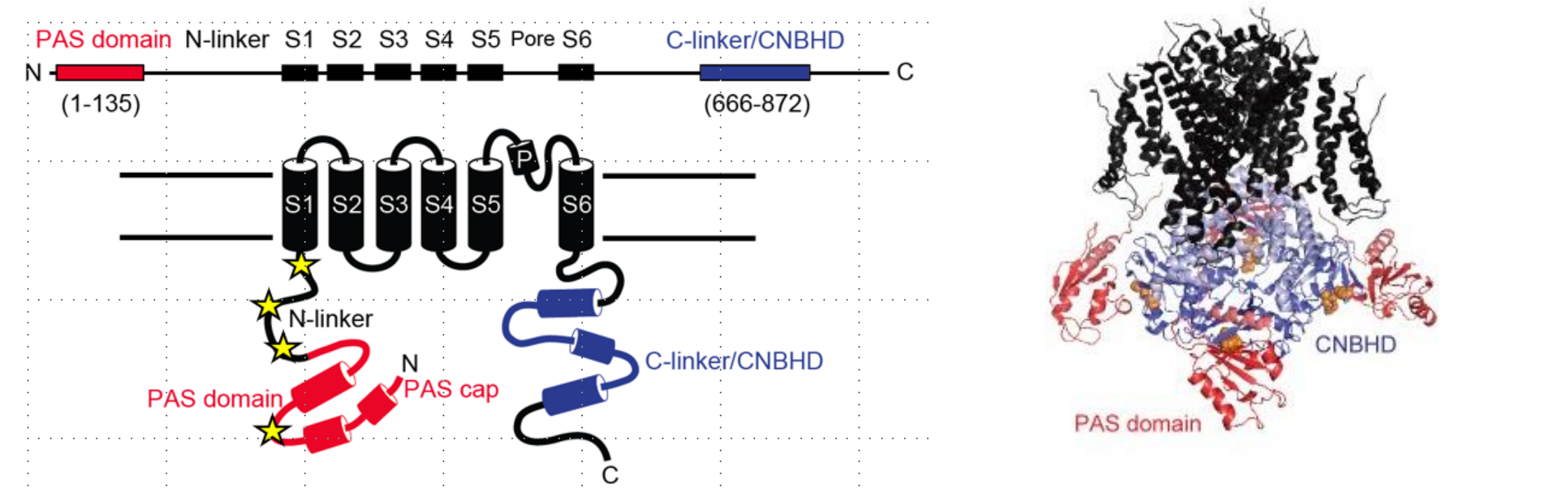


## ABSTRACT

The human Ether-à-go-go (hERG) potassium channels produce the IKr cardiac current, which drives the repolarization of membrane potential and terminates the action potential. Point mutations that introduce a TAG stop codon in hERG channels have been found in patients of LQT syndrome type 2. LQT2 syndrome patients have an increased risk of cardiac arrhythmias that may result in sudden cardiac arrest. We propose that the increased action potential duration (APD) that characterizes LQT2 can be explained by the loss of hERG function and thus a decreased IKr.

