Inter-α/β subunits coupling mediating pre-inactivation and augmented activation of BKCa(β2)

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Large-conductance calcium-activated potassium (BK) channels regulate the electric properties and neurotransmitter release in excitable cells. Its auxiliary β2 subunits not only enhance gating, but also confer inactivation via a short-lived preinactivated state. However, the mechanism of enhancement and preinactivation of BK channels by β2 remains elusive. Using our newly developed methods, we demonstrated that electrostatic forces played a crucial role in forming multiple complementary pairs of binding sites between α and β subunits including a “PI site” required for channel preinactivation, an “E site” enhancing calcium sensitivity and an “Ecalt” coupling site transferring force to gate from the Ca2+-bowl via the β2(K33, R34, K35), E site and S6-C linker, independent of another Ca2+ binding site mSlo1(D362,D367).

A comprehensive structural model of the BK(β2) complex was reconstructed based on these functional studies, which paves the way for a clearer understanding of the structural mechanisms of activation and preinactivation of other BK(β) complexes.
To fully understand the mechanisms of α/β interactions, four approaches were jointly used to identify multiple complementary pairs of a preinactivation PI site and an enhancing calcium sensitivity E site with a signal transduction pathway via an E\textsubscript{Cab} site coupling to the calcium-bowl in the cytosolic region of α and β subunits, which provides a comprehensive model of how the mSlo1α subunit functions in concert with the β2 subunit. Our methods may be usefully extended to reveal and confirm interaction sites between subunits in other multimeric membrane protein complexes.

**Results**

Modulation of BK-type channel gating by β2 subunits involves both membrane-spanning and cytoplasmic domains of mSlo1\textsuperscript{23}. To better understand the dynamic structural interactions within the BK(β2) complex during gating, we focused on putative cytosolic binding sites formed by the β2 N-terminal segment and the mSlo1α subunit.

**Specific β2 mutations change the structural configuration of β2-mSlo1 complex.** In previous studies, we scanned the entire N-terminal of the β2 subunit and created three interesting β2 mutants, β2(D16R,E17K), β2(E44K,D45R) and β2(D16R,E17K, E44K,D45R) (Figure 1)\textsuperscript{18}. Wild-type (WT) BK(β2) channels co-assembled by mSlo1α and auxiliary β2 subunits show rapid inactivation with a significant left-shift of the G–V curve, compared with BK(mSlo1α) channels (Figure 2A–C). When similarly coexpressed with mSlo1α, β2(D16R,E17K) significantly slowed the inactivation time constant (τ\textsubscript{i} = 43.0 ms at 100 mV) with little change in G–V curve compared with WT β2 (τ\textsubscript{i} = 16.8 ms). By contrast, β2(E44K,D45R) markedly shifted the G–V of activation (+60.3 mV) with kinetics of inactivation (τ\textsubscript{i} = 26.3 ms) similar to WT β2. The third mutant β2(D16R,E17K,E44K,D45R) not only shifted the G–V back to that of mSlo1α, but also virtually eliminated inactivation (Figure 2A–C). For evaluating changes in G–V curves more accurately, the ID was deleted from the WT β2 and its mutational variants. Thus, two constructs were built for WT β2 and each mutation variant here. One with ID (β2(D16R,E17K)) was used for comparing the inactivation time constant and the other without ID (Δ3–β2(D16R,E17K)) for comparing the G–V curves. To exclude the possibility that changes in both the G–V and τ\textsubscript{i} resulted from an altered stoichiometry of β2 per channel due to insufficient expression of β subunit, we carried out experiments varying the expression ratio of β2 and mSlo1α subunits. Results in Figure S1A indicated that the G–V curve of BK(Δ3–β2) did not obviously shift until the co-transfected ratio of Δ3–β2:mSlo1 decreased to 0.1:1 from the 2:1 ratio used in this study, suggesting that the amount of β2 co-expressed with mSlo1 was about 20-fold more sufficient than necessary to maintain a saturating stoichiometry of β2 per channel for all experiments, consistent with the results reported by Wang et al\textsuperscript{13}. These results with the mutant β2(E44K,D45R) are consistent with a decreased binding affinity to mSlo1α subunits compared with WT β2.

