Supplementary Table 1.

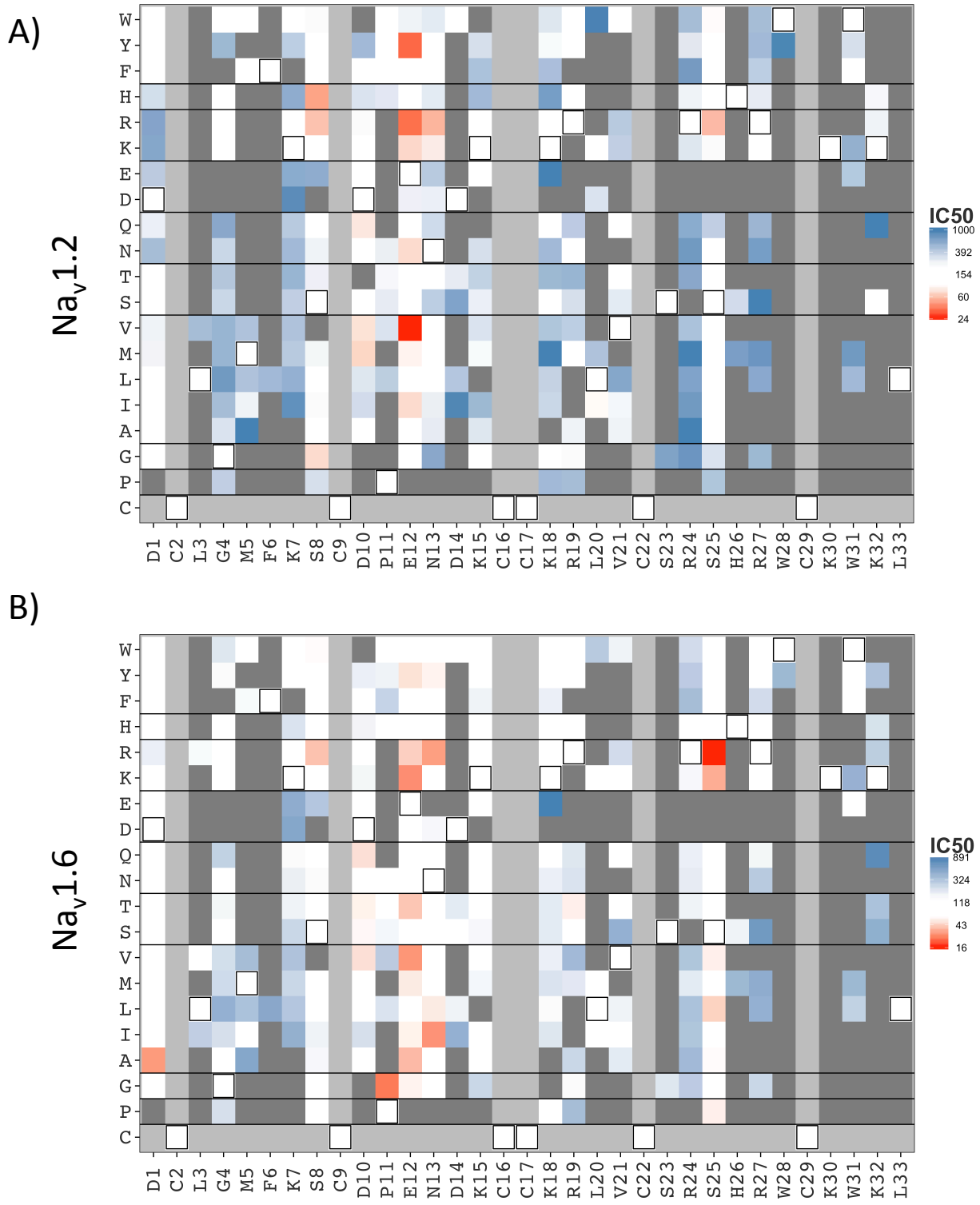
Crystallography data and refinement statistics **Data collection: 2670-Fab structure**	
Space group	$P2_12_12_1$
Cell dimensions	
a, b, c (Å)	97.32, 98.44, 107.35
α, β, γ (°)	90.00, 90.00, 90.00
Resolution range (Å)	47.00–1.75 (1.8-1.75)
R_{merge}(%)	0.057 (0.271)
⟨I/σ(I)⟩	49.57 (6.07)
Completeness (%)	99.8 (99.1)
Redundancy	7.1 (6.0)
Reflections measured	738,250
Unique reflections	103,911 (5,056)
Wilson B-factor (Å2)	20.8
Refinement	
Resolution range (Å)	47.00–1.75
Reflections (total)	98,659
Reflections (Rfree)	5,172
Rwork/Rfree (%)	19.0/21.9
NCS groups	0
TLS groups	10
No. of non-hydrogen protein atoms	8540
No. of ligand atoms	0
No. of water molecules	948
Average B-factor (Å2)	13.7
RMS deviations	
Bonds (Å)	0.007
Angles (°)	1.27
Ramachandran plot	
Preferred (%)	97
Allowed (%)	3
Disallowed (%)	0