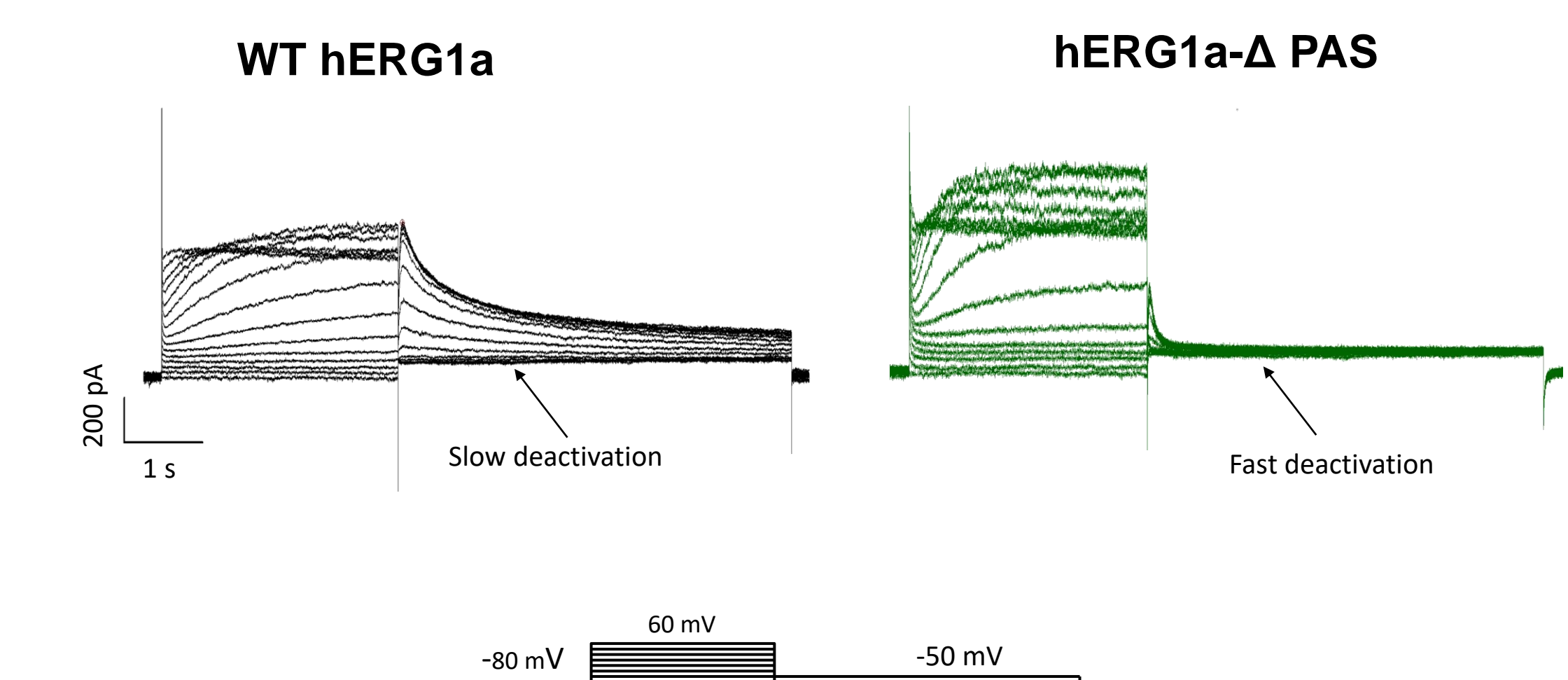
This work studies the effect of point mutations that introduce the stop codon TAG in hERG channels and how it affects its expression and electrical properties as well as the rescue of function by incorporating non-canonical amino acids (ncAA) in hERG TAG LQT2 mutants. When transfected in HEK293 cells, hERG-Q81X produces a functional channel with a faster deactivation rate and smaller currents than the wild type channel, while hERG1-S182X, E229X and W398X transfected cells do not produce current. With the use of an ortholog set of aaRS and tRNA, we were able to incorporate L-ANAP, a ncAA, in hERG LQT2 mutants and found that the incorporation of the ncAA into hERG-Q81X restores the WT phenotype of hERG currents. Meanwhile, the incorporation of a ncAA into hERG-182X and E229X but not in W398X results in the production of a full-length WT-like channel. We propose that the effect of hERG TAG mutants in LQT2 syndrome can be due to changes in the channel kinetics or a diminished channel expression and that the WT channel expression and function can be rescued by the incorporation of a ncAA.

## hERG is a voltage activated K<sup>+</sup> Channel



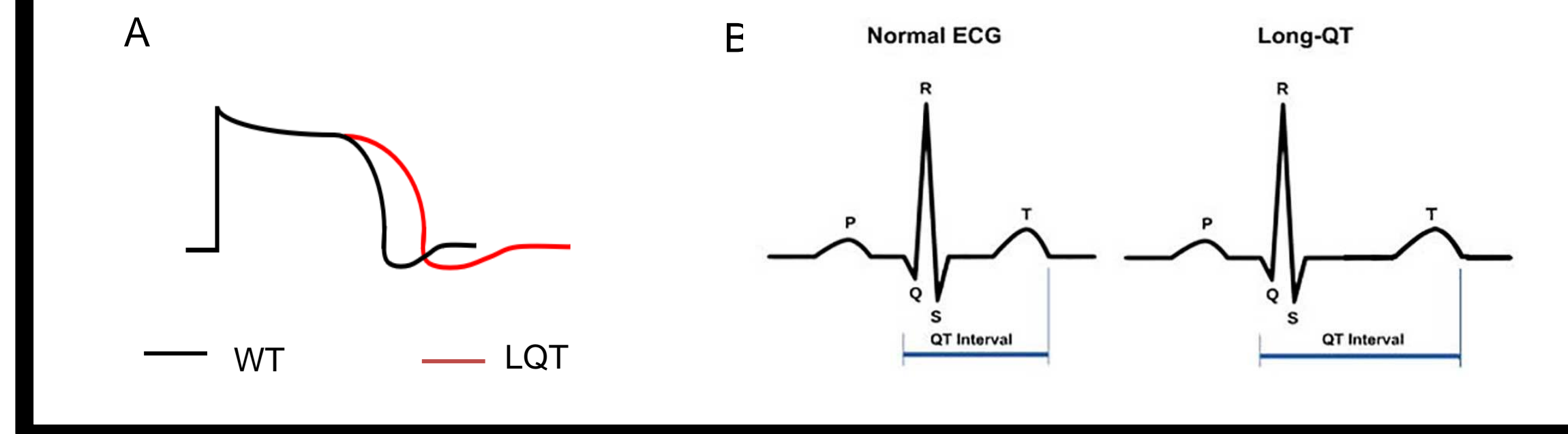
**Figure 1.** hERG channels are voltage-activated K<sup>+</sup> channels. They are tetramers where each subunit has six transmembrane (S1-S6) domains, a pore (P) domain which forms the ion conduction pathway and intracellular N- and C-terminal regions. hERG channels have a distinctive N-terminal PAS domain and a C-terminal CNBDH domain. Yellow stars indicate the position of point mutations that introduce a stop codon (TAG) associated to long QT syndrome.

