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).