AMDCC II
Update for Fall 07

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Hypothesis

Adiponectin and Nox4 are key modifier genes for diabetic nephropathy and diabetic cardiovascular complications

Proposed Models

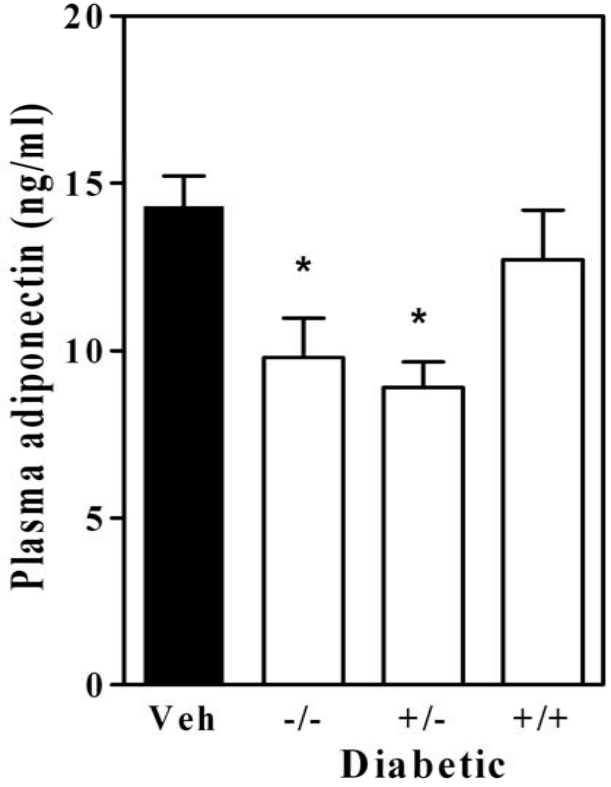
- Model 1: ***Apn/Dcn*** **dKO mouse with diabetes**
  - B6.129-Dcn^{tm1loz}-Adipoq^{tm1Chan}/Kxs
  - Data thus far in AdipoKO mice +/- diabetes

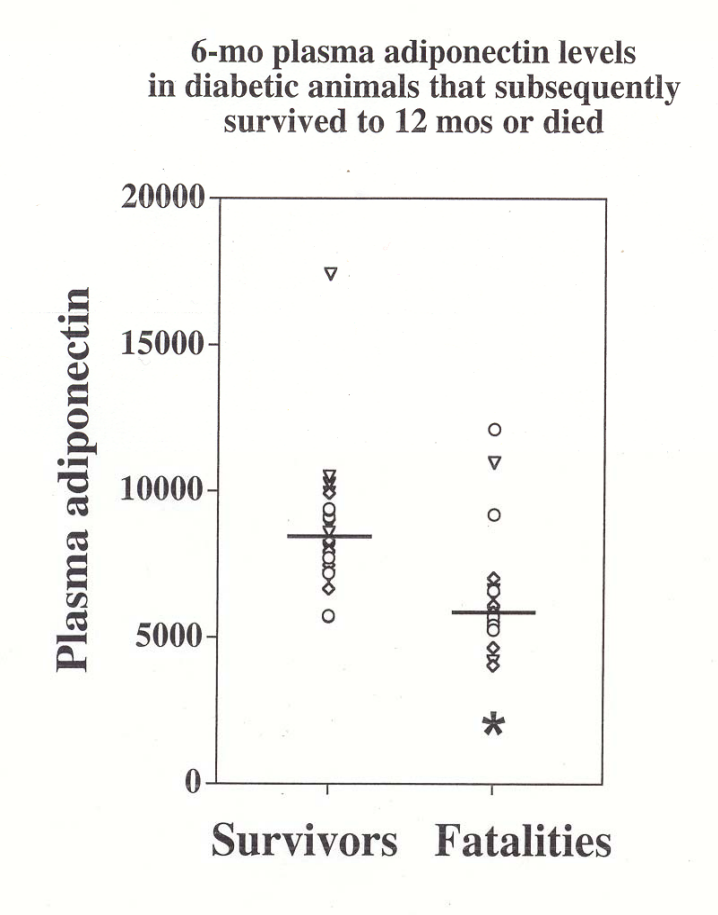
- Model 2: **Nox4 transgenic with SM22α promoter**
  - B6;129-Tg(Nox4-SM22α)/Kxs
  - New proposal (podocyte specific inducible Nox4 tg)

Diabetic *Dcn* dKO Mice

- Decorin (Dcn) is a small leucine rich proteoglycan which is an endogenous inhibitor of TGF-β and increased in mouse and human diabetic kidney disease
- Diabetic *Dcn* KO mice was characterized by progressive albuminuria, increased mesangial matrix accumulation, glomerular fibrin cap formation, GBM and TBM thickening, deterioration in renal function, and increased mortality
- *Dcn* KO diabetic mice with increased mortality had high plasma creatinine and low adiponectin levels
- *Dcn* KO diabetic mice have increased Nox4 in kidneys and glomeruli (in press Am J Pathol)

