Supplemental Data

Solution structure of the Kv4.3 N-terminus (residues 1-40)

The highly conserved N-terminus of Kv4 channels is a critical determinant for their interaction with KChIPs (Fig. 7A). The structural basis of these effects was investigated by determining the solution structure of a synthetic peptide Kv4.3(1-40) representing the first 40 amino acids of Kv4.3 that precede the T1 domain. Due to the hydrophobicity of the peptide it was dissolved in 42% trifluroroethanol-d_3 (v/v). ^{1}H NMR spectra were measured at 295 and 301 K on a Bruker Avance 600 with a room-temperature pulsed-field gradient probe. The concentration of the peptide was 1.2 mM in 3 mM DTT, pH ~3. Homonuclear NOESY (100 ms mixing time), CLEAN-TOCSY (60 and 80 ms mixing time) and DQF-COSY spectra were recorded to assign the ^{1}H resonances of the peptide and to obtain constraints for the structure calculations with DYANA (v. 1.5) (1).

The ^{1}H NMR data in Fig. 1A clearly identify a helix between Pro10 and Ala16. There is also a strong helical propensity at the N-terminus of Kv4.3(1-40) as indicated by $^3J(H^N\text{-}H\alpha)$ coupling constants $\leq$ 6 Hz and several medium-range NOEs that are typically observed in helices (2). Thus, structure calculations show a helix in the sequential stretch Ala6-Ala16 (Figs. 1C and 1D). The C-terminal half of the peptide is unstructured and the absence of long-range NOEs implies that Kv4.3(1-40) does not form tertiary structure. These results are fully consistent with the structural properties of the Kv4.3 N-terminus in the recently published crystal structures of the Kv4.3T1-KChIP1 complex. In both cases, the N-terminus of Kv4.3 forms a short α-helix for interaction with KChIP1 that is followed by an extended loop without secondary structure connecting to the T1 domain.

Supplemental References

1. Güntert, P., Mumenthaler, C., and Wuthrich, K. (1997) *J Mol Biol* **273**, 283-298
2. Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, John Wiley & Sons, New York
3. Wishart, D. S., Sykes, B. D., and Richards, F. M. (1992) *Biochemistry* **31**, 1647-1651

Supplemental Figure Legends

Figure 1: NMR data and solution structure of Kv4.3(1-40) in 42% trifluorethanol-d_3 (v/v), 295 K.

