Supplementary Material

Sequential Delivery of Cyclopeptide RA-V and Doxorubicin for Combination Therapy on Resistant Tumor and *In Situ* Monitoring of Cytochrome c Release

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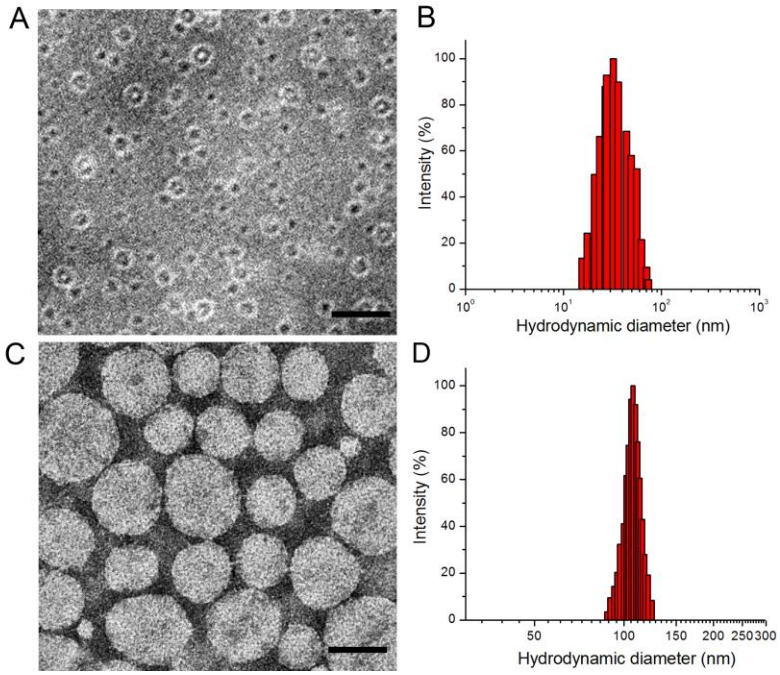
Supplementary figures

1. **Figure S1.** (A) TEM micrograph of Dox/MPP-DGL. Scale bar: 100 nm. (B) Size distribution of the Dox/MPP-DGL characterized by DLS at 25 °C. (C) TEM micrograph of DGLipo NPs. Scale bar: 100 nm. (D) Size distribution of the DGLipo NPs characterized by DLS at 25 °C.
2. **Figure S2.** TEM micrograph of degraded DGLipo NPs in acidic environment. Scale bar: 100 nm.
3. **Figure S3.** Long-term-stability study of Dox/MPP-DGL in RPMI 1640 or DMEM with 10% FBS, and DGLipo NPs in RPMI 1640 or DMEM with 10% FBS.

