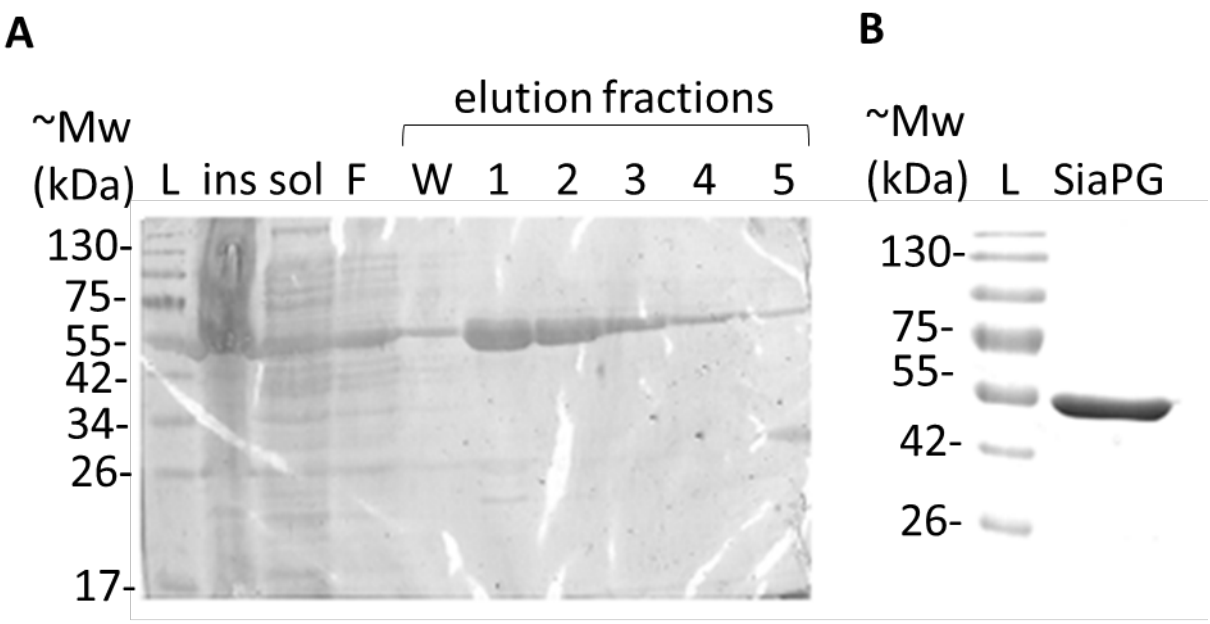
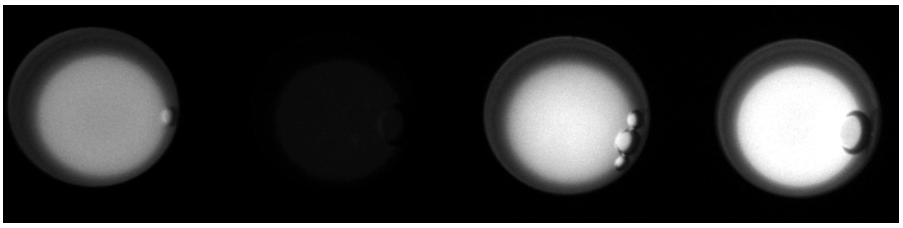


