SCHU S4 PIlA (FLAG-tag)

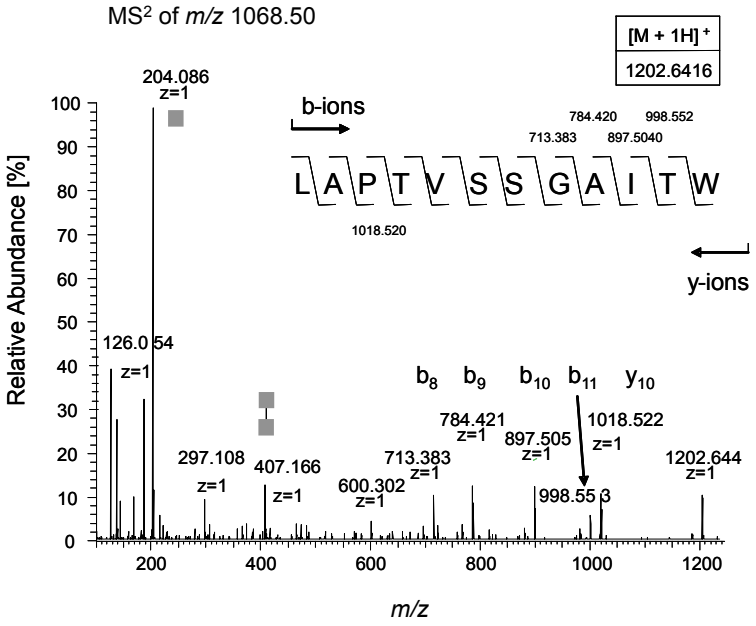
FSLVELMVVIAIIAILAAVAIPMYSNYTTRAQLGSDLSALGGAKATVAE**R**IANNN
GDASQVTILQANAAANGLPSGASVAAGTISYPSTVSGATIQLAPTVSSGAITWTC
NISGVSASQVPSNCNAIDYKDDDDK

Sequence coverage: (AA) 67%

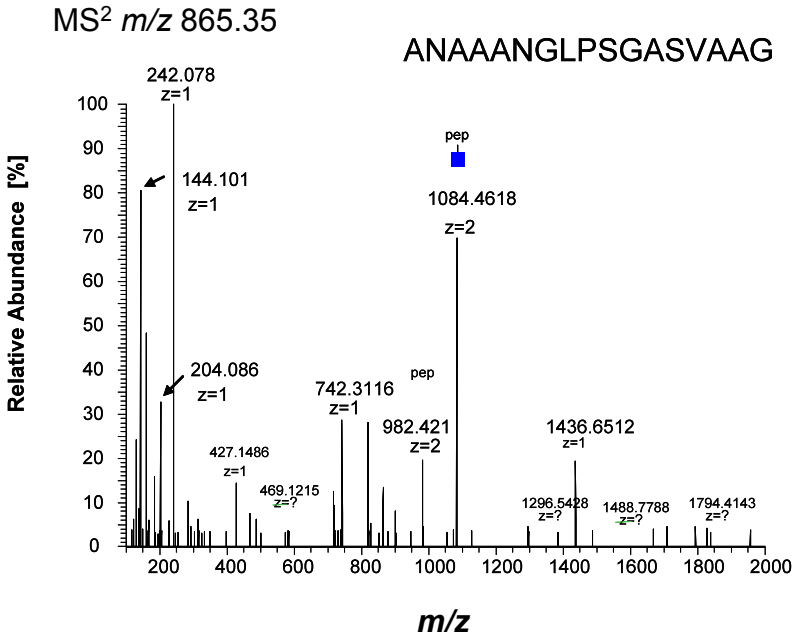
Peptide (detected)	MH^+
GLPSGA*	501.2667
KDDDDK	735.3155
SALGGAKAT	775.4308
VSSGAITW*	820.4199
VAERIANN	858.4679
RAQLGSDL	859.4632
GGAKATVAEK	931.5207
AQLGSDLSAL	974.5153
GSDLSALGGAK	975.5105
TTRAQLGSDL	1061.5585
RAQLGSDLSAL	1130.6164
AIDYKDDDDK	1197.5269
LAPTVSSGAITW*	1202.6415
AQLGSDLSALGGAK	1287.6903
TTRAQLGSDLSAL	1332.7117
ANAAANGLPSGASVAAG*	1398.6972
IANNNGDASQVTIL	1429.7281
AGTISYPSTVSGATIQ	1552.7853
VAEKIANNNGDASQVT	1630.8031
VAEKIANNNGDASQVTIL	1856.9712
GGAKATVAEKIANNNGDASQVTIL	2342.2310
SALGGAKATVAEKIANNNGDASQVTIL	2613.3842

