The role of Nox derived Oxidative stress in diabetic complications

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Disclosures

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- Research grant support:
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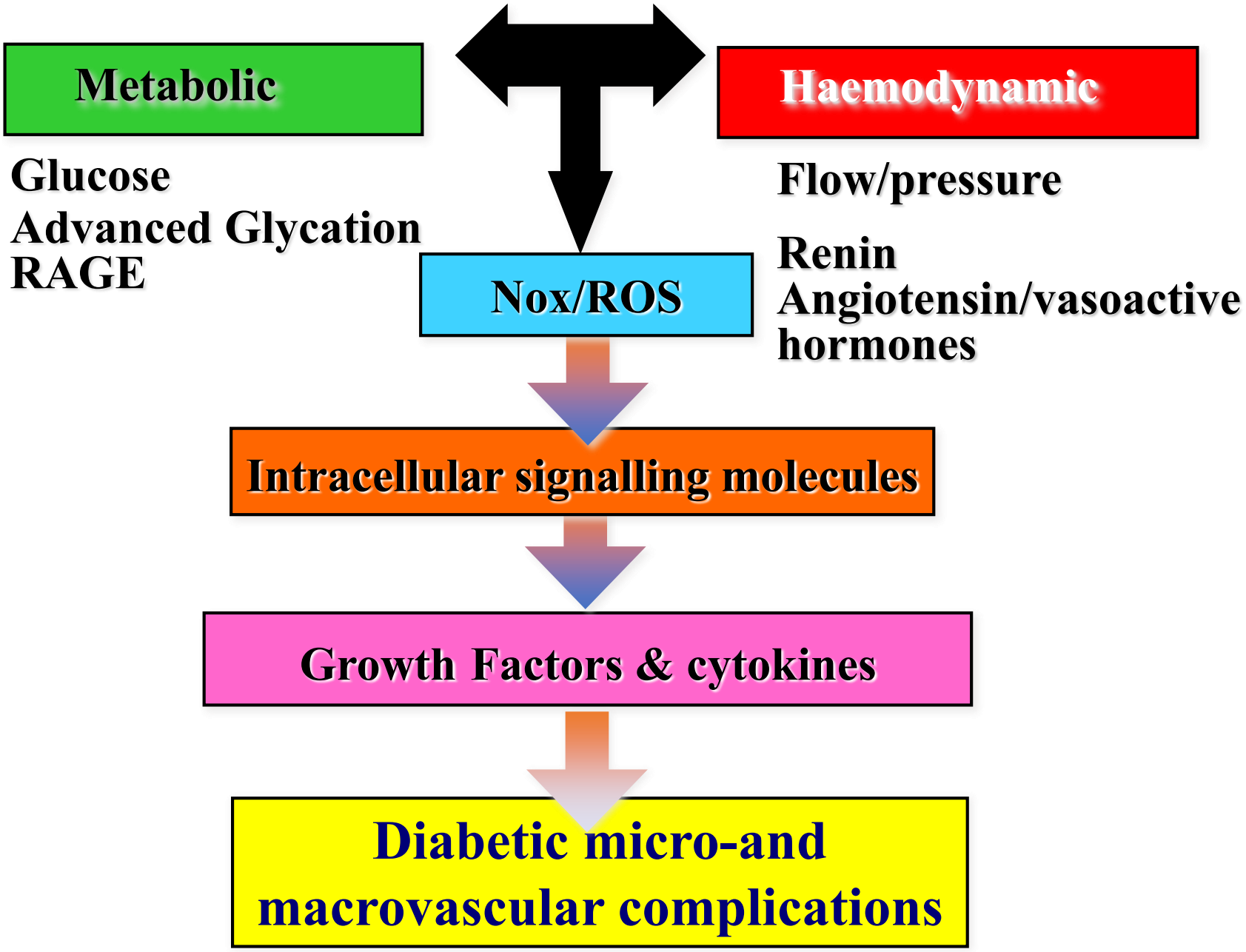
- Glaxo Smith Kline
- Boehringer INgelheim



Adapted from Cooper, Diabetologia, 44:1957, 2001

Mediators of the toxic environment of diabetes

Oxidative stress

Cardiovascular Risk Factors

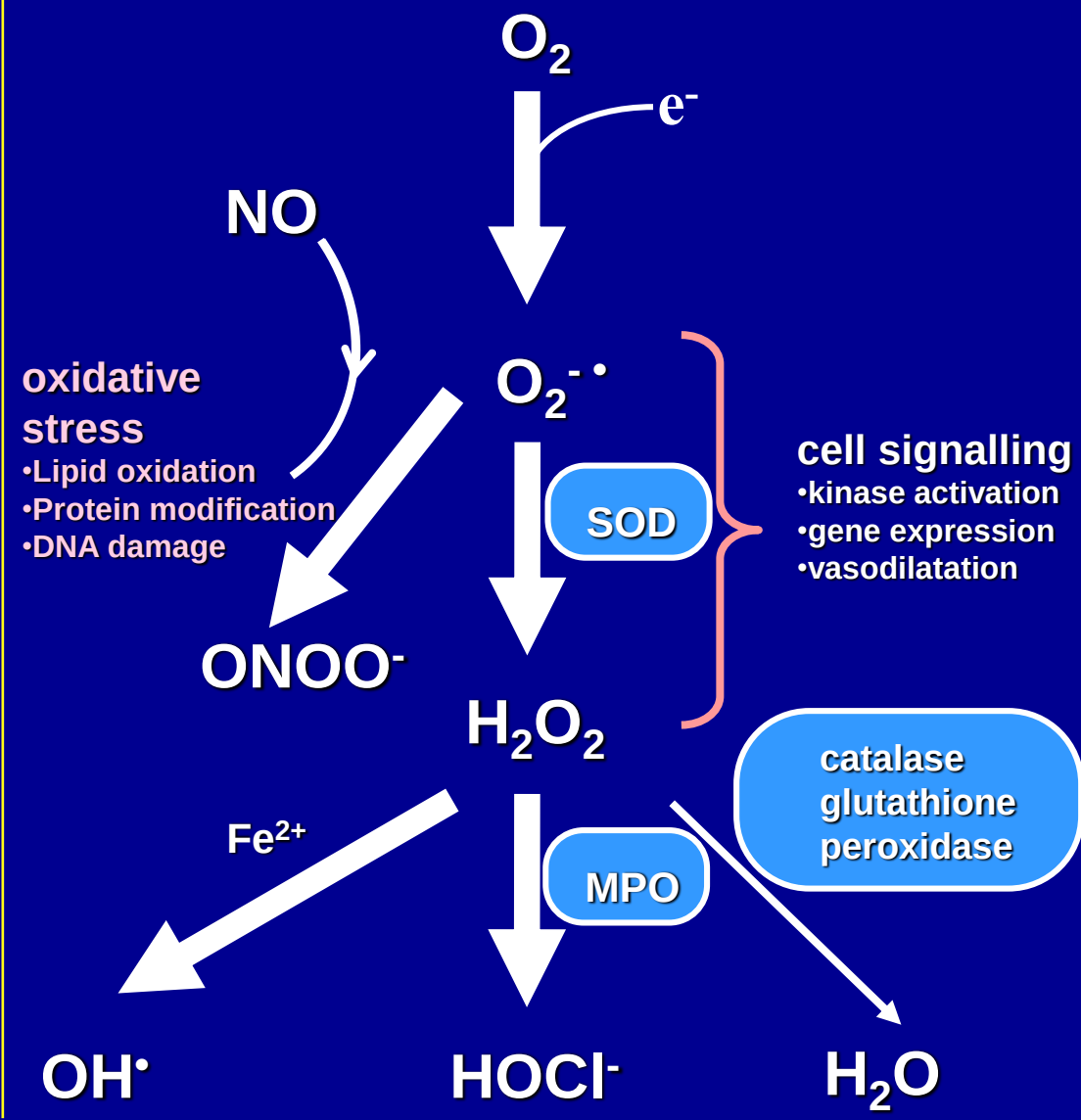
hypertension,
hypercholesterolaemia,
diabetes,
smoking

ROS

($O_2^{-\bullet}$, H_2O_2, $OH^{\bullet}$, HOCl)

Adapted from G Drummond

Reactive Oxygen Species



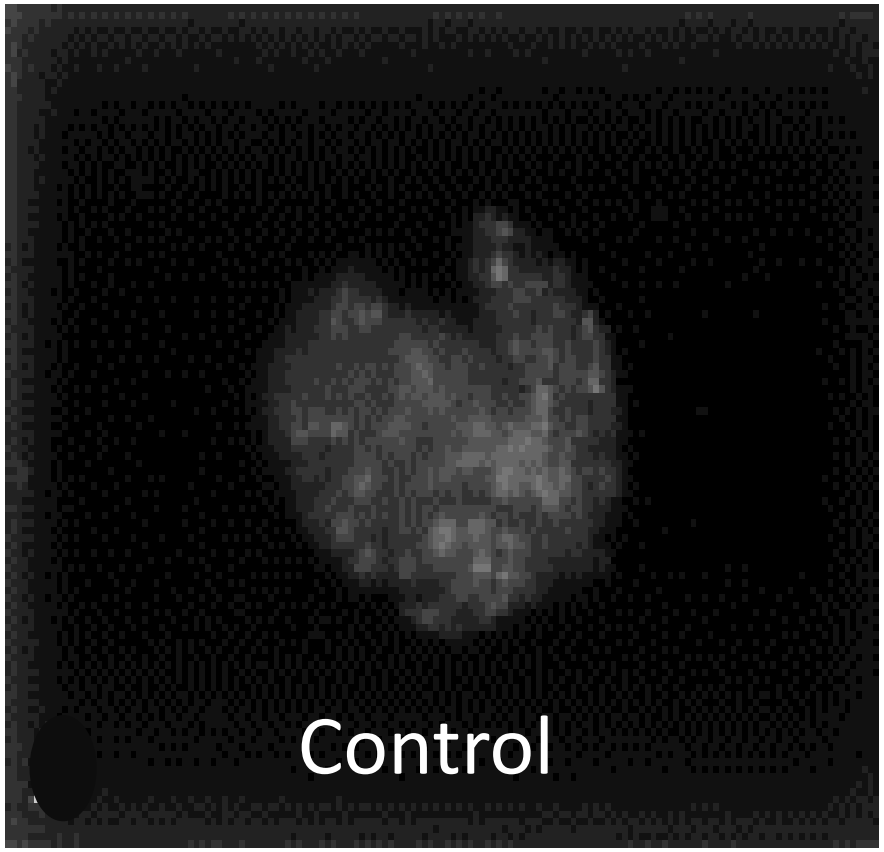
The Janus faced role of ROS

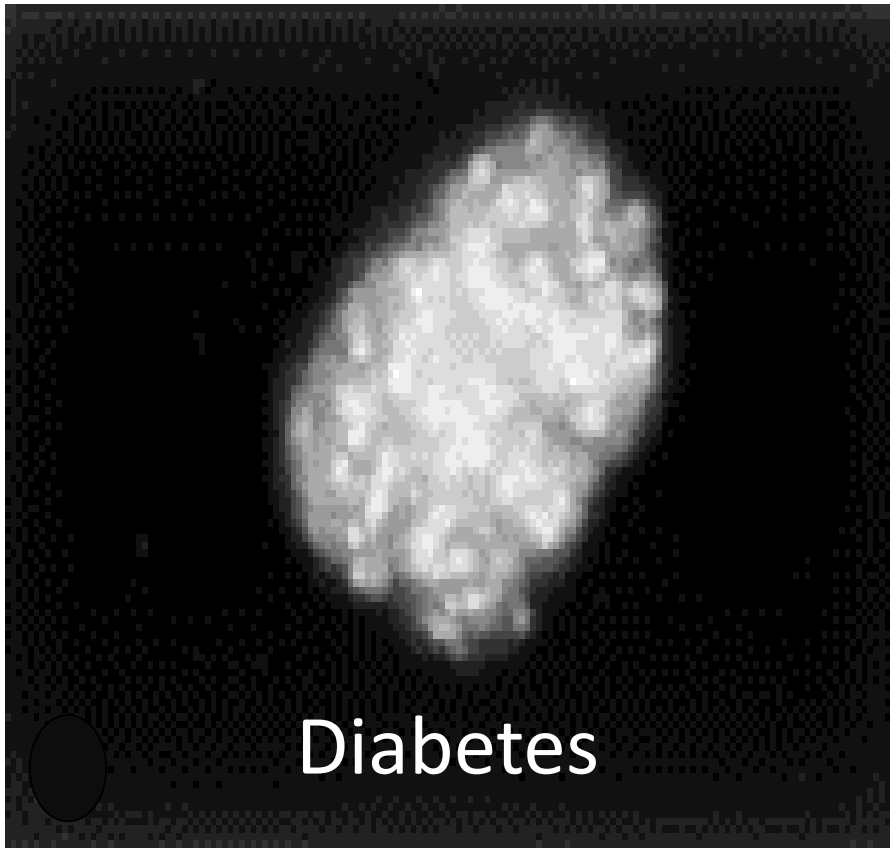
- **Physiological role**
  - signalling molecules
  - transcription factors
  - activation of
    - G-protein-coupled receptors,
    - ion channels
    - kinases/phosphatases.

Pathological role

- Modify targets
- Cellular dysfunction
- Inflammation

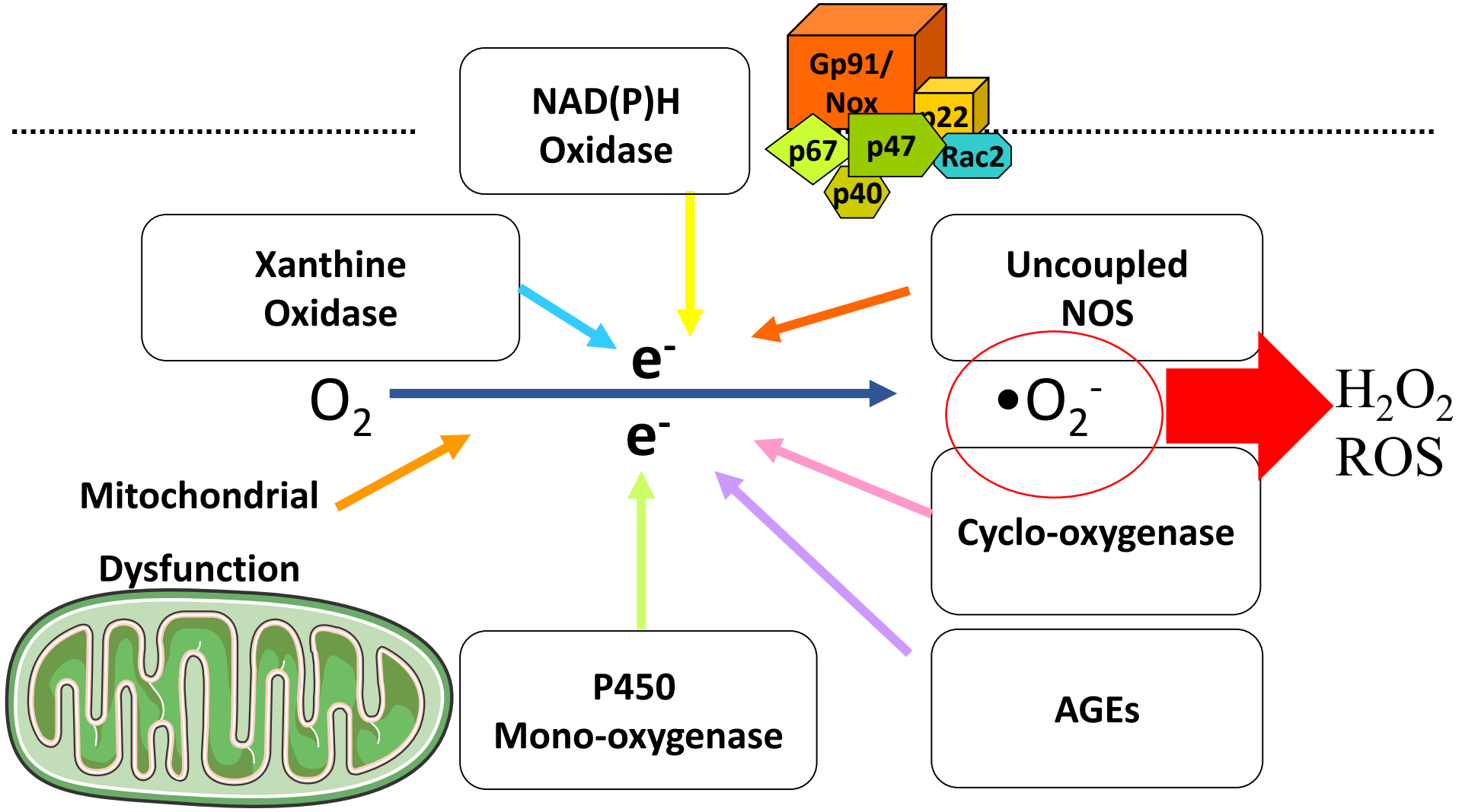
Most intracellular ROS come from superoxide



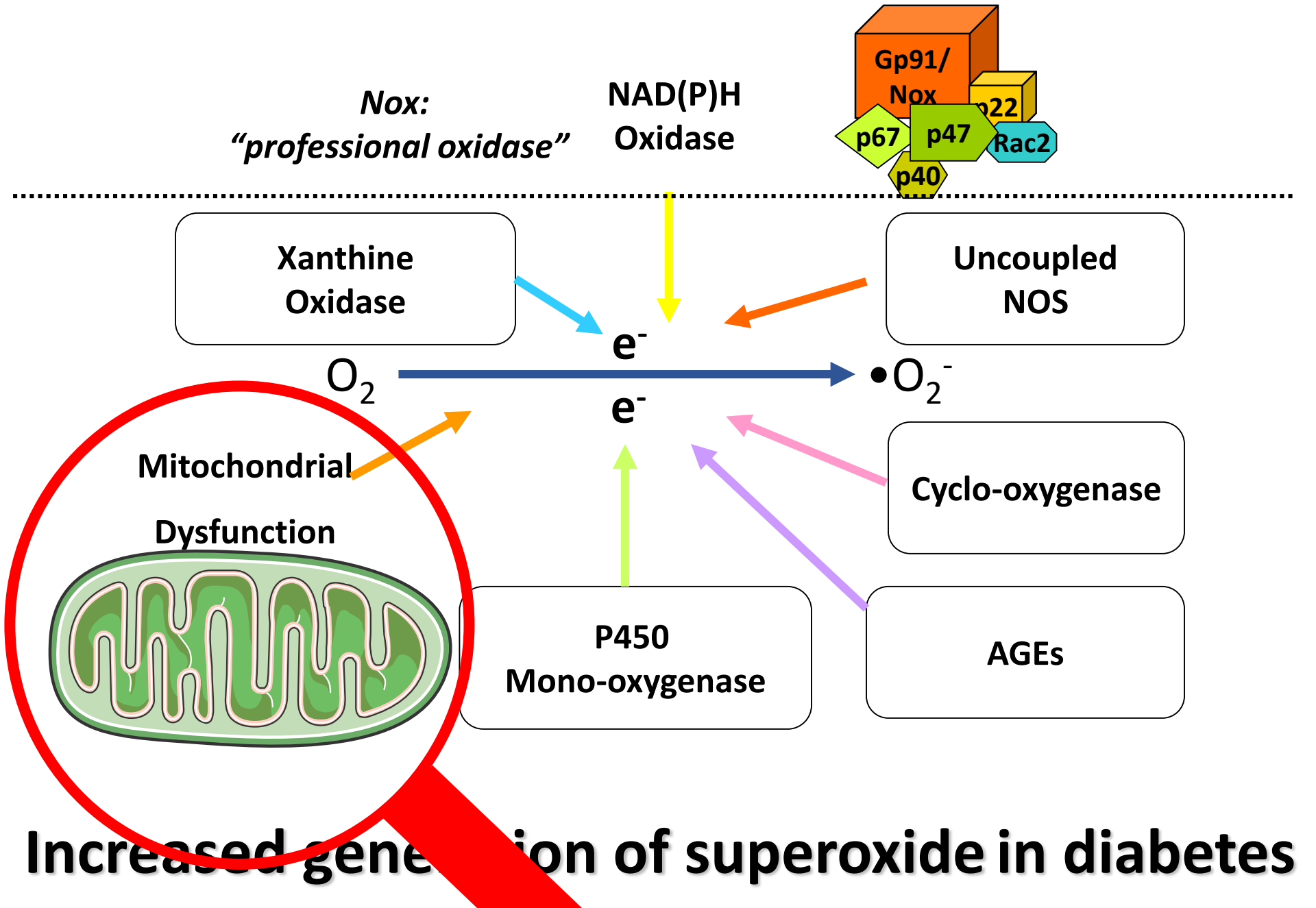


Superoxide production in diabetic glomeruli

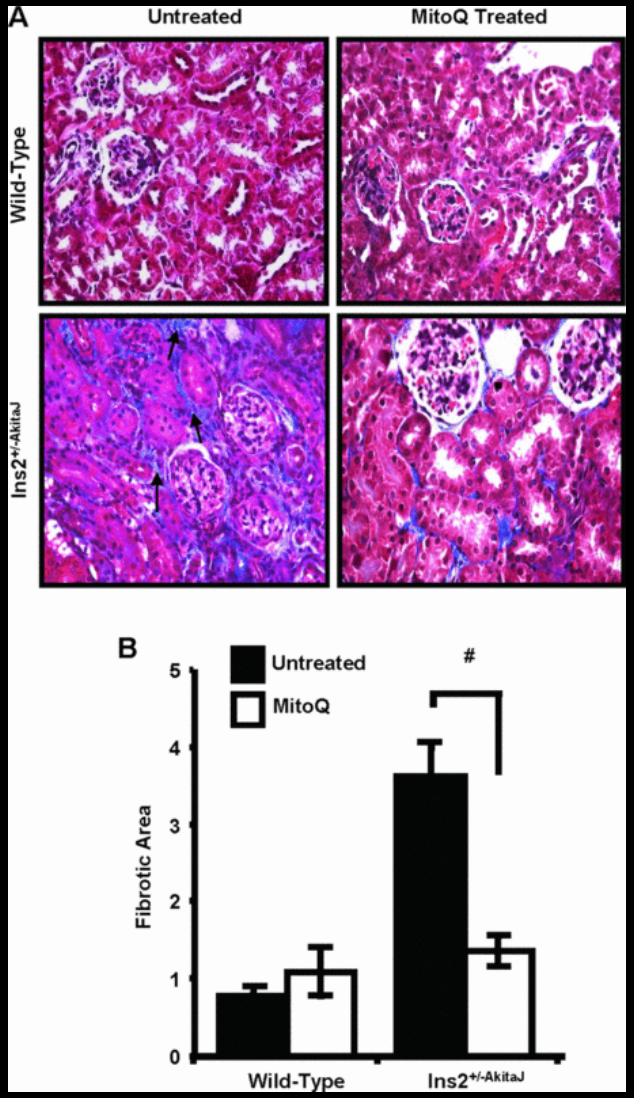
Asaba et al. KI 2005, Satoh M et al. Am J Physiol Renal Physiol 2005

