Supplementary materials

Synthesis of DNA template and dsRNAs

The primers for synthesis of DNA template for dsRNA against PI4Kβ of *Xenopus* oocytes contained a T7 promoter attached to the 5'-end of both the forward and reverse primers. The primer pairs were:

5'-TAATACGACTCACTATAGGNAGTGGAGAACGACGACGACGATGAG-3´

5'-TCGGGGTCCCTGCGGAACGAACGTAGTTG-3' and

5'-AGTGGAGAACGACGACGACGATGAG-3'

5'-TAATACGACTCACTATAGGNTCGGGGTCCCTGCGGAACGAACGTAGTTG -3'

All primers were produced by Sangon Biotech (Sangon-BIOTECH, Shanghai).The protocol used for PCR amplification was: initial denaturation at 94ºC for 3 minutes followed by 30 cycles of 94ºC for 30 s, 60ºC for 30 s, and 72ºC for 45 s, followed by a 10 minute incubation at 72ºC.

RNA was transcribed from the template using a T7 RiboMAX™ Express RNAi System (Promega, Madison, WI). The transcripts were assessed for integrity on a 1.5% agarose gel, and diluted in injection buffer (5-10 ng/oocyte) and stored at –80ºC until use.

Target sequence information of siRNA for PI4Kβ of *Xenopus* oocytes:

5'-GGATCCTAATACGACTCACTATAGCCAGAG CATCAACTTCTCCC -3'

5'-AAGGGAGAAGTTGATGCTCTGGCTATAGTGAGT CGTATTAGGATCC -3'

5'-GGATCCTAATACGACTCACTATAGGGAGAAGTT GATGCTCTGGC -3'

5'-AAGCCAGAGCATCAACTTCTCCCTATAGTGAG TCGTATTAGGATCC -3'

The suquence of scrambled siRNA were:

5'-GGATCCTAATACGACTCACTATAGTACATA ACCTCCGCTCACGC -3'

5'-AAGCGTGAGCGGAGGTTATGTACTATAGTGA GTCGTATTAGGATCC -3'

5'-GGATCCTAATACGACTCACTATAGCGTGAG CGGAGGTTATGTAC -3'

5'-AAGTACATAACCTCCGCTCACGCTATAGT GAGTCGTATTAGGATCC -3'

Figure S1. Voltage- and time-dependent activity of Ci-VSP

(A), KCNQ2/Q3 channels were expressed alone (left) or co-expressed with Ci-VSP (right) in *Xenopus* oocytes. KCNQ2/Q3 currents were elicited by step depolarizing voltages from -70 mV~100 mV, with 10 mV increments. The activated KCNQ2/Q3 currents were inhibited at relative large depolarizing voltages in Ci-VSP expressing oocytes, indicating activation of Ci-VSP. (B), I-V curves of KCNQ2/3 currents with (open circle) or without (Filled circle) co-expression of Ci-VSP. Ci-VSP was activated at membrane potentials more positive than +40 mV, manifested by the inhibition of KCNQ2/Q3 currents. (C), Negative membrane potentials inactivated activity of Ci-VSP in less 30 s. The left panel shows the normalized peak current amplitudes induced by 6 repeated depolarization voltage of + 40 mV but separated by different length of repolarization of -80 mV. Depolarization to +40 mV from -80 mV for 5 s activated both KCNQ2/Q3 and Ci-VSP, resulting an otherwise non-inactivating KCNQ2/Q3 currents inactivating (right panel, the first current trace). The second and following depolarization after short (5 s) repolarization to -80 mV produced KCNQ2/Q3 currents with diminished peaks; however, when repolarization time was increased to longer than 30 s, the second and following depolarization produced KCNQ2/Q3 currents with same peak amplitudes as the first one, indicating inactivation of Ci-VSP by the repolarization.

Figure S2. The mass spectrum of phosphoinositides extracted from the resolved dots of TLC

(A), The ESI (electrospray ionization) mass spectrum of 18:0/20:4 PIP extracted from dots of TLC showing in Figure 4E. The major ions were at m/z 339($[M-2H-R_2'CH=CO]^{2-}$) and m/z 681($[M-2H-R_1CO_2^-]^-$).

