Supporting information

Bacterial Toxicity of Germanium Nanocrystals Induced by Doping with Boron and Phosphorous

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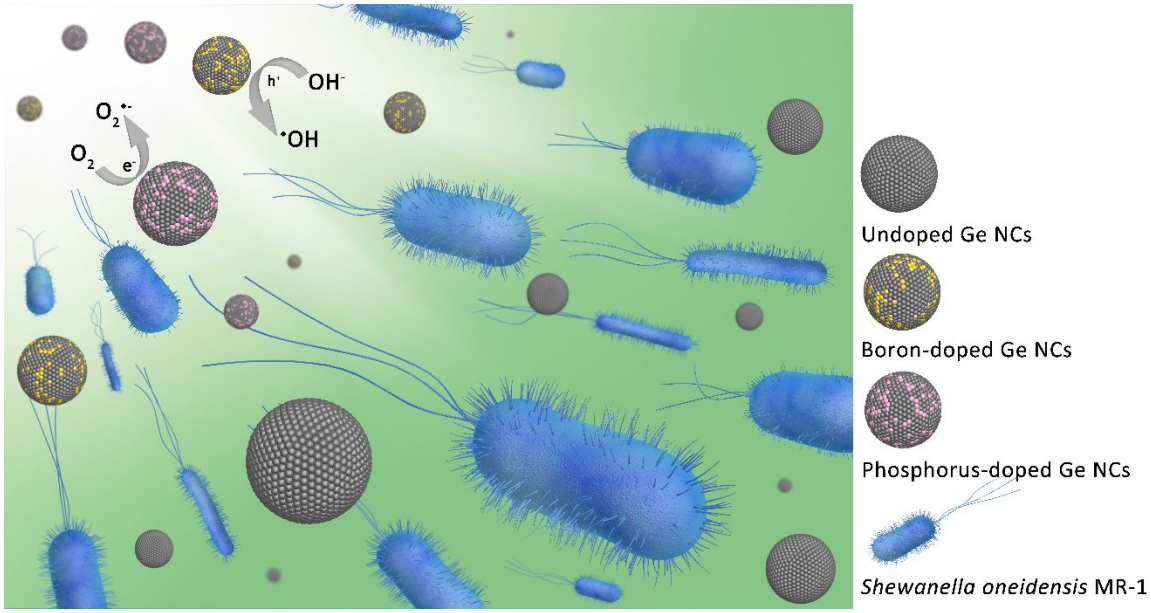

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Supporting information list

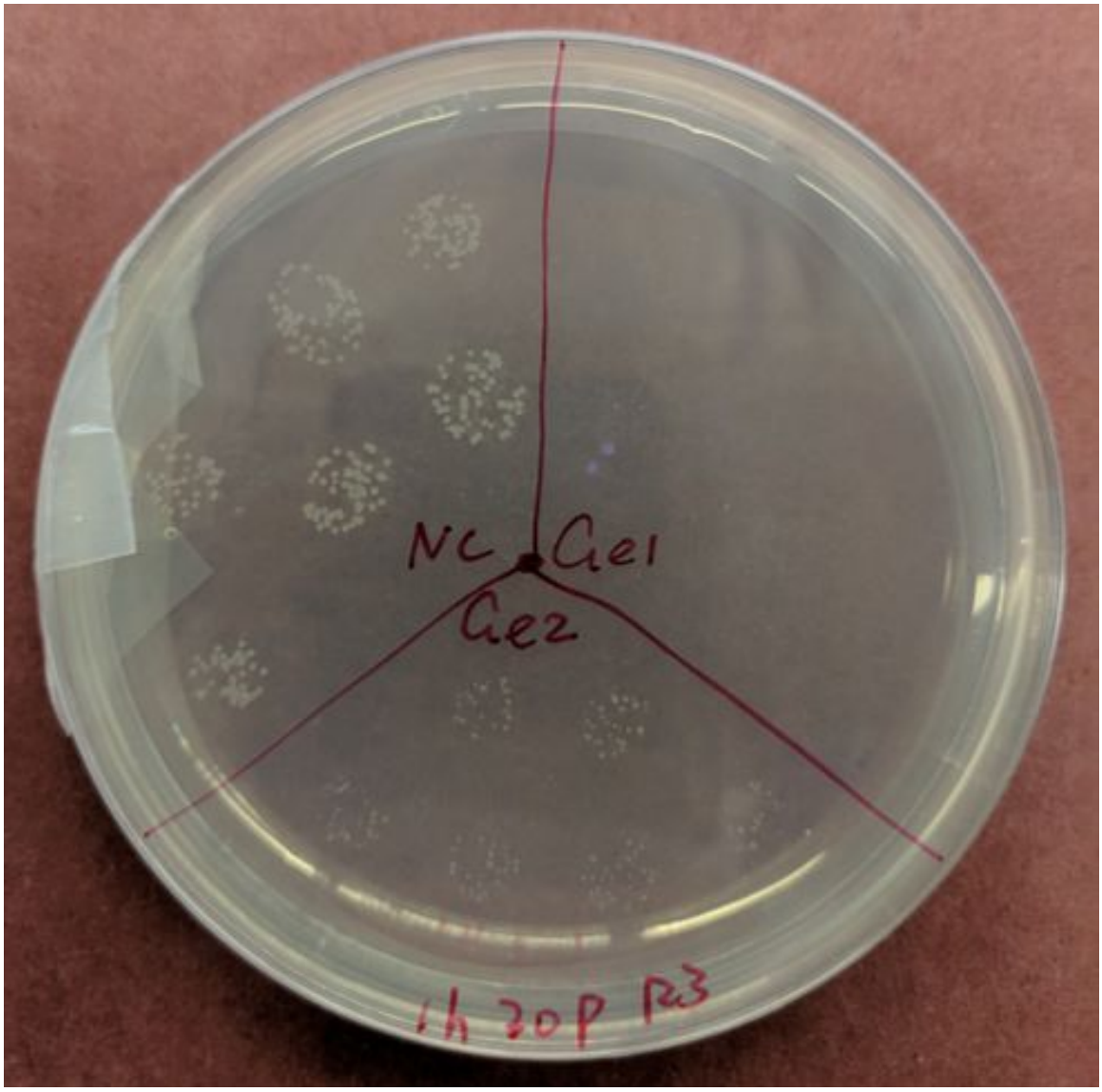

Figure S1. Photographic image representing the layout of drop plate colony counting for *S. oneidensis*. Each small white/gray drop on the plate is a bacterial colony.

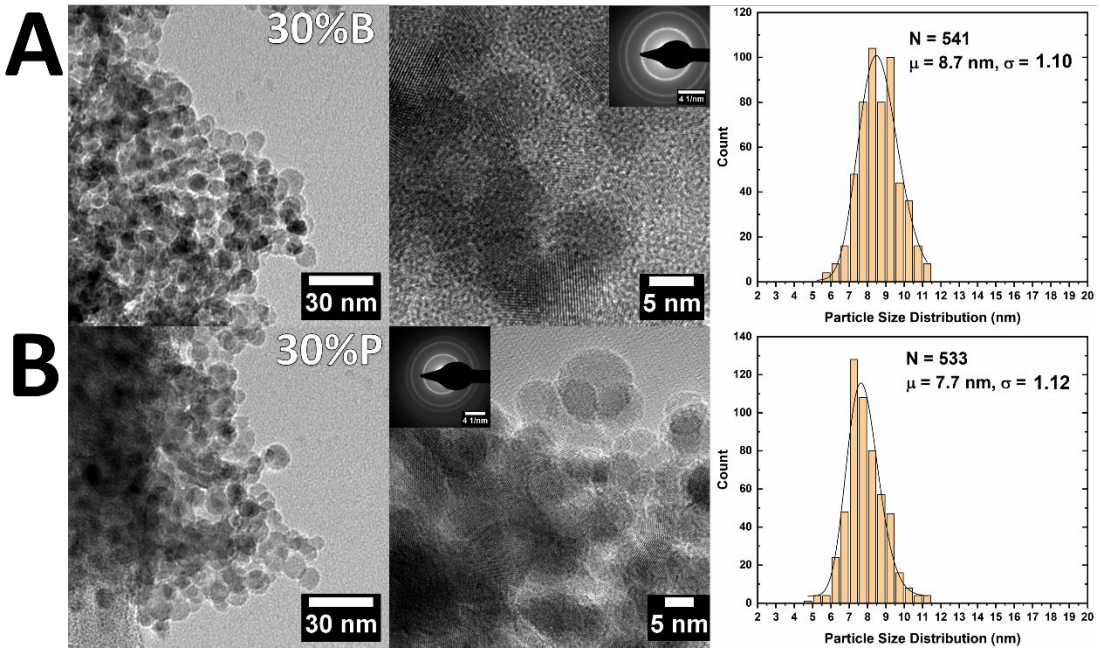

Figure S2. TEM images and size analysis of 30% B-doped Ge NCs and 30% P-doped Ge NCs with N = 500+ particles counted.

Table S1. *d*-spacing values of undoped Ge NCs (calculated using the SAED pattern).

Ring radius (nm^{-1})	***d*-spacing (nm)**	***(hkl)***
3.021	**0.331**	(111)
5.124	0.195	(220)
5.989	0.167	(311)

Table S2. *d*-spacing values of 10% B-doped Ge NCs (calculated using the SAED pattern).

Ring radius (nm^{-1})	***d*-spacing (nm)**	***(hkl)***
3.135	0.319	(111)
5.291	0.189	(220)
6.211	0.161	(311)

Table S3. *d*-spacing values of 30% B-doped Ge NCs (calculated using the SAED pattern).

Ring radius (nm^{-1})	***d*-spacing (nm)**	***(hkl)***
3.031	0.330	(111)
5.076	0.197	(220)
5.780	0.173	(311)

Table S4. *d*-spacing values of 10% P-doped Ge NCs (calculated using the SAED pattern).

Ring radius (nm^{-1})	***d*-spacing (nm)**	***(hkl)***
3.049	0.328	(111)
4.926	0.203	(220)
5.747	0.174	(311)

Table S5. *d*-spacing values of 30% P-doped Ge NCs (calculated using the SAED pattern).

Ring radius (nm^{-1})	***d*-spacing (nm)**	***(hkl)***
3.077	0.325	(111)
5.464	0.183	(220)
5.883	0.170	(311)

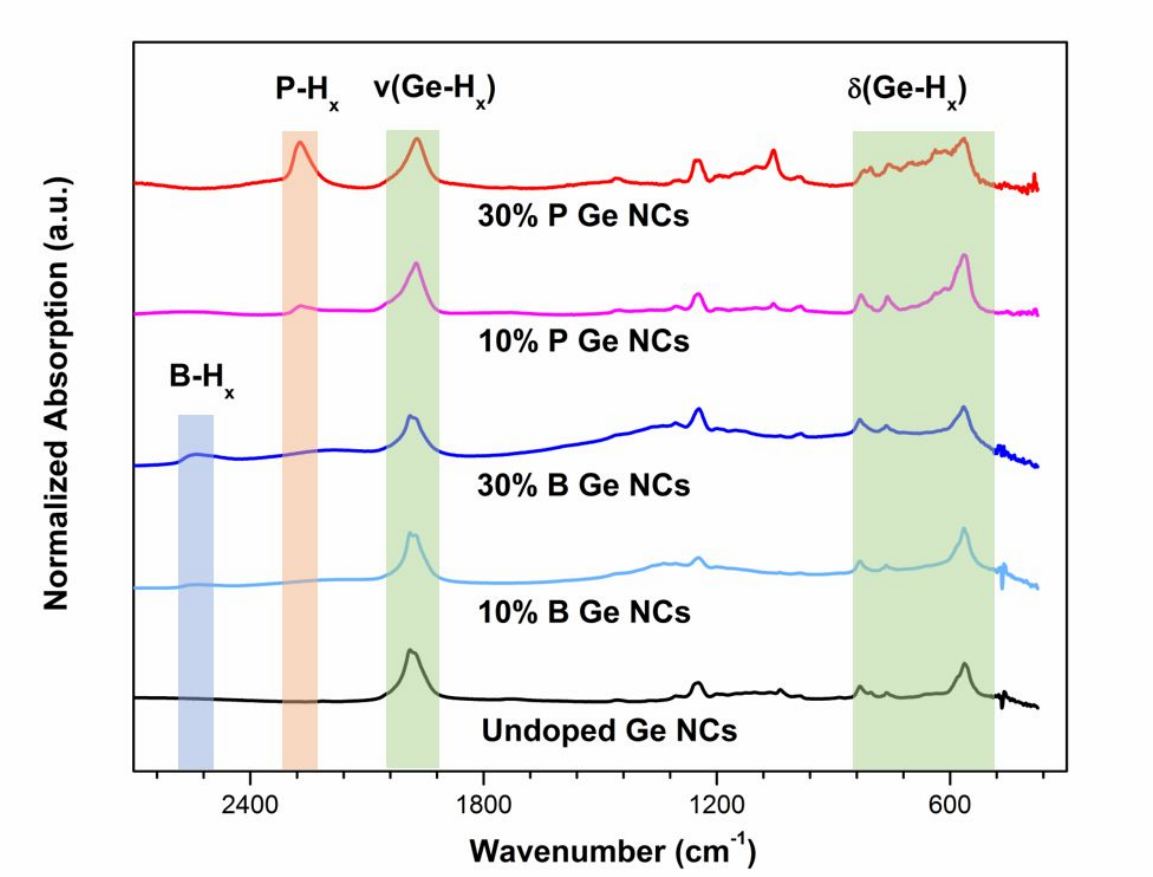

Figure S3. FTIR spectra for freshly synthesized Ge NCs.

