Overview of oxidative stress and antioxidant defence system

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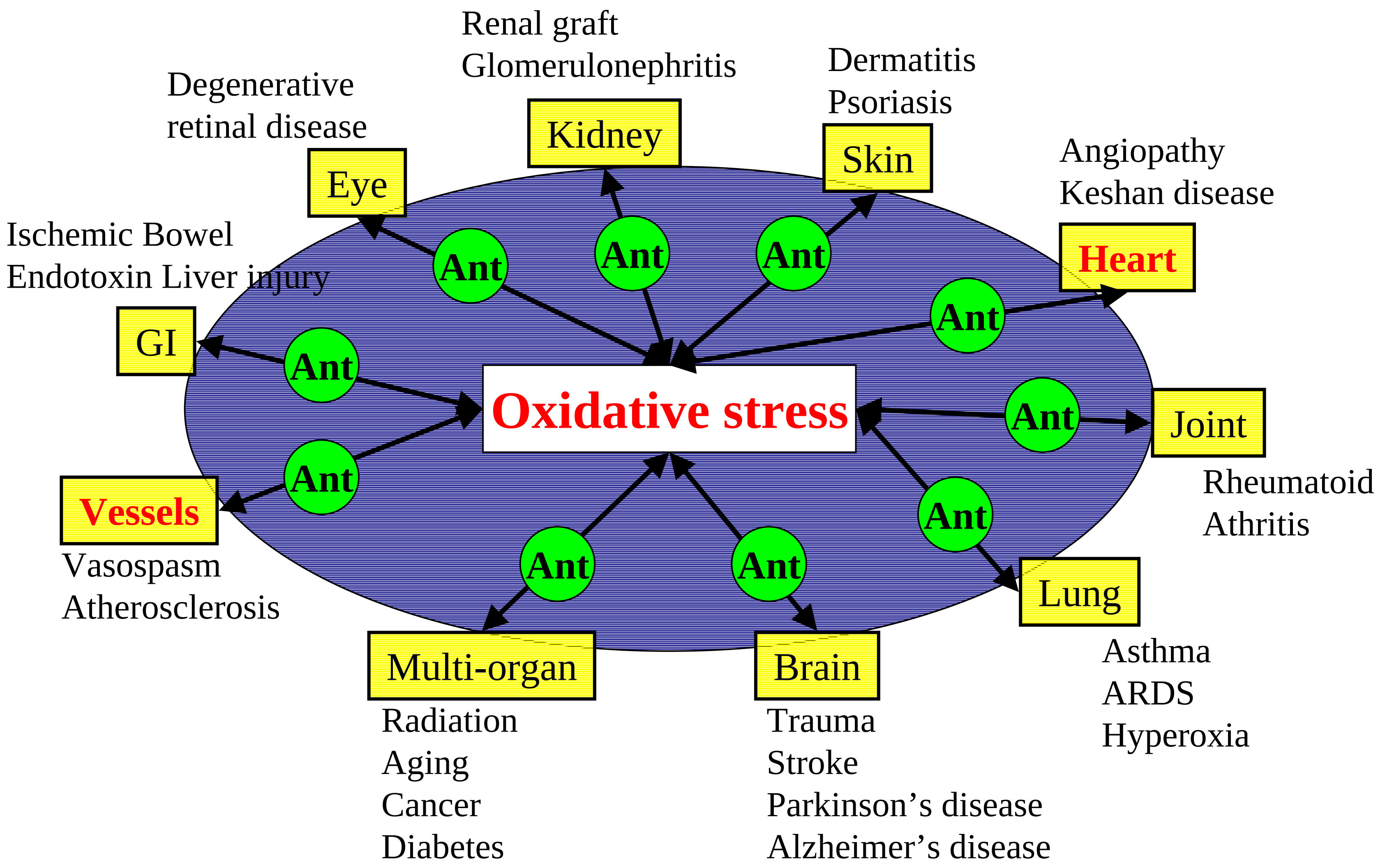
Today's presentation

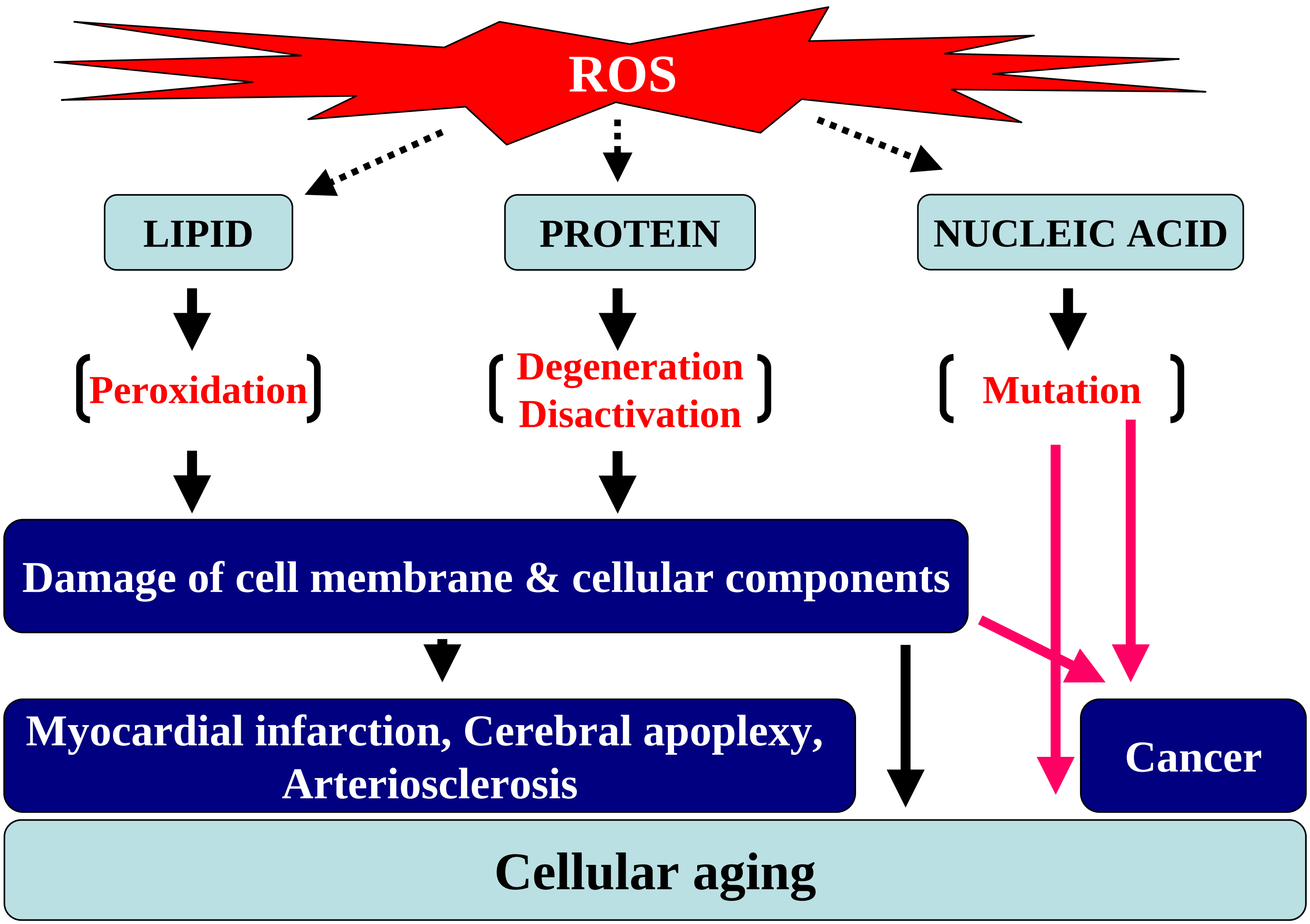


Oxidative Stress

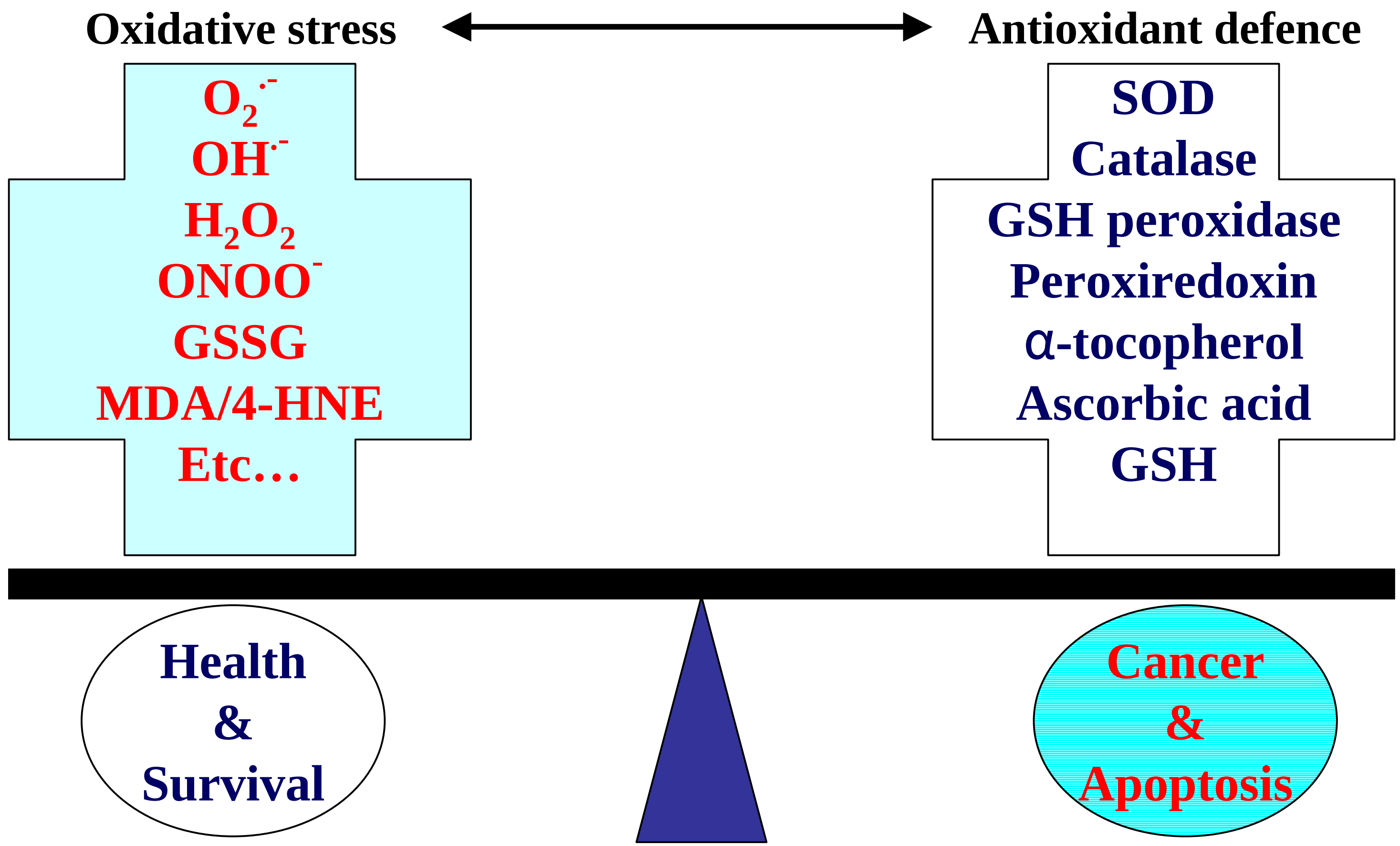
- **The O_2 is major promoters of radical reaction.**
- **Oxidative stress is imposed on cells as a result of one of three factors:**

  1) an increase in oxidant generation,
  2) a decrease in antioxidant protection,
  3) a failure to repair oxidative damage.

