



Supplementary Note 1

Sequence alignment of SV2s in the loop 4 region.

Representative sequences of SV2A, SV2B, and SV2C were selected from different species: human SV2A (NP_055664.3), 2B (CAG33367.1), 2C (AAI00828.1); rat SV2A (NP_476558.2), 2B (NP_476555.1), 2C (NP_113781.1); dog SV2A (XP_003639668.1), 2B (XP_005618386.1), 2C (XP_546060.2); bovine SV2A (NP_776387.1), 2B (NP_001076917.1), 2C (NP_001178948.1); chicken SV2B (XP_425081.2), 2C (XP_429151.3). Sequence alignments were made using Clustal Omega (Sievers, F. et al., *Mol Syst Biol* **7**, 539, 2011) and ESPrict (Gouet, P. et al., *Bioinformatics* **15**, 305–8, 1999). Only the truncated loop 4 of SV2 is shown for clarity. Identical residues are indicated with white letters on a red background, similar conserved residues are in red letters, varied residues are in black letters. The residues of SV2C that directly bind to H_cA are indicated by blue ovals; the pentapeptide-repeat is labeled by green triangles. The N-linked glycosylation site, N559, is highly conserved, but not F563 (blue arrows).

880 890 900 910 920

BoNT/A1 NIINTSILNLRYES.NHLIDLSRYASKINIGSRVNFDPIDKNOIQOLFPLESSKIEVILK
 BoNT/A2 NIVNTSILSIVYKK.DDLIDLSRYGAKINIGDRVYYSIDKNOIKLPLESSKIEVILK
 BoNT/A3 NIVNTSILSIVYKK.DDLIDLSRYGAKINIGDRVYYSIDKNOIKLPLESSKIEVILK
 BoNT/A4 NITNASILSIVYKD.DDLIDLSRYGAEIYNGDKVYNSIDKNOIKLPLESSKIEVILK
 BoNT/A5 NIINTSILNLRYES.NHLIDLSRYASEINIGSRVNFDPIDKNOIQOLFPLESSKIEVILK
 BoNT/A6 NIINTSILSLRYEN.NHLIDLSRYASKINIGSRVNFDPIDKNOIQOLFPLESSKIEVILK
 BoNT/A7 NIINTSILNLRYES.NHLIDLSRYASKINIGSRVNFDPIDKNOIQOLFPLESSKIEVILK
 BoNT/A8 NITNTSILSIVVDK.DGRIDLSRYGAEIYNGDKVSYNSIDKNOIKLPLESSKIEVILK
 BoNT/HA ELKYNCILNLIKYEEMDRDKLVDSSGYRSRINIGTGVKFSIDKNOIQOLFPLESSKIEVILK

930 940 950 960 970 980

BoNT/A1 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A2 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A3 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A4 KAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A5 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A6 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A7 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/A8 NAIVNSMYENFSTSFWRIPKYFNSISLNNEYTINCIMENNSGWKVSINYG...EIIW
 BoNT/HA NGVIYNSMYENFSTSFWRIPKYFRN...INNEYKITSMONGNSGWEVSLNFSNMNSKIIW

990 1000 1010 1020 1030 1040

BoNT/A1 TLDQTQEIQRVVFKYSQMINISDYINRWIEVITINNRLNNSKIYINGRLIDQKPISNLG
 BoNT/A2 TLDQNKQNIQRVVFKYSQMVNISDYINRWIEVITINNRLTKSKYIINGRLIDQKPISNLG
 BoNT/A3 TLDQNKQNIQRVVFKYSQMVNISDYINRWIEVITINNRLTKSKYIINGRLIDQKPISNLG
 BoNT/A4 TLDQTQEIQRVVFKYSQMINISDYINRWIEVITINNRLTKSKYIINGRLIDQKPISNLG
 BoNT/A5 TLDQNKQNIQRVVFKYSQMVNISDYINRWIEVITINNRLNNSKIYINGRLIDQKPISNLG
 BoNT/A6 TLDQNKQNIQRVVFKYSQMVNISDYINRWIEVITINNRLTKSKYIINGRLIDQKPISNLG
 BoNT/A7 TLDQNKQNIQRVVFKYSQMVNISDYINRWIEVITINNRLTKSKYIINGRLIDQKPISNLG
 BoNT/A8 TLDQNKQNIQRVVFKYSQMVNISDYINRWIEVITINNRLTKSKYIINGRLIDQKPISNLG
 BoNT/HA TLDTEGIKKTVVVQYTCNINISDYINRWIEVITINNRLNNSKIYINGRLINEESISDLG

