**Supplementary Materials**

**Short-wavelength Blue Light Exposure Promotes Cataract Formation via Pyroptosis Activation**

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Contents

1. Supplementary materials and methods

1. **Supplementary Materials and Methods**

**1.1 The option of concentration of caspase-1 inhibitor,** **AC-YVAD-CMK**

The concentration of AC-YVAD-CMK was detected by cell counting kit-8(CCK-8) assay (SAB, USA). HLE-B3 cells (5 ×104) were seeded into 96-well plates. Each plate was divided into different groups according to the concentration of HLE-B3 hLECs: 0 μmol/L group, 5μmol/L group, 10μmol/L group, 20μmol/L group, 40μmol/L group and 60μmol/L group and cultured for 0, 24, 48, and 72 h. CCK-8 solution (1:10) was mixed into each well and incubated at 37°C in a humidiﬁed atmosphere of 5% Co2 for 1 h. A microplate reader (Bio-Rad, USA) was used to detect the optical density (OD) of each well at 450 nm. Each time point was tested in triplicate. The final concentration of 20μmol/L was added to for subsequent experiments.

**1.2 Short-wavelength blue light exposure**

Experimental illuminating systems (455-460 nm) were installed on the top of the cell culture incubator, which maintained a temperature of 37°C in a humidiﬁed atmosphere of 5% CO2 and 95% air. To reduce the interference of the medium on the illumination effect, the cell culture medium was changed to DMEM/F12 (without phenol red) containing only 1% FBS to reduce the chromophores present in the culture medium. We adopted an illuminance of 2500 lux to study the potential influence of blue LED light on hLECs**,** which were assessed at the position of the culture plates by a digital light meter (TES Electrical Electronic Corp., Taiwan).

**1.3 Enzyme-linked immunosorbent assay**

The protein expressions of caspase-1, -4, and GSDMD in the three groups were decided by using individual ELISA kits. Detailed methods are provided in the supplementary materials. Briefly, HLE-B3 hLECs were seeded onto 96-well plates and then treated under different conditions. At the appropriate times, the supernatant was collected for ELISA. The absorbance was determined at a wavelength of 450 nm with a microplate reader (Bio-Rad, USA), and the protein levels of caspase-1, caspase-4, and GSDMD were measured by comparing the OD of the samples to the standard curve.

**1.4 Flow cytometry**

To assess pyroptosis in HLE-B3 hLECs, cell viability assay of LECs was detected by flow cytometry (BD FACSCalibur, Franklin Lakes, USA) with Annexin V-APC and propidium iodide (PI) in 6-well plates according to the manufacturer’s instructions for 5min at 4°C in the dark. The assay was repeated 3 times. when pyroptosis occurs in cells, membrane permeability increased. PI can enter the cells and the DNA is stained. while phosphatidylserine (PS) is on the inside of the cell membrane, which can be stained by an antibody (Annexin V). Hence, the integrity of the cell membrane is not affected and PI cannot enter the cell to stain the DNA inside the cell when apoptosis occurs. However, PS will be fliped from the inside of the cell membrane to the outside when apoptosis occurs, which will be stained by its antibodies. Therefore, when flow cytometry was used to distinguish pyroptosis from apoptosis, apoptosis could only be observed Annexin V monopositive. On the other hand, the double-positive of Annexin V and PI occurs in cell pyroptosis.

**1.5** **Immunofluorescence localization**

Cells were fixed in 4% paraformaldehyde. After washing with PBS 3 times for 3 minutes each time, the HLE-B3 hLECs were blocked using 200 ml blocking solution for 60 min at 37°C in the dark, and then incubated with primary antibody including anti-rabbit caspase-1 p20 (1:200， Bioss Antibodies, Beijing, China), or anti- mouse GSDMD (1:200, Santa Cruz Biotechology, Inc. USA) at 4 °C overnight. The cells were rinsed with PBS three times at 37°C. Goat anti-rabbit (1:100, ZSGB-BIO, Beijing, China) and anti-mouse(1:100, ZSGB-BIO, Beijing, China) IgG secondary antibody was added and followed by an incubation for 40 min at 37 °C. Then, the pre-formed developer DAPI-containing working solution was added and continued to incubate for 20 min at 37 °C, and immediately rinsed with PBS. Finally, the slice was dehydrated, sealed and mounted with neutral gum. The representative image acquisition was performed using the laser scanning confocal microscope (Nikon C2, Tokyo, Japan). The fluorescence images were further processed using Image-Pro plus 6.0. Six random sections per smear using a magnification of × 400 over a microscopic field were observed within 1 hour.

**1.6 Western blotting analysis** **and antibodies**

Western blotting was used to quantify protein expression as previously described 32. The total proteins in the lenses were extracted using a BCA kit (Beyotime, Biotechnology, Shanghai, China). Proteins were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)(Bio-Rad Laboratories, CA, USA), transferred to polyvinylidene difluoride (PVDF) membranes and incubated with a primary mouse anti-caspase-1 antibody (1:1000) (Abcam, Cambridge, London, United Kingdom), anti-GSDMD antibody (1:1000) (Abbexa Biotechnology Co., Ltd., United Kingdom), and anti-GAPDH antibody (1:10000) (Kangchen Biotechnology Co., Ltd., China) at 4°C for overnight. Membranes were washed 3 times in 5% BSA and incubated with peroxidase-conjugated goat anti-rabbit and goat anti-mouse secondary antibody (1:10000) (ZSGB-BIO, Beijing, China) for 1 h. Detection was carried out with ECL detection reagent (Beyotime Biotechnology，Shanghai, China). After secondary antibody incubation, membranes were developed using the Chemiluminescence Plus Western blot analysis kit (Thermo Fisher Scientific, Sunnyvale, CA, USA). Gray band values were analyzed with Image J software (National Institute of Health, Bethesda, MD, USA).