Dcn Deficient Diabetic Mice Associated with Decreased Adiponectin

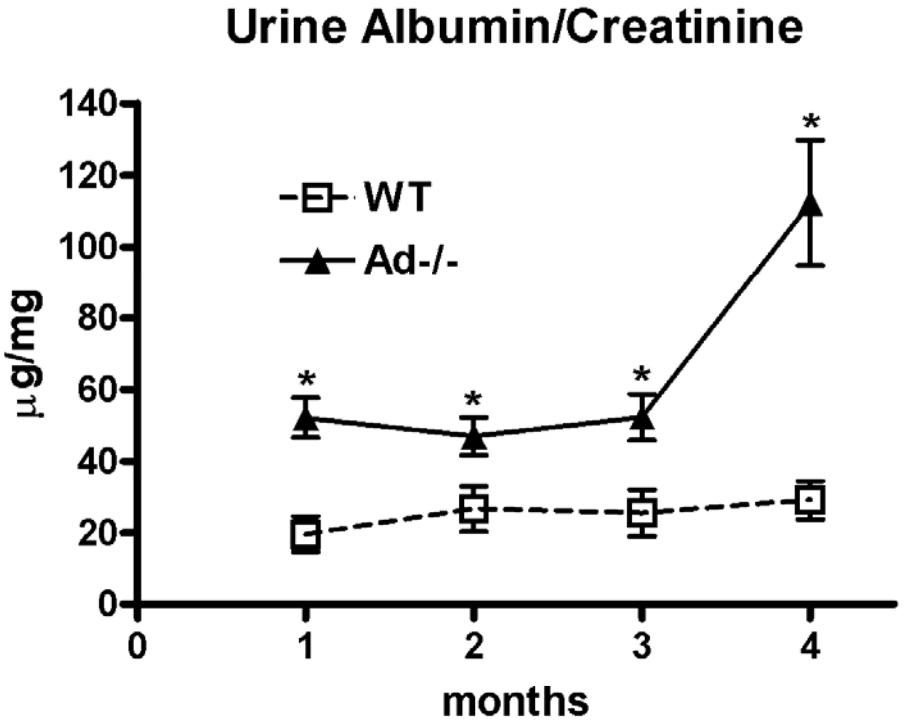




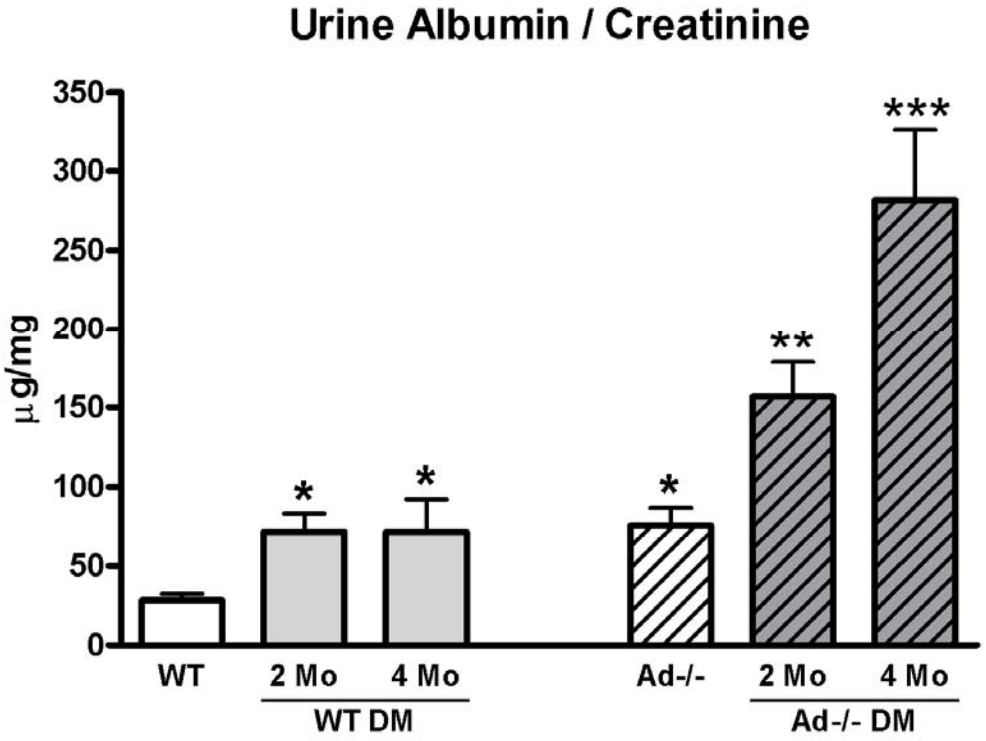
Adiponectin and Kidney Disease

- Adiponectin (also called AdipoQ, ACRP30, and GBP28) is an abundant circulating protein, signals via AdipoR1 and R2 and is expressed primarily by adipocytes
- Adiponectin enhances insulin sensitivity, suppresses inflammation, and may protect against vascular disease
- Adiponectin levels have been found to be an important variable linked to cardiovascular disease in patients with kidney disease and type 1 diabetes
- Adiponectin promoter SNPs associated with type 1 DN
- Adiponectin KO mice have increased glomerular disease with sub-total nephrectomy (ATVB 2007)

