

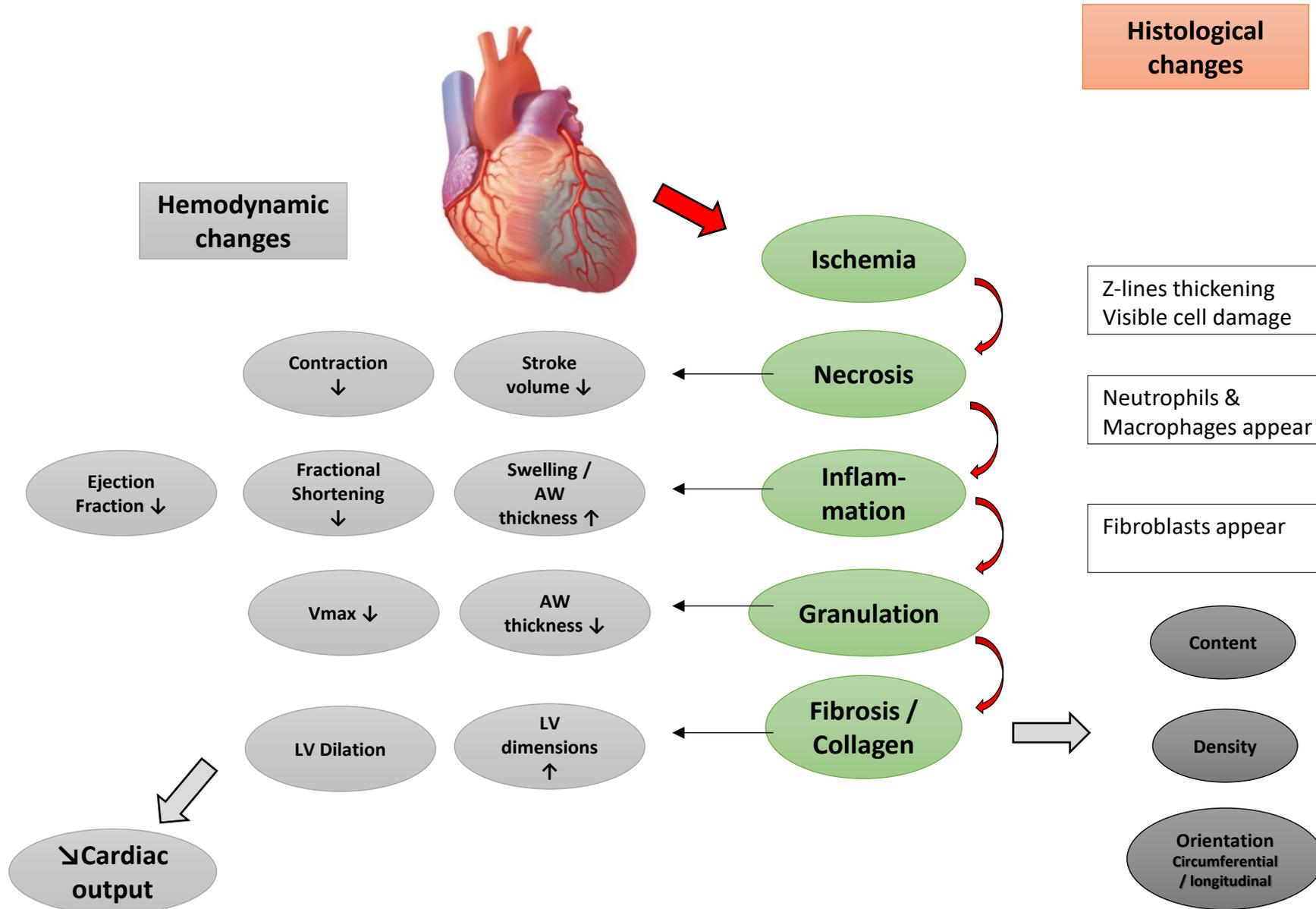
Preclinical heart failure models
IPS Therapeutique Inc.
2025



Pathological processes in Heart Failure (HF)

- Heterogeneous pathogenesis involving:
 1. Acute injury
 1. Ischemia/hypoxia
 2. Coronary dysfunction
 3. Myocardial infarction
 4. Sepsis
 2. Chronic, long-standing compromise
 1. Hemodynamic overload
 2. Valve regurgitation
 3. Stenosis
 3. Genetic variation
 1. Loss of contraction
 2. Sarcolemmal fragility
 3. Dysregulated calcium handling
 4. Chemical / drug toxicity

Dan's cartoon for MI – *Thanks Marge!*



Some models used at IPST	Description
Transverse Aortic Constriction (TAC)	Induces pressure overload leading to hypertrophy and HFrEF.
Myocardial Infarction (MI)	Coronary artery ligation produces ischemic HFrEF.
Isoproterenol-induced HF	Chronic β -adrenergic stress causes cardiomyopathy.
Doxorubicin-induced HF	Chemotherapy cardiotoxicity leading to HFrEF.
Pulmonary Artery Banding	Right ventricular pressure overload leading to right HF.
Valve regurgitation/stenosis	Staph Aureus endocarditis due to mechanical valve damage
Monocrotaline-induced PAH	Pulmonary hypertension progressing to right HF.
ZSF1 Obese Rat	Metabolic syndrome model; diastolic dysfunction and HFpEF with preserved EF.
Dahl Salt-Sensitive Rat (DOCA salt model)	Hypertension-induced concentric hypertrophy progressing to HFpEF.
Spontaneously Hypertensive Rat (SHR)	Chronic hypertension model; HFpEF phenotype in older animals.
db/db and ob/ob Mice	Genetic obesity and diabetes models, recapitulating HFpEF features.
HFpEF double-hit models	High-fat diet + vasopressor (ANGII, L-NAME), etc.

Myocardial Infarction

- **Induction:**

- Surgical ligation of LAD ± reperfusion
- Ligation can be permanent or last for a fixed time (30, 60, 90 minutes, etc.)

- **Heart failure:**

- Results from acute/chronic targeted ischemia
- May be accompanied by arrhythmia/dyskinesia
- Decreased cardiac output associated with \searrow fractional shortening (approx. 30-50%), and \searrow aortic Vmax
- Detectable within 2 hours post MI/IR by echocardiography

- **Endpoints:**

- Cardiac echography (ultrasound)
- Biomarkers (troponins, CKMB, ANP, NT-Pro-BNP)
- Invasive hemodynamics (BP, HR, ECG, P-V Loop)
- Histology

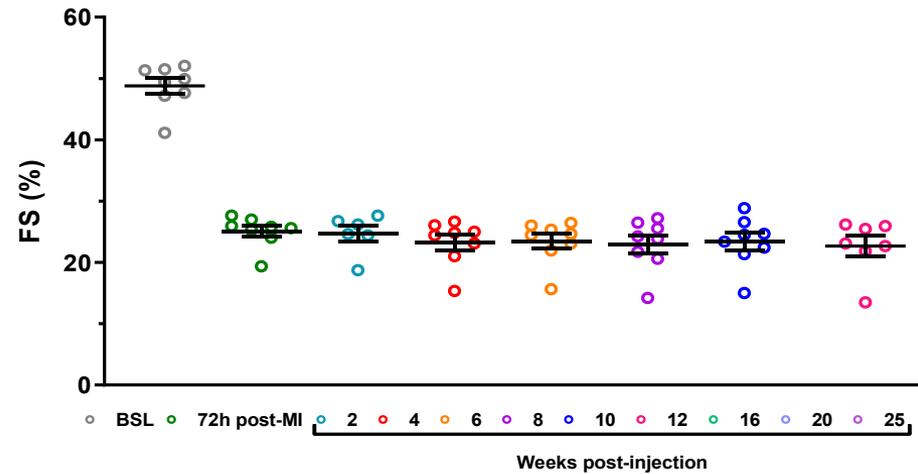
- **Major Limitations:**

- Area-at-risk cannot \geq 35% of LV area leads to \nearrow mortality

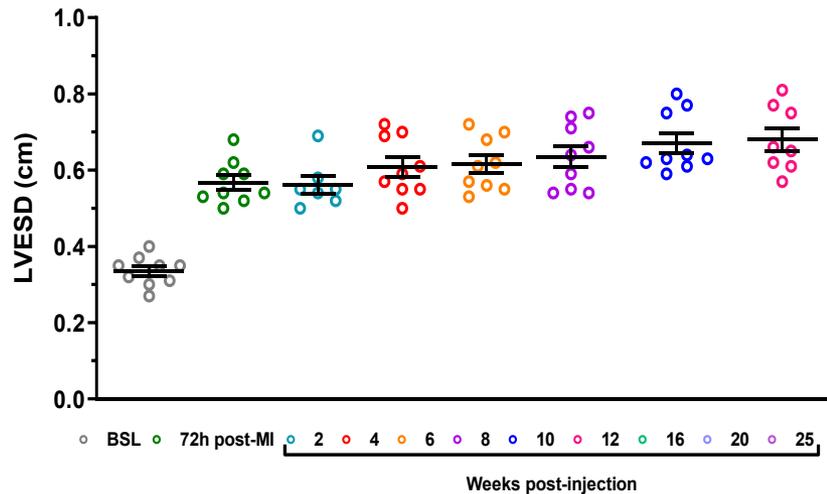
Progression of cardiac remodeling post MI



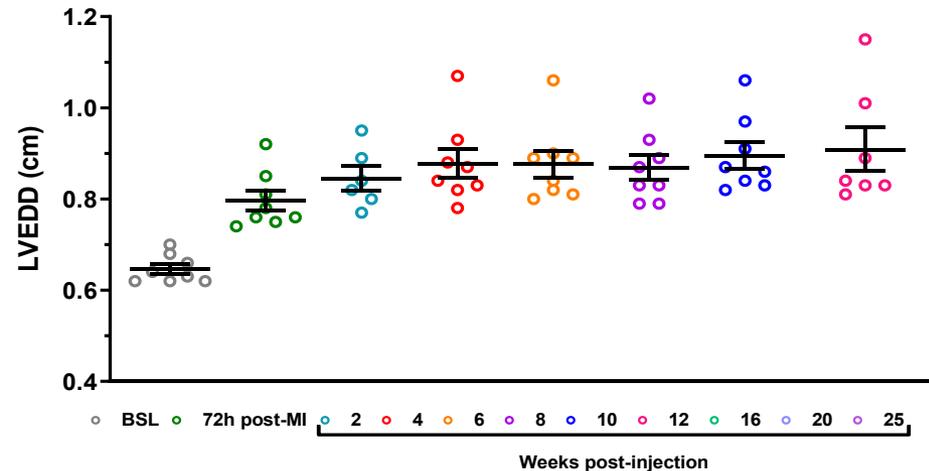
Fractional Shortening



Left Ventricular End Systolic Dimensions



Left Ventricular End Diastolic Dimensions



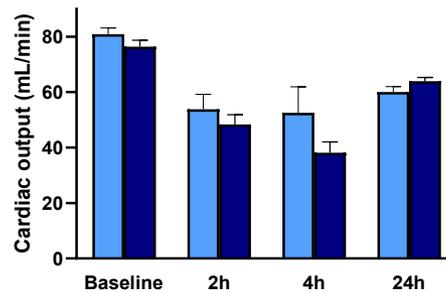
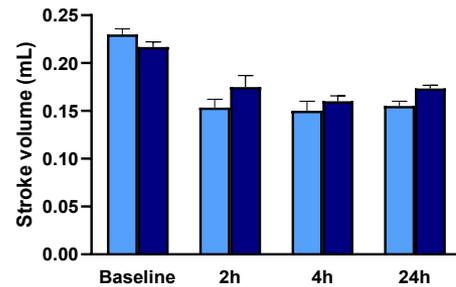
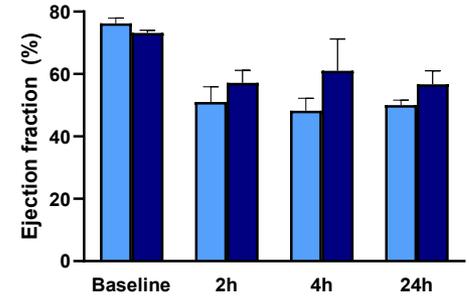
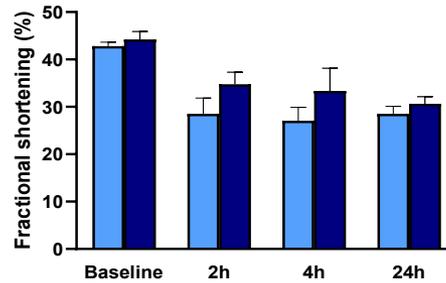
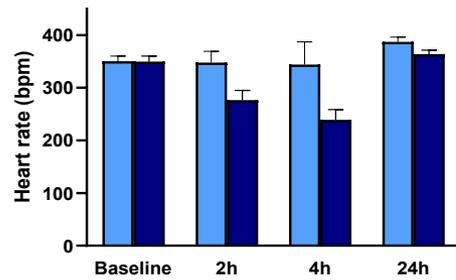
How long before we can measure the effect of an MI?

Protocol summary

- MI induction by LAD ligation (30 min of ischemia) followed by reperfusion
- Carvedilol (1 mg/kg) or vehicle injected iv 5 min pre-reperfusion and 4 hrs post reperfusion
- Echocardiography made at baseline, 2, 4 and 24 hrs post-MI
- Heart harvested 2 and 24 hrs post-MI for Evan's blue and TTC staining

Group	Treatment	Echocardiography	Evans Blue/TTC	n
1	Vehicle	Baseline and 2h post-MI	2h post-MI	3
2	Carvedilol 1 mg/kg	Baseline and 2h post-MI	2h post-MI	3
3	Vehicle	Baseline, 2, 4 and 24h post-MI	24h post-MI	3
4	Carvedilol 1 mg/kg	Baseline, 2, 4 and 24h post-MI	24h post-MI	3

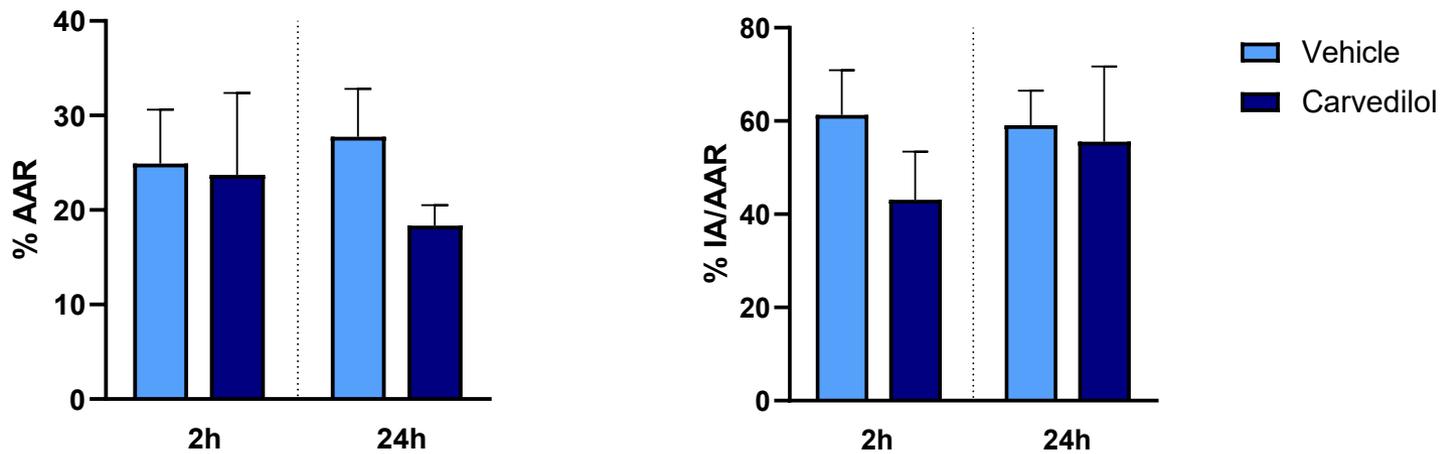
Baseline and post-MI Echocardiography



Vehicle
Carvedilol

- The loss in cardiac functions measured 2 hrs post-MI is the same as the loss measured 24 hrs post-MI for Vehicle animals.

