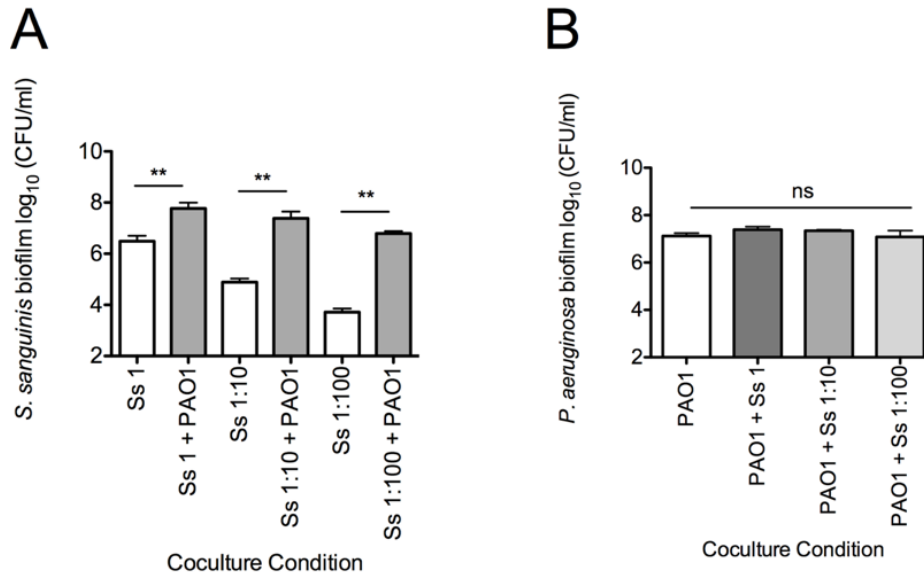


1 **Supplemental Figures**

2

3 **Figure S1**



4 **Figure S1: Growth of *S. sanguinis* SK36 and *P. aeruginosa* PAO1 in coculture.** (A

5 and B) The dilution series of *S. sanguinis* SK36 from an OD₆₀₀ of 0.1 (Ss 1 = undiluted)

6 and the response of each indicated dilution (1:10, 1:100) to coculture with *P. aeruginosa*

7 PAO1 (A) and the corresponding *P. aeruginosa* PAO1 (PAO1) biofilm growth in

8 coculture with each *S. sanguinis* dilution (B). The data shown in Figure 1A and Figure

9 S1 panels A and B are from the same experiments. Each bar represents an average of

10 three biological replicates with three technical replicates. Error bars represent SD. ns,

11 not significant, **, P < 0.01 by repeated measures two-tailed student's *t*-test (A) or

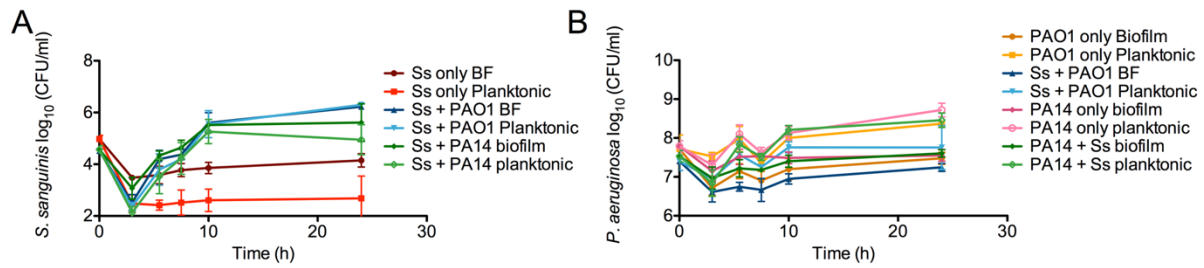
12 repeated measures one-way analysis of variance (ANOVA) with Dunnett's post-test

13 using PAO1 as the control (B).