C

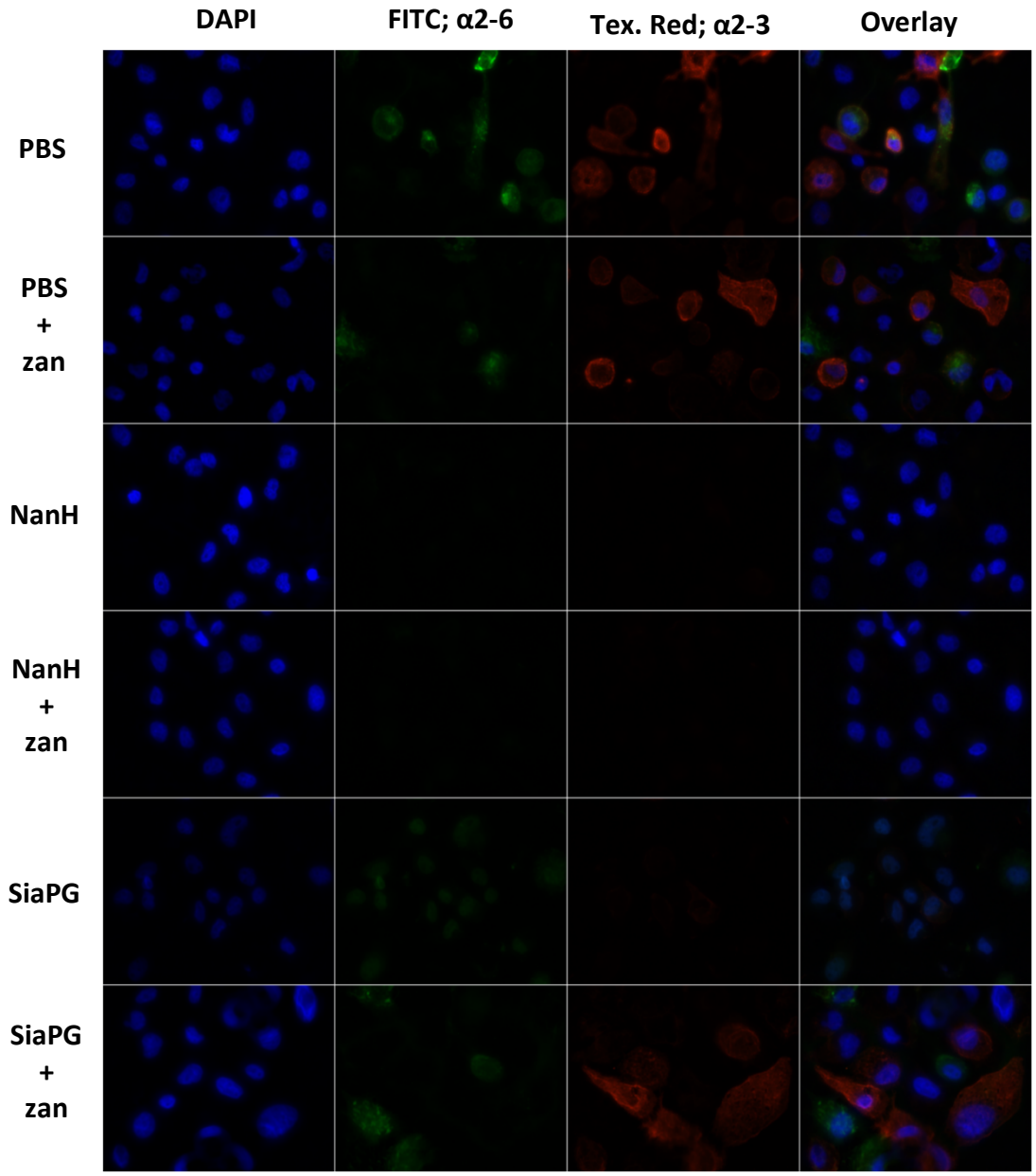
Native protein

MANNTLLAKTRRYVCLVVFCCLMAMMMHLSGQEVTMWGDSHGVAPNQVRRTLVKVALSESLPPGAKQIRIGFSLP
KETEEKVTALYLLVSDSLAVRDLPDYKGRVSYDSFPISKEDRTTALSADSVAGRCFFYLAADIGPVASFSRSDTLTARVE
ELAVDGRPLPLKELSPASRRLYREYEALFVPGDGGSRNYRIPSILKTANGTLIAMADRRKYNQTDLPEDIDIVMRRST
DGGKSWSDPRIIVQGEGRNHGFGDVALVQTQAGKLLMIFVGGVGLWQSTPDRPQRTYISESRDEGLTWSPPRDI
THFIFGKDCADPGRSRWLASFCASGQGLVLPSGRVMFVAAIRESGQEYVLNNYVLYSDDEGGTWQLSDCAYHRG
DEAKLSLMPDGRVLMSVRNQGRQESRQRFFALSSDDGLTWERAKQFEGIHDPGCNGAMLQVKRNGRNQMLHS
LPLGPDGRRDGAVYLFDHVSGRWSAPVVVNSGSSAYSDMTLLADGTIGYFVEEDDEISLVFIRFVLDDLFDARQ

Supplemental figure S1. Purification of SiaPG A) SiaPG purification via affinity chromatography. L= Molecular mass ladder, ins = insoluble fraction of whole bacterial lysate, sol = soluble fraction of whole bacterial lysate, F = flow through, W = wash, elution fractions were 1 ml each. B) Purified SiaPG post-dialysis. C) Native gene encoding SiaPG and resulting encoded protein (PG_0352, obtained from UniProt; Q7MX62), 100 % protein identity between W83 and ATCC 33277 strains). Red = secretion signal site, absent in the codon optimized protein expressed here using *E. coli*.

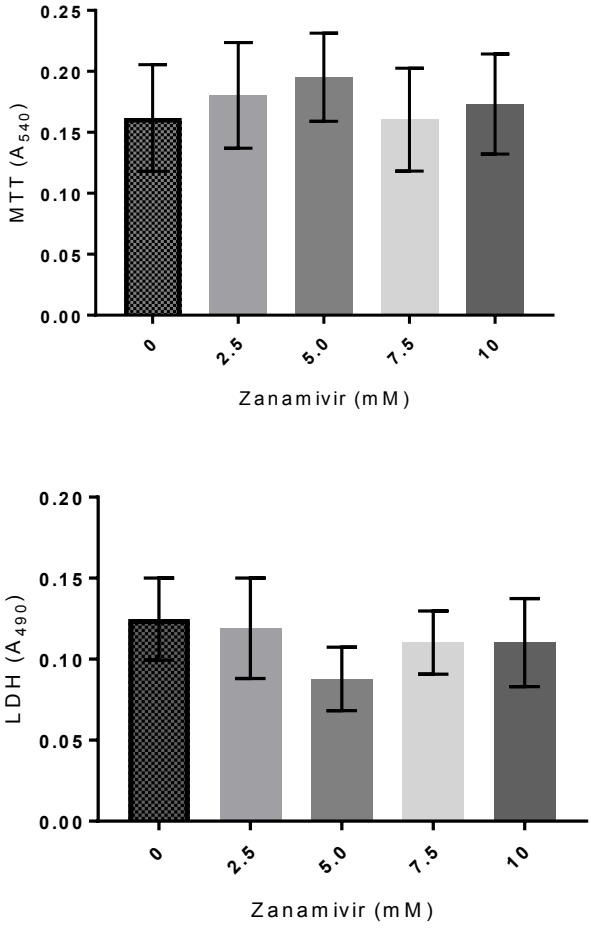


Supplemental figure S2: Sialidase activity of SiaPG, *P. gingivalis* SiaPG-inactivated isogenic mutant (Pg381ΔsiaPG) and its complemented strain (ΔsiaPG^{+})



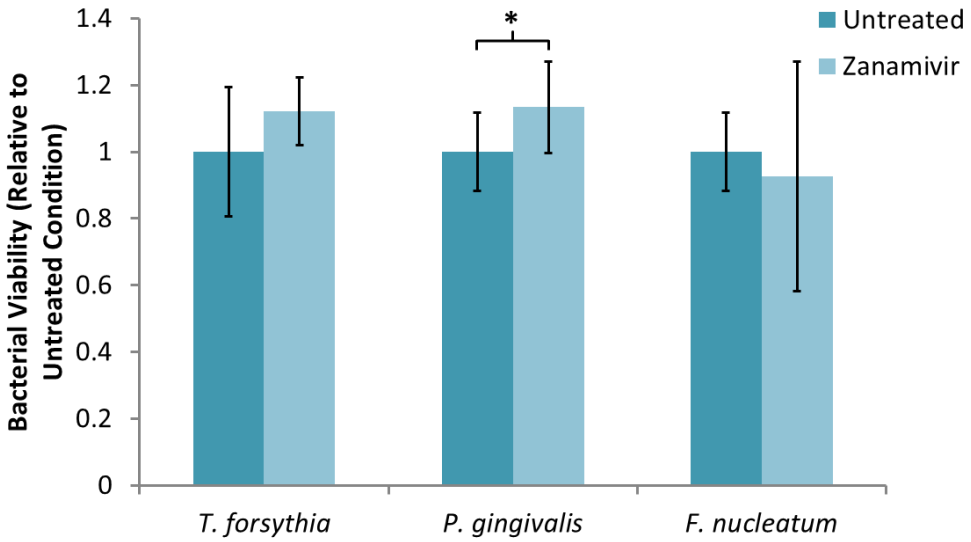
Supplemental figure S3. Purified sialidases desialylate oral epithelial cell surfaces.

Cells were stained with lectins for α2-3 and α2-6 linked sialic acid in red and green, respectively. Prior to staining, cells were treated with NanH and SiaPG in PBS, in the presence or absence of 10mM zanamivir, as indicated. All images were visualised using the same microscopy and image processing parameters (fluorescence intensity, exposure time and background subtraction). Images were captured in three fields of view, and this was repeated in three separate experiments. Images shown are representative of each condition.



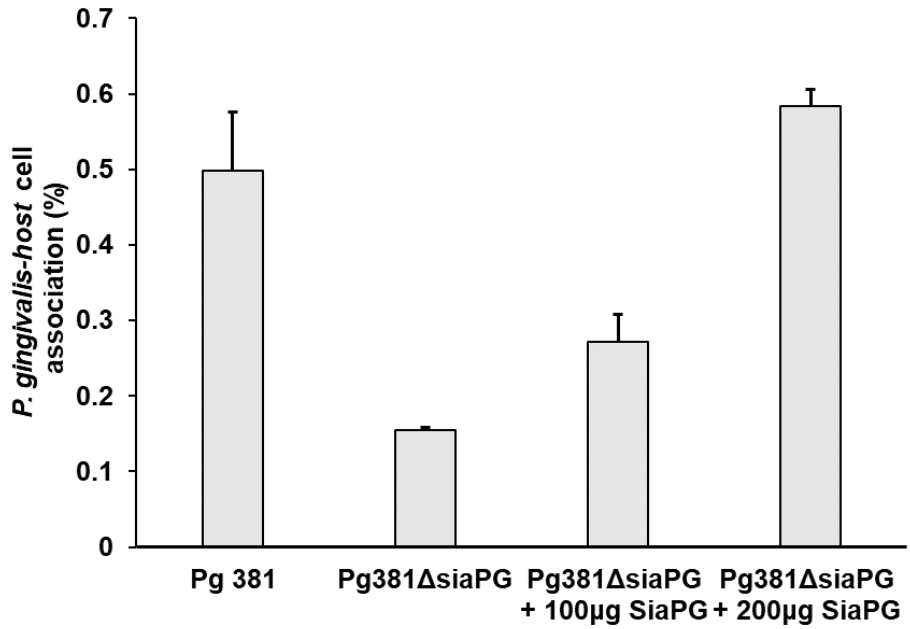
Supplemental figure S4. Viability testing of OKF6 oral epithelial cells exposed to zanamivir.

The oral epithelial cell line OKF6 was exposed to *T. forsythia* in the presence and absence of 10 mM zanamivir for 2.5 hours, followed by an MTT assay on cells, or an LDH assay on culture supernatant, with the absorbance at 490 and 540 nm for LDH and MTT, respectively, used to relatively quantify levels of MTT and LDH. Data shown represent the mean of two experiments, where each condition was repeated three times per experiment. Error bars=SEM. No significant differences were found (where p=<0.05), as determined by one way ANOVA, with repeated measures and Tukey's correction for multiple comparisons.

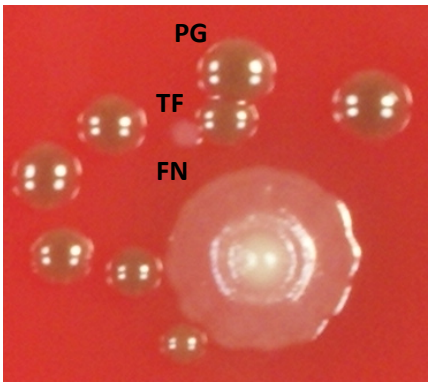


Supplemental figure S5. Viability testing of periodontal pathogens exposed to zanamivir.

T. forsythia, P. gingivalis, and F. nucleatum were incubated in the presence and absence of 10mM zanamivir for 2.5 hours, followed by enumeration of viable organisms by agar plate counts, and data were expressed as the change in cell numbers relative to the untreated condition. Data shown represent the mean of three experiments, where each condition was repeated three times per experiment. Error bars=SD. Significance determined by T-test (*p=<0.05).

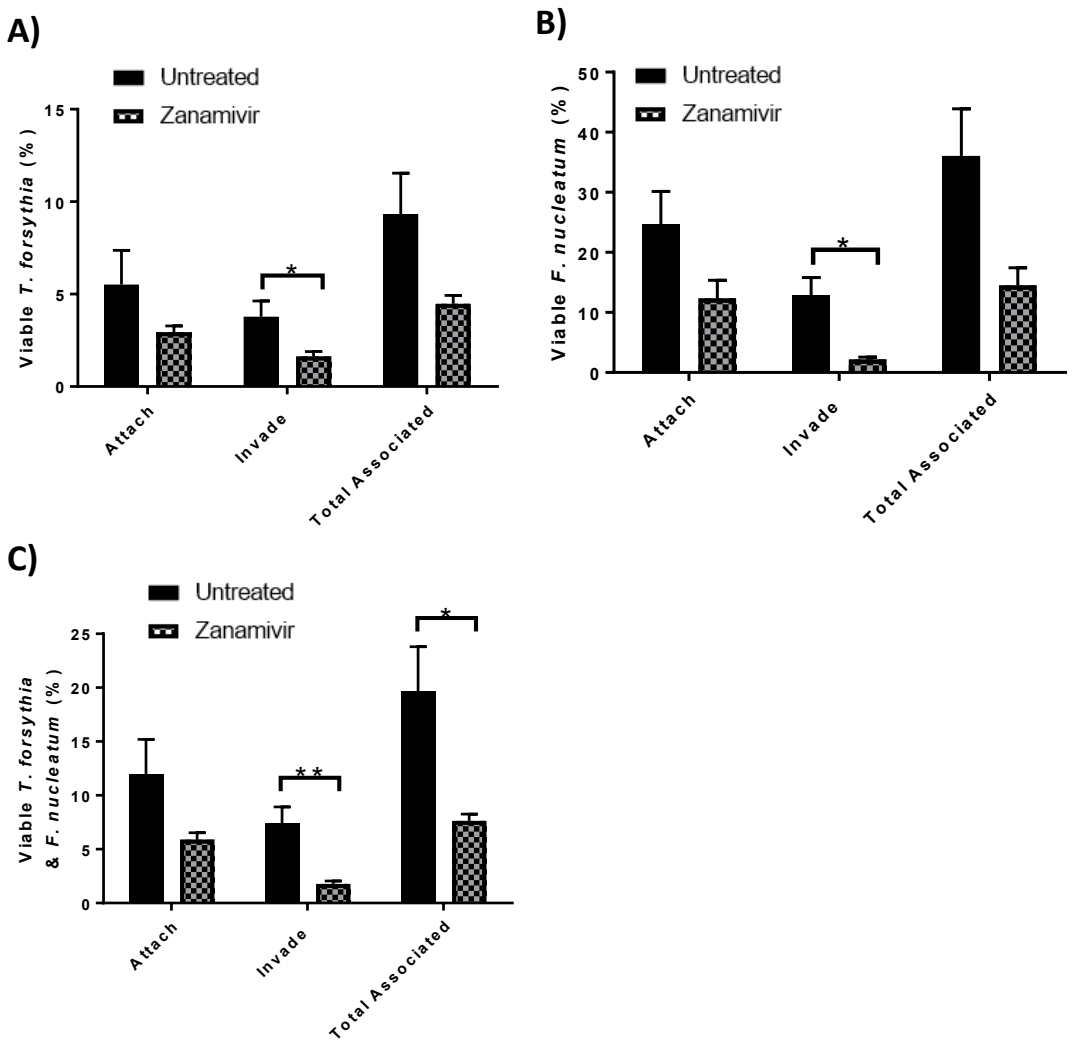


Supplemental Figure S6. Association of *P. gingivalis* with OBA9 cells. OBA-9 cells were incubated with P. gingivalis 381 or the SiaPG inactivated mutant (ΔPG352), in the presence or absence of 100 and 200 µg of SiaPG. Bacterial association was calculated as percentage of input bacteria. Data represent the mean of two experiments, where each condition was repeated three times per experiment. Error bars = SEM, significant differences between Pg 381 and the other conditions were determined by paired T-test, *p=<0.05, **p=<0.01).



