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Supplementary Materials for

Comprehensive vaccine design for commensal disease progression

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Supplementary Materials

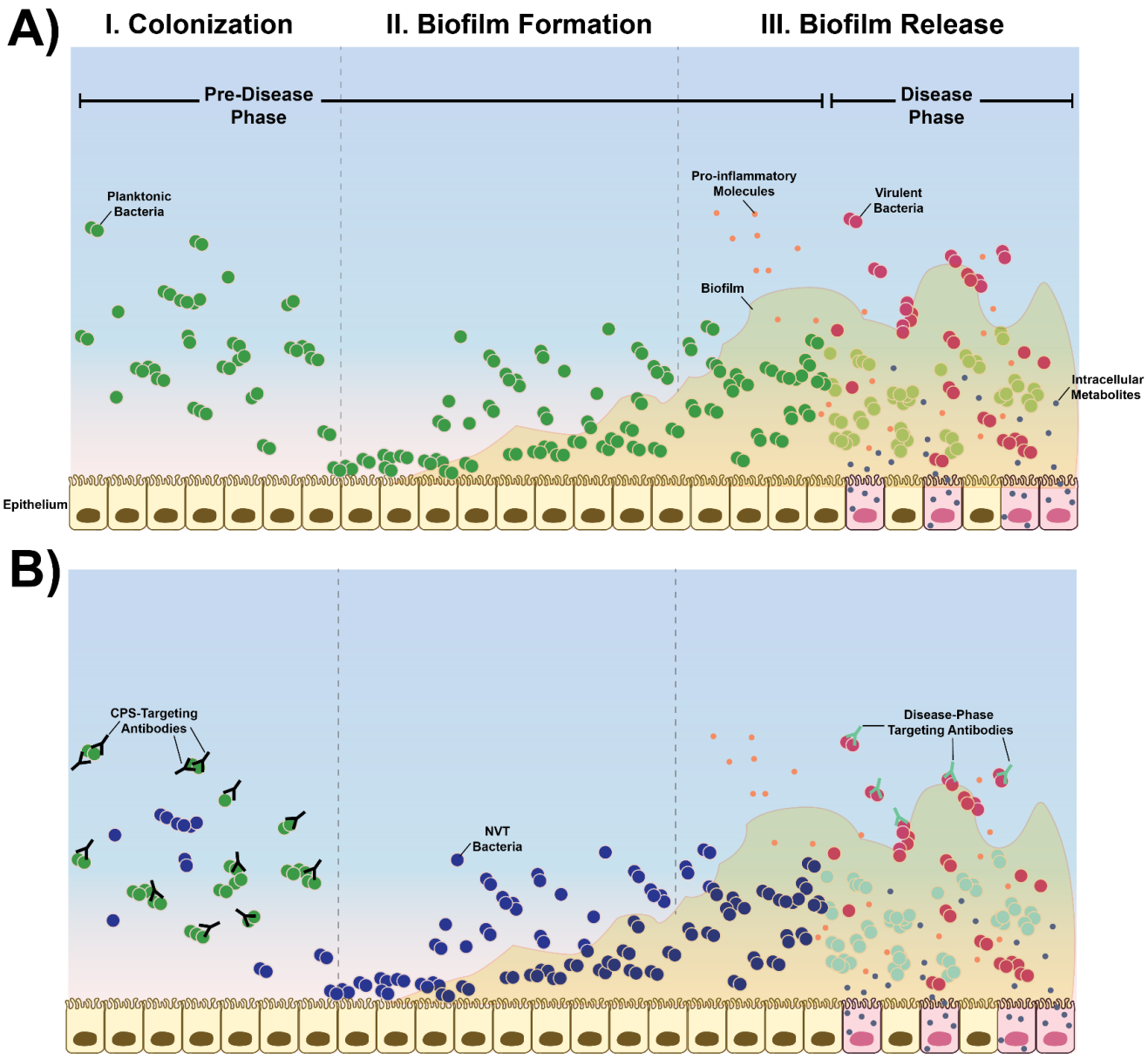

fig. S1. Commensal disease progression model featuring pneumococcal disease. (**A**) *S. pneumoniae* colonization, biofilm establishment, environmental stimulation to virulence, and biofilm release and disease symptoms. (**B**) Immune targets that span current vaccine options (Prevnar and Pneumovax; capsular polysaccharide [CPS] targeting of specific serotypes) and the LEPS platform (addressing both colonization of currently-covered vaccine serotypes and biofilm release of nonvaccine-type [NVT] serotypes).

