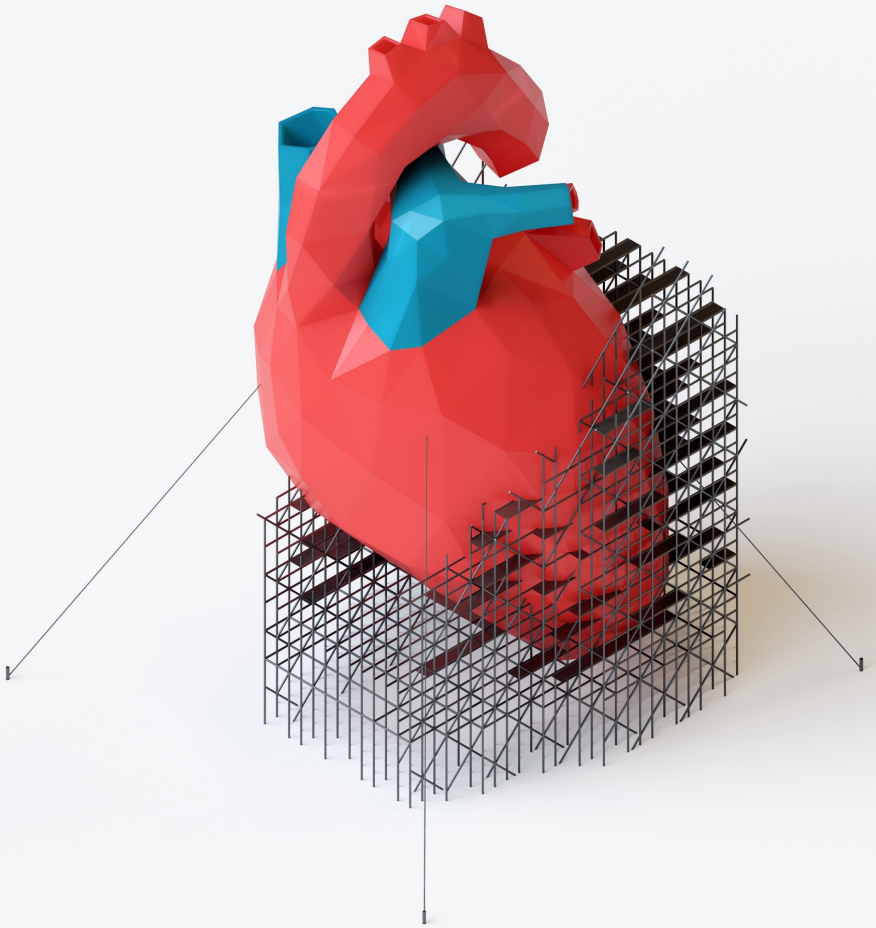
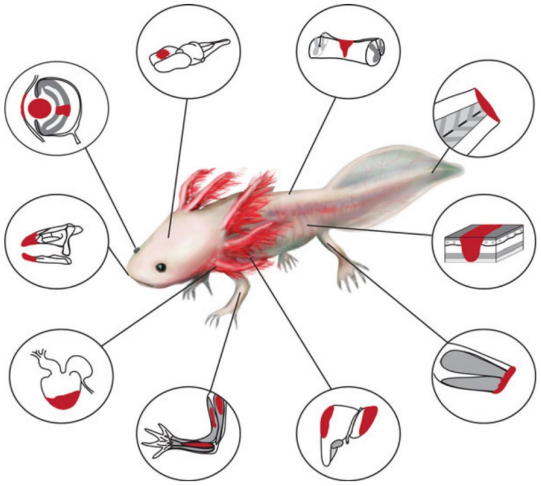
Regenerating Tissue to Treat Disease

Michael J. Bollong
Associate Professor
Early Career Endowed Roon Chair
Department of Chemistry



Regeneration of limbs and organs in lower species

Multi-organ and limb regeneration in salamanders



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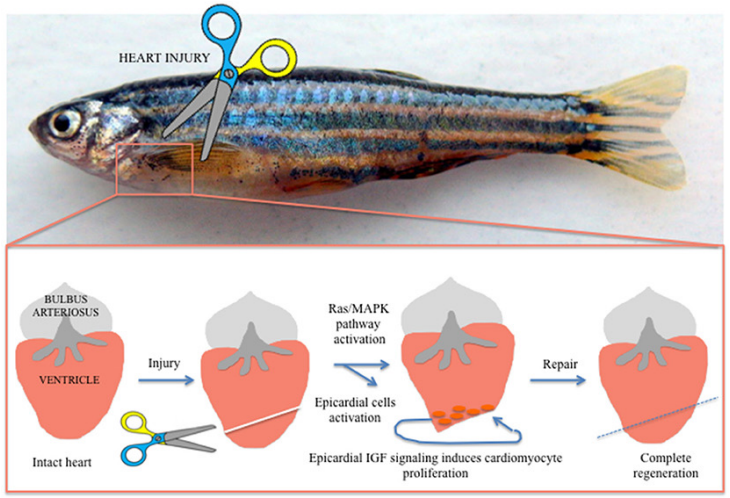
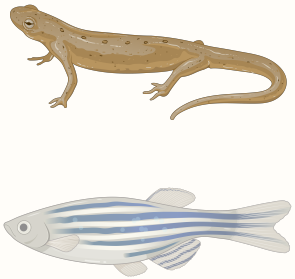
Cardiac regeneration in zebrafish



PMID: 31448289

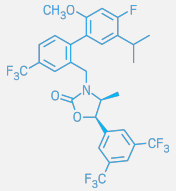
Small molecule drugs for regenerative medicine

Regenerative capacity in lower species



Resilience to disease

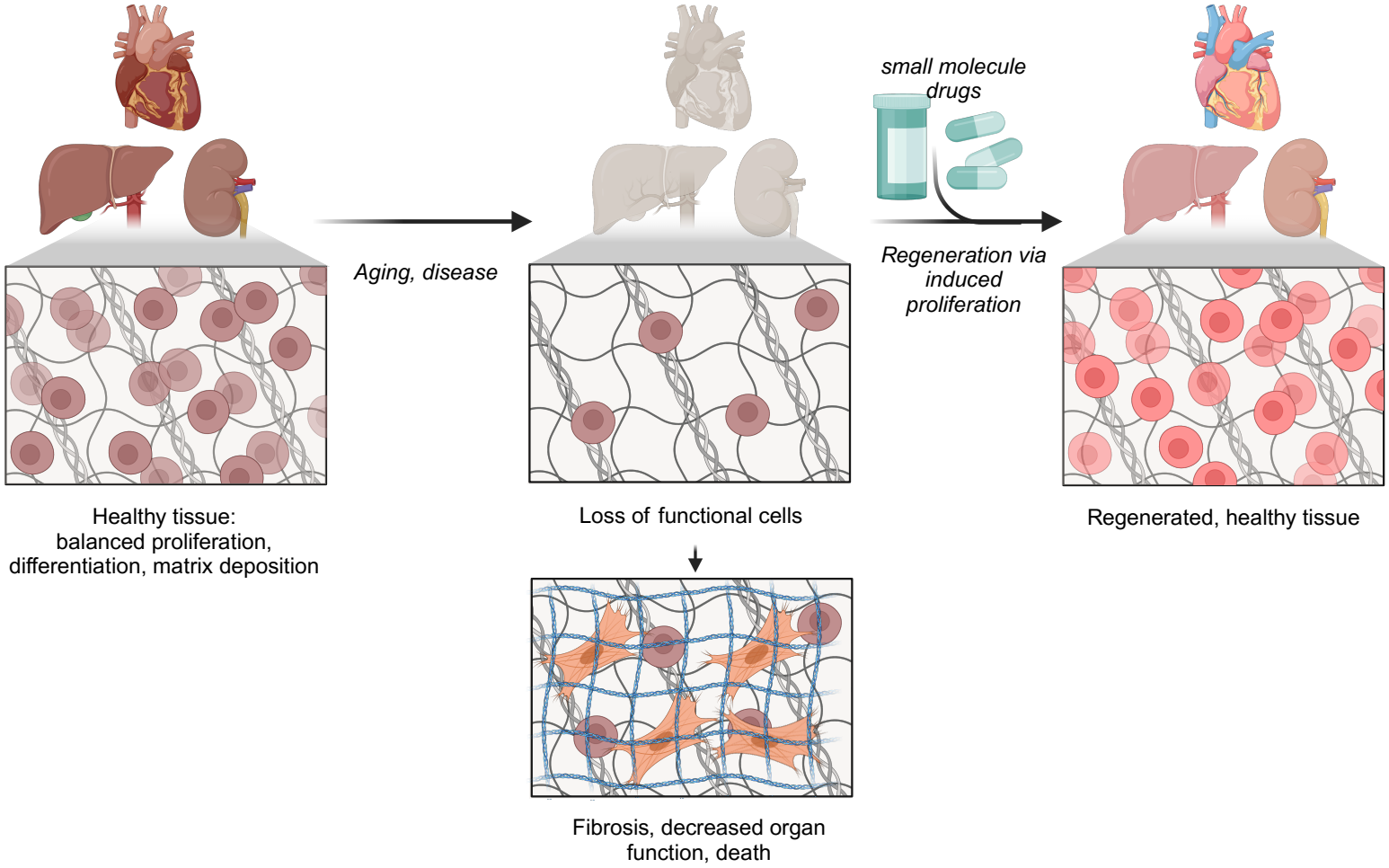
Small molecules identified by phenotypic chemical screens