Adiponectin KO Mice Have Increased Albuminuria



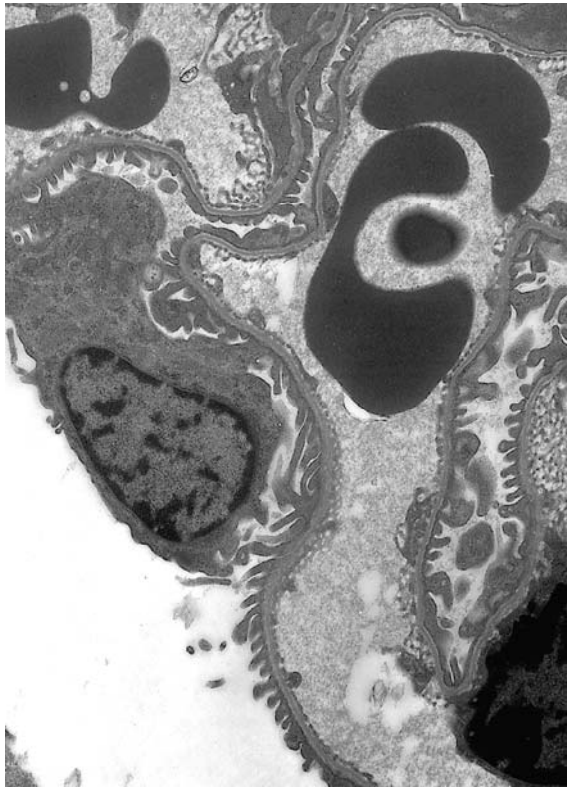
Increased Albuminuria in Diabetic Adiponectin KO Mice on Bl6



