

Supplemental results

To examine the consequence of each sequence alteration required to generate the SCN variants BK₀ and BK_{SRKR} from the parent construct, mbr5, we constructed a series of clones that differed by stepwise addition and deletion of the indicated sequences (Fig. S1 A). We found that the addition of the “MANG” N-terminal sequence found in the SCN clones (C2), removal of IYF at splice site 2 (C3), and switching the “VEDEC” C-terminal sequence to “VYR” (C4) had no significant effects on the G-V relationships or kinetics of activation or deactivation (Fig. S1, B–D). However, comparing mbr5 currents to BK₀, the addition of the Ca²⁺-bowl insert at splice site 3 right-shifted the G-V curve at 0, 1, and 100 μM Ca²⁺ (Fig. S2 A and Table S1). There was no effect of adding the Ca²⁺-bowl exon at 10 μM Ca²⁺. The addition of the Ca²⁺-bowl exon also influenced the kinetics of activation and deactivation. At 10 and 100 μM Ca²⁺, BK₀ had slowed activation compared with mbr5 (Fig. S2 B). BK₀ deactivation was faster at 0 Ca²⁺, but slower at 100 μM Ca²⁺, than mbr5, with no change seen at 1 and 10 μM Ca²⁺ (Fig. S2 C). Thus, among the sequences tested here, the addition of the Ca²⁺-bowl exon is the first alteration to produce significant changes in BK current properties. Notably, the changes in G-V and kinetics reported in this study for the addition of the Ca²⁺-bowl exon are larger than reported previously (Ha et al. 2000. *Eur. J. Biochem.* 267:910–918). However, the back-

ground variants used in the respective studies were also different.

The addition of the SRKR exon at site 1 (BK_{SRKR}) produced a further right-shift of the G-V at 0, 1, and 100 μM, but not 10 μM Ca²⁺ (Fig. S2 A and Table S1). To determine whether the addition of the Ca²⁺ bowl at site 3 or the SRKR exon at site 1 had a larger impact on BK current properties, the magnitudes of the V_{1/2} shifts were compared ($\Delta V_{1/2}$ for C4-BK₀ vs. BK₀-BK_{SRKR}). At 1 and 100 μM Ca²⁺, the $\Delta V_{1/2}$ values produced by the addition of the Ca²⁺ bowl and SRKR exons were similar, being 29 and 33 mV at 1 μM Ca²⁺, and both 3 mV at 10 μM Ca²⁺ (Table S1). However, the addition of SRKR produced a larger $\Delta V_{1/2}$ at 0 Ca²⁺ μM (49 mV compared with 34 mV for adding Ca²⁺ bowl). Overall, the addition of SRKR had the largest impact on BK current properties of the sequences tested in this study and produced the most right-shifted G-V curves (see also Results in the main text).