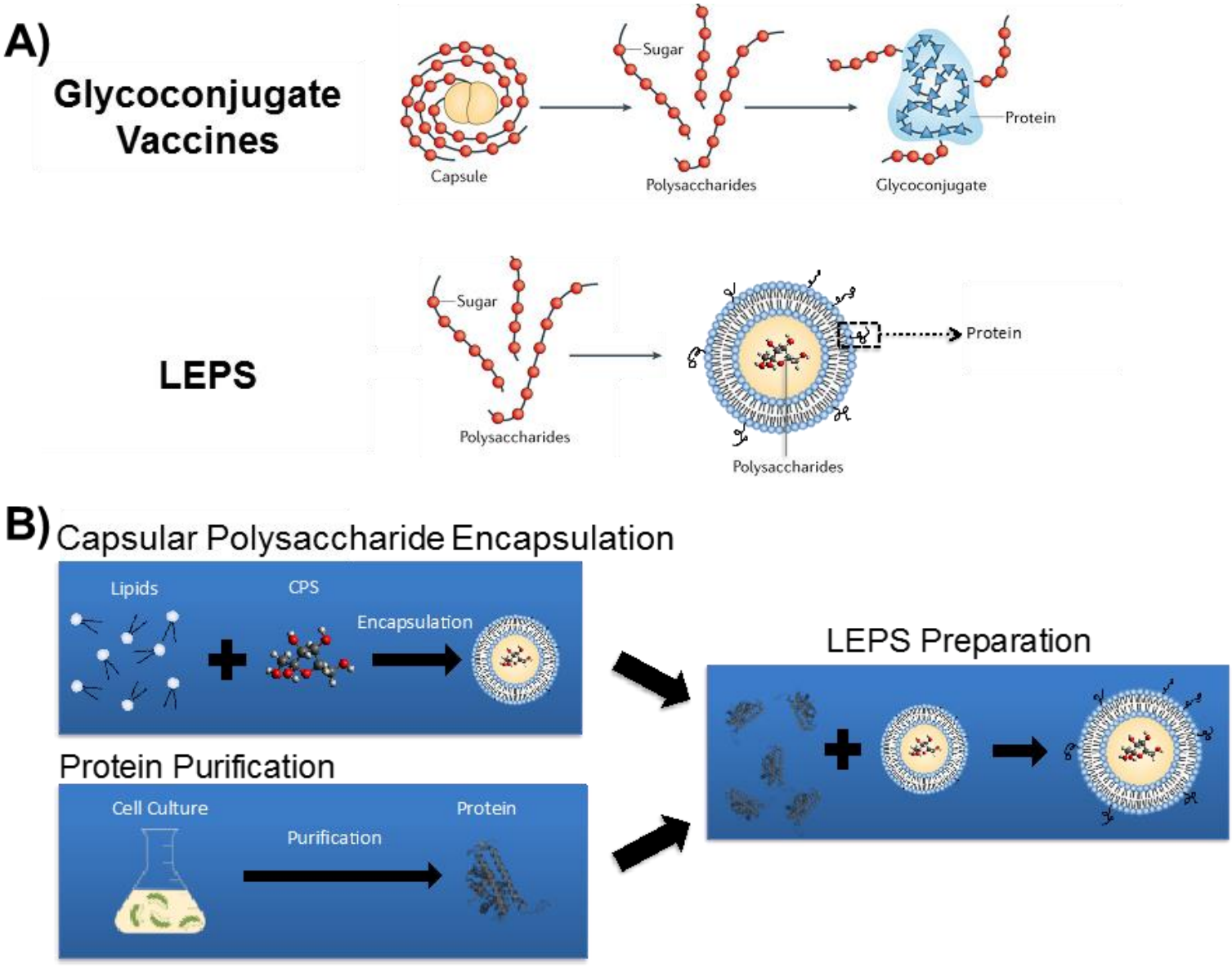

fig. S2. The LEPS platform. (**A**) Schematic comparison of a glycoconjugate vaccine (e.g., the Prevnar family) and LEPS. (**B**) Schematic representation of the formulation procedure to generate the LEPS particle.