Podocyte Effacement in Ad KO Mice: Reversal with Ad Treatment

WT

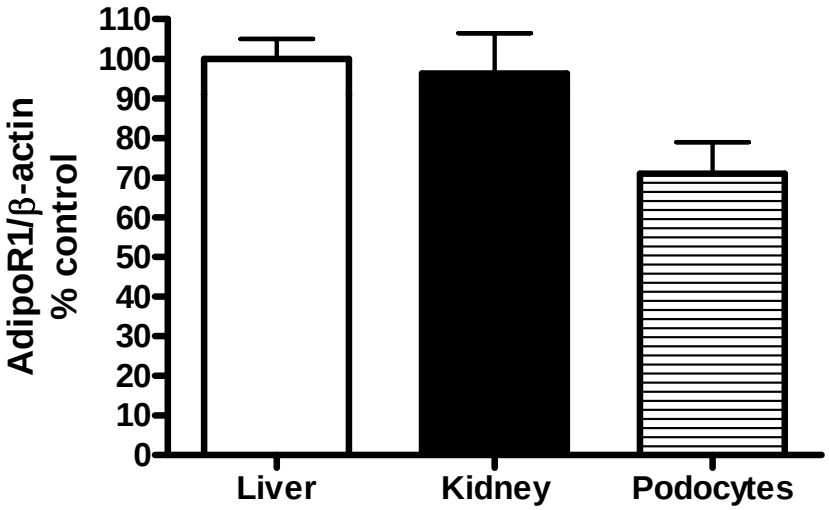
Ad-/-

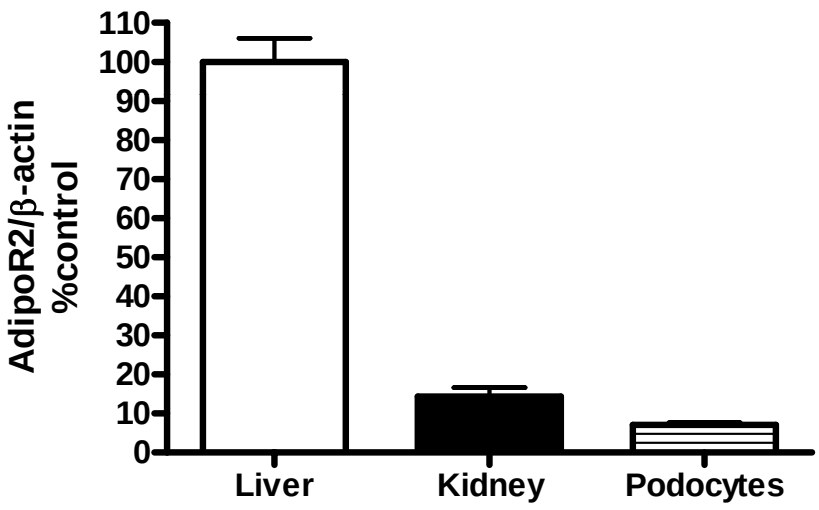


Ad-/-

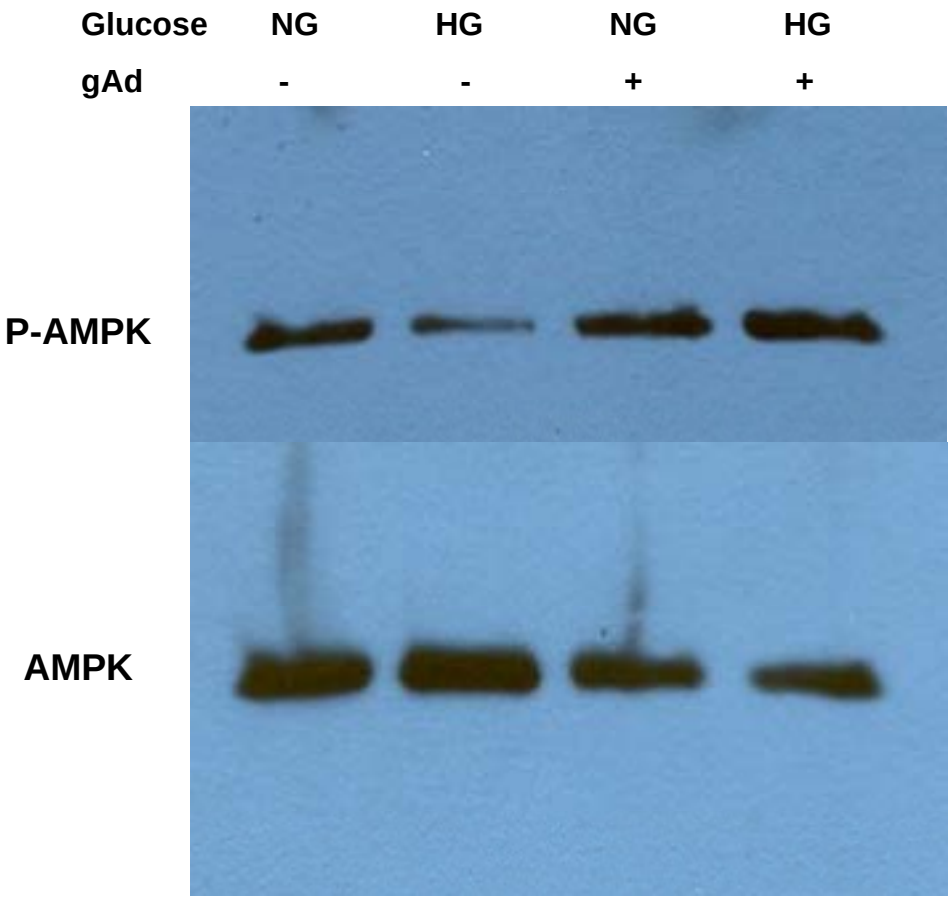
+gAd

Kidney and Podocytes Express Adipo R1 but not R2