Values in parentheseses correspond to the highest-resolution shell

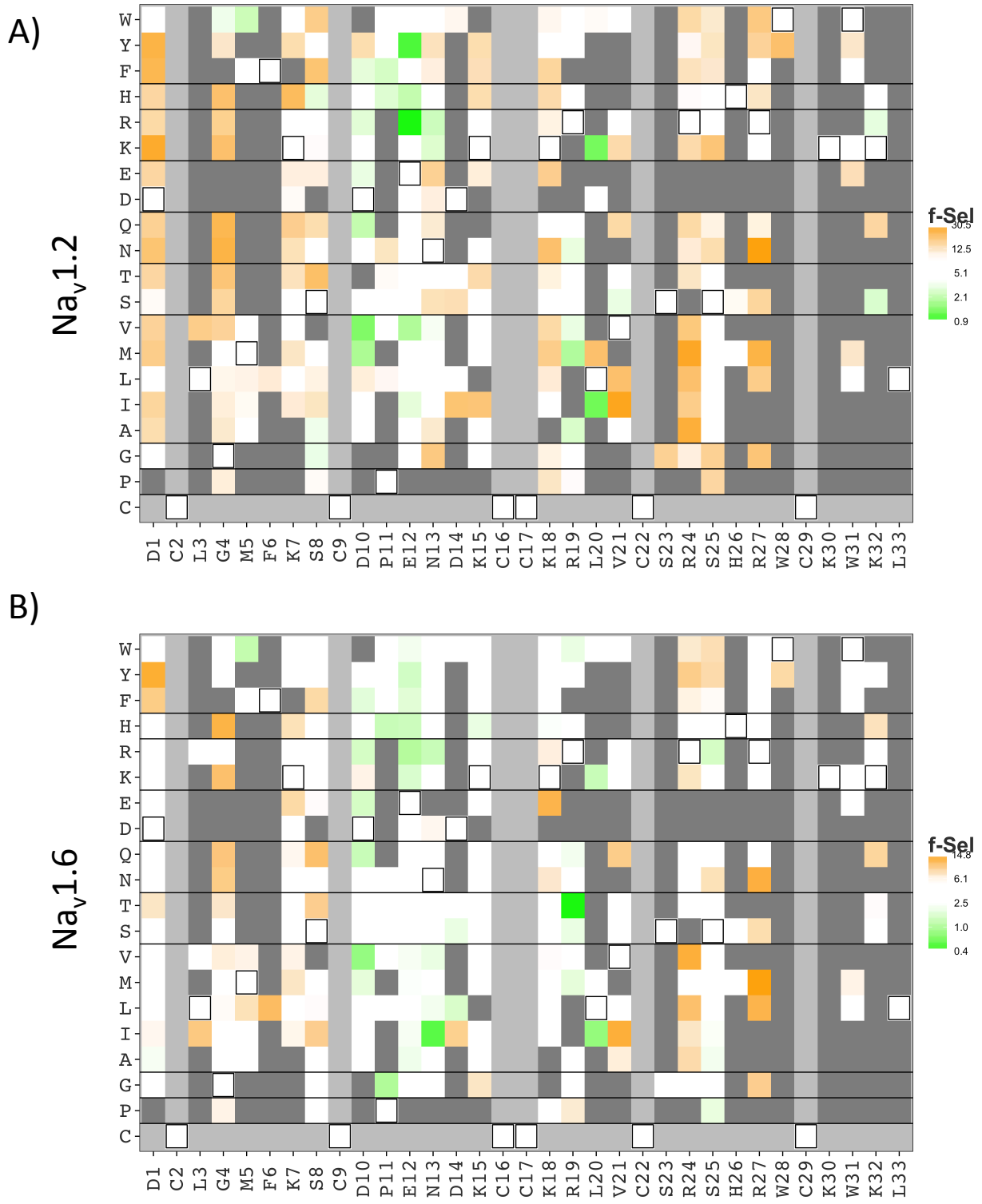
Supplementary Table 2

	Nav1.7				Nav1.2				Nav1.6			
Compound	**n**	**IC50 (nM)**	**STE (nM)**	**f.d.**	**n**	**IC50 (nM)**	**STE (nM)**	**f.d.**	**n**	**IC50 (nM)**	**STE (nM)**	**f.d.**
2670	226	25.1	1.3		65	146.3	9.9		71	92.1	6.8	
2670a	11	4.1	1.7	6.2	5	58.8	9.4	2.5	11	13.9	2.6	6.6
D1I	31	10.6	1.8		12	162.9	52.1		20	65.5	9.2	
D1Ia	84	2.0	0.2	5.3	20	77.3	8.6	2.1	32	15.1	1.0	4.3

STE: Standard error, f.d. = fold difference of amidated vs. non-amidated, n=number of measurements