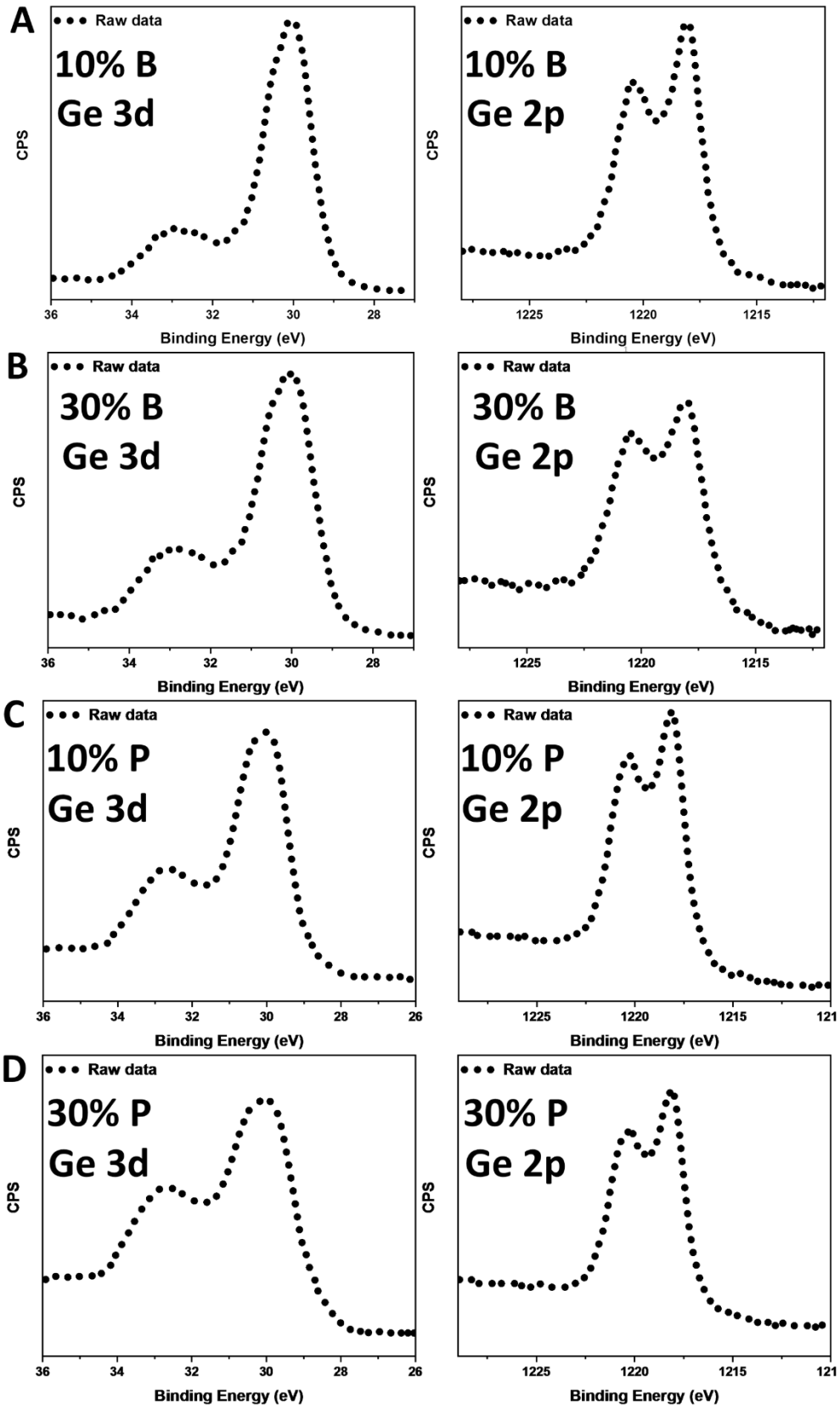

Figure S4. High-resolution XPS spectra for doped Ge NCs.

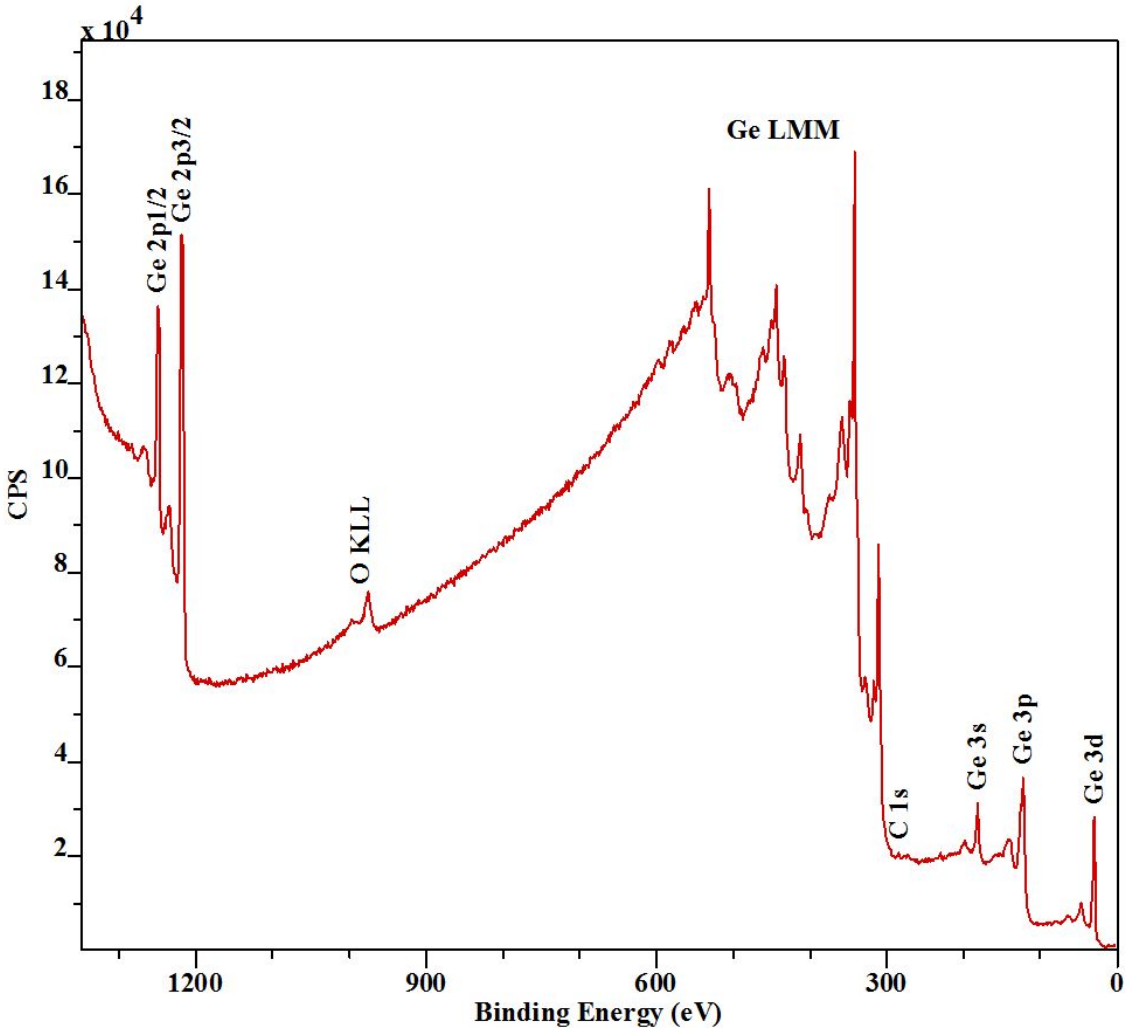

Figure S5. XPS survey spectrum for undoped Ge NCs.

It is challenging to assess the atomic percentage of oxygen, boron, and phosphorus because Ge LMM peak groups (300-600 eV) overlap with O1s (531 eV).

Similarly, for doped Ge NCs, it is also difficult to gauge boron and phosphorus incorporation because Ge3s (180 eV) overlaps with B1s (188 eV) and P2s (189 eV) while Ge 3p (120-125 eV) overlaps with P2p (135-136 eV).

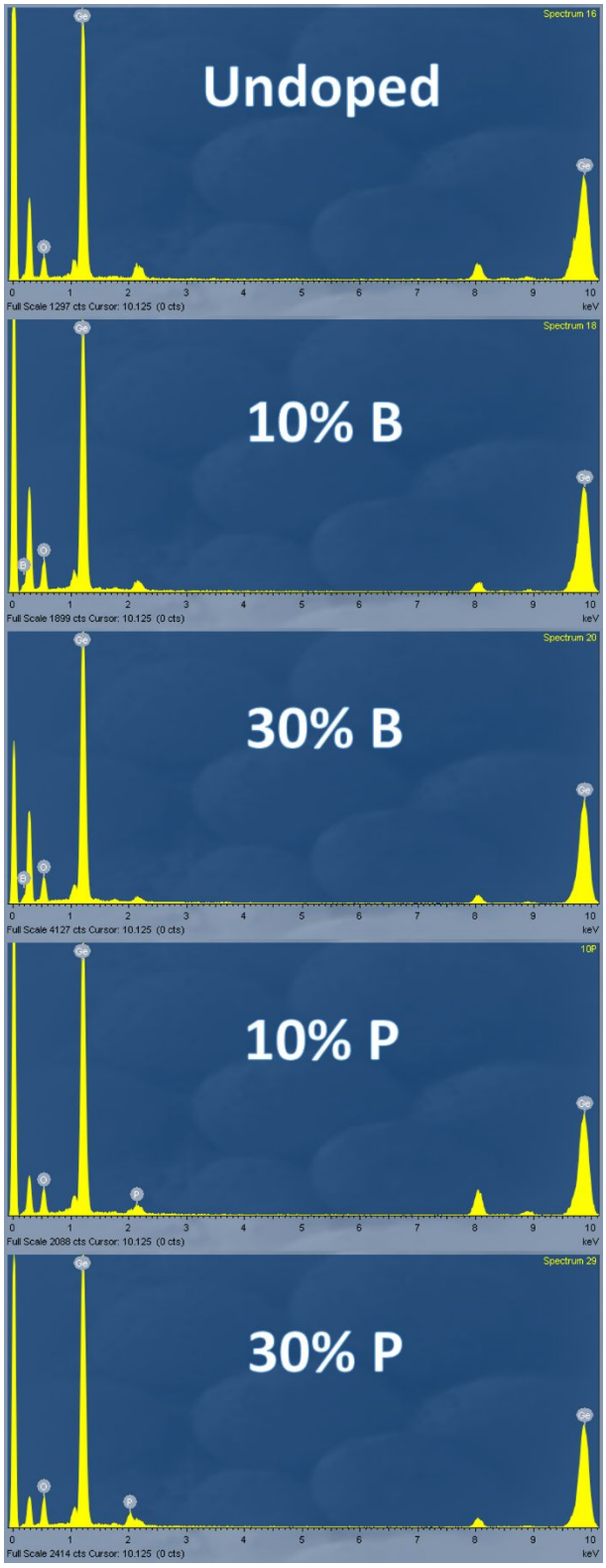

Figure S6. Boron and phosphorus doping levels of Ge NCs evaluated by EDS (Energy dispersive x-ray spectroscopy).

