Regeneración cardíaca utilizando nano y microtecnología

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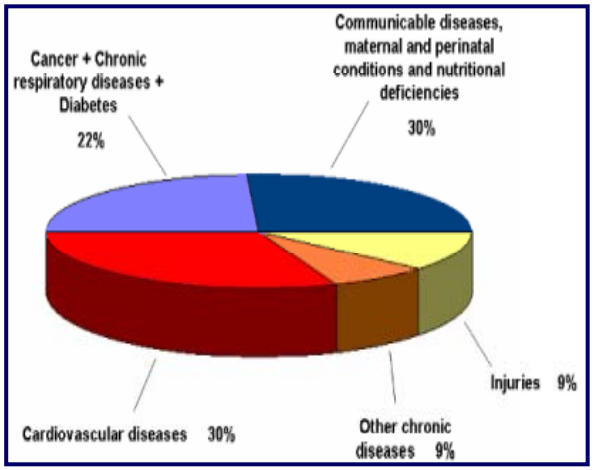
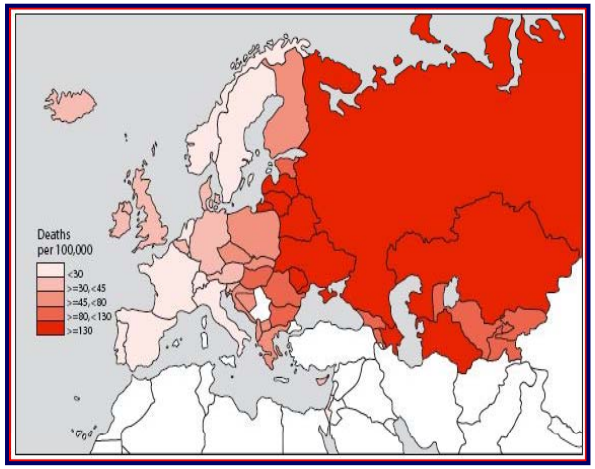
Universidad de Navarra

Cardiovascular Diseases (CVD)

- Number one cause of death in the world: 30% of all global deaths
- Nearly half (48%) of all deaths in Europe
- Spain: 31.2% of all deaths



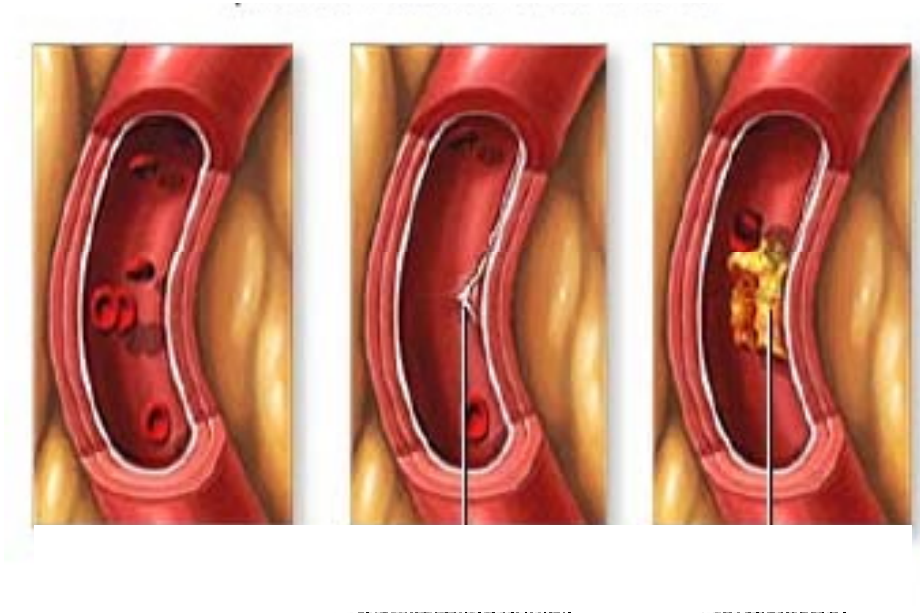


Cardiovascular Diseases, World Health Organization (WHO). Estimates for 2005. Available at http://www.who.int/cardiovascular_diseases

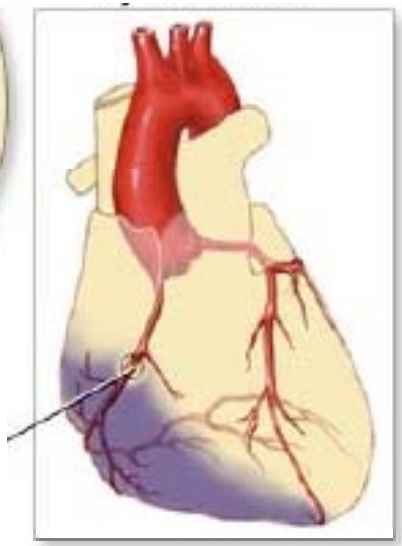
British Heart Foundation. Estimates for 2008. Available at http://www.heartstats.org.

Instituto Nacional de Estadística (INE). Defunciones según la causa de muerte 2009. Available at http://www.ine.es.

Physiopathological Aspects



Coronary blockage

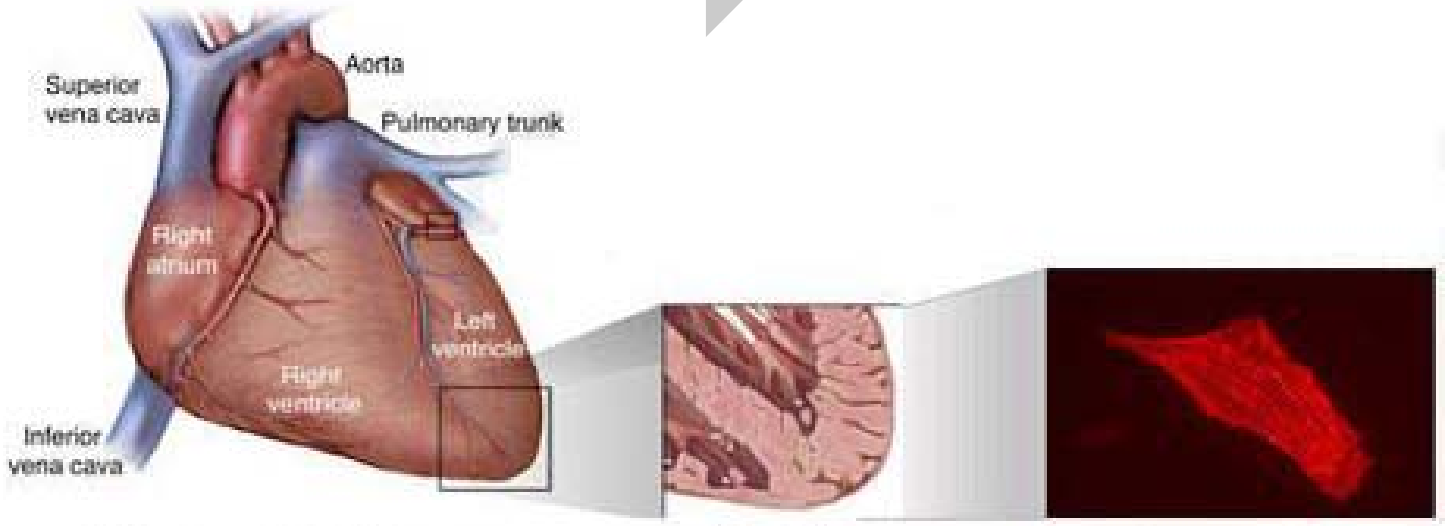


Myocardial infarction

Physiopathological Aspects

Ischemia (hypoxia) → Cardiomyocyte deficiency → Unworkable scarred tissue → Hypertrophy

INCREASED RISK OF ARRHYTHMIA, VENTRICULAR FIBRILLATION AND HEART ATTACK



ischemia | **Cardiac hypertrophy** | **Cardiomyocyte**

Cardiac Regeneration

Current standard therapies

- Drugs
- Reperfusion
- Vascular and bypass surgeries
- Organ transplantation

Modern approaches

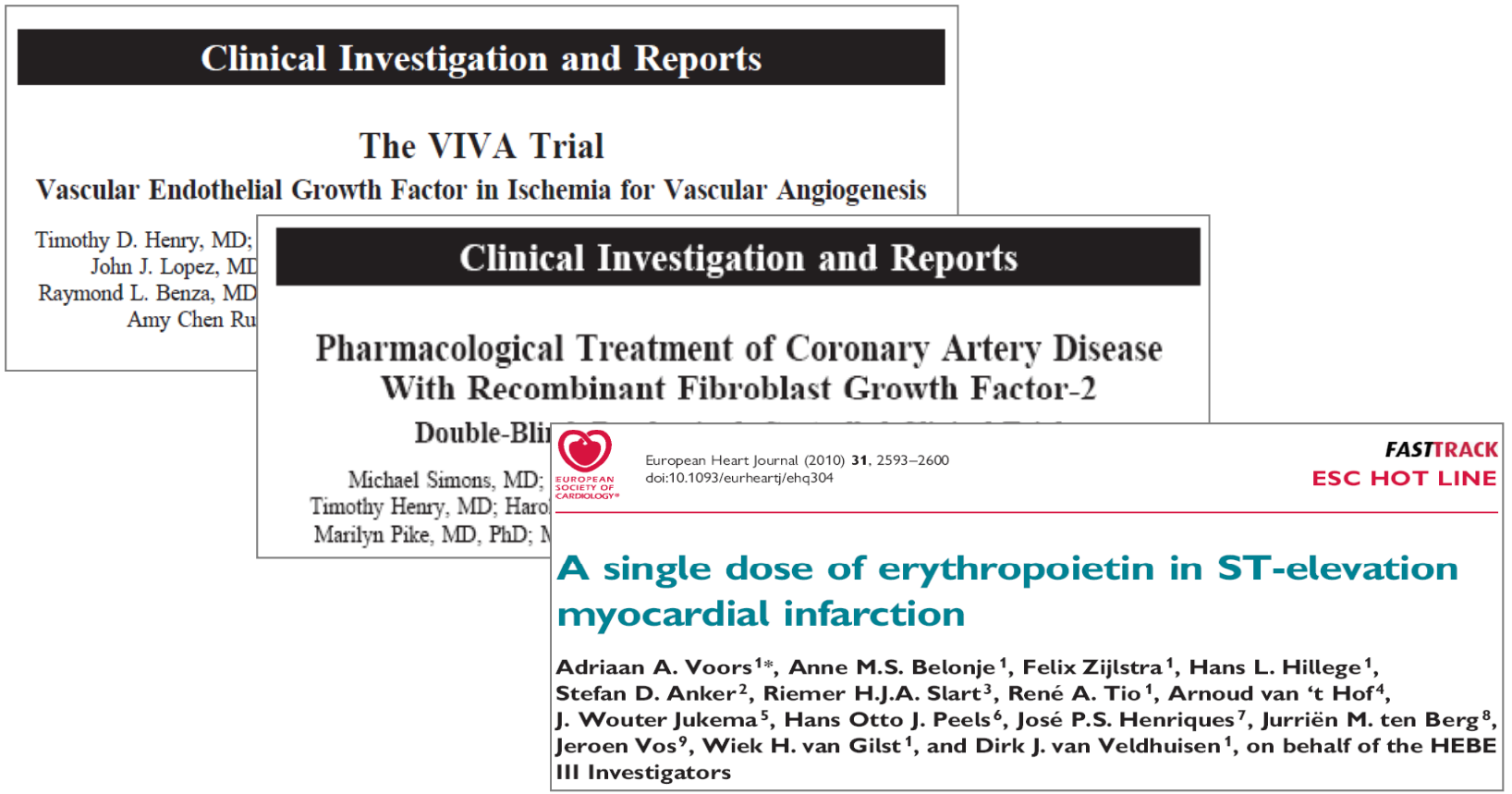
- Cell-based therapies
- Gene therapy
- Growth factor therapy

Clinical Trials in Cardiac Diseases

Protein	Route	Trial	n	Outcomes	Study
FGF-1	IM	Phase I	20	↑Capillary density, but no evidence of improved ventricular function	Schumacher *et al.*, 1998
FGF-2	IC	Phase I	25	Well tolerated dose-escalation trial in subjets with stable angina	Unger *et al.*, 2000
	IC	Phase I	52	↑Exercise tolerance, ↓size of ischemic area	Laham *et al.*, 2000
	IC/ IV	Phase I	59	↑Perfusion and attenuation of stress-induced ischemia; no control group	Udelson *et al.*, 2000
	IC	Phase II	337	FIRST study; ↓symptoms of angina at 90 days follow-up, but no longer detectable at 180 days; no improvement in myocardial perfusion	Simons *et al.*, 2002
VEGF	IC	Phase I	14	↑Perfusion in patients treated with low-dose	Hendel *et al.*, 2000
	IC	Phase I	15	Well tolerated dose-escalation trial; ↑myocardial perfusion in half of patients at 60 days	Henry *et al.*, 2001
	IV	Phase I	28	↑Myocardial perfusion and collateral density	Gibson *et al.*, 1999
	IC/ IV	Phase II	178	VIVA study; safe and well tolerated; no improvements in exercise tolerance and myocardial perfusion	Henry *et al.*, 2003
NRG-1	IV	Phase I	15	Safe and well tolerated; short-term improvements in cardiac function; no control group	Jabbour *et al.*, 2010
	IV	Phase II	44	Progressive improvements of cardiac function and anti-remodeling effect in patients with chronic heart failure, but no statistically significance differences	Gao *et al.*, 2010
EPO	IV	Phase I	44	Evidence of safety and biologic activity of erythropoietin in patients with acute myocardial infarction; increased expression of angiogenesis signaling proteins	Tang *et al.*, 2009
	IV	Phase II	529	A single high dose of EPO did not improve cardiac function after 6 weeks	Voors *et al.*, 2010

Formiga *et al.*, Heart Failure Reviews 17(3): 449-73, 2012

Clinical Trials in Cardiac Diseases



The results from the three larger clinical trials were disappointing

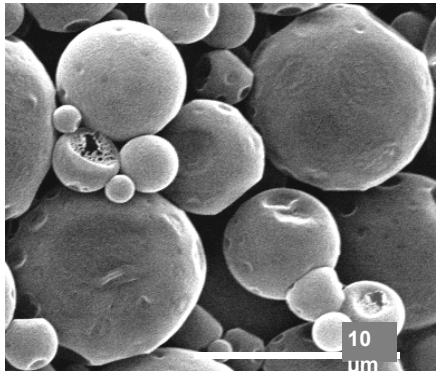
Clinical Trials in Cardiac Diseases

Clinical trials failure

- short *in vivo* half-life (30-90 min)
- protein instability *in vivo*
- undesired vascularization in non-target sites

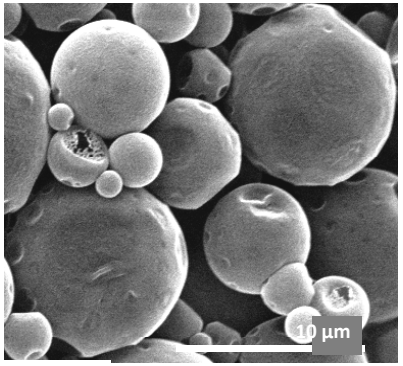
Lessons from Clinical Trials

- No availability of the protein on myocardium after bolus injection
- Protein therapy requires local administration and continuous exposure of protein

