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Supplementary Materials for

In situ pneumococcal vaccine production and delivery through a hybrid biological-biomaterial vector

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This PDF file includes:

Supplementary Materials

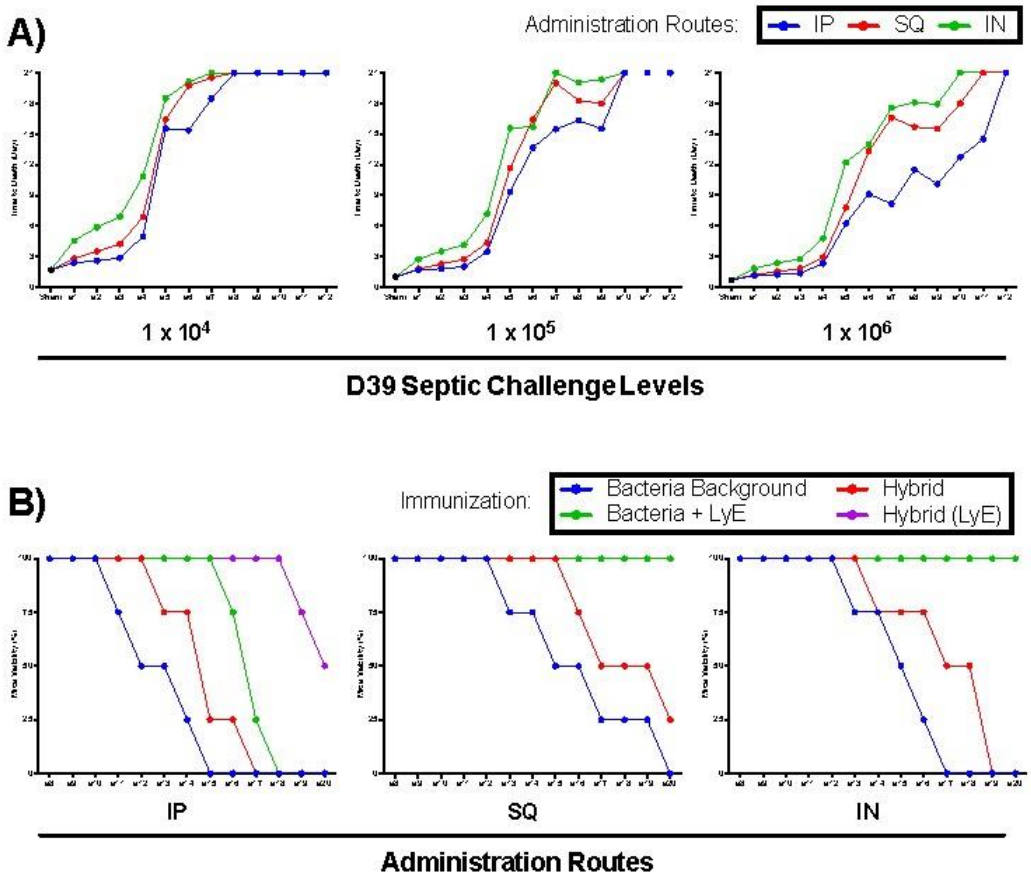


fig. S1. Dosing and toxicity assessment of hybrid and bacterial vectors. (**A**) Hybrid vectors expressing PspA were administered at 12 doses (10^1 to 10^{12}; X-axis of each individual plot) either IP, SQ, or IN and challenged with three levels of D39 in a sepsis model. (**B**) Bacterial and hybrid vectors were administered over 13 doses (10^8 to 10^{20}; X-axis of each individual plot) IP, SQ, and IN and mouse subjects monitored for viability.

