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SUPPLEMENTAL MATERIALS:

Table S1. Macroscopic Gap Junctional Conductance Properties of Cx40 AF mutations

Connexin	Cx40	G38D	M163V	G38D+M163V
g_j (nS)	4.82	9.69	6.81	10.31
SEM	1.31	3.77	2.11	2.01
n	6	5	6	5

Data were obtained for WTCx40 and 3 of the G38D cell pairs using N2a cells that were transiently transfected with untagged constructs. Many of the other cell pairs were so highly coupled that V_j-dependent gating was not observed, and they were not included in this analysis. Tagging the CT domain with EGFP or mCherry produced cell pairs with lower g_j values wherein V_j-dependent gating was readily observed and were used for the remainder of the data. n, number of cell pairs included in this analysis.

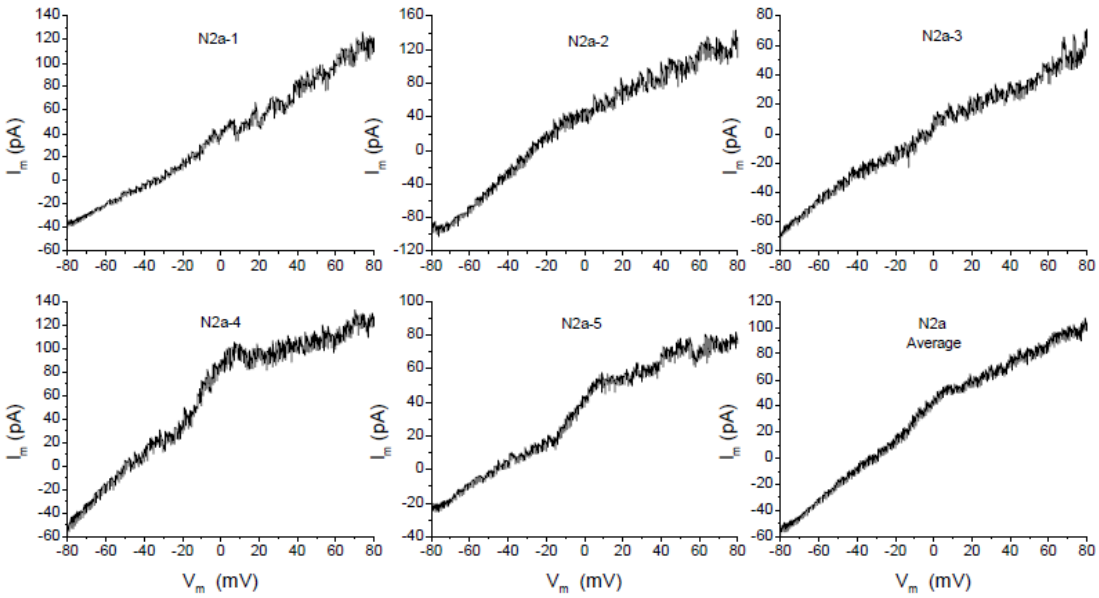

Figure S1. Whole cell current traces from five untransfected N2a cells bathed in 0 mM $CaCl_2$ saline during a -80 to +80 mV, 8 sec (50 ms/mV) staircase ramp of membrane potential (V_m). The V_m protocol was repeated 6 times per cell and one representative trace is shown for each experiment. The ensemble averaged whole cell current for all five cells is illustrated in the last (lower right) panel.