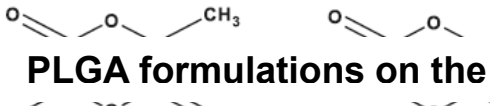


PROTEIN ENCAPSULATION INTO POLYMERIC PARTICLES

Polymeric Particles



Poly (lactic-co-glycolic acid) - PLGA



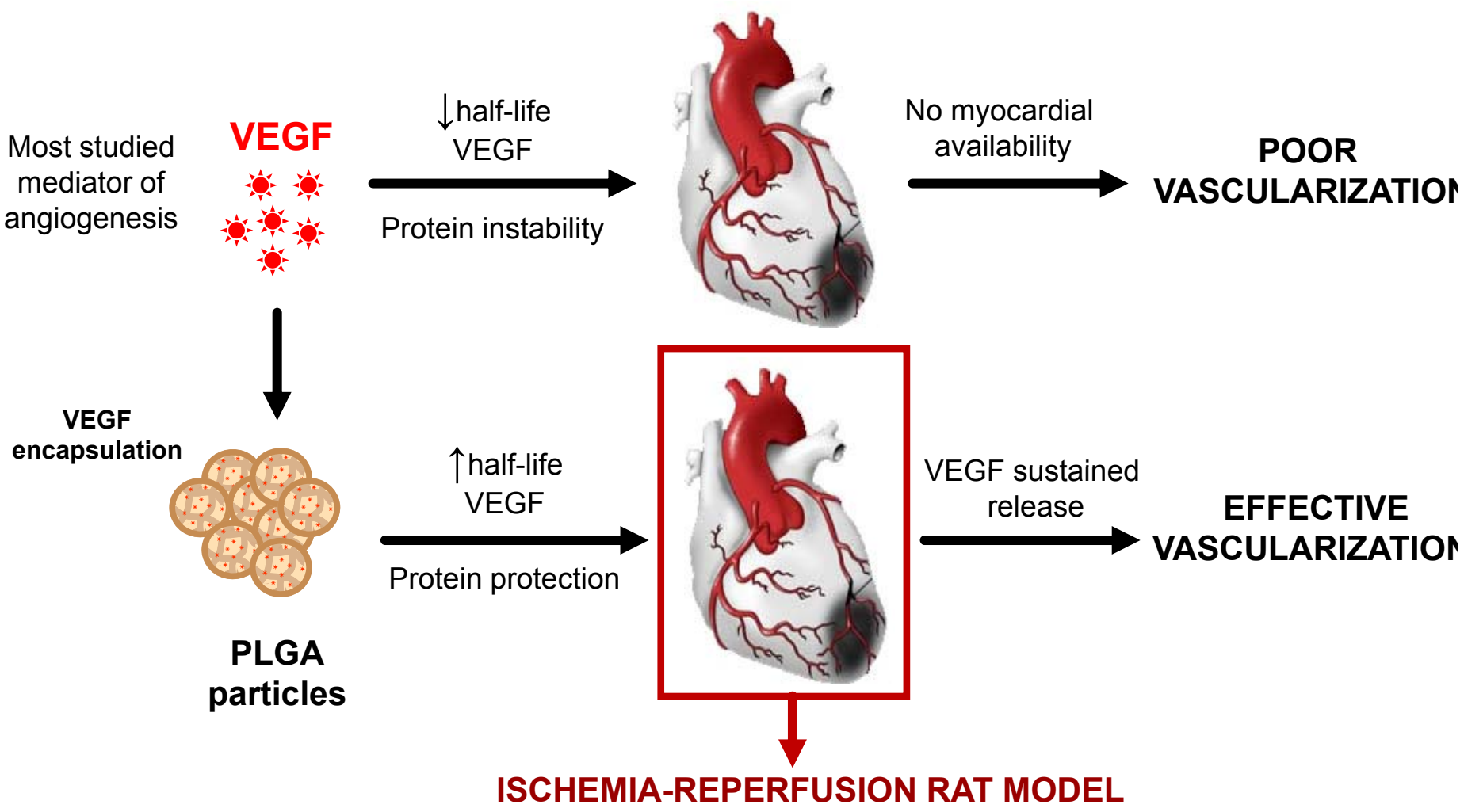
PLGA formulations on the market

Drug	*Trade name*	*Company*	*Polymer*	*Route*	*Application*
buserelin acetate	Profact®Depot, Suprefact®Depot	Hoechst Marion Roussel	PLGA	s/c implant	prostate cancer
goserelin acetate	Zoladex®Depot	Astra Zeneca	PLGA	s/c implant	prostate cancer, endometrioses
leupr… ace…	Trenantone®		PLA	suspension	…te …er, …ioses
octreotide acetate	Sandostatin LAR®Depot	Novartis Pharma	PLGA	s/c suspension	GH suppression, anti cancer
triptorelin	Decapeptyl® Depot	Debiopharma	PLGA	s/c depot injection	LHRH agonist, prostate cancer
recombinant human growth hormone	Nutropin®Depot, [discontinued commercialisation since 06/2004]	Genentech-Alkermes	PLGA	monthly s/c injection	growth hormone deficiency

FDA approved product for drug delivery

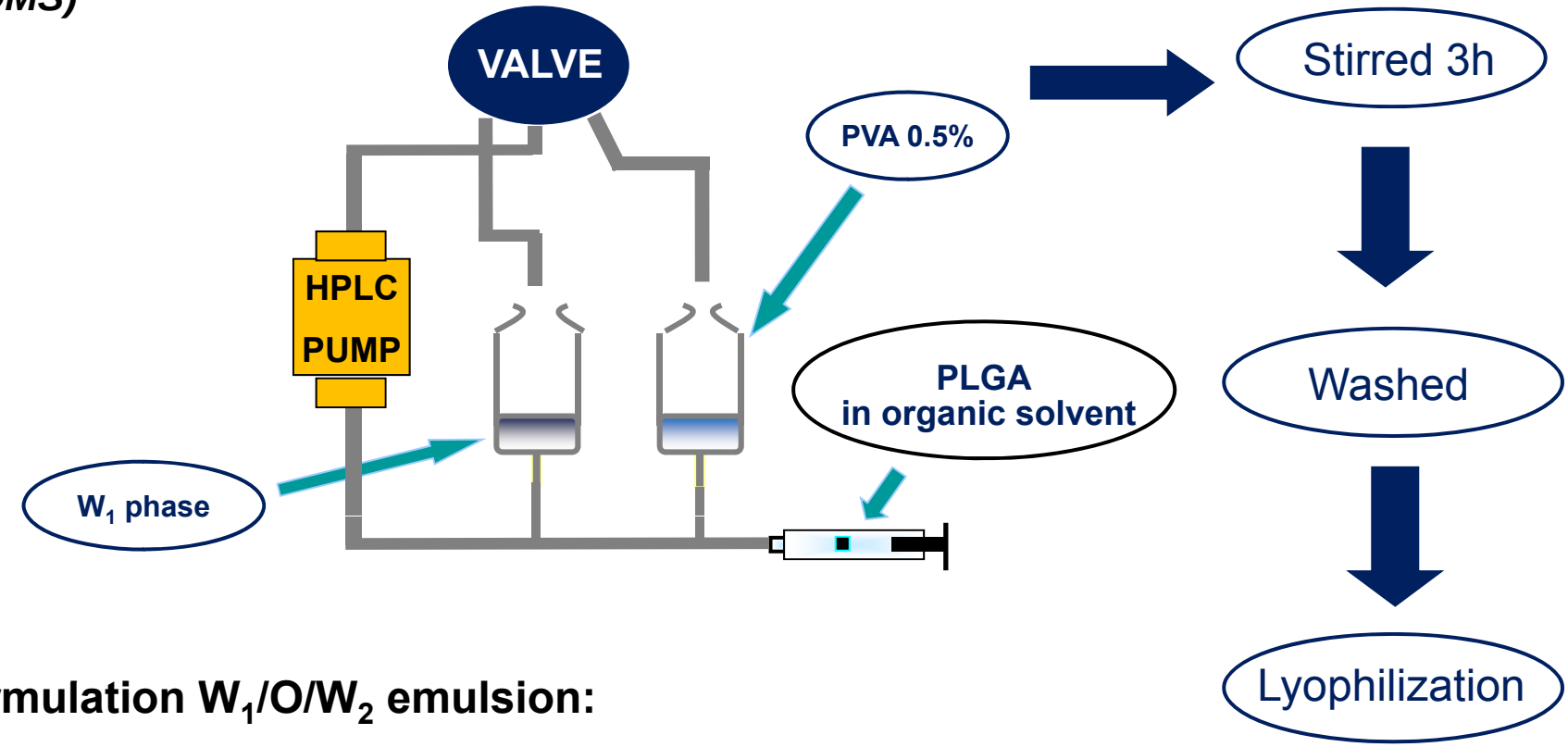
$CO_2 + H_2O$

Growth Factors (VEGF) and Cardiac Repair



Formulation

Total Recirculation One-Machine System (TROMS)



Formulation $W_1/O/W_2$ emulsion:

W_1: HSA + PEG 400 in PBS

Organic Phase: PLGA in dichloromethane/acetone mixture

W_2: PVA 0.5%

Formulation

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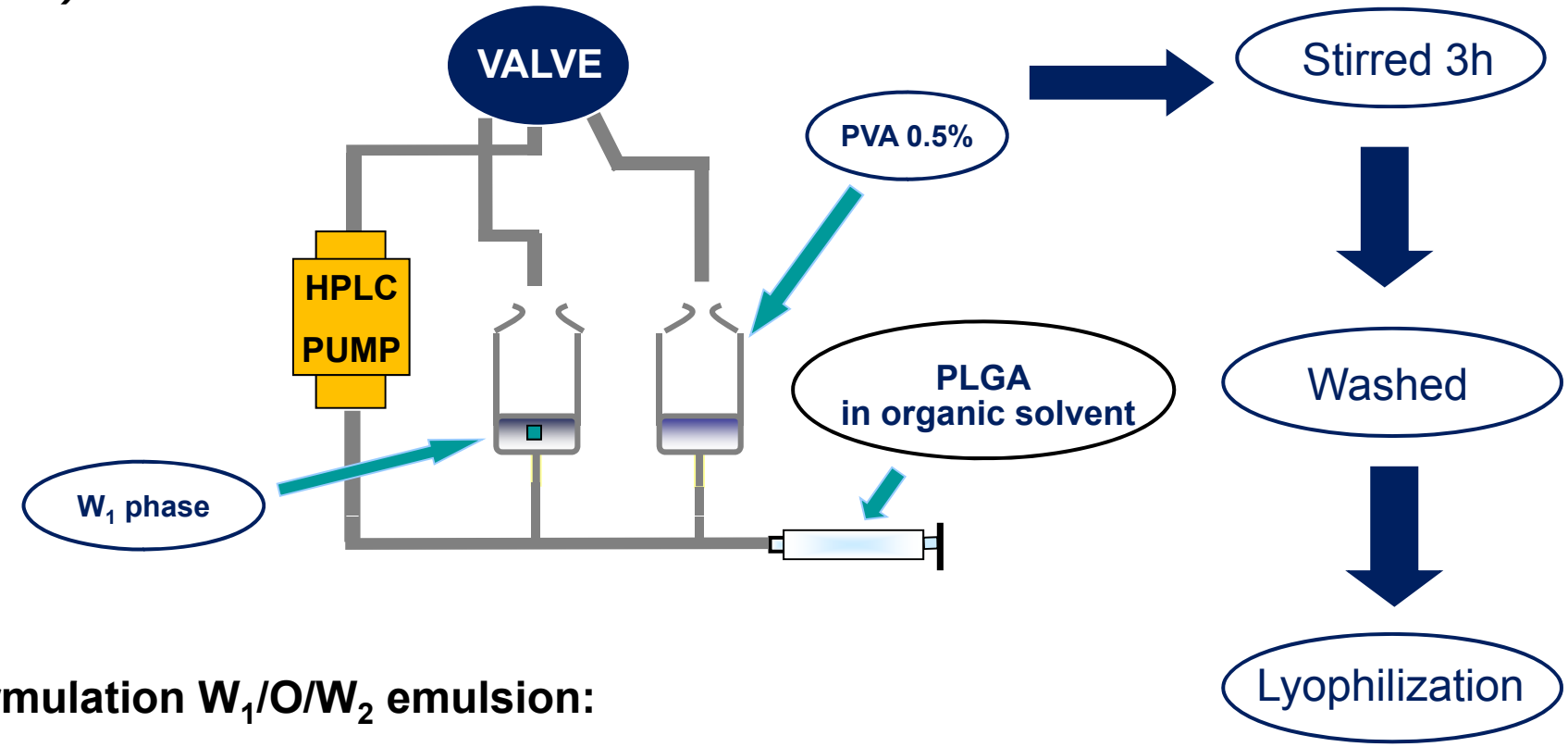

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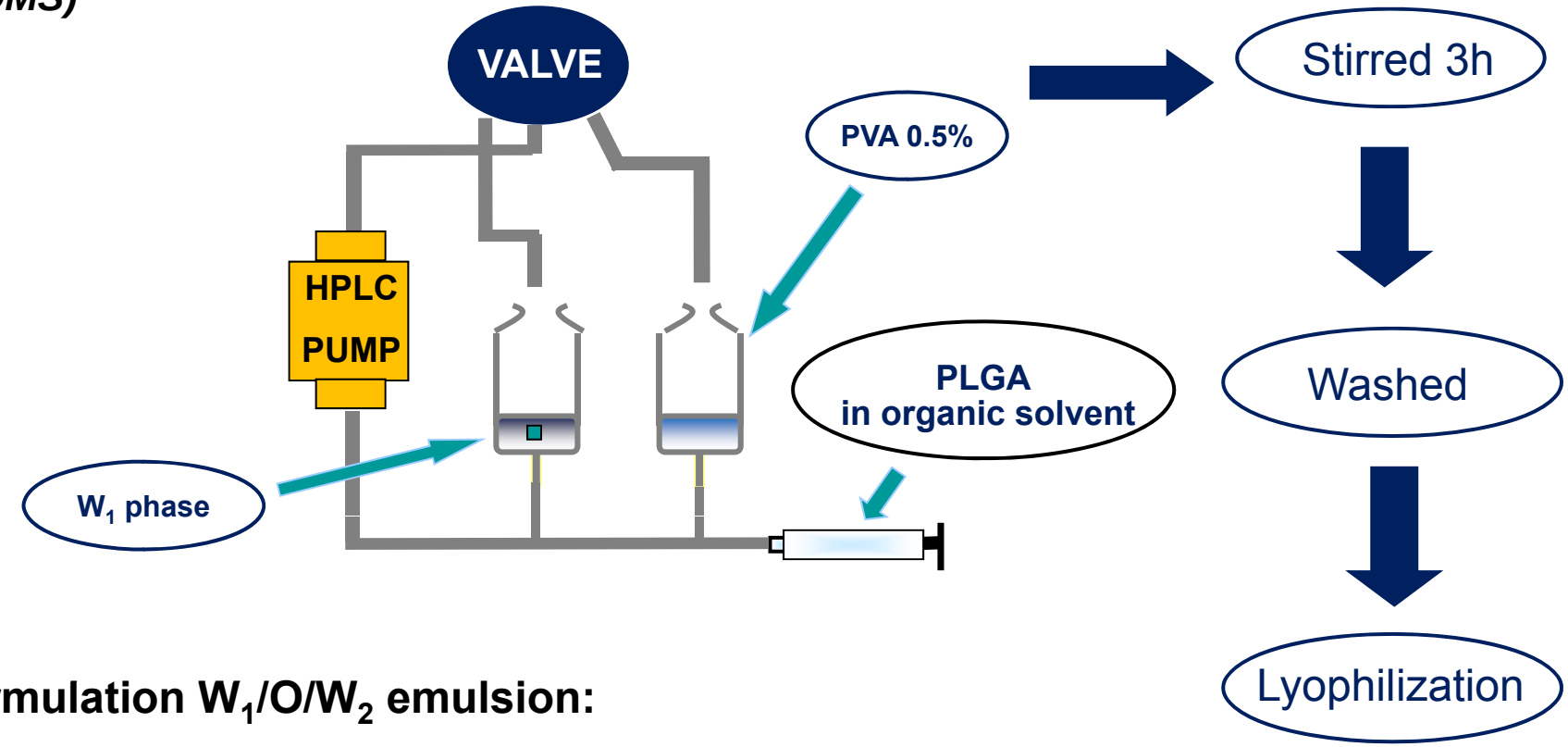

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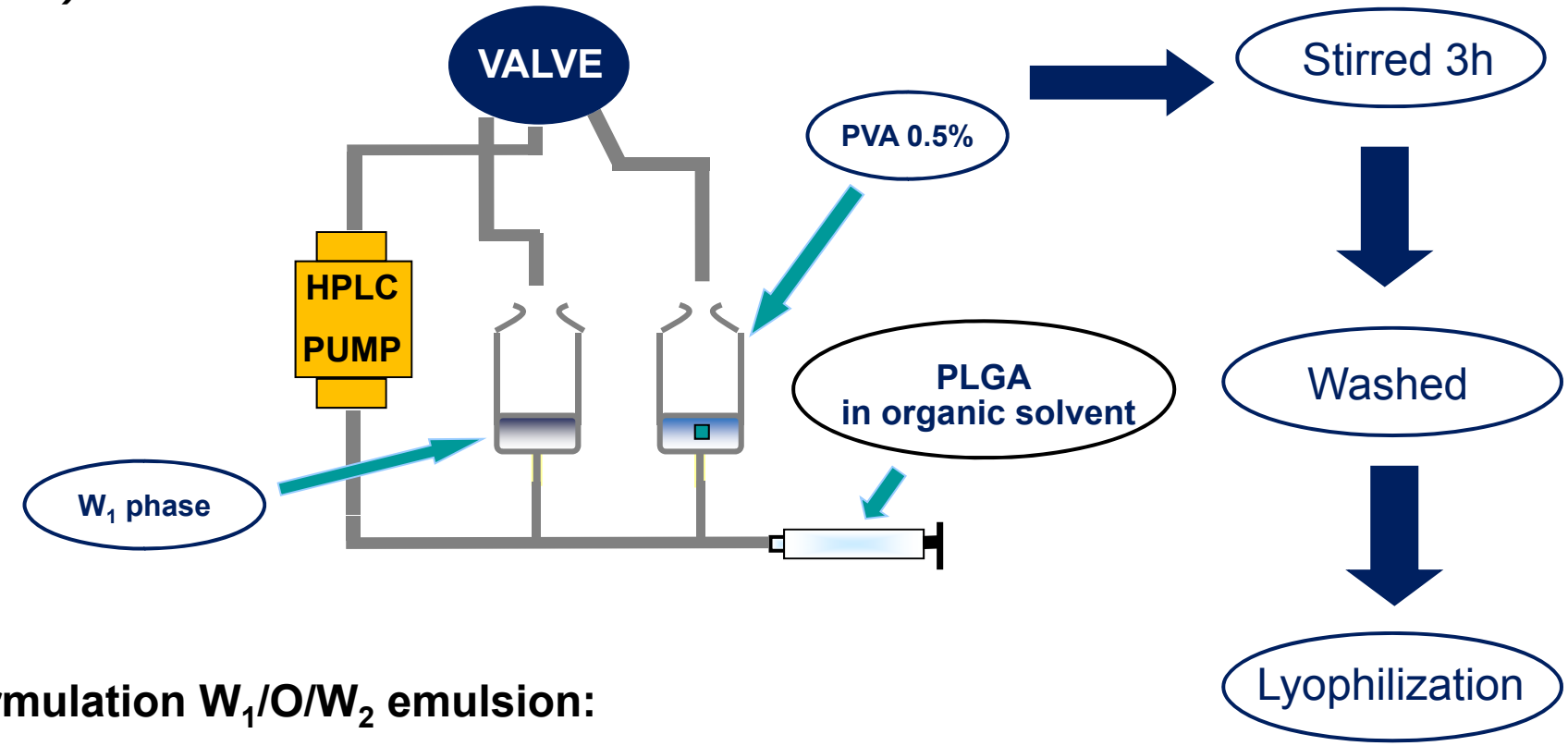

