# Phenotypic Screening Uncovers Eicosanoid Degradation in Fibroblasts as a Therapeutic Target for Cardiac Fibrosis

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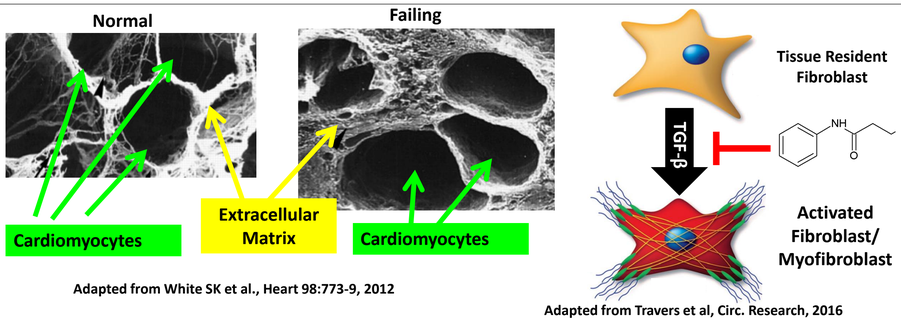
## Abstract

Cardiac fibrosis is defined as the existence of excess collagen-rich fibrotic tissue in the myocardium, which leads to adverse outcomes such as fatal arrhythmias and heart failure via abnormal muscle relaxation and contraction. Cardiac fibrosis is a major unmet medical need, and the elucidation of novel mechanisms involved in fibrogenesis in the heart is required for the development of new therapies for this deadly process. Fibrosis is mainly driven by fibroblasts, which secrete extracellular matrix proteins such as collagen. Using a high content imaging platform, we performed a high throughput phenotypic screen of 546 target focused small molecules using three different types of fibroblasts (cardiac, kidney and lung) to discover inhibitors of fibroblast activation driven by the pro-fibrotic growth factor, TGF-β. Nine overlapping 'hit' compounds were identified that blocked myofibroblast activation without overt toxicity. Follow-up studies were performed with **SW033291**, a compound that inhibits 15-PGDH, an enzyme that degrades arachidonic acid-derived eicosanoids. Inhibiting eicosanoid degradation with SW033291 blocked cardiac fibroblast activation *in vitro* and ameliorated angiotensin II-mediated cardiac interstitial fibrosis *in vivo*. RNA-seq data showed that treating activated fibroblasts with SW033291 induced a global dampening of pro-fibrotic gene expression in association with reduced TGF-β signaling, and augmented ERK kinase signaling. **Follow-up functional studies demonstrated that SW033291-mediated inhibition of cardiac fibroblast activation is dependent on stimulation of ERK signaling**. Finally, screening of different eicosanoids implicated 12-(S)-HETE as the anti-fibrotic factor that is induced by SW033291. The findings reveal a critical role for eicosanoid degradation in the pathogenesis of cardiac disease, and suggest potential for 15-PGDH inhibitors for anti-fibrotic therapy.

## Background

Heart failure, the clinical manifestation of numerous forms of cardiovascular disease, is a devastating disorder characterized by interstitial fibrosis, chamber remodeling, and reduced ventricular compliance. Heart disease remains the predominant cause of mortality in the United States, accounting for nearly 800,000 deaths per year. Myocardial fibrosis is a significant global health problem associated with nearly all forms of heart disease. Cardiac fibroblasts (CF) are responsible for the homeostasis of the extracellular matrix; however, upon injury, these cells transform into a myofibroblast phenotype and contribute to cardiac fibrosis. This remodeling involves pathological changes that include chamber dilation, cardiomyocyte hypertrophy and apoptosis, and ultimately leads to the progression to heart failure. In general, the critical phases of this response consist of inflammation, proliferation of non-myocytes, and scar maturation, with the CF intimately involved in all of these processes.

**Finding novel, druggable targets for therapeutic intervention remains a top priority.**
**Despite substantial improvements in therapeutic strategies, cardiovascular disease remains the leading cause of death worldwide indicating an urgent need for innovative treatment strategies.**




Adapted from White SK et al., Heart 98:773-9, 2012

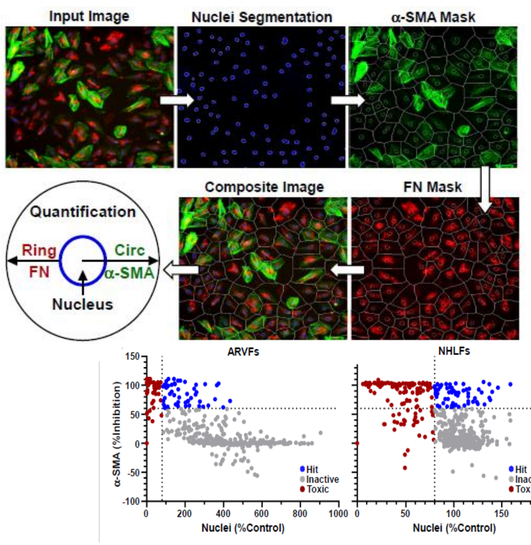



Adapted from Travers et al, Circ. Research, 2016

## method: High Content Screening (HCS) Assay

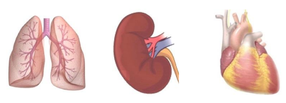
**High content screening:** Thermo CellInsight CX7 **Data analysis:** CDD Vault

