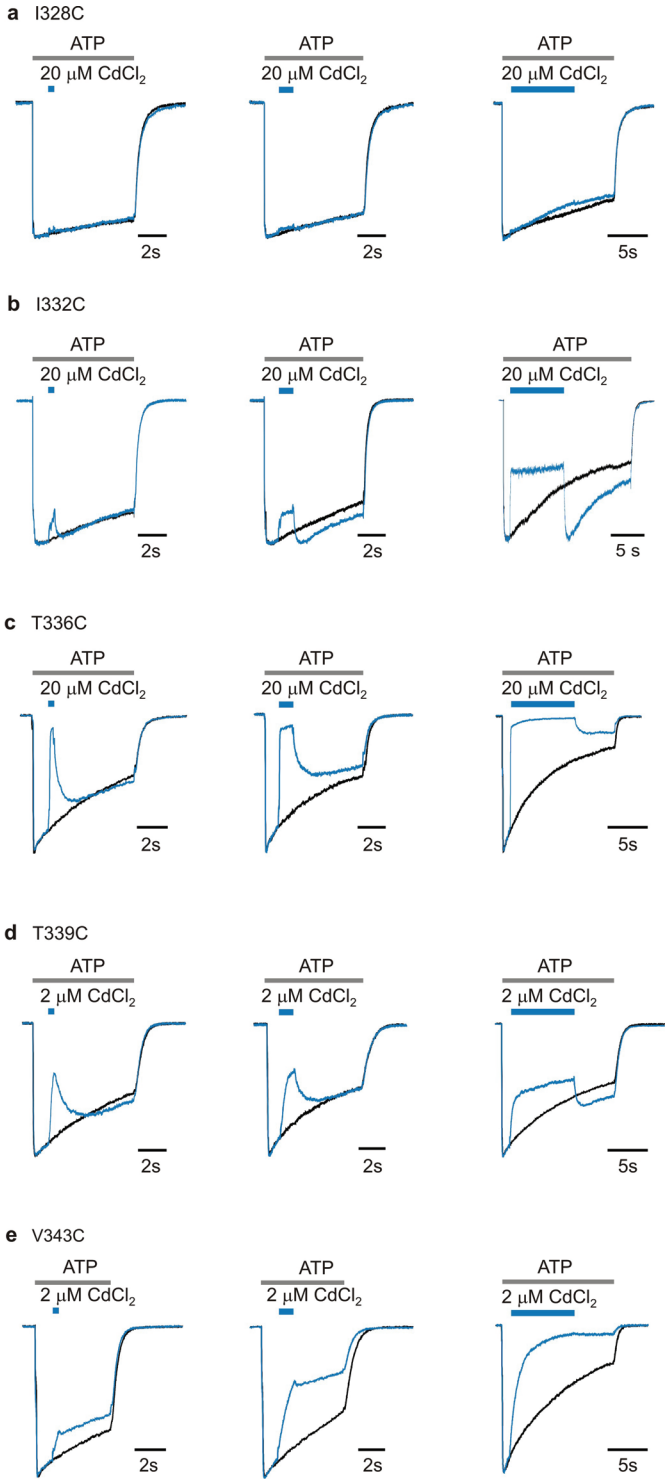
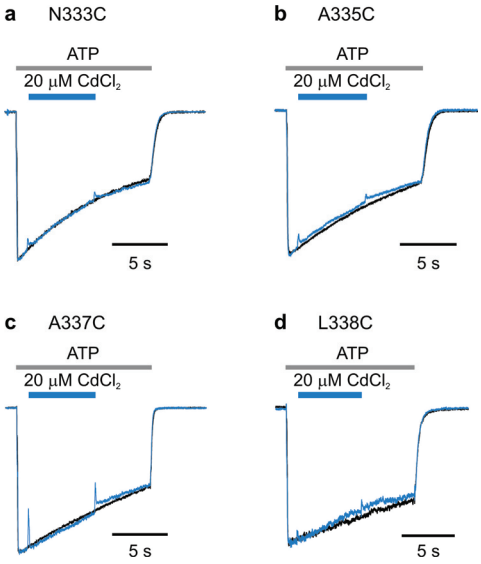


