Voltage-Gated HCN Channels

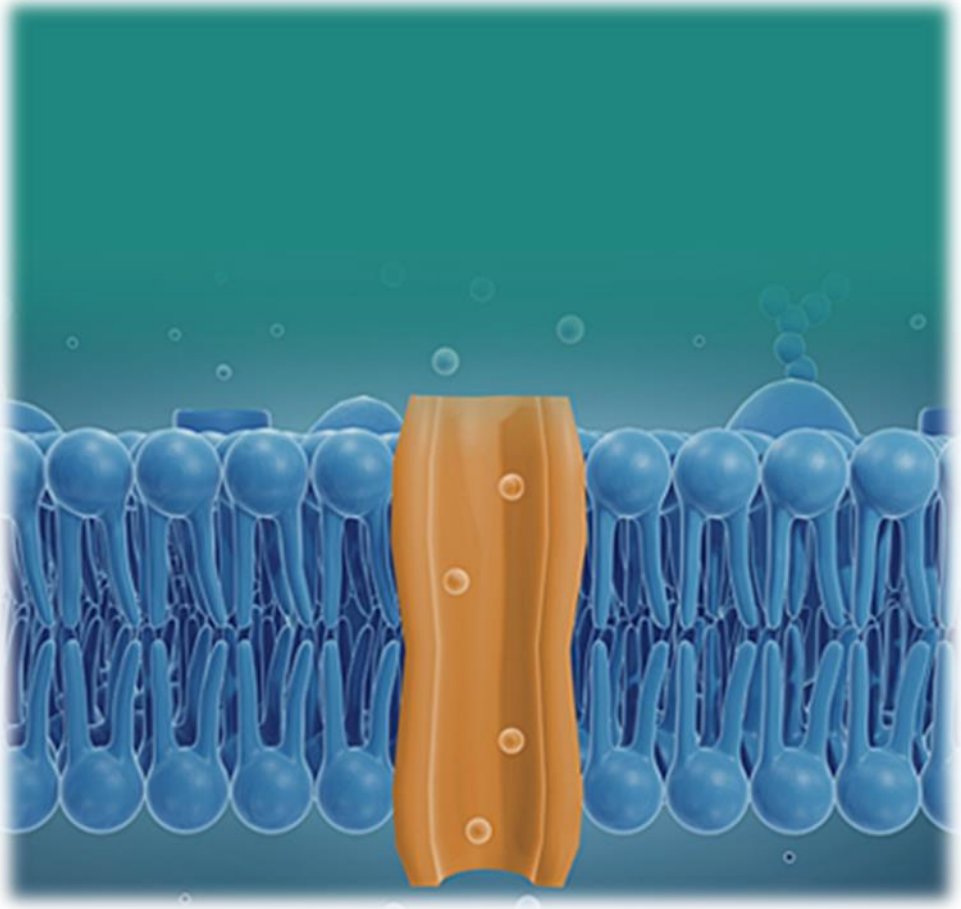
- Introduction to HCN Channels
- HCN Channelopathies and Drugs
- HCN References

Please press the "Back" button to return to the previous menu

BACK

Click Channel Type to Access Validation Data:

HCN1
HCN2
HCN3
HCN4



Introduction to HCN Channels

BACK

HCN channels are hyperpolarization activated cation-selective channels opened at hyperpolarized voltages more negative than about -50 mV. They are members of the cyclic nucleotide-gated (CNG) ion channel family. Cyclic AMP and cyclic GMP bind directly to the cyclic nucleotide binding domain (CNBD) and shift the channels voltage activation curves to more positive values, enhancing channel activity. They differ from CNG channels which are not gated by voltage. HCN channels are largely responsible for the pacemaker currents found in neurons, photoreceptors and cardiac cells in the sinoatrial (SA) node [1-5]. Historically, in native tissue these channels have been called I_q, I_f and I_h. The HCN channels consist of six transmembrane domains and they form tetramers, HCN1 and HCN4 have been shown to form heteromers [6].

Physiologically, sympathetic stimulation of SA node cells raises cAMP levels and increases HCN currents, this leads to depolarization between heartbeats and an increase in the heart rate during the fight-or-flight response. Parasympathetic release of acetylcholine binding to muscarinic acetylcholine receptors reduces the heart rate by hyperpolarizing pacemaker cells. HCN channels in neurons generate pacemaker potentials, are responsible for setting the resting potential, control synaptic transmission and plasticity, and electrically integrate dendritic activity [3,5].

There are four mammalian members of the HCN channel family (HCN1-HCN4) with approximately 60% sequence identity shared [7-10]. As with other voltage gated channels, the S4 segment of the channels largely contains the voltage sensor [47]. HCN channels when expressed in heterological systems generate currents typical of native I_h-like currents. They are activated by membrane hyperpolarization, are cation selective with a P_{Na}/P_K permeability ratio of ~0.2, they respond to cyclic nucleotide binding with a rightward shift in voltage-dependence, and are blocked by external Cs^+.

The voltage-activation of HCN2 and HCN4 are shifted by cyclic nucleotides, whereas HCN1 and HCN3 are only weakly affected [3,5]. HCN1 through HCN4 channels differ in their speed of activation with HCN1 being the fastest channel, followed by HCN2, HCN3, and HCN4. In mammalian cardiac SA node, HCN4 makes up 70% and HCN1 30% of the total I_h current [12-14]. HCN2 is the most abundant neuronal HCN channel, expressed widely in the brain while HCN1 is more localized in the hippocampus and HCN4 in the thalamus, HCN3 in the olfactory bulb and some hypothalamic nuclei [15].

HCN Channelopathies and Drugs

BACK

The HCN4 gene has been linked to sinus node dysfunction with over 20 mutations causing channelopathies including atrial fibrillation, AV block, QT prolongation, tachycardia-bradycardia syndrome sinus bradycardia [16]. HCN1 and HCN2 have been associated with human epilepsy in diverse ways including gain and loss of channel function [17]

Due to the involvement of cardiac HCN channels in pace making, these channels are promising therapeutic drug targets for the treatment of cardiac arrhythmias and ischemic heart disease. Blockers of I_h and HCN channels include ZD7288 which exhibits voltage-dependence of blockade [18]. Use-dependent blockers include ivabradine, zatebradine, and cilobradine [19-21]. Ivabradine is FDA approved for angina pectoris to lower the heart rate.

HCN References

BACK

1) DiFrancesco D. (1993) Pacemaker mechanisms in cardiac tissue. Annu Rev Physiol, 55: 455-72. [PMID:7682045]

2) Pape HC. (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu Rev Physiol, 58: 299-327. [PMID:8815797]

3) Biel M, Wahl-Schott C, Michalakis S, Zong X. (2009) Hyperpolarization-activated cation channels: from genes to function. Physiol Rev, 89 (3): 847-85. [PMID:19584315]

4) Pape HC. (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu Rev Physiol, 58: 299-327. [PMID:8815797]

5) Robinson RB, Siegelbaum SA. (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol, 65: 453-80. [PMID:12471170]

6) Altomare C, Terragni B, Brioschi C, Milanesi R, Pagliuca C, Viscomi C, Moroni A, Baruscotti M, DiFrancesco D. (2003) Heteromeric HCN1-HCN4 channels: a comparison with native pacemaker channels from the rabbit sinoatrial node. J Physiol (Lond.), 549 (Pt 2): 347-59. [PMID:12702747]

7) Gauss R, Seifert R, Kaupp UB. (1998) Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. Nature, 393 (6685): 583-7. [PMID:9634235]

8) Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M. (1998) A family of hyperpolarization-activated mammalian cation channels. Nature, 393 (6685): 587-91. [PMID:9634236]

9) Ludwig A, Zong X, Stieber J, Hullin R, Hofmann F, Biel M. (1999) Two pacemaker channels from human heart with profoundly different activation kinetics. EMBO J, 18 (9): 2323-9. [PMID:10228147]

10) Santoro B, Liu DT, Yao H, Bartsch D, Kandel ER, Siegelbaum SA, Tibbs GR. (1998) Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. Cell, 93 (5): 717-29. [PMID:9630217]

11) Männikkö R, Elinder F, Larsson HP. (2002) Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. Nature, 419 (6909): 837-41. [PMID:12397358]

12) Fenske S, Krause SC, Hassan SI, Becirovic E, Auer F, Bernard R, Kupatt C, Lange P, Ziegler T, Wotjak CT et al.. (2013) Sick sinus syndrome in HCN1-deficient mice. Circulation, 128 (24): 2585-94. [PMID:24218458]

HCN References

BACK

13) Ishii TM, Takano M, Xie LH, Noma A, Ohmori H. (1999) Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. J Biol Chem, 274 (18): 12835-9. [PMID:10212270]

14) Stieber J, Herrmann S, Feil S, Löster J, Feil R, Biel M, Hofmann F, Ludwig A. (2003) The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. Proc Natl Acad Sci USA, 100 (25): 15235-40. [PMID:14657344]

15) Cao-Ehlker X, Zong X, Hammelmann V, Gruner C, Fenske S, Michalakis S, Wahl-Schott C, Biel M. (2013) Up-regulation of hyperpolarization-activated cyclic nucleotide-gated channel 3 (HCN3) by specific interaction with K+ channel tetramerization domain-containing protein 3 (KCTD3). J Biol Chem, 288 (11): 7580-9. [PMID:23382386]

16) Verkerk AO, Wilders R. (2015) Pacemaker activity of the human sinoatrial node: an update on the effects of mutations in HCN4 on the hyperpolarization-activated current. Int J Mol Sci, 16 (2): 3071-94. [PMID:25642760]

17) DiFrancesco JC, DiFrancesco D. (2015) Dysfunctional HCN ion channels in neurological diseases. Front Cell Neurosci, 6: 174. [PMID:25805968]

18) BoSmith RE, Briggs I, Sturgess NC. (1993) Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (If) in guinea-pig dissociated sinoatrial node cells. Br J Pharmacol, 110 (1): 343-9. [PMID:7693281]

19) Bois P, Bescond J, Renaudon B, Lenfant J. (1996) Mode of action of bradycardic agent, S 16257, on ionic currents of rabbit sinoatrial node cells. Br J Pharmacol, 118 (4): 1051-7. [PMID:8799581]

20) Raes A, Van de Vijver G, Goethals M, van Bogaert PP. (1998) Use-dependent block of Ih in mouse dorsal root ganglion neurons by sinus node inhibitors. Br J Pharmacol, 125 (4): 741-50. [PMID:9831910]