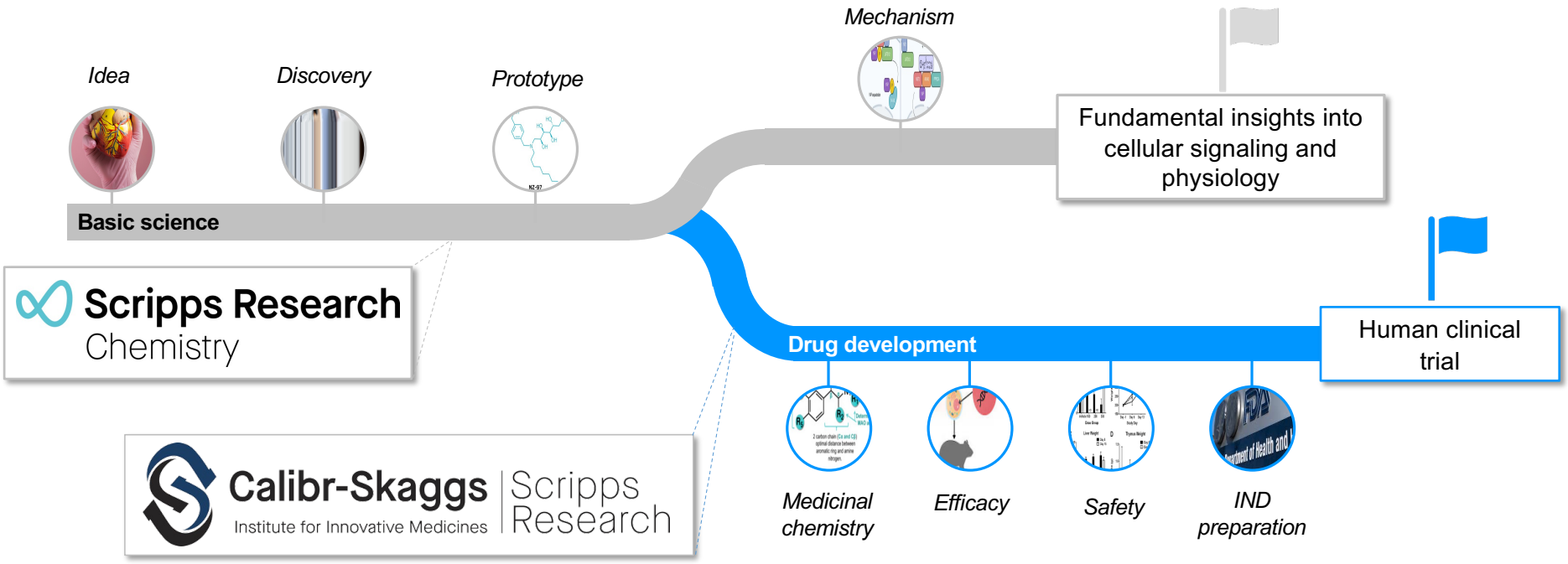
Induced proliferation, differentiation, remodeling

Human tissue degeneration with aging

Regenerative organ repair requires cellular proliferation



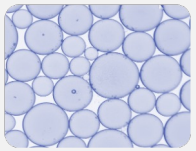
Bollong Lab: all phases of small molecule drug development



Overview of talk

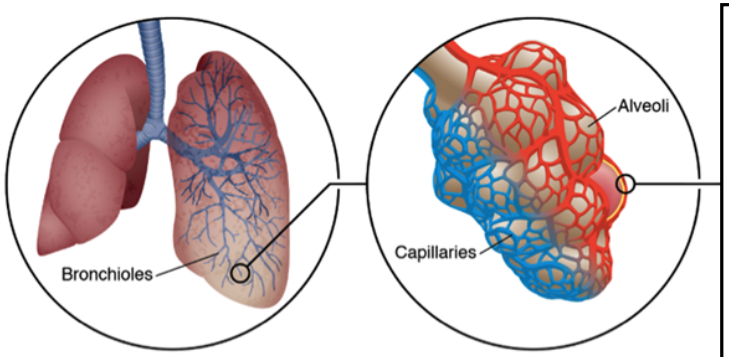
A drug that regenerates the lower airway

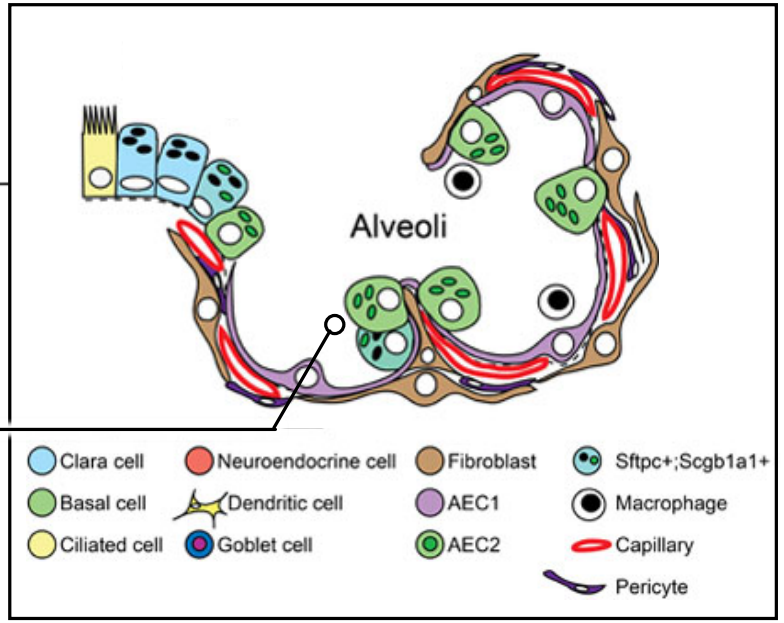
Manipulating organ size control for cardiac repair

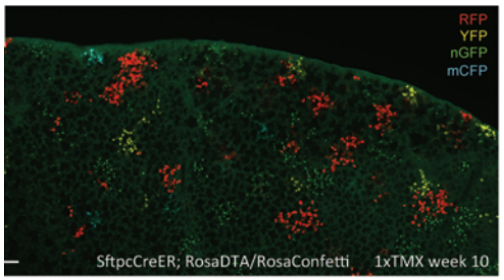


Drugging the cellular causes of aging

AEC2s are the stem cells of the lower airway







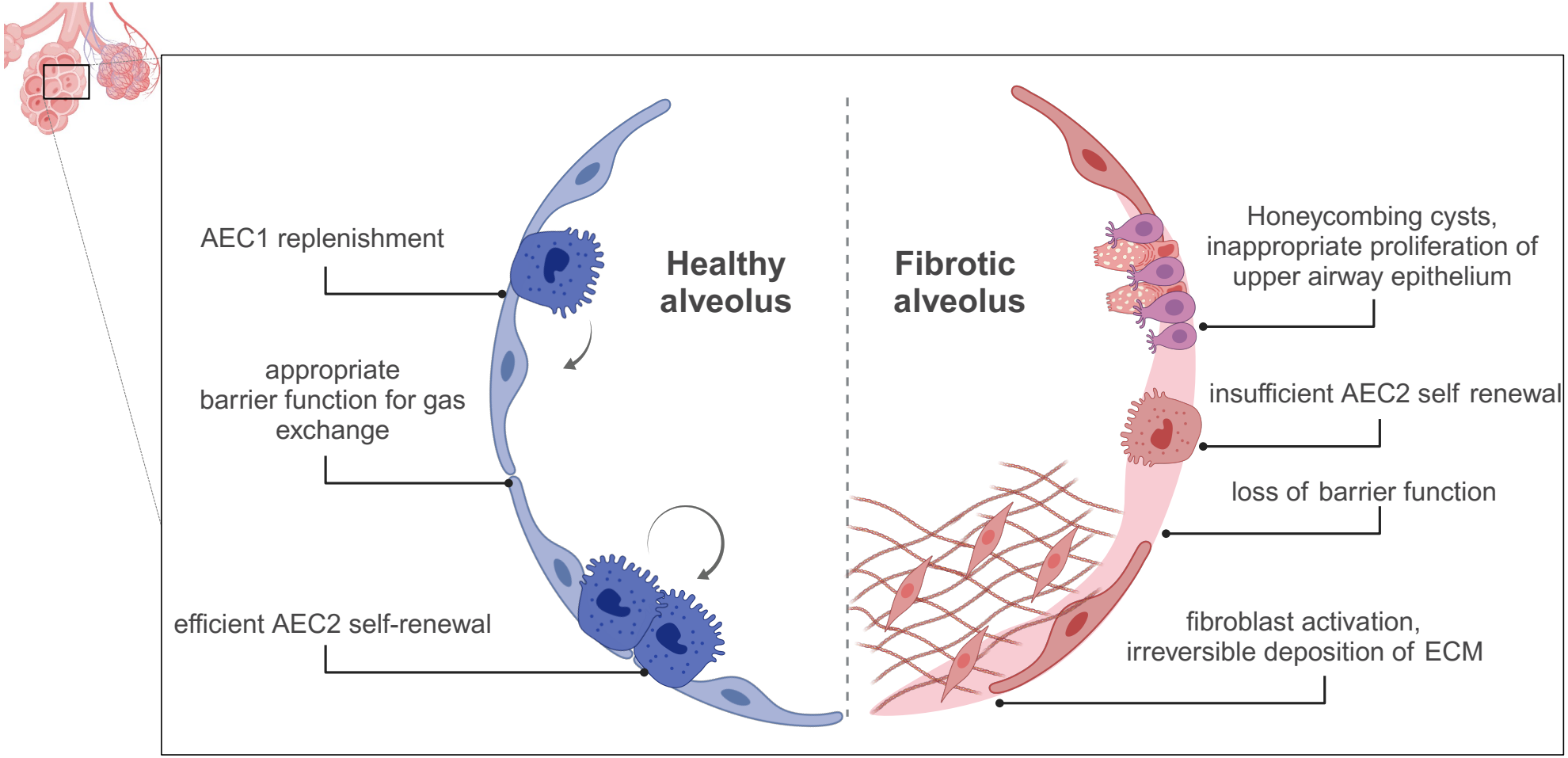
AEC2 – <10% area, 60% of total cells, cuboidal, surfactant production, source of AEC1 cells