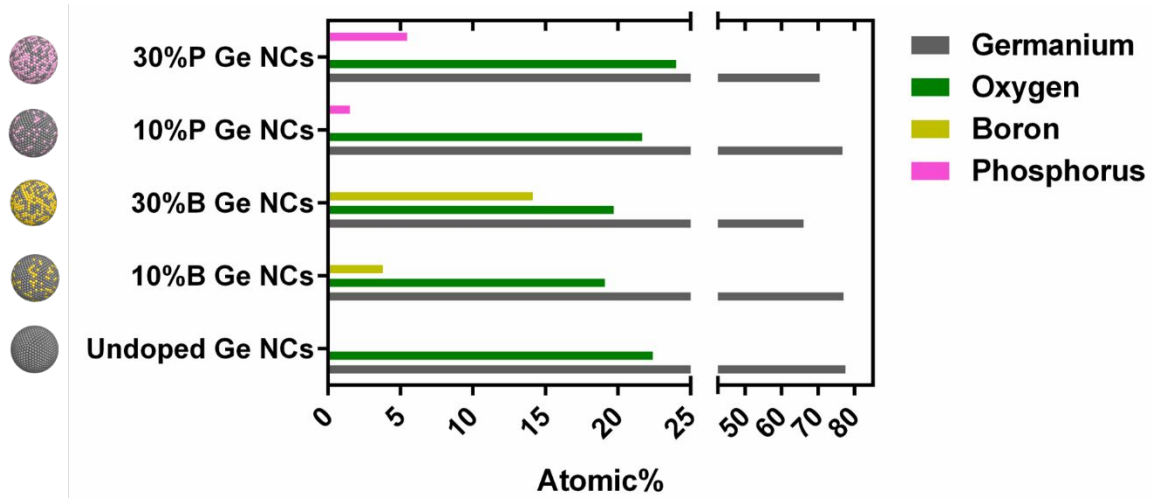

Figure S7. Comparison of doping levels of Ge NCs.

Table S6. Atomic percentage summary of Ge NCs.

Atomic% (N = 4 or 5)	Germanium	Oxygen	Boron	Phosphorus
Undoped Ge NCs	77.6 ± 1.1	22.4 ± 1.1	N/A	N/A
10%B Ge NCs	77.1 ± 3.9	19.1 ± 3.7	3.8 ± 0.7	N/A
30%B Ge NCs	66.1 ± 1.8	19.7 ± 1.8	14.1 ± 1.9	N/A
10%P Ge NCs	76.8 ± 0.7	21.7 ± 0.6	N/A	1.5 ± 0.1
30%P Ge NCs	70.5 ± 0.3	24.0 ± 0.5	N/A	5.5 ± 0.2

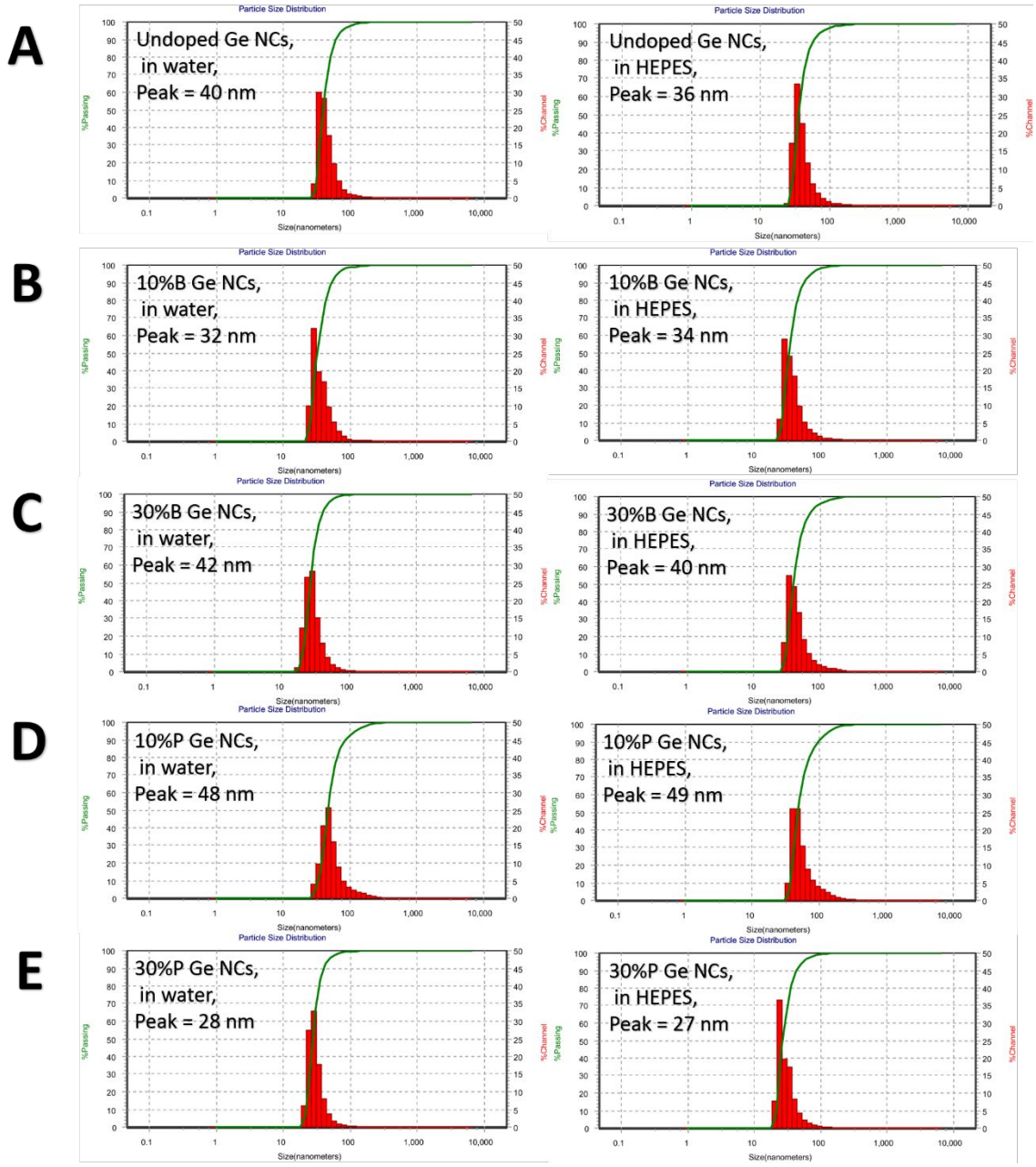

Figure S8. Dynamic light scattering (DLS) results for Ge NCs in both water (left) or HEPES buffer (right).

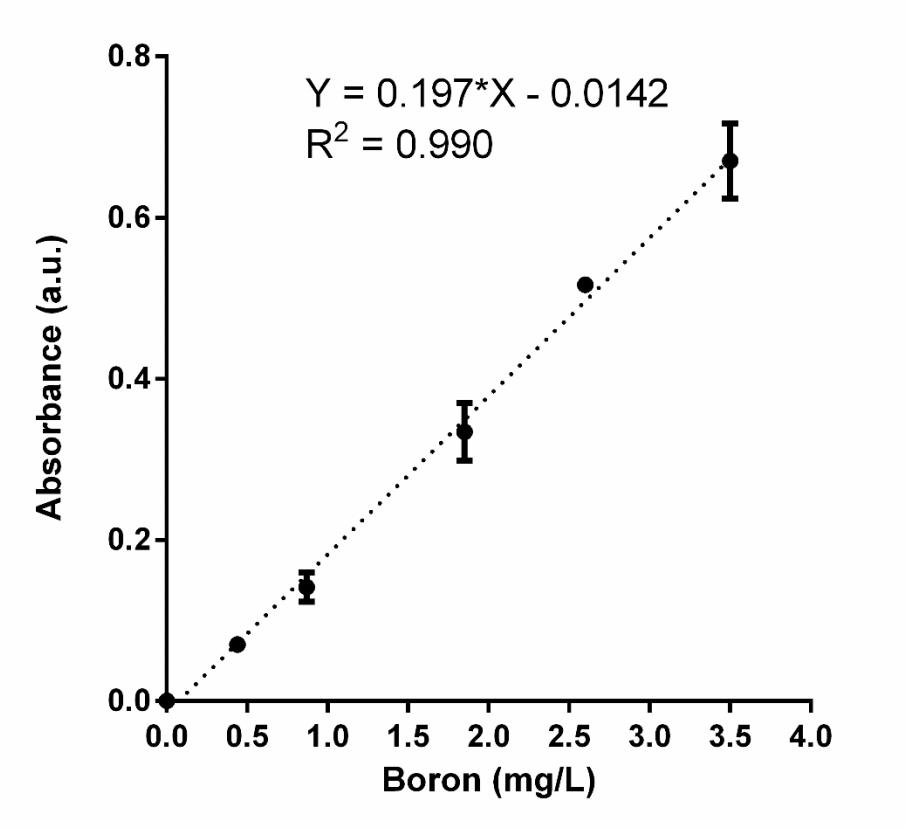

Figure S9. Calibration curve to determine the concentration of boron. Curcumin reacts with borates to form red colored rosocyanine, which absorbs at 540 nm. (n = 3)

Table S7. Boron standards conversion.

Boric acid mg/L	Boron mg/L
0	0
2.5	0.4
5.0	0.9
10.0	1.85
15.0	2.6
20.0	3.5

Based on the calibration equation (Y = 0.197*X - 0.0142), it can be back-calculated that for 10% B-doped Ge NCs (1000 mg/L), the supernatant boron concentration is 1.5 ± 0.2 mg/L while for 30% B-doped Ge NCs (1000 mg/L), it is 3.5 ± 0.1 mg/L.

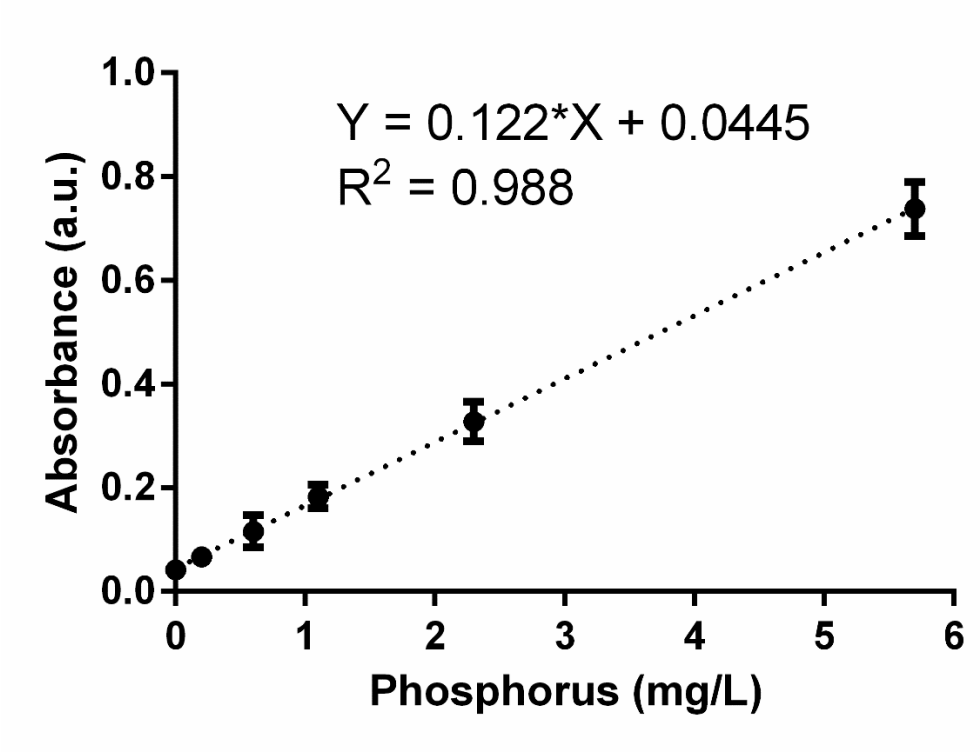

Figure S10. Calibration curve to determine the concentration of phosphorus. Antimony potassium tartrate and ammonium molybdate react with orthophosphate to form an antimony-phospho-molybdate complex, which can be reduced to a blue complex by ascorbic acid. Its absorbance is measured at 670 nm. (n = 3)

Table S8. Phosphorus standards conversion

KH_2PO_4 mg/L	Phosphorus mg/L
0	0
1	0.2
2.5	0.6
5	1.1
10	2.3
25	5.7

Based on the calibration equation (Y = 0.122*X + 0.0445), it can be back-calculated that for 10% P-doped Ge NCs (1000 mg/L), the supernatant phosphorus concentration is 1.8 ± 0.1 mg/L while for 30% P-doped Ge NCs (1000 mg/L), it is 2.3 ± 0.1 mg/L.

