Nuclear BK Channels Regulate Gene Expression via the Control of Nuclear Calcium Signaling

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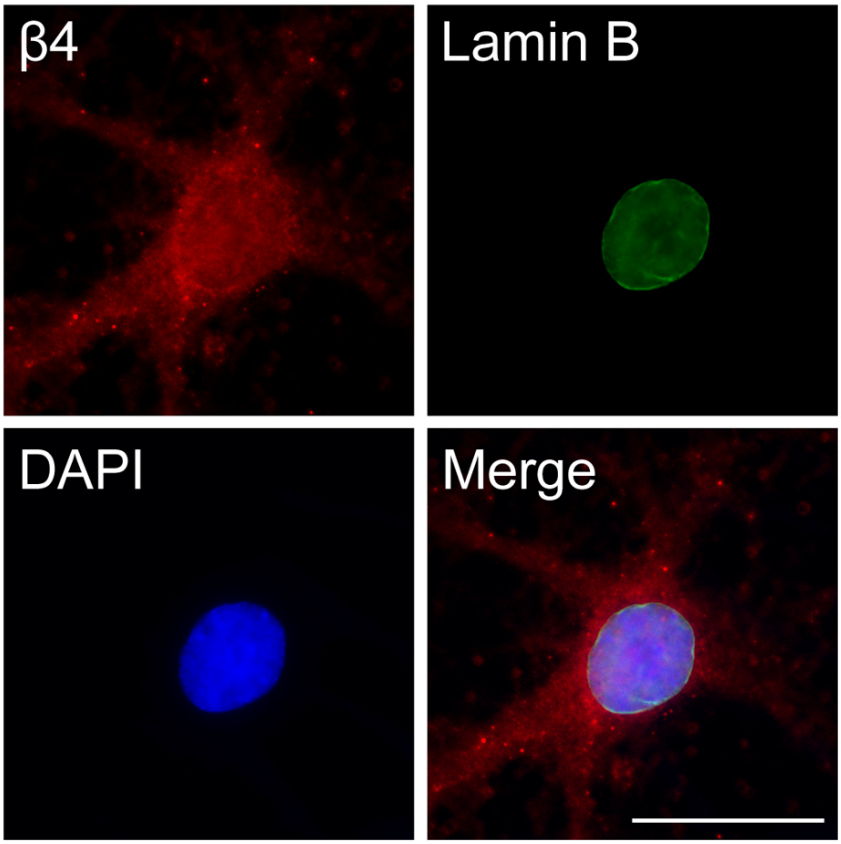
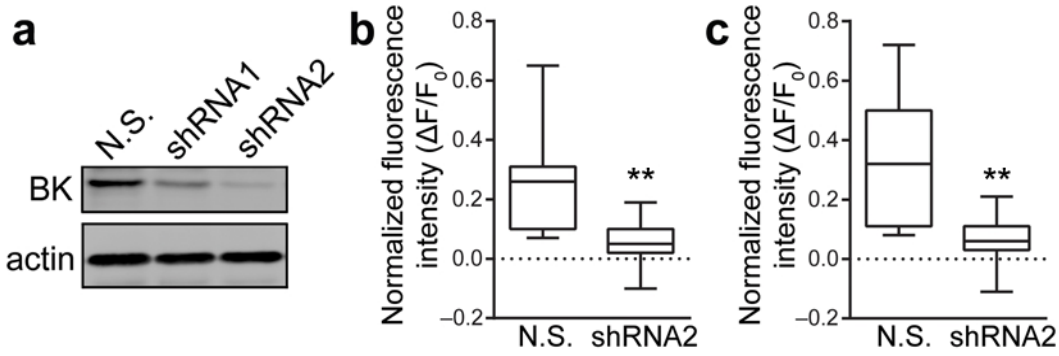
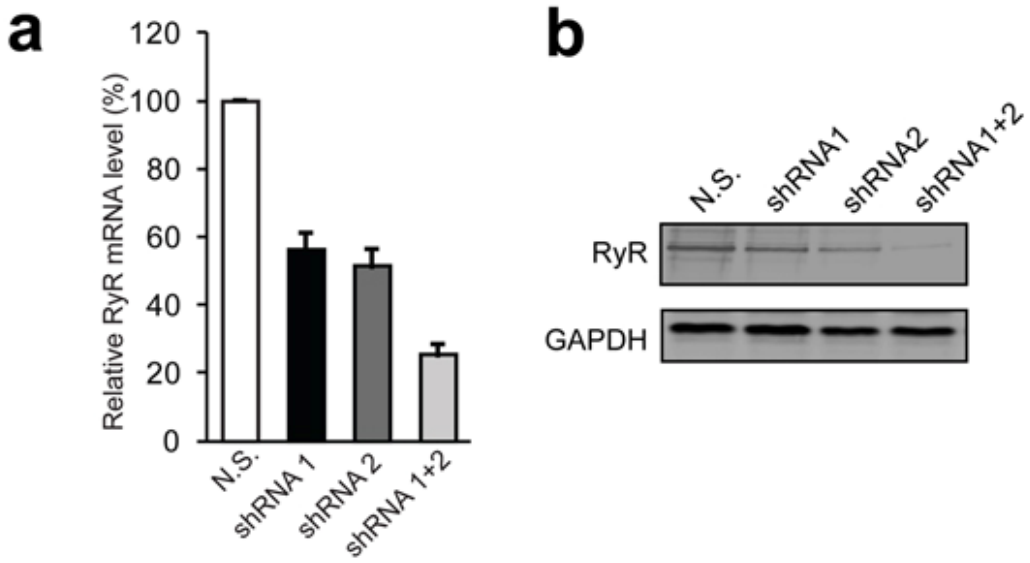


