

**Title:** Engineering a next-generation glycoconjugate-like *Streptococcus pneumoniae* vaccine

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**Supplementary Table 1: Microfloral bacteria with GIpO homologs tested for cross-reactivity with percent homology of protein regions indicated**

Species	Colonization Location	Full-Length	Average Homology	
			Surface-Accessible	Epitope
<i>Streptococcus mitis</i> *	Oral Cavity, Throat, Nasopharynx	98%	92%	99%
<i>Streptococcus oralis</i>	Oral Cavity	97%	97%	99%
<i>Streptococcus infantis</i> *	Upper Respiratory	97%	97%	97%
<i>Streptococcus cristatus</i>	Oral Cavity	95%	95%	95%
<i>Streptococcus parasanguinis</i>	Oral Cavity	93%	92%	95%
<i>Streptococcus sanguinis</i>	Oral Cavity	94%	94%	95%
<i>Streptococcus gordonii</i>	Oral Cavity	95%	94%	94%
<i>Streptococcus salivarius</i>	Oral Cavity, Upper Respiratory	94%	94%	93%
<i>Streptococcus dysgalactiae</i> *	Gastrointestinal and Genitourinary Tract	75%	75%	73%
<i>Streptococcus pyogenes</i>	Upper Respiratory, Skin	75%	74%	72%
<i>Lactobacillus iners</i>	Vaginal Tract	63%	64%	70%
<i>Streptococcus agalactiae</i> **	Gastrointestinal and Genitourinary Tract	71%	72%	68%
<i>Alloiococcus otitis</i> *	Nasopharynx	64%	64%	64%
<i>Enterococcus durans</i>	Gastrointestinal Tract	61%	61%	64%
<i>Lactobacillus plantarum</i> **	Gastrointestinal Tract	62%	63%	62%
<i>Enterococcus faecalis</i> *	Gastrointestinal Tract	63%	63%	62%
<i>Lactobacillus salivarius</i> **	Gastrointestinal Tract	60%	59%	61%
<i>Aerococcus christensenii</i> *	Vaginal Tract	60%	62%	61%
<i>Pediococcus acidilactici</i> **	Gastrointestinal Tract	58%	58%	58%
<i>Lactobacillus pentosus</i>	Vaginal Tract, Gastrointestinal Tract	63%	62%	58%

\*Evaluated for antibody binding via immunofluorescence assay

\*\*Evaluated for antibody binding and neutralization activity using OPA assay

**Supplementary Table 2: Complete OPA data for LEPS(20V):PncO dose escalation**

Serotype	PCV13		PPSV23		LEPS(20V):PncO (34 µg)		LEPS(20V):PncO (68 µg)		LEPS(20V):PncO (136 µg)	
	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR
1	150	---	126	---	120	45	205	150	189	128
2	---	---	101	---	110	145	136	150	172	208
3	120	---	30	---	112	182	136	196	132	230
4	48	---	51	---	182	486	233	192	224	571
5	56	---	92	---	53	366	73	608	62	545
6A	59	---	---	---	211	88	285	491	257	111
6B	50	---	30	---	178	18	235	96	207	30
6C	---	---	---	---	---	317	---	23	---	358
6D	---	---	---	---	---	440	---	289	---	489
7A	---	---	---	---	---	162	---	489	---	231
7B	---	---	---	---	---	94	---	174	---	120
7C	---	---	---	---	---	152	---	115	---	188
7F	180	---	33	---	171	44	234	172	227	72
8	---	---	81	---	135	70	168	56	198	81
9A	---	---	---	---	---	100	---	67	---	117
9N	---	---	59	---	20	397	24	117	27	529
9V	187	---	125	---	149	435	165	468	193	435
10A	---	---	150	---	---	47	---	392	---	83
10F	---	---	---	---	---	30	---	62	---	44
11A	---	---	96	---	---	59	---	40	---	76
11B	---	---	---	---	---	66	---	63	---	110
11C	---	---	---	---	---	82	---	86	---	96
12A	---	---	---	---	---	102	---	86	---	108
12B	---	---	---	---	---	354	---	95	---	450
12F	---	---	38	---	161	366	190	429	221	515
13	---	---	---	---	---	126	---	472	---	158
14	105	---	37	---	51	221	63	122	58	230
15A	---	---	---	---	---	61	---	234	---	88
15B	---	---	172	---	---	386	---	80	---	382
15C	---	---	---	---	---	306	---	375	---	485
15F	---	---	---	---	---	196	---	401	---	305
16A	---	---	---	---	---	182	---	229	---	295
16F	---	---	---	---	---	230	---	246	---	311

