Elucidating the Molecular Mechanisms and Cellular Specificity of HDAC Inhibitor Efficacy in Diastolic Dysfunction

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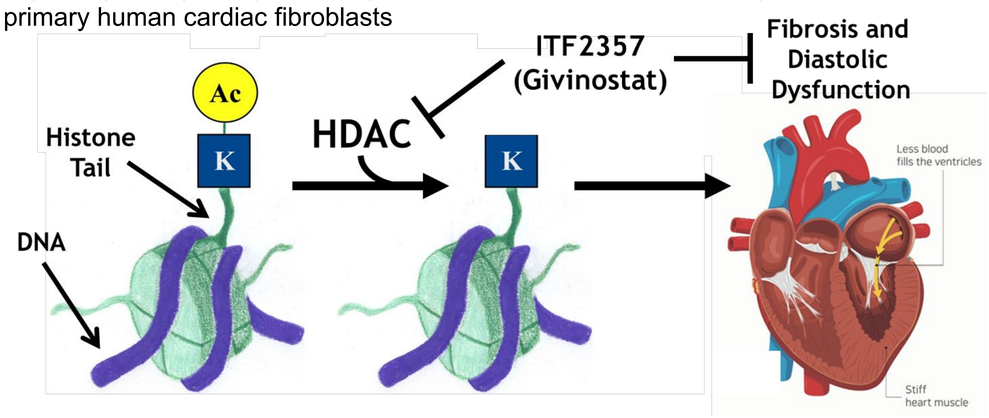
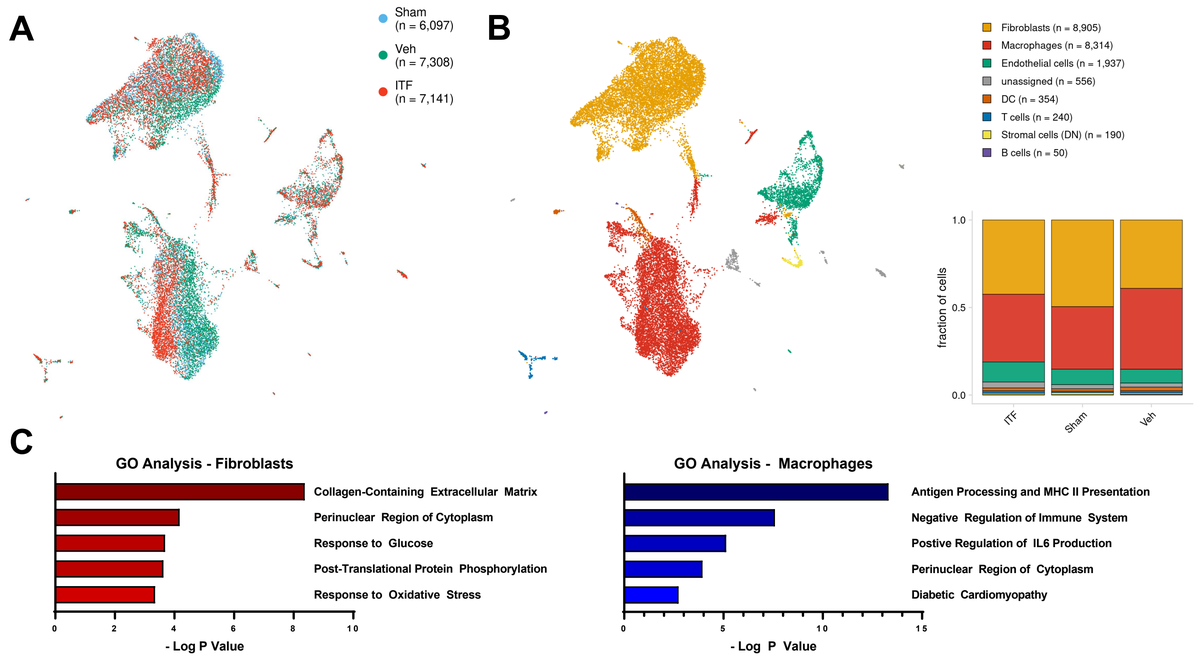
Introduction

