Nephropathy Group Update 10-09

AMDCC

THE ANIMAL MODELS OF DIABETIC
COMPLICATIONS CONSORTIUM

Current AMDCC Nephropathy Investigators/Projects

Erwin Bottinger/Katalin Susztek– Role and Mechanisms of Epithelial Injury in Diabetic Nephropathy

Chip Brosius/Mattheus Kretzler– Recapitulating transcriptional pathways of human diabetic nephropathy in mice

Tom Coffman/Susan Quaggin– Angiogenic Signals in Diabetic Complications

Ray Harris/Takamune Takahashi– Endothelial Dysfunction in Diabetic Nephropathy

Moshe Levi– Novel Models of Diabetic Nephropathy

Kumar Sharma– Adiponectin and Nox 4 in Diabetic Kidney Disease

Oliver Smithies/Nobuyuki Takahashi– Bradykinin, Nitric Oxide and Mitochondrial DNA Damage in Diabetic Complications

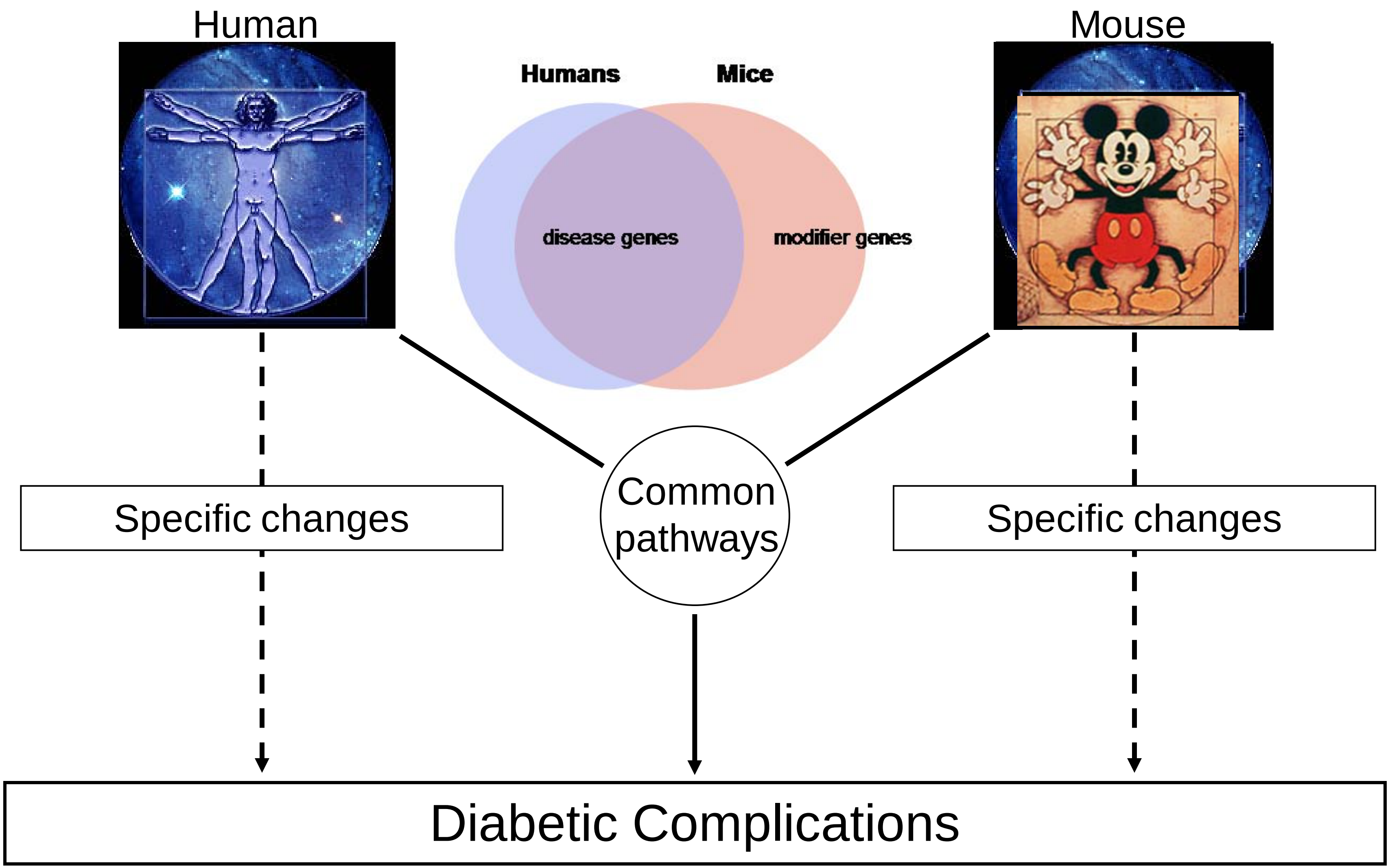
Ongoing Consortial Characterization Studies of Models of Diabetic Nephropathy

Ins2^{Akita} model - strain optimization
Breyer, Brosius, Coffman, Harris, Takahashi

Streptozotocin model- strain optimization
Coffman, Susztek, Takahashi

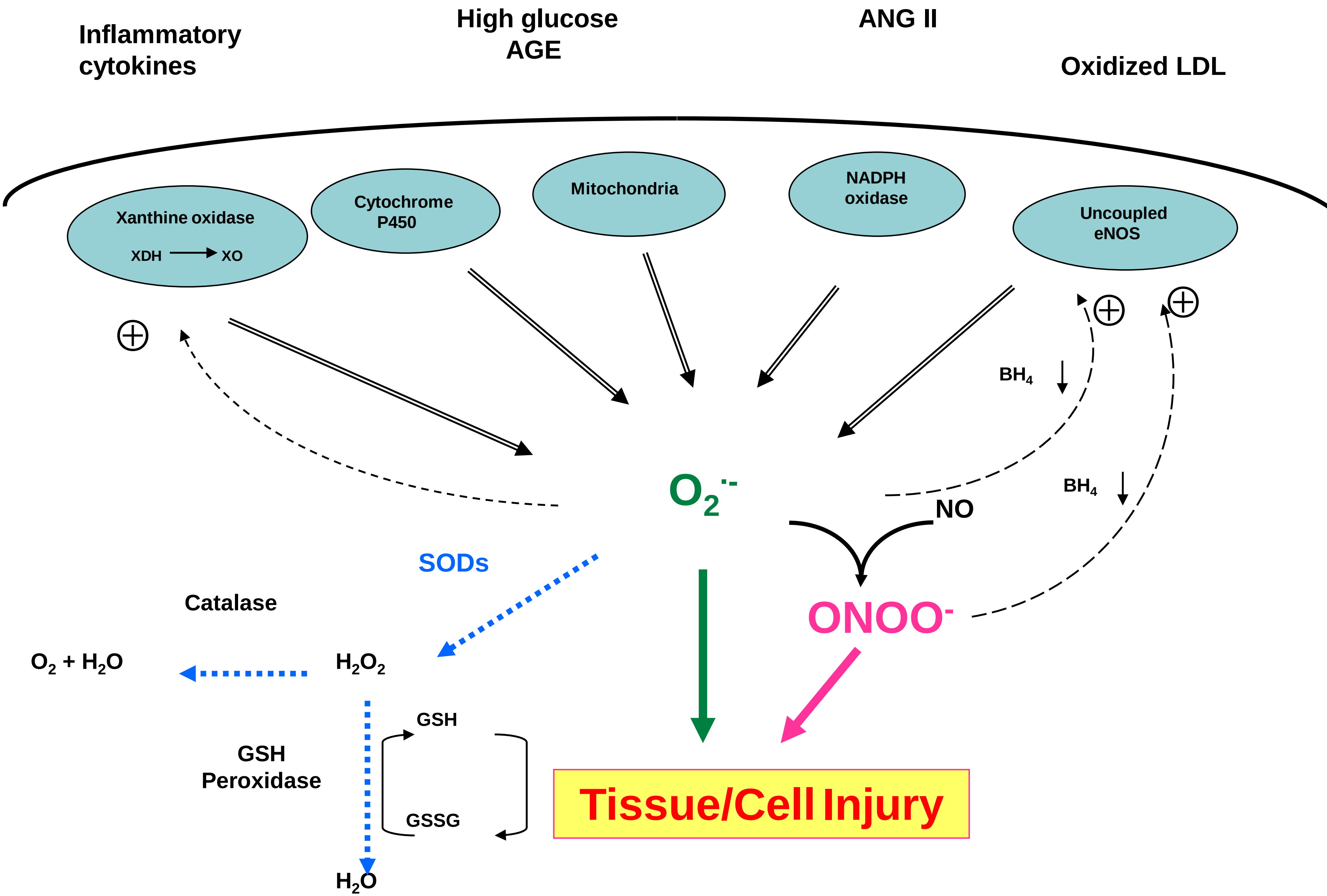
Maneuvers to accelerate nephropathy
Breyer, Coffman, Harris

AMDCC approach to complications

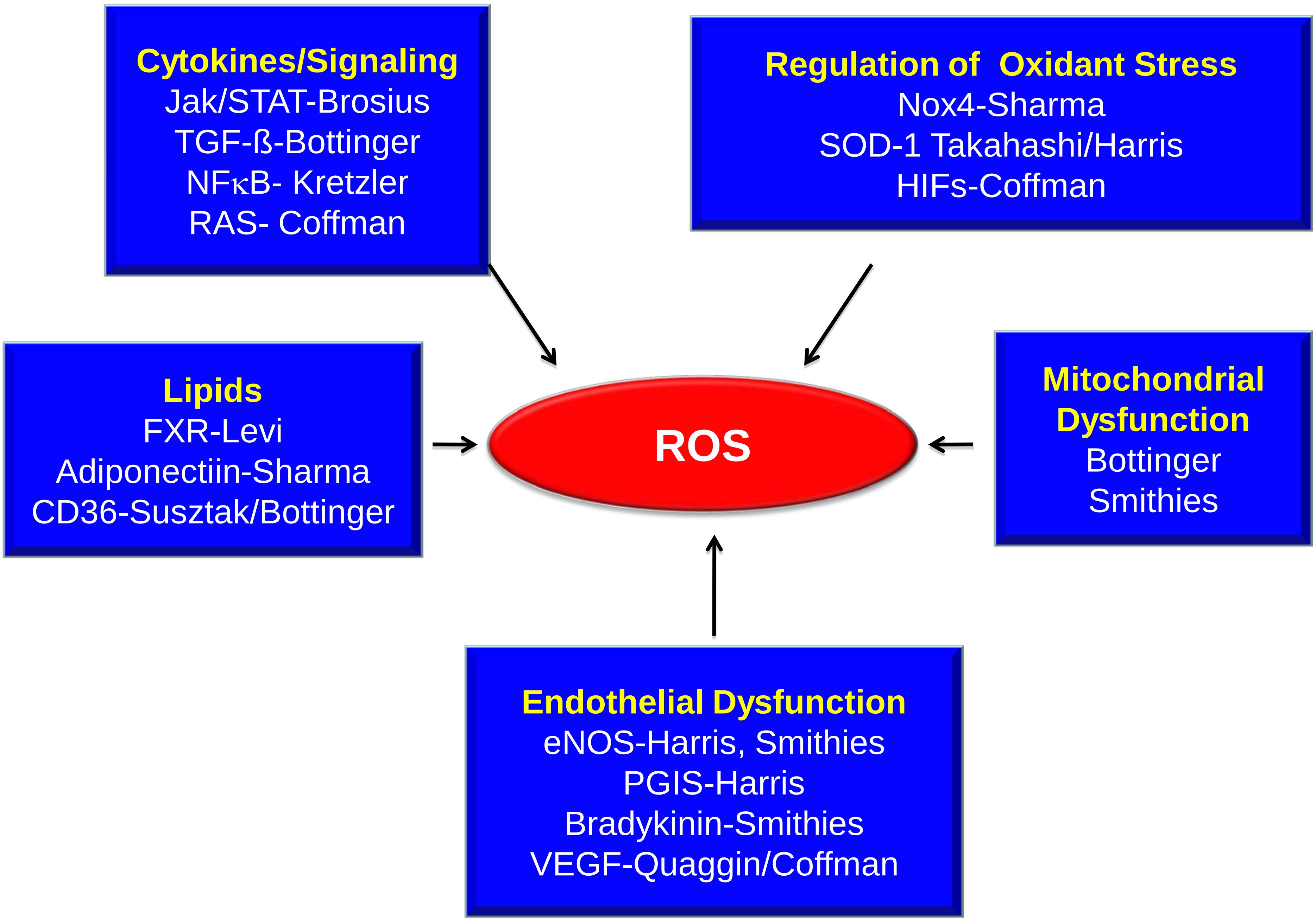


(apologies to Chip Brosius)

