

Mechanisms of hypertension and diabetic nephropathy

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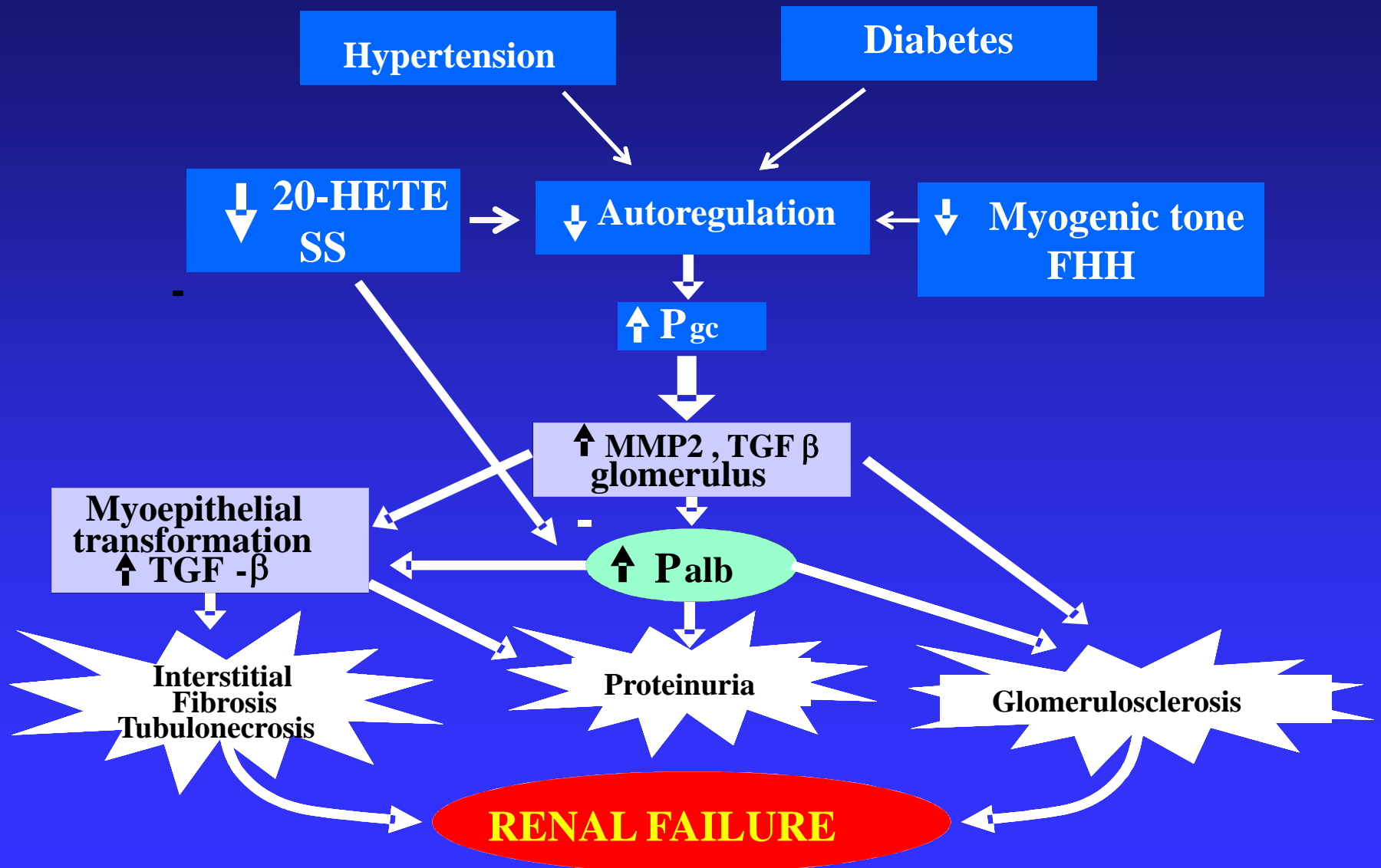


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MEDICAL CENTER**

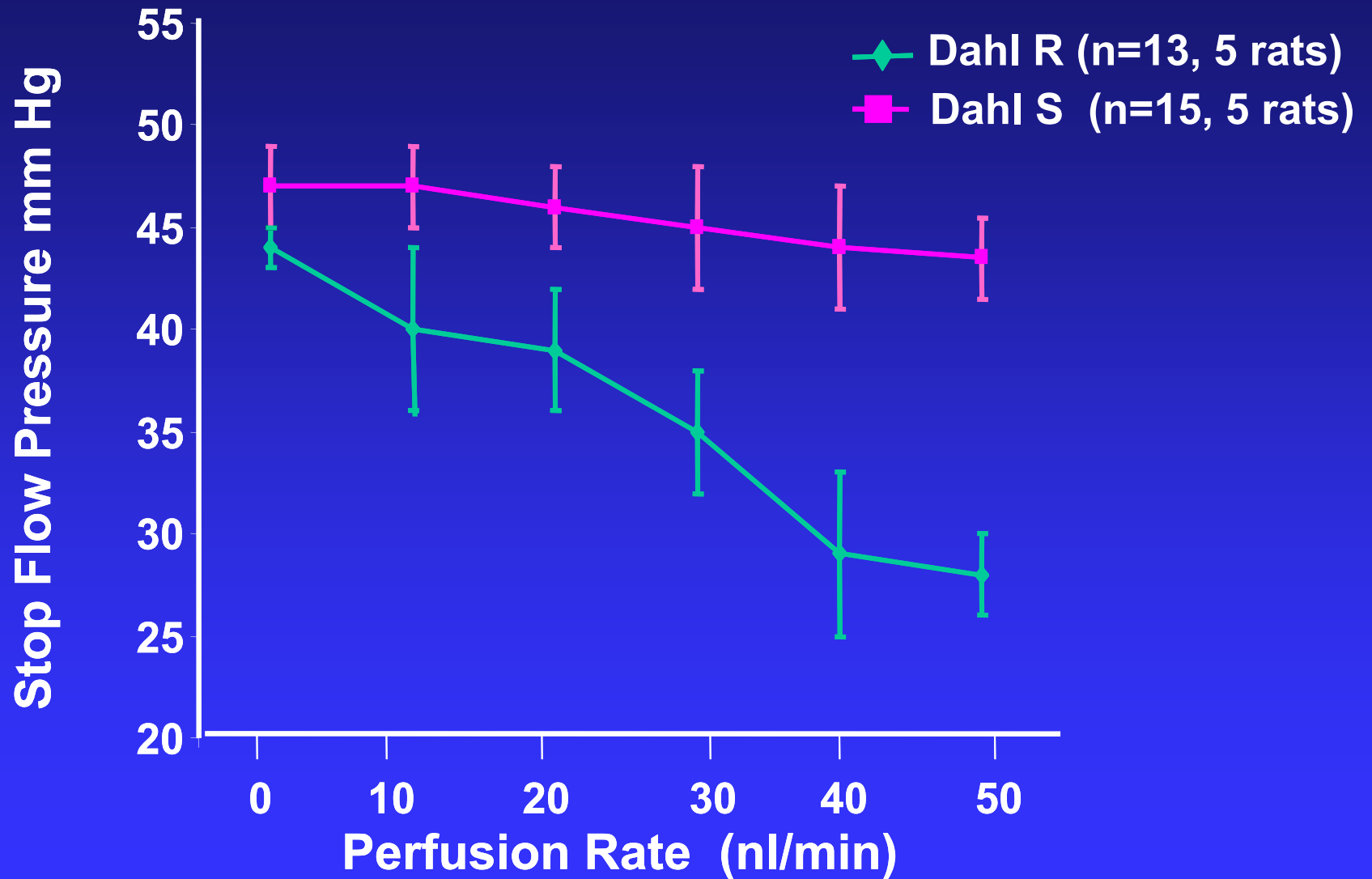
The economic burden of kidney disease

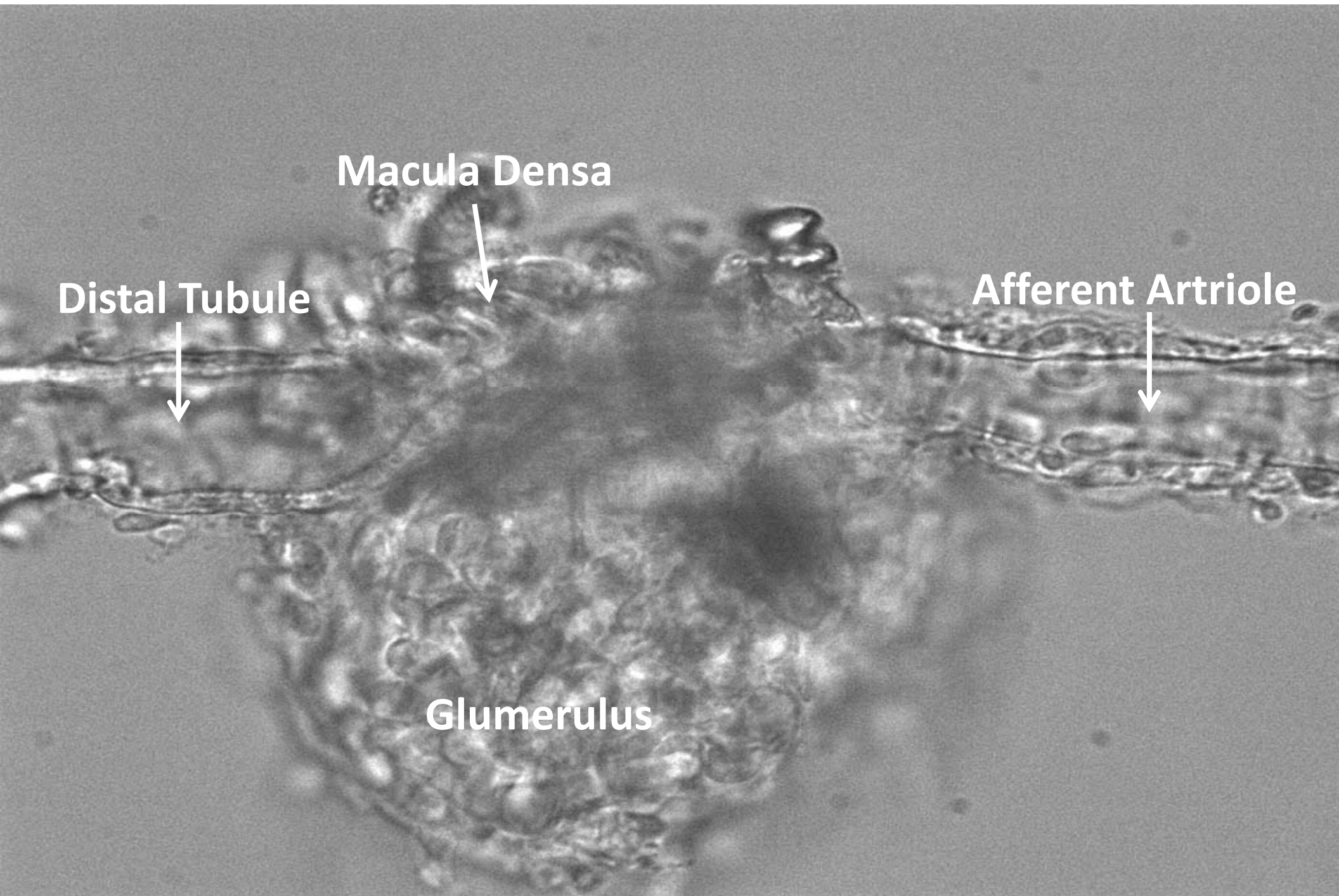
- Hypertension and Diabetes are the leading cause of end stage renal disease. 66% of new cases.
- Number of patients with chronic renal disease is exploding currently estimated at 30 million or 13% of the population.
- Currently, approximately 500,000 Americans have been diagnosed with kidney failure. The number of Americans with this advanced stage of the disease is expected to grow to 785,000 by 2020.
- The annual cost of treating kidney disease is \$42 billion, 25% of Medicare spending.
- **There is a critical need for development of new therapies to slow or reverse the progression of chronic kidney disease.**

Impaired autoregulation and 20-HETE, MMP2, TGF- β Interaction in the Pathogenesis of Renal Disease