4. **Figure S4.** (A) Fluorescence spectra of Dox/Duplex with increasing concentrations of Cyt c (from bottom to top: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM) after 5 min of incubation in the HEPES buffer (5 mM). (B) Fluorescence intensity at 595 nm of Dox/Duplex toward various analytes: Cyt c (10 μM); other analytes (10 μM and 100 μM). Data are means ± SD (n = 3), ***P < 0.001 compared to other groups using a one-way ANOVA.
5. **Figure S5.** Real-time confocal fluorescence imaging of HeLa cells incubated with DGLipo NPs at 37 °C for 2 h, and further incubated with fresh culture medium for additional 4 h. Scale bars: 20 μm.
6. **Figure S6.** Co-localization images of DGLipo NPs in HeLa cells. HeLa cells incubated with DGLipo NPs at 37 °C for 2 h, and further incubated with fresh culture medium for additional 1 h, and then incubated with 100 nM LysoTracker Blue, ER Tracker Blue, Hoechst 33342 and MitoTracker Blue 10 min. Scale bars: 20 μm.
7. **Figure S7.** Co-localization images of DGLipo NPs in HeLa cells. HeLa cells incubated with DGLipo NPs at 37 °C for 2 h, and further incubated with fresh culture medium for additional 4 h, and then incubated with 100 nM Hoechst 33342 for 10 min. Scale bars: 20 μm.
8. **Figure S8.** Confocal fluorescence images of apoptosis by the JC-1 assay in HeLa cells treated with 50 μg mL^{-1} DGLipo NPs or 50 μg mL^{-1} DGLipo NPs (no RA-V). Scale bars: 20 μm. In healthy cells with high mitochondrial membrane potential, JC-1 spontaneously forms complexes known as J-aggregates with intense red fluorescence. In apoptotic or unhealthy cells with low mitochondrial membrane potential, JC-1 remains in the monomeric form, which shows only green fluorescence. The fluorescence of green channel was excited at 488 nm and the emission was collected between 510-545 nm. The fluorescence of red channel was excited at 543 nm and the emission was collected between 575-630 nm.
9. **Figure S9.** Confocal images of immunostaining show the release of Cyt c in the cells. HeLa cells incubated with 50 μg mL^{-1} DGLipo NPs for 2 h, and further incubated with fresh culture medium for additional (A) 1 h, (B) 1.5 h and (C) 2 h. And then fixed and stained with Cyt c antibody and FITC-conjugated secondary antibody. Cells were observed by confocal microscopy. Scale bars: 20 μm.
10. **Figure S10.** Real-time fluorescence imaging of Cyt c release in HeLa cells incubated with 50 μg mL^{-1} DGLipo NPs for 2 h, and further incubated with fresh culture medium for additional 1 h. The mitochondria were stained by MitoTracker Blue. Scale bars: 20 μm.
11. **Figure S11.** Real-time fluorescence imaging of HeLa cells incubated with 50 μg mL^{-1} cDGLipo NPs for 2 h, and further incubated with fresh culture medium for additional 2 h. Scale bars: 20 μm.
12. **Figure S12.** MTT assay of HeLa cells and HeLa/MDR cells in the presence of different concentrations of free Dox. The half-maximal inhibitory concentration (IC_{50}) of Dox on HeLa cells and HeLa/MDR cells were 1.8 μM and 10 μM, respectively.
13. **Figure S13.** Change of relative tumor volume (V/V_0) upon treatments with different concentrations of DGLipo NPs on HeLa tumor-bearing mice. Data are means ± SD (n = 6), **P < 0.01, ***P < 0.001.
14. **Figure S14.** Change of relative tumor volume (V/V_0) upon different treatments on HeLa/MDR tumor-bearing mice. Data are means ± SD (n = 6), ***P < 0.001 compared to other groups using a one-way ANOVA.

Supplementary video

Video S1. Real-time fluorescence imaging of Cyt c release in HeLa cells incubated with 50 μg mL^{-1} DGLipo NPs for 2 h, and further incubated with fresh culture medium for additional 1 h.



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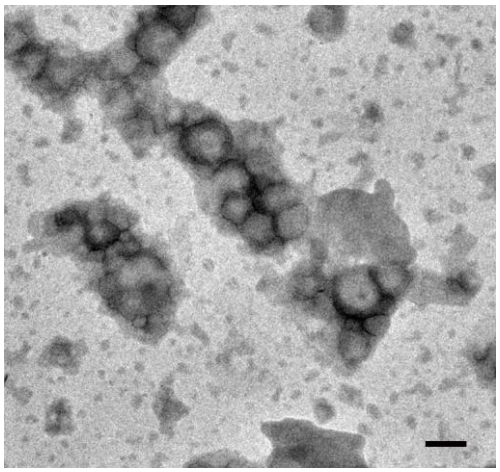


Figure S2. TEM micrograph of degraded DGLipo NPs in acidic environment. Scale bar: 100 nm.