Cell & tissue damage by ROS

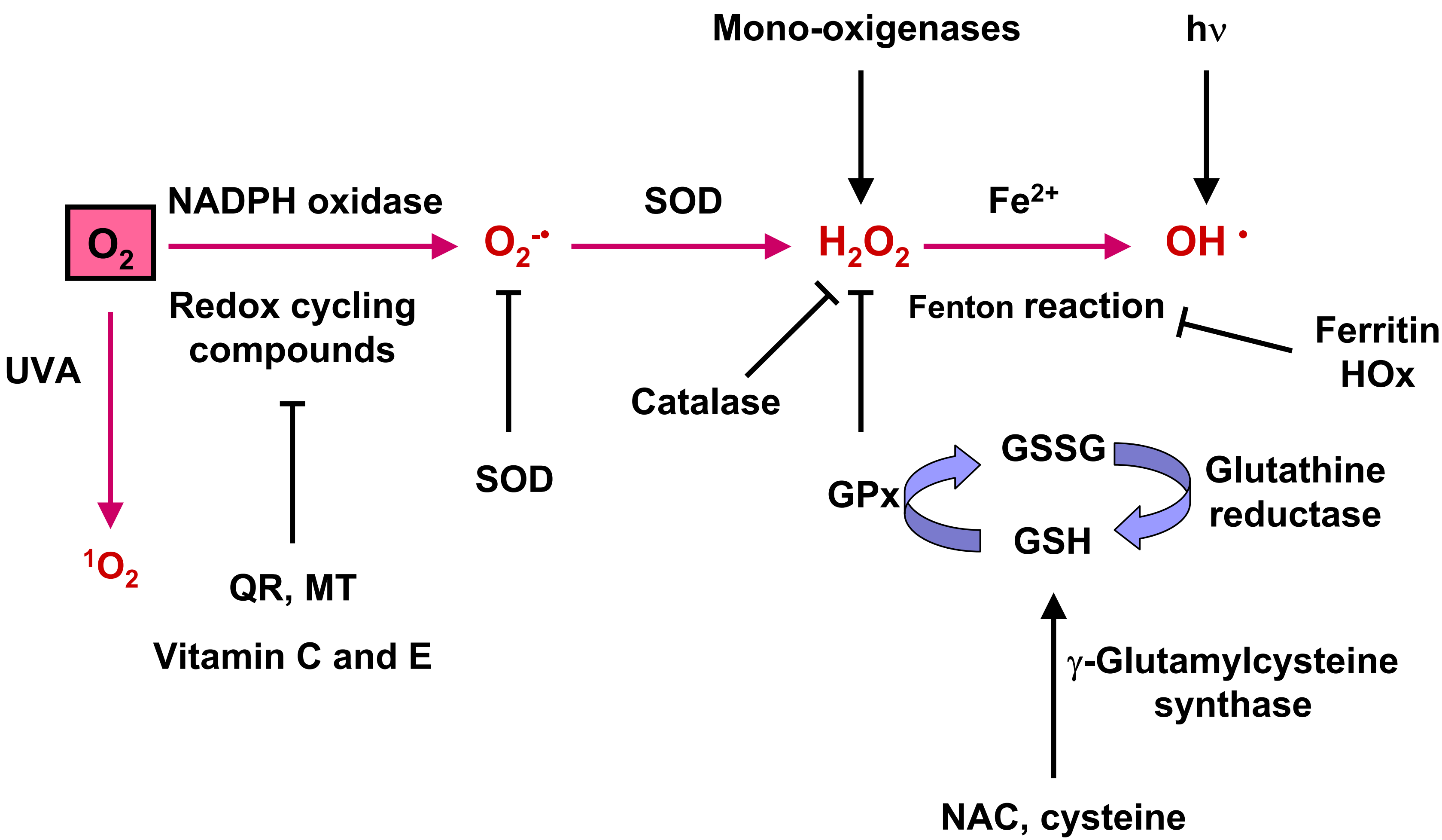


Redox balance



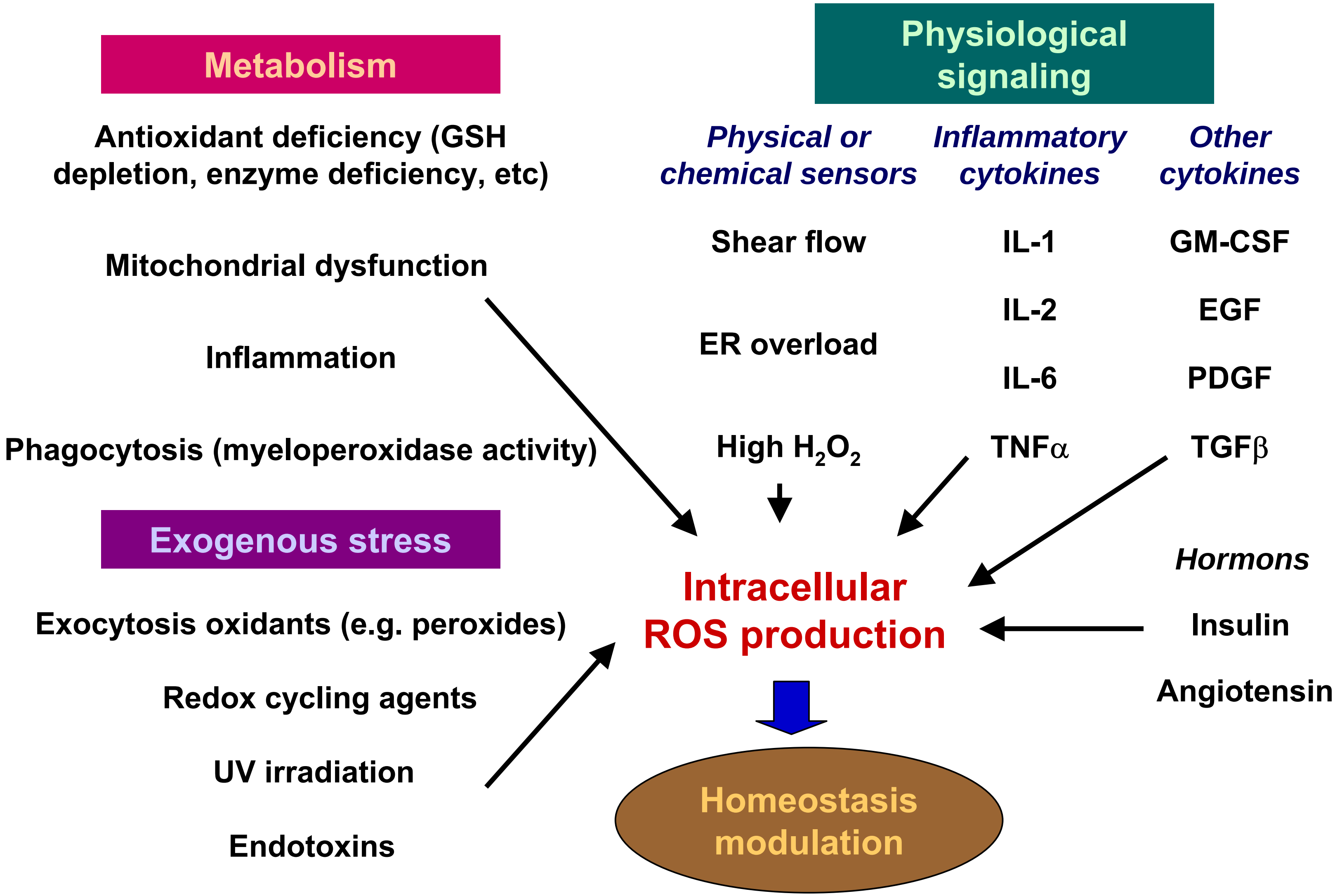
European Journal of Biochemistry 267 (16): 4904.

ROS generation and detoxification



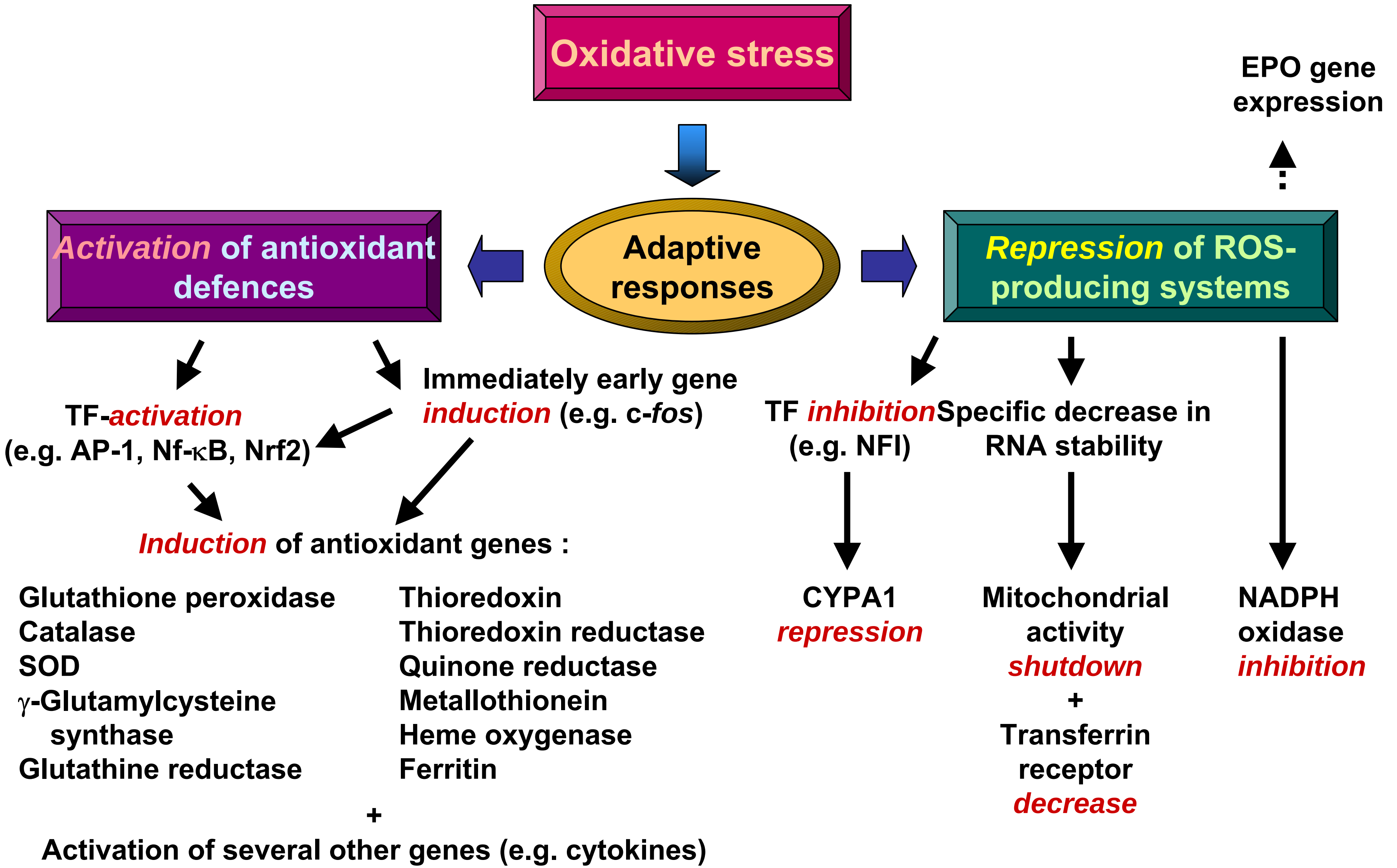
Biochem. J. (1999) 342, 481.

Various factors elicit intracellular ROS production



Biochem. J. (1999) 342, 481.

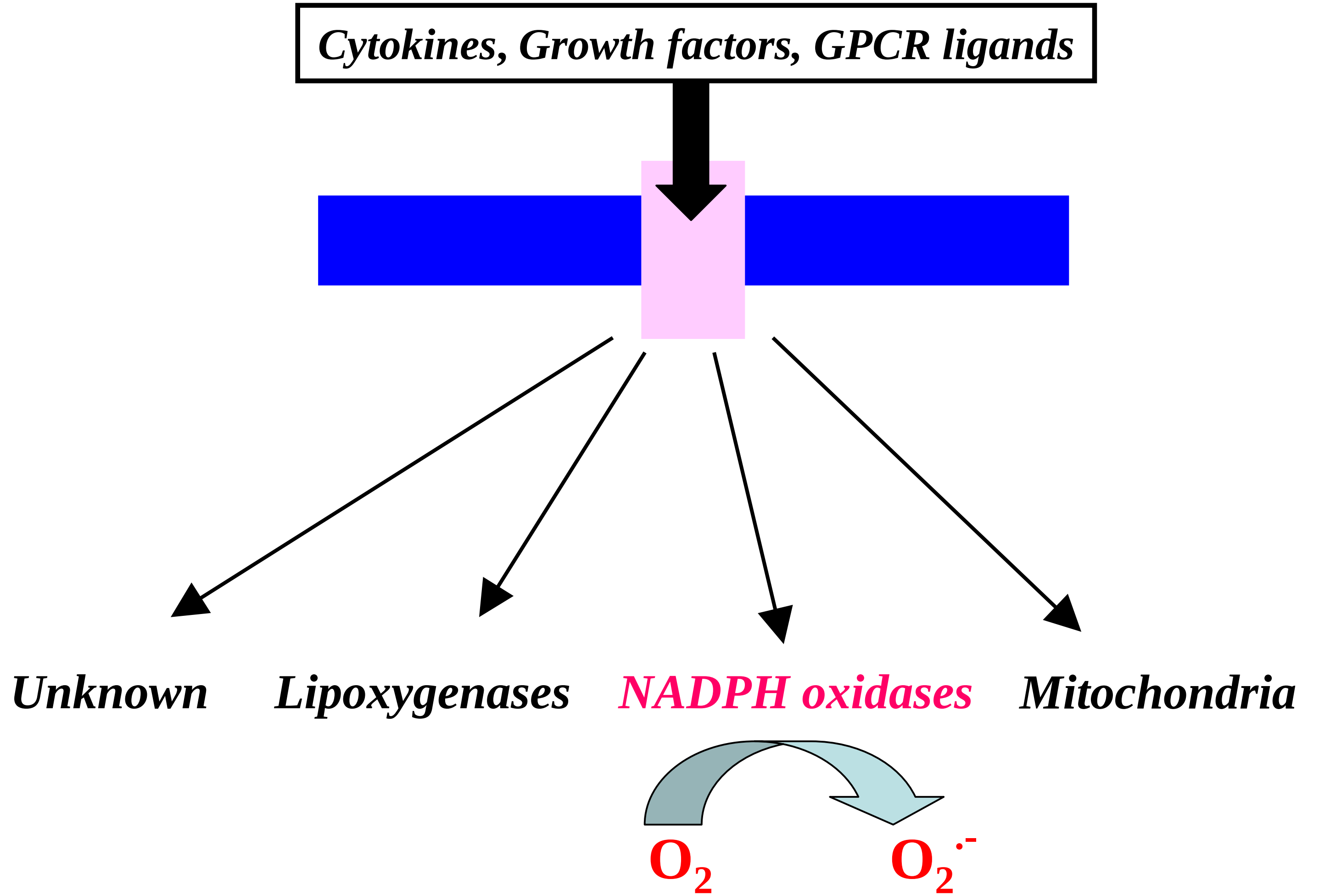
Adaptive response to oxidative stress



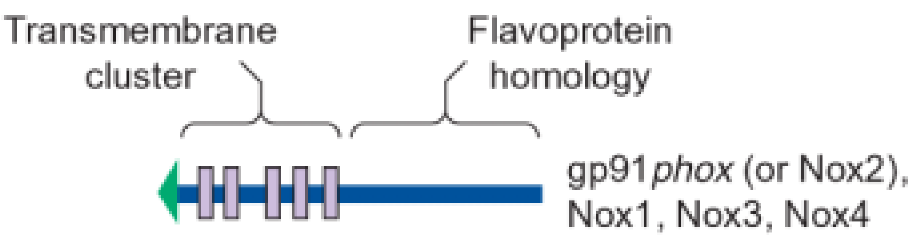
Biochem. J. (1999) 342, 481.