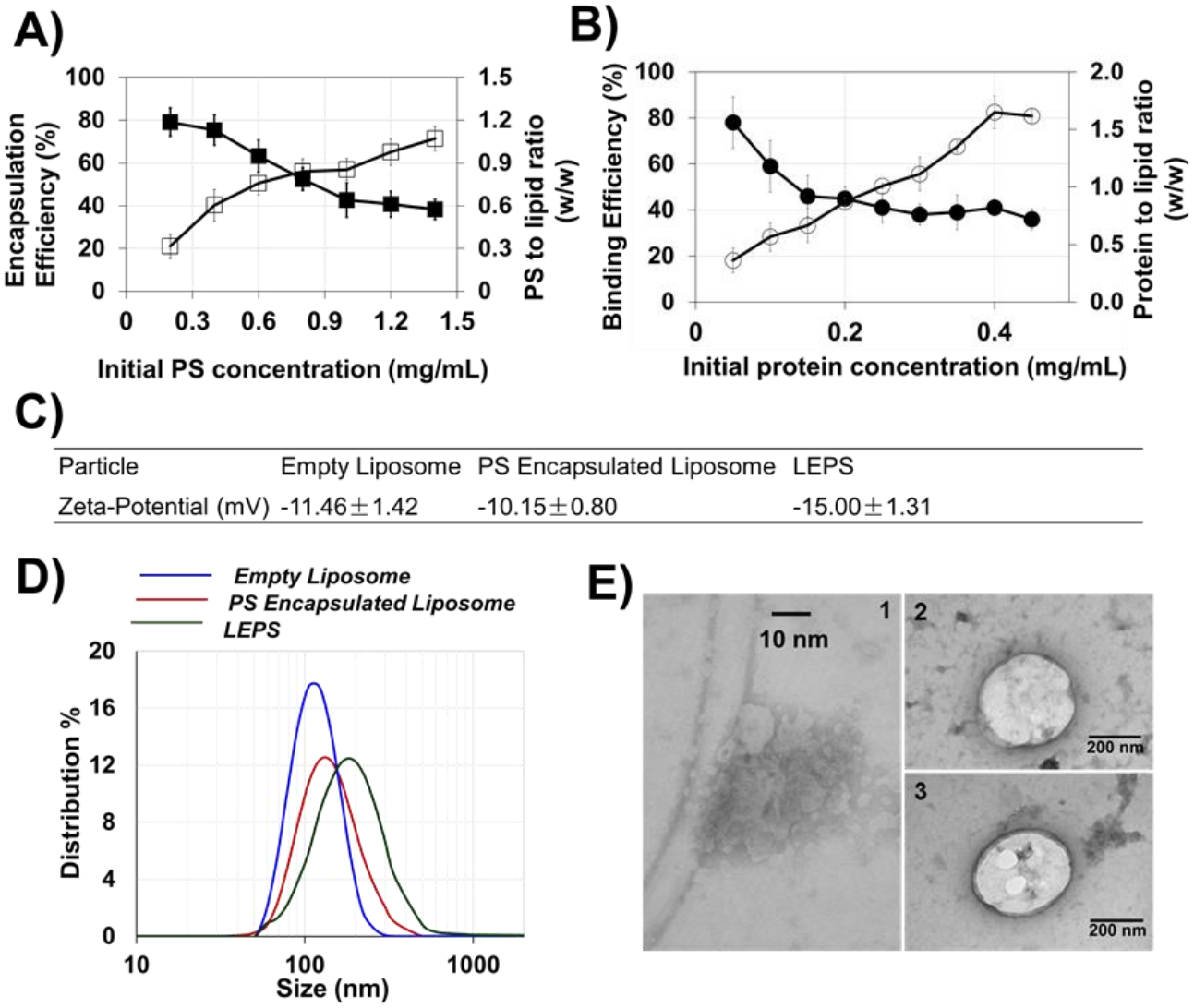

fig. S3. LEPS characterization. Polysaccharide (PS) encapsulation efficiency (**A**; solid squares) or protein surface binding efficiency (**B**; solid circles) vs. initial concentration. Particle surface charge evaluation (**C**) and size distribution (**D**). (**E**) LEPS particle image by transmission electron microscopy; 1: LEPS surface; 2: PS Encapsulated Liposome; 3: LEPS.

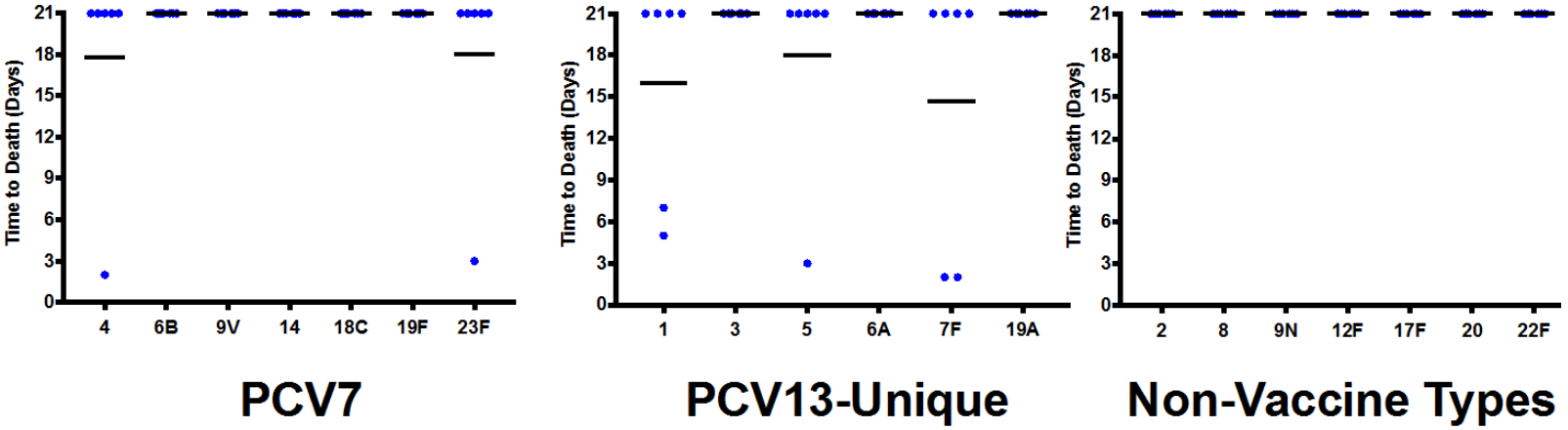

fig. S4. Protective capabilities of LEPS immunizations when challenged with serotypes that span the current Prevnar 7 and Prevnar 13 treatment options. Mice immunized with a LEPS formulation containing 20 polysaccharides (i.e., LEPS20) were evaluated in sepsis models and challenge strains (x-axis) are grouped into categories based upon inclusion in respective glycoconjugate vaccines. Specifically, the "PCV7" label includes bacterial serotypes covered by both Prevnar 7 and Prevnar 13; whereas, the "PCV13-Unique" grouping presents six additional serotypes covered by Prevnar 13. The "Non-Vaccine Types" label corresponds to serotypes not included in commercial glycoconjugate vaccines.

