

Table S1. MALDI-TOF characterization of novel nAChR-targeting toxins purified from *Metridium senile* venom and their established N-terminal sequences (cysteine residues are shown with bold).

Peptide name	Average MW measured by MALDI-TOF (Da)	Calculated average MW of the respective predicted target peptides deduced from cDNAs after proposed posttranslational modifications (Da)	N-terminal sequence established by Edman degradation
α-AnmTX-Ms11a-1 (short name Ms11a-1)	4594.8	4594.38	G C KKLNSN C TRQYRE C CHGLV C RRPNYGNRGILWR
α-AnmTX-Ms11a-2 (Ms11a-2)	4653.4	4653.41	G C KNLNSH C YRQHRE C CHGLV C R
α-AnmTX-Ms11a-3 (Ms11a-3)	4918.4	4918.82	G C KKLNSY C TRQHRE C CHGLV C RRPDYGIG
α-AnmTX-Ms11a-4 (Ms11a-4)	3274.8	3274.79	C AQTGGT C SKSKD C CIVTAI C STA

(a) Ms11a-1

DNA: gattcgggtgtgaacatcaccaagatagcaacgtttcaagcttcacactcaaccgaactg

+1fr: ·D·S·G·V·N·I·T·K·I·A·T·F·Q·A·S·H·S·T·E·L·

DNA: gatattacactcaaccgaactggatattactattataagggattcgaa**atg**gcttcaaag

+1fr: ·D·I·T·L·N·R·T·G·Y·Y·Y·Y·K·G·F·E·M·A·S·K·

DNA: attttctttgtccttgcagtgtttcttgtgatgtcggctgtgctccccgaatctttcgcg

+1fr: ·I·F·F·V·L·A·V·F·L·V·M·S·A·V·L·P·E·S·F·A·

DNA: ggctgcaagaaattgaattcgaattgttcgagacaataaccgtgagtgttgcatgggtttg

+1fr: ·G·C·K·K·L·N·S·N·C·S·R·Q·Y·R·E·C·C·H·G·L·

DNA: gtgtgcagaaggcctaactatggcaatggcagaggattctttggagatgtgtcaaggcc

+1fr: ·V·C·R·R·P·N·Y·G·N·G·R·G·I·L·W·R·C·V·K·A·

DNA: aagaagtgaatggatgcttataataaaggataagctcttcattggttaagaataataacta

+1fr: **K**·**K**·*·M·D·A·Y·N·K·G·*·A·L·H·W·L·R·I·I·L·

DNA: atactaataactaatgtttttcaggagggttgctctaaagaactaaaattcaaactgtatta

+1fr: ·I··L··I··L··M··F··F··R··R··L··L··*··R··T··K··I··Q··T··V··L·

DNA: acactgtaattcaaaaagggaaataaactatcatagttgattcaaaaaaaaaaaaaaaaaaaaaa

+1fr: ·T··L··*··F··K··K··E··I··N··Y··H··S··*··F··K··K··K··K··K··K·

(b) Ms11a-4

DNA: agatacaagtgaacattgaagtcgttcttgcaagtgaaatctctcgagcttgataacc**atg**

+1fr: ·R··Y··K··*··T··L··K··S··F··L··Q··V··N··L··S··S··L··I··T··M·

DNA: aaggtccttggtgcagtccttgctcttctgctctactgatgtgtatgtttgttgacatcgcg

+1fr: ·K··V··L··V··A··V··L··V··F··A··L··L··M··C··M··F··V··D··I··A·

DNA: gaaagtcgtcgtcgagacaaccagaataccccagtgggcttcgatatgacgaggagatg

+1fr: E · S · R · R · R · D · N · P · E · Y · P · S · G · L · R · Y · D · E · E · M ·

DNA: ggtgttttcaaacggtgcgctcaaacgggagggacatgctccaaatctaaagattgctgt

+1fr: ·G··V··F··K··R··C··A··Q··T··G··G··T··C··S··K··S··K··D··C··C·

DNA: attgtaacagctatctgcagtacagcaacaagtcctaaaacatgtttttatggctagtga

+1fr: ·I·V·T·A·I·C·S·T·A·T·S·P·K·T·C·F·Y·G·*·*·*·

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DNA:  aagagcgggaatatagtgtgtgcatgcagatTTTacaattgaagatgtctttcacttagc
+1fr:  ·K·S·G·N·I·V·C·A·C·R·F·Y·N·*·R·C·L·S·L·S·

