

Supplementary Materials: Purification and Characterization of Recombinant Botulinum Neurotoxin Serotype FA, Also Known as Serotype H

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Supplemental Figure 1

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rBoNT-FA(0)-his	MPVVINSFNYYDDPVNDNTIIYIRPPYYETSNTYFKAFQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGSD	70
mrBoNT-FA(0)-his	MPVVINSFNYYDDPVNDNTIIYIRPPYYETSNTYFKAFQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGSD	70
mrBoNT-FA-his	MPVVINSFNYYDDPVNDNTIIYIRPPYYETSNTYFKAFQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGSD	70
rBoNT-FA(0)-his	GYFDPNYLSTNTEKNKYLQIMIKLFKRINSKPAGQILLEEKNAIPYLGNSYQEEQFTTNNRTVSFNVK	140
mrBoNT-FA(0)-his	GYFDPNYLSTNTEKNKYLQIMIKLFKRINSKPAGQILLEEKNAIPYLGNSYQEEQFTTNNRTVSFNVK	140
mrBoNT-FA-his	GYFDPNYLSTNTEKNKYLQIMIKLFKRINSKPAGQILLEEKNAIPYLGNSYQEEQFTTNNRTVSFNVK	140
rBoNT-FA(0)-his	LANGNIVQQMANLI IWGPGPDLTTNKGGIIYSPYQSMEATPYKDGFGSMTVEFSPEYATAFNDISIAS	210
mrBoNT-FA(0)-his	LANGNIVQQMANLI IWGPGPDLTTNKGGIIYSPYQSMEATPYKDGFGSMTVEFSPEYATAFNDISIAS	210
mrBoNT-FA-his	LANGNIVQQMANLI IWGPGPDLTTNKGGIIYSPYQSMEATPYKDGFGSMTVEFSPEYATAFNDISIAS	210
rBoNT-FA(0)-his	HSPSLFIKDPALILMHQLIYVLHGLYGYITEYKIPNVVQSYMKVTKPITSAEFLTFGGRDRNIVPQSI	280
mrBoNT-FA(0)-his	HSPSLFIKDPALILMHQLIYVLHGLYGYITEYKIPNVVQSYMKVTKPITSAEFLTFGGRDRNIVPQSI	280
mrBoNT-FA-his	HSPSLFIKDPALILMHELIHVLHGLYGYITEYKIPNVVQSYMKVTKPITSAEFLTFGGRDRNIVPQSI	280
rBoNT-FA(0)-his	QSQLYNKVLSYKRIASRLNKVNTATALINIDEFKNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFT	350
mrBoNT-FA(0)-his	QSQLYNKVLSYKRIASRLNKVNTATALINIDEFKNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFT	350
mrBoNT-FA-his	QSQLYNKVLSYKRIASRLNKVNTATALINIDEFKNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFT	350
rBoNT-FA(0)-his	EFNLAYEFKIKTRLGYLAENFGPFYLPNLLDDSIYTEVDGFNIGALSINYQGQNGSDINSIKKLQGGV	420
mrBoNT-FA(0)-his	EFNLAYEFKIKTRLGYLAENFGPFYLPNLLDDSIYTEVDGFNIGALSINYQGQNGSDINSIKKLQGGV	420
mrBoNT-FA-his	EFNLAYEFKIKTRLGYLAENFGPFYLPNLLDDSIYTEVDGFNIGALSINYQGQNGSDINSIKKLQGGV	420
rBoNT-FA(0)-his	VSRVVRLCNSNSN-TKN--S---LCITVNNRDLFFIASQESYAGENTINTYKEIDDTTTLDPSPFEDILDKVI	484
mrBoNT-FA(0)-his	VSRVVRLCNSVI PRKGTKAPPRLCITVNNRDLFFIASQESYAGENTINTYKEIDDTTTLDPSPFEDILDKVI	490
mrBoNT-FA-his	VSRVVRLCNSVI PRKGTKAPPRLCITVNNRDLFFIASQESYAGENTINTYKEIDDTTTLDPSPFEDILDKVI	490
rBoNT-FA(0)-his	LNFEQVIVQMPNRRVSTDIQKDNYPKYDYNRTDIIDSYEVRNYNTFFYLNAQKFSNPESNITLTSSF	554
mrBoNT-FA(0)-his	LNFEQVIVQMPNRRVSTDIQKDNYPKYDYNRTDIIDSYEVRNYNTFFYLNAQKFSNPESNITLTSSF	560
mrBoNT-FA-his	LNFEQVIVQMPNRRVSTDIQKDNYPKYDYNRTDIIDSYEVRNYNTFFYLNAQKFSNPESNITLTSSF	560