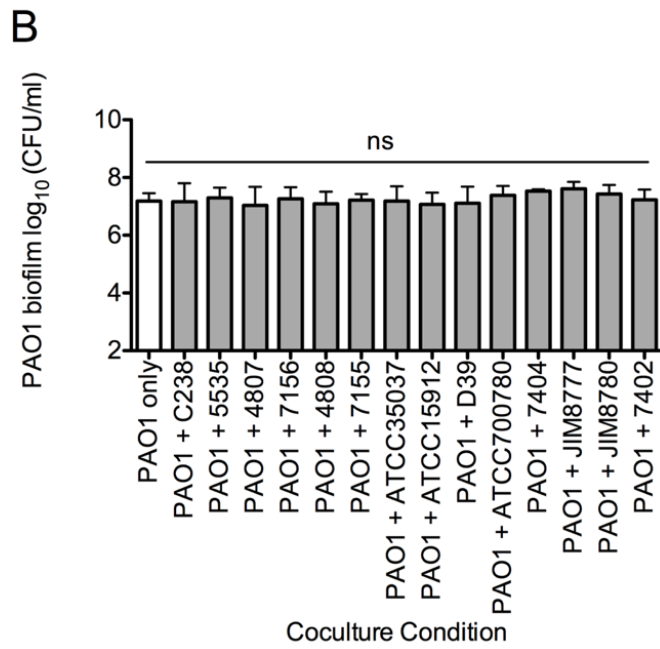
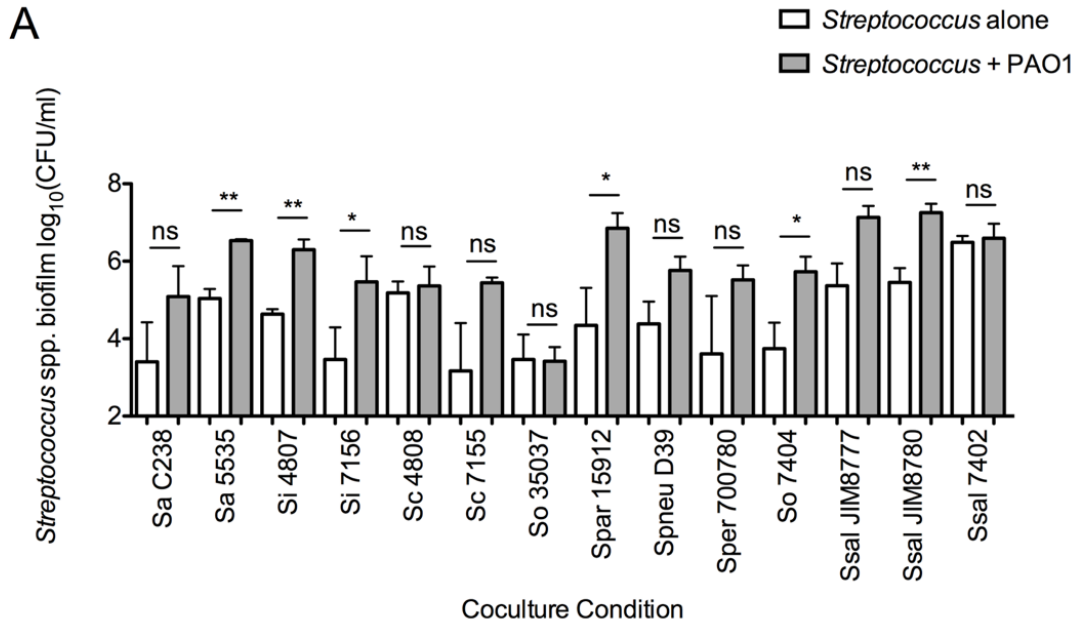
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16 **Figure S2**

