Updated on 29 November 2023. Raw data of Figure 1G, 1H, 6A, 6B, 6F added.

Materials and Methods

Materials

2-methylimidazole, cetyltrimethylammonium bromide (CTAB), Zn(NO3)2·6H2O, $NaBH_4$, and $RuCl_3$ were purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium **(**DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). MTT was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). SH-SY5Y cells were purchased from Wuhan Pricella Life Technology Co. Ltd. (Wuhan, PRC). DCFH-DA and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) HCl were purchased from Sigma-Aldrich. Fluorescein isothiocyanate (FITC), Lyso Tracker, and Mito Tracker were purchased from Thermo Fisher Scientific (Rockford, IL, USA). All reagents were used as received.

Mito-Ru MOF preparation

The synthesized cube BSA@ZIF:2-methylimidazole (740 mM) and 1 mg BSA were dissolved in 2.0 mL deionized (DI) water containing 0.025% (w/v) cetyltrimethylammonium bromide (CTAB) by stirring at 500 rpm for 5 min. Then 0.5 mL of 97.5 mM aqueous $Zn(NO_3)_2 \bullet 6H_2O$ was added to the foregoing mixture and stirring was resumed for 5 min. The solution was left to stand at room temperature for 3 h and the prepared cube BSA@ZIF was collected by centrifugation.

Cube Mito-Ru MOF synthesis

Lecithin (10 mg), DSPE-PEG-NH_2 (1.0 mg), and cholesterol (2.0 mg) were dissolved in 3.0 mL of a 1:1 (v/v) chloroform/methanol solvent mixture and the latter was removed by vacuum. Then 2 mg Ru MOF solution (2 mL) was added to the residue and subjected to 300 W ultrasonic treatment for 5 min to obtain the Ru MOF@Liposome. Then 10 mM carbodiimide-HCl (EDC) and 25 mM *N*-hydroxysuccinimide (NHS) were added to 10 mM TPP (pH 7.2) solution. The mixture was incubated at 30 °C with

shaking for 15 min and added to the Ru MOF@Liposome solution to obtain the Mito-Ru MOF.

Animals

Male mice aged 6–8 weeks and each weighing 18–21 g was provided by the Animal Laboratories Center of Zhengzhou University, Zhengzhou, Henan, PRC, and housed at the central facility of the Zhengzhou University School of Medicine under a standard 12 h light/12 h dark cycle. They had *ad libitum* food and water access. In all behavioral experiments, mice were acclimated in our animal facility for at least one week before the experiment and in the laboratory for at least 30-60 minutes before the test. The study protocol was approved by the Institutional Ethics Review Committee of Zhengzhou University (No.2021062). The number of animals used and animal suffering were minimized to the greatest extent possible.

Mouse CFA-induced TMD pain model and drug treatment

Intra-TMJ injection of CFA (10 μL; 5 mg/mL; Chondrex Inc., Woodinville, WA, USA) or saline (10 μL) was conducted under isoflurane anesthesia. CFA or saline was unilaterally injected into the superior joint space of the TMJ as previously reported [15]. Mito-Ru MOF (8 mg/kg) or vehicle (phosphate-buffered saline (PBS)) was injected through the tail vein 30 min after the CFA or saline injection.

Orofacial mechanical hypersensitivity test

Calibrated von Frey filaments were used to test orofacial mechanical hypersensitivity before the treatments and at different time points after intra-TMJ CFA or saline injection. The mice were restrained in Plexiglas® cylinders 10 cm in length. The animals were free to extend their heads and forepaws but could not turn around. The mice were left to acclimate for 5 min. Each von Frey filament was applied five times to the trigeminal nerve-innervated skin area for 1–2 s with a 10-s interval between applications. The first application delivered the lowest filament force (0.08 g) while the subsequent four applications incrementally increased in force magnitude. Sharp withdrawal of the head upon stimulation was scored as a positive response. The head

withdrawal threshold was calculated as the force at which a positive response was elicited by three of the five consecutive stimuli.

H&E staining of the TMJ

CFA or normal saline was injected into the temporomandibular canal under isoflurane anesthesia. The mice were euthanized on day 3 after caudal vein administration of Mito-Ru MOF or PBS. Intact TMJ tissues including the synovium, articular disk, cartilage, and mandibular condyle were fixed with 4% (v/v) paraformaldehyde (PFA) and decalcified with 10% (w/v) ethylenediaminetetraacetic acid (EDTA). The TMJ tissue was then dehydrated and embedded in paraffin. All paraffin-embedded specimens were sliced into 4-mm serial sections and stained with H&E.

