NQO1, A Versatile Cytoprotective Enzyme in Response to Age-Related Diseases

Dong-Hoon HYUN, PhD
Department of Life Science
Ewha Womans University

Oxidative Stress, Mitochondrial Dysfunction & Ageing

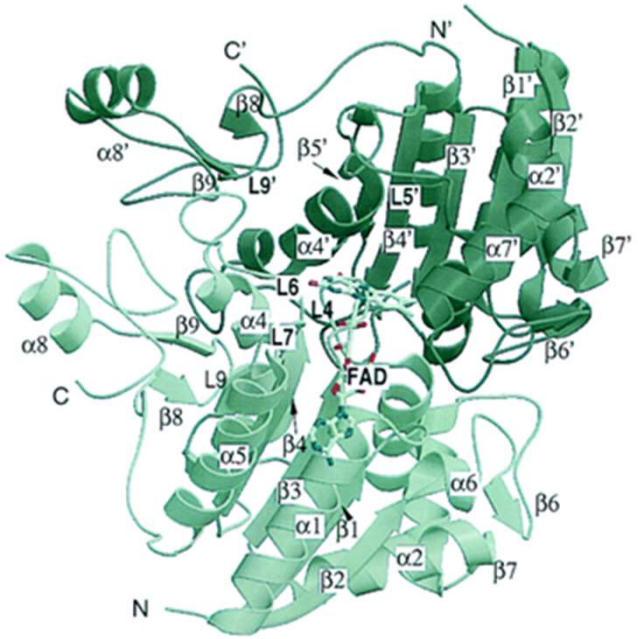
- Mitochondrial dysfunction occurs with age & may be one of causes of neurodegenerative diseases
- Oxidative stress is involved in the pathology of aging and age-related diseases at an early stage in its development
- The pathology is confirmed by decreased antioxidant defenses & increased oxidative damage
- Accumulation of mitochondrial DNA mutations, commonly identified in age-related diseases, induce impairments of mitochondrial complexes
  - Mitochondrial complex I deficiency in PD
  - Defective complex II & IV activity in ALS
  - Decreased complex III activity in aged heart
- Impaired mitochondrial function causes a shortage of ATP supply, resulting in induction of further problems in biochemical pathways

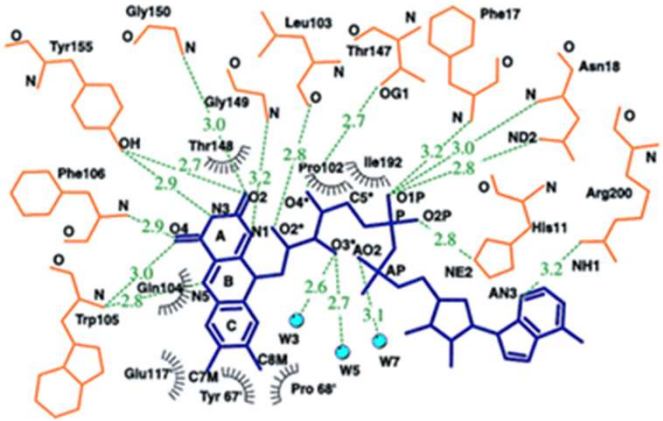
Alternative Mechanisms following Energy Depletion

- Mitochondrial dysfunction, strenuous muscle activity, diseases, etc
- Alternative pathway to produce energy by
  - Activated glycolysis & fermentation in the cytosol
  - Plasma membrane redox system (PMRS)
- Regulation of the cellular redox homeostasis via maintenance of NAD^+/NADH ratio, which can modulate Sir2/SIRT1 (e.g. calorie restriction (CR)
- Supplemented ubiquinol & CoQ, whose metabolism is altered in aged brain and Alzheimer's disease, can protect mitochondria from oxidative stress

NADH-Quinone Oxidoreductase 1 (NQO1)

- 33 kDa FAD-containing homodimer
- Involved in the more efficient 2-electron reduction using NAD(P)H in the PMRS, subsequently causing no formation of $CoQ^{\bullet -}$
- Largely a cytosolic protein & is translocated into the inner surface of the PM under oxidative stress
- Inhibited by dicoumarol
- Detoxifying enzyme induced by Nrf2 in association with ARE under stress conditions





NQO1

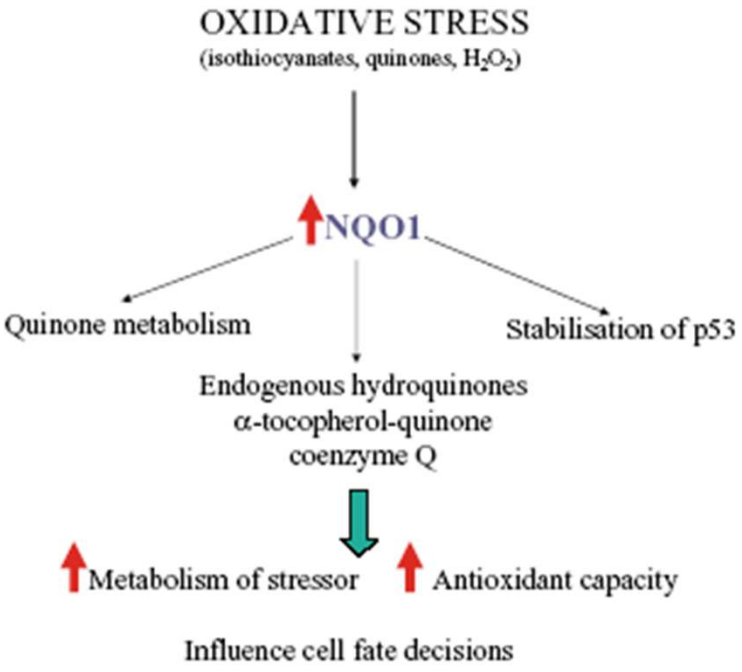


Fig. 2. Cytoprotective functions of NQO1. A summary of the mechanisms by which NQO1 can protect cells against both oxidative stress and neoplasia is presented.

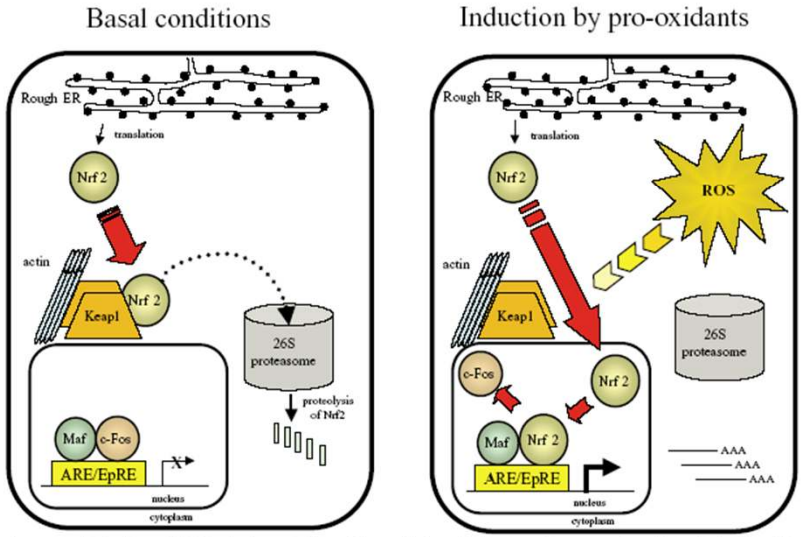


Fig. 6. Transcription factors recruited to the *nqo1*-ARE under homeostatic conditions or during oxidative stress. The cartoon shows that under basal conditions small Maf protein binds to the ARE along with another bZip factor. The identity of the other binding partner for small Maf is not known, but it may be c-Fos, other Maf proteins, or Nrf1. Under normal homeostatic conditions, little Nrf2 is associated with the ARE [71] as it is targeted for proteasomal degradation by Keap1 [118,119,123]. However, under conditions of oxidative stress, Nrf2 is stabilised, translocates to the nucleus, and displaces repressor complexes.

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Nutrition, Metabolism & Cardiovascular Diseases

Induction of HO-1 and redox signaling in endothelial cells by advanced glycation end products: A role for Nrf2 in vascular protection in diabetes

M. He [a,1], R.C.M. Siow [a,1], D. Sugden [b], L. Gao [a], X. Cheng [a], G.E. Mann [a,*]

[a] *Cardiovascular Division, School of Medicine, King's College London, 150 Stamford Street, London SE1 9NH, UK*
[b] *Reproduction & Endocrinology Division, School of Biomedical & Health Sciences, King's College London, London SE1 9UL, UK*

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Redox signaling;
Nrf2-Keap1;
Heme oxygenase-1;
NQO1;
c-Jun terminal kinase

Abstract *Background and aims:* Hyperglycemia and diabetes are associated with increased formation of advanced glycation end products and enhanced oxidative stress, leading to the progression of diabetic vascular disease. We have investigated the mechanisms by which AGE-modified bovine albumin (AGE-BSA) induces reactive oxygen species (ROS) generation, leading to nuclear factor-erythroid 2-related factor (Nrf2) dependent induction of the antioxidant genes heme oxygenase-1 (HO-1) and NADPH:quinone oxidoreductase 1 (NQO1) in bovine aortic endothelial cells.

Methods and results: AGE-BSA (100 $\mu g\,ml^{-1}$, 0–24 h), but not native BSA, elicited time-dependent increases in ROS generation, Nrf2 nuclear translocation and enhanced mRNA and protein expression of HO-1 and NQO1, but not glutathione peroxidase-1. Inhibition of ROS production with the superoxide scavenger Tiron or inhibitors of flavoproteins (diphenylene iodonium) and NADPH oxidase (apocynin), but not eNOS (L-NAME) or mitochondria complex I (rotenone) abrogated HO-1 induction by AGE-BSA. Although AGE-BSA induced rapid phosphorylation of JNK and Akt, only inhibition of JNK abrogated HO-1 expression, implicating the involvement of the JNK signaling pathway in AGEs activation of Nrf2/ARE-linked antioxidant gene expression.

Supplemental Material can be found at:
http://jpet.aspetjournals.org/content/suppl/2010/01/19/jpet.109.162271.DC1.html

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Nuclear Factor Erythroid 2-Related Factor 2 Deletion Impairs Glucose Tolerance and Exacerbates Hyperglycemia in Type 1 Diabetic Mice[S]

Lauren M. Aleksunes, Scott A. Reisman, Ronnie L. Yeager, Michael J. Goedken, and Curtis D. Klaassen

Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas (L.M.A., S.A.R., R.L.Y., C.D.K.); and Department of Pathology, Schering-Plough Research Institute, Lafayette, New Jersey (M.J.G.)

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ABSTRACT

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) induces a battery of cytoprotective genes after oxidative stress. Nrf2 aids in liver regeneration by altering insulin signaling; however, whether Nrf2 participates in hepatic glucose homeostasis is unknown. Compared with wild-type mice, mice lacking Nrf2 (Nrf2-null) have lower basal serum insulin and prolonged hyperglycemia in response to an intraperitoneal glucose challenge. In the present study, blood glucose, serum insulin, urine flow rate, and hepatic expression of glucose-related genes were quantified in male diabetic wild-type and Nrf2-null mice. Type 1 diabetes was induced with a single intraperitoneal dose (200 mg/kg) of streptozotocin (STZ). Histopathology and serum insulin levels confirmed depleted pancreatic β-cells in STZ-treated mice of both genotypes. Five days after STZ, Nrf2-null mice had higher blood glucose levels than wild-type mice. Nine days after STZ, polyuria occurred in both genotypes with more urine output from Nrf2-null mice (11-fold) than wild-type mice (7-fold). Moreover, STZ-treated Nrf2-null mice had higher levels of serum β-hydroxybutyrate, triglycerides, and fatty acids 10 days after STZ compared with wild-type mice. STZ reduced hepatic glycogen in both genotypes, with less observed in Nrf2-null mice. Increased urine output and blood glucose in STZ-treated Nrf2-null mice corresponded with enhanced gluconeogenesis (glucose-6-phosphatase and phosphoenolpyruvate carboxykinase)- and reduced glycolysis (pyruvate kinase)-related mRNA expression in their livers. Furthermore, the Nrf2 activator oltipraz lowered blood glucose in wild-type but not Nrf2-null mice administered STZ. Collectively, these data indicate that the absence of Nrf2 worsens hyperglycemia in type I diabetic mice and Nrf2 may represent a therapeutic target for reducing circulating glucose levels.

Contents lists available at ScienceDirect

Atherosclerosis

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Protective effects of magnesium lithospermate B against diabetic atherosclerosis via Nrf2-ARE-NQO1 transcriptional pathway

Kyu Yeon Hur[a,b,i], Soo Hyun Kim[b], Min-Ah Choi[b], Darren R. Williams[a,b,j], Yong-ho Lee[c], Sang Won Kang[d], Umesh C.S. Yadav[e], Satish K. Srivastava[e], Mankil Jung[f], Jin Won Cho[g], Sang Geon Kim[h], Eun Seok Kang[a,b,c], Eun Jig Lee[a,b,c], Hyun Chul Lee[a,b,c,*]

[a] Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, 120-752, Republic of Korea
[b] Institute of Endocrine Research, Yonsei University College of Medicine, Seoul, 120-752, Republic of Korea
[c] Department of Internal Medicine, Yonsei University College of Medicine, Division of Endocrinology and Metabolism, 134, Shinchon-Dong Seodaemun-Gu, Seoul, 120-752, Republic of Korea
[d] Department of Life Science, Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Republic of Korea
[e] Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555, USA
[f] Department of Chemistry, Yonsei University, Seoul, 120-749, Republic of Korea
[g] Department of Biology, Yonsei University, Seoul, 120-749, Republic of Korea
[h] College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea
[i] Department of Internal Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, 135-710, Republic of Korea
[j] Department of Life Sciences, Gwangju Institue of Science and Technology, Gwangju 500-712, Republic of Korea