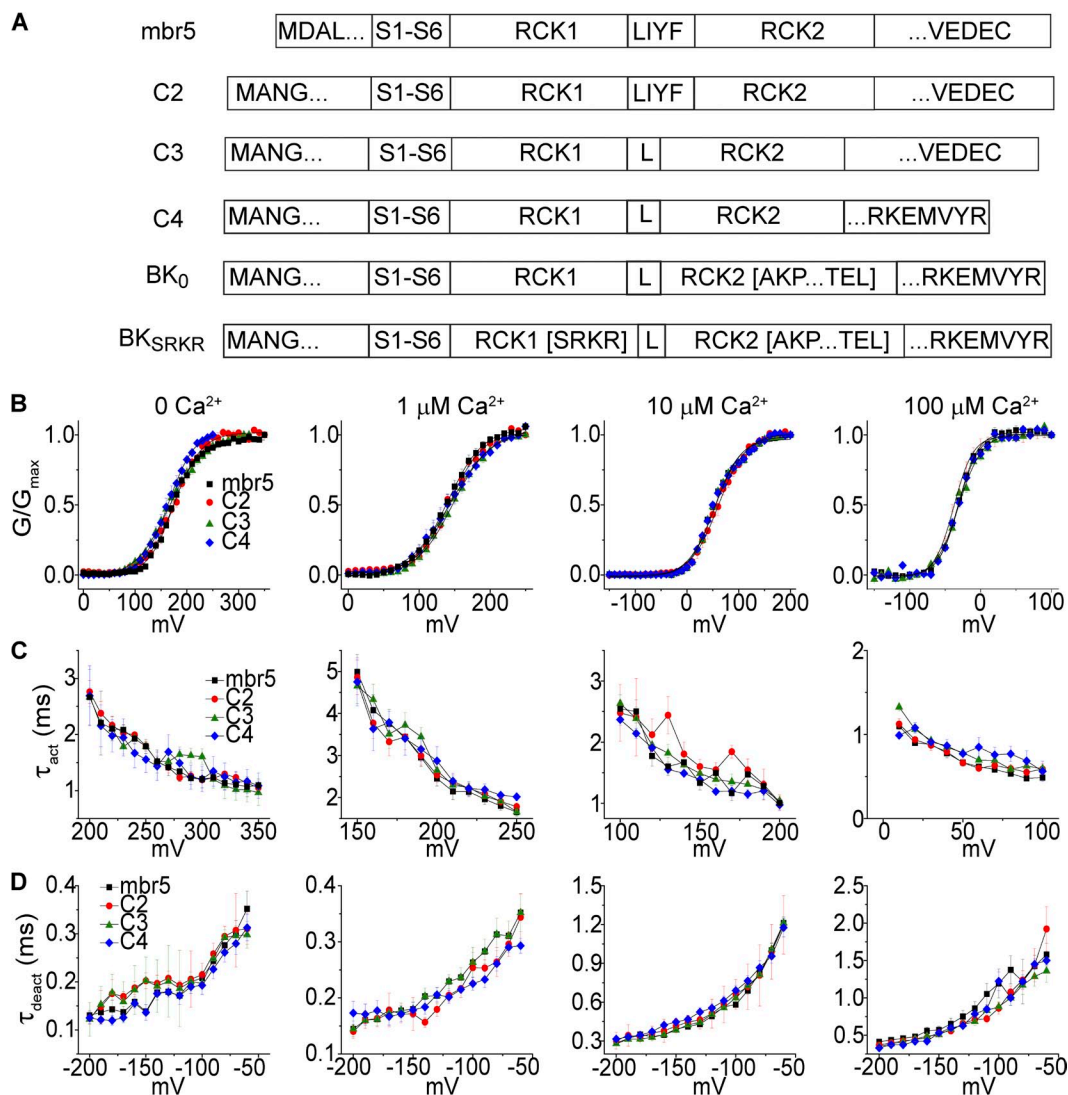


Figure S1. Properties of BK currents from clones mbr5, C2, C3, and C4. (A) Schematic diagram of mbr5, BK₀, BK_{SRKR}, and the intermediate clones used to examine the functional consequences of stepwise additions or deletions of particular inserts. (B) G-V relationship for constructs mbr5, C2, C3, and C4, at the indicated Ca²⁺ concentrations. The alternative N terminus, deletion of IYF, and the alternative C terminus had no effect on the G-V curves. V_{1/2} values are given in Table S1. (C and D) Plot of τ_{act} or τ_{deact} versus voltage for constructs at the indicated Ca²⁺; as with the G-V curves, no differences in the time constants of activation or deactivation were seen for any of the constructs.

