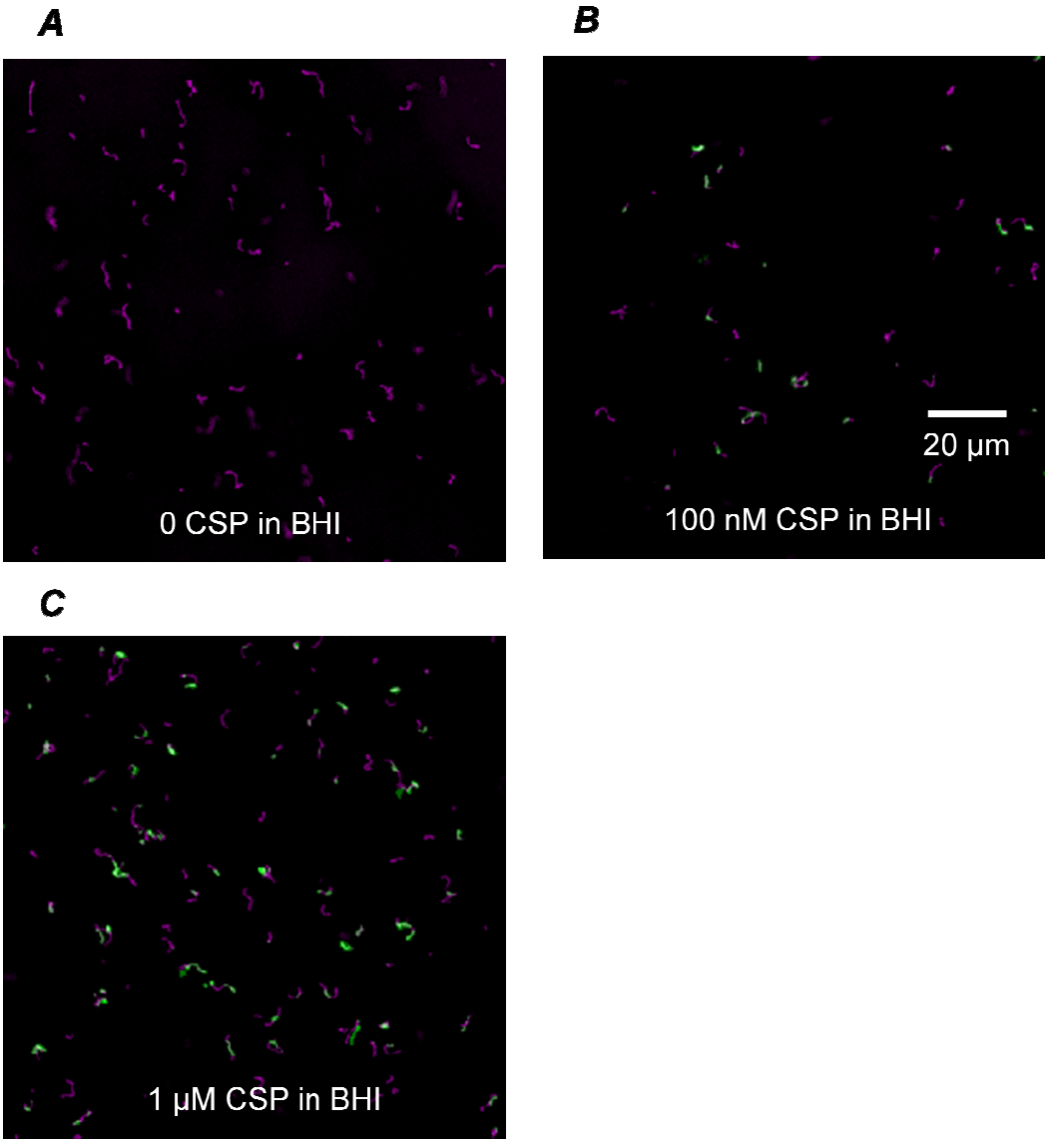
