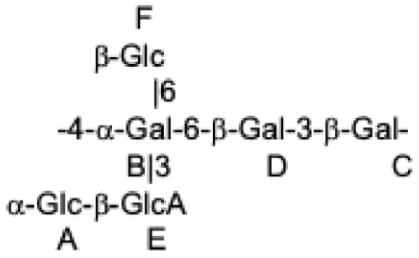
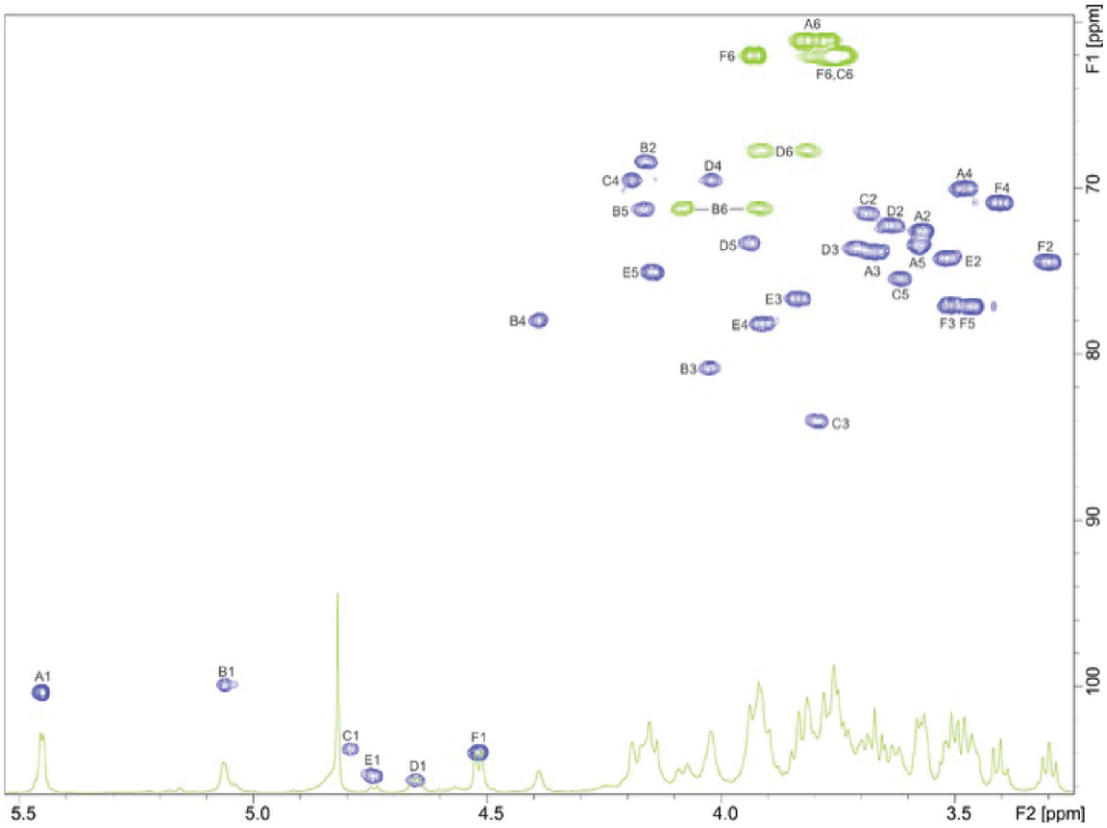
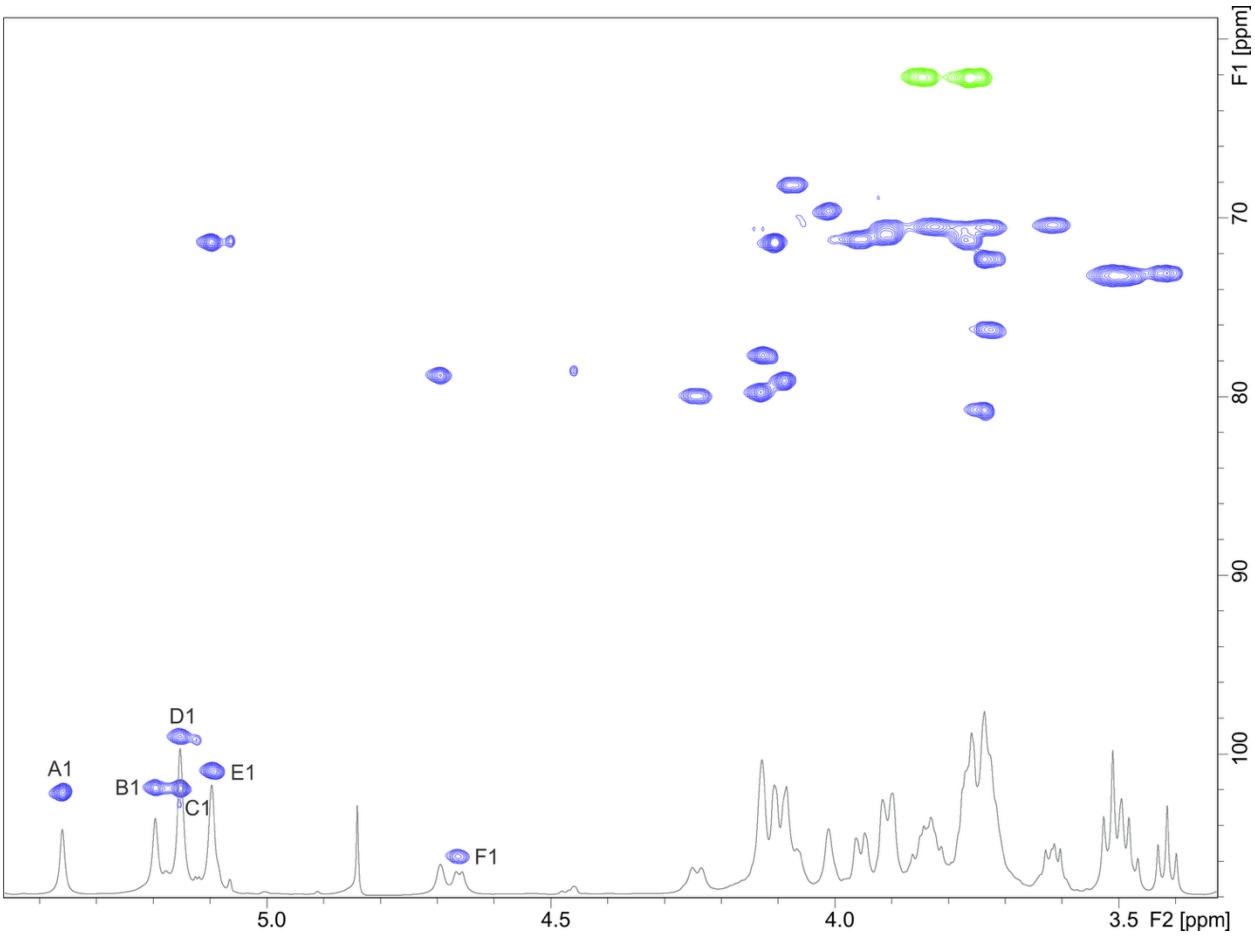
Supplementary Figures and Tables



