Supplementary Materials: Selective Closed-State Nav1.7 Blocker JZTX-34 Exhibits Analgesic Effects against Pain

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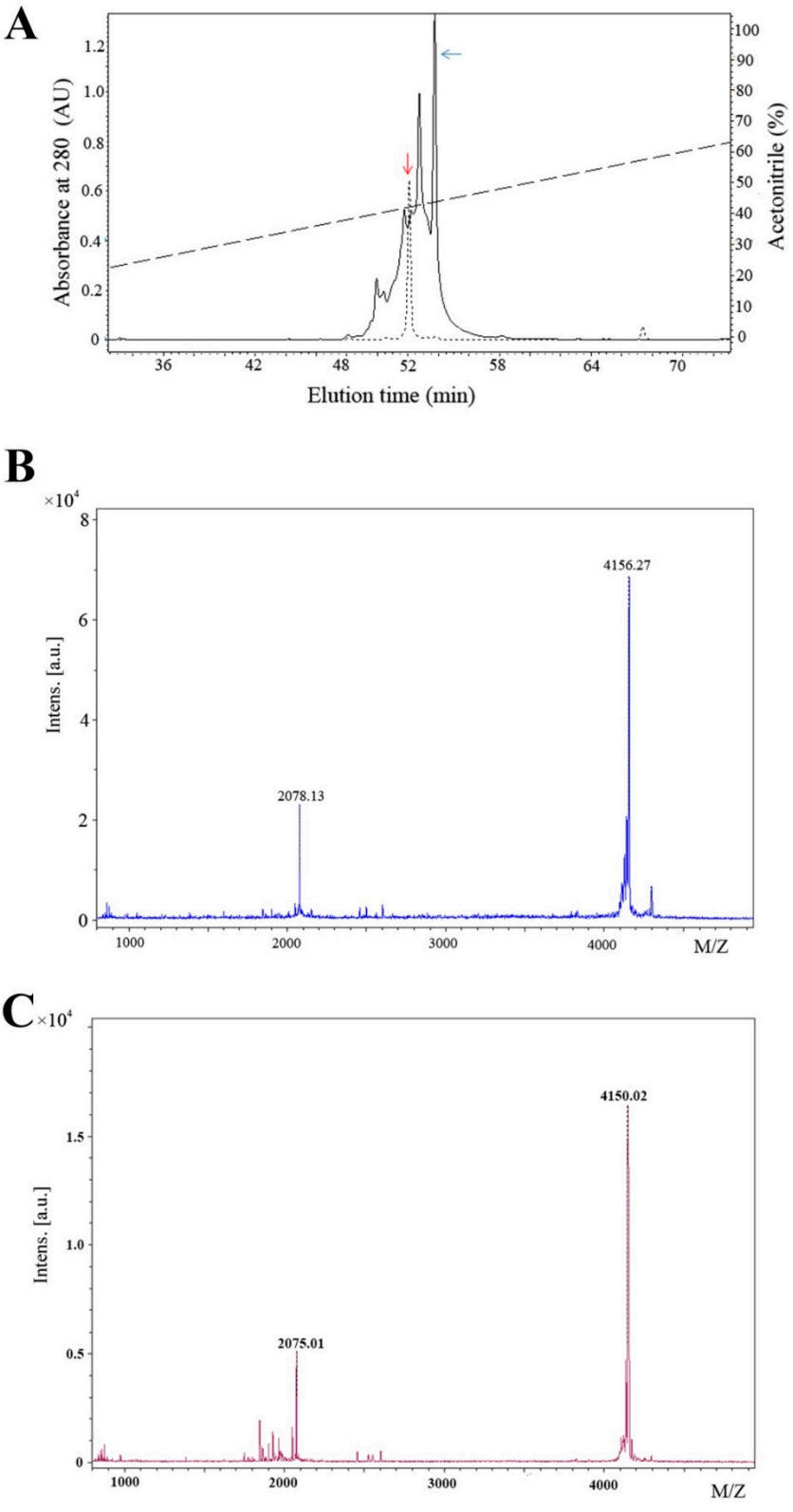


Figure S1. Identification of linear and refolded JZTX-34. (**A**) Linear and refolded JZTX-34 was separated by analytical reverse-phase HPLC (monitored at 280 nm). (**B**) MALDI-TOF mass spectra of synthetic JZTX-34. (**C**) MALDI-TOF mass spectra of refold JZTX-34.