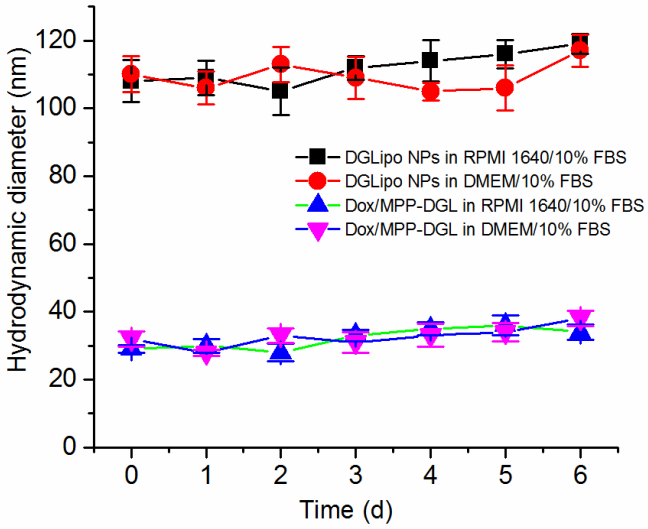
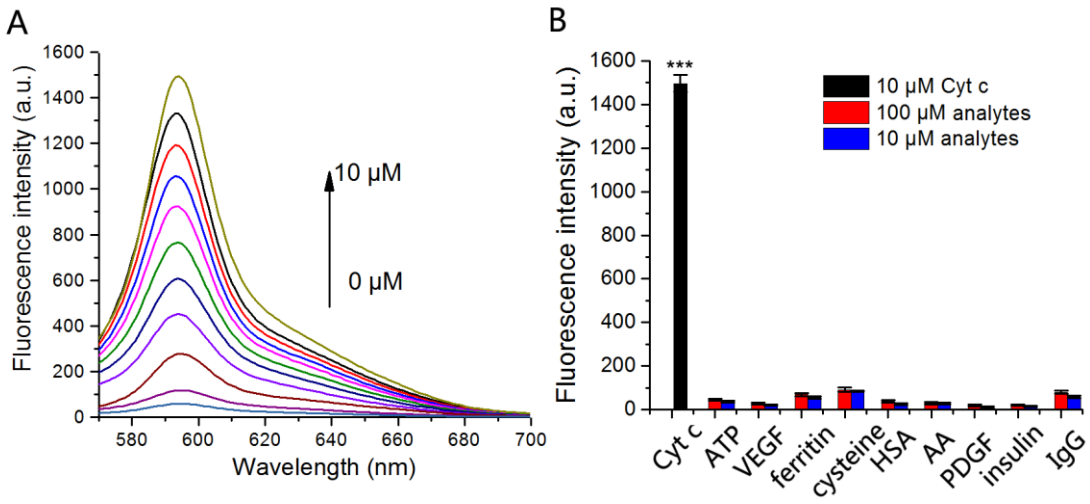


Figure S3. Long-term-stability study of Dox/MPP-DGL in RPMI 1640 or DMEM with 10% FBS, and DGLipo NPs in RPMI 1640 or DMEM with 10% FBS.



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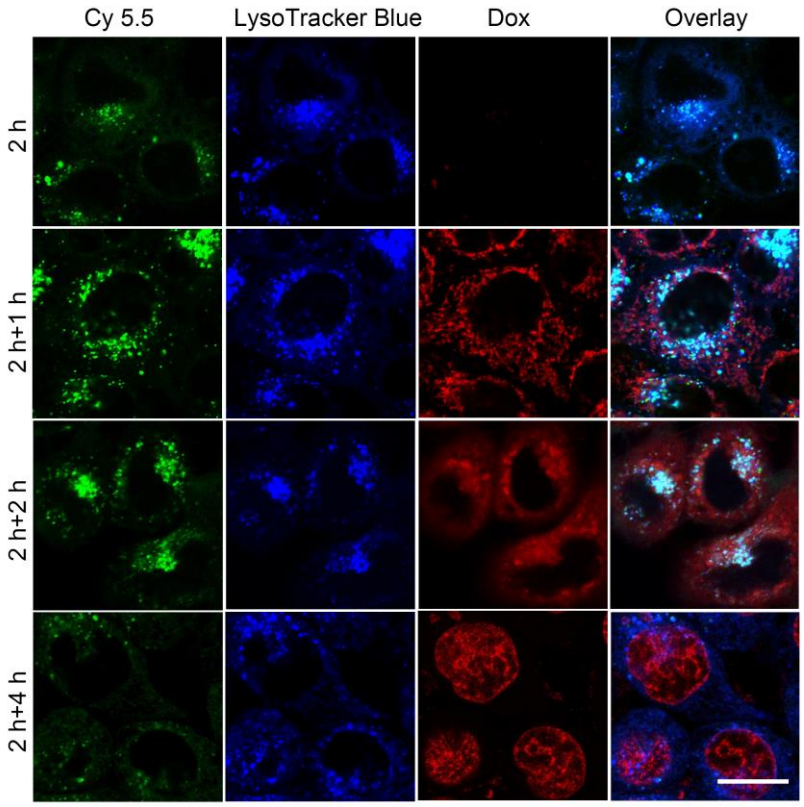


