Extracellular hemin is a reverse use-dependent gating modifier of cardiac voltage-gated Na^+ channels

– Supplementary Information –

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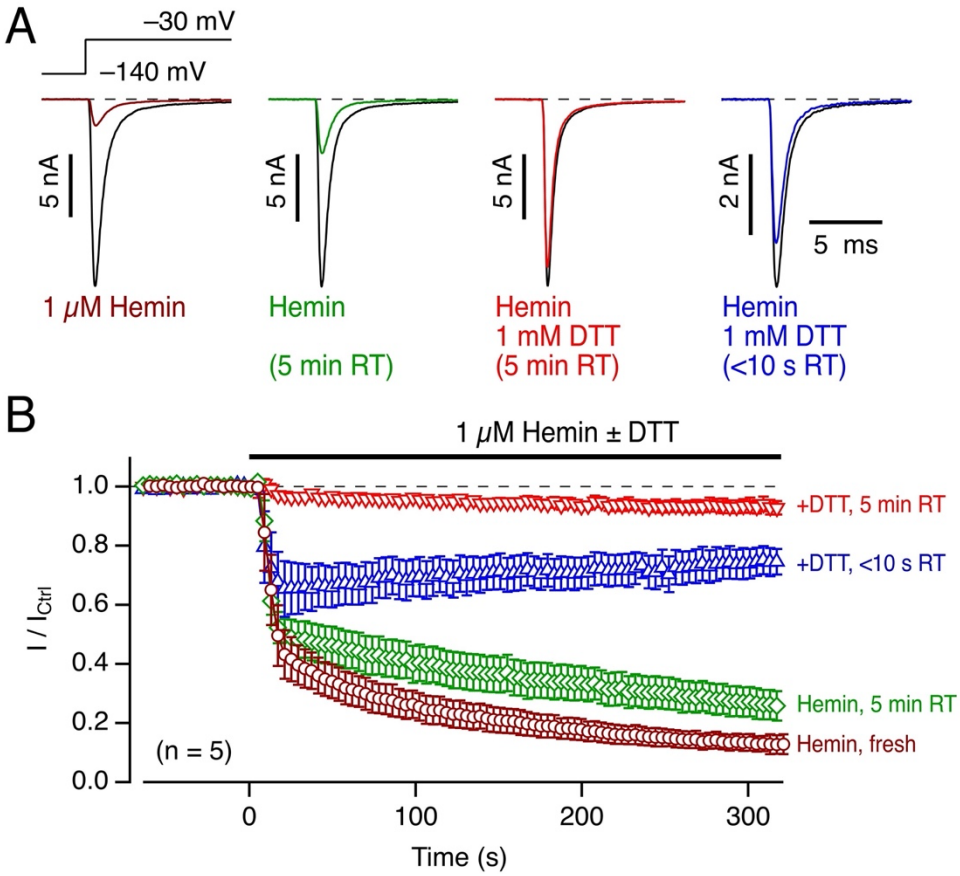
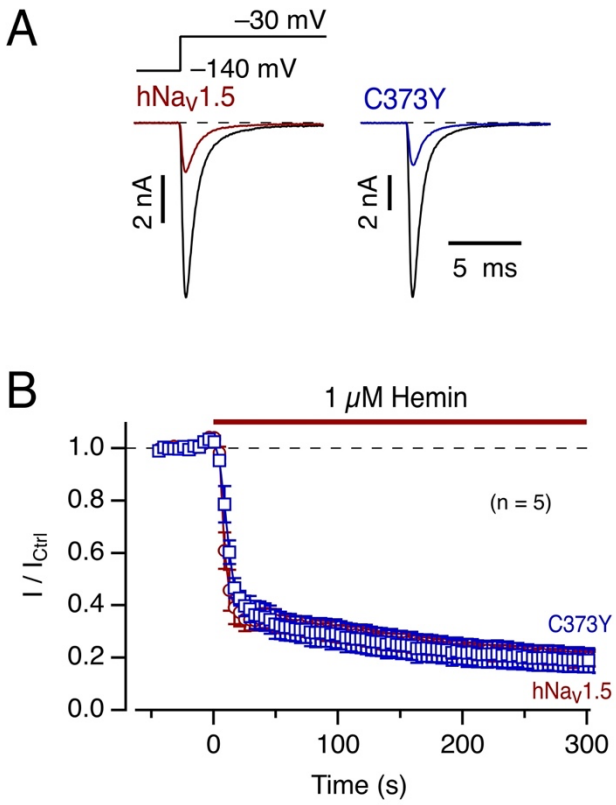
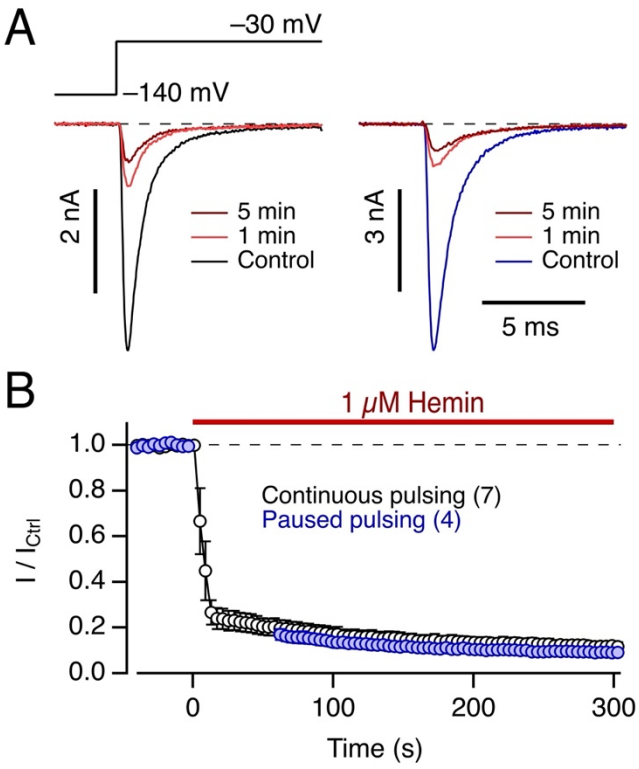
Supplementary Figures