## hERG characteristic slow deactivation requires the interaction of the PAS and CNBDH domains



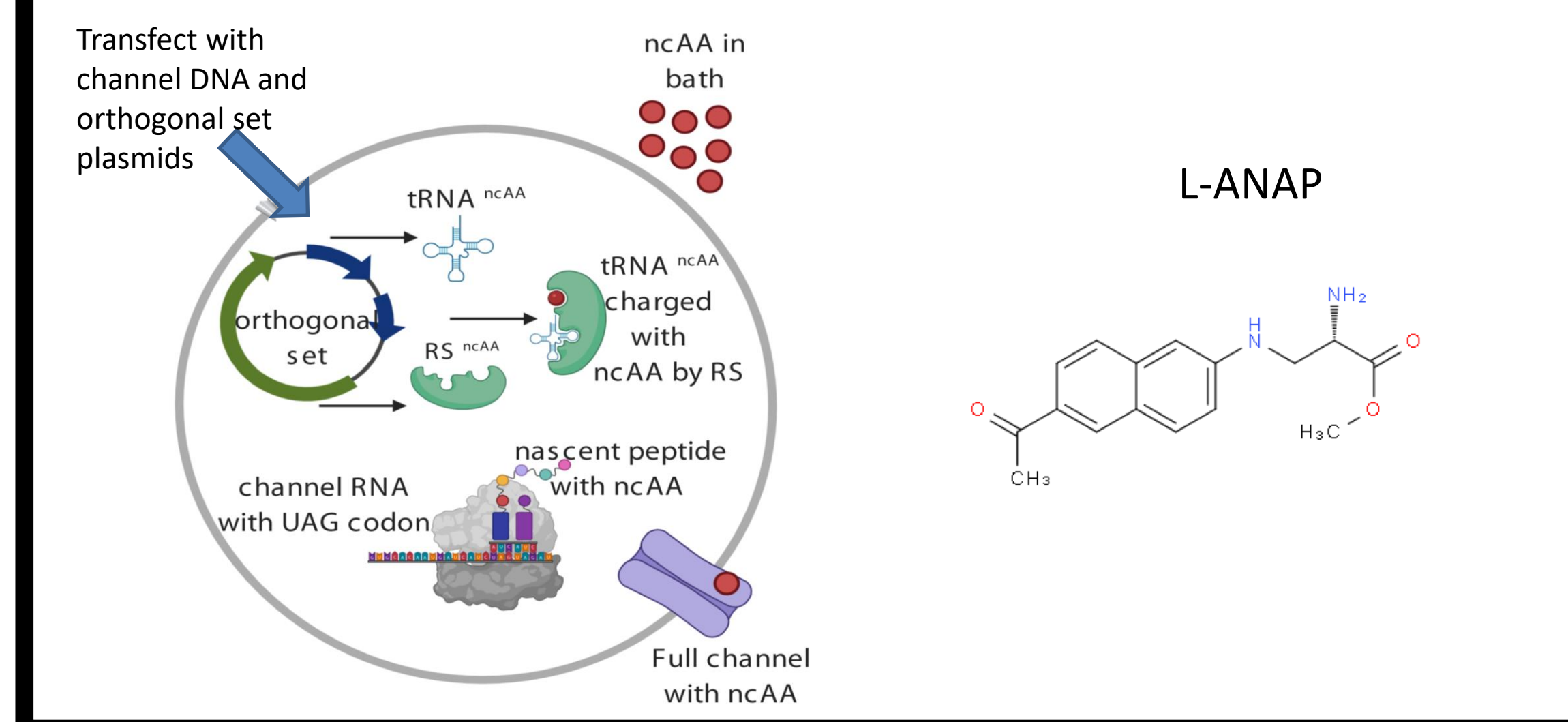
**Figure 2.** Representative family of currents elicited by series of voltage steps in HEK293 cells transfected with WT hERG1a or hERG Δ PAS. WT hERG channels present a characteristic slow deactivation, regulated by direct interactions between the PAS and CNBDH domains. Deletion of the 135 amino acids corresponding to the PAS domain results in fast inactivation of the channel.