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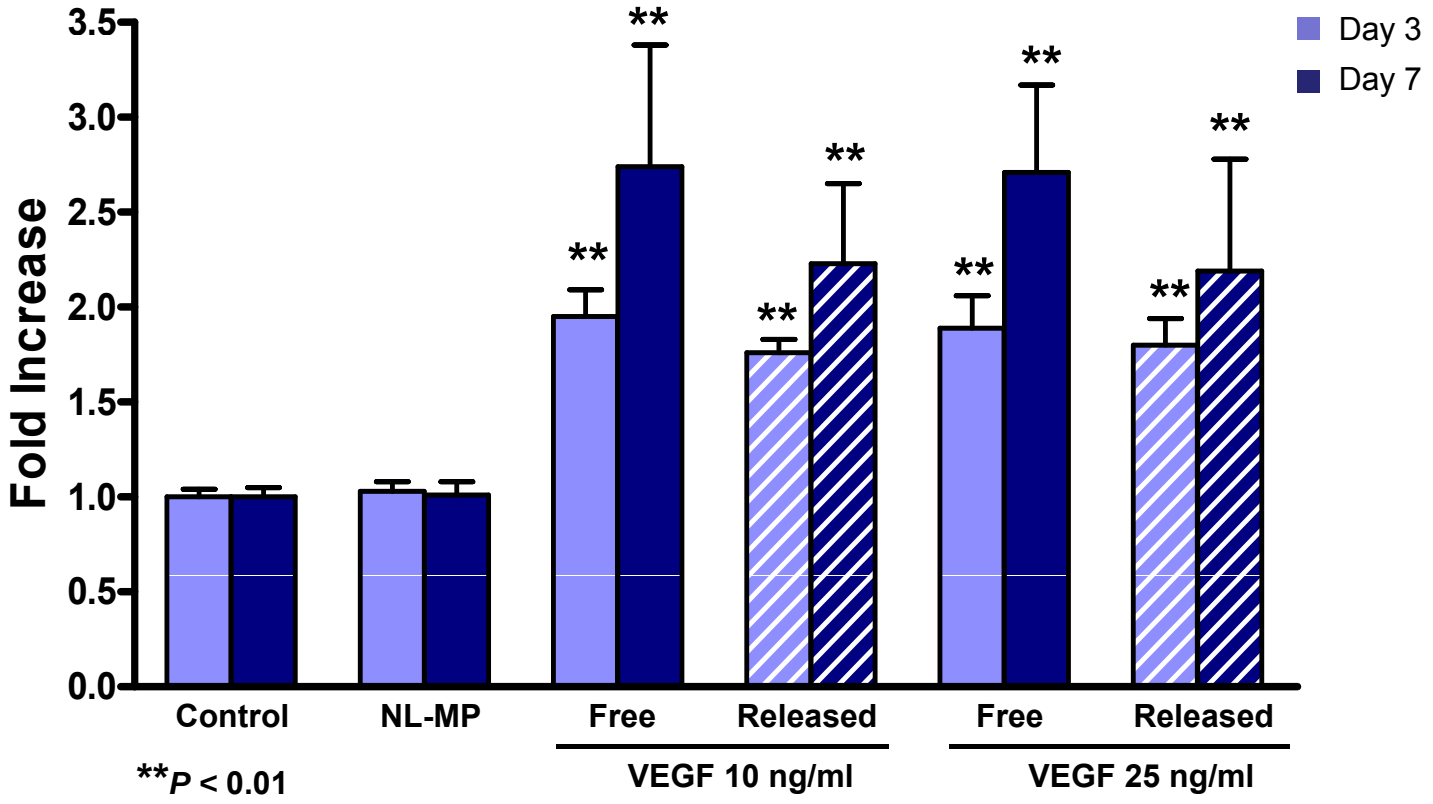
In Vitro VEGF Bioactivity

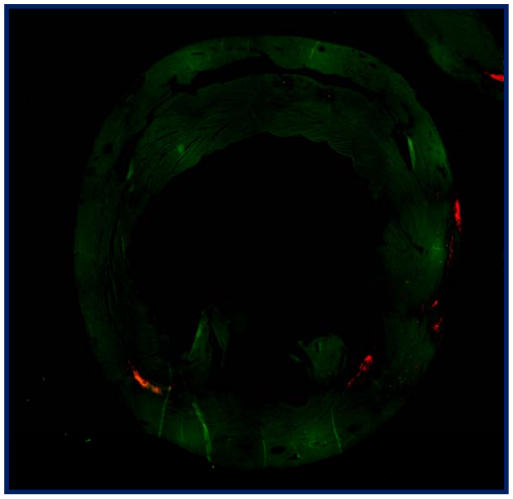
Human Iliac Artery Endothelial Cell line (HIAEC) proliferation assay



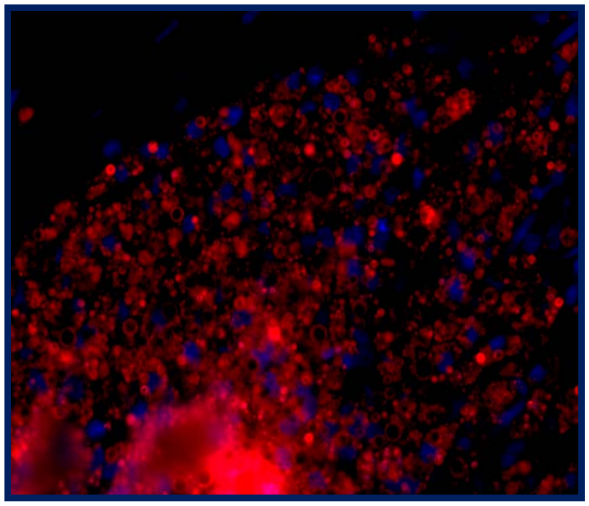
In Vivo Biocompatibility of PLGA-Particles

Persistence of 5 µm-sized particles after the intramyocardial injection:

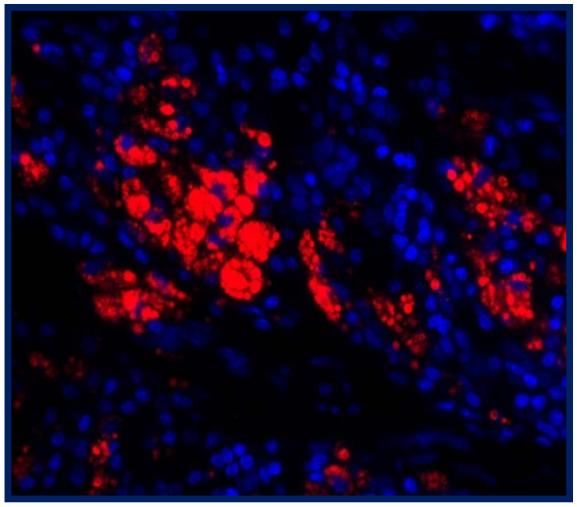
Histological study using Rhodamine-labeled-MP



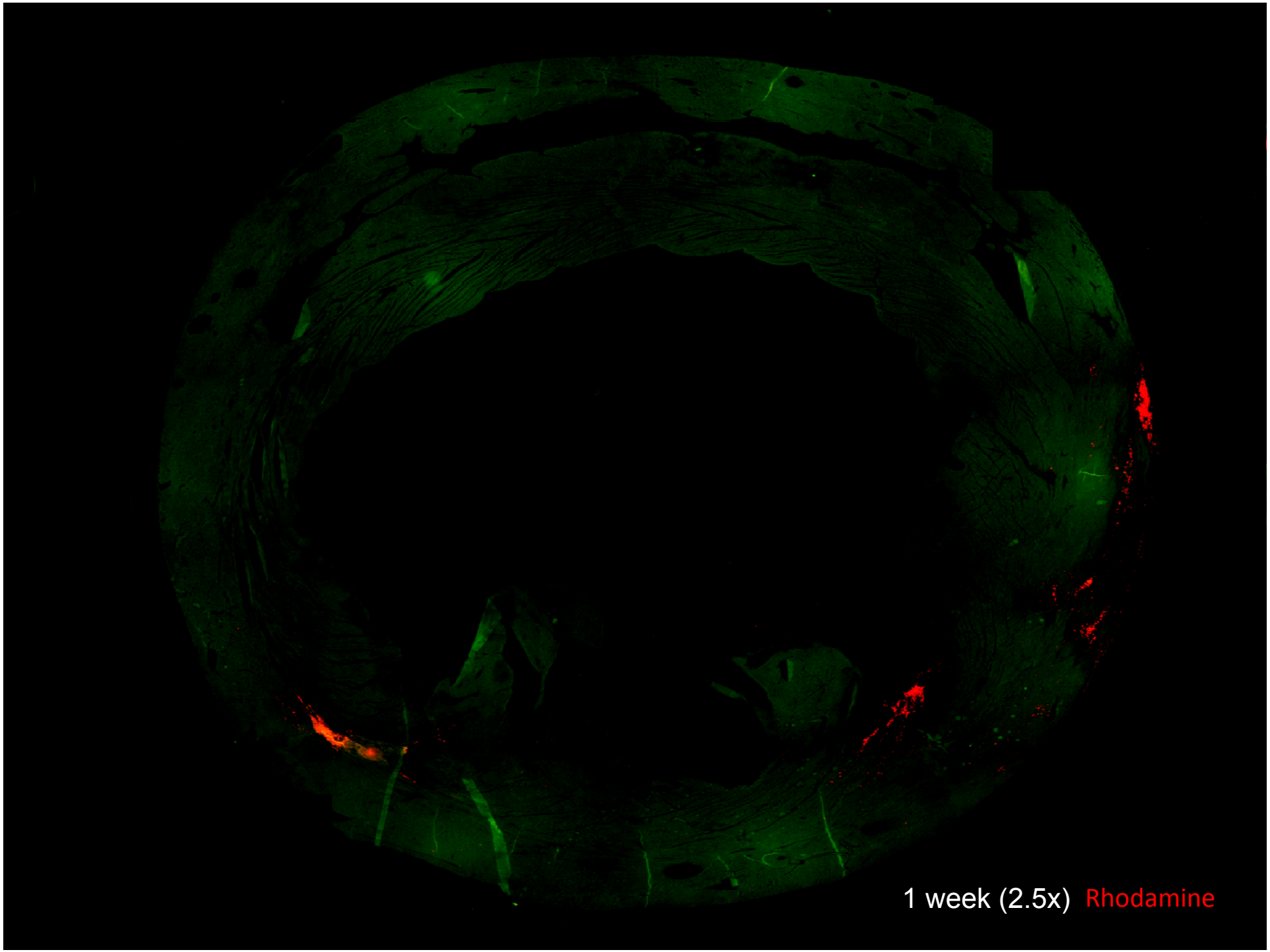
1 week (Rhodamine, 2.5x)

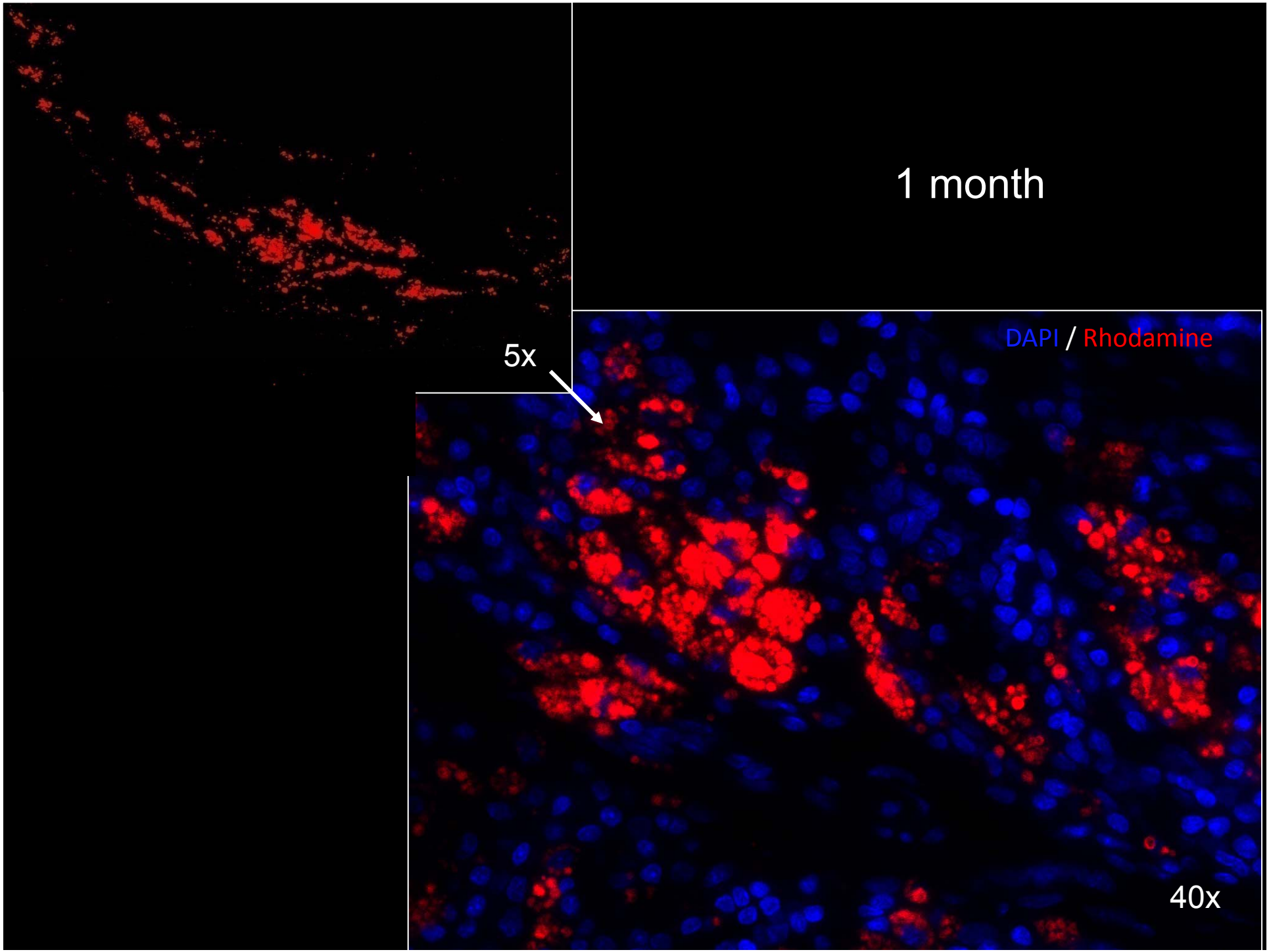


1 week (DAPI/Rhodamine, 40x)

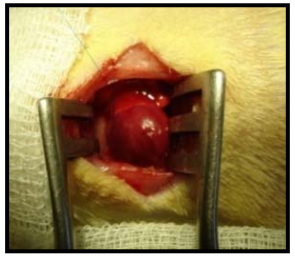


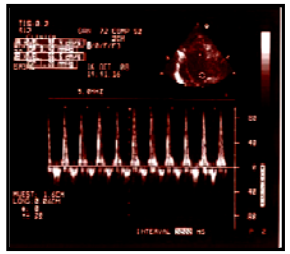
1 month (DAPI/Rhodamine, 40x)

