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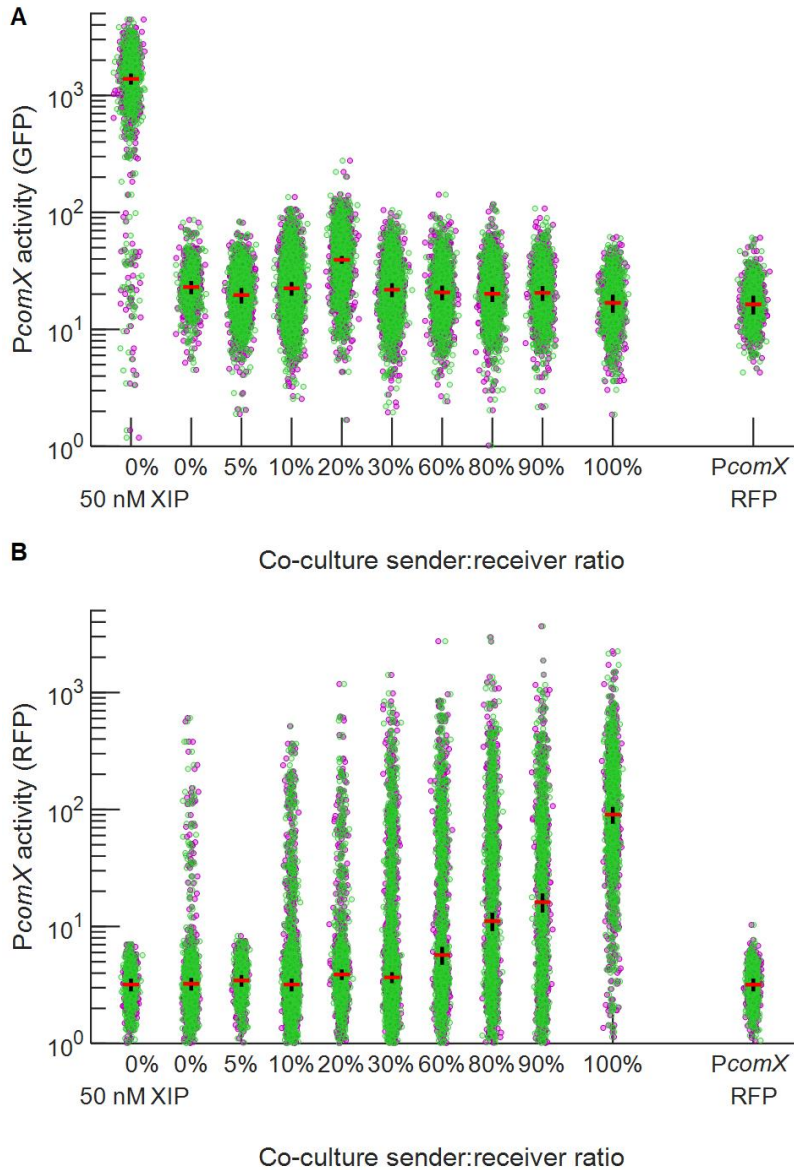
Supporting Information

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

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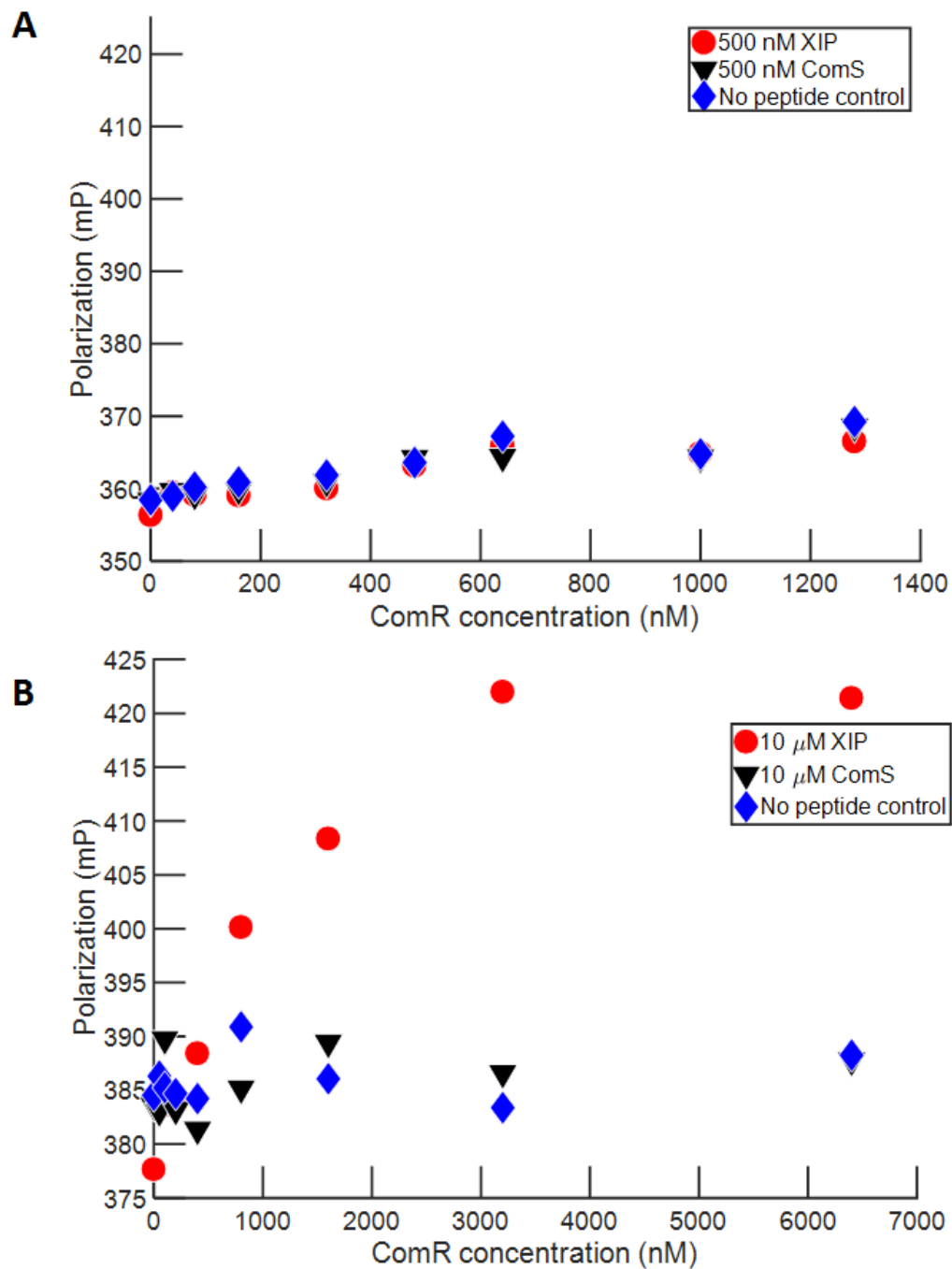
20 **Figure S1 – Response of cocultures is time-independent**

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

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

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

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

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

34 fluorescent DNA and 0.05 mg ml⁻¹ salmon DNA were present along with no signal
 35 peptides (black), XIP (blue) or ComS (green).

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

38 The system of ODEs used to fit microfluidic data is given below. X represents ComX, Z
 39 the internal XIP concentration, S the internal ComS concentration, R the (constant)
 40 ComR concentration and Exo the exogenous XIP level. All units are in nM and seconds
 41 where appropriate. Other symbols are parameters describing the reaction kinetics. A
 42 star indicates one of the V parameters contributing to feedback, while unstarred Vs
 43 indicate a maximum rate of production of ComX. Hill kinetics corresponding to inferred
 44 cooperativity from FP assays were used. Calculated parameters are given in Table S2.
 45 A 200-iteration bootstrap analysis of the data was performed in order to estimate
 46 parameter robustness, with the 10th and 90th percentiles of parameter values reported.
 47 These percentile values demonstrate preservation of the relative order of magnitude
 48 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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$$\frac{dS}{dt} = \alpha_0 - \beta_S S - \gamma S + \frac{V_1^* R^2 Z^2}{R^2 Z^2 + K_X^4} + \frac{V_2^* RS}{RS + K_S^2} - \frac{V_2 RS}{RS + K_S^2} \quad (2)$$

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

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

[XIP]	<i>a</i>	<i>b</i>
Wild type	-	-
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
$\Delta comS$	-	-
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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59 **Table S2: Fitted values for the 12 parameters of the model and statistical**
60 **measurement of their robustness from bootstrap process.**

Parameter	Best fit value – used for Fig. 7	10 th percentile from bootstrap	90 th percentile from bootstrap	Units
α_0	7.85	1.78	19.1	nM s ⁻¹
β_S	7.17×10^{-3}	7.97×10^{-4}	1.32×10^{-2}	s ⁻¹
γ	0.452	0.227	2.74	s ⁻¹
V_1^*	1.07×10^4	3.8×10^3	2.46×10^4	nM s ⁻¹
V_2^*	1.33×10^4	3.22×10^3	2.65×10^4	nM s ⁻¹
K_x	148	42	210	nM
K_S	2740	849	3000	nM
V_1	3.55	0.988	558	nM s ⁻¹
V_2	778	309	2190	s ⁻¹
J	9.39	6.14×10^{-2}	17.6	s ⁻¹
β_Z	1.28	0.264	3.07	nM s ⁻¹
β_X	10.5	1.71	16.2	s ⁻¹

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63 **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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