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Supplemental Information

Gating Currents in the Hv1 Proton Channel

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Effect of varying voltage prepulse duration on I_{TAIL} -V and Q_{ON} -V relations in W207A-N214R.

A) The absolute value of I_{TAIL} measured at $V_{TAIL} = -90$ mV after V_{STEP} for the indicated duration to voltages between -60 mV and $+50$ mV (V_{STEP} : -60 mV, red circles; -50 mV, orange circles; -40 mV, yellow circles; -30 mV, olive circles; -20 mV, green circles; -10 mV, aqua circles; 0 mV, cyan circles; $+10$ mV, blue circles; $+20$ mV, indigo circles; $+30$ mV, violet circles; $+40$ mV, black circles; $+50$ mV, gray circles) in a representative cell expressing W207A-N214R is shown. Solid lines represent fits to a single exponential function (-60 mV: $\tau = 102.5$ ms, red line; -50 mV: $\tau = 155.6$ ms, orange line; -40 mV, $\tau = 301.3$ ms, yellow line; -30 mV, $\tau = 355.3$ ms, olive line; -20 mV, $\tau = 236.4$ ms, green line; -10 mV, $\tau = 153.2$ ms, aqua line; 0 mV, $\tau = 102.4$ ms, cyan line; $+10$ mV, $\tau = 73.3$ ms, blue line; $+20$ mV, $\tau = 52.9$ ms, indigo line; $+30$ mV, $\tau = 37.4$ ms, violet line; $+40$ mV, $\tau = 27.1$ ms, black line; $+50$ mV, $\tau = 21$ ms, gray line). Note that the Legend applies to panels A and B.

B) Q_{ON} in the same cell as shown in panel A is measured at $V_{STEP} = +100$ mV after V_{PP} for the indicated duration to voltages between -70 mV and $+40$ mV (V_{PP} : -70 mV, magenta circles; -60 mV, red circles; -50 mV, orange circles; -40 mV, yellow circles; -30 mV, olive circles; -20 mV, green circles; -10 mV, aqua circles; 0 mV, cyan circles; $+10$ mV, blue circles; $+20$ mV, indigo circles; $+30$ mV, violet circles; $+40$ mV, black circles). Solid lines represent fits to a single exponential function (-70 mV: $\tau = 2.4 \times 10^8$ ms, magenta line; -60 mV: $\tau = 407.6$ ms, red line; -50 mV: $\tau = 333.5$ ms, orange line; -40 mV, $\tau = 365.5$ ms, yellow line; -30 mV, $\tau = 273.1$ ms, olive line; -20 mV, $\tau = 161.1$ ms, green line; -10 mV, $\tau = 105.4$ ms, aqua line; 0 mV, $\tau = 64.3$ ms, cyan line; $+10$ mV, $\tau = 43.1$ ms, blue line; $+20$ mV, $\tau = 28.7$ ms, indigo line; $+30$ mV, $\tau = 20.9$ ms, violet line; $+40$ mV, $\tau = 19.5$ ms, black line).

C, D) Linear leak-subtracted I_{TAIL} -V (**C**) or Q_{ON} -V (**D**) relations are normalized to their respective maxima and plotted in function of V_{STEP} or V_{PP} duration (50 ms, red circles; 100 ms, blue triangles; 200 ms, magenta triangles; 500 ms, green diamonds; 1000 ms, indigo triangles; 2000 ms, violet triangles).