21) Stieber J, Wieland K, Stöckl G, Ludwig A, Hofmann F. (2006) Bradycardic and proarrhythmic properties of sinus node inhibitors. Mol Pharmacol, 69 (4): 1328-37. [PMID:16387796]

HCN1 (CYL3040)

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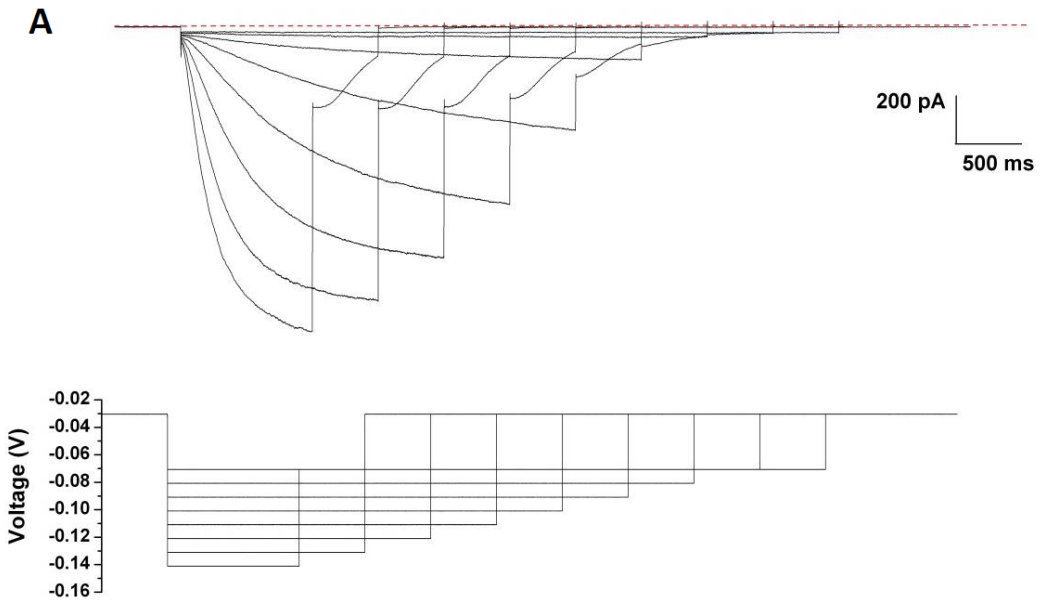
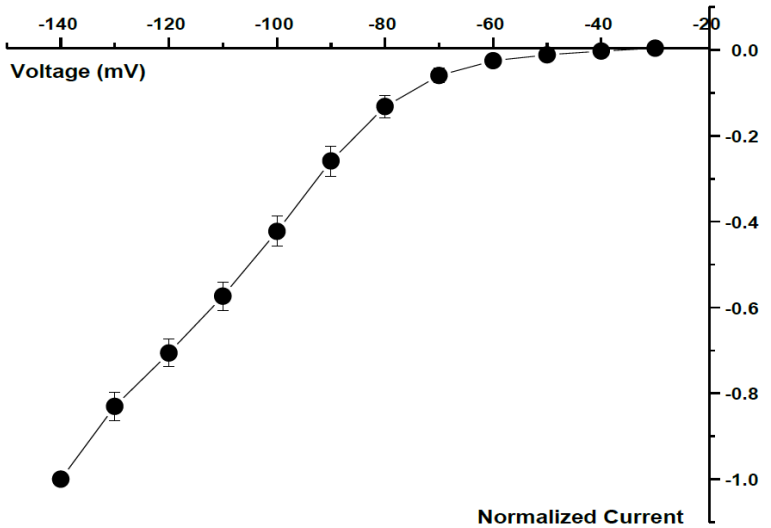
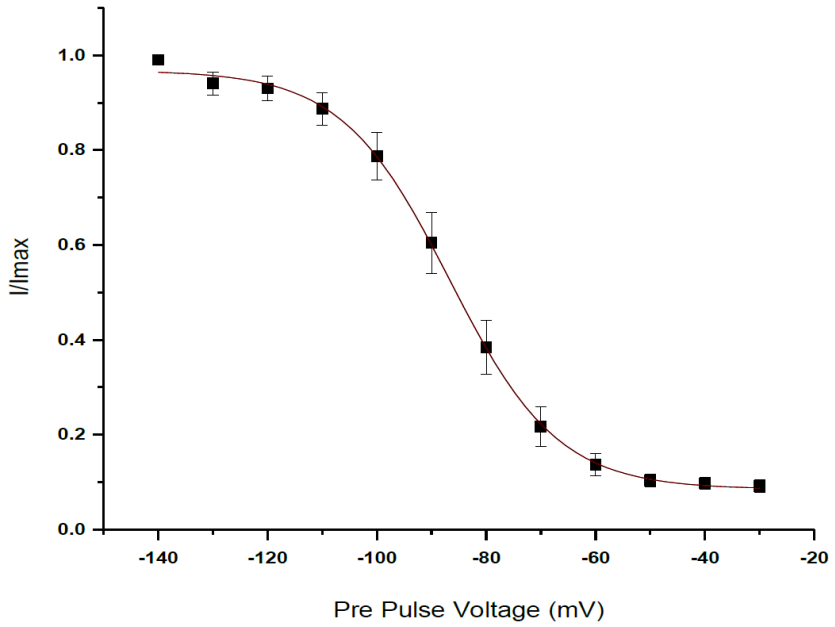
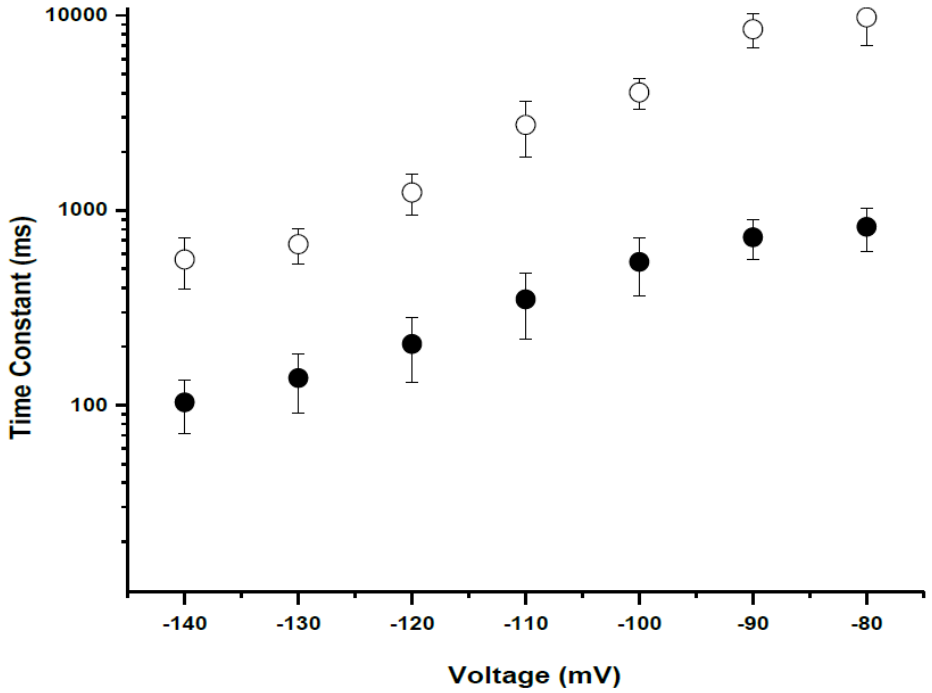




HCN1 Raw Data Currents and Current-Voltage (I/V) Relationship channels: **(Left)** Raw HCN1 currents elicited by the voltage protocol shown below the currents. Red dotted line indicates zero current. (**Right)** Current-Voltage (I/V) relationship. Maximum negative current amplitudes were measured and normalized to the maximum current amplitude obtained at -140 mV. Mean data from 10 cells is plotted (Manual patch-Clamp Data)

HCN1





BACK

HCN1 Voltage Dependence of Activation and Time Constants of Activation: **Left:** The instantaneous current on stepping back to -70 mV from various test potentials was measured for each cell and normalized to the instantaneous current obtained with a pre pulse to -140 mV. The mean data from 11 cells is shown that could be described by a Boltzmann equation giving an estimated V½ of -86.3 ± 3mV and a slope of 7.8 ± 0.6 mV. **Right:** Time Constants of Activation. On stepping to various hyperpolarizing voltages from a holding potential of -30 mV, hHCN currents activated following an exponential time course after an initial delay. hHCN1 time constants obtained by exponential fits decreased with increasing hyperpolarizing steps (solid circles) and at a given voltage were around 4-5x less than hHCN2 time constants (open circles) (Manual Patch Clamp Data).

HCN1

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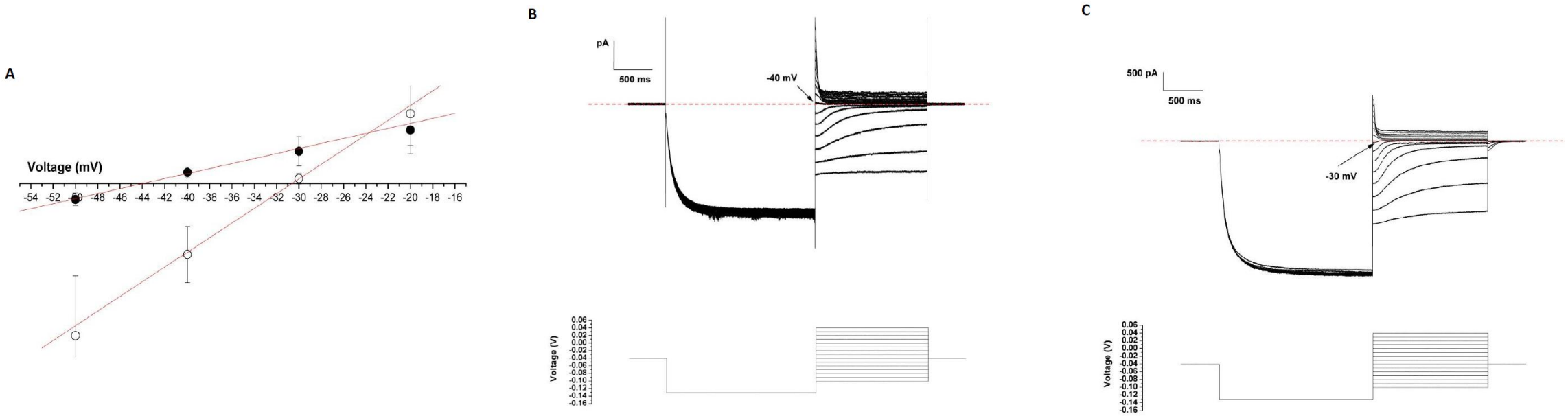

