Electronic Supplementary Material (ESI) for Nanoscale Advances.

Photoactivated antibiotics to treat intracellular infection of bacteria

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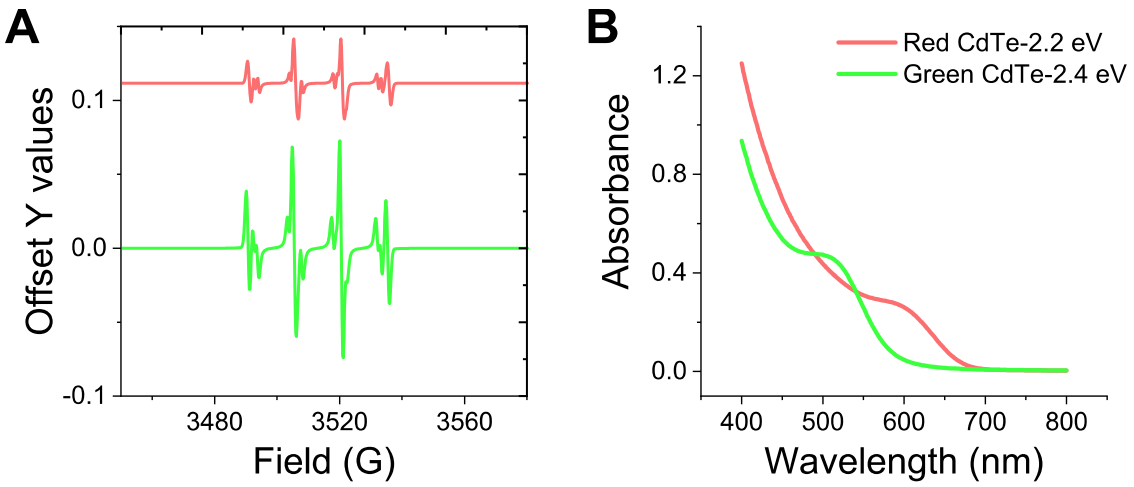

Figure S1. Characterization of CdTe Nanoparticles. (A) Electro paramagnetic resonance (EPR) measurement of light activated CdTe-2.4 eV (green) and CdTe-2.2 eV (red). (B) UV-Vis absorbance spectra of green and red CdTe showing a shift in absorbance to higher wavelengths for the red CdTe as compared to green CdTe which correlates with their difference in size.

