

Supplementary Information for

**The well-coordinated linkage between acidogenicity and aciduricity via insoluble glucans
on the surface of *Streptococcus mutans***

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1) Supplementary Figures:



Fig. S1: Genes with log₂ 1.5 fold increase in the WT pH 5.5 relative to pH 7.5. Dashed vertical lines indicate significant fold change. Colored bars indicate log₂ fold changes for differential expression. Filled colored circles indicate normalized expression values for each condition tested with sucrose. gtfBC mutant with sucrose pH 5.5 (bcw5), gtfBC mutant with sucrose pH 7.5 (bcw7), WT with sucrose pH 5.5 (Wtw5), WT with sucrose pH 7.5 (wtw7.5).

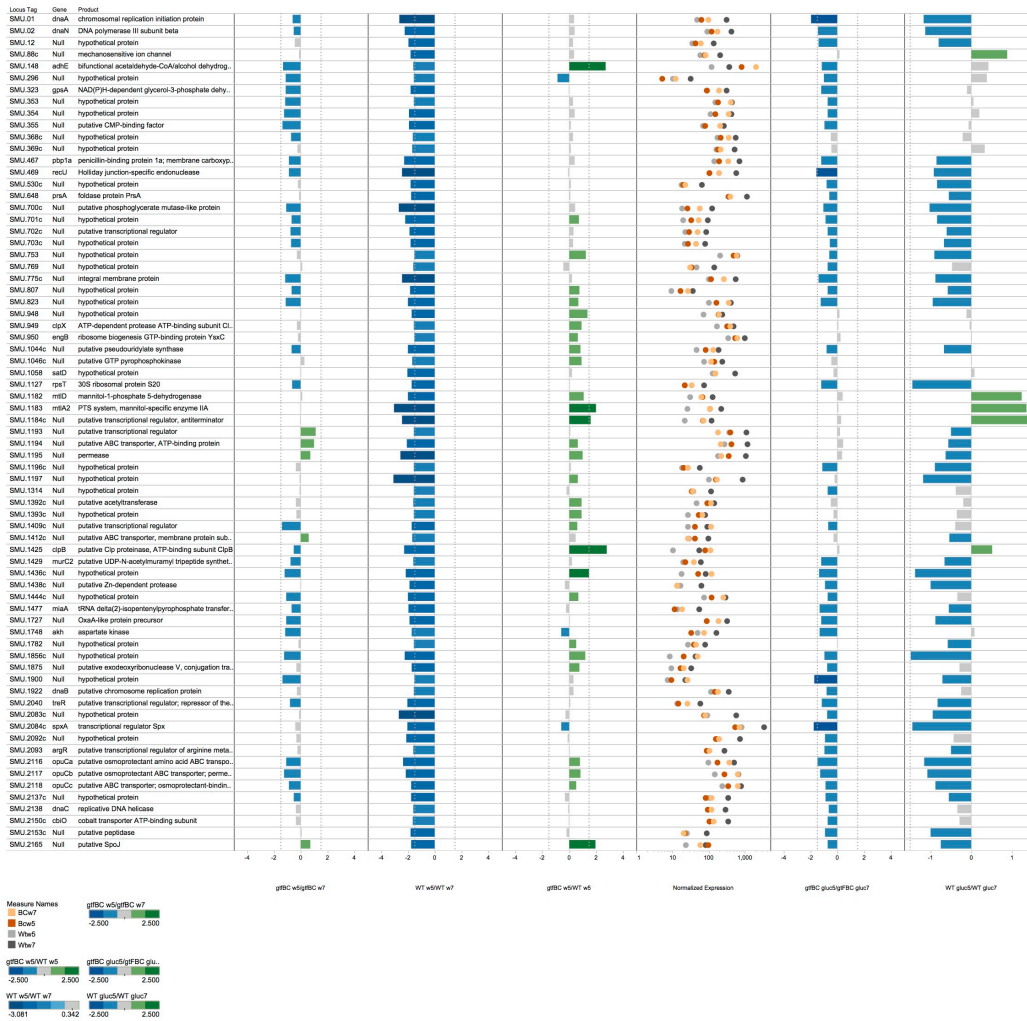


Fig. S2. Genes with greater than $\log_2 1.5$ fold decrease in the WT at pH 5.5 relative to pH 7.5. Dashed vertical lines indicate significant fold change. Colored bars indicate \log_2 fold changes for differential expression. Filled colored circles indicate normalized expression values for each condition tested with sucrose. gtfBC mutant with sucrose pH 5.5 (bcw5), gtfBC mutant with sucrose pH 7.5 (bcw7), WT with sucrose pH 5.5 (Wtw5), WT with sucrose pH 7.5 (Wtw7).



Fig. S3: Genes with log₂ 1.5 fold increase in the gtfBC mutant at pH 5.5 relative to pH 7.5. Dashed vertical lines indicate significant fold change. Colored bars indicate log₂ fold changes for differential expression. Filled colored circles indicate normalized expression values for each condition tested with sucrose. gtfBC mutant with sucrose pH 5.5 (bcw5), gtfBC mutant with sucrose pH 7.5 (bcw7), WT with sucrose pH 5.5 (Wtw5), WT with sucrose pH 7.5 (wtw7.5).

