Supporting Information: Sphingomyelin Liposomes Containing Porphyrin-phospholipid for Irinotecan Chemophototherapy

Authors: Kevin A Carter[1], Dandan Luo[1], Aida Razi[2], Jumin Geng[1], Shuai Shao[1], Joaquin Ortega[2], Jonathan F Lovell[1,*]

[1]Department of Biomedical Engineering[1], University at Buffalo, State University of New York, Buffalo, NY, 14260. [2]Department of Biochemistry and Biomedical Sciences and M. G. DeGroote Institute for Infectious Diseases Research, McMaster University, Hamilton, ON L8S4L8, Canada

*Email: jflovell@buffalo.edu

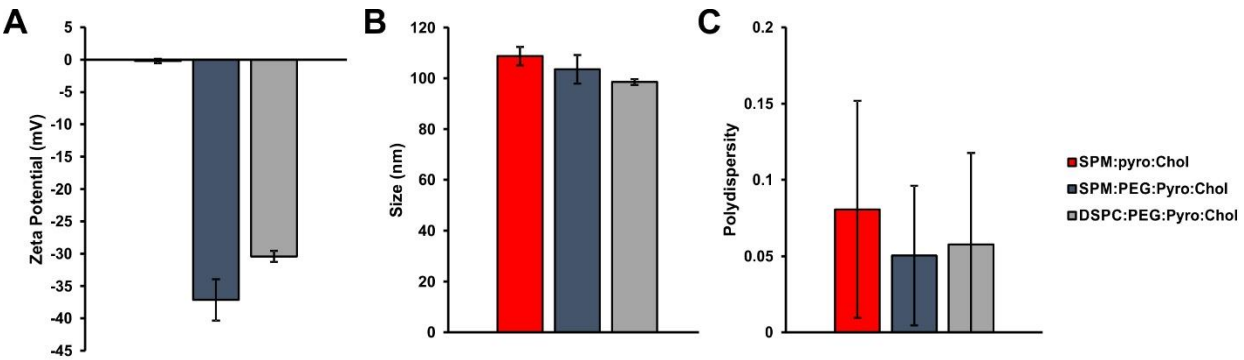

Figure S1: Liposome characterization by dynamic light scattering. A) DLS zeta potential of liposomes in 1 mM NaCl **B)** Size and **C)** polydispersity in PBS.

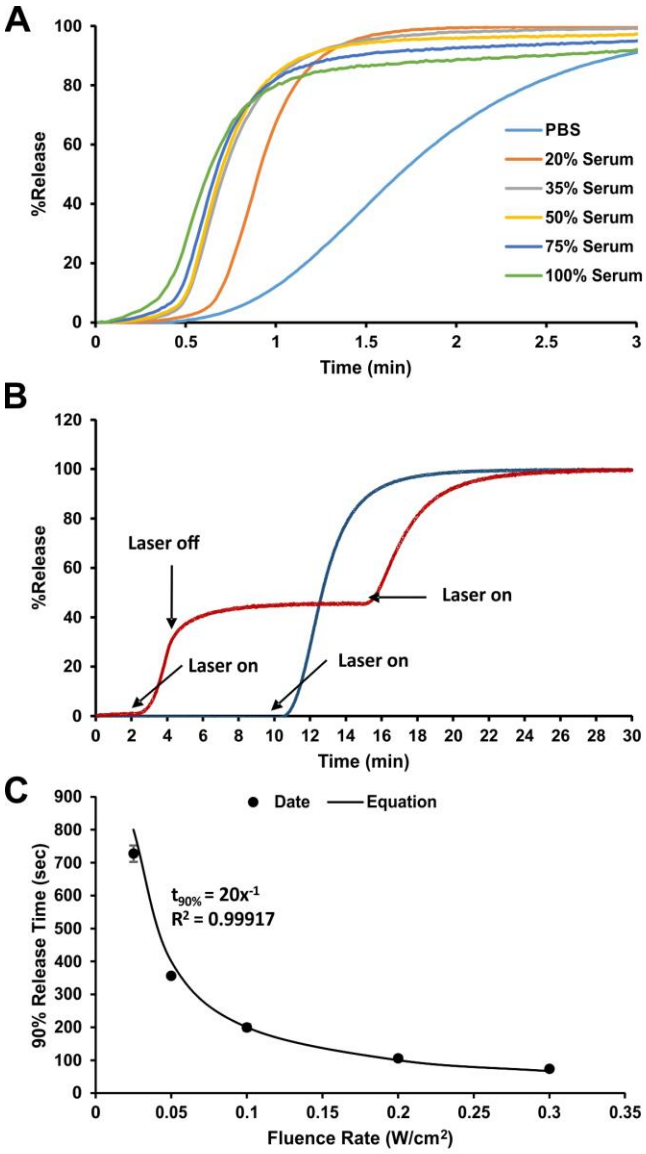

Figure S2 Release properties of IRT PoP liposomes. A) Laser-induced release of IRT from PoP liposomes (SPM:pyro-lipid:chol (53:2:45)) was tested in PBS containing different amounts of serum. The release was found to be slowest in pure PBS and accelerated as serum content was increased. **B)** Effect of laser treatment on release of IRT. Liposomes were diluted in PBS and IRT fluorescence monitored in real time. In case #1 the laser was turned on after 10 minutes (blue line) showing that there was no release until the laser was turned on. In the second case the laser was turned on after two minutes and then turned off after 4 minutes. At 15 minutes the laser was turned on again. When the laser is initially turned on the drug is released and stops when it is turned off, then continues to release when the laser is turned on a second time. In both cases a fluence rate of 200 mW/cm2 was used. **C)** Effect of fluence rate on IRT release. Fluence rates were varied between 25-300 mW/cm^2 and the time required for 90% release calculated. When the time (in sec.) is plotted against the fluence rate (in W/cm^2) the data points can be fit to a curve with the formula $20x^{-1}$ which represents the energy required to obtain 90% release (approximately 20 J/cm^2) divided by the fluence rate (in W/cm^2). This shows that the release of IRT is a function of the applied energy and the release rate can be tuned by varying the fluence rate for faster or slower release.

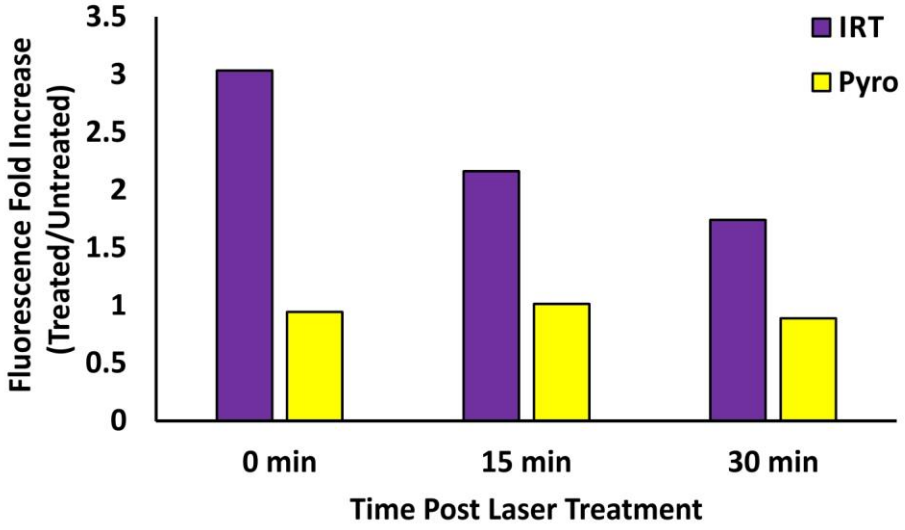

Figure S3 Relative IRT and Pyro fluorescence in treated and untreated ears. Histograms of the treated and untreated ears were taken to measure the channel values of IRT and pyro channels of images shown in Fig 4B. The values of the treated ear was divided by the values if the untreated ear. IRT was shown to be 2-3 folds higher in the treated ear versus the untreated ear. Pyro however is the same in both ears suggesting there is no release or accumulation of pyro in the treated region.

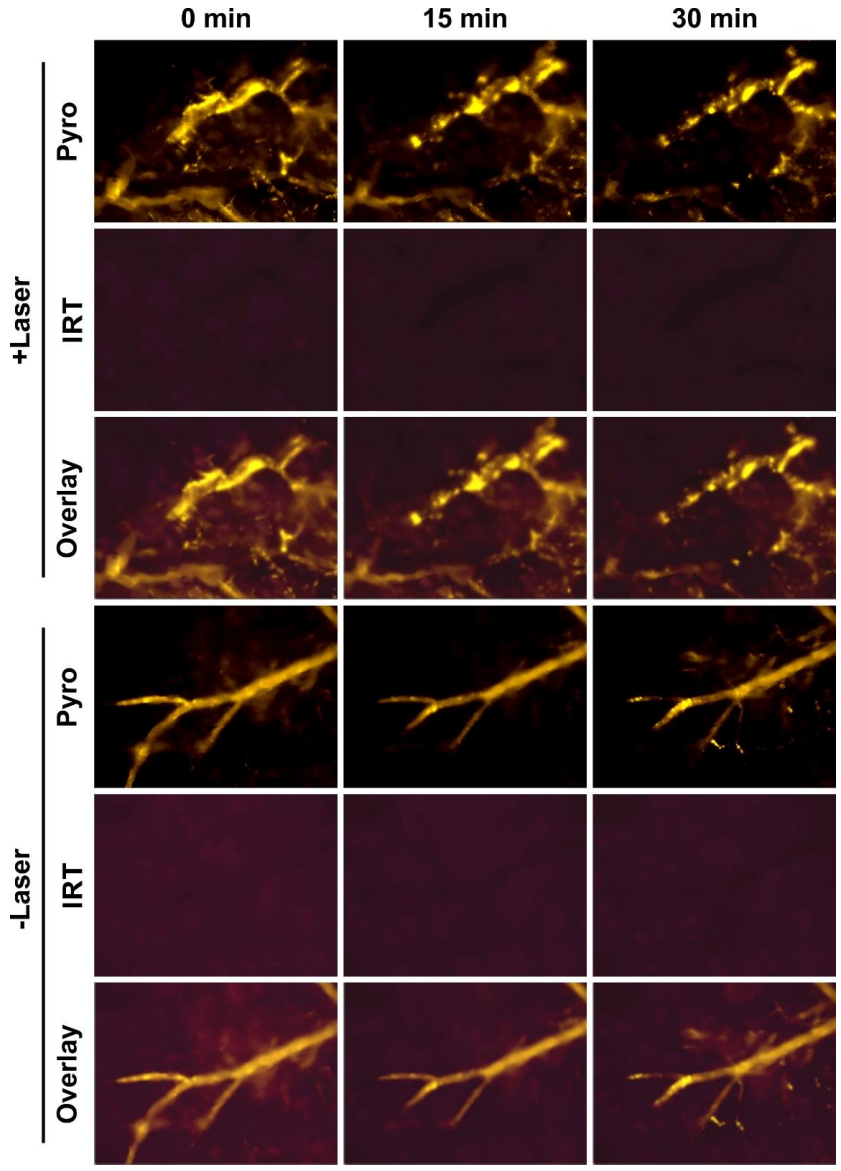

Figure S4: Intravital imaging following phototreatment with empty PoP liposomes and IRT-loaded SPM liposomes. Nude mice were injected with empty SPM:pyro-lipid:chol liposomes (10 mg/kg equivalent IRT dose) and pyro free IRT loaded liposomes. Following injection mice were anaesthetized with isoflurane and treated on one ear with a 665 nm laser at a fluence rate of 300 mW/cm^2 for 10 minutes. Following light treatment IRT and pyro in the treated and untreated ears were imaged using a fluorescent microscope. Unlike mice injected with IRT loaded PoP-liposomes there was no significant increase in IRT fluorescence observed suggesting the release observed in the IRT loaded PoP-liposomes was due to release of the drug from circulating and/or extravasated liposomes and not liposome accumulation.

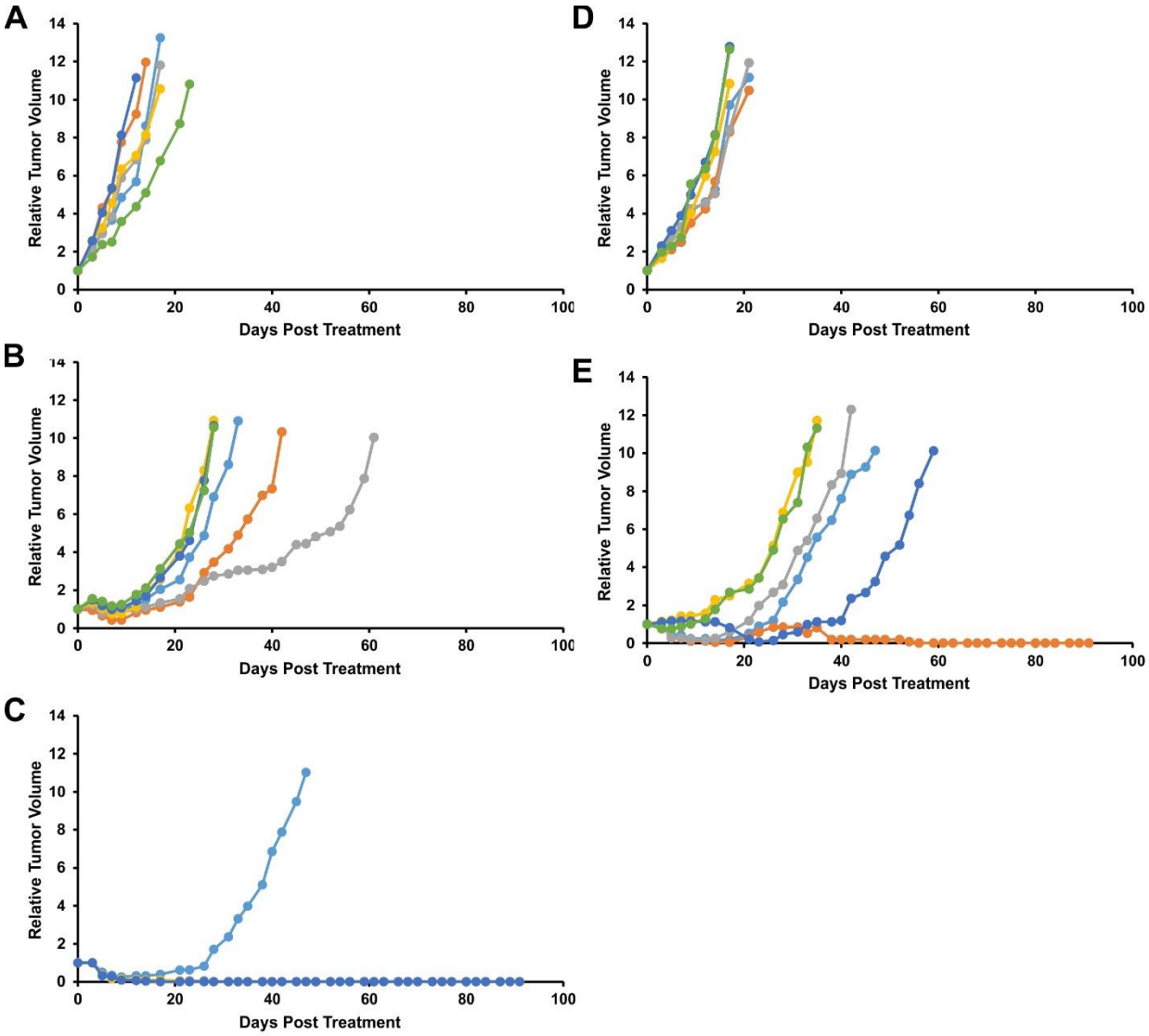

Figure S5: Individual tumor growth curves for mice treated **A)** saline, **B)** IRT PoP –laser, **C)** IRT PoP +laser, **D)** free IRT, and **E)** empty PoP +laser.