Sp5C microinjection

NF-κB p65-siRNA or Scramble-siRNA was microinjected into the unilateral Sp5C of normal mice. The animals were anesthetized with 5% isoflurane until they no longer responded to the tail contraction test. Thereafter, 1.5% isoflurane was administered to maintain anesthesia. All mice were weighed and those nearest the average weight were included in the experiment. After the mice had reached the appropriate level of general anesthesia, the hair on the tops of their heads was removed with scissors and sterilized. Erythromycin eye ointment was applied to both corneas to prevent desiccation. The mice were placed prone on a stereotactic device and a midline incision was made to expose the skull. A 40 microsyringe fitted with microelectrodes was positioned above the trigeminal ganglion using previously determined coordinates [21], namely, -8.0 mm in the anterior and posterior parts (AP), 1.5 mm in the medial lateral (ML), and 4.5 mm in the ventral dorsal (DV). SiRNA solution (40×10^{-6} M; 1 μL/Sp5C) was injected into the Sp5C at the rate of 100 nL/min using the aforementioned microsyringe. The microelectrodes were left *in situ* for 10 min to allow the siRNA solution to diffuse. The skin incision was disinfected and sutured with 5-0 nylon non-absorbent monofilament thread. The treatment groups were Scramble-siRNA and NF-κB p65-siRNA.

Western blotting

The mice were sacrificed 3 d after the intra-TMJ CFA or saline injection with or without subsequent Mito-Ru MOF treatment under isoflurane anesthesia,and their Sp5C tissues were harvested. The NF-κB and phosphor-NF-κB expression levels were measured by quantitative western blotting. Affinity-purified antibodies against phospho-NF-κB p65 (phospho S536) (No. Ab76302; 1:1,000; Abcam, Cambridge, UK) and NF-κB p65 (No. 66535-1-Ig; 1:1,000; Proteintech, Rosemont, IL, USA) were used. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the loading control. Band intensities were quantified by densitometry.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Mouse Sp5C RNA was extracted 3 d after intra-TMJ CFA or saline injection with or without subsequent Mito-Ru MOF treatment. The PCR was conducted using Advanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) in a Bio-Rad CFX96 Real-Time PCR System. The ratios of the mRNA levels of each treatment group to that of the control were calculated. GAPDH was used for data normalization. The primer sequences used in this study were NEAT1 Forward: GTTCACAGCCCTGCTCAGAT; NEAT1 Reverse: TTCCAGGCACAATCCTCACC. GAPDH Forward: CAGTGGCAAAGTGGAGATTGTTG; GAPDH Reverse: TCGCTCCTGGAAGATGGTGAT.

Immunofluorescence

Frozen Sp5C tissue sections were incubated overnight with mouse anti-p-p65 antibody or anti-8-OHdG monoclonal antibody at 4 ℃. The sections were then incubated with fluorescent secondary antibodies and 1RU 500 diluent at room temperature for 1 h. The tissue sections were mounted on slides and observed under a fluorescence microscope. Immunofluorescence intensity was measured with ImageJ (National Institutes of Health [NIH], Bethesda, MD, USA).

Statistical analysis

Data were presented as means ± standard deviation (SD). Statistically significant differences between group pairs were determined with Student's *t*-test. Two-way analysis of variance (ANOVA) followed by the Bonferroni test detected statistically significant differences among multiple groups. Differences were considered significant at $P < 0.05$. GraphPad Prism v. 7.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for all data processing.

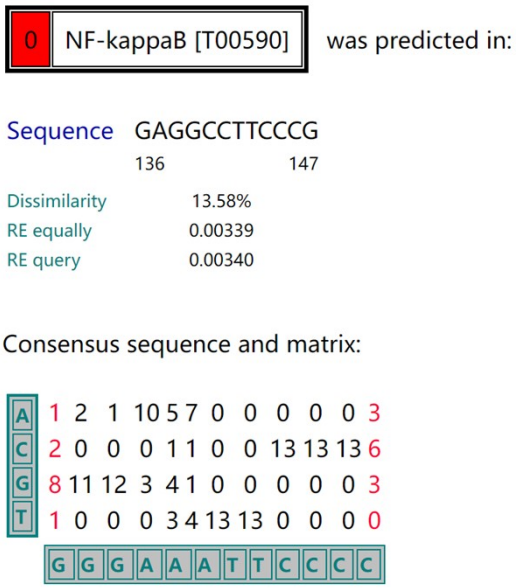

Figure S1. Predicted binding sites of NF-KB to NEAT1 through UCSC online website and PROMO database.