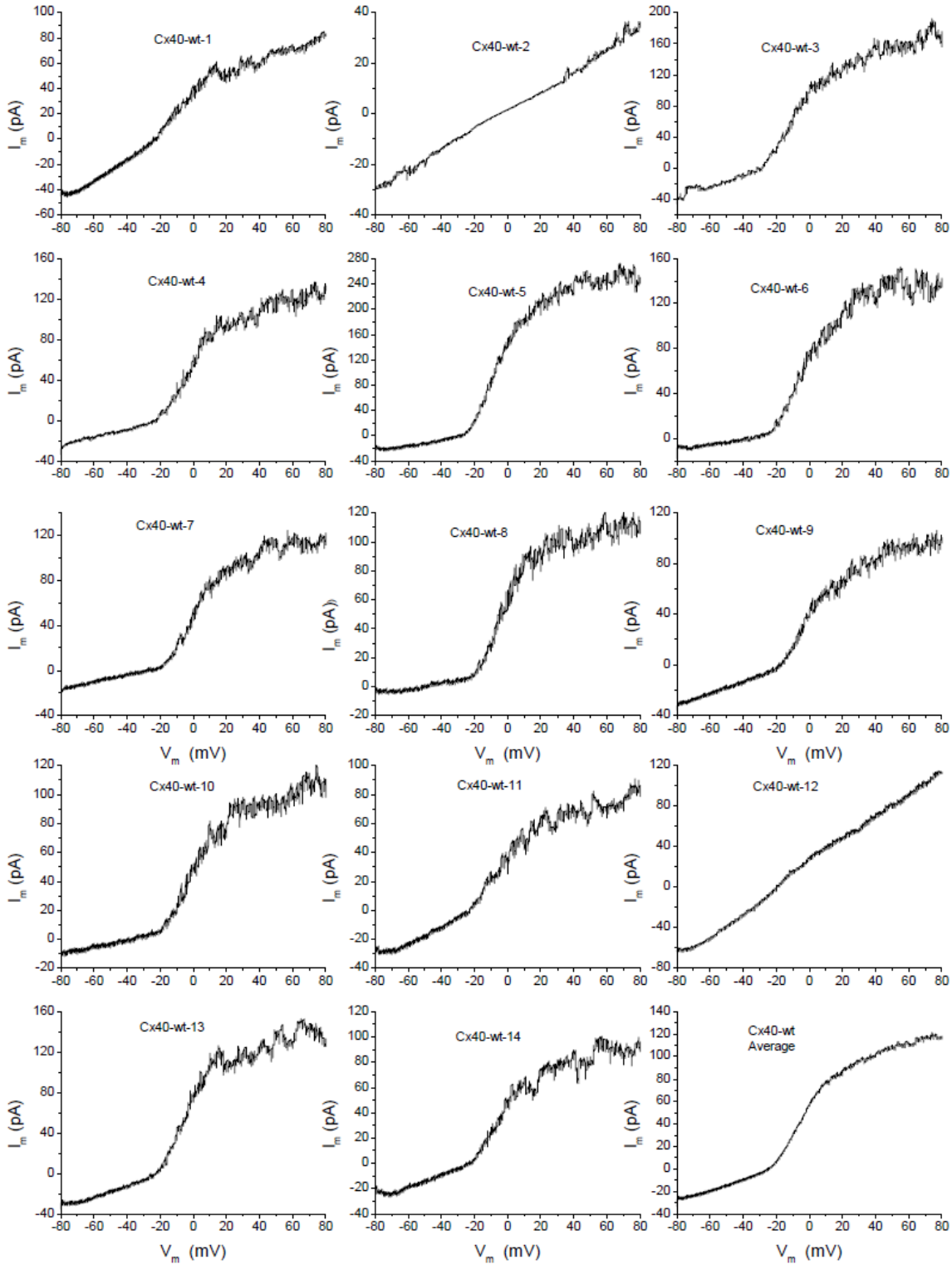

Figure S2. Whole cell current traces from fourteen N2a cells stably transfected with wild-type Cx40 bathed in 0 mM $CaCl_2$ saline during the same 160 mV, 8 sec V_m protocol. The ensemble averaged current for these 14 experiments is illustrated in the fifteenth (lower right) panel.