- Diastolic dysfunction is associated with the development of heart failure with preserved ejection fraction (HFpEF) and contributes to the pathogenesis of numerous other cardiac maladies
- HFpEF is resistant to standard-of-care medications for the treatment of HF with reduced ejection fraction, highlighting an urgent unmet medical need
- We addressed the therapeutic potential of ITF2357 (Givinostat), a clinical-stage inhibitor of histone deacetylase (HDAC) catalytic activity, in a murine model of diastolic dysfunction with preserved EF and primary human cardiac fibroblasts



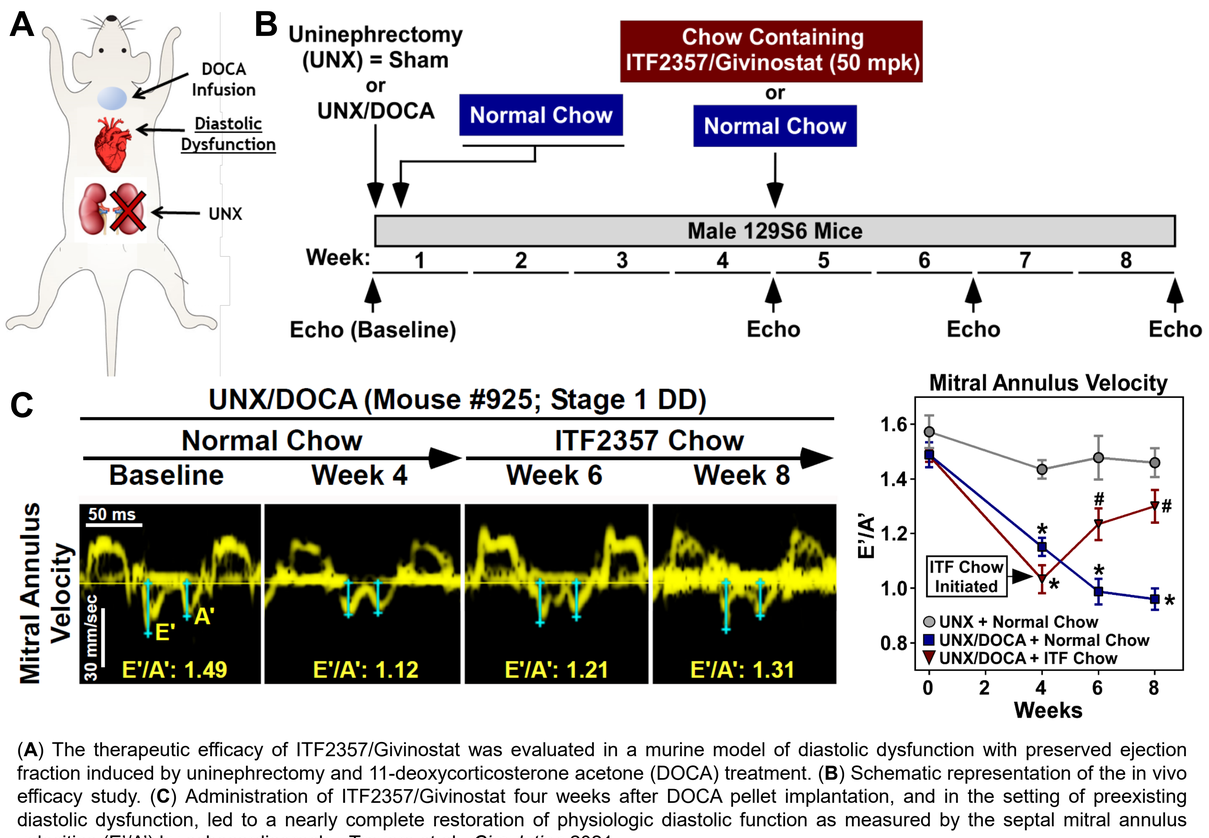
Diastolic heart failure, a major cause of morbidity and mortality, is defined as symptoms of heart failure in patients with preserved LV contractility. It is characterized by LV stiffness with decreased compliance and impaired relaxation, and is the final common end point of many types of pathologic stress including hypertension. Dynamic acetylation of nucleosomal histones represents an important component of chromatin-dependent signal transduction. HDACs are a family of enzymes that catalyze the removal of acetyl groups from lysine residues, resulting in fibrosis and diastolic dysfunction. ITF2357/Givinostat is a clinical-stage inhibitor of HDAC catalytic activity.

