Supplementary Materials for

Enhanced Anti-Tumor Efficacy through a Combination of Integrin αvβ6-Targeted Photodynamic Therapy and Immune Checkpoint Inhibition

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Supplementary Methods

Integrin αvβ6 expression of 4T1 tumor cells

The expression status of murine integrin αvβ6 in 4T1 cell was tested by cell immunofluorescence staining. Briefly, 4T1 cells or HEK293 cells (negative control) grown in 35-mm MatTek glass bottomed culture dishes were fixed using 4% paraformaldehyde. After blocking with 10% fetal bovine serum in PBS, cells were incubated with an anti-mouse integrin β6 primary antibody (R&D Systems, Minneapolis, MN) for 1 h and then visualized with a dye-labeled secondary antibody using a Leica TCS-NT confocal microscope (Wetzler, Heidelberg, Germany).

Detection of singlet oxygen

IRDye700 (1.0 μM) or DSAB-HK (1.0 μM IRDye700 equivalent concentration) solution was mixed with 1.0 μM singlet oxygen sensor green (SOSG) (Invitrogen, Carlsbad, CA) and then irradiated with a 690-nm laser (Shanghai Laser & Optics Century Co., Ltd., Shanghai, China) for various periods of time. SOSG fluorescence was measured with the IVIS optical imaging system (Xenogen, Alameda, CA; excitation wavelength = 465 nm; emission wavelength = 520 nm). For the specificity experiments, 50 mM singlet oxygen quencher NaN_3 was added to the solution and singlet oxygen molecules generated by DSAB-HK were detected using the same protocol.

In vitro PDT

Cell viability assay was performed to determine the effect of DSAB-HK PDT on tumor growth. Briefly, 4T1 cells (5×10^3/well) grown in 96-well plates were incubated with PBS (vehicle control), 100 nM DSAB-HK or DSAB for 1 h at 37°C. After washing with PBS, cells were irradiated at 0, 4, 8, and 16 J/cm^2 with a 690-nm laser. Cell viability was then determined using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan).

Ex vivo NIRF imaging

4T1 tumor-bearing mice (n = 5 per group) were injected with 0.5 nmol DSAB-HK or DSAB with or without a blocking dose (300 μg) of HK peptide through the tail vein. At 8 h p.i., the mice were sacrificed. The tumors and major tissues/organs were harvested, placed on the black papers, and then sujected to NIRF imaging using the IVIS optical imaging system.

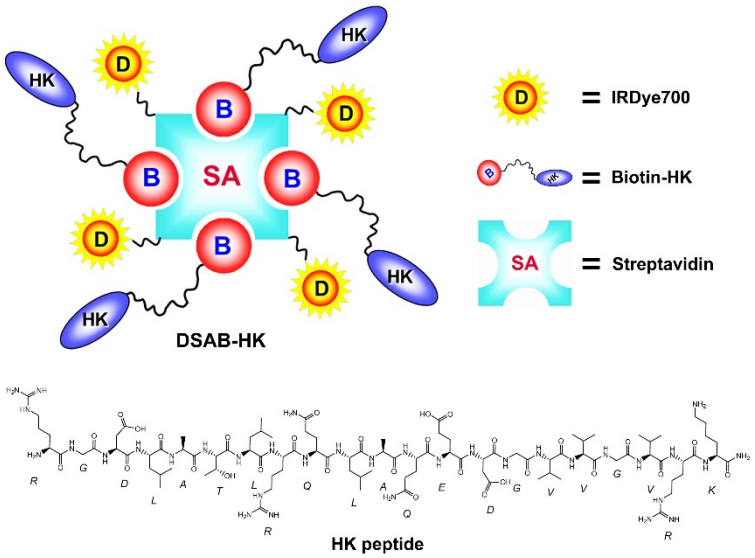

Figure S1. Schematic illustration of the integrin αvβ6-targeting NIRF probe DSAB-HK.