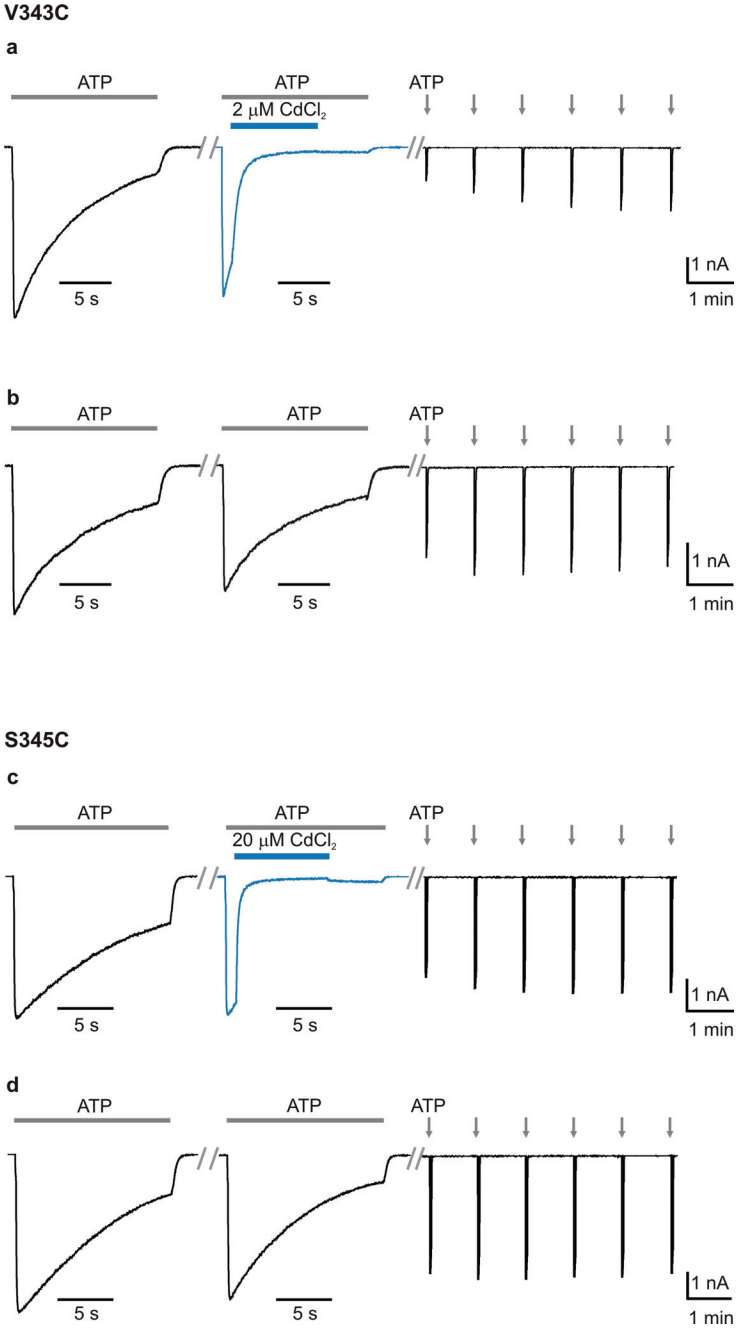
Supplementary Figure S1| Effects of extracellular Cd^{2+} on Cys mutants in TM2.

Black current traces show ATP-activated currents under control conditions, while those in blue show the effects of external Cd^{2+} application in the presence of ATP. Blue traces have been scaled to superimpose with control traces in black. Cd^{2+} was applied for 300 ms (left panel), 1 s (middle panel) or 8 s (right panel). **a)** I328C was insensitive to extracellular Cd^{2+}. **b and d)** I332C and T339C currents were rapidly inhibited by Cd^{2+}, and the inhibition could be reversed rapidly after removing the metal from the external solution. At both of these positions, longer Cd^{2+} application seem to prevent current desensitization. **c)** T336C current was inhibited by Cd^{2+}, and the inhibition recovered rapidly if Cd^{2+} was applied for only 300 ms. Longer Cd^{2+} application resulted in stable coordination as indicated by a fraction of current that recovers slowly. Under control conditions, substantial desensitization occurs during long ATP applications, suggesting that stable coordination of Cd^{2+} may occur in a desensitized state. **e)** V343C currents were inhibited by Cd^{2+} regardless of the application time, and the inhibition recovered slowly after removing Cd^{2+}. Saturating ATP concentrations (30 or 100 μM) were used in these experiments. Similar results were observed in 4 to 6 different experiments.