Figure S5. Real-time confocal fluorescence imaging of HeLa cells incubated with DGLipo NPs at 37 °C for 2 h, and further incubated with fresh culture medium for additional 4 h. Scale bars: 20 μm.

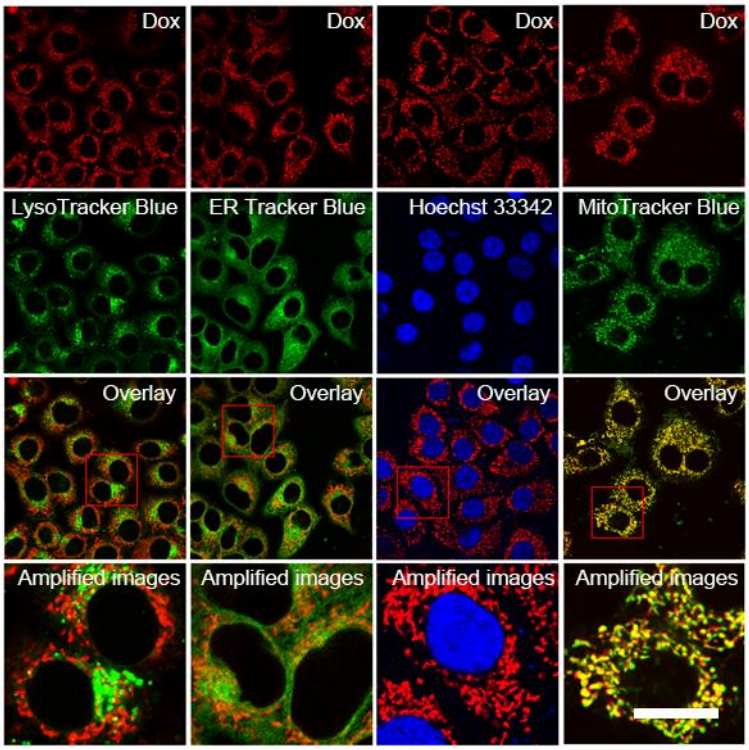
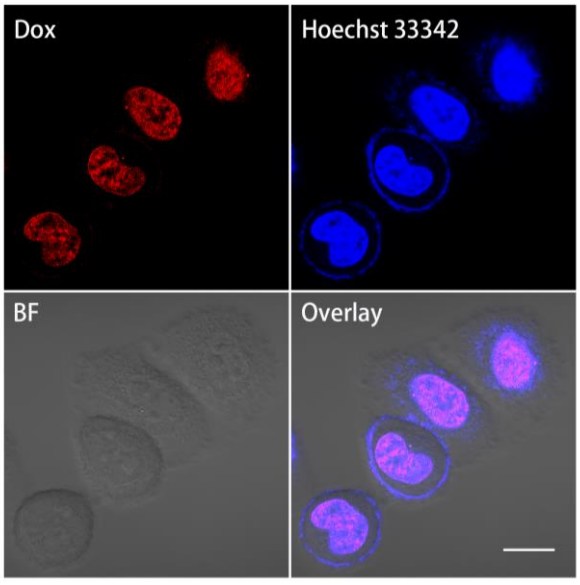


Figure S6. Co-localization images of DGLipo NPs in HeLa cells. HeLa cells incubated with DGLipo NPs at 37 °C for 2 h, and further incubated with fresh culture medium for additional 1 h, and then incubated with 100 nM LysoTracker Blue, ER Tracker Blue, Hoechst 33342 and MitoTracker Blue 10 min. Scale bars: 20 μm.