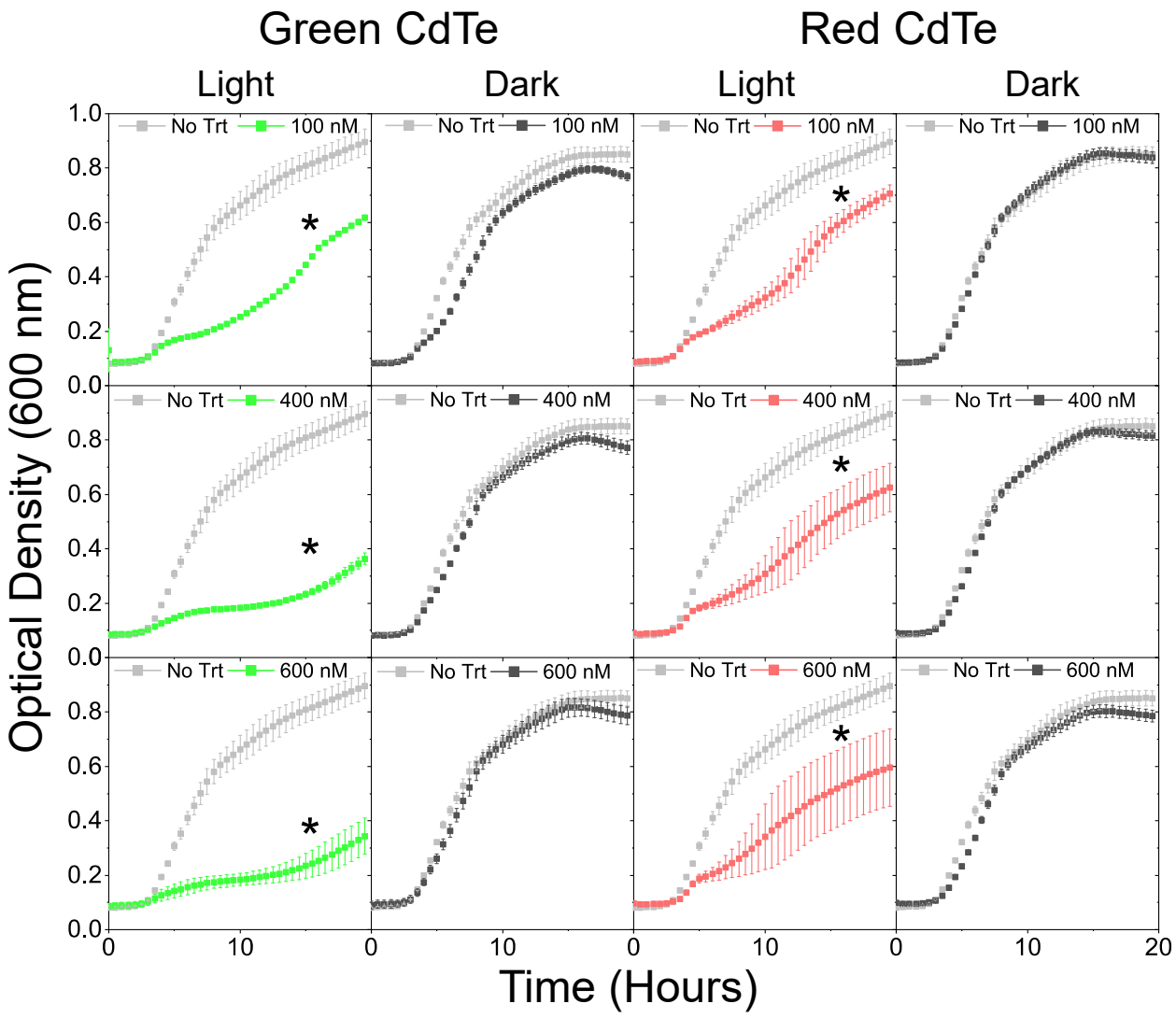

Figure S2. Growth Curves of SL1344 treated with CdTe. Raw OD 600nm growth curves of 1:1,000 dilution of SL1344 from overnight growth treated with varying concentrations of CdTe (red and green) under light activation or in dark. An asterisk (*) indicates a statistical difference (unpaired t-test, equal variance, $\alpha = 0.05$) between no treatment and treatment at 16 hours of growth.

