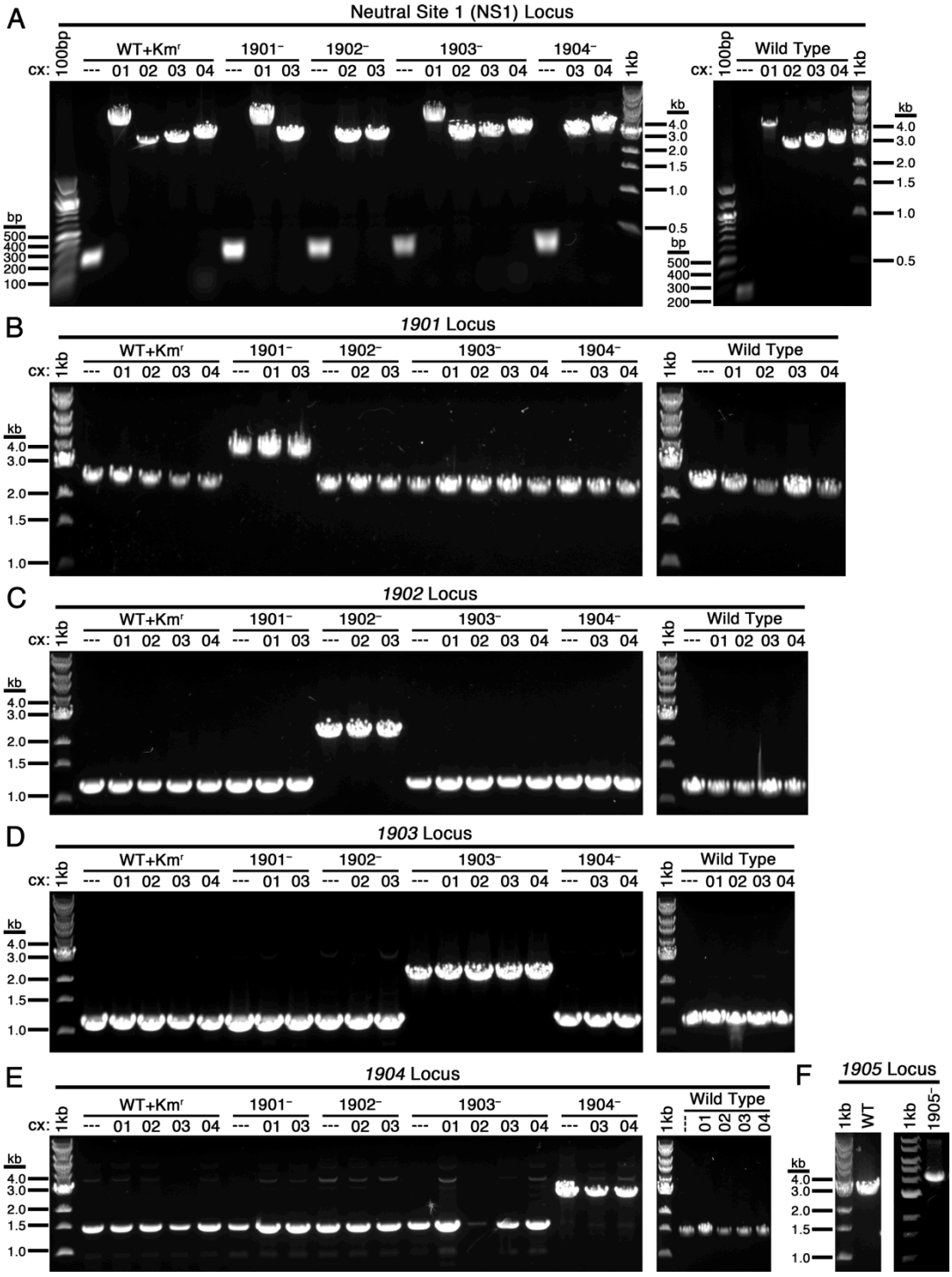

FIG S1 Genotype confirmation via PCR amplification of genomic loci relevant to *1901 – 1905* mutants. PCR was performed on whole cells of *S. elongatus* strains using primers designed to test the composition and segregation of each locus of

interest (Table 2). The sample loaded in each lane is identified by a combination of the parental strain on top and the constitutive expression (cx) open reading frame (ORF) below (--- = WT NS1; 01 = 1901^{cx}; 02 = 1902^{cx}; 03 = 1903^{cx}; 04 = 1904^{cx}). In all PCR reactions, only a single band of the expected size was observed, confirming the genotype and segregation of the strains. A) PCR analysis of the NS1 locus. The expected size of the PCR products are: WT NS1 = 266 bp; 1901^{cx} = 4.1 kb; 1902^{cx} = 2.8 kb; 1903^{cx} = 2.9 kb; and 1904^{cx} = 3.1 kb. B) *1901* locus: WT = 2.5 kb; 1901^{-} = 3.7 kb. C) *1902* locus: WT = 1.2 kb; 1902^{-} = 2.4 kb. D) *1903* locus: WT = 1.2 kb; 1903^{-} = 2.4 kb. E) *1904* locus: WT = 1.5 kb; 1904^{-} = 2.7 kb. F) *1905* locus: WT = 3.0 kb; 1905^{-} = 4.2 kb.

