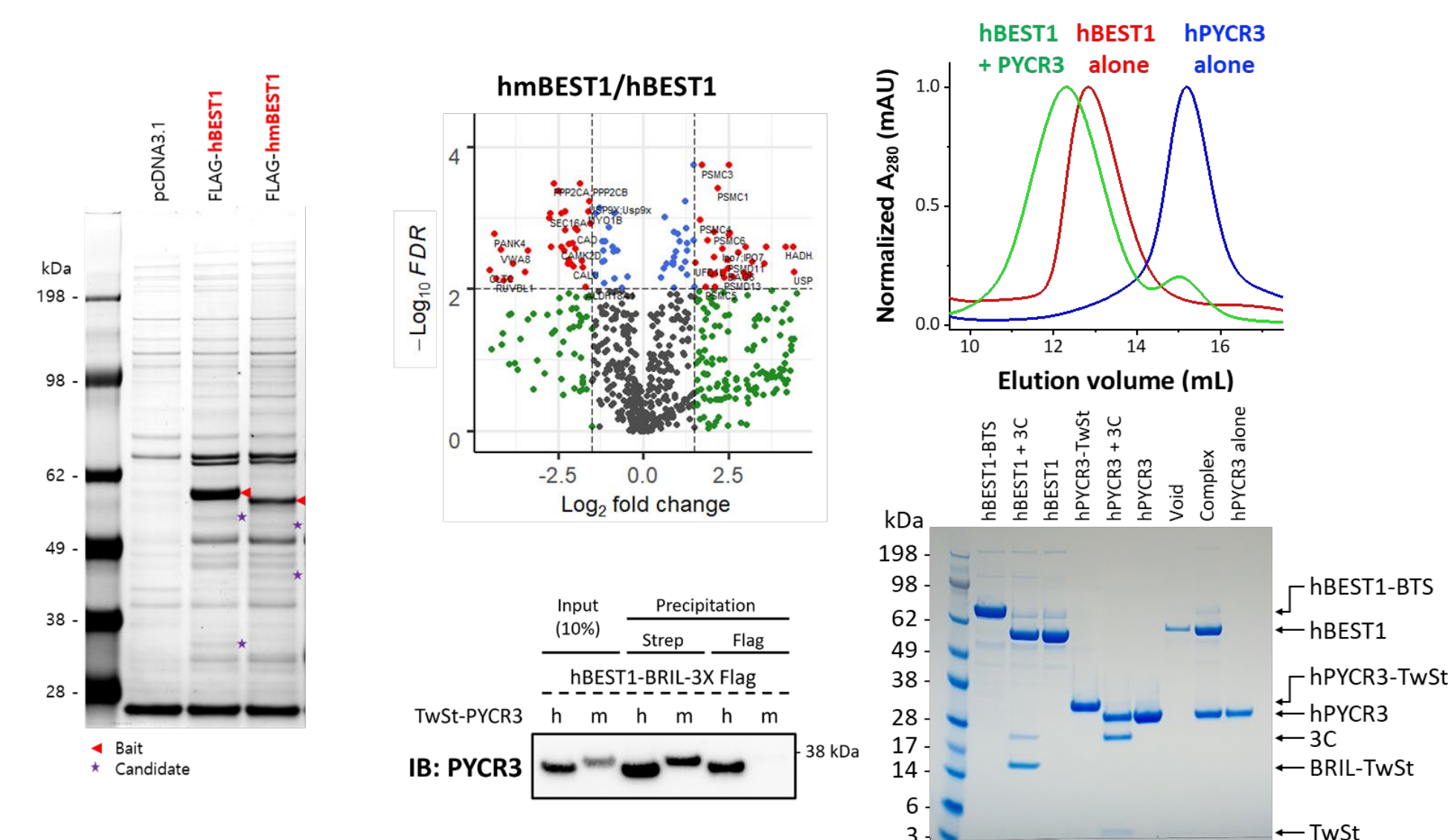


• Influence of GADs (glutamic acid decarboxylases) on BEST1

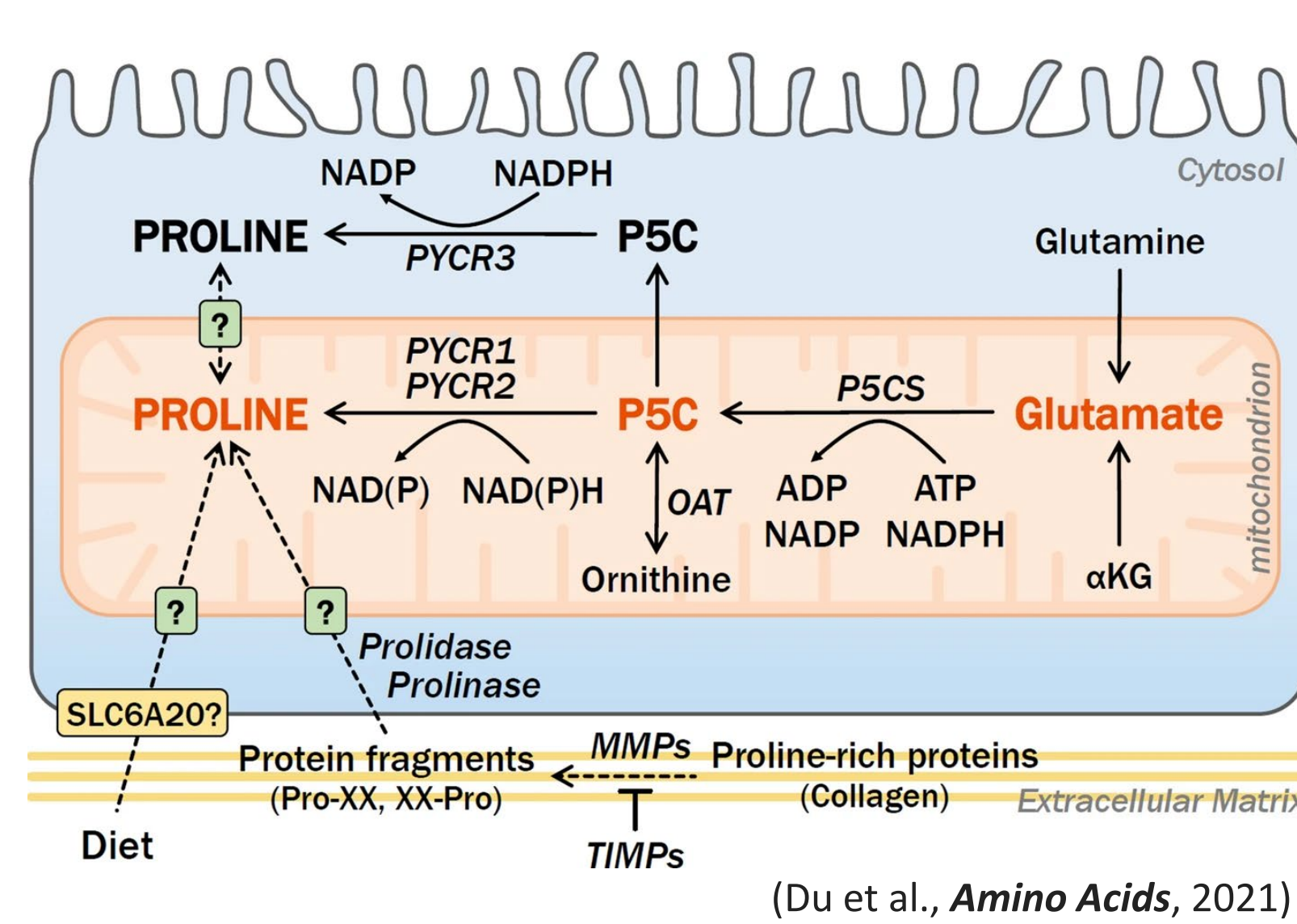