HCN1 Reversal Potential Measurements: The reversal potential was calculated either in low K^+ external solutions (solid circles in A) or high K^+ external solutions (open circles in A) by using the voltage protocols in B and C. The data is the mean of 3 and 4 cells respectively. Currents were activated by a hyperpolarizing voltage prior to stepping to various voltages negative and positive to the reversal potential i.e. where the instantaneous current crosses the zero current level (red dotted lines in B and C). In B, in low external K+ conditions, the current reverses near -40 mV whereas in high K+ external conditions in C, the current reverses near -30 mV (Manual Patch Clamp Data)

HCN1

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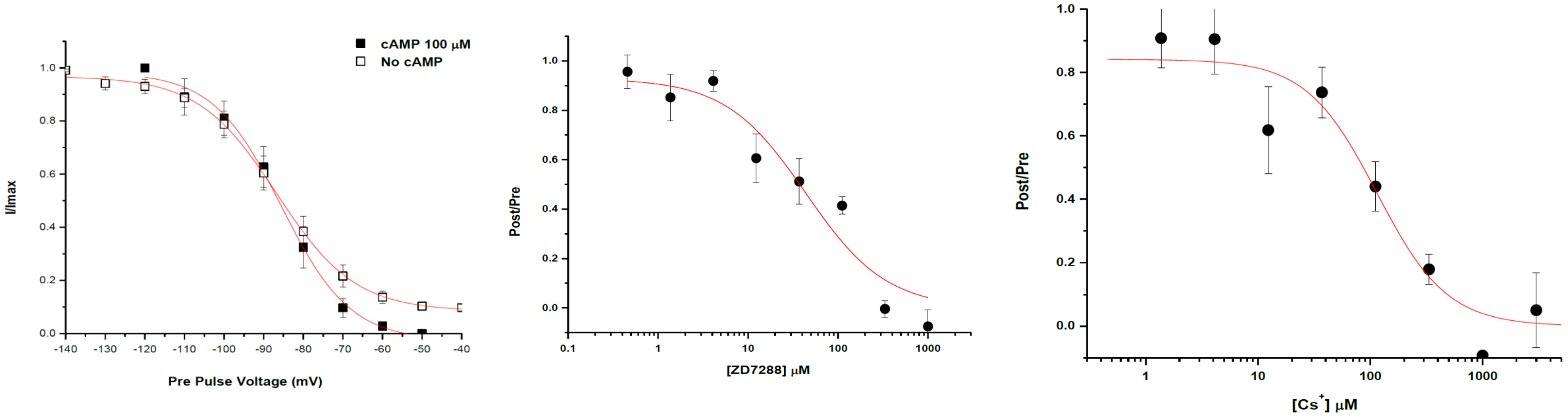

HCN1 Pharmacology: **Left:** The instantaneous current on stepping back to either -70 mV (no cAMP, open squares) or -50 mV (cAMP 100 µM, solid squares) from various test potentials was measured for each cell and normalized to the instantaneous current obtained with a pre pulse to -140 mV (no cAMP) or -120 mV (cAMP 100 µM). The control data (no cAMP) is from Figure 2 and the mean data in the presence of cAMP is from 3 cells. The data, in the presence of cAMP, could be described by a Boltzmann equation giving an estimated V½ of -84.3 ± 2.2mV and a slope of 7.1 ± 0.12 mV. (Manual Patch Clamp Data) **Middle:** Effect of ZD7288 on HCN1 currents. The effect of a 10 min incubation of various concentrations of ZD7288 was assessed on the amplitude of hHCN1 currents. Each data point represents the mean of 3-8 cells (IonWorks HT Data). **Right:** Effect of Cs+ on hHCN1 currents. The effect of a 10 min incubation of various concentrations of Cs+ was assessed on the amplitude of hHCN1 currents. Each data point represents the mean of 3-8 cells (IonWorks HT Data)

HCN1

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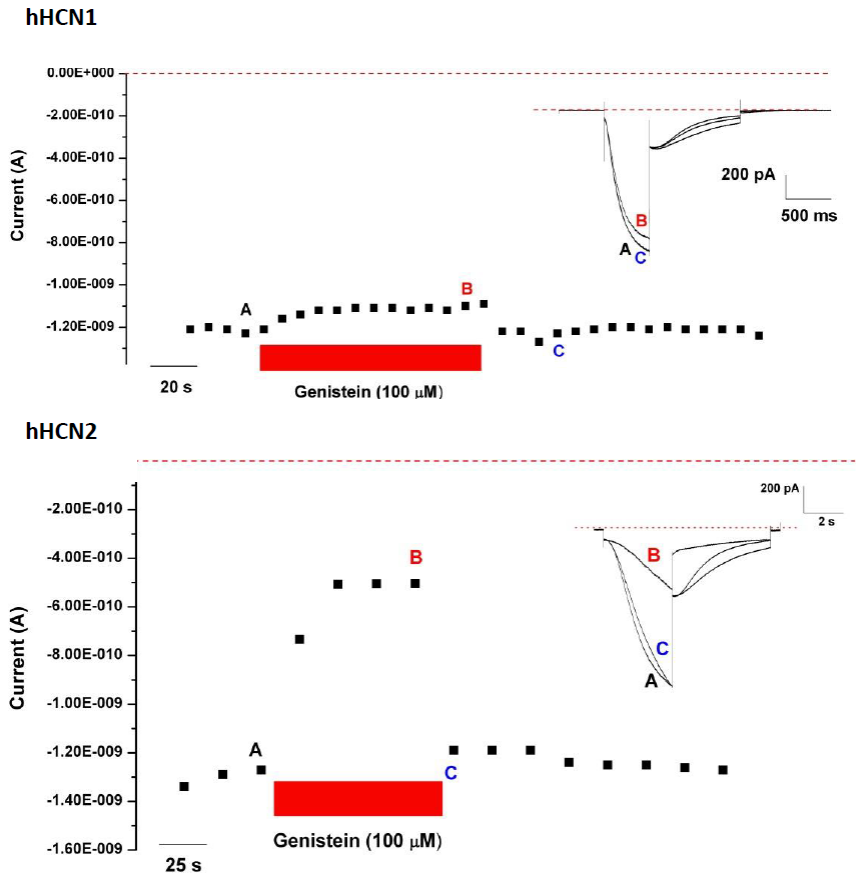

Effect of Genistein on hHCN1 and hHCN2 Currents: The voltage protocol used in the upper panel, to evoke hHCN1 currents, involved stepping from the holding potential of -30 mV to -140 mV then stepping back to -70 mV for 1 s prior to returning to the holding potential. Pulses were applied every 10 s. Peak currents at the end of the -140 mV step were measured before, during and after 100 µM genistein and plotted versus pulse number where the presence of genistein is depicted by the red bar. The inset shows current traces recorded before (A) during (B) and after (C) genistein application. The voltage protocol used to evoke hHCN2 currents in the lower panel was similar although the durations of the pulses were longer and peak current was assessed at -110 mV instead of -140 mV (Manual Patch Clamp Data).