Furthermore, we recorded the instantaneous tail currents of WT and mutant β2 to measure the rectification ratio R (Figure 2D-left and Figure S1B), which fully depends upon the number of positively charged lysine in the outer loop of β2 subunits\textsuperscript{17}. A summary of the R values revealed that the R of mSlo1/Δ3–β2(D16R,E17K,E44K,D45R) formed by coexpression of mSlo1 and Δ3–β2(D16R,E17K, E44K,D45R) was similar to that of mSlo1 alone (Figure 2D-right), suggesting that the structural configuration of α/β complex is markedly changed.

**Preinactivation site of the BK(β2) complex.** In Figure 2B, we noticed that the inactivation time constant of mSlo1/β2(D16R,E17K) was slowed substantially to 42.5 ms from 16.8 ms (mSlo1/β2) without shifting the G–V curves, suggesting that β2(D16,E17) may act as a preinactivation site to shorten the inactivation time constant. Previous studies reported that a preactivated state (O*) exists in Kv1.2(Kvβ) and BK(β2) channels\textsuperscript{22–24,26}. The acidic residues at the N-terminal of β2 subunits first bind to basic residues located at the cytosolic surface just outside the pore, producing a preactivated state, followed by insertion of a hydrophobic N-terminal domain into the ionic conduction pathway to block the pore\textsuperscript{23,24,27}.

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**Figure 1 | A Schematic of Different Mutations of mSlo1 and β2 Subunits.** Topological maps for mutations of mSlo1α (bottom) and β2 subunits (top) are displayed. The inactivation domain (ID), FIW and FIWF1W (dFIW) are highlighted in slateblue. Tdimer2 (red) and EGFP (green) were attached to the C-terminus of β2 and mSlo1α subunits, respectively, and Myc (green) was inserted at the N-terminus of the mSlo1α subunit. The central panel shows a multi-sequence alignment of β2, dFIW–β2, Δ3–β2, Δ30–β2 and the partial sequence of mSlo1. The boxed amino acids (cyan, orange and pink) in mSlo1 and in the N-terminal of β2, dFIW–β2, Δ3–β2 and Δ30–β2 indicate regions of α/β interaction, termed the preinactivation (PI), electrostatic coupling (E\textsubscript{Cab}) and enhancing (E) sites as indicated.
To identify the interaction site on mSlo1 corresponding to β2(D16,E17), thermodynamic double-mutant cycle analysis was adopted here. Changes of pairwise coupling in free energy between mutations in pairs of residues located in different subunits were respectively calculated by using a thermodynamic square conformational change (DD) scheme here. Changes of pairwise coupling in free energy proportion to $V_{50}$, we can measure the corresponding $V_{50}$ instead. The values of $R$ are 1.13 ± 0.06 (n = 10) for mSlo1 alone(gray), 1.78 ± 0.04 (n = 8) for mSlo1/Δ3-β2 (black), 1.80 ± 0.05 (n = 7) for mSlo1/Δ3-β2(D16,E17K) (blue), 1.61 ± 0.1 (n = 14) for mSlo1/Δ30-β2 (E44K,D45R) (red) and 1.21 ± 0.06 (n = 10) for mSlo1/Δ3-β2(D16R,E17K, E44K,D45R) (green).

