Kruse et al., http://www.jgp.org/cgi/content/full/jgp.201210806/DC1

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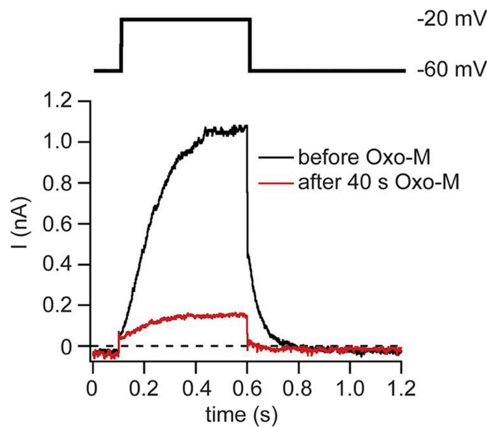

Figure S1. Activation of M_1R strongly decreases $K_V7.2/K_V7.3$ current amplitudes. Current traces in $K_V7.2/K_V7.3$ channels coexpressed with M_1R before Oxo-M (black) and after 40 s application of 10 µM Oxo-M (red).

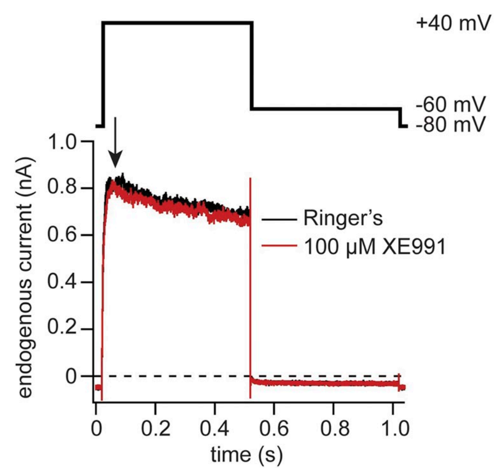

Figure S2. Endogenous potassium currents in tsA-201 cells are not blocked by XE991. Endogenous potassium currents in mock-transfected tsA-201 cells were elicited by the pulse protocol given. Black indicates current trace in Ringer's. Red indicates current trace 1 s after the end of a 20-s application of 100 µM XE991. With the 40-mV pulse, current amplitudes of endogenous potassium currents were 1037 ± 206 pA (n = 5, measured at time point indicated by the arrow).

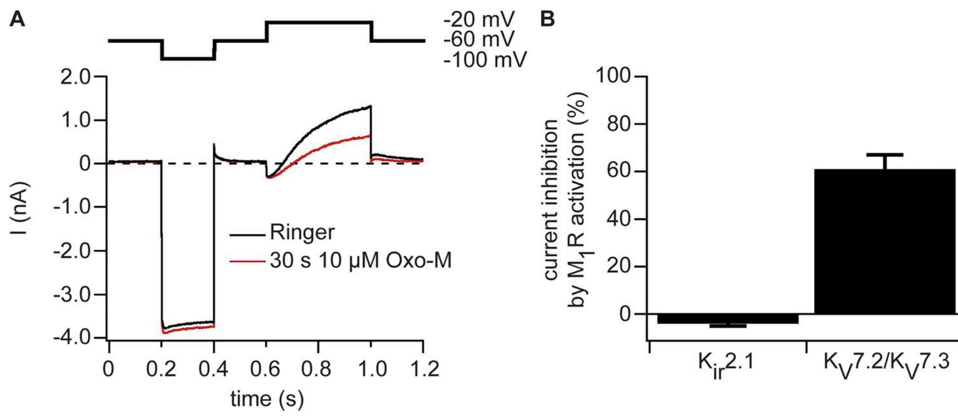

Figure S3. Depletion of PI(4,5)P_2 by M_1R activation inhibits $K_V7.2/K_V7.3$ channels but not $K_{ir}2.1$ channels. (A) $K_{ir}2.1$- and $K_V7.2/K_V7.3$-mediated currents were recorded before (black) and after activation of M_1R (red). Pulse protocol is given above current traces. (B) Decrease in $K_{ir}2.1$- and $K_V7.2/K_V7.3$-mediated currents after activation of M_1R (n = 5). Error bars represent ±SEM.

