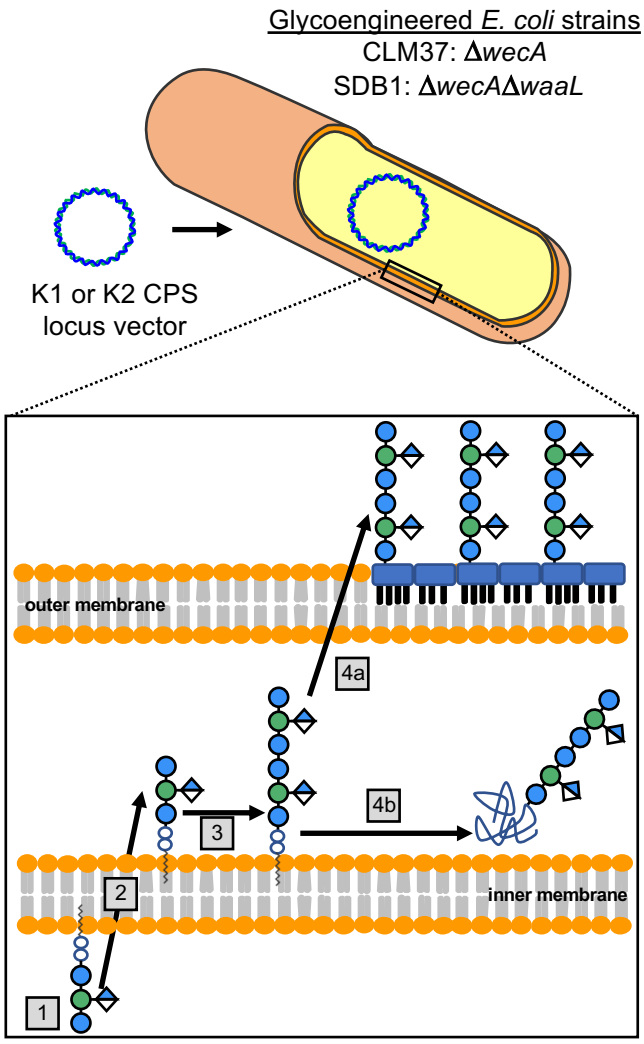
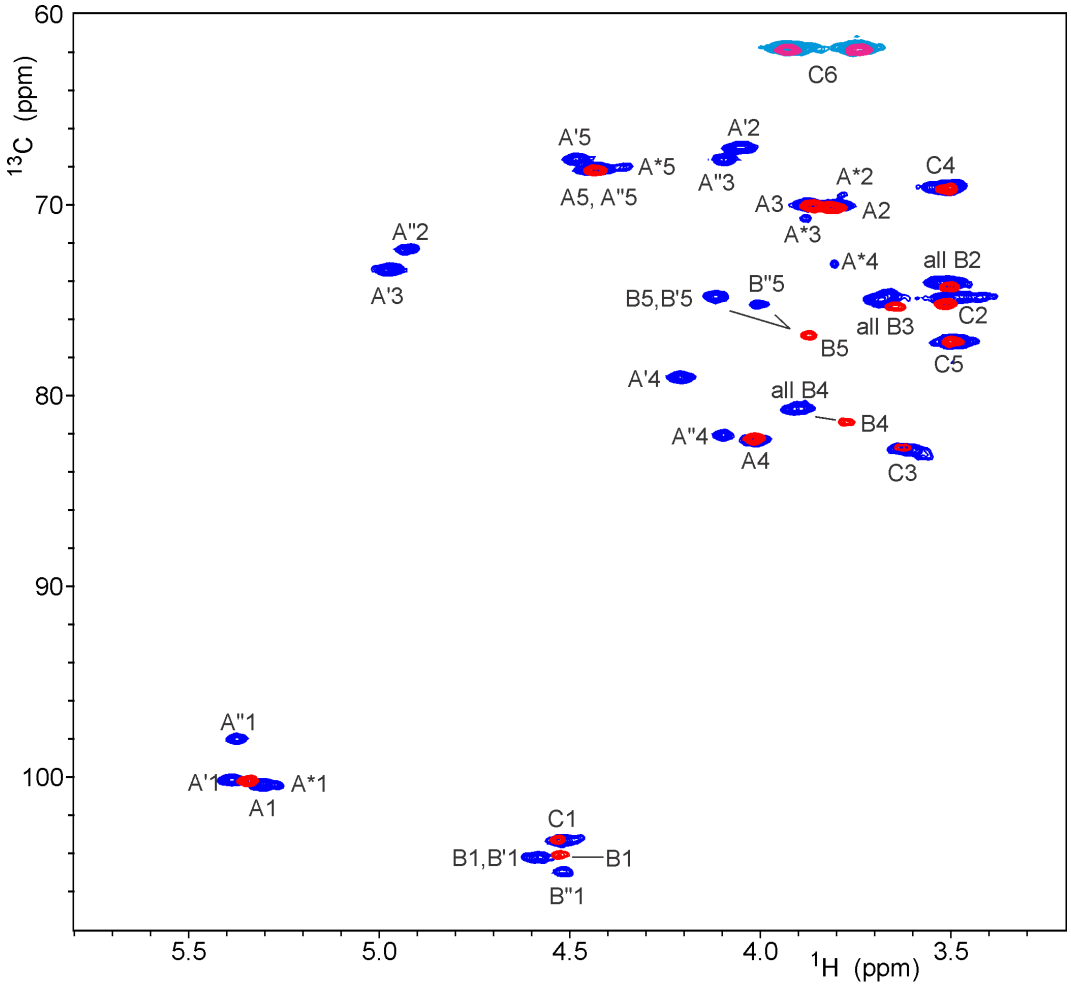



Supplemental Figure 1. Glycoengineering schematic in *E. coli*. The *E. coli* strains CLM37 or SDB1 are commonly used for heterologous glycan assembly and transfer. A plasmid encoding for the biosynthetic machinery of a particular glycan is transferred into *E. coli*. The glycan is then assembled sequentially onto a monophosphorylated undecaprenol precursor (1), flipped to the periplasm (2), and polymerized into a lipid-linked polysaccharide (3). The lipid-linked polysaccharide can be subsequently transferred to the outer core saccharide to generate an LPS molecule by the WaaL ligase (4a) or transferred by an oligosacchryltransferase to a target protein (4b).