A mutation β2(W4E) was previously used to support the existence of preinactivated state (O*) of BK(β2) based on the fact that it has an enhanced probability of very brief openings at steady-state. However, the structural basis of O* was not further identified. To better understand the preinactivation process, we recorded macropatches from the mutation β2(W4E,D16R,E17K), which showed slower inactivation with a higher steady-state offset currents relative to β2(W4E) (Figure S3 and Figure 3E–F). To confirm the role of β2(D16,E17) in preinactivation, single-channel recordings were carried out for β2(W4E) (Figure 3E) and β2(W4E,D16R,E17K) (Figure 3F). In Figure 3E, β2(W4E) produced single channels that exhibited a high frequency of brief openings, indicative of a two-step inactivation scheme. In Figure 3F, however, the brief openings were reduced to minimum with β2(W4E,D16R,E17K), consistent with removal of the preinactivation site. Distributions of open times were well-fitted by the sum of two exponential components (Figure 3G–H). The fast and slow components confer an averaged $\tau$ of 0.31 ms for β2(W4E) and $\tau = 2.0$ ms for β2(W4E,D16R,E17K), consistent with what we predicted previously. The two major characteristics of preinactivation are acceleration of inactivation and brief openings caused by rapid transitions between I and O* states due to shortening the inactivation-chain length of β2 via formation of $\pi$β2 contacts at the preinactivation sites (Fig. 3E–F, sFig. 3A–C). Similarly, it also happens in the case of mSlo1(K330E,K331E)/β2(W4E,D16R,E17K) (Fig. 3G–H) in which residues in both $\pi$ and β2 mediating PI formation are mutated but complementary in polarity. In Figure 3G, mSlo1(K330E,K331E)/β2(W4E,D16R,E17K) showed no inactivation and no swift transitions, but with a very flickery opening more similar to the burst-like opening of mSlo1(K330E,K331E) itself at +100 mV (sFig. 3C). Considering that binding occurs just before channel inactivated, we name the pairwise residues mSlo1(K330,E331)/β2(D16,E17) as a preinactivated (PI) site. Noticeably, the PI site is a transitory binding site, which is either non-functional or inaccessible when the channel is closed.
when coexpressed with mSlo1\textsuperscript{x}. Interestingly, a mutant dFIW-\beta2, engineered with an additional N-terminal FIW sequence before \beta2 (Figure 1), was firmly retained in ER and BK currents were eliminated from the plasma membrane even when coexpressed with either mSlo1-EGFP or Myc-mSlo1 (Figure 4A), suggesting that dFIW is an enhanced retention signal for mSlo1/\beta2 complexes. Based on these results, we hypothesized that immunofluorescence could be used to employ for assaying a physical separation of \alpha and \beta subunits within the mSlo1/\beta2 complex, after deletion or mutation of candidate binding sites required for their co-assembly. For developing this assay, we demonstrated that the fused fluorescent proteins (-EGFP, Myc- and -Tdimer2) did not alter the gating properties of WT channels (Figure S4–S5).

Taking advantage of the enhanced retention signal dFIW, we devised a visual assay for \alpha/\beta association. We reasoned that mutations in either mSlo1/\alpha or \beta2 subunits which reduced the affinity of \alpha/\beta association would be directly reflected by the fraction of mSlo1\textsuperscript{x} released from ER-retention to the plasma membrane. The first candidate \alpha/\beta binding site on \beta2 tested was composed of residues \beta2(E44,D45), based upon the observation that \beta2(E44K,D45R) fully abolished the enhancing Ca\textsuperscript{2+} sensitivity of \beta2. Where is the corresponding site on mSlo1\textsuperscript{x} which directly interacts with \beta2(E44,D45)? Several studies suggest that TM1 and TM2 of the \beta subunit are closely located with Slo1\textsuperscript{x} S2 and S0, respectively\textsuperscript{20,21}. Moreover, the crystal structure of the entire cytoplasmic region of BK(hSlo1) has been determined by two distinguished laboratories\textsuperscript{22,23}. Therefore, we reasoned that a candidate \alpha/\beta association site on mSlo1\textsuperscript{x} corresponding to \beta2(E44,D45) should possess at least some of three features: 1) Positive charges (Arginine (R) or Lysine (K)), 2) Location near the C-terminal end of S2, 3) Location near to or within the RCK1 domain. Based on the above criteria, a series of possible binding sites were tested experimentally (Figure 4). After screening mSlo1\textsuperscript{x} residues (K47), (K59), (K65), (K330,K331), (K392,K393) and (K623,R624), we finally focused on the interaction between mSlo1(K392,K393) and \beta2(E44,D45), which satisfied the above criteria. After co-transfecting with dFIW-\beta2(E44K,D45R)-Tdimer2, mSlo1\textsuperscript{x} appeared on the plasma membrane as we predicted (Figure 4B-left). Similarly, co-transfecting Myc-mSlo1 (K392E, R393D) with dFIW-\beta2-Tdimer2 conferred the same result (Figure 4C-left). The non-inactivated currents recorded from these cells indicated that the affinity of \alpha/\beta association was significantly decreased by either mSlo1(K392E,R393D) or \beta2(E44K,D45R) (Figure 4B-C-right). Additionally, we also noticed that dFIW-\beta2(E44K,D45R)-Tdimer2 was still restricted to the cytoplasm. To further test if \alpha/\beta association was mediated at this site, we coexpressed Myc-mSlo1(K392E,R393D) with dFIW-\beta2(E44K,D45R)-Tdimer2, in which the charge polarities in mSlo1\textsuperscript{x} and \beta2 subunits at the putative \alpha/\beta association site were reversed but complementary. In this case, we saw small green puncta at the plasma membrane and null currents from recordings (Figure 4D), indicating that most of the mSlo1\textsuperscript{x} complexed with \beta2 and was retained within the cells. After permeabilizing the cell membrane with Triton X-100, we found that both Myc-mSlo1(K392E,R393D) and dFIW-\beta2(E44K,D45R)-Tdimer2 were indeed retained and colocalized inside the cell (Figure 4D-left), just as effectively as Myc-mSlo1/dFIW-\beta2-Tdimer2 (Figure 4A).

