JAGIELLONIAN UNIVERSITY
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Oxidative stress in vascular system: basic concepts & novel ideas

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Oxidative stress, by resulting in a maladaptive endothelial cell phenotype, is one of the common means by which conditions such as hyperlipidemia, hypertension, advanced age, hyperhomocysteinemia and diabetes, all of awhich are associated with an increased risk of vascular diseases, lead to a dysfunctional endothelium

K. Irani, in Endothelial Biomedicine, 2007

Oxidative stress

1. Definition

2. Reactive oxygen species

3. Sources of reactive oxygen species in the vasculature

4. Antioxidant enzymes

5. Non-canonical functions of some anti-oxidant systems

Oxidative stress

Imbalance in the production and inactivation of reactive oxygen species

Role of oxidative stress

1. Physiological signaling
2. Pathophysiological

Influence of oxidative stress is determined by:

a) site of the production - the same enzymes may play a different role in various cells (eg. NADPH oxidase in professional phagocytic cells and vascular wall cells)
b) amount of ROS generated
c) activity of antioxidant systems

Reactive oxygen species

- **ROS** are chemical species containing oxygen atoms with unpaired electrons that may be charged or uncharged. The unpaired electrons render the molecule unstable, allowing the ROS to react with electrons, other chemical species or macromolecules such as proteins and nucleic acids

- Molecular oxygen serves as the primary electron acceptor yielding the superoxide anion

- Subsequent univalent reductive steps can lead to the generation of the whole range of ROS

- The distinct physical and chemical properties of each of these ROS determine its toxicity as well as its suitability as a signaling intermediary

Types of reactive oxygen species

1. **Free radicals**
   a. Superoxide - $O_2^{-\cdot}$
   b. Hydroxyl radical - $OH^{\cdot}$
   c. Peroxy-radicals - $ROO^{\cdot}$
   d. nitric oxide – $NO^{\cdot}$

2. **Not free radicals**
   a. Hydrogen peroxide - H_2O_2
   b. Peroxynitrite - $ONOO^-$

Sources of reactive oxygen species

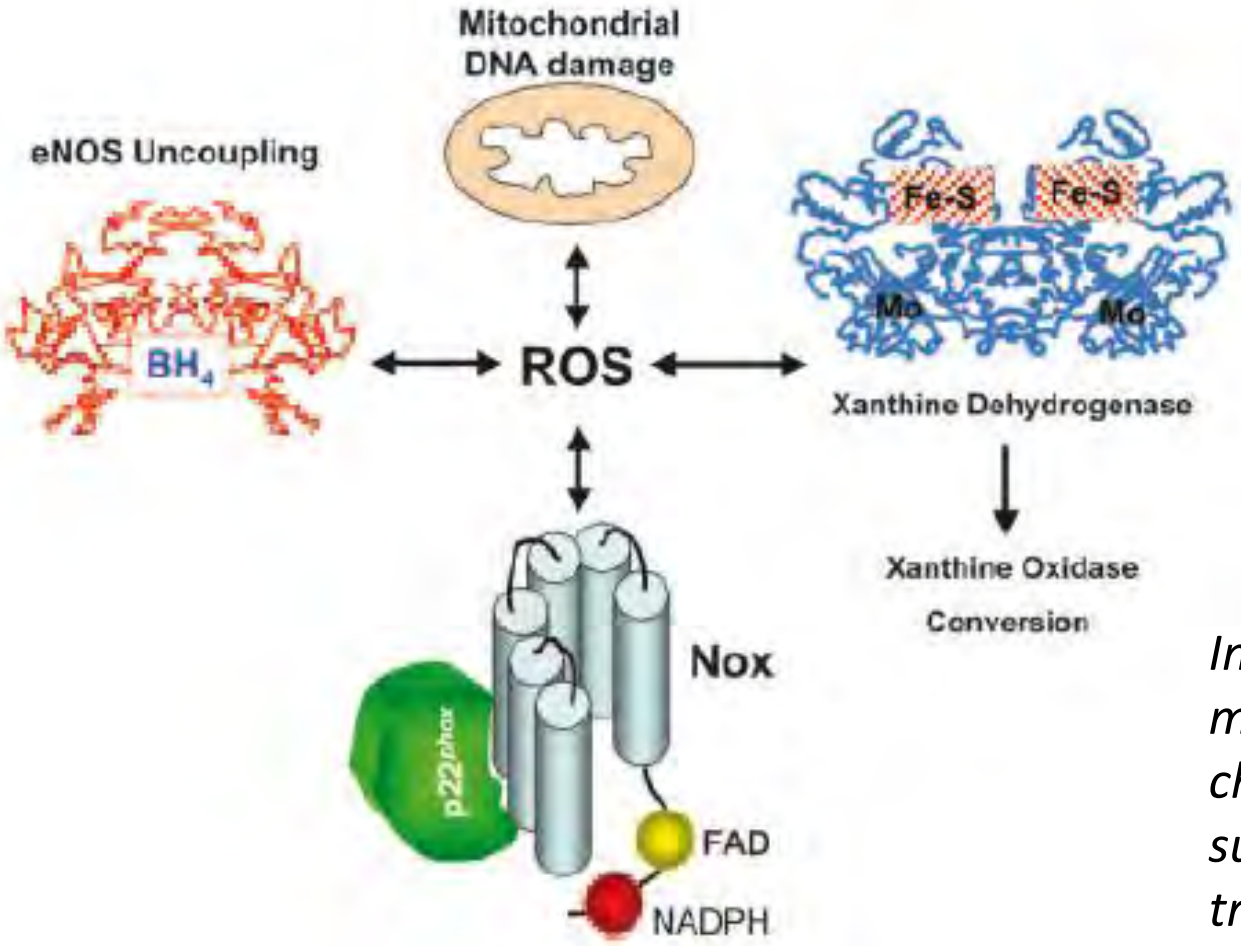
Sources of $O_2^{-\cdot}$ in ECs:

1. NADPH oxidases
2. Xanthine oxidoreductase
3. Uncoupled endothelial nitric oxide synthase
4. The cytochrome P450-based enzymes
5. Mitochondrial respiration

Other mechanisms:

1. Catalytic conversion of other ROS, such as H_2O_2 by dismutation of $O_2^{-\cdot}$
2. Conversion of H_2O_2 to hypochlorus acid
3. Reduction of ROS by transition metals such as th eferrous ion to the highly reactive hydroxyl radical

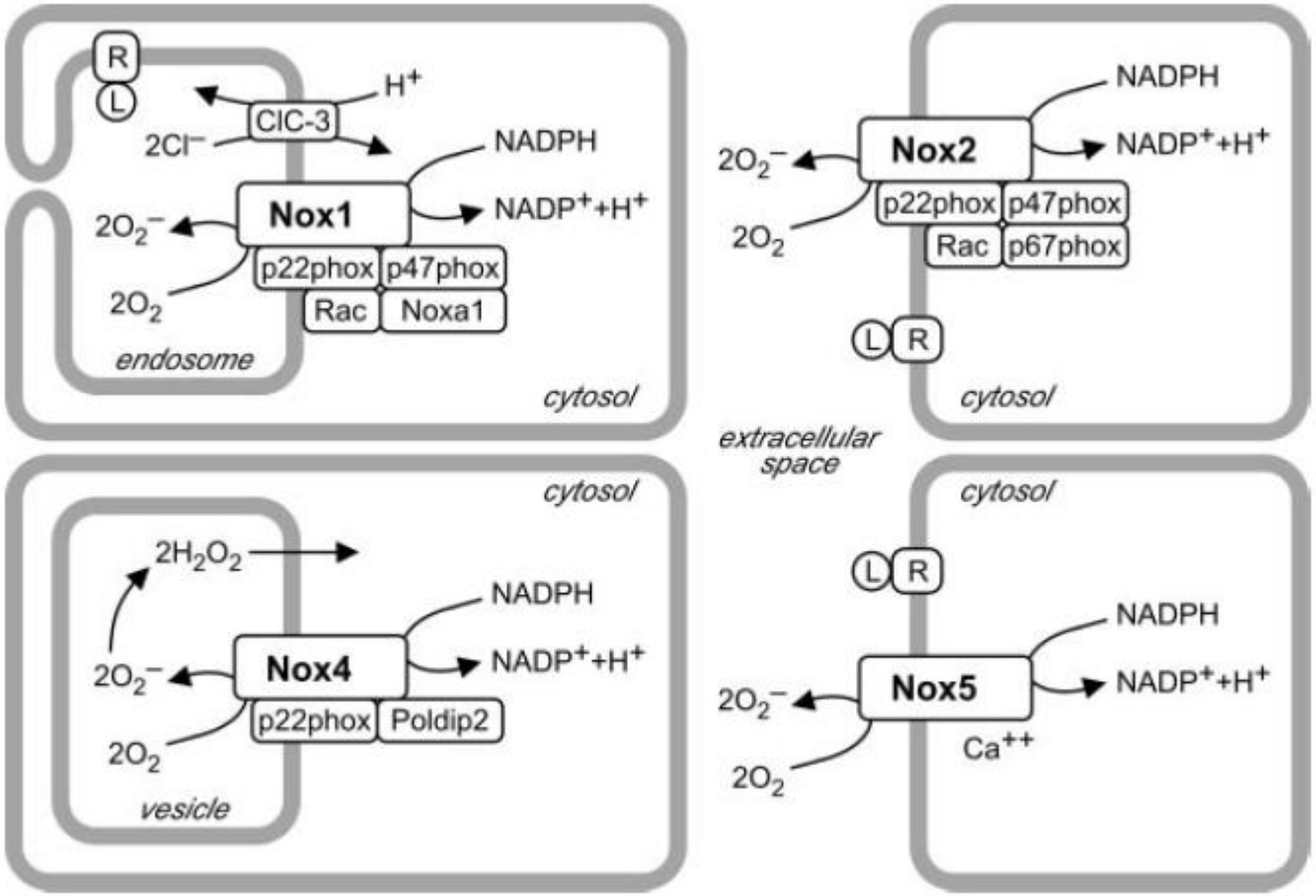
Sources of reactive oxygen species



In physiological conditions mitochondrial respiratory chain is the main source of superoxide radical, transforming about 5% of molecular oxygen to $O_2^{-\cdot}$

Mueller et al., ATVB 2005

Different subcellular localisation of vascular NADPH oxidases



Lassegue & Griendling, ATVB, 2010;30:653-661

Molecular targets of ROS within vascular wall cells

1. Detrimental –
   targeting macromolecules – lipids, nucleic acids – may result in damage

2. Physiological – involvement in signal transduction

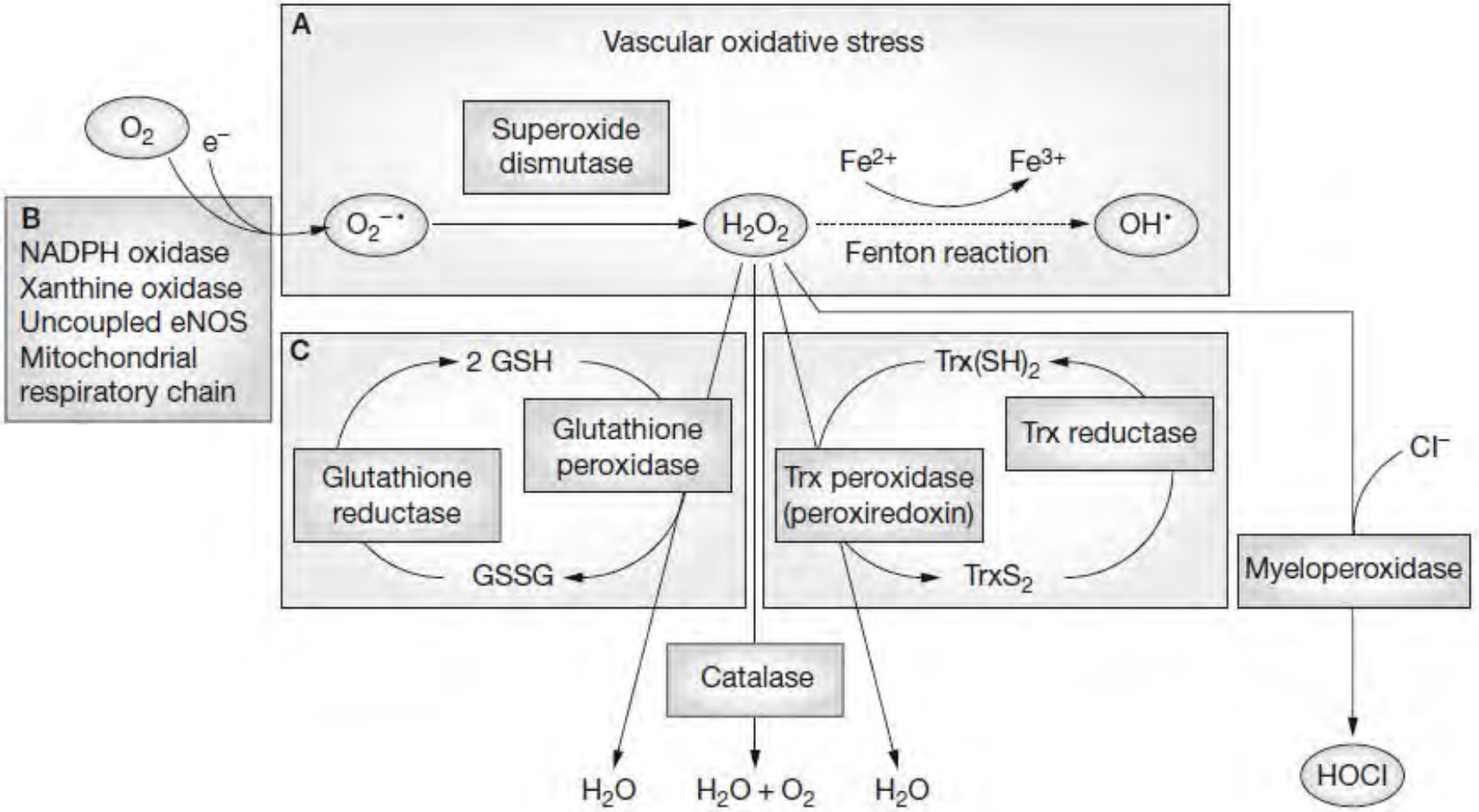
   a.Tyrosine kinases - PDGF
   b.Tyrosine phosphatases
   c.Serine/threonine kinases

Antioxidant systems

1. **Enzymatic systems**
   a.Superoxide dismutases
   b. Catalase
   c. Glutathione peroxidases
   d. Heme oxygenases
   e. Thioredoxin/thioredoxin reductase

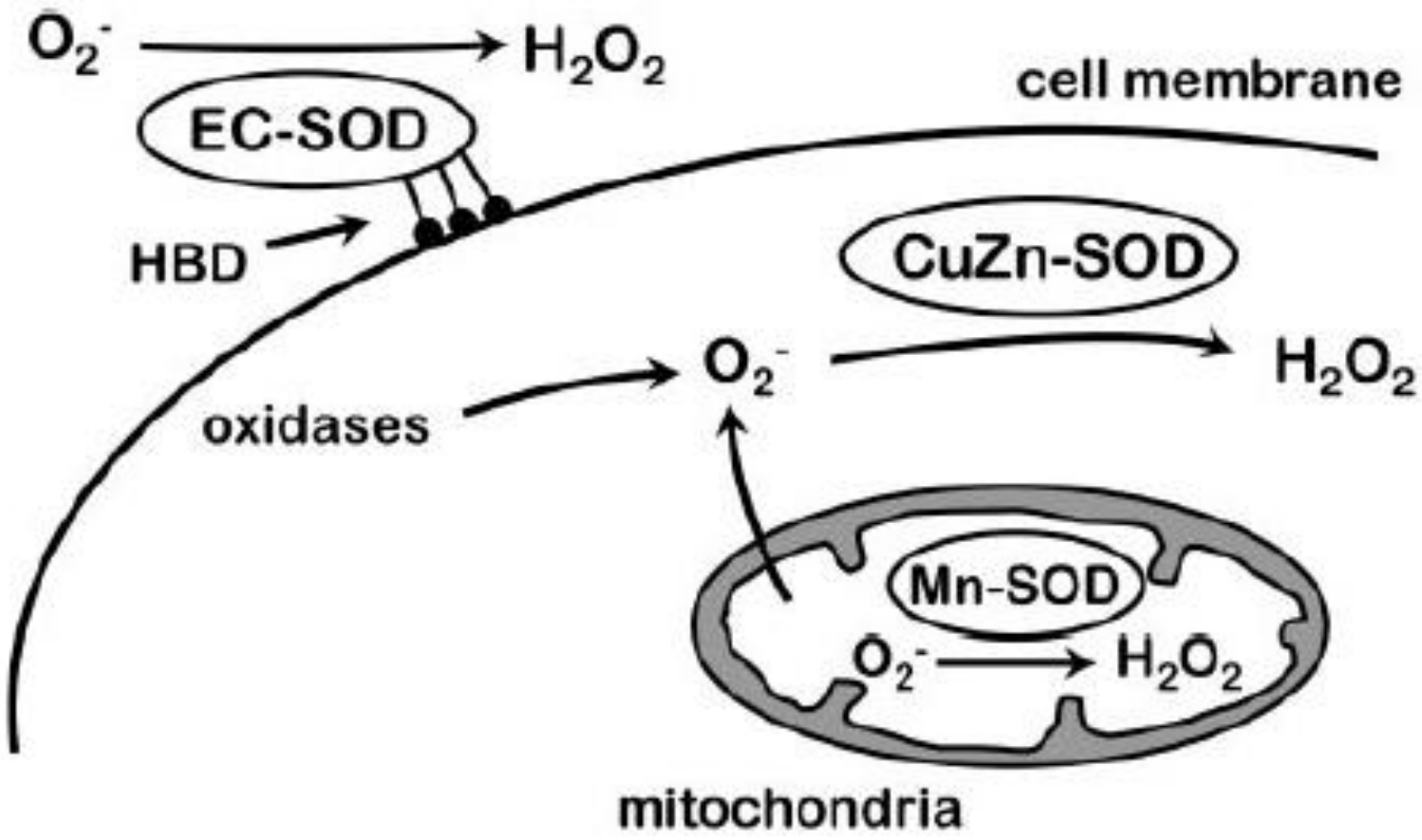
2. **Non-enzymatic systems**
   a.GSH
   b.TRX
   c.Heme byproducts: CO, ferritin, biliverdin and bilirubin
   d.Vitamin A and C