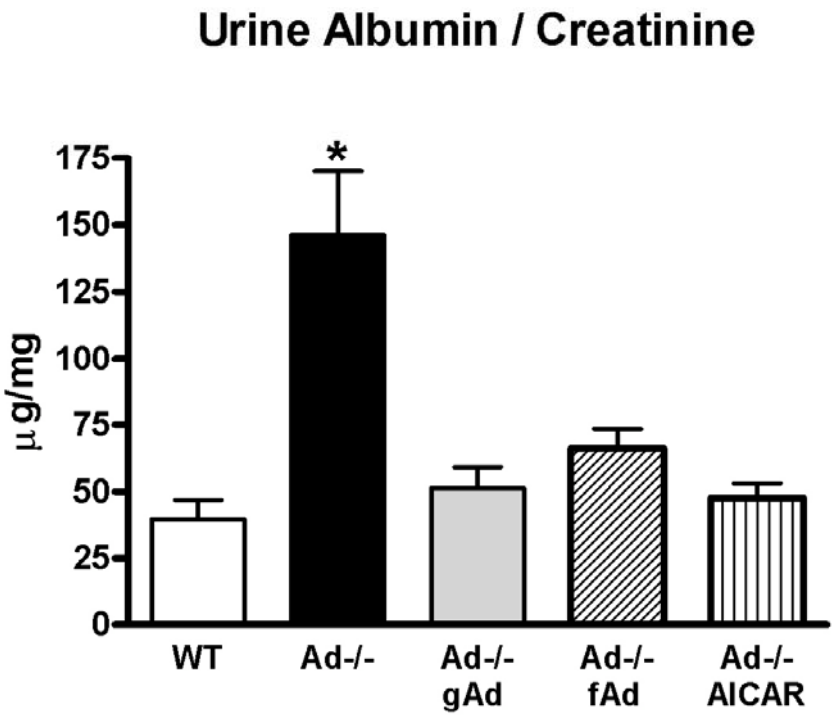




High Glucose Suppression of AMPK in Podocytes is Prevented by Adiponectin



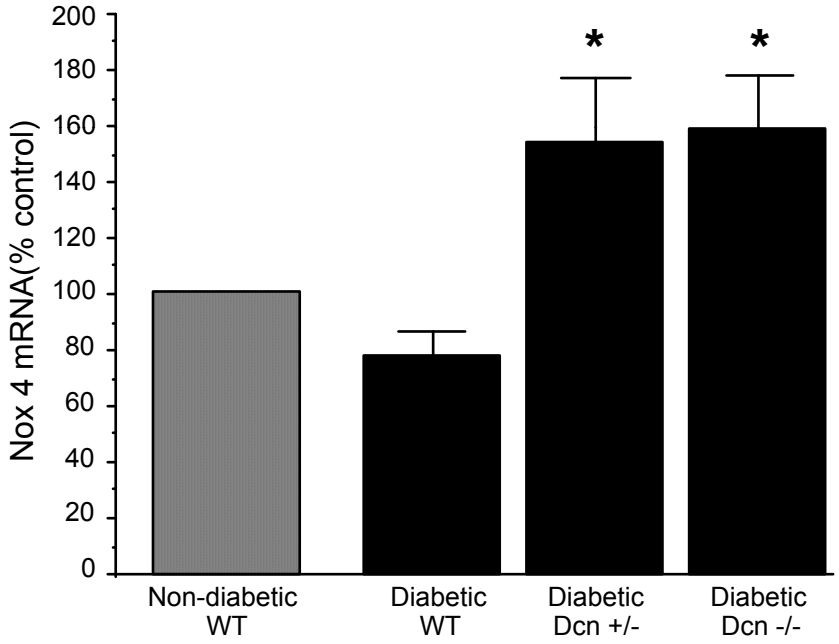
Adiponectin and AMPK Stimulation Normalizes Albuminuria in AdKO Mice