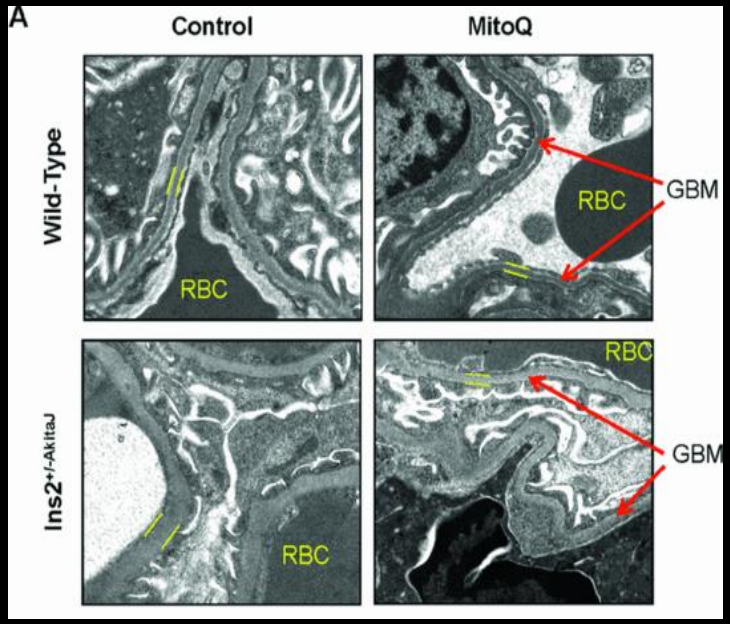


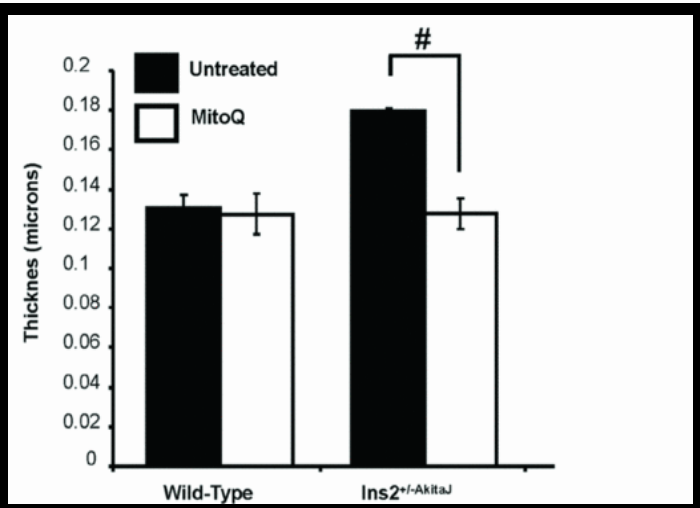
Increased generation of superoxide in diabetes



Increased gene[illegible]ion of superoxide in diabetes



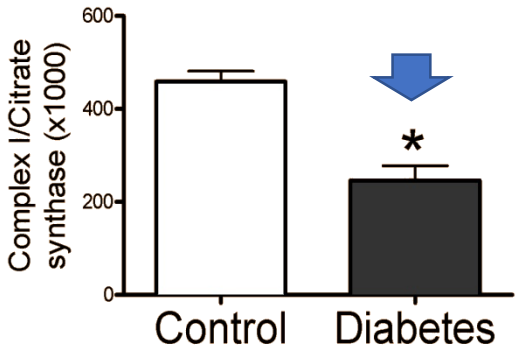




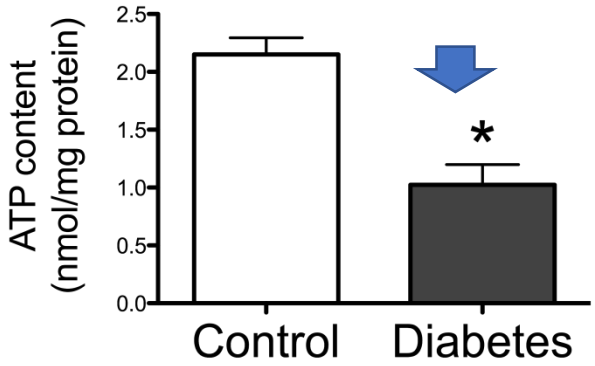
Chacko et al. Biochem J. 2010 Nov 15;432(1):9-19

Mitochondria-targeted ubiquinone (MitoQ) reduces Interstitial fibrosis and GBM thickening in Ins2(+/)$^{-}$(AkitaJ) mice

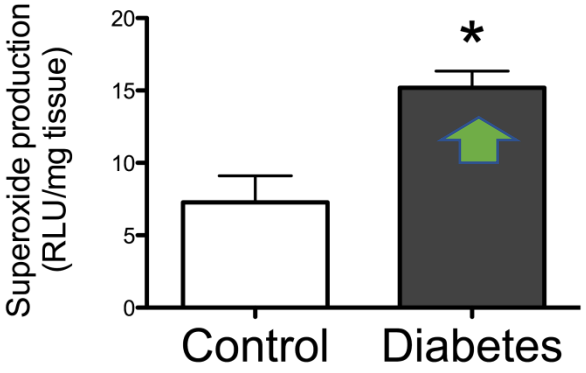
Respiratory chain dysfunction



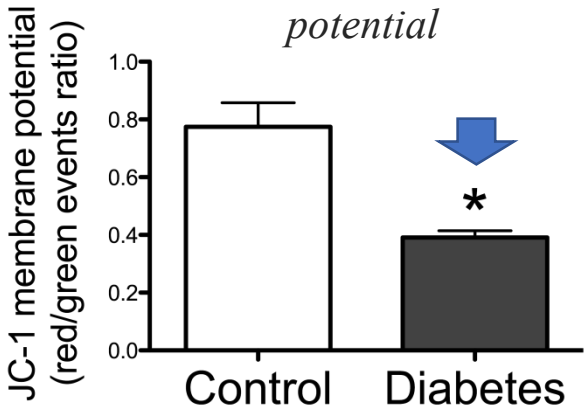
ATP depletion

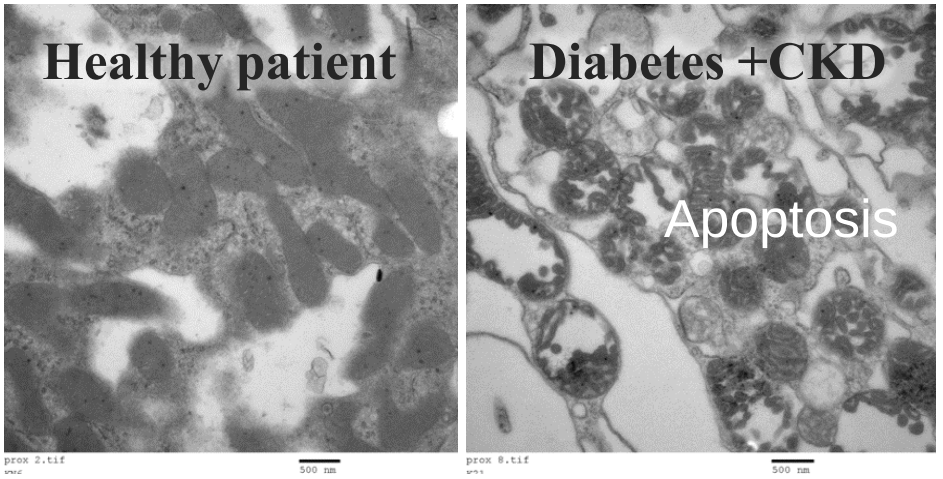


Increased mitochondrial ROS



Reduced membrane potential

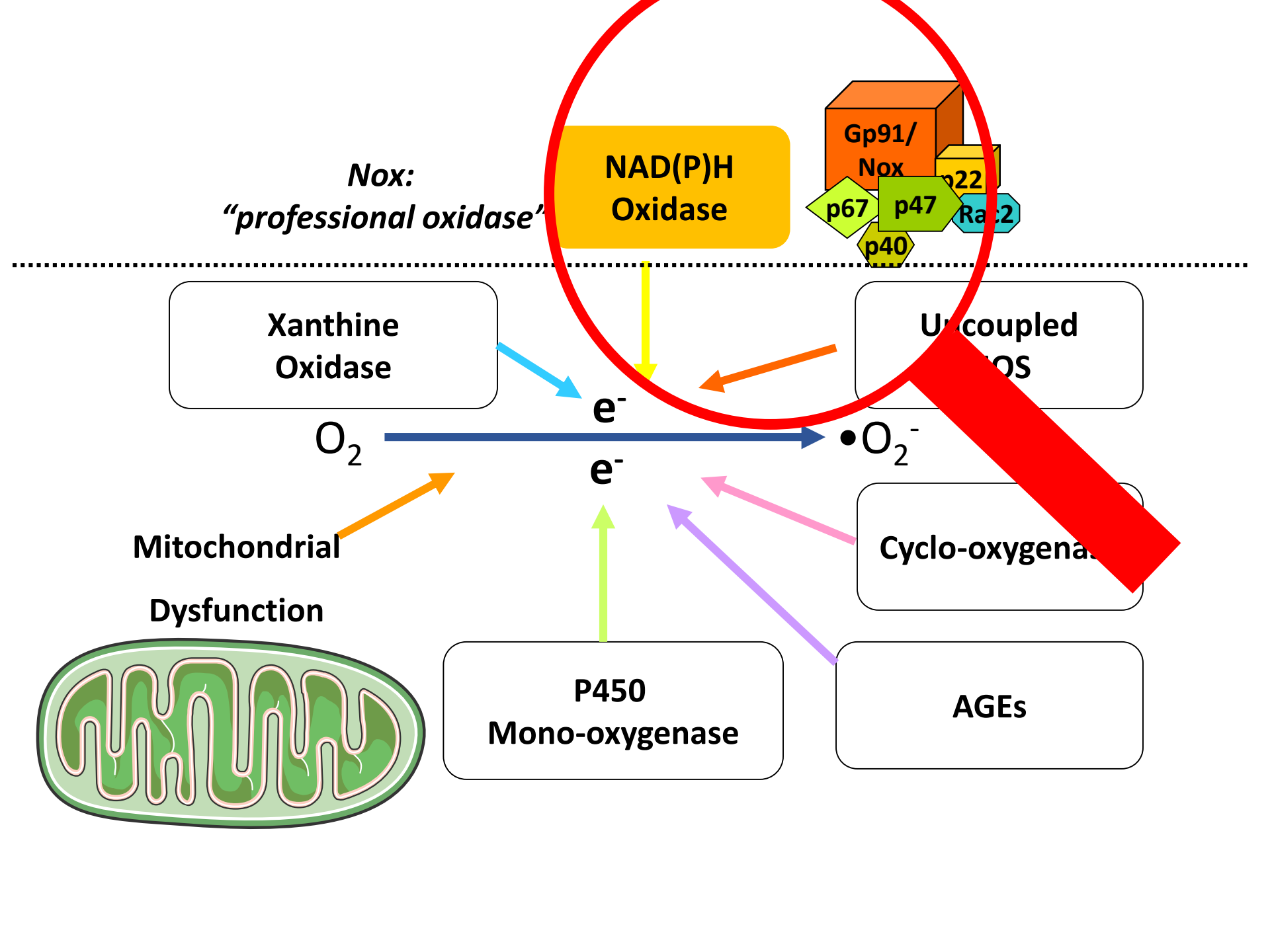




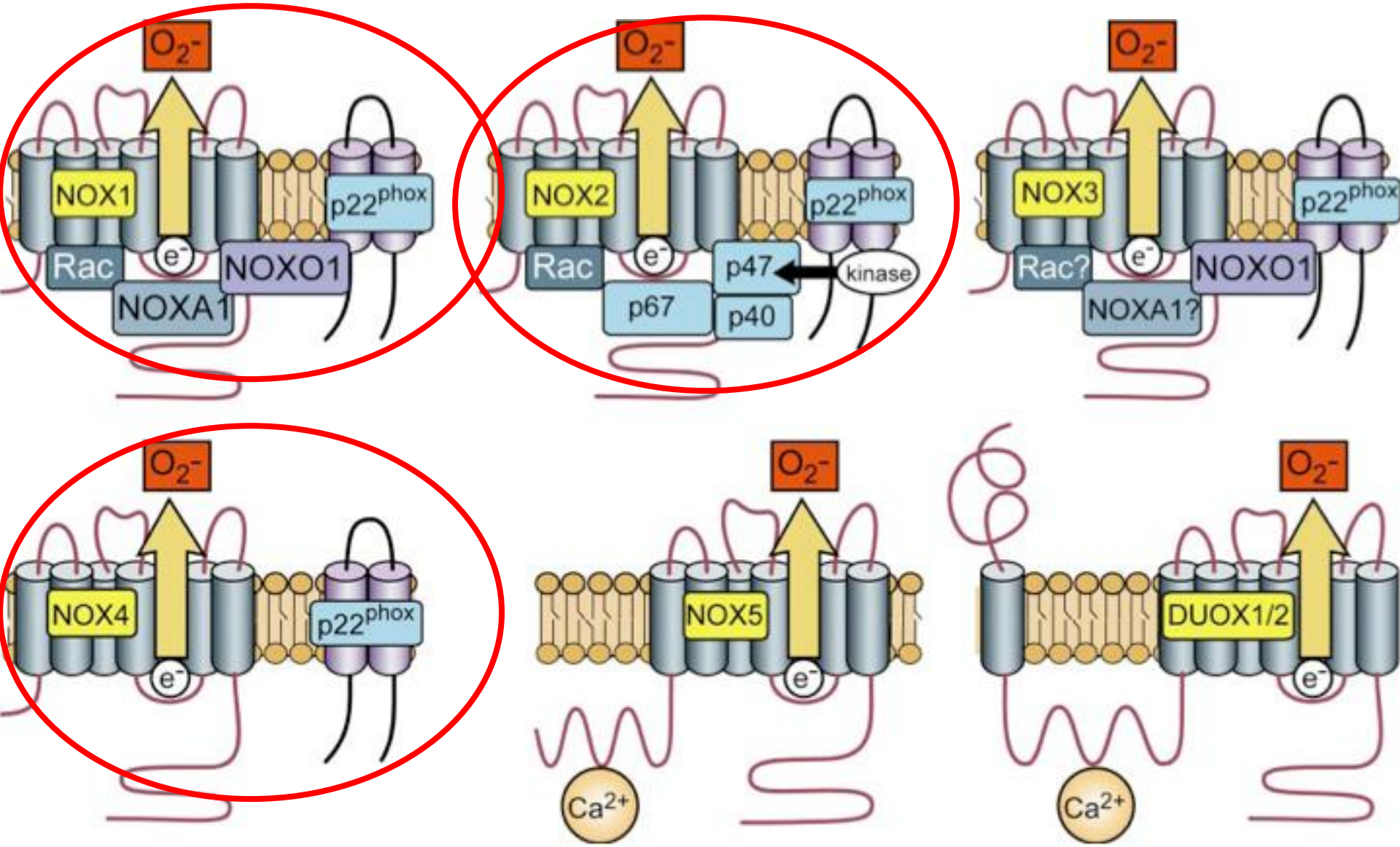
EM Proximal tubular cells

Coughlan MT et al., J Am Soc Nephrol 2009

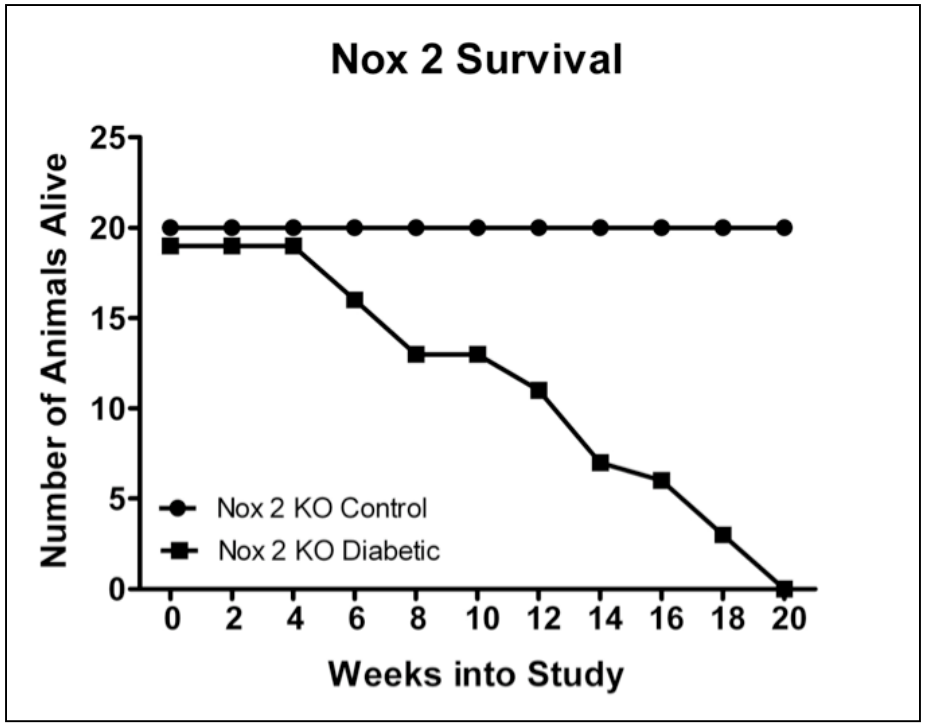
Mitochondrial disturbances in diabetes: altered mitochondrial bioenergetics


Nox:
"professional oxidase"
NAD(P)H
Oxidase
Gp91/
Nox
p22
p67
p47
Rac2
p40
Xanthine
Oxidase
Uncoupled
eNOS
e-
O2
•O2-
e-
Mitochondrial
Dysfunction
Cyclo-oxygenase
P450
Mono-oxygenase
AGEs

Nox isoforms

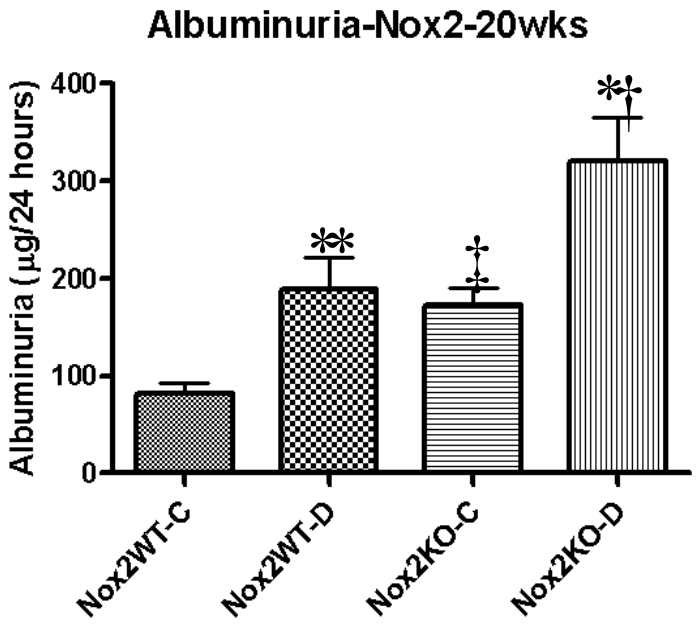


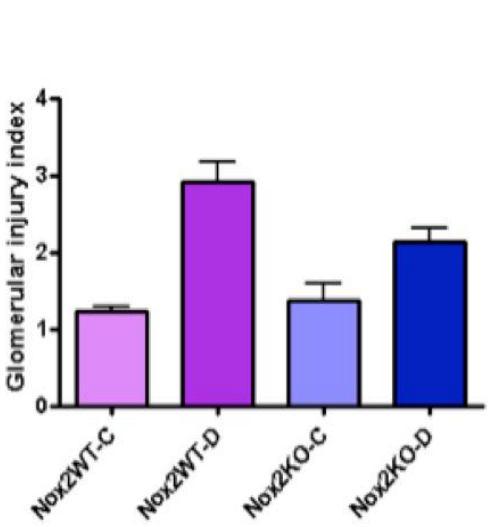
Nox2 deletion increases susceptibility to infections

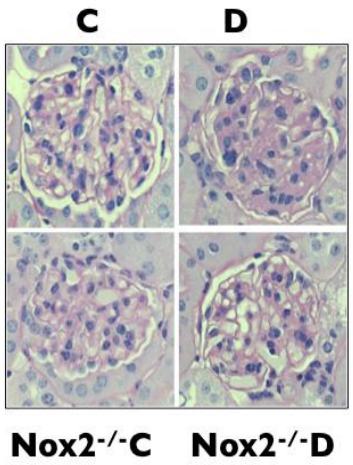


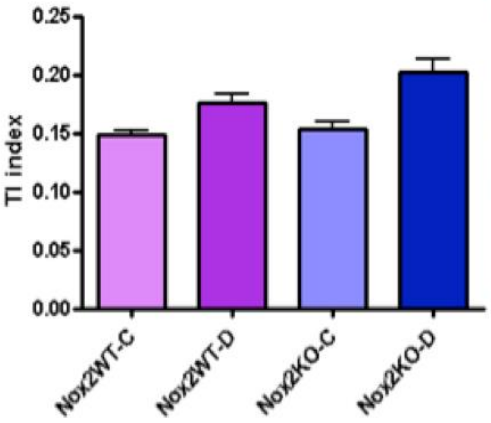
S Gray et al, Circulation, 2013,127:1888-902

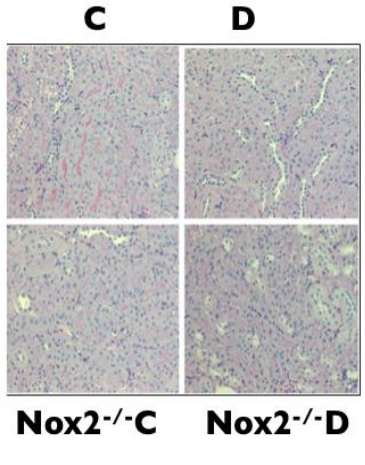
NOX2 deficiency does not protect from diabetic nephropathy