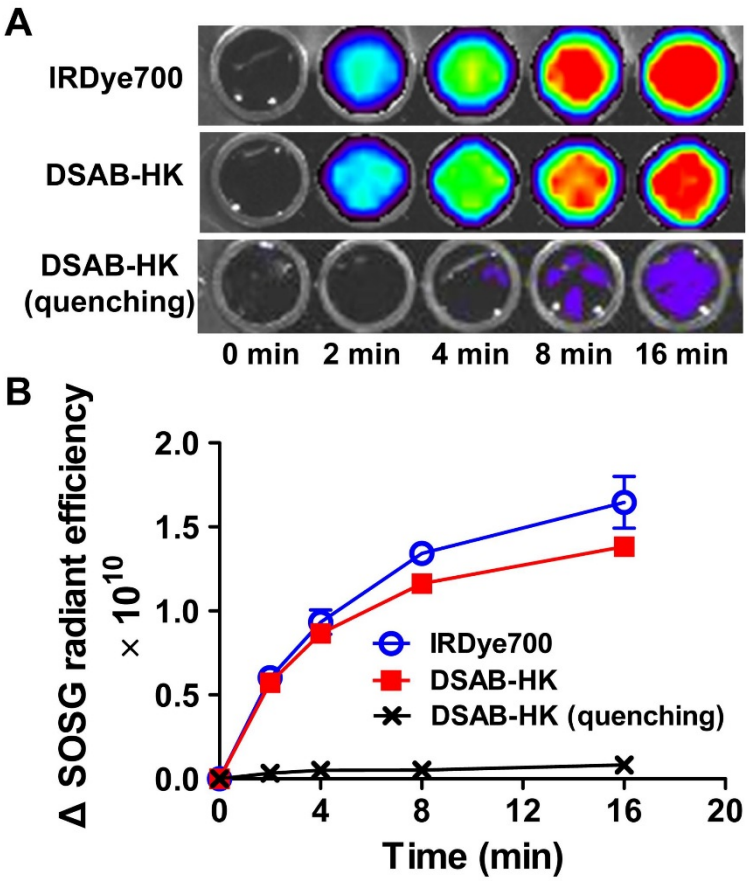

Figure S2. Singlet oxygen generation of IRDye700 and DSAB-HK (with or without 50 mM NaN_3 quenching) after irradiation for different periods of time as determined by the SOSG assay.

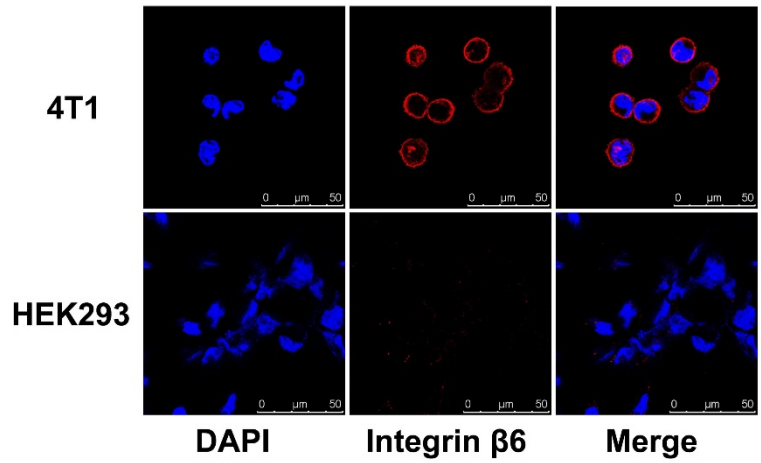

Figure S3. Immunofluorescence staining of 4T1 and HEK293 (negative control) cells for murine integrin β6 using an anti-integrin β6 primary antibody followed by visualization using a dye-labeled secondary antibody.

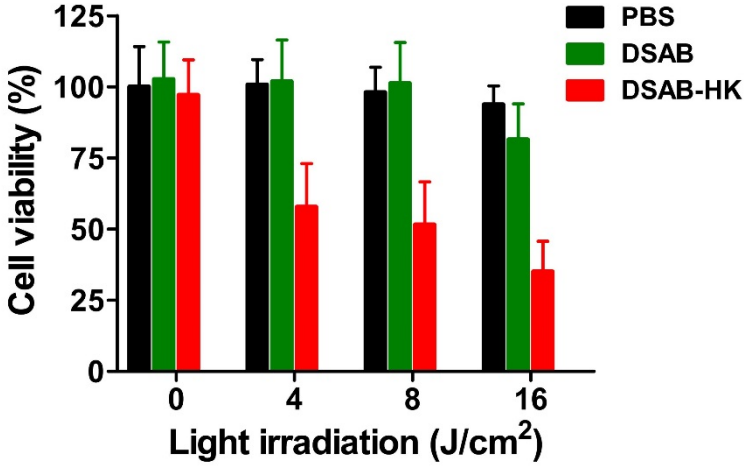

Figure S4. Tumor-specific cytotoxicity of DSAB-HK PDT in vitro as determined by the Cell Counting Kit-8 assay using a kit.