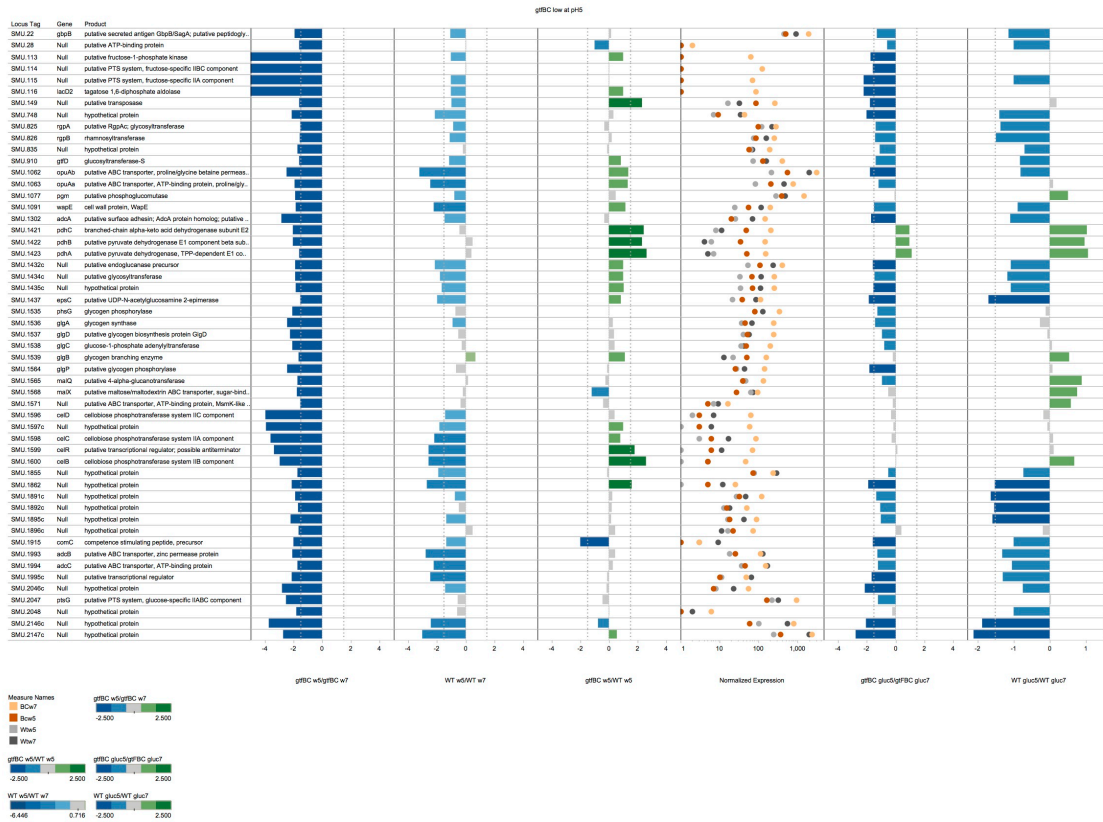


Fig. S4: Genes with \log_2 1.5 fold decrease in the gtfBC mutant at pH 5.5 relative to pH 7.5. Dashed vertical lines indicate significant fold change. Colored bars indicate \log_2 fold changes for differential expression. Filled colored circles indicate normalized expression values for each condition tested with sucrose. gtfBC mutant with sucrose pH 5.5 (bcw5), gtfBC mutant with sucrose pH 7.5 (bcw7), WT with sucrose pH 5.5 (Wtw5), WT with sucrose pH 7.5 (wtw7.5).