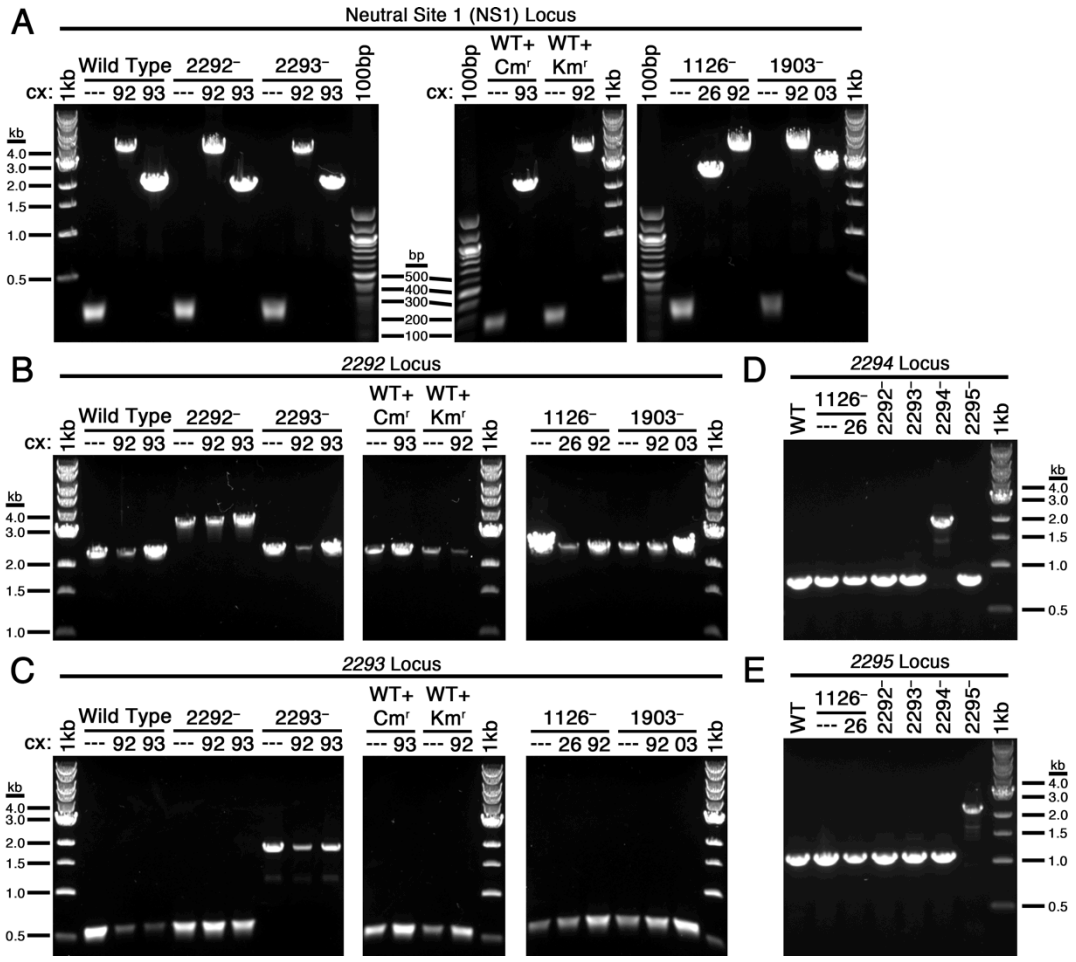

FIG S2 Genotype confirmation via PCR amplification of genomic loci relevant to *2292* and *2293* mutants. PCR was performed as in Fig. S1. Gels are labeled as in Fig. S1 (--- = WT NS1; 92 = 2292^{cx}; 93 = 2293^{cx}; 03 = 1903^{cx}; 26 = 1126^{cx}). In all PCR reactions, only a single band of the expected size was observed, confirming the genotype and segregation of the strains. A) PCR analysis of the NS1 locus. The expected size of the PCR products are: WT NS1 = 266 bp; 2292^{cx} = 4.0 kb; 2293^{cx} = 2.2 kb; 1126^{cx} = 2.6 kb; and 1903^{cx} = 2.9 kb. B) *2292* locus: WT = 2.4 kb; 2292^{-} = 3.6 kb. C) *2293* locus: WT = 553 bp; 2293^{-} = 1.8 kb.

D) *2294* locus: WT = 837 bp; 2294^{-} = 2.0 kb. E) *2295* locus: WT = 1.1 kb; 2295^{-} = 2.3 kb.