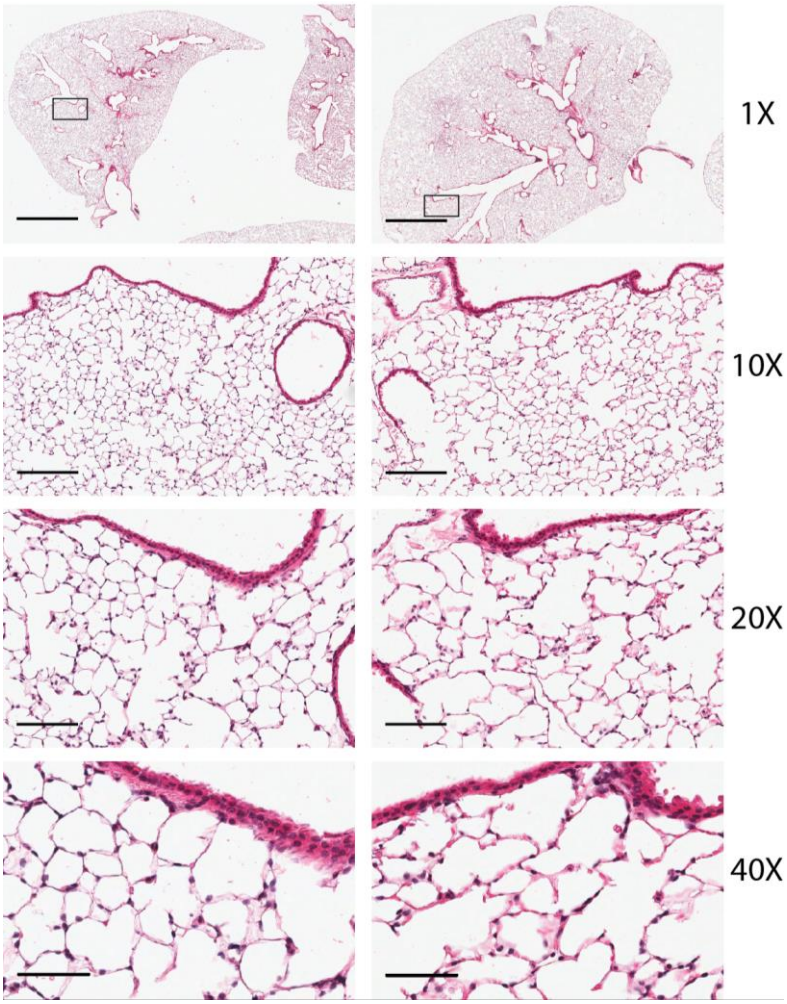


fig. S2. Histological intrasanal toxicity evaluation of hybrid devices. Specifically, mice were unvaccinated (left column) or vaccinated on days 0 and 14 by IN administration of 10^{13} hybrid devices containing the consolidated antigens (right column). On day 28, the mice were challenged with a sham injury (IN instillation of 50 µL normal saline), sacrificed 24 hours later by exsanguination, the lung vasculature flushed, and the lungs fixed. The hematoxylin-eosin-stained 5 µm sections above are from the right cranial lobe of the lungs. The boxes in the 1× images represent the areas that were subsequently acquired at 10×, 20×, and 40×. Scale bars represent 2 mm for 1×, 200 µm for 10×, 100 µm for 20×, and 60 µm for 40×. Both samples display normal histology with no evidence of tissue damage or inflammatory reaction. The bronchial and bronchiolar mucosa and alveoli are intact with typical architecture and no indication of necrosis, edema, or inflammatory infiltrate. Images represent typical findings from all lung lobes from two mice in each group.

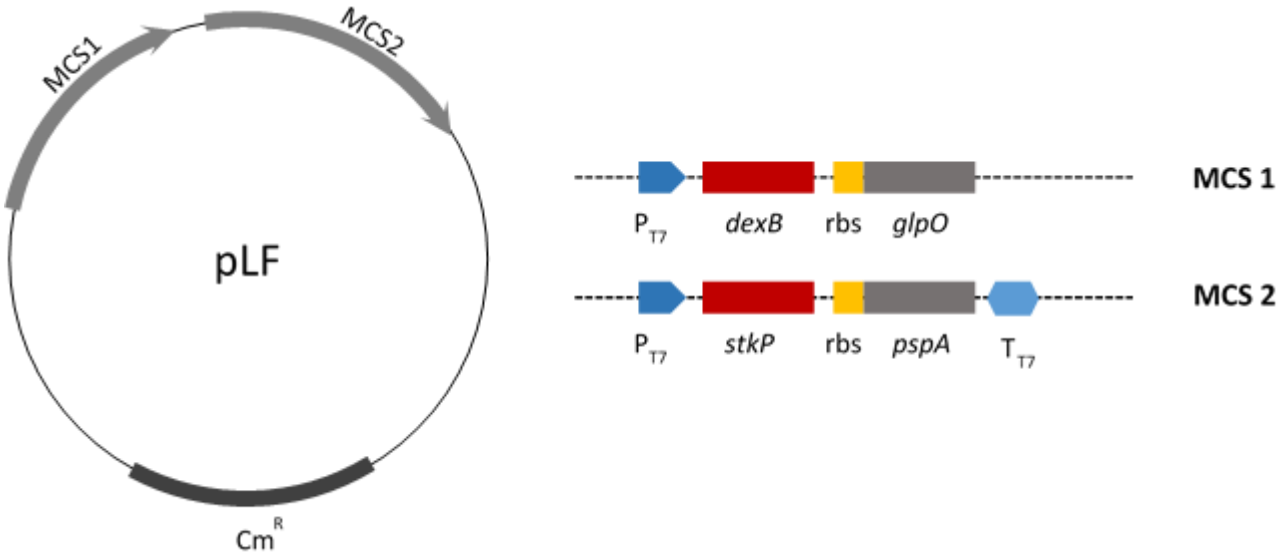


fig. S3. The pLF consolidation design and organization. MCS: multiple cloning site; Cm^R: chloramphenicol resistance; P_{T7}: T7 promoter; rbs: ribosomal binding site; T_{T7}: T7 terminator.

