Oxidant stress:

endothelial mechanisms

J-L Balligand

UCL, Brussels

jl.balligand@uclouvain.be

Funding by FONDATION LEDUCQ

EUGeneHeart

Genomics of Cardiomyocyte Signaling
to Treat and Prevent Heart Failure

Reactions of oxidants with biological targets

Oxidants can damage virtually all biological molecules

DNA, RNA, cholesterol, lipids, carbohydrates, proteins and antioxidants

Extent of target damage targets depends on many factors

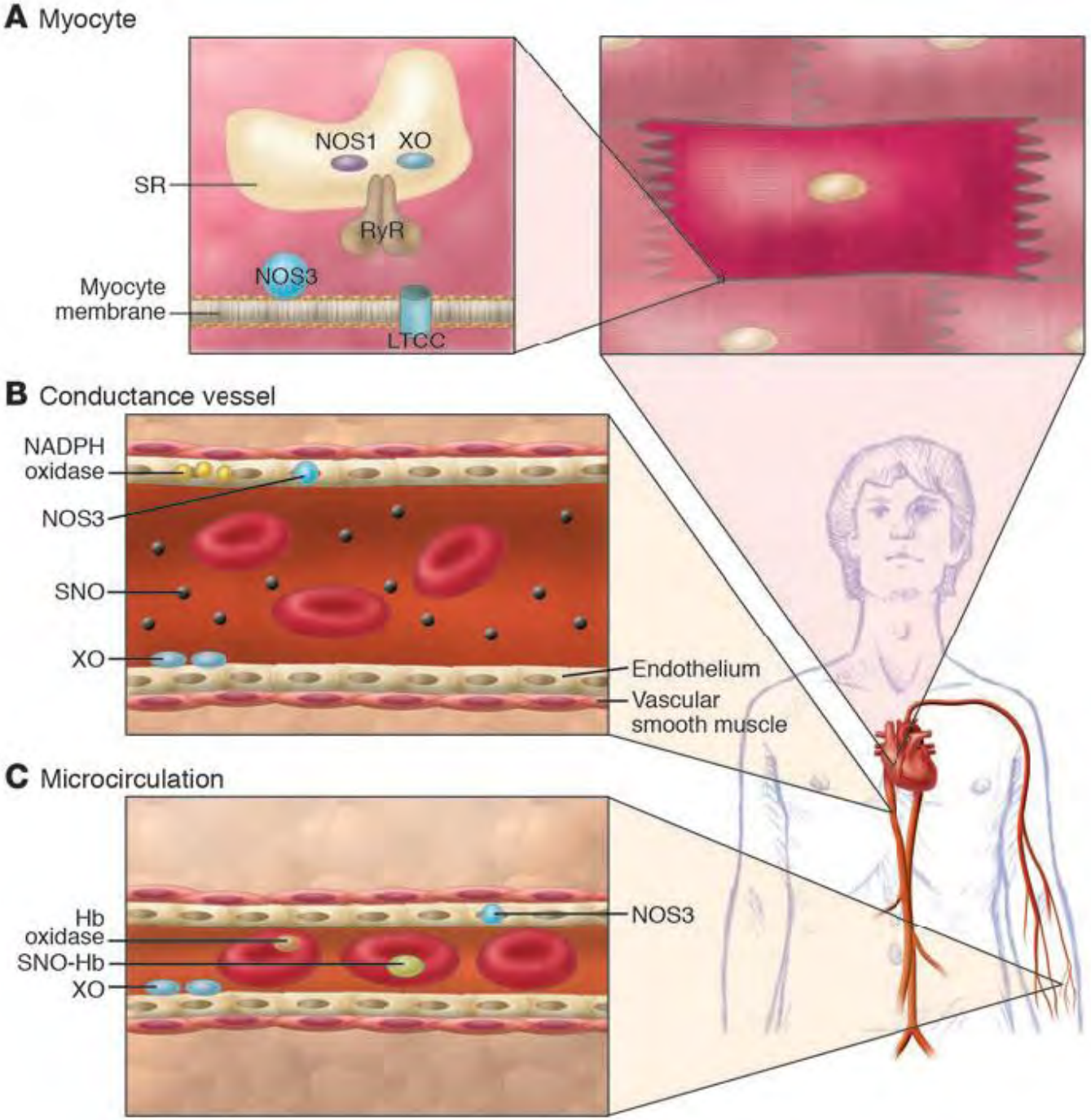
concentration of target
rate constant for reaction of oxidant with target
location of target versus oxidant
occurrence of secondary damaging events
occurrence of transfer reactions
repair and scavenging reactions

Biochem. J., 1997, 324, 1-18; Biochim. Biophys. Acta, 2001, 1504, 196-219

CONCENTRATION !

Oxidative **SIGNALING** ⟷ Oxidative **DAMAGE**

LOCATION !


A Myocyte
NOS1
XO
SR
RyR
NOS3
Myocyte membrane
LTCC
B Conductance vessel
NADPH oxidase
NOS3
SNO
XO
Endothelium
Vascular smooth muscle
C Microcirculation
Hb oxidase
SNO-Hb
XO
NOS3

Meta-Analysis: High-Dosage Vitamin E Supplementation May Increase All-Cause Mortality

Edgar R. Miller III, MD, PhD; Roberto Pastor-Barriuso, PhD; Darshan Dalal, MD, MPH; Rudolph A. Riemersma, PhD, FRCPE; Lawrence J. Appel, MD, MPH; and Eliseo Guallar, MD, DrPH

Background: Experimental models and observational studies suggest that vitamin E supplementation may prevent cardiovascular disease and cancer. However, several trials of high-dosage vitamin E supplementation showed non–statistically significant increases in total mortality.

Purpose: To perform a meta-analysis of the dose–response relationship between vitamin E supplementation and total mortality by using data from randomized, controlled trials.

Patients: 135 967 participants in 19 clinical trials. Of these trials, 9 tested vitamin E alone and 10 tested vitamin E combined with other vitamins or minerals. The dosages of vitamin E ranged from 16.5 to 2000 IU/d (median, 400 IU/d).

Data Sources: PubMed search from 1966 through August 2004, complemented by a search of the Cochrane Clinical Trials Database and review of citations of published reviews and meta-analyses. No language restrictions were applied.

Data Extraction: 3 investigators independently abstracted study reports. The investigators of the original publications were contacted if required information was not available.

Data Synthesis: 9 of 11 trials testing high-dosage vitamin E (≥400 IU/d) showed increased risk (risk difference > 0) for all-cause mortality in comparisons of vitamin E versus control. The pooled all-cause mortality risk difference in high-dosage vitamin E trials was 39 per 10 000 persons (95% CI, 3 to 74 per 10 000 persons; $P = 0.035$). For low-dosage vitamin E trials, the risk difference was −16 per 10 000 persons (CI, −41 to 10 per 10 000 persons; $P > 0.2$). A dose–response analysis showed a statistically significant relationship between vitamin E dosage and all-cause mortality, with increased risk of dosages greater than 150 IU/d.

Limitations: High-dosage (≥400 IU/d) trials were often small and were performed in patients with chronic diseases. The generalizability of the findings to healthy adults is uncertain. Precise estimation of the threshold at which risk increases is difficult.

Conclusion: High-dosage (≥400 IU/d) vitamin E supplements may increase all-cause mortality and should be avoided.

Ann Intern Med. 2005;142:37-46. www.annals.org

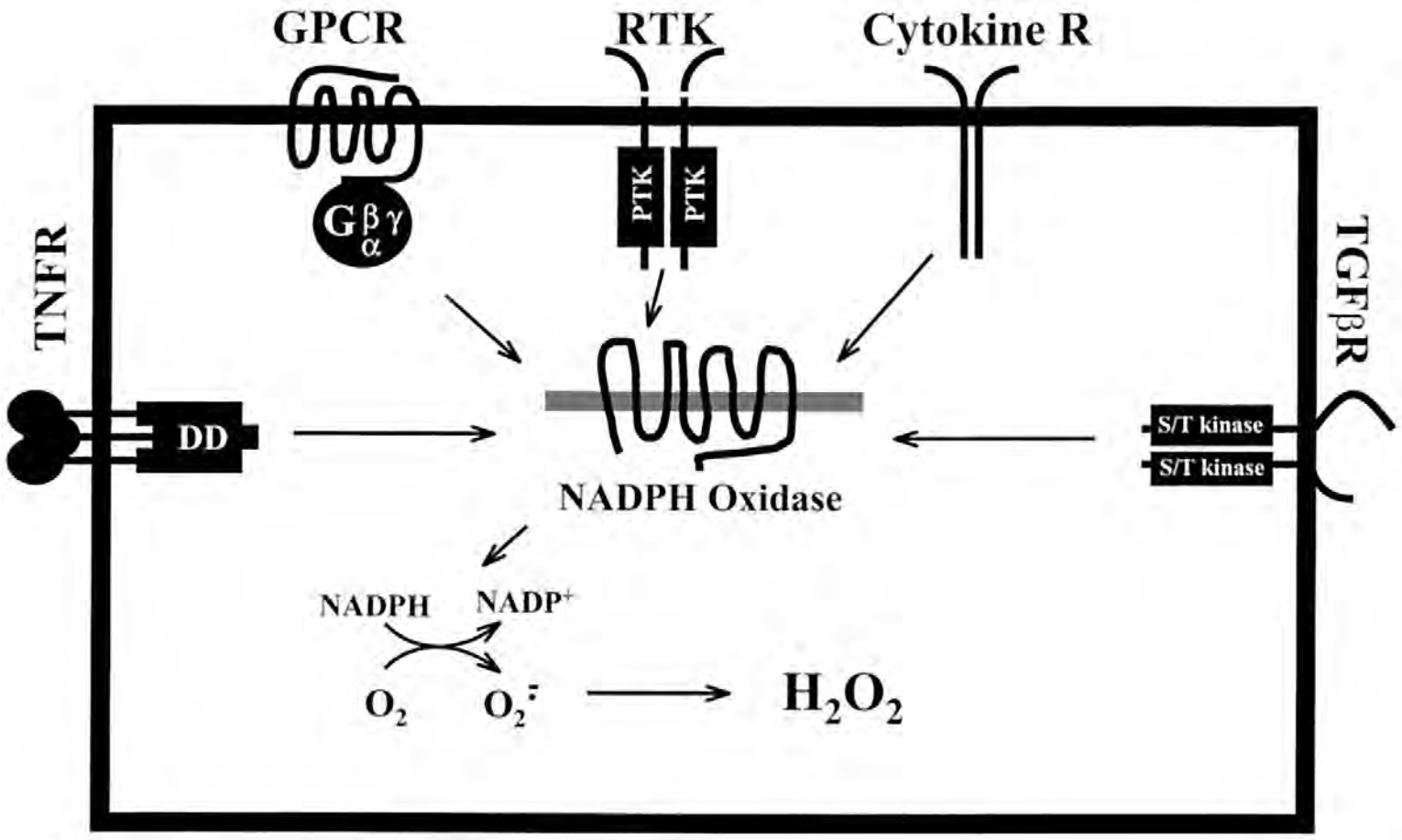
For author affiliations, see end of text.

Re-evaluated 19 vitamin E antioxidant trials between1994 and 2004

136,000 patients in North America, Europe and China

Death rate increased: 1 in 20 chance of dying earlier if 200IU Vitamin E

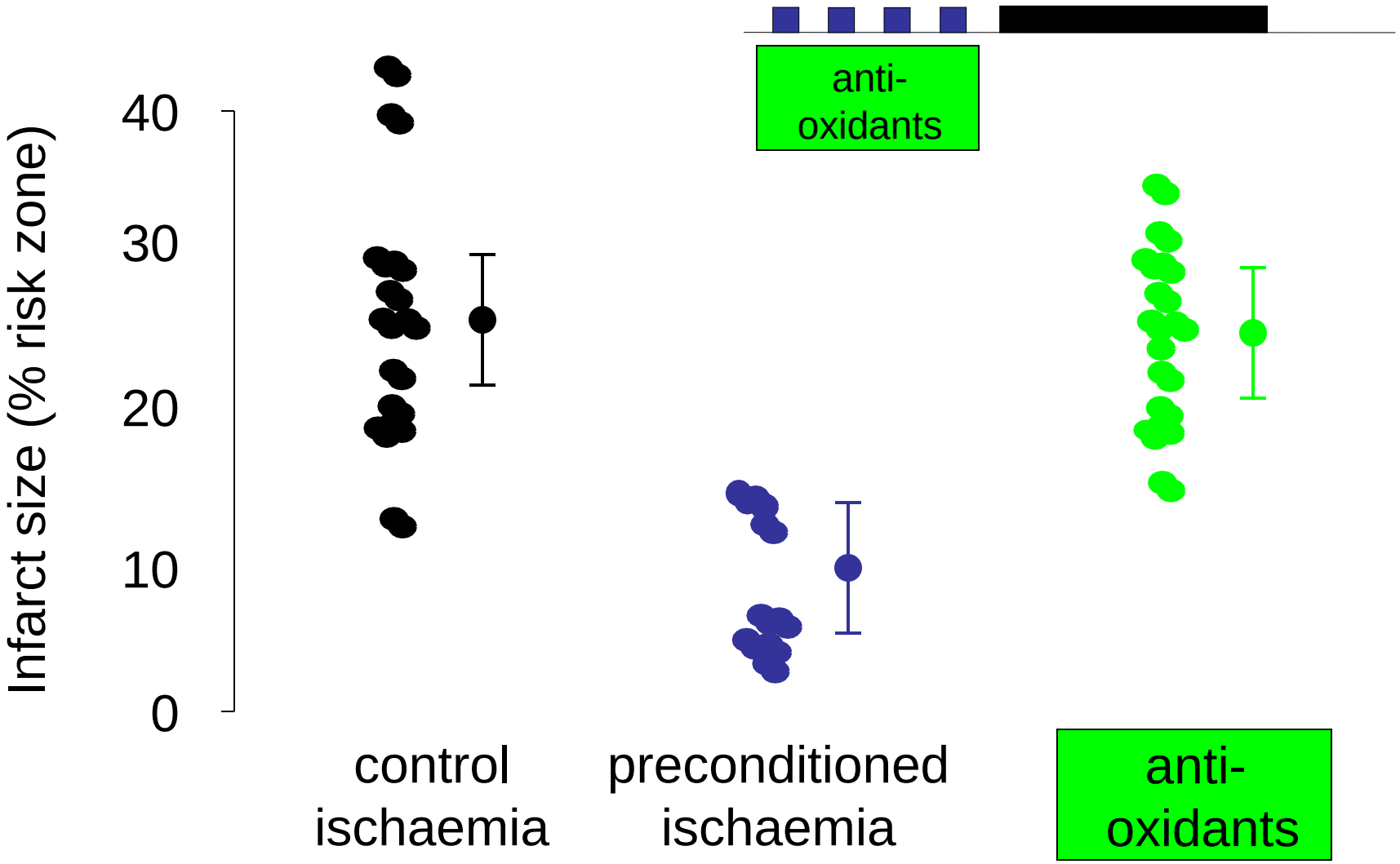
Oxidant production can occur as part of normal function



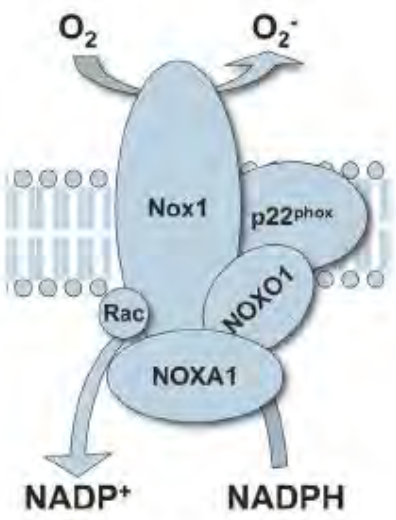
Many classes of surface receptor couple to oxidant production

Rhee *et al.*, J Am Soc Nephrol 14: S211–S215, 2003

Antioxidants block protection by ischemic preconditioning

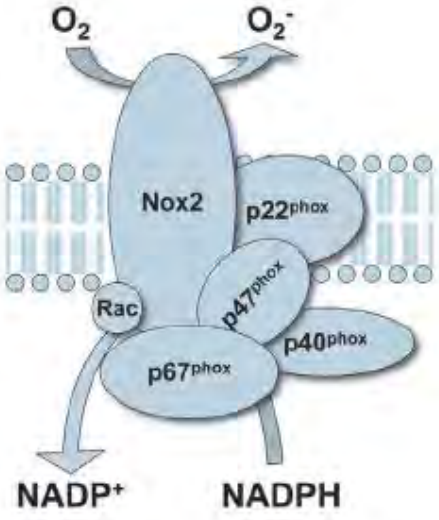


Nox1



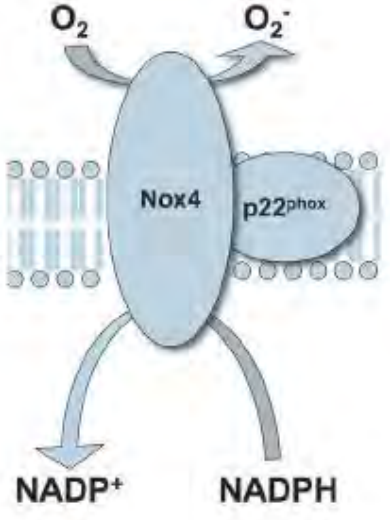
VSMC
(EC)

Nox2



EC
Fibroblast
Inflammatory cells
Human VSMC

Nox4

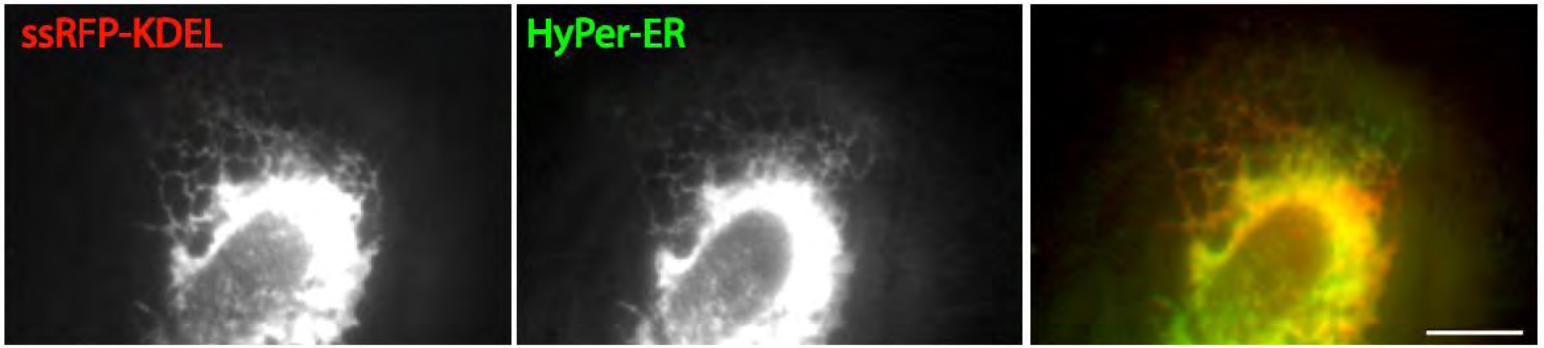


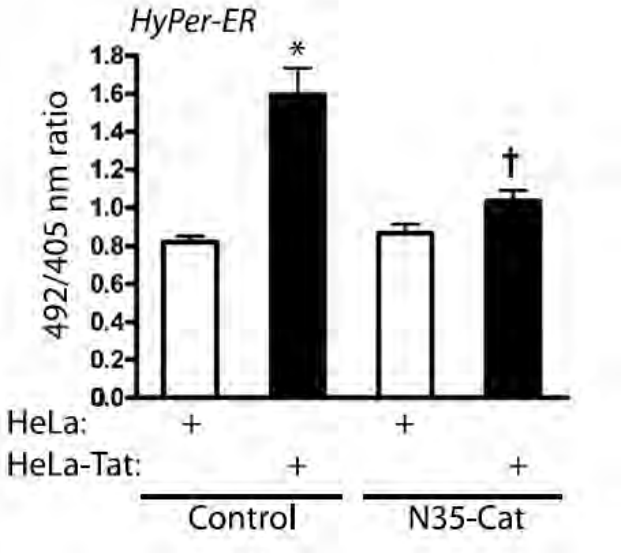
EC
VSMC
Fibroblast

Pharmacological Reports, 2008, 60, 21–28

Nox4-Derived H_2O_2 Mediates Endoplasmic Reticulum Signaling through Local Ras Activation[▽]

Ru-Feng Wu, Zhenyi Ma, Zhe Liu, and Lance S. Terada*

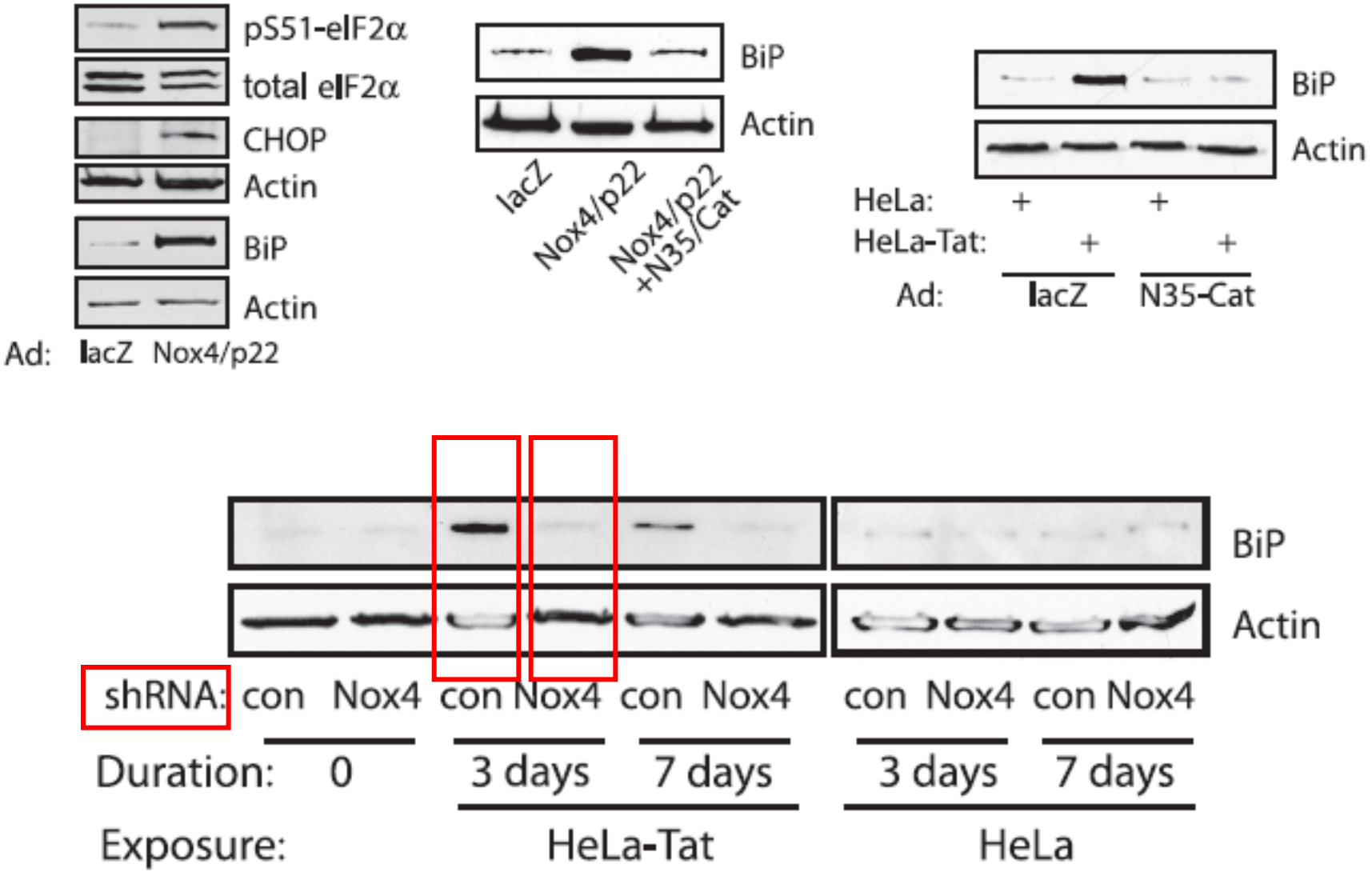




HyPer-ER: H2O2-sensitive fluorescent ratiometric sensor targeted to ER

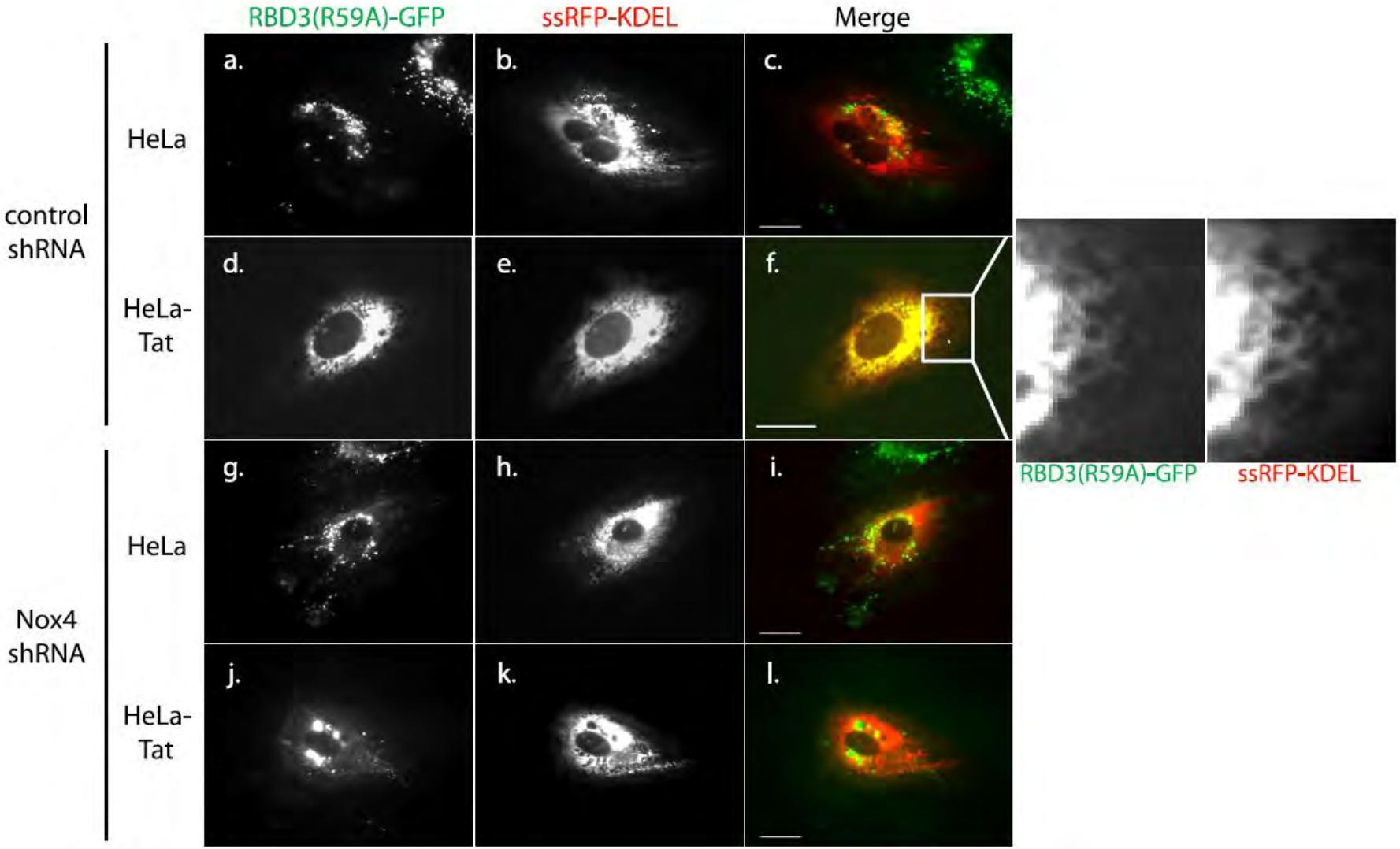
Mol Cell Biol 2010; 30: 3553-3568

Tat >>>NOX4>>>ER stress response



Mol Cell Biol 2010; 30: 3553-3568

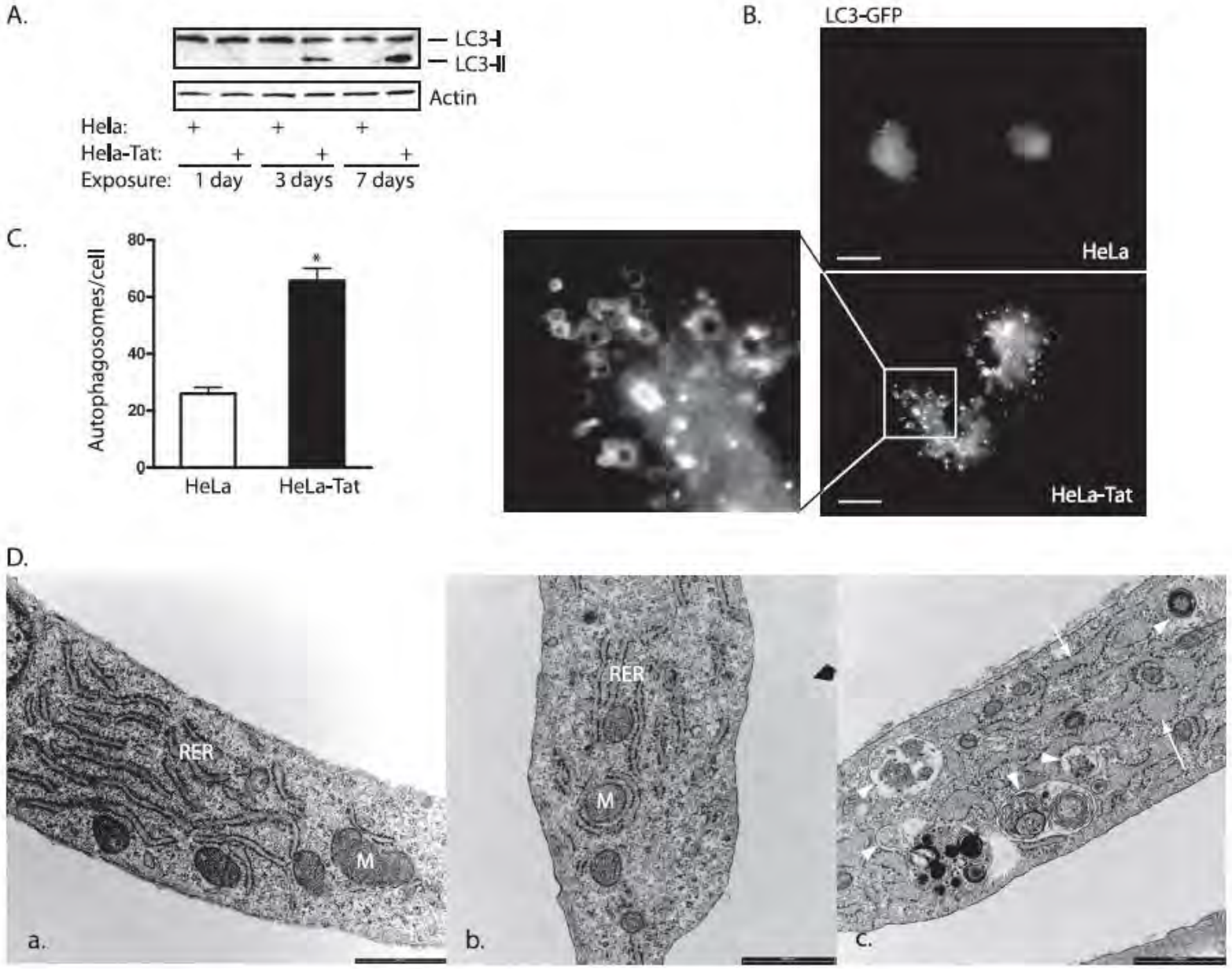
Tat activates Ras through NOX4 specifically in the ER



RBD3-GFP: fluorescent reporter for activated Ras

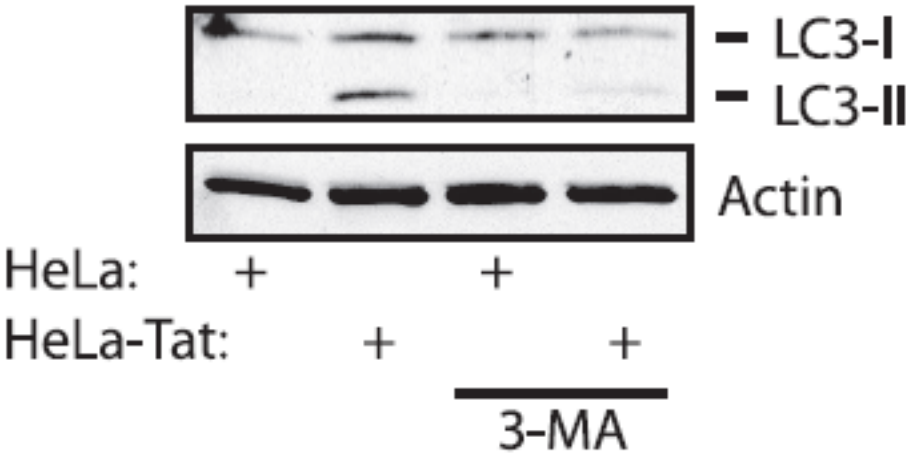
Mol Cell Biol 2010; 30: 3553-3568

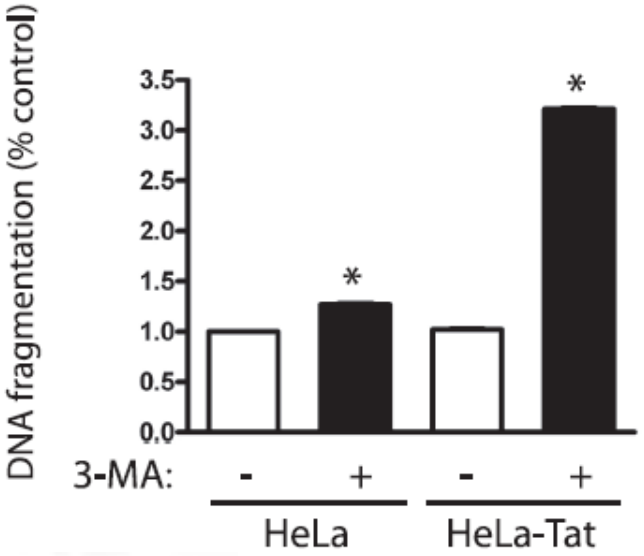
Tat >>>NOX4>>>ER stress response >>>EC autophagy

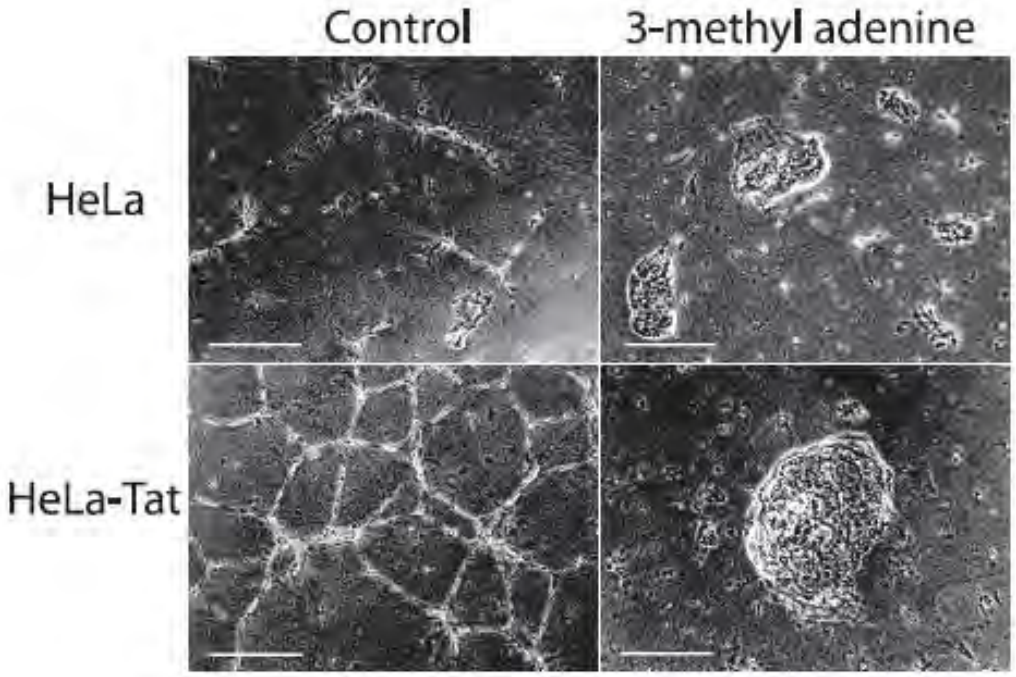


Mol Cell Biol 2010; 30: 3553-3568

NOX4-induced autophagy protects EC against cell death



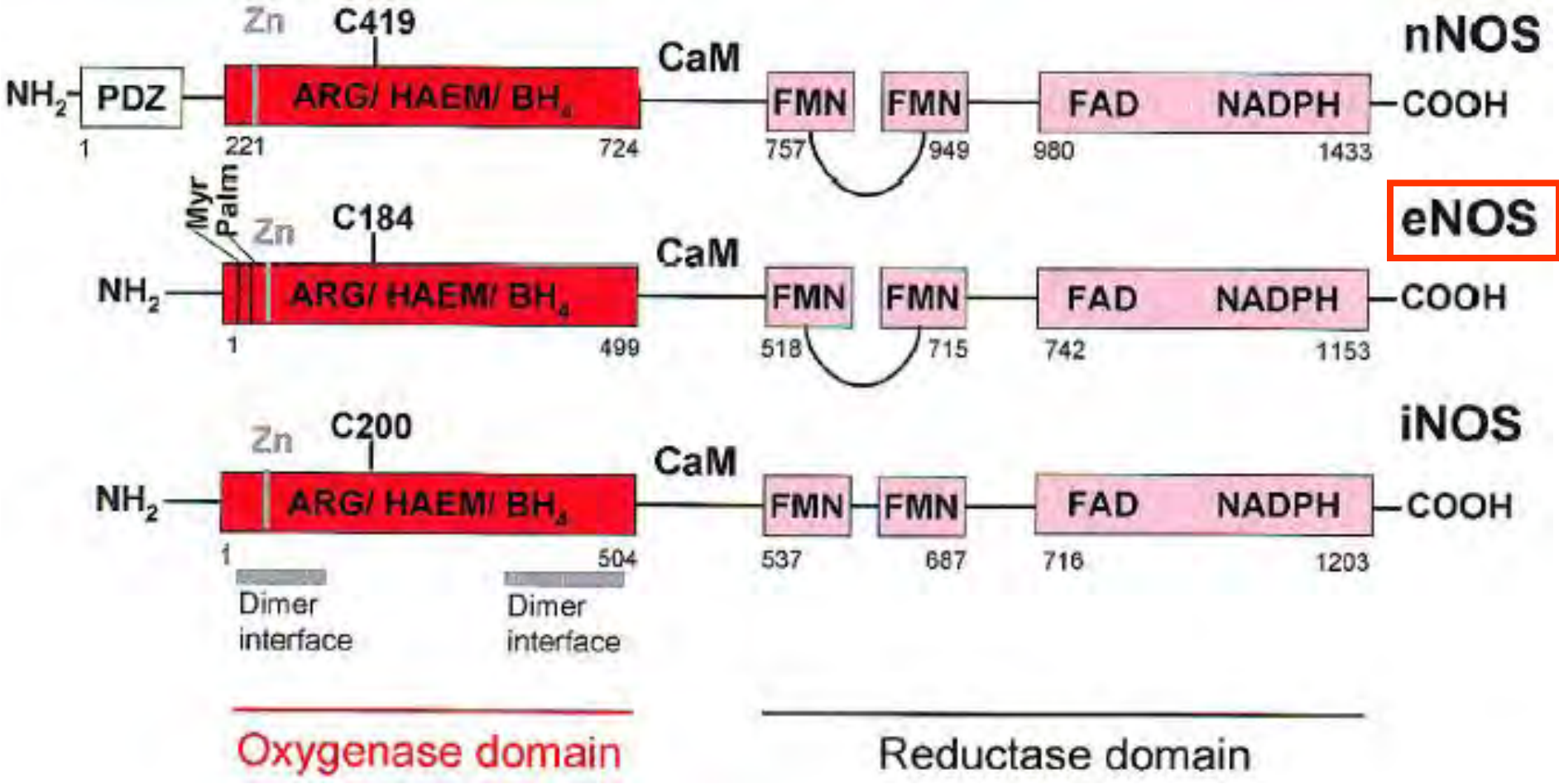


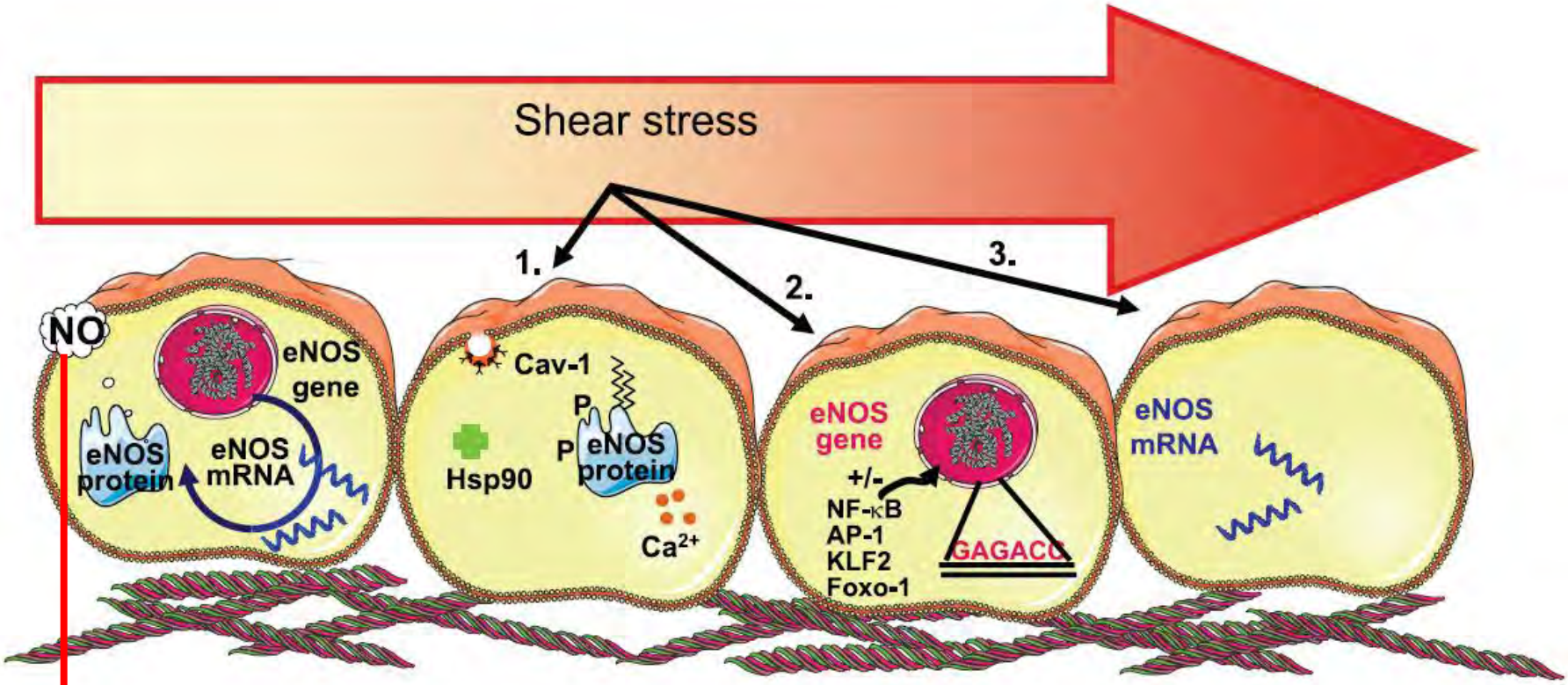


Mol Cell Biol 2010; 30: 3553-3568

Summary I

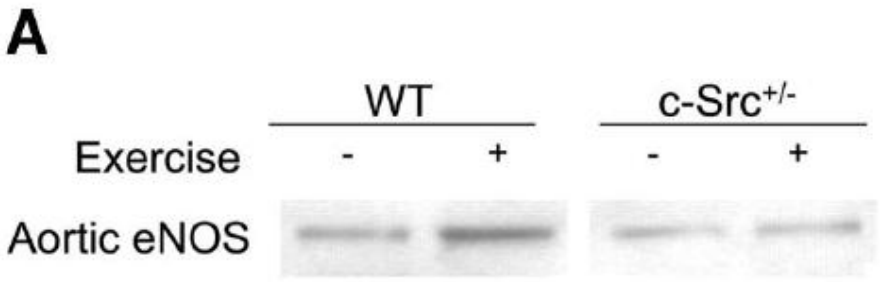
- EC express different NOX isoforms
- Each NOX has a specific subcellular localization
- NOX4 produces H2O2 in the ER, that mediates ER stress response but also autophagy
- H2O2-mediated autophagy protects EC against cell death

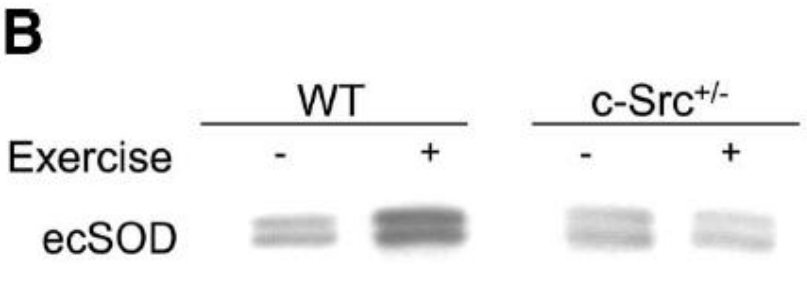

nNOS
Zn
C419
CaM
NH2
PDZ
ARG/ HAEM/ BH4
FMN
FMN
FAD
NADPH
COOH
1
221
724
757
949
980
1433
eNOS
Myr
Palm
Zn
C184
CaM
NH2
ARG/ HAEM/ BH4
FMN
FMN
FAD
NADPH
COOH
1
499
518
715
742
1153
iNOS
Zn
C200
CaM
NH2
ARG/ HAEM/ BH4
FMN
FMN
FAD
NADPH
COOH
1
504
537
687
716
1203
Dimer interface
Dimer interface
Oxygenase domain
Reductase domain

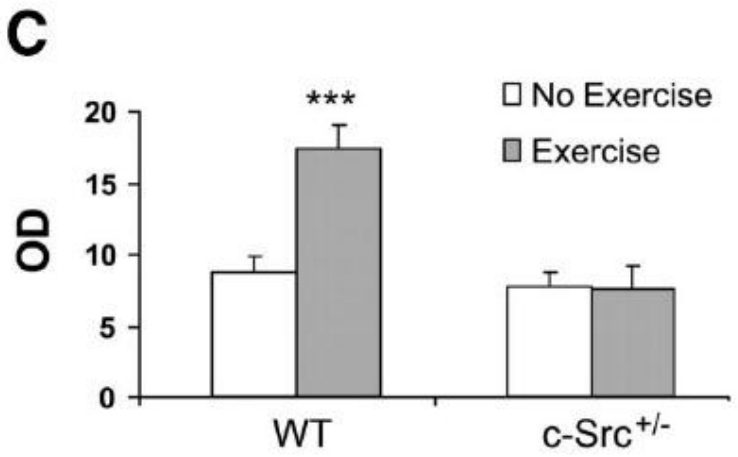


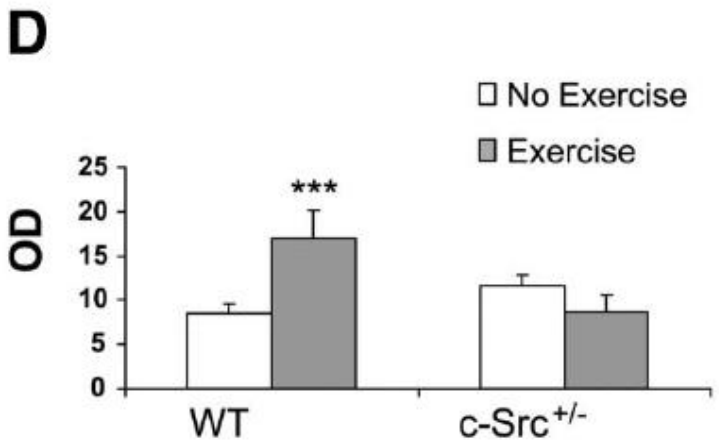
Balligand JL et al ***Physiol Rev*** 2009

ROS-sensitive c-Src mediates exercise-induced upregulation of eNOS and ecSOD

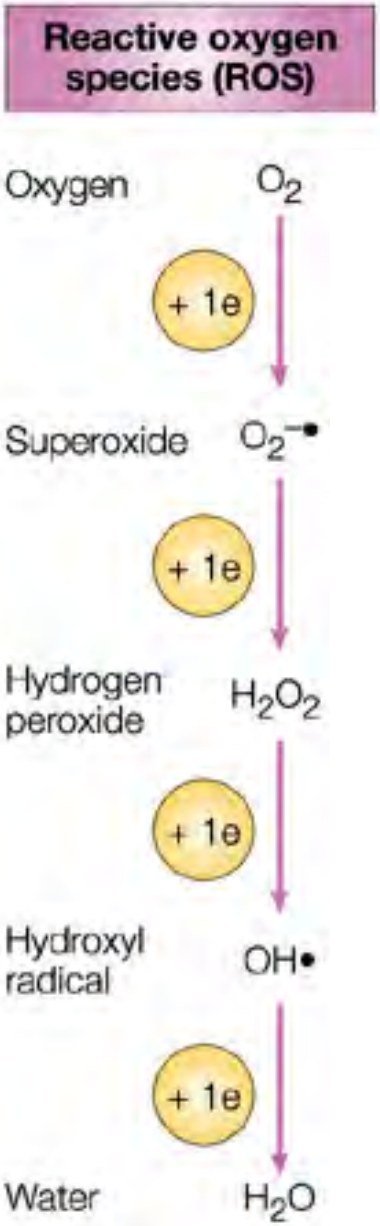




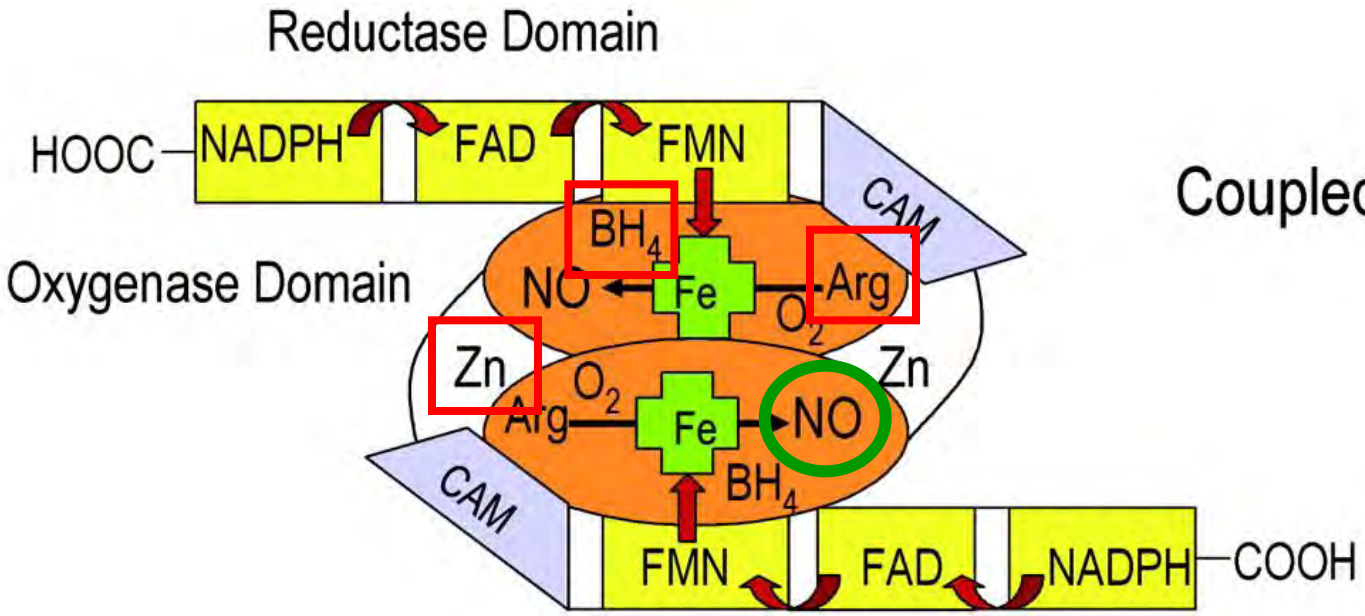




Davis ME, Cai H, McCann L, Fukai T, Harrison DG.
Am J Physiol Heart Circ Physiol 284: H1449–H1453, 2003.

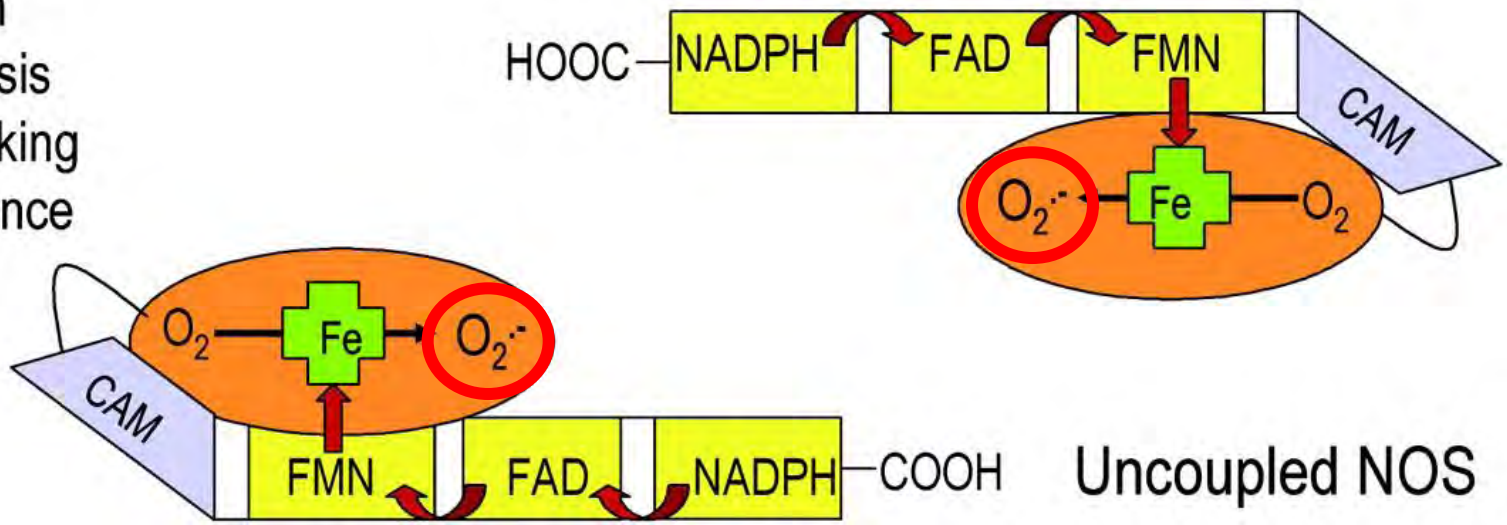


Nature Reviews Microbiology 2, 820-832 (2004)

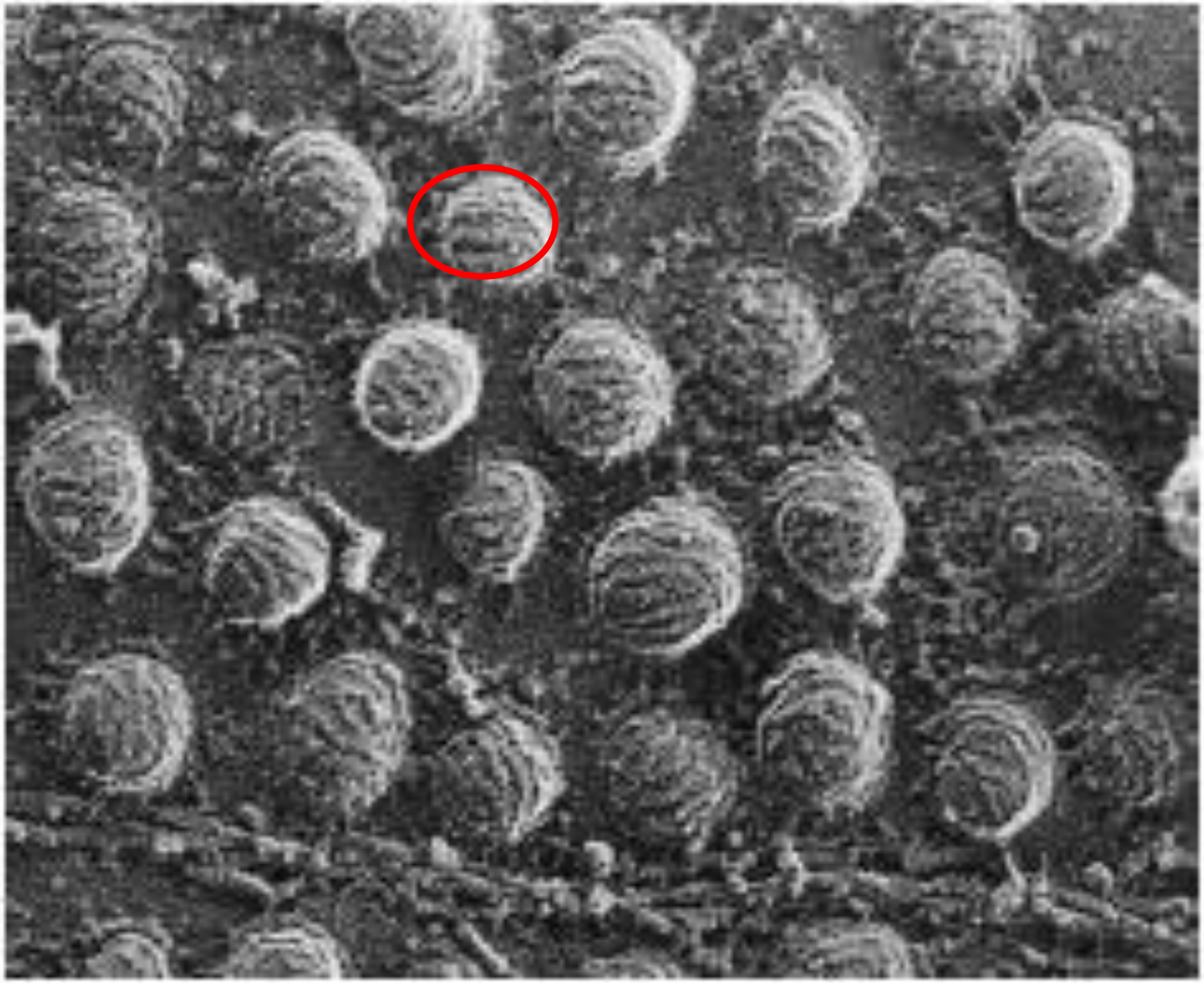


Coupled NOS

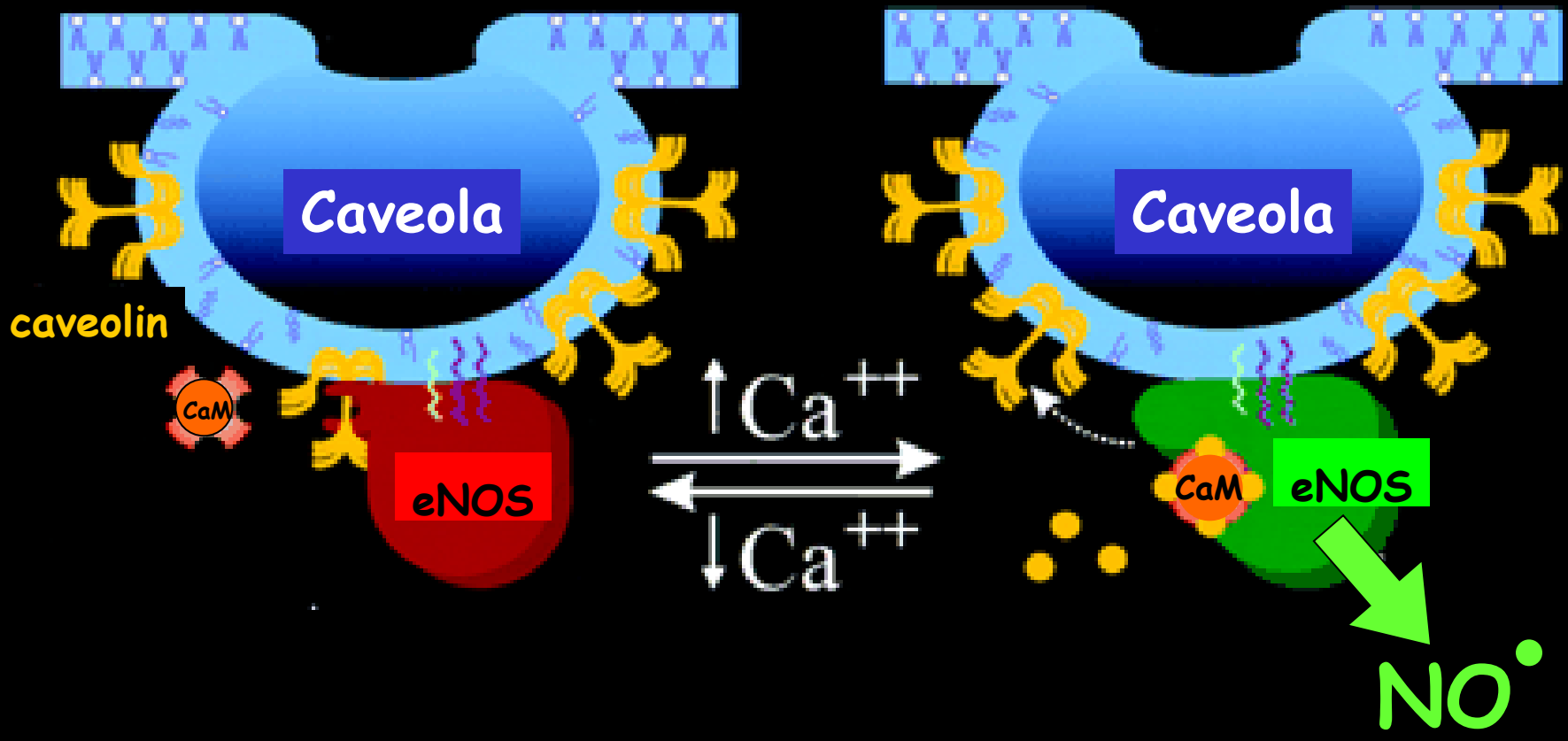
Diabetes mellitus
Hypertension
Atherosclerosis
Chronic smoking
Nitrate tolerance



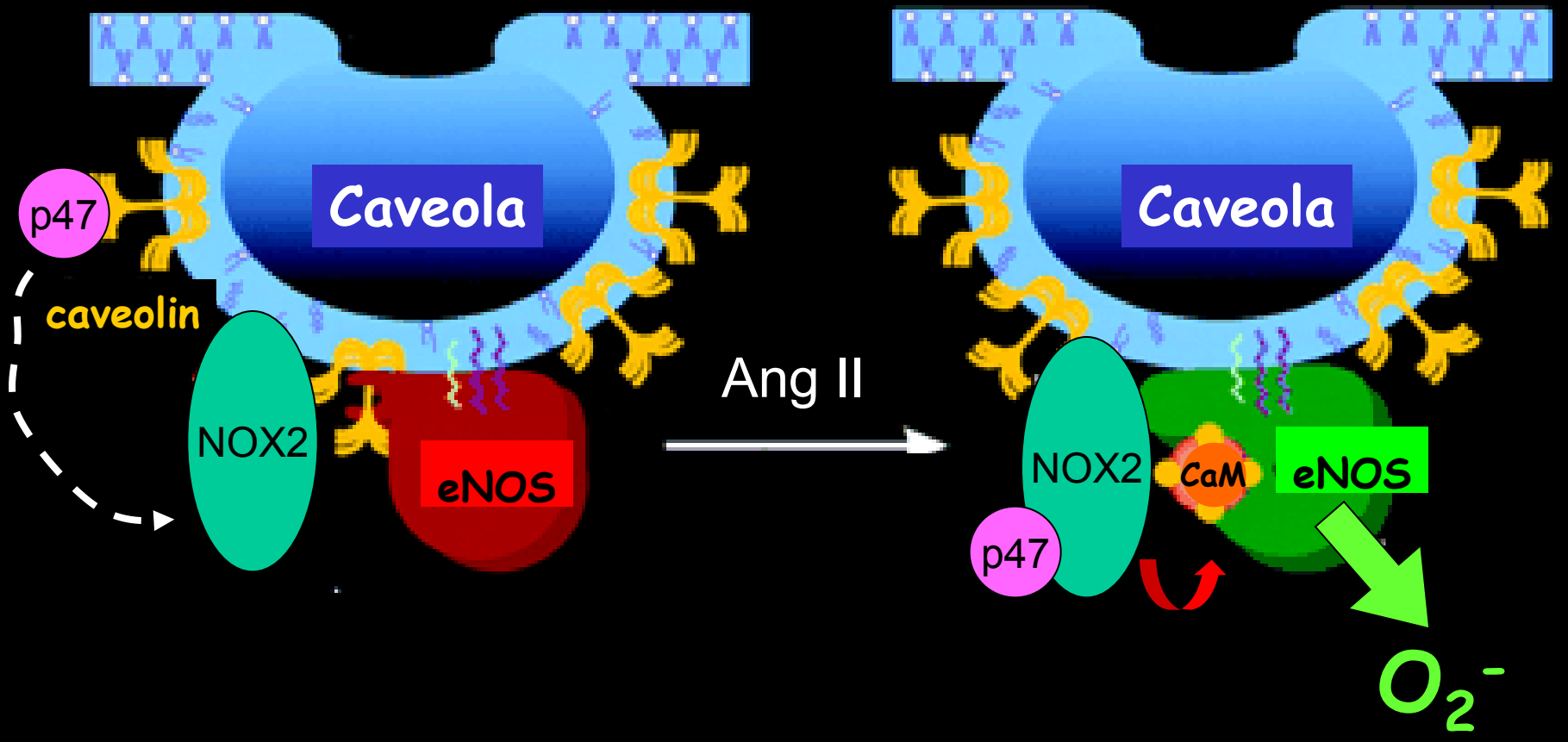
Uncoupled NOS



Caveolin versus calmodulin: Counterbalancing allosteric modulators of eNOS



Co-localization of eNOS and NADPH oxidase: caveolin-dependent eNOS uncoupling



Review

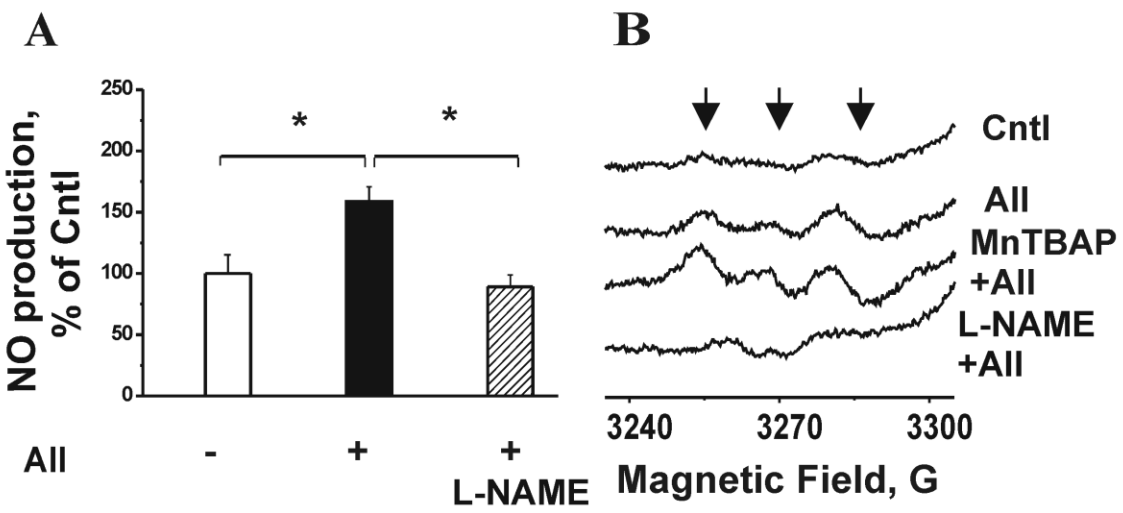
Nature Reviews Drug Discovery 10, 453-471 (June 2011)

Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets

Grant R. Drummond, Stavros Selemidis, Kathy K. Griendling & Christopher G. Sobey

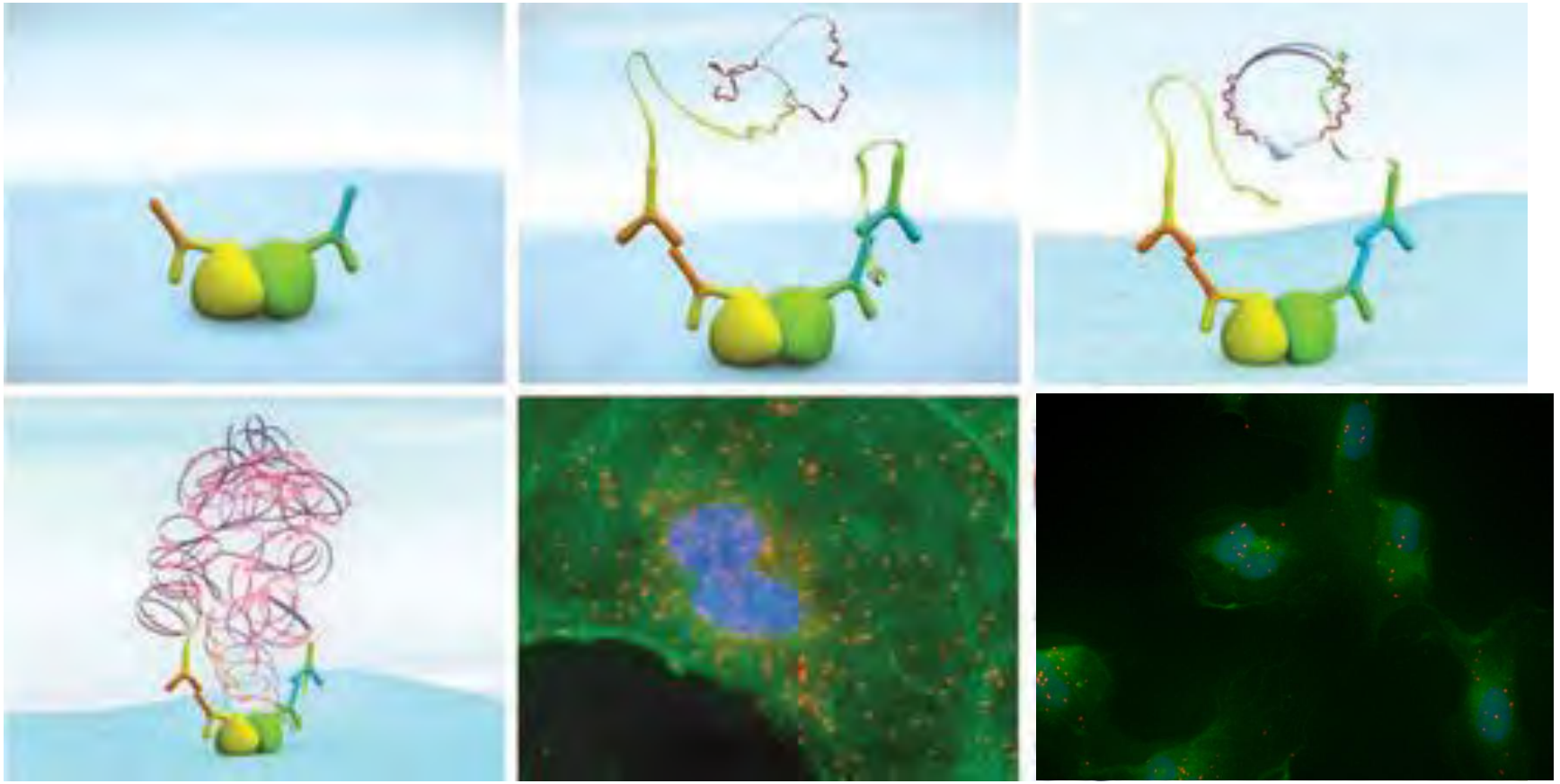
Abstract

NADPH oxidases are a family of enzymes that generate reactive oxygen species (ROS). The NOX1 (NADPH oxidase 1) and NOX2 oxidases are the major sources of ROS in the artery wall in conditions such as hypertension, hypercholesterolaemia, diabetes and ageing, and so they are important contributors to the oxidative stress, endothelial dysfunction and vascular inflammation that underlies arterial remodelling and atherogenesis. In this Review, we advance the concept that compared to the use of conventional antioxidants, inhibiting NOX1 and NOX2 oxidases is a superior approach for combating oxidative stress. We briefly describe some common and emerging putative NADPH oxidase inhibitors. In addition, we highlight the crucial role of the NADPH oxidase regulatory subunit, p47phox, in the activity of vascular NOX1 and NOX2 oxidases, and suggest how a better understanding of its specific molecular interactions may enable the development of novel isoform-selective drugs to prevent or treat cardiovascular diseases.

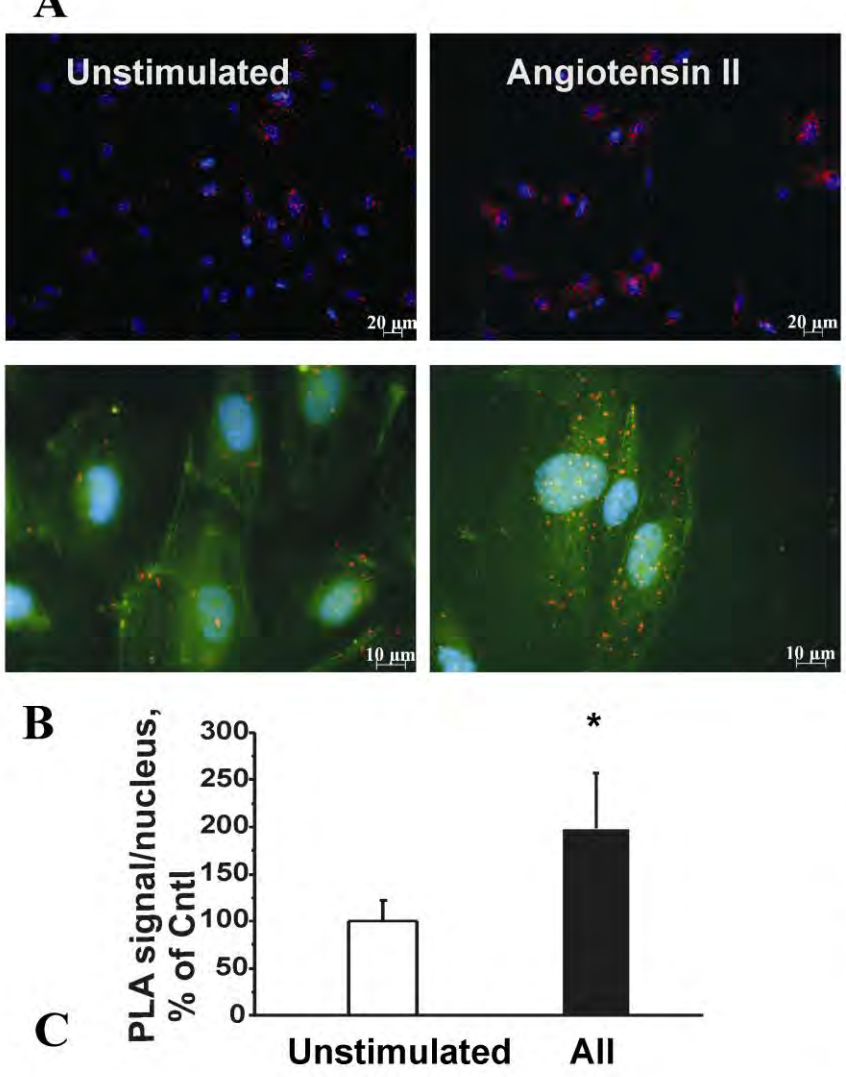


ATVB 2011, in press

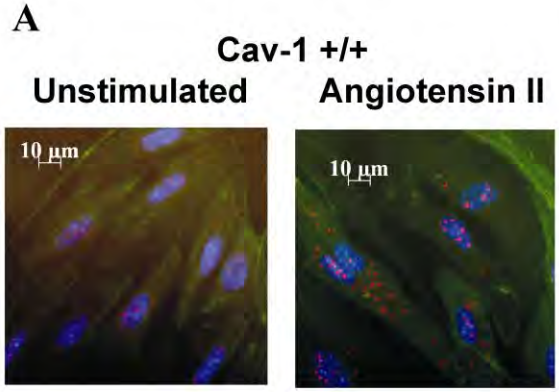
In situ Proximity Ligation Assay



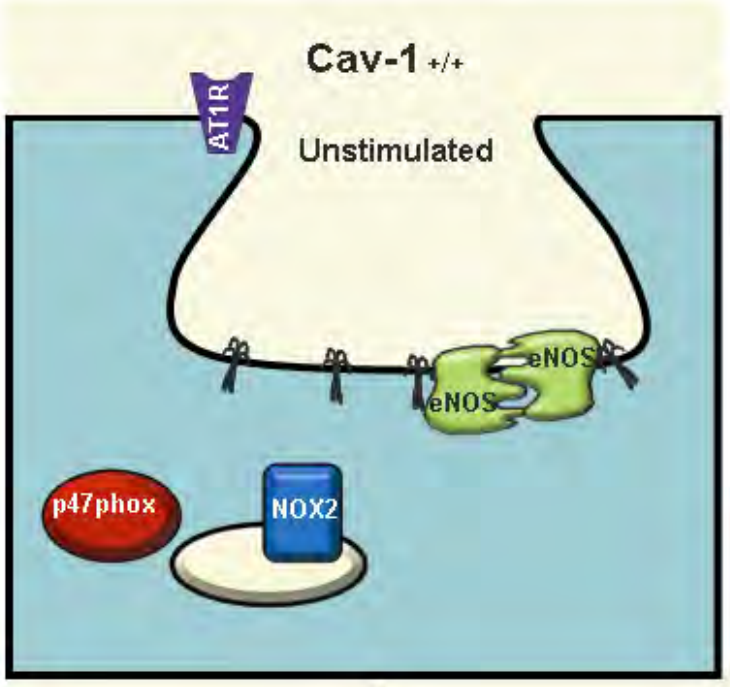
nature methods | september 2009 |



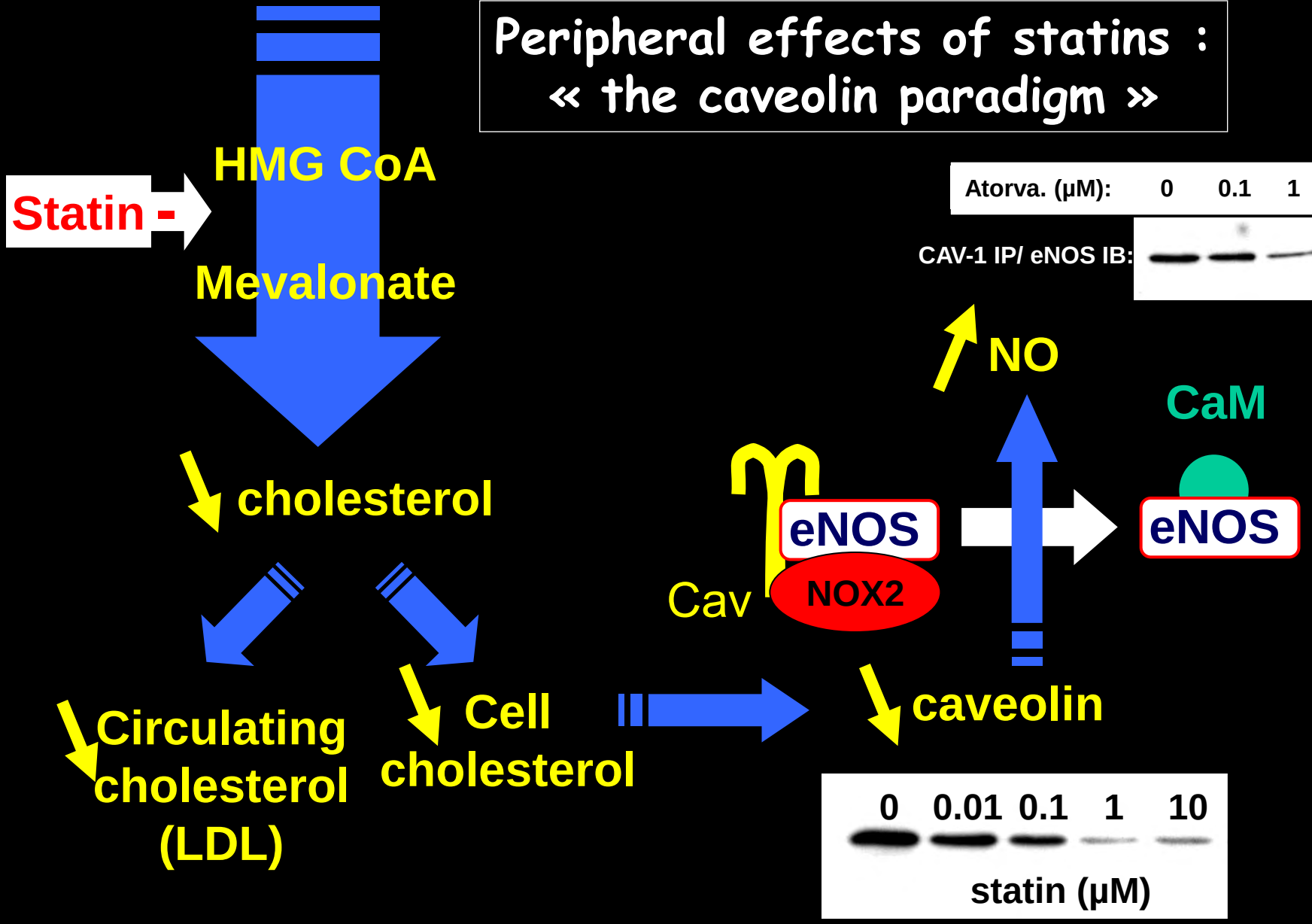
ATVB 2011, in press



ATVB 2011, in press


Cav-1 +/+
AT1R
Unstimulated
eNOS
eNOS
p47phox
NOX2

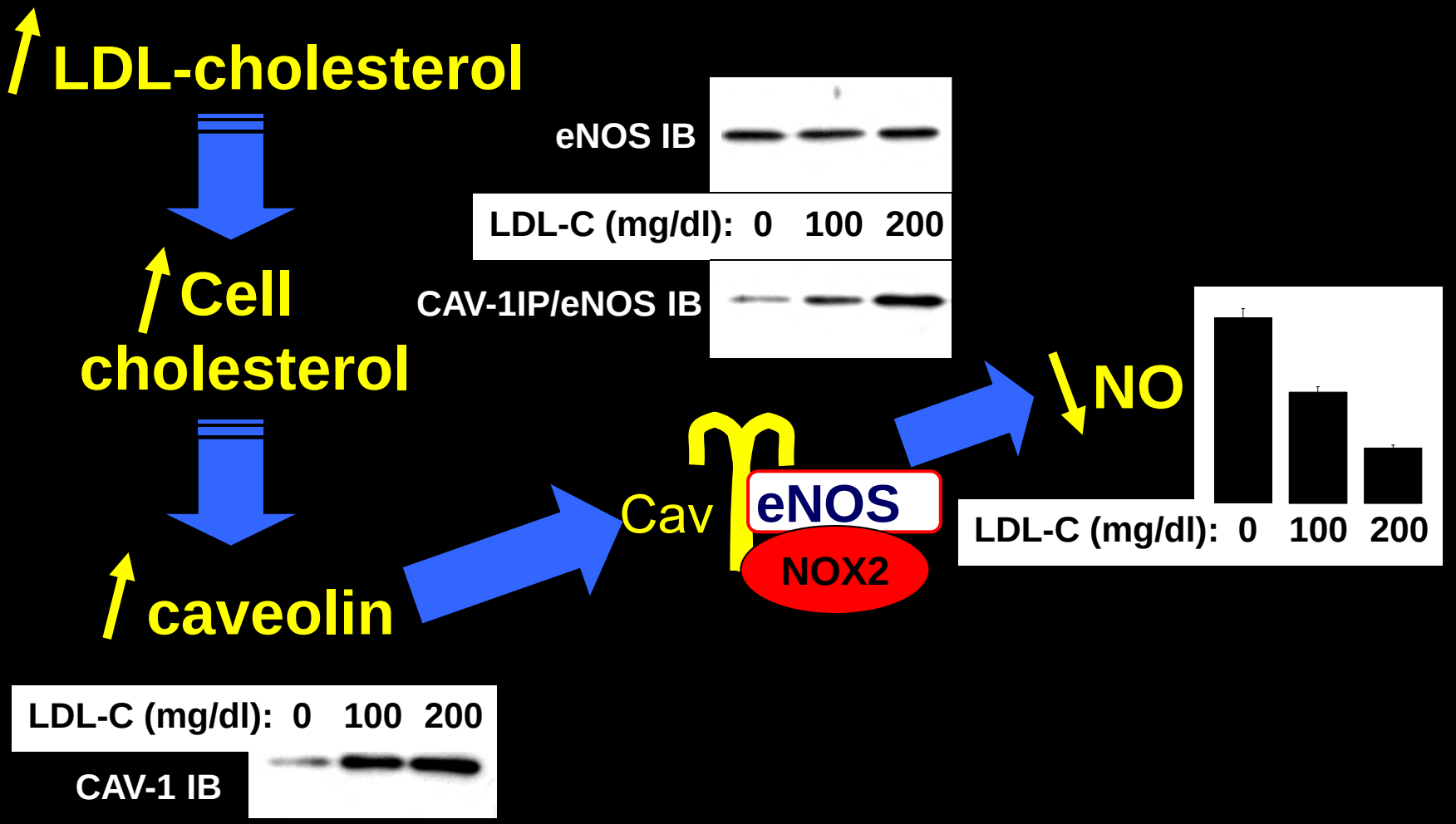
Peripheral effects of statins : « the caveolin paradigm »



