Supporting Information

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SI Materials and Methods

Transfection of CHO-K1 Cells. Cells were grown in culture flasks (BD Falcon - −75 cm^2, BD Bioscience, San Jose, CA) in F12-Kaighn's modification medium (HyClone, Logan, UT) supplemented with 10% FBS (Sigma, St. Louis, MO) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA) at 37°C in a humidified 5% CO_2 atmosphere. Twice a week, cells were detached by treatment with trypsin (Sigma) and EDTA (Invitrogen) and subcultured at at ratio of 1:10. For electrophysiological experiments cells were subcultured on slides covered with 50 μg/ml PolyD-lysine (Sigma) for 16–24 h before use. Cells were transfected with plasmid DNA using calcium-phosphate. After 5 min of incubation, a total of 200 μl mixture in 1.8 ml medium was applied to the cells. After 7–8 h of incubation, cells were washed with EGTA (5 mM in PBS, pH 7.4 adjusted with NaOH) and the cells were then treated with 10% glycerol in PBS for 1 min. This procedure was repeated twice and finally cells were incubated in fresh F12-Kaighn's modification medium for 16–24 h. The transfection efficiency was confirmed by cotransfection of pHluorin. CHO cells transfected with pHluorin only showed no detectable pH-sensitive endogenous currents (pH 5.5: −0.61 ± 1.4 pA, $n = 7$; pH 5.5 with amantadine: 1.7 ± 0.7 pA, $n = 7$).

Single-Electrode Voltage Clamp – SEVC. Macroscopic currents were recorded in the whole-cell configuration at room temperature (24–28°C) with the patch-clamp technique using an Axonpatch 200A amplifier (Axon Instruments, Sunnyvale, CA) connected to a Digidata 1440A computer interface. The holding potential was 0 mV. Patch pipettes were made of soft glass capillaries (Corning 8161, Corning, NY) using a P-87 horizontal puller (Sutter Instruments, Novato, CA), and filled with an internal solution containing (in mM): N-methyl-D-glucamine(NMDG) 135; EGTA 5; Hepes 25; pH 7.2 (adjusted with CH3SO3H). The pipette resistance filled with this internal solution yielded 5.2 ± 0.2 MΩ ($n = 26$). An external solution containing (in mM) NMDG 135; CaCl2 5; Hepes 25 and glucose 10 (adjusted with CH3SO3H) was applied to the transfected cells before recording. Currents were low-pass filtered at a cut-off frequency of 2 kHz and acquired at 10 kHz. Series resistance was measured for WT A/M2 and the mutants. The values were A/M2: 4.4 ± 0.6 MΩ, $n = 5$; D44A: 5.4 ± 0.3 MΩ, $n = 7$; S31N: 5.6 ± 0.8 MΩ, $n = 6$; D44A/S31N: 5.6 ± 0.5 MΩ, $n = 8$; and mock cells: 5.8 ± 1.1 MΩ, $n = 7$. Series resistance was compensated by 40% in all experiments. The access resistance was verified before, during, and after each recording and cells that showed 15% change in the access resistance were not included in the analysis. Currents were analyzed using the pCLAMP 10.0 software package (Axon Instruments).

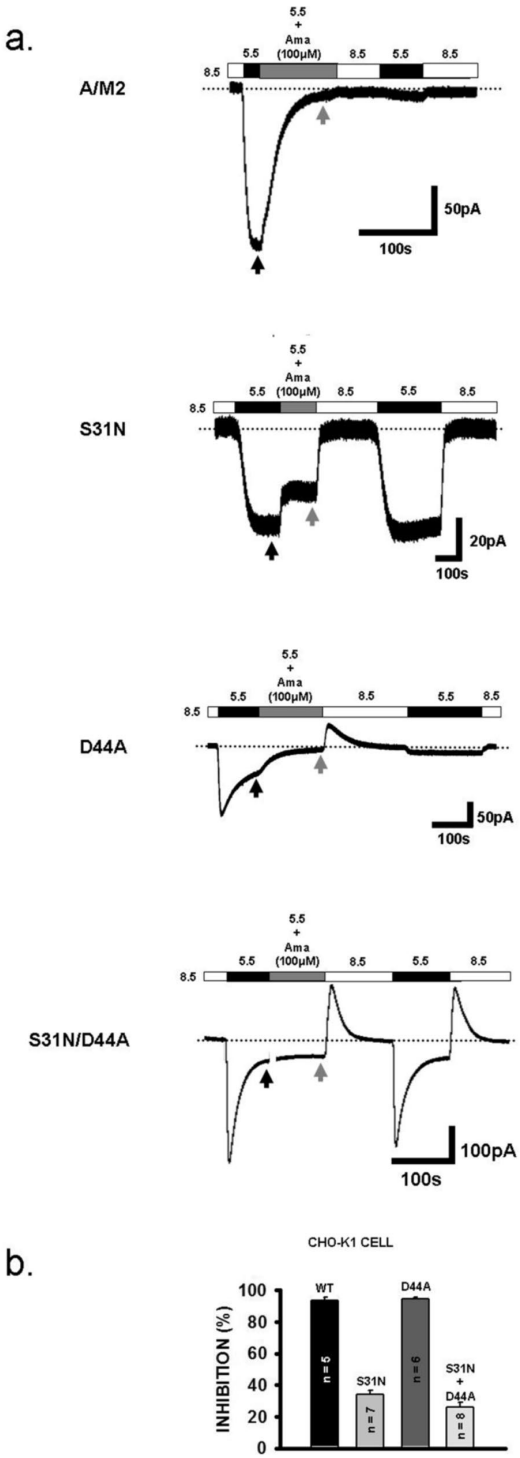

Fig. S1. (*a*) Representative currents of WT A/M2 and S31N, D44A and S31N/D44A mutant ion channels in CHO-K1 cells. Amantadine (100 μM) applied extracellularly inhibited the inward currents of cells expressing WT and D44A mutant ion channels. In contrast, the S31N mutant and the double-mutant S31N/D44A ion channels were only partially inhibited by amantadine (100 μM) and this inhibition was reversible. All cells were permitted to recover at least 600 s in pH 8.5 bathing solution before a second application of pH 5.5. Artifacts caused by solution changes are not shown. (*b*) Percentage inhibition (mean $\pm$ SEM) of WT A/M2 and mutants by amantadine (100 μM). The percentage inhibition of WT A/M2 variants were calculated using the ratio of current during amantadine application (mean current during the final 300 ms, second arrow in panel *a*) to the peak current recorded with pH 5.5 (first arrow in panel a). The currents of D44A and S31N/D44A mutant ion channels decreased while exposed to low pH bathing medium. For these mutants the percentage of inhibition was quantified using the mean current amplitude for the final 300 ms of a 2-minute application of amantadine (second arrow) and the mean amplitude for the final 300 ms before amantadine application (first arrow).