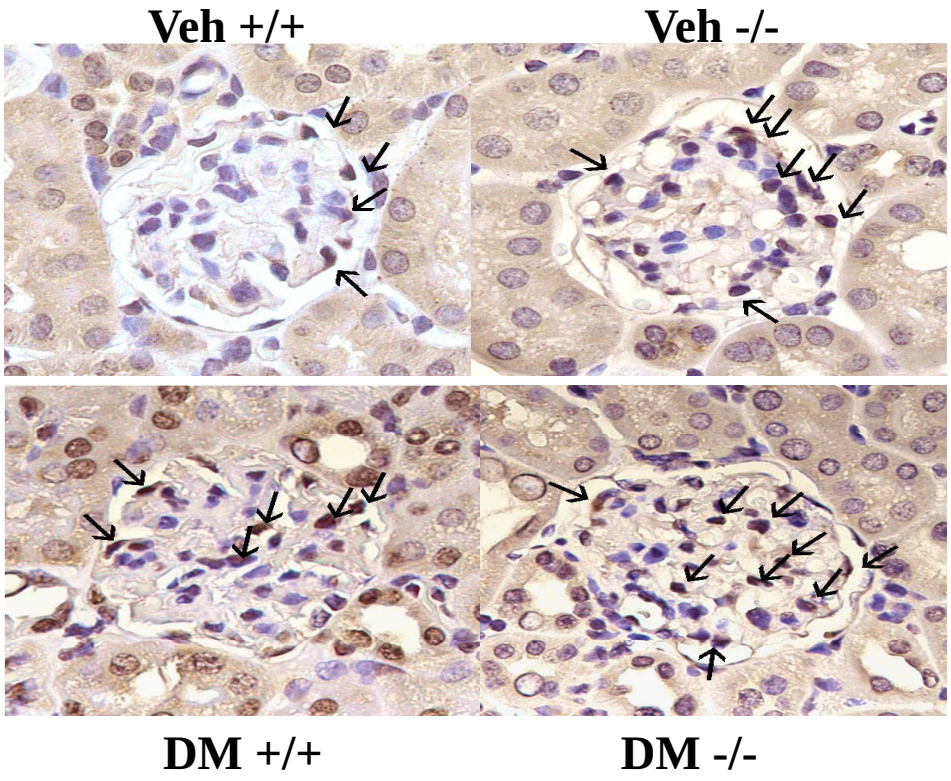
Summary

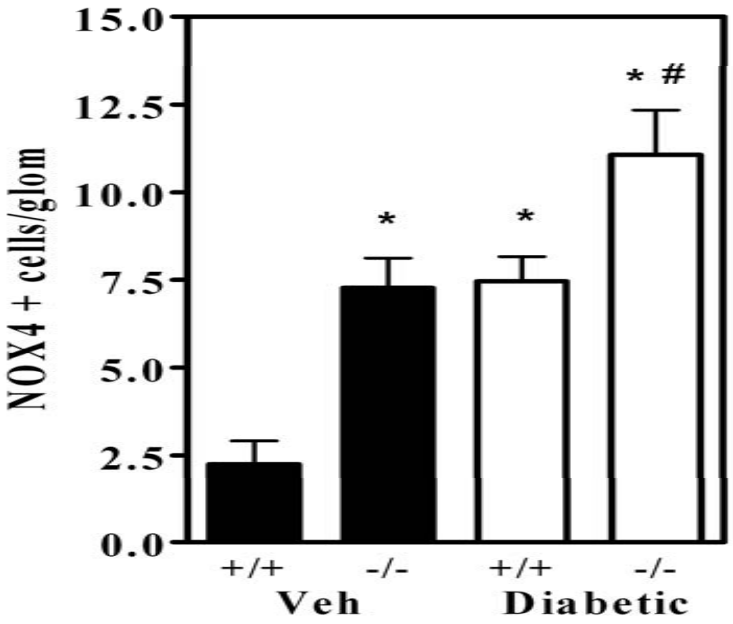
- Adiponectin KO mice have increased albuminuria and podocyte foot process effacement
- Podocytes express AdipoR1 and respond to adiponectin with AMPK activation
- Treatment of AdKO mice with Adiponectin or AMPK activators normalizes albuminuria
- Study long-term diabetes (12 months) with Akita in AdKO mice, backcross onto DBA
- Will study downstream targets of AMPK in podocytes and relationship to albuminuria and diabetes
- Crossing of AdKO mice with DcnKO, AdipoR1 KO or other disease modifiers may enhance progressive diabetic nephropathy in mice
- Role of Adiponectin in other diabetic vascular complications, may be worthwhile to include in standard phenotyping