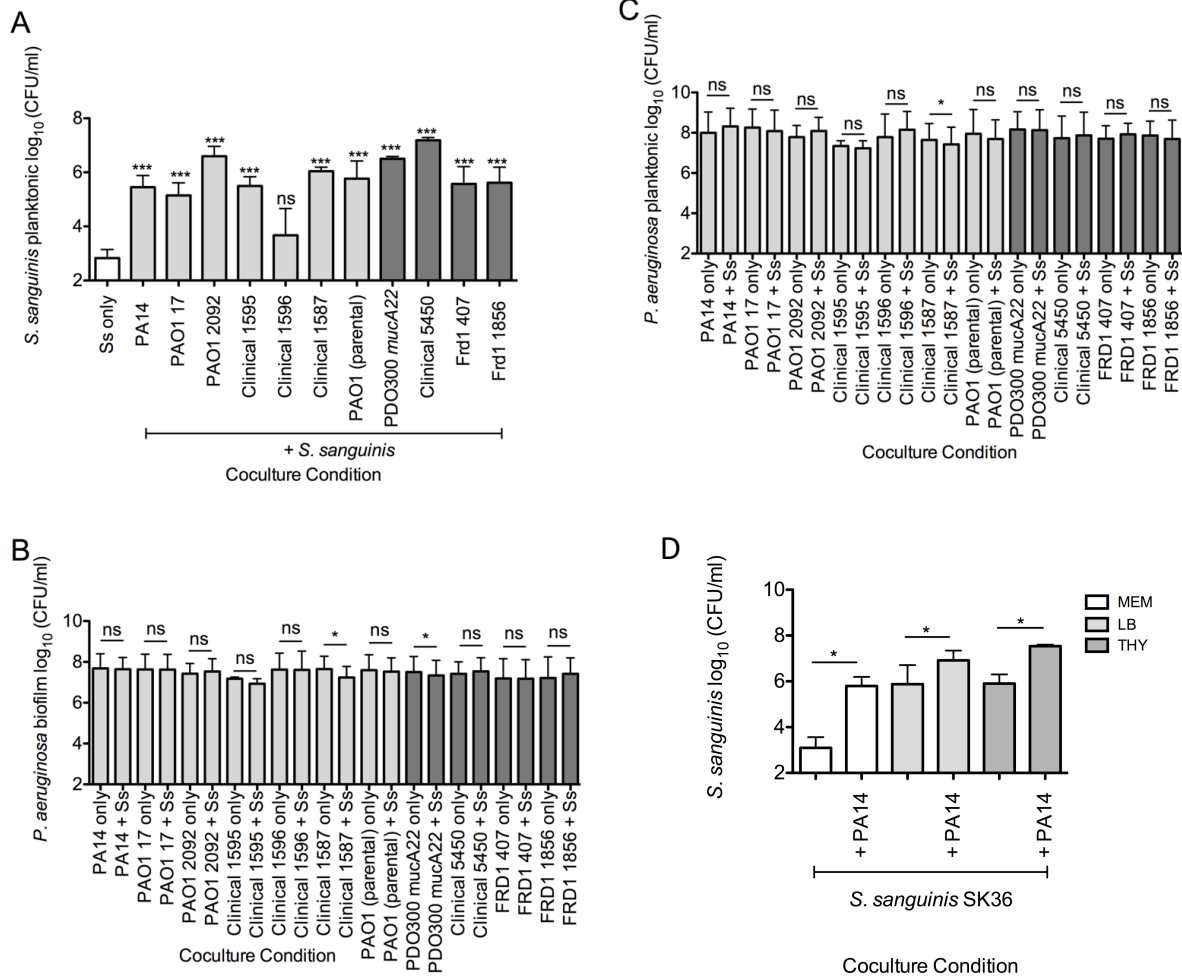


17 **Figure S2: Coculture growth kinetics of *S. sanguinis* and *P. aeruginosa* PAO1 and**
18 **PA14 over 24 hours.** (A and B) The growth kinetics of the indicated bacterial species in
19 coculture was investigated over 24 hours, with *S. sanguinis* biofilm and planktonic
20 growth from coculture with *P. aeruginosa* PA14 and PAO1 (A), and the corresponding
21 *P. aeruginosa* PA14 and PAO1 biofilm and planktonic growth (B). The data shown in
22 Figure 1B and Figure S2 panels A and B are from the same experiments. Each time
23 point represents the average of three biological replicates and three technical replicates.



26 **Figure S3: All oral *Streptococcus spp.* tested in coculture with *P. aeruginosa***
27 **PAO1 and the corresponding *P. aeruginosa* growth.** (A) Coculture biofilm growth of
28 every oral *Streptococcus spp.* strain tested here with *P. aeruginosa* PAO1.
29 *Streptococcus spp.* are indicated by their strain number and correspond to the following
30 strains: *S. anginosus* C238, *S. anginosus* 5535, *S. intermedius* 4807, *S. intermedius*
31 7156, *S. constellatus* 4808, *S. constellatus* 7155, *S. oralis* ATCC35037, *S.*
32 *parasanguinis* ATCC15912, *S. pneumoniae* D39, *S. peroris* ATCC700780, *S. oralis*
33 7404, *S. salivarius* JIM8777, *S. salivarius* JIM8780, and *S. salivarius* 7402. (B) The
34 growth of *P. aeruginosa* PAO1 in coculture with each oral *Streptococcus spp.* strain
35 tested here. The data shown in Figure S3A and B, and Figure 1C are from the same
36 experiments. Each bar represents the average of three biological replicates with three
37 technical replicates. ns, not significant, *, $P < 0.05$, **, $P < 0.01$ by repeated measures
38 two-tailed student's *t*-test (A) or by repeated measures ANOVA with Dunnett's posttest
39 using PAO1 as the control (B).

40 **Figure S4**



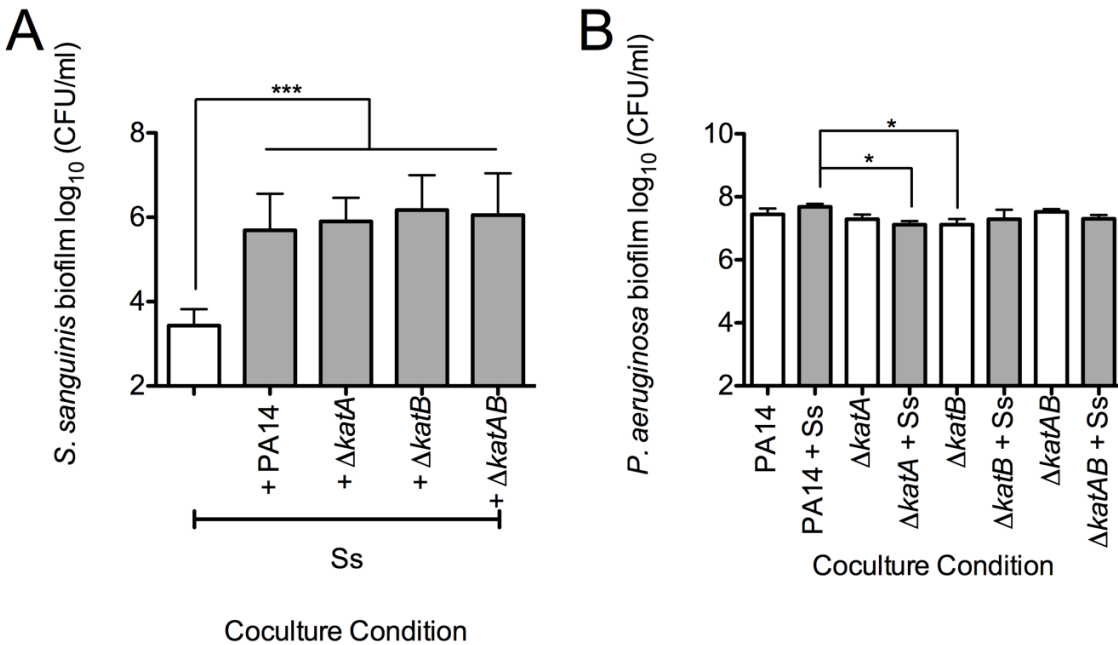
41 **Figure S4: *S. sanguinis* SK36 planktonic growth, and *P. aeruginosa* biofilm and**
 42 **planktonic growth corresponding to Figure 1. (A to C) Coculture experiments were**
 43 **conducted with *S. sanguinis* SK36 with different *P. aeruginosa* clinical and laboratory**
 44 **strains to investigate the effects on *S. sanguinis* planktonic growth (A), and *P.***
 45 ***aeruginosa* biofilm (B) and *P. aeruginosa* planktonic growth (C). The data shown in**
 46 **Figure 1D and Figure S4 panels A to C are from the same experiments. Bars represent**
 47 **the average of three biological replicates with three technical replicates. Error bars**
 48 **indicate SD. ns, not significant, *, P < 0.05, **, P < 0.01, ***, P < 0.001 by repeated**

49 measures ANOVA with Dunnett's posttest for multiple comparisons using Ss only as the
50 control (A), or by repeated measures two-tailed student's *t*-test (B and C). (D) S.