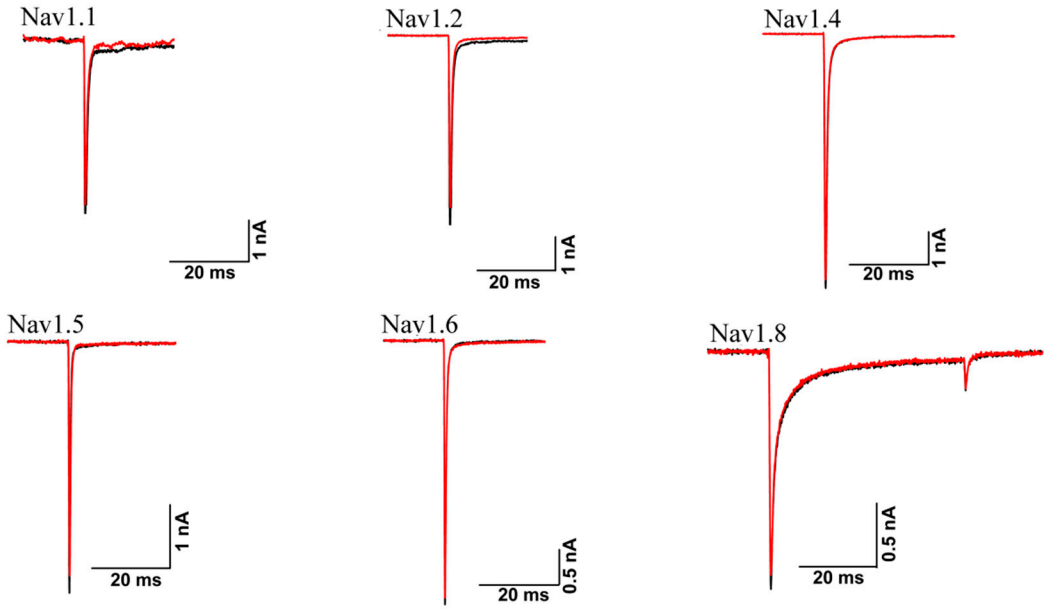
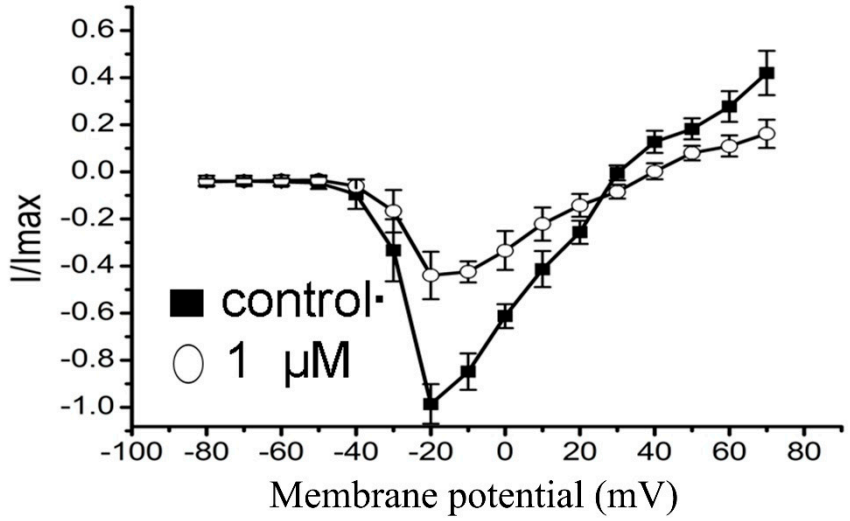
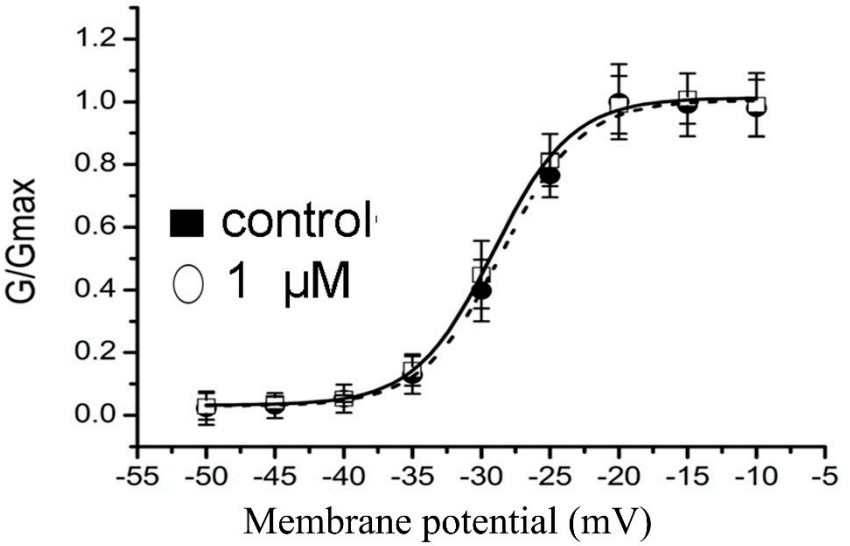


