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Busan, Korea, April 16, 2010

**Significance of Nitric Oxide Synthases (NOSs)  
in the Cardiovascular System  
- Lessons from Mice Lacking All NOSs -**

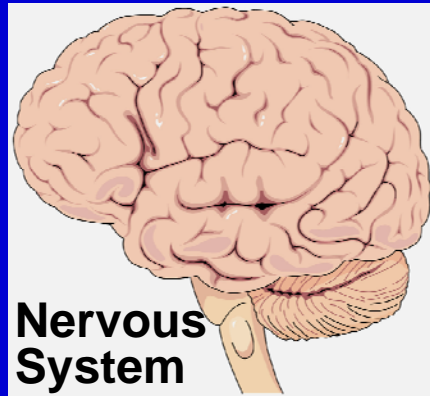
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Yutaka Otsuji<sup>4</sup>

# Significance of Nitric Oxide Synthases (NOSs) in the Cardiovascular System - Lessons from Mice Lacking All NOSs -

1. Vasculoprotective role of nNOS
2. NO-independent long-term vascular action of L-arginine analogues
3. Cardiovascular phenotypes in mice lacking all NOSs

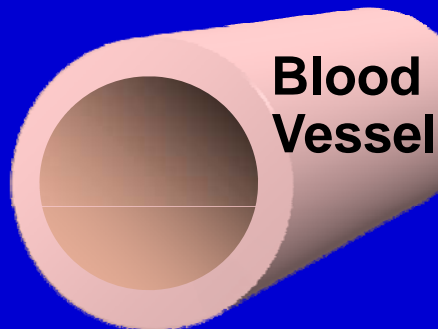
# The Nitric Oxide Synthase (NOS) System



Neuronal NOS (nNOS)

Inflammation

Inducible NOS (iNOS)

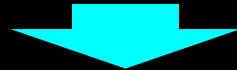


Endothelial NOS (eNOS)

# Role of Each NOS Isoform in Vascular Lesion Formation

## eNOS

- eNOS-KO mice/Carotid artery ligation model (*JCI* 1998)
  - Vascular lesion is exacerbated
- eNOS-TG mice/Carotid artery ligation model (*ATVB* 2001)
  - Vascular lesion is ameliorated



eNOS plays a vasculoprotective role

## iNOS

- iNOS-KO mice/Cardiac transplantation model (*Circulation* 1998)
  - Vascular lesion is exacerbated
- iNOS-KO mice/Carotid cuff placement model (*Circ Res* 1999)
  - Vascular lesion is ameliorated



iNOS has dual roles

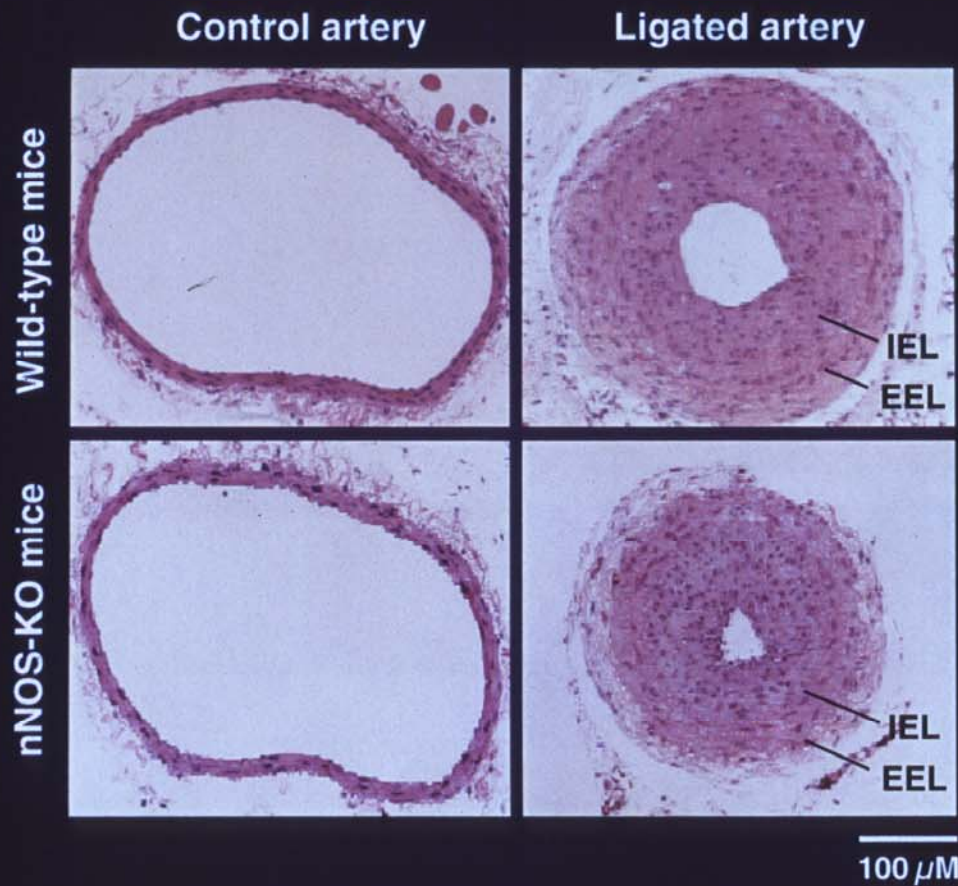
## nNOS

Role of nNOS is unknown

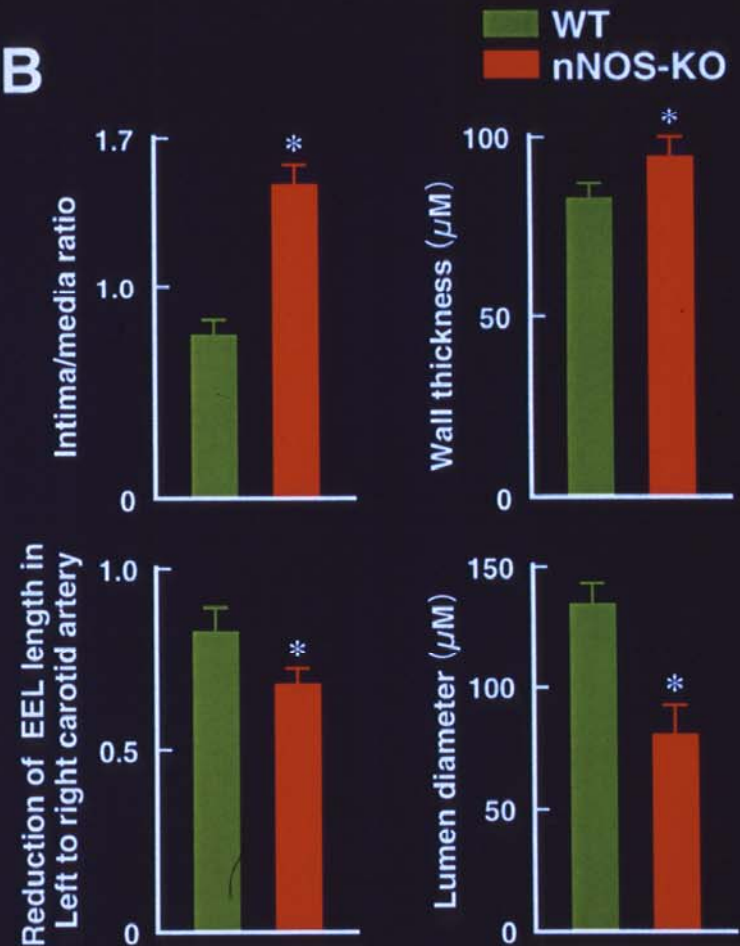


# Vascular Lesion Formation in Wild-Type and nNOS-KO Mice at 4 Weeks After Carotid Artery Ligation

**A**



**B**



# Factors That Induce Vascular nNOS Upregulation

**PDGF**

Nakata S, Tsutsui M, et al.  
*ATVB* 2005;25:2502

**Angiotensin II**

Morishita T, Tsutsui M, et al.  
*FASEB J* 2002;16:1994

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graph TD; PDGF --> Vascular_nNOS_upregulation; Angiotensin_II --> Vascular_nNOS_upregulation; Interleukin_1_beta --> Vascular_nNOS_upregulation; Statin --> Vascular_nNOS_upregulation;
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**Vascular nNOS upregulation**

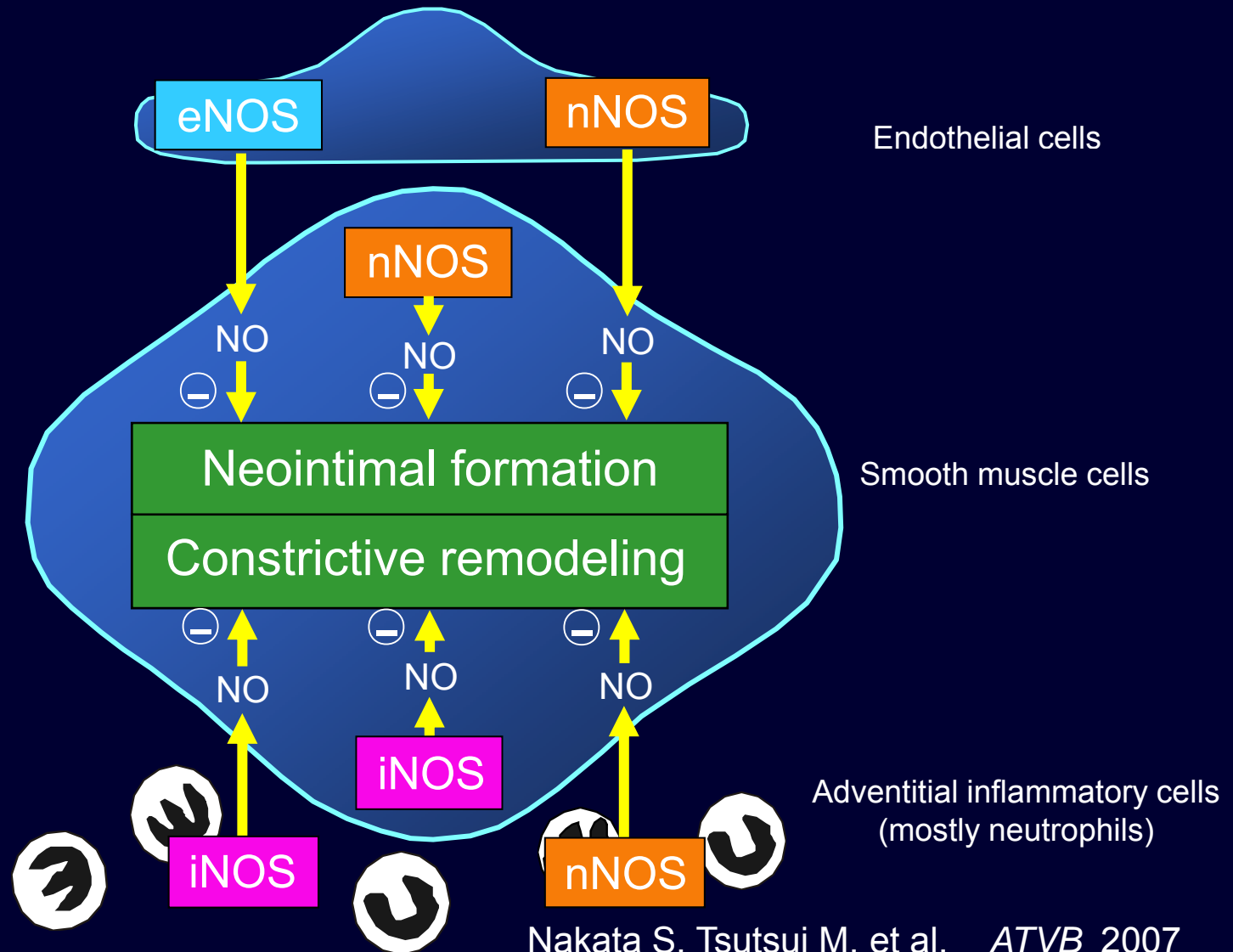
**Interleukin-1 $\beta$**

Morishita T, Tsutsui M, et al.  
*FASEB J* 2002;16:1994

**Statin**

Nakata S, Tsutsui M, et al.  
*ATVB* 2006, in press

# Vasculoprotective Role of Each NOS Isoform in Mouse Carotid Artery Ligation Model



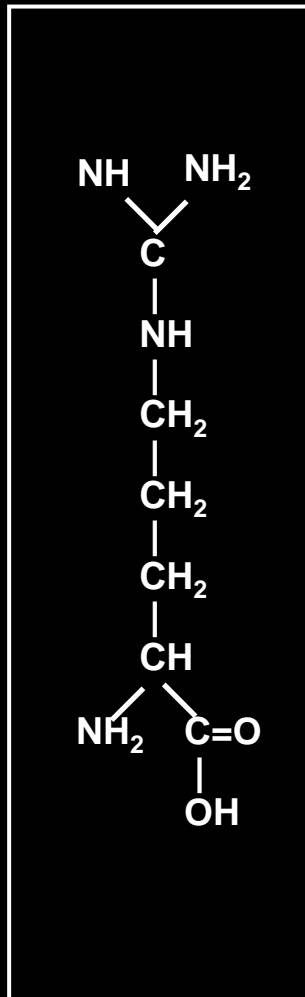
Nakata S, Tsutsui M, et al. *ATVB* 2007

Nakata S, Tsutsui M, et al. *ATVB* 2005

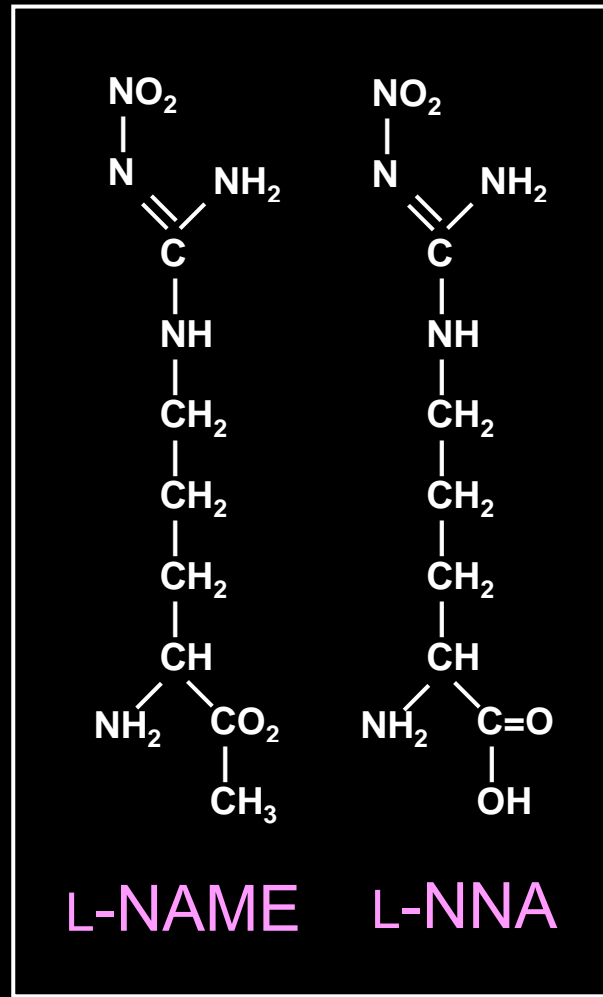
Morishita T, Tsutsui M, et al. *FASEB J* 2002

# L-Arginine and its Synthetic and Endogenous Analogues

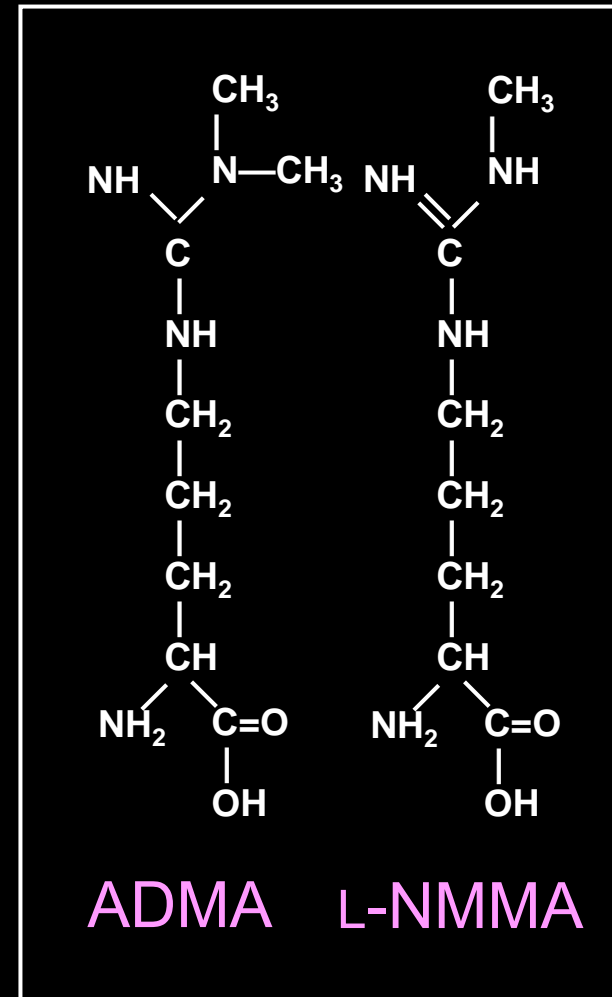
L-Arginine



Synthetic analogues



Endogenous analogues





# Previously Assumed Mechanism for Arteriosclerotic Vascular Lesion Formation Caused by Long-Term Treatment with L-Arginine Analogue

Long-term treatment with L-arginine analogue

L-NAME, ADMA, etc

Inhibition of endothelial NOS activity

Inhibition of endothelial NO synthesis

Arteriosclerotic vascular lesion formation

# Coronary Vascular Lesion Formation Caused by Long-Term Treatment with L-NAME in Wild-Type and eNOS-KO Mice

Wild-type mice

eNOS-KO mice

Control

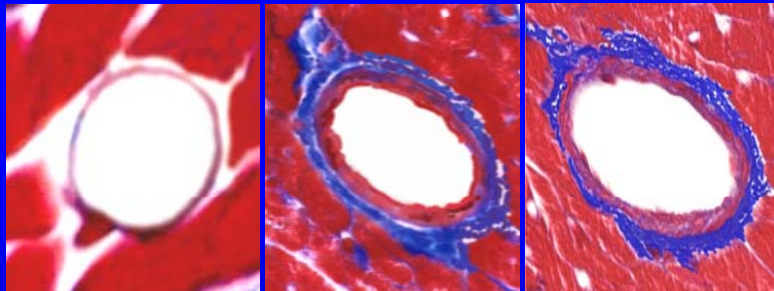
L-NAME

L-NAME  
+L-arginine

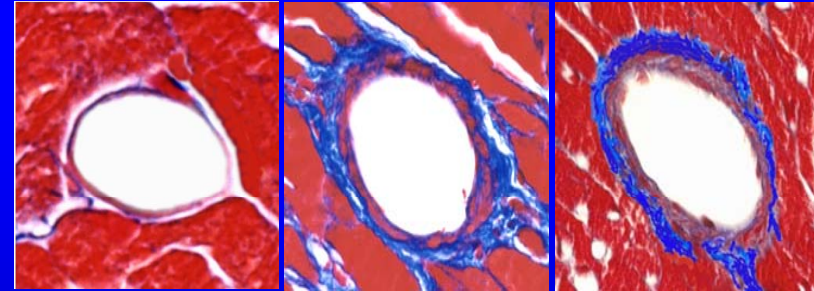
Control

L-NAME

L-NAME  
+L-arginine



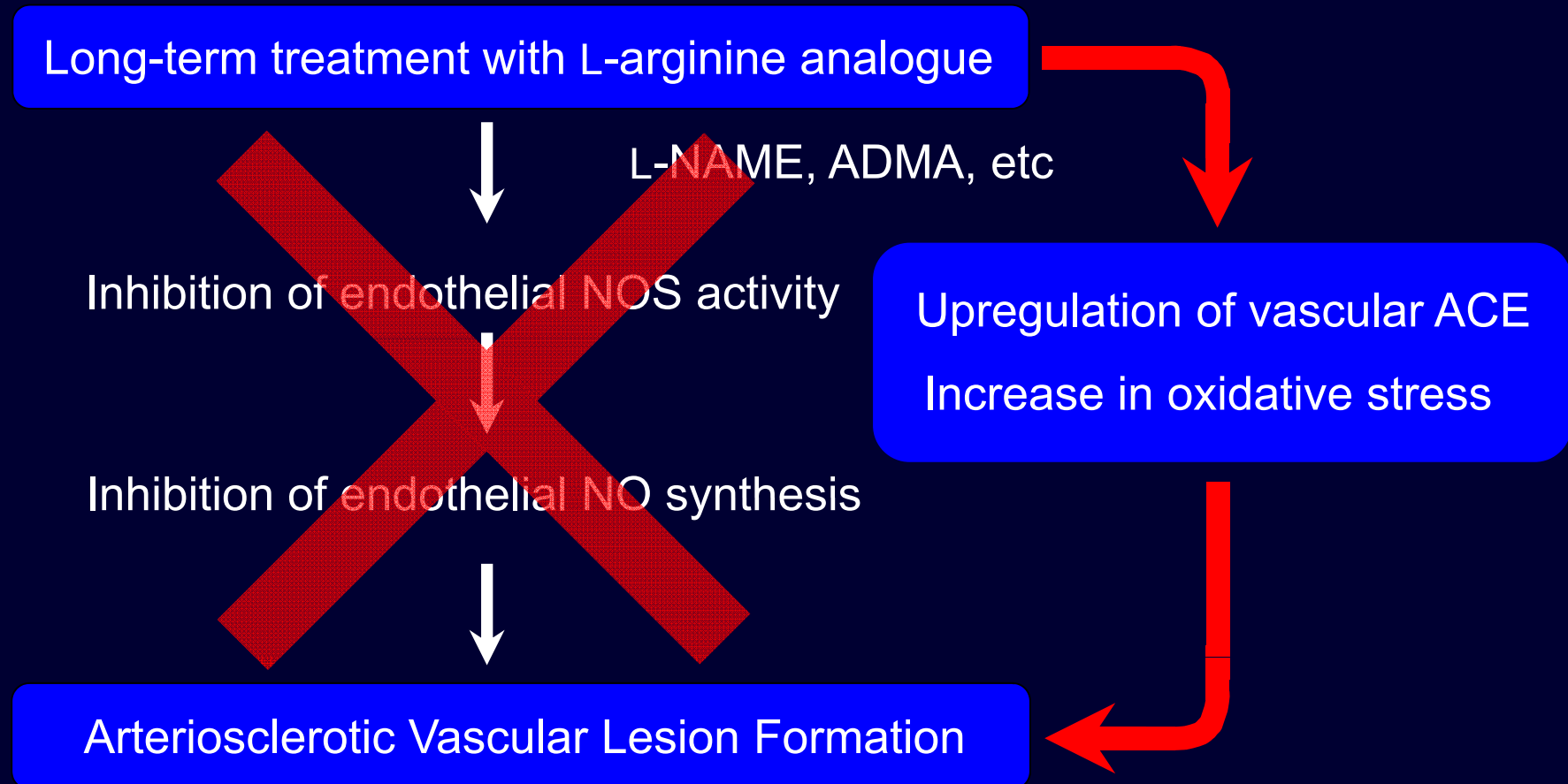
50  $\mu$ m



50  $\mu$ m

Suda, Tsutsui, et al. *Circulation* 2002

# Recently Clarified Mechanism for Arteriosclerotic Vascular Lesion Formation Caused by Long-Term Treatment with L-Arginine Analogue



Suda O, Tsutsui M, et al. *Circulation* 2002

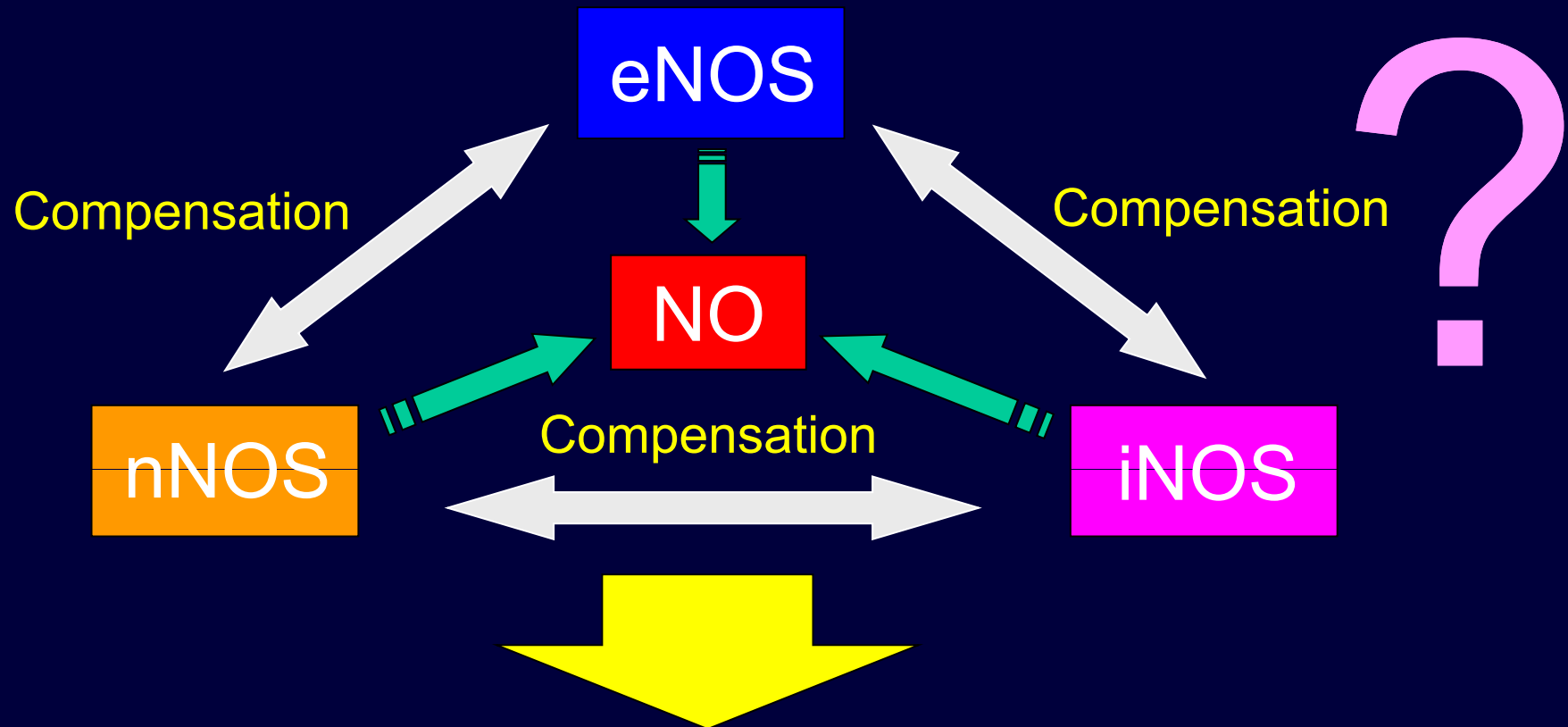
Suda O, Tsutsui M, et al. *ATVB* 2004

Pharmacological studies with NOS inhibitors

→ NOS inhibitors are non-specific

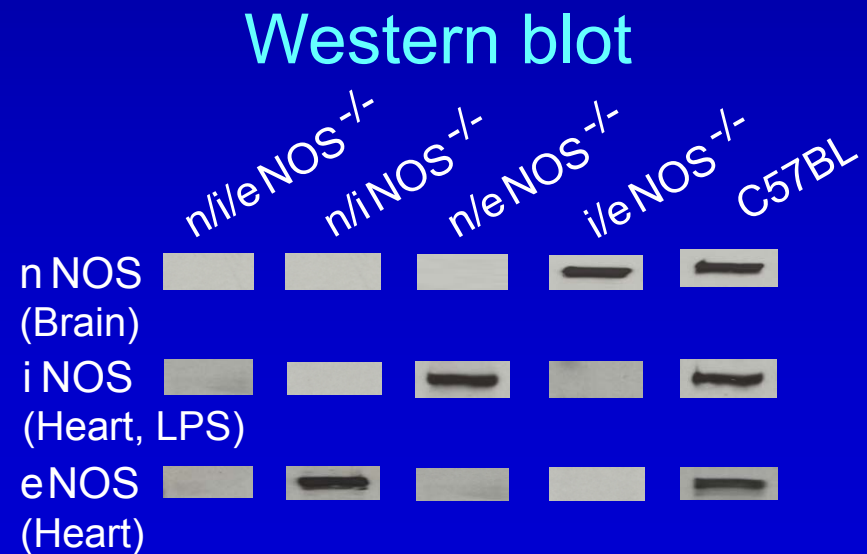
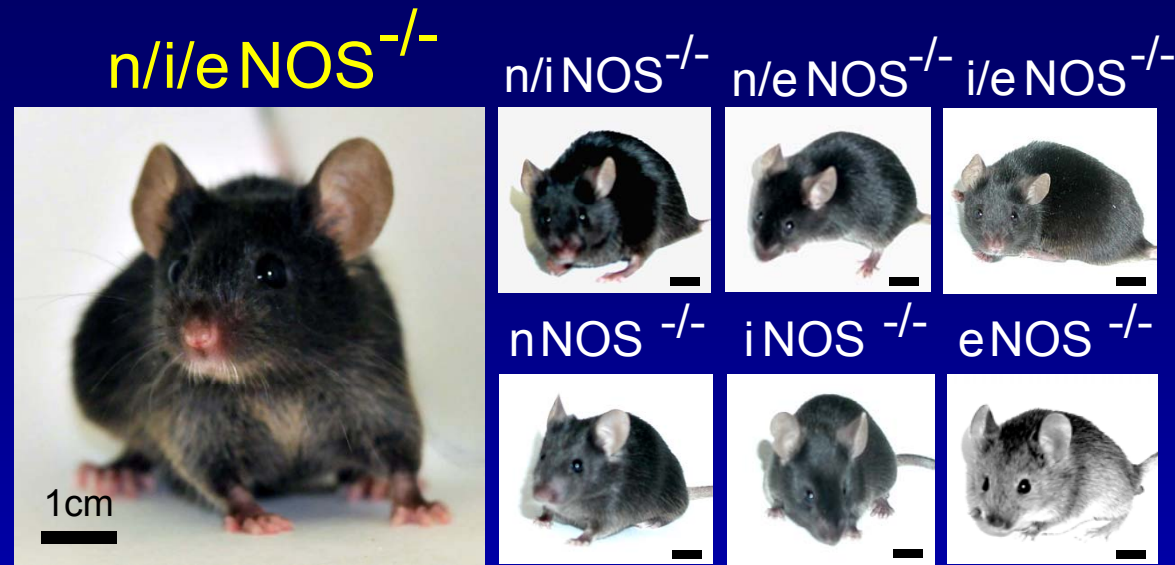
Studies with mice that lack each NOS isoform

→ Compensatory mechanism operates



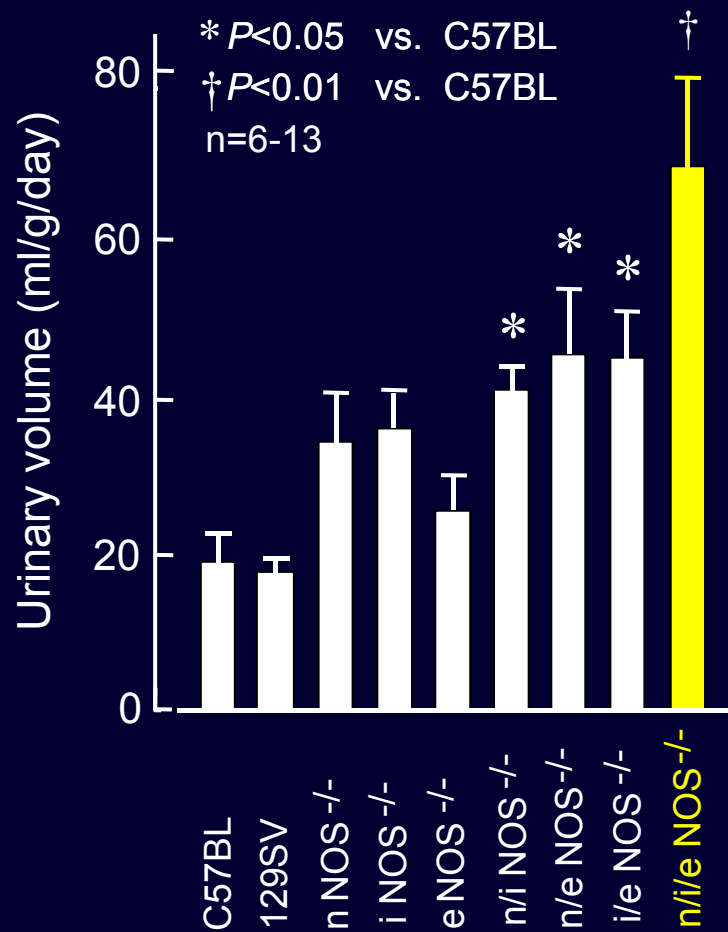
The Idea of Generating Triply NOSs<sup>-/-</sup> Mice

# Development of Mice Lacking All NOS Isoforms

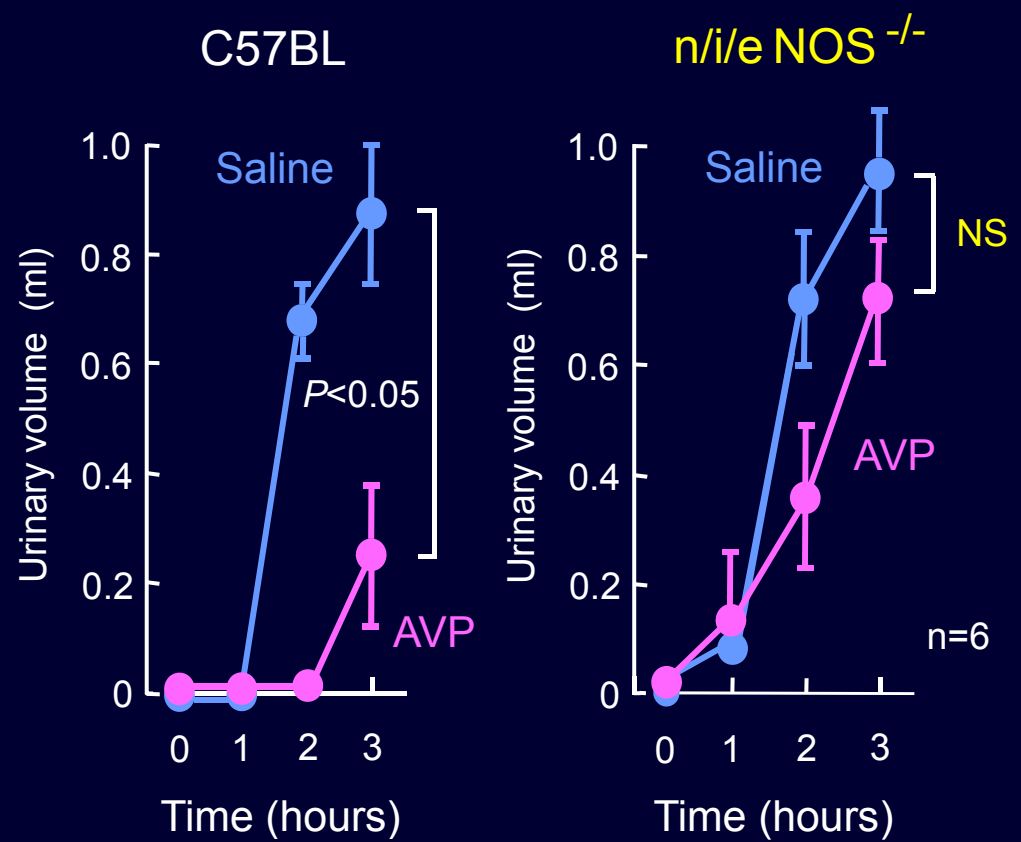


# Nephrogenic Diabetes Insipidus in Mice Lacking All Nitric Oxide Synthase Isoforms

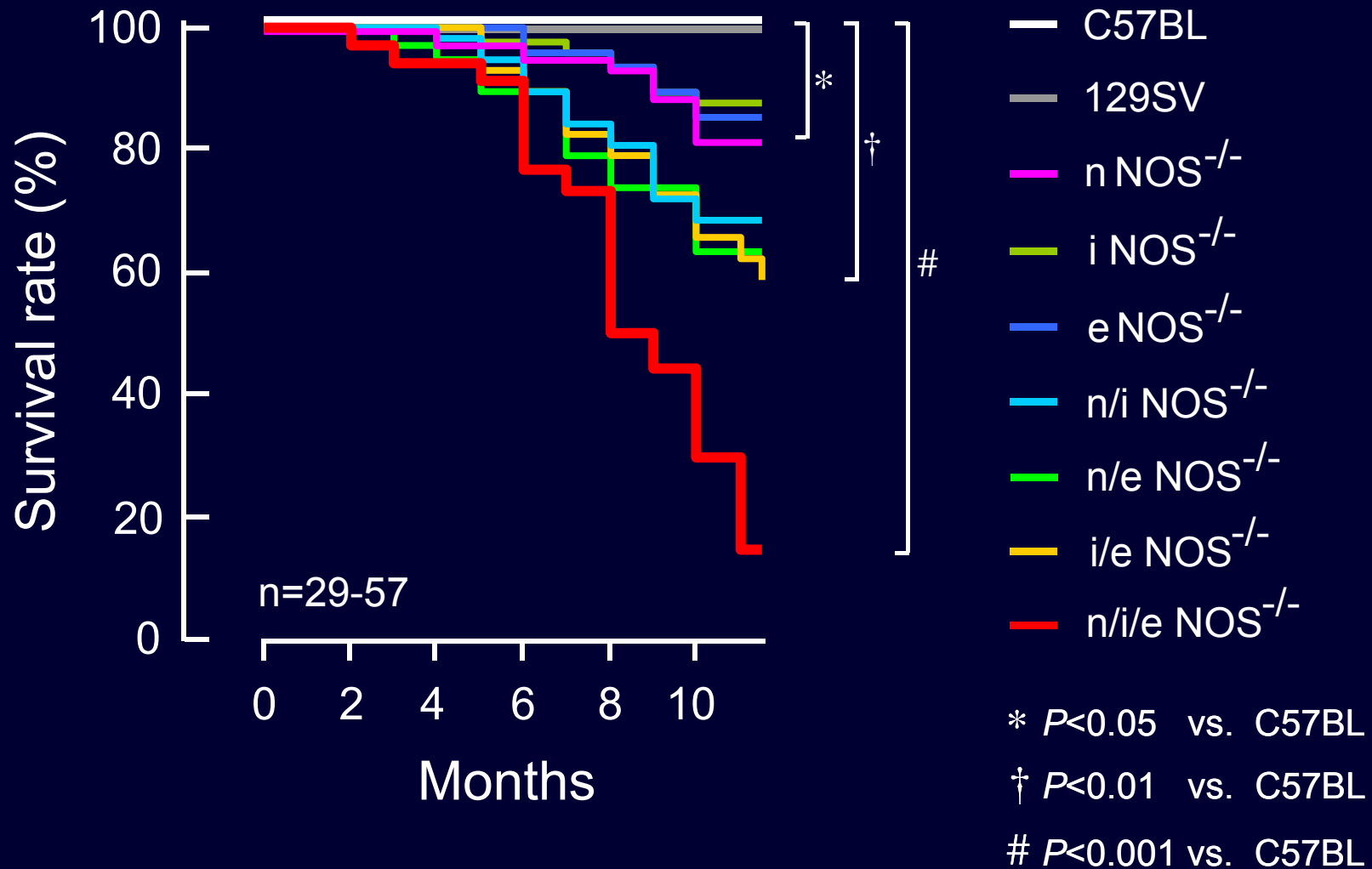
Urinary Volume



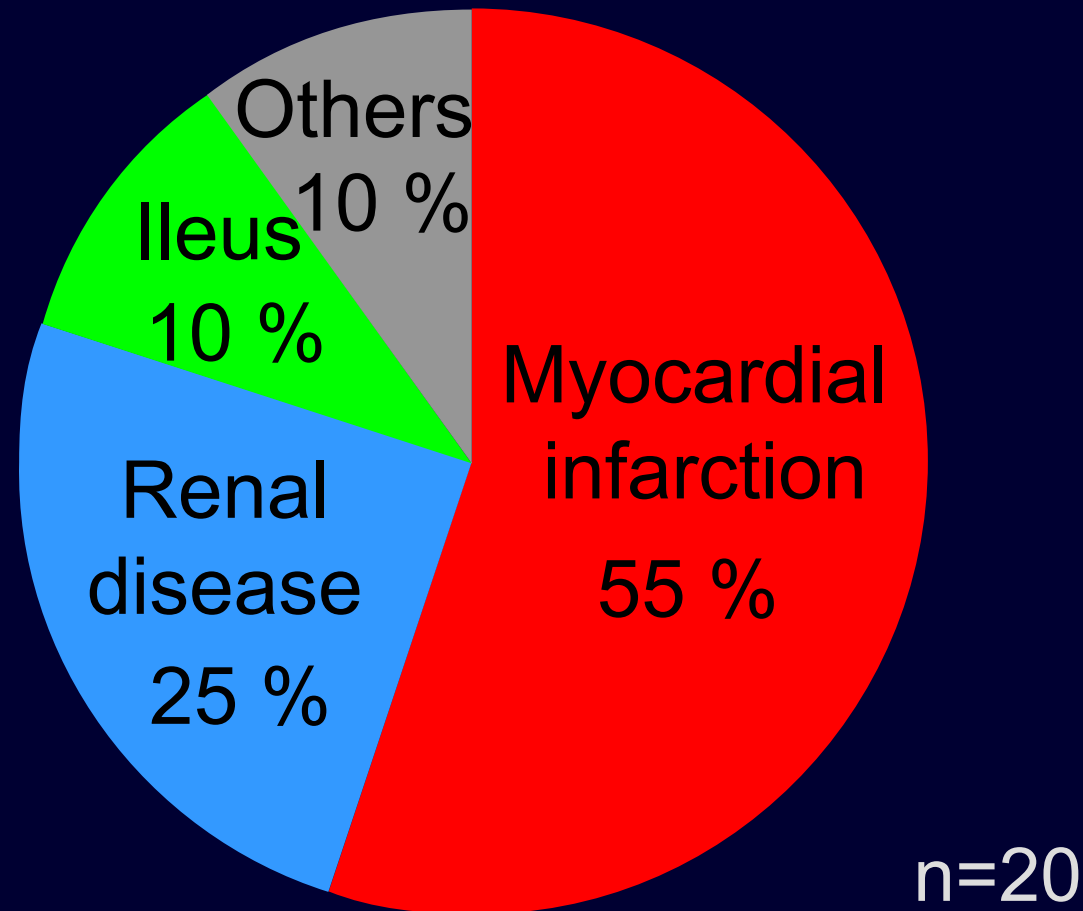
Renal Sensitivity to Exogenous Vasopressin



# Survival Rate in Male Wild-Type and NOS<sup>-/-</sup> Mice



# Cause of Death in Male Triply n/i/eNOS<sup>-/-</sup> Mice



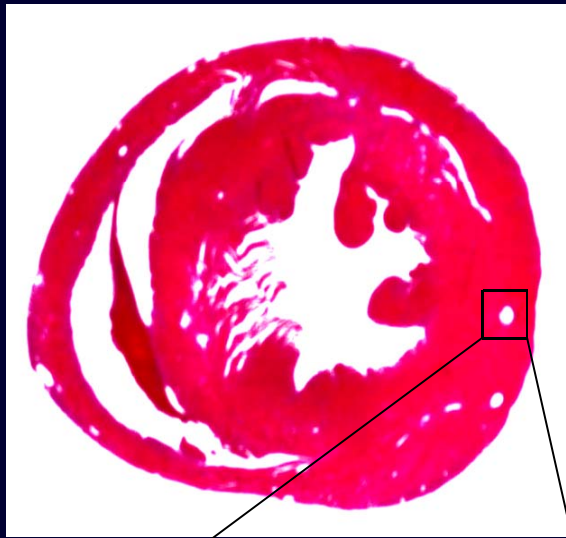


# Acute Myocardial Infarction in Male Triply $n/i/eNOS^{-/-}$ Mice that Died at 8 Months of Age

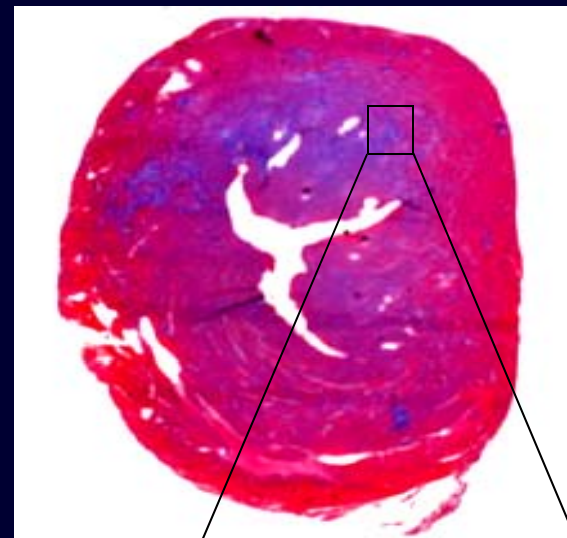
Normal wild-type

Dead triply  $n/i/eNOS^{-/-}$

Heart

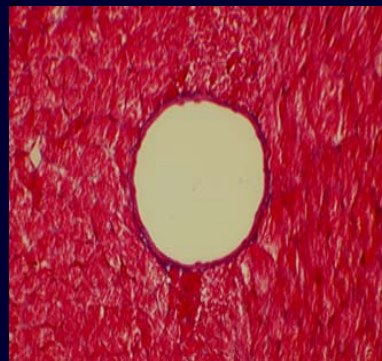


1 mm

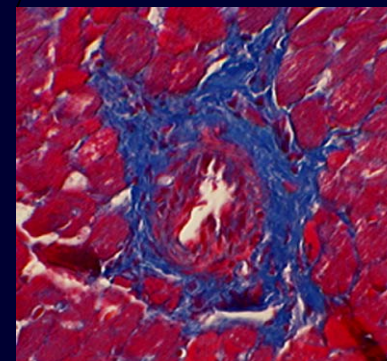


1 mm

Coronary artery



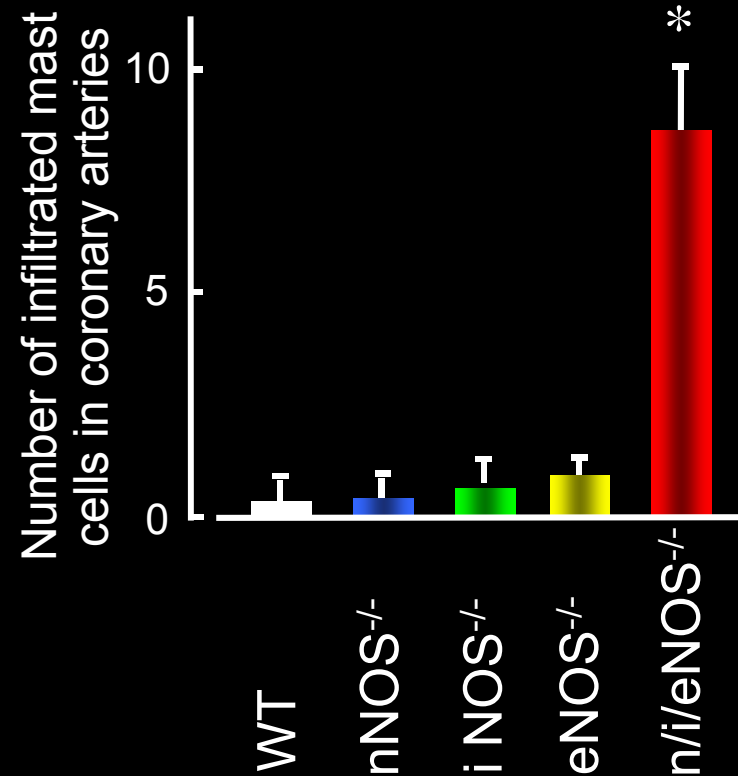
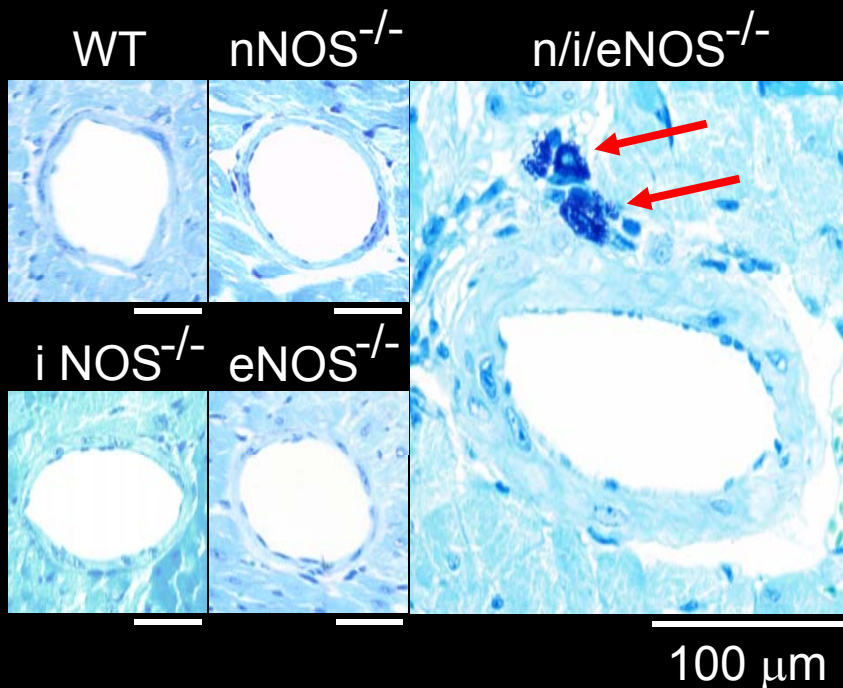
100  $\mu$ m



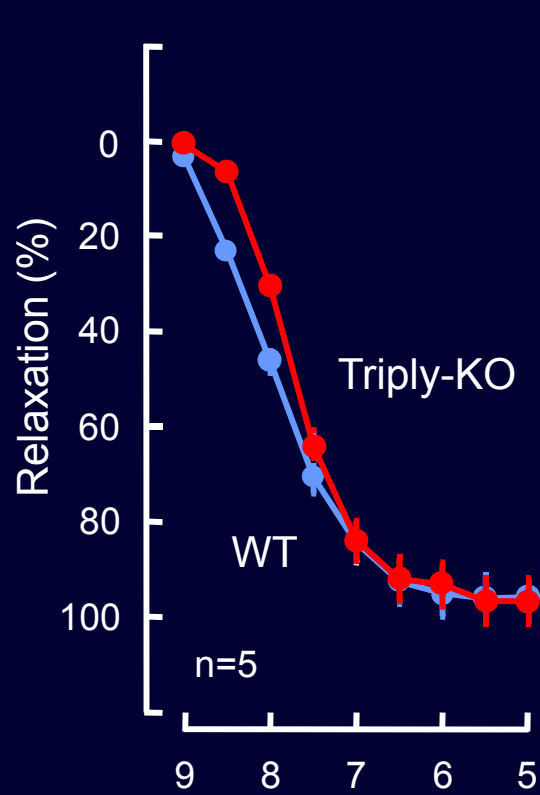
100  $\mu$ m

# Mast Cell Infiltration in the Coronary Artery Adventitia of Dead Triply $n/i/eNOS^{-/-}$ Mice

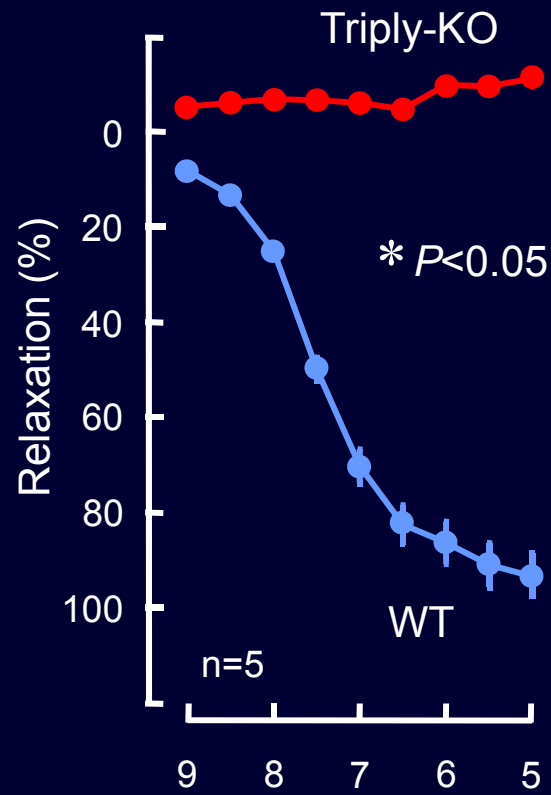
Coronary artery  
(Toluidine-blue staining)



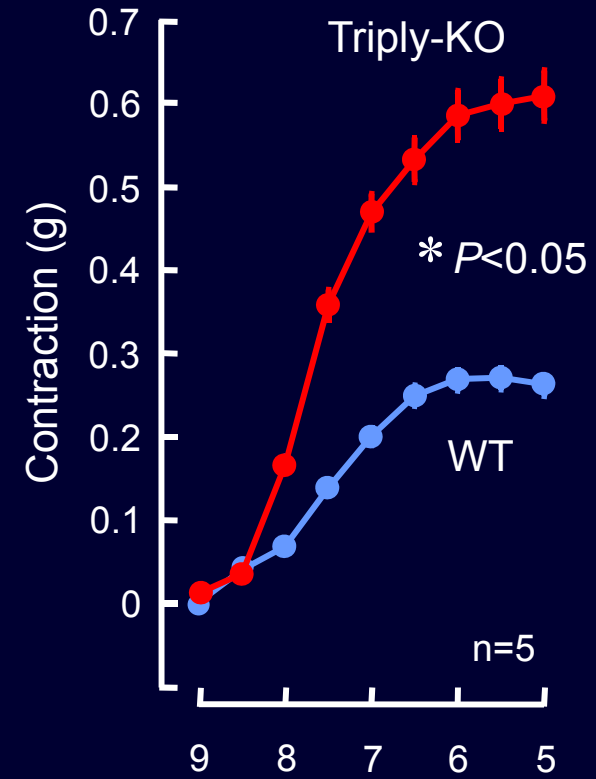
# Vascular Reactivities in Isolated Aortas of Wild-Type and Triply $n/i/eNOS^{-/-}$ Mice



Forskolin  
(-log mol/L)



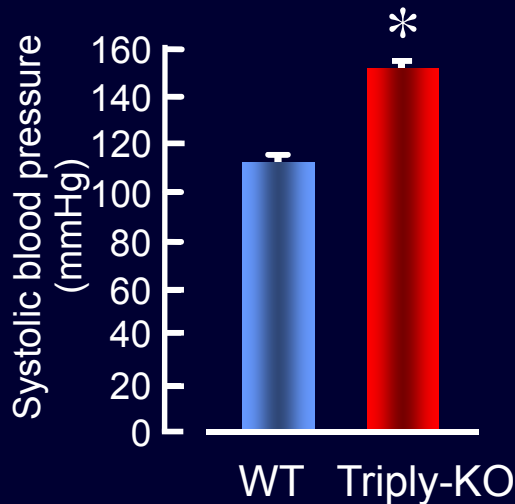
Acetylcholine  
(-log mol/L)



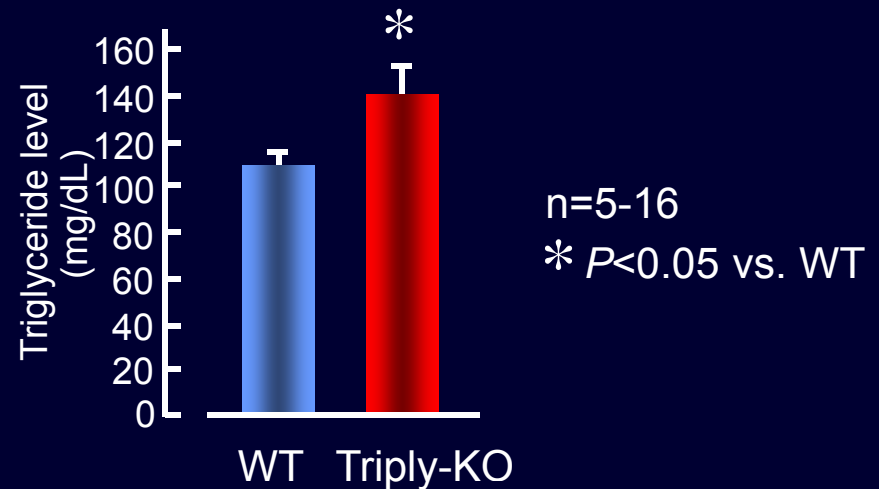
Phenylephrine  
(-log mol/L)

# Metabolic Phenotypes in Triply n/i/eNOS<sup>-/-</sup> Mice

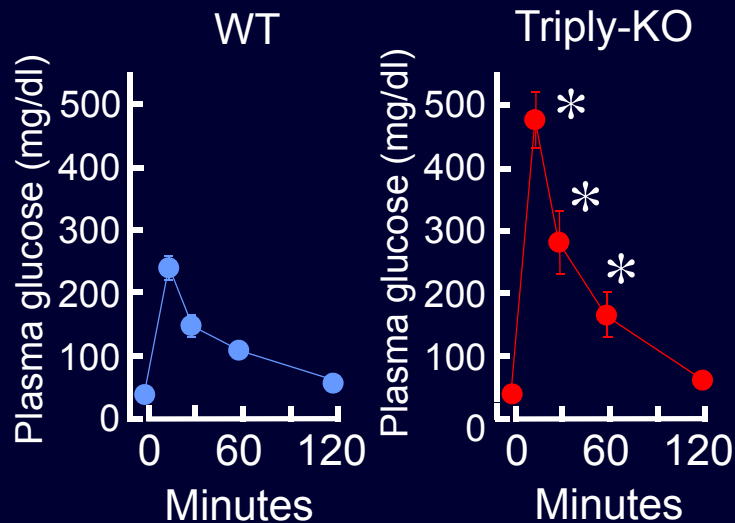
## Hypertension



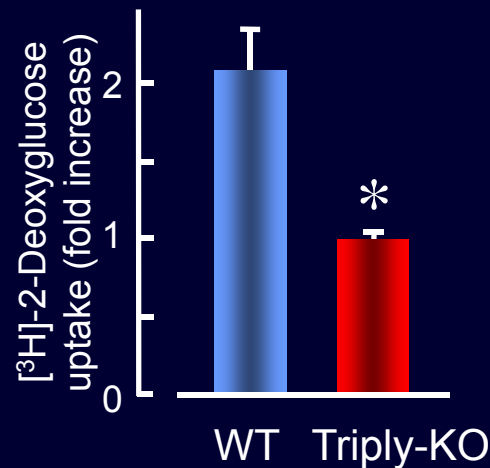
## Hypertriglyceridemia



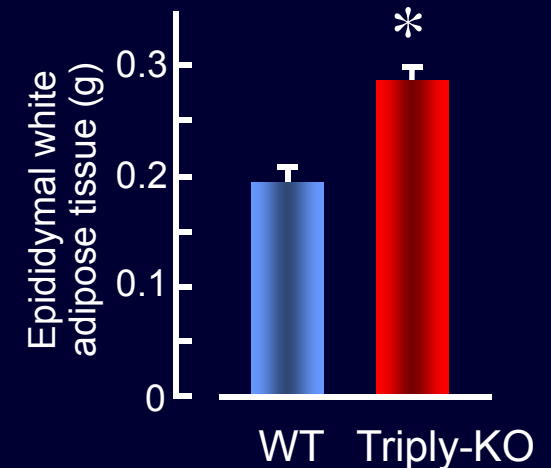
## Glucose Intolerance



## Insulin Resistance

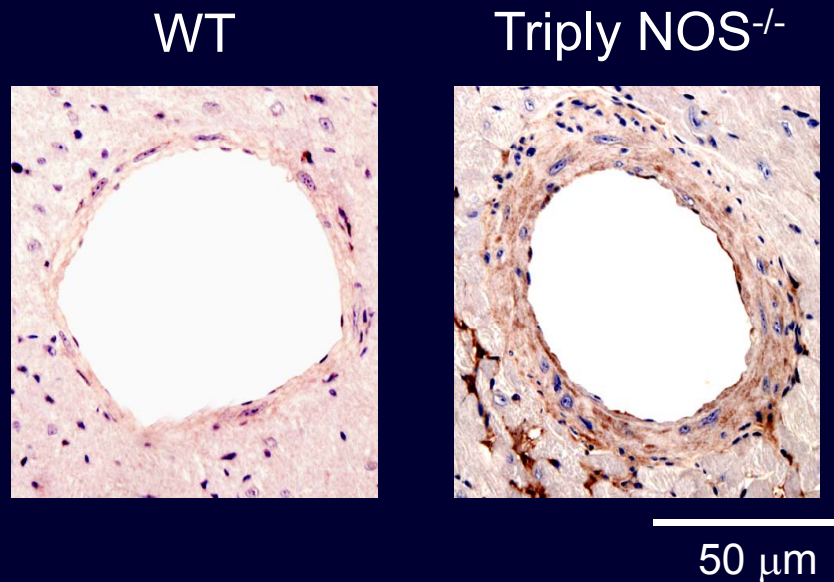


## Visceral Obesity

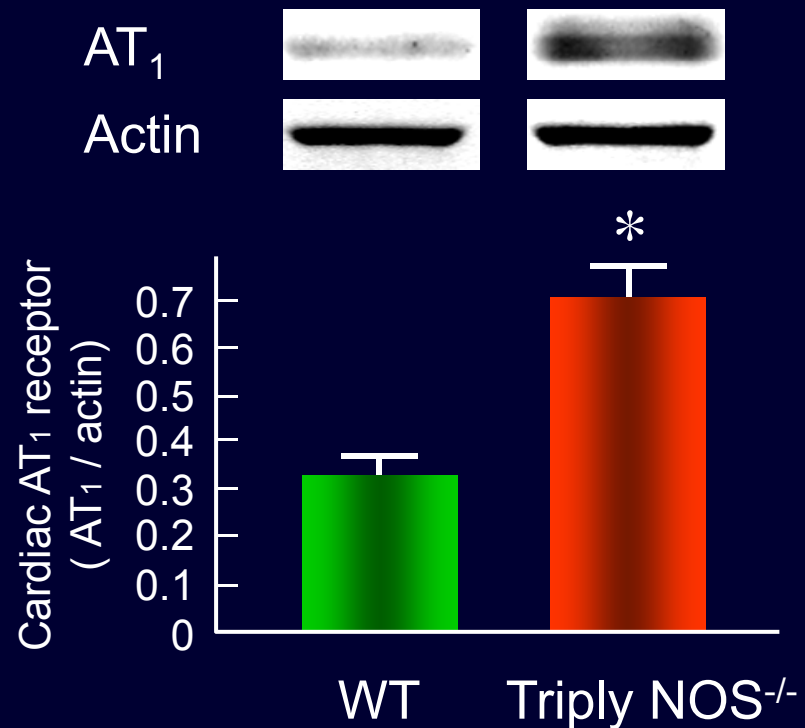


# Expression Levels of Angiotensin II Type 1 (AT<sub>1</sub>) Receptor in the Heart of Wild-Type and Triply n/i/eNOS<sup>-/-</sup> Mice

Immunostaining  
(Coronary artery)



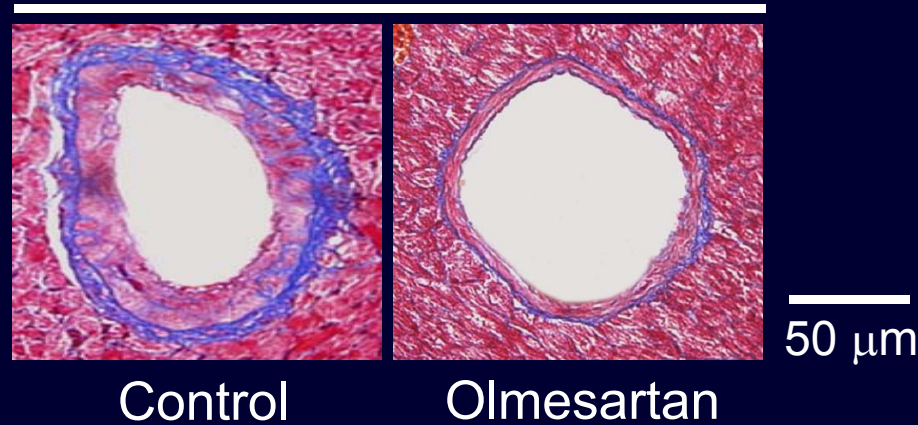
Western blot



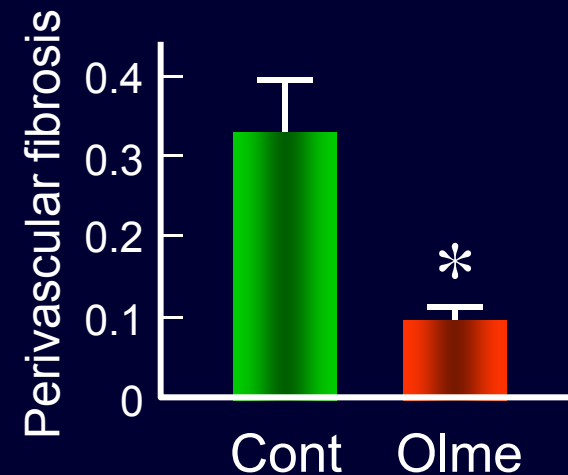
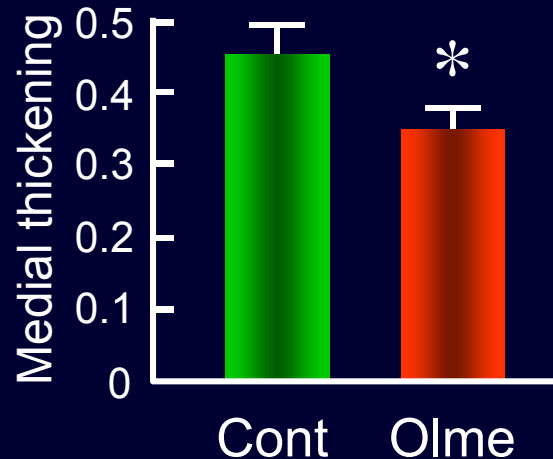
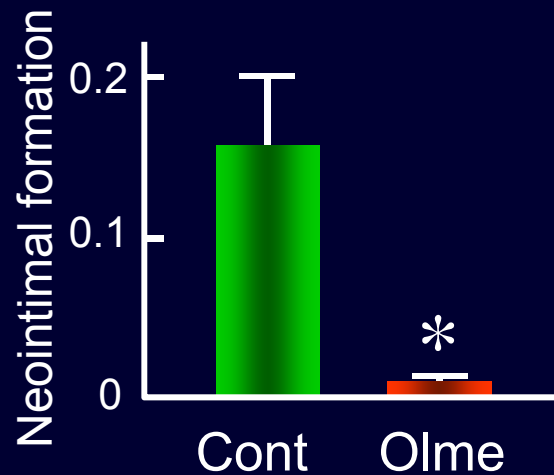
# Effect of Long-Term Treatment with Olmesartan on Coronary Arteriosclerotic Lesion Formation in Triply $n/i/eNOS^{-/-}$ Mice

Triply  $NOS^{-/-}$

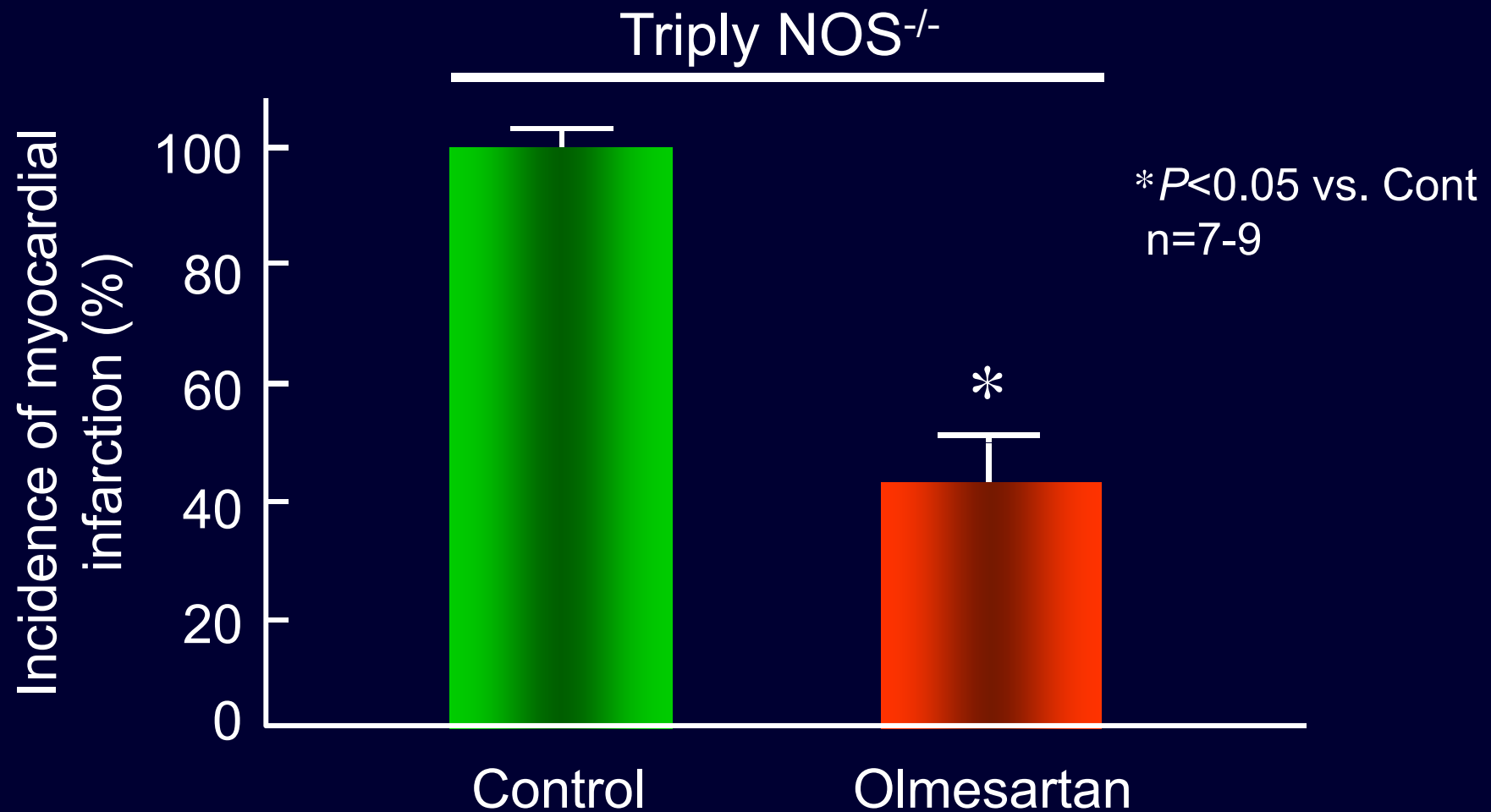
Coronary artery



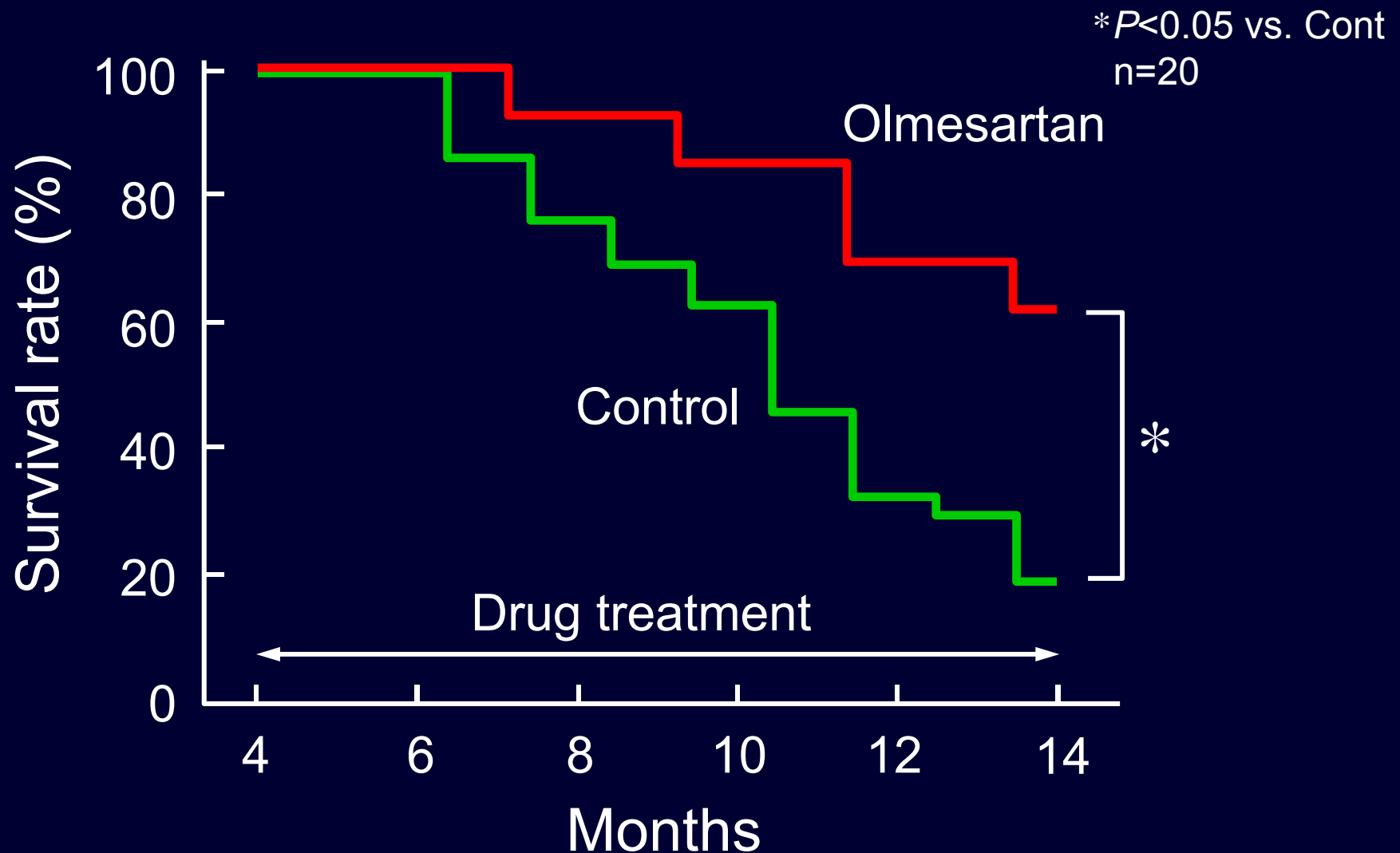
\* $P < 0.05$  vs. Cont  
n=6



# Effect of Long-Term Treatment with Olmesartan on the Incidence of Myocardial Infarction in Triply n/i/eNOS<sup>-/-</sup> Mice

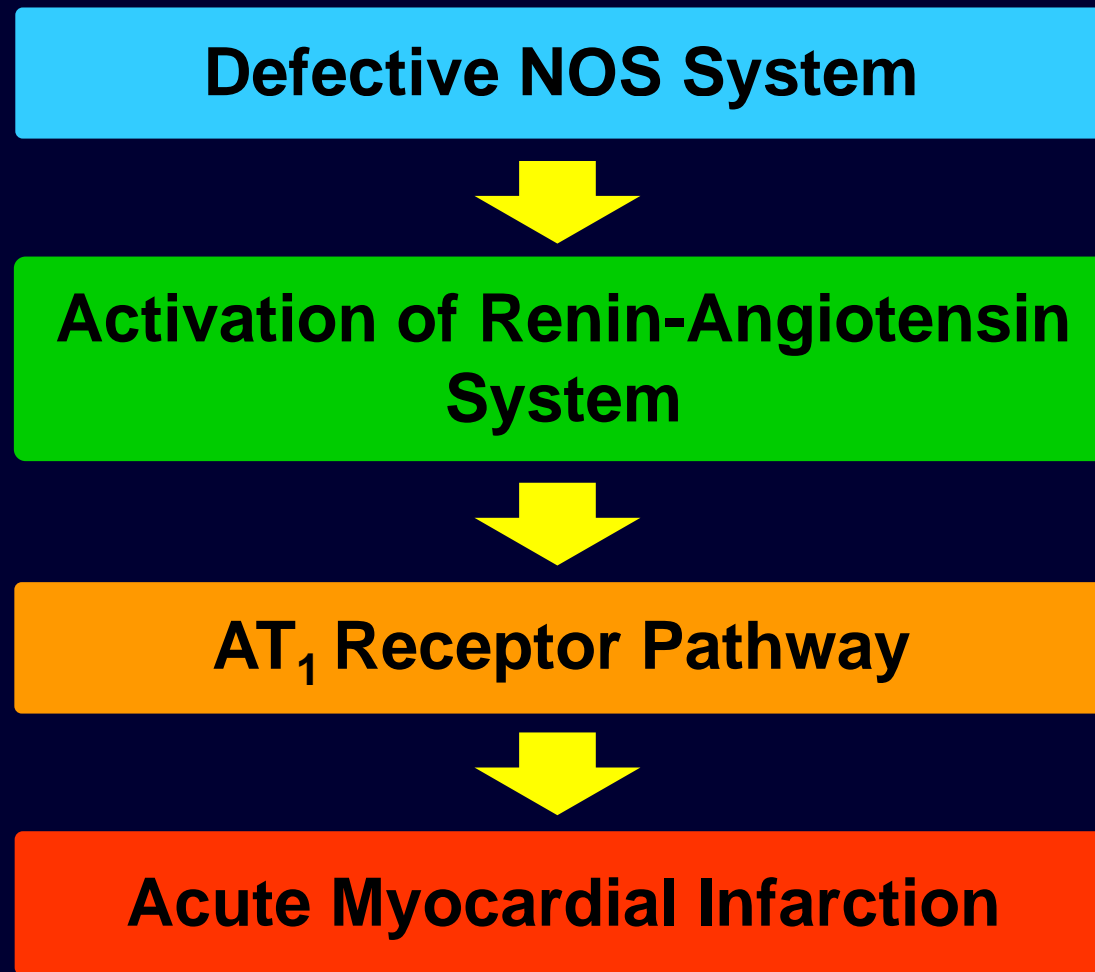


# Effect of Long-Term Treatment with Olmesartan on Survival Rate in Triply *n/i/eNOS*<sup>-/-</sup> Mice



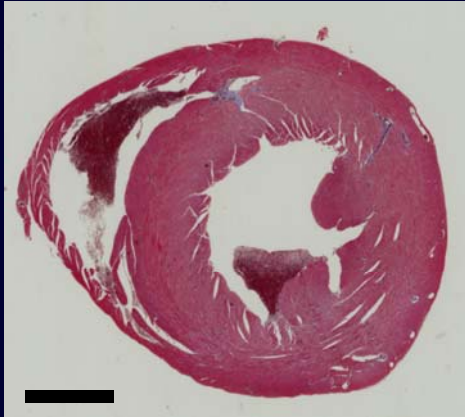


# Involvement of AT<sub>1</sub> Receptor Pathway in the Pathogenesis of Myocardial Infarction in Triply NOS<sup>-/-</sup> Mice

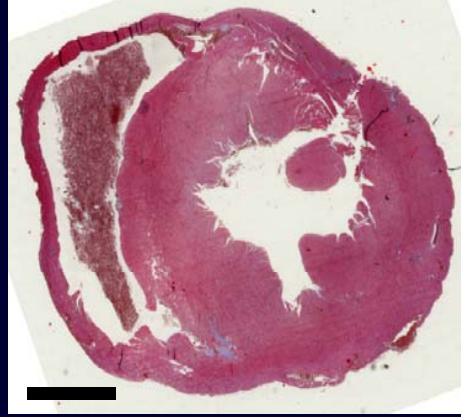


# Cross Section of the Heart in Wild-Type and NOS<sup>-/-</sup> Mice at 5 Months of Age

WT



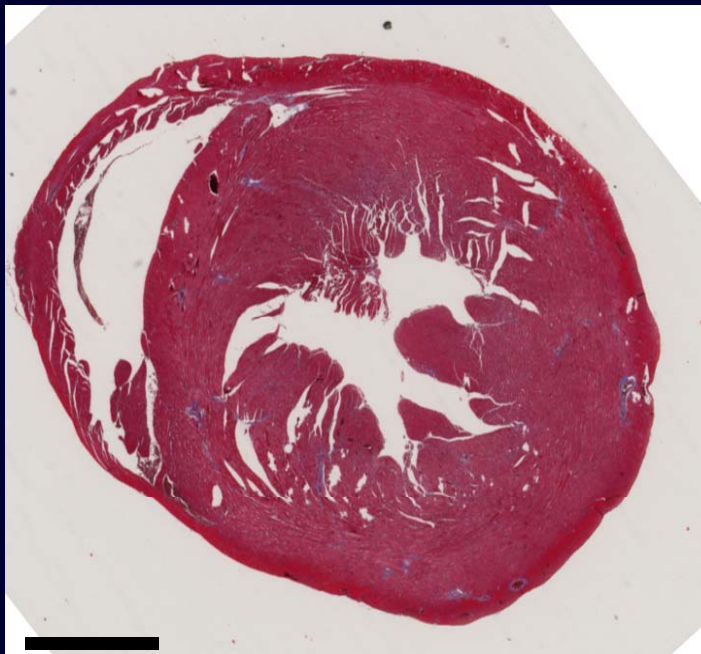
nNOS<sup>-/-</sup>



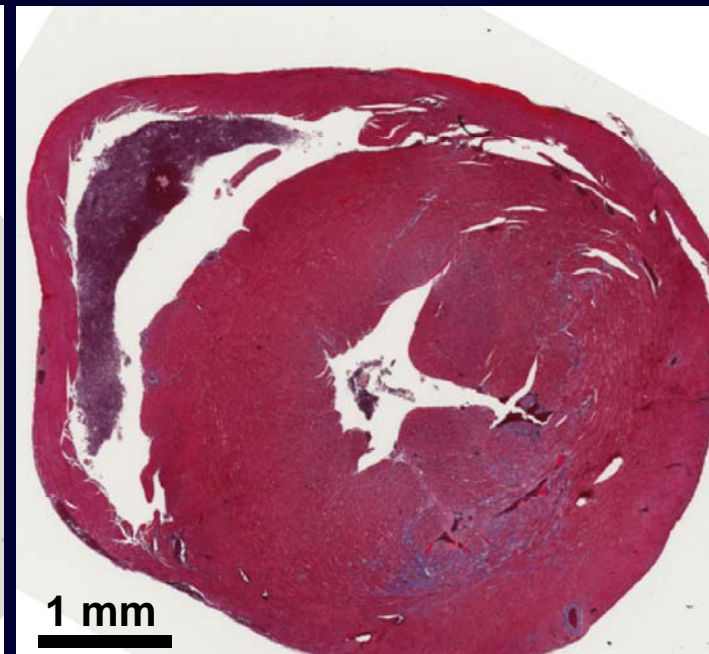
iNOS<sup>-/-</sup>



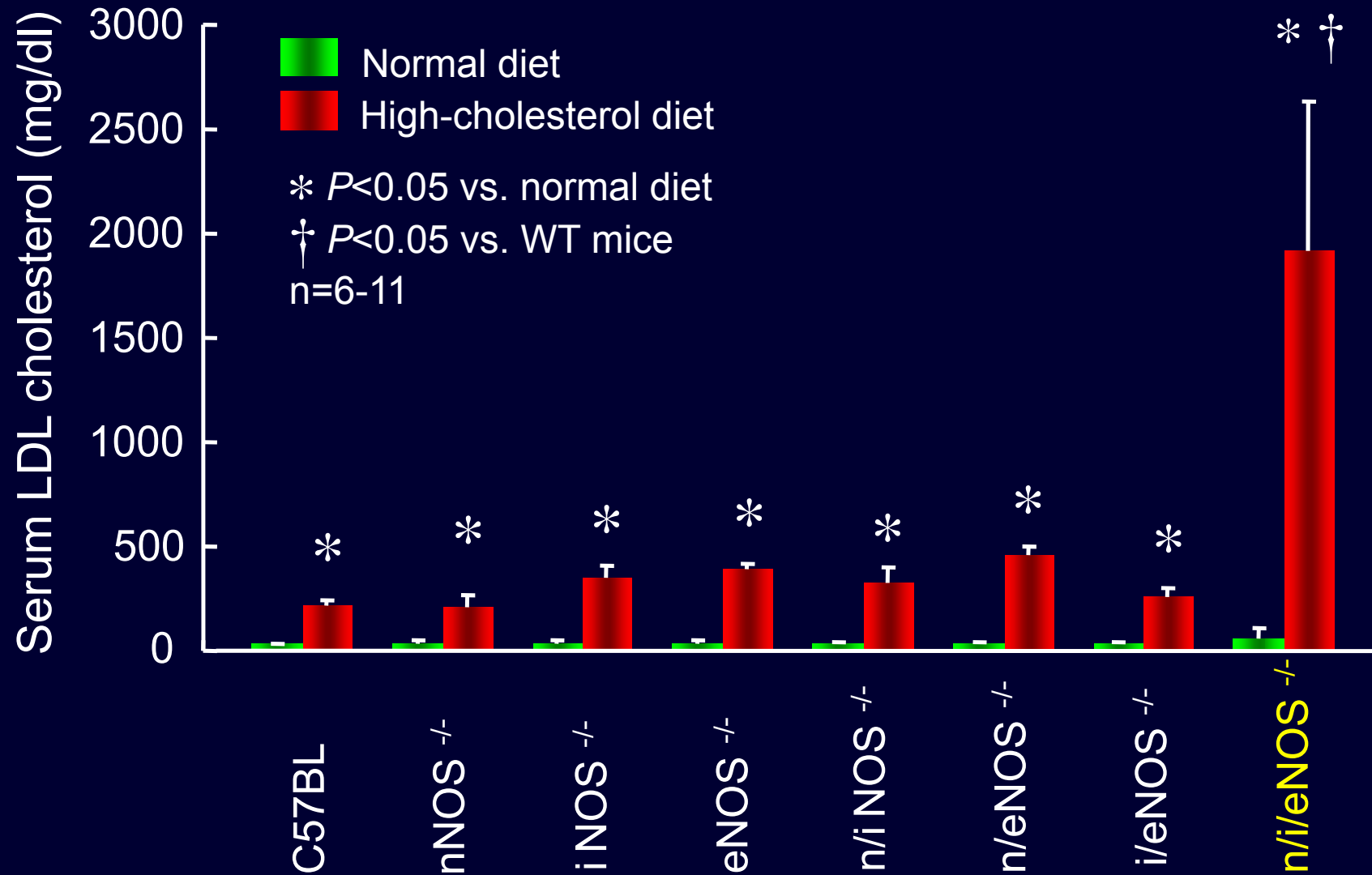
eNOS<sup>-/-</sup>



n/i/e NOS<sup>-/-</sup>



## Serum Low Density Lipoprotein (LDL) Cholesterol Levels in Wild-Type and NOS<sup>-/-</sup> Mice Fed Either a Normal or a High-Cholesterol Diet for 3 Months



# Oil-Red O Staining in Longitudinally Opened Aorta of Wild-Type and NOS <sup>-/-</sup> Mice Fed a High-Cholesterol Diet for 3 Months

Triply n/i/eNOS <sup>-/-</sup>



nNOS <sup>-/-</sup>



n/i NOS <sup>-/-</sup>



i NOS <sup>-/-</sup>



n/eNOS <sup>-/-</sup>



eNOS <sup>-/-</sup>



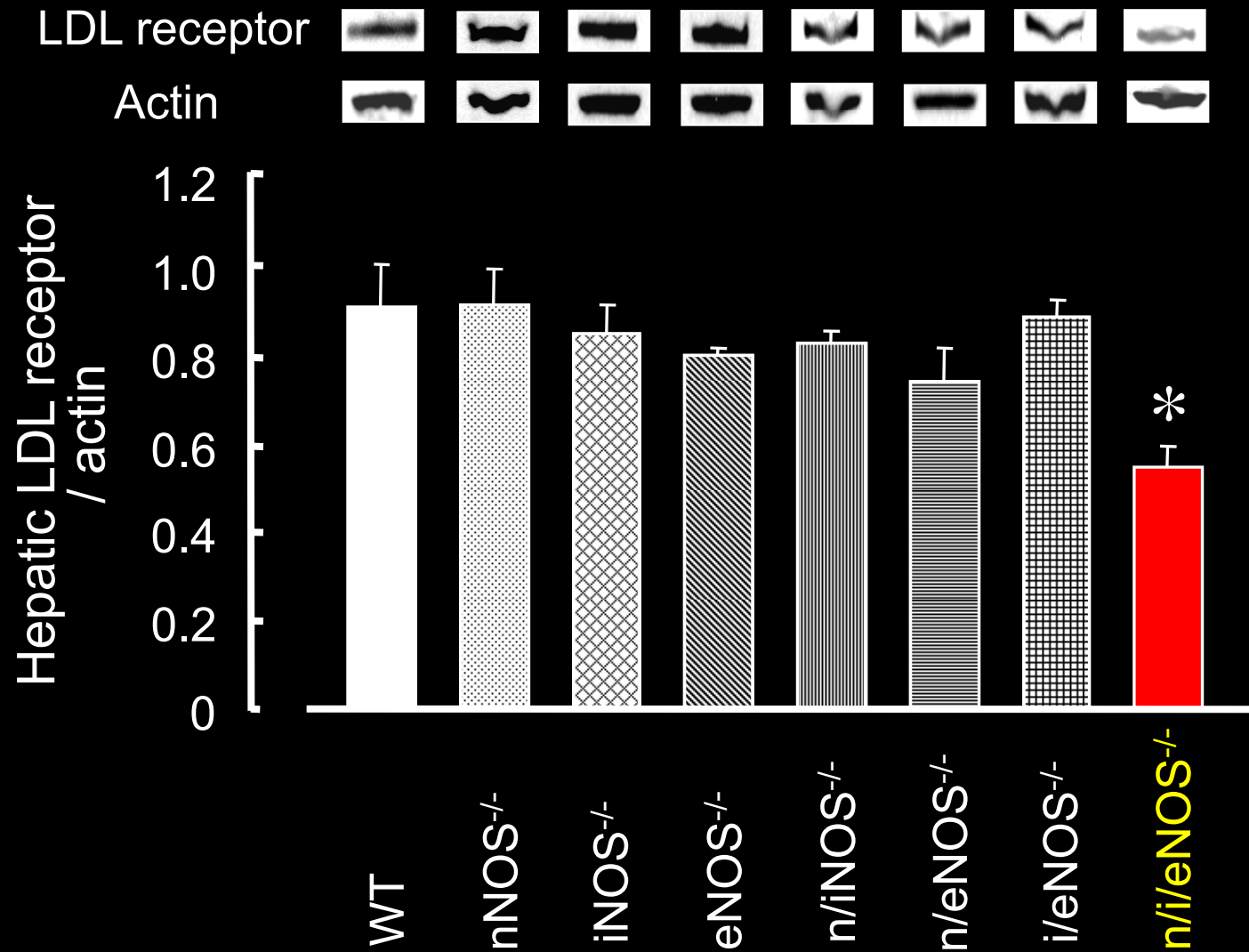
i/eNOS <sup>-/-</sup>



WT



# Expression levels of Hepatic Low-Density Lipoprotein (LDL) Receptor in Wild-Type and NOS<sup>-/-</sup> Mice



# A Variety of Cardiovascular Phenotypes in Mice Lacking All NOS Isoforms

