



KSC 2015
10/17/2015 @Goyang

Mechanism of Angiogenesis in the Damaged Heart

The University of Tokyo Graduate School of Medicine
CREST

Issei Komuro, MD, PhD,
FAHA, FESC, FISHR, FJCC

Disclosure of COI

Issei Komuro

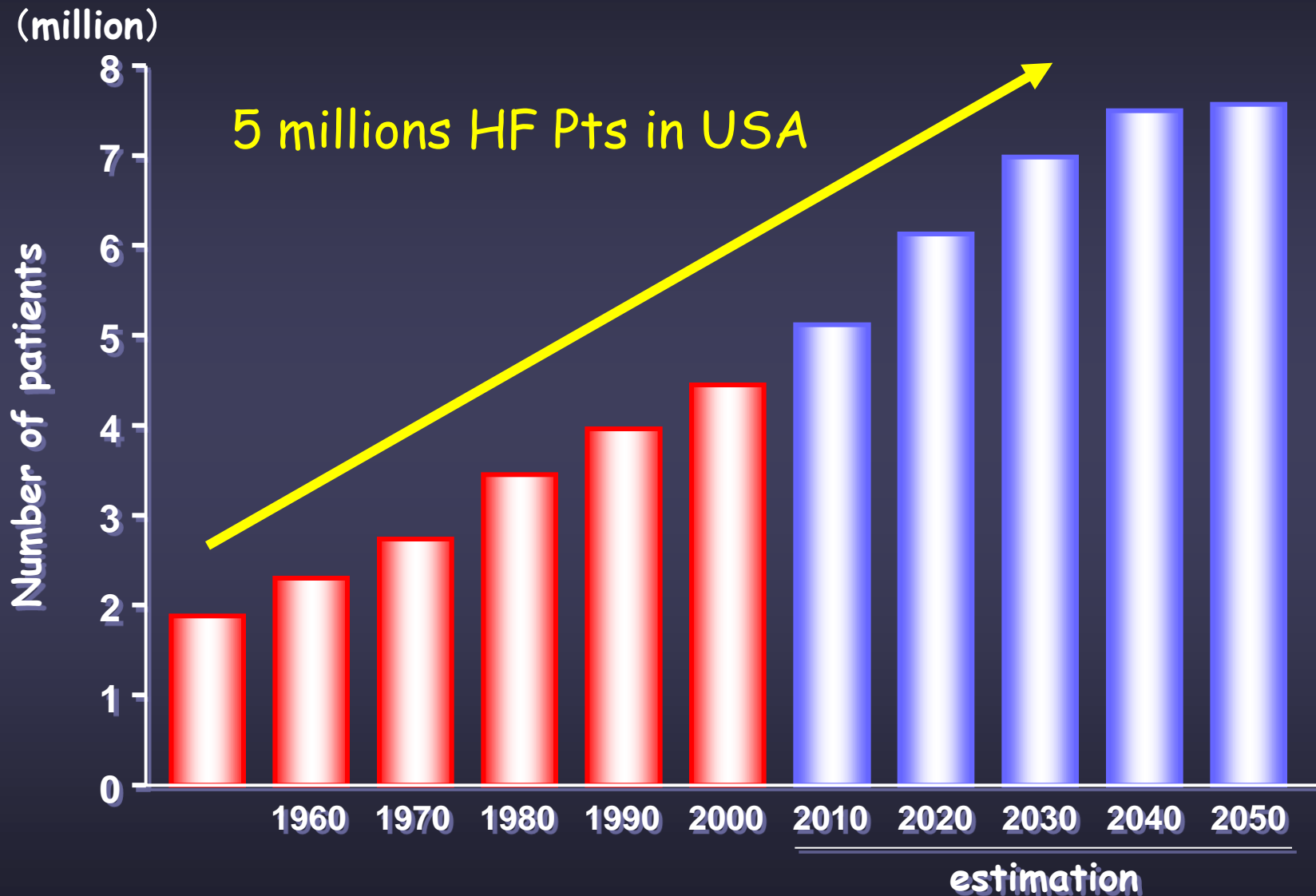
Speaker's fees

Takeda Pharmaceutical Company Limited. • Nippon
BoehringerIngelheim Co., Ltd. • AstellasPharma Inc. • DAIICHI
SANKYO COMPANY, LIMITED

Grants

Takeda Pharmaceutical Company Limited. • Nippon
BoehringerIngelheim Co., Ltd. • MSD K.K. • AstellasPharma Inc.
• Genzyme Japan K.K. • Dainippon Sumitomo Pharma Co., Ltd
• Mitsubishi Tanabe Pharma Corporation • Bristol-Myers
Squibb Company • DAIICHI SANKYO COMPANY, LIMITED

Number of Heart Failure Patients in USA



National Health and Nutrition Examination Survey III 1980 and, US Bureau of the Census data and projections In Hayflick L:Ballantine Books, 1994. より改変

Mechanisms of Heart Failure

Stem cells

Ischemia
Nature 2007

Ep

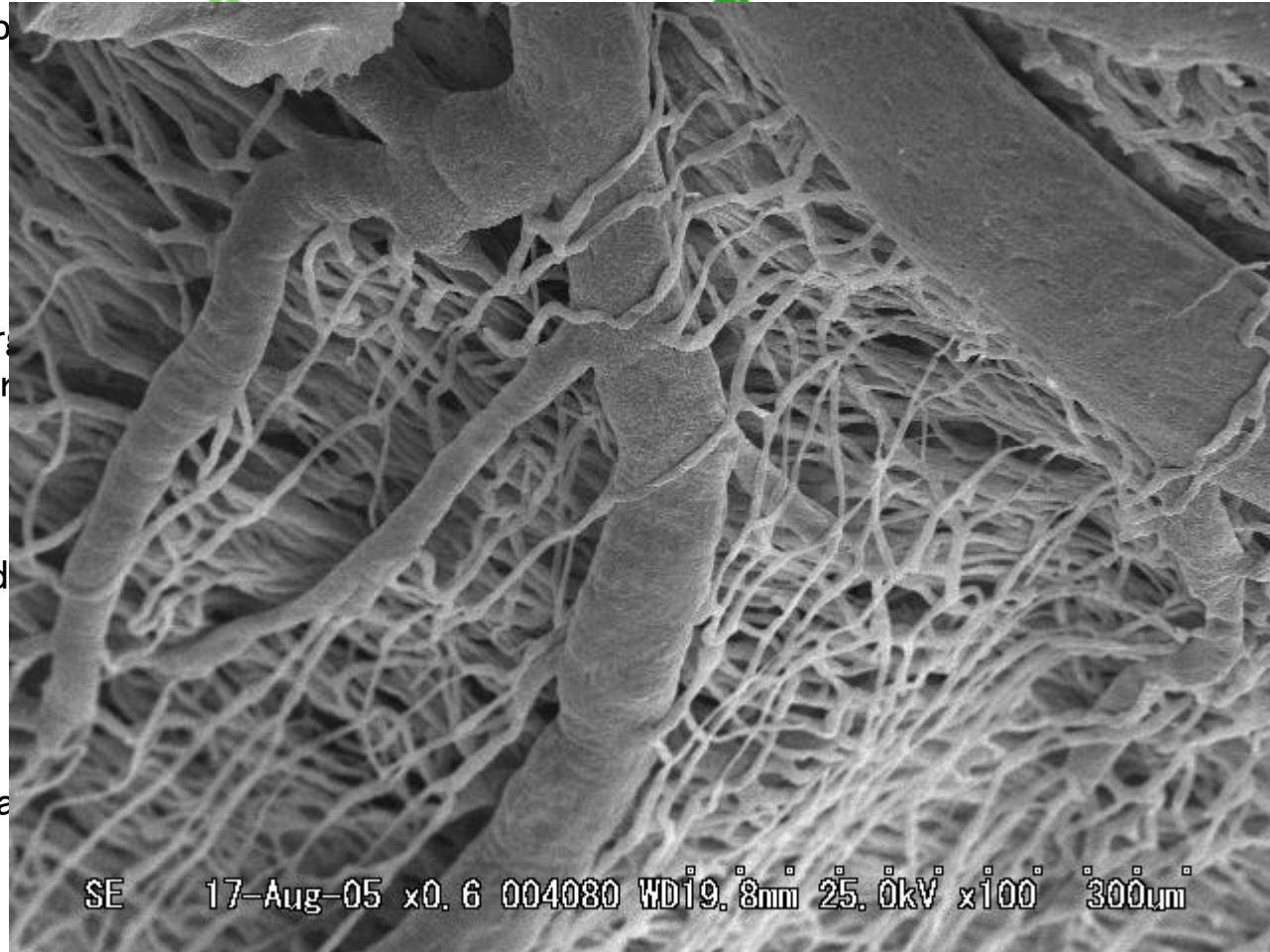
Adrenergic
receptor

Ca hand

Signa

Inflammation
Nature 2012

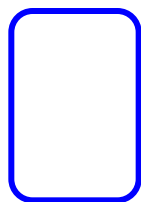
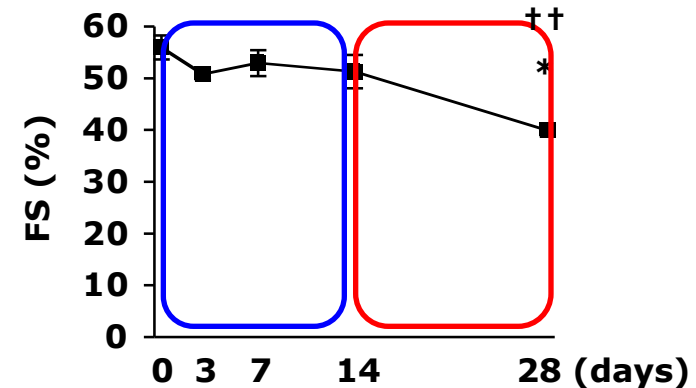
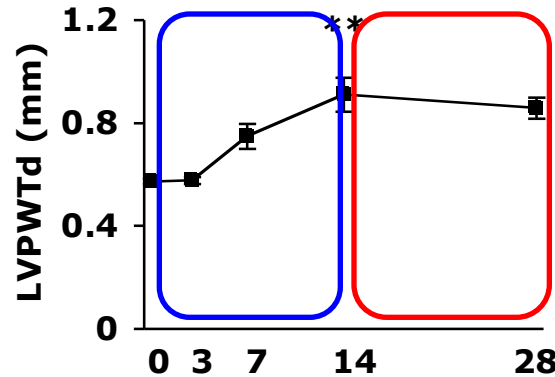
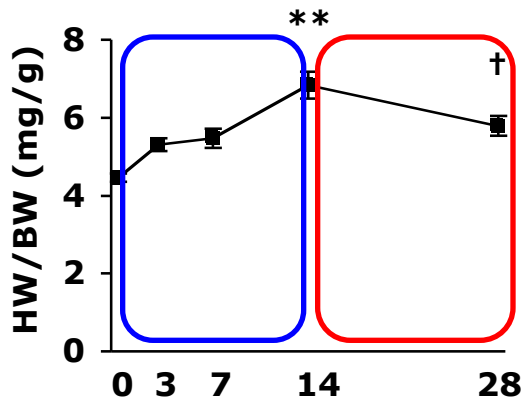
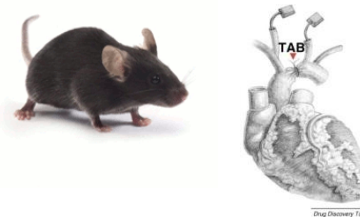
stress



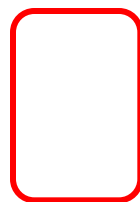
Adaptive and Maladaptive Phases of Cardiac Hypertrophy

Time-series analysis after thoracic aortic constriction (TAC)

TAC operation

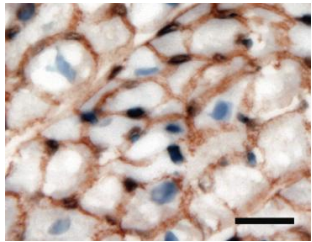


adaptive phase

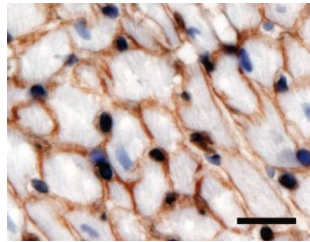


maladaptive phase

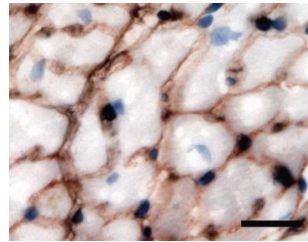
Number of Vessels in Hearts



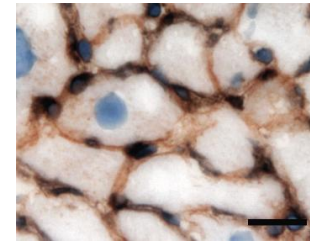
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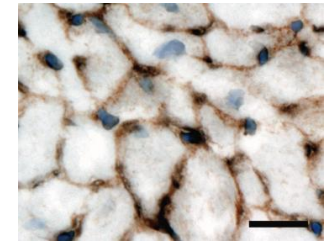
3