17A	---	---	---	---	---	283	---	251	---	312
17F	---	---	110	---	126	229	139	260	125	345
18A	---	---	---	---	---	133	---	264	---	163
18B	---	---	---	---	---	303	---	163	---	379
18C	180	---	48	---	212	393	282	333	245	612
18F	---	---	---	---	---	173	---	523	---	198
19A	180	---	134	---	180	76	243	185	224	96
19C	---	---	---	---	---	172	---	75	---	267
19F	200	---	55	---	326	322	365	217	325	519
20	---	---	43	---	102	201	122	425	122	245
21	---	---	---	---	---	322	---	211	---	490
22A	---	---	---	---	---	406	---	412	---	655
22F	---	---	79	---	201	432	272	504	253	545
23A	---	---	---	---	---	72	---	510	---	70
23B	---	---	---	---	---	353	---	66	---	615
23F	170	---	15	---	81	333	97	455	106	365
24A	---	---	---	---	---	23	---	376	---	22
24B	---	---	---	---	---	284	---	21	---	256
24F	---	---	---	---	---	80	---	272	---	96
25F	---	---	---	---	---	269	---	100	---	328
27	---	---	---	---	---	108	---	288	---	151
28A	---	---	---	---	---	214	---	141	---	193
28F	---	---	---	---	---	358	---	197	---	470
29	---	---	---	---	---	82	---	351	---	121
30	---	---	---	---	---	274	---	96	---	325
31	---	---	---	---	---	199	---	346	---	240
33A	---	---	---	---	---	401	---	261	---	430
33F	---	---	20	---	---	199	---	361	---	241
34	---	---	---	---	---	320	---	217	---	414
35A	---	---	---	---	---	109	---	324	---	118
35B	---	---	---	---	---	61	---	109	---	100
35C	---	---	---	---	---	212	---	80	---	304
35F	---	---	---	---	---	32	---	240	---	42
37	---	---	---	---	---	161	---	42	---	211
38	---	---	---	---	---	92	---	187	---	118
39	---	---	---	---	---	231	---	109	---	266
41A	---	---	---	---	---	320	---	274	---	415
42F	---	---	---	---	---	188	---	349	---	279

**Supplementary Table 3: Complete OPA data for LEPS(20V):GlpO+PncO dose escalation**

Serotype	PCV13		PPSV23		LEPS(20V):GlpO+PncO (68 µg)		LEPS(20V):GlpO+PncO (136 µg)		LEPS(20V):GlpO+PncO (272 µg)	
	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR
1	150	---	126	---	176	45	219	150	146	128
2	---	---	101	---	110	145	212	150	143	208
3	120	---	30	---	144	182	143	196	184	230
4	48	---	51	---	222	486	233	192	191	571
5	56	---	92	---	63	366	52	608	63	545
6A	59	---	---	---	302	88	287	491	269	111
6B	50	---	30	---	289	18	263	96	347	30
6C	---	---	---	---	---	317	---	23	---	358
6D	---	---	---	---	---	440	---	289	---	489
7A	---	---	---	---	---	162	---	489	---	231
7B	---	---	---	---	---	94	---	174	---	120
7C	---	---	---	---	---	152	---	115	---	188
7F	180	---	33	---	245	44	247	172	196	72
8	---	---	81	---	154	70	178	56	171	81
9A	---	---	---	---	---	100	---	67	---	117
9N	---	---	59	---	28	397	23	117	26	529
9V	187	---	125	---	208	435	189	468	181	435
10A	---	---	150	---	---	47	---	392	---	83
10F	---	---	---	---	---	30	---	62	---	44
11A	---	---	96	---	---	59	---	40	---	76
11B	---	---	---	---	---	66	---	63	---	110
11C	---	---	---	---	---	82	---	86	---	96
12A	---	---	---	---	---	102	---	86	---	108
12B	---	---	---	---	---	354	---	95	---	450
12F	---	---	38	---	225	366	241	429	247	515
13	---	---	---	---	---	126	---	472	---	158
14	105	---	37	---	57	221	100	122	157	230
15A	---	---	---	---	---	61	---	234	---	88
15B	---	---	172	---	---	386	---	80	---	382
15C	---	---	---	---	---	306	---	375	---	485
15F	---	---	---	---	---	196	---	401	---	305
16A	---	---	---	---	---	182	---	229	---	295
16F	---	---	---	---	---	230	---	246	---	311