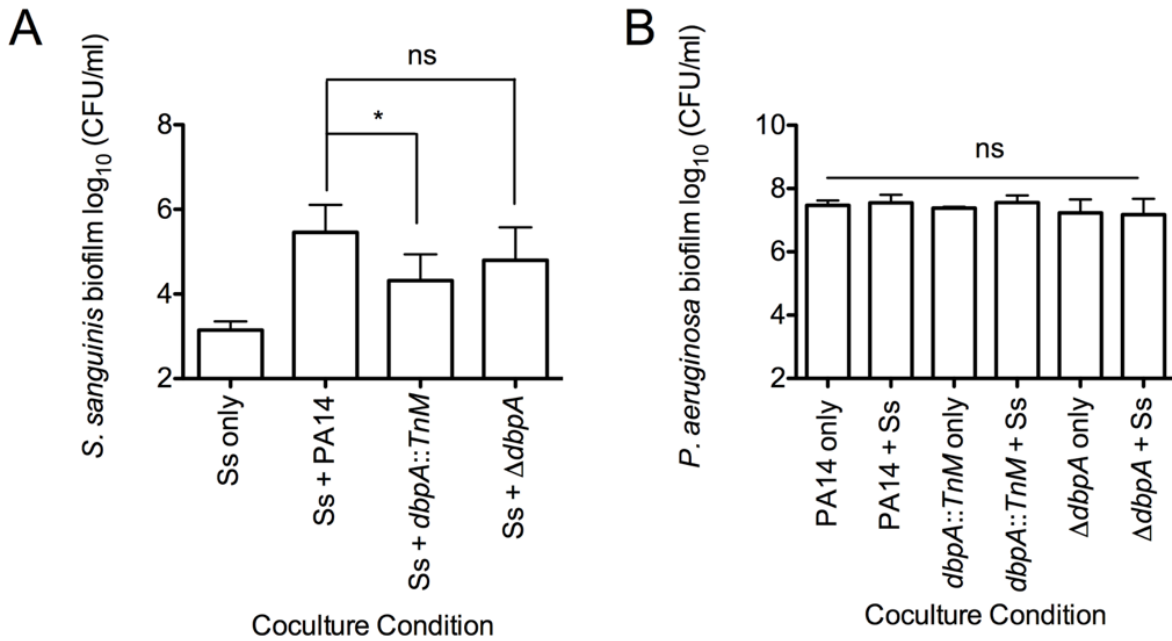
51 *sanguinis* SK36 was tested in coculture with *P. aeruginosa* PA14 in the indicated rich
52 media. The error bars indicate the standard deviation. ns, not significant, *, $P < 0.05$ by
53 paired two-tailed student's *t*-test.

54

55



57 **Figure S5: *P. aeruginosa* catalase mutants do not have a defect in *S. sanguinis***
 58 **SK36 growth enhancement.** (A) *S. sanguinis* SK36 biofilm growth data from coculture
 59 with *P. aeruginosa* PA14 $\Delta katA$, $\Delta katB$, and $\Delta katAB$ mutants. (B) Corresponding *P.*
 60 *aeruginosa* biofilm growth data indicates no significant growth defects of *P. aeruginosa*
 61 catalase mutants in coculture with *S. sanguinis* SK36. Bars represent the average of
 62 three biological replicates with three technical replicates. Error bars indicate SD. ns, not
 63 significant by repeated measures ANOVA with Dunnett's post-test for multiple
 64 comparisons using Ss only as the positive control (A) and by repeated measures
 65 ANOVA with Tukey's post-test for multiple comparisons (B).



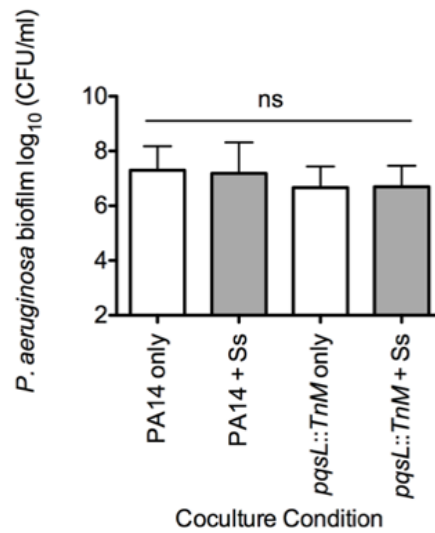
67

68 **Figure S6: The *P. aeruginosa* PA14 Δ *dbpA* mutant does not have a defect in *S.***
 69 ***sanguinis* SK36 growth enhancement.** (A) *S. sanguinis* SK36 biofilm growth data
 70 from coculture with the *P. aeruginosa* PA14 *dbpA::TnM* and the Δ *dbpA* mutants. (B) *P.*
 71 *aeruginosa* biofilm growth data from coculture with *S. sanguinis* SK36. Bars represent
 72 the average of four biological replicates with three technical replicates. Error bars
 73 indicate SD. ns, not significant, *, $P < 0.05$ by repeated measures ANOVA with
 74 Dunnett's posttest for multiple comparisons using Ss + PA14 as the control condition.

