Supporting Information

Castillo et al. 10.1073/pnas.1504378112

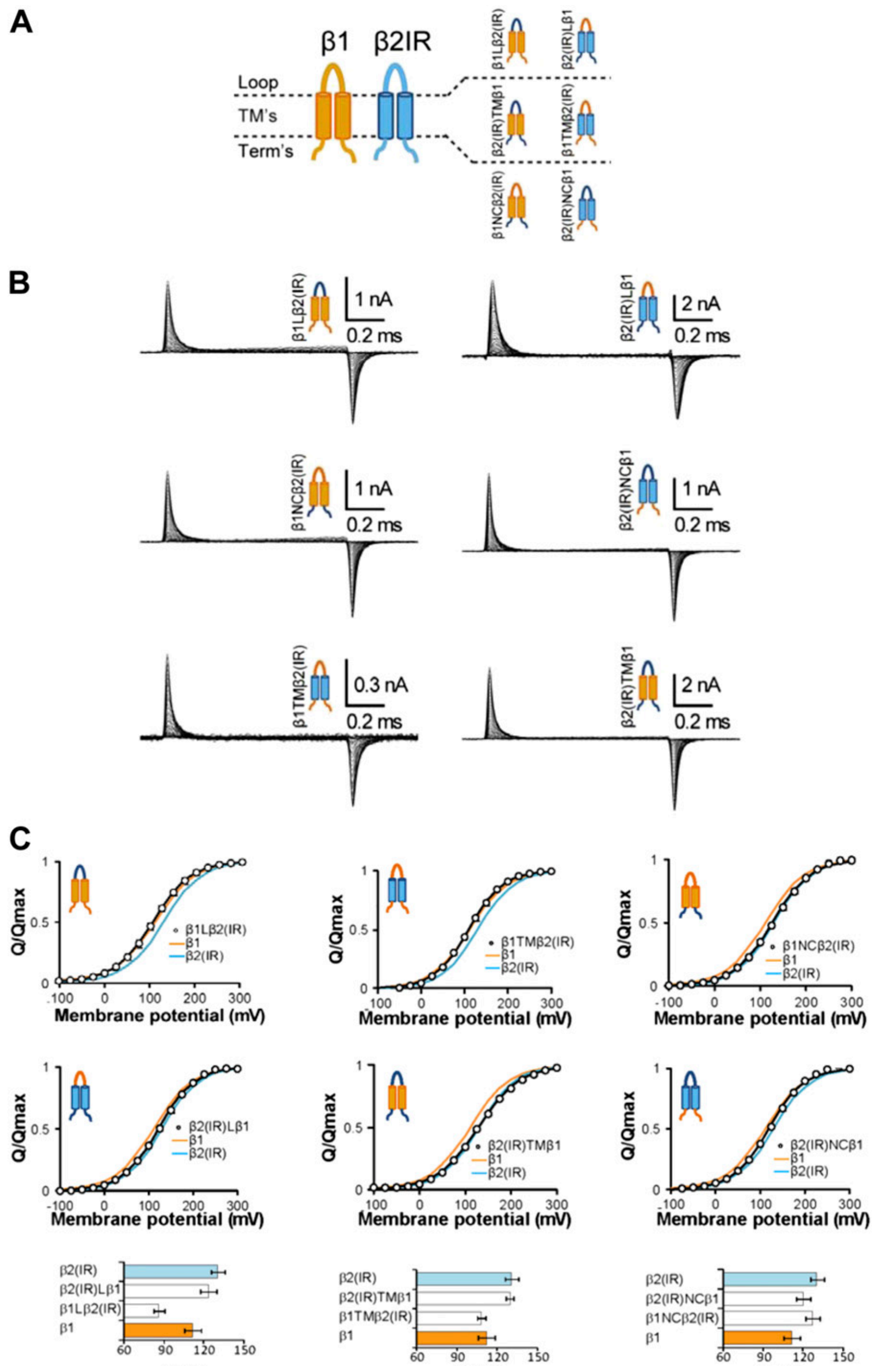

Fig. S1. Gating currents of BK channels coexpressed with chimeric β1/β2-subunits. (*A*) Topological model of chimeric β1/β2-subunit constructs, in which we exchanged the main regions of the auxiliary protein, namely the extracellular loop, transmembrane domains, and N and C termini. The β1-subunit is shown in orange and β2 is in blue. (*B*) Representative recordings of BK channels with β1/β2 chimeric constructs, as indicated. (*C*) Gating charge–voltage relationship for the indicated BK+β1/β2 chimeric complexes. For comparison, all Q–V plots include the curve from channels formed by BK/β1 (orange) and BK/β2 (blue). (*Lower*) For all chimeras, quantification of $V_{0.5}$ obtained from the Q–V curves is presented (mean ± SEM).

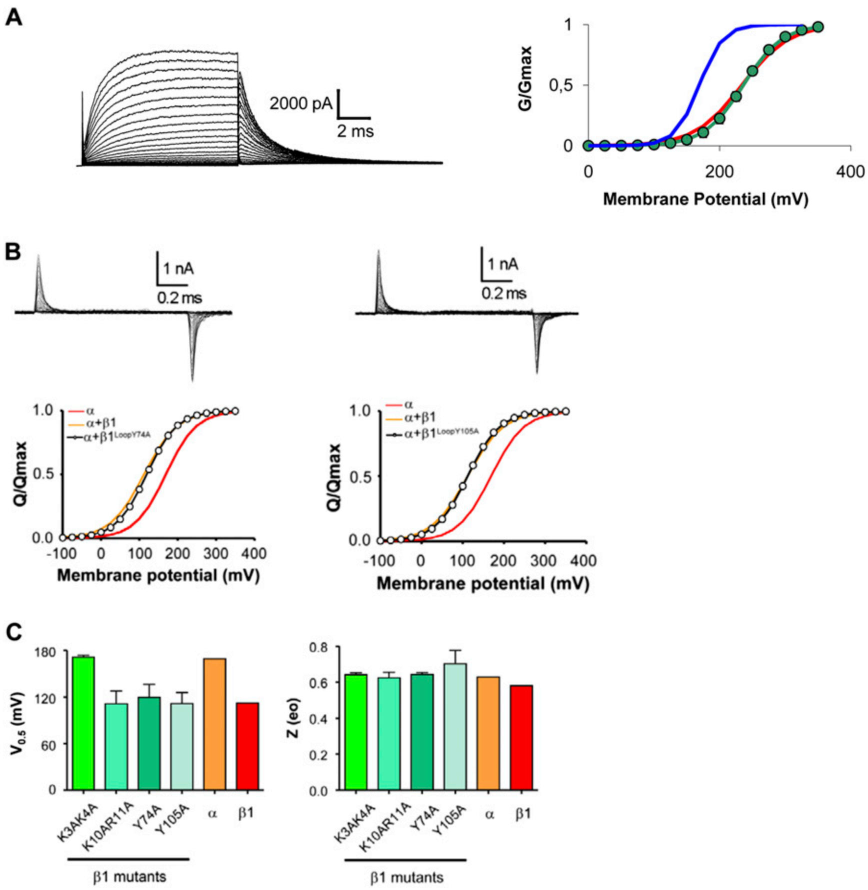