DNA:  tggaatcatgtaaaataaaaccactctttgcttgtaatggtgatcaggattattataata
+1fr:  ·W·N·H·V·K·*·N·H·S·L·L·V·M·V·I·R·I·I·I·I·

DNA:  aaacatgtaatggtgtcga
+1fr:  ·K·H·V·M·V·S·

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Figure S1. Structural organization of precursor proteins. (a) nucleotide sequence of *ms11.1* cDNA accompanied by deduced amino acid sequence. (b) nucleotide sequence of *ms11.4* cDNA accompanied by deduced amino acid sequence. The signal peptide sequences are *underlined*, determined mature peptide sequences are bold and highlighted gray, and the propeptide sequences are shown in italics.

Table S2. 3D-alignment of Ms11a-3 with proteins in PDB database.

No	PDB ID (Ref.)	Name	Q-score (RMSD)	N _{align} (N _{alt})	Source organism	Target (that was in the corre- sponding Ref.)	No (or weak) effect
1	2MXM (doi.org/10.1124/mol.115.098178)	TRTX-Tp1a	0.66 (1.07Å)	32(33)	<i>Thrixopelma pruriens</i>	hNa _v 1.1 (IC ₅₀ 101±30 nM); hNa _v 1.3 (IC ₅₀ 1.3±4.7 nM); hNa _v 1.5 (IC ₅₀ >500 nM); hNa _v 1.7 (IC ₅₀ 2.1±1.3 nM)	human voltage-gated calcium channels (5 μM); nAChR (5 μM)
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNHRECHGLVCR-RPDYGIGRGILWKCTRARK</div> <div>2MXM :DCLKFGWKCNPRNDKCCSGLKCGSNH-----NWCKLHL-</div> <div>102030</div> </div>							
2	2MQF (doi.org/10.1016/S0014-5793(03)01303-6; doi.org/10.1124/mol.115.100784)	TRTX-HHN2B	0.64 (1.06Å)	32(34)	<i>Haplopetelma hainanum</i>	para/tipE (IC ₅₀ 4.3±0.3 μM) rNa _v 1.2/β ₁ (IC ₅₀ 68±6 μM)	
<div> <div>10203040</div> <div>Ms11a-3:-GCKKLNSYCTRNHRECHGLVCR-RPDYGIGRGILWKCTRARK</div> <div>2MQF :AECCKGFGKSCVPGKNECCSGYACNSRD-----KWCKVLL-</div> <div>102030</div> </div>							
3	6BR0 (doi.org/10.1074/jbc.RA118.002553)	CCOTX-I	0.63 (1.26Å)	32(33)	<i>Ceratogyrus marshalli</i>	hNa _v 1.2 (EC ₅₀ 6.1±0.7 nM); hNa _v 1.4 (EC ₅₀ 263±45 nM); hNa _v 1.5 (EC ₅₀ 188±20 nM); hNa _v 1.6 (EC ₅₀ 40.6±5.8 nM); hNa _v 1.7 (IC ₅₀ 129.1±6.5 nM)	
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNHRECHGLVCR-RPDYGIGRGILWKCTRARK</div> <div>6BR0 :DCLGWFKSCDPKNDKCKKNYTCSRDR-----RWCKYDL-</div> <div>102030</div> </div>							
4	1MB6 (doi.org/10.1074/jbc.M204063200)	HWTX-IV	0.59 (0.95Å)	31(35)	<i>Haplopetelma schmidtii</i>	neuronal tetrodotoxin-sensi- tive voltage-gated sodium channel of adult rat dorsal root gan- glion neurons (IC ₅₀ 30 nM)	tetrodotoxin-resistant voltage-gated sodium channel

