- 1 Anti-Psl Targeting of *Pseudomonas aeruginosa* Biofilms for Neutrophil-Mediated
- 2 Disruption
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20 Supplemental Information

21	Supplementary Fig. S1. Structure of Psl and synthetic Psl oligosaccharides. (A) Cell-
22	free Psl is a pentasaccharide repeat unit. (B-E) Synthetic Psl oligosaccharides used to
23	evaluate binding of anti-Psl mAbs which recognized unique epitopes (class I, II and III) 1 .
24	(B) Psl tetrasaccharide – bound by the class II mAb. (C) Psl pentasaccharide – bound by
25	the class II mAb, (D) Psl hexasaccharide – bound by class II and class III mAbs. (E) Psl
26	di-pentasaccharide – bound by the class II mAb and weakly by the class III mAb.
27	
28	Supplementary Fig. S2. Individual anti-Psl mAbs stain PAO1 biofilms. Flow grown
29	biofilms of PAO1 stained with either (A) class I (green), (B) class II (orange), or (C)
30	class III (red) anti-Psl mAbs, imaged via CLSM at 10x magnification, and processed via
31	IMARIS. Scale bars represent 150 µm.
32	
33	Supplementary Fig. S3. Thermally injured porcine tissue does not react with an
34	isotype control IgG mAb. P. aeruginosa infected skin from thermally injured pigs was
35	imaged via CLSM at 10x magnification. (A) DIC image (same as Fig. 3), (B) DAPI
36	staining, and (C) control IgG mAb staining. (D-F) The wound bed is below the white
37	dotted line and white dashed line represents below the wound bed. Scale bars represent
38	100 μm.

39

40 Supplementary Figure S4. Anti-Psl mAbs elicit a phagocytic burst response from
41 human neutrophils. Planktonic PAO1 was incubated with neutrophils and PMA
42 (positive control), non-opsonized or IgG isotype control antibody (negative controls), or

43 opsonized with human serum, individual anti-Psl mAbs, or a combination of all three44 mAbs.

45

46 Supplementary Movie S1. Anti-Psl mAbs differentially stain the biofilm. IMARIS

47 generated movie from image shown in Figure 2B with removal of layers (class I – green,

48 class II – yellow/orange, and class III – red) and rotation to demonstrate differential
49 staining.

- 50
- 51

52 Supplementary Fig. S1



54 Supplementary Fig. 2



56 Supplementary Fig. S3





Supplementary TABLE S1. Raw data from biofilm attachment assays.

63	Experiment 1			
64 (「				
65	Condition	<u>O.D. A₅₄₀</u>	Condition	<u>O.D. A₅₄₀</u>
67	Anal #1	0.022	DAO1 #1	0.121
68	$\Delta psi \#1$	0.035	PAO1 #1	0.121
69	$\Delta psi \# 2$ Ansl #3	0.058	ΡΔΟ1 #3	0.138
70	$\Delta p s i \pi s$	0.050	1 AO1 #5	0.120
71	IgG Control #1	0.111	Class I #1	0.037
72	IgG Control #2	0.150	Class I #2	0.056
73	IgG Control #3	0.135	Class I #2	0.070
74	190 0011101 110	0120		01070
75	Class II #1	0.070	Class III #1	0.080
76	Class II #2	0.126	Class III #2	0.081
77	Class II #3	0.141	Class III #3	0.059
78				
79	All 3 mAbs #1	0.102		
80	All 3 mAbs #2	0.121		
81	All 3 mAbs #3	0.106		
82				
83	Experiment 2			
84				
85	Condition		Condition	
86	<u>Condition</u>	<u>0.D. A</u> 540_	<u>Condition</u>	<u>0.D. A</u> 540
87	$\Delta nsl \#1$	0.049	PAO1 #1	0 146
88	$\Delta psi \#1$ $\Delta nsl \#2$	0.060	PAO1 #2	0.140
89	$\Delta pst \# 2$ $\Delta nsl \# 3$	0.057	PAO1 #2	0.155
90	Apor no	0.027	11101 110	0.100
91	IgG Control #1	0.143	Class I #1	0.066
92	IgG Control #2	0.160	Class I #2	0.129
93	IgG Control #3	0.150	Class I #3	0.116
94	8			
95	Class II #1	0.128	Class III #1	0.162
96	Class II #2	0.112	Class III #2	0.088
97	Class II #3	0.137	Class III #3	0.071
98				
99	All 3 mAbs #1	0.122		
100	All 3 mAbs #2	0.134		
101	All 3 mAbs #3	0.105		
102				
103	Experiment 3			
104	*			
105	Condition	O.D. A ₅₄₀	Condition	O.D. A540
106			<u></u>	
107	$\Delta psl \#1$	0.031	PAO1 #1	0.155
108	$\Delta psl \#2$	0.059	PAO1 #2	0.174
109	$\Delta psl \#3$	0.061	PAO1 #3	0.168
110	*		-	
111	IgG Control #1	0.164	Class I #1	0.080
112	IgG Control #2	0.173	Class I #2	0.122
113	IgG Control #3	0.159	Class I #3	0.047
114				

