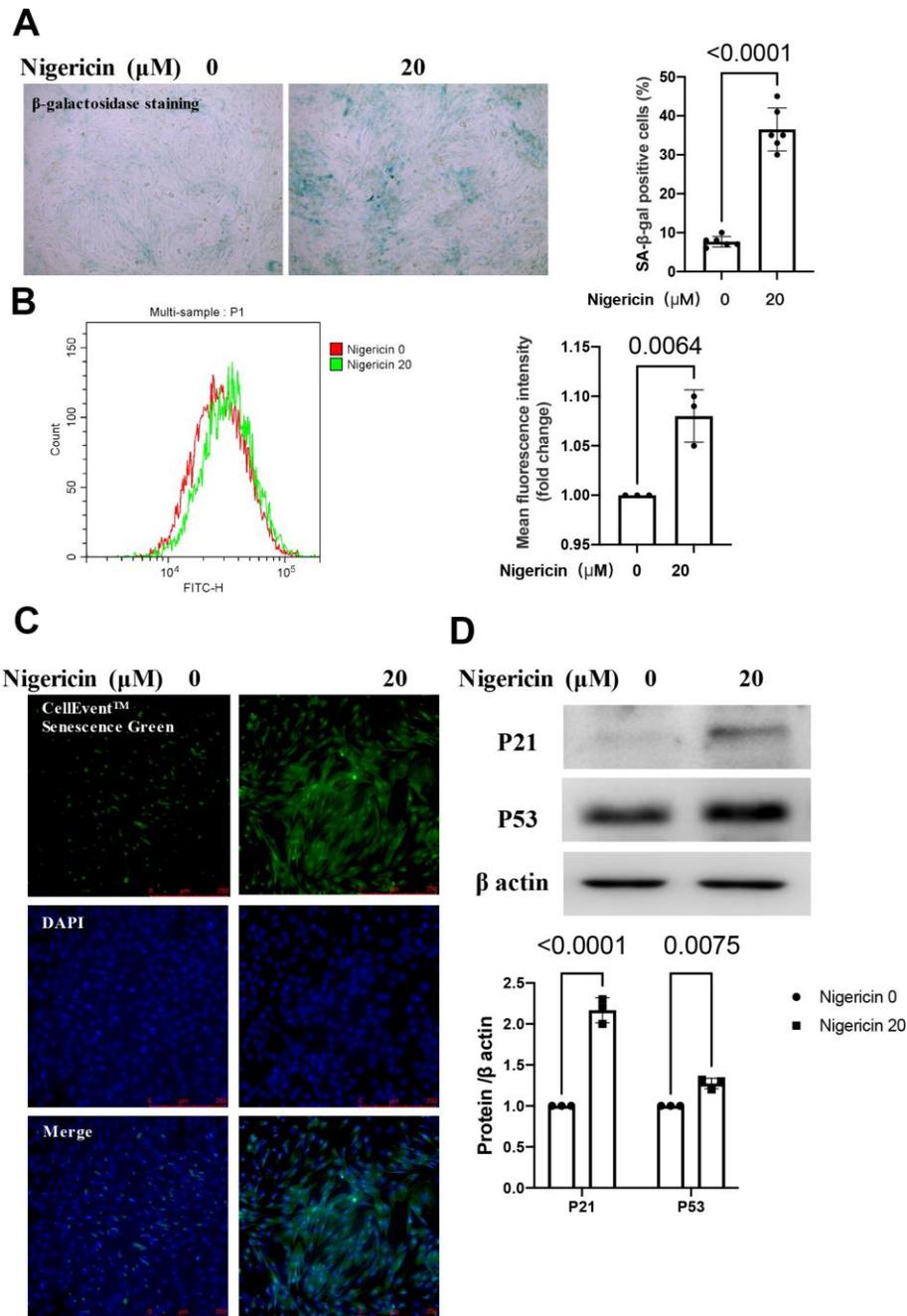
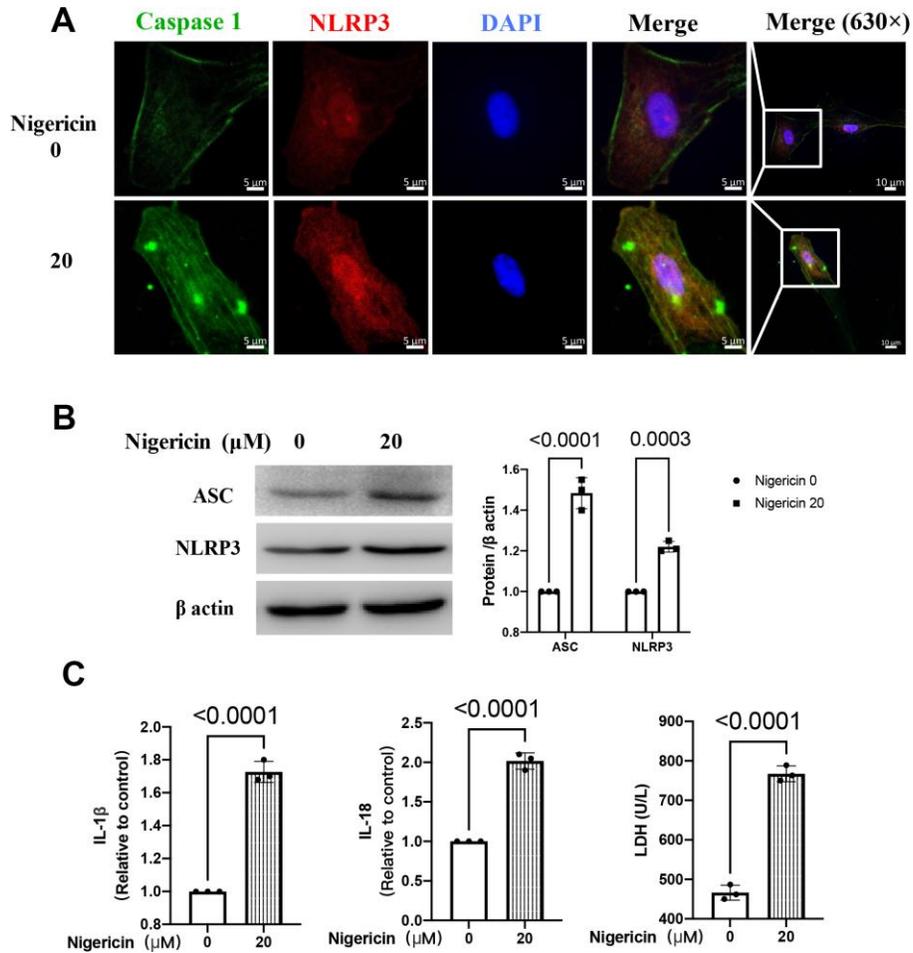