TGF Responses in Dahl S and R Rats





Macula Densa



Distal Tubule

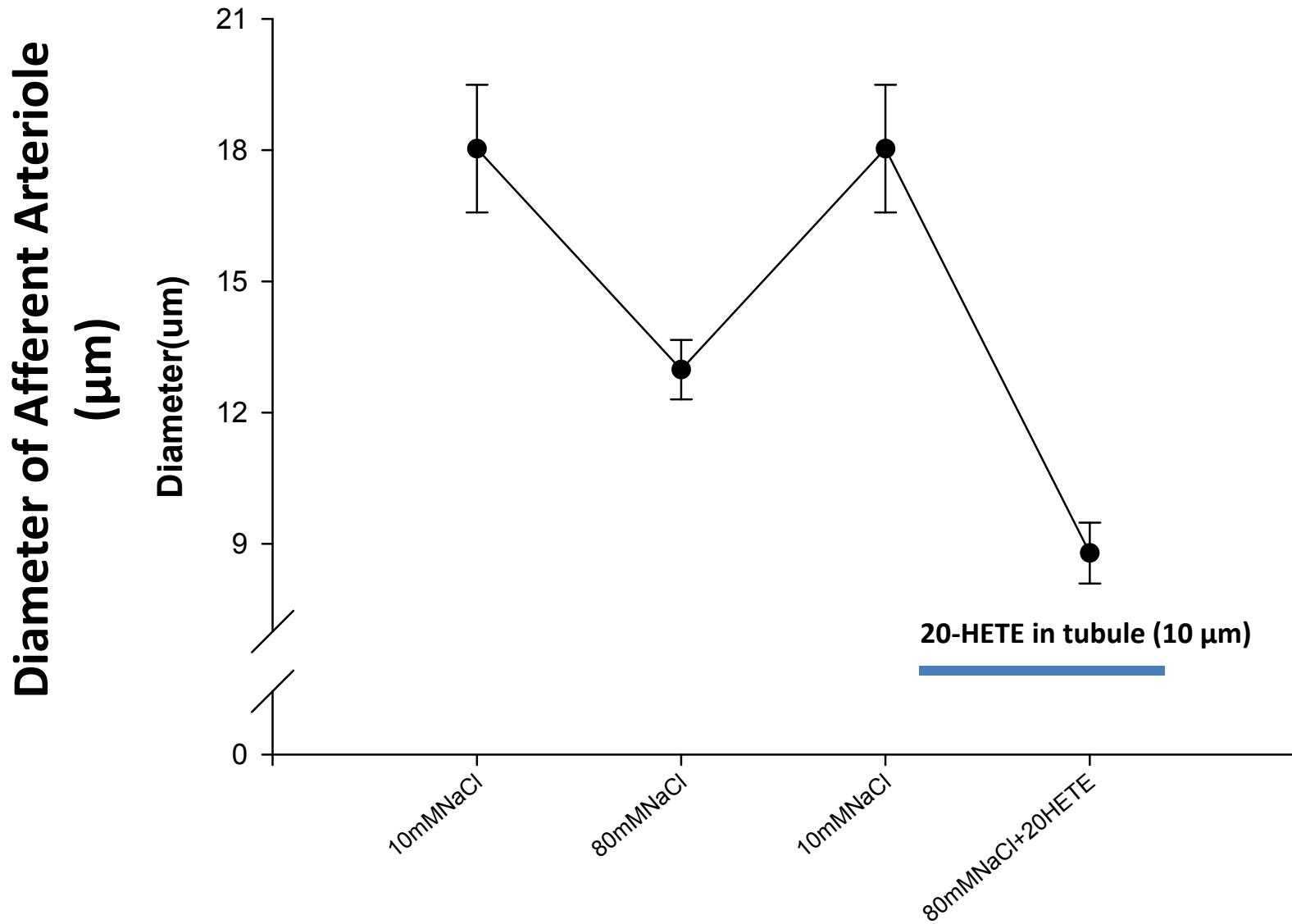


Afferent Artriole

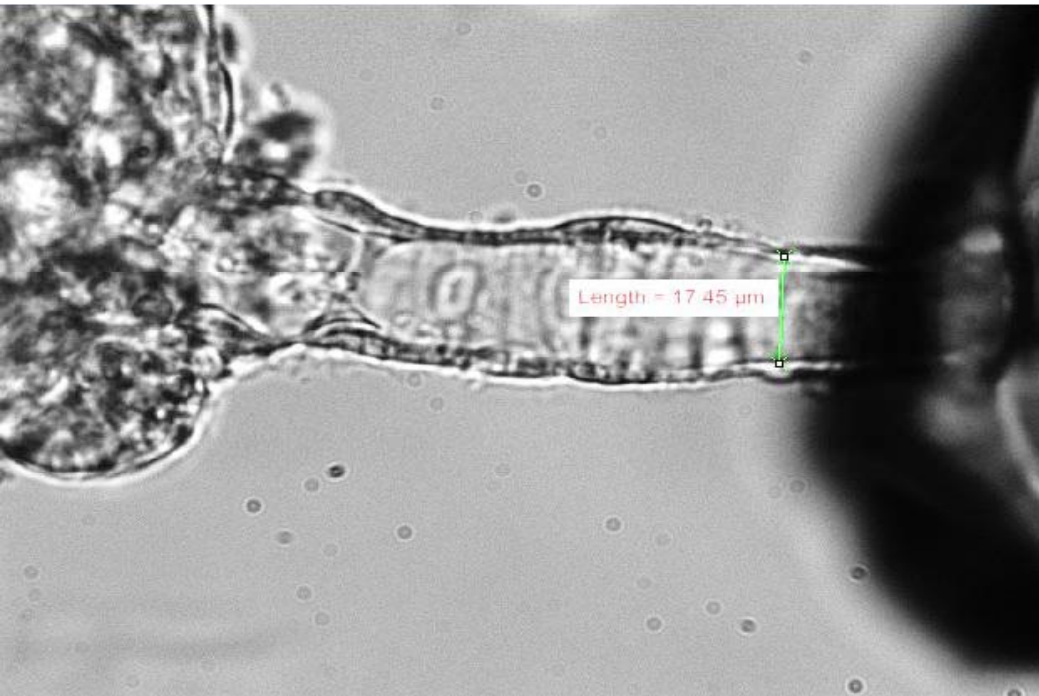


Glumerulus

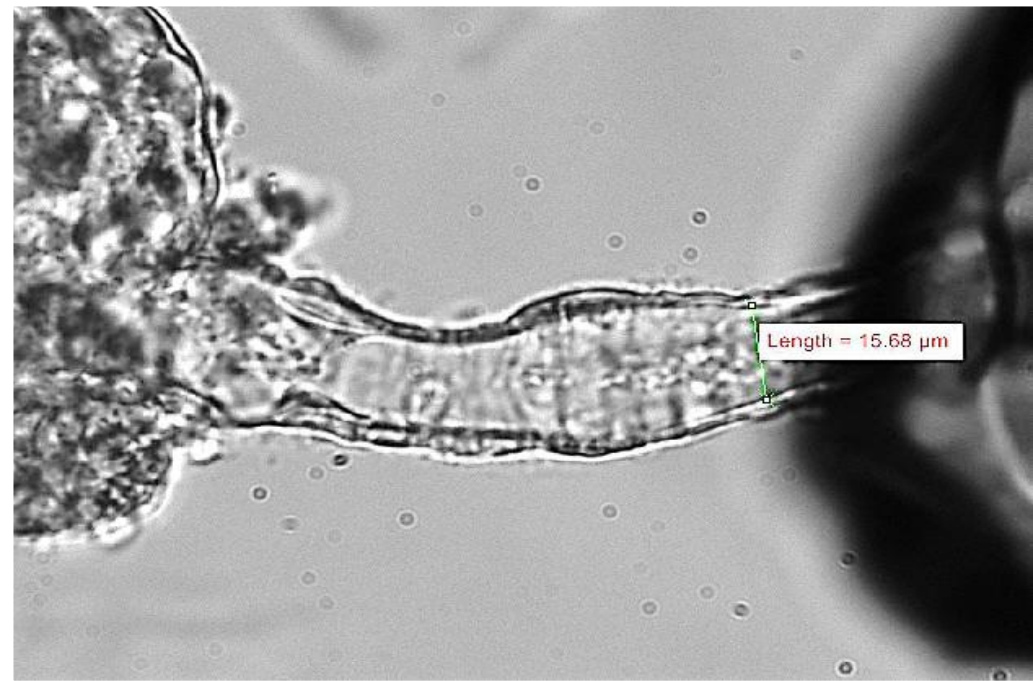
Effect of 20-HETE on TGF



Control

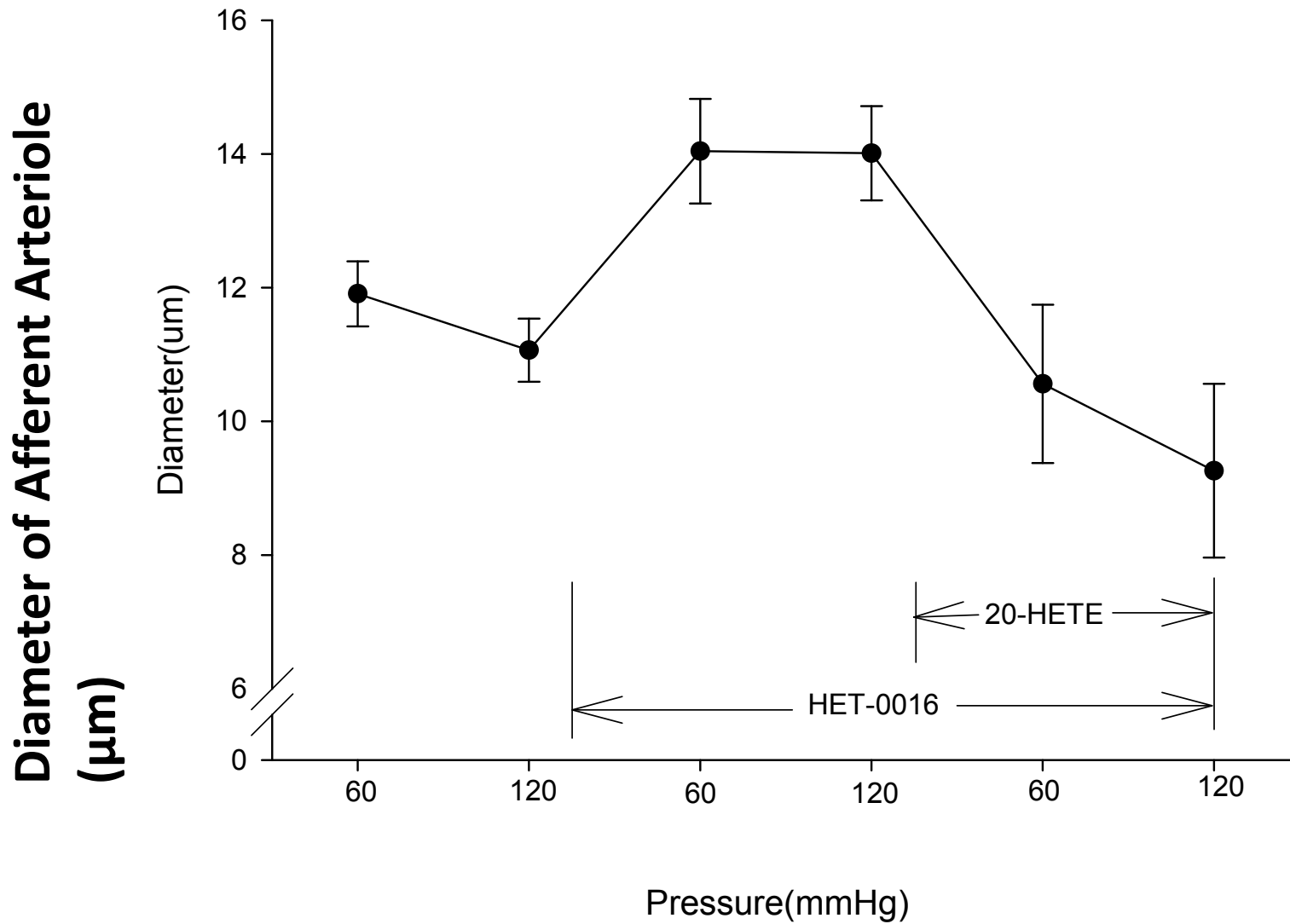


60mmHG

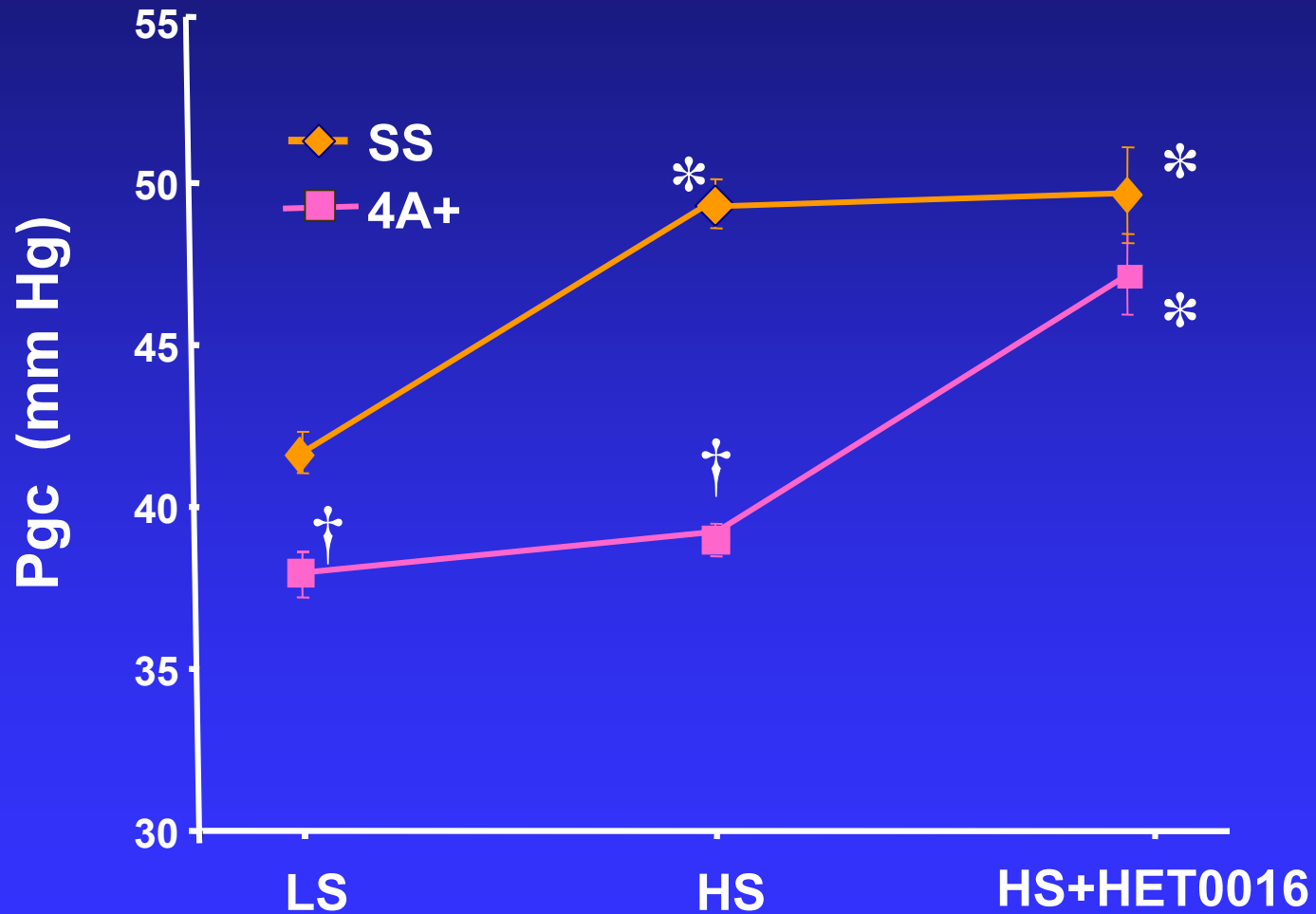


120mmHG

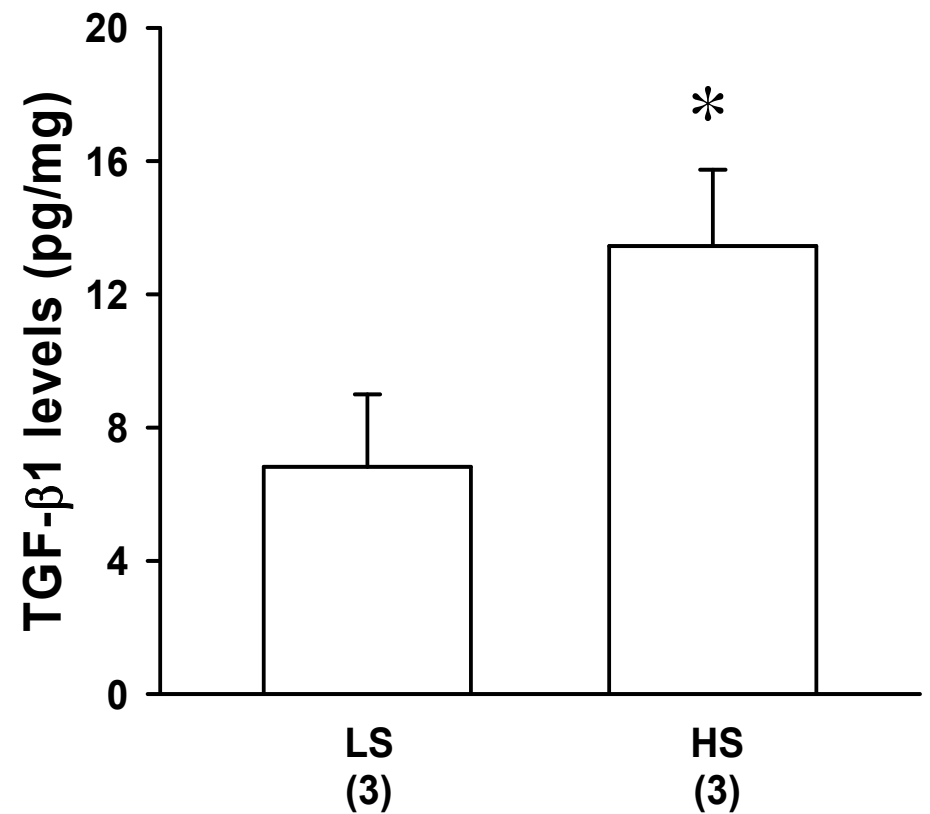
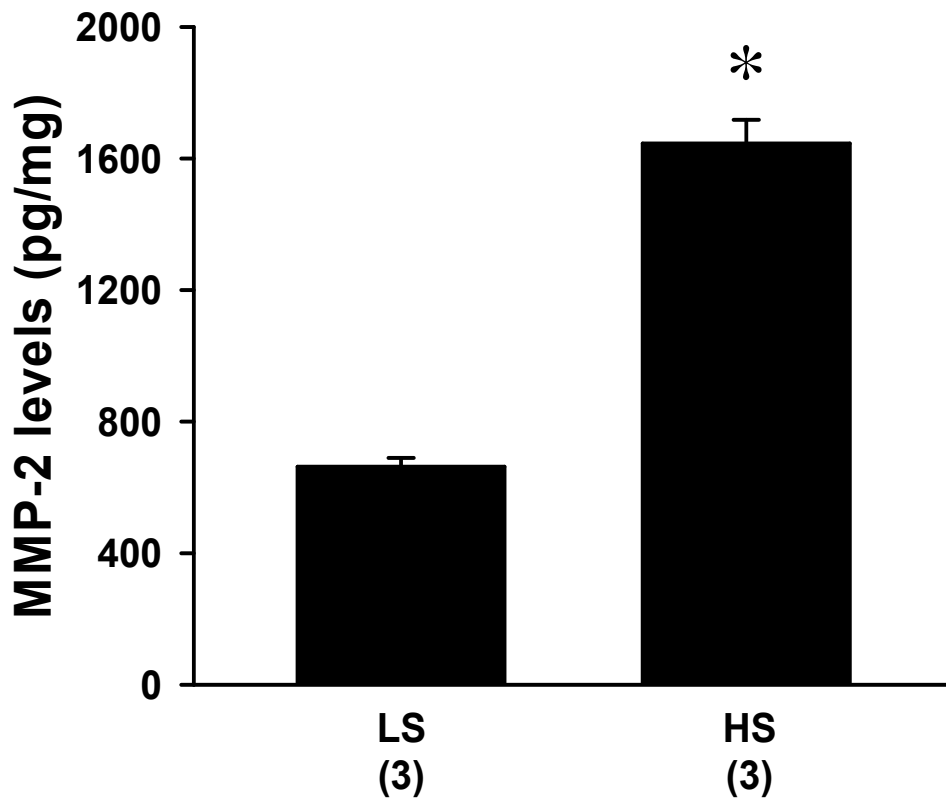
Effect of 20-HETE on Myogenic Response



Pgc during the During the Development of Hypertension in Dahl S rats

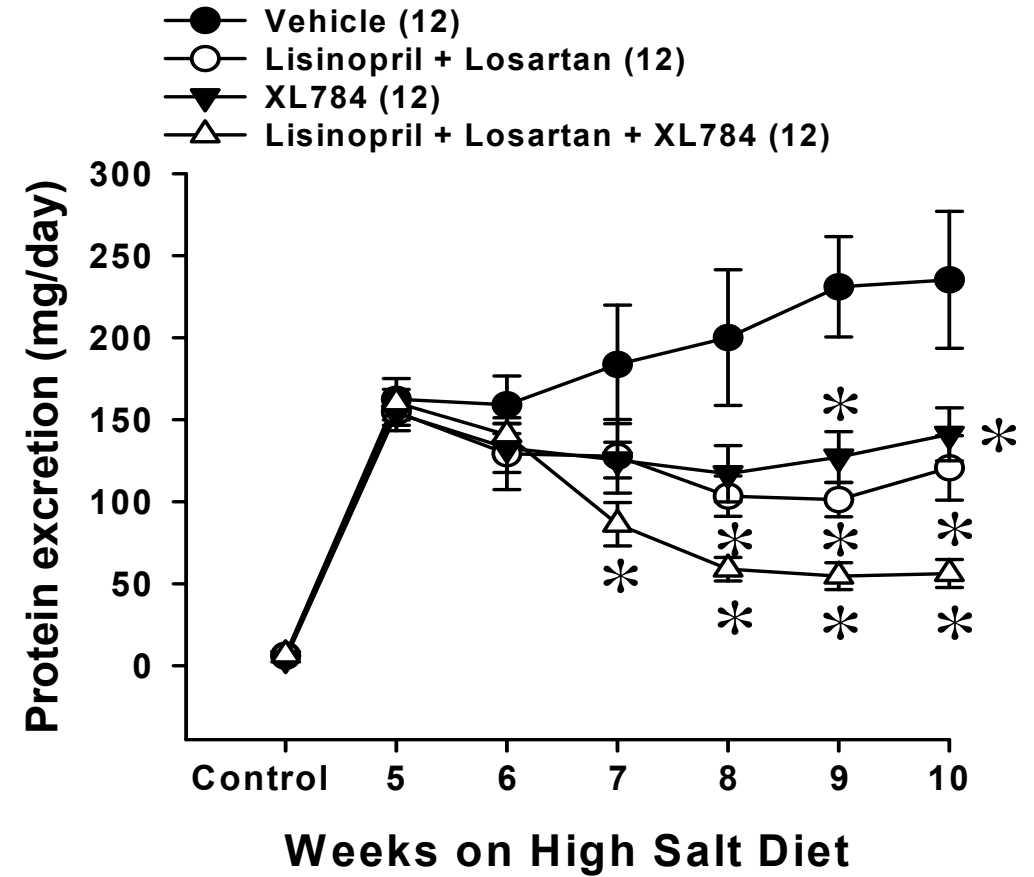
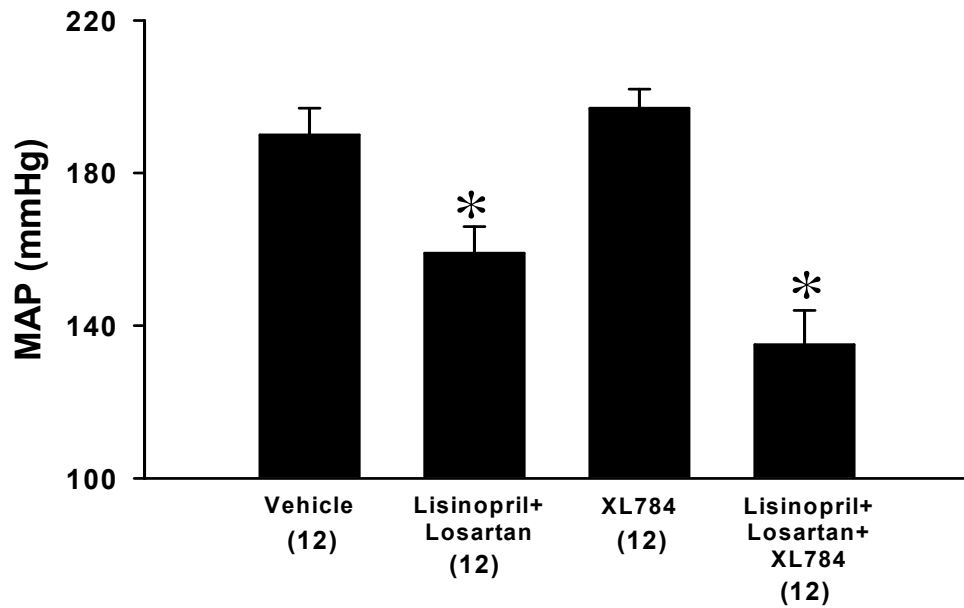


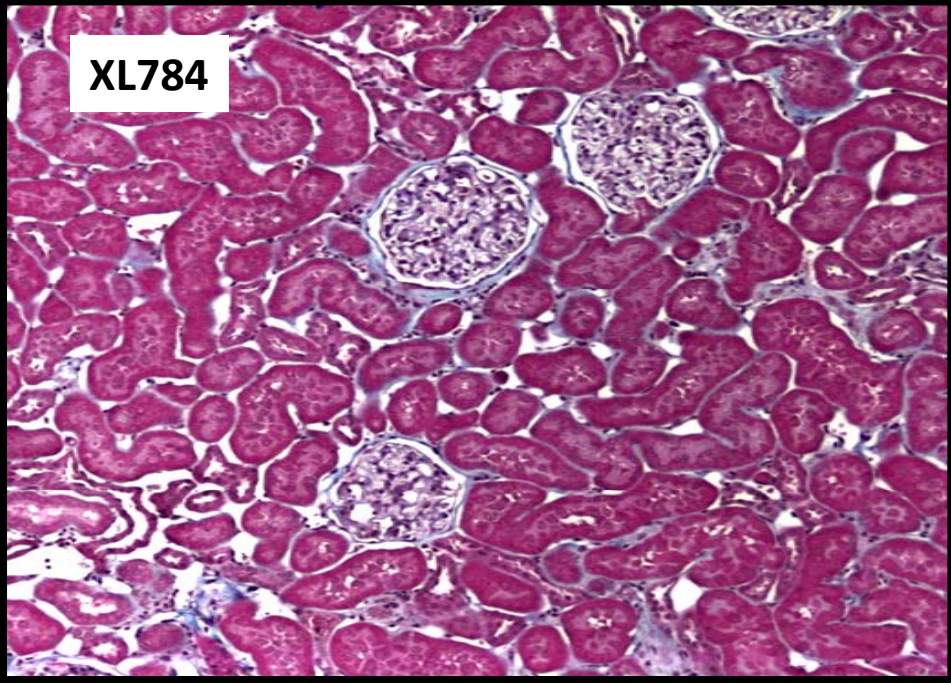
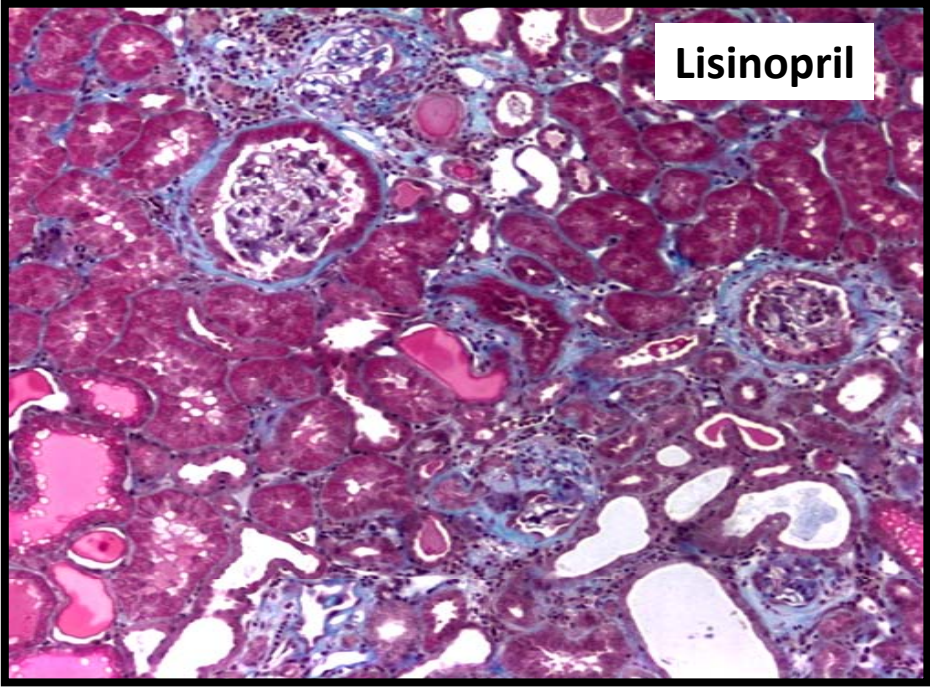
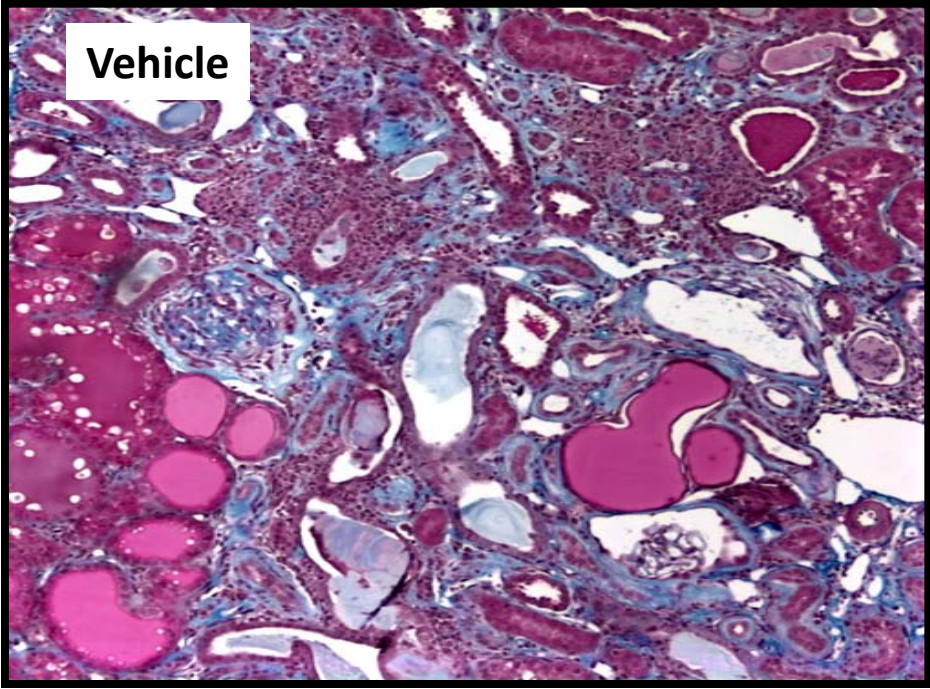
MMP-2 and TGF- β 1 levels in Dahl S rats fed either a low salt or high salt diet



Hypertension

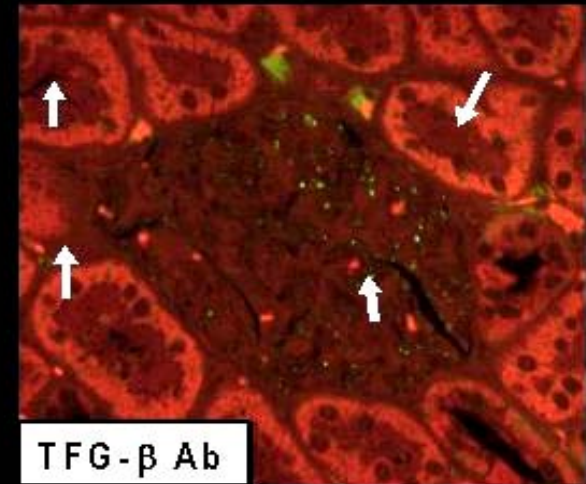
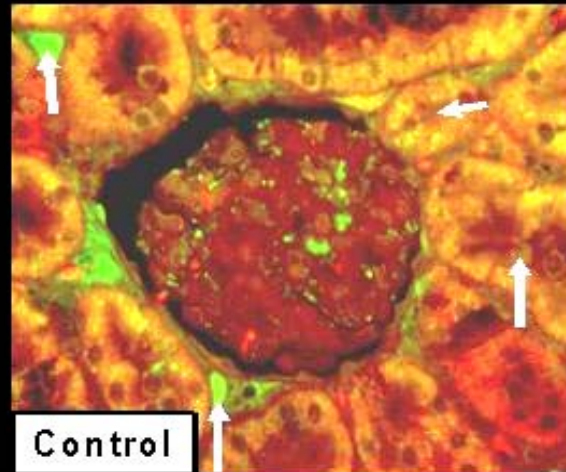
XL784 reversibility study in Dahl S rats



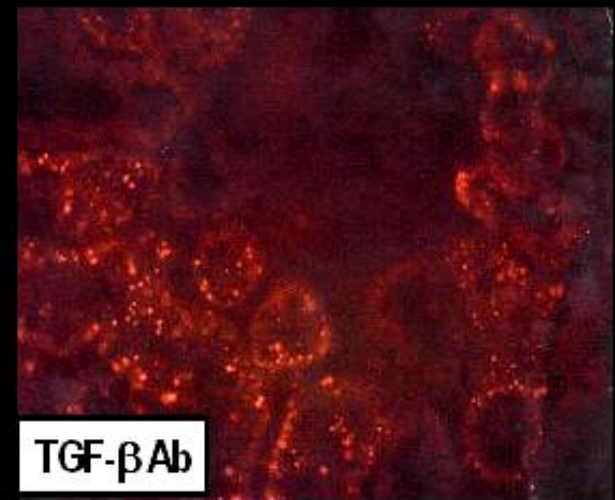
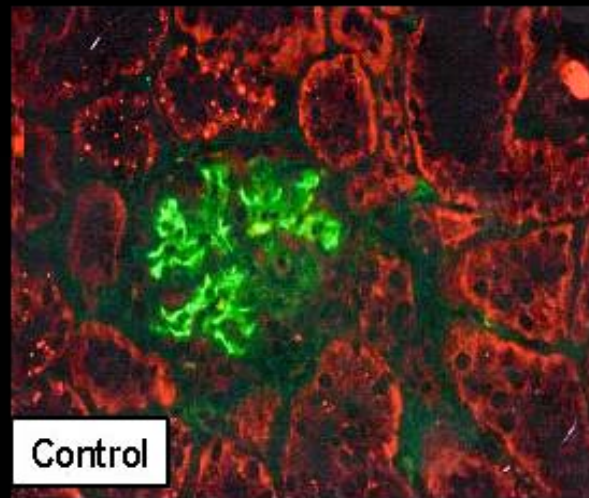


Effect of Hypertension on the Expression of TGF- β in Dahl S Rats

TGF- β 1



TGF- β 2



Effect of TGF- β Ab on Proteinuria and Glomerular Injury in Dahl S Rats fed a High Salt Diet for 3 Weeks.

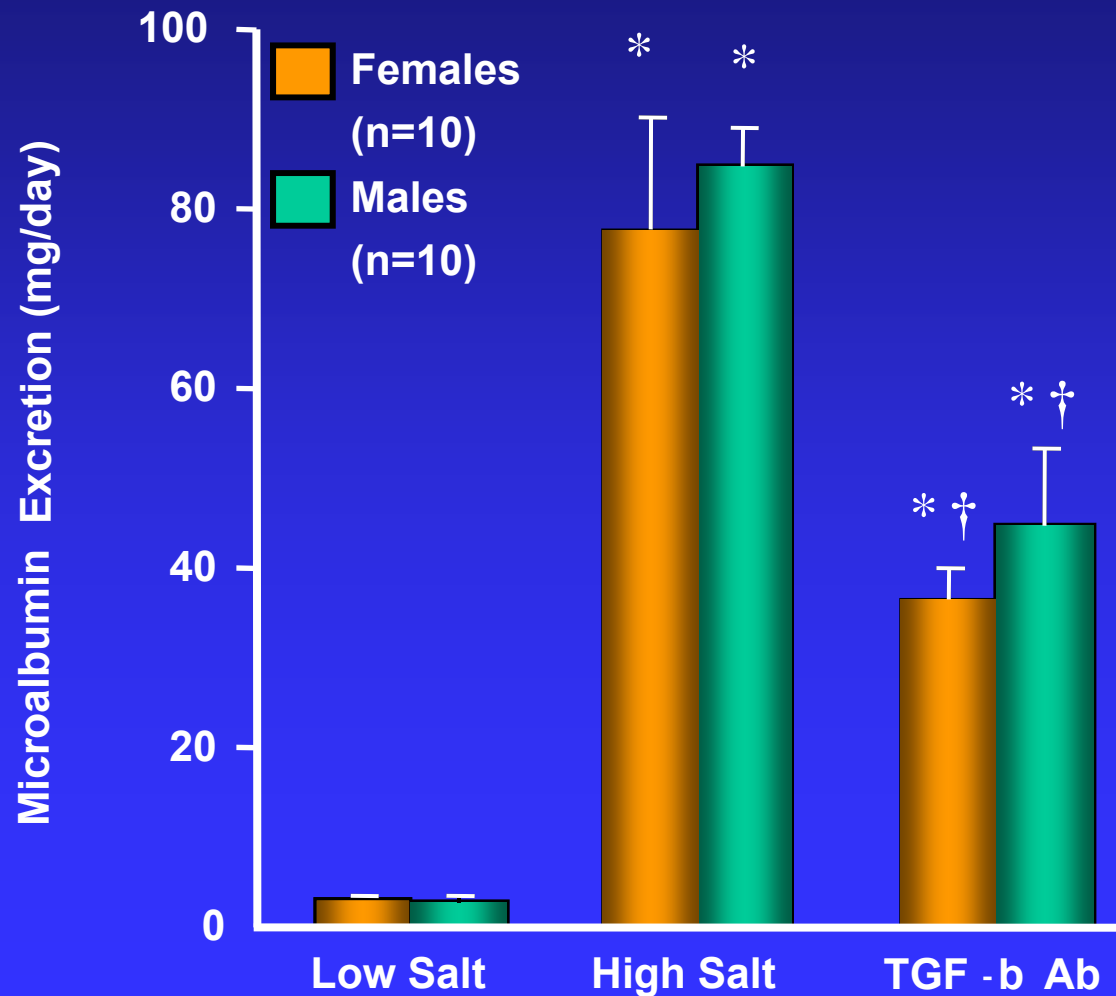
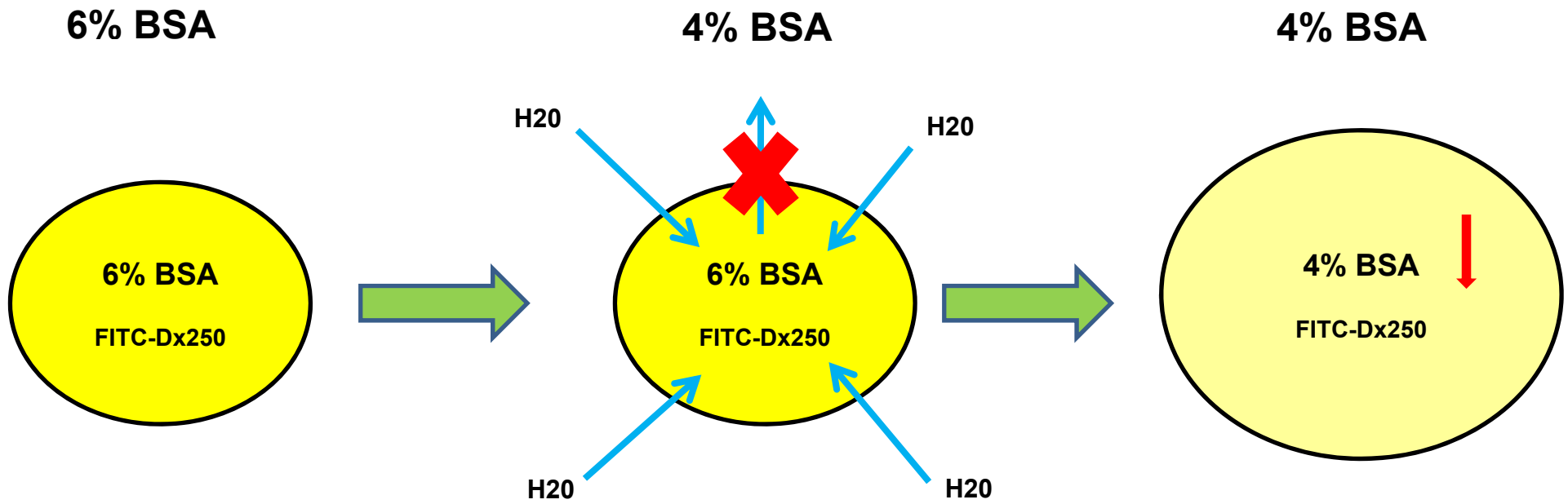


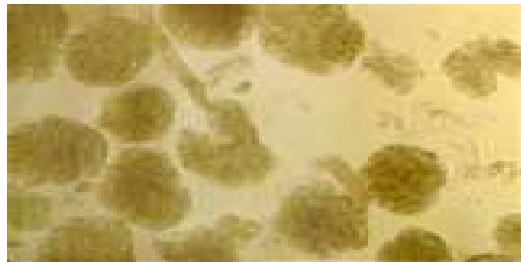
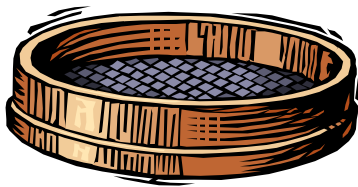
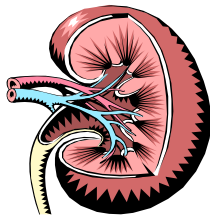
Figure 1 B