AEC1 – >90% area, gas exchange

AEC2s are stem/progenitors of the lower airway

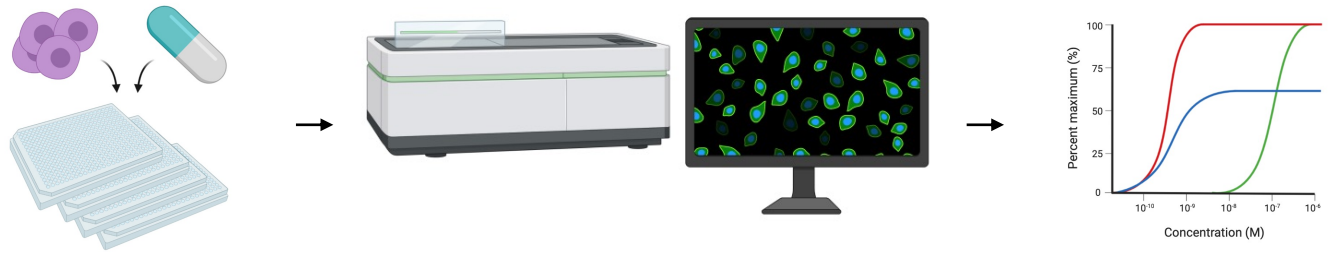
- Clonal proliferation after partial genetic ablation
- 3D clonal growth and differentiation *in vitro*

Ineffective AEC2 proliferation is causative of **Idiopathic Pulmonary Fibrosis (IPF)**

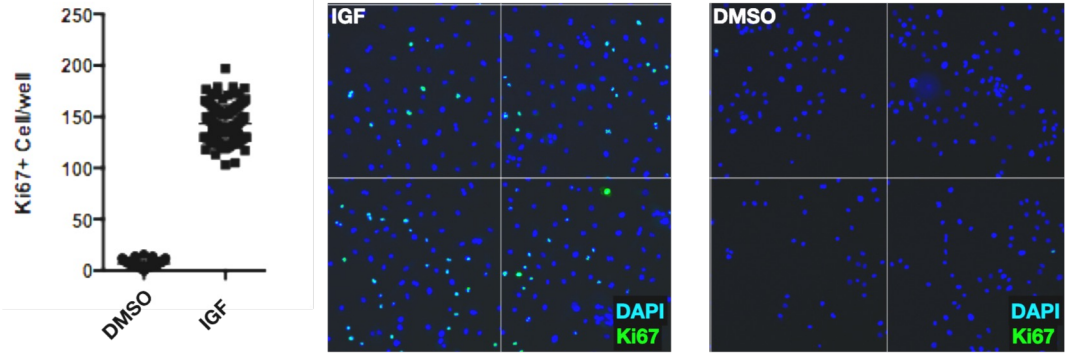


A repurposed drug screen for molecules that expand AEC2s

High content imaging-based screening of >12,000 repurposed drugs



A high content imaging assay for identifying AEC2-expanding molecules



A screen for drug molecules that accelerate AEC2 expansion and repair

A repurposed drug mechanism promotes proliferation of AEC2s

DPP4 inhibitors are widely used medications for T2D

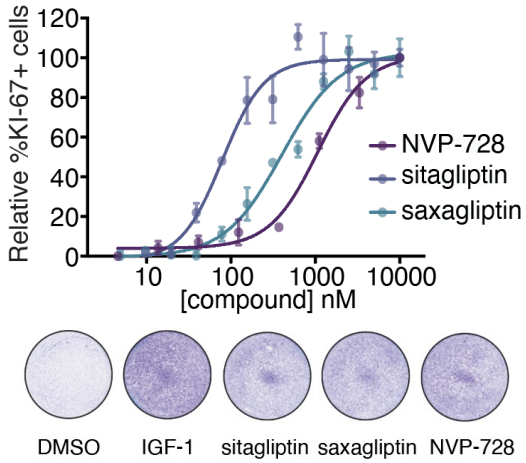
8.9M prescriptions in USA

Dipeptidyl peptidase-4 (DPP4) inhibitors

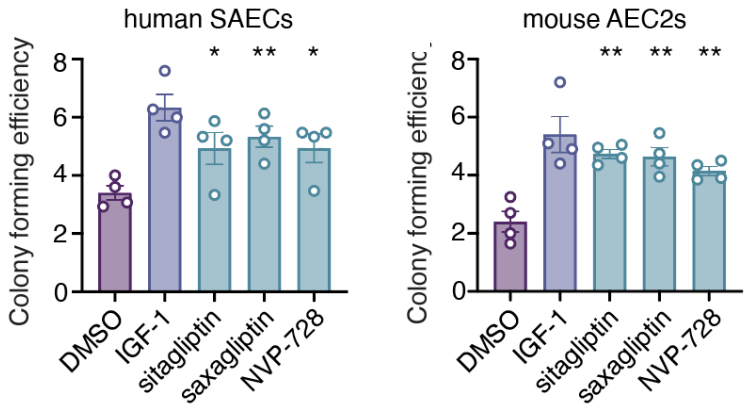
- Increase incretin hormone levels to control blood glucose in Type 2 Diabetes
- Front line T2D medication (>0.4 Billion patients worldwide)
- 4 FDA approved ‘gliptins’:
  - Januvia (Sitagliptin)
  - Onglyza (Saxagliptin)
  - Tradjenta (Linagliptin)
  - Nesina (Alogliptin)
- Very widely used; millions of patients in USA
- Minimal adverse effects

