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Supplemental information

Pseudomonas aeruginosa aggregates in cystic fibrosis sputum produce exopolysaccharides that likely impede current therapies

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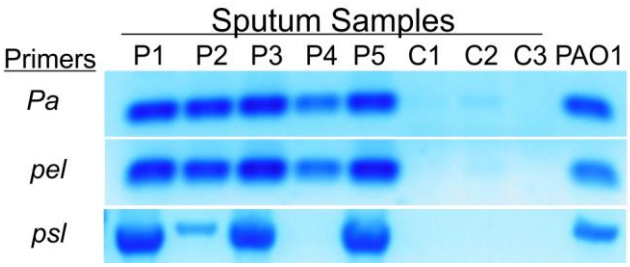

Fig. S1. *P. aeruginosa* sputum samples show heterogeneity in the genetic capability to produce Pel and Psl. Related to STAR Methods, "Detection of Pel, Psl, and *P. aeruginosa* Genes in Sputum". Primers specific for *pel*, *psl*, and *P. aeruginosa* were used to amplify genes in CF sputum samples in which the clinical laboratory indicated were positive (P1-P5) or negative (C1-C3) for *P. aeruginosa*. PAO1 genomic DNA was used as a positive control.

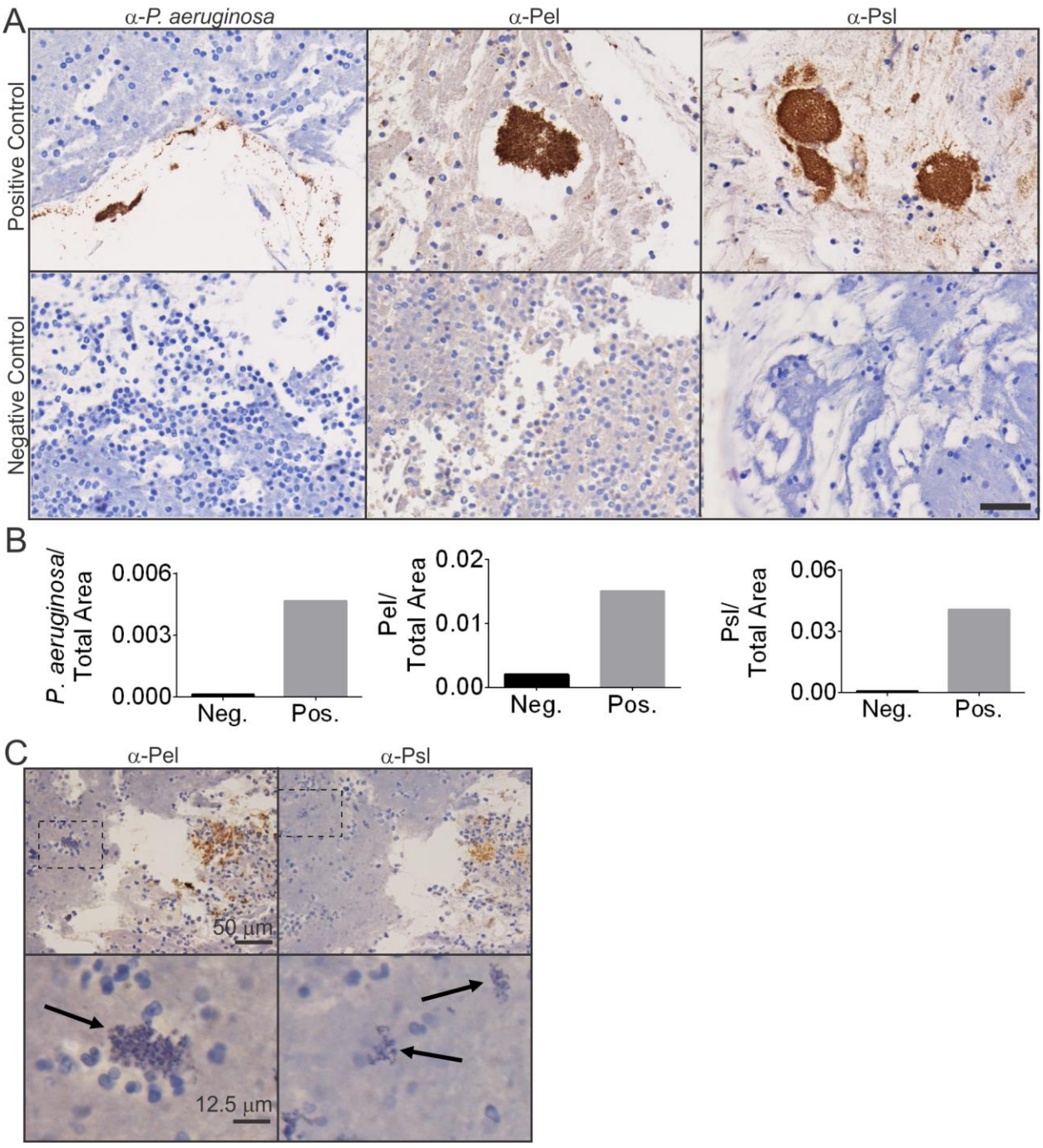

Fig. S2. IHC staining of Pel and Psl is specific for target antigens. Related to Figures 1 and 2. (A/B) Positive controls (Pos.) are non-*P. aeruginosa* sputum spiked with *P. aeruginosa* strains that overproduce Pel or Psl. Negative controls (Neg.) are CF sputum that does not contain *P. aeruginosa*, Pel or Psl. (A) Representative IHC images of Pel, Psl, or *P. aeruginosa* (brown) and counterstain (blue). Scale bar 50 µm. (B) Global quantification of the total area of positive signal normalized to the total area of the sputum specimen. (C) Pel and Psl antibodies (brown) do not stain Gram-positive cocci (arrows) in sputum containing *P. aeruginosa.* Second row contains enlargements of dashed rectangles in first row.

