

Supplementary Figure 1: Selective and effective local perfusion of TEA.

A series of control experiments were conducted to ensure that local perfusion of TEA by micropipette pressure injection to Purkinje cell soma or dendrites provided effective drug concentrations that were limited to the targeted compartment. The overall strategy was to use relatively modest drug concentrations and short duration perfusions while optimizing orientation of the perfusion pipette with respect to the orientation of the Purkinje cell and outflow of the recording chamber (see Methods). In this series, 1 mM TEA was locally applied to a Purkinje cell soma or dendrites for 2 seconds prior to somatic current injection. (A) DC current injection of 0.2 nA induced tonic firing in Purkinje cells. Selective somatic perfusion caused spike broadening and reduction of the AHP, consistent with block of a somatic conductance contributing to spike repolarization. (B) Selective dendritic perfusion of TEA did not alter the tonic firing waveform. (C) Prolonged current injection of 0.8 nA was used to elicit failure of tonic firing, yet was below the threshold for evoking dendritically generated spike-bursts. Somatic perfusion caused the spikes to fail more rapidly, but did not change the plateau voltage following spike failure. (D) Selective dendritic perfusion did not affect initial spiking but induced repetitive spike-bursts, which are known to be initiated by dendritic Ca^{2+} spikes. This demonstrates that somatic and dendritic Kv3 channels differentially affect Purkinje cell excitability, and selective TEA perfusion may be used to characterize the contributions of each population separately.

Supplementary Figure 2: Simple spikes recorded in vivo in Kv3.3 KO mice have increased width and prolonged duration. Fifty consecutive simple spikes were averaged from unfiltered extracellular recordings for each Purkinje cell analyzed. Shown are representative examples of averaged simple spikes recorded from a wild type (A) and Kv3.3 KO (B) Purkinje cell, normalized in amplitude from baseline to peak of positive-going phase. (C) Traces from (A) and (B) are overlaid and expanded in time. As evident in this example, simple spikes from Kv3.3 KO mice tended to have longer half-amplitude duration (arrows), a relatively deeper negative going phase and prolonged return to baseline. (D) Quantification of half-amplitude duration for the population of wild type and Kv3.3 KO Purkinje cells (*, $p < 0.01$).