Stable Expression of Modified Green Fluorescent Protein in Group B Streptococcus to Enable Visualization in Experimental Systems

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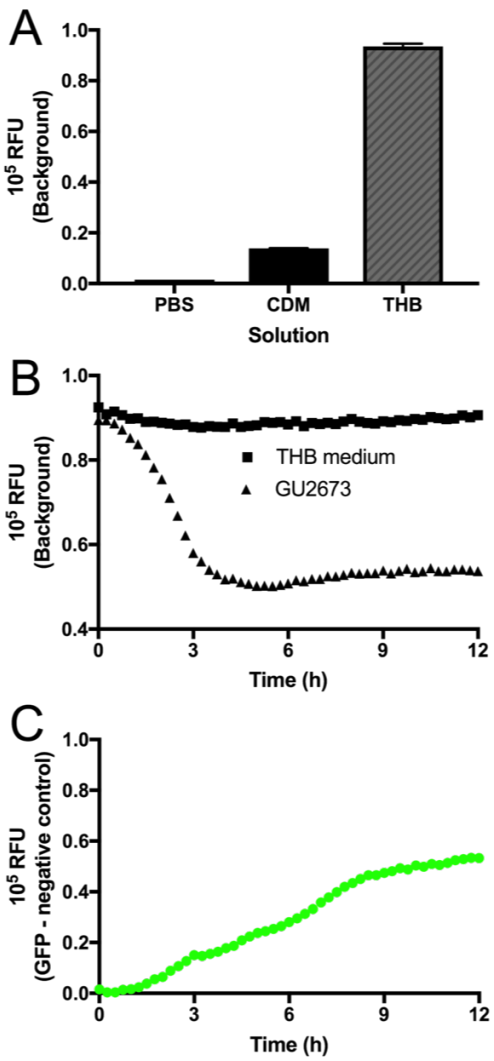
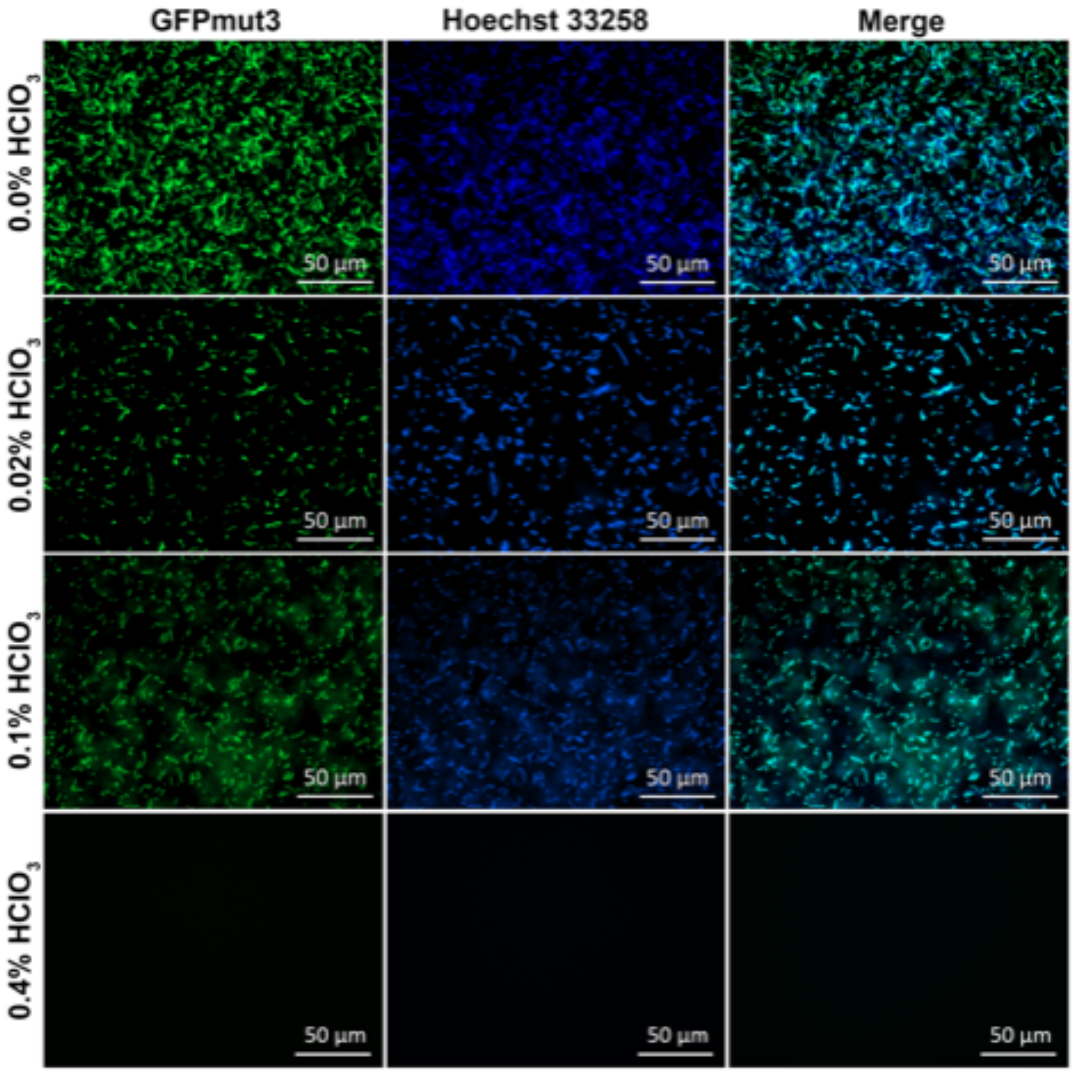
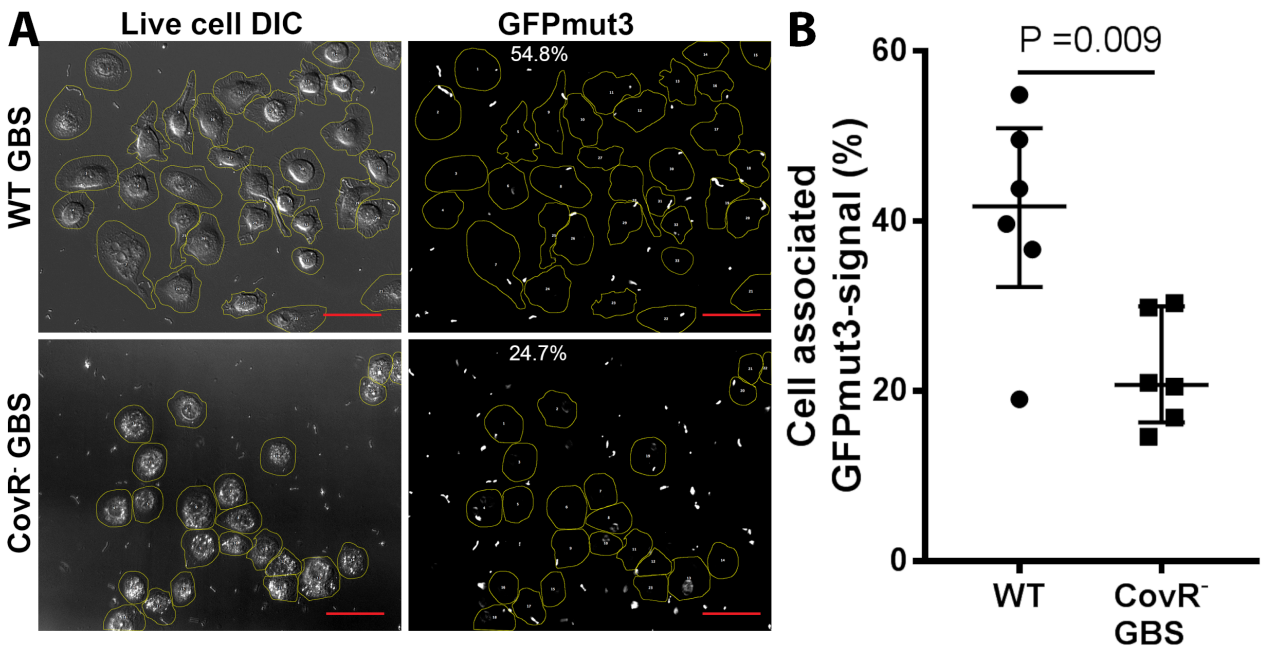
SUPPLEMENTAL MATERIAL