-3-β-Glc-4-β-GlcA-4-α-L-Fuc2/3OAc-
C B A

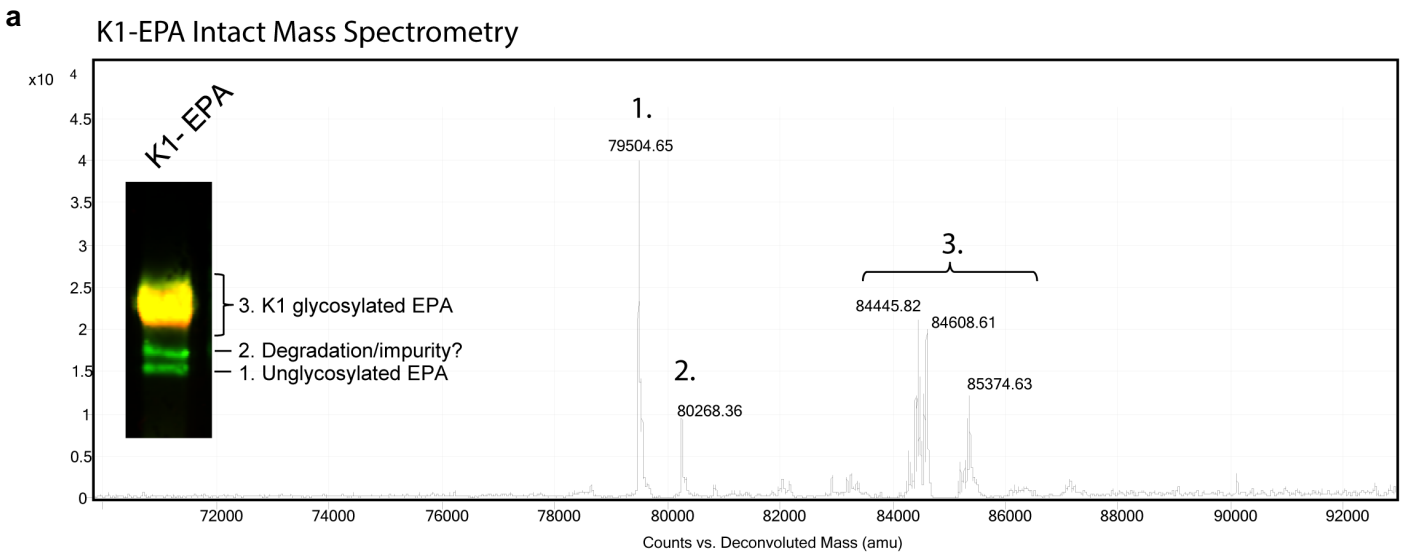


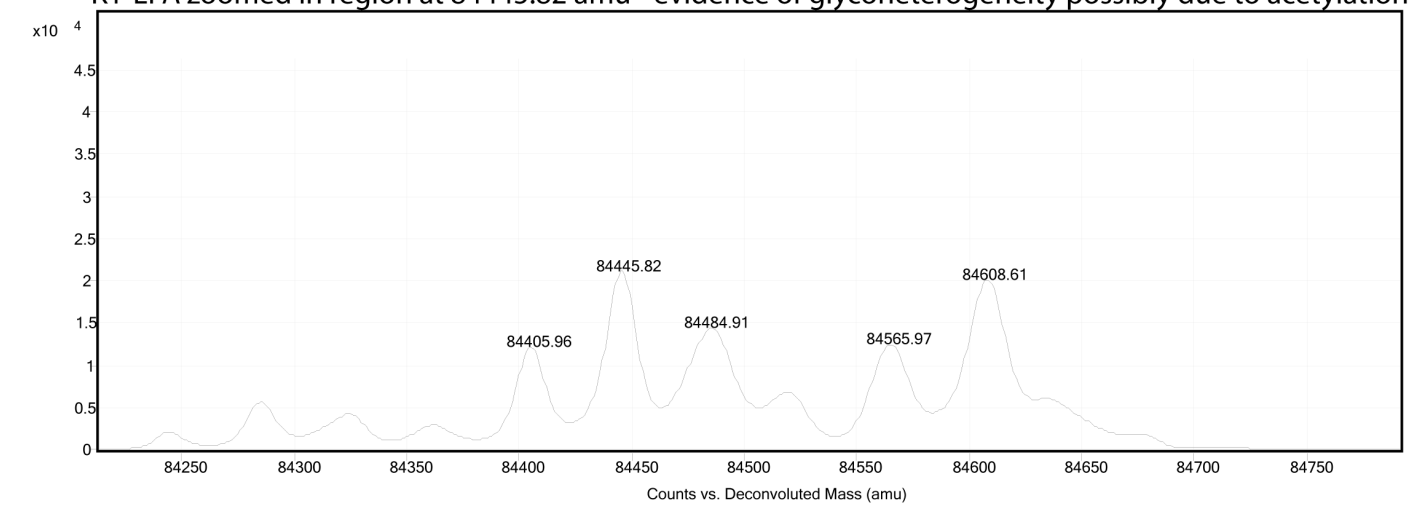

Supplemental Figure 2. Overlap of $^{1}$H-$^{13}$C HSQC spectra of native (blue-cyan) and deacylated (red-magenta) *K. pneumoniae* NTUH K-2044 capsular polysaccharide.