1050 1060 1070 1080 1090 1100

BoNT/A1 NIHASNNIMFKLDGCRDTHRYIWKIYFNLFDKELNEKEIKDLYDNOSNSGILKDFWGDYL
 BoNT/A2 NIHASNKIMFKLDGCRDPRRYIMIKYFNLFDKELNEKEIKDLYDSOSNSGILKDFWGNYL
 BoNT/A3 NIHASNKIMFKLDGCRDPRRYIMIKYFNLFDKELNEKEIKDLYDSOSNSGILKDFWGNYL
 BoNT/A4 NIHASNKIMFKLDGCRDPRRYIVIKYFNLFDKELSEKEIKDLYDNOSNSGILKDFWGDYL
 BoNT/A5 NIHASNNIMFKLDGCRDTHRYIWKIYFNLFDKELNEKEIKDLYDNOSNSGILKDFWGNYL
 BoNT/A6 NIHASNKIMFKLDGCRDPRRYIMIKYFNLFDKELNEKEIKDLYDSOSNSGILKDFWGNYL
 BoNT/A7 NIHASNKIMFKLDGCRDPRRYILIKYFNLFDKELNEKEIKDLYDNOSNSGILKDFWGDYL
 BoNT/A8 NIHASNNIMFKLDGCRDPRRYIVIKYFNLFDKELNEKEIKDLYDNOSNSGILKDFWGDYL
 BoNT/HA NIHASNNIMFKLDGCRDPRRYIWKIYFNLFDKELNKEIKDLYDNOSNSGILKDFWGDYL

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1110 1120 1130 1140 1150 1160

BoNT/A1 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGSVMTTNIYLNLSLYRGTKFFIHKY
 BoNT/A2 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGSVMTTNIYLNLSLYEGTKFFIHKY
 BoNT/A3 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGSVMTTNIYLNLSLYMGTKFFIHKY
 BoNT/A4 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRDNVMTTNIYLNLSLYMGTKFFIHKY
 BoNT/A5 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGSIVTTNIYLNLSLYMGTKFFIHKY
 BoNT/A6 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRSTLTTNIYLNLSLYMGTKFFIHKY
 BoNT/A7 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGSVTTNIYLNLSLYMGTKFFIHKH
 BoNT/A8 QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGSVMTTNIYLNLSLYMGTKFFIHKY
 BoNT/HA QYDKFYMLNLYDPNKYVDVNNVGIIRGYMYLKGPRGRIVTTNIYLNLSLYMGTKFFIHKY

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1170 1180 1190 1200 1210 1220

BoNT/A1 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A2 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A3 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A4 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A5 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A6 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A7 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/A8 ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS
 BoNT/HA ASGNKDNIVRNNDRVYINVVVKNKEYRLATNASOAGVEKILSALEIPDVGNLSQVVMKS

1230 1240 1250 1260 1270 1280

BoNT/A1 KNDQGITNKCKMNLQDNNNGNDIGFIFGHQFNNTAKLVASNWNRYQIERSSRTLGCSEWFI
 BoNT/A2 KDDQGITRNKCKMNLQDNNNGNDIGFIFGHLYDNIAKLVASNWNRYQVGRASRTFGCSWEFI
 BoNT/A3 KDDQGITRNKCKMNLQDNNNGNDIGFVGFHLYDNIAKLVASNWNRYQVGRASRTFGCSWEFI
 BoNT/A4 KNDQGITNKCKMNLQDNNNGNDIGFIFGHQFNNTAKLVASNWNRYQIERSSRTLGCSEWFI
 BoNT/A5 KNDQGITRNKCKMNLQDNNNGNDIGFIFGHQFNNTDKLVASNWNRYQIERSSRTFGCSWEFI
 BoNT/A6 KNDQGITRNKCKMNLQDNNNGNDIGFIFGHKFNIDYKLVASNWNRYQIEISRTFGCSWEFI
 BoNT/A7 KNDQGITRNKCKMNLQDNNNGNDIGFIFGHQFNNTAKLVASNWNRYQIGKTSVTFGCSWELI
 BoNT/A8 KNDQGITRNKCKMNLQDNNNGNDIGLIFGHQFNNTAKLVASNWNRYQVGRASRTFGCSWEFI
 BoNT/HA ENDQGITRNKCKMNLQDNNNGNDIGFIFGHQFNNTAKLVASNWNRYQIGKASRTFGCSWEFI

1290

BoNT/A1 PVDDCWGERPFL
 BoNT/A2 PVDDCWGESLFL
 BoNT/A3 PVDDCWGESLFL
 BoNT/A4 PVDDCWGERPFL
 BoNT/A5 PVDDCWGESFLL
 BoNT/A6 PVDDCWGESFLL
 BoNT/A7 PVDCWGESLFL
 BoNT/A8 PVDDCWGESLFL
 BoNT/HA PVDDCWGESLFL

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Supplementary Note 2

Sequence alignment among eight BoNT/A subtypes and BoNT/HA.

The amino acid sequence of BoNT/A1-A8 and HA are taken from GenBank: AAQ06331.1 (A1), ACO83782.1 (A2), ABA29017.1 (A3), ACQ51417.1 (A4), ACG50065.1 (A5), ACW83608.1 (A6), AFV13854.1 (A7), AJA05787.1 (A8), and KGO15617.1 (HA). Key H_CA residues that are recognized by antibody CR1 (PDB code: 2NYY) (Garcia-Rodriguez, C. et al., *Nat Biotechnol* **25**, 107–16, 2007) are indicated by black stars. H_CA residues that directly interact with SV2C peptide or the N559-glycan are labeled by blue triangles or yellow ovals, respectively.