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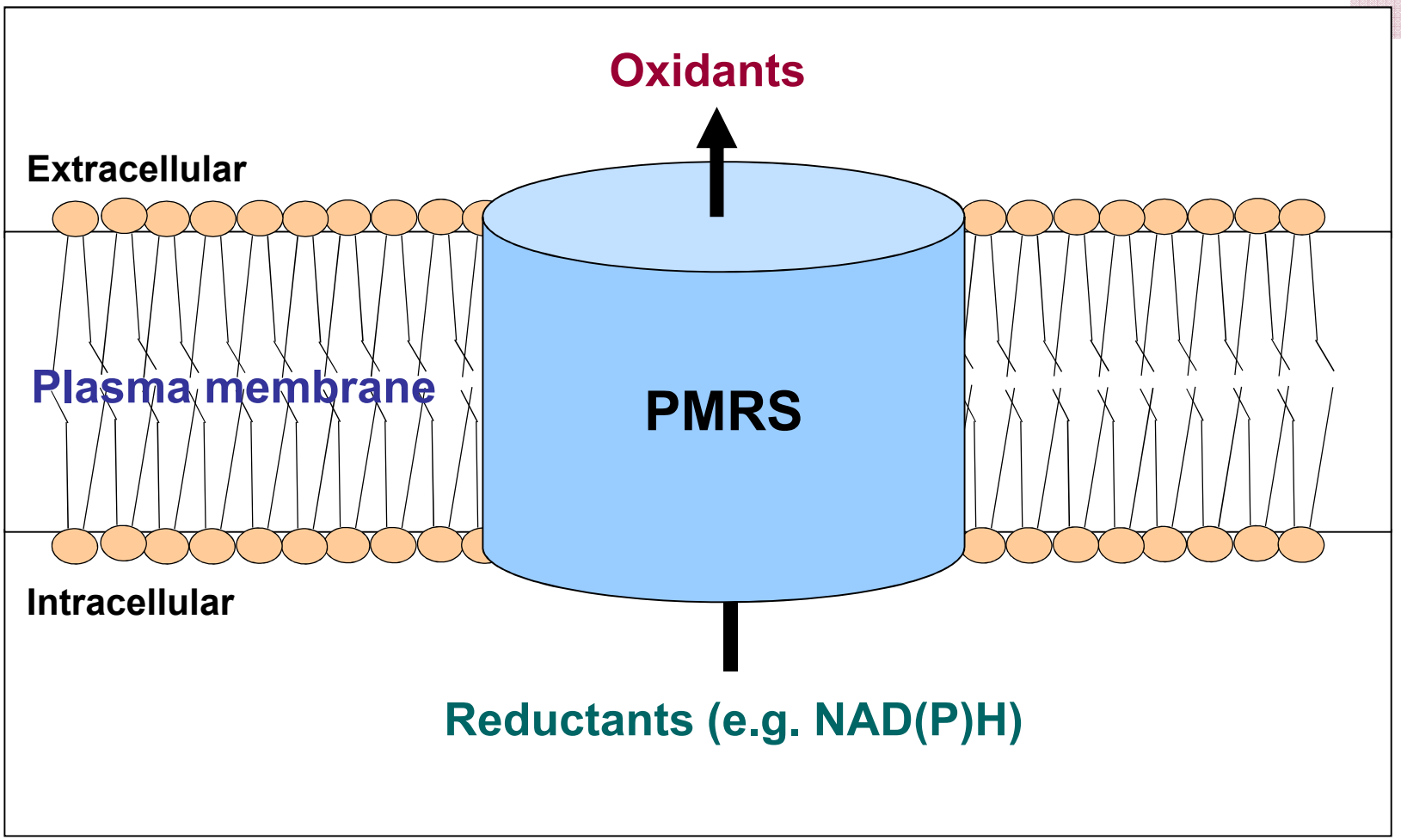
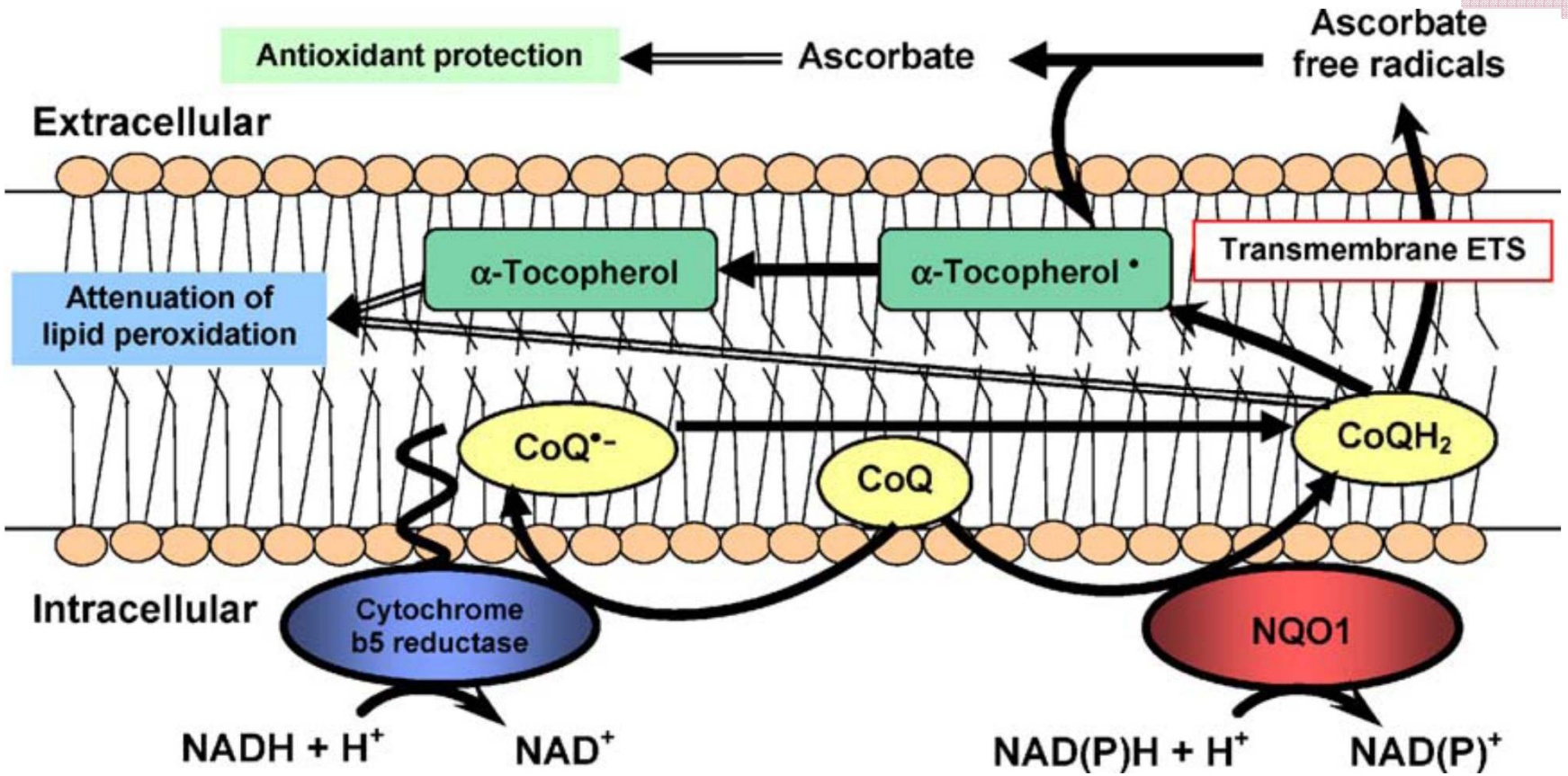
ABSTRACT

Hyperglycemia-induced oxidative stress is known to play an important role in the development of several diabetic complications, including atherosclerosis. Although a number of antioxidants are available, none have been found to be suitable for regulating the oxidative stress response and enhancing antioxidative defense mechanisms. In this study, we evaluated the effects of magnesium lithospermate B (LAB) against oxidative stress. We also endeavored to identify the target molecule of LAB in vascular smooth muscle cells (VSMCs) and the underlying biochemical pathways related to diabetic atherosclerosis. Modified MTT and transwell assays showed that the increased proliferation and migration of rat aortic VSMCs in culture with high glucose was significantly inhibited by LAB. LAB also attenuated neointimal hyperplasia after balloon catheter injury in diabetic rat carotid arteries. To determine molecular targets of LAB, we studied the effects of LAB on aldose reductase (AR) activity, O-GlcNAcylation, and protein kinase C (PKC) activity in VSMCs under normoglycemic or hyperglycemic conditions and showed the improvement of major biochemical pathways by LAB. Potential involvement of the nuclear factor erythroid 2-related factor-2 (Nrf2) – antioxidant responsive element (ARE)-NAD(P)H: quinone oxidoreductase-1 (NQO1) pathway was assessed using siRNA methods. We found that LAB activates the NQO1 via the Nrf2-ARE pathway, which plays an important role in inhibition of the major molecular mechanisms that lead to vascular damage and the proliferation and migration of VSMCs. Together, these findings demonstrate that the induction of the Nrf2-ARE-NQO1 pathway by LAB could be a new therapeutic strategy to prevent diabetic atherosclerosis.

Simplified Plasma Membrane Redox System (PMRS)

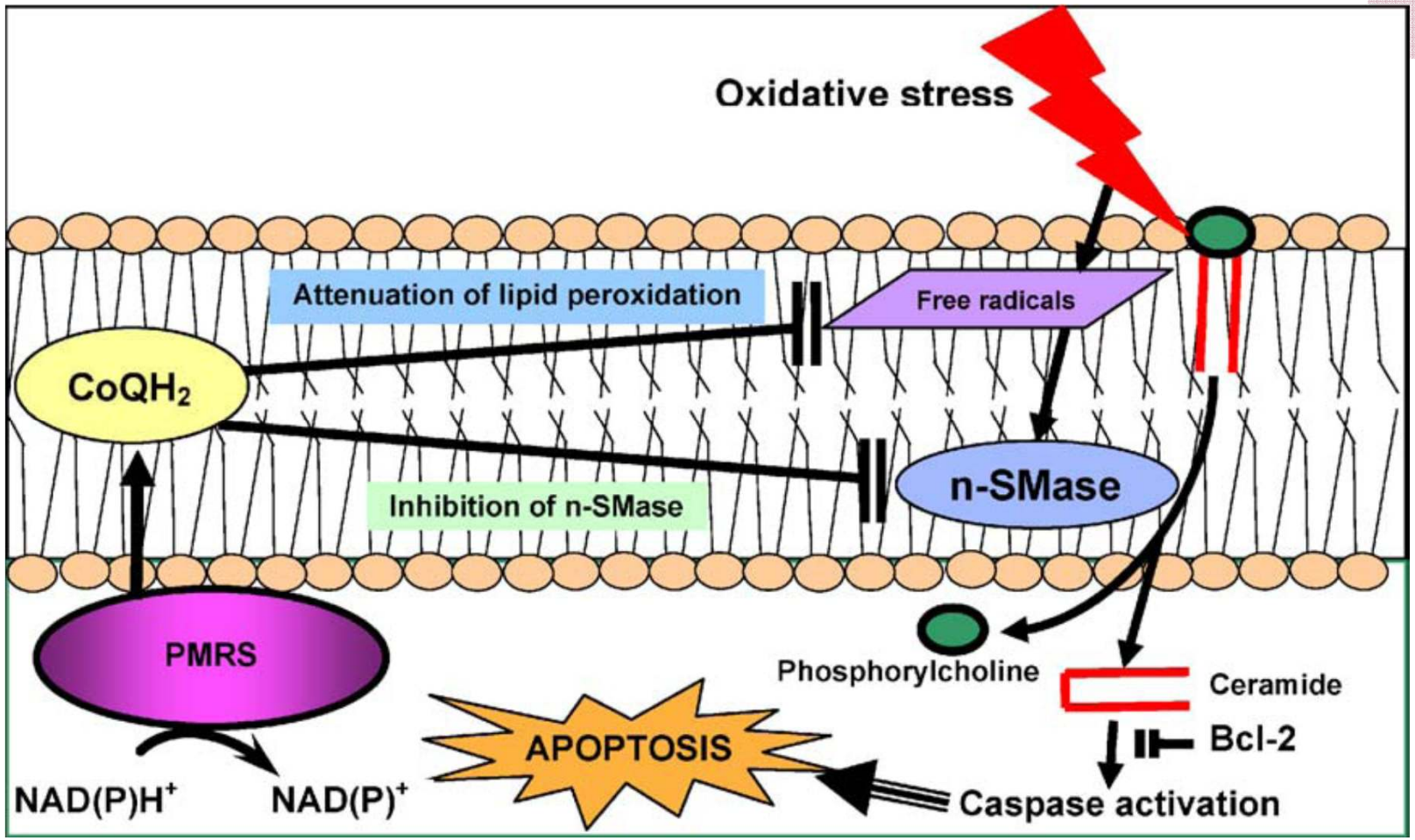


Schematic Diagram of the PMRS



Hyun et al. (2006) *Ageing Res. Rev.* **5**: 209-220

PMRS & Sphingomyelin Signalling



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Compensation Mechanisms in Response to Impairment of Energy Metabolism

- Increased PMRS (e.g. DT-diaphorase) activity in lymphocytes from IDDM patients
- Enhanced glycolysis & PMRS activity in mitochondria-deficient cells (e.g. ρ^o cells)
- Elevated PMRS → Anti-ageing (e.g. CR)
- Declined PMRS → Progression of diseases (e.g. AD)
- Overexpressed PMRS → Cytoprotection

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Enhanced Activity of the Plasma Membrane Oxidoreductase in Circulating Lymphocytes from Insulin-Dependent Diabetes Mellitus Patients

Giorgio Lenaz,*[,1] Ugo Paolucci,* Romana Fato,* Marilena D'Aurelio,* Giovanna Parenti Castelli,* Gianluca Sgarbi,* Graziella Biagini,† Luca Ragni,‡ Silvana Salardi,‡ and Emanuele Cacciari‡

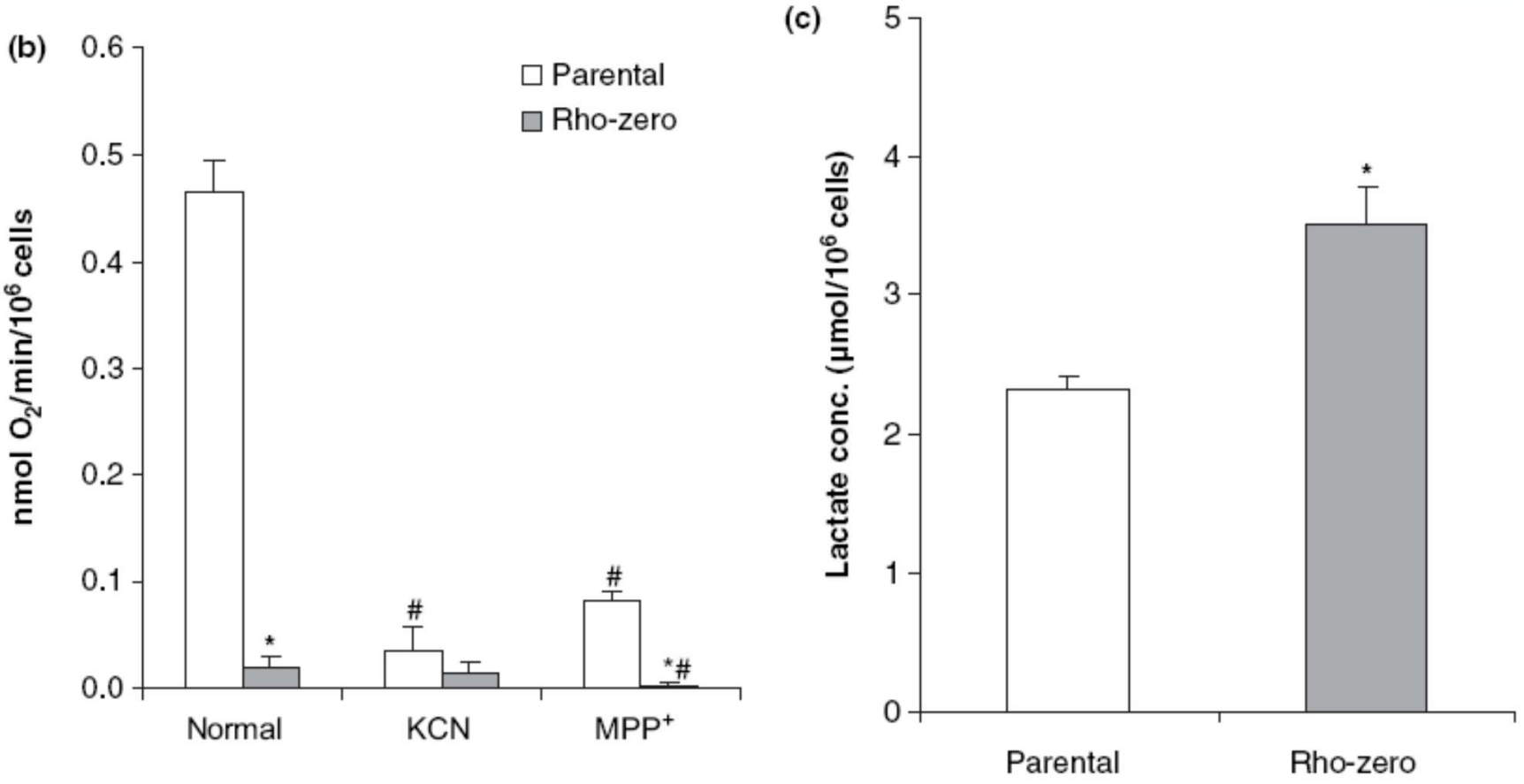
**Dipartimento di Biochimica, Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy; †Istituto di Morfologia Normale, Università di Ancona, Via Ranieri, 60100 Ancona, Italy; and ‡Clinica Pediatrica I, Università di Bologna, Via Massarenti 11, 40138 Bologna, Italy*