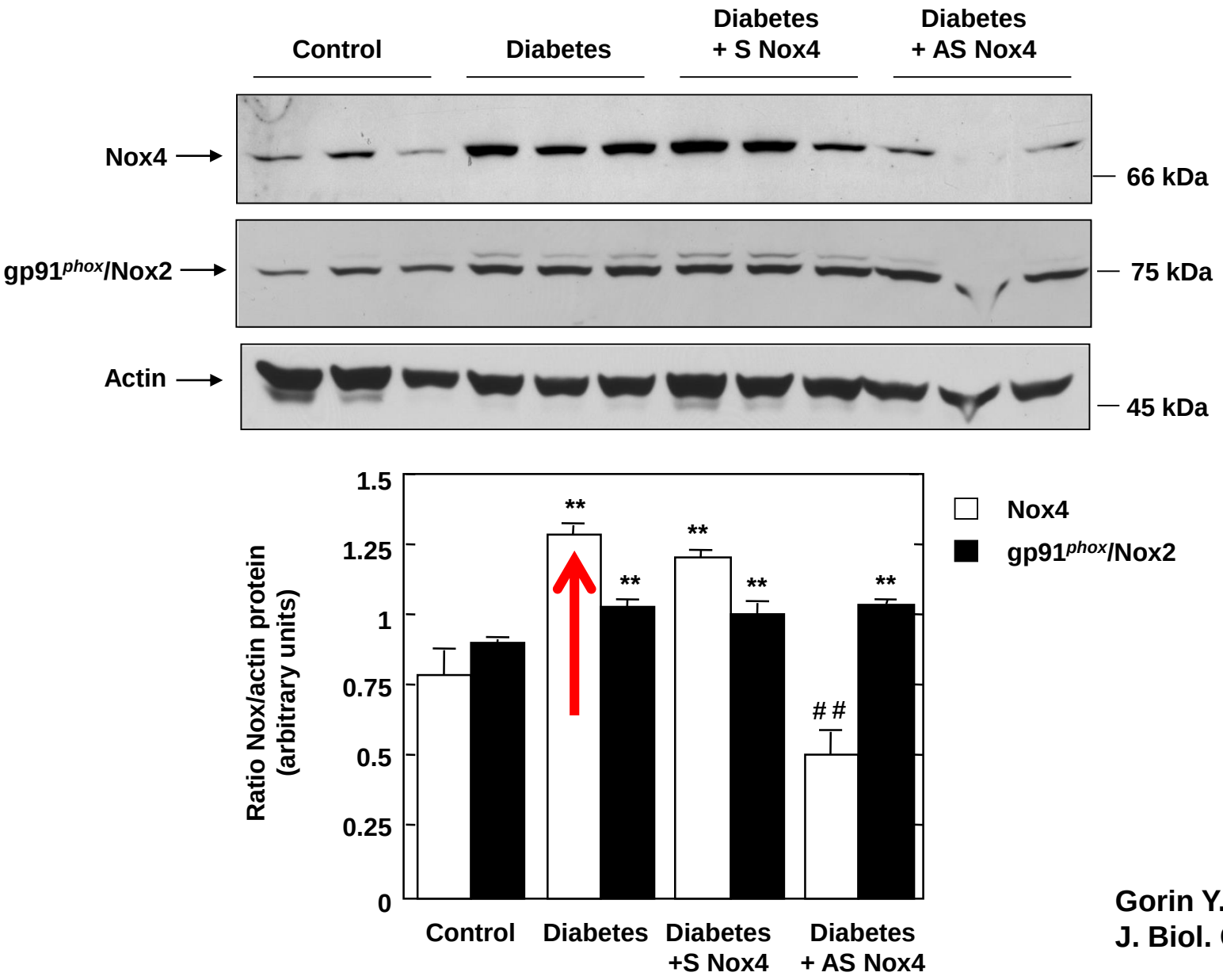






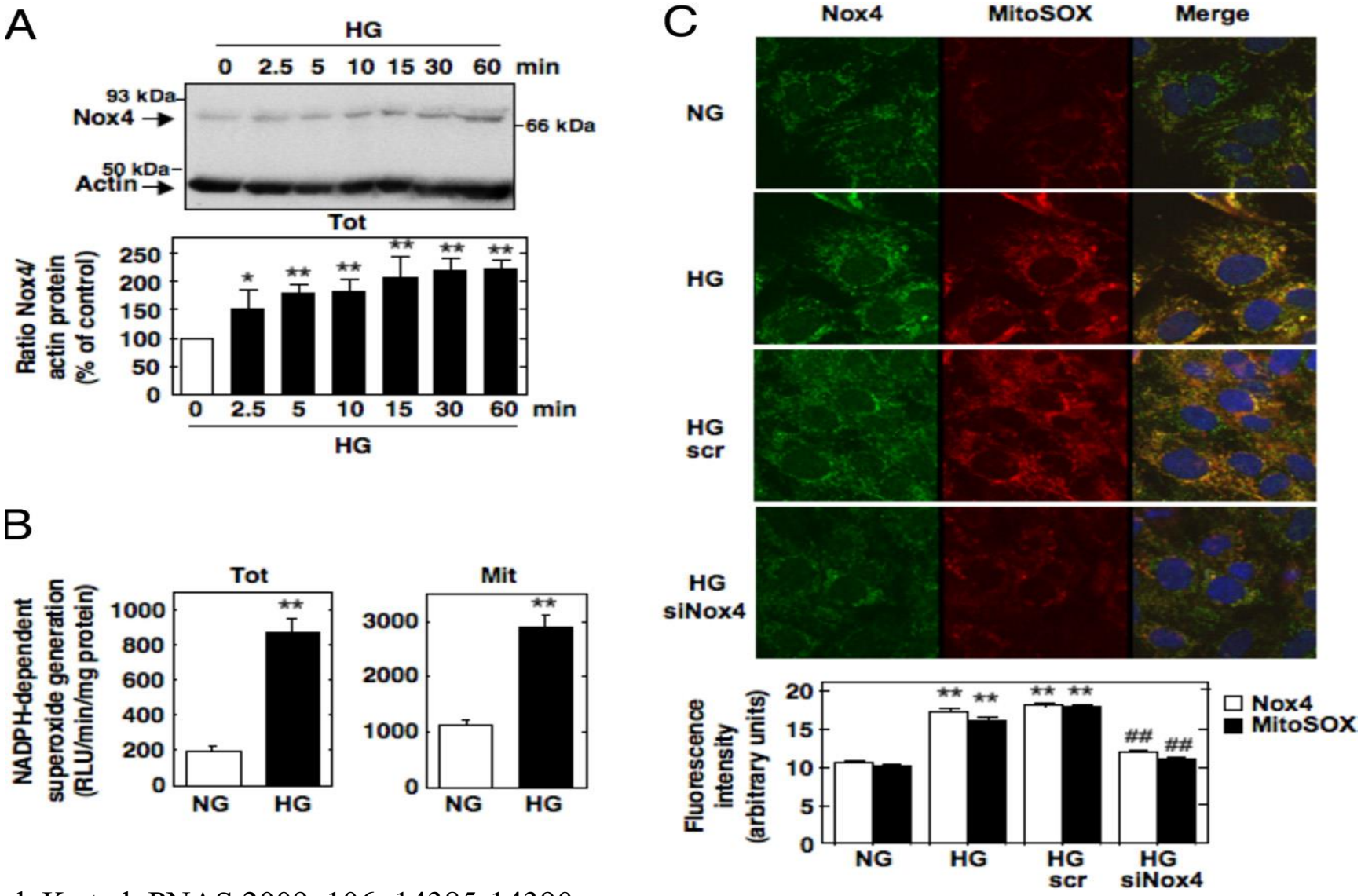


Y You et al, Am J Physiol 2013



Gorin Y. et *al.*,
J. Biol. Chem. 2005

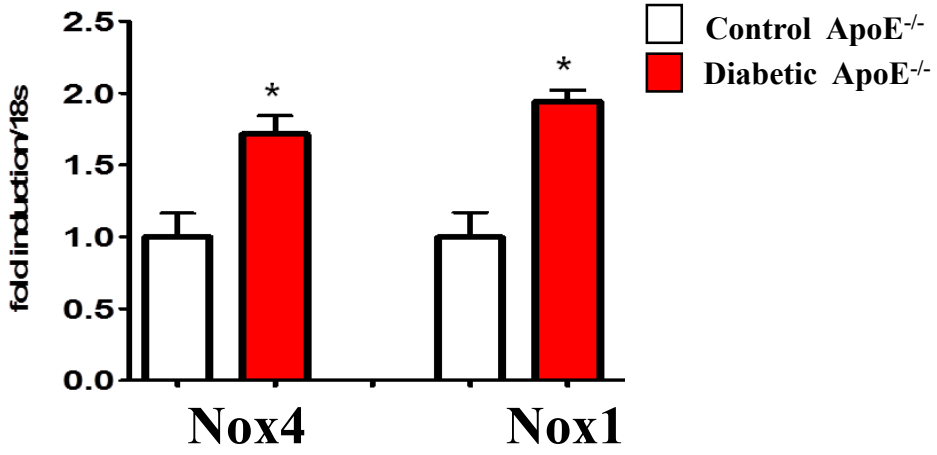
Upregulation of Nox4 in the diabetic kidney



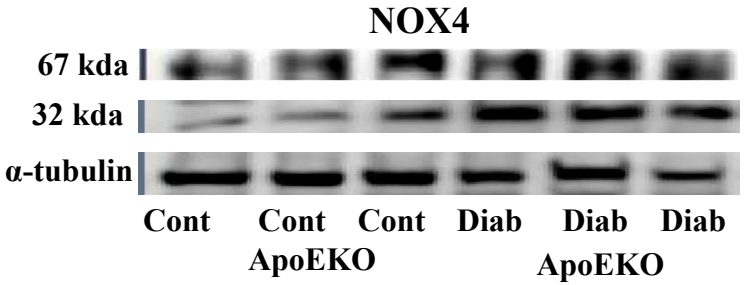
Block K et al, PNAS 2009, 106: 14385-14390

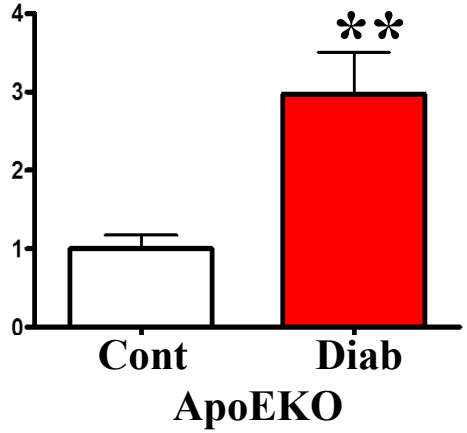
Nox4 also localizes to mitochondria

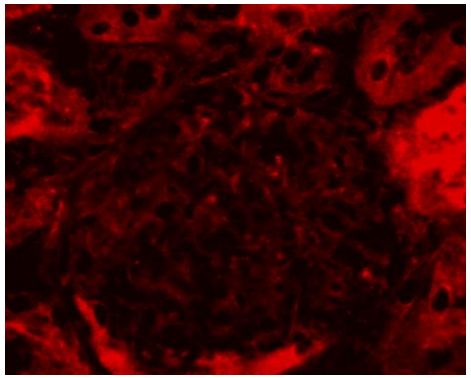
NOX4 in diabetic nephropathy



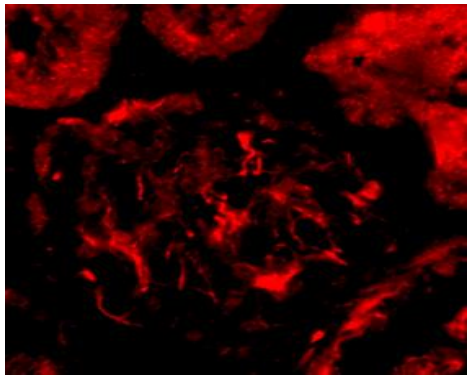
Protein expression of Nox4 in renal cortex







Control ApoE$^{-/-}$



Diabetic ApoE$^{-/-}$

Jha et al. *JASN, 2014, 25(6):1237-54*

Experimental design

STZ: 55mg/kg
5 daily injections ip

Week 10 and 20

Metabolic caging for 24hrs
Urine collection
Blood sample & Cull
Kidney tissue collection

± STZ — ***Nox4*$^{+/+}$ *ApoE*$^{-/-}$ or *Nox4*$^{-/-}$ *ApoE*$^{-/-}$ mice**

± STZ — ***Nox1*$^{+/y}$ *ApoE*$^{-/-}$ or *Nox1*$^{-/y}$ *ApoE*$^{-/-}$ mice**

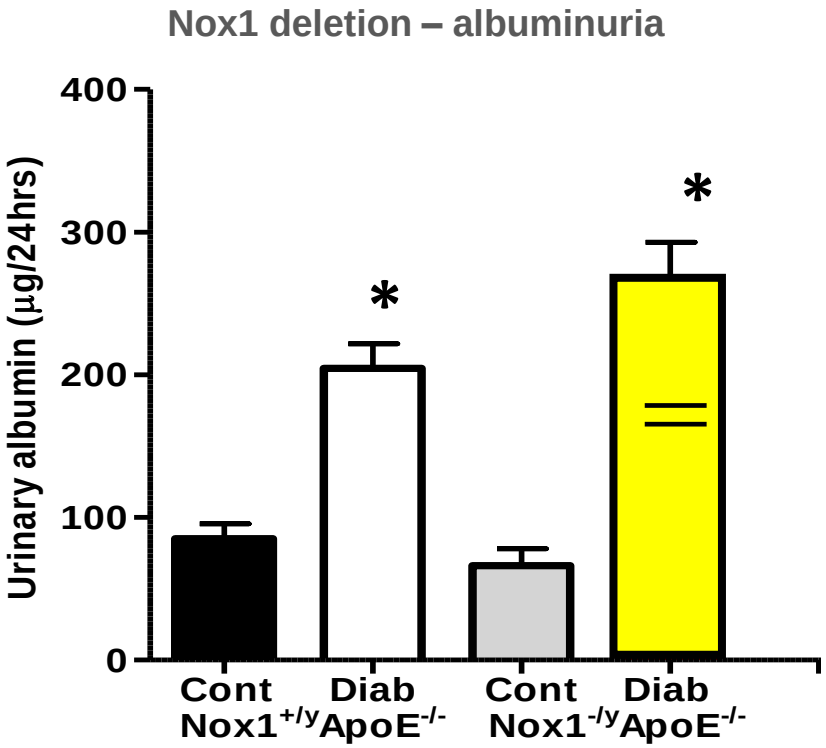
Male mice
6 wks of age

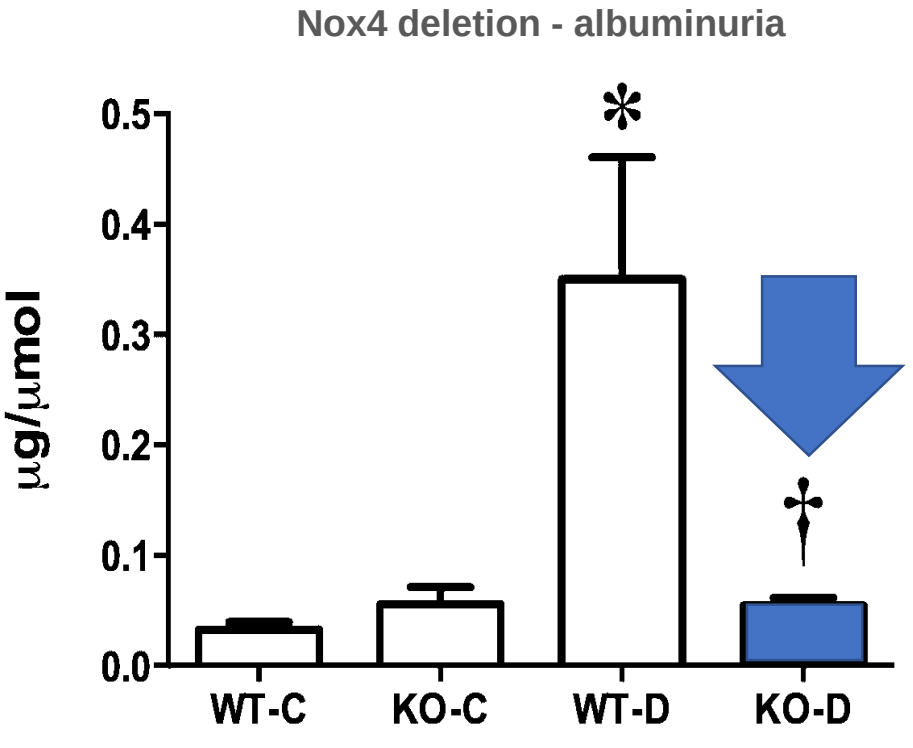
0 week

10 weeks

20 weeks

Deletion of NOX4 but not NOX1 reduces albuminuria in diabetic apoE KO mice



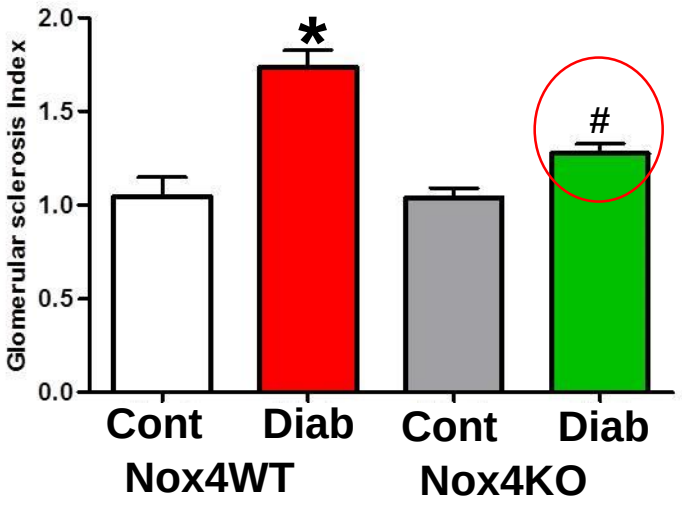


Jha et al. *JASN, 2014, 25(6):1237-54*

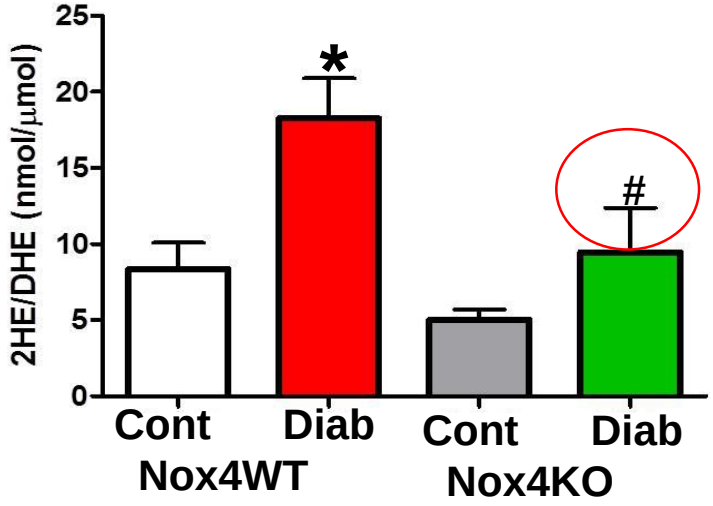
NOX4 in diabetic nephropathy

NOX 4 deficiency attenuates glomerulosclerosis

Glomerulosclerotic index (GSI)

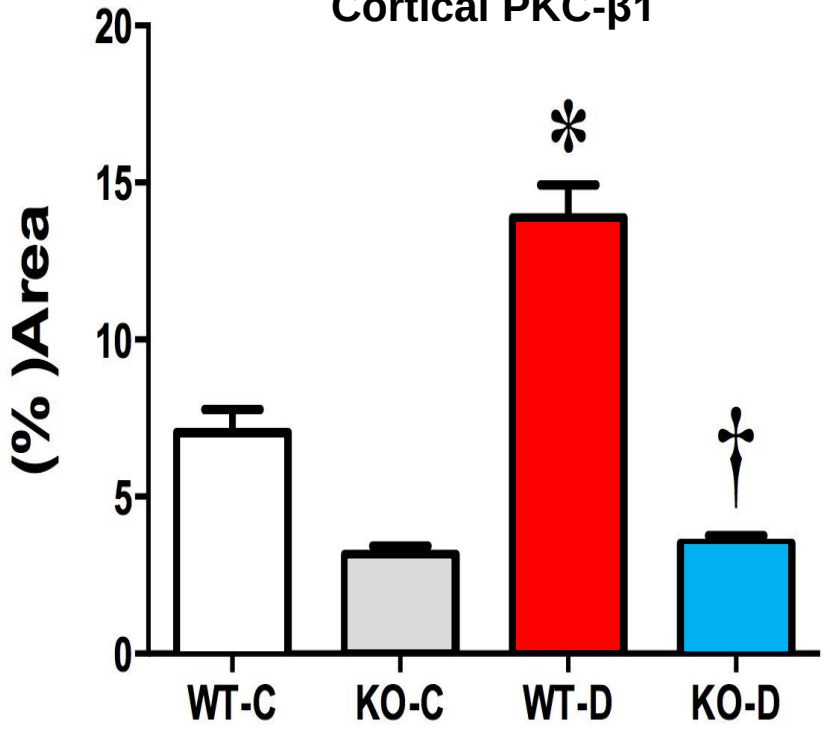


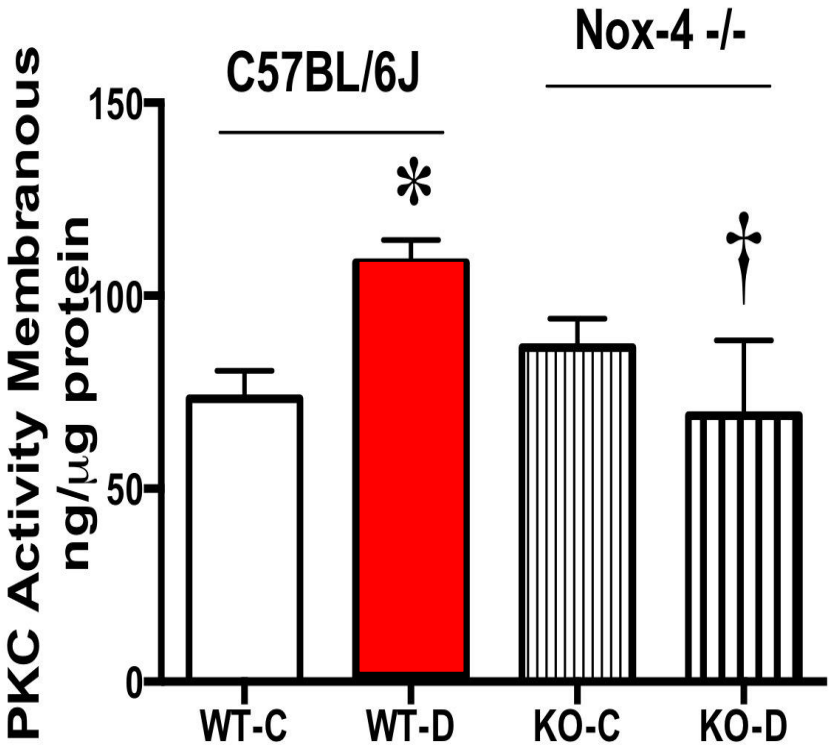
Renal superoxide production



NOX4 in diabetic nephropathy

Nox-4 KO mice also have reduced PKCα & PKC-β1





*p<0.001 vs WT-C; †p<0.001 vs WT-D

V Thallas-Bonke et al, Physiol Rep 2014

NOX4 deletion: direct or indirect mediator?