Evan's Blue and TTC staining



The infarct size measured 2 hrs post-MI is the same as the infarct size measured 24 hrs post-MI.

AAR = Area At Risk

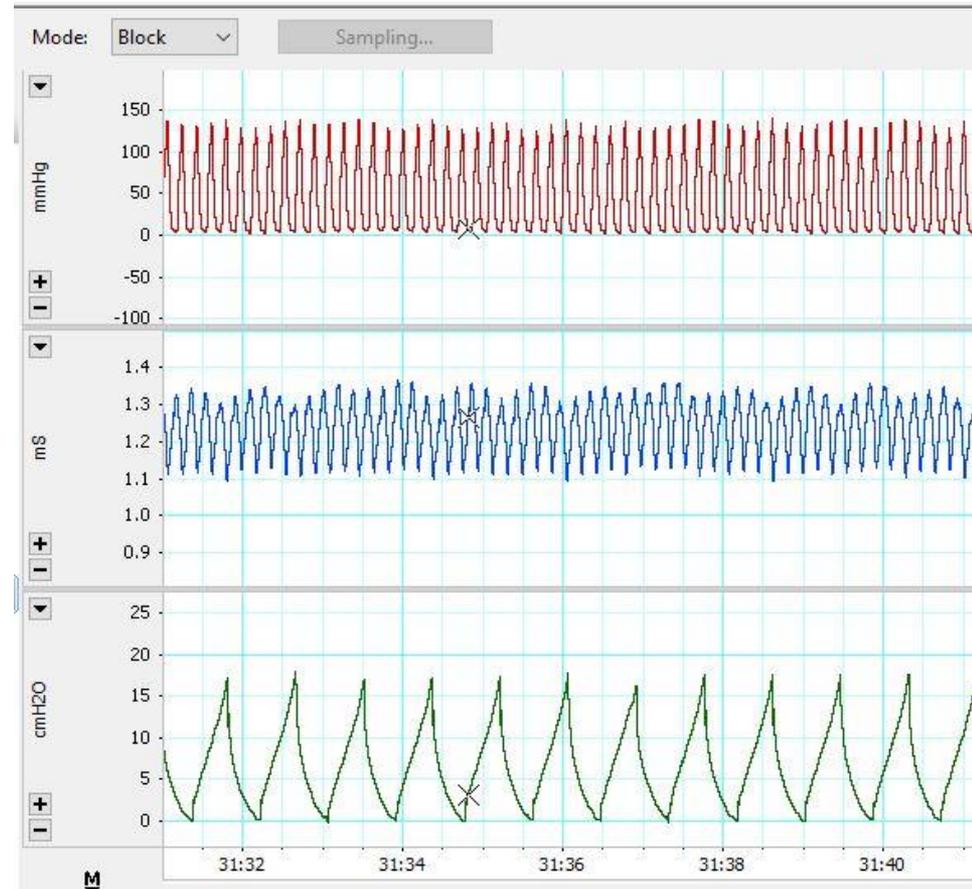
IA = Infarcted Area

Progression of cardiac remodeling post MI

What happens to the LV compliance?

PV Loop measurements

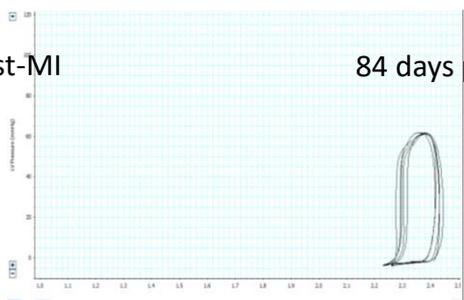
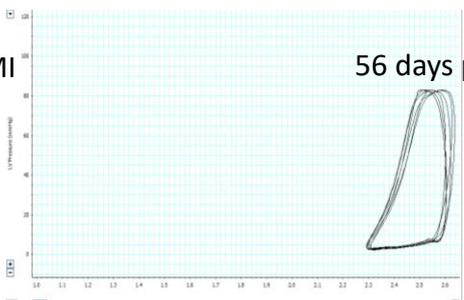
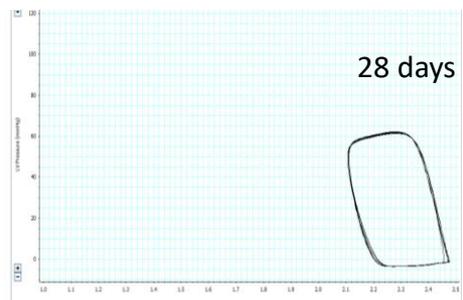
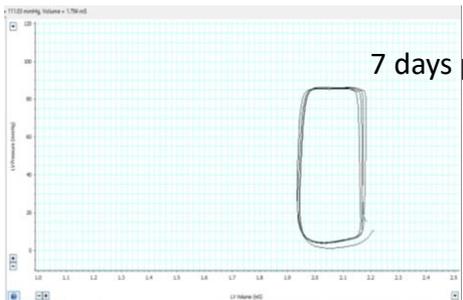
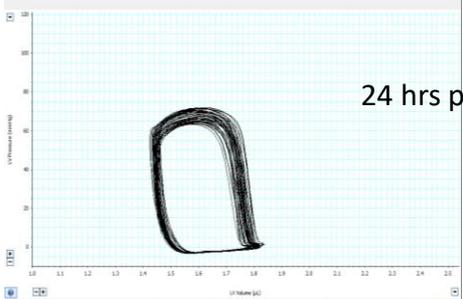
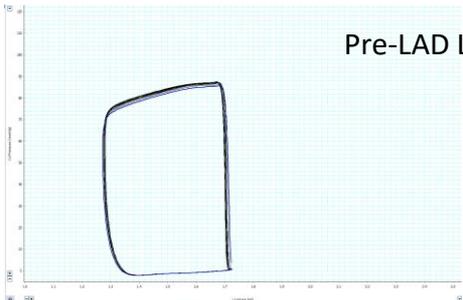
- PV loops were measured using AD Instruments LabChart connected to a Millar solid-state Mikro-Tip rat PV catheter
- The catheter is inserted into the carotid artery of an anesthetized animal and pushed through the aortic valve into the left ventricle (*timing is everything*)
- Alternatively, a thoracotomy can be performed to insert the PV catheter directly through the myocardium at the apex of the heart.



Progression of cardiac remodeling post MI

What happens to the LV compliance?

- Loss of contractility is almost immediate in MI
- Follows dilation (7+ days) with reduced ejection fraction
- Fibrosis becomes visible after 28+ days leading to further losses in compliance and fractional shortening

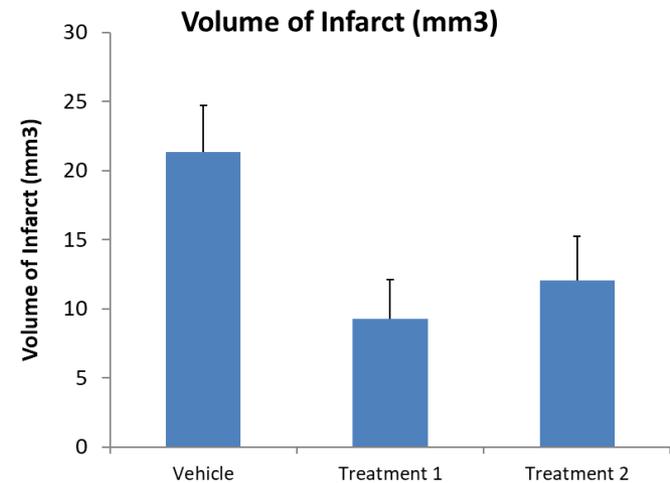
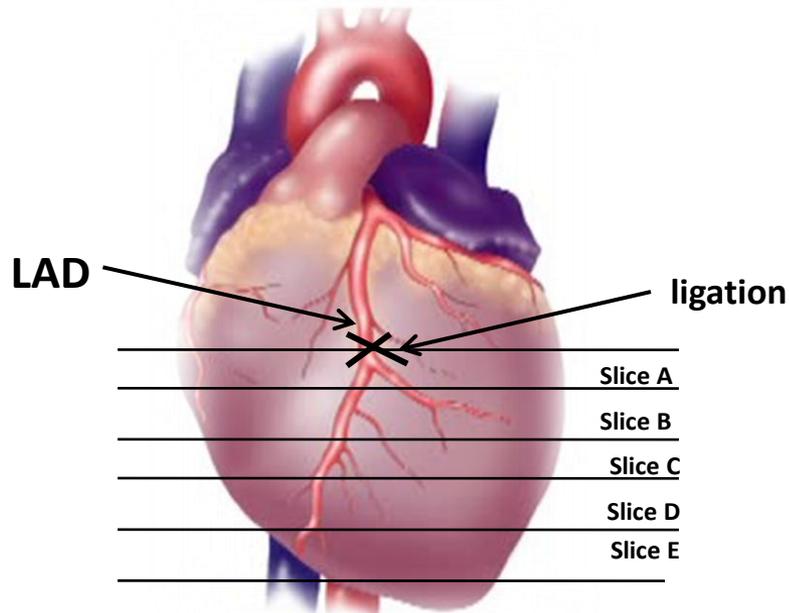
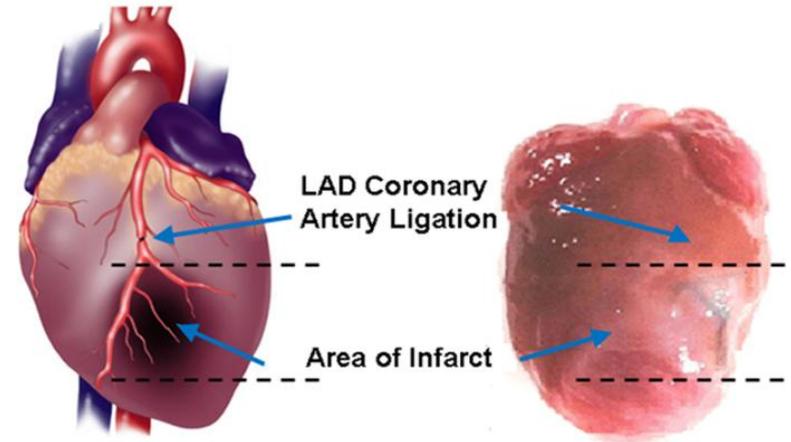


Reconstruction of ventricular MI volume from axial slices

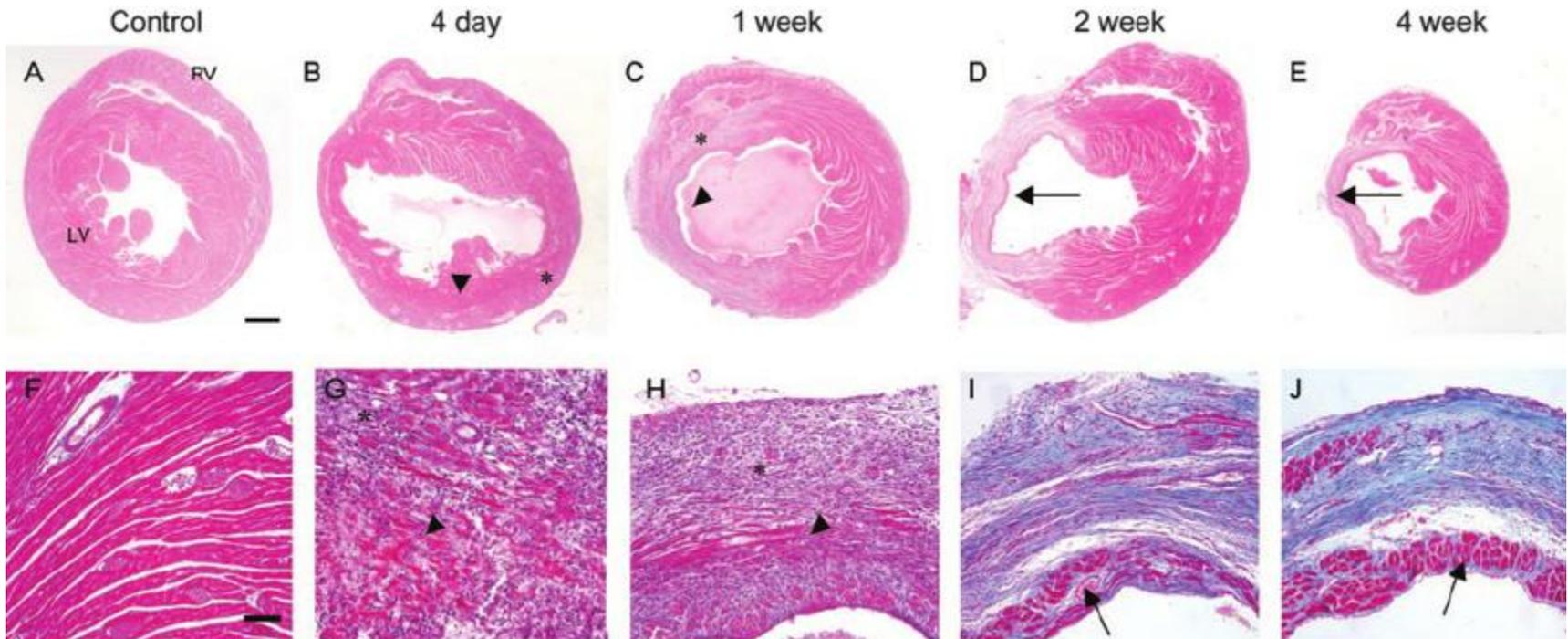
Area-at-risk determination (Evans' Blue)



Infarcted area (TTC staining)