RESULTS

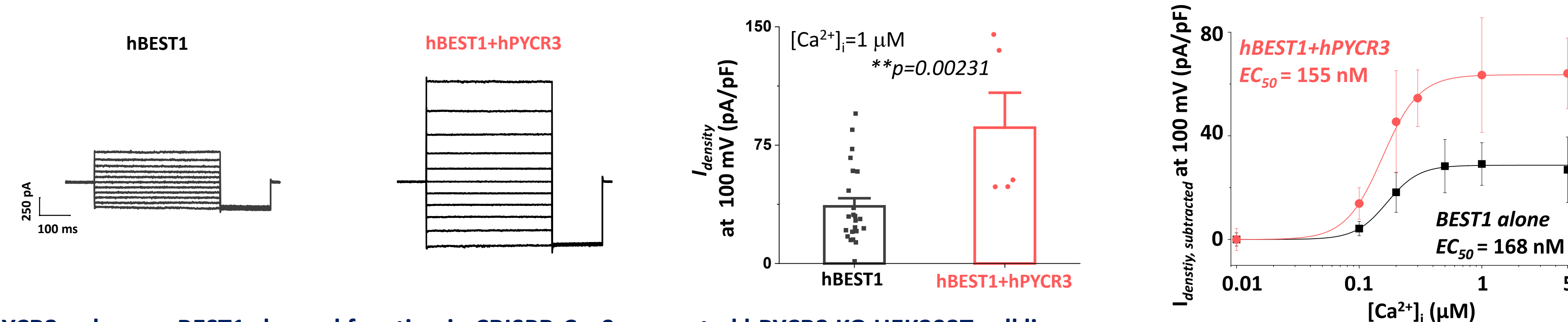
• IP-MS fished out hPYCR3 as an interacting protein to hBEST1.