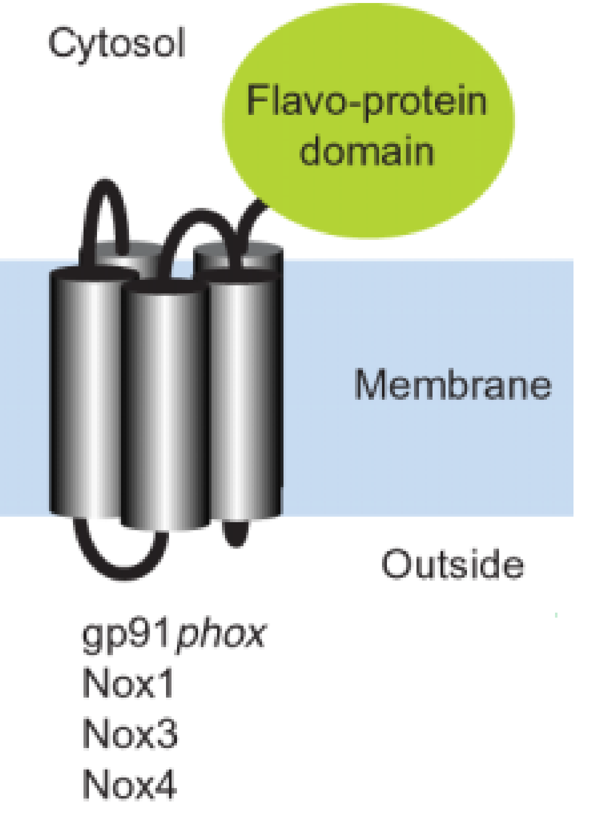
Reactive oxygen species

ROS generation

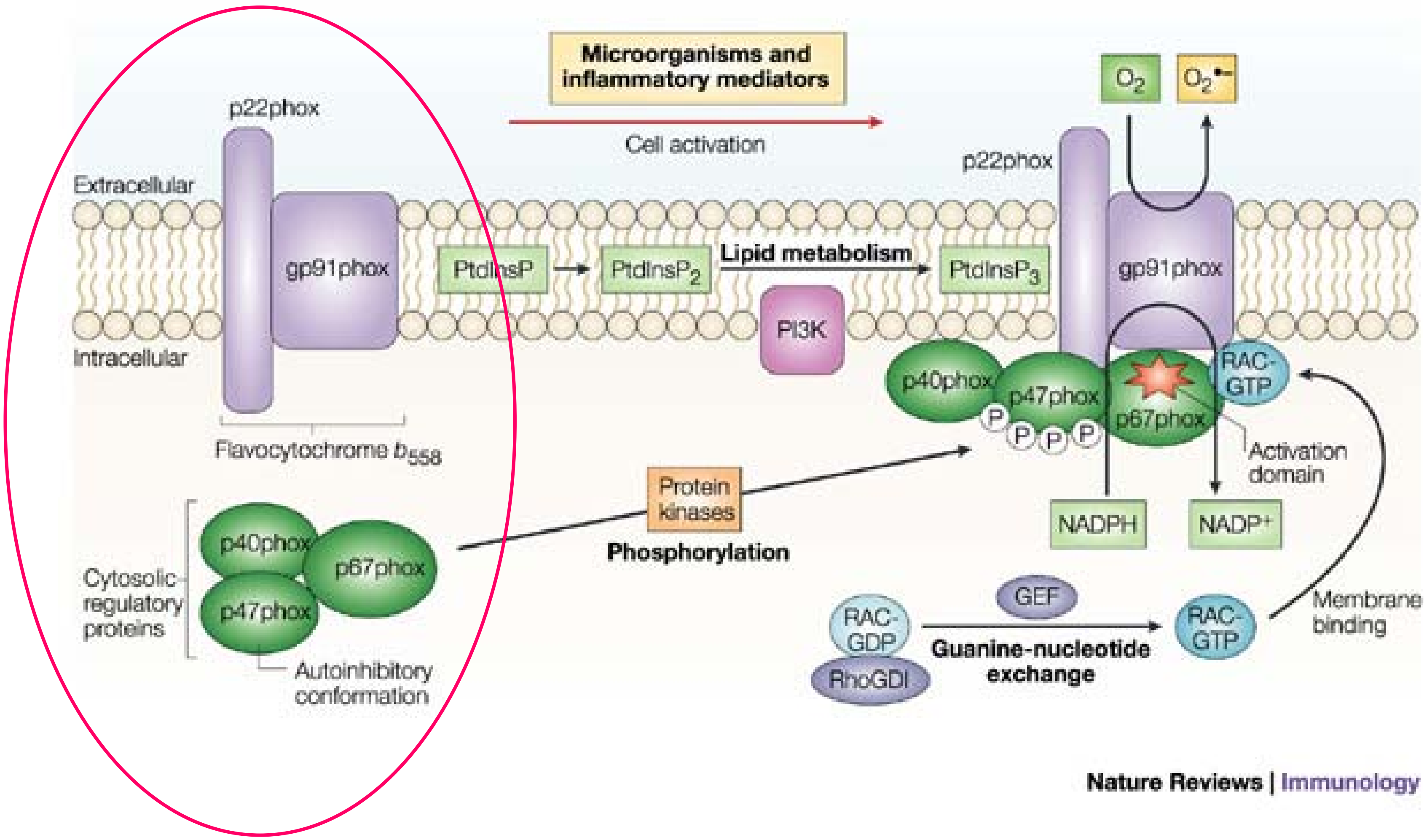


Topology of Nox enzymes

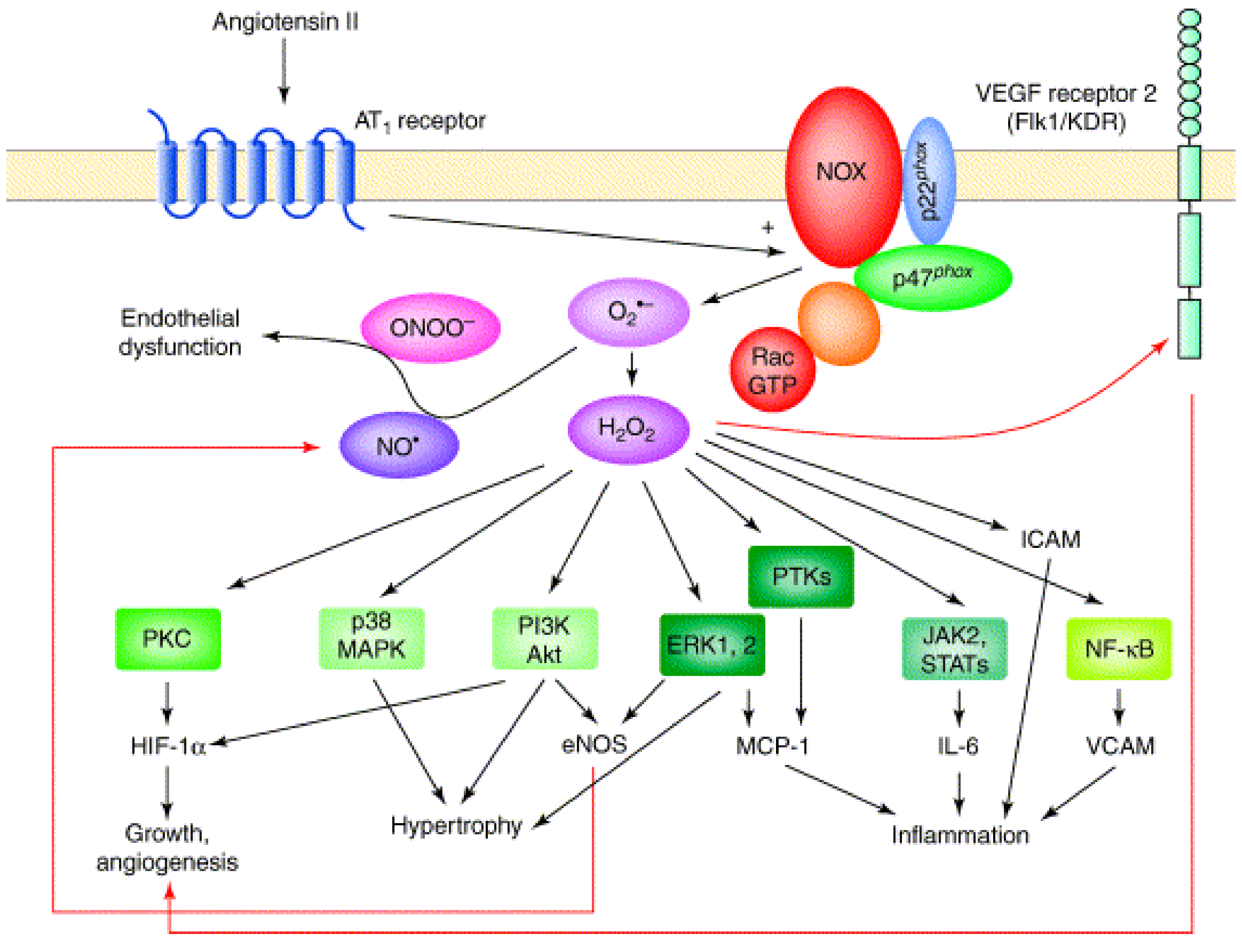




Nox activation signal



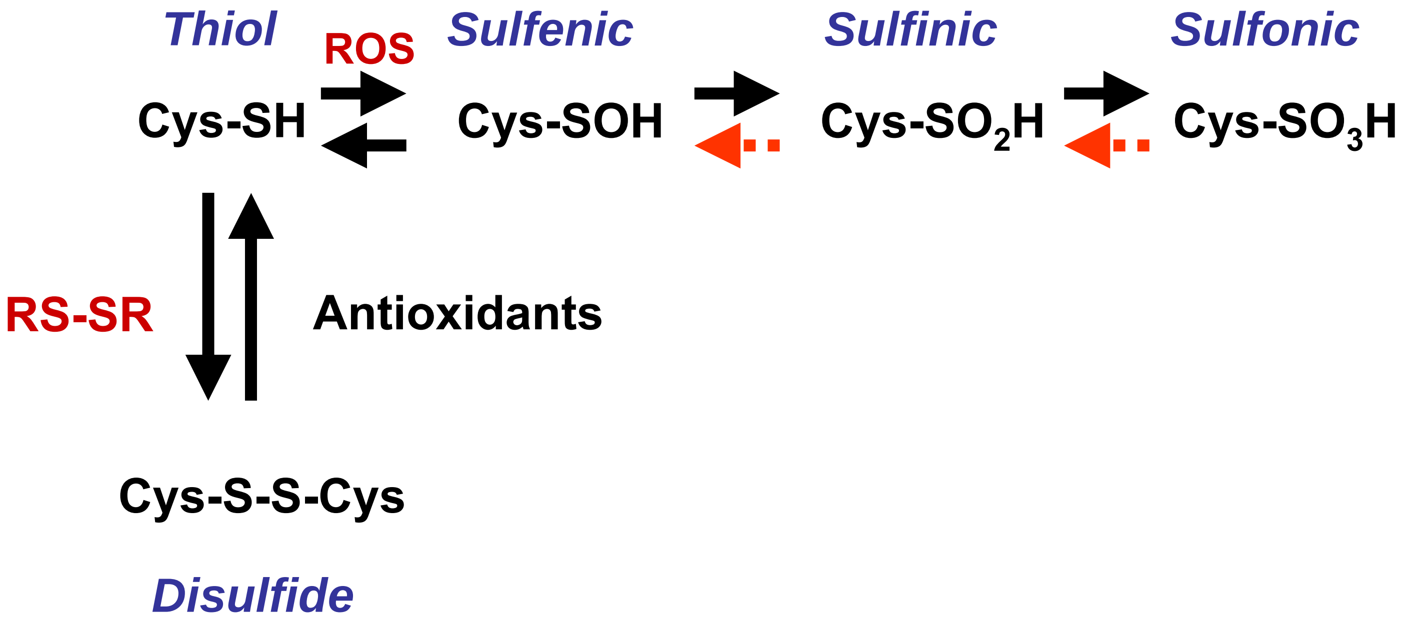
Nox-dependent signalings in vascular cells



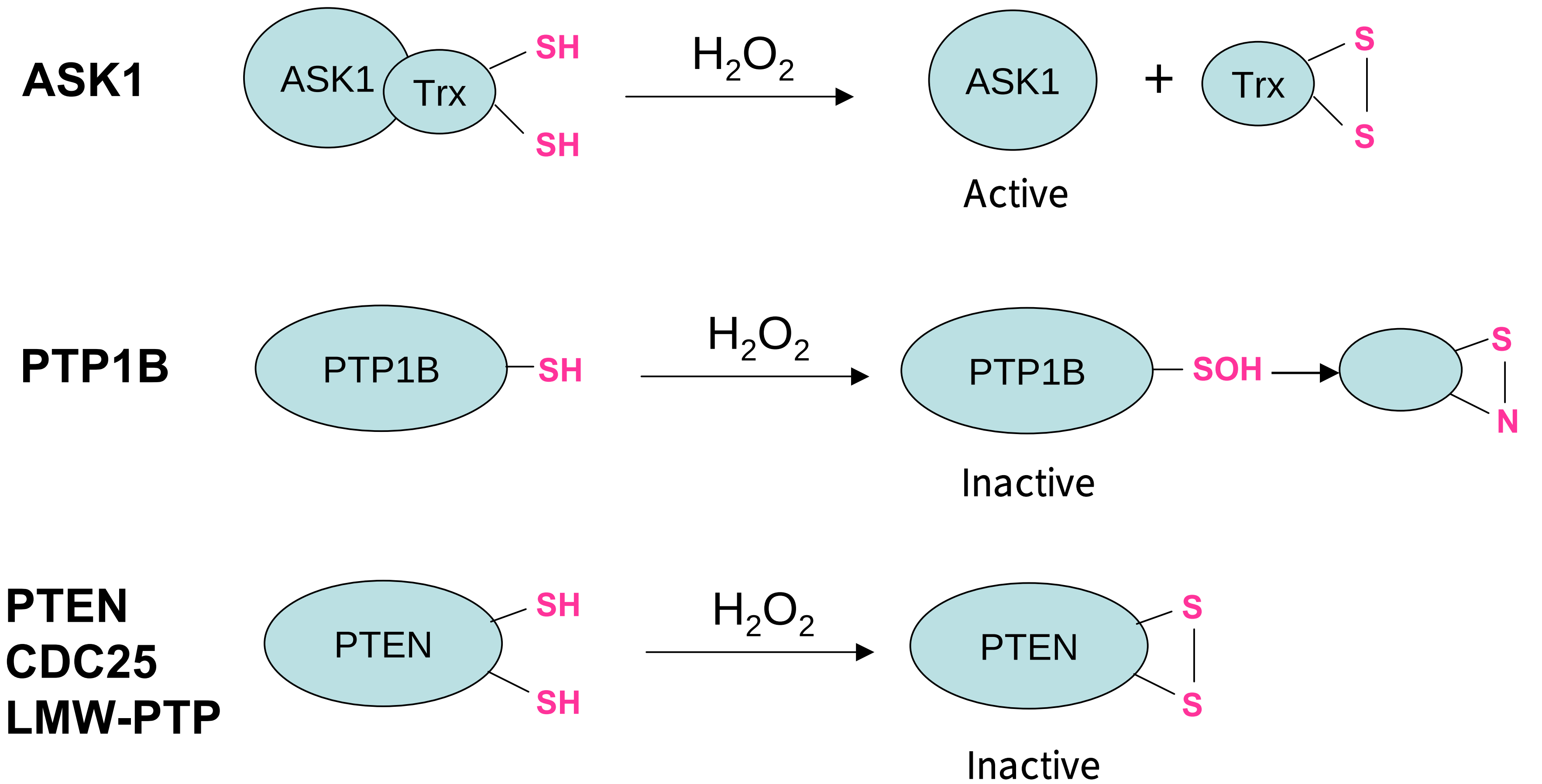
TRENDS in Pharmacological Sciences

ROS targets: Requirement

- It must have a low pKa and so highly reactive cysteine residue.
- A cysteine residue should be able to be oxidized easily by either air or H_2O_2, which in turn causes a conformational change or an activity loss.



Ways that ROS work



ROS

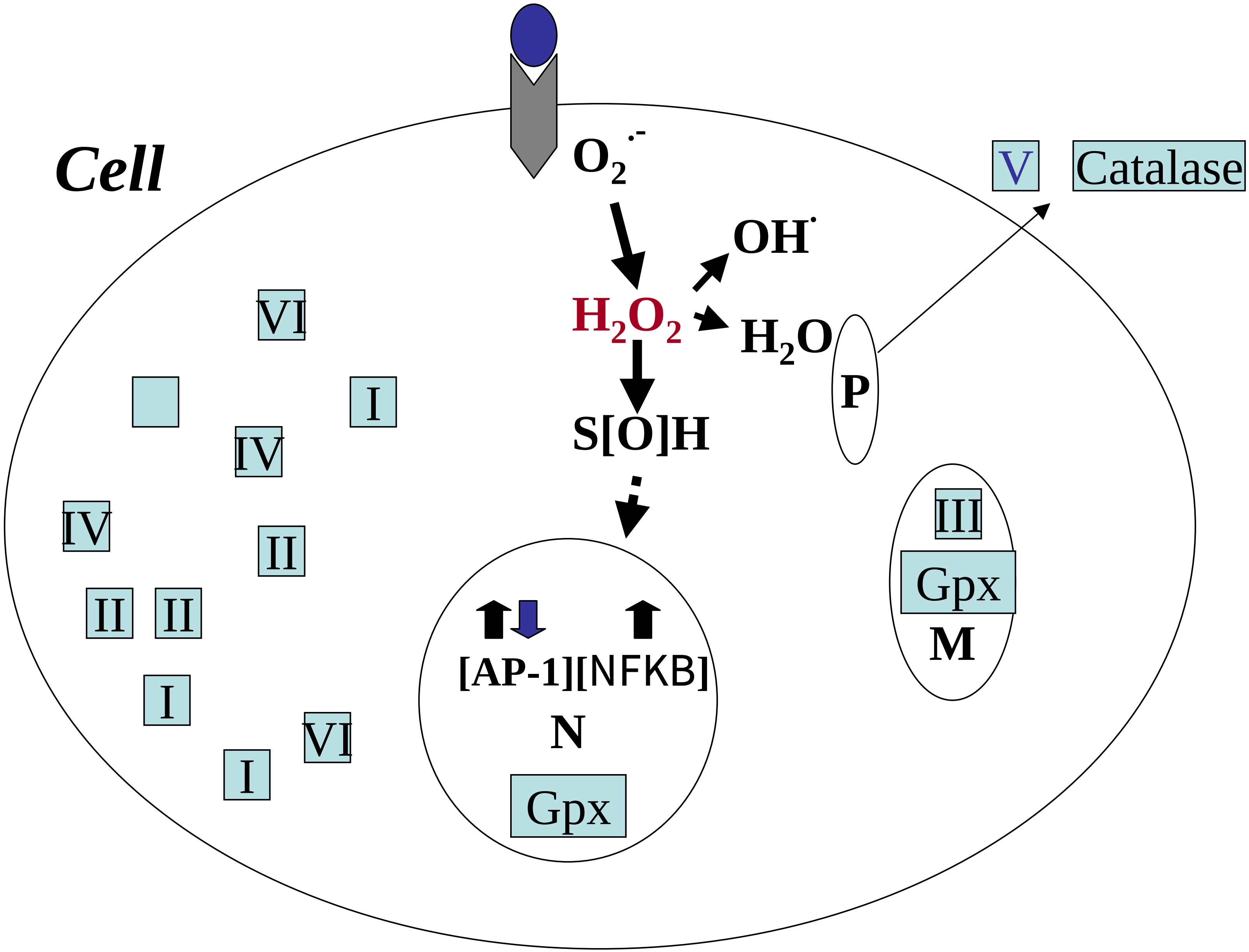
2nd messenger	**Oxidative stress**
Well-controlled	Uncontrollable
Receptor-mediated	External sources
Intracellular targets	Non-specific (carpet bombing)
Rapidly eliminated	Usually followed by oxidative burst
Localized action	Diffusible

