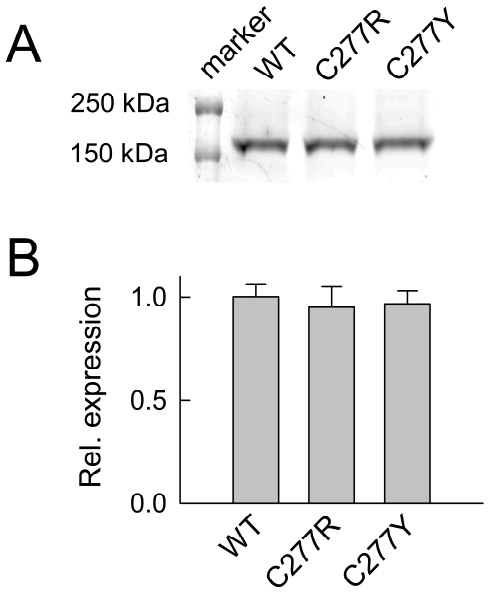
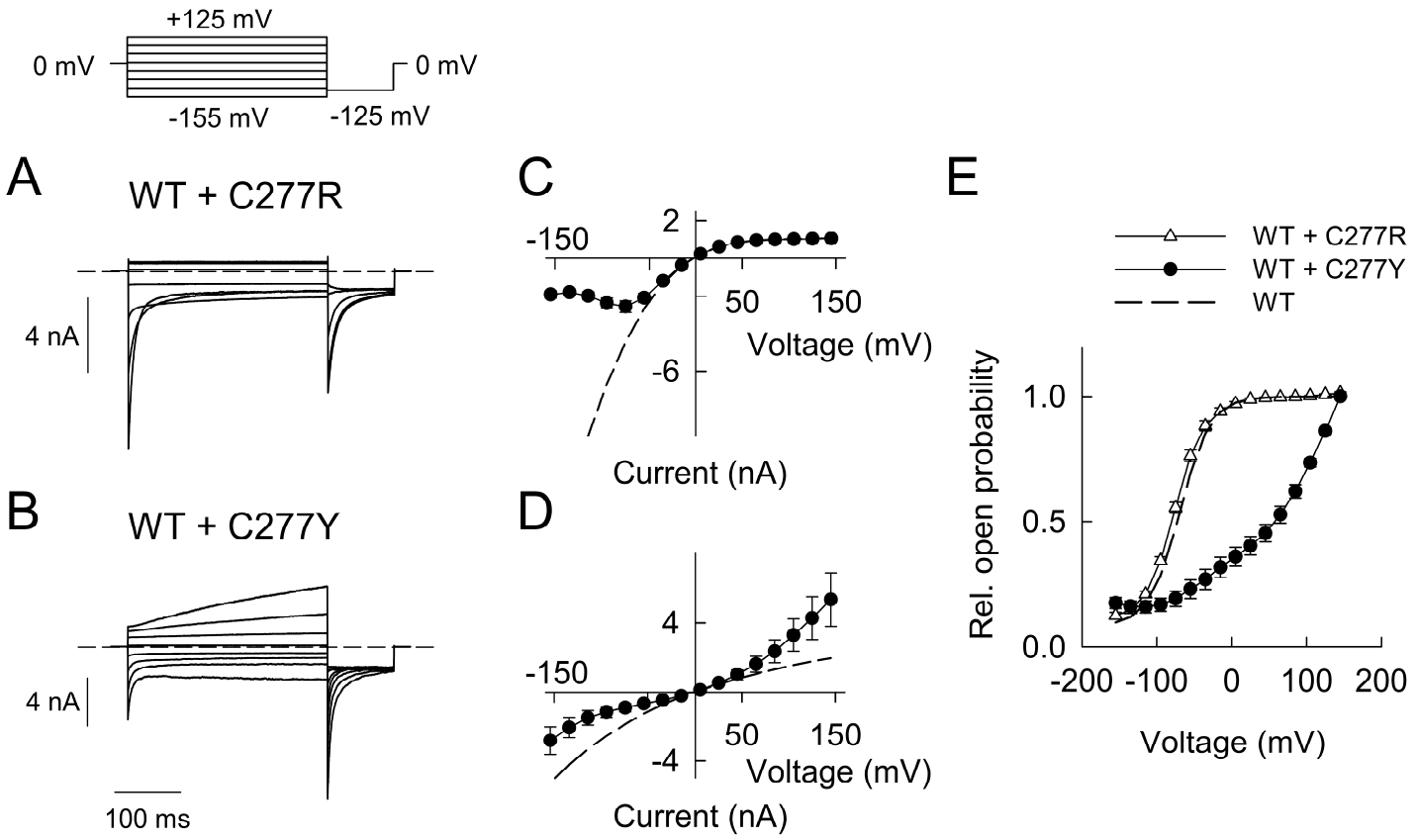