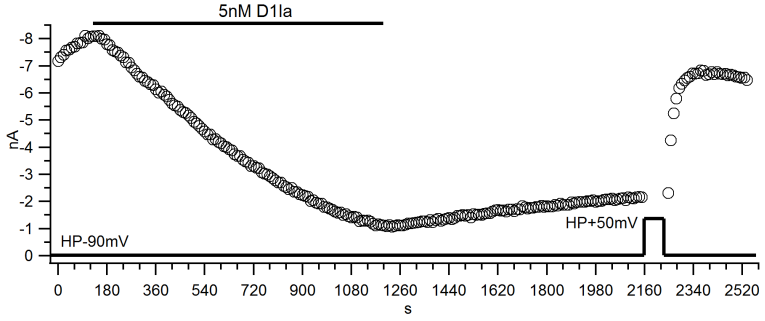


Suppl Fig 1. Affinity table for $Na_v1.2$ and $Na_v1.6$. Substitutions with improved potency are in red, substitutions with worse selectivity in blue and neutral or almost neutral substitutions in white. Amino acid are group by chemical similarity.

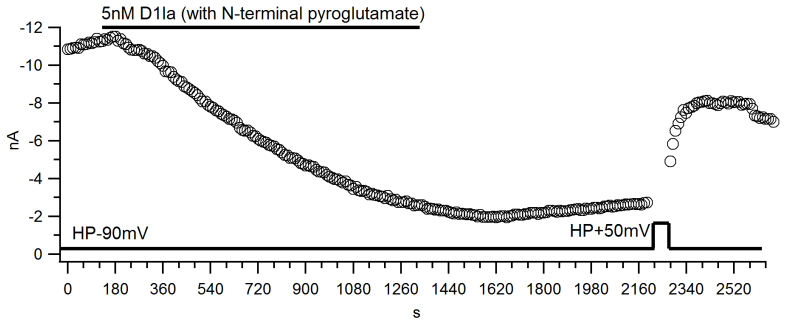


Suppl Fig 2. Selectivity table for Na$_v$1.2 and Na$_v$1.6. Substitutions with improved selectivity are in orange, substitutions with worse selectivity in green and neutral or almost neutral substitutions in white. Amino acid are group by chemical similarity.