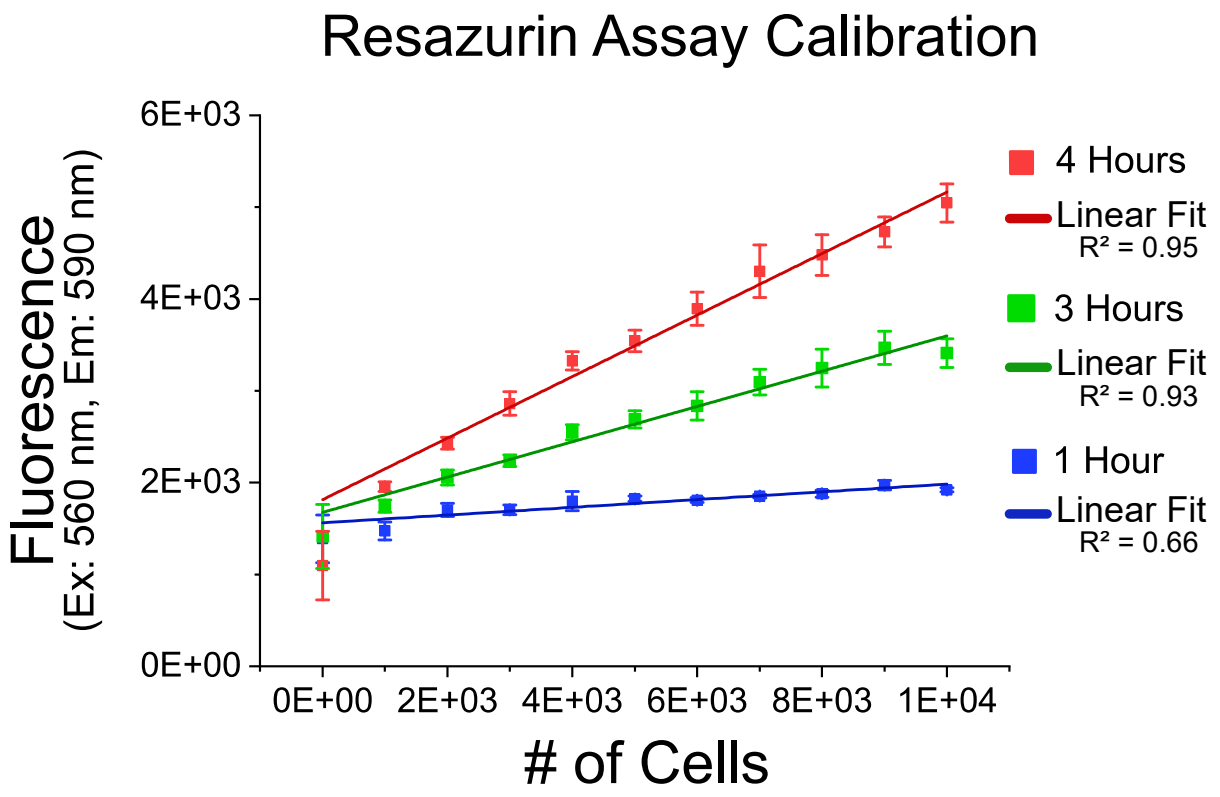

Figure S3. Resazurin calibration for preosteoblast cells. Varying amounts of preosteoblast cells were seeded (x axis) and incubated for 42 hours, the amount of time from seeding to lysing in an infection experiment, before measuring viability using resazurin. Resazurin was incubated for varying amounts of time (1, 3, and 4 hours) before measuring fluorescence to establish a calibration curve. A strong linear trend was seen at 3 and 4 hours of incubation and all viability experiments were subsequently done using 3 to 4 hours of resazurin incubation.