E, F) $V_{0.5}$ (**E**) and z_G (**F**) values determined from Boltzmann fits to I_{TAIL} -V (black stars; see panel **C**) or Q_{ON} -V (red stars; see panel **C**) relations are shown in function of V_{PP} (for Q_{ON}) or V_{STEP} (for I_{TAIL}) duration. Solid lines in panel **E** represent fits to a double exponential function of the form $V_{0.5} = V_{0.5(0)} + A_1 \cdot e^{t/\tau_1} + A_2 \cdot e^{t/\tau_2}$ (red line: Q_{ON} : $V_{0.5(0)} = -44.7$ mV, $A_1 = 136$ mV, $\tau_1 = 20.6$ ms, $A_2 = 29.8$ mV, $\tau_2 = 310.4$ ms; black line, I_{TAIL} : $V_{0.5(0)} = -34.4$ mV, $A_1 = 142.1$ mV, $\tau_1 = 21.3$ ms, $A_2 = 50.6$ mV, $\tau_2 = 211.8$ ms). Solid lines in panel **F** represent fits to a single exponential function of the form $z_G = z_{G(0)} + A \cdot e^{t/\tau}$ (red line: Q_{ON} : $z_{G(0)} = 2.3 e_0$, $A = -1.5 e_0$, $\tau = 260.3$ ms; black line, I_{TAIL} : $z_{G(0)} = 3.2 e_0$, $A = -2.3 e_0$, $\tau = 261.5$ ms).

Supplementary Figure 2. Steady-state I_{STEP} -V relations in W207A-N214R.

A, B) Representative currents elicited by the indicated double-pulse voltage protocols (**A**: to measure I_G at +140 mV; **B**: to measure I_{TAIL} at -90 mV) in a cell expressing R205A-N214R are shown. *Inset* shows outward transient current attributed to I_G at higher resolution.

C) Normalized mean (\pm SEM) I_{TAIL} -V and Q_{ON} -V relations expressing R205A-N214R are shown ($n = 3$ cells). Mean $V_{0.5}$, z_G and V_M values are reported in Table 1.

D) Plots of maximal Q_{ON} ($Q_{ON\ max}$) vs. maximal I_{TAIL} ($I_{TAIL\ max}$) measured in individual cells expressing R205A-N214R (filled red circles), W207A-N214R data (Figure 3) is shown for comparison. Linear fit to the data: slope 2.5 pC/nA; $R^2 = 0.93$.

E) The number of channels (N) is calculated as described in Fig. 3. Linear fit to the data yield an estimate of the unitary conductance.

Supplementary Figure 3.

A) Representative currents in a representative cell expressing W207A-N214R- Δ C elicited by the indicated voltage step protocol ($V_{\text{HOLD}} = -80$ mV; $V_{\text{STEP}} = 50$ mV for 0 to 200 ms in 5 ms intervals; $V_{\text{TAIL}} = -90$ mV) are shown.

B) I_{TAIL} activation time courses determined from experiments like that shown in panel **A** are measured over the range of V_{STEP} indicated by the legend are shown. Data are fit to a single exponential without a delay (see *Methods*).

C) I_{STEP} elicited by a voltage step to +50 mV (black line; data from panel **A**) is shown together with the fitted I_{TAIL} activation time course (red line; data from panel **B**) scaled to the amplitude of the steady-state I_{STEP} .

D) I_{STEP} at various V_{STEP} is determined after subtraction of a scaled exponential fit to the I_{TAIL} activation timecourse measured at the indicated V_{STEP} , as shown in panel **C** for data at +50 mV.

E) $Q_{\text{ON}}-V$ relation resulting from integrating the subtracted I_{STEP} records (panel **D**) is shown (solid black squares). A Boltzmann fit of the data yields parameters ($V_{0.5} = -33.5$ mV; $z_G = 1.81 e_0$) that are similar to values reported for the $Q_{\text{ON}}-V$ relation in W207A-N214R obtained without subtraction of the fitted and scaled I_{TAIL} timecourse (Table 1).

