

SUPPLEMENTARY MATERIAL

PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP₂) CONTROLS MAGNESIUM GATEKEEPER TRPM6 ACTIVITY

Jia Xie¹, Baonan Sun¹, Jianyang Du¹, Wenzhong Yang¹, Hsiang-Chin Chen², Jeffrey D. Overton², Loren W. Runnels², Lixia Yue^{1*}

From Calhoun Cardiology Center, Department of Cell Biology, University of Connecticut Health Center, Farmington, CT¹, and Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey², USA.

Supplemental Figure 1. Effects of CCh on TRPM6 current in the HEK293 cells without M1 receptor. **A**, Representative traces in the absence and presence of 200 μ M CCh. **B**, Time course of TRPM6 current development and response to the CCh in the HEK293 without M1 receptor over-expression.

Supplemental Figure 2. PLC inhibitor directly blocks both TRPM6 and TRPM7 currents. **A**, Representative traces of TRPM6 in response to PLC inhibitor U73122 (5 μ M) and its inactive analogue U73343 (100 μ M). **B**, Normalized TRPM6 current before and after PLC inhibitor U73122 (5 μ M) and its inactive analogue U73343 (100 μ M) perfusion. **C**, Representative traces of TRPM7 in response to PLC inhibitor U73122 and its inactive analogue U73343. **D**, Averaged inhibition of TRPM7 by PLC inhibitor U73122 and its analogue U73343.

Supplemental Figure 3. Effects of downstream pathways of GTP γ S on TRPM6. **A**, Effects of 5 μ M Forskolin and 1 μ M PDBu on TRPM6 currents. **B**, Normalized current amplitude of TRPM6 after treatment with Forskolin and PDBu (n=6). Note TRPM6 currents were not influenced by cAMP activator Forskolin or PKC activator PDBu. **C**, Translocation of PH-GFP from plasma membrane to cytosol induced by 0.5 μ M GTP γ S included in the pipette solution. Method for measurement of PH-GFP translocation is detailed in Fig. 3. **D**, Translocation of PH-GFP

from plasma membrane to cytosol induced by application of CCh. Note the translocation of PH-GFP by GTP γ S is slower than that induced by CCh because GTP γ S needs to be dialyzed into the cells.

Supplemental Figure 4. Effects of Rap on TRPM6 in cells transfected with phosphatase-dead mutant D281.

A, Representative traces before and after Rap application in cells transfected with TRPM6, lyn-FRB (LDR), and CF-InP-D281A. **B**, Time course of the TRPM6 current before and after 10 μ M Rap. **C**, Normalized current density comparison before and after Rap.

Supplemental Figure 5. Inclusion of PIP₂ slows down inactivation of TRPM6 caused by activation of Ci-VSP.

The protocol used for Ci-VSP activation was as same as shown in Fig. 3A. TRPM6 was almost completely inactivated within 100 s; whereas less than 50% of TRPM6 was inactivated within 200 s and that inactivation seemed to have reached a plateau when pipette solution contained 10 μ M PIP₂.

Supplemental Figure 6. Inactivation of TRPM6 by PIP₂ depletion under perforated-patch configuration. A,

Representative traces of TRPM6 recorded before and after depolarization. The protocol used for these recordings was similar to that in Fig. 3A except the holding potential after each ramp was +60mV to maintain the Ci-VSP activity in order to out-beat PIP₂ re-synthesis process. **B**, Time course of TRPM6 current inactivation induced by Ci-VSP activation in cells co-transfected with TRPM6 and Ci-VSP. **C**, Mean current amplitude of TRPM6 before and after Ci-VSP activation (n=9, * p<0.05).

Supplemental Figure 7. Inactivation of TRPM7 by PIP₂ depletion under perforated-patch configuration. A,

Representative traces of TRPM7 recorded before and after depolarization, using the protocol detailed in Fig. 3. **B**, Mean current amplitude of TRPM7 before and after Ci-VSP activation (n=9, * p<0.05).