17A	---	---	---	---	---	283	---	251	---	312
17F	---	---	110	---	168	229	111	260	208	345
18A	---	---	---	---	---	133	---	264	---	163
18B	---	---	---	---	---	303	---	163	---	379
18C	180	---	48	---	260	393	216	333	278	612
18F	---	---	---	---	---	173	---	523	---	198
19A	180	---	134	---	216	76	190	185	182	96
19C	---	---	---	---	---	172	---	75	---	267
19F	200	---	55	---	372	322	273	217	420	519
20	---	---	43	---	129	201	103	425	123	245
21	---	---	---	---	---	322	---	211	---	490
22A	---	---	---	---	---	406	---	412	---	655
22F	---	---	79	---	285	432	258	504	285	545
23A	---	---	---	---	---	72	---	510	---	70
23B	---	---	---	---	---	353	---	66	---	615
23F	170	---	15	---	115	333	125	455	138	365
24A	---	---	---	---	---	23	---	376	---	22
24B	---	---	---	---	---	284	---	21	---	256
24F	---	---	---	---	---	80	---	272	---	96
25F	---	---	---	---	---	269	---	100	---	328
27	---	---	---	---	---	108	---	288	---	151
28A	---	---	---	---	---	214	---	141	---	193
28F	---	---	---	---	---	358	---	197	---	470
29	---	---	---	---	---	82	---	351	---	121
30	---	---	---	---	---	274	---	96	---	325
31	---	---	---	---	---	199	---	346	---	240
33A	---	---	---	---	---	401	---	261	---	430
33F	---	---	20	---	---	199	---	361	---	241
34	---	---	---	---	---	320	---	217	---	414
35A	---	---	---	---	---	109	---	324	---	118
35B	---	---	---	---	---	61	---	109	---	100
35C	---	---	---	---	---	212	---	80	---	304
35F	---	---	---	---	---	32	---	240	---	42
37	---	---	---	---	---	161	---	42	---	211
38	---	---	---	---	---	92	---	187	---	118
39	---	---	---	---	---	231	---	109	---	266
41A	---	---	---	---	---	320	---	274	---	415
42F	---	---	---	---	---	188	---	349	---	279

**Supplementary Table 4: LEPS(24V):PncO planktonic serotype OPA data**

Serotype	PCV13	PPSV23	LEPS(24V):CRM197	LEPS(24V):PncO
1	150	126	156	178
2	---	101	129	134
3	120	30	110	113
4	48	51	211	209
5	56	92	105	210
6A	59	---	254	312
6B	50	30	217	217
7F	180	33	169	186
8	---	81	147	181
9N	---	59	88	108
9V	187	125	165	148
10A	---	150	178	196
11A	---	96	205	205
12F	---	38	179	186
14	105	37	165	172
15B	---	172	146	146
17F	---	110	155	154
18C	180	48	257	272
19A	180	134	211	232
19F	200	55	210	189
20	---	43	114	139
22F	---	79	255	232
23F	170	15	182	189
33F	---	20	205	262