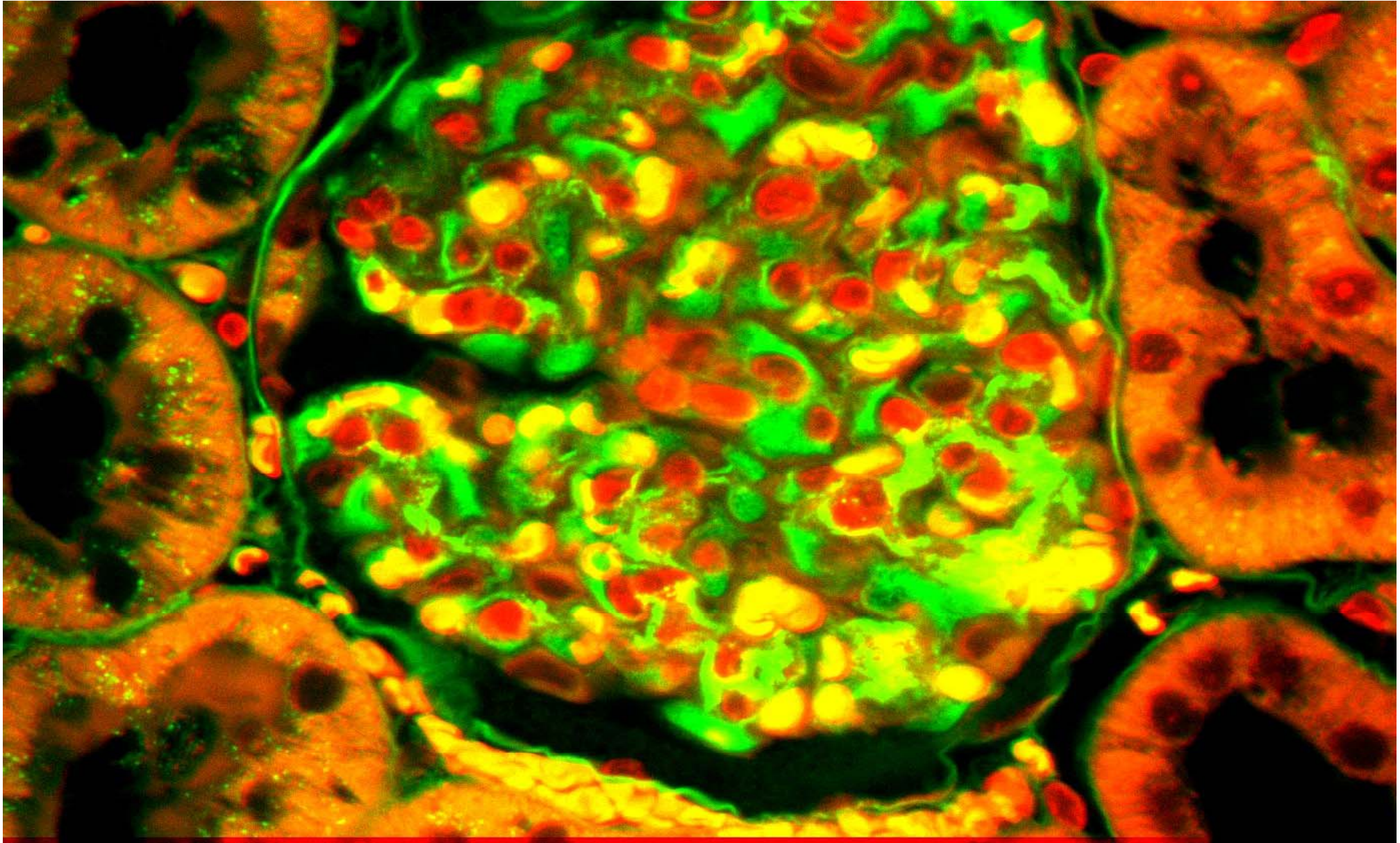
Labeling and Isolation of Glomeruli and Imaging



FITC-Dextran 250 kD



SD glomerulus albumin and dextran labeled



Live Image of glomeruli

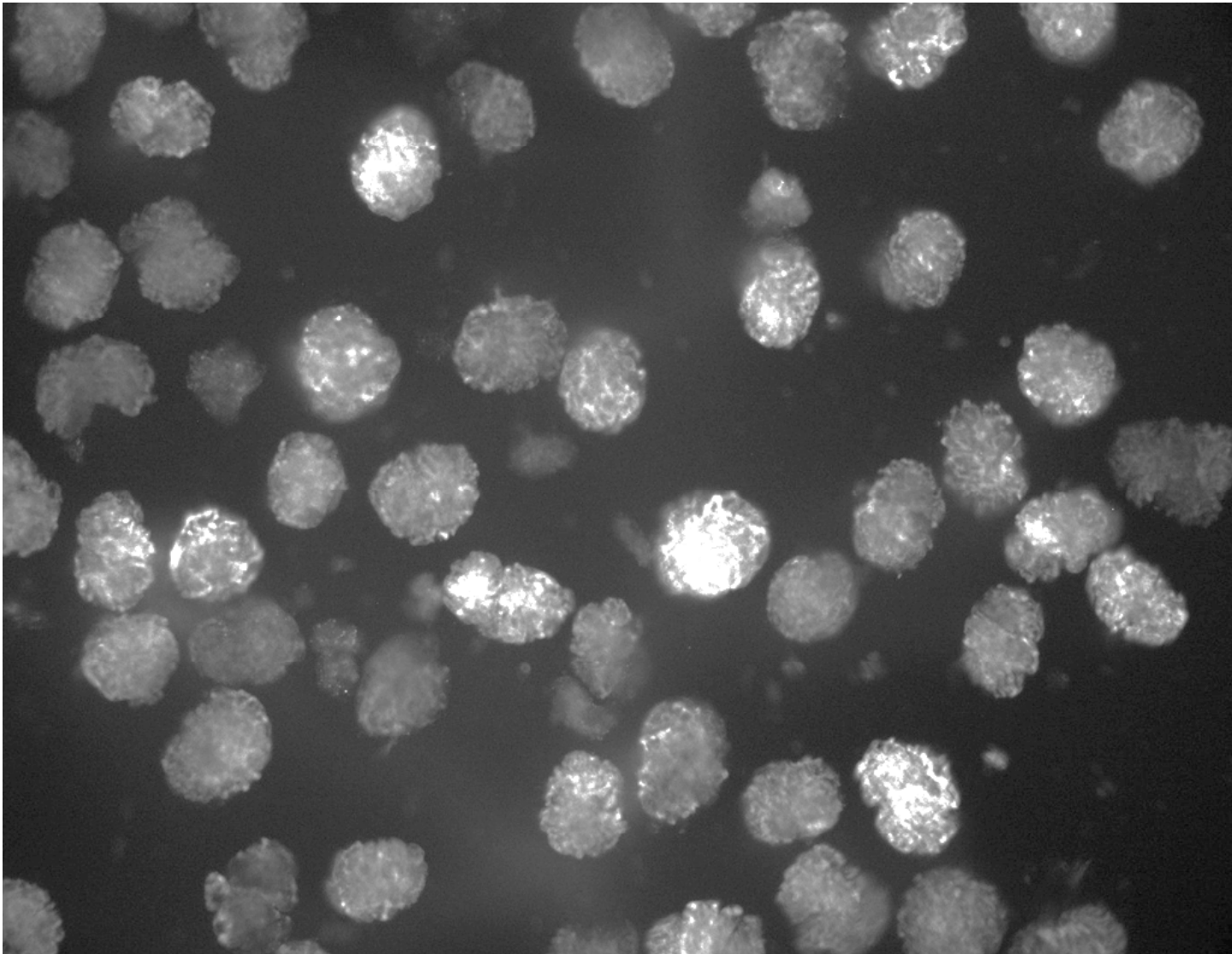


Figure 3A

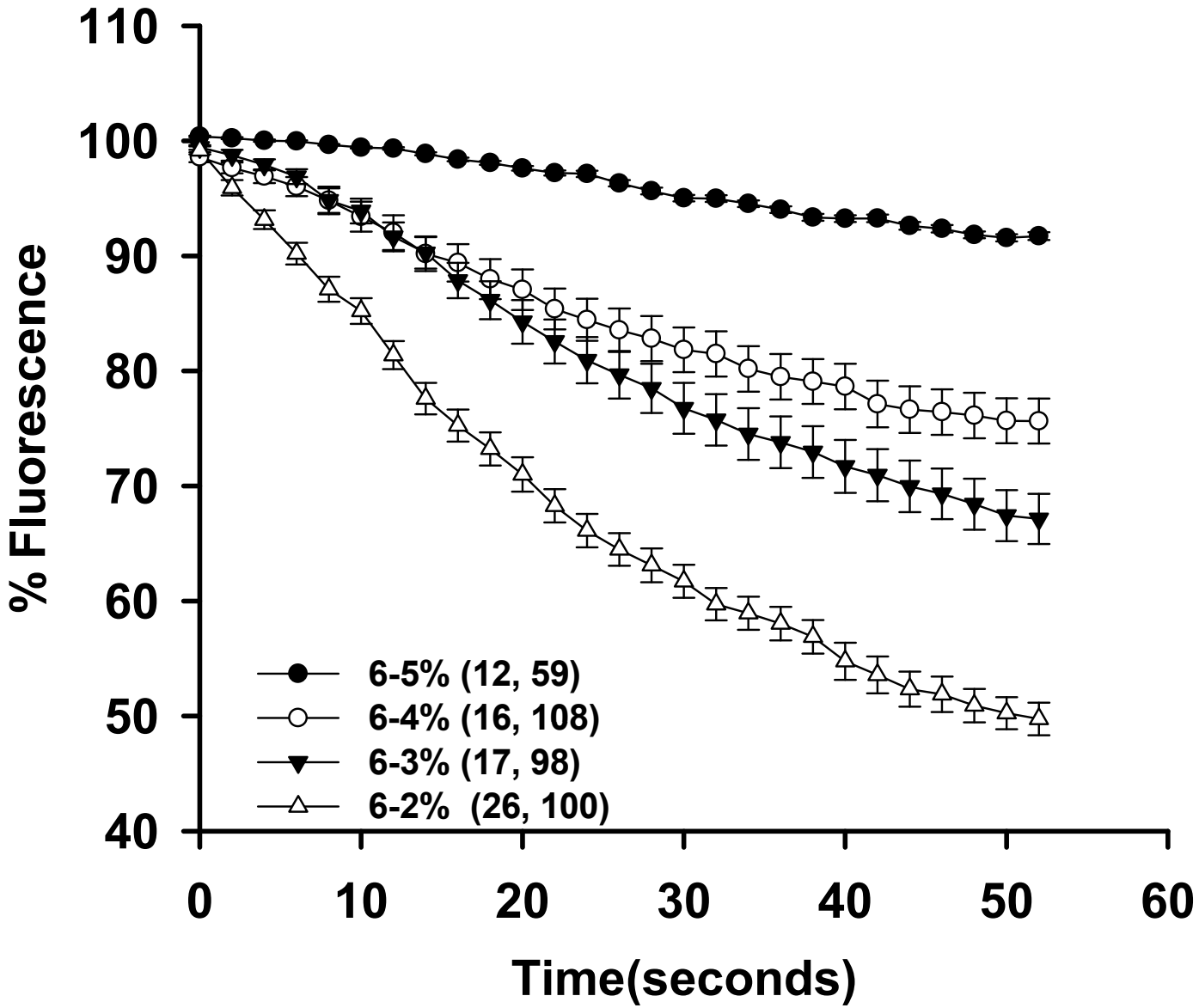
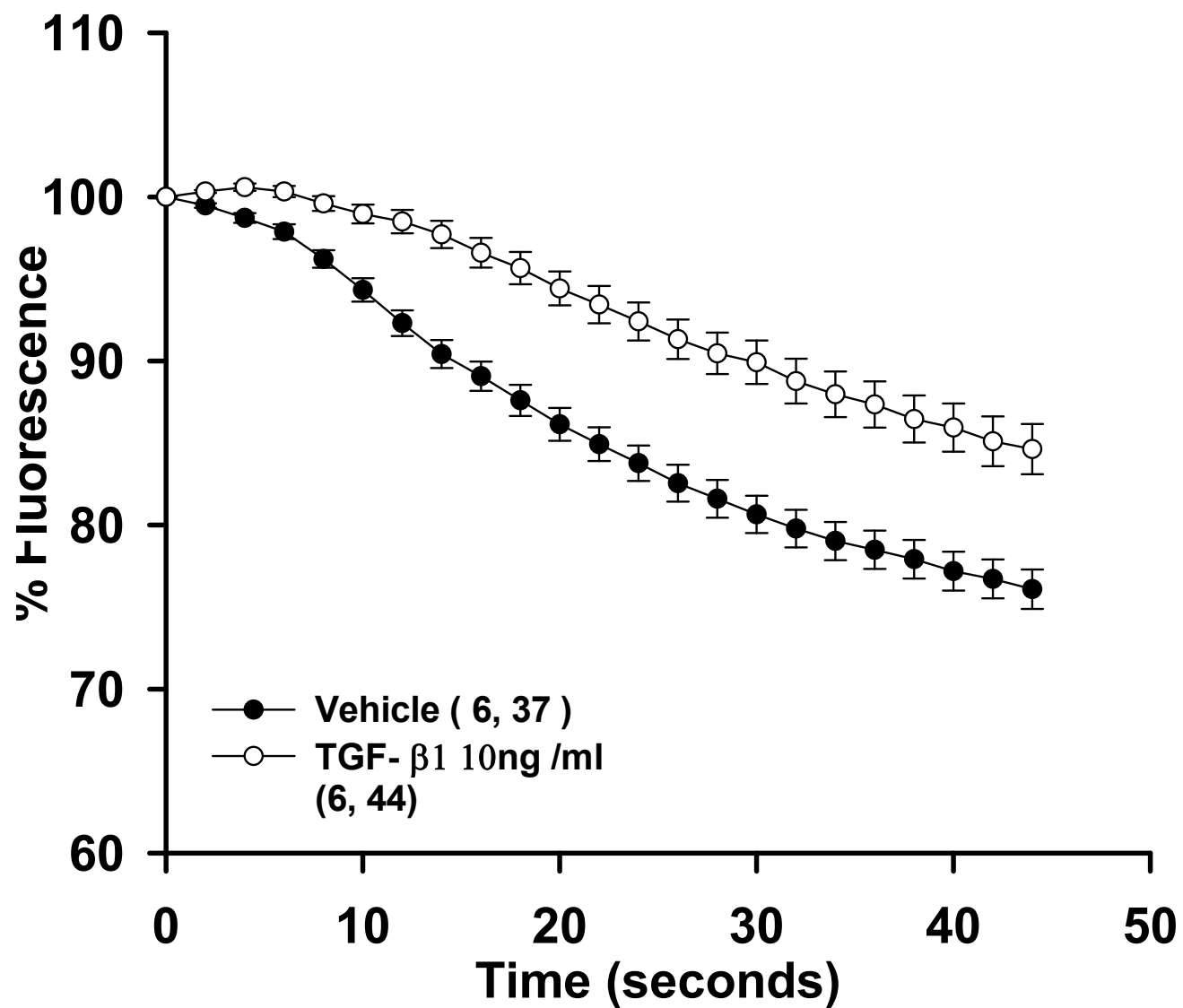
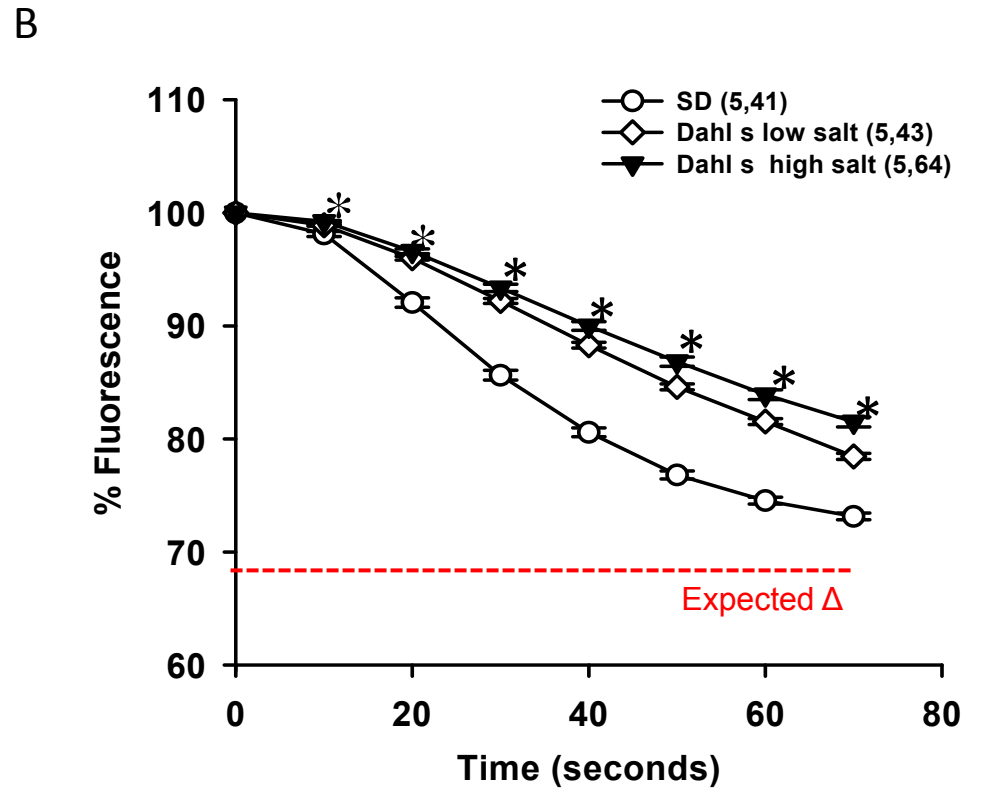
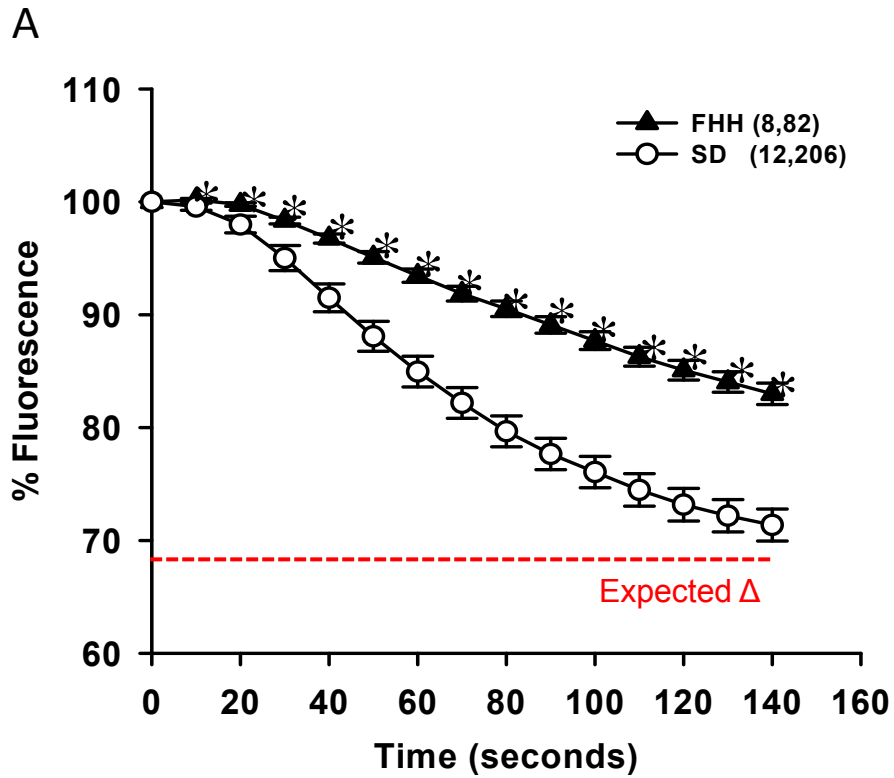