ROS elimination

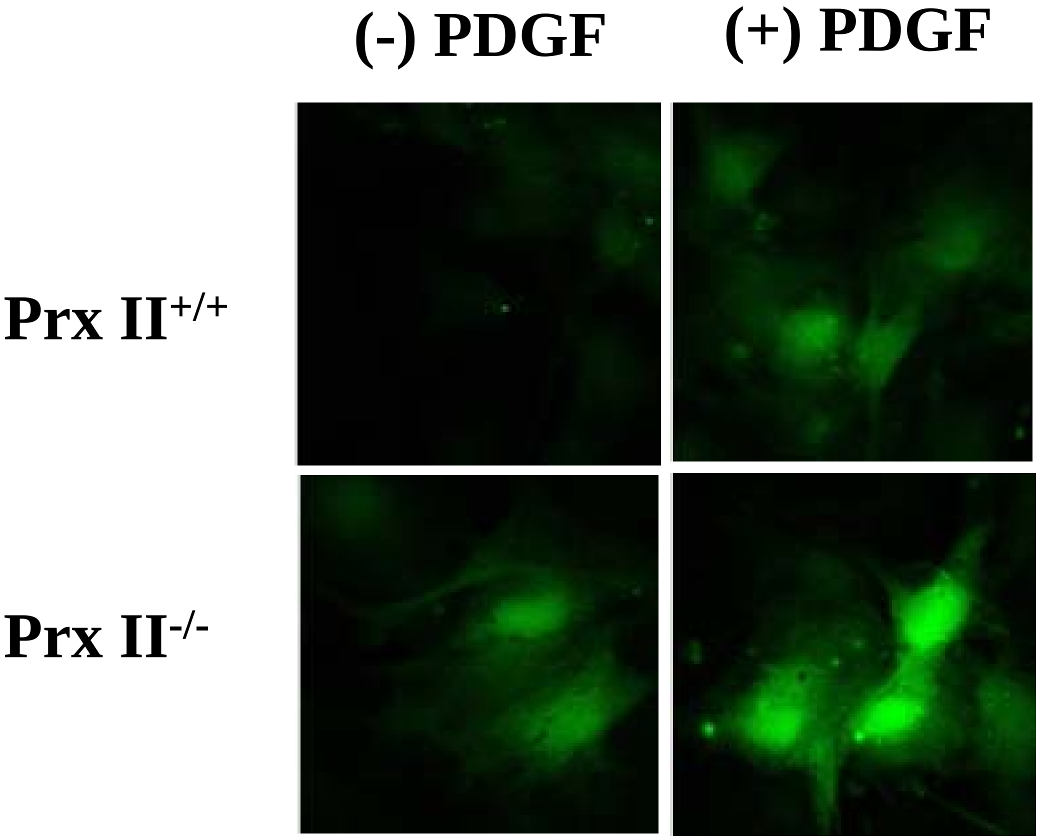
Table 1. ROS scavenging and detoxifying enzymes

Enzyme	Reaction catalysed
Superoxide dismutase	$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$
Glutathione peroxidase	$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$ $LOOH + 2GSH \rightarrow GSSG + H_2O + LOH$
Glutathione S-transferase	$RX + GSH \rightarrow RSG + HX^*$
Glutathione reductase	NADPH +GSSG □ $NADP^+$+2GSH
Thioredoxin	$Trx\text{-}(SH)_2$ +Protein-S_2 □ Trx-S_2 +Protein-$(SH)_2$
Cytochrome c peroxidase	complex+2cyt-c(Fe^{2+}) $\rightarrow$ enzyme+2cyt-c(Fe^{2+}) +2OH^-

Peroxiredoxin



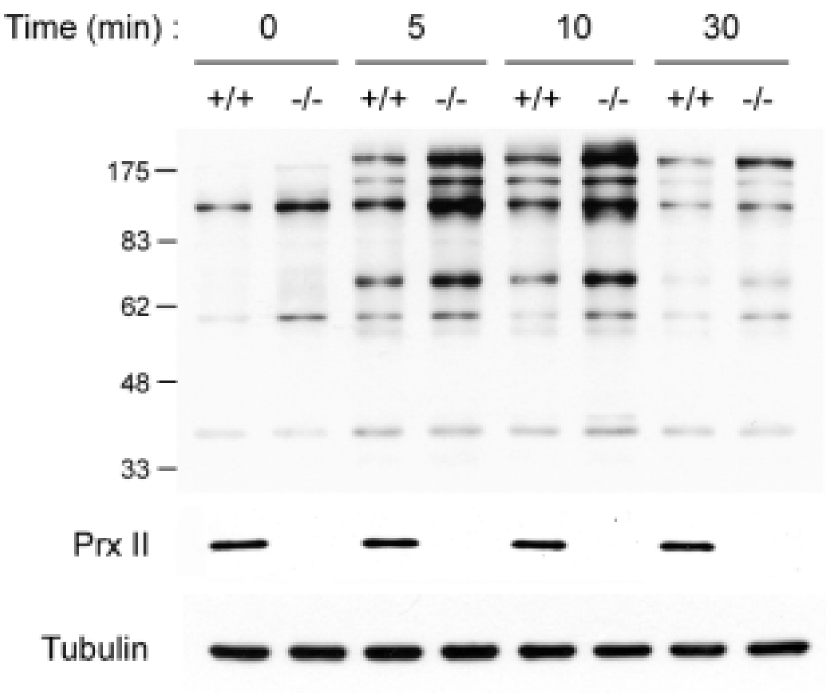
PDGF-induced H_2O_2 production in MEFs



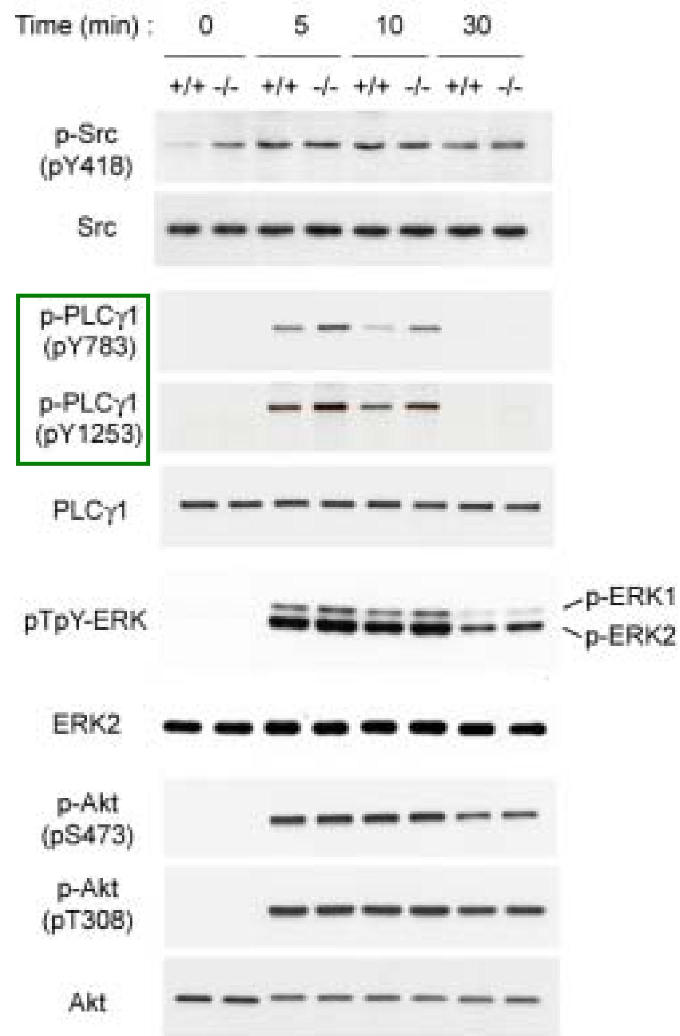
(Kang et al., in Ehwa Univ.)

Specific amplification of PLCγ1 activation

Total phosphotyrosine

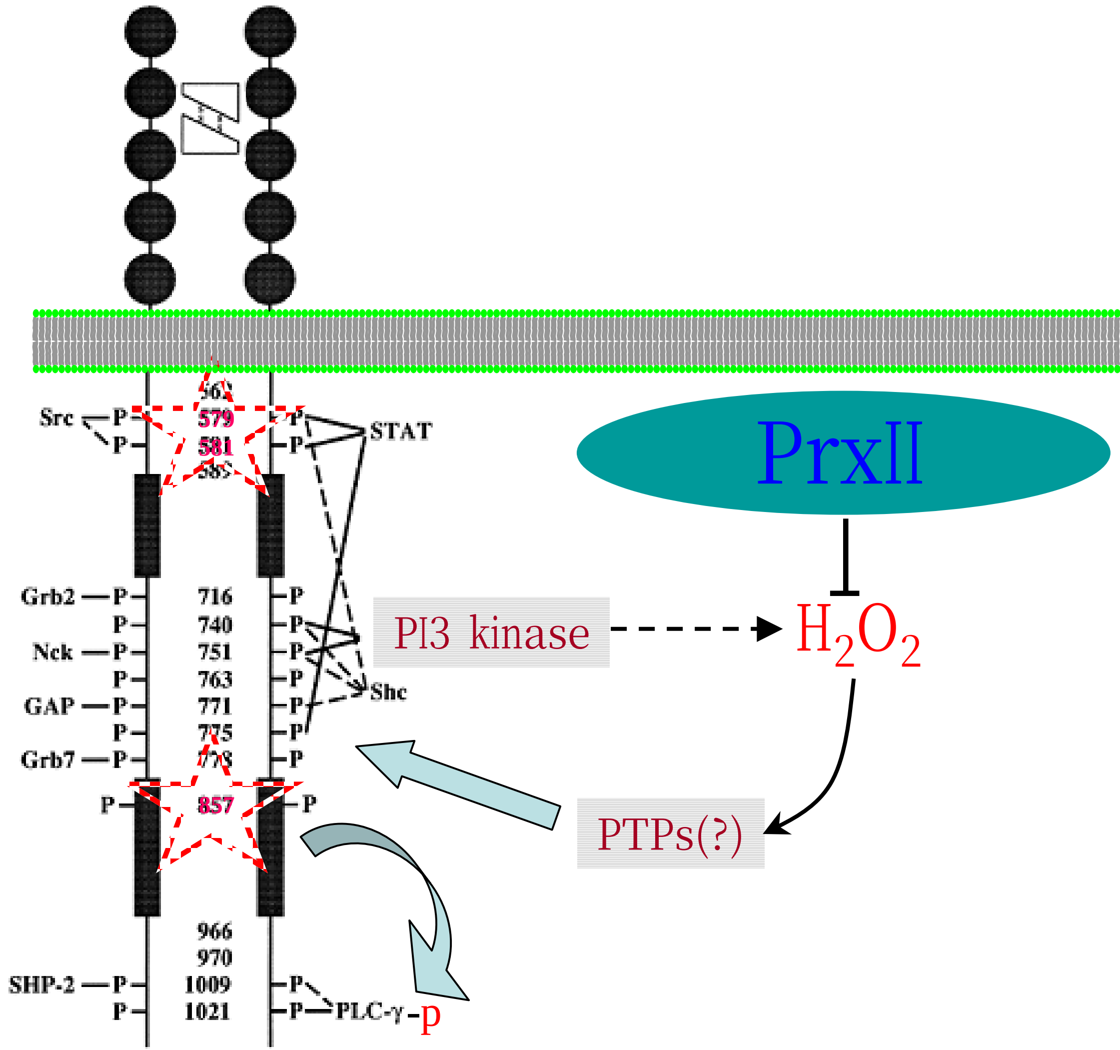


Individual receptor-interacting proteins



(Kang et al., in Ehwa Univ.)

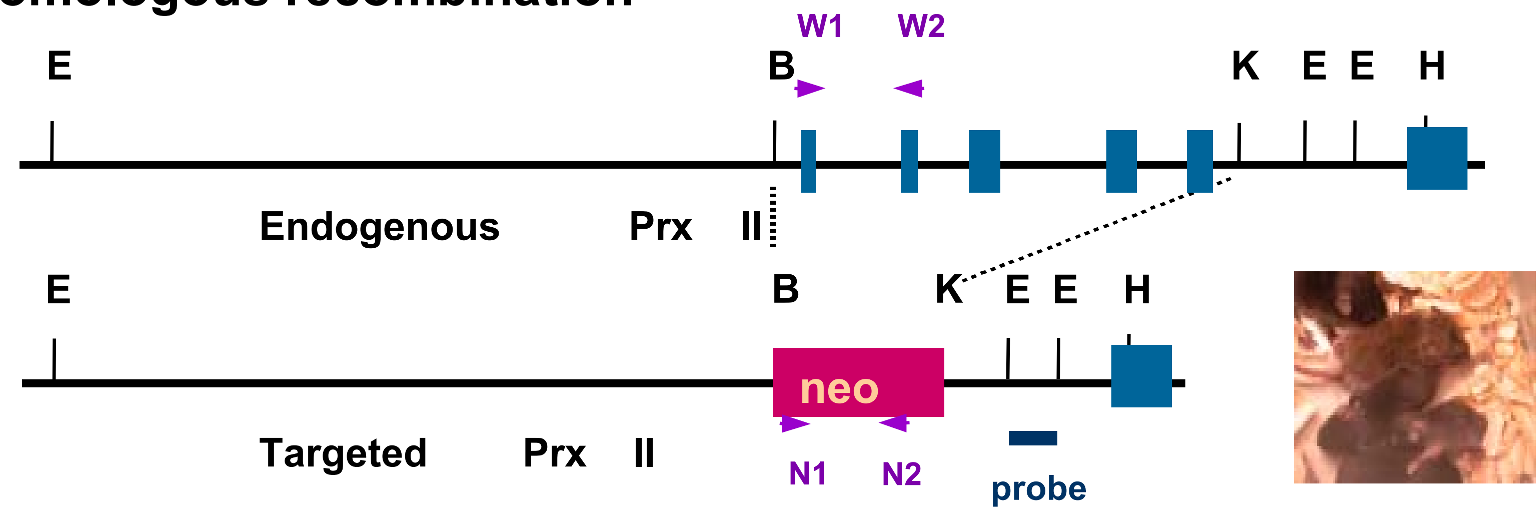
Prx II determines amplitude of PDGFRβ-PLCγ1 activation pathway



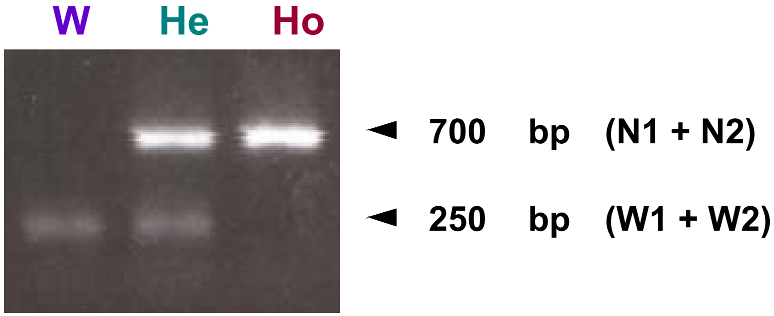
The identification of ROS target molecules in PrxII-deficient mice