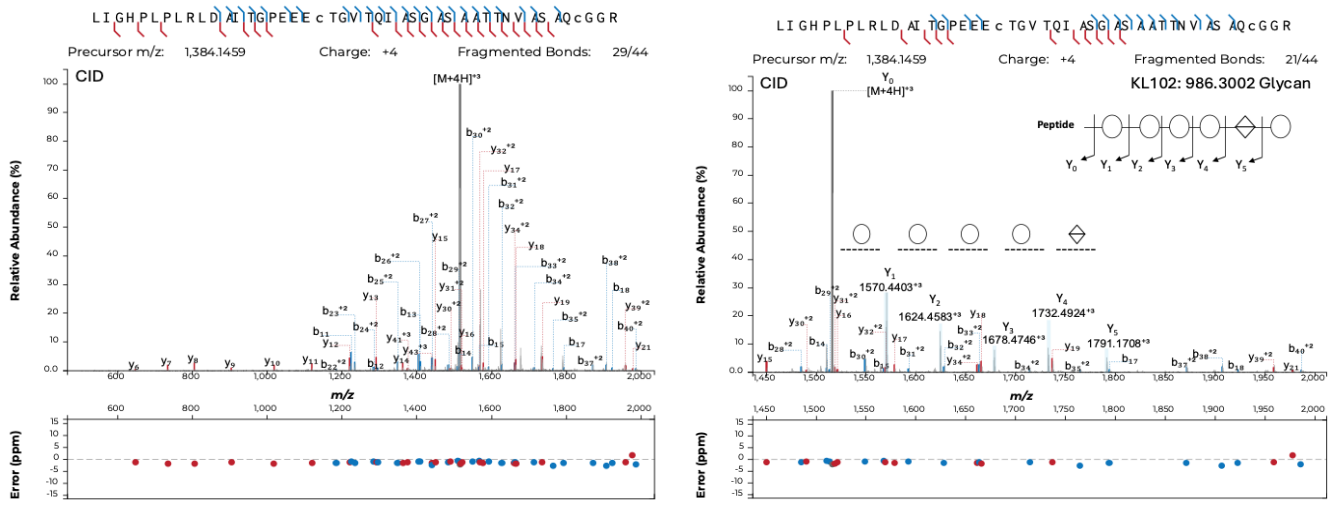
Sugar		H/C 1	H/C 2	H/C 3	H/C 4	H/C 5	H/C 6
α-Glc A	H	5.45	3.57	3.67	3.48	3.58	3.78; 3.82
	C	100.4	72.6	73.8	70.0	73.5	61.1
α-Gal B	H	5.06	4.16	4.02	4.39	4.16	3.92; 4.08
	C	99.9	68.4	80.8	78.0	71.3	71.2
β-Gal C	H	4.79	3.68	3.79	4.19	3.62	3.75; 3.75
	C	103.8	71.6	84.0	69.5	75.5	62.1
β-Gal D	H	4.65	3.64	3.70	4.02	3.94	3.82; 3.91
	C	105.6	72.3	73.7	69.5	73.4	67.8
β-GlcA E	H	4.74	3.51	3.83	3.91	4.14	
	C	105.4	74.2	76.7	78.2	75.1	172.7
β-Glc F	H	4.52	3.30	3.51	3.40	3.46	3.93; 3.74
	C	104.0	74.4	77.0	70.9	77.2	62.1