Figure 5B

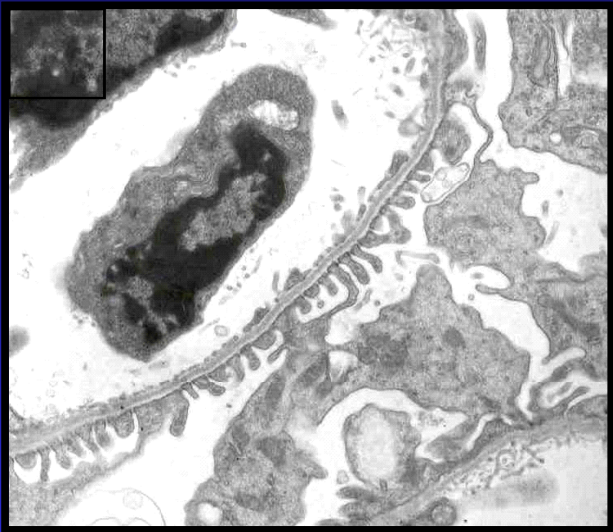


Hypertensive model

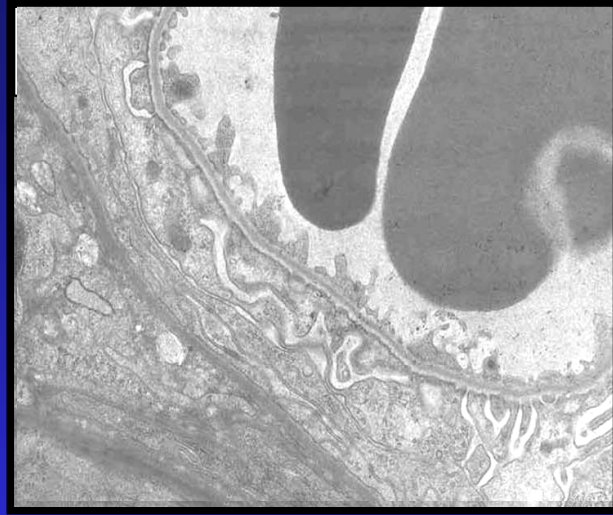


Effect of Hypertension on Glomerular Capillaries in Dahl S Rats

Control



High salt



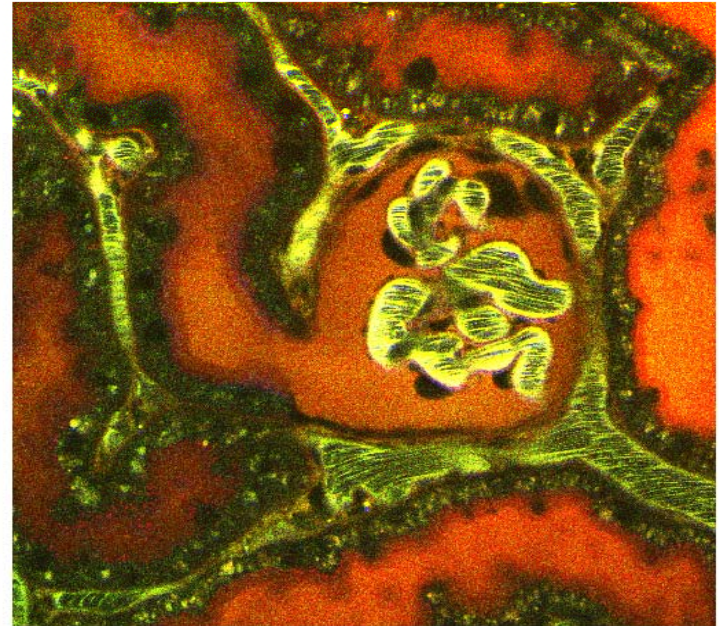
High salt + anti TGFb therapy



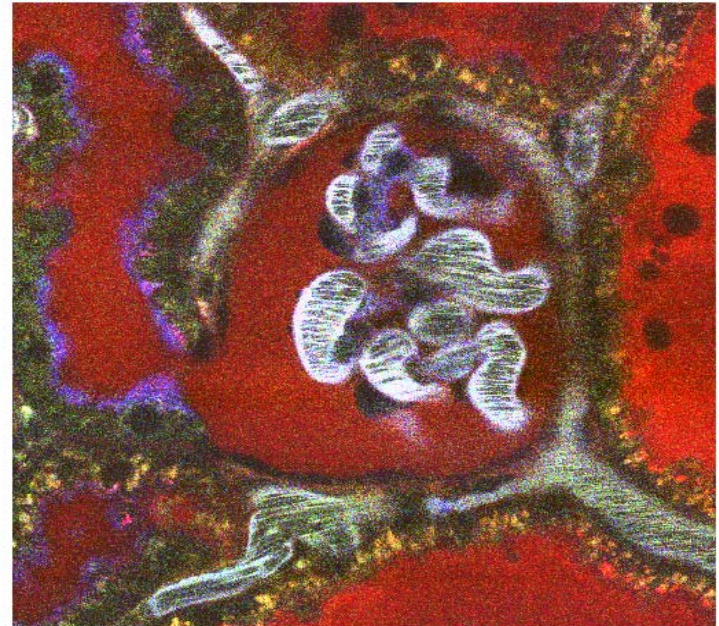
2 photon verification of TGF b effect

Fitc dextran
Low mw dextran
Albumin blue

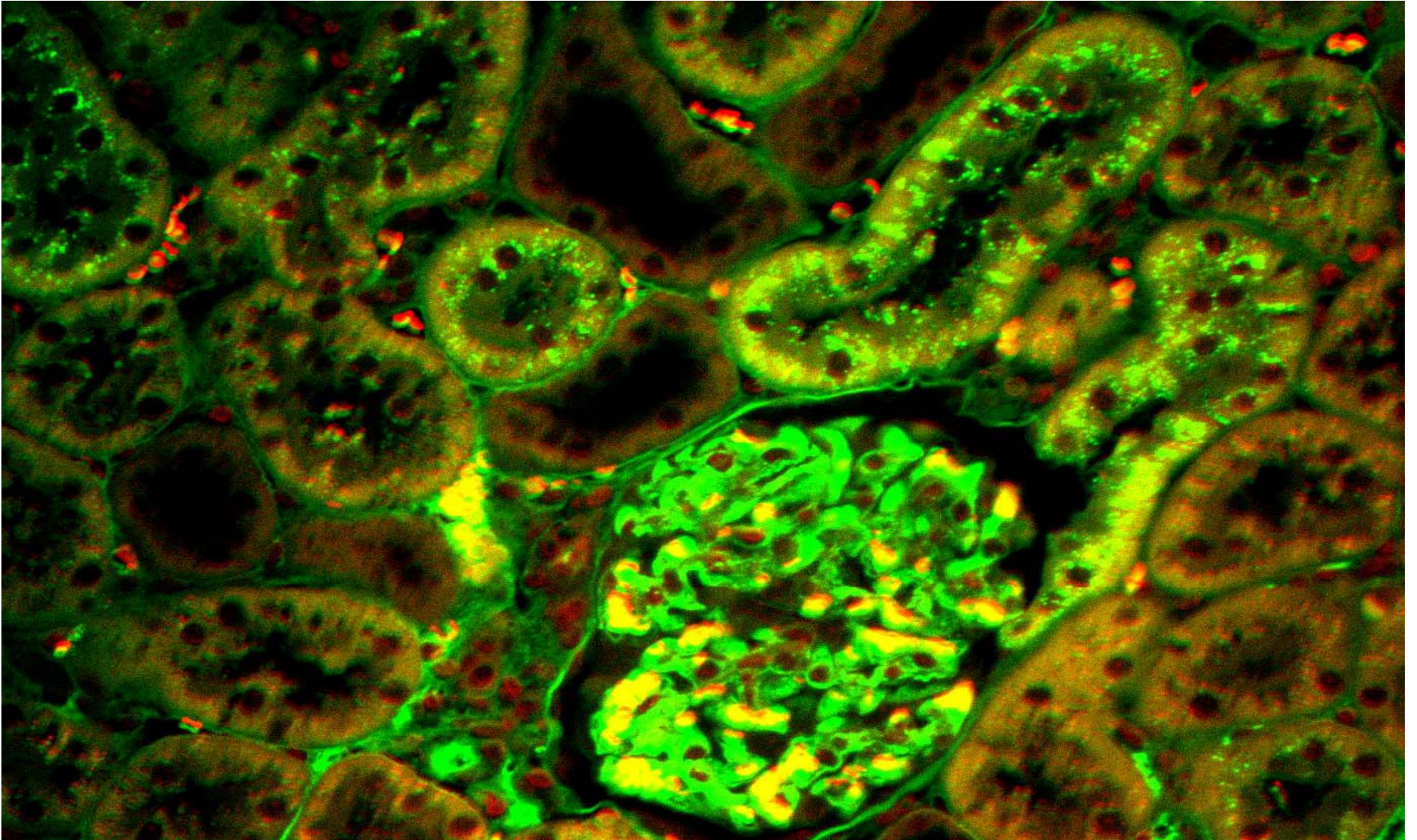
Control



TGF-B



Dahl S high salt 20X

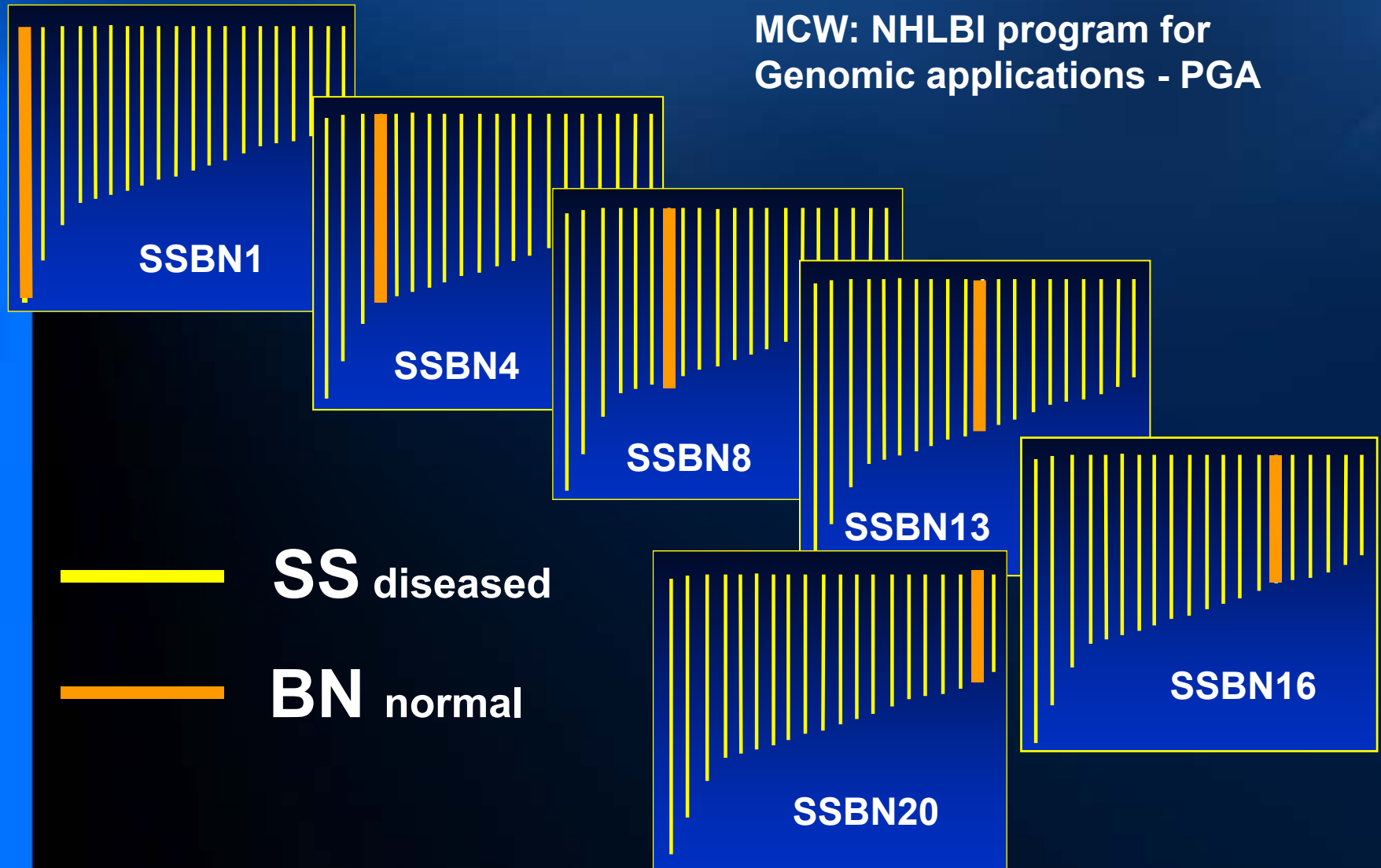


Evidence Linking P4504A to salt sensitive Hypertension in Dahl S Rats

- The formation of 20-HETE is decreased in the kidney in Dahl S rats compared to other normotensive and hypertensive strains of rats.
- 20-HETE production in OM is reduced in Dahl S rats which contributes to elevated loop Cl- reabsorption in TALH. 20-HETE production is decreased in glomerulus but vascular production does not seem to lower than other strains.
- Chronic treatment with Fibrates increases the renal formation of 20-HETE, improves pressure natriuresis, and reduces blood pressure in Dahl S rats.
- The CYP4A region on chromosome 5 cosegregates with hypertension in a Dahl S X Lewis F2 cross.
- CYP4A genes are located on rat chromosome 5. Do they contribute to Hypertension in Dahl S rats?

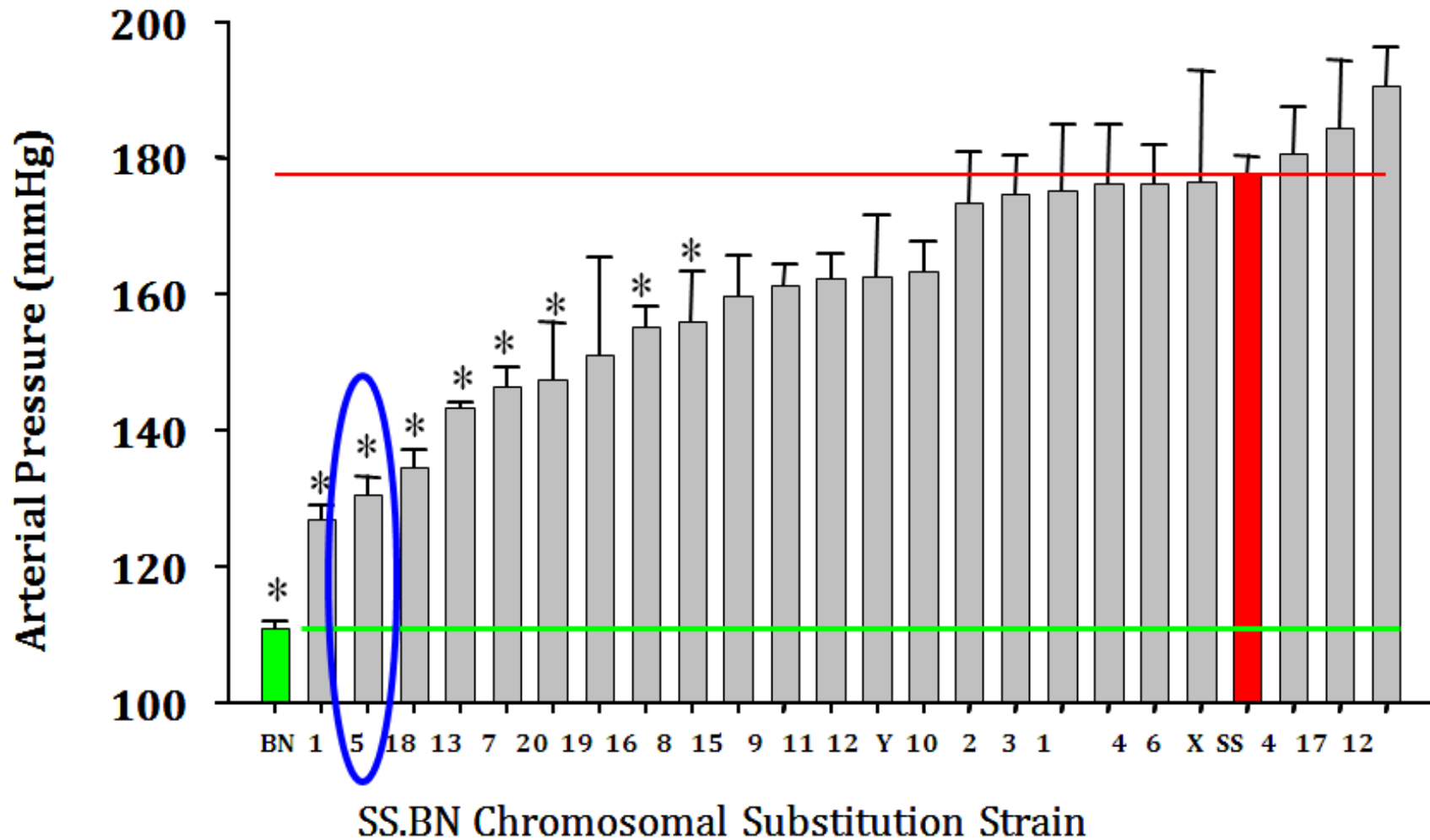
Consomic rats are single chromosome substitutions

MCW: NHLBI program for Genomic applications - PGA



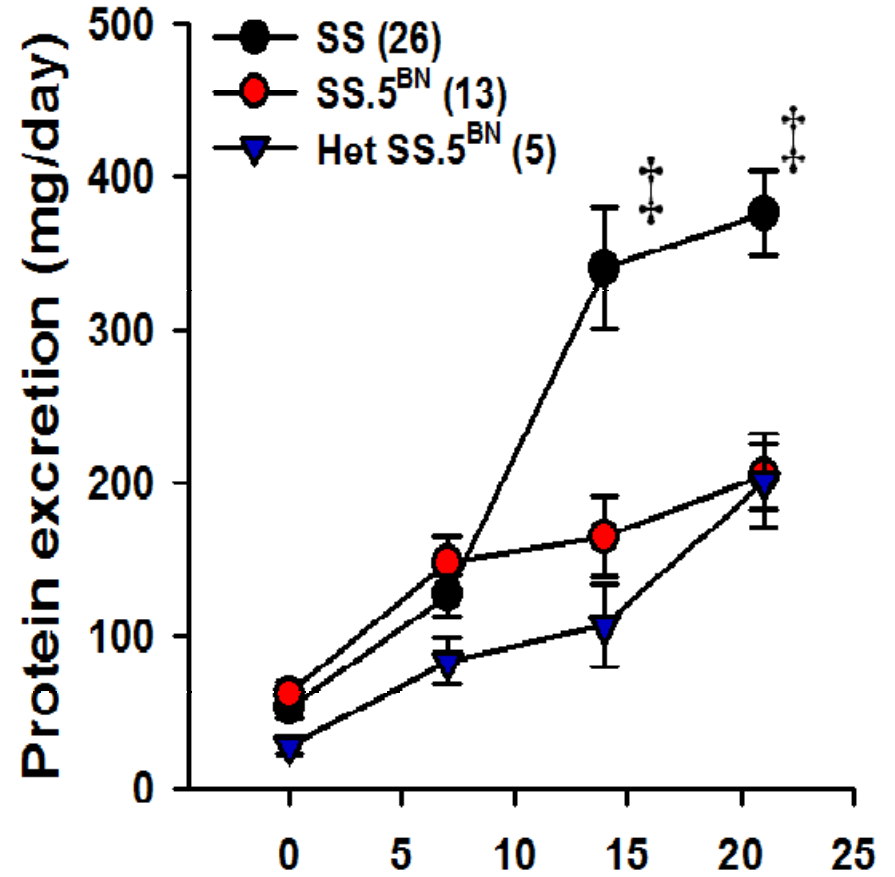
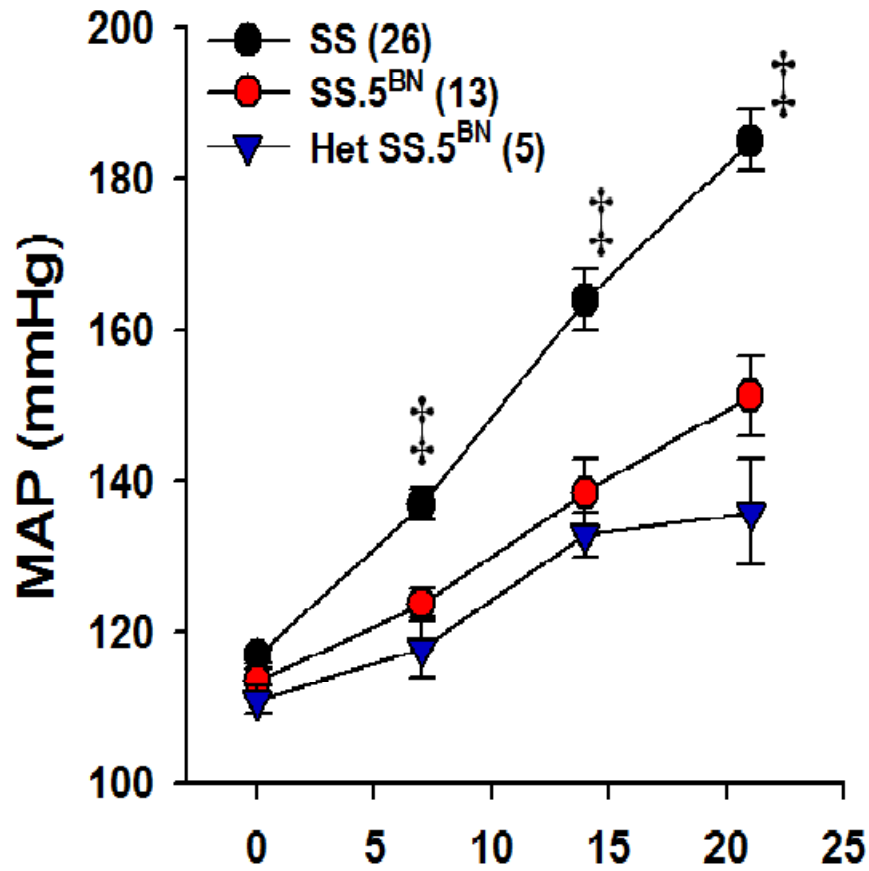
Note: the strain sequenced—is this BN

Blood pressure targets captured in the SS.BN Consomic strains



Mattson et al. AJP Renal Physiol 295: F837-F842, 2008.

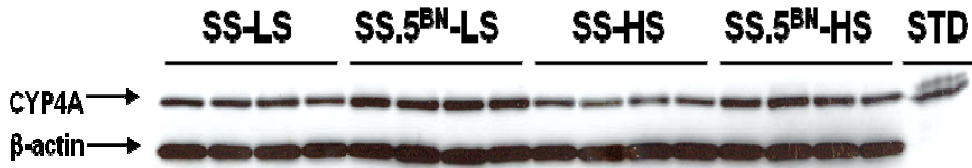
Telemetry Confirmation of renoprotective phenotype in SS.5^{BN} rats



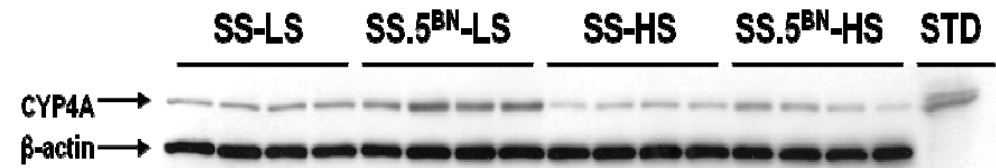
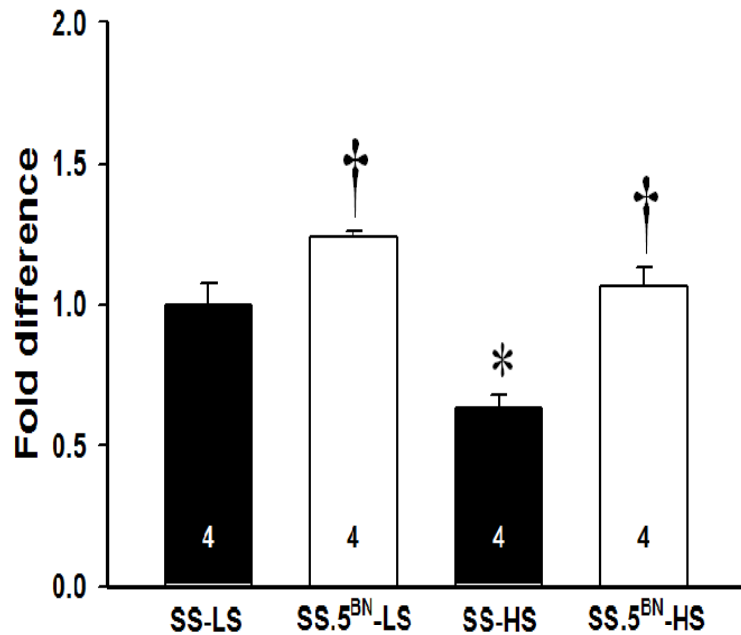
‡ P<0.05 vs. other strains

Days on High Salt Diet

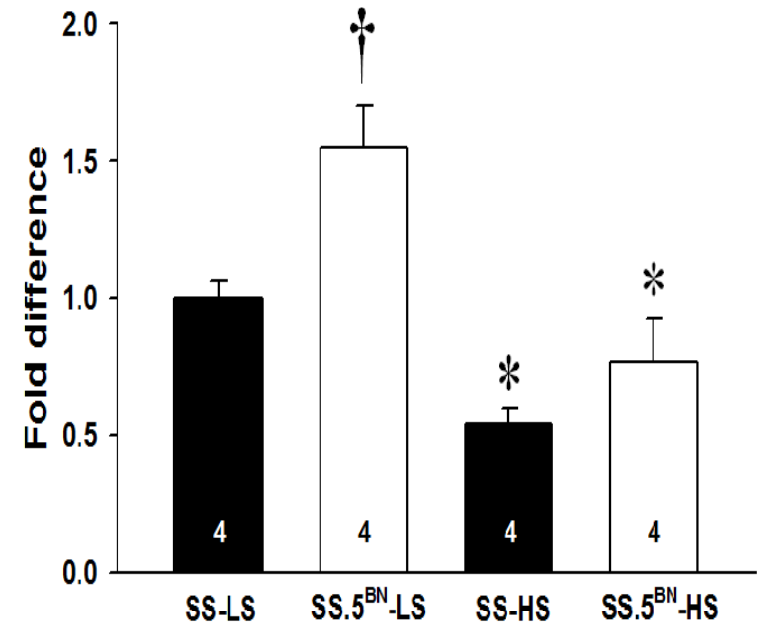
CYP4A expression in the kidney of SS and SS.5^{BN} rats



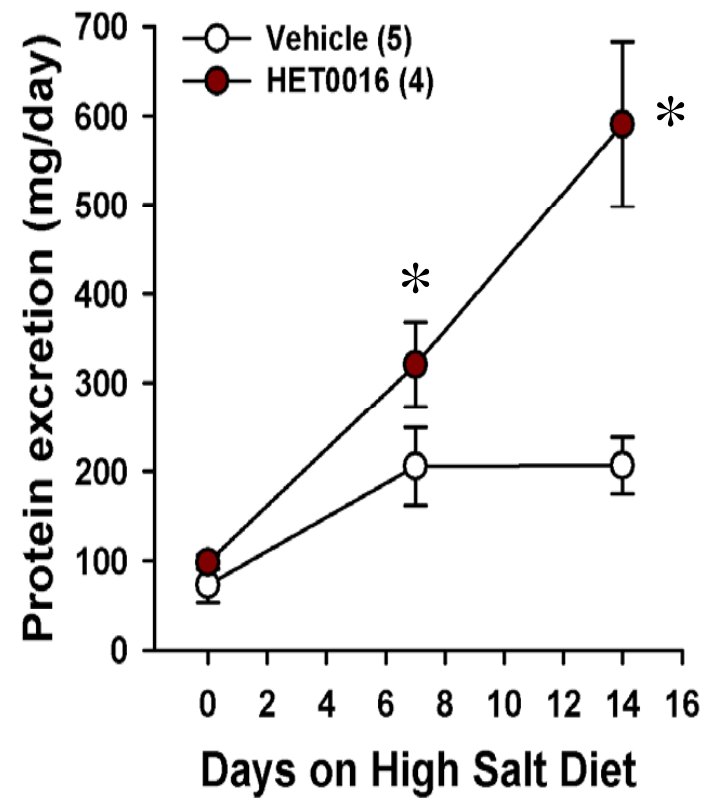
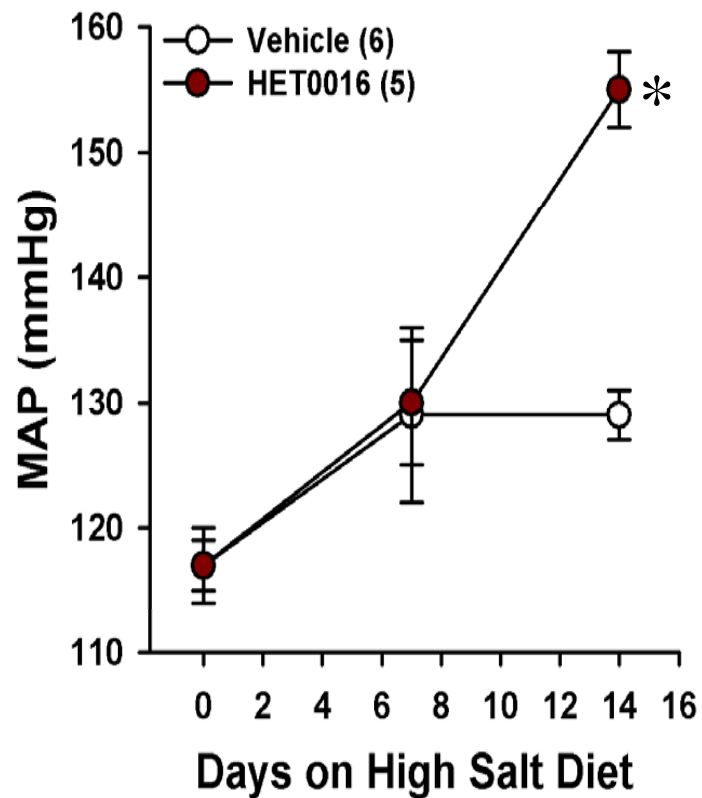
Renal cortex



Renal outer medulla

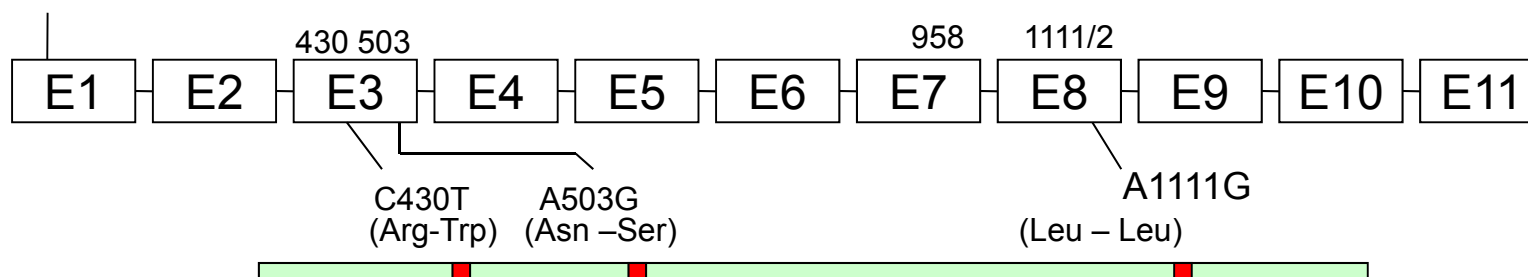
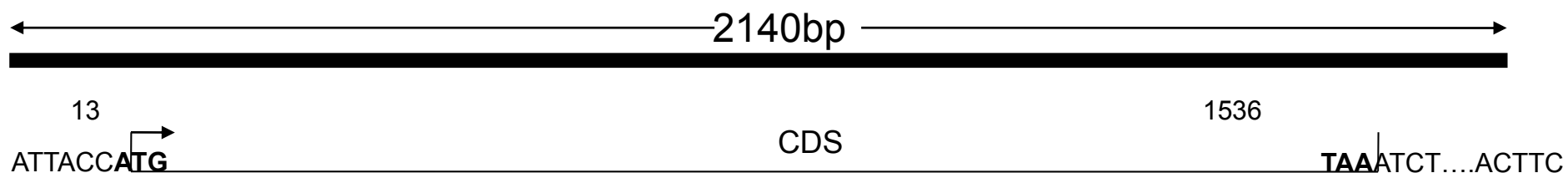


