Supplemental Materials

Immunofluorescence protocol:
For whole-mount immunofluorescence, the RPE-choroid-sclera sample was fixed in Histochoice (AMRESCO, Solon, OH) at room temperature for 3-4 hours. The sample was washed in phosphate-buffered saline (PBS) with 0.5% Triton X-100 and 0.1% sodium azide at room temperature and blocked in the same washing buffer containing 3% BSA overnight at 4°C. The tissue was incubated in washing buffer containing 1% BSA and 10 μg/mL of mouse anti-HSP70/72 (C92F3A-5 no. ADI-SPA-810-F; ENZO Life Sciences, Farmingdale, NY, USA) and 2.5 μg/mL of rabbit anti-ZO-1 (40-2200, Invitrogen, Carlsbad, CA) primary antibodies for 3 days at 4°C. After washing, the sample was incubated with 2 μg/mL of Alexa Fluor 594 Goat anti-mouse IgG (405326, Biolegend, San Diego, CA) and 8 μg/mL dilution of AlexaFluor 488 Donkey anti-rabbit IgG (A-21206, Invitrogen) secondary antibodies and 2 μg/mL of 4’,6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) in washing buffer with 1% BSA for 1 day at 4°C. For samples that were previously stained with EthD-III, a secondary antibody solution of 2 μg/mL of DyLight 488 Goat anti-mouse IgG (405310, Biolegend) and 4μg/mL of AlexaFluor 594 Goat anti-rabbit IgG (A-11072, Invitrogen) and 2μg/mL DAPI was used instead to avoid overlapping with the persistent red EthD-III signal.

Captions for Supplemental Videos
Video 1. Temperature rise (top) and Arrhenius integral (bottom) at FA threshold power for 20 ms exposure with CW (left) and 5% duty cycle (right) settings from 577 nm model with 140 μm laser spot diameter, overlaid on rabbit retina histology. Temperature during 20 ms exposure and 20 ms of cooling after the laser pulse is shown with 1 frame per 0.1 ms. Red contour represents damage threshold (Ω = 1) and green contour represents the threshold of HSP expression (Ω = 0.1).

Video 2. Temperature rise (top) and Arrhenius integral (bottom) with 20 ms exposures at the average power 33.3mW, corresponding to FA threshold with 5% duty cycle. Temperature during 20 ms exposure and 20 ms of cooling after the laser pulse is shown with 1 frame per 0.1 ms. Red contour represents the damage threshold (Ω = 1) and green contour represents the threshold of HSP expression (Ω = 0.1).

Video 3. Temperature rise (top) and Arrhenius integral (bottom) at ideal NRT power, just below the damage threshold, for 20 ms exposure with CW (left) and 5% duty cycle (right) settings from rabbit 577 nm model with 140 μm laser diameter. Temperature during 20 ms exposure and 20 ms of cooling after the laser pulse is shown with 1 frame per 0.1 ms. Green contour represents the threshold of HSP expression (Ω = 0.1).
Video 4. Temperature rise (top) and Arrhenius integral (bottom) at FA threshold power for 200 ms exposure with CW (left) and 5% duty cycle (right) settings from rabbit 577 nm model with 140 μm laser diameter. Temperature during 200 ms exposure is shown with 1 frame / 1 ms, which makes the 0.1 ms micropulse appear 10x longer than it is, relative to the micropulse cycle. Red contour represents the damage threshold ($\Omega = 3.04$) and green contour represents the threshold of HSP expression ($\Omega = 0.304$).

Video 5. Temperature rise (top) and Arrhenius integral (bottom) at the average power of 20.1 mW, corresponding to FA threshold for 200 ms exposure with 5% duty cycle. Temperature during 200 ms exposure is shown with 1 frame / 1 ms, which makes the 0.1 ms micropulse appear 10x longer than it is, relative to the micropulse cycle. Red contour represents the damage threshold ($\Omega = 3.04$) and green contour represents the threshold of HSP expression ($\Omega = 0.304$).

Video 6. Temperature rise (top) and Arrhenius integral (bottom) at ideal NRT power, just below the damage threshold, for 200 ms exposure with CW (left) and 5% duty cycle (right) settings from rabbit 577 nm model with 140 μm laser diameter. Temperature during 200 ms exposure is shown with 1 frame / 1 ms, which makes the 0.1 ms micropulse appear 10x longer than it is, relative to the micropulse cycle. Green contour represents the threshold of HSP expression ($\Omega = 0.304$).