Generation of PrxII deficient mice

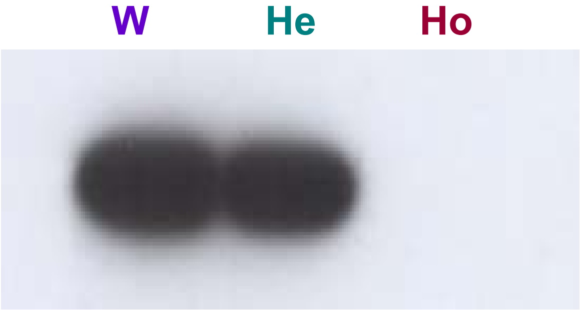
A Homologous recombination



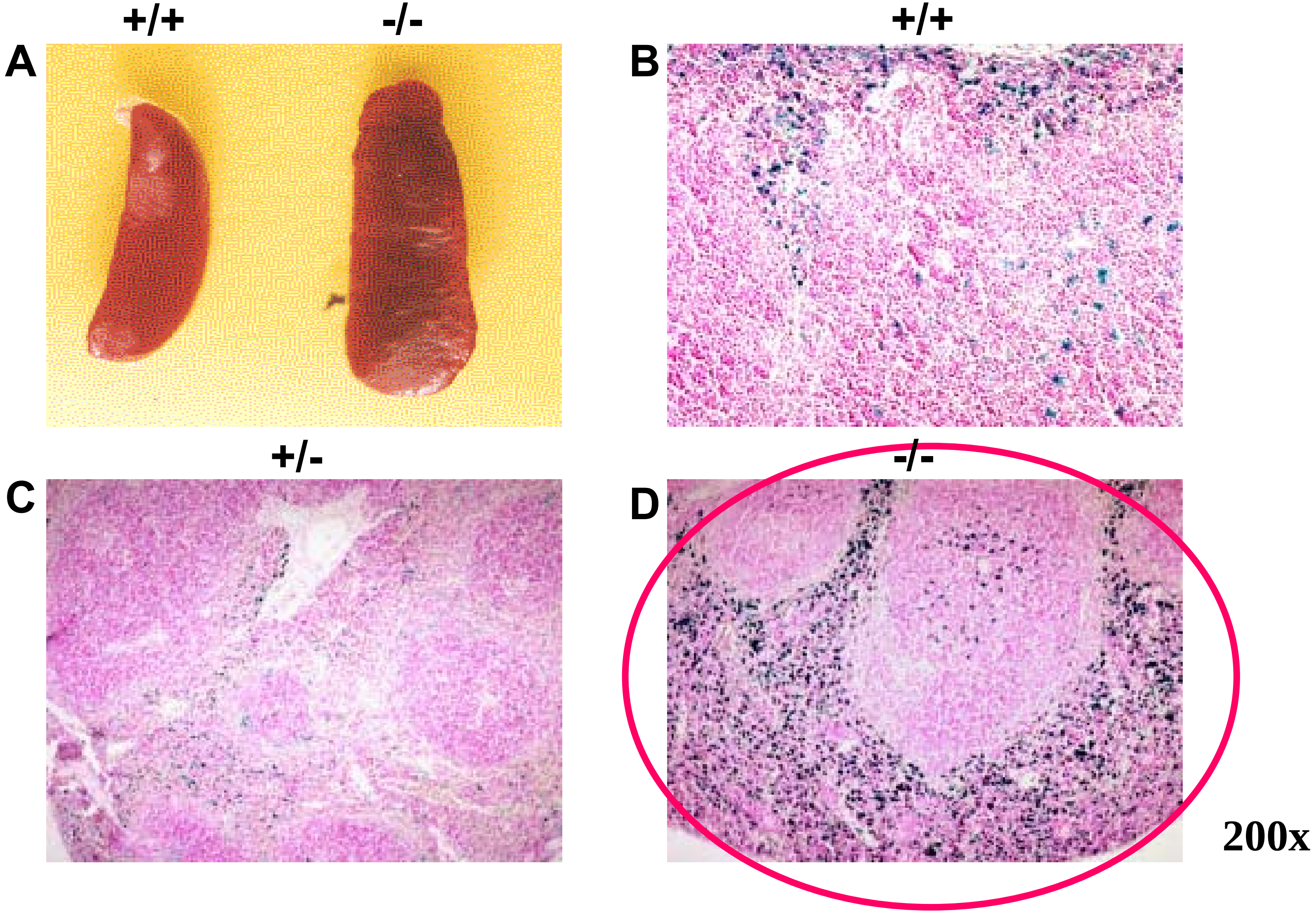
B PCR analysis



C Western analysis

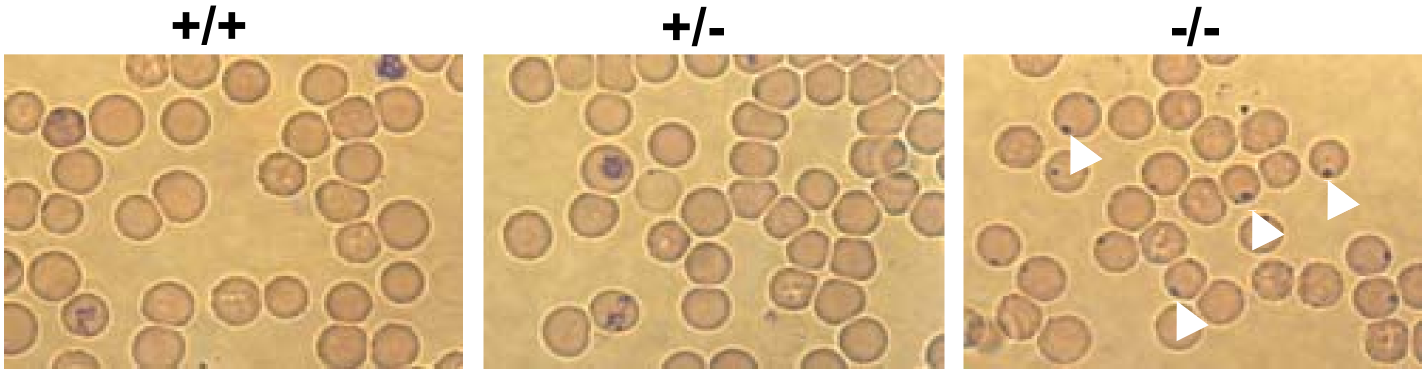


Chronic hypertrophy in spleen



Heinz body formation in red blood cells

A



B

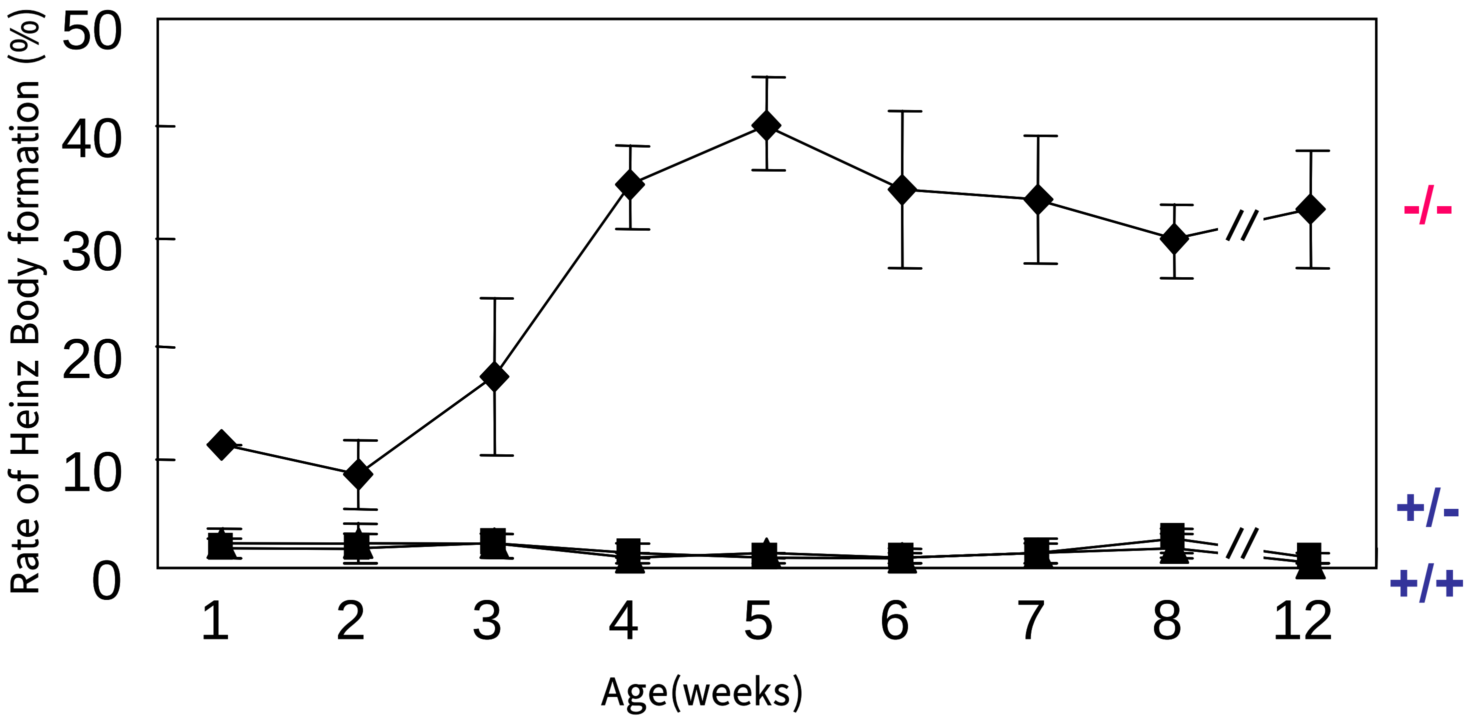
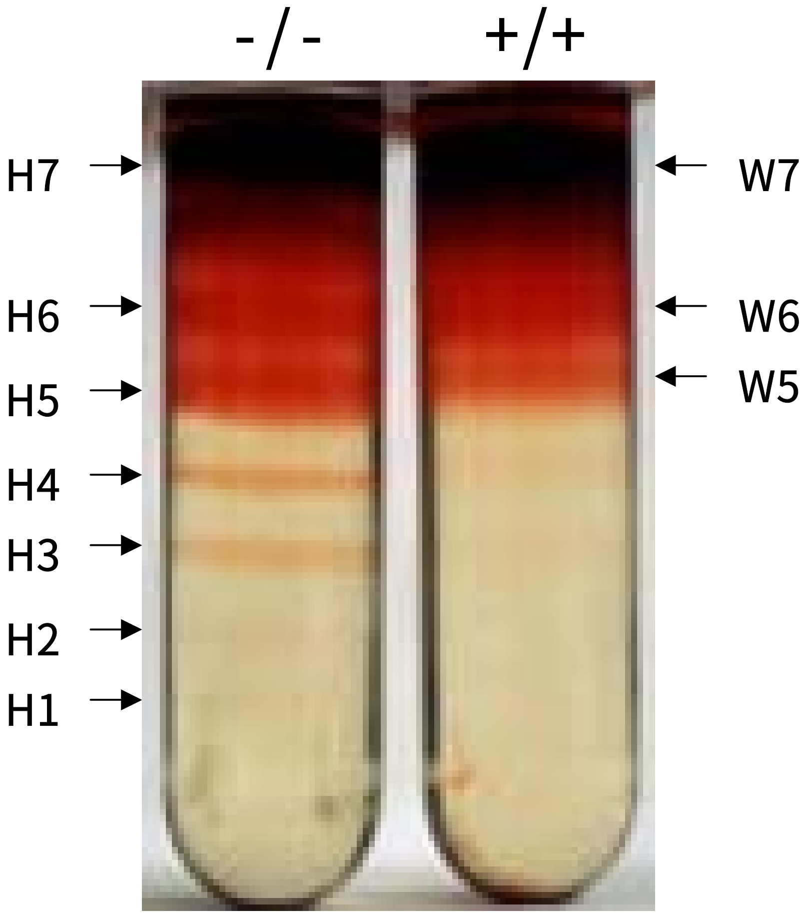


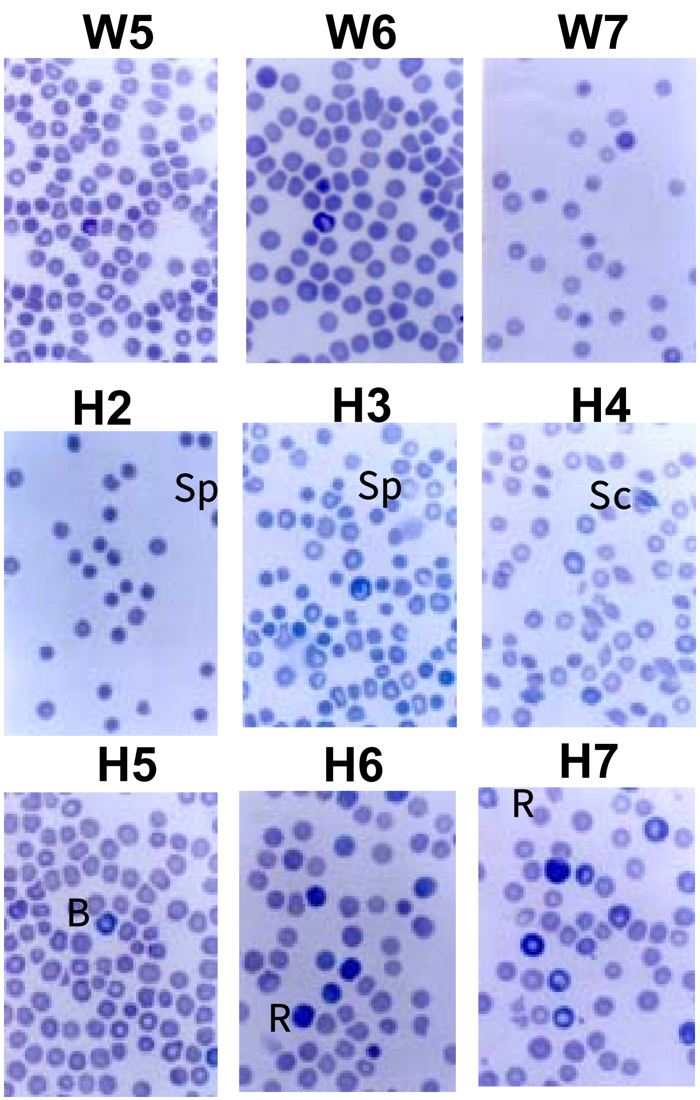
Table 1. Hematology and cation contents of PrxII knock-out mice

	+/+ (n=14)	-/- (n=18)
Hematology		
WBC ($10^3/\mu l$)	5.1±2.5	5.7±2.9
RBC ($10^6/\mu l$)	**10.4±0.6**	**9.3±0.9**
Hemoglobin (g/dl) $^{+}$	16.5±0.9	14.7±1.5
Hematocrit(%) $^{+}$	54.4±3.1	48.8±5.8
Reticulocyte(%) $^{++}$	**1.8±0.7**	**3.5±1.0**
MCV(fL)	52.0±2.6	52.2±2.4
Spleen weight(SW/BW)	0.002±0.0002	0.003±0.0003

+, $P<0.05$; ++, $P<0.0001$

RBC abnormality in PrxII$^{-/-}$ mice



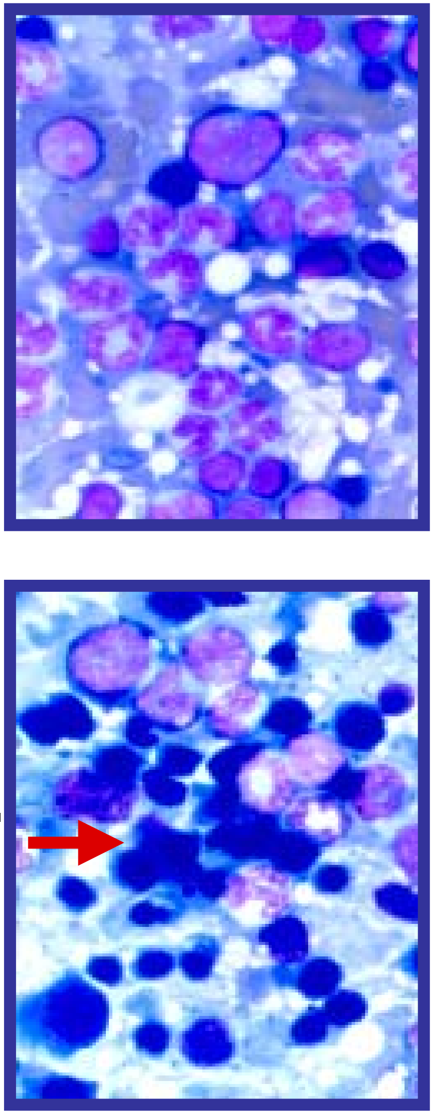


Myeloid Cell Comparison in Bone Marrow

A

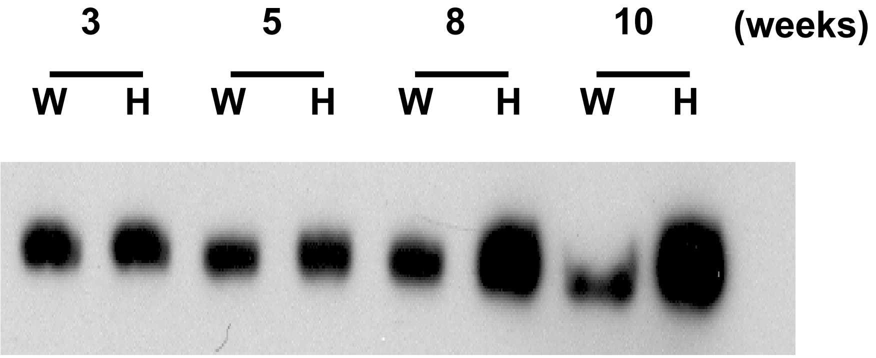
Wild (+/+)