-3-β-Glc-4-β-GlcA-4-α-L-Fuc2/3OAc-
C B A

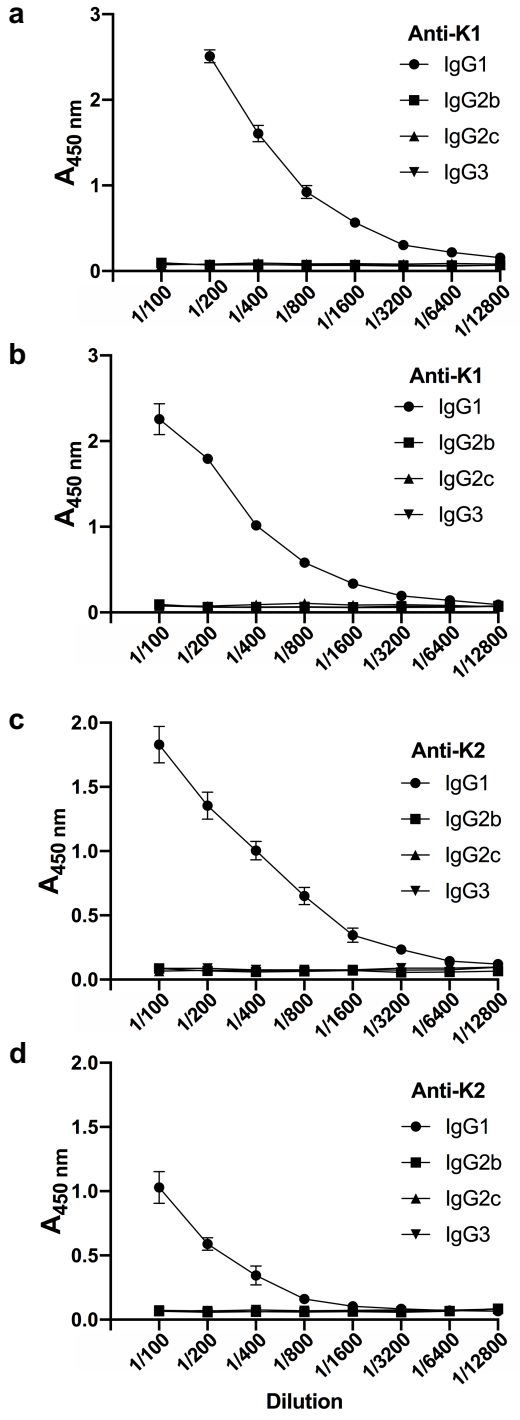
| Unit | | H/C-1 | H/C-2 | H/C-3 | H/C-4 | H/C-5 | H/C-6a;b |
|---|---|---|---|---|---|---|---|
| α-Fuc A" 2OAc | H | 5.37 | 4.93 | 4.09 | 4.10 | 4.45 | 1.27 |
| | C | 98.0 | 72.3 | 67.6 | 82.0 | 68.1 | 16.1 |
| α-Fuc A' 3OAc | H | 5.39 | 4.05 | 4.97 | 4.21 | 4.49 | 1.27 |
| | C | 100.2 | 67.0 | 73.4 | 79.0 | 67.6 | 16.1 |
| α-Fuc A no Ac | H | 5.30 | 3.80 | 3.87 | 4.01 | 4.43 | 1.27 |
| | C | 100.4 | 70.0 | 70.0 | 82.3 | 68.1 | 16.1 |
| α-Fuc A* term. | H | 5.27 | 3.79 | 3.88 | 3.80 | 4.37 | 1.18 |
| | C | 100.4 | 69.5 | 70.7 | 73.1 | 68.0 | 16.4 |
| β-GlcA B" | H | 4.60 | 3.53 | 3.68 | 3.95 | 4.11 | |
| | C | 104.2 | 74.0 | 74.9 | 80.7 | 74.8 | |
| β-GlcA B' | H | 4.52 | 3.51 | 3.67 | 3.92 | 4.01 | |
| | C | 104.9 | 74.0 | 74.8 | 80.7 | 75.2 | |
| β-GlcA B | H | 4.58 | 3.51 | 3.69 | 3.89 | 4.12 | |
| | C | 104.2 | 74.0 | 74.9 | 80.7 | 74.8 | 178.0 |
| β-Glc C | H | 4.52 | 3.51 | 3.61 | 3.50 | 3.49 | 3.74; 3.92 |
| | C | 103.3 | 74.9 | 82.8 | 69.1 | 77.1 | 61.7 |

Supplemental Table 1. NMR data for the K1 capsular polysaccharide extracted from *K. pneumoniae* NTUH K-2044 ($D_2O$, 25 °C, 600 MHz). B-A, B'-A', B"-A". OAc: 2.09/21.6, 2.07/21.6 ppm .