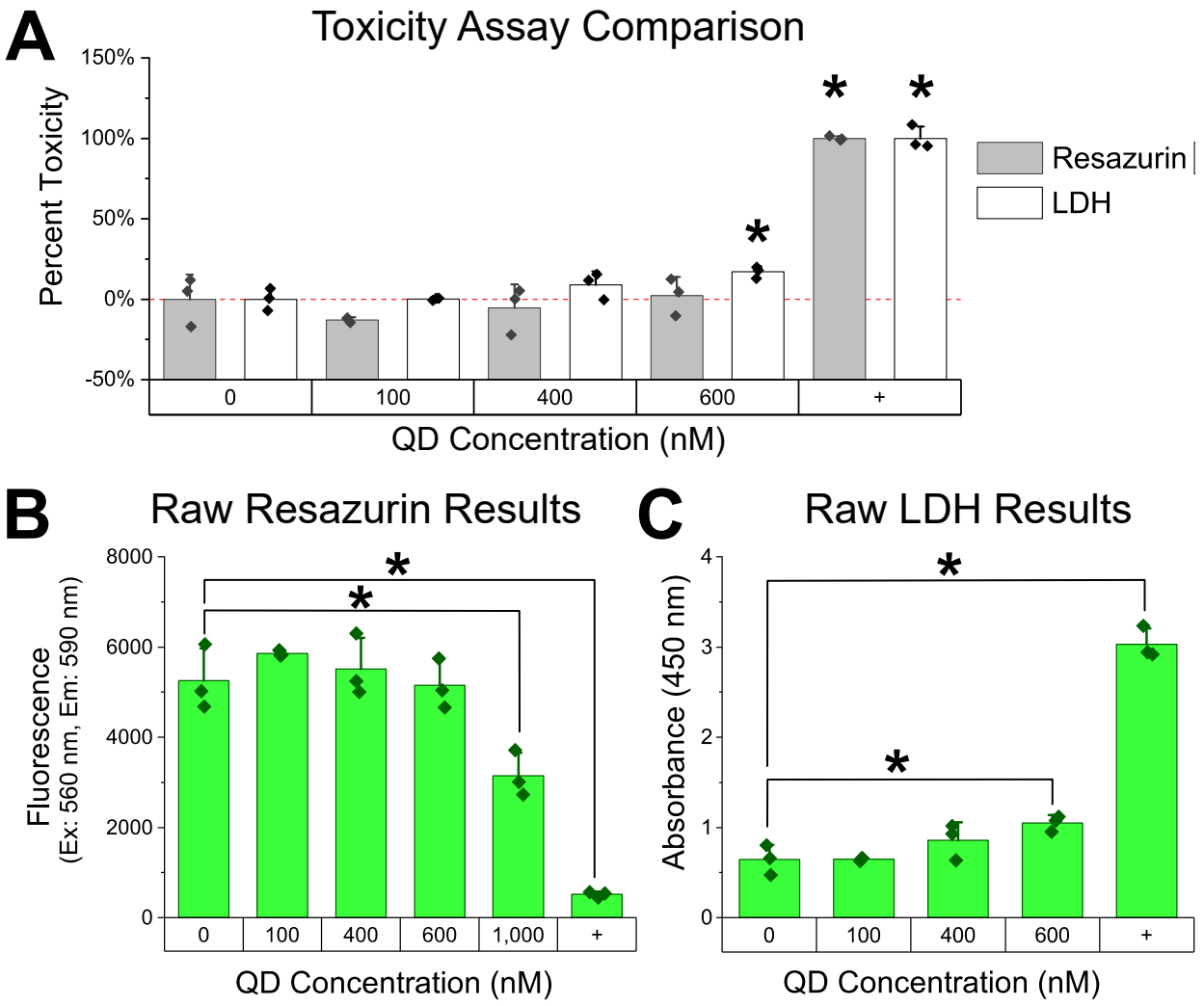

Figure S4 CdTe toxicity data and comparison. (A) Toxicity of CdTe-2.4 eV on preosteobast cells was tested using two assays: Lactate Dehydrogenase (LDH) and Resazurin. Both assays showed no statistically significant difference in percent toxicity of CdTe-2.4 eV compared to no treatment up to 400 nM. However, LDH assay showed statistically significant toxicity at 600 nM while Resazurin did not. This can be explained by the fact that percent toxicity is measured relative to each assay's positive and negative controls. Both negative controls are the same, no treatment, but for an LDH assay the positive control is induced toxicity by Triton addition and a Resazurin assay's

positive control is no cells, meaning that the Resazurin assay has definitive controls whereas the LDH assay's positive control may not represent full toxicity. Toxicity measurements were compared to their respective no treatment controls and significance indicated with an asterisk (*). (B) Raw fluorescence results from a Resazurin viability assay measuring the viability of the preosteoblast cells after 18 hours of incubation with light activated CdTe-2.4 eV. (C) Raw absorbance results from a LDH toxicity assay measuring the toxicity of the preosteoblast cells after 18 hours of incubation with light activated CdTe-2.4 eV. An asterisk (*) indicates a statistically significant difference (unpaired t-test, equal variance, $\alpha = 0.05$) to no treatment.