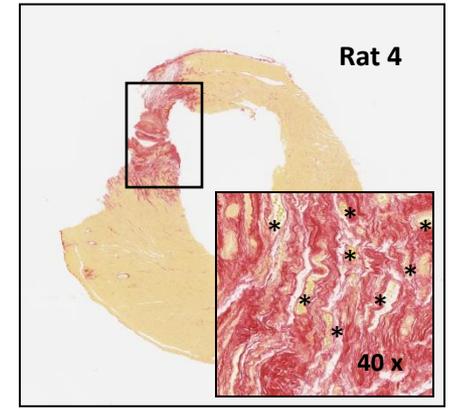
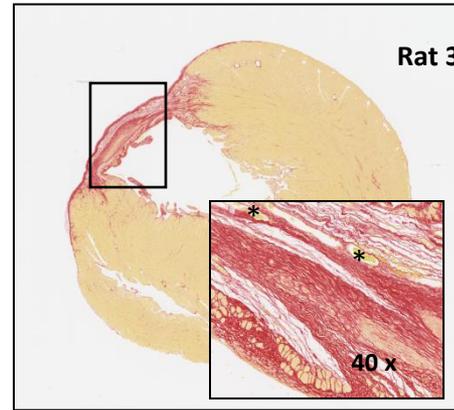
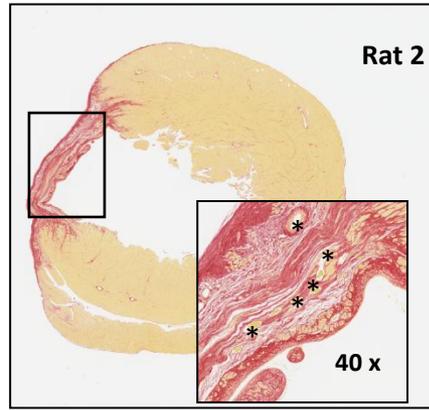
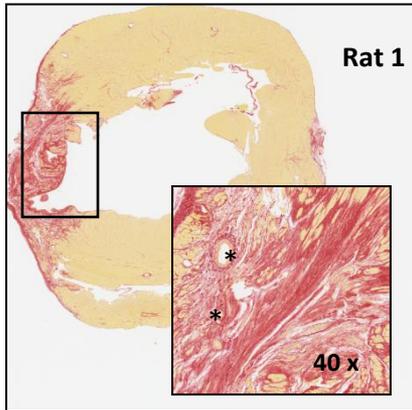
Histological changes appear with time in rodent MI models



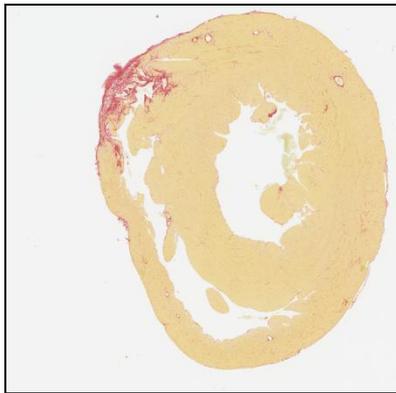
Histology of infarct repair. Hematoxylin and eosin stains (top row; $\times 20$; bar , 0.5 mm) and Masson trichrome (bottom row; $\times 200$; bar , 100 μm ; pink - viable tissue; red - necrosis; blue - collagen)

- intense inflammatory response that occurs at 4 days
- encapsulation of necrosis (arrowheads) by granulation tissue (*)
- progressive thinning of the ventricular wall
- ventricular chamber dilation
- progressive collagen (blue) deposition, and subendocardial sparing (arrows).

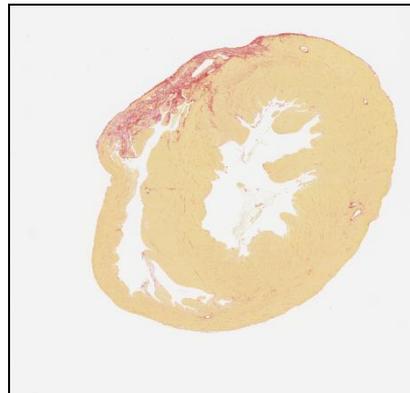
Microphotographs stained with Picro Sirius Red 28 days post ischemia-reperfusion



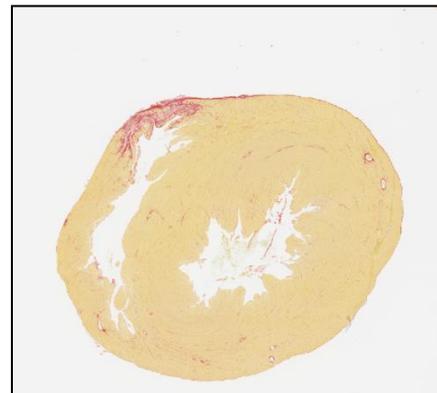
Slice A



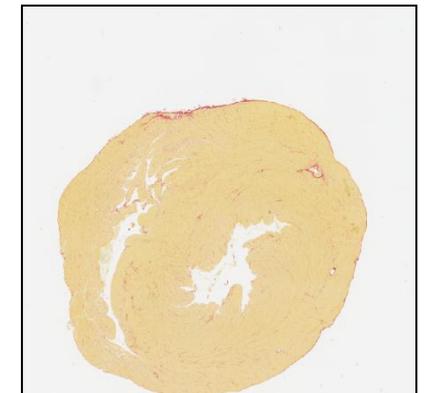
Slice B



Slice C

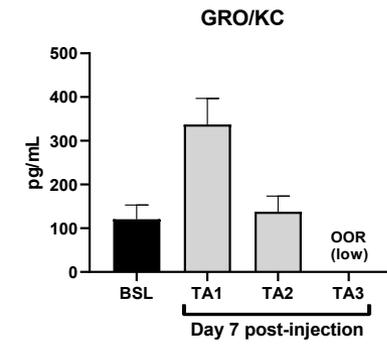
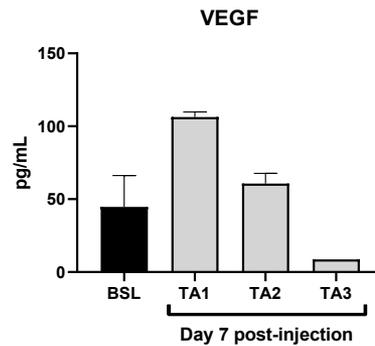
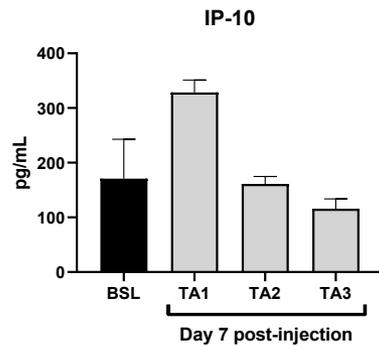
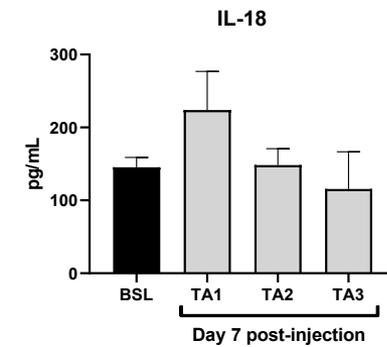
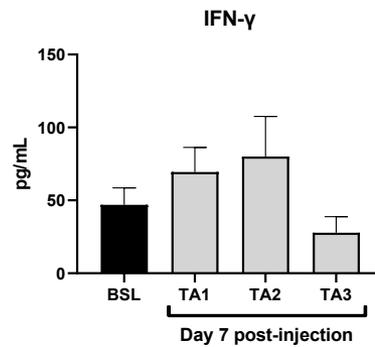
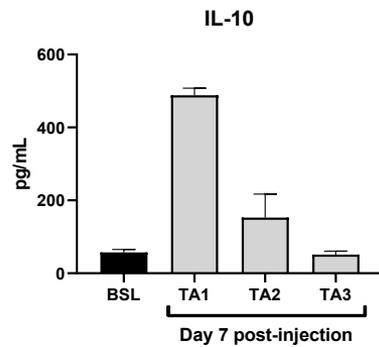
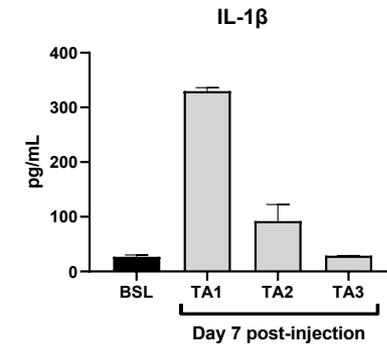
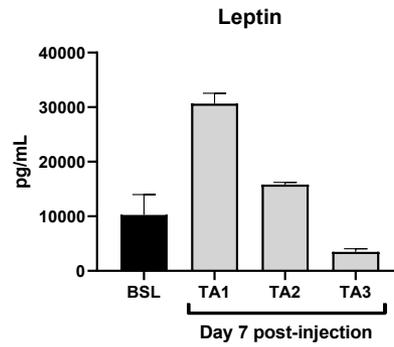
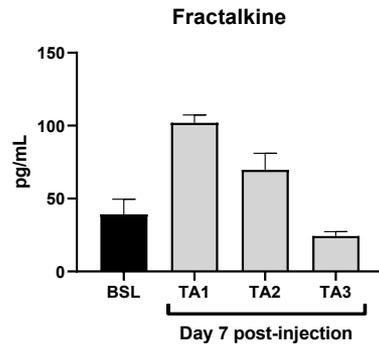


Slice D

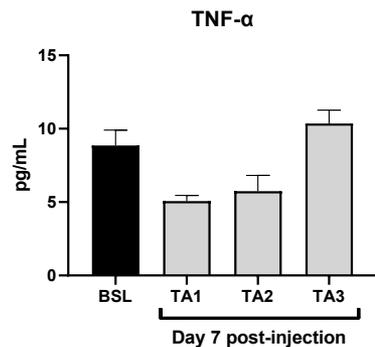
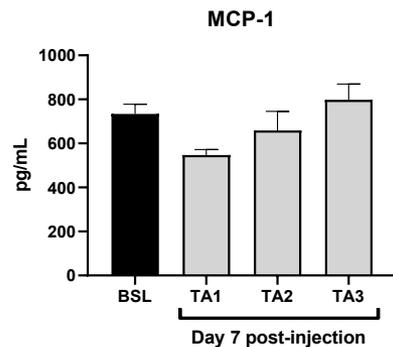
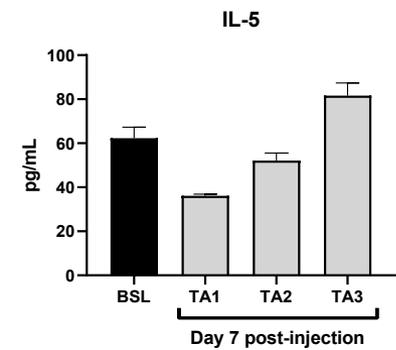
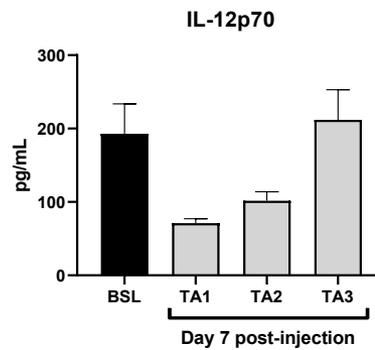
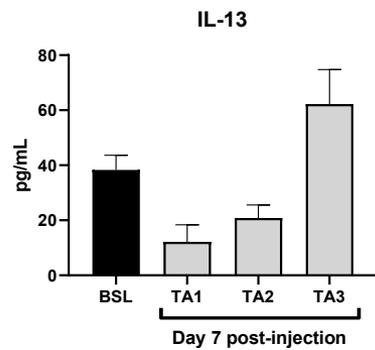
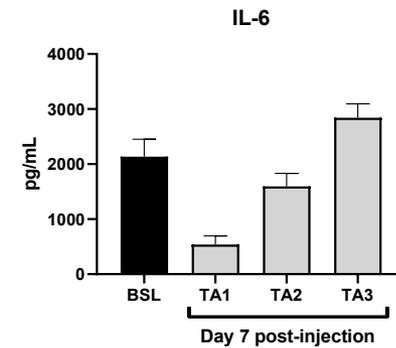
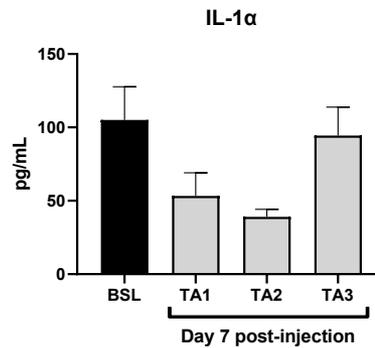
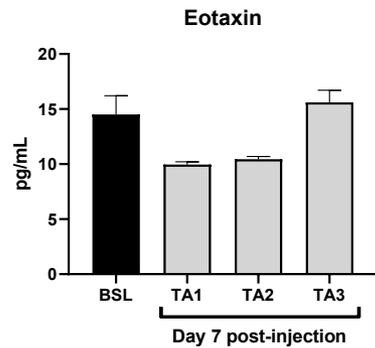


- Collagen very dense and generally well organized
- Expansion between myocytes increased
- Very numerous small blood vessels in the infarcted zone (abundant neovascularization) *
- Transcardiac scarring

