MASTVRTDMEVSSELFSNGCTFWQRVLLTASLLTCWLLPTTARVTIESLP 50

PQVVEGENVLLRVDNMPENLLVFGWYRGMTNMRHAIALHSLYYSVTAKGL 100

KHSGRETLYINGTLWIQNVTQEDTGYYTFQTISKQREMVSNTSLYLHVYS 150
GLHTSNTNLYLH

SLFICGRPTTLVPPTIELVPASVAVGGSILLLVHNIPKYLQSLFWYKGLI 200

VFNKVEIARYRTAKNSWEPGPAHSGRETVYSNGSLLLQNVTWKDTGFYTL 250

RTLTRYQKMELAHIYLQVDTSSSLCCDTLDSAQLGIDPVPRHAAEGGSVL 300

LQVHNLPEDVQTISWYKGVLSTKDFKIAEYSILTKSIISGRAHSRRETGY 350

TNGSLLLQDVTEKDSGLYTLVTIDSNVRVVTAHVQVNIHKLVTQPAMRVT 400
SGLYKVAYDWQH

DSTVRVQSSVVFTCFSDNTGISIRWLFNNQRLQLTERMTLSPSKCQLRIH 450

TVRKEDAGDYQCEAFNPVSSKTSLPISLTVMNE 483

**Figure S1. Similarity between C1 or C2 and a protein called carcinoembryonic antigen-related cell adhesion molecule 3-like isoform X1 (NCBI Reference Sequence: XP_021010770.1).** The C1 and C2 are aligned under the full sequence of the protein with the identical amino acids highlighted in red and yellow, respectively. C1 and the protein share a motif of SNTXLYLH (X=unshared amino acid) (highlighted in red). C2 and the protein share a motif of SGLY (highlighted in yellow). To know more about this protein, visit this website: https://www.ncbi.nlm.nih.gov/protein/1195666353/

## *Experimental Procedures*

***Cells and mice.*** The cells used in the experiment included melanoma cells A375 (ATCC-CRL-1619) and human epidermal melanocytes (HEM, ATCC-PCS-200-013) from ATCC (The Global Bioresource Center). The cells were incubated at 37℃ with 5% $CO_2$ with the proper medium. For A375 cells, the medium used was DMEM (ATCC-PCS-30-2002) supplemented with 10% (v/v) fetal bovine serum. For HEM cells, the medium used was Dermal cell basal medium (ATCC-PCS-200-030) supplemented with an adult melanocyte growth kit (ATCC-PCS-200-042). A375 cells were harvested with 0.25% trypsin and 1 mM EDTA while HEM cells were harvested with 0.05% trypsin and 0.02% EDTA (ATCC-PCS-999-003). BALB/c mice (female, 4-6 weeks old) were purchased for in vivo biopanning and therapy. Melanoma tumor bearing mice were prepared by injecting A375 cells ($2\times10^6$) into the flank of nude mice subcutaneously. The tumor volume was checked twice every week. Once the tumor diameter reached about 0.5 cm, tumor bearing mice were ready for use. The protocols for animal studies were approved by the Institutional Animal Care and Use Committee of Zhejiang University.

***Phage library.*** The phage library we used was Ph.D.-12 from NEW ENGLAND Biolabs. This library is made of billions of M13 phage clones with each clone displaying a 12-mer peptide at the N-terminus of p3 minor coat protein.

***In vitro biopanning.*** We followed our reported in vitro biopanning protocol to select melanoma cell-targeting peptides with slight modification [9]. Briefly, $10^{11}$ phage virions

diluted in 1 ml blocking buffer (0.5 % BSA (w/v) +DMEM) were incubated with empty 60×15 mm Petri dishes at room temperature for 1 h first to deplete the phages which bound to the Petri dishes. The depleted phage library was then incubated with control cells (normal skin cells, HEM) at room temperature for 1 h to remove the phages which could specifically bind to the normal skin cells. The depleted phage library (input) was transferred to and incubated with the target A375 cells at room temperature for 1 h. The medium was shaken slightly every 10 min. Phages that did not bind to the target A375 cells were removed by discarding the supernatant. Phages that weakly bound to the target cells were washed away from the target cells by washing for 10 times using a washing buffer (0.5% BSA (w/v) + 0.1% Tween (v/v) + DMEM). Phages (output) that tightly bound to the target cells were eluted from the cells using an elution buffer (pH 2.2 Glycine-HCl + 1 mg/ml BSA) on ice for 10 min. The eluted solution was neutralized with 1M Tris-HCl (pH=9.1). The output was then used as a new input for the next round of biopanning.