Decorin and Renal Nox4

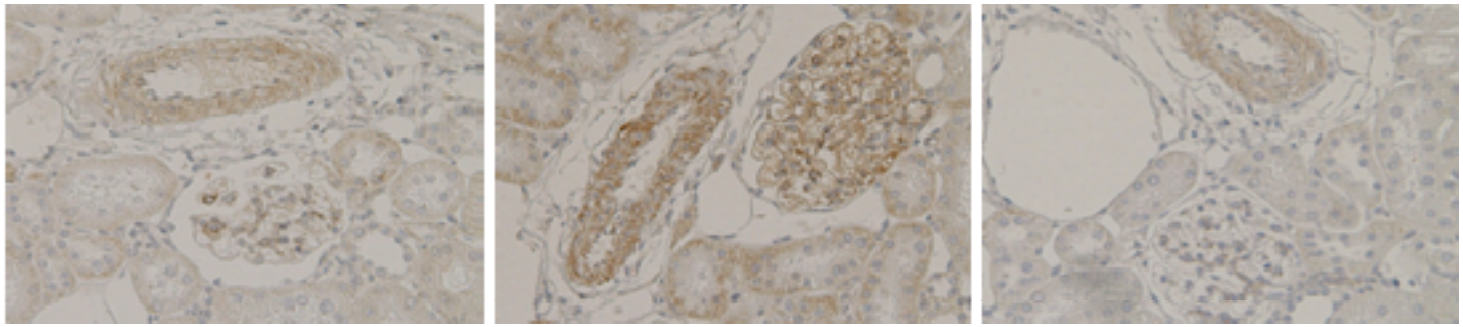


Diabetic Dcn KO Mice Have Increased Glomerular Nox4

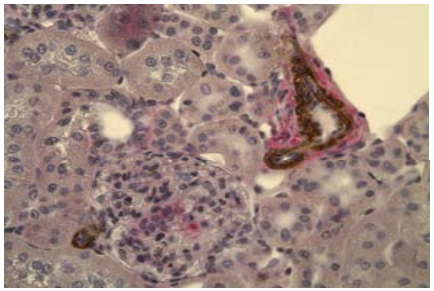




Nox 4 and Diabetic Kidney

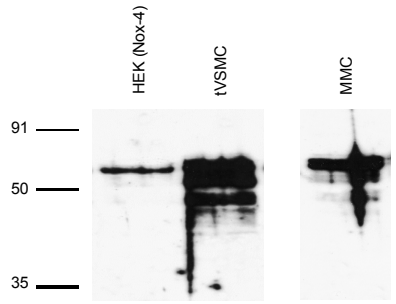


Normal Rat STZ-Diabetic-2 wks STZ-Diabetes + aT



Nox4 and

SM α-actin



NADPH Oxidase Isoforms

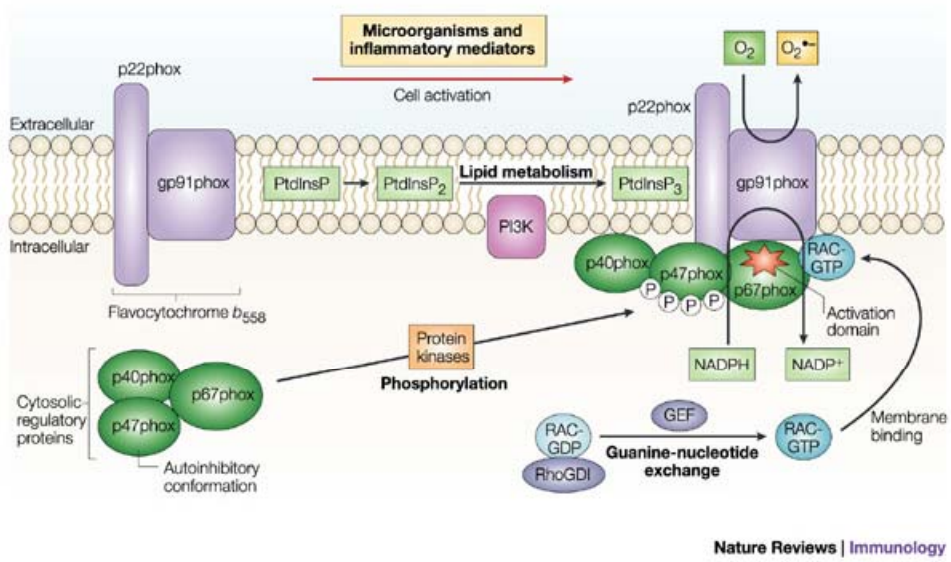
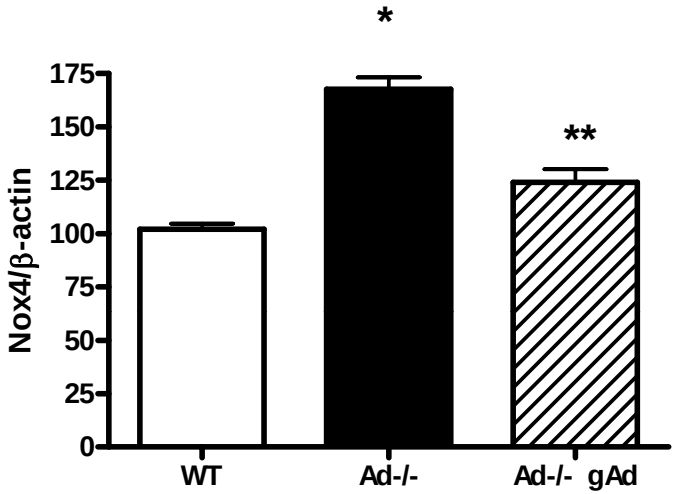


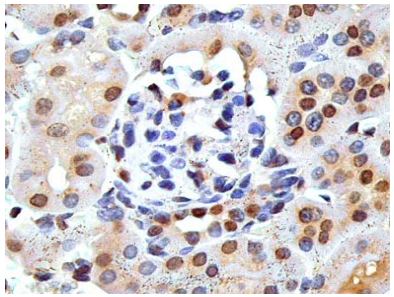
Table 1 | **Human NOX/DUOX enzymes**

Enzyme	Highest level of expression	Known regulatory factors
gp91phox (NOX2)	Phagocytes	p47phox, p67phox, p40phox and RAC1/RAC2
NOX1	Inducible: colon and vascular smooth muscle	NOXO1, NOXA1 and p22phox
NOX3	Fetal kidney	N.D.
NOX4	Kidney, osteoclasts, ovary and eye; widespread	N.D.
NOX5	Spleen, sperm, mammary glands and cerebrum	Calcium
DUOX1	Thyroid, cerebellum and lungs	Calcium
DUOX2	Thyroid, colon, pancreatic islets and prostate	Calcium

Nature Reviews Immunol, 2004

Ad KO Mice have Increased Renal and Glomerular Nox4





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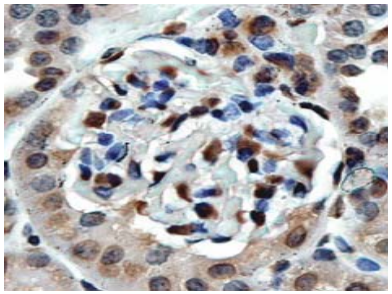
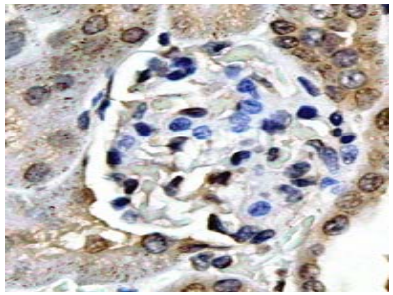
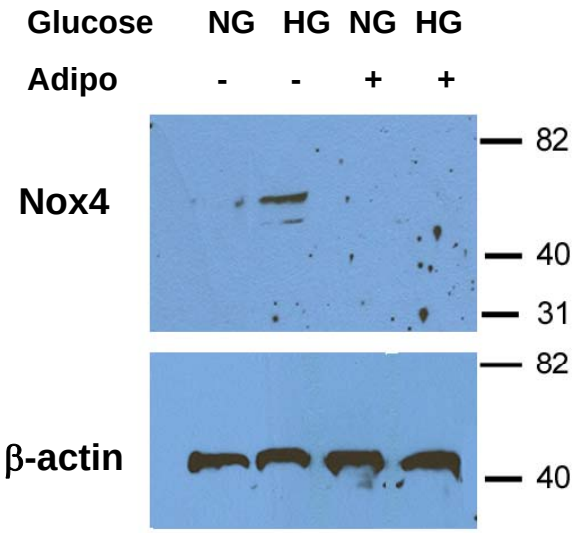