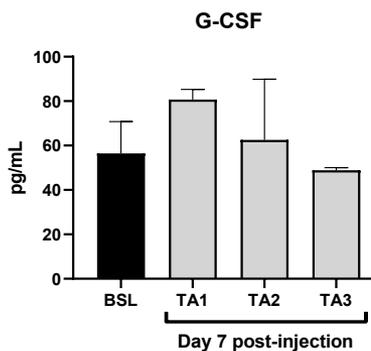
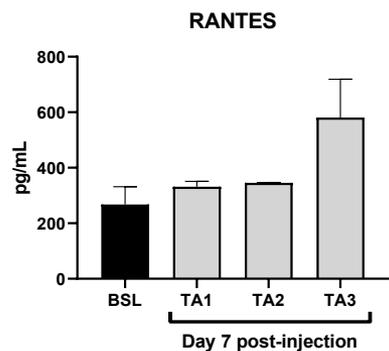
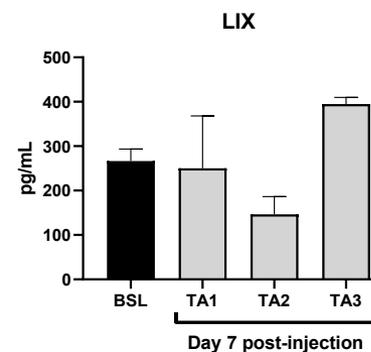
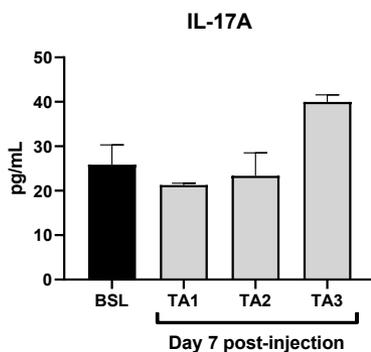
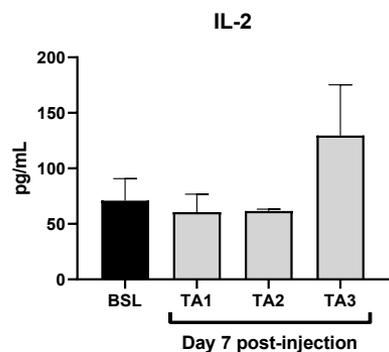
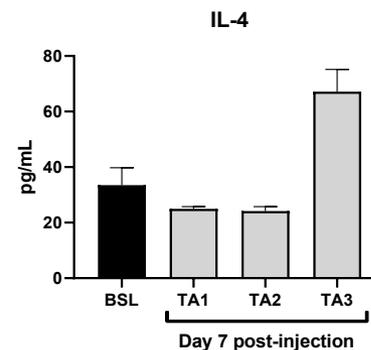
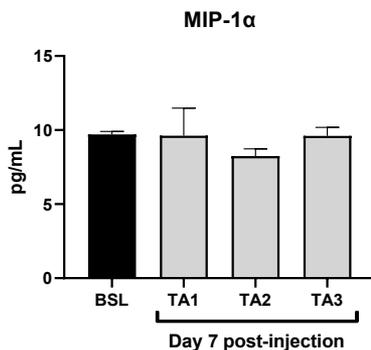
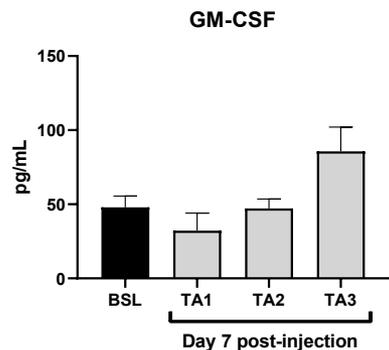
Cytokine, chemokines, leukotrienes count 7 days post MI - UPREGULATED



Cytokine, chemokines, leukotrienes count 7 days post MI – DOWN REGULATED



Cytokine, chemokines, leukotrienes count 7 days post MI – UNCHANGED



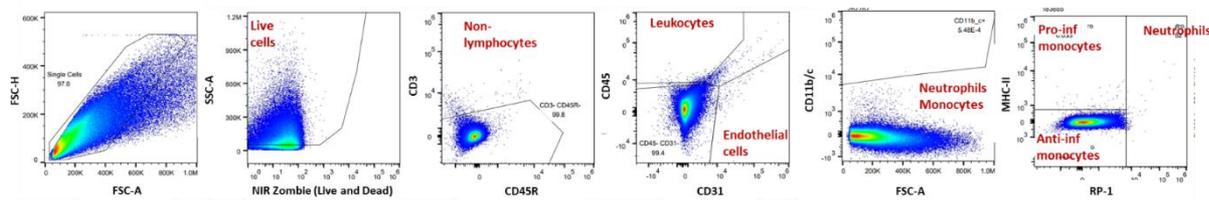
Immune cell count in myocardial tissues 24 and 72 hrs post MI

Characterization of cell population in cardiac samples

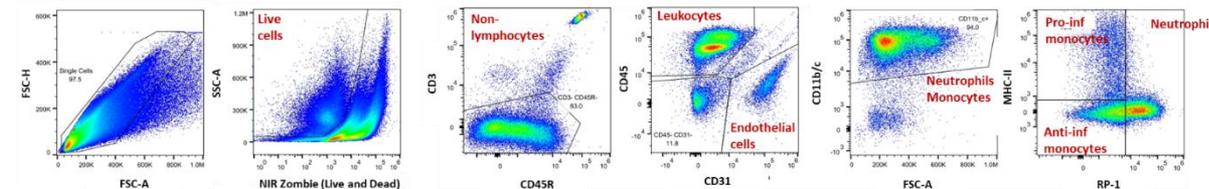
Cell type	Markers	Parent population
Non-lymphocytes	CD3 ⁻ , CD45R ⁻	Live cells
Non-lymphocytes leukocytes	CD45 ⁺ , CD31 ⁻	Non-lymphocytes
Endothelial cells	CD45 ⁻ , CD31 ⁺	Non-lymphocytes
Monocytes	CD11b/c ⁺ , RP-1 ⁻	Leukocytes
Anti-inflammatory monocytes	MHC-II ⁻	Monocytes
Pro-inflammatory monocytes	MHC-II ⁺	Monocytes
Neutrophils	CD45 ⁺ , CD11b/c ⁺ , RP-1 ⁺	Leukocytes

Illustration of the different gates used in unstained (A) stained (B) cardiac samples.

A)

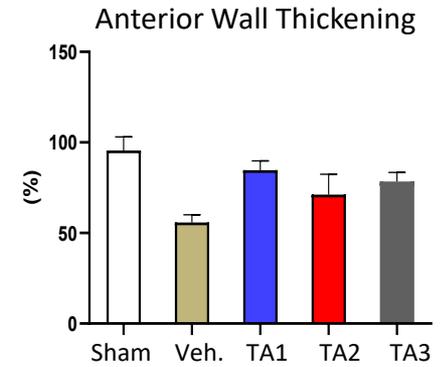
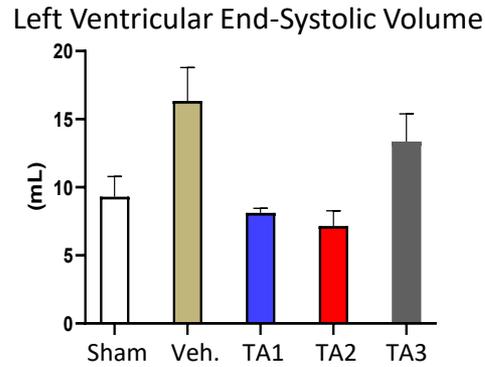
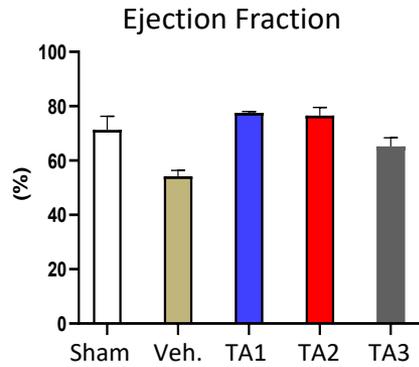


B)



MI in small and large animals

± telemetry monitoring



Endpoints common to all HF models

1. Functional

1. Echography
2. Hemodynamics
3. PV Loops

2. Biochemical

1. Troponins
2. CK-MB
3. NT-proBNP
4. MMP-2, MMP-9
5. TNF- α , IL-1 β , IL-6

3. Histological

1. H&E
2. PicroSirius Red
3. Masson's
4. Van Gieson's
5. Periodic Acid-Schiff
6. CD31 and α -SMA
7. CD45 and LY6G

On a per-model basis:

- Exercise tolerance (treadmill)
- Respiratory function assessment
- Indirect calorimetry
- Glucose clamp
- Telemetry implants for pressure or ECG monitoring

- MRI –imaging

Volume overload induced by aortic valve endocarditis

- **Induction:**

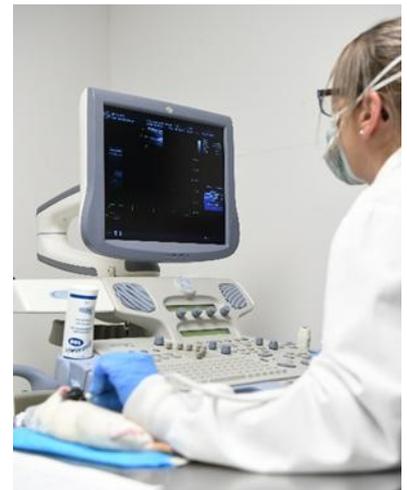
- Localized injection of 1M Staph Aureus CFU across the valve
- Valve dysfunction appears within 48-72 hours, model lasts from 96h to 14 days

- **Heart failure:**

- Results from valve regurgitation
- Gradual ventricular eccentric hypertrophy with dyskinesia
- Decreased cardiac output associated with \searrow ejection fraction, \nearrow TAPSE
- Detectable within 48h post injection

- **Endpoints:**

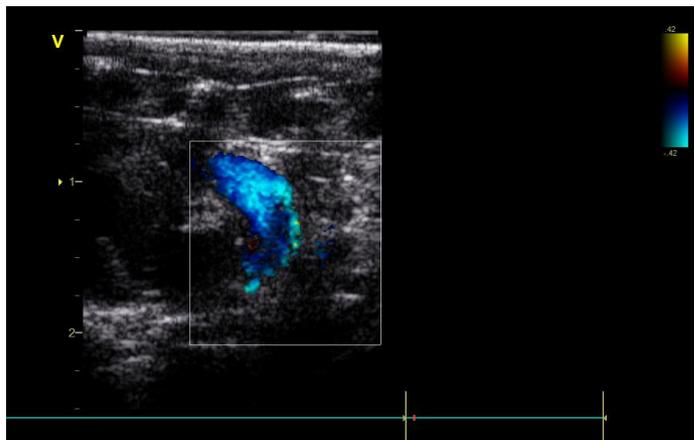
- Cardiac echography (ultrasound shows clear regurgitation)
- CFU determination in valve (terminal)
- Invasive hemodynamics (BP, HR, ECG)
- Histology



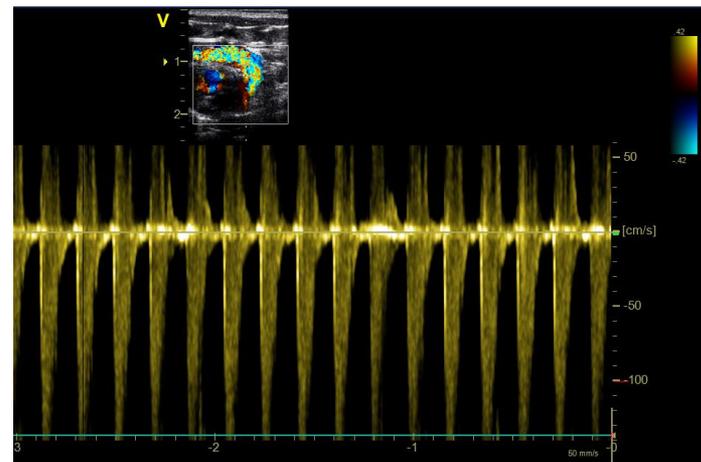
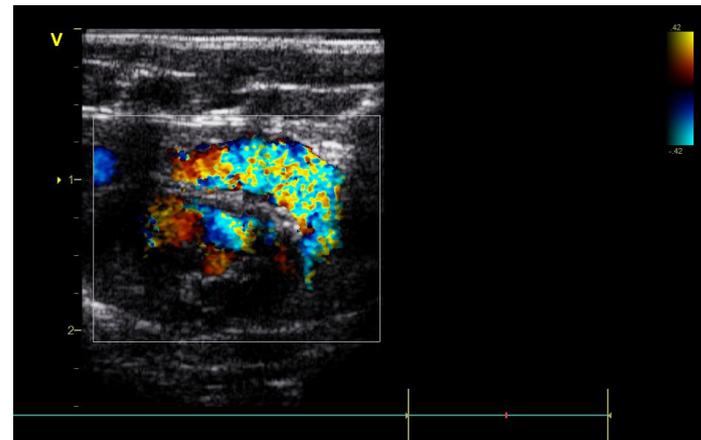
Volume overload induced by aortic valve endocarditis

High-resolution cardiac echography is used to measure valve regurgitation

Healthy valve



Regurgitating valve after 14 days



Pressure overload induced by Transverse Aortic Constriction (TAC)

- **Induction:**

- Surgical partial ligation of aorta with approx. 20-30% remaining blood flow in juvenile animals (they grow into the banding)
- 6-8 weeks of banding necessary for full LV remodeling

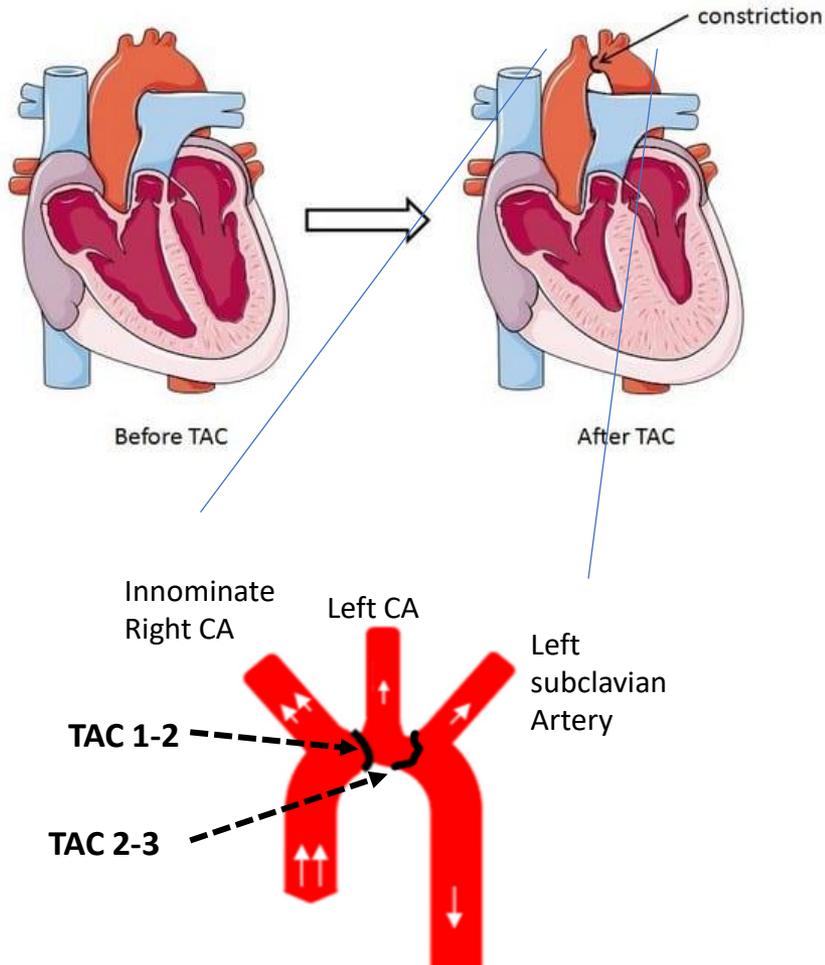
- **Heart failure:**

- Results from pressure overload
- LV hypertrophy
- **Dilation**, some fibrosis after 6-8 weeks
- Decreased cardiac output associated with \searrow fractional shortening, and \searrow ejection volume, \searrow function with \nearrow volume (dilation)
- Detectable within 5-7days post-banding

- **Endpoints:**

- Cardiac echography (ultrasound)
- Biomarkers (troponins, NT-Pro-BNP)
- Invasive hemodynamics (BP, HR, ECG, PV loops)
- Histology

