Supporting Information

Innate Immune Activation by Conditioned Medium of Cancer Cells Following Combined Phototherapy with Photosensitizer-loaded Gold Nanorods

Malvin Wei Cherng Kang[1], Haiyan Liu[1,2,3], James Chen Yong Kah[1,4]*

[1]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, University Hall, Tan Chin Tuan Wing, Level 04, #04-02, 21 Lower Kent Ridge, Singapore 119077.

[2]Deparment of Microbiology & Immunology, National University of Singapore, 5 Science Drive 2, Block MD4 Level 3, Singapore 117545

[3]Life Sciences Institute Immunology Programme, National University of Singapore, 28 Medical Drive, Centre for Life Sciences, Level 3, Singapore 117456

[4]Department of Biomedical Engineering, National University of Singapore, 4 Engineering Drive 3, Block E4, #04-08, Singapore 117583.

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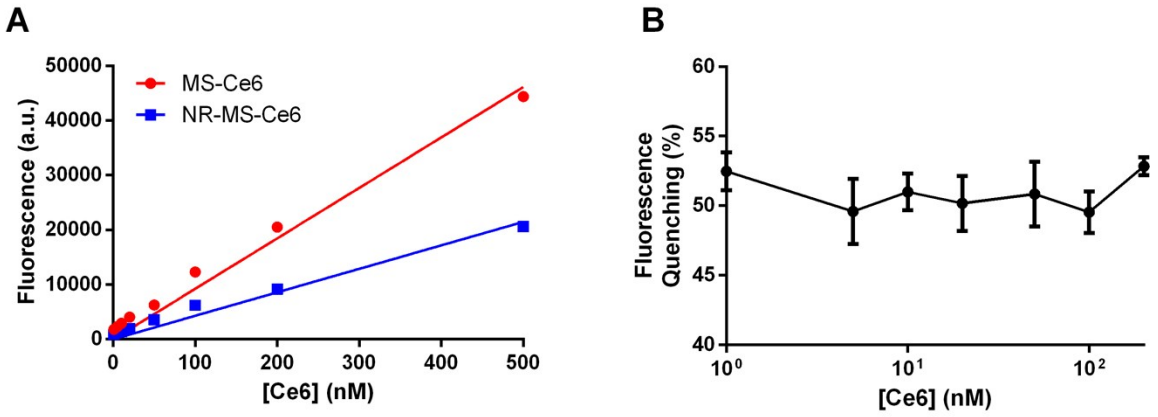

Figure S1. (A) As NR-CTAB possesses a UV-Vis absorption peak at 664 nm which matches the 665 nm emission wavelength of Ce6 when excited at 405 nm, two linear calibrations of Ce6 fluorescence against Ce6 concentrations of up to 500 nM were plotted for both MS-Ce6 and NR-MS-Ce6 (i.e. MS-Ce6 introduced to NR-CTAB to form NR-MS-Ce6), which showed a decrease in Ce6 fluorescence signal in NR-MS-Ce6 associated with quenching by NRs. (B) Fluorescence quenching of Ce6 from its loading on NRs did not vary significantly across a range of concentrations with a constant Ce6 fluorescence quenching of 50.9 ± 0.6 % observed when Ce6 was loaded on NR-MS-Ce6 at 200 nM Ce6 or less. The amount of Ce6 loaded to NR-MS-Ce6 was subsequently corrected for Ce6 fluorescence quenching.