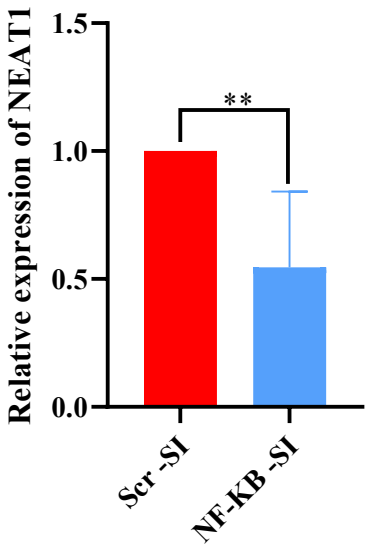

Figure S2. Expression of NEAT1.

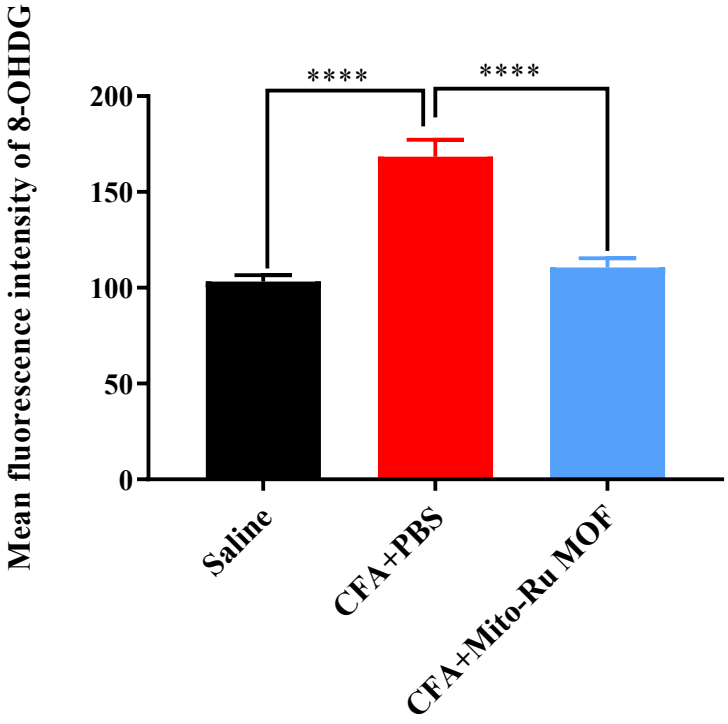

Figure S3. Fluorescence intensity statistics of 8-OHDG.

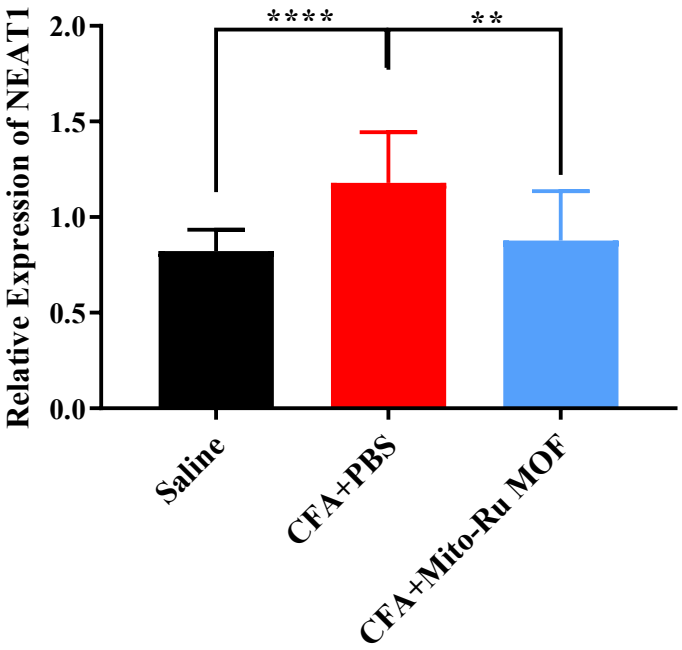

Figure S4. Expression of NEAT1 treated by Saline, CFA + PBS, and CFA + Mito-Ru MOF.

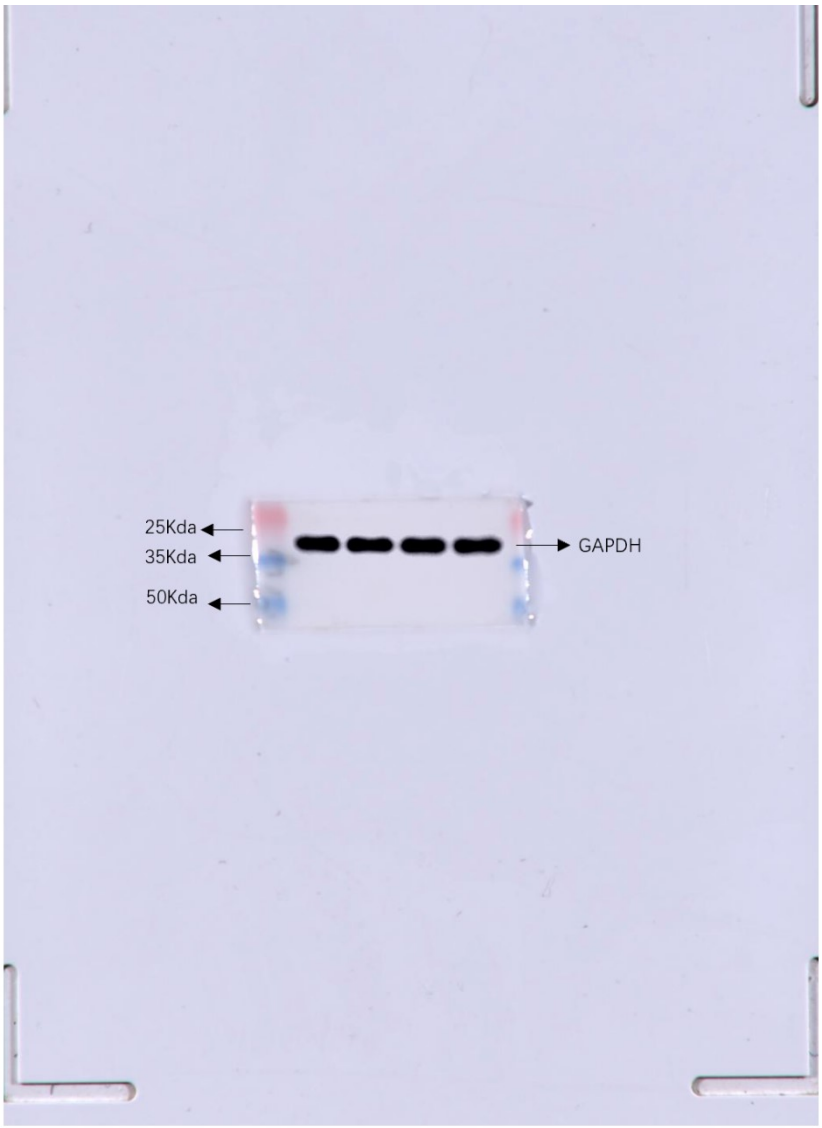

Figure 1G GAPDH

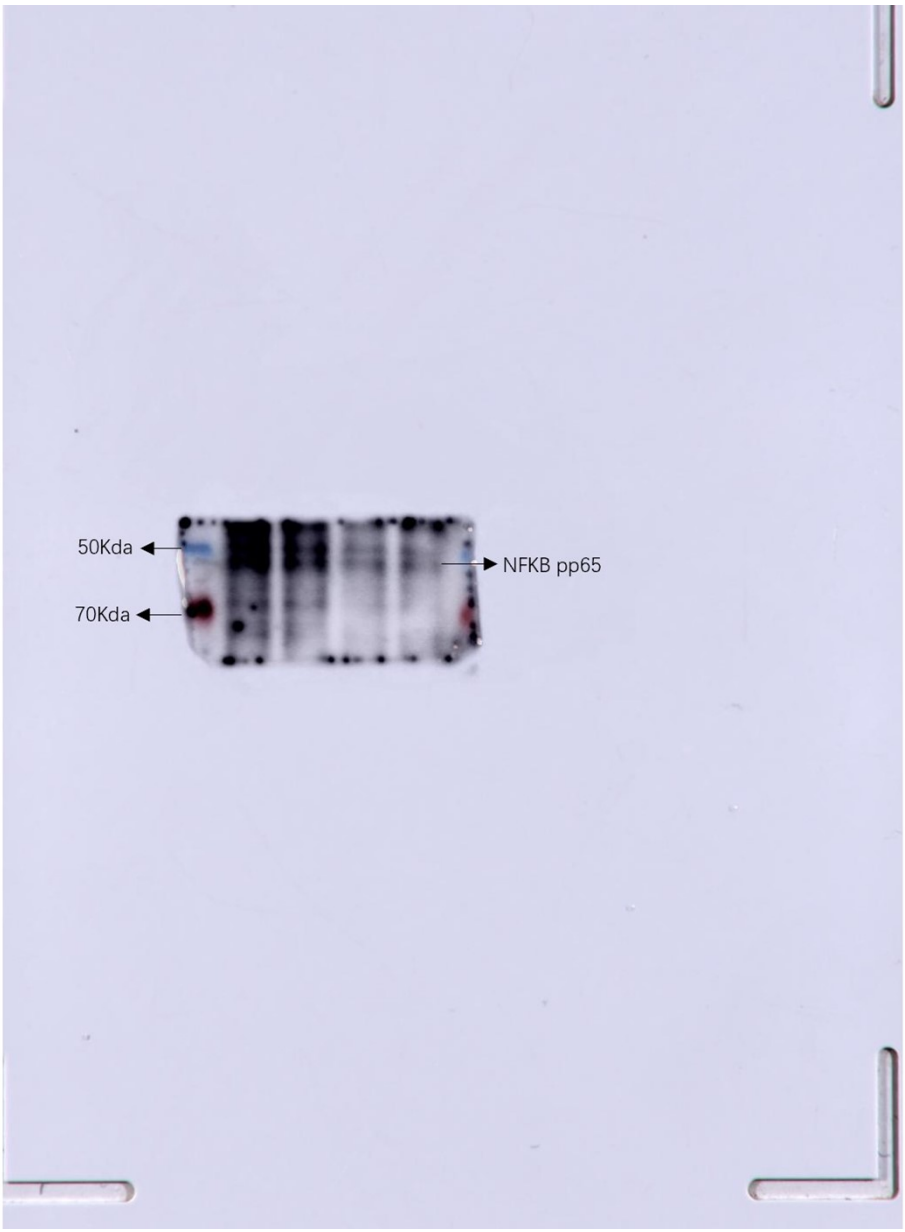