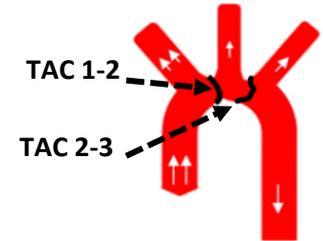
Hypertrophic cardiomyopathy induced by TAC (pressure overload)



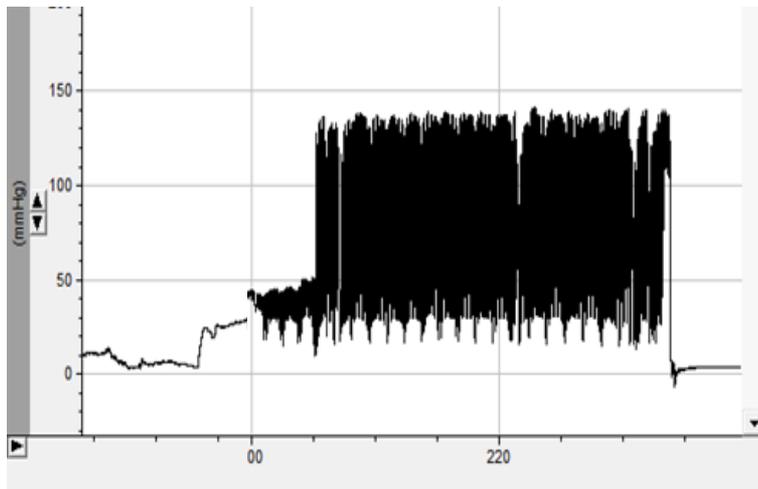
- Juvenile animals
- TAC = ligation in one location of the aorta
- Wait 6-8 weeks; the animals “grow” in the ligation
- Weekly echocardiography
- Blood sampling for biomarkers: Troponin I, NT-Pro BNP
- Invasive hemodynamics by jugular or aortic catheterization
- Telemetry implantation for non-invasive monitoring of LV pressure
- Pressure-Volume loop (usually terminal)
- Histology / microCT scans/Morphometry
- Snap-freezing of tissues for gene testing (up/down regulation)

Concentric hypertrophy induced by TAC

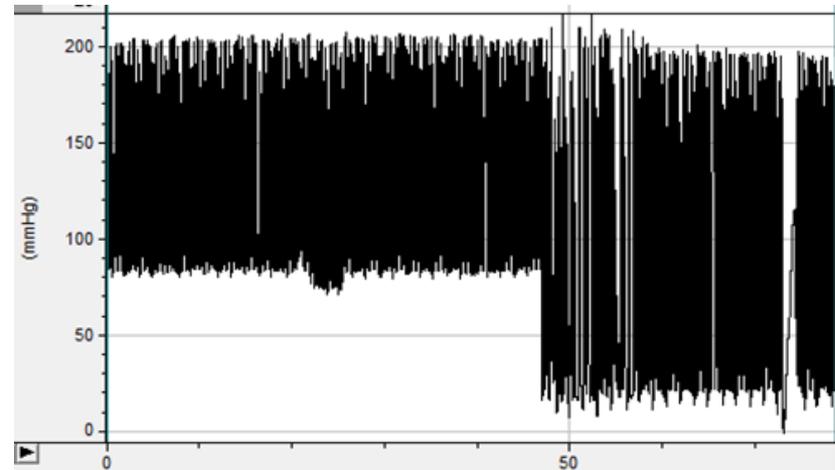
Carotid catheterization makes it possible to measure systemic and intraventricular pressures.



Pre-TAC



Week 8 post-TAC 1-2

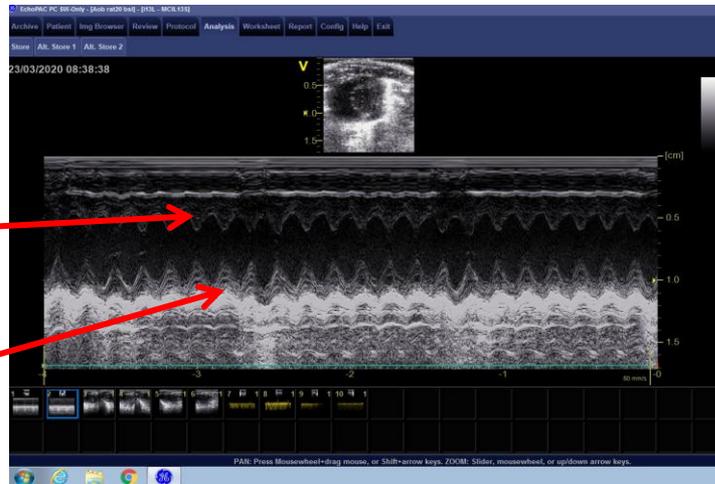


	Body Weight (g)		Weight gain	Heart rate	Sat'n	Arterial pressure			Left Ventricular pressure			Pulse Press.	LV weight	RV weight	Heart weight (g)
	Day 0	End of exp. phase	(g)	(bpm)	(%)	Diastol (mmHg)	Systol (mmHg)	Mean (mmHg)	Diastol (mmHg)	Systol (mmHg)	Mean (mmHg)	(mmHg)	(g)	(g)	(g)
SHAM	129.00	527.50	378.67	278.25	98.83	76.99	106.01	86.66	7.00	78.00	30.66	29.01	1.05	0.49	1.54
TAC 1-2	127.00	574.33	447.33	267.67	98.50	89.75	100.07	93.19	25.94	177.20	76.36	10.32	1.62	0.45	2.07
TAC 2-3	140.67	506.33	365.67	241.00	98.17	78.90	91.27	83.02	11.63	151.48	58.25	8.24	1.45	0.37	1.82

TAC induced LV hypertrophy

	Baseline (Pre-TAC)	Day 28
Anterior wall thickness (AWT) in end diastole, mm	1.08	2.30
Posterior wall thickness (PWT) in end diastole, mm	1.15	2.10
LV End Diastolic Dimensions, cm	0.55	0.60
LV End Systolic Dimensions, cm	0.30	0.24
Fractional Shortening (%)	45.45	60.60

TAC 2-3 Rat 20 Day 0 (Pre-TAC)



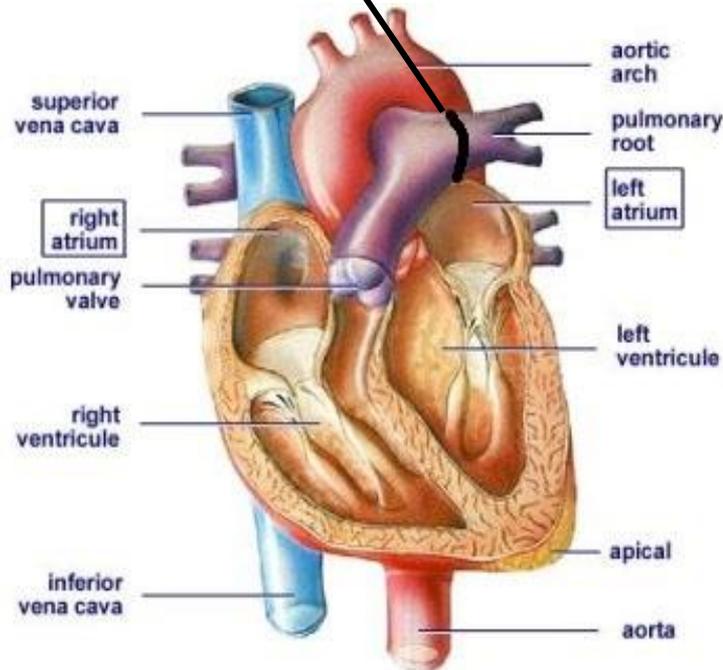
TAC 2-3 Rat 20 Day 28



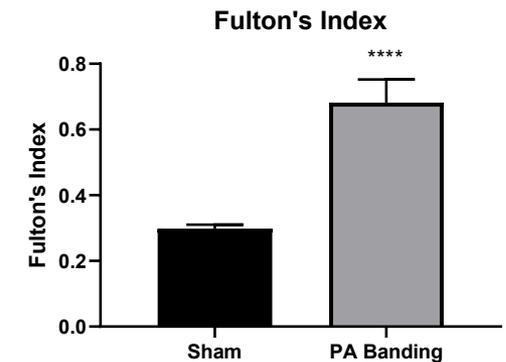
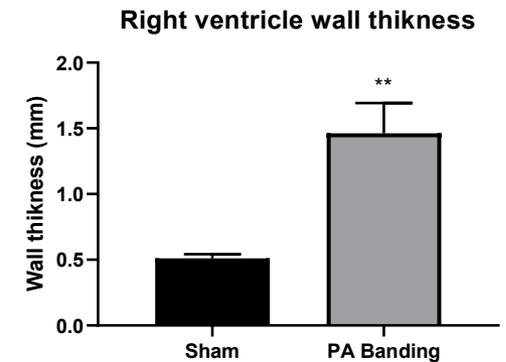
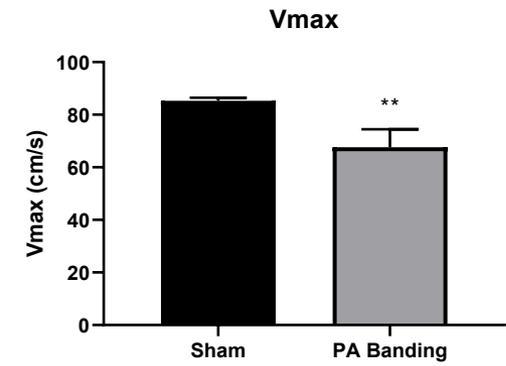
M-mode recording of mid left ventricle from a 2-D parasternal short axis view. Measurement locations for chamber dimensions in diastole and systole are labeled. The same rat (#20) was subjected to weekly echocardiography examinations.

RV hypertrophy induced by pulmonary artery banding (pressure overload)

Ligation on pulmonary artery using thread and a 20G needle.

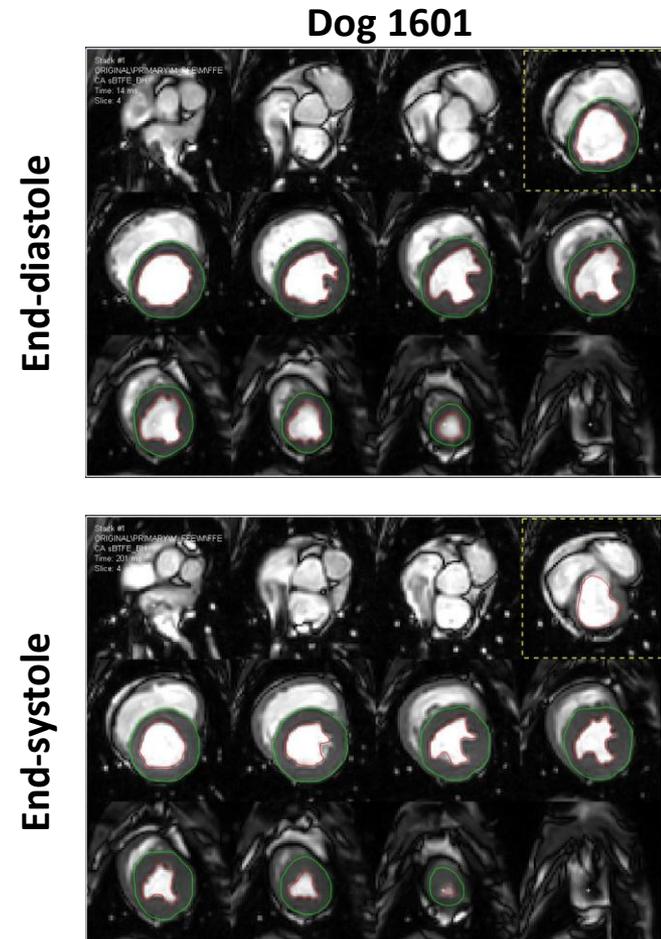


All data collected 8 weeks post-banding



Volume overload induced by high-salt diet

- **Induction:**
 - Dahl salt-sensitive male rats
 - High salt diet (8%) from age 7 weeks for up to 26 weeks
 - Upregulated Renin-Angiotensin System
- **Heart failure:**
 - Results from volume overload
 - Compensatory hypertrophy at 8 weeks with preserved EF
 - Dilation starts after 8-10 weeks: Long, so long.
- **Endpoints:**
 - Cardiac echography (ultrasound measured every 4 weeks)
 - Biomarkers (troponins, CKMB, ANP, NT-Pro-BNP)
 - Terminal invasive hemodynamics (BP, HR, ECG)
 - Histology

