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Supplementary Materials for

Engineering a light-responsive, quorum quenching biofilm to mitigate biofouling on water purification membranes

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This PDF file includes:

Table S1. Sequence details for the gene circuits.

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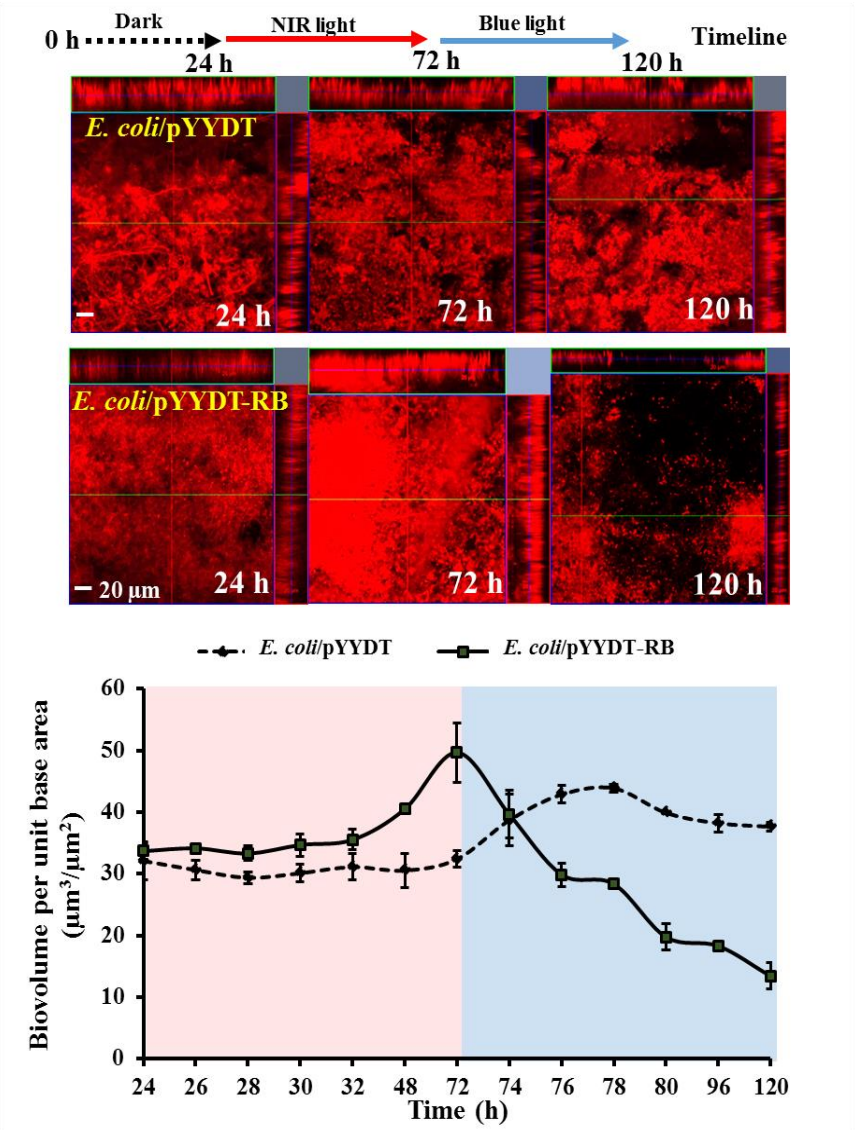

Fig. S1. Biofilm formation of the control *E. coli*/pYYDT and light-responsive *E. coli*/pYYDT-RB strains in biofilm flow cell. (**A**) CLSM images of biofilms at 24, 72 and 120 h. The red line represents biofilms formed under NIR light and the blue line represents biofilms formed under blue light. Each CLSM image contains one top-down view (x−y plane) and two side views (x−z and y−z planes). The scale bar is 20 μm. (**B**) Biofilm biovolume (expressed as biovolume per unit base area). The red and blue zones represent incubation under NIR light and blue light, respectively. The error bar represents the standard deviation.