Reactive Oxygen Species Overproduction in Diabetes



Current AMDCC Nephropathy Investigators/Projects



Vanderbilt AMDCC

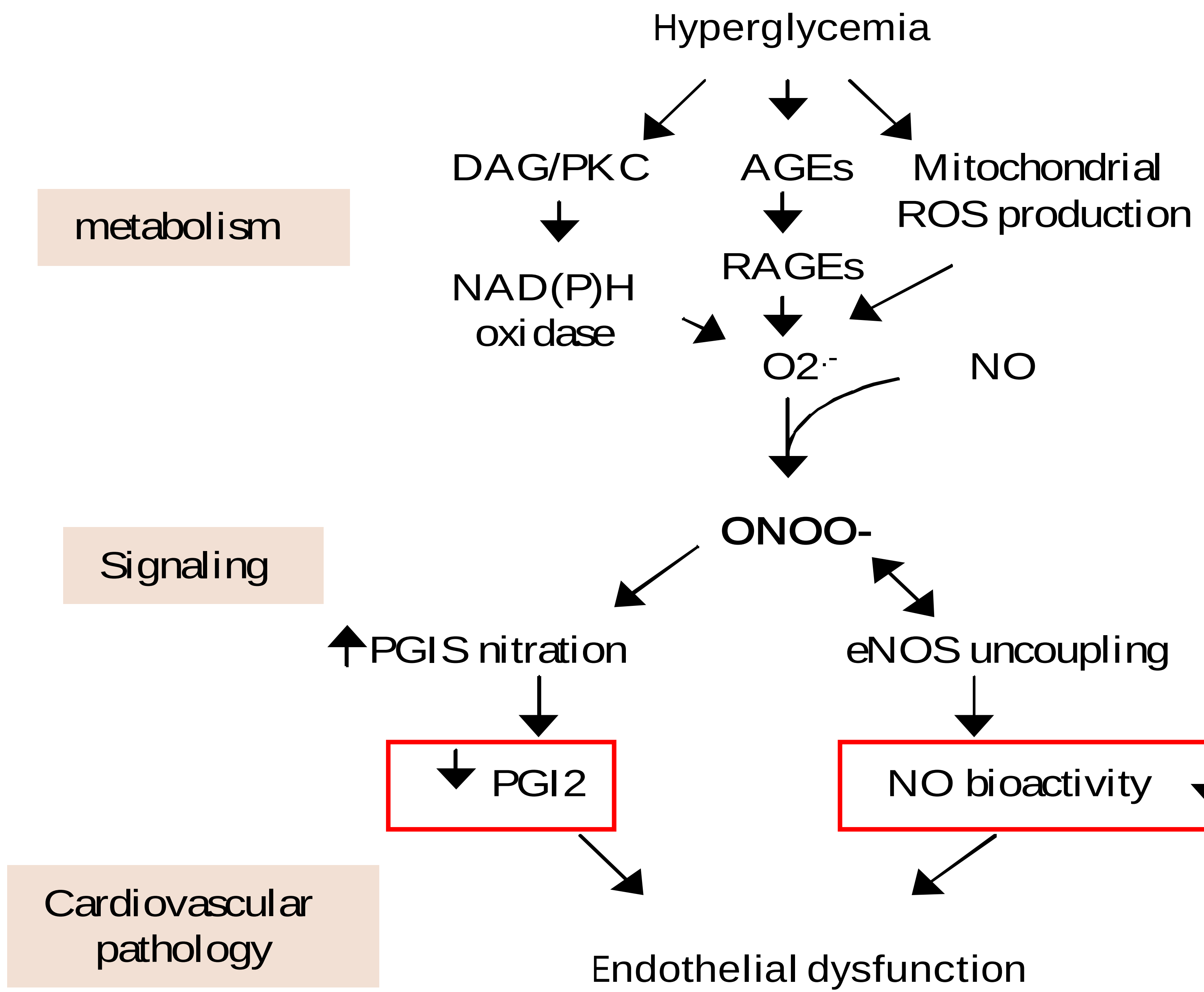
Generating Endothelial-Specific Knockouts of Endothelial Nitric Oxide Synthase and Prostacyclin Synthase

Ray Harris

Takamune Takahashi

Chuanming Hao

9-18-2007


Hyperglycemia
DAG/PKC
AGEs
Mitochondrial ROS production
metabolism
NAD(P)H oxidase
RAGEs
O2·-
NO
ONOO-
Signaling
PGIS nitration
eNOS uncoupling
PGI2
NO bioactivity
Cardiovascular pathology
Endothelial dysfunction

eNOS

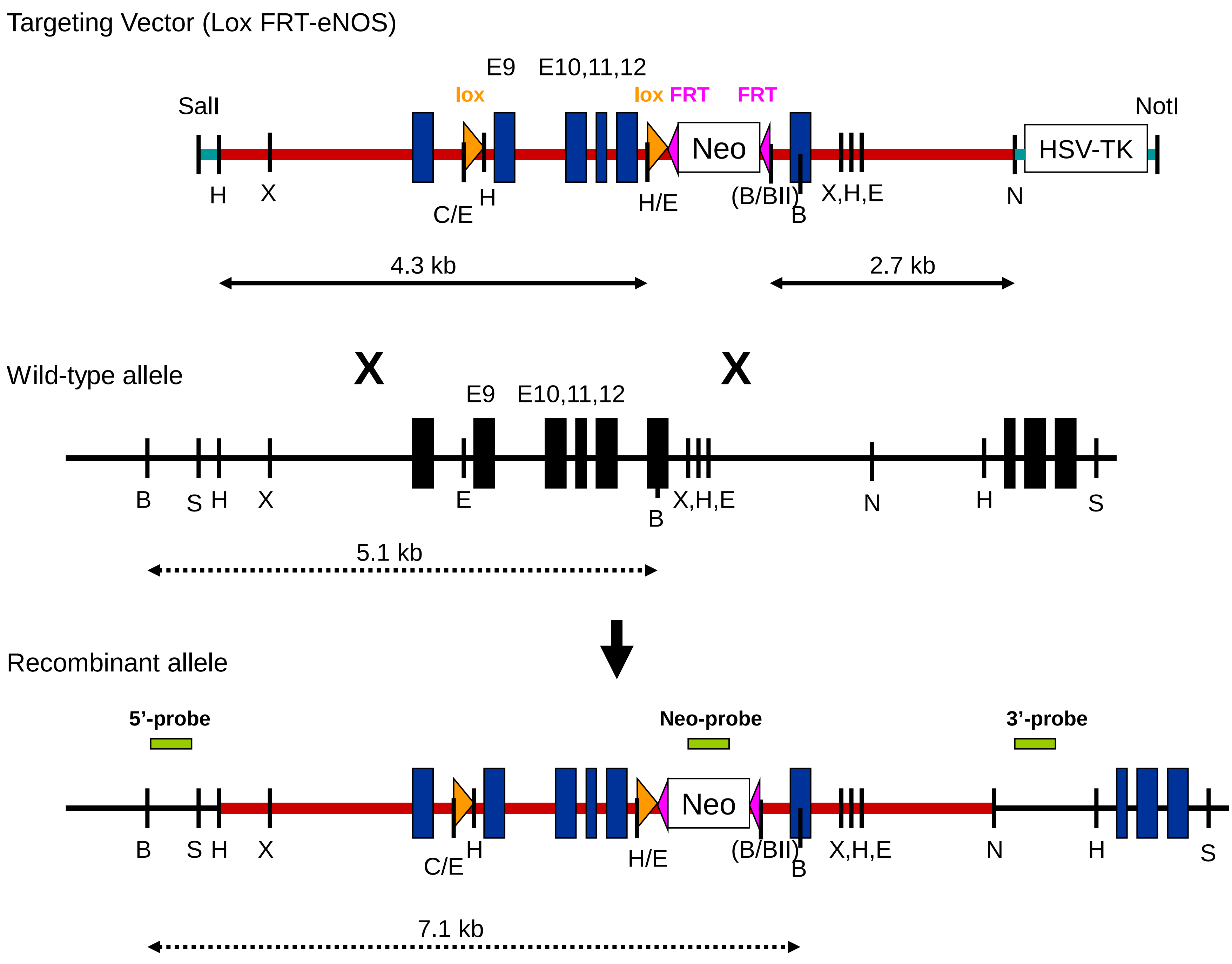
In microvasculature, eNOS-derived NO controls basal tone and vascular permeability and inhibits platelet aggregation and neutrophil adhesion.Hyperglycemia *per se* produces reductions in eNOS expression, eNOS activity and NO availability in cultured cells

In early diabetes eNOS mRNA and protein expression increases in the vasculature in early diabetes but activity markedly decreases.

In established diabetes of longer duration, vascular eNOS expression decreases.

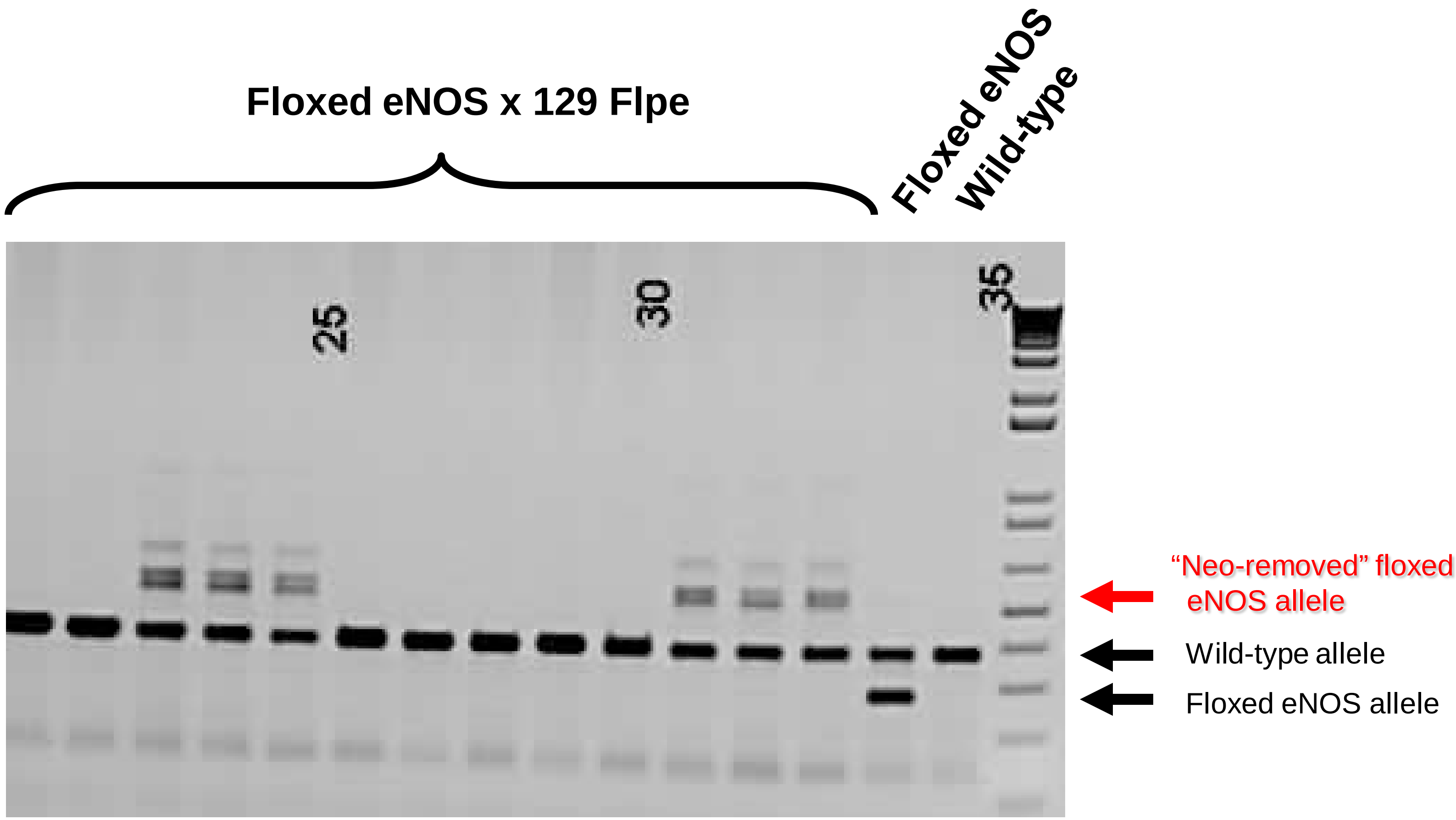
In addition to vasculature, eNOS is also expressed in:

Cardiac myocytes
Thick ascending limb
Pulmonary epithelial cells
Adipocytes
Platelets
Hippocampus
Dorsal root ganglia