Chronic administration of HET0016 reverses the phenotype of SS.5^{BN} rats



14 Days on High Salt Diet

Sequencing of CYP4A3 cDNA



Ref Seq

NM 175760 SD

SS1

SS2

SS3

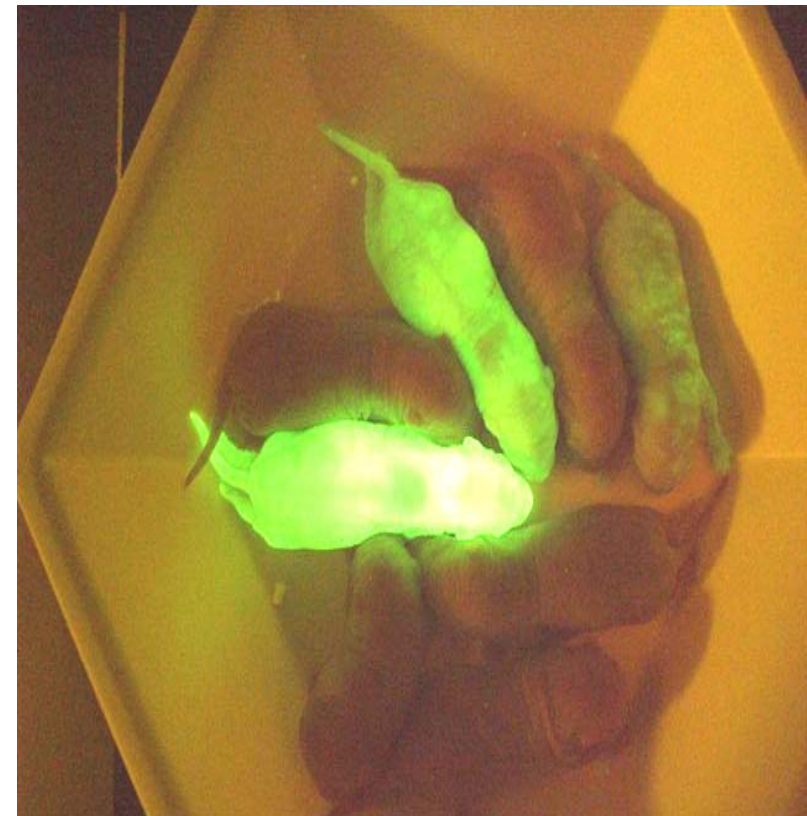
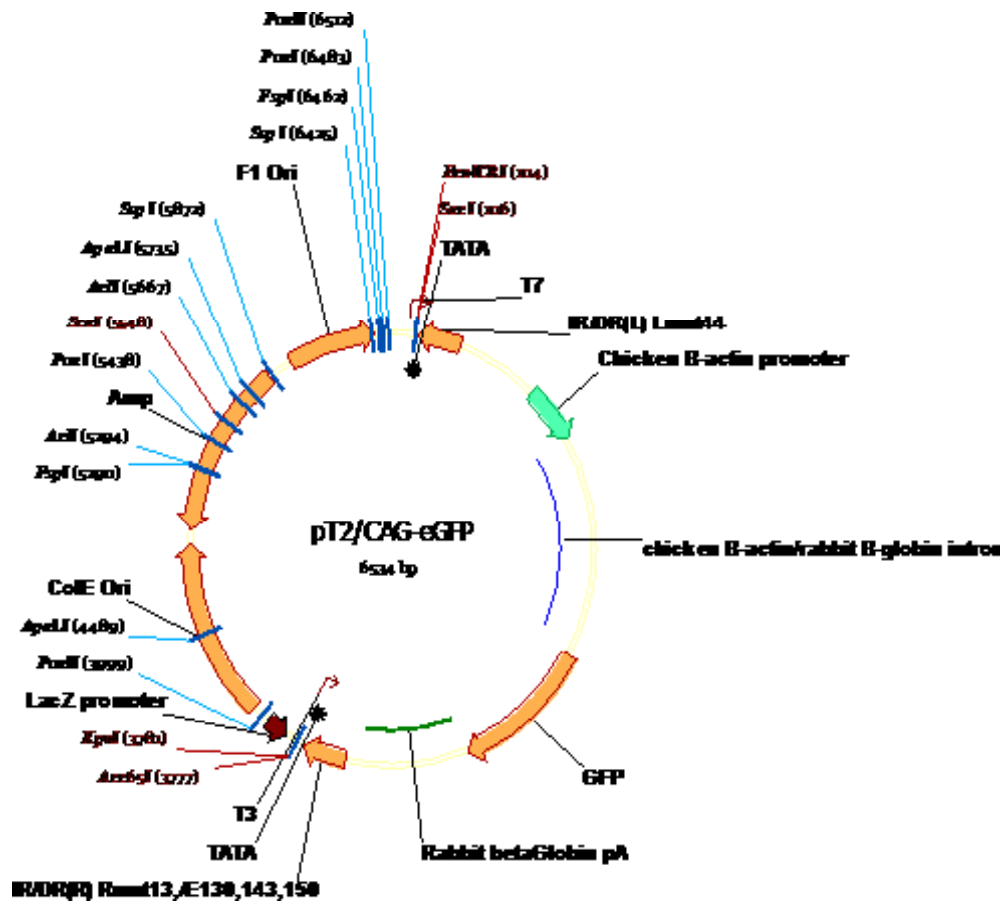
SSBN51

SSBN52

SSBN53

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ccagcaatc ...	gtcag g tataatatcatgttt...	gat g ccttacacc
ccagcaatc ...	gtcag g tataatatcatgttt...	gat g ccttacacc
ccagcaatc ...	gtcag g tataatatcatgttt...	gat g ccttacacc
ccagcaat t ..	gtcaatataat	atcatgttt...	gataccttacacc
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ccagcaat t ..	gtcaatataatatcatgttt...	gataccttacacc

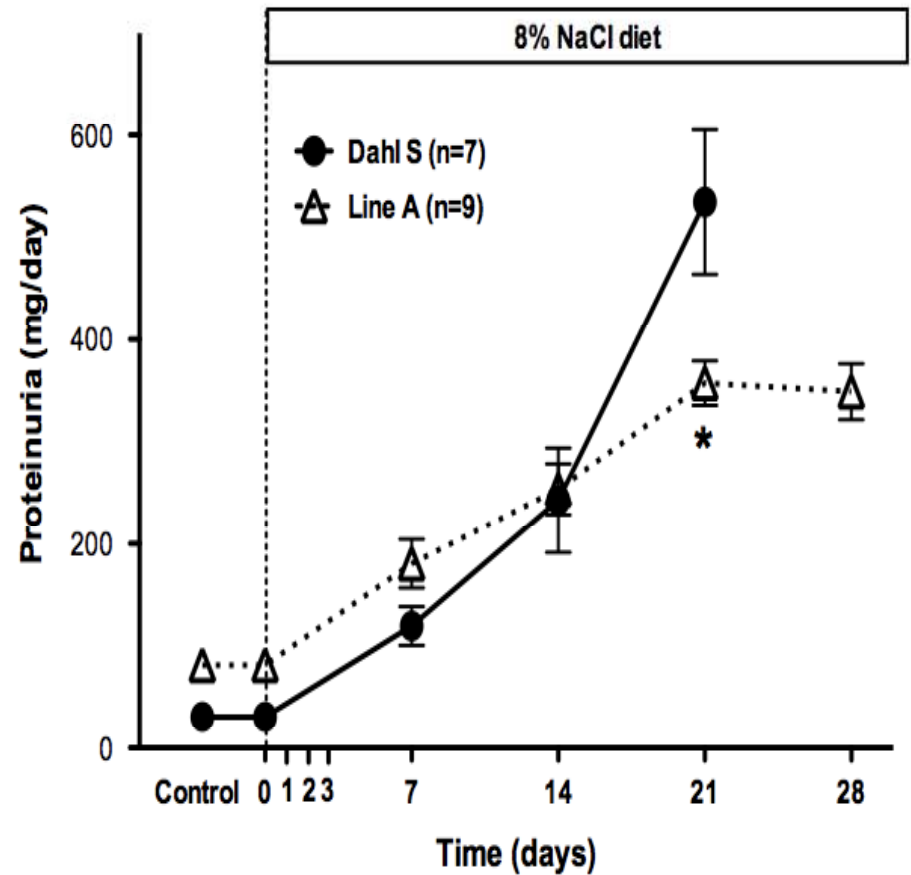
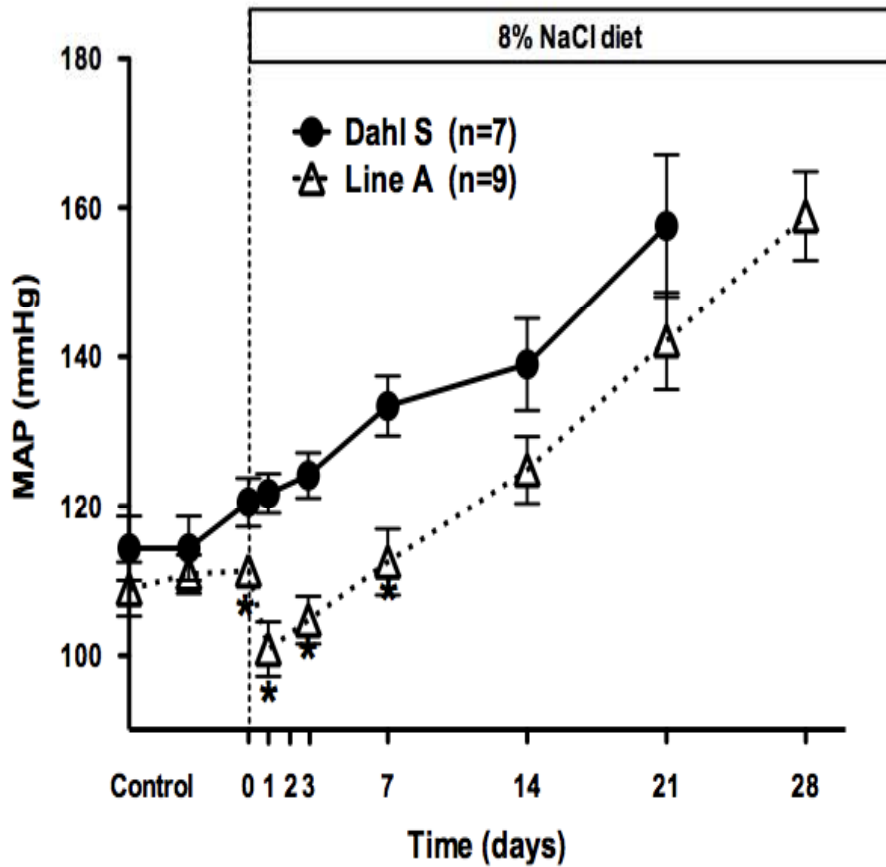
Creation of GFP transgenic rats using Sleeping Beauty transposon



MCW, Guertz and Jacob

Add3, Dusp5 in FHH, 4A1 in SS rats

MAP and Proteinuria- SB Trasposon- CYP4A Transgenic rats vs Dahl S rats



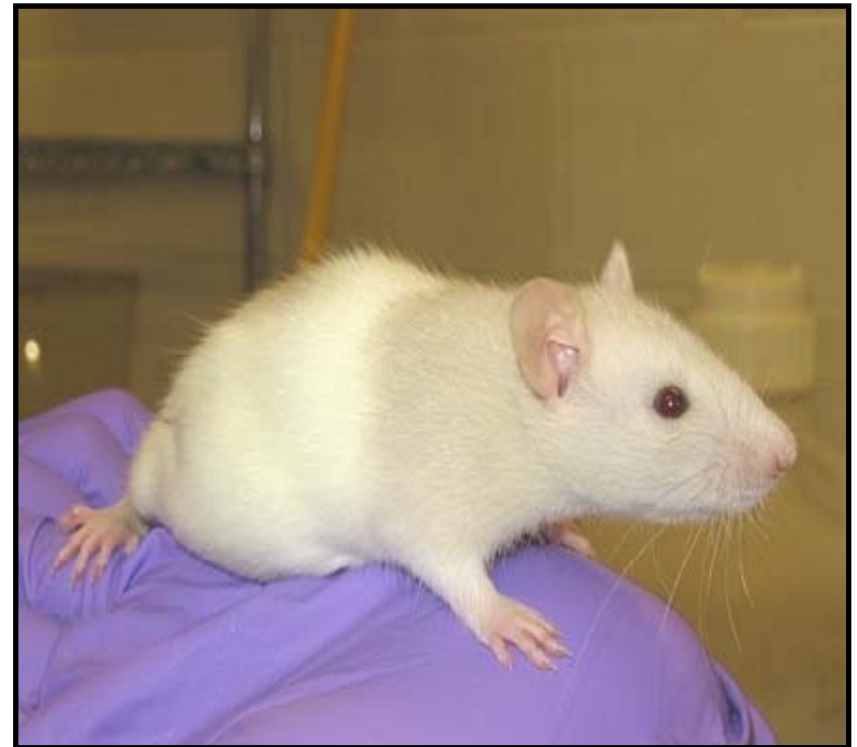
* vs Dahl SSJr, $p < 0.05$

The FHH rat

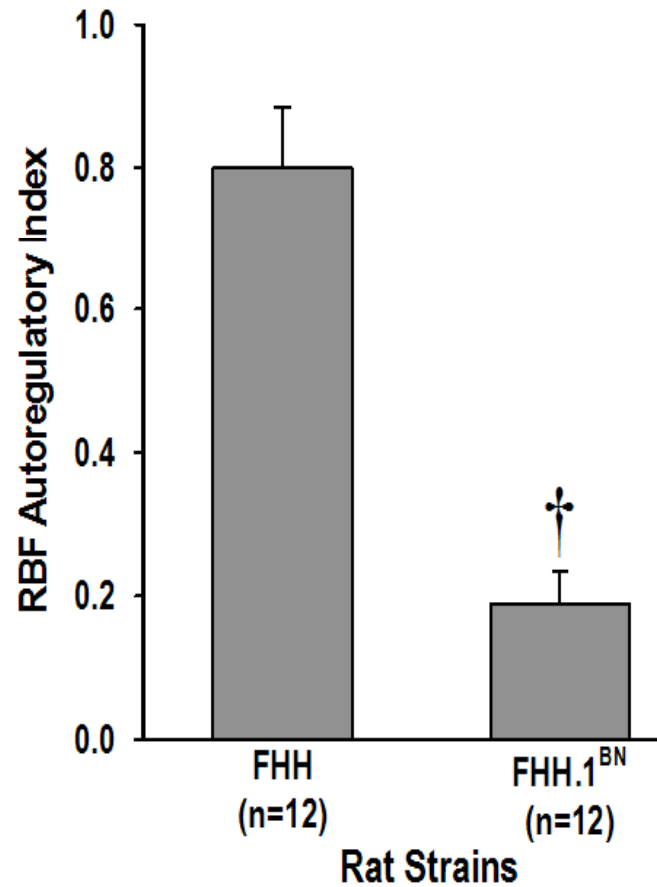
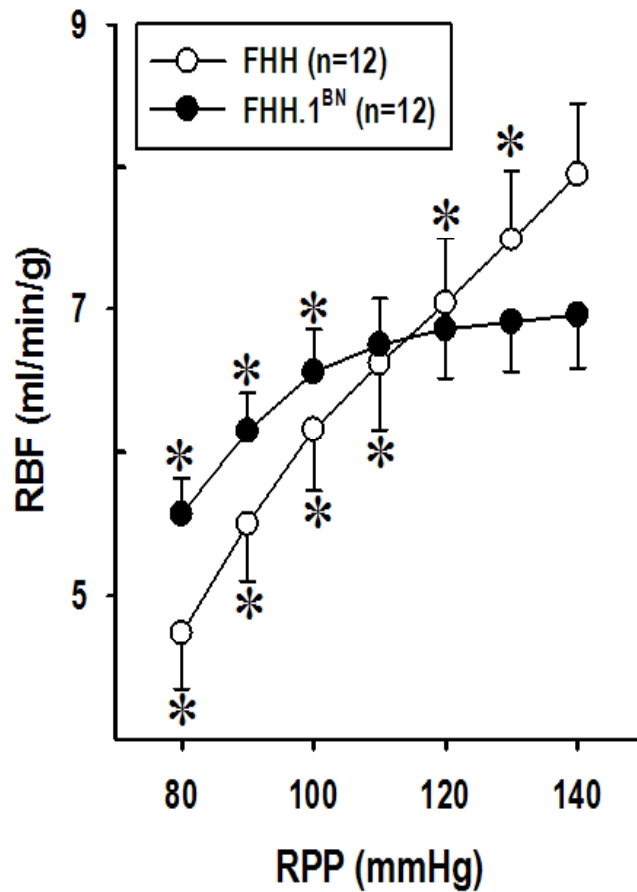
The FHH rat is a genetic model of hypertension-induced renal disease

- Proteinuria- QTL chr 1
- Focal glomerulosclerosis
- Systolic hypertension (late)
- Pulmonary Hypertension
- Bleeding disorder
- Coat Color

Kidney Int Suppl 45: S2-S5, 1994

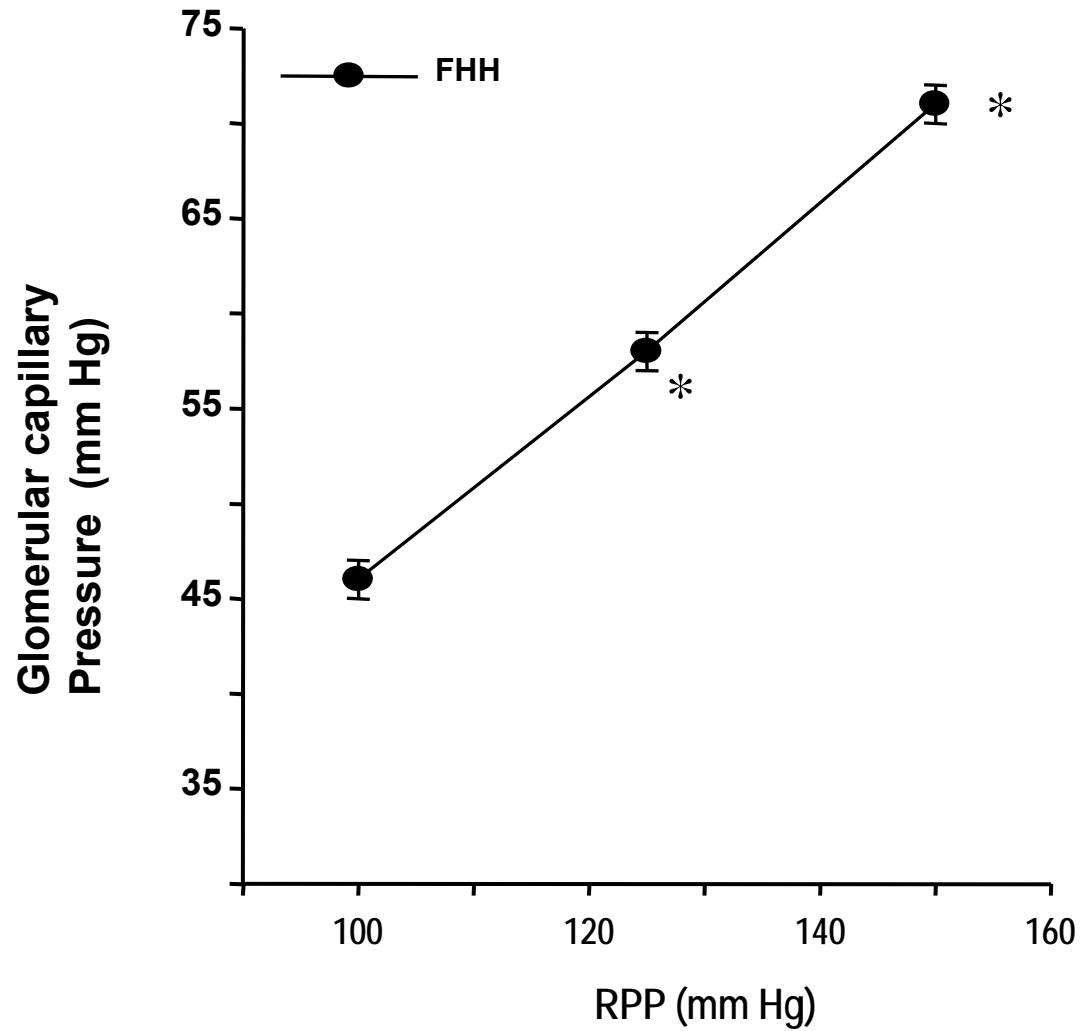


Substitution of Chromosome 1 from the BN rat restores the autoregulation of RBF in FHH rats



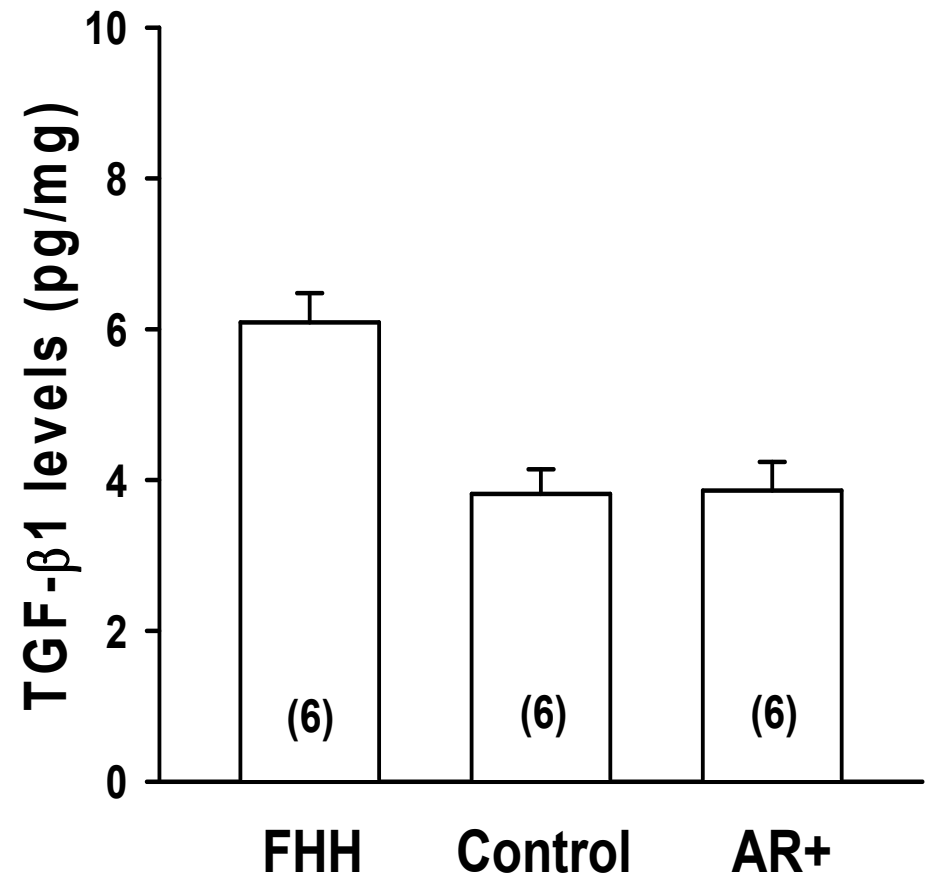
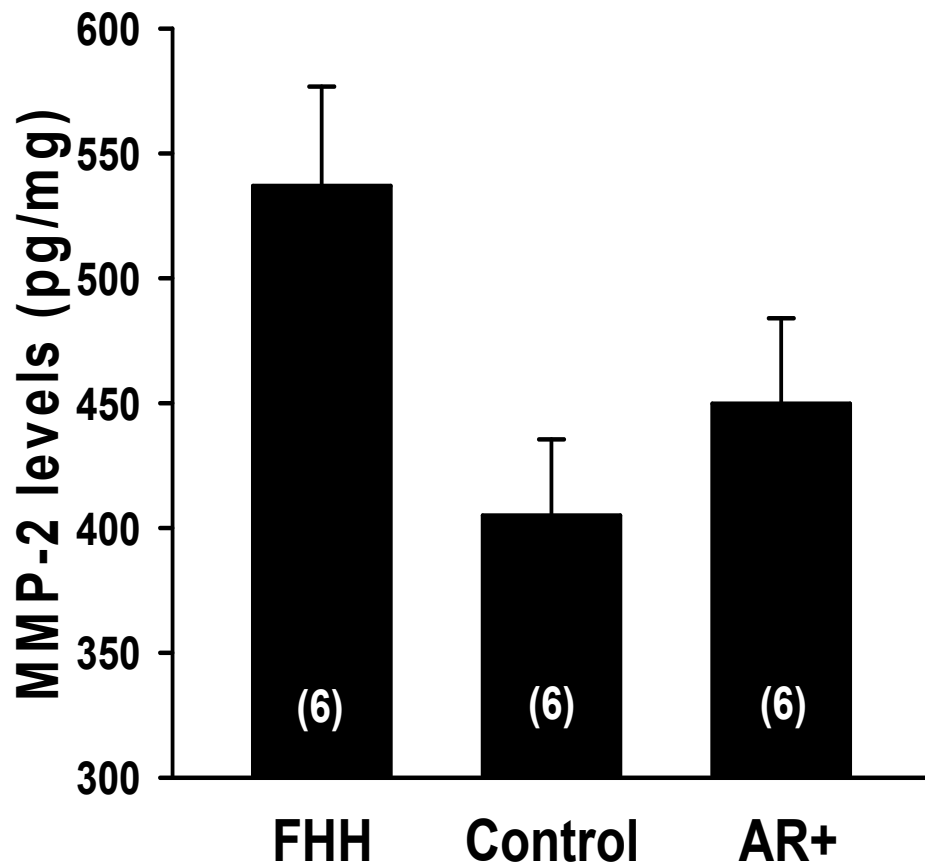
AJP Renal Physiol 290: F1213-F1221, 2006.

Pgc is elevated in FHH rats

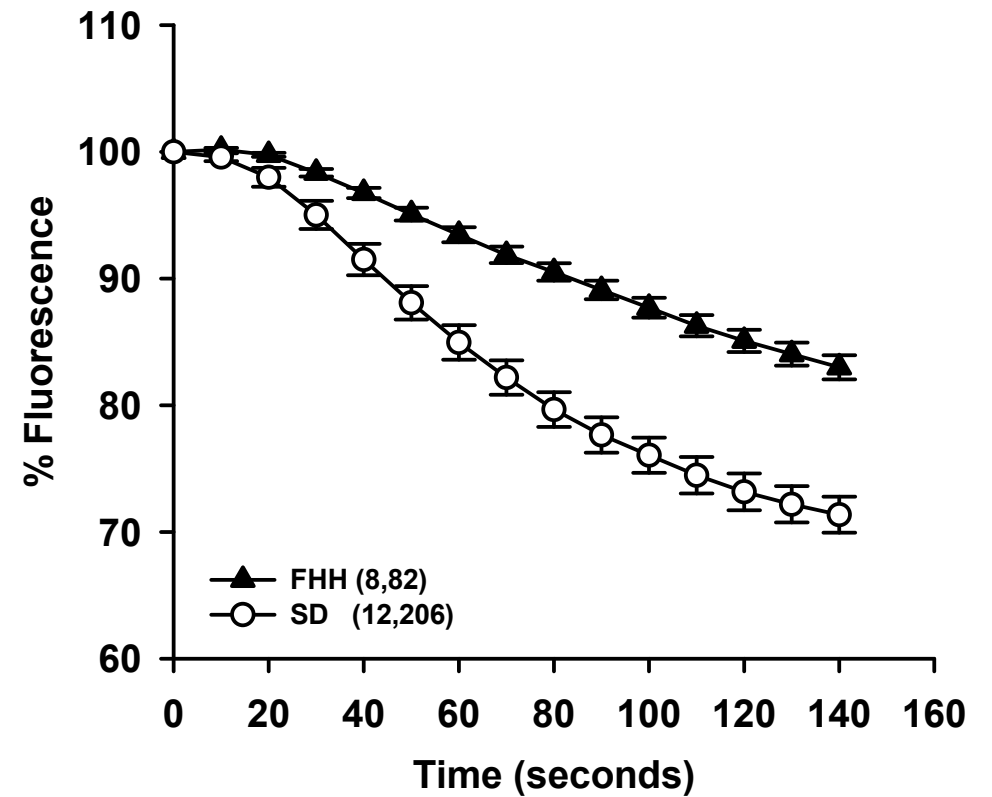
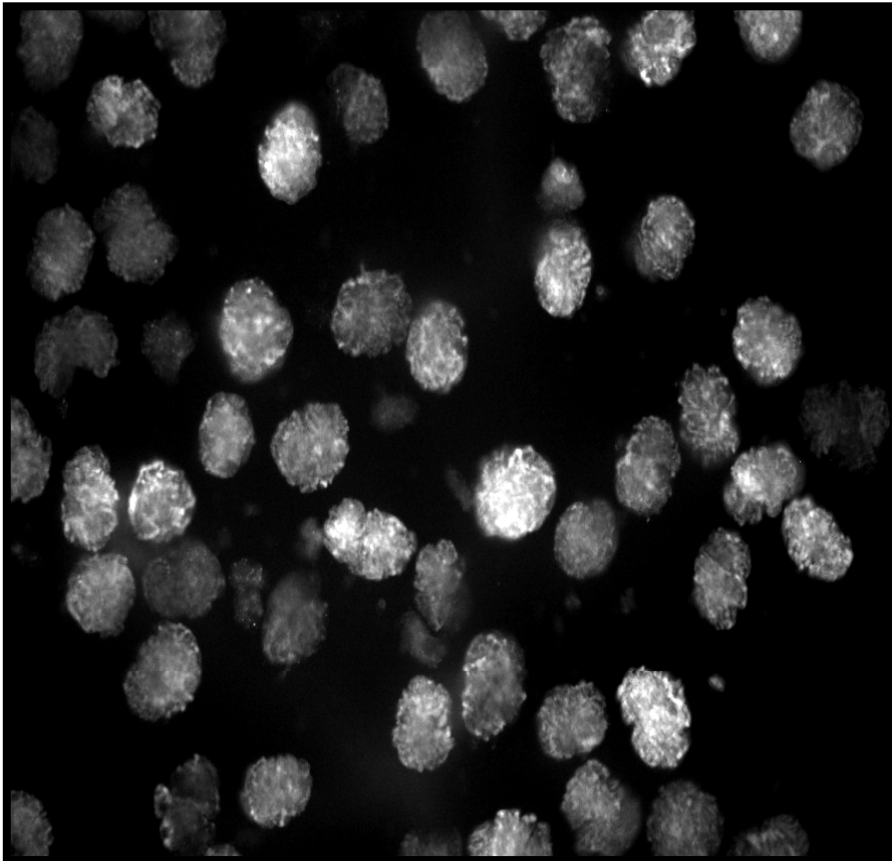


AJP Regul Integr Comp Physiol 276: R855-R863, 1999.

