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Supporting Material

Modulation of conductance-voltage relationship of BKCa channel by shortening the cytosolic loop connecting two RCK domains

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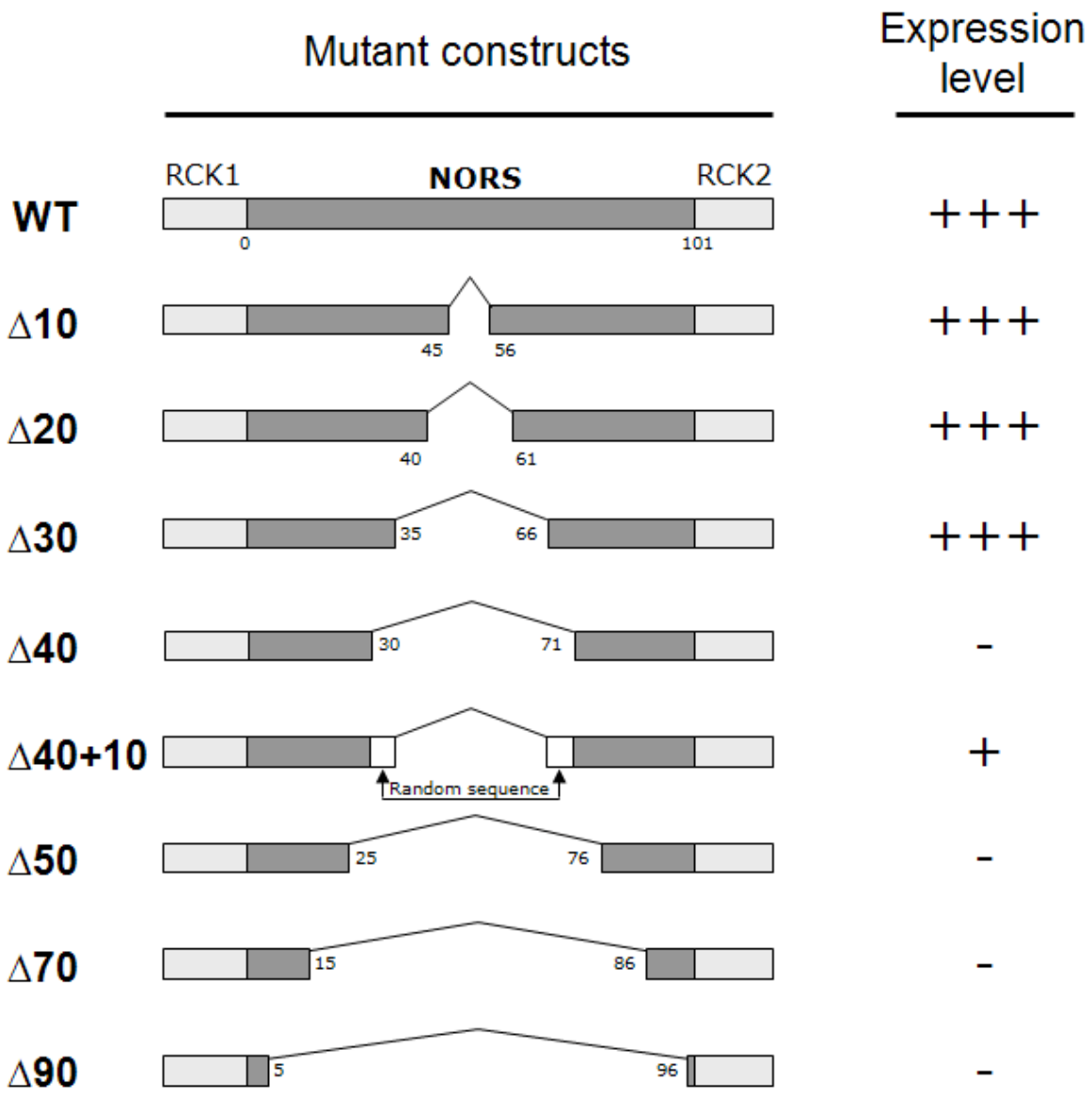
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Serial deletions in NORS connecting two RCK domains.
Representation of NORS deletion constructs and expression level of each construct. Schematic diagrams of NORS between RCK1 and RCK2 are shown. Individual deletions with indicated amino acid positions were constructed and expressed in CHO-K1 cells. Expression level was estimated by recording ionic currents of mutant channels in excised inside-out configuration (+ : <1nA, ++ : 1nA~5nA, +++ : >5nA). ***none*** indicates that the construct generates no detectable currents, even under extreme conditions. Shortening the NORS by deleting 10, 20, or 30 amino acids gave functional BK_{Ca} channel, but deleting more than 40 amino acids gave no functional BK_{Ca} channel when expressed in CHO-K1 cells. However, when random 10 amino acids are ins**e**rted into 40-deletion mutant, that mutant channel became functional.

Supplementary Figure 2. Positional deletions of 30-amino acids in NORS connecting two RCK domains.
Representation of NORS deletion constructs and expression level of each construct. Schematic diagrams of NORS between RCK1 and RCK2 are shown. Individual deletions with indicated amino acid positions were constructed and expressed in CHO-K1 cells. Expression level was estimated by recording ionic currents of mutant channels in excised inside-out configuration (+ : <1nA, ++ : 1nA~5nA, +++ : >5nA). ***none*** indicates that the construct generates no detectable currents, even under extreme conditions. Two deletions at the most peripheral region on both sides of the loop (Δ30-1 and Δ30-7) resulted in non-functional channels.