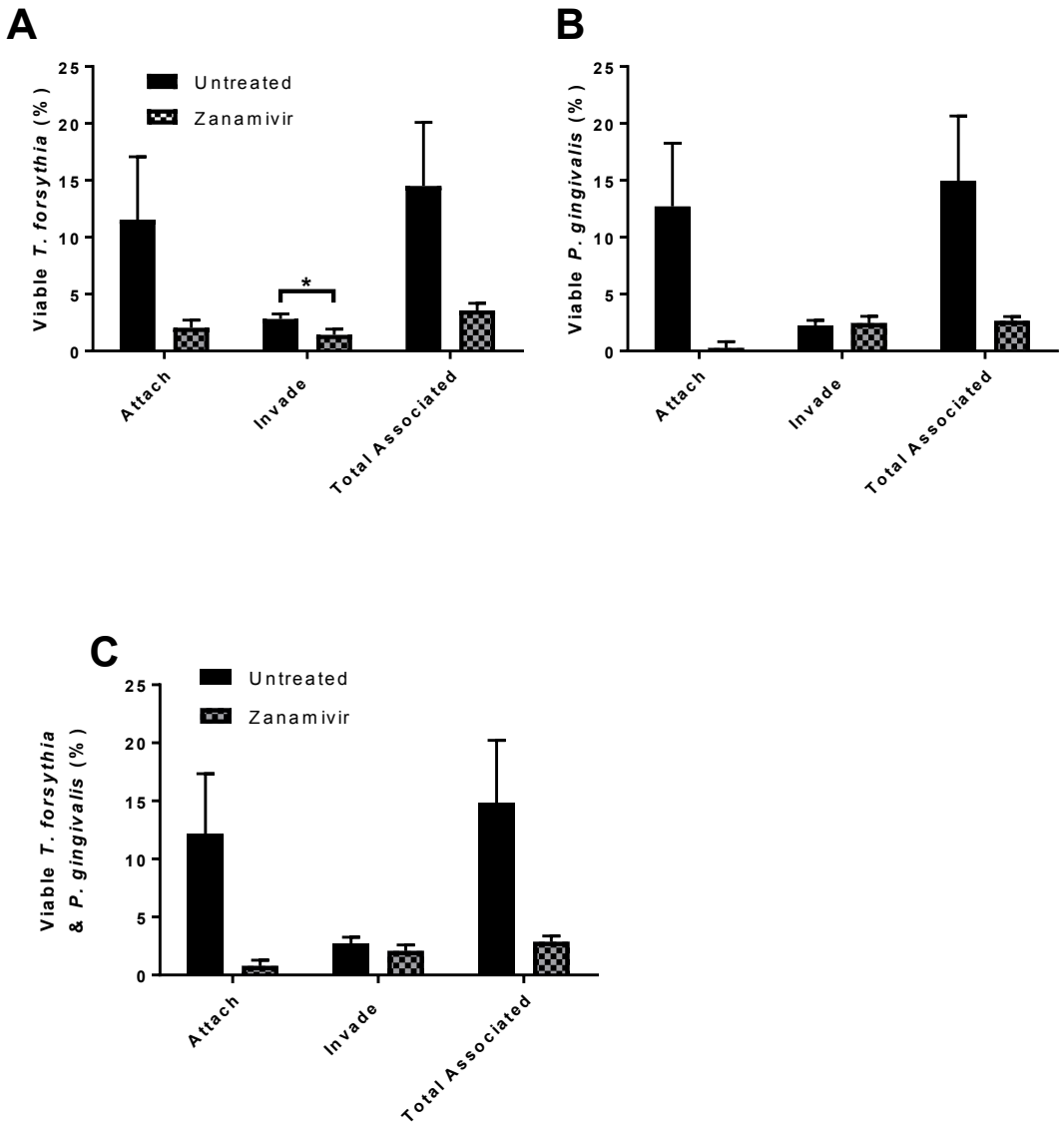
Supplemental figure S7. Colony morphology of *P. gingivalis*, *F. nucleatum*, and *T. forsythia* during mixed-species enumeration.

Images of mixed-species agar cultures to highlight differences in colony morphology. Left panel = unassisted view, right panel = colony counter microscope view. One colony representative of each species is labelled on the images. *P. gingivalis* (PG) forms opaque black-pigmented colonies, *T. forsythia* (TF) forms translucent grey colonies, and *F. nucleatum* (FN) forms large beige colonies, translucent at the edges with a raised, opaque centre.