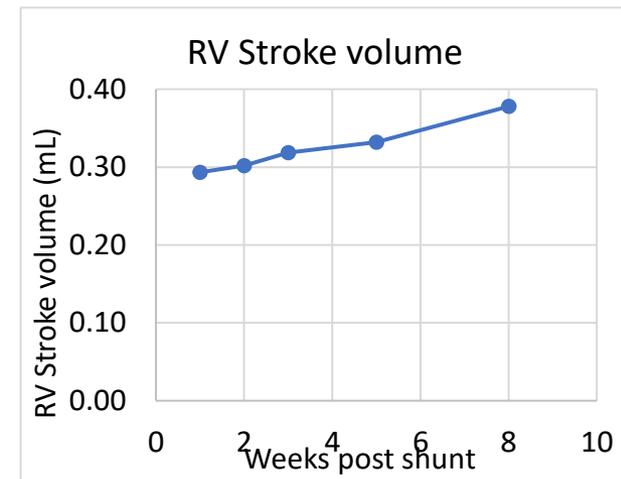
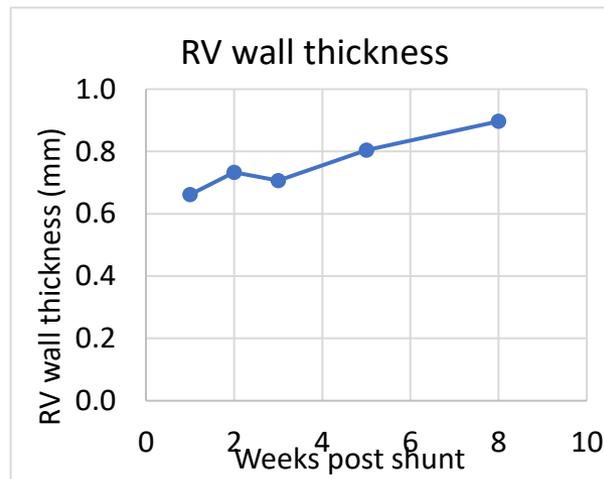
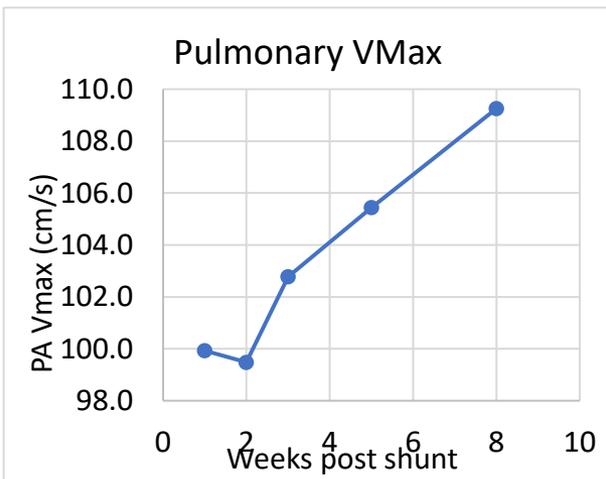
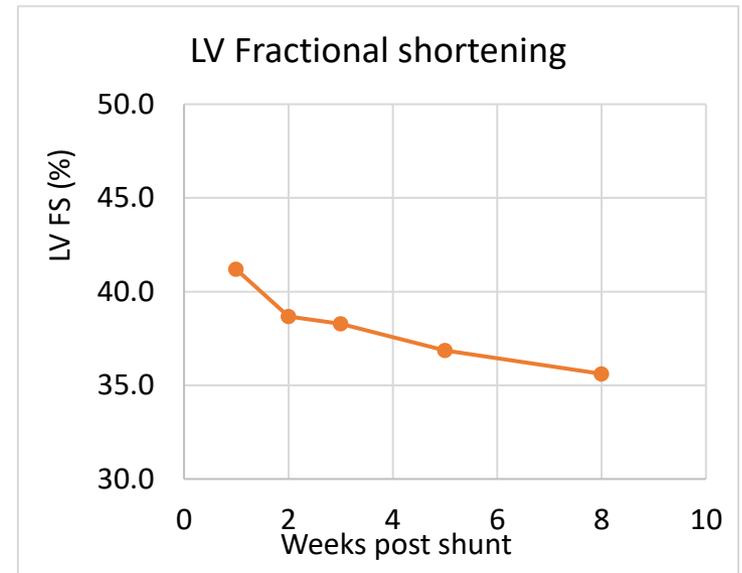
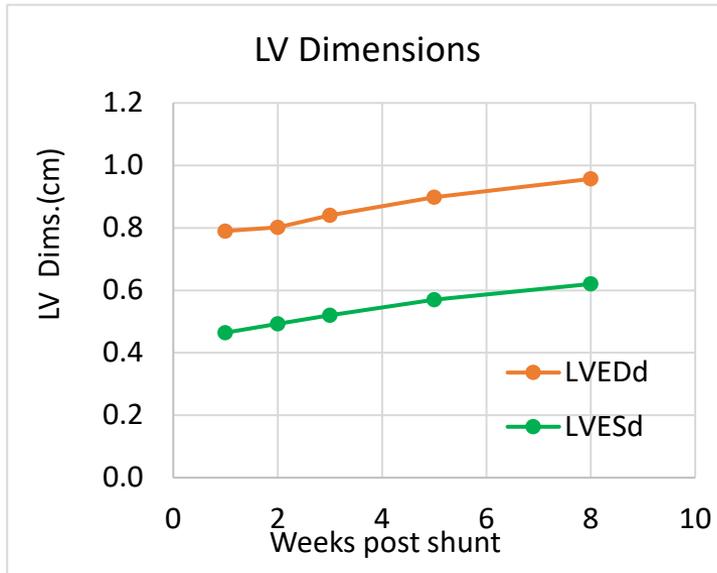


Volume overload-induced by aorto-caval shunt

- **Induction:**
 - Create aortic-vena cava shunt using a needle
- **Heart failure:**
 - Results from volume overload
 - HF at week 8 with preserved EF?
 - *Are we generating HF here?*
 - Some ventricular hypertrophy (starts at Wk20)
- **Endpoints:**
 - Cardiac echography (ultrasound measured every 4 weeks)
 - Biomarkers (troponins, CKMB, ANP, NT-Pro-BNP)
 - Terminal invasive hemodynamics (BP, HR, ECG)
 - Propranolol and atropine challenges
 - Histology



Volume overload-induced by aorto-caval shunt



Volume overload-induced by aorto-caval shunt

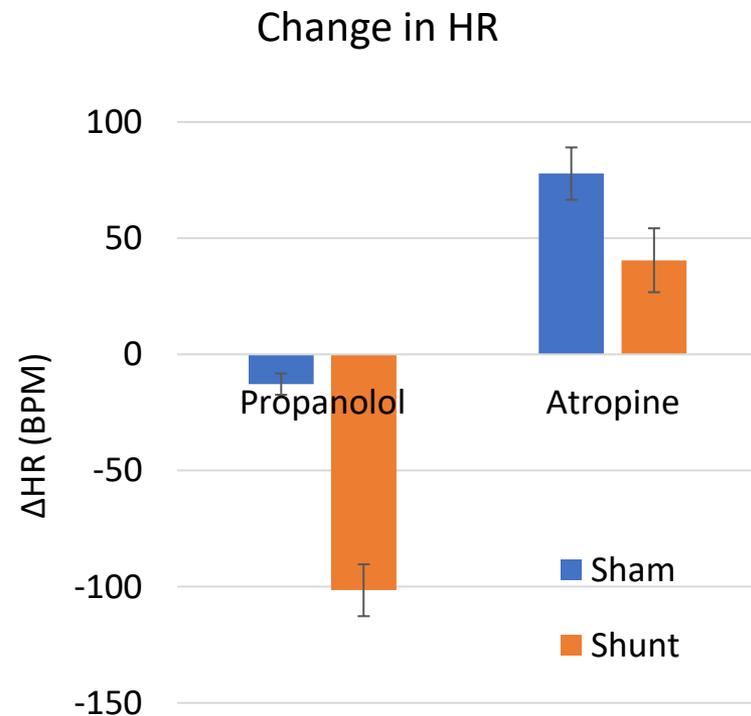
Assessment of sympathetic and parasympathetic tones 8 weeks post-shunt

Shunt animals exhibited enhanced transient bradycardia in response to 1 mg/kg i.p. propranolol compared to small Δ HR in sham animals

→ gain in sympathetic tone compatible with HF

Shunt animals exhibited decreased response to atropine compared to sham animals

→ loss of parasympathetic tone compatible with HF



Induction of arrhythmias by overpacing

- **Induction:**

- Implant pacemaker
- Pace the heart 24/7 for 4 weeks at 800 bpm (rats), 650 bpm (g-p)

- **Heart failure:**

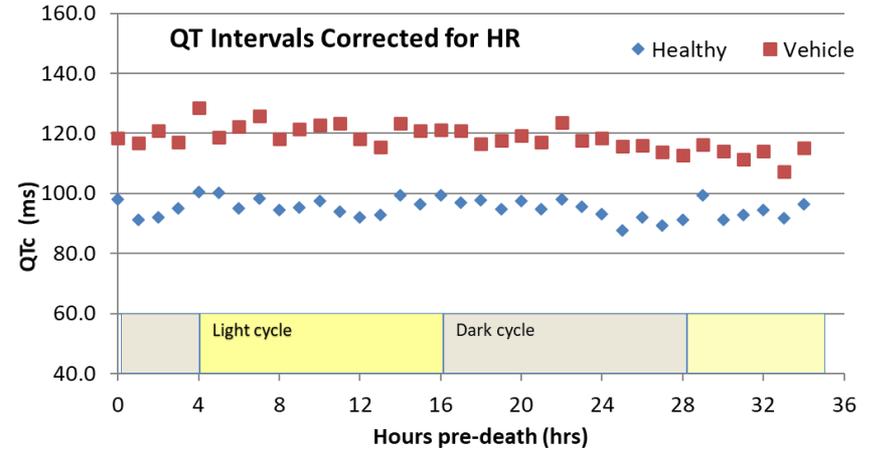
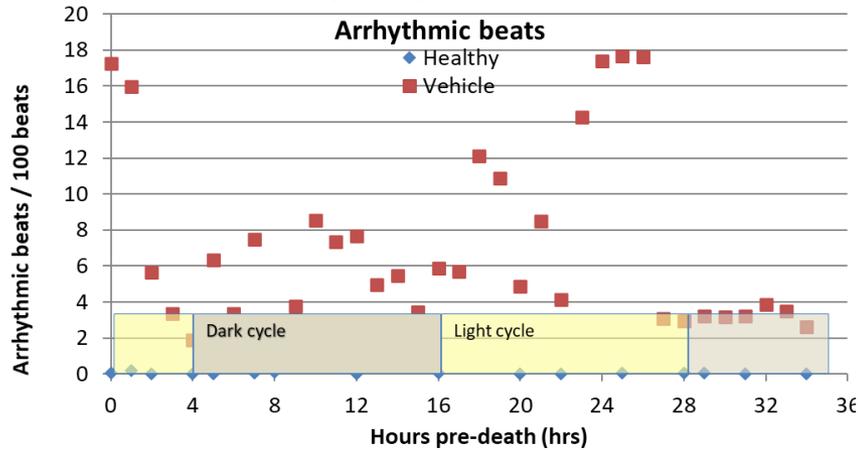
- Results from ventricular fatigue and remodeling
- Usually accompanied by arrhythmia
- Some ventricular hypertrophy
- Decreased cardiac output associated with \searrow fractional shortening, and \searrow end-diastolic volume and \searrow end-systolic volume

- **Endpoints:**

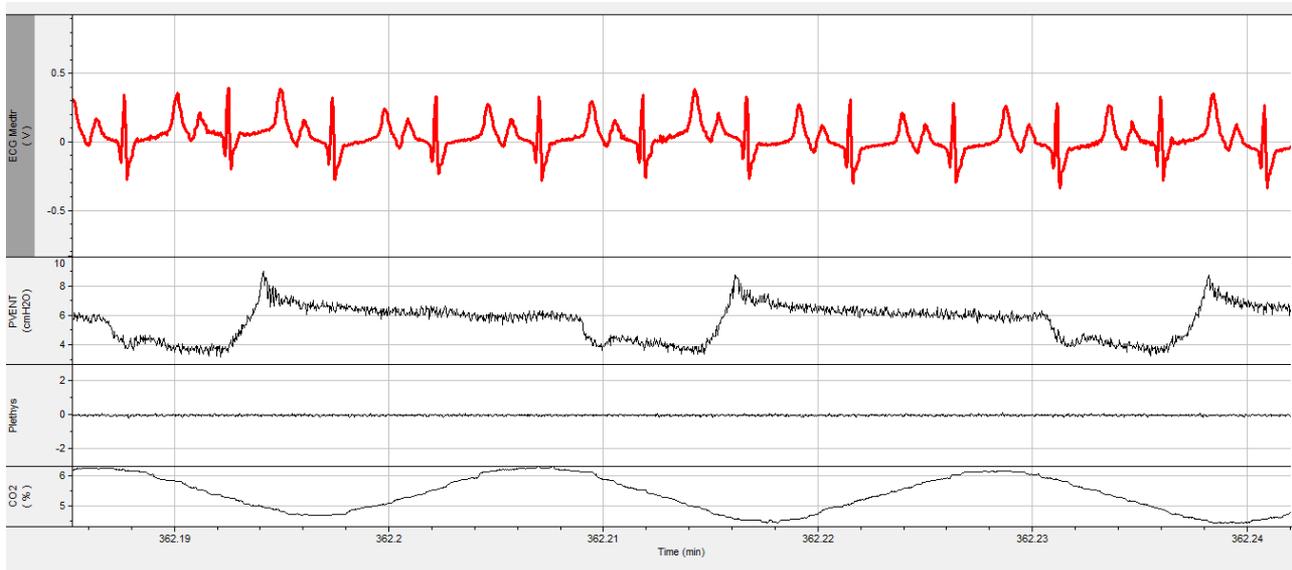
- Cardiac echography (ultrasound)
- Biomarkers (troponins, CKMB, ANP, NT-Pro-BNP)
- Invasive hemodynamics (BP, HR, ECG)
- Histology

Arrhythmias induced by overpacing

In telemetry-implanted rats

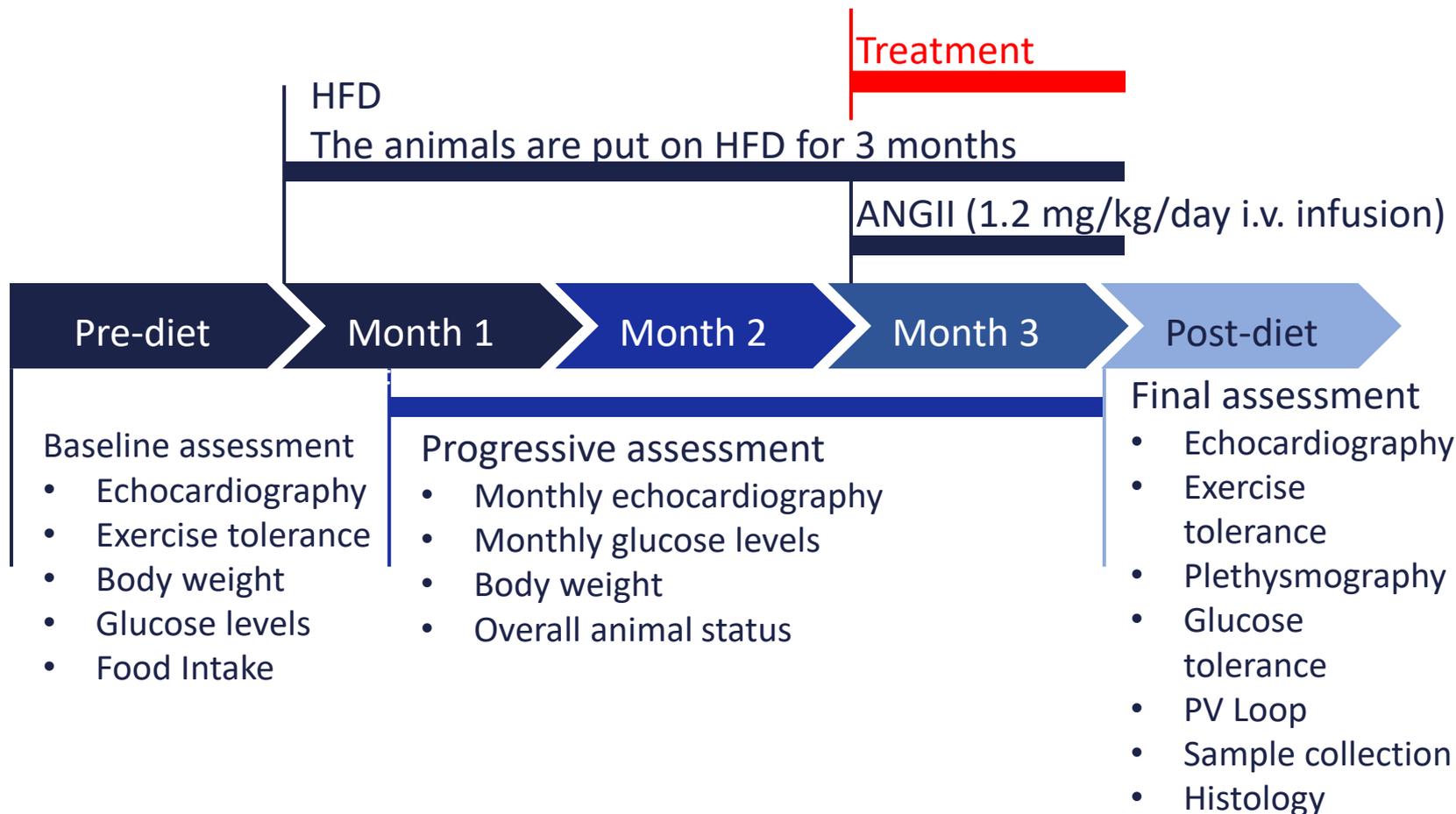


In telemetry-implanted pigs



Heart Failure with Preserved Ejection Fraction

“It’s about the double hit”



