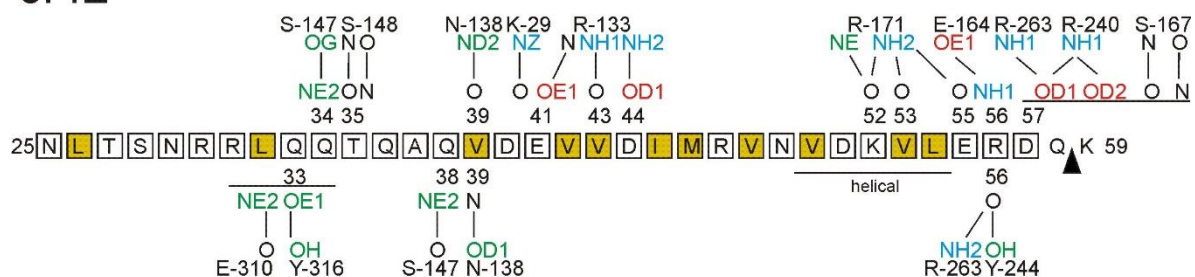
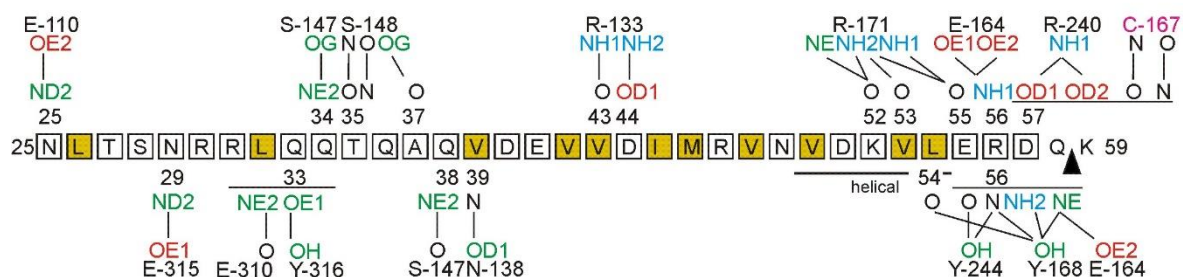


**Figure S1.** Cleavage of VAMP-1 by LC/F1, LC/F6, LC/F7, and LC/F9. Rat VAMP-1 (1-118) was generated by *in vitro* transcription/translation and incubated with various LCF subtypes applied at 3, 30, and 300 nM final concentrations for 1 hour at 37 °C in toxin assay buffer and subsequently subjected to SDS-PAGE. [<sup>35</sup>S-Met]-labeled VAMP-1 and its cleavage fragments were visualized by phosphorimaging. The position of intact VAMP-1 is indicated by a filled arrowhead. The position of the C-terminal cleavage fragments is indicated by an open arrowhead. The N-terminal cleavage fragment does not contain methionine and is therefore not detectable. The asterisk marks the position of hemoglobin non-specifically associated with [<sup>35</sup>S-Met].

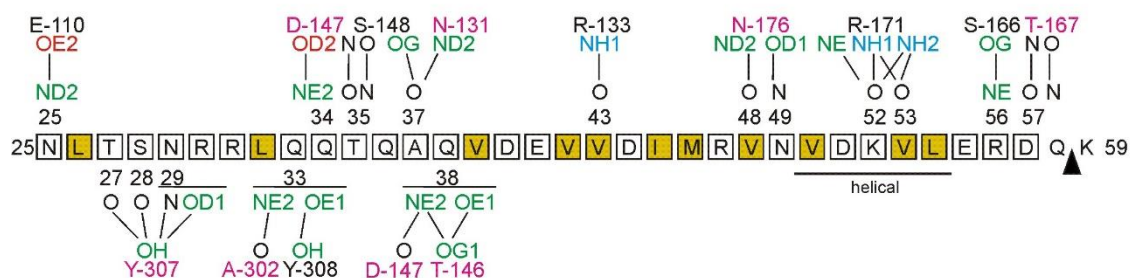
### 3FIE



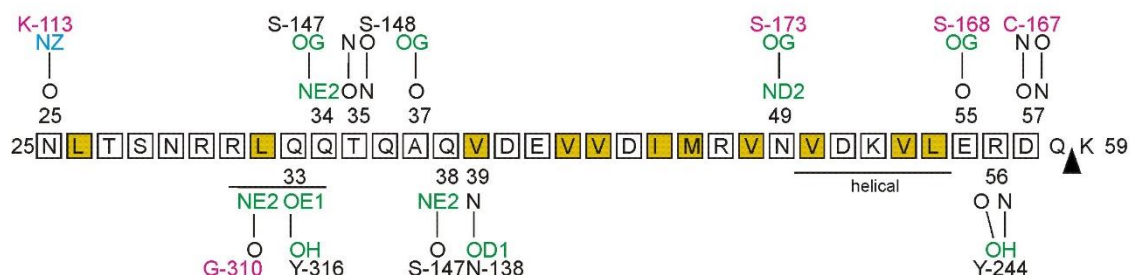
### F6



### F7



### F9



**Figure S2.** Proposed H-bond interactions of LC/F6, LC/F7, and LC/F9 with VAMP-2 in comparison to LC/F1. Structures for LC/F6, LC/F7, and LC/F9 were predicted applying the SWISS MODEL software [43] and then superimposed on the structure of the LC/F1/VAMP-2 inhibitor peptide 1 complex (pdb: 3FIE) [35]) based on sequence alignments using the DS visualizer 2.5 software. Intermolecular H-bonds to VAMP-2 were calculated by using DS visualizer 2.5. VAMP-2 inhibitor peptide amino acids are boxed and shown in single letter code. Residues carrying hydrophobic side chains are highlighted yellow. Positions and atoms or functional groups are specified for those residues of VAMP-2 that form interactions with LC/F. LC/F amino acids proposed to H-bond to VAMP-2 are indicated

including their participating atoms or functional groups. They are highlighted magenta for LC/F6, LC/F7, and LC/F9, if they differ from their counterpart in LC/F1. Uncharged side chain groups are presented in green, negatively charged groups in red, and positively charged groups in blue.

**Table S1.** Nucleotide and amino acid identity comparisons of BoNT/F LC subtypes.

| Light chain |             | subtype     |             |             |             |             |             |             |             |             |             |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| subtype     | strain      | F1          | F2          | F3          | F4          | F5          | F6          | F7          | F8          | F9          | H           |
| F1          | Langeland   |             | 82.0        | 82.9        | 96.4        | 47.0        | 94.1        | 63.3        | 97.7        | <b>82.5</b> | 49.1        |
| F2          | CDC3281     | <i>91.3</i> |             | 97.5        | 82.9        | 45.9        | 81.5        | 59.7        | 81.5        | <b>85.4</b> | 46.8        |
| F3          | VPI4257     | <i>91.8</i> | <i>98.9</i> |             | 83.8        | 45.9        | 82.7        | 60.8        | 82.5        | <b>86.6</b> | 47.0        |
| F4          | CDC54089    | <i>98.3</i> | <i>91.9</i> | <i>92.3</i> |             | 47.3        | 93.4        | 64.0        | 96.4        | <b>82.2</b> | 49.3        |
| F5          | CDC54075    | <i>64.8</i> | <i>63.6</i> | <i>63.7</i> | <i>64.7</i> |             | 47.3        | 46.5        | 46.4        | <b>46.4</b> | 79.7        |
| F6          | 202F        | <i>97.6</i> | <i>91.3</i> | <i>91.9</i> | <i>97.4</i> | <i>64.8</i> |             | 63.3        | 94.1        | <b>80.6</b> | 48.0        |
| F7          | CNM1212/11  | <i>75.6</i> | <i>73.1</i> | <i>73.5</i> | <i>75.9</i> | <i>62.5</i> | <i>76.1</i> |             | 62.9        | <b>60.4</b> | 48.5        |
| F8          | 357         | <i>99.0</i> | <i>91.3</i> | <i>91.6</i> | <i>98.3</i> | <i>64.5</i> | <i>97.6</i> | <i>75.4</i> |             | <b>82.0</b> | 49.3        |
| F9          | H078-01     | <i>91.4</i> | <i>93.4</i> | <i>93.9</i> | <i>91.6</i> | <i>64.2</i> | <i>91.2</i> | <i>73.9</i> | <i>91.5</i> |             | <b>47.5</b> |
| H(F5/A1)    | CFSAN024410 | <i>63.8</i> | <i>63.2</i> | <i>63.0</i> | <i>63.9</i> | <i>86.2</i> | <i>63.5</i> | <i>61.7</i> | <i>63.7</i> | <b>63.2</b> |             |

Shown are the percentages of nucleic acid (*italic*, lower left gray triangle) and amino acid (upper right triangle) identities among LC/F subtypes. GenBank accession numbers used are: F1: ABS41202 and GU213203, F2: CAA73972 and Y13631, F3: ADA79575 and GU213227, F4: GU213221, F5: GU213212, F6: AAA23263 and CP006903, F7: KX671958, F8: AUZC01000000, and H (FA mosaic): KGOO15617 and JSCF01000000.