**Supplementary Table 5: Size and charge characterization of LEPS variants**

Attachment Strategy	Size (nm)	Zeta Potential (mV)
<b>[Ni-NTA]</b>		
Empty	110.0 ± 17.6	-11.0 ± 1.4
20-Valent	193.5 ± 13.8	-10.2 ± 2.8
20-Valent + Protein	179.2 ± 20.6	-17.9 ± 2.2
24-Valent	249.1 ± 9.0	-12.3 ± 1.6
24-Valent + Protein	286.7 ± 20.2	-15.9 ± 2.7
<b>[Co-NTA]</b>		
Empty	113.3 ± 22.5	-9.8 ± 1.7
20-Valent	181.9 ± 14.2	-11.8 ± 3.2
20-Valent + Protein	225.8 ± 16.5	-17.5 ± 2.4
24-Valent	221.3 ± 10.8	-11.8 ± 1.6
24-Valent + Protein	237.9 ± 17.8	-13.5 ± 3.4
<b>BSB</b>		
Empty	128.5 ± 14.2	-13.7 ± 2.3
20-Valent	249.2 ± 18.9	-16.4 ± 2.0
20-Valent + Protein	207.1 ± 19.7	-23.1 ± 2.7
24-Valent	255.3 ± 13.6	-16.4 ± 1.9
24-Valent + Protein	275.4 ± 21.9	-22.2 ± 1.8
<b>BS</b>		
Empty	138.0 ± 16.3	-15.5 ± 2.6
20-Valent	225.3 ± 11.6	-14.1 ± 2.0
20-Valent + Protein	232.5 ± 19.2	-20.5 ± 1.4
24-Valent	256.3 ± 16.0	-16.6 ± 2.4
24-Valent + Protein	265.1 ± 15.5	-22.0 ± 1.2