Supplementary FIG. S1. Media used for bacteriology exhibited different levels of autofluorescence at the excitation emission wavelengths used for the detection of GFPmut3 (A). Autofluorescence detected in THB medium remains stable in the absence of bacteria (squares) but in the presence of GBS (triangles) declines in a manner inversely proportional to culture density (shown in Fig 6). Negative control correction of fluorescence intensity can be used to adjust for the autofluorescence background interference detected in THB medium to provide a fluorescence profile (C) similar to that detected using fluorescence polarization intensity (shown in Fig 6C).



Supplementary FIG. S2. Effect of increasing concentrations of sodium hypochlorite on GBS cell morphology and fluorescence. GBS strain 874391 (2, 4, 5) were rendered non-viable following exposure to sodium hypochlorite ≥0.02%, according to colony count assays (Fig 7B). However, GBS continued to emit high levels of fluorescence at this level of sodium hypochlorite (upper panels). Sodium hypochlorite 0.4% abolished the fluorescence and destroyed the bacteria (bottom panel), while 0.1% attenuated fluorescence in intact GBS cells (Fig 7A), which were rendered non-viable, according to colony count assays.



43 **Supplementary FIG. S3. Relative quantitation of cell-associated fluorescence in**
44 **assays comparing the adhesion of WT GBS and CovR mutant to human cells.**
45 Inoculation of 5637 uroepithelial cells with WT and CovR mutant GBS (3) (both carrying
46 plasmid pGU2664) shows the attenuated adherence phenotype of the CovR mutant based
47 on the fluorescence detection of GFPmut3; representative cell masks used to quantitate
48 pixels is shown in (A). Quantitation of the fluorescence signals in areas co-located with
49 the human cells (cell-associated fluorescence) was achieved using ImageJ software 1.51
50 (1), and compared to acellular areas (slide-associated fluorescence); six fields-of-view
51 (one representative shown for WT and mutant) were analyzed to generate quantitative
52 data and revealed significantly more fluorescence signal co-located with epithelial cells
53 inoculated with WT GBS versus the CovR mutant (B). Scale bars = 50 µm; % shown is
54 cell-associated fluorescence detected for that image.
55

Supplementary Movie. S1. Visualization of adhesion of GBS to human cells. 5637 uroepithelial cells were inoculated with GU2666 (GFP+) containing plasmid pGU2664 and video was captured after 2 h of static incubation and subsequent initiation of flow. The video (30 s total, looped 5x) shows human cells with bound GBS (red arrows) amid a background of media and bacteria flowing through the chamber. The video highlights many chains of non-adhered GBS passing in the flow-through (red box). The video represents ~0.8 chamber-volumes of media using medium flow conditions, as described in Materials and Methods.

Supplemental References

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