HCN1

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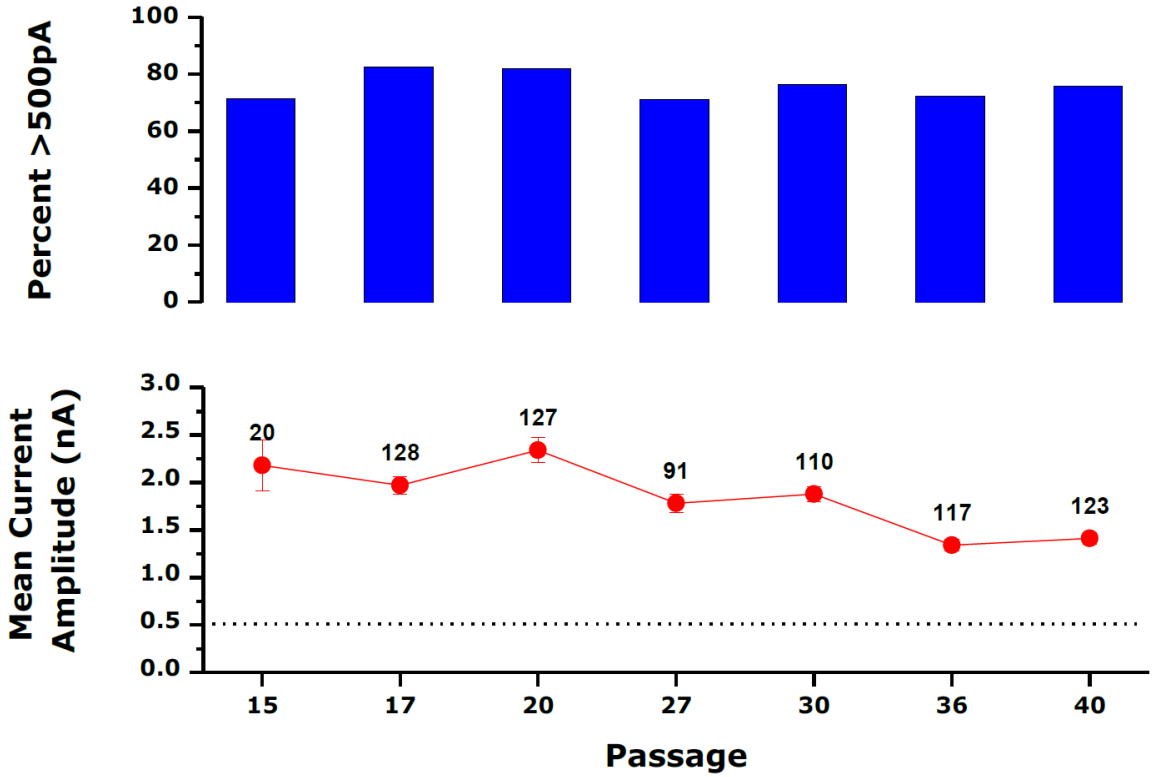
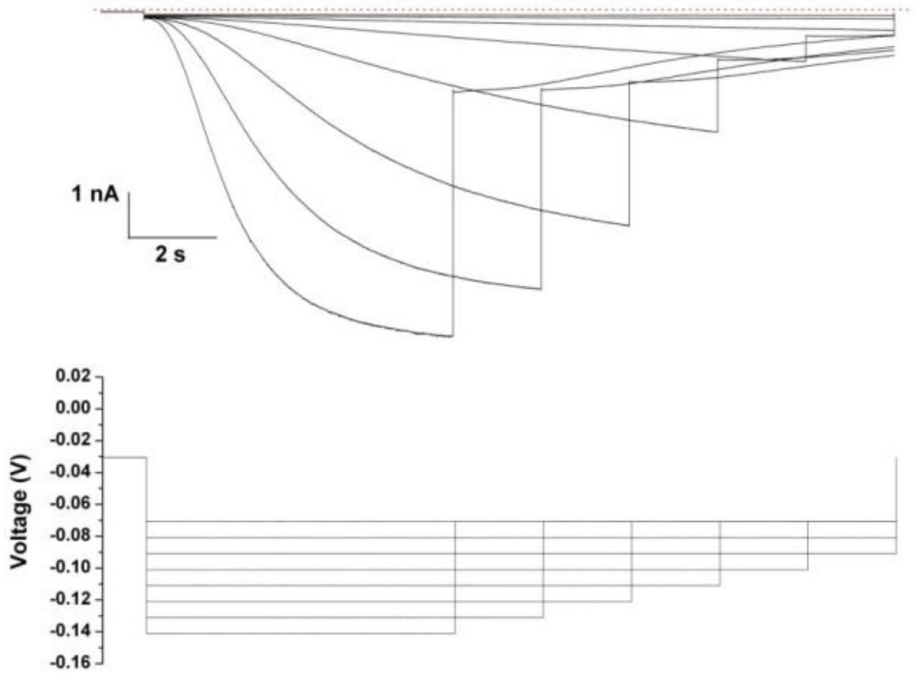
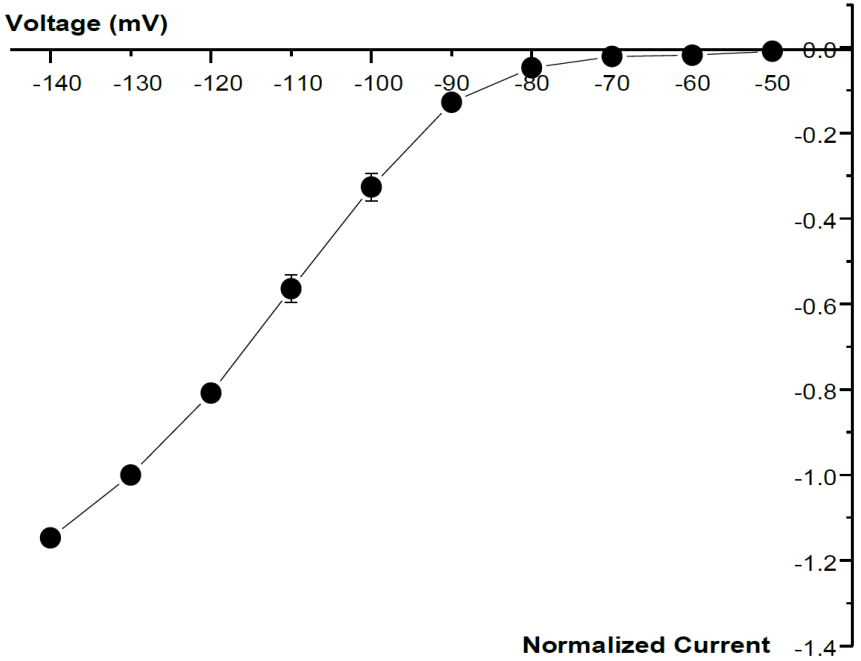


Stability of hHCN1 Over Passage Number: The upper panel shows the percentage of cells expressing a mean peak current >500 pA at -120 mV at cell passages 15, 17, 20, 27, 30, 36, and 40. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles - out of 32 cells for passage 15 and out of 192 cells for all other passages) (IonWorks HT Data).

HCN2 (CYL3041)

BACK





hHCN2 Raw Data Currents and Current-Voltage (I/V) relationship: Left: The upper panel represents typical current traces obtained by applying the voltage protocol in the lower panel. Red dotted line indicates zero current level. The maximum current amplitude was measured on stepping to the various hyperpolarizing potentials and normalized to the maximum current amplitude, obtained at -140 mV. **Right:** Mean data from 7 cells is plotted. (Manual Patch Clamp Data)

HCN2

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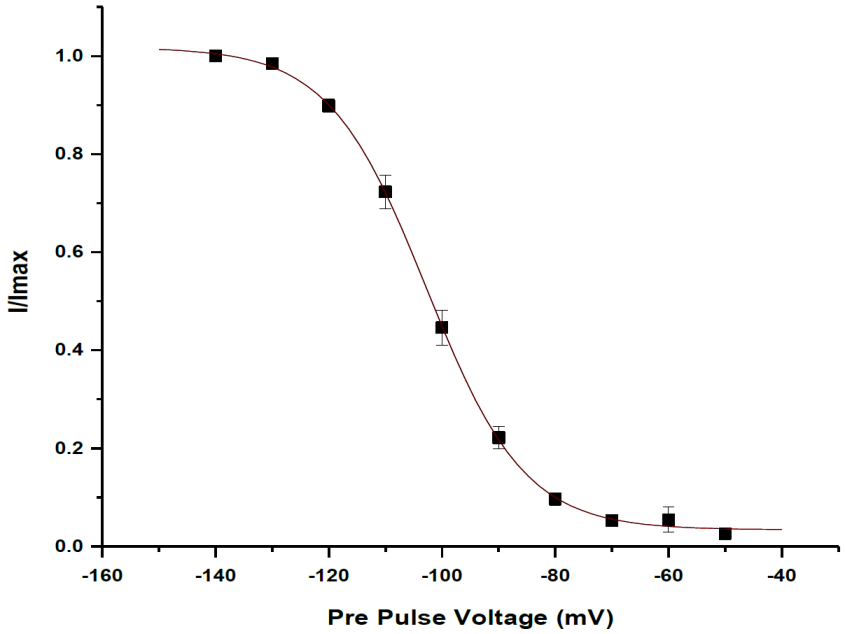

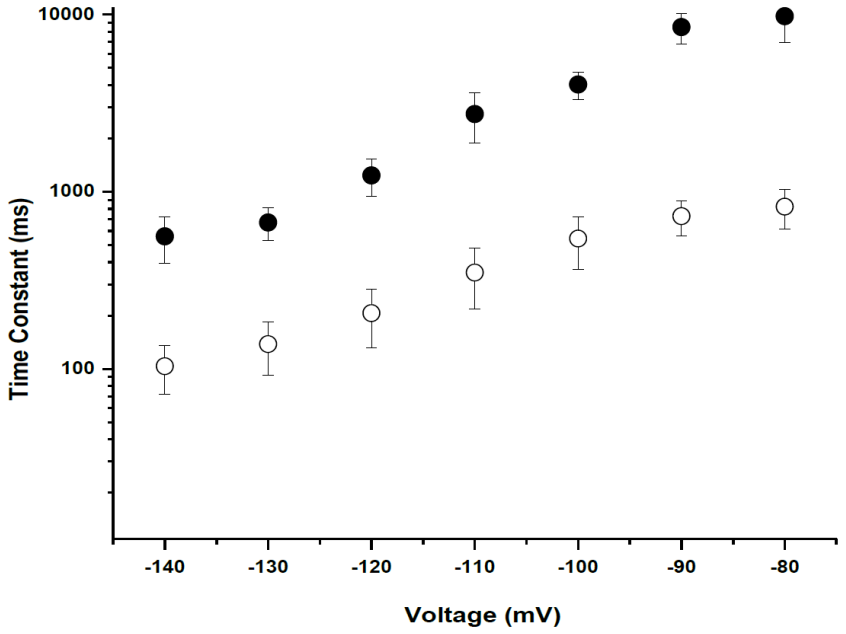

HCN2 Voltage Dependence of Activation and Time Constants of Activation: **Left:** The instantaneous current on stepping back to -70 mV from various test potentials was measured for each cell and normalized to the instantaneous current obtained with a pre pulse to -140 mV. The mean data from 7 cells is shown that could be described by a Boltzmann equation giving an estimated V½ of -102.6 ± 1.3mV and a slope of 8.4 ± 0.4 mV. **Right:** Time Constants of Activation. On stepping to various hyperpolarizing voltages from a holding potential of -30 mV, hHCN currents activated following an exponential time course after an initial delay. hHCN2 time constants obtained by exponential fits decreased with increasing hyperpolarizing steps (solid circles) and at a given voltage were around 4-5x greater than hHCN1 time constants (open circles) (Manual Patch Clamp Data)