***In vivo biopanning.*** We followed a previously reported in vivo biopanning protocol to search for tumor tissue-homing peptides [10]. Briefly, the phage library ($10^{11}$ pfu) in 200 μl PBS was used as an input and injected into the melanoma tumor-bearing mice intravenously. After the phage library was circulated in the mice for 24 h, the mice were euthanized by $CO_2$ asphyxiation. PBS buffer was used to remove the phages not associated with the tumors by heart perfusion. Then the tumors were excised and ground. The tumor tissue-homing phages (output) in the ground mixture were amplified by infecting bacteria and their genome was commercially sequenced to determine the tumor

tissue-homing peptides displayed at the tips of the homing phages. The output was then used as an input and injected into new tumor-bearing mice for the next rounding of in vivo biopanning.

***Phage Amplification.*** The output phages eluted from the melanoma cells or separated from the melanoma tumors were amplified by infecting the early-log phase ER2738 ($OD_{600}$=0.01-0.05) with vigorous shaking for 4.5 h at 37 ℃. Then the phages in the supernatant of the culture solution were precipitated twice with 20% polyethylene glycol (PEG)/NaCl. The purified phages were used as the new input phage library for the next round of biopanning.

***Phage titering.*** After 3 rounds of selection, the eluted output phages were titrated by infecting the bacteria ER2738 and then being poured on the LB/PTG/Xgal plate to develop colonies. Random colonies were picked up and sequenced to determine the sequence of targeting peptides displayed at the tip of the targeting phages.

***Binding affinity assessment.*** The A375 cell binding affinity and specificity of the peptides displayed at the tip of the selected phages were determined by an output/input assay and enzyme linked immunosorbent assay (ELISA). The output/input assay was similar with the biopanning process. Briefly, phages displaying a particular peptide sequence ($10^{10}$ pfu) were incubated with targeted A375 cells for 1 h at room temperature. Then unbound phages were removed by washing with a washing buffer (0.5% BSA (w/v)

+ 0.1% Tween (v/v) + DMEM) for 10 times. Subsequently, the bound phages were eluted from the cell surface using an elution buffer (pH 2.2 Glycine-HCl + 1 mg/ml BSA) on ice for 10 min, followed by neutralization with 1 M Tris-HCl (pH=9.1). Then the eluted phages were titrated by infecting the bacteria ER2738 on the LB/PTG/Xgal plates.

Phage ELISA was performed following our previously reported protocol [9]. Briefly, A375 cells were cultured in 96 well plates overnight and fixed by 4% paraformaldehyde (Sigma-Aldrich Co. LLC, USA) for 15 min, followed by washing with PBS for 3 times. Subsequently, the cells were blocked with 0.5%BSA+PBS for 0.5 h at room temperature. Phages displaying a specific peptide ($2\times10^9$ pfu) in 0.5%BSA+PBS were added and incubated with the cells for 1 h at room temperature. After washing with PBST (PBS+0.1% Tween 20) for 3 times and PBS for 2 times, the cells were incubated with anti-M13 phage antibody (SCBT, US) that recognized the major coat of the phages for 1 h at room temperature. After the cells were washed with PBST and PBS as before, a secondary antibody, anti-mouse IgG-Alkaline Phosphatase antibody (Sigma, US), was added to each well and incubated for 1 h at room temperature. After each well was washed with PBST for 3 times and PBS for 2 times, p-nitrophenyl phosphate (pNPP) was then added and the absorption of each well was determined as optical density (OD) at 405 nm.

***Immunofluorescence assay.*** A375 cells were cultured in 24 well plates overnight, blocked with 0.5% BSA+DMEM for 0.5 h at room temperature, and then incubated with

different phages ($2\times10^{10}$ pfu) in 0.5% BSA+DMEM for 1 h at 37℃. After the cells were washed with PBST for 3 times and PBS for 2 times, the cells were incubated with anti-M13 phage antibody (SCBT, US) for 1 h at 37 ℃ and washed with PBST and PBS as the previous steps. Then anti-mouse IgG-R (SCBT, US) was added to each well and incubated for 1 h at 37 ℃. DAPI was used to stain nuclei. Finally, the cells were observed under a Nikon fluorescence microscope.

**In vivo photodynamic therapy**. The tumor-bearing mice were divided into the following groups (n=8): (1) Phage nanofibers with C1 displayed at the tips and with PPa conjugated on the side walls; (2) Phage nanofibers with C2 displayed at the tips and with PPa conjugated on the side walls; (3) Phage nanofibers with C3 displayed at the tips and with PPa conjugated on the side walls; (4) Phage nanofibers with T1 displayed at the tips and with PPa conjugated on the side walls; (5) Wild type phage nanofibers with no peptide displayed at the tips but with PPa conjugated on the side walls; (6) Phosphate buffered saline. One day after *i.v.* injection of the therapeutic agents at the phage dose of $2\times10^{14}$ PFU into the mice, the tumor sites of the mice were irradiated by the 658 nm laser for 5 min under a power density of 1.25 W/cm$^2$ to trigger PDT. Then the tumor volume was monitored for a period of 25 days.