

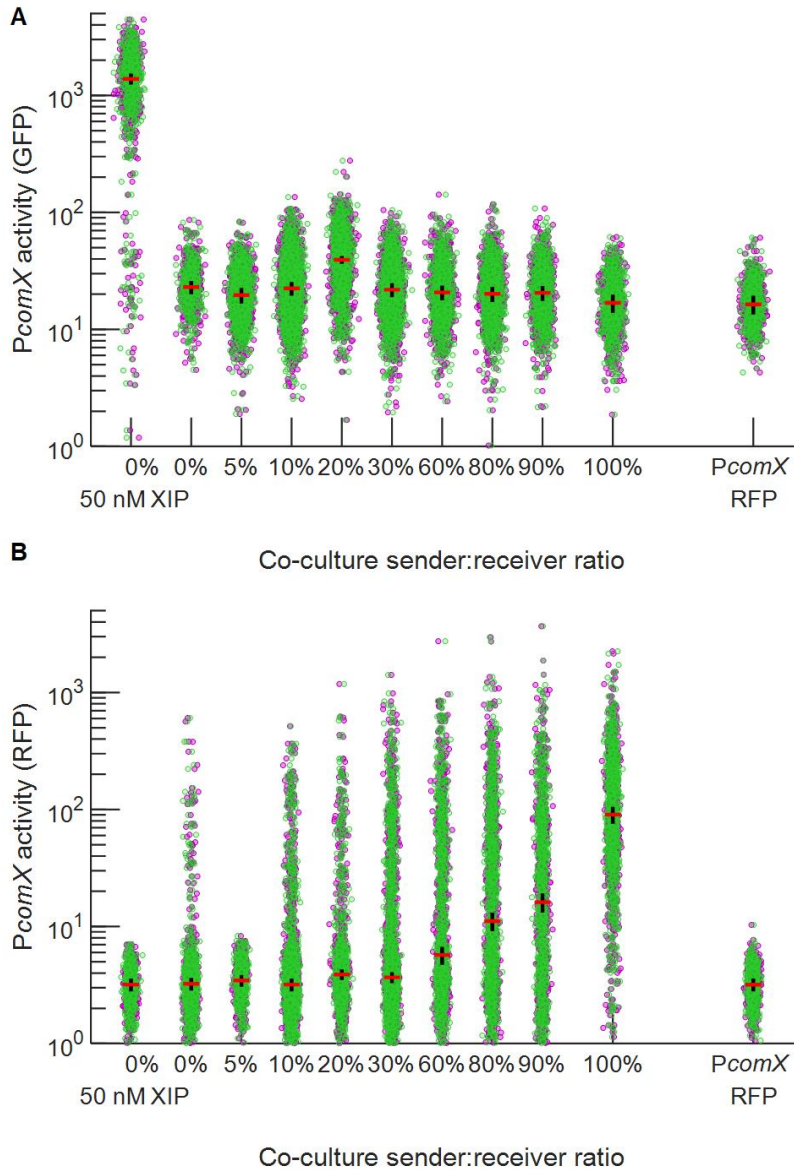
## Supporting Information

Intracellular signaling through the *comRS* system in *Streptococcus mutans* genetic competence