Supplemental Figure 8. PIP₂ depletion abolishes potentiation effects of 2-APB on TRPM6. A,

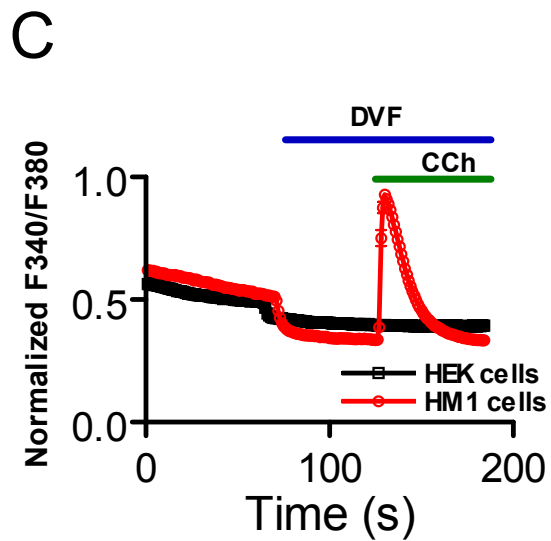
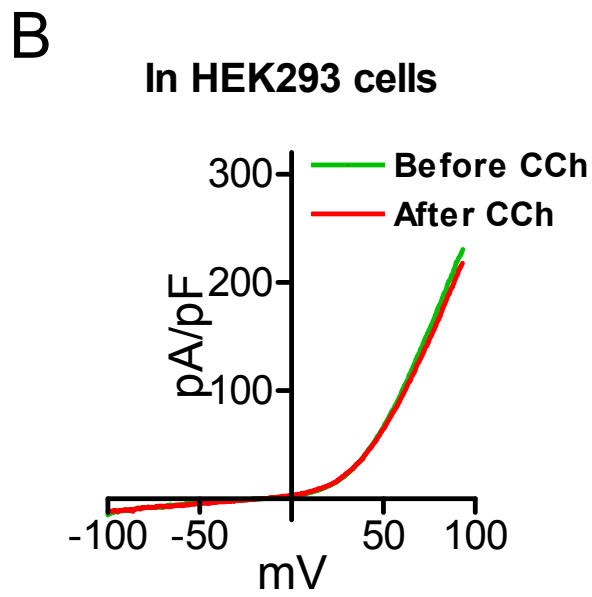
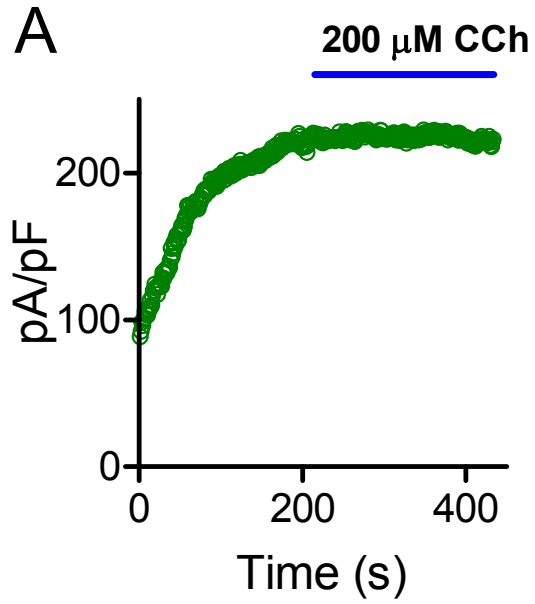
Time-dependent inactivation of TRPM6 induced by activation of Ci-VSP in a TRPM6 and Ci-VSP co-transfected cell. Experimental protocol for activating Ci-VSP is as same that shown in Fig 3A. Application of 2-APB (200 μ M) at time point “a” was able to potentiate TRPM6, but 2-APB failed to increase TRPM6 current when applied at time point “c” when TRPM6 was completely inactivated by Ci-VSP activation. **B**, Representative currents of TRPM6 before (“a”) and after application of 2-APB (“b” and “c”). Similar results were obtained in another 5 experiments.

Supplemental Figure 9. Kinase domain of TRPM6 interacts with PLC isoforms, but the interaction is not necessary for PLC induced inactivation of TRPM6 and TRPM7. A,