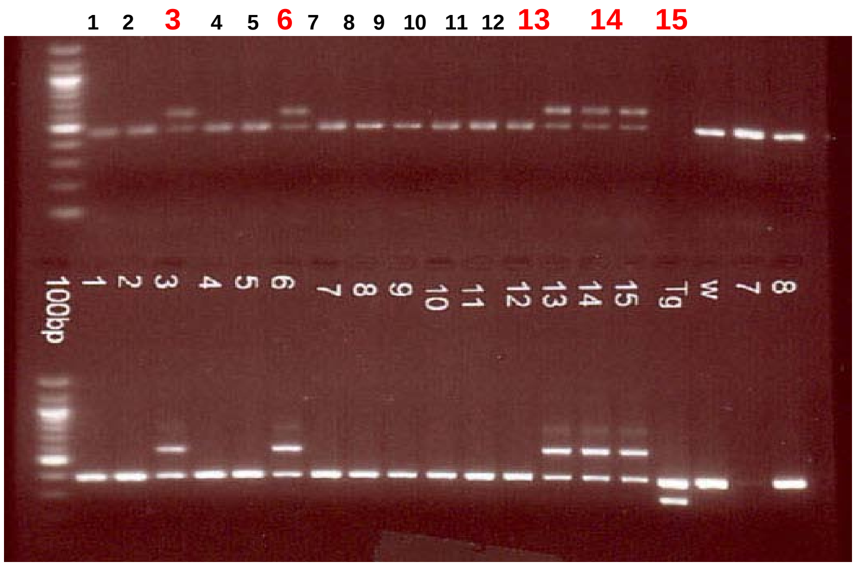
Targeting Vector (Lox FRT-eNOS)
E9
E10,11,12
lox
lox
FRT
FRT
SalI
NotI
Neo
HSV-TK
H
X
C/E
H
H/E
(B/BII)
B
X,H,E
N
4.3 kb
2.7 kb
Wild-type allele
X
X
E9
E10,11,12
B
S
H
X
E
B
X,H,E
N
H
S
5.1 kb
Recombinant allele
5'-probe
Neo-probe
3'-probe
Neo
B
S
H
X
C/E
H
H/E
(B/BII)
B
X,H,E
N
H
S
7.1 kb

Generation of Floxed eNOS mouse

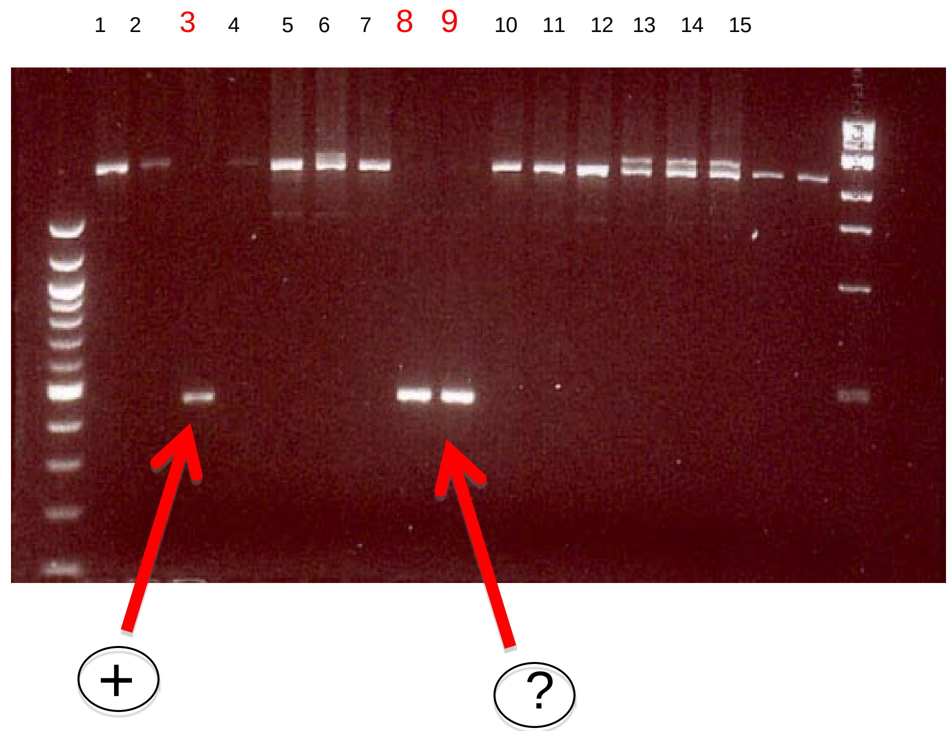


eNOS$^{flox/flox}$ mice were crossed with Tie-2 Cre Mice

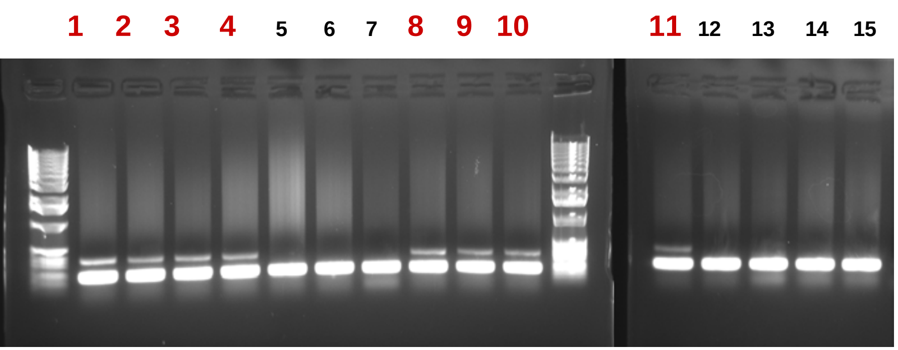
eNOS$^{flox/flox}$ genotyping



eNOS deletion in Lung



Cre genotyping



Floxed PGIS

Targeting Prostacyclin Synthase

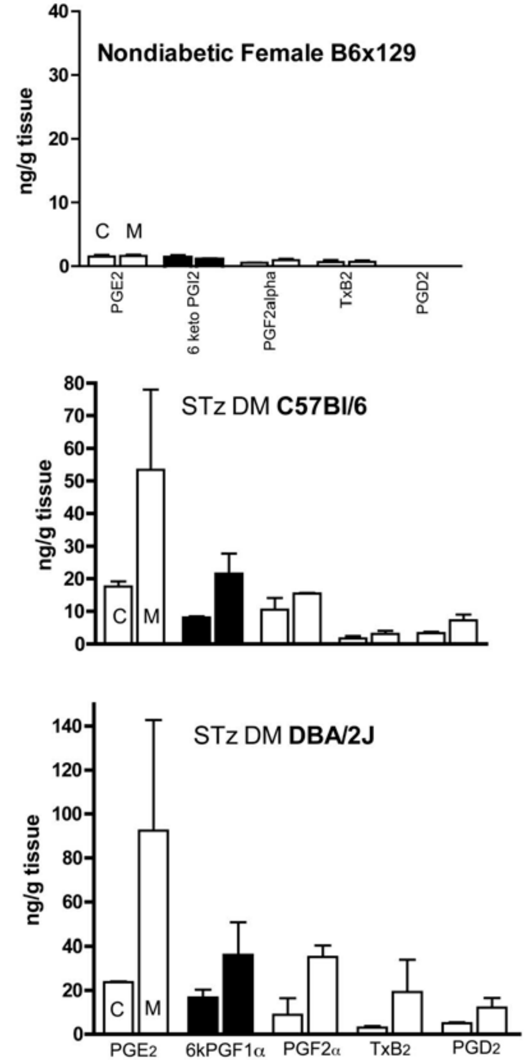
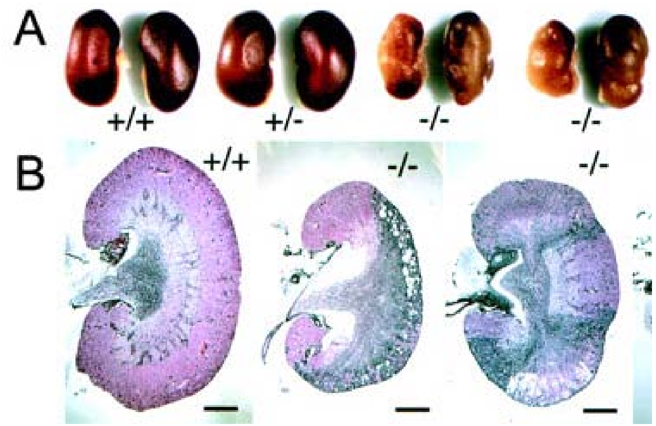
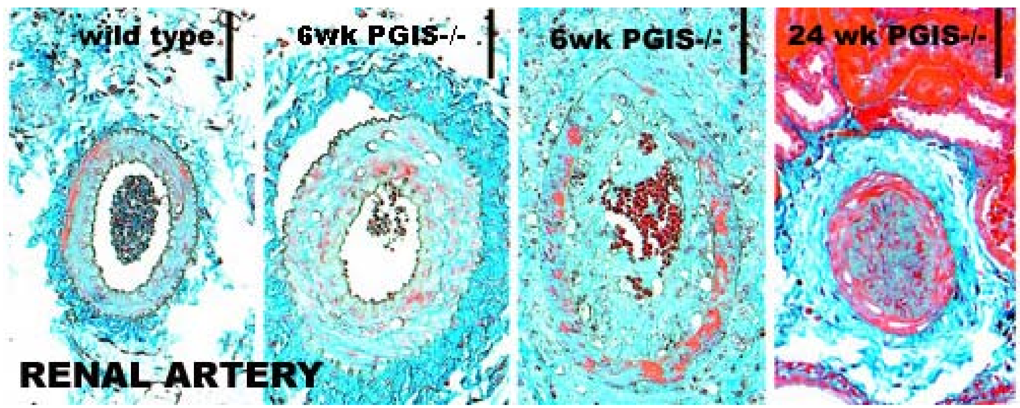
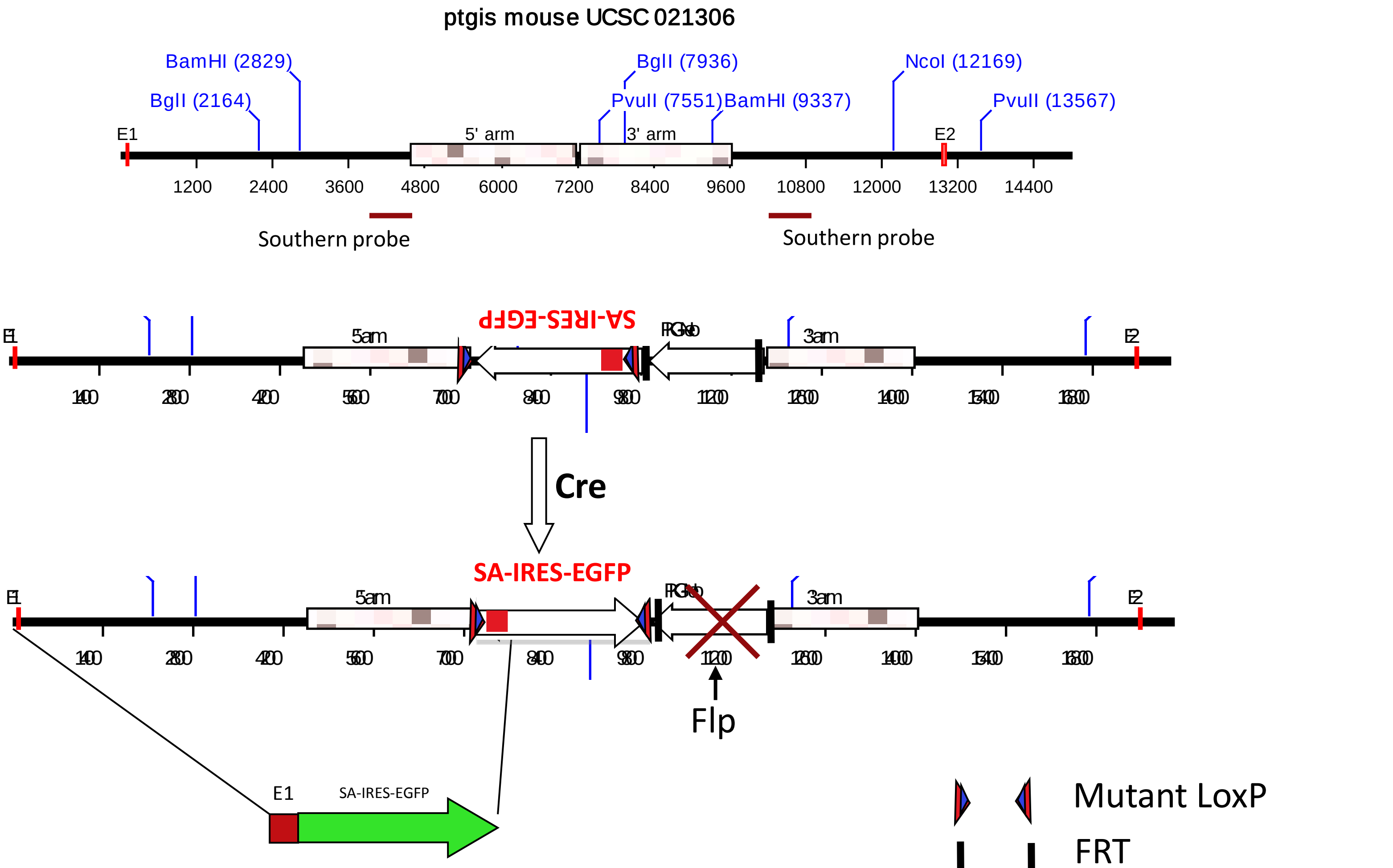


Figure 4: Prostaglandin profile in r enal cortex (C) and medulla (M). Top panel: non-diabetic mice. Middle panel: diabetic C57BL/6J; Bottom panel Diabetic DBA2/J mice.