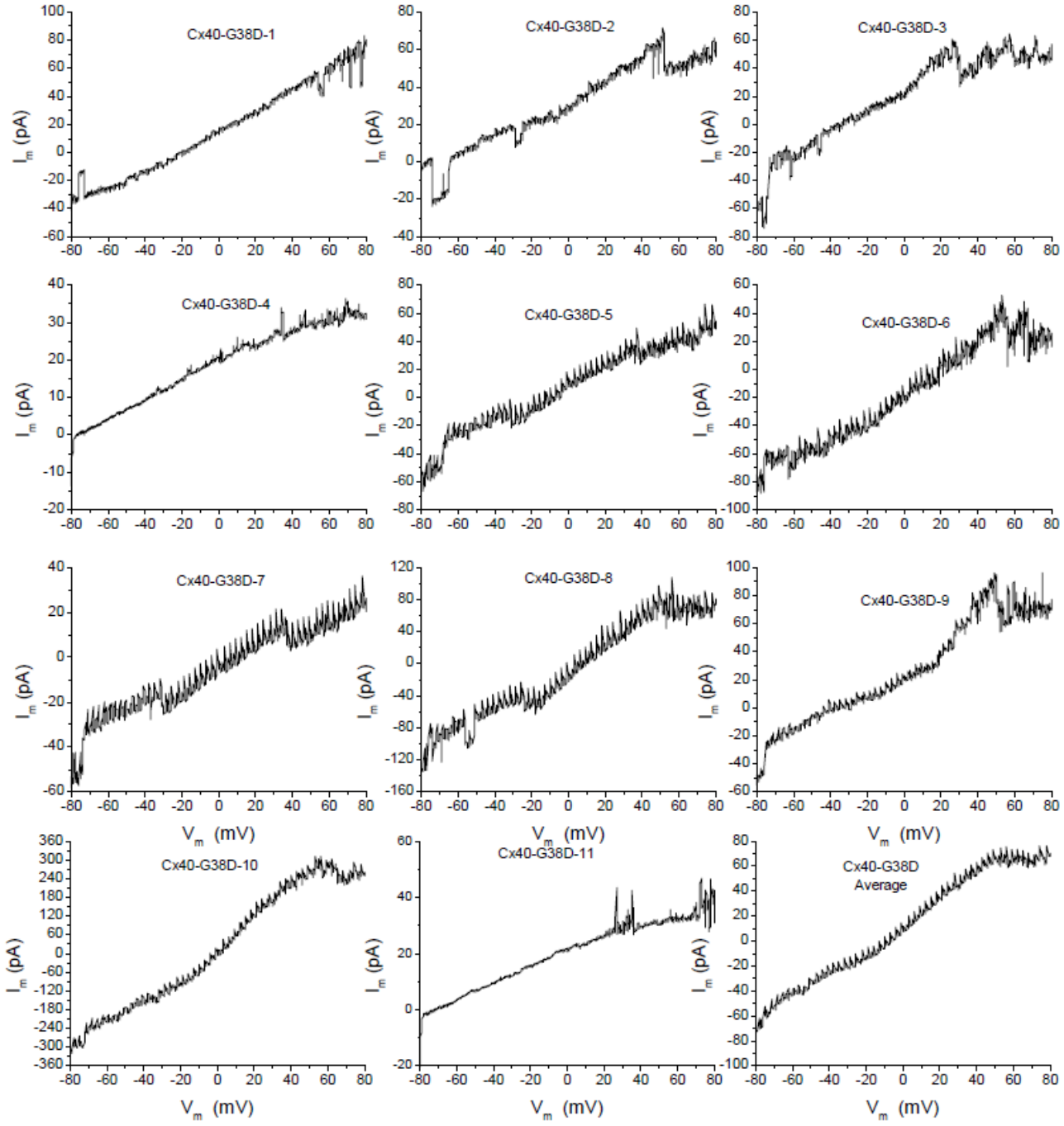

Figure S3. Whole cell current traces from eleven N2a cells transiently transfected with G38D bathed in 0 mM $CaCl_2$ saline during the sameV_m protocol. The ensemble averaged current for these 11 experiments is illustrated in the twelfth (lower right) panel. Hemichannel currents are visible in all 11 individual current traces, but not in the ensemble averaged whole cell current traces.

