Northwestern Medicine®

Feinberg School of Medicine Chicago IL
Division of Nephrology and Hypertension

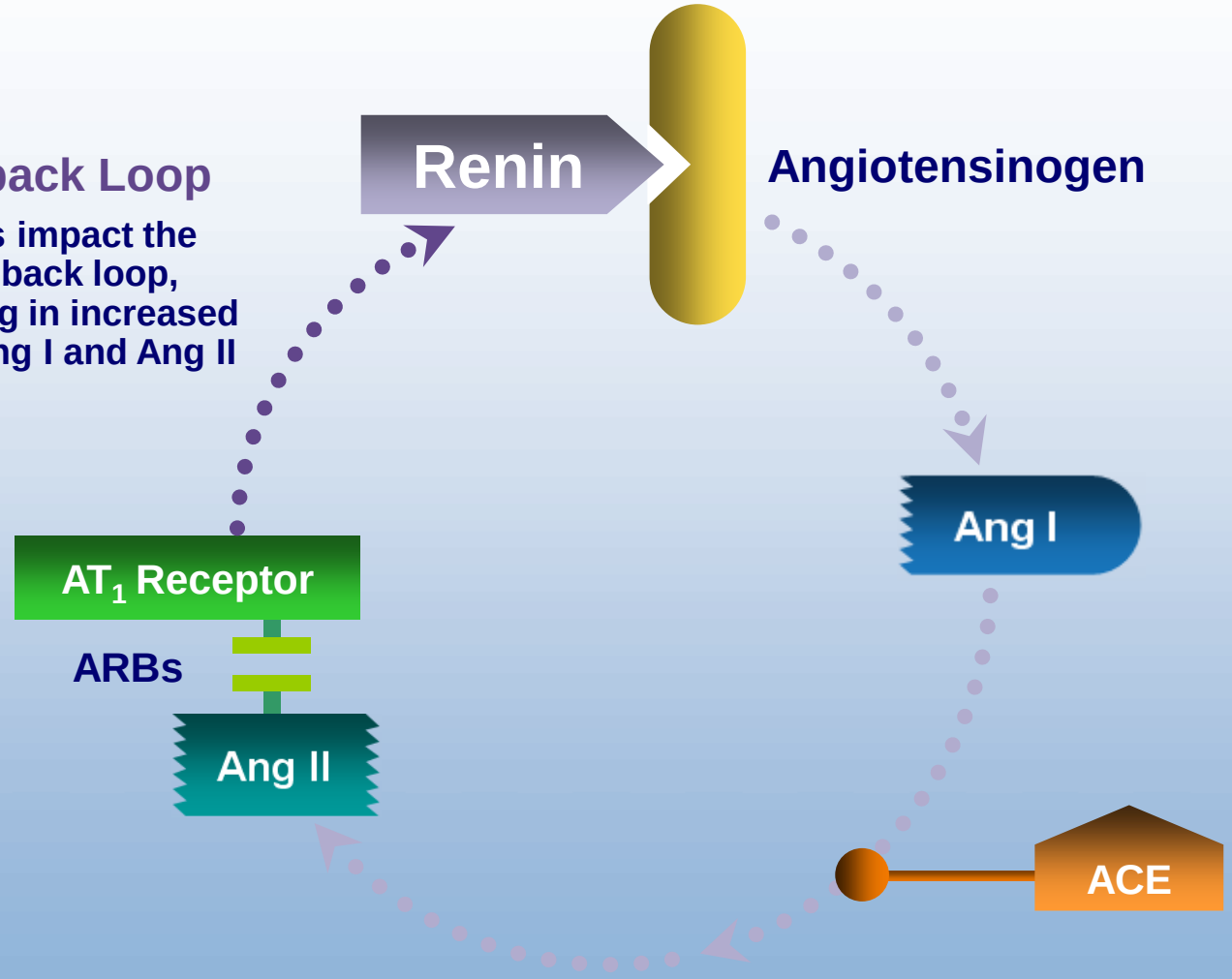
Daniel Batlle MD
Earle, del Greco
Levin Professor of Medicine
Division of Nephrology and Hypertension
Northwestern University Feinberg School of Medicine
Chicago IL

The great refugee: How Laszlo Kubala became a Barcelona legend

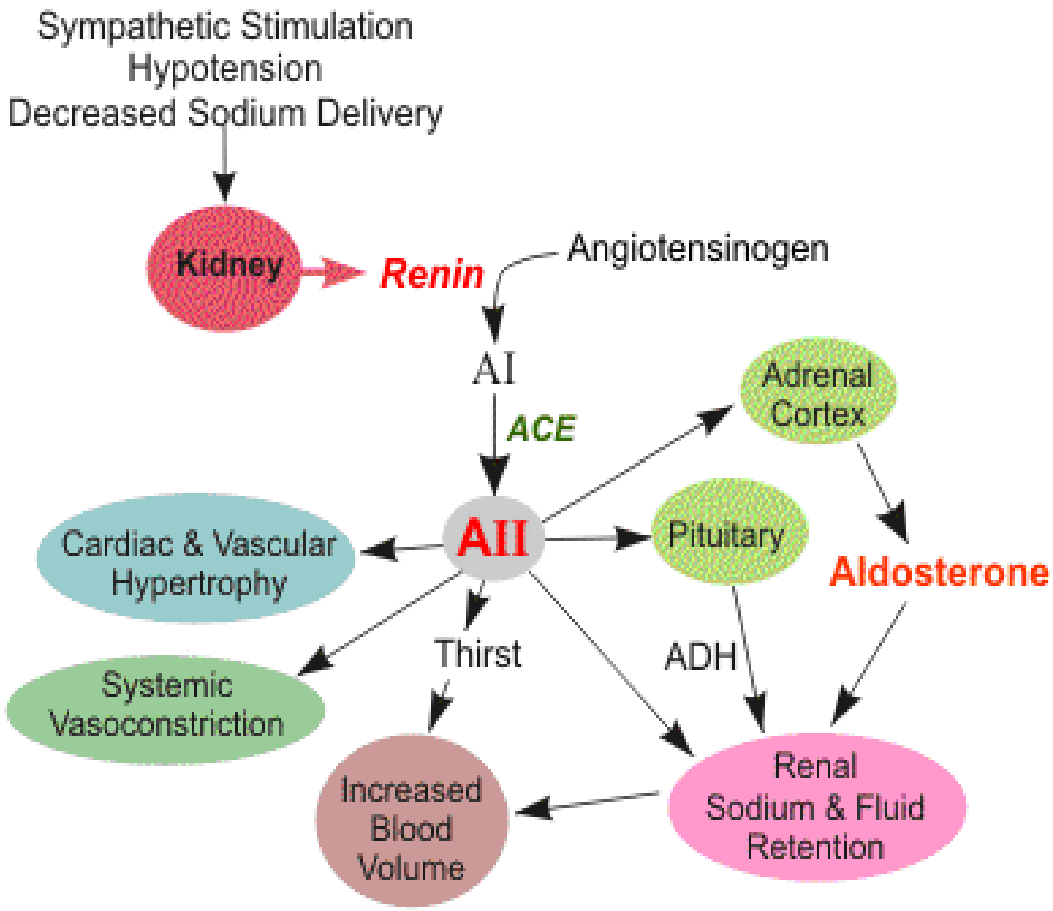
Blockade of the Renin Angiotensin System

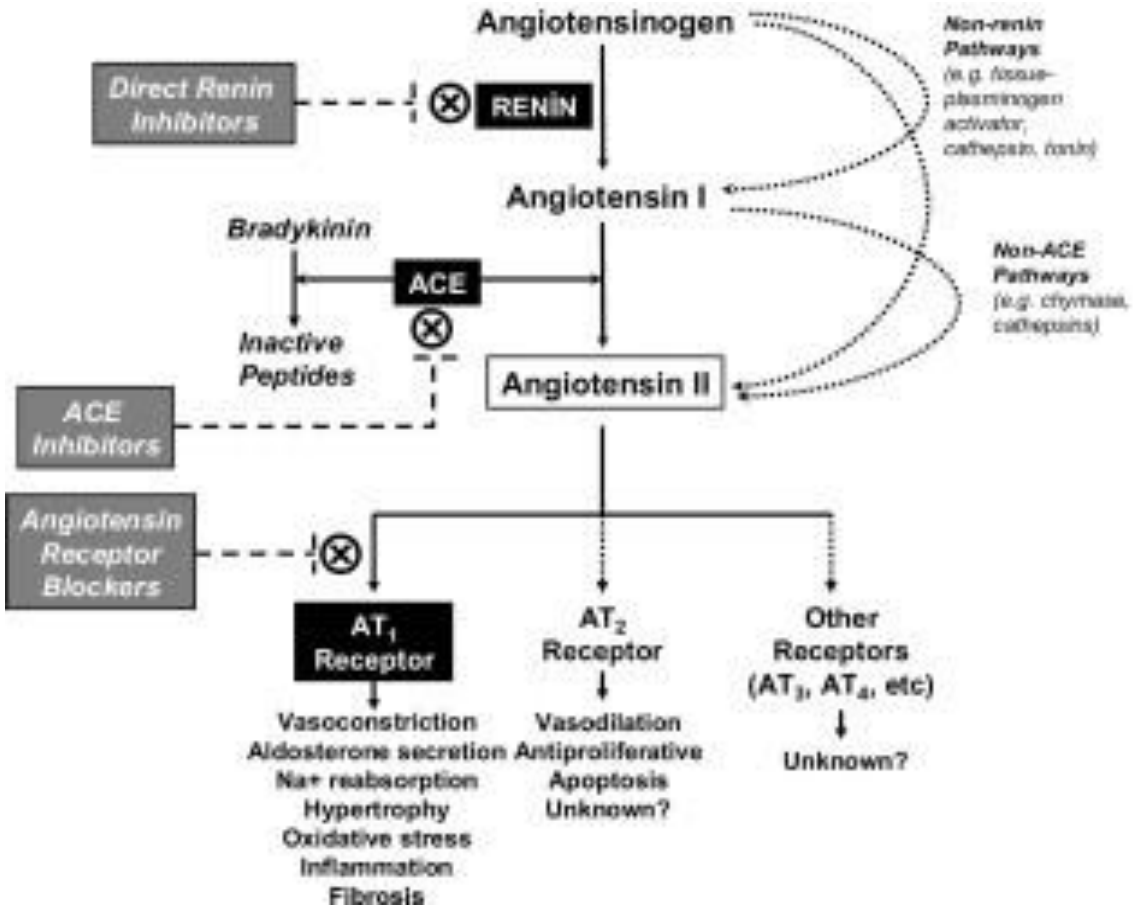
Feedback Loop

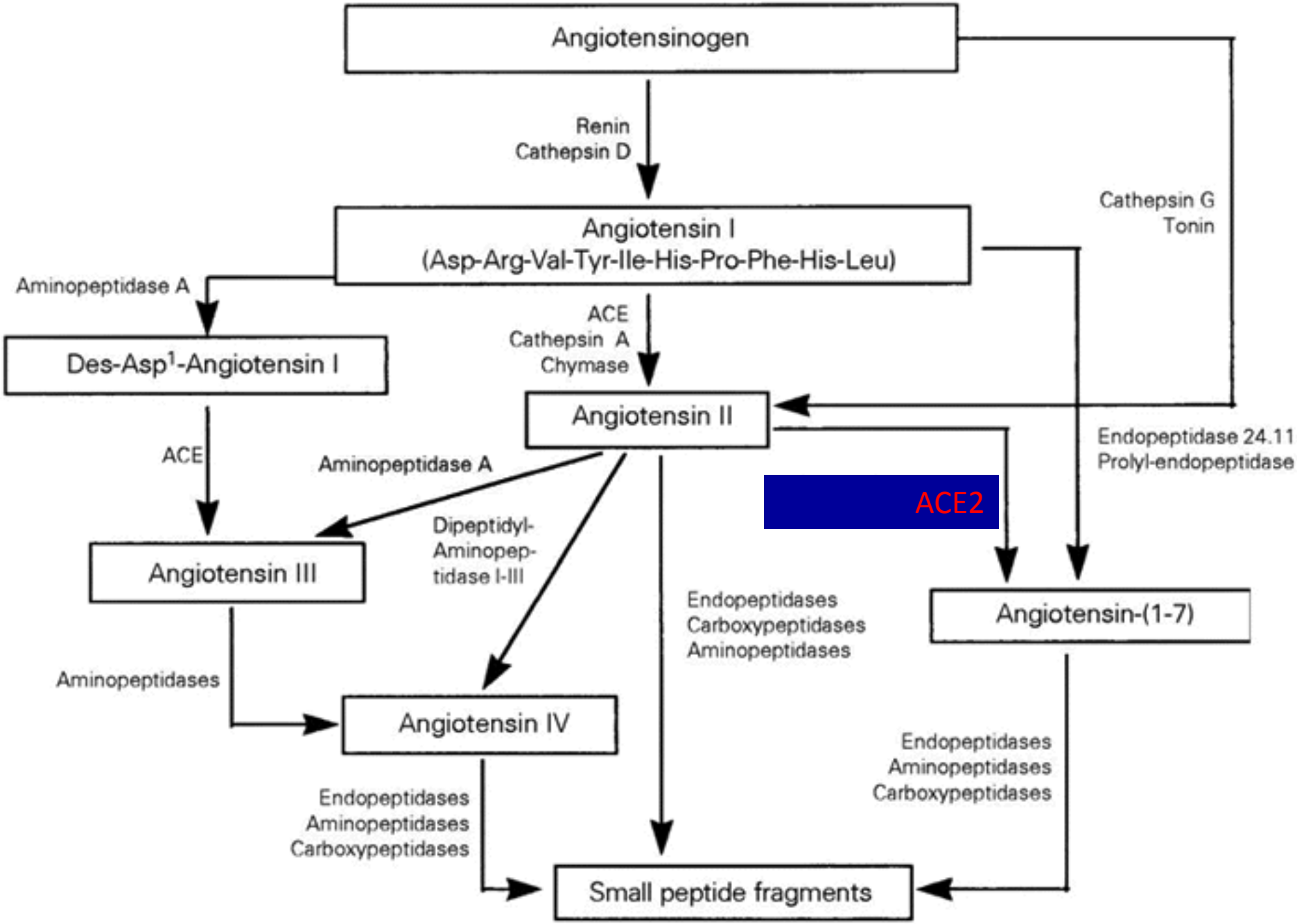
- ARBs impact the feedback loop, resulting in increased PRA, Ang I and Ang II



ARB, angiotensin receptor blocker; PRA, plasma renin activity; Ang, angiotensin; AT_1 Receptor, angiotensin I receptor; ACE, angiotensin-converting enzyme.


Sympathetic Stimulation
Hypotension
Decreased Sodium Delivery
Kidney
Renin
Angiotensinogen
AI
ACE
Adrenal Cortex
Cardiac & Vascular Hypertrophy
AII
Pituitary
Aldosterone
Thirst
ADH
Systemic Vasoconstriction
Increased Blood Volume
Renal Sodium & Fluid Retention


Angiotensinogen
Direct Renin Inhibitors
RENIN
Non-renin Pathways
(e.g. tissue-plasminogen activator, cathepsin, tonin)
Angiotensin I
Bradykinin
ACE
Inactive Peptides
Non-ACE Pathways
(e.g. chymase, cathepsins)
Angiotensin II
ACE Inhibitors
Angiotensin Receptor Blockers
AT1 Receptor
AT2 Receptor
Other Receptors (AT3, AT4, etc)
Vasoconstriction
Aldosterone secretion
Na+ reabsorption
Hypertrophy
Oxidative stress
Inflammation
Fibrosis
Vasodilation
Antiproliferative
Apoptosis
Unknown?
Unknown?


