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SUPPLEMENTARY INFORMATION

Folic acid functionalized hollow nanoparticles for selective photodynamic therapy of cutaneous squamous cell carcinoma

Duncan Hieu M. Dam,[a] Lingzhi Zhao,[bc] Sophia A. Jelsma,[a] Yanli Zhao,[b]* and Amy S. Paller[a]*

[a]Department of Dermatology, Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611 USA

[b]Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 637371, Singapore

[c]State Key Laboratory of Natural Medicine, School of Basic Medical Sciences and Clinical Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu 211198, P. R. China

Email: apaller@northwestern.edu, zhaoyanli@ntu.edu.sg

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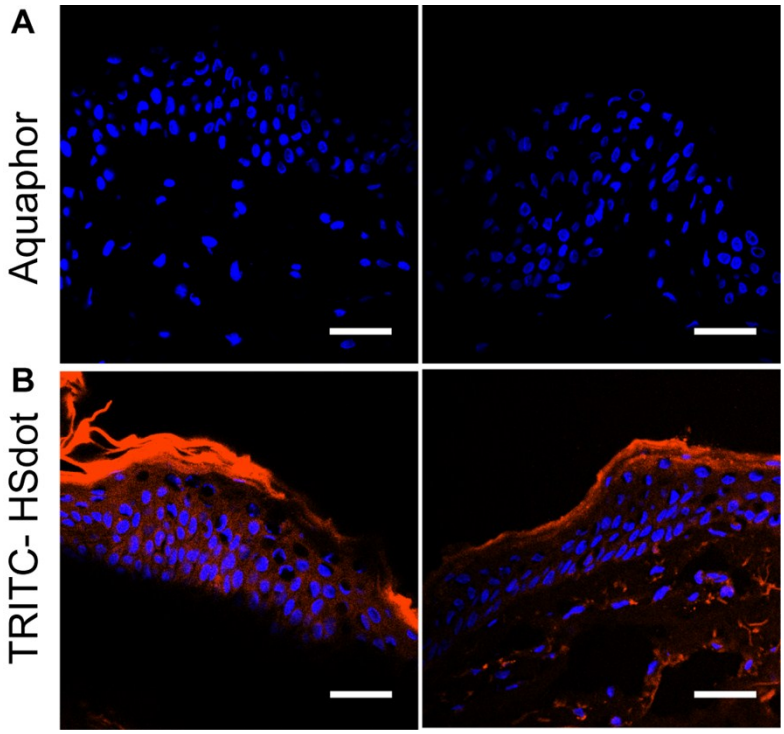

Figure S1: Penetration of nanocarrier HSdots through human intact skin. 5 mg/mL of TRITC-labeled HSdots were mixed with Aquaphor (1:1) to make a final concentration of 2.5 mg/mL HSdots. The HSdots were then topically applied on the surface of human abdominoplasty skin *ex vivo* for 72 hours. Confocal images of abdominoplasty sections showed increased TRITC signals in samples applied with TRITC-HSdots compared to samples only treated with Aquaphor. Scale bar: 100 μm.

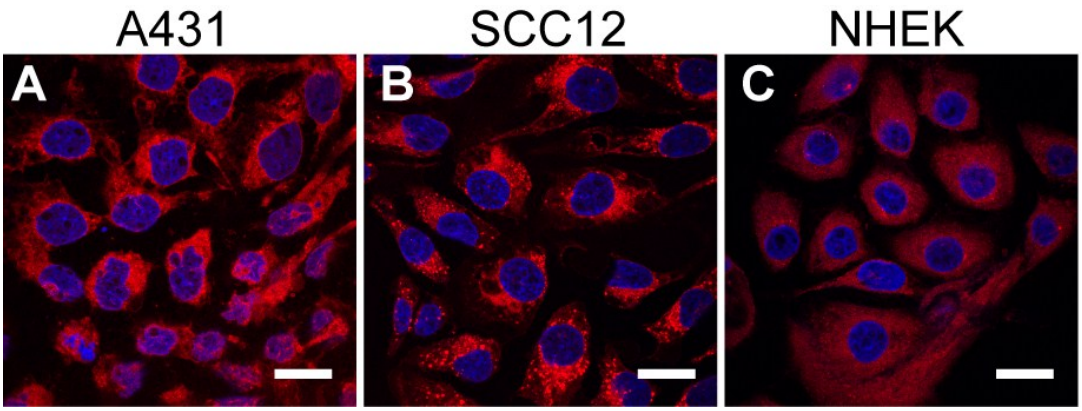

Figure S2: Uptake of ZnPC-loaded HSdots in SCC and normal cells. ZnPC-loaded TRITC-labeled HSdots were observed in (A) A431 and (B) SCC12 after 48 hours of incubation. (C) ZnPC-loaded TRITC-labeled HSdots were also found in NHEK after 48 hours of incubation. Scale bar: 20 μm.