Homo. (-/-)

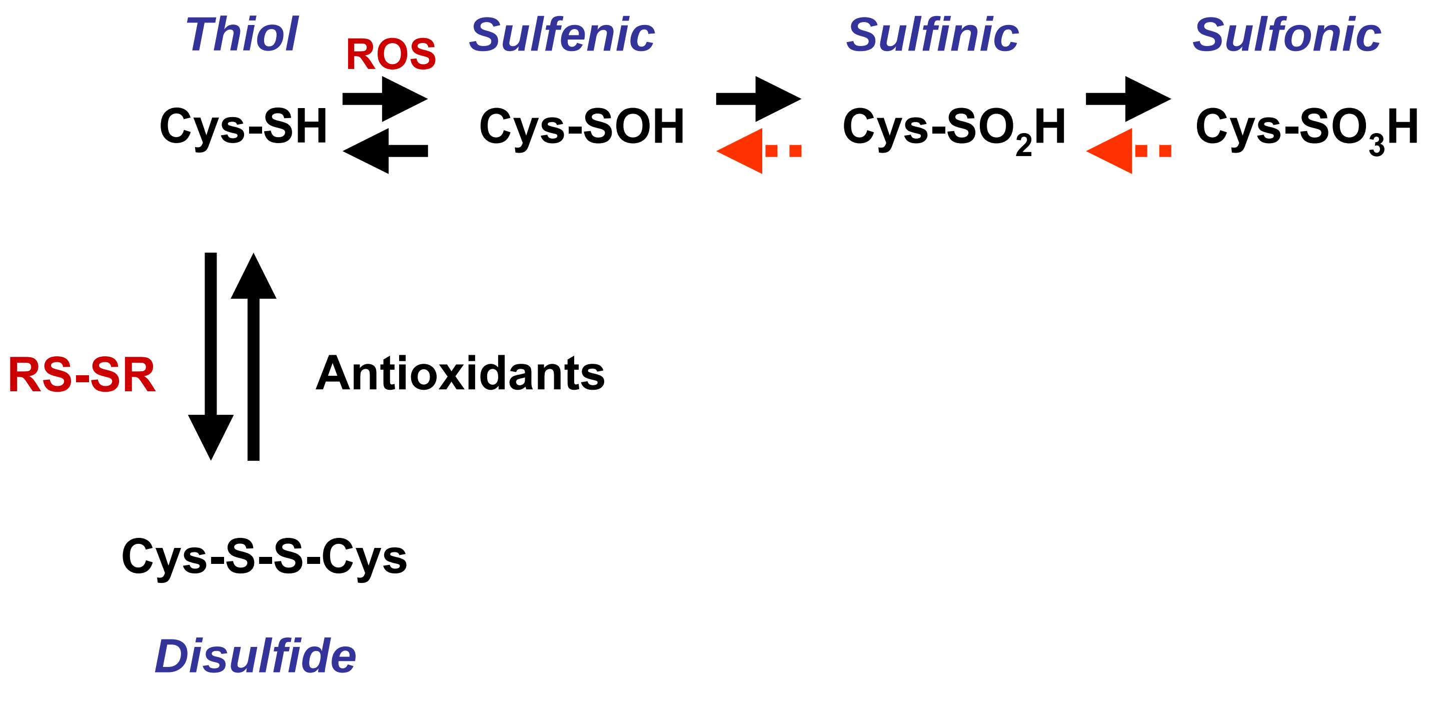


B

EPO level in serum



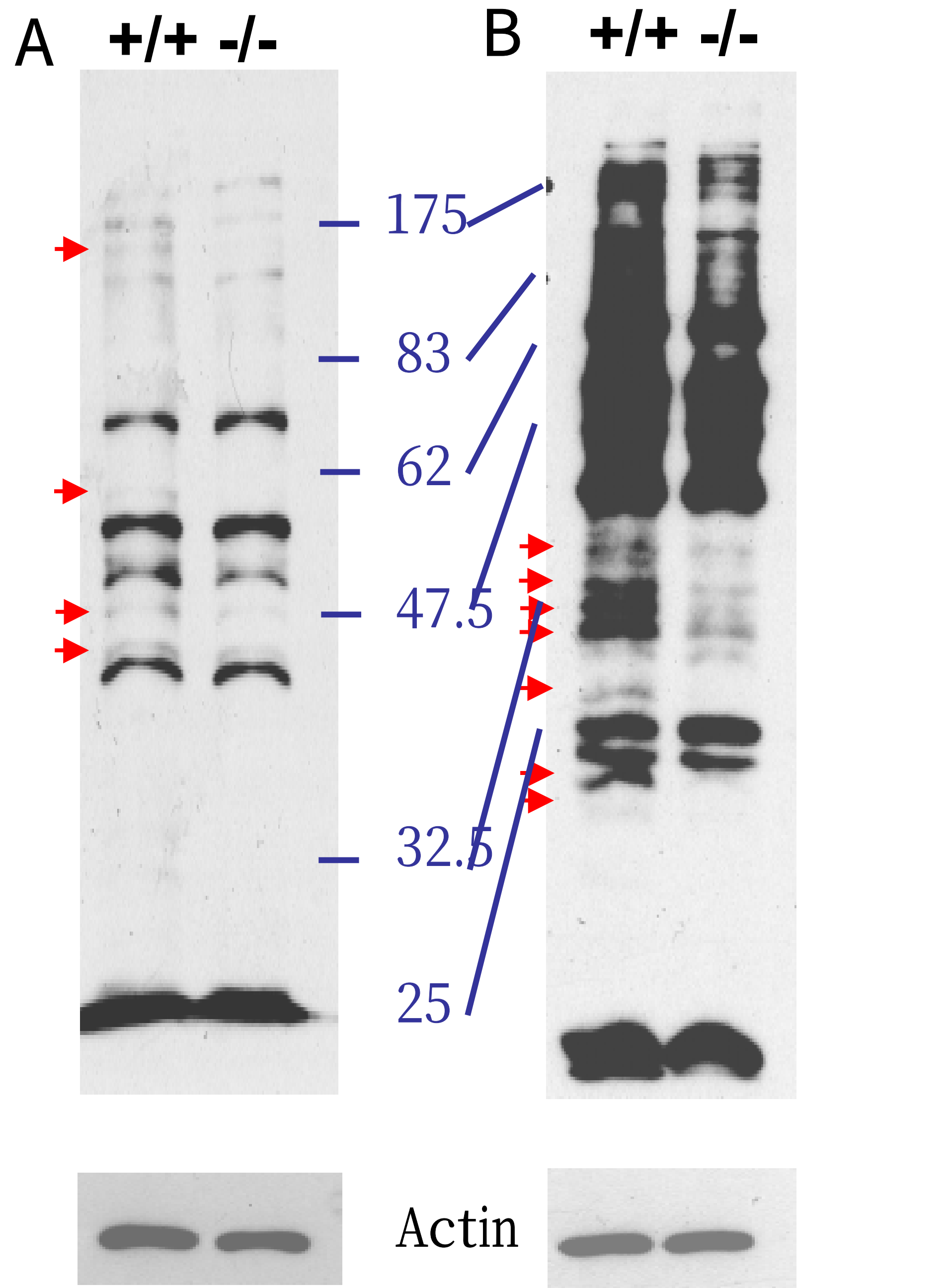
Protein target validation in PrxII KO mice



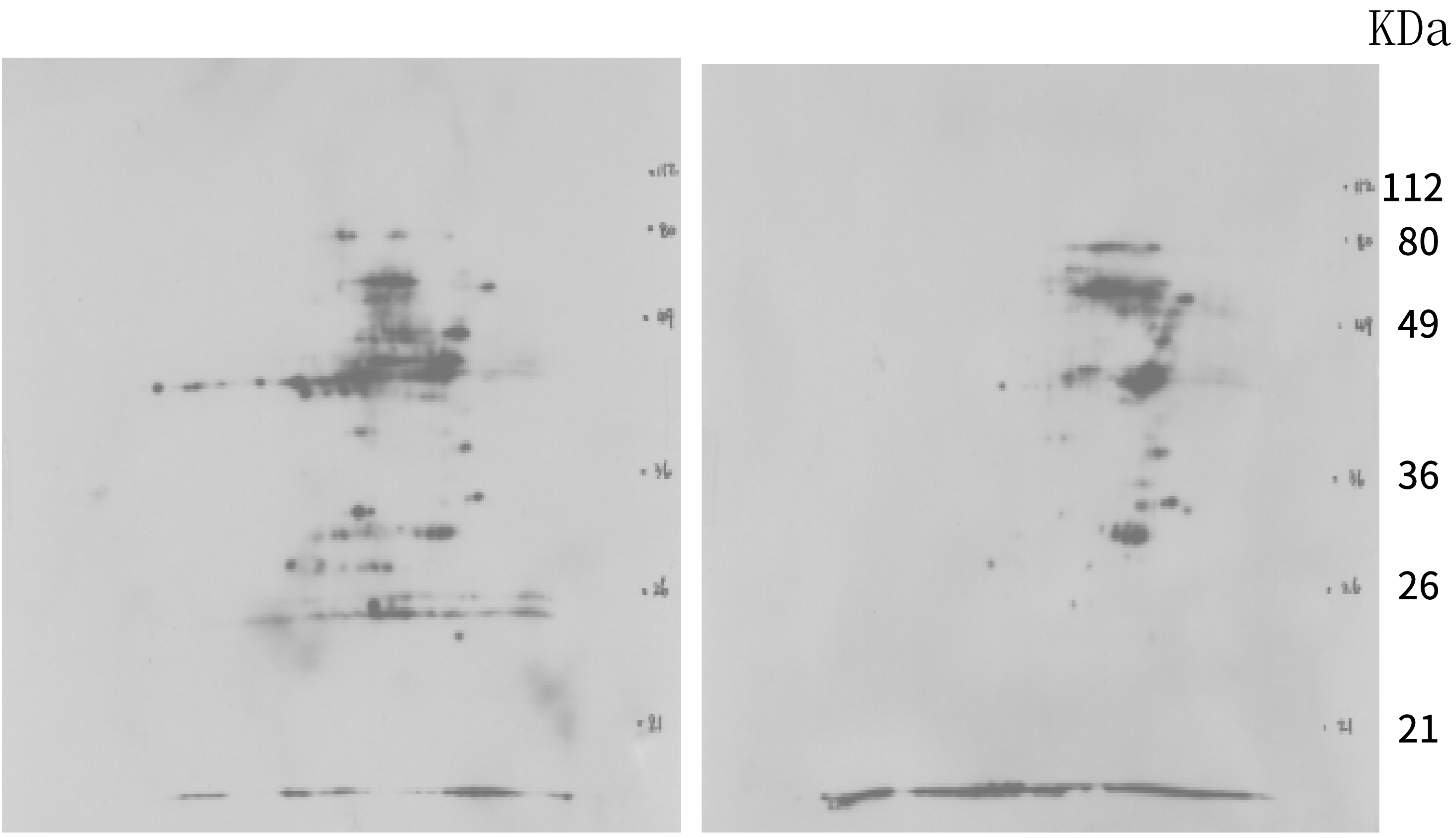
Detecting membrane proteins that contain sensitive cysteine residues to oxidative stress.

(A) The BIAM indicates a biotin labeled iodoactic amide. The BIAM was decreased in PrxII deficient RBC.

(B) The ^{14}C-IAM also showed that oxidized proteins were increased in RBC of PrxII-deficient mice.