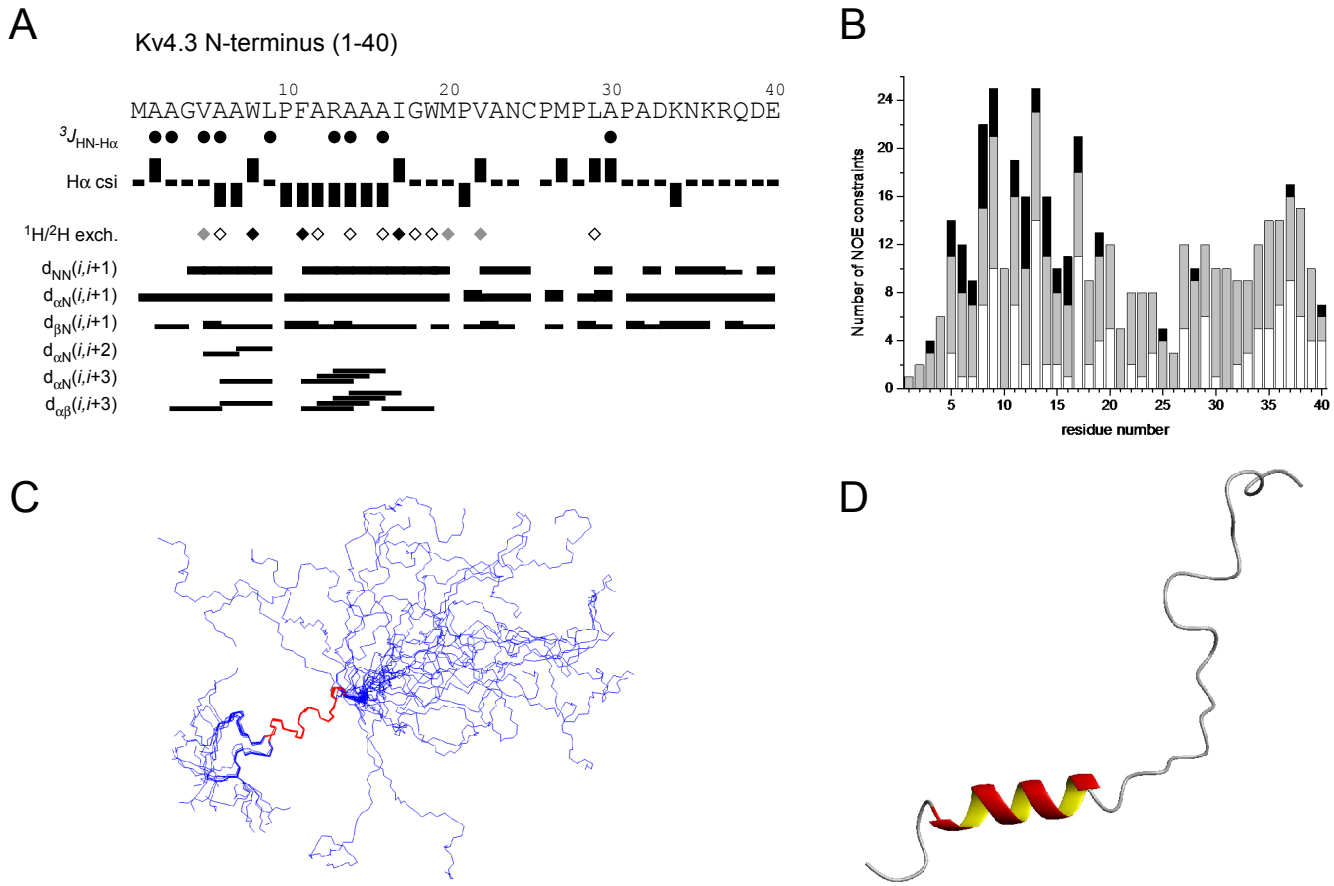
(A) Sequence and schematic representation of NMR data of Kv4.3(1-40). Amino acids with a $^3J(H^N\text{-}H\alpha)$ coupling constant $\leq$ 6 Hz diagnostic of a helical backbone conformation are marked with a black dot. The Hα chemical shift index (csi) (3) is shown on the second line below the sequence. Black bars pointing downwards represent a csi value of -1 as typically observed for Hα in helices, bars pointing upwards represent a csi value of +1 as it occurs in β-strands and small black rectangles represent a csi value of 0, i.e. a Hα chemical shift value close to random coil chemical shifts. The third line shows the result of a $^1H/^2H$ exchange

experiment (dissolution of a lyophilized peptide sample in 100% ^{2}H2O). Diamonds identify backbone amide protons that exchange slowly with $^{2}H_2O$. Open, grey, and black symbols correspond to $^{1}H^{N}$ resonances that are still visible after exchange times of 90, 150, and 360 min, respectively. The following lines show important sequential (d(*i,i+1*)) and medium-range NOEs observed for Kv4.3(1-40). Line thickness represents strong (< 2.7 Å), medium (2.7-4 Å), and weak (> 4 Å) NOEs.
(B) Plot of the number of NOEs per residue as a function of the Kv4.3(1-40) sequence. Intraresidual NOEs are white, sequential NOEs grey, and medium-range NOEs black.
(C) Backbone superposition of the final family of 25 structures of Kv4.3(1-40) between Trp8 and Ala16 (highlighted in red). Two subfamilies of structures with different orientation of the N-terminus before Trp8 are seen.
(D) Ribbon representation of the backbone of the first structure of the family. The helical secondary structure between Ala6 and Ala16 is emphasized. 14 of the 25 structures in the final family show a helical conformation between Val5 and Ala7.
The structures were calculated with DYANA (v. 1.5) (1) on the basis of 295 NOEs (135 intraresidual, 134 sequential, 26 medium-range), 7 constraints for dihedral angles Φ which are based on $^{3}J(H^{N}\text{-}H\alpha)$ coupling constants, and 6 stereospecific assignments. The average value of the target function for the 25 best structures was 0.52 ± 0.07 Å^{2}.