*= glycopeptide

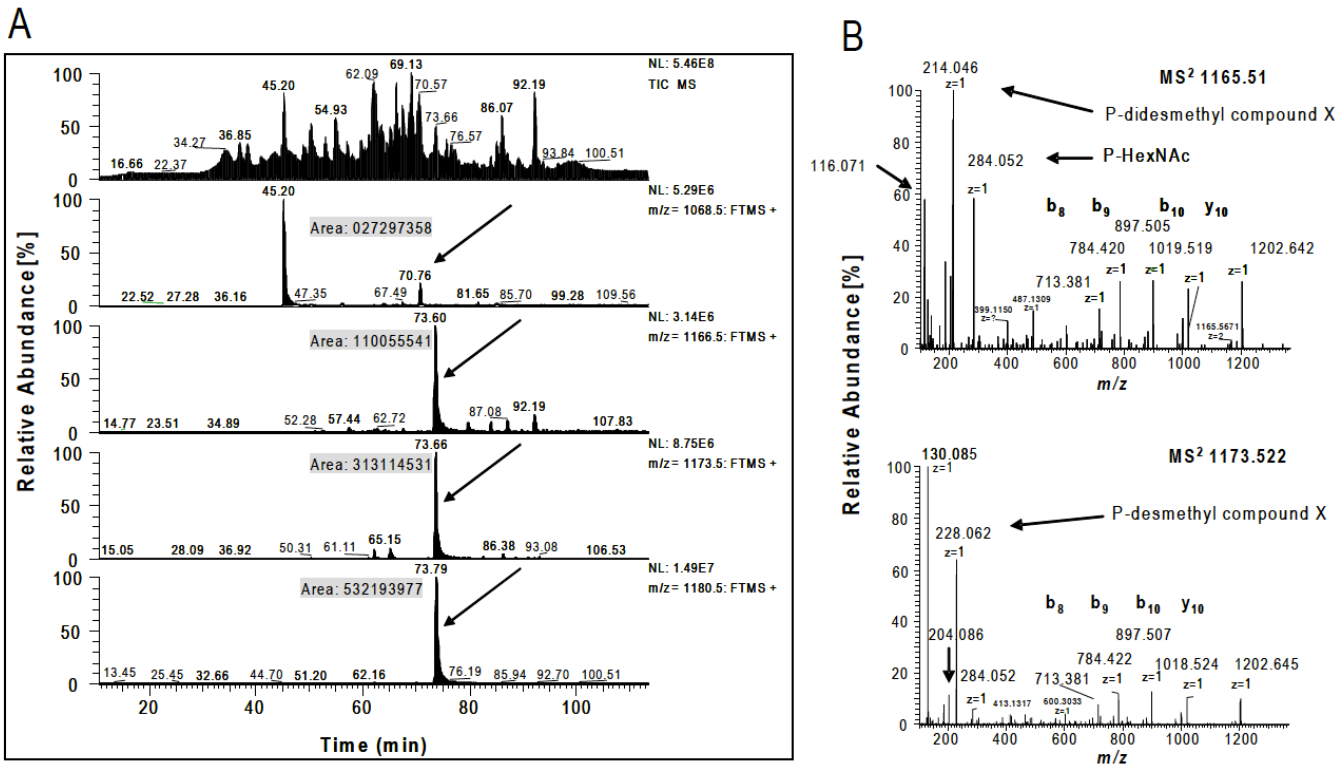
Supplementary Fig. 1. Top: Primary structure of mature (prepilin peptidase processed) PilA from Type A strain SCHU S4 showing coverage of peptides identified by MS (red) and serine attachment sites (underlined). Gray highlighting indicates SGA motifs associated with attachment sites (underlined serines - note that there is a third SGA glycosylation motif between the two noted here). The sole difference between PilA from Type B strain FSC200 and that shown here is the presence of a lysine as opposed to the arginine shown here in bold.