Fig. S2. G–V and Q–V relationships for BK channels coexpressed with different β1-mutants. (*A*, *Left*) Representative macroscopic current recordings from the $\alpha + \beta 1^{N\_K3AK4A}$ mutant in 0 Ca^{2+} and 1 mM K^+. (*Right*) G–V curves from tail currents, comparing the G–V relationship between α (blue) and α + β1 (red) with the mutant $\alpha + \beta 1^{N\_K3AK4A}$ channel (green). (*B*, *Top*) Representative I_g recordings $\alpha + \beta 1_{Y74A}$ (*Right*) and $\alpha + \beta 1_{Y105A}$ channels (*Left*) measured in 0 Ca^{2+}. (*Bottom*) Gating charge–voltage relationship for the $\alpha + \beta 1_{Y74A}$ (*Right*) and $\alpha + \beta 1_{Y105A}$ (*Left*) channels. Gating currents were elicited by 1-ms pulses between −90 and 350 mV in increments of 20 mV. For comparison, Q–V plots include the curve from channels formed by α (red) and α + β1 (orange). (*C*) Quantification of $V_{0.5}$ and *z* values obtained from the Q–V curves (mean ± SEM). A nonparametric *t* test was used to compare statistical significance between wild-type β1 and the mutant β-subunits. For comparison, plots include the quantification of channels formed by α (red) and α + β1 (orange).