75

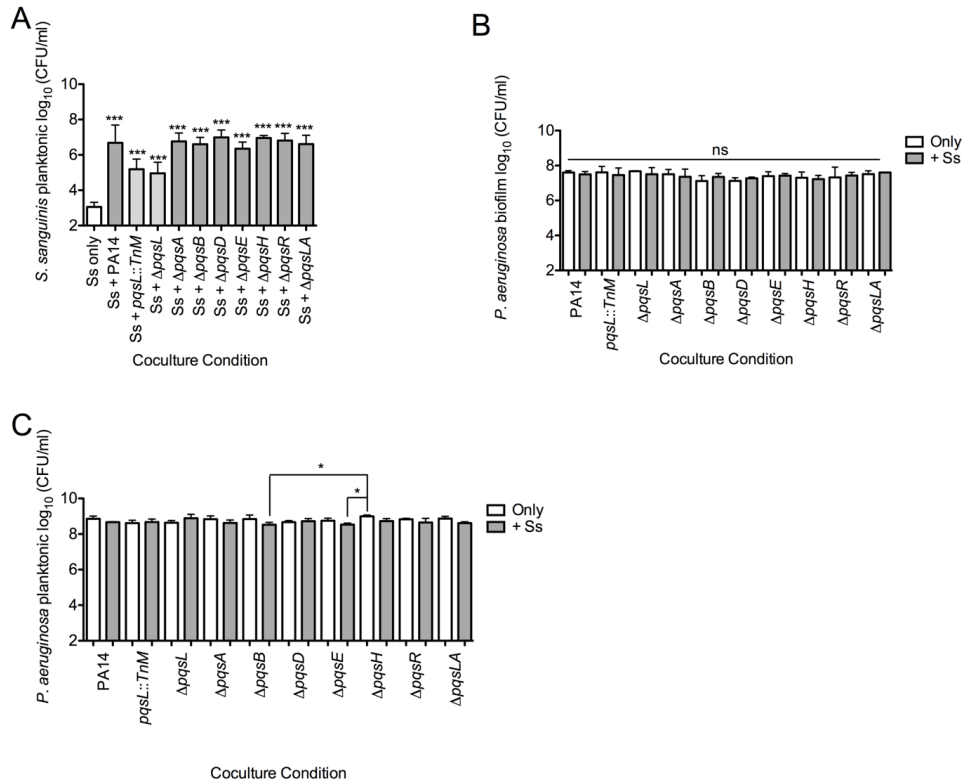
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77 **Figure S7**

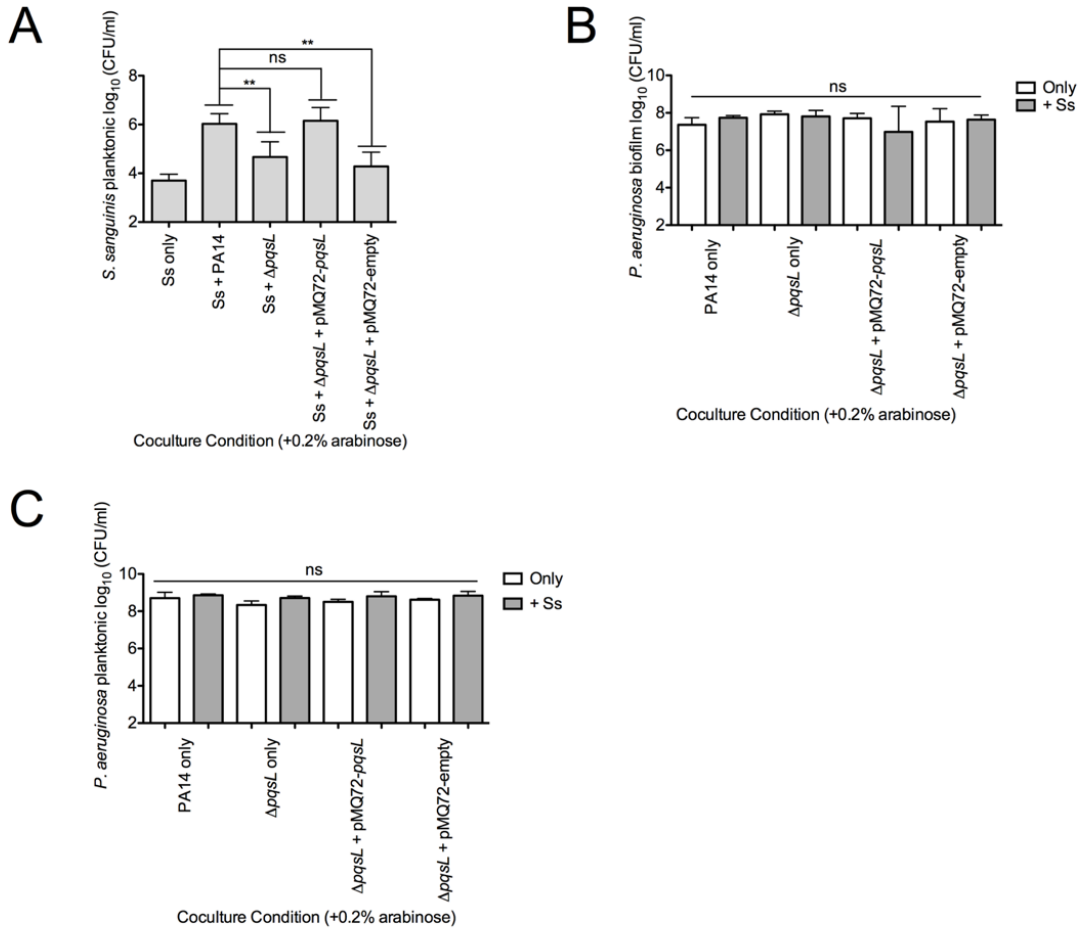


78 **Figure S7: The *P. aeruginosa* PA14 *pqsL::TnM* mutant does not have a growth**
79 **defect compared to wild-type *P. aeruginosa* PA14.** *P. aeruginosa* PA14 biofilm
80 growth data from coculture with *S. sanguinis* SK36. The data shown in Figure S7 and
81 Figure 2B are from the same experiments. Bars represent the average of three
82 biological replicates with three technical replicates. Error bars indicate SD. ns, not
83 significant by repeated measures ANOVA with Dunnett's posttest for multiple
84 comparisons using *P. aeruginosa* PA14 only as the control condition.

