

Fig. 3A



Fig. 3A NLRP3

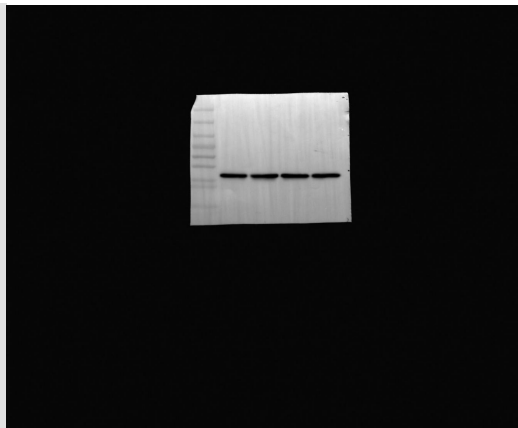


Fig. 3A GAPDH



Fig. 3A GSDMD

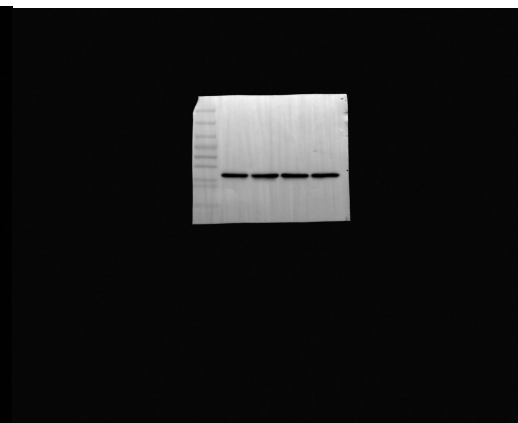


Fig. 3A GAPDH

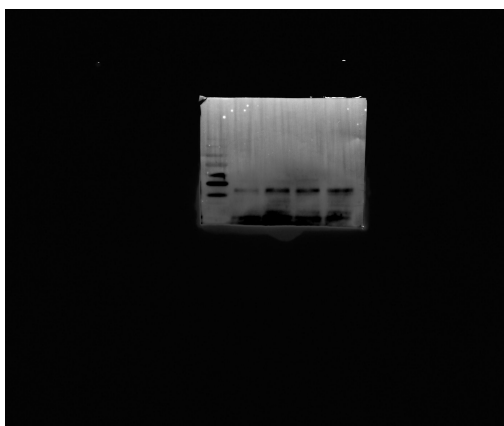


Fig. 3A GSDMDN

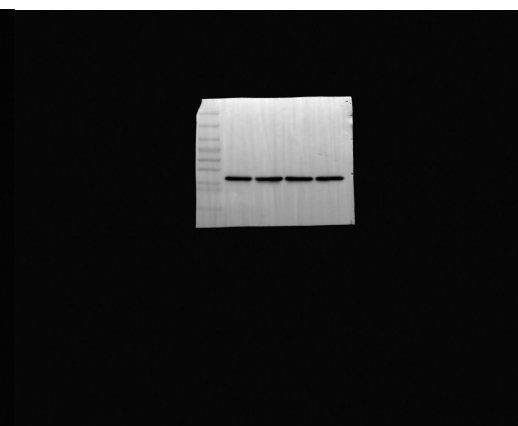


Fig. 3A GAPDH

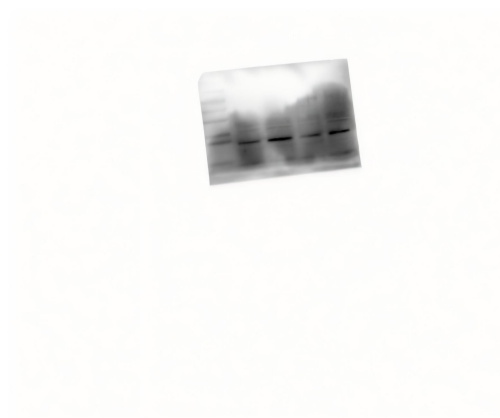


Fig. 3A IL-1 β

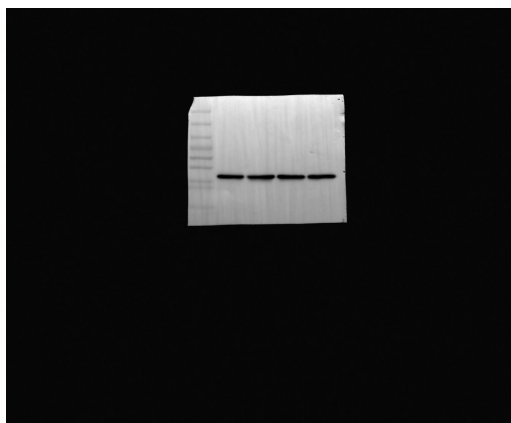


Fig. 3A GAPDH

Fig. 3D

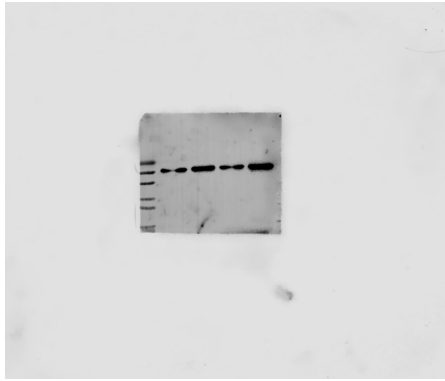


Fig. 3D Beclin1

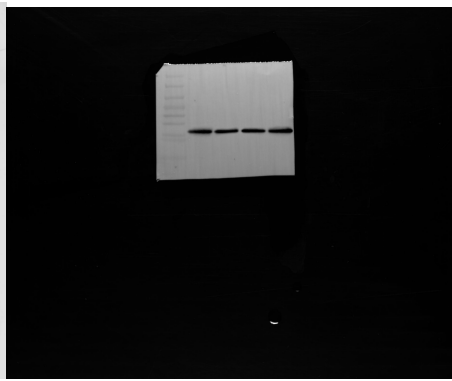


Fig. 3D GAPDH

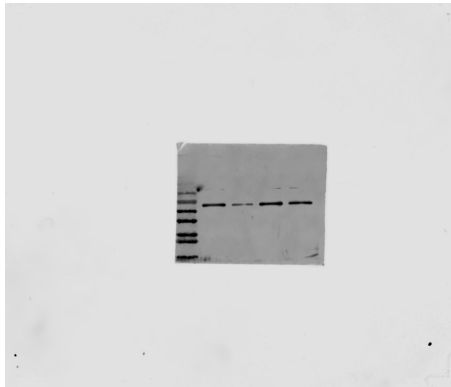


Fig. 3D P62

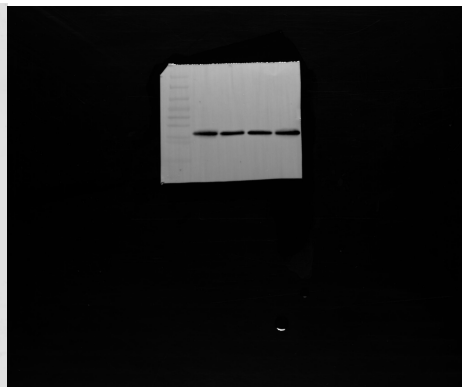


Fig. 3D GAPDH

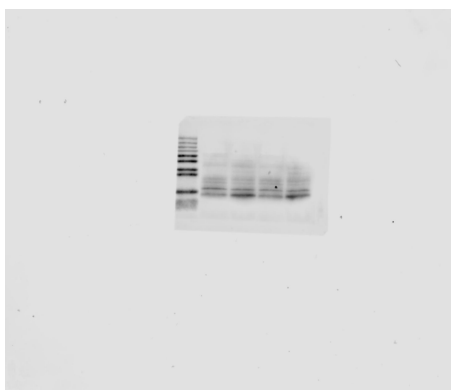


Fig. 3D lc3

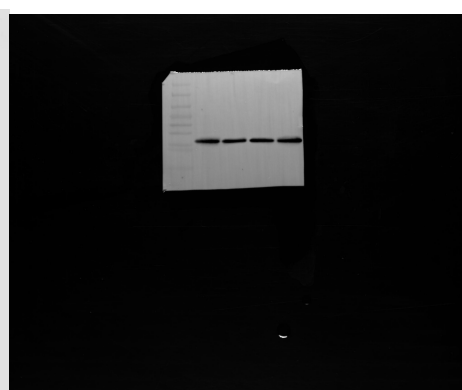


Fig. 3D GAPDH

Fig. 4A

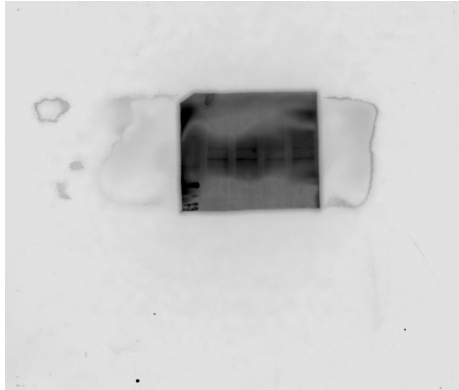


Fig. 4A NLRP3

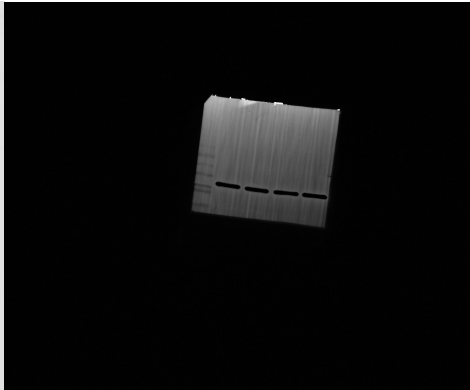


Fig. 4A GAPDH

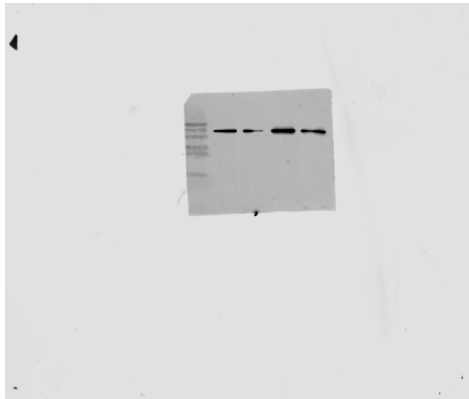


Fig. 4A GSDMD

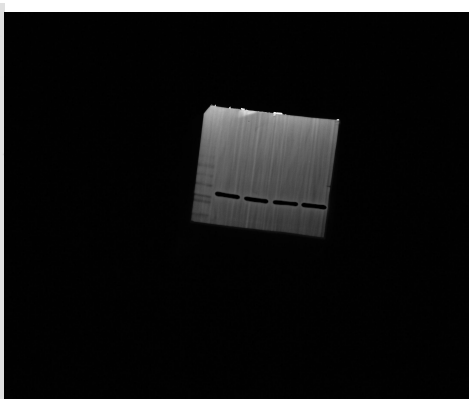


Fig. 4A GAPDH



Fig. 4A GSDMDN

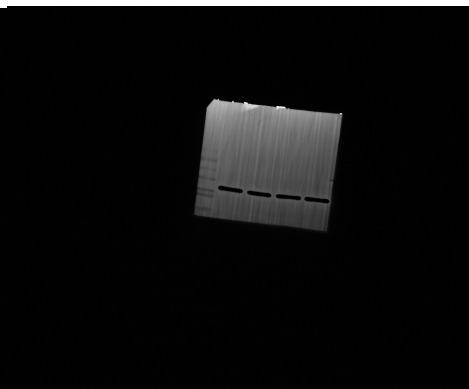


Fig. 4A GAPDH

Fig. 4B