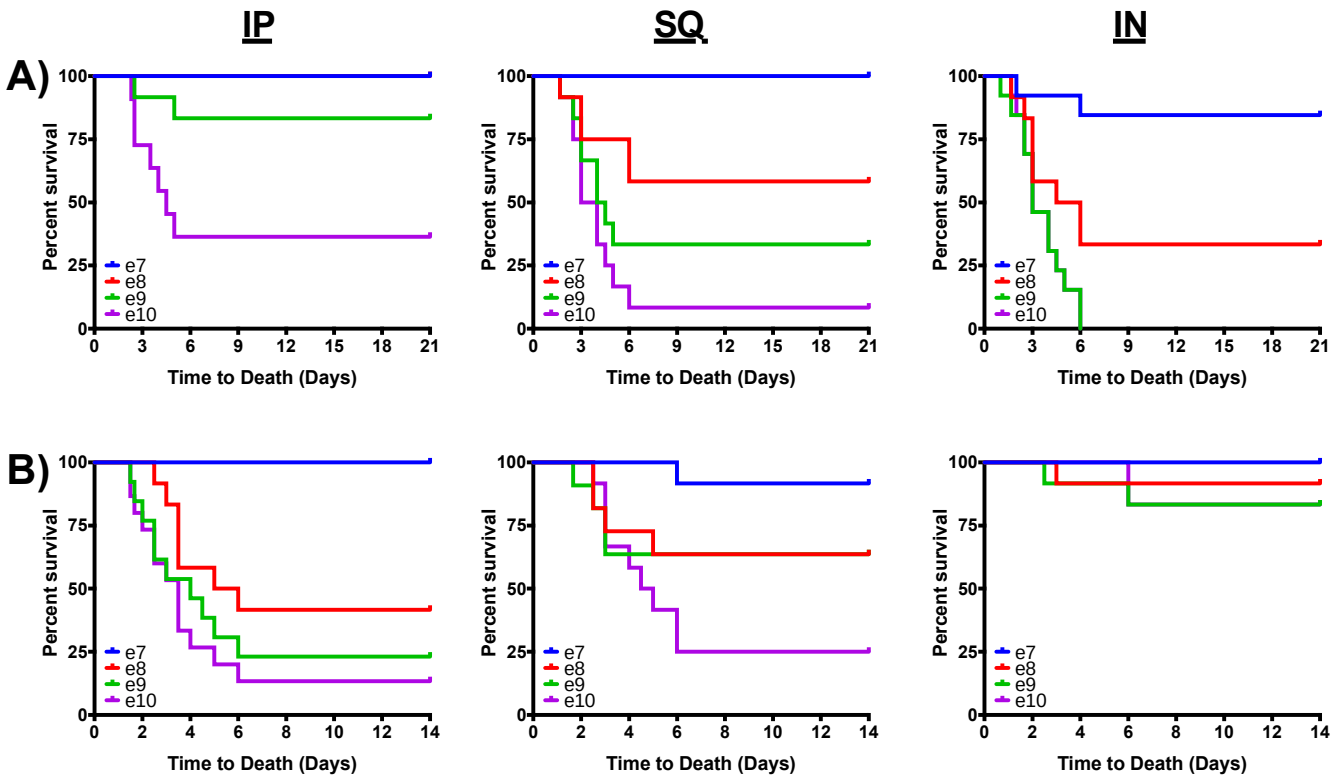


fig. S4. Challenge levels of D39. Hybrid vectors expressing antigens PspA, GlpO, PncO, StkP, and DexB were administered IP, SQ, or IN and challenged with four levels of D39 in sepsis (**A**) or pneumonia (**B**) models.

