Badheka et al., http://www.jgp.org/cgi/content/full/jgp.201411336/DC1

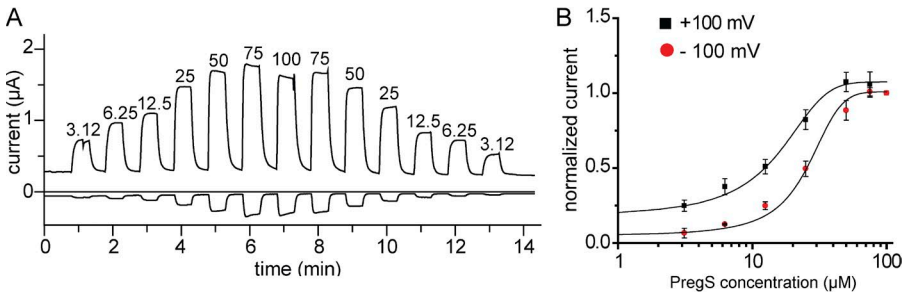

Figure S1. Concentration-dependent effects of PregS on hTRPM3. (A) Representative data from a TEVC measurement in a *Xenopus* oocyte showing the effects of the indicated concentrations of PregS (μM) on the hTRPM3. Data were plotted from −100 to 100–mV ramp measurements as described in Materials and methods. (B) Concentration-dependent activation of hTRPM3 by PregS; all values normalized to 100 μM ($n = 5$), EC_{50} = 19.3 μM at 100 mV and 28.5 μM at −100 mV. Error bars represent SEM.

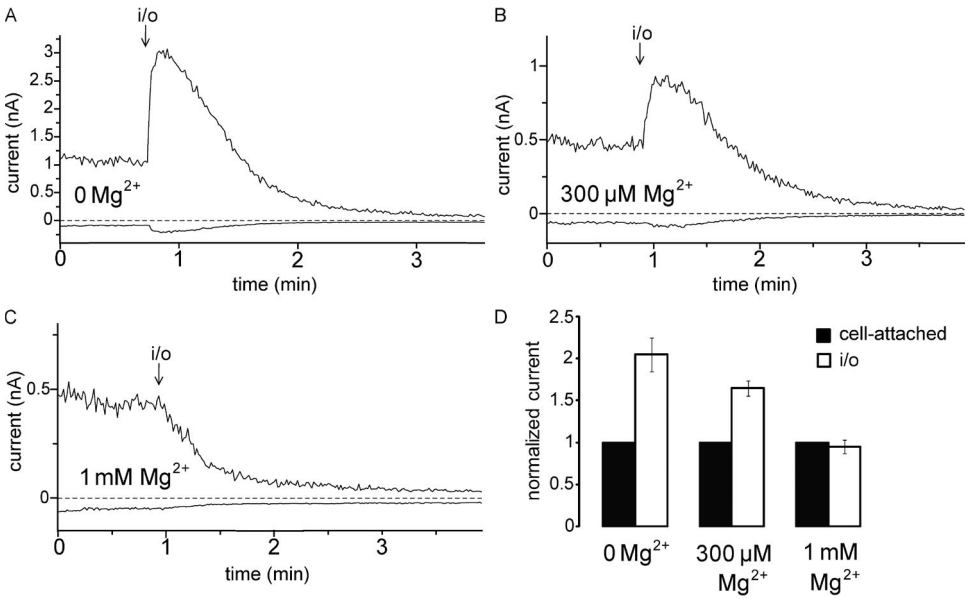

Figure S2. The effect of Mg^{2+} on the current increase after patch excision. (A–C) Representative traces at 100 and −100 mV; experiments were performed on hTRPM3-expressing *Xenopus* oocytes with 100 μM PregS in the patch pipette as described in Materials and methods. Patches were excised into a bath solution with no Mg^{2+}, 300 μM Mg^{2+}, or 1 mM Mg^{2+}, as indicated. (D) Statistics; the mean of the currents at 100 mV in the first 8 s after excision was measured and normalized to the currents before excision ($n = 6–7$). Error bars represent SEM.