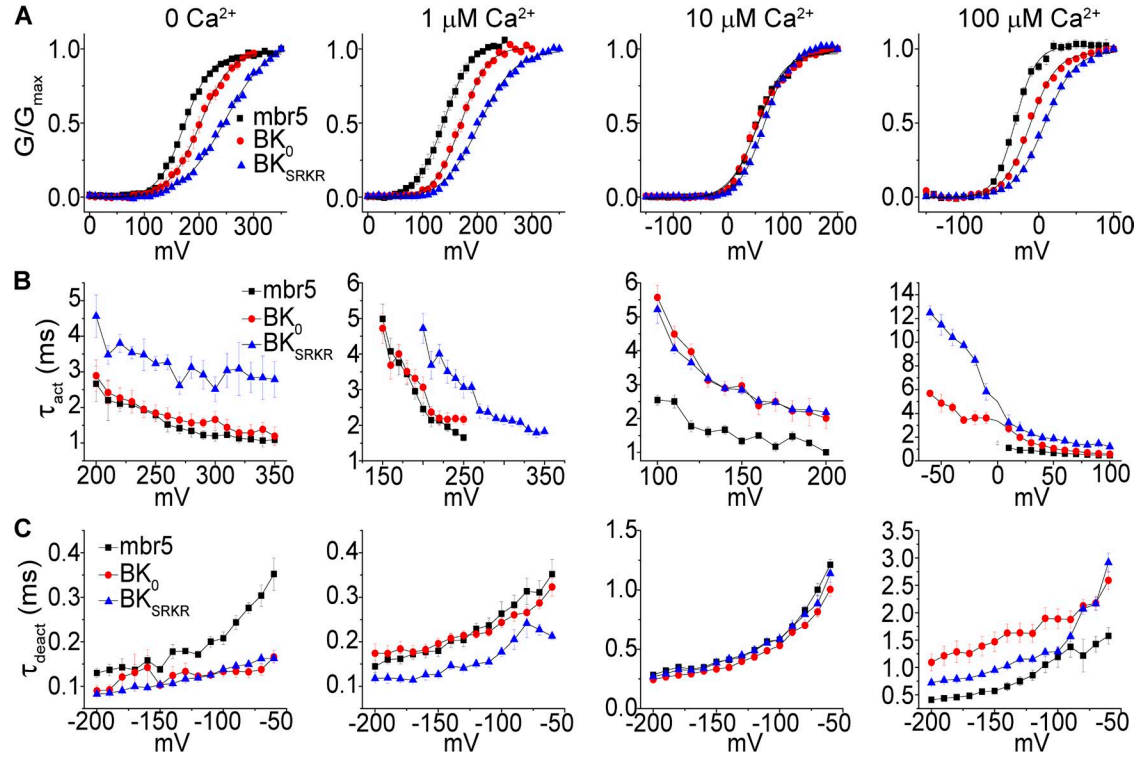


Figure S2. Properties of BK currents from mbr5, BK₀, and BK_{SRKR}. (A) G-V relationship for mbr5, BK₀, and BK_{SRKR}. The addition of the Ca²⁺-bowl exon (BK₀) and SRKR (BK_{SRKR}) right-shifts the G-V relationship at all Ca²⁺ except for 10 μM Ca²⁺, where no shift is observed. The magnitude of the shift was similar between BK₀ and mbr5, and between BK_{SRKR} and BK₀ at 1 and 100 μM Ca²⁺, but was larger for BK_{SRKR} at 0 Ca²⁺. V_{1/2} values are given in Table S1. (B and C) Plot of τ_{act} or τ_{deact} versus voltage for constructs at the indicated Ca²⁺. BK_{SRKR} is slower to activate than mbr5 at all Ca²⁺ concentrations, whereas BK₀ only activates slower than mbr5 at 10 and 100 μM Ca²⁺. At 0 Ca²⁺, mbr5 deactivates slower than BK₀ and BK_{SRKR}; however, as Ca²⁺ is increased, the rate of deactivation of mbr5 increases compared with BK₀ and BK_{SRKR}.