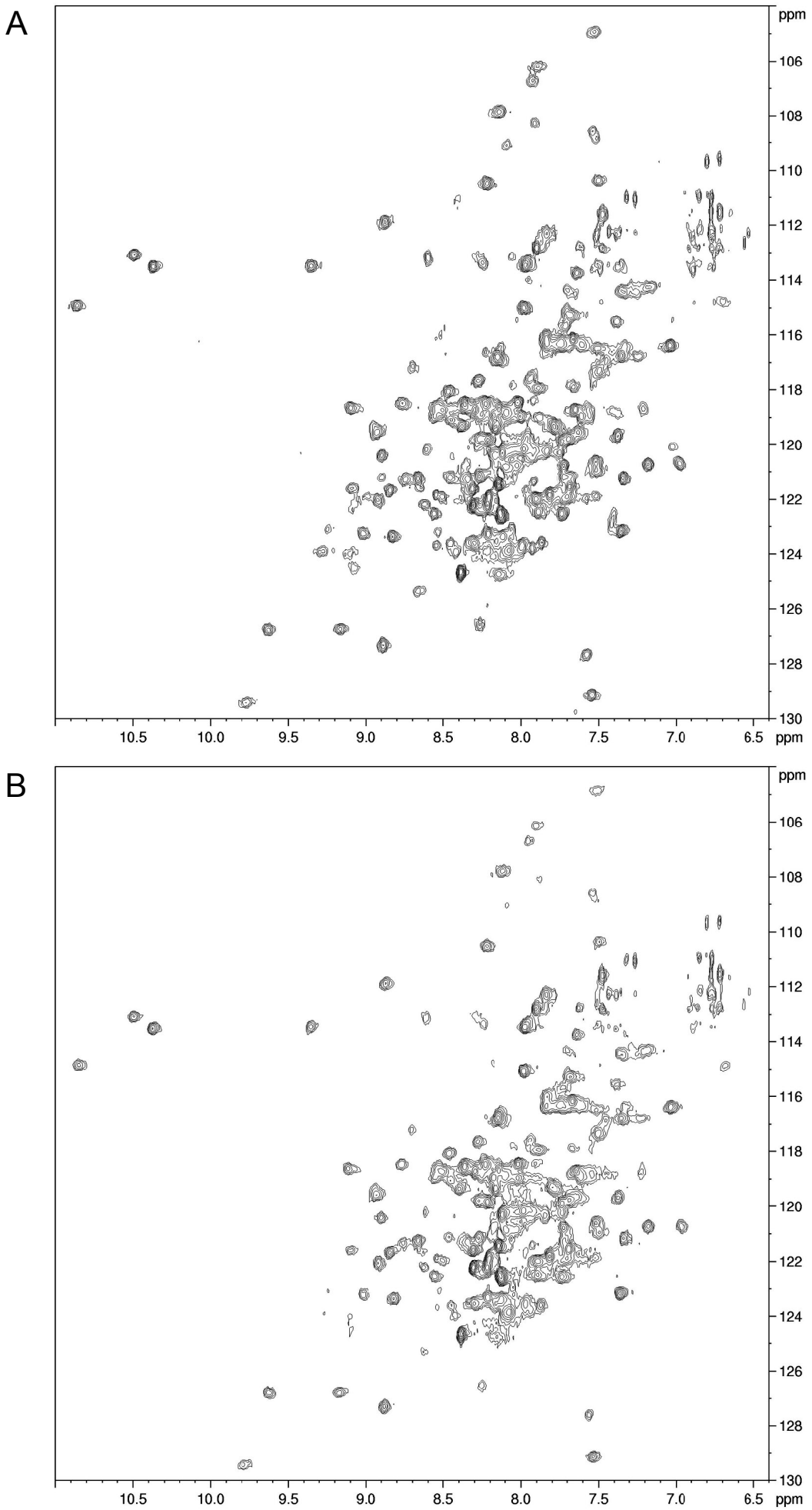
Figure 2: Interaction of KChIP4a with Kv4.3(1-40).
(A) $^{1}H\text{-}^{15}N$ TROSY spectrum of 0.1 mM ^{15}N labeled, Ca^{2+} and Mg^{2+} loaded KChIP4a at 300 K in 10 mM MOPS (pH 7.4), 4 mM DTT, 5 mM N-octylglucoside and 2 mM $MgCl_2$ (number of transients 80).
(B) Same as in (A), but after addition of 21 µl of a 3.78 mM Kv4.3(1-40) stock solution in the same buffer as the protein (number of transients 96). The protein-peptide ratio is 1:1.5.