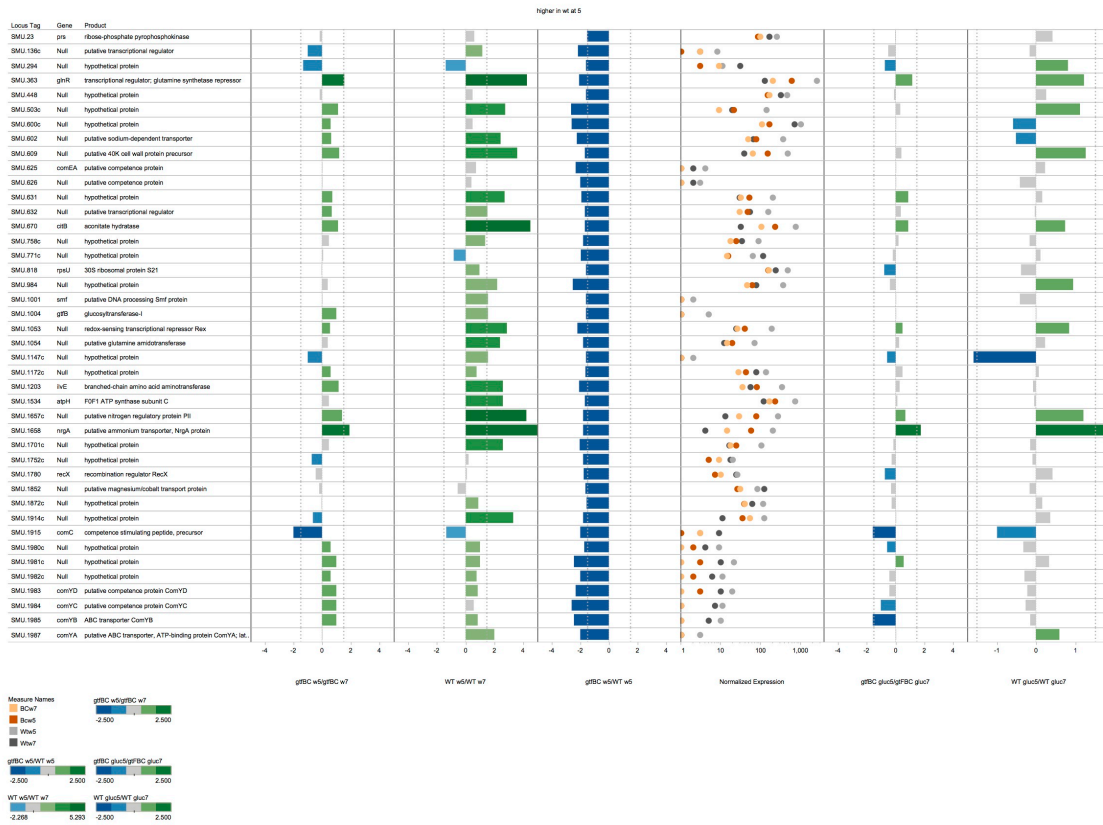


Fig. S5: Genes with \log_2 1.5 fold increase in the WT at pH 5.5 relative to gtfBC mutant pH 5.5. Dashed vertical lines indicate significant fold change. Colored bars indicate \log_2 fold changes for differential expression. Filled colored circles indicate normalized expression values for each condition tested with sucrose. gtfBC mutant with sucrose pH 5.5 (bcw5), gtfBC mutant with sucrose pH 7.5 (bcw7), WT with sucrose pH 5.5 (Wtw5), WT with sucrose pH 7.5 (wtw7.5).

