Supplementary information, Data S1

Materials and Methods

Animals

Normal adult male BALB/c mice (Taconic Farms, Germantown, NY) were used for the experiments. All mice were treated according to ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocols were approved by the Animal Care and Use Committee, University of California, Berkeley. Mice were anesthetized using a mixture of ketamine, xylazine, and acepromazine (50 mg, 10 mg, and 1 mg/kg body weight, respectively) for each surgical procedure.

Induction of corneal inflammatory lymphangiogenesis and hemangiogenesis

Suture-induced inflammatory neovascularization model was used. Briefly, 3 to 8 sutures (11-0 nylon, AROSurgical, Newport Beach, CA) were placed into corneal stroma without penetrating into the anterior chamber, followed by application of antibiotic ointment.

Ophthalmic slit-lamp microscopic examination of corneal blood vessels

Eyes before and 14 days after suture placement were examined by an ophthalmic slit-lamp with an integrated digital camera system (SL-D4 and DC-3; Topcon Medical Systems, Japan).

Non-contact live imaging and videos of corneal blood and lymphatic vessels with fluorescent

stereomicroscopes

Mice were anesthetized and kept warm with isothermal pad (Braintree Scientific, Braintree, MA) throughout the imaging process. FITC-labeled dextran (2,000 kDa molecular weight, Sigma-Aldrich, St. Louis, MO) was injected into the subconjunctival space with the assistance of an adjustable eye and head holder. Dye uptake was continuously monitored under a fluorescent stereomicroscope connected to a camera and a computer station with imaging software and screen. Digital pictures were taken under two light exposure conditions: FITC excitation light alone or combined with LED bright field light. For Supplementary Video 1, series of time-lapse images were taken under both FITC excitation light and LED bright field light sources using a Leica M165F FC stereomicroscope (JH Technology, San Jose, CA) in a time frame of 3 images per second. A total of 120 images taken within 40 seconds were streamed with the NIH Image J software.

Live imaging of lymphatic vessels in the skin

The experiment was performed similarly as described above except that the FITC-labeled dextran dye was injected intradermally into tail tip. Digital images were taken to demonstrate the cross-sections of lymphatic vasculatures.

Live imaging and videos of the cornea with the advanced two-photon excitation fluorescence microscope

A custom-built two-photon microscope based on the Movable Objective Microscope (Sutter Instrument Co., CA) was used. It is enclosed in a Faraday cage to minimize electrical noise and stray light. The mouse was positioned on a portable platform with feedback-regulated heating

pad and the adjustable eye and head holder. The excitation laser pulses were focused onto the cornea by a high numerical-aperture, water-immersion objective. Fluorescence emission was collected with a GaAsP photomultiplier tube behind an optical filter. The objective is controlled by a micromanipulator and can be translated in x-y-z axes in fine micron-scale spatial resolution. This system can reach a z-resolution of 0.5 microns. In some cases, large field-of-view images were taken with an air objective. The entire system is driven by the ScanImage software developed at Janelia Farm and fluorescence images were assigned pseudo-colors.
Two-photon videos (Supplementary Video 2, 3, and 4) were taken and streamed with the NIH Image J software. For Supplementary Video 2, a stack of 100 image slices, each an average of 3 frames, was taken with a z-step of 10 µm. For Supplementary Video 3**,** 70 image frames were acquired with a z-step of 1 µm with no averaging. Supplementary Video 4 was generated with a z-step of 0.5 µm.

Pharmaceutical Interventions

Mice were randomized to receive systemic administrations of anti-VEGFR-2 monoclonal antibodies (800 µg; DC101; ImClone Systems Corporation, wholly-owned subsidiary of Eli Lilly and Company, New York, NY) or their isotype controls on Day 0 and Day 3 after 4 suture placement.