Podocyte-specific Nox4KO

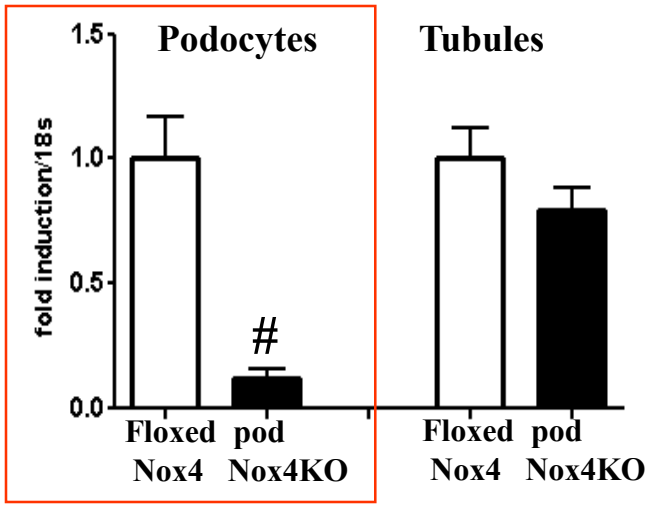
Floxed Nox4

X

Pod cre

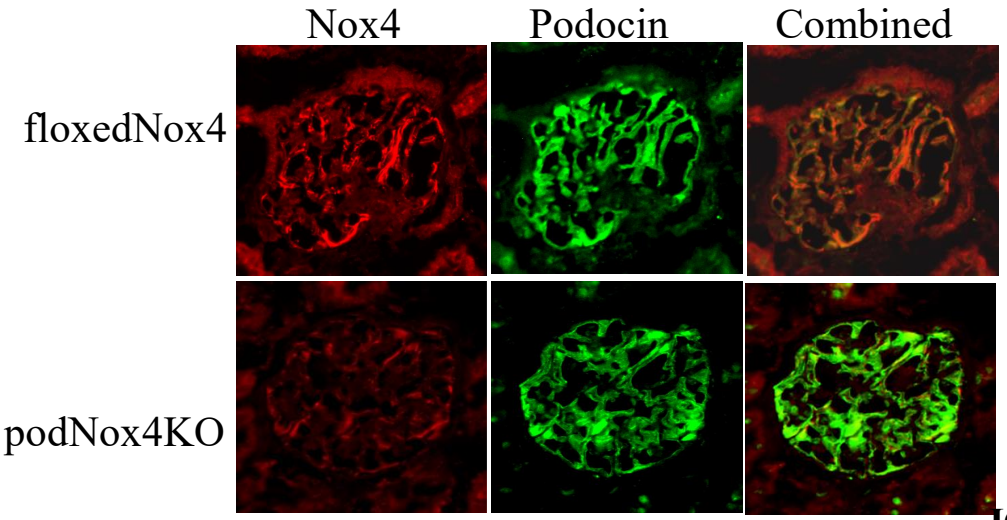
Podocyte specific Nox4 KO mouse
(podNox4KO)

Nox4 gene expression



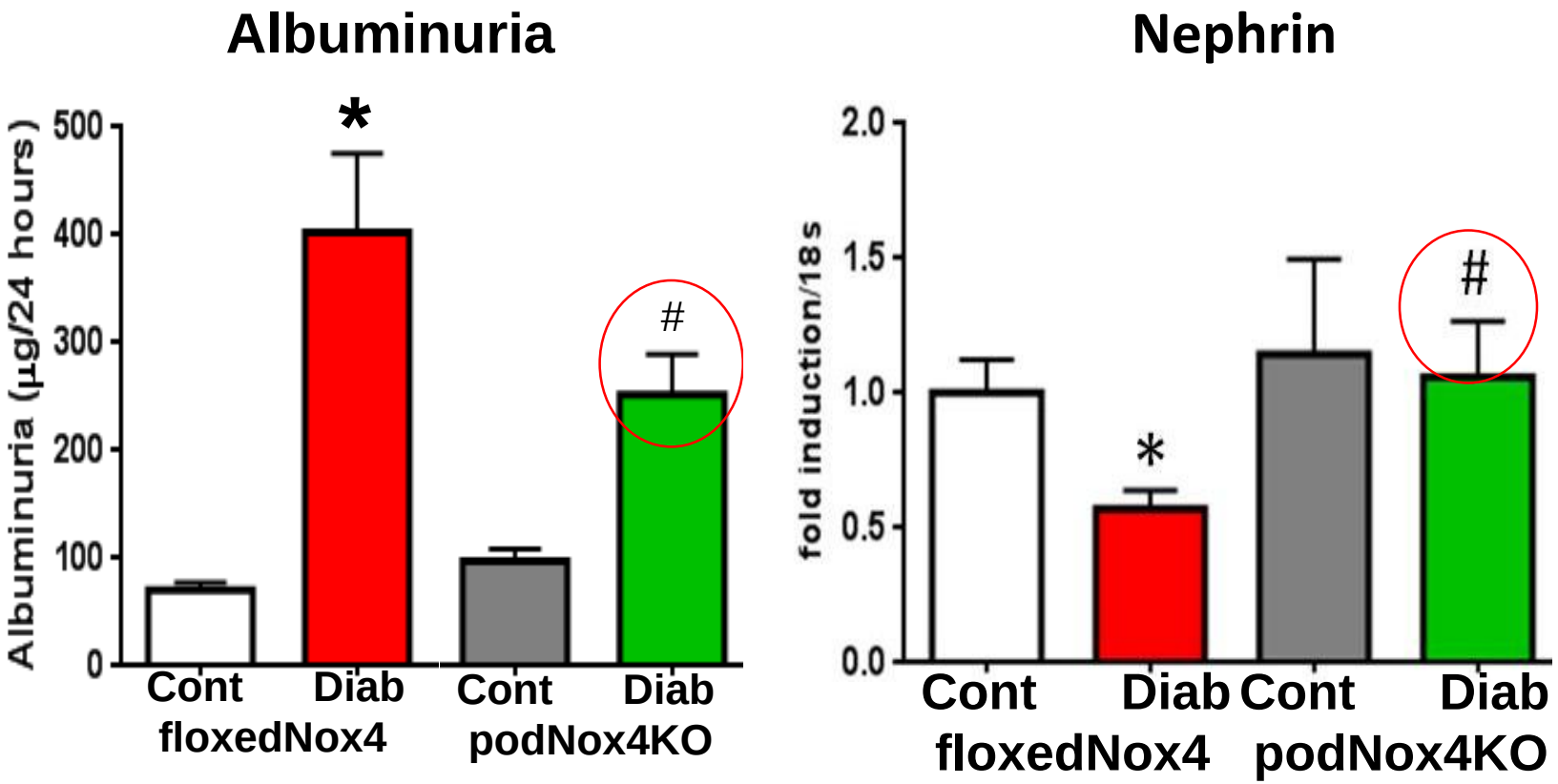
#p<0.05 vs Flox-Nox4

Colocalisation of Nox4 and podocin



JC Jha, Diabetologia 2016

Podocyte specific Nox4 deletion attenuates albuminuria in diabetes

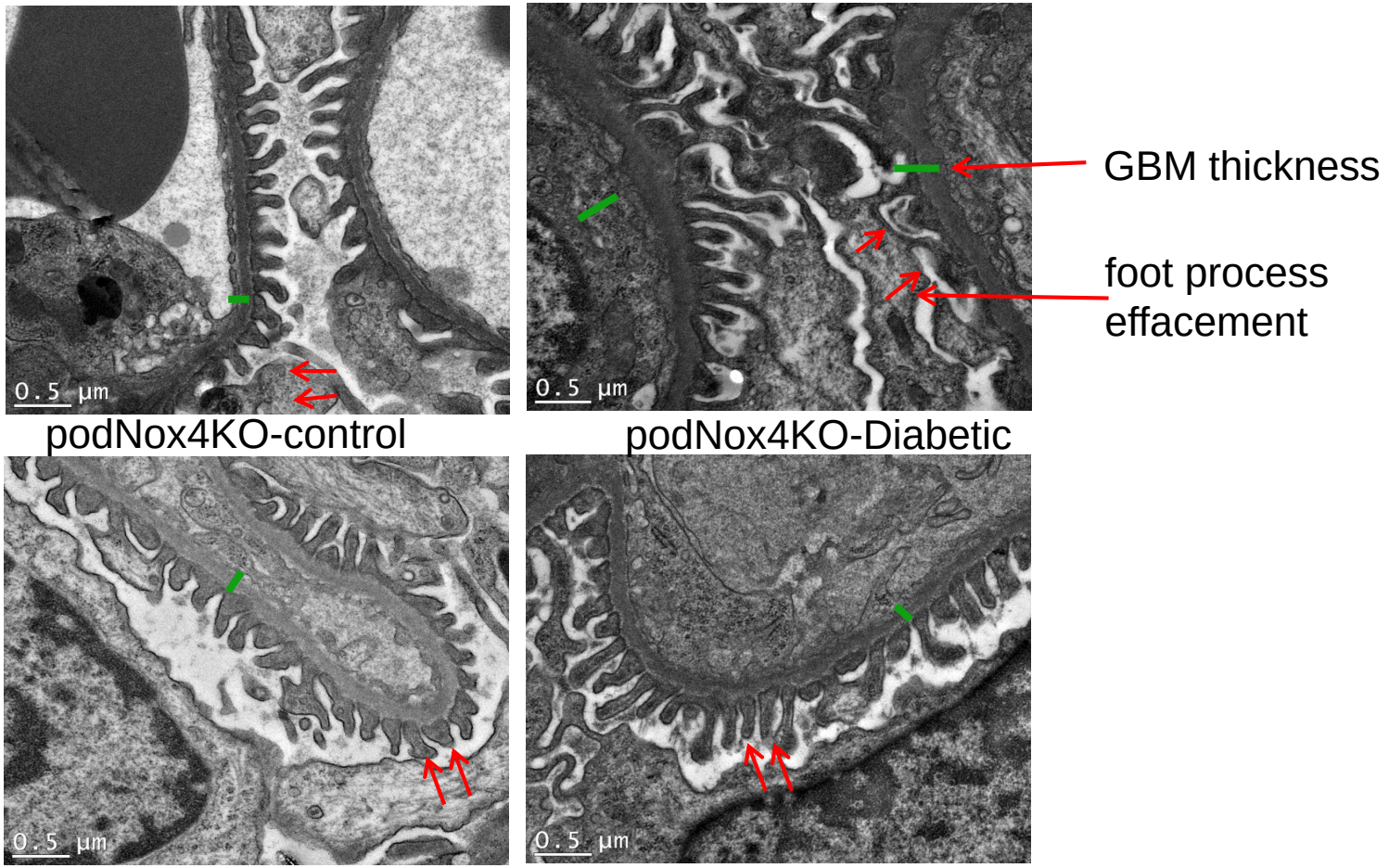


*p <0.05 vs respective non-diabetic controls
#p<0.05 vs floxedNox4 diabetic mice

JC Jha, Diabetologia 2016

Podocyte NOX4 in diabetes

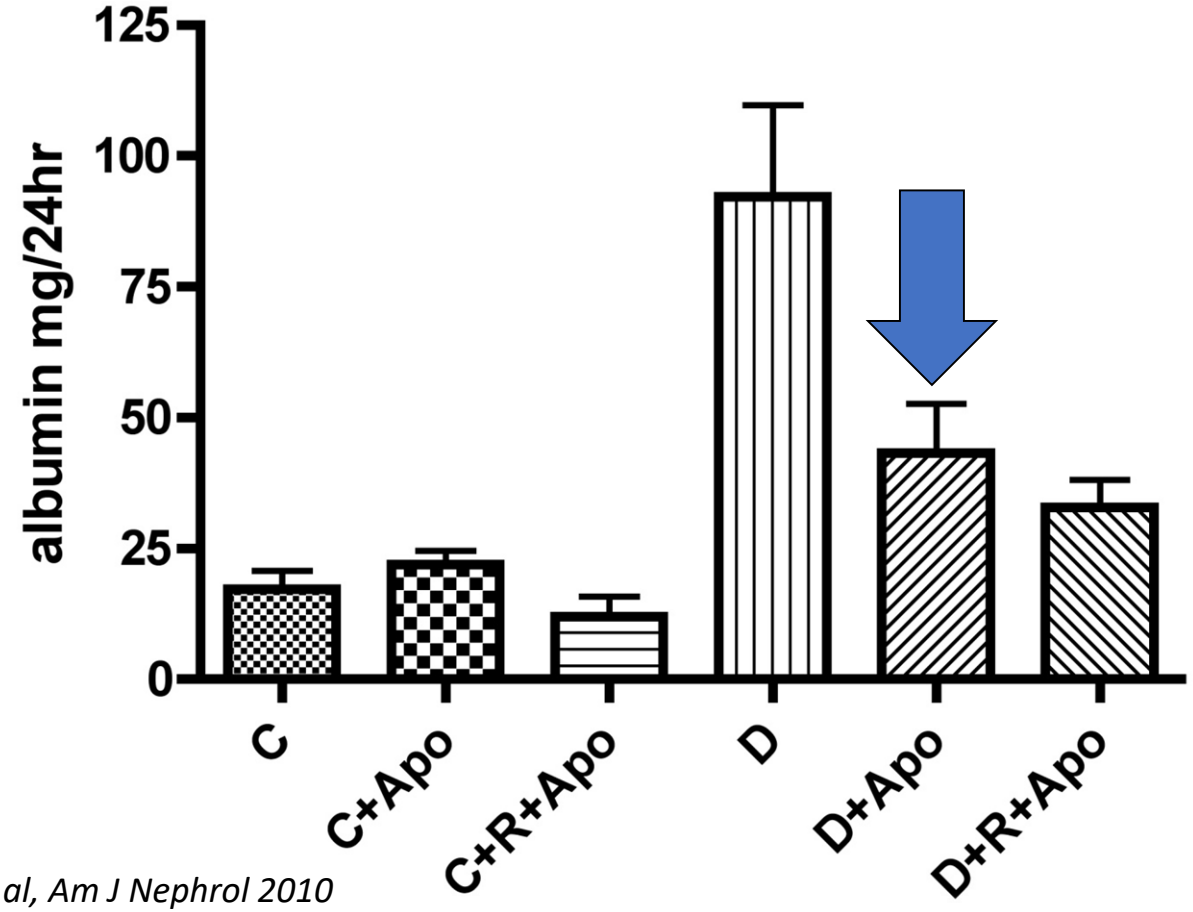
Podocyte specific Nox4 deletion attenuates glomerular basement membrane (GBM) thickness as well as reduces podocyte foot process effacement in diabetes



JC Jha, Diabetologia 2016

Podocyte NOX4 in diabetes

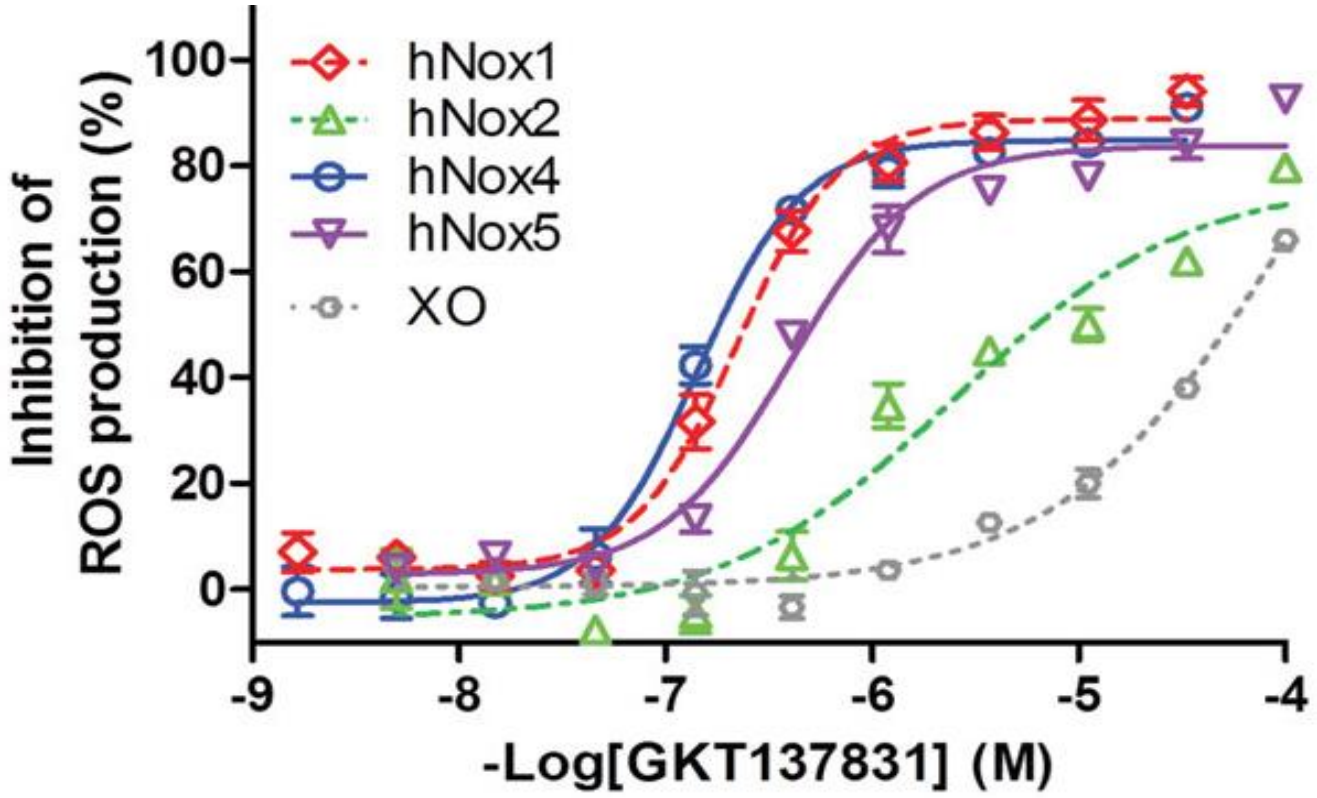
Non-specific ROS inhibition with apocynin reduces albuminuria in mice with STZ diabetes



Thallas-Bonke et al, Am J Nephrol 2010

NOX inhibition in diabetes?

First in- class Nox1/Nox4 Inhibitor: GKT137831 (Genkyotex SA, Switzerland)



•Specific •Selective •Orally bioavailable

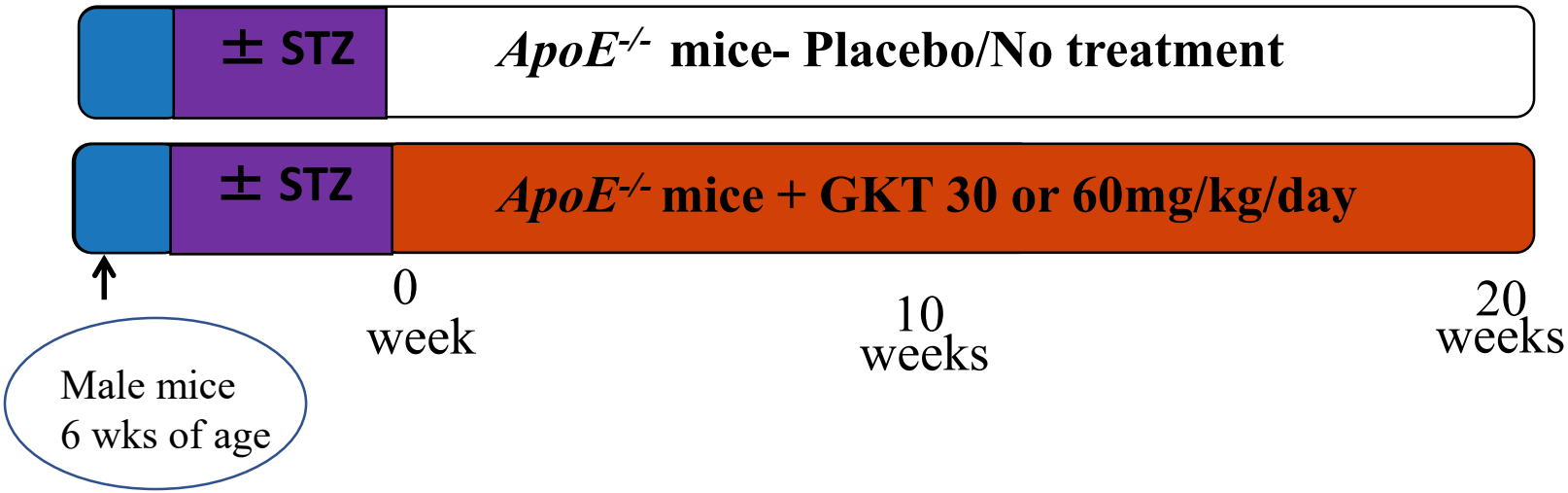
Aoyama T et al, Hepatology, 2012, 56:2311-27

Experimental design

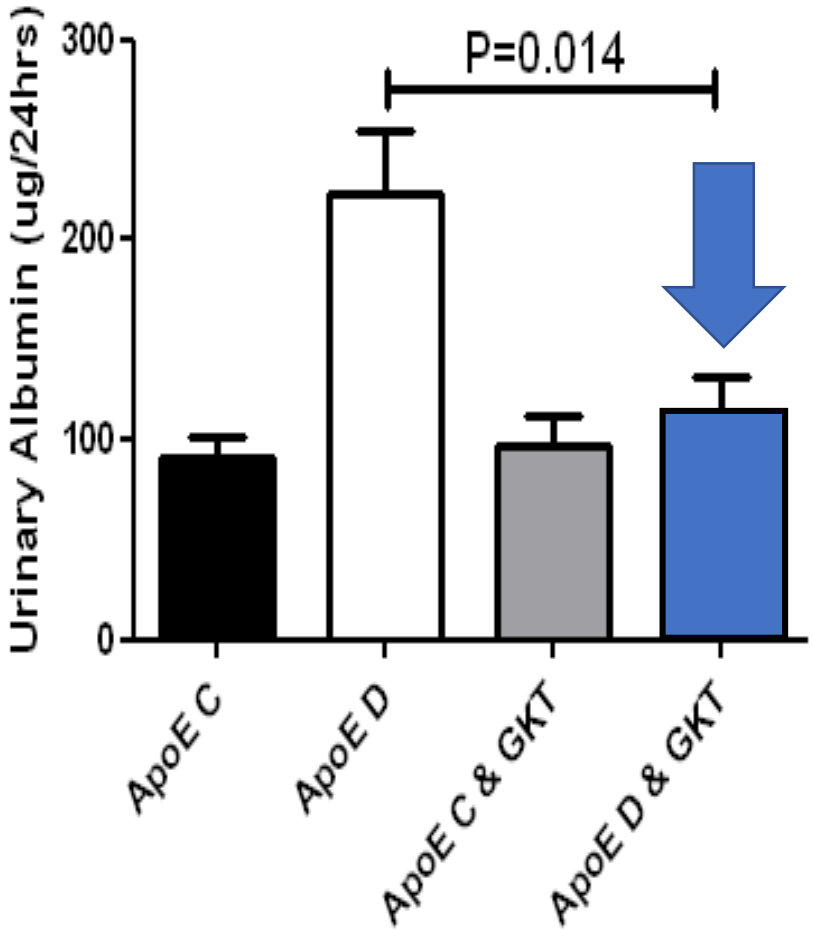
STZ: 55mg/kg
5 daily injections ip

Week 10 and 20

Metabolic caging for 24hrs
Urine collection
Blood sample & Cull
Kidney tissue collection