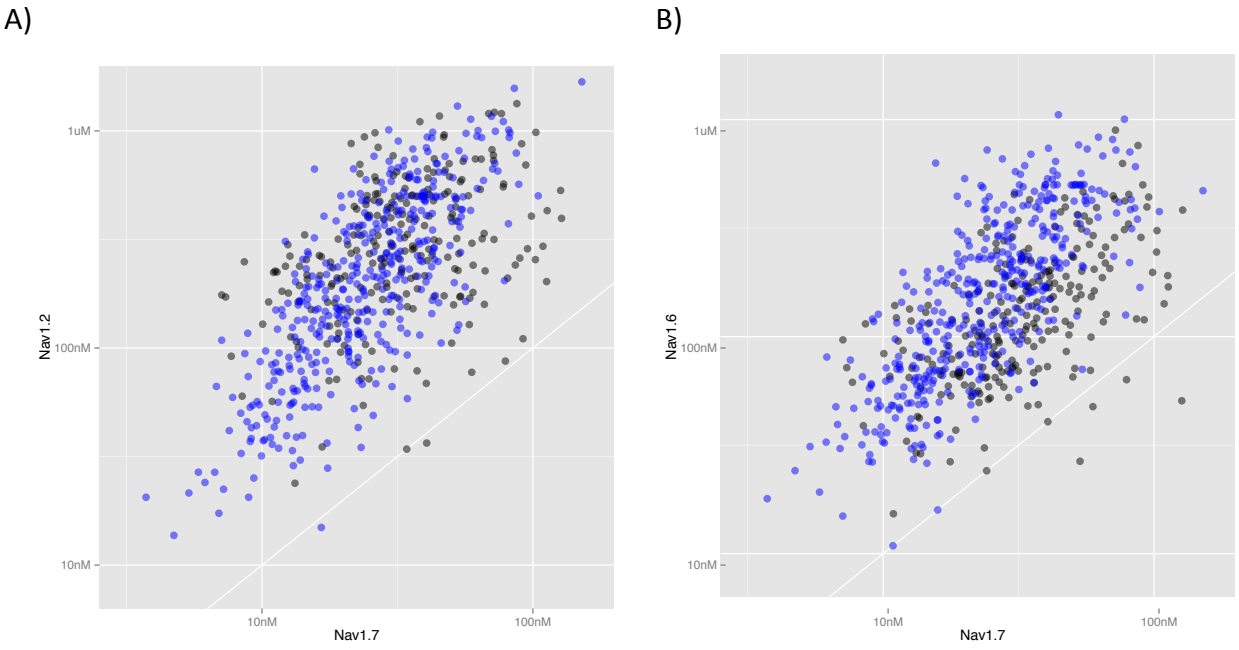
A)



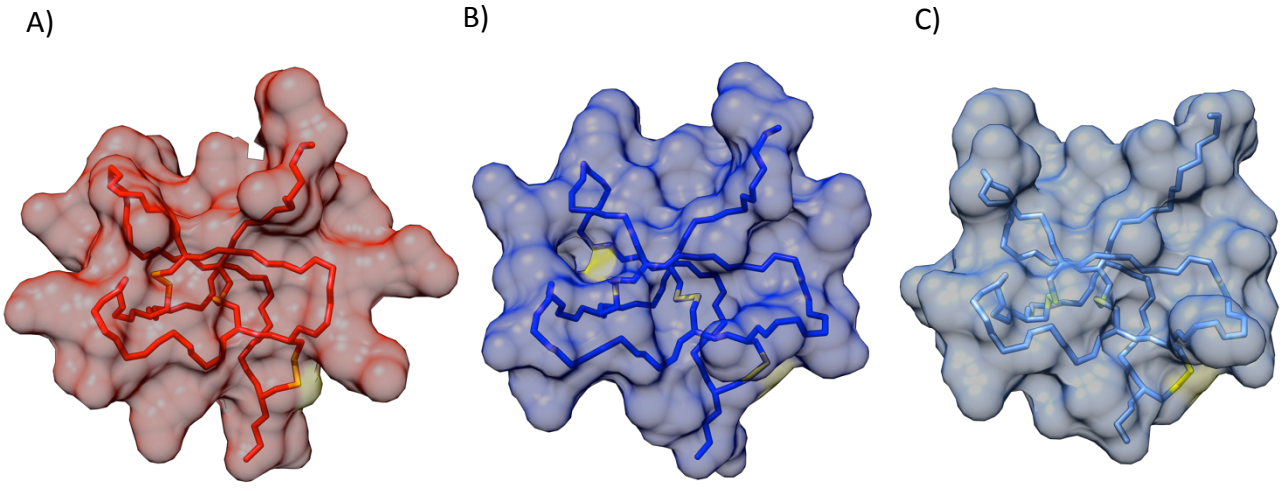
B)



Suppl Fig 3. Time course of Nav1.7 current block by **A)** D1Ia and **B)** by N-terminal pyroglutamated variant of D1Ia in HEK293 cells stably expressing the human Nav1.7 channel. Nav1.7 currents were evoked by a 15ms step depolarization to 0 mV every 10 s from a holding potential of −90 mV. The time of compound application is indicated by solid line. The Nav1.7 current block was relieved during compound's washout phase by depolarizing the cell for one minute from a holding potential of -90mV to +50mV. Subsequent depolarizing 15ms pulses to 0 mV every 10 s from a holding potential of −90 mV revealed significant recovery of the current amplitude. The manual whole-cell patch clamp technique was used to record Nav1.7 currents.