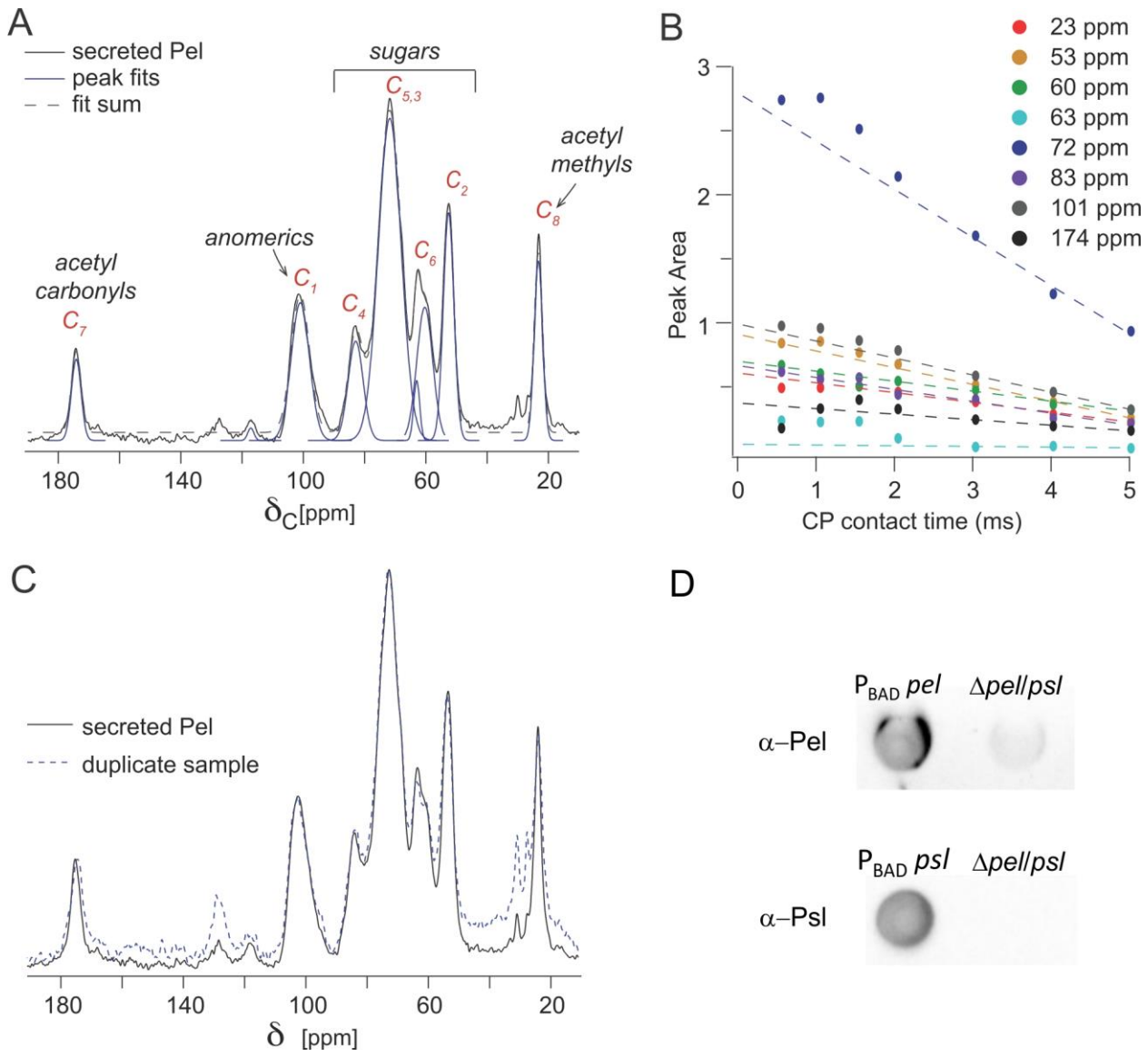

Fig. S3. Half of the Pel sugar residues are deacetylated. Related to Figure 3A. (A) The ^{13}C-CPMAS spectrum of Pel contained peaks (23, 53, 60, 63, 72, 101, and 174 ppm) consistent with GlcNAc/GalNAc. The methyl peak at 23 ppm and the carbonyl peak at 174 ppm indicated that Pel was an acetylated polysaccharide. The intensity due to the C2 carbon is shifted upfield to 53 ppm as expected based upon its predicted amination. The spectral fits are shown to highlight the peaks that were used for subsequent analysis. (B) The CP contact time was arrayed, and the peak areas were fit to determine that there were approximately 0.5 acetyl groups per sugar (based on stoichiometry of acetyl methyls and carbonyls relative to the other sugar carbons; peak areas were normalized to the anomeric peak area). (C) ^{13}C-CPMAS spectra of two biological replicates of secreted Pel were compared, and the secreted Pel sample and its extent of acetylation were found to be reproducible. One of the samples (*blue-dotted line*) retained more DNA, evidenced by the peak intensity