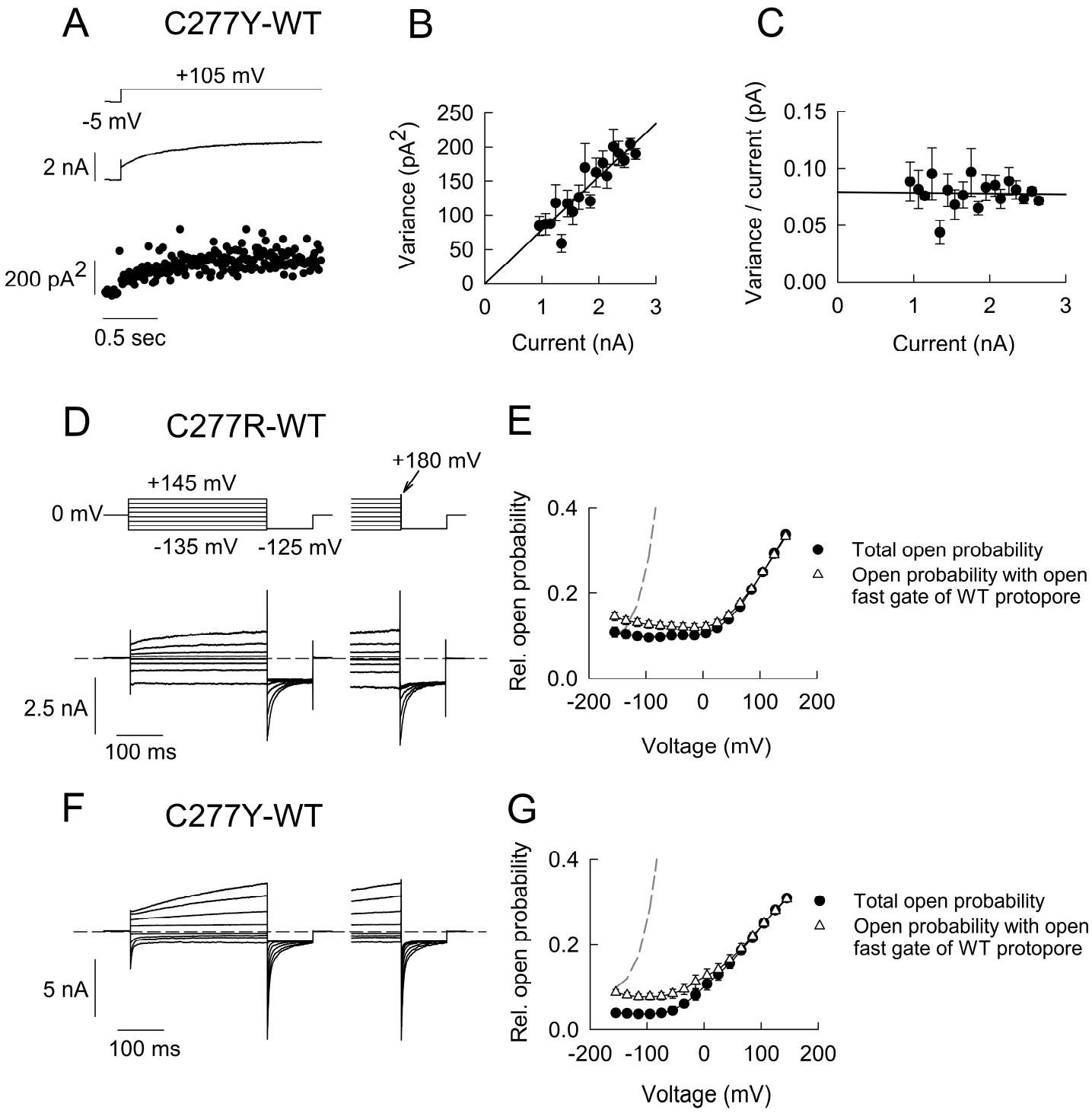