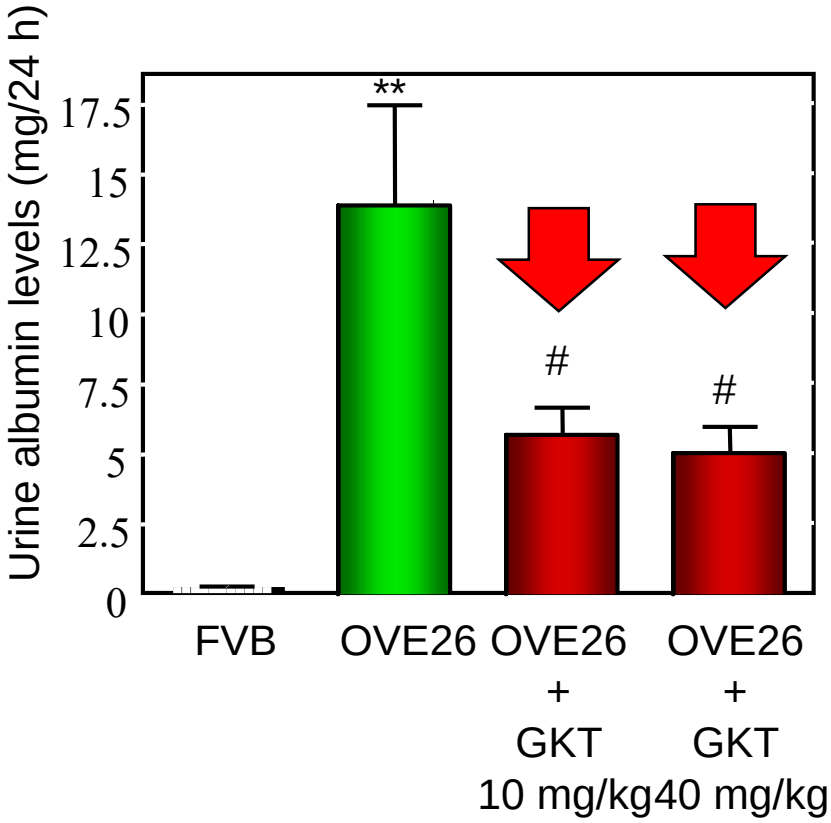


Nox inhibition with GKT reduces albuminuria in diabetic apoE KO mice



Jha et al. *JASN, 2014, 25(6):1237-54*

in OVE26 mice

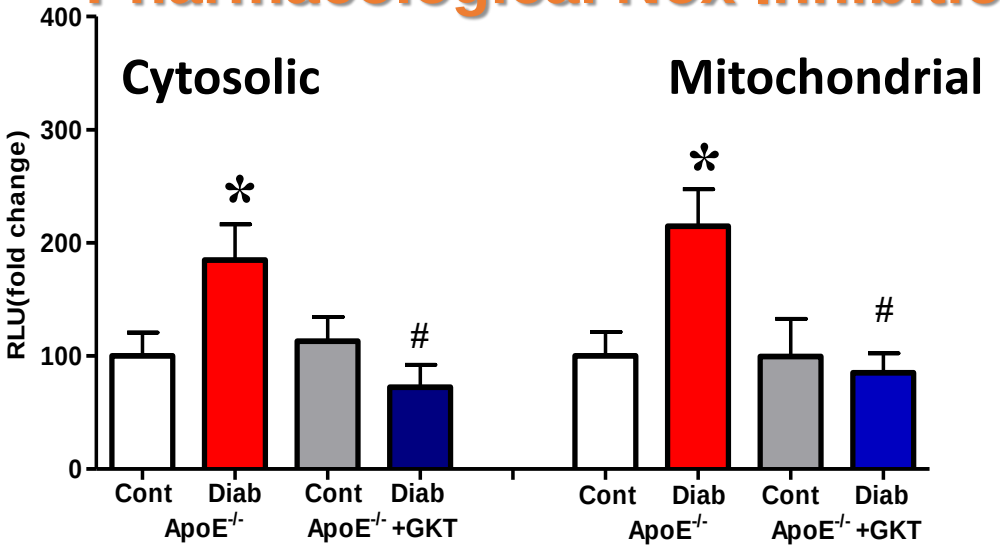


Gorin et al. *Am J Physiol, 2015, 308:F1276-87*

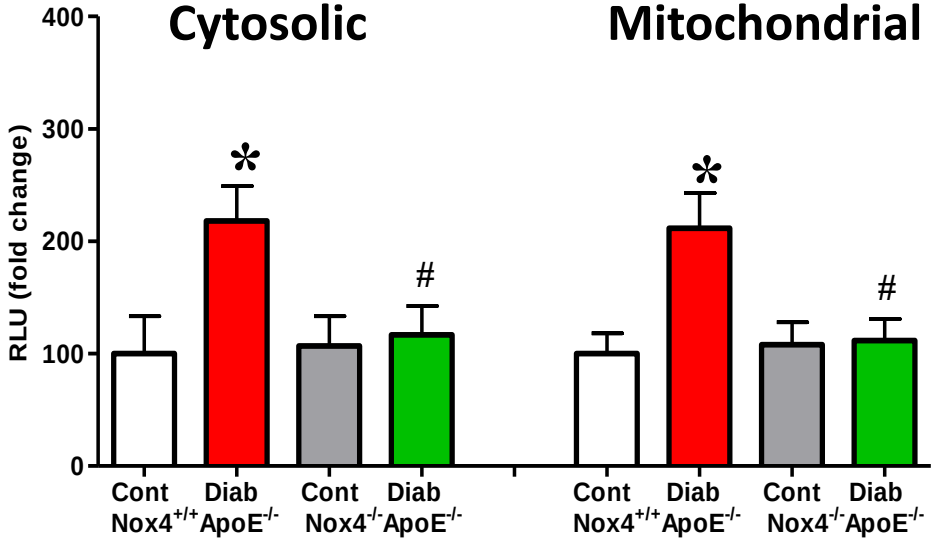
NOX inhibition in diabetic nephropathy

Reactive Oxygen Species (ROS)

Pharmacological Nox Inhibition



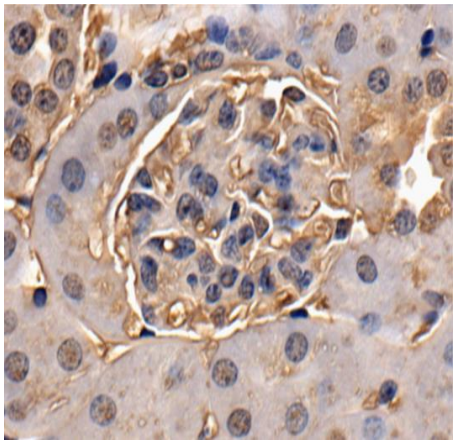
Nox4 Deletion



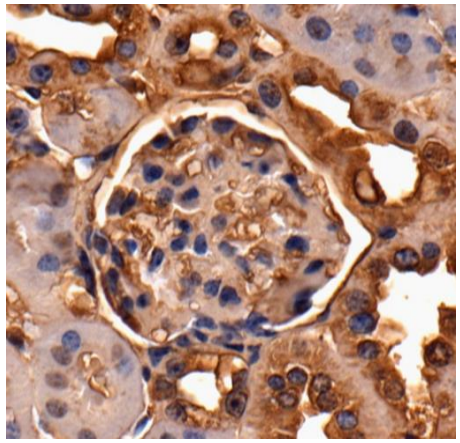
NOX inhibition attenuates renal fibrosis

Fibronectin

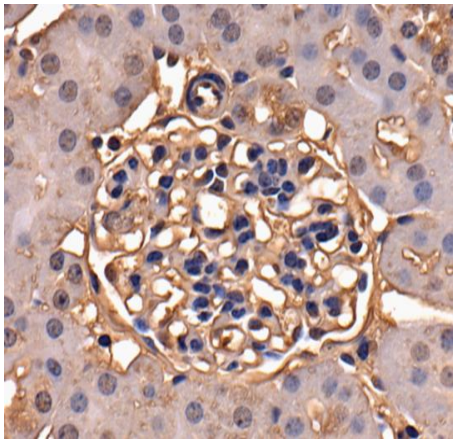
Control



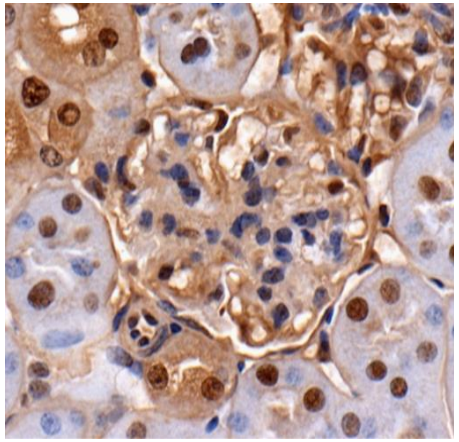
Diabetes



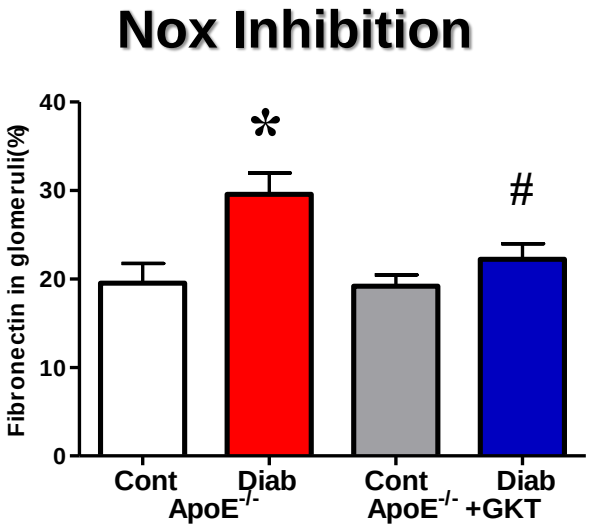
Nox inhibition (GKT137831)



Control

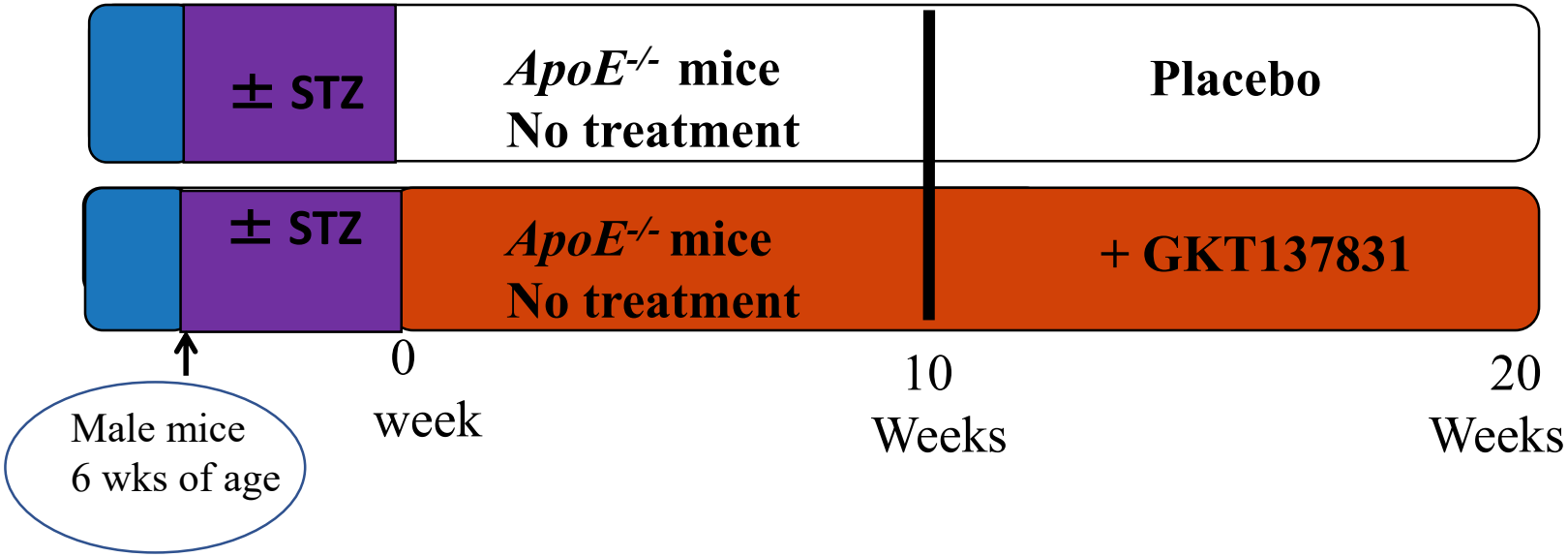


Diabetes

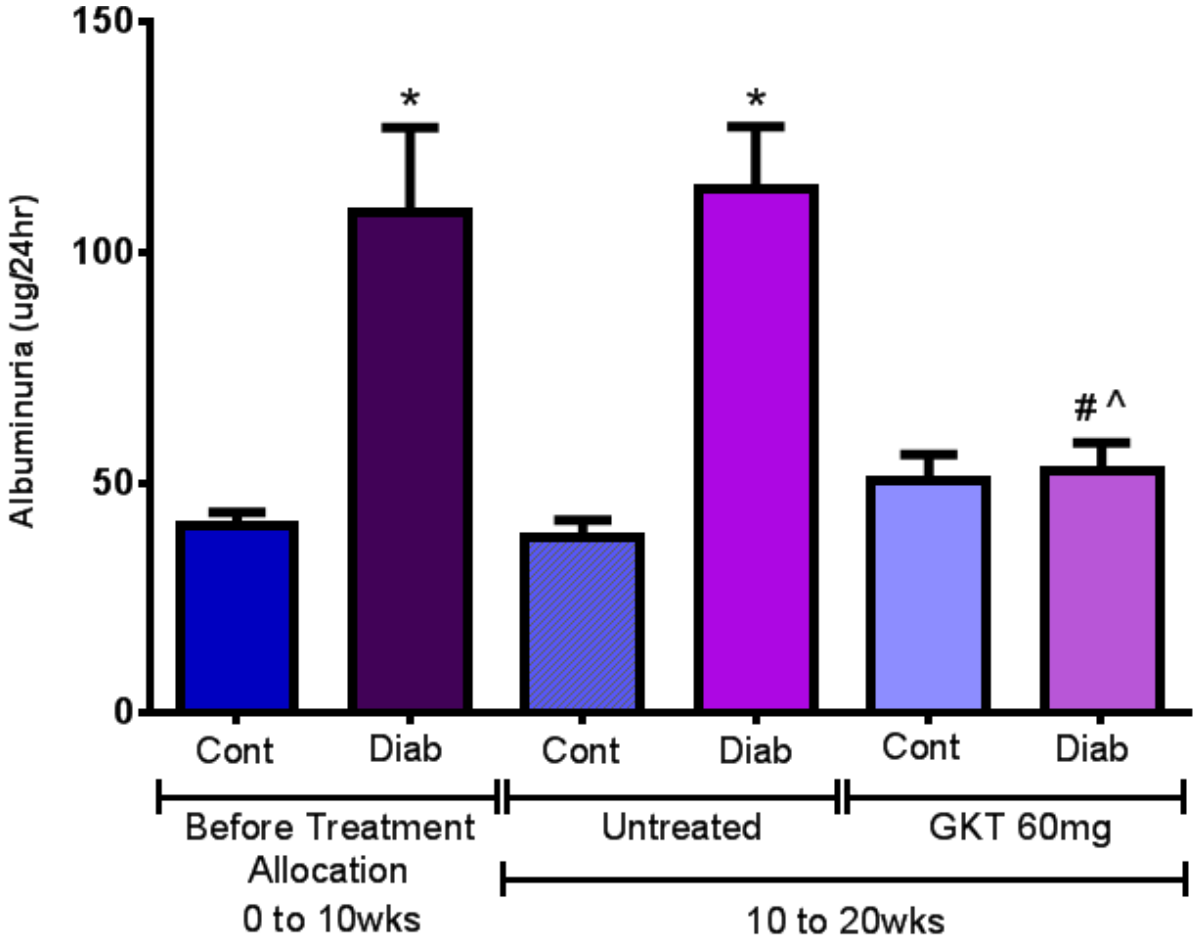


Jha et al, JASN (2014)

Delayed intervention in established nephropathy



Nox inhibition attenuates albuminuria in established disease



*P<0.05 v Control before allocation. #P<0.05 v Diabetic before allocation. ^P<0.05 v Untreated Diabetic

S Gray et al, Diabetologia 2017

Clinical translation

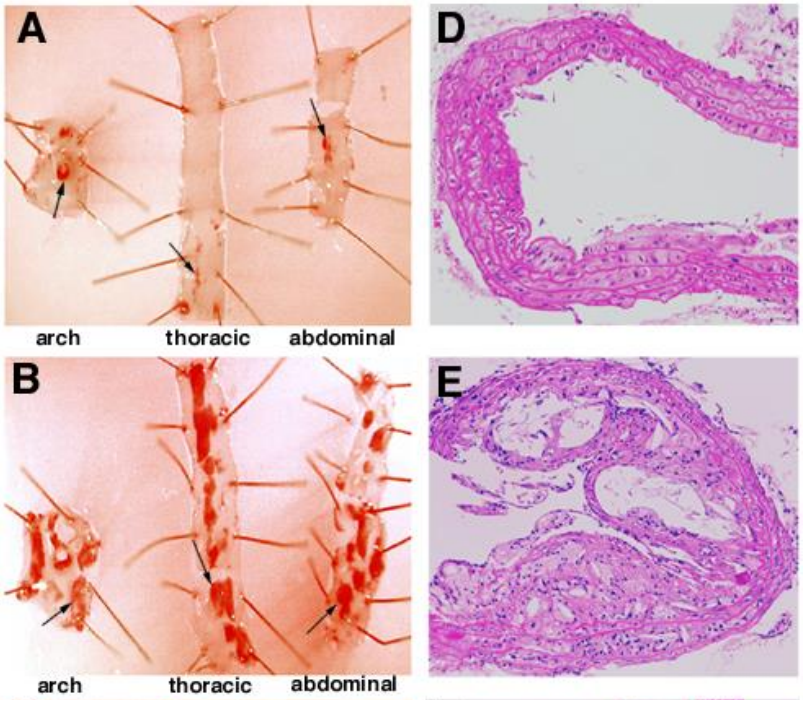
GKT137831 in phase IIb clinical trial in type2 diabetic nephropathy with albuminuria despite maximal RAS blockade

NCT02010242, Genkyotex SA

9th Sept2015: Safety confirmed, reduction in inflammatory markers, albuminuria unchanged

JDRF/NHMRC Australia funded CONCEPT-study:
A double-blind randomised placebo-controlled Phase IIa study evaluating the safety and efficacy of inhibition of NADPH oxidase With GKT137831 in adults with type1 diabetes and elevated albumin excretion

Macrovascular Disease:
Diabetes- accelerated atherosclerosis

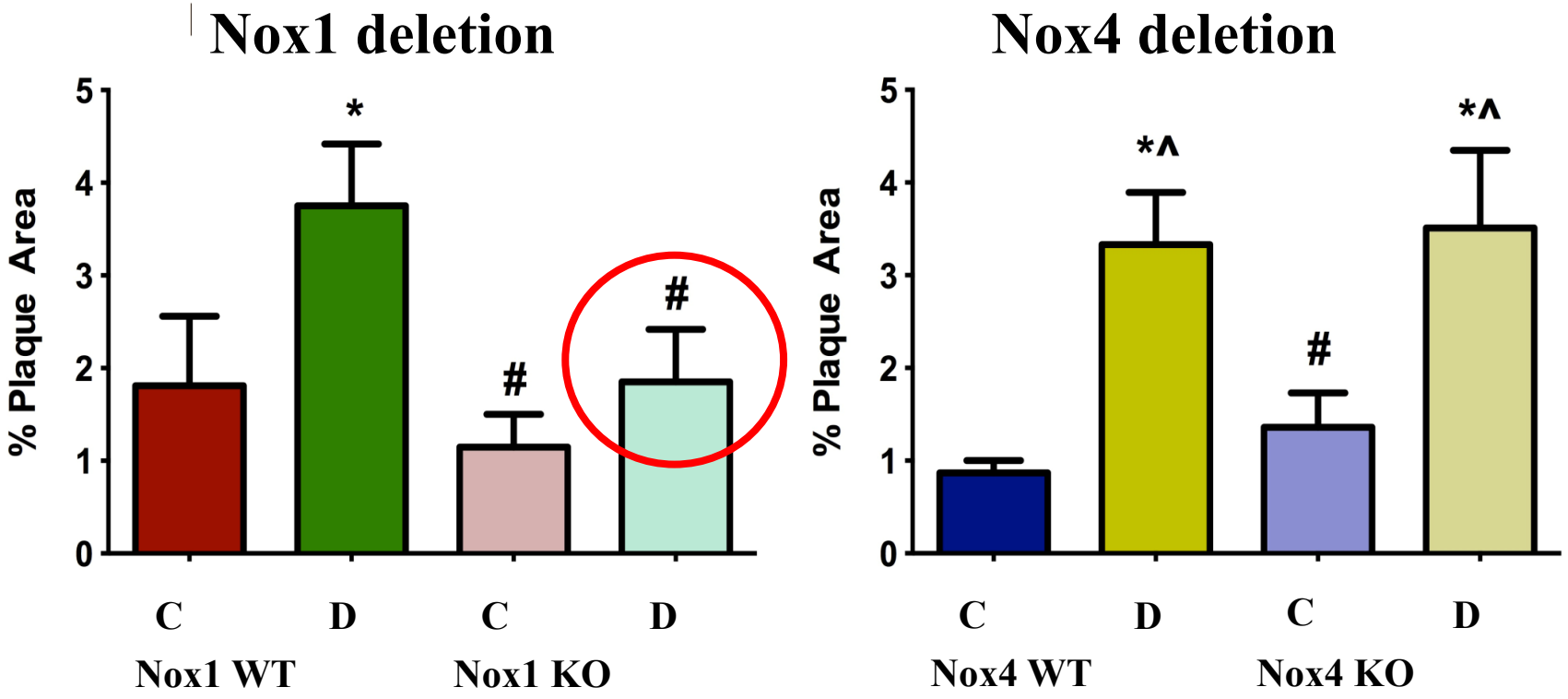


Control

Diabetes

Candido R et al, Circulation, 2012

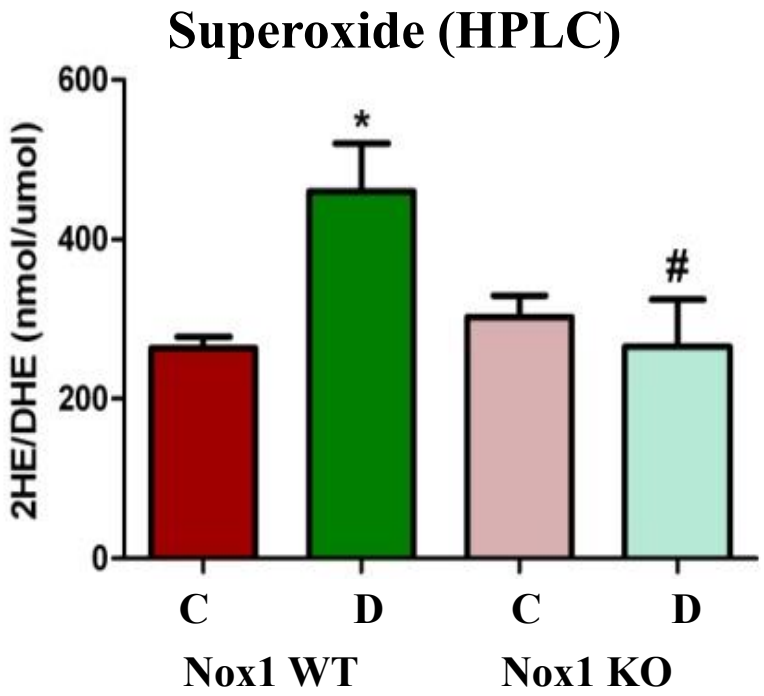
Atherosclerosis is attenuated in diabetic Nox1 deficient mice