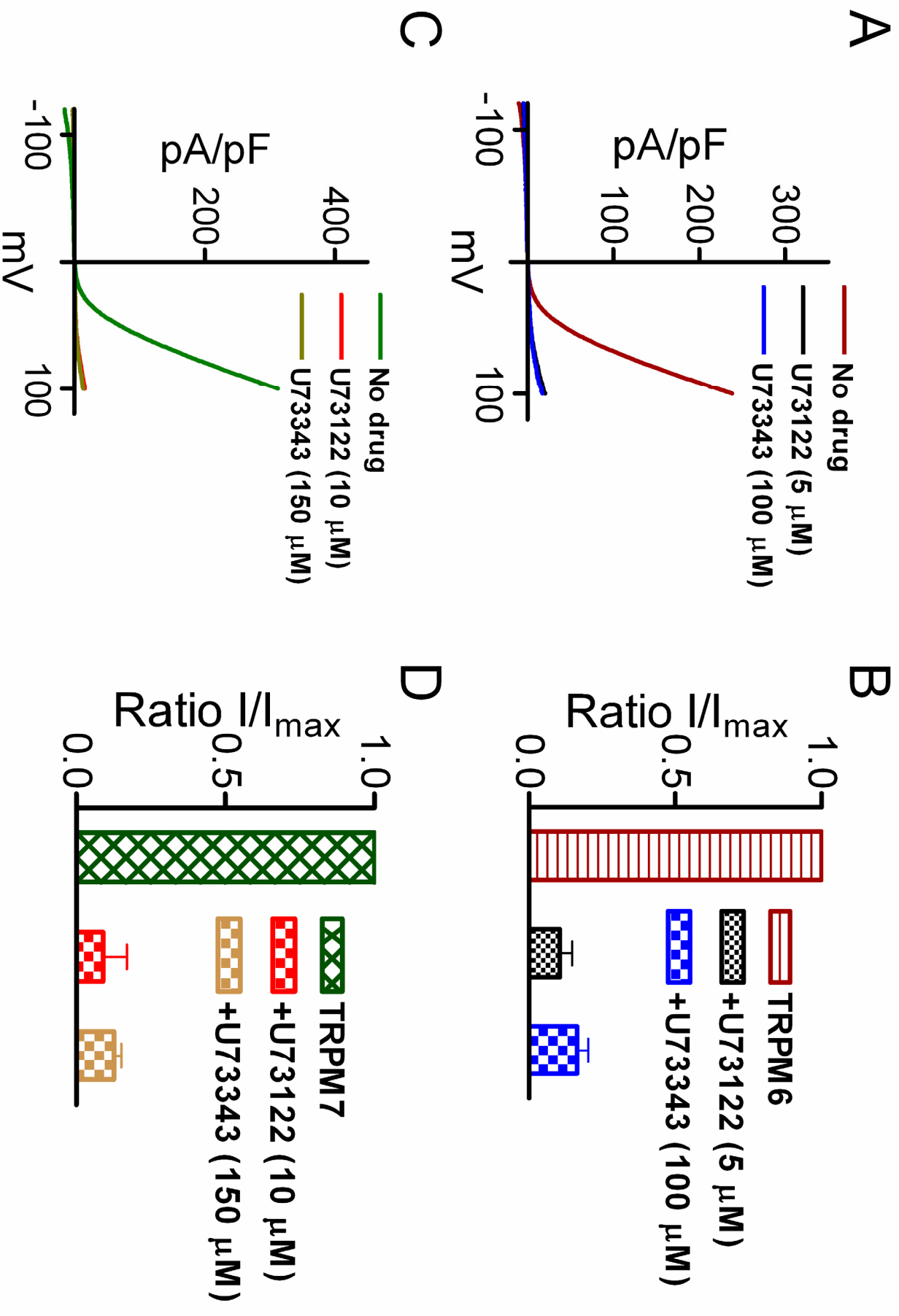
Coomassie blue staining of purified GST tagged kinase domain of TRPM6 and TRPM7 from bacteria. **B**, A GST-pulldown purification assay using purified GST tagged kinase domain with the cell lysates from HEK-293 cells transfected with various isoforms of PLC.

GST tagged TRPM6 kinase domain (GST-M6 KIN) and TRPM7 kinase domain (GST-M7 KIN) were expressed and purified from bacteria. Different isoforms of PLC were transfected to HEK293 cells. Antibodies against different isoforms were used to detect the bound PLC protein. **C**, Representative traces of TRPM6- Δ kinase recorded in cells dialyzed with or without 1 mM GTP γ S in the pipette solution. 2-APB (200 μ M) was used to verify that the currents recorded were TRPM6 but not TRPM7 currents. **D**, Comparison of the TRPM6- Δ kinase current density in cells with or without 1mM GTP γ S in the pipette solution (n=7, *p<0.05). **E**, Representative traces of the TRPM7- Δ kinase recorded in cells dialyzed with or without 1 mM GTP γ S in the pipette solution. **F**, Normalized current amplitude of TRPM7- Δ kinase with or without 1mM GTP γ S treatment (n=8,* p<0.05).