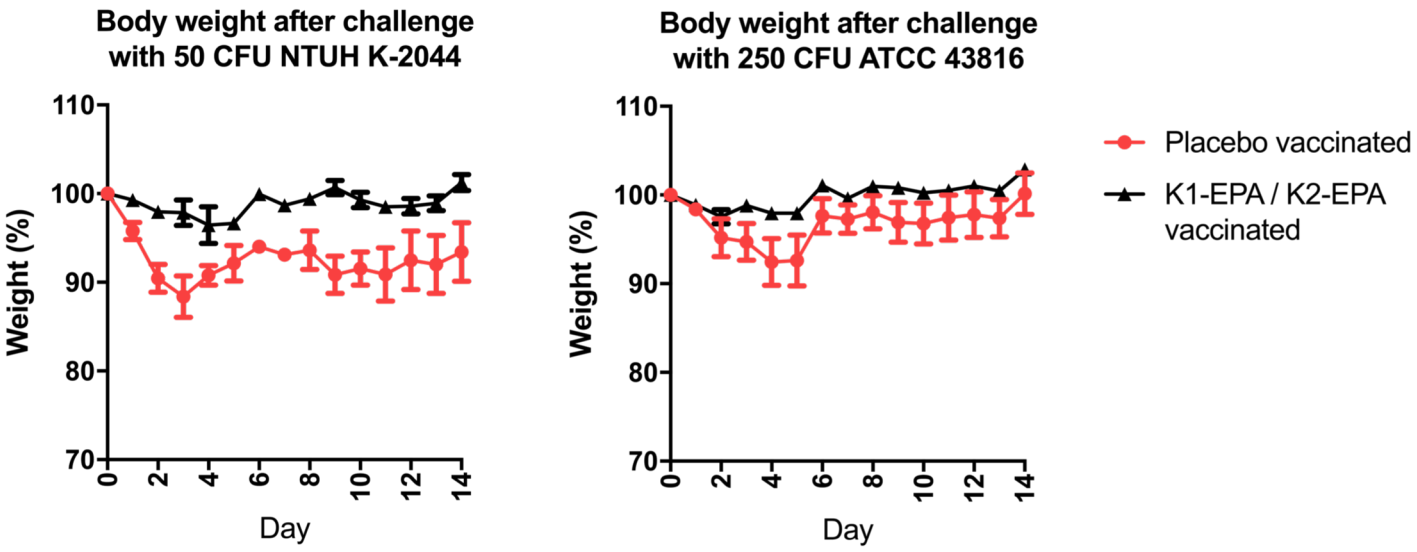



Supplemental Figure 3. Intact Mass Spectrometry of K1-EPA. **a**, Intact protein mass spectrometry analysis showing the MS1 mass spectra for K1-EPA. The EPA fusion protein has a theoretical mass of 79,526.15 Da and can be observed as the peak at 79,504.65 Da. The EPA fusion protein was also observed as a series of peaks compatible with different glycoforms containing the inherently heterogenous K1 repeat units, which is non-stoichiometrically modified with acetylation at the fucose residue and/or pyruvylated at the glucuronic acid residue. The inset of panel **a** is the from Figure 3 showing the western blot analysis of the K1-EPA bioconjugate. **b,** The MS1 spectra zoomed in at 84445.82 atomic mas units showing evidence for possible aceylation. The acetyl group has mass of 43 amu.




Supplemental Figure 4. Serotype specific IgG subclass responses. Day 42 sera samples were pooled and analyzed for IgG subclasses via ELISA. **a**, K1-EPA vaccinated pooled sera IgG subclass distribution and specificity to the K1 antigen. The 1/100 dilution was excluded from graphical presentation as the TMB substrate formed formed a black precipitate as a result of highly reactive sera at this dilution. **b**, K1-EPA/K2-EPA vaccinated pooled sera IgG subclass distribution and specificity to the K1 antigen. **c**, K2-EPA vaccinated pooled sera IgG subclass distribution and specificity to the K2 antigen. **d**, K1-EPA/K2-EPA vaccinated pooled sera IgG subclass distribution and specificity to the K2 antigen. Pooled sera samples were analyzed in triplicate and graphed to show the standard error of the mean.




Supplemental Figure 5. Body weights for surviving placebo- or bioconjugate-vaccinated mice challenged with a low dose of either *K. pneumoniae* NTUH K-2044 or ATCC 43816. Bioconjugate-vaccinated mice challenged with the K1 strain (NTUH K-2044) had statistically significant increases in body weights when compared to placebo-vaccinated mice after challenge on days 1-3 and 5-14. No statistically significant differences were observed between the body weights of K2 challenged mice groups. Statistical significance was determined using two-tailed t-tests with the Holm-Sidak method for multiple comparisons.

## SI Materials and Methods

**Bacterial strains, plasmids, and growth conditions.** Strains and plasmids used in this work are listed in Supplemental Table 2. *E. coli* strains were grown in Super Optimal Broth (SOB) at 30 °C overnight for LPS and bioconjugate vaccine production. *Klebsiella pneumoniae* strains were grown in Luria broth or on L-agar plates at 37 °C for infection studies and at 30˚C in SOB for capsular polysaccharide extraction. For plasmid selection, the antibiotics were used at the following concentrations: ampicillin (100 μg $mL^{-1}$), chloramphenicol (12.5 μg $mL^{-1}$), and kanamycin (20 μg $mL^{-1}$). Oligonucleotides used in this study are listed in Supplementary Table 3.

**Construction of the K1 and K2 capsule locus containing plasmids.** The K1 and K2 capsule locus clusters were individually cloned into the vector pBBR1MCS2 using a Gibson Assembly strategy (New England Biolabs). Briefly, the K1 cluster was PCR amplified using *K. pneumoniae* NTUH K-2044 genomic DNA as template. The 5' region of the K1 cluster from *wzx* to *wcaI* was first PCR amplified with a BamHI 3' overhang and 20 base pairs of homology to pBBR1MCS2 vector immediately surrounding the BamHI site. BamHI digested pBBR1MCS2 was then used with this PCR product in a Gibson Assembly reaction and transformed into DH10b cells. Transformants were selected on L-agar supplemented with kanamycin. Plasmid DNA was extracted, digested with BamHI, and then used in another Gibson Assembly reaction with the PCR product containing the remaining 3' region of the K1 cluster (*wcaJ* to *ugd*) with 20 bp of homology to the newly generated pBBR1MCS2-5'-K1 cluster plasmid. Transformants were selected on L-agar supplemented with kanamycin. Plasmid DNA was extracted and sequenced. A similar strategy was employed to clone the K2 cluster; however, *K. pneumoniae* 52.145 genomic DNA was used as template and the 5' K2 cluster region PCR was amplified

to contain the genes *wcuF* to immediately upstream of *wcaJ* and the 3' K2 cluster region PCR was amplified to contain *wcaJ* to *ugd*.