**Supplementary Table 6: Body and organ weight of mice post vaccination**

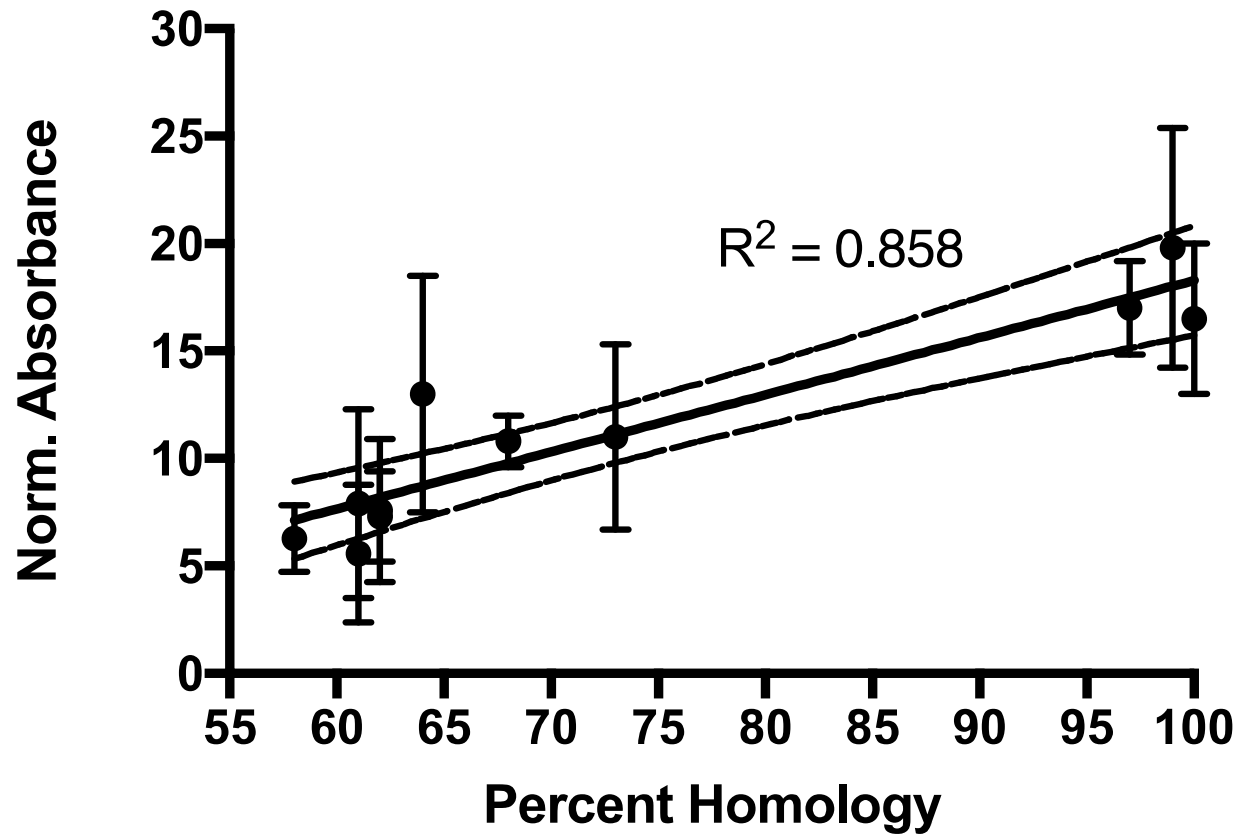
Animal	Sham	Alum:PncO			LEPS(20V):CRM197		
		1×	5×	10×	1×	5×	10×
Terminal Weight	-						
<b>Male CD-1 Mice (N=5)</b>							
Body Weight (g)	30.3 ± 3.1	30.6 ± 2.1	29.7 ± 2.3	31.8 ± 2.4	31.5 ± 2.9	30.3 ± 1.8	31.5 ± 2.5
Brain Weight (mg)	472.0 ± 26	462.6 ± 20	472.0 ± 25	463.4 ± 26	467.8 ± 16	472.6 ± 19	468.6 ± 20
Lung Weight (mg)	180.0 ± 15	185.4 ± 23	174.6 ± 25	174.6 ± 6	171.0 ± 17	185.4 ± 18	171.0 ± 15
Heart Weight (mg)	175.0 ± 23	169.8 ± 15	180.3 ± 28	166.3 ± 17	171.5 ± 15	173.3 ± 26	182.0 ± 21
Liver Weight (g)	1.8 ± 0.23	1.8 ± 0.22	1.8 ± 0.18	1.8 ± 0.25	1.7 ± 0.17	1.9 ± 0.15	1.7 ± 0.3
Kidney Weight (mg)	440.0 ± 62	422.4 ± 30	421.3 ± 41	453.3 ± 20	465.1 ± 32	453.2 ± 31	449.7 ± 22
<b>Female CD-1 Mice (N=5)</b>							
Body Weight (g)	26.0 ± 1.5	27.0 ± 2.8	25.7 ± 2.8	26.3 ± 1.7	26.3 ± 2.3	26.7 ± 2.5	26.5 ± 2.1
Brain Weight (mg)	460.0 ± 20	469.2 ± 28	433.4 ± 17	446.2 ± 30	461.1 ± 17	473.8 ± 16	455.4 ± 17
Lung Weight (mg)	170.0 ± 21	176.8 ± 25	161.5 ± 20	173.4 ± 25	178.5 ± 23	161.5 ± 30	173.4 ± 16
Heart Weight (mg)	150.0 ± 20	156.0 ± 25	154.5 ± 27	157.5 ± 29	144.0 ± 30	148.5 ± 29	145.5 ± 19
Liver Weight (g)	1.3 ± 0.11	1.3 ± 0.29	1.3 ± 0.26	1.3 ± 0.15	1.2 ± 0.16	1.3 ± 0.23	1.3 ± 0.18
Kidney Weight (mg)	280.0 ± 33	282.8 ± 55	282.8 ± 28	280.0 ± 51	294.0 ± 27	294.0 ± 37	282.2 ± 53

**Supplementary Table 7: Percent change in OPA 50% killing dilution of planktonic *S. pneumoniae* for LEPS(20V):PncO protein dose escalation**

