## SUPPLEMENTAL MATERIAL

Pless et al., http://www.jgp.org/cgi/content/full/jgp.201311036/DC1



**Figure S1.** Analysis of Nha incorporation. Bar graph showing the average current size observed when TAG stop codons replaced the codons for acidic residues in S2 and S3 in Nav1.4; red bars show average current sizes after coinjection of Na<sub>v</sub>1.4-TAG mRNA with (Nha)acylated tRNA (Nha-pdCpA-tRNA; abbreviated as Nha in the figure), and gray bars show current sizes obtained after coinjection of Na<sub>v</sub>1.4-TAG mRNA with pdCpA-tRNA; abbreviated as Nha in the figure), and gray bars show current sizes obtained after coinjection of Na<sub>v</sub>1.4-TAG mRNA with pdCpA-tRNA lacking a conjugated amino acid (abbreviated as pdCpA in the figure). \*, statistical difference to values obtained with pdCpA in an unpaired *t* test (P < 0.05). Note that Glu624TAG elicited currents when injected with Nha-coupled tRNA but not amino acid–free tRNA. However, given the limiting rescued current size obtained with Nha-pdCpA-tRNA (<200 nA), this substitution was not characterized but instead the conventional Glu624Gln mutant was analyzed (see Fig. 2). Furthermore, Glu598TAG and Glu1373TAG generated large currents in the presence of pdCpA-tRNA lacking a conjugated amino acid and were thus not further investigated. Note the break in the y axis.

-