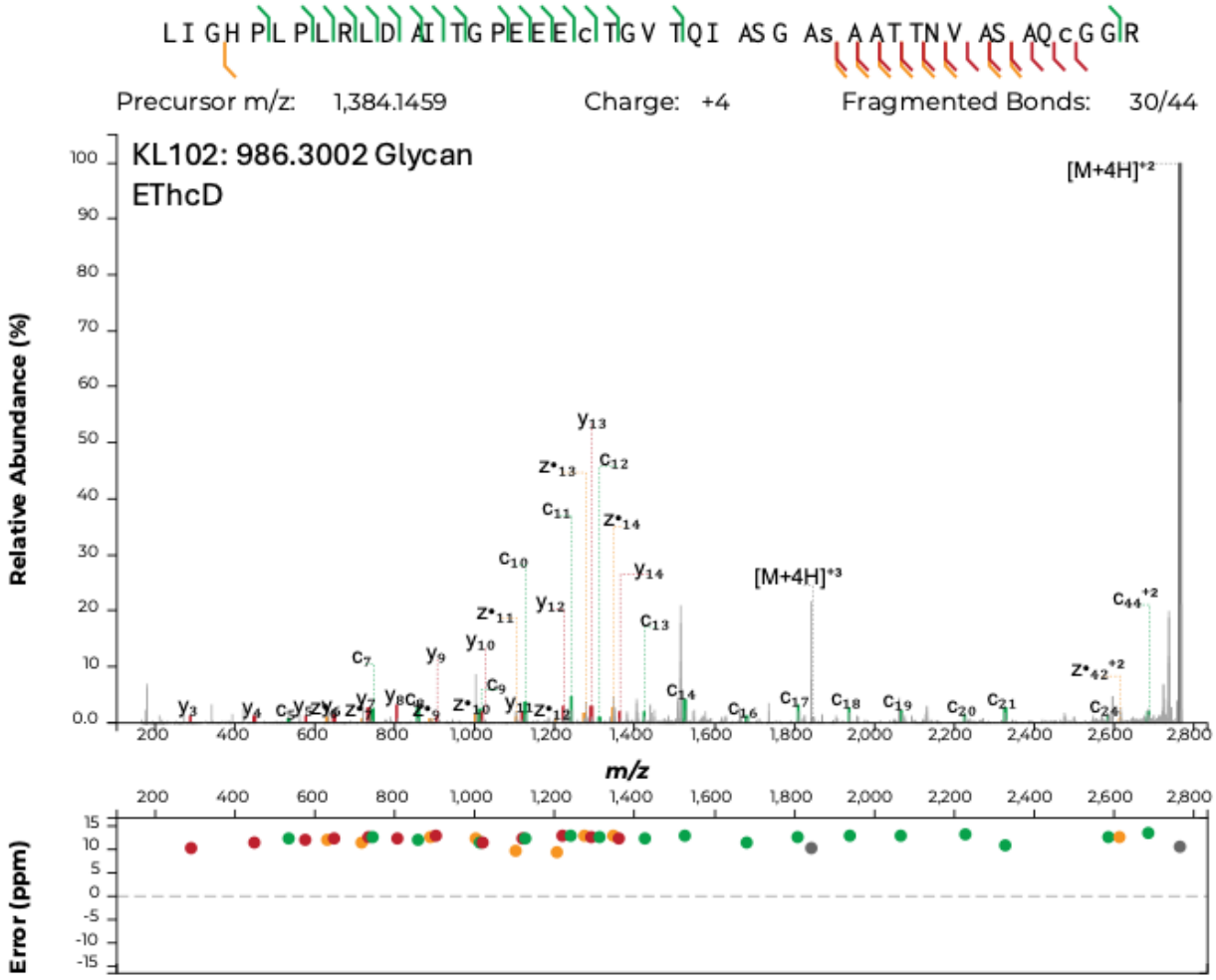
Supplemental Figure 1. Solved structure and NMR data for the surface exposed polysaccharide extracted from *E. coli* W3110 heterologously expressing the KL102 polysaccharide (600 MHz, 25 °C, δ ppm).



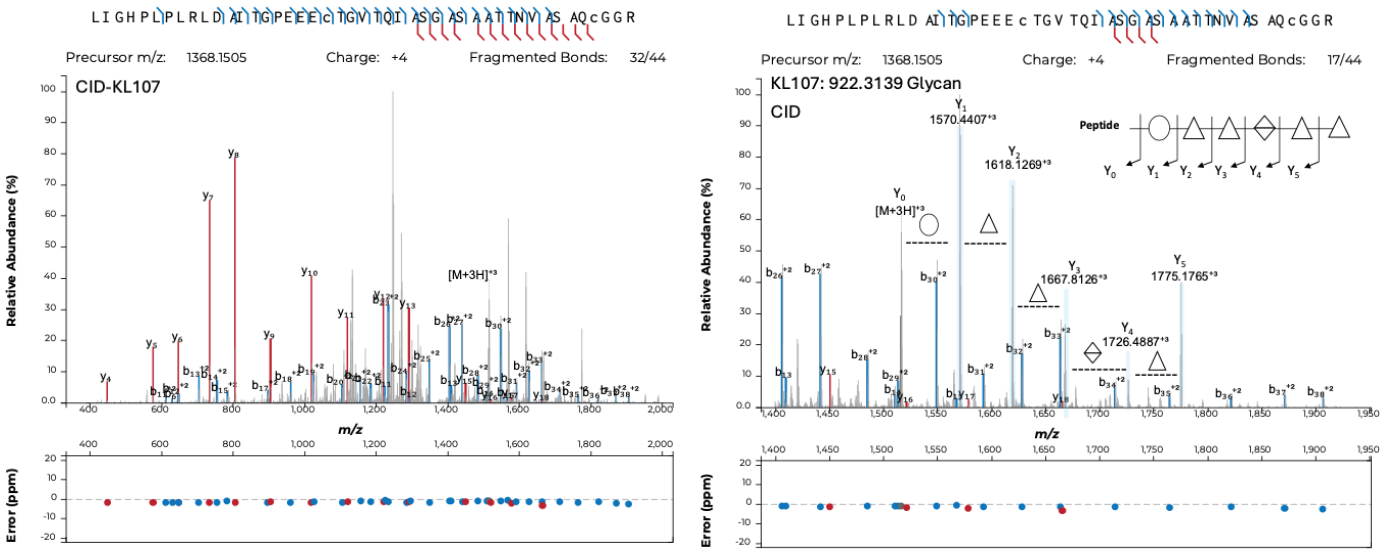
Supplemental Figure 2. ^{1}H-^{13}C HSQC spectrum of the surface exposed polysaccharide extracted from *E. coli* W3110 heterologously expressing the KL107 polysaccharide. We noted that HSQC spectrum shown in the publication for the original KL107 HSQC spectrum[19] contains errors in the signal labels where D2 and D3 are shown twice, whereas those on the right were for F2 and F3.