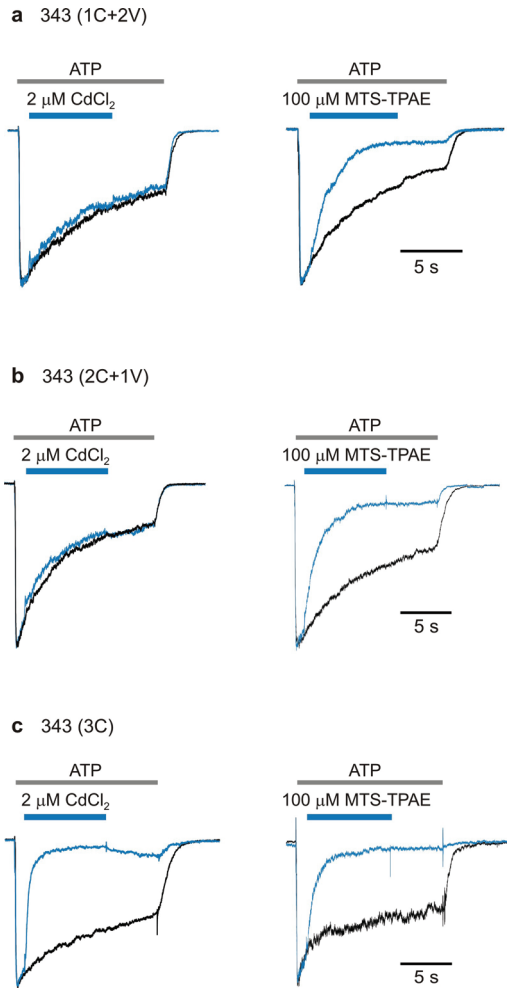
Supplementary Figure S2|
Extracellular Cd^{2+} has no effect on four Cys mutants in the external region of TM2.

Black current traces show ATP-activated currents under control conditions, while those in blue show the effects of external Cd^{2+} application in the presence of ATP. Blue traces have been scaled to superimpose with control traces in black. Saturating concentrations of ATP (30 to 100 μM) were used in each case, and similar results were obtained in 4 different experiments.



Supplementary Figure S3| Recovery of V343C and S345C channels after removal of external Cd^{2+}.

a) and c) ATP (100 μM) was first applied alone as a control, and then Cd^{2+} was applied for 8 sec. during a second ATP application (given 2 min later). Subsequent ATP applications were given every minute, beginning 1 min after the second ATP application. Similar results were obtained in 4 different experiments. **b) and d)** Control experiments using the same protocol as in a) but without Cd^{2+} application.



Supplementary Figure S4|
Effects of extracellular Cd^{2+} and MTS-TPAE on concatamers containing one to three Cys substitutions at position V343.

Black current traces show ATP-activated currents under control conditions, while those in blue show the effects of external Cd^{2+} application in the presence of ATP. Blue traces have been scaled to superimpose with control traces in black.
a) A trimeric concatamer with 1 Cys and 2 Val at 343 is insensitive to 2 μM Cd^{2+}, but can be inhibited by 100 μM MTS-TPAE (with a modification rate of $4.8\pm0.4\times10^{4}$ $M^{-1}s^{-1}$), indicating that the Cys mutation is accessible.
b) A concatamer with 2 Cys and 1 Val at 343 is insensitive to 2 μM Cd^{2+}, but can be inhibited by 100 μM MTS-TPAE with a modification rate of $5.9\pm0.6\times10^{4}$ $M^{-1}s^{-1}$. **c)** A concatamer with 3 Cys at 343 is inhibited by applying either 2 μM Cd^{2+} or 100 μM MTS-TPAE (with a modification rate of $1.1\pm0.1\times10^{5}$ $M^{-1}s^{-1}$). Saturating concentrations of ATP (30 μM) were used in each case, and similar results were obtained in 4 different experiments.