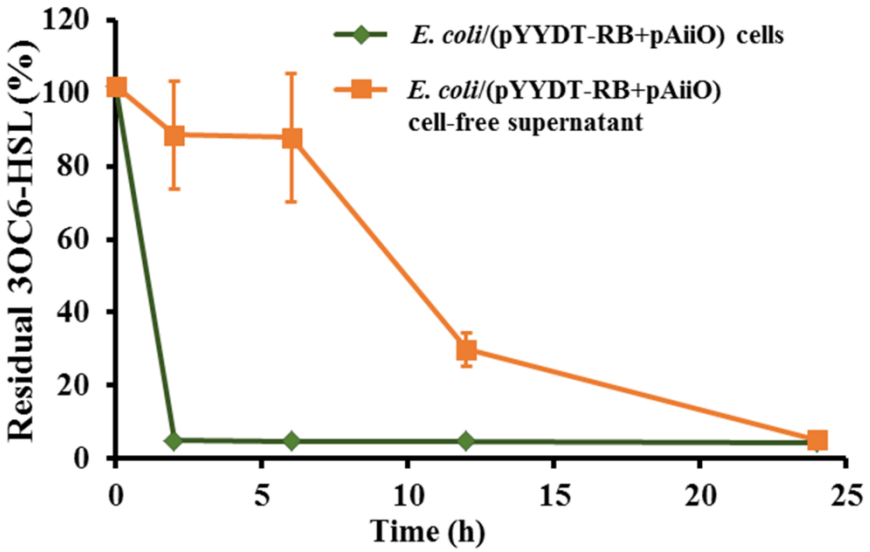

Fig. S2. Inactivation of exogenous 3OC6-HSL by *E. coli*/(pYYDT-RB + pAiiO) cells and the cell-free supernatant. Residual 3OC6-HSL was quantified using the *E. coli* JB525 bioassay at 0, 2, 6, 12 and 24 h post incubation. Experiments were conducted in triplicate.

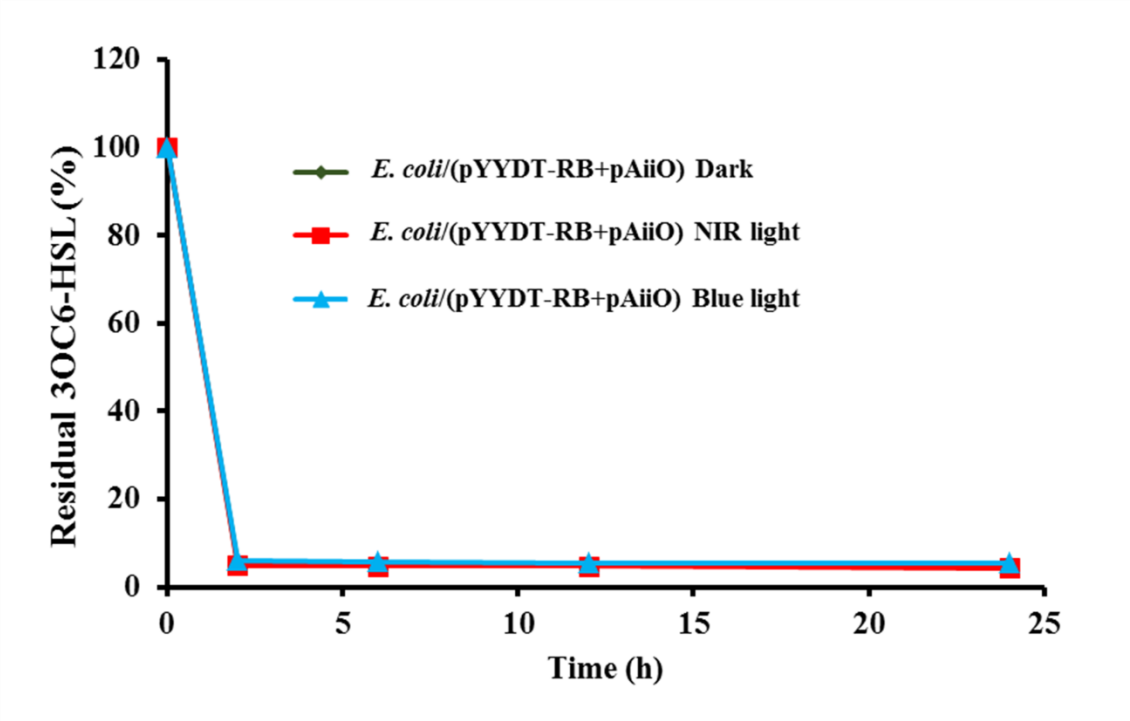

Fig. S3. Inactivation of exogenous 3OC6-HSL by *E. coli*/(pYYDT-RB + pAiiO) cells incubated in the dark, under NIR light and blue light. Residual 3OC6-HSL was quantified using the *E. coli* JB525 bioassay at 0, 2, 6, 12 and 24 h post incubation. Experiments were conducted in triplicate.