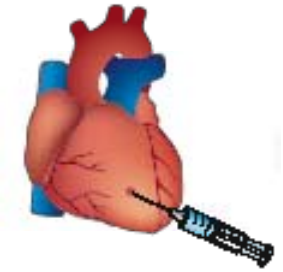

1 week (2.5x) Rhodamine


1 month
5x
DAPI / Rhodamine
40x

In Vivo Study: Experimental Design







- 4 day

Ischemia-Reperfusion

- 2 day

Baseline ECHO (LVEF<55%)

0 day

Intramyocardial administration

+30 day

SACRIFICE

Morphometric and histological analysis

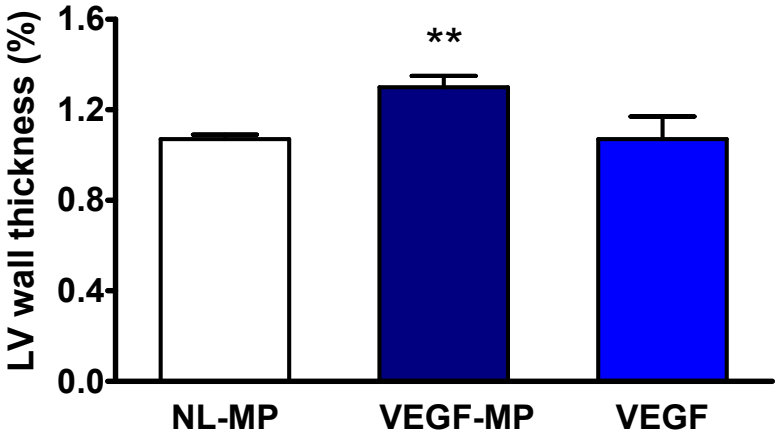
TREATMENTS
(n = 6 per group)

NL- MP

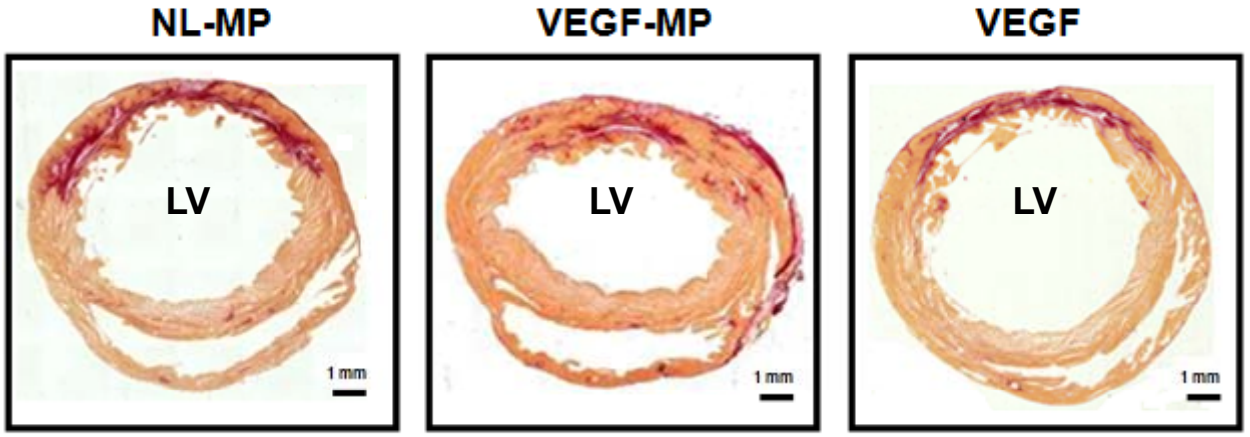
VEGF-MP

VEGF

In Vivo study: Morphometric Analysis

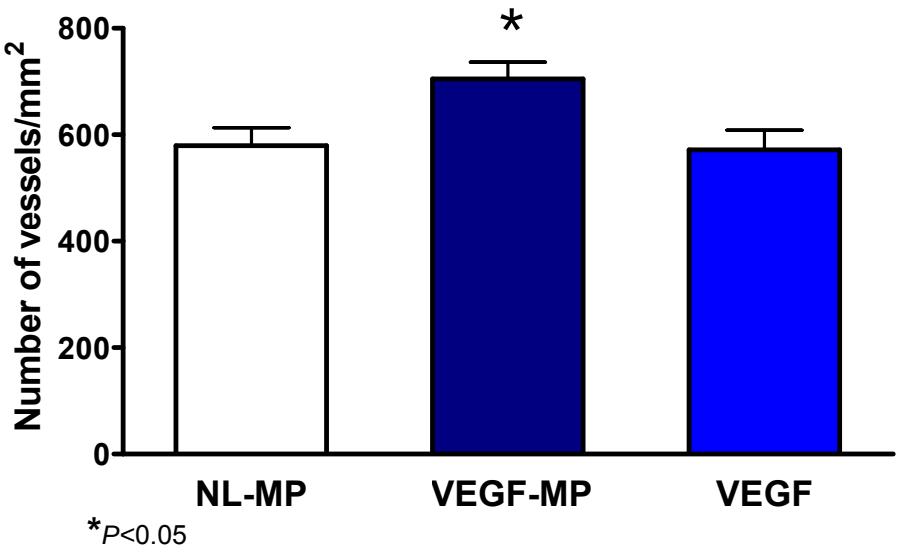


**$P<0.01$

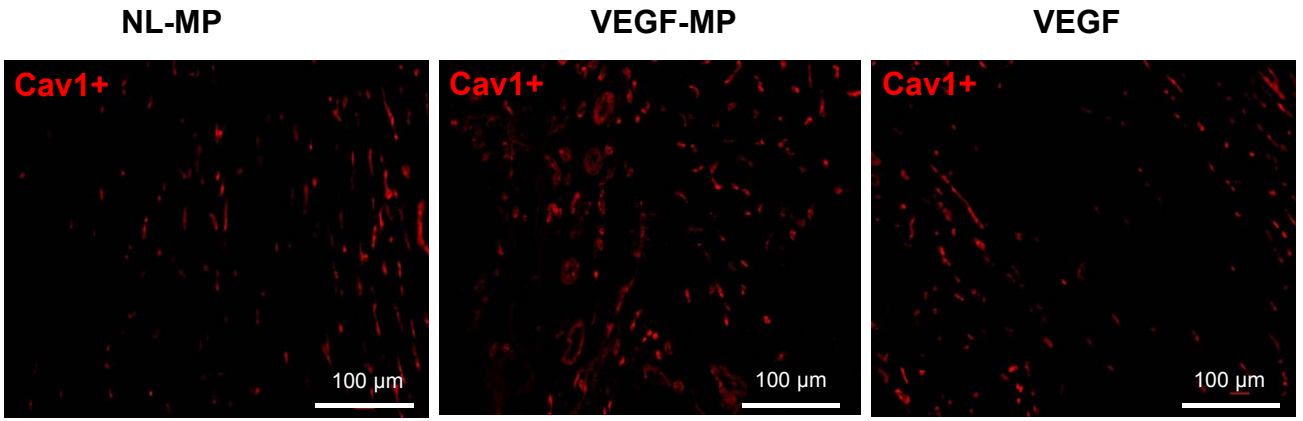


Sirius red staining (Left Ventricle, LV)

In Vivo Study: Capillary Density

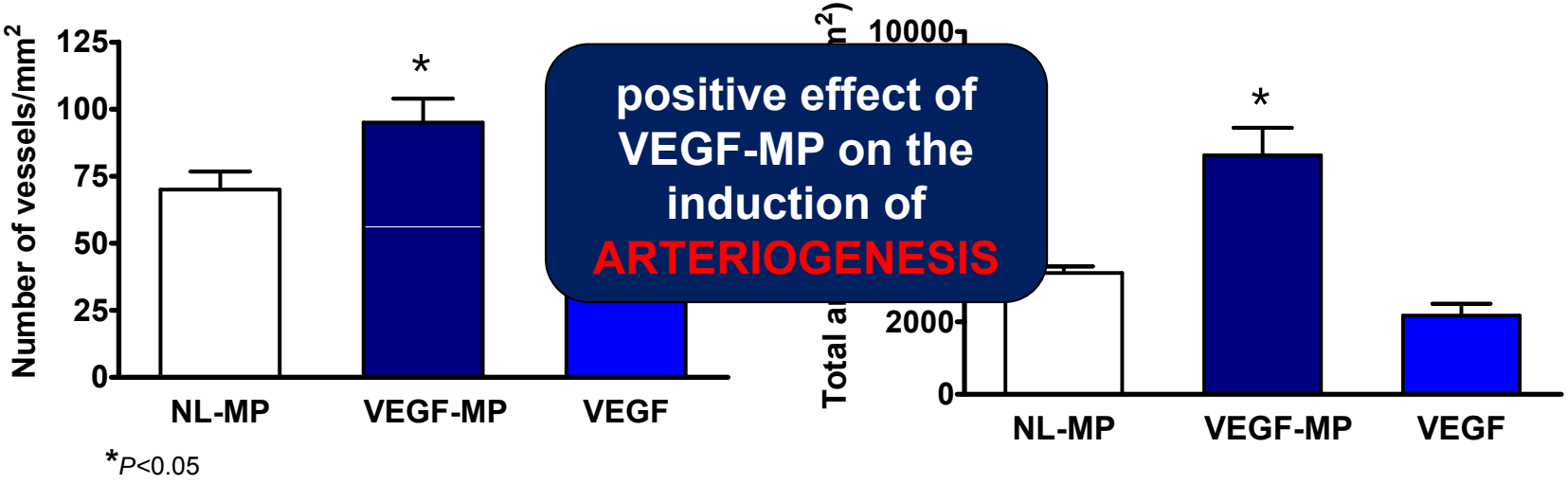


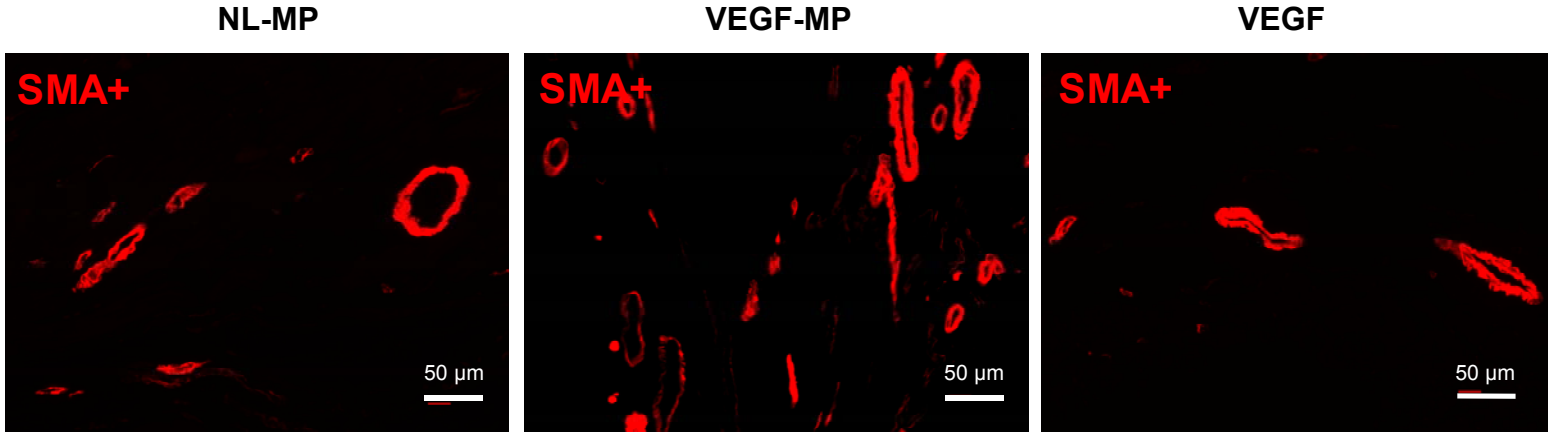
positive effect of VEGF-MP on the induction of ANGIOGENESIS



Caveolin-1: Endothelial cells marker (small capillary vessels)