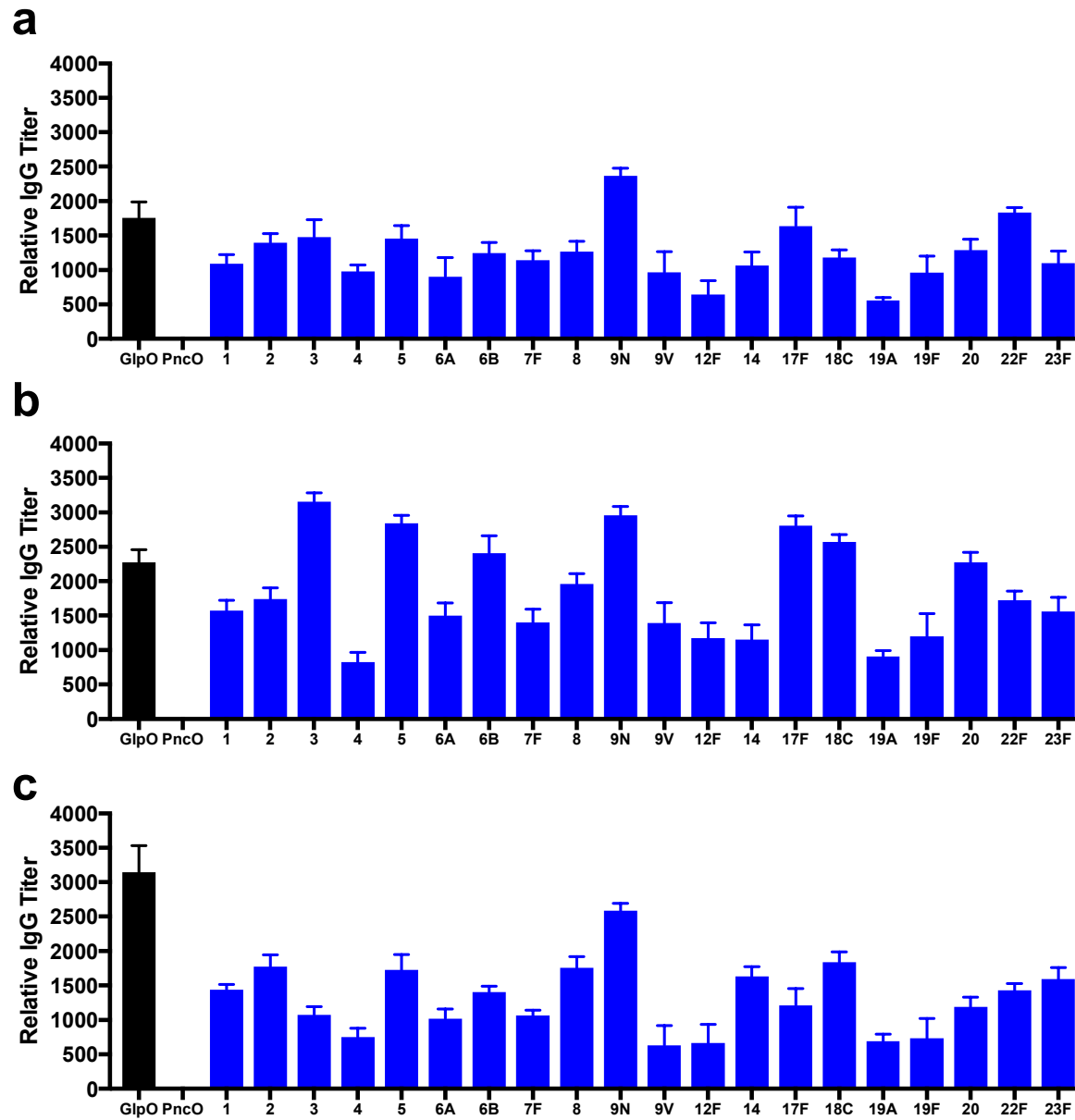
Serotype	LEPS(20V):PncO OPA Titer Percent Change	
	34 → 68 µg	68 → 136 µg
1	71%	-8%
2	24%	26%
3	21%	-3%
4	28%	-4%
5	39%	-16%
6A	35%	-10%
6B	32%	-12%
7F	37%	-3%
8	24%	18%
9N	17%	14%
9V	11%	17%
12F	18%	16%
14	23%	-7%
17F	10%	-10%
18C	33%	-13%
19A	35%	-8%
19F	12%	-11%
20	20%	0%
22F	35%	-7%
23F	20%	9%

