Supporting Information for

IR-808 Loaded Nanoethosomes for Aggregation-Enhanced Synergistic Transdermal Photodynamic/Photothermal Treatment of Hypertrophic Scars

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S1 The preparation of carbopol gel

The carbopol gel matrix was prepared as follows: (a) 1.6 g of carbopol and 4 mL of propanetriol were added to 42 mL of double-distilled water; (b) the mixture was stirred for 2 h at room temperature, and then neutralized to pH 7.4 by using 2.5 mL of triethanolamine; (c) carbopol gel matrix was obtained after hydrating overnight.

S2 The details of western blot

1×10^6 HSFs were seeded and were incubated for 24h. The pretreated HSFs were incubated for 4 h after PDT/PTT procedure. Total cellular proteins were extracted using RIPA lysis buffer containing phosphatase and protease inhibitors (Beyotime Biotechnology, China). The concentration of total protein was detected with a BCA Protein Assay kit (Beyotime Biotechnology, China). Equal amounts (20 μg) of protein were separated using 4-20% SDS-PAGE gels. The proteins were then transferred to nitrocellulose membranes (0.45 μm; Millipore, USA). The membranes were blocked with 5% nonfat milk for 1 h at room temperature and incubated with primary antibodies (anti-Caspase3, anti-Caspase9, anti-cytochrome C, anti-β actin, rabbit monoclonal antibodies) (1:1000; Abcam, USA) overnight at 4 °C. After washing, the membranes were incubated with goat anti-rabbit secondary antibodies (1:5000; LI-COR Biosciences, USA). Finally, the membranes were visualized using the Odyssey CLx Infrared Imaging System (LI-COR Biosciences, Lincoln, Nebraska, USA). β actin protein intensity was used as an internal control.

Fresh tissues were dissected and homogenized for protein extraction. The total proteins were extracted and the western blot analysis was conducted as described. The

membranes were blocked with 5% nonfat milk for 1 h at room temperature and incubated with primary antibodies (anti-Caspase3, anti-Caspase9, anti-HSP70, anti-HSP90, anti-β actin, goat anti-rabbit antibodies) (1:3000; Servicebio, China) overnight at 4 °C. After washing, the membranes were incubated with donkey anti-goat secondary antibodies (1:3000; Servicebio, China).

S3 Construction of rabbit HS model

Ten adult New Zealand white rabbits (2.0−2.5 kg, Si-Lai-Ke, Shanghai, China), were single-housed in a regulated environment (22 ± 2 °C), and provided ad libitum access to feed and water. The rabbits were anesthetized using pentobarbital sodium delivered intravenously via ear veins (30 $mgkg^{-1}$). Four wounds (10 mm diameter) were created down to bare cartilage and the perichondrium was removed on the ventral surface of each ear. The wounds were more than 10 mm apart from each other to prevent potential interference. After surgery, the rabbits were returned to their cages and the wounds were covered using sterile gauzes for 1 day. After 30 days, the HS models were established with the thickness ratio above 1.5 between HS tissue and healthy skin.

S4 *in vivo* Visualization of IR-808-ES

After administrated with IR-808-ES gels for 3 h, HS tissues were harvested and washed using PBS. Subsequently, HS tissue pieces (1 × 1 × 1 mm) were prefixed using 2.5% glutaraldehyde overnight, dehydrated using graded ethanol for 20 min, and infiltrated and post fixed using 2% osmium tetroxide. After they were embedded in epoxyresin, they were cut into ultrasections perpendicular to the epidermis (70 nm thickness), and then, the sections were examined using a JEM-2010 TEM at an

accelerating voltage of 120 kV.