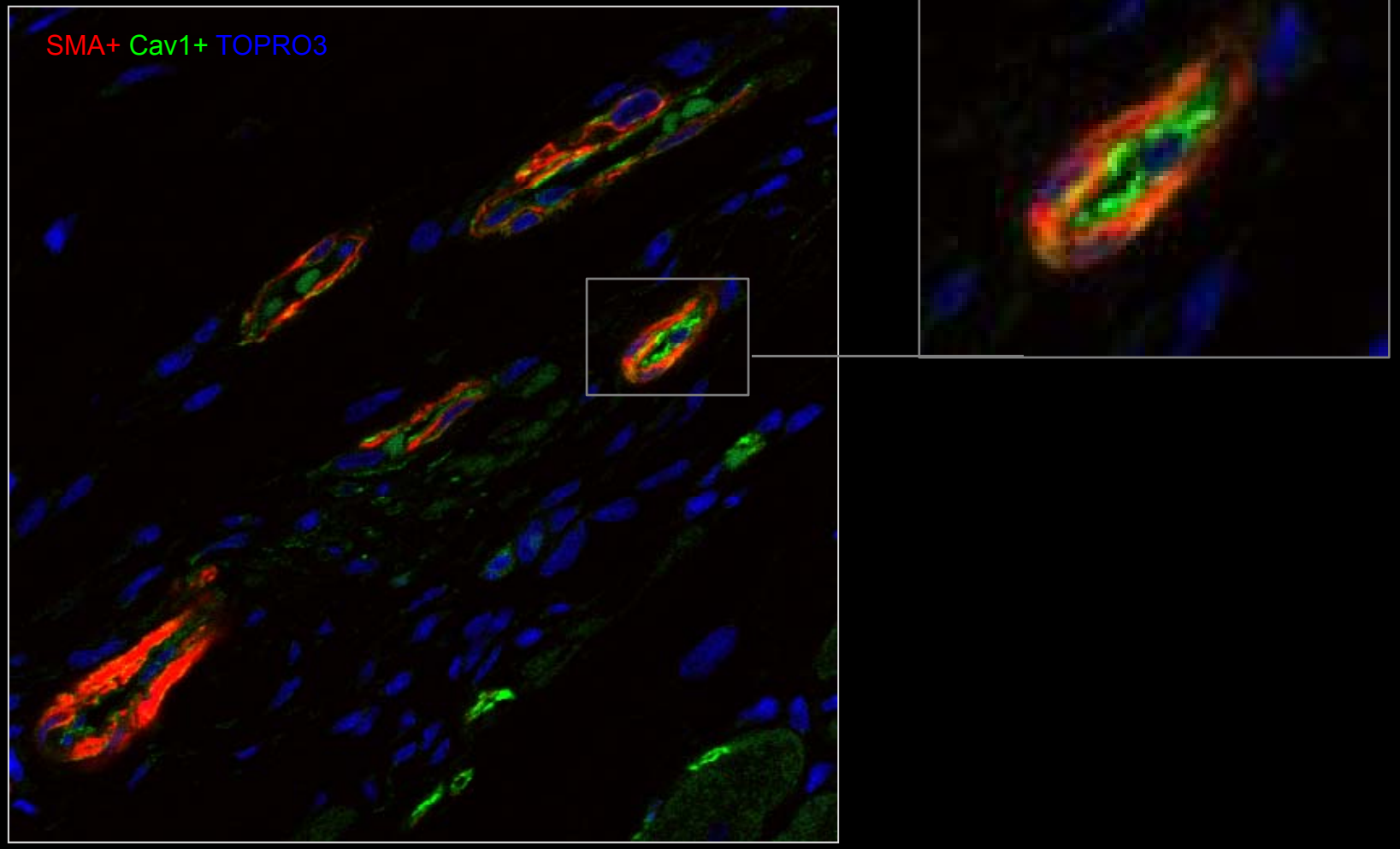
In Vivo Study: Arteriolar Density



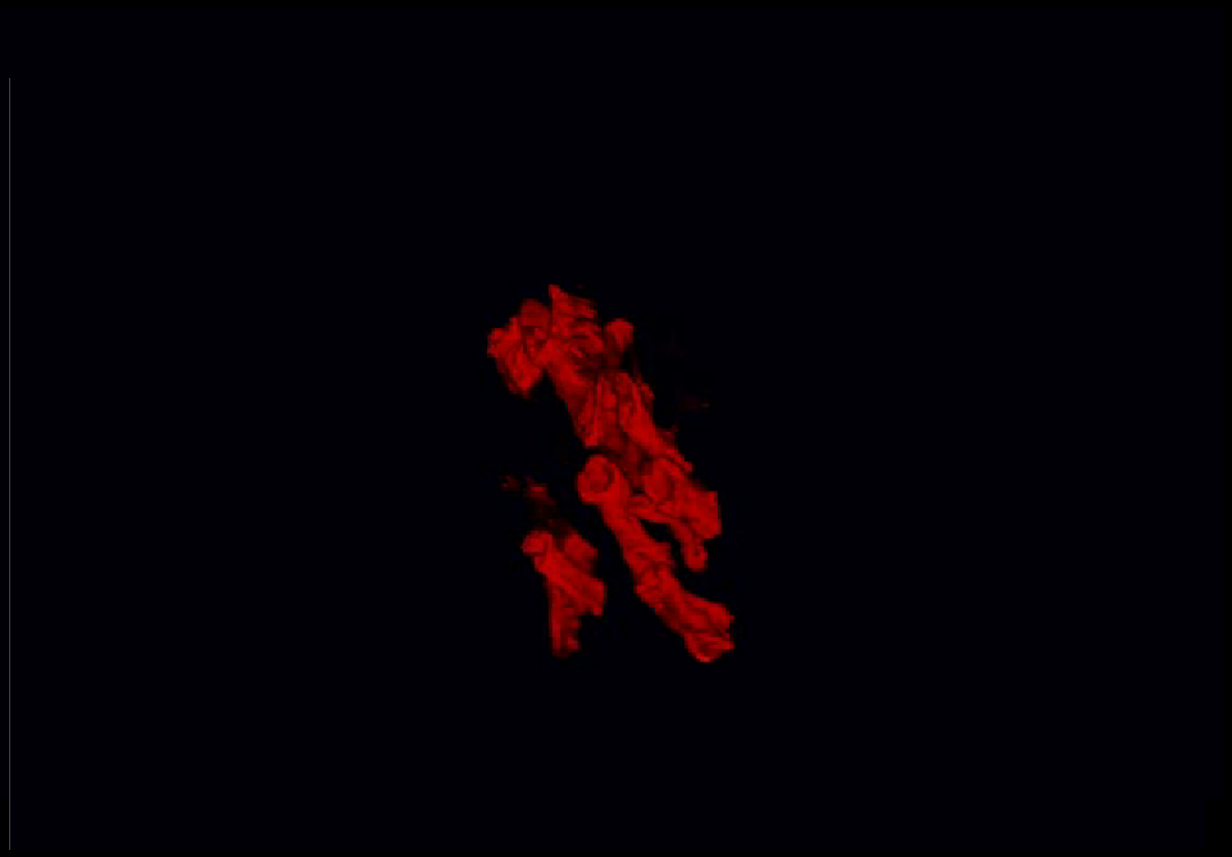


Smooth muscle alpha actin (SMA): arterioles/arteries marker

In Vivo Study: Vessel Structure

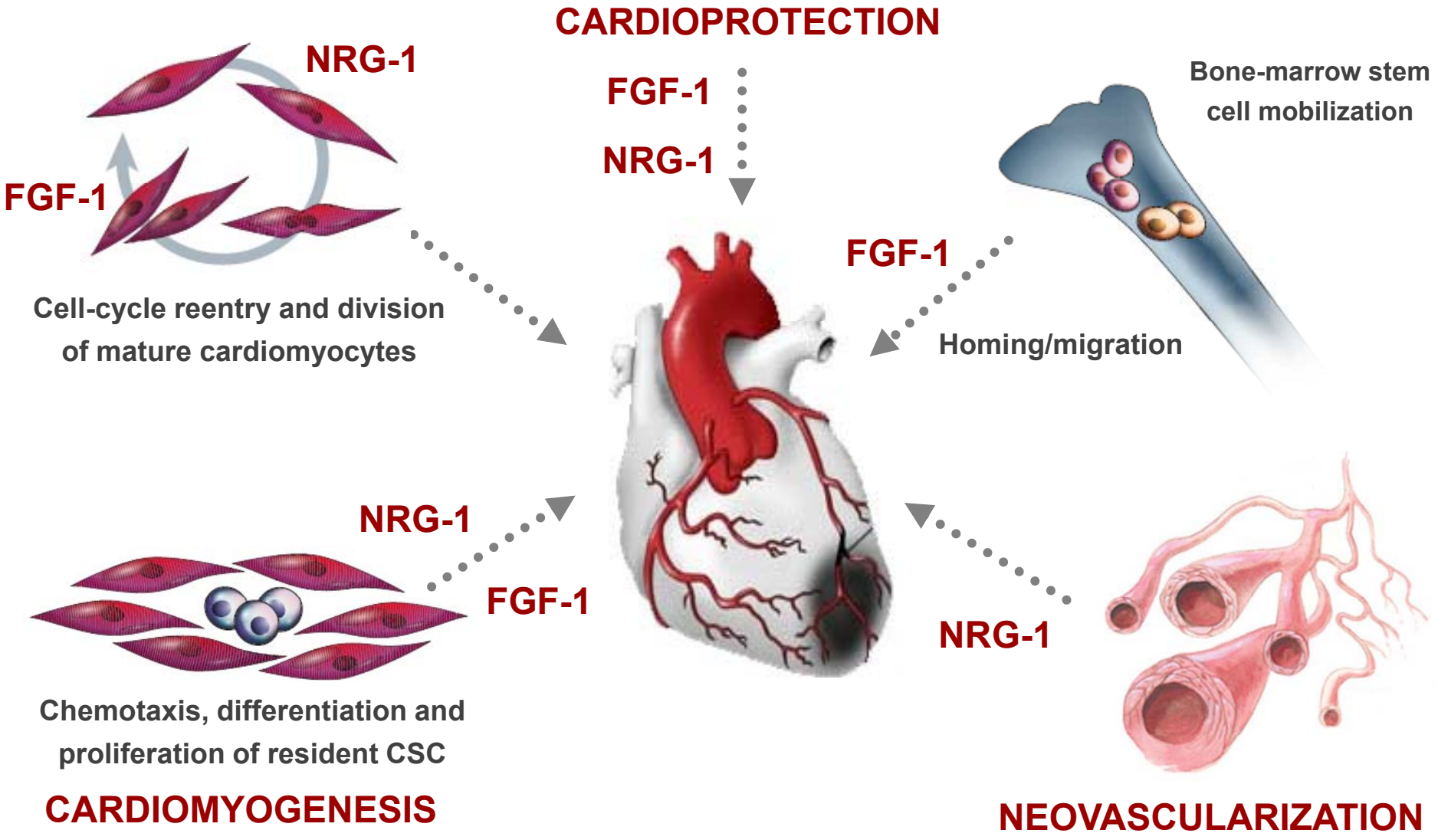


In vivo Study: Vessel Structure



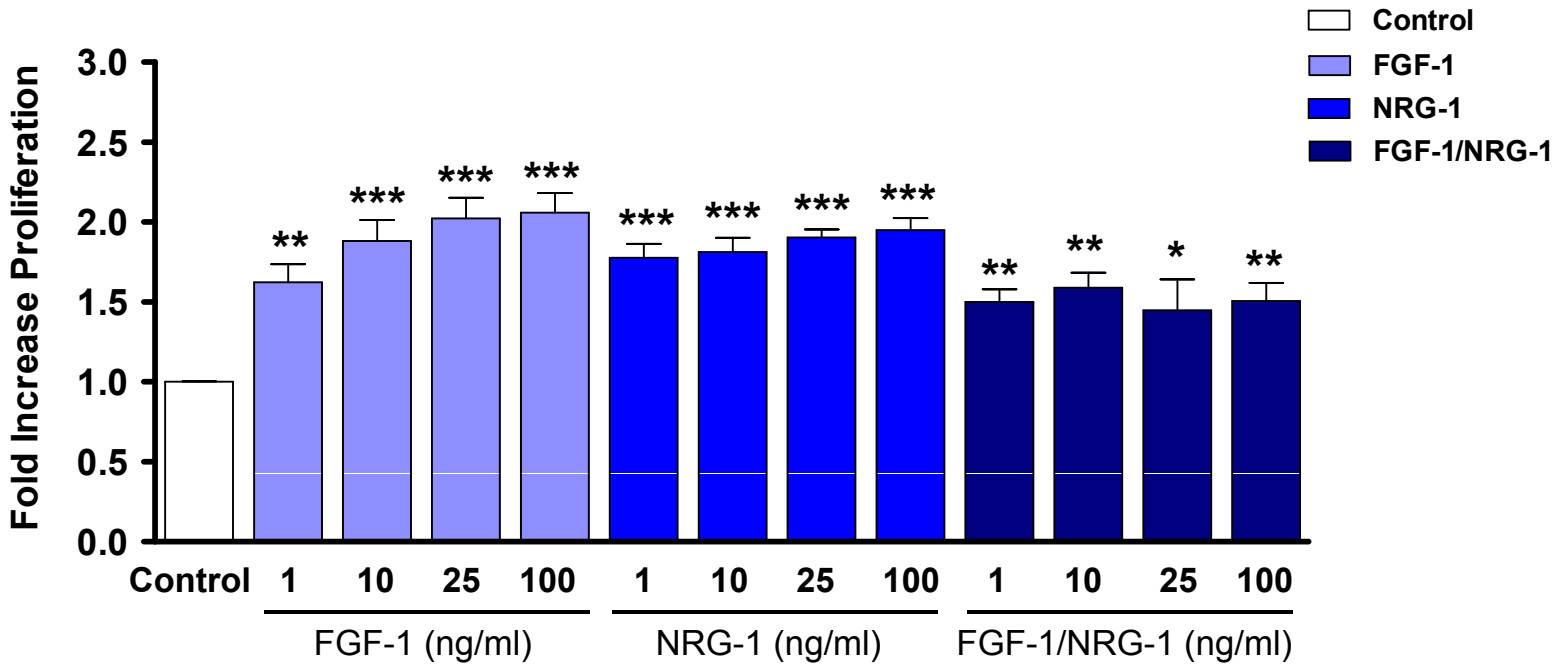
3D confocal images after immunofluorescence caveolin staining

Growth Factors



Cardiomyocyte Proliferation Assay

HL-1 cardiomyocytes Proliferation

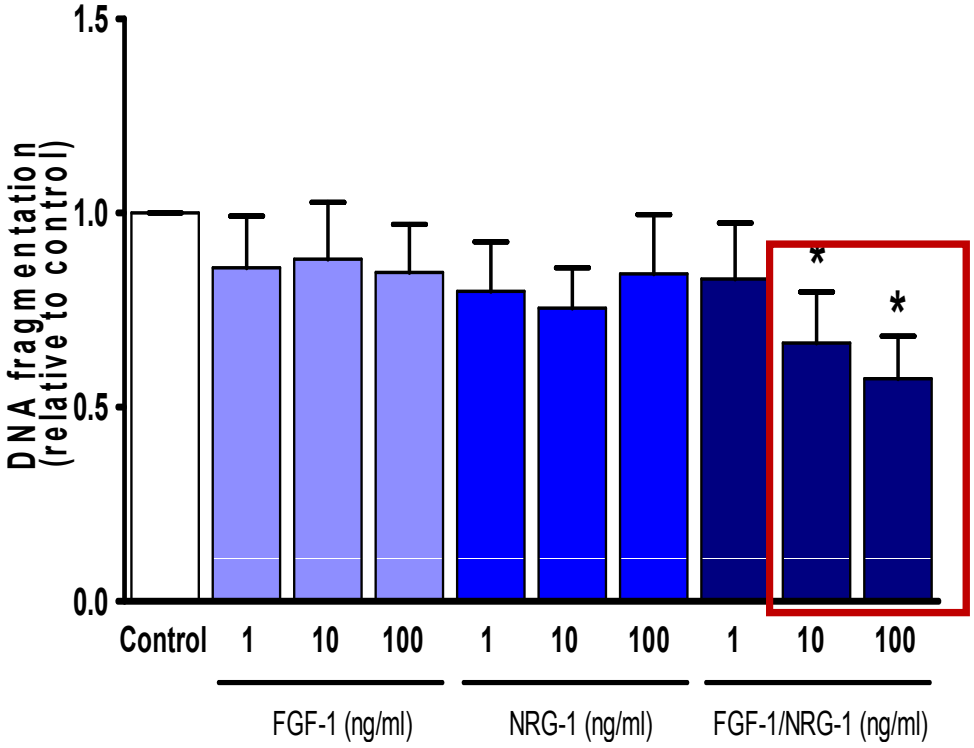


*P<0.05, **P<0.01, ***P<0.001 *vs*. control

FGF-1 and NRG-1 induce adult cardiomyocyte proliferation *in vitro*

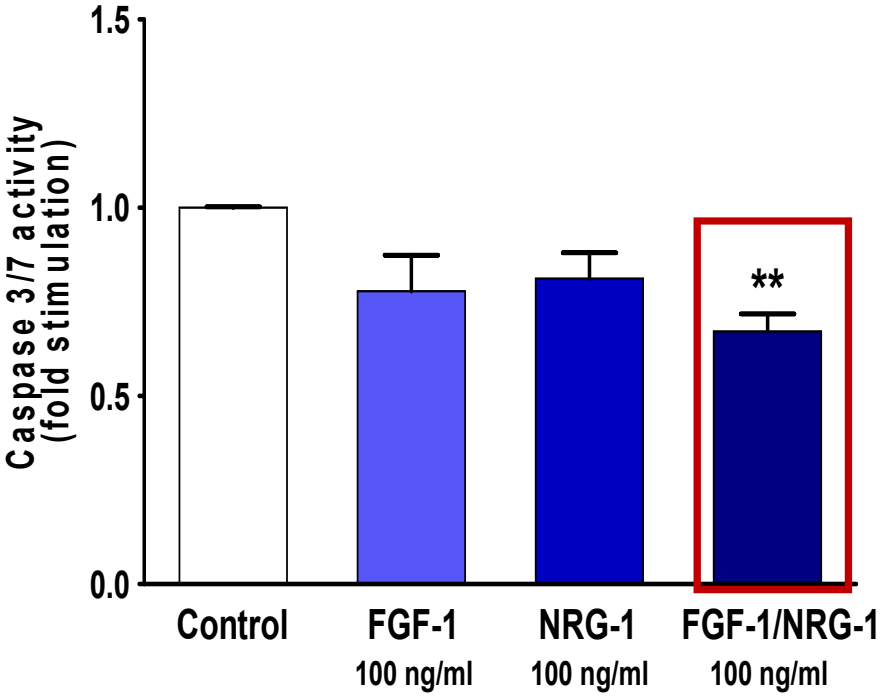
Cardioprotection Assays

Hypoxia-induced Apoptosis (ELISA detection of histone-associated DNA fragmentation)