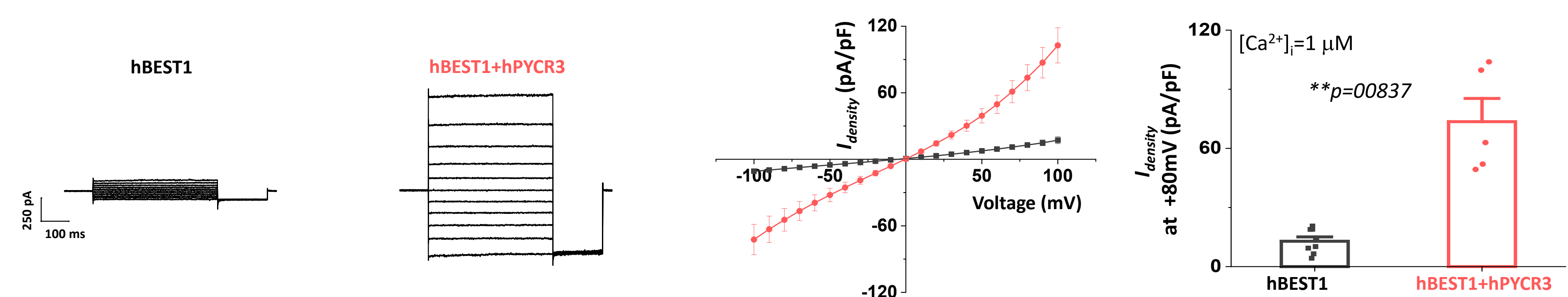
• Proline biosynthesis



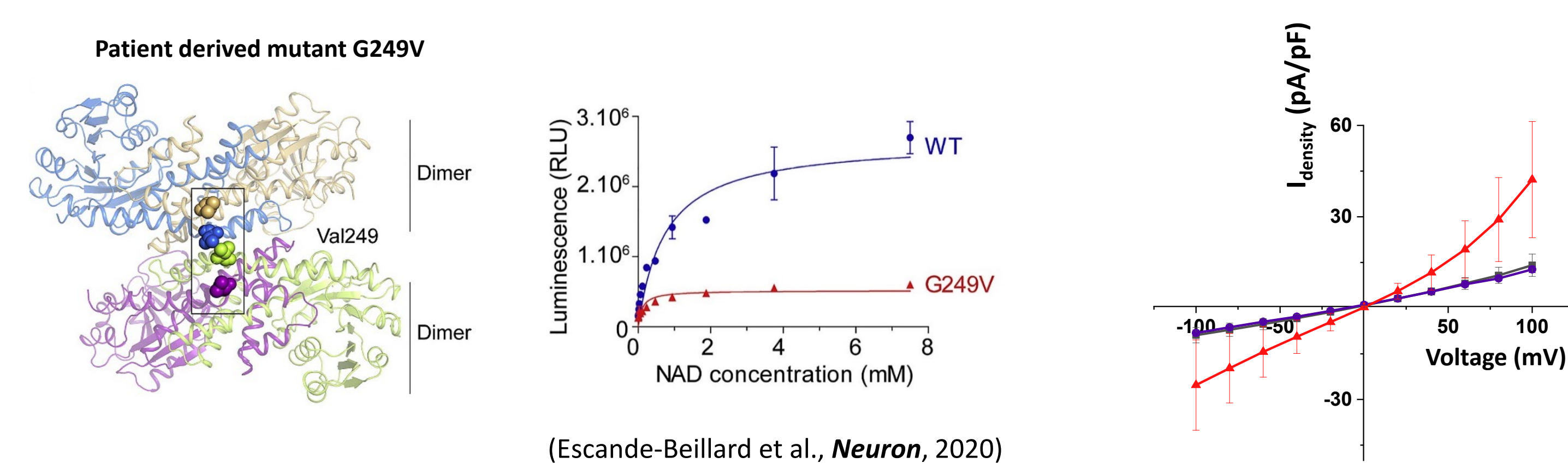
• hPYCR3 enhances BEST1 channel function in HEK293T.