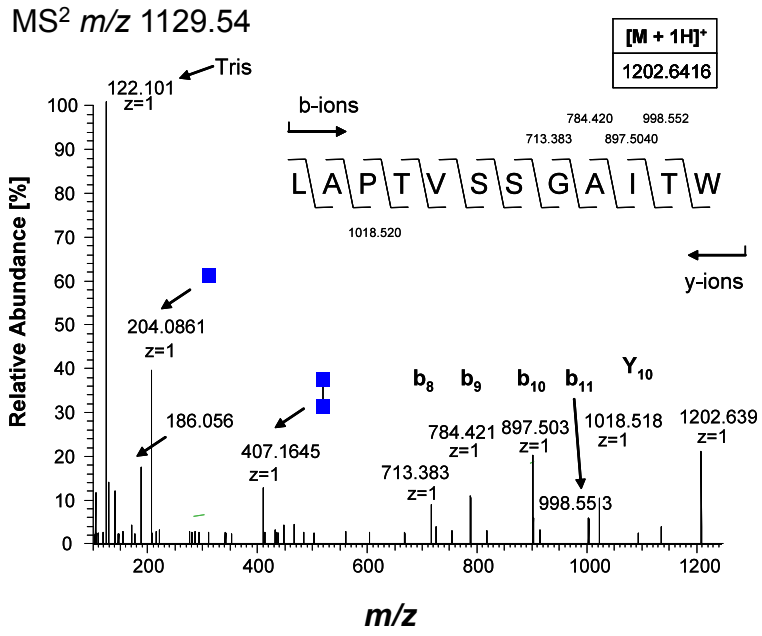
Supplementary Fig. 2. MS^2 spectrum of the doubly charged peptide at *m/z* 1068.50^{2+} eluting at 70.76 minutes confirms that the peptide ^{97}LAPTVSSGAITW^{108} is modified with HexNAc-Hex-Hex-HexNAc-HexNAc. Identity of the peptide is confirmed by the fragment ions b_8 to b_{11} at *m/z* 713.383 to 998.553, respectively, as well as the accurate peptide mass of 1202.642 Da (+2.5 ppm).



Supplementary Fig. 3. Identification the PilA peptide ^{66}ANAAANGLPSGASVAG^{81} purified from *F. tularensis subsp. tularensis* SCHU S4 carrying the pentasaccharide plus the 242.067 Da moiety. Note the presence of the oxonium ions at *m/z* 204.086 and 242.078 corresponding to hexose and compound X respectively. The signal at *m/z* 1084.461 corresponds to the peptide carrying the HexNAc moiety (blue square).



Supplementary Fig 4. MS detection of microheterogeneity related to PilA glycosylation. (A) Top panel represents the total ion chromatogram (*TIC*), and lower panels show the selected ion (*SIC*) chromatogram of fragments yielding ions at *m/z* 204.086, since glycopeptides containing HexNAc gives rise to this diagnostic oxonium ion by HCD-induced fragmentation. (B) MS^2 spectra of glycopeptide with increased retention times (eluting at 73.60 minutes - *m/z* 1166.51^{2+} and 73.66 minutes - *m/z* 1173.52^{2+}) showing that they represent the peptide ^{97}LAPTVSSGAITW^{108} with altered forms of the glycan. Note the common fragment ions as well as unique ions in the lower molecular *m/z* range at *m/z* 242.067 / 144.100, *m/z* 228.062 / 130.085 and *m/z* 214.046 / 116.071 that suggest differences relating to varying degrees of methylation of an otherwise identical compound X.



Supplementary Fig. 5. MS^2 characterization of PilA-derived glycopeptide purified from *F. tularensis* subsp. *holarctica* FSC749 (wildtype). Identity of the peptide is confirmed by the fragment ions b_8 to b_{11} at *m/z* 713.383 to 998.553, respectively, as well as the accurate peptide mass of 1202.642 Da (+2.5 ppm).

The presence of signals related to HexNAc are noted with blue squares.