<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNRHRECCH--GLVCRRPDYGIGRGILWKCTRARK</div> <div>1MB6 :ECLEIFKACNPSNDNCCKSSKLVCSRK-----TRWCKYNI-</div> <div>102030</div> </div>							
5	2MPQ (doi.org/10.1111/bph.13081)	TRTX-Hd1a	0.59 (1.13Å)	32(36)	<i>Haplopelma doriae</i>	hNav, especially hNav1.1 and hNav1.7	
<div> <div>10203040</div> <div>Ms11a-3:-GCKKLNSYCTRNRHRECCH--GLVCR-RPDYGIGRGILWKCTRARK</div> <div>2MPQ :GACLGFGKSCNPSNDNCCKSSSLACSTKH-----KWCKYEL-</div> <div>102030</div> </div>							
6	6GFT (doi.org/10.1111/bph.14628)	CyrTx-1a	0.58 (1.07Å)	30(33)	<i>Cyriopagopus schioedtei</i>	hNav1.1 (IC ₅₀ 72±10 nM); hNav1.2 (IC ₅₀ 75.5±4.3 nM); hNav1.3 (IC ₅₀ 307±15 nM); hNav1.6 (IC ₅₀ 115±7.5 nM); hNav1.7 (IC ₅₀ 130±2 nM); hNav1.4 (IC ₅₀ 7.7±0.2 µM); hNav1.5 (IC ₅₀ >10µM); hNav1.8 (IC ₅₀ >10 µM);	hCa _v 1.2, 3.1, 3.2 hK _v 7.1, 11.1 hK _{ir} 2.1
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYC-TRNHR-ECCHGLVCR-RPDYGIGRGILWKCTRARK</div> <div>6GFT :ECKGFGKSCVP--GKNECCSGLTCSNKH-----KWCKVLL-</div> <div>102030</div> </div>							
7	2N1N (doi.org/10.1038/nature01473)	VSTx1	0.56 (1.72Å)	33(35)	<i>Grammostola rosea</i>	K _v AP	
<div> <div>10203040</div> <div>Ms11a-3:-GCKKLNSYCTRNRHRECCHGLVCRRPDYGIGRGILWKCTRARK-</div> <div>2N1N :SECGKFMWKCKN-SNDCCKDLVCSSRW-----KWCVLASPF</div> <div>102030</div> </div>							
8	1EIT (doi.org/10.1021/bi952605r)	µ-Aga-I	0.55 (1.65Å)	33(36)	<i>Agelenopsis aperta</i>	Voltage-gated sodium channels	
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNRH-RECCHGLVCRRPDYGIGRGILWKCTRARK</div> <div>1EIT :ECVPENGHCRDWYDE-CCEGFYCSCRN-----PPKCICRNNN-</div> <div>102030</div> </div>							

9	2M9L (doi.org/10.1016/j.cub.2014.01.013; doi.org/10.1016/j.toxicon.2006.09.014)	ProTx-I	0.54 (1.37Å)	31(35)	<i>Thrixopelma pruriens</i>	TRPA1 (IC ₅₀ 389±77 nM); Na _v 1.2, Na _v 1.5, Na _v 1.7, Na _v 1.8	
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNHRECCHGLVCR-RPDYGIGRGILWKCTRARK--</div> <div>2M9L :ECRYWLGGCS-AGNTCCKHLVCSRRH-----GWCVWDGTFS</div> <div>102030</div> </div>							
10	1LU8 (doi.org/10.1016/j.toxicon.2006.09.030)	GsMTx-4	0.54 (1.72Å)	32(34)	<i>Grammostola rosea</i>	TRPC1	voltage-gated channels
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNHRECC-HGLVCRRPDYGIGRGILWKCTRARK</div> <div>1LU8 :GCLEFWWKCNPNDDKCCRPKLKCSKLF-----KLCNFSF-</div> <div>102030</div> </div>							
11	7A64 (doi.org/10.1021/acspts.c.1c00072)	Tsp1a	0.53 (1.55Å)	28(28)	<i>Thrixopelma sp.</i>	hNaV1.7 (IC ₅₀ 10 nM); hNaV1.1 (IC ₅₀ 450 nM); hNaV1.2 ((IC ₅₀ 250 nM)	
<div> <div>10203040</div> <div>Ms11a-3:GCKKLNSYCTRNHRECCHGLVCRRPDYGIGRGILWKCTRARK</div> <div>7A64 :YCNKFLWTCD-SERPCCEGLVCRL-----WCKIN--</div> <div>1020</div> </div>							