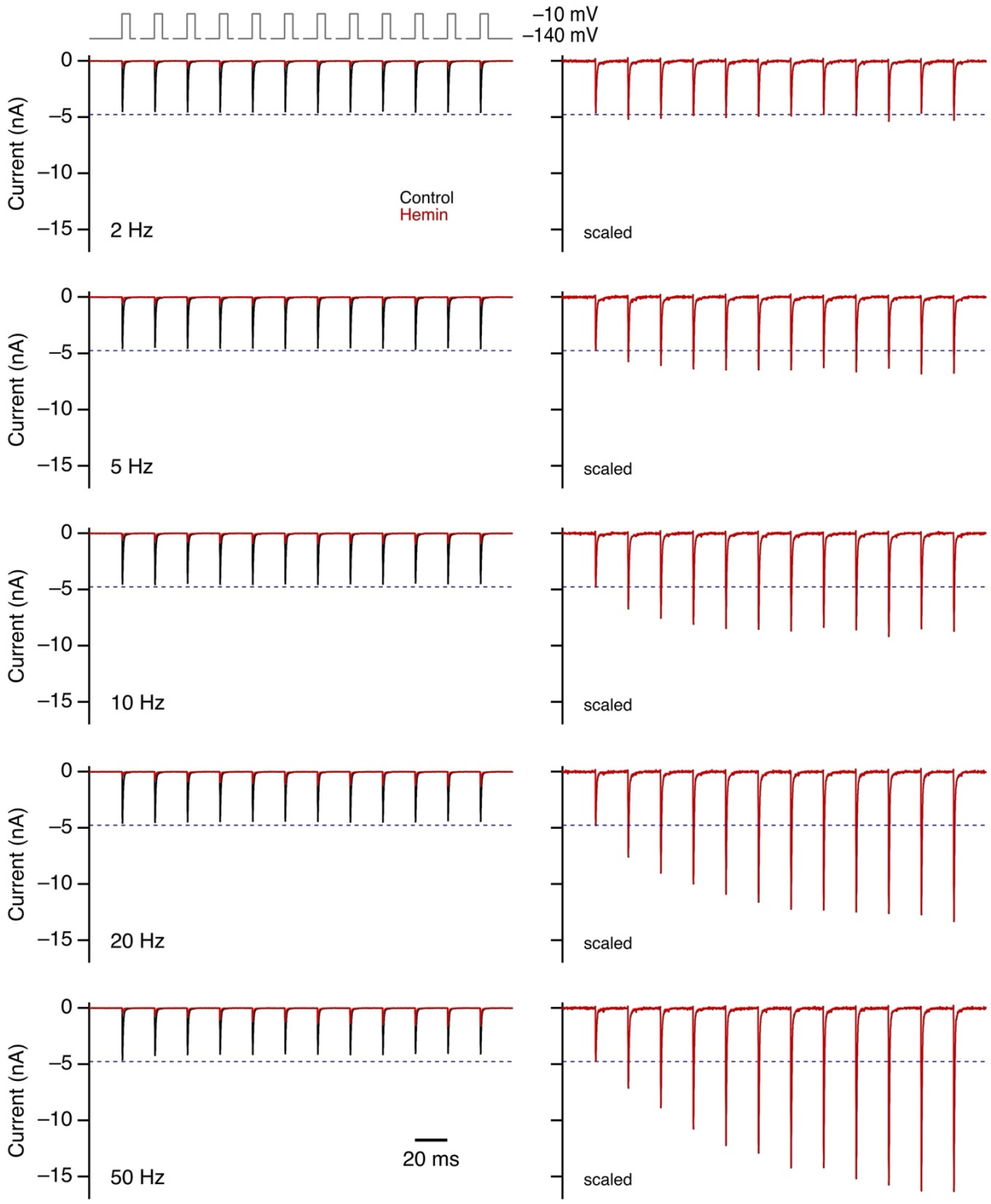
Supplementary Figure 1: Reduction of hemin in the presence of DTT.
(**A**) Whole-cell currents mediated by $Na_V1.5$ channels at –30 mV before (black) and about 5 min after (color) application of fresh 1 µM hemin, hemin after an episode of 5 min at room temperature (RT, green), hemin incubated with 1 mM DTT 5 min prior application (red), or incubated with 1 mM DTT <10 s prior application (blue). Preincubation was performed at 5x the concentration to yield 1 µM hemin after bath application. (**B**) Mean time courses of peak current at –30 mV with hemin application under the conditions of (A): hemin brown circles, hemin after 5 min at RT (green squares), hemin for 5 min in 1 mM DTT (red downward triangles), hemin mixed with 1 mM DTT <10 s prior to application to the cells. Data are mean ± sem.