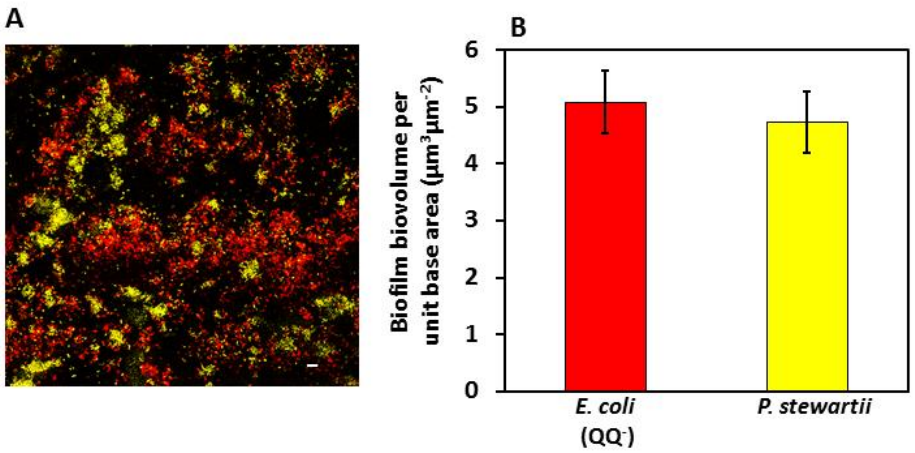

Fig. S4. Coculture biofilm formation by the mCHERRY-tagged control *E. coli* (QQ negative) and YFP-tagged *P. stewartii*. (**A**) CLSM image of co-culture biofilm at 24 h. The scale bar is 20 µm. (**B**) Biofilm biovolume (expressed as biovolume per unit base area). The error bar represents the standard deviation.

Method details to observe residual EPS after blue light-mediated dispersal

To observe the presence of the residual EPS, in particular, extracellular polysaccharides, mCHERRY-tagged QQ biofilms were allowed to grow in each channel of the flow cell for 2 days and then subjected to two different conditions: i) incubated in the dark for 24 h (control) and ii) incubated under blue light for 24 h. After the incubation, 0.2 mg mL^{-1} of Concanavalin A-FITC conjugate (Molecular Probes, Invitrogen) was pumped through the flow cell followed by incubation for 30 min in the dark. Then sterile 0.9 % NaCl was pumped through respective channels for 10 min to remove excess ConA stain. The ConA-stained polysaccharides were observed with excitation at 488 nm and emission at 509 nm. The mCHERRY-tagged cells were excited at 550 nm and observed at 650 nm for emission. The images were split into different channels in the x-y direction to separately observe the differently fluorescent components.