With the help of fluorescence imaging, a physical separation of mSlo1\textsuperscript{x} and \beta2 subunits was achieved by mutations, suggesting that mSlo1(K392,K393) and \beta2(E44,D45) formed a binding site to enhance the calcium sensitivity of BK(\beta2) channels. To quantify these results, we collected more data for statistical analysis (Figure 4E). The membrane fluorescence intensity of Myc-mSlo1 for each combination was normalized to the intensity of Myc-mSlo1/\beta2-Tdimer2, by immunohistochemistry under non-permeabilized condition. By this criteria, fluorescence intensities were: 100%
Determination of the binding site mSlo1(K392,R393)::β2(E44,D45) and the enhancing calcium sensitivity pathway. To confirm and quantitate the interaction between these two residues, we similarly introduced double-mutant cycle analysis. All G-V curves of the mSlo1(K392,R393) vs β2(E44,D45) cycle were shown in Figure 5A. The total change of ΔAV50 was about +70 mV, suggesting that a strong coupling exists between the mSlo1 (K392,R393) and β2(E44,D45). A summary of ΔAV50 shows that it is independent of Ca2+ again (Figure 5D). Altogether, considering that both mSlo1 (K392,E,R393D) and Δ30-β2(E44K,D45R) exhibited a significant change in BK channel activation, and that the mutant Δ30-β2(E44K,D45R) abolished the enhancing effect on calcium sensitivity of β2 at 10 μM Ca2+ (Figure 2C). The same results were also obtained at 0-300 μM Ca2+ (Figure 6). Therefore, we defined the complementary paired residues mSlo1(K392,R393)::β2(E44,D45) as an enhancing (E) site.

It is interesting to further explore how the E site enhances the calcium sensitivity of BK(β2). Using the mutants mSlo1(5D5N) and mSlo1(D362A,D367A) to remove the calcium binding sites, we examined whether the enhancement by E site was blocked. In Figure 6, a series of activation currents are plotted for the mutants mSlo1(5D5N) and mSlo1(D362A,D367A) to combine with the E site enhanced the calcium sensitivity of BK by a functional E site (Figure 6B). Thus, we inferred that the calcium sensitivity of BK when the E site is formed (Figure 6C–D). In Figure 6E, a series of activation currents are plotted for the mutants mSlo1(5D5N) and mSlo1(D362A,D367A) combined with the Δ30-β2 and Δ30-β2(E44K,D45R). The V50-[Ca2+] curve indicates that the calcium bowl changes the calcium sensitivity of BK when the E site is formed (Figure 6A). On the contrary, the V50-[Ca2+] curve indicates that mSlo1(D362,D367) does not alter the calcium sensitivity of BK by a functional E site (Figure 6B). Thus, we inferred that the E site enhanced the calcium sensitivity of BK(β2) only via the Ca2+ bowl pathway.

