AMDCC Progress Report Duke/UNC/Stanford Unit

Program Director

Thomas M. Coffman, MD

Susceptibility Mutation ← *Model Development*

+ ApoE-/-

+STZ

Kidney Phenotype Screen

Vascular & Kidney Phenotype Screen

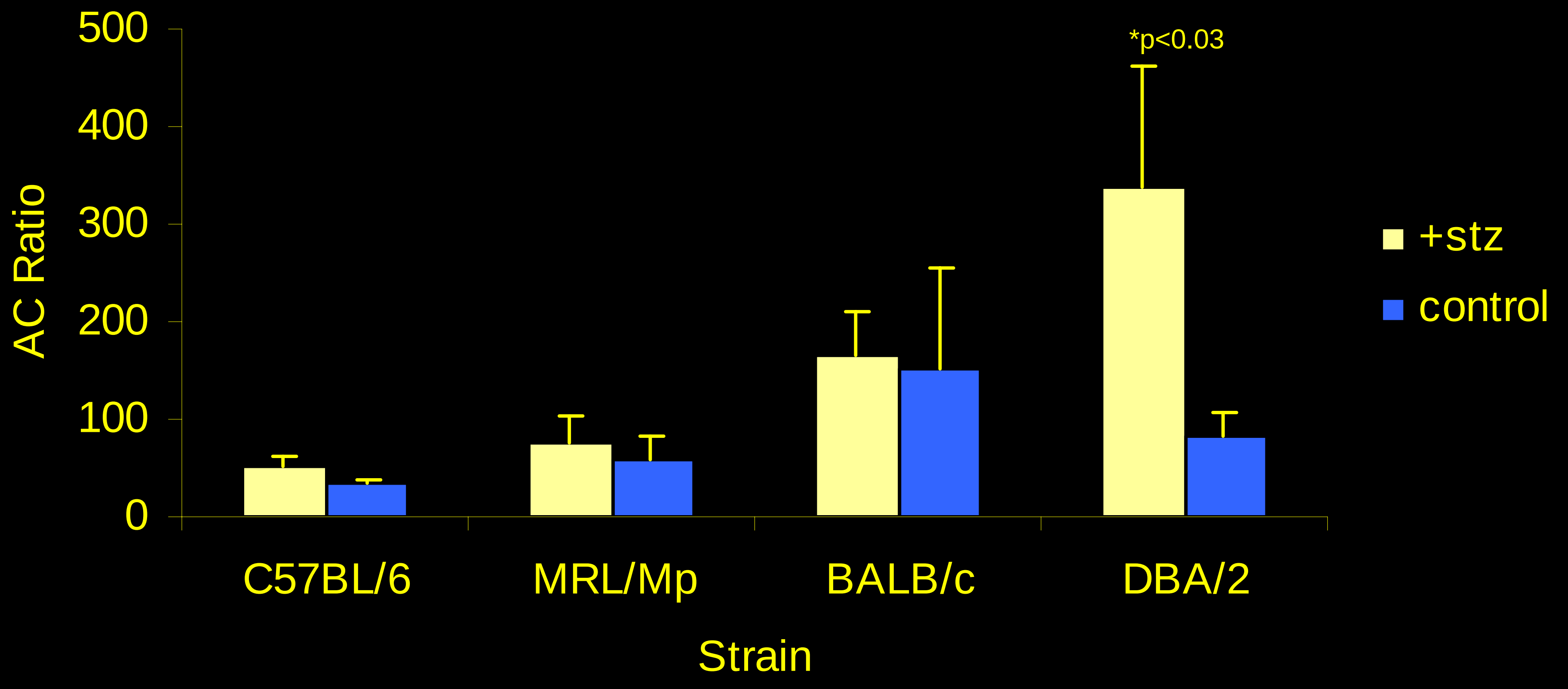
Transfer mutations to diabetic backgrounds

Generate ES Cell Lines From Diabetic Mice →

Strain Comparison

- Of the 5 strains tested, DBA/2 found to be most susceptible in STZ model

Albumin-to-Creatinine Ratio in Male Mice



Mesangial Volumes in Male Mice

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Model Comparison

Advantages of the Akita ($Ins2^{+/C96Y}$) model

16 Week Blood Glucose Values

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(*p=0.0018)

Differential Blood Pressure Responses

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(*p=0.0018 vs Akita Control)

Increased Proteinuria in Akita Mice

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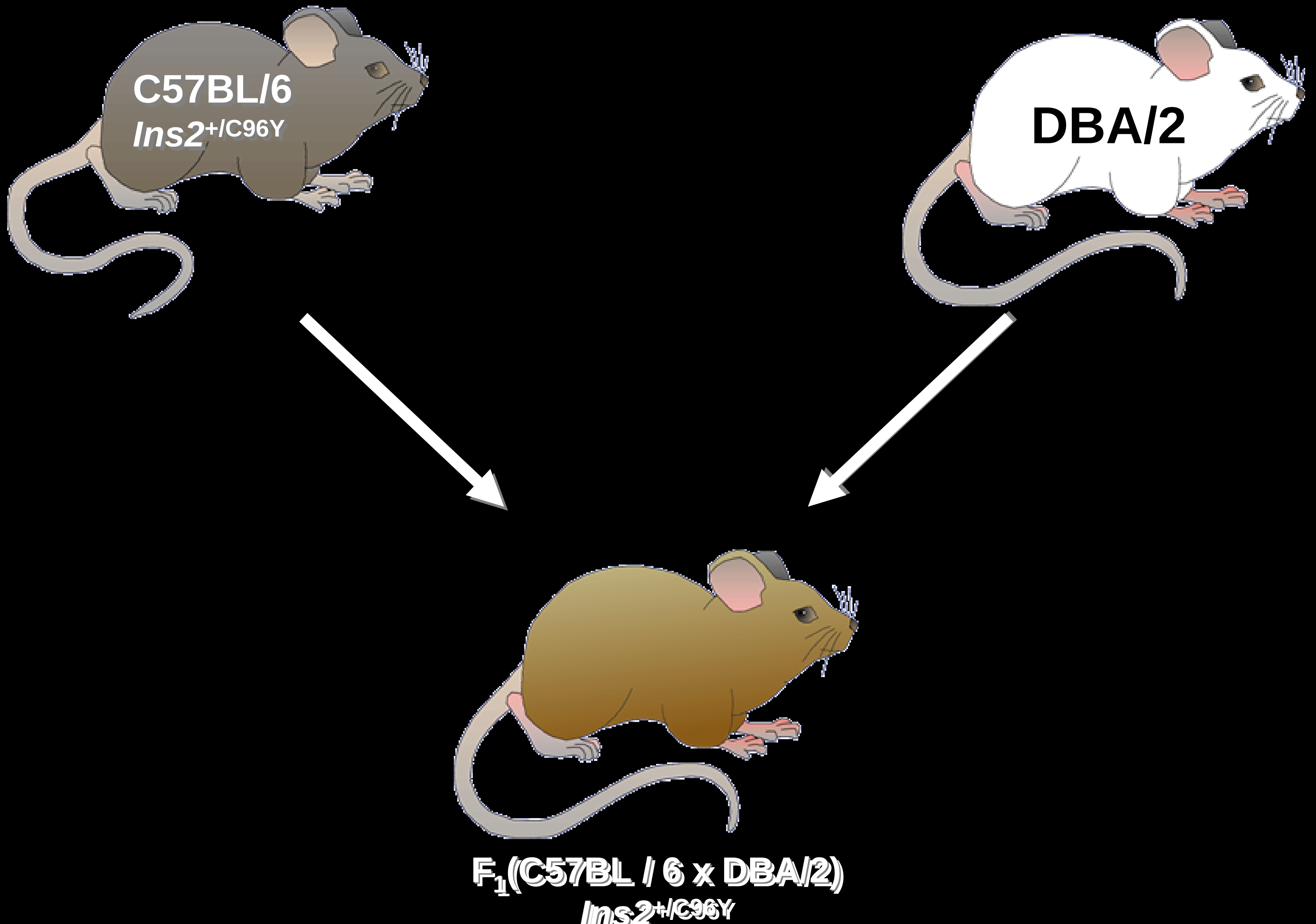
(*p=0.018 vs. STZ)

Mesangial Volumes in Diabetic Mice


C57BL/6
Ins2+/C96Y
DBA/2
F1(C57BL / 6 x DBA/2)
Ins2+/C96Y

Blood Glucose Levels in Mice

*

(*p=0.011)

Enhanced Proteinuria in
F_1 (DBA/2 x C57BL/6)*Ins2*$^{+/C96Y}$ Mice

*

(*p<0.001 vs. B6-Akita)

Mesangial Expansion in
F_1 (DBA/2 x C57BL/6)*Ins2*$^{+/C96Y}$ Mice

*

(*p<0.001 vs. B6-Akita)

Studies in Progress

- Large group of animals for long-term studies
- Back-crossing of ***Ins2***$^{+/C96Y}$ onto DBA/2 and other backgrounds

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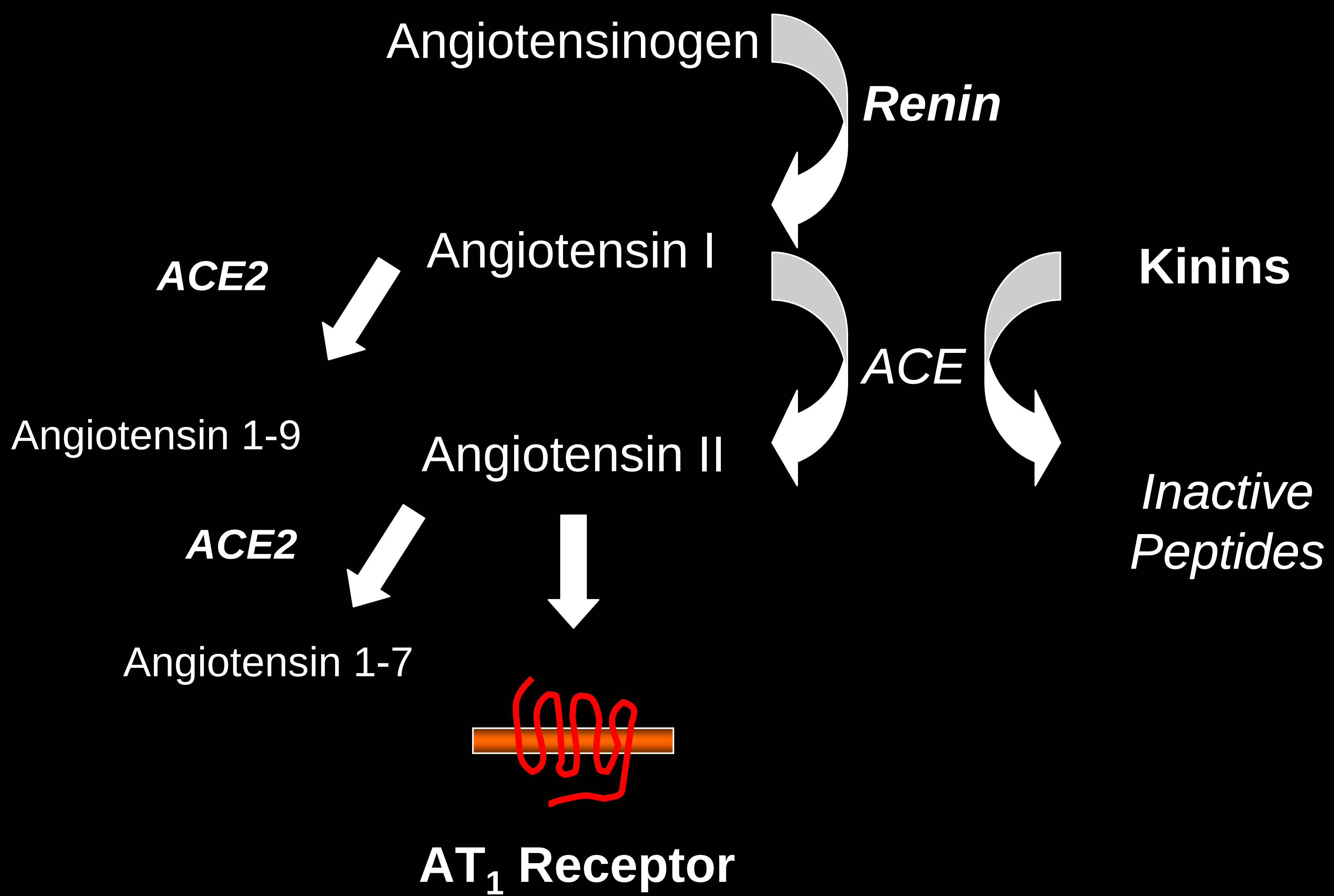
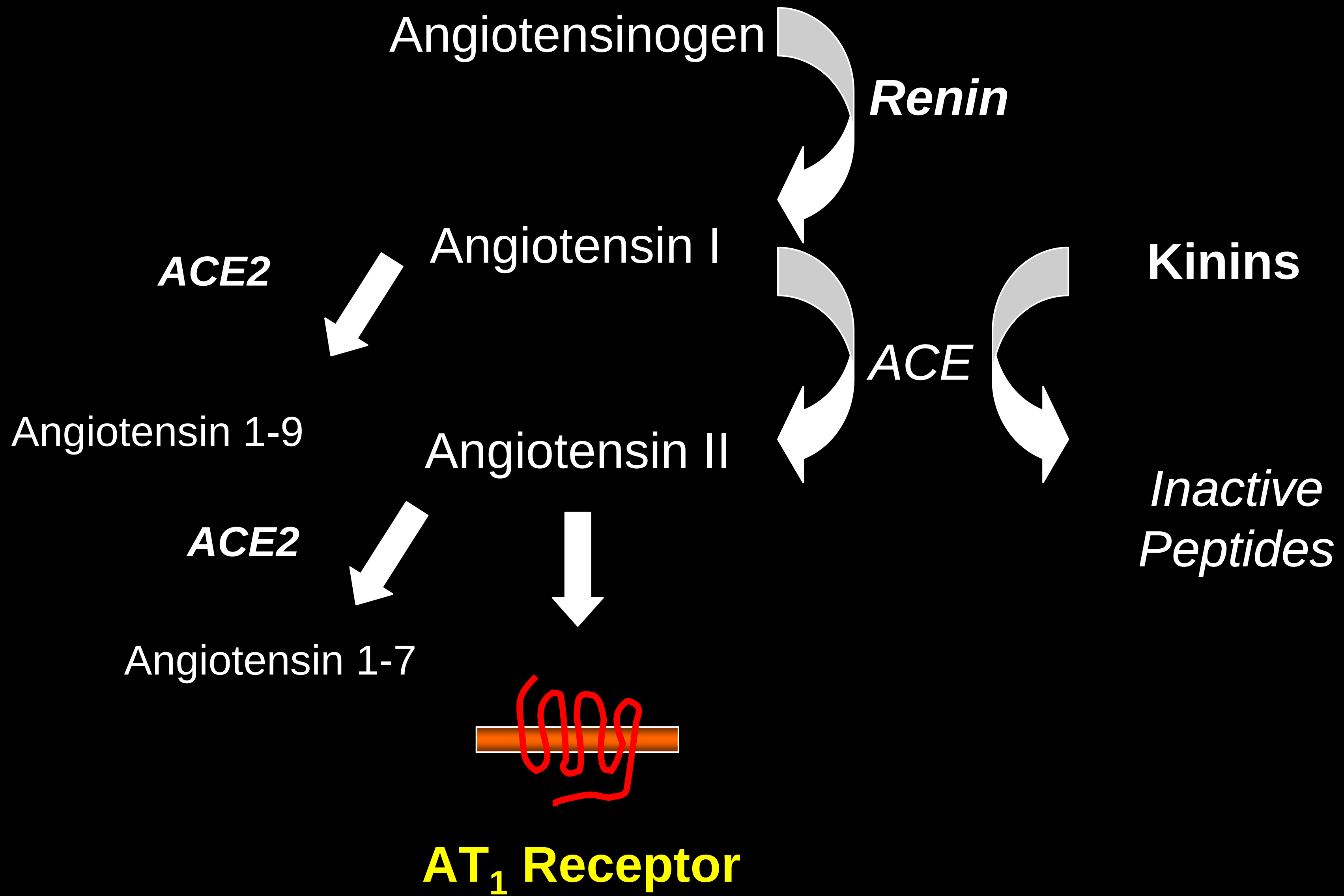


Angiotensinogen
Renin
Angiotensin I
ACE2
Kinins
ACE
Angiotensin 1-9
Angiotensin II
Inactive Peptides
ACE2
Angiotensin 1-7
AT1 Receptor


Angiotensinogen
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ACE
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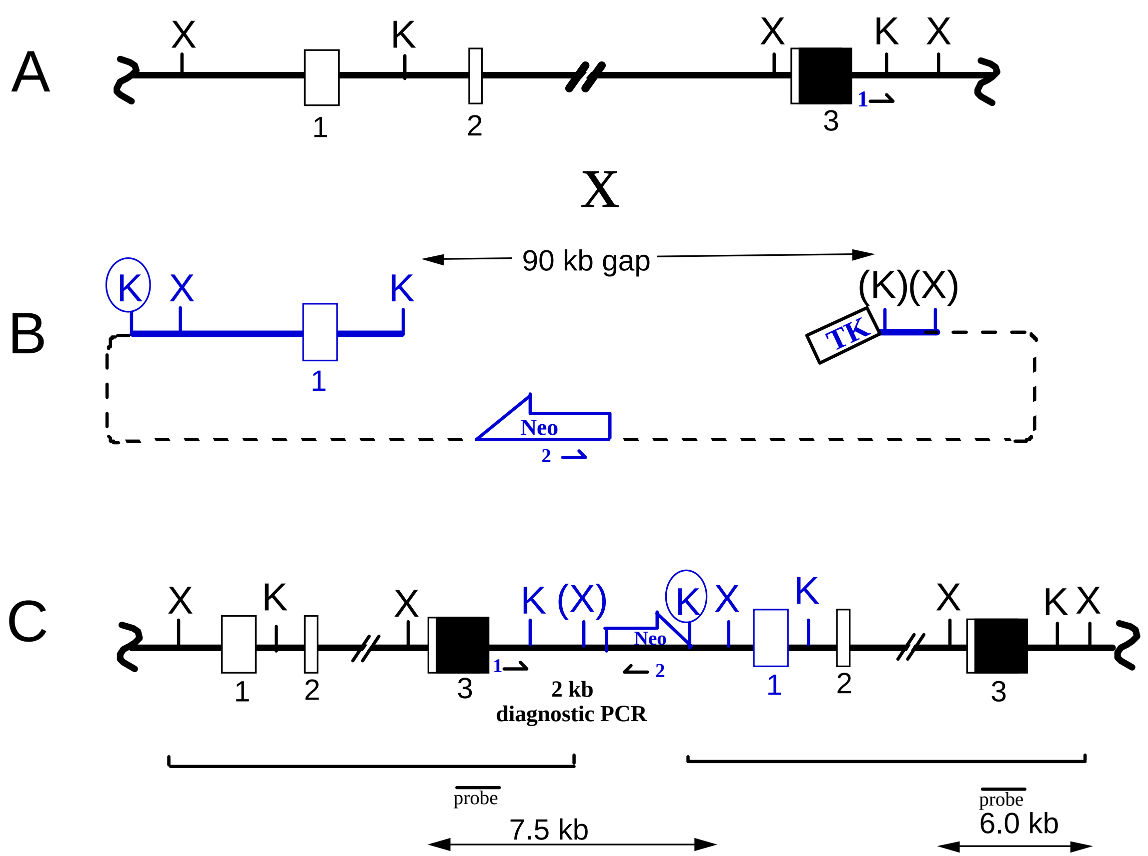
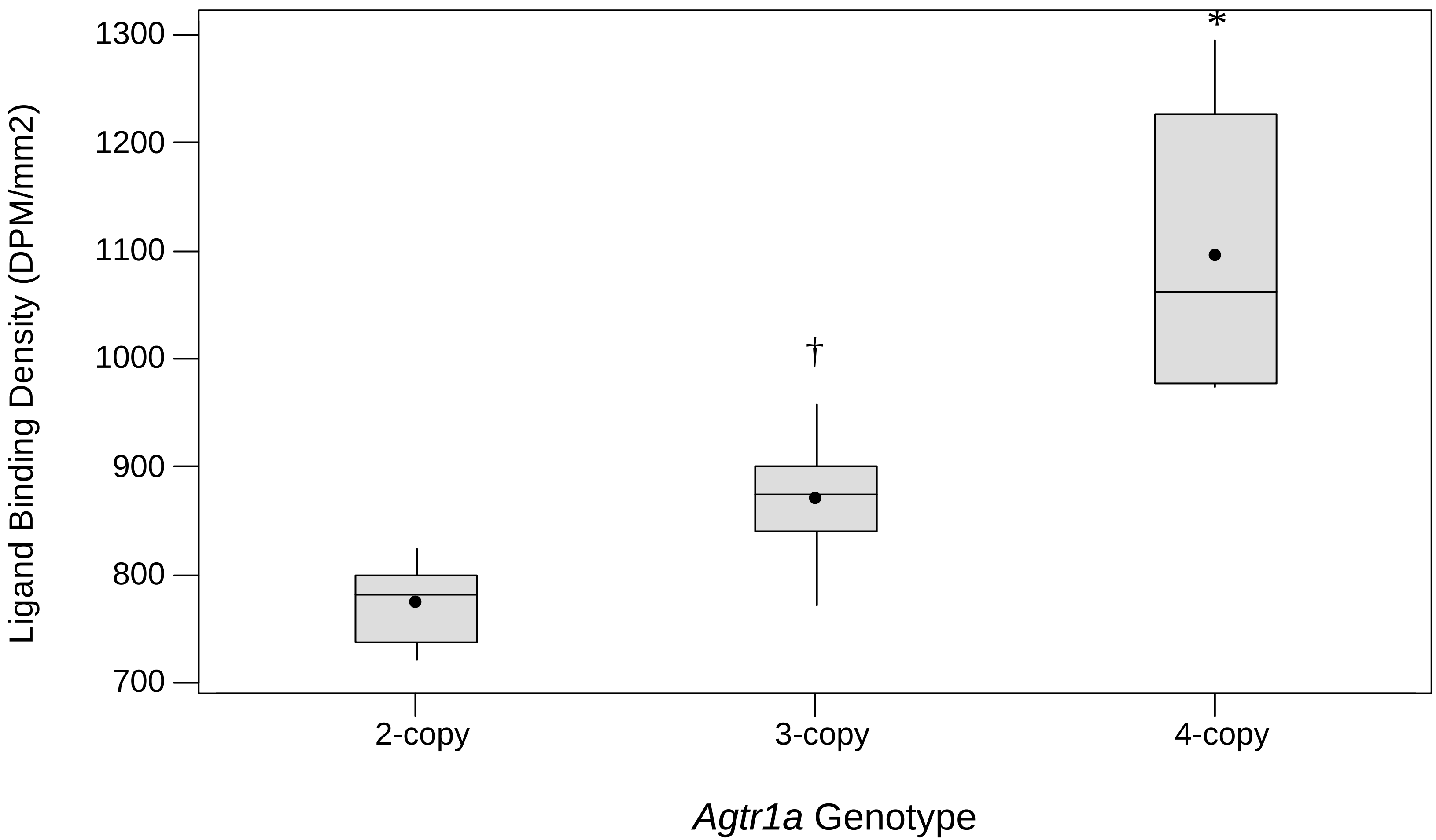
Angiotensin 1-9
Angiotensin II
Inactive Peptides
ACE2
Angiotensin 1-7
AT1 Receptor

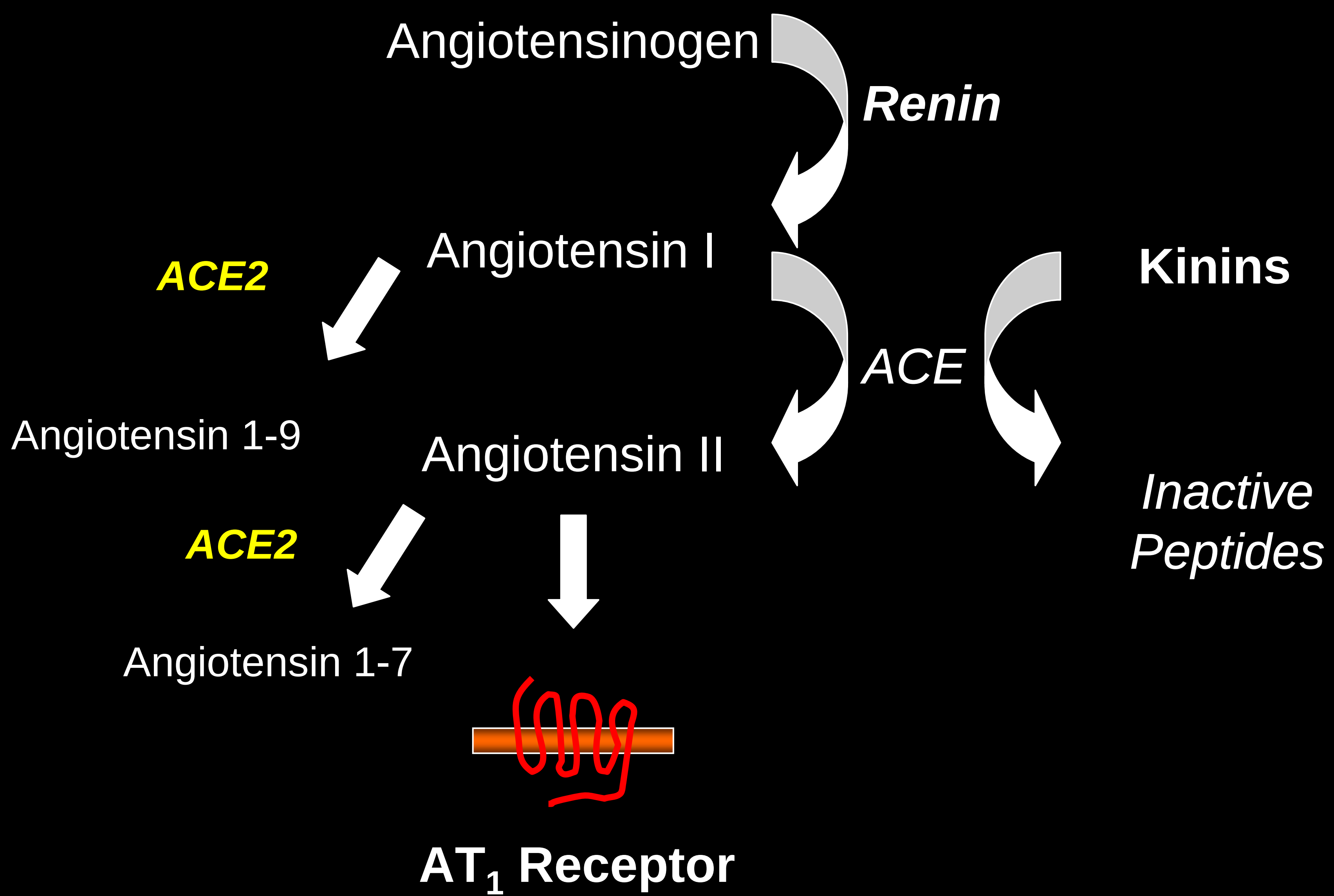
Agtr1a Gene Duplication





Ligand Binding Density (DPM/mm2)
1300
1200
1100
1000
900
800
700
*
†
2-copy
3-copy
4-copy
Agtr1a Genotype

ACR in *Agtr1a* 4-Copy Mice


Angiotensinogen
Renin
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ACE
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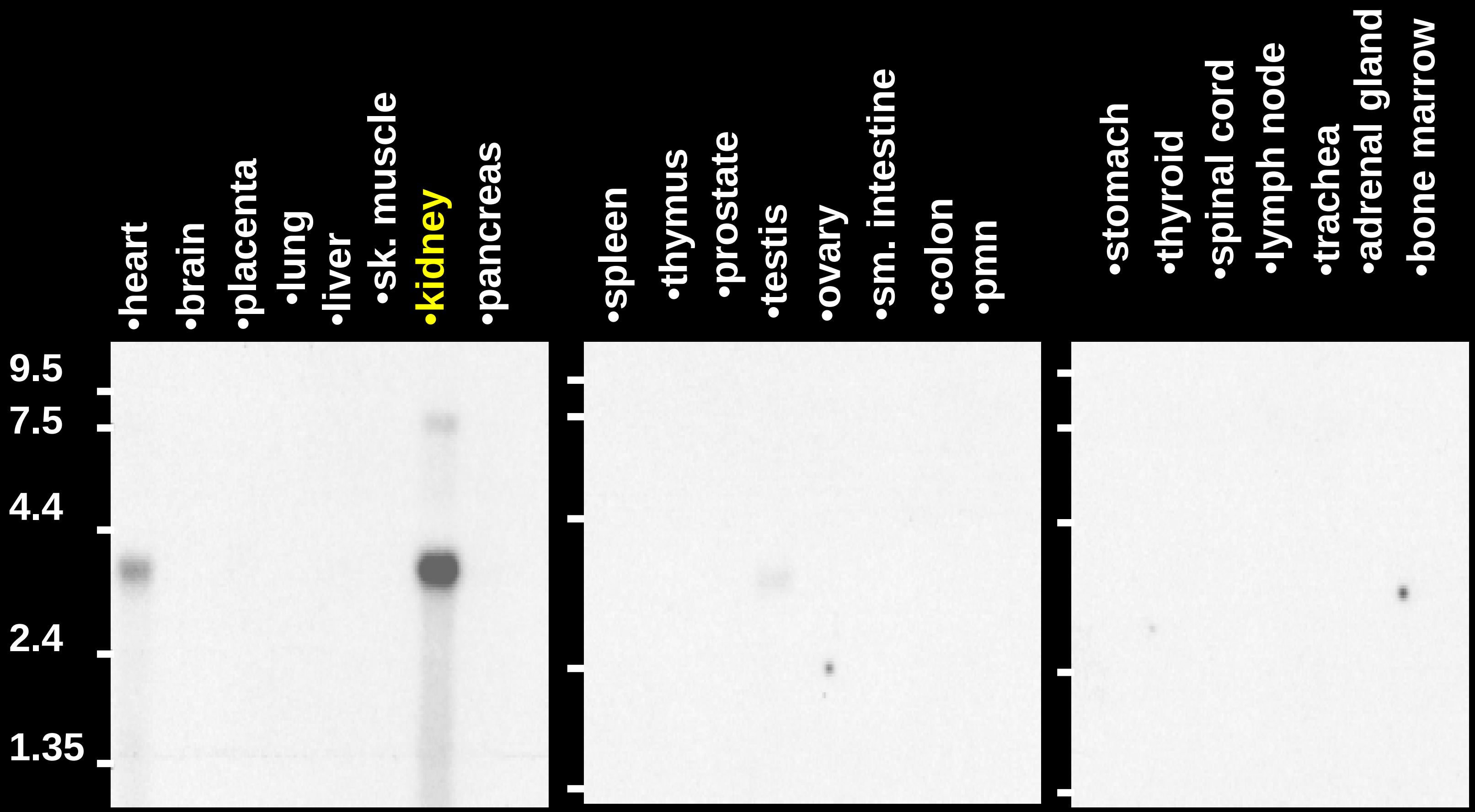
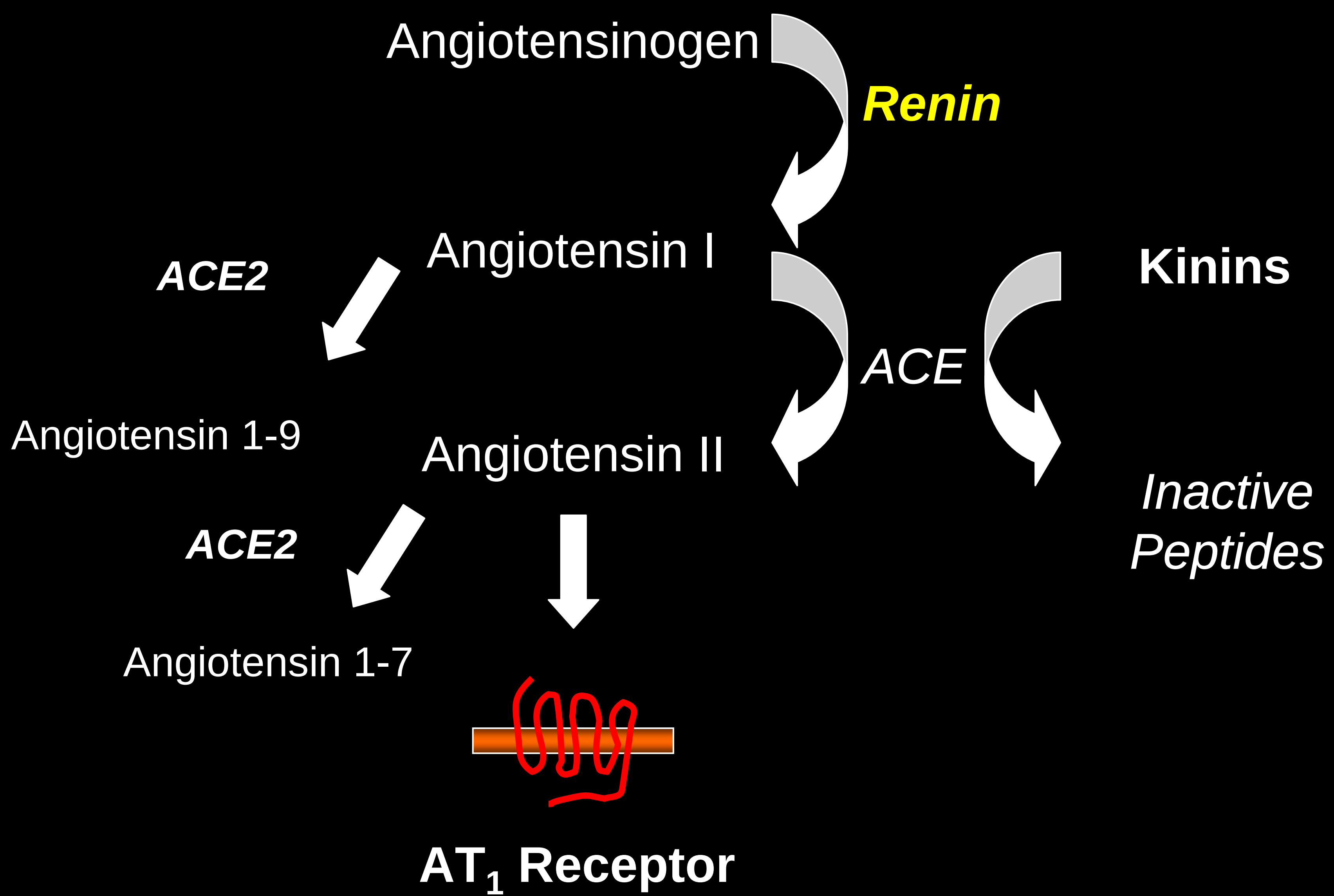
Inactive
Peptides
Angiotensin 1-7
AT1 Receptor

ACE2 mRNA Expression



Donoghue et al, *Circ Res* 87:1, 2000

ACR in *Ace2*-Knockout Mice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AT1 Receptor

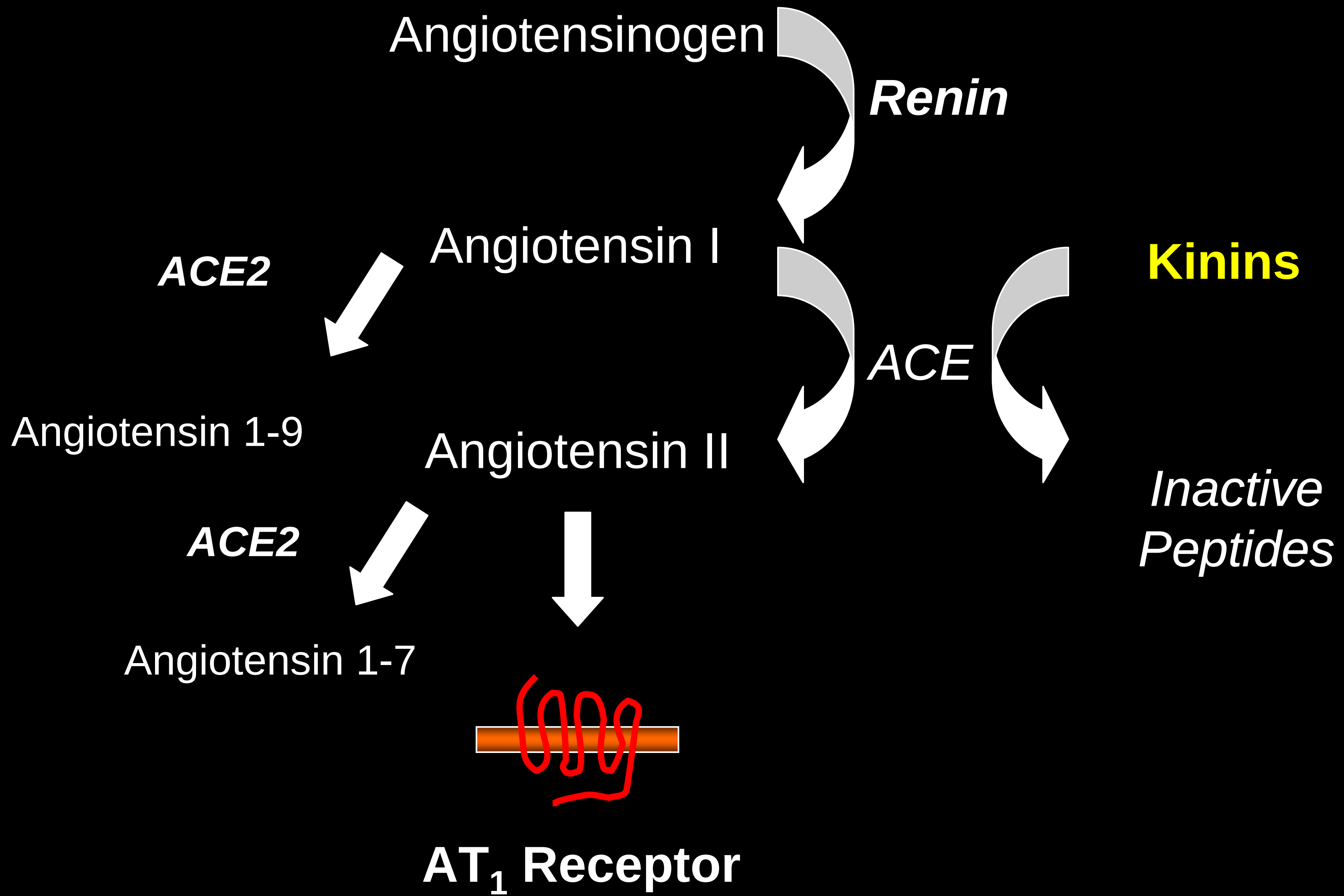
Renin Transgenic Mice

- Single-copy transgene on C57BL/6 background
- Renin expression driven by albumin promoter

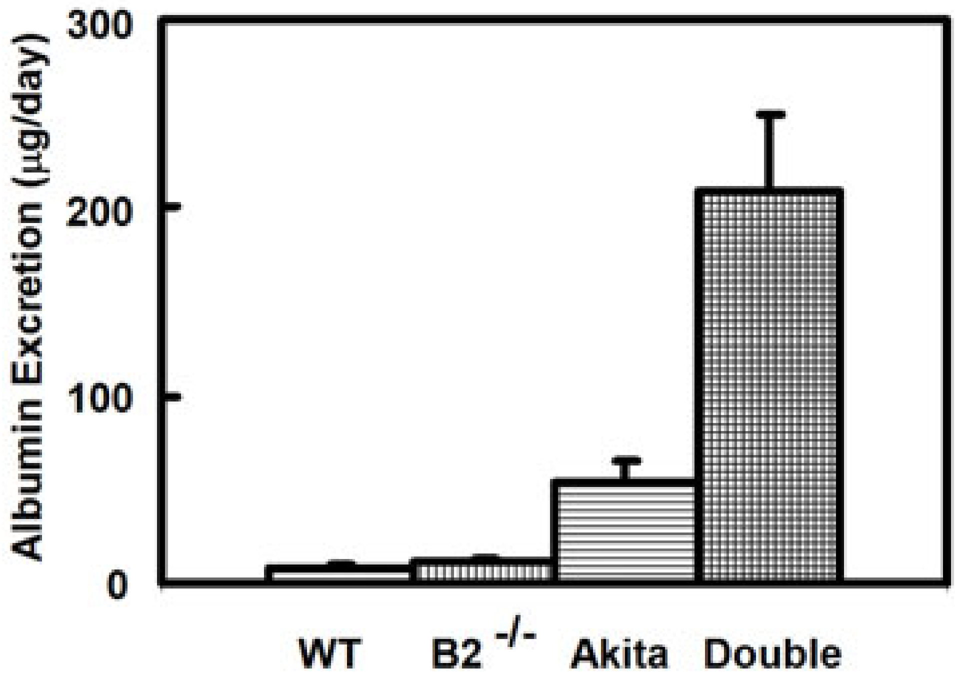
Blood Glucose in Renin Tg Mice

Blood Pressures in Renin Tg Mice

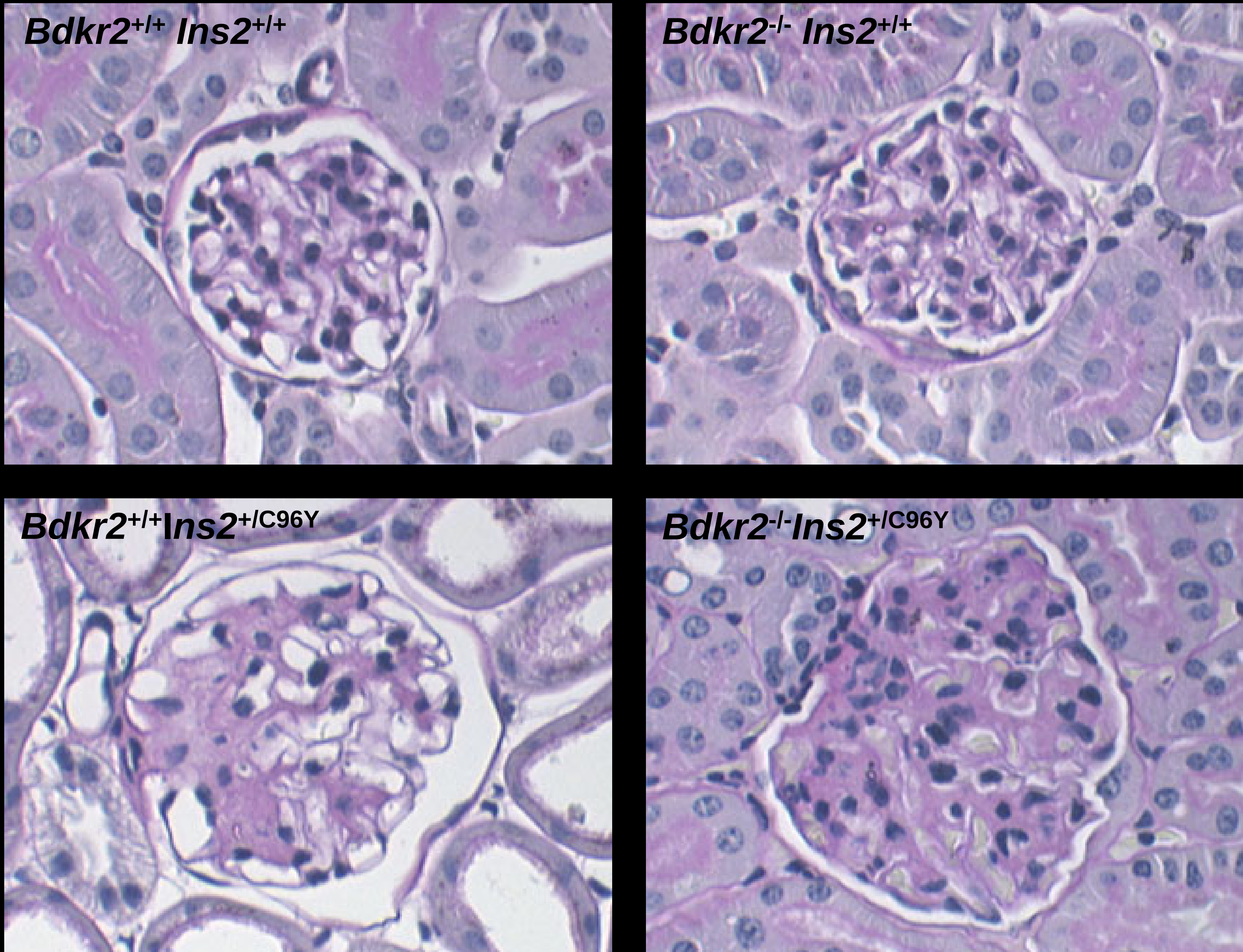
Albuminuria in Renin Tg Mice


Angiotensinogen
Renin
Angiotensin I
ACE2
Kinins
ACE
Angiotensin 1-9
Angiotensin II
ACE2
Inactive Peptides
Angiotensin 1-7
AT1 Receptor

Absence of Bradykinin B2 receptor increases diabetic albuminuria



PNAS 101:13302-13305, 2004



Bdkr2$^{+/+}$ *Ins2*$^{+/+}$
Bdkr2$^{-/-}$ *Ins2*$^{+/+}$
Bdkr2$^{+/+}$*Ins2*$^{+/C96Y}$
Bdkr2$^{-/-}$*Ins2*$^{+/C96Y}$

Completed Screens

Strain	Diabetes Induction	Blood Glucose	Blood Pressure	AC Ratio	Pathology
Tp$^{-/-}$ (C57BL/6)	STZ	x	x	x	(Renal)
Tp$^{-/-}$ (BALB/c)	STZ	x	x	x	(Renal)
TIMP3$^{-/-}$	STZ	x	x	x	(Renal)
Agtr1a dupl (2/2)	STZ	x	x	x	(Renal)
Bdkr2$^{-/-}$	STZ	x	-	x	(Renal)
Bdkr2$^{-/-}$ x *Ins2*^{Akita}	genetic	x	x	x	((Renal)
Nos3$^{-/-}$	STZ	(x)	-	(x)	(Renal)
Apoe$^{-/-}$(B6) 2mo old	STZ	x	x	x	Renal/Vascular
Apoe$^{-/-}$(B6) 6mo old	STZ	x	x	x	(Renal)/Vascular
Cbs$^{-/-}$*Apoe*$^{-/-}$ (w/o Met)	STZ	x	x	x	(Renal)/Vascular
Cbs$^{-/-}$*Apoe*$^{-/-}$ (w Met)	STZ	x	x	x	(Renal)/Vascular
F_1(DBA/2 x *Ins2*^{Akita})	genetic	x	x	(x)	(Renal)
Ppar$^{P435L/+}$ (129xB6 F1)	STZ	(x)	(x)	(x)	(Renal/Vascular)
129xB6 F1	STZ	(x)	(x)	(x)	(Renal Renal)

Susceptibility Mutation

Model Development

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+STZ

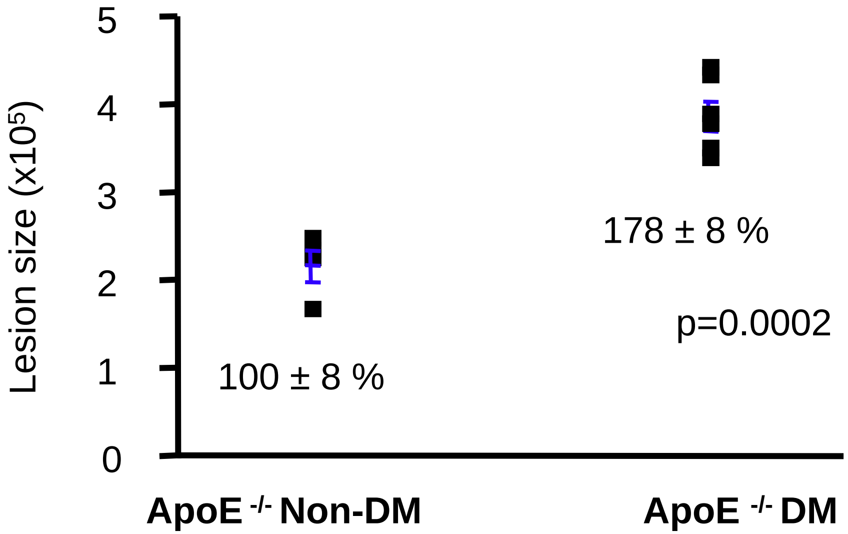
Kidney Phenotype Screen

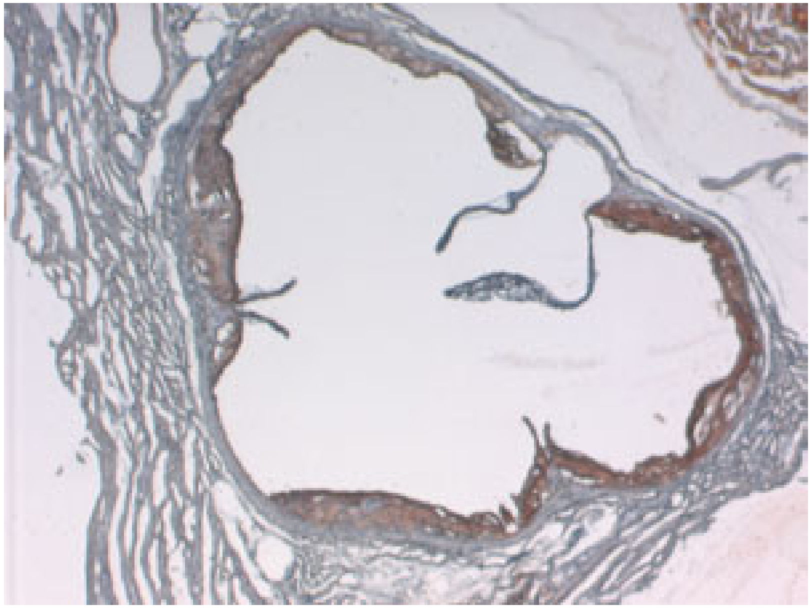
Vascular & Kidney Phenotype Screen

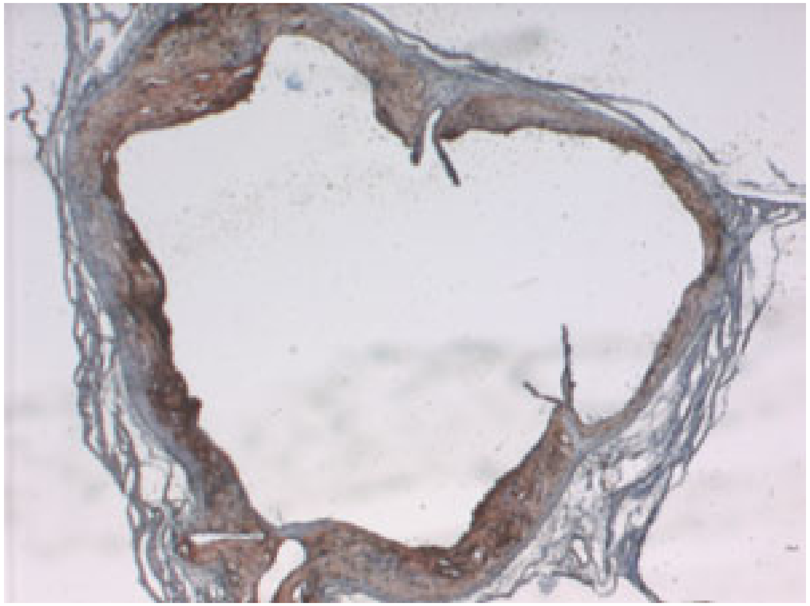
Transfer mutations to diabetic backgrounds

Generate ES Cell Lines From Diabetic Mice

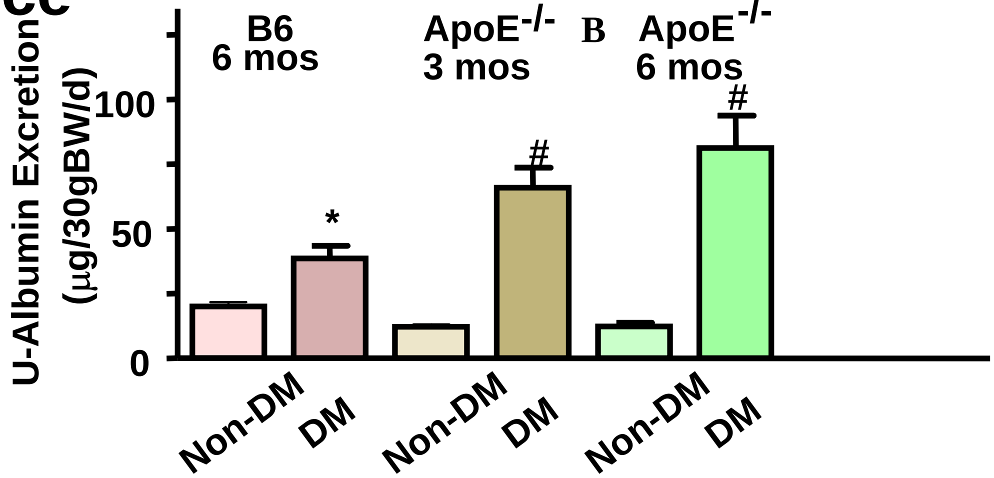
Diabetes exacerbates the atherosclerotic lesion in ApoE-/- mice



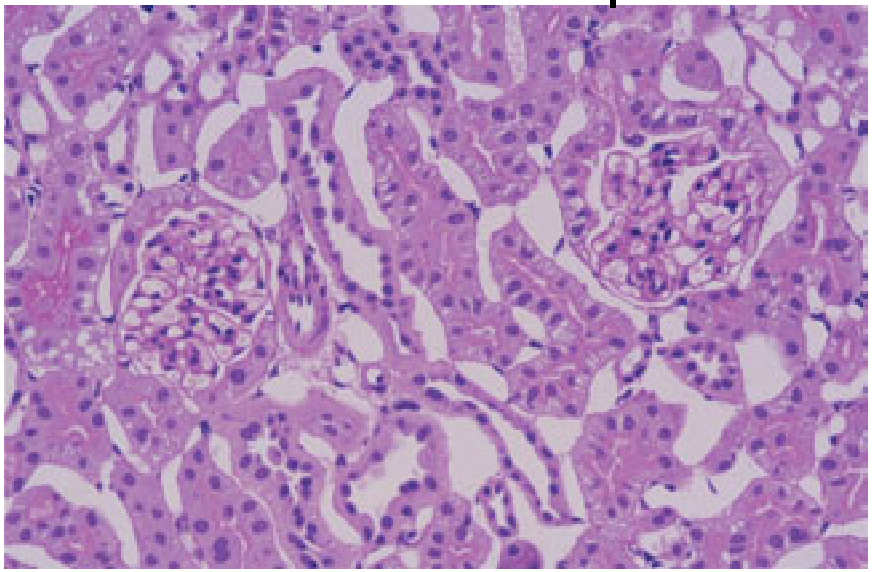




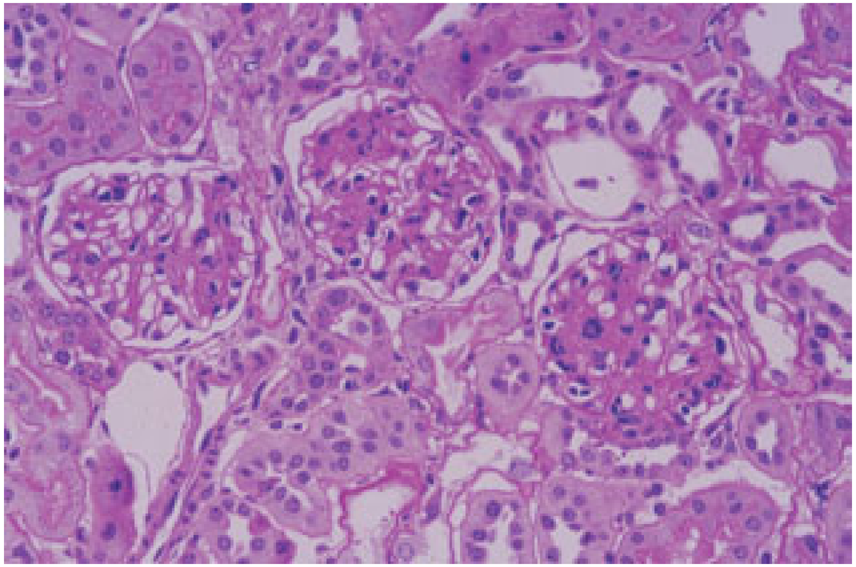
Diabetes exacerbates diabetic nephropathy in ApoE-/- mice

