An optogenetic controllable T cell system for hepatocellular carcinoma immunotherapy

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Supplementary information

Supplementary Figures

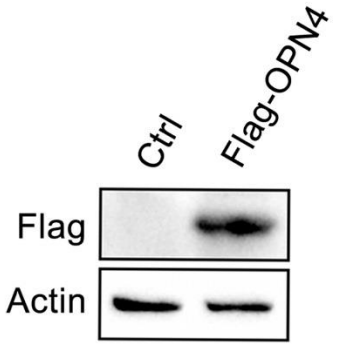


Figure S1. The expression of melanopsin in 293T cells detected by western blot. Flag-OPN4 was transfected into 293T cells. After transfection, cell lysates were prepared and analyzed by Western blot using anti-Flag antibody.

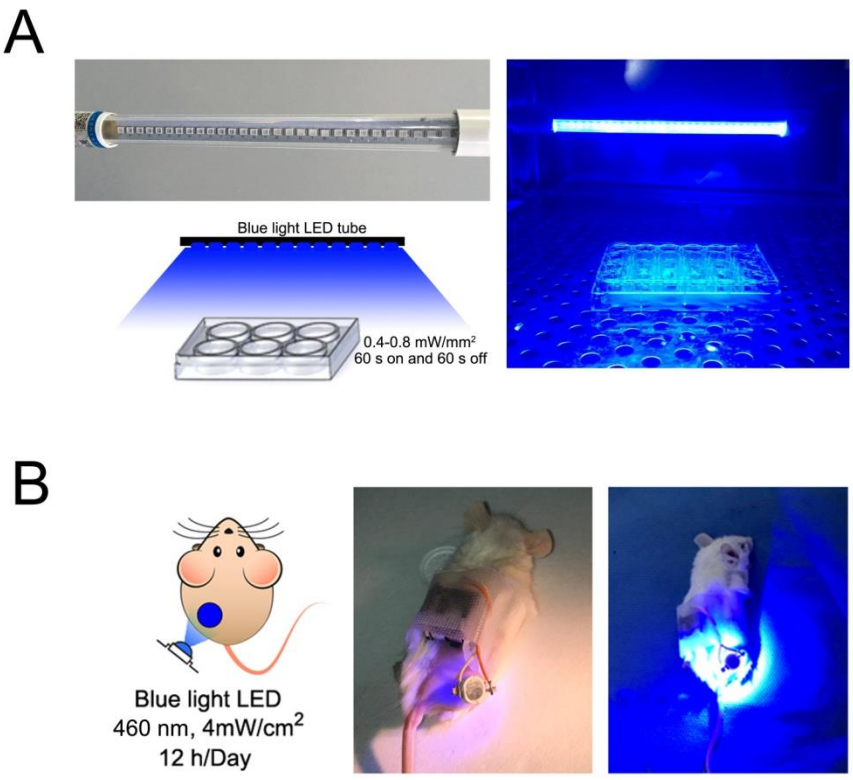


Figure S2: Custom-designed blue light LED devices for (A) cultured cells or (B)mice.

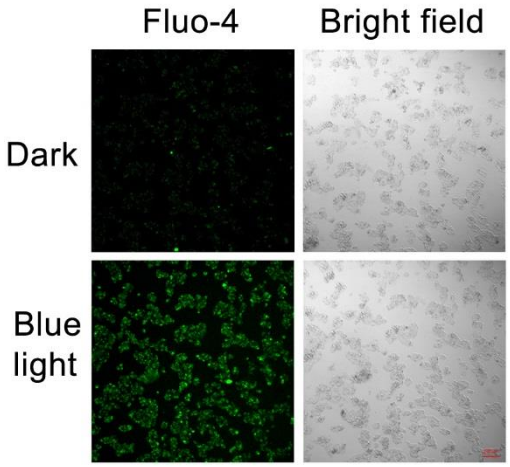


Figure S3. Confocal microscopy-based visualization of intracellular Ca^{2+} indicated by fluo-4. OPN4 stable expression 293T cells were exposed to blue-light illumination with repeated dark-light cycle (0.5mW/cm^2, 60 s ON and 60 s OFF) for 24 hours and the cells were stained with fluo-4 then visualized by the confocal microscope.