MMP-2 and TGF- β 1 levels are increased in the kidneys of FHH rats at 21 weeks

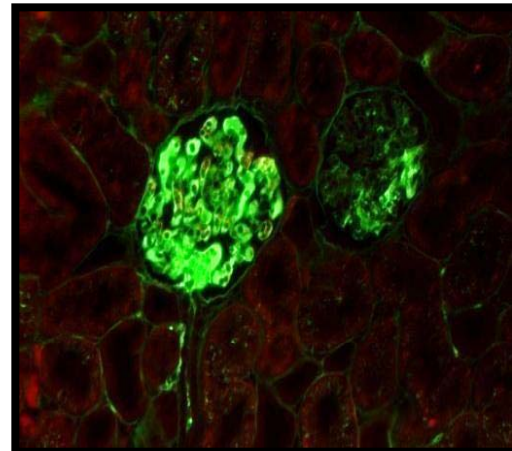
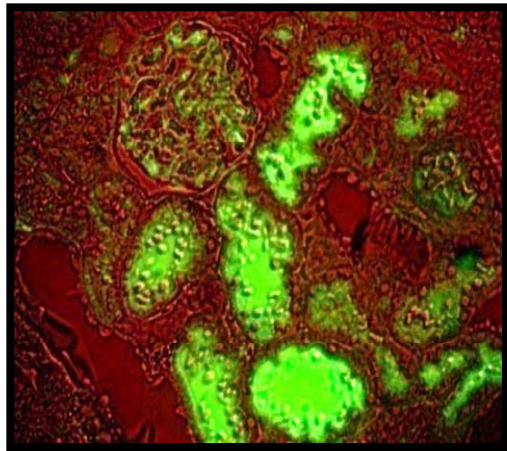


Dilutional Palb measurements in SD and FHH rats

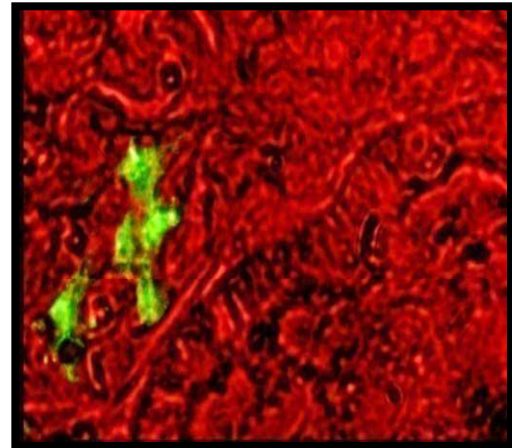
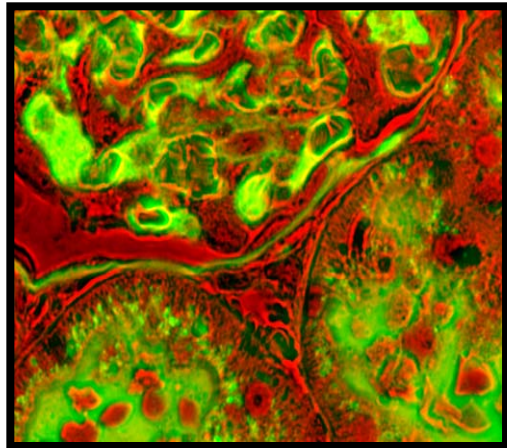


Infusion of FITC albumin in FHH and FHH.1^{BN} congenic rats

10x



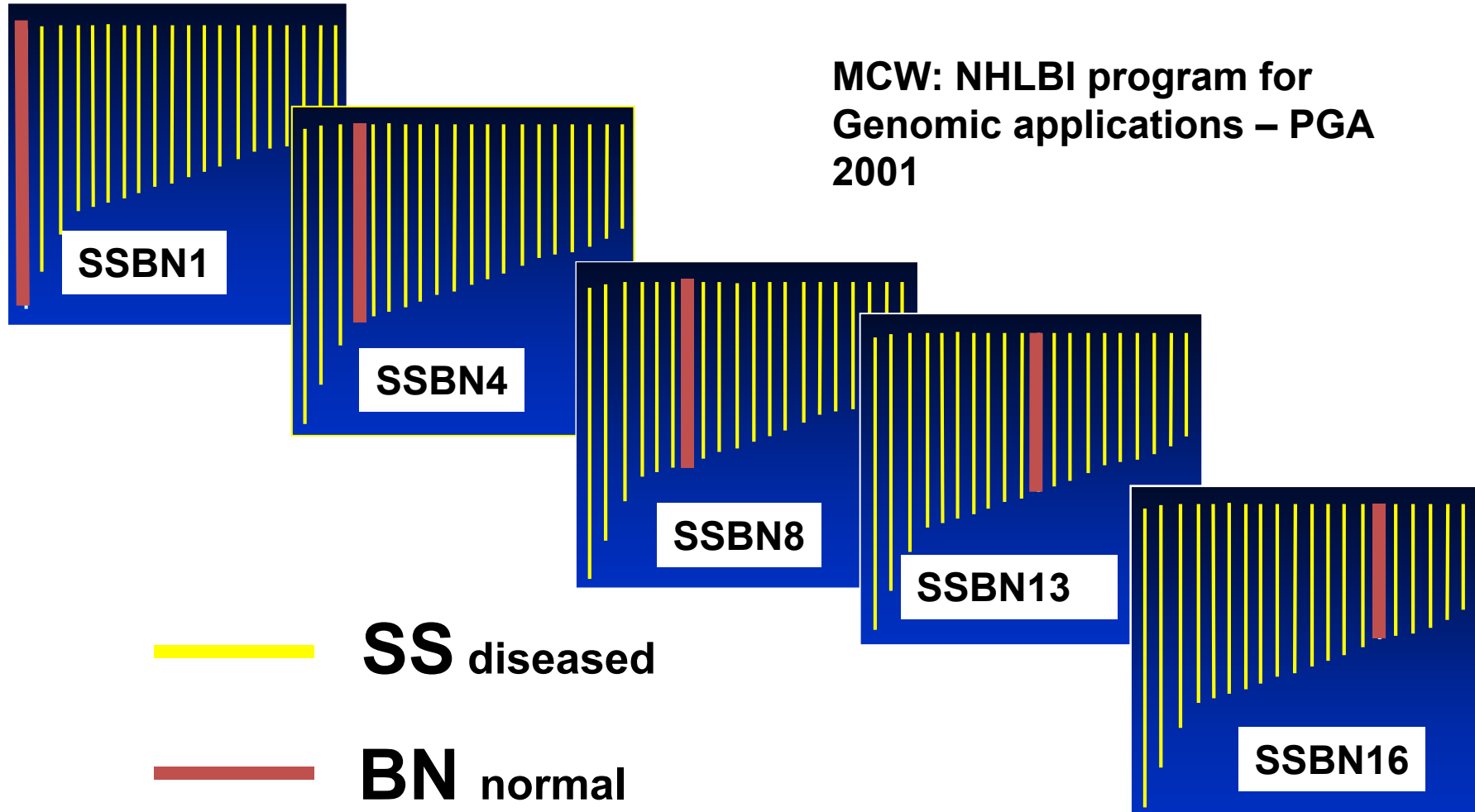
40x



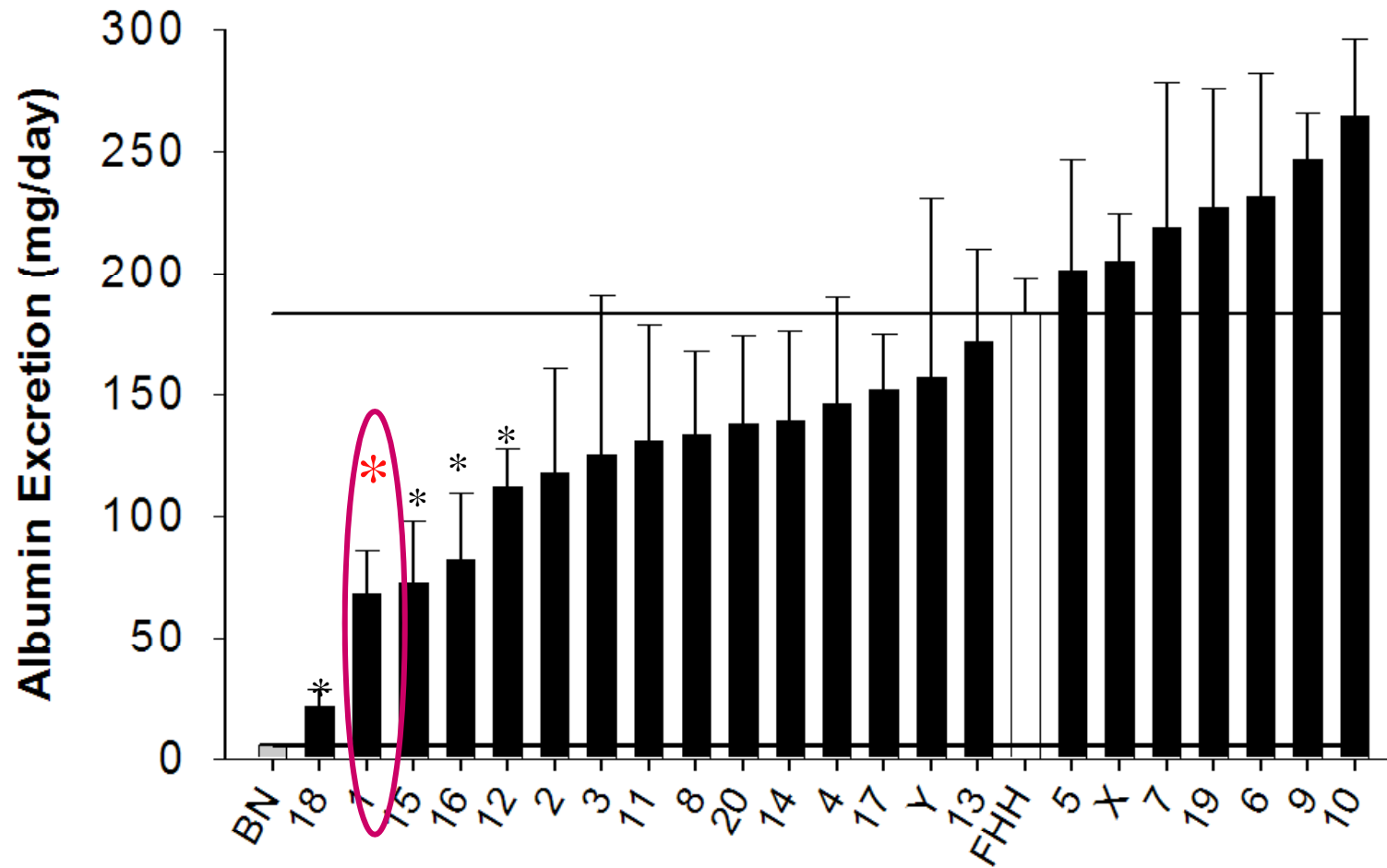
FHH

AR+

Unbiased Discovery Based Approach: Consomic rats are single chromosome substitutions

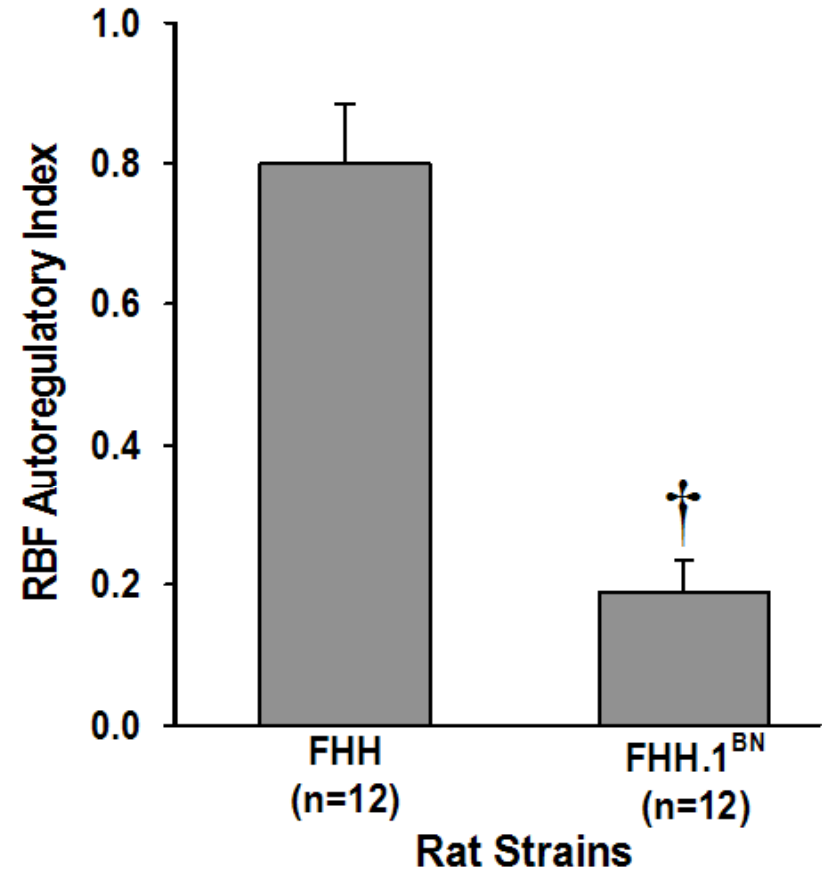
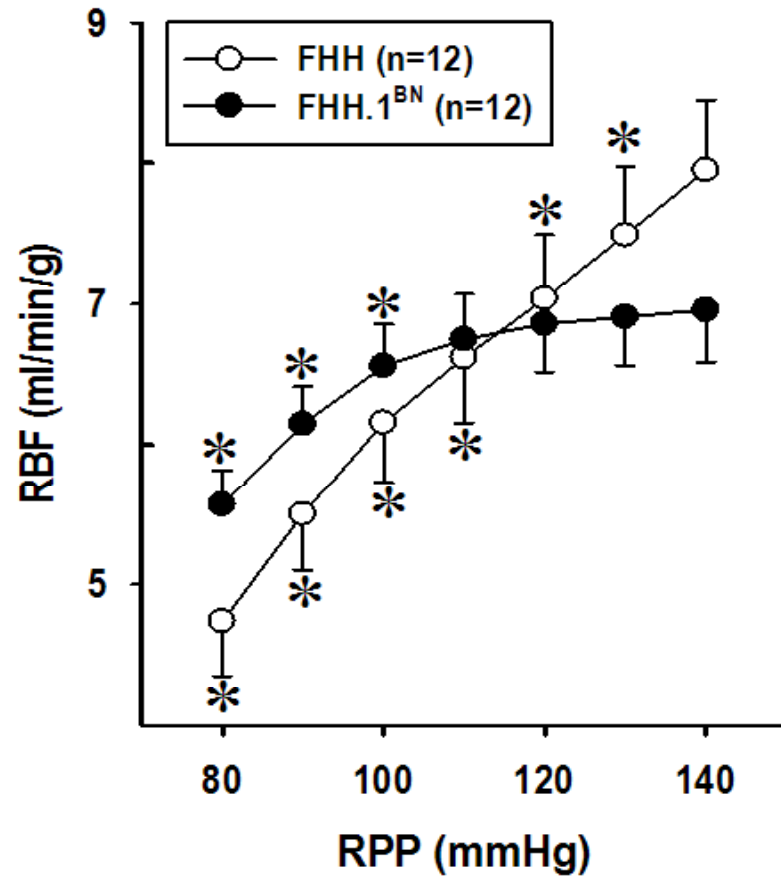


Albuminuria in FHH treated with LNAME

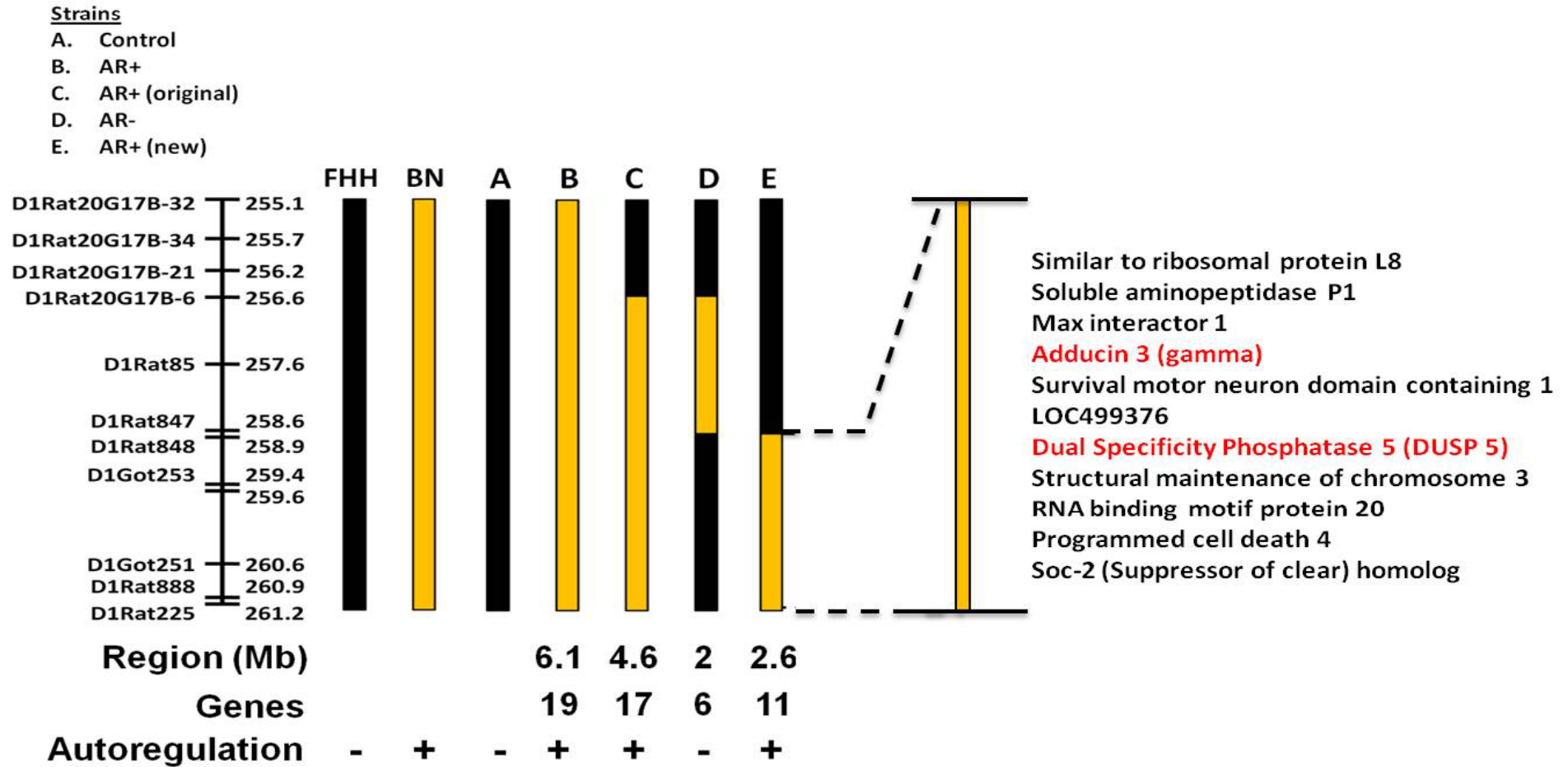


Mattson et al. AJP Renal Physiol 295: F1905-F1914, 2007.

Transfer of Chrm 1 restores autoregulation of RBF in the FHH rat



Genetic map illustrating the introgressed regions in FHH.1^{BN} AR+ congenic strains

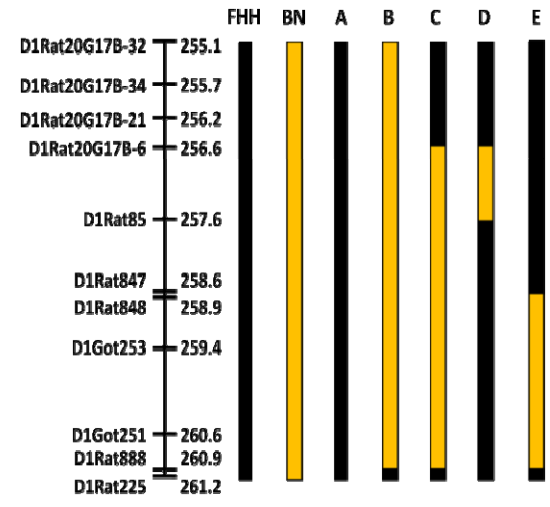
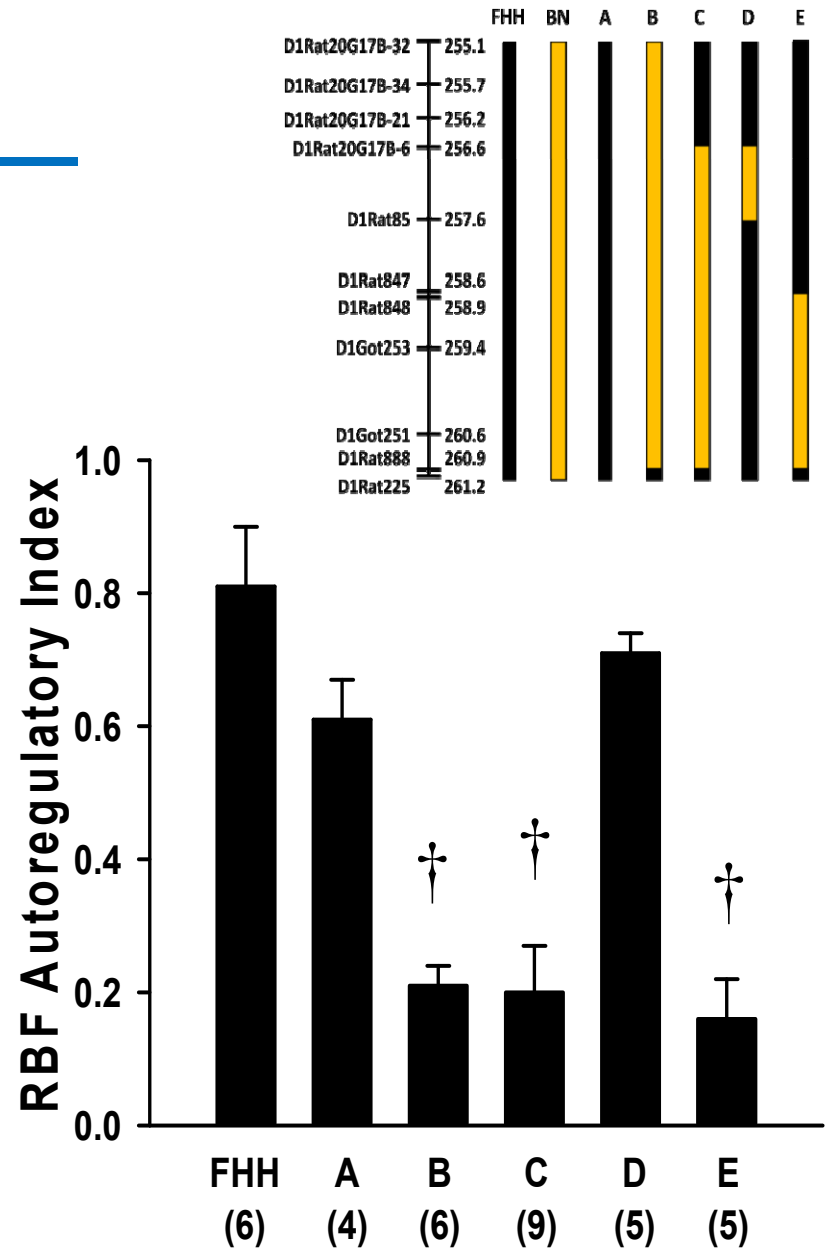
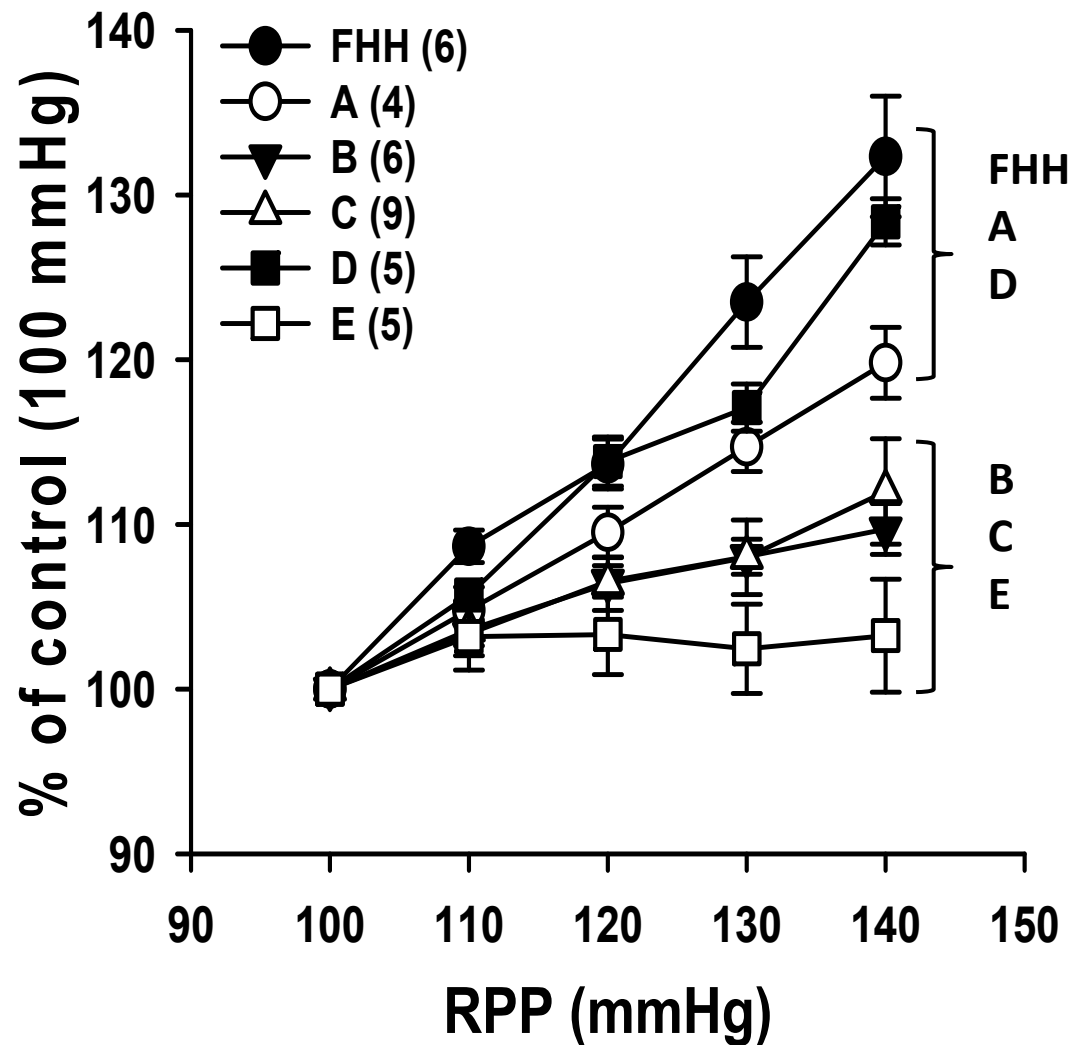


Left: indicates the location of genetic markers used to genotype the animals on chromosome 1 in FHH rats.