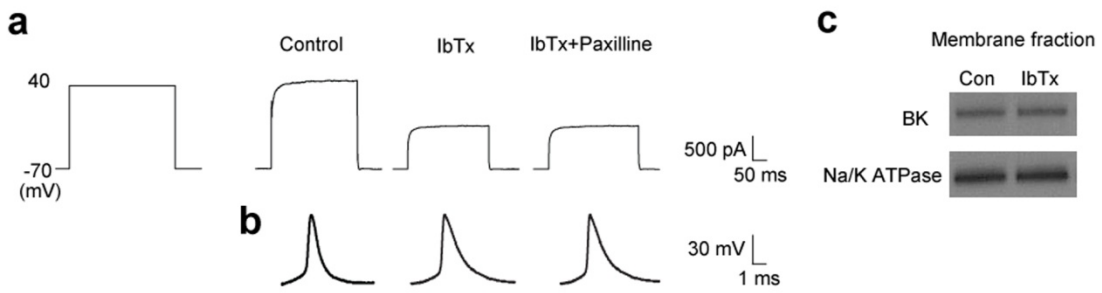
Supplementary Figure 1. BK β4 subunits are expressed in nuclear envelope. The distribution of BK channel β4 subunits (green) was examined by confocal immunofluorescence in cultured hippocampal neurons. Intracellular ring-like labeling around the nucleus was observed and co-localized with lamin B (red). Scale bar represents 20 μm.



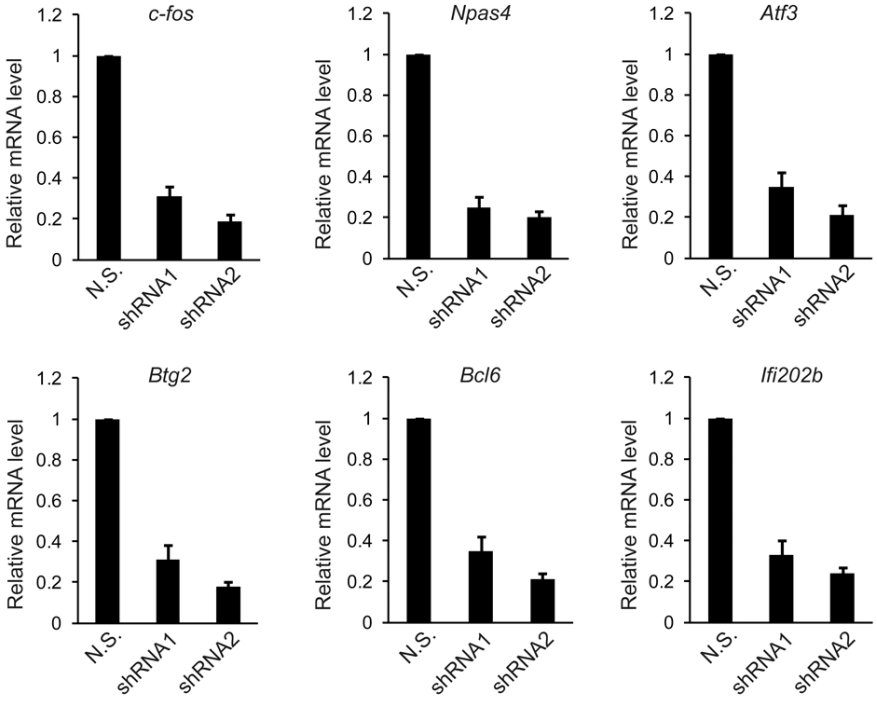
Supplementary Figure 2. The effects of BK channel shRNAs on nBK channel expression and nuclear Ca^{2+} signal. (**a**) BK channel expression after knockdown by two pairs of lentiviral shRNAs. (**b**) Paxilline-induced changes in Fluo-4/AM fluorescence intensity in the isolated nuclei after knockdown of BK channel (n = 25 for each group, unpaired t-test, $P = 1.83 \times 10^{-7}$, $t_{48} = 6.09$). (**c**) Paxilline-induced changes in Fluo-4/dextran fluorescence intensity in the isolated nuclei after knockdown of BK channel (n = 25 for each group, unpaired t-test, $P = 2.67 \times 10^{-7}$, $t_{48} = 5.98$). The bottom and top of the box represent the first and third quartiles, and the band inside the box is the median. The whisker represents the minimum and maximum of all of the data. $^{**}P < 0.01$. The full-length blots for **a** are presented in **Supplementary Figure 7**.