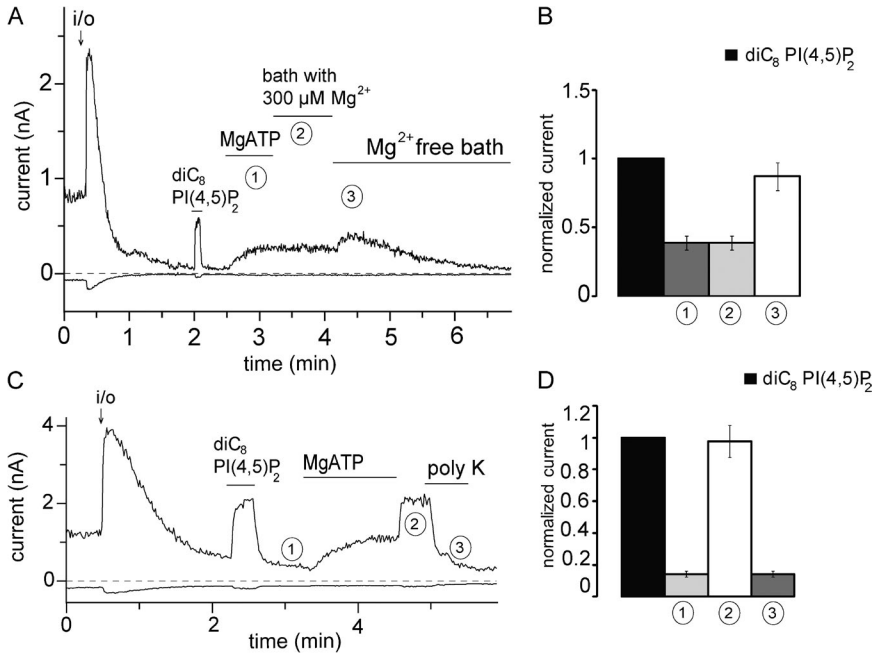

Figure S3. The effect of Mg^{2+} on the current increase after washout of MgATP and the effect of Poly-Lys after MgATP. Experiments were performed on hTRPM3-expressing *Xenopus* oocytes with 100 µM PregS in the patch pipette as described in Materials and methods; data are shown at 100 and −100 mV. (A) Representative trace; the applications of 25 µM diC_8 $PI(4,5)P_2$, 2 mM MgATP (free [Mg] = 300 µM), bath solution containing 300 µM Mg^{2+}, and Mg^{2+}-free bath solution are indicated by the horizontal lines. (B) Summary of the data in A ($n = 5$). (C) Representative measurement showing the effect of 25 µM diC_8 $PI(4,5)P_2$, 2 mM MgATP, and 30 µg/ml Poly-Lys. (D) Summary of the data in C ($n = 3$). Error bars represent SEM.

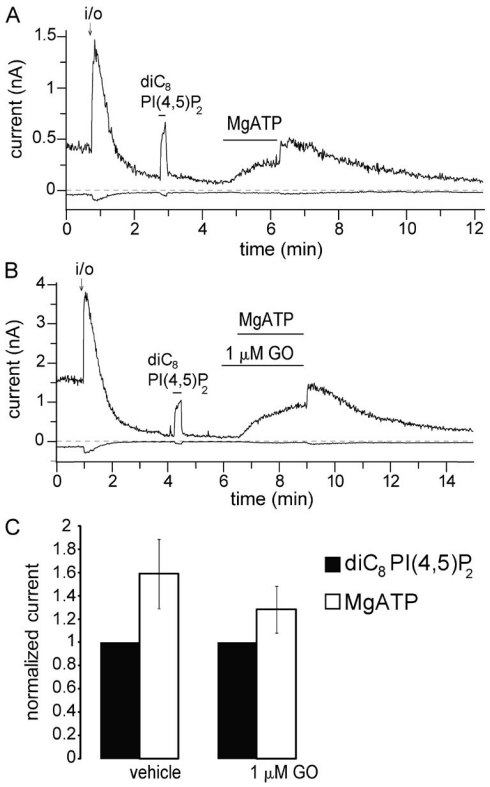

Figure S4. PKC inhibition does not affect MgATP. Excised inside-out patch measurements have been performed on hTRPM3-expressing *Xenopus* oocytes with 100 μM PregS in the patch pipette as described in Materials and methods; data are plotted at 100 and −100 mV. (A and B) Representative traces for the effects of MgATP in the absence and presence of the PKC inhibitor Gö6976 (GO); the applications of 2 mM MgATP, 1 μM Gö6976, and 25 μM diC_8 $PI(4,5)P_2$ are indicated by the horizontal lines. (C) Summary of the data for control and PI-PLC–treated patches ($n = 5$). Error bars represent SEM.

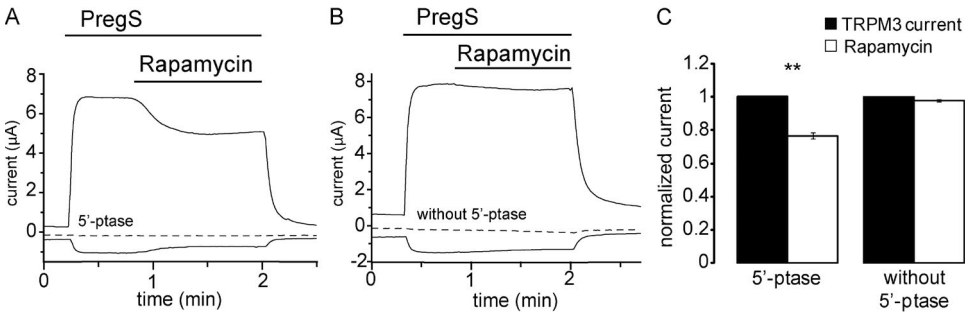

Figure S5. The effect of rapamycin-inducible 5′-phosphatase in *Xenopus* oocytes. (A) Representative TEVC measurement in a *Xenopus* oocyte expressing hTRPM3 and the components of the rapamycin-inducible 5′-phosphatase system. Measurements were performed using a ramp protocol from −100 to 100 mV, and current amplitudes are plotted at 100 and −100 mV. The applications of 100 μM PregS and 1 μM rapamycin are indicated by the horizontal lines. (B) Similar experiment as in A in a control oocyte, expressing TRPM3 and the components of the rapamycin-inducible system without the 5′-phosphatase. (C) Statistical summary of the data at 100 and −100 mV ($n = 4$–5). Error bars represent SEM. **, $P < 0.01$.