



### **Supplementary Figure S1. Dynamics of $V_j$ -gating during intracellular alkalization**

(A)  $I_j$  recordings in response to repeated  $V_j$  ramps from 0 to -110 mV before and during application of  $\text{NH}_4\text{Cl}$  (5 and 20 mM). Green, blue and red colors (A-F) represent data from control conditions, 5 and 20 mM of  $\text{NH}_4\text{Cl}$ , respectively. Voltage steps of -20 mV were used to measure  $g_j$  in between voltage ramps. (B)  $g_j$ - $V_j$  dependencies calculated from  $V_j$  and  $I_j$  records shown in A. (C) Averaged  $g_j$ - $V_j$  dependencies for control conditions and during application of 5 and 20 mM  $\text{NH}_4\text{Cl}$  from plots shown in (B). Lines in grey are fitting curves obtained by using the model of contingent gating. (D)  $P_{o-o}$  dependence on  $V_j$  for averaged  $g_j$ - $V_j$  plots shown in (C). (E) Changes of  $V_{h,o}$  (open circles) and  $N_f$  (filled circles) derived from averaged  $g_j$ - $V_j$  plots shown in (C). The regression lines of second order (solid lines) show that  $V_{h,o}$  and  $N_f$  changed from -11.5 mV and 340 channels to 87 mV and 525 channels, respectively.