Feron O, Dessy C, Desager JP, Balligand J-L Circulation 2001

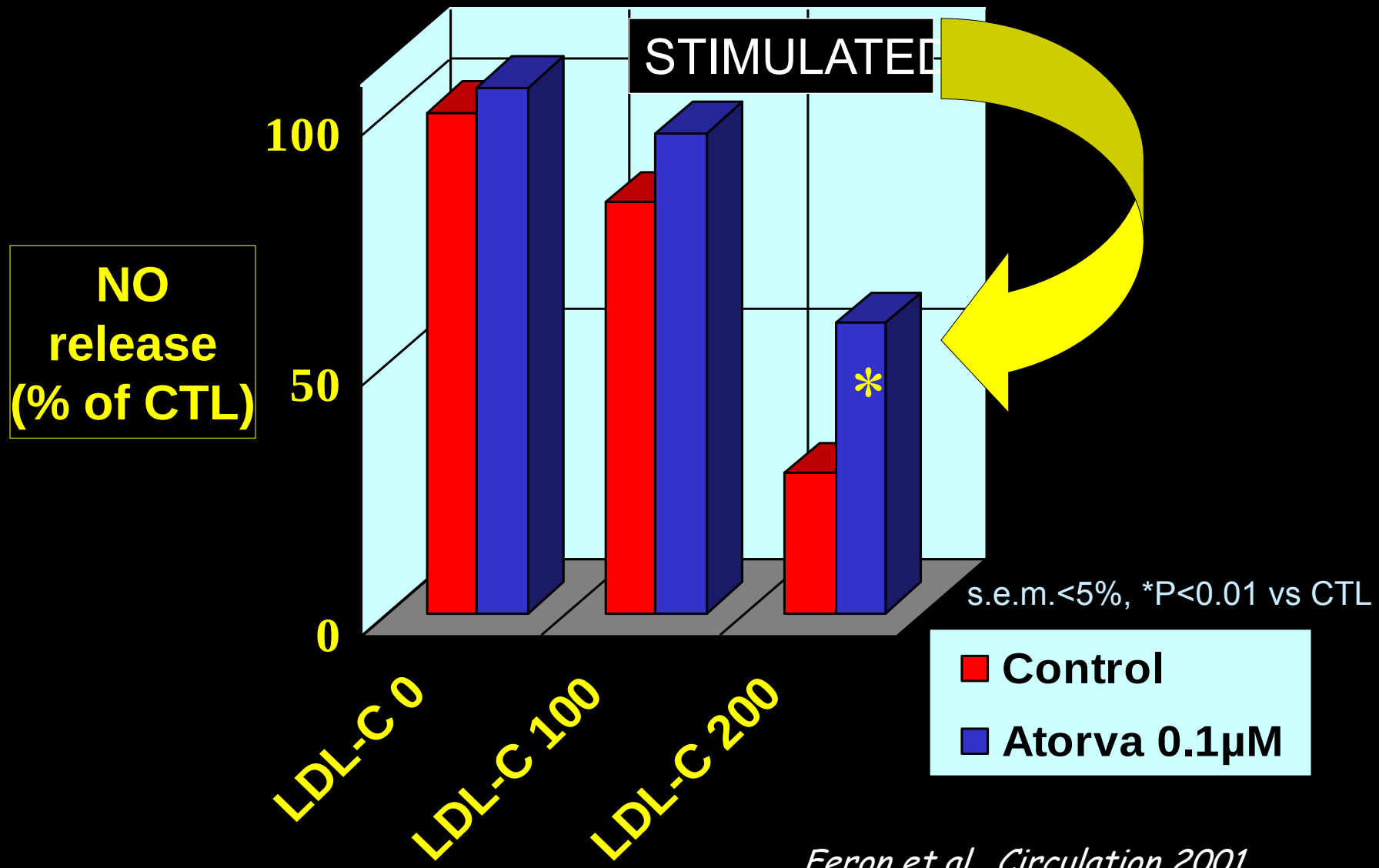
High LDL cholesterol decreases NO production by promoting the interaction of caveolin and eNOS

and possibly more assembly of NADPH oxidase



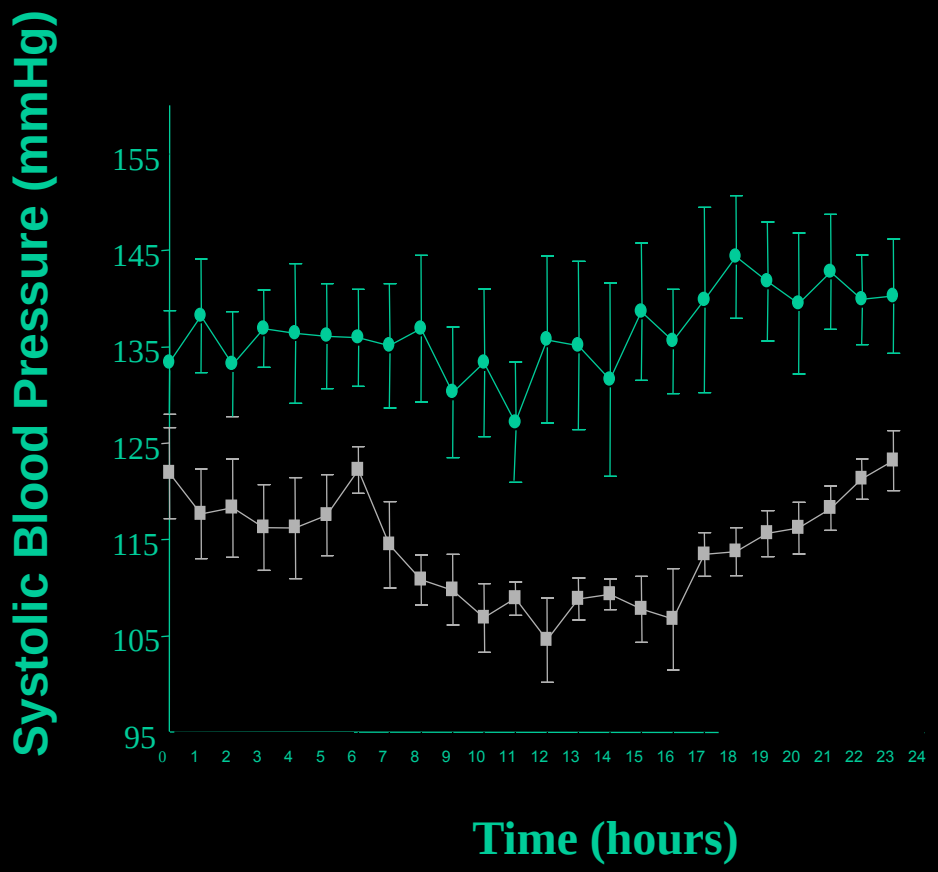
Feron O, Dessy C, Moniotte S, Desager JP, Balligand J-L J Clin Invest 1999

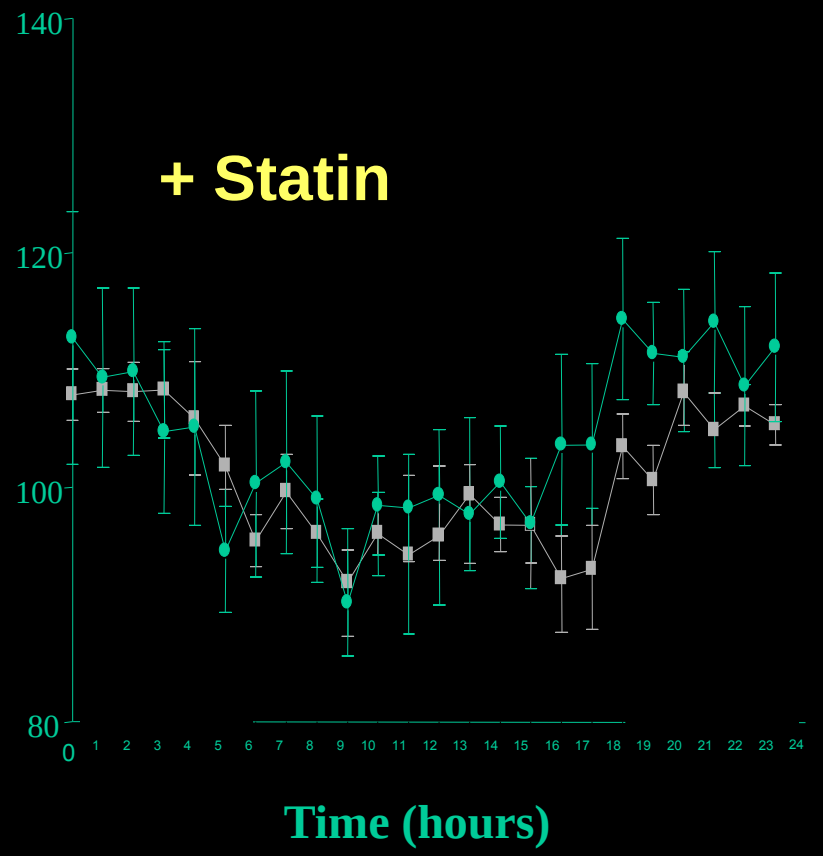
Statin promotes agonist-stimulated NO release at high LDL-Chol.



Feron et al., Circulation 2001

Effect of chronic statin treatment on systolic Blood Pressure





- C57 Bl6, control mice
- APOE KO Mice

at unchanged ***plasma*** chol !

Pelat M, Feron O, Balligand JL, *Circulation* 2003

Summary II

- eNOS and H2O2 can cooperatively enhance vasodilation
- Caveolin-1 mediates NADPH oxidase assembly and activation in caveolae
- Activation of NADPH oxidase in caveolae both mediates eNOS activation with Ang II and promotes eNOS uncoupling
- Moderate Cav-1 downregulation (as obtained with statins) inhibits NADPH oxidase activation, prevents eNOS uncoupling and restores NO bioavailability

Institut de Recherche Expérimentale et Clinique (IREC)

UCL

I. Lobysheva
B. Sekkali
J. Hammond
A. Friart
B. Manoury
N. Hermida Blanco
A. De Pauw
S. Moniotte
C. Bouzin
C. Dessy
O. Feron

REMA/UCL
B. Gallez

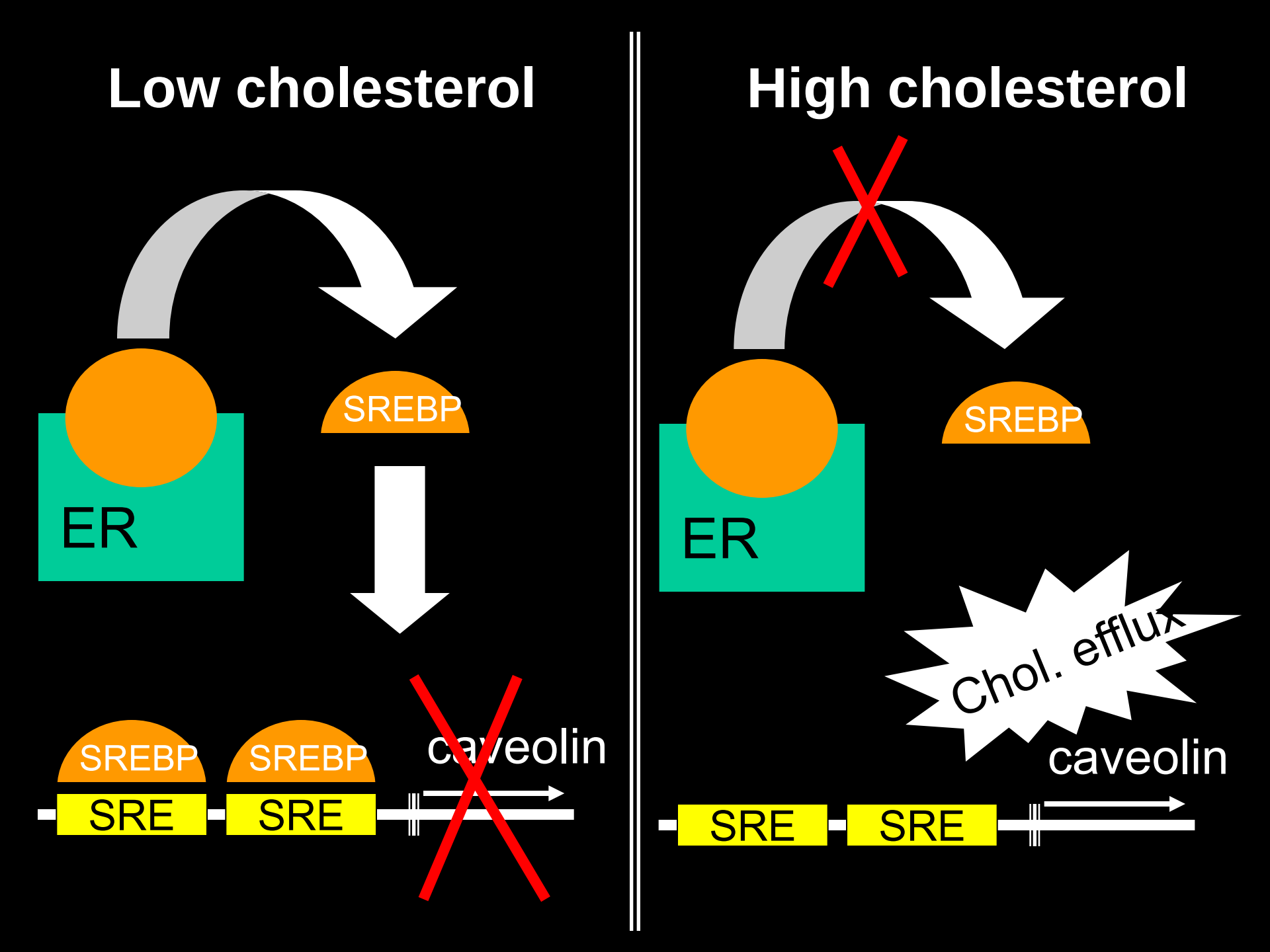
EUGeneHeart

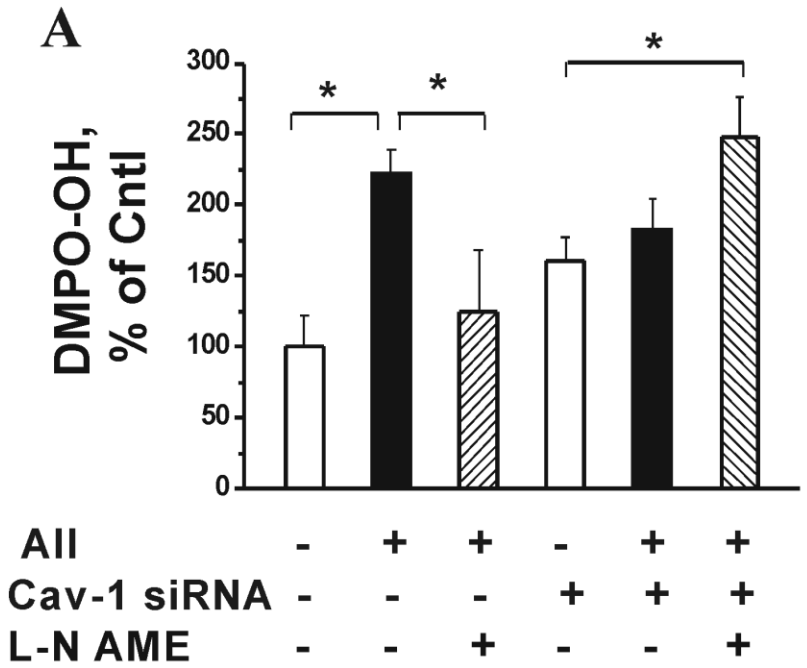
Genomics of Cardiomyocyte Signaling to Treat and Prevent Heart Failure

Funding by FONDATION LEDUCQ

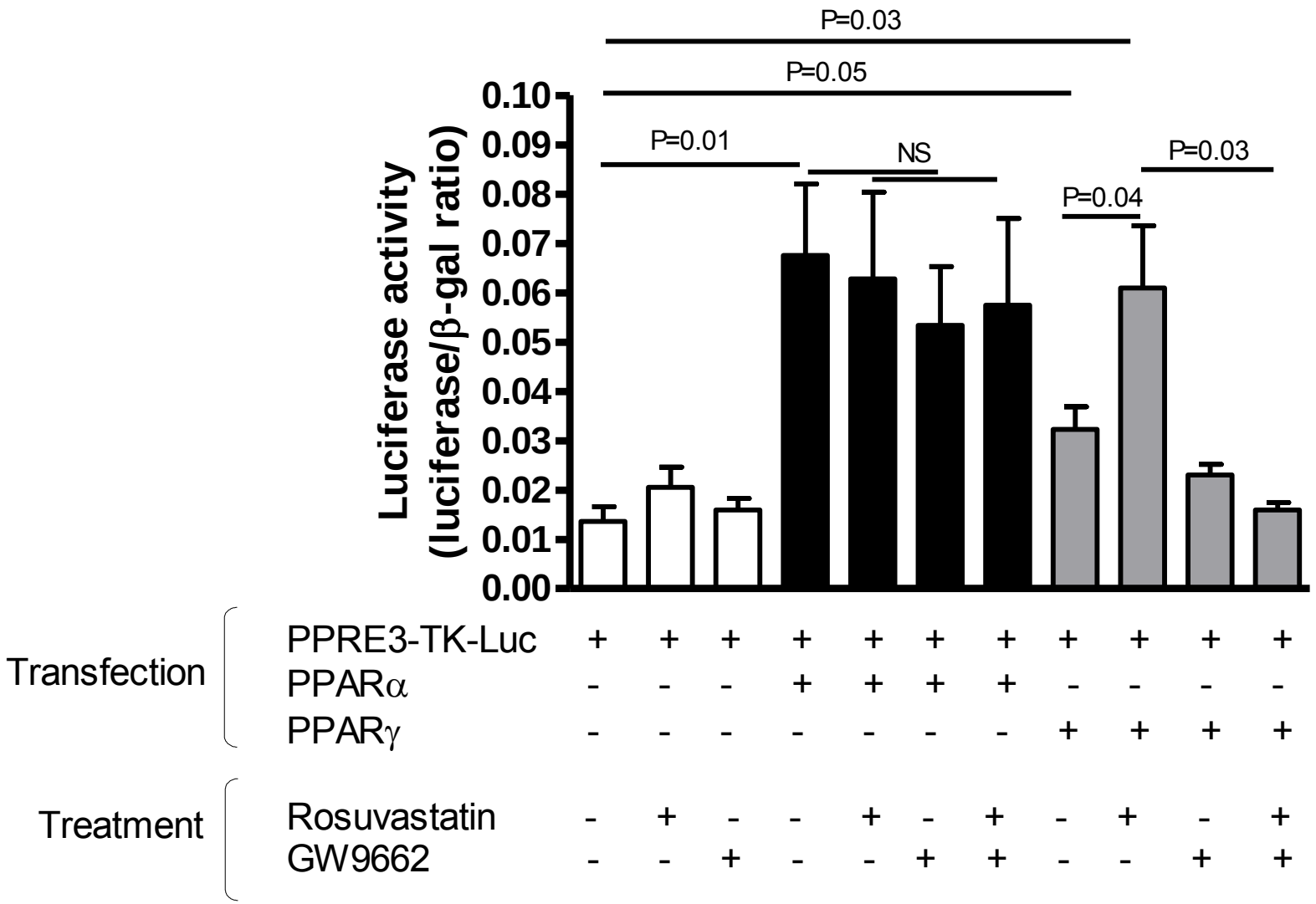
Université catholique de Louvain

JL.Balligand@uclouvain.be

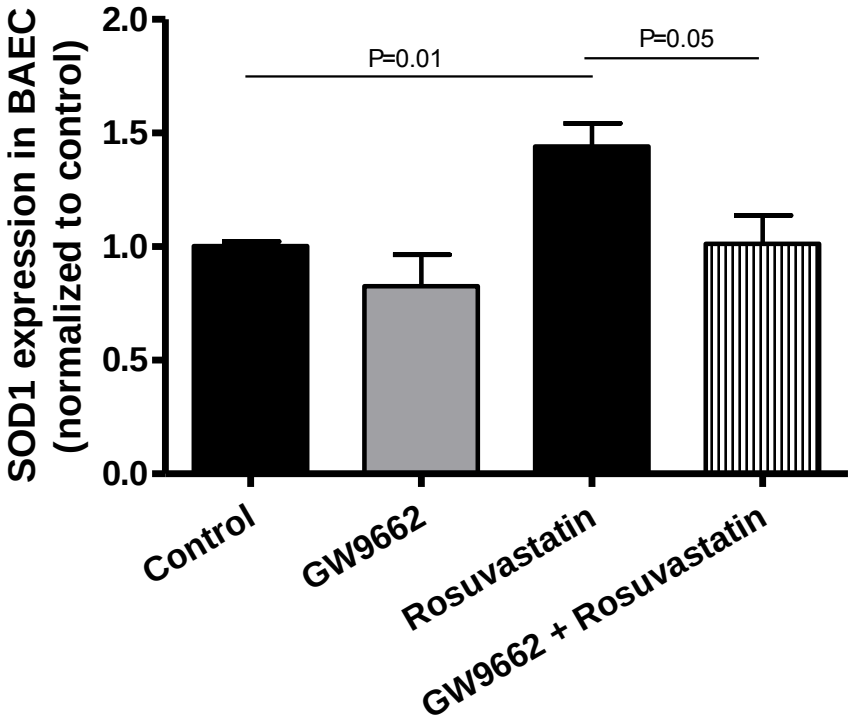

Low cholesterol
High cholesterol
SREBP
ER
SREBP
ER
SREBP
SREBP
caveolin
SRE
SRE
Chol. efflux
caveolin
SRE
SRE

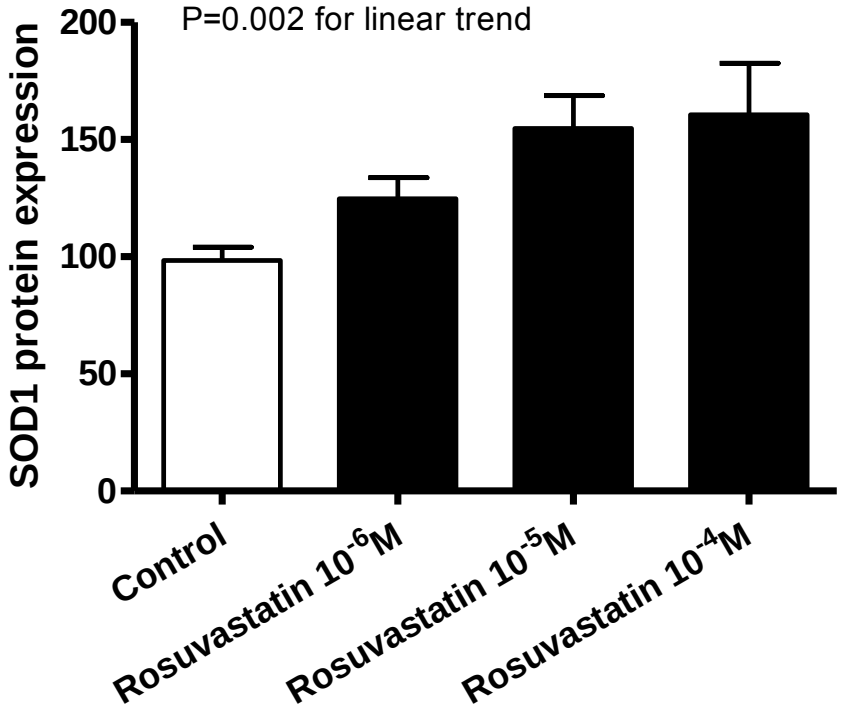

A
DMPO-OH, % of Cntl
300
250
200
150
100
50
0
*
*
*
AII - + + - + +
Cav-1 siRNA - - - + + +
L-N AME - - + - - +
B
NO production, % of Cntl
500
400
300
200
100
0
*
*
*
*
*
AII - + + - + +
Cav-1 siRNA - - - + + +
PEG-SOD - - + - - +

Rosuvastatin activates PPAR-gamma transcriptional activity



Desjardins F et al Eur Heart J 2008





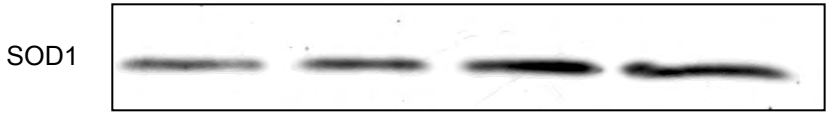


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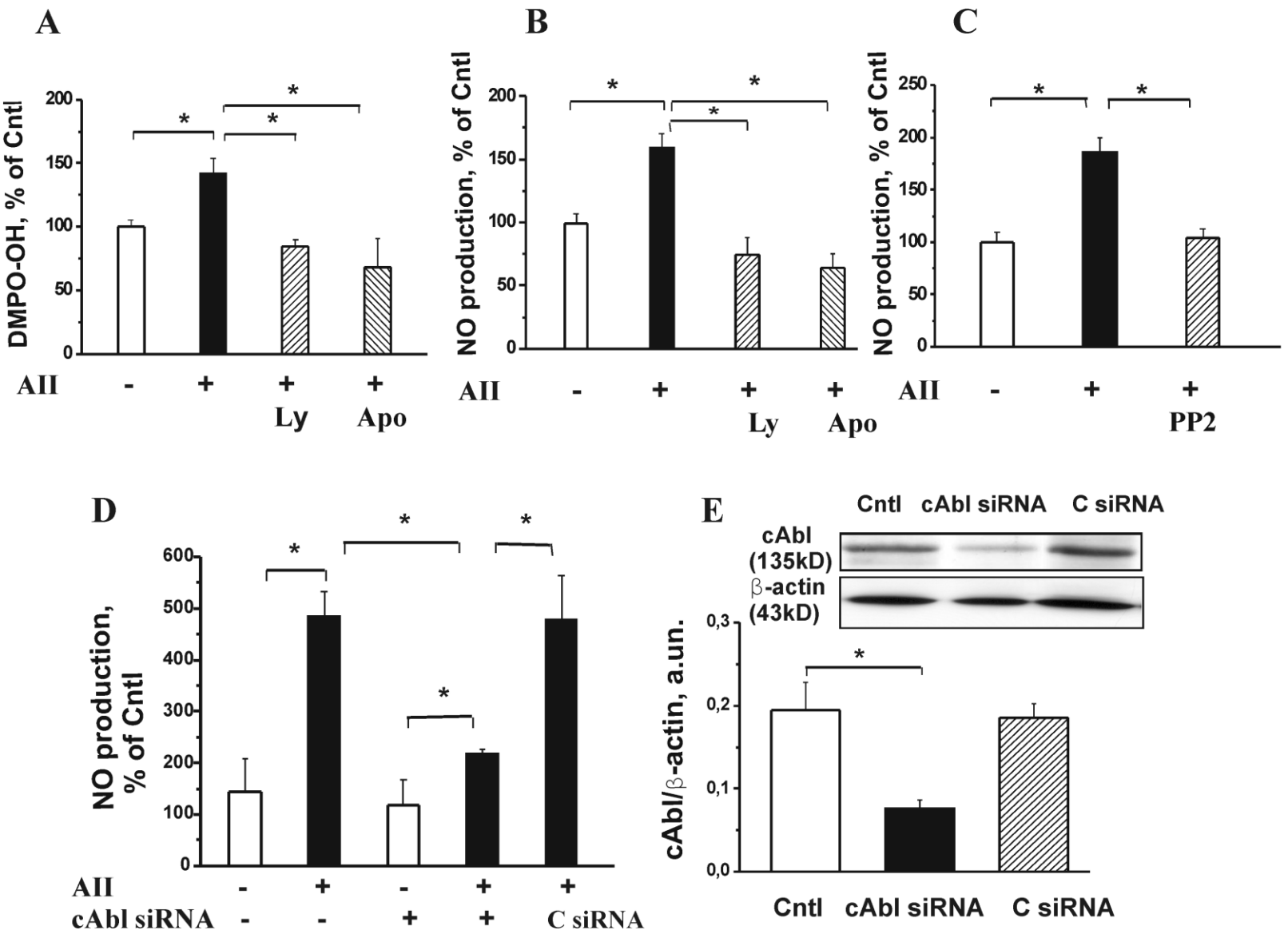
Figure 2.



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