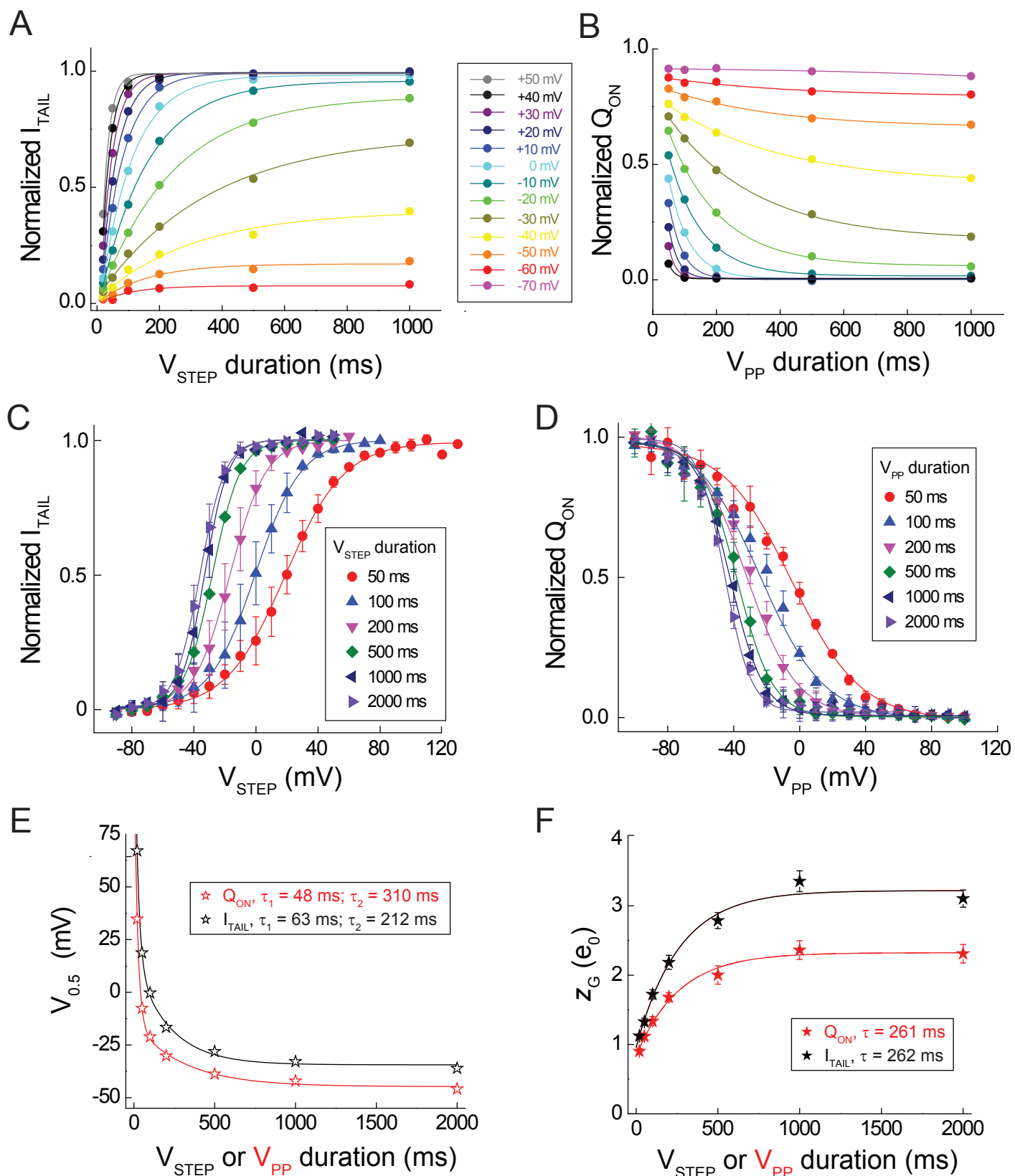
F) The decaying phase of subtracted I_{STEP} records (panel **D**) are fit to a single exponential function without a delay (see *Methods*) and the fitted time constant (τ_{DEACT}) from is shown in function of V_{STEP} (open black squares).

Supplementary Figure 4.

A, B) Representative currents in a cell expressing W207A-N214R are elicited by the indicated double-pulse voltage protocols at pH_O 6.5 (**A**) and pH_O 5.5 (**B**). I_G is measured at +50 mV (**A**) or +90 mV (**B**) and I_{TAIL} (square symbols) is measured at -90 mV (**A**) or -50 mV (**B**).

C) $I_{\text{STEP}}-V$ relations from $n = 9$ (pH_o 6.5, open black squares) or $n = 4$ (pH_o 5.5, filled red squares) experiments are linear leak-subtracted and normalized to the amplitude of the maximal inward current; the mean (\pm SEM) values are plotted in function of V_{STEP} .