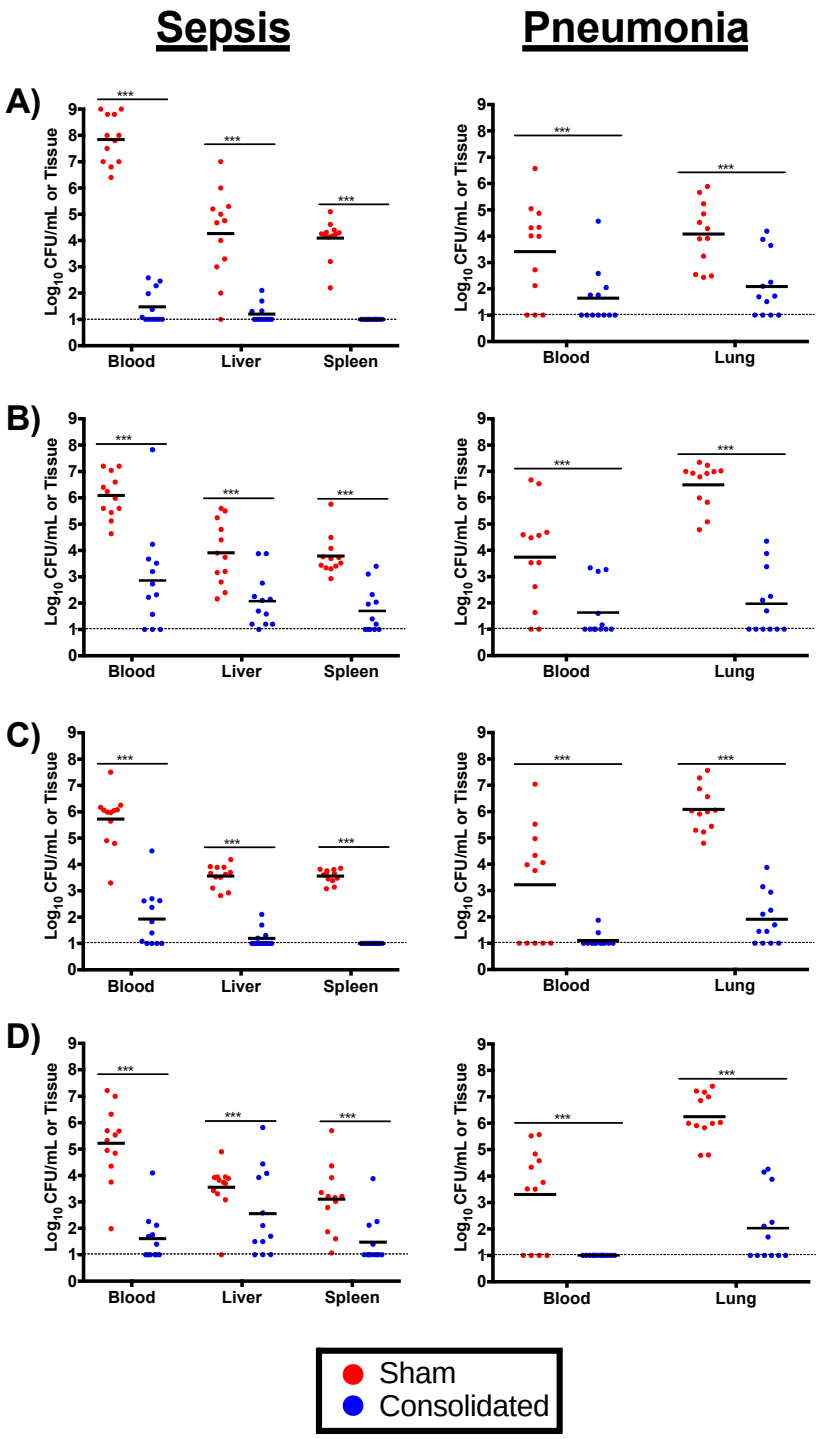


fig. S5. Bacterial burden assessed for hybrid vector vaccination with the consolidated antigens against pneumococcal challenge strains that included D39 (**A**), A66.1 (**B**), WU2 (**C**), and TIGR4 (**D**) in either a sepsis or pneumonia model. Dotted line represents limit of detection for bacterial counts; ***$P < 0.001$.

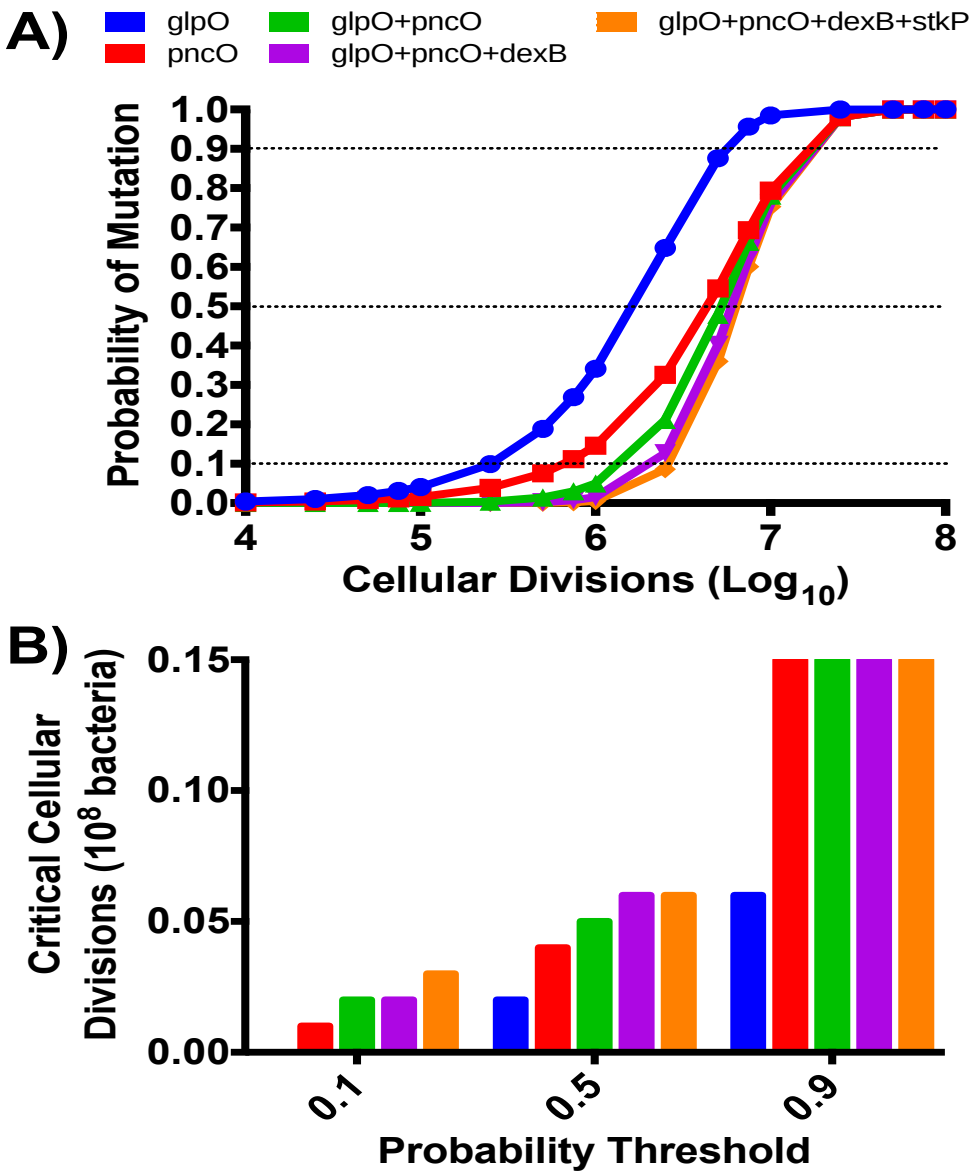


fig. S6. Probability of mutations occurring in *S. pneumoniae* genes (*glpO*, *pncO*, *dexB*, and *stkP*) through cellular division. Probability of at least one mutation simultaneously occurring in *glpO* (blue); *pncO* (red); *glpO* and *pncO* (green); *glpO*, *pncO*, and *dexB* (purple); and *glpO*, *pncO*, *dexB*, and *stkP* (orange) over 10^8 cellular divisions (**A**). Critical number of cellular divisions required to achieve a 10%, 50%, and 90% probability that at least one mutation will occur in each gene (**B**). A rate of 4.8×10^{-4} beneficial mutations per genome per duplication (*23*) and a genome size of 2.1 Mbp were used in these calculations.