SUPPLEMENTARY FIGURES



Supplementary Figure 1. NLRP3 inflammasome activation by nigericin induced cardiocytes aging. H9c2 cells were pre-treated with or without nigericin (20 μM), a commonly used NLRP3 activator, for 1 hour, and then incubated with standard medium for 24 hours. (A) Representative bright-field photomicrographs showed that nigericin treatment increased the percentage of cells expressing β -galactosidase. (B) Flow cytometry analysis was applied to detect the β -galactosidase mean fluorescence intensity after the nigericin treatment. (C) The CellEvent™ Senescence Green staining showed that nigericin treatment increased the senescence-associated β -galactosidase expression. (D) The aging-associated proteins (P53, P21) were detected by western blot, and the corresponding quantification was present.



Supplementary Figure 2. NLRP3 inflammasomes were activated by nigericin in cardiocytes. H9c2 cells were pre-treated with or without nigericin (20μM), a commonly used NLRP3 activator, for 1 hour, and then incubated with standard medium for 24 hours. (A) Representative confocal fluorescent images showed that nigericin treatment increased the colocalization of NLRP3 (red) and caspase-1 (green) proteins in a concentration-dependent manner. (B) Representative immunoblots of the NLRP3 and ASC proteins and the corresponding quantification were shown. (C) IL-1β, IL-18 and LDH release levels in cell culture were detected. NLRP3, Nod-like receptor family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein.