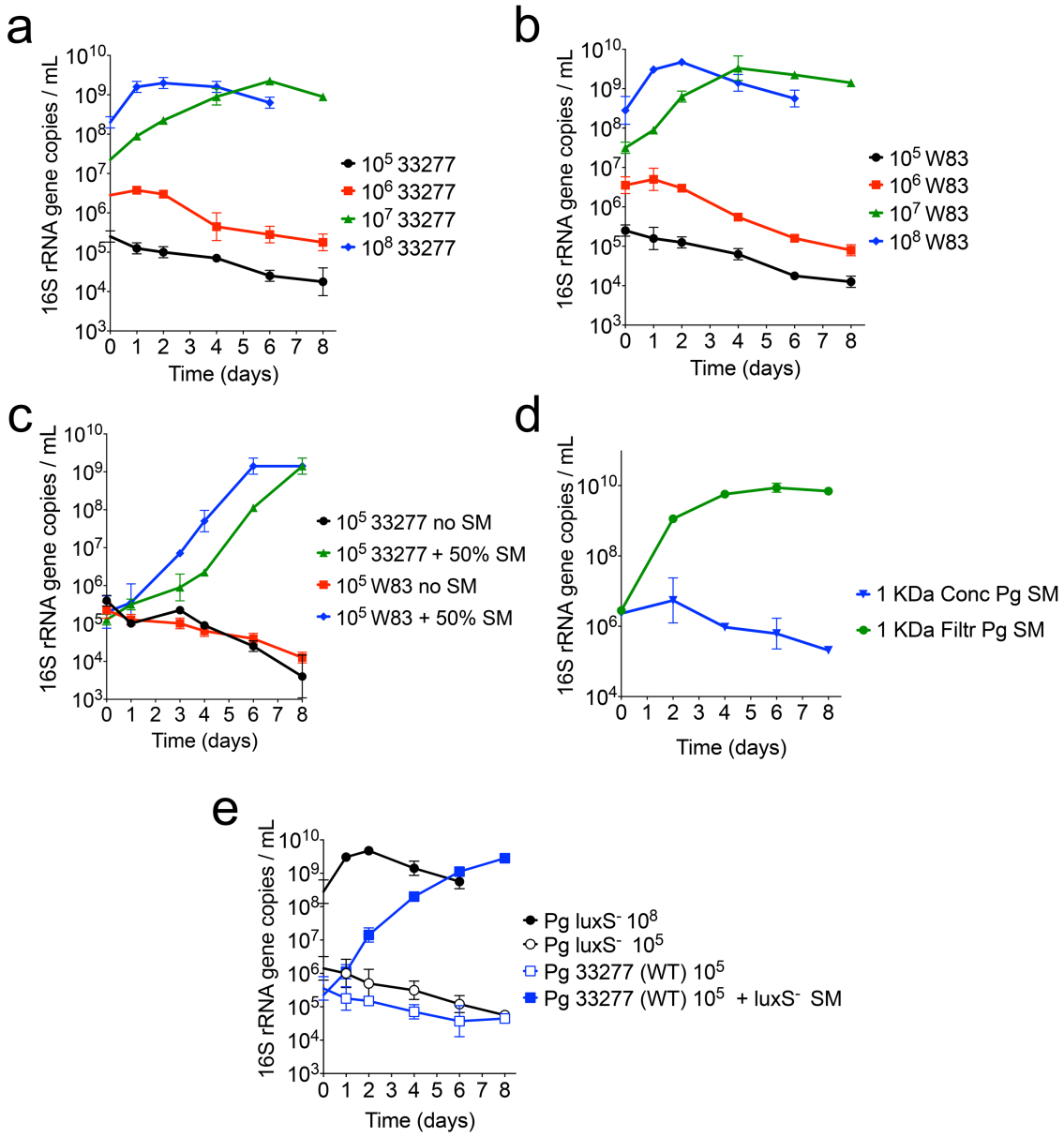
Article:
Hoare, A, Wang, H, Meethil, A et al. (6 more authors) (2021) A cross-species interaction with a symbiotic commensal enables cell-density-dependent growth and in vivo virulence of an oral pathogen. The ISME Journal, 15. pp. 1490-1504. ISSN 1751-7362