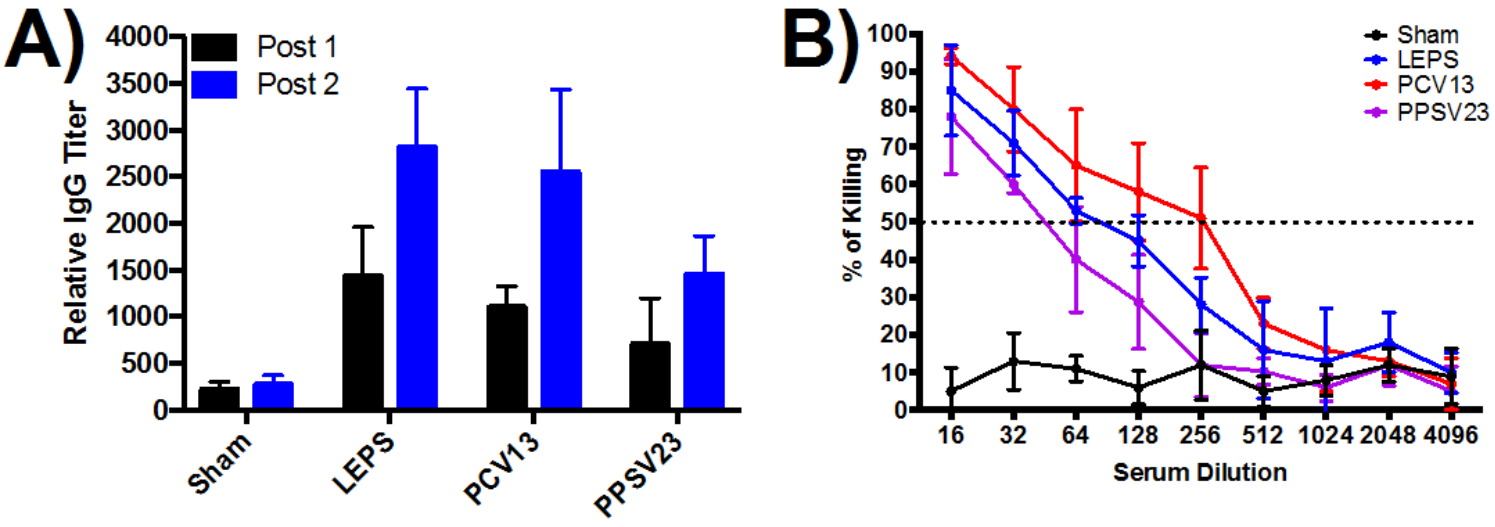

fig. S5. LEPS vaccine strategy in rabbits. New Zealand White rabbits were immunized with LEPS particles containing *S. pneumoniae* serotype 19F CPS (day 0) and boosted (day 14) and peripheral blood samples analyzed for (**A**) total IgG titers at day 14 (Post 1) and 28 (Post 2) and (**B**) functional antibody activity (Post 2 samples) against serotype 19F via OPA assay.