Supplemental Figure 1

rBoNT-FA(0)-his	DTGLEGSKVYTFSSDFINNINKPVQALLFIEWVKQVIRDFTTEATKTSTVDKDKDISLVVPI IGLALN	624
mrBoNT-FA(0)-his	DTGLEGSKVYTFSSDFINNINKPVQALLFIEWVKQVIRDFTTEATKTSTVDKDKDISLVVPI IGLALN	630
mrBoNT-FA-his	DTGLEGSKVYTFSSDFINNINKPVQALLFIEWVKQVIRDFTTEATKTSTVDKDKDISLVVPI IGLALN	630
rBoNT-FA(0)-his	IGDEIYKQHFAEAVELVGAGLLEFSPEFLIPTLLIFTIKGYLTGSIRDKDKI IKTLDNALNVRDQKWKE	694
mrBoNT-FA(0)-his	IGDEIYKQHFAEAVELVGAGLLEFSPEFLIPTLLIFTIKGYLTGSIRDKDKI IKTLDNALNVRDQKWKE	700
mrBoNT-FA-his	IGDEIYKQHFAEAVELVGAGLLEFSPEFLIPTLLIFTIKGYLTGSIRDKDKI IKTLDNALNVRDQKWKE	700
rBoNT-FA(0)-his	LYRWVSKWLTINTQFNKRKEQMYKALKNQATAIKKI IENKYNNTTDEKSKIDSSYNINEIERTLNEK	764
mrBoNT-FA(0)-his	LYRWVSKWLTINTQFNKRKEQMYKALKNQATAIKKI IENKYNNTTDEKSKIDSSYNINEIERTLNEK	770
mrBoNT-FA-his	LYRWVSKWLTINTQFNKRKEQMYKALKNQATAIKKI IENKYNNTTDEKSKIDSSYNINEIERTLNEK	770
rBoNT-FA(0)-his	INLAMKNI EQFITESS IAYLINI INNETIQKLSYDDLVRRYLLGYIRNHSSILGNSVEELNSKVNHL D	834
mrBoNT-FA(0)-his	INLAMKNI EQFITESS IAYLINI INNETIQKLSYDDLVRRYLLGYIRNHSSILGNSVEELNSKVNHL D	840
mrBoNT-FA-his	INLAMKNI EQFITESS IAYLINI INNETIQKLSYDDLVRRYLLGYIRNHSSILGNSVEELNSKVNHL D	840
rBoNT-FA(0)-his	NGIPFELSSYTND SLLIRYFNKNY GELKYNCLINIKYEMDRDKLVDSSGYRSRINIGTGKVFSEIDKNQV	904
mrBoNT-FA(0)-his	NGIPFELSSYTND SLLIRYFNKNY GELKYNCLINIKYEMDRDKLVDSSGYRSRINIGTGKVFSEIDKNQV	910
mrBoNT-FA-his	NGIPFELSSYTND SLLIRYFNKNY GELKYNCLINIKYEMDRDKLVDSSGYRSRINIGTGKVFSEIDKNQV	910
rBoNT-FA(0)-his	QLSNLESSKIEVILNNGVIYNSMYENFSTFWIRIPKYFRNINNEYKI ISCMQNNSGWEVSLNFSNMNSK	974
mrBoNT-FA(0)-his	QLSNLESSKIEVILNNGVIYNSMYENFSTFWIRIPKYFRNINNEYKI ISCMQNNSGWEVSLNFSNMNSK	980
mrBoNT-FA-his	QLSNLESSKIEVILNNGVIYNSMYENFSTFWIRIPKYFRNINNEYKI ISCMQNNSGWEVSLNFSNMNSK	980
rBoNT-FA(0)-his	IIWTLQDTEGIKKT VV FQYTQ NINISDY INRWIFVTITNNRLS NSKIY INGRLINEESISDLGN I HASNN	1044
mrBoNT-FA(0)-his	IIWTLQDTEGIKKT VV FQYTQ NINISDY INRWIFVTITNNRLS NSKIY INGRLINEESISDLGN I HASNN	1050
mrBoNT-FA-his	IIWTLQDTEGIKKT VV FQYTQ NINISDY INRWIFVTITNNRLS NSKIY INGRLINEESISDLGN I HASNN	1050
rBoNT-FA(0)-his	IMFKLDGCRDPHRYIWI KYFNLF DKELNKKEIKDLYDNQSN SGI LKDFWGDYLYQDKPYYMLNLYDPNKY	1114
mrBoNT-FA(0)-his	IMFKLDGCRDPHRYIWI KYFNLF DKELNKKEIKDLYDNQSN SGI LKDFWGDYLYQDKPYYMLNLYDPNKY	1120
mrBoNT-FA-his	IMFKLDGCRDPHRYIWI KYFNLF DKELNKKEIKDLYDNQSN SGI LKDFWGDYLYQDKPYYMLNLYDPNKY	1120