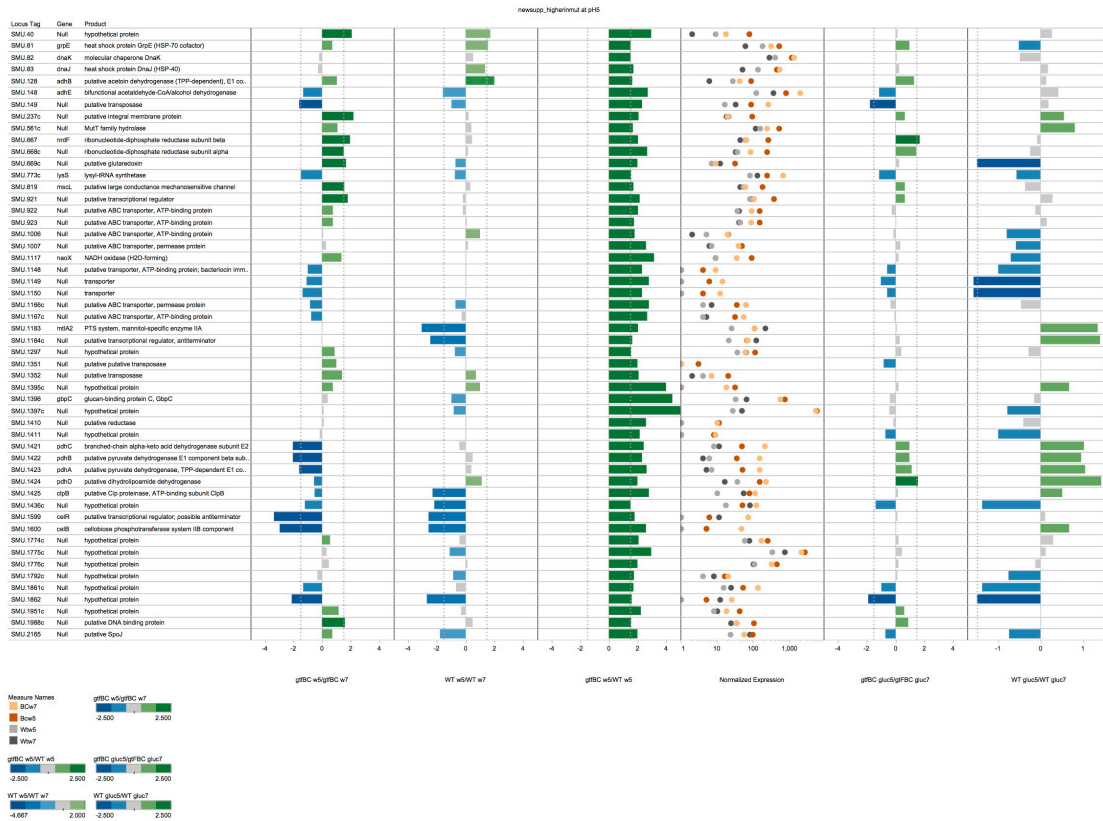


Fig. S6: Genes with \log_2 1.5 fold decrease in the WT at pH 5.5 relative to gtfBC mutant pH 5.5. Dashed vertical lines indicate significant fold change. Colored bars indicate \log_2 fold changes for differential expression. Filled colored circles indicate normalized expression values for each condition tested with sucrose. gtfBC mutant with sucrose pH 5.5 (bcw5), gtfBC mutant with sucrose pH 7.5 (bcw7), WT with sucrose pH 5.5 (Wtw5), WT with sucrose pH 7.5 (wtw7.5).

