**Supplemental Table 1** Testing of GI modification across different regions of GI. The amino acids encoded by the amplified region or the CT5 amino acid mutations are listed in the first column. The forward and reverse primers used to make the GI fragments by PCR amplification are listed. The results of testing whether SEC modified the fragments (shown in Figure 1) are summarized in the last column.

|  |  |  |
| --- | --- | --- |
| Region expressed/mutated | Primers  | O-GlcNac modified by SEC |
| 1-200 | Lynn/GiBam Atg32aCggatccatggctagttcatcttcatctgaGi600FcagcggccgctaAGCAAGTAGTATATCACTGAT | No |
| 188-400 | Gi562FcggatccAGGCCTTTGTCTCCATGGATCGi1200RcagcggccgctaCGGCTGTGAGAGTATGCGGAA | No |
| 388-600 | Gi1162FCggatccGCTGCAGCCGCTTTGCTTTTCGi1800RcagcggccgctaTTCATGGCTAACACATACAGT | No |
| 589-698 | Lynn/Gi1765FcggatccCTTTTTGTTGTGTTGACTGLynn/Gi2093RcagcggccgctaTCCATACTCTTTAGATGACCC | No |
| 685-828 | Lynn/Gi2053FcggatccACAAAGCCTGTAAAGATAAATGGGGi2464RcagcggccgctaGCTTGCACATGTGTTCTCTTG | No |
| 685-851 | Lynn/Gi2053FcggatccACAAAGCCTGTAAAGATAAATGGGGi2551RcagcggccgctaCTTATGGTTTCCTCTTGGATT | Yes |
| 789-1041 | Gi2365FcggatccAAAGTTGTTGCCTCCATTGTTGACGi3123RcagcggccgctaGGCTTCAAGTAGCTCAACTTG | Yes |
| 822-1041 | Gi2464FcggatccCAAGAGAACACATGTGCAAGCGi3123RcagcggccgctaGGCTTCAAGTAGCTCAACTTG | Yes |
| 840-1041 | Gi2518FcggatccTCAAGGACTGAAATGAATCCLynn/Gi3123RcagcggccgctaGGCTTCAAGTAGCTCAACTTG | No |
| 1034-1173 End | Lynn/Gi3100FcggatccCCTCAACTTGAGCTACTTGALynn/GitermNot32a2gtgcggccgctattgggacaaggatatagt | No |
| 789-893 | Lynn/GI-CT5for (NcoI)accatggcCAAAGTTGTTGCCTCCATTGTTGACALynn/GI-CT5rev (XhoI)TCTCGAGTTAACCACAATAGAACCCTGCGAGTCTAT | Yes |
| T825A | Lynn/GI-mut6fCTGGAAACAAGAGAACgcaTGTGCAAGCACCACLynn/GI-mut6rGTGGTGCTTGCACATGCGTTCTCTTGTTTCCAG | Yes |
| T829A |  Lynn/GI-mut11fAACACATGTGCAAGCgccACATGCTTTGATACAGC Lynn/GI-mut11rGCTGTATCAAAGCATGTGGCGCTTGCACATGTGTT  | No |
| T830A |  Lynn/GI-mut12fACATGTGCAAGCACCgCATGCTTTGATACAGCG Lynn/GI-mut12rCGCTGTATCAAAGCATGCGGTGCTTGCACATGT | Yes |
| T837A |  Lynn/GI-mut13fGATACAGCGGTGgCATCCGCCTCAAGG  Lynn/GI-mut13rCCTTGAGGCGGATGCCACCGCTGTATC  | Yes |
| S838A | Lynn/GI-mut14fCAGCGGTGACAgCCGCCTCAAGGACLynn/GI-mut14rGTCCTTGAGGCGGCTGTCACCGCTG | Yes |
| S840A | Lynn/GI-mut7fGGTGACATCCGCCGCAAGGACTGAAATGAATCCLynn/GI-mut7rGGATTCATTTCAGTCCTTGCGGCGGATGTCACC | Yes |
| T834A | Lynn/GI-mut9fACCACATGCTTTGATgCAGCGGTGACATCCGLynn/GI-mut9rCGGATGTCACCGCTGCATCAAAGCATGTGGT | Yes |
| S828A | Lynn/GI-mut10fACAAGAGAACACATGTGCAGCCACCACATGCTTTGATACAGLynn/GI-mut10rCTGTATCAAAGCATGTGGTGGCTGCACATGTGTTCTCTTGT  | Yes |

789 – KVVASIVDKAEPLEAYLKNTPVQKDSVTCLNWKQENTCAS**T**TCFDTAVTSAS

RTEMNPRGNHKYARHSDEGSGRPSEKGIKDFLLDASDLANFLTADRLAGFYCG - 893

**Supplemental Figure 1** Sequence of GI fragment expressed in CT5. The amino acid numbering is based on GenBank: AAT80910.1. The underlined T corresponds to T829 in intact GI.



**Supplemental Figure 2** O-GlcNAc modified CT5 peptides were enriched by RCA I affinity chromatography. **(a)** MALDI-TOF analysis of trypsinized CT5 before capping with galactose and enrichment. Unmodified and modified QENTCASTTCFDTAVTSASR peptides with single O-GlcNAc (203 Da) were observed at *m/z* 2209 and 2412, respectively. The insert shows a protein blot demonstrating that CT5 co-expressed with SEC in *E. coli* was O-GlcNAc modified. The left two lanes show a portion of the stained gel with molecular weight markers followed by purified CT5 stained with Coomassie Brilliant blue (CBB). The blot (GalT) shows CT5 after labeling of GlcNAc residues with [3H] galactose. **(b)** Peptides produced with trypsin and enriched by RCA I column chromatography were analyzed using MALDI-TOF. CT5 QENTCASTTCFDTAVTSASR peptide (*m/z* 2209) containing single modification +365 Da (LacNAc) was observed at *m/z* 2573. The insert shows that QENTCASTTCFDTAVTSASRTEMNPRGNHK peptides (*m/z* 3371) containing a single modification (+365 Da) was observed at *m/z* 3736 after Lys-C digestion and enrichment.