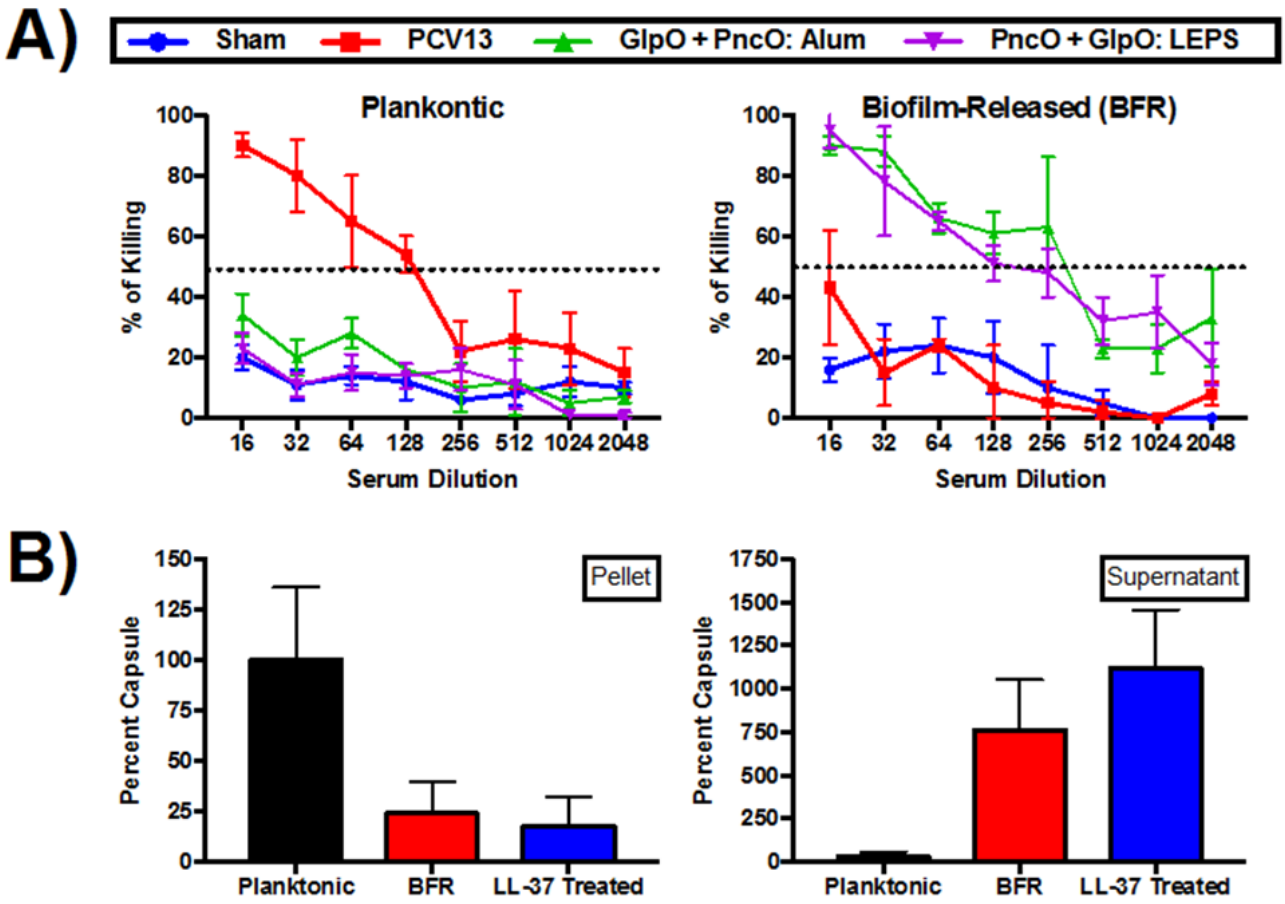

fig. S6. OPA assay for GlpO and PncO directed against specific *S. pneumoniae* cell types. (**A**) OPA for planktonic (left) and biofilm-released (BFR) (right) serotype 19F bacteria mediated by serum from mice immunized with Prevnar 13 (PCV13), GlpO + PncO with Alum adjuvant, and GlpO + PncO with empty LEPS. (**B**) Capsular polysaccharide (CPS) content in the pellet (left) and supernatant (right) for planktonic bacteria, BFR bacteria, and planktonic bacteria treated with LL-37 to remove CPS; "Percent Capsule" values are relative to the planktonic samples in each sub-figure.