Figure 1G p-p65

Figure 1H GADPH

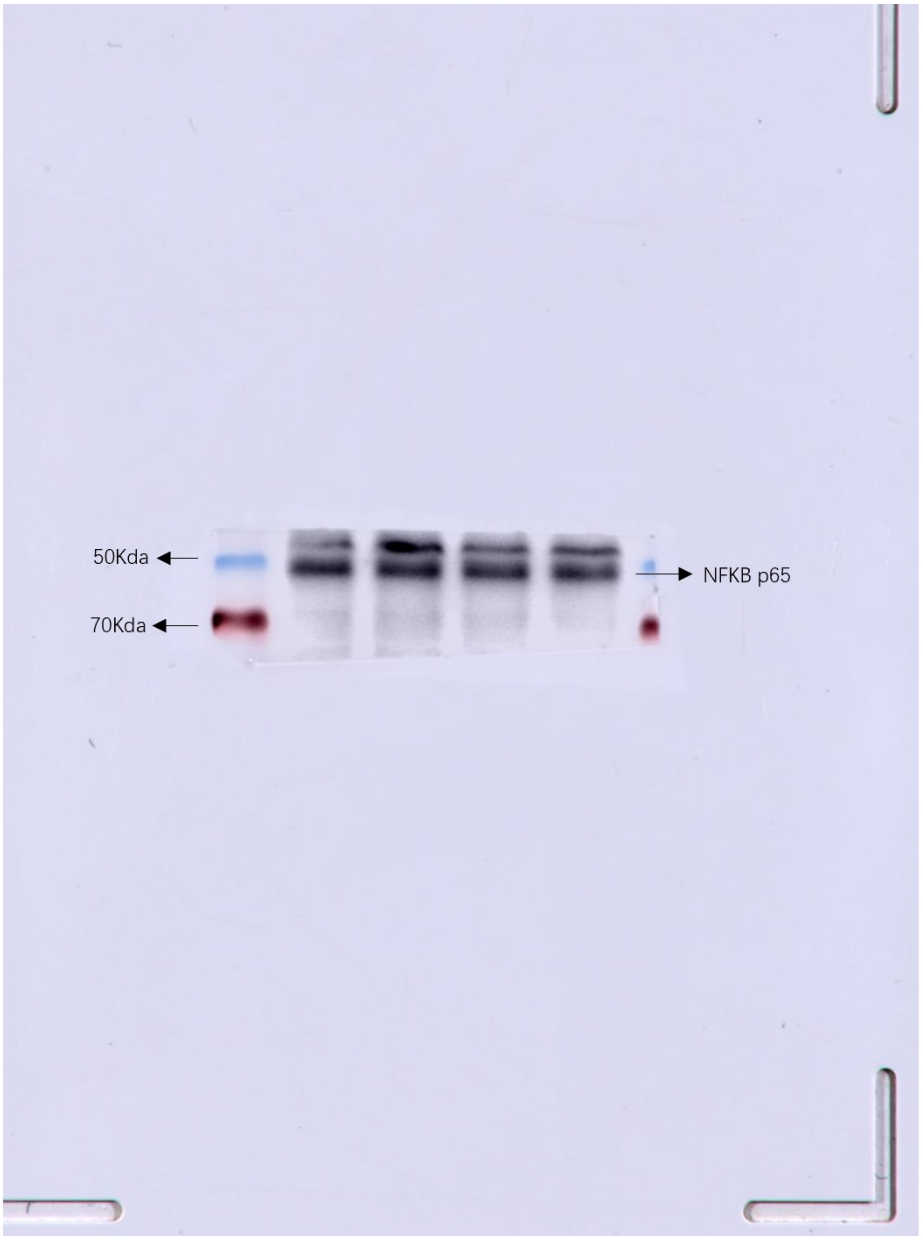

Figure 1H p65