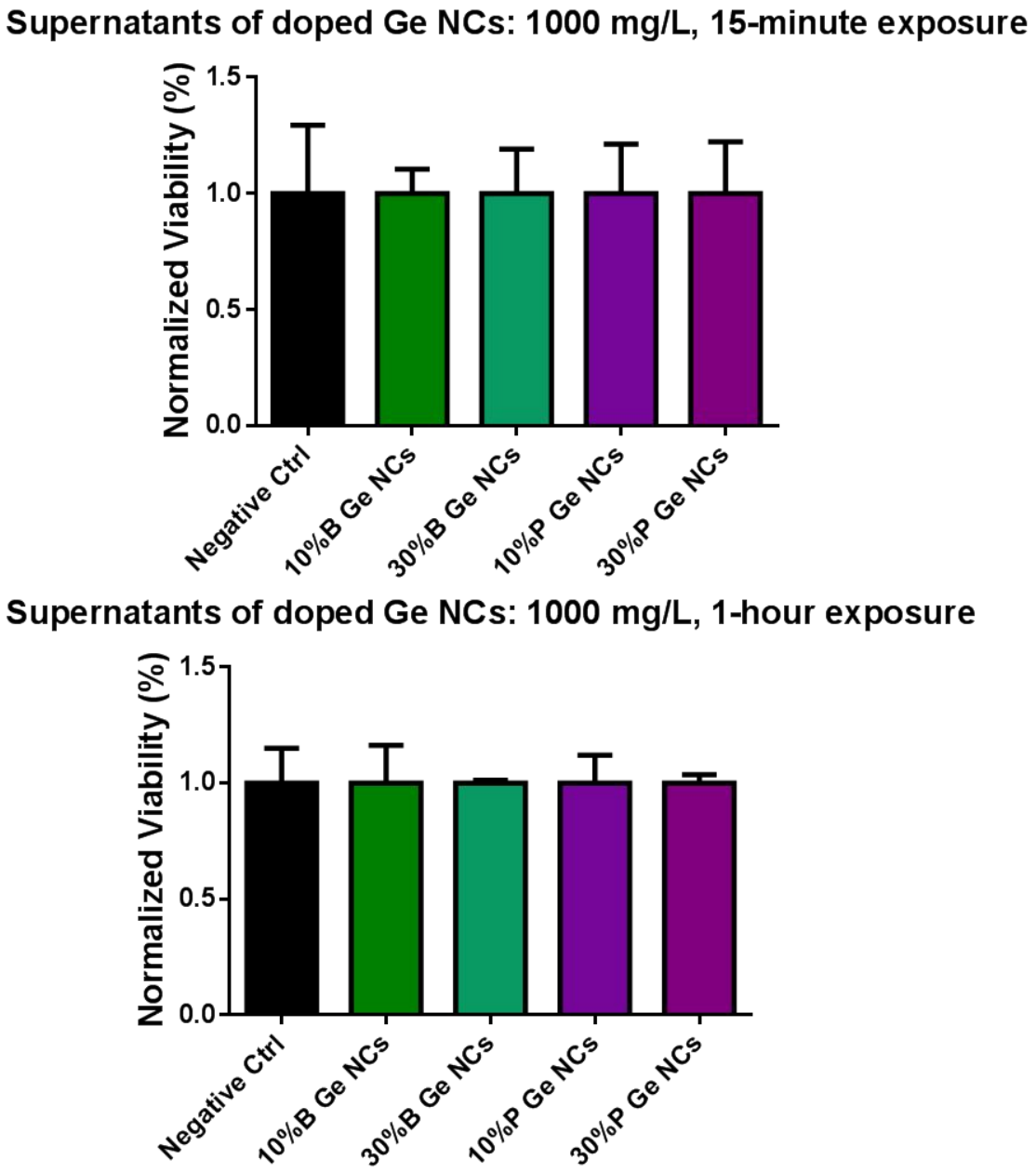

Figure S11. Colony counting experiments using the supernatants of Ge NCs. There are no significant differences among the different experimental conditions.

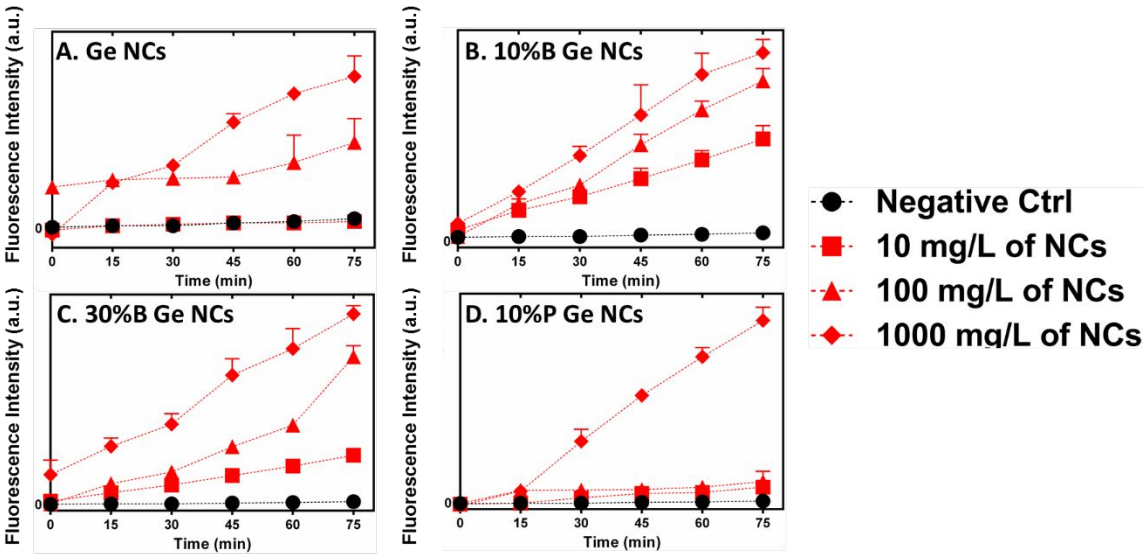

Figure S12. Enlarged views for the abiotic ROS generation of varied Ge NCs, determined by the DCFDA assay (incubation time 1.25 h, n= 3).

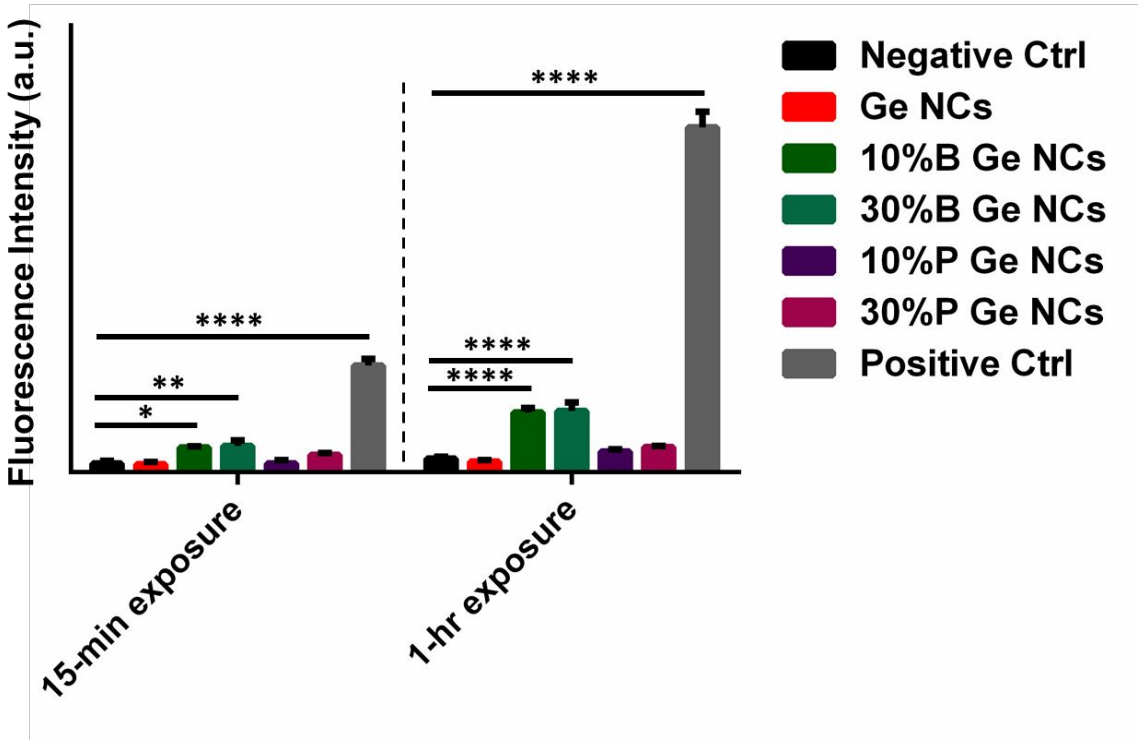

Figure S13. Hydroxyl radical generation at 15-minute and 1-hour time intervals, as measured by cell-free HPF assays. The asterisks indicate statistical significance level: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (examined by two-way ANOVA, followed by Sidak's multiple comparison tests, n = 3).

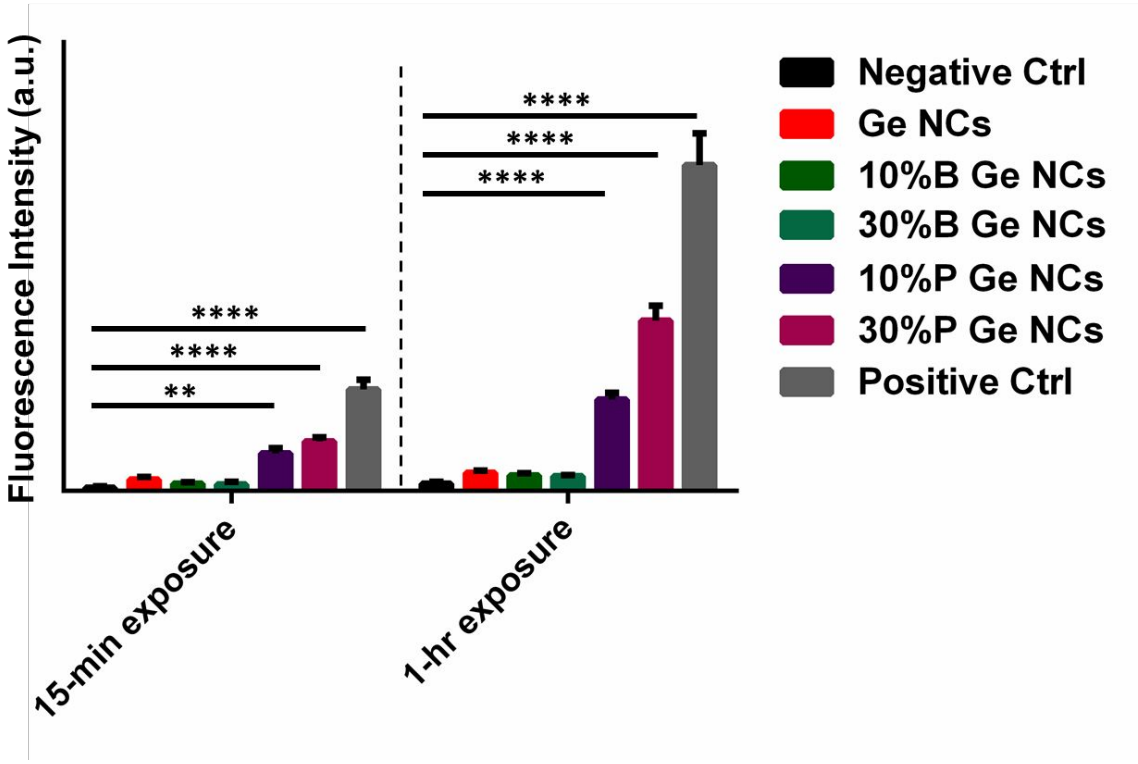

Figure S14. Superoxide radical generation at 15-minute and 1-hour time intervals, as measured by DHE assays. The asterisks indicate statistical significance level: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (examined by two-way ANOVA, followed by Sidak's multiple comparison tests, n = 3).