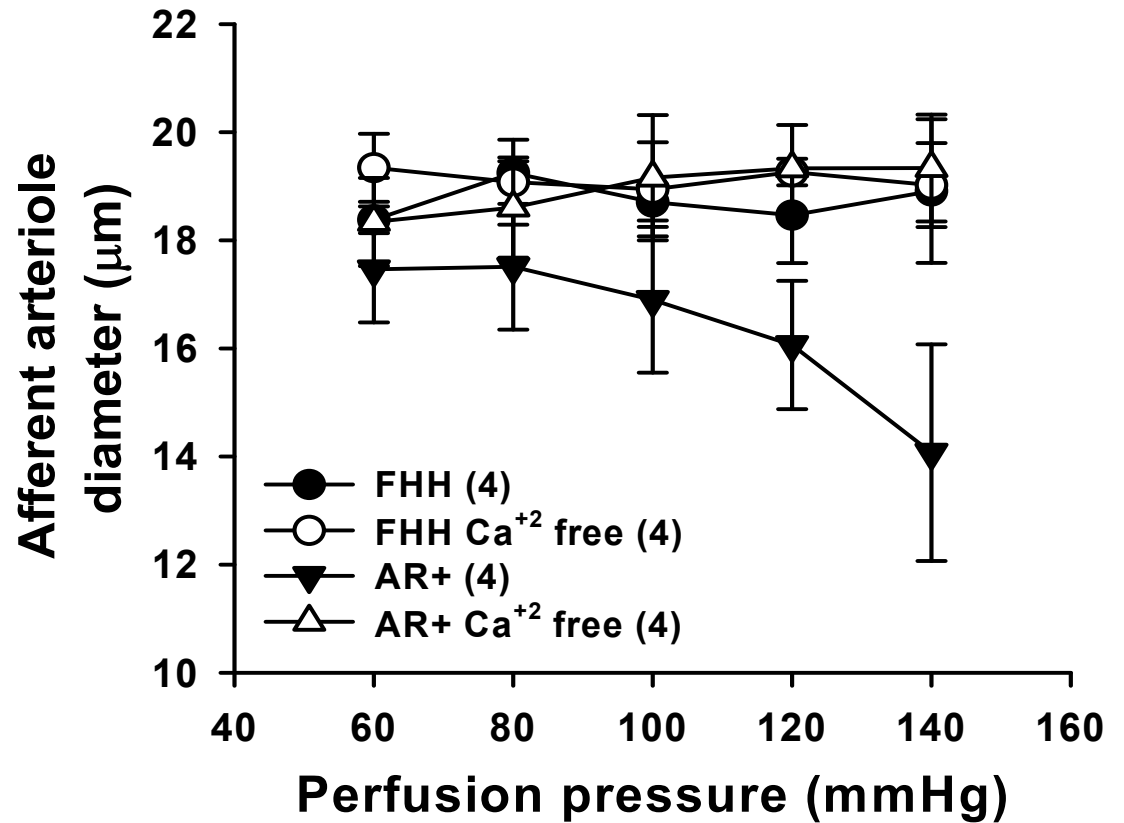
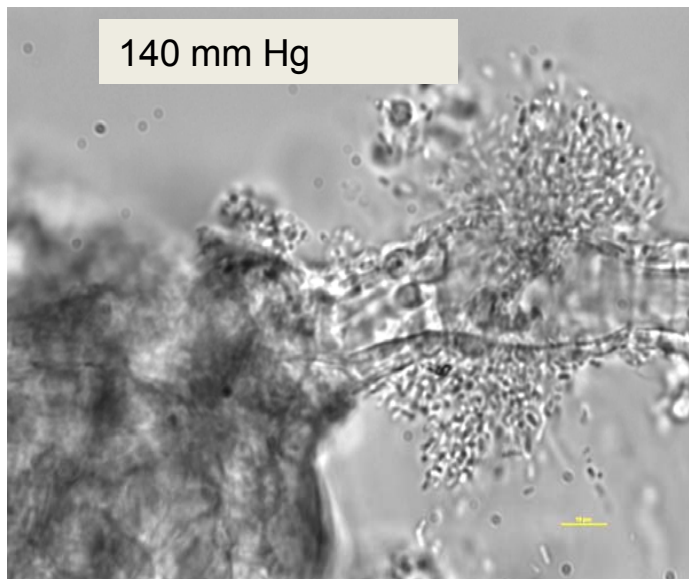
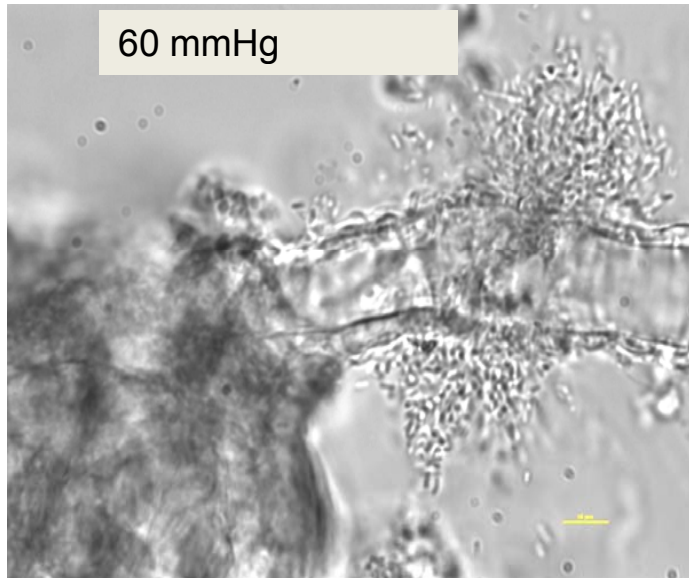
The closed and open filled bars refer to Fawn hooded-hypertensive (FHH) and Brown Norway (BN) genomes, respectively.

Right: indicates known candidate genes in the 2.6 Mb region of interest. AR= Auto regulation; + present; - present

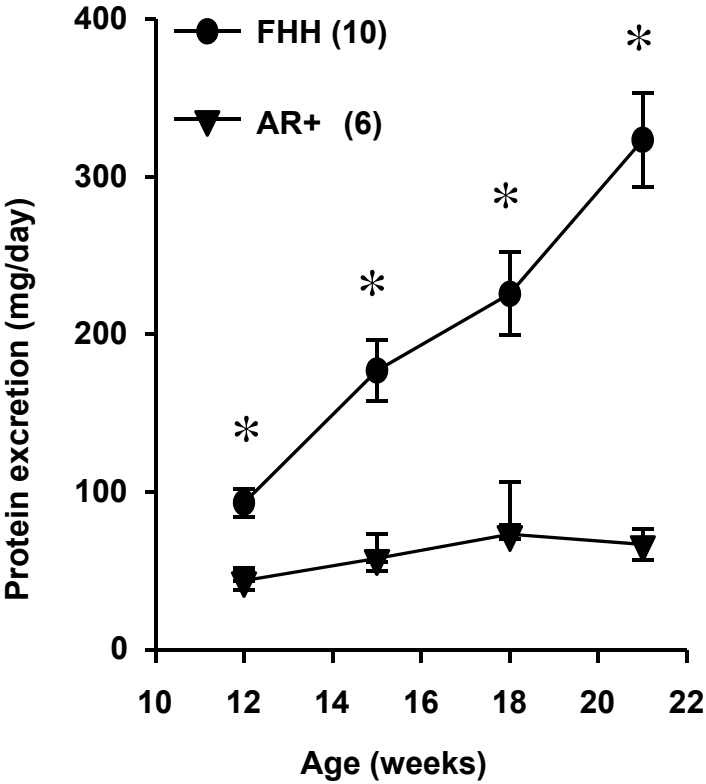
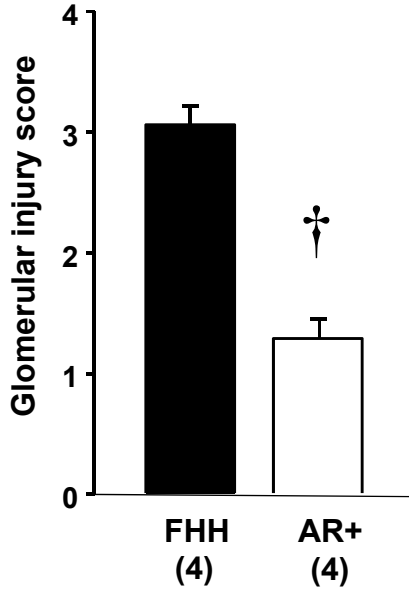
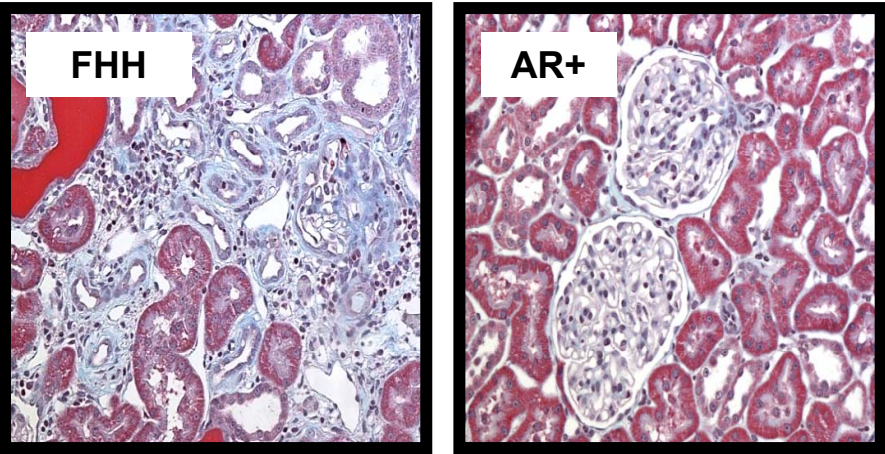
FHH and FHH.1^{BN} congenic rats



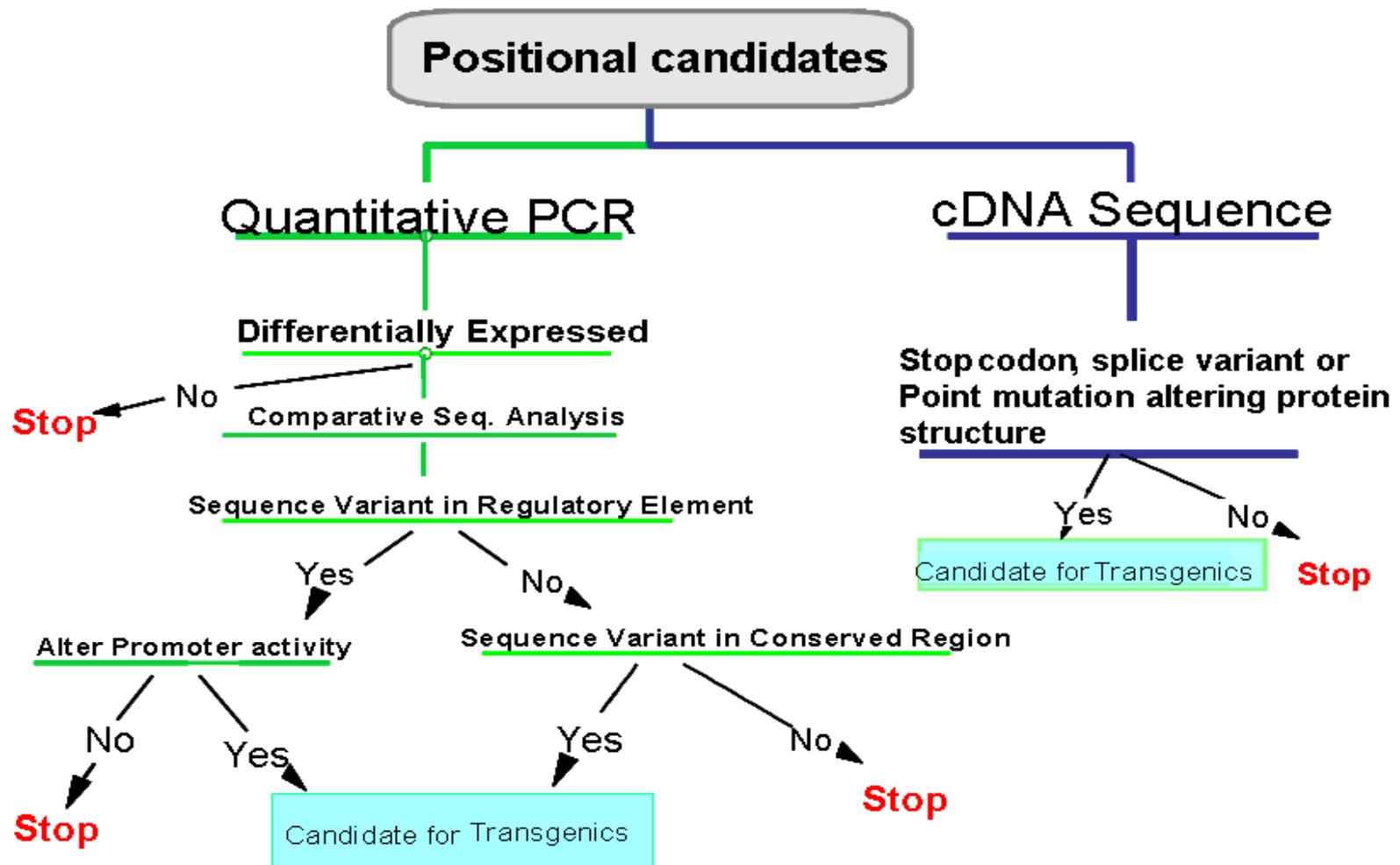
Restoration of myogenic response in AR+ FHH.1BN congenic strain E



Restoration of RBF autoregulation mitigates proteinuria and renal injury



Decision tree for the prioritization of candidate genes for transgenic studies.

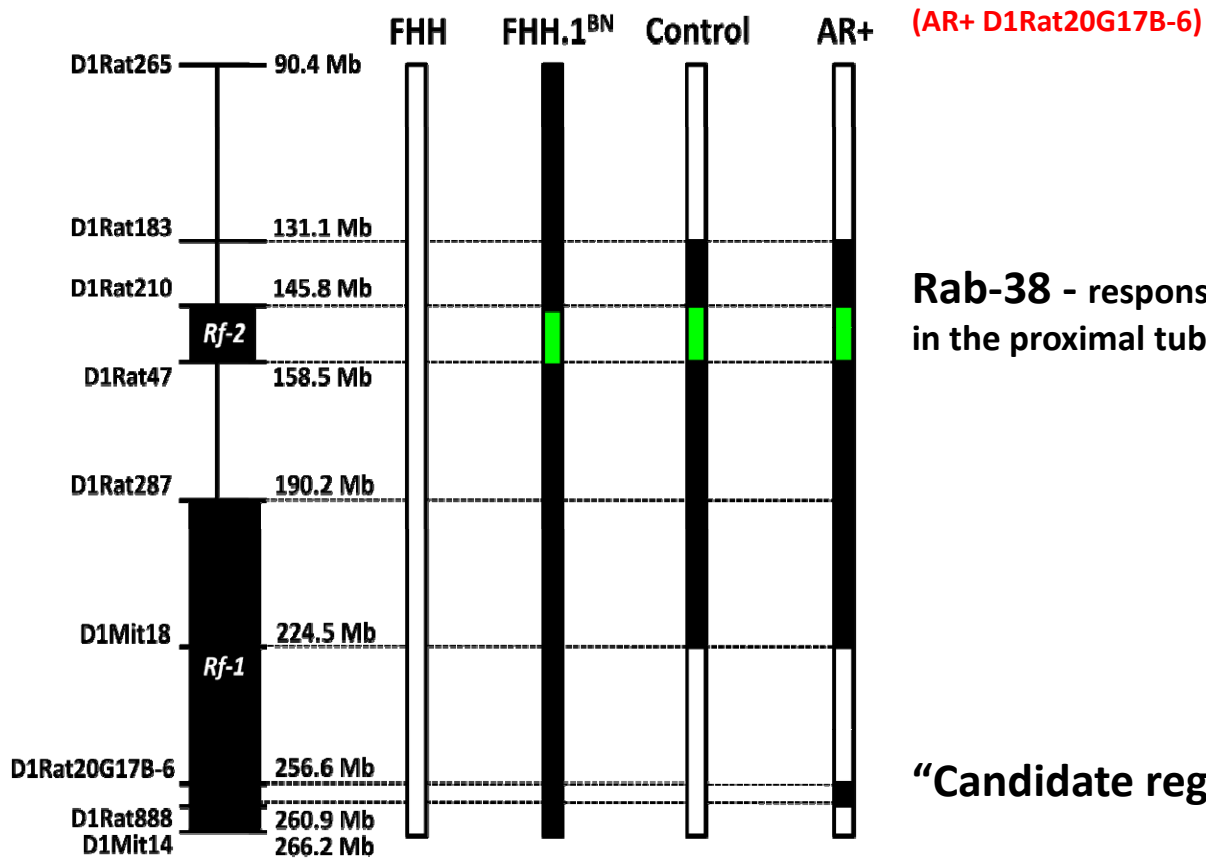


SNP analysis in coding region of Add3 gene: 37 SNPs

Exon/Intron mRNA Position of SNP	Exon2 147	Exon2 229	Exon13 1847
ENSRNOT00000017600(BN) NC-0015100.2(SD) NM-031552(SD)	C/Threonine C/Threonine G/Serine	C/Aspartic Acid C/Aspartic Acid T/Aspartic Acid	A/Lysine A/Lysine C/Glutamine
BN gDNA	C	C	A
ACI gDNA	C	T	A
FHH gDNA	G	T	C
SHR gDNA	G	T	A
FHHRV mRNA	G	T	C
FHHCV mRNA	G	T	C
FHM8RV mRNA	C	C	A
FHM8CV mRNA	C	C	A
SHR mRNA	G	T	A

19 SNPs in Dusp5 gene

FHH and FHH.1^{BN} congenic rats



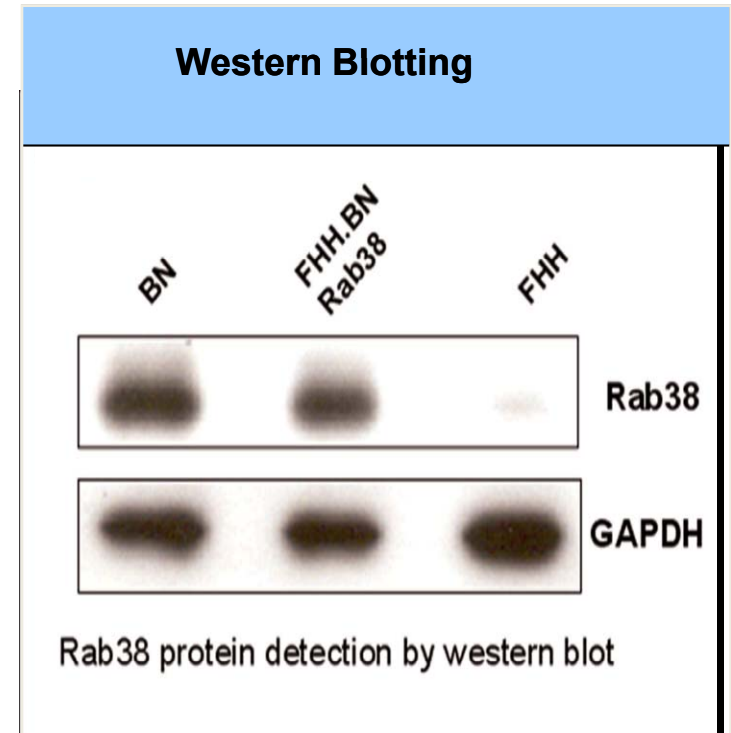
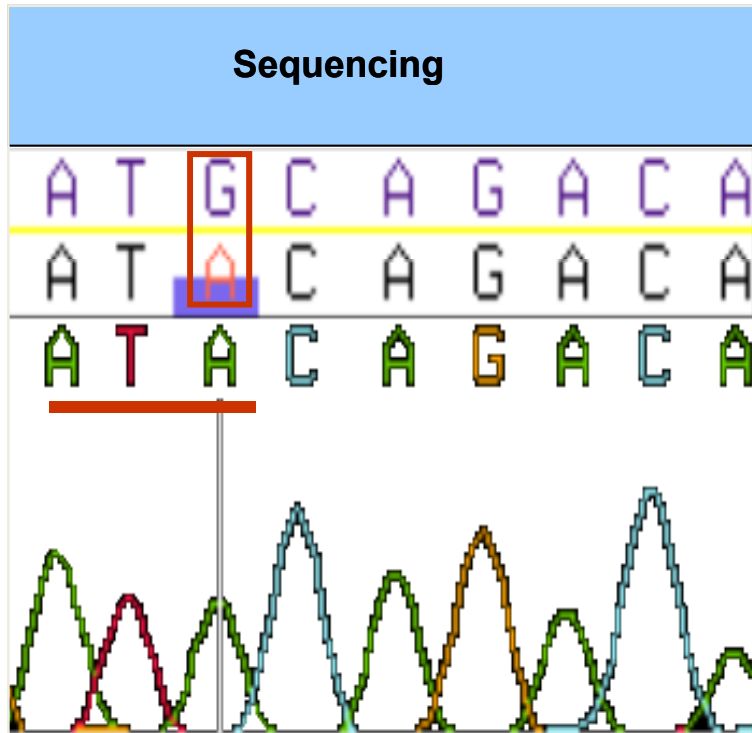
Rab-38 - responsible for the re-uptake of filtered protein in the proximal tubules

“Candidate region”

Autoregulation of RBF	-	+	-	+
Proteinuria	+	-	+	?

Rab38 Sequencing

FHH.BNRab38
FHH



Rab 38 regulates reuptake and processing of filtered albumin

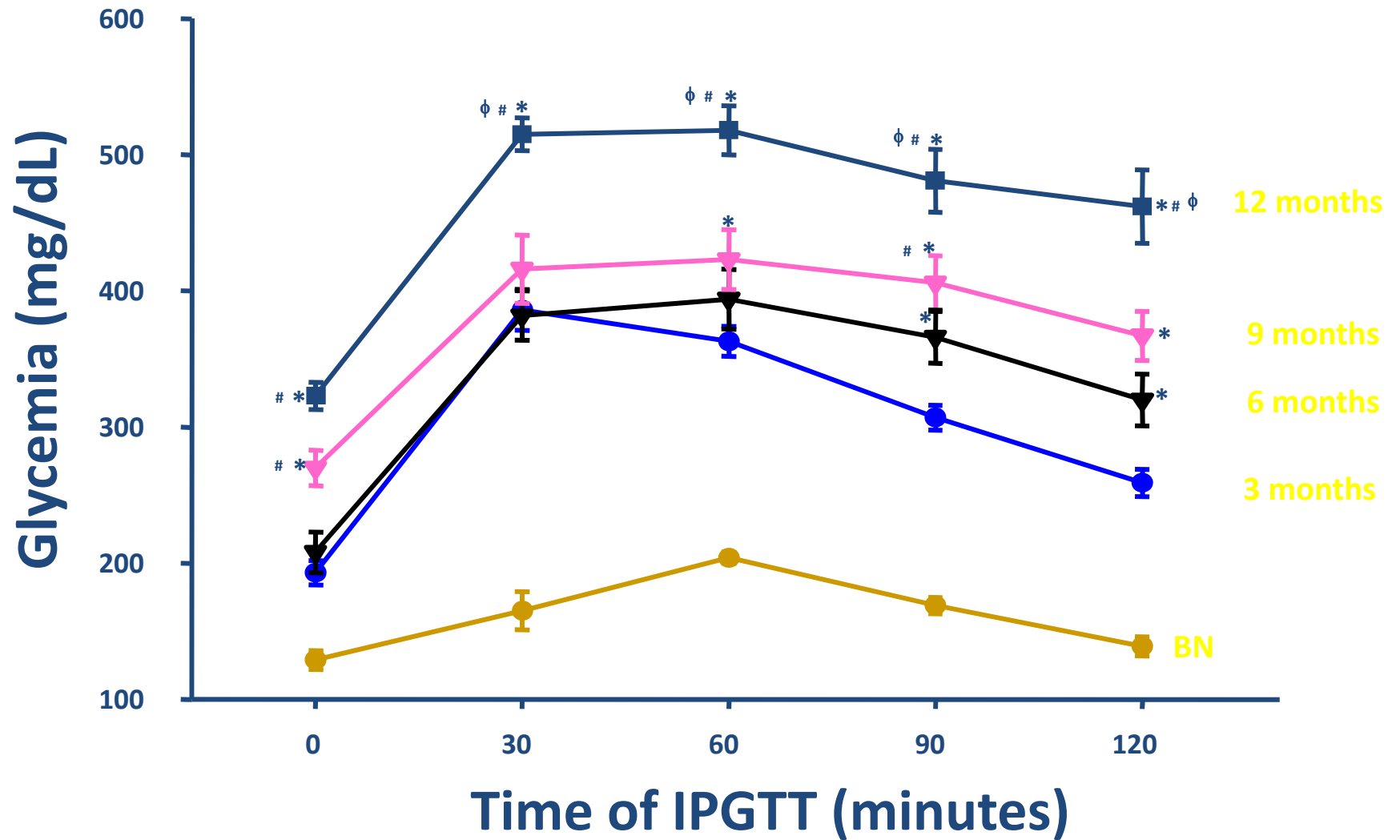
Reasons for lack of progress in preventing diabetic nephropathy

- ***Problem:***

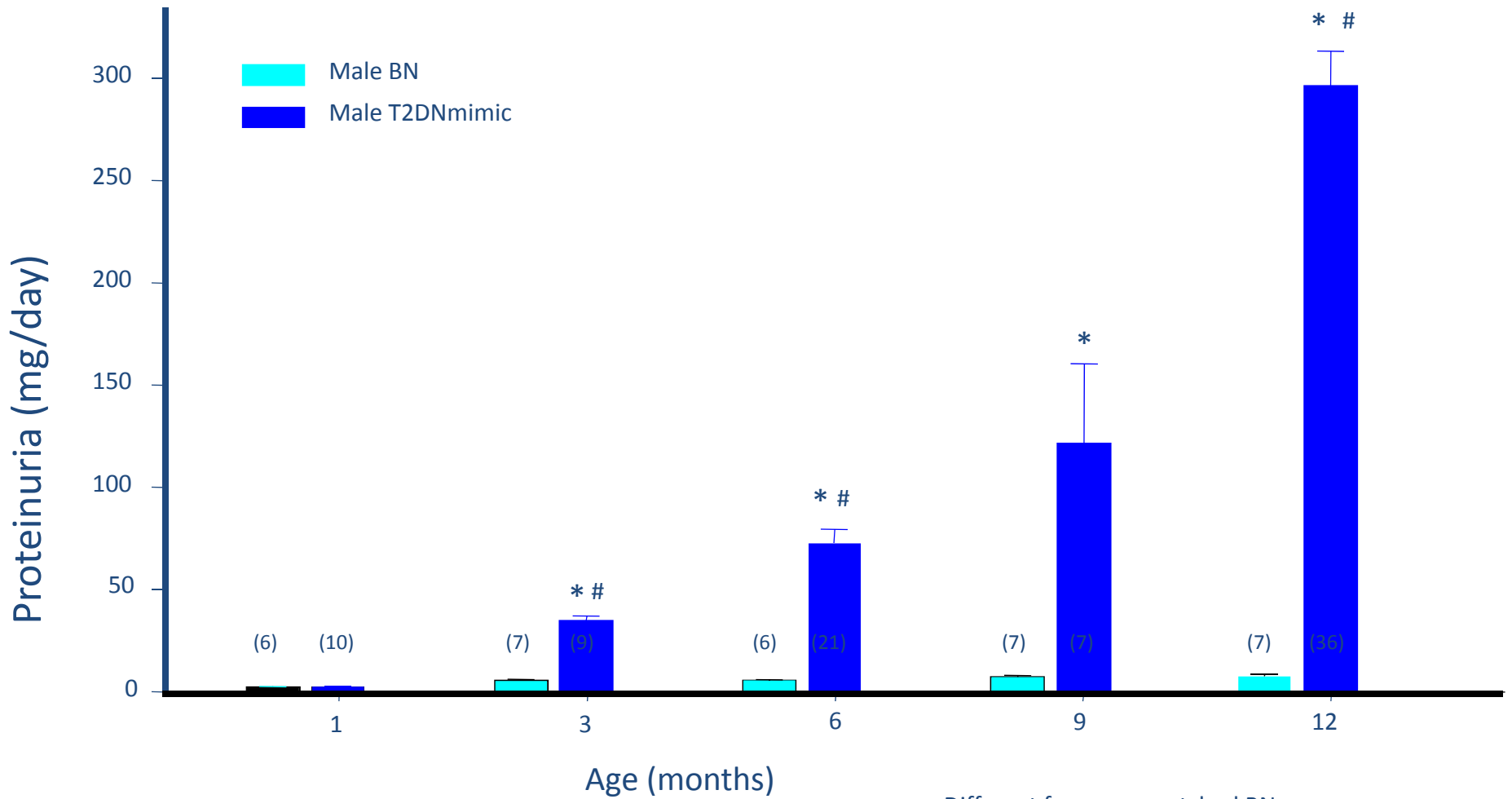
No rodent model of diabetic nephropathy exhibits progressive renal disease and lesions resembling those seen in man. Cell models not particularly informative.