**Compound library (~2,500 compounds) I thought you only screened ~550 compounds (yes 546)**
- Selleckchem Target Selective Library

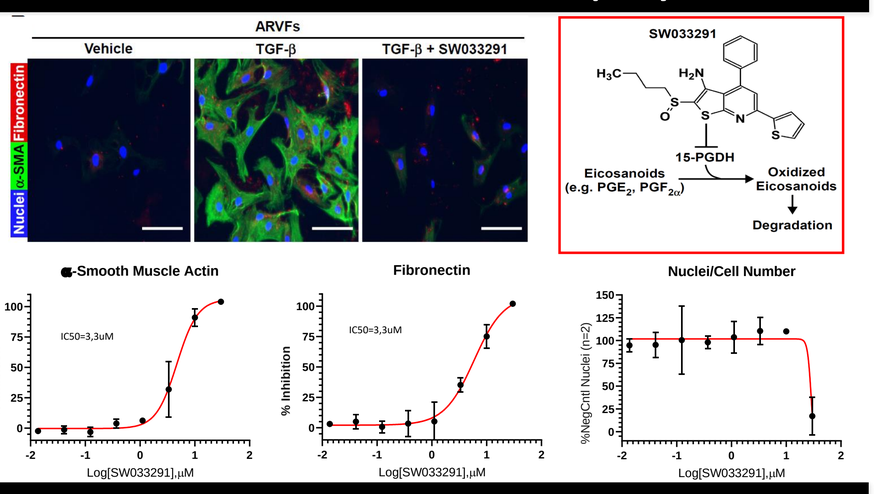



**Cell-based fibrosis HCS assays under development**

-Human: **NHLF**- Human Lung Fibroblasts
-Rat: **NRK-49F** (rat renal fibroblasts)
**ARVF** (Adult Rat Ventricular Fibroblasts)