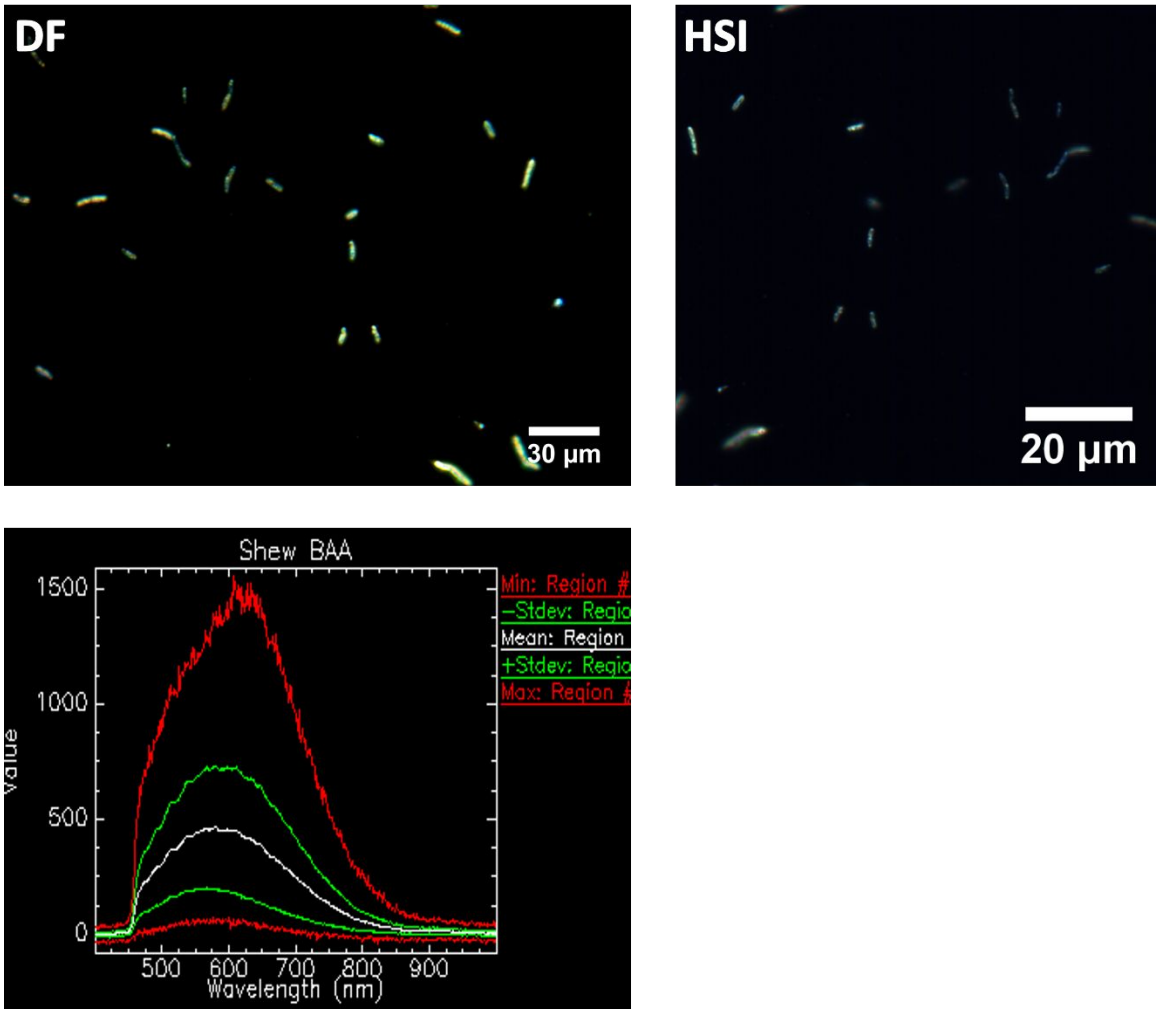

Figure S15. Cytoviva analysis of *S. oneidensis* cells: dark-field (DF) image, hyperspectral (HSI) image, and spectral library for *S. oneidensis* cells, generated using the region-of-interest (ROI) tool.

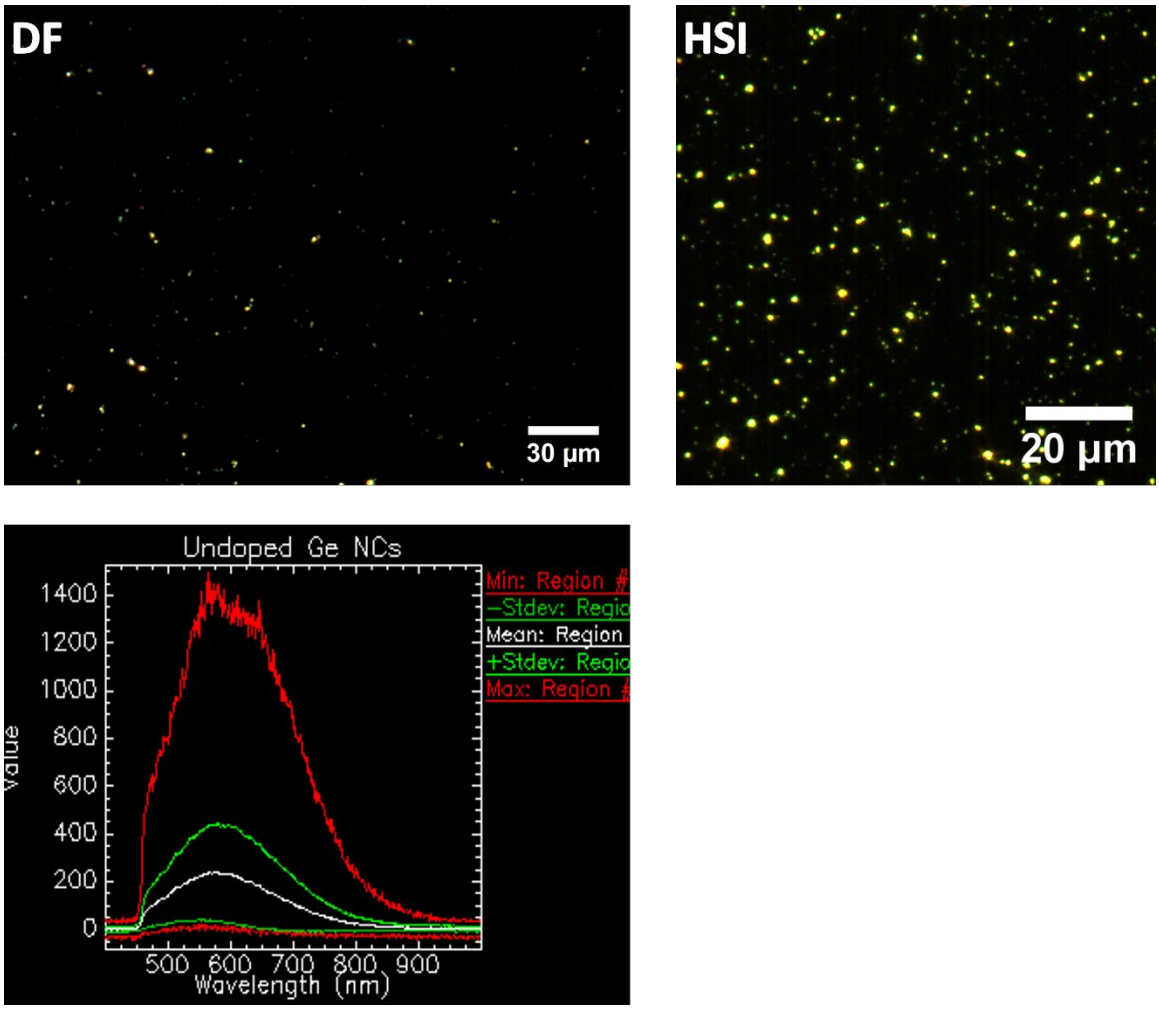

Figure S16. Cytoviva analysis of undoped Ge NCs: dark-field (DF) image, hyperspectral (HSI) image, and spectral library for undoped Ge NCs, generated using the ROI tool.

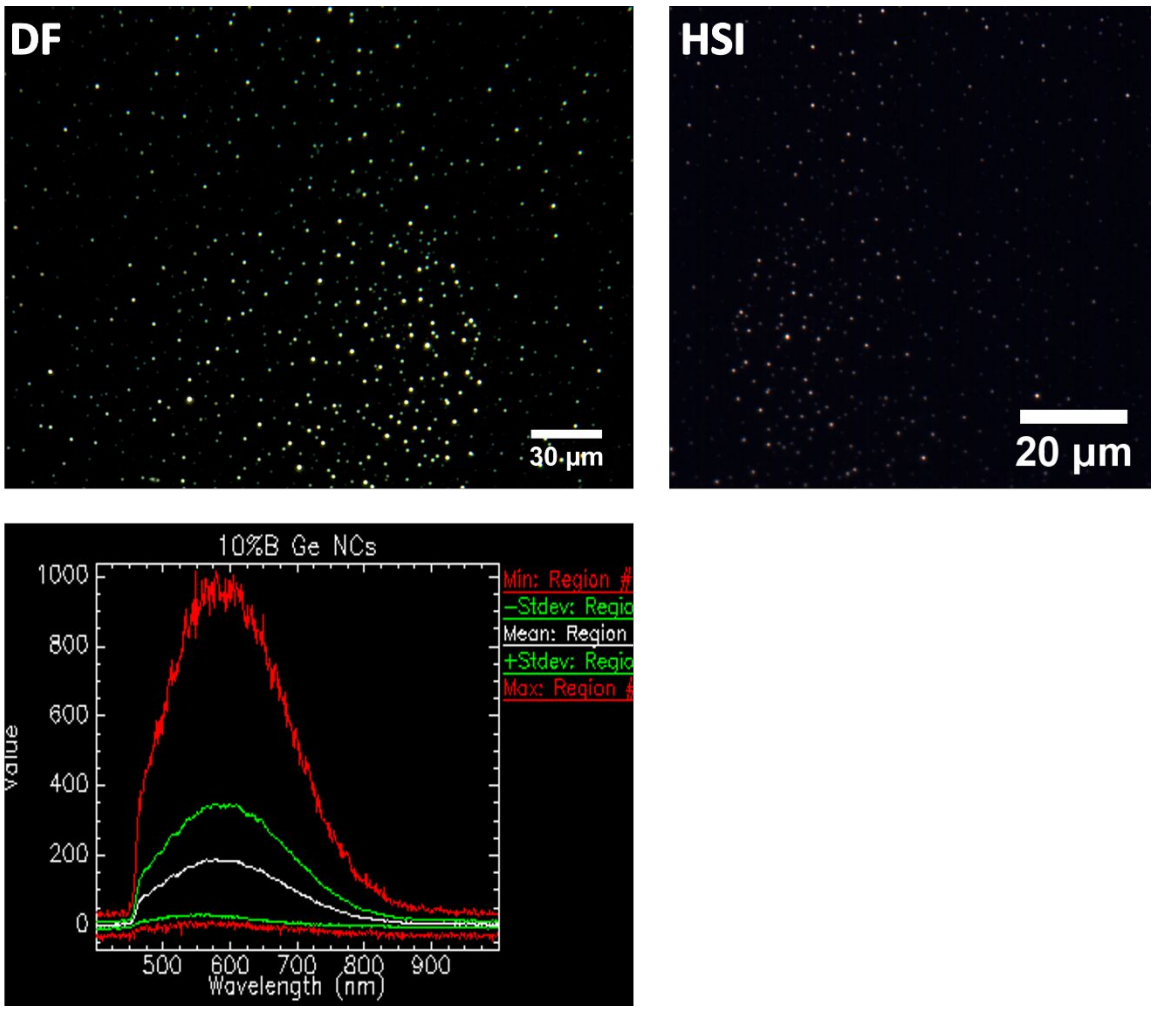

Figure S17. Cytoviva analysis of 10% B-doped Ge NCs: dark-field (DF) image, hyperspectral (HSI) image, and spectral library for 10% B-doped Ge NCs, generated using the ROI tool.