*P<0.05 *vs*. control

Caspase 3/7 activity

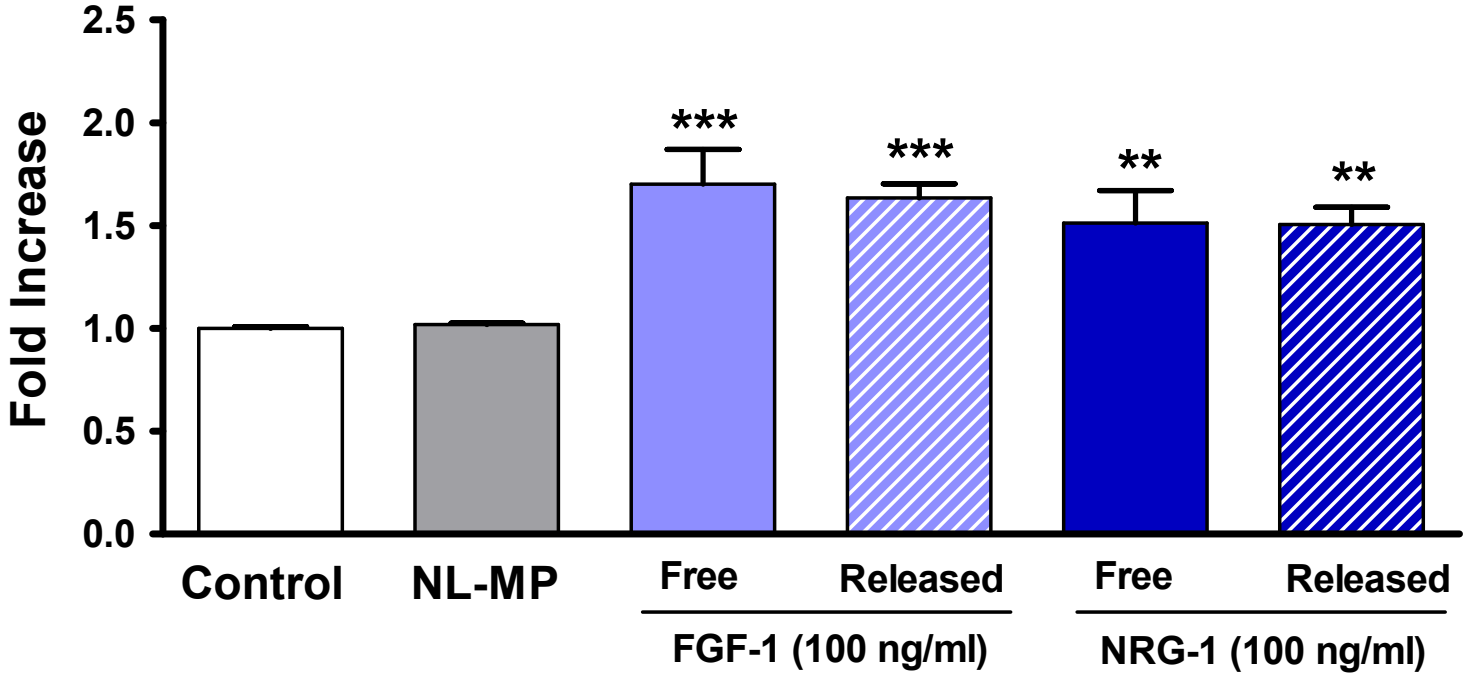


**P<0.01 *vs*. control

FGF-1/NRG-1 combined treatment decreases cardiomyocyte apoptosis *in vitro*

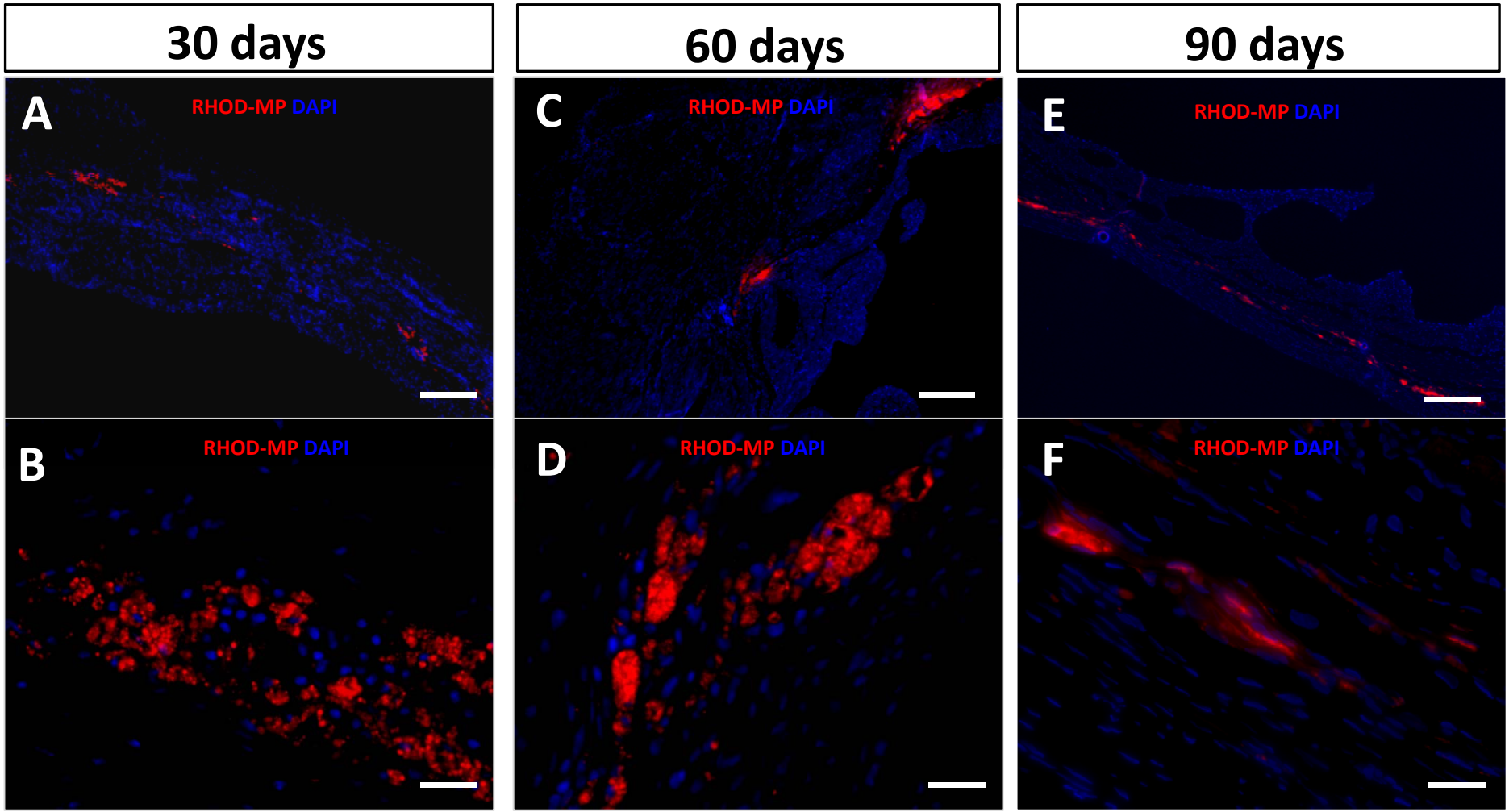
Bioactivity of FGF-1/NRG-1 Released from MP

HL-1 cardiomyocytes Proliferation

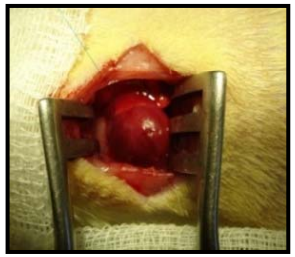


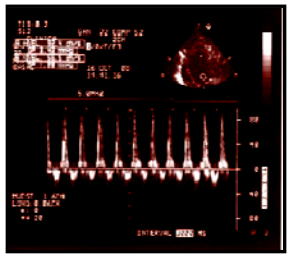
**P<0.01, ***P<0.001 *vs*. control

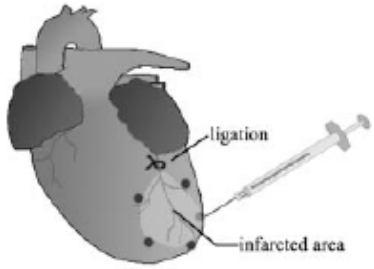
In vivo characterization of MP: engraftment

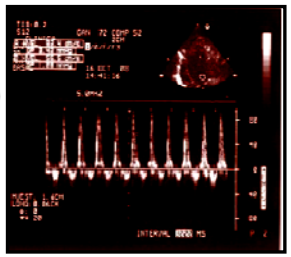


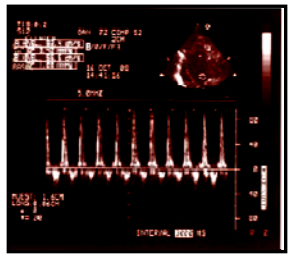
Design of *in vivo* studies









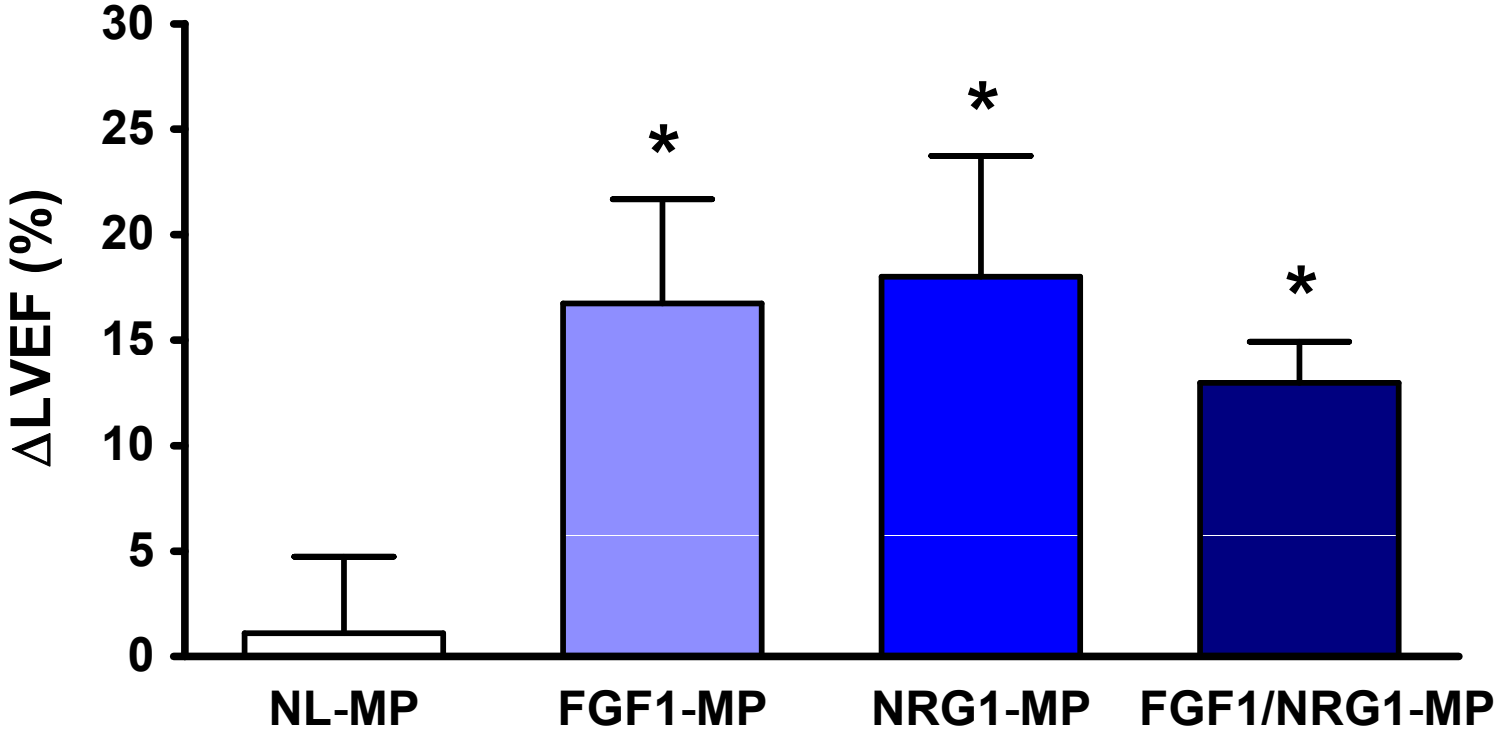


- 4 day	- 2 day	0 day	+30 day	+90 day 3 months ECHO SACRIFICE
Permanent ligation MI	Baseline ECHO (LVEF<50%)	Intramyocardial administration	1 month ECHO	

TREATMENTS
(n = 10 per group)

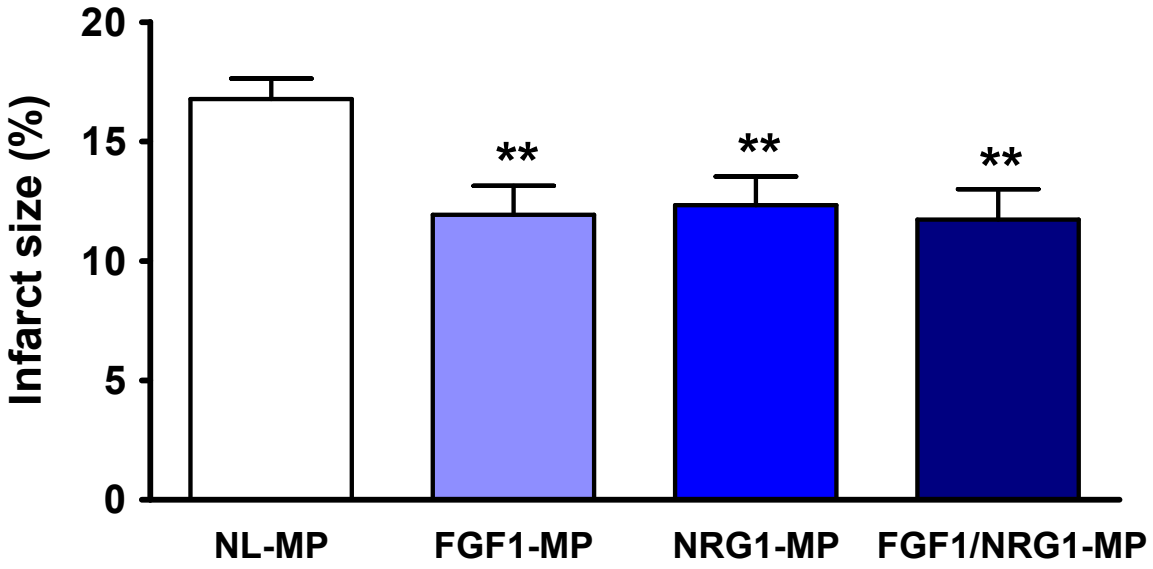
- Empty MP
- FGF-1 MP 1740 ng
- NRG-1 MP 1300 ng
- FGF1+NRG1 MP

Functional Results: Cardiac Function

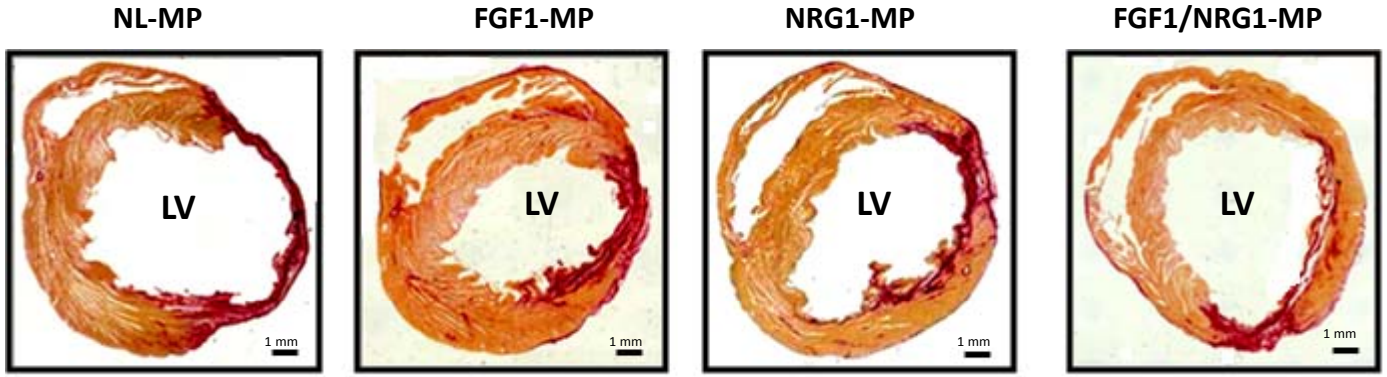


**P*<0.05 *vs*. NL-MP (control)

Functional Results: Infarct Size

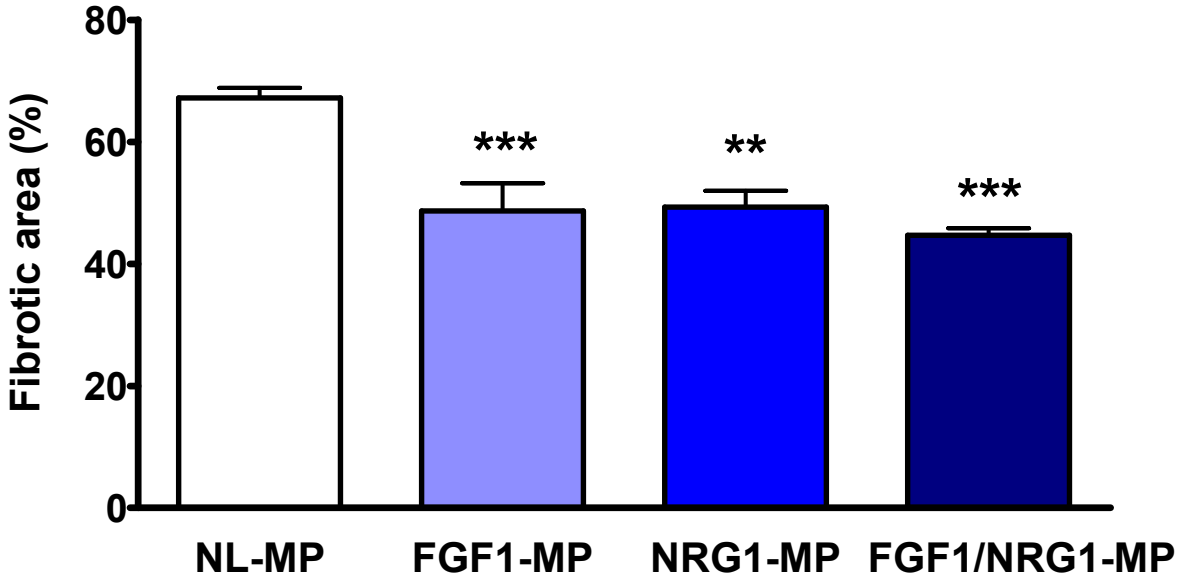


***P*<0.01 *vs*. NL-MP (control)

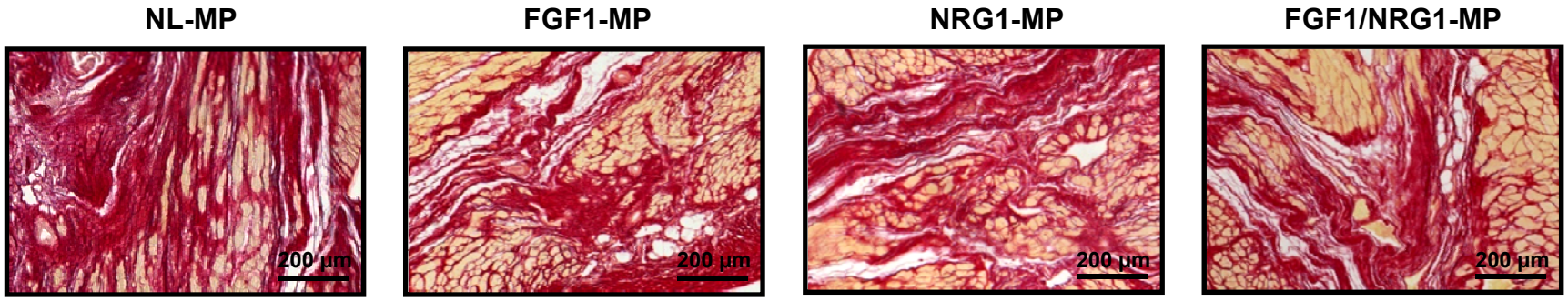


Sirius red staining (Left Ventricle, LV)

