1 **SUPPLEMENTAL INFORMATION**

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5 **Title**: RgpF is required for maintenance of stress tolerance and virulence in
6 *Streptococcus mutans.*

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24 SUPPLEMENTAL METHODS

25 **Quantitative real-time PCR.** RNA was extracted from three independent
26 cultures grown at steady state conditions of pH 7 and pH 5, according to
27 previously described methods (1). The High Capacity cDNA Reverse
28 Transcription kit (Applied Biosystems, Carlsbad, CA) was used to generate cDNA
29 from RNA samples using random primers. Specific primers to *rgpF* were used
30 (RT-forward: 5'-GCGAGATGGAATGGTCTT-3' and RT-reverse: 5'-
31 CACGGTTGTTGGTCAATC-3') with Power SYBR Green Master Mix (Applied
32 Biosystems) and reactions were carried out in a Step One Plus Real-time PCR
33 System (Applied Biosystems). The mRNA copy number was quantified based on
34 a standard curve of PCR products for specific gene targets.

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36 **Growth curves**. Growth rates were determined using a BioScreen C plate
37 reader (Growth Curves USA, Piscataway, NJ). Overnight cultures of *S. mutans*
38 UA159, Δ*rgpF*, and $rgpF^+$ strains grown in TY medium supplemented with 1%
39 (w/v) glucose were subcultured 1:20 (UA159 and $rgpF^+$) or 1:10 (Δ*rgpF*) into
40 fresh medium and incubated at 37°C in a 5% (v/v) CO_2/95% air atmosphere until
41 cultures reached an OD_{600} of 0.3. A 10 μL aliquot was used to inoculate wells of
42 a microtiter plate containing 300 μL test medium. Assays were performed at 37°C
43 and OD_{600} was continually read at 30 minute intervals following 10 seconds of
44 shaking at medium amplitude. Generation times were calculated using the
45 formula $0.3/[(N–N_0)/(T–T_0)]$, where N represents the mean OD_{600} value at the end
46 of exponential phase and N_0 represents the mean OD_{600} at the beginning of

47 exponential phase. T and T_0 refer to the times in minutes that correspond to the
48 OD_{600} values for N and N_0, respectively. Statistical significance (†; $p \leq 0.02$) was
49 determined by pairwise comparison using Student's *t*-test, comparing Δ*rgpF* to
50 both UA159 and *rgpF*$^+$.
51
52 **Interspecies competition spot assay controls.** *Streptococcus gordonii* DL1
53 and *Streptococcus sanguinis* 10904, members of the peroxigenic streptococcal
54 group, were used as primary cultures in peroxigenic competition assays (2).
55 Bacteria were grown overnight in BHI medium at 37°C in a 5% (v/v) CO_2/95% air
56 atmosphere, then subcultured into fresh BHI medium until cultures reached an
57 OD_{600} of 0.4. An 8.0 µL aliquot was then spotted onto pre-warmed BHI agar and
58 incubated overnight anaerobically at 37°C. Anaerobic conditions were achieved
59 by utilizing a GasPak jar apparatus containing a BD GasPak EZ Anaerobe
60 Container System sachet (BD, Sparks, MD, USA) to deplete oxygen gas within
61 the sealed vessel, verified by the inclusion of a dry anaerobic indicator (BD
62 GasPak). Concurrently, competing bacteria were similarly grown overnight and
63 subcultured (i.e. at 37°C in a 5% (v/v) CO_2/95% air atmosphere), and an 8.0 µL
64 aliquot was spotted immediately adjacent to the primary spot. Plates were then
65 returned to GasPak jars for anaerobic outgrowth. A *S. mutans smx* mutant strain
66 (SMU.1649; responsible for production of the base-excision repair enzyme Smx)
67 was included as a positive control for oxidative stress susceptibility (3).
68 Separately, acidogenic competition assays were performed using *S.*
69 *mutans* UA159 and Δ*rgpF* as primary cultures, and both primary and competing

70 cultures were grown as described above. Spots were plated onto BHI agar
71 medium buffered to pH 7.0 using 50 mM KPO_4 buffer to control for acidification of
72 agar and incubated at 37°C in a 5% (v/v) CO_2/95% air atmosphere.

