Supplemental Material

DI/S5-SS1

DmNa_v1:   MGV*LTQKCIKKFPLDGSWGNLTDE*------------------*NWDYHNRNSSNWYSEDEG*

rNa_v1.2a: MGN*LRNKCLQWPPDNSTFEINITSFFNNSLDWNGTAFNRTVNMFNWDEYIEDKSHFYFLEGQ*

DmNa_v1:   *ISFPLCGNISGAGQCDDDYVCLQGFGPNPNYGYTSF*DSF

rNa_v1.2a: *NDALLCGNSSDAGQCPEGYICVKA-GRNPNYGYTSF*DTF

DI/SS2-S6

DmNa_v1:   VL*RAAGP*WH

rNa_v1.2a: TL*RAAGK*TY

DIV/S1-S2

DmNa_v1:   MTLDR*YDASDTYNA*VLDY

rNa_v1.2a: MMVET*DDQSQEMTN*ILYW

DIV/S3-S4

DmNa_v1:   LGLVLS*DIIEKYFVSPTL*LRVVR

rNa_v1.2a: VGMFLA*ELIEKYFVSPTL*FRVIR

SUPPLEMENTAL FIGURE 1: Sequence alignment of extracellular loops (in italics) of the Pore-module of DI and Gating-module of DIV of DmNa_v1 and Na_v1.2a. Sequences used in the swap experiments are underlined.

SUPPLEMENTAL TABLE 1: Biophysical properties of channel chimeras. Voltage dependence of activation was measured by fitting the GV curve with one component Boltzmann distribution (see Methods), yielding half-maximal activation ($V_{1/2}$) and the slope factor ($k_{1/2}$). Steady-state inactivation was measured as depicted in the Methods, with V_h representing the membrane potential at which half-maximal inactivation was achieved; k_h is the slope factor. *n* for all experiments is at least 6.

Channel	Activation		Steady-state inactivation	
	$V_{1/2}$, mV	$k_{1/2}$	V_h, mV	k_h
DmNa$_v$1 unmodified	-23.2±1.4	5.3±0.1	-48.9±1.1	5.2±0.1
DmNa$_v$1$^{rNa_v1.2a(DIV/S1-S2)}$	-20.7±1.1	5.0±0.3	-45.2±0.9	5.5±0.1
DmNa$_v$1$^{rNa_v1.2a(DIV/S1-S2 + S3-S4)}$	-27.5±2	4.5±0.5	-43.8±1.2	5.4±0.1
DmNa$_v$1$^{rNa_v1.2a (DIV/S3-MFLA)}$	-24.5±3	4.8±0.4	-42.1±1	5.1±0.2
DmNa$_v$1$^{rNa_v1.2a(DI/SS2-S6)}$	-22.0±1.7	5.5±0.5	-46.0±1.2	5.4±0.1
DmNa$_v$1$^{rNa_v1.2a(D1/S5-SS1 + SS2-S6)}$	-21.1±1	4.6±0.4	-43.7±1.2	4.8±0.2
DmNa$_v$1$^{rNa_v1.2a (site-3 face)}$	-25.8±2.2	4.5±0.2	-46.0±1.8	5.4±0.1
DmNa$_v$1$^{rNa_v1.2a(Site-3 face + E1613D)}$	-21.9±0.8	4.8±0.2	-40.7±1.3	5.4±0.1
rNa$_v$1.2a unmodified	-25±1.6	4.2±0.2	-43.9±0.8	6.4±0.1
rNa$_v$1.2a^{E1613D}	-25.3±1.8	4.13±0.3	-43.6±0.7	6.8±0.2