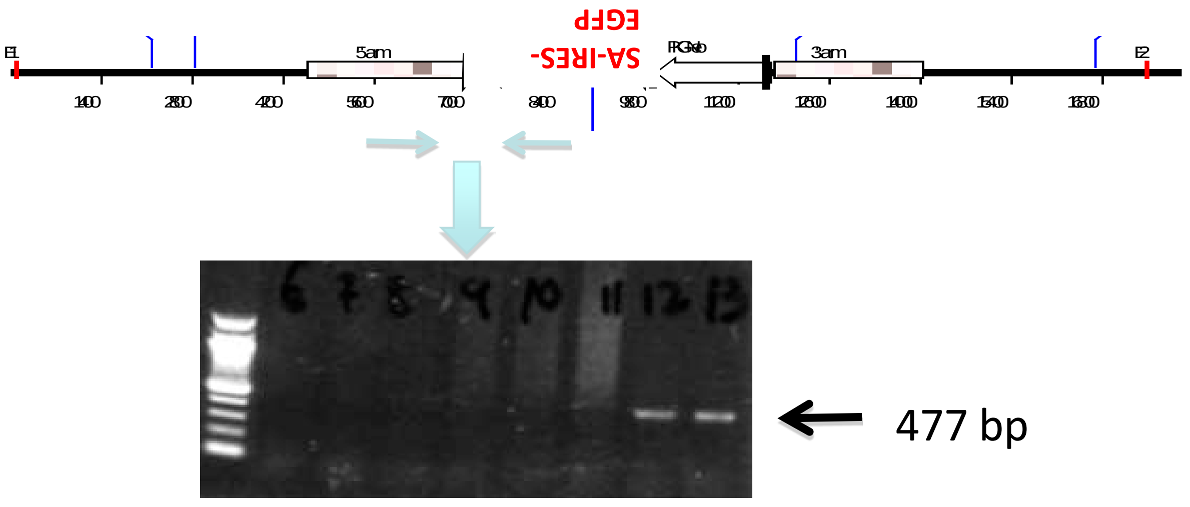




Floxed ptgis Mouse



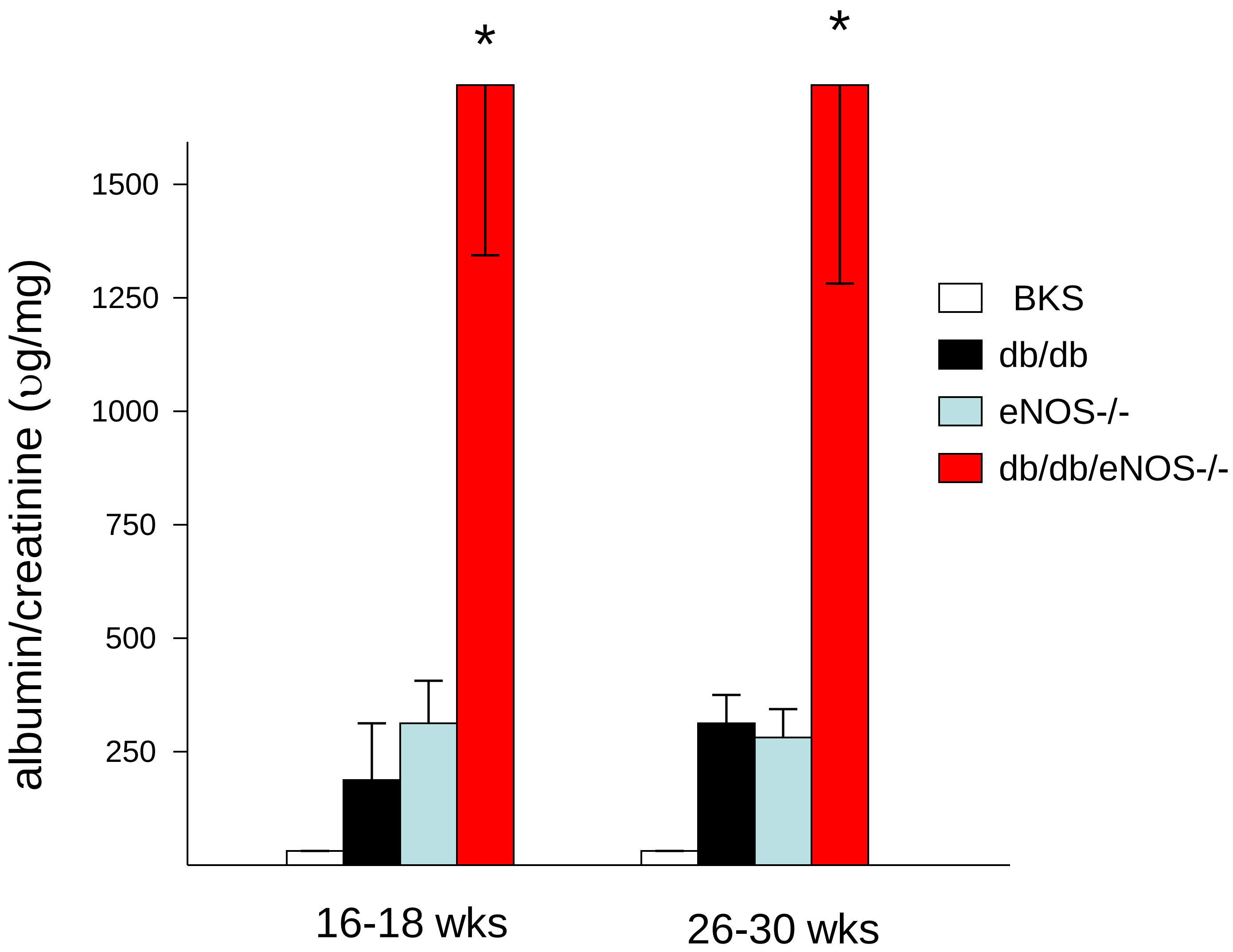
- Two targeted ES cells were picked for cystoblast injection (1C11 and 3D8)
- First injection (2007)
  - 1C11, 3 chimeras (2 male and 1 female). One male chimera had color coat germline transmission, but no transgene transmission.
  - 3D8, 4 chimeras (3 male and 1 female). None had germline transmission.
- Second injection (2009)
  - 1C11, 4 chimeras (male). One had both color coat transmission and germline transmission of transgene.
  - 3D8, 5 chimeras (male). None had germline transmission.

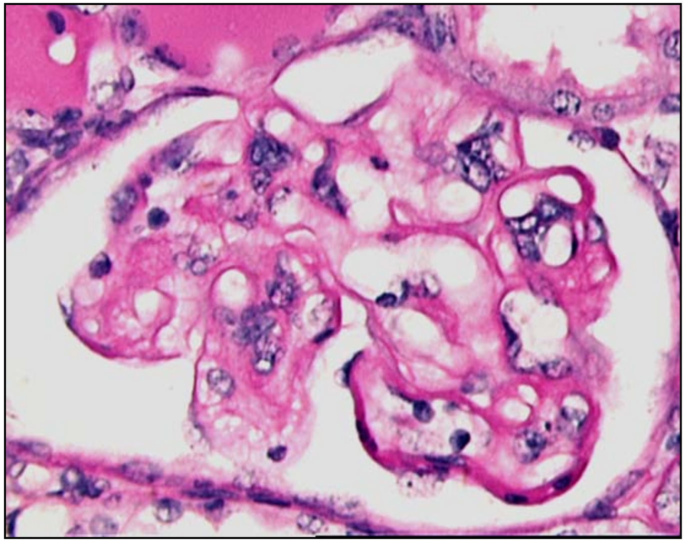


Ongoing Characterization Studies

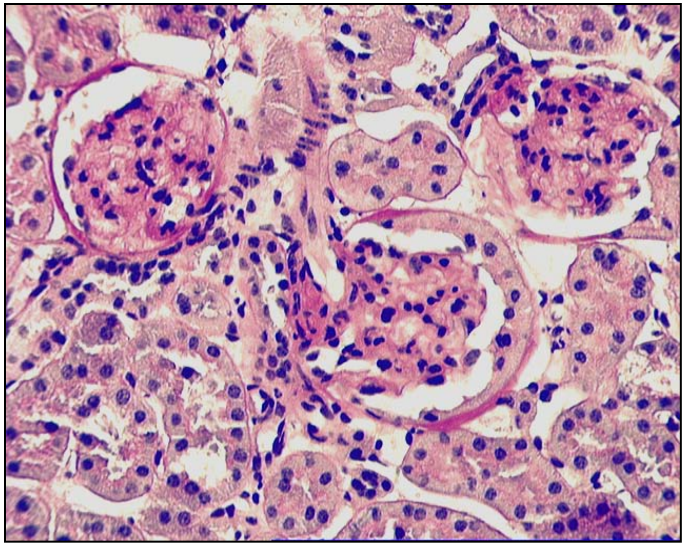
1 Characterization of eNOS-/-*db/db* Mice