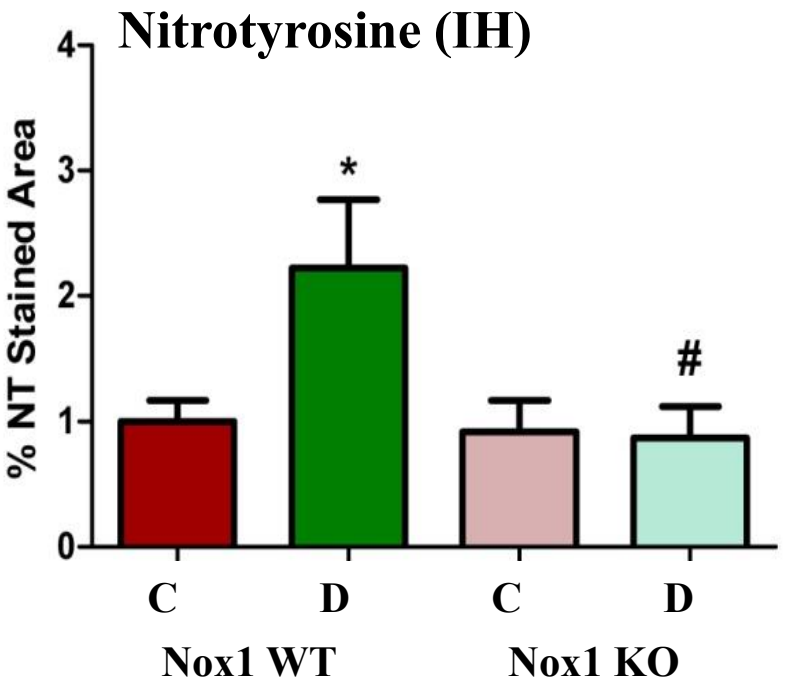


S Gray et al, Circulation, 2013,127:1888-902

All data are Mean±SEM, N=8-10 per group, each study analysed seperatley using one-way ANOVA with a LSD post-hoc test. $P<0.05$ * v WT Control, # v WT Diabetic.

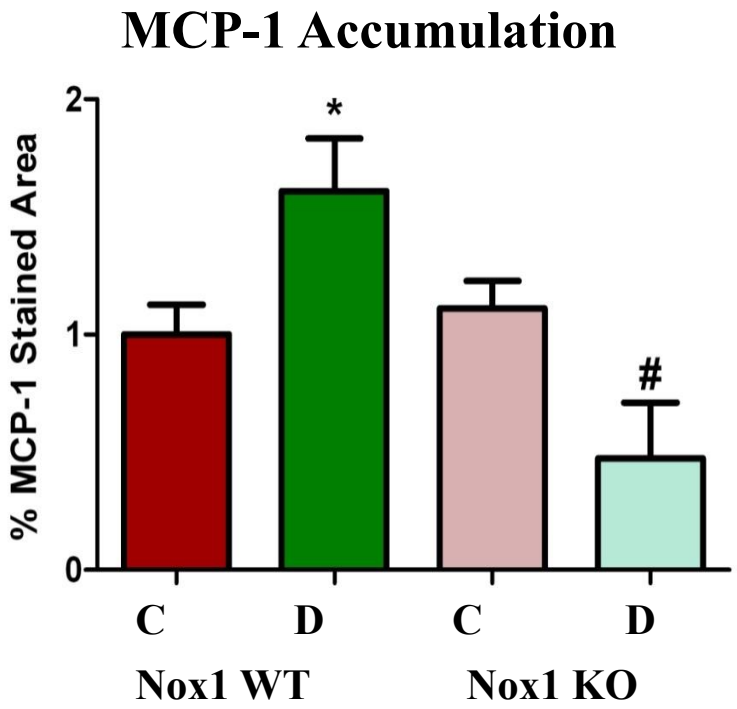
Nox1 deletion: reduced aortic ROS

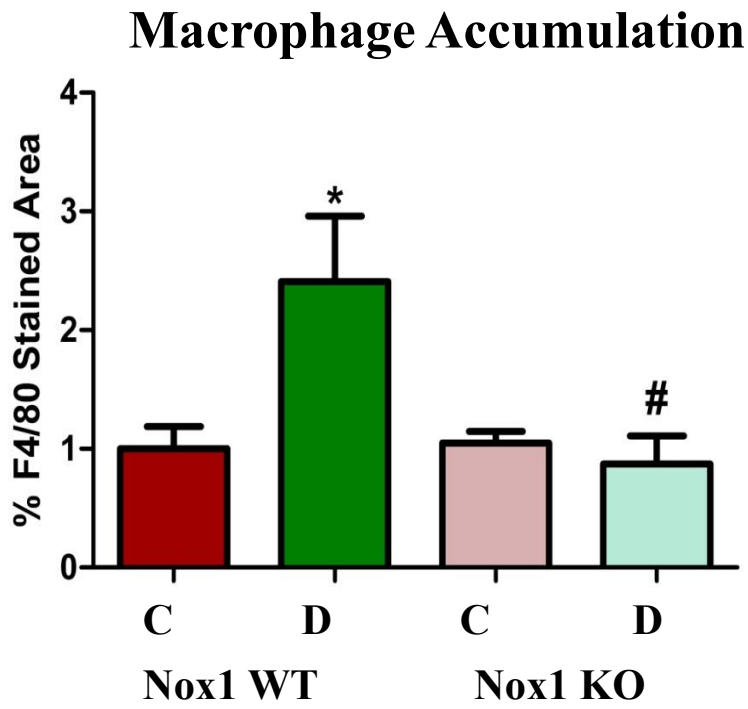




All data are Mean±SEM, N=8-10 per group, each study analysed seperatley using one-way ANOVA with a LSD post-hoc test. $P<0.05$ * v WT Control, # v WT Diabetic.

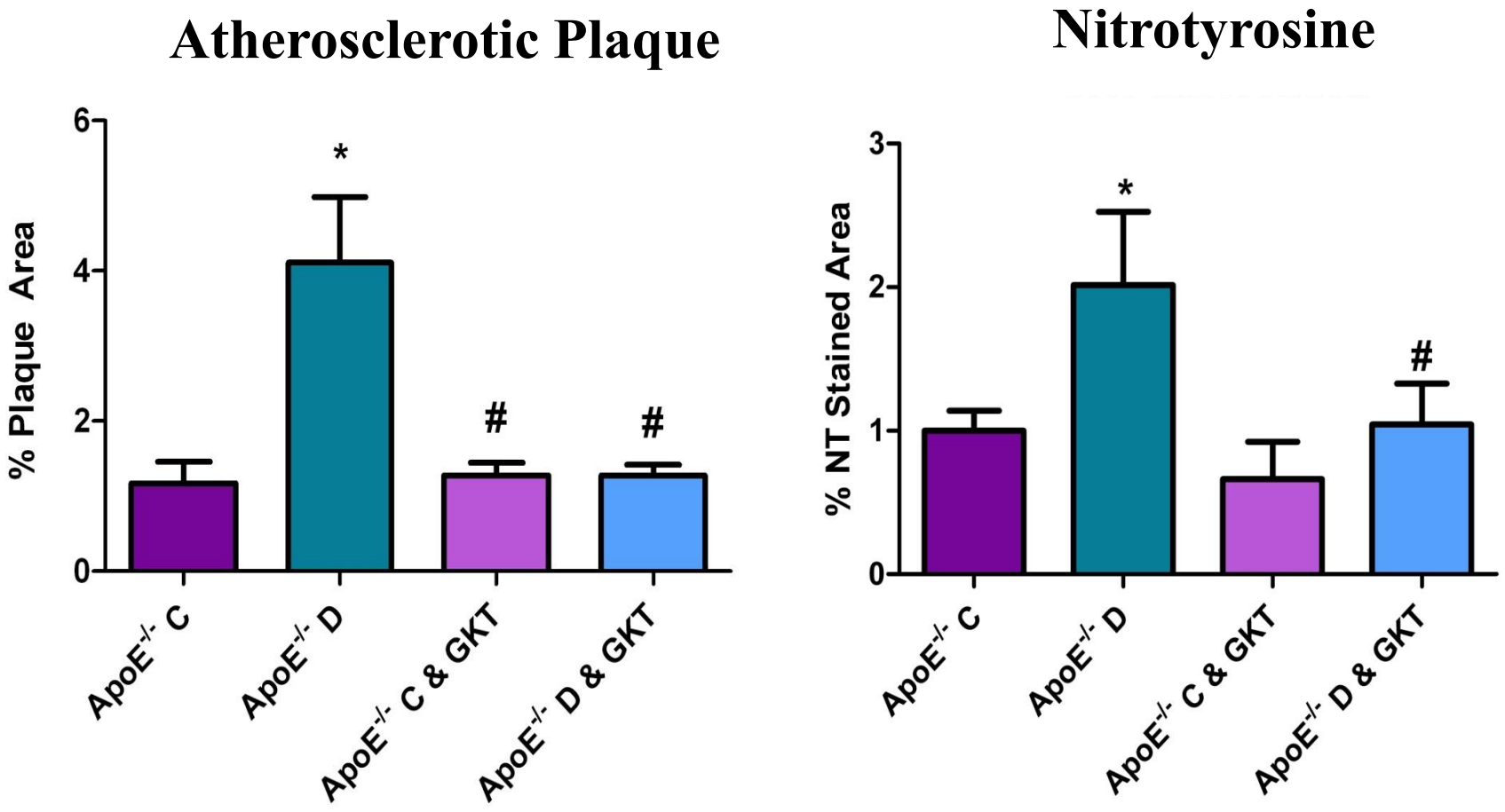
Nox 1 Deletion: attenuation of MCP-1 and macrophage accumulation



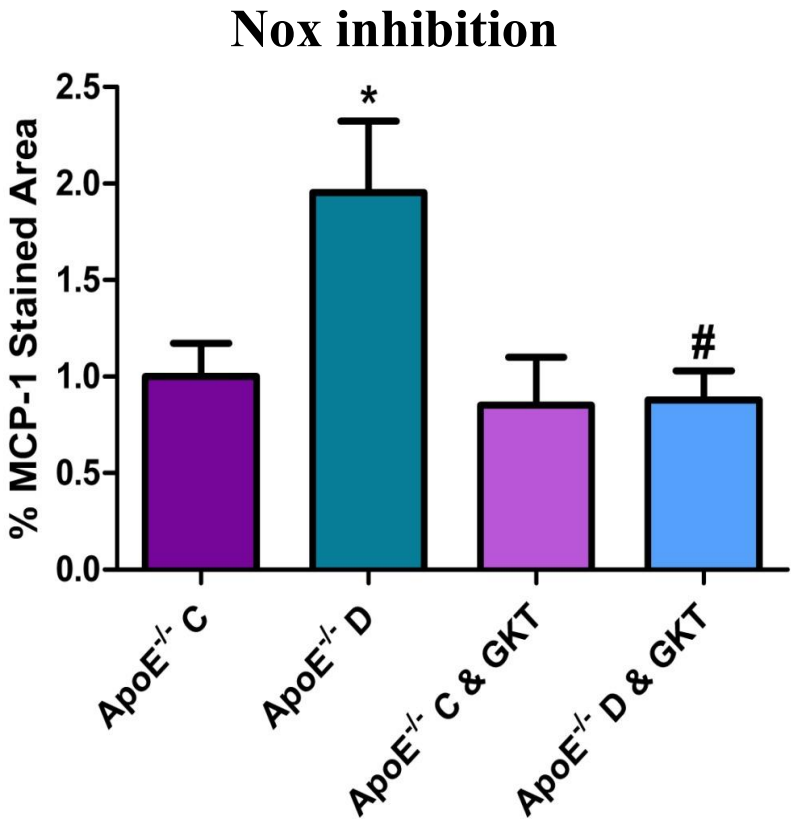


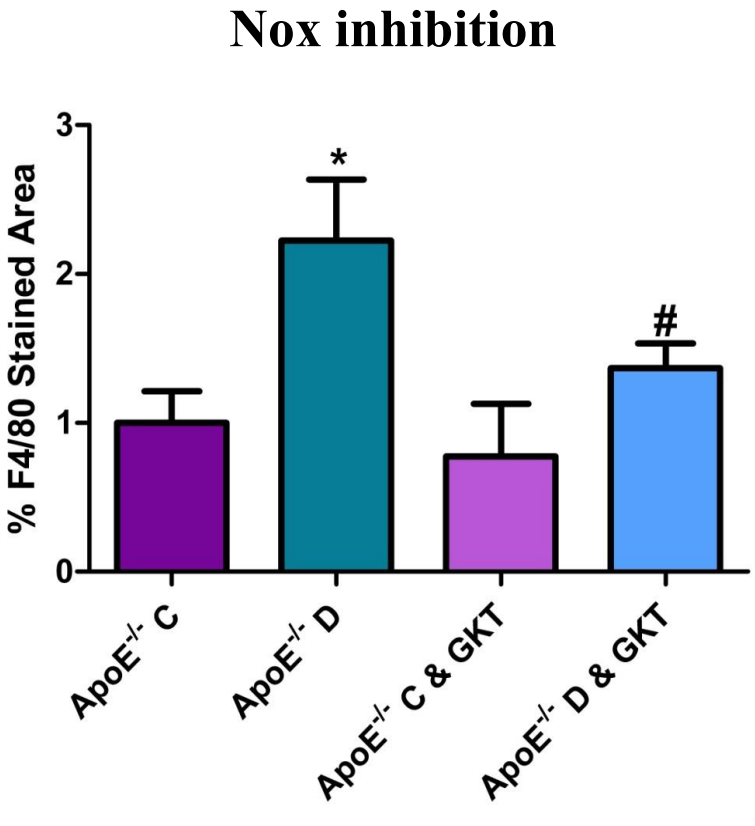
All data are Mean±SEM, N=8-10 per group, each study analysed seperatley using one-way ANOVA with a LSD post-hoc test. $P<0.05$ * v WT Control, # v WT Diabetic.

Pharmacological Nox inhibition attenuates plaque formation



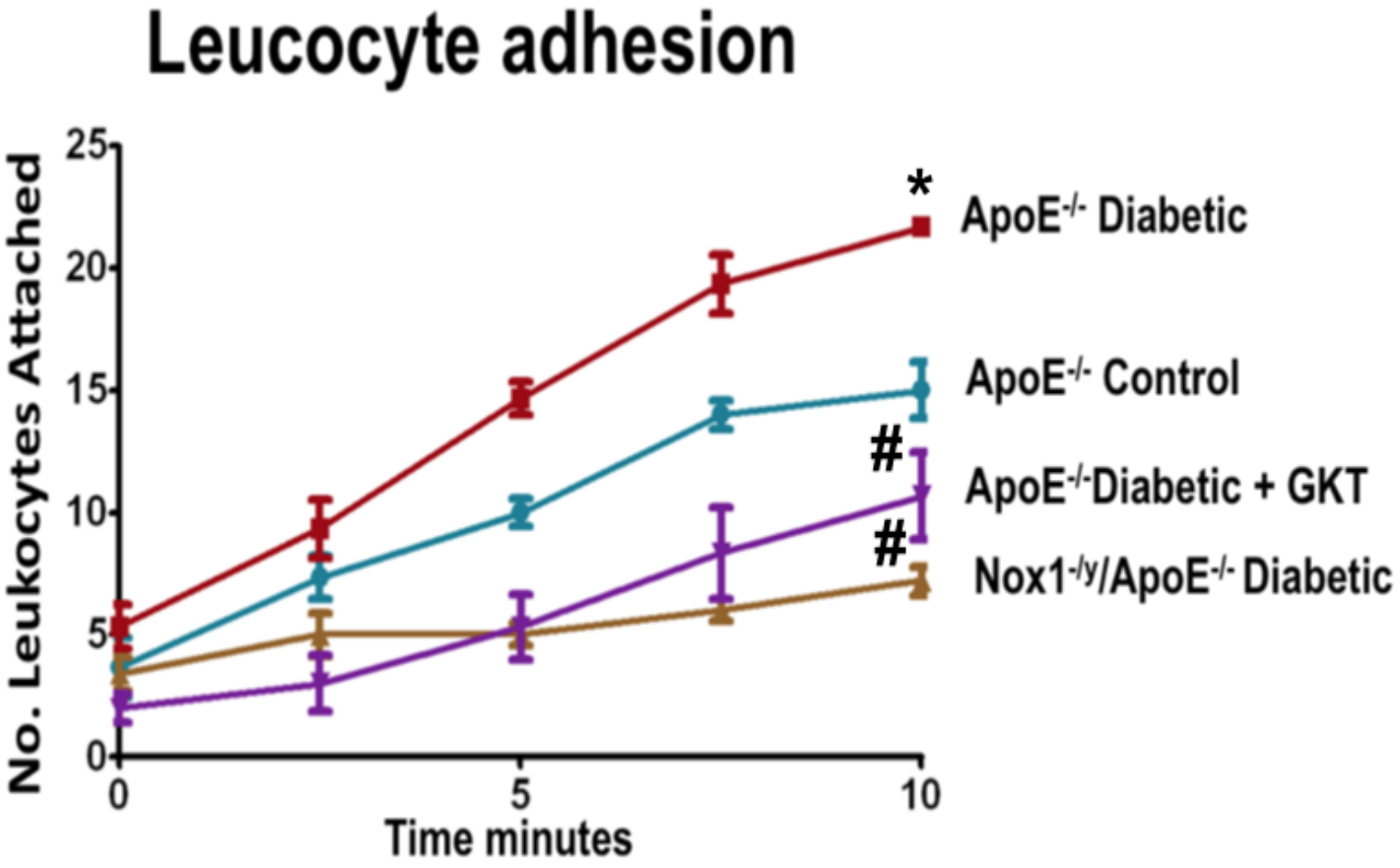
MCP-1 Macrophage accumulation





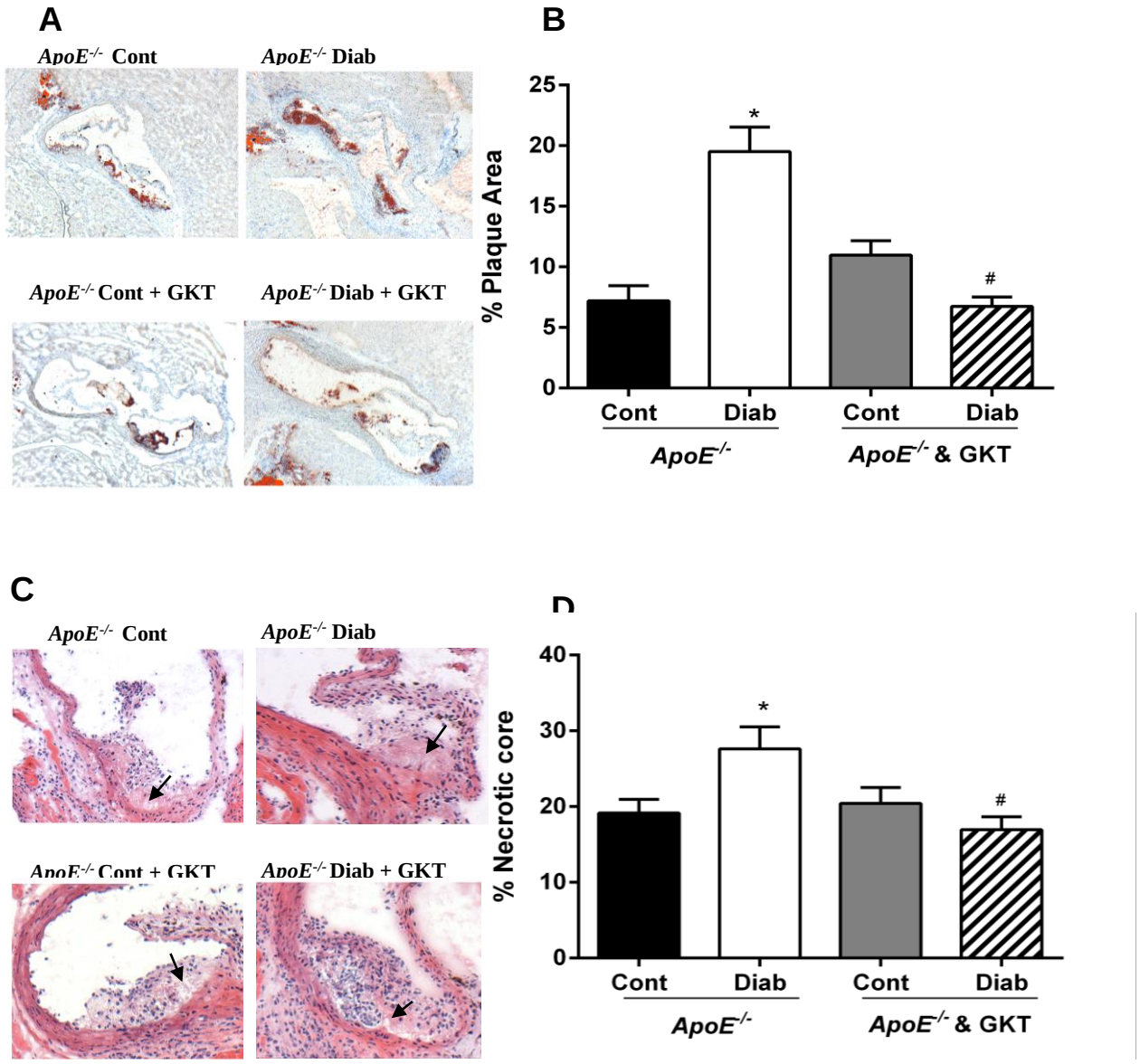
All data are Mean±SEM, N=8-10 per group, data analysed using one-way ANOVA with a LSD post-hoc test. $P<0.05$ * v Normal Glucose Non-Target, # v High Glucose Non-Target.