73 Data is shown as representative images of outgrowth inhibition
74 from three independent experiments performed in triplicate.

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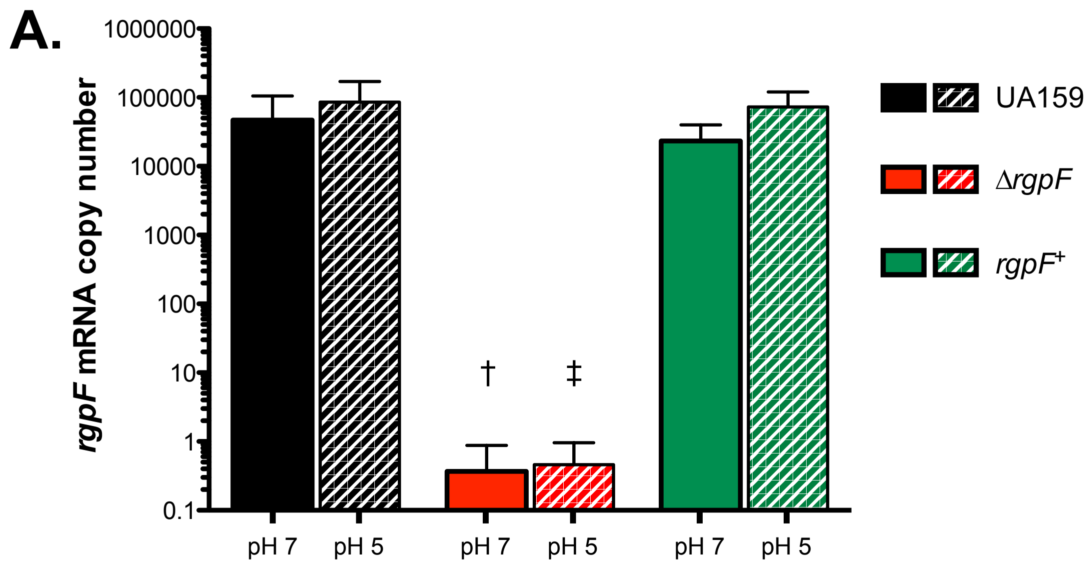

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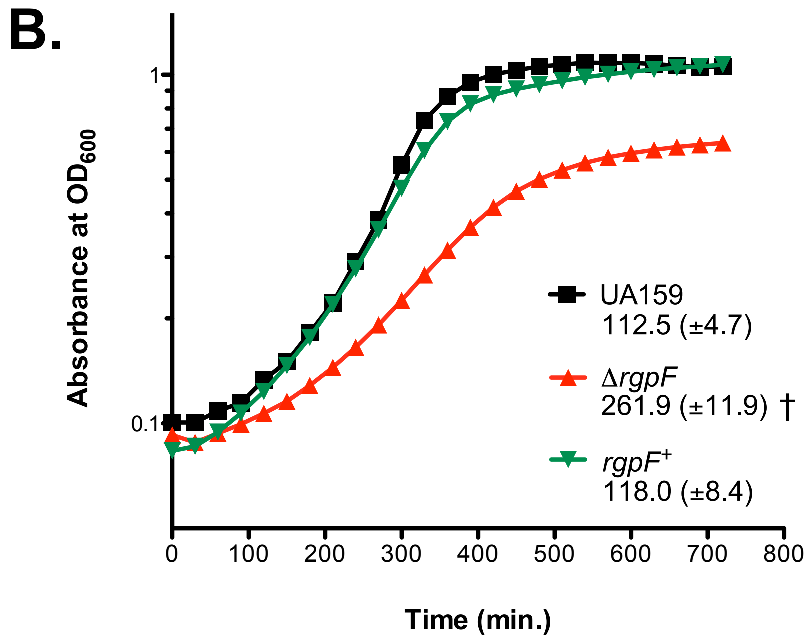