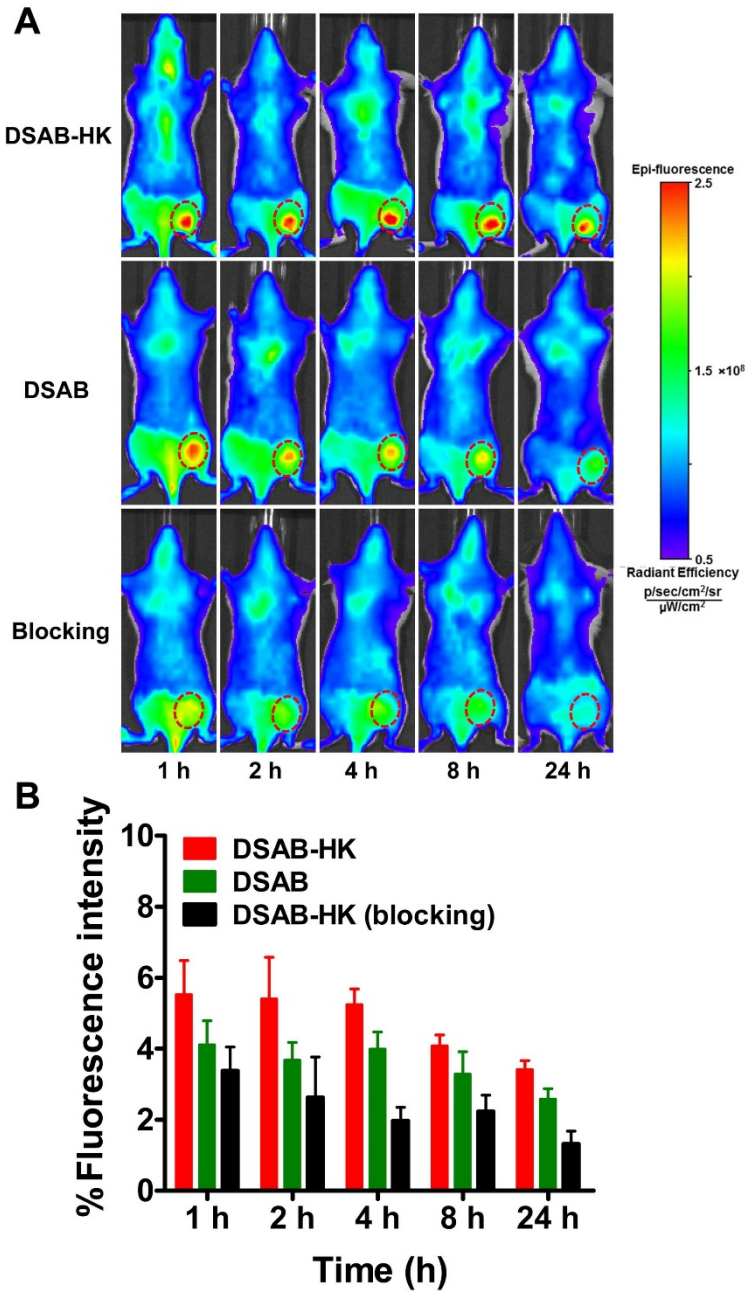

Figure S5. In vivo NIRF imaging (A) and quantified tumor uptake (B) of 4T1 tumor-bearing mice at 1, 2, 4, 8 and 24 h after injection of DSAB-HK (with or without a blocking dose of the HK peptide) or DSAB. Tumors are indicated with circles.