Single Cell RNA Sequencing in the UNX/DOCA Diastolic Dysfunction Model



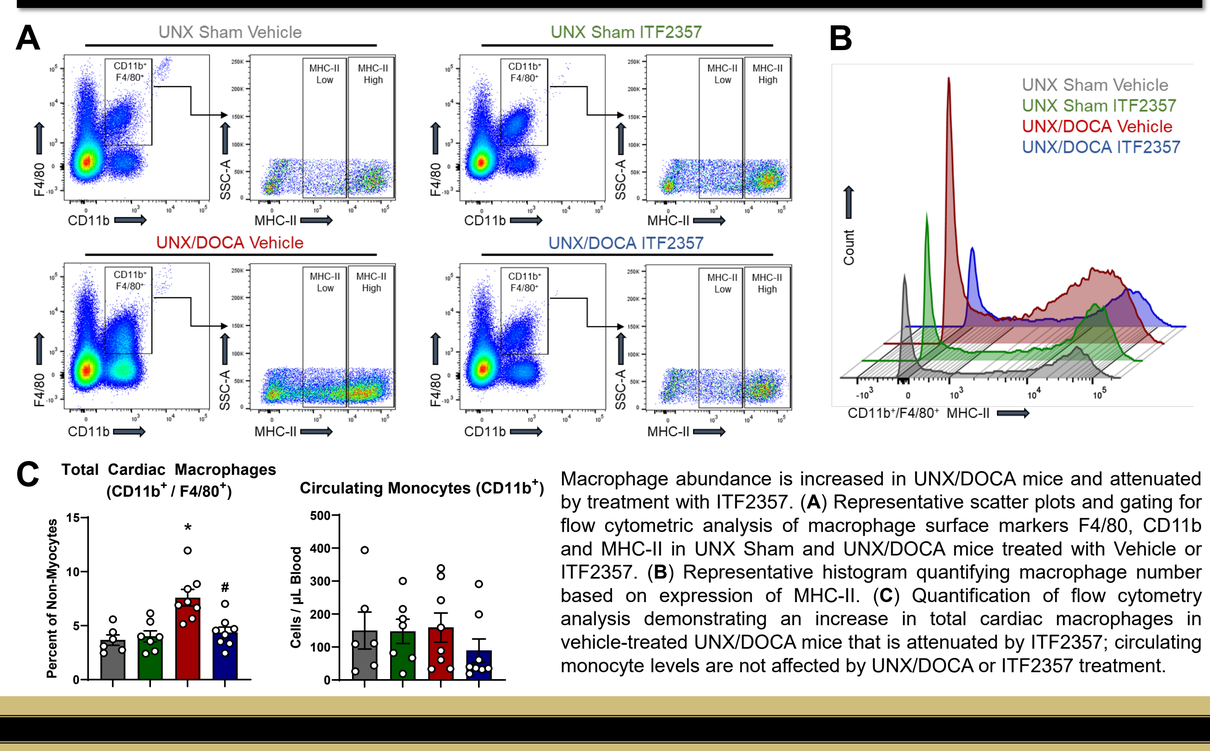
(**A**) UMAP projections are shown for clustering and visualizing differences between cells. After filtering cells to only include those with between 250 and 6,000 detected genes (20,546 cells total), the top 2,000 genes that show the most cell-to-cell variation were identified. (**B**) UMAP projections are shown for the selected cell type annotations (clustering resolution 3.4) with cells colored by type. The fraction of cells belonging to each type is shown on the right. (**C**) Gene Ontology analysis of genes upregulated in the vehicle-treated UNX/DOCA mouse when compared to Sham and downregulated by ITF2357 for cardiac fibroblasts and macrophages.