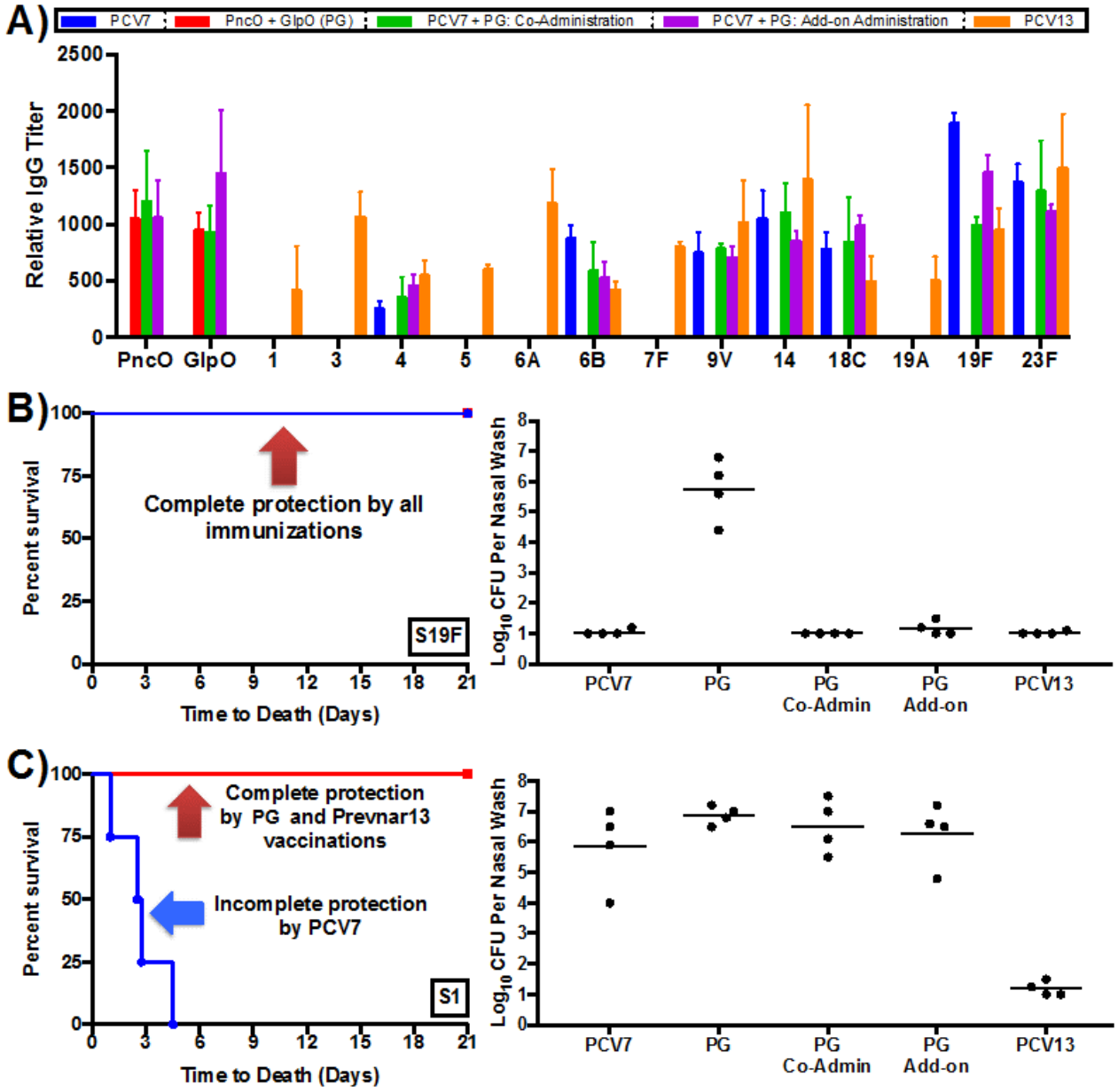

fig. S7. Evaluation of immunogenicity of GlpO and PncO administered either jointly (coadministration) or as a booster (add-on) with Prevnar 7. (**A**) IgG titers against PncO, GlpO, and specific serotypes resulting from color-coded vaccination samples. (**B**) Mouse protection (left; sepsis challenge model) and bacterial burden (right; measured 5 days post-colonization) upon GlpO and PncO (PG) administration either jointly or as a booster with Prevnar 7. Mice were challenged with serotype 19F (covered by Prevnar 7 and Prevnar 13 [PCV13]) or 1 (not covered by Prevnar 7 but covered by Prevnar 13).

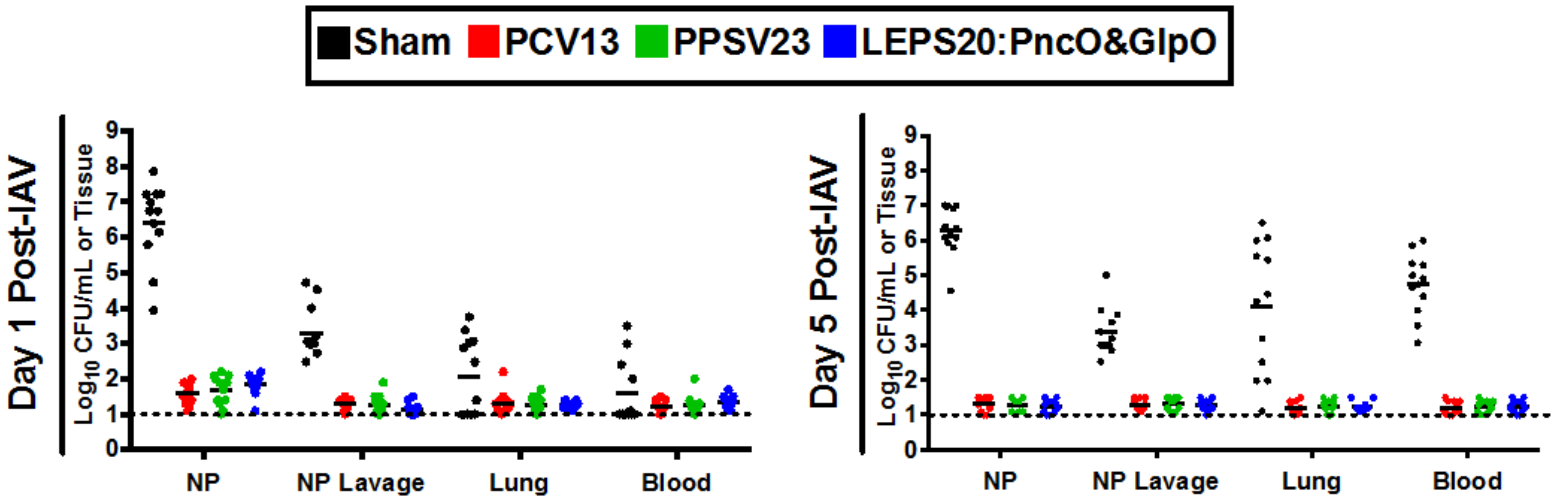

fig. S8. Additional assessment of LEPS20/PncO and GlpO when using a murine IAV-induced pneumonia model with serotype 19F. Bacterial counts from the nasopharynx surface (NP), nasopharynx wash (lavage), lungs, and blood 1 and 5 days post-IAV administration. Prevnar 13 (PCV13); Pneumovax 23 (PPSV23).