Heart Failure with Preserved Ejection Fraction

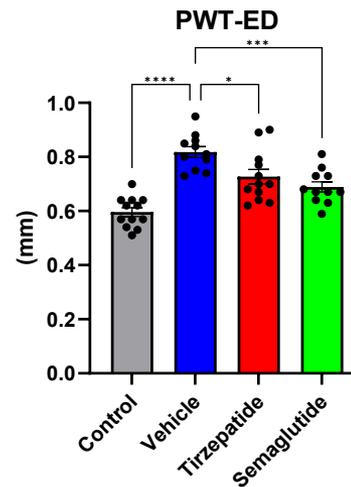
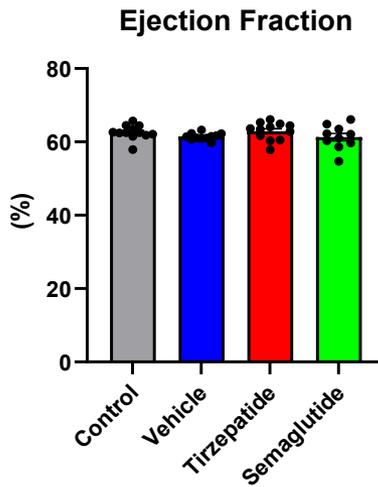
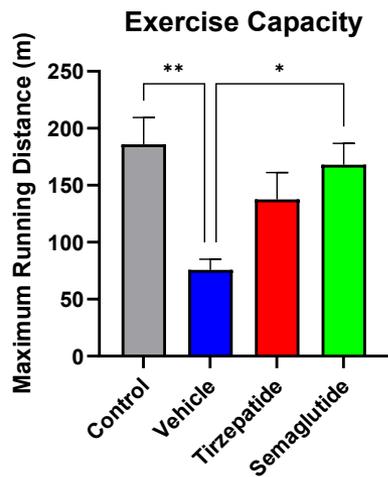
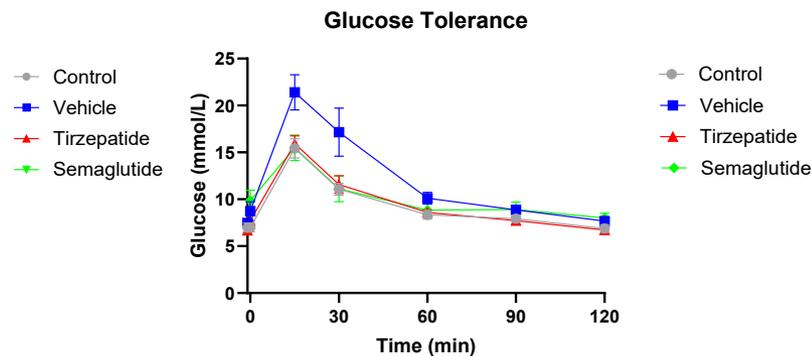
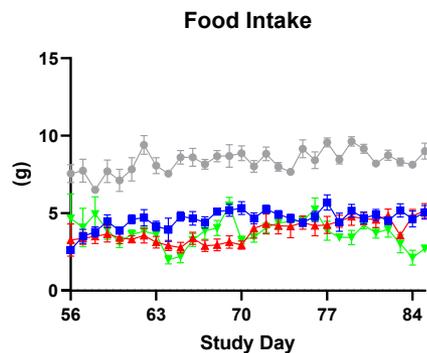
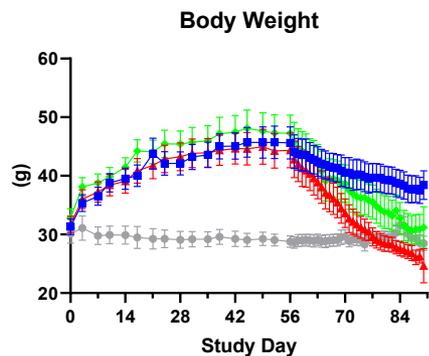
“It’s about the double hit”

Protocol

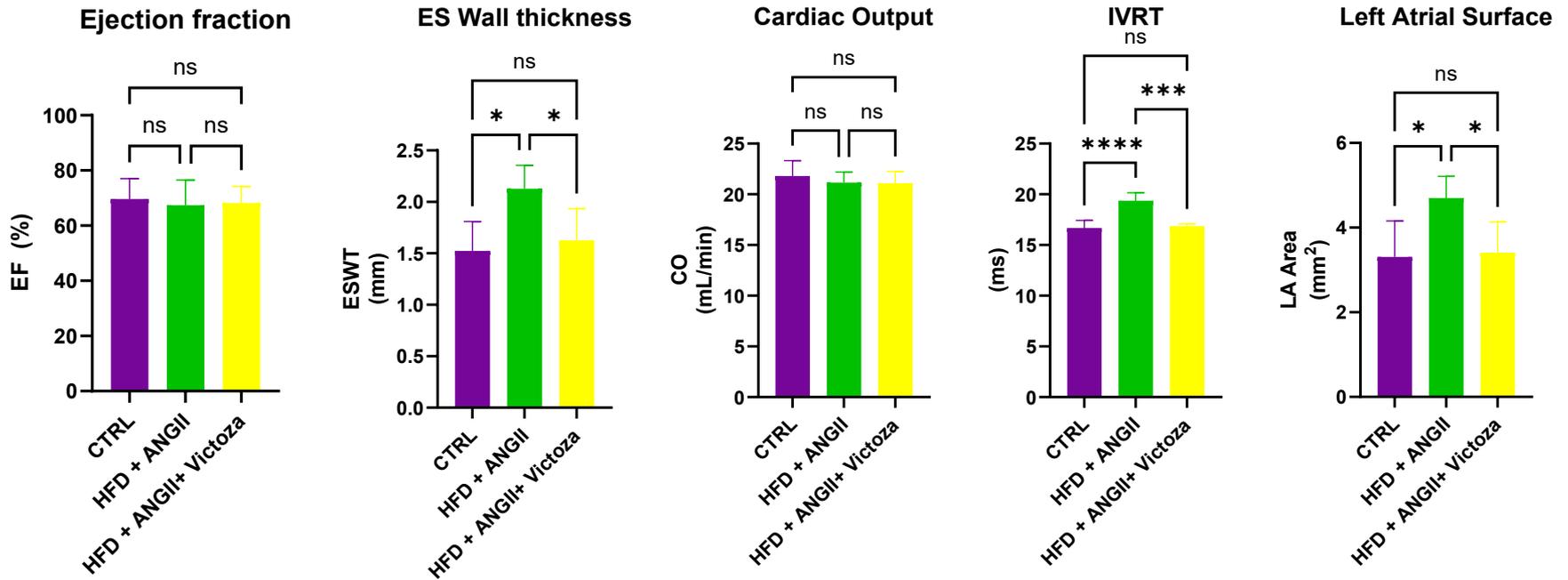
- C57BL/6J mice of 18- to 22- month-old from Jackson Laboratory
- Weekly body weight and food intake monitoring
- High-fat diet for 12 weeks (84 days)
- Continuous subcutaneous infusion of Angiotensin II (ANGII) from Day 56 to 84
- Test article or vehicle administered subcutaneously once per day as of Day 56
- Exercise capacity evaluated on week 12
- Echography performed on Day 0, 55 (for Randomization) and 82
- Fasting glucose levels and glucose tolerance test performed on Day 83

Heart Failure with Preserved Ejection Fraction

“It’s about the double hit”

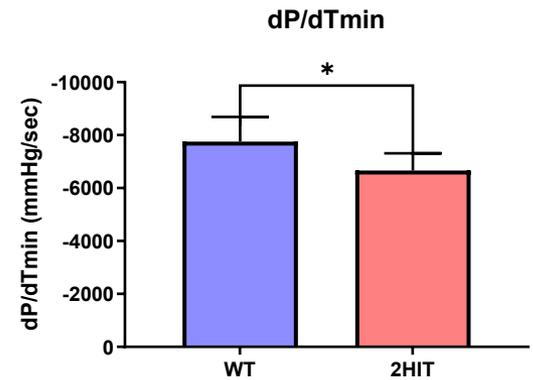
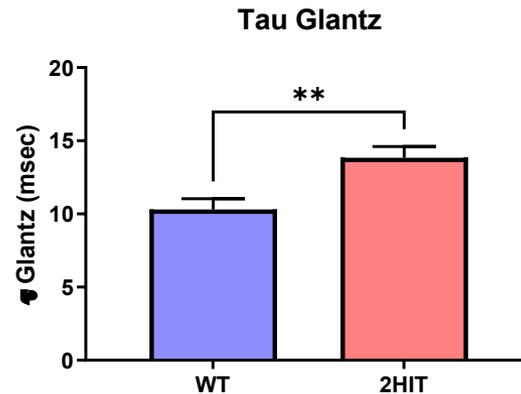
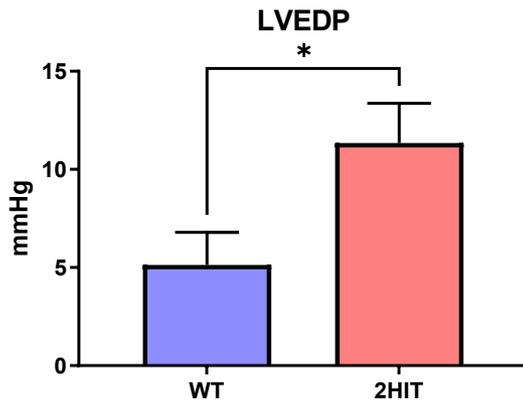


Echocardiography: Mice



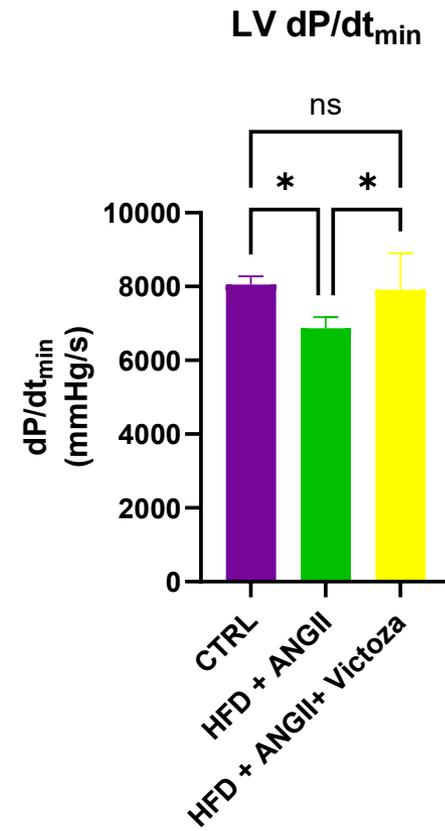
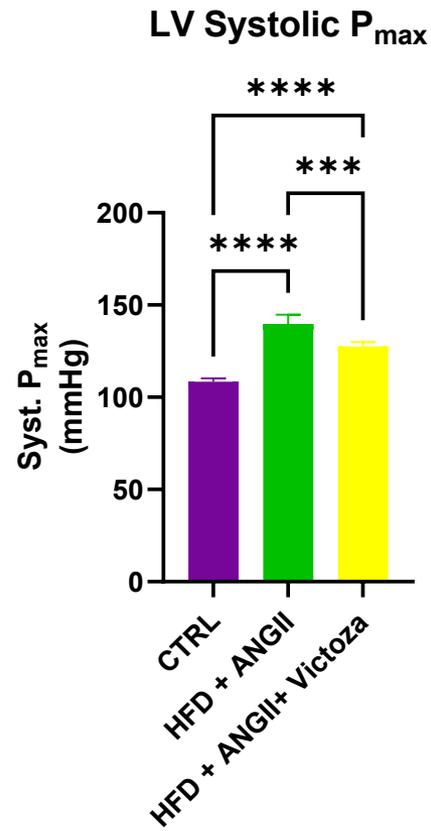
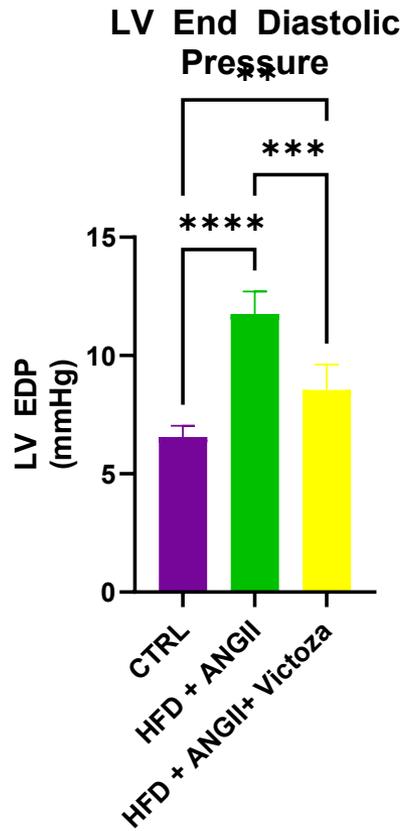
Mitral valve E velocity and mitral valve septal annulus velocity E', indicating diastolic dysfunction, are difficult to measure in the mouse.

