## A HCD-MS/MS of m/z 752.89, +2 $L_{137}\,L\,T\,G\,T\,F\,S_{143}\,A\,G\,K_{146}$ 100 475.1924 [●-① -2 H<sub>2</sub>O]<sup>+</sup> 90 257.1135 ( -H<sub>2</sub>O 80 493 2035 70 Abundance 05 09 05 ●-() -H<sub>2</sub>O) 275.1239 40 30 $b_2$ 20 $y_5$ 10 200 250 300 350 500 550 150 400 В H<sub>2</sub>O, NH<sub>2</sub>-CH=CH H<sub>2</sub>O H<sub>2</sub>O 100 90 HO 80 H<sub>2</sub>N 60 50 257.1135 40 Chemical Formula: C<sub>11</sub>H<sub>19</sub>N<sub>2</sub>O<sub>6</sub> Exact Mass: 275.12376 30 $b_2$ 180.0654 20 239.1025 10 100 160 180 200 220 240 260 280

**Supplemental Figure 4: HCD-MS/MS spectrum from L**<sub>137</sub>**LTGTFS@AGK**<sub>146</sub> **plus a 510 Da modification** (@represents the site of modification). The HCD-MS/MS spectrum showed the accurate mass of glycan-related ions corresponding to dehydrated ions of the 510 Da modification, and an oxonium ion from the 274 Da moiety of the 510 Da modification (A). Figure 3 B represents the zoomed HCD spectrum between m/z 100 to 300. It should be noted that the signals observed from the peptide with 510 Da were very low compared to those with 524 Da or 538 Da modifications, due to low abundance of the 510-Da modification occuring in the flagellin. However, we detected main fragment ions from 274-Da modification. An inset in the spectrum B shows a proposed structure of the 274-Da moiety (observed m/z of 274 Da moiety as an oxonium ion is m/z 275). The pentagon cartoon and black-colored circle cartoons in the figure represent the 274 Da- and 236 modifications, respectively.