Functional Results: Fibrosis

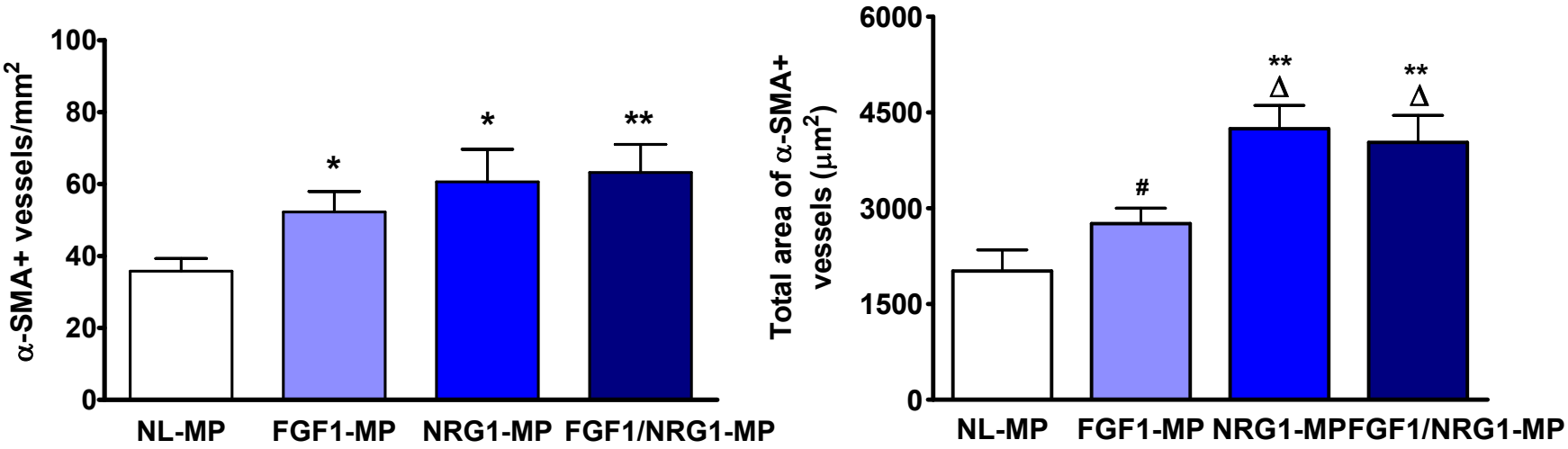


**P<0.01 and ***P<0.001 *vs.* NL-MP (control)

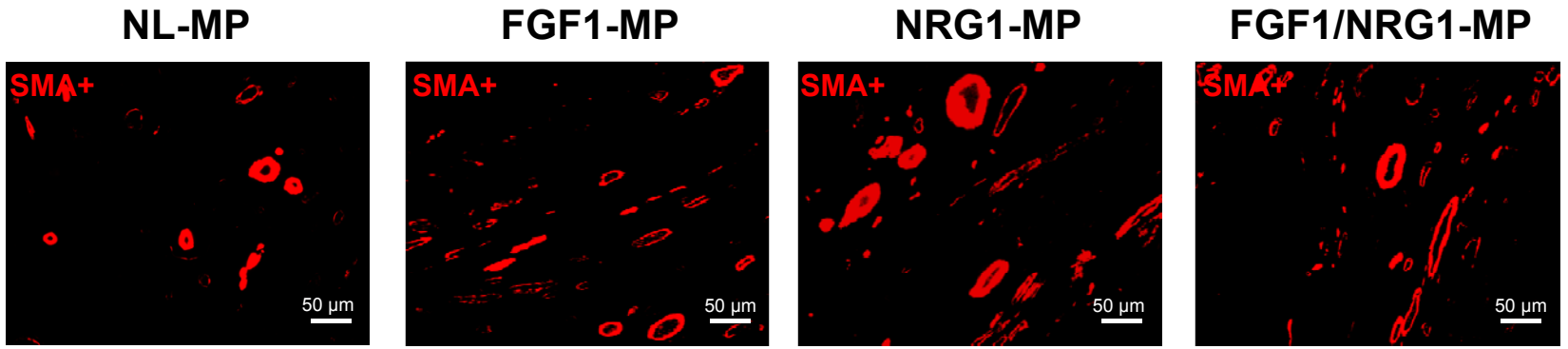


Sirius red staining

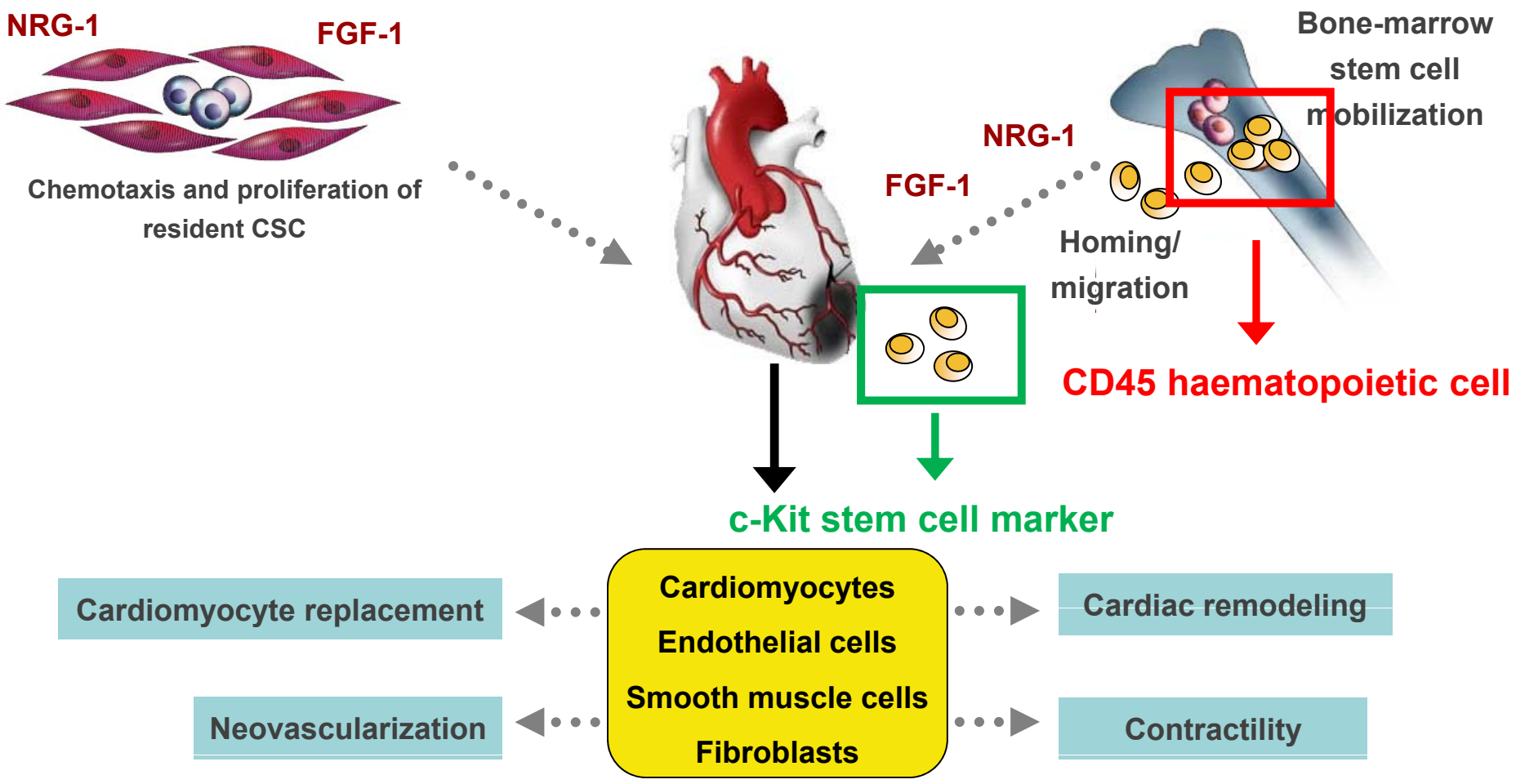
Vascular Density and Area



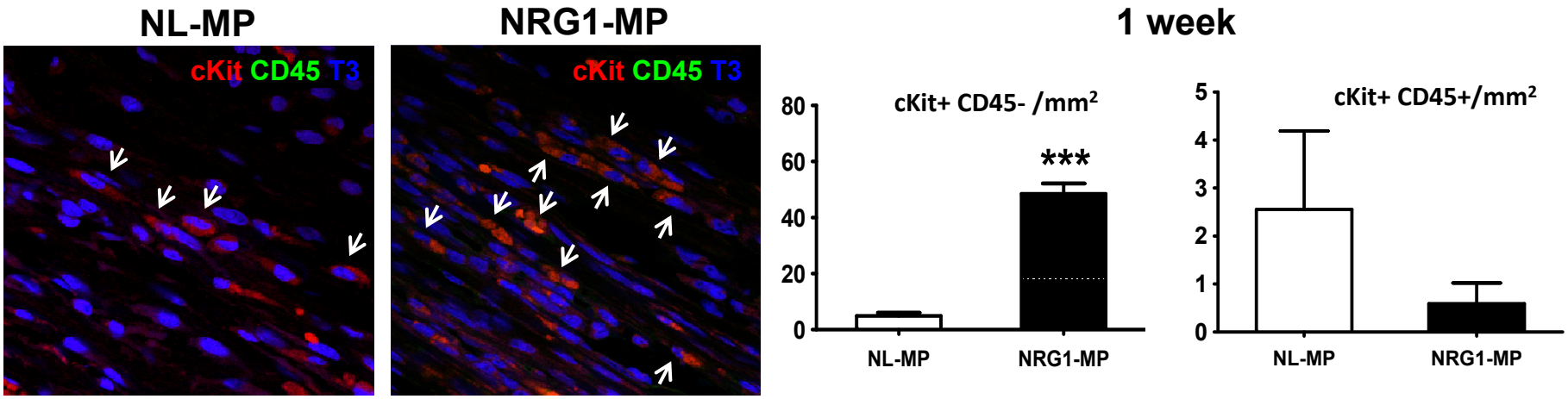
*P<0.05, **P<0.01 and #P=0.08 *vs.* NL-MP (control), ΔP<0.05 *vs*. FGF1-MP group