85 **Figure S8**



86 **Figure S8: *S. sanguinis* SK36 planktonic growth in coculture with the *P.***
 87 ***aeruginosa* PA14 *pqs* mutant strains, and the corresponding *P. aeruginosa pqs***
 88 **mutant strain biofilm and planktonic viable counts. (A to C) *S. sanguinis* SK36**
 89 **planktonic growth from coculture with *P. aeruginosa* PA14 PQS biosynthetic mutants**
 90 **(A) and the corresponding *P. aeruginosa* PQS biosynthetic mutants' biofilm (B) and**
 91 **planktonic growth data (C). The data shown in Figure 3B and Figure S8 panels A-C are**
 92 **from the same experiments. Bars represent the average of three biological replicates**
 93 **and three technical replicates. Error bars indicate SD. *, P < 0.05, ***, P < 0.001 by**
 94 **repeated measures ANOVA with Dunnett's posttest for multiple comparisons using Ss**
 95 **only as the control condition (A) or by repeated measures ANOVA with Tukey's posttest**
 96 **for multiple comparisons (B and C).**



98

99 **Figure S9: Quantifying growth from complementation studies.** (A) *S. sanguinis*

100 SK36 planktonic growth data from coculture with wild type *P. aeruginosa* PA14, the

101 $\Delta pqsL$ mutant, complementation strain and vector control. (B and C) There is no

102 significant difference in *P. aeruginosa* biofilm (B) and planktonic (C) cells recovered

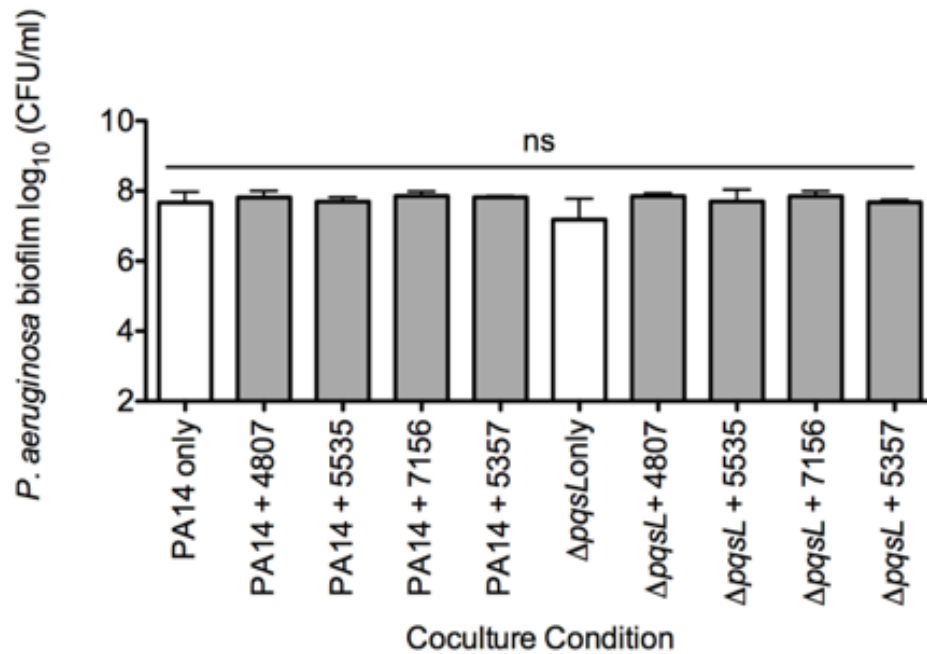
103 from coculture with *S. sanguinis* SK36. The data shown in Figure 3C and Figure S9

104 panels A-C are from the same experiments. Bars represent three biological replicates

105 with three technical replicates. Error bars indicate SD. ns, not significant, **, P < 0.01,

106 by repeated measures ANOVA with Tukey's posttest for multiple comparisons.