Enzymes generating and inactivating reactive oxygen species



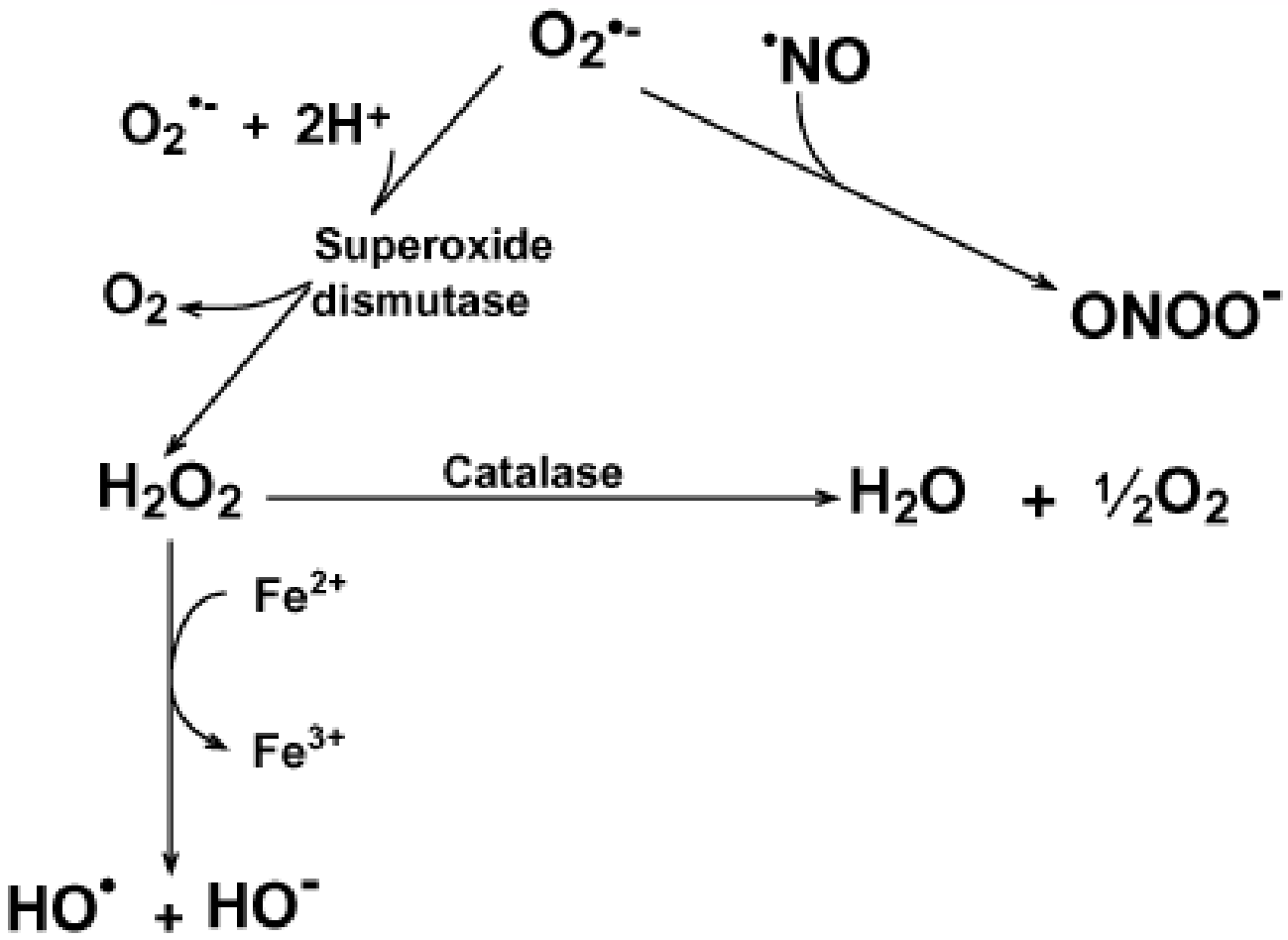
Forstermann U, Nature Rev Cardiol 2008

Subcellular localisation of superoxide dismutases

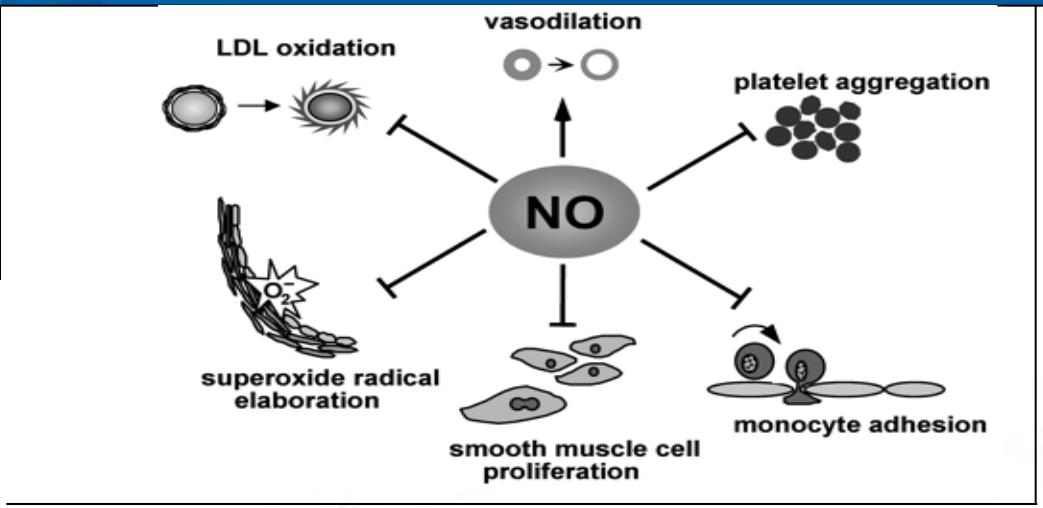


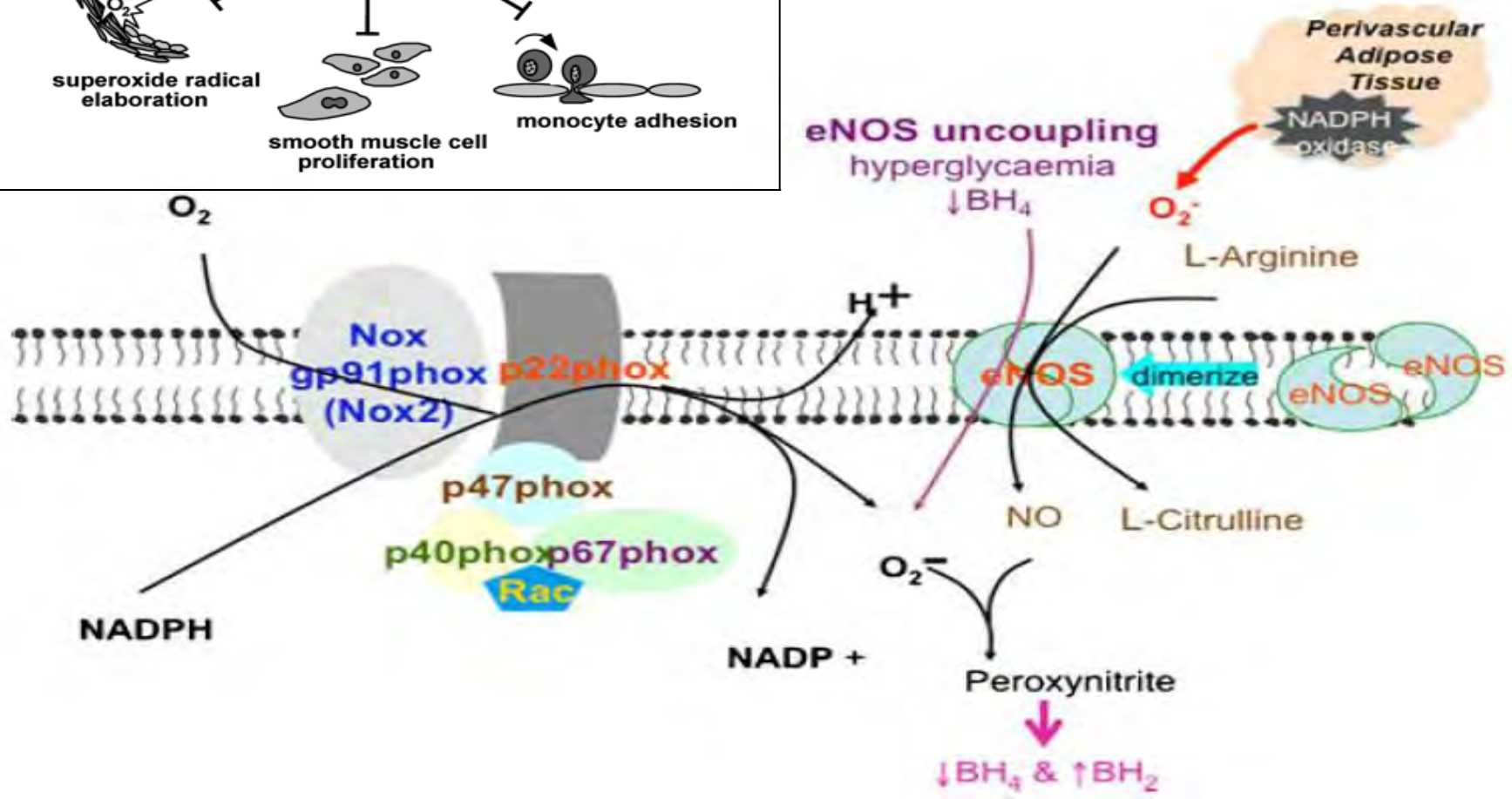
Faraci and Didion, ATVB 2004

SOD and nitric oxide compete for superoxide



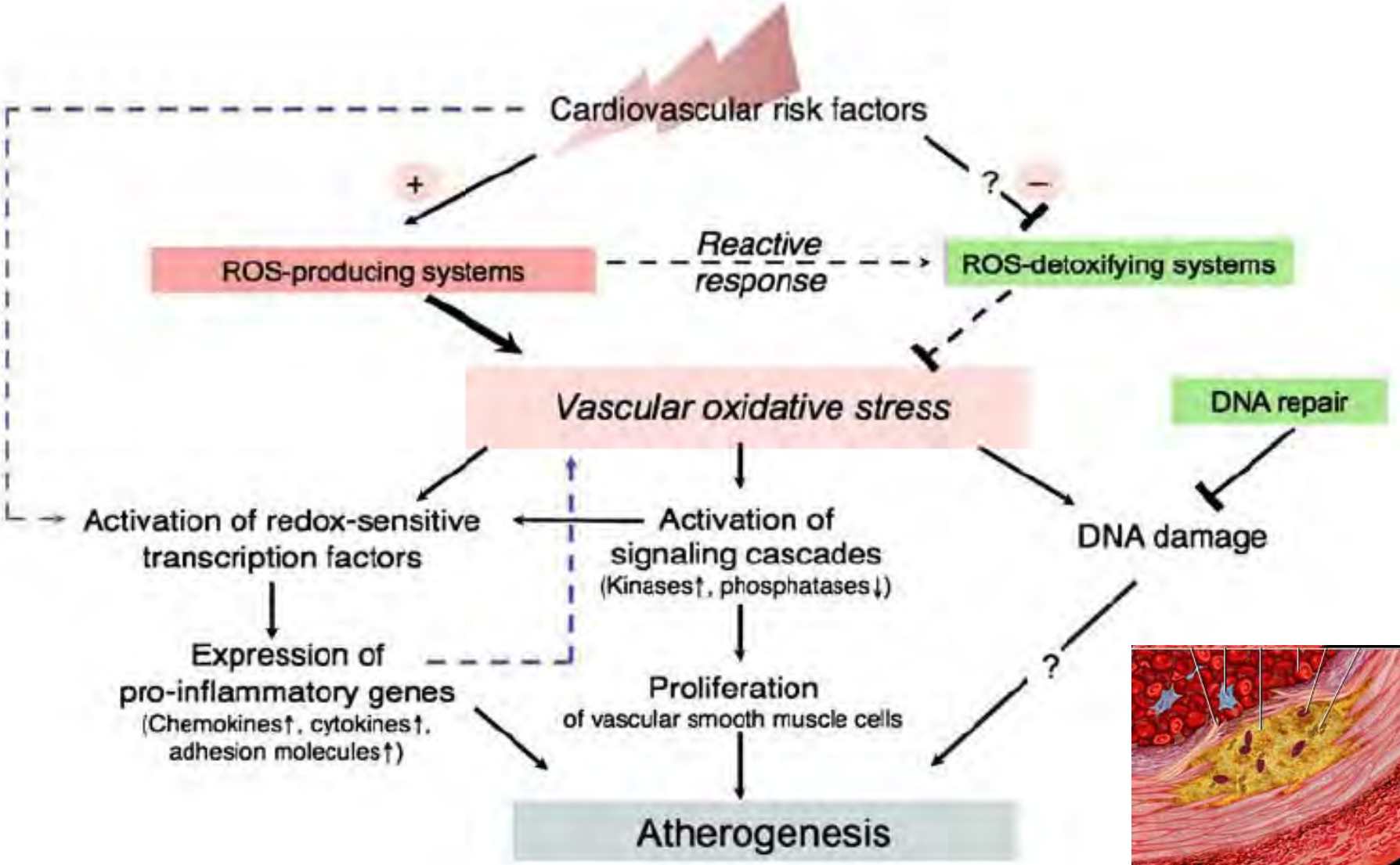
Oxidative stress causes endothelial dysfunction

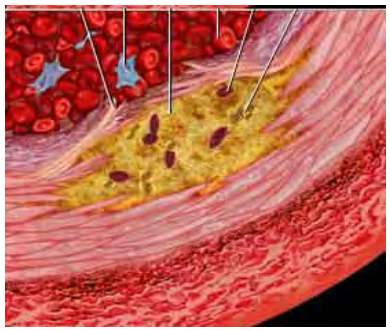




Ding and Triggle. Uer J Physiol 2010.

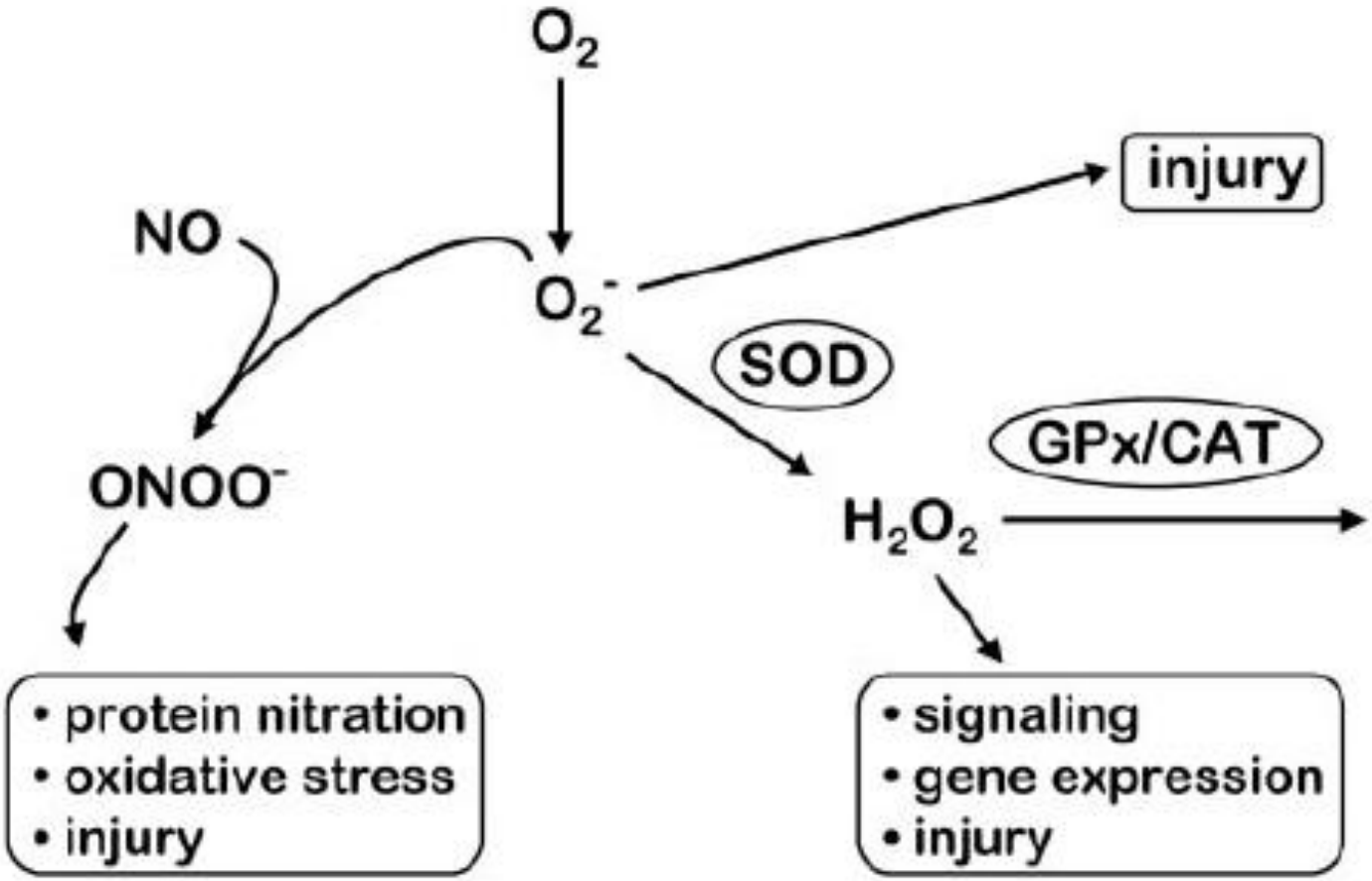
Link between oxidative stress and atherosclerosis





Forstermann U. Eur J Physiol 2010

Signaling and damage induced by ROS and NO·



Faraci and Didion, ATVB 2004

Glutathione peroxidase

Diminishes oxidative stress by using GSH to reduce H_2O_2 and lipid peroxides to their corresponding alcohols

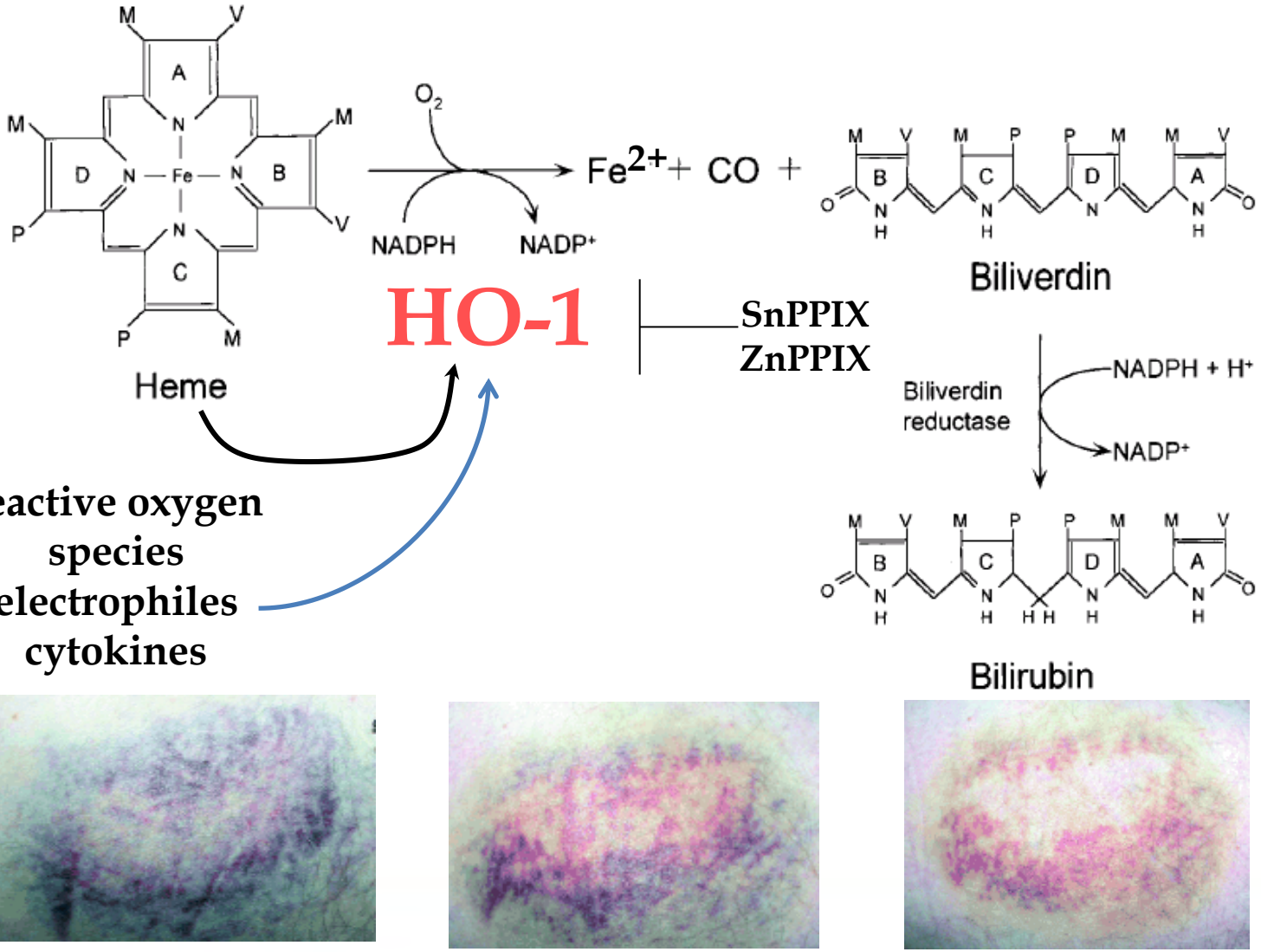
$$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$$

$$GS–SG + NADPH + H+ \rightarrow 2\ GSH + NADP+$$

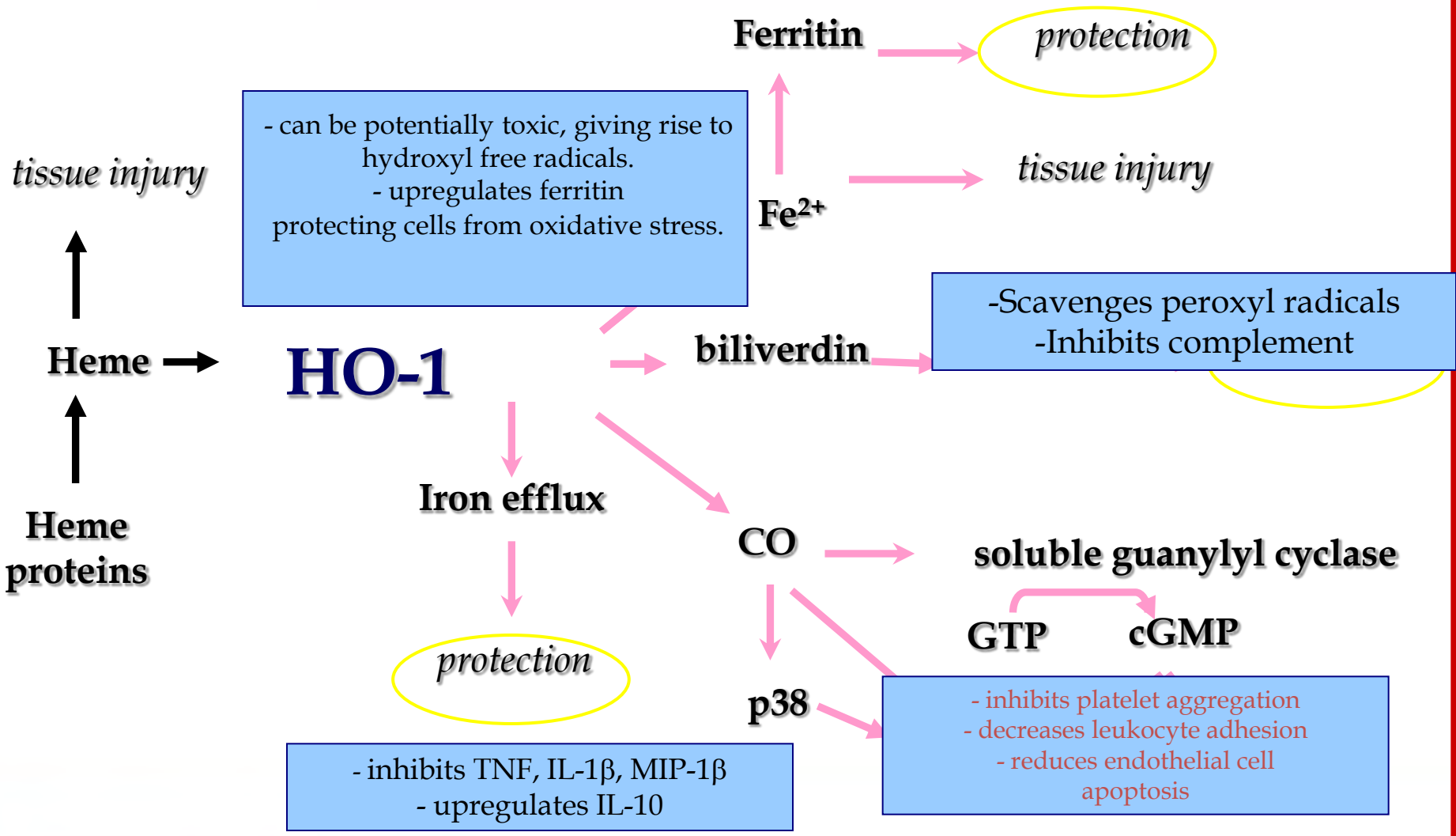
Gpx-1: the most abundant intracellular isoform of glutathione peroxidases