Angiotensinogen
Renin
Cathepsin D
Cathepsin G
Tonin
Angiotensin I
(Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu)
Aminopeptidase A
Des-Asp1-Angiotensin I
ACE
Cathepsin A
Chymase
Angiotensin II
ACE
Aminopeptidase A
Endopeptidase 24.11
Prolyl-endopeptidase
ACE2
Dipeptidyl-
Aminopep-
tidase I-III
Angiotensin III
Endopeptidases
Carboxypeptidases
Aminopeptidases
Angiotensin-(1-7)
Aminopeptidases
Angiotensin IV
Endopeptidases
Aminopeptidases
Carboxypeptidases
Endopeptidases
Aminopeptidases
Carboxypeptidases
Small peptide fragments

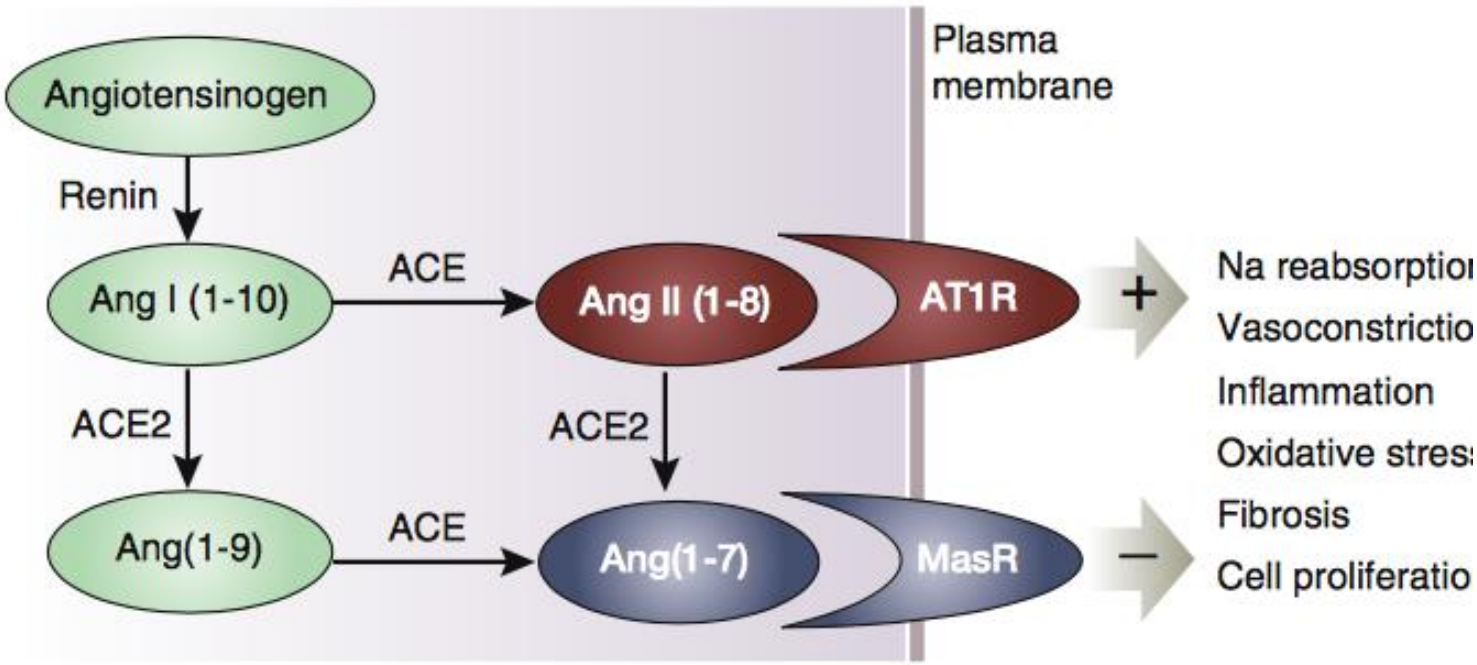
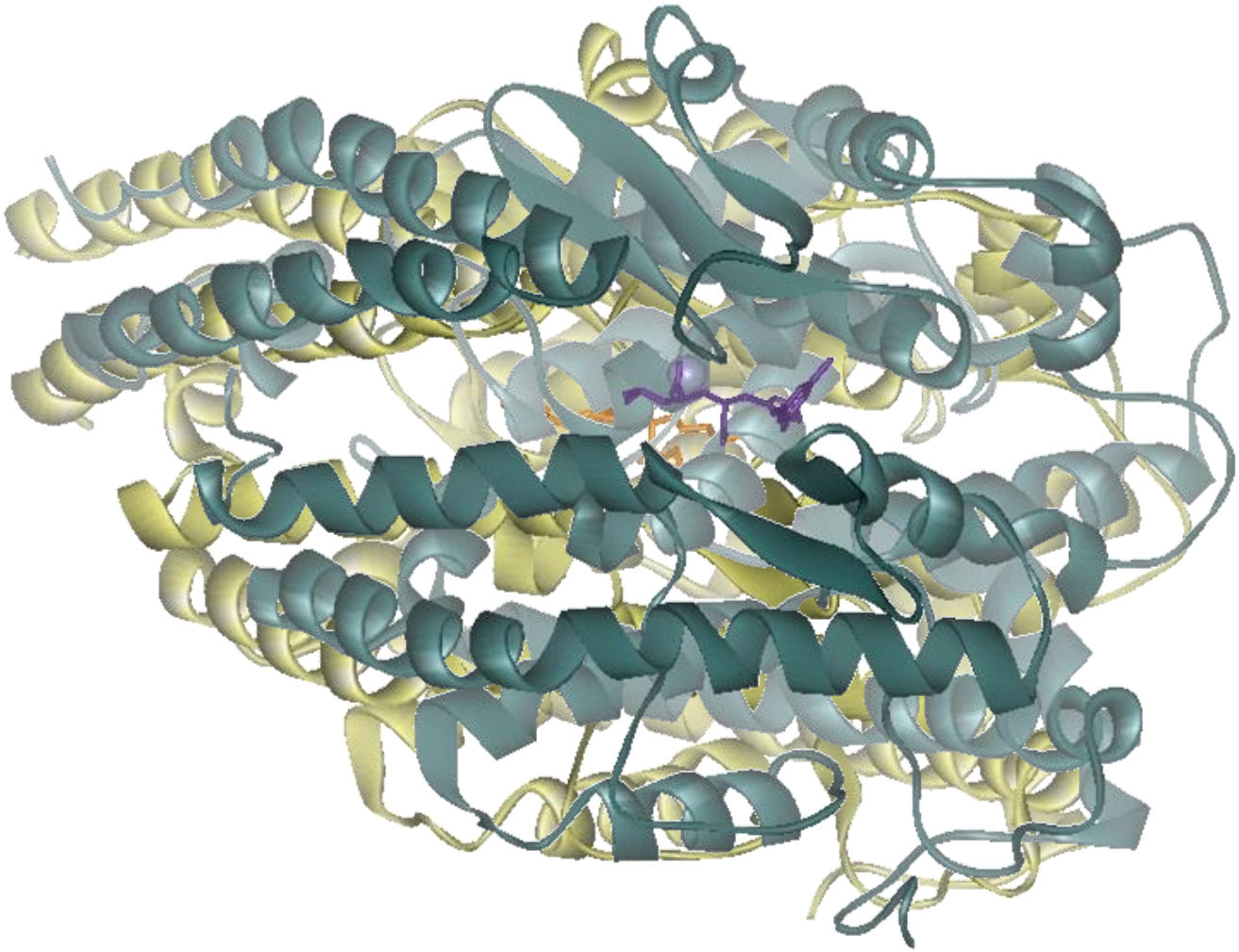


Figure 1 | Schematic representation summarizing important sites of action of angiotensin-converting enzyme (ACE) and ACE2.

Structural Conservation: ACE-ACE2



Monika Rella, Richard Jackson, Tony Turner

Amino acid sequences and ACE/ACE2 cleavage sites of RAS mediator peptides

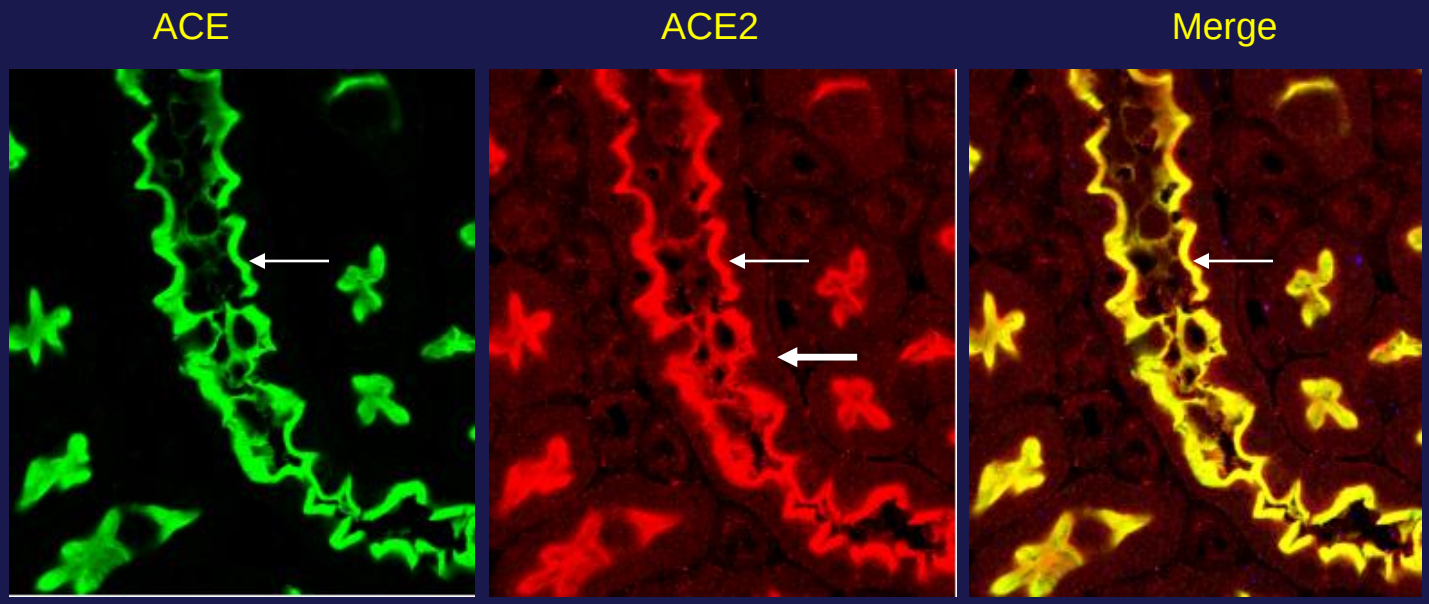
Angiotensin I	Asp Arg Val Tyr Ile His Pro Phe His Leu
Angiotensin II	Asp Arg Val Tyr Ile His Pro Phe
Angiotensin III	Arg Val Tyr Ile His Pro Phe
Angiotensin IV	Val Tyr Ile His Pro Phe
Angiotensin(1–7)	Asp Arg Val Tyr Ile His Pro
Angiotensin(1-–9)	Asp Arg Val Tyr Ile His Pro Phe His
Apelin-13	Gln Arg Pro Arg Leu Ser His Lys Gly Pro Met Pro Phe
Bradykinin	Arg Pro Pro Gly Phe Ser Pro Phe Arg
[Des-Arg]^{9} Bradykinin	Arg Pro Pro Gly Phe Ser Pro Phe
Dynorphin A	Tyr Gly Gly PheLeu Arg Arg Ile Arg Pro Lys Leu Lys
β-Casomorphin	Tyr Pro PheVal Glu Ile

Current Biology

Eriksson et al. Current Biology, Vol. 12, R745–R752, 2002

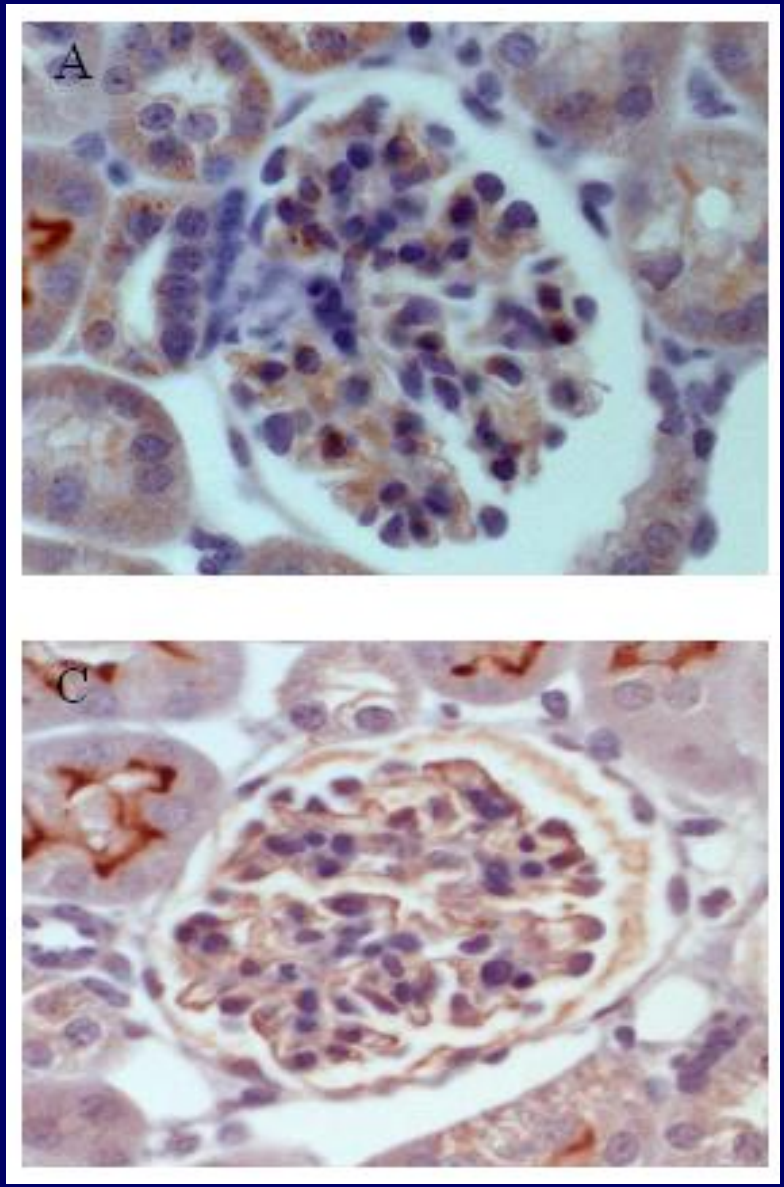
Objectives

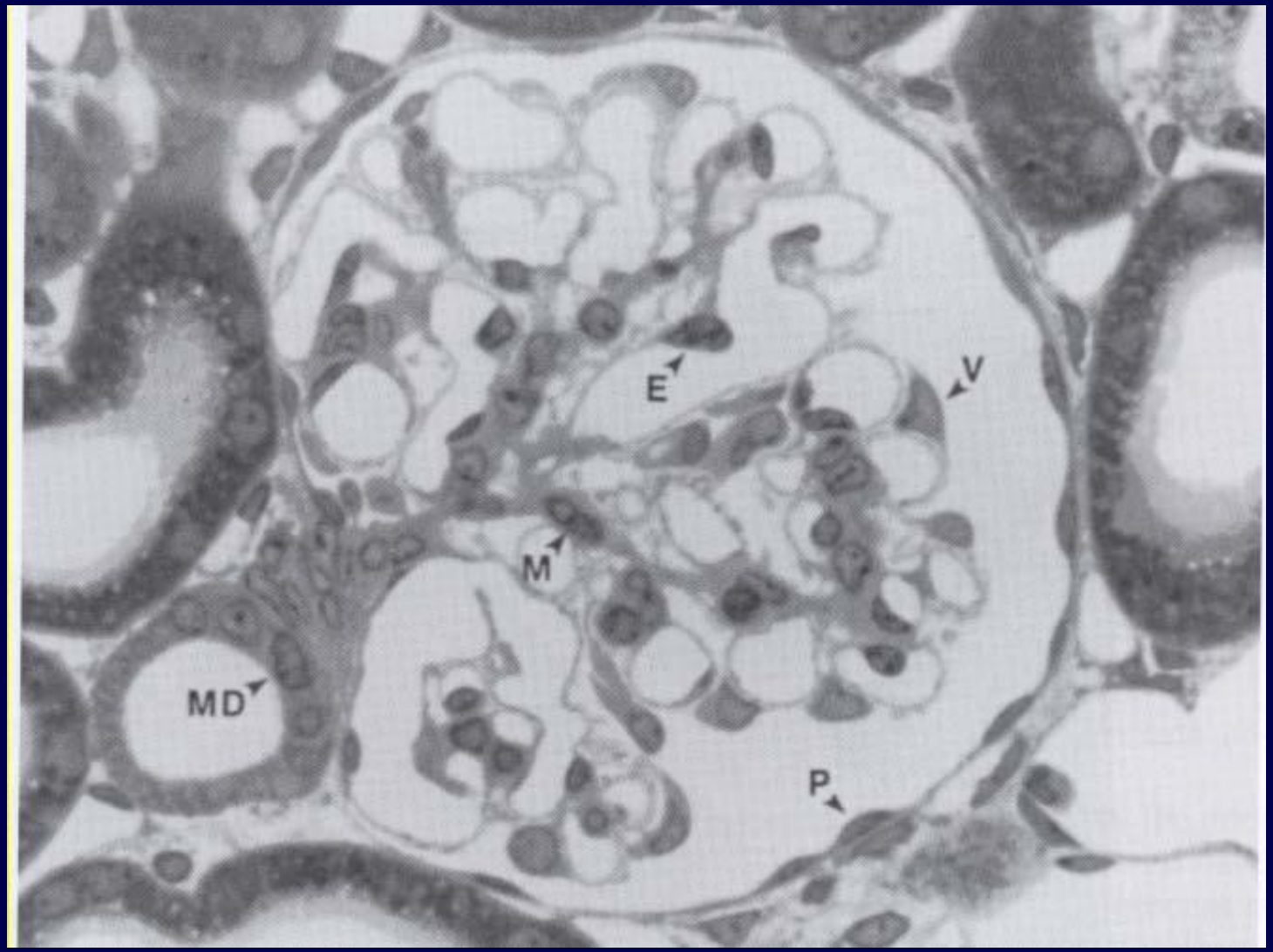
- ACE2 localization within the kidney
- Alteration in ACE2 expression in mouse models of diabetic kidney disease.
- Impact of ACE2 inhibition and genetic ablation on diabetic kidney disease.
- Effect of recombinant ACE2 on blood pressure and Angiotensin II metabolism.
- Potential therapeutic effect of ACE2 amplification.


ACE
ACE2
Merge

ACE

ACE2

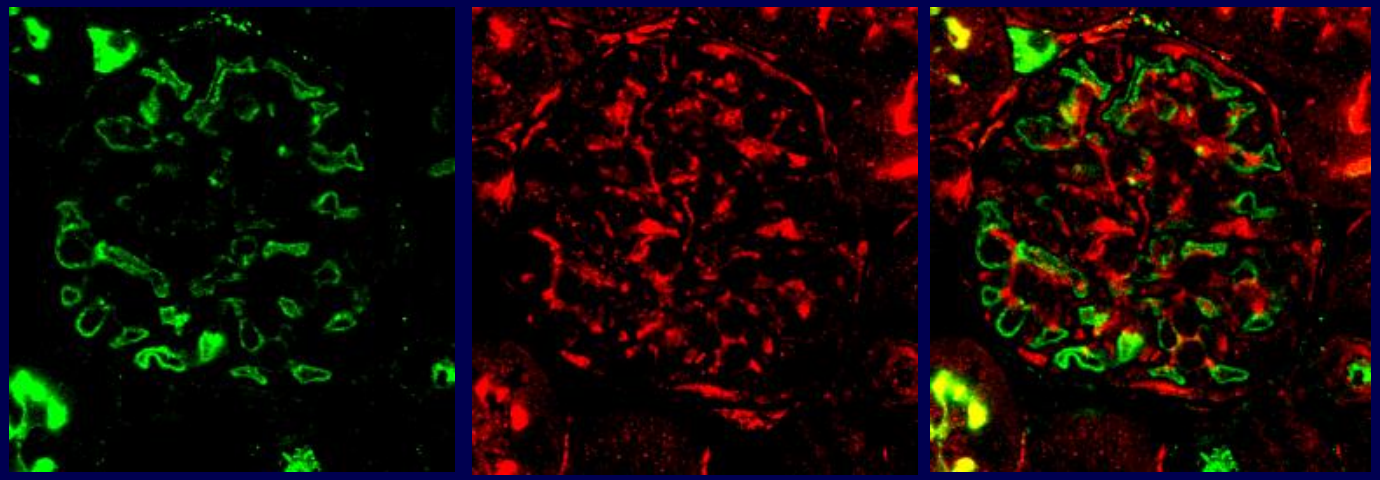



E
V
M
MD
P

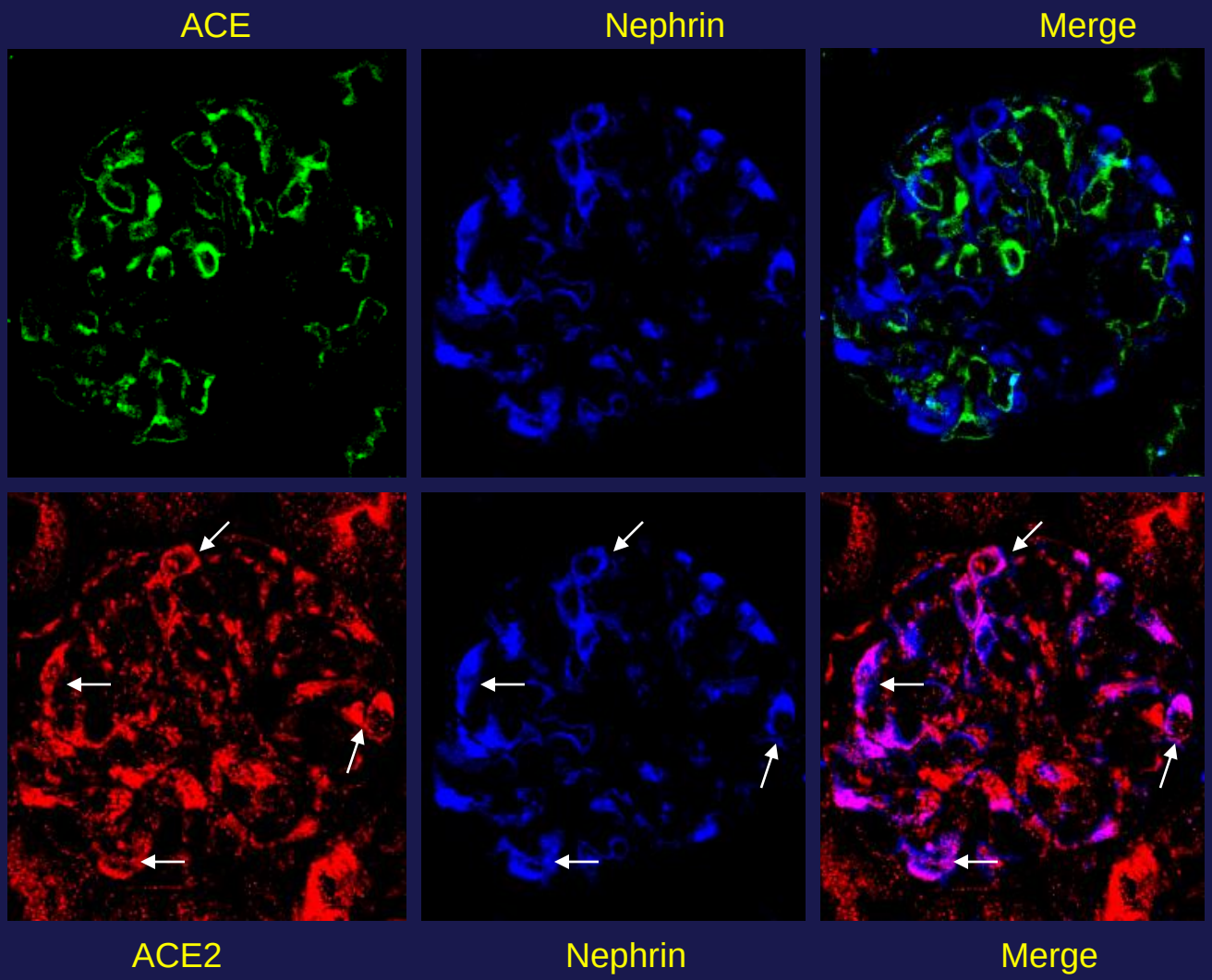
Glomerular Cell Markers

	ACE2	ACE
Podocyte		
Nephrin	+++	-
Podocin	++	-
Synaptopodin	++	-
Endothelial Cell		
Pecam-1	+/-	+++
Mesangial Cell		
α-smooth muscle actin	++	+

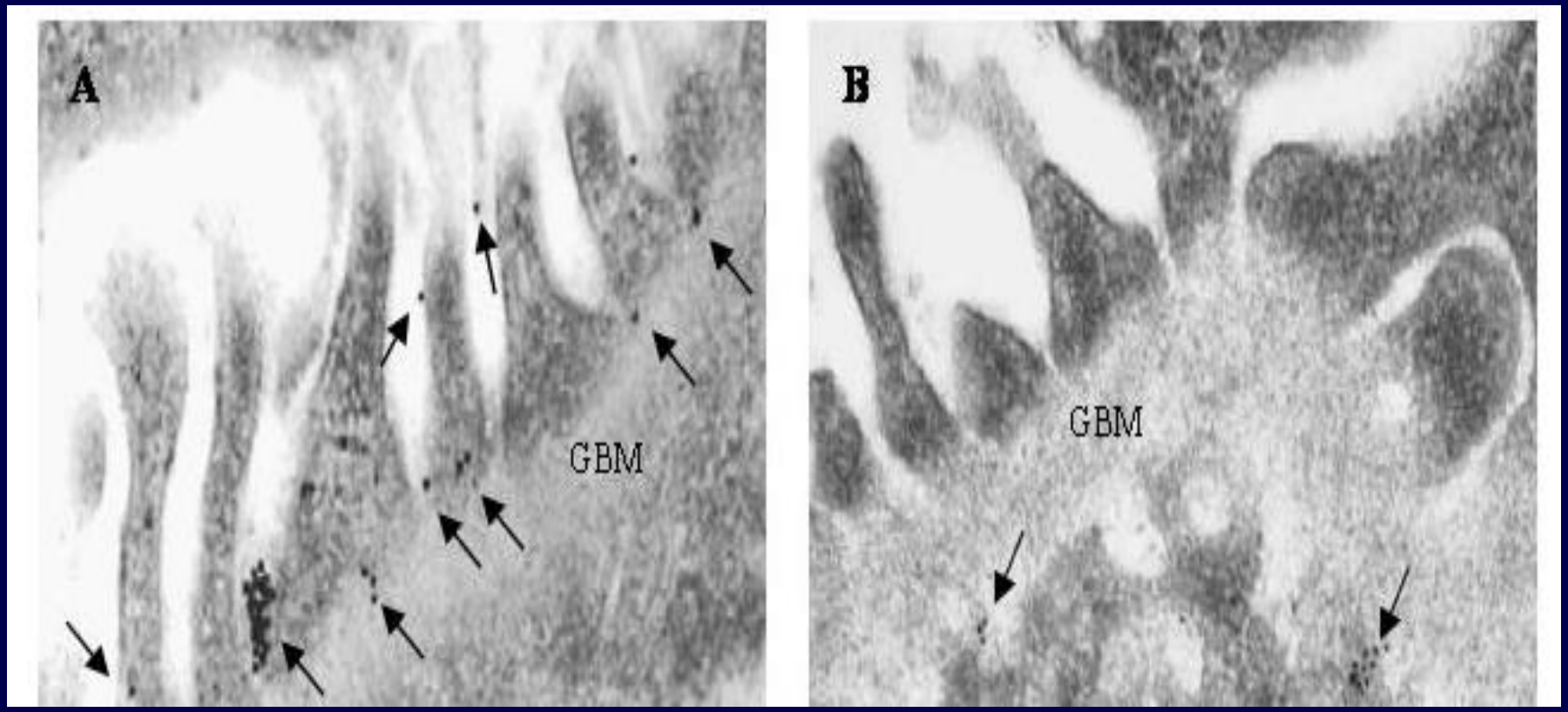
ACE ACE2 Merge



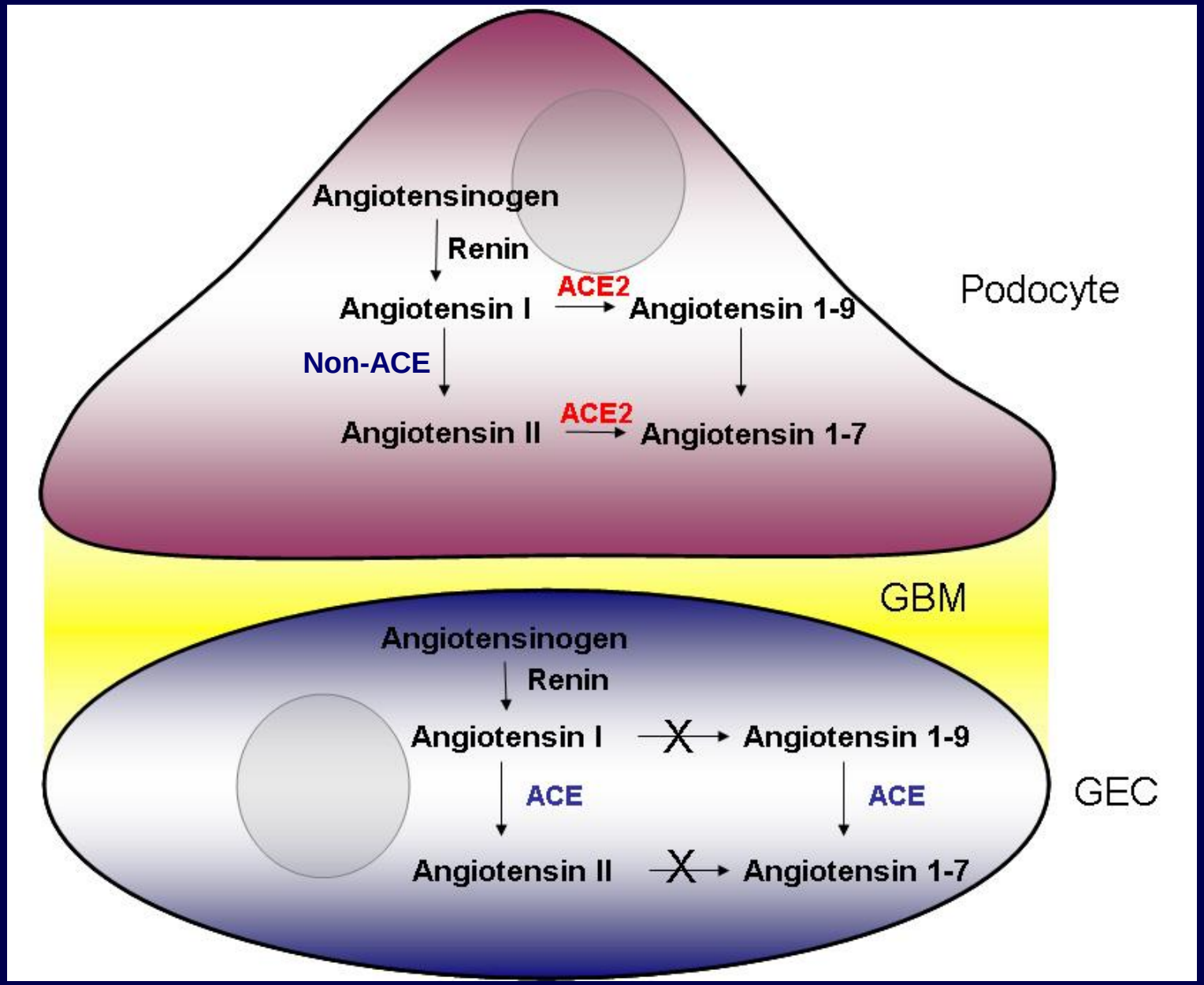
Ye et al. JASN 2006


ACE
Nephrin
Merge
ACE2
Nephrin
Merge

ACE2 ACE



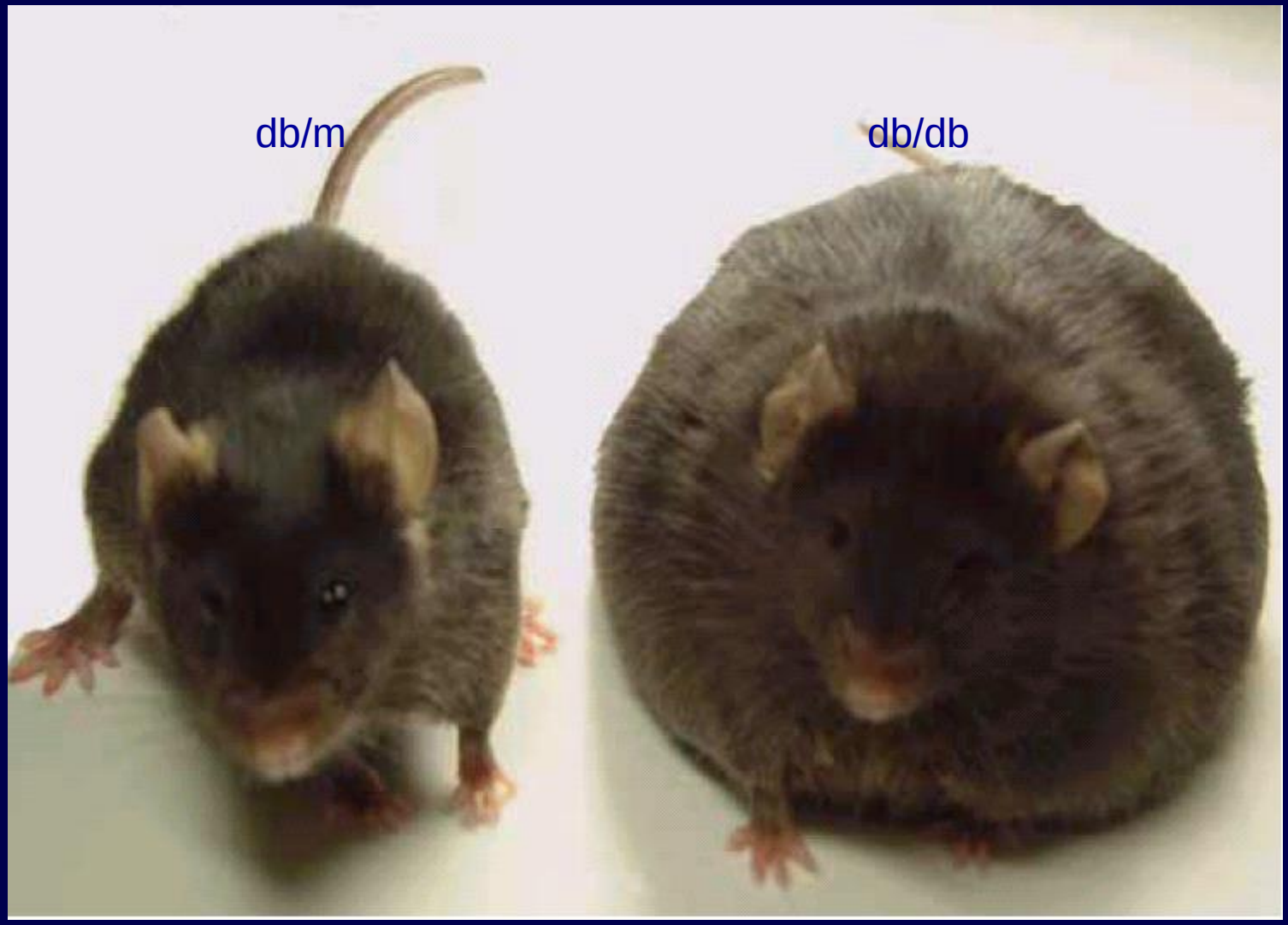
Ye et al JASN 2006



Batlle D, Soler MJ, Wysocki J,
Curr Opinion Nephrol and Hypertension2008

Objectives

- ACE2 localization within the kidney
- Alteration in ACE2 expression in mouse models of diabetic kidney disease.
- Impact of ACE2 inhibition and genetic ablation on diabetic kidney disease.
- Effect of recombinant ACE2 on blood pressure and Angiotensin II metabolism.
- Potential therapeutic effect of ACE2 amplification.

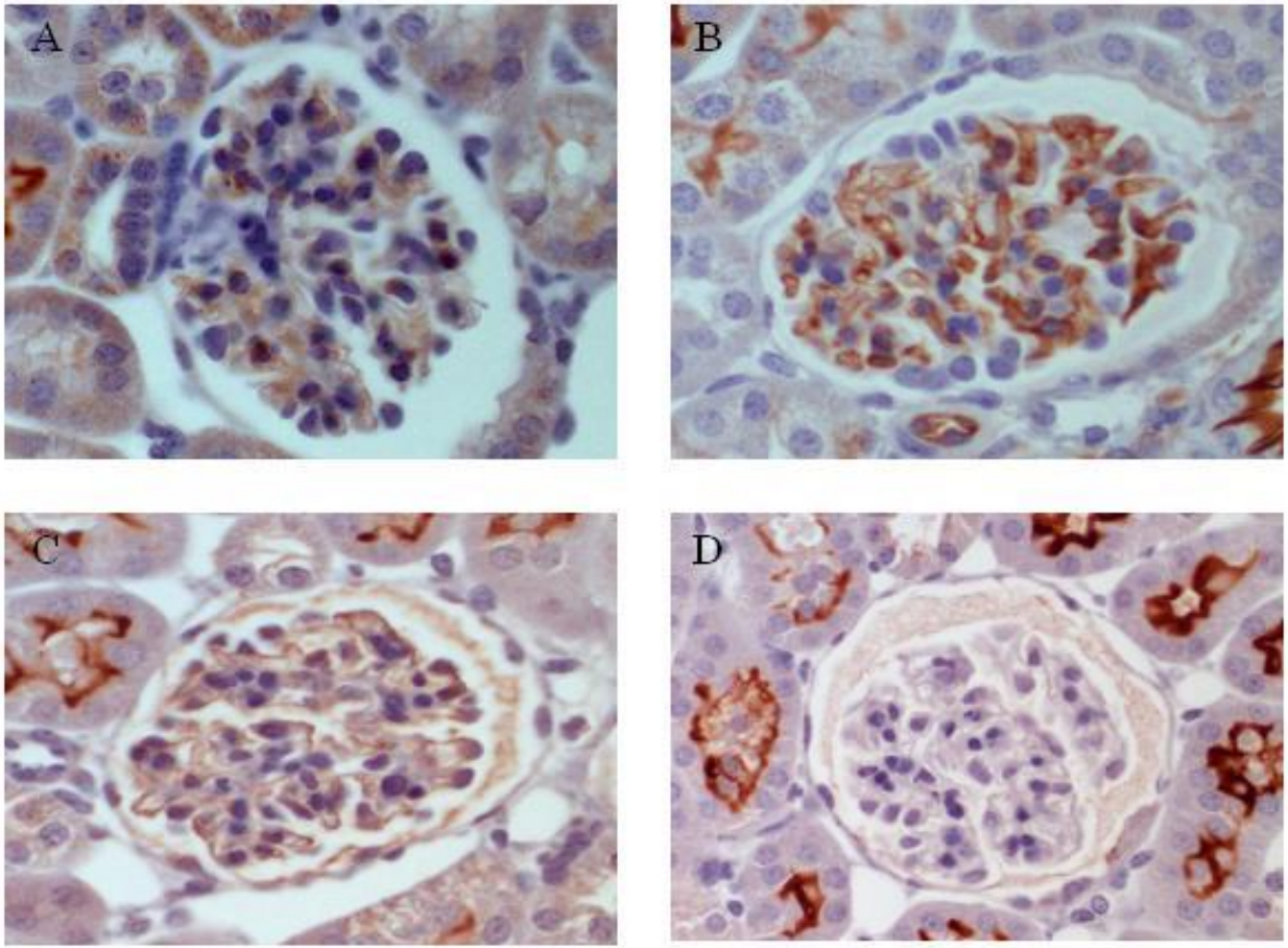

db/m
db/db

Control

Diabetic db/db

ACE

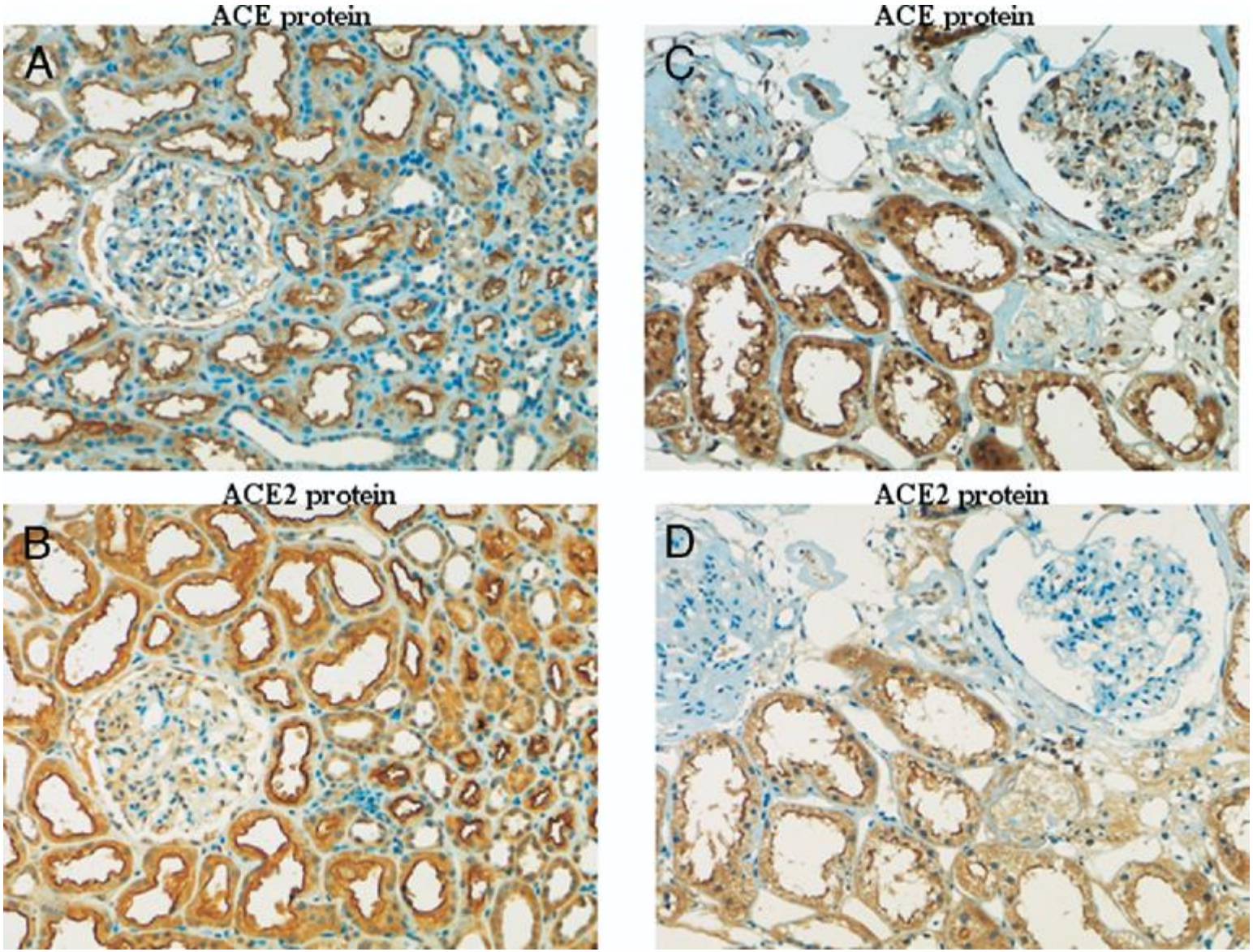
ACE2



Ye et al. JASN 2006

Control

Diabetic nephropathy



Mizuiri S Am J Kidney disease 2008

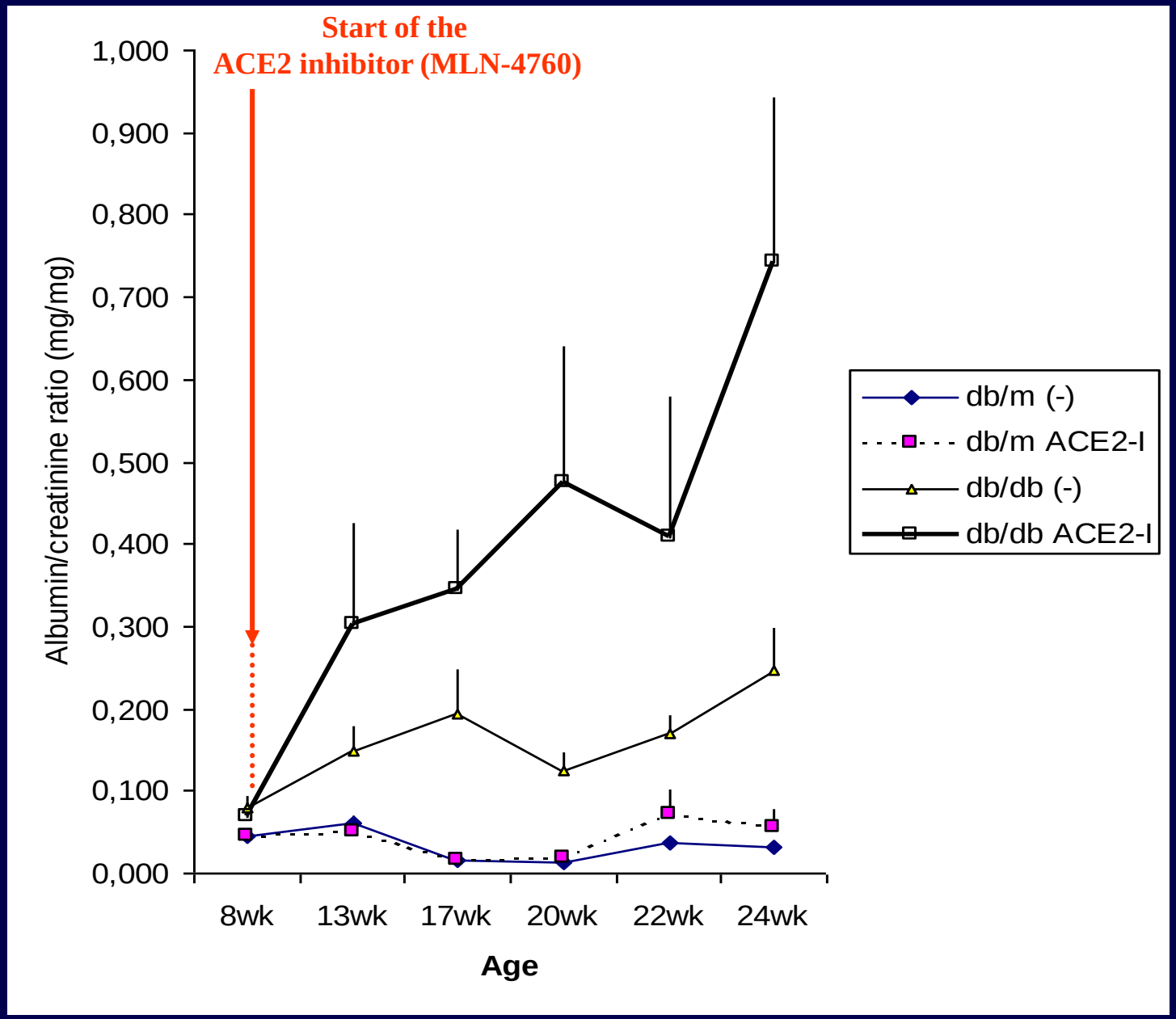
HYPOTHESIS

Glomerular Expression

$$\frac{\text{High ACE}}{\text{Low ACE2}} = \uparrow \text{ANG II} = \text{Albuminuria}$$

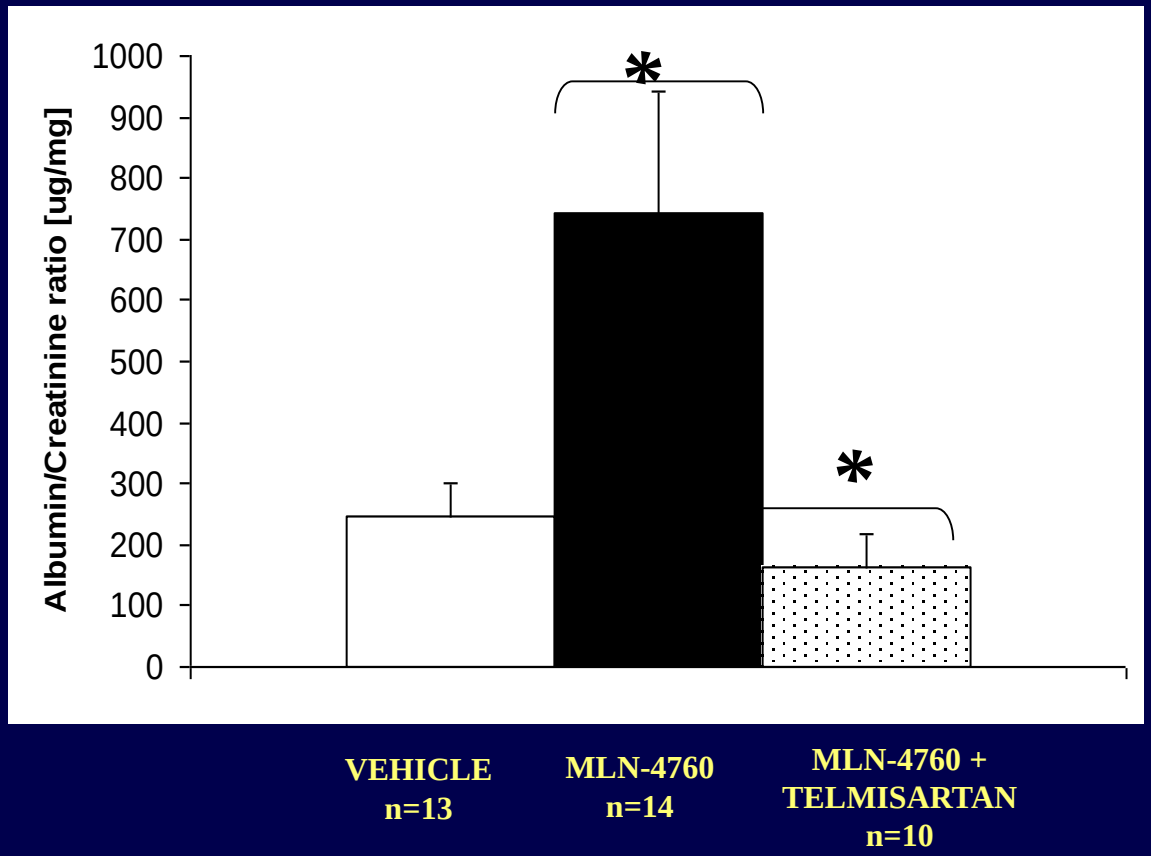
Objectives

- ACE2 localization within the kidney
- Alteration in ACE2 expression in mouse models of diabetic kidney disease.
- Impact of ACE2 inhibition pharmacologically and genetic ablation on diabetic kidney disease.
- Effect of recombinant ACE2 on blood pressure and Angiotensin II metabolism.
- Potential therapeutic effect of ACE2 amplification.


Start of the
ACE2 inhibitor (MLN-4760)
1,000
0,900
0,800
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
Albumin/creatinine ratio (mg/mg)
8wk
13wk
17wk
20wk
22wk
24wk
Age
db/m (-)
db/m ACE2-I
db/db (-)
db/db ACE2-I

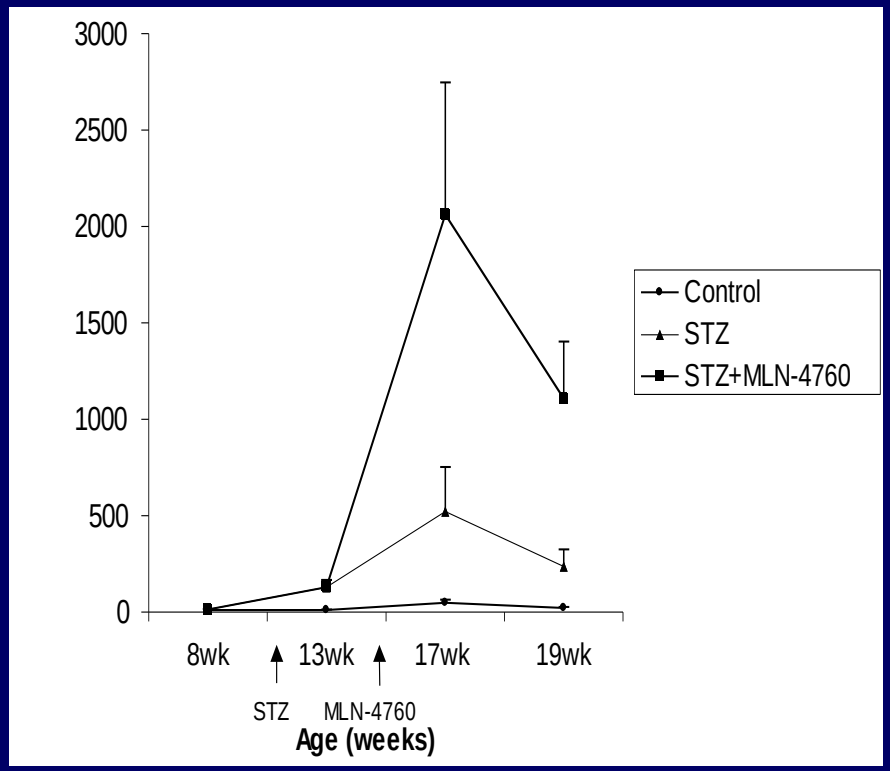
Ye et al 2006

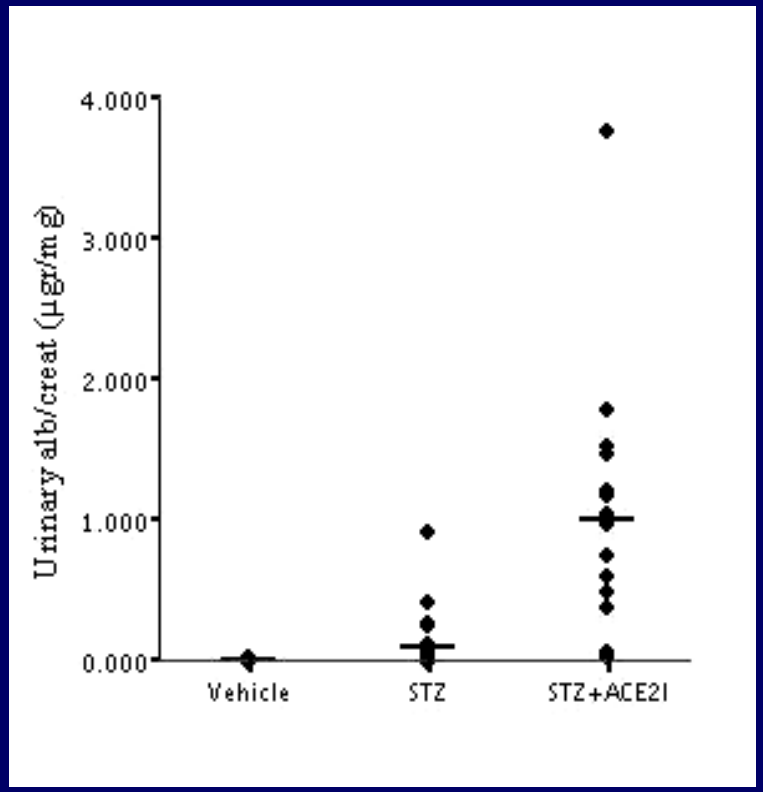
Rescue of effect of ACE2 inhibition (MLN 4760) on albuminuria by Angiotensin II blockade(Telmisartan)



Ye et al 2006

Effect of ACE2 inhibition (MLN4760) on albuminuria in Streptozotocin (STZ) model

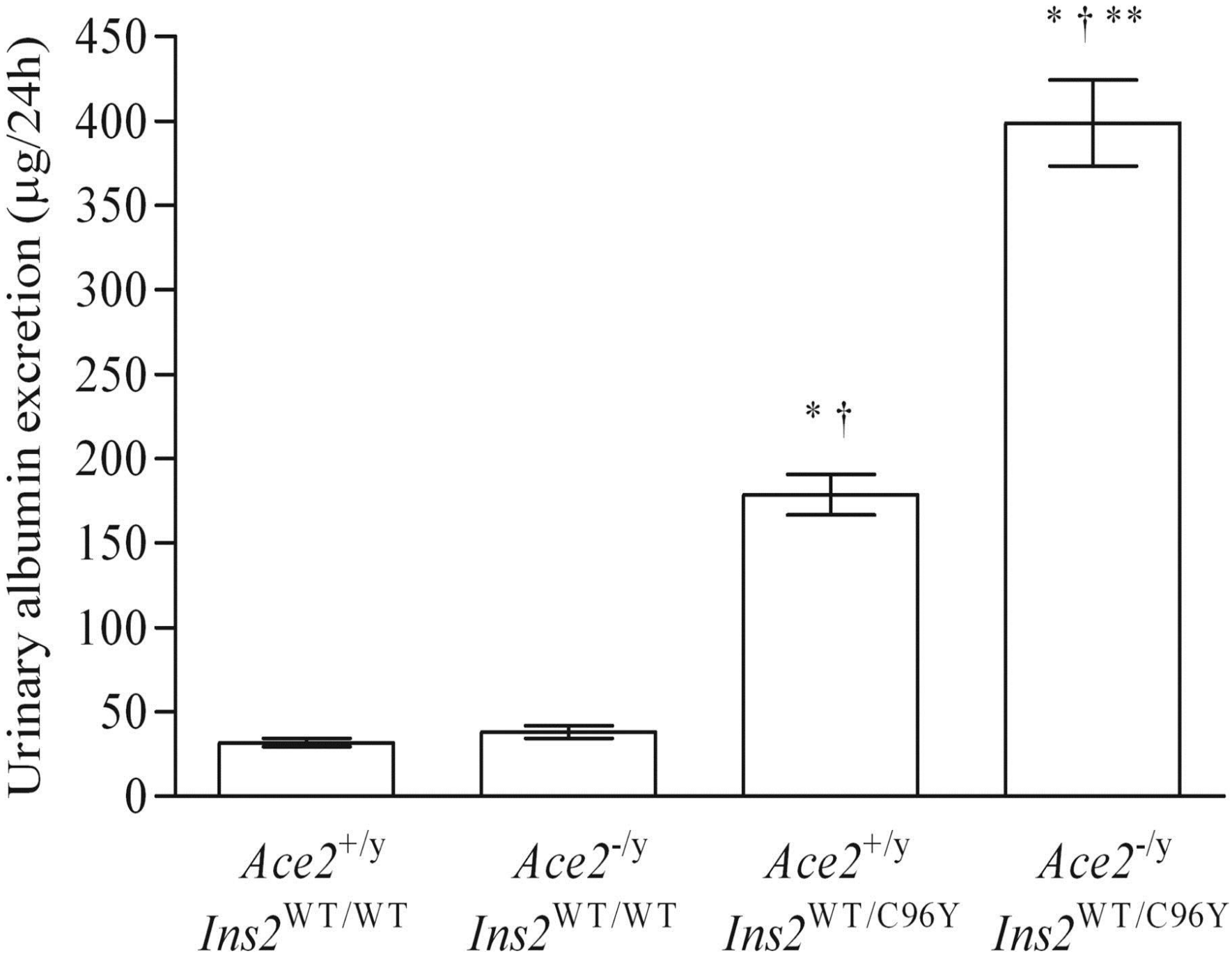




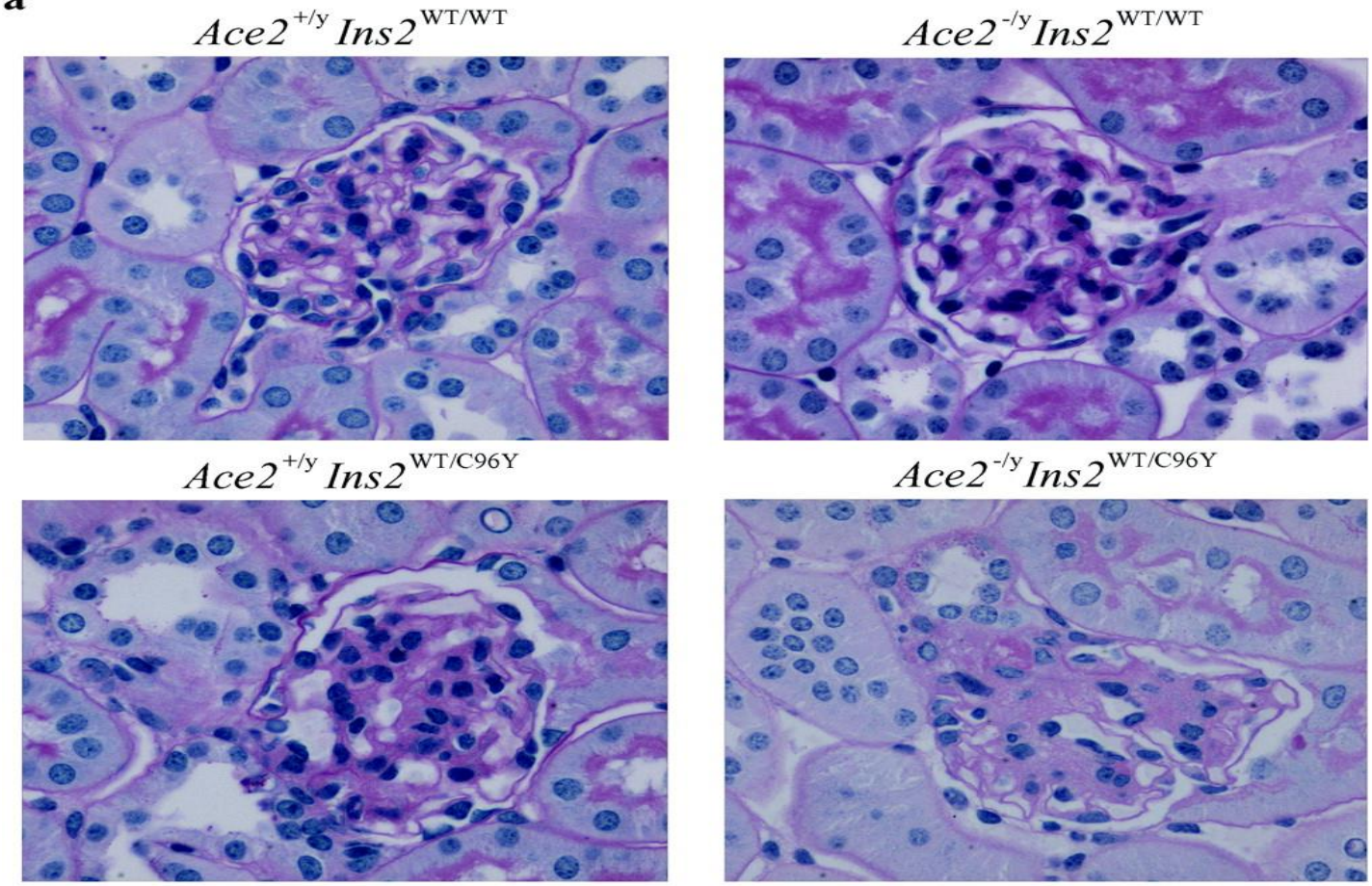
Soler et al KI 2008

Summary

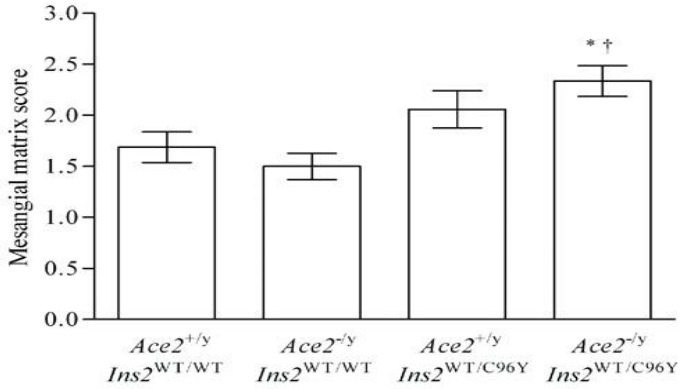
- ACE2 is localized in the glomerular epithelial cell (podocyte)
- ACE2 expression is down-regulated whereas ACE is up-regulated in glomeruli from diabetic mice
- ACE2 inhibition exacerbates kidney disease in diabetic mice.
- **Studies in ACE2 knockout.**


Urinary albumin excretion (μg/24h)
450
400
350
300
250
200
150
100
50
0
* †
* † **
Ace2+/y
Ins2WT/WT
Ace2-/y
Ins2WT/WT
Ace2+/y
Ins2WT/C96Y
Ace2-/y
Ins2WT/C96Y

a

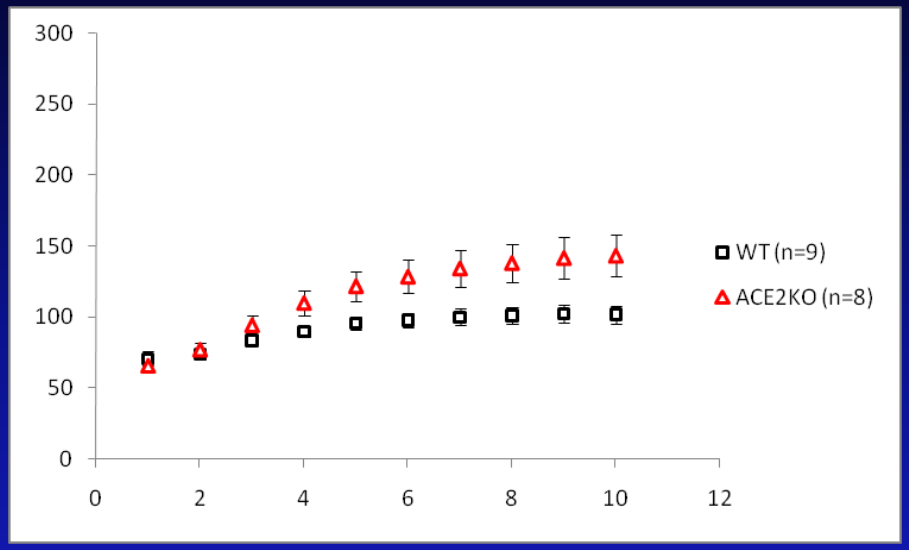

Ace2+/y Ins2WT/WT
Ace2-/y Ins2WT/WT
Ace2+/y Ins2WT/C96Y
Ace2-/y Ins2WT/C96Y

b

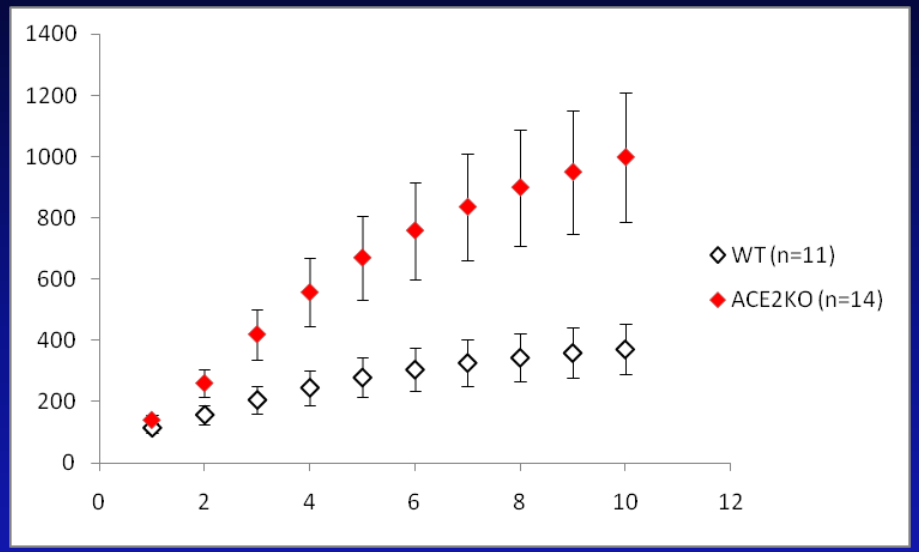

Mesangial matrix score
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* †
Ace2+/y Ins2WT/WT
Ace2-/y Ins2WT/WT
Ace2+/y Ins2WT/C96Y
Ace2-/y Ins2WT/C96Y

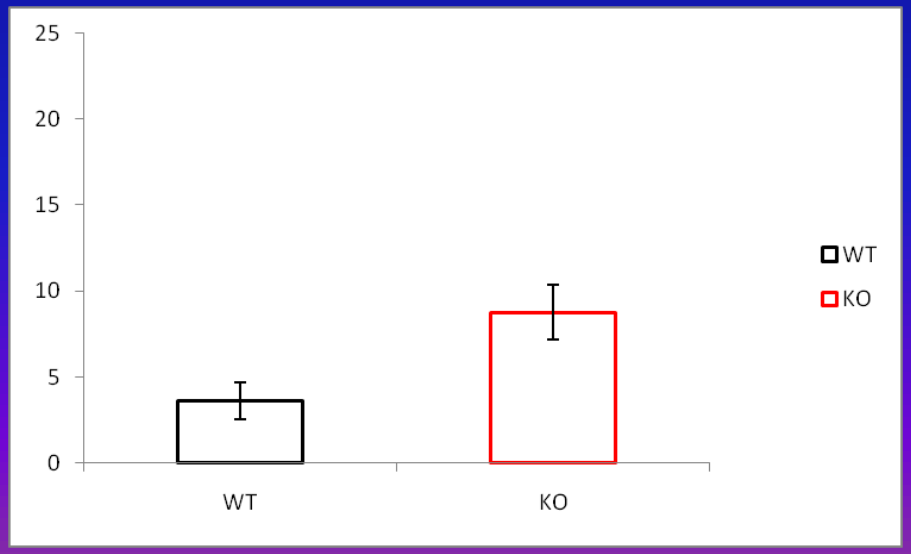
Kidney NADPH oxidase activity
Males

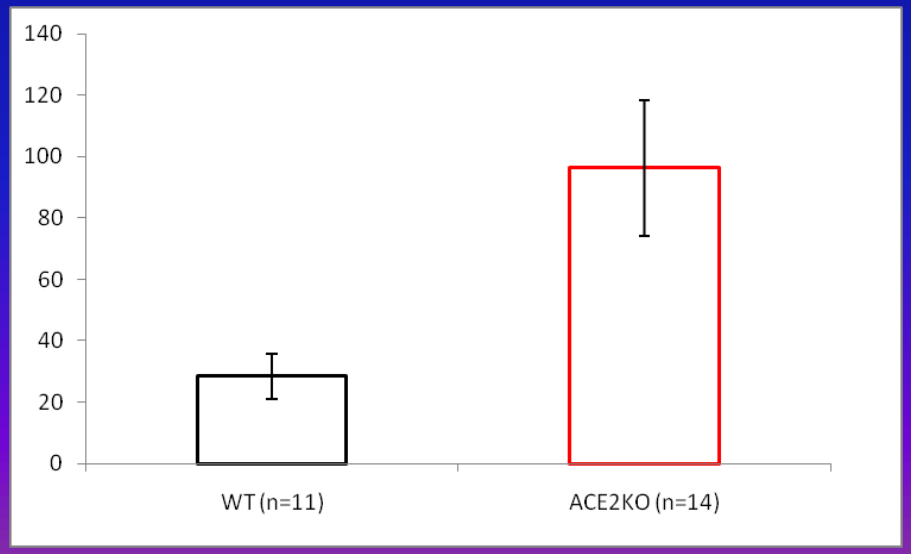
14 wks



40 wks

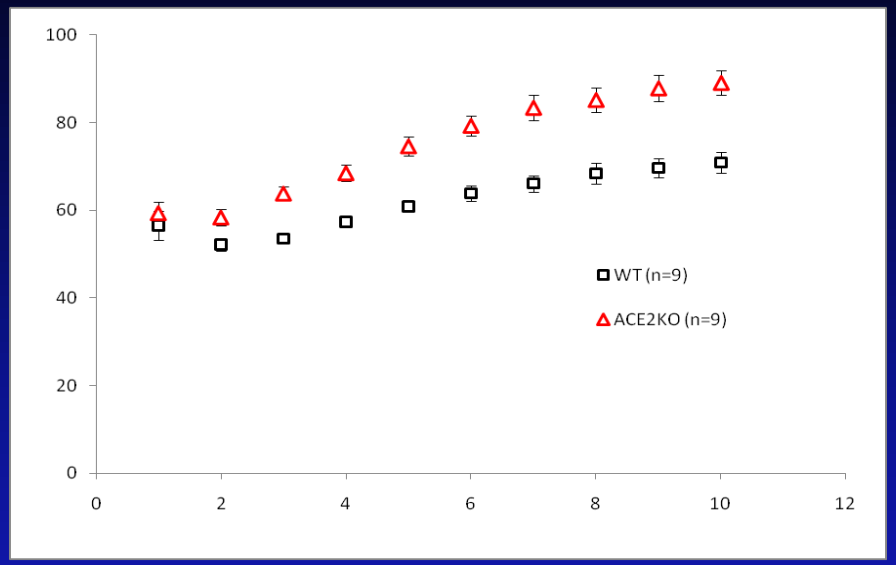


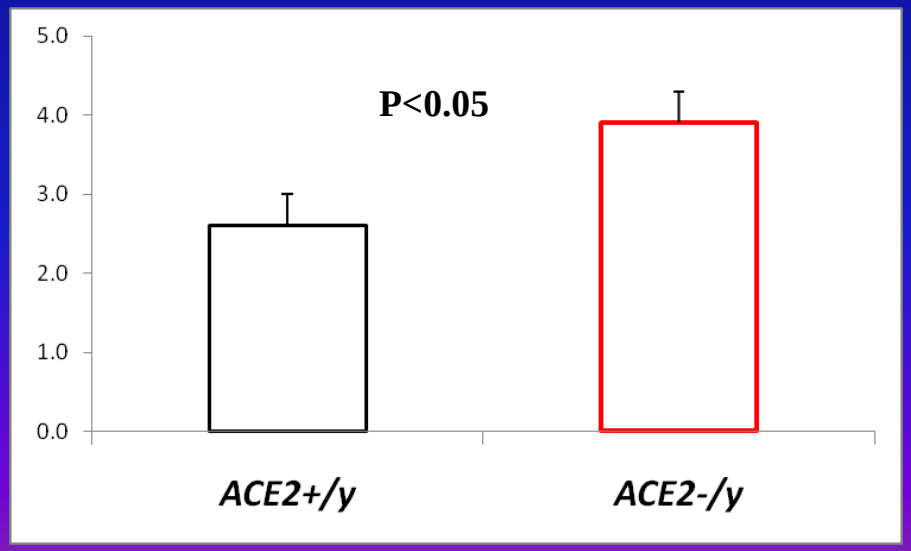




Heart NADPH oxidase activity in male mice

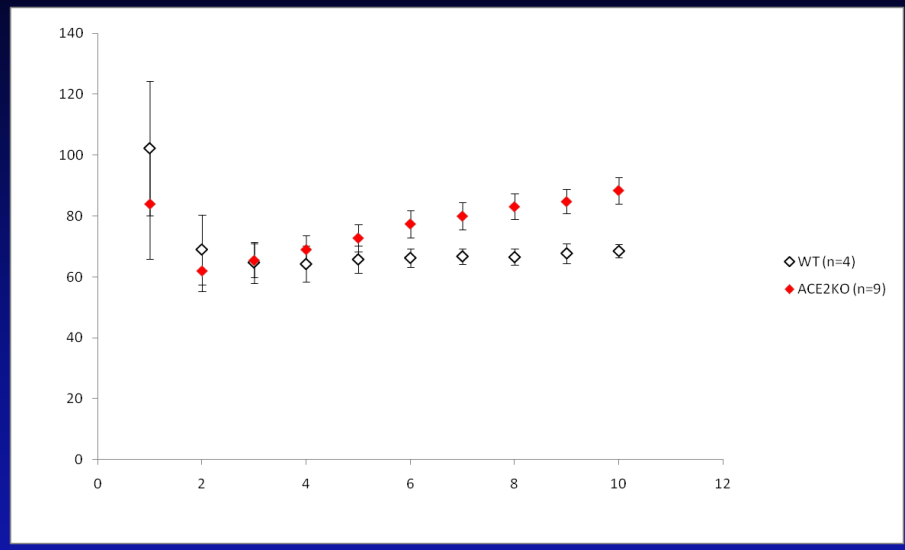
14 wks

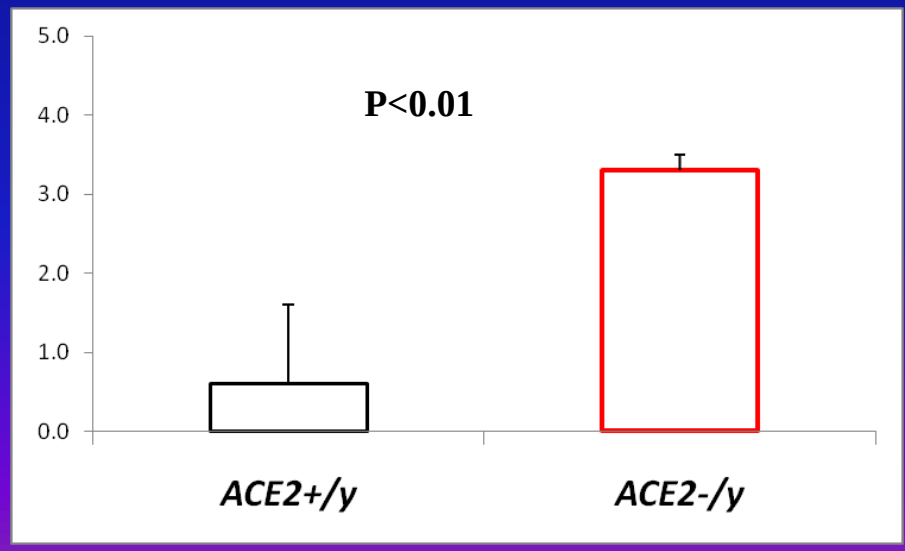




n=9 n=9

40 wks



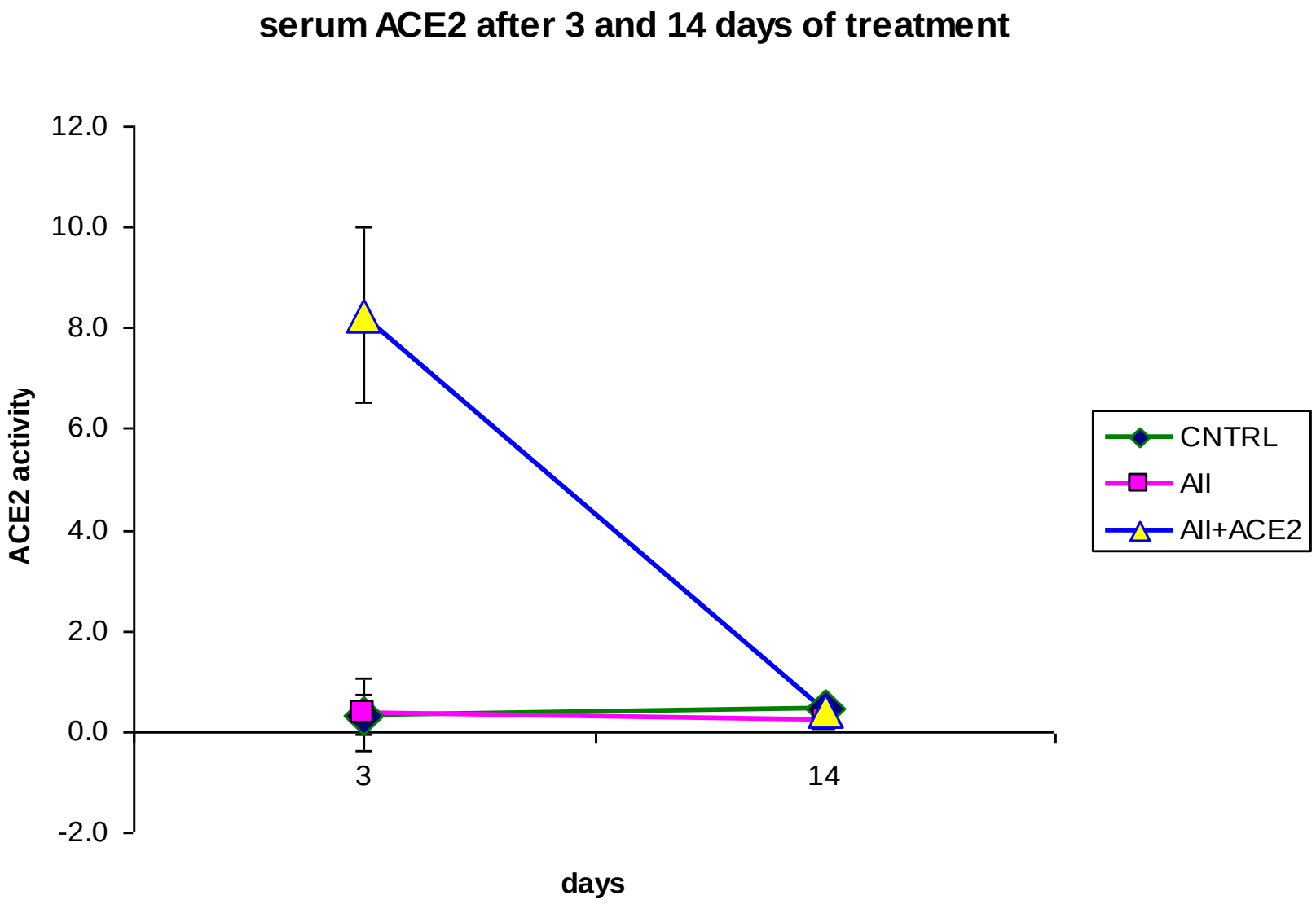


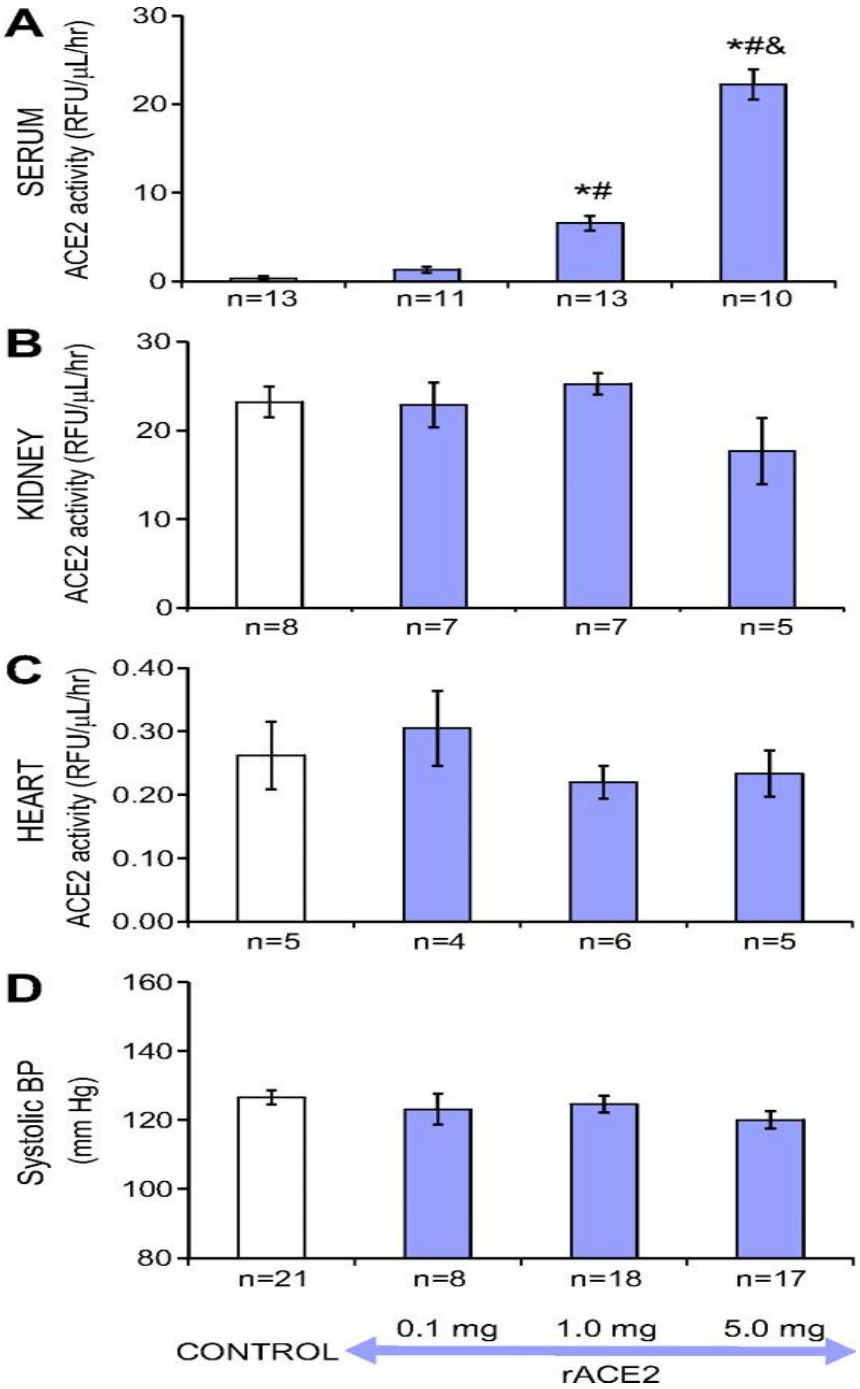
n=4 n=9

Wysocki et al. unpublished

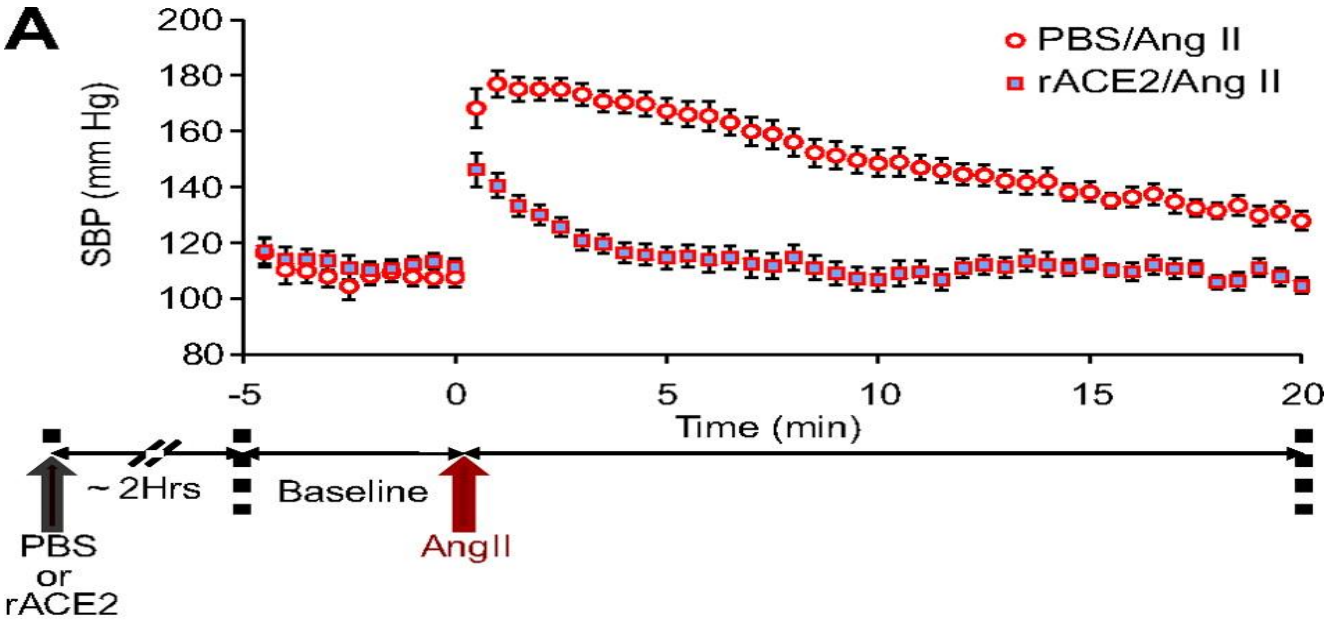
Objectives

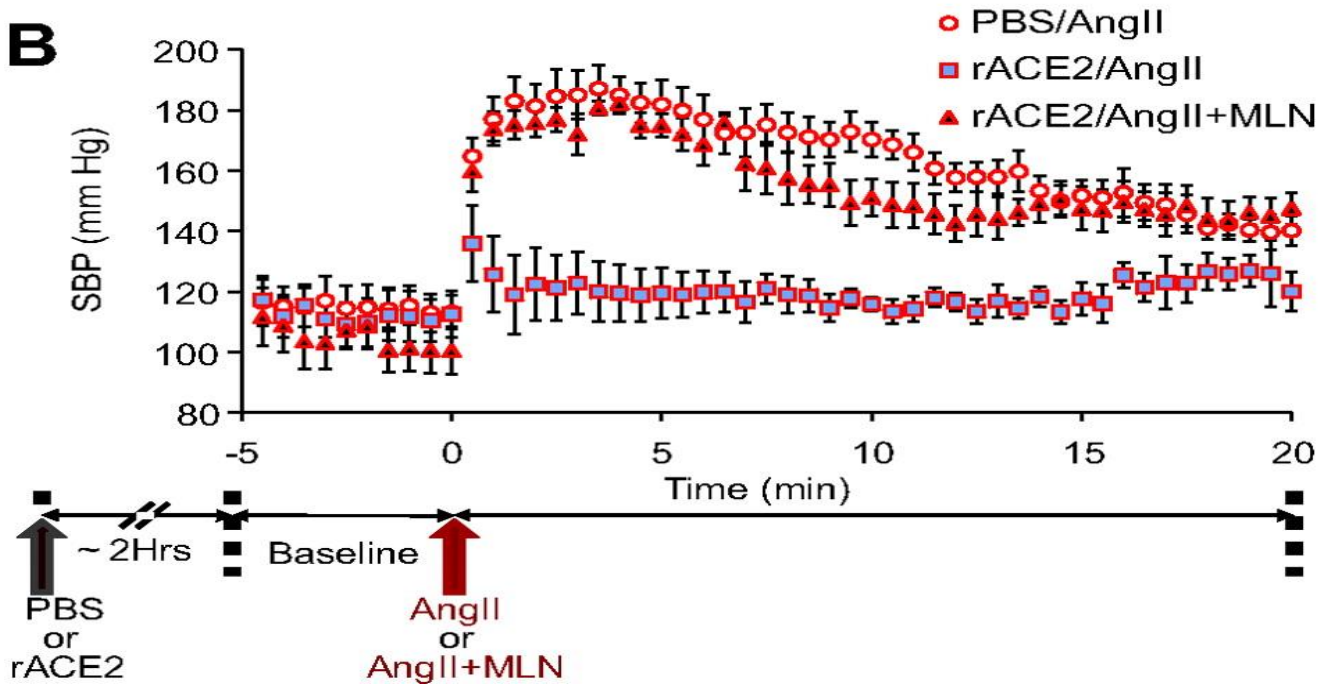
- ACE2 localization within the kidney
- Alteration in ACE2 expression in mouse models of diabetic kidney disease.
- Impact of ACE2 inhibition and genetic ablation on diabetic kidney disease.
- Effect of recombinant ACE2 on blood pressure and Angiotensin II metabolism.
- Potential therapeutic effect of ACE2 amplification.

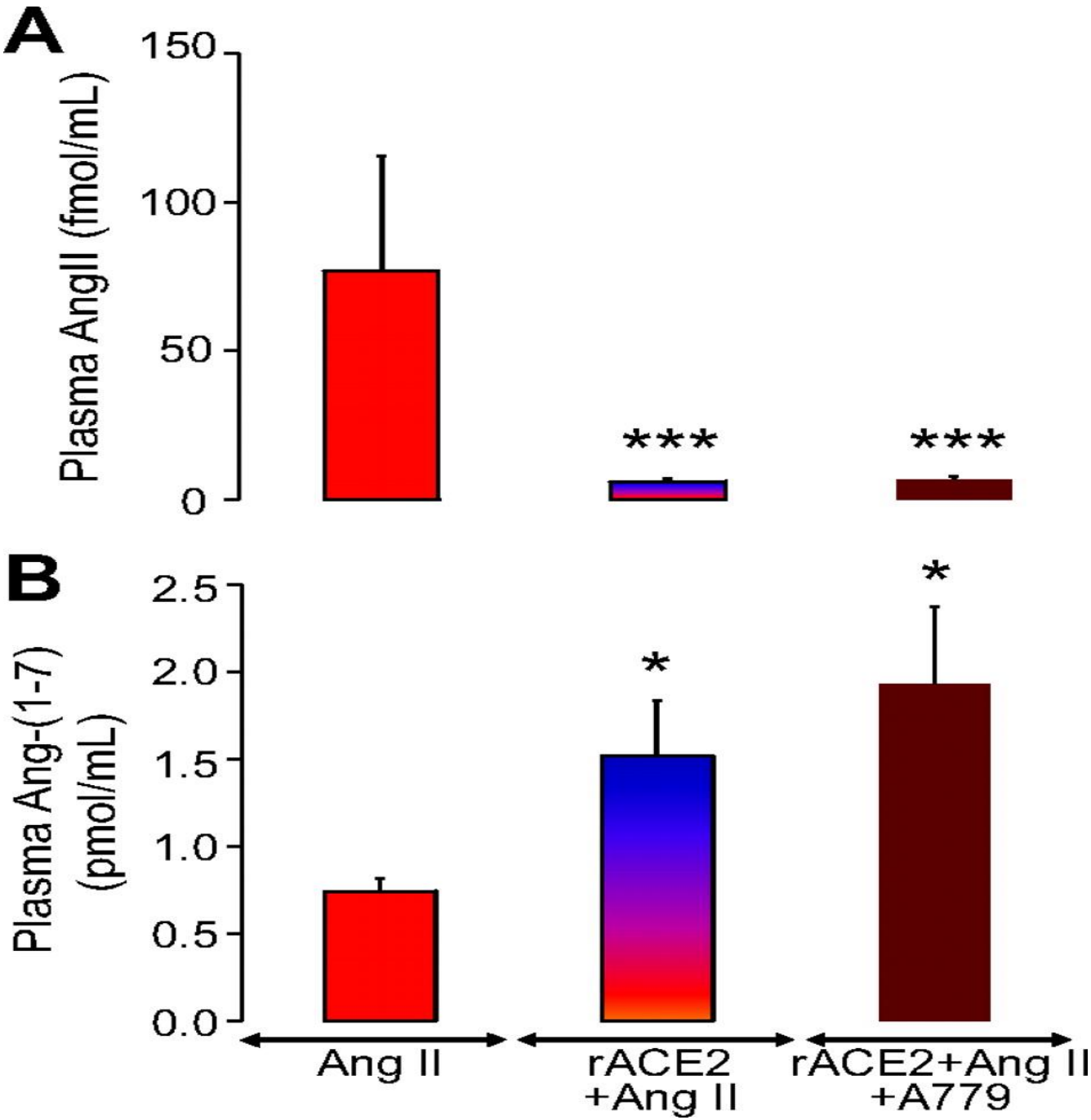

serum ACE2 after 3 and 14 days of treatment
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
ACE2 activity
3
14
days
CNTRL
AII
AII+ACE2



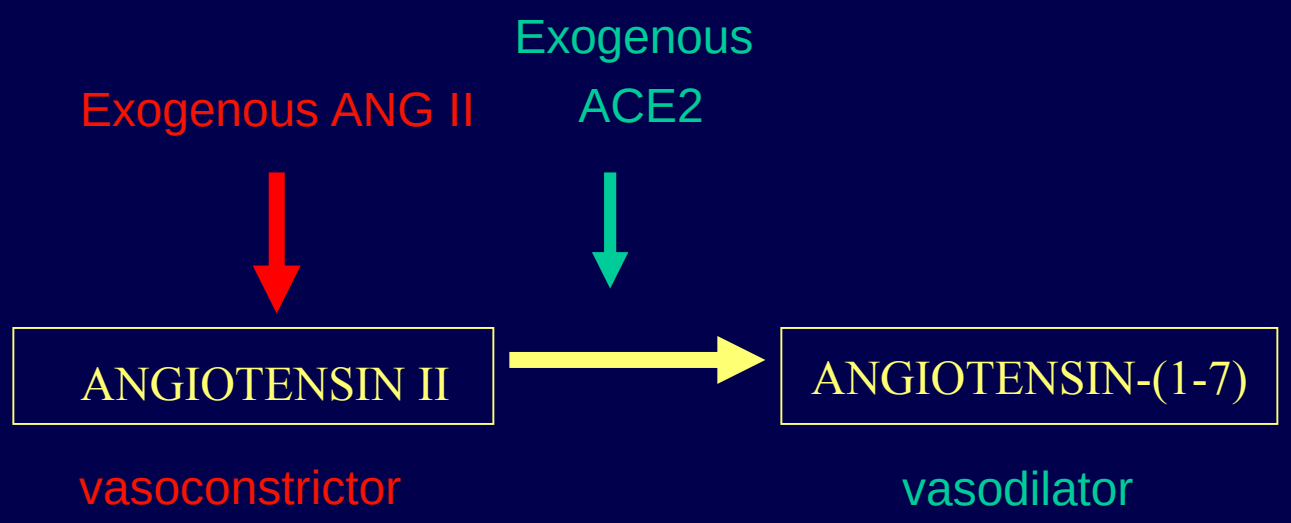
Wysocki et al Hypertension 2010

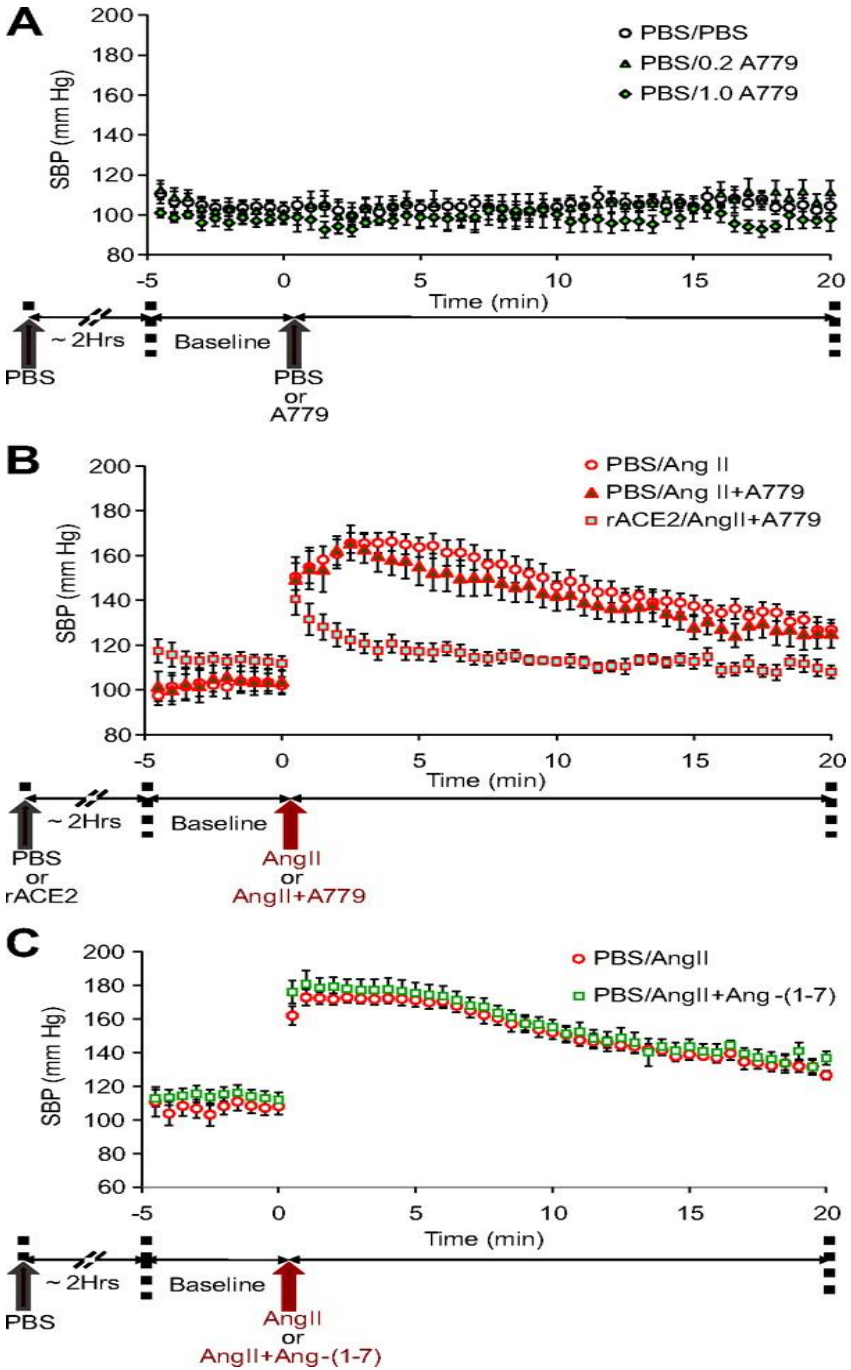

A
PBS/Ang II
rACE2/Ang II
SBP (mm Hg)
200
180
160
140
120
100
80
-5
0
5
10
15
20
Time (min)
~ 2Hrs
Baseline
PBS
or
rACE2
AngII


B
PBS/AngII
rACE2/AngII
rACE2/AngII+MLN
SBP (mm Hg)
200
180
160
140
120
100
80
-5
0
5
10
15
20
Time (min)
~ 2Hrs
Baseline
PBS
or
rACE2
AngII
or
AngII+MLN

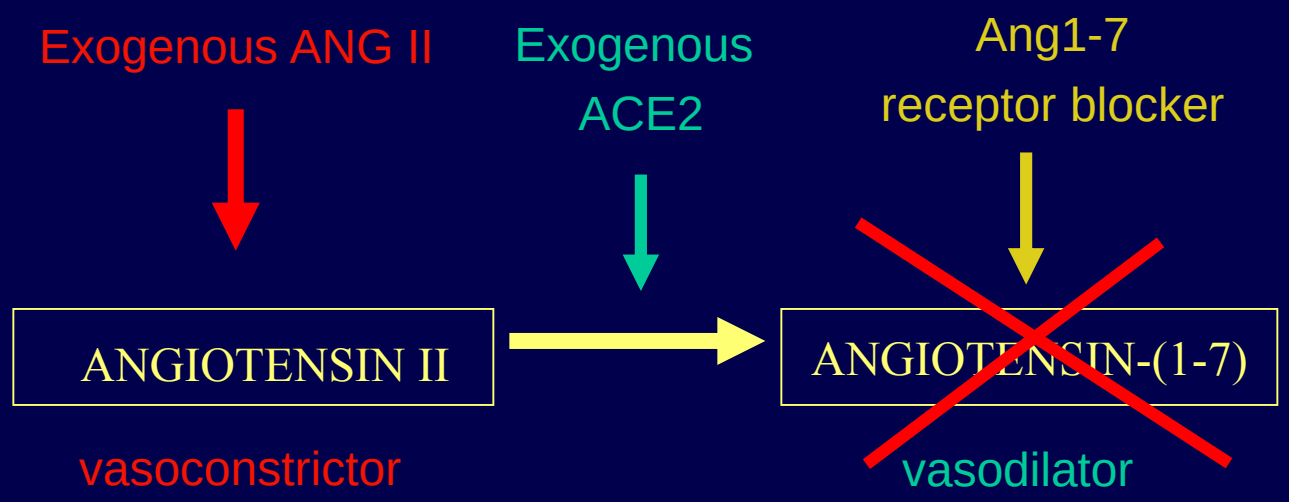

A
Plasma AngII (fmol/mL)
150
100
50
0
***
***
B
Plasma Ang-(1-7) (pmol/mL)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
Ang II
rACE2 +Ang II
rACE2+Ang II +A779

Effect of rACE2 on blood pressure




A
PBS/PBS
PBS/0.2 A779
PBS/1.0 A779
SBP (mm Hg)
Time (min)
~ 2Hrs
Baseline
PBS
PBS
or
A779
B
PBS/Ang II
PBS/Ang II+A779
rACE2/AngII+A779
SBP (mm Hg)
Time (min)
~ 2Hrs
Baseline
PBS
or
rACE2
AngII
or
AngII+A779
C
PBS/AngII
PBS/AngII+Ang-(1-7)
SBP (mm Hg)
Time (min)
~ 2Hrs
Baseline
PBS
AngII
or
AngII+Ang-(1-7)

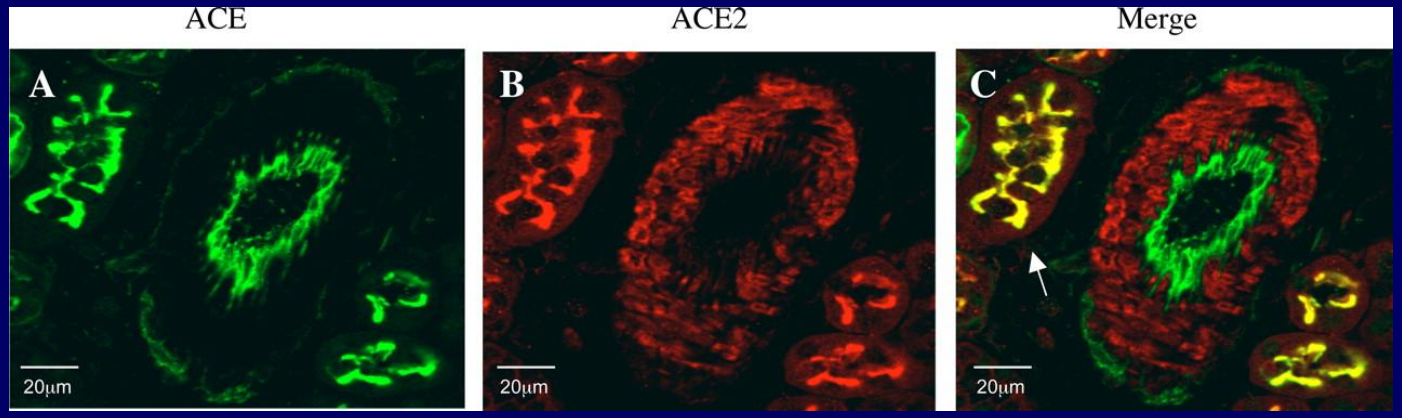
Effect of ACE2 on blood pressure



Objectives

- ACE2 localization within the kidney
- Alteration in ACE2 expression in mouse models of diabetic kidney disease.
- Impact of ACE2 inhibition and genetic ablation on diabetic kidney disease.
- Effect of recombinant ACE2 on blood pressure and Angiotensin II metabolism.
- Potential therapeutic effect of ACE2 amplification.