## Long QT syndrome type 2



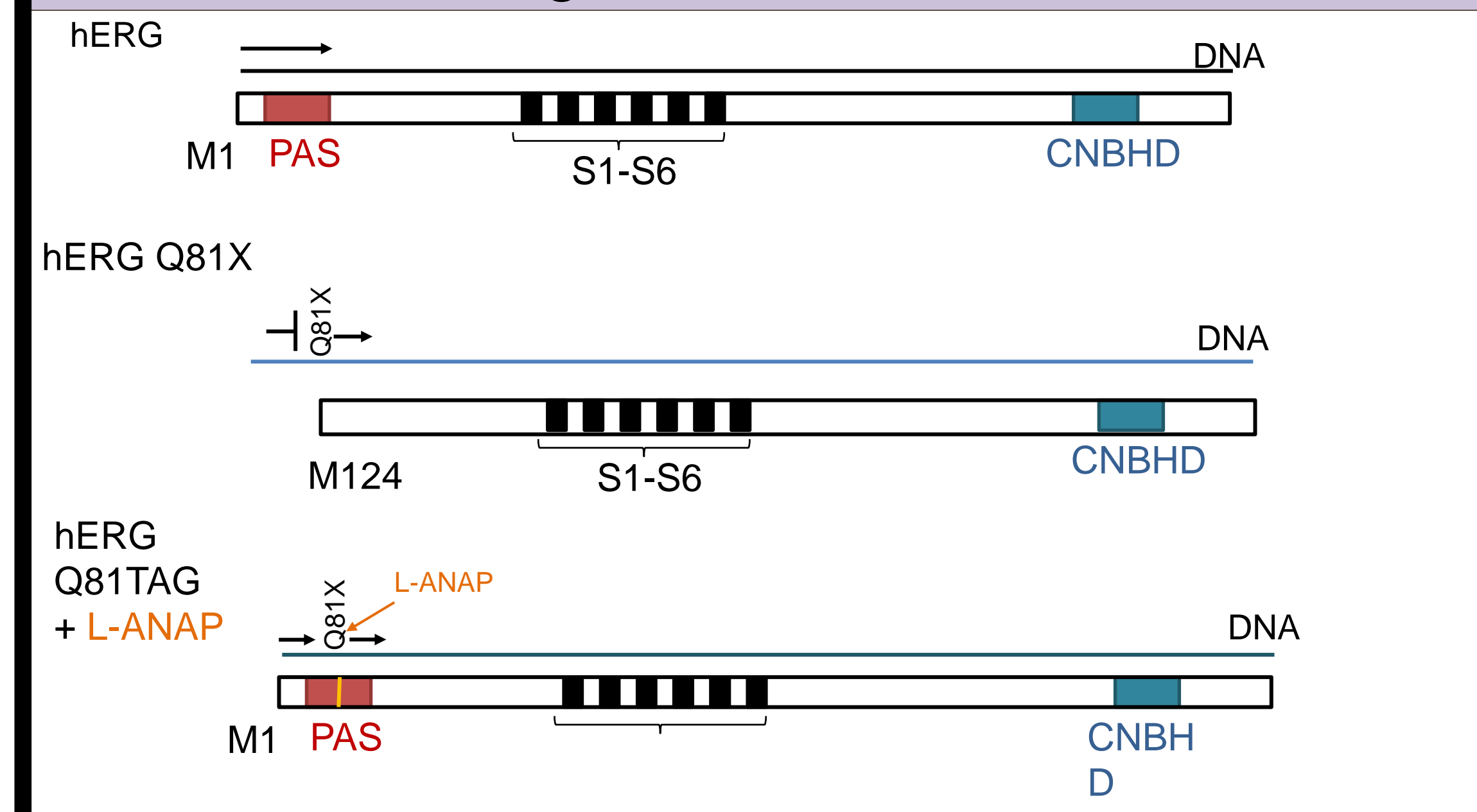
**Figure 3.** The slow deactivation of hERG channels is necessary for the termination of the ventricular action potential, disruptions in its function can lead to an elongation of the QT interval and potentially to arrhythmias or sudden cardiac arrest. A) Schematic of the ventricular action potential between WT (black) and LQT (red) phenotypes. B) Schematic of electrocardiograms in healthy (left) and LQT (right), showing the elongated QT interval.

## L-ANAP, a non-canonical amino acid



**Figure 4.** Schematic of the ortholog set of tRNA, and aminoacyl tRNA synthetase (RS) modified to introduce a ncAA using the amber stop codon (TAG). By transfecting HEK293 cells with the plasmids containing the ortholog set and the hERG TAG LQT2 mutants and exposing the cells to L-ANAP in the bath we can test the incorporation of the ncAA in the mutant channels and its effect on hERG currents.

## hERG Q81X produces a truncated channel by initiating translation at M124

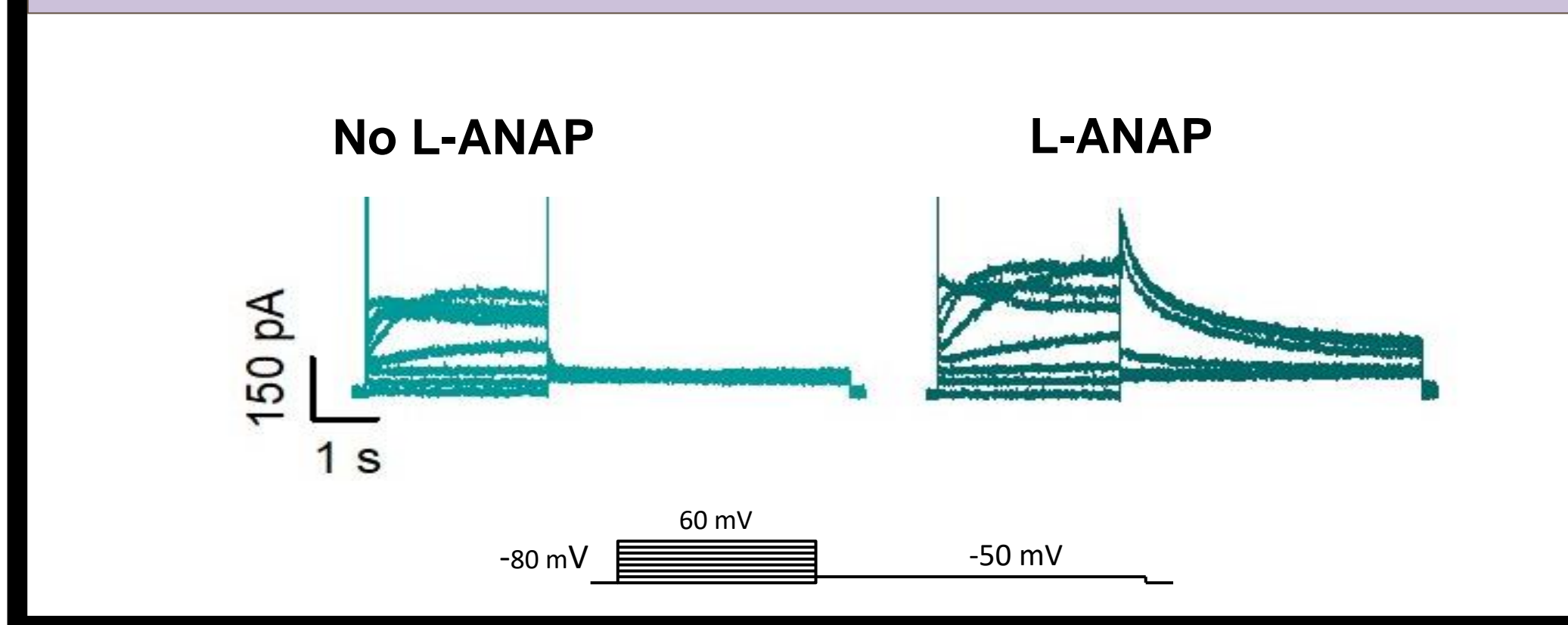


**Figure 5.** Scheme of DNA and proteins encoding (top) wild-type hERG (center) hERG Q81 TAG and (lower) hERG Q81 + L-ANAP. hERG can produce a truncated channel that starts at M124 and has fast deactivation similar to hERG ΔPAS. M1= initiating Met start site, M124= putative alternative Met start site.