a selenocysteine-containing protein

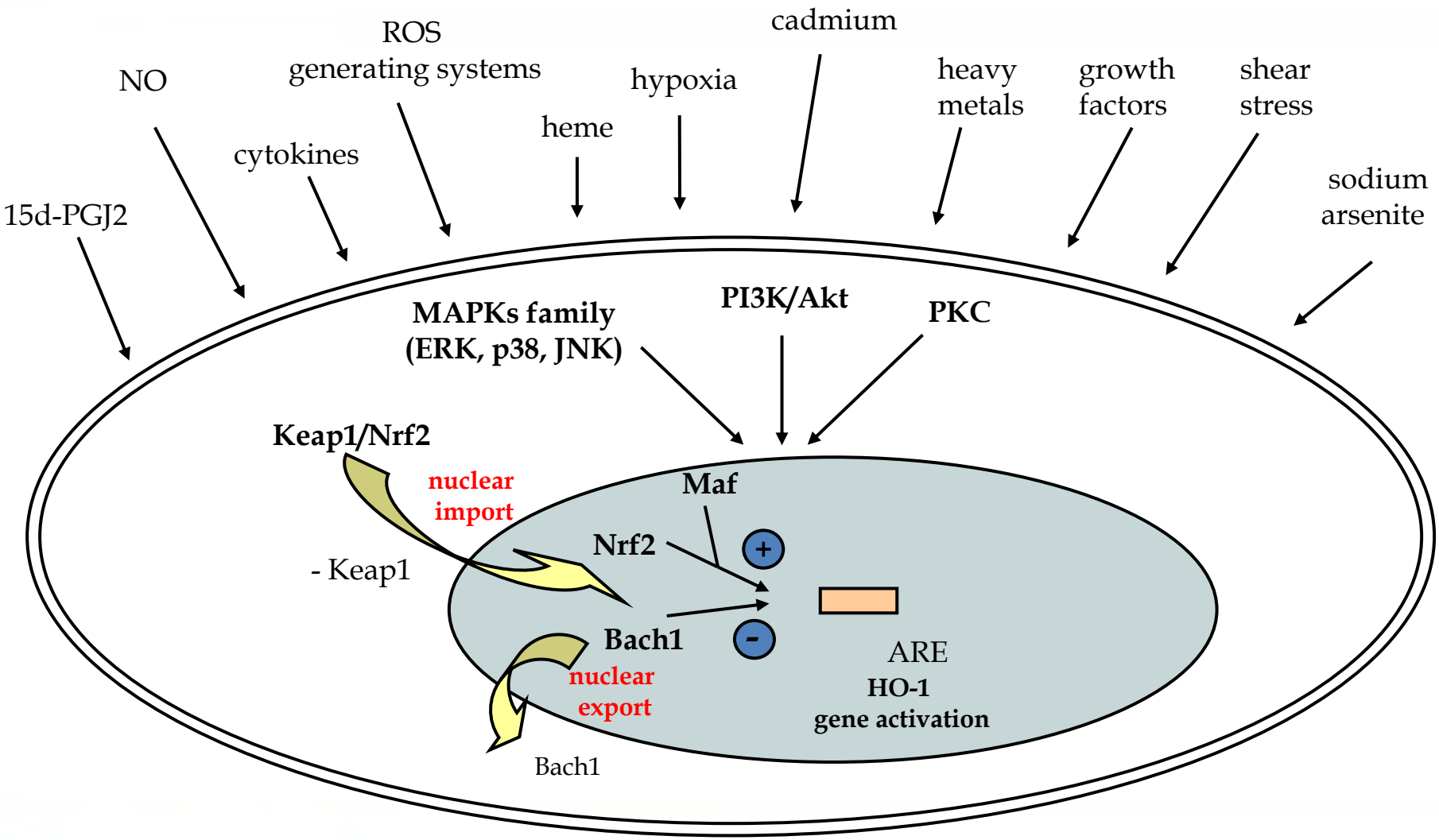
Heme oxygenase-1 activity



Mechanisms of protective effects of HO-1

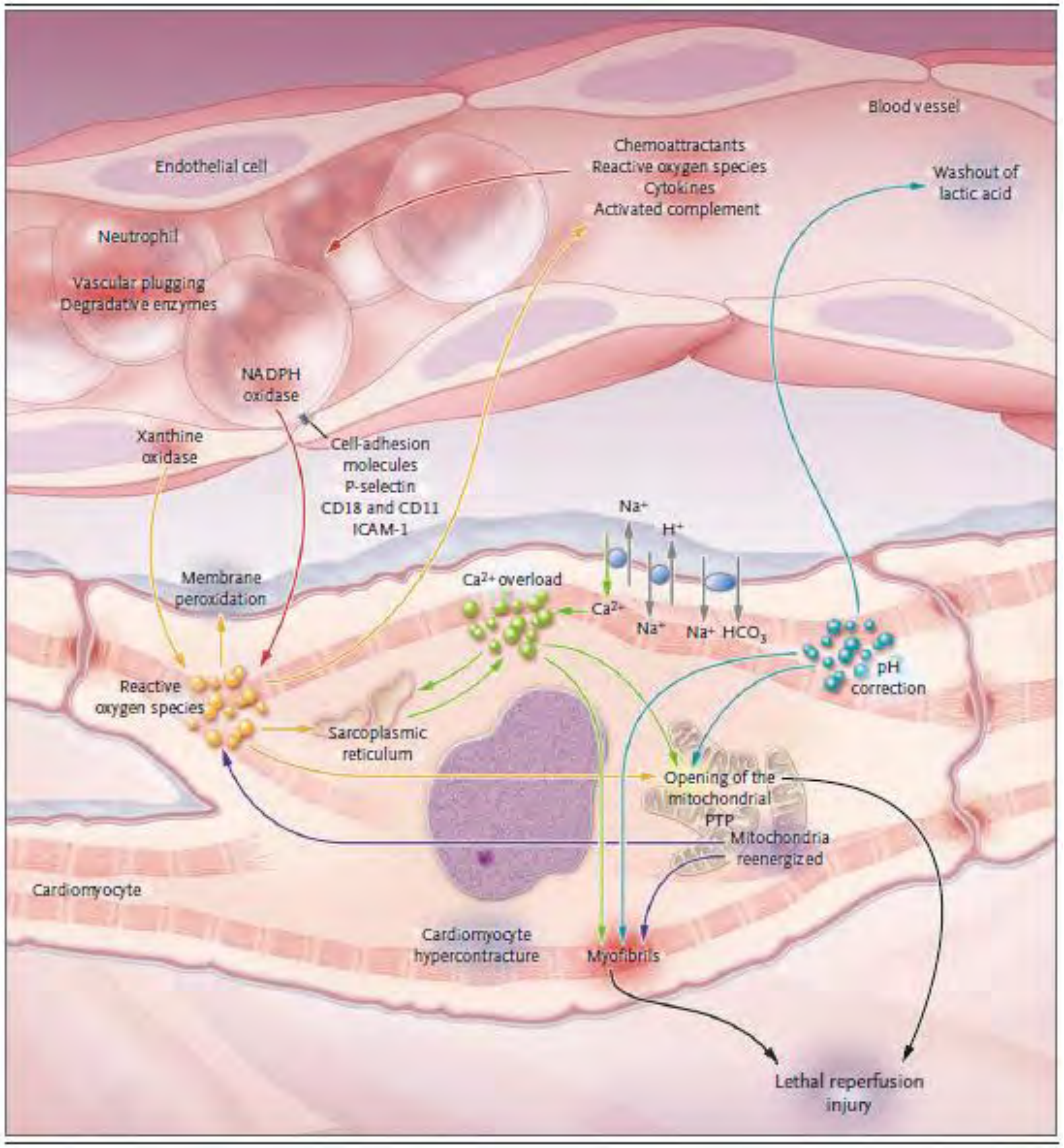


Nrf2- a major mediator of HO-1 expression



Loboda et al. (Dulak) , ARS 2008

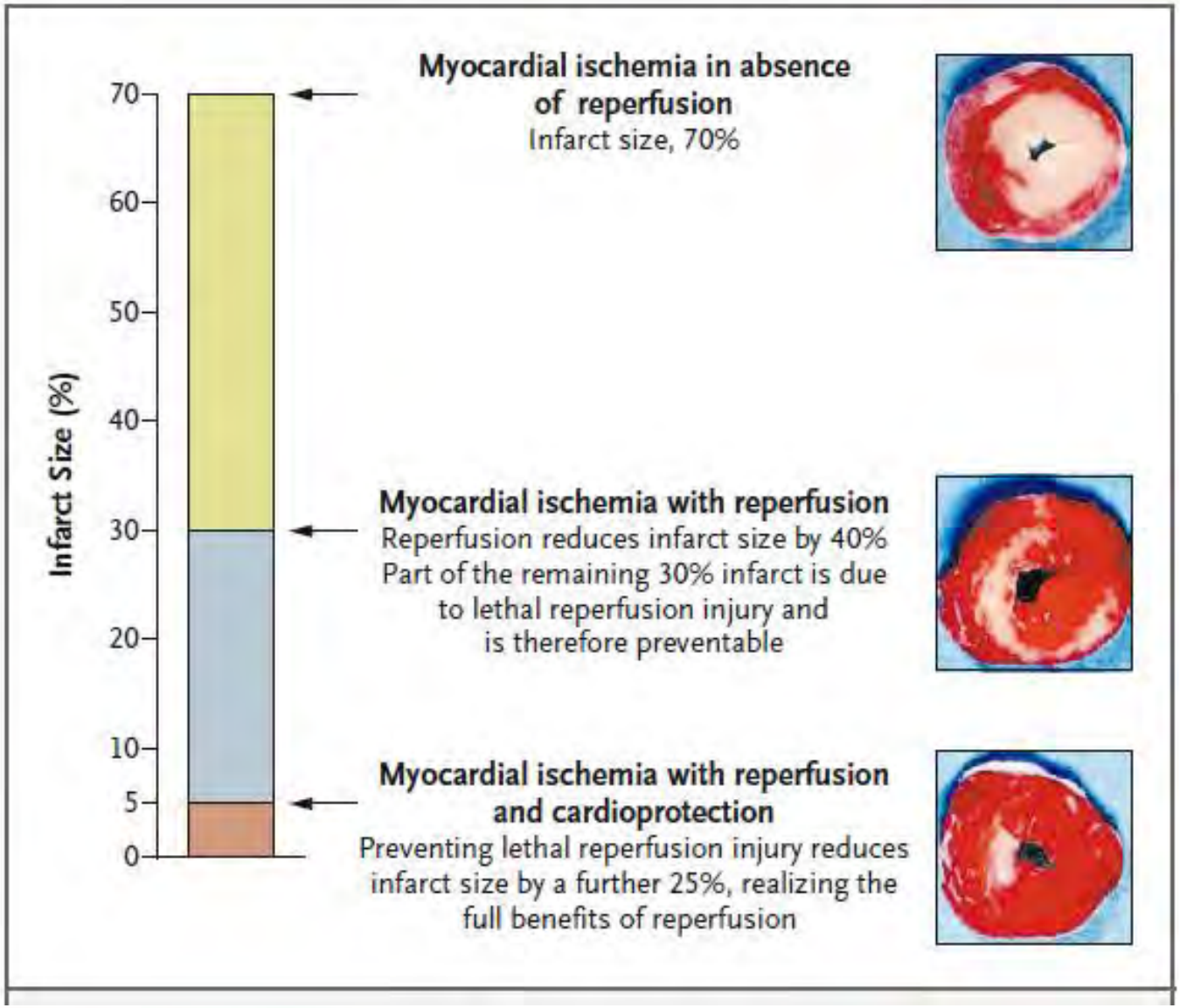
Major mediators of ichemia-reperfusion injury



1. **Increased reactive oxygen species production from parenchymal, endothelial cells and inflitrating leukocytes**
   - incomplete reduction of oxygen by damaged mitochondria
   - action of oxidases
   - decreased antioxidant reponse – can be additionally compromised by hypoxia
2. **Inflammation –** due to increased production of cytokines and increased expression of adhesion molecules by hypoxia parenchymal and endothelial cells : leads to recrutiment of leukocytes and aggravation of injury
2. **Activation of complement**

Yellon & Hausenloy, NEJM 2007

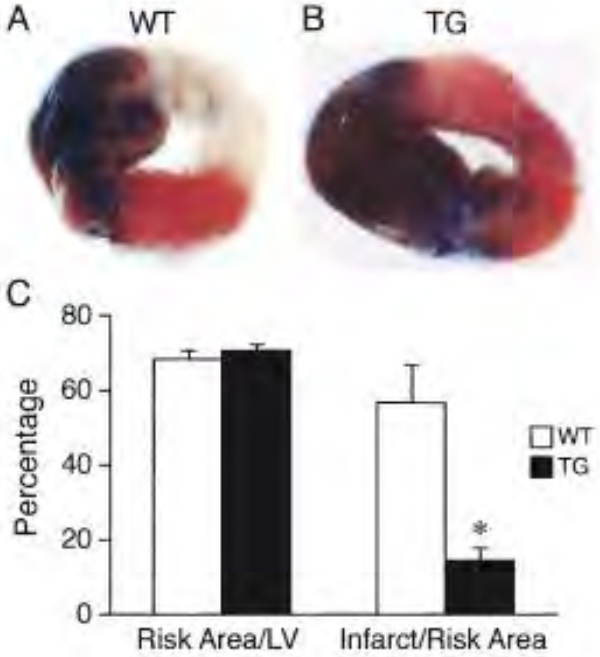
Contribution of Lethal Reperfusion Injury to Final Myocardial Infarct Size

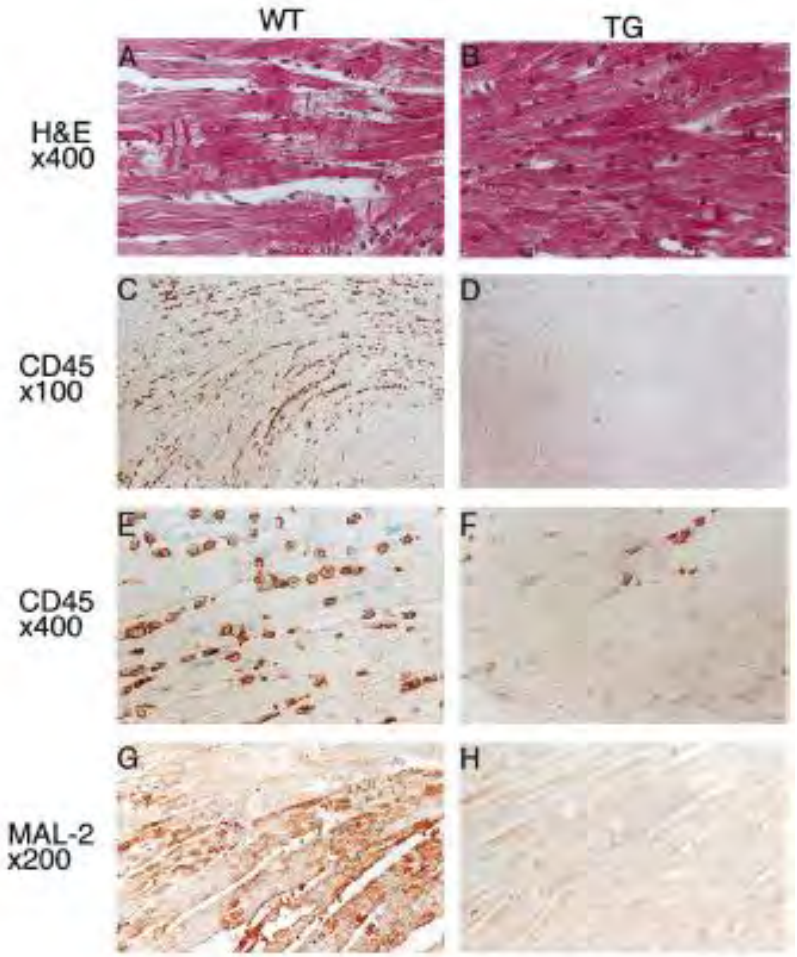


Yellon & Hausenloy, NEJM 2007

Heme oxygenase-1 protects against myocardial ischemia and reperfusion injury

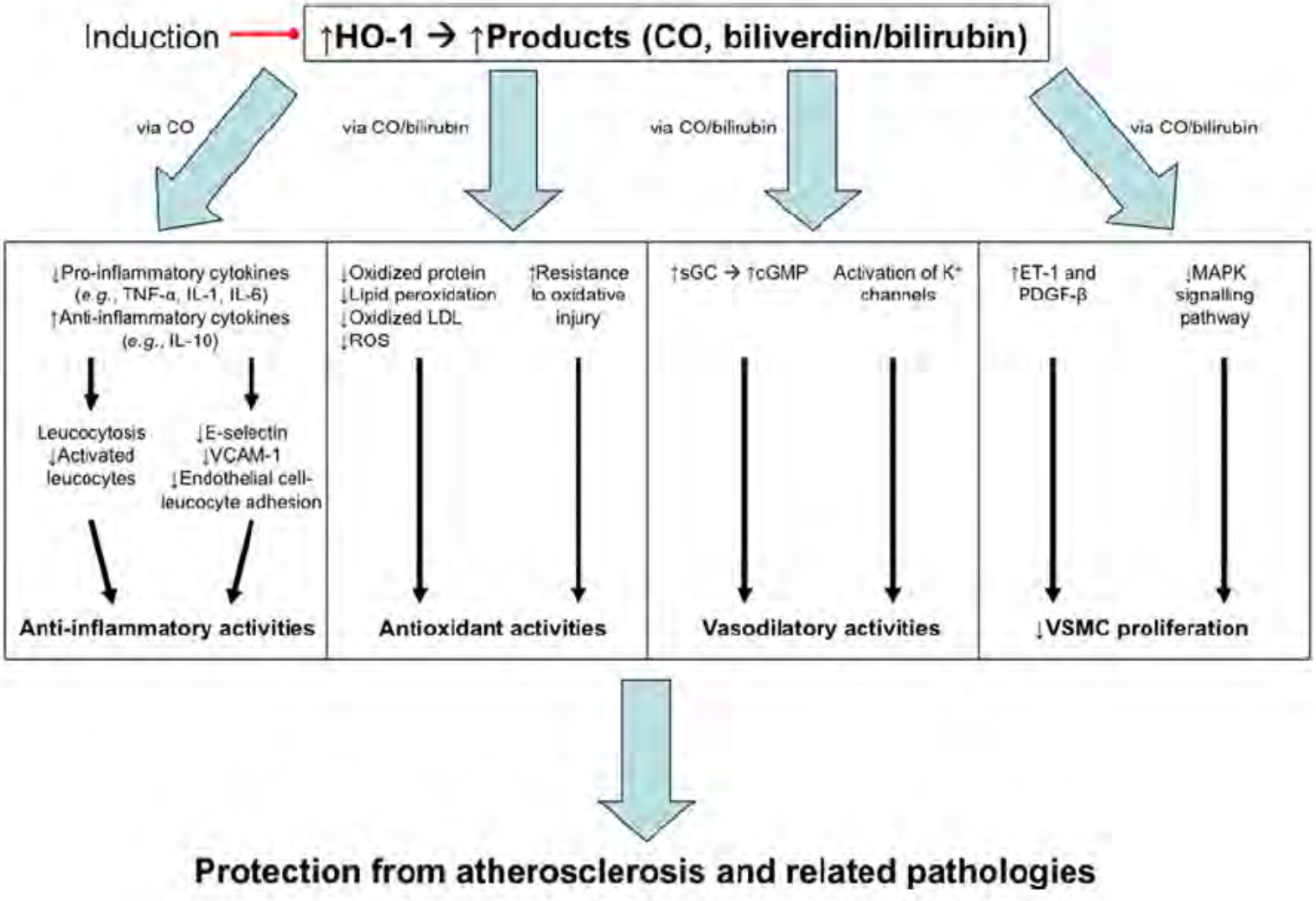
Cardiac-Specific Expression of Heme Oxygenase-1 Protects Against Ischemia and Reperfusion Injury in Transgenic Mice