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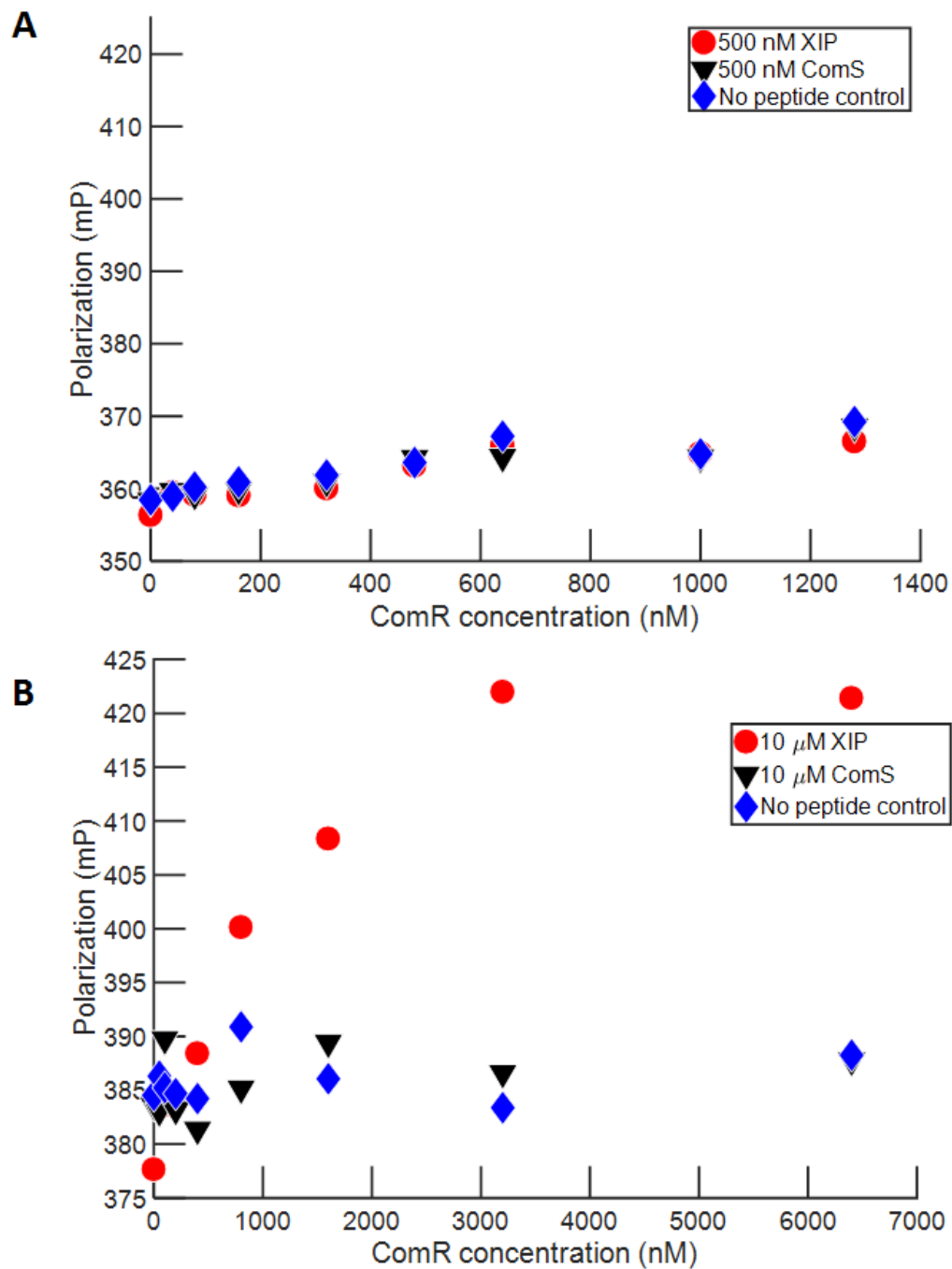
19 **Figure S1 – Response of cocultures is time-independent**

20 (A) GFP (*comX* reporter) and (B) RFP (*comY*) fluorescence of individual cells in co-  
 21 cultures of sender (184*comS* *PcomX-rfp*) and receiver (*PcomX-gfp*  $\Delta$ *comS*) strains of *S.*  
 22 *mutans*. Samples are labeled by percentage by volume of 184*comS* (sender) culture in  
 23 the initial preparation of the coculture. Fluorescence was measured immediately (0 h,  
 24 green) after mixing, or 4 h (magenta) after mixing the coculture. The red horizontal bars

25 show the median fluorescence immediately after mixing (0 h); the black vertical bars

26 show the median at 4 h. Data are from the coculture experiment of Figure 4.

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29 **Figure S2: Effect of histidine tag on ComR binding of ComS and XIP**

30 Fluorescence polarization study of ComS and XIP interaction with ComR that was N- or  
 31 C-terminally tagged with 6X-histidine. Polarization is plotted versus [ComR] for (A) C-  
 32 terminally tagged ComR and (B) N-terminally tagged ComR. In each case 1 nM

33 fluorescent DNA and 0.05 mg ml<sup>-1</sup> salmon DNA were present along with no signal  
 34 peptides (black), XIP (blue) or ComS (green).