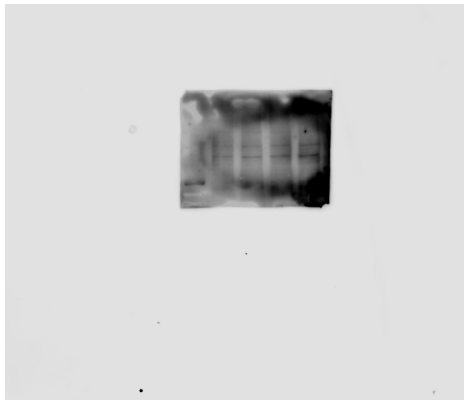


Fig. 4B NLRP3

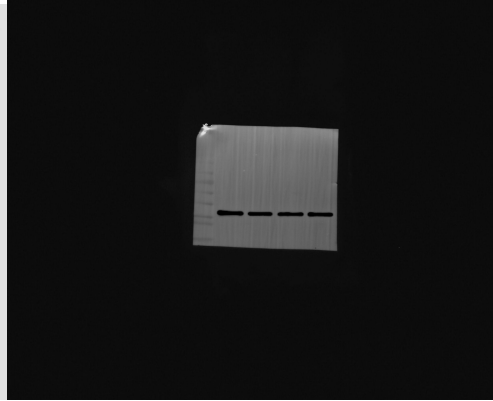


Fig. 4B GAPDH

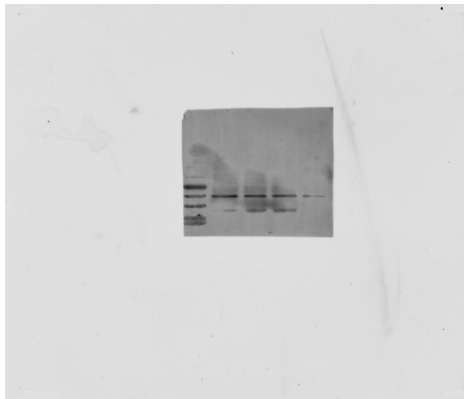


Fig. 4B GSDMD

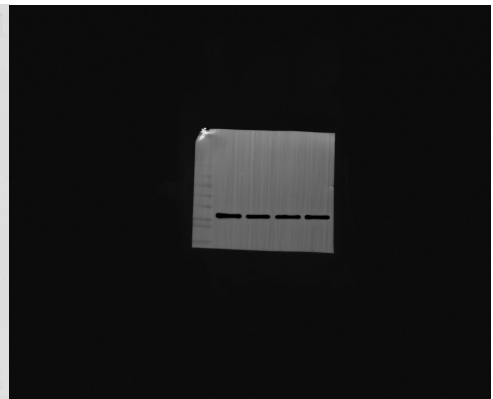


Fig. 4B GAPDH

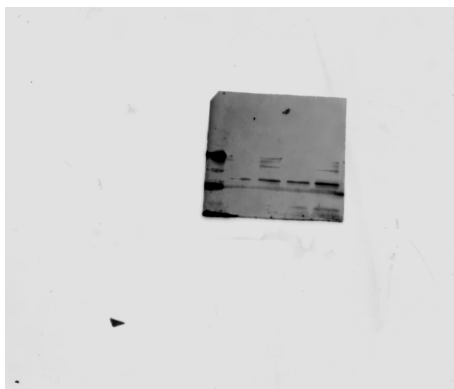


Fig. 4B GSDMDN

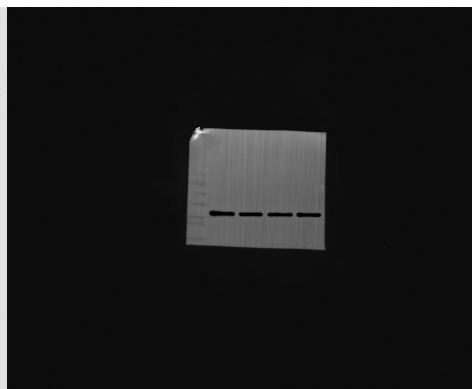


Fig. 4B GAPDH

Fig. 5E

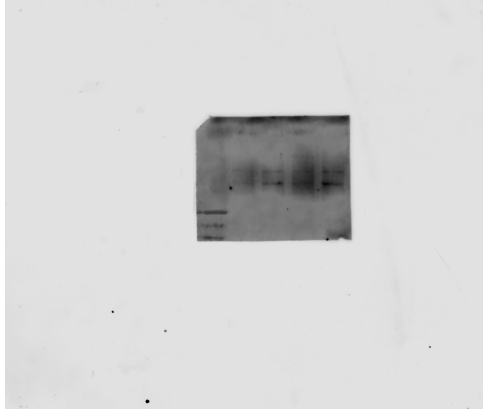


Fig. 5E NLRP3

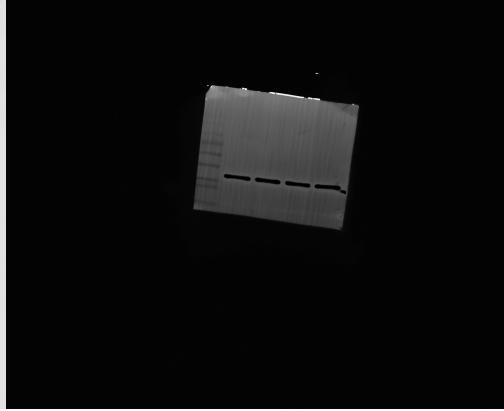


Fig. 5E GAPDH

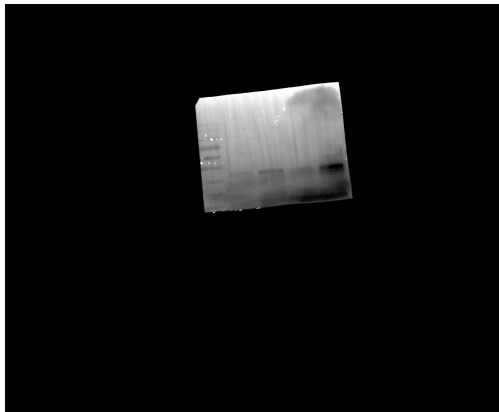


Fig. 5E GSDMDN

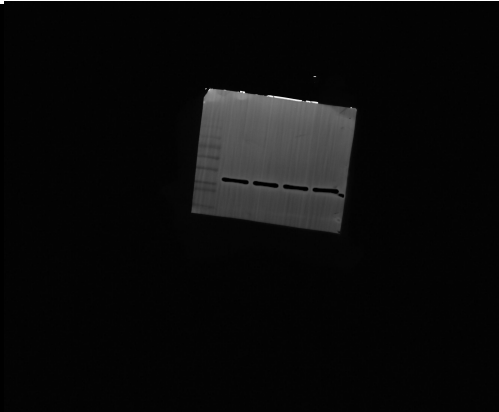


Fig. 5E GAPDH

Qingdao University Laboratory Animal Welfare Ethics Committee Approval

Project title: Inhibition of Anlotinib-induced autophagy enhances pyroptosis and increases osteosarcoma sensitivity to anlotinib

Responsible Unit: Qingdao University

Project Leader: Bingxin Zheng Yue Bin

Project Profile: In this project, all personnel participating in this animal experiment project will pass the animal operation assessment, and all experimental procedures will be implemented in strict accordance with the animal welfare and ethical standards formulated by Qingdao University. BALB/c-nu nude mice will be ideal carriers for rapidly proliferating tumor cells, which will be applicable to the construction of the in vivo tumor model. All experimental mice will be purchased from Beijing Huafukang Biotechnology Co., Ltd. (License No.: SCXK (Jing) 2024-0003), and the whole transportation process will be performed in full compliance with the transportation specifications for SPF-grade laboratory animals.

All mice will be housed in the Laboratory Animal Platform of the Biomedical Center at Qingdao University, and all animal manipulations will be conducted after one week of adaptive rearing. The objective of this project will be to clarify the regulatory effects of anlotinib on apoptosis, pyroptosis and autophagy in osteosarcoma cells, and the dual functional mechanism of anlotinib against osteosarcoma will be further explored. Intratumoral injection therapy will be administered to mice synchronously during the establishment of tumor-bearing mouse models.

Based on the sample size calculation formula, the theoretical number of experimental animals required for each group will be calculated as 10. After taking accidental animal loss during tumor modeling and experimental operation into comprehensive consideration, a minimum of 11 mice per group will be confirmed for the formal experiment. A sterile syringe will be adopted to inject 100 μ L of 143B osteosarcoma cell suspension (1.0×10^6 cells/mL) into the subcutaneous tissue of the right limb of each mouse. Sixty-six tumor-bearing nude mice will be randomly assigned to four groups, as listed below: Control group: Equal volumes of 0.5% CMC-Na will be injected via the tail vein, together with equal-volume normal saline via intraperitoneal administration every day, without additional drug intervention; Anlotinib

monotherapy group: Anlotinib will be injected via tail vein at a dose of 2 mg/kg once daily, which will be the in vivo equivalent dosage matched to the minimum in vitro IC_{50} ; CQ monotherapy group: The autophagy inhibitor chloroquine (CQ) will be delivered by intraperitoneal injection at 25 mg/kg every day; Combined treatment group (CQ + anlotinib): Mice will be pretreated with intraperitoneal CQ (25 mg/kg) for 1 h, followed by tail vein injection of anlotinib (2 mg/kg) on a daily basis. Both drugs will be given at in vivo dosages corresponding to their respective minimum effective in vitro IC_{50} values. After the commencement of drug administration, the tumor volume and body weight of each mouse will be recorded once every two days for a total of two consecutive weeks. Upon the completion of the experiment, all mice from each group will be anesthetized and humanely euthanized. Blood samples will be collected, and tumor tissues as well as major visceral organs will be isolated for subsequent analytical detections.

Comments from the Laboratory Animal Welfare Ethics Committee

In this project, the rights and interests of mice were fully protected, which met the requirements of the Laboratory Animal Welfare Ethics Committee. Agree on the research protocol.

Laboratory Animal Welfare Ethics Committee (seal):