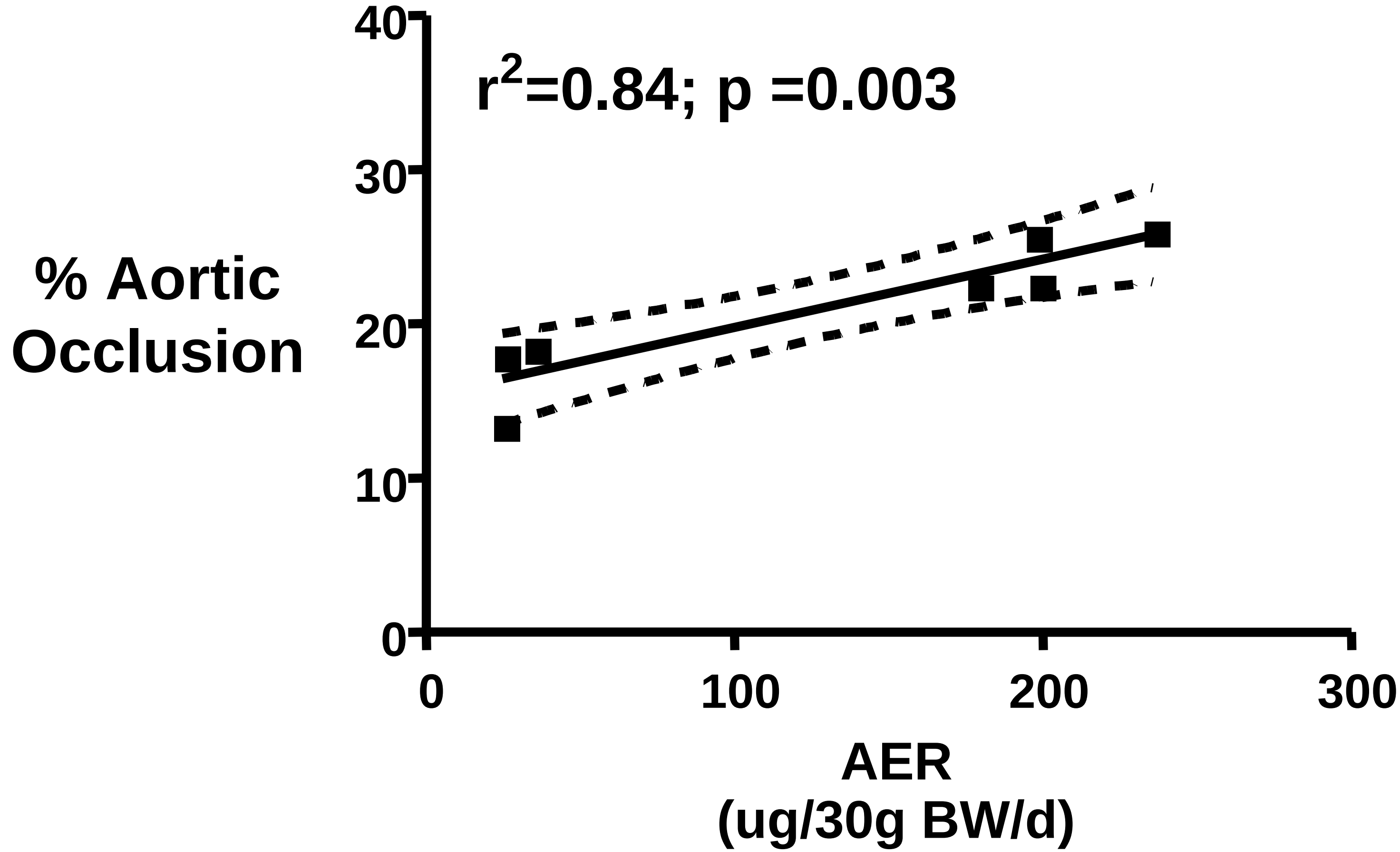


Non-diabetic ApoE-/-

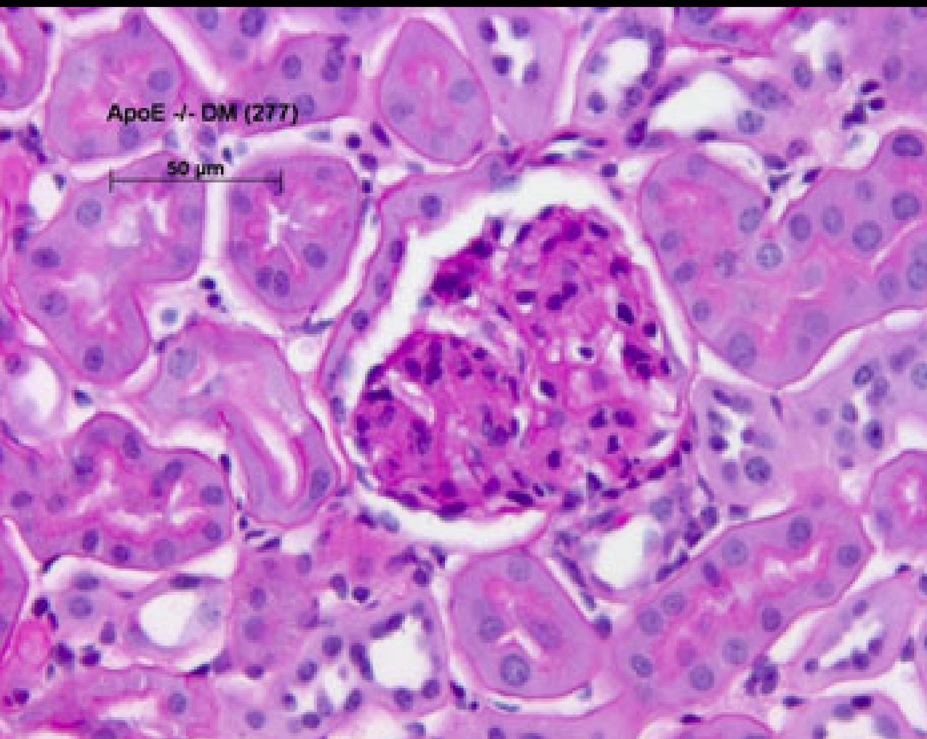
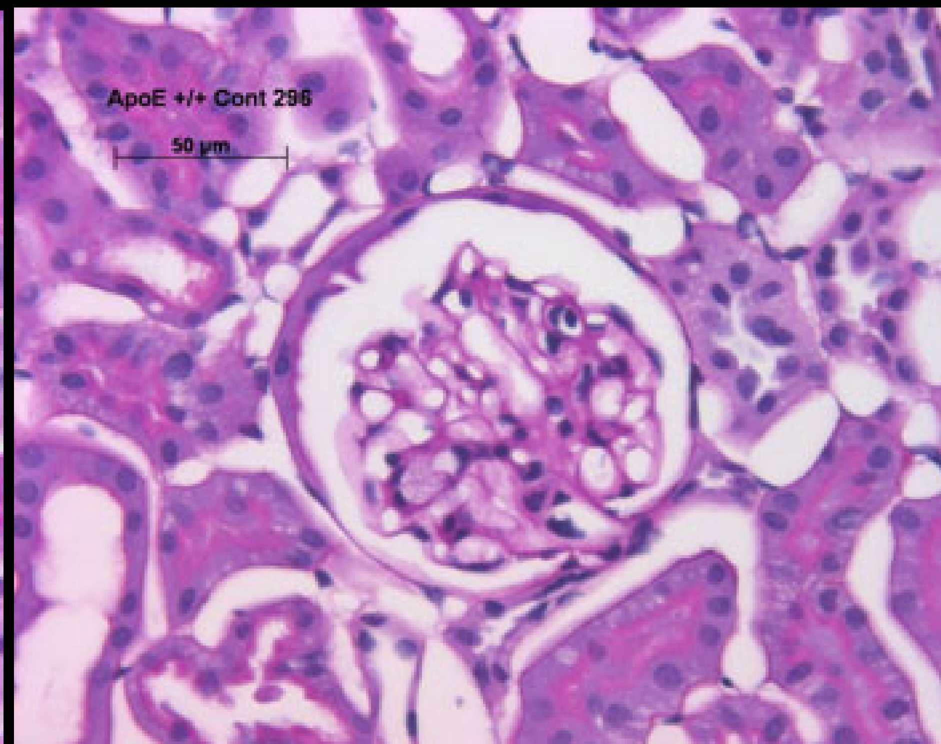