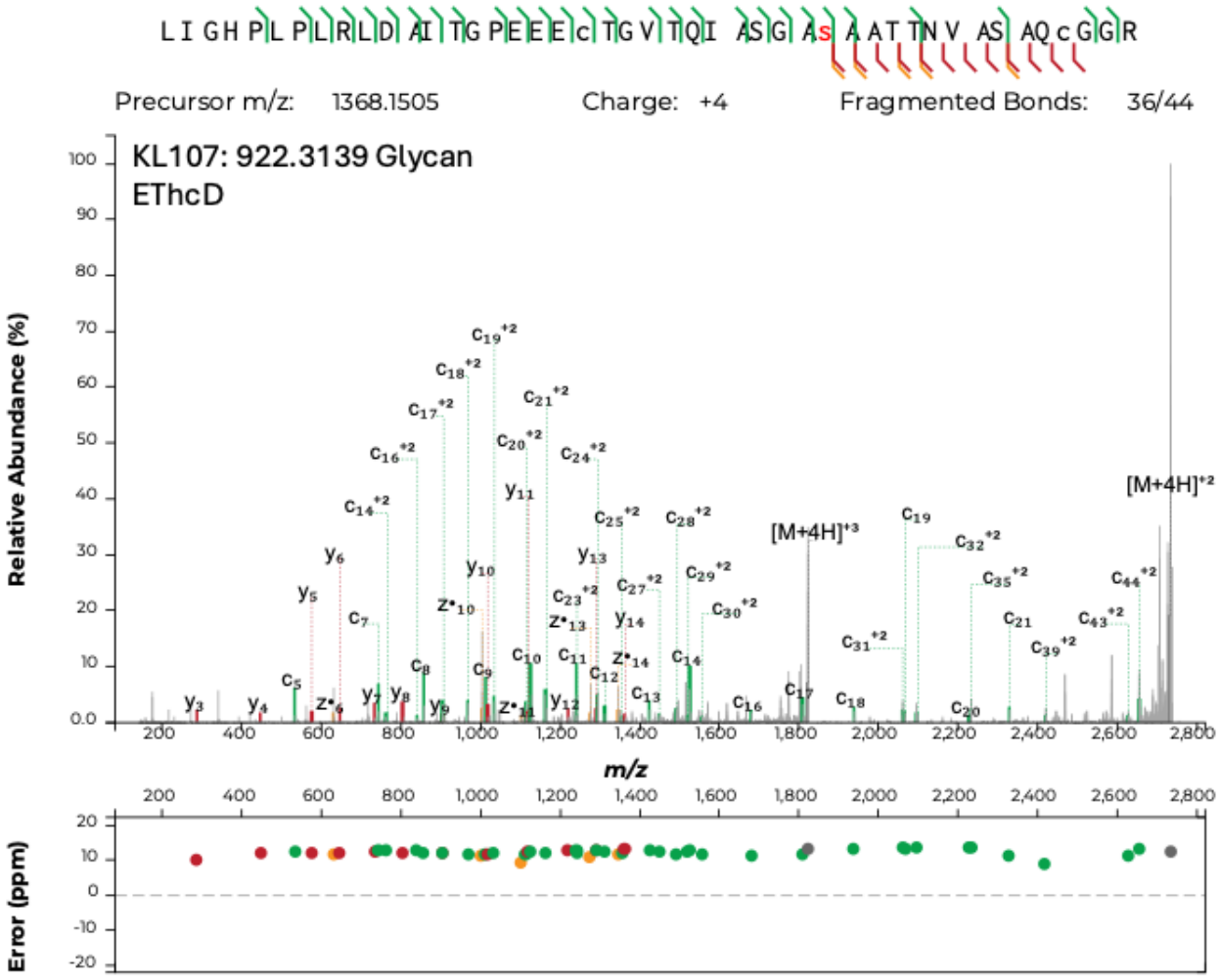
Supplemental Figure 3. CID spectra of KL102-EPA2 glycopeptides. Collision-induced dissociation (CID) spectra demonstrating the presence of a hexasaccharide unit attached to the shown tryptic peptide. CID glycan fragmentation showed the presence of a 986 Da modification attached to the peptide with the following composition: Hex-Hex-Hex-Hex-HexA-Hex.