PV loops: Rats

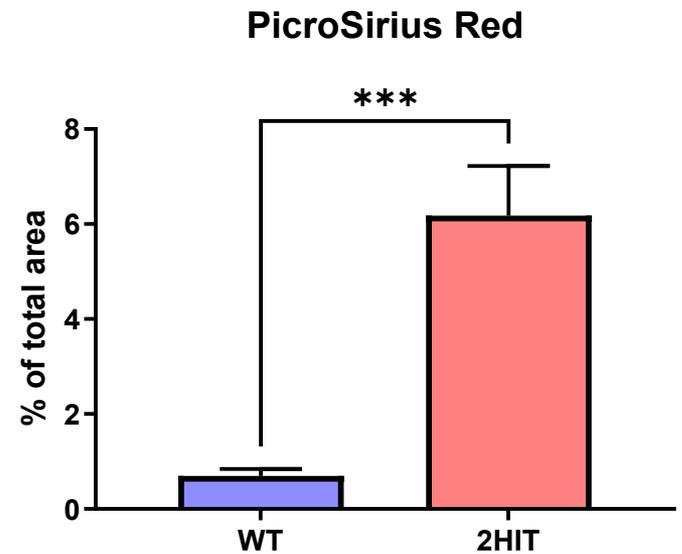
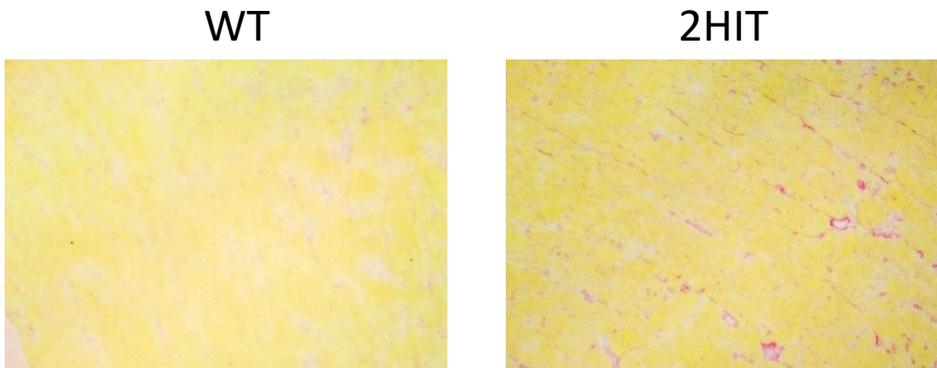


Pressure-volume loop analysis → comprehensive assessment of cardiac systolic and diastolic function. The increased end-diastolic pressure suggest LV diastolic myocardial stiffness. Tau Glantz and decreased rate of relaxation indicate a stiff left ventricle.

PV loops: Mice

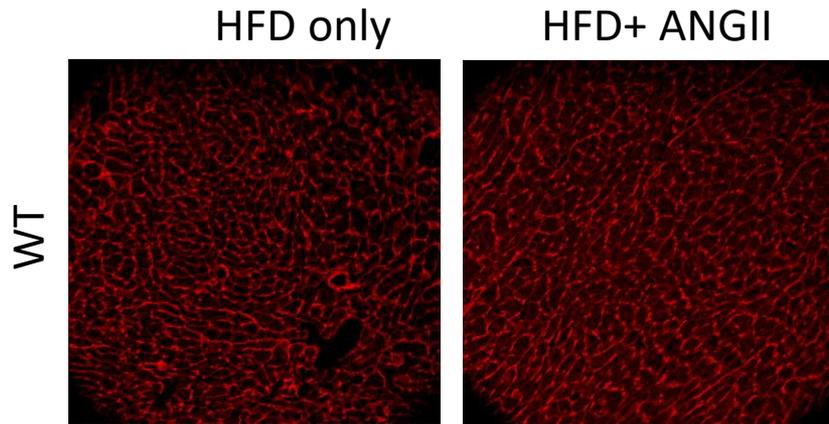


Histology: Rats and mice

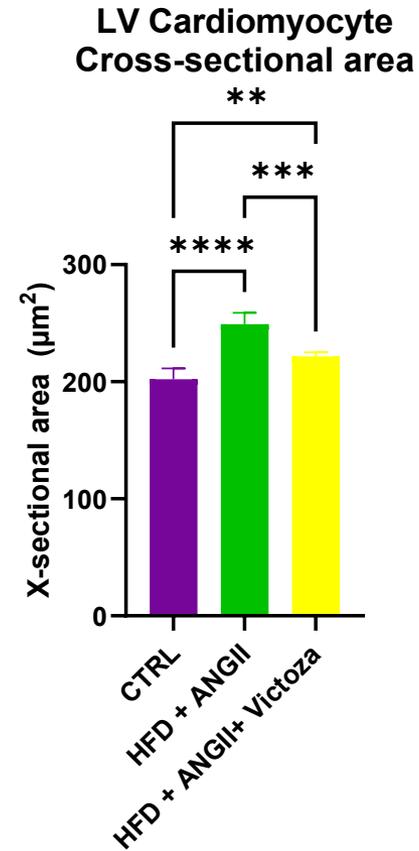


2-hit rats develop LV myocardial fibrosis.

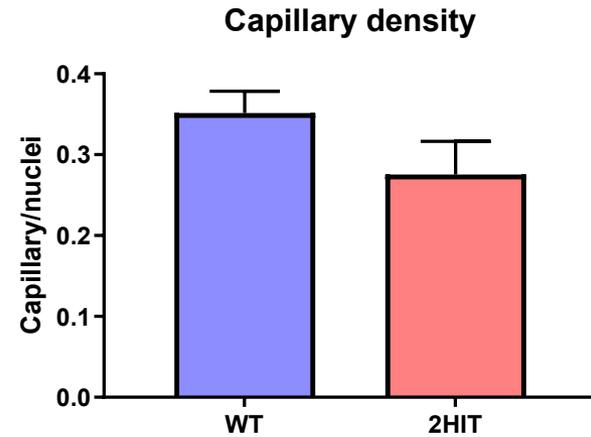
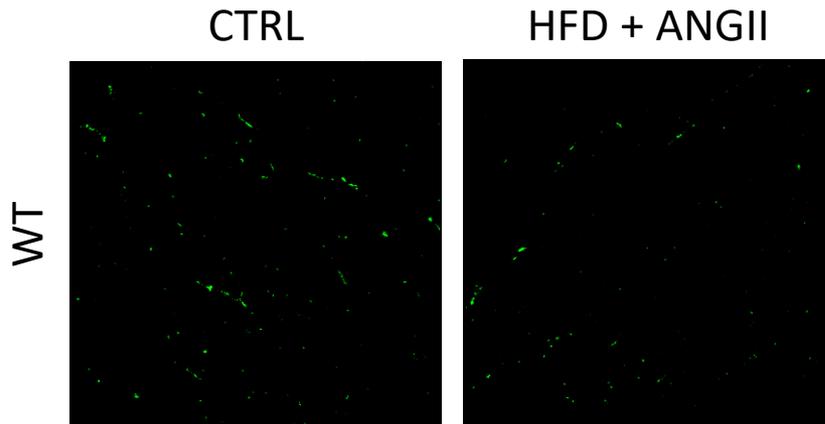
Histology: Rats & Mice



2-hit mice develop cardiomyocyte hypertrophy

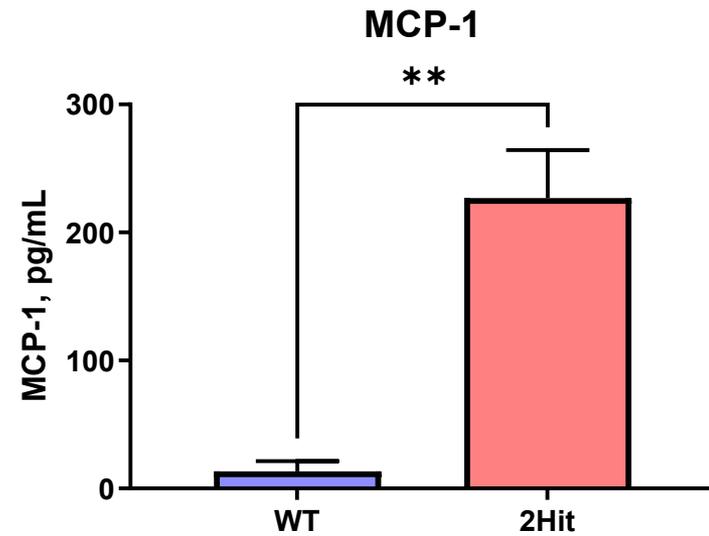
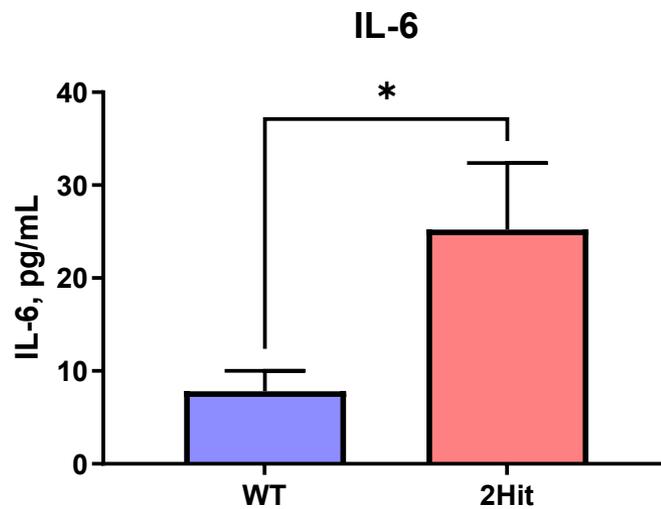


Histology: Rats & Mice



2-hit animals show trend of capillary density reduction.

Biomarkers: Rats



Biomarkers suggest presence of inflammation in the LV myocardium of HFD+ANGII animals.

Heart failure model: Anthracyclin toxicity

- **Induction:**

- Intraperitoneal injection of 2 mg/kg doxorubicin every 2 days for 7 days
- Cumulative dose of doxorubicin determines the extent of the damage

- **Heart failure:**

- Results from classical myocardial toxicity + DNA damage without repairs
- Loss of myocardial filament organization
- Decreased cardiac output associated with \searrow ejection fraction, \searrow fractional shortening, \searrow everything
- Detectable within 72 hours post injection #1
- Severe, irreversible, essentially a survival model

- **Endpoints:**

- Cardiac echography (ultrasound)
- Invasive hemodynamics (BP, HR, P-V loop, ECG)
- Biomarkers (troponins, CKMB, ANP, NT-Pro-BNP)
- Histology

- **Major Limitations:**

- Extremely variable from one animal to the next
- Irreversible: efficacy means a mitigation of damage, right-shift in doses of anthracyclin which can be used before toxicity becomes crippling.

Heart failure model: CLP sepsis

- **Induction:**

- Cecal ligation and perforation in adult rats leads to acute sepsis
- 12 hours to introduce treatment, model is generally over 72h post-CLP

- **Heart failure:**

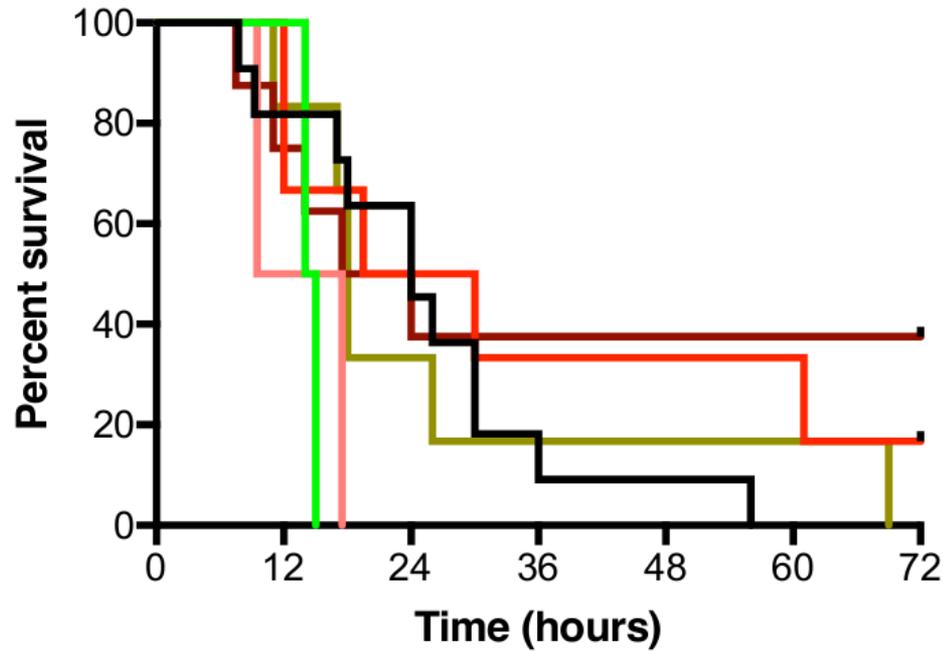
- Results from complete septic shock symptoms
- Hypovolemia, exaggerated immune response, global ischemia
- Decreased cardiac output associated with \searrow ejection fraction, \searrow fractional shortening, \searrow everything
- Severe, acute, essentially a survival model

- **Endpoints:**

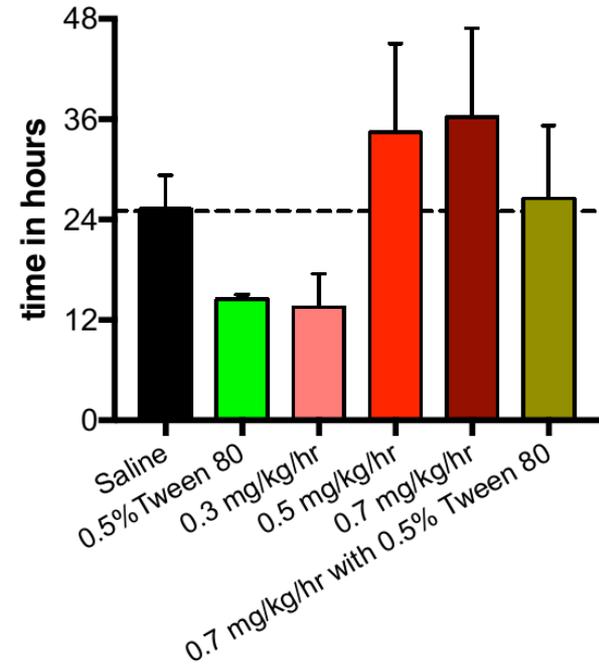
- Cardiac echography (ultrasound)
- Biomarkers (troponins, CKMB, BNP)
- Histology

Heart failure model: CLP sepsis

Survival rate



Survival time



Questions, comments, complaints?



Dan Salvail

Vice-President

- dsalvail@ipsttherapeutique.com
- Tel: 1-819-820-1515 x 232



Annie Bouchard

Director of preclinical strategy & surgical programs

- abouchard@ipsttherapeutique.com
- Tel: 1-819-820-1515 x231



Some of IPST's preclinical models of HF

Induction of dysfunction	Current preclinical models used at IPST
Ischemia/hypoxia/coronary dysfunction (TLR4 & TLR2 activation)	Coronary ligation (usually the LAD) FeCl ₃ surface application (coronary thrombus)
Valve regurgitation/stenosis	Staph Aureus endocarditis Mechanical valve damage
Increased afterload/hypervolemia	TAC or pulmonary artery banding (PAB) or DOCA + salt diet (WT or Dahl SS rats) Angiotensin-II + phenylephrine infusions
Chemical / drug toxicity	Anthracyclin-induced cardiomyopathy
Acute heart failure / sepsis	Cecal ligation and perforation (CLP)-induced acute cardiomyopathy
Hypertrophic cardiomyopathy	Isoproterenol-induced ventricular hypertrophy DBA/2JD2 mice and other genetic models
Dilated cardiomyopathy	TNF α infusion to degrade collagen ECM
Arrhythmias	Overpacing Surgical induction of atrial fibrillation
HFpEF	Diabetes T2 + High salt diet High fat diet + L-NAME or ANGII