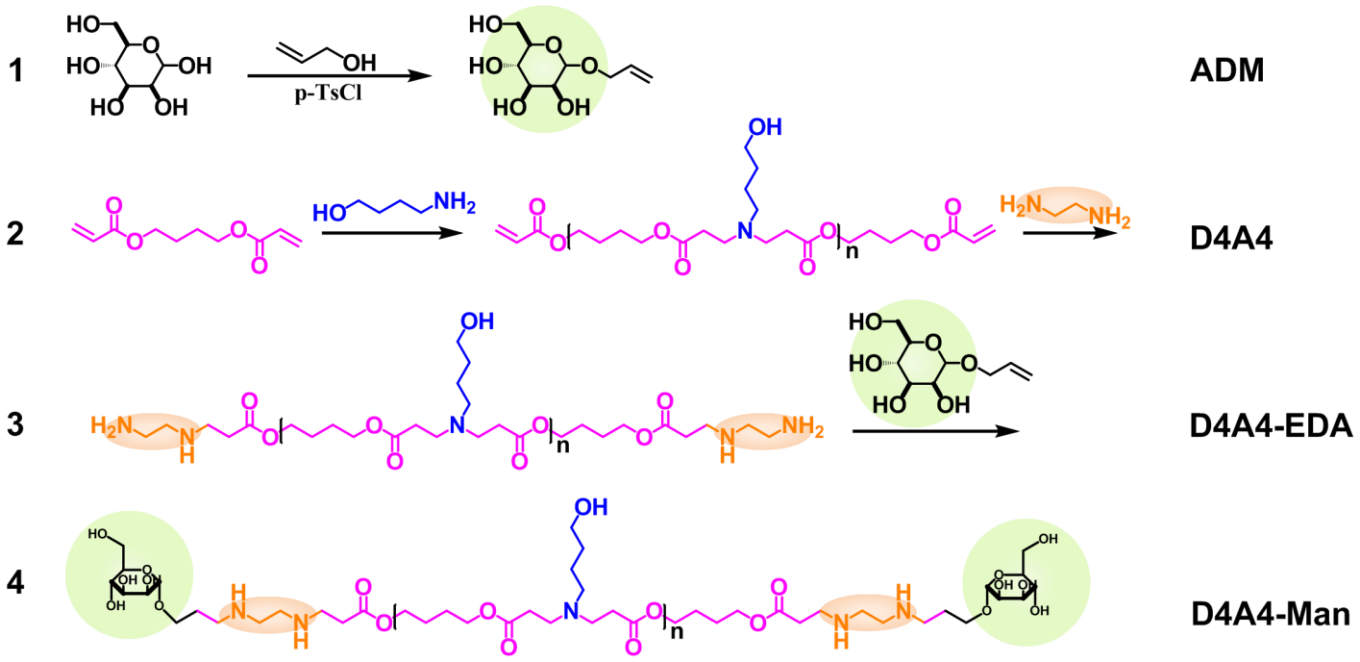
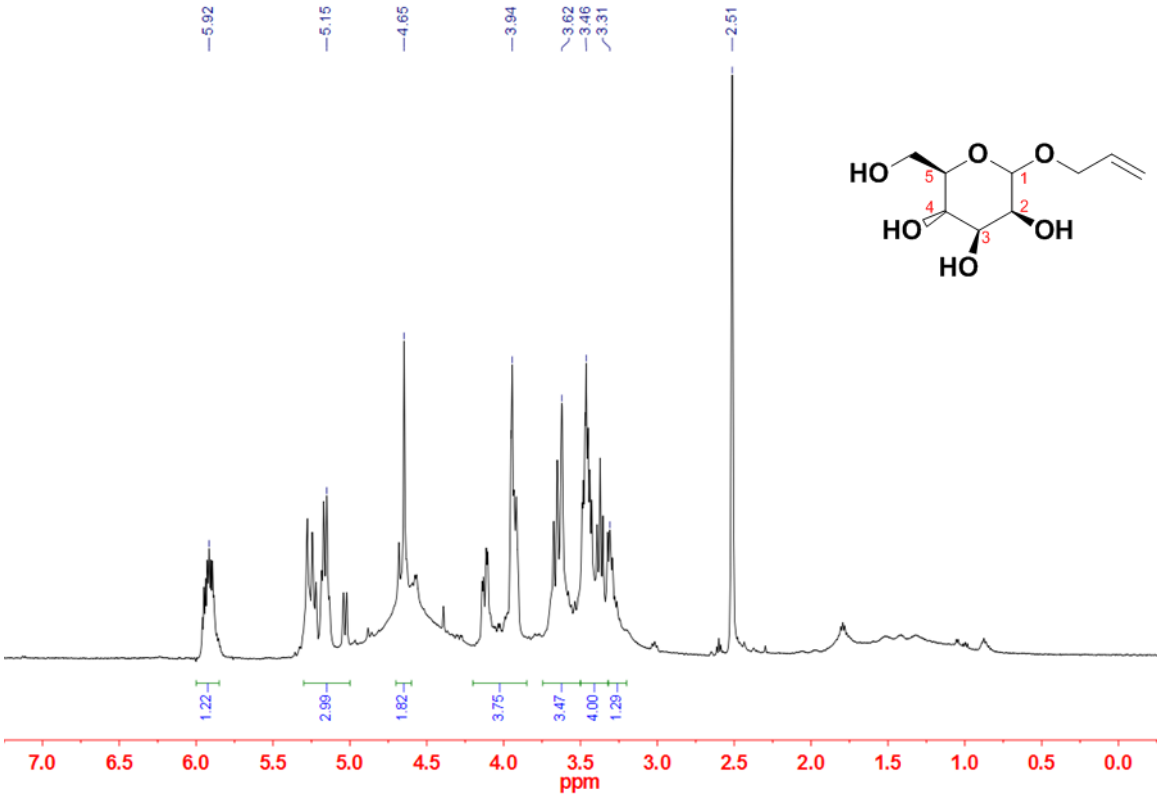


fig. S7. Synthetic scheme for PBAE D4A4-Man. Once generated, D4A4-Man was used as the coating of the hybrid vector. ^{1}H-NMR characterization data is provided below and was used to calculate purity of the final D4A4-Man polymer by comparing the hydrogen content of mannose signals in the 3.55-3.7 ppm region to backbone polymer signals in the 1.3-1.5, 2.55-2.8, and 3.9-4.15 ppm regions (similar to the approach used in reference 19); resulting polymer purity was calculated at 96%.