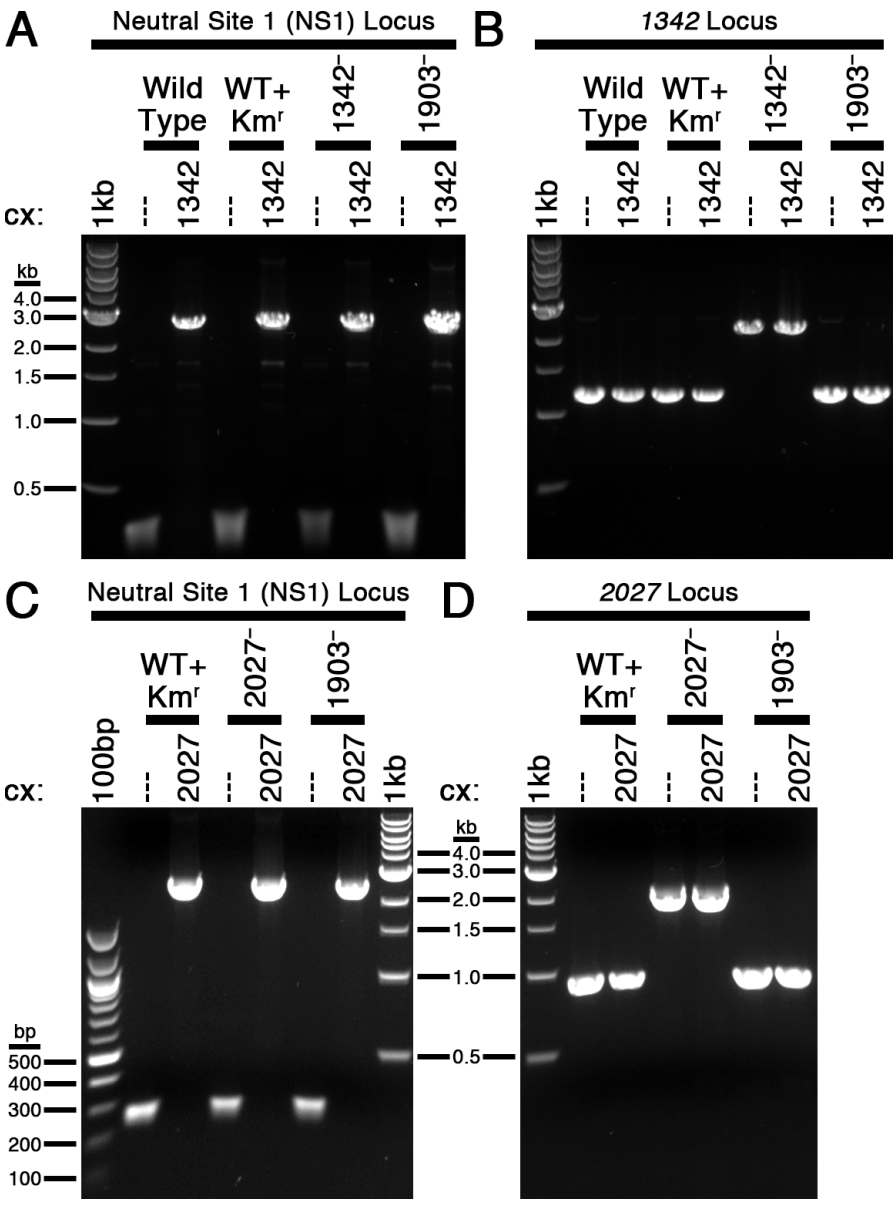

FIG S3 Genotype confirmation via PCR amplification of genomic loci relevant to *1342* and *2027* mutants. PCR was performed as in Fig. S1. Gels are labeled as in Fig. S1. In all PCR reactions, only a single band of the expected size was observed, confirming the genotype and segregation of the strains. A) PCR analysis of the NS1 locus for *1342*-related strains. The expected size of the PCR products are: WT NS1 = 266 bp; 1342^{cx} = 2.8 kb. B) *1342* locus: WT = 1.1 kb;

1342^{-} = 2.3 kb. C) NS1 locus for *2027*-related strains: WT = 266 bp; 2027^{cx} = 2.5 kb. D) *2027* locus: WT = 751 bp; 2027^{-} = 2.0 kb.