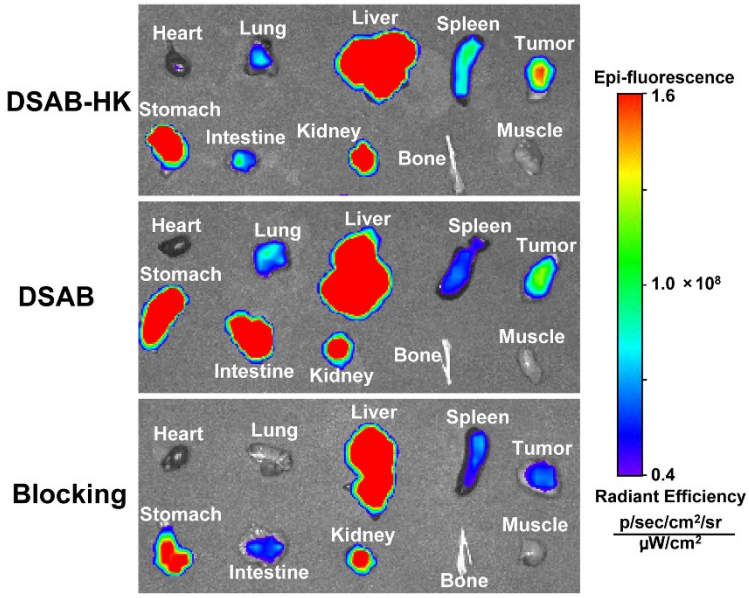

Figure S6. Ex vivo NIRF imaging of major organs at 8 h postinjection of DSAB-HK (with or without the HK peptide blocking) or DSAB in subcutaneous 4T1 tumor mice.

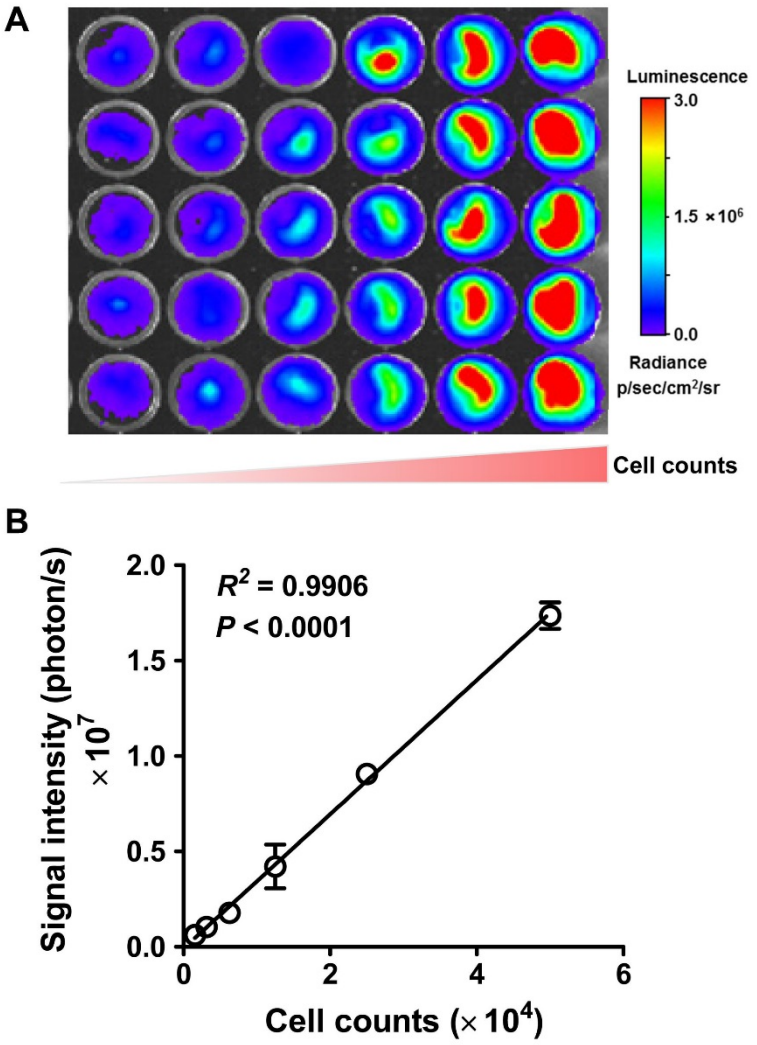

Figure S7. Bioluminescence imaging of varying numbers of 4T1-fLuc cells plated on 96-well plates showed a linear correlation between the bioluminescence signal intensity and tumor cell number.