S-F Yet et al., Circ Res 2001

Atheroprotective properties of heme oxygenase



Chan et al. (Stocker) Clinical Science 2011: 120: 493-504,

Vascular disorders associated with HO/CO

Aneurysms
Arteriovenous fistula restenosis
Atherosclerosis
Cancer
Hypertension
Impaired wound healing
Ischemia/reperfusion injury
Peripheral vascular disease
Preeclampsia
Psoriasis
Sickle cell disease
Transplant-related arteriosclerosis
Vascular restenosis

Dulak et al., Circulation 2008: 117: 231-241

Support for the protective role of HO-1 against atherosclerosis and related diseases

Evidence	Reference
Presence of HO-1 in human and experimental atherosclerotic lesions	19
High serum bilirubin levels associated with decreased risk of coronary artery disease	8
Low serum bilirubin levels associated with increased risk of coronary artery disease	7
HO-1 induction/upregulation decreases experimental atherosclerosis	36–38
HO-1 inhibition/downregulation increases experimental atherosclerosis	36, 37
Increased atherosclerosis in $ApoE^{-/-}HO\text{-}1^{-/-}$ vs $ApoE^{-/-}HO\text{-}1^{+/+}$ mice	9
Increased vein graft stenosis in $ApoE^{-/-}HO\text{-}1^{-/-}$ vs $ApoE^{-/-}HO\text{-}1^{+/+}$ mice	9
HO-1 overexpression decreases intimal thickening in experimental restenosis	10, 40, 46
HO-1 deficiency/downregulation increases intimal thickening in experimental restenosis	10, 120
Cardiac-specific HO-1 overexpression protects against experimental ischemia/reperfusion injury	11, 85
Lung-specific overexpression of HO-1 ameliorates pulmonary vascular hypertension in a model of chronic hypoxia	86

Stocker R, Perella M, Circulation 2006: 114: 2178-2189

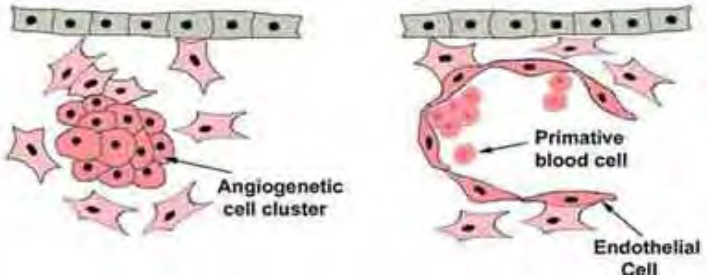
Non-canonical functions of pro- and antioxidant pathways

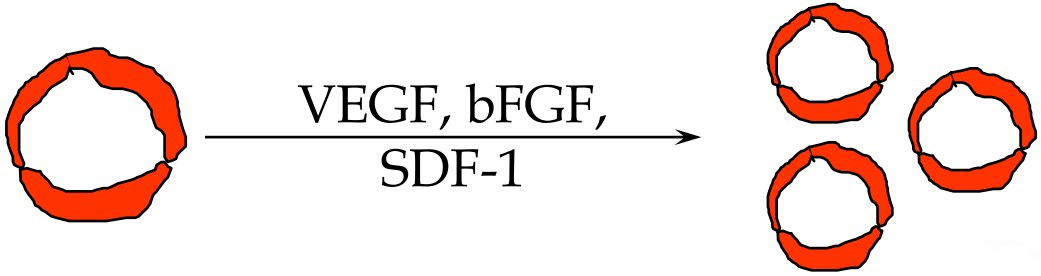
Oxidative stress and blood vessel formation

Mechanisms of blood vessel formation

Vasculogenesis

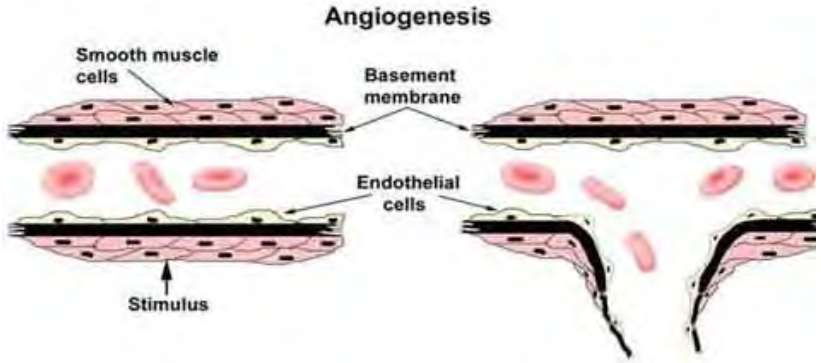
capillaries are formed from vascular progenitor cells



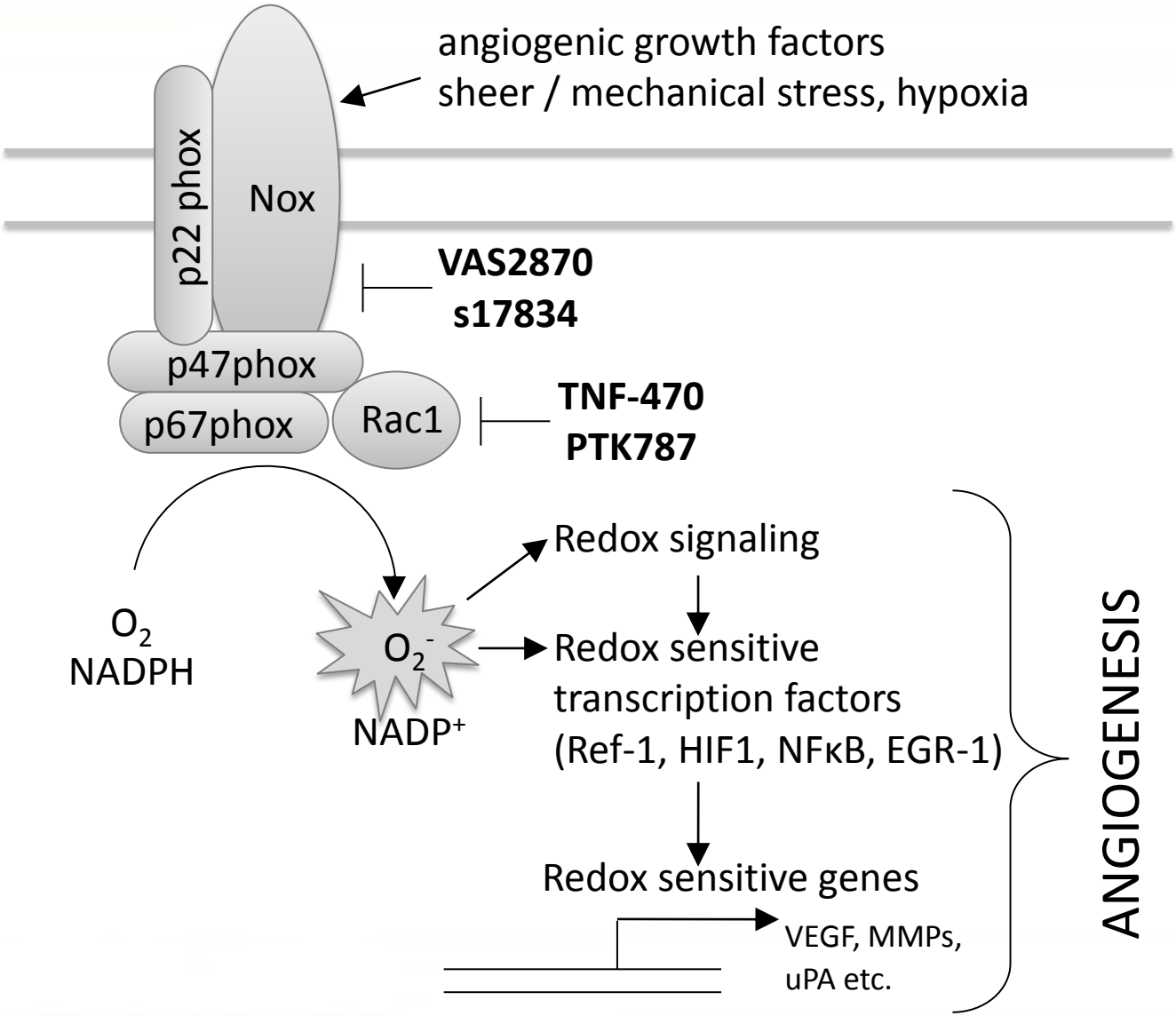


Angiogenesis

formation of new blood vessels from pre-existing capillaries

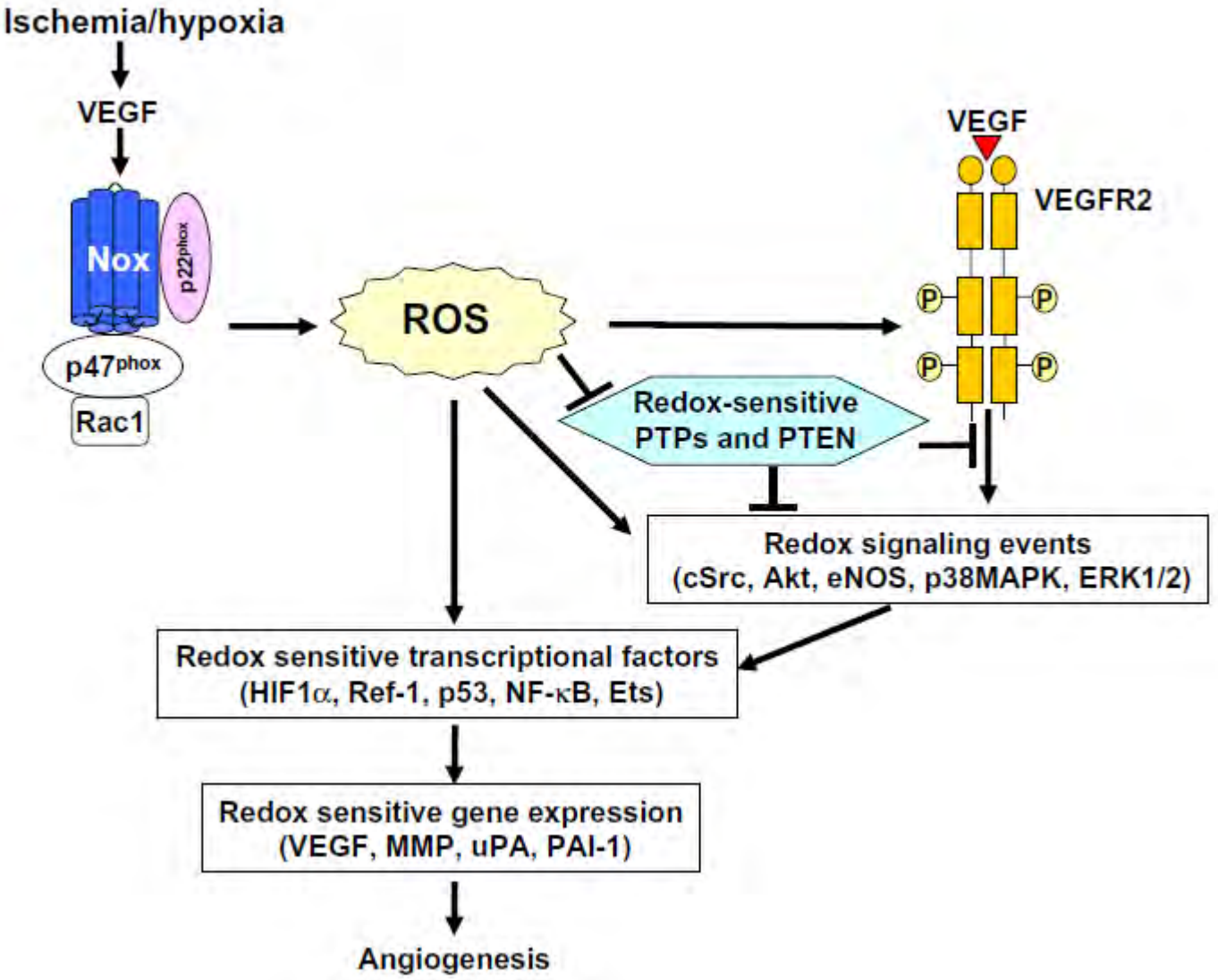


Involvement of reactive oxygen species in angiogenesis



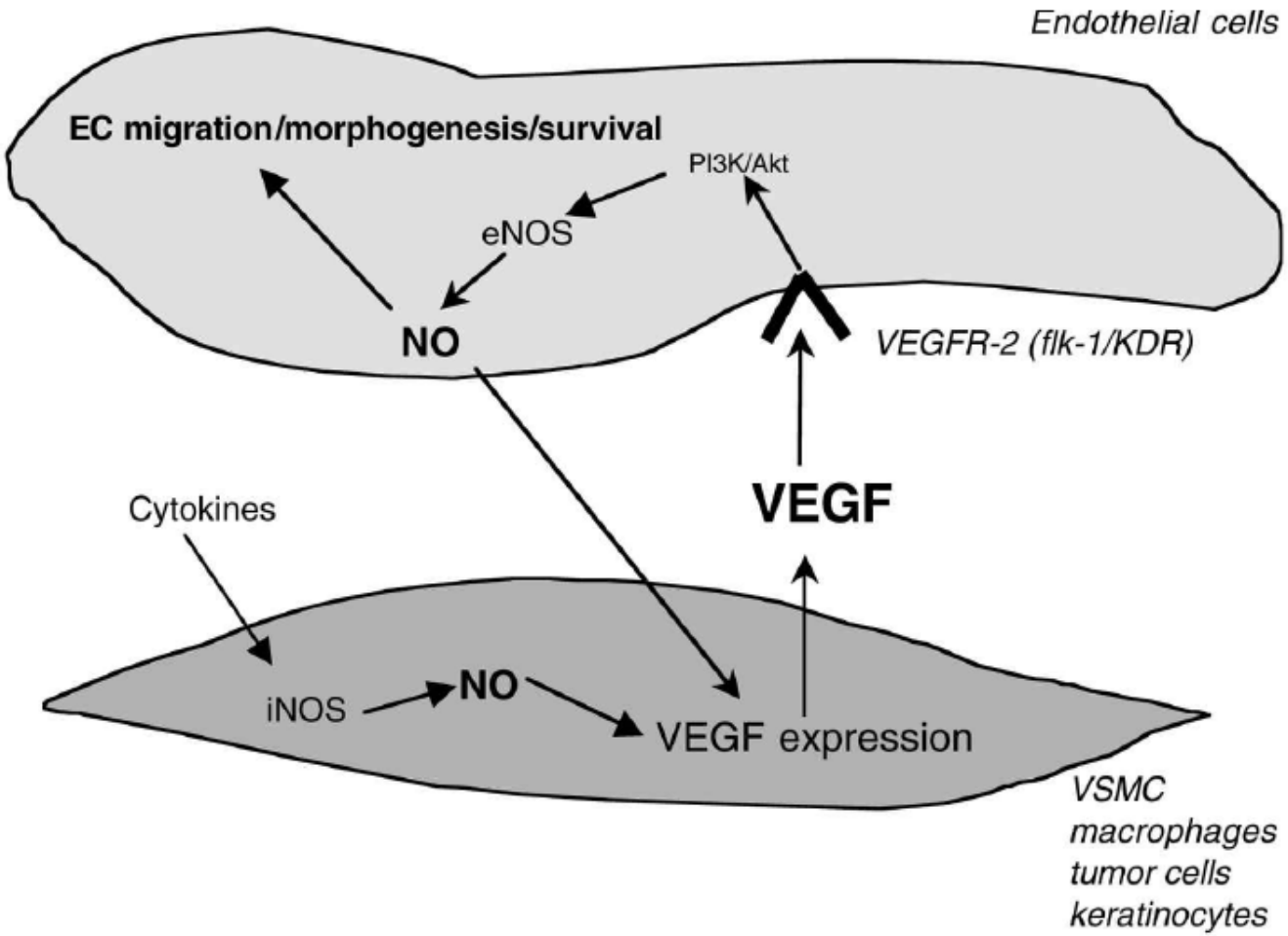
Tertil et al., Curr Pharm Des, 2010: 16: 3877-3894

Involvement of reactive oxygen species in angiogenesis



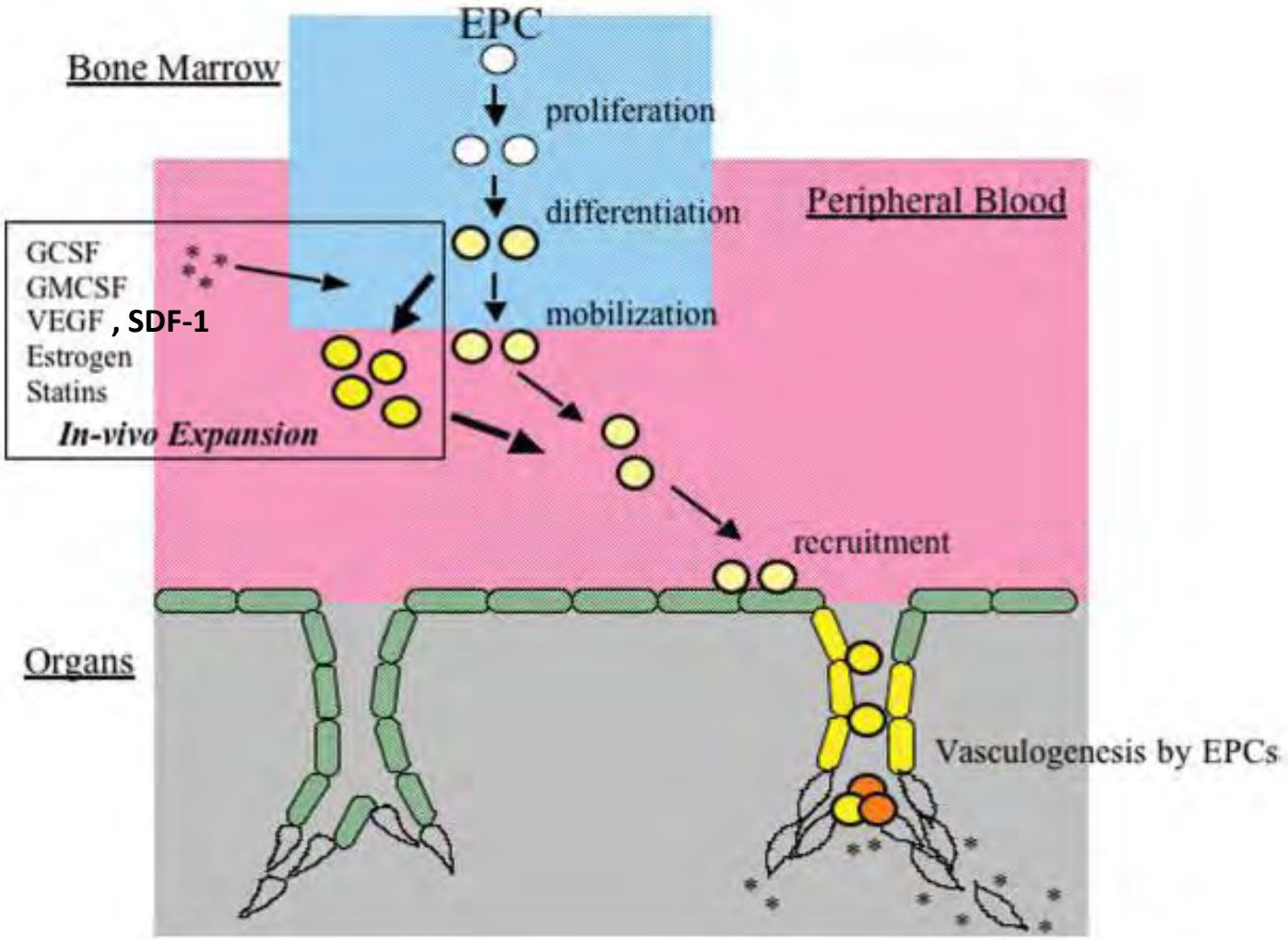
Ushio-Fukai & Nakamura, Cancer Letters 2008: 266: 37–52

NO works upstream and downstream of VEGF



Dulak & Jozkowicz, Antioxid Redox Signal, 2003: 5:123-32

Endothelial progenitor cells and postnatal vasculogenesis

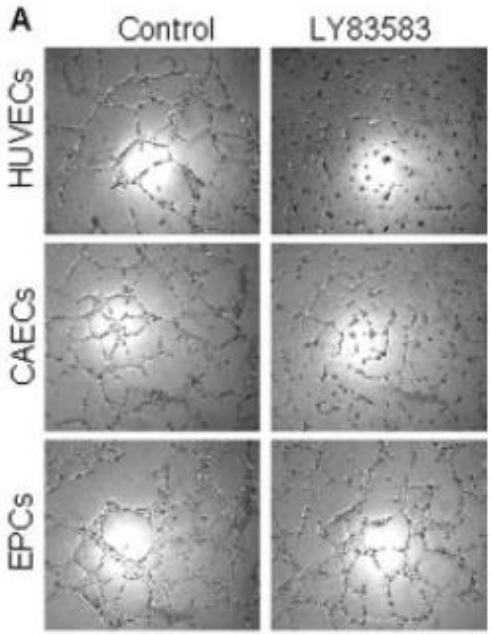


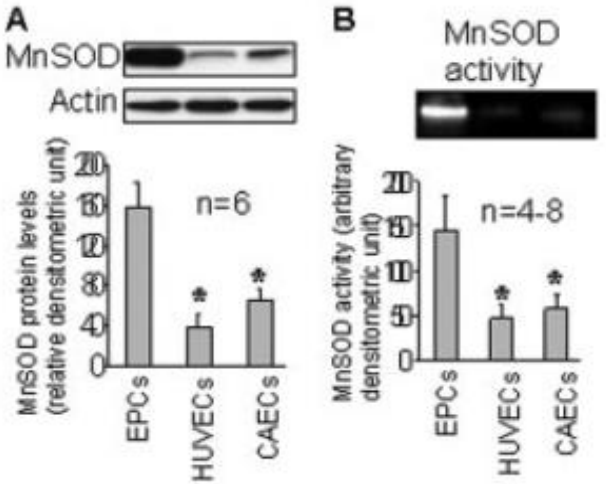
Urbich et al., 2005

EPC express manganese superoxide dismutase

Human Endothelial Progenitor Cells Tolerate Oxidative Stress Due to Intrinsically High Expression of Manganese Superoxide Dismutase