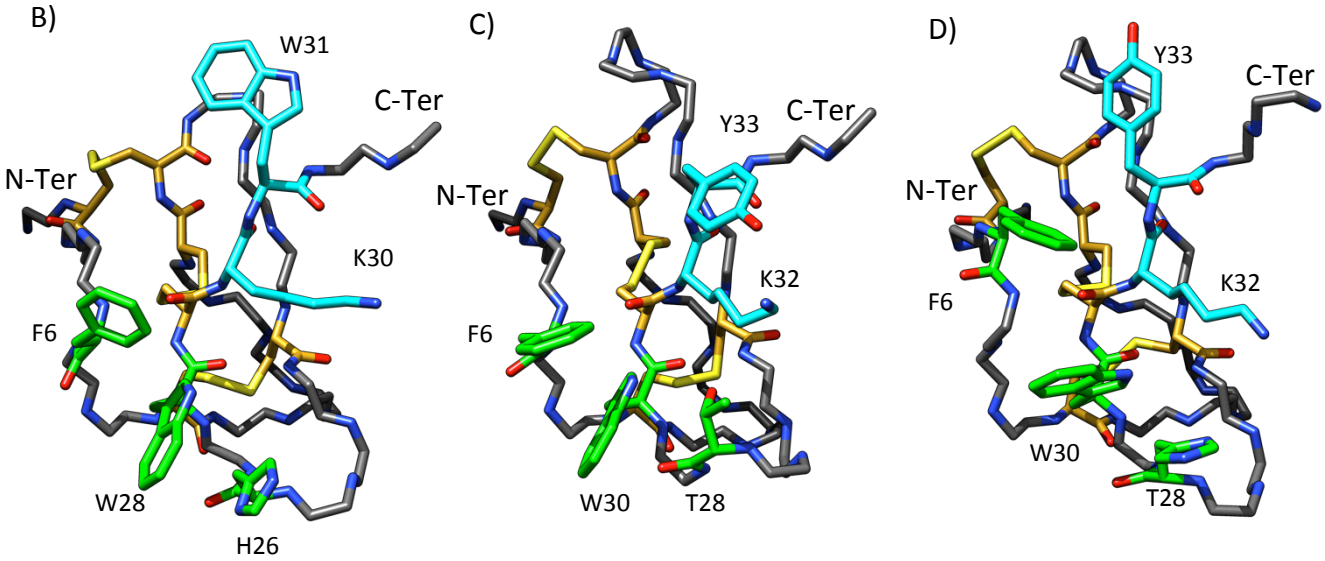
Suppl Fig 4. A) Potency scatter plot (IC_{50} $Na_v1.7$ vs. IC_{50} counter screening channel Nav1.2) of molecules generated by saturated mutagenesis experiments. B) Same plot as in panel A, but with Nav1.6 as counter screening channel. Single mutants of 2670 are shown in gray, while double mutants are shown in blue.



Suppl Fig 5. Molecular surfaces of **A)** 2670 microprotein compared to **B)** Huwentoxin-IV and **C)** Hainantoxin-IV. Due to the small size of the proteins, relatively small number of amino acid changes can have significant effects on the surface topology.

A)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	18a	18b	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
2670	D	C	L	G	M	F	K	S	C	D	P	E	N	D	K	C	C	K	-	-	R	L	V	C	S	R	S	H	R	W	C	K	W	K	L
Huwentoxin-4	E	C	L	E	I	F	K	A	C	N	P	S	N	D	Q	C	C	K	S	S	K	L	V	C	S	R	K	T	R	W	C	K	Y	Q	I
Hainantoxin-4	E	C	L	G	F	G	K	G	C	N	P	S	N	D	Q	C	C	K	S	S	N	L	V	C	S	R	K	H	R	W	C	K	Y	E	I



2670

Huwentoxin-IV
(PDB: 1MB6)

Hainantoxin-IV
(PDB: 1NIY)

Suppl Fig 6. Comparison of previously reported interacting residues in Huwentoxin-IV and Hainantoxin-IV to corresponding residues in microprotein 2670. A) Sequence alignment of 2670, Huwentoxin-IV and Hainantoxin-IV. Structures of B) 2670 residues, full list of interacting residues is shown in main Figure 5E and 5F C) Huwentoxin-IV, and D) Hainantoxin-IV shown in same orientation with main interacting residues shown in green and cyan. The data shows that all three proteins interact via same face of the molecule with Nav1.7 channel.