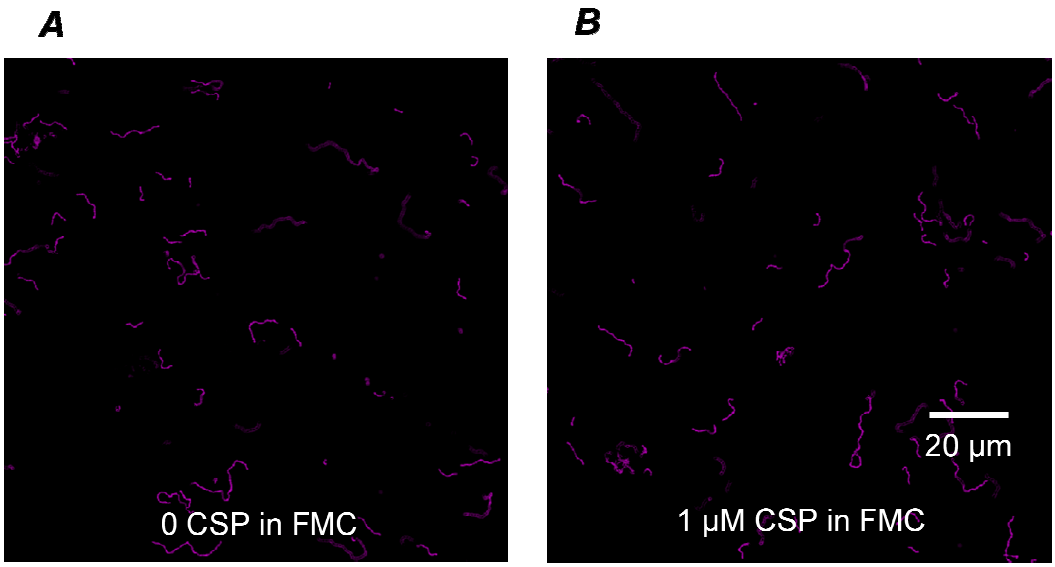
## Supporting Figure S1