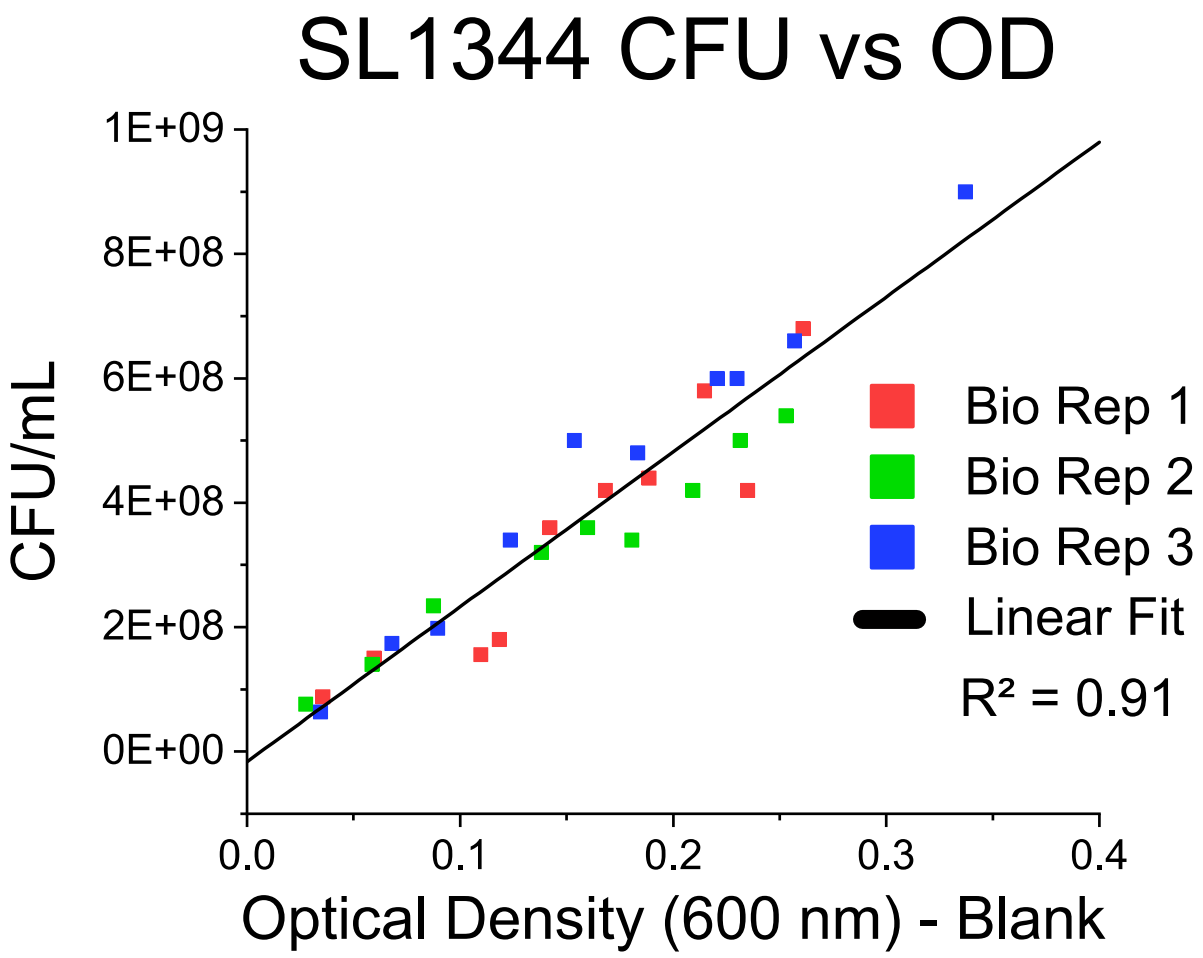

Figure S5 SL1344 optical density compared to CFU characterization. Optical density at 600 nm of SL1344 after 3 hours of regrowth from an overnight and dispersed in PBS, with PBS blank subtracted, was compared to the resulting CFU/mL to create a calibration that could be used to ensure the correct MOI was used. Three biological replicates of SL1344 were measured and a linear regression was performed on all three replicates showing a strong linear trend.