DPP4 inhibitors expand AEC2s in cell culture

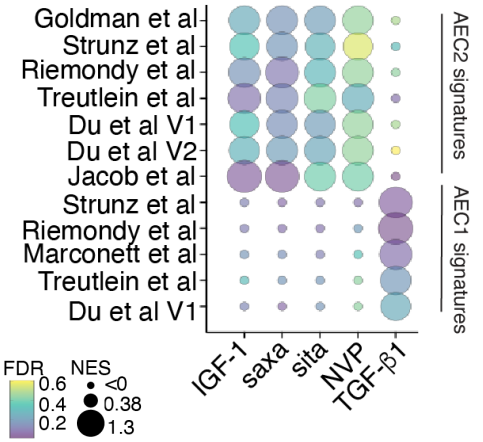
2D growth in cell culture



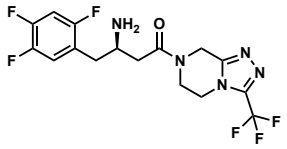
3D growth as alveolospheres



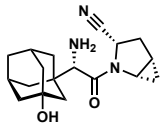
Maintained stemness



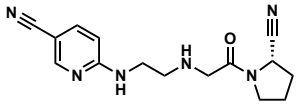
sitagliptin



saxagliptin

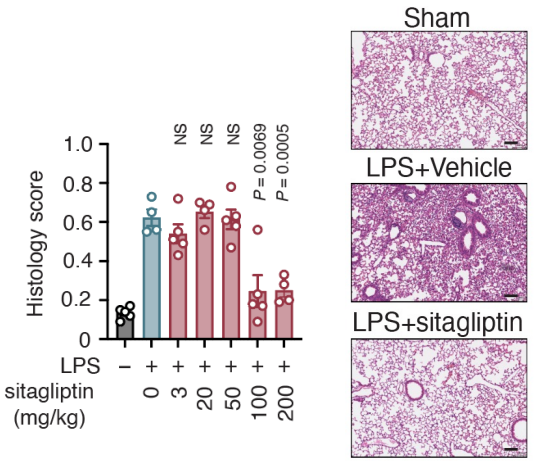


NVP-728

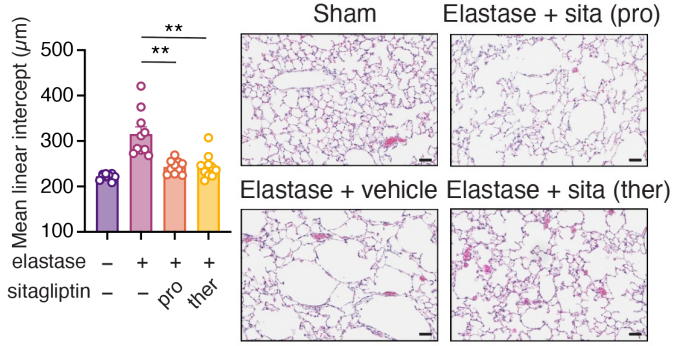


High dose sitagliptin inhibits lung disease in mouse

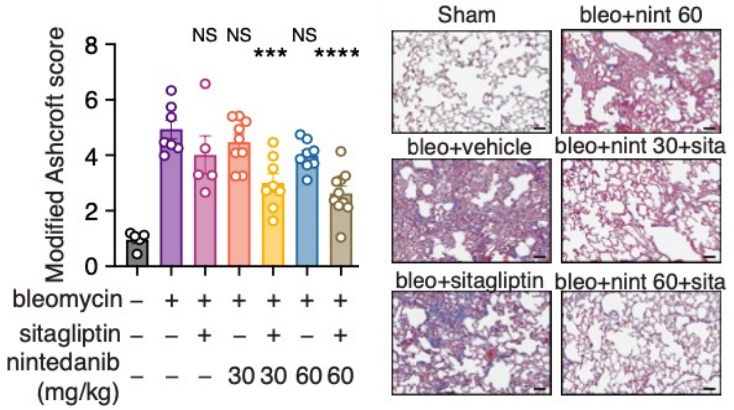
Acute lung injury with LPS



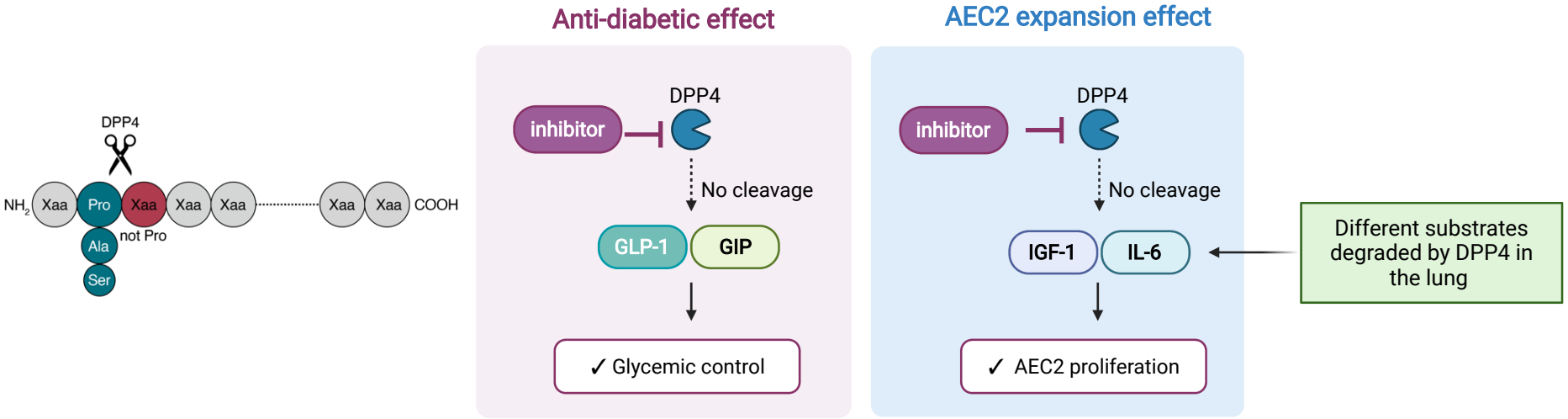
Elastase-induced COPD model



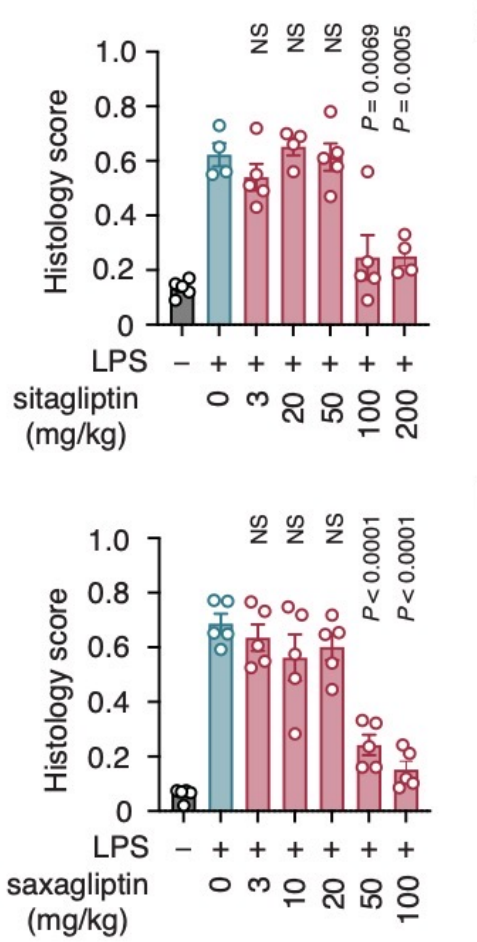
Bleomycin-induced lung fibrosis (a model of IPF)



A different set of factors are degraded by DPP4 in the lung



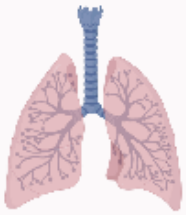
>10x human equivalent oral dose is required for efficacy in animal models



Comparative oral QD MEDs

	glucose tolerance	lung repair	fold
sitagliptin	0.3-3 mg/kg	100 mg/kg	33-333
saxagliptin	0.1-1 mg/kg	50 mg/kg	50-500

How to overcome limitations with oral gliptins



Problem

- DPP4 activity in the lung compartment is critical
- 10-500x the approved clinical dose to achieve therapeutic drug exposure in lung

Solution

- Direct delivery of a lung retained molecule for continuous drug exposure at the site of action
- >10x less drug required
- Very minimal peripheral exposure
- Once weekly dosing; < 10 m nebulization

Identification of lung retained therapeutic candidate CMR316

Medicinal chemistry strategy

Iterative evaluation of covalent and noncovalent DPP4 inhibitor warheads derived from clinical or investigational gliptins

gliptin core

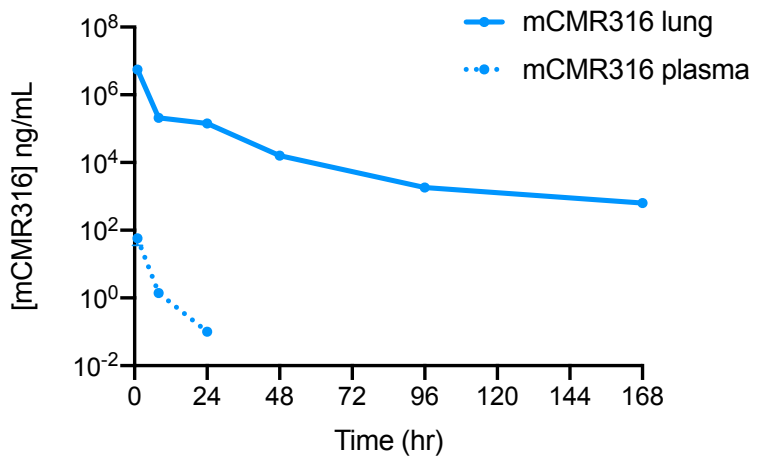
linker

lung retention group

Flexible linker moieties to exit the cavernous DPP4 active site

Clinically validated moieties with mixed polarity discourage membrane permeability, enabling long term lung retention consistent with QW dosing

Therapeutic candidate CMR316

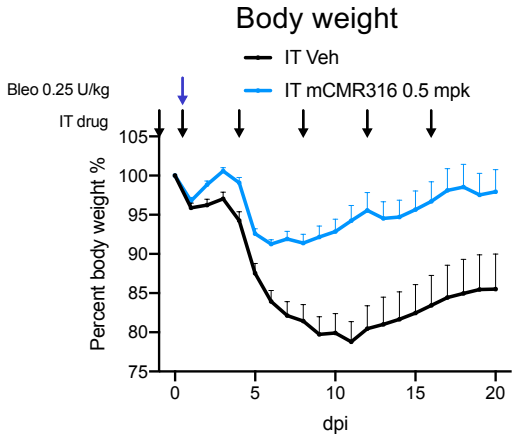


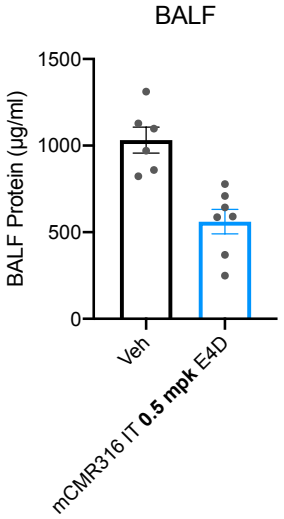
Flow scheme to identify lead candidates

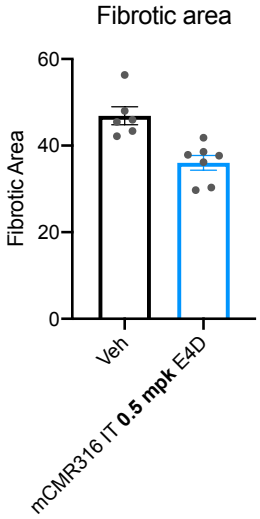
DPP4 enzyme activity (<10 nM) → LPS-induced acute lung injury model (0.01 mg/kg EOD IT) → Rodent pharmacokinetics (PO/IT) lung v. plasma partitioning (>10^3) → Bleomycin-induced fibrosis model (< 1 mg/kg E4D IT) → CMR316

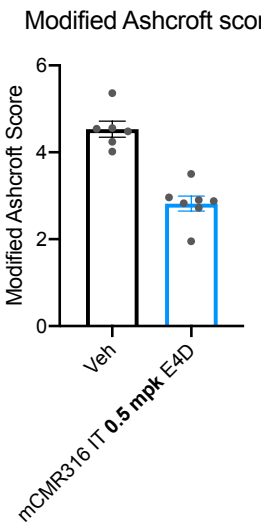
Lung-targeted, persistent CMR316 is efficacious in a lung fibrosis mouse model

Metrics of efficacy in the bleomycin model



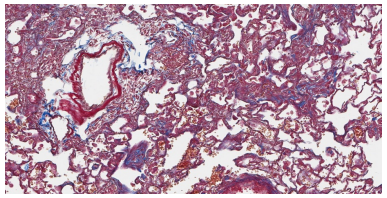




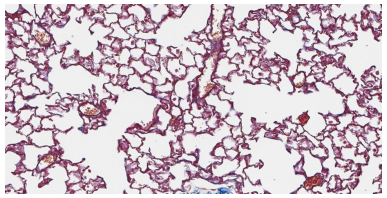


Lung histology

Bleo + Vehicle

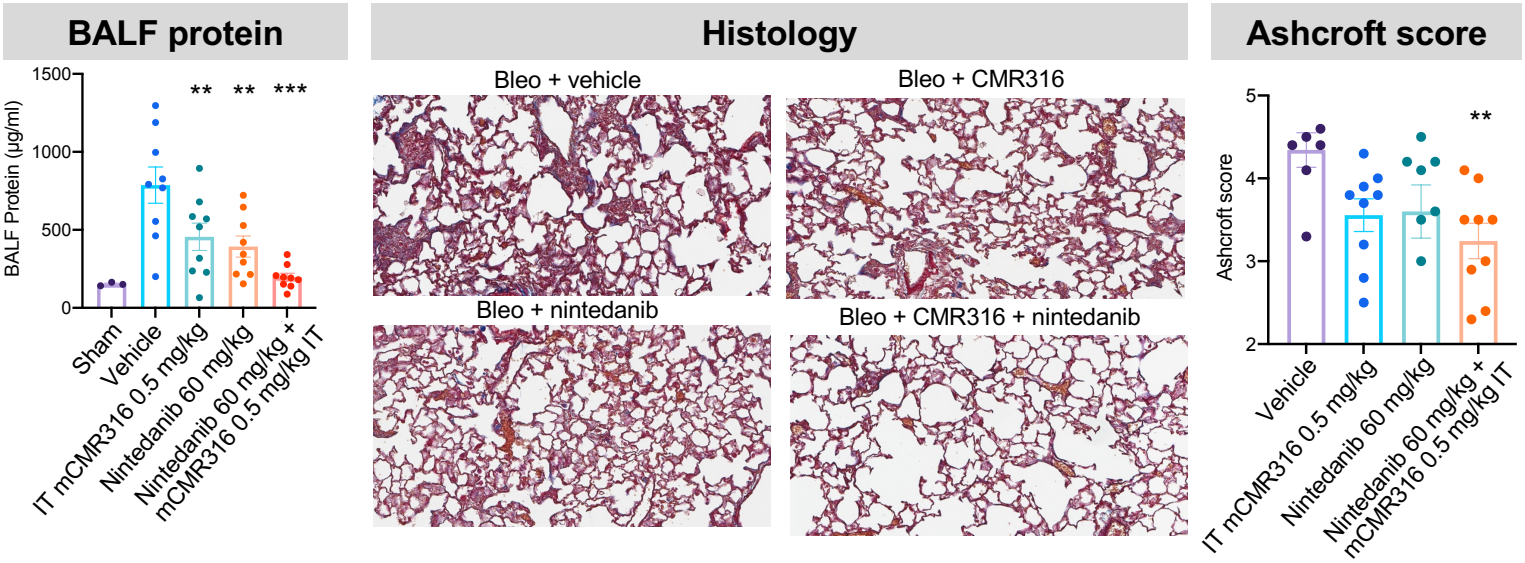


Bleo + CMR316



- CMR316 (0.5 mg/kg E4D IT; **800-fold lower delivered dose** vs Sitagliptin) evaluated in bleomycin-induced lung fibrosis mouse model significantly decreased severity (body weight, BALF protein levels, histological profiling of fibrotic area and Ashcroft scoring of fibrosis induction)