## Hypothesis

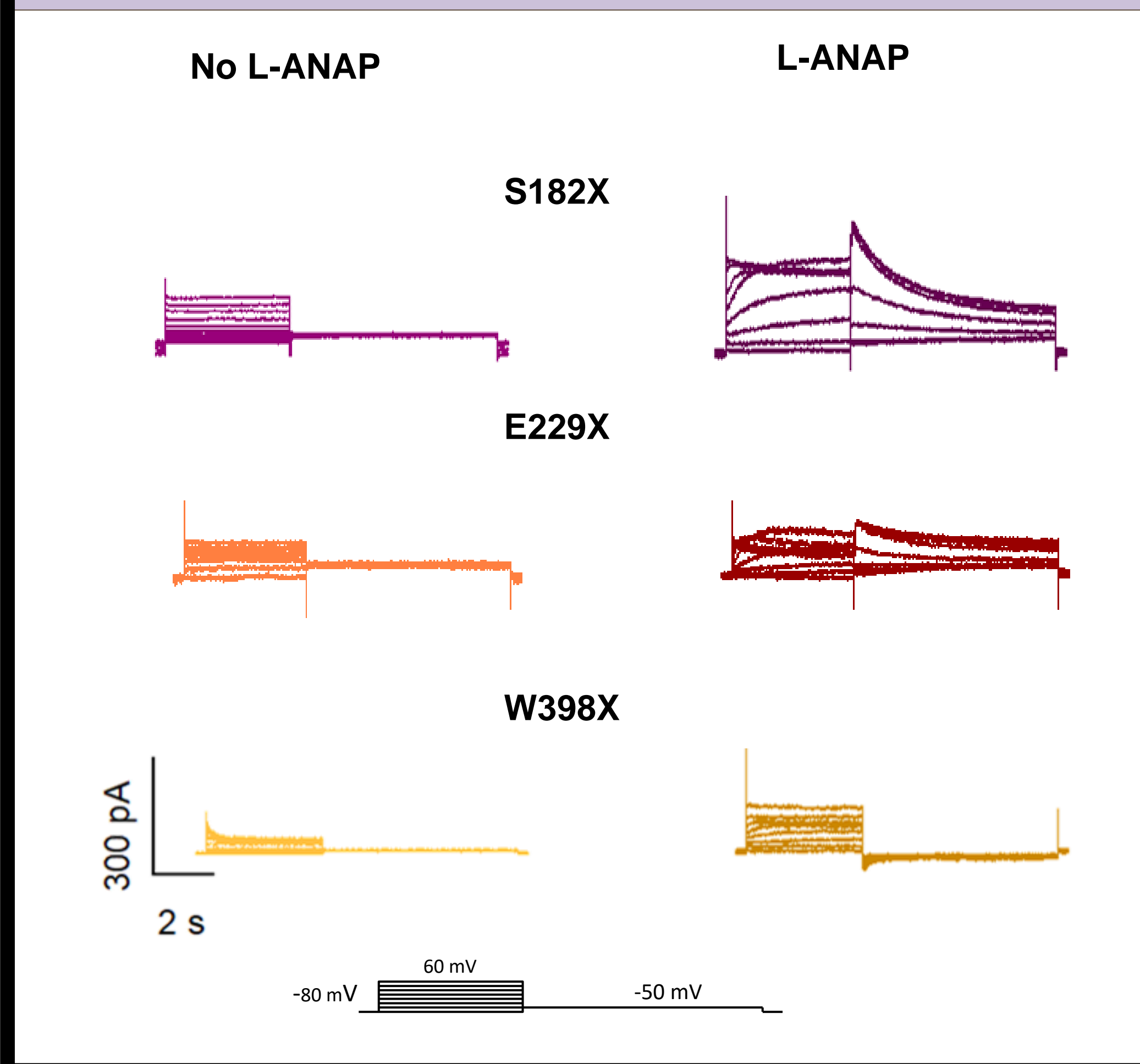
We hypothesize that LQT2-linked hERG TAG mutants present loss of function due to lack of protein translation or abnormal deactivation and that the WT current can be restored with the incorporation of ncAA such as L-ANAP.