Albuminuria

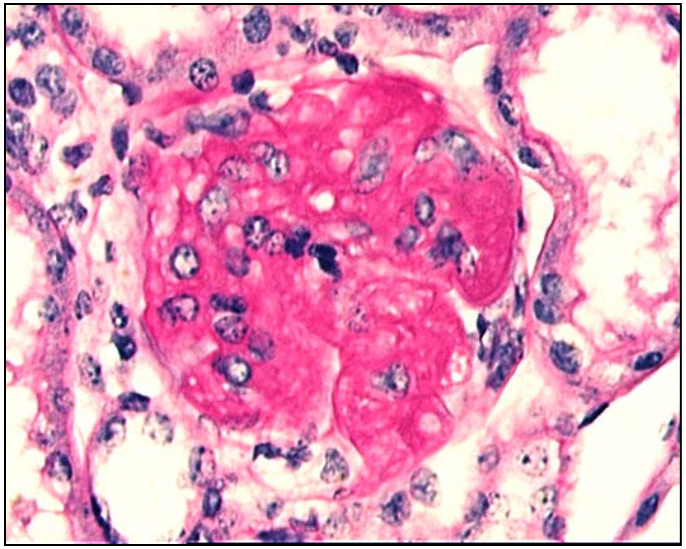




db/db eNOS-/- 16 wks



db/db eNOS-/- 26 wks

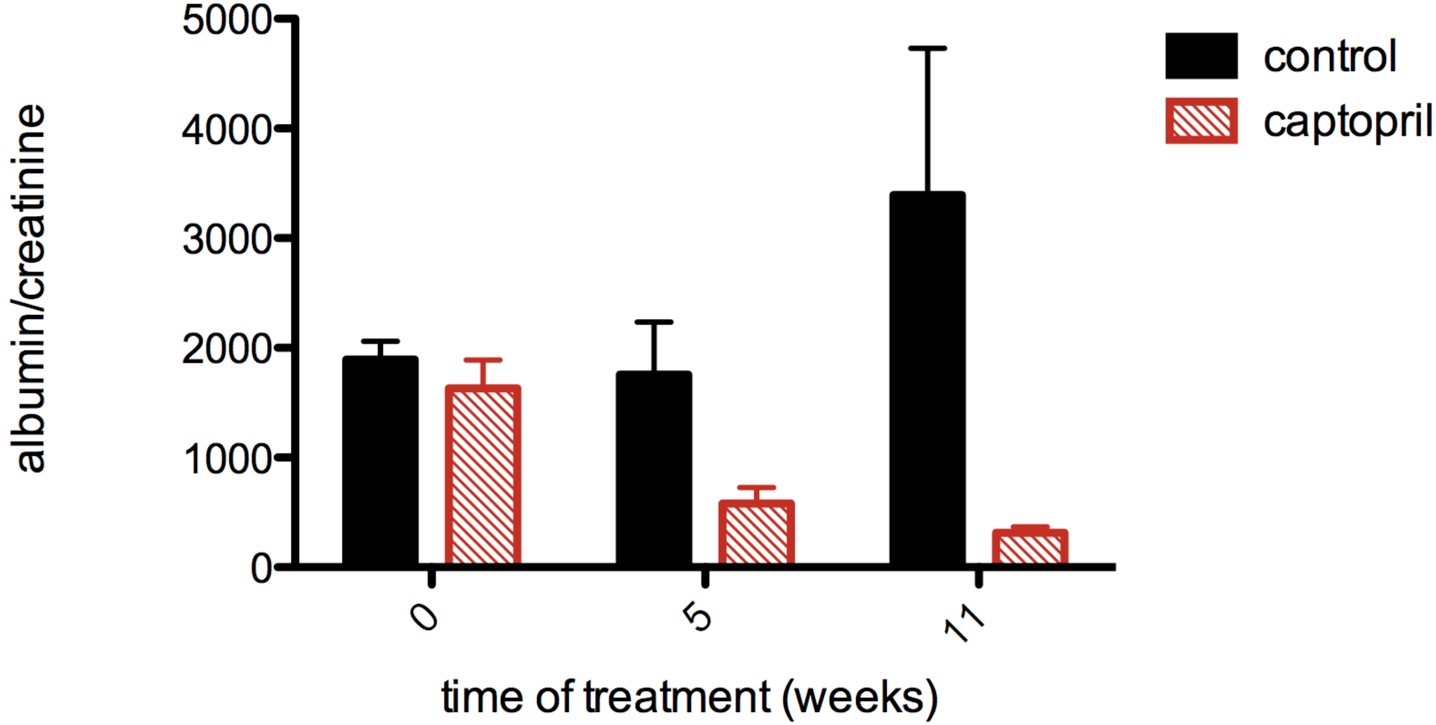


db/db eNOS-/- 26 wks

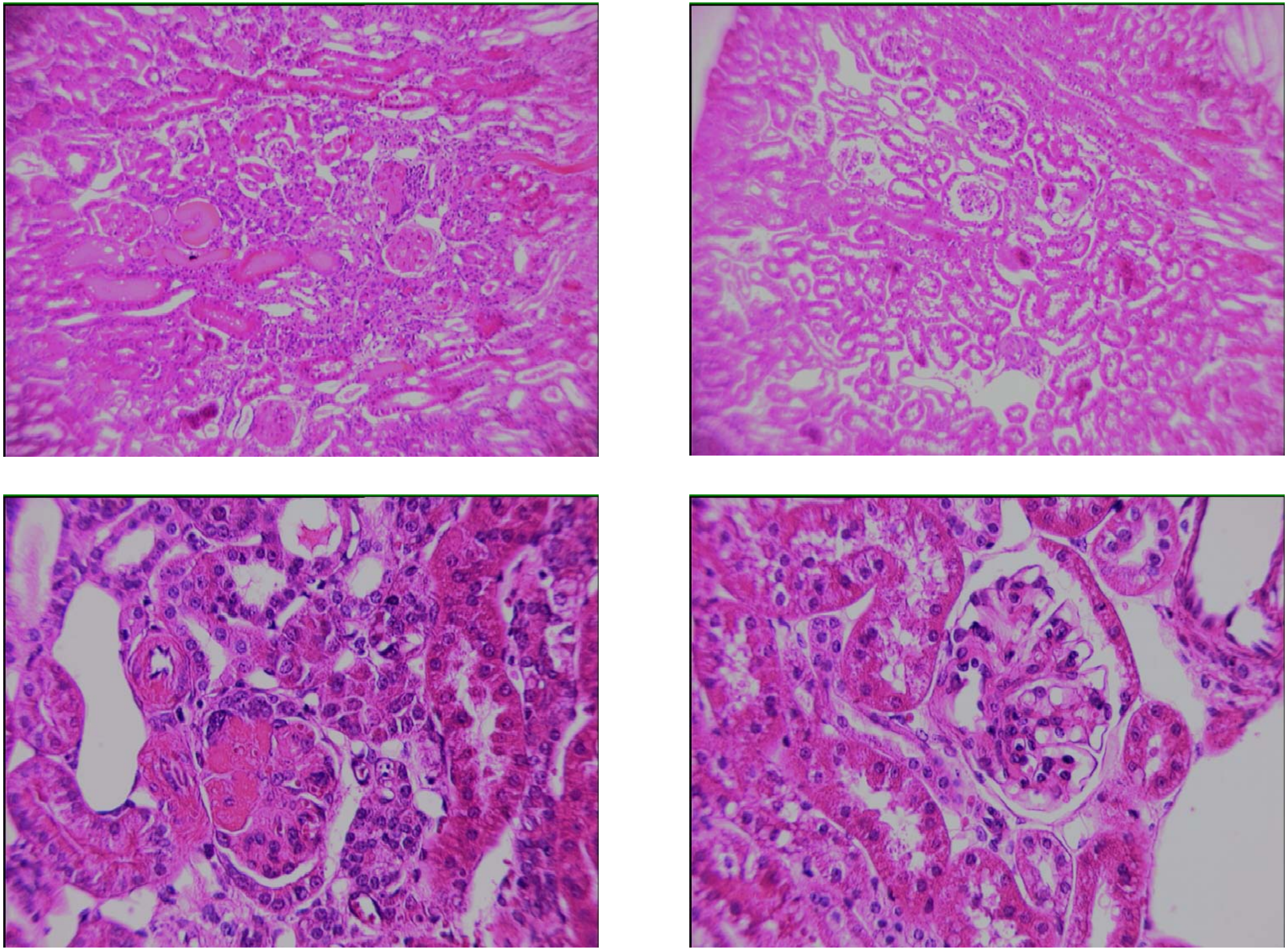
ACE Inhibition Decreases Glomerulopathy in eNOS db/db Mice

eNOS db/db mice were 8-13 weeks at beginning of treatment (9.6±1 (cap) (n=7) vs 9.9±.5 (con) (n=9))

one captopril treated mouse died after 6 weeks of treatment
two control mice died at 12 weeks and one died at 14 weeks



ACE Inhibition Decreases Glomerulopathy in the eNOS db/db Model



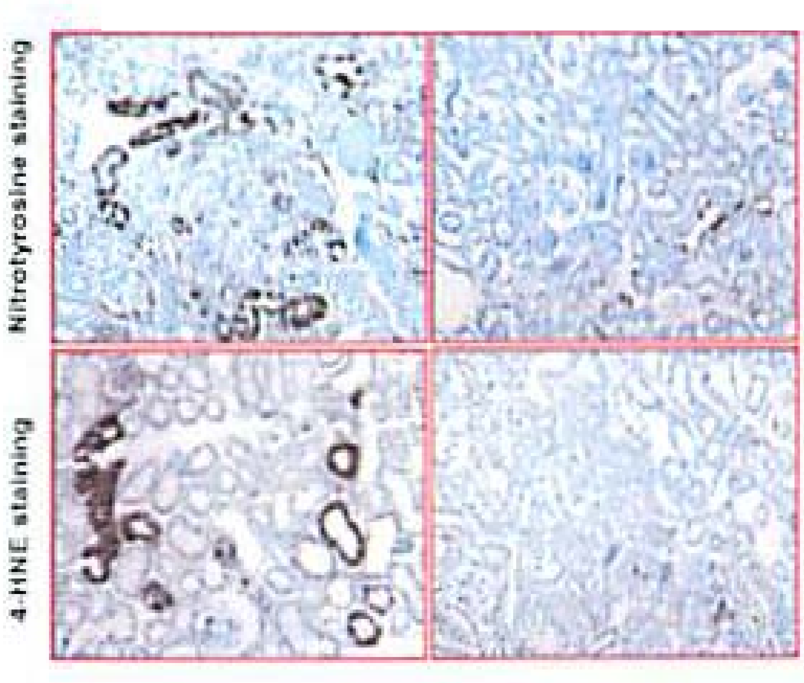
eNOS db/db

eNOS db/db + captopril

ACE Inhibition Decreased Oxidant Stress and Macrophage Infiltration in eNOS *db/db* Kidneys

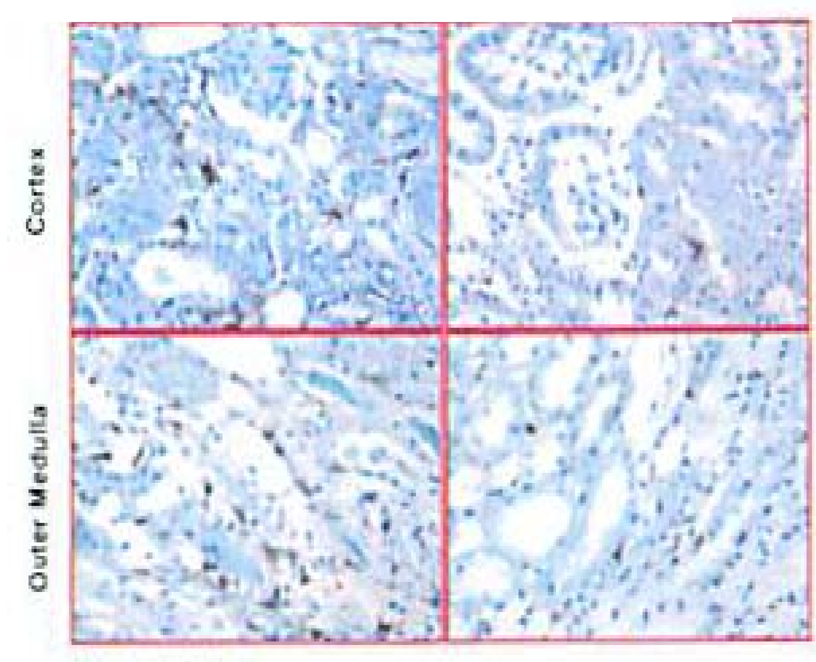
Nitrotyrosine and 4-HNE

control captopril

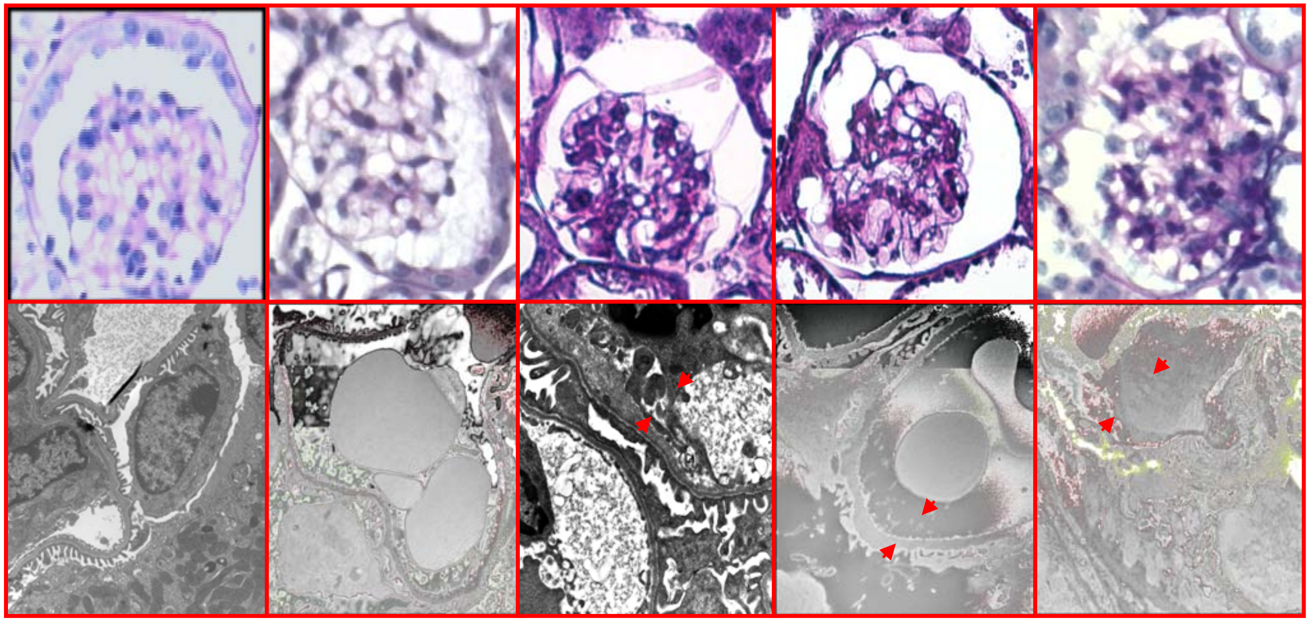


F480

control captopril

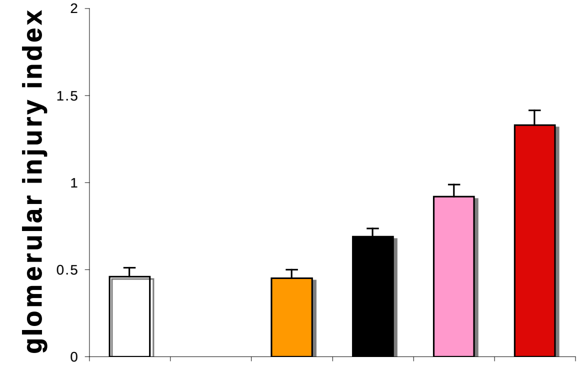


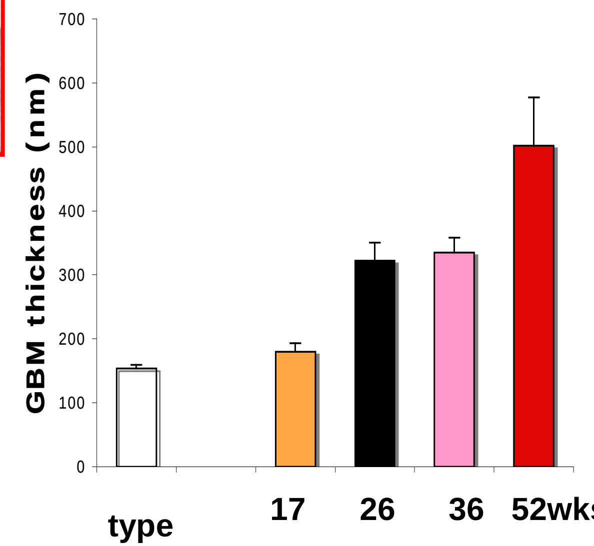
Progression of Nephropathy in *db/db* Mice