CMR316 synergizes with Nintedanib in the bleomycin mouse model



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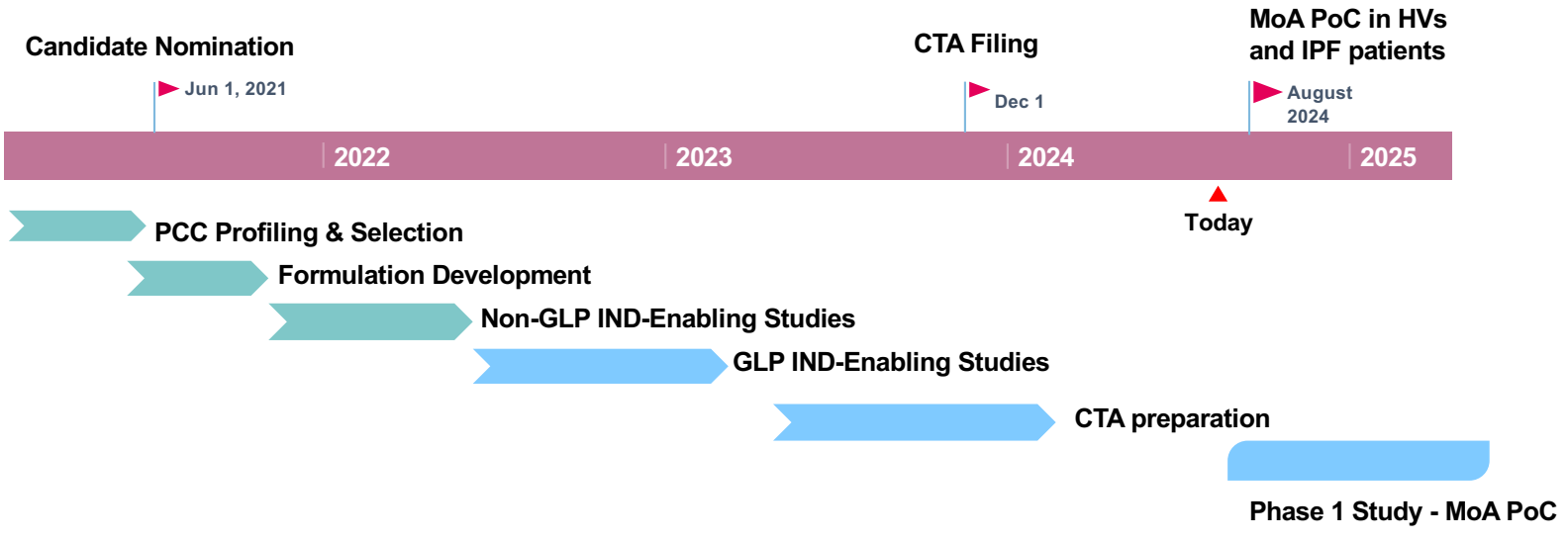
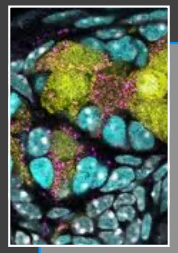
Timeline to the clinic

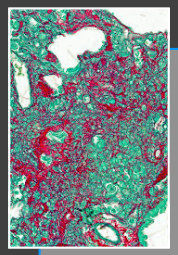


Impact

The only regenerative approach to treating IPF



Augmenting the body's endogenous repair capacity through a safe repurposing mechanism



Potential therapies for other lung diseases: COVID 19, COPD, ARDS

Team responsible for bringing CMR316 to the clinic

Sida Shao

Peter Schultz

Sean Joseph

Jeff Chen

BACHRACH FAMILY FOUNDATION

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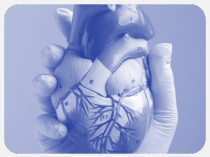
Calibr team:

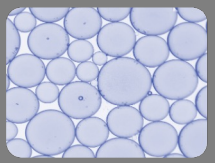
- Arnab Chatterjee
- Jenny Remeeva
- Jing Li
- Gregory Specht
- Van Nguyen Tran
- Vadim Kluyshnichenko
- Shuangwei Li
- Lirui Song
- Alice Cooper
- Jason Brittain
- Victor Chi
- Chan Beals
- Kit Bonin
- Julius Arceo
- Arnie Garcia
- Alex Brooks
- Zon Wang
- Geneva Hargis
- Isy Goldwasser
- Mary Sullivan

Overview of talk

A drug that regenerates the lower airway



Manipulating organ size control for cardiac repair



Drugging the cellular causes of aging

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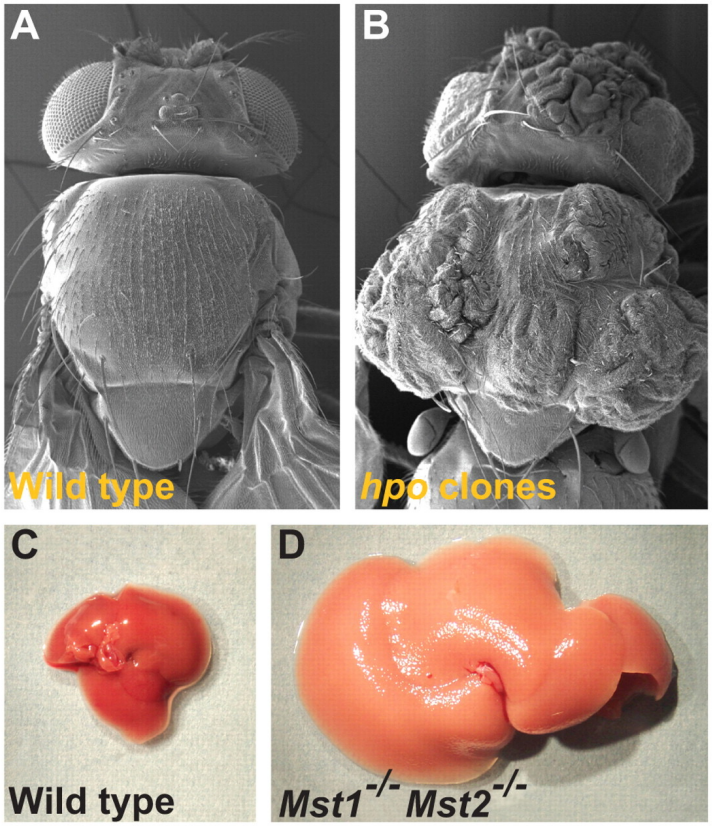
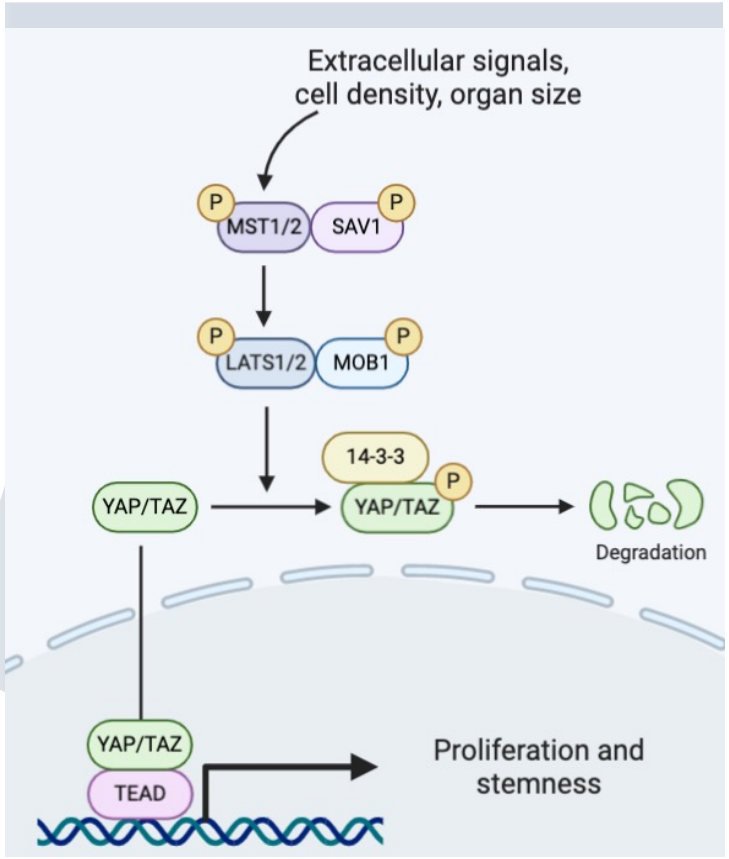
The Hippo-YAP pathway controls organ size in animals

