



## **Supplemental Material to:**

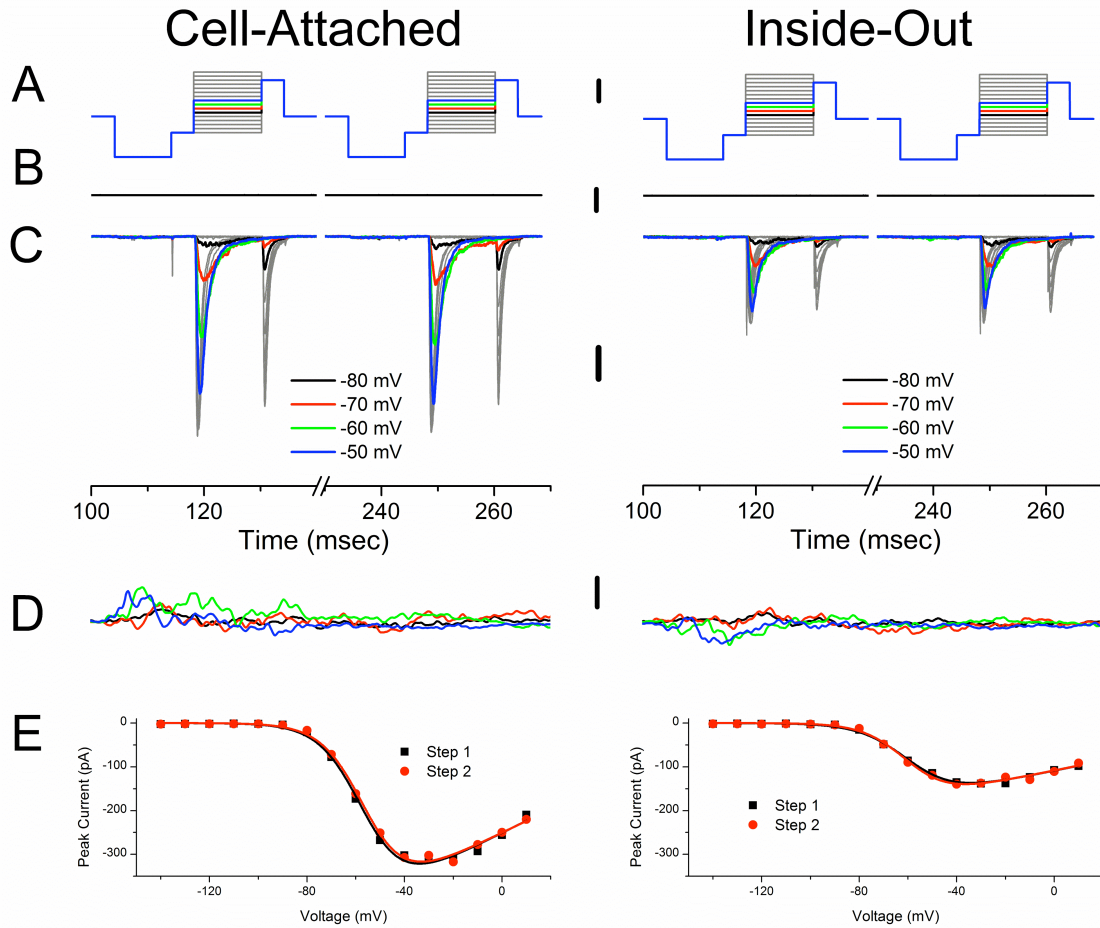
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and Gianrico Farrugia**

**Membrane permeable local anesthetics modulate NaV1.5  
mechanosensitivity**

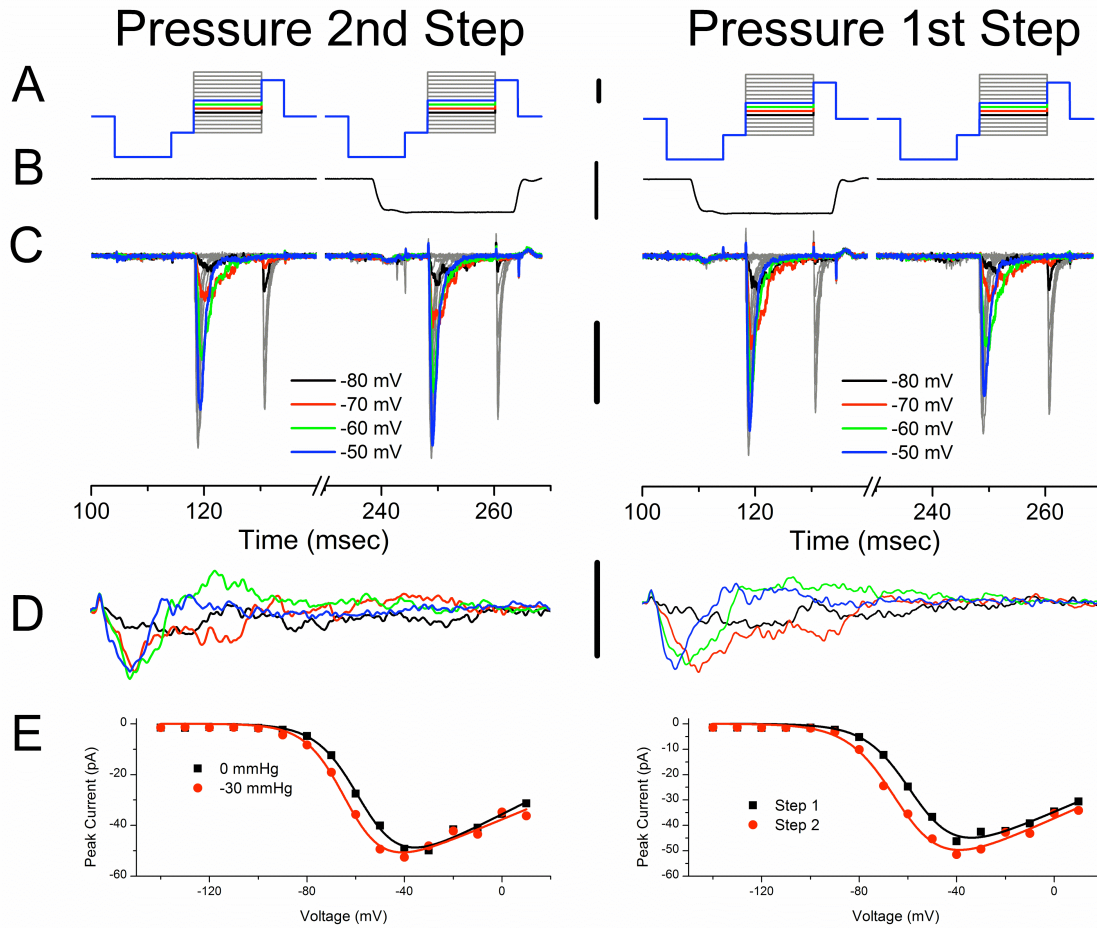
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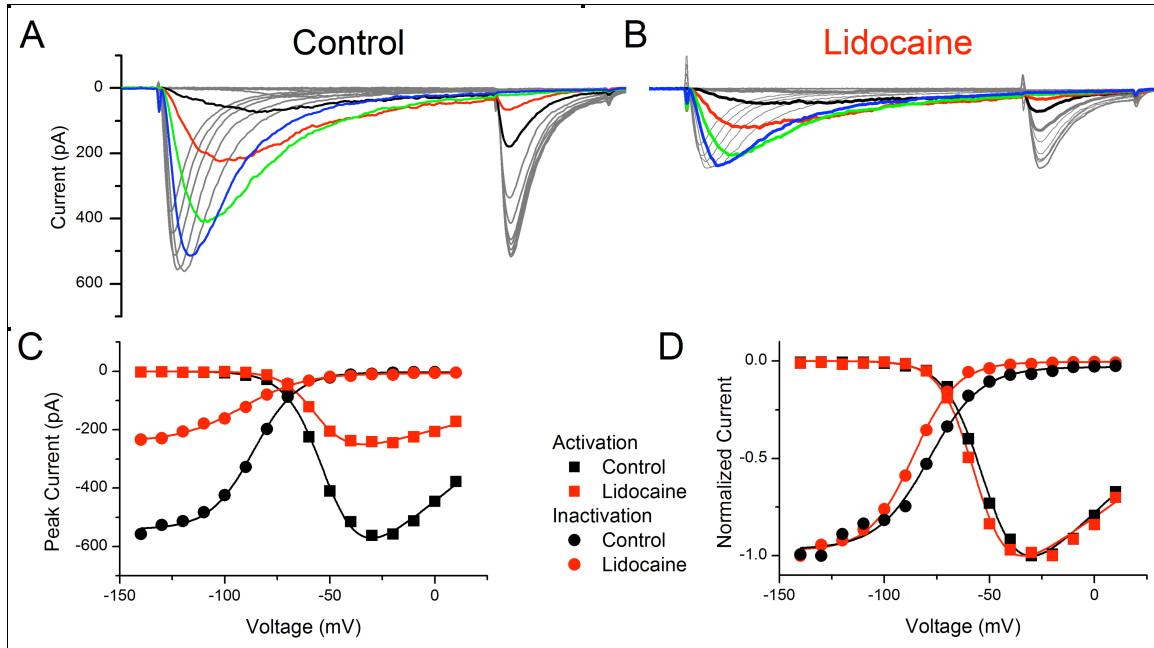
**<http://www.landesbioscience.com/journals/channels/article/21202/>**



Supplementary Figure 1. In cell-attached (left) and inside-out (right) configurations the two ladder protocol results in reproducible and overlapping voltage-dependent currents. A) Voltage protocol detailed in the Methods section includes two voltage-ladders (-140 to +10 mV by 10 mV) separated by 100 msec at HP (-100 mV) and pre-pulses to -200 mV (10 msec) and -140 mV Following the pre-pulse steps were 16 test voltage steps in 10 mV increments from -140 mV (4 msec). Test steps were -140 mV to 10 mV for 12 msec per step to test activation and a step to -10 mV for 4 msec to test availability. Scale bar is 50 mV. B) No pressure was applied during this protocol. Scale bar is 50 mmHg. C) Voltage-dependent currents from first and second voltage ladders in cell-attached and the same patch as excised inside-out. Scale bar is 50 pA. D) Difference currents  $I_{diff} = I_{step1} - I_{step2}$ . Scale bar is 20 pA. E) Peak current versus voltage (IV) curves comparing ladder 1 to ladder 2 for cell-attached and inside-out patches.



Supplementary Figure 2. The application of pressure to cell-attached patches in a two-ladder protocol can be done either at the second voltage ladder (left panels) or first voltage ladder (right panels). A) Voltage ladders are the same for both protocols. B) Pressure steps of same duration applied either to second ladder (left) or first ladder (right). C) Voltage-dependent currents. D) Difference currents for activation  $I_{diff} = I_{30\text{mmHg}} - I_{0\text{mmHg}}$  are similar regardless if pressure is applied second or first. E) Current-voltage (IV) curves showing a similar shift in the half-point of voltage-dependence of activation whether pressure is applied second or first.



Supplementary Figure 3. Lidocaine (50  $\mu$ M) applied to inside out patches decreases peak current and hyperpolarizes voltage-dependence of inactivation. A) In an inside-out patch voltage-dependent activation and inactivation were obtained from the first ladder of the two-ladder protocol as discussed above. B) Lidocaine was (50  $\mu$ M) then added to the bath and voltage-dependent currents were obtained. C) Voltage-dependent peak currents for activation and inactivation (IVs) were fit with two-state Boltzmann functions, showing the decrease in peak currents with addition of lidocaine. D) Normalized IVs demonstrate a hyperpolarizing shift in the voltage-dependence of inactivation.