7

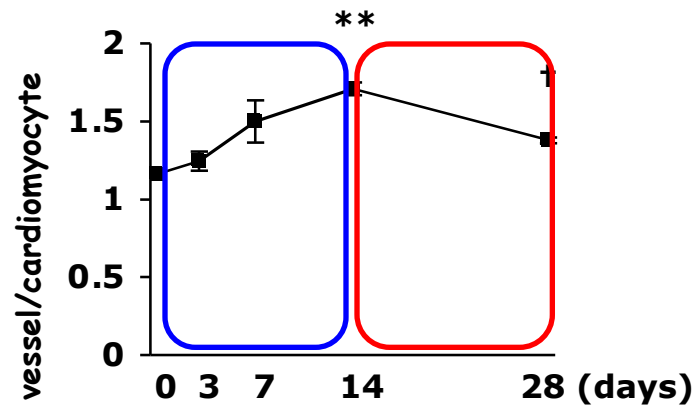


14



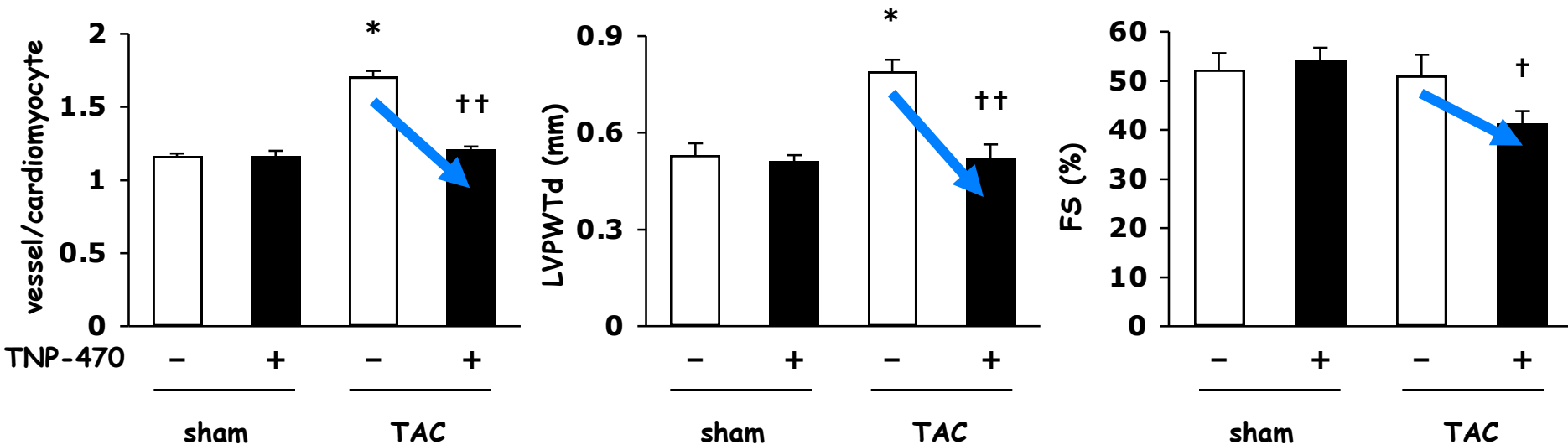
28 (days)

x400
bar = 5 μ m
brown; dystrophin
black; PECAM
blue; nucleus



Inhibition of Angiogenesis

**TNP-470: angiogenic inhibitor
2 weeks after TAC (adaptive phase)**



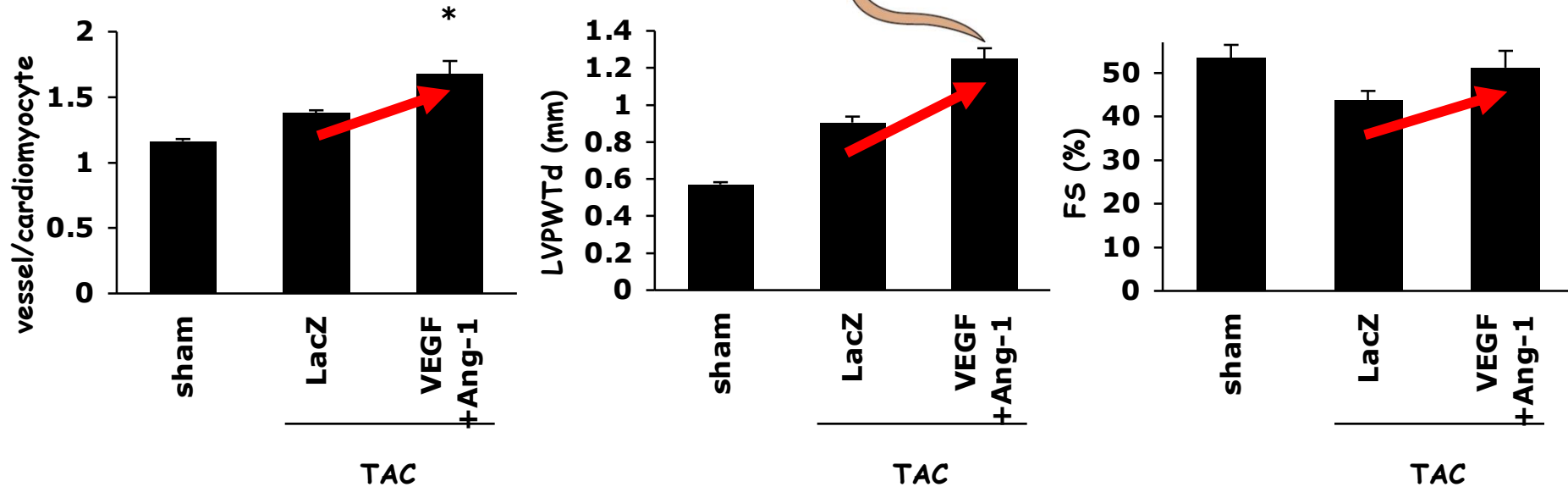
Angiogenesis is necessary for development of cardiac hypertrophy and maintenance of cardiac function

Promotion of Angiogenesis

4 weeks after TAC (maladaptive phase)



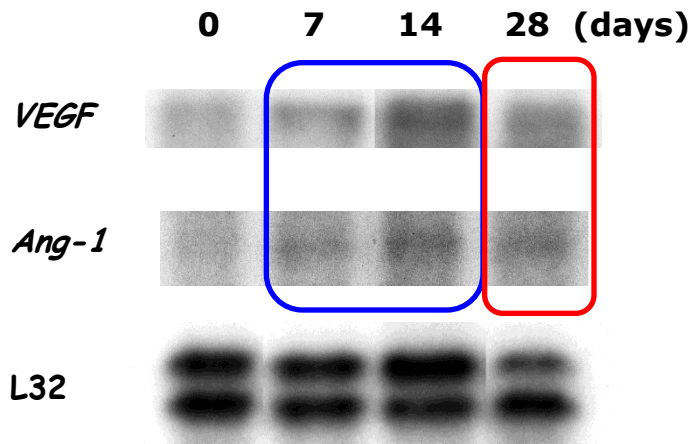
Adenoviral vectors coding: VEGF&Ang-1



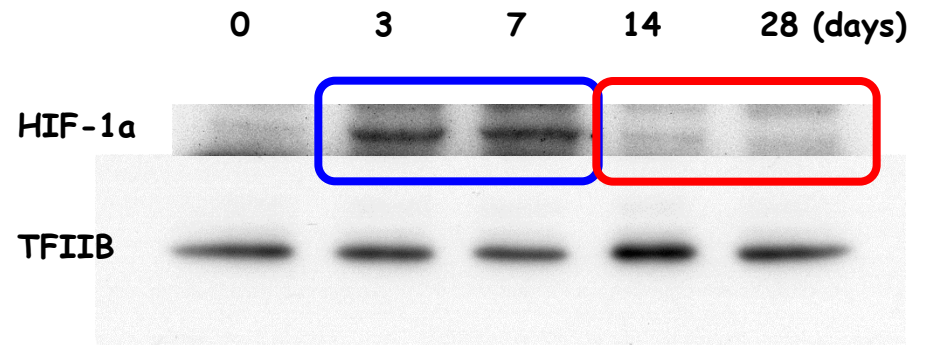
Angiogenesis is enough for development of cardiac hypertrophy and maintenance of cardiac function