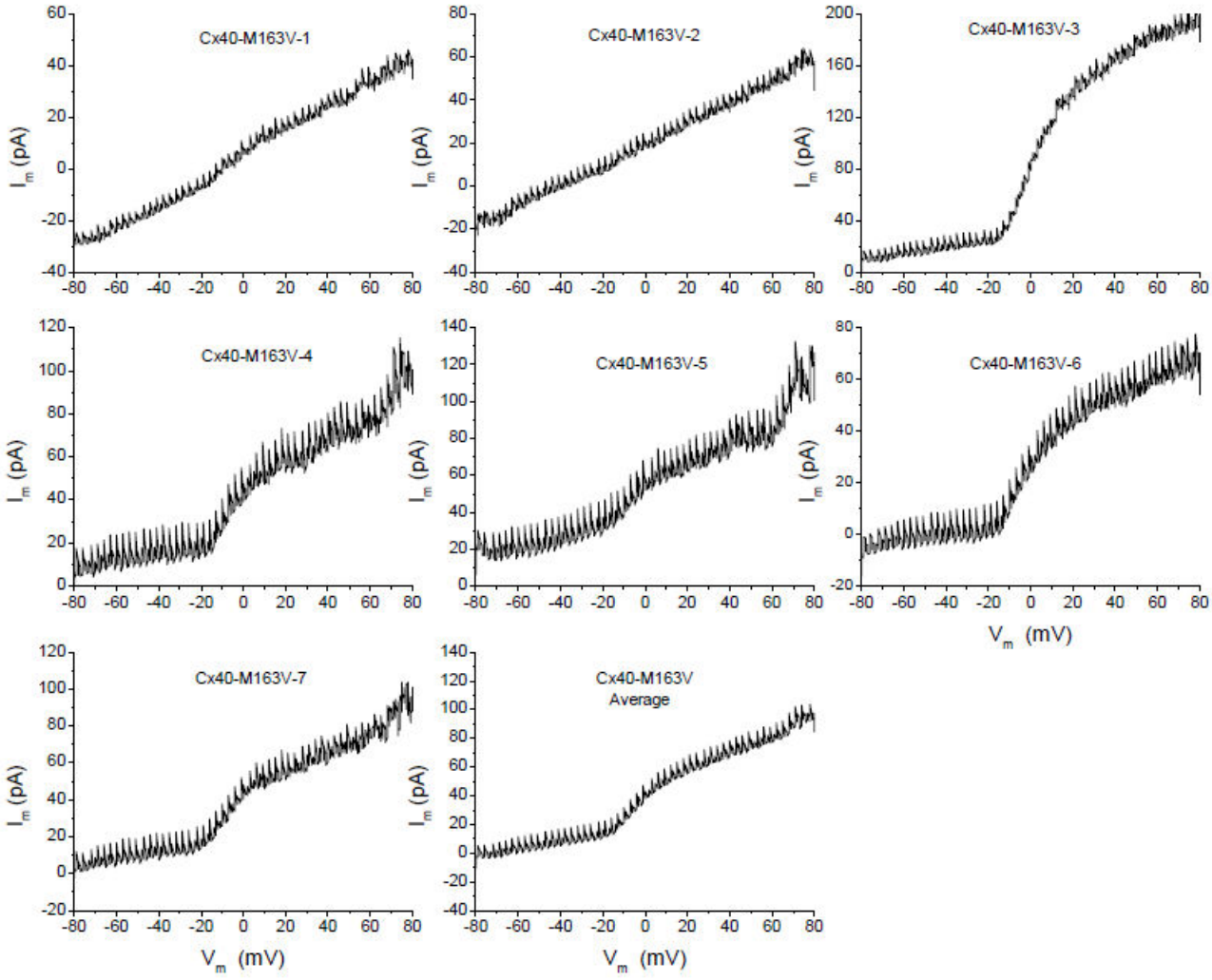

Figure S4. Whole cell current traces from seven N2a cells transiently transfected with M163V bathed in 0 mM $CaCl_2$ saline during the sameV_m protocol. The ensemble averaged current for these 7 experiments is illustrated in the eighth (lower right) panel. Hemichannel currents are not readily visible in these individual current traces or the ensemble averaged whole cell current traces.