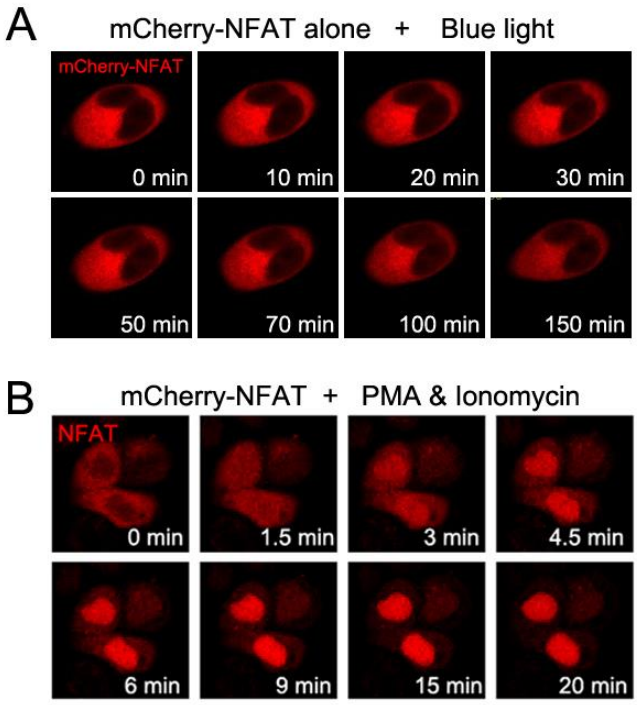


Figure S4. Negative and positive control for NFAT translocation assay. HeLa cells were transient transfected with mCherry-NFAT1 for 24h, and then exposed to blue light (A) or treated with Ionomycin and PMA (B)**.** Representative snapshots of cells during mCherry-NFAT1 nuclear translocation were imaged by confocal microscope.

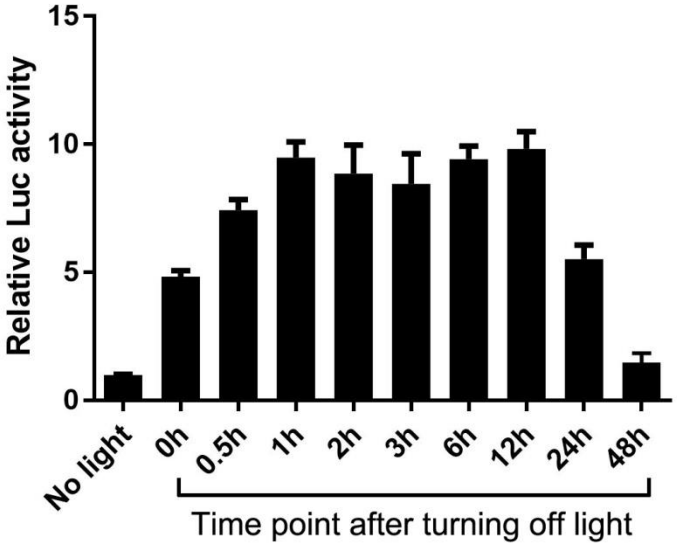


Figure S5. The relative Luciferase activity of engineered 293T cells after turning off the blue light. OPN4+ 293T cells were transfected with pNFAT-Luc vector, and the cells were exposed to pulsed blue light (0.5 mW/cm^2, 60 s ON with 60 s OFF interval) for 24 h, and then followed by culture in dark for different times as indicated (ranging from 0.5 to 48 hours) prior to cell lysis to quantify luciferase activity.

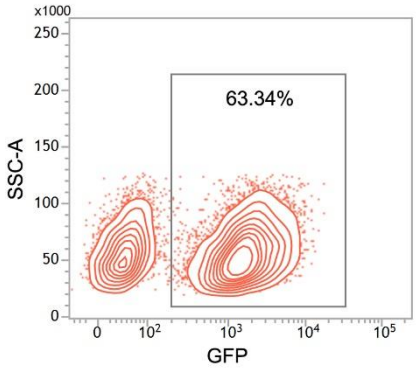


Figure S6. Flow cytometric analysis of the transduction efficiency of lentiviral vectors to PAN T cells. PCDN-OPN4 was transduced into PAN T cells, resulting in GFP expression, which was used to detect the transduction efficiency by flow cytometry.