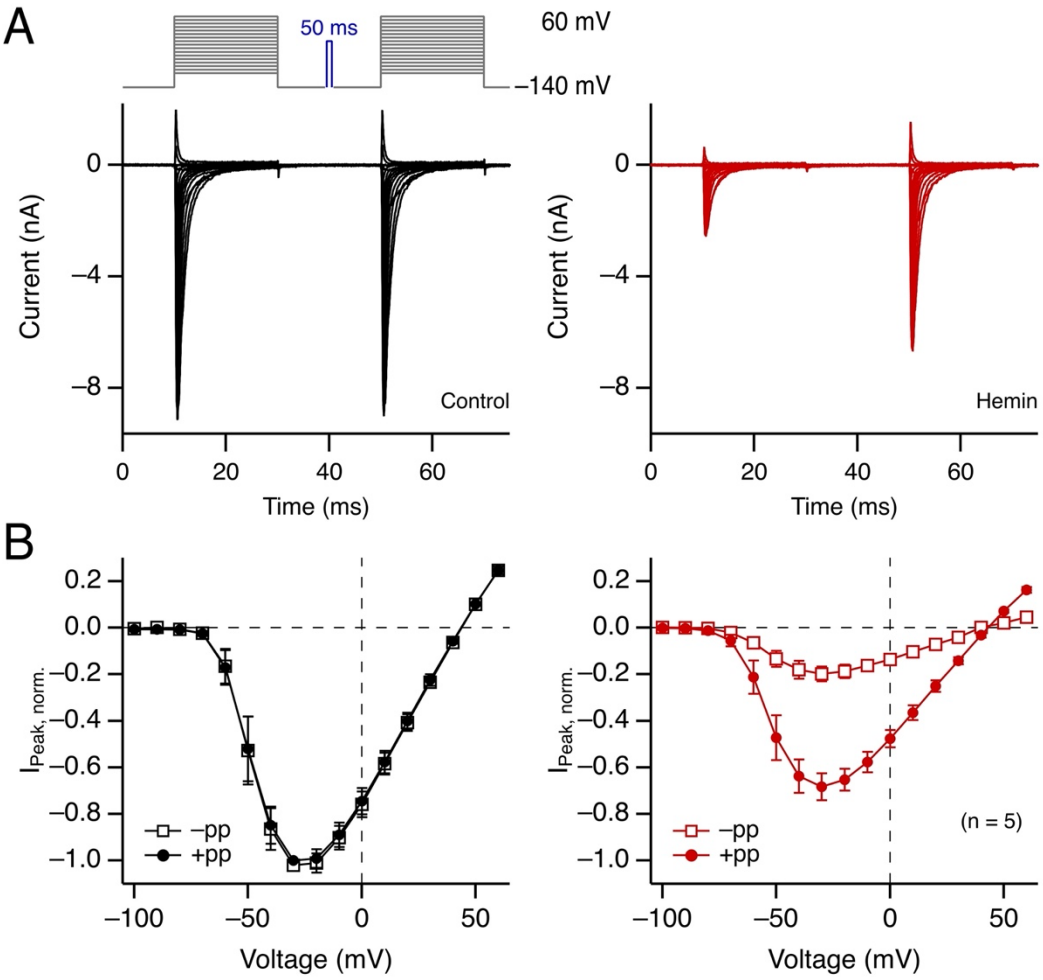
Supplementary Figure 2: Hemin sensitivity of hNa$_V$1.5-C373Y.
(**A**) Representative whole-cell currents mediated by Na$_V$1.5 channels and the variant C373Y at –30 mV before (black) and about 5 min after (color) application of 1 µM hemin. (**B**) Mean time courses of peak current at –30 mV with hemin application under the conditions of (A) for hNa$_V$1.5 (brown circles) and variant C373Y (blue squares). Data are mean ± sem.