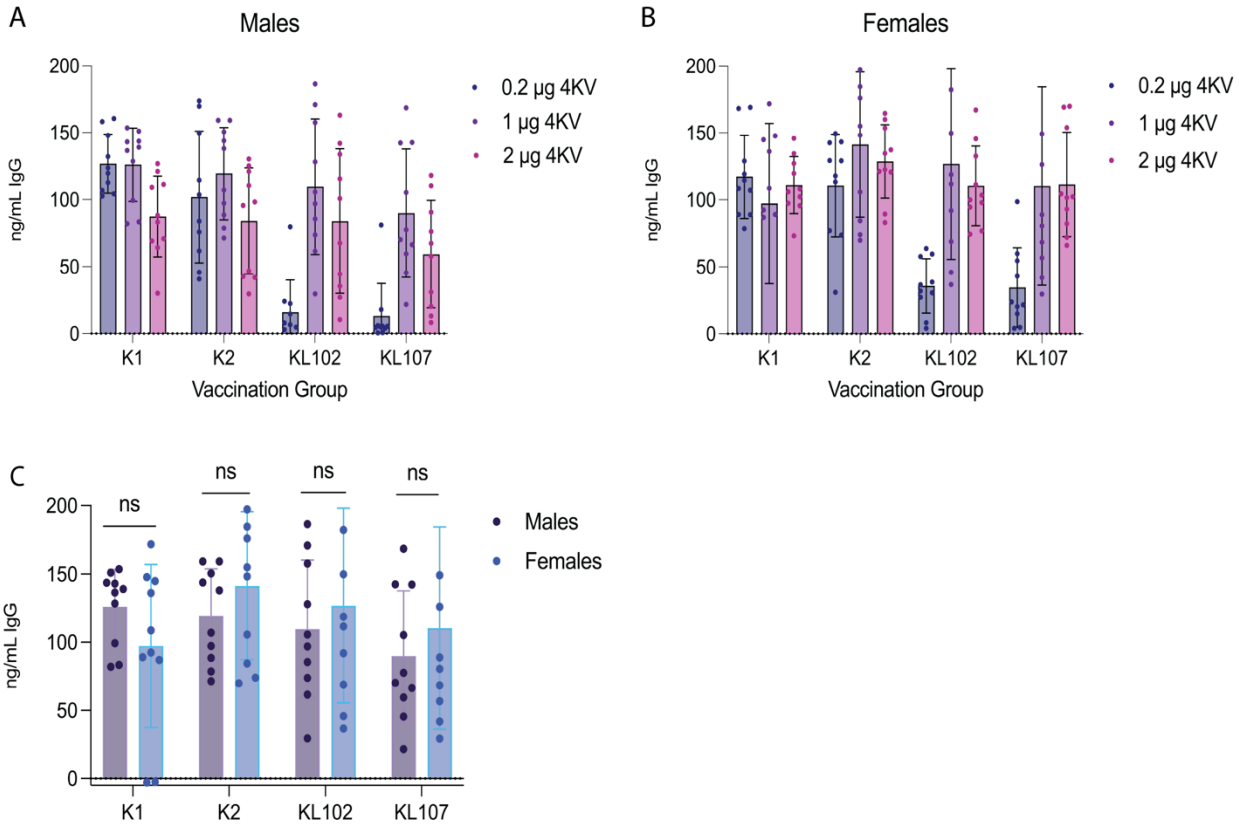
Supplemental Figure 4. EThcD spectrum of KL102-EPA2 glycopeptide. Electron-transfer/higher-energy collisional dissociation (EThcD) fragmentation of the same tryptic peptide revealed a single site of glycosylation (the red serine residue) containing the 986 Da modification.



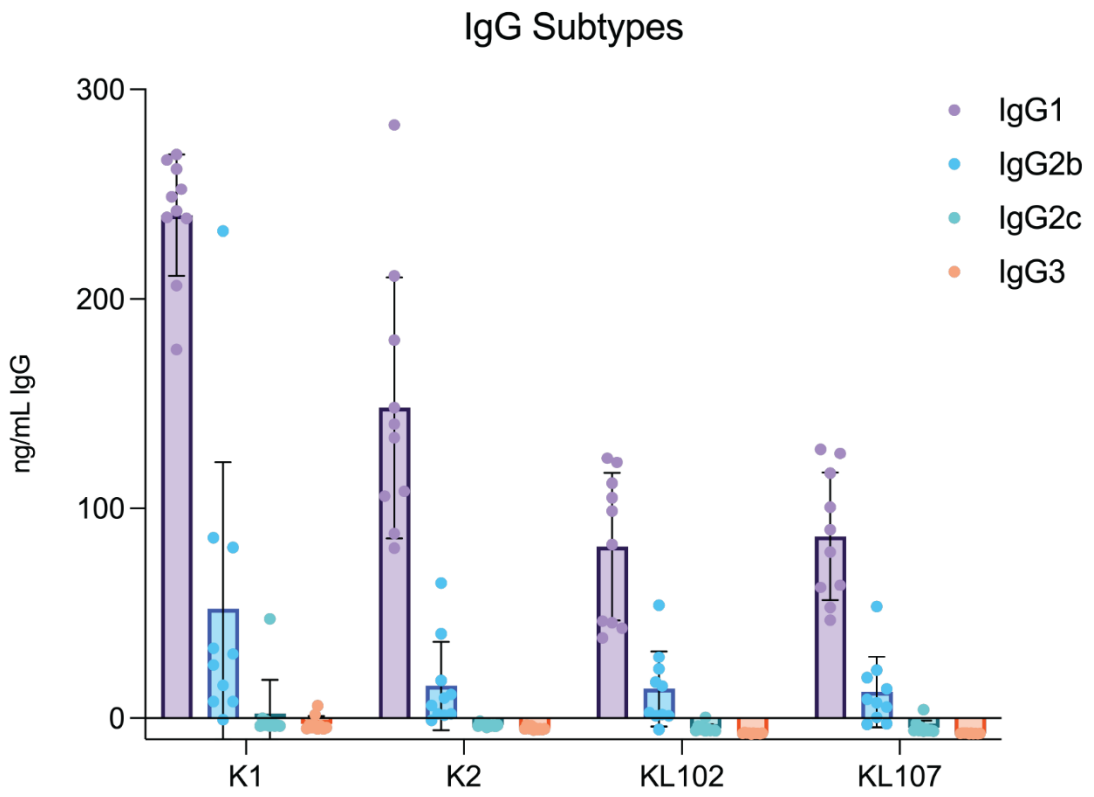
Supplemental Figure 5. CID spectra of KL107-EPA2 glycopeptides. Collision-induced dissociation (CID) spectra demonstrating the presence of a hexasaccharide unit attached to the shown tryptic peptide. CID glycan fragmentation showed the presence of a 922 Da modification attached to the peptide with the following composition: Hex-dHex-dHex-HexA-dHex-dHex.