115	Class II #1	0 293	Class III #1	0.155
116	Class II #2	0.108	Class III #2	0.107
117	Class II #2	0.115	Class III #2	0.102
118				01102
119	All 3 mAbs #1	0 104		
120	All 3 mAbs #2	0.092		
121	All 3 mAbs #3	0.104		
122	111 5 111 105 115	0.101		
122	Experiment A			
123	Елрентені 4			
124	~		~	
125	Condition	<u>O.D. A₅₄₀</u>	Condition	<u>O.D. A₅₄₀</u>
126				
127	$\Delta psl \#1$	0.067	PAO1 #1	0.129
128	Δpsl #2	0.067	PAO1 #2	0.152
129	Δpsl #3	0.048	PAO1 #3	0.147
130	•			
131	IgG Control #1	0.105	Class I #1	0.103
132	IgG Control #2	0.125	Class I #2	0.117
133	IgG Control #3	0.155	Class I #3	0.127
134	C			
135	Class II #1	0.111	Class III #1	0.110
136	Class II #2	0.122	Class III #2	0.112
137	Class II #3	0.133	Class III #3	0.133
138				
139	All 3 mAbs #1	0.106		
140	All 3 mAbs #2	0.121		
141	All 3 mAbs #3	0.120		

Supplementary TABLE S2. Raw data from biofilm aggregation assays.

*Experiment 1*146

147	Condition_	<u>O.D. A₆₀₀</u>	<u>O.D.A₄₉₀</u>	Aggregation Index (A ₄₉₀ /A ₆₀₀)
148		0.001		10.170
149	$\Delta psl \#1$	0.091	1.134	12.462
150	$\Delta psl \#2$	0.093	1.122	12.065
151 152	Δpsl #3	0.093	1.142	12.280
153	WFPA 801 #1	0.072	1.196	16.611
154	WFPA 801 #2	0.071	1.114	15.690
155	WFPA 801 #3	0.074	1.160	15.676
156				
157	WFPA 801 + ara #1	0.040	0.940	23.500
158	WFPA 801 + ara #2	0.043	1.000	23.256
159	WFPA 801 + ara #3	0.045	1.044	23.200
160				
161	IgG Control #1	0.044	1.000	22.727
162	IgG Control #2	0.042	0.982	23.381
163	IgG Control #3	0.044	1.018	23.136
164		0.011	11010	201100
165	Class I #1	0.054	1 068	19 778
166	Class I #2	0.053	1.036	19 547
167	Class I #2	0.055	1.090	19 500
168		0.050	1.072	17.500
169	Class II #1	0.053	1 066	20 113
170		0.055	1.000	10 236
171	Class II $\#2$	0.033	1.038	21 625
172		0.040	1.050	21.025
173	Class III #1	0.0/19	1 100	22 1/19
174	Class III #1	0.049	1.100	22.77
175	Class III $\#2$	0.040	1.000	22.200
176		0.050	1.114	22.280
177	A11.3 mAbs #1	0.066	1 210	18 333
170	All $3 \text{ mAbs } \#1$	0.000	1.210	18.555
170	All 3 mAbs $#2$	0.059	1.008	18.022
100	All 5 IIIA08 #5	0.000	1.130	18.335
100				
101	F			
182	Experiment 2			
183				
184	Condition	<u>O.D. A₆₀₀</u>	<u>O.D.A₄₉₀</u>	Aggregation Index (A ₄₉₀ /A ₆₀₀)
185				
186	$\Delta psl \#1$	0.090	1.016	11.289
187	Δpsl #2	0.094	1.111	11.819
188	Δpsl #3	0.103	1.158	11.243
189				
190	WFPA 801 #1	0.071	1.098	15.465
191	WFPA 801 #2	0.074	1.128	15.243
192	WFPA 801 #3	0.080	1.210	15.125
193				
194	WFPA 801 + ara #1	0.053	1.106	20.868
195	WFPA 801 + ara #2	0.055	1.130	20.545
196	WFPA 801 + ara #3	0.059	1.186	20.102
197				