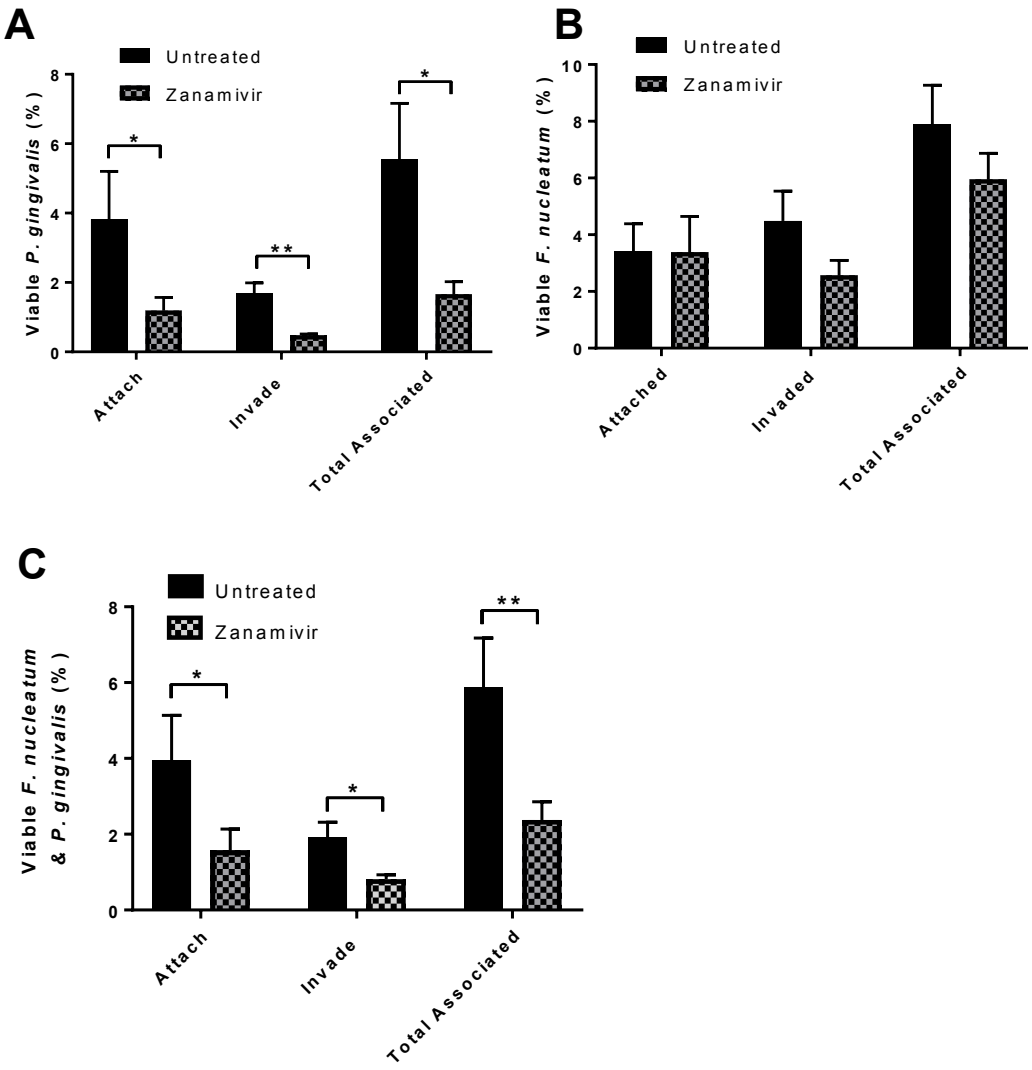
Supplemental figure S8. The effect of zanamivir on attachment and invasion of epithelial cells co-infected with *T. forsythia* and *F. nucleatum*.

Antibiotic protection assays were performed in the presence or absence of zanamivir with *T. forsythia* and *F. nucleatum.* A) Level of *T. forsythia*-host cell association, B) Level of *F. nucleatum*-host cell association, or C) Level of both *T. forsythia* and *F. nucleatum*-host cell association. Bacterial attachment, invasion, and total association with host cells was normalised to the number of bacteria that were used to infect each condition that had survived the duration of the assay (the percentage of viable bacteria). Zanamivir= 10mM zanamivir present during host cell exposure to bacteria. Data represent the mean from three experimental repeats, and each condition was repeated in triplicate during each experiment. Error bars=SEM, Significance determined by paired T-test, *p=<0.05, **p=<0.01.



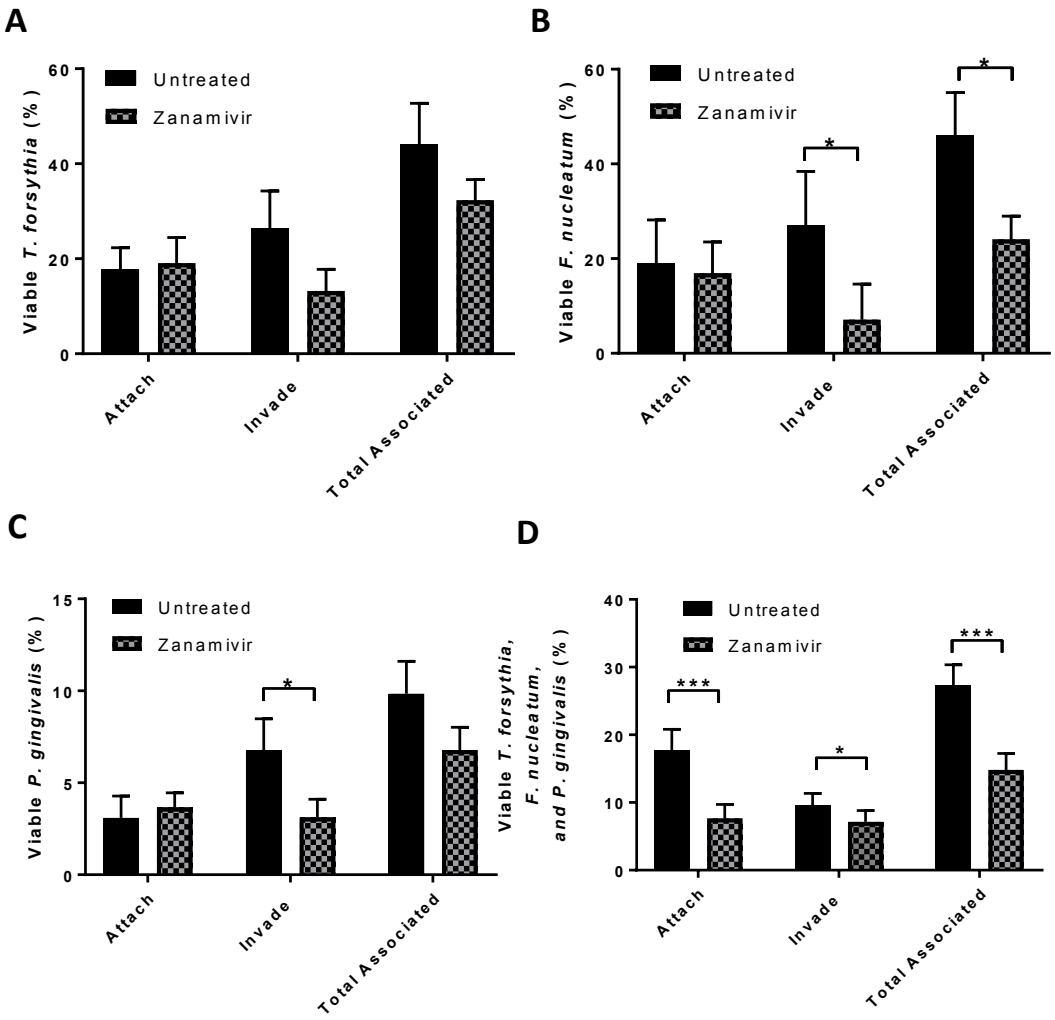
Supplemental figure S9. The effect of zanamivir on attachment and invasion of epithelial cells co-infected with *T. forsythia* and *P. gingivalis*.

Antibiotic protection assays were performed in the presence or absence of zanamivir with *T. forsythia* and *P. gingivalis A*) Level of *T. forsythia*-host cell association, B) Level of *P. gingivalis*-host cell association, or C) Level of both *T. forsythia* and *P. gingivalis*-host cell association. Bacterial attachment, invasion, and total association with host cells was normalised to the number of bacteria that were used to infect each condition that had survived the duration of the assay (the percentage of viable bacteria). Zanamivir= 10 mM zanamivir present during host cell exposure to bacteria. Data represent the mean from three experimental repeats, and each condition was repeated in triplicate during each experiment. Error bars=SEM. Significance determined by paired T-test, *p=<0.05.



Supplemental figure S10. The Effect of Zanamivir on Attachment and Invasion of Epithelial Cells Co-infected with *P. gingivalis* and *F. nucleatum*.

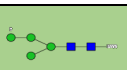
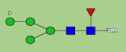
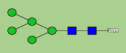
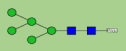
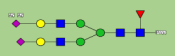
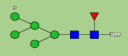
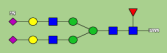
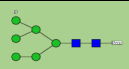
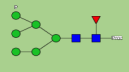
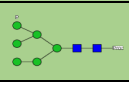
Antibiotic protection assays were performed in the presence or absence of zanamivir with *T. forsythia* and *F. nucleatum* A) Level of *T. forsythia*-host cell association, B) Level of *F. nucleatum*-host cell association, or C) Level of both *P. gingivalis* and *F. nucleatum*-host cell association. Bacterial attachment, invasion, and total association with host cells was normalised to the number of bacteria that were used to infect each condition that had survived the duration of the assay (the percentage of viable bacteria). Zanamivir=10mM zanamivir present during host cell exposure to bacteria. Data represent the mean from three experimental repeats, and each condition was repeated in triplicate during each experiment. Error bars=SEM, Significance determined by paired T-test, *p=<0.05, **p=<0.01.

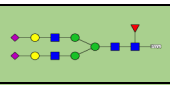
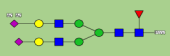
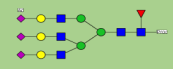
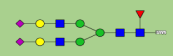
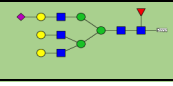


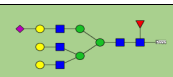
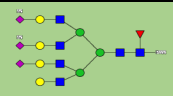
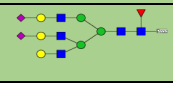
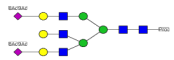
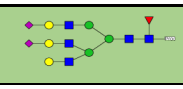
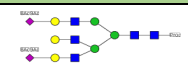
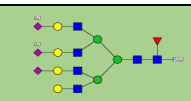
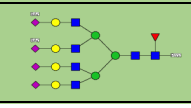
Supplemental figure S11. The effect of zanamivir on attachment and invasion of epithelial cells co-infected with *T. forsythia, F. nucleatum*, and *P. gingivalis*.

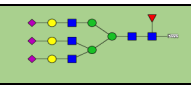
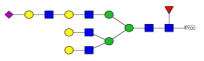
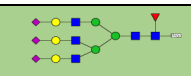
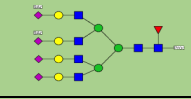
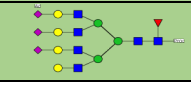
Antibiotic protection assays were performed in the presence or absence of zanamivir with *T. forsythia, F. nucleatum,* and *P. gingivalis.* A) *T. forsythia*-host cell association, B) *F. nucleatum*-host cell association, C) *P. gingivalis*-host cell association D) *T. forsythia, F. nucleatum,* and *P. gingivalis*-host cell association. Bacterial attachment, invasion, and total association with host cells was normalised to the number of bacteria that were used to infect each condition that had survived the duration of the assay (the percentage of viable bacteria). Zanamivir=10mM zanamivir present during host cell exposure to bacteria. Data represent the mean from three experimental repeats, and each condition was repeated in triplicate during each experiment. Error bars=SEM, Significance determined by paired T-test, *p=<0.05, **p=<0.01. ***p=<0.001.

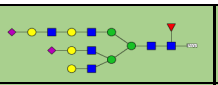
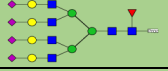
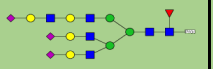
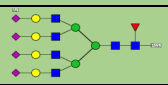
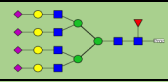
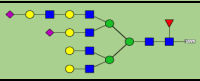
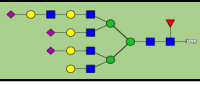
Supplemental table 1. Composition of EPO glycans.

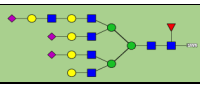
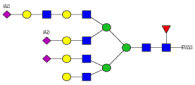
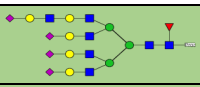
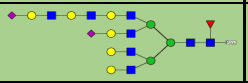
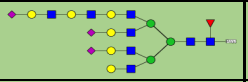
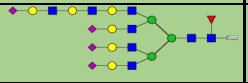
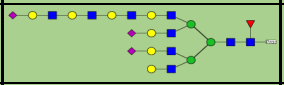
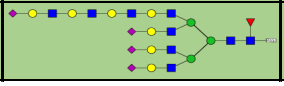
UHPLC Peak ID	% Area	ESI-LC/MS								
		Composition								
		Possible structure	Example Glycan cartoon	Hex (H)	HexNAc (N)	Fuc (F)	Neu5Ac (S)			potential sulphate or phosphate
							0 OAc	1 OAc	2 OAc	
1	0.16	Man4+P		4	2	0	0	0	0	1
2	0.45	FMan4+P		4	2	1	0	0	0	1
3	1.23	Man5+P		5	2	0	0	0	0	1
4	0.15	Man5+P		5	2	0	0	0	0	1
		FA2G2S2(Ac)2		5	4	1	1	0	1	0
5	0.70	Man5+P		5	2	0	0	0	0	1
		FMan5+P		5	2	1	0	0	0	1
6	0.47	FMan5+P		5	2	1	0	0	0	1
		FA2G2S2(Ac)1		5	4	1	1	1	0	0
7	1.67	Man6+P		6	2	0	0	0	0	1
8,9	1.16	FA2G2S1		5	4	1	0	0	0	0
		Man6+P		6	2	0	0	0	0	1
10,11	0.62	FMan6+P		6	2	1	0	0	0	1
		Man6+P		6	2	0	0	0	0	1

12	3.05	FA2G2S2		5	4	1	2	0	0	0
13	0.48	FA2G2S1S1(Ac)2		5	4	1	1	0	1	0
		FA3G3S2(Ac)1		6	5	1	1	1	0	0
		FA2G2S2		5	4	1	2	0	0	0
		FA3G3S3(Ac)2		6	5	1	3	0	1	0
14,15	0.46	FA2G2S2		5	4	1	2	0	0	0
16	0.94	FA3G3S1		6	5	1	1	0	0	0

17	0.54	FA3G3S1		6	5	1	1	0	0	0
		FA4G4S3(Ac)2.		7	6	1	1	2	0	0
18	1.96	FA3G3S2		6	5	1	2	0	0	0
		A3G3S2(Ac)4		6	5	0	0	0	2	0
19	1.62	FA3G3S2		6	5	1	2	0	0	0
		A3G3S2(Ac)4		6	5	0	0	0	2	0
		FA4G4S3(Ac)2		7	6	1	1	2	0	0
		FA4G4S4(Ac)2		7	6	1	2	2	0	0

20	5.32	FA3G3S3		6	5	1	3	0	0	0
21	1.76	FA4G4S1 or FA3G3S1(LacNAc)1		7	6	1	1	0	0	0
		FA3G3S3		6	5	1	3	0	0	0
		FA4G4S4(Ac)2		7	6	1	2	2	0	0
		FA4G4S3Ac		7	6	1	2	1	0	0

22	6.19	FA4G4S2 or FA3G3S2(LacNAc)1		7	6	1	2	0	0	0
		FA4G4S4		7	6	1	3	1	0	0
23	11.24	FA4G4S3 or FA3G3S3(LacNAc)1		7	6	1	3	0	0	0
		FA4G4S4Ac		7	6	1	3	1	0	0
24	12.96	FA4G4S4		7	6	1	4	0	0	0
25,26	2.78	FA4G4S2(LacNac)1		8	7	1	2	0	0	0
27	9.83	FA4G4S3(LacNac)1		8	7	1	3	0	0	0

28	1.31	FA4G4S3(LacNac)1		8	7	1	3	0	0	0
		FA4G4S3Ac2(LacNac)1		8	7	1	1	2	0	0
29	13.67	FA4G4S4(LacNac)1		7	6	1	4	0	0	0
30	1.76	FA4G4S2(LacNac)2		7	6	1	2	0	0	0
31	5.50	FA4G4S3(LacNAc)2		9	8	1	3	0	0	0
32	8.26	FA4G4S4(LacNAc)2		9	8	1	4	0	0	0
33	1.33	FA4G4S3(LacNAc)3		10	9	1	3	0	0	0
34	1.72	FA4G4S4(LacNac)3		10	9	1	4	0	0	0