HDAC Inhibition Reverses Preexisting Diastolic Dysfunction



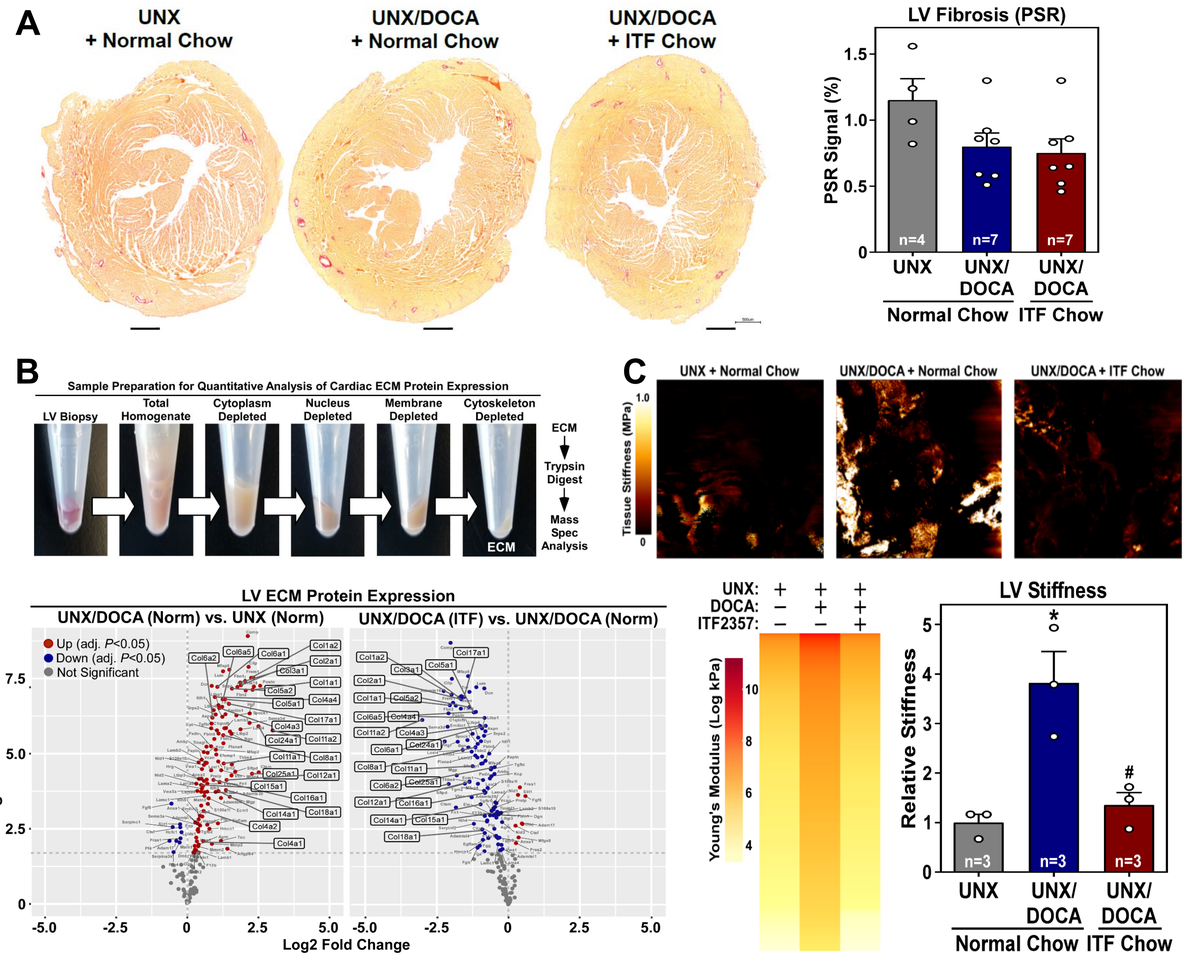
(**A**) The therapeutic efficacy of ITF2357/Givinostat was evaluated in a murine model of diastolic dysfunction with preserved ejection fraction induced by uninephrectomy and 11-deoxycorticosterone acetone (DOCA) treatment. (**B**) Schematic representation of the in vivo efficacy study. (**C**) Administration of ITF2357/Givinostat four weeks after DOCA pellet implantation, and in the setting of preexisting diastolic dysfunction, led to a nearly complete restoration of physiologic diastolic function as measured by the septal mitral annulus velocities (E'/A') by echocardiography. Travers et al., *Circulation* 2021.