TABLE 1

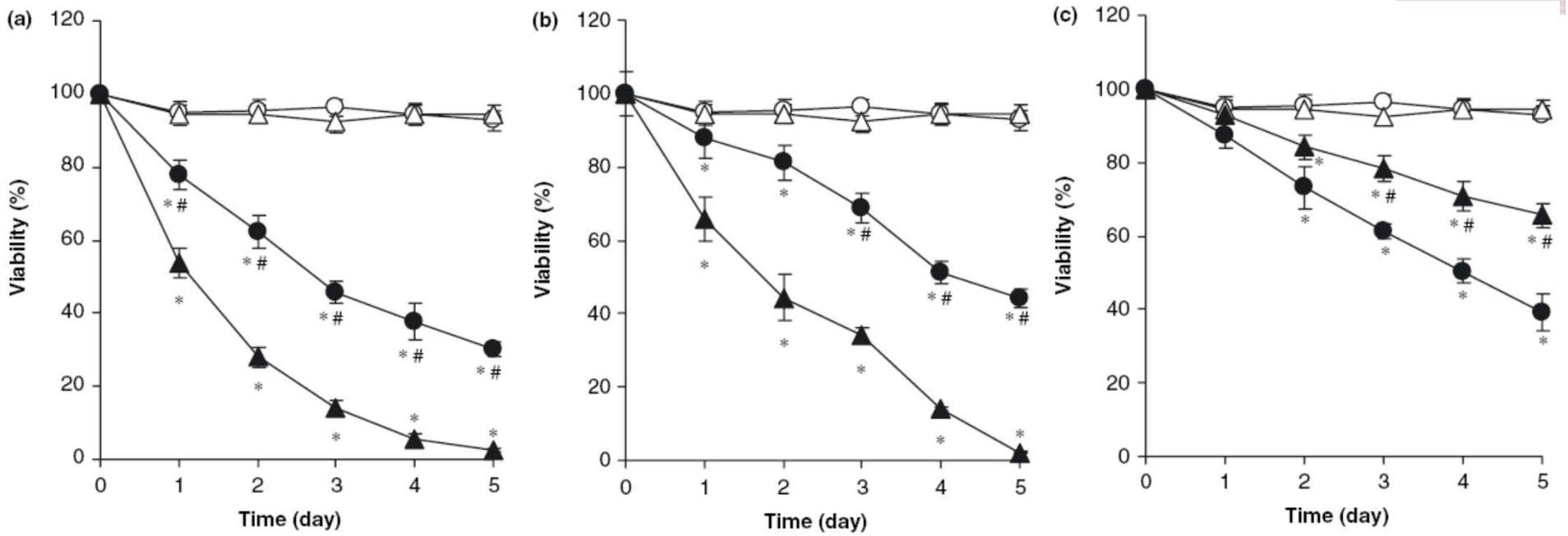
DCIP Reductase Activity in Intact Lymphocytes from IDDM Patients and Age-matched Controls

	Total activity	+ Dicoumarol	DT-diaphorase (total minus dicoumarol)
Controls	0.93 ± 0.21	0.91 ± 0.21	0.10 ± 0.16
Patients	1.72 ± 0.53	1.25 ± 0.30	0.48 ± 0.34

Note. See text for details. Activity is expressed in nmol DCIP reduced per 10^7 cells. All differences between patients and controls were statistically significant ($P < 0.001$).



Hyun et al. (2007) *J. Neurochem.* **100**: 1364-1374



2-Deoxyglucose Iodoacetamide H_2O_2

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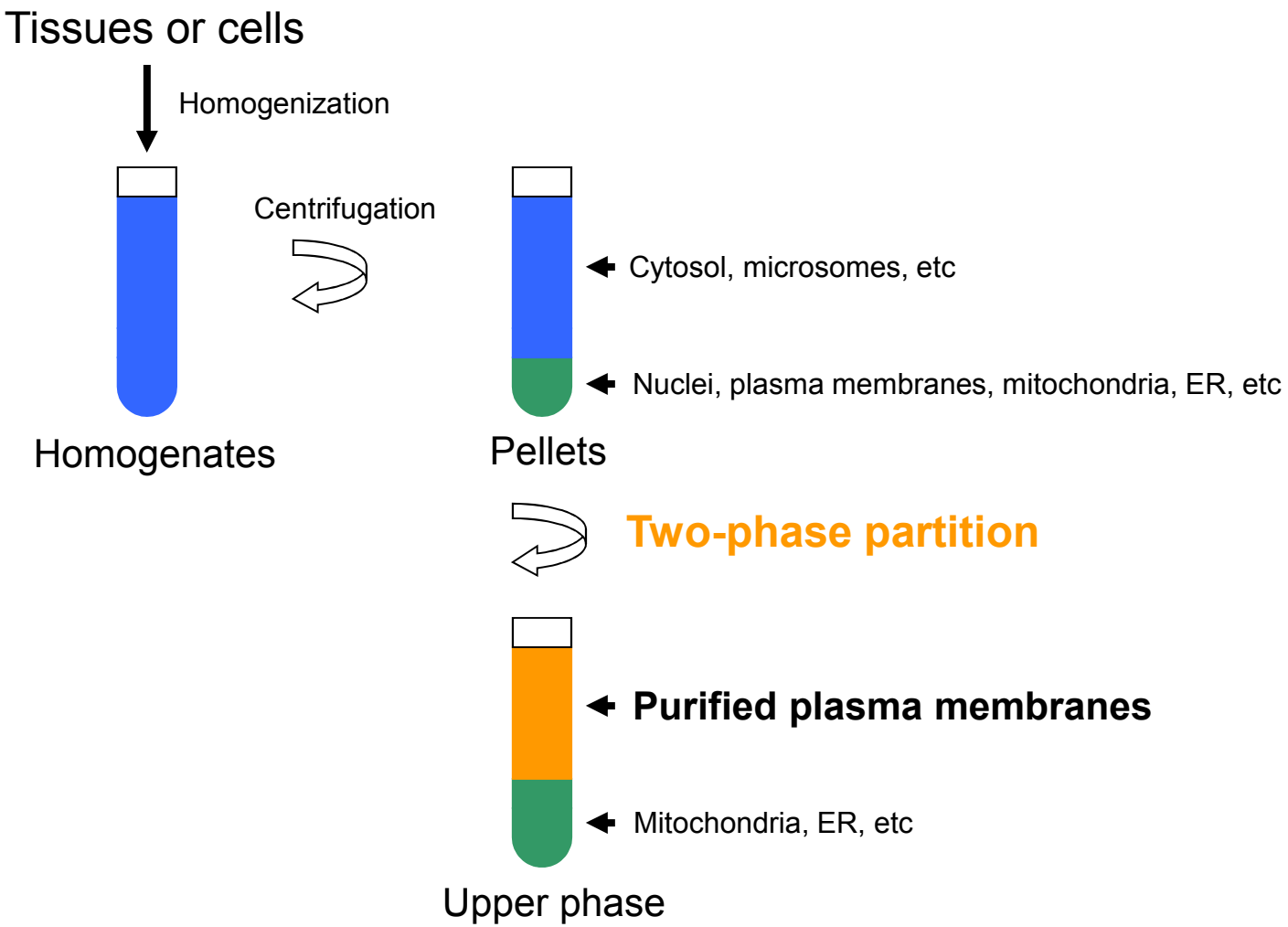
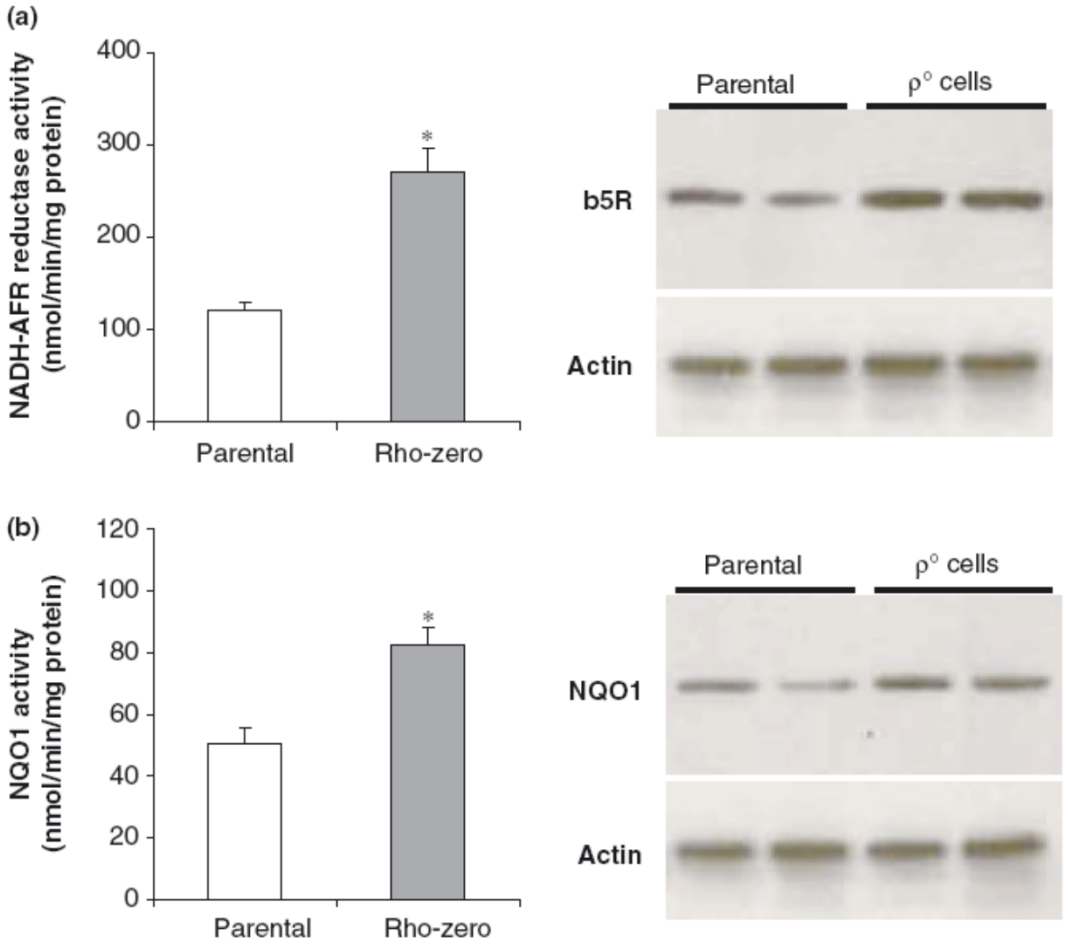
The Procedure for the Isolation of the PMs





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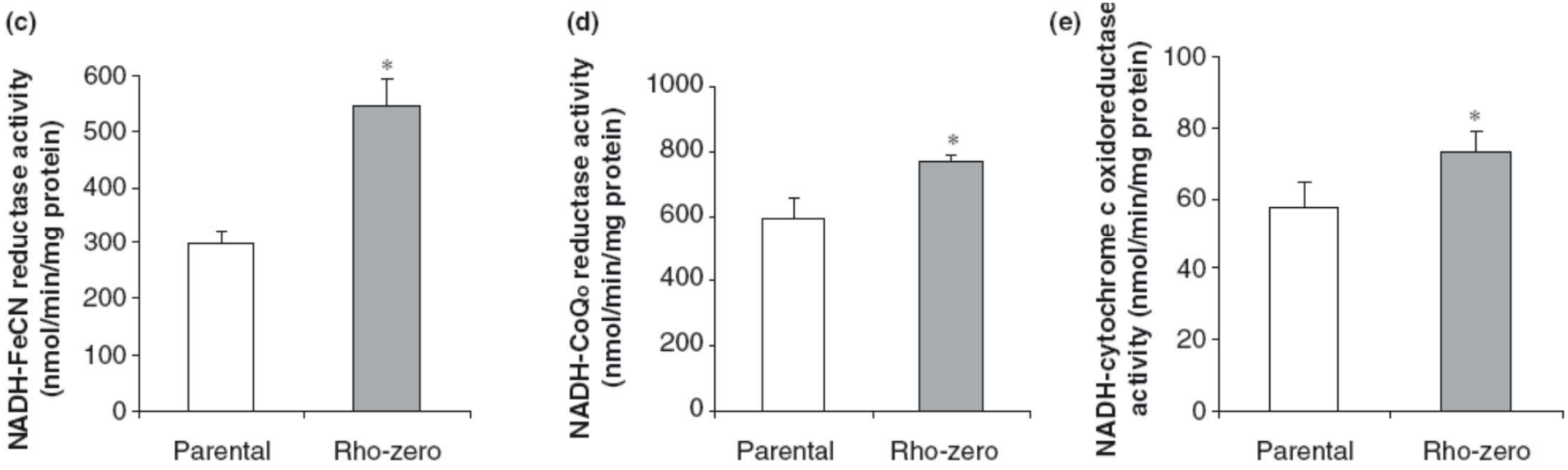

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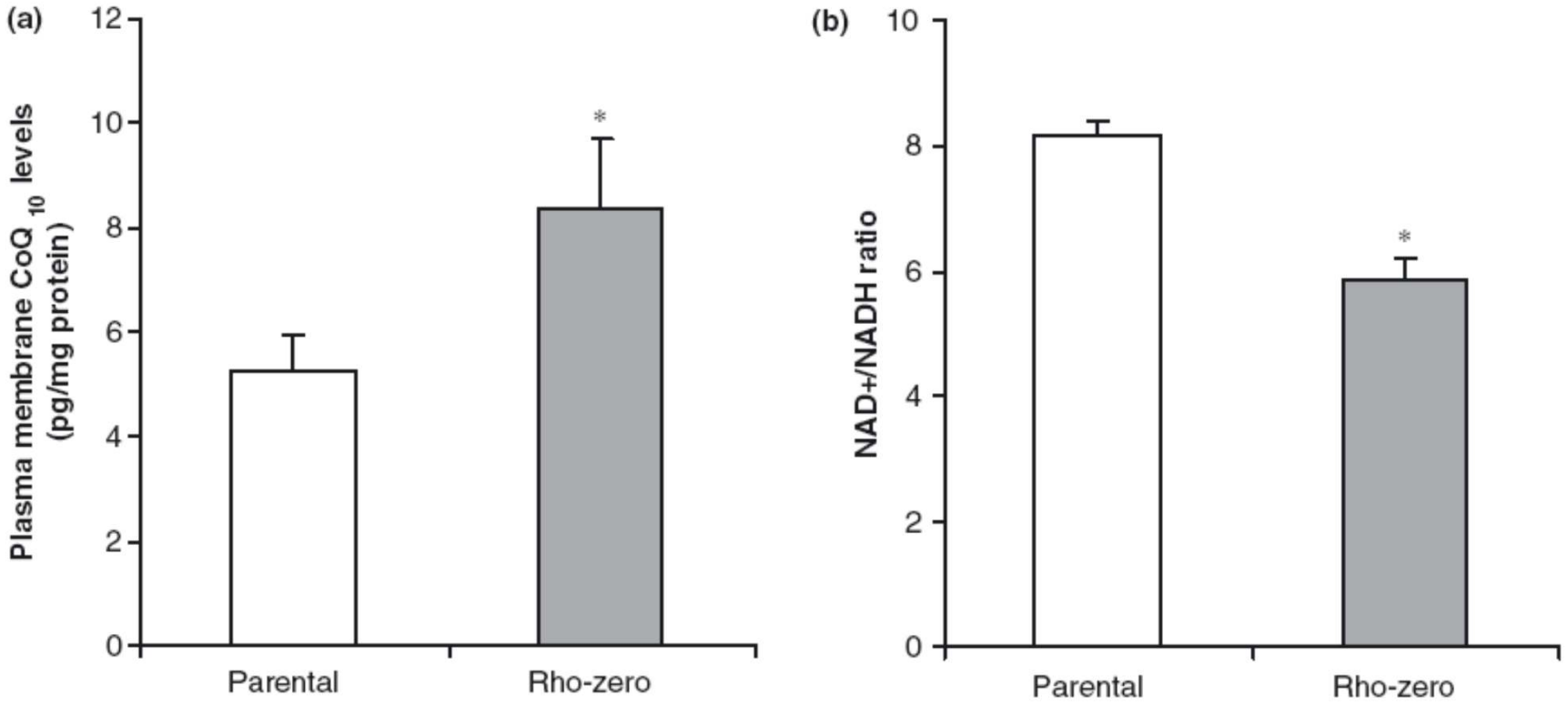

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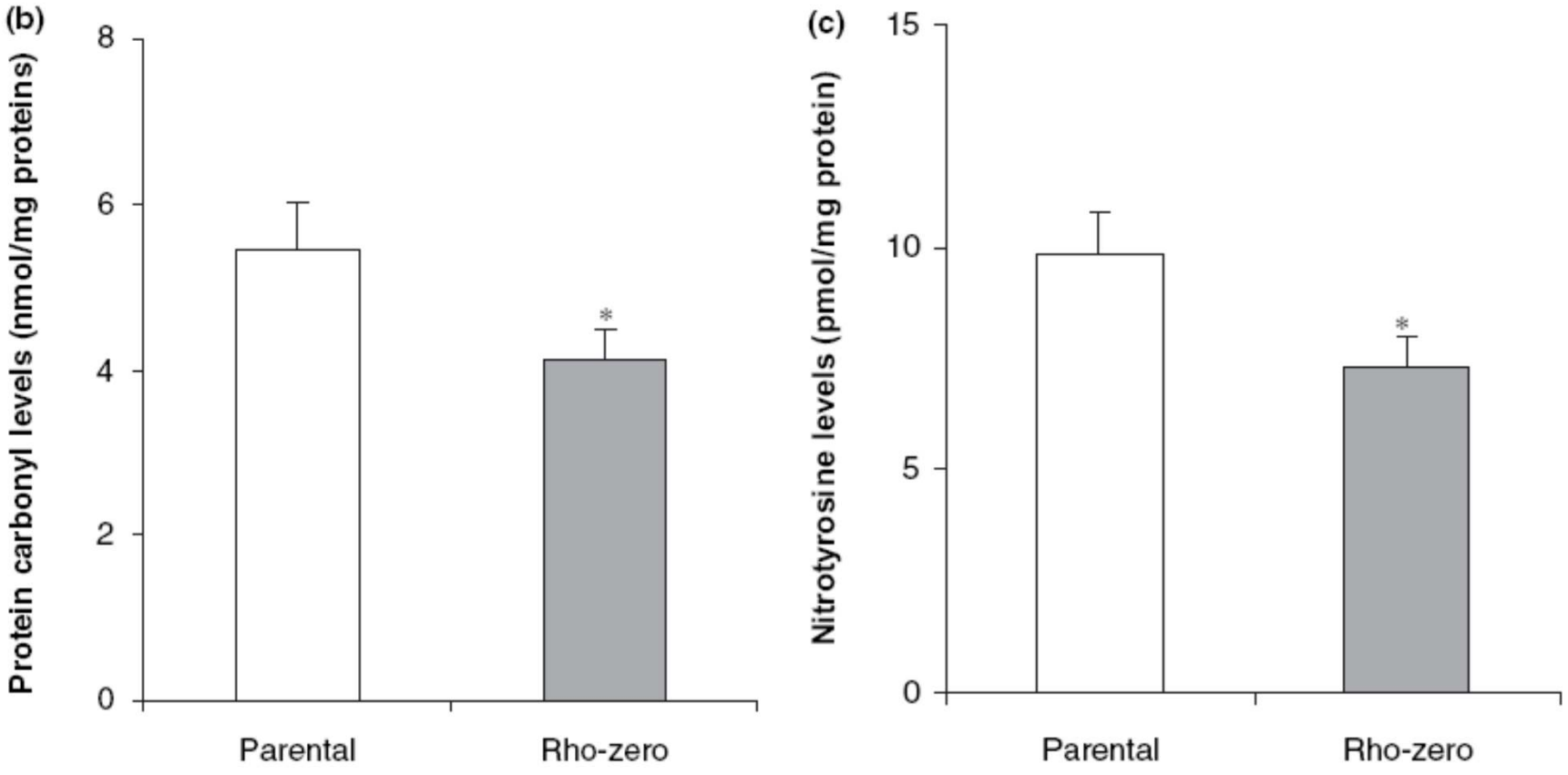

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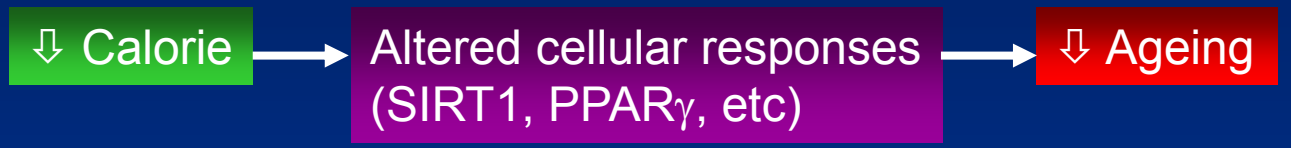
Calorie Restriction

- Undernutrition (NOT malnutrition) with reduced (20-40%) total energy intake
- Slows physiological aging in many systems
  - up to 50% by CR from yeast and nematodes to rodents and monkeys
  - Lower production of ROS & attenuate oxidative stress
- Life span extension is observed with diverse diet compositions as long as calories are reduced (e.g. Intermittent fasting)

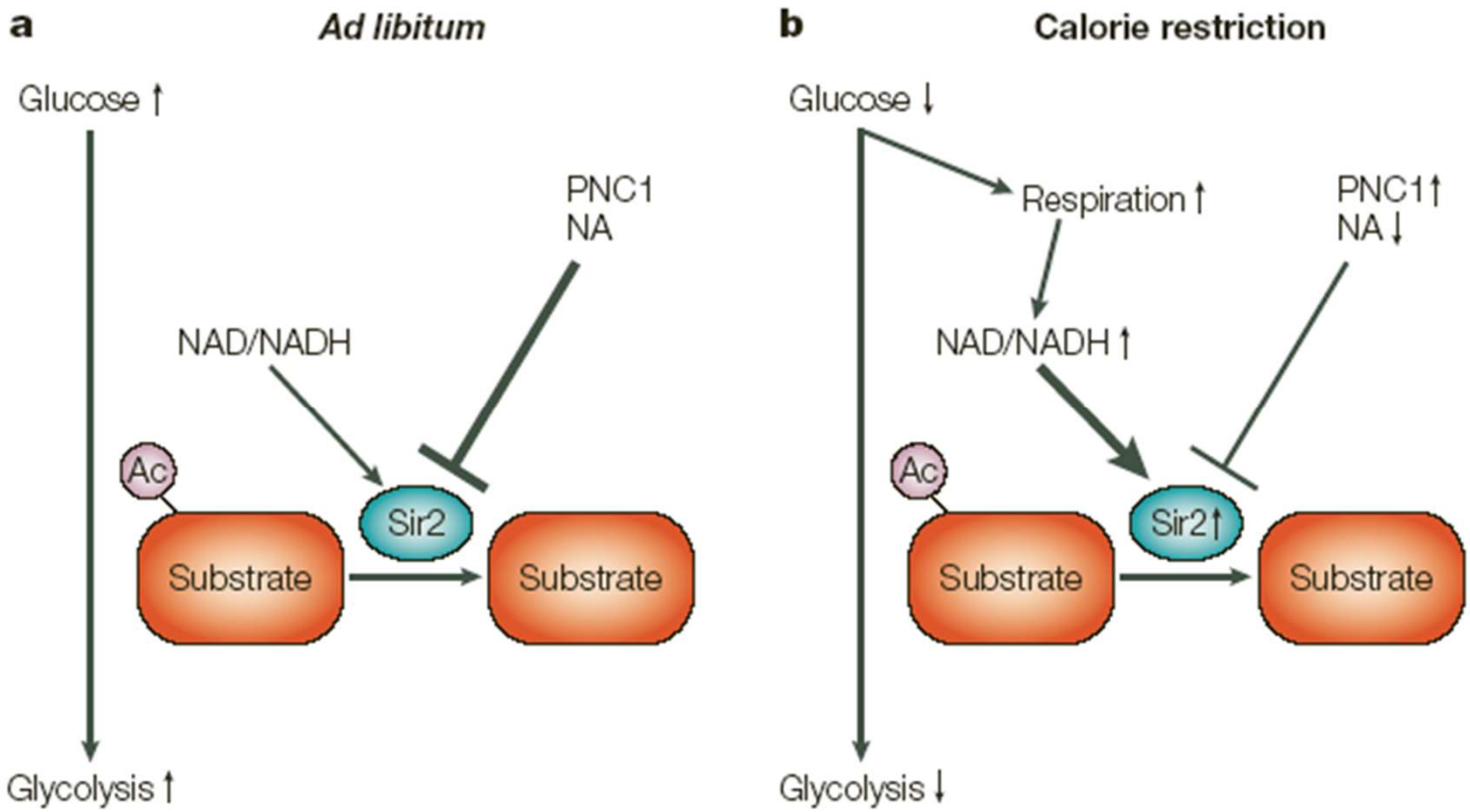
Two General Mechanisms How CR Extends Life Span

Mechanical

Regulated

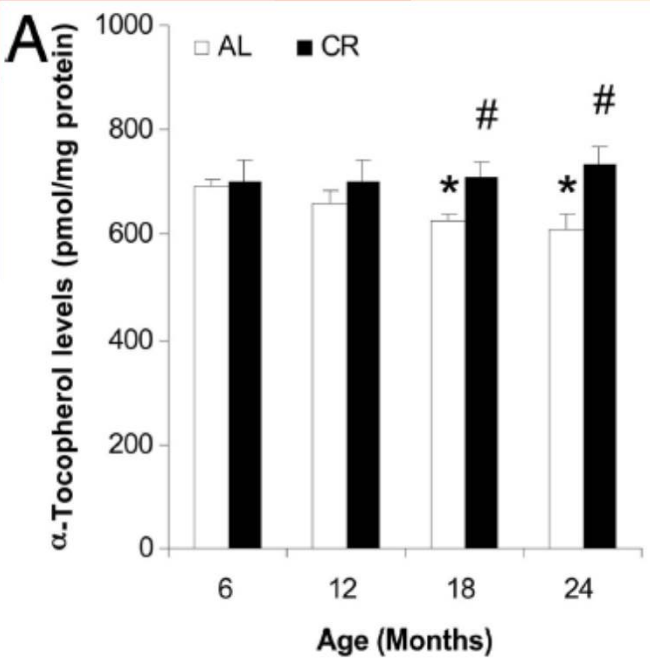


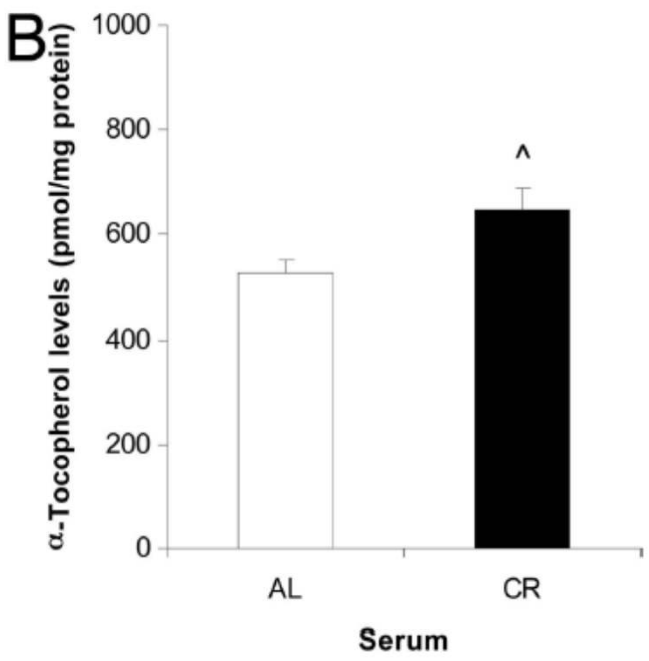
CR, SIR2 & Metabolism in Yeasts

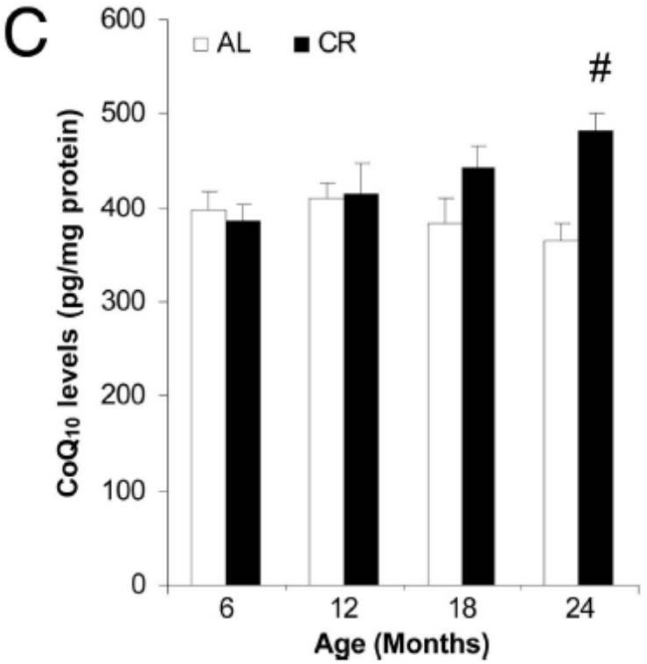


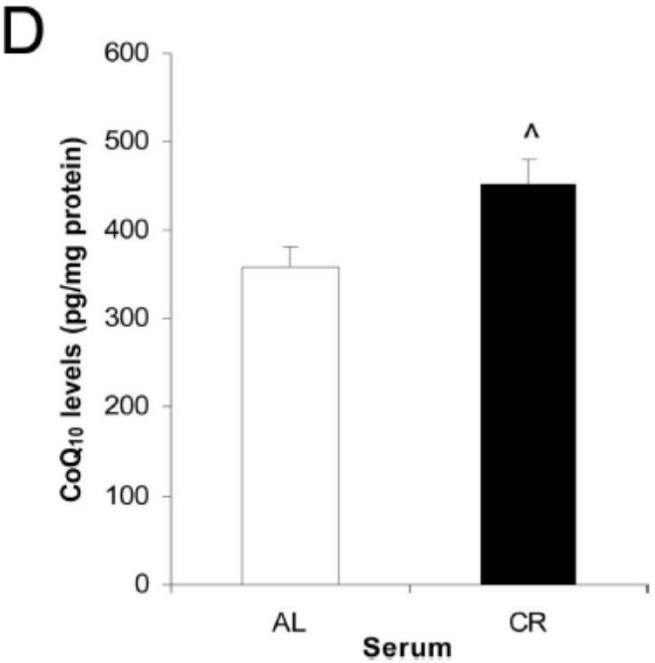
PNC1: pyraninamidase-1
NA: nicontinamidase

Bordone & Guarente (2005)