Where M (molecular weight of PIP) = 966; $R_2'CH=CO$: $C_{18}H_{29}CH=CO$; $R_1CO_2^-$: $C_{17}H_{35}CO_2^-$

(B), The ESI mass spectrum of 18:0/20:4 PIP_2 extracted from dots of TLC showing in Figure 4E. The major ions were at m/z 268(R_1CHO), m/z 347($[M-3H]^{3-}$), m/z725($M-C_6H_8O_3(OPO_3H_2)_2$), m/z 886($[M-H-2HPO_3]^-$) and m/z 1029($M-H_2O$).

Where M (molecular weight of PIP_2) =1047; R_1CHO: $C_{17}H_{35}$ CHO.

Figure S3. The depolarization increased KCNQ2(H328C)/Q3 currents

KCNQ2(H328C)/Q3 currents were increased by a 15 min depolarization (0 mV) to a greater extent than KCNQ2/Q3 currents, although the difference did not reach statistical significance.

Figure S4. Extracellular calcium did not contribute to the depolarization-induced potentiation of KCNQ2/Q3 currents

Occytes were either superfused with a Ca^{2+}-containing ND 96 solution or Ca^{2+}-free solutions with or without added EGTA. Folds of current increases after 15 min depolarization at 0 mV were compared. No difference was found between experimental groups.

Fig.S1

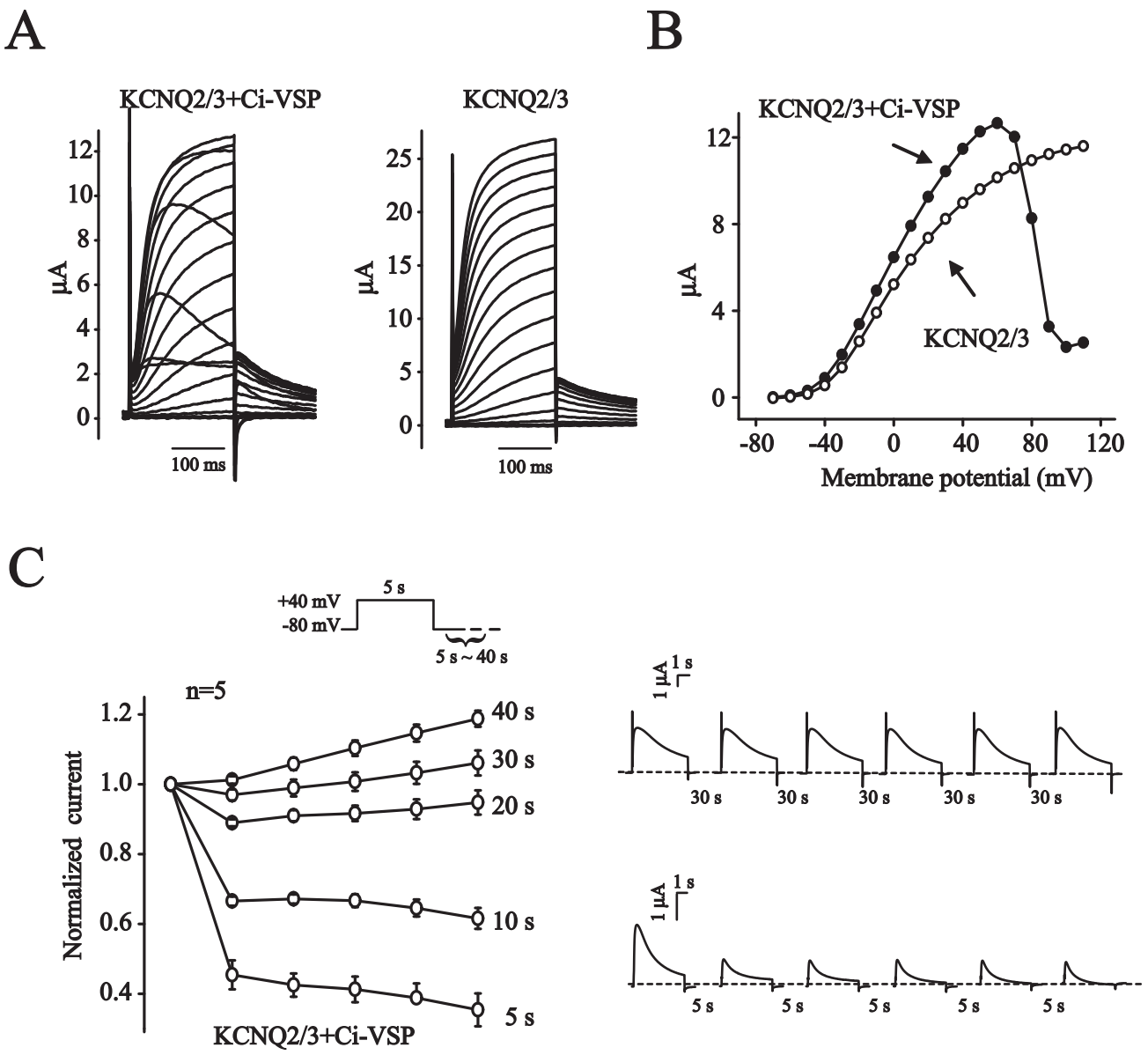
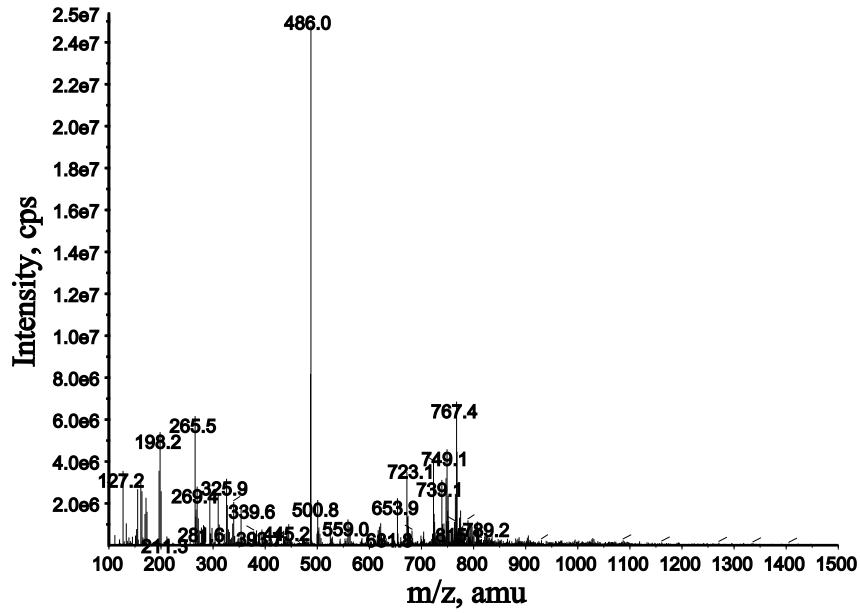

A
KCNQ2/3+Ci-VSP
KCNQ2/3
μA
100 ms
B
KCNQ2/3+Ci-VSP
KCNQ2/3
μA
Membrane potential (mV)
C
+40 mV
-80 mV
5 s
5 s ~ 40 s
n=5
Normalized current
40 s
30 s
20 s
10 s
5 s
KCNQ2/3+Ci-VSP
1 μA
1 s
30 s
5 s

Fig. S2

A

PIP



B

PIP_2

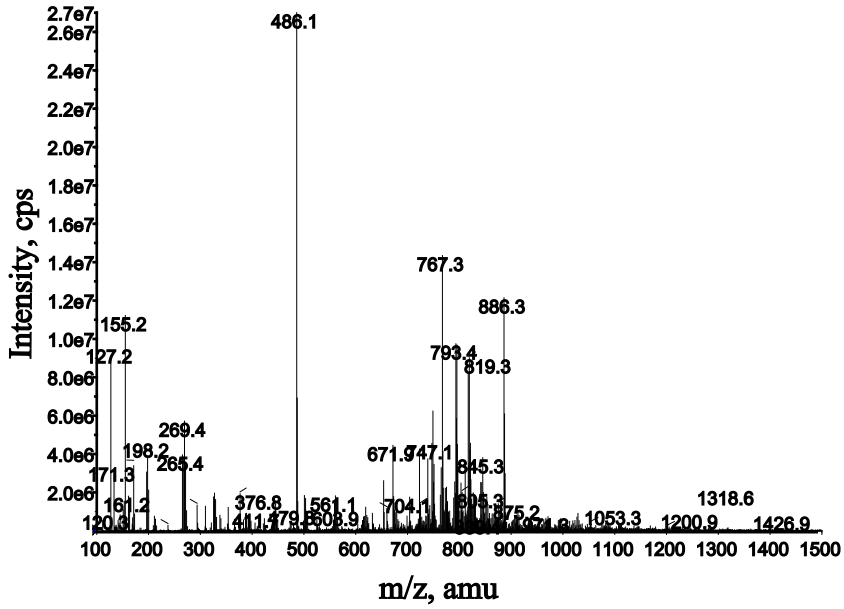


Fig. S3

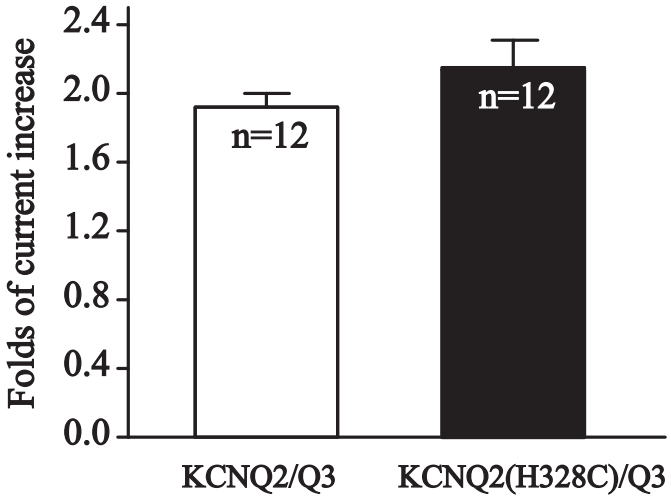
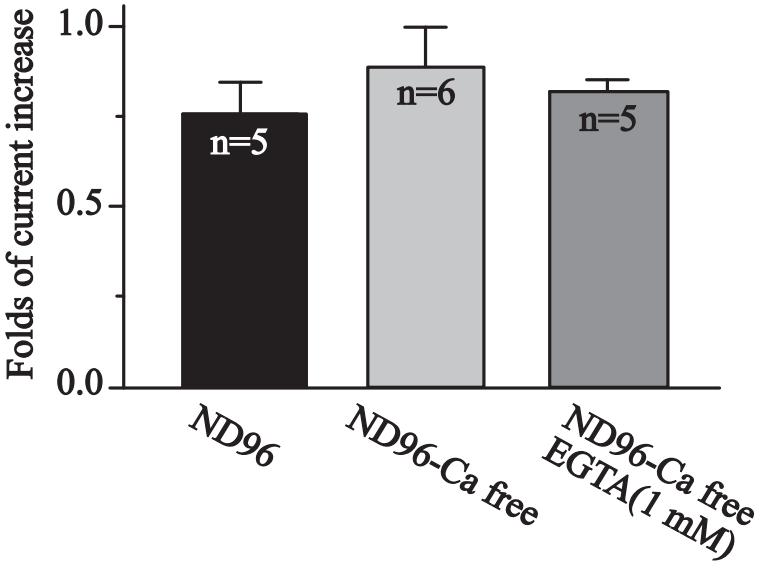

Folds of current increase
2.4
2.0
1.6
1.2
0.8
0.4
0.0
n=12
n=12
KCNQ2/Q3
KCNQ2(H328C)/Q3

Fig. S4


Folds of current increase
1.0
0.5
0.0
n=5
n=6
n=5
ND96
ND96-Ca free
ND96-Ca free
EGTA(1 mM)