Figure 3: Interaction of KChIP4a(Δ1-42) with Kv4.3(1-40).
(A) $^{1}H\text{-}^{15}N$ TROSY spectrum of 0.27 mM ^{15}N labeled, Ca^{2+} and Mg^{2+} loaded KChIP4a(Δ1-42) at 300 K in 10 mM MOPS (pH 7.4), 4 mM DTT, 5 mM N-octylglucoside and 2 mM $MgCl_2$ (number of transients 48).
(B) Same as in (A), but after addition of 17.8 µl of a 3.85 mM Kv4.3(1-40) stock solution in the same buffer as the protein (number of transients 64). The protein-peptide ratio is 1:1.

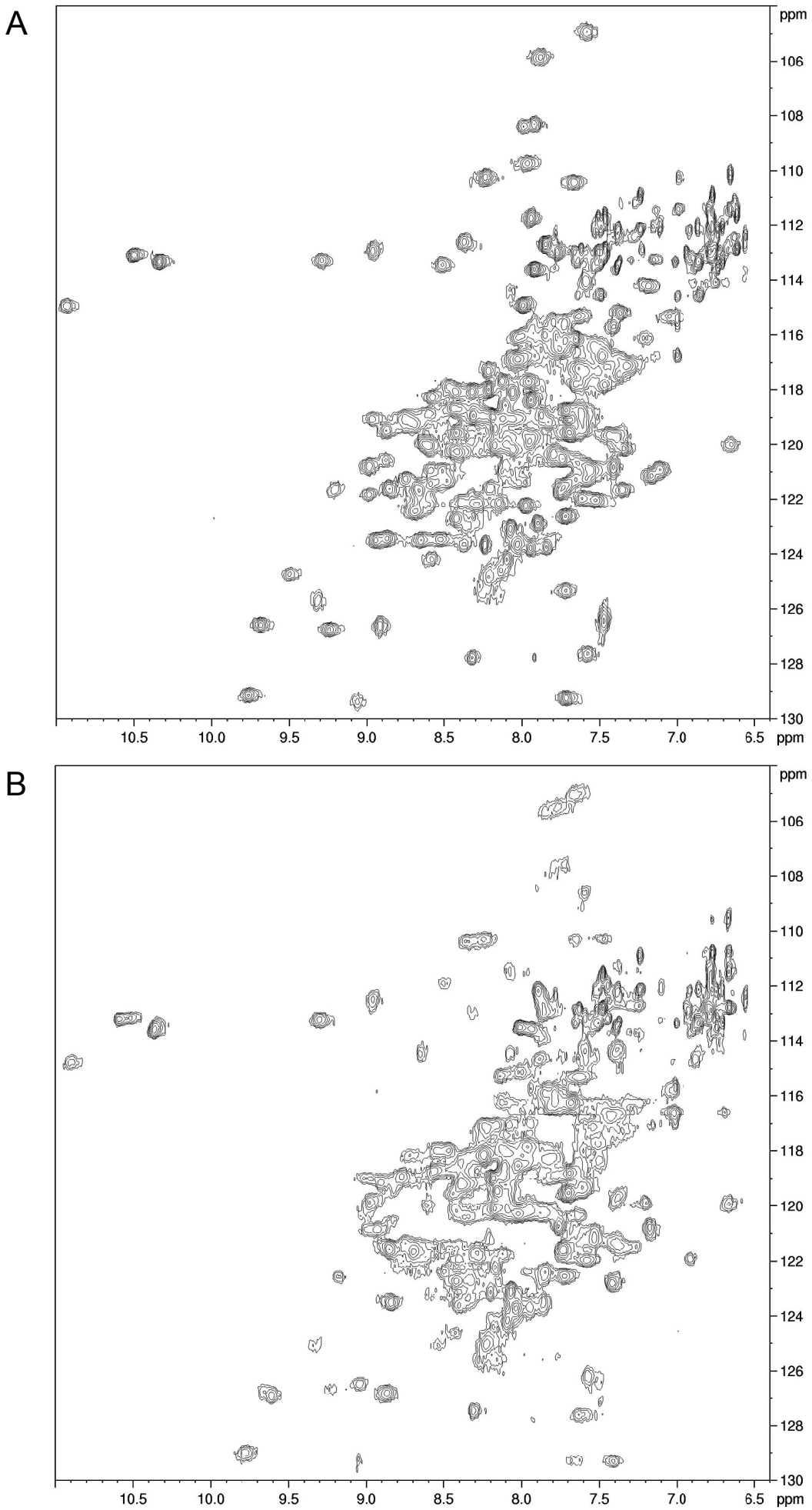
Figure 4: Activation of Kv4.3 channels is altered upon association with KChIP4a-delN.
Currents recorded in response to 250 ms voltage steps from -100 to voltages between -80 mV and 30 mV in giant inside-out patches excised from oocytes expressing the indicated channels were normalized to the current amplitude at 0 mV and plotted as a function of membrane potential. Note the difference of about 15 mV in the activation threshold between both channels.