**K1 and K2 LPS expression in *E. coli* CLM37.** *E. coli* CLM37 was made electrocompetent by growing cells to mid-logarithmic stage followed by two rounds of washing in 10% glycerol and a final resuspension in 1/250th of the original culture volume. Cells were electroporated with either the K1 or K2 expressing plasmid and selected on L-agar supplemented with kanamycin. The pACT3-*rmpA* plasmid was introduced to CLM37 cells expressing the K1 or K2 expressing plasmid following the same procedure with transformants selected on L-agar supplemented with kanamycin and chloramphenicol. Colonies were picked and used to inoculate starter cultures of SOB which were grown overnight at 30˚C with 225 rpm. One liter of SOB media supplemented with antibiotics and 0.2 mM IPTG and was inoculated to an optical density at 600 nm ($OD_{600}$) at 0.08 with CLM37 cells from starter cultures and grown at 30˚C with 225 rpm for 20 hours. Cells were pelleted, washed in phosphate buffer saline (PBS) and either used immediately for LPS extraction or lyophilized for subsequent NMR analysis.

**LPS extraction and silver staining for silver staining and western blot analysis.** LPS was extracted following the methods of Marolda *et al* (1). Briefly, LPS was extracted using a hot phenol method from 2.0 OD units of stationary phase grown *E. coli* cells. Precipitated LPS was resuspended in 50 µL of Laemmli buffer. A 10 µL aliquot of LPS was separated on a 15% sodium dodecasulfate (SDS) polyacrylamide gel. Silver staining of SDS gels was performed according to the methods of Tsai and Frasch (2).

**Bioconjugate vaccine expression in *E. coli* SDB1.** *E. coli* SDB1 was made electrocompetent by growing cells to mid-logarithmic stage followed by two rounds of washing in 10% glycerol and a final resuspension in 1/250th of the original culture volume. Cells were electroporated

with either the K1 or K2 CPS expressing plasmid and selected on L-agar supplemented with kanamycin. Colonies were picked made electrocompetent as before and then electroporated with the pACT3-*rmpa-pglS* plasmids and transformants selected on L-agar supplemented with kanamycin and chloramphenicol. Colonies were picked, made electrocompetent again and then electroporated with pCH4 and transformants selected on L-agar supplemented kanamycin, chlorophenol, and ampicillin. Five to ten colonies were swabbed and used to inoculate 250 mL starter cultures in SOB supplemented with antibiotics for overnight growth at 30˚C with 225 rpm. Starter cultures were then used to inoculate multiple batches of 2 L Erlenmeyer flasks containing 1 L of SOB media supplemented with antibiotics and 0.2 mM IPTG. Cultures were grown for 24 hours at 30˚C with 225 rpm. Cells were pelleted by centrifugation and resuspended in 20 mM Tris 500 mM NaCl pH 8.0 and stored at -80˚C.

**EPA, EPA-K1 and EPA-K2 purification.** *E. coli* SDB1 cells were lysed by sonication and lysates were clarified by centrifugation. Clarified lysates were passed over 3 mL of Nickel NTA resin, washed with 100 mL of 20 mM Tris 500mM NaCl 10 mM Imidazole pH 8.0, and eluted with 5 mL of 20 mM Tris 500 mM NaCl 300 mM Imidazole pH 8.0. Eluted proteins were concentrated to 2.5 mL using Amicon Ultra Centrifugal Filters with a 50 kDA cutoff and then buffered exchanged into 20 mM Tris pH 8.0 using PD-10 columns. Buffer exchanged proteins were loaded onto a Mono Q 5/50 GL column. Proteins were eluted using a linear gradient of buffer containing 20 mM Tris 1 M NaCl pH 8.0. Fractions containing EPA, EPA-K1, of EPA-K2 were pooled, concentrated and separated on a Superdex 200 10/300 GL column equilibrated with 20 mM Tris 150 mM NaCl pH 8.0. Fractions enriched for EPA, EPA-K1, of EPA-K2 were pooled, concentrated and buffer exchanged into sterile PBS using Amicon Ultra Centrifugal Filters with a 50 kDa. EPA, EPA-K1, and EPA-K2 were analyzed for total protein content using a DC Protein Assay (Bio-Rad). EPA-K1 and EPA-K2 were analyzed for total carbohydrate content using a modified anthrone sulfuric acid method, whereby, 4 mL of a 2 mg $mL^{-1}$

anthrone-sulfuric acid was added to 1 mL of glucose standards or EPA-K1 or EPA-K2 samples. Samples were heated in a boiling water bath for 10 minutes and measured for absorbance at 620 nm. Total carbohydrate (μg $mL^{-1}$) content was extrapolated from a glucose standard curve. EPA, EPA-K1, and EPA-K2 were formulated as approximately 200 ng of polysaccharide conjugated to 5 μg of carrier protein per vaccine dose.

