Supplementary Materials I

Novel Caffeic Acid Phenethyl Ester-Mortalin Antibody Nanoparticles Offer Enhanced Selective Cytotoxicity to Cancer Cells

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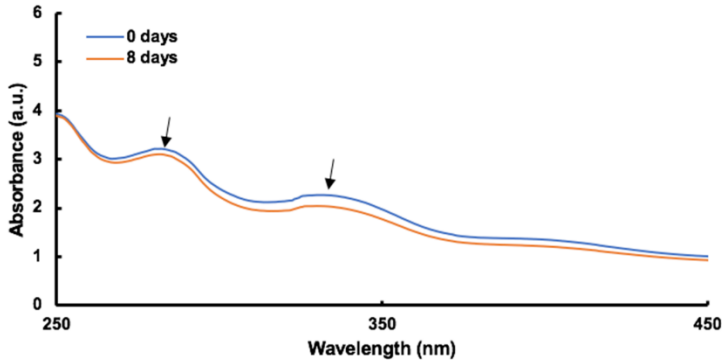

Figure S1. UV-Vis-NIR absorption spectra of CAPE-MotAb nanoparticles at different time points (0 and 8 days). Absorbance of MotAb at 280 nm and CAPE at 335 nm (marked with black arrows) didn't show significant difference from 0 to 8 days.

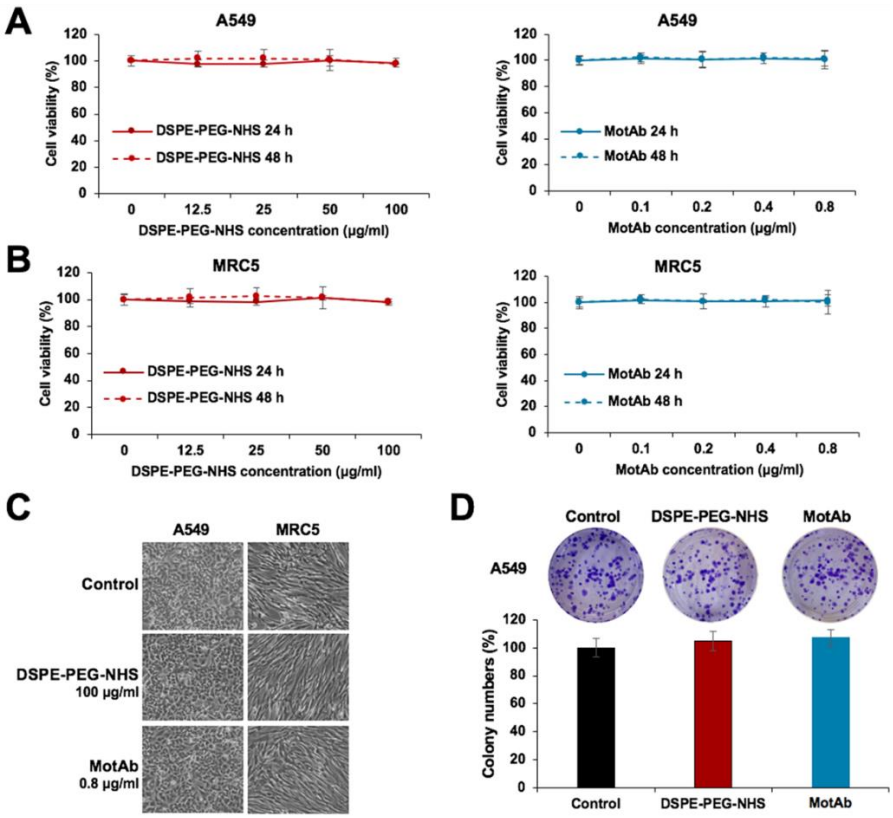

Figure S2. Viability of **(A)** A549 and **(B)** MRC5 cells treated with DSPE-PEG-NHS and MotAb (at the concentrations indicated) for 24 h and 48 h. No cytotoxic effect of DSPE-PEG-NHS and MotAb was obtained on A549 and MRC5 cells (mean ± SD, n = 3). **(C)** Phase contrast images show no toxicity

when A549 and MRC5 cells exposed to DSPE-PEG-NHS and MotAb. **(D)** No reduction in colony forming ability was observed in treated cells after 12 h incubation. DSPE-PEG-NHS concentration: 100 µg/mL, MotAb concentration: 0.8 µg/ml. Quantitation from three independent experiments (mean ± SD, $n = 3$).