HCN2

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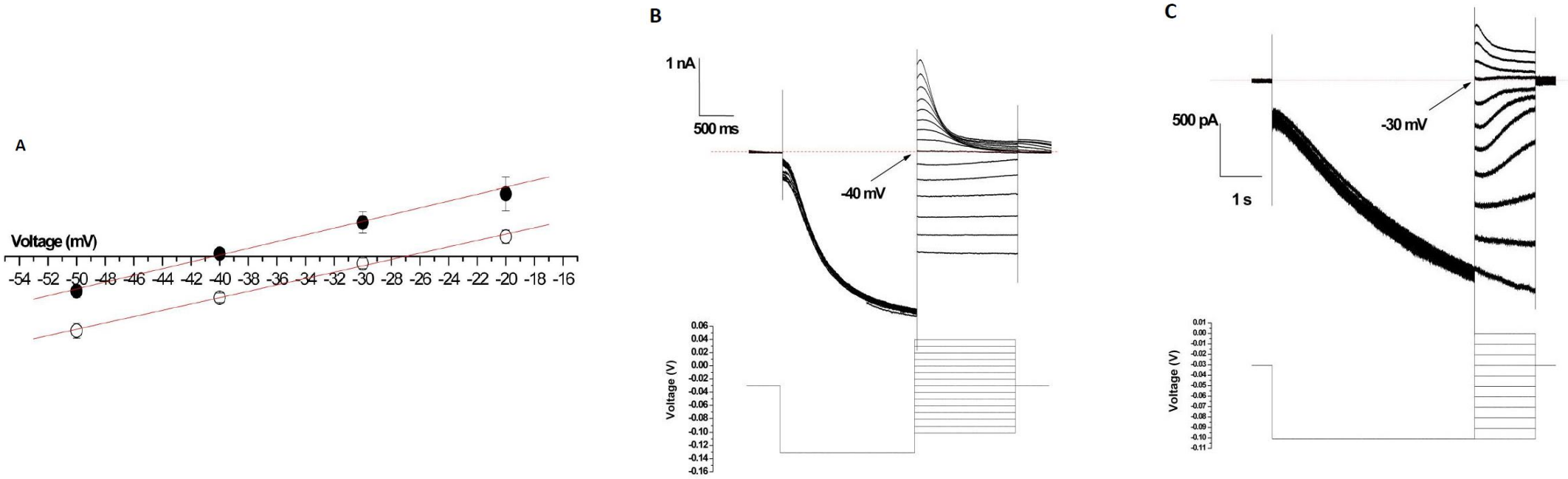
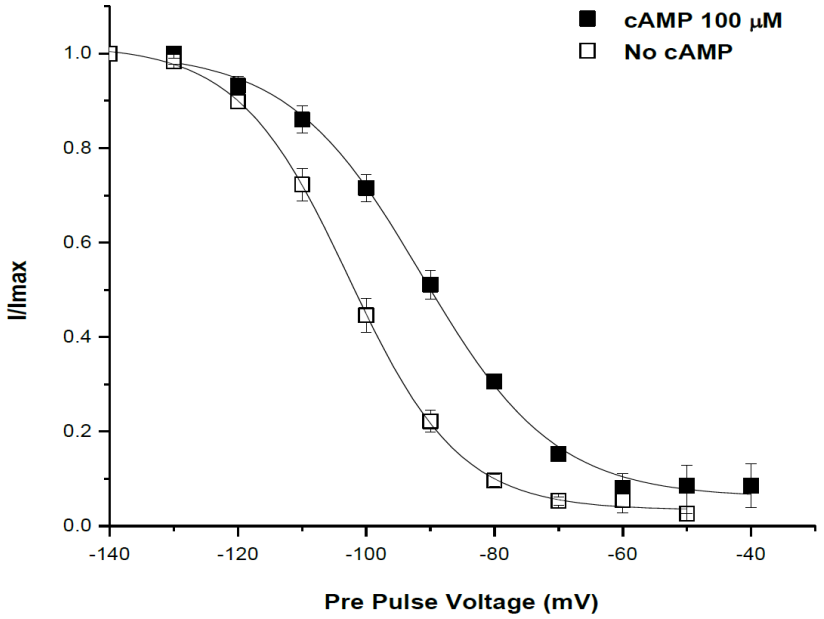
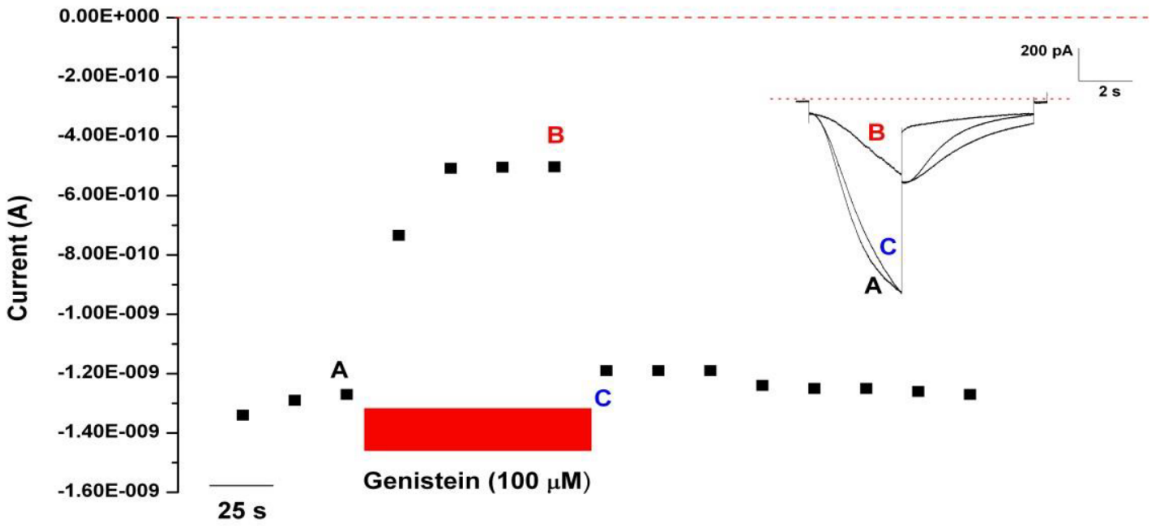


Reversal Potential Measurements. The reversal potential was calculated either in low K+ external solutions (solid circles in A) or high K+ external solutions (open circles in A) by using the voltage protocols in B and C. The data is the mean of 3 and 4 cells respectively. Currents were activated by a hyperpolarizing voltage prior to stepping to various voltages negative and positive to the reversal potential i.e. where the instantaneous current crosses the zero current level (red dotted lines in B and C). In B, in low external K+ conditions, the current clearly reverses at -40 mV whereas in high K+ external conditions in C, the current reverses at -30 mV (Manual Patch Clamp Data)

HCN2





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hHCN2 Pharmacology: **Left:** The instantaneous current on stepping back to either -70 mV (no cAMP, open squares) or -50 mV (cAMP 100 µM, solid squares) from various test potentials was measured for each cell and normalized to the instantaneous current obtained with a pre pulse to -140 mV (no cAMP) or -130 mV (cAMP 100 µM). The control data (no cAMP) and the mean data in the presence of cAMP is from 4 cells. The data could be described by a Boltzmann equation giving an estimated V½ of -90.3 ± 1.8mV and a slope of 11.6 ± 2.2 mV. **Right:** Effect of genistein on hHCN2 currents. The voltage protocol used in upper panel A, to evoke HCN2 currents, involved stepping from the holding potential of -30 mV to -110 mV for 3.5 s then stepping back to -70 mV for 5 s prior to returning to the holding potential. Pulses were applied every 20 s. Peak currents at the end of the -110 mV step were measured before, during and after 100 µM genistein and plotted versus pulse number where the presence of genistein is depicted by the red bar. The inset shows current traces recorded before (A) during (B) and after (C) genistein application. (Manual Patch Clamp Data)

HCN2

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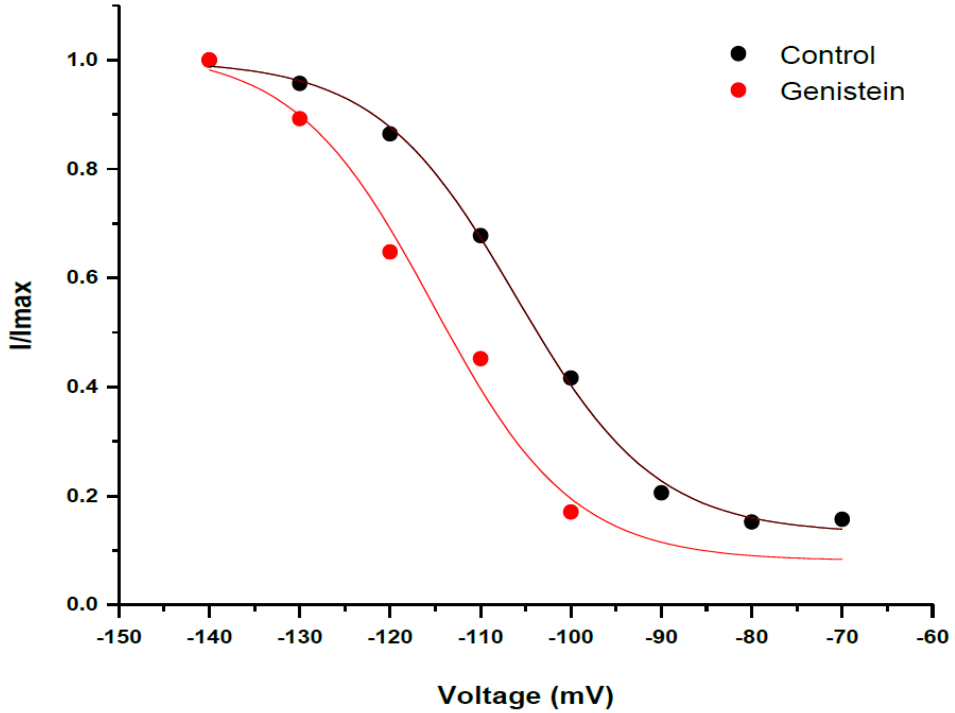

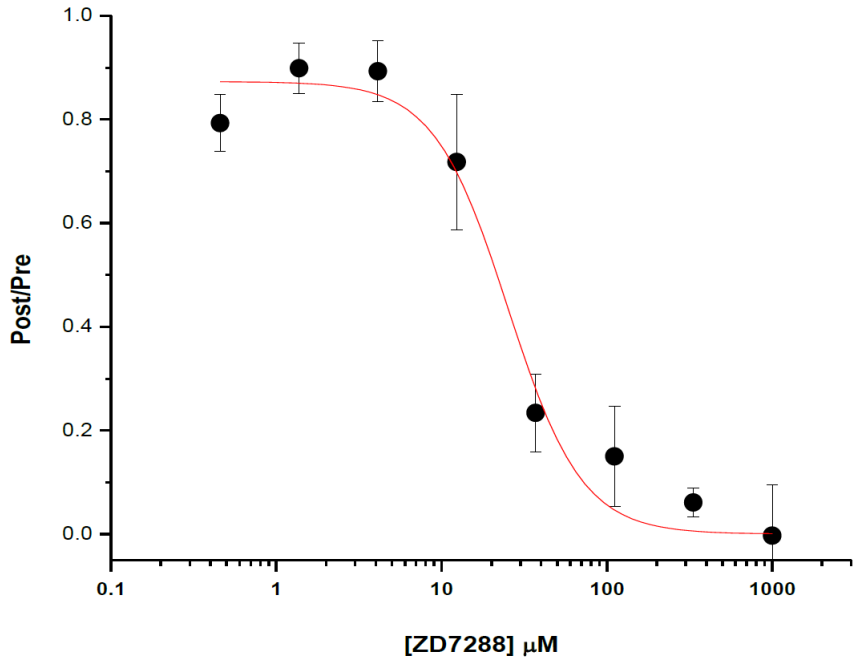

hHCN2 Pharmacology: **Left:** Genistein (100 µM) shifted the V½ of activation from -106 mV to -115 mV (Manual Patch Clamp Data) **Right:** Effect of ZD7288 on HCN2 currents. The effect of a 10 min incubation of various concentrations of ZD7288 was assessed on the amplitude of hHCN2 currents. Each data point represents the mean of 3-8 cells. (IonWorks HT Data)

