**Introduction:** Cardiovascular disease is a burden on the healthcare system, costing **$351.2** **Billion** and causing **17.6 million** global deaths each year1. Heart disease, characterized by irreparable damage resulting in fibrosis and lost tissue function, accounts for **43%** of cardiovascular disease deaths1. Suffering an acute myocardial infarction (MI – AKA heart attack) leads to rapid progression of heart disease due to early tissue injury and later pathologic tissue remodeling2. Existing treatments for MI aim to reduce heart damage (statins, ACE inhibitors) or address the subsequent pathologic remodeling (fibrinolytics, ventricular assist devices). These approaches are ineffective and indiscriminately target symptoms rather than precisely correct the dysregulated cellular processes (inflammation, apoptosis) that cause these symptoms. **Localized therapies that target the underlying signaling cascades responsible for heart remodeling could change the way this disease is treated.**

Following acute MI, cardiomyocytes (CM) lost to inflammation and ischemia are replaced by activated myofibroblasts that deposit collagen to stabilize the damaged heart. Deposited collagen becomes cross-linked and causes the tissue to stiffen, diminishing contractility and heart function. Cross-linked collagen cannot be cleared by natural processes and CMs do not regenerate; thus lost tissue function is permanent2. Myofibroblasts are activated through cellular signaling (Inflammation, CM apoptosis) and increased mechanical stress. *Inflammatory signaling reduction, CM apoptosis reduction, and interruption of tissue stiffening are key therapeutic targets to treat the underlying causes of this disease*. Recent studies have identified IL-13 and IL-33 as factors that can reduce CM loss and regulate immune responses following MI3. **The described work intends to determine if IL-13 and IL-33 can confer the same degree of protection in an in vitro model of MI.**

**Methods:** *Cell culture:* H9C2 rat heart myoblasts (Sigma #88092904-1VL) were cultured at 37°C & 5% CO2 in DMEM/F12 complete media with 2 mM L-glutamate and 10% FBS (Sigma #SLM-243-B), and supplemented with 100 mM Gibco antibiotic-antimycotic (ThermoFisher #15240062). Cells were passaged 7 times, reaching 70-80% confluency, and then seeded in a 96 well plate at a density of 5e4 cells/well. *Simulated Hypoxia:* Seeded cells were incubated for 72 hours before subjecting to simulated hypoxia. Seeded cells were placed inside a hypoxic chamber (37°C, 1% O2) and the wells were aspirated and 200 of hypoxic media (DMEM/F12 media equilibrated inside the hypoxic chamber for 12 hours) was transferred to each well. Cells were subjected to simulated hypoxia for 3 hours, after which the wells were aspirated and 200 of DMEM/F12 media supplemented with 100 mM IncuCyte® Caspase-3/7 Green Apoptosis Assay Reagent (Sartorius #4440) and test factors. Test factors include 20 ng/mL TNF (Apoptosis Control), 100 ng/mL IL-13 (Apoptosis Protection), 100 ng/mL IL-33 (Apoptosis Protection), Factor Control (No factors), and hypoxia control (Double Control). The hypoxia control group followed the same cell culturing and seeding procedure, but these cells were neither subjected to hypoxia nor exposed to any factors. *Apoptosis Assay:* Apoptosis rate was tracked for each well seeded with cells in the 96 well plate using IncuCyte® Live-Cell Analyzer, which tracked the total count of green labeled (apoptotic) cells at 2-hour intervals. Experimental groups (N=6) were compared at the 24-hour time point. *Statistical Analysis:* Comparisons between multiple treatment groups were performed using one-way ANOVA, followed by Holm correction for multiple comparisons, and p < 0.05 was considered statistically significant. Statistical tests were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

**Results:**

**References:** [1]Benjamin, E., et al. (2018) Circulation. [2]Konstarn, M., et al. (2011) JACC Cardiovasc. Imaging. [3]Wodsedalek, D., et al. (2019) Am. J. Physiol. Heart Circ. Physiol. [4]Wang , Y., et al. (2019) Circulation. [5]Jung, M., et al. (2017) Basic Res. Cardiol. [6] Liu, Q., et al. (2019) JCI Insight. [7]El Hajj, E., et al. (2018) Am. J. Physiol. Heart Circ. Physiol. [8]Matsumura, Y., et al. (2019) Biomaterials. [9]Tang, J., et al. (2018) Sci. Adv. [10]JhunJhunwala, S., et al. (2012) Adv. Mater. [11]Curaj, A., et al. (2015) J. Vis. Exp.