Additional experiments were performed to demonstrate that the Δ30-β2(K33,R34,K35) interacted directly with the Ca2+-bowl based on the ΔAV50 = 58 mV at 10 μM Ca2+ (Figure 6C–D). In Figure 6E, β2(K33,R34,K35) is just located immediately above the Ca2+-bowl, within distance to form the Ecaß site. Consequently, a model revised from Magelby’s lab is used to illustrate the mechanism of enhancing...
calcium sensitivity by β2 (Figure 6F): the Ca\(^{2+}\)-bowl exerts a force onto the BK S6 gate, augmented by formation of the $E_{Ca}$ site. By this model, the E site acts in a similar fashion to exert supplemental lateral force onto the gating ring to open the gate via the S6-C linker. This model may help to explain both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent mechanisms for enhancing gating by β2.

More details about the mechanisms of pre-inactivation and augmented activation of large conductance Ca\(^{2+}\)-activated (BK/β2) K\(^{+}\) channel complexes can be found in the Movie S1.

**Discussion**

There is a significant diversity in the functional characteristics of BK-type channels mainly due to their association with β subunits which underlies their diverse physiological roles in neural cells. Owing to the lack of detailed structural information of the mSlo1/β complex, their coupling mechanism has been a major long-standing puzzle. In this study, we unequivocally demonstrated that three complementary pairs of residues played critical roles in activation pathway and preinactivation of the mSlo1/β complex and developed two novel approaches for further exploring the nature of inter-subunit interactions in ion channels.

Conceivably, this E site, serving as an extra pivot or a scaffold between the membrane-spanning and RCK domains of mSlo1, boosts the channel gating force from the calcium bowl via the C-linker of S6 segment, but mSlo1(D362,D367) site delivers the force independently probably due to lack of electrostatic connection as E site does. This also explains why β2 does not potentiate BK channel gating at 0 Ca\(^{2+}\). Considering that the β2(E44,D45) residues are conservative within the BKβ family, it is interesting to investigate different mechanisms of enhancing Ca\(^{2+}\) sensitivity of BK channels between β1 and β2.

To form the PI site at least four conditions must be satisfied: 1) the PI site of mSlo1 must be near the pore; 2) It must move while the pore opens; 3) It must be positively charged; 4) The PI site on the N-terminal of β must be negatively charged. Based on that, we hypothesize that β3b should have a PI site near the region of β3b (D17 or D19), mimicking that of β2(D16,E17). However, the positive charges of the PI site may accelerate inactivation due to electrostatic effects, if the ID of β subunits is negatively charged. Compared with WT β2 (Figure 2B), for instance, W4E shows faster inactivation (Figure 3E). Therefore, changes in inactivation time constants cannot be an exclusive criterion for conclusively identifying PI sites. Without satisfying the above conditions, inactivation of channels cannot proceed according to the two-step inactivation mechanism.
In this study, we developed a novel approach (Retention-Release) to directly visualize a dissociating process of the mSlo1/dFIW-β2 complex stably retained in ER, after mutating one of binding sites. Here the retention signal is the hydrophobic FIW-FIW motif in β2, which might anchor to the unknown trafficking or ER-resident proteins to prevent the mSlo1/dFIW-β2 complex from exiting the ER. This approach opens access for the identification of binding sites in vivo by a simple visual screen for spatial separation after mutating the binding site. Therefore, this may be a very useful general approach for studying in vivo protein-protein interactions, especially for multimeric membrane proteins. This completely differs from the standard co-immunoprecipitation method, which may give artifactual results. The second approach (Rectification) is to utilize the changes in the S0 helix of BK and the TM1 of another “FIW” to the N-terminus of mSlo1-EGFP, EGFP in pEGFP-N1 (Clontech) was cut by two restriction enzymes and subcloned into pcDNA3.1 (+) (Invitrogen). Then, mSlo1 was fused in-frame to the N-terminus of the EGFP using appropriate restriction enzymes. Using the same method, a series of constructs in hβ2 were obtained. β2-Tdimere2 was ligated into the pcDNA3.1 vector. Tdimere2 is a red fluorescence protein derived from DsRed2. For dFIW-β2-Tdimere2, we added another “FIW” to the N-terminus of β2-Tdimere2 and subcloned into pCDNA1 using PCR-based techniques. The Myc-mSlo1 construct was created by adding the sequence of Myc (EQKLISEEDL) in the forward primer and then subcloned into pcDNA3.1. Truncations of the β2 subunit, Δβ3-β2 and Δβ2-β2, were generated by removing amino acids from positions 2 through 4 and from positions 2 through 31, respectively. Mutations were introduced using QuickChange Site-Directed Mutagenesis Kit (Stratagene). All constructs and point mutations were verified by direct DNA sequence analysis. Figure 1 shows the topological map of the constructs and mutations for all the experiments.

