Supporting information

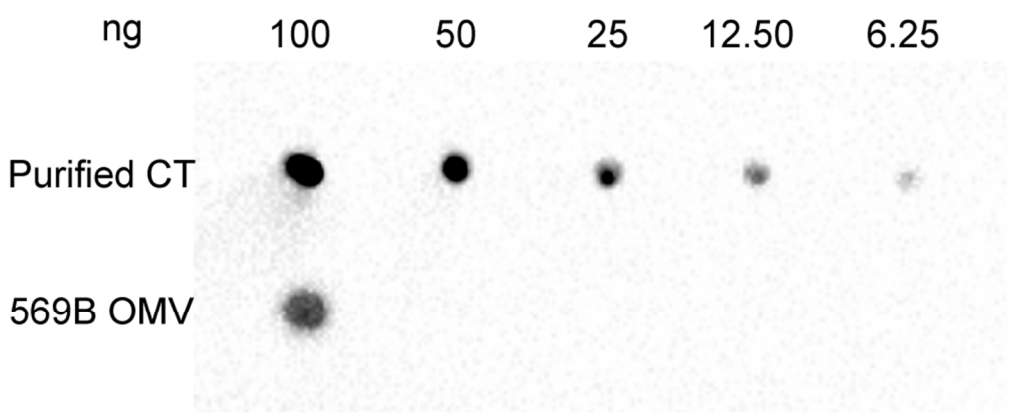
S1 Fig. Detection of CT in *V. cholerae* 569B OMVs. Dot blot analysis for determination of CT concentration in 569B OMVs using purified CT as a standard. The mass of CT present in 569B OMVs was calculated by comparing the intensity of the antibody-reactive spot from 569B OMVs to that of purified CT with known mass.

S2 Fig. Cytotoxicity of OMVs and OMV-free supernatant. Representative phase contrast image of FHs 74 intestinal epithelial cells treated with (i) OMVs and (ii) OMV-free supernatant.

Figure legends

FIGURE 1. Characterization of *V. cholerae* OMVs. (A) Scanning electron micrographs of *V. cholerae* 569B OMVs. (B) Western blot analysis of *V. cholerae* 569B OMVs. Two strong anti-CT polyclonal antibody-reactive bands were detected, demonstrating the presence of CT in the OMVs. (C) Relative CT concentration in 569B OMVs and OMV-free supernatant. The mass of CT present in purified OMVs or the OMV-free supernatant was determined by comparing the intensity of an antibody-reactive spot to that of CT standards with known mass.

FIGURE 2. *V. cholerae* 569B OMV-mediated cytotoxicity. (A) Representative phase contrast image of OMV-treated FHs 74 intestinal epithelial cells. (i) Untreated cells and (ii) OMV-treated cells (0.4 ng/ μL of CT, 4 hr). Enlarged view of the regions in small boxes are shown in the insets. (B) Quantitative analysis of the OMV-treated cell morphology. Cells were treated with a serial dilution of 569B OMVs (5 to 40 ng CT/well), and the resulting morphology scored on a scale of 1 (spindly) to 4 (rounded), based on the percentage of cell rounding. Data are expressed as mean ± SD (N=3). One-way ANOVA followed by Bonferroni's post hoc test was used to compare differences between treated and untreated samples. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.



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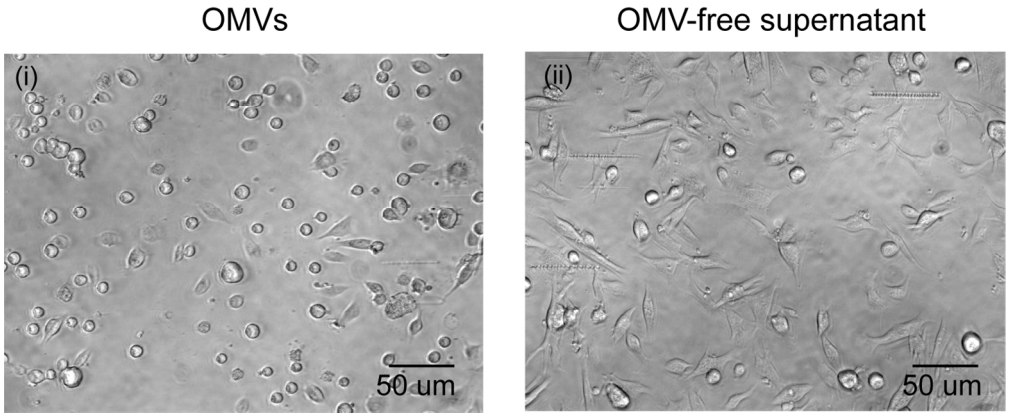

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