## L-ANAP incorporation rescues WT slow deactivation in hERG Q81X



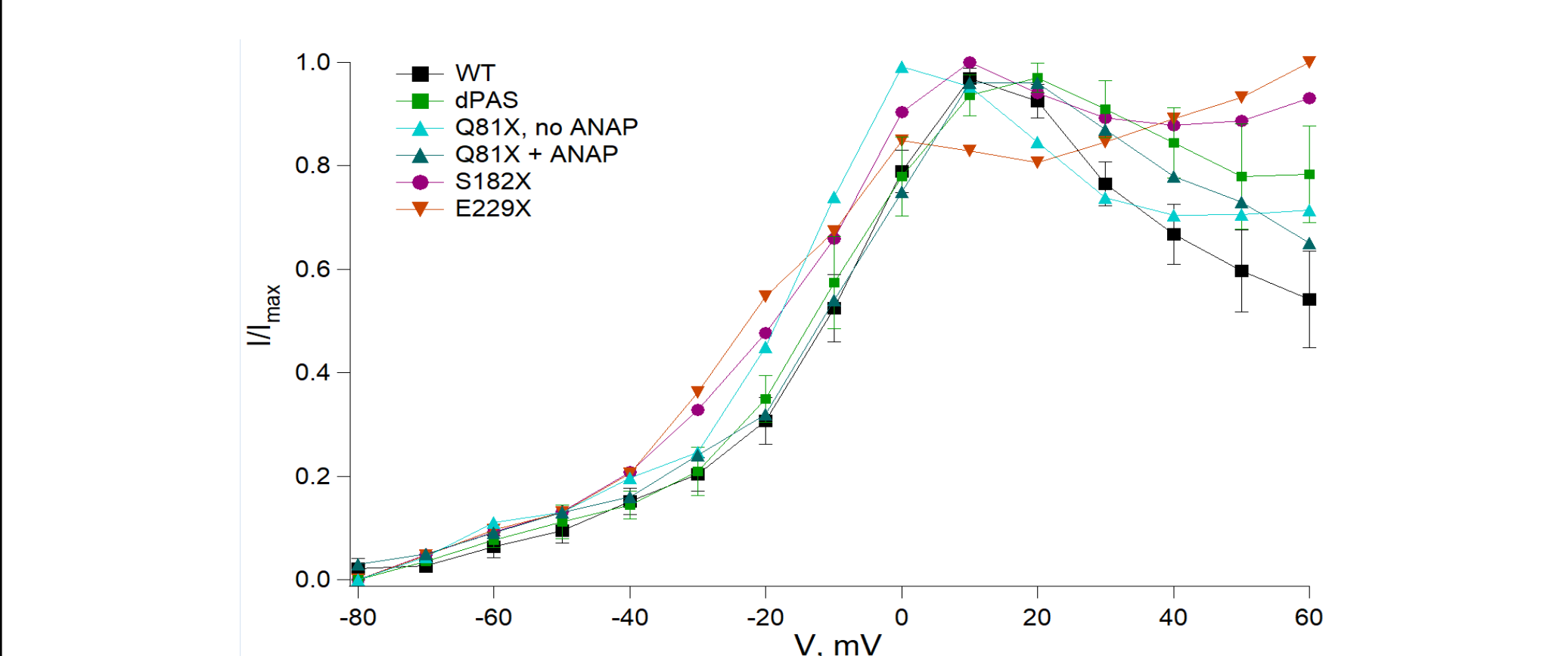
**Figure 6.** Representative family of currents elicited by series of voltage steps in HEK293 cells transfected with WT hERG Q81X. On the left, currents from cells incubated without L-ANAP, on the right, currents from cells incubated with L-ANAP in the cell culture media. In the absence of L-ANAP, hERG Q81X produces a channel with fast deactivation similar to that of hERG Δ PAS. When the HEK293 cells are incubated in the presence of L-ANAP the channel presents the fast deactivation typical of WT hERG.

## L-ANAP incorporation rescues phenotype of some hERG TAG LQT2 mutants



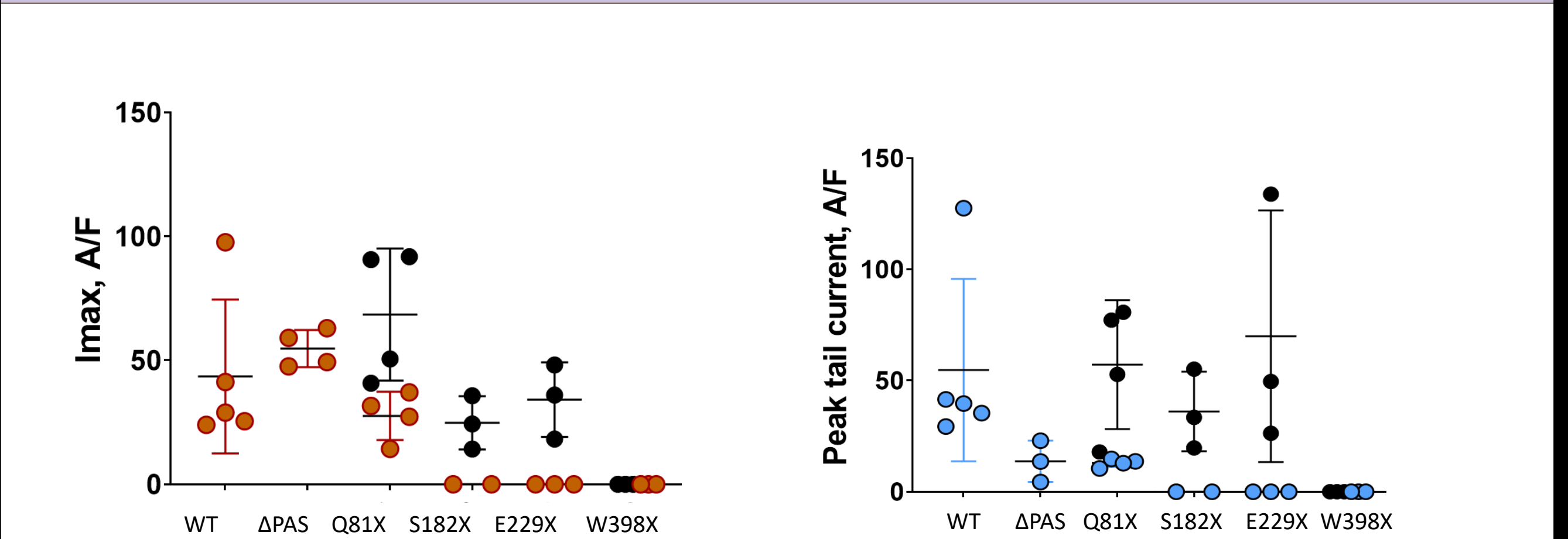
**Figure 7.** Representative family of currents elicited by series of voltage steps in HEK293 cells transfected with different hERG TAG LQT2 mutants and incubated with (right) or without (left) L-ANAP in the bath. L-ANAP incorporation produces S182X and E229X currents, but not W289X.