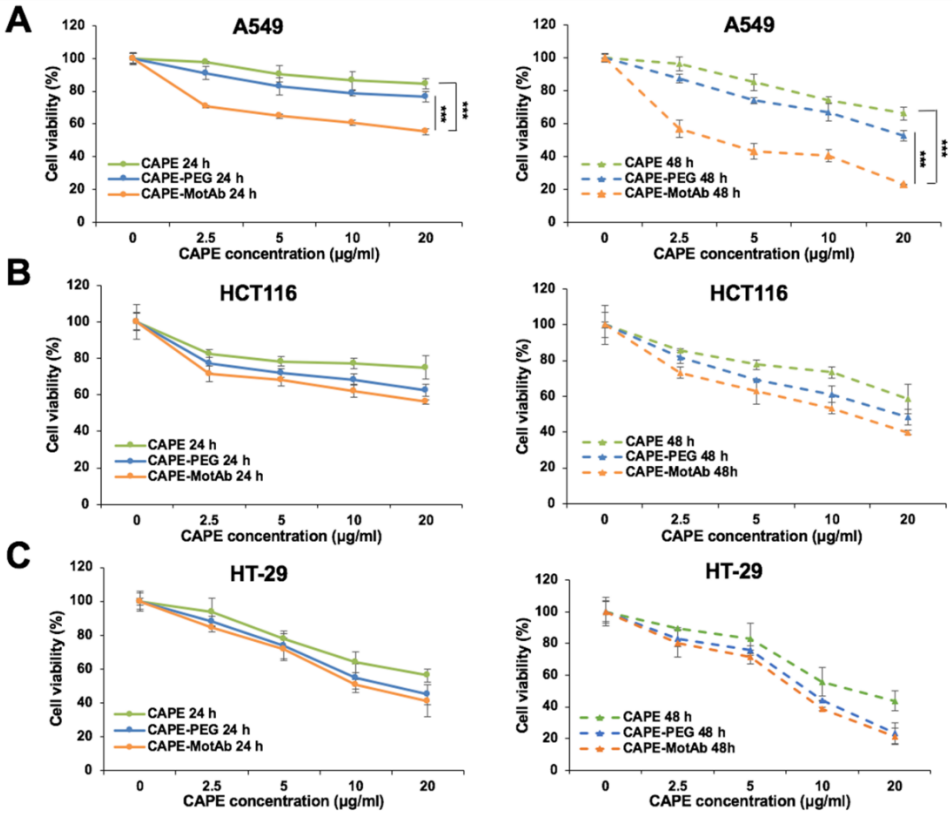

Figure S3. Cytotoxic effect of CAPE, CAPE-PEG and CAPE-MotAb on (**A**) A549, (**B**) HCT116 and (**C**) HT-29 cells at the concentrations indicated for 24 h and 48 h, respectively. These three cell lines treated with CAPE, CAPE-PEG and CAPE-MotAb showed dose- and time-dependent decrease in cell viability. CAPE-MotAb treated A549 cells showed enhanced cytotoxicity as compared to CAPE and CAPE-PEG treatments; HCT116 and HT-29 cells did not show much difference. Quantitation from three independent experiments (mean ± SD, $n = 3$), *** $p < 0.001$ (Student's t-test).

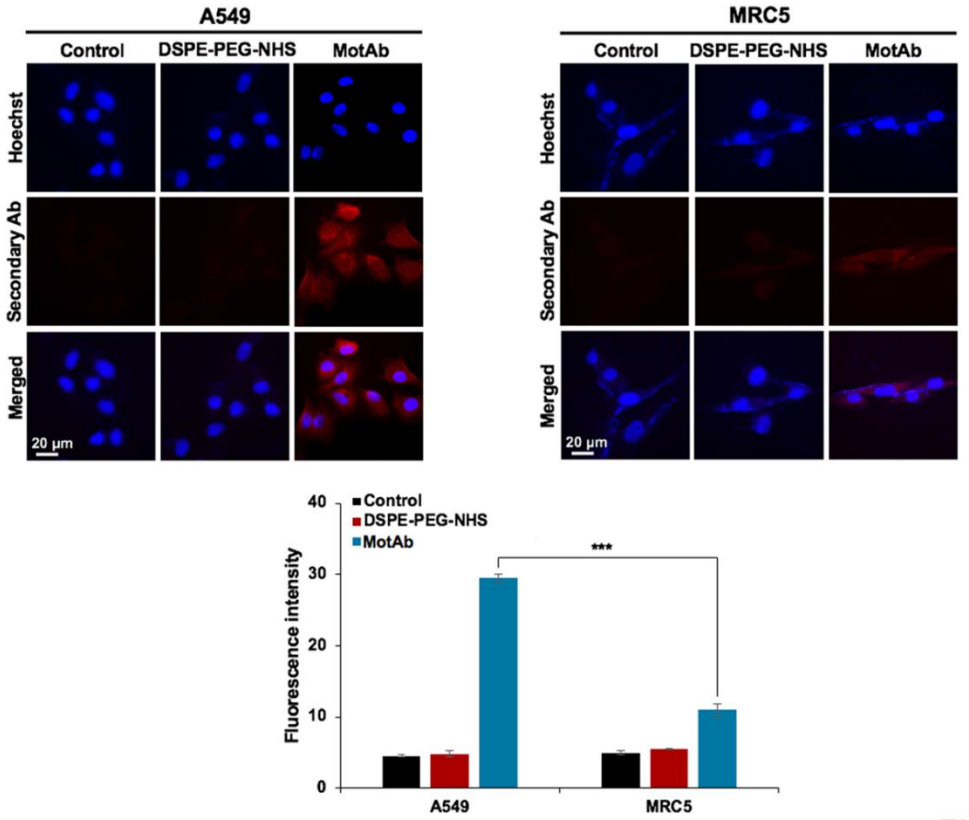

Figure S4. Fluorescence microscopy images of A549 and MRC5 cells treated with DSPE-PEG-NHS and MotAb for 12 h followed by staining with Alexa Fluor™ 594-tagged secondary antibody. The nuclei were stained with Hoechst. DSPE-PEG-NHS concentration: 100 μg/mL, MotAb concentration: 0.8 μg/mL. Quantitation of mortalin expression from fluorescence images (mean ± SD, n = 3), *** $p < 0.001$ (Student's t-test).

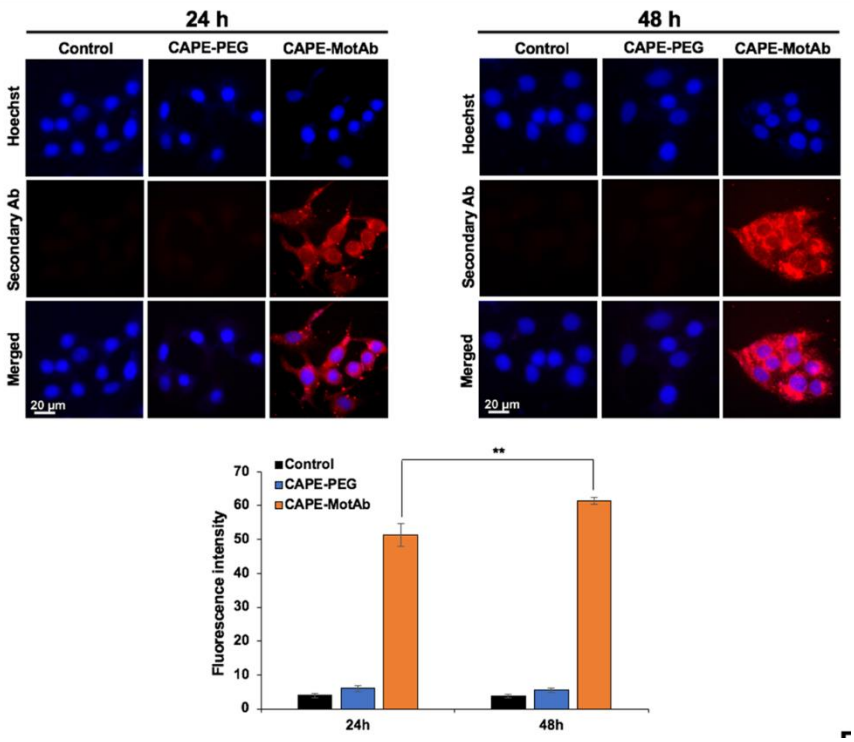

Figure S5. Fluorescence microscopy images of A549 cells treated with CAPE-PEG and CAPE-MotAb for 24 and 48 h respectively, followed by staining with Alexa Fluor™ 594-tagged secondary antibody. The nuclei were stained with Hoechst. Cellular uptake of CAPE-MotAb by A549 cells was in a time-

dependent manner. CAPE concentration: 20 μg/mL. Quantitation of mortalin expression from fluorescence images (mean ± SD, $n = 3$), ** $p < 0.01$ (Student's t-test).

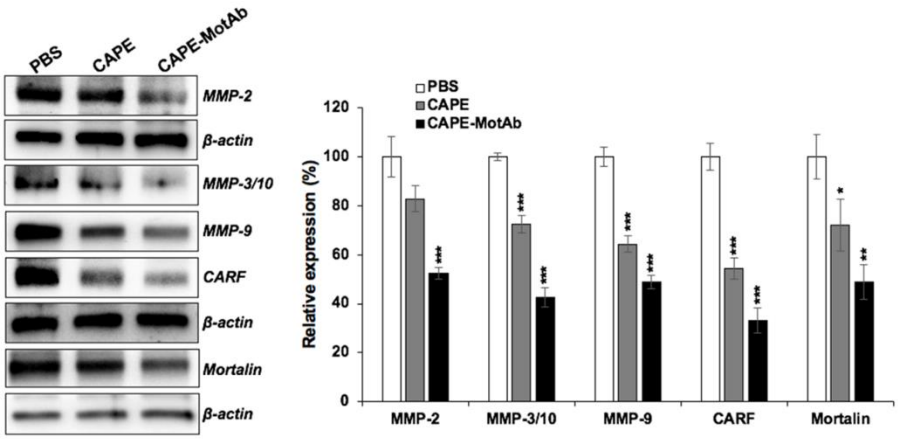

Figure S6. Western blotting analysis of tumor lysates. CAPE-MotAb treated group showed significant downregulation of MMP-2, MMP-3/10, MMP-9, CARF and mortalin levels. Quantitation from three independent experiments (mean ± SD, $n = 3$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's t-test to control).