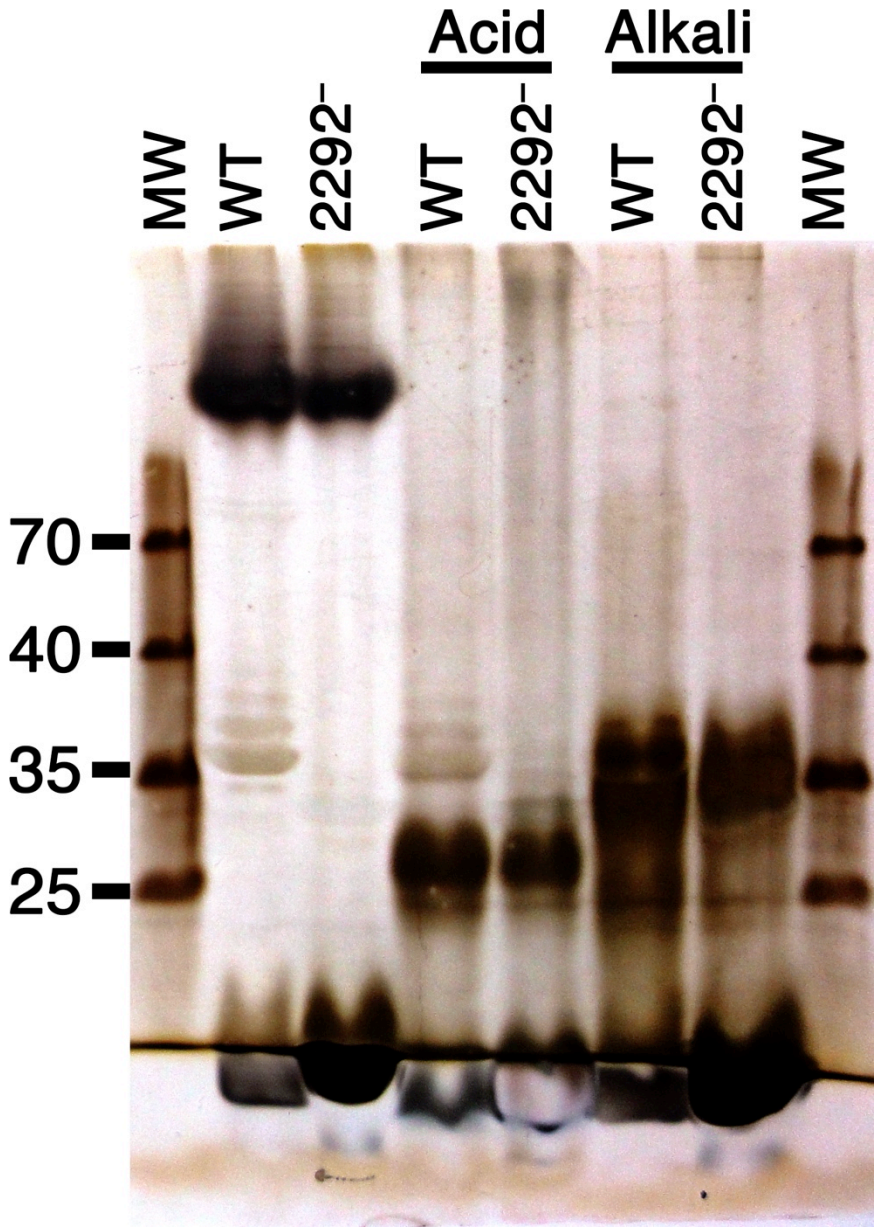

FIG S4 Mild acid and alkali treatment of outer membrane purifications of WT and 2292^{-}. Outer membrane samples were generated by the ultracentrifugation method of Simkovsky, et al. (1) and were treated with a mild acid or mild alkali treatment, as described in Rick, et al. (2). Samples were run on a SDS Tris-glycine gel as labeled. The molecular weight standard (MW) is marked in kilodaltons on the left.