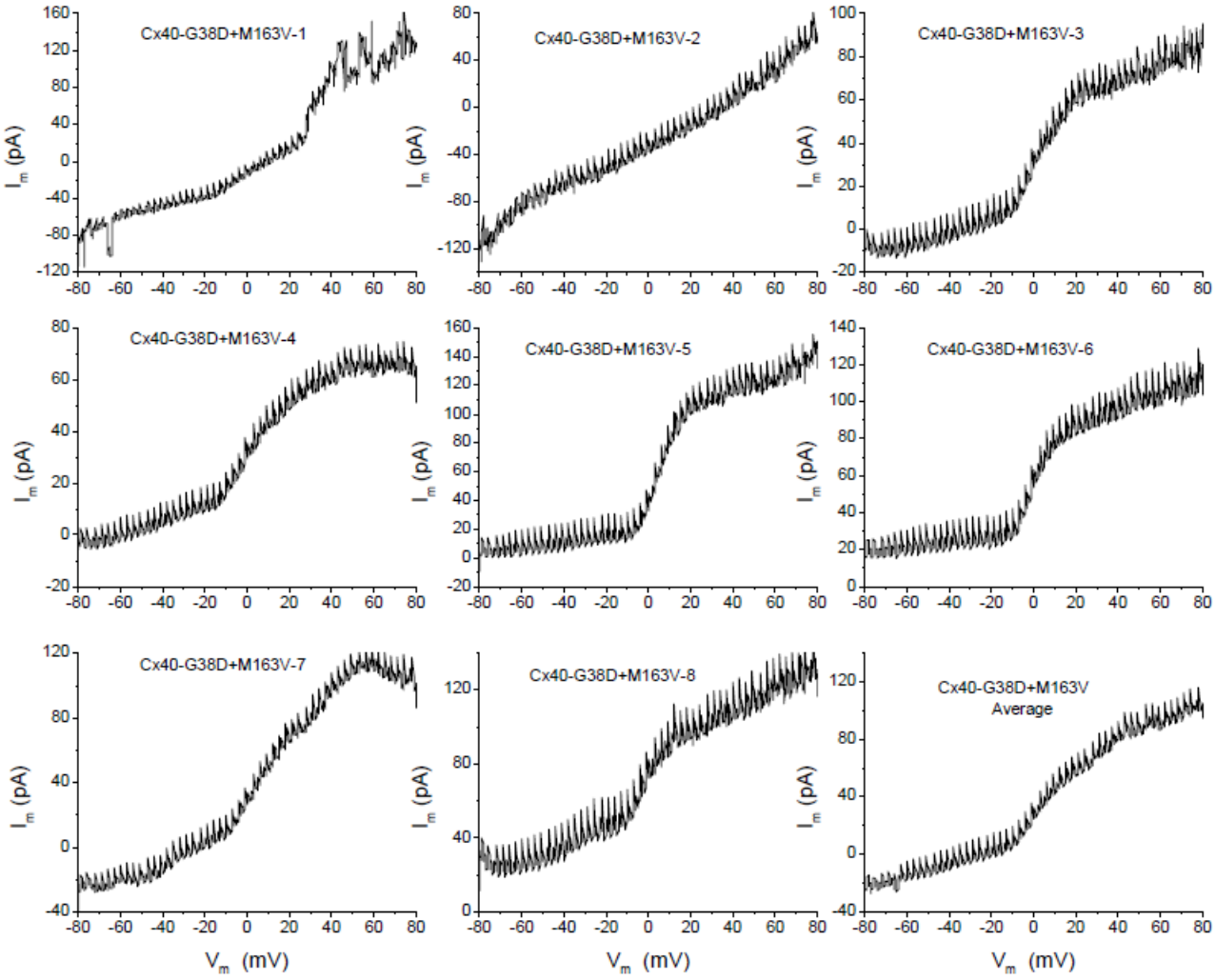

Figure S5. Whole cell current traces from eight N2a cells transiently transfected with G38D+M163V bathed in 0 mM $CaCl_2$ saline during the typical 160 mV V_m protocol. The ensemble averaged current for these 8 experiments is illustrated in the ninth (lower right) panel. Hemichannel currents were visible in one individual current trace, but not in the ensemble averaged whole cell current traces.