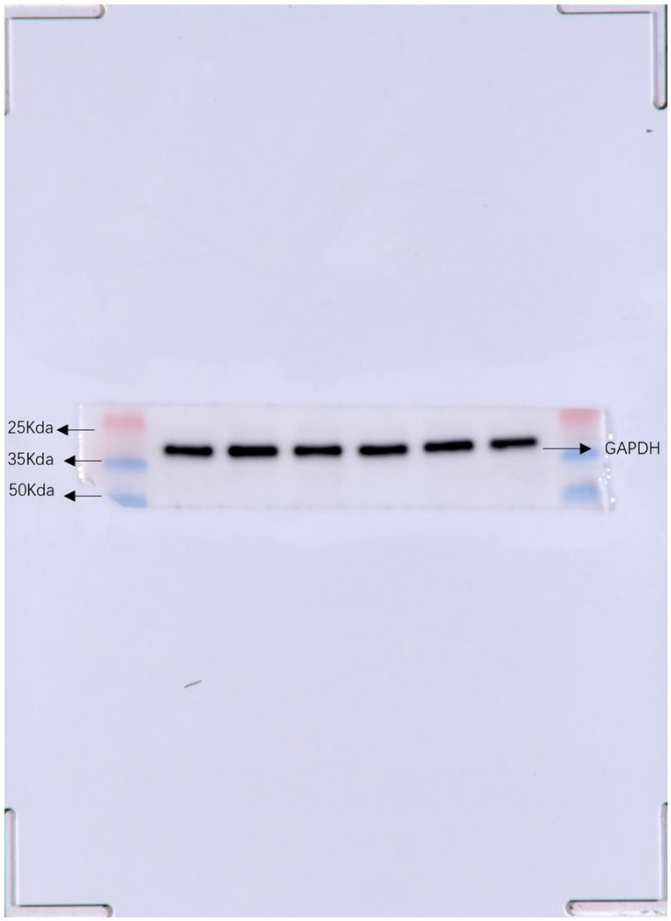

Figure 6A GAPDH

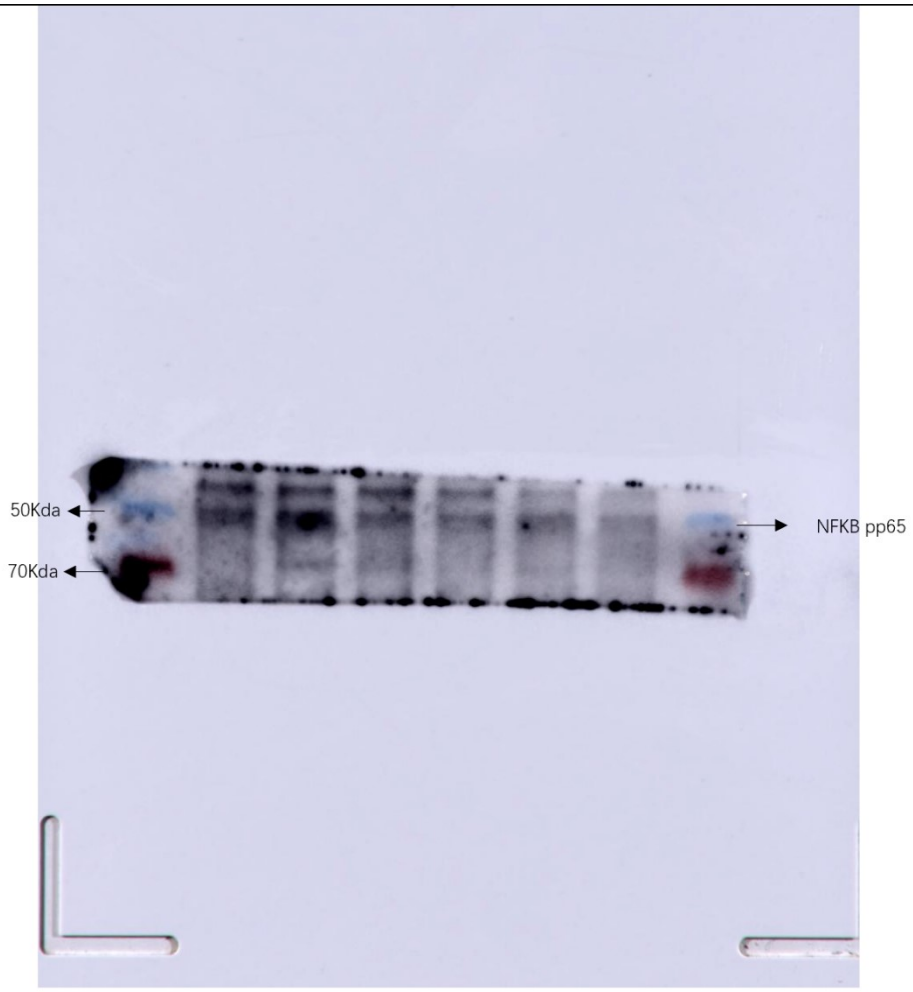