Tongrong He, Timothy E. Peterson, Ekhson L. Holmuhamedov, Andre Terzic, Noel M. Caplice, Larry W. Oberley, Zvonimir S. Katusic

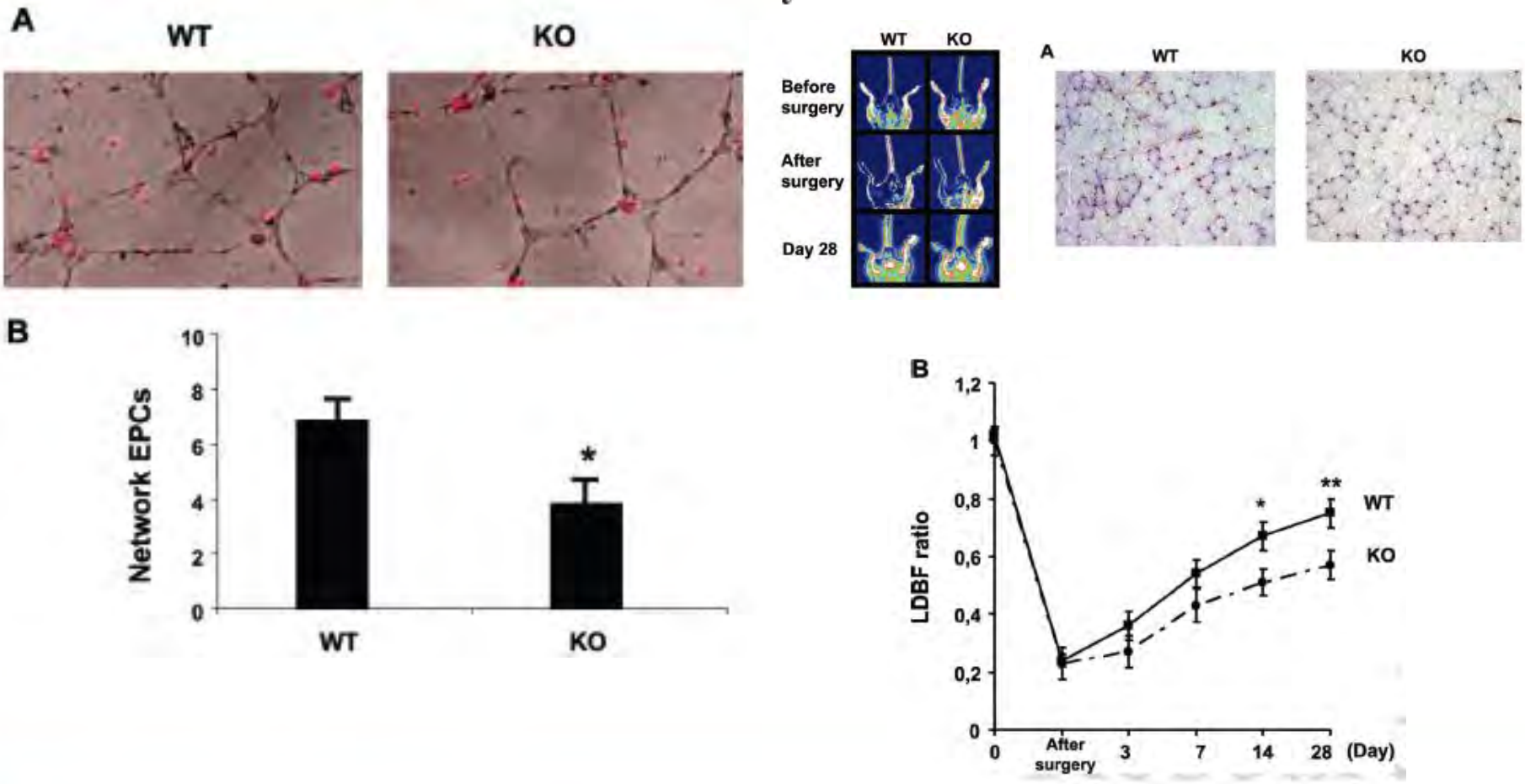




ATVB 2004: 24: 2021-2027

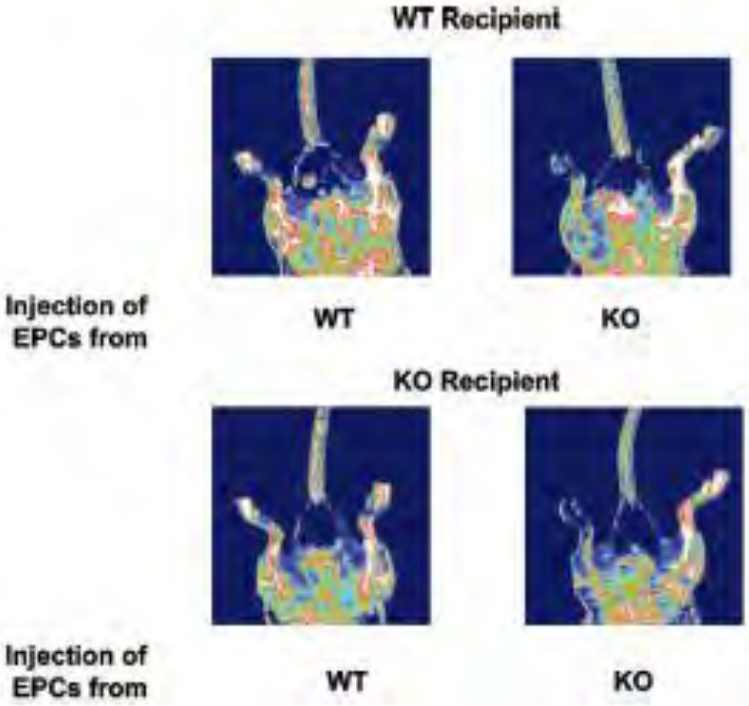
EPC dysfunction in Gpx-1 deficient mice

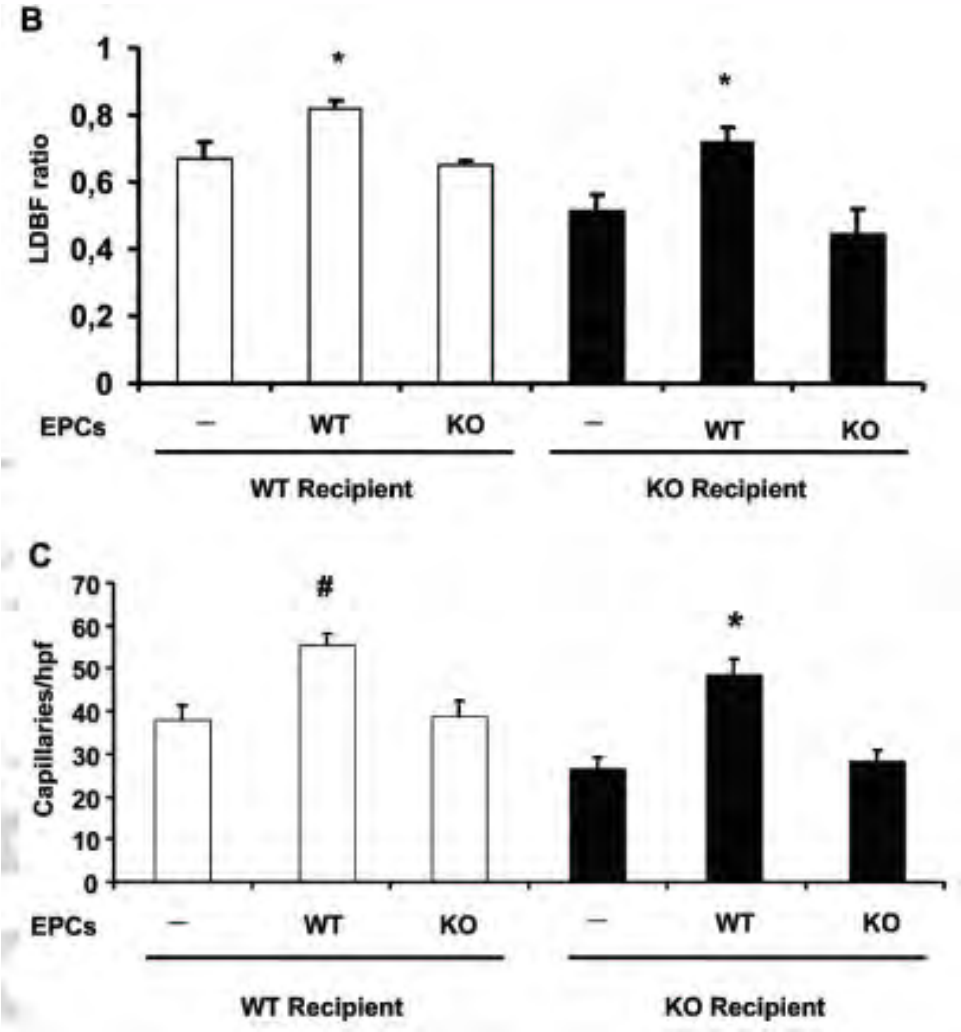
Impaired Angiogenesis in Glutathione Peroxidase-1–Deficient Mice Is Associated With Endothelial Progenitor Cell Dysfunction



Galasso et al. Circ Res 2006

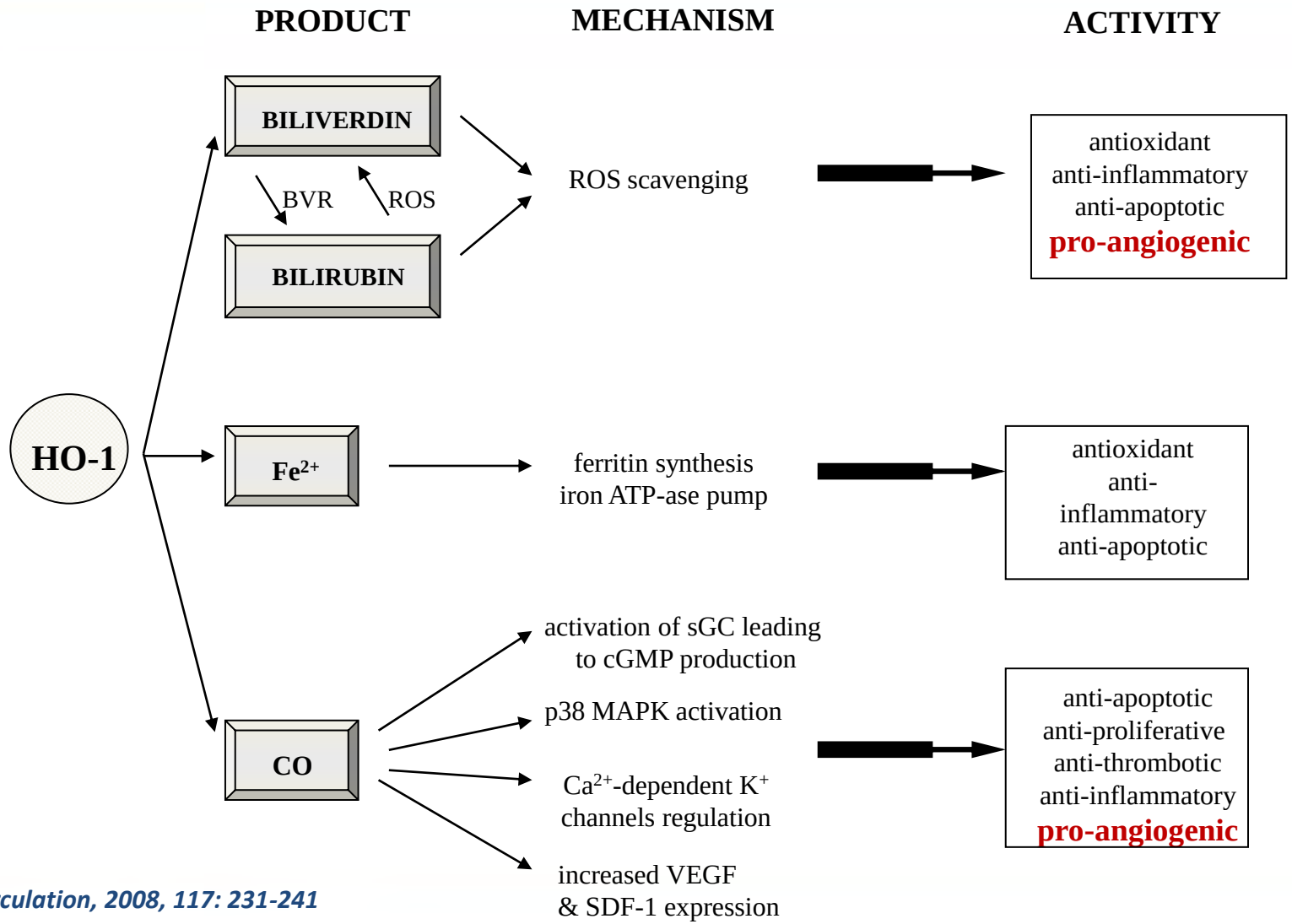
EPC dysfunction in Gpx-1 deficient mice





Galasso et al. Circ Res 2006

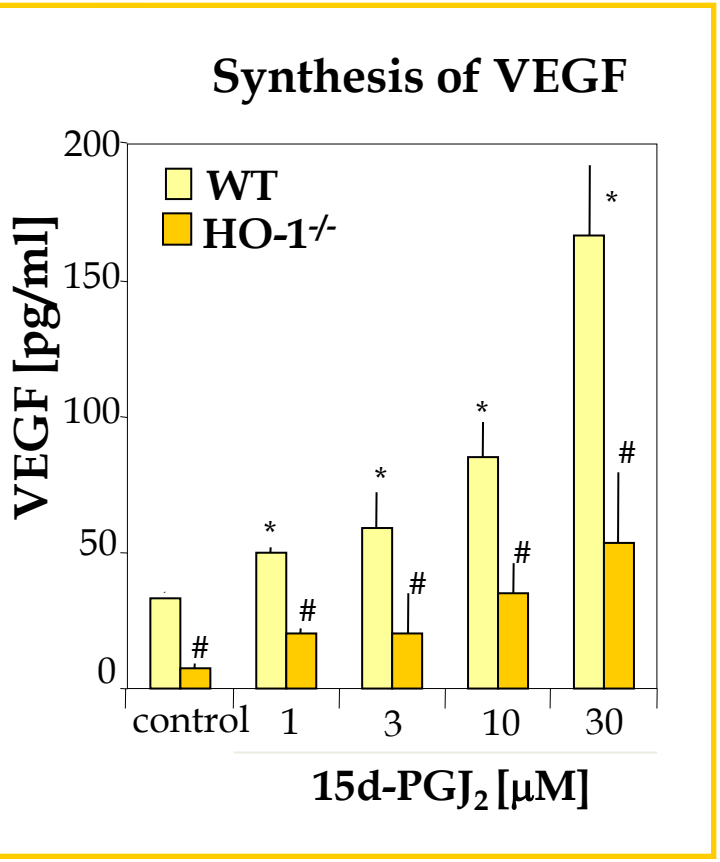
Pleiotropic effects of heme oxygenase-1 and its products

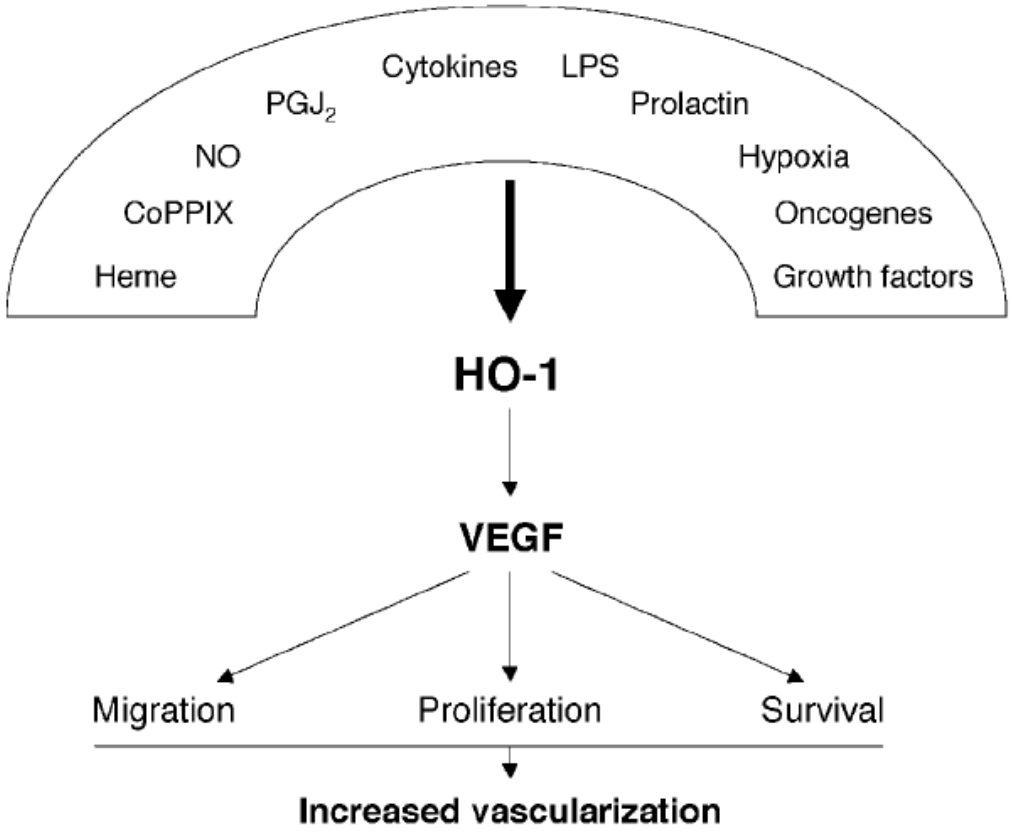


Dulak et al. - *Circulation, 2008, 117: 231-241*

Dulak et al. - *Current Opinion Lipidol, 2008;19:505-12*.