Bimodal activation of *comX* by CSP in static (no flow) BHI medium. Wild-type *S. mutans* harboring a *PcomX::gfp* reporter plasmid was incubated at 37 ˚C in BHI containing exogenous CSP concentrations of (*A*) 0, (*B*) 100 nM, or (*C*) 1 µM. The figures show GFP fluorescence (green) microscopy images overlaid on reversed phase contrast (magenta) images. Images were collected after 2.5 h. In the presence of 100 nM or 1 µM CSP approximately 30-50% of cells have activated *comX*. The remainder of cells show little fluorescence, like the untreated controls at 0 CSP.



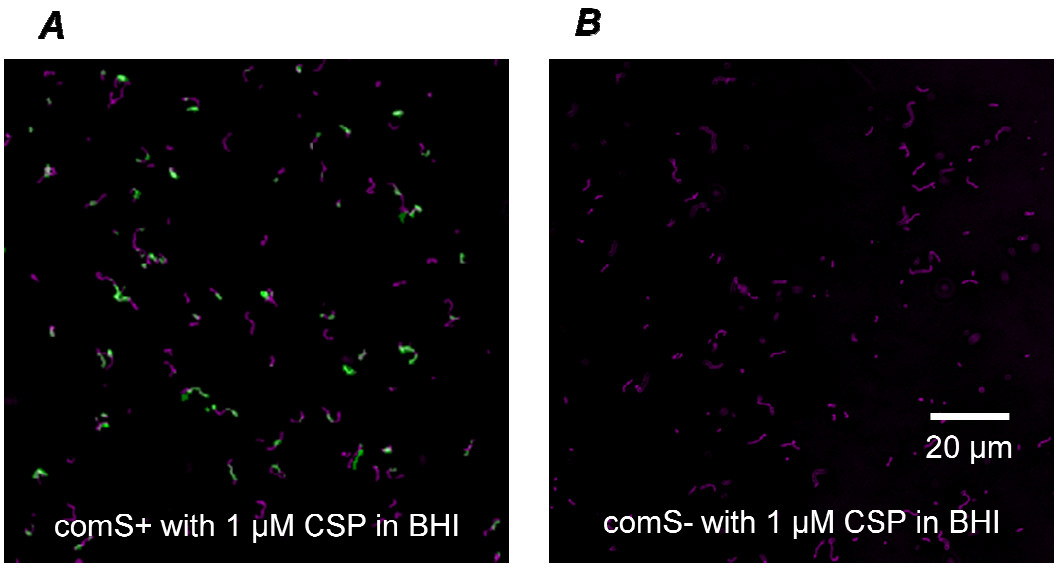
## Supporting Figure S2

CSP induces no response from *comX* in static (no flow) FMC medium. The *PcomX::gfp* reporting strain of *S. mutans* was incubated at 37 ˚C for 3 hrs in FMC medium containing CSP concentrations of (*A*) 0 and (B) 1 µM. In overlays of GFP fluorescence (green) with reverse phase contrast (magenta), neither sample shows significant activation of *comX*.



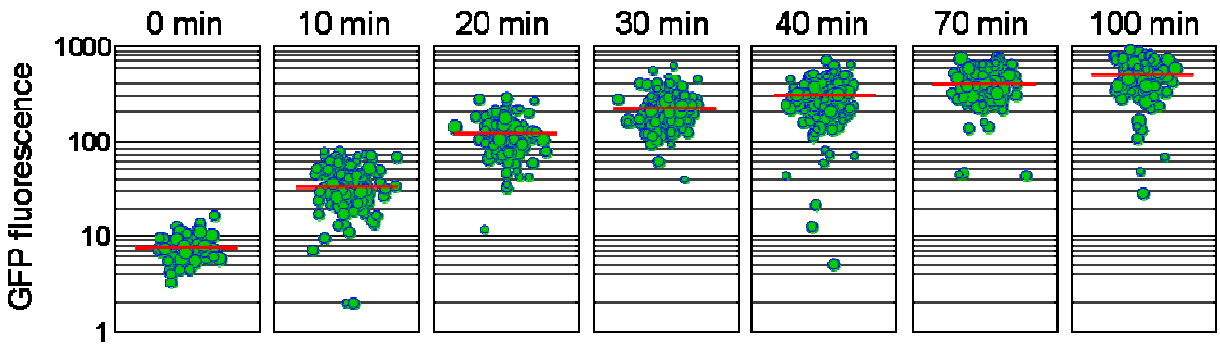
## Supporting Figure S3

Bimodal response to *comX* requires an intact *comS.* (A) Wild-type and (B) *comS* mutant strains of *S. mutans* carrying the *PcomX::gfp* reporter were incubated for 2.5 h in static BHI containing 1 µM CSP. The bimodal *comX* response observed in the wild-type genetic background is absent in the *comS* mutant.



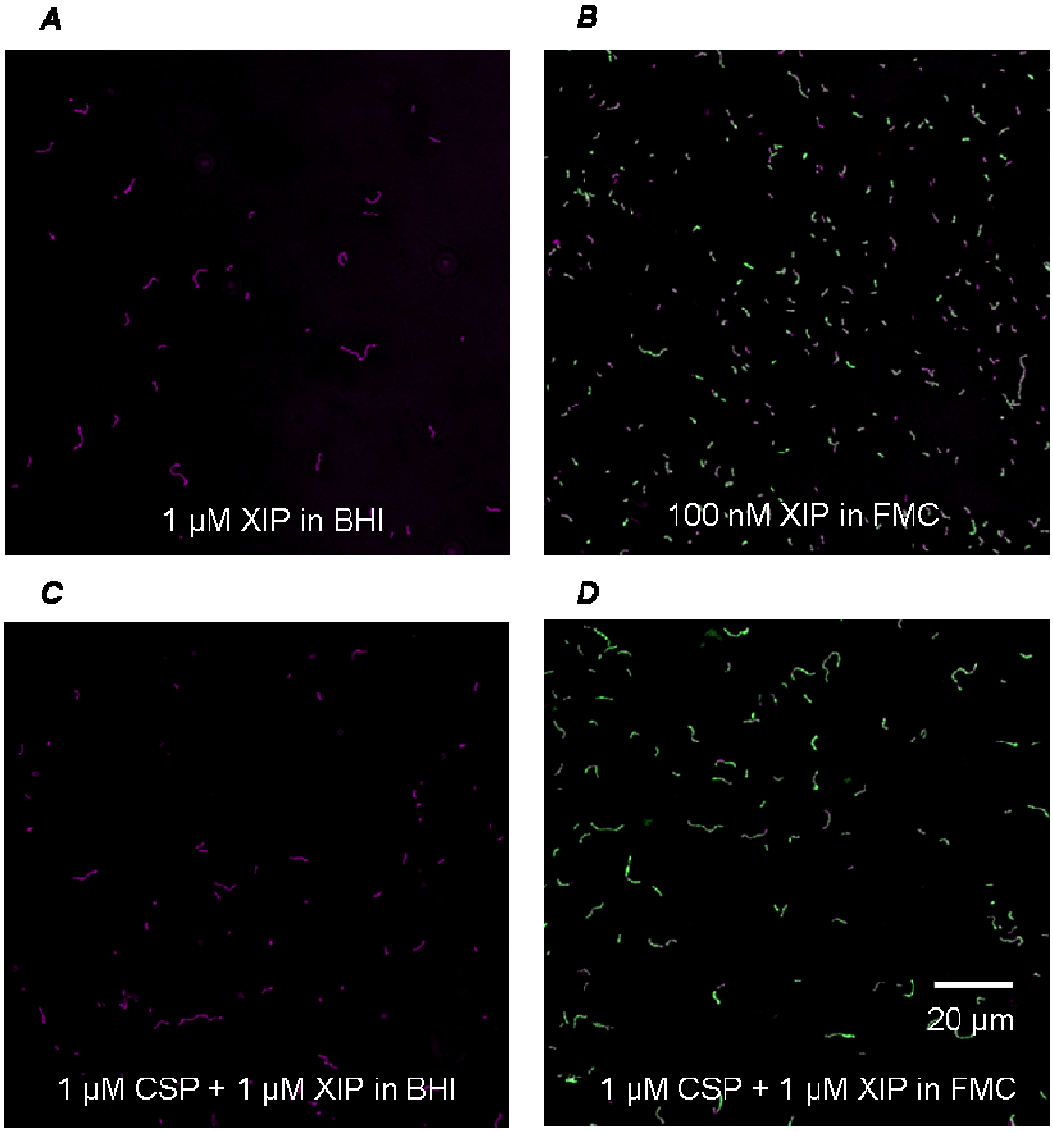
## Supporting Figure S4

Kinetics of *comX* response to exogenous XIP in cells exposed to FMC. A *comS* mutant is prepared in FMC medium and supplied with 500 nM XIP at time *t* = 0. The figure shows the GFP fluorescence of individual cells at subsequent times ranging from *t* = 10 min to *t* = 100 min. The red horizontal bar indicates the median fluorescence of each group of cells. The figure indicates that *comX* expression approaches steady-state levels after approximately 20-30 minutes.



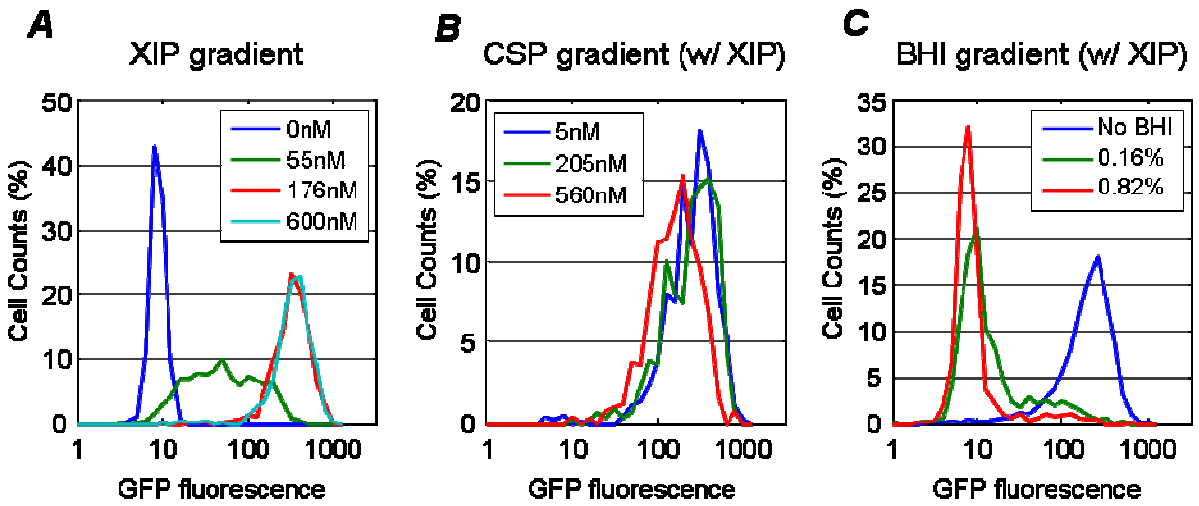
## Supporting Figure S5

XIP induces a unimodal response in a *comS* mutant in FMC, but no response in BHI medium. Microscopy images show the response of a *PcomX::gfp* reporter in a *comS* mutant that is incubated with (a) 1 µM XIP in BHI, (B) 100 nM XIP in FMC, (C) 1 µM XIP + 1 µM CSP in BHI, and (D) 1 µM XIP + 1 µM CSP in FMC. Regardless of whether CSP is provided, the *comS* mutant does not express *comX* in BHI medium. In FMC medium the *comS* mutant shows unimodal (population-wide) response to XIP or XIP+CSP.