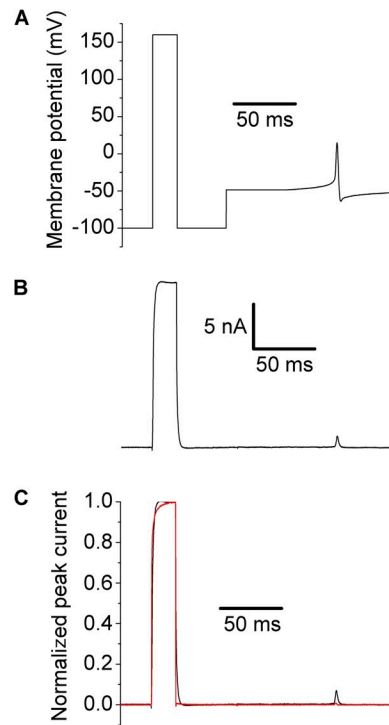


Figure S3. BK currents are far from maximally activated in response to APs. (A) The complete day voltage protocol used to activate channels, consisting of a maximally activating voltage jump to +160 mV, followed by a holding potential identical to the mean daytime neuronal resting membrane potential, and the day AP waveform. (B) Example BK₀ current in response to A, clearly showing that the AP does not maximally activate the channel. (C) Overlay of normalized currents from BK₀ (black) and BK_{SRKR} (red) channels showing the reduced AP current and slower activation of BK_{SRKR} currents compared with BK₀ currents in response to the day AP command waveform.

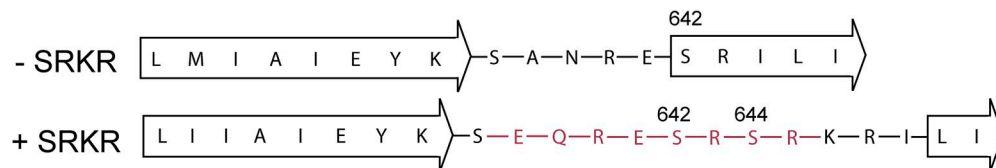


Figure S4. Structural model for the alternate splice site 1 region of BK₀ and BK_{SRKR} variants. The SRKR insert shifts S642 from a β-strand region into an extended loop region in the BK channel. Comparison between the cytosolic domains of a human BK channel crystal structure that lacks the SRKR insert (Protein Data Bank accession no. 3NAF; Wu et al. 2010. *Nature*. 466:393–397) and a zebrafish structure that contains the SRKR insert (Protein Data Bank accession no. 3U6N; Yuan et al. 2012. *Nature*. 481:94–97) reveals that the SRKR insert enlarges the loop region between two β strands. Arrows denote residues within β strands. Atomic coordinates of residues marked in red were not determined.

Table S1
V_{1/2} values for BK variants

BK variant	V _{1/2} (mV)			
	0 μM Ca ²⁺	1 μM	10 μM	100 μM
mbr5	173 \pm 2	138 \pm 5	58 \pm 3	-31 \pm 2
C2	174 \pm 3	144 \pm 3	58 \pm 6	-34 \pm 2
C3	167 \pm 5	144 \pm 5	54 \pm 3	-30 \pm 2
C4	166 \pm 6	142 \pm 7	55 \pm 2	-27 \pm 2
BK ₀	200 \pm 11	171 \pm 4	58 \pm 3	-11 \pm 2
BK _{SRKR}	249 \pm 3	204 \pm 4	61 \pm 2	8 \pm 2
BK ₀ + Alk P		166 \pm 2	59 \pm 4	-8 \pm 3
BK _{SRKR} + Alk P		166 \pm 6	62 \pm 2	-7 \pm 3
BK _{SRKR} -S644A		206 \pm 6	62 \pm 3	4 \pm 3
BK _{SRKR} -S642A		172 \pm 6	54 \pm 4	-11 \pm 3
BK ₀ -S642D		208 \pm 3	59 \pm 2	11 \pm 6
BK _{SRKR} -S642A/S644A		167 \pm 4	62 \pm 2	-15 \pm 2
BK ₀ + β 4			92 \pm 5	-23 \pm 3
BK _{SRKR} + β 4			151 \pm 12	15 \pm 8

G-V relationships were constructed from voltage-clamp recordings as described in Materials and methods in the main text. V_{1/2} values at the indicated [Ca²⁺]_i were determined from Boltzmann fits to G-V data obtained from inside-out patches using standard square voltage pulses to activate currents in symmetrical K⁺ conditions ($n \geq 8$ for each condition). Alk P, patches treated with alkaline phosphatase.