HO-1 regulates VEGF synthesis in response to different stimuli

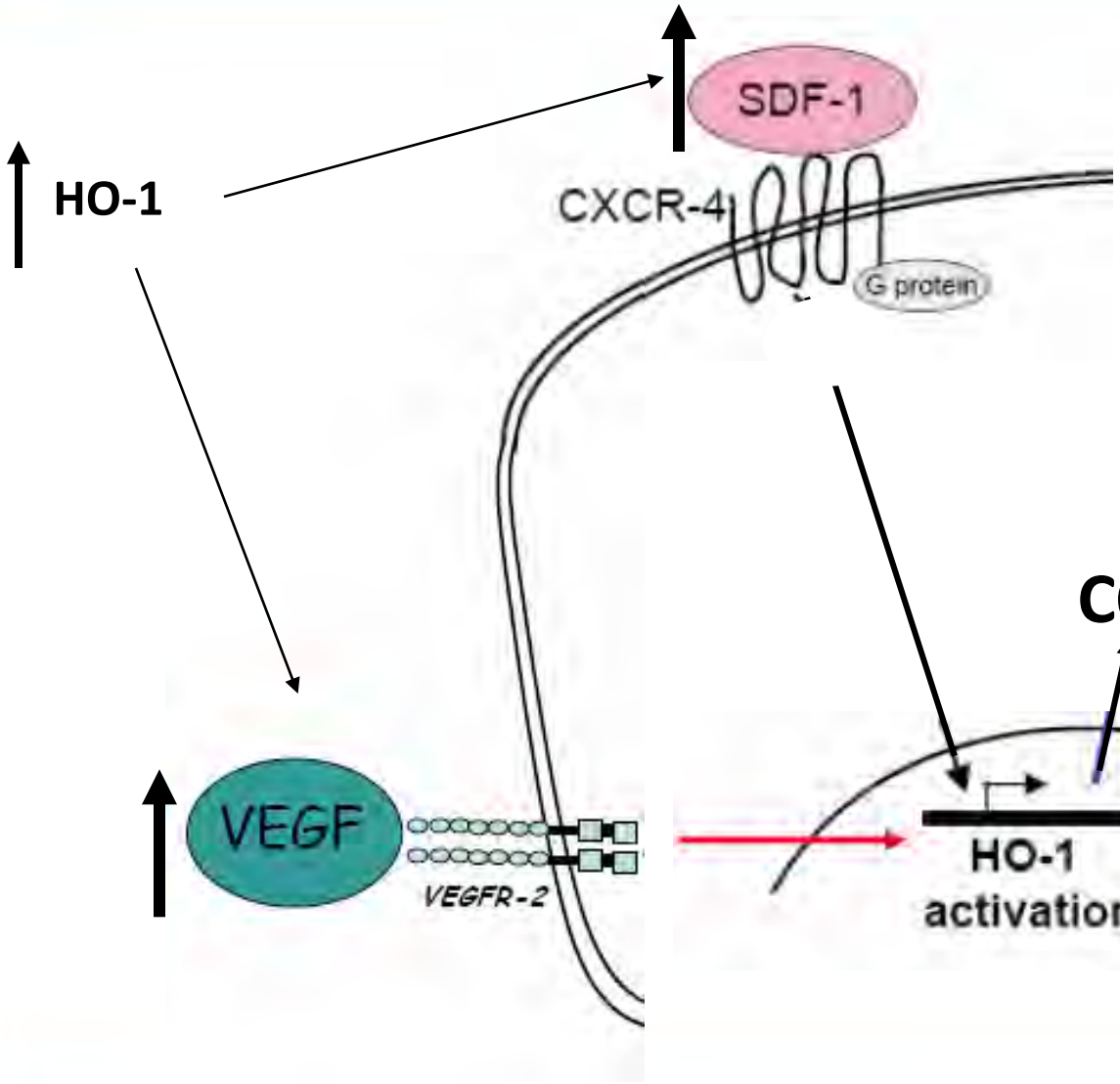




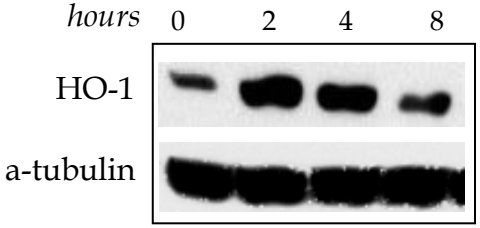
Cisowski et al., BBRC 2005

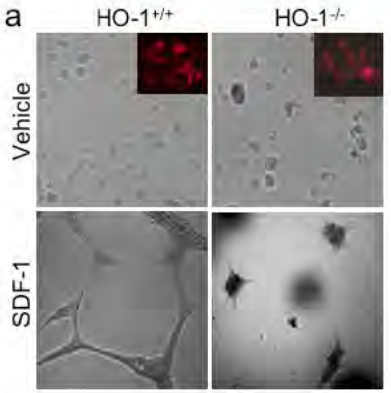
Dulak et al., Circulation, 2008: 117: 231-241

HO-1 works upstream and downstream of angiogenic growth factors



VEGF 50 ng/ml

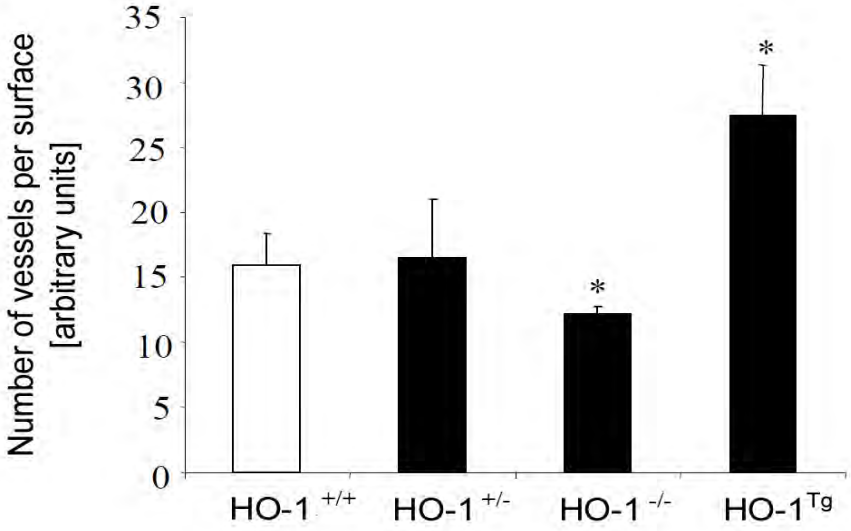


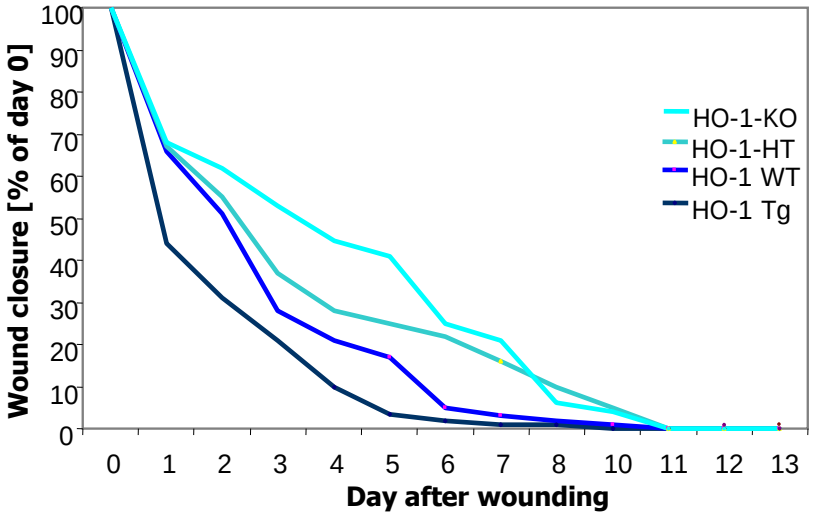


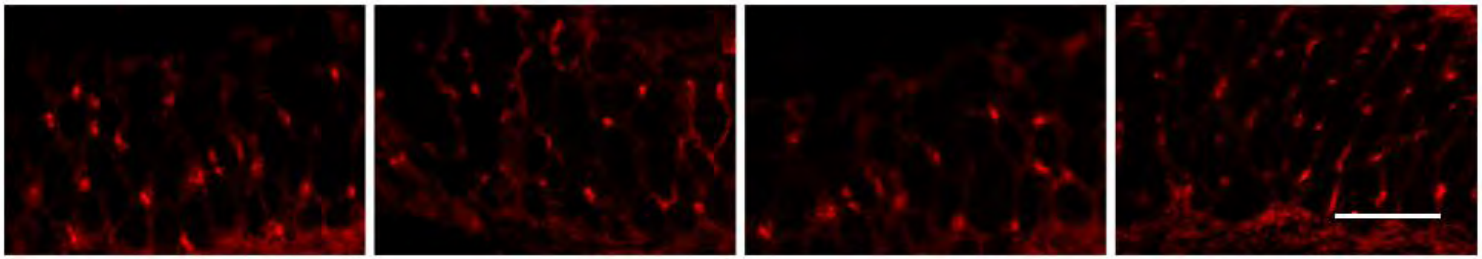
Reviewed in: Dulak et al., Circulation 2008;
Grochot Przeczek et al. Thrombosis & Hemostasis, 2010

HO-1 is required for blood vessel formation

Skin wound healing



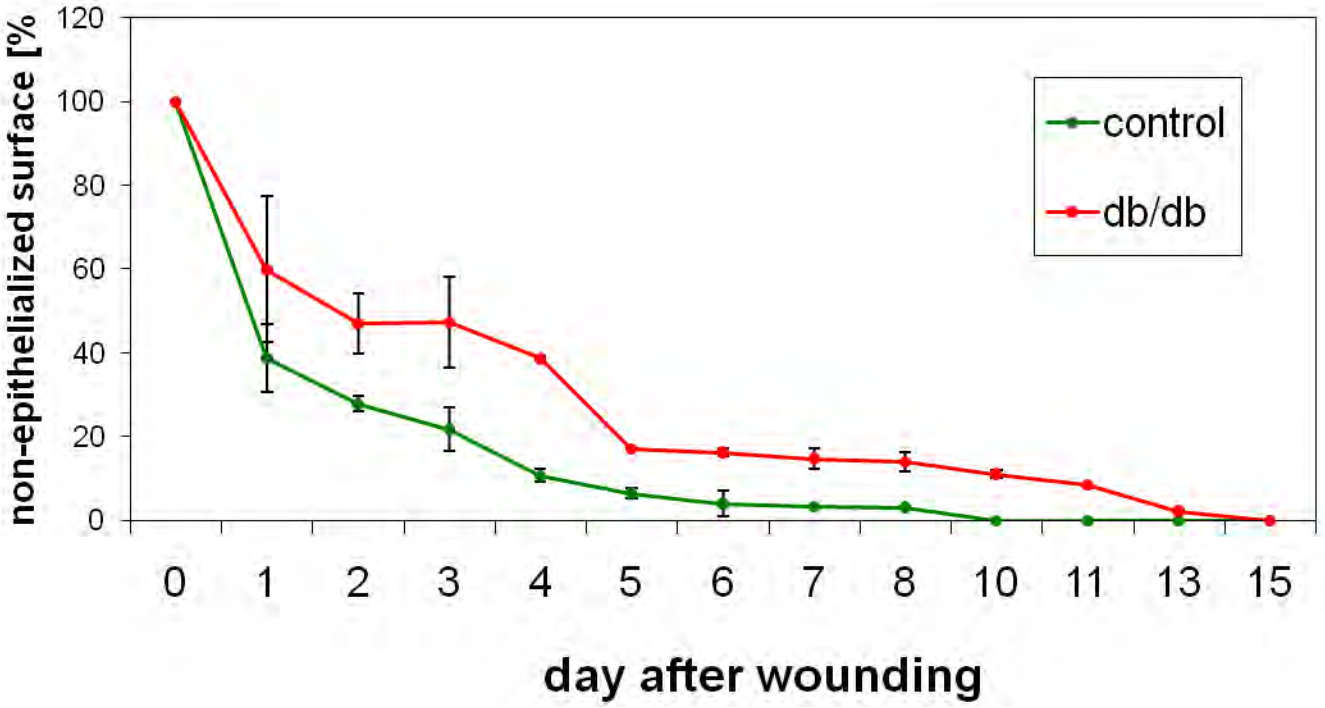


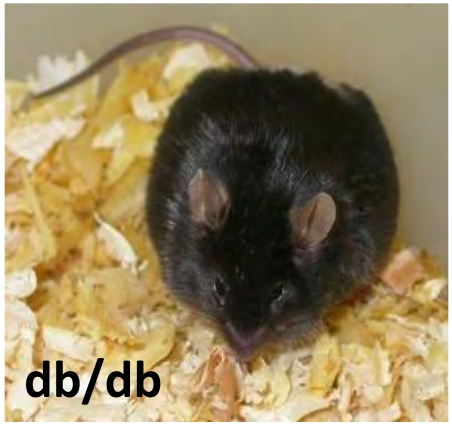


HO-1 +/+ HO-1 +/- HO-1 -/- HO-1Tg

Grochot-Przeczek et al. , PLoS ONE 4(6): e5803; 2009

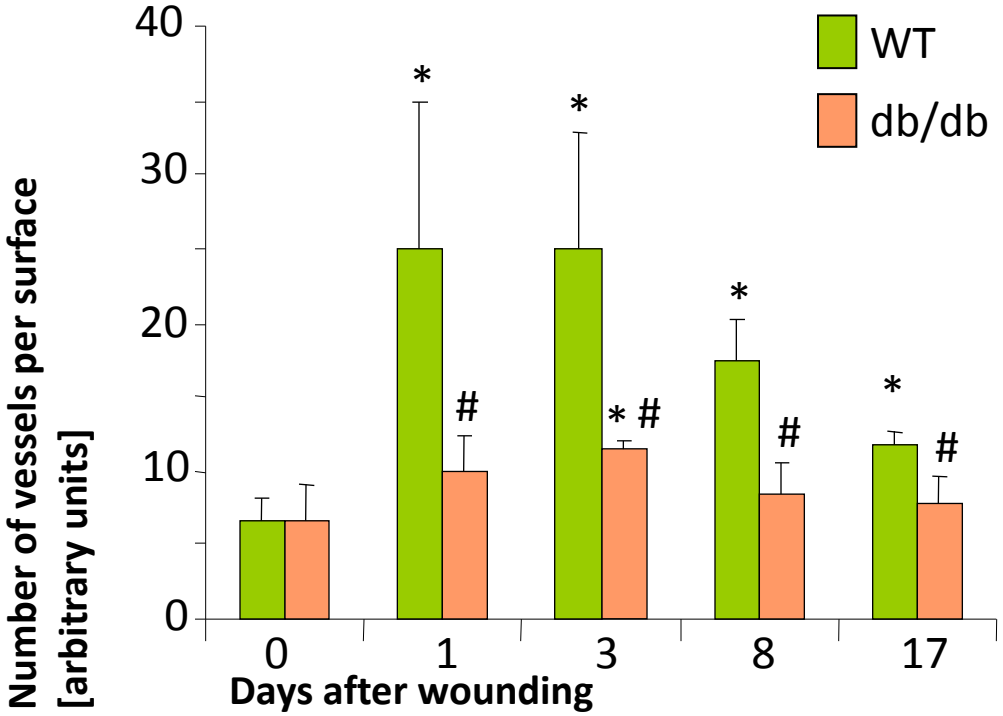
Impaired wound healing in diabetic mice

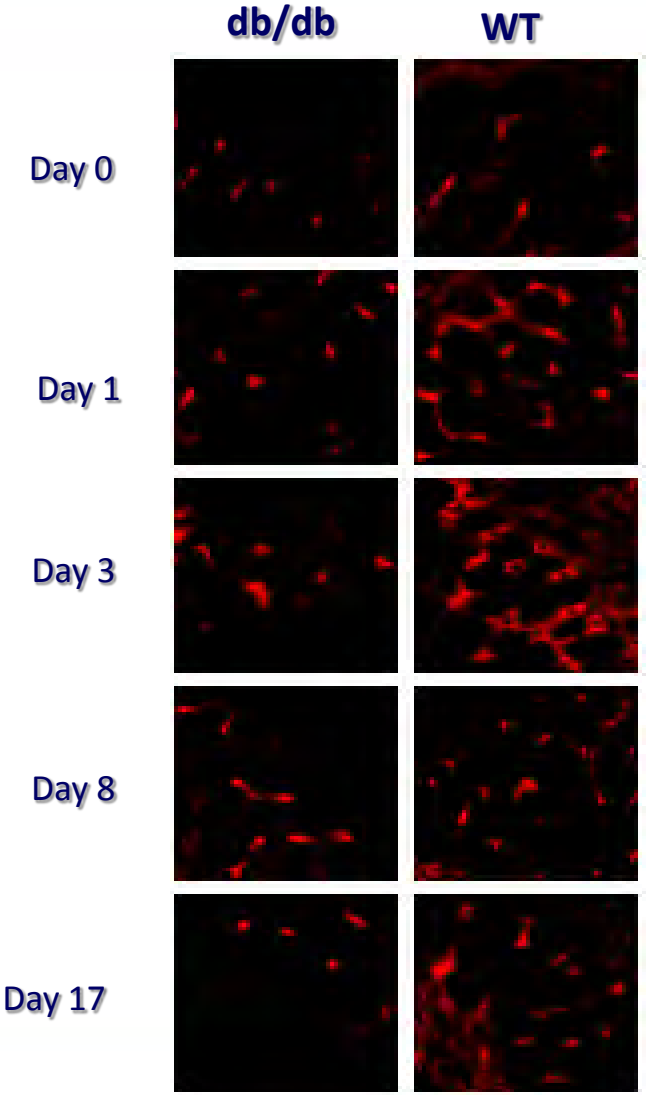




Grochot-Przeczek et al. , PLoS ONE 4(6): e5803; 2009

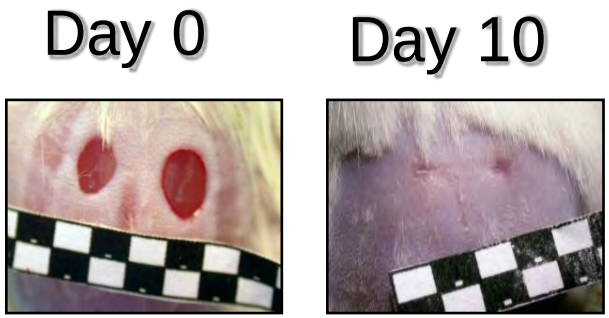
Neovascularization of the wounded skin in diabetic mice

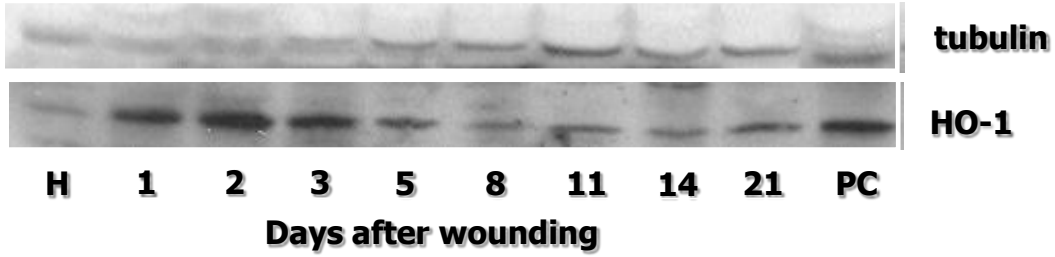


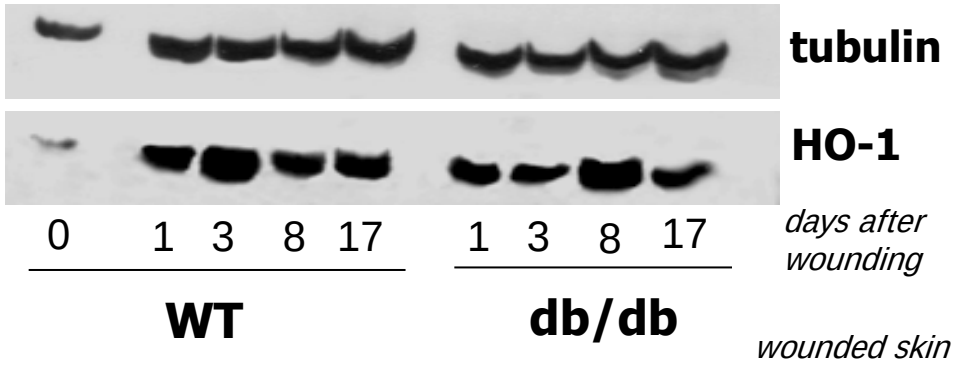


Grochot-Przeczek et al. , PLoS ONE 4(6): e5803; 2009

Expression of HO-1 in the skin after injury is delayed in diabetic mice

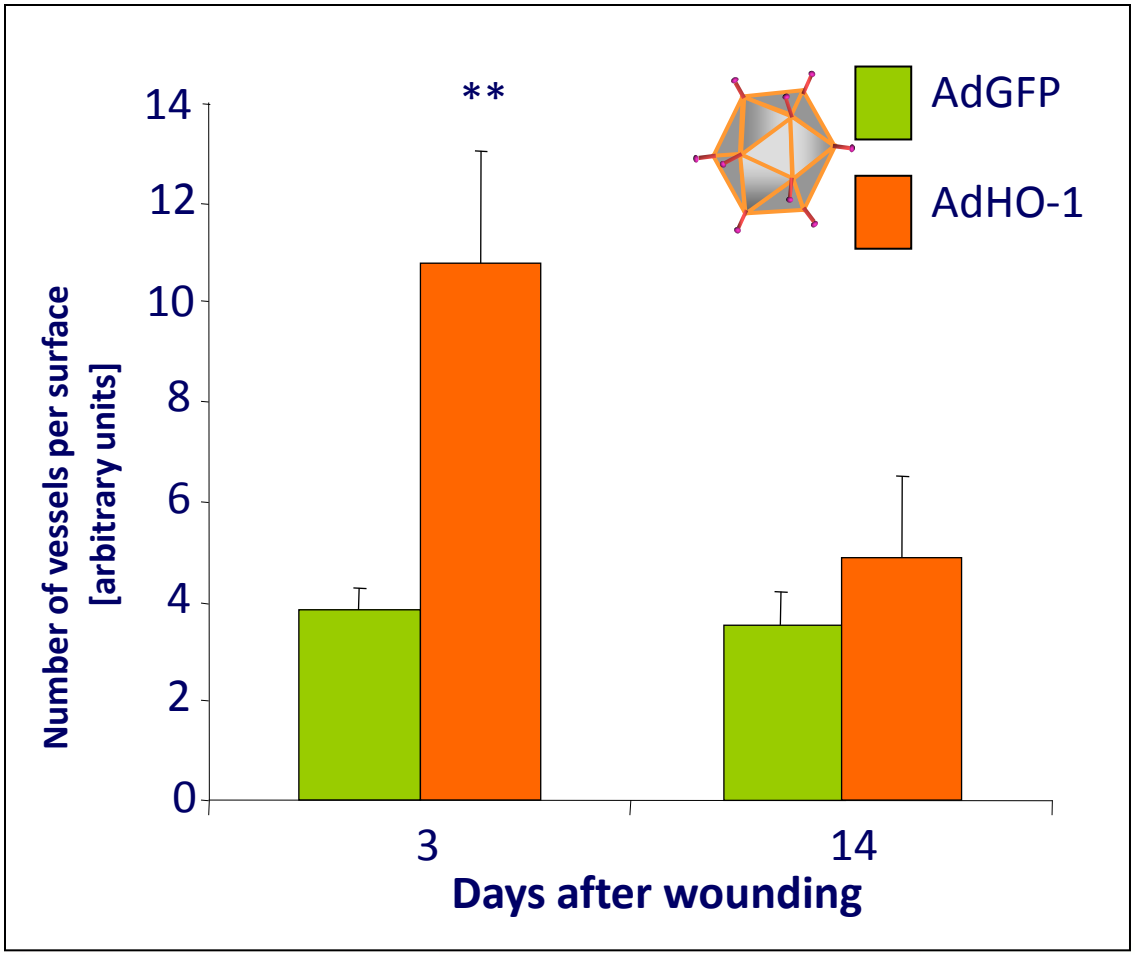






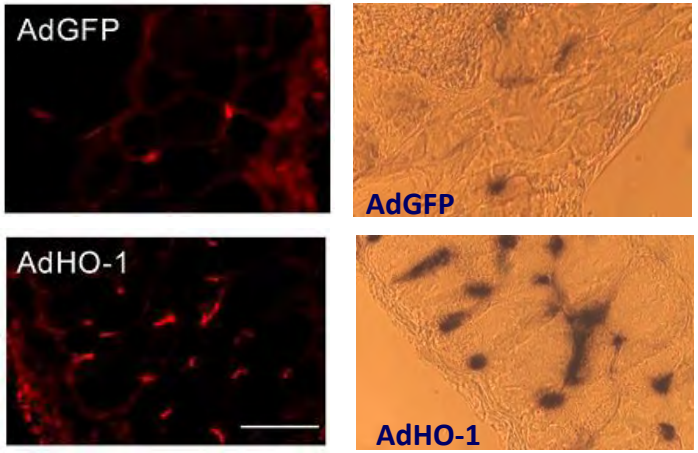
Grochot-Przeczek et al., PLoS One. 2009;4(6):e5803