Vascular Adhesion is reduced in Nox1 deleted mice and in *ApoE*$^{-/-}$ mice treated with the Nox inhibitor GKT137831

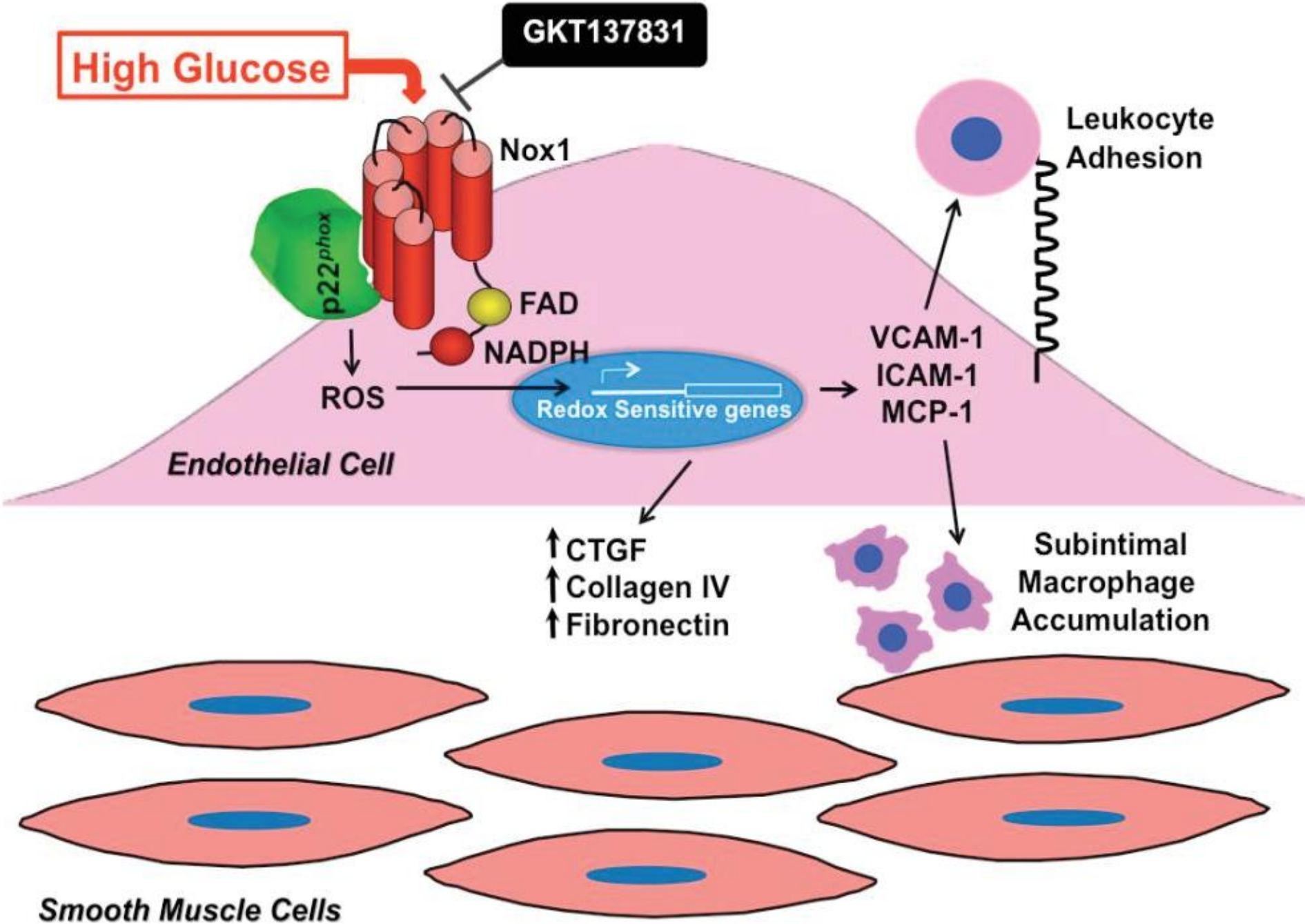


S Gray et al, Circulation, 2013,127:1888-902

Nox inhibition improves plaque stability

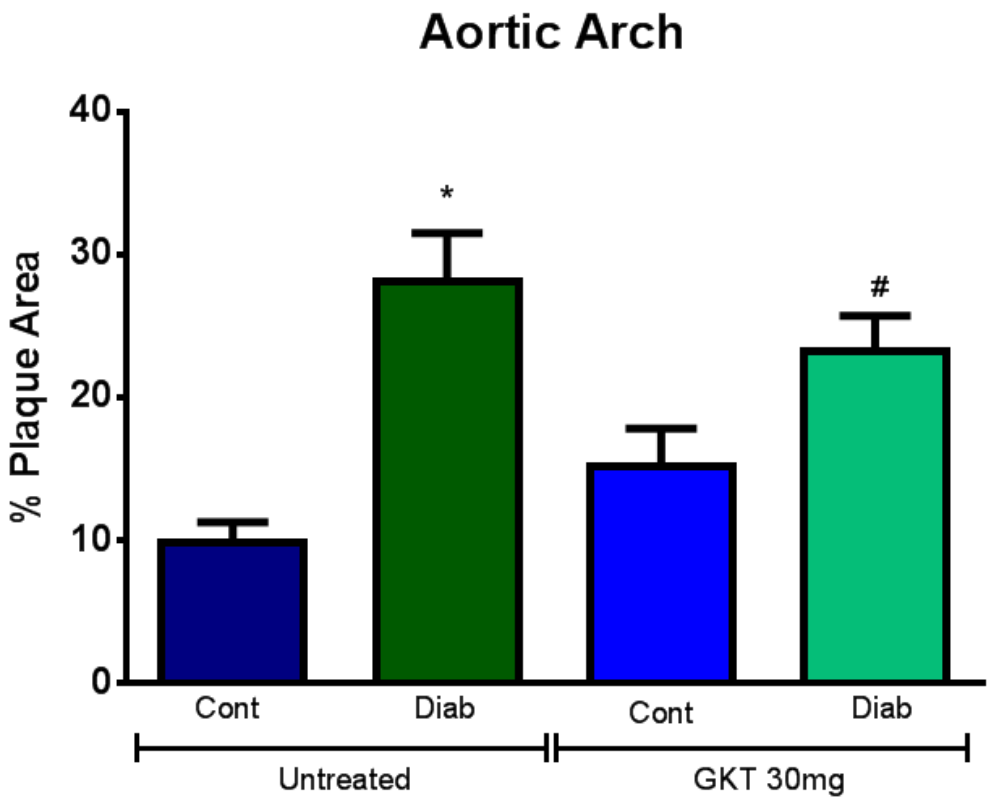


E DiMarco et al, Diabetologia, 2014, 57:633-42



Itani HA et al, Circulation, 2013, 127:1850-2

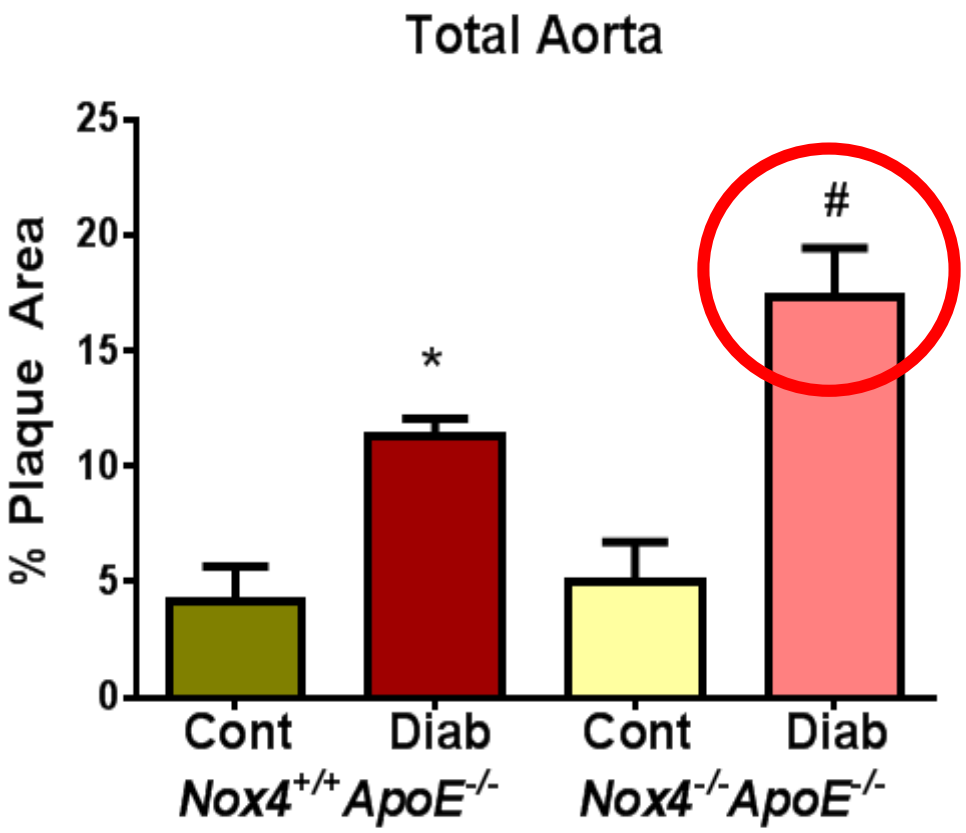
Nox inhibition in established atherosclerosis

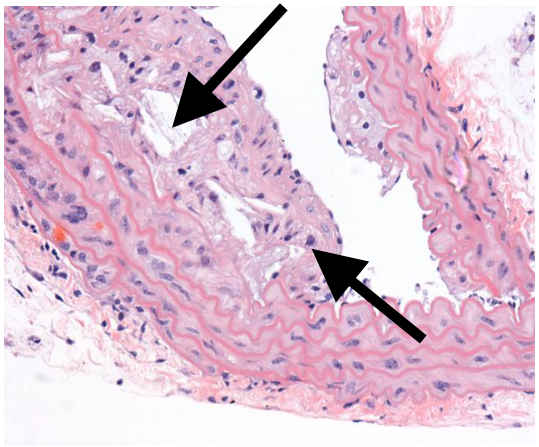


Stats - *P<0.05 v Untreated Control. #P<0.05 v Untreated Diabetic

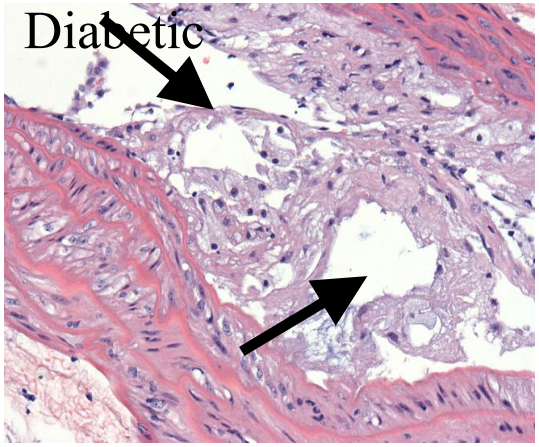
S Gray et al, Diabetologia 2017

However, in longterm diabetes, deletion of Nox4 accelerates atherosclerosis





Nox4$^{+/+}$*ApoE*$^{-/-}$ Diabetic

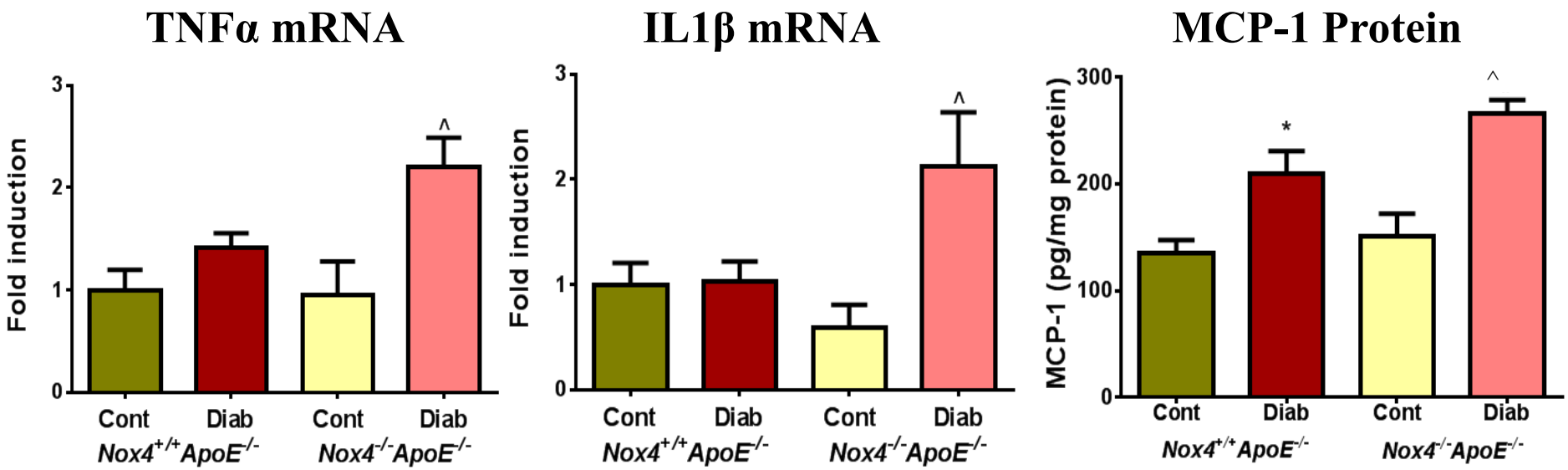


Nox4$^{-/-}$*ApoE*$^{-/-}$ Diabetic

Gray S et al, ATVB 2016

All data are Mean±SEM, N=8-10 per group, analysed using one-way ANOVA with a LSD post-hoc test. P<0.05 * v WT Control, ^ v WT Diabetic.

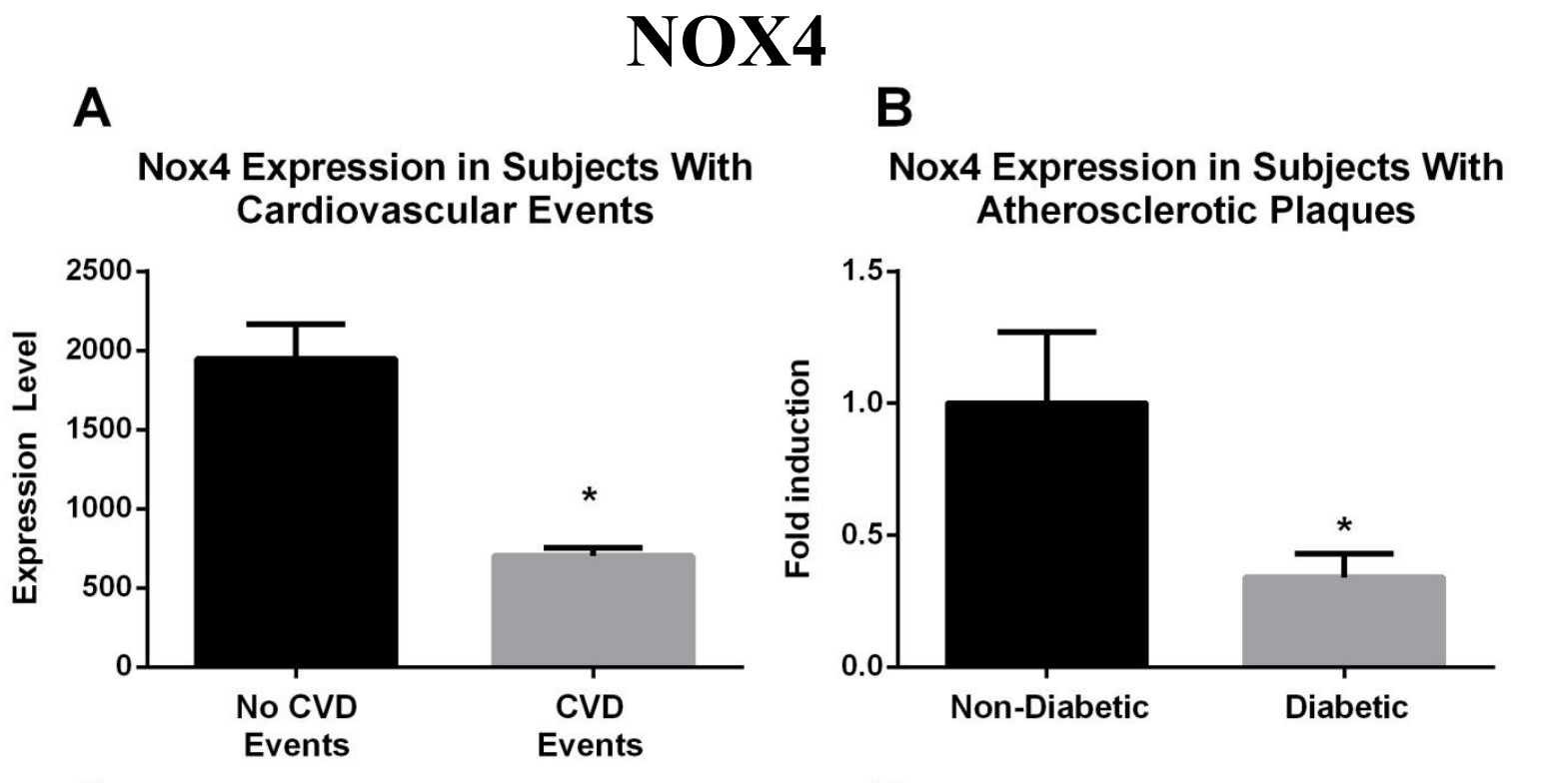
Increased aortic expression of pro-inflammatory and chemotaxis markers



Gray S et al, ATVB 2016

All data are Mean±SEM, N=8-10 per group, each study analysed separately using one-way ANOVA with a LSD post-hoc test. $P<0.05$ * v WT Control, ^ v WT Diabetic.

Nox 4 is reduced in advanced plaques in *patients*



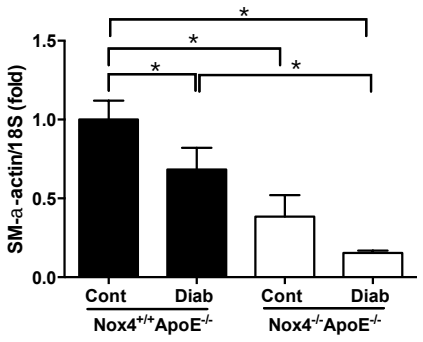
University of Maastricht

Biobank, Baker IDI Heart & Diabetes Institute

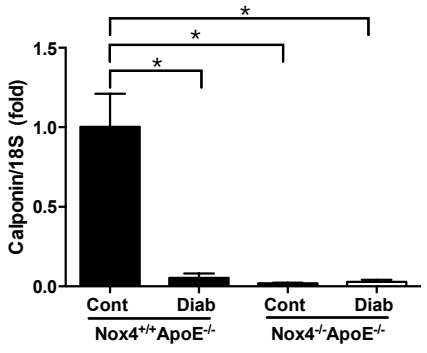
Nox4 deletion mediates a phenotypic switch in VSMCs

Contractile → Synthetic

SM- actin



Calponin



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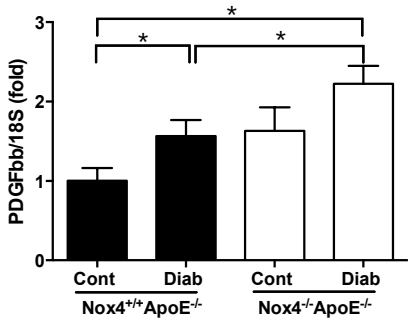
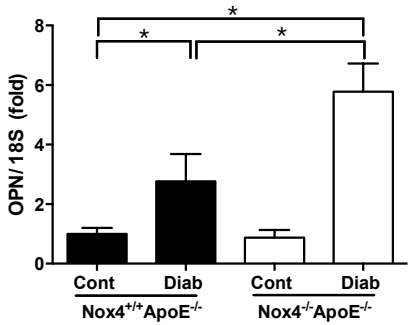
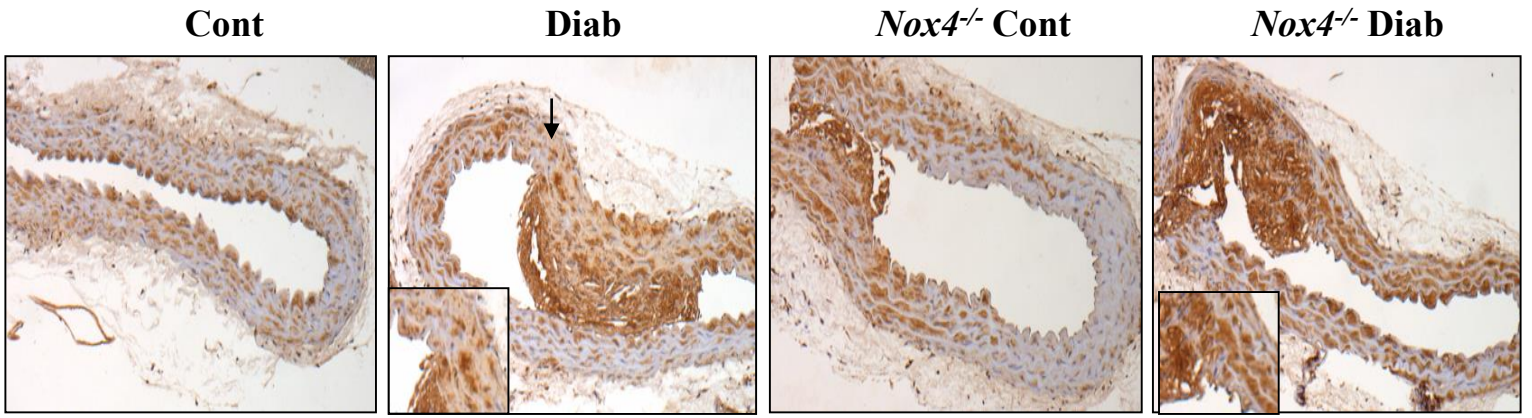