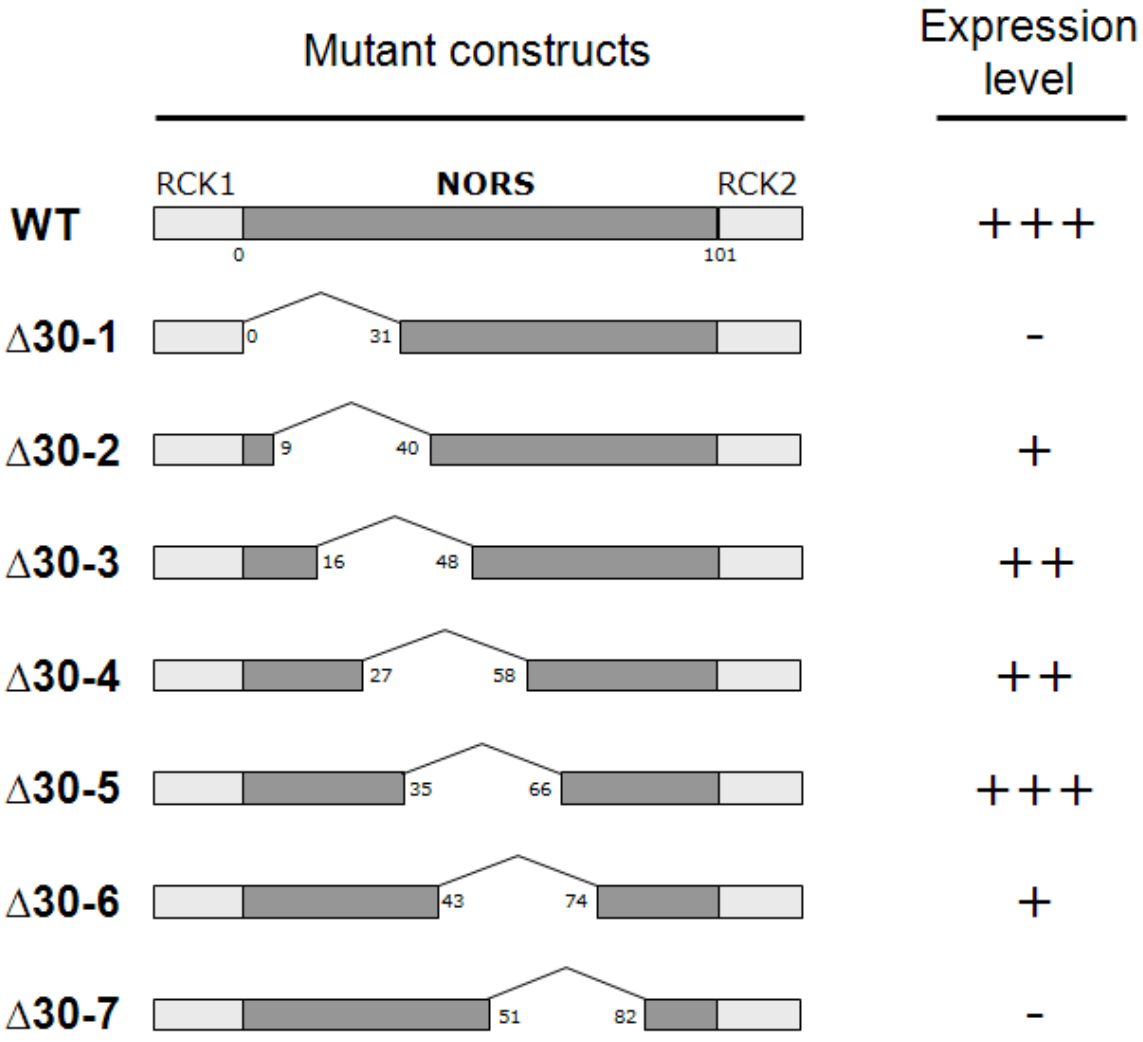
Supplementary Figure 3. Functional effects of low-expressed 30-deletion mutants.
A. Representative macroscopic current traces of the wild-type and low-expressed 30-deletion mutants (Δ30-2 and Δ30-6). The functional activity of each construct was tested in a whole-cell configuration due to the low expression level of the mutant proteins. Wild type channels were also recorded in whole-cell mode to compare with mutant channels. Ionic currents were induced by voltage steps ranging from -10 mV to 150 mV at 40 mV increments from the holding voltage of -100 mV. **B.** Normalized conductance-voltage relations (G/G_{max} vs. V_m) of the wild-type (empty symbols) and mutant channels (filled symbols). The membrane was held at -100mV and then stepped from -80mV to 200mV in 10mV increment for 100 ms. The symbols for each mutant channel are indicated in the inset. Each channel was recorded with 2μM $[Ca^{2+}]_i$. Data points were fitted with the Boltzmann function. **C.** Membrane potential of half-maximal activation ($V_{1/2}$) determined at 2 μM $[Ca^{2+}]_i$. Each data point represents the mean±S.E.M. Values differing from the wild type by the paired Student's t-test at $p<0.05$ (*) or $p<0.01$ (**) are indicated.

Supplementary Figure 1



NORS:
DDVTDPKRIKKCGCRRLEDEQPPTLSPKKKQRNGGMRNSPNTSPKL MRHDPLLIPGNDQIDNMDSNVKKYDSTGMFHWCAPKEIEKVILTRSEA AMTVLSG

Supplementary Figure 2



Supplementary Figure 3