Suppl. Figure 1 Expression levels of hClC-1 are not altered by mutations C277R and C277Y. (A) Fluorescence scan of a SDS-PAGE gel of YFP-tagged WT and mutant hClC-1 channels in the total cleared lysate of cells that were transfected with equal amounts of plasmid DNA. **(B)** Relative expression levels of WT (n = 11), C277R (n = 8) and C277Y (n = 12) hClC-1 channels are given from relative fluorescent values obtained in gels as shown in (A).

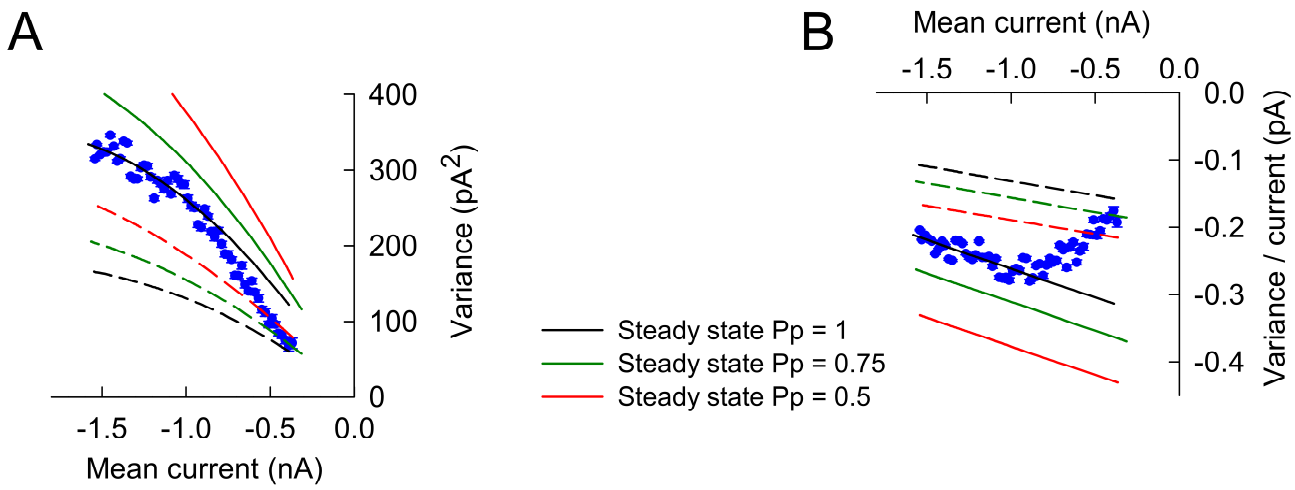


Suppl. Figure 2 Heterodimeric channels modify current responses of cells co-expressing WT and mutant hClC-1. (A, B) Representative current recordings from HEK293T cells co-transfected with equal amounts of plasmid DNA encoding for WT and mutant hClC-1. Dashed lines indicate zero current. **(C, D)** Voltage dependence of instantaneous (dashed lines) and late current amplitudes (symbols) (C, WT + C277R, n = 7; D, WT + C277Y, n = 6). **(E)** Voltage dependence of relative open probabilities (WT + C277R, n = 9; WT + C277Y, n = 6). The dashed line represents the activation curve of WT hClC-1 homodimers.