HCN2

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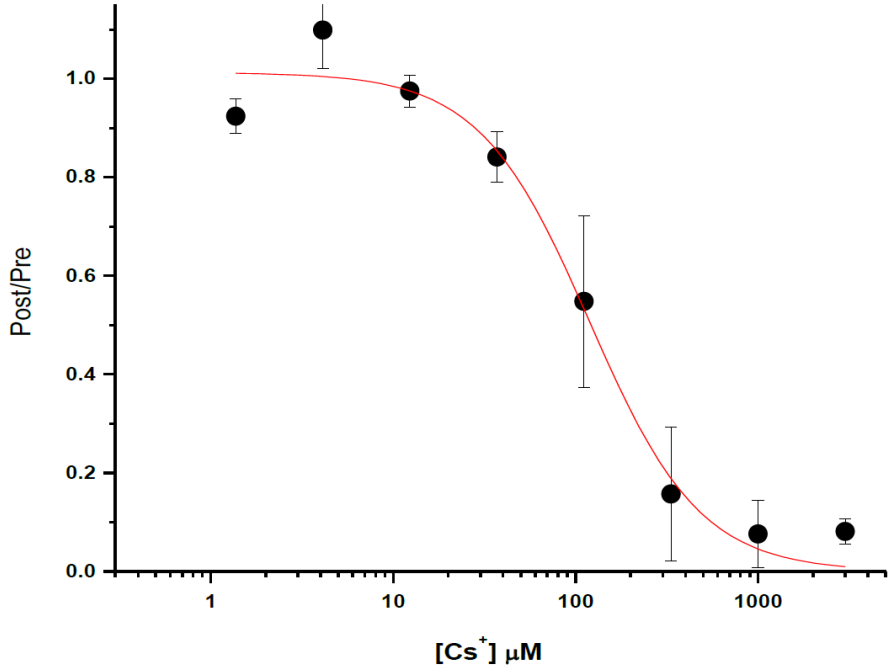

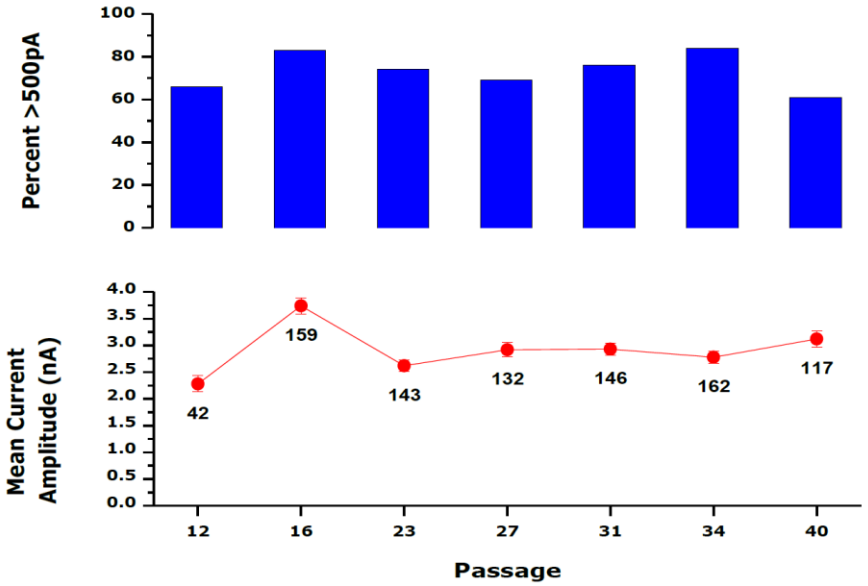

hHCN2 Pharmacology and Stability of Expression Over Passage: **Left:** Effect of Cs+ on hHCN2 currents. The effect of a 10 min incubation of various concentrations of Cs+ was assessed on the amplitude of hHCN2 currents. Each data point represents the mean of 3-8 cells. **Right:** The upper panel shows the percentage of cells expressing a mean peak current >500 pA at –120 mV at cell passages 12, 16, 23, 27, 31, 34, and 40. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers adjacent to red circles - out of 64 cells for passage 12 and out of 192 cells for all other passages) (IonWorks HT Data)

HCN3 (CYL3042)

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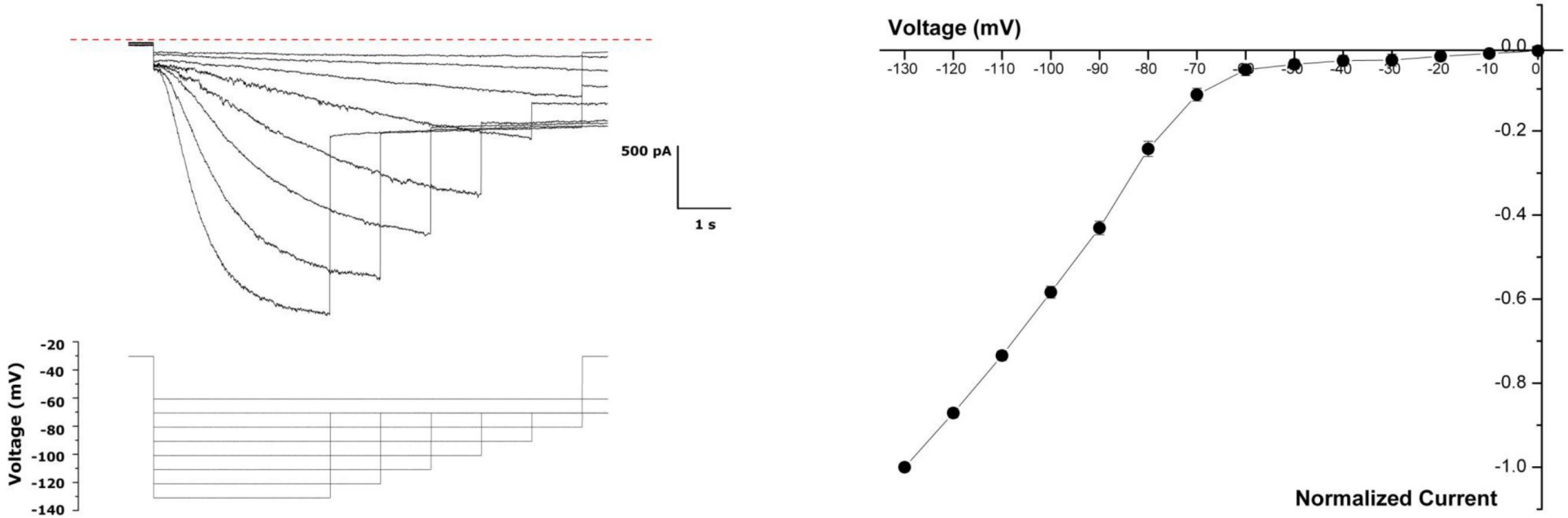

hHCN3 Raw Data Currents and Current-Voltage (I/V) Relationship: **Left:** The upper panel represents typical current traces obtained by applying the voltage protocol in the lower panel (see text for details). Red dotted line indicates zero current level. The maximum current amplitude was measured on stepping to the various hyperpolarizing potentials and normalized to the maximum current amplitude, obtained at -130 mV. **Right:** Mean data from 7 cells is plotted (Manual Patch Clamp Data)

HCN3

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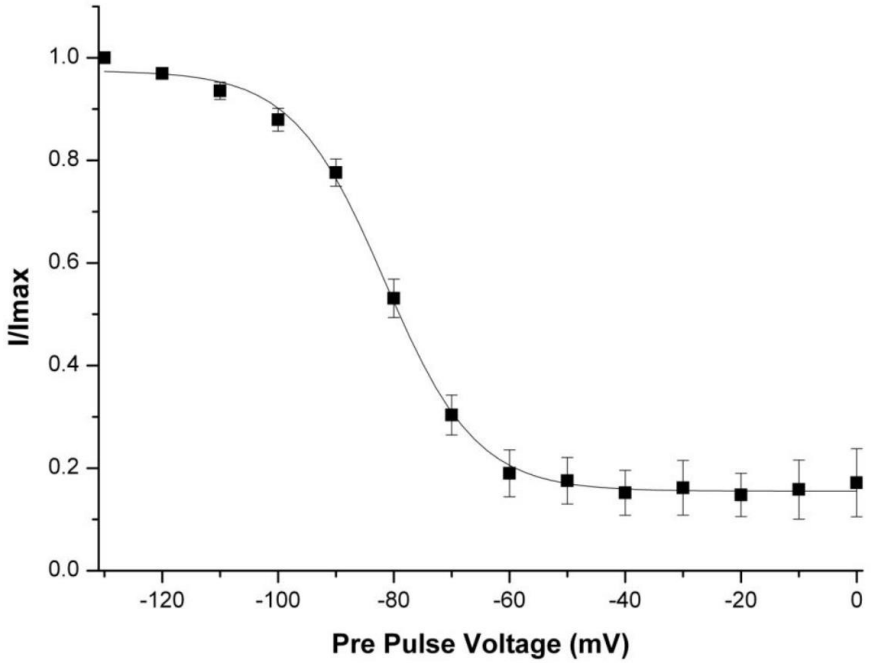