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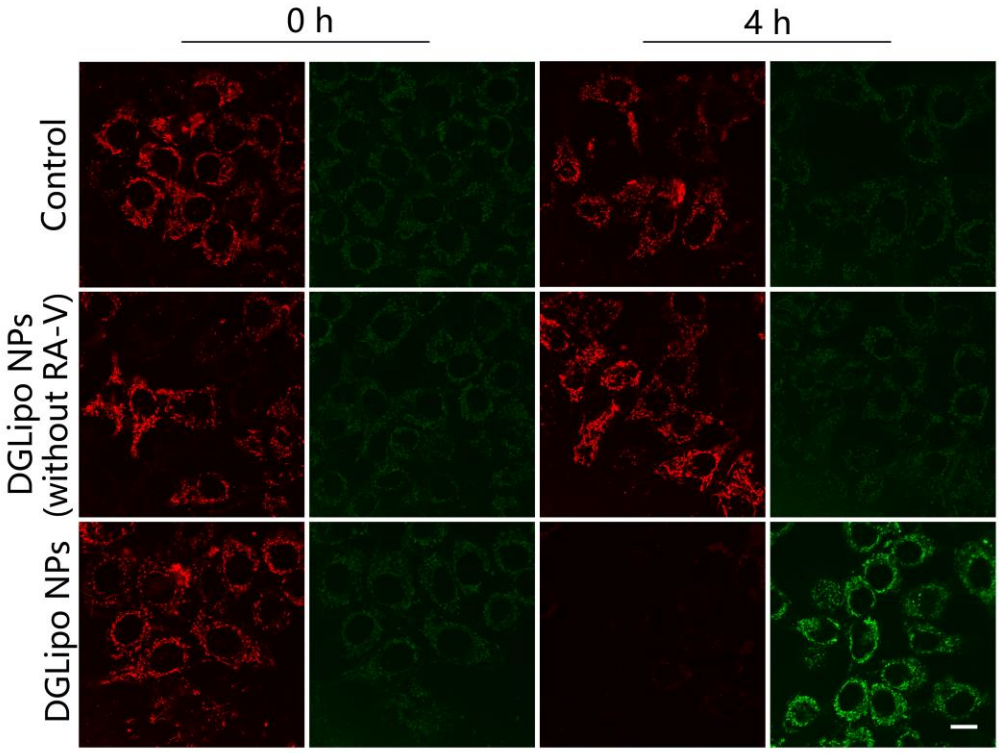

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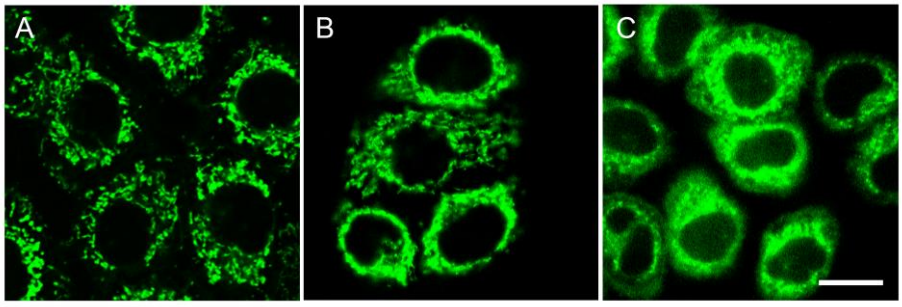