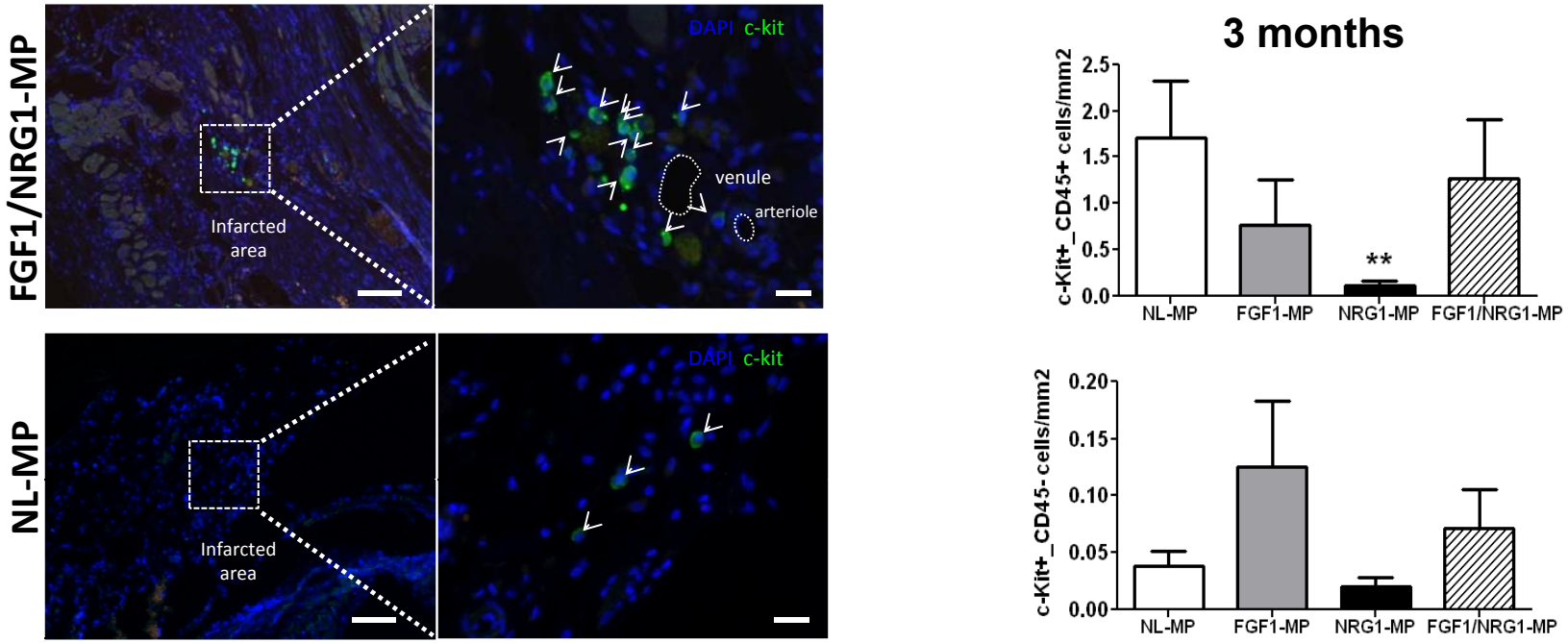


Recruitment c-kit^{+} progenitor cells

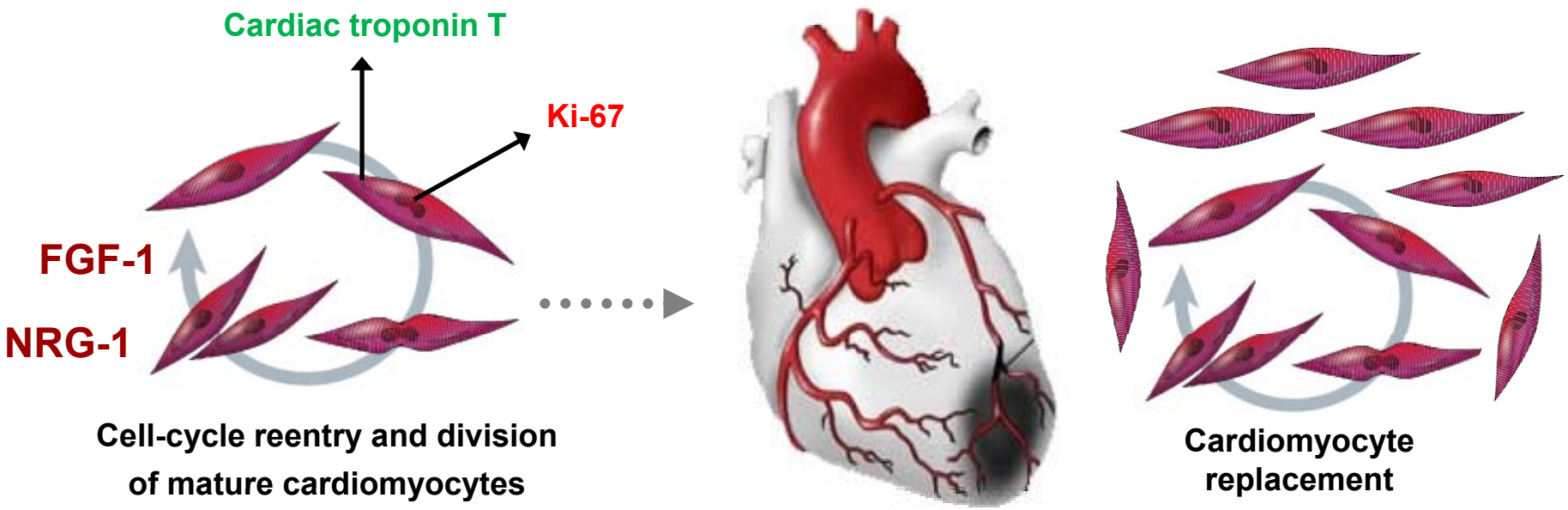


Recruitment c-kit^{+} progenitor cells

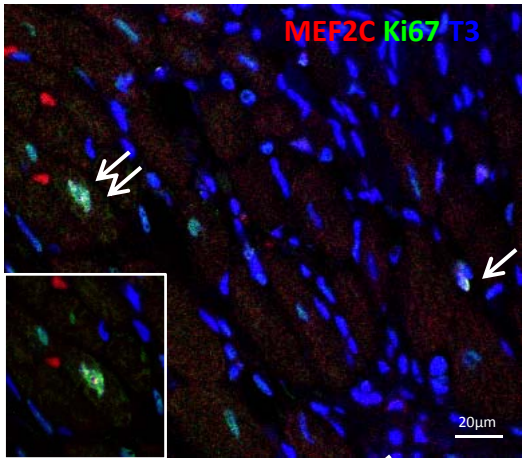


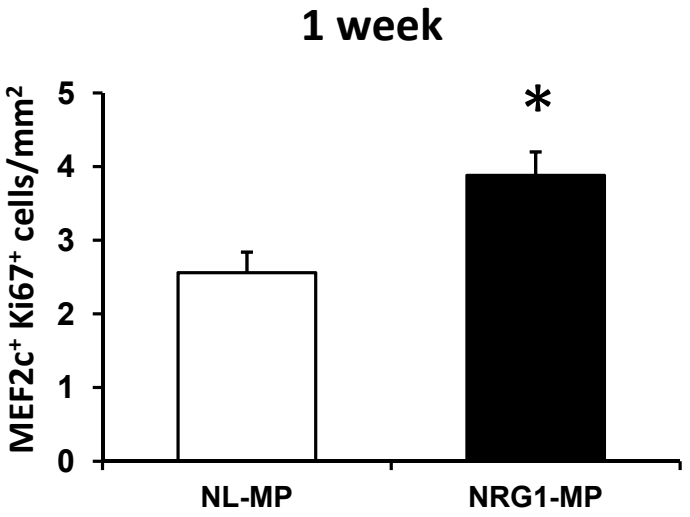


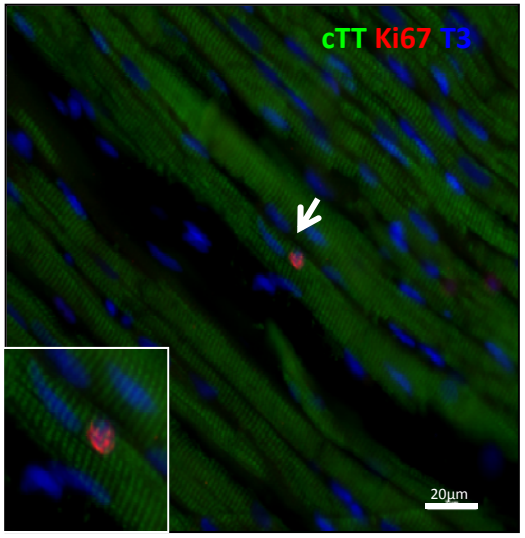
Cardiomyocyte Proliferation

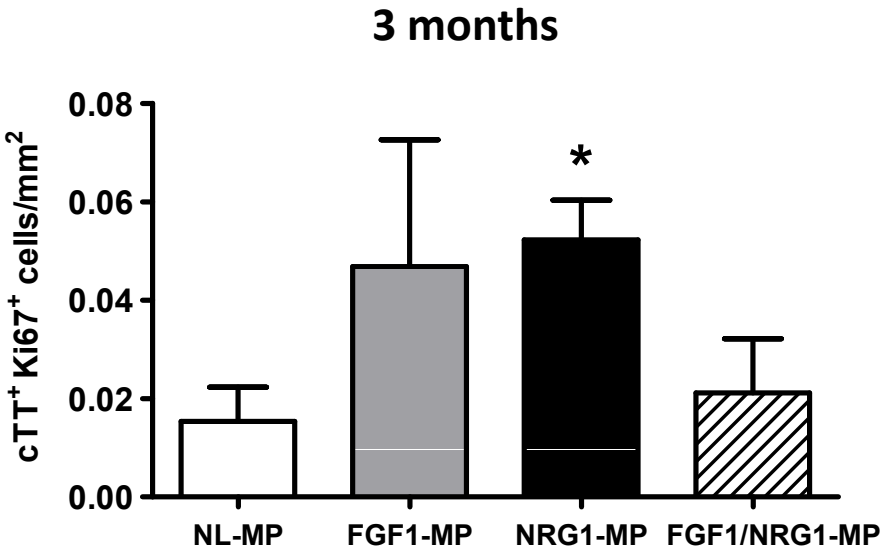


Cardiomyocyte Proliferation

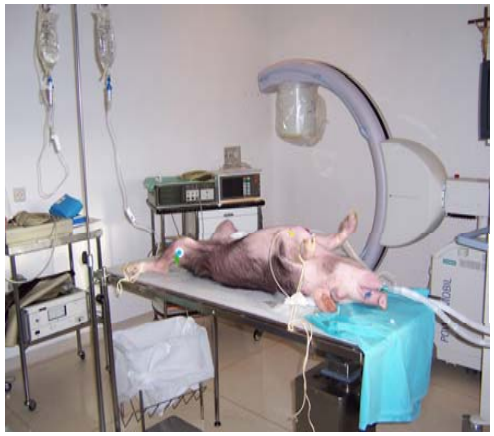








Preclinical Model



- 7 day

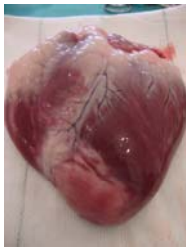
Permanent Oclussion

0 day

NOGA Guided intramyocardial

+ 120 day

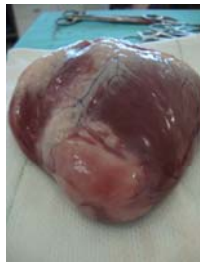
ECHO, NOGA, Sacrifice



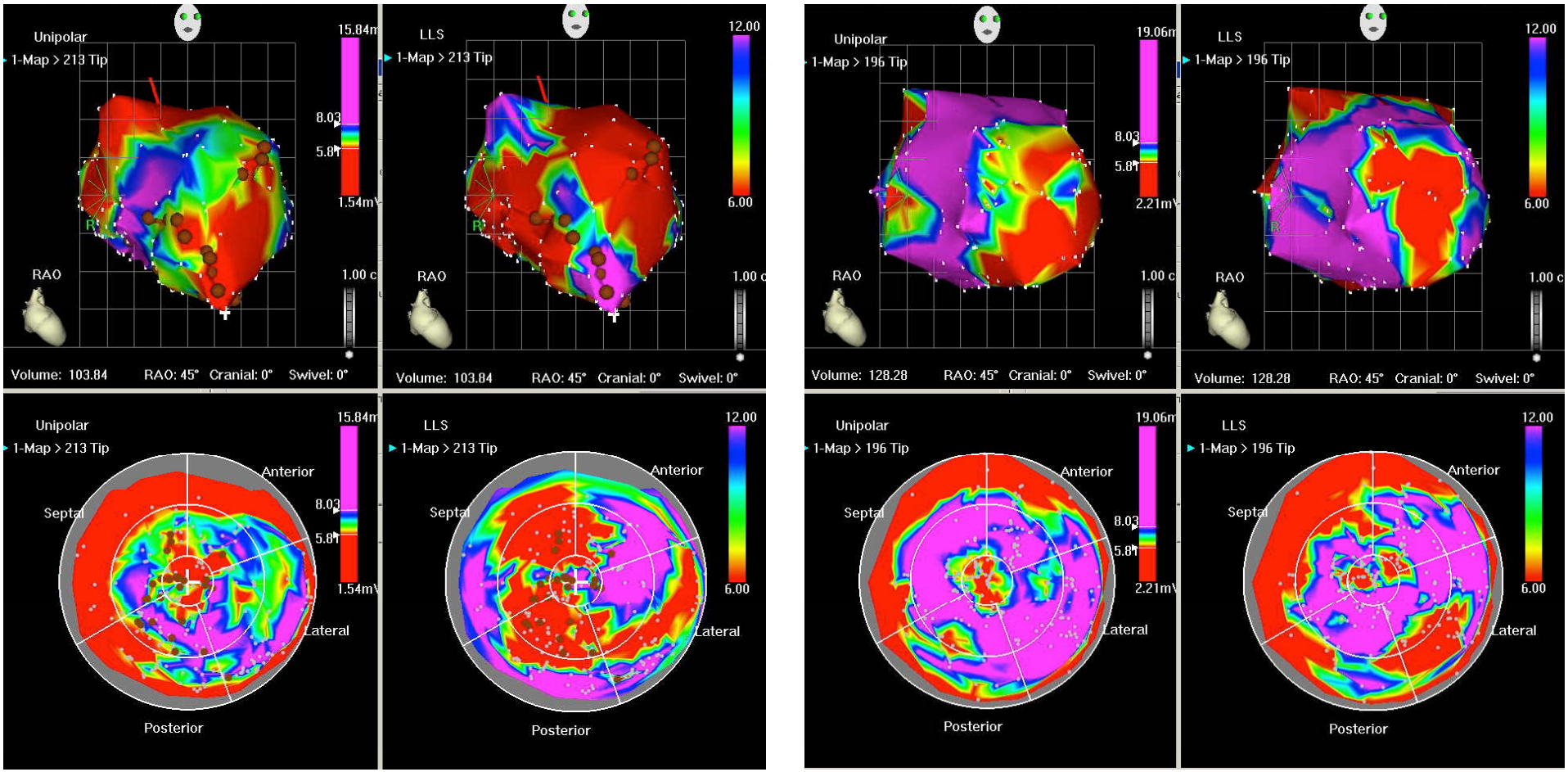
TREATMENTS

(n = 8 per group)

- **Contr**
- **FGF-1 MP**
- **NRG-1 MP**

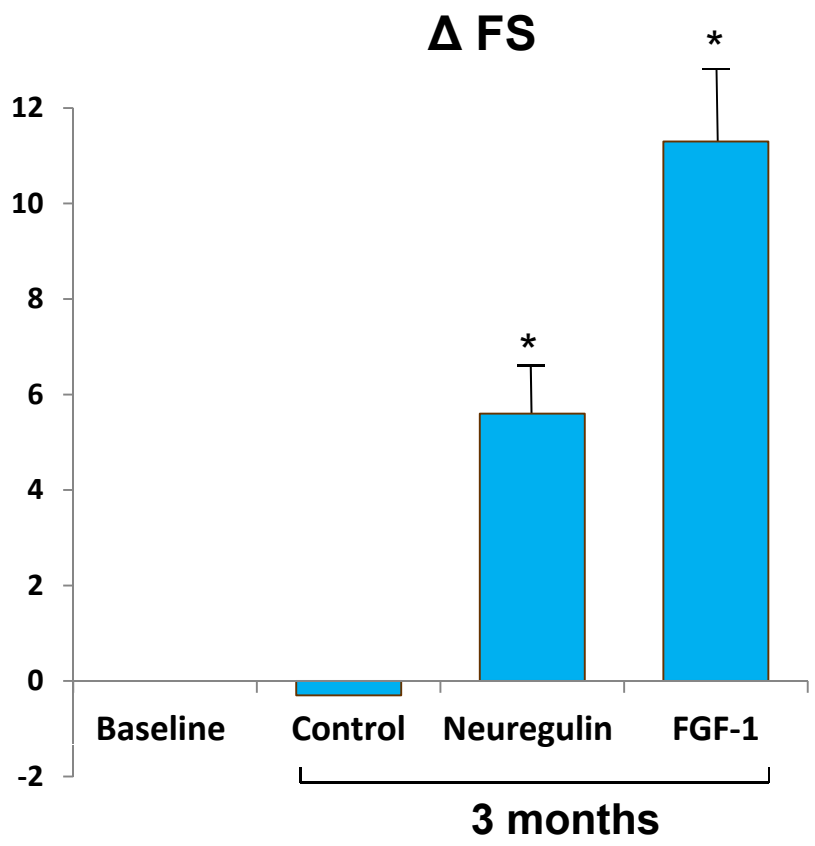


Preclinical Model

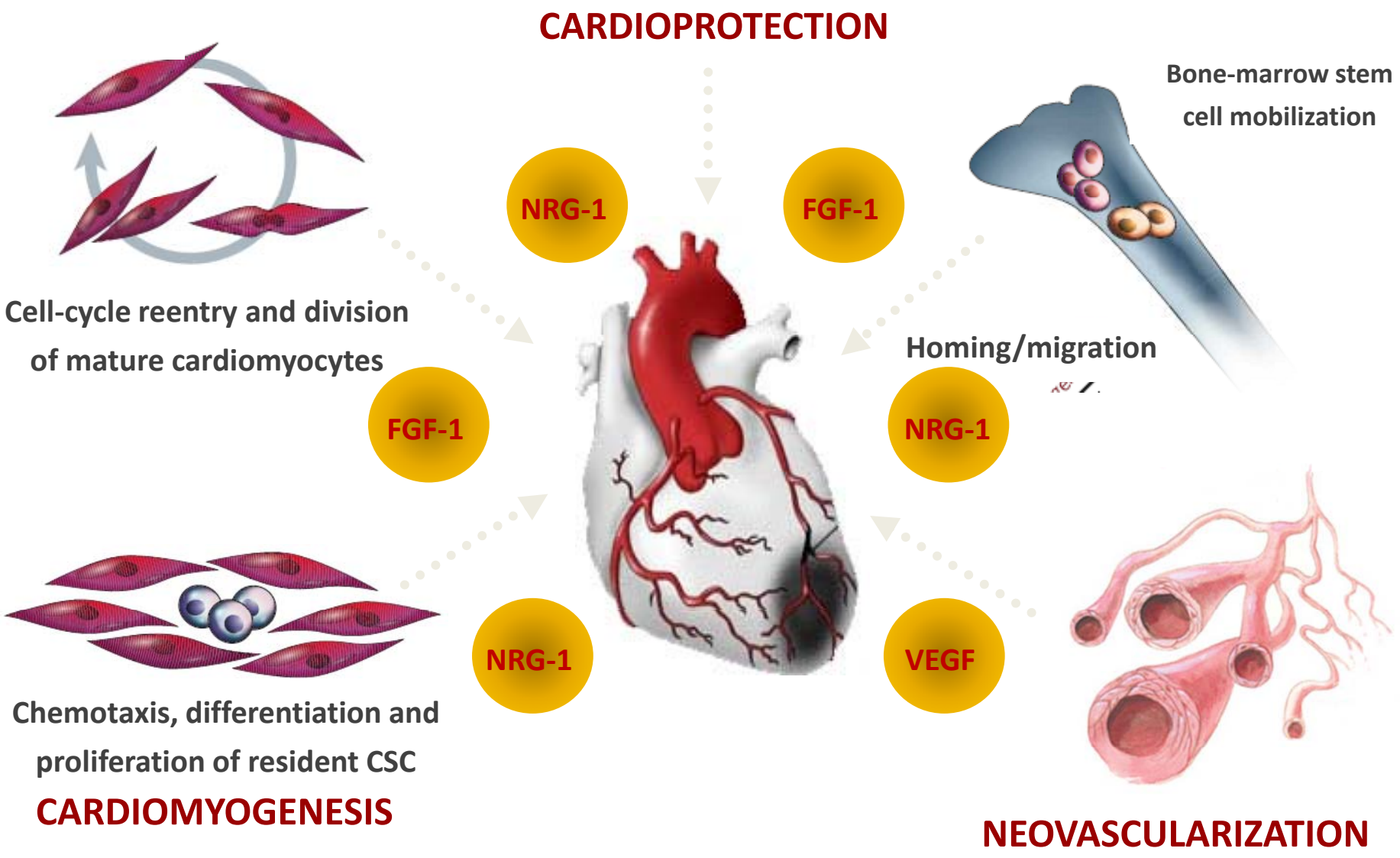


Injection **Follow up**

Preclinical Model



Summary


CARDIOPROTECTION
Bone-marrow stem cell mobilization
NRG-1
FGF-1
Cell-cycle reentry and division of mature cardiomyocytes
Homing/migration
FGF-1
NRG-1
NRG-1
VEGF
Chemotaxis, differentiation and proliferation of resident CSC
CARDIOMYOGENESIS
NEOVASCULARIZATION

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canfundación
lo que tú has decidido

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