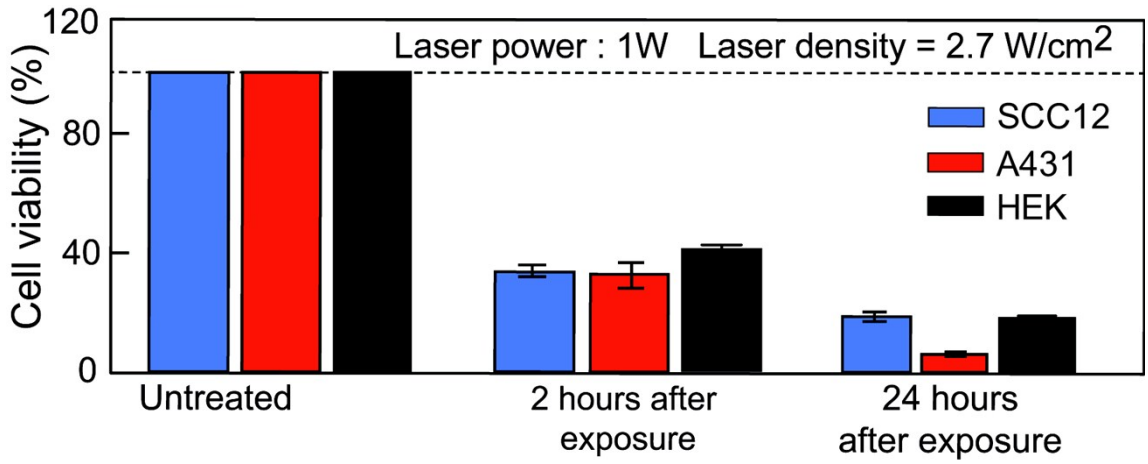

Figure S3: Toxicity of ZnPC-loaded HSdots after laser exposure. Cells were treated with ZnPC-loaded HSdots at 2mg/mL and exposed to 671 nm laser for 2 min. Two hours after exposure, death was observed in more than 60% of SCC cells and NHEKs. Cell death increased to 80% after 24 hours of exposure.

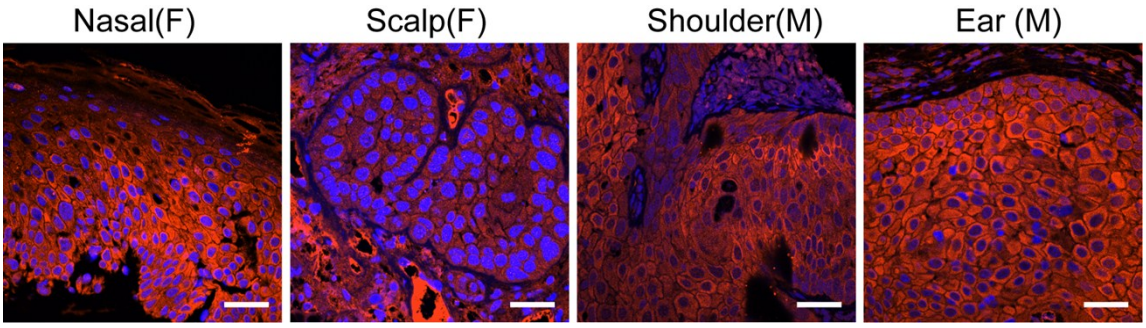

Figure S4: Expression of folate receptors in different SCC tissues. Expression of folate receptors was observed in different SCC tissues in both female and male patients. Scale bar: 50 μm.