107 **Figure S10**

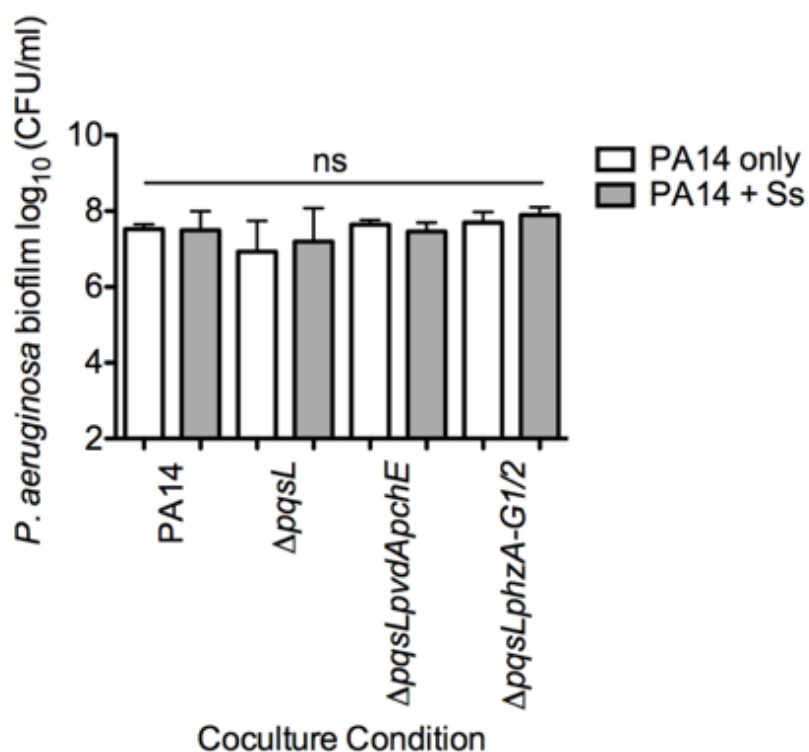


108 **Figure S10: There is no significant difference between the growth of *P.***
109 ***aeruginosa* strains in the presence of different *Streptococcus* spp.** Growth of the
110 wild-type and mutant *P. aeruginosa* strains in the presence of different *Streptococcus*
111 spp. The data shown in Figures 3D and Figure S10 are from the same experiments.
112 Bars represent three biological replicates with three technical replicates. Error bars
113 indicate SD. ns, not significant by repeated measures ANOVA with Tukey's posttest for
114 multiple comparisons.

115

116

117 **Figure S11**



118

119 **Figure S11: There is no significant difference between the growth of *P.***

120 ***aeruginosa* PA14 wild type and mutant strains in coculture with *S. sanguinis***

121 **SK36.** Growth of the wild type and mutant *P. aeruginosa* PA14 as biofilms from

122 coculture with *S. sanguinis* SK36 (Ss). The data shown in Figures 4A and Figure S11

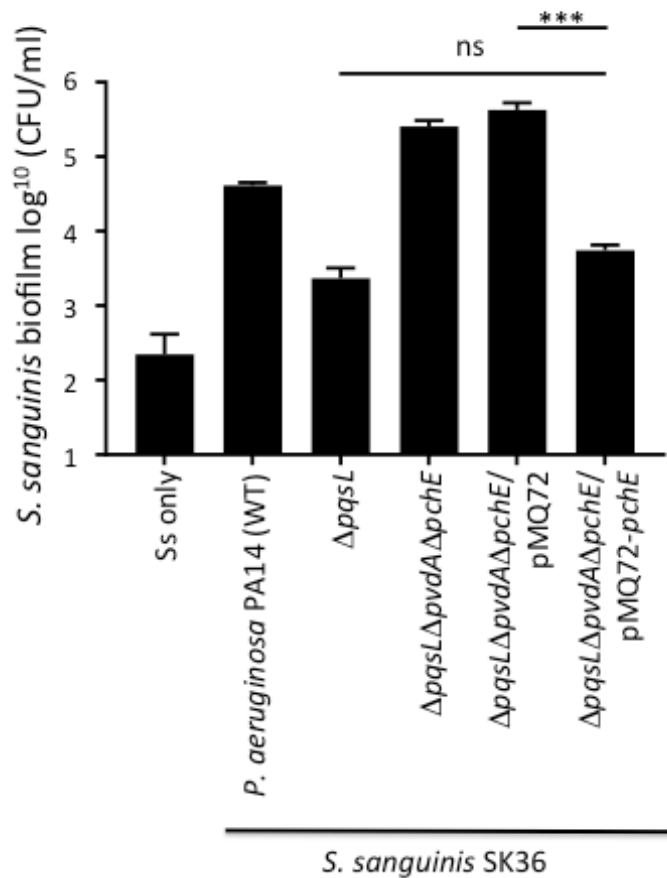
123 are from the same experiments. Bars represent three biological replicates with three

124 technical replicates. Error bars indicate SD. ns, not significant by repeated measures

125 ANOVA with Tukey's posttest for multiple comparisons.

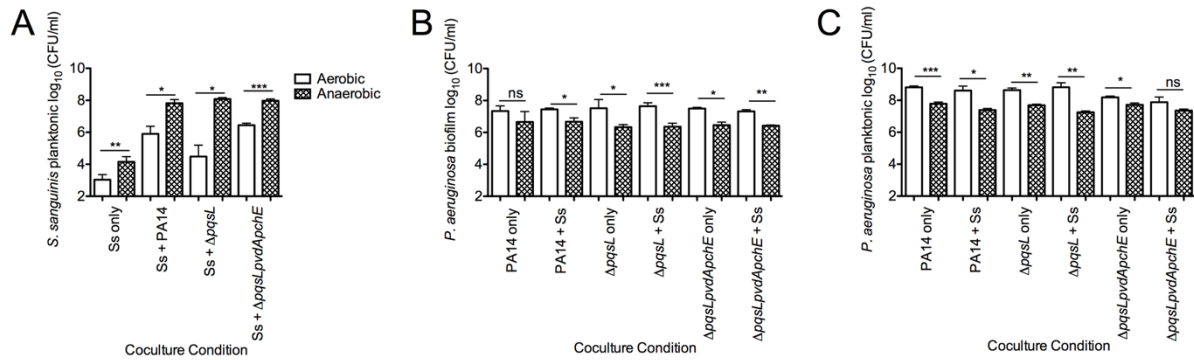
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129 **Figure S12: Complementation analysis.** Shown is the complementation analysis of
 130 the $\Delta pqsLpvdApchE$ mutant in coculture with *S. sanguinis* SK36. Introducing the
 131 pMQ72 plasmid carrying the *pchE* gene (pMQ72-*pchE*) but not the vector control
 132 (pMQ72) restores the reduced viability of *S. sanguinis* SK36 observed for the $\Delta pqsL$
 133 mutant. ***, $P < 0.001$ comparing $\Delta pqsL\Delta pvdA\Delta pchE/pMQ72$ to
 134 $\Delta pqsL\Delta pvdA\Delta pchE/pMQ72-pqsE$. Significance was determined with a one-way ANOVA
 135 followed by Tukey's multiple comparison. There was no significant difference (ns)
 136 between the $\Delta pqsL$ mutant and the $\Delta pqsL\Delta pvdA\Delta pchE/pMQ72-pqsE$ strain using this
 137 same test.