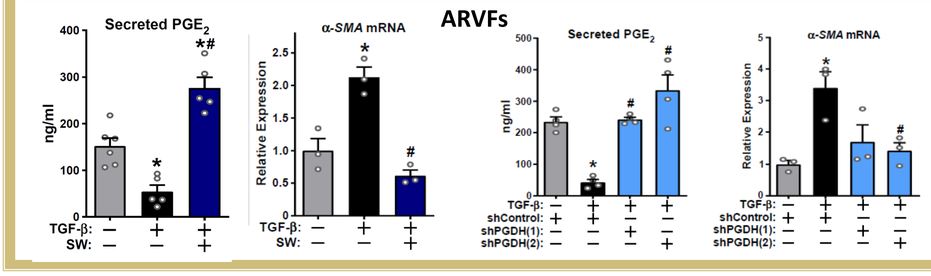
## HCS *Results: SW033291 (SW)*



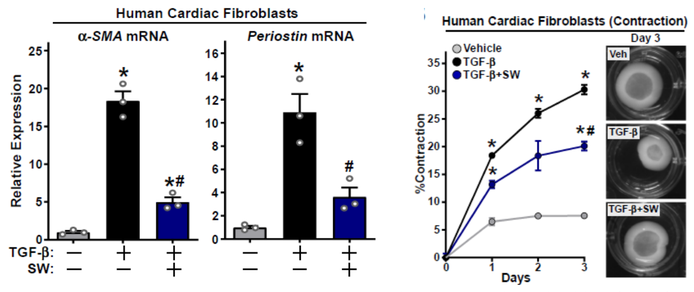




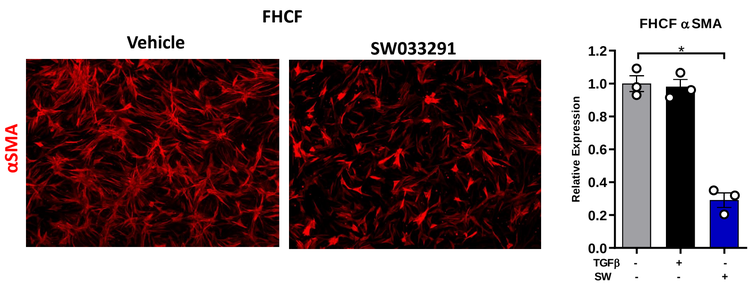
## SW: eicosanoids and fibrotic genes




## Results: SW in Failing Human Cardiac Fibroblasts (FHCF)

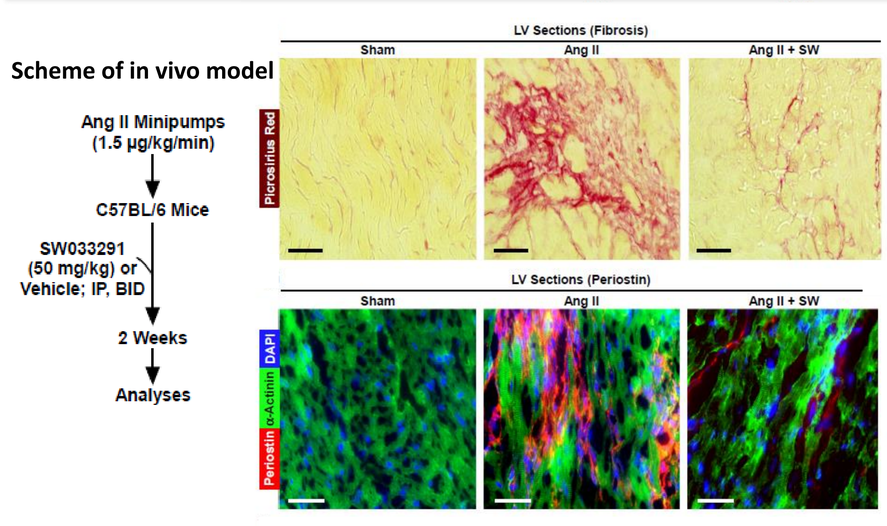



Gene expression and contraction assay in Normal Human Cardiac Fibroblast (NHCF) from LONZA



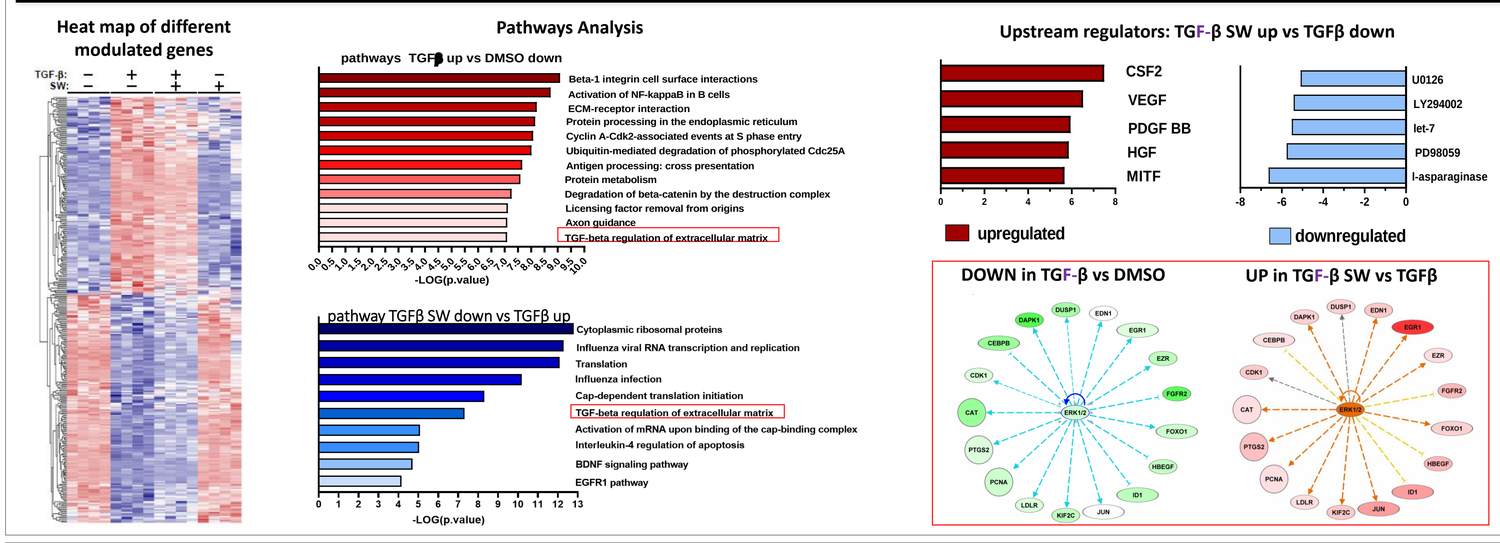

Immune Fluorescence and gene expression in fibroblasts isolated from a piece of human LV with left ventricular assist device (LVAD): **end-stage heart failure**