Ad-/-



Ad-/- gAd

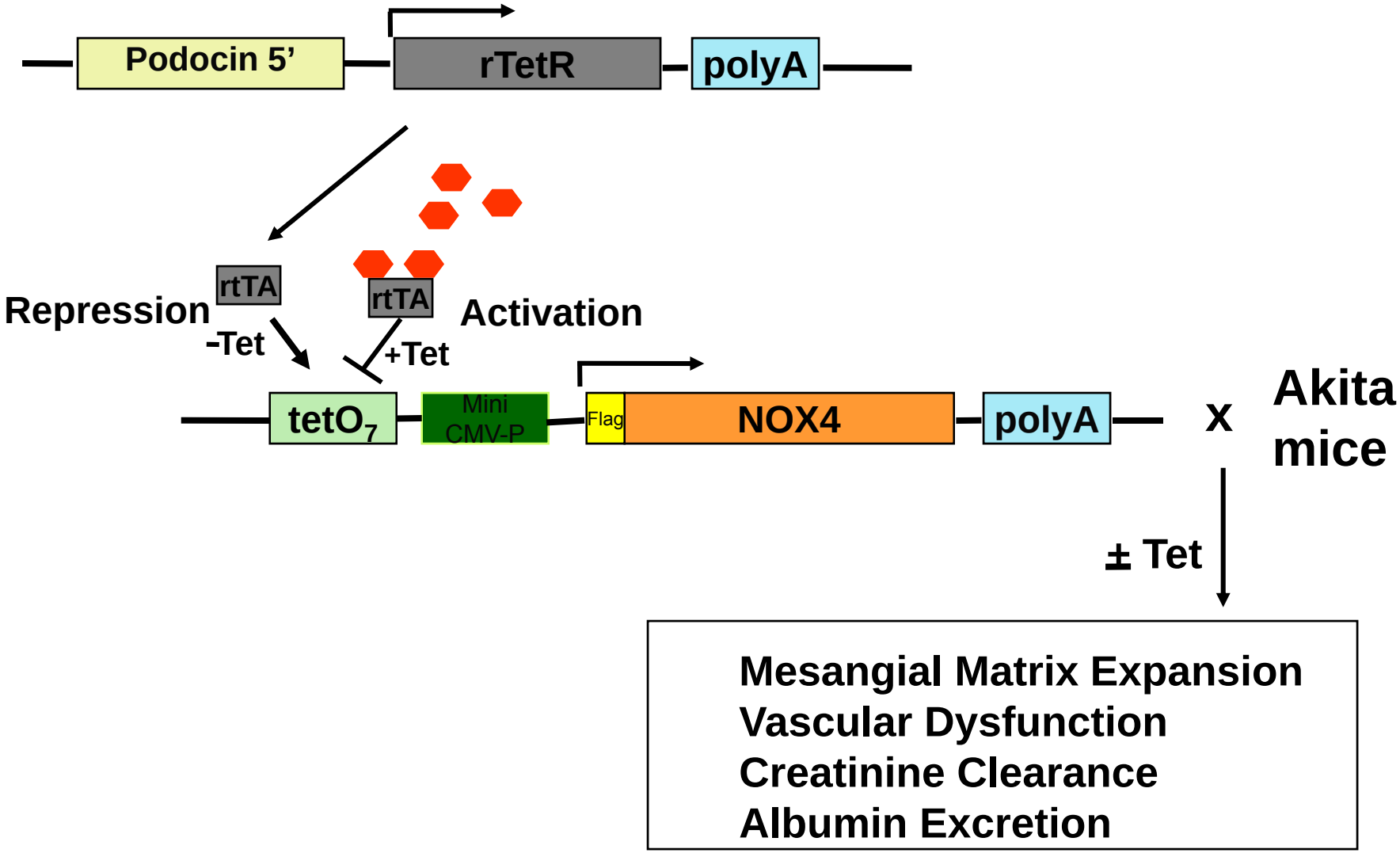
Upregulation of Nox4 in Podocytes by High Glucose is Regulated by Adiponectin



Rationale for tg SM22α/Nox4

- NADPH oxidase involved in impaired autoregulation of diabetes
- Nox4 highly expressed in kidney and increased in smooth muscle cells of diabetic kidney
- SM22α promoter specific for vascular smooth muscle cells and 2-3x increase over basal
- Difficulty in inserting Nox4 in Sm22α vector
- Concern regarding sustained over-expression of Nox4 independent of diabetes

Proposal for Inducible Targeted tg podocyte-Nox4



Planned Studies in Next 1-2 years

- Akita Inducible Podocyte-Nox4tg mice will be phenotyped for diabetic nephropathy
- Plan to study regulation of Nox4 in podocytes in cell culture
- Inducible Nox4 may be applicable to diabetic cardiovascular disease, retinopathy, neuropathy

Acknowledgements

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