Neovascularization of the wounds is improved in diabetic mice injected with AdHO-1



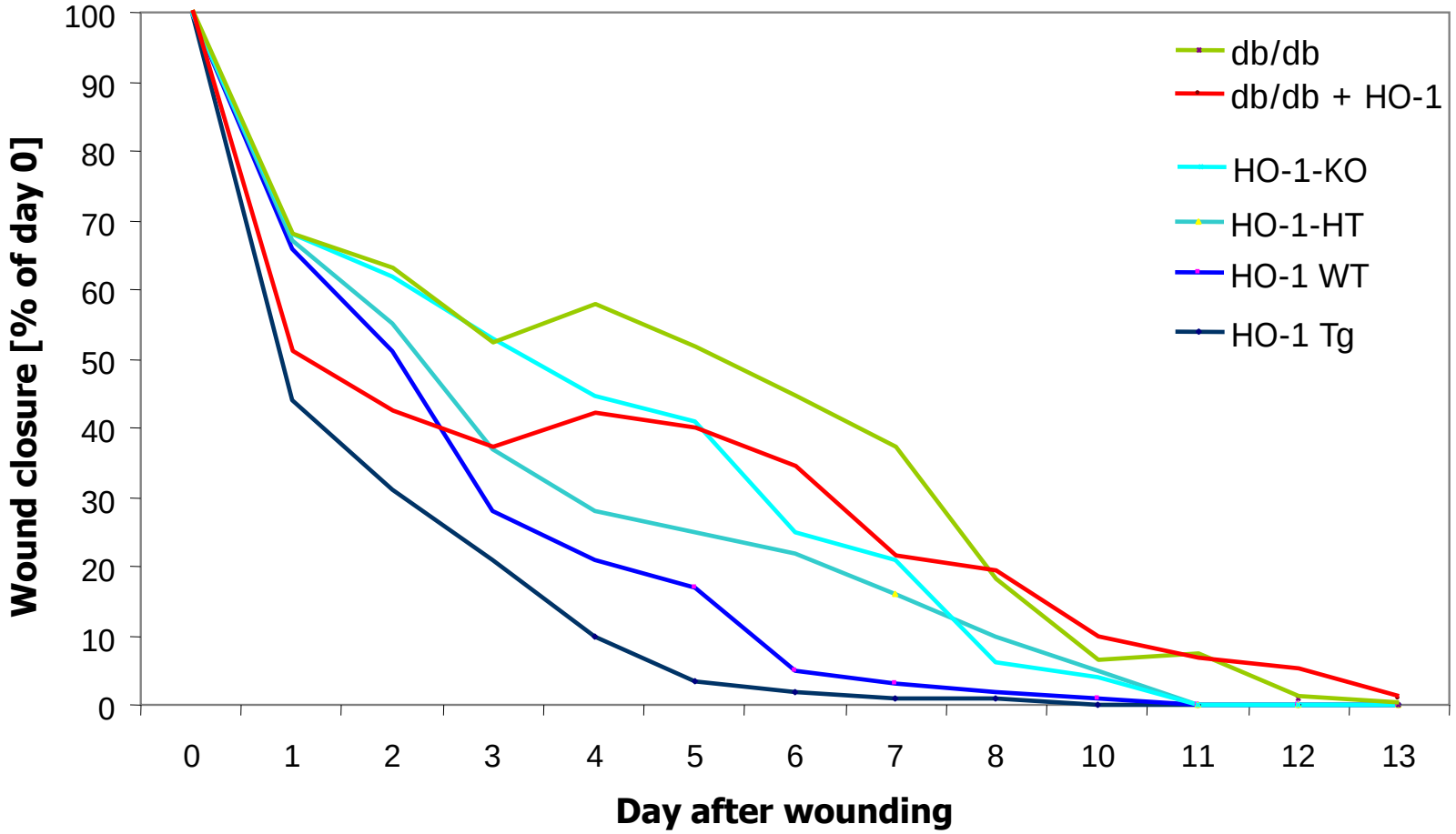
3 days after wounding

CD 31 — *alkaline phosphatase*



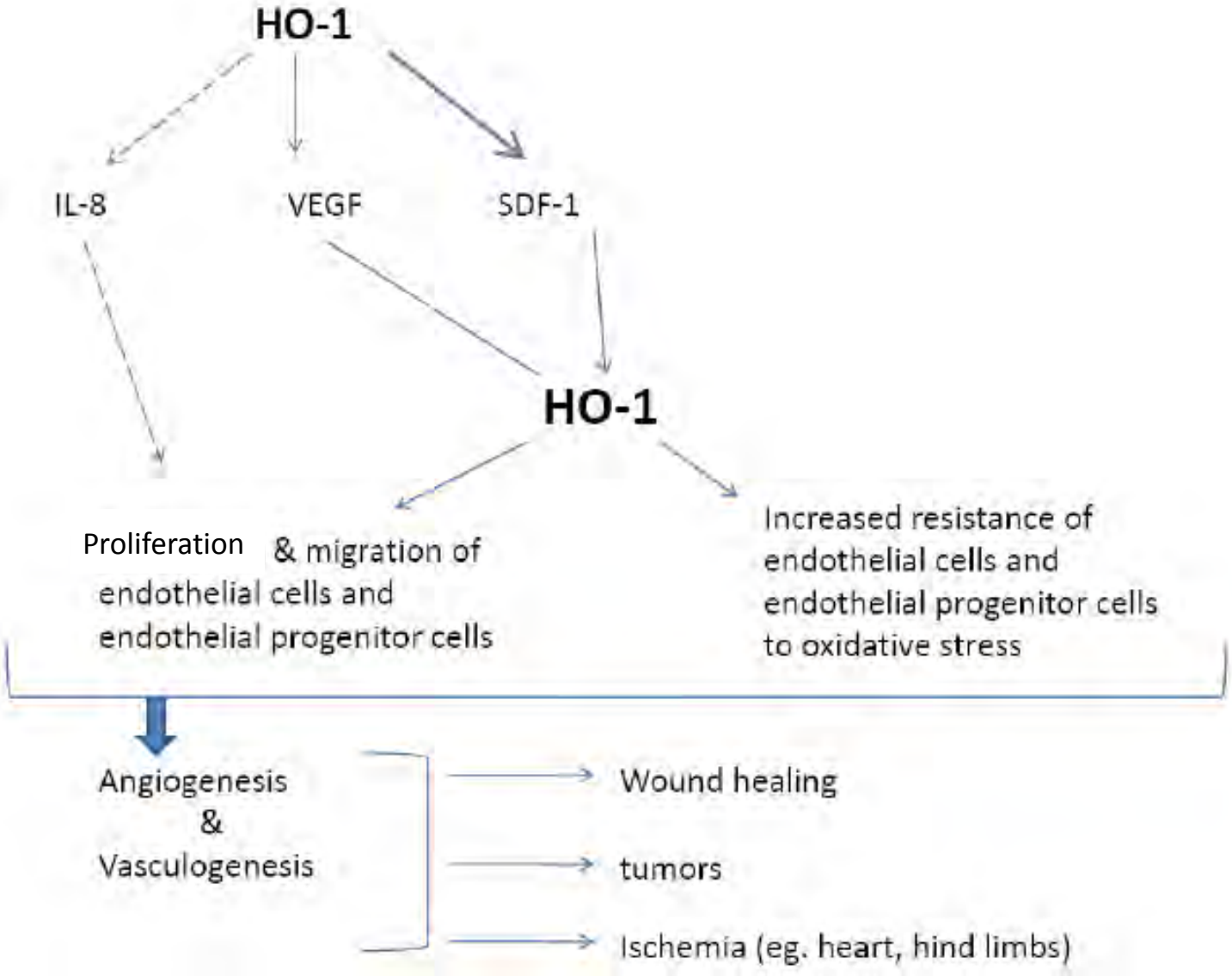
Grochot-Przeczek et al. , PLoS ONE 4(6): e5803; 2009

HO-1 and wound healing



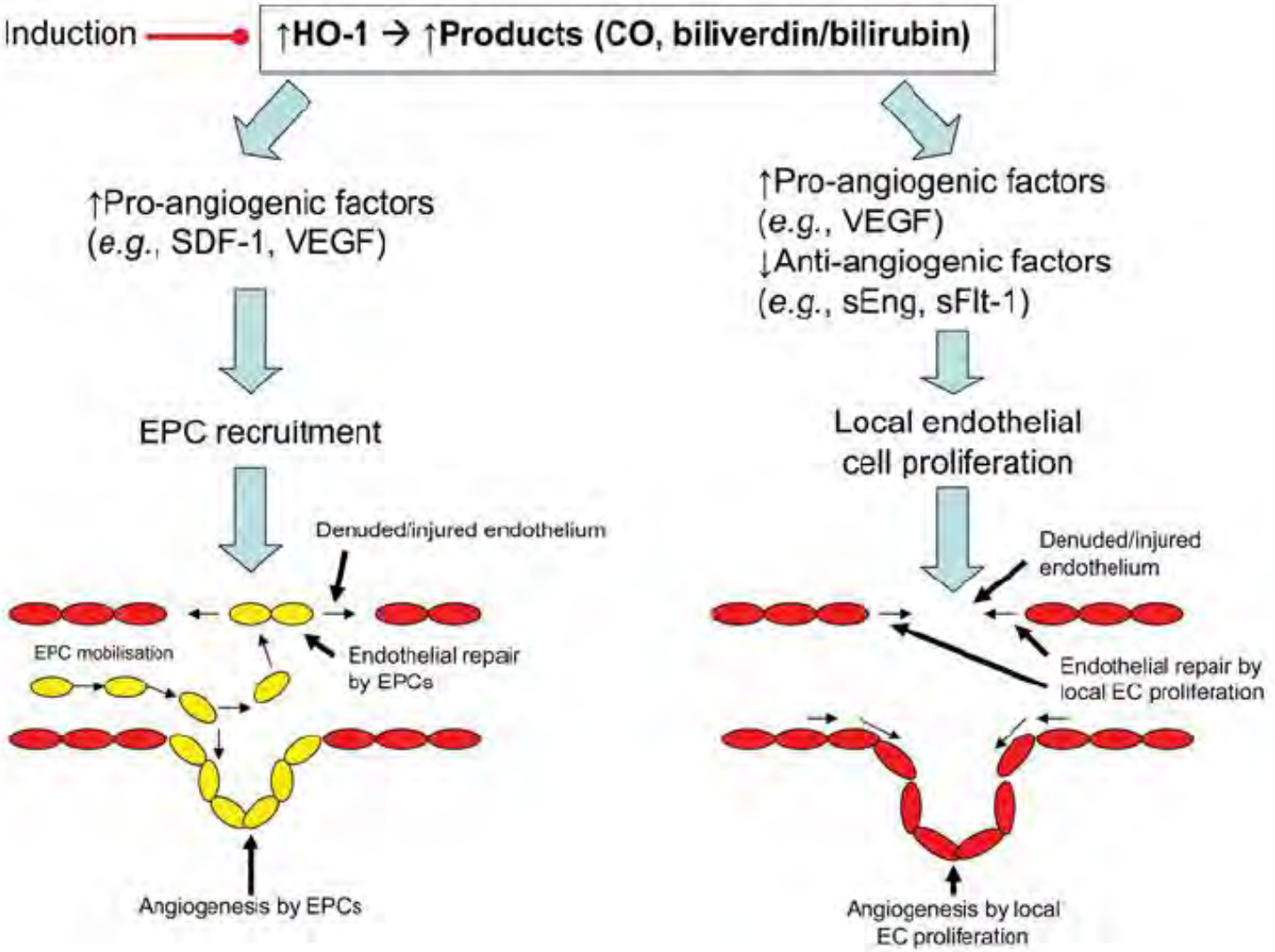
Grochot-Przeczek et al. , PLoS ONE 4(6): e5803; 2009

Role of heme oxygenase-1 in blood vessel formation



Dulak et al., Circulation, 2008: 117: 231-241
Grochot-Przeczek et al., Thrombosis & Hemostasis, 2010 ,104: 424-31

Role of heme oxygenase in vascular repair



Chan et al. (Stocker) Clinical Science 2011: 120: 493-504,

ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 10, 2008
© Mary Ann Liebert, Inc.
DOI: 10.1089/ars.2008.2043

Comprehensive Invited Review

Heme Oxygenase-1 and the Vascular Bed: From Molecular Mechanisms to Therapeutic Opportunities

Agnieszka Loboda,[1] Agnieszka Jazwa,[1] Anna Grochot-Przeczek,[1] Andrzej J. Rutkowski,[1] Jaroslaw Cisowski,[1] Anupam Agarwal,[2] Alicja Jozkowicz,[1] and Jozef Dulak[1]

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
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Heme Oxygenase-1 and Carbon Monoxide in Vascular Pathobiology

Focus on Angiogenesis

Jozef Dulak, PhD; Jessy Deshane, PhD; Alicja Jozkowicz, PhD; Anupam Agarwal, MD

(*Circulation*. 2008;117:231-241.)

Effect of heme oxygenase-1 on vascular function and disease

Jozef Dulak, Agnieszka Loboda and Alicja Jozkowicz

Current Opinion in Lipidology 2008, 19:505–512

Heme oxygenase-1 in neovascularisation: A diabetic perspective

Anna Grochot-Przeczek; Jozef Dulak; Alicja Jozkowicz
Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

doi:10.1160/TH09-12-0825
Thromb Haemost 2010; 104: 424–431

HO-1: clinical relevance?

Arteriosclerosis, Thrombosis, and Vascular Biology
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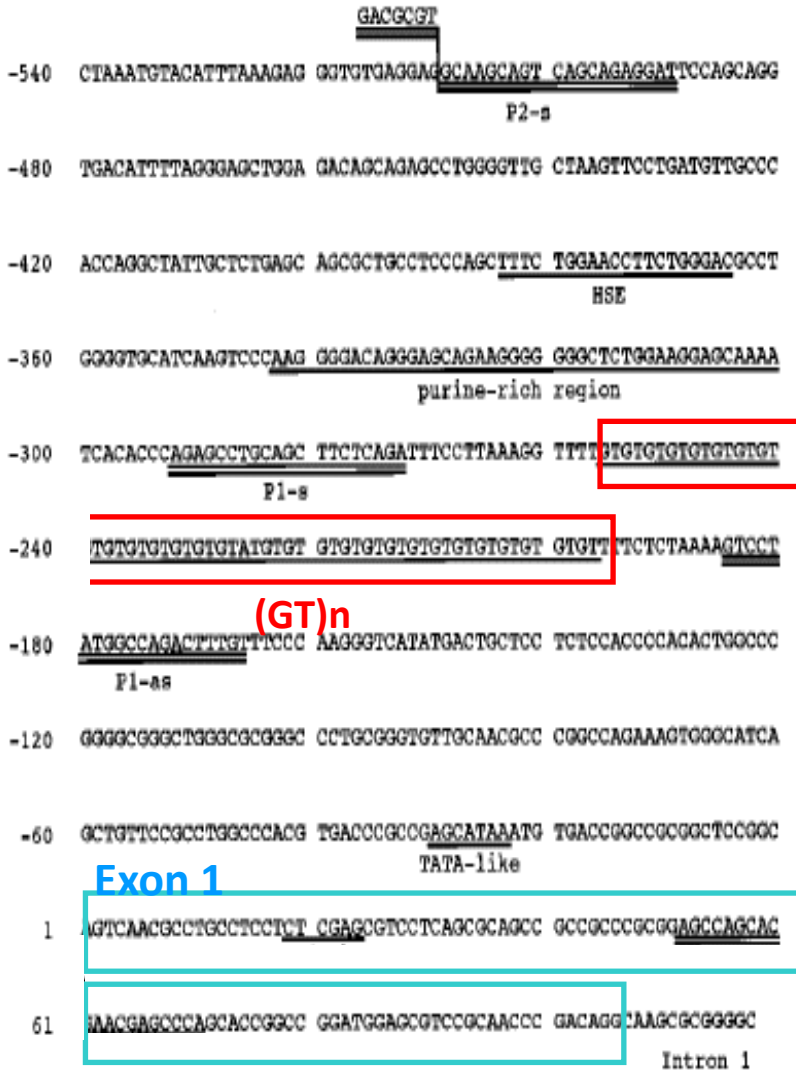
Role of Heme Oxygenase-1 in Human Endothelial Cells: Lesson From the Promoter Allelic Variants

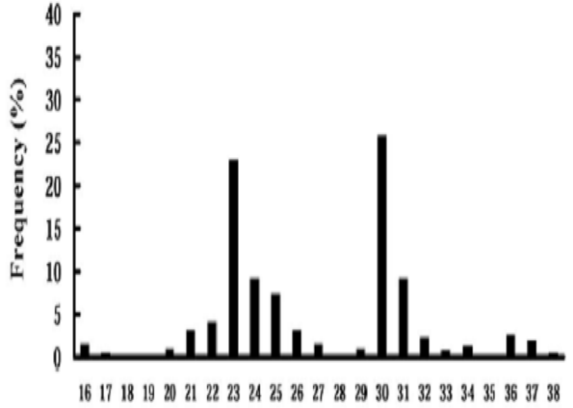
Hevidar Taha, Klaudia Skrzypek, Ibeth Guevara, Anneliese Nigisch, Stefan Mustafa, Anna Grochot-Przeczek, Pawel Ferdek, Halina Was, Jerzy Kotlinowski, Magdalena Kozakowska, Aneta Balcerczyk, Lucie Muchova, Libor Vitek, Guenter Weigel, Jozef Dulak and Alicja Jozkowicz

Arterioscler Thromb Vasc Biol 2010;30;1634-1641; originally published online May 27, 2010;
DOI: 10.1161/ATVBAHA.110.207316

Human HMOX-1 promoter polymorphism



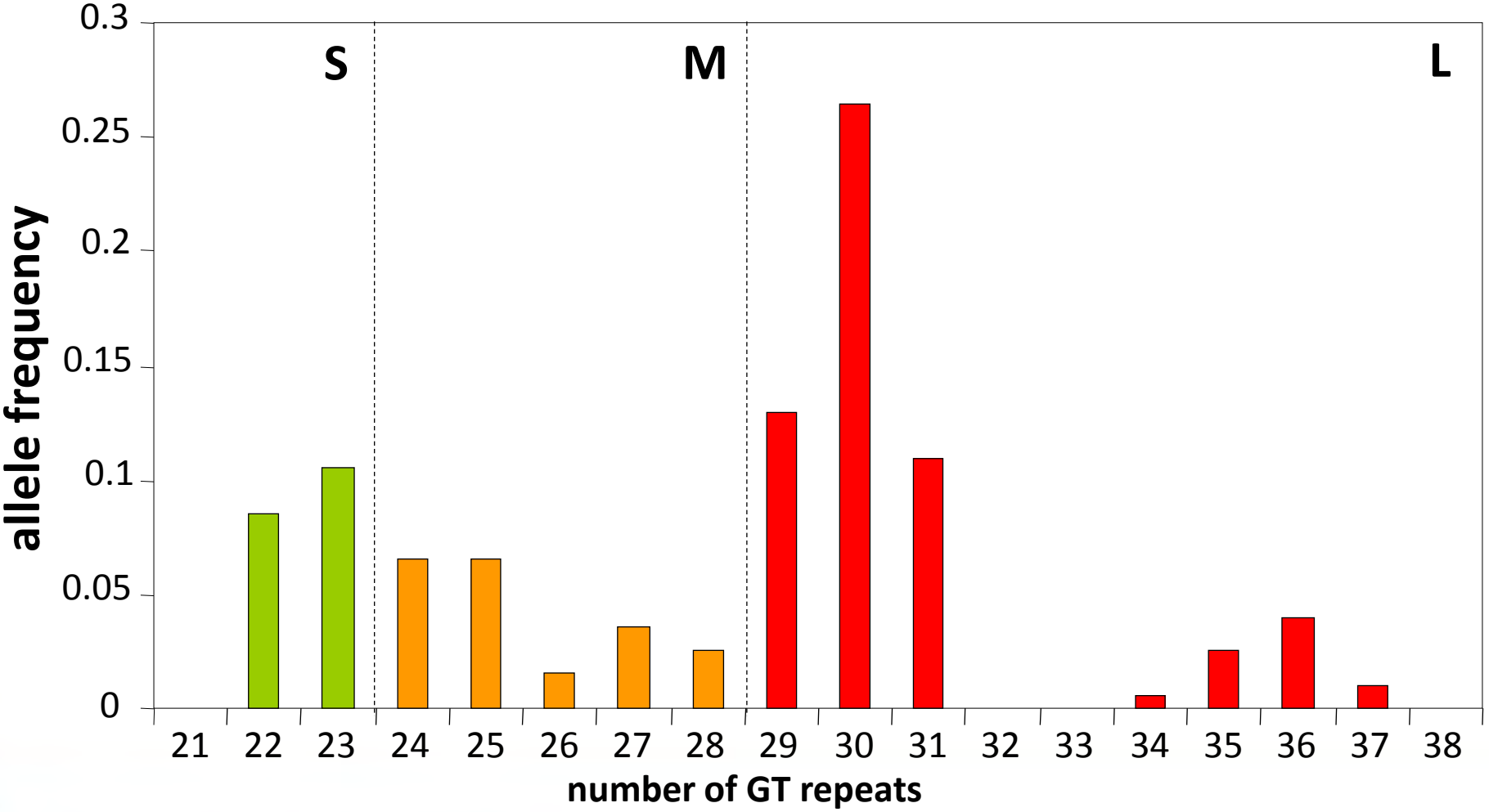
(GT)n promoter polymorphism of the human HO-1 gene



Yamada et al. Am J Hum Genet 2000; Chen et al. Eur Heart J 2004.