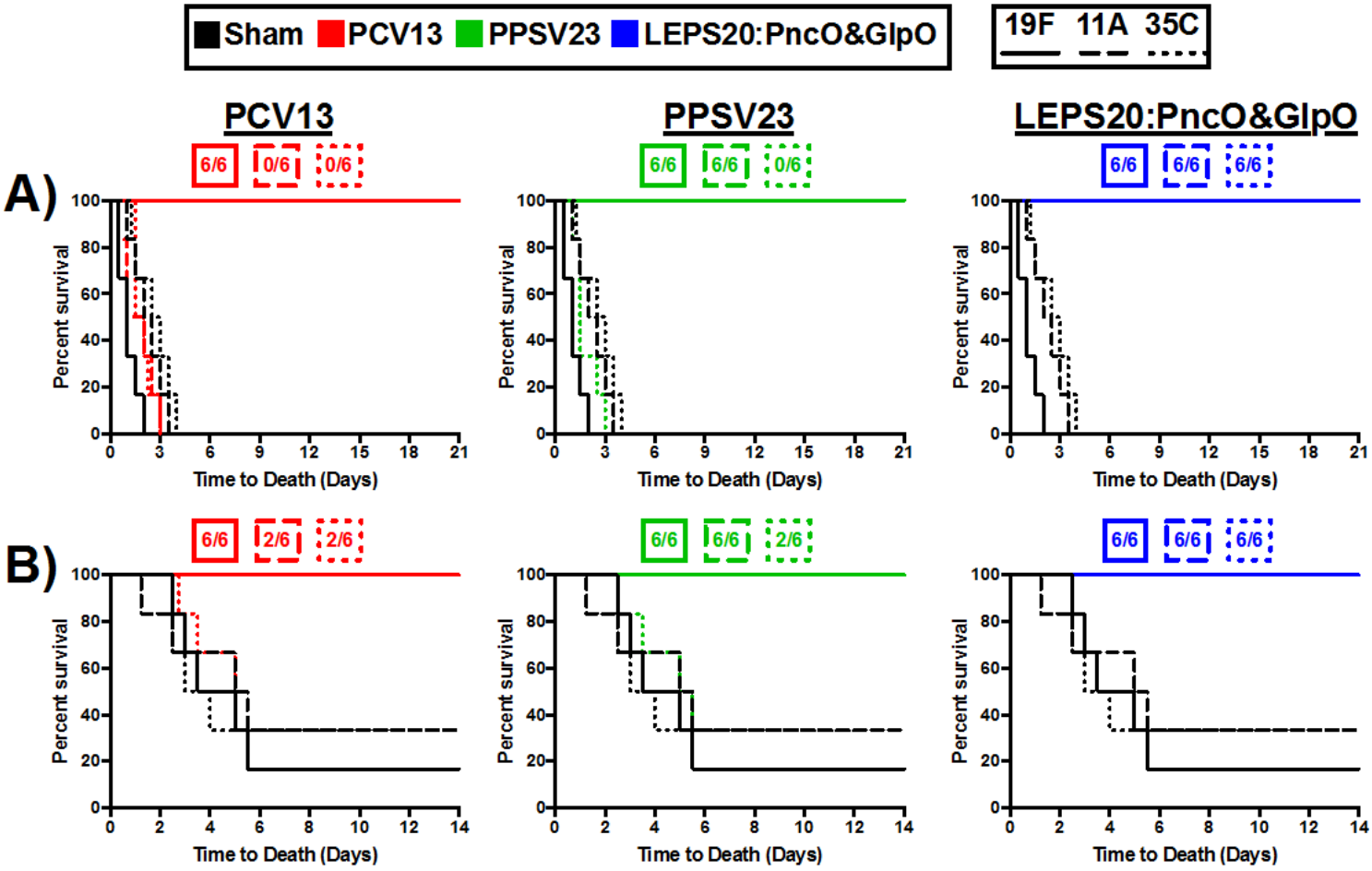

fig. S9. Additional murine disease model assessment of LEPS20/PncO and GlpO. Mouse challenge-protection data comparison between Prevnar 13 (PCV13), Pneumovax 23 (PPSV23), and the complete LEPS system using (A) sepsis and (B) pneumonia disease models. Survival (animal numbers boxed) is indicated across vaccination options when challenged with the indicated serotypes, which are partly covered by Prevnar 13 (19F) and Pneumovax (19F and 11A) and fully covered by LEPS20:PncO&GlpO.

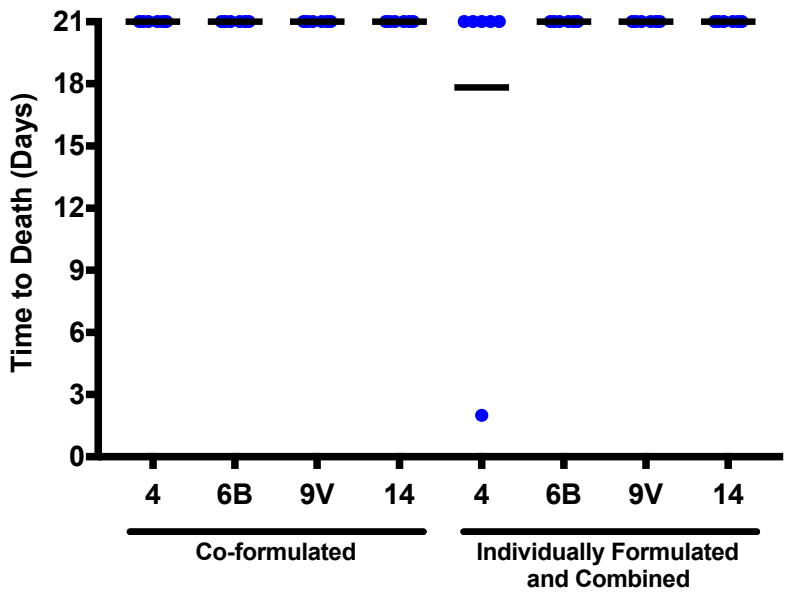

fig. S10. Alternative LEPS formulation procedures and comparison in murine challenge protection assays. Polysaccharides from *S. pneumoniae* serotypes 4, 6B, 9V, and 14 were either mixed and co-formulated during liposomal preparation or individually formulated within liposomal particles that were then combined prior to mouse vaccination. A sepsis disease model was then used to challenge the mice subjects with the individual serotypes (x-axis).