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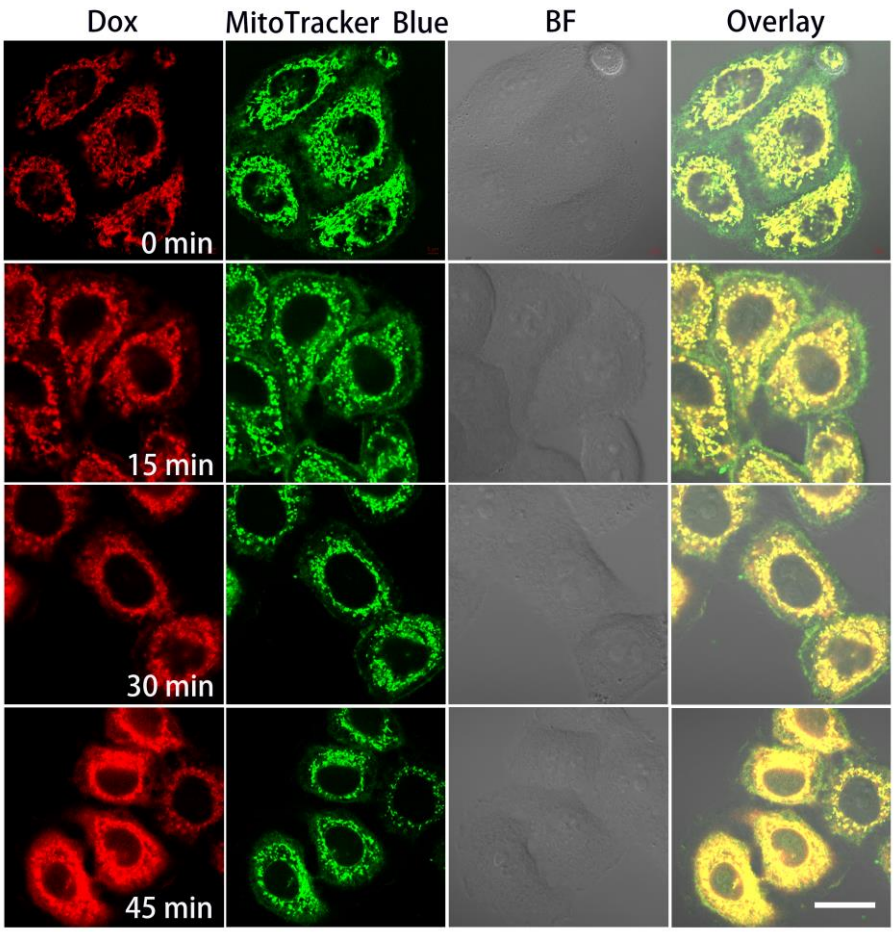

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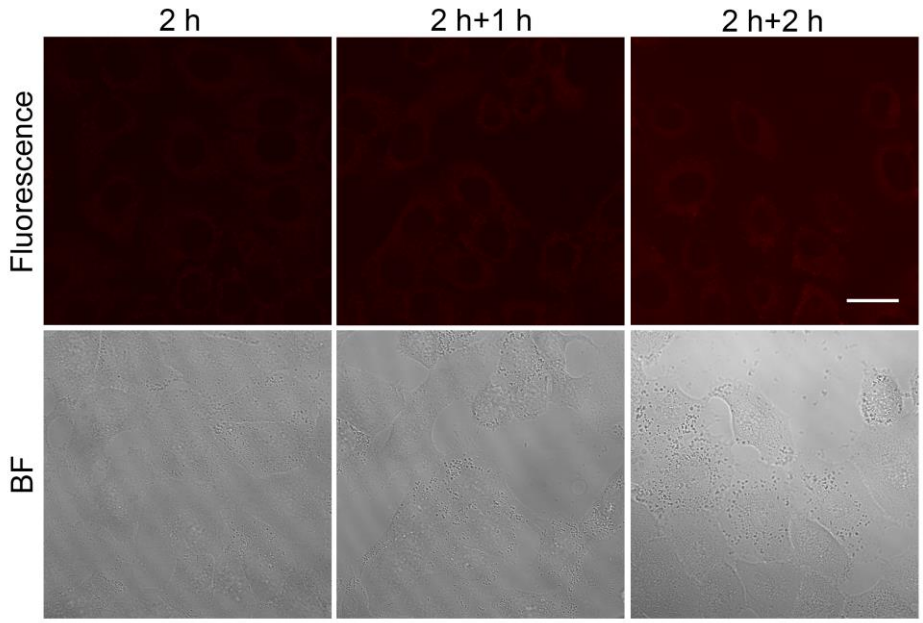