near 130 ppm. (D) α-Pel and α-Psl immunoblots show supernatant of P_{BAD} *pel* and P_{BAD} *psl* cultures have similar amount of polysaccharide. Five microliters of supernatant from cultures was blotted onto nitrocellulose membrane and probed with Pel or Psl antisera.

Table S1. Cystic fibrosis patient characteristics. Related to Figure 2.

ID[1]	Age	FEV1[2] (L)	FEV1[2] (%pred)	Clinical Lab Microbiology	CFTR Genotype
P1	31	0.80	19%	*P. aeruginosa, S. aureus, Pandorea*	G551D/Unknown
P2	29	0.59	15%	*P. aeruginosa* (some mucoid), *S. aureus*	ΔF508/N1303K
P3	26	2.00	50%	*P. aeruginosa* (some mucoid)	ΔF508/621+1G>T
P4	23	3.03	101%	*P. aeruginosa* (mucoid), *S. aureus*	ΔF508/c.3883_3886del
P5	37	3.84	84%	*P. aeruginosa* (mucoid), *Chryseobacterium*	ΔF508/621+1G>T
C1	27	1.68	52%	*S. aureus, Achromobacter*	ΔF508 homozygous
C2	48	1.62	55%	*Staphylococcus, Achromobacter, Klebsiella*	ΔF508/2857delG
C3	53	1.23	32%	*Achromobacter, S. aureus*	ΔF508/1717-IG

[1]Identification numbers indicate patient sputum samples with *Pseudomonas* (P1-5) or controls without *Pseudomonas* (C1-3).

[2]Forced expiratory volume.