**Western blotting.** Purified LPS or glycoproteins were separated by 15% or 8% sodium dodecasulfate polyacrylamide gels, respectively. Samples were transferred to nitrocellulose membranes (Bio-Rad). Nitrocellulose membranes were blocked with Licor TBS blocking buffer, incubated with primary antibodies for 30 min, washed three times in TBS supplemented with Tween-20, incubated with secondary antibodies for 30 min, washed three times with TBS supplemented with Tween-20, and then visualized using an Odyssey Infrared Imaging System (LiCor Biosciences, USA). Primary antibodies included the monoclonal antibody 4C5 specific to the *K. pneumoniae* K1 CPS and the anti-*Pseudomonas* exotoxin A antibody (P2318-1ML) both used at 1:5000. Secondary antibodies included Licor IRDye 680RD goat anti-mouse (925-68070) and goat anti-rabbit 800CW (926-32211) used at 1:10000 dilutions.

**Preparation of extracts for NMR analysis.** The K1 and K2 LPS O polysaccharides were extracted from *E. coli* CLM37 by stirring cells in 45% phenol at 75°C for 20 min (2–3 g of dry cells in 200 mL). The mixture was dialyzed against running tap water after which acetic acid was added to a final concentration of 10%. The precipitates were removed by centrifugation for 30 min at 8000 rpm. The supernatant was dialyzed against distilled water then lyophilized. Lyophilized polysaccharides were dissolved in 10 mL of 2% acetic acid and heated for 1–2 hour at 100°C until a clear solution and precipitate formed. The reaction was cooled and the precipitate was removed by centrifugation at 10,000 rpm. The supernatant was loaded onto a Hitrap Q column and eluted using a linear gradient of water to 1 M NaCl final concentration over 1 h.

The native K1 capsular polysaccharide was extracted from *K. pneumoniae* NTUH K-2044 grown in SOB at 30˚C for 20 hours. Cells were pelleted and washed twice in PBS, flash frozen and lyophilized. *K. pneumoniae* lyophilized cells were boiled in water and the precipitate was removed by centrifugation. To reduce viscosity for analysis, the solution was heated for 30 minutes with 2% acetic acid. The precipitate was removed by centrifugation and the soluble polysaccharides were separated on a Biogel P6 column (2.5 × 60 $cm^2$) in 1% acetic acid and subsequently lyophilized. The polysaccharide fractions were a mixture of the native OPS from the LPS and the K1 CPS determined to be in a ratio of approximately 1:2. The CPS and OPS were loaded onto a Hitrap Q column where both the OPS and the CPS were retained. OPS and CPS were eluted using a linear gradient of water to 1 M NaCl final concentration over 1 h. Fractions containing the K1 CPS were pooled and desalted on Sephadex G15 column. CPS was analyzed by NMR as is and after *O*-deacetylation

**NMR analysis.** NMR experiments were carried out on a Bruker AVANCE III 600 MHz (1 H) spectrometer with 5 mm Z-gradient probe with acetone internal reference (2.23 ppm for 1 H and 31.45 ppm for 13C) using standard pulse sequences cosygpprqf (gCOSY), mlevphpr (TOCSY, mixing time 120 ms), roesyphpr (ROESY, mixing time 500 ms), hsqcedetgp (HSQC), hsqcetgpml (HSQC-TOCSY, 80 ms TOCSY delay) and hmbcgplpndqf (HMBC, 100 ms long range transfer delay). Resolution was kept at < 3 Hz/pt in F2 in protonproton correlations and < 5 Hz/pt in F2 of H-C correlations. The spectra were processed and analyzed using the Bruker Topspin 2.1 program.

**Enzyme-linked immunosorbent assays (ELISA).** For IgG endpoint, kinetic, and subclass ELISAs, 96-well plates were coated with $8x10^8$ CFU $mL^{-1}$ of either *K. pneumoniae* NTUH K-2044 (K1) or ATCC 43816 (K2). Strains were first grown overnight in LB broth at 26˚C, washed twice in PBS, and then resuspended in water prior to coating. Plates were air dried for 48 h in

a biological safety cabinet. Fifty microliters of methanol was then added to each well and the plates were air dried in a biological safety cabinet for another 24 hours. To perform the IgG titration, day 42 mouse sera from each immunization group was serially diluted two-fold in PBS supplemented with tween 20 and antibodies were detected with an anti-mouse, HRP-linked IgG (Cell Signaling Technology #7076) diluted 1:4000. For mouse serum titrations, the reciprocal of the last serum dilution that resulted in an optical density at 450 nm equal to or lower than 0.1 was considered the titer of that serum. For representation purposes, negative titers (less than or equal to the cutoff) were given an arbitrary titer value of 10. Inter-plate variations were controlled by including an internal reference positive control on each plate consisting of pooled sera from each immunization group. To perform the titration of mouse immunoglobulin subclasses, day 42 sera were pooled and serially diluted (2-fold) in PBST, and antibodies were detected using the following HRP-conjugated goat anti-mouse secondaries purchased from Southern Biotech: goat anti-IgG1, goat anti-IgG2b, goat antiIgG2c, or goat anti-IgG3 all diluted 1:2000.

**Intact protein analysis.** Intact mass analysis was performed as described previously (3). Briefly, samples were re-suspended in 2% acetonitrile 0.1% trifluroacetic acid and loaded onto a Jupiter 300 C5 column (Phenomenex) using an Agilent 1200. The EPA-K2 sample was de-salted by washing with 2% acetonitirile 0.1% formic acid and separated using a linear gradient of 80% acetonitirile 0.1% formic acid (2%-80% acetonitrile over 12 minutes using 0.2mL/min). MS1 mass spectra were acquired at 1 Hz between a mass range of 300–3000*m/z*. Intact mass analysis and deconvolution was performed using MassHunter B.06.00 (Agilent).