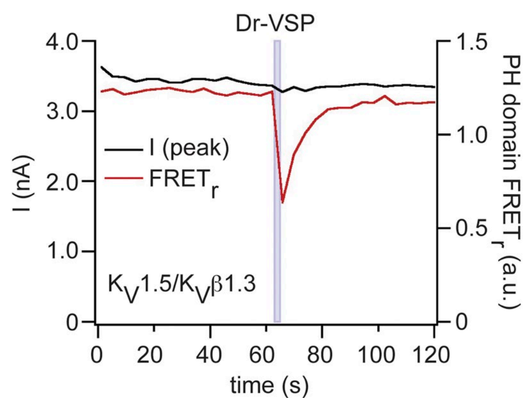

Figure S4. PH probes indicate a transient drop of PI(4,5)P_2 levels from VSP activation. To test for a decrease in PI(4,5)P_2 levels after activation of Dr-VSP, we expressed CFP- and YFP-tagged PH probes together with K_V channels and Dr-VSP and monitored changes in FRET ratio as a reporter of changes in PI(4,5)P_2 levels at the plasma membrane. Traces show time courses of FRET ratio (red) in a representative experiment (expression of $K_V1.5$, $K_V\beta1.3$, Dr-VSP, eCFP-PH, and eYFP-PH) together with peak current (black). Bar indicates period of Dr-VSP activation by a 2-s depolarizing pulse to 100 mV. The recovery of FRET ratio after the initial drop indicates PI(4,5)P_2 resynthesis at the plasma membrane. No alteration in peak current amplitude was observed.