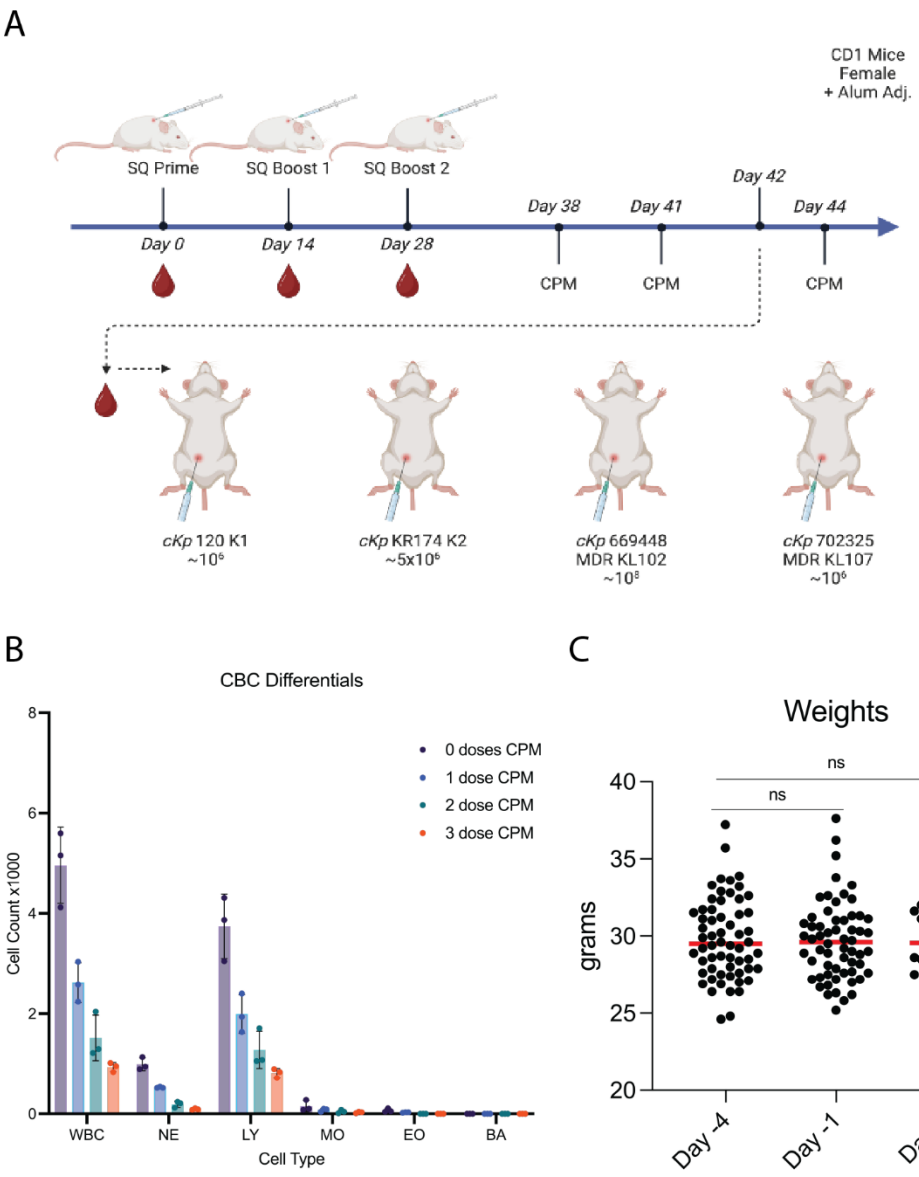
Supplemental Figure 6. EThcD spectrum of KL107-EPA2 glycopeptide. Electron-transfer/higher-energy collisional dissociation (EThcD) fragmentation of the same tryptic peptide revealed a single site of glycosylation (the red serine residue) containing the 922 Da modification.