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36 **Deterministic fit: Equation system and calculated parameter values**

37 The system of ODEs used to fit microfluidic data is given below. X represents ComX, Z  
 38 the internal XIP concentration, S the internal ComS concentration, R the (constant)  
 39 ComR concentration and Exo the exogenous XIP level. All units are in nM and seconds  
 40 where appropriate. Other symbols are parameters describing the reaction kinetics. A  
 41 star indicates one of the V parameters contributing to feedback, while unstarred Vs  
 42 indicate a maximum rate of production of ComX. Hill kinetics corresponding to inferred  
 43 cooperativity from FP assays were used. Calculated parameters are given in Table S2.  
 44 A 200-iteration bootstrap analysis of the data was performed in order to estimate  
 45 parameter robustness, with the 10<sup>th</sup> and 90<sup>th</sup> percentiles of parameter values reported.  
 46 These percentile values demonstrate preservation of the relative order of magnitude  
 47 between dissociation constants for XIP-ComR and ComS-ComR.

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$$\frac{dX}{dt} = \frac{V_1 R^2 Z^2}{R^2 Z^2 + K_X^4} + \frac{V_2 RS}{RS + K_S^2} - \beta_X X \quad (1)$$

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$$\frac{dS}{dt} = \alpha_0 - \beta_S S - \gamma S + \frac{V_1^* R^2 Z^2}{R^2 Z^2 + K_X^4} + \frac{V_2^* RS}{RS + K_S^2} - \frac{V_2 RS}{RS + K_S^2} \quad (2)$$

50 
$$\frac{dZ}{dt} = J(Exo - Z) - \beta_Z Z + \gamma S - \frac{V_1 R^2 Z^2}{R^2 Z^2 + K_X^4} - \frac{V_1^* R^2 Z^2}{R^2 Z^2 + K_X^4} \quad (3)$$

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54 **Table S1: Parameters for gamma distribution fits to single cell *PcomX* GFP**  
 55 **fluorescence distributions in microfluidic experiments.**

[XIP]	<i>a</i>	<i>b</i>
<b>Wild type</b>	-	-
0	4.09	5.51
280	5.50	172
700	9.62	153
1840	11.0	184
3250	12.5	168
5230	12.4	174
6000	11.7	172
<b><math>\Delta comS</math></b>	-	-
0	6.14	2.29
30	7.10	2.44
850	4.24	166
940	5.11	148
3000	7.44	160
4020	7.61	172
6000	7.45	180

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58 **Table S2: Fitted values for the 12 parameters of the model and statistical**  
 59 **measurement of their robustness from bootstrap process.**

Parameter	Best fit value – used for Fig. 7	10 <sup>th</sup> percentile from bootstrap	90 <sup>th</sup> percentile from bootstrap	Units
$\alpha_0$	7.85	1.78	19.1	nM s <sup>-1</sup>
$\beta_S$	$7.17 \times 10^{-3}$	$7.97 \times 10^{-4}$	$1.32 \times 10^{-2}$	s <sup>-1</sup>
$\gamma$	0.452	0.227	2.74	s <sup>-1</sup>
$V_1^*$	$1.07 \times 10^4$	$3.8 \times 10^3$	$2.46 \times 10^4$	nM s <sup>-1</sup>
$V_2^*$	$1.33 \times 10^4$	$3.22 \times 10^3$	$2.65 \times 10^4$	nM s <sup>-1</sup>
$K_x$	148	42	210	nM
$K_S$	2740	849	3000	nM
$V_1$	3.55	0.988	558	nM s <sup>-1</sup>
$V_2$	778	309	2190	s <sup>-1</sup>
$J$	9.39	$6.14 \times 10^{-2}$	17.6	s <sup>-1</sup>
$\beta_Z$	1.28	0.264	3.07	nM s <sup>-1</sup>
$\beta_X$	10.5	1.71	16.2	s <sup>-1</sup>

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62 **Table S3: RT-qPCR primer sequences**

Gene and direction	Primer sequence
<i>comX</i> forward	5'-CGTCAGCAAGAAAGTCAGAAA C-3'
<i>comX</i> reverse	5'-ATACCGCCACTTGACAAACAG-3'
<i>comS</i> forward	5'-TCAAAAAGAAAGGAGAATAACA-3'
<i>comS</i> reverse	5'-TCATCTGAGATAAGGGCTGT-3'
16S rRNA forward	5'-CACACCGCCCGTCACACC-3'
16S rRNA reverse	5'-CAGCCGCACCTTCCGATACG-3'

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