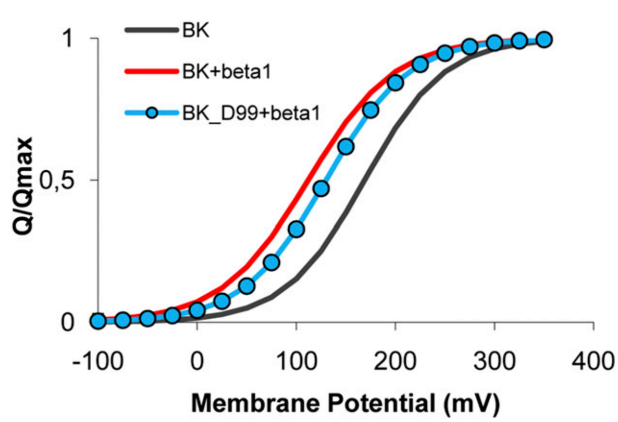

Fig. S3. Mg^{2+}-coordinating residue in BK partially modifying Q–V relationships. Q–V relationships for the BK^{S0_D99A} mutant coexpressed with β1 (blue; $n = 8$) compared with BK alone (black) and BK+β1 (red). Two-way ANOVA was used to compare statistical significance between BK wild type coexpressed with β1 and the BK mutant protein coexpressed with the auxiliary subunit.

Table S1. Summary of gating properties for β1/β2 chimeras

BK channel type	$V_{0.5}$, mV	z, e_0	n
α + β1*	112 ± 5	0.58 ± 0.02	8
α + β2*	130 ± 7	0.61 ± 0.02	7
α + β1Lβ2	107 ± 9	0.61 ± 0.06	8
α + β2Lβ1	123 ± 6	0.62 ± 0.01	10
α + β1TMβ2	108 ± 3	0.65 ± 0.02	8
α + β2TMβ1	129 ± 3	0.56 ± 0.01	9
α + β1NCβ2	127 ± 5	0.60 ± 0.03	7
α + β2NCβ1	120 ± 5	0.64 ± 0.06	5

*$V_{0.5}$ and z values were determined as in Contreras et al. (1).

1. Contreras GF, Neely A, Alvarez O, Gonzalez C, Latorre R (2012) Modulation of BK channel voltage gating by different auxiliary β subunits. *Proc Natl Acad Sci USA* 109(46):18991–18996.

Table S2. Summary of gating properties for β1/β3 chimeras

BK channel type	$V_{0.5}$, mV	z, e_0	n
α*	169 ± 12	0.63 ± 0.06	14
α + β1*	112 ± 5	0.58 ± 0.02	8
α + β3*	189 ± 4	0.56 ± 0.02	8
α + β1Lβ3	119 ± 1	0.67 ± 0.01	6
α + β3Lβ1	174 ± 14	0.61 ± 0.01	9
α + β1TMβ3	116 ± 6	0.68 ± 0.02	7
α + β3TMβ1	165 ± 8	0.64 ± 0.02	8
α + β1NCβ3	171 ± 2	0.67 ± 0.01	10
α + β3NCβ1	112 ± 5	0.57 ± 0.01	8
α + β1Nβ3	177 ± 2	0.63 ± 0.01	7
α + β3Cβ1	177 ± 2	0.64 ± 0.02	7
α + β3Nβ1	129 ± 4	0.64 ± 0.02	6

*$V_{0.5}$ and z values were determined as in Contreras et al. (1).