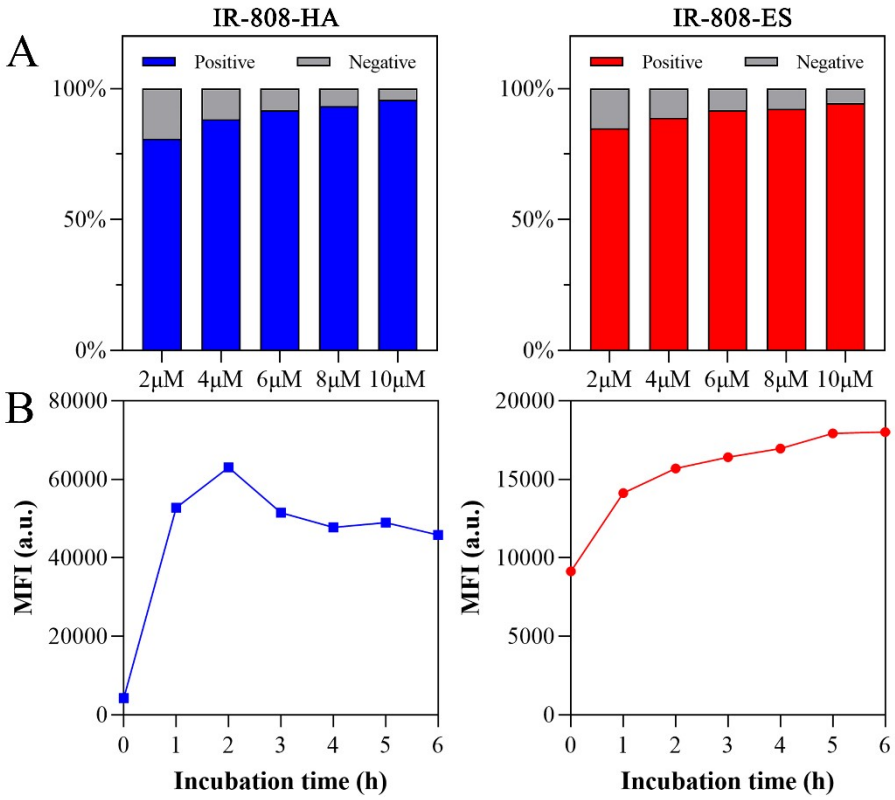

Figure S1. (A)The percentage of positive HSFs and (B) MFI values with different formulations.

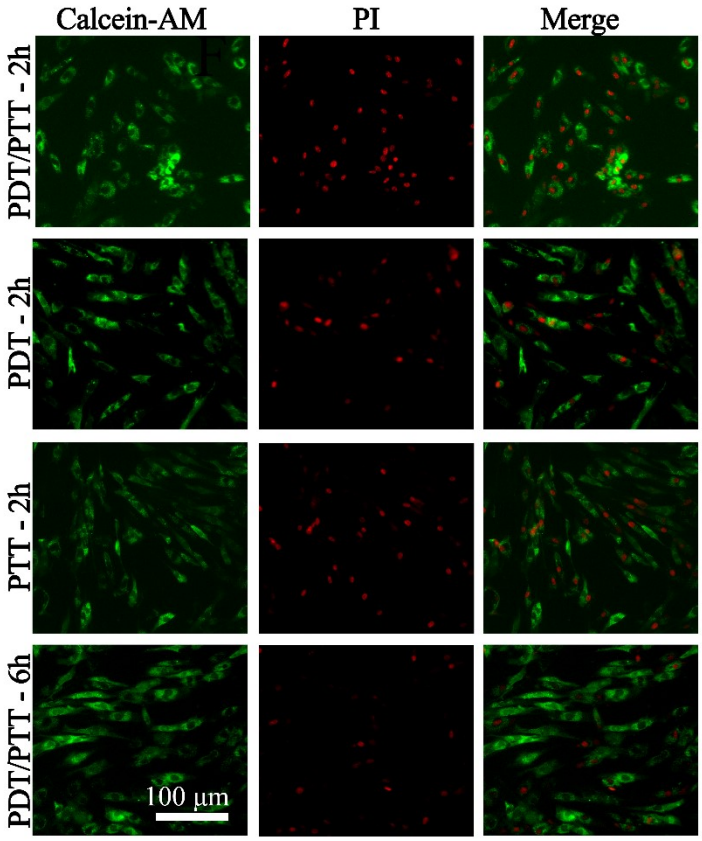

Figure S2. Fluorescent images of Calcein AM/PI co-stained HSFs after laser irradiation.

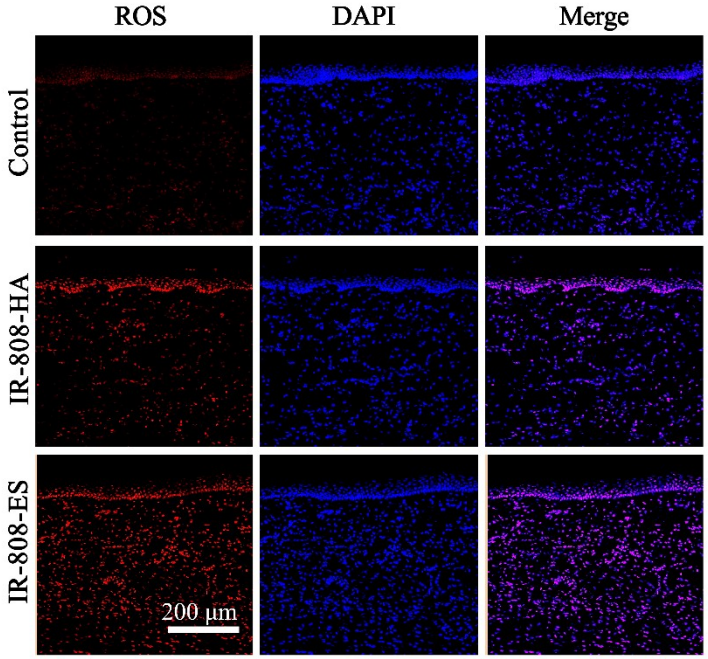

Figure S3. Fluorescent images of *in vivo* ROS generation in HS tissues.