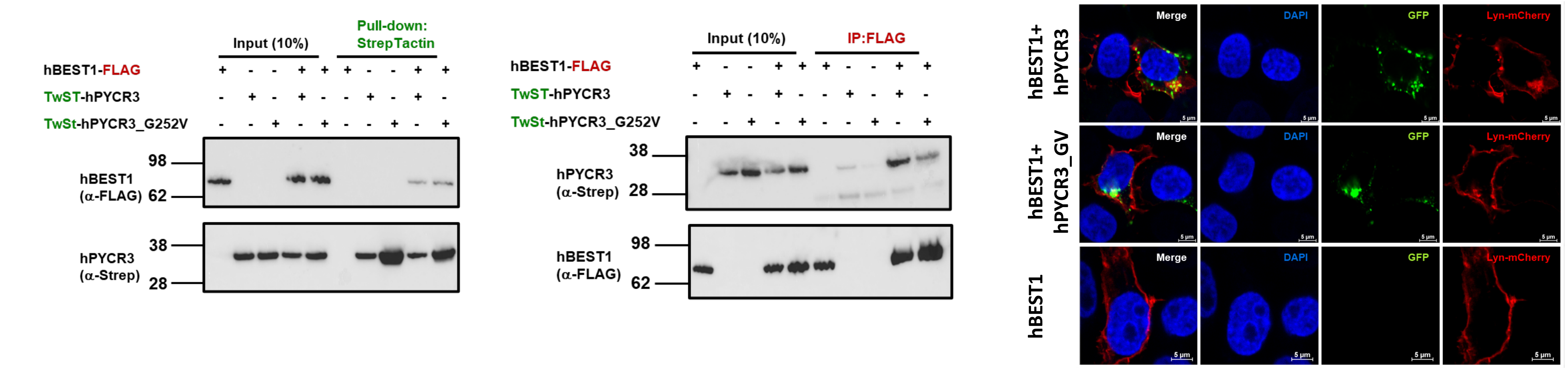
• hPYCR3 enhances BEST1 channel function in CRISPR-Cas9-generated hPYCR3 KO HEK293T cell line.