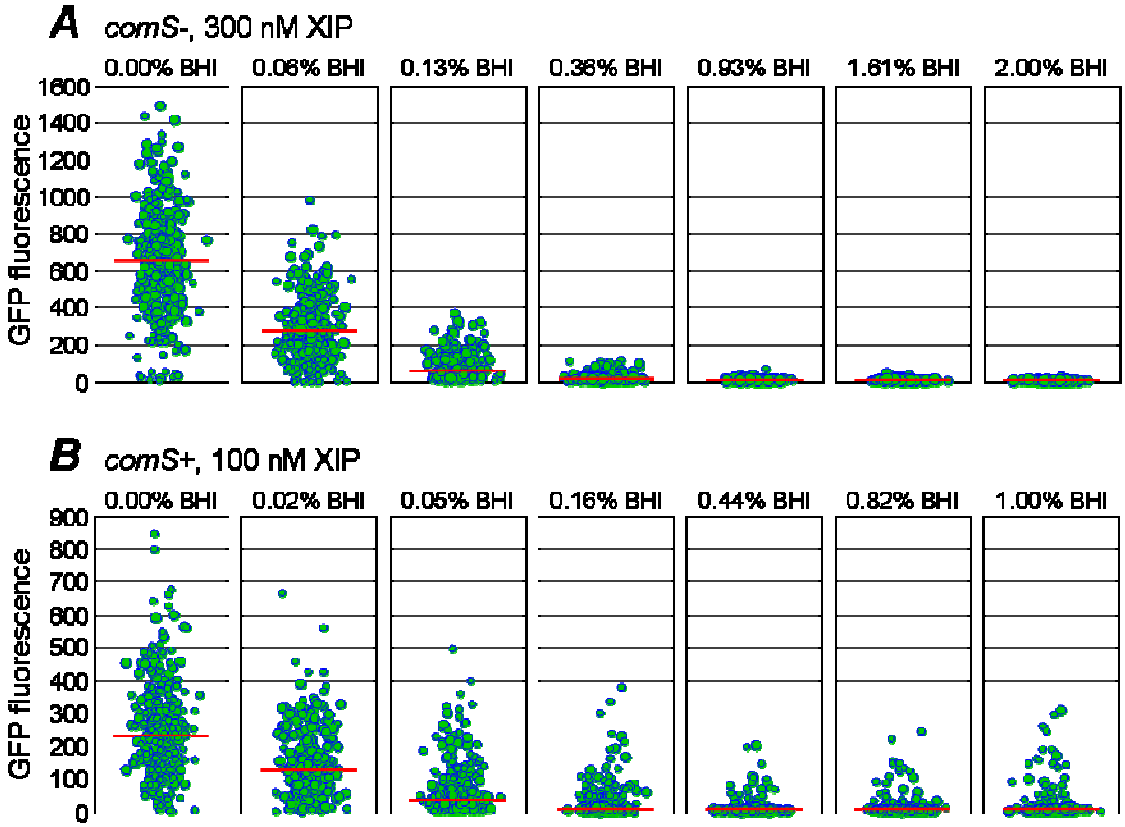
## Supporting Figure S6

Histograms of *comX* expression levels observed in individual cells during microfluidic studies. All cells carry an intact *comS* gene and a P*comX::gfp* reporter. (*A)* When cells were subject to different XIP levels in FMC medium, the distribution of *comX* expression is unimodal: all cells are activated to similar levels, and the average degree of activation increases in a graded fashion as the XIP concentration rises. (*B)* The addition of CSP to cells in FMC containing 50 nM XIP does not change the unimodal character of the distribution of *comX* levels. However the average activation declines slightly at the highest CSP concentration. (*C*) The *comX* response to 100 nM XIP in FMC changes when small amounts of BHI are mixed into the FMC. In the absence of any BHI, a unimodal response is observed. At 0.16% BHI (v:v), most cells switch off, although some continue to fluoresce at levels (~30-300) similar to those observed in the absence of BHI. These cells comprise the long, high-fluorescence tail of the green curve. At 0.82% BHI, most cells show no fluorescence above background levels, but a few percent continue to fluoresce at levels near 100, as if in BHI were absent. Hence in the strain with an intact *comS* gene the addition of BHI quenches the response to XIP while also converting it from a unimodal to a bimodal response.



## Supporting Figure S7

Effect of BHI on response to XIP in (*A*) a *comS* mutant and (*B*) the wild-type genetic background. (A) *comS*-deficient *S. mutans* is supplied with a flow of FMC medium and varying amounts of BHI (v:v), both containing 300 nM XIP. The presence of BHI reduces the median (horizontal red line) level of *PcomX* expression in a unimodal (graded) fashion. At 1-2% BHI no cells are observed to express at levels significantly greater than the baseline. (*B*) In the wild-type background strain, BHI is equally effective in suppressing the average *PcomX* response to XIP, but the response is bimodal. At 1% BHI the median expression has decreased to baseline levels, but a subpopulation of cells continues to express almost as strongly as in the absence of BHI.



## Supporting Figure S8

High concentrations of environmental XIP can overcome the inhibiting effect of BHI. A *comS*-deficient mutant with a P*comX-gfp* reporter was subjected to varying concentrations of exogenous XIP in a microfluidic device supplying 0.15% BHI in FMC. GFP fluorescence was measured after 2 h exposure to the medium and XIP. Although the BHI/FMC admixture suppresses the *comX* response to 100 nM XIP, the figure shows that *comX* becomes activated as the environmental XIP concentration approaches 1 µM. These data suggest that the inhibiting effect of BHI is due to small peptides that are present in BHI and compete with environmental XIP for access to the Opp peptide permease.