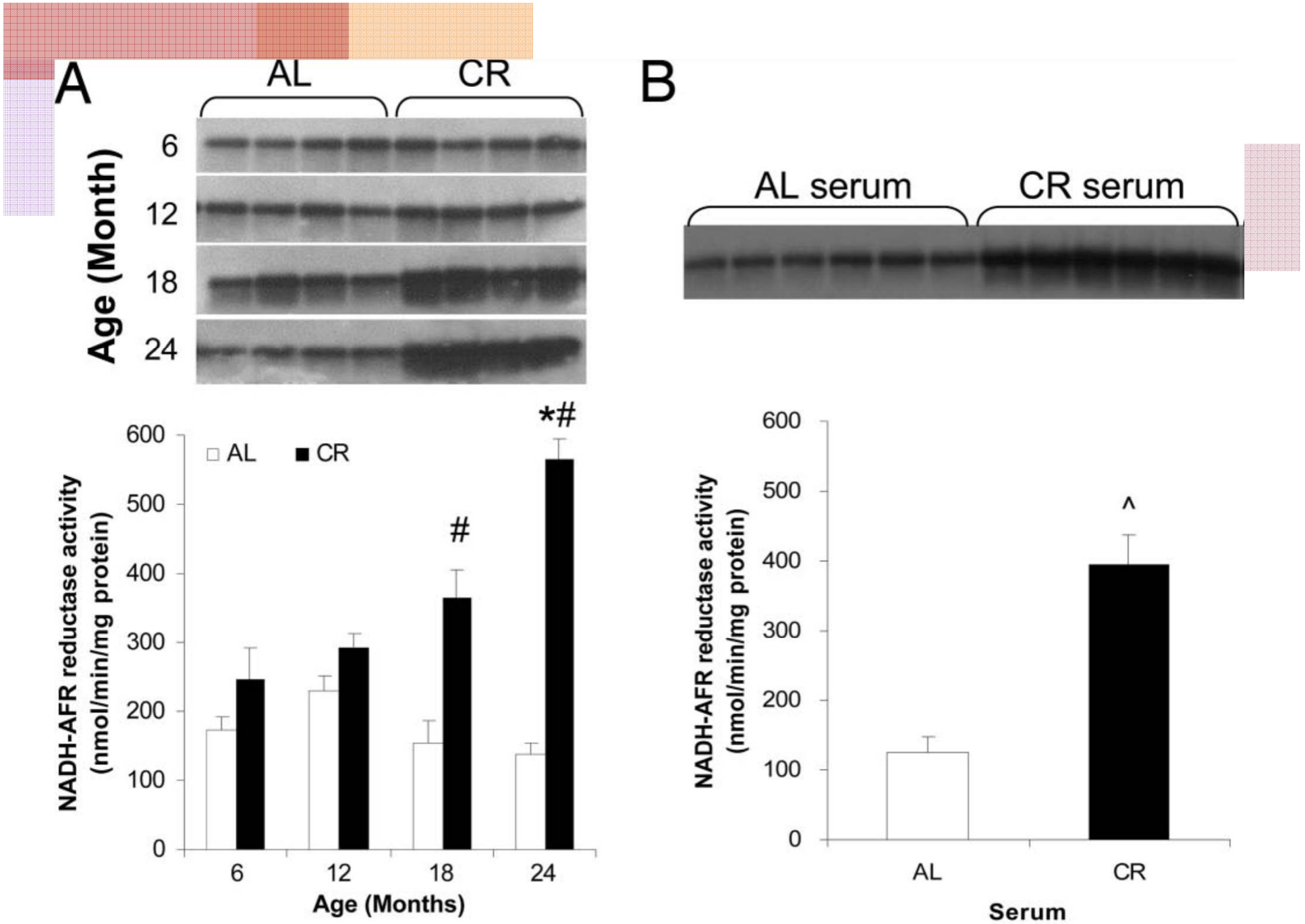








Hyun et al. (2006) *PNAS* **103**: 19908-19912



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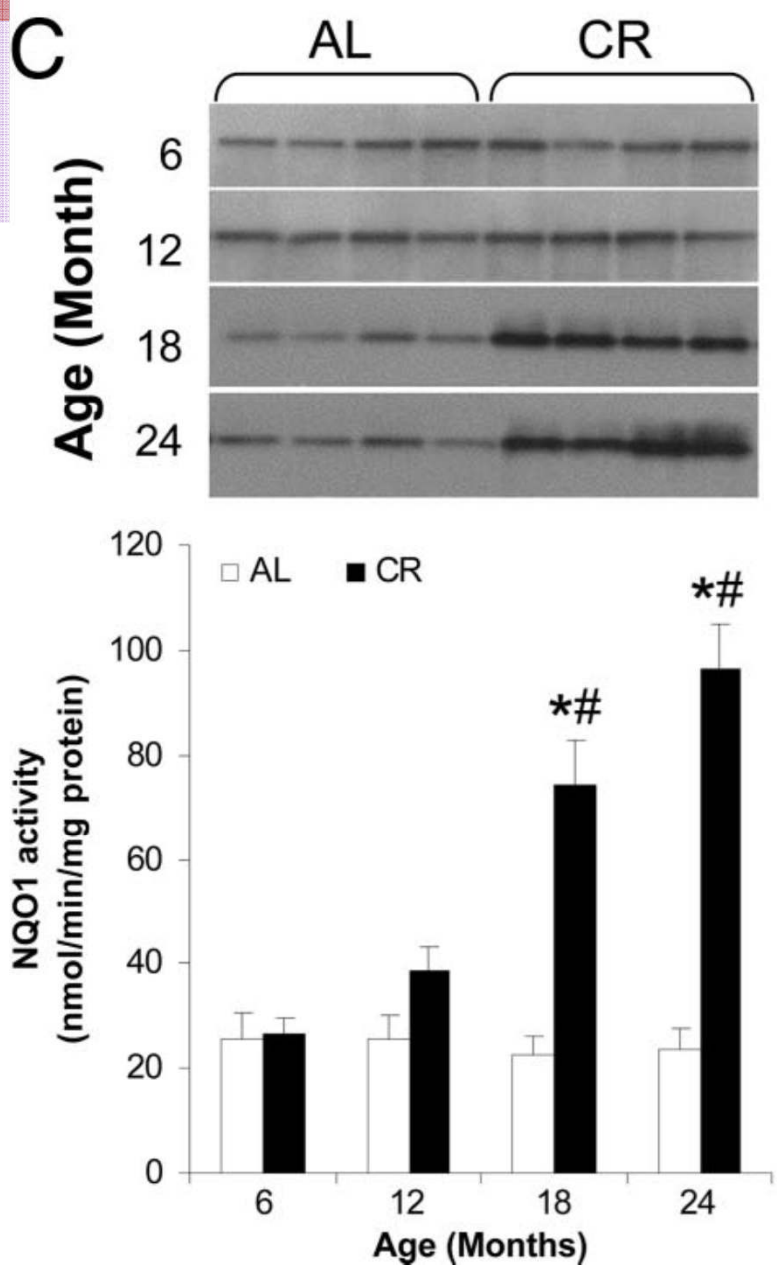

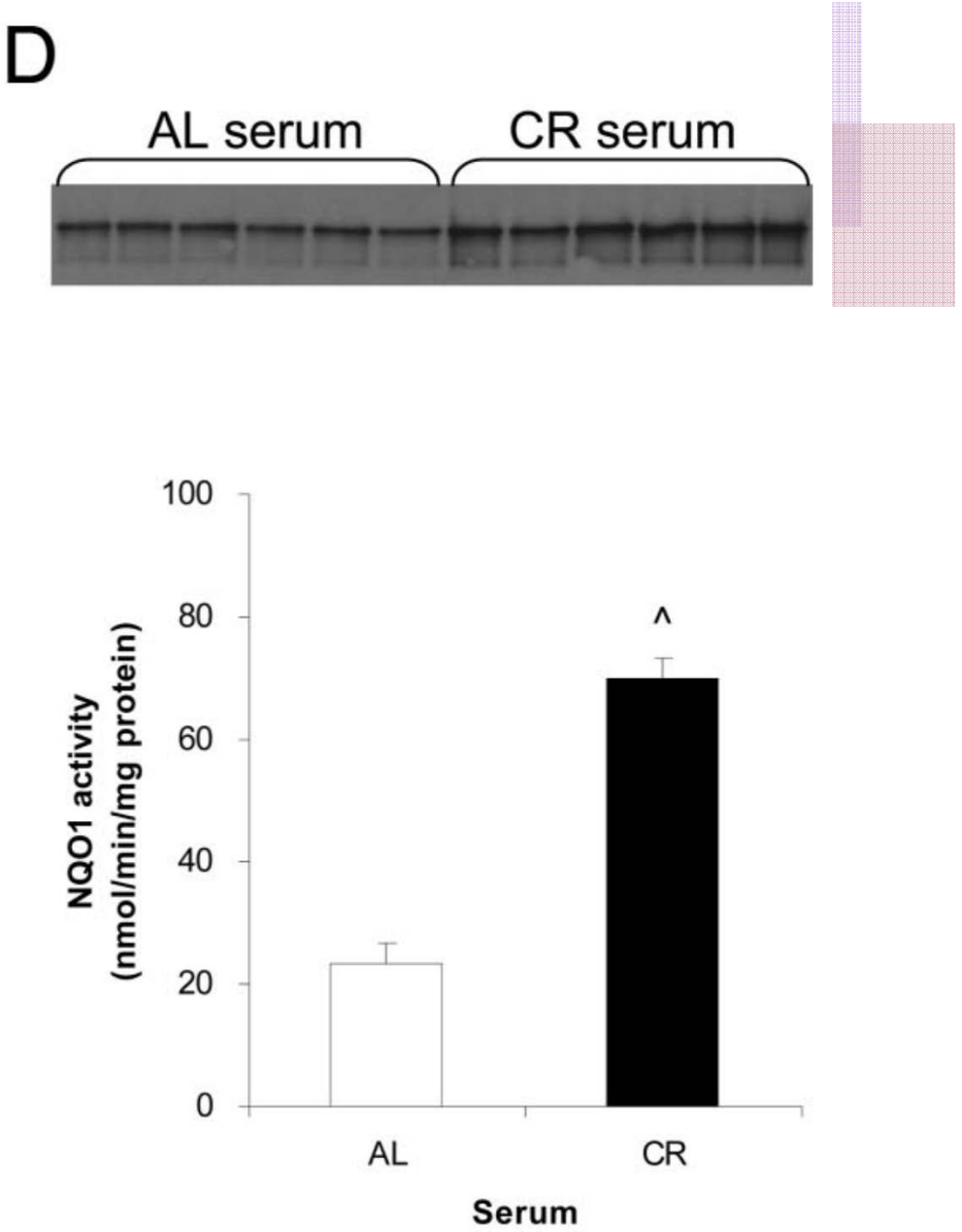

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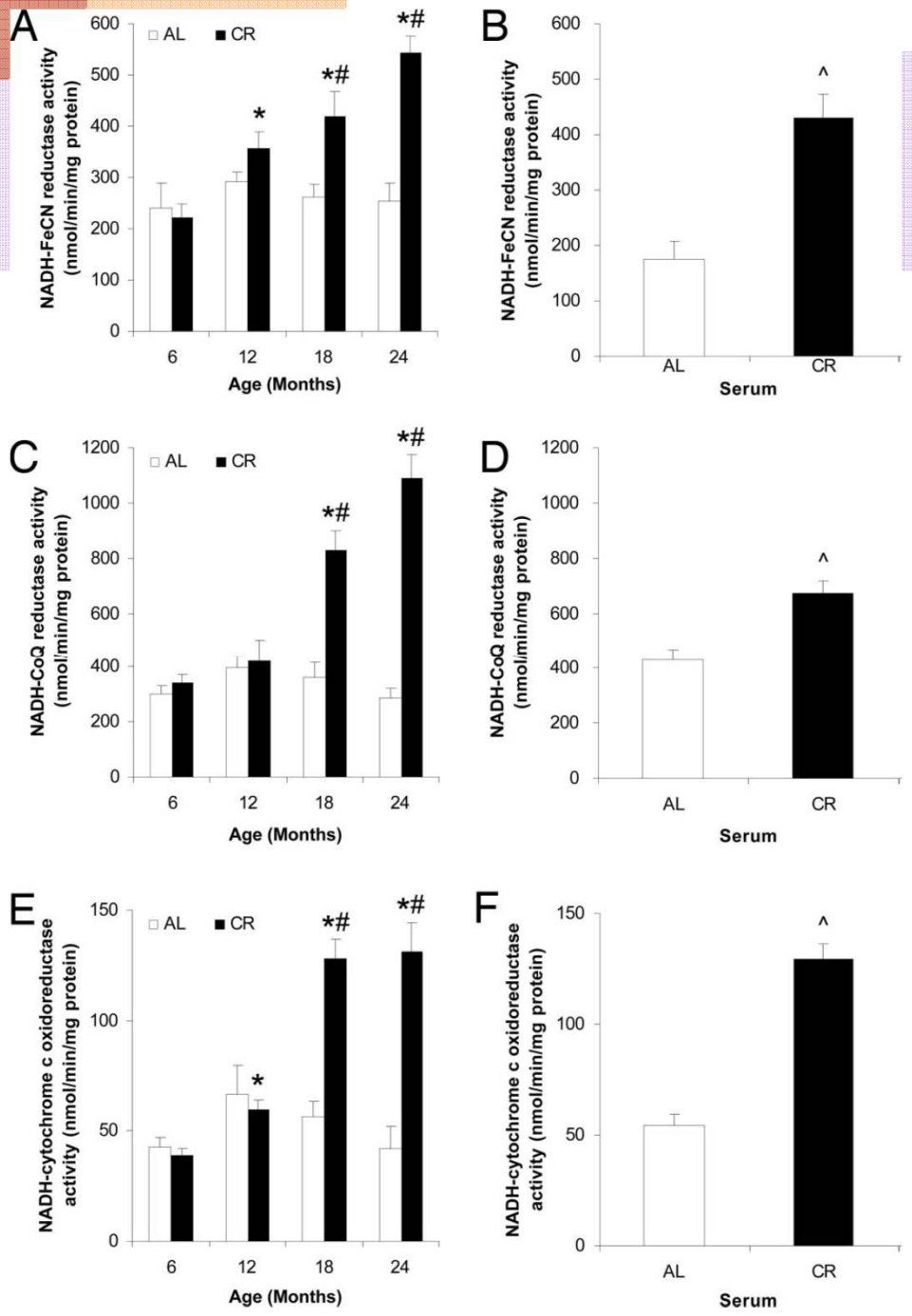

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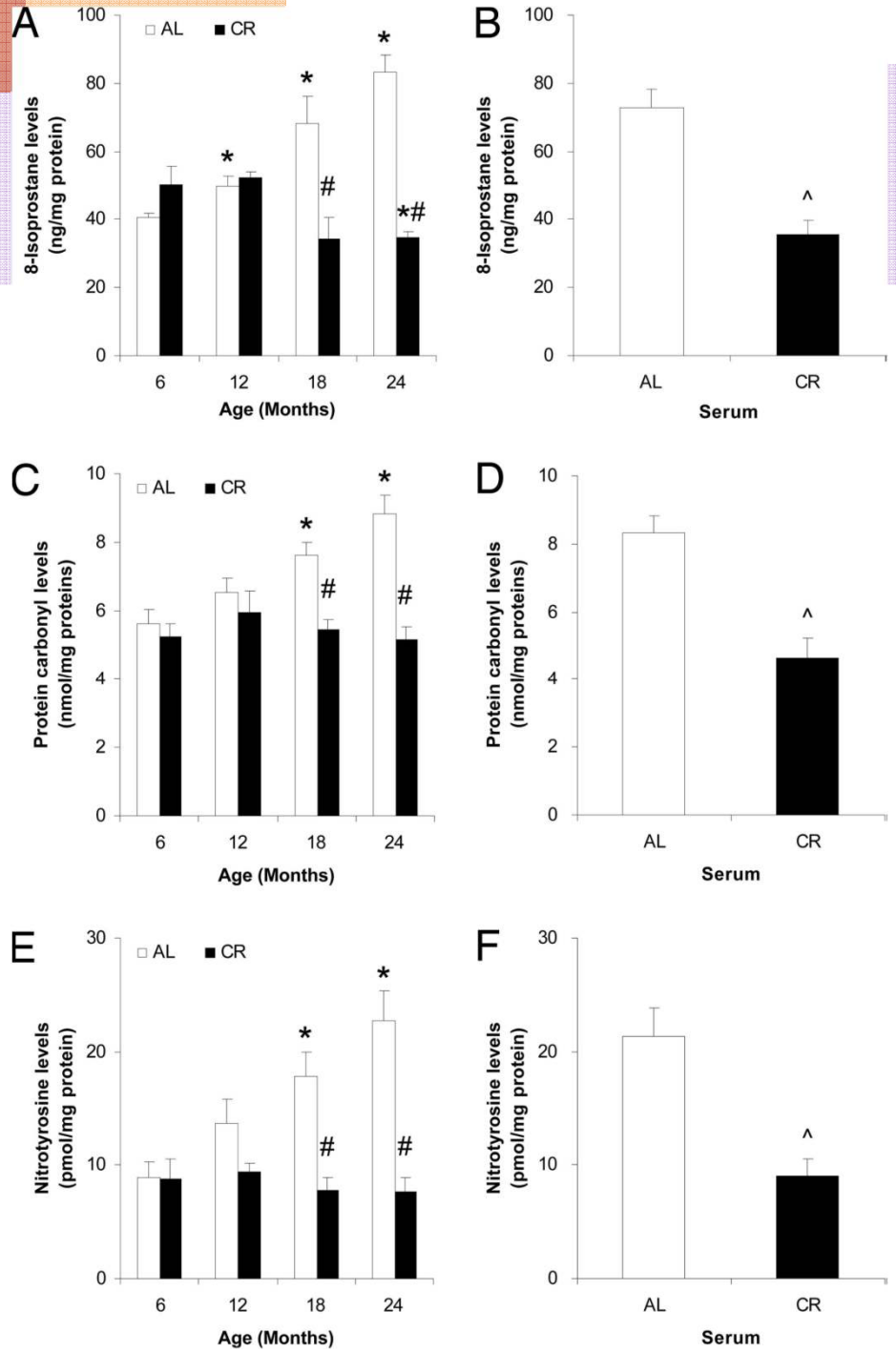