198	IgG Control #1	0.056	1.112	19.857
199	IgG Control #2	0.059	1.100	18.644
200	IgG Control #3	0.057	1.168	20.491
201	-			
202	Class I #1	0.057	1.054	18.491
203	Class I #2	0.057	1.096	19.228
204	Class I #3	0.059	1.102	18.678
205				
206	Class II #1	0.056	1.044	18.643
207	Class II #2	0.058	1.138	19.621
208	Class II #3	0.058	1.162	20.034
209				
210	Class III #1	0.051	1.038	20.353
211	Class III #2	0.058	1.344	23.172
212	Class III #3	0.057	1.122	19.684
213				
214	All 3 mAbs #1	0.062	0.994	16.032
215	All 3 mAbs #2	0.063	1.008	16.000
216	All 3 mAbs #3	0.066	1.060	16.061
217				
218				
219				
220	Experiment 3			
221	2			
221	Condition			Λ aggregation Index (Λ / Λ)
222	Condition	<u>U.D. A₆₀₀</u>	$0.D.A_{490}$	Aggregation index (A_{490}/A_{600})
223	And #1	0.120	0.840	7.075
224	$\Delta psi \#1$	0.120	0.849	7.073
225	$\Delta psi \# 2$	0.110	0.877	6.680
220	$\Delta psi \# s$	0.122	0.810	0.089
227	WEDA 801 #1	0.107	1.076	10.056
220	WEDA 801 #2	0.107	1.070	0.786
220	WEDA 801 #2	0.112	1.090	9.780
230	WITA 001 #3	0.120	1.124	8.321
231	WFDA 801 + ara #1	0.084	1.050	12 500
232	WFDA $801 \pm ara \#2$	0.084	1.030	12.300
233	WFPA $801 \pm ara \#3$	0.080	1.140	13.320
235	W11A001 + a1a #3	0.090	1.130	12.007
235				
230	IgG Control #1	0.088	0.006	10 205
227	IgG Control #1	0.088	0.906	10.295
237	IgG Control #1 IgG Control #2	0.088 0.089 0.081	0.906 0.988 0.892	10.295 11.101
237 238 239	IgG Control #1 IgG Control #2 IgG Control #3	0.088 0.089 0.081	0.906 0.988 0.892	10.295 11.101 11.012
237 238 239 240	IgG Control #1 IgG Control #2 IgG Control #3	0.088 0.089 0.081	0.906 0.988 0.892	10.295 11.101 11.012
237 238 239 240 241	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2	0.088 0.089 0.081 0.097	0.906 0.988 0.892 1.064	10.295 11.101 11.012 10.969 11 573
237 238 239 240 241 242	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #2	0.088 0.089 0.081 0.097 0.089	0.906 0.988 0.892 1.064 1.030 1.132	10.295 11.101 11.012 10.969 11.573 11.792
237 238 239 240 241 242 243	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3	0.088 0.089 0.081 0.097 0.089 0.096	0.906 0.988 0.892 1.064 1.030 1.132	10.295 11.101 11.012 10.969 11.573 11.792
237 238 239 240 241 242 243 244	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3	0.088 0.089 0.081 0.097 0.089 0.096	0.906 0.988 0.892 1.064 1.030 1.132	10.295 11.101 11.012 10.969 11.573 11.792
237 238 239 240 241 242 243 244 244	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #1	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.085	0.906 0.988 0.892 1.064 1.030 1.132 0.986	10.295 11.101 11.012 10.969 11.573 11.792 12.024
237 238 239 240 241 242 243 244 245 246	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #2 Class II #2 Class II #2	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.085 0.090	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378
237 238 239 240 241 242 243 244 245 246 247	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #1 Class II #2 Class II #3	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.085 0.090	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014 1.024	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378
237 238 239 240 241 242 243 244 245 244 245 246 247 248	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #2 Class II #3	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.082 0.085 0.090	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014 1.024	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378
237 238 239 240 241 242 243 244 245 244 245 246 247 248 249	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #2 Class II #3 Class III #1 Class III #1 Class III #1	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.082 0.085 0.090 0.086	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014 1.024 1.032	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378 12.000 12.000
237 238 239 240 241 242 243 244 245 246 247 248 249 250	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #2 Class II #3 Class III #1 Class III #2 Class III #2 Class III #2 Class III #2	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.085 0.090 0.086 0.085 0.084	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014 1.024 1.032 1.028 1.018	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378 12.000 12.094 12.110
237 238 239 240 241 242 243 244 245 246 247 246 247 248 249 250 251	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #2 Class II #3 Class III #1 Class III #1 Class III #2 Class III #3	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.085 0.090 0.086 0.085 0.085	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014 1.024 1.032 1.028 1.018	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378 12.000 12.094 12.119
237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252	IgG Control #1 IgG Control #2 IgG Control #3 Class I #1 Class I #2 Class I #3 Class II #1 Class II #2 Class II #3 Class III #1 Class III #2 Class III #3 All 3 mAbs #1	0.088 0.089 0.081 0.097 0.089 0.096 0.082 0.085 0.090 0.086 0.085 0.084	0.906 0.988 0.892 1.064 1.030 1.132 0.986 1.014 1.024 1.032 1.028 1.018	10.295 11.101 11.012 10.969 11.573 11.792 12.024 11.929 11.378 12.000 12.094 12.119 12.247