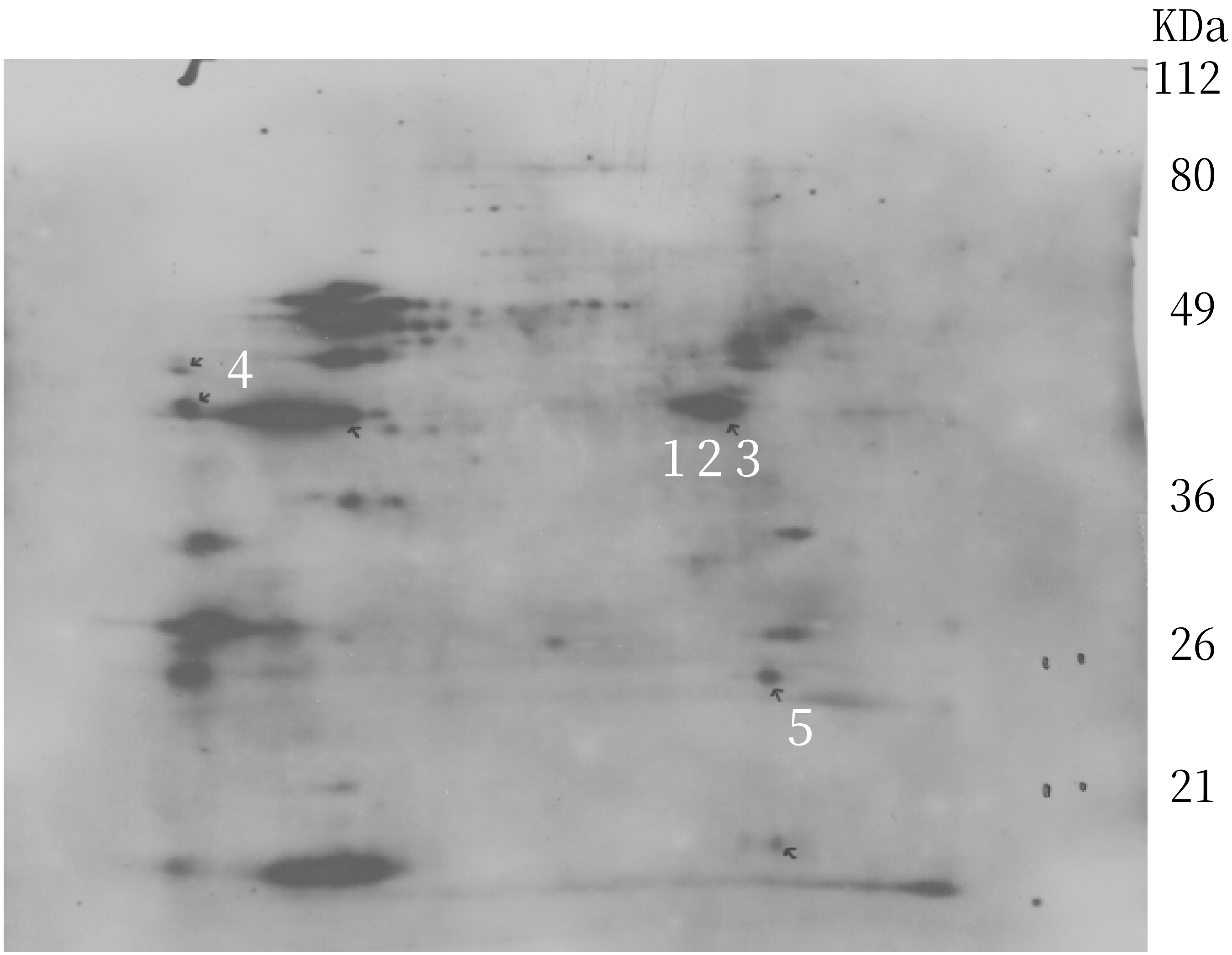


Comparison of oxidized proteins



+/+ -/-

Selection of oxidized proteins



PI 10 to 3

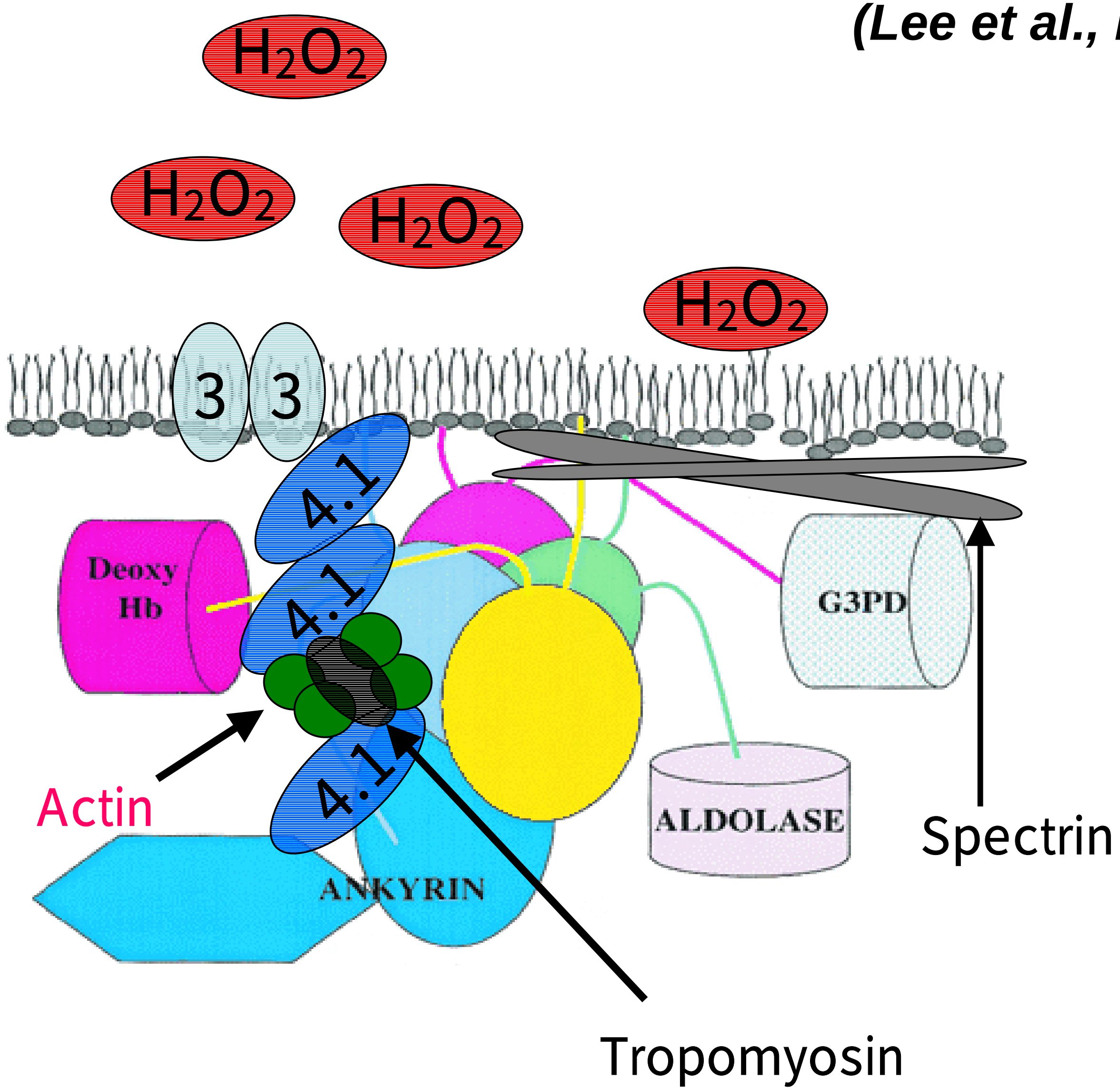
Identification of oxidized proteins

Number	Approximate quantity*	Approximate size (KDa)	The reason that the spot is selected	
1	5	45	Decreased IAB labelling (WT > HO)	actin
2	5	45	Decreased IAB labelling (WT > HO)	actin
3	5	45	Decreased IAB labelling (WT > HO)	actin
4	2	50	Decreased IAB labelling (WT > HO)	EF
5	1	25	Decreased IAB labelling (WT > HO)	GST P

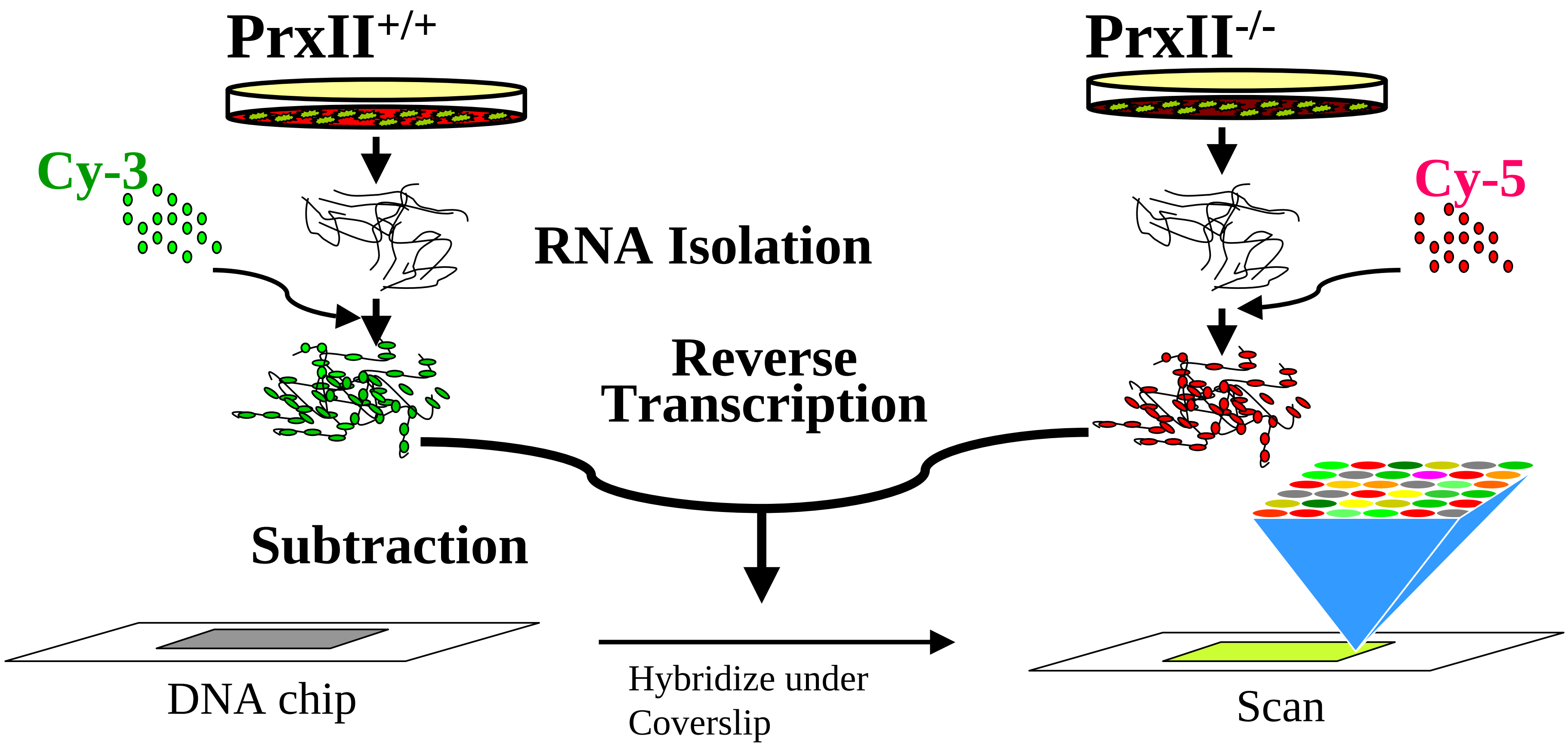
***1 100 ng**

Cytoskeletal network

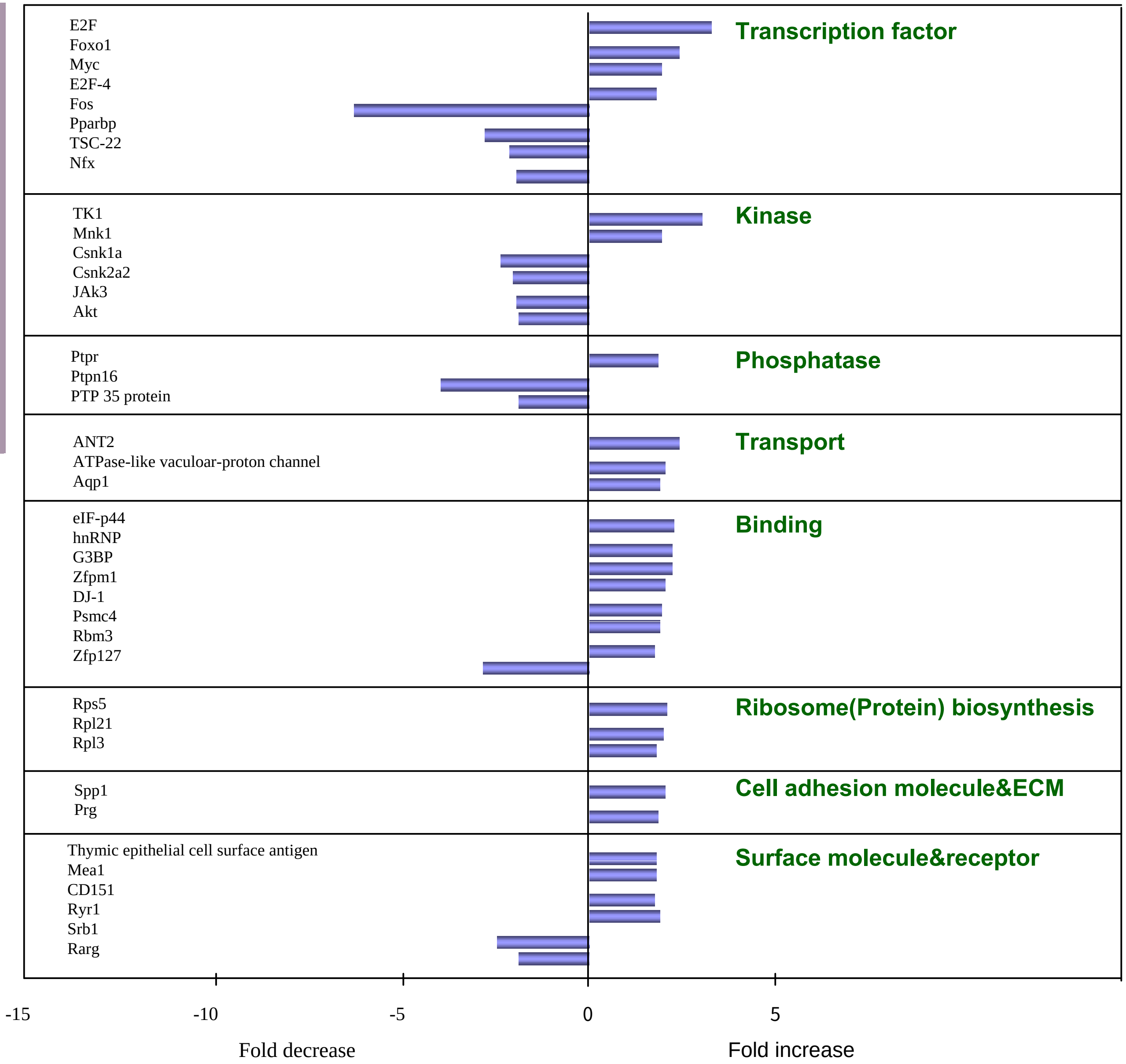
(Lee et al., Blood, 2003)



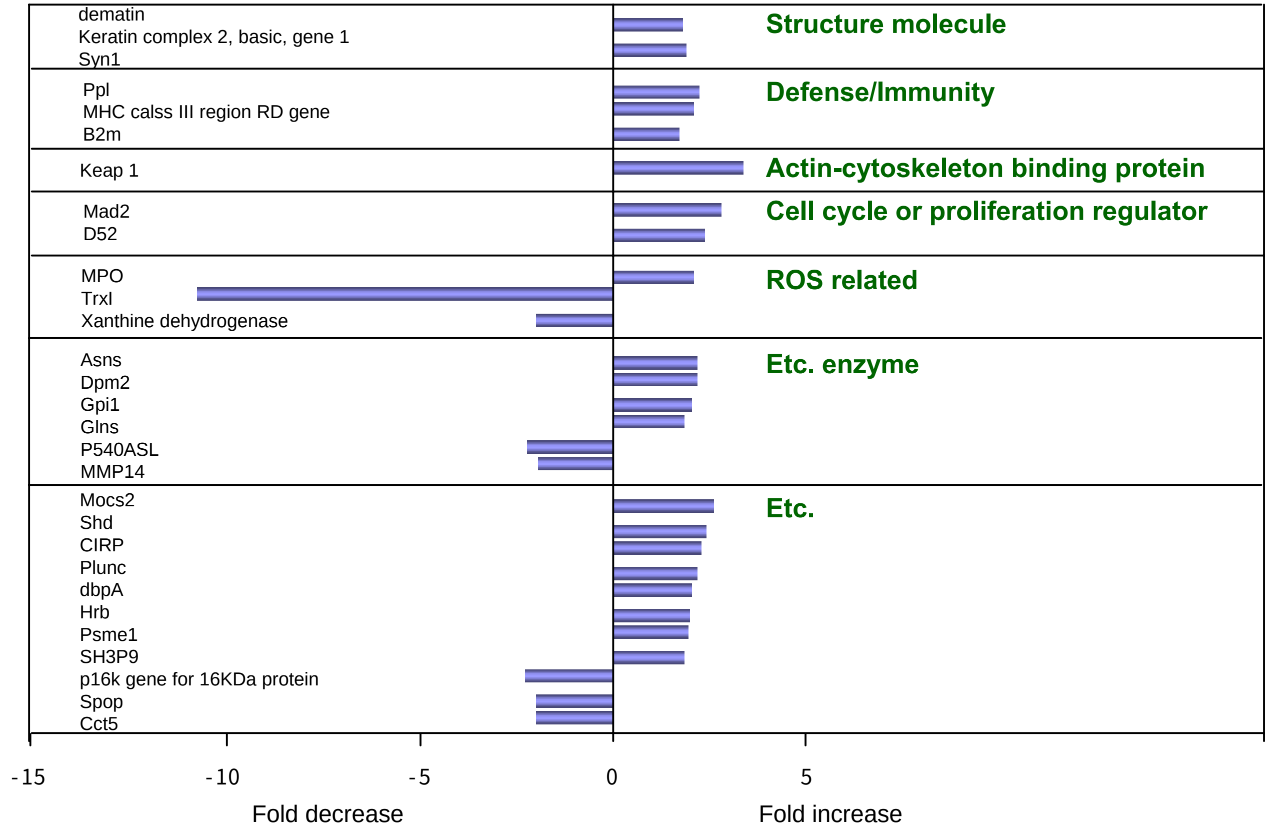
Differentially expressed genes in PrxII$^{-/-}$ BMCs



List of differentially regulated genes in PrxII$^{-/-}$ BM cells (1)



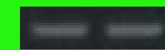
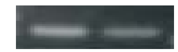
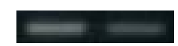
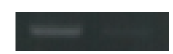
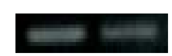
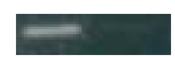
List of differentially regulated genes in PrxII$^{-/-}$ BM cells (2)