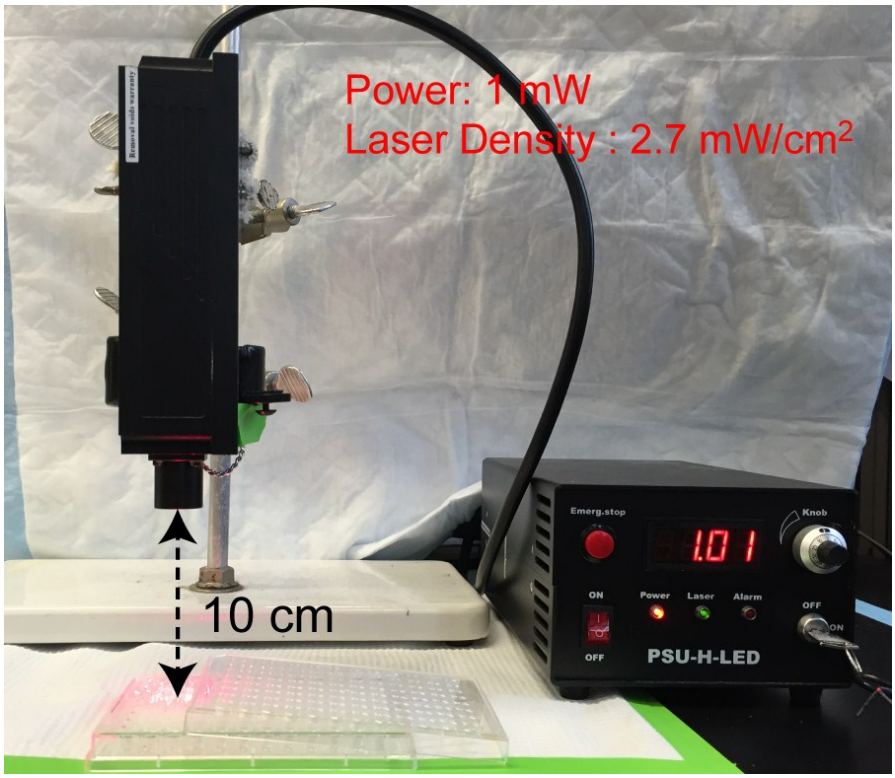

Figure S5: Laser setup for laser exposure. The 671-nm diode laser was mounted 10 cm directly above the sample for irradiation. The laser was set to irradiate 1 mW. The size of the laser beam was adjusted to fit the well size of the 96-well plate. Laser was exposed for 2 min per well.

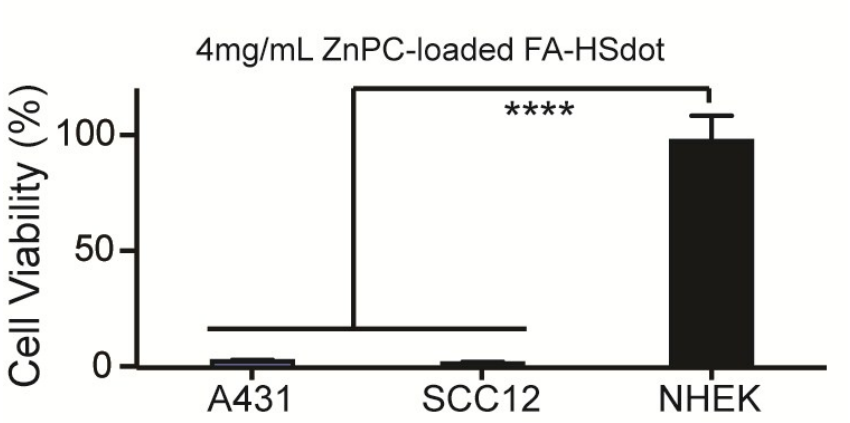

Figure S6: Effect of ZnPC-loaded FA-HSdots 24 hours after laser exposure. 24 hours after 2 min of 671-nm laser exposure, more than 98% of ZnPC-loaded FA-HSdot-treated SCC cells were killed, while minimal toxicity was observed in NHEKs under the same treatment.