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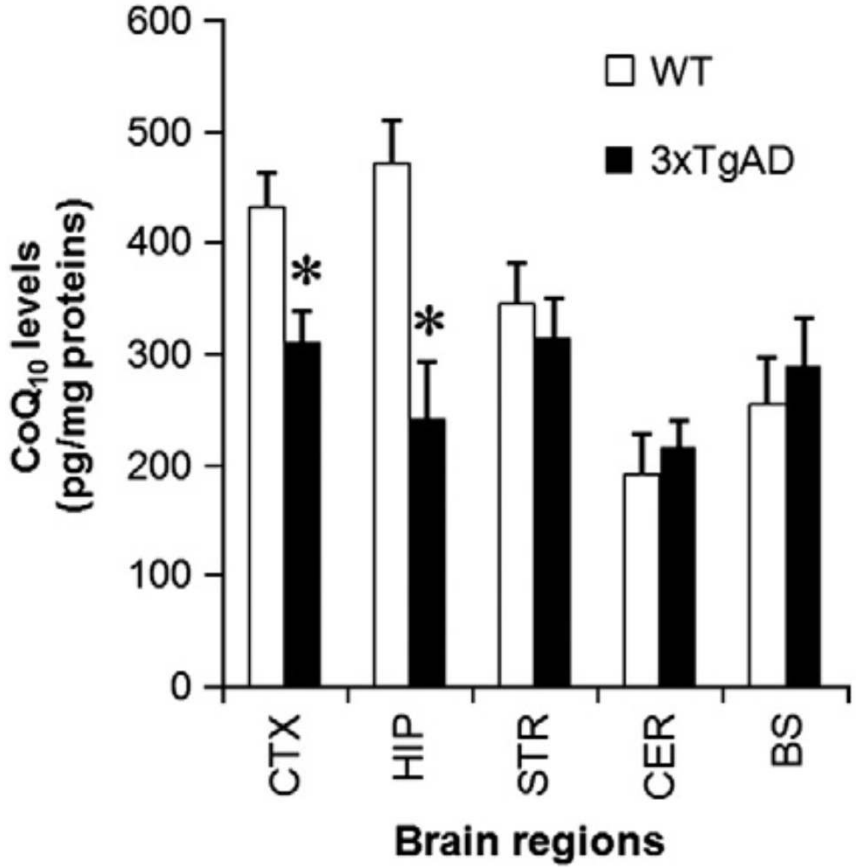
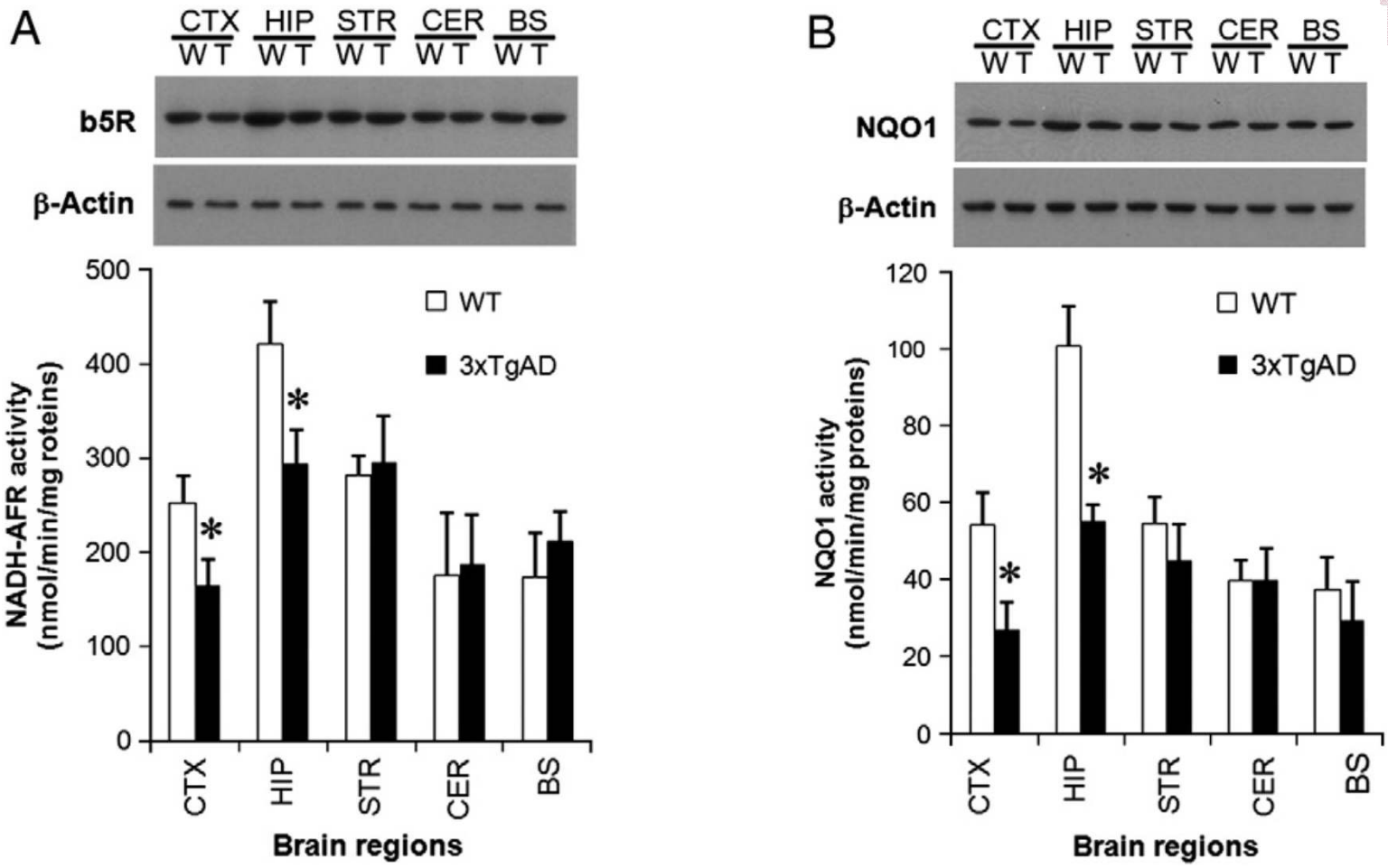
Down-Regulation of NQO1 in Age-Related Diseases



Hyun et al. (2010) *Exp. Neurol.* **225**: 423-420



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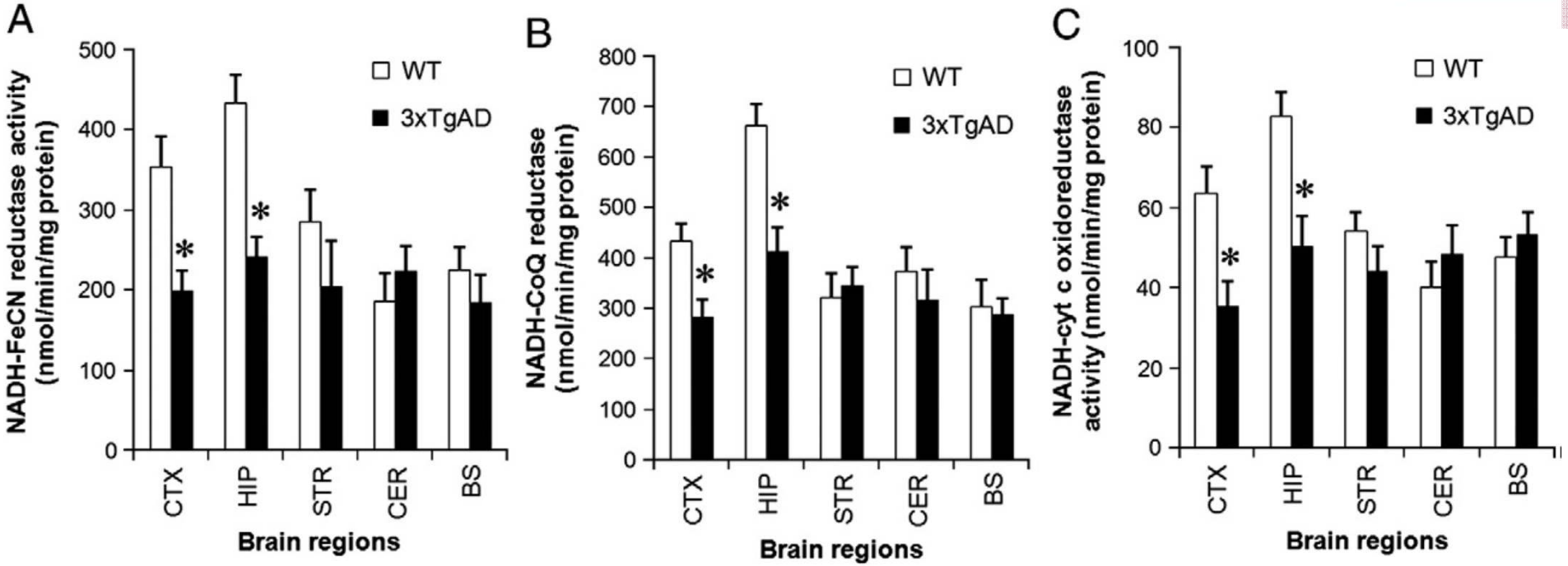

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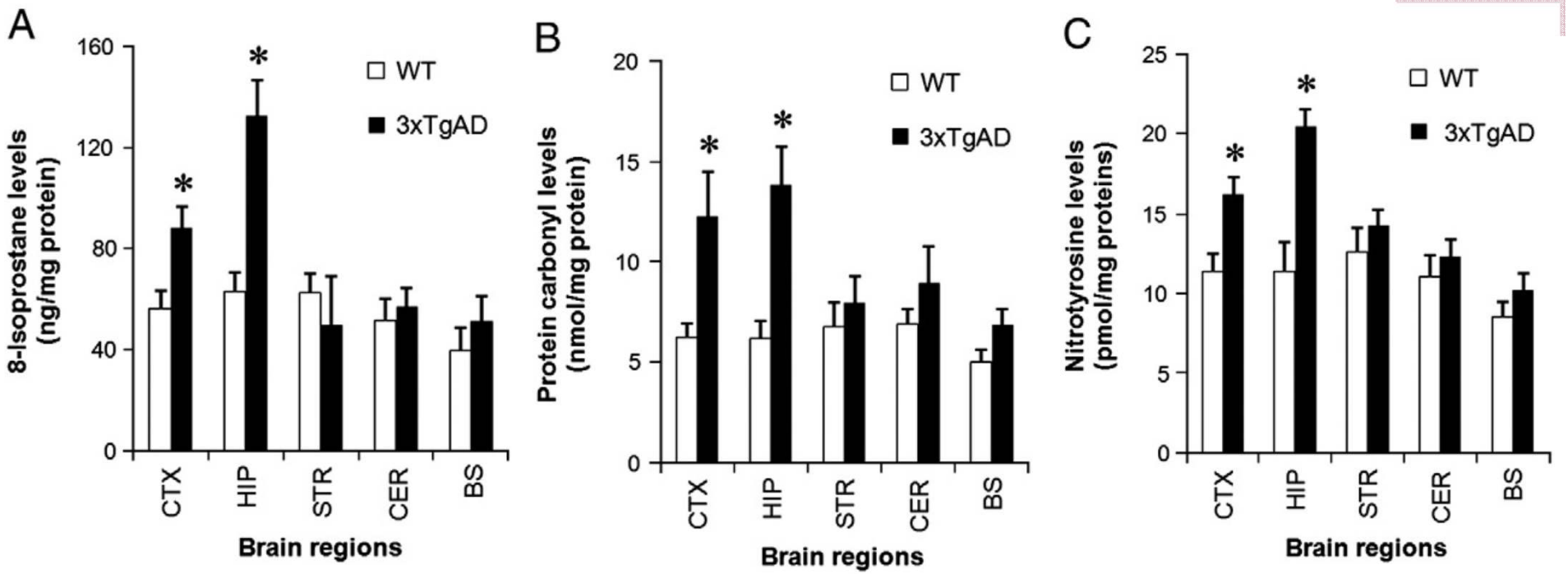

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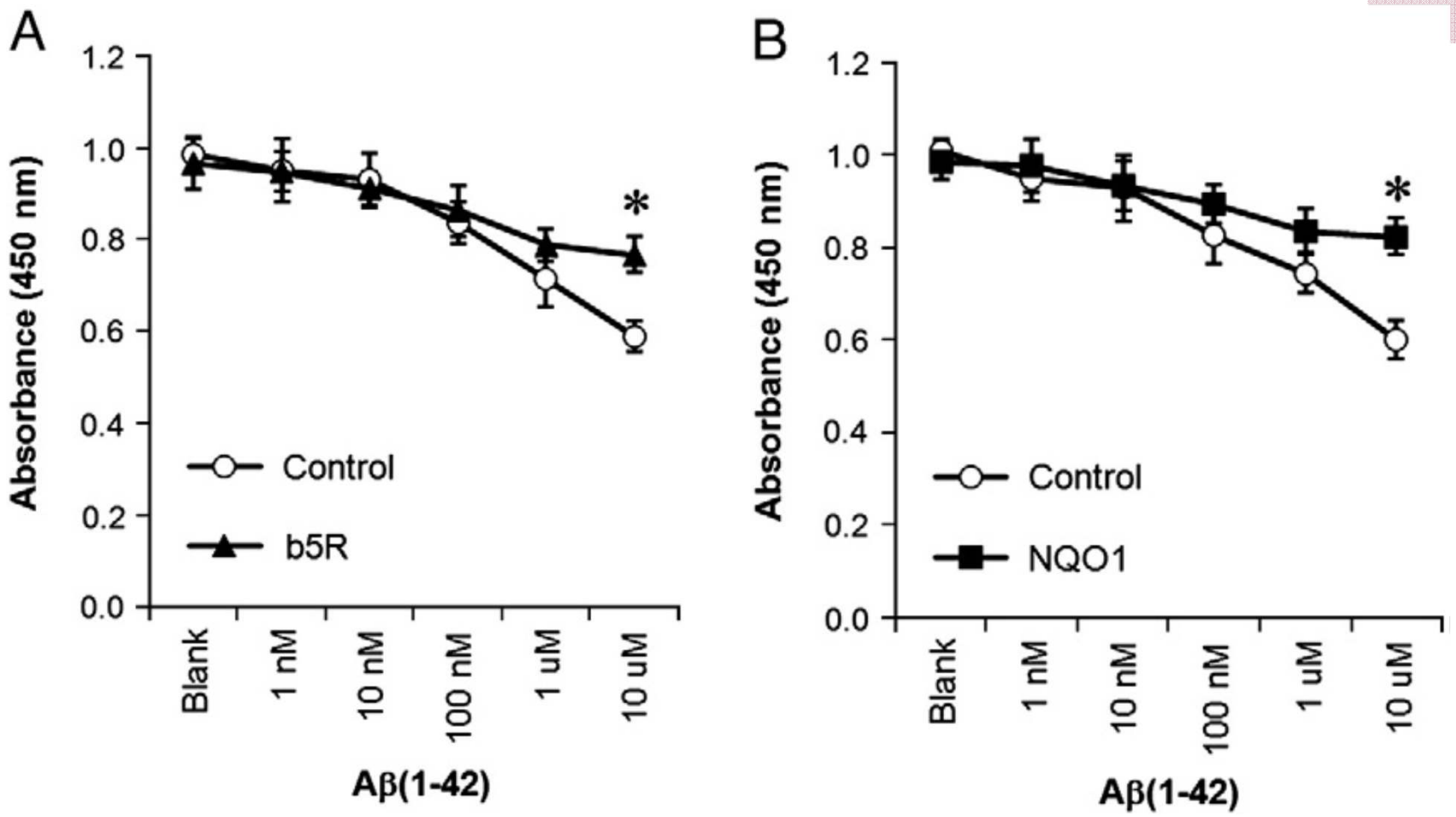
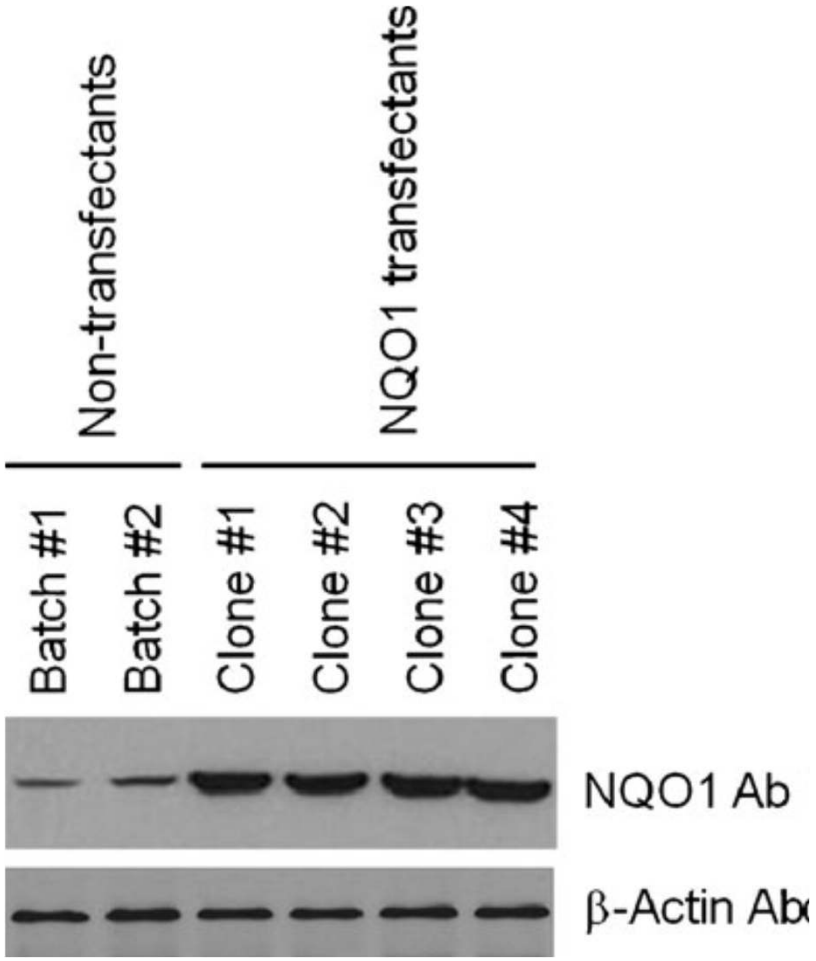
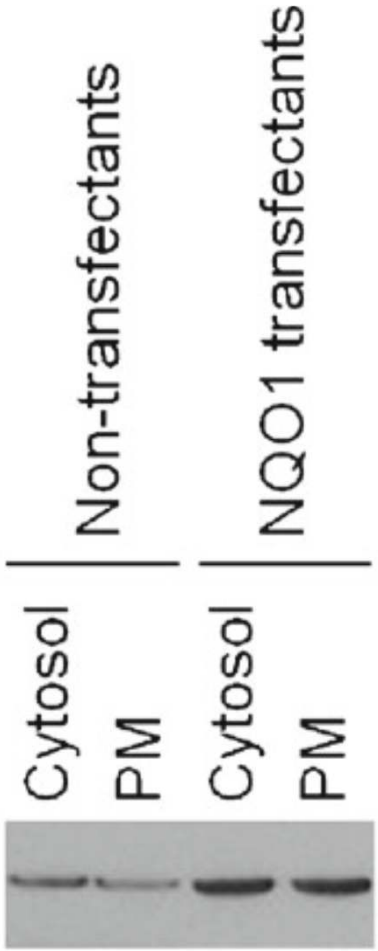