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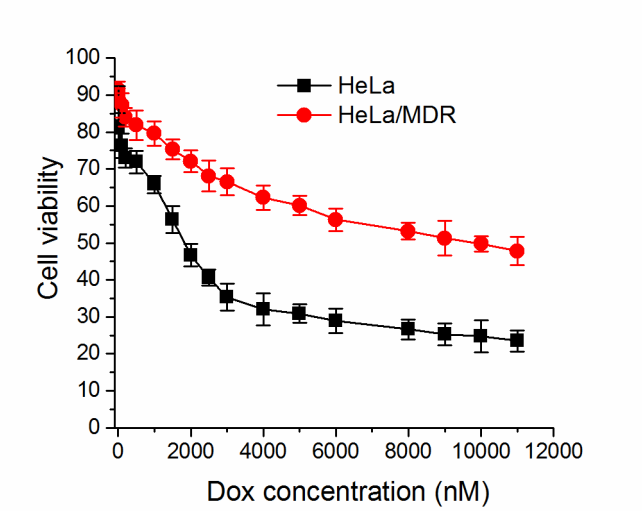

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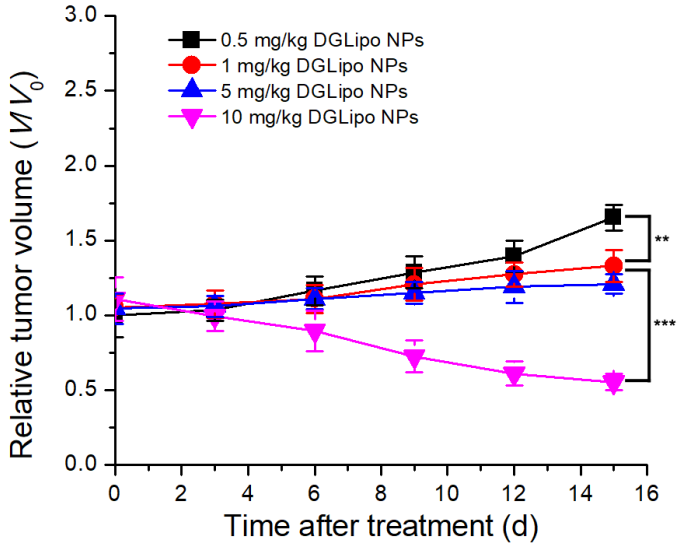

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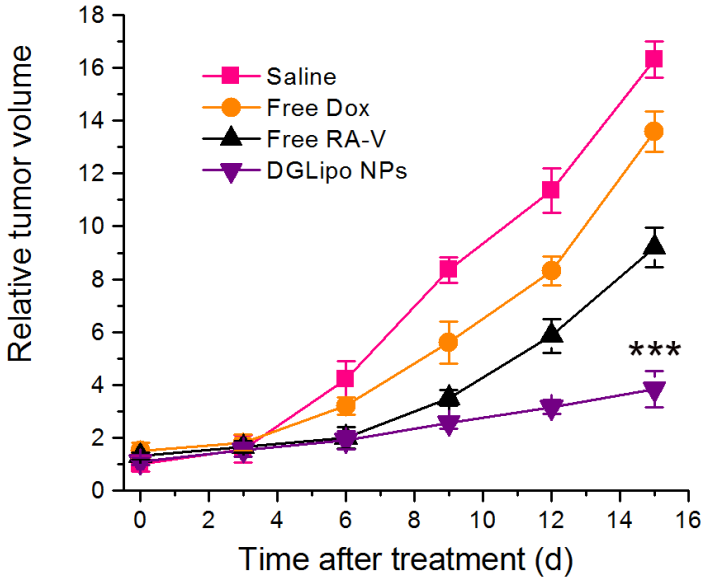

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