**Methods**

**Constructs and mutations.** For construct of mSlo1-EGFP, EGFP in pEGFP-N1 (Clontech) was cut by two restriction enzymes and subcloned into pcDNA3.1 (+) (Invitrogen). Then, mSlo1 was fused in-frame to the N-terminus of the EGFP using appropriate restriction enzymes. Using the same method, a series of constructs in hβ2 were obtained. β2-Tdimere2 was ligated into the pcDNA3.1 vector. Tdimere2 is a red fluorescence protein derived from DsRed2. For dFIW-β2-Tdimere2, we added another “FIW” to the N-terminus of β2-Tdimere2 and subcloned into pCDNA1 using PCR-based techniques. The Myc-mSlo1 construct was created by adding the sequence of Myc (EQKLISEEDL) in the forward primer and then subcloned into pcDNA3.1. Truncations of the β2 subunit, Δβ3-β2 and Δβ2-β2, were generated by removing amino acids from positions 2 through 4 and from positions 2 through 31, respectively. Mutations were introduced using QuickChange Site-Directed Mutagenesis Kit (Stratagene). All constructs and point mutations were verified by direct DNA sequence analysis. Figure 1 shows the topological map of the constructs and mutations for all the experiments.

**Immunofluorescence and confocal microscopy.** About 20 hours after transfection, cells were fixed with 2% paraformaldehyde in PBS for 10 min. For cell permeabilization, 0.1% Triton X-100 was added for 10 min. After blocking with 5% FBS for 1 h, cells were incubated with mouse monoclonal anti-human Myc antibody (1:3000) (Abcam) overnight at 4°C. Then, cells were washed (2.5% FBS in PBS, 5 min, x3), and incubated with FITC-conjugated goat anti-mouse IgG (H + L) (1:300) (Proteintech) for 2 h at RT. Non-specific secondary antibodies were blocked with 1% BSA (SU6N). The S0 helix of BK and the TM1 of another “FIW” to the N-terminus of mSlo1-EGFP, EGFP in pEGFP-N1 (Clontech) was cut by two restriction enzymes and subcloned into pcDNA3.1 (+) (Invitrogen). Then, mSlo1 was fused in-frame to the N-terminus of the EGFP using appropriate restriction enzymes. Using the same method, a series of constructs in hβ2 were obtained. β2-Tdimere2 was ligated into the pcDNA3.1 vector. Tdimere2 is a red fluorescence protein derived from DsRed2. For dFIW-β2-Tdimere2, we added another “FIW” to the N-terminus of β2-Tdimere2 and subcloned into pCDNA1 using PCR-based techniques. The Myc-mSlo1 construct was created by adding the sequence of Myc (EQKLISEEDL) in the forward primer and then subcloned into pcDNA3.1. Truncations of the β2 subunit, Δβ3-β2 and Δβ2-β2, were generated by removing amino acids from positions 2 through 4 and from positions 2 through 31, respectively. Mutations were introduced using QuickChange Site-Directed Mutagenesis Kit (Stratagene). All constructs and point mutations were verified by direct DNA sequence analysis. Figure 1 shows the topological map of the constructs and mutations for all the experiments.