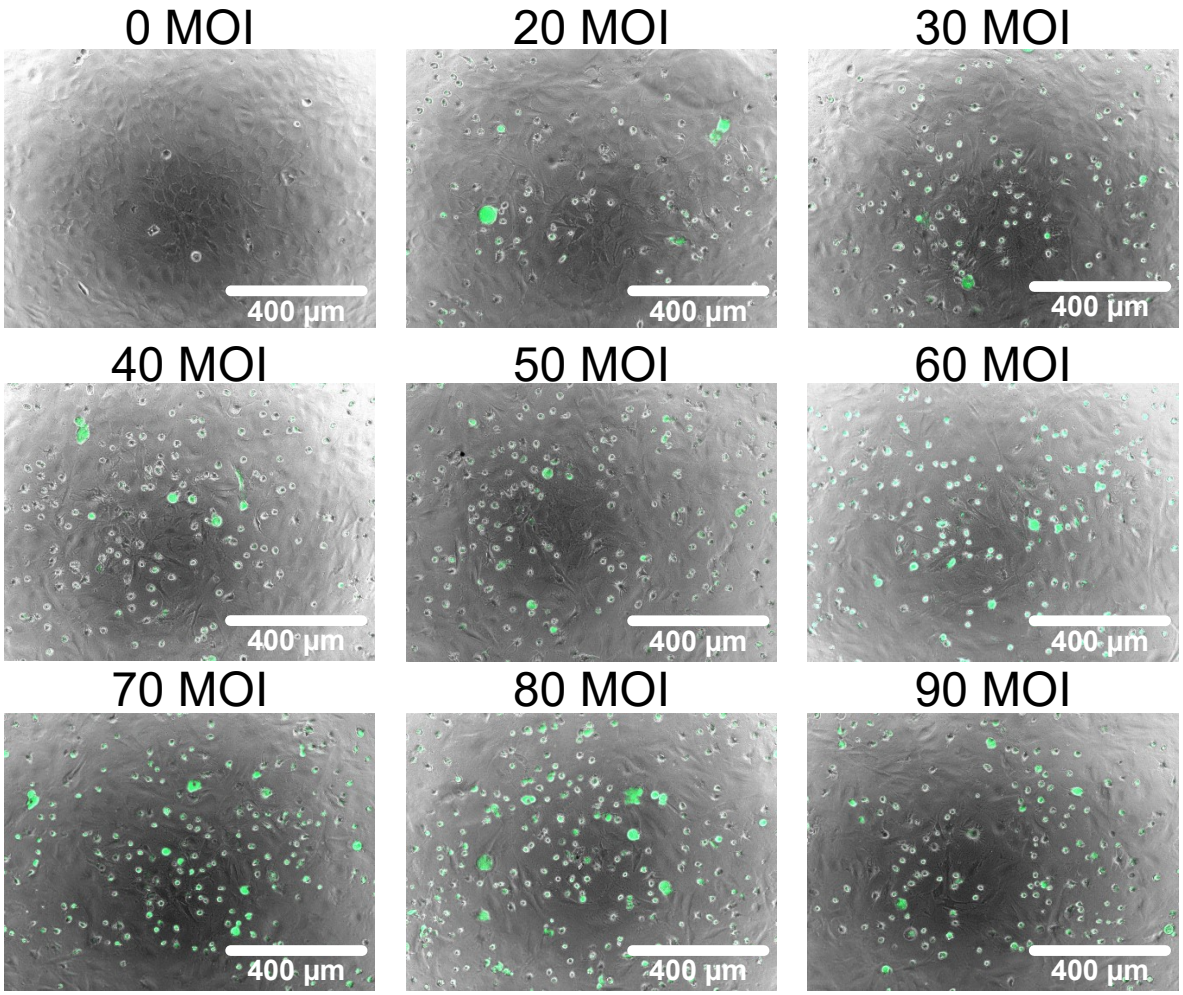

Figure S6 Microscopy of varying MOIs in preosteoblast cells. Confocal microscopy was performed after 18 hours of infection by SL1344 with varying MOIs. Due to SL1344 expressing GFP infected preosteoblast cells were visualized by imaging preosteoblast cells in bright field (grey) and overlaying the image with the GFP fluorescence from intracellular SL1344 (green).

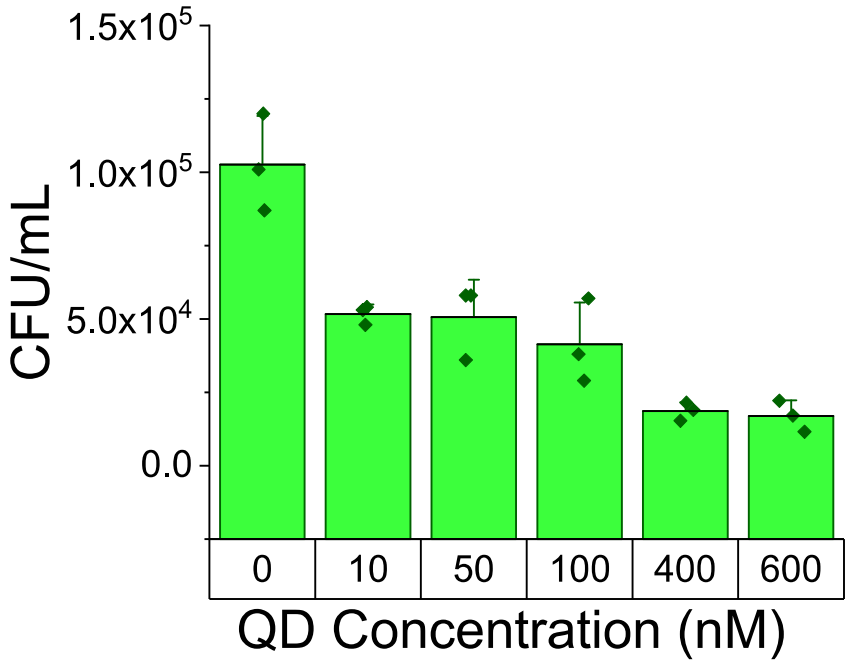

Figure S7 Raw CFU/mL of lysed SL1344 after CdTe treatment. Intracellular SL1344 were enumerated by CFU after 18 hours of treatment with varying concentrations of CdTe-2.4 eV.