## IN VIVO: ANG II model

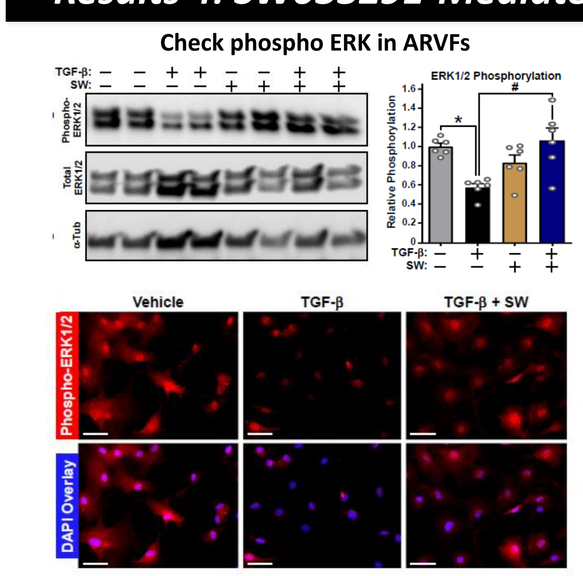



**ELISA in LV lysate** : SW injection is able to increase $PGE_2$ levels in the whole LV

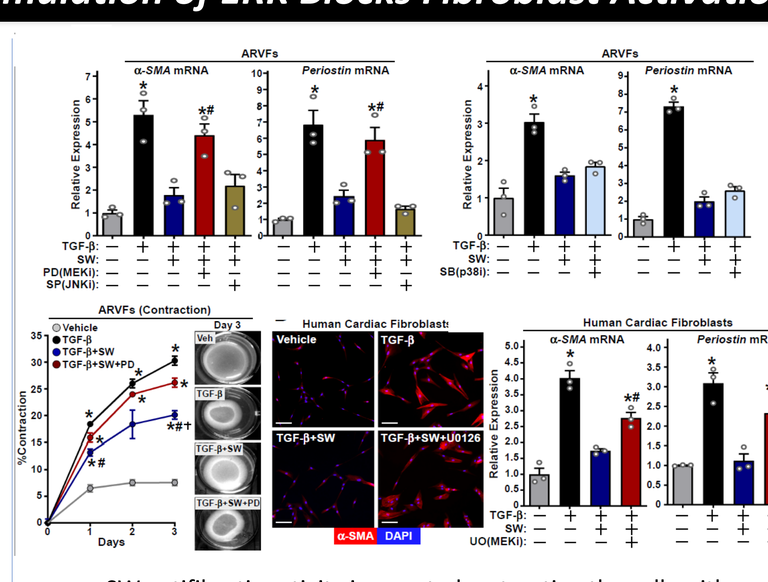
## Results 3: RNA-SEQ Analysis in ARVFs




## Results 4: SW033291-Mediated Stimulation of ERK Blocks Fibroblast Activation

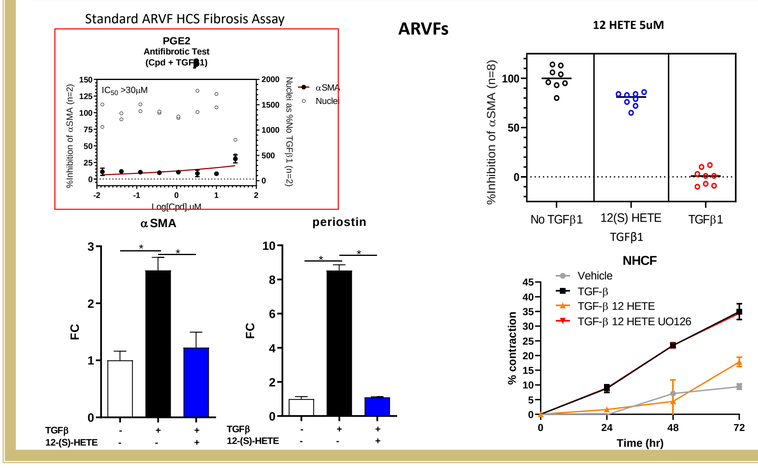



ERK activation decreases in activated fibroblasts and is restored by SW treatment

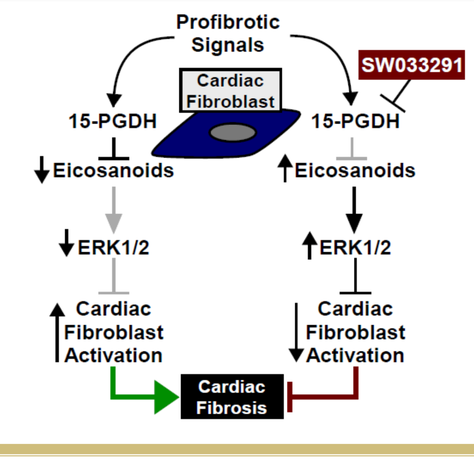



SW antifibrotic activity is reverted co-treating the cells with MEK inhibitor PD98 or UO126

## Results 5: 12-HETE




## Proposed mechanism

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