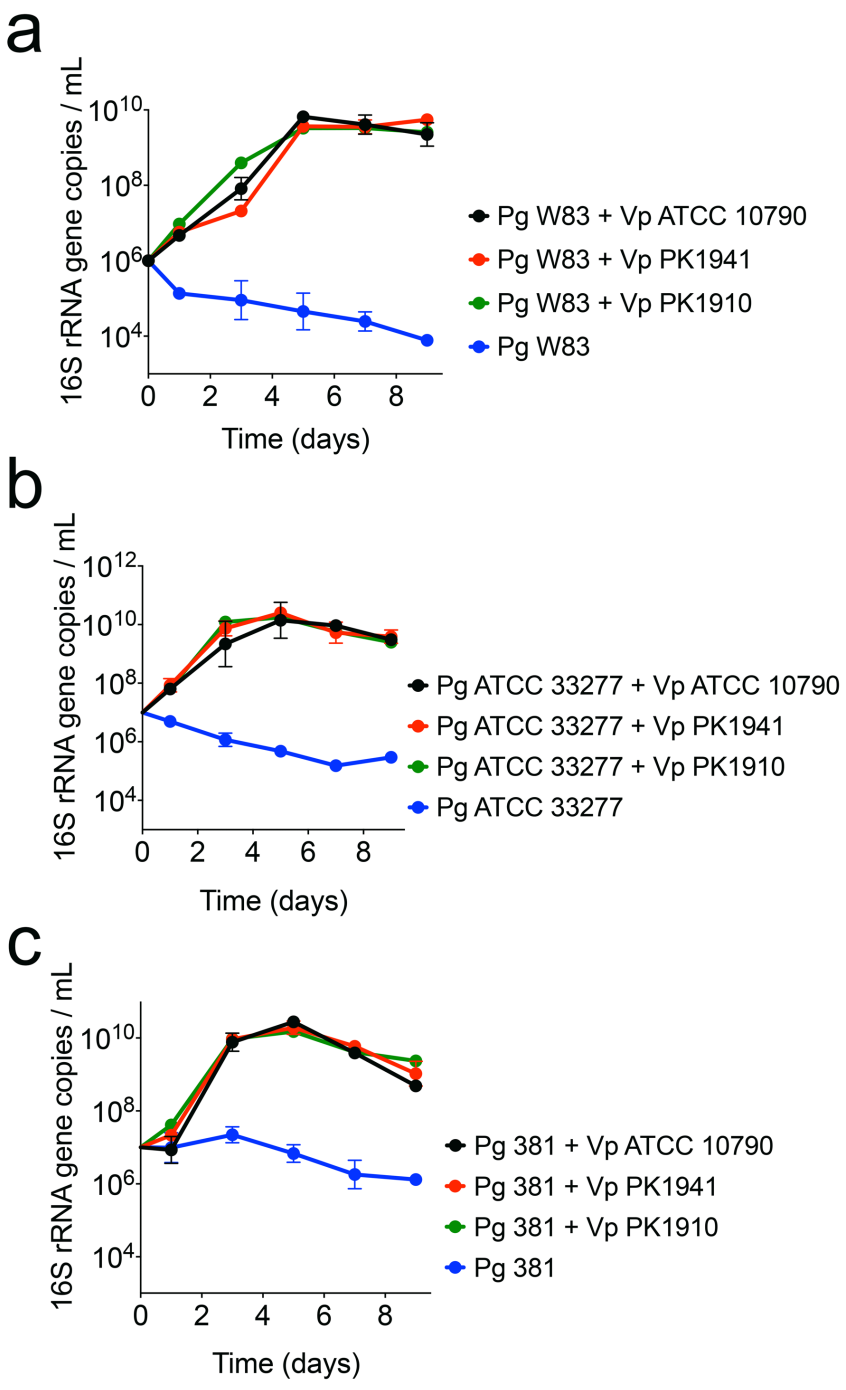
https://doi.org/10.1038/s41396-020-00865-y

Supplementary Material



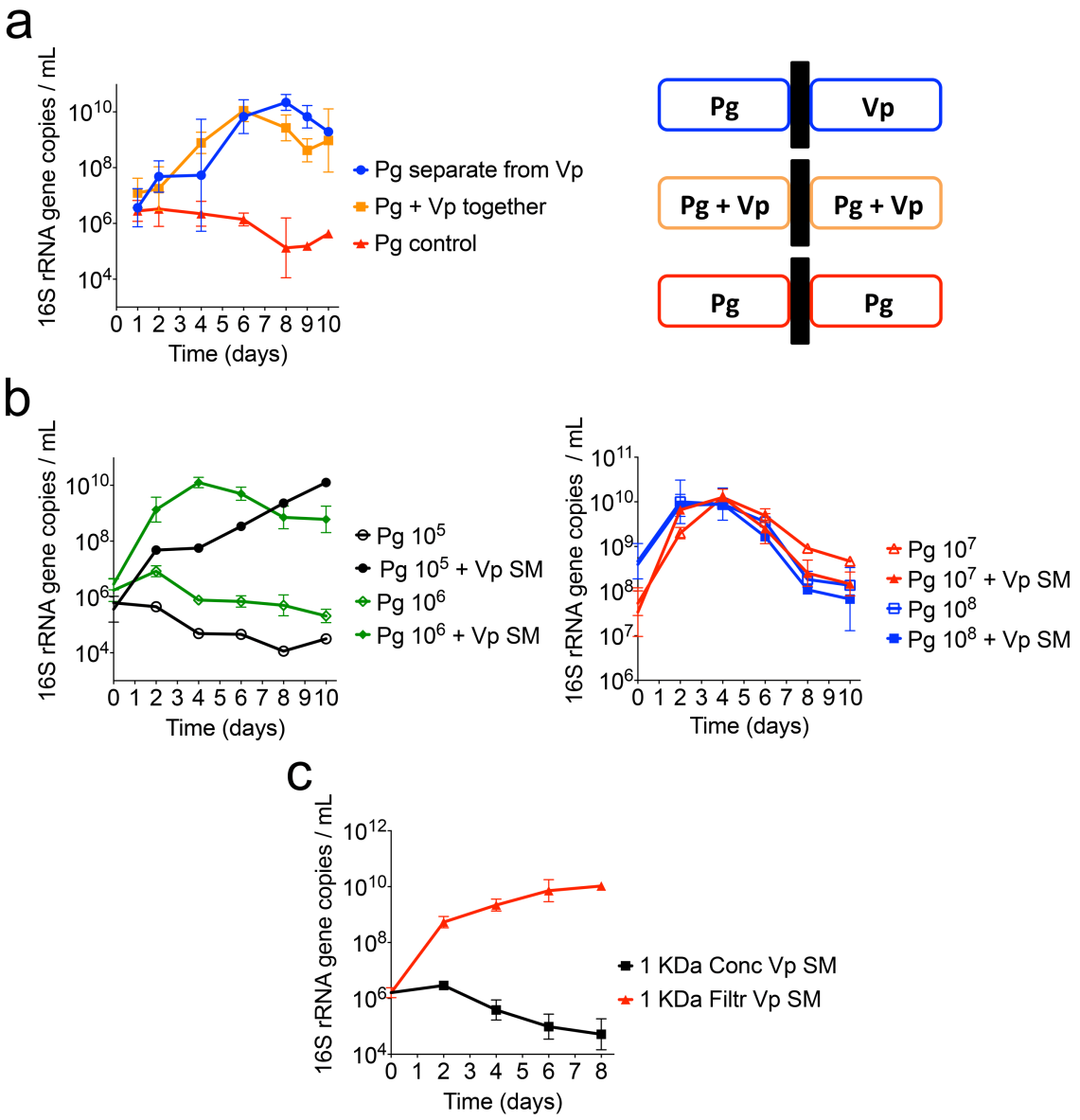
Supplemental Figure 1. Cell-density dependent growth and effect of spent medium (SM) on different strains of *P. gingivalis* (Pg). Growth of Pg ATCC 33277 (**a**) and Pg W83 (**b**) at different cell densities in mucin-serum. **c.** Effect of SM on the growth of Pg ATCC 33277 and Pg W83 in mucin-serum. **c.** Pg 381 soluble factor capable of supporting growth of low-cell-density Pg is smaller than 1 kDa. SM was obtained from