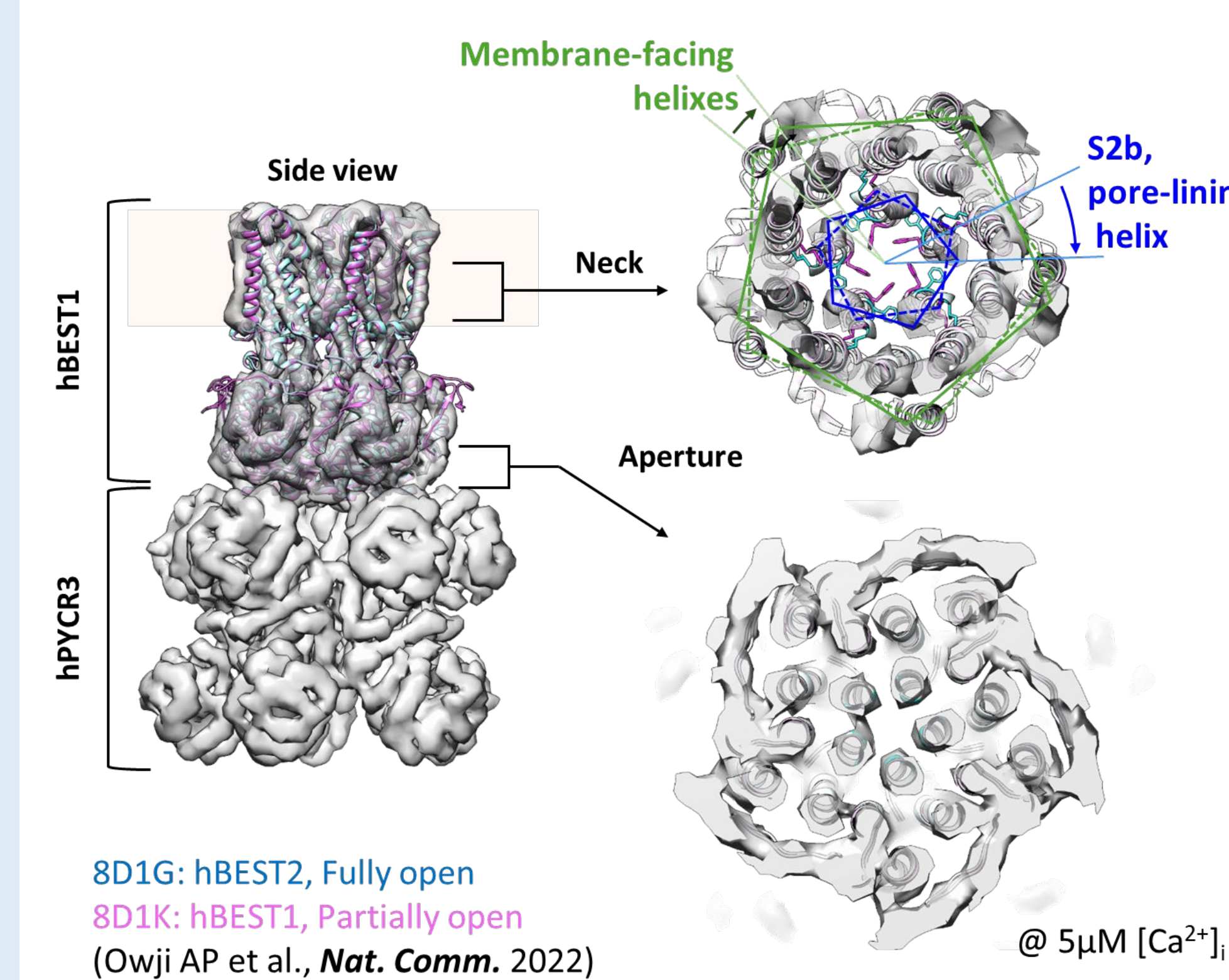
• Loss-of-function mutation



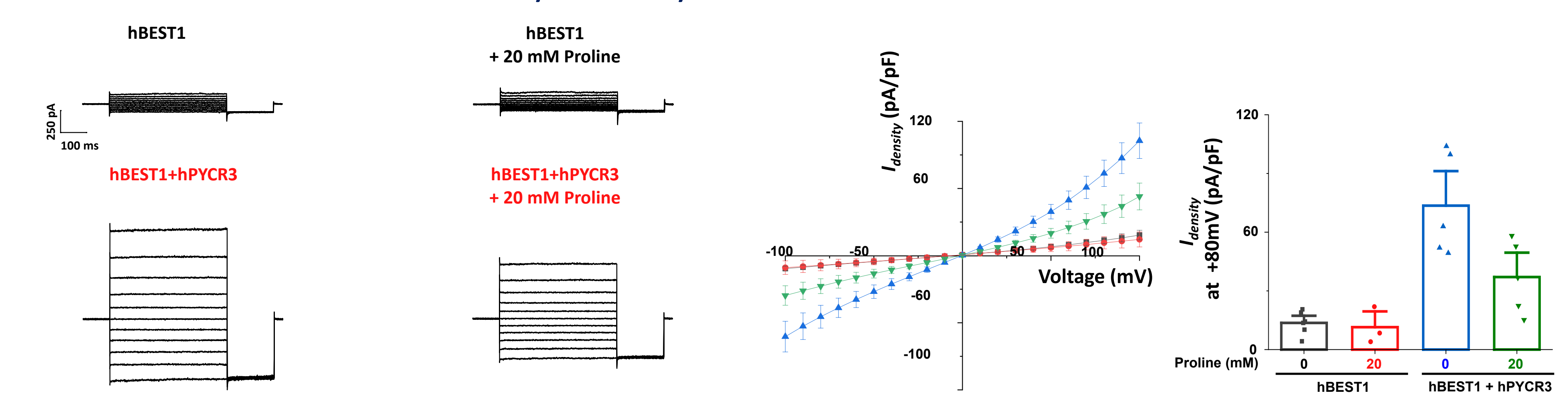
• hPYCR3 Interacts with BEST1 channel in HEK293T.



• Rotation of entire TM helices in hBEST1-hPYCR3 complex vs. S2b helix rotation in hBEST1 and hBEST2.



• The correlation between hBEST1 and hPYCR3 enzymatic activity in hPYCR3 KO cell line.



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).