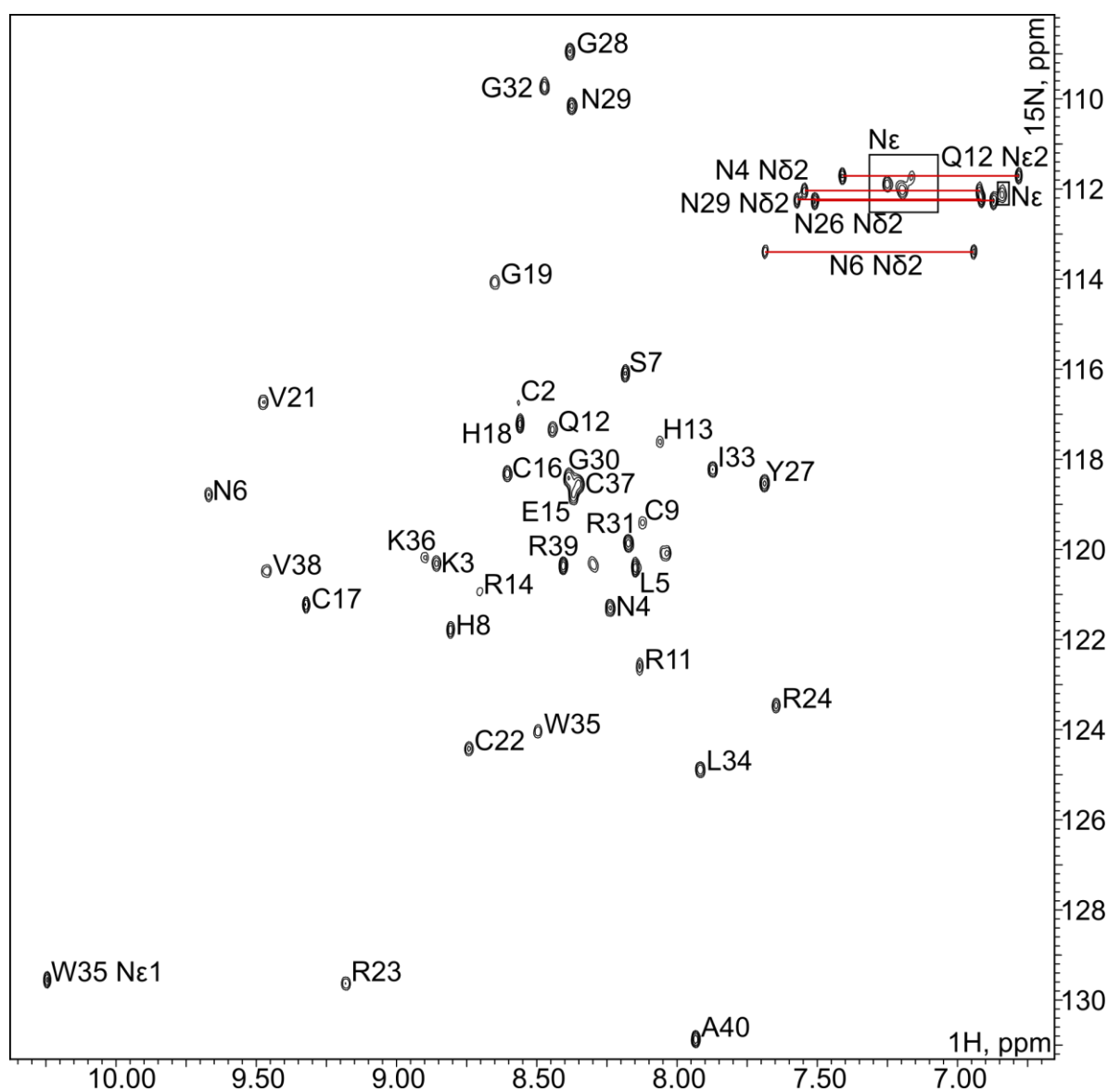


Figure S2. ^1H - ^{15}N -HSQC spectrum of Ms11a-2. Spectrum was recorded at 30 °C, pH 5.2. The assignment of cross-peaks is indicated.