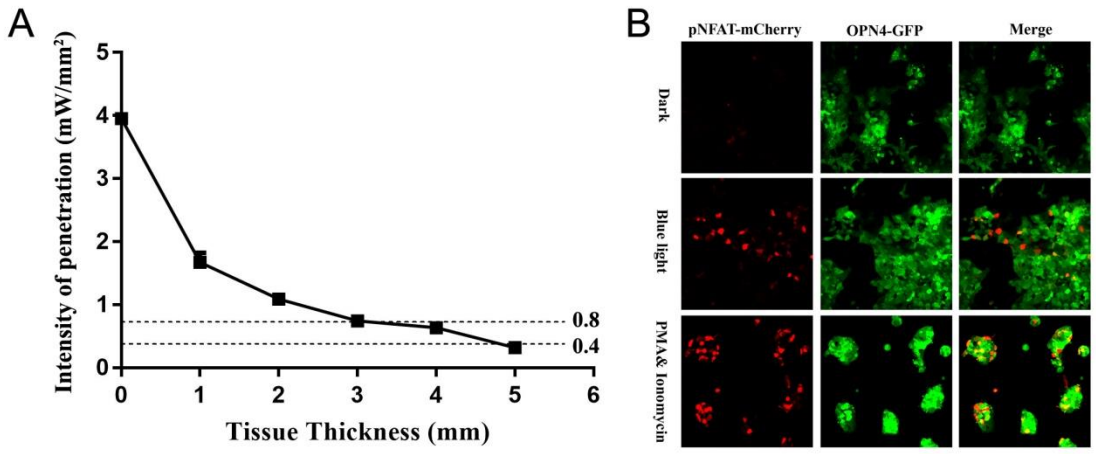


Figure S7. (A) The remained light intensity after blue light penetrating different thickness of tissues. Optical power meter (Thorlabs PM200) equipped with a thermal power sensor (S120VC) was used to detect the light intensity. Sensor, tissue and LED lamp were sequentially stacked to form a sandwich-like structure. The absorption wavelength was set at 460nm and the reading for each thickness of tissue was independently repeated three times. The area between two dash lines was the actual intensity used in vitro cell experiment. Additionally, light intensity still retained 0.3mW/cm^2, as blue light penetrated 5mm tissue. (B) Our optogenetic control system was triggered by blue light with intensity of 0.3mW/cm^2. Engineering modified 293T cells transfected with pNFAT-mCherry vector was illuminated with 0.3mW/cm^2 pulsed blue light for 24 hours to simulate the blue light initiated transgenic system after penetrating 5mm tissue. PMA and ionomycin treatment were also used as positive control as described in manuscript.

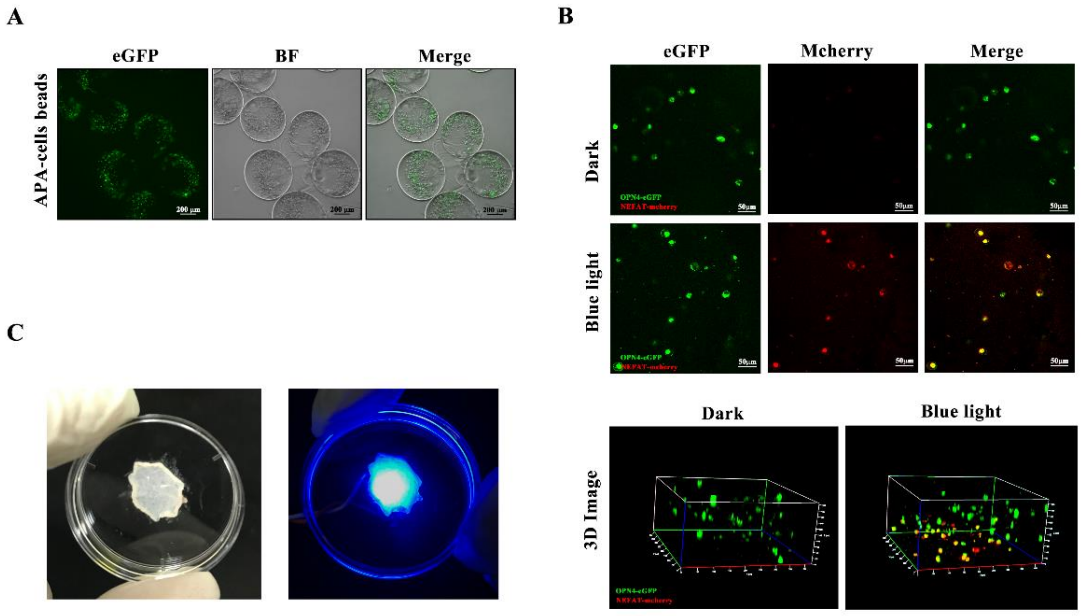


Figure S8. Ex vivo study confirmed that our light controlled optogenetic system could be triggered by external blue light. (A) Transgenic 293T cells were encapsulated into alginate/poly-L-lysine/alginate beads. $5*10^5$ of 293T cells co-transfected with OPN4-eGFP and pNFAT-mCherry vector were suspended in 1.5% (w/v) sodium alginate buffer to a final concentration of 5 × 105 cells/ml. Afterwards, 300 μL of this suspension was extruded via a 25-gauge needle into a receiver petri containing 100mM 1.1% $CaCl_2$ and incubated for 10 min. The droplet size was controlled by parallel airflow (diameter: around 500 μm). Around 500 APA droplets including 300 cells per droplet were generated. After wash with saline, the beads were incubated for 10 min in 0.05% (w/v) poly- L -lysine solution and for another 10 min in 0.6% (w/v) sodium alginate. (B) Encapsulated transgenic cells were activated by blue light. The beads containing transgenic cells were subcutaneously implanted into mice (200 beads each mouse), and locally irradiated with blue light LED for 24 hours. Then, the beads were harvested from mice and visualized under the microscope. (C) Blue light could easily penetrate skin. The isolated mouse skin is placed on a petri dish and exposed to blue light. Blue light can be easily collected on the other side of the skin.

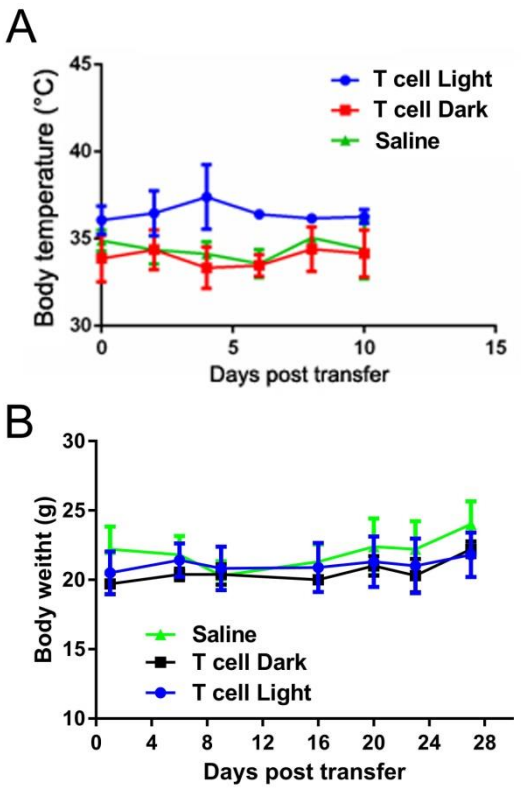
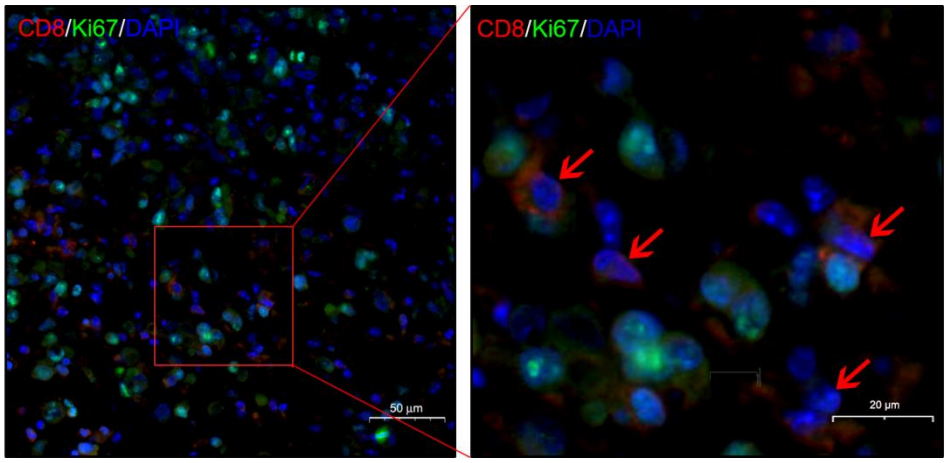