A conserved pathway controls organ size



Molecular logic of Hippo signaling



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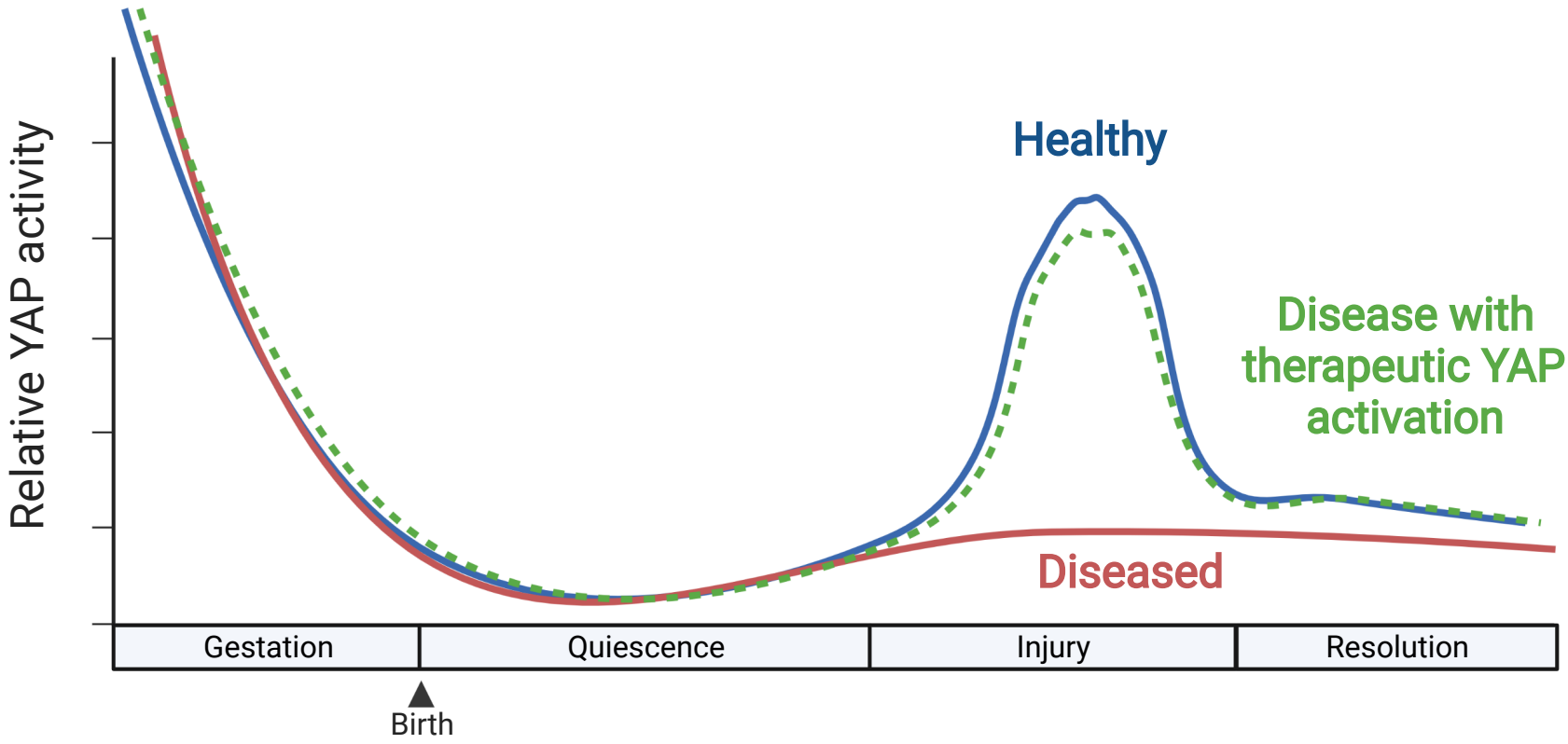
YAP is transiently activated for normal repair and regeneration



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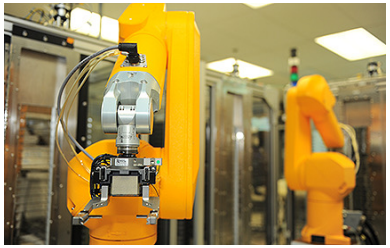
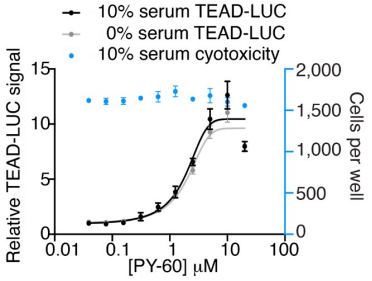
Identifying drug-like small molecules that activate YAP

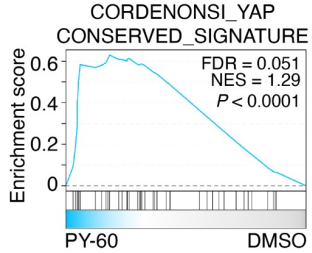
Testing >700k compounds



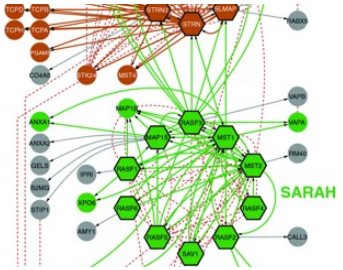
Identification of small molecule activator of YAP



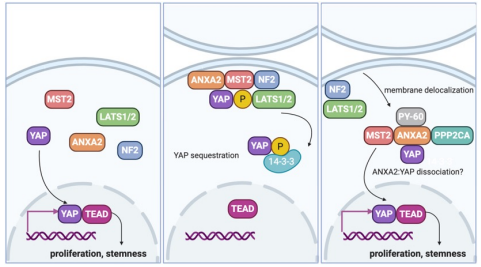
Selective activation of a YAP transcriptional program



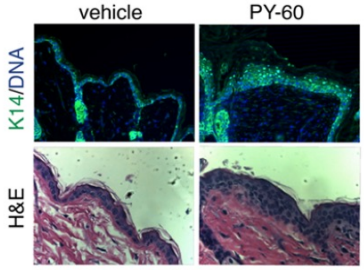
Target deconvolution via chemical proteomics

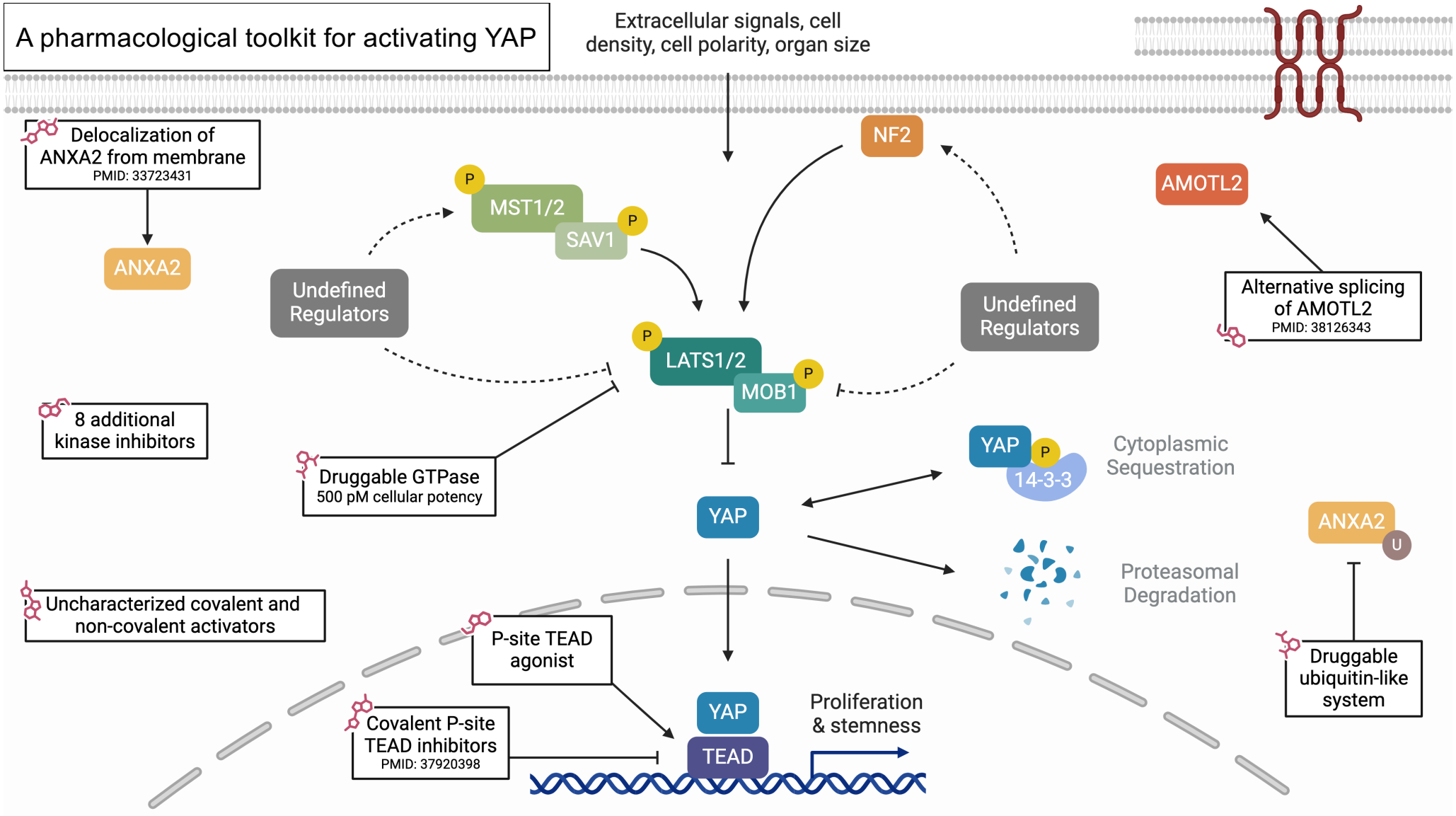


Understanding cellular mechanism

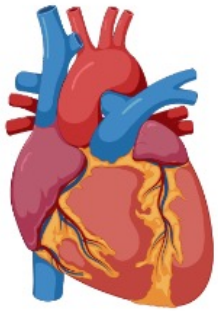


Animal proof of concept

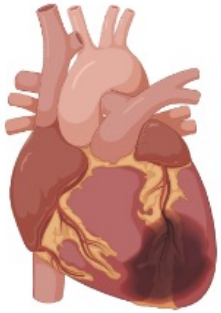



A pharmacological toolkit for activating YAP
Extracellular signals, cell density, cell polarity, organ size
Delocalization of ANXA2 from membrane
PMID: 33723431
ANXA2
NF2
AMOTL2
Alternative splicing of AMOTL2
PMID: 38126343
MST1/2
SAV1
P
Undefined Regulators
Undefined Regulators
LATS1/2
MOB1
8 additional kinase inhibitors
Druggable GTPase
500 pM cellular potency
YAP
14-3-3
Cytoplasmic Sequestration
YAP
Proteasomal Degradation
ANXA2
U
Druggable ubiquitin-like system
Uncharacterized covalent and non-covalent activators
P-site TEAD agonist
Covalent P-site TEAD inhibitors
PMID: 37920398
YAP
TEAD
Proliferation & stemness