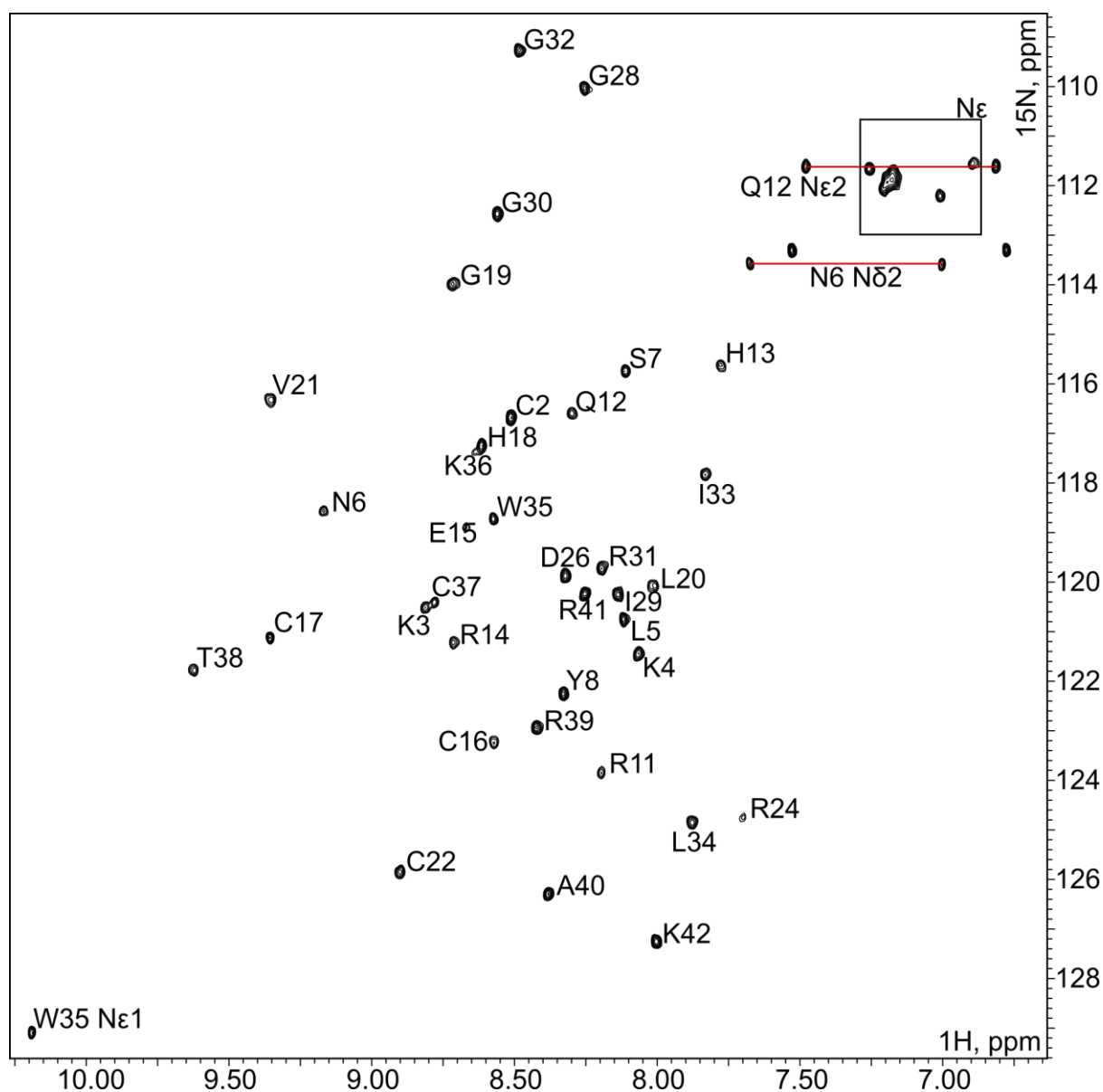


Figure S3. ^1H - ^{15}N -HSQC spectrum of Ms11a-3. Spectrum was recorded at 30 °C, pH 4.5. The assignment of cross-peaks is indicated.