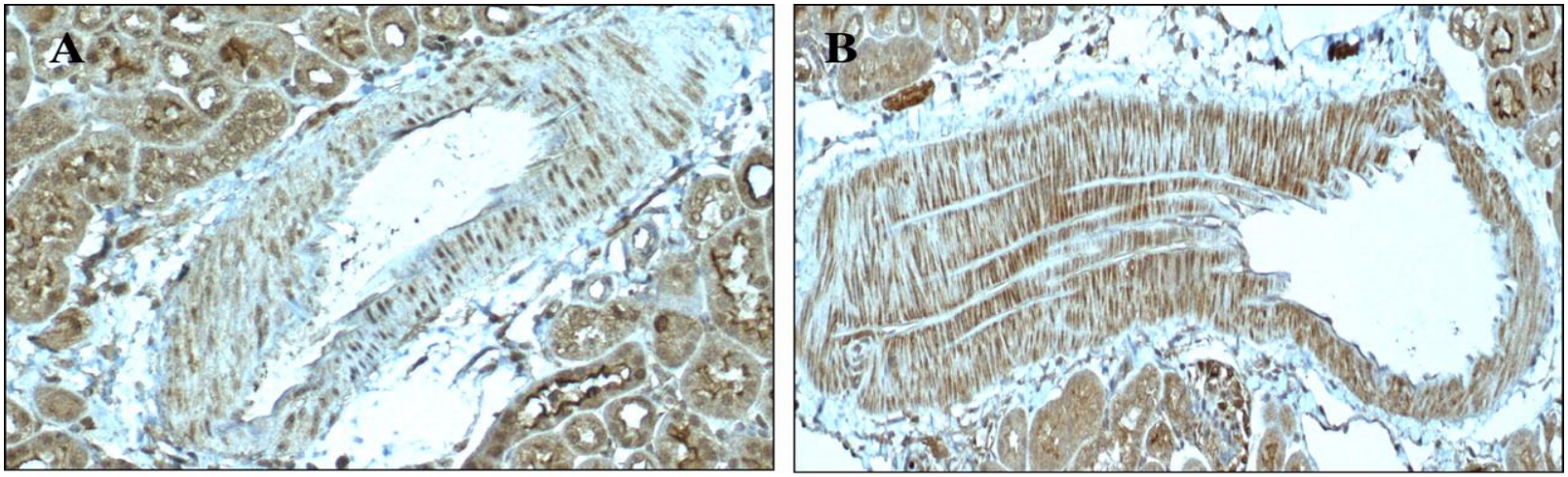
Fig. 1. Angiotensin-converting enzyme (ACE) and ACE2 localization in renal vasculature: immunofluorescence staining of ACE (green; A) and ACE2 (red; B) in a kidney arteriole from mouse kidney

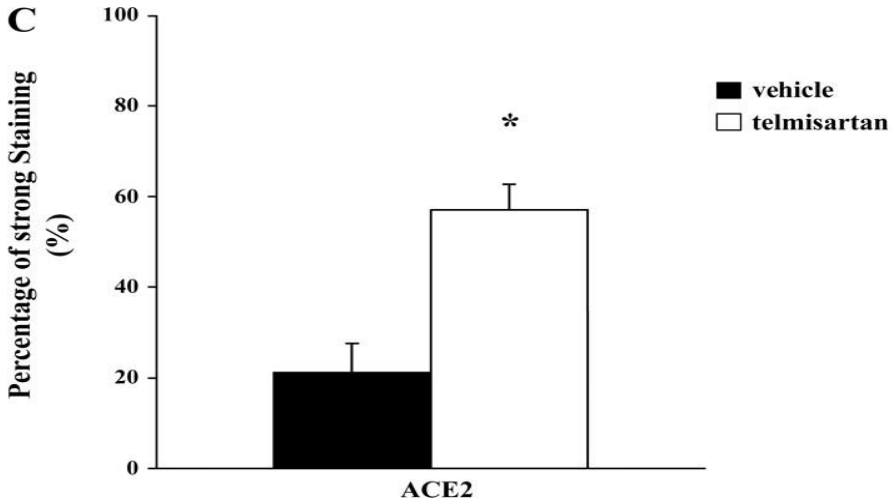


Soler, M. J. et al. Am J Physiol Renal Physiol 296: F398-F405 2009; doi:10.1152/ajprenal.90488.2008

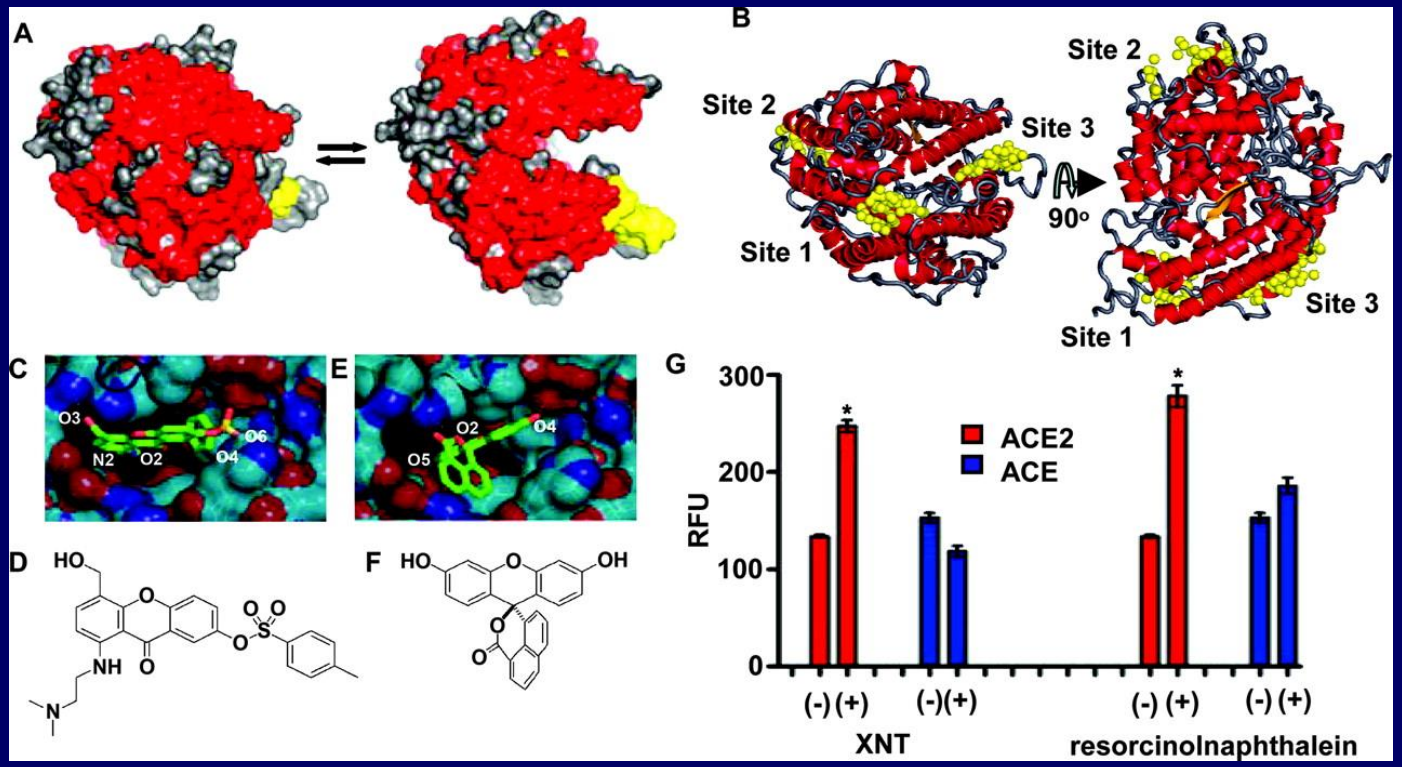
AJP - Renal Physiology

Soler et al AJP 2008





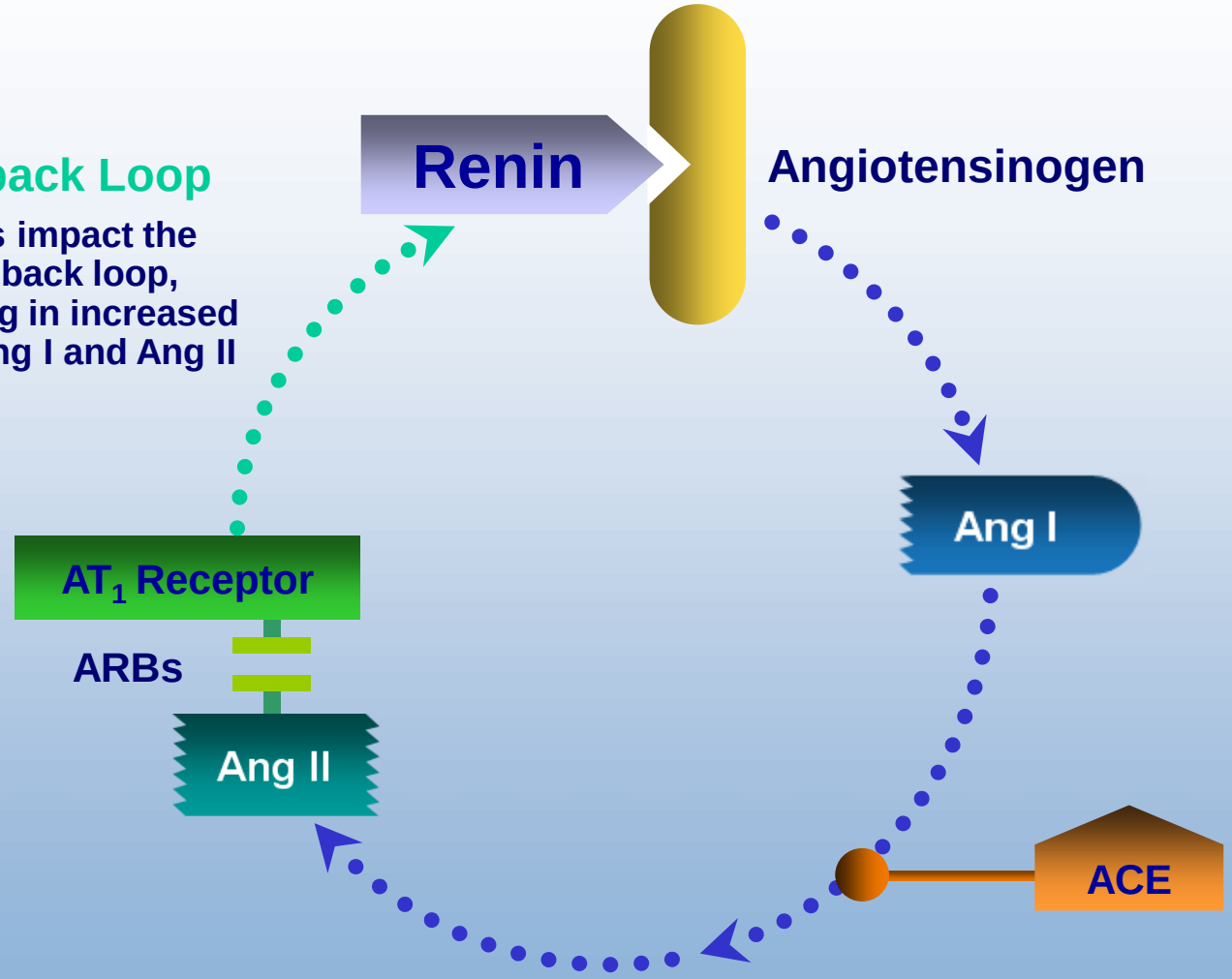
Structure-based identification of an ACE2 activator



Blockade of the Renin Angiotensin System

Feedback Loop

- ARBs impact the feedback loop, resulting in increased PRA, Ang I and Ang II



ARB, angiotensin receptor blocker; PRA, plasma renin activity; Ang, angiotensin; AT_1 Receptor, angiotensin I receptor; ACE, angiotensin-converting enzyme.

Only Direct Renin Inhibition Inhibits the Entire Renin Angiotensin System[1-6]

Direct renin inhibitors are currently in development

Class	PRA	Ang I	Ang II
Diuretic	↑	↑	↑
ACEI	↑	↑	↓
ARB	↑	↑	↑
Direct Renin Inhibitor (DRI)	↓	↓	↓

Increased peptide levels have not been shown to overcome the blood pressure-lowering effect of these agents.

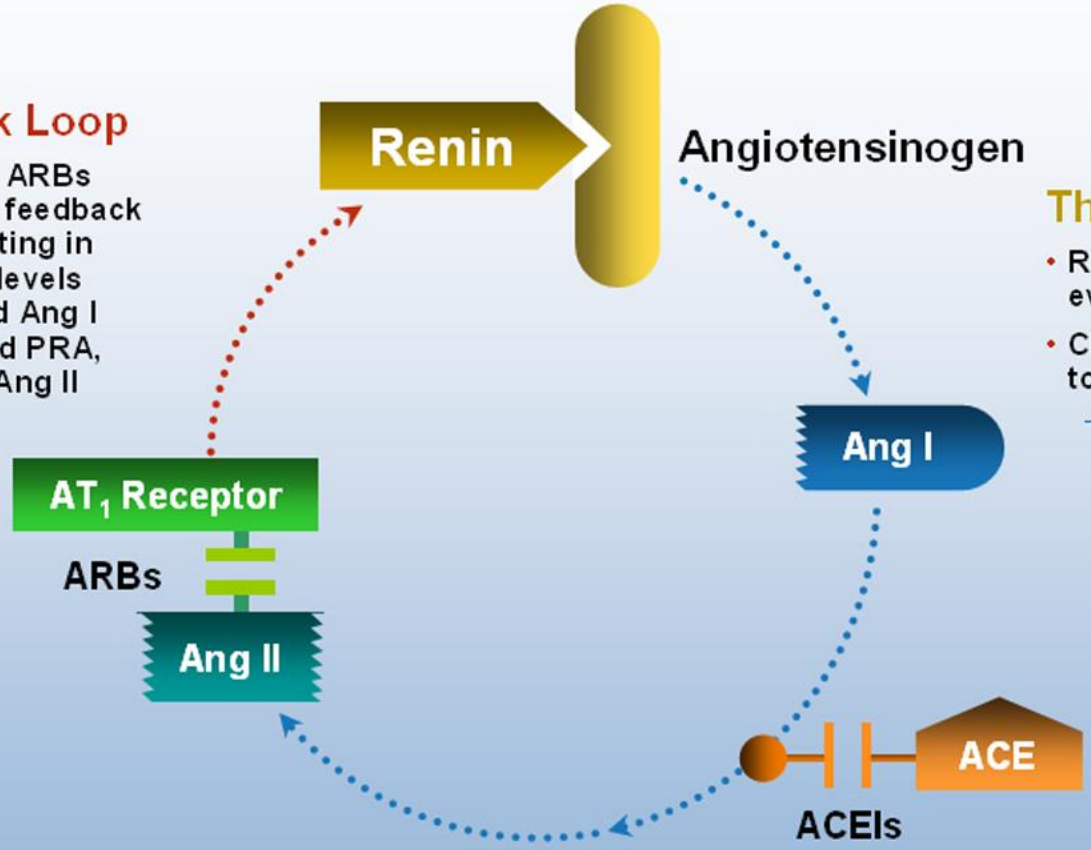
PRA, plasma renin activity; Ang, angiotensin; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

1. Johnston CI. *Blood Pressure*. 2000;9(suppl 1):9-13.
2. Widdop RE et al. *Hypertension*. 2002;40:516-520.
3. Fabiani ME et al. In: *Angiotensin II Receptor Antagonists*. 2001:263-278.
4. Waybill MM et al. *J Vasc Interv Radiol*. 2003;14:961-975.
5. Reid IA. *Adv Physiol Ed*. 1998;20:S236-S245.
6. Lin C et al. *Am Heart J*. 1996;131:1024-1034.

An Efficient Strategy to Achieve Interruption of the Renin Angiotensin System Is Direct Renin Inhibition[1-3]

Feedback Loop

- ACEIs and ARBs impact the feedback loop, resulting in increased levels of PRA and Ang I (ACEIs) and PRA, Ang I and Ang II (ARBs)*



The Point of Activation

- Renin initiates a chain of events within the system
- Cleaves angiotensinogen to form Ang I
  - Ang I is then converted to Ang II

*Increased peptide levels have not been shown to overcome the blood pressure-lowering effect of these agents.