Angiogenic Factors and HIF-1

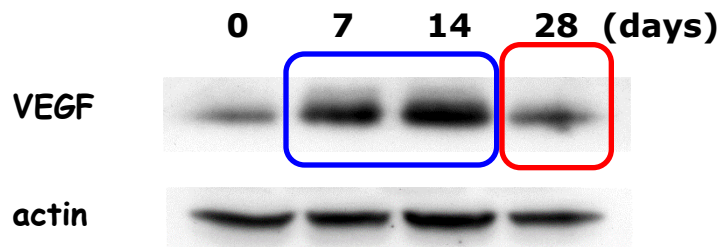
RPA



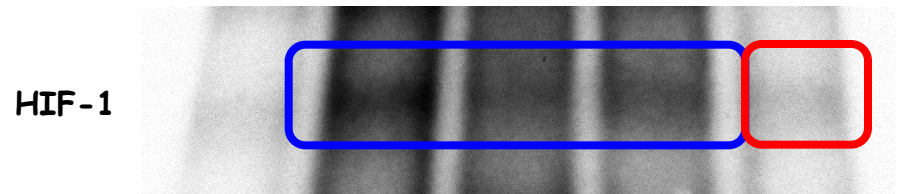
Western Blot Analysis



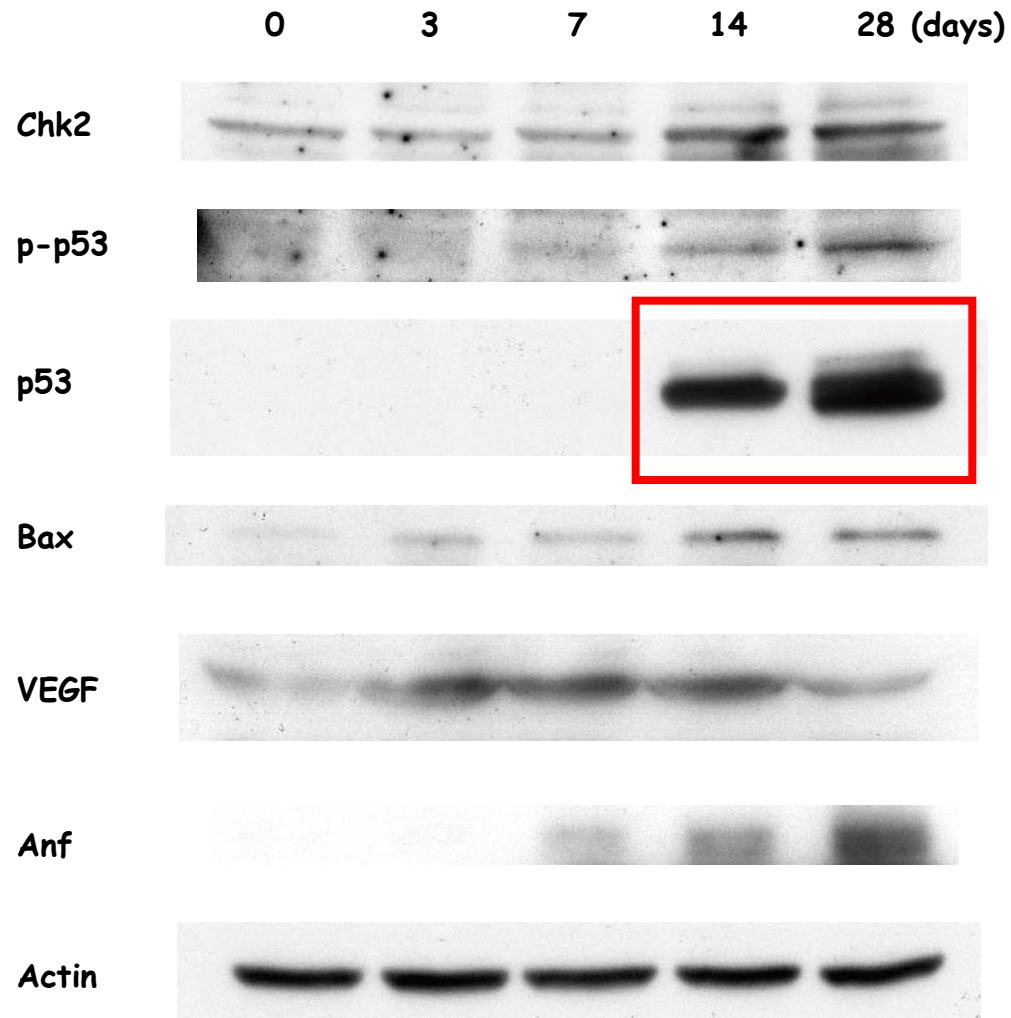
Western Blot Analysis



EMSA

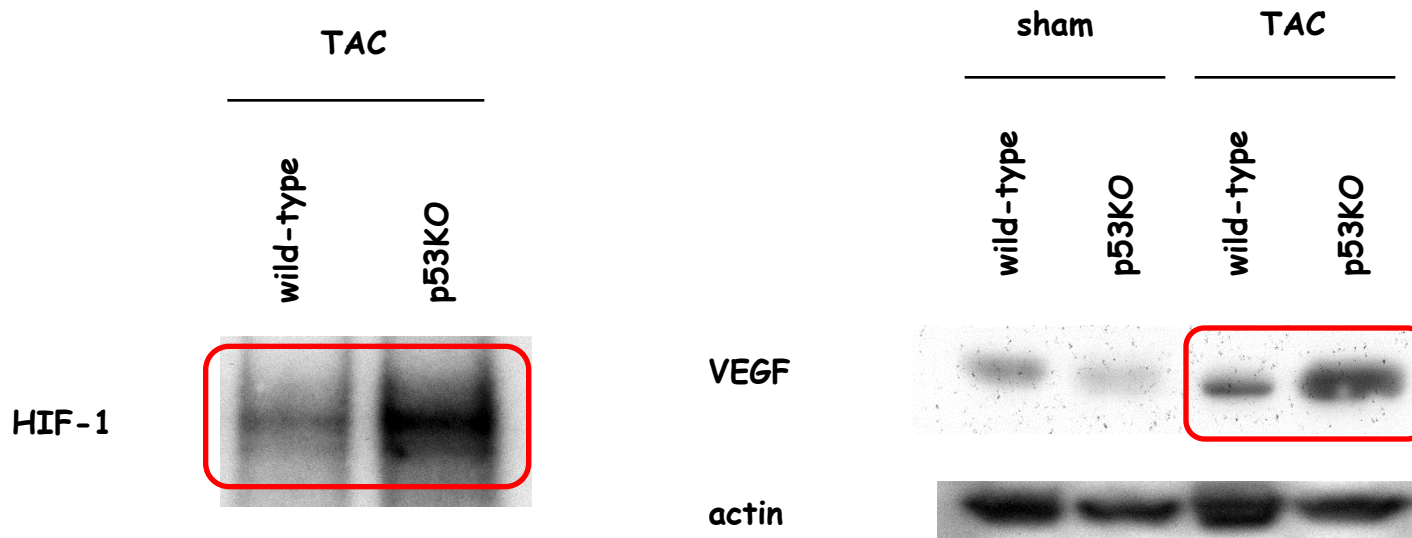


Chk2 activation before p53 Expression



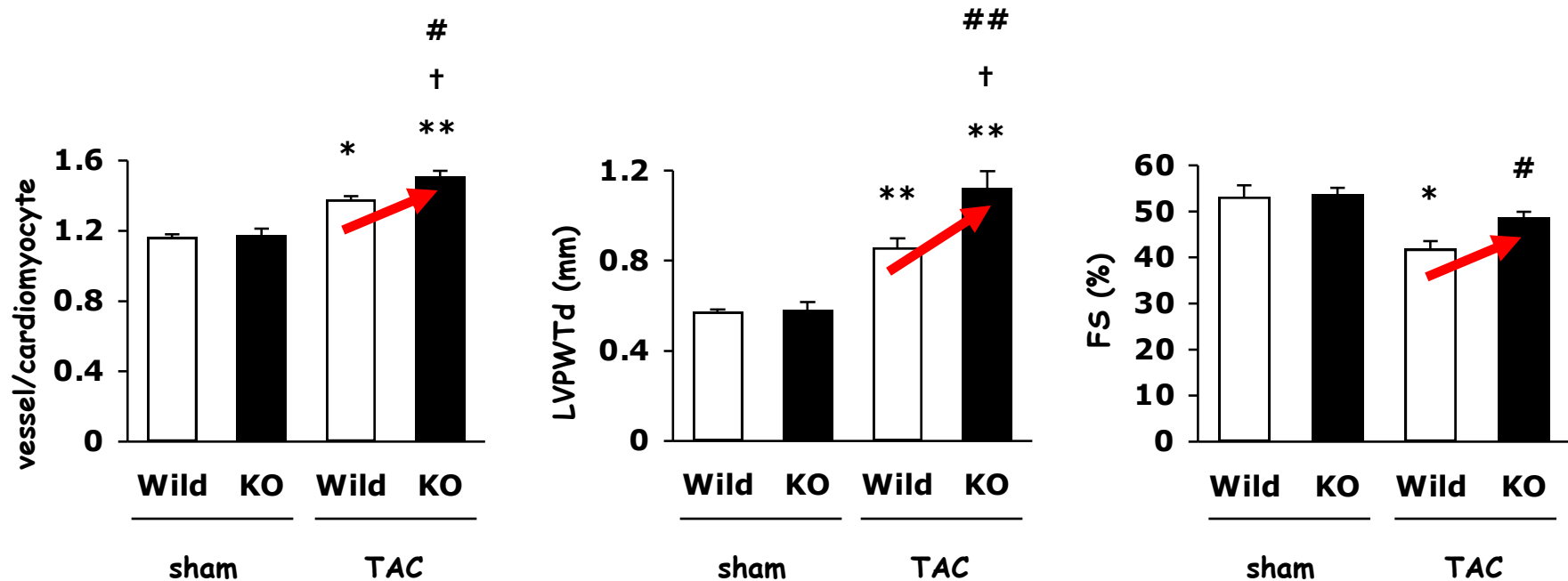
Expression of HIF-1 and VEGF in p53 KO Mice

4 weeks after TAC (maladaptive phase)

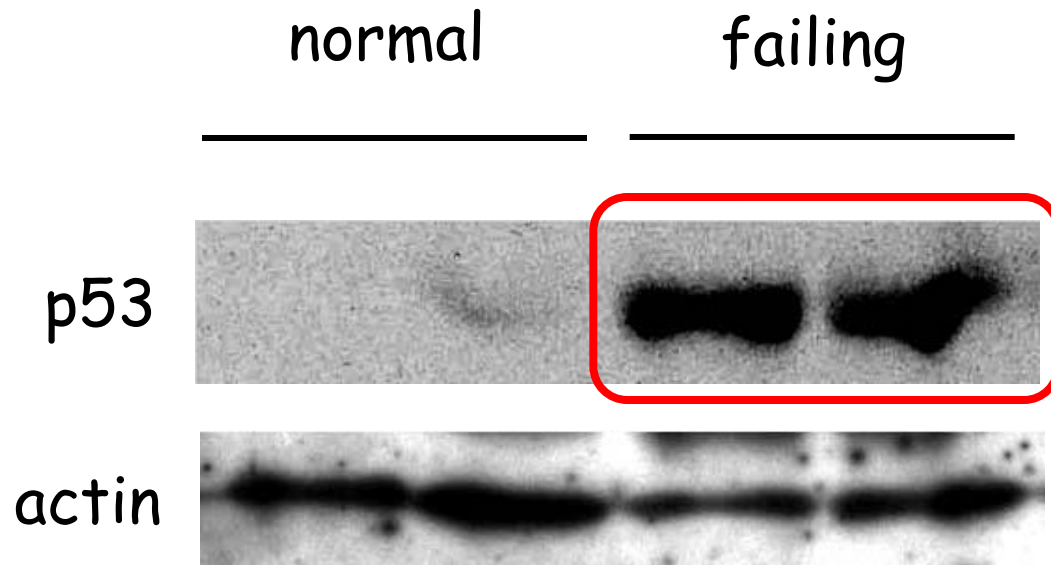


Cardiac Function is preserved in p53 KO Mice

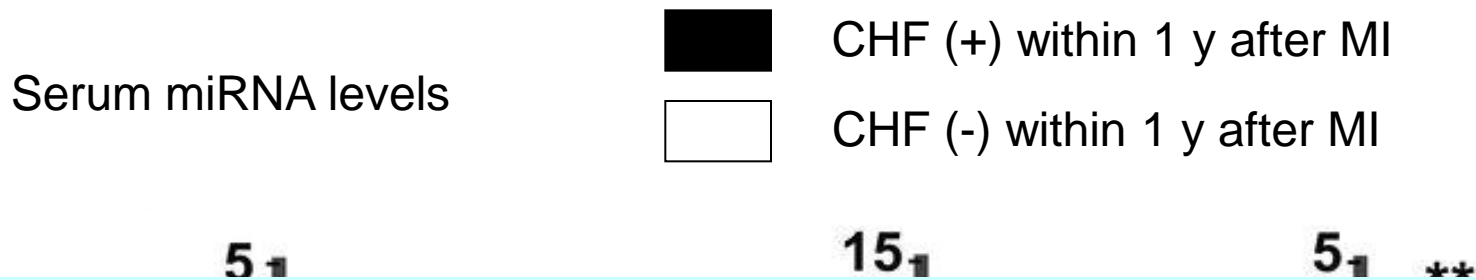
4 weeks after TAC (maladaptive phase)



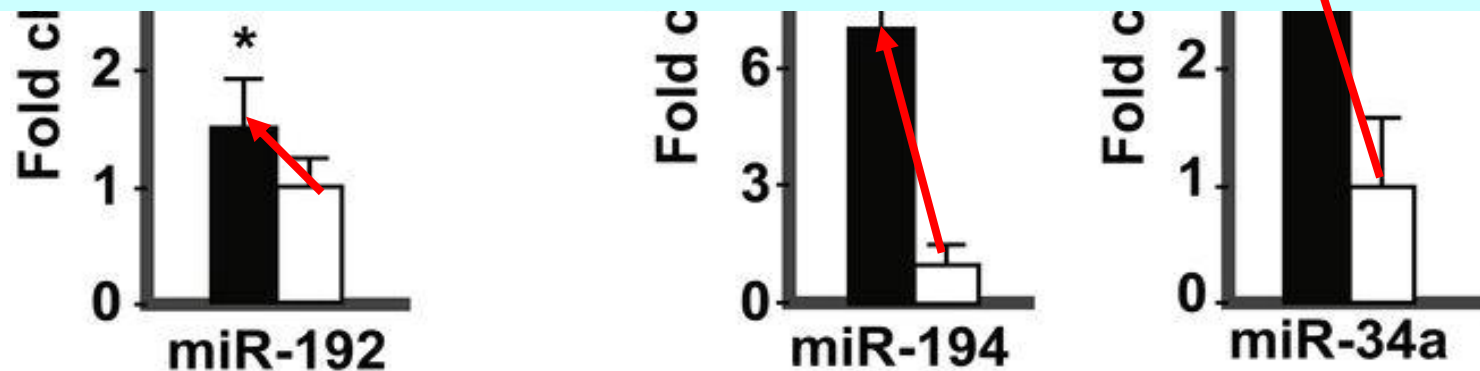
p53 is expressed in human failing heart



miR-192,194,34a are increased in serum of patients with heart failure after MI



miR-192, -194, -34a are targets of p53!



miR-194 and 34a levels at discharge
are well-correlated with left ventricular size

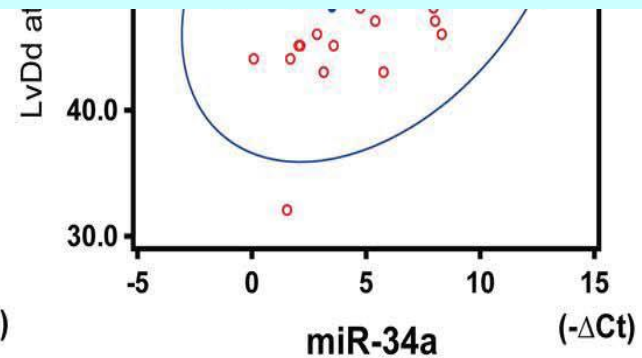
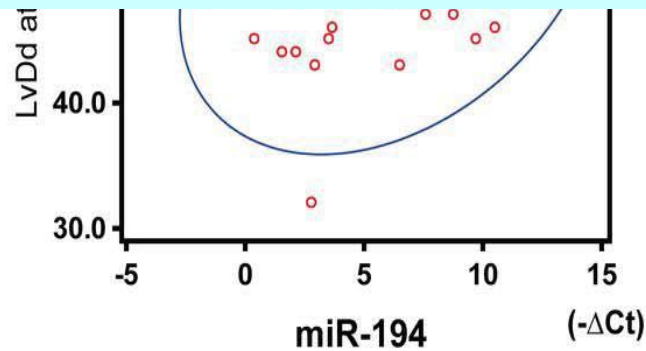
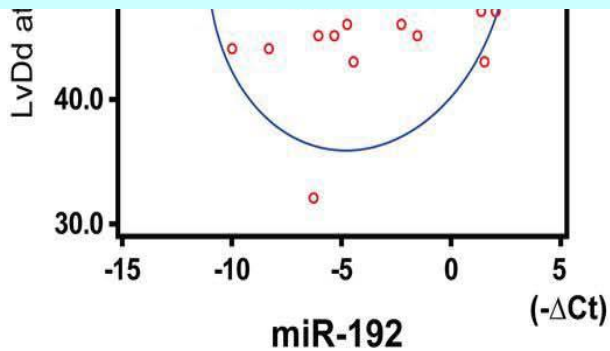
(mm) $r=0.09$ (95%CI -0.18, 0.34), $P=0.52$

(mm) $r=0.33$ (95%CI 0.08, 0.54), $P=0.01$

(mm) $r=0.38$ (95%CI 0.14, 0.58), $P=0.003$



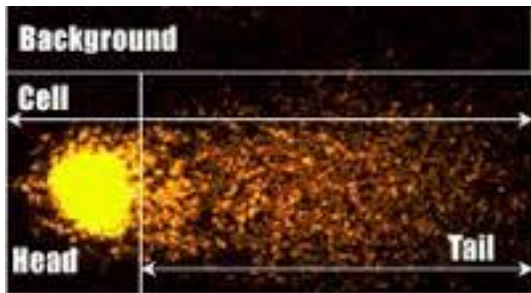
miR-194 and -34a are predictive markers for heart failure



Comet Assay

Comet assay is a method for measuring DNA damages in eukaryotic cells. The intensity of the comet tail relative to the head reflects the number of DNA breaks.

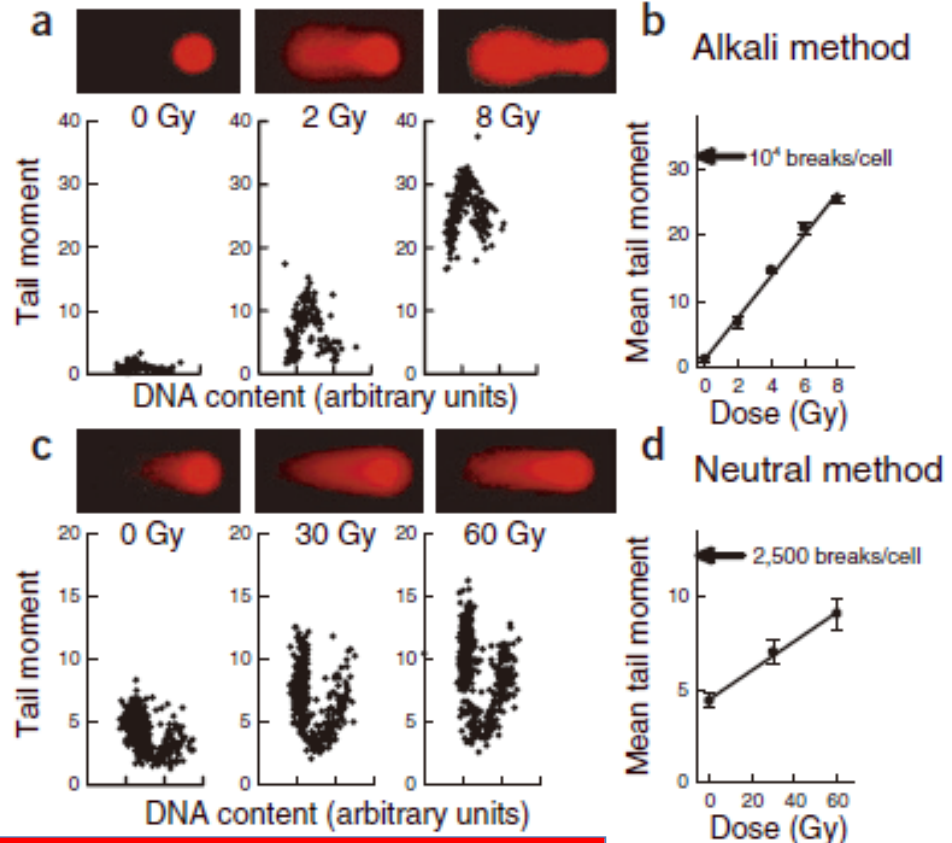
Alkaline comet detects all types of DNA damage.



Tail moment

$$= (\% \text{DNA in Tail} \times \text{Tail length}) / 100$$

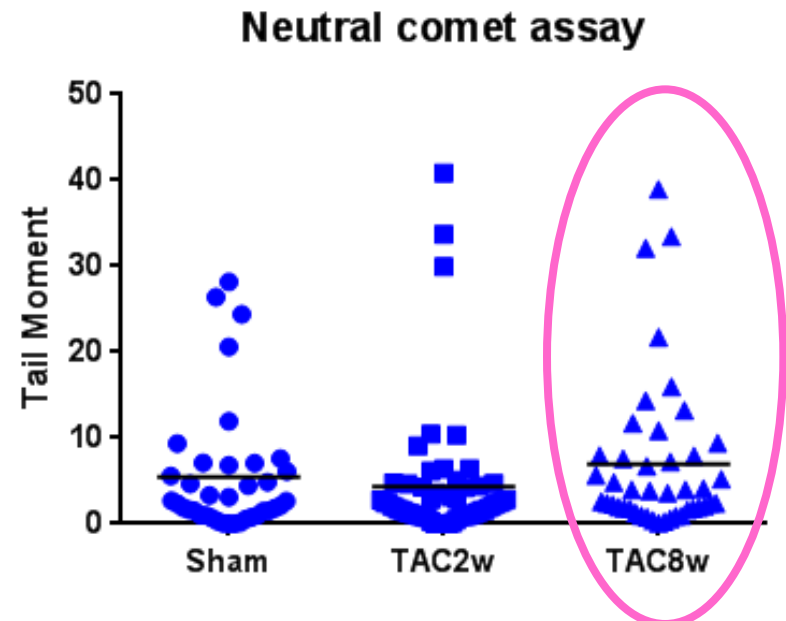
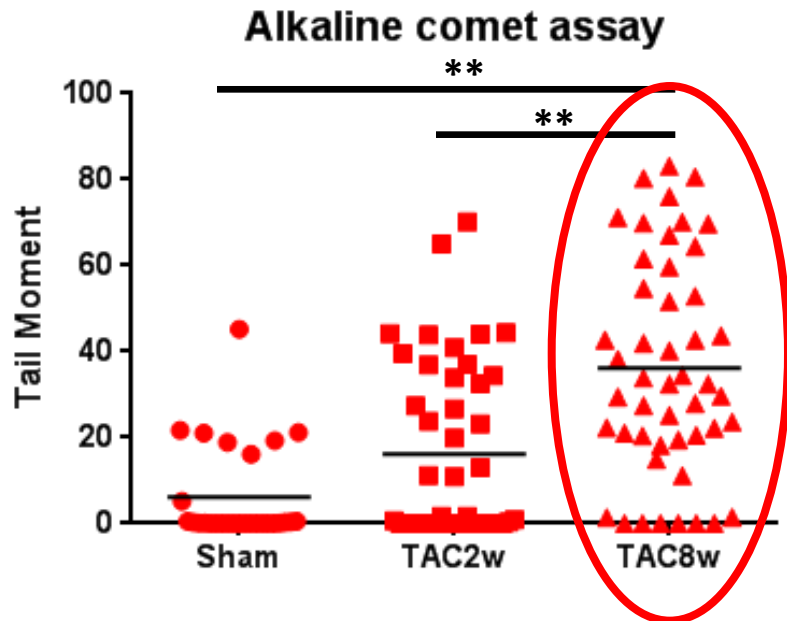
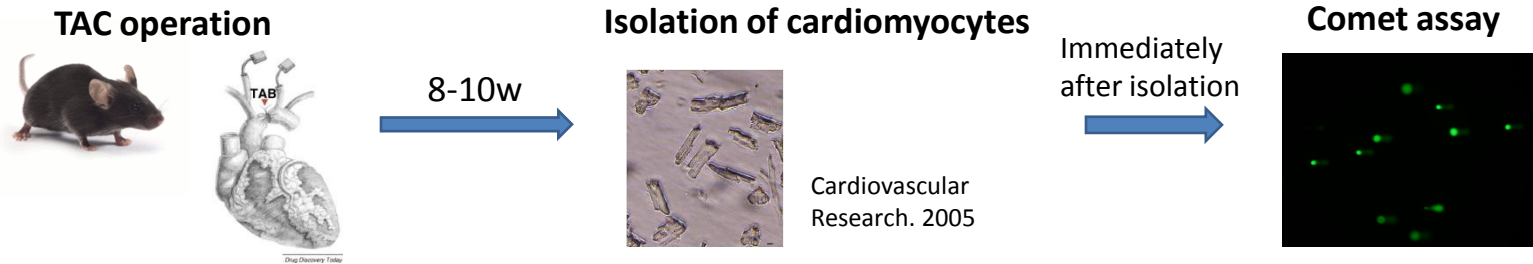
<http://www.cometassayindia.org/index.htm>



Neutral comet only detects DSBs.

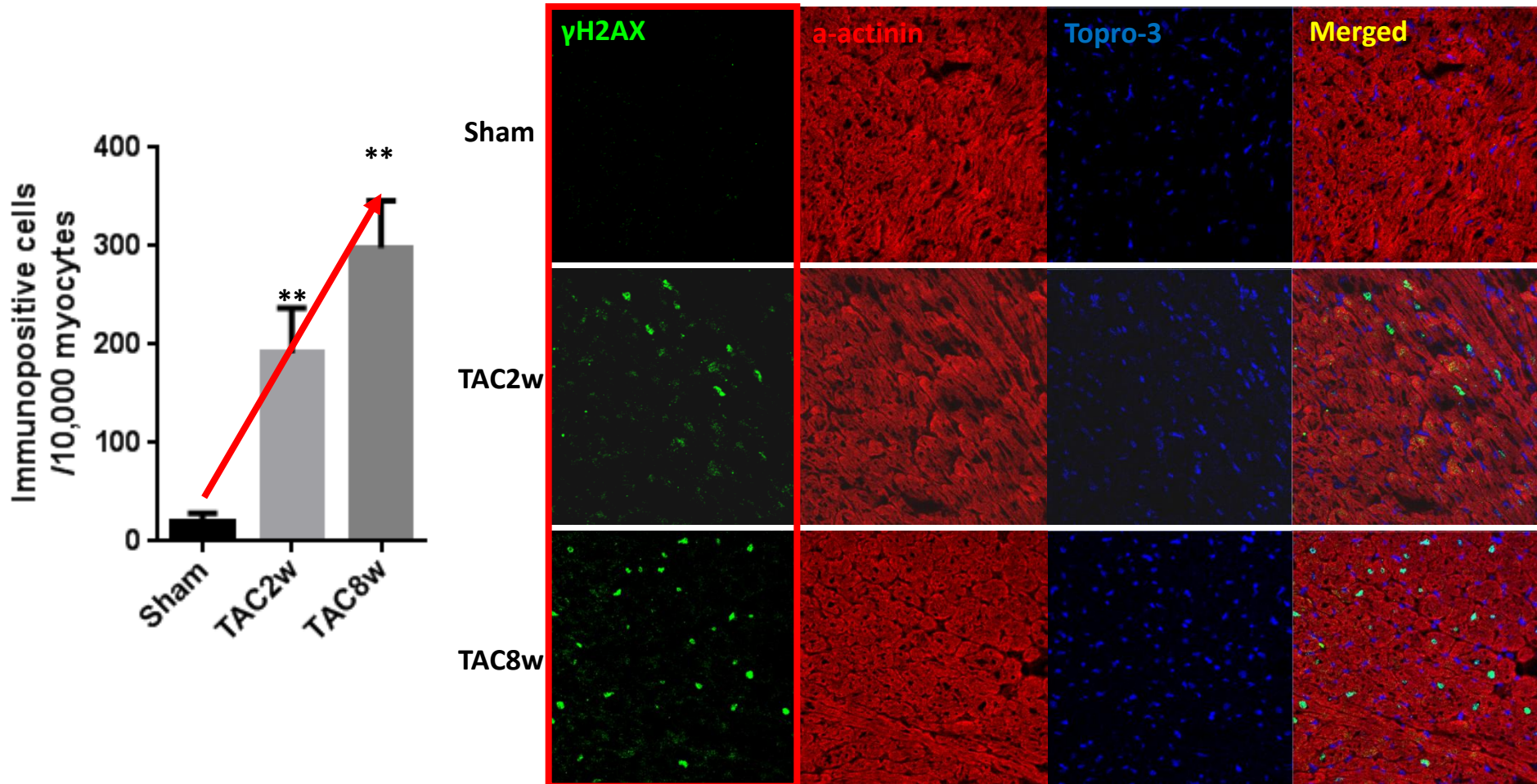
Olive PL et al. Nature Prot 2006

DNA SSBs accumulate in the failing heart

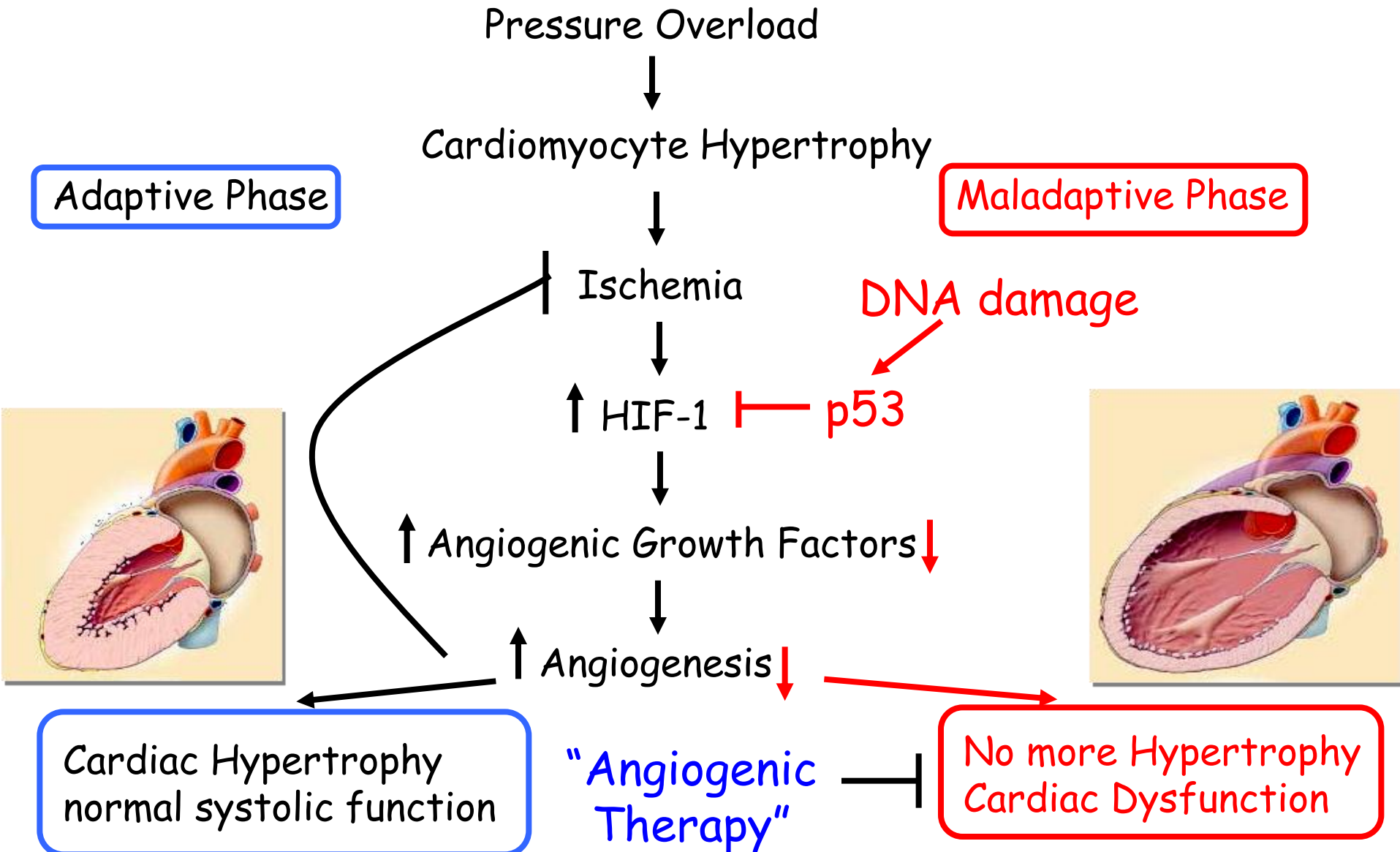


* $P < 0.05$; ** $p < 0.01$ by Steel-Dwass analysis

γ H2AX positive CMs increased after pressure overload

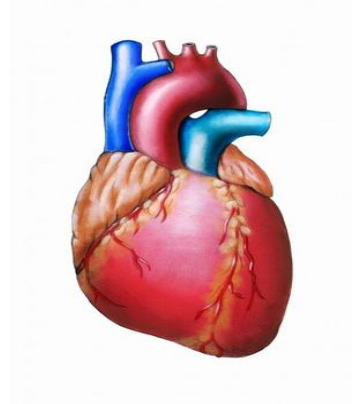


Molecular mechanisms of Heart Failure



How we would induce angiogenesis?

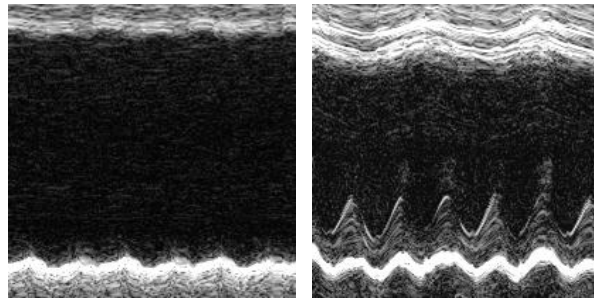
Erythropoietin (EPO)



EPO prevents LV remodeling after MI

EPO (10000u/kg) s.c 5days after MI

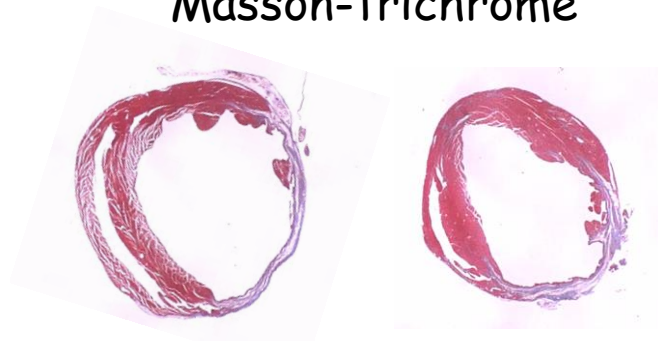
UCG



control

EPO

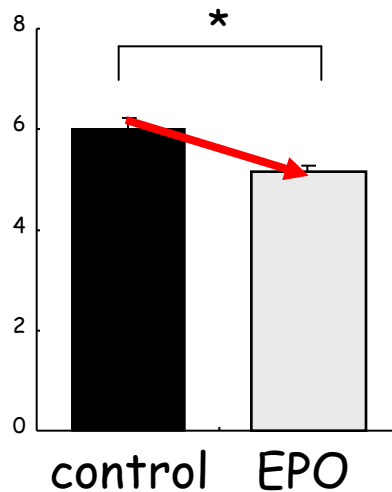
Masson-trichrome



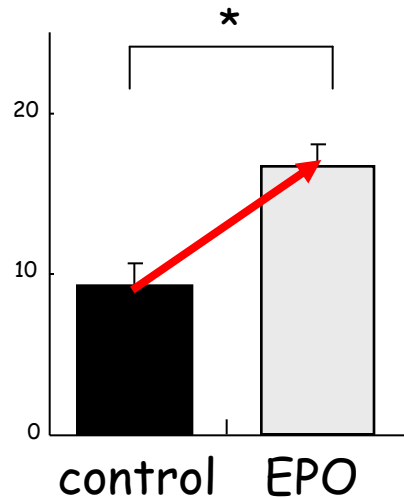
control

EPO

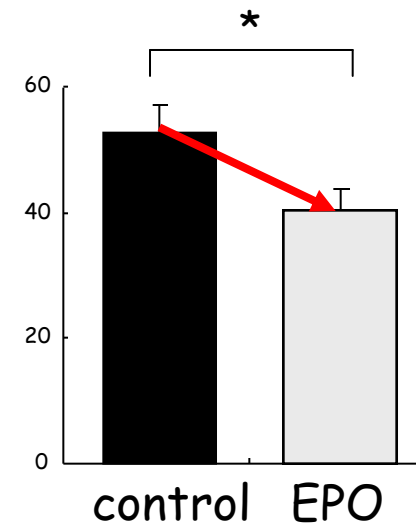
LVEDD (mm)



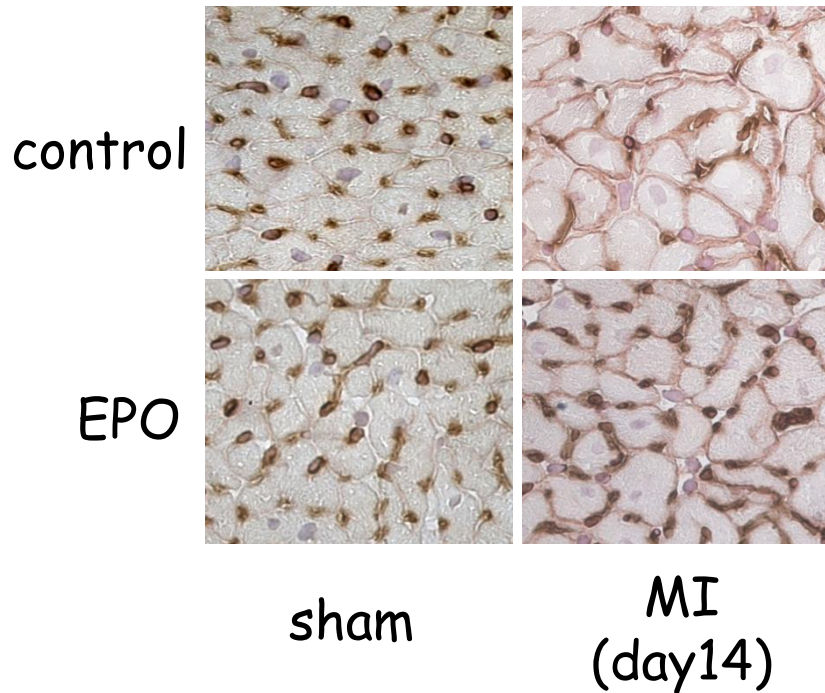
FS (%)



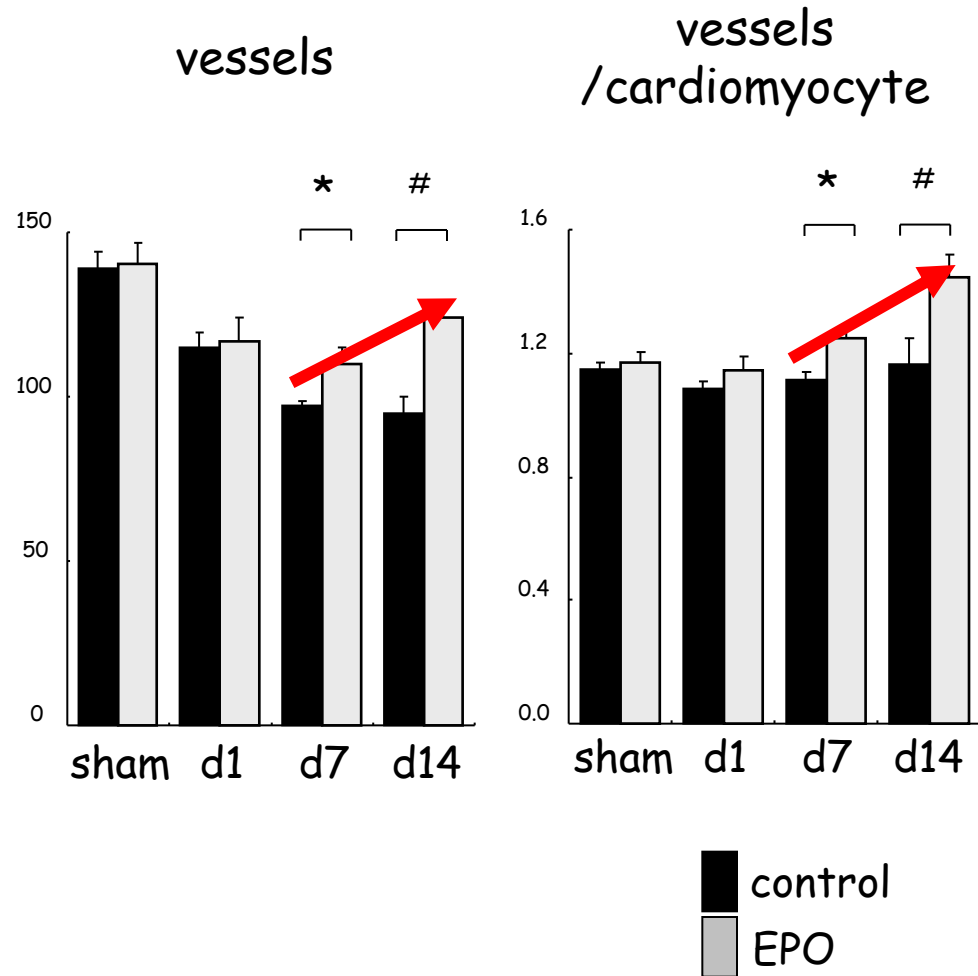
Infarct size (%)



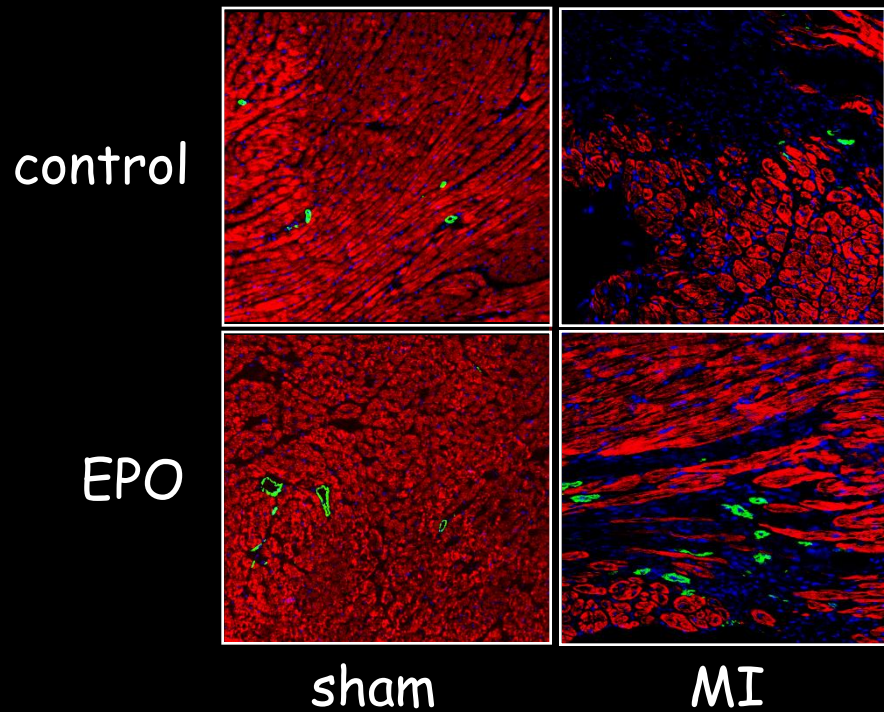
EPO promotes angiogenesis in infarcted hearts



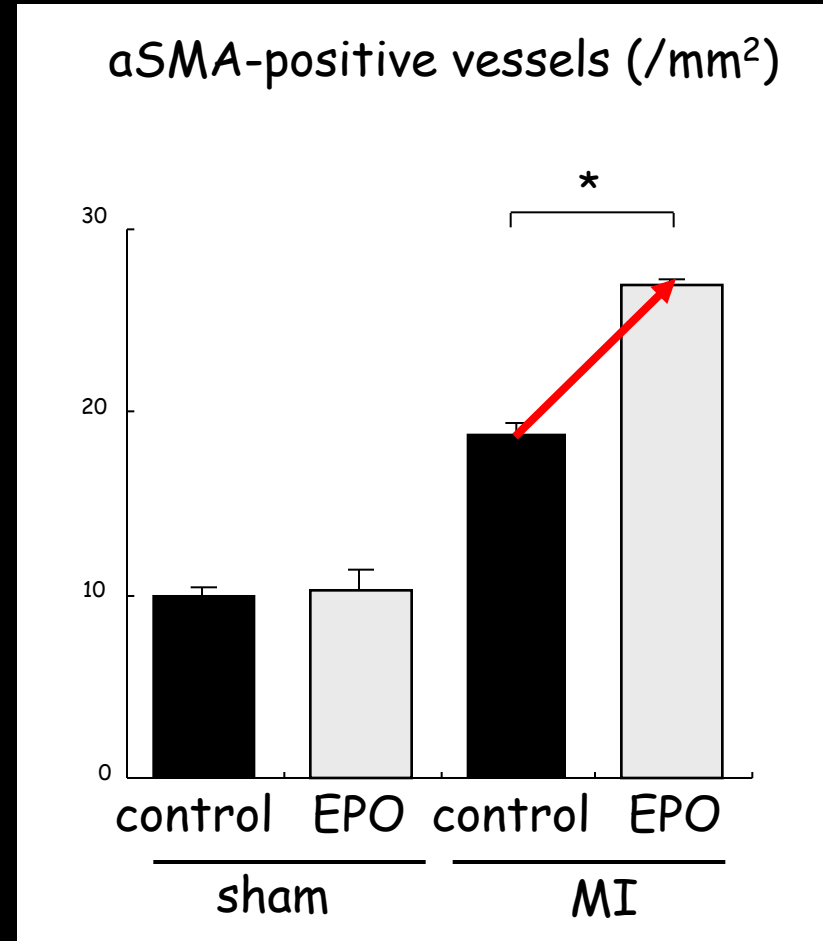
 vessels (PECAM)
 cell membrane (dystrophin)



EPO induces mature vessel formation



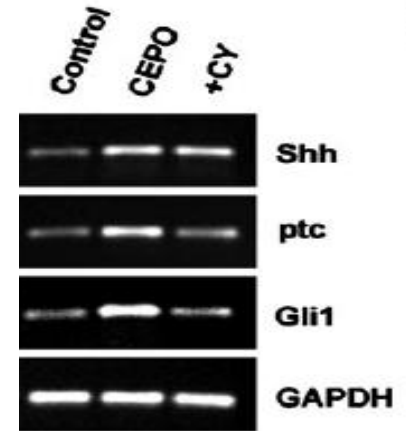
α -smooth muscle actin (α SMA)
phalloidin
Topro3



Sonic hedgehog (Shh)

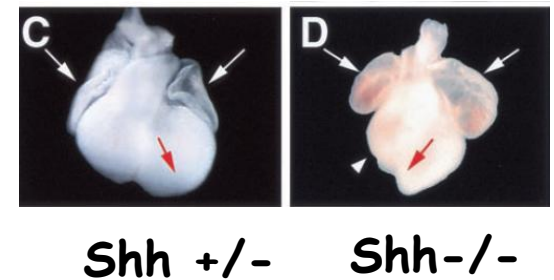
Carbamylated EPO (CEPO) promotes neural progenitor cell proliferation through an upregulation of Shh expression

Wang L et al. J.Bio.Chem 2007



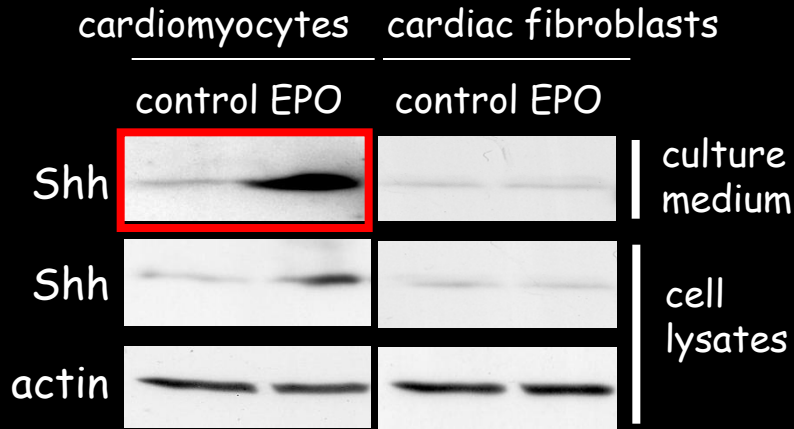
Shh is a critical regulator of cardiac growth and vasculature during embryogenesis

Tsukui et al. PNAS 1999

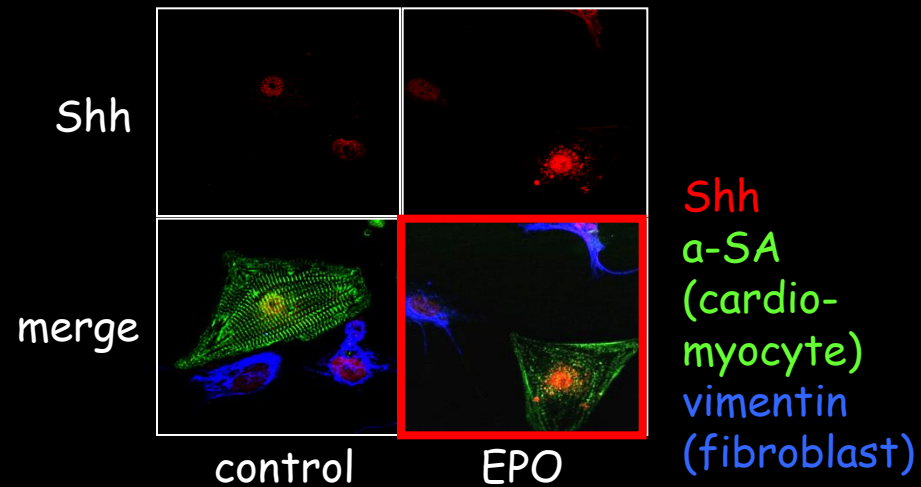


EPO upregulates Shh expression in cardiomyocytes and infarcted hearts

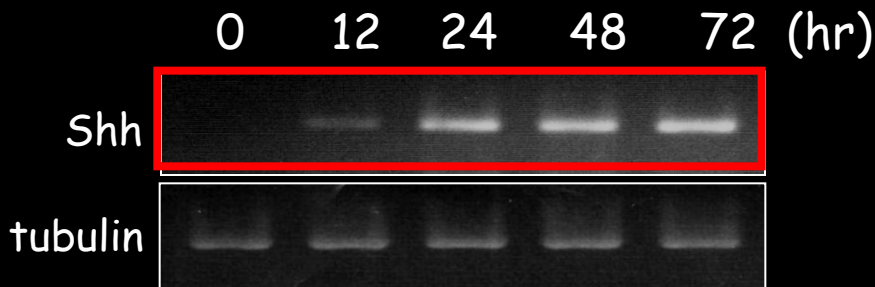
western blot



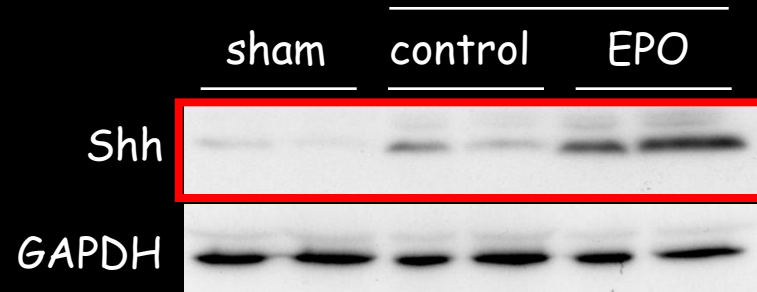
immunocytochemistry



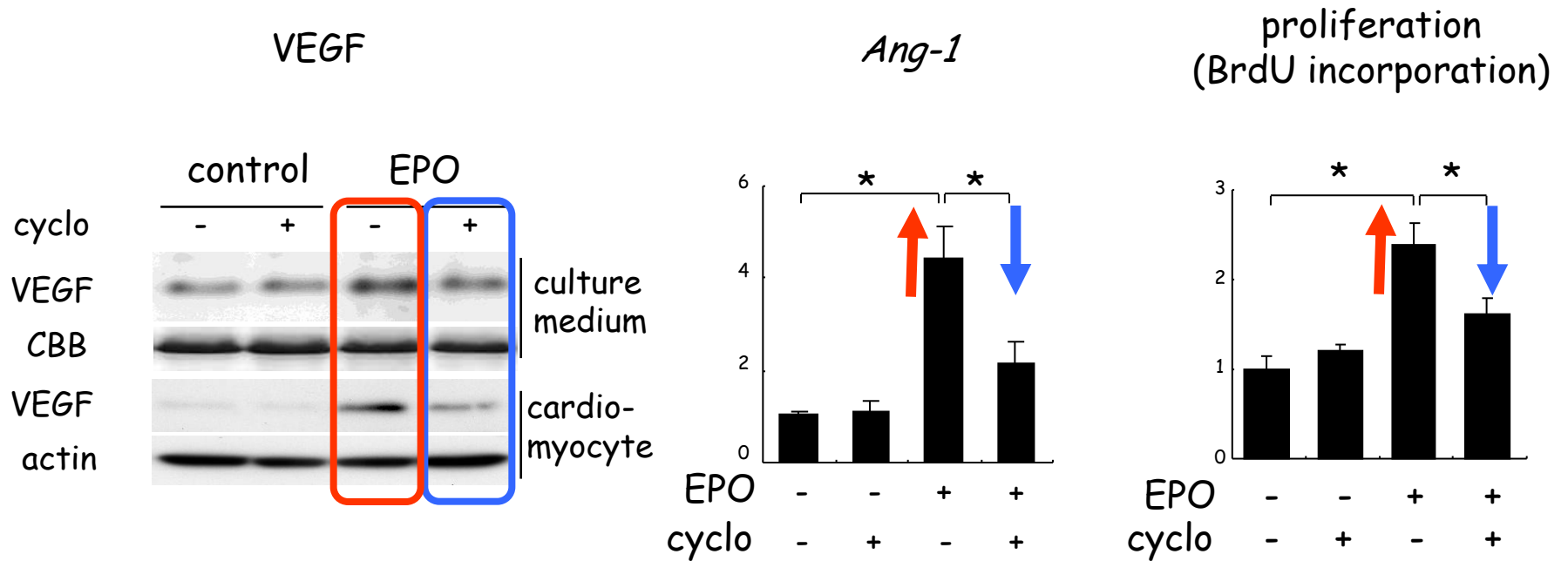
RT-PCR (cardiomyocytes)