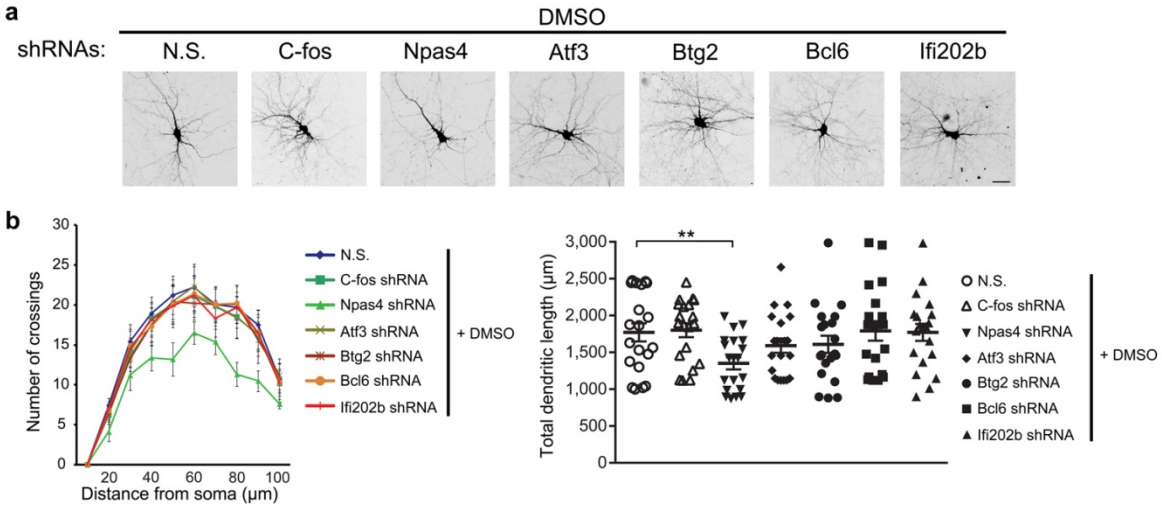
Supplementary Figure 3. RyRs expression was down-regulated by RyRs shRNA. (**a**) RyRs mRNA level after knockdown by shRNAs. GAPDH was used as control. One-way ANOVA ($N = 3$ for each group, $P = 9.47 \times 10^{-6}$, $F_{3,8} = 57.27$) and *post hoc* test. (**b**) RyRs protein level after knockdown by shRNAs. Fisher's least significant difference test was used for *post hoc* test in one-way ANOVA. Error bars represent the mean ± s.e.m.. $^{**}P < 0.01$. The full-length blots for **b** are presented in **Supplementary Figure 7**.



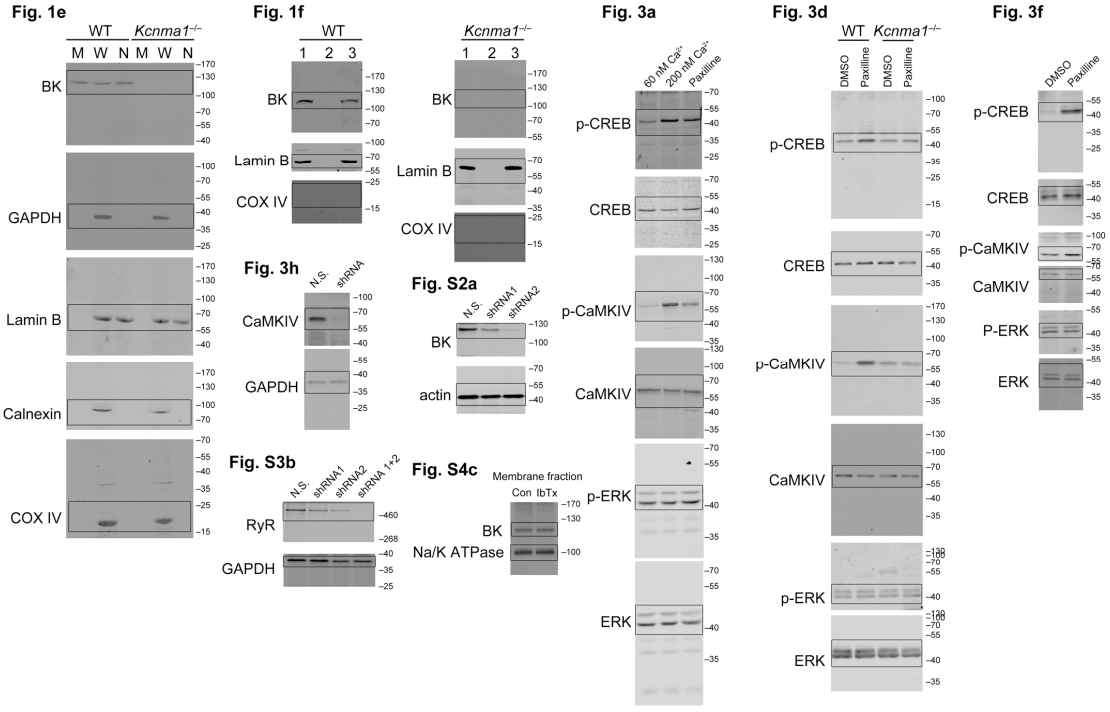
Supplementary Figure 4. The effects of IbTx on pmBK channel activity and expression. (**a**) Outward potassium channel currents were recorded in whole-cell patch clamp to show that paxilline (10 μM) had no additive effect on pmBK channel blockade by IbTx (100 μM). The recording solution contains 1mM 4-AP, 1μM TTX, and 5μM glybenclamide. (**b**) The effects of IbTx (100 μM) and IbTx (100 μM)+paxilline (10 μM) on action potentials. IbTx broadened the action potential by blocking BK channels. Paxilline did not show additive effects. (**c**) Pre-incubation (10 min) of IbTx did not affect BK channel expression on plasma membrane. Membrane proteins were isolated as described in Methods section. Na/K ATPase was used as loading control. The full-length blots for **c** are presented in **Supplementary Figure 7**.



Supplementary Figure 5. Validation for shRNAs against nBK channel-regulated genes. mRNA level of *c-fos, Npas4, Atf3, Btg2, Bcl6 and Ifi202b* after knockdown by shRNAs as indicated. One-way ANOVA (N = 3 independent cultures from at least 3 litters for each group; $P = 1.77 \times 10^{-5}$, $F_{2,6} = 112.05$ for *c-fos*; $P = 1.70 \times 10^{-6}$, $F_{2,6} = 248.60$ for *Npas4*; $P = 2.47 \times 10^{-5}$, $F_{2,6} = 99.99$ for *Atf3*; $P = 1.09 \times 10^{-5}$, $F_{2,6} = 132.38$ for *Btg2*; $P = 2.84 \times 10^{-5}$, $F_{2,6} = 95.38$ for *Bcl6*; $P = 1.09 \times 10^{-5}$, $F_{2,6} = 132.29$ for *Ifi202b*) and *post hoc* test. Fisher's least significant difference test was used for *post hoc* test in one-way ANOVA. Error bars represent the mean ± s.e.m..



Supplementary Figure 6. The effects of nBK channel-regulated genes on dendritic arborization. (a) Representative micrographs of hippocampal neurons transfected with GFP-tagged plasmids containing gene-specific shRNAs or non-silencing shRNAs with the treatment of DMSO. (b) Sholl analysis (left) and quantification of total dendritic length (right) in hippocampal neurons treated as indicated. Right, one-way ANOVA (P = 0.042, $F_{6,133}$ = 2.26) and *post hoc* test. N = 20 cells from 4 independent cultures from at least 4 litters for each group. Fisher's least significant difference test was used for *post hoc* test in one-way ANOVA. Error bars represent the mean ± s.e.m.. Scale bar = 30 μm. **P < 0.01. N.S., non-silencing shRNAs.



Supplementary Figure 7. The full length images of the blots presented in the figures.