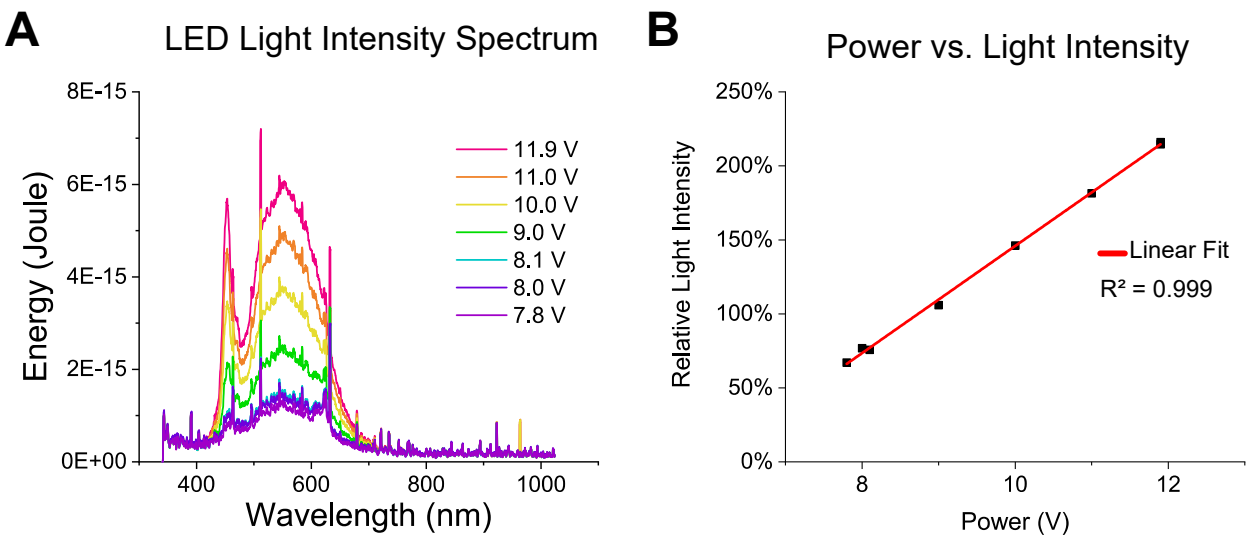

Figure S8 LED Sheet Light Intensity. (A) Light intensity (μW/cm^2) measurement of the LED sheet used to activate the nanoparticles at varying degrees of power. (B) Percent total light intensity, light intensity normalized to the light intensity at 8.7 V, compared to power shows a strong linear correlation. All experiments are conducted at a power of 8.7 V, corresponding to 100% light intensity, unless noted. Low light intensity was conducted at 7.8 V and 67% of normal light intensity.

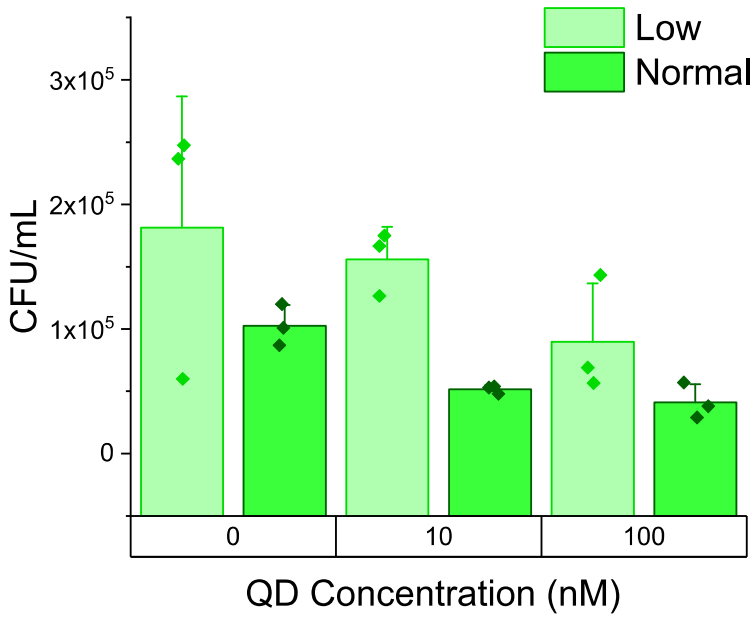

Figure S9 Raw CFU/mL of lysed SL1344 after varying light intensity treatment. Intracellular SL1344 were enumerated by CFU after 18 hours of treatment with varying concentrations of CdTe-2.4 eV. Low light intensity is approximately 67% the intensity of the normal light intensity used.