Supplemental Figure 1

rBoNT-FA(0)-his	LDVNNVGIRGYMYLKGPRGRIVTTNI YLNSTLYMGTKFI IKKYASGNKDNIVRNNDRVYINVVVKNKEYR	1184
mrBoNT-FA(0)-his	LDVNNVGIRGYMYLKGPRGRIVTTNI YLNSTLYMGTKFI IKKYASGNKDNIVRNNDRVYINVVVKNKEYR	1190
mrBoNT-FA-his	LDVNNVGIRGYMYLKGPRGRIVTTNI YLNSTLYMGTKFI IKKYASGNKDNIVRNNDRVYINVVVKNKEYR	1190
rBoNT-FA(0)-his	LATNASQAGVEKILSAVEIPDVGNLSQVVVMKSENDQGIRNKCKMNLQDNNNGNDIGFIGFHQFN NIAKLV	1254
mrBoNT-FA(0)-his	LATNASQAGVEKILSAVEIPDVGNLSQVVVMKSENDQGIRNKCKMNLQDNNNGNDIGFIGFHQFN NIAKLV	1260
mrBoNT-FA-his	LATNASQAGVEKILSAVEIPDVGNLSQVVVMKSENDQGIRNKCKMNLQDNNNGNDIGFIGFHQFN NIAKLV	1260
rBoNT-FA(0)-his	ASNWYNRQIGKASRTFGCSWEFIPVDDGWGESSL HHHHHHHHHH	1298
mrBoNT-FA(0)-his	ASNWYNRQIGKASRTFGCSWEFIPVDDGWGESSL HHHHHHHHHH	1304
mrBoNT-FA-his	ASNWYNRQIGKASRTFGCSWEFIPVDDGWGESSL HHHHHHHHHH	1304

Figure S1. Multiple sequence alignment of rBoNT/FA(0)-his, mrBoNT/FA(0)-his, and mrBoNT/FA-his. Positions of the E227Q and H230Y catalytic site inactivating mutations, the S429 to L437 activation loop substitution, and C-terminal ten-histidine affinity tag, are boxed in grey.