**Supplementary Table 8: Percent change in antibody titer for LEPS(20V):PncO protein dose escalation**

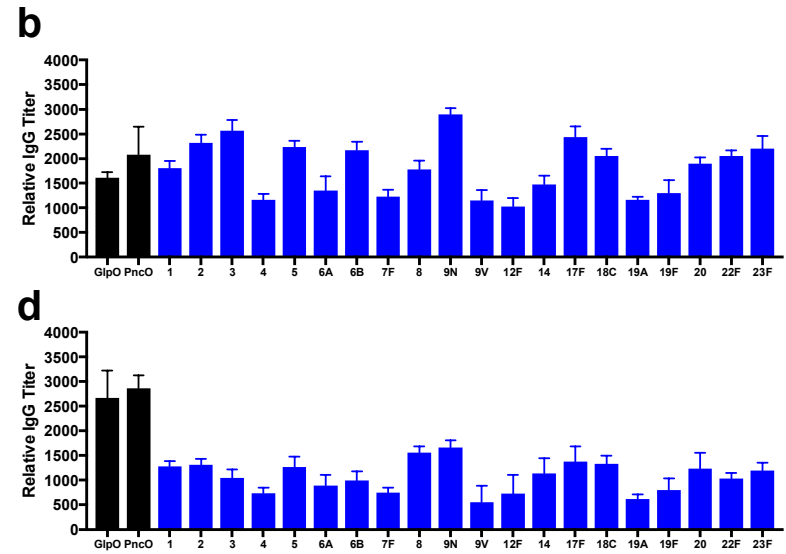
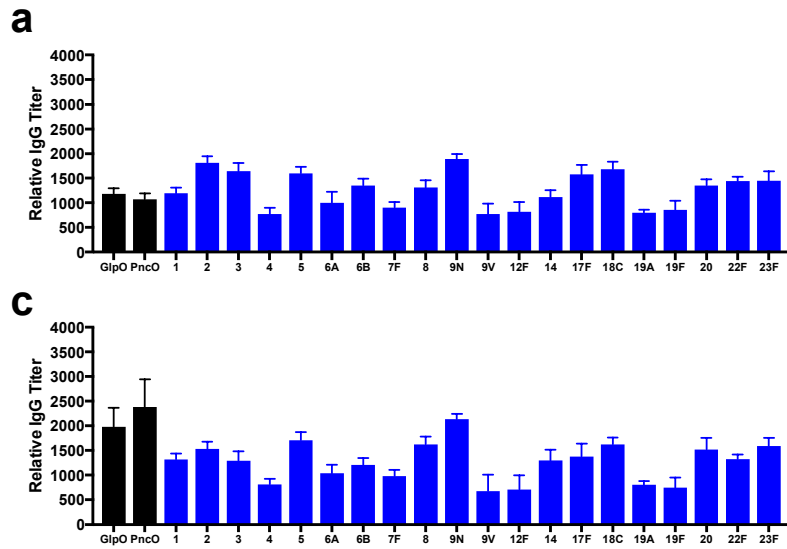
Serotype	LEPS(20V):PncO Antibody Titer Percent Change	
	34 → 68 µg	68 → 136 µg
PncO	34%	49%
1	106%	-12%
2	38%	35%
3	70%	-69%
4	-42%	5%
5	119%	-57%
6A	97%	-42%
6B	76%	-43%
7F	66%	-40%
8	19%	17%
9N	-1%	-7%
9V	24%	-37%
12F	0%	-20%
14	47%	0%
17F	33%	-52%
18C	87%	-21%
19A	85%	-25%
19F	-20%	9%
20	1%	-21%
22F	-6%	-15%
23F	80%	-14%



**Supplementary Figure 1:** Correlation between immunosorbent assay results and bacterial homology with GlpO. Dotted lines and error bars represent the 95% confidence interval of four technical replicates.



**Supplementary Figure 2:** GlpO dose escalation antibody titers. Concentration of protein (black) and CPS (blue) antibodies in mice vaccinated with 34 (a), 68 (b), or 136 (c) µg of protein in LEPS(20V):GlpO formulation. Error bars represent the 95% confidence interval of four biological replicates.



**Supplementary Figure 3:** GlpO+PncO dose escalation antibody titers. Concentration of protein (black) and CPS (blue) antibodies in mice vaccinated with 34 (a), 68 (b), 136 (c), or 272 (d) µg of total protein in LEPS(20V):GlpO+PncO formulation. Error bars represent the 95% confidence interval of four biological replicates.