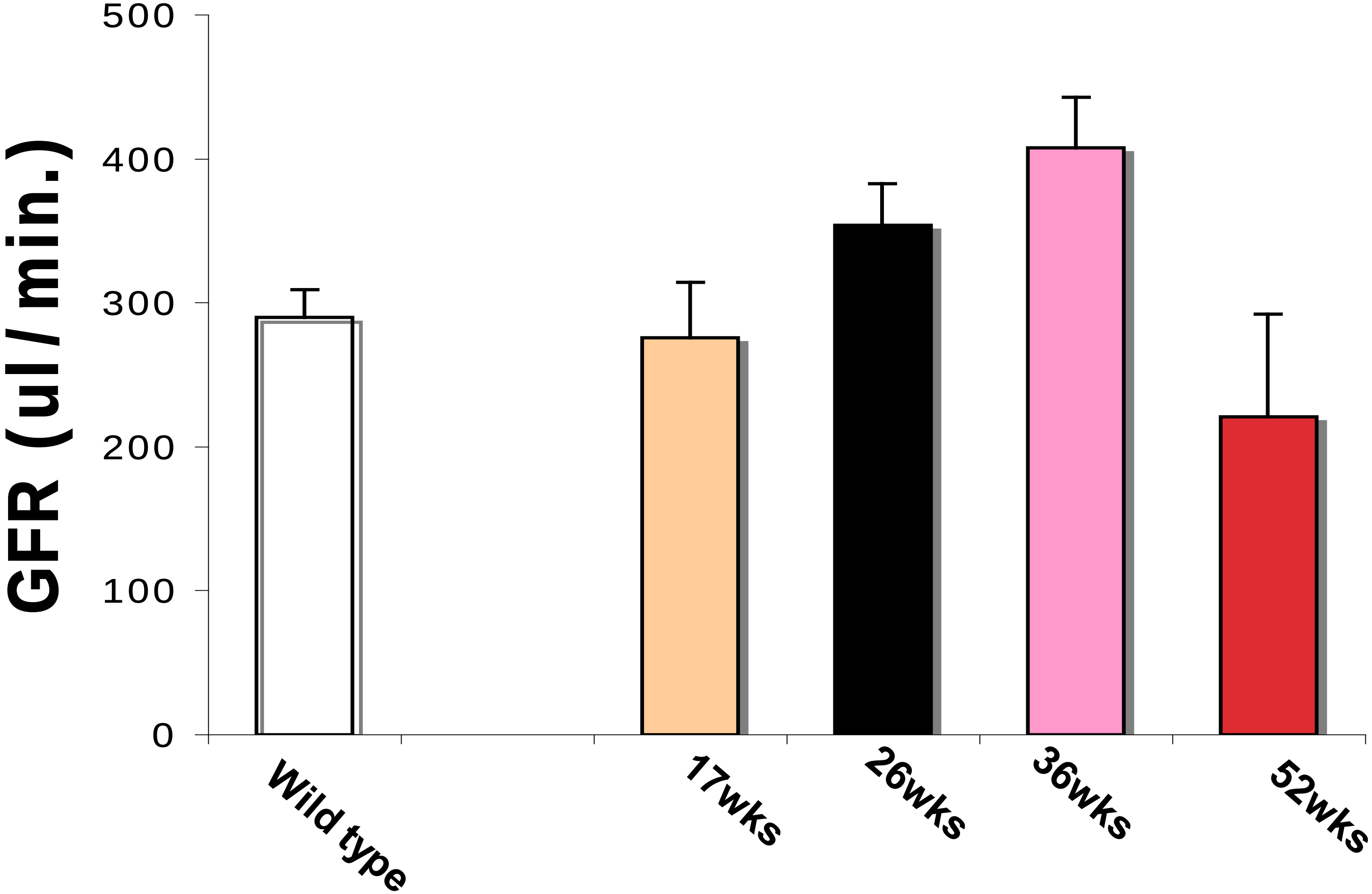
Wild type **17** **26** **36** **52**

db/db (wks)

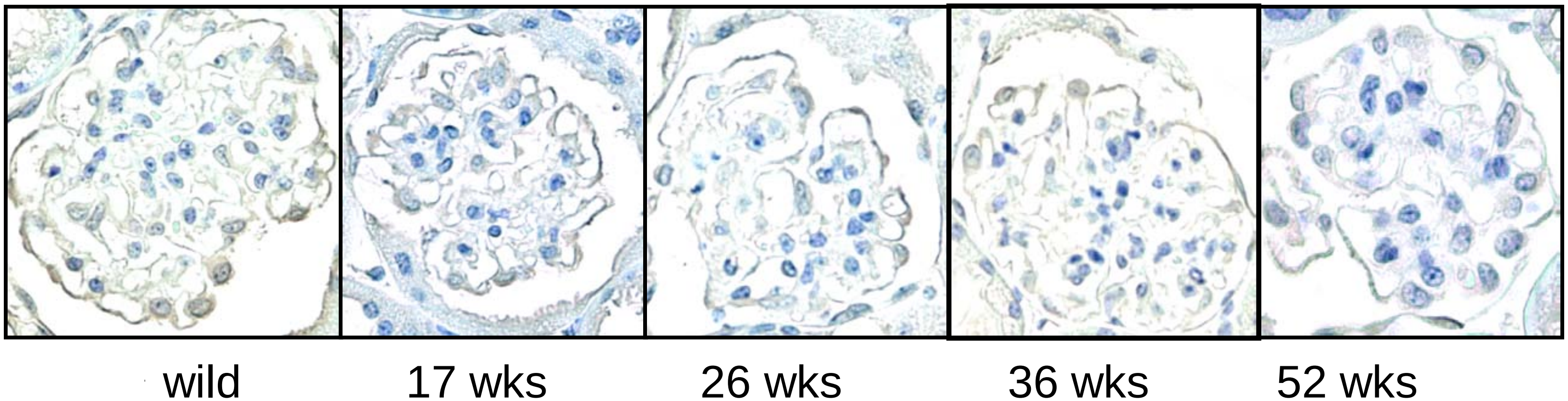


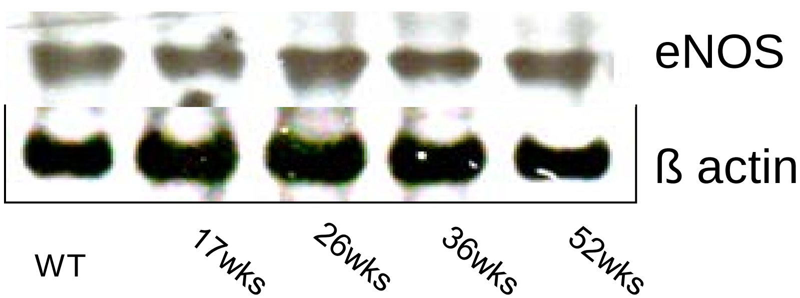


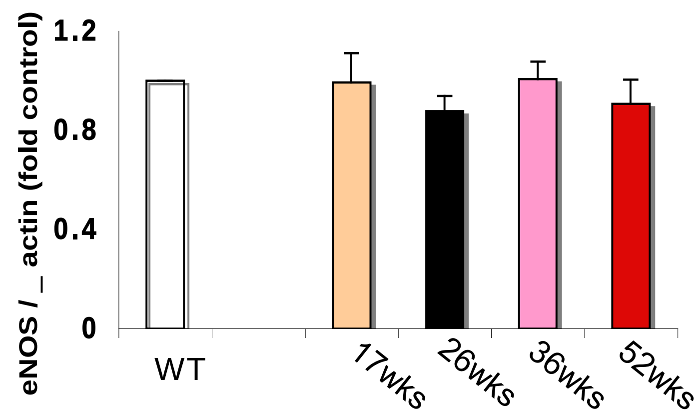
Glomerular Filtration Rate in *db/db* Mice



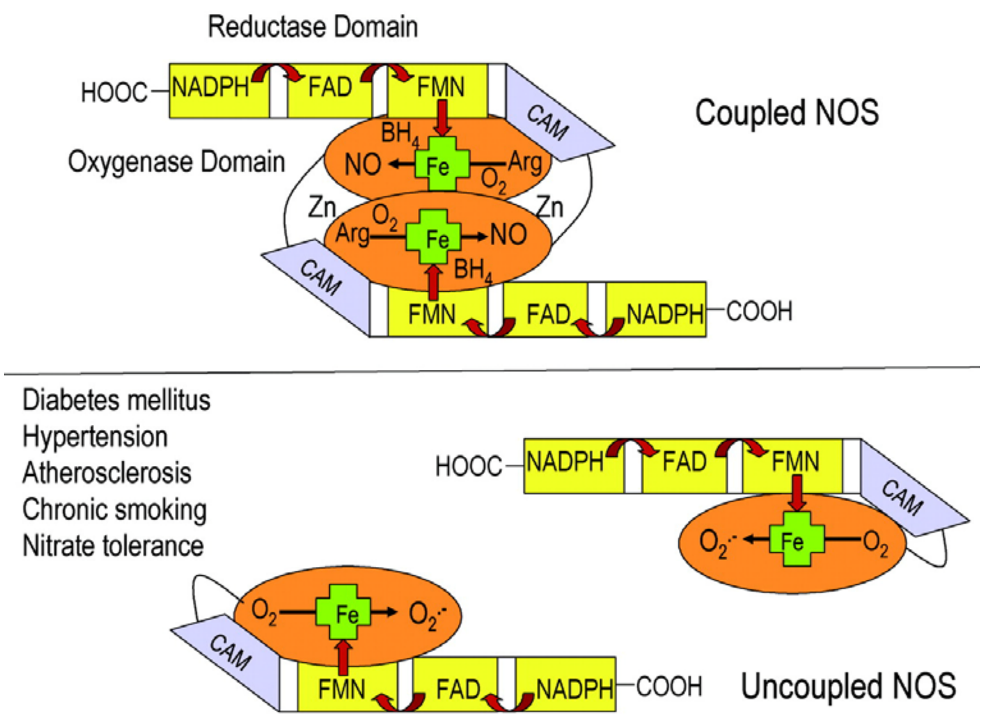
There Was No Significant Change in Glomerular eNOS Expression in *db/db* Mice