Heart failure is a tremendous unmet medical need



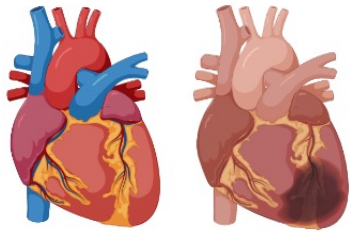
Healthy



Myocardial Infarct

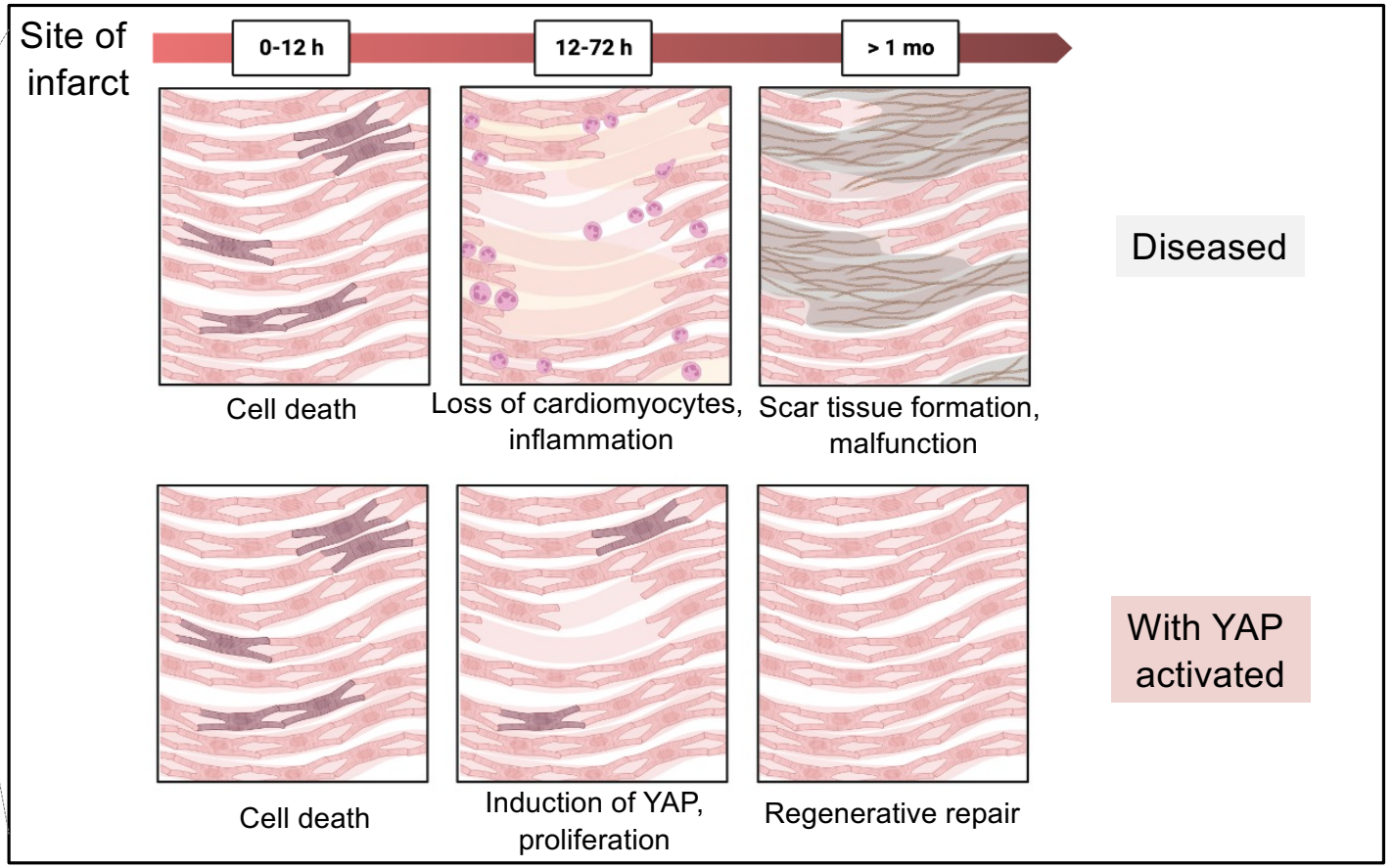
- Nearly 5 million Americans (1-2% of the population) suffer from heart failure; 10% of those over 70 yrs. old
- Heart failure is a $30 billion dollar US healthcare burden
- Heart disease is currently number one killer of men and women
- Current drugs only delay progression but do not stop disease or heal damage

Heart failure derives from an irreversible loss of cardiomyocytes after MI



Healthy

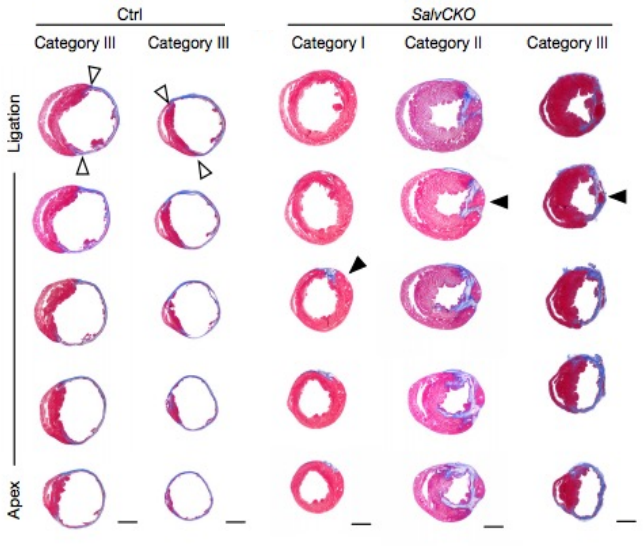
Myocardial Infarct

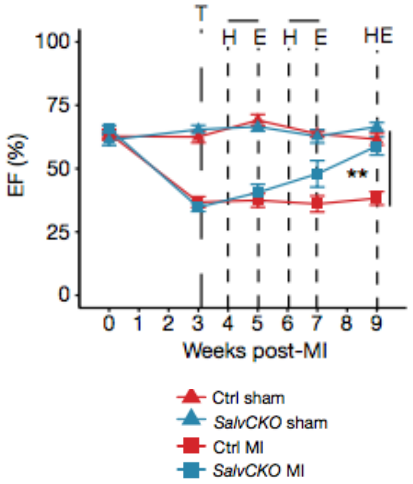


Scripps Research

Genetic activation of YAP promotes regenerative cardiac repair in mice and pigs

Martin lab, Nature 2017
Genetic YAP activation ***reverses*** heart failure in mouse through regenerative cardiomyocyte growth (3 weeks after myocardial infarction)





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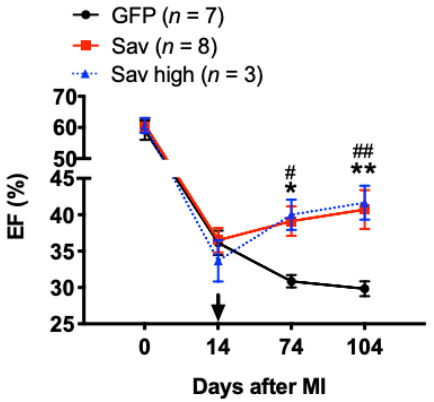
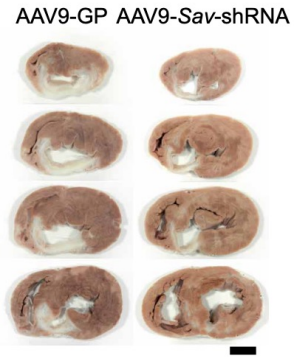
Martin lab, Science Translational Medicine 2021
Genetic YAP activation ***reverses*** heart failure in pigs through regenerative cardiomyocyte growth (2 weeks after MI, durable reversal over 100 days)