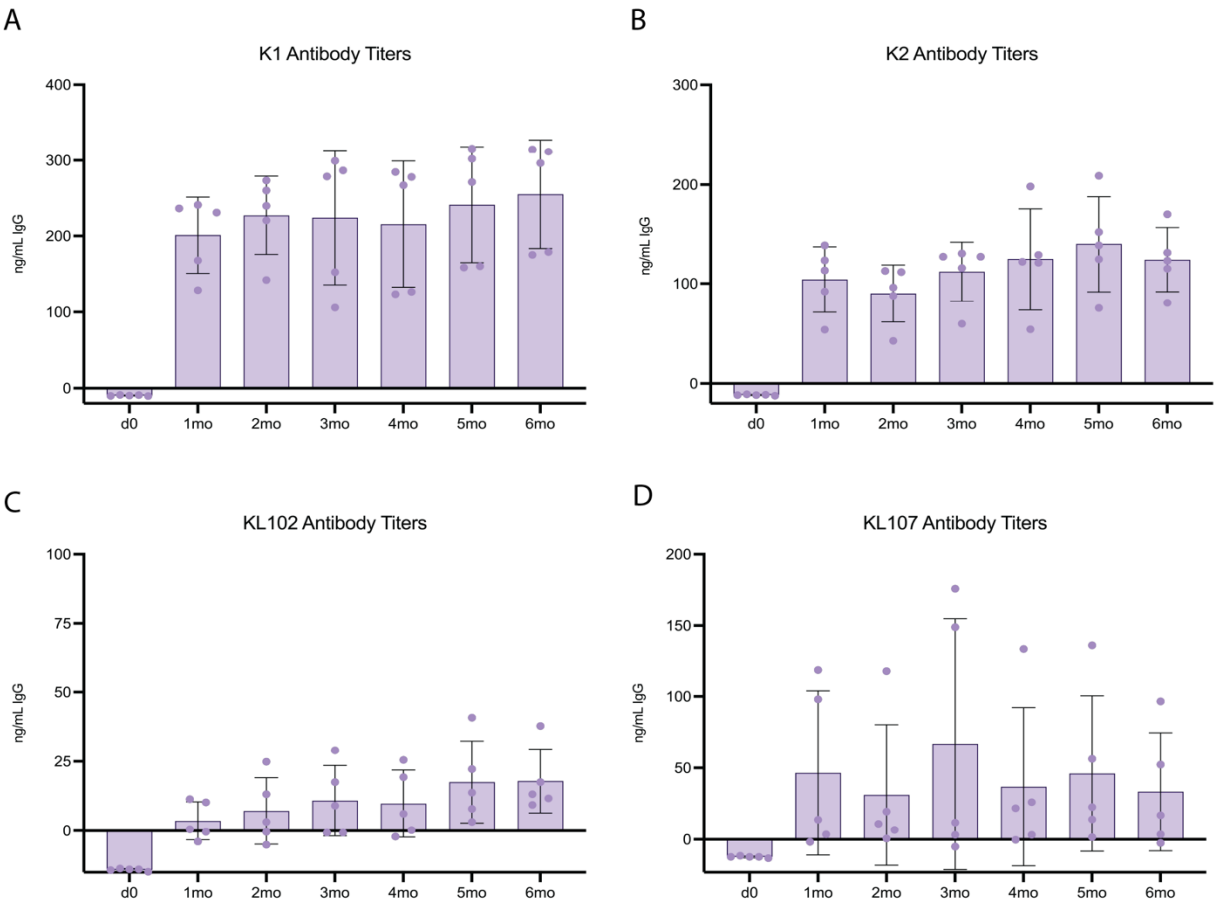
Supplemental Figure 7: Dose comparison of K4V formulations in male and female mice. Male (A) and female (B) CD-1 mice were immunized with either 0.2 μg, 1 μg, or 2μg of polysaccharide K4V-EPA bioconjugate vaccine. Day 42 serum was tested against each capsule type via ELISA on plates coated with glycoengineered *E. coli* expressing each capsule type. Graphs display immunoglobulin concentrations of 1:100 serum dilutions as calculated from a standard curve. (C) The 1 μg polysaccharide day 42 serum from male and female mice were compared and not found to be statistically different. Statistical analyses were performed via Mann-Whitney U test. ns not significant. Error bars represent standard deviation.



Supplemental Figure 8: IgG subtypes generated after vaccination with K4V-EPA. IgG subtypes were determined from day 42 sera via ELISA using plates coated with glycoengineered *E. coli* expressing each individual capsule type and HRP conjugated secondary antibodies for each IgG subtype. Graphs display immunoglobulin concentrations of 1:100 serum dilutions as calculated from a standard curve.



Supplemental Figure 9: Immunocompromised model and assessment of immunocompromised mice. (A) Timeline schematic of vaccinations followed by cyclophosphamide (CPM) treatment to induce an immunocompromised state. (B) A blood sample was taken following each treatment with CPM from three mice and tested via CBC panel. (C) Additionally, mice were weighed during the CPM treatment regimen to look for alternations in weight. Each dot represents a single mouse. Statistical analyses were performed via ANOVA and Dunnett multiple comparison test, ns not significant. Error bars represent standard deviation. CPM: cyclophosphamide; WBC: white blood count; NE: neutrophil; LY: lymphocyte; MO: monocyte; EO: eosinophil; BA: basophil



Supplemental Figure 10: IgG kinetics over six-month period following vaccination. Mice were immunized with monovalent formulations of K1-EPA, K2-EPA, KL102-EPA, or KL107-EPA on days 0, 14, and 28. Serum samples were taken once a month for six months following vaccination. Polysaccharide specific IgG titers were measured via ELISA on plates coated with either glycoengineered *E. coli.* Graphs display immunoglobulin concentrations of 1:100 serum dilutions as calculated from a standard curve.

Supplemental Table 1: Additional strains used in this study.

Strains	Description	Reference
AR0362	Classical multidrug resistant KL107 isolate from the CDC CTV Bank[48] encoding KPC	37
KR32	Classical multidrug resistant KL102 isolate from the Rosen Lab *Klebsiella* repository from the ascites fluid of a 20-year-old male	This study
VNM47	*E. coli* expression strain W3110 Δ*waaL*, Δ*gtrABS*, Δ*rfe-rffM*, Δ*glf - wbbk*::*manCB*	This study
VNM71	*E. coli* expression strain W3110 Δ*gtrABS*, Δ*rfe - rffM*, ∆*recA*, ∆*waaL*	This study
pVNM162	KL107 expression plasmid with *K. pneumoniae* AR0362 *wbaP – wzy* in pWKS130 backbone	This study
pVNM299	K2 expression plasmid with *K. pneumoniae* ATCC 43816 *wcuF - wzx* in pBBR1MCS3 backbone	17
pVNM303	Dual-purpose K1 or K2 expression plasmid with *K. pneumoniae* ATCC 43816 *wcaJ – ugd* in pWKS130 backbone	17
pVNM340	K1 expression plasmid with *K. pneumoniae* NTUH-K2044 *wzx – wcaI* in pBBR1MCS3 backbone	This study
pVNM374	Bioconjugation plasmid expressing *P. aeruginosa* EPA with two fused sequons and *A. baylyi* ADP1 PglS oligosaccharyltransferase in pEXT20 backbone	This study
pVNM379	KL102 expression plasmid with *K. pneumoniae* Kp32 *wbaP – ugd* in pBBR1MCS2 backbone	This study
pVNM403	pDOC-O16 antigen plasmid	This study
pVNM661	pDOC-O16 antigen plasmid with *K. pneumoniae manCB*	This study

Supplemental Table 2: Primers used in this study.

