

SUPPLEMENTARY MATERIALS

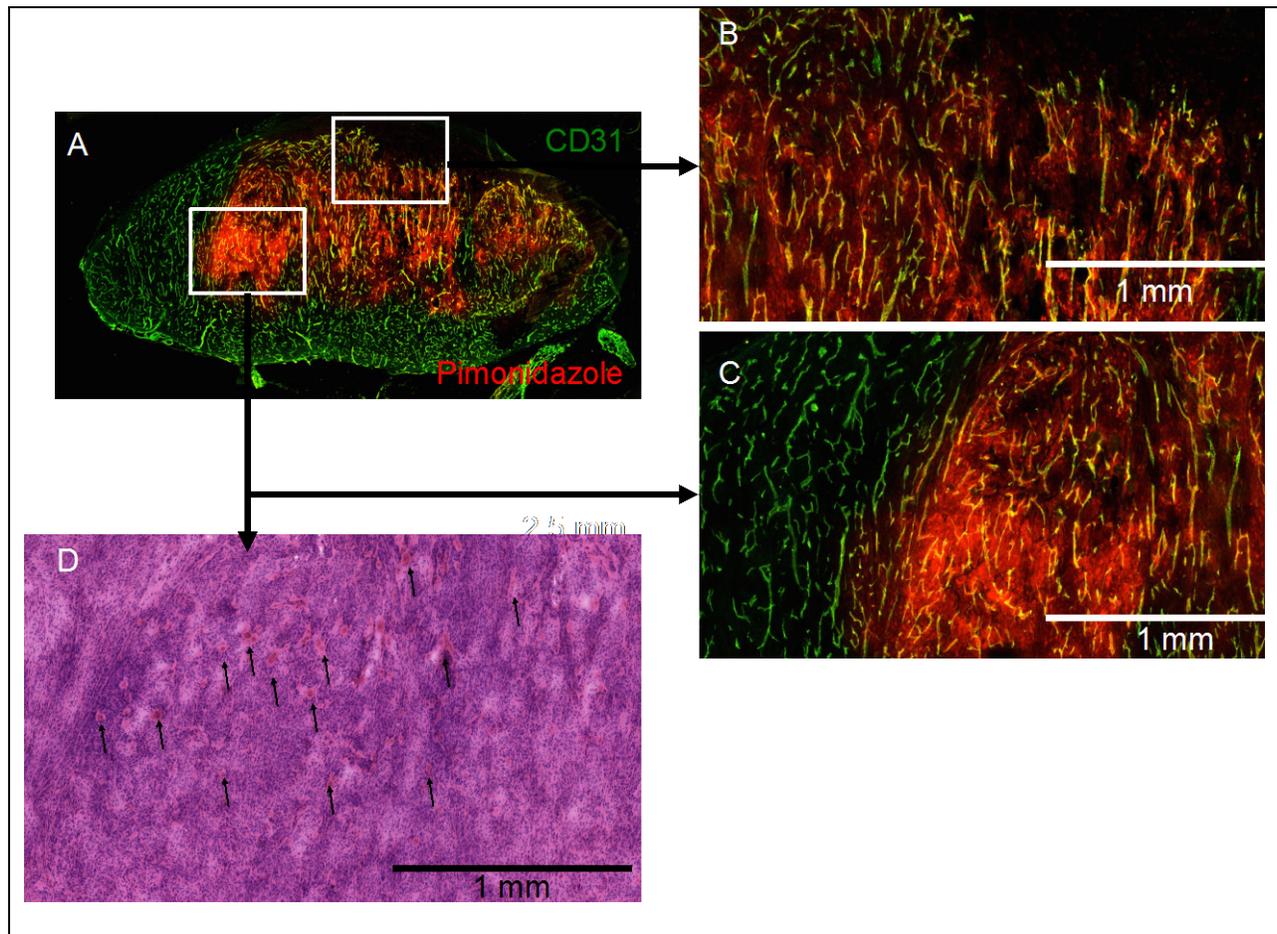


Figure S1: A. Immunofluorescence (IF) image of a tumor post-PDT in the 1-hr DLI group. Vasculature (CD31) is stained in green and hypoxia (pimonidazole stain) is shown in red. B & C are zoomed insets of regions highlighted in white rectangle in the IF image. D. H&E image of adjacent tissue section corresponding to the area shown in inset C. The black arrows indicate vascular congestion in the area of hypoxia.

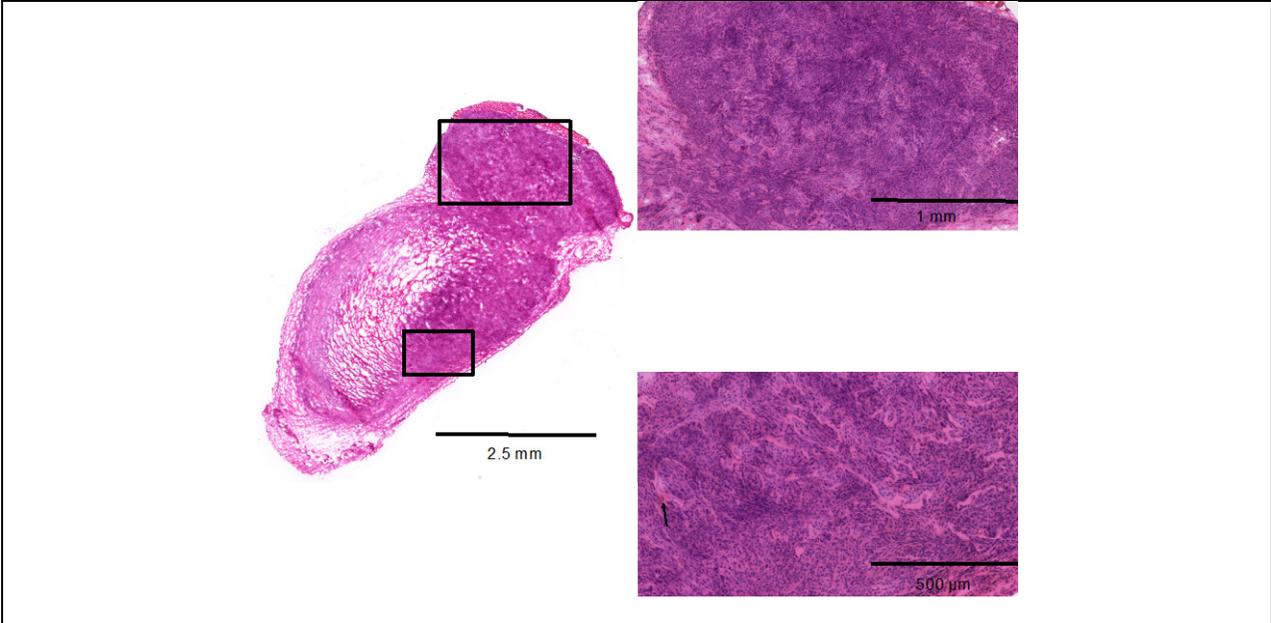


Figure S2: H&E image of a tumor in the 3-hr DLI group (from Fig. 4 bottom panel). Zoomed insets do not show significant vascular congestion. The zoomed regions are the areas with positive pimonidazole stain as shown in Fig. 4 bottom panel.

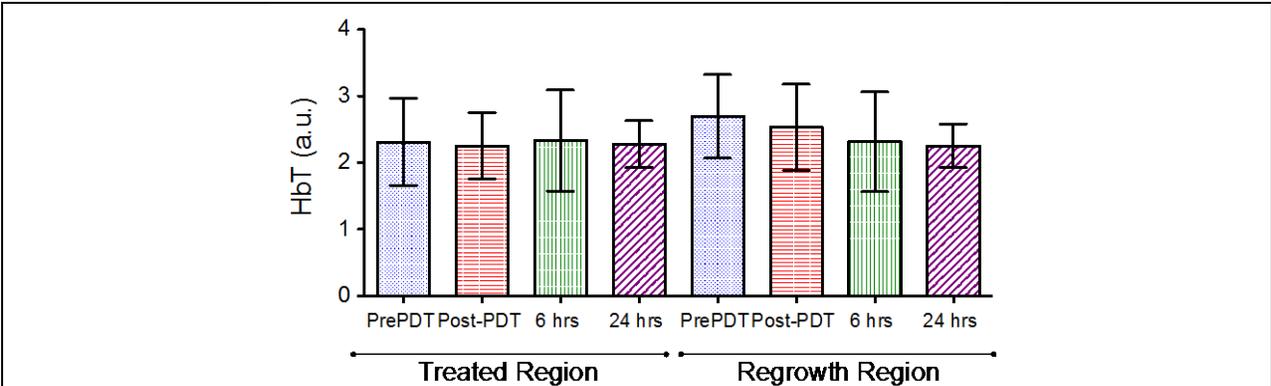


Figure S3. Mean HbT values at various time points (Pre-PDT, Post-PDT, 6-hrs and 24-hrs post-PDT) in tumors with regrowth in the 1-hr DLI group. Error bars indicate standard deviation.

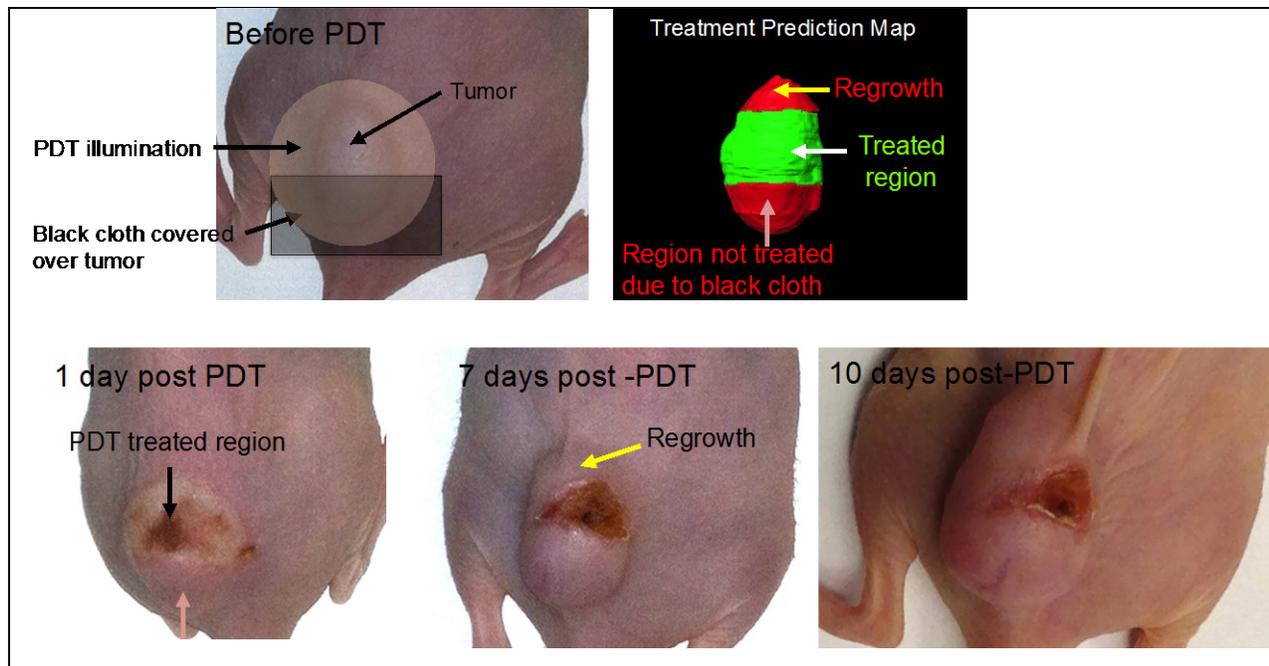


Figure S4: Photographs and prediction map in a tumor that was treated with PDT with bottom half of the tumor covered with black cloth. 1 day post treatment no PDT damage was observed in the region covered with black cloth (i.e., the area did not receive light, hence no PDT action). The prediction map obtained by 24-hrs post-PDT using oxygen saturation maps from PAI showcased the region not treated due to obstruction of light by black cloth (indicated by pink arrows). The 1 day post-treatment photograph showcases PDT damage in the illuminated region (black arrows). However, 7 days post-PDT regrowth in the tumor (yellow arrow) was observed. Indeed the prediction map showed that the top region of area of the tumor did not have sufficient treatment and hence would regrow. 10 days post-PDT photograph of the tumor shows aggressive regrowth in the top region of the tumor as indicated in the prediction map obtained within 24-hrs post-treatment.

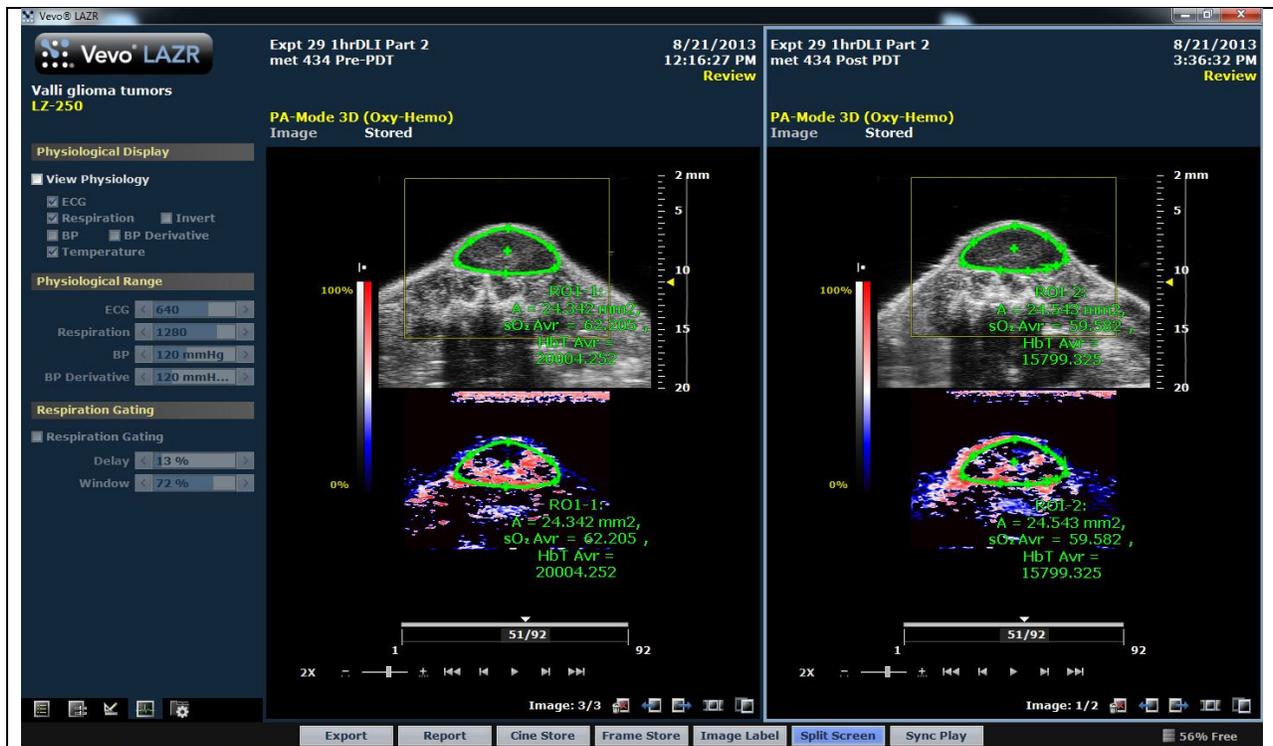


Figure S5: A screenshot of the split screen capability in the Vevo LAZR system software. The split screen feature enables real-time adjustment of the tumor location similar to that acquired prior to PDT.

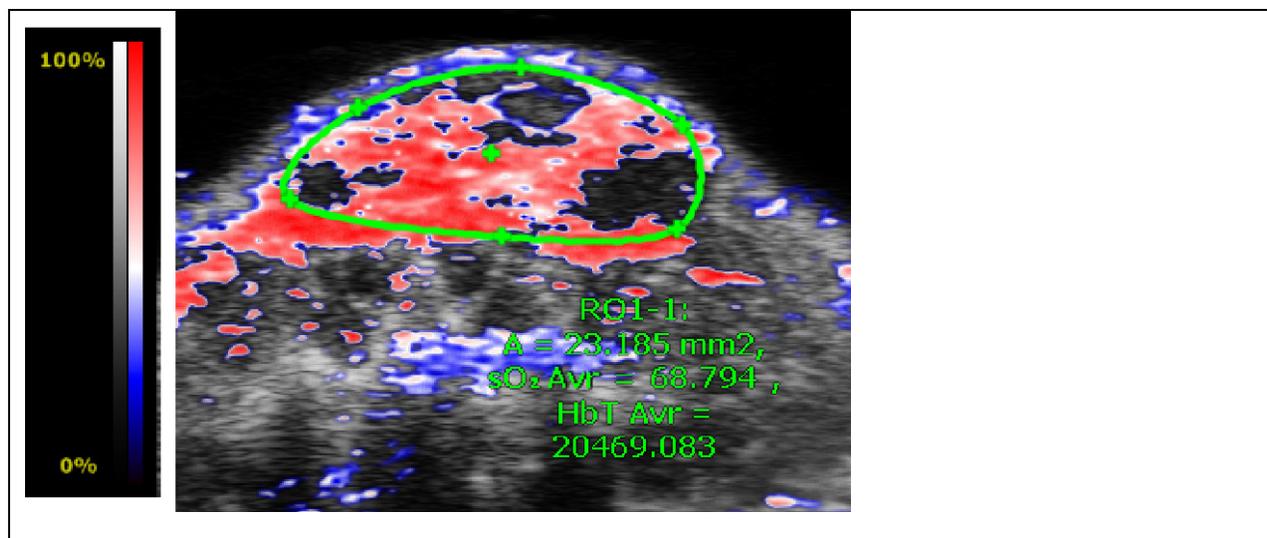


Figure S6: A representative screenshot of the ultrasound (grayscale) image overlaid with a StO₂ image of the subcutaneous tumor. The StO₂ image was pseudo-colored such that red represented oxygenated regions while blue represented hypoxic regions. The of the Vevo LAZR workstation software gave the area (A), average StO₂ (sO₂ Avr) and HbT values (HbT Avr) in the region of interest (ROI is shown in green).

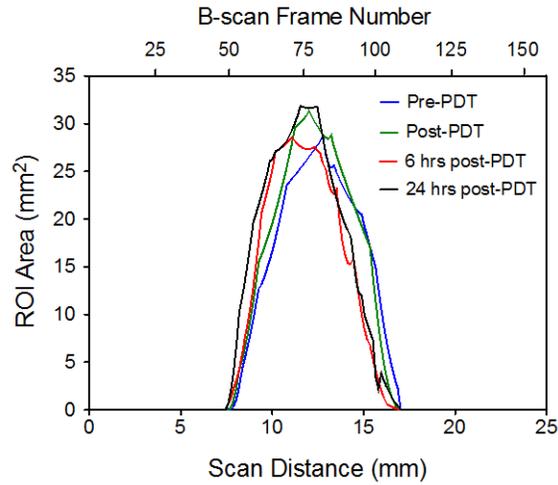


Figure S7. An example of the tumor ROI area as a function of 3D scan distance at different time points in the same mouse. As seen in Movie S2, the tumor ROI area has a normal distribution with increase in area as the scan moves towards the center of the tumor and decrease in area as the scan progress away from the tumor.

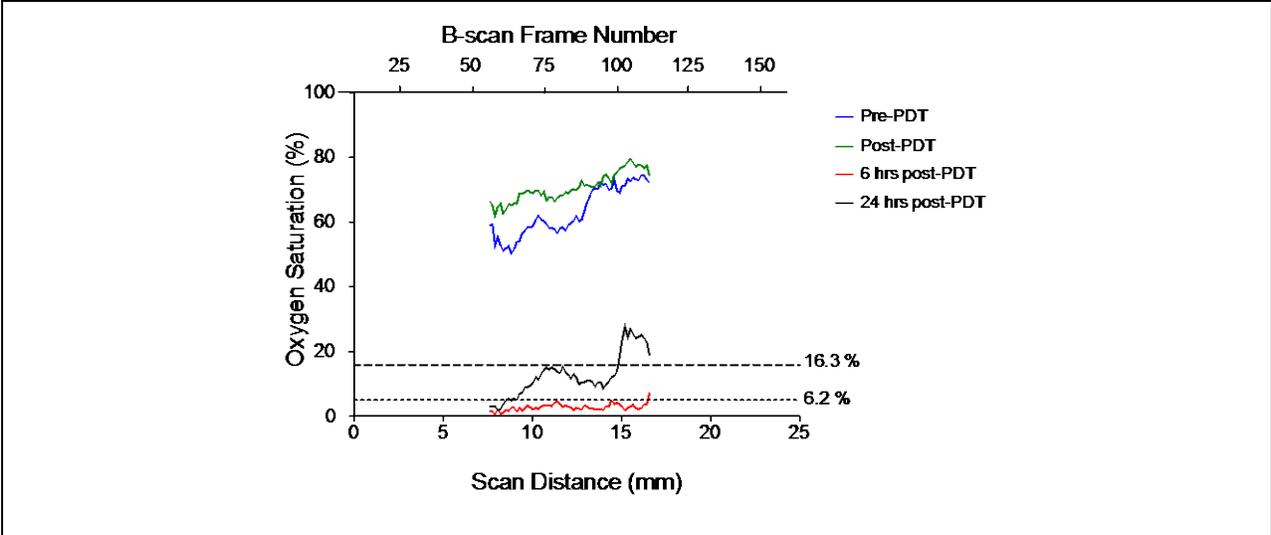


Figure S8: A representative StO₂ graph as a function of scan distance for a tumor at different time points. A customized MATLAB routine is written to verify if the StO₂ values at a particular B-scan frame is less than 6.2% and 16.3% at 6 hours and 24 hours post-PDT respectively. For a particular B-scan image (frame number), if the algorithm yielded a value of 1 (true case), the tumor ROI of that image is pseudo-colored green (representing treated region) in the 3D visualization software AMIRA else the ROI is pseudo-colored red (representing non-treated region).

Supplementary Tables

Table S1: Comparison of StO₂ values from the 1-hr DLI (responders) and 3-hr DLI (non-responders) groups at various time points using one-way ANOVA Tukey's multiple comparison test

Tukey's Multiple Comparison Test	Difference in the mean	Significant? P < 0.001?
Pre-PDT 3-hr DLI vs Pre-PDT 1-hr DLI	3.41	No
Post-PDT 3-hr DLI vs Post-PDT 1-hr DLI	-0.43	No
Pre-PDT 3-hr DLI vs Post-PDT 3-hr DLI	-0.63	No
Pre-PDT 1-hr DLI vs Post-PDT 1-hr DLI	-4.47	Yes
Pre-PDT 3-hr DLI vs 6-hrs 3-hr DLI	8.53	Yes
Pre-PDT 3-hr DLI vs 24-hrs 3-hr DLI	0.91	No
Pre-PDT 1-hr DLI vs 6-hrs 1-hr DLI	49.80	Yes
Pre-PDT 1-hr DLI vs 24-hrs 1-hr DLI	44.74	Yes
Post-PDT 3-hr DLI vs 6-hrs 3-hr DLI	9.17	Yes
Post-PDT 3-hr DLI vs 24-hrs 3-hr DLI	1.55	No
Post-PDT 1-hr DLI vs 6-hrs 1-hr DLI	54.27	Yes
Post-PDT 1-hr DLI vs 24-hrs 1-hr DLI	49.22	Yes
6-hrs 3-hr DLI vs 6-hrs 1-hr DLI	44.67	Yes
24-hrs 3-hr DLI vs 24-hrs 1-hr DLI	47.24	Yes
6-hrs 3-hr DLI vs 24-hrs 3-hr DLI	-7.62	Yes
6-hrs 1-hr DLI vs 24-hrs 1-hr DLI	-5.06	Yes

Table S2: Comparison of HbT values from the 1-hr DLI (responders) and 3-hr DLI (non-responders) groups at various time points using one-way ANOVA Tukey's multiple comparison test

Tukey's Multiple Comparison Test	Mean Diff.	Significant? P < 0.001?
Pre-PDT 3-hr DLI vs Pre-PDT 1-hr DLI	-0.086	No
Post-PDT 3-hr DLI vs Post-PDT 1-hr DLI	-0.17	No
Pre-PDT 3-hr DLI vs Post-PDT 3-hr DLI	-0.038	No
Pre-PDT 1-hr DLI vs Post-PDT 1-hr DLI	2.22	No
Pre-PDT 3-hr DLI vs 6-hrs 3-hr DLI	0.18	No
Pre-PDT 3-hr DLI vs 24-hrs 3-hr DLI	0.22	No
Pre-PDT 1-hr DLI vs 6-hrs 1-hr DLI	-0.5838	Yes
Pre-PDT 1-hr DLI vs 24-hrs 1-hr DLI	0.01	No
Post-PDT 3-hr DLI vs 6-hrs 3-hr DLI	0.22	No
Post-PDT 3-hr DLI vs 24-hrs 3-hr DLI	0.25	No
Post-PDT 1-hr DLI vs 6-hrs 1-hr DLI	-0.80	Yes
Post-PDT 1-hr DLI vs 24-hrs 1-hr DLI	-0.21	No
6-hrs 3-hr DLI vs 6-hrs 1-hr DLI	-0.85	Yes
24-hrs 3-hr DLI vs 24-hrs 1-hr DLI	-0.3	No
6-hrs 3-hr DLI vs 24-hrs 3-hr DLI	0.03	No
6-hrs 1-hr DLI vs 24-hrs 1-hr DLI	0.59	Yes

TABLE S3: Receiver-operating-curve analysis of the two parameters StO₂ at 6-hrs and StO₂ at 24-hrs

Parameter	Area under curve	Standard Error	95% Confidence Interval
StO ₂ at 6-hrs post PDT	0.978	0.00530	0.967 to 0.988
StO ₂ at 24-hrs post PDT	0.991	0.00344	0.984 to 0.998

TABLE S4: Pair wise comparison of ROC curves of the two parameters StO₂ at 6-hrs and StO₂ at 24-hrs

Difference between ROC areas	0.0131
Standard Error	0.00640
95% Confidence Interval	0.000575 to 0.0257
Z statistic	2.050
Significance level	P = 0.0404

Table S5: Comparison of HbT values from the treated regions and possible regrowth regions in the 1-hr DLI groups at various time points using one-way ANOVA Tukey's multiple comparison test.

Tukey's Multiple Comparison Test	Difference in the mean	Significant? P < 0.001?
Pre-PDT treated region vs Pre-PDT regrowth region	-0.38	No
Post-PDT treated region vs Post-PDT regrowth region	-0.27	No
Pre-PDT treated region vs Post-PDT treated region	0.06	No
Pre-PDT regrowth region vs Post-PDT regrowth region	0.16	No
Pre-PDT treated region vs 6-hrs treated region	-0.02	No
Pre-PDT treated region vs 24-hrs treated region	0.035	No
Pre-PDT regrowth region vs 6-hrs regrowth region	0.38	No
Pre-PDT regrowth region vs 24-hrs regrowth region	0.44	No
Post-PDT treated region vs 6-hrs treated region	-0.08	No
Post-PDT treated region vs 24-hrs treated region	-0.02	No
Post-PDT regrowth region vs 6-hrs regrowth region	0.22	No
Post-PDT regrowth region vs 24-hrs regrowth region	0.27	No
6-hrs treated region vs 6-hrs regrowth region	0.017	No
24-hrs treated region vs 24-hrs regrowth region	0.025	No
6-hrs treated region vs 24-hrs treated region	0.05	No
6-hrs regrowth region vs 24-hrs regrowth region	0.06	No

Table S6: Physical characteristics of liposomes encapsulating BPD

Concentration (μM)	Size (nm)	Zeta Potential (mV)	Polydispersity Index
238.7	134.16	15.26	0.0744

Supplementary Movie Legend

Movie S1. Movie showing 3D scan of the tumor along the Y- axis (Blue color). The X- axis and Z- axis are shown in red and green color respectively. A 3D scan consists of several B-scan images of ultrasound images (grayscale) overlaid with StO₂ images. The StO₂ image was pseudocolored such that red represented oxygenated regions while blue represented hypoxic regions.

Movie S2. The movie shows the tumor ROI area at different frames on the 3D scan. The area of the ROI (A), average StO₂ and HbT values within the ROI (Fig. S6), are calculated using the OxyZated™ tool and the HemoMeaZure™ tool of the Oxy-Hemo feature of the Vevo LAZR software (VisualSonics, Canada).