fig. S7 (cont.). NMR spectra: compounds were characterized in d-DMSO by ^{1}H-NMR spectroscopy using a Varian INOVA-500 (500 MHz) spectrometer maintained at 25ºC.

Legend: br: broad; d: doublet; t: triplet; q: quartet; m: multiple



ADM:

3.2-3.35 (H, m, C***H*** at 3 position)

3.35-3.5 (4H, m, C***H*** at 4 and 5 positions and CHC***H*****$_2$**OH)

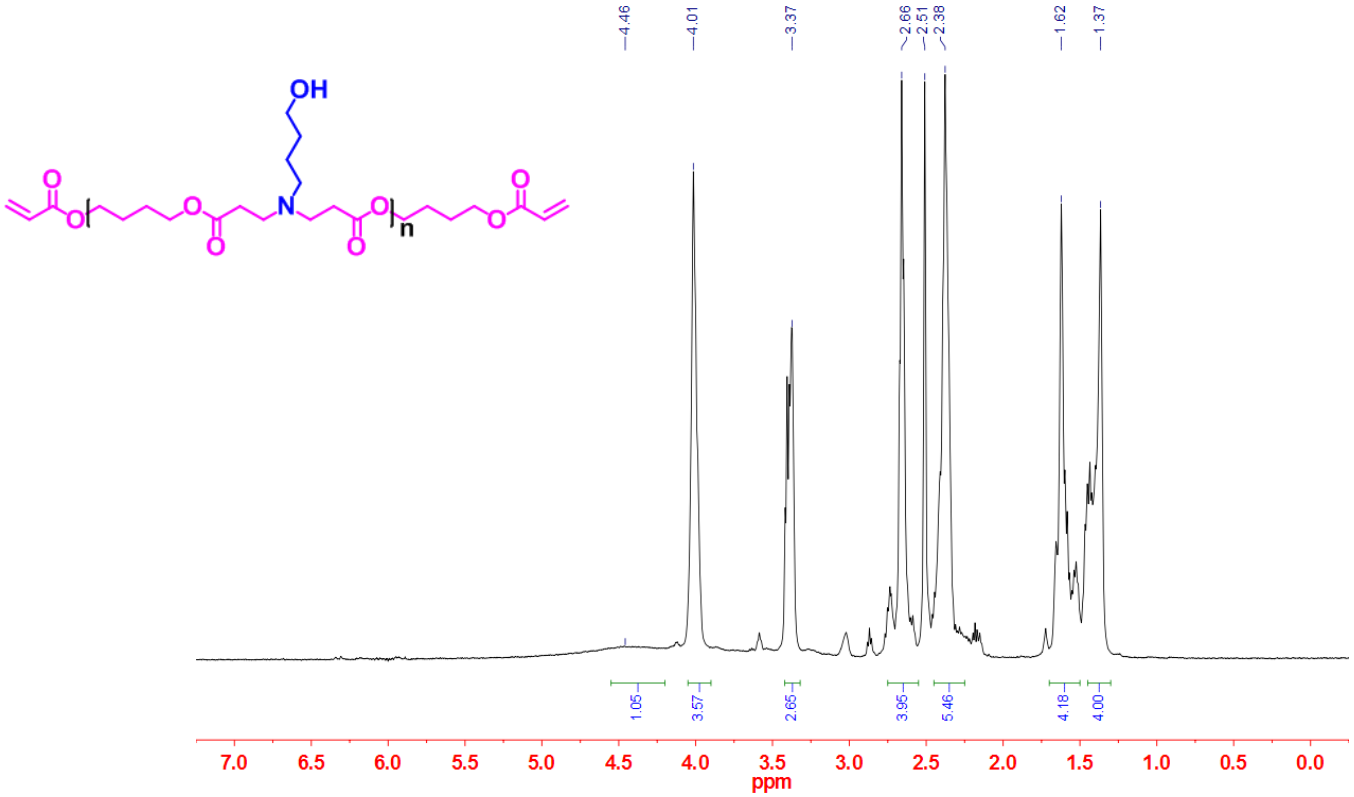
3.5-3.75 (4H, q, 3×CHO***H*** and CHCH$_2$O***H***)

3.85-4.2 (3H, m, C***H*** at 2 position and CHOC***H*****$_2$**CH=CH$_2$)

4.6-4.7 (H, d, C***H*** at 1 position: C***H***OCH$_2$CH=CH$_2$)

5.0-5.3 (2H, m, CHOCH$_2$CH=C***H*****$_2$**)

5.8-6.0 (H, m, CHOCH$_2$C***H***=CH$_2$)



D4A4:

1.3-1.45 (4H, br, $NCH_2C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2OH$)

1.5-1.7 (4H, br, $CH_2C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2(COO)CH_2CH_2NCH_2CH_2(COO)$)