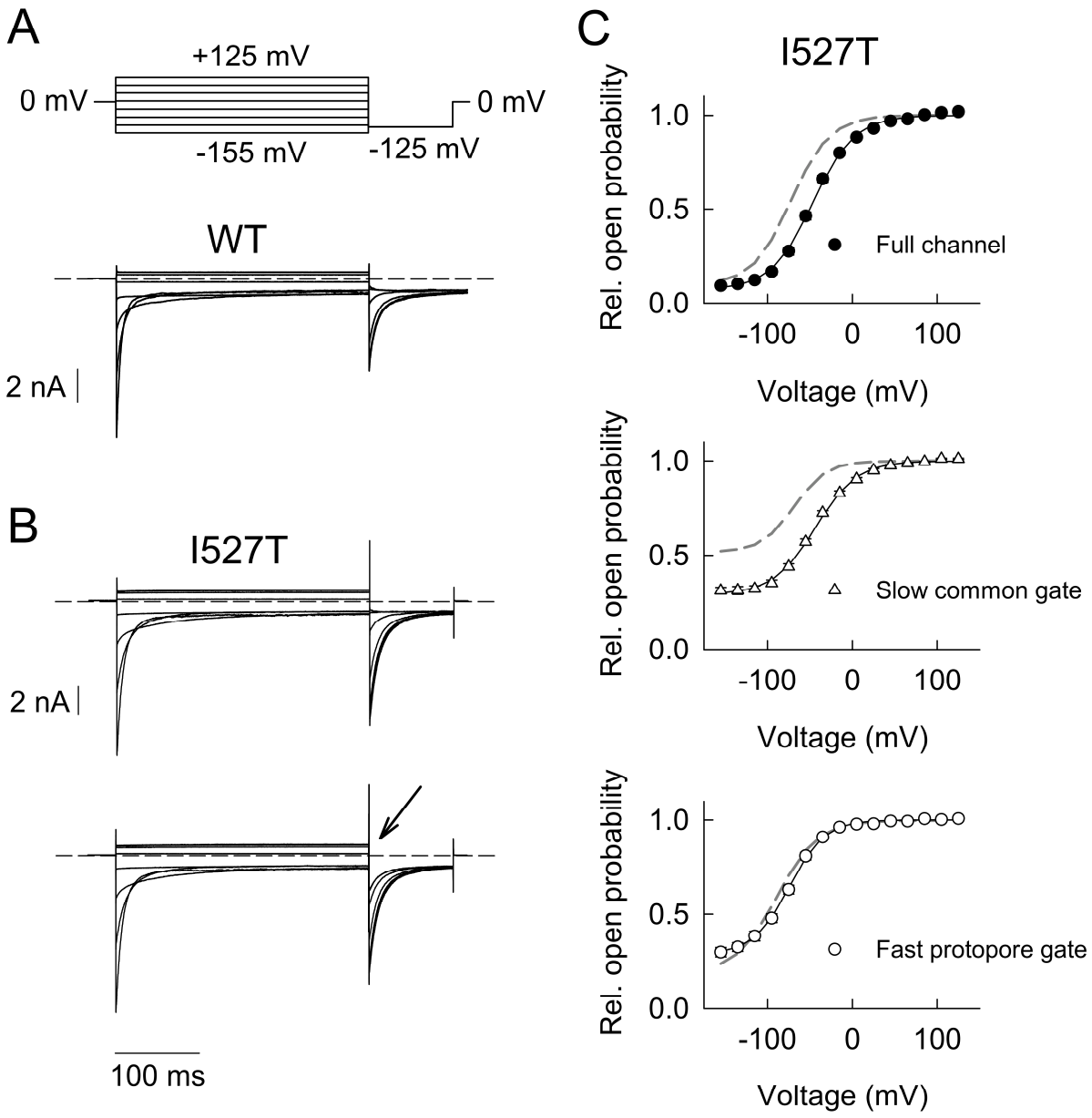


Suppl. Figure 3 C277 mutations modify common gating in concatenated heterodimers. (A-C) Non-stationary noise analysis of concatamer C277Y-WT obtained from very slow current activation upon a single voltage step to +105 mV. **(A)** Time dependence of current amplitudes and variances. Variance points are given as mean values calculated from time intervals with a bin width of 10 ms. **(B,C)** Plots of current variances or ratio of variance by current amplitude versus current amplitudes from the same cells. Variance points at individual sampling times were sorted into evenly-spaced current bins and given as mean ± SEM using a bin width of 0.1 nA. **(D, F)** Pulse protocol and representative current responses of HEK293T cells expressing

concatameric channels C277R-WT (D) and C277Y-WT (F). A short interpulse to +180 mV (0.5 ms, arrow) fully opens the fast gate of WT protopores and modifies tail current amplitudes at the test potential of -125 mV. Dashed lines indicate zero current. **(E, G)** Voltage dependence of relative open probabilities of C277R-WT (E, n = 5) and C277Y-WT concatamers (G, n = 5). Activation curves are normalized to 0.25 at +105 mV. The dashed line represents the activation curve of WT hClC-1 homodimers.



Suppl. Figure 4 Predicted current-variance relationships allow estimation of absolute steady state protopore open probability of C277Y hClC-1. (A) Current variance versus mean current amplitude plots as already given in Fig. 5E. Lines display predicted current variance relationships for two extreme cases, constitutively open protopore gates (solid line) or constitutively open common gates (dashed line), respectively. Parabolas were calculated using equation (3), assuming a steady state open probability of the protopore gate *Pp* = 1 (black lines), *Pp* = 0.75 (green lines) or *Pp* = 0.5 (red lines). Experimental data will fall in between the parabolas only if steady state *Pp* is larger than 0.75. **(B)** Ratio of variance by current amplitude plotted versus mean current amplitude (same data as in A).



Suppl. Figure 5 I527T shifts activation curve of hClC-1 channels. (A-B) Pulse protocol and representative current responses of HEK293T cells expressing WT (A) or mutant I527T hClC-1 (B; upper panel: responses to standard pulse protocol; lower panel: responses to modified pulse protocol with a short interpulse to +180 mV (0.5 ms) prior to test potential (-125 mV) that fully opens the fast gate and modifies the tail current amplitudes (arrow)). Dashed lines indicate zero current. **(C)** Voltage dependence of the total relative open probabilities of I527T channels (upper panel) and relative open probabilities of the slow common gate (middle panel) and the fast protopore gate (lower panel) (n = 5). Dashed lines display the activation curves of WT hClC-1 (n = 6). Mutation I527T shifts slow gate activation curve by about 25 mV to more positive potentials.