

## Supplemental Material

### S1: MATLAB<sup>®</sup> R2012a codes for image analysis

(1) Fluorescence intensity

```
im = imread('image name');
```

```
im_g = im(:,:,Nψ);
```

```
ans = sum(sum(im_g));
```

```
ans/(K*Kδ)
```

(2) Fluorescence coverage

```
im = imread('image name');
```

```
im_g = im(:,:,Nψ);
```

```
result = im_g > Mζ;
```

```
ans = sum(sum(result));
```

```
ans/(K*Kδ)
```

Footnotes:

<sup>ψ</sup>N = 1 for red fluorescence (EPS), N = 2 for green fluorescence (bacteria)

<sup>ζ</sup>M is the background color intensity, which varies in different experiments. In the present study, M = 30 and 35 for green and red fluorescence, respectively.

<sup>δ</sup>K is the pixel value in each dimension of an image.

**S2: *S. gordonii* gene expression with nicotine using the traditional  $2^{-\Delta\Delta Ct}$  method.**

Overnight *S. gordonii* cells were treated with 0 and 2 mg/ml nicotine for 8 hours in TSBS, and planktonic cells were harvested for qRT-PCR.  $2^{-\Delta\Delta Ct}$  of each sample was calculated.

\*P<0.05.

