Ultrasound Enhanced Matrix Metalloproteinase-9 Triggered Release of Contents from Echogenic Liposomes

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I. Optical Microscopy:

To support particle size distribution findings of DLS Zeta Sizer instrument, we observed ELIPs under an optical microscope (Olympus IX81® motorized inverted microscope). The ELIPs contained 1 mol% 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) and all the remaining procedure was followed as described in main manuscript. A glass slide was used and 30-50 µL of liposomal solution (0.2 mg/mL) was added. A coverslip then placed on to the top of the sample. Care was taken while putting coverslip to avoid entrapment any air bubbles and extra solution coming out from the coverslip was wiped using a tissue paper. All images were viewed using 60X objective and captured using CellSens Standard software (version 1.6).

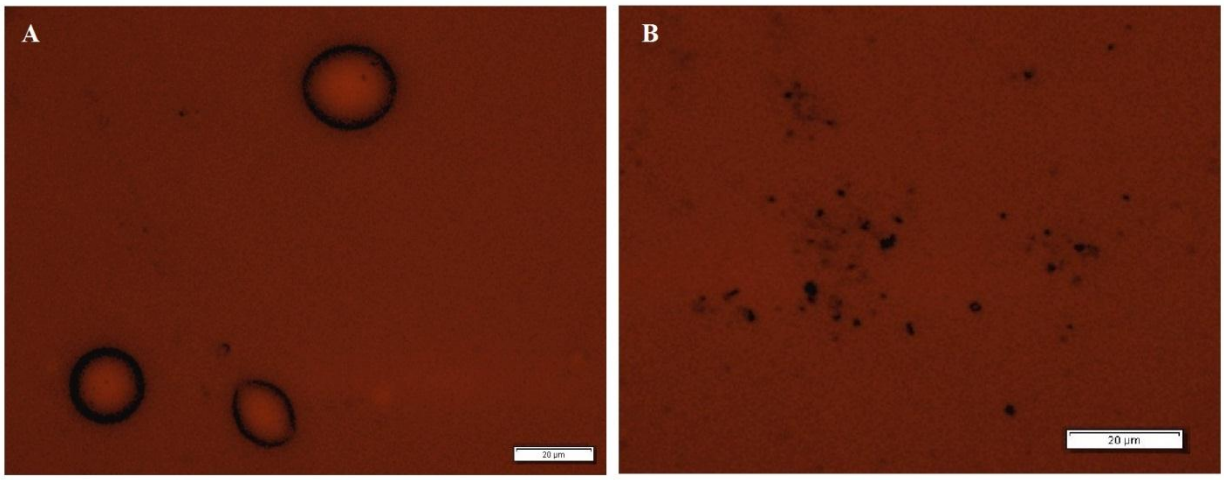

Figure S1. Optical microscopic images of ELIPs observed using 60X lens.

II. Quenching effect of ELIPs on carboxyfluorescein fluorescence:

To study effect of echogenic liposomes on the fluorescence of carboxyfluorescein, two batches of liposomes were prepared (regular liposomes and echogenic liposomes without encapsulation of dye) following the same protocol as described in main manuscript. Reconstituted liposomal solutions (0.02 mg/mL of total lipid) were then incubated in 0.5 µM carboxyfluorescein in 25 mM HEPES buffer pH 8 with added Ca^{2+} and Zn^{2+} for an hour. The fluorescence emission was monitored (excitation at 480 nm) from 350 nm to 750 nm at different time intervals (0 min, 30 min and 60 min; Figures S2 – S4).