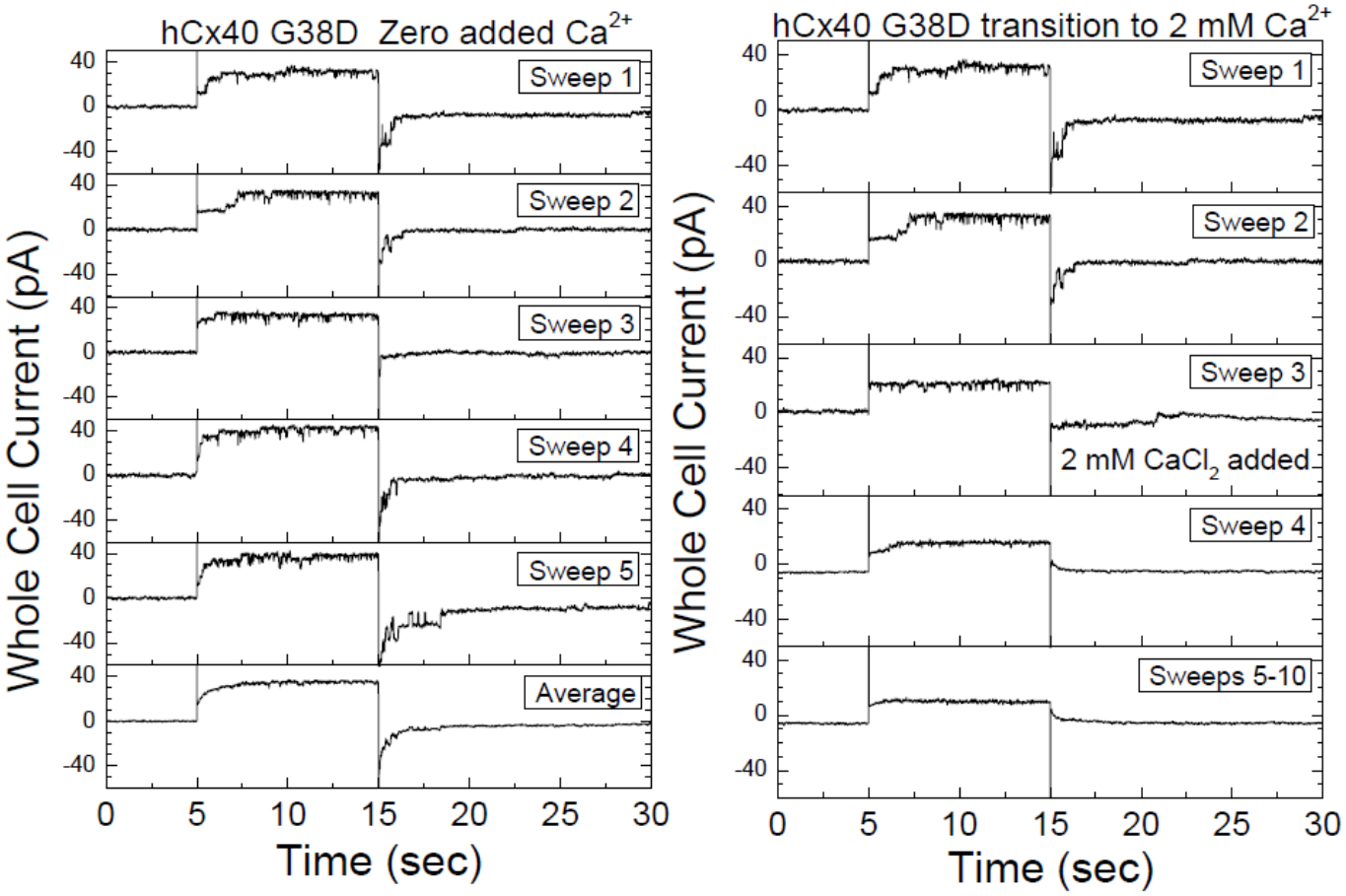

Figure S6. Whole cell current traces recorded during a -40 to +20 mV, 10 sec duration V_m step applied to one N2a cell transiently transfected with G38D. The V_m step was repeated five times and the ensemble averaged current trace (bottom panel) illustrates the hemichannel current activation at +20 mV and deactivation upon return to -40 mV V_m in the absence of external calcium. The addition of 2 mM $CaCl_2$ to the bath during a train of ten +20 mV steps resulted in the progressive closure of the G38D hemichannels in this experiment.