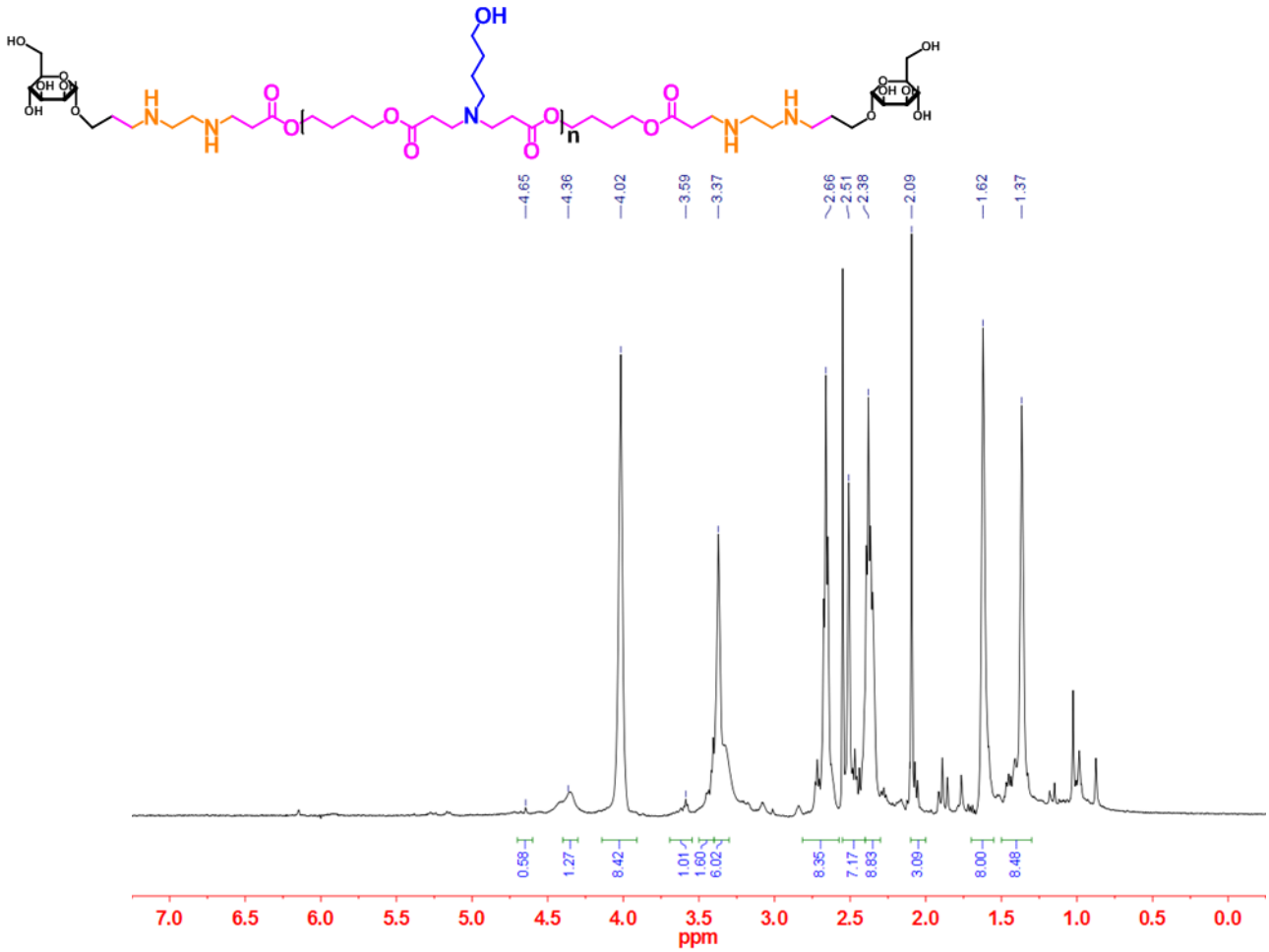
2.25-2.45 (6H, br, $NC\boldsymbol{H_2}CH_2CH_2CH_2OH$ and $CH_2CH_2CH_2CH_2(COO)C\boldsymbol{H_2}CH_2NCH_2C\boldsymbol{H_2}(COO)$)

2.55-2.75 (4H, t, $CH_2CH_2CH_2CH_2(COO)CH_2C\boldsymbol{H_2}NC\boldsymbol{H_2}CH_2(COO)$)

3.3-3.4 (2H, br, $NCH_2CH_2CH_2C\boldsymbol{H_2}OH$)

3.9-4.05 (4H, br, $C\boldsymbol{H_2}CH_2CH_2C\boldsymbol{H_2}(COO)CH_2CH_2NCH_2CH_2(COO)$)

4.2-4.55 (H, br, $NCH_2CH_2CH_2O\boldsymbol{H}$)



D4A4-Man:

1.3-1.5 (4H, br, $NCH_2C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2OH$)

1.55-1.7 (6H, m, $CH_2C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2(COO)CH_2CH_2NCH_2CH_2(COO)$ and $NHCH_2CH_2NHC\boldsymbol{H_2}CH_2$)

2.0-2.1 (H, br, N***H***)

2.3-2.4 (6H, br, $NC\boldsymbol{H_2}CH_2CH_2CH_2OH$) and $CH_2CH_2CH_2CH_2(COO)C\boldsymbol{H_2}CH_2NCH_2C\boldsymbol{H_2}(COO)$)

2.4-2.55 (6H, m, $NHC\boldsymbol{H_2}C\boldsymbol{H_2}NHC\boldsymbol{H_2}CH_2$)