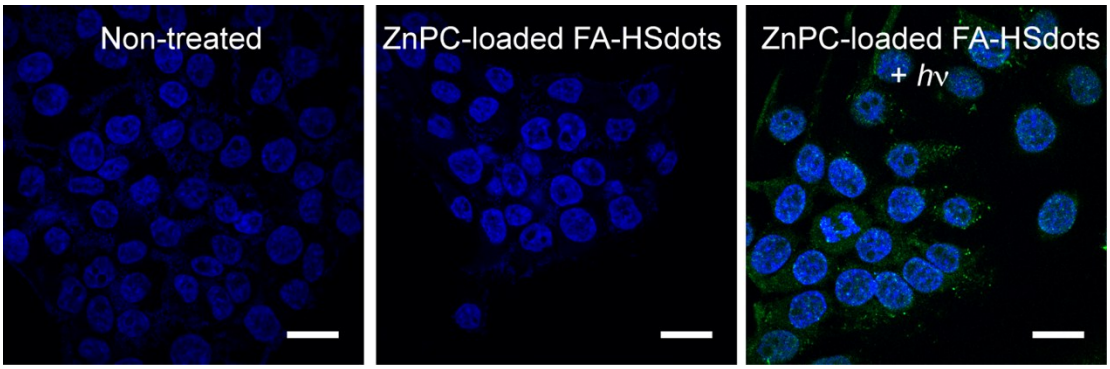

Figure S7: TUNEL assay in CAL27 cells. TUNEL assay shows an increase in number of nuclear DNA fragmentation in SCC cells treated with ZnPC-loaded FA-HSdots and laser, but not in NHEKs. Scale bar: 20 μm.

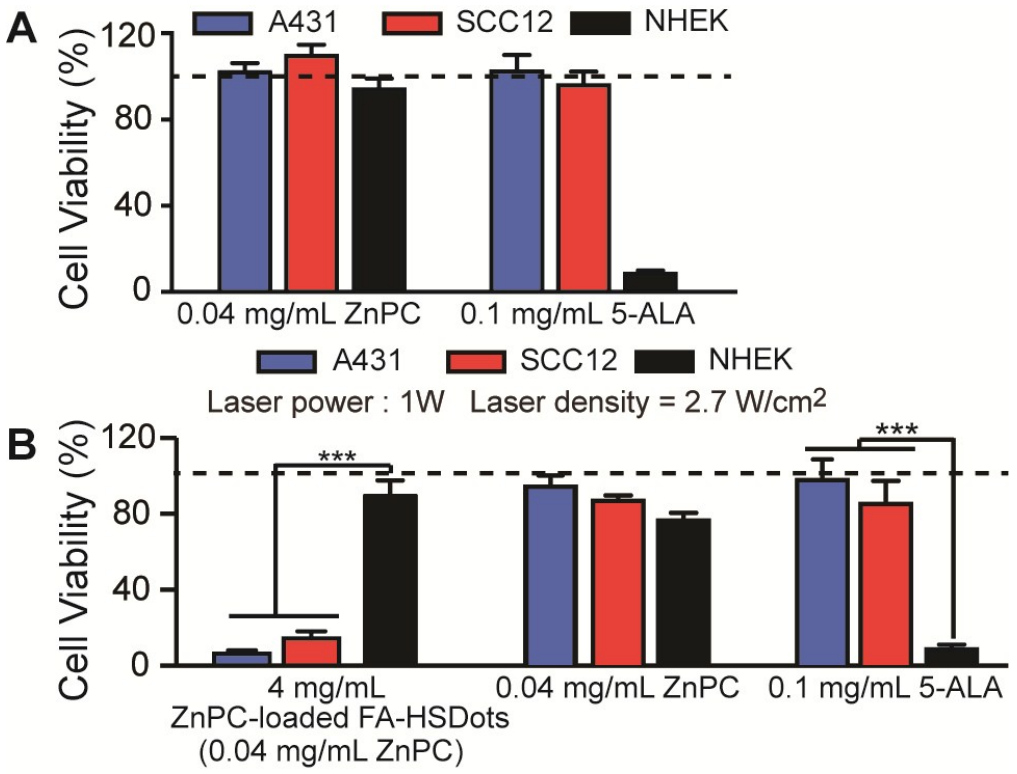

Figure S8: Compared therapeutic efficacy of ZnPC-loaded HSdots with commercially available products. (A) At 0.04 mg/mL of free ZnPC and 0.1 mg/mL of 5-ALA, no toxicity was observed in SCC. However, significant cell death (>90%) was observed in NHEK after treated with 5-ALA. (B) After 2 mins. of 671-nm laser exposure, we observed > 90% cell death in ZnPC-loaded FA-HSdots-treated SCC, but minimal cell death (< 5%) in both free ZnPC-treated and 5-ALA-treated SCC. However, in NHEK, we observed no toxicity after treatment with our nanosystems, < 15% cell death after treatment with free ZnPC, and > 90% cell death after treatment with 5-ALA.