MI

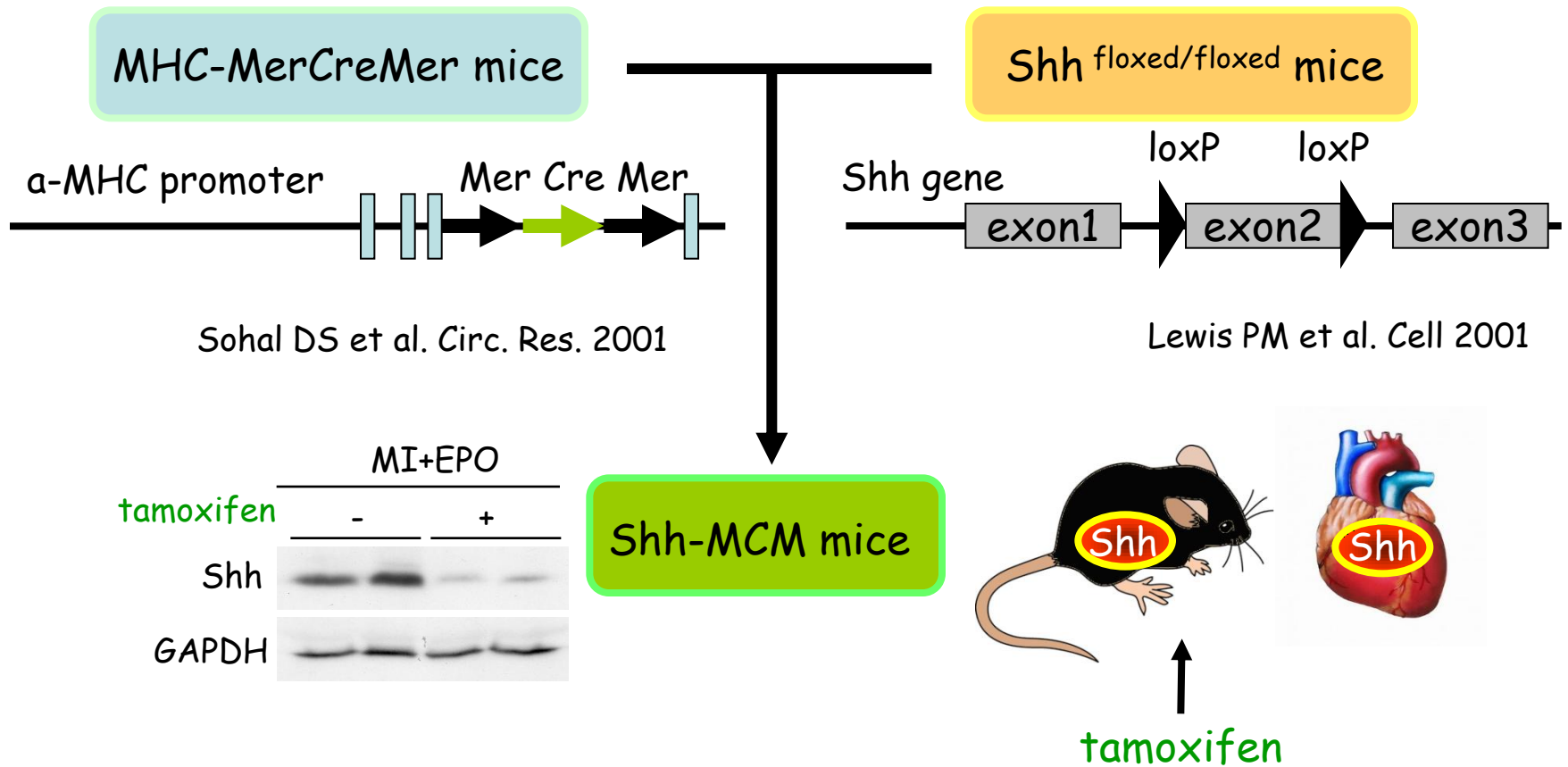


Shh signaling is critical for angiogenic effects of EPO (in vitro)



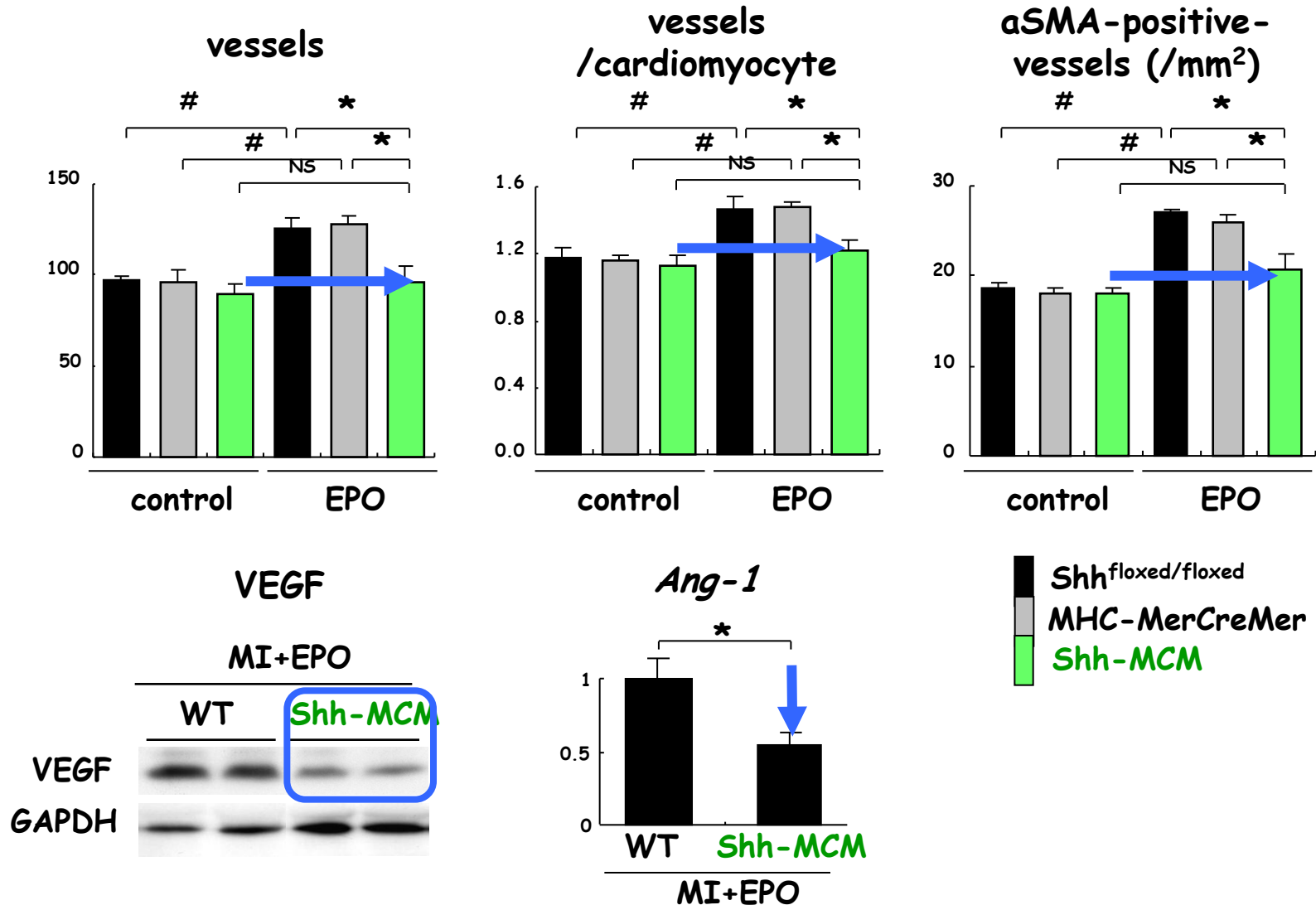
cyclopamine (cyclo) : a specific inhibitor of Shh signaling

α MHC-MerCreMer; $Shh^{fl/fl}$ mice (Shh-MCM mice)