diabetic ApoE-/-

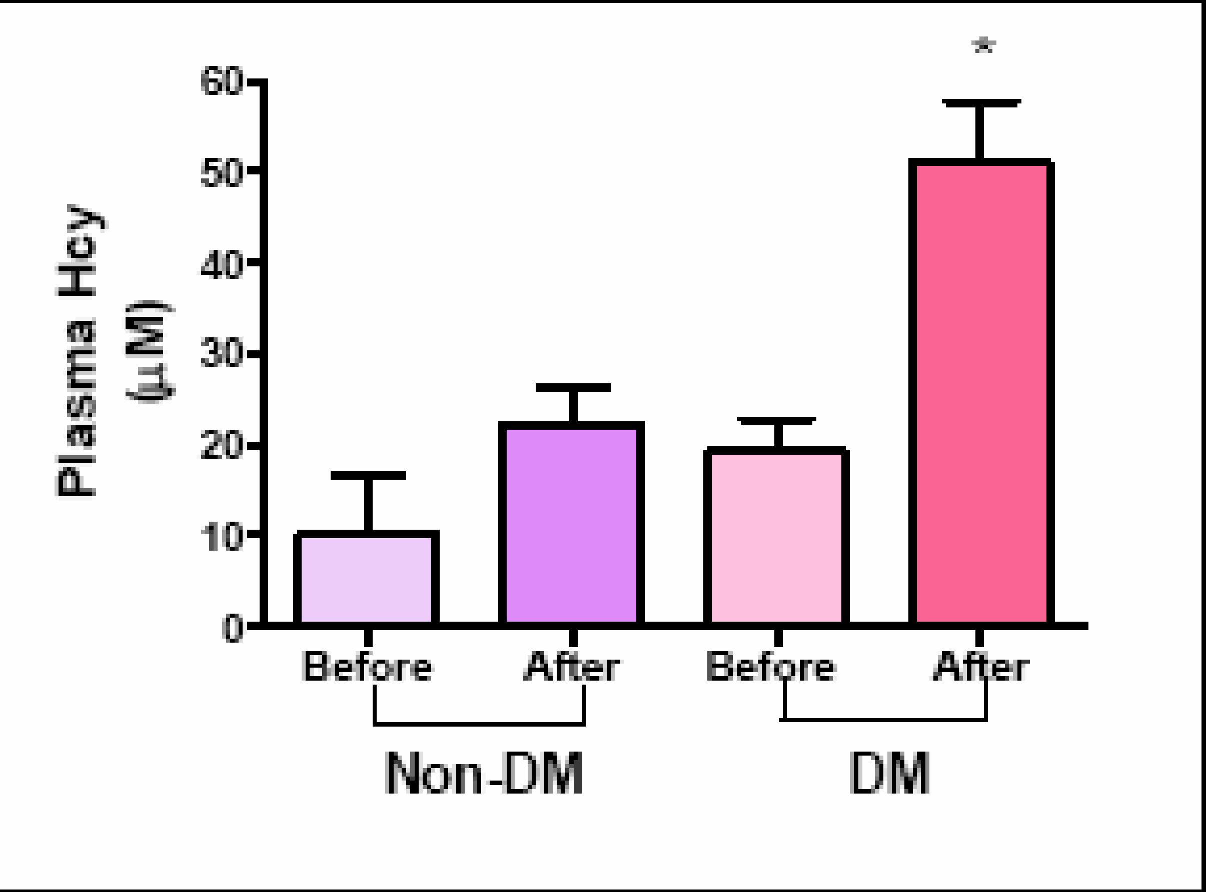




r^2=0.84; p =0.003
% Aortic Occlusion
40
30
20
10
0
0
100
200
300
AER
(ug/30g BW/d)


ApoE -/- DM (277)
50 µm

ApoE +/+ Cont 296
50 µm

-CBS+/-ApoE-/- given STZ
-Supplemented with L-methionine (0.5%) or L-methionine (0.5%)/Folate (200μg/L)



Plasma Hcy levels in apoE-/-CBS+/- Non diabetic (Non-DM) and Diabetic (DM) mice at study initiation (2 months old) and conclusion (8 months old). P<0.05, n=6.

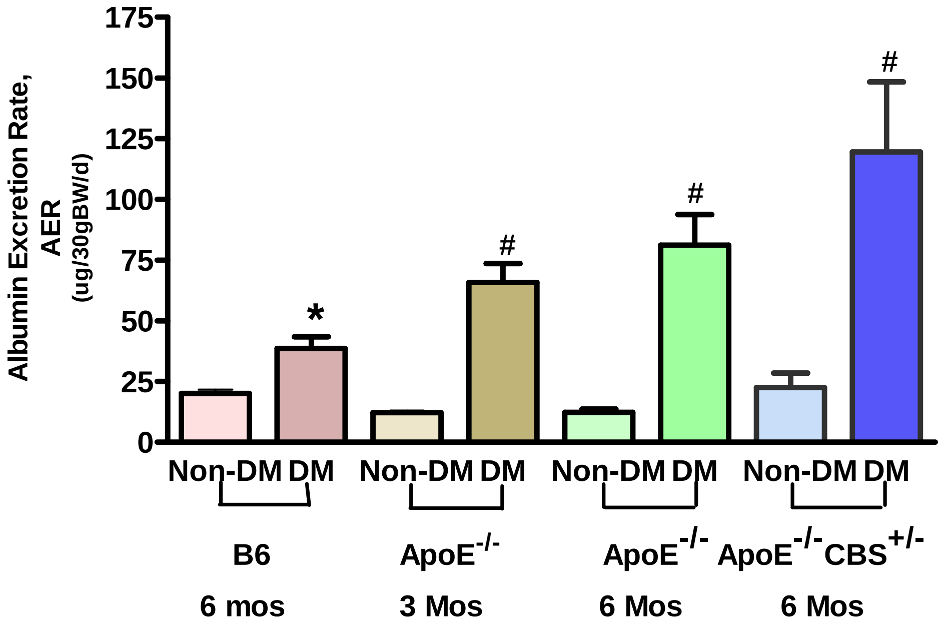
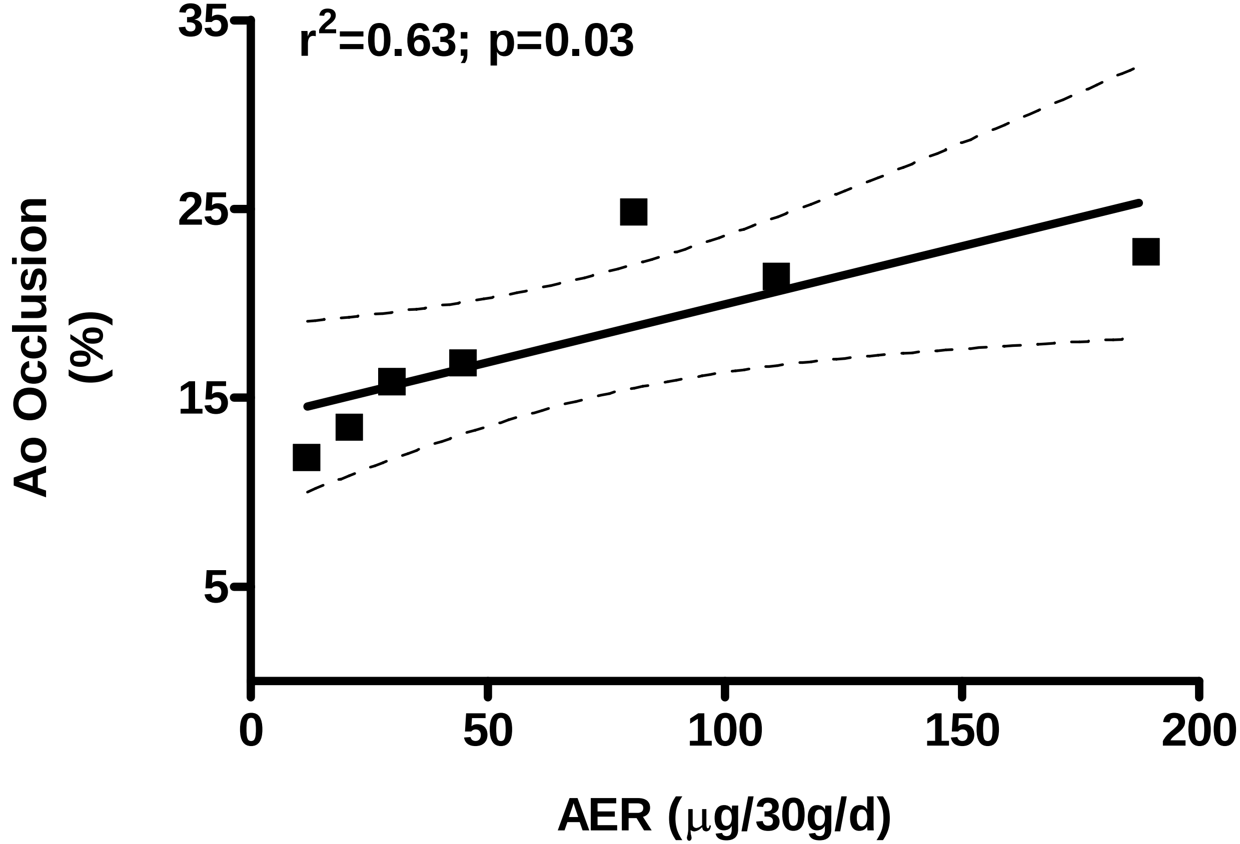


Figure 2. Albumin Excretion rate (AER) at the end of 3 or 6 months



Proximal Aortic occlusion is related to AER in ApoE-/-CBS+/- Mice

New Model Development

- *Ppar*$^{P465L/+}$ mouse line

  (Tsai et al., *J Clin Invest*, 2004)
  - Increased B-cell insulin production
  - Hypertension
  - Abdominal fat distribution
- 3'UTR modification of the CTGF Gene