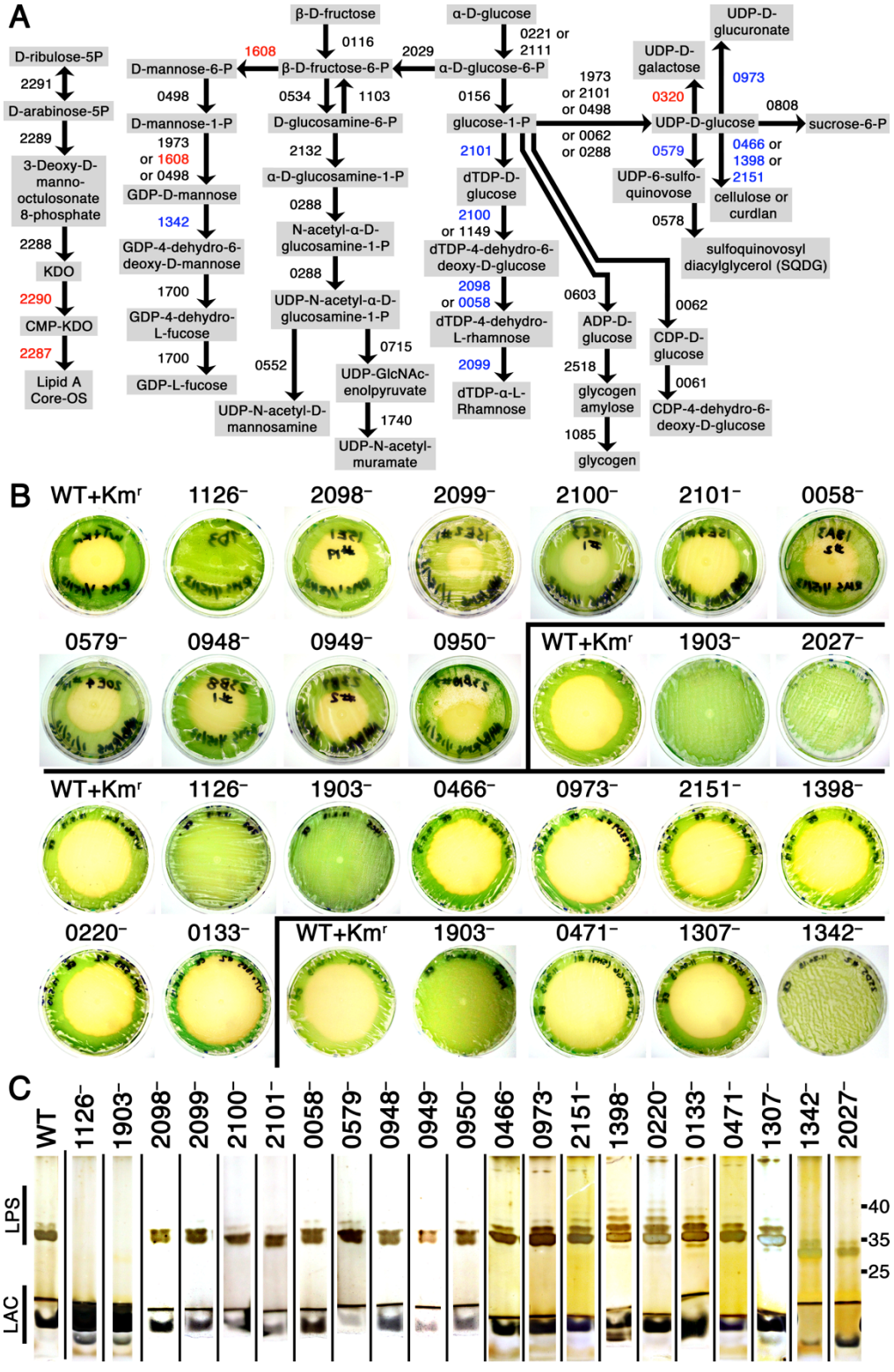

FIG S5 Insertional transposon mutagenesis and phenotype analysis of putatitve sugar-nucleotide synthesis and polymerization genes. A) Metabolic map of steps

involved in sugar-nucleotide synthesis and polymerization based on KEGG pathways (3). Genes encoding *S. elongatus* PCC 7942 homologs of enzymes involved in each step are listed adjacent to the appropriate arrow. Gene names are colored blue if a segregated mutant was generated and confirmed, black if no mutations were attempted, and red if mutations were attempted but transformants or segregated clones could not be recovered. B) Amoeba grazing assays of segregated mutants and appropriate controls (WT+Km^r, 1126^-, and 1903^-). Plates are grouped with appropriate controls by experimental batch due to slight variations in experimental conditions per batch, such as plate size or amoebae incubation time. C) SDS-PAGE analysis of outer membrane preparations from successfully generated mutants and previously characterized controls**.** The regions containing banding patterns that represent lipopolysaccharide (LPS) and lipid A-core (LAC) are indicated on the left while the positions of molecular weight standards are indicated on the right in kilodaltons.