Evaluation of Macrophage Content and Abundance in UNX/DOCA by Flow Cytometry



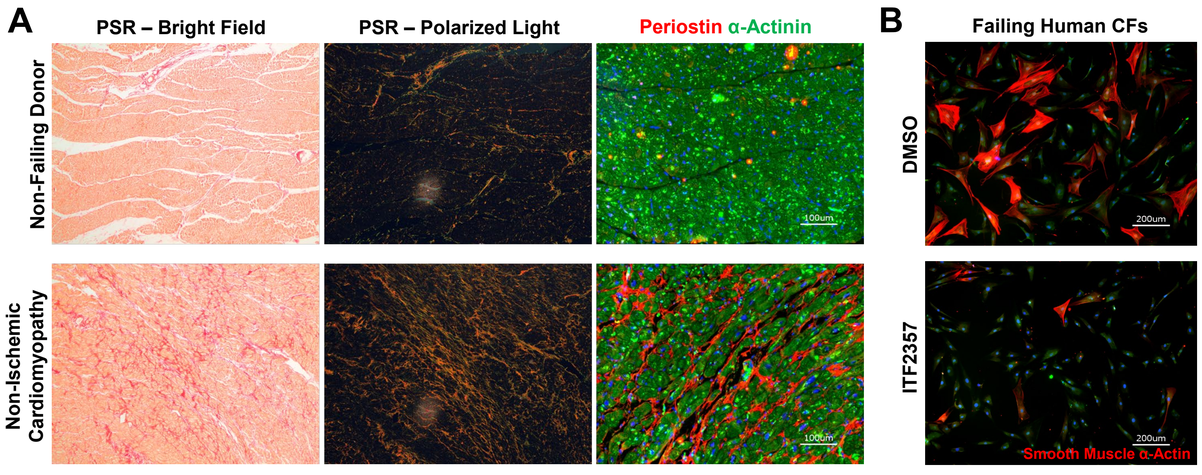
Macrophage abundance is increased in UNX/DOCA mice and attenuated by treatment with ITF2357. (**A**) Representative scatter plots and gating for flow cytometric analysis of macrophage surface markers F4/80, CD11b and MHC-II in UNX Sham and UNX/DOCA mice treated with Vehicle or ITF2357. (**B**) Representative histogram quantifying macrophage number based on expression of MHC-II. (**C**) Quantification of flow cytometry analysis demonstrating an increase in total cardiac macrophages in vehicle-treated UNX/DOCA mice that is attenuated by ITF2357; circulating monocyte levels are not affected by UNX/DOCA or ITF2357 treatment.

Evaluation of ECM Remodeling Reveals "Hidden" Fibrosis Blocked by HDAC Inhibition



(**A**) No evidence of cardiac fibrosis was detected in cardiac sections from UNX/DOCA animals based on histological analysis using picrosirius red staining. (**B**) Decellularization of left ventricular (LV) biopsy tissue yields an ECM enriched fraction that was subsequently analyzed by quantitative mass spectrometry. Volcano plots show magnitude and significance of ECM proteins altered in LVs of UNX/DOCA+normal chow vs UNX+normal chow mice (left) and UNX/DOCA+ITF chow mice vs UNX/DOCA+normal chow mice (right), with collagens highlighted. This analysis revealed substantial ECM compositional alterations in UNX/DOCA mice that were abolished by treatment with ITF2357/Givinostat. (**C**) Atomic force microscopy reveals LV stiffening that is blocked by ITF2357/Givinostat. Representative stiffness maps in kPa (top) and Young's modulus heat map (bottom) show the stiffness distribution across groups. Travers et al., *Circulation* 2021.

HDAC Inhibition in Cardiac Fibroblasts from Explanted Human Heart Tissue



(**A**) Cardiac tissue was collected from a human non-failing donor heart not suitable for transplant, as well as from an explanted failing heart from a non-ischemic cardiomyopathy patient. Fibrotic remodeling was evaluated by picrosirius red staining under both bright field, or alternatively by evaluating the birefringence of the PSR signal using polarized light microscopy; cardiac sections were also labeled using antibodies directed against periostin. (**B**) Failing human cardiac fibroblasts were treated with the HDAC inhibitor ITF2357/Givinostat and myofibroblast activation was evaluated by assessing smooth muscle α-actin expression.

Conclusions

- HDAC inhibition is a powerful means of reducing ECM-mediated stiffening of the heart to restore physiological diastolic function
- "Hidden fibrosis" not detected by standard histological methodologies is pathophysiologically relevant in the development of diastolic dysfunction
- UNX/DOCA causes dramatic shifts in fibroblasts and macrophages that are blunted by ITF2357
- HDAC inhibition suppresses the activation of failing human cardiac fibroblasts

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