Figure S1. Concentration dependence and slow reversibility of the PIP2 action. (A) Peak outward current size at 150 mV as a function of time before the application of PIP2 (blue), in the presence of 10 and 30 µM PIP2 (red), and after washout of PIP2 (black) in Slo1 channels. (B) Current-enhancing effect of PIP2 in Slo1+ β1 is functionally saturated at 10 µM. Representative currents at 120 mV before and after the application of 10 and 30 µM PIP2 (top) and peak outward current size at 120 mV (bottom). (C) Illustrative currents (top) and peak outward current size (bottom) recorded from Slo1+ β1 before the application of PIP2 (blue), in the presence of 10 µM PIP2 (red), and after washout of PIP2 (black). Currents were recorded without Ca2+.

Figure S2. Inhibitory effect of PIP2 on Slo1 channels expressed in Xenopus oocytes. (A) Representative currents through Slo1 from a patch taken from a Xenopus oocyte before (blue) and after (red) the application of 10 µM PIP2. (B) Representative peak I-V curves from Slo1 expressed in a Xenopus oocyte before (blue) and after (red) the application of 10 µM PIP2. (C) G-V curves from Slo1 expressed in Xenopus oocytes before (blue) and after (red) the application of 10 µM PIP2. The V0.5 and Qapp values for the control group are 177.6 ± 5.5 mV and 1.45 ± 0.15, and for the PIP2 group, they are 192.6 ± 4.5 mV and 1.00 ± 0.06, respectively; n = 4.
Figure S3.  diC8 PIP2 is less effective than brain-derived PIP2 on both Slo1 and Slo1 + β1. (A) Representative currents through Slo1 before (blue) and after (red) the application of 10 µM diC8. (B) G-V curves of Slo1 before (blue) and after (red) the application of 10 µM diC8 PIP2. The smooth curves are Boltzmann fits to the results. The $V_{0.5}$ and $Q_{app}$ values are 168.4 ± 1.8 mV and 1.17 ± 0.06 for control and 173.3 ± 3.8 mV and 1.19 ± 0.06 for the application of diC8 PIP2 ($n$ = 6). (C) Represent currents through Slo1 + β1 before (blue) and after (red) the application of 10 µM diC8. (D) Peak outward currents through Slo1 + β1 elicited by pulses to 120 mV as a function of time. The red bar indicates the diC8 PIP2 application period. (E) Illustrative Slo1 + β1 G-V curves before (blue), during application of 10 µM diC8 PIP2 (red), and after washout (black). The $V_{0.5}$ and $Q_{app}$ values are 188 mV and 0.84 for the control group, 158 mV and 0.95 for the PIP2 group, and 174 mV and 0.84 for the washout group, respectively. (F) Comparison of $V_{0.5}$ in Slo1 + β1 by 10 µM of brain-derived PIP2 (blue) and 10 µM diC8 PIP2 (red). All results shown were obtained without Ca2+. Error bars represent mean ± SEM.

Figure S4.  A high concentration of Ca2+ antagonizes the stimulatory effect of PIP2 on Slo1 D362A:D367A:E399A:Δ894–895 + β1. (A) Peak outward currents through Slo1 D362A:D367A:E399A:Δ894–895 + β1 elicited by pulses to 160 mV. The application of 2 mM Ca2+ to the intracellular side antagonizes the stimulatory effect of 10 µM PIP2, and the antagonistic effect of Ca2+ is relieved by the Ca2+ chelator EGTA (11 mM). (B) Illustrative G-V curves from a patch expressing Slo1 D362A:D367A:E399A:Δ894–895 + β1 channels. Blue, control; red, 10 µM PIP2; gray, 10 µM PIP2 + 2 mM Ca2+; green, wash. Similar results were obtained in 10 patches all together ($ΔV_{0.5} = −15.7 ± 4.2$ mV). (C) Changes in $V_{0.5}$ by PIP2, PIP2 + Ca2+, and wash. Error bars represent mean ± SEM.
Figure S5. Scaled currents through Slo1 complexes with auxiliary subunits with point mutations. (A) Scaled currents at 200 (top) and −200 mV (bottom) from Slo1 + β1 with β1-to-β2 point mutations (red). For each mutant, the currents obtained from Slo1 alone (black) and Slo1 + wild-type β1 (blue) are shown for comparison. (B) Scaled currents at 200 (top) and −200 mV (bottom) in Slo1 + β1 with mutations at position 11. For each mutant, the currents obtained from Slo1 alone (black) and Slo1 + wild-type β1 (blue) are shown for comparison. (C) Scaled currents at 200 (top) and −200 mV (bottom) in Slo1 + β2 Δ2–32 with β2-to-β1 point mutations (red). For comparison, scaled currents from Slo1 alone (black) and Slo1+ wild-type β2 Δ2–32 (blue) are shown. (D) Scaled currents at 200 (top) and −200 mV (bottom) in Slo1 + β4 with β4-to-β1 point mutations (red). For comparison, scaled currents from Slo1 alone (black) and Slo1 + wild-type β4 (blue) are shown. The sweep width represents mean ± SEM; n = 4–12. All results were obtained without Ca²⁺.
Figure S6. PIP2 enhances currents through divalent-insensitive Slo1+β1 channels. (A) Representative currents through Ca2+– and Mg2+–insensitive Slo1 D362A:D367A:E399A:Δ894–895 + β1 channels before and after the application of 10 µM PIP2. (B) G-V curves in Slo1 D362A:D367A: E399A:Δ894–895 + β1 channels before (blue) and after (red) the application of 10 µM PIP2. The curves represent Boltzmann fits with $V_{0.5} = 201.8 \pm 2.1$ mV and $Q_{app} = 1.03 \pm 0.05$ (Control), and $V_{0.5} = 148.5 \pm 1.4$ mV and $Q_{app} = 1.02 \pm 0.05$ (10 µM PIP2); n = 14.