Supplementary Figure 3: Time course of hemin-induced current inhibition.
(**A**) *left*, Whole-cell currents according to the indicated pulse protocol before (black) and 1 and 5 min after application of 1 µM hemin (color). *right*, As in the left panel but with an interruption of current measurements for 1 min starting from hemin application. (**B**) Mean time courses of the peak currents at –30 mV from experiments as in (A) with continuous pulsing at an interval of 4 s (open, black) and with a 1-min interruption of pulsing (filled, blue). Data are means ± sem with n in parentheses.



Supplementary Figure 4: Reverse use dependence of hemin-induced current inhibition.
left, Superimposed $Na_V1.5$ whole-cell currents before (black) and about 5 min after application of 1 µM hemin (red), stimulated with the indicated pulse protocol. Depolarizing steps to –10 mV always lasted 5 ms, while the interpulse interval at –140 mV (gaps) was adjusted to yield the indicated pulse frequencies. *right*, Current traces in the presence of hemin from the left panels, scaled to match the peak current of the first pulse to the control value (also indicated by the dashed lines).



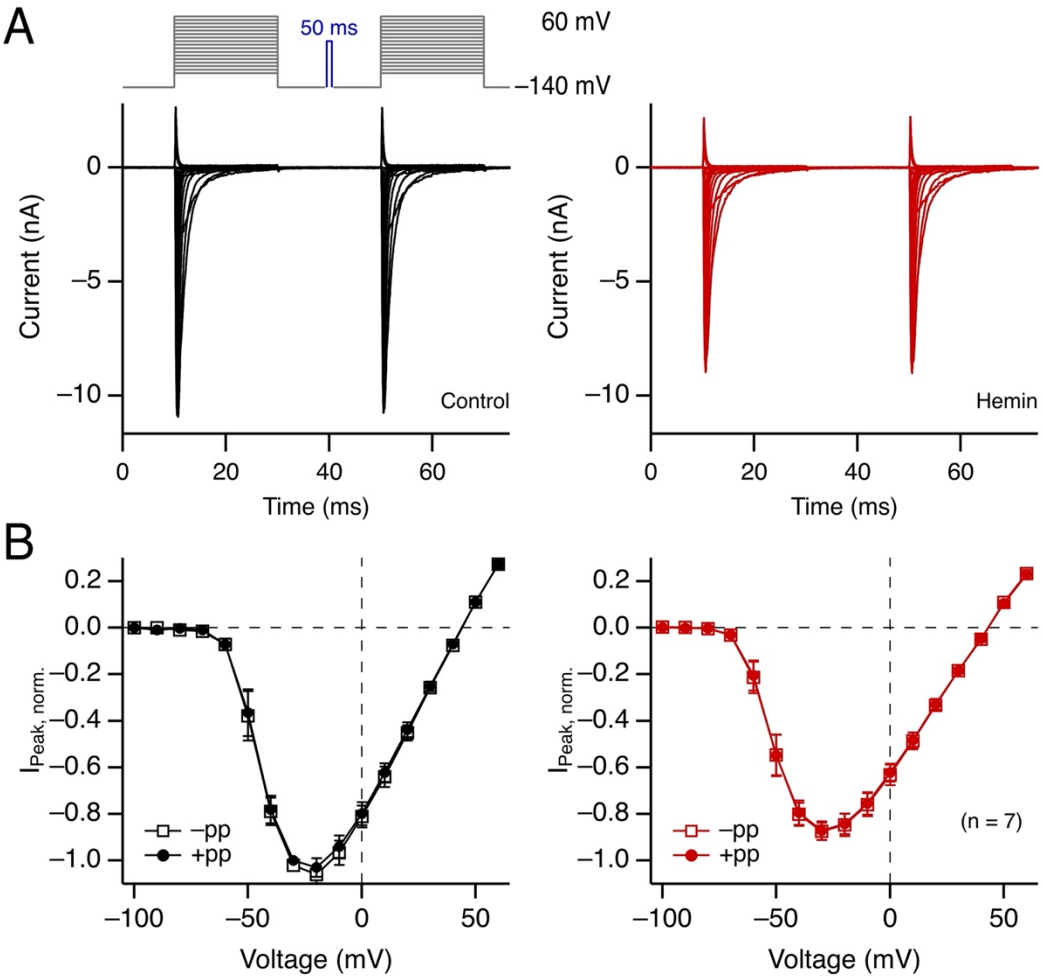
Supplementary Figure 5: Comparison of current-voltage relationships without and with depolarizing prepulses.

(**A**) Superposition of representative hNa$_V$1.5 whole-cell currents measured with the indicated two-pulse current-voltage protocol with a prepulse in the center (indicated in blue) of 50 ms at –10 mV; *left* control, *right* with 1 µM hemin. (**B**) Mean peak currents from experiments as in (A), normalized to the current value at –30 mV with (filled circles) and without prepulse (open squares) as a function of voltage without (*left*) and with 1 µM hemin (*right*). Straight lines connect data points for clarity. Data are mean ± sem.

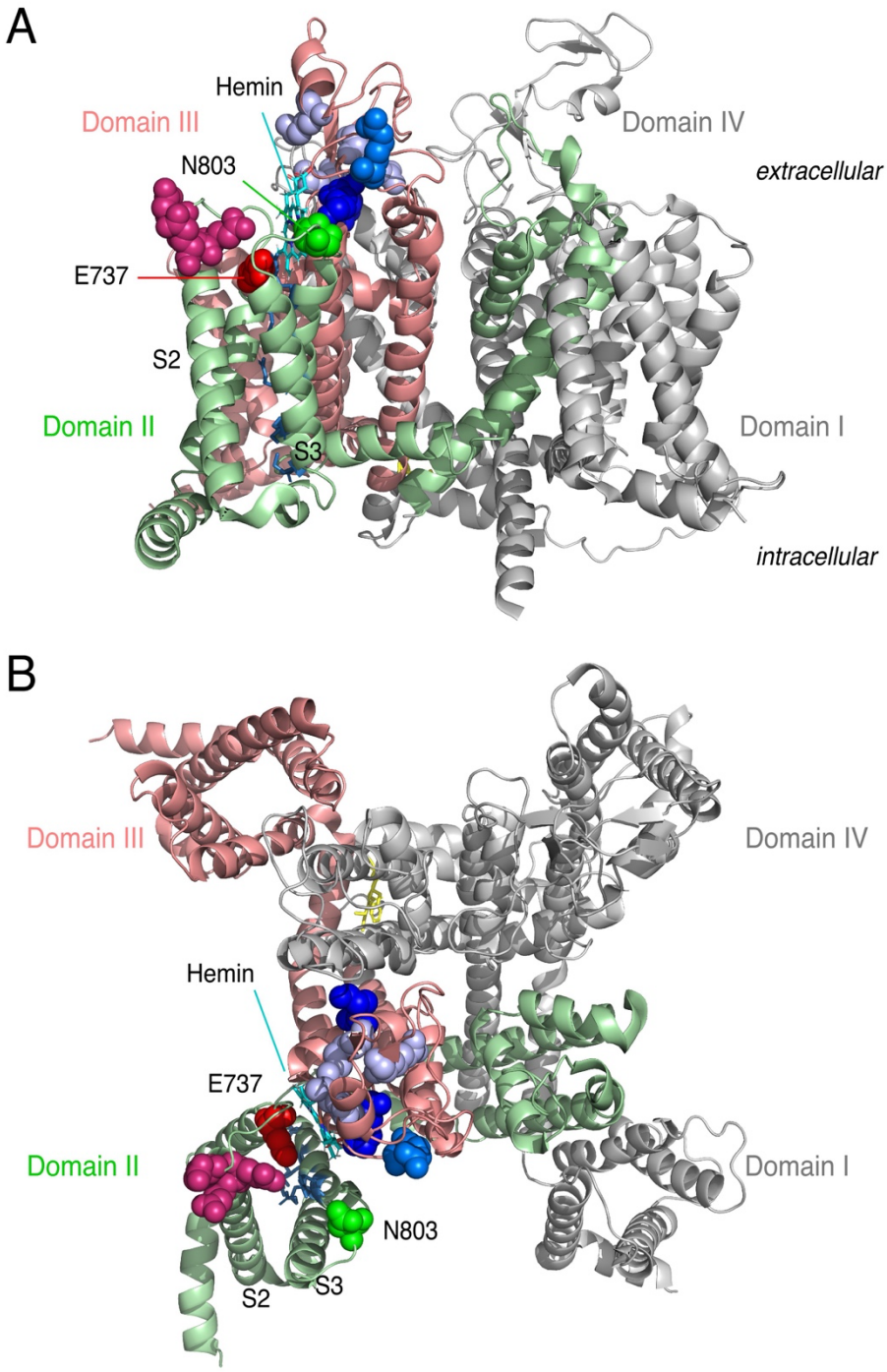
hNa$_V$1.5	VILSLMELGL SRMSNLSVLR	808
hNa$_V$1.2	.SL..M..GL ANVEG.....	850
rNa$_V$1.2	.SL..M..GL ANVEG.....	850
hNa$_V$1.1	.TL..V..GL ANVEG.....	859
hNa$_V$1.3	.SL..M..GL SNVEG.....	851
hNa$_V$1.4	.TL..M..GL ANVQG.....	669
hNa$_V$1.6	.SL..M..SL ADVEG.....	844
hNa$_V$1.7	.TL..V..FL ADVEG.....	835
hNa$_V$1.8	.TV..L..GV AKKGS.....	756

Supplementary Figure 6: Multiple sequence alignment encompassing Na$_V$ channel domain-II S3/S4 linker segments.

The numbers refer to the last amino acid shown in the peptide sequences, which is the outmost charged residue of the S4 segment (blue). The first 6 residues (green) are part of S3. The relevant residues for this study are highlighted in red (hNa$_V$1.5-N803), orange (homologous glycine), or violet (hNa$_V$1.8-S751). Dots denote conserved residues within this alignment.



Supplementary Figure 7: Lack of use dependence in variant hNa$_V$1.5-N803G.
(**A**) Superposition of representative hNa$_V$1.5-N803G whole-cell currents measured with the indicated two-pulse current-voltage protocol with a prepulse in the center (indicated in blue) of 50 ms at –10 mV; *left* control, *right* with 1 µM hemin. (**B**) Mean peak currents from experiments as in (A), normalized to the current value at –30 mV with (filled circles) and without prepulse (open squares) as a function of voltage without (*left*) and with 1 µM hemin (*right*). Straight lines connect data points for clarity. Data are mean ± sem.



Supplementary Figure 8: Hypothetical model of hemin–hNa$_V$1.5 interaction.
(**A**, **B**) Cryo-EM structural data of human Na$_V$1.5 according to pdb 6LQA with a hypothetical placement of hemin in the cleft between the domain–II voltage sensor and the pore loop of domain III in side view (A) and top/extracellular view (B). Pale green: domain II; salmon: domain III; grey: domains I and IV; green spheres: N803; red spheres: conserved negative charge in domain-II S1/S2 (E737); magenta spheres: negative charges specific for Na$_V$1.5; dark blue spheres: conserved positive charges (K1359, K1399); lighter blue spheres: non-conserved positives charges in Na$_V$1.5; blue sticks: gating charges (3x Arg, 2x Lys) of domain-II S4; yellow sticks: inactivation motif "IFM" in the linker between domains III and IV. Hemin is shown as light blue sticks with red carboxylate groups. No structural optimization performed.