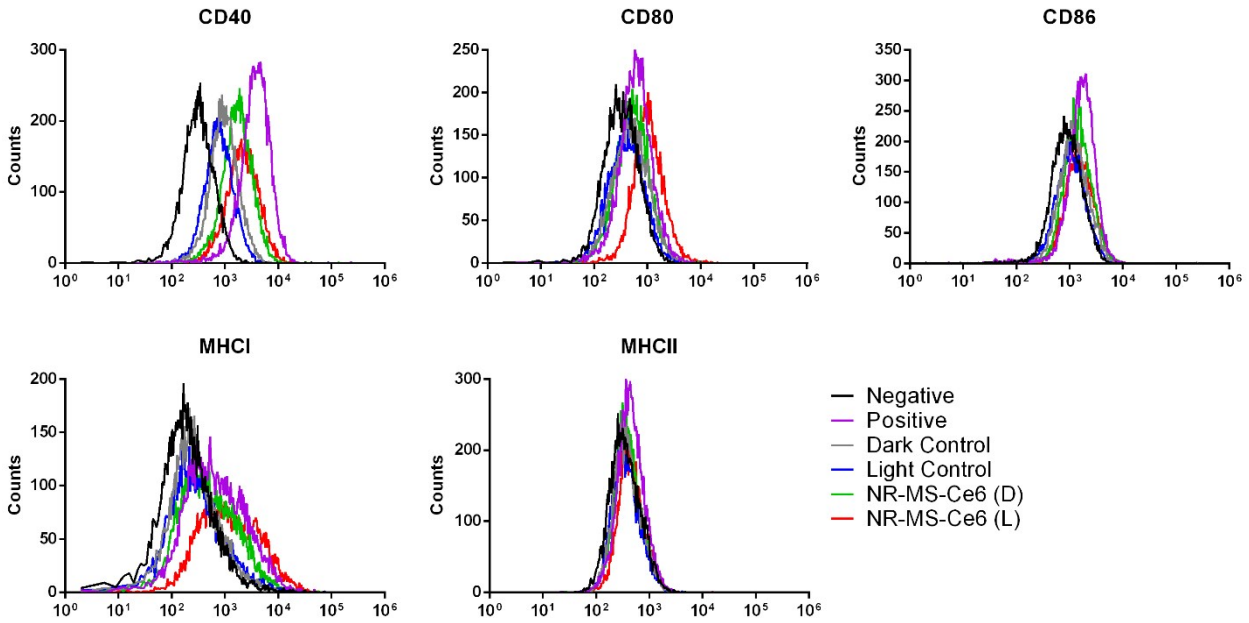

Figure S2. Raw fluorescence histogram profiles obtained from flow cytometry indicating the expression levels of CD40, CD80, CD86, MHCI and MHCII surface markers on J774A.1 murine macrophages dosed with conditioned medium from 9.0 x 10^4 EMT6 cells following combined PDT+PTT with 0.2 nM NR-MS-Ce6. Note: L and D denotes EMT6 cells dosed with NR-MS-Ce6 and irradiated (red) or kept in the dark (green) respectively, light control refers to laser-irradiated EMT6 cells (blue) and dark control refers to non-laser irradiated EMT6 cells (grey), both in the absence of NR-MS-Ce6. Negative control (black) refers to unstimulated immune cells, while positive control (purple) were stimulated with LPS.