Figure 6A p-p65

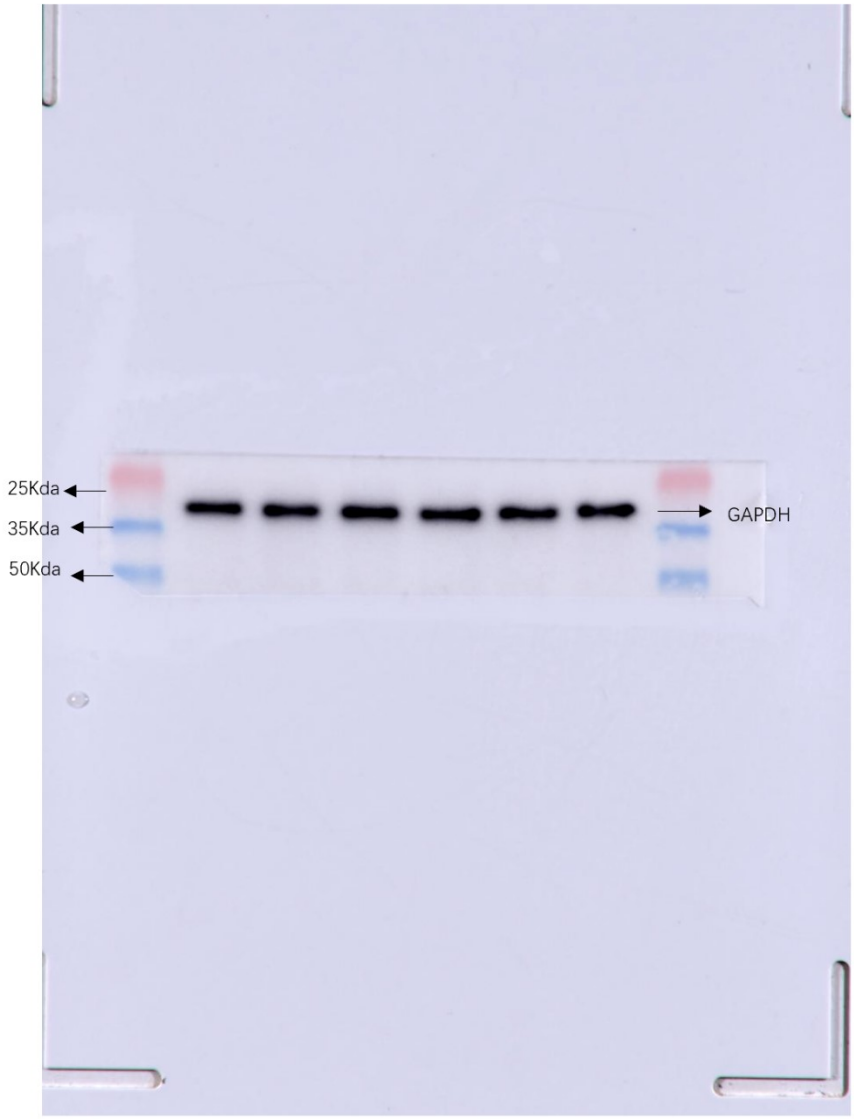

Figure 6B GAPDH

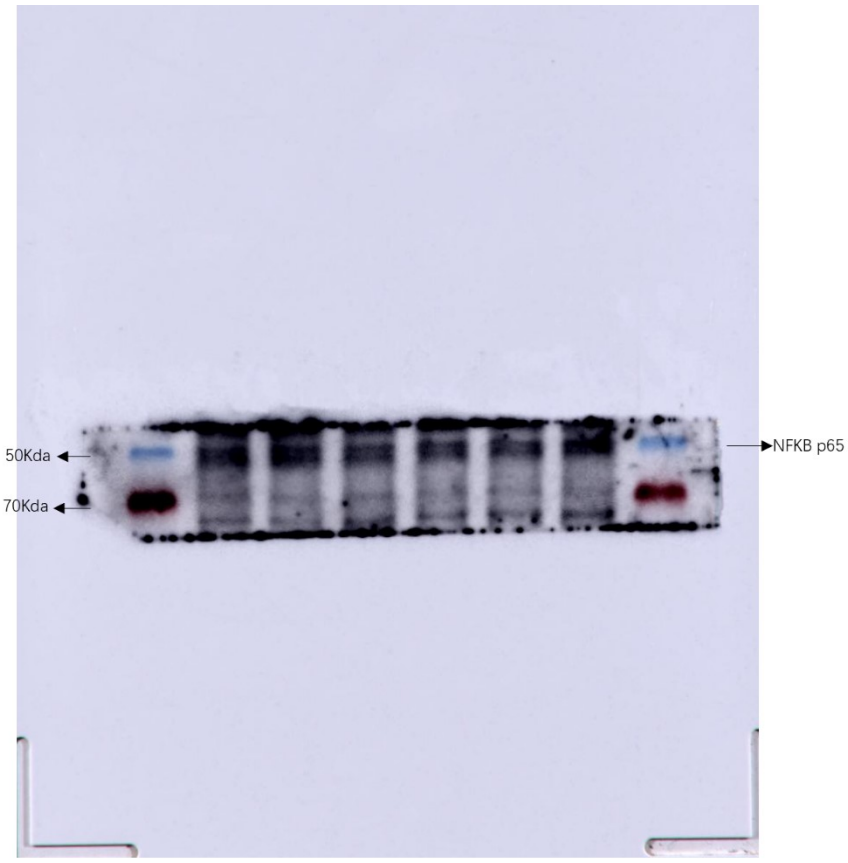

Figure 6B p65

Figure 6F GAPDH

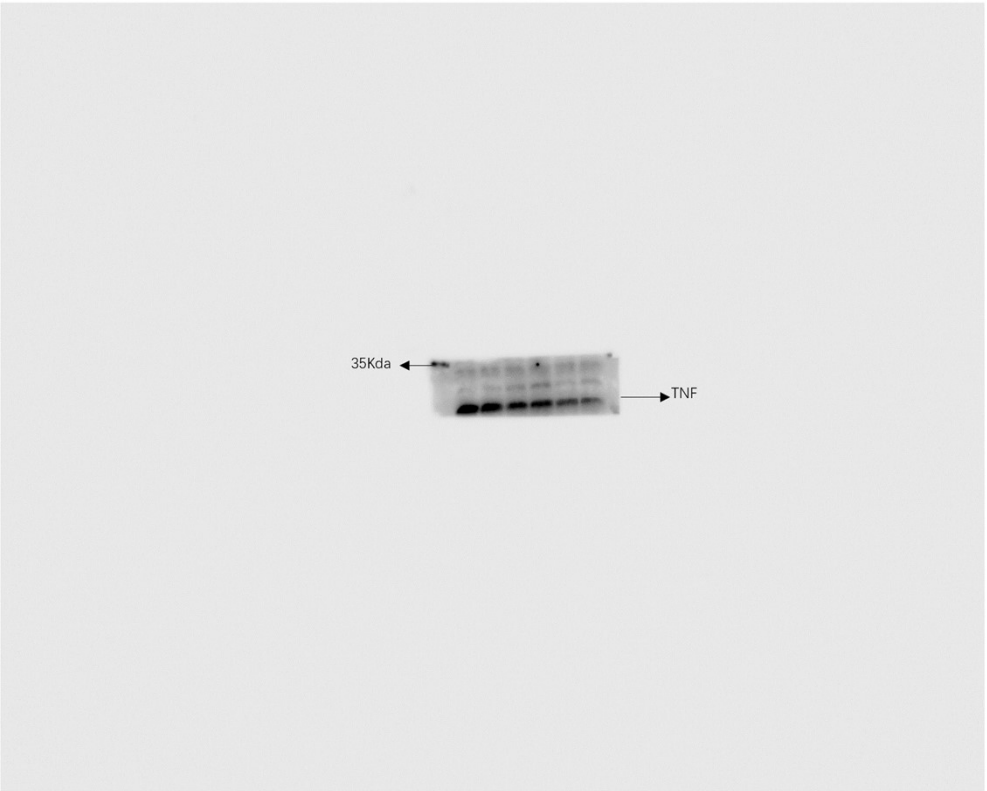

Figure 6F TNF