**Murine vaccination.** All murine immunizations complied with ethical regulations for animal testing and research. Experiments were conducted at Washington University School of Medicine in St. Louis according to institutional guidelines and received approval from the

Institutional Animal Care and Use Committee at Washington University in St. Louis. Four- to six-week-old female BALB/c mice were subcutaneously injected with 100 µL of a vaccine formulation on days 0, 14, and 28. Vaccine groups consisted of EPA alone (5 µg), K1-EPA (5 µg protein, ~222 µg polysaccharide), K2-EPA (5 µg protein, 195 µg polysaccharide) or K1-EPA/K2-EPA mixture (10 µg protein, 417 µg polysaccharide). Mice were either sacrificed and terminally bleed on day 42 or challenged with *K. pneumoniae* (described below). Mice vaccinated for non-challenge experiments had sera collected on days 0, 14, 28 and 42. Vaccines were formulated with Imject Alum in a 1:1 ratio (50 µL vaccine to 50 µL Imject Alum).

***Klebsiella pneumoniae* murine infection model.** Groups of vaccinated mice were anesthetized with isoflurane and aspirated with 50 µL challenge dose of either *K. pneumoniae* NTUH K-2044 or *K. pneumoniae* ATCC 43816 as previously described (4). Bacteria were prepared as follows: *K. pneumoniae* strains were grown statically in 20 mL of LB broth for 16 hours at 37˚C. Bacterial cultures were centrifuged at 8000 x *g* for 10 mins. The supernatants were discarded and the bacterial pellets were subsequently resuspended to an $OD_{600}$ of 0.2 in sterile PBS. Cultures were then serially diluted 1:2,000 in PBS to obtain the desired final concentration for the high dose inoculum. A 50 µL aliquot was then used to challenge anesthetized mice for the high dose challenge (actual challenge dose - 4,700 and 4,300 CFU for NTUH K-2044 and ATCC 43816, respectively). For the low dose challenge, an additional dilution was performed and a 50µL aliquot was then used as to inoculate mice (actual challenge dose – 50 and 250 CFU for NTUH K-2044 and ATCC 43816, respectively. Mouse survival and weight were monitored for two weeks. Each experiment was performed a single time with n=10 mice per group. Survival differences were determined by the Log-rank (Mantel-Cox) test and weight differences were determined using two-tailed t-tests with the Holm-Sidak method for multiple comparisons

1. Marolda CL, Lahiry P, Vines E, Saldias S, & Valvano MA (2006) Micromethods for the characterization of lipid A-core and O-antigen lipopolysaccharide. *Methods Mol Biol* 347:237-252.
2. Tsai CM & Frasch CE (1982) A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. *Anal Biochem* 119(1):115-119.
3. Harding CM*, et al.* (2019) A platform for glycoengineering a polyvalent pneumococcal bioconjugate vaccine using *E. coli* as a host. *Nat Commun* 10(1):891.
4. Kudva A*, et al.* (2011) Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. *J Immunol* 186(3):1666-1674.

**Supplemental Table 2**

| Strains and Plasmids | Description | Source |
|---|---|---|
| Strains | | |
| E. coli SDB1 | W3110, ΔwaaLΔwecA | [1] |
| E. coli DH10B | General cloning strain | Invitrogen |
| K. pneumoniae NTUH K-2044 | Hypervirulent, hypermucoviscous K1 isolate | [2] |
| K. pneumoniae ATCC 43816 | Hypervirulent, hypermucoviscous K2 isolate | [3] |
| K. pneumoniae 52.145 | K2 isolate | [4] |
| Plasmids | | |
| pEXT20 | Cloning vector | [5] |
| pACT3 | Cloning vector | [5] |
| pBBR1-MCS2 | Cloning vector | [6] |
| pCH4 | pEXT20 containing the ΔE553 variant of exotoxin A of P. aeruginosa (EPA) with an N-terminal DsbA signal sequence and a C-terminal glycotag consisting of ComP110264 without the first 28 amino acids. A peptide linker (GGGS) fuses EPA to the ComP fragment. | [7] |
| pACT3-rmpA$_{K1_PL}$ | pACT3 containing the rmpA gene from the virulence plasmid of K-2044 NTUH (Accession BAH65944) | This study |
| pACT3-rmpA$_{K1_PL}$-pglS$_{ADP1}$ | pACT3-rmpA containing the pglS gene from A. baylyi ADP1 | Thus study |
| pBBR1MCS2-K1 | pBBR1MCS2 containing the K. pneumoniae NTUH K-2044 K1 capsular polysaccharide cluster from genes wzx to ugd | This study |
| pBBR1MCS2-K2 | pBBR1MCS2 containing the K. pneumoniae 52.145 capsular polysaccharide cluster from genes from wcuF to ugd | This study |


1 Garcia-Quintanilla, F., Iwashkiw, J. A., Price, N. L., Stratilo, C. & Feldman, M. F. Production of a recombinant vaccine candidate against *Burkholderia pseudomallei* exploiting the bacterial *N*-glycosylation machinery. *Front Microbiol* **5**, 381, doi:10.3389/fmicb.2014.00381 (2014).

2 Chou, H. C. *et al.* Isolation of a chromosomal region of *Klebsiella pneumoniae* associated with allantoin metabolism and liver infection. *Infect Immun* **72**, 3783-3792, doi:10.1128/IAI.72.7.3783-3792.2004 (2004).

3 Bakker-Woudenberg, I. A., van den Berg, J. C., Vree, T. B., Baars, A. M. & Michel, M. F. Relevance of serum protein binding of cefoxitin and cefazolin to their activities against *Klebsiella pneumoniae* pneumonia in rats. *Antimicrob Agents Chemother* **28**, 654-659 (1985).