Osteopontin

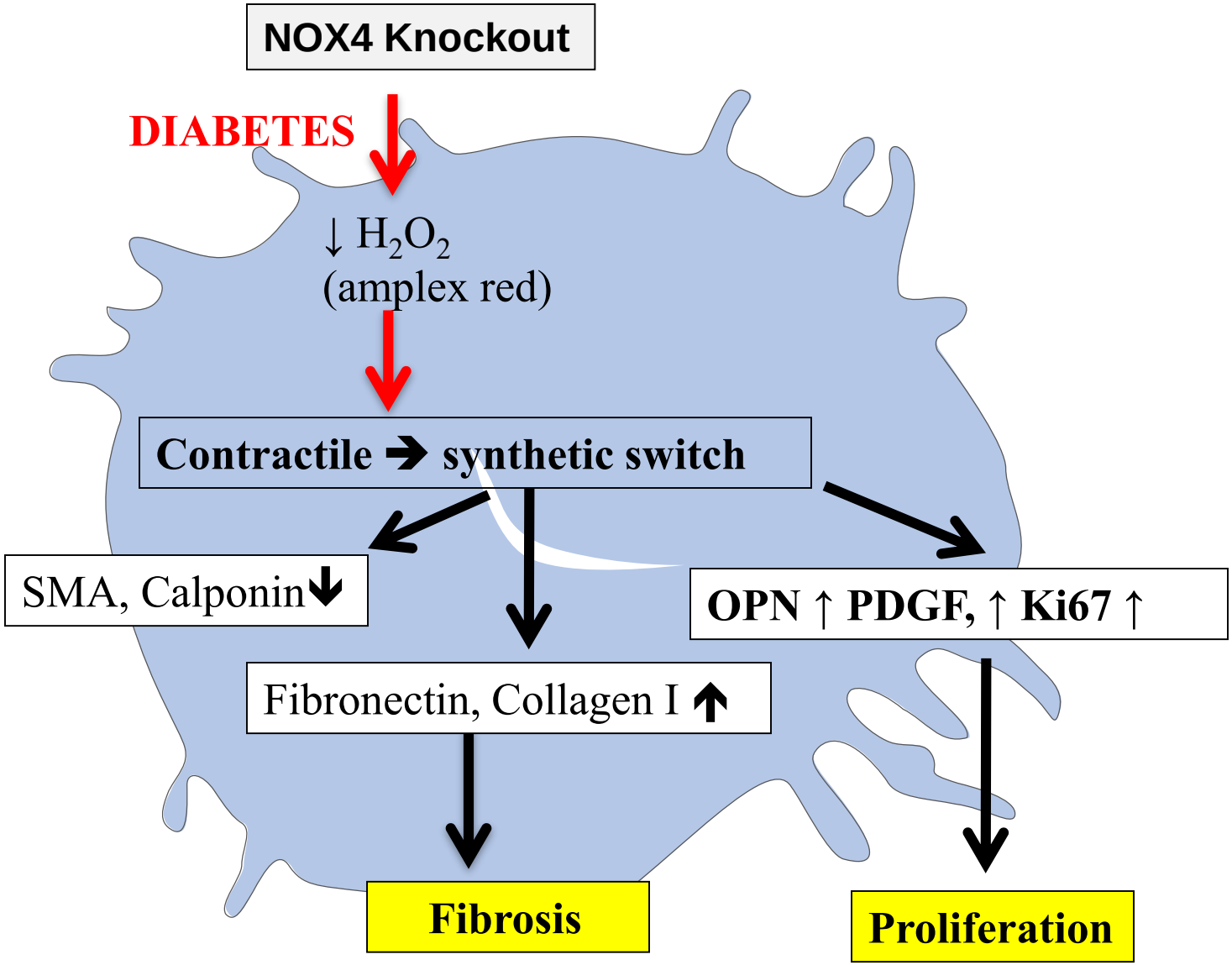


Osteopontin



E Di Marco et al, Free Radic Biol med 2016

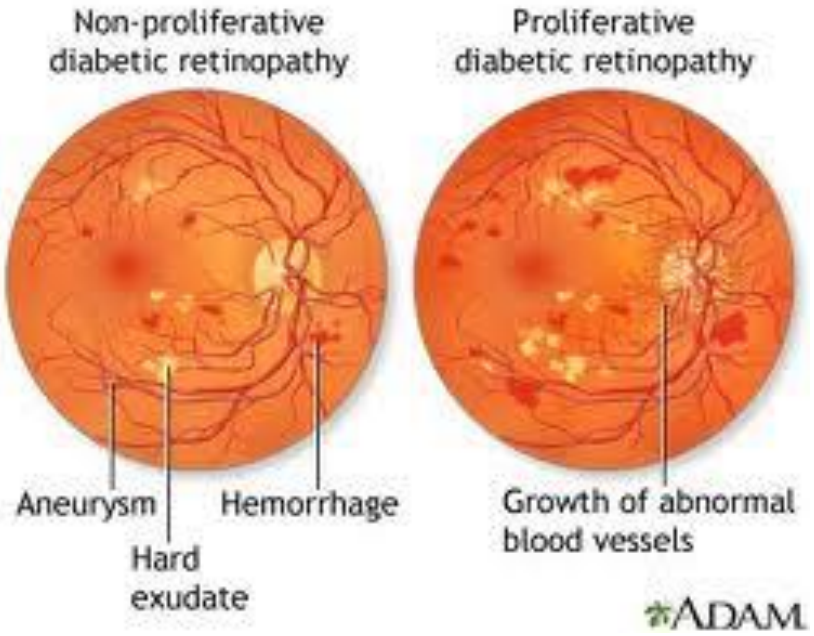
Vascular smooth muscle cell



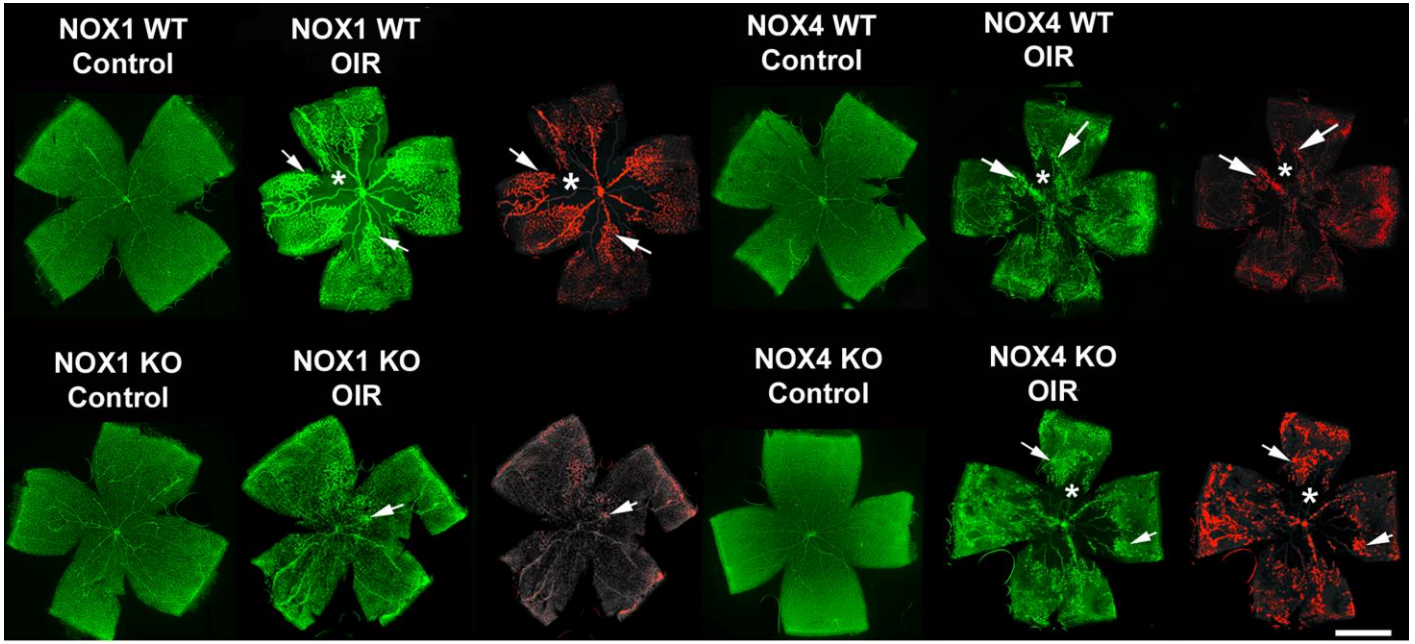
E Di Marco et al, Free Radic Biol med 2016

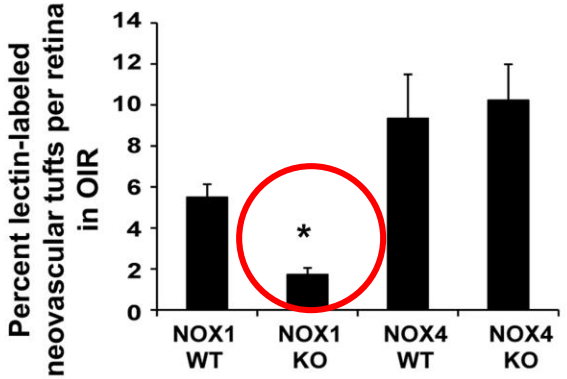
Retinopathy

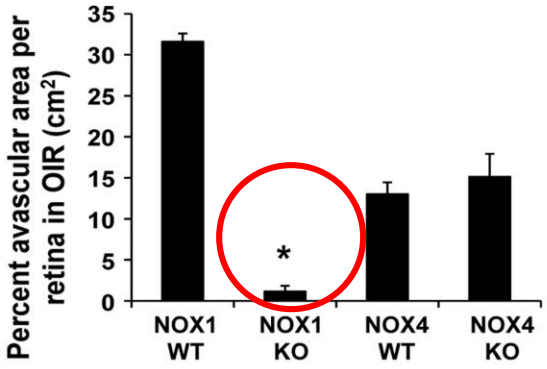
Almost everyone with type 1 diabetes and 60% of those with type 2 diabetes will develop some form of diabetic eye disease within 20 years of diagnosis



Deficiency in Nox1 reduces neovascularization in ischemic retinopathy

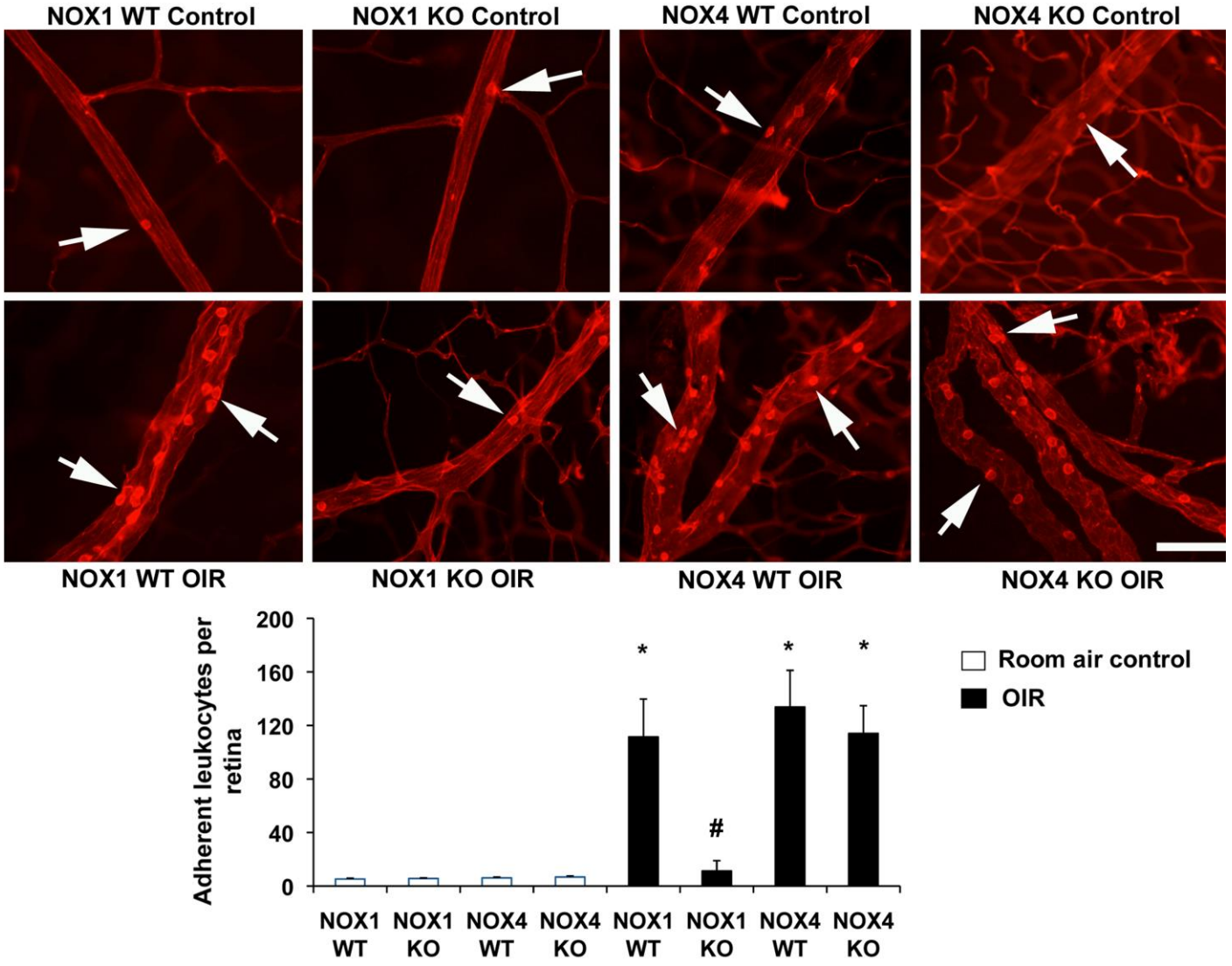






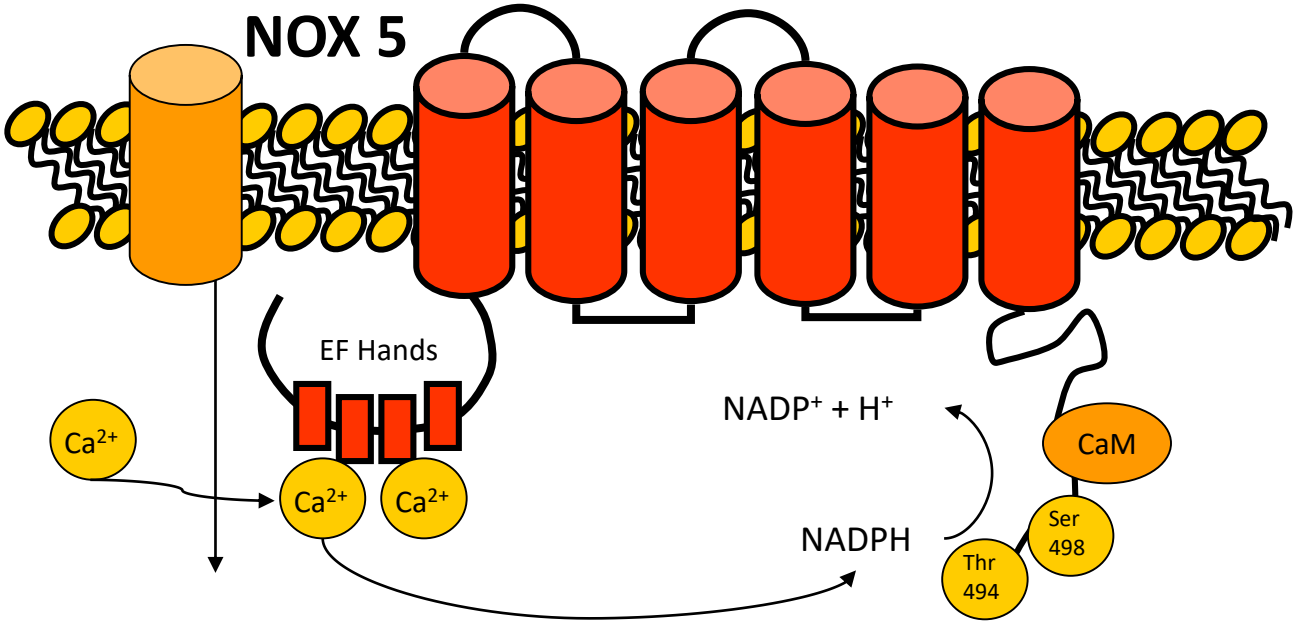
J Wilkinson-Berka, Antiox Redox Signal 2014

Leucocyte adhesion



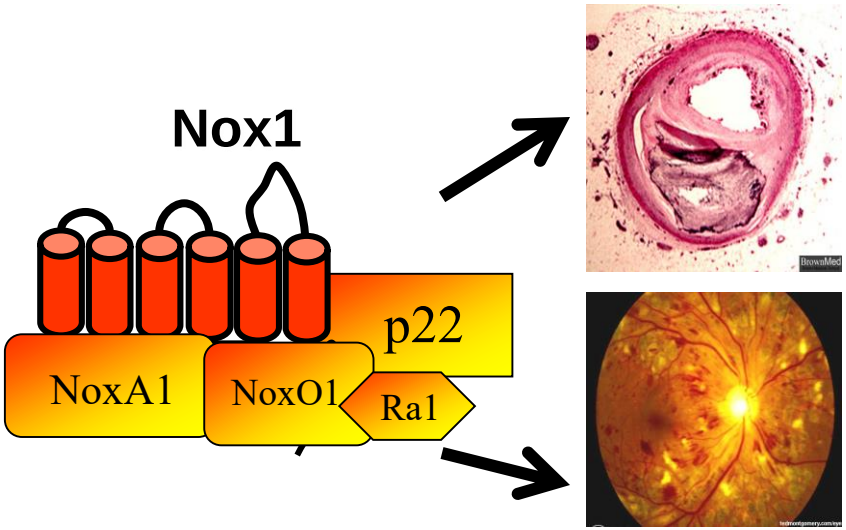
The human isoform: Nox5

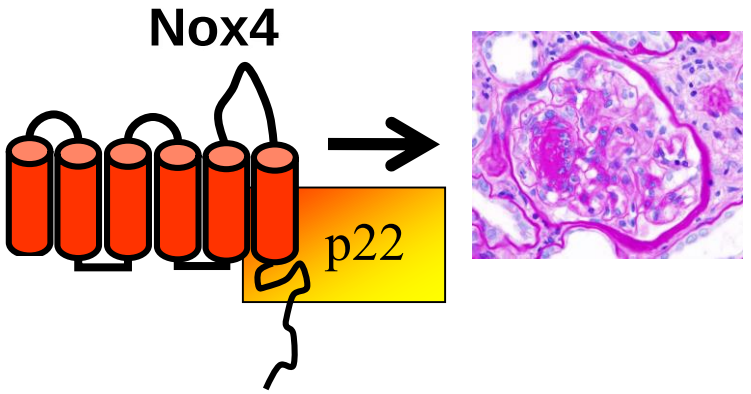
- Nox5 gene is absent in rodents
- Nox5 does not require NADPH oxidase subunits
- Nox5 is Calcium-sensitive

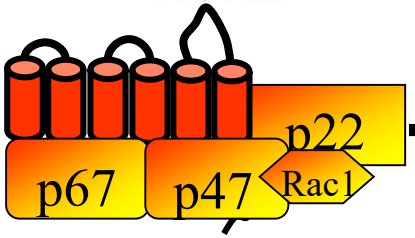


Jha JC et al, Clinical Science 2017

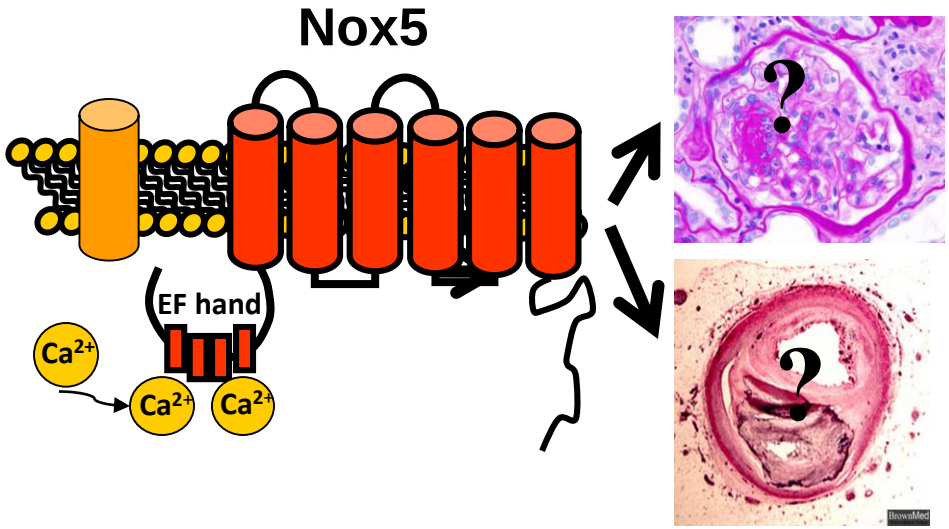
Summary







Infection defense Immune system



GKT137831 $\xrightarrow{\text{Nox1/4}}$ Atherosclerosis↓, Nephro-& Retinopathy↓

Thanks to …

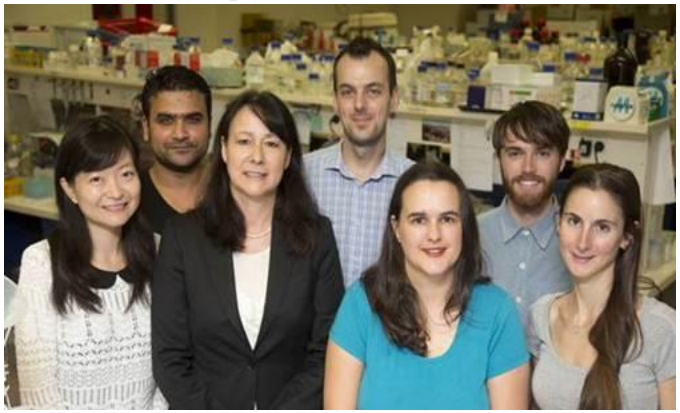
Baker IDI Heart and Diabetes Institute

Stephen Gray
Jay Cha Chandra
Elyse DiMarco
Mark Cooper

MONASH University
Monash Diabetes

Kidney Research Centre, Ottawa Hospital Research Institute & Institute of Cardiovascular and Medical Sciences, University of Glasgow

Rhian Touyz
Chris J Kennedy



Australian Government
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