Supplemental Materials

MATERIALS AND METHODS

RT-PCR to evaluate gene expression

To examine expression of *gap2* and *gap3*, total RNA was extracted from *S. parasanguinis* wild-type, *gap* mutants, and Gap3 overexpressing strains. Standard protocol for ZR RNA MiniPrep (Zymo Research) was followed, with the exception of the lysing step in which 1.5 ml of pelleted cells (OD_{470}= 0.6) were vortexed in 1 mL of TRIzol® Reagent (Invitrogen) and Lysing Matrix B (MP Bio) for 5 minutes on high. Following extraction, RNA was treated with RQ1 DNase (Promega) and then subjected to reverse transcription using M-MLV (Promega) standard protocol and Random Primers (Promega) for cDNA synthesis. cDNA was subsequently used as a template for PCR amplification using a *gap2* primer set (Gap2-1191-F, Gap2-1584-R) and a *gap3* primer set (Gap3-1-F, Gap3-534-R). Another primer set (Gap3-1-F, GFP-101-R) was used to detect only Gap3 expressed by the plasmid in the overexpressing strains. Expression of *fimA* was also detected by RT-PCR with FimA-1-F and FimA-465-R, serving as a control.

TABLES

Table S1. Primers used in this study

Primers	Sequences
Gap2-1191-F	GATCGTCGACCCGACGGCTGTAATTGTAGGTAAG
Gap2-1584-R	GGCGCCGGGATCCTCTTCCAAACTGATCTTCTAGAAT
Gap3-1-F	GCGCCGGCCATGGATGACTAAACAGTTAATTTCTG
Gap3-534-R	GCGGCGCCGGCGGATCCAATATATTCTATTAAATTTTTCACC
GFP-101-F	TCACCCTCTCCACTGACAGAAAATTTGTG
FimA-1-F	GGCATGAAAAAAATCGCTTCTGTCCTCGCCC
FimA-465-R	GGCAATGTTTTTAGCGTAGAGGATCCCG

FIGURES

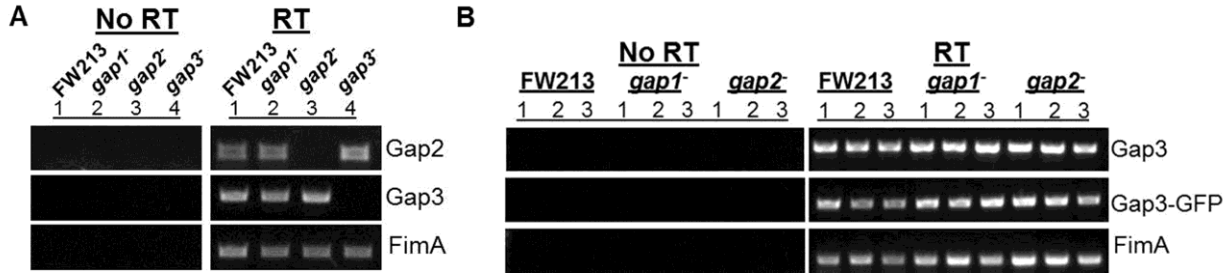

Figure S1. No difference is observed in transcription of *gap2* and *gap3* among strains. RT-PCR analysis in wild-type, *gap* mutants, and Gap3 overexpressing strains. **(A)** Strains include FW213 wild-type and insertional mutants of *gap1*, *gap2*, and *gap3*. Transcription levels of *gap2* and *gap3* were determined. **(B)** Strains include FW213 wild-type, *gap1* mutant, and *gap2* mutant overexpressing Gap3 alone (lanes 1, 4, and 7), Gap2 and Gap3 (lanes 2, 5, and 8), or Gap1, Gap2, and Gap3 (lanes 3, 6, and 9) in the pIB184-*gfp* vector, where Gap3 is tagged with GFP in all strains. Transcription levels of *gap3* and *gap3* transcribed from the plasmid (*gap3-gfp*) were determined. Transcription of FimA was used a control.