W207A-N214R



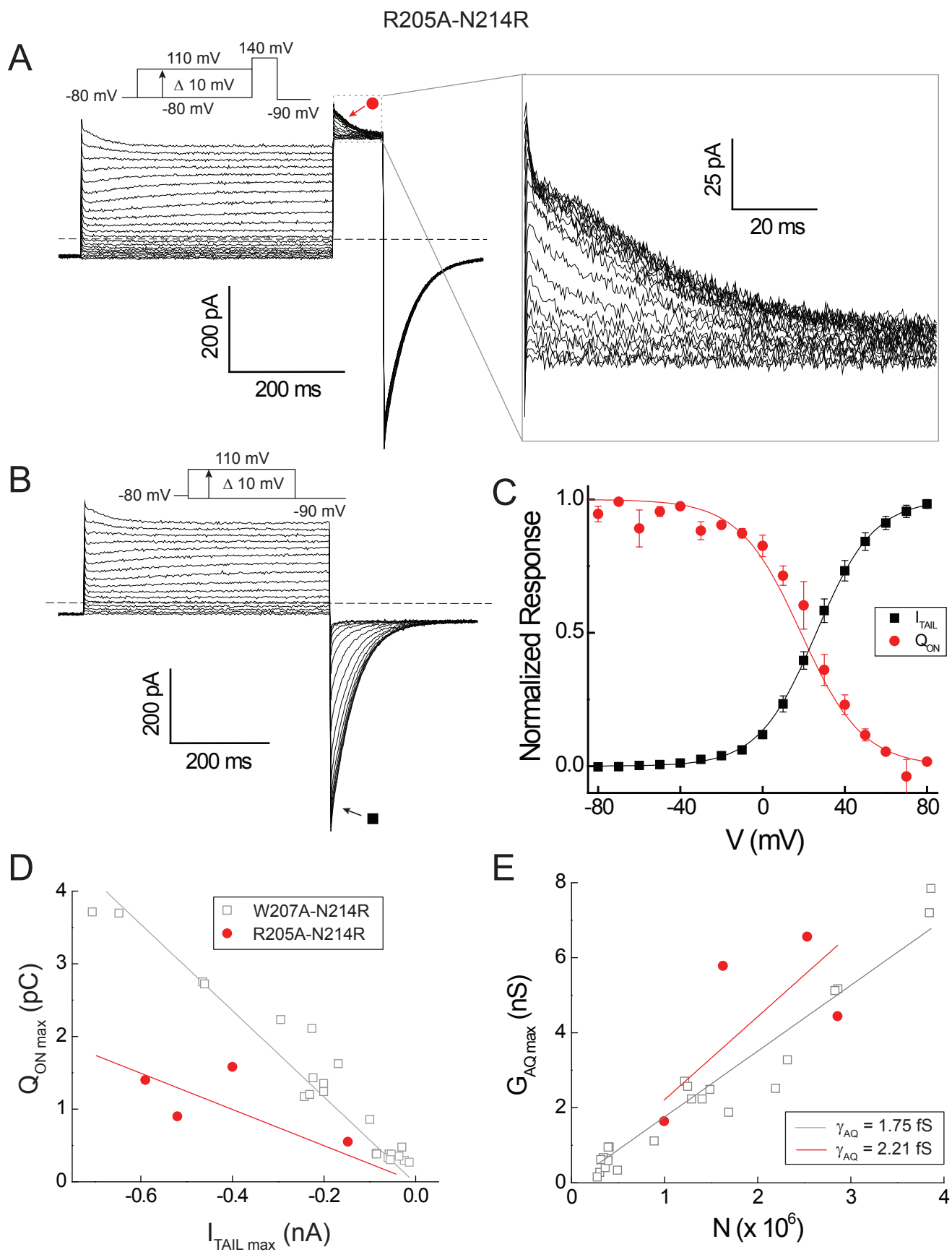


Figure S3

W207A-N214R- Δ C