2.55-2.8 (4H, t, $CH_2CH_2CH_2CH_2(COO)CH_2C\boldsymbol{H_2}NC\boldsymbol{H_2}CH_2(COO)$)

3.3-3.4 (5H, br, $NCH_2CH_2CH_2C\boldsymbol{H_2}OH$, $NHCH_2CH_2NHCH_2CH_2C\boldsymbol{H_2}$, and C***H*** at 3 position of mannose)

3.4-3.5 (4H, m, C***H*** at 4 and 5 position and $CHC\boldsymbol{H_2}OH$ of mannose)

3.55-3.7 (4H, q, 3×CHO***H*** and $CHCH_2O\boldsymbol{H}$ of mannose)

3.9-4.15 (4H, m, $C\boldsymbol{H_2}CH_2CH_2C\boldsymbol{H_2}(COO)CH_2CH_2NCH_2CH_2(COO)$ and C***H*** at 2 position of mannose)

4.3-4.4 (H, br, $NCH_2CH_2CH_2O\boldsymbol{H}$)

4.6-4.7 (H, d, C***H*** at 1 position of mannose)

table S1. DexB, GlpO, StkP, and PncO antigen description and analysis.

			Virulent Gene Expression (log2) Relative to				Strain Conservation (% Homology)		
Gene	**Size (bp)**	**Function**	**Planktonic**	**Biofilm**	**Average log2 Fold Change**	**Surface Accessible**	**Full Protein**	**Surface Accessible Regions**	**Surface Accessible Epitopes**
dexB	1,608	Glucan 1,6-α-glucosidase	1.5	1.5	1.5	No	98%	N/A	N/A
glpO	1,827	α-glycerophosphate oxidase	9	5.9	7.4	Yes	99%	98%	98%
stkP	1,980	Serine/threonine protein kinase	0.7	0.7	0.7	Yes	99%	99%	99.4%
pncO	690	Bacteriocin ABC transporter transmembrane protein	8.5	4.8	6.6	Yes	95%	93%	97%

table S2. Antigen cloning summary.

Antigen Gene	Primers	Source	Restriction Sites	Plasmid	Name
dexB	F: TAAGCACATATGCAAGAAAAATGGTGGCATAATGCCGTAG R: TAAGCACTCGAGTTCCACACAGAAAGCATCCCA	D39	*Nde*I/*Xho*I	pET21c	pCJ05
glpO	F: TAAGCAGAGCTCGAATTTTCAAAAAAAACACGTGAATTGTC R: TAAGCACTCGAGATTTTTTAATTCTGCTAAATCGTTGTTAG	D39	*Sac*I/*Xho*I	pET21c	pCJ07
stkP	F: TAAGCACATATGATCCAAATCGGCAAGATTTT R: TAAGCAGCGGCCGCAGGAGTAGCTGAAGTTGTTTTA	D39	*Nde*I/*Not*I	pET21c	pCJ08
pncO	F: TAAGCACATATGAAAAAGTATCAACTTCTATT R: TAAGCACTCGAGCCCCAAGACCCTATGTAGAAAA	EF3030	*Nde*I/*Xho*I	pET21c	pCJ10

table S3. Consolidated plasmid (pLF) cloning summary.

Antigen Gene	Primers	Source	Restriction Sites
dexB	F: GCGGGATCCCAAGAAAAATGGTGGCATAATGCCGTAG R: ATAGGCGCGCCTTATAGTAATTCCACACAG	pCJ05	*Bam*HI/*Asc*I
glpO	F: GCGGTCGACAAGGAGATATAATGGAATTTTCAAAAAAAAC R: GCGGCGGCCGCTTAATTTTTTAATTCTGC	pCJ07	*Sal*I/*Not*I
stkP	F: GCGCATATGATCCAAATCGGCAAGATTTTTG R: GCGCAATTGTTAAGGAGTAGCTGAAGTTGTTTTAG	pCJ08	*Nde*I/*Mun*I
pspA	F: ATAGGCCGGCCAAGGAGATATAATGGAAGAATCTCCCGTAGCCA R: ATACTCGAGTTATTCTGGGGCTGGAGTTTCTGGA	pUAB055	*Fse*I/*Xho*I

table S4. *S. pneumoniae* strains used in the current study. Green highlighted strains are not currently included in commercial vaccine formulations.

Strain	Capsule Type (Serotype)	Virulence Pattern	Included in Current Vaccines	This Study Protection %
D39	2	1	Yes	100%
DBL2	2	2	Yes	66%
A66.1	3	1	Yes	100%
WU2	3	1	Yes	100%
ATCC6303	3	2	Yes	100%
3JYP2670	3	2	Yes	33%
TIGR4	4	2	Yes	100%
DBL5	5	2	Yes	100%
WCH16 – Heat Released	6A	4	Yes	50%
DBL6A	6B	2	Yes	100%
ATCC-6312 – Heat Released	12F	4	No	100%
ATCC-10354 – Heat Released	15B	4	No	100%
EF3030	19F	3	Yes	100%
EF3030 – Heat Released	19F	4	Yes	100%
ATCC-6324 – Heat Released	24	4	No	100%
ATCC-6327 – Heat Released	27	4	No	33%

Virulence Pattern Classification	Carriage	Lung Infection	Sepsis	Comments
1	-	++	+++	Bacteria carries poorly and transitions to the blood wherever placed
2	±	++	++	Bacteria causes strong infection wherever placed; occasionally will transition to the blood and cause death
3	++	+++	±	Bacteria carries well and infects locally but unlikely to kill; rarely transitions to the blood and causes death
4	++	+++	++	Bacteria carries well and causes strong infection wherever placed and can transition to the blood