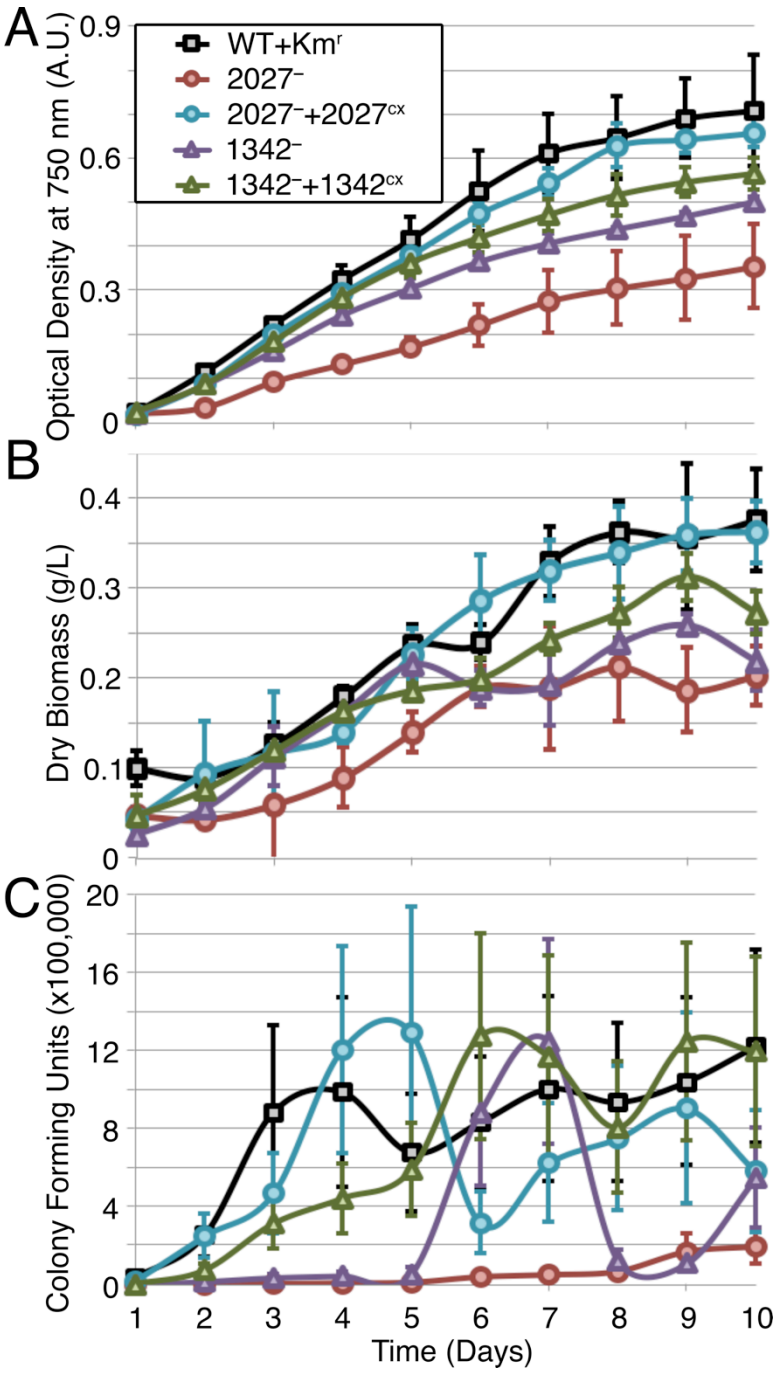

FIG S6 Growth analysis of 2027⁻ and 1342⁻. (A) Growth of flask cultures was measured by optical density at 750 nm over the course of 10 days. Because reductions in absorbance measurements may be due to the autoflocculation or clumping associated with previously characterized mutants (4), cell densities

were also measured by (B) dry biomass as previously described (1) and (C) colony forming units. Markers represent the average of biological triplicates; error bars represent the 95% confidence interval. Counts for 2027^{-} and 1342^{-} do not include larger, WT-like colonies (Fig. S7C).