254 255All 3 mAbs #3 0.087 1.032 11.862 255 256 257 $Experiment 4$ 258 259 $Condition$ $O.D. A_{600}$ $O.D.A_{490}$ Aggregation Index (A ₄₉₀ /A ₆₀₀)260 $\Delta psl #1$ 0.198 0.973 4.914 262 $\Delta psl #2$ 0.209 0.903 4.321 263 $\Delta psl #3$ 0.208 0.991 4.764 264 265 WFPA 801 #1 0.213 1.078 5.061 266WFPA 801 #1 0.215 1.184 5.507 267WFPA 801 #3 0.249 1.166 4.683 268 269 WFPA 801 + ara #1 0.104 1.002 9.635 270WFPA 801 + ara #3 0.098 1.042 10.633 273IgG Control #1 0.109 1.022 9.376 274IgG Control #2 0.101 1.018 10.079 275IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278Class I #2 0.124 0.838 6.758 279Class I #3 0.134 0.928 6.925
256Experiment 4258ConditionO.D. A_{600} O.D. A_{490} Aggregation Index (A_{490}/A_{600})260 $\Delta psl \#1$ 0.1980.9734.914262 $\Delta psl \#2$ 0.2090.9034.321263 $\Delta psl \#3$ 0.2080.9914.764264
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
$\begin{array}{cccccccccccccccccccccccccccccccccccc$
264 265 WFPA 801 #1 0.213 1.078 5.061 266 WFPA 801 #2 0.215 1.184 5.507 267 WFPA 801 #3 0.249 1.166 4.683 268
265 WFPA 801 #1 0.213 1.078 5.061 266 WFPA 801 #2 0.215 1.184 5.507 267 WFPA 801 #3 0.249 1.166 4.683 268 269 WFPA 801 + ara #1 0.104 1.002 9.635 270 WFPA 801 + ara #2 0.106 0.980 9.245 271 WFPA 801 + ara #3 0.098 1.042 10.633 272 273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
266 WFPA 801 #2 0.215 1.184 5.507 267 WFPA 801 #3 0.249 1.166 4.683 268
267 WFPA 801 #3 0.249 1.166 4.683 268 269 WFPA 801 + ara #1 0.104 1.002 9.635 270 WFPA 801 + ara #2 0.106 0.980 9.245 271 WFPA 801 + ara #3 0.098 1.042 10.633 272 273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
268 269 WFPA 801 + ara #1 0.104 1.002 9.635 270 WFPA 801 + ara #2 0.106 0.980 9.245 271 WFPA 801 + ara #3 0.098 1.042 10.633 272 273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
269 WFPA 801 + ara #1 0.104 1.002 9.635 270 WFPA 801 + ara #2 0.106 0.980 9.245 271 WFPA 801 + ara #3 0.098 1.042 10.633 272 273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
270 WFPA 801 + ara #2 0.106 0.980 9.245 271 WFPA 801 + ara #3 0.098 1.042 10.633 272 273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
271 WFPA 801 + ara #3 0.098 1.042 10.633 272 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
272 273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
273 IgG Control #1 0.109 1.022 9.376 274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
274 IgG Control #2 0.101 1.018 10.079 275 IgG Control #3 0.105 1.058 10.076 276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
275 IgG Control #3 0.105 1.058 10.076 276
276 277 Class I #1 0.132 0.894 6.773 278 Class I #2 0.124 0.838 6.758 279 Class I #3 0.134 0.928 6.925
277Class I #10.1320.8946.773278Class I #20.1240.8386.758279Class I #30.1340.9286.925
278Class I #20.1240.8386.758279Class I #30.1340.9286.925
279 Class I #3 0.134 0.928 6.925
200
200
281 Class II #1 0.132 0.950 7.197
282 Class II #2 0.142 0.966 6.803
283 Class II #3 0.138 1.114 8.072
284
285 Class III #1 0.140 1.098 7.843
286 Class III #2 0.133 1.050 7.895
287 Class III #3 0.130 1.062 8.169
288
289 All 3 mAbs #1 0.153 1.006 6.575
290 All 3 mAbs #2 0.161 1.106 6.870
291 All 3 mAbs #3 0.164 1.080 6.585
292

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