Primer	Sequence	Description
pWKS-K1 wzx WT RBS F1	CTAAAGGGAACAAAAGCTGGTAAGGACT GGGTGTGAATAAAAG	For cloning the K1 capsule cluster into pBBR1 MCS3
MCS3-K1 wcaI R1	GGCATTTGAGAAGCACACGGAATCCATT CCACCTAACG	For cloning the K1 capsule cluster into pBBR1MCS3
pWKS-Kp 102 F1	CTAAAGGGAACAAAAGCTGGTAATGACT CAGTTCGGCAAG	For cloning the KL102 capsule cluster into pBBR1MCS2
MCS2-KL102 ugd R1	CCTCAGGCATTTGAGAAGCACACGGGC AGAATTAATCGTTACCAAACAG	For cloning the KL102 capsule cluster into pBBR1MCS2
pWKS-Kp 107 F1	CTAAAGGGAACAAAAGCTGGTAATGAAA ACACTAAAGCAAAATATTATGAG	For cloning the KL107 capsule cluster into pWKS130
pWKS-Kp 107 R1	CGACCTCGAGGGGGGGCCCGTTTTAAG GCCTCACGTTGC	For cloning the KL107 capsule cluster into pWKS130
waaL-del-1	AGATAAGAAGTGAGTTTTAACTCACTTCTT AAACTTGTTTATTCAGGCTGGAGCTGCTTC	For deleting *waaL* from *E. coli* W3110
waaL-del-2	AGCAGTTTTGGAAAAGTTATCATCATTATA AAGGTAAAACTCCTCCTTAGTTCCTATTCC	For deleting *waaL* from *E. coli* W3110
yfd 5’ KO F1	GGATGATAGACTTCATTCCTTTGATTATTAG CTGATAGAAGAAAGGCTGGAGCTGCTTC	For deleting *gtrABS* from *E. coli* W3110
yfd 3’ KO R1	TTATAGCTTGTGCGCGCCATGATTGGCG CGCAATTTAAACTCCTCCTTAGTTCCTATTCC	For deleting *gtrABS* from *E. coli* W3110
ECA-del-1	TTAAATAAATTGCGCGTTTGGTTACGGTTT GTGCGTTTTGCTGCAGGCTGGAGCTGCTTC	For deleting *wecA - yifK* from *E. coli* W3110
ECA-del-2	GTTATACTTCTGCTAATAATTTTCTCTGAG AGCATGCATTTCCTCCTTAGTTCCTATTCC	For deleting *wecA - yifK* from *E. coli* W3110
ECA-del-4	GACGGAGTGACCACTCCGTCGCTTTACAA AGAGAGGAAAATCCTCCTTAGTTCCTATTCC	For deleting *wecA - rffM* from *E. coli* W3110
ECA-del-5	CGTGGTTATACTTCTGCTAATAATTTTCTCT GAGAGCATGCATTAGGCTGGAGCTGCTTC	For deleting *wecA - rffM* from *E. coli* W3110
recA KO CmR F1	GAACATATTGACTATCCGGTATTACCCGG CATGACAGGAGTCCTCCTTAGTTCCTATTCC	For deleting *recA* from *E. coli* W3110
recA KO CmR R1	GCAAAAGGGCCGCAGATGCGACCCTTGT GTATCAAACAAGACGAGGCTGGAGCTGCTTC	For deleting *recA* from *E. coli* W3110
pDOC-OA V2 5’ F1	GGTAATCGATGAATTCAAGCTTGGATCCATTG CCAAAGCAGCAGATAG	For amplifying the 5’ homology arm of the pDOC-O16 antigen plasmid
pDOC-OA V2 5’ R1	GAACTTCGAAGCAGCTCCAGCCTCATAAAA TCCTCAGCAAACCAG	For amplifying the 5’ homology arm of the pDOC-O16 antigen plasmid
pDOC-OA 3’ HA F1	GGAATAGGAACTAAGGAGGAATGGTATATAT AATAATCGTTTCCCAC	For amplifying the 3’ homology arm of the pDOC-O16 antigen plasmid
pDOC-OA 3’ HA R1	AGTCGACGCTAGCATATGAGTGCCTTTCTTGG TCTGATG	For amplifying the 3’ homology arm of the pDOC-O16 antigen plasmid
pKD4 inverse F1	AGGCTGGAGCTGCTTC	For amplifying the kanR cassette of the pDOC-O16 antigen plasmid, and for linearizing the plasmid
pKD4 KanR R1	TCCTCCTTAGTTCCTATTCC	For amplifying the kanR cassette of the pDOC-O16 antigen plasmid

pDOC inverse F1	CTCATATGCTAGCGTCGACTAG	For amplifying the sacB gene and ori for the pDOC-O16 antigen plasmid
pDOC inverse R1	GGATCCAAGCTTGAATTCATCG	For amplifying the sacB gene and ori for the pDOC-O16 antigen plasmid
rfbX 3' R1	CATAAAATCCTCAGCAAACCAG	For linearizing the pDOC-O16 antigen plasmid
pDOC-OA-manC F1	CTGGTTTGCTGAGGATTTTATGTTGCTTCCTG TGATTATGG	For cloning *K. pneumoniae manCB* into the pDOC-O16 antigen plasmid
pDOC-K2 manB R1	CTTCGAAGCAGCTCCAGCCTGGGTAATGCA TTACTTGAGAAGAG	For cloning *K. pneumoniae manCB* into the pDOC-O16 antigen plasmid