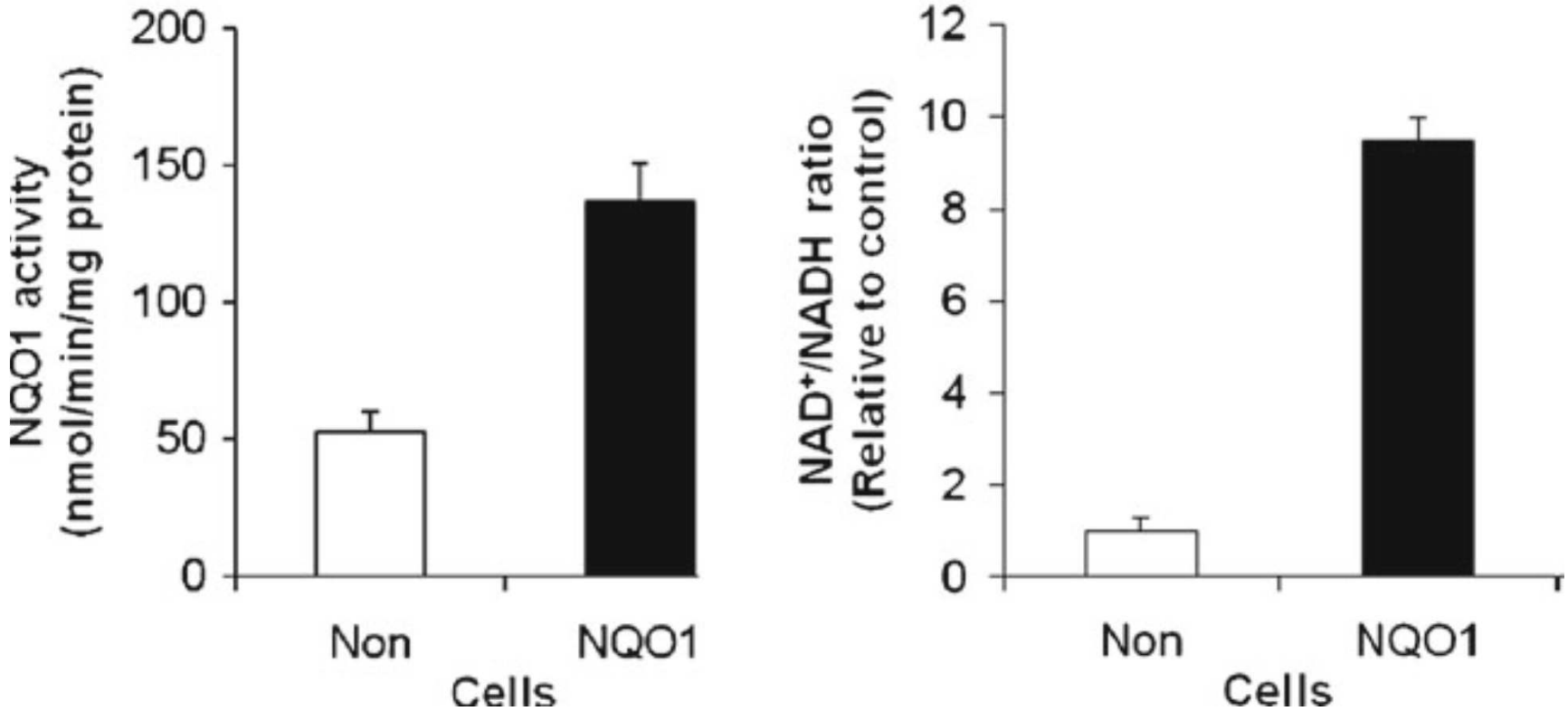
Hyun et al. (2010) *Exp. Neurol.* **225**: 423-420