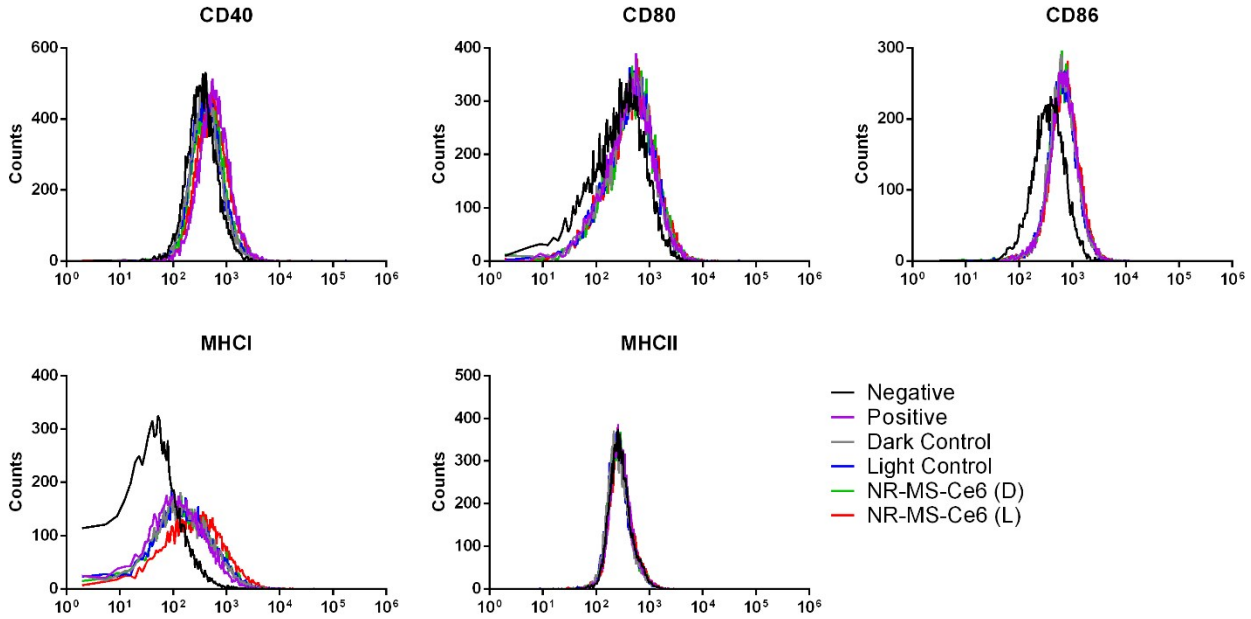

Figure S3. Raw fluorescence histogram profiles obtained from flow cytometry indicating the expression levels of CD40, CD80, CD86, MHCI and MHCII surface markers on DC2.4 murine macrophages dosed with conditioned medium from 9.0 x 10^4 EMT6 cells following combined PDT+PTT with 0.2 nM NR-MS-Ce6. Note: L and D denotes EMT6 cells dosed with NR-MS-Ce6 and irradiated (red) or kept in the dark (green) respectively, light control refers to laser-irradiated EMT6 cells (blue) and dark control refers to non-laser irradiated EMT6 cells (grey), both in the absence of NR-MS-Ce6. Negative control (black) refers to unstimulated immune cells, while positive control (purple) were stimulated with LPS.

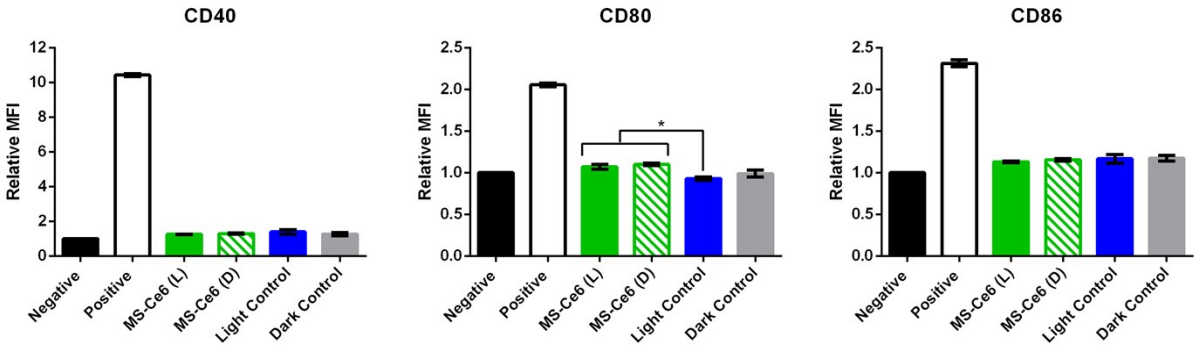

Figure S4. Relative mean fluorescence intensities (MFI) obtained from flow cytometry analysis indicating the expression levels of CD40, CD80, CD86 surface markers on J774A.1 macrophages dosed with conditioned medium from 3.0 x 10^4 EMT6 cells following PDT treatment with 20 nM of MS-Ce6. Note: L and D denote EMT6 cells dosed with MS-Ce6 and irradiated (green) or kept in the dark (shaded green) respectively, light control refers to laser-irradiated EMT6 cells (blue) and dark control refers to non-laser irradiated EMT6 cells (gray), both in the absence of MS-Ce6. Negative control (black) refers to unstimulated J774A.1 cells, while positive control (white) refers to J774A.1 cells stimulated with LPS. Error bar indicates standard error based on N = 3 (* $p < 0.05$).