1. Contreras GF, Neely A, Alvarez O, Gonzalez C, Latorre R (2012) Modulation of BK channel voltage gating by different auxiliary β subunits. *Proc Natl Acad Sci USA* 109(46):18991–18996.

Table S3. Summary of ΔΔ*G* for BK (β1/β3) channels

BK channel type	Δ*G*, kcal/mol	ΔΔ*G*, kcal/mol
α	2.4 ± 0.1	1.0 ± 0.2**
α + β1	1.5 ± 0.1	0
α + β1Lβ3	1.8 ± 0.1	0.3 ± 0.2
α + β3Lβ1	2.4 ± 0.7	0.9 ± 0.7
α + β1TMβ3	1.8 ± 0.2	0.3 ± 0.2
α + β3TMβ1	2.4 ± 0.4	1.0 ± 0.4**
α + β1NCβ3	2.6 ± 0.1	1.1 ± 0.2*
α + β3NCβ1	1.5 ± 0.2	0.0 ± 0.3
α + β1Nβ3	2.6 ± 0.1	1.1 ± 0.2*
α + β3Cβ1	2.6 ± 0.2	1.1 ± 0.2*
α + β3Nβ1	1.9 ± 0.2	0.4 ± 0.2

Free energy was calculated as $\Delta G = zFV_{0.5}$, with the z and $V_{0.5}$ values determined from Q–V relationships. Changes in free energy were determined by $\Delta\Delta G = F(z_i V_{0.5\ i} - z_0 V_{0.50})$, with z_0 and $V_{0.50}$ as the values for α + β1 (reference value) and z_i and $V_{0.5\ i}$ as the values for the other α + β combinations.

**$P < 0.01$, *$P < 0.05$, nonparametric pairwise Wilcoxon test.

Table S4. Summary of gating properties for mutant channels

BK channel type	$V_{0.5}$, mV	z, e_0	n
α*	169 ± 3	0.63 ± 0.06	14
$\alpha+\beta1$*	112 ± 5	0.58 ± 0.02	8
$\alpha+\beta1^{N\_K3AK4A}$	171 ± 3	0.64 ± 0.01	16
$\alpha+\beta1^{N\_K10AR11A}$	111 ± 18	0.62 ± 0.04	5
$\alpha+\beta1^{Loop\_Y74A}$	119 ± 19	0.64 ± 0.01	4
$\alpha+\beta1^{Loop\_Y105A}$	111 ± 16	0.70 ± 0.09	4
$\alpha^{D99A}+\beta1$	130 ± 3	0.61 ± 0.02	6

*$V_{0.5}$ and z values were determined as in Contreras et al. (1).

1. Contreras GF, Neely A, Alvarez O, Gonzalez C, Latorre R (2012) Modulation of BK channel voltage gating by different auxiliary β subunits. *Proc Natl Acad Sci USA* 109(46):18991–18996.

Table S5. Summary of $\Delta\Delta G$ for BK/$\beta1^{mut}$ channels

BK channel type	ΔG, kcal/mol	$\Delta\Delta G$, kcal/mol
$\alpha+\beta1$	1.5 ± 0.1	0
$\alpha+\beta1^{N\_K3AK4A}$	2.5 ± 0.2	1.1 ± 0.2**
$\alpha+\beta1^{N\_K10AR11A}$	1.6 ± 0.5	0.2 ± 0.5
$\alpha+\beta1^{Loop\_Y74A}$	1.8 ± 0.5	0.3 ± 0.6
$\alpha+\beta1^{Loop\_Y105A}$	1.8 ± 0.7	0.4 ± 0.7

Free energy was calculated as $\Delta G = zFV_{0.5}$, with the z and $V_{0.5}$ values determined from Q–V relationships. Changes in free energy were determined by $\Delta\Delta G = F(z_i V_{0.5\ i} - z_0 V_{0.50})$, with z_0 and $V_{0.50}$ as the values for $\alpha+\beta1$ (reference value) and z_i and $V_{0.5\ i}$ as the values for the other $\alpha+\beta$ combinations.
**$p < 0.01$, nonparametric pairwise Wilcoxon test.