Pg 381 cultures grown in mucin-serum, then filtered through 3 kDa and 1 kDa membranes, followed by lyophilization and reconstitution (10x) in dIH_20. Reconstituted filtrates were added to fresh mucin-serum medium (1:3, vol:vol) to evaluate growth of low-cell-density Pg (10^5 cells mL^{-1}). **e.** Growth of a Pg Δ *luxS*::*ermF* mutant ($luxS^-$) at different cell densities in mucin-serum and effect of SM from the *luxS*$^-$ strain on growth of Pg ATCC 33277 (wild type).



Supplemental Figure 2. The interaction between *V. parvula* (Vp) and *P. gingivalis* (Pg) is not strain-specific. Graphs show growth of Pg W83 (**a**), Pg ATCC 33277 (**b**) or Pg 381 (**c**) as monocultures and in the presence of either Vp ATCC 10790, PK 1941 or

PK 1910. Inoculum size was 10^5 cells $mL^{-1}$and all experiments were conducted in mucin-serum medium. Data represent mean and standard deviations from three independent experiments.



Supplemental Figure 3. Characterization of the soluble factor mediating the support of *V. parvula* (Vp) on the growth of *P. gingivalis* (Pg). a. Physical contact between Pg and Vp is not necessary to allow growth of a low-cell-density Pg inoculum. Pg and Vp were grown in chambers separated by a filter membrane with a pore-size of 0.22 µm. As controls, Pg + Vp were inoculated together or Pg was inoculated in monoculture at both

sides of the membrane. Inoculation-density was 10^5 cells mL^{-1} for both species. **b.** Effect of Vp SM (24 h) on different Pg inoculum sizes. Left graph shows effect of Vp SM on Pg inoculated at 10^5 or 10^6 cells mL^{-1}, and right graph shows effect of Vp SM on Pg inoculated at 10^7 or 10^8 cells mL^{-1}. **c.** Vp soluble factor capable of supporting growth of low-cell-density Pg is smaller than 1 kDa. SM from Vp grown in mucin-serum was filtered through 3 kDa and then 1 kDa membranes, followed by lyophilization and reconstitution (10X) in dIH_20. Reconstituted fractions (Conc= fraction >1kDa and Filtr=fraction < 1kDa) were added to fresh mucin-serum medium (1:3, vol:vol) to evaluate growth of low-cell-density Pg (10^5 cells mL^{-1}). Data in all panels represent replicates (mean and standard deviation) from at least three independent experiments.

Supplementary Table 1. List of compounds tested for their ability to induce growth of a low-cell-density inoculum of *P. gingivalis*. Experiments were performed with commercially available compounds in the indicated concentrations (middle column).

Compound	Tested concentration (mM)	Effect on *P. gingivalis*
D-pantothenic acid (D-PA)	0.005, 0.05 and 0.50	No growth
D-panthenol	0.005, 0.05 and 0.50	No growth
β-alanine	0.01, 0.10 and 1.0	No growth
tyrosol	0.002, 0.02 and 0.20	No growth
spermidine	0.069, 0.69 and 6.90	No growth
spermine	0.000049, 0.00049 and 0.0049	No growth
cadaverine	0.0979, 0.979 and 9.79	No growth
putrescine	0.1134, 1.134 and 11.34	No growth
4-aminobenzoate/para-amino benzoic acid (pABA)	0.0729, 0.729 and 7.29	No growth