4 Nassif, X. & Sansonetti, P. J. Correlation of the virulence of *Klebsiella pneumoniae* K1 and K2 with the presence of a plasmid encoding aerobactin. *Infect Immun* **54**, 603-608 (1986).

5 Dykxhoorn, D. M., St Pierre, R. & Linn, T. A set of compatible tac promoter expression vectors. *Gene* **177**, 133-136 (1996).

6 Kovach, M. E. *et al.* Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**, 175-176 (1995).

7 Harding, C. M. *et al.* A platform for glycoengineering a polyvalent pneumococcal bioconjugate vaccine using *E. coli* as a host. *Nat Commun* **10**, 891, doi:10.1038/s41467-019-08869-9 (2019).

## Supplemental Table 3

| | |
|---|---|
| 5'_K1_+_3'_wzc F2 | gatatcgaattcctgcagcccggggGGTAGATTACGAGAAGGCTG |
| 5'_K1_+_3'_wzc R2 | ggtggcggccgctctagaactagtgggatccCTAAGAAACATCCATAAAATAAGC |
| 3'_K1_+_3'_wzc F2 | gcttattttatggatgtttcttaggGACTTTTAGGTATGAAATTACTTGTATATG |
| 3'_K1_+_3'_wzc R2 | gtggcggccgctctagaactagtggTTAATCGTTACCAAACAGATC |
| K1 seq 2 | ccggcggttttagcttttgt |
| K1 seq 3 | cctttatatataaaccgggg |
| K1 seq 4 | ccgttacgaacttgaacgag |
| K1 seq 5 | gccgcaaatacgagaagtgt |
| K1 seq 6 | cgtagttggtgaagagtattttg |
| K1 seq 7 | tttggtgtgcctatgttggt |
| K1 seq 8 | gctctgattaccggtatcac |
| K1 seq 9 | ctctgtacgtcaattcgtag |
| K1 seq 10 | gccggtataaaactctgcga |
| K1 seq 11 | gagatgctgagaaatgaagccac |
| K1 seq 12 | ggtgtttaacgtctgcagac |
| K1 seq 13 | gggattagcaattagattacg |
| K1 seq 14 | ggttctttacctgcgggtattaa |
| K1 seq 15 | ggggtatatgcttcgtcata |
| K1 seq 16 | cggtaccggtgtttccggtg |
| K1 seq 17 | tcactgatgcttatgcgcaaaacg |
| K1 seq 18 | cattattcttgagccggtcg |
| K1 seq 19 | gagcgttcccaggacgtgaa |
| K1 seq 20 | gcgaagcccatcagcggc |
| K1 seq 21 | acacaacggacggtatca |
| K1 seq 22 | ttcttgcgtgaaggacgt |
| 5'_K2_52.145 Fwd | gatatcgaattcctgcagcccggggATGAACGTCATCCATATCG |
| 5'_K2_52.145 Rev | ggtggcggccgctctagaactagtgggatccTTAGTGTTGCCATGATATATTTC |
| 3'_Kp_52_145 Fwd | agaaatatatcatggcaacactaagGAGTTTTTCATCTCTTGTTTG |
| 3'_Kp_52_145 Rev | gtggcggccgctctagaactagtggTTAATCGTTACCAAACAGATC |
| pBBR1_MCS2 seq1 | tgagcggataacaatttcacac |
| K2 Seq 1 | taggttactcaagaaggggacgaa |
| K2 Seq 2 | aatgcagtttcgctagatggacaa |
| K2 Seq 3 | aaggggcattcggtaacag |
| K2 Seq 4 | cgggcggttgacttacga |
| K2 Seq 5 | ggagcgaatttgtaaaaatgtttac |
| K2 Seq 6 | cactggaactaatcatggagaaca |
| K2 Seq 7 | ctctcttgaagctagtgtattt |
| K2 Seq 8 | aaaatcgggccgaaaatgacatc |
| K2 Seq 9 | gttcttggtgctatttatgttggg |
| K2 Seq 10 | cctgccaaatcattcaggggg |
| K2 Seq 11 | gattcaggggtggcgtactacac |
| K2 Seq 12 | ggtatactgtcgggaggaatgatg |
| K2 Seq 13 | ttgccttatagggaacaatgttgc |
| K2 Seq 14 | ccttggttagggtttgaggttaaa |
| K2 Seq 15 | cacaaattaatggttggcgtggtg |
| K2 Seq 16 | ccatcatgcctggtgggcag |
| K2 Seq 17 | tgctggcaccgtacttcaaa |
| K2 Seq 18 | ccccggccatcgccctgg |
| K2 Seq 19 | tgctggtgatgaaccgcga |
| K2 Seq 20 | acaacccgatgaactacaa |
| K2 Seq 21 | tgctggtggcggagctgc |
| K2 Seq 22 | tacgatcccaaaaccaactac |
| K2 Seq 23 | ccggttattatctatgaaccg |
| rmpA + pACT3 F | attcgagctcggtacccgggATGGAAAAATATATTTACTTTATATGTAAC |
| rmpA + pACT3 R | cctgcaggtcgactctagagCTAAATACTTGGCATGAGCC |
| PglS ADP1 + SalI F | ATAgtcgacATGAATAGTATTTTTAAAAAAATCAAAAAT |
| PglS ADP1 + HindIII R | ATAaagcttTCAATATTCTATTGAACAAAATTTTAACTT |
| rmpA seq 1 | ggttgatgaaagatggctca |
| pglS adp1 seq 1 | GCTCCTCATATAATGGTCGT |
| pgls adp1 seq 2 | CTCACACAATTTGTTCTTAGAT |
| pgls adp1 seq 3 | GCTTATCGATATCCAAATGCATC |