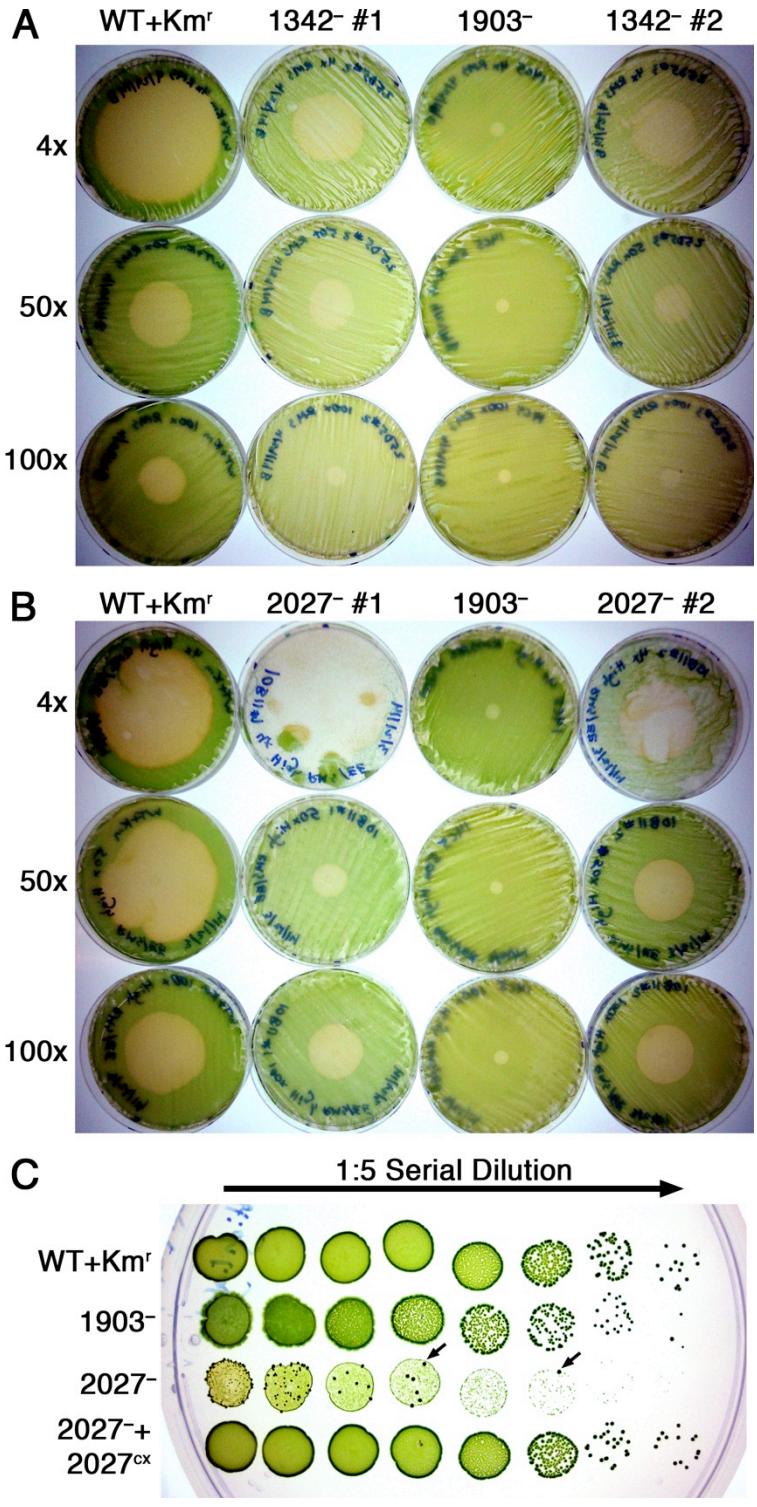

FIG S7 Effects of initial cyanobacterial cell plating density and suppression of impaired growth phenotype on amoeba resistance. Lawn plates of replicate

clones of (A) 1342^{-} and (B) 2027^{-} mutants and control strains were seeded using 1 mL (4x), 12.5 mL (50x), pr 25 mL (100x) of culture concentrated to a final volume of 250 μl. After 6 d of growth, all plates were exposed to ~20,000 amoebae added to the center of each plate as a 4 μl spot and incubated at 30 °C for 6 d prior to photographing the plates. (C) 1:5 serial dilution plate of 2027^{-} and related strains generated for CFU analysis in Fig. S6C. In contrast to the small colonies observed in early time point samples of 2027^{-} (Fig. 4B), colonies (arrows) growing like WT or the complemented strain 2027^{-}+2027^{cx} are present in the 2027^{-} samples on this day 10 plate.