138 **Figure S13**



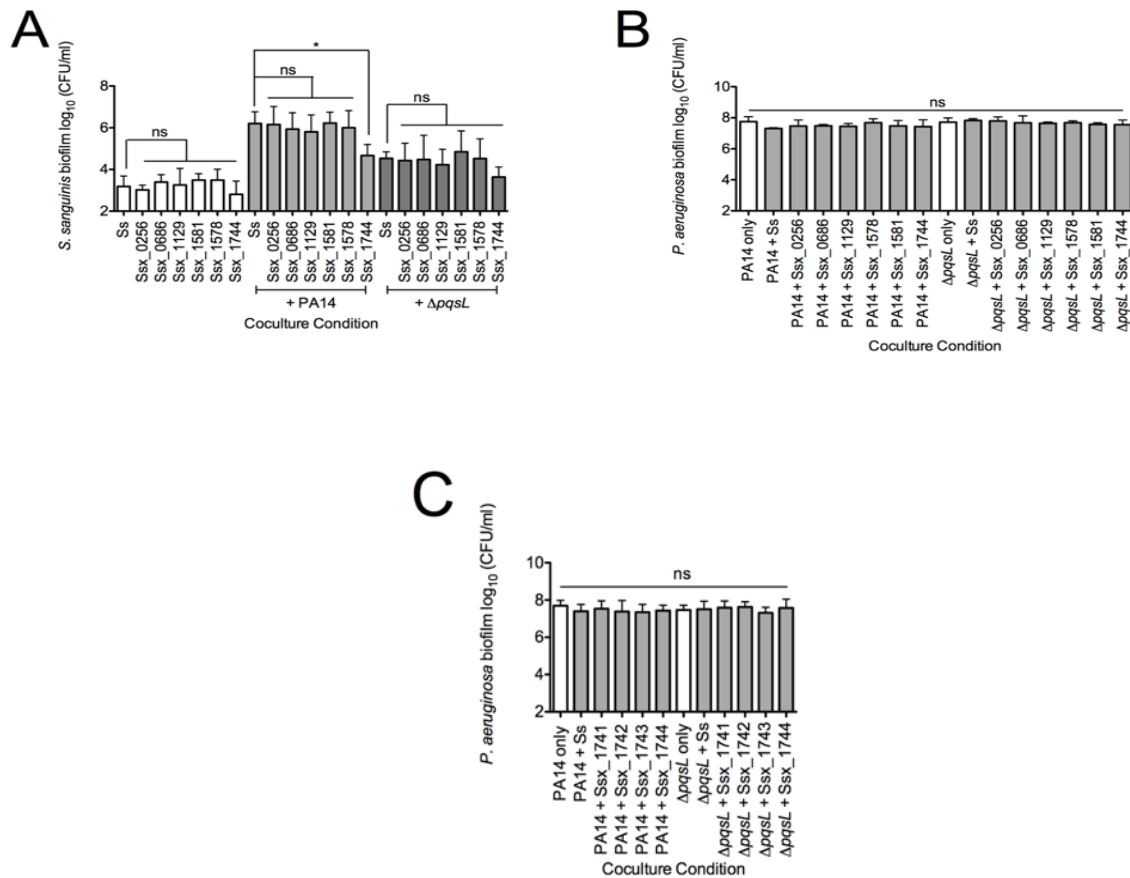
139 **Figure S13: Growth under anaerobic conditions.** (A) *S. sanguinis* SK36 planktonic
 140 growth increases upon coculture with *P. aeruginosa* PA14 in anaerobic conditions. (B
 141 and C) *P. aeruginosa* biofilm (B) and planktonic growth in coculture with *S. sanguinis*
 142 SK36 in anaerobic conditions are lower (C) compared to *P. aeruginosa* grown in air.
 143 The data in Figure 4B and Figure S13 panels A-C are from the same experiments. Bars
 144 represent the average of three biological replicates with three technical replicates. Error
 145 bars indicate SD. ns, not significant, *, P < 0.05, **, P < 0.01, ***, P < 0.001 by repeated
 146 measures two-tailed student's *t*-test.

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148

149

150 **Figure S14**



151

152 **Figure S14: *S. sanguinis* SK36 iron acquisition mutant strain coculture growth.**

153 (A) *S. sanguinis* SK36 iron acquisition mutants tested in coculture with *P. aeruginosa*

154 PA14 and the $\Delta pqsL$ mutant strain. (B and C) *P. aeruginosa* biofilm growth from

155 coculture with *S. sanguinis* mutant strains lacking genes for iron acquisition. Figures

156 S14A and B are from the same experiment, and Figures 4C and S14C are from the

157 same experiment. Bars represent the average of three biological replicates with at least

158 three technical replicates. Error bars indicate SD. ns, not significant, *, $P < 0.05$ by one

159 way ANOVA with Dunnett's posttest for multiple comparisons using the Ss condition as

160 the control (A) or with Tukey's posttest for multiple comparisons (B and C).