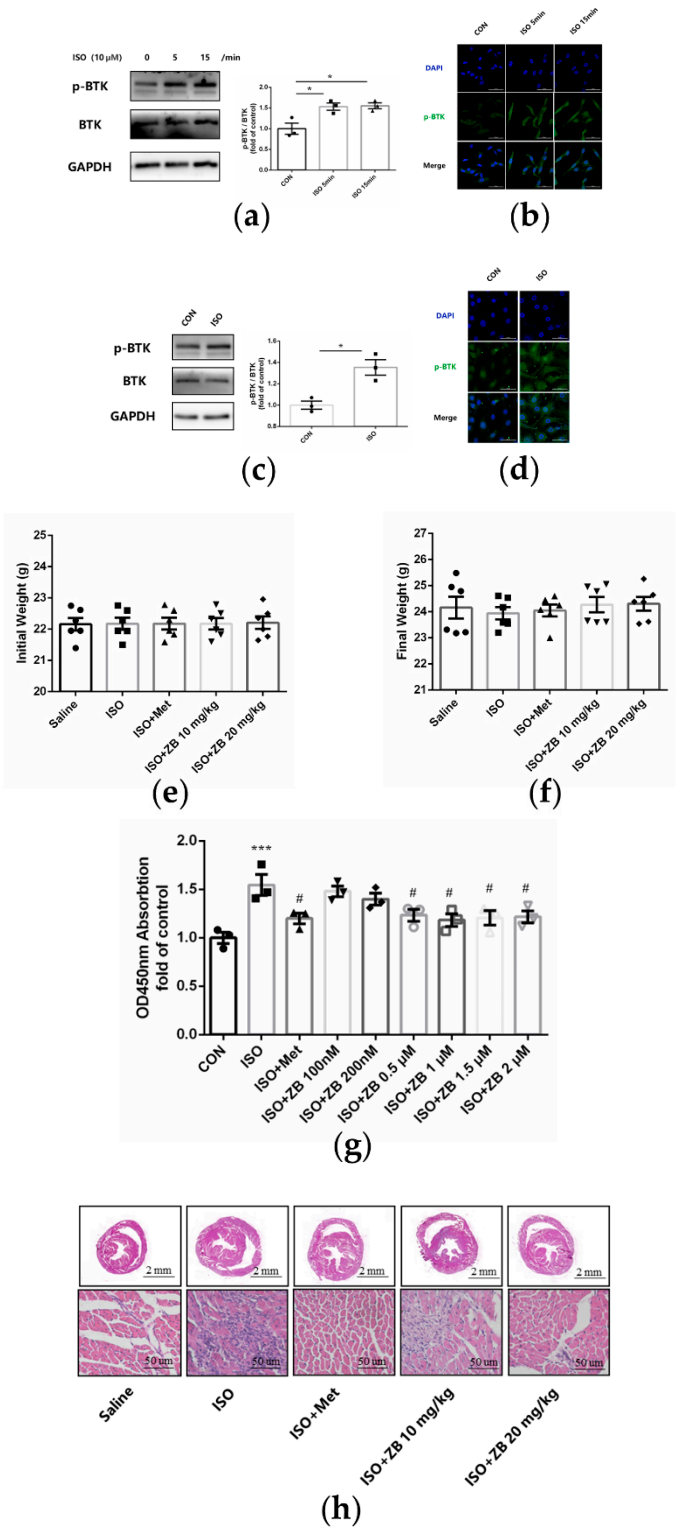


Supplementary Material

Zanubrutinib Ameliorates Cardiac Fibrosis and Inflammation Induced by Chronic Sympathetic Activation



Supplemental Figure S1. Phosphorylation of BTK with short term ISO treated cardiac fibroblasts. **(a)** Western Blot analysis for phospho-BTK level after 5 and 15 min ISO ($10\ \mu\text{mol L}^{-1}$) treatment in NRCFs ($n=3$); **(b)** Immunofluorescence staining of phospho-BTK after 5 and 15 min ISO ($10\ \mu\text{mol L}^{-1}$) treatment in NRCFs. Scale bar: $50\ \mu\text{m}$. NRCFs were starved for 24 h, then treated with ISO ($10\ \mu\text{mol L}^{-1}$) for 5 min and 15min. Phosphorylation of BTK with long term ISO treated cardiac fibroblasts. **(c)** Western Blot analysis for phospho-BTK level after 24 h ISO ($10\ \mu\text{mol L}^{-1}$) treatment in NRCFs ($n=3$); **(d)** Immunofluorescence staining of phospho-BTK after 24 h ISO ($10\ \mu\text{mol L}^{-1}$) treatment in NRCFs. Scale bar: $50\ \mu\text{m}$. NRCFs were starved for 24 h, then treated with ISO ($10\ \mu\text{mol L}^{-1}$) for 24 h. Mice weight of each group. **(e)** Initial body weight of animal experiment from each group ($n=6$); **(f)** Final body weight of animal experiment from each group ($n=6$). Cell viability in different doses of ZB in cardiac fibroblasts. **(g)** Analysis of cell viability in NRCFs by CCK-8. NRCFs were starved for 24 h, incubated with ZB ($0.1\text{-}2\ \mu\text{mol L}^{-1}$) or equal DMSO for 1 h and then treated with ISO ($10\ \mu\text{mol L}^{-1}$) for 24 h. Met was used to be a positive control. H&E staining in heart tissue in cardiac inflammation model. **(h)** Representative $1\times$ and $40\times$ images and quantification of H&E staining in heart tissue ($n=6$). Scale bar (upper): $2\ \text{mm}$; scale bar (lower): $50\ \mu\text{m}$. The data are shown as $\text{mean}\pm\text{SEM}$ (one-way ANOVA with Tukey's post-hoc multiple comparison tests).