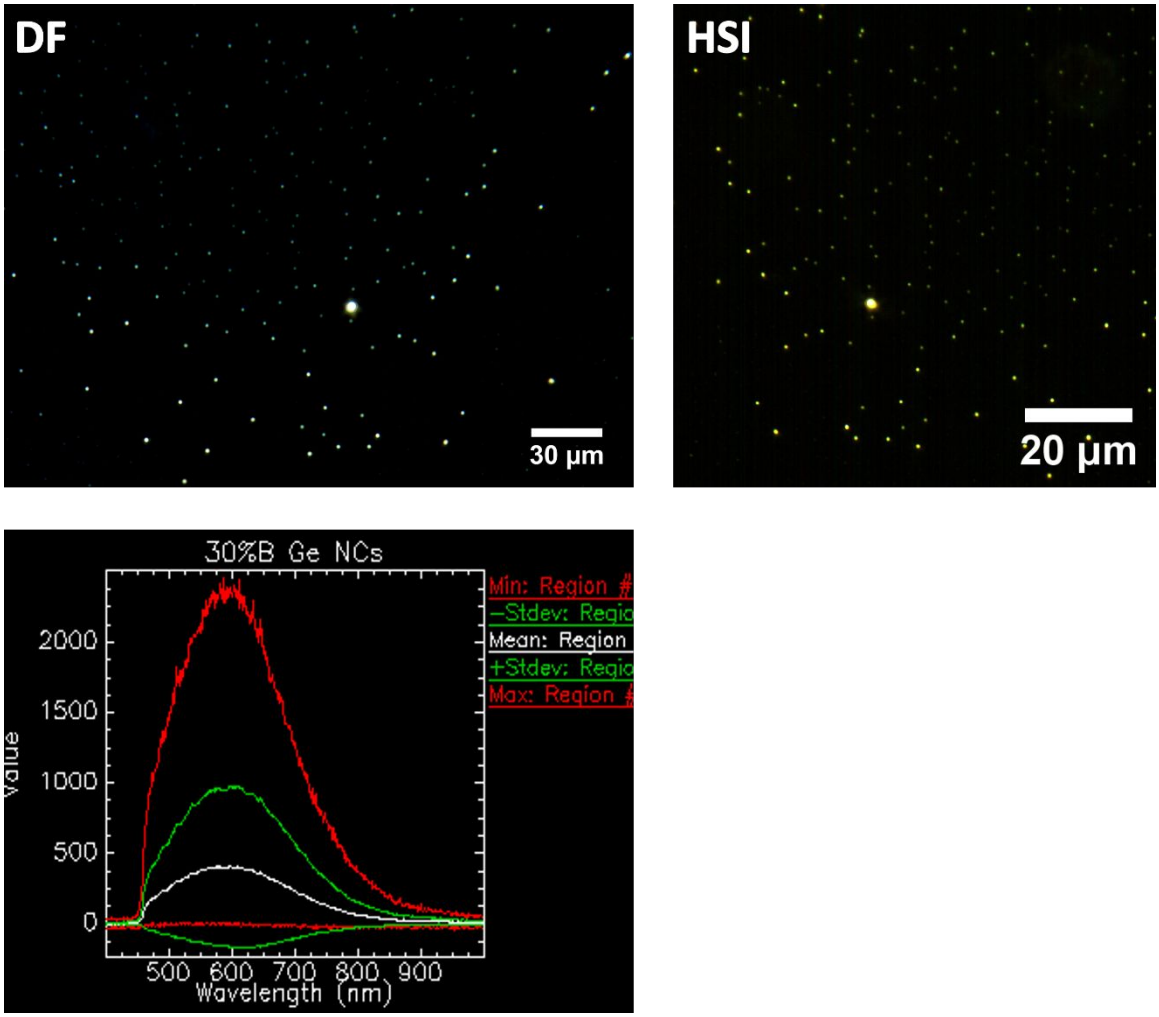

Figure S18. Cytoviva analysis of 30% B-doped Ge NCs: dark-field (DF) image, hyperspectral (HSI) image, and spectral library for 30% B-doped Ge NCs, generated using the ROI tool.

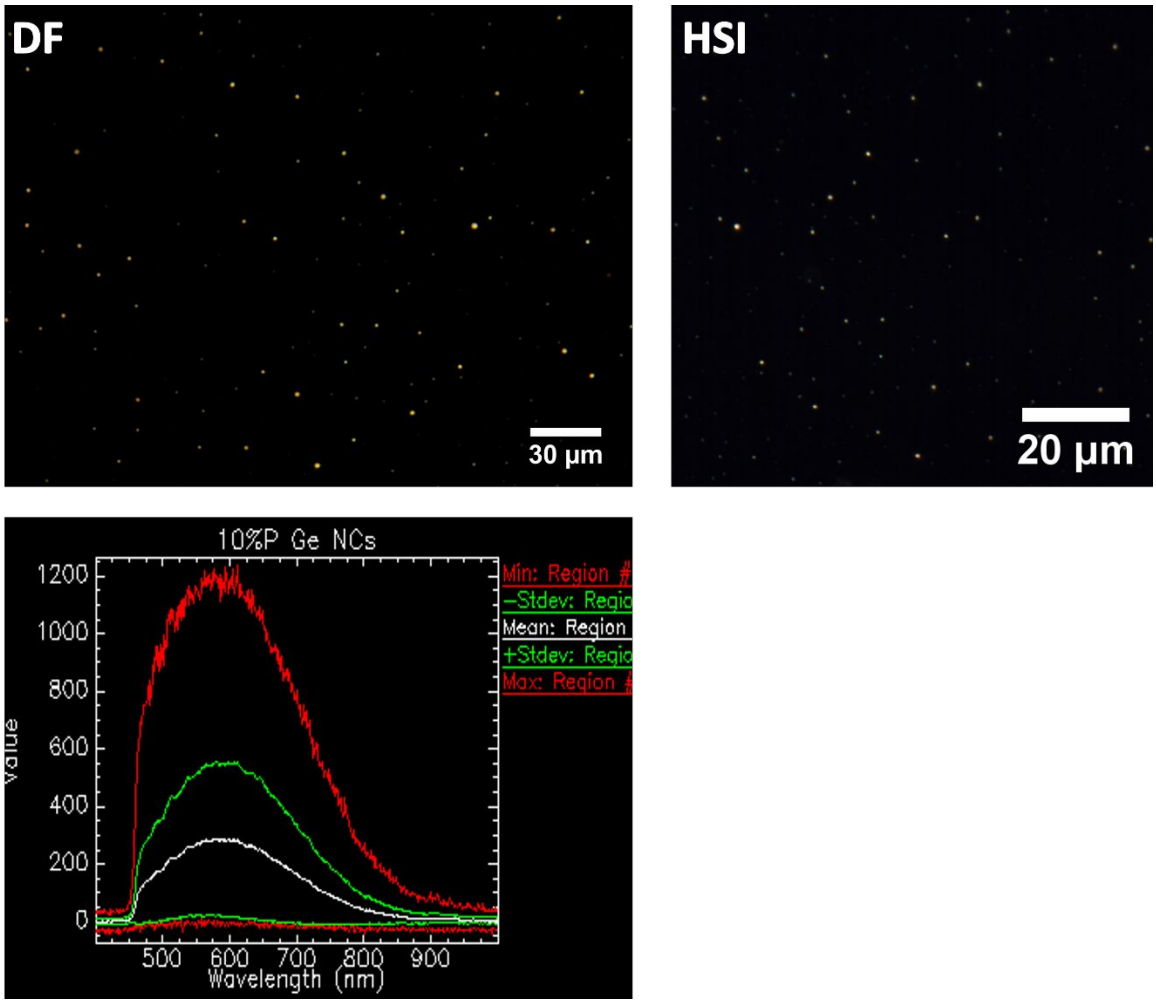

Figure S19. Cytoviva analysis of 10% P-doped Ge NCs: dark-field (DF) image, hyperspectral (HSI) image, and spectral library for 10% P-doped Ge NCs, generated using the ROI tool.

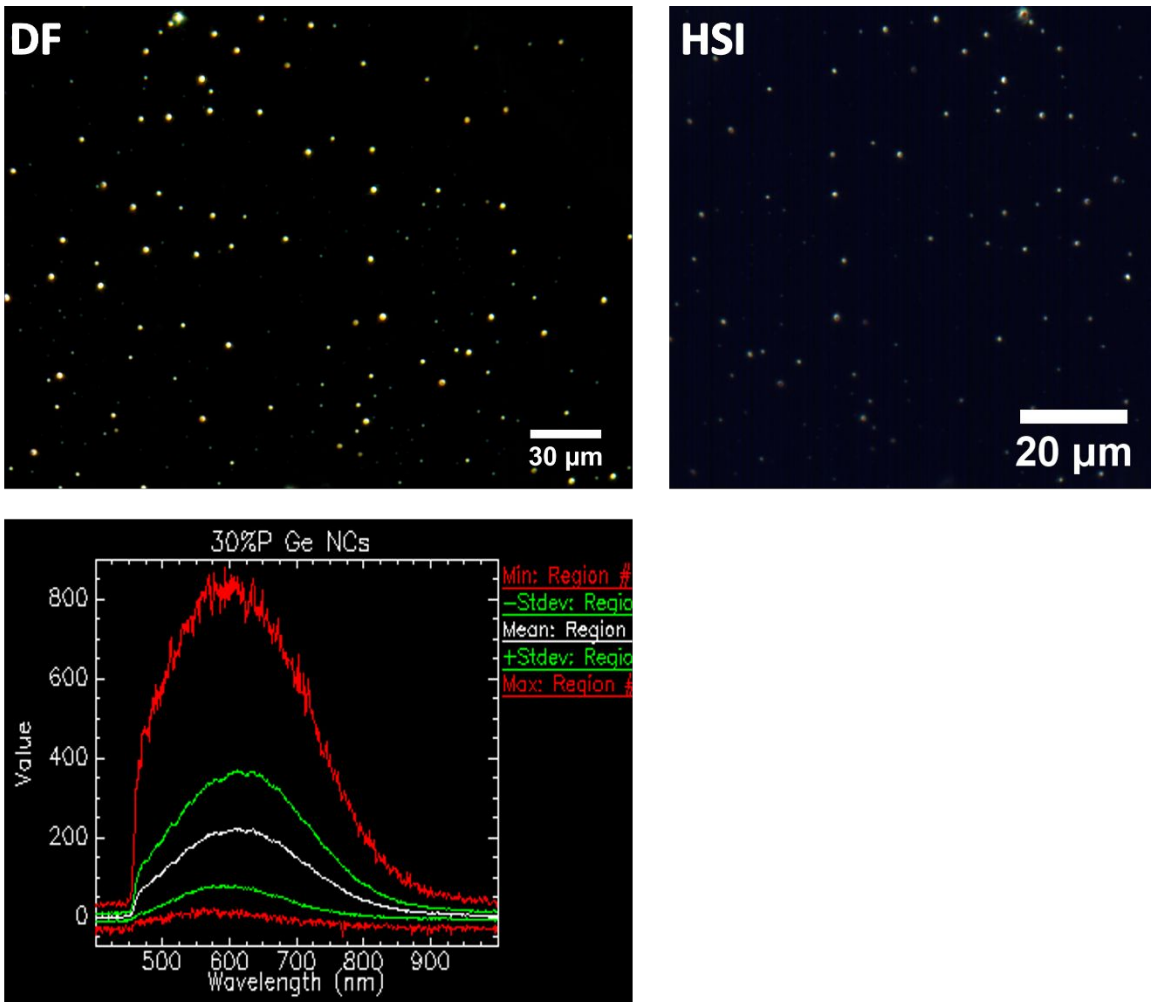

Figure S20. Cytoviva analysis of 30% P-doped Ge NCs: dark-field (DF) image, hyperspectral (HSI) image, and spectral library for 30% P-doped Ge NCs, generated using the ROI tool.

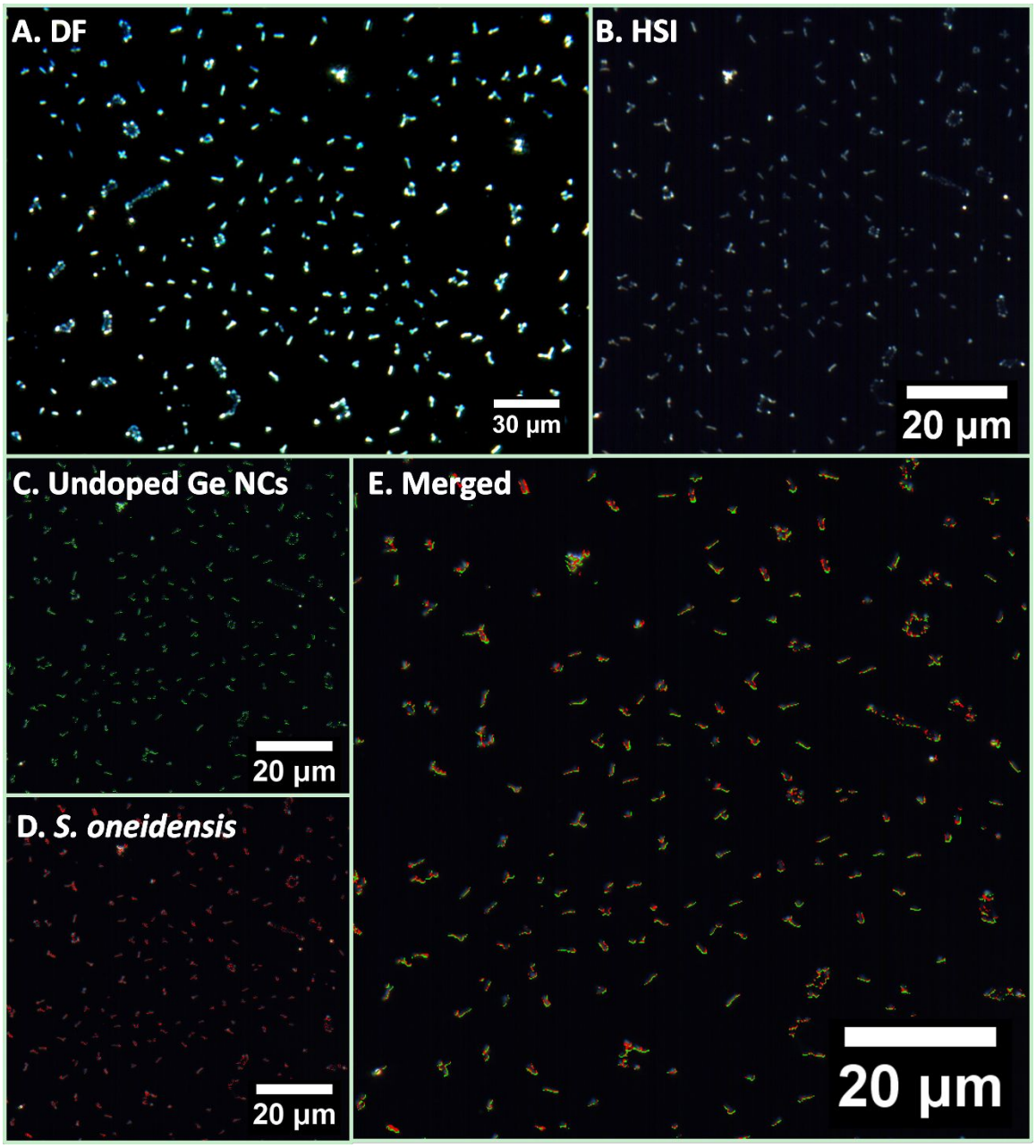

Figure S21. Enhanced dark-field analysis of the association between *S. oneidensis* cells with undoped Ge NCs: (A) dark-field image of bacteria exposure solution, (B) hyperspectral image of region of interest (ROI) in A. Mapping (C) undoped Ge NCs alone and (D) *S. oneidensis* cells alone in the hyperspectral data. (E) Merged mapping results of (C) and (D) where bacteria cells are false-colored red while undoped Ge NCs with green.

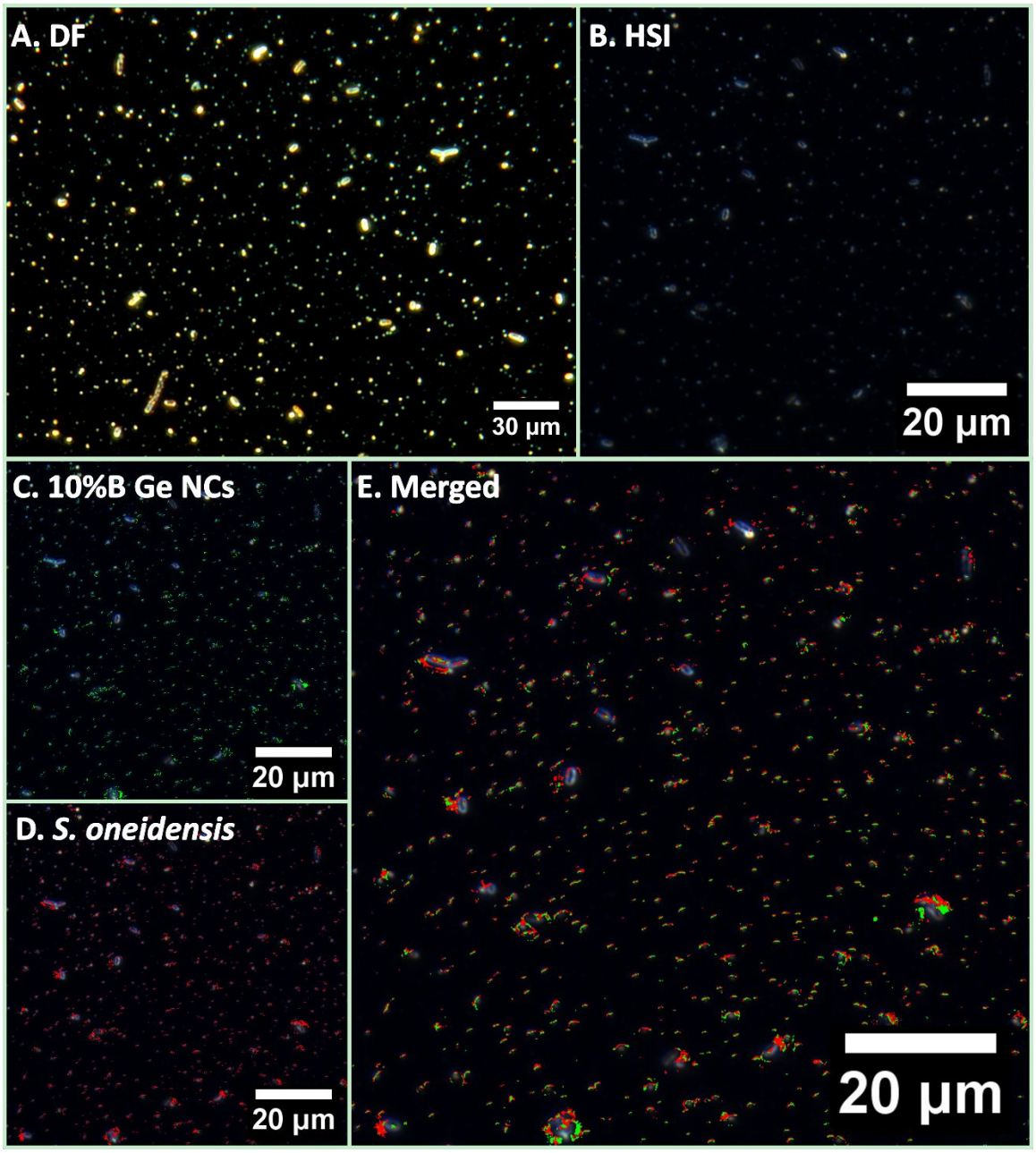

Figure S22. Cytoviva enhanced dark-field analysis of the association between 10% B-doped Ge NCs with *S. oneidensis*. (A) dark-field image of bacteria exposure solution, (B) hyperspectral image of bacteria exposure solution. Mapping (C) 10% B-doped Ge NCs alone and (D) bacteria cells alone in the hyperspectral data. (E) The merge of mapping results (C) and (D) (10% B-doped Ge NCs are false-colored with green while bacteria cells with red).

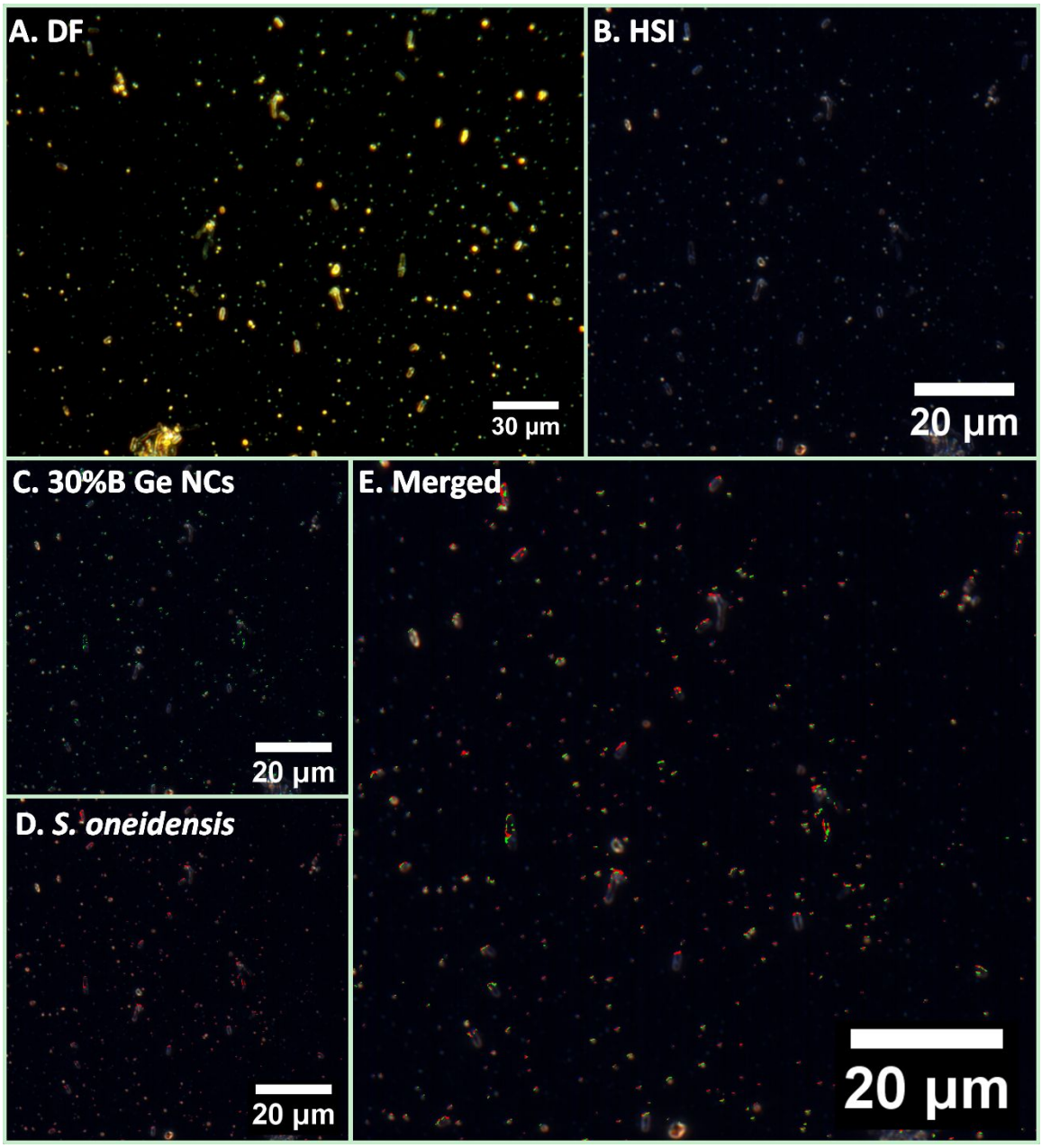

Figure S23. Cytoviva enhanced dark-field analysis of the association between 30% B-doped Ge NCs with *S. oneidensis*. (A) dark-field image of bacteria exposure solution, (B) hyperspectral image of bacteria exposure solution. Mapping (C) 30% B-doped Ge NCs alone and (D) bacteria cells alone in the hyperspectral data. (E) The merge of mapping results (C) and (D) (30% B-doped Ge NCs are false-colored with green while bacteria cells with red).

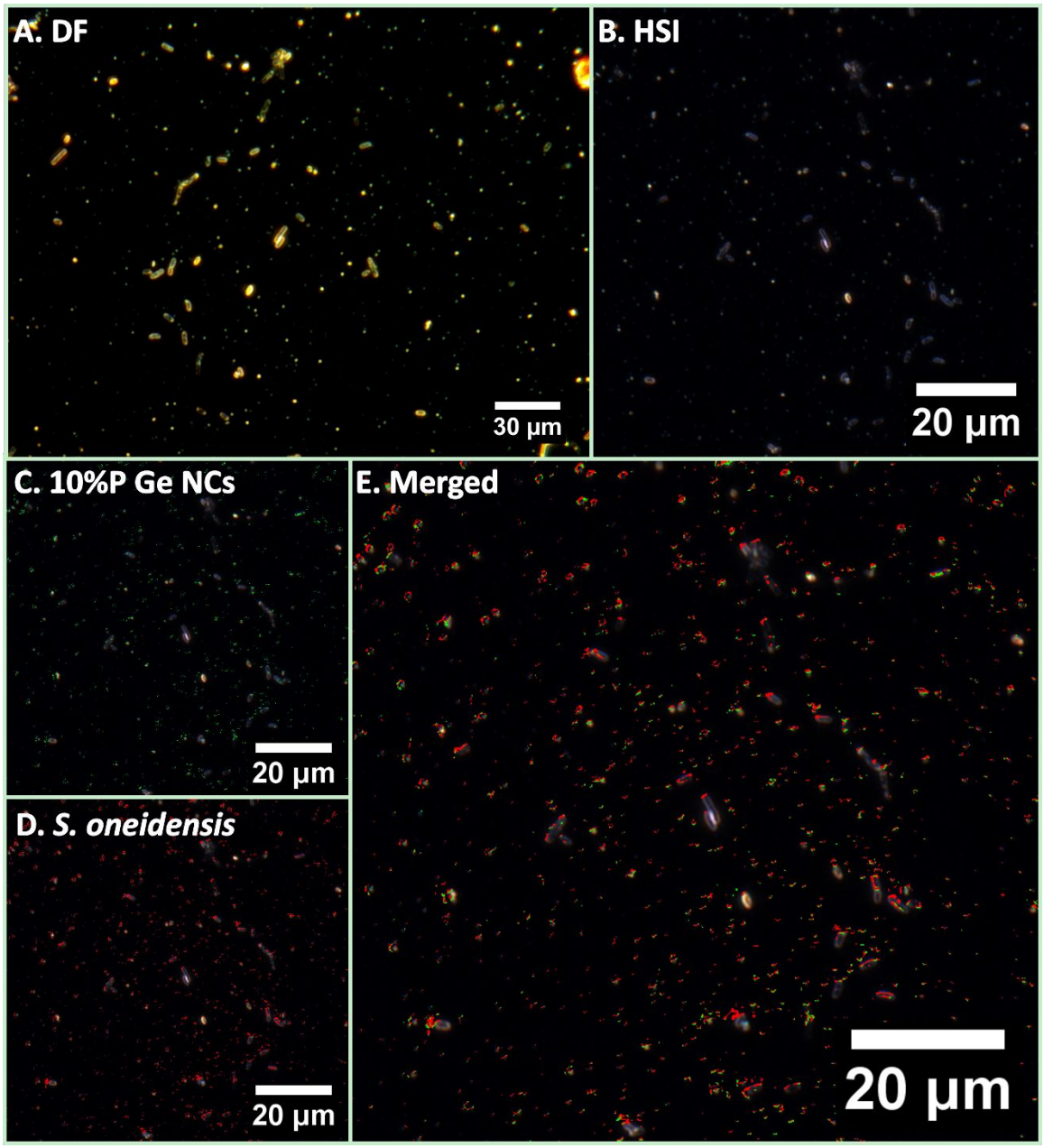

Figure S24. Cytoviva enhanced dark-field analysis of the association between 10% P-doped Ge NCs with *S. oneidensis*. (A) dark-field image of bacteria exposure solution, (B) hyperspectral image of bacteria exposure solution. Mapping (C) 10% P-doped Ge NCs alone and (D) bacteria cells alone in the hyperspectral data. (E) The merge of mapping results (C) and (D) (10% P-doped Ge NCs are false-colored with green while bacteria cells with red).

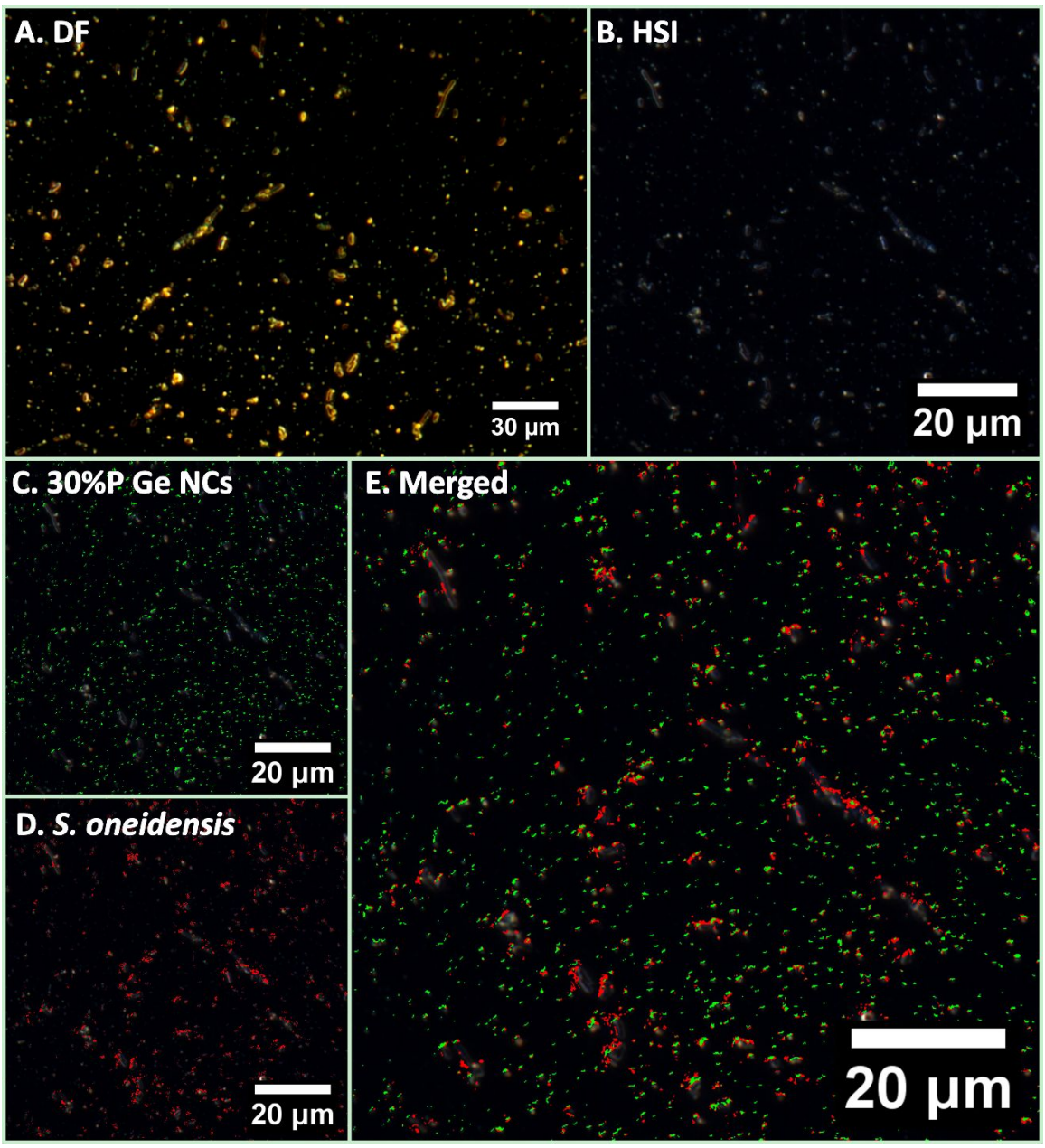

Figure S25. Cytoviva enhanced dark-field analysis of the association between 30% P-doped Ge NCs with *S. oneidensis*. (A) dark-field image of bacteria exposure solution, (B) hyperspectral image of bacteria exposure solution. Mapping (C) 30% P-doped Ge NCs alone and (D) bacteria cells alone in the hyperspectral data. (E) The merge of mapping results (C) and (D) (30% P-doped Ge NCs are false-colored with green while bacteria cells with red).

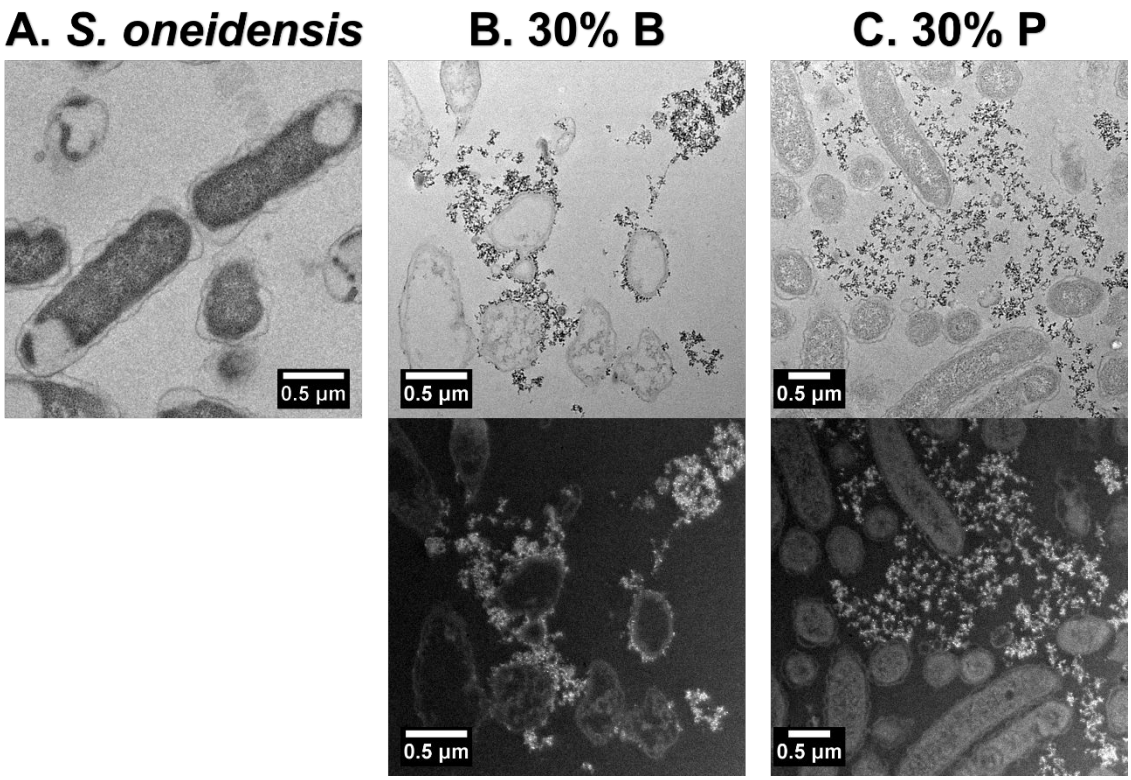

Figure S26. (upper level) Bright-field TEM images for (A) S. oneidensis cells and bacteria cells exposed to (B) 30% B-doped Ge NCs and (C) 30% P-doped Ge NCs; (lower level) Dark-field TEM images for bacteria cells exposed to (B) 30% B-doped Ge NCs and (C) 30% P-doped Ge NCs.