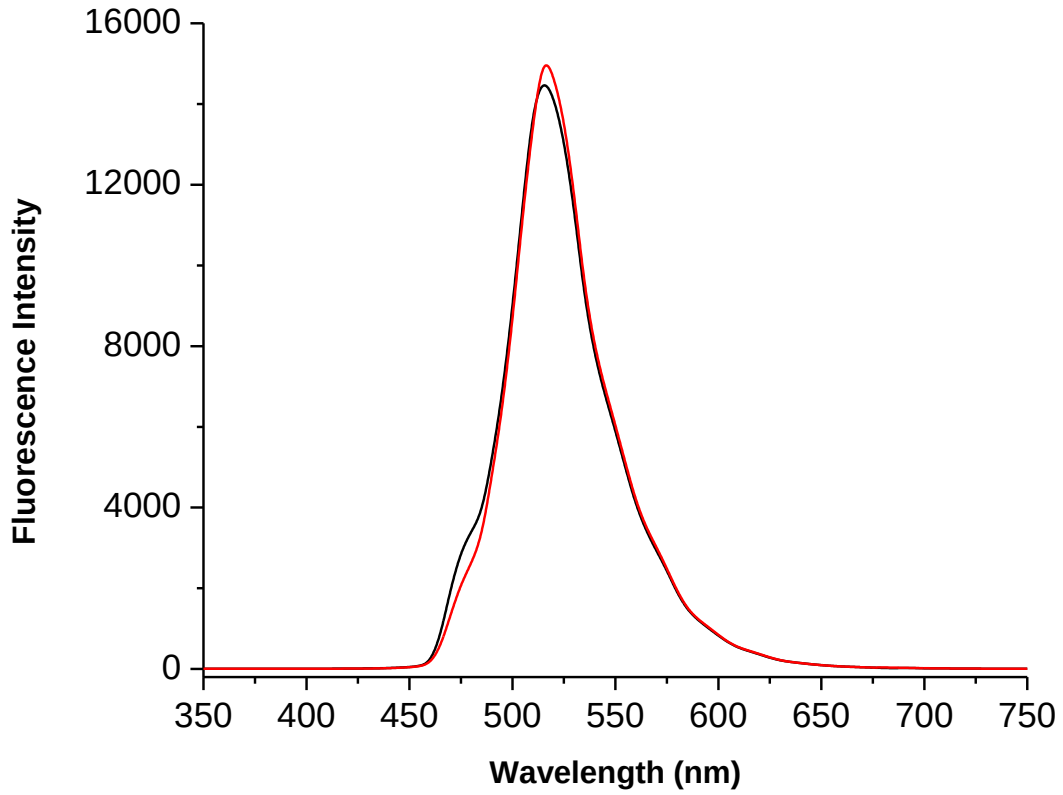

Figure S2. Initial (t = 0 min) emission spectra for carboxyfluorescein incubated with regular liposomes (black trace) and ELIPs (red trace).