MPVIVNSFNYYDDPVDNDNTIIYIRPPYYETSNTYFKAQIMDNVWIIIPERYRLGIDPSLFNPPVSLKAGSDGYFDPNYLSTNTEKNKYLQIMIKLFRINSKPAGQIILEEIKNAIPYLGNSYTQEEQFTTNNRTVSFNVKLANGNIVQQMANLI IWGPGDPLTNTKGGI IYSPYQSMEATPYKDFGFSIMTVEFSPEYATAFNDISIASHSPSLFKDPALILMHELIVLHGLYGYITTEYKITPNVVQSYMVKTPITSAEFLTFGGRDRNIVPQSIQSQLYNKVLSYKRIASRLNKVNTATALINI DEFKNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFTEFNLAYEFKIKTRLGYLEAENFGPFYLPNLLDDSIYTEVDGFGNIGALSINYQGQNIGSDINSIKKLQGGQGVVSRVRLC₄₂₈KSVIPRKGTKAPPRLC₄₄₄ITVNNRDLFFIASQESYGEINTYKEIDDTTTLDPSEFEDILDKVILNFNQVVPQMPNRRVSTDIQKDNYPKYDYNRTDIIDSYEVGRNYNTFFYLNAQKFSPNESNITLTSFDTGLEGSKVYTFSSDFINNINKPVQALLFIEWVKQVIRDFTTEATKTSTVDKLDKDISLVVPIYGLALNIGDEIYKQHFVAEVLVAGLLELFSPEFLIPTLLIFTIKGYLTGSIRDKDKIKITLDNALNVRDQKWKELYRWVSKWLTINTQFNKRKEQMYKALKNQATAIKKI IENKYNNTTDEKSKIDSSYNINEIERTLNEKINLAMKNIEQFITESSIAYLINI INNETIQKLKSYDDLVRRYLLGYIRNHSSILGNSVEELNSKVNHLNNGI PFELSSYTNDLIRYFNKNYGEIKYNCILNIKYEMDRDKLVDSSGYRSRINIGTVKFSEIDKNQVQLSNLESSKIEVILNNGVIYNSMYENFSTSFWRIRIPKYFRNINNEYKILSCMQNNSGWEVSLNFSNMNSKI IWTLQDTEGIKKTVVFQYQYNINISDYINRWIFVTTNNRLSNSKIYINGRLINEESISDLGNIHASNNIMFKLDGCRDPHRYIWKYFNLFDKELNKKELI KDLYDNQSNSGILKDFWGDYLYQYDKPYYMLNLYDPNKYLDVNNVGI RGYMYLKGPRGRIVTNTNIYLNSTLYMGTKFI IKKYASGNKDNI VRNDRVYINVVVKNEYRLATNASQAGVEKILSAVEIPDVGNLSQVVVMKSENDQGIRNKCKMNLQDNNGNDIGFIFGHQFNNAIKLVASNWNRYQIGKASRTFGCSWEFIPVDDGWGESSLHHHHHHHHHH

Light chain, LC/FA
BoNT/F1 activation loop
Translocation domain, H_N/FA
Unknown function, H_{CN}/FA
Receptor binding domain, H_{CC}/FA
 Affinity tag (His₁₀)

Figure S2. Primary amino acid sequence of mrBoNT/FA-his. Light chain, LC/FA, amino acids shown in black. Substituted BoNT/F1 activation loop amino acids shown underlined. *Translocation domain, H_N/FA*, amino acids shown in grey italic. *Unknown function, H_{CN}/FA*, amino acids shown in black italic. **Receptor binding domain, H_{CC}/FA**, amino acids shown in bold black italic. Affinity tag (His₁₀), amino acids shown in grey.

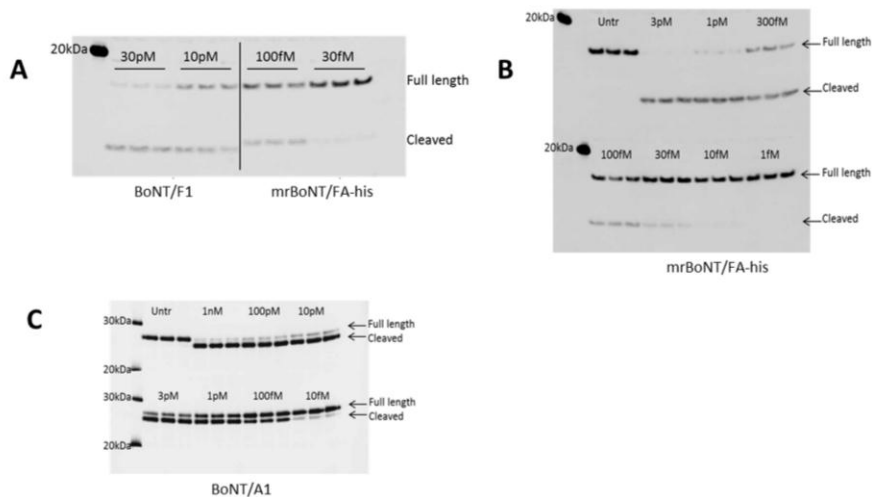


Figure S3. SNARE cleavage in rat cortical neurons panel (A) shows Western blot analysis of full-length and cleaved VAMP-2 from rat cortical neurons treated with nBoNT/F1 or mrBoNT/FA-his for 24 hours. Panel (B) shows Western blot analysis of full-length and cleaved VAMP-2 from rat cortical neurons treated with a concentration range of mrBoNT/FA-his for 24 hours. Panel (C) shows Western blot analysis of full-length and cleaved SNAP-25 from rat cortical neurons treated with a concentration range of nBoNT/A1 for 24 hours.