Targeting of *Streptococcus mutans* Biofilms by a Novel Small Molecule Prevents Dental Caries and Preserves the Oral Microbiome

S.S. Garcia, M.S. Blackledge, S. Michalek, L. Su, T. Ptacek, P. Eipers, C. Morrow, E.J. Lefkowitz, C. Melander, and H. Wu

APPENDIX

Appendix Table 1. Summary of results from a rat caries study after 4 weeks of either repeated topical application or no treatment.

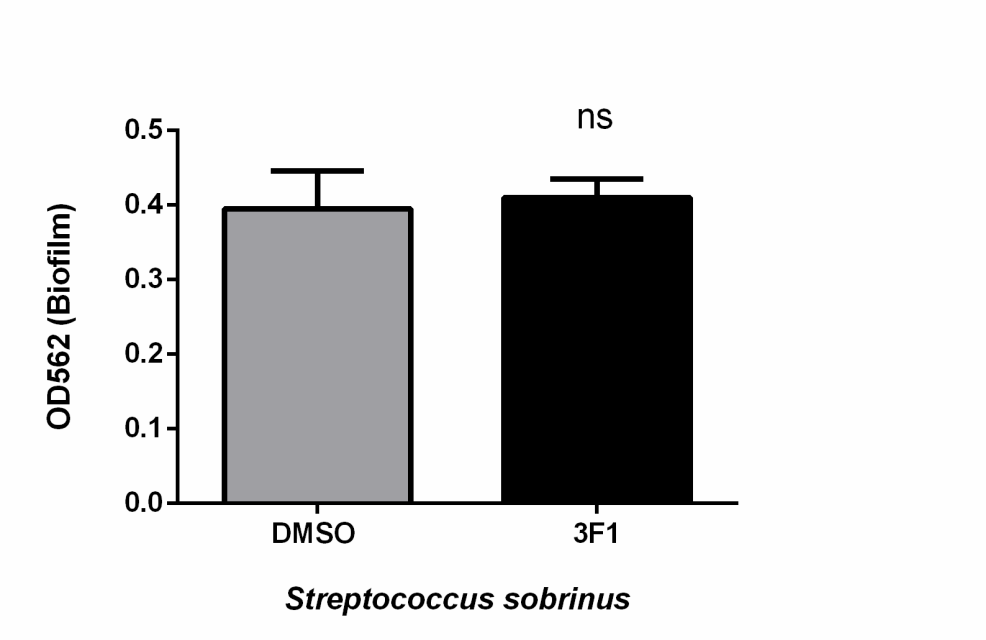
	Topical Application of Vehicle Control	No Treatment
Mean enamel caries score	Buccal: 13.4±0.6 Sulcal: 19.6±0.5 Proximal: 6.8±0.5	Buccal: 14.8±0.3 [ns] Sulcal: 21.2±0.4 [ns] Proximal: 7.7±0.3 [ns]
Weight (g)	160±14	149±10 [ns]
CFUs recovered *S. mutans* UA159 ($x10^6$)	4.7±1.1	4.8±0.8 [ns]

Samples were collected at the end of the study. n=6 per group. Groups were compared by ANOVA. ns = not significant.

<u>Appendix Table 2.</u> Mean relative abundance of phylum in Sm+CPD and End samples in different rat groups.