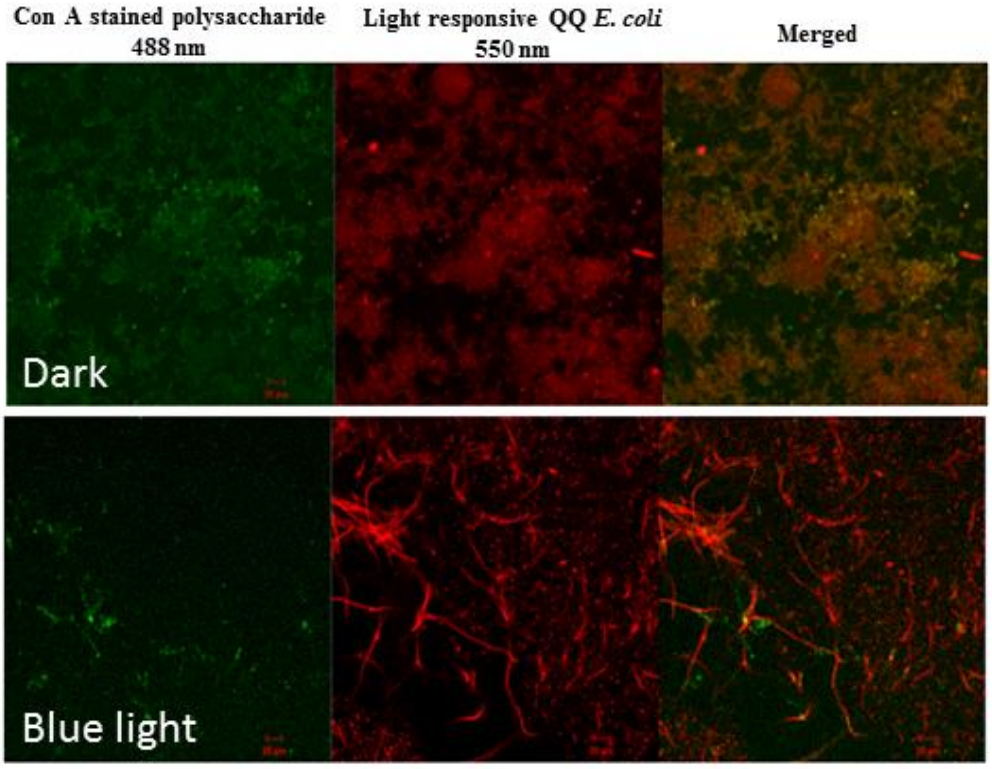

Fig. S5. CLSM images of mCHERRY-tagged light-responsive QQ *E. coli* biofilm and ConA-stained polysaccharide in the flow cell. The top panel illustrates the incubation of biofilm in dark (control), whereas the bottom panel represents biofilm exposure under blue light. The scale bar is 20 μm. Results are representative of three separate experiments.

Method details to observe dead bacteria after blue light-mediated dispersal

The light-responsive QQ strain was allowed to grow in a flow cell for 2 days, followed by challenging the pre-formed QQ biofilm with dead *P. stewartii* for 24 h. Briefly, to prepare the dead *P. stewartii*, 12 h grown culture of planktonic YFP tagged *P. stewartii* (OD_{600} ~ 0.5) were fixed with 2% paraformaldehyde for 15 min followed by PBS wash to remove the residual paraformaldehyde. The relative amount of dead *P. stewartti* on the QQ biofilms was determined under two different conditions, i) after incubating in the dark for 24 h (control) and ii) after incubating under blue light for 24 h. The YFP-tagged *P. stewartii* was observed with excitation at 514 nm and emission at 527 nm. The mCHERRY-tagged *E. coli* was observed with excitation at 550 nm and emission at 650 nm. The CLSM images were analyzed using Imaris to calculate *E. coli* biofilm biovolume per unit base area and the relative amount of *P. stewartti* (dead cells) on pre-formed *E. coli*.

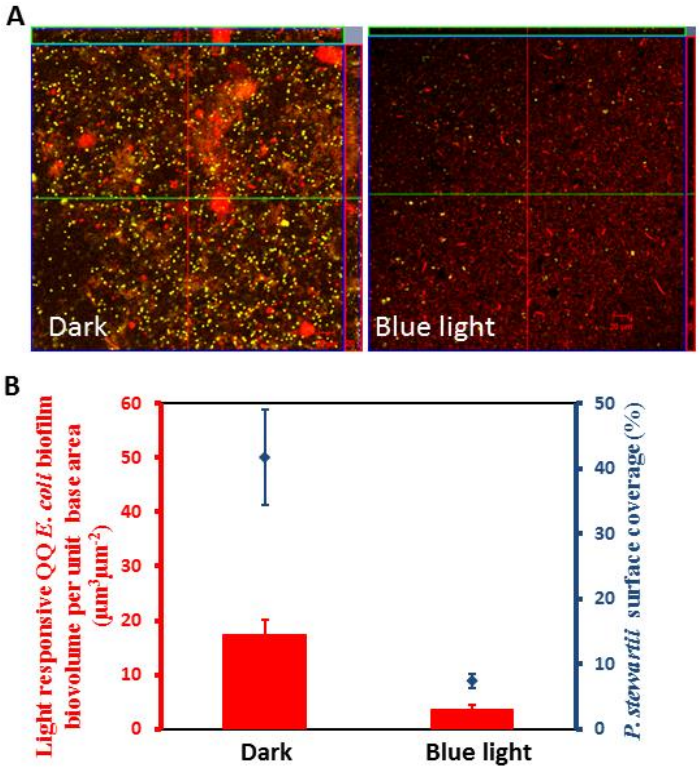

Fig. S6. CLSM images of dead *P. stewartii* (YFP tagged) on preformed light-responsive QQ *E. coli* biofilm. (**A**) The right panel illustrates the incubation of biofilm in dark (control), whereas the left panel represents biofilm exposure under blue light. The scale bar is 20 μm (**B**) Primary Y-axis represents biofilm biovolume of light-responsive QQ *E. coli* biofilm. Secondary Y- axis represents surface coverage by *P. stewartii*. The error bar represents the standard deviation. Results are representative of three separate experiments.