Supplementary Materials: Engineering Gain-of-Function Analogues of the Spider Venom Peptide HNTX-I, A Potent Blocker of the hNav1.7 Sodium Channel

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Figure S1. Synthesis and oxidative folding of wild type HNTX-I. (**A**) RP-HPLC purification of crude linear HNTX-I, and asterisk indicated the peak containing HNTX-I linear peptide; (**B**) MALDI-TOF MS analysis of purified linear HNTX-I; (**C**) Analytical RP-HPLC purification of crude folded HNTX-I, and asterisk indicated the correctly folded peptide; (**D**) MALDI-TOF MS analysis of the purified folded HNTX-I, and inset was an enlarged view of the peak.



Figure S2. Synthesis and oxidative folding of HNTX-I analogue E1G-N23S-D26H-L32W. (**A**) RP-HPLC purification of crude linear HNTX-I analogue E1G-N23S-D26H-L32W, and asterisk indicated the peak containing E1G-N23S-D26H-L32W linear peptide; (**B**) MALDI-TOF MS analysis of purified linear E1G-N23S-D26H-L32W; (**C**) Analytical RP-HPLC purification of crude folded E1G-N23S-D26H-L32W, and asterisk indicated the correctly folded peptide; (**D**) MALDI-TOF MS analysis of the purified folded HNTX-I, and inset was an enlarged view of the peak.



Figure S3. Effect of E1G-N23S-D26H-L32W on Nav1.2 and Nav1.6 expressed in HEK 293 cells. Concentrationresponse curves of E1G-N23S-D26H-L32W at Nav1.2 and Nav1.6 assessed by whole-cell patch-clamp experiments. IC₅₀ value of E1G-N23S-D26H-L32W on Nav1.2 and Nav1.6 werev Data are mean \pm SEM, with n = 3-5 cells per data point.