SUPPLEMENTARY INFORMATION

ARCHITECTURE OF THE HCN SELECTIVITY FILTER AND CONTROL OF CATION PERMEATION

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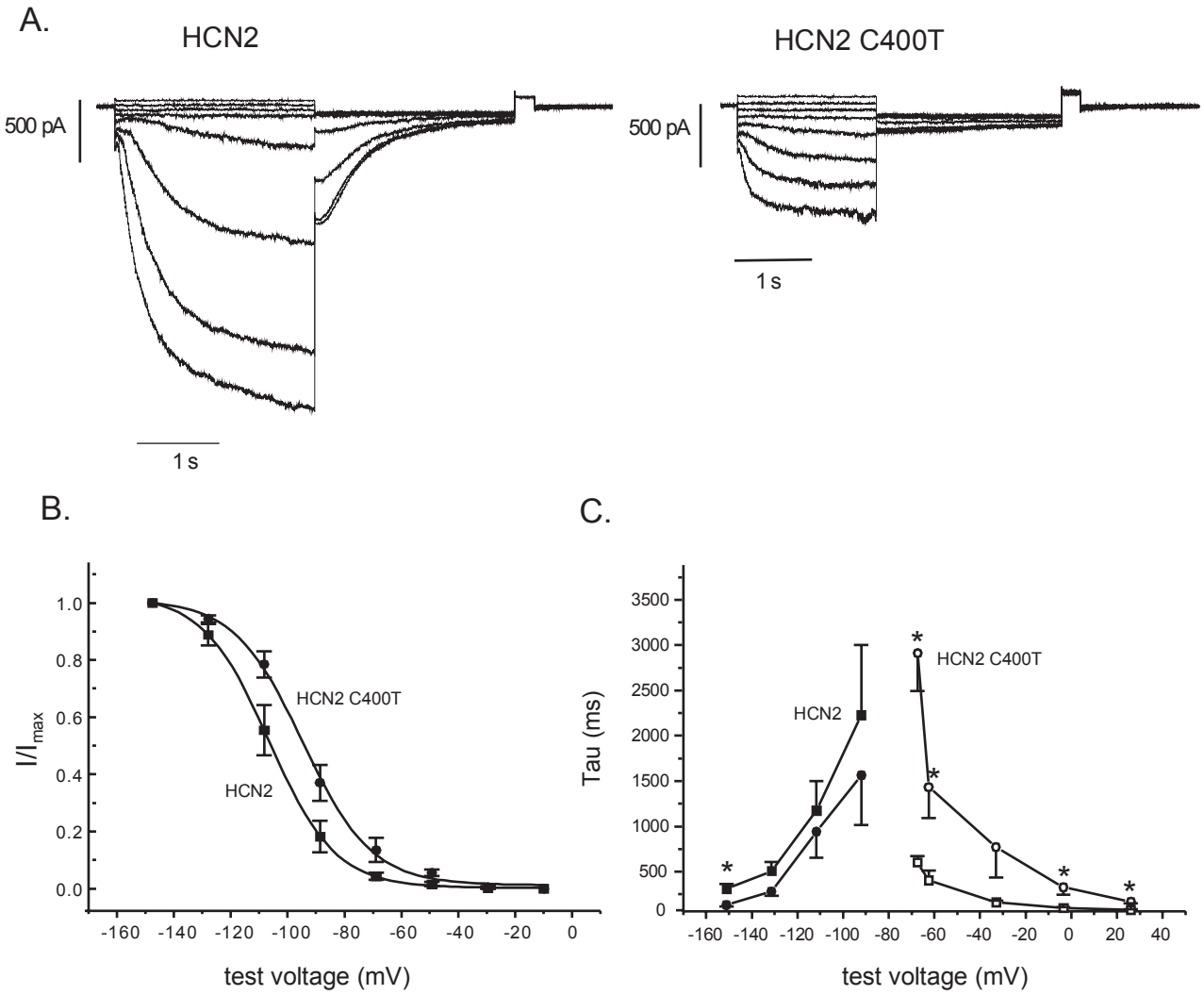
Supplemental Figure 1



Supplemental Figure 1. Substitution of cysteine 400 of HCN2 with threonine shifts the I_f activation curve to more positive potentials.

A. Current traces recorded from two representative cells in response to 2-second test voltage steps ranging from -50 mV to –150 mV, in 20 mV increments from a holding potential of -35 mV, and returned to –65 mV to generate deactivating tail currents. Note the slower rates of deactivation in the mutant channel.

B. I_f activation curves generated from tail current amplitudes recorded in CHO cells expressing HCN2 (squares) or HCN2 C400T (circles) at –65 mV and normalized to the largest tail current amplitude elicited, as indicated in 'A' (black arrow). The data were fit with a first order Boltzmann equation, $f(V) = I_{max}/(1 + e^{(V_{1/2}-V)/k})$. This fitting yielded V½ and k values of –107.7 ± 4.1 mV and 9.7 ± 1.0 mV (n = 5 cells) and –95.3 ± 3.2 mV and 11.7 ± 1.7 mV (n = 6 cells) for cells transfected with HCN2 or HCNC400T, respectively. Between the two groups of cells, V½ values were significantly different whereas the k values were not (unpaired t-test, $p<0.05$).

C. Time constants (τ) of I_f activation (filled symbols) or deactivation (closed symbols) versus test voltage determined from current traces recorded from CHO cells expressing HCN2 (squares) or HCN2 C400T (circles) (see Methods). Single asterisks indicate a significant difference between values for cells expressing HCN2 or HCN2 C400T.