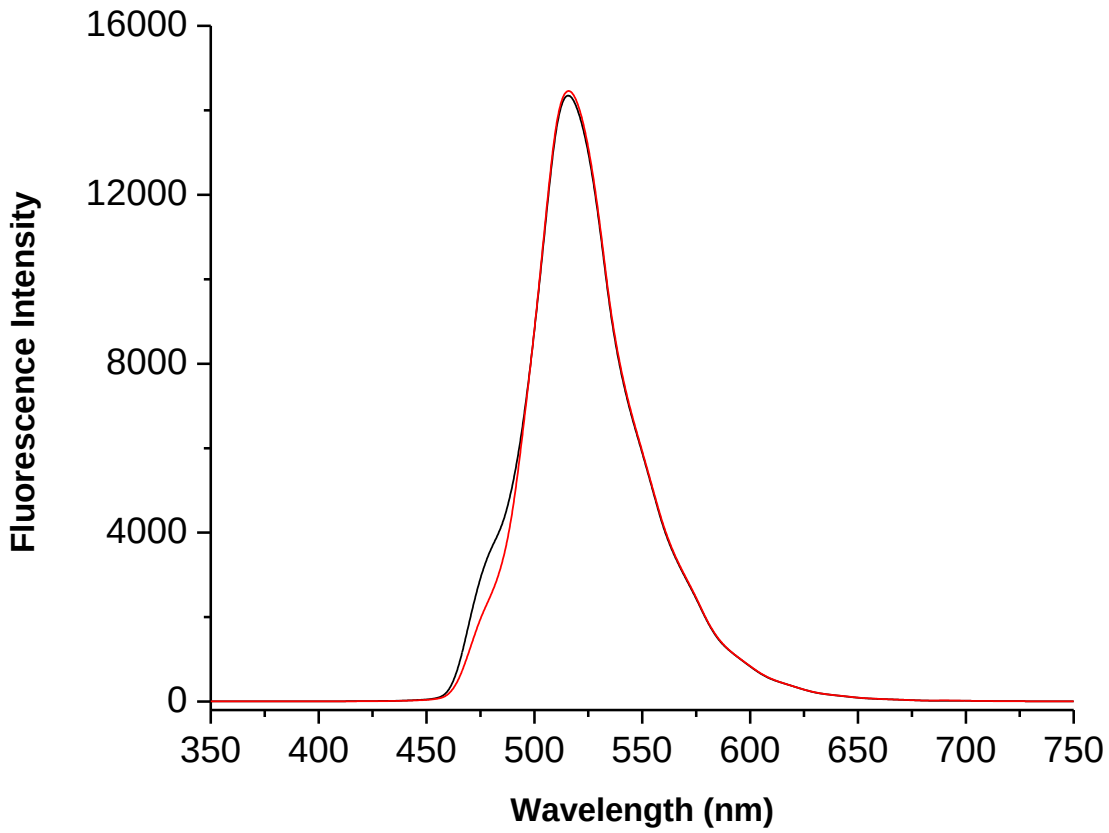

Figure S3. Emission spectra for carboxyfluorescein incubated with regular liposomes (black trace) and ELIPs (red trace) after 30 minutes.

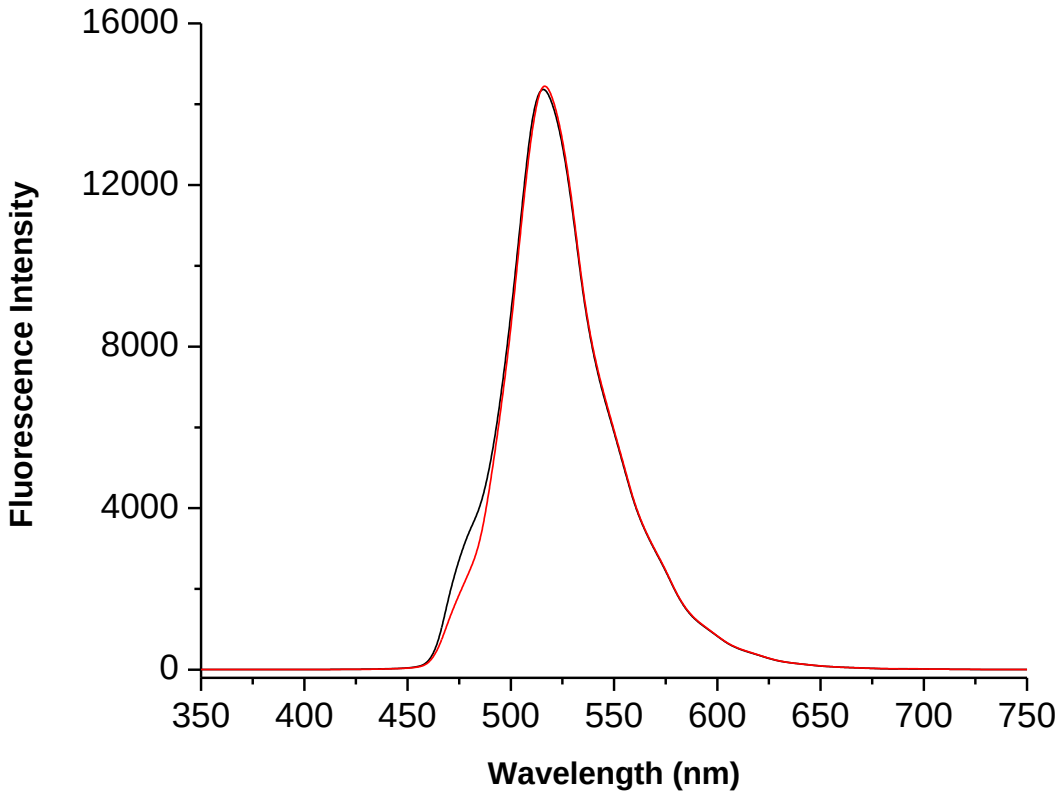

Figure S4. Emission spectra for carboxyfluorescein incubated with regular liposomes (black trace) and ELIPs (red trace) after 60 minutes.