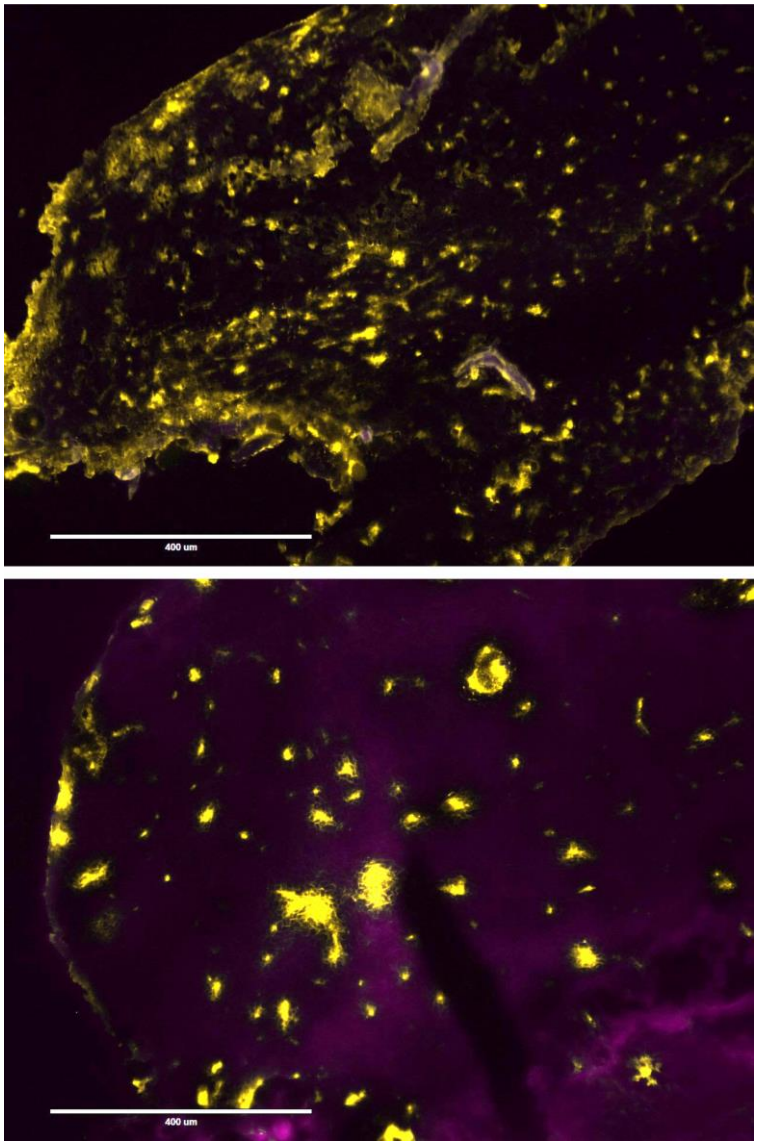

Figure S6 Tumor drug microdistribution. BALB/c mice bearing C26 tumors on both rear flanks were injected with 15 mg/kg IRT PoP liposomes. Following injection mice were anesthetized using isoflurane and one tumor was treated with a 665 nm laser at a fluence rate of 300 mW/cm^2 for 16.75 minutes. Mice were then sacrificed immediately and 24 hours after laser treatment and tumors surgically removed. Excised tumors were flash frozen in OCT compound using liquid nitrogen. The frozen tumors were cut into 10 µm slices using a cryostat and slices imaged in a EVOS FL Auto microscope using a DAPI filter cube (357 nm excitation; 477 nm emission) for IRT and a custom filter cube (400 nm excitation; 679 nm emission) for pyro.

Supporting Video 1 (see online): Intravital Imaging of IRT loaded PoP-liposomes. Mice were injected with 10 mg/kg IRT loaded PoP-liposomes. Following injection, the mice were anaesthetized with isoflurane positioned on the microscope stage. The treated ear was positioned between a glass slide and a glass coverslip. The ear was treated with a 665 nm laser at a fluence rate of 300 mW/cm^2 for 10 minutes (300 J/cm^2) and images obtained using the time-lapse feature of the microscope with 1 image taken every 3 seconds and assembled into a single video at a frame rate of 30 FPS. The video shows the first five minutes in which the laser was off, 10 minutes in which the laser was on and 10 minutes after the laser was turned off. IRT release can be seen immediately following the activation of the laser and decreases after the laser was turned off, which may be the result of the IRT washing away after release. Video caption shows time (in minutes) and laser status (on/off).

Supporting Video 2 (see online): Intravital Imaging of empty PoP-liposomes and IRT loaded std. liposomes. Mice were injected with empty PoP-liposomes (10 mg/kg equivalent IRT) and IRT loaded std. liposomes. Following injection, the mice were anaesthetized with isoflurane positioned on the microscope stage. The treated ear was positioned between a glass slide and a glass coverslip. The ear was treated with a 665 nm laser at a fluence rate of 300 mW/cm^2 for 10 minutes (300 J/cm^2) and images obtained using the time-lapse feature of the microscope with 1 image taken every 3 seconds and assembled into a single video at a frame rate of 30 FPS. The video shows the first five minutes in which the laser was off, 10 minutes in which the laser was on and 10 minutes after the laser was turned off. There is no notably increase in IRT fluorescence showing that IRT must be loaded in PoP-liposomes to obtain release. Video caption shows time (in minutes) and laser status (on/off).