## Rescue of hERG currents by incorporation of L-ANAP



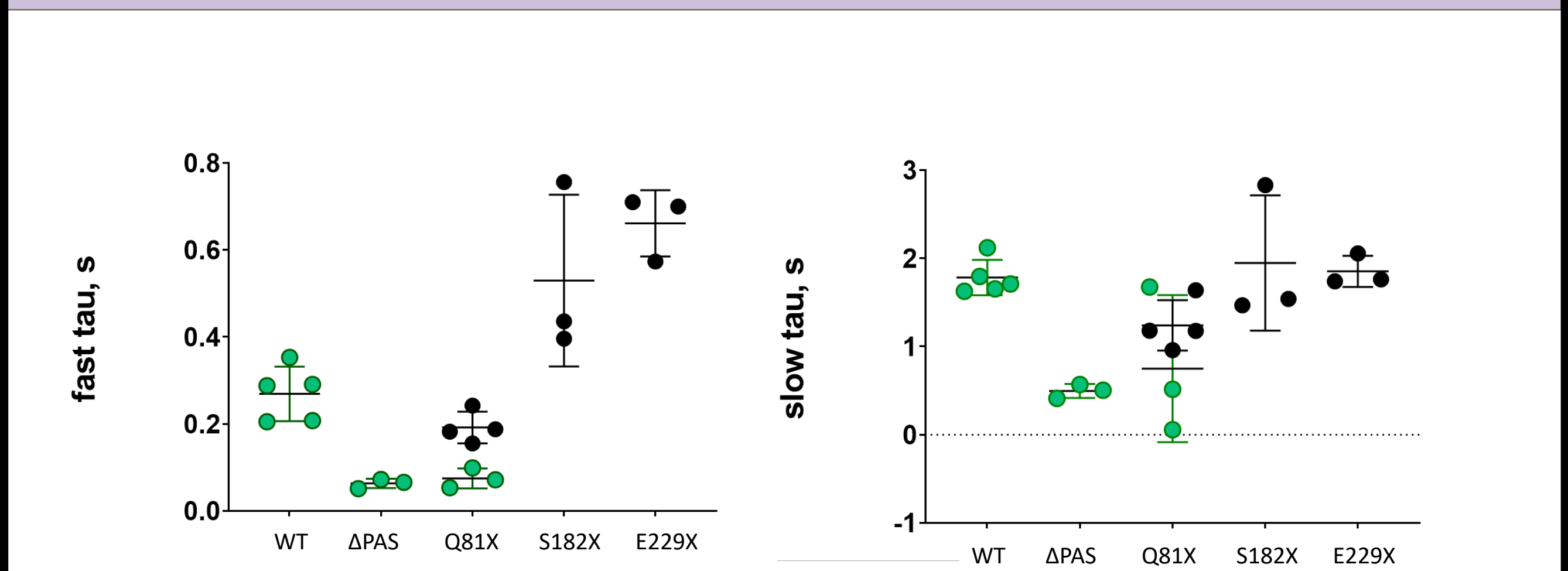
**Figure 8.** The incorporation of L-ANAP produces Q81X, S82X and E229X mutant channels currents that activate in a similar way to hERG WT

## Maximum activation and inactivation currents before and after the incorporation of L-ANAP



**Figure 9.** On the left comparison of maximum current densities during the activation, on the right peak tail currents. Orange or blue symbols represent WT channel, hERG-dPAS or the mutant channels in the absence of L-ANAP, black symbols represent the currents in the presence of L-ANAP.

## Deactivation time constants



**Figure 10.** Comparison of deactivation time constants before and after the incorporation of L-ANAP. Green symbols represent WT channel, hERG-dPAS or the mutant channels in the absence of L-ANAP, black symbols represent the currents in the presence of L-ANAP.

## Conclusions and further direction

The incorporation of a ncAA such as L-ANAP can rescue the WT current of some hERG TAG LQT2 mutant channels in mammal cells (HEK293). It recovers both the expression and the slow deactivation necessary for the proper termination of the ventricular action potential.

The next step will be to test the incorporation of ncAA in cardiomyocytes derived from human induced pluripotent stem cells (CM-hiPSC) in which LQT2 TAG mutations have been incorporated by CRISPR. This model will allow us to determine if the rescue of hERG WT currents correlate with a rescue of action potential duration.

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## Acknowledgements

This work was supported by MSCRF post-doctoral training grant 2022-MSCRFF-5931 (to IH) and MSCRF-Discovery Grant 5621 (to MCT) the University of Maryland, Baltimore