Decreased : 20 genes

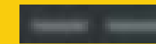
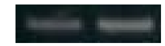
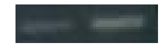
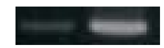
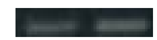
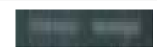
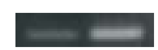
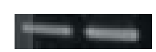
Increased : 47 genes

A. Increased candidate in Prx$^{-/-}$ bone marrow cells

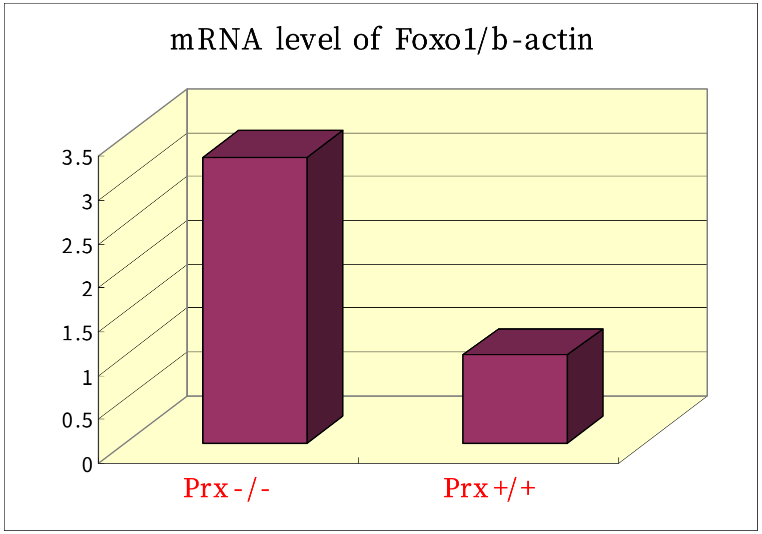
		-/- +/+
	β-Actin	
Transcription factor	Idh3b	
	Foxo1	
	Myc	
Kinase	TK1	
	Csnk1a1	
	MAPK8	
Phosphatase	Ptpr	
Protease	Psmc4	
	MMP14	
Transport	Slc25a5	
	Aqp1	
ROS-related	Keap1	
Defense / Immunity	Ppl	
	Hrb	
	Psme1	
	B2m	

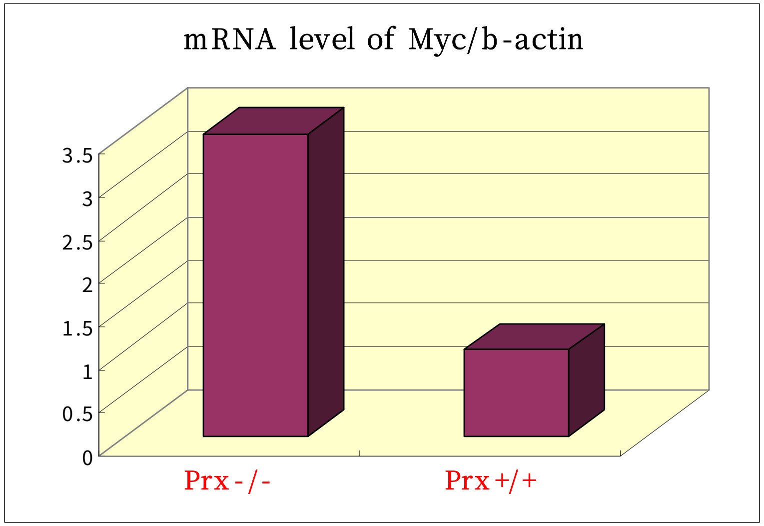
	β-Actin	
Binding	eIF3s4	
	hnRNPH1	
	G3bp	
	Zfpm1	
	DJ1	
	Rbm3	
Ribosome biosynthesis	Rps5	
	Rpl3	
Cell adhesion	Prg	
Cell cycle	Cirbp	
	Mad2L	
Surface molecule	Mea1	
	Thymic epithelial cell surface antigen	
RNA helicase	DbpA	
Enzyme	Asns	
Etc..	Plunc	
	p16kDa protein	

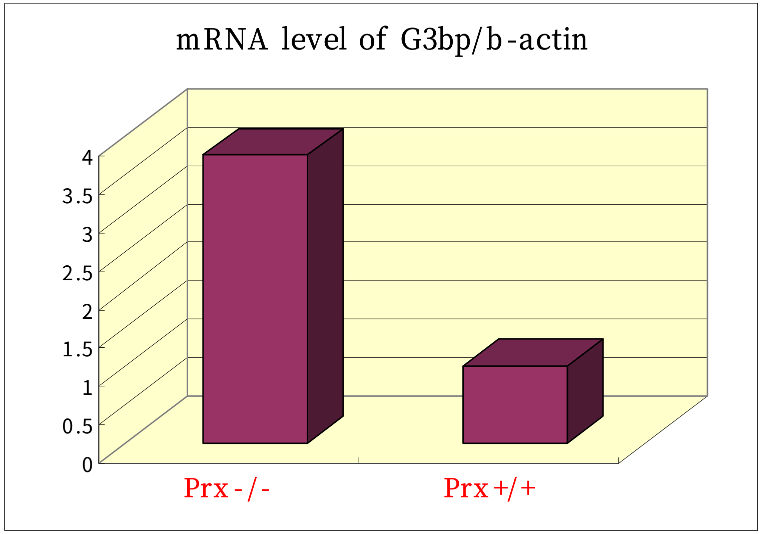
B. Decreased candidate in Prx$^{-/-}$ bone marrow cells

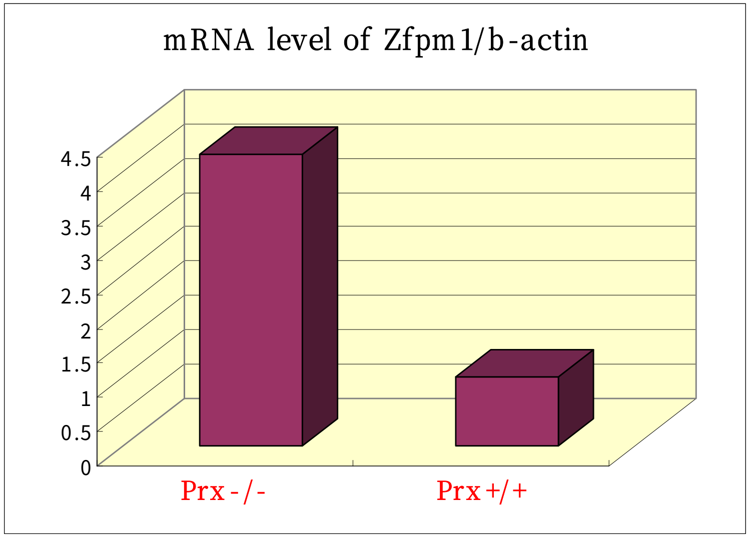
		-/- +/+
	β-Actin	
Transcription factor	Fos	
	Pparbp	
	Gilz	
	RARg	
Kinase	Mnk1	
	Csnk2a2	
	Serine/Threonine protein kinase	
Phosphatase	Ptpn16	
Cell adhesion	Spp1	
Surface molecule	Srb1	
Structure molecule	Syn1	
ROS-related	MPO	
	PrxII	
	Xanthine dehydrogenase	
Enzyme	Mocs2	
	Gpi1	
Etc..	Shd	
	Bin3	
	Spop	

C. Real-time PCR in bone marrow cells of Prx$^{-/-}$ & Prx$^{+/+}$ mice

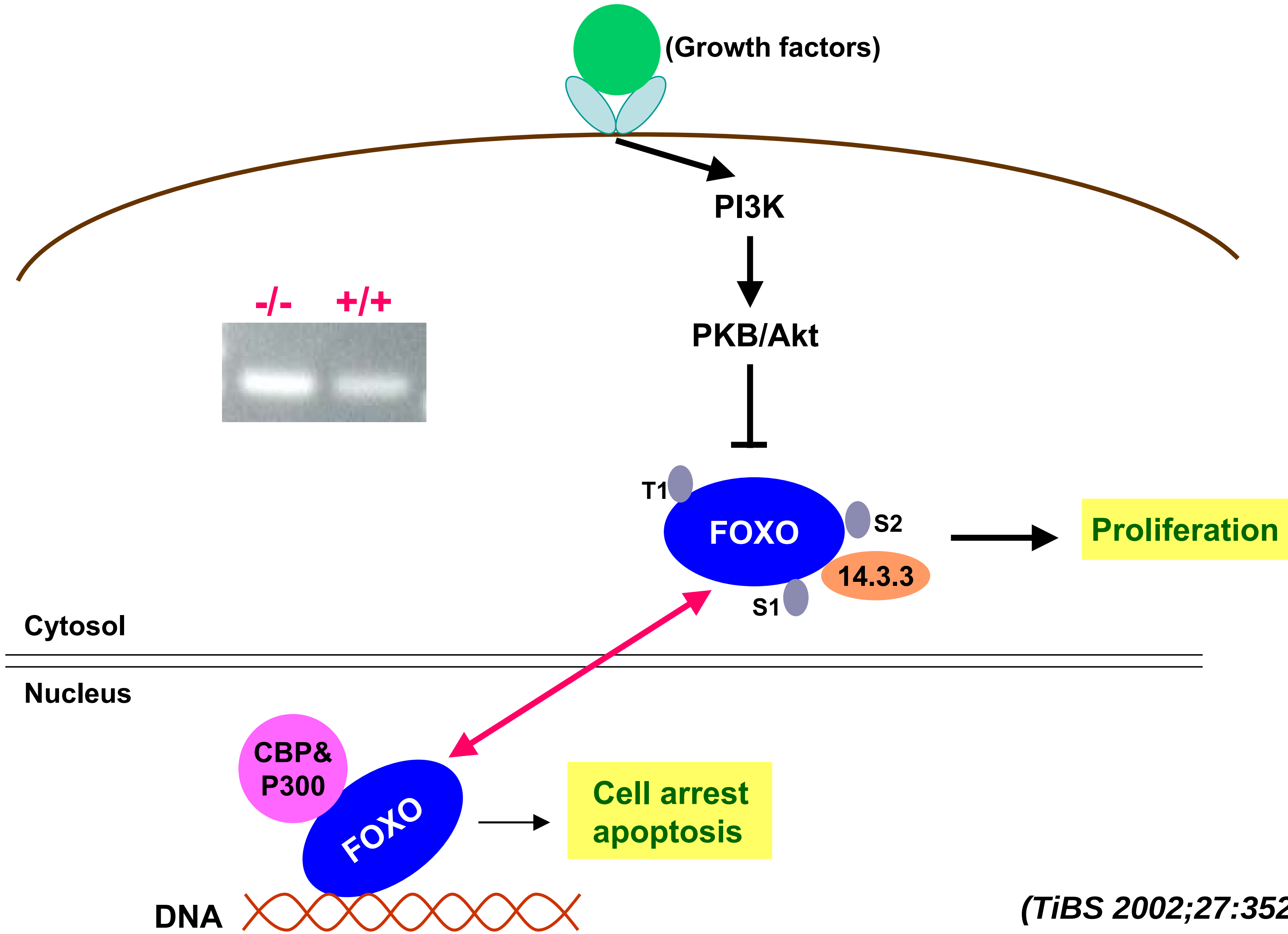








Regulations of FOXO1

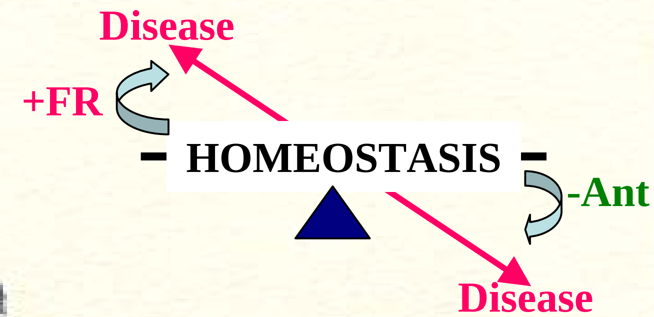


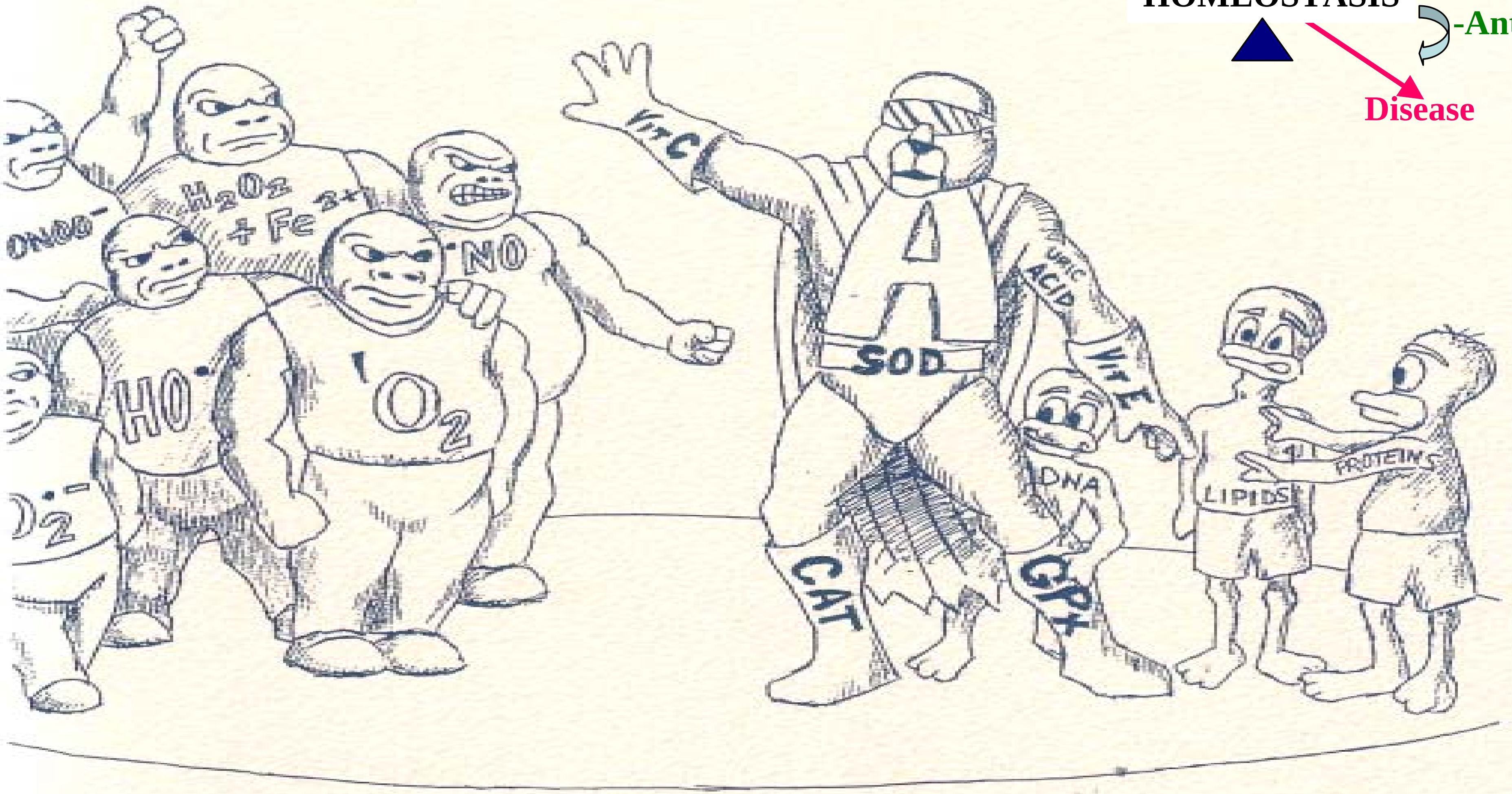
(TiBS 2002;27:352)

OXYGEN '97

SUNRISE FREE RADICAL SCHOOL

November 21-24, 1997
San Francisco, CA





Acknowledgments

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  이동석 박사, 김선욱, 김수희

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  김동준 (DNA chip analysis)

  박성구 박사 Lab.
  고창원 (Proteomics)

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  도소희 (Protein analysis)

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