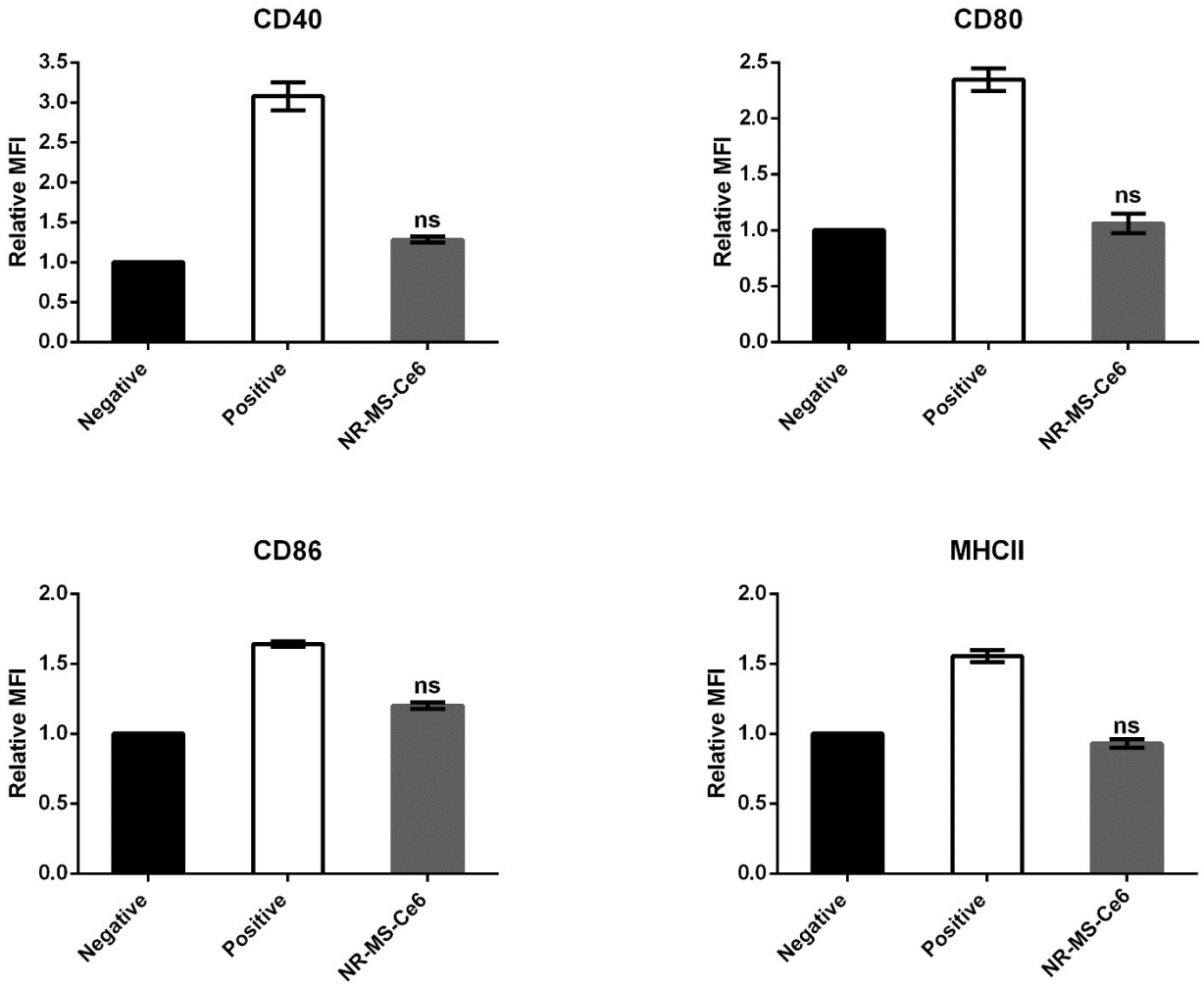

Figure S5. Relative mean fluorescence intensities (MFI) obtained from flow cytometry analysis indicating the expression levels of CD40, CD80, CD86, MHCII surface markers on J774A.1 macrophages dosed directly with 0.25nM NR-MS-Ce6. Negative control (black) refers to unstimulated J774A.1 cells, while positive control (white) refers to J774A.1 cells stimulated with LPS. Error bar indicates standard error based on N = 3. (ns: $p > 0.05$)