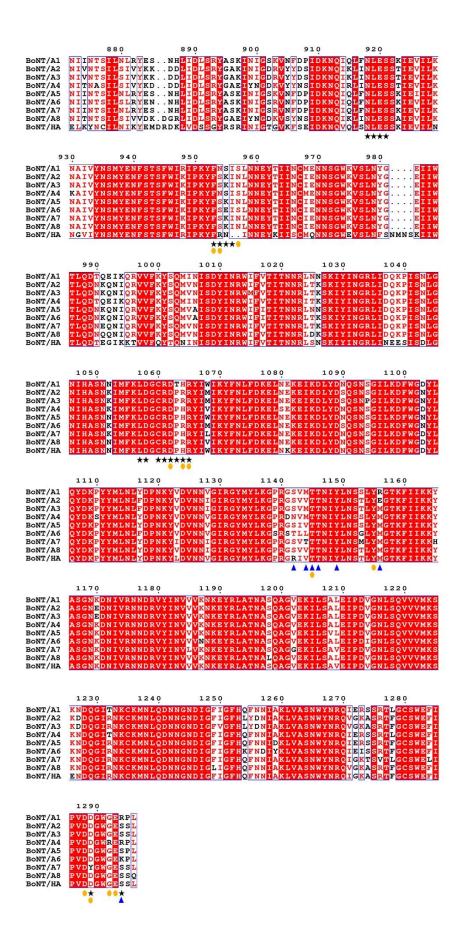


## **Supplementary Note 1**

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 ESPript (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<sub>C</sub>A 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).



## **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<sub>C</sub>A 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<sub>C</sub>A residues that directly interact with SV2C peptide or the N559-glycan are labeled by blue triangles or yellow ovals, respectively.