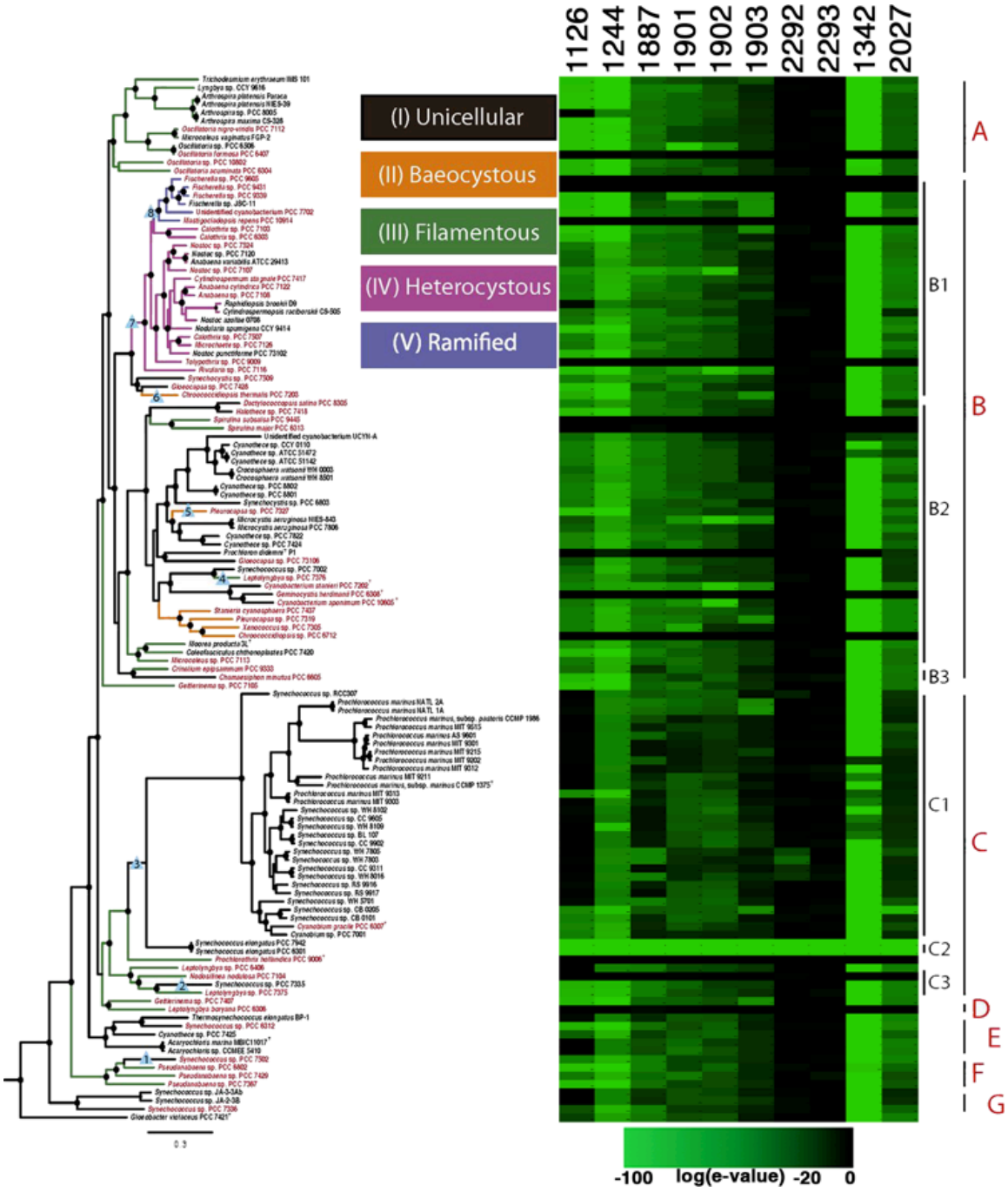

FIG S8 Conservation of LPS synthesis genes across the sequenced cyanobacteria. Each *S. elongatus* PCC 7942 LPS synthesis gene (x-axis of the heat map) was used as a BLAST query to identify the most similar gene in every sequenced cyanobacteria (phylogenetic tree on the left,

adapted with permission from Shih, et al. (5)). The heat map is colored based upon the logarithm of the e-value of the top hit, with bright green representing a highly similar sequence and black representing the inability to discover any similar genes in that species.

TABLE S1 Mutants impairing nucleotide-sugar production or polymerization that were attempted and their resulting phenotypes.

Gene	UGS Mutant	Predicted Function	Segregated Mutant Generated?	Mutant is resistant to HGG1 amoeba?	Mutant has altered LPS banding pattern?
0058	18A3	Synthesis of nucleotide activated rhamnose	Yes	No	No
0133	18E5	Conserved transmembrane protein; conserved synteny with *0134*	Yes	No	No
0134	18E7	Wzy-like	No	--	--
0220	5A3	Putative Wzc protein	Yes	No	No
0281	19A8	Glycosyltransferase, WecA-like	No	--	--
0320	19C2	Nucleotide activation of galactose	No	--	--
0357	19E4	Wzy-like	No	--	--
0463	19G5	Wzb-like	No	--	--
0466	19G7	Synthesis of cellulose or curdlan	Yes	No	No
0471	5H9	Permease protein of sugar ABC transporter	Yes	No	No
0579	20E4	sqdX, synthesis of sulfoquinovosyl diacylglycerol (6)	Yes	No	No
0948	23B8	Permease protein of sugar ABC transporter	Yes	No	No
0949	23B9	Permease protein of sugar ABC transporter	Yes	No	No
0950	23B10	Sugar transport substrate binding protein	Yes	No	No
0973	23D9	Synthesis of nucleotide activated glucuronate	Yes	No	No
1307	25A7	ABC transport permease, possibly of oligosaccharides	Yes	No	No
1342	25D5	Synthesis of nucleotide activated fucose	Yes	Yes	Yes
1398	25H6	Synthesis of cellulose or curdlan	Yes	No	No
1608	12D5	Nucleotide activation or synthesis of mannose	No	--	--
1761	13G6	TolC-like Type I secretion protein	No	--	--
2025	14H11	Glycosyltransferase type 1	No	--	--
2027	10B11	Glycosyltransferase type 2	Yes	Yes	Yes
2028	10B12	Glycosyltransferase type 1	No	--	--
2098	15E1	Synthesis of nucleotide activated rhamnose	Yes	No	No
2099	15E2	Synthesis of nucleotide activated rhamnose	Yes	No	No
2100	15E3	Synthesis of nucleotide activated rhamnose	Yes	No	No
2101	15E4	Nucleotide activation of glucose	Yes	No	No
2148	10D4	ATPase of ABC transporter system; conserved synteny with 2149 transporter	No	--	--
2149	2E11	ABC transport permease, possibly of oligosaccharides	No	--	--
2150	2E12	Choloylglycine hydrolase, affecting envelope and host-interaction (7)	No	--	--
2151	2F1	Synthesis of cellulose or curdlan	Yes	No	No
2287	3C1	Glycosyltransferase or polymerase of KDO	No	--	--
2290	10E7	Nucleotide activation of KDO	No	--	--

Supplementary References

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