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82 **Figure S1. Complementation of *rgpF* restores expression and growth.**

83 (Panel A) Transcription of *rgpF* was determined by qRT-PCR using RNA isolated

84 from cultures of *S. mutans* UA159 (black bars), Δ*rgpF* (red bars) and $rgpF^+$

85 (green bars) grown in continuous culture to steady-state pH values of 7 (solid

86 bars) and 5 (hashed bars). Samples from three independent chemostat cultures

87 were measured in triplicate and are represented as mean values ± SD. (Panel

88 B) Cultures of *S. mutans* UA159 (■), Δ*rgpF* (▲) and $rgpF^+$ (▼), were grown

89 overnight in TY + 1% (w/v) glucose medium. The overnight cultures were used to

90 inoculate fresh TY + 1% (w/v) glucose and cultures were grown to OD_{600} ~ 0.3,

91 then used to inoculate a plate containing fresh medium. Growth was assessed in

92 a Bioscreen C plate reader by measuring OD_{600}. Data are shown as mean values

93 ± SD, with calculated doubling times ± SD depicted in legend (n=10). Statistical

94 significance (†; $p \leq 0.02$) was determined by pairwise comparison using

95 Student's *t*-test, comparing Δ*rgpF* to both UA159) and $rgpF^+$.

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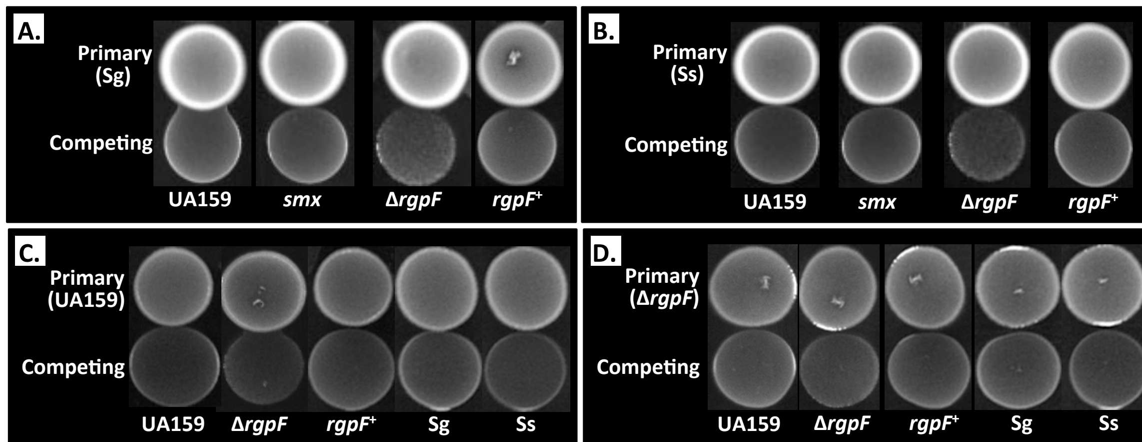

100 **Figure S2. Interspecies competition assay controls.** Competitive spot plating
101 was performed under anaerobic conditions for oxidative stress control
102 experiments using *S. gordonii* DL-1 (Sg) (Panel A) and *S. sanguinis* 10904 (Ss)
103 (Panel B) as primary spots. *S. mutans* UA159 (Panel C) and *S. mutans* Δ*rgpF*
104 (Panel D) were used as primary spots in acidic stress control experiments where
105 spots were plated on BHI agar medium buffered to neutral pH using 50 mM KPO_4
106 buffer. The *S. mutans* strain *smx* was included as a positive oxidative stress-
107 sensitive control and carries a mutation in SMU.1649 encoding a base excision
108 repair enzyme (3).

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110 SUPPLEMENTAL REFERENCES

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