Effect of HO-1 promoter polymorphism on endothelial cells

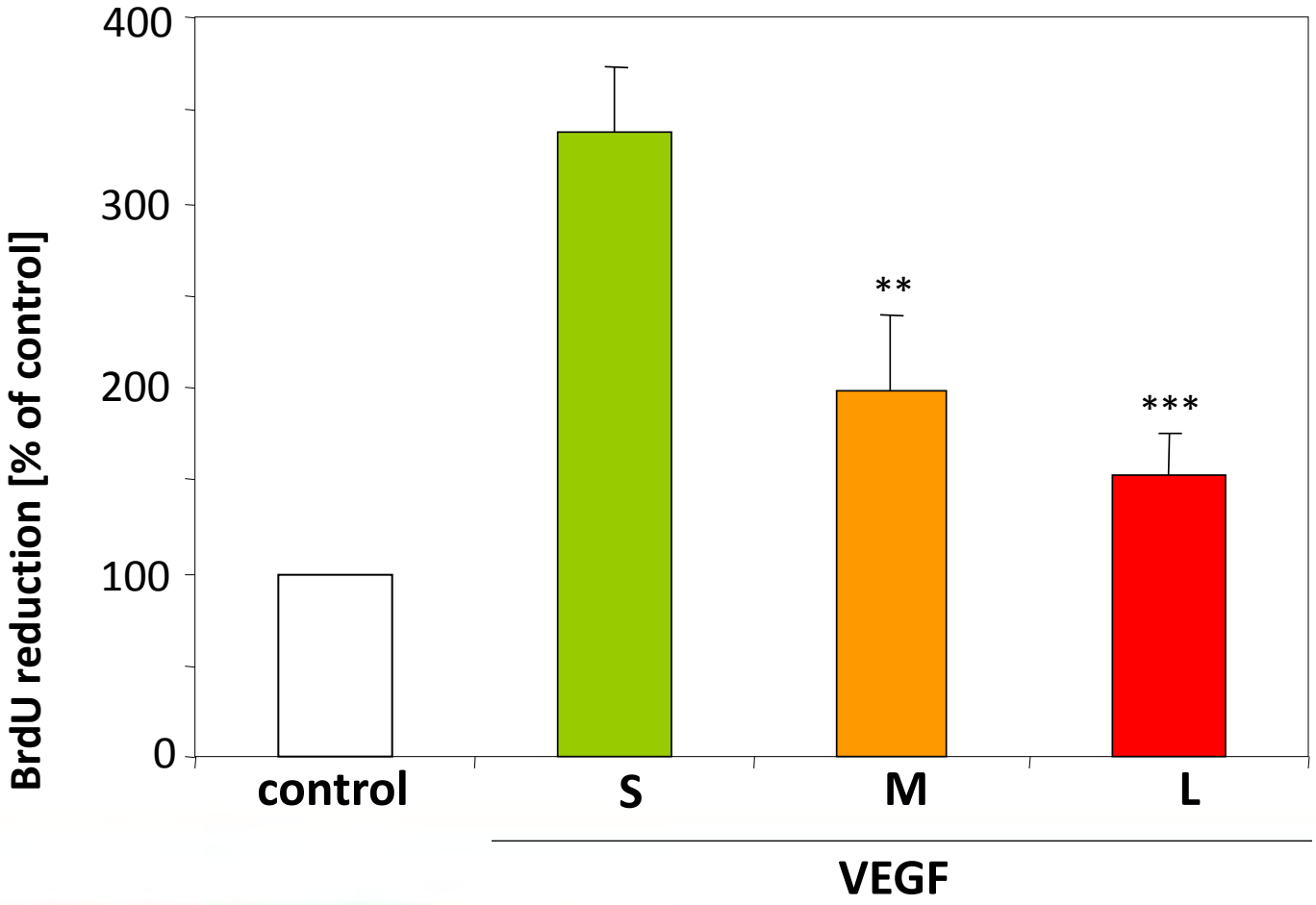
Allele frequency distribution



Taha et al., Arterioscler Thromb Vasc Biol. 2010 Aug; 30(8):1634-41.

Effect of HO-1 promoter polymorphism on endothelial cells

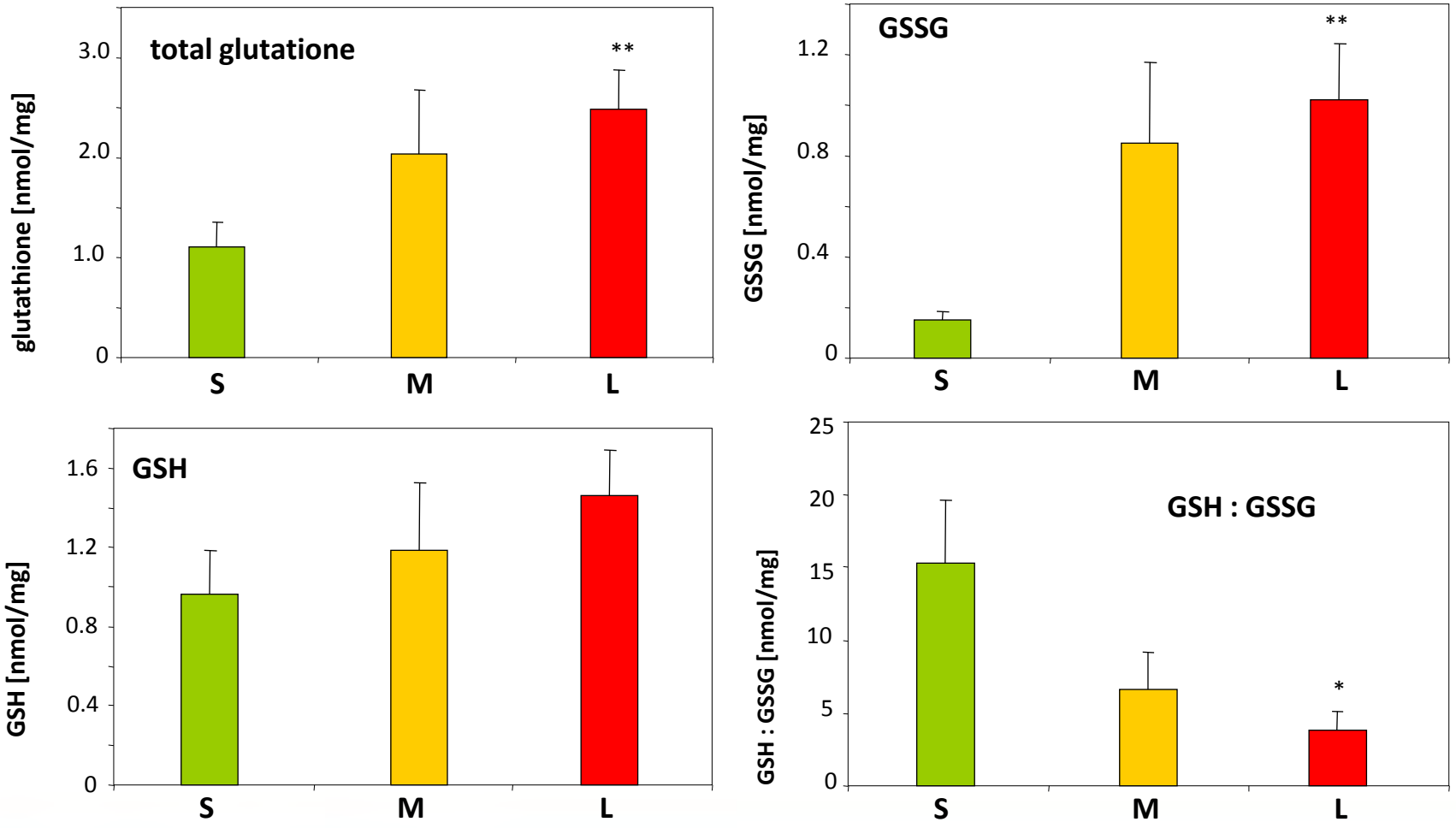
HUVEC cell proliferation



Taha et al., Arterioscler Thromb Vasc Biol. 2010 Aug; 30(8):1634-41.

Effect of HO-1 promoter polymorphism on endothelial cells

Glutathione concentration in endothelial cells



H_2O_2 (100 μM, 16 h)

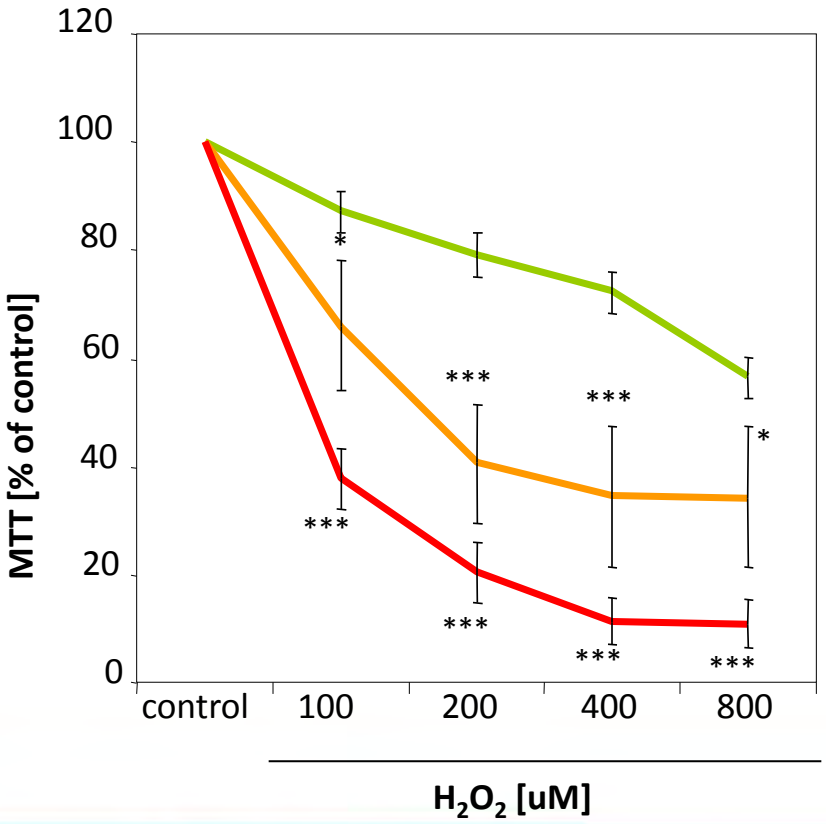
Taha et al., Arteroscler Thromb Vasc Biol. 2010 Aug; 30(8):1634-41.

Effect of HO-1 promoter polymorphism on endothelial cells

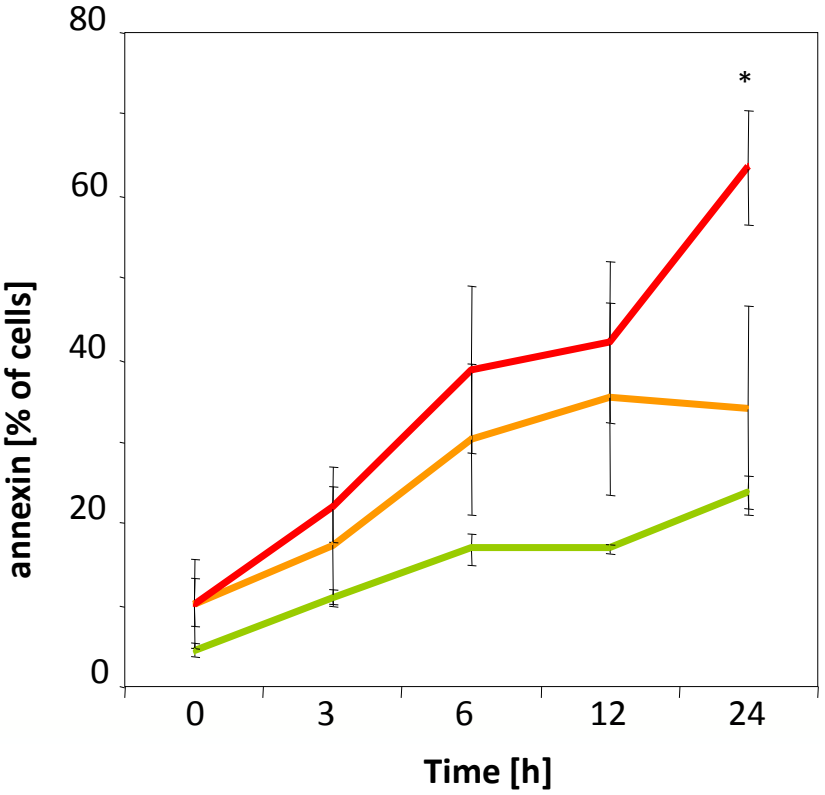
Endothelial cell viability

S M L

MTT reduction assay

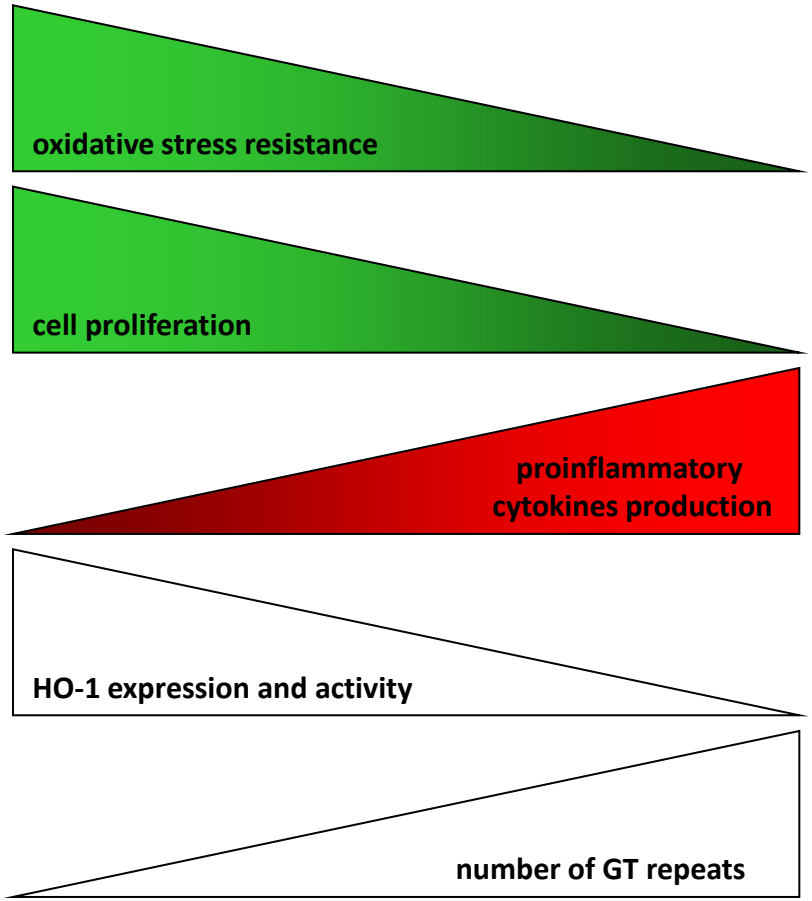


Annexin-V staining



Taha et al., Arterioscler Thromb Vasc Biol. 2010 Aug; 30(8):1634-41.

Significance of HO-1 and its promoter polymorphisms in humans



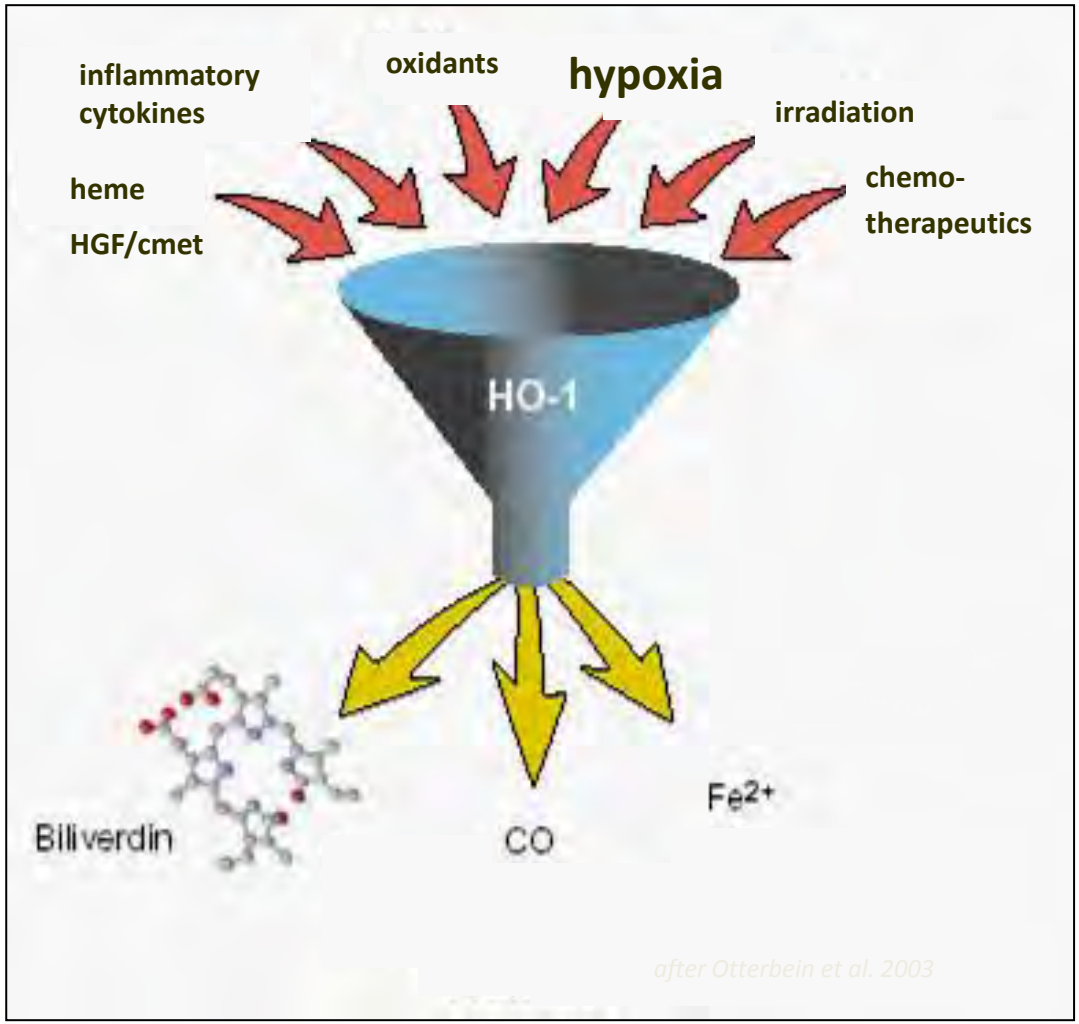
Grochot-Przeczek et al., Clinical Science, in press

Evidence supporting the vascular protective role of HO-1 in humans

Evidence
Shorter GT repeats (i.e. higher HO-1 expression) associated with lower prevalence of CAD and coronary events
Shorter GT repeats associated with lower risk of restenosis after coronary stenting
Increased HO-1 expression in human carotid endarterectomy specimens associated with reduced plaque vulnerability
High bilirubin levels associated with reduced prevalence and incidence of CAD and ischaemic stroke
Low bilirubin levels associated with endothelial dysfunction assessed via flow-mediated dilatation
Low bilirubin levels associated with increased carotid intima-media thickness
Human HO-1 deficiency exhibited hallmarks of severe endothelial injury and oxidative stress

Chan et al. (Stocker) Clinical Science 2011: 120: 493-504,

Heme oxygenase-1: a master of gene regulation…



Effect of HO-1 induction:

- Cytoprotection (resistance to oxidative stress)
- Immunomodulation (inhibition of inflammation, induction of Treg cells)
- **Modulation of angiogenesis**

after Otterbein et al., *Trends Mol Med. 2003*

Final conclusions

- Reactive oxygen species are produced by a number of enzymes which are regulated by environmental, chemical and mechanical stimuli

- The synthesis and level of ROS in vascular system is determined by the interactions between the pro- and anti-oxidant pathways

- ROS can exert both physiological and pathophysiological effects on endothelial and vascular functions

- Redox homeostasis is perturbed in many cardiovascular diseases states - may result in oxidative stress

However, the „non-canonical „ functions of anti-oxidative enzymes are recognized to play more and more important role

6th European Meeting for Vascular Biology and Medicine

Krakow, 21-24 September 2011

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