table S1. OPA comparison between Prevnar 13, Pneumovax 23, and a LEPS formulation containing 20 polysaccharides (that is, 20 valent). Values represent serum dilution at which 50% of cellular killing occurred in the OPA assay. Dashed lines indicate nonvaccine serotypes; higher OPA readings are attributed to a stronger antibody response (i.e., higher titers) for the particular polysaccharide.

Serotype / Vaccine	PCV13	PPSV23	LEPS (20V)
1	129	88	40
2	---	77	85
3	98	23	101
4	34	45	136
5	47	67	38
6A	55	---	169
6B	39	26	151
7F	240	29	151
8	---	65	98
9N	---	52	15
9V	176	114	117
10A	---	128	---
11A	---	94	---
12F	---	34	128
14	88	36	44
15B	---	143	---
17F	---	112	101
18C	222	46	156
19A	120	147	154
19F	144	49	243
20	---	37	75
22F	---	77	149
23F	331	13	69
33F	---	16	---

table S2. OPA comparison between Prevnar 13, Pneumovax 23, and the PncO + GlpO protein antigens (administered with alum adjuvant). Values represent serum dilution at which 50% of cellular killing occurred in the OPA assay. Dashed lines indicate non-vaccine serotypes; higher OPA readings are attributed to a stronger antibody response (i.e., higher titers) for that particular polysaccharide. Serotype-specific *S. pneumoniae* strains utilized in the OPA assay include cellular phenotypes associated with initial colonization (planktonic) and disease (i.e., biofilm-released [BFR]).

	PCV13		PPSV23		PncO + GlpO: Alum	
Serotype	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR
1	129	---	88	---	---	15
2	---	---	77	---	---	122
3	98	---	23	---	---	160
4	34	---	45	---	---	450
5	47	---	67	---	---	339
6A	55	---	---	---	---	87
6B	39	---	26	---	---	18
6C	---	---	---	---	---	276
6D	---	---	---	---	---	370
7A	---	---	---	---	---	169
7B	---	---	---	---	---	99
7C	---	---	---	---	---	145
7F	240	---	29	---	---	45
8	---	---	65	---	---	61
9A	---	---	---	---	---	96
9N	---	---	52	---	---	348
9V	176	---	114	---	---	382
10A	---	---	128	---	---	51
10F	---	---	---	---	---	26
11A	---	---	94	---	---	63
11B	---	---	---	---	---	61
11C	---	---	---	---	---	85
12A	---	---	---	---	---	94
12B	---	---	---	---	---	288
12F	---	---	34	---	---	366
13	---	---	---	---	---	103
14	88	---	36	---	---	221
15A	---	---	---	---	---	51
15B	---	---	143	---	---	368
15C	---	---	---	---	---	253
15F	---	---	---	---	---	166
16A	---	---	---	---	---	184
16F	---	---	---	---	---	202
17A	---	---	---	---	---	248
17F	---	---	112	---	---	196
18A	---	---	---	---	---	139

	PCV13		PPSV23		PncO + GlpO: Alum	
Serotype	Planktonic	BFR	Planktonic	BFR	Planktonic	BFR
18B	---	---	---	---	---	275
18C	222	---	46	---	---	339
18F	---	---	---	---	---	190
19A	120	---	147	---	---	62
19C	---	---	---	---	---	183
19F	144	---	49	---	---	339
20	---	---	37	---	---	214
21	---	---	---	---	---	266
22A	---	---	---	---	---	325
22F	---	---	77	---	---	366
23A	---	---	---	---	---	77
23B	---	---	---	---	---	353
23F	331	---	13	---	---	273
24A	---	---	---	---	---	24
24B	---	---	---	---	---	281
24F	---	---	---	---	---	75
25F	---	---	---	---	---	236
27	---	---	---	---	---	110
28A	---	---	---	---	---	235
28F	---	---	---	---	---	351
29	---	---	---	---	---	80
30	---	---	---	---	---	292
31	---	---	---	---	---	186
33A	---	---	---	---	---	352
33F	---	---	16	---	---	186
34	---	---	---	---	---	352
35A	---	---	---	---	---	97
35B	---	---	---	---	---	52
35C	---	---	---	---	---	186
35F	---	---	---	---	---	31
37	---	---	---	---	---	177
38	---	---	---	---	---	81
39	---	---	---	---	---	186
41A	---	---	---	---	---	352
42F	---	---	---	---	---	186

table S3. GlpO and PncO summary.

Gene	Size (aa)	Function
GlpO	609	α-glycerophosphate oxidase
PncO	230	Bacteriocin ABC transporter transmembrane protein