Dai et al., http://www.jgp.org/cgi/content/full/jgp.201210944/DC1

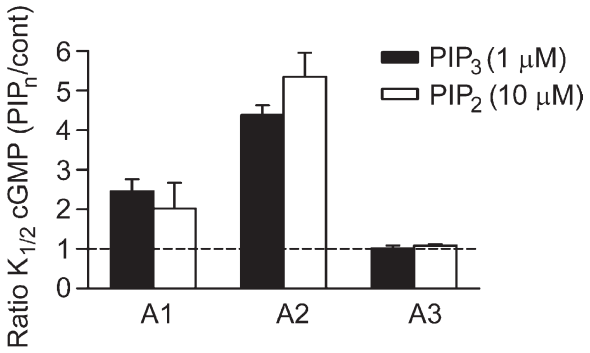

Figure S1. Subunit-specific regulation of homomeric CNG channels by phosphoinositides. Effects of 10 μM PIP_2 or 1 μM PIP_3 on apparent cGMP affinity of homomeric (bovine) CNGA1, (rat) CNGA2, or (human) CNGA3 channels expressed as the ratio of $K_{1/2}$ cGMP after PIP_2 or PIP_3 divided by control $K_{1/2}$ cGMP (PIP_n/cont). The broken horizontal line refers to a ratio of one, equivalent to no change in the parameter after PIP_n application. Error bars indicate mean ± SEM.

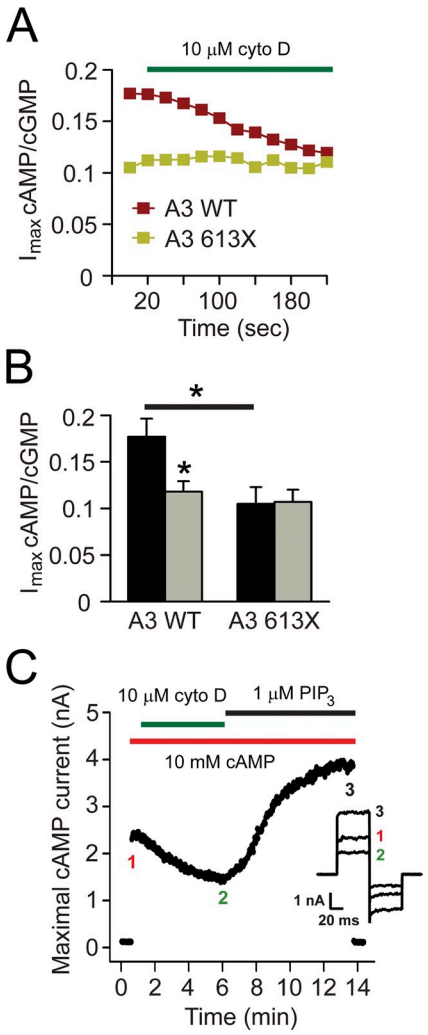

Figure S2. Disruption of the cytoskeleton does not prevent PIP_3 regulation of cAMP efficacy. (A) Representative time courses showing that direct application of 10 μM cyto D to inside-out patches diminished cAMP efficacy relative to cGMP for A3 wild-type channels, but not for A3-613X channels. (B) Summary illustrating the effect of direct application of cyto D (gray bars) on patches excised from cells expressing A3 wild-type or 613X channels, as shown in A. Cyto D reduced the I_{max} cAMP/cGMP ratio for wild-type A3, but not 613X channels; *, $P < 0.05$ compared with the control (black bars). The initial ratios of I_{max} cAMP/cGMP for A3 wild-type and 613X channels also were significantly different (*, $P < 0.05$). Error bars indicate mean ± SEM. (C) Time course showing the effect of 1 μM PIP_3 on the saturating cAMP current after cyto D application. Horizontal bars indicate application of the respective agents. The inset displays representative current traces before (1), after cyto D (2), and after PIP_3 (3), corresponding to the numbers below the time course for I_{max} cAMP current.

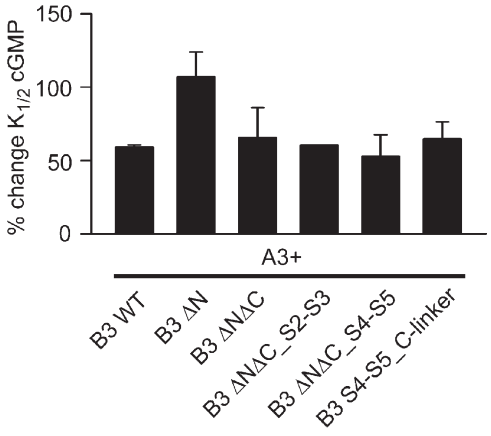

Figure S3. CNGB3 truncations and mutations do not prevent PIP_3 regulation of CNGA3+CNGB3 channels. Percentage change in $K_{1/2}$ cGMP is shown after application of 1 µM PIP_3 for heteromeric CNGA3+CNGB3 channels having CNGB3 deletions and/or charge-neutralizing mutations in potential PIP_n-binding sites: B3 wild type (WT; $n = 3$), ΔN (Δ2–205; $n = 4$), ΔNΔC (Δ2–205, Δ669–809; $n = 4$), ΔNΔC_S2-S3 (Δ2–205, Δ669–809, R291Q, K292Q, R295Q; $n = 1$), ΔNΔC_S4-S5 (Δ2–205, Δ669–809, K347Q, R352Q, R355Q; $n = 2$), and S4-S5_C-linker (K347Q, R352Q, R355Q, K473Q, K477N; $n = 4$). Error bars indicate mean ± SEM.