**Image analysis and statistics.** In experiments with coexpressed Myc-mSlo1 (green) and β2-Tdimere2 (red), four cases were seen: No fluorescence, green fringe, red cytoplasm, and red cytoplasm with green fringe. For statistical analysis, we ignored cells with no fluorescence or with only green fringe, due to no expression or expression of only Myc-mSlo1 in HEK293 cells. Fluorescence intensity of the green fringe was calculated by Image J software under non-permeabilized condition, and normalized to the intensity of Myc-mSlo1/β2-Tdimere2. Representative images in figures were dealt with AutoQuantX2. The error bar is S.E.M.

**Patch clamp recording.** For recordings, transfected HEK293 cells were transferred one day after transfection to 160 K concentration solutions were made in three resin types: 1 M sucrose, 1 M mannitol and 0.25 M sucrose. After blocking with 5% FBS for 1 h, cells were incubated with mouse monoclonal anti-human Myc antibody (1:3000) (Abcam) overnight at 4°C. Then, cells were washed (2.5% FBS in PBS, 5 min, x3), and incubated with FITC-conjugated goat anti-mouse IgG (H + L) (1:300) (Proteintech) for 2 h at RT. Non-specific secondary antibodies were removed by washing with PBS (5 min, x6), followed by a final soak in PBS. Cells were visualized by confocal laser scanning microscopy (Olympus IX71) using a 100× oil immersion lens (NA1.30). Parameter selection, sample scanning and image acquisition were all controlled by Andor Iq 2 software.

**Homology modeling.** Full models of BK channels were built by Homology Modeling from known partial crystal structure of ion channels. The complexes of mSlo1 and β2 were assembled and then optimized to a local minimal in energy. During the modeling process, the S0 helix domain was built from MthK (PDB:1LNQ) and KcsA (PDB:1K4C). The closed state model was from the closed RCK by Yunkun Wu et al. (PDB:3NAF). The open state model was from the open RCK by Peng Yuan et al. (PDB:3U6N). The S0 helix of BK and the TM1 of β2 was oriented manually according to Guoxia Liu et al. The missed loops of RCK and the linker of S0-S1 were also rebuilt and refined. The partial structure of β2 was from the NMR structure (PDB:1062) and TM1 helix was built manually and refined by Molecular Dynamics (Gromacs). All of structural models were prepared and rendered by PyMol suites (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.).
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Acknowledgments

We thank Dr. Luyang Wang for valuable suggestions on this work. This work was supported by grants from the National Basic Research Program of China (2010CB529804), the National Science Foundation of China (30971179, 31170814, 31028006). The authors have no conflicts to disclose.

Author contributions

W. Z., C. L. and X. G. contributed to the biochemical assays. S. W. contributed to Homology Modeling. P. H., G. G., Z. Z., H. L., J. Y. and Y. W. performed electrophysiological experiments. P. H. and J. D. designed the experiments. W. Z. and P. H. analyzed the data. A. D. W. and J. D. wrote the paper. All authors reviewed the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Hou, P.P. et al. Inter-α/β subunits coupling mediating pre-inactivation and augmented activation of BKCa(β2). *Sci. Rep.* 3, 1666; DOI:10.1038/srep01666 (2013).
CORRIGENDUM: Inter-α/β subunits coupling mediating pre-inactivation and augmented activation of BK_{Ca}(β2)

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This Article contains a typographical error in Figure 1. “…FASYVPEIIELIGNRKKYGGSNFLKDFLH…LEALFKHRHTQVEFYQGSVL” should read “…FASYVPEIELIGNRKKYGGSYAVSGRK…LEALFKHRHTQVEFYQGSVL” The correct Figure 1 appears below.