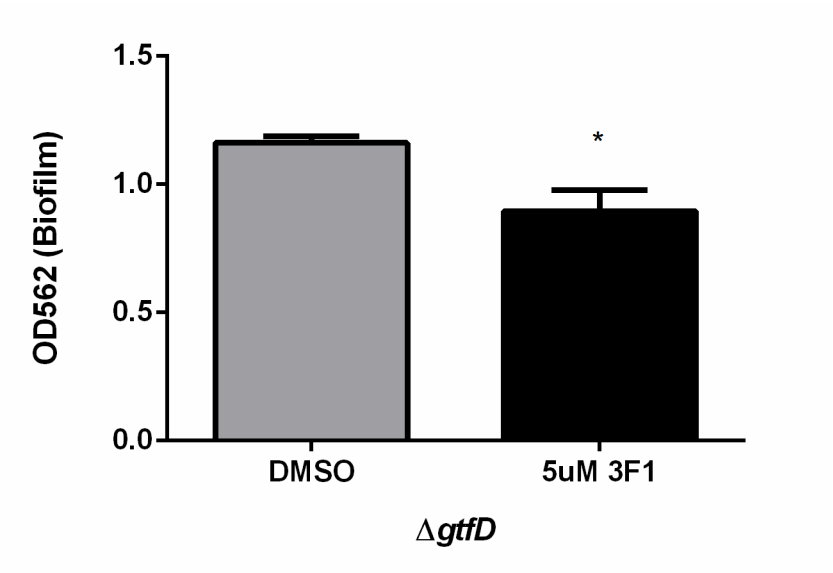
Treatment			
3F1	**Taxonomy-Phylum**	**Mean-Sm+CPD**	**Mean-End**
	TM7	0	0.27*
	Gemmatimonadetes	0.00105	0.06*
	Firmicutes	59.61	51.59
	Proteobacteria	24.15	28.17
	Bacteriodetes	9.08	15.77
	Actinobacteria	2.58	1.96
	Verrucomicrobia	2.78	0.87
	Cyanobacteria	0.11	0.16
	[Thermi]	0.5	0.36
	Fusobacteria	0.09	0.47
	Spirochaetes	0.52	0.13
	Deferribacteres	0.31	0.01
	Tenericutes	0.09	0.04
No Treatment	TM7	0	0.15*
	Gemmatimonadetes	0.00109	0.06*
	Firmicutes	72.23	56
	Proteobacteria	17.07	23.11
	Bacteriodetes	3.9	15.2
	Actinobacteria	1.9	3.24
	Verrucomicrobia	3.38	1.08
	Cyanobacteria	0.21	0.27
	[Thermi]	0.33	0.3
	Fusobacteria	0.22	0.32
	Spirochaetes	0.64	0.07
	Deferribacteres	0.04	0.03
	Tenericutes	0.04	0.08
Fluoride	TM7	0.05	0.24 ^{ns}
	Gemmatimonadetes	0.06	0.03 ^{ns}
	Firmicutes	55.57	56.91
	Proteobacteria	30.04	24.81
	Bacteriodetes	6.08	14.04
	Actinobacteria	2.18	2.01
	Verrucomicrobia	0.5	0.75
	Cyanobacteria	4.62	0.2
	[Thermi]	0.26	0.28
	Fusobacteria	0.27	0.48
	Spirochaetes	0.03	0.03
	Deferribacteres	0.08	0.01
	Tenericutes	0.18	0.07

*$p < 0.05$ after FDR correction, ns = not significant



<u>Appendix Figure 1.</u> Small molecule 3F1 does not disperse *Streptococcus sobrinus* biofilms *in vitro*.

S. sobrinus biofilms were formed for 6 h. Biofilms were then washed and treated with DMSO, 5µM 3F1, or 5µM 3F2 for 14 h. After 14 h, cells released into the media were measured at OD_{470}. Loosely adhered cells were then washed off and remaining biomass quantitated by crystal violet staining at OD_{562}. Bars represent the mean of 3 independent experiments. Differences were statistically compared by ANOVA. Error bars represent standard error. ns = not significant.



<u>Appendix Figure 2.</u> Small molecule 3F1 disperses *S. mutans* biofilm independent of GtfD. *S. mutans* single-gene knock-out mutant lacking GtfD (Δ*gtfD*) was allowed to form biofilms for 6 h. Biofilms were then washed and treated with DMSO or 5μM 3F1 for 14 h. Loosely adhered cells were then washed off and remaining biomass quantitated by crystal violet staining at OD_{562}. Bars represent the mean of 3 independent experiments. Differences were statistically compared by ANOVA. Error bars represent standard error. *$p < 0.05$.