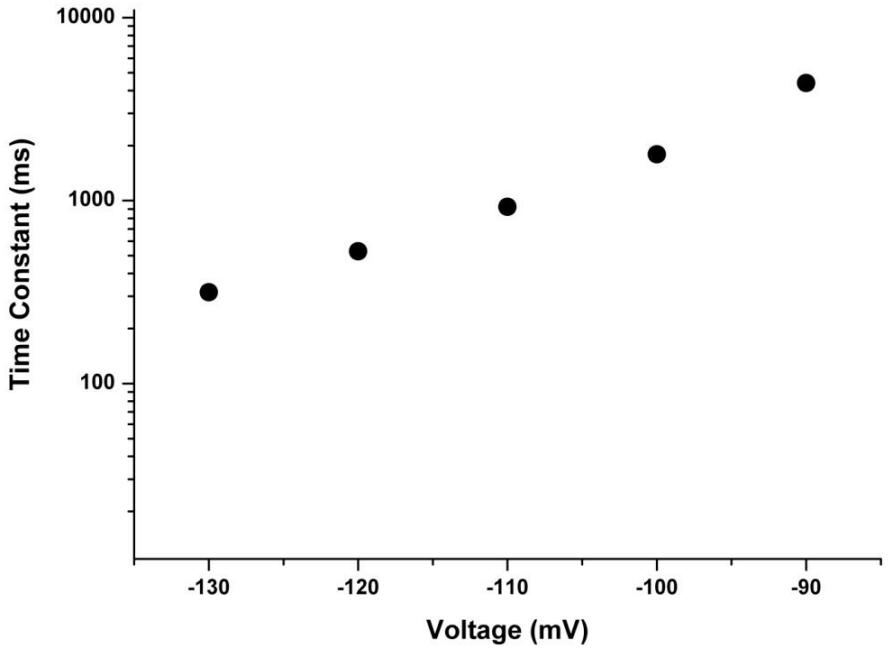

hHCN3 Voltage-Dependence and Time Constants of Activation: **Left**: The instantaneous current on stepping back to -70 mV from various test potentials was measured for each cell and normalized to the instantaneous current obtained with a pre pulse to -130 mV. The mean data from 7 cells is shown that could be described by a Boltzmann equation giving an estimated V½ of –81.6 ± 0.53 mV and a slope of 7.9 ± 0.5 mV. **Right:** On stepping to various hyperpolarizing voltages from a holding potential of -30 mV, hHCN3 currents activated following an exponential time course after an initial delay. The time constants of activation, obtained by exponential fits of the data, decreased with increasing hyperpolarizing steps (Manual Patch Clamp Data)

HCN3

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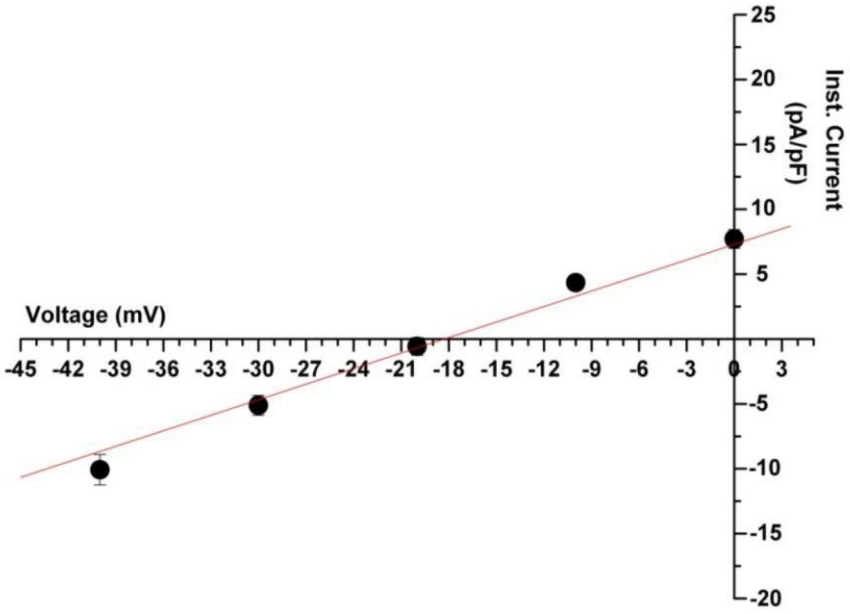
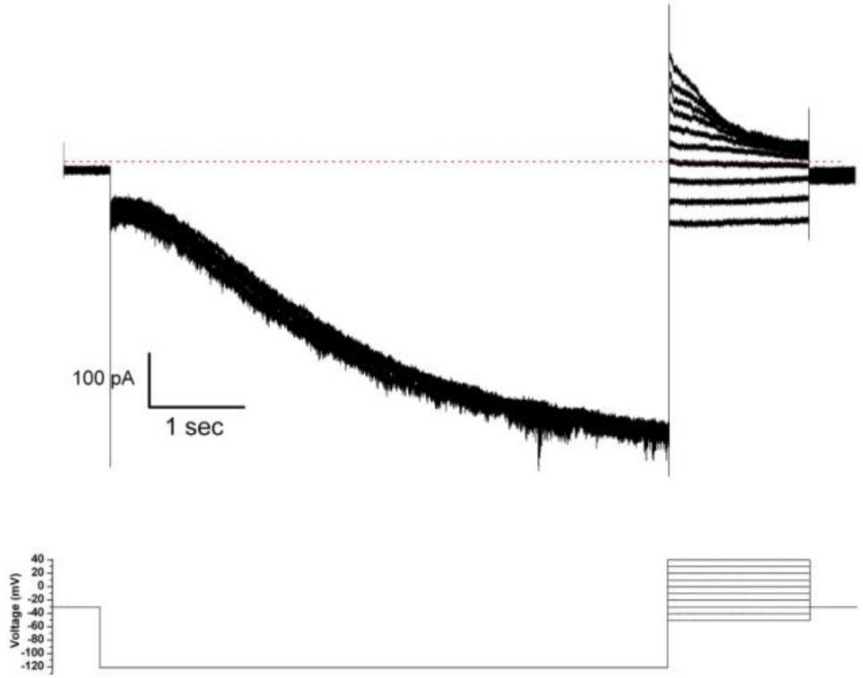
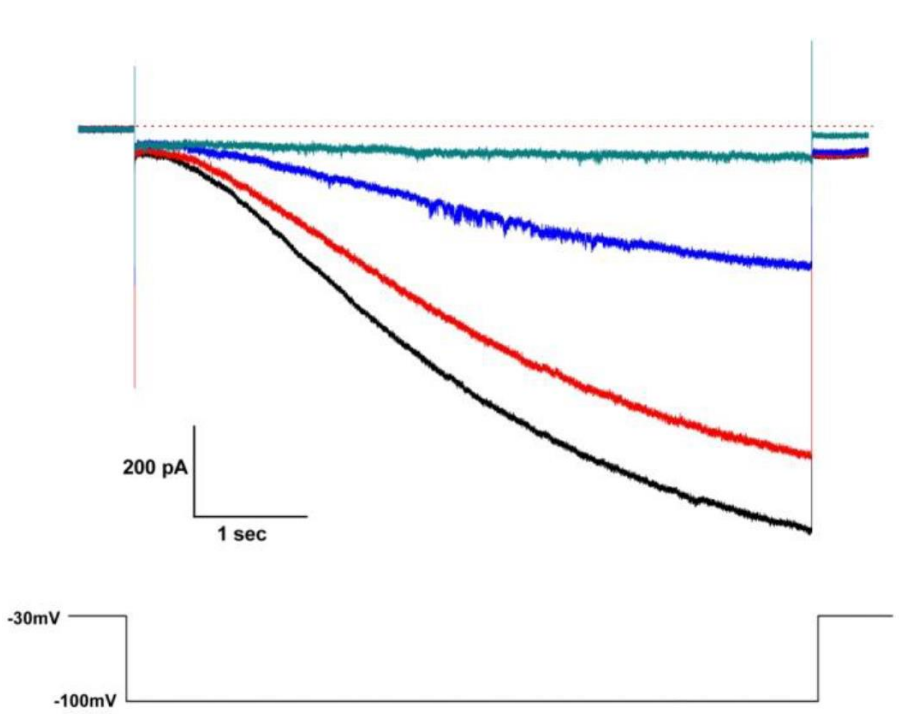


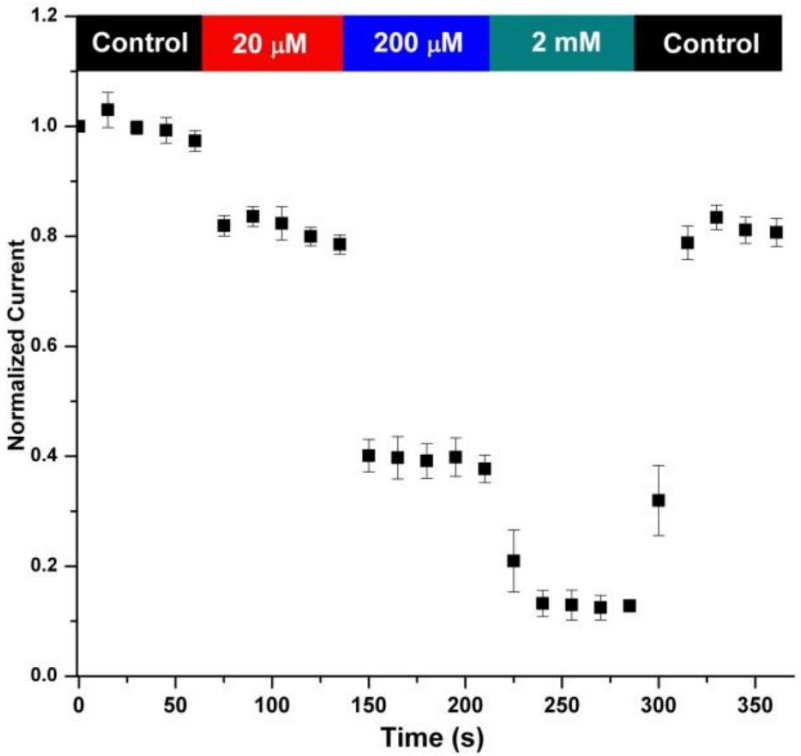


hHCN3 Reversal Potential Measurements: **Left:** Cells were held at –30mV and currents activated by a 6 second hyperpolarizing step to –120 mV prior to stepping to various voltages negative and positive to the reversal potential i.e. where the instantaneous current crosses the zero current level. The instantaneous current (pA/pF) is plotted against the corresponding test voltage in the lower panel (n = 4). A linear fit of the data gave a reversal potential of around -18 mV. **Right**: Typical traces from a representative cell. (Manual Patch Clamp Data)

HCN3

BACK





Effect of Cs^+ on hHCN3 Ionic Currents: Left: Sample trace of a cell held at –30 mV and pulsed to -100 mV for 6 seconds once every 15 seconds, increasing concentrations of Cs^+ were added as per the color coding in the right panel. **Right**: Increasing concentrations of Cs+ were applied to the cell in a cumulative manner until a stable response was seen. Peak current amplitudes in the presence of 20 µM, 200 µM and 2 mM Cs^+ were normalized to controls dose and plotted against time (upper panel, n = 4) (Manual Patch Clamp Data)

