Animal care and use statement

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Manuscript title: DSS-induced acute colitis impairs dermal lymphatic function in mice

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The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 ℃, 12h/12h light/dark, 50% humidity, ad libitum access to food and water) for two weeks prior to experimentation. Experimental colitis was induced by giving 4 % (wt/vol) DSS solution to replace drinking water *ad libitum* for 7 days. Control mice received drinking water. On day 7 the animals were euthanized via overdose of isoflurane and then cervical dislocation as a secondary method.

For imaging mesenteric lymphatic vessels, 1ml of a long-chain fatty acid, Bodipy-FL-C16 (Life Technologies) was orally administered to control mice and 7-day DSS-treated mice. Intragastric gavage administration was carried out with conscious animals, using straight gavage needles (15-17 g body weight: 22 gauge, 1 inch length, 1.25 mm ball diameter). At 30 mins after oral administration, mice were euthanized and fluorescence imaging was performed to visualize fluorescent lymphatic vessels in the mesentery.

For in vivo near-infrared fluorescence imaging, mice were anesthetized with isoflurane and maintained at 37°C on a warming pad. A volume of 10 or 2 μl of 645 μM of ICG (Akorn, Inc.)

dissolved in mixture of distilled water and 0.9 % sodium chloride in a volume ratio of 1:9 was injected intradermally at the base of the tail or to the dorsal aspect of left hind paw, respectively, using 31 gauge needles or 34 gauge needles. Fluorescence images were acquired immediately before and for up to 20 min after i.d. injection using a custom-built NIRF imaging system as described previously.

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