- ***Solution:*** We combined the genome of the FHH rat that develops renal disease but not diabetes with that of the GK rat that develops type II diabetes but not renal disease.

Glucose Intolerance in T2DN Rats



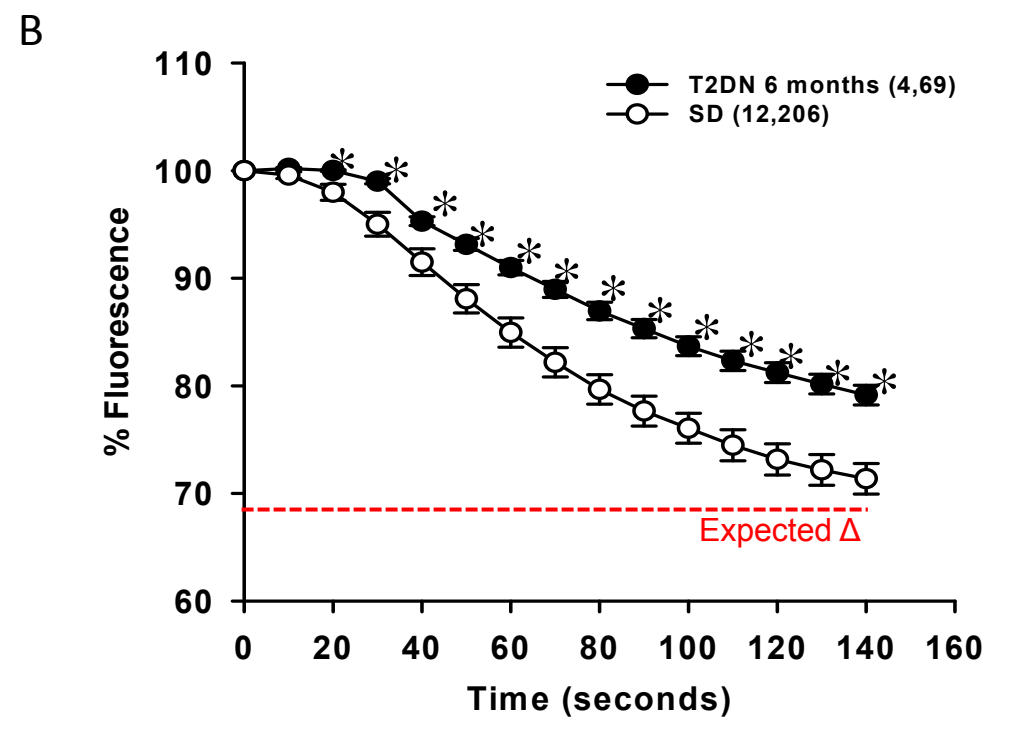
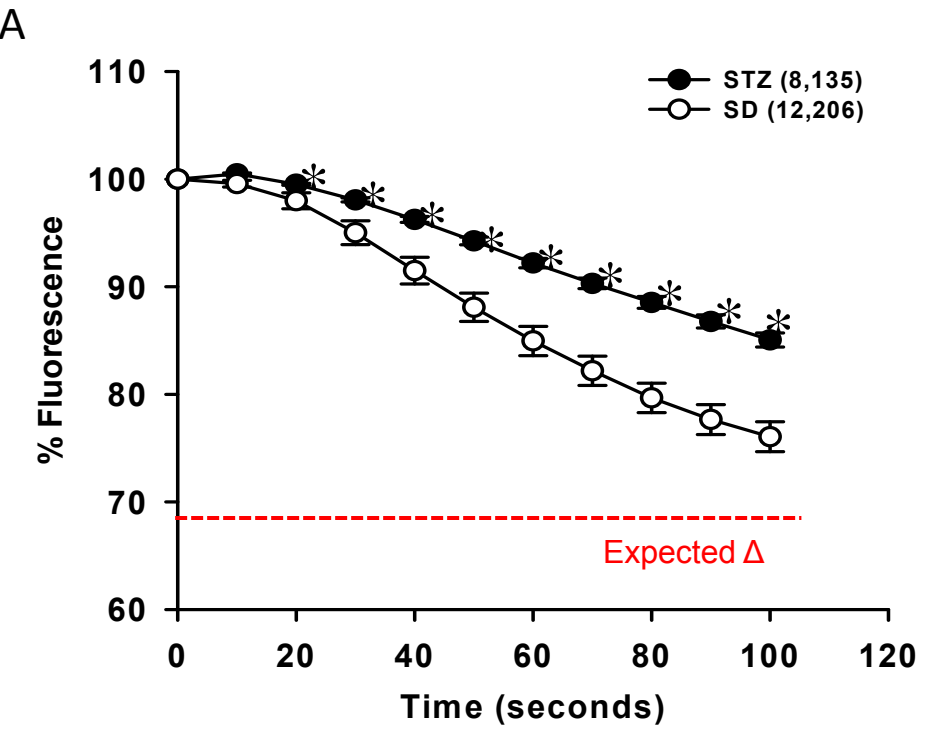
Progressive Proteinuria in T2DN Rats



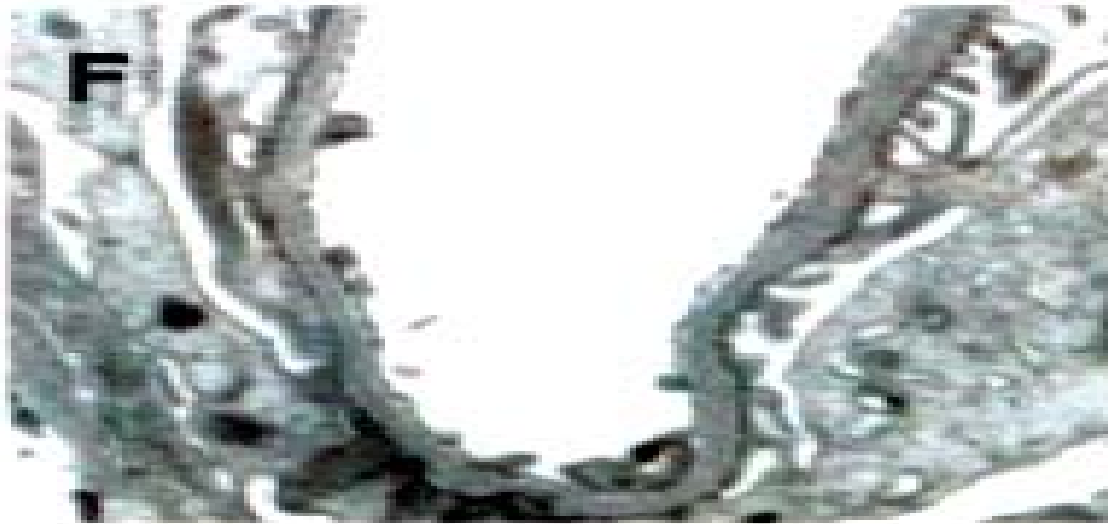
* - Different from age-matched BN

- Different from previous age in group-matched

Diabetes model

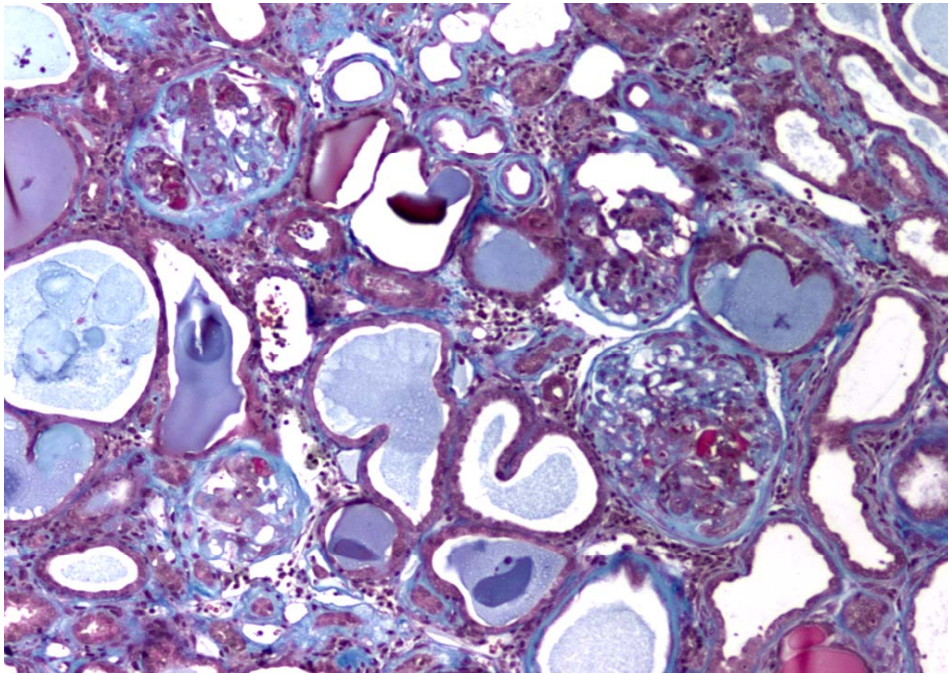


Glomerular Basement Membrane in 12 month T2DN Rats

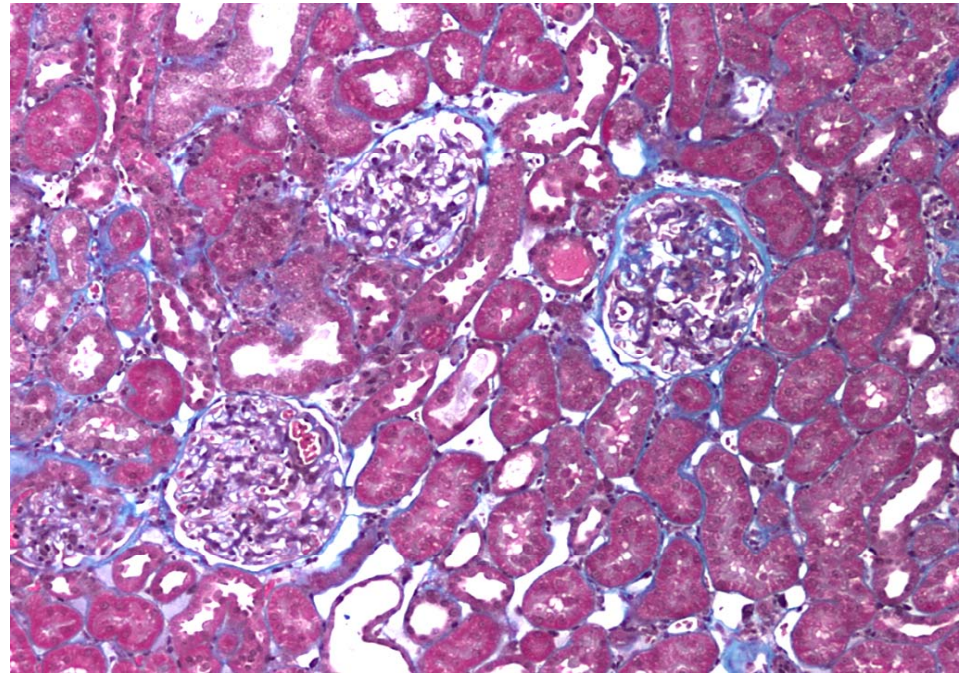


Effect of tight diabetic control on diabetic nephropathy

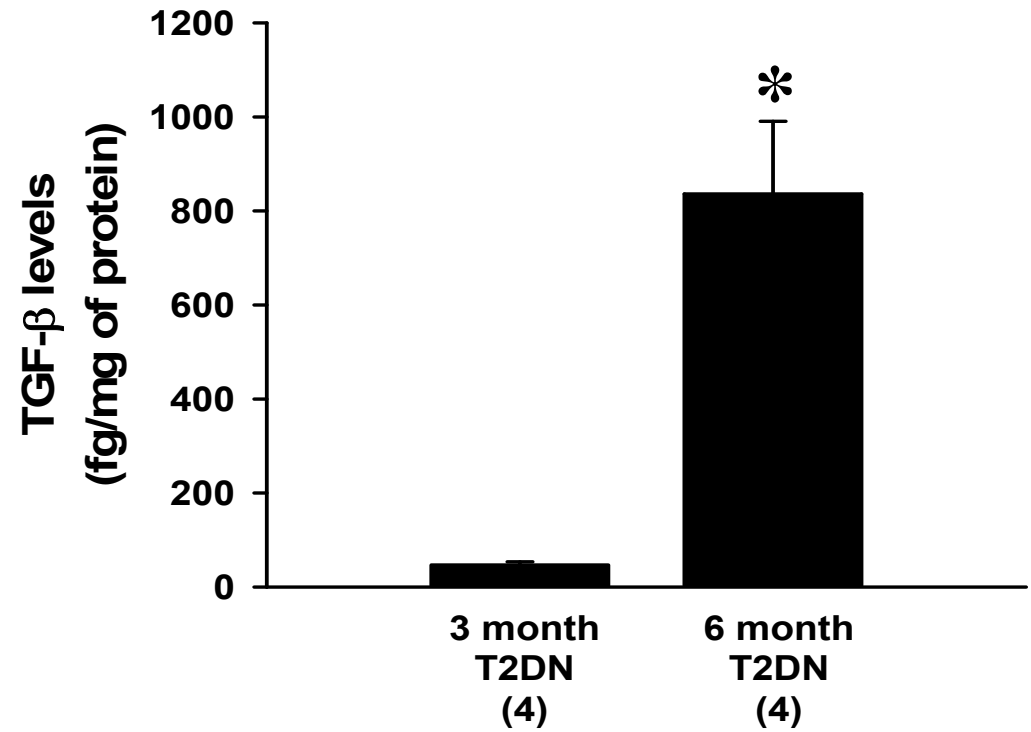
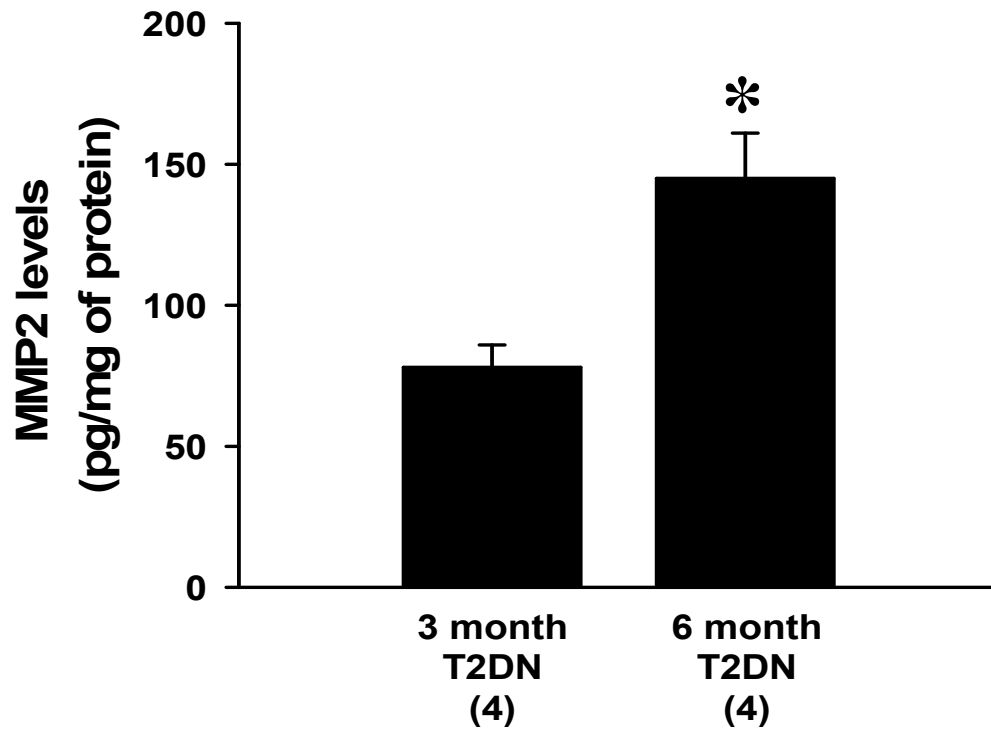
Vehicle



Insulin

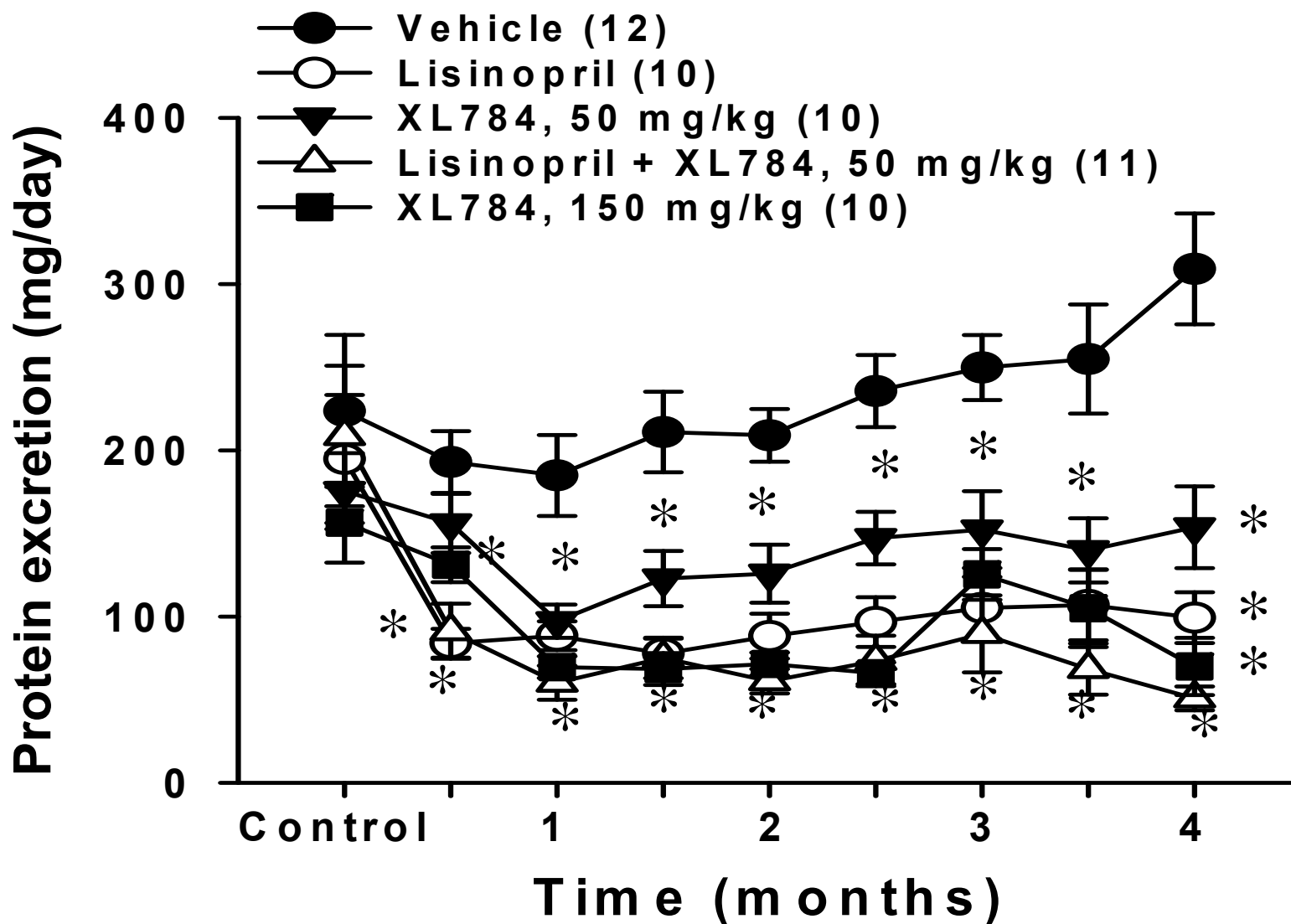


MMP-2 and TGF- β 1 levels in T2DN rats



Type-2 diabetes

Effects of XL784 on proteinuria in T2DN rats

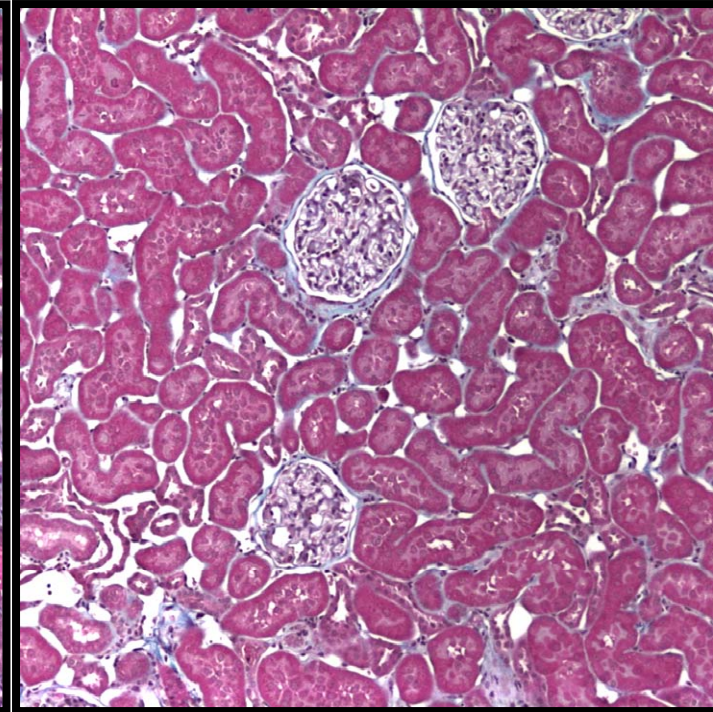
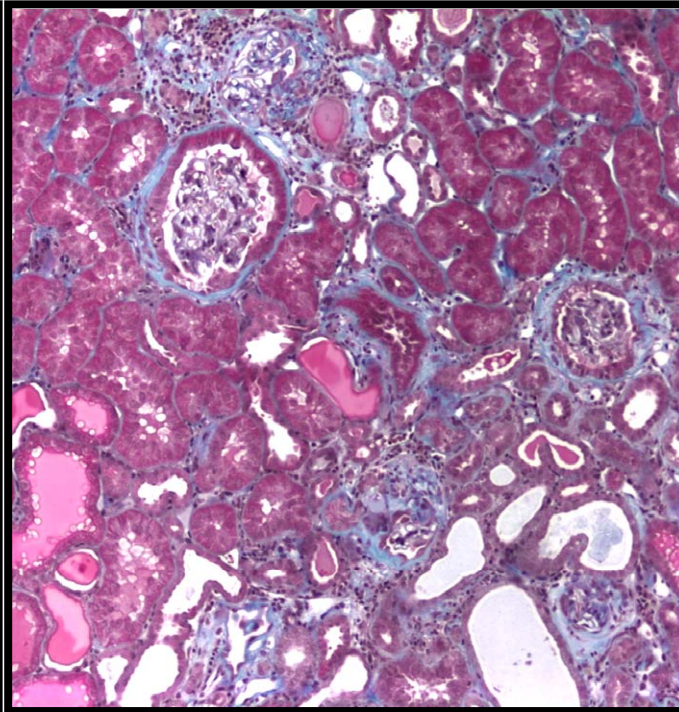
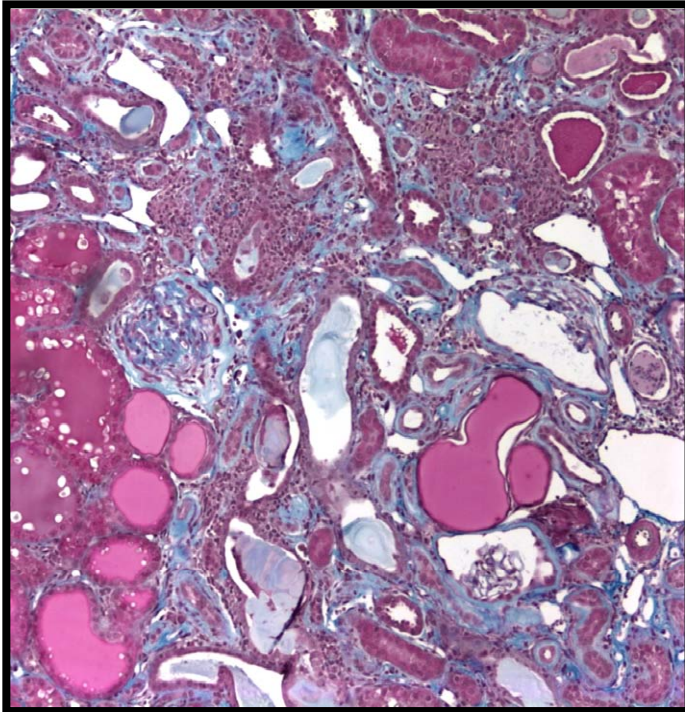


*Effect of an ACE Inhibitor and MMP on
diabetic nephropathy*

Vehicle

Lisinopril

XL784



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