Overexpression or Kocking-Down of NQO1 & Cytoprotection

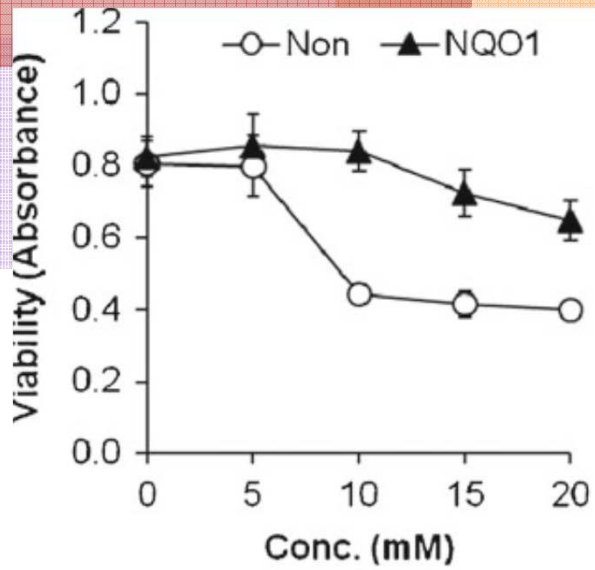




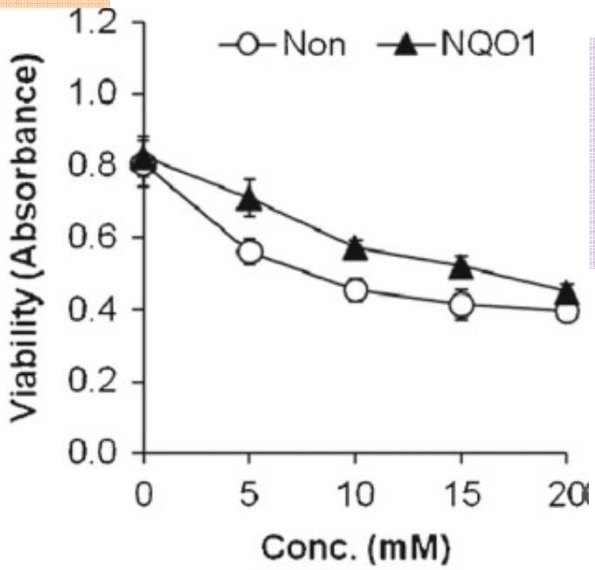
Hyun et al. (2011) *AGE (in press)*



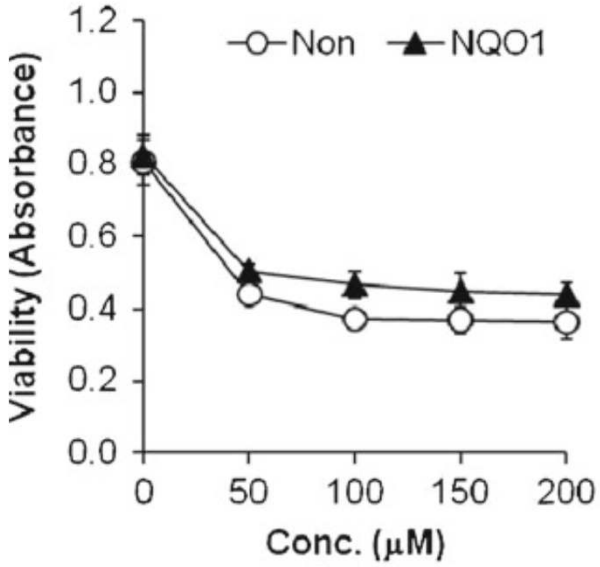
Hyun et al. (2011) *AGE (in press)*



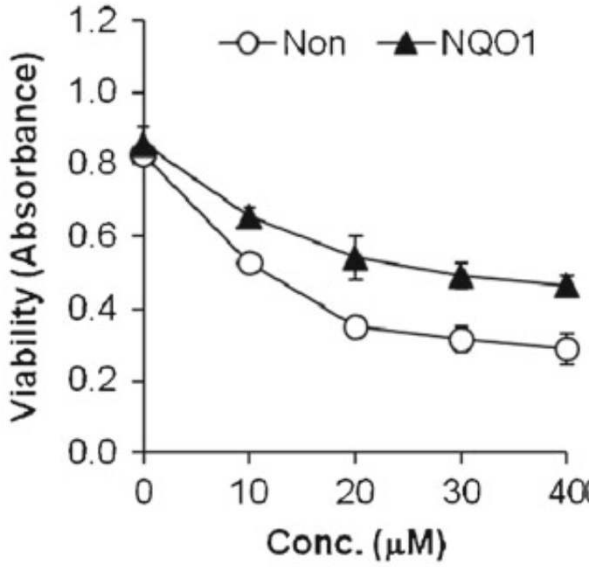
2-DG



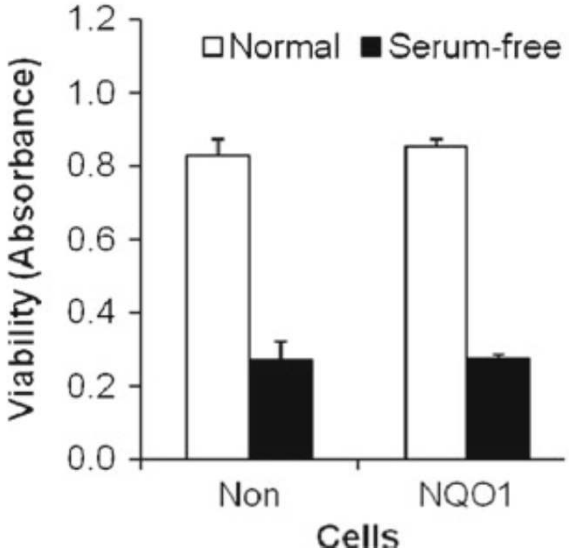
KCN



H_2O_2

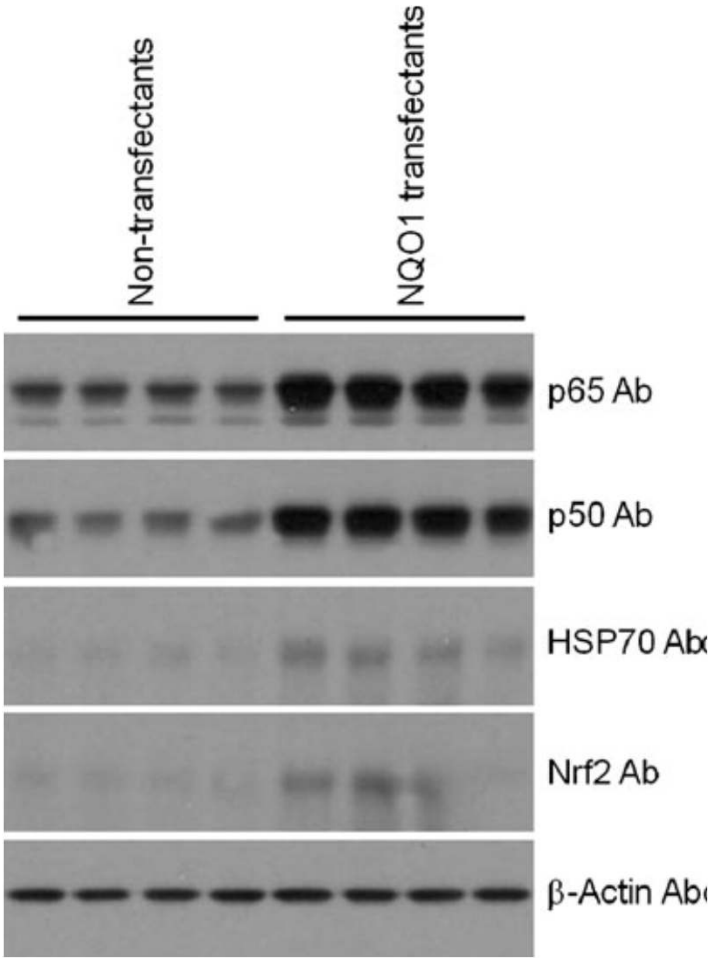


Lactacystin

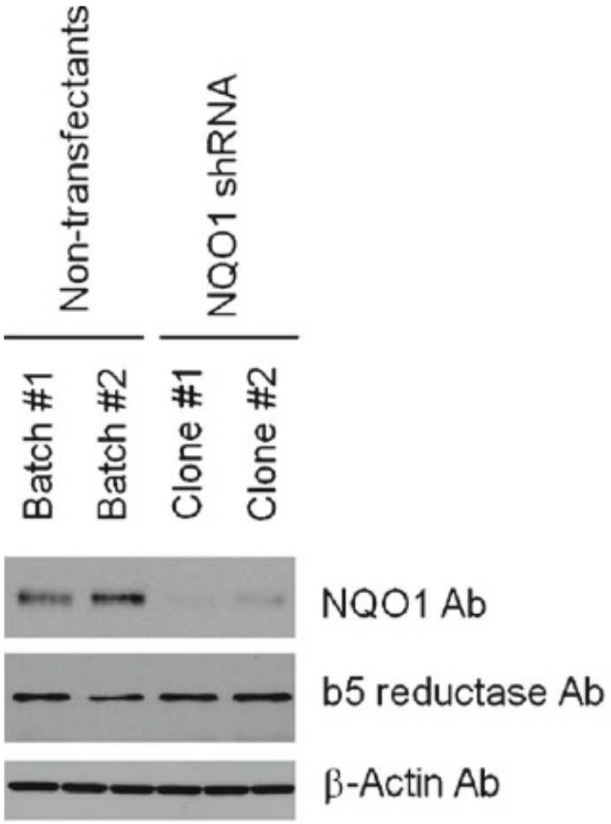


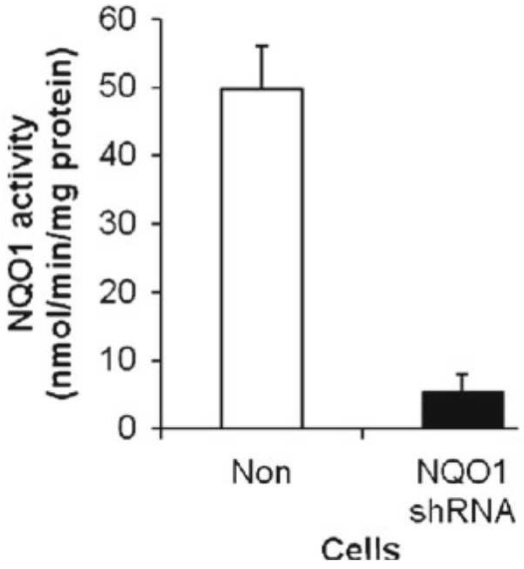
Serum-free

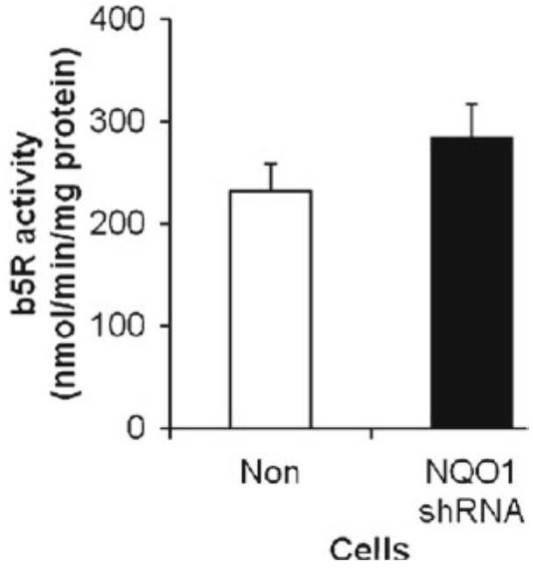
Hyun et al. (2011) *AGE (in press)*



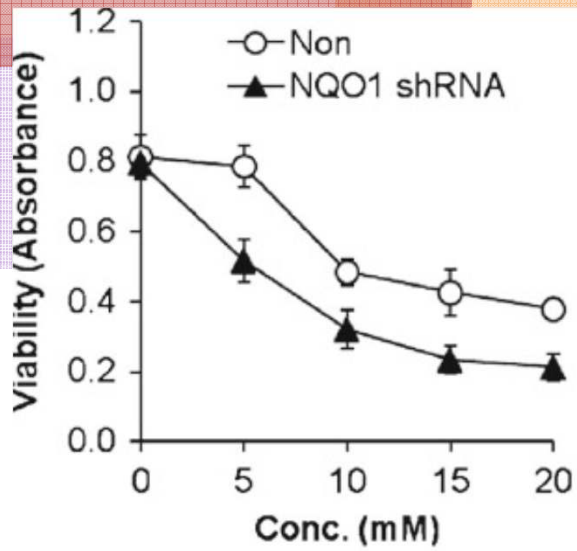
Hyun et al. (2011) *AGE (in press)*







Hyun et al. (2011) *AGE (in press)*



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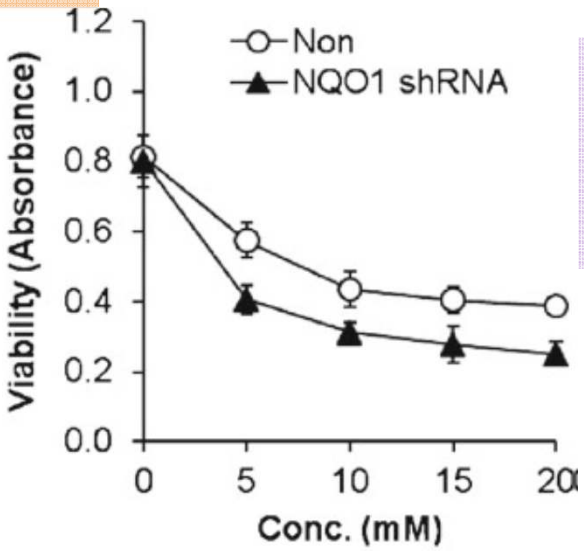

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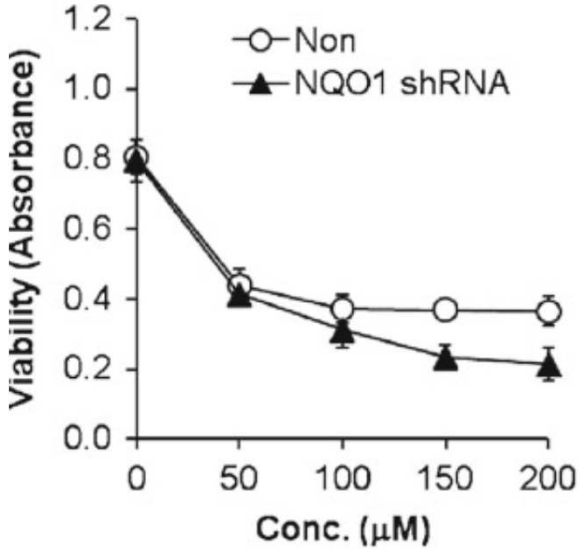
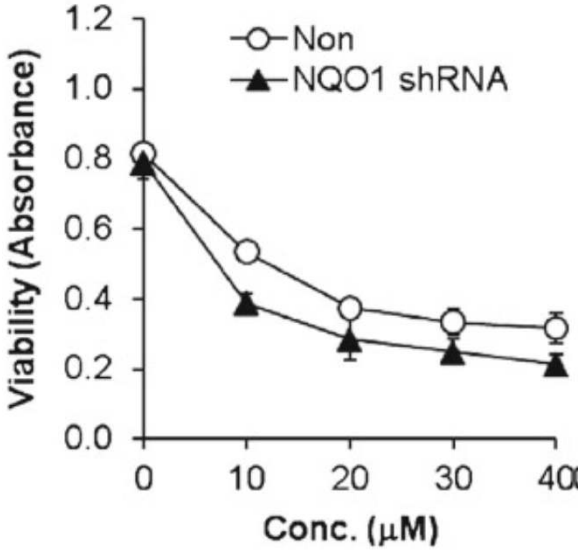


H_2O_2



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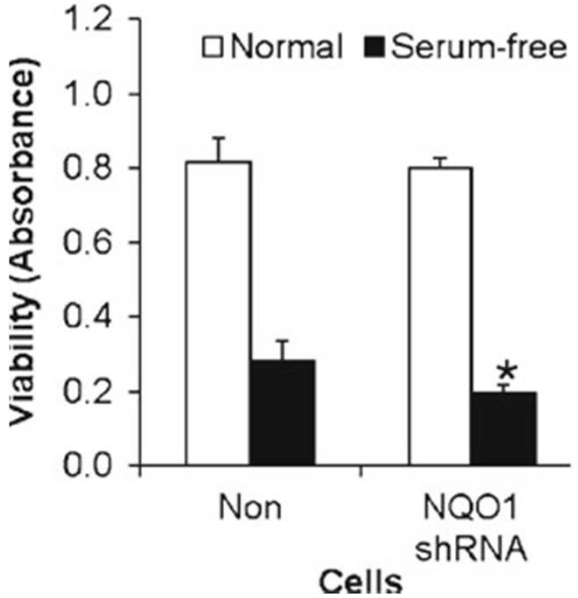


Serum-free

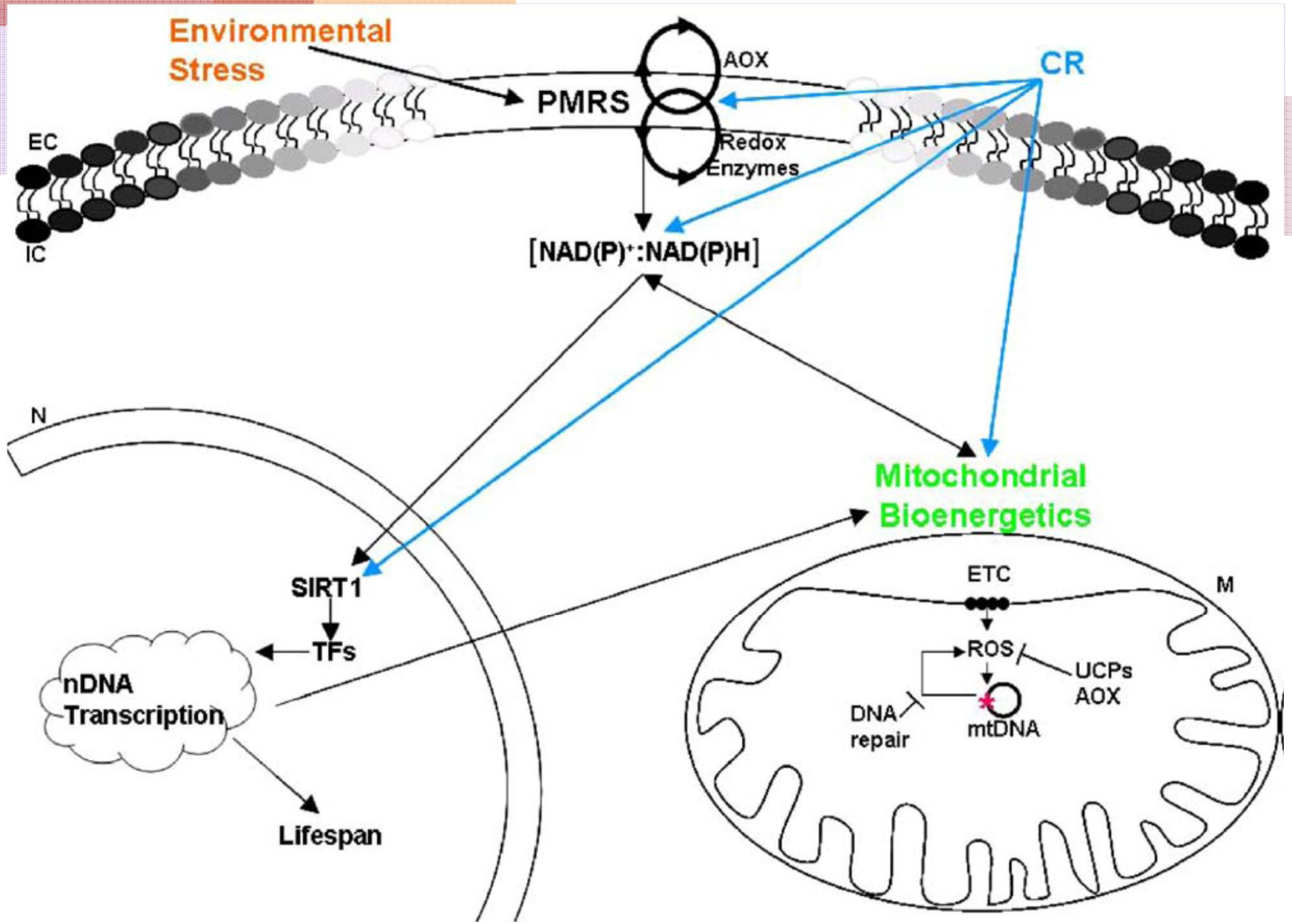
Hyun et al. (2011) *AGE (in press)*

Conclusion

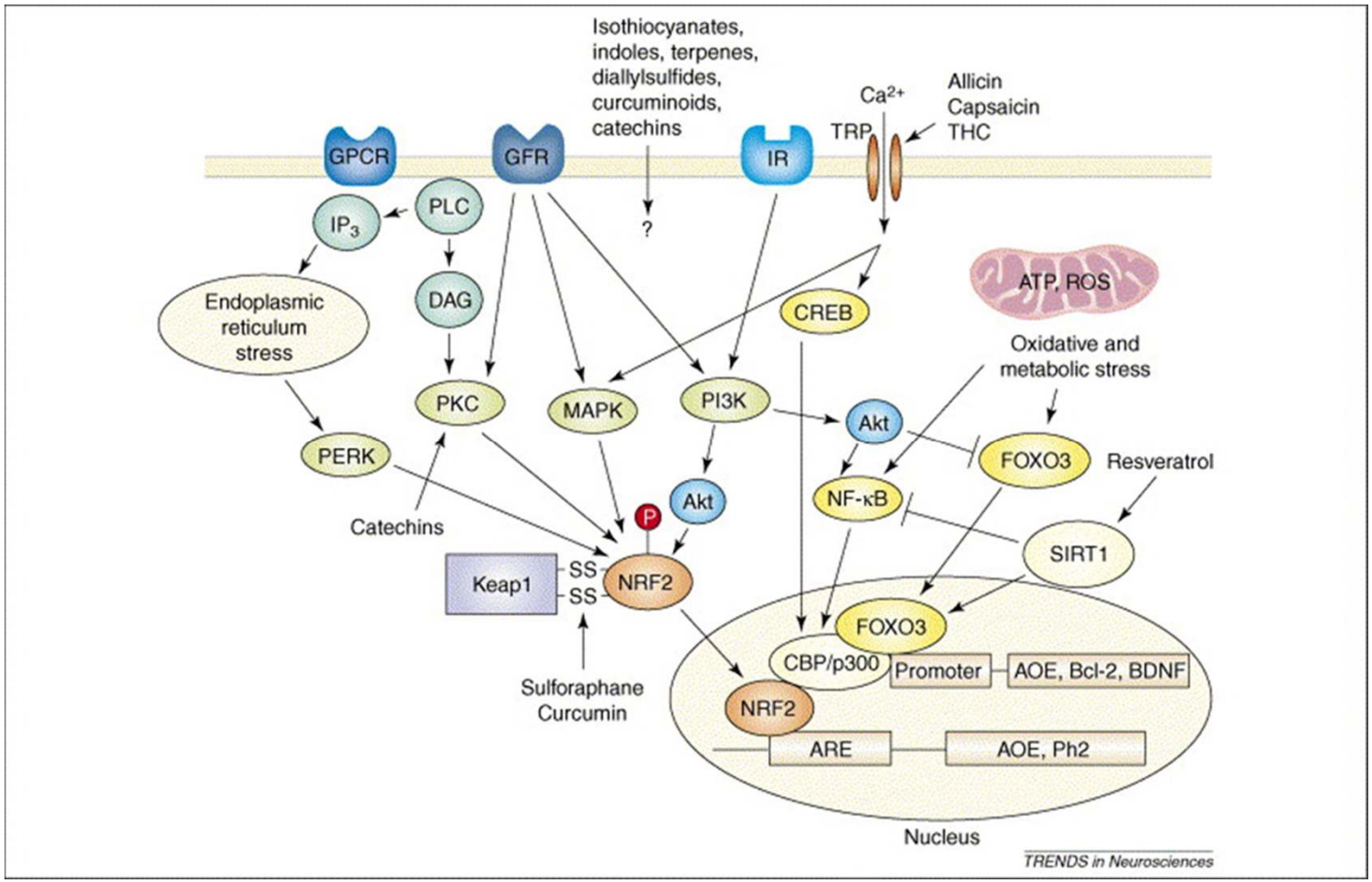
- A compensation mechanism (PMRS) is altered in response to mitochondrial dysfunction, CR & progression of AD

- Enhanced PMRS activity in mitochondria-deficient cells (e.g. ρ^o cells)
- Elevated PMRS → Anti-ageing (e.g. CR)
- Declined PMRS → Progression of diseases (e.g. AD)

- **Overall, our data support the growing view that enhanced PMRS is a key step in maintaining normal cellular function and delaying the ageing process**



Hunt et al. (2006) *Ageing Res. Rev.* **5**: 125-143



Mattson & Cheng (2006) *Trends in Neurosci.* **29**: 632-639

Thank You for Your Attention!!!