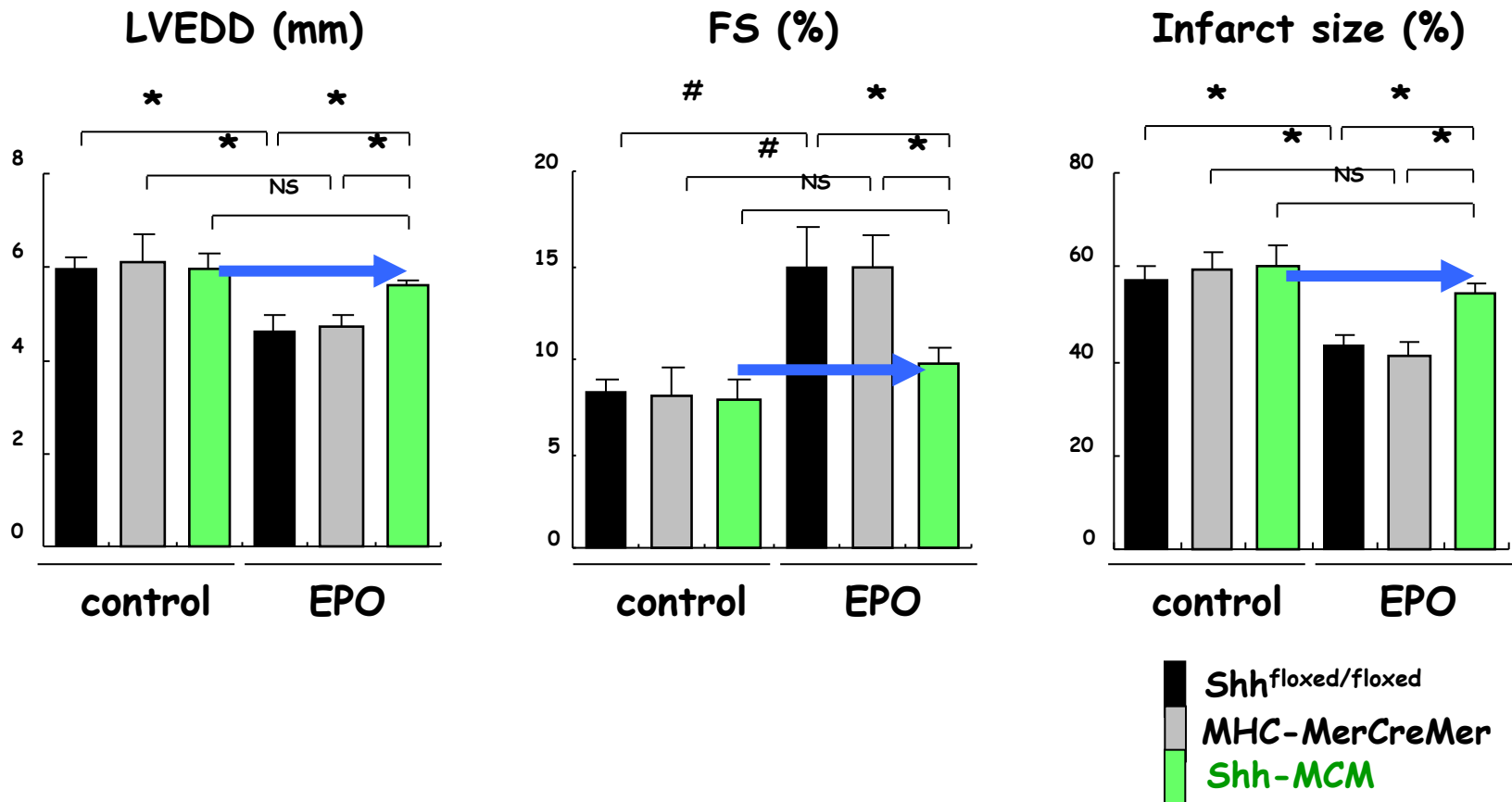


Shh is deleted only in cardiomyocytes after tamoxifen treatment

Cardiomyocyte-specific Shh deletion abolishes EPO-induced angiogenesis

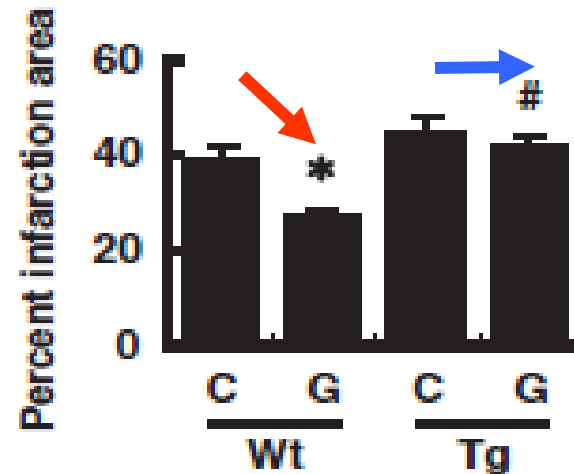
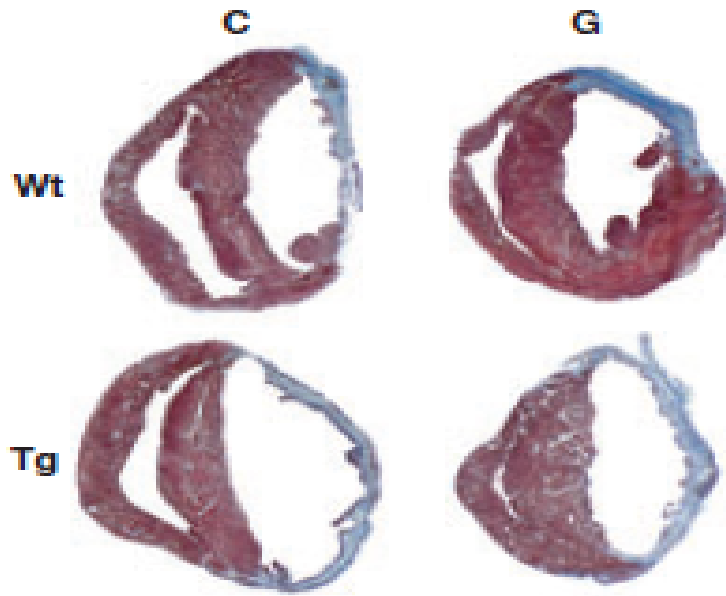
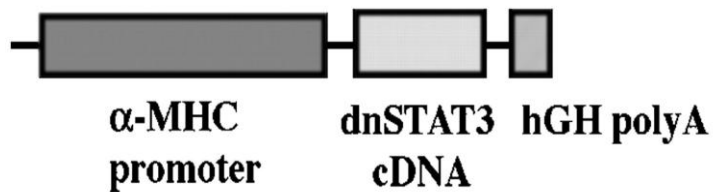


Cardiomyocyte-specific Shh deletion abolishes EPO-induced cardioprotection



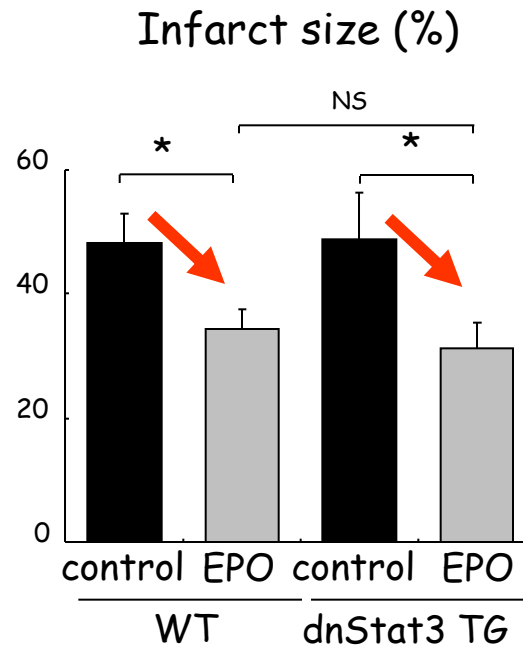
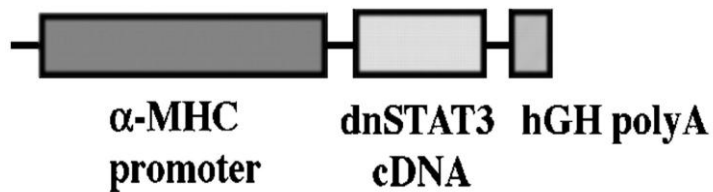
G-CSF protects heart from MI through Stat3-mediated antiapoptotic effects

dnSTAT3-Tg mice

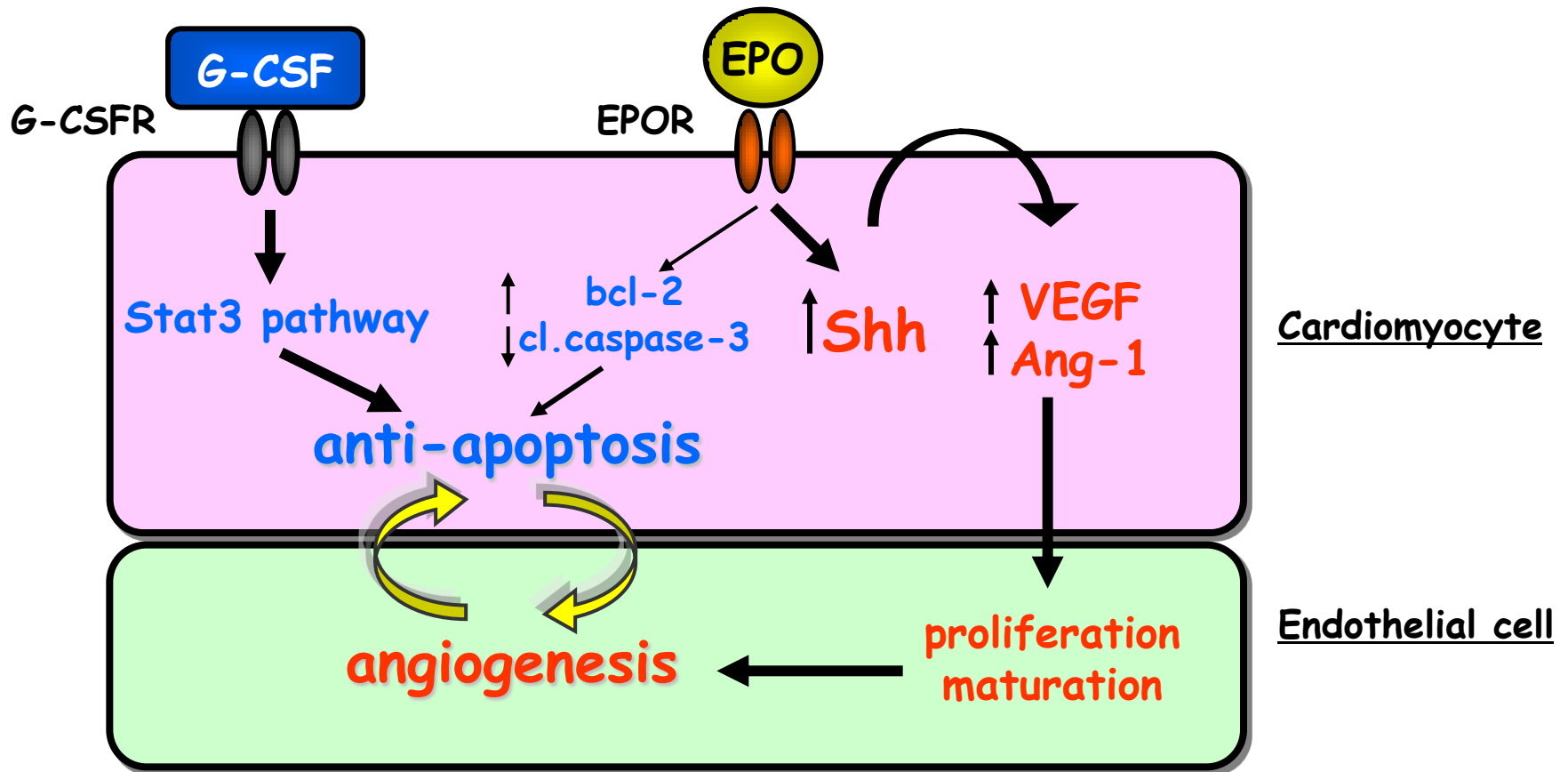


Stat3 activation is *not* involved in EPO-induced cardioprotection

dnSTAT3-Tg mice

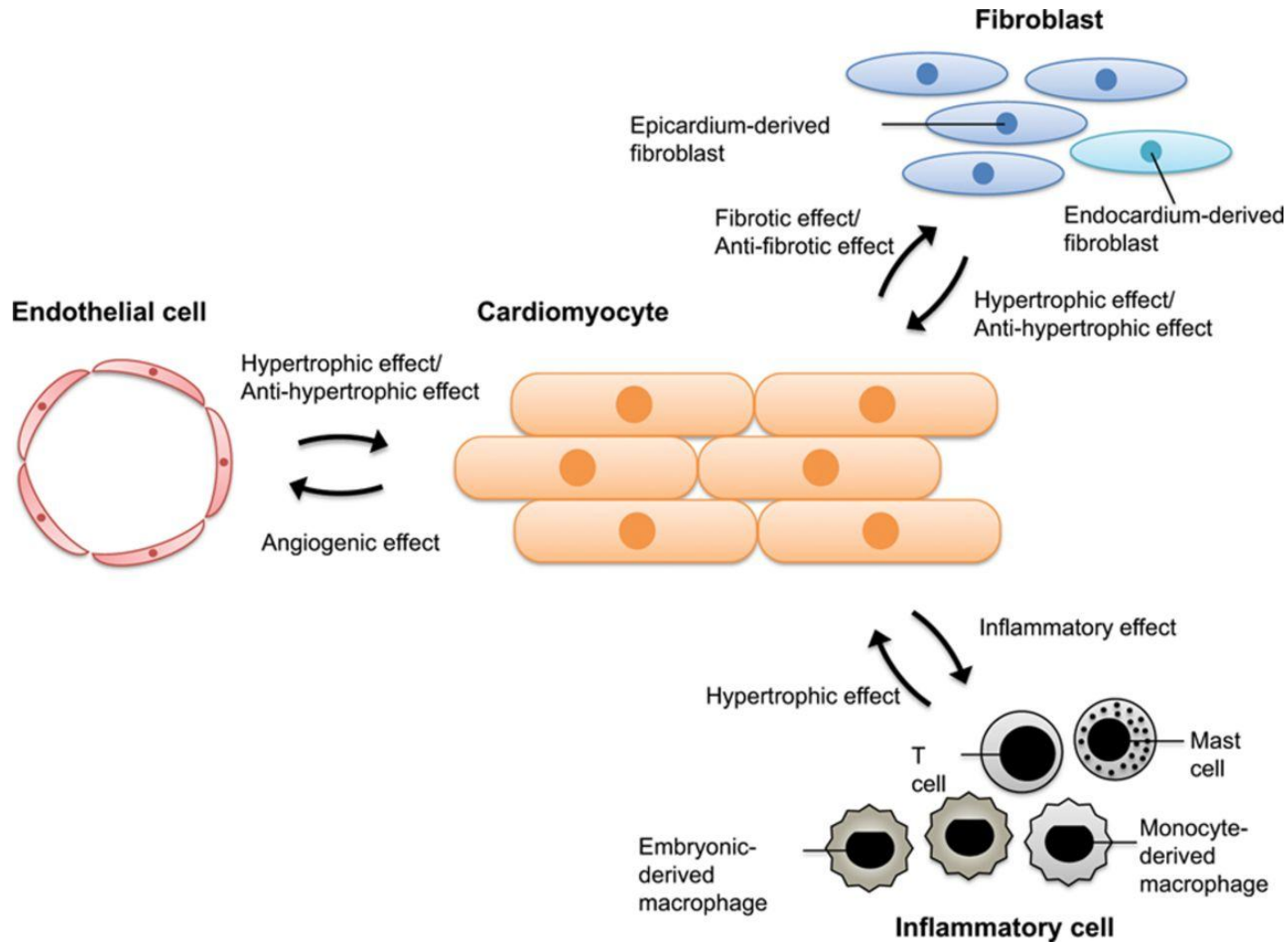


Mechanism of EPO-induced cardioprotection at MI



We have been conducting a clinical study using epo for AMI, EPO-AMI II.

Cardiac Nonmyocytes in the Hub of Cardiac Hypertrophy



Acknowledgement

Chiba University Graduate School of Medicine
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Department of Cardiovascular Medicine

Tomoaki Higo

The University of Tokyo Graduate School of Medicine
Department of Cardiovascular Medicine

Kazutaka Ueda



CREST

The Creation of Basic Medical Technologies to
Clarify and Control the Mechanisms Underlying Chronic Inflammation