Figure S9. Body temperature and body weight of SK-HEK-1 xenograft mice. B-NDG mice (8 weeks, n=5) bearing Sk-HEK-1 (nano-Luc+) orthotopic tumor were intra-tumorally injected with 5×10^6 engineered T cells on the day 1 and 7, respectively. After the first treatment, mice received blue light irradiation (4mW/cm^2, 12 h everyday) in the experimental group. Mice in the other two groups were feed normally. Body temperature (A) and Body weights (B) of SK-Hep-1 (nano-Luc+) xenograft mice treated either with PBS or engineered T cells in the presence or absence of pulsed blue light were measured at different time points as indicated.



Figures S10. Fluorescent IHC co-staining of CD8 and Ki67 in tumor tissue. Tumor tissues were harvested from mice infused with engineered T cells then with blue light illumination. Slides were stained with CD8 (red) and Ki67 (green), respectively. Primary antibody against CD8 (Invitrogen, Cat # MA5-14548, USA) and donkey anti-Rabbit secondary antibody conjugated by alexa 555（Invitrogen， Cat # A-31572, USA）were used to labelled CD8. Meanwhile, primary antibody against Ki67 (eBioscience, Cat # 14-5699-82) and goat anti-mouse antibody conjugated by alexa 488 (Invitrogen, Cat # A-11001, USA) were used to stain Ki-67.

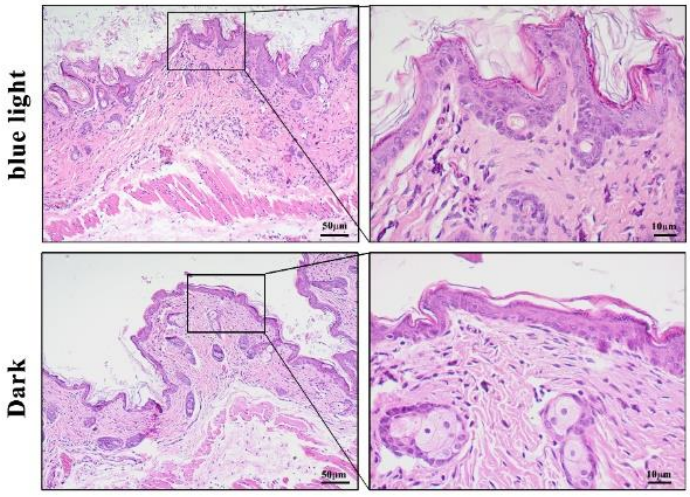


Figure S11. Histochemical staining of mouse skin after blue light irradiation. Blue light emitting diodes (LED) were glued at the right side of mouse back (diameter ≈1cm), and the left side of mouse back was ensured to be kept in dark, which was defined as control. Irradiation at 460 nm was lasted for 2 weeks, 12 hours daily. The right and left side of back skins were removed respectively to conduct the pathological evaluation and DNA sequencing, respectively.

Movie Legend

Movie 1. Blue light induced Ca^{2+} influx reported by Ca^{2+} indicator GCaMP6s. Melanopsin stable expression 293T cells were further transfected with GCaMP6s vector. After transfection, Ca^{2+} change was monitored by confocal microscope following extra blue light stimulation (0.5mW).

Movie 2-4. Light-induced nuclear translocation of mCherry-NFAT. OPN4 stable expression HeLa cells were transient transfected with mCherry-NFAT1. After transfection, cells were exposed to repeated light-dark cycles (0.5mW/cm^2, 60 s ON and 60 s OFF). The translocation of mCherry-NFAT was monitored by confocal microscope.

Movie 5. NFAT transfection alone cannot translocate into nuclear upon blue light illumination. HeLa cells were transient transfected with mCherry-NFAT1. After transfection, cells were exposed to repeated light-dark cycles (0.5mW/cm^2, 60 s ON and 60 s OFF). mCherry-NFAT was monitored by confocal microscope.

Movie 6. PMA and Ionomycin treatment induced nuclear translocation of NFAT. HeLa cells were transient transfected with mCherry-NFAT1. After transfection, cells were treated with PMA and Ionomycin, The translocation of mCherry-NFAT was monitored by confocal microscope.