2) Supplementary Tables:

S1 Table. Primers used in this study

Gene name	Primer sequence (5'-3', forward and reverse)
<i>gtfB</i>	AGCAATGCAGCCAATCTACAAAT ACGAACTTTGCCGTTATTGTCA
<i>gtfC</i>	GGTTTAACGTCAAAATTAGCTGTATTAGC CTCAACCAACCGCCACTGTT
<i>atpA</i>	TATTGCTCGTGCTTGCGGAC TTTCACCCAGACCATCAACAGG
<i>atpD</i>	GGCGACAAGTCTCAAAGAATTG AACCATCAGTTGACTCCATAGC
<i>msmK</i>	CCTTTATATTGATGATAAACTCA CATATTTTCATAAACGCTCAT
<i>dexB</i>	AATGGCAGACTGAGTTGGA CACGCATAAGGTGAAGAAG
16S rRNA	CTTACCAGGTCTTGACATCCCG ACCCAACATCTCACGACACGAG

S2 Table. The number of differentially regulated genes in *S. mutans* wt and *gtfBC* under different conditions.

	# genes up-regulated	# genes down-regulated
^a wt sucrose pH5.5/wt sucrose pH7	192	96
^b gtfBC sucrose pH5.5/ gtfBC sucrose pH7	47	57
^c gtfBC glucose pH5.5/ gtfBC glucose pH7	11	31
^d wt glucose pH5.5/ wt glucose pH7	14	17

^a wild type *S. mutans* cells pre-grown in the presence of sucrose were subjected to 3-hr exposure to buffered (pH5.5 and pH7)MDM medium;

^b gtfBC mutant cells pre-grown in the presence of sucrose were subjected to 3hr exposure to buffered (pH5.5 and pH7) MDM medium;

^c gtfBC mutant cells pre-grown in the presence of glucose were subjected to 3-hr exposure to buffered (pH5.5 and pH7)MDM medium;

^d wild type *S. mutans* cells pre-grown in the presence of glucose were subjected to 3hr exposure to buffered (pH5.5 and pH7) MDM medium;

S3 Table. qPCR analysis of relative expression of selected genes in *S. mutans* wild type and *gtfBC*-deficient mutant grown at neutral or acidic pH in the presence of sucrose or glucose.

Functional class annotation	Gene Name	Sugar added	Wild-type	<i>gtfBC</i> mutant
			Fold change (pH 5.5 vs pH7.5) ^a	
			qPCR (avg ± SD)	
Insoluble glucans synthesis	<i>gtfB</i>	sucrose	2.63±0.55	
		glucose	0.83±0.22	
Insoluble glucans synthesis	<i>gtfC</i>	sucrose	2.27±0.71	
		glucose	0.46±0.13	
F ₁ F ₀ -H/ATPase	<i>atp</i> operon	sucrose	<i>atpA</i> :2.61±1.27 <i>atpD</i> :8.10±2.56	<i>atpA</i> :1.02±0.68 <i>atpD</i> :1.00±0.16
		glucose	<i>atpA</i> :1.51±0.31 <i>atpD</i> :1.07±0.21	<i>atpA</i> :0.95±0.53 <i>atpD</i> :0.88±0.22
Multiple-sugar metabolism (msm)	<i>msmK</i>	sucrose	4.75±1.44	-2.64±0.89
		glucose	1.71±0.15	-3.16±1.45
	<i>dexB</i>	sucrose	3.42±1.69	-3.52±1.71
		glucose	1.87±0.42	-2.67±0.42

^a Normalized expression was calculated by dividing the expression in *S. mutans* at pH 5.5 by the expression at pH 7.5.