HCN3

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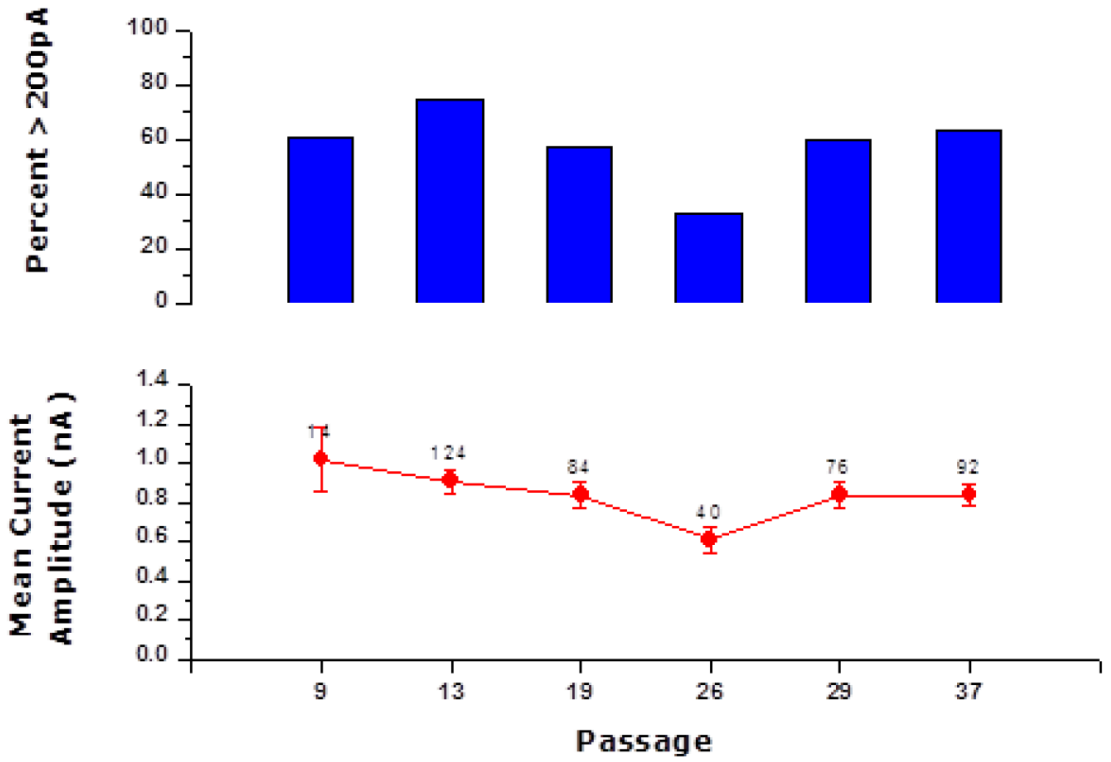

hHCN3 Stability of Expression Over Passage: The upper panel shows the percentage of cells expressing a mean peak current >200 pA at –120 mV at cell passages 9, 13, 19, 26, 29 and 37. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers adjacent to red circles - out of 32 cells for passage 9 and out of 192 cells for all other passages). (IonWorks HT Data)

HCN4 (CYL3012)

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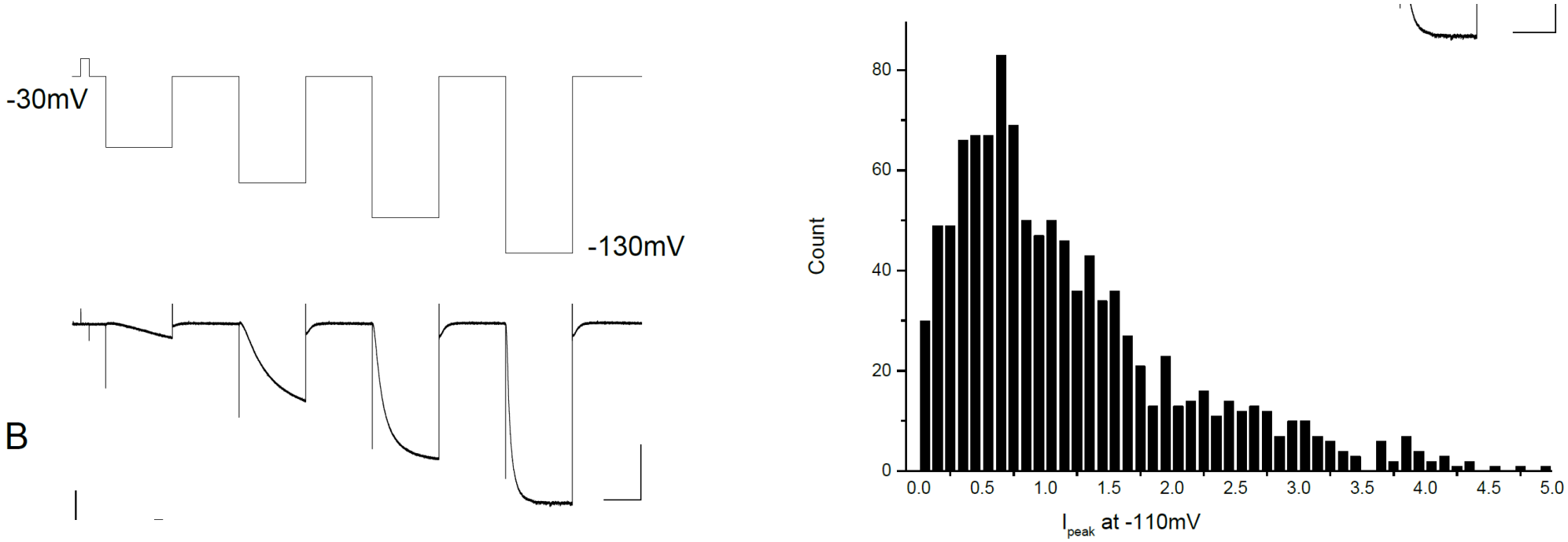

hHCN4 Ionic Currents: Left: Representative currents evoked from the voltage-command shown in the upper panel. Note the slowly activating inward current in response to hyperpolarising steps. Calibration bars 2 s and 500 pA. **Right:** Population histogram for peak (inward) current amplitude at –110 mV obtained from 1099 cells. (IonWorks HT Data).

HCN4

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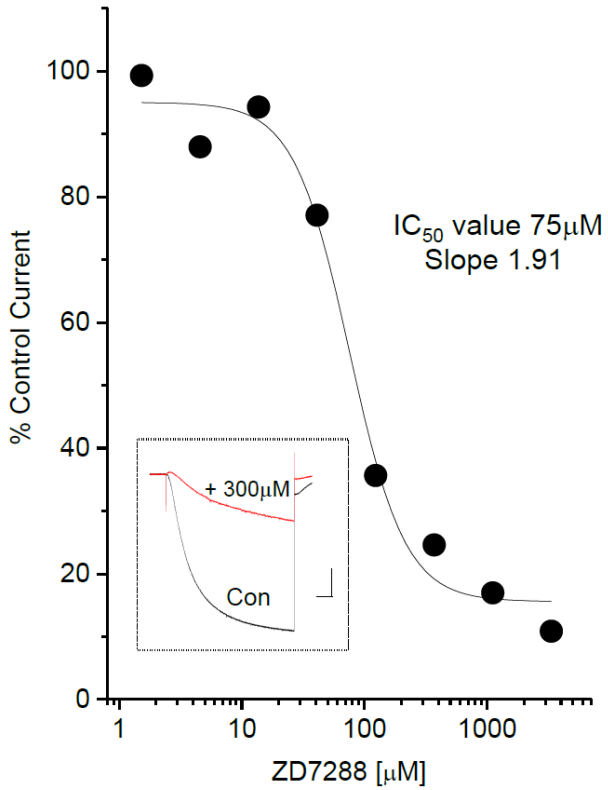

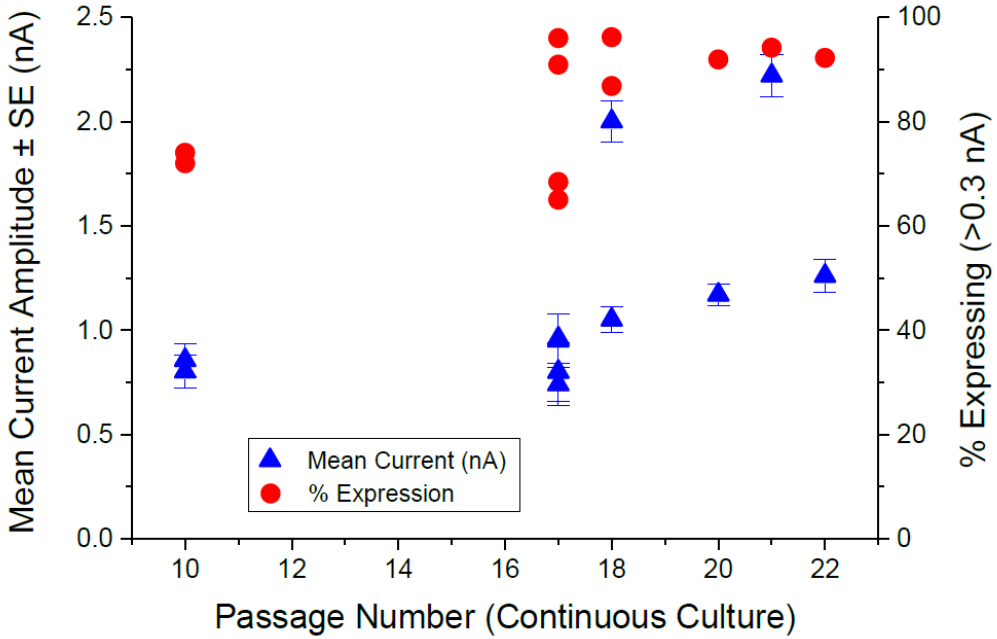

Inhibition of hHCN4 Currents by ZD7288 and Stability of Expression over Passage: **Left:** Concentration-response curve and representative current record (inset) for inhibition of hHCN4 currents by ZD7288 in stably expressing CHO-K1 cells. Each point represents the median value from 2-4 determinations (different cells). From the four parameter logistic equation the IC_{50} value was 75 µM and the Hill slope 1.91. hHCN4 currents were evoked by a test step of 4 s from –30 mV to –110 mV. The scale bars in the inset are 500 ms (x) and 500 pA (y), respectively. **Right:** Mean peak current for expressors (blue triangles) and % of cells expressing (red circles)). Each point represents the results from a single IonWorks HT run. (IonWorksHT Data).