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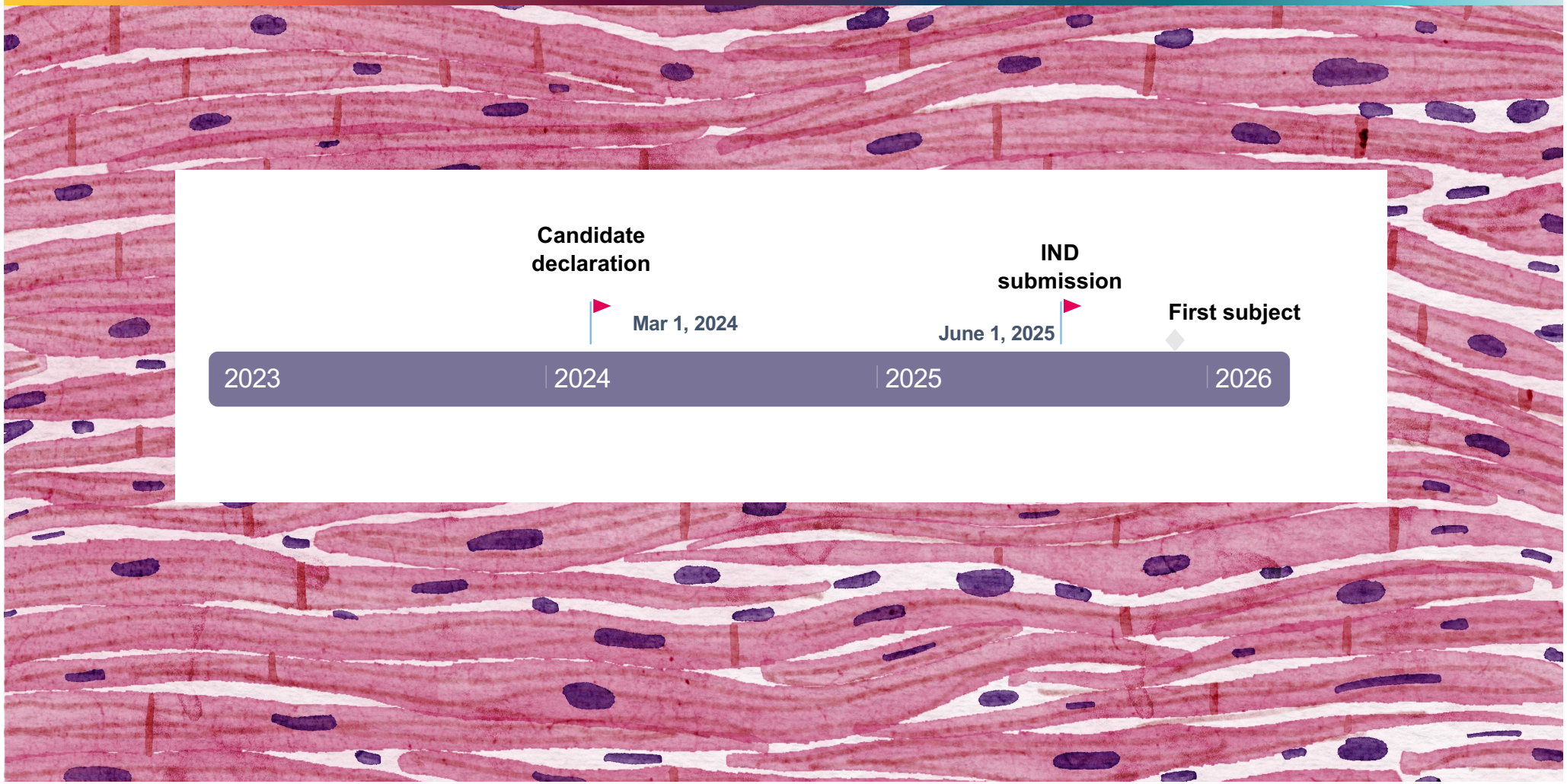
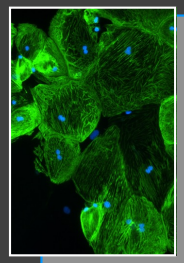
Scripps Research

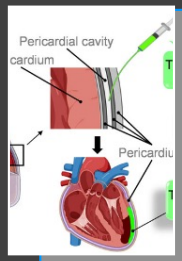
Timeline to the clinic



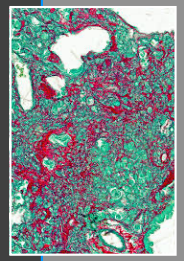
Impact



The only pathway that promotes proliferation of adult cardiomyocytes



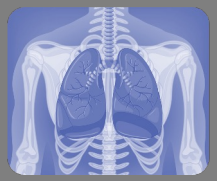
The first pharmacological approach to regenerating the heart



Potential for broad cardiac applicability:

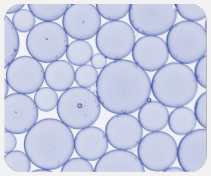
Post MI, congestive HF, dilative cardiomyopathy, others

Overview of talk



A drug that regenerates the lower airway

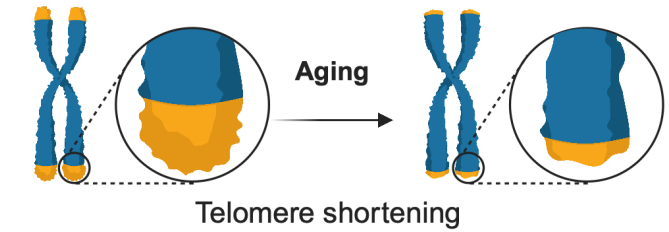
Manipulating organ size control for cardiac repair



Drugging the cellular causes of aging

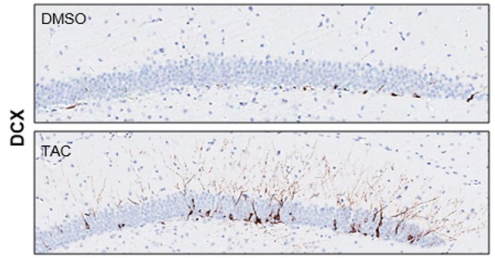
Longevity: Activation of Telomerase to Rejuvenate Aged Tissues

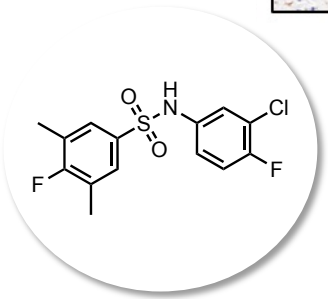
Lack of telomerase leads to telomere shortening with age



Cell division stops
Increased senescence
Decreased stem cell function

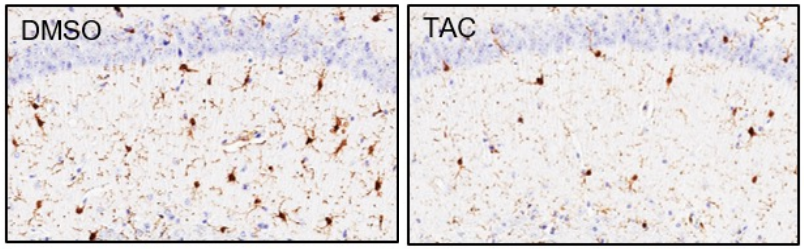
Increased neurogenesis in aged animals with telomerase reactivation



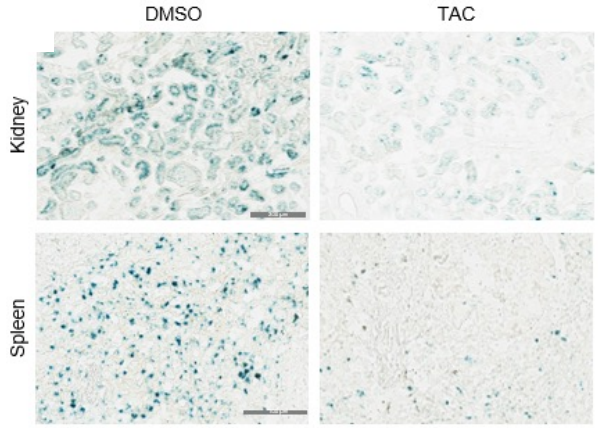


Telomerase activating compound TAC

Decreased inflammation in brain in aged animals



Loss of senescent dysfunctional cells in aged animals



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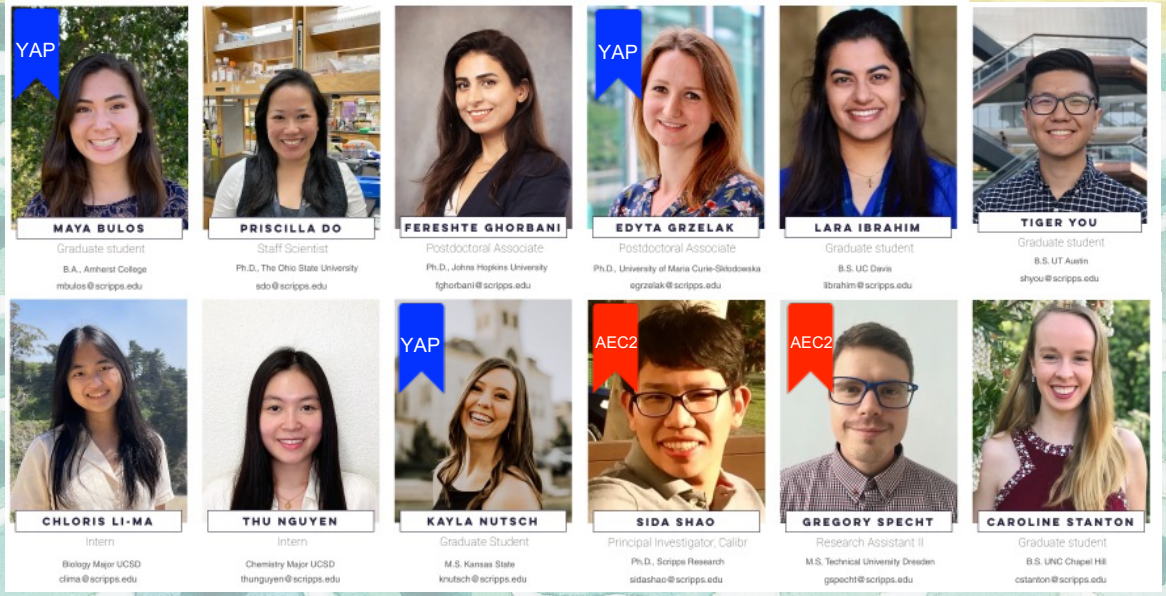
Collaboration with Ron DePinho and Pete Schultz

Acknowledging the Bollong Lab, Scripps, and Calibr



Calibr collaborators:
Arnab Chatterjee
Jeff Chen
Sean Joseph
Case McNamara
Kristen Johnson
Chan Beals
Weijun Shen
Travis Young
Victor Chi
Heather McGee
Elshan Nakath
Lirui Song
Manoj Kumar
Katy Wilson
Van Nguyen-Tran
Ashley Woods
Janelle Stricker
Jing Li
Mary Sullivan
Jenny Remeeva
Qiangwei Fu
Kit Bonin

Scripps Collaborators:
Schultz Lab, TSRI
Wiseman Lab, TSRI
Lamia Lab, TSRI
Chanda Lab, TSRI
Ye Lab, TSRI
Cravatt Lab, TSRI
Kelly Lab, TSRI
Teijaro Lab, TSRI
Erb Lab, TSRI
Lairson Lab, TSRI
Saez Lab, TSRI
Parker Lab, TSRI
Saghatelian Lab, Salk

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