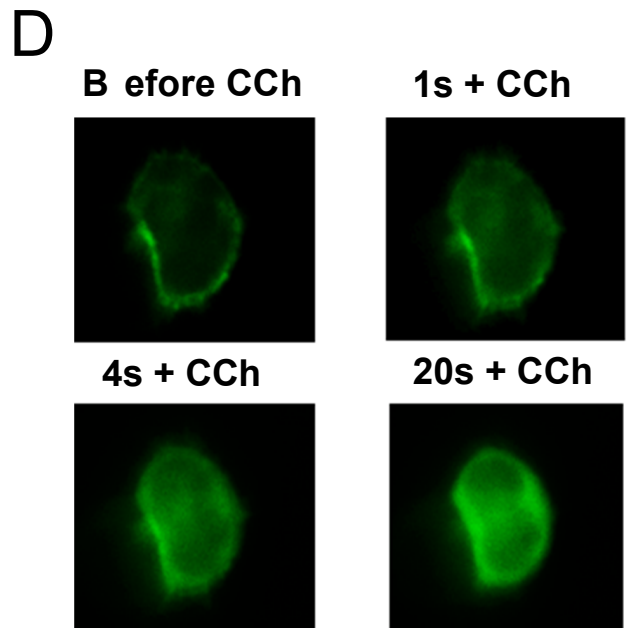
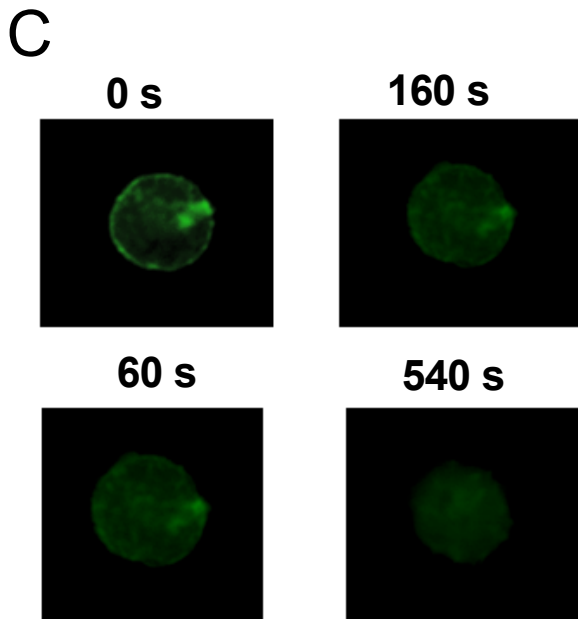
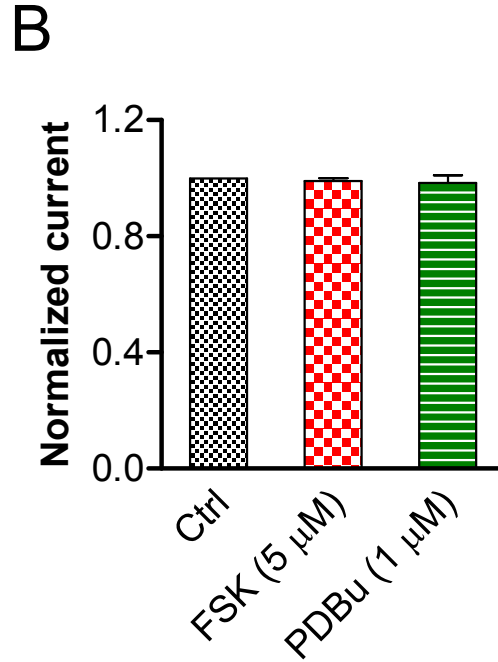
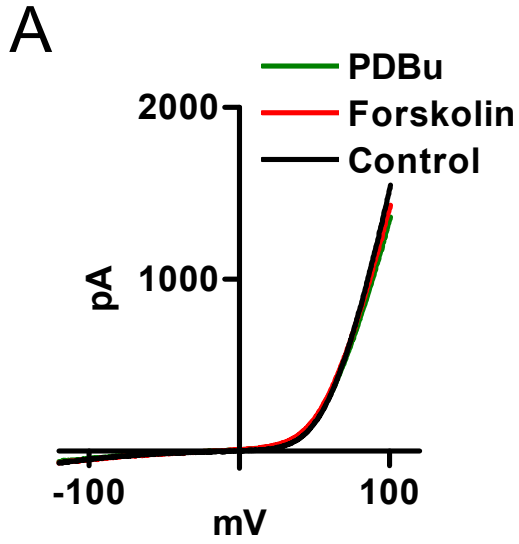
Supplemental Fig.1



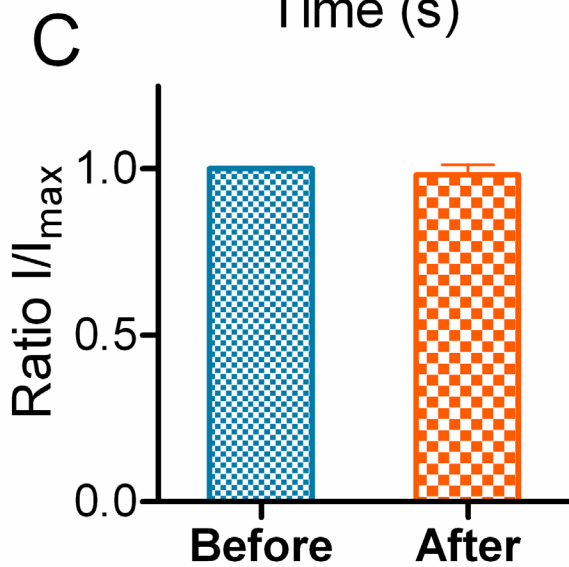
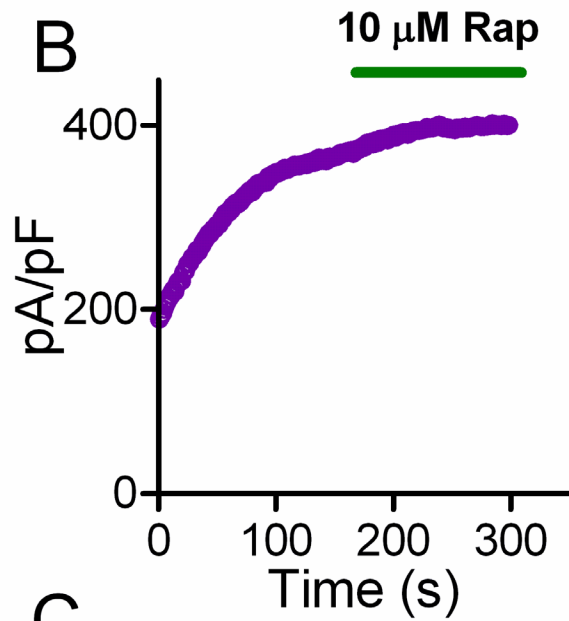
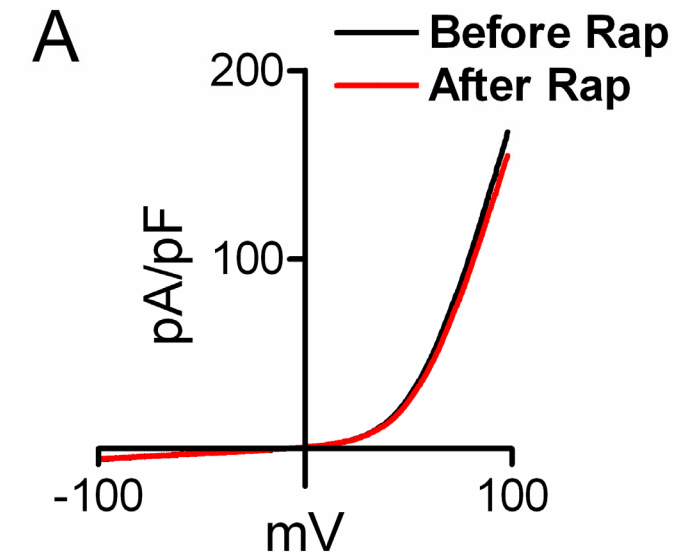
Supplemental Fig. 2



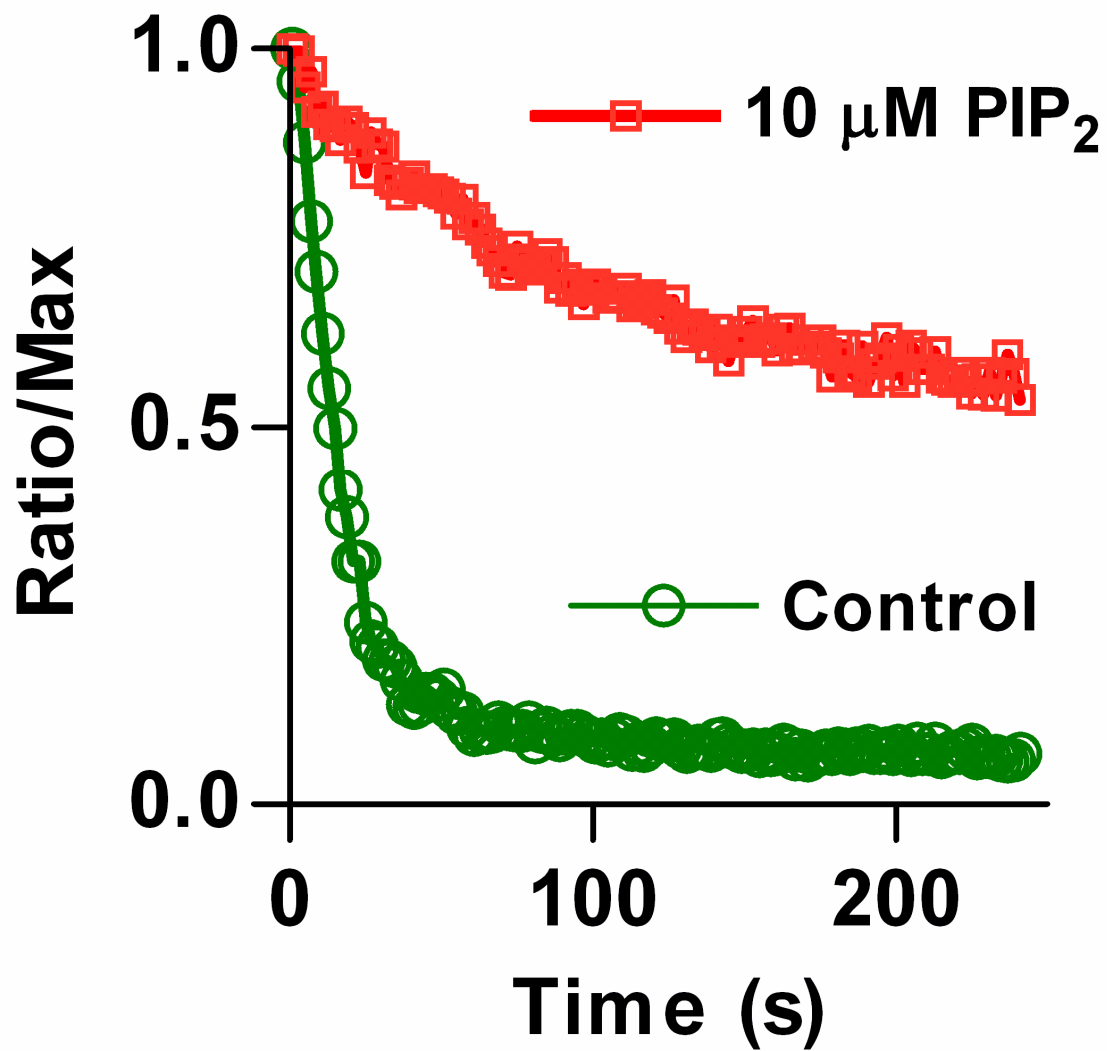
Supplemental Fig. 3



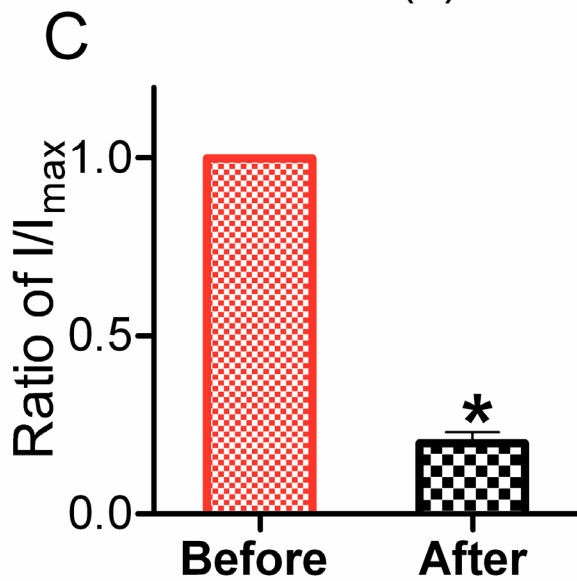
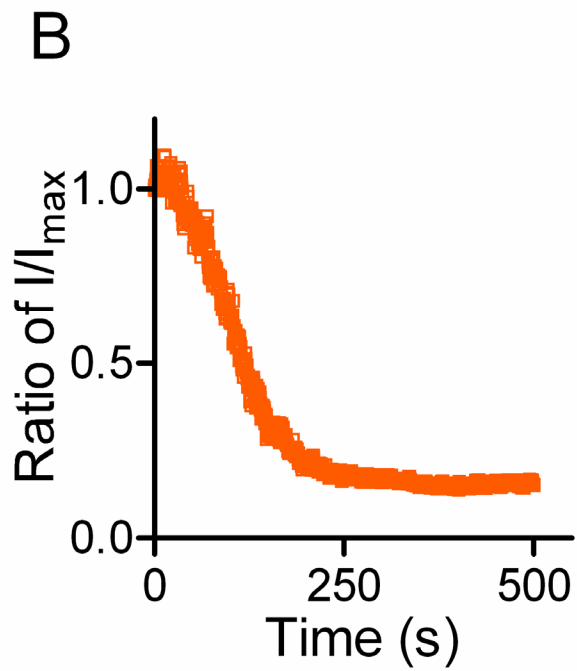
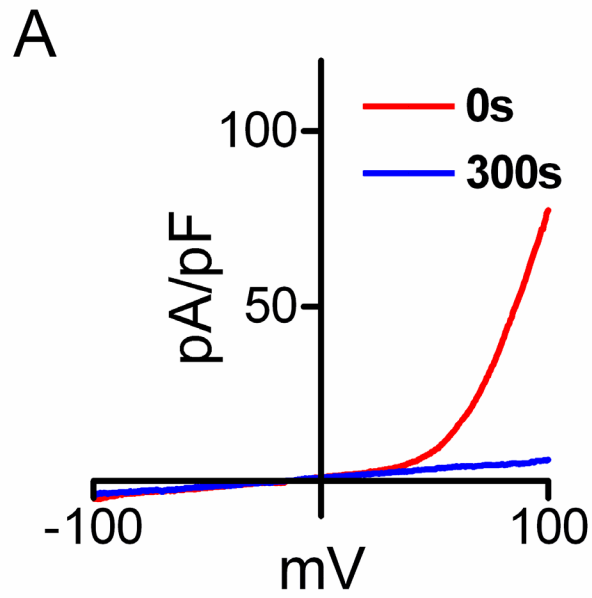
Supplemental Fig. 4



Supplemental Fig. 5

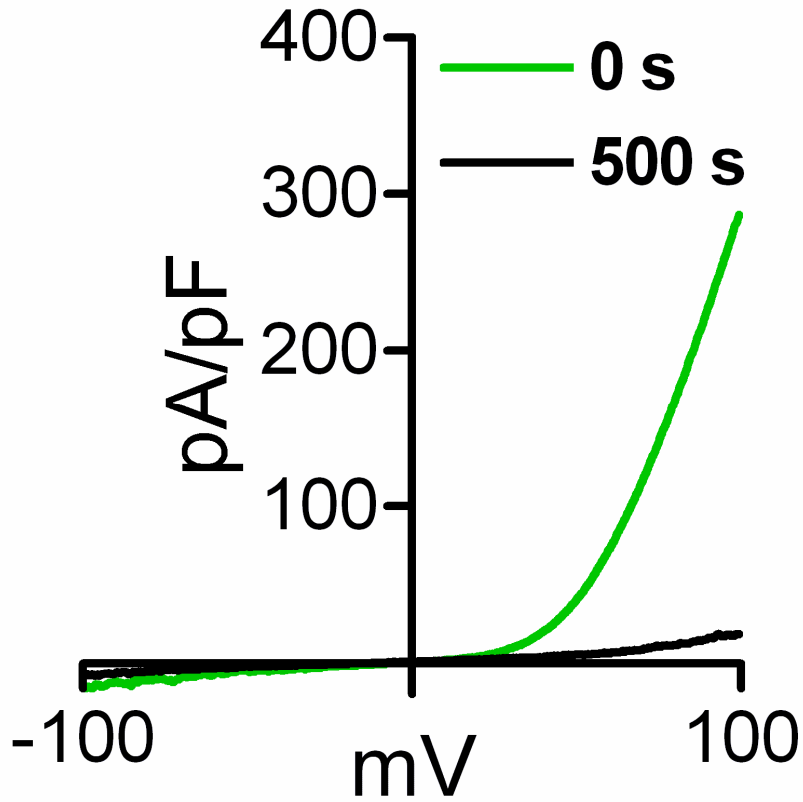


Supplemental Fig. 6

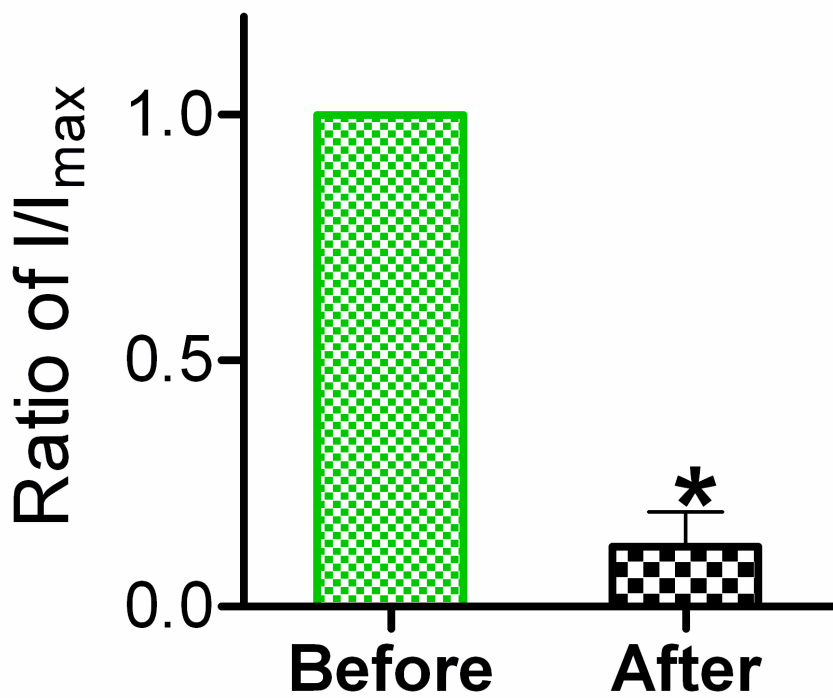


Supplemental Fig. 7

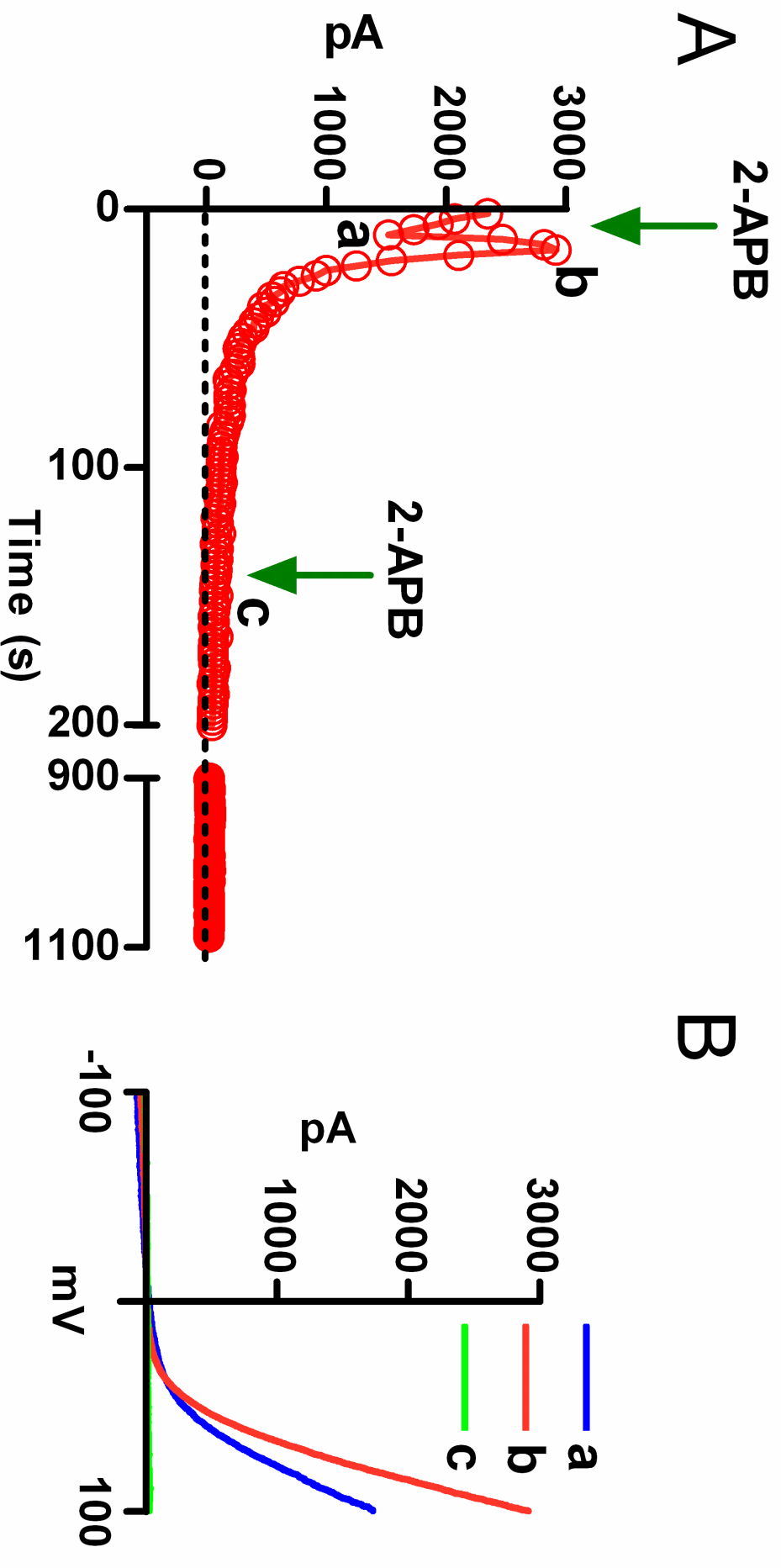
A



B



Supplemental Fig. 8



Supplemental Fig. 9