Suppl. Fig. 1, Schwenk et al.



Suppl. Fig. 2, Schwenk et al.



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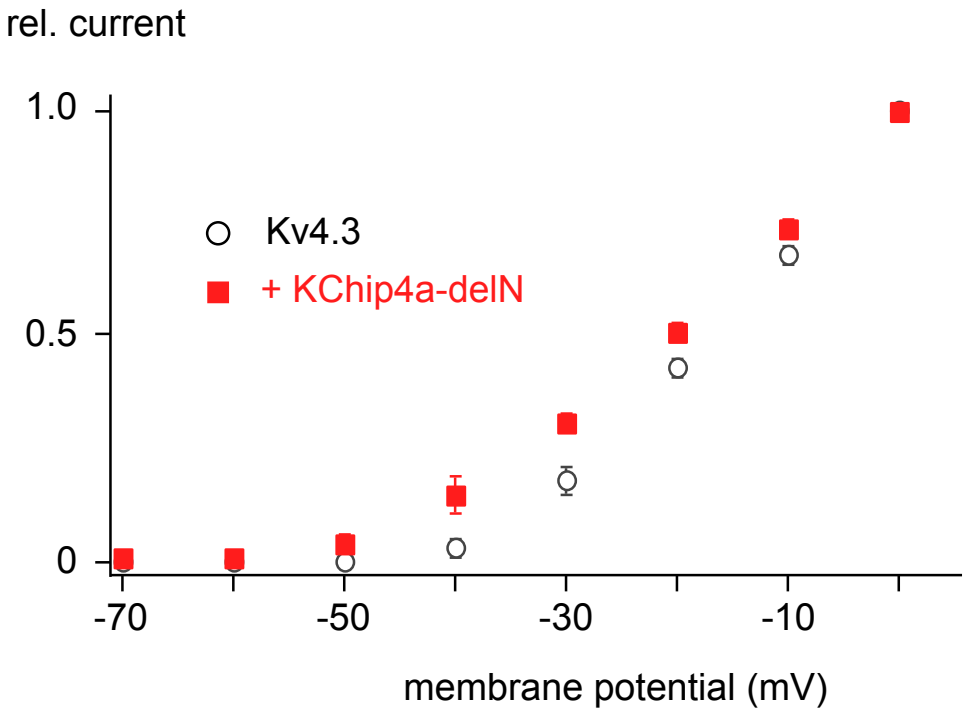

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