Figure S2. 1 μM JZTX-34 showed no effect on Nav1.1, 1.2, 1.4, 1.5, 1.6 and 1.8. All inward sodium channels were elicited by a 50 ms depolarizing potential of −10 mV from a holding potential of −80 mV every 5 s.


I/Imax
control
1 μM
Membrane potential (mV)


G/Gmax
control
1 μM
Membrane potential (mV)

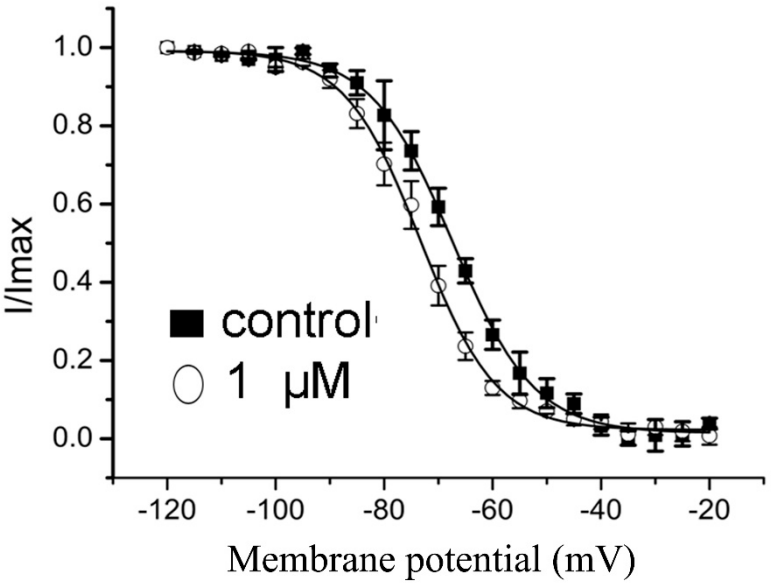

I/Imax
control
1 μM
Membrane potential (mV)

Figure S3. Effects of JZTX-34 on the current-voltage relationship and the steady-activation and inactivation of sodium channel subtypes Nav1.7. (**A**) Effect of 1 μM toxin on the current-voltage relationship of sodium currents. Cells were held at −80 mV, and sodium currents were induced by 50 ms depolarizing steps to various potentials ranging from −80 to +80 mV with 10 mV increments. (**B**) Effect of 1 μM toxin on the steady-activation of Nav1.7. The steady-activation kinetics was estimated based on the data from A. The conductance was calculated using the equation G(Nav) = I/(V-$Vrev$) in which I, V, $Vrev$ represent inward current value, membrane potential, and reversal potential, respectively. Data were plotted as a fraction of the maximum conductance. (**C**) Effect of 1μM toxin on steady-state inactivation of Nav1.7. The voltage dependence of steady-state inactivation was estimated using a standard double pulse protocol in which sodium currents were induced by a 20 ms depolarizing potential of -10mV following a 500 ms prepulse at potentials that ranged from −100 mV to −10 mV. Currents were plotted as a fraction of the maximum peak current. Data points (mean±S.E.) were fitted according to the boltzmann equation (n=5).