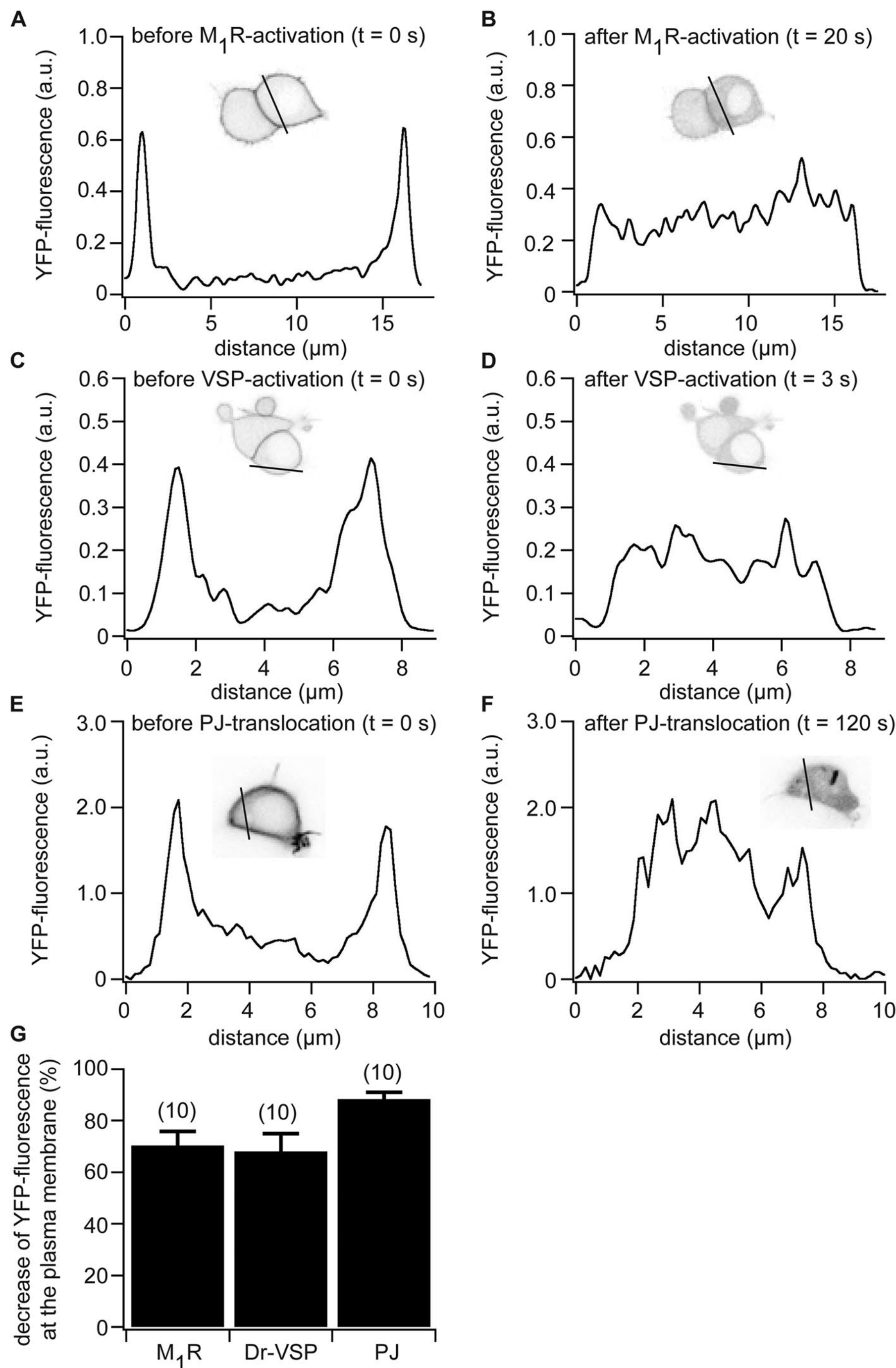

Figure S5. Depletion of $PI(4,5)P_2$ by activation of M_1R or Dr-VSP or recruitment of pseudojanin leads to translocation of YFP-tagged PH probes from the plasma membrane into the cytoplasm. (A) Line scan measuring YFP fluorescence of a cell expressing YFP-tagged PH probes and M_1R before activation of the receptor by Oxo-M. (B) Same as in A but after activation of M_1R. (C–F) Same protocol as in A and B but with coexpression of Dr-VSP (C and D) or pseudojanin (PJ; E and F). Insets show analyzed cells, and black lines indicate area used for the line scan. Plots A–F were generated from the mean of three neighboring line scans for each cell. To compensate for short exposure times, plots A–D were treated with the smooth function of Igor Pro (binomial smooth, setting of 2). (G) Decrease in YFP fluorescence of YFP-tagged PH probes at the plasma membrane after activation of M_1R, Dr-VSP, or pseudojanin. Numbers in parentheses indicate individual experiments. Error bars represent ±SEM.

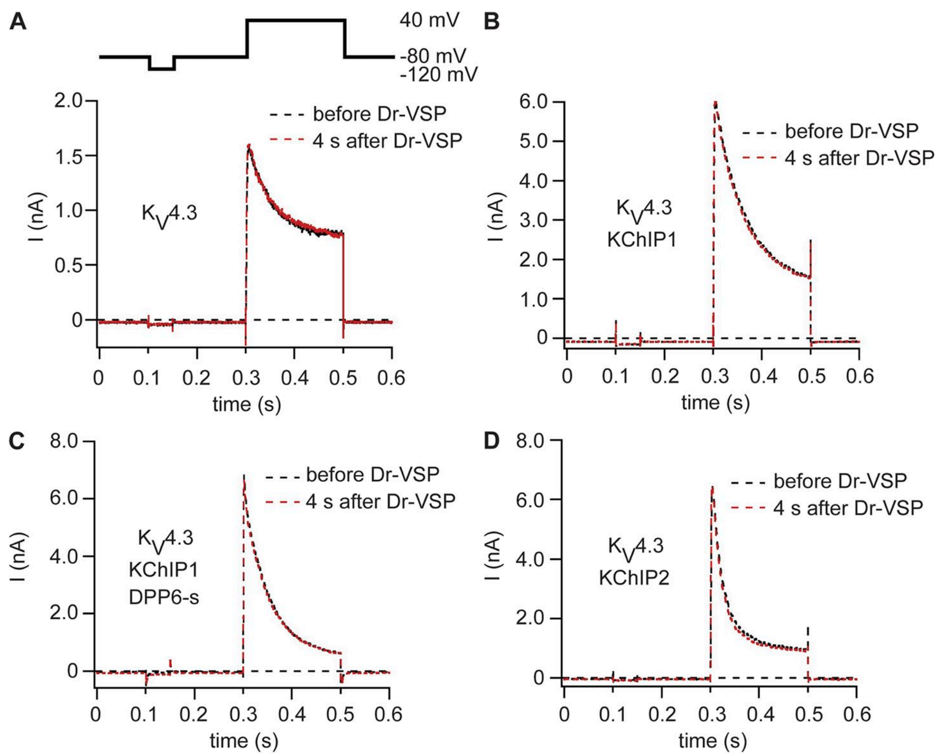

Figure S6. Activation of Dr-VSP shows insensitivity of $K_V4.3$ channels to depletion of $PI(4,5)P_2$. (A) Current traces of a cell expressing $K_V4.3$ and Dr-VSP. Black indicates before activation of Dr-VSP, and red indicates 4 s after activation of Dr-VSP. (B–D) Same protocol as in A but with coexpression of KChIP1 (B), KChIP1 and DPP6-s (C), or KChIP2 (D).