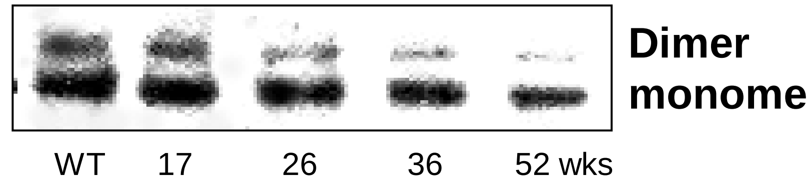


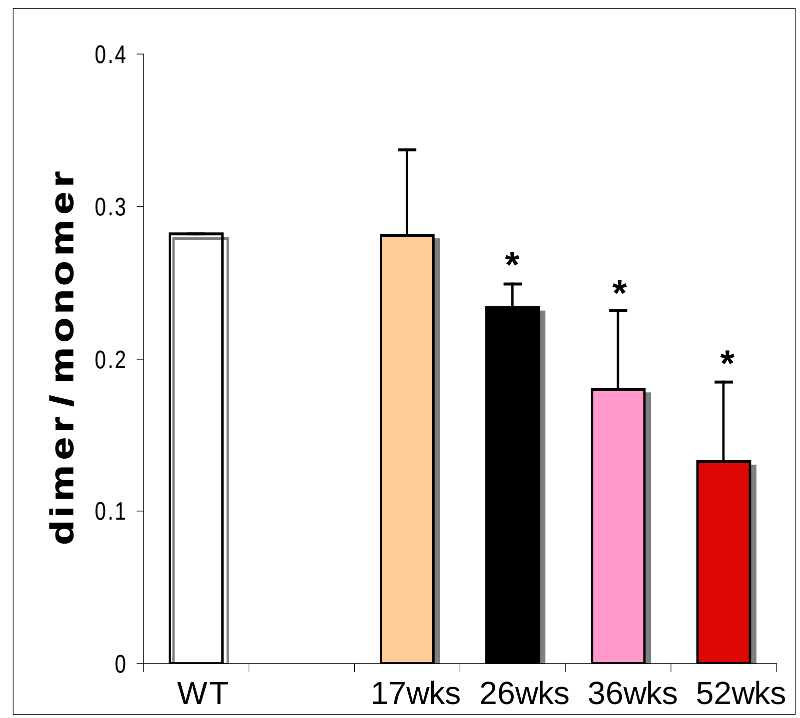


There Was Increasing eNOS Uncoupling With Age in *db/db* Mice

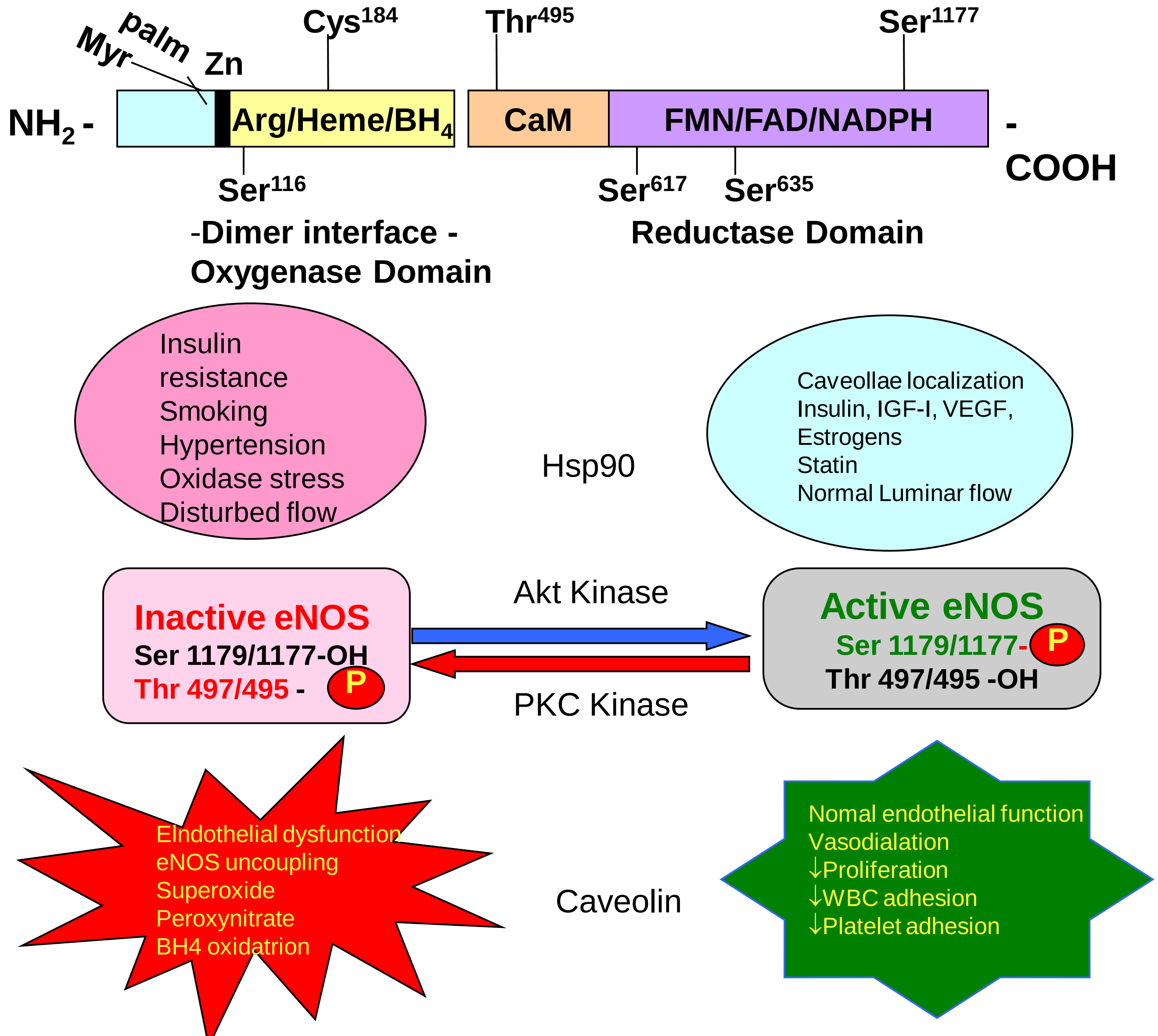


Munzel, T. et al. Arterioscler Thromb Vasc Biol 2005;25:1551



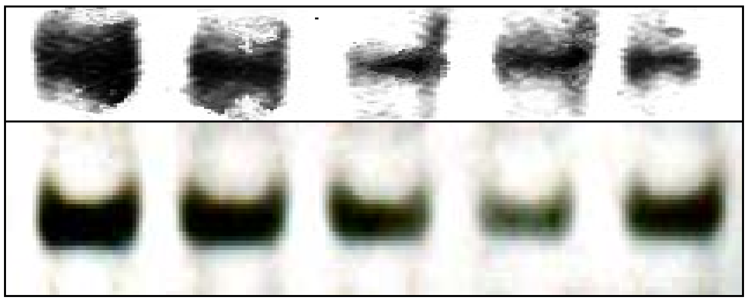


Effect of Phosphorylation on eNOS Activity



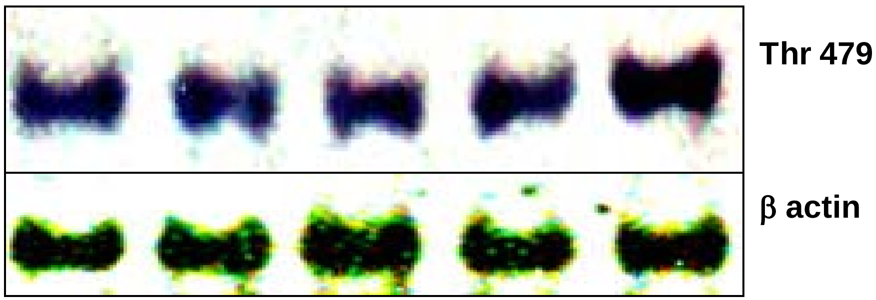
There Was Reduced Ser 1179 Phosphorylation in Aging *db/db* Mice, While Thr 479 Phosphorylation Was Not Altered

Ser 1179 phosphorylation



WT 17 26 36 52 wks

Thr 479 phosphorylation



WT 17 26 36 52 wks

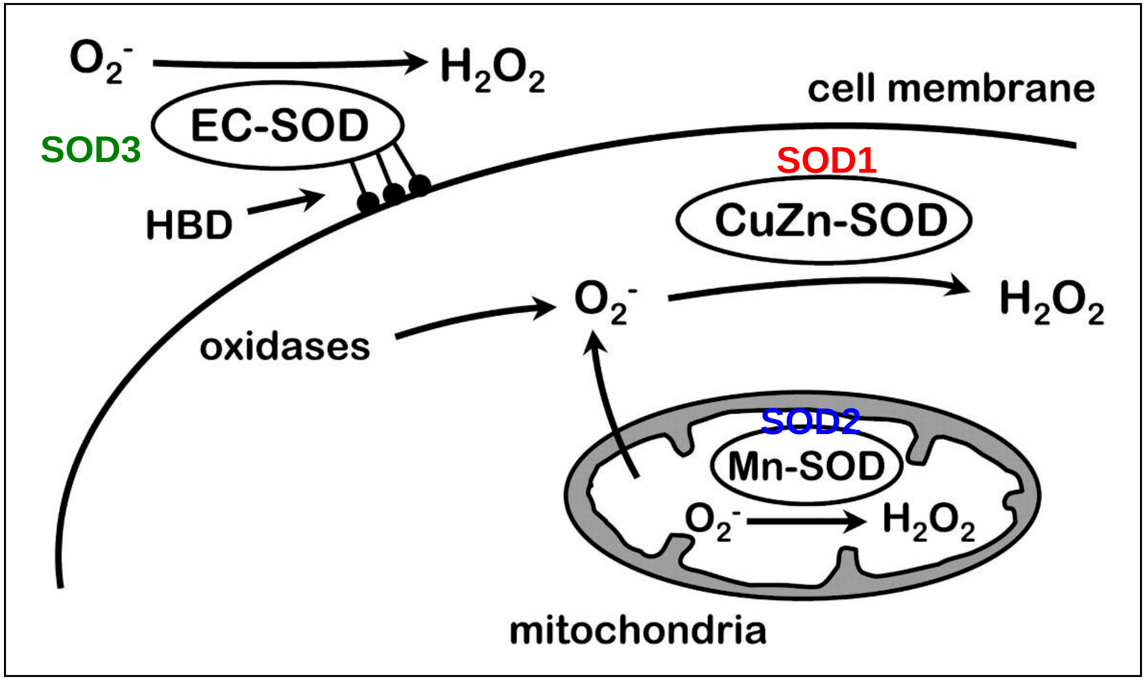
2 Characterization of AkitaSOD1-/- Mice

Isoforms of Superoxide Dismutase (SOD)

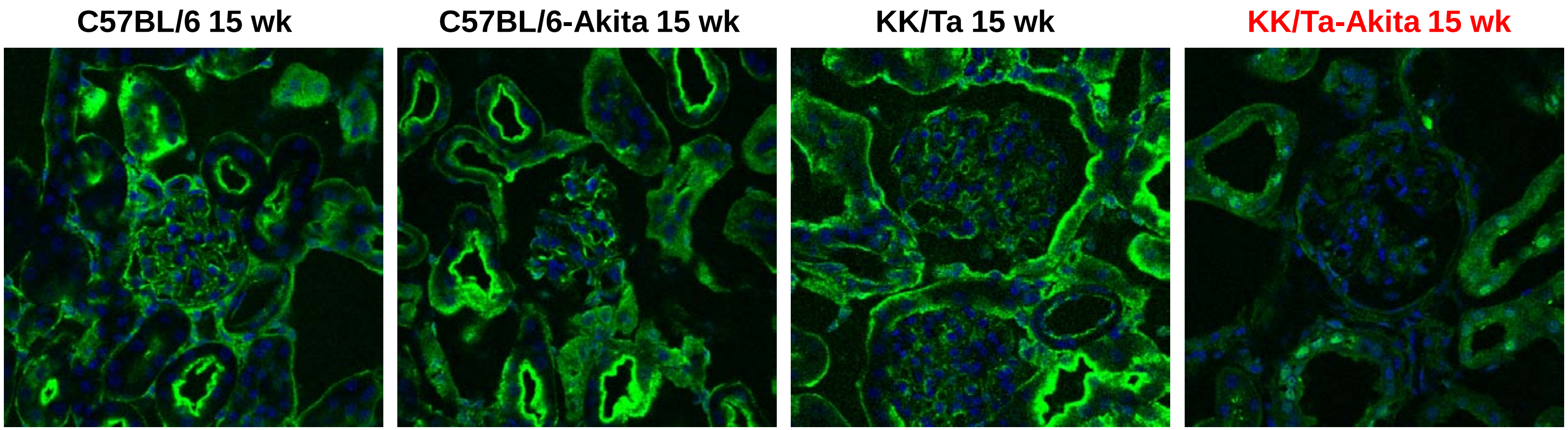
1) SOD1 (Cu/Zn SOD): Cytosol (and Mitochondria)

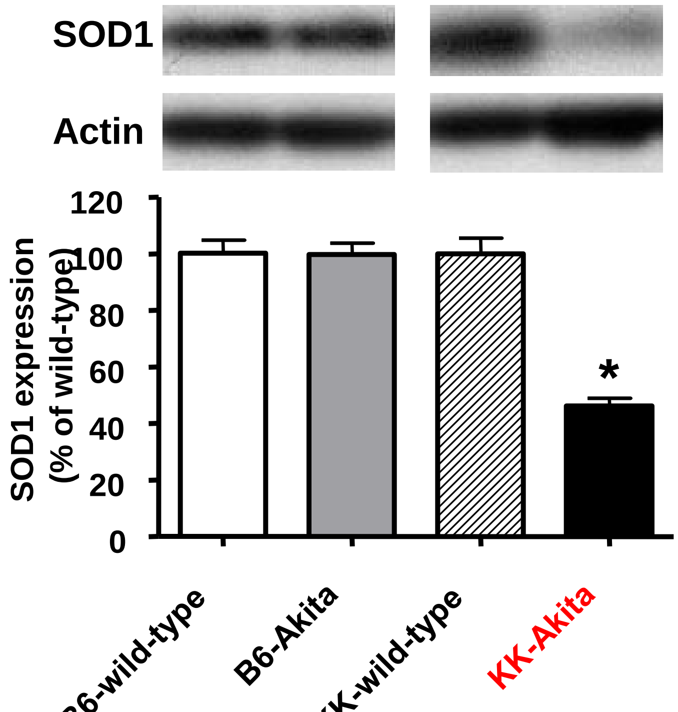
2) SOD2 (MnSOD): Mitochondria

3) SOD3 (ecSOD): Extracellular space



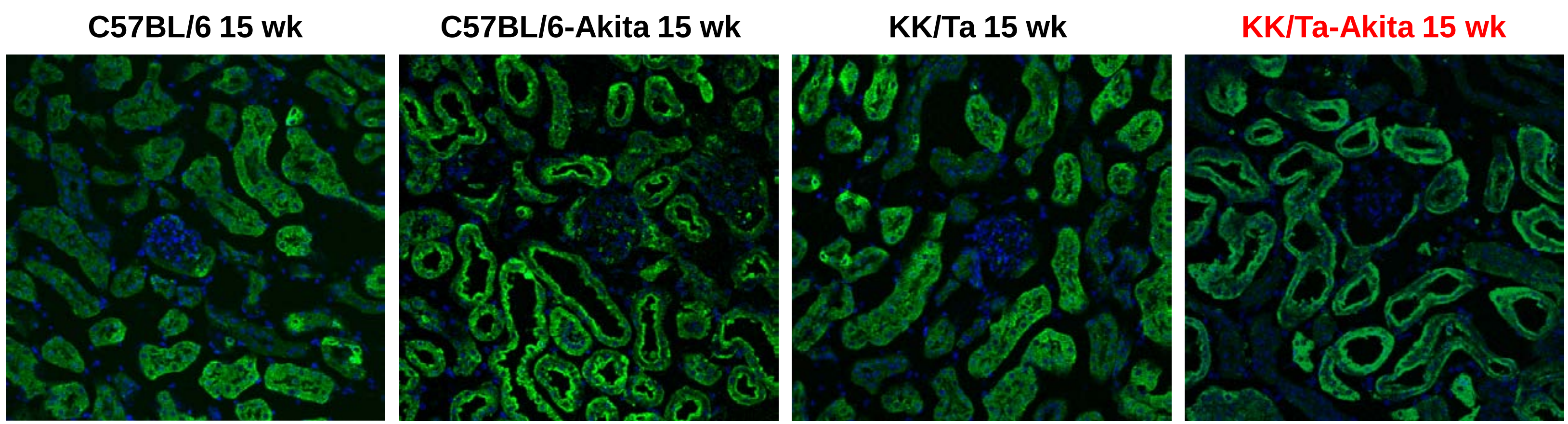
Immunofluorescence histochemistry and Western blot for SOD1 (cytoplasmic CuZn-SOD) in 15-week-old male mouse kidneys

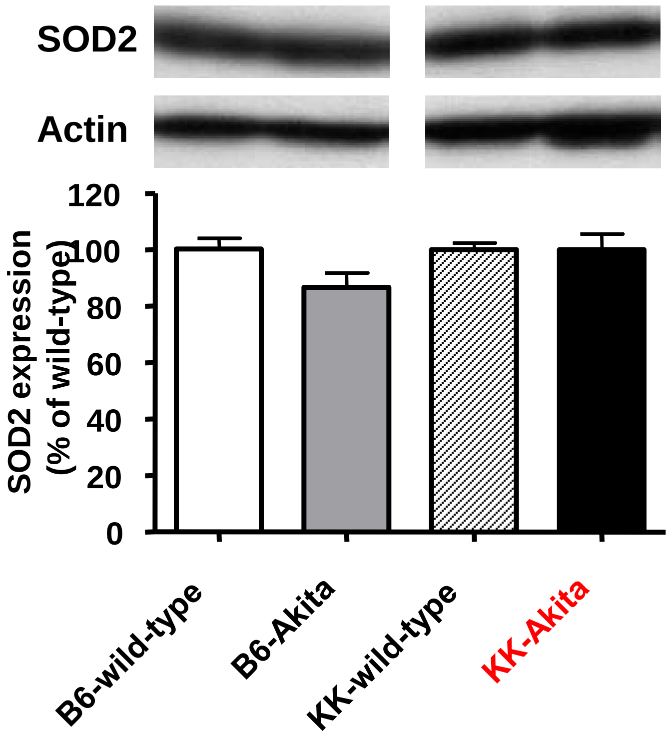




* P < 0.001 vs. KK-wild-type; n = 4 for each group.

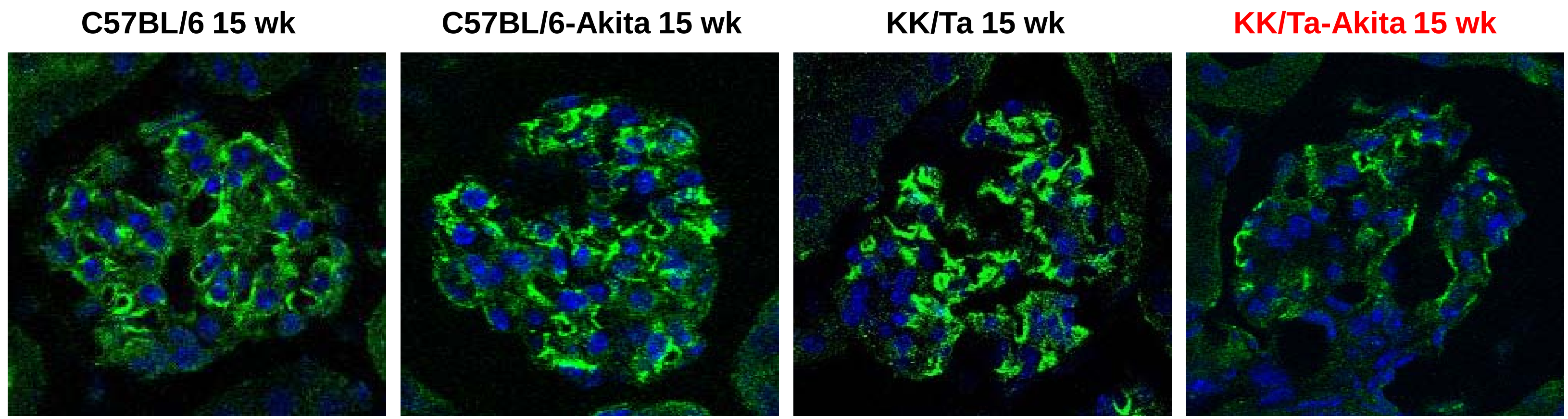
Immunofluorescence histochemistry and Western blot for SOD2 (mitochondrial Mn-SOD) in 15-week-old male mouse kidneys

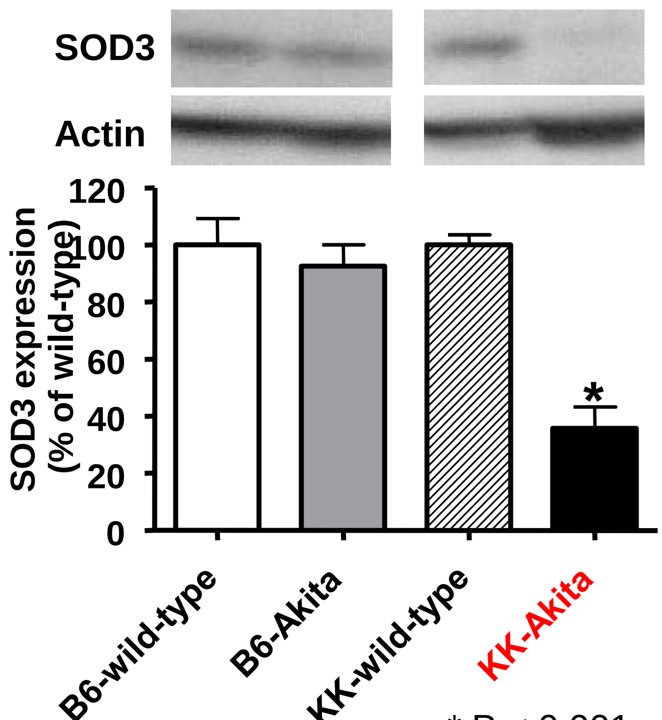




n = 4 for each group

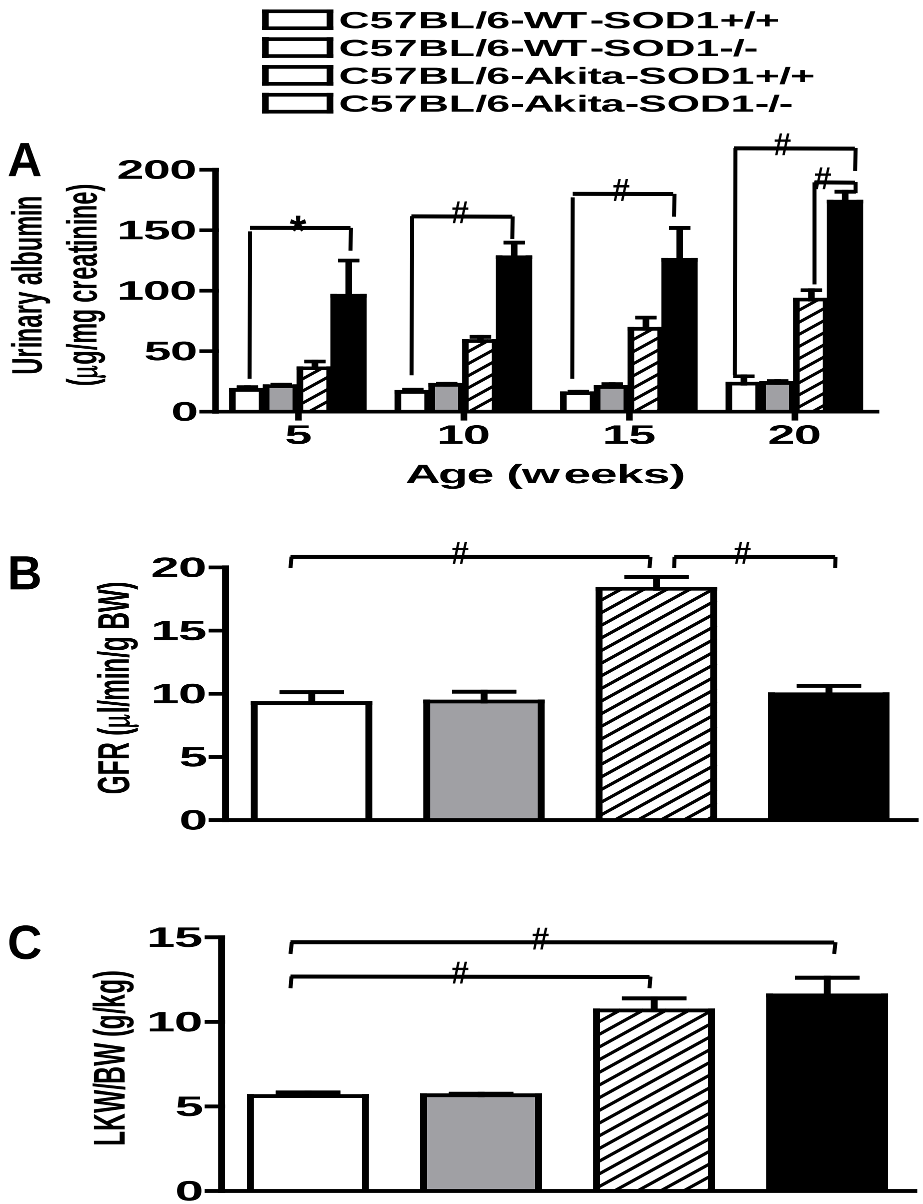
Immunofluorescence histochemistry and Western blot for SOD3 (extracellular SOD) in 15-week-old male mouse kidneys



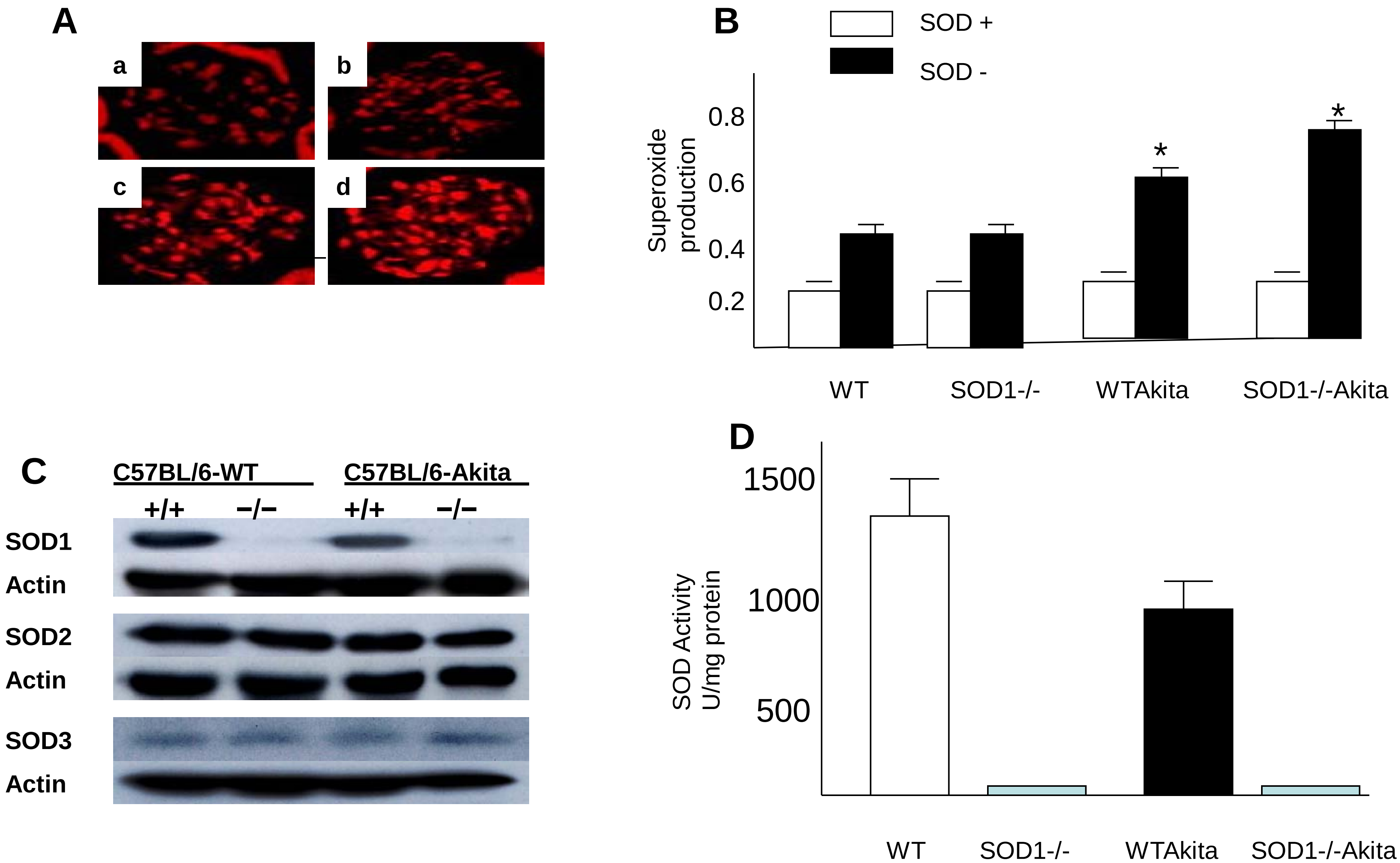


* P < 0.001 vs. KK-wild-type; n = 4 for each group

Increased Albuminuria and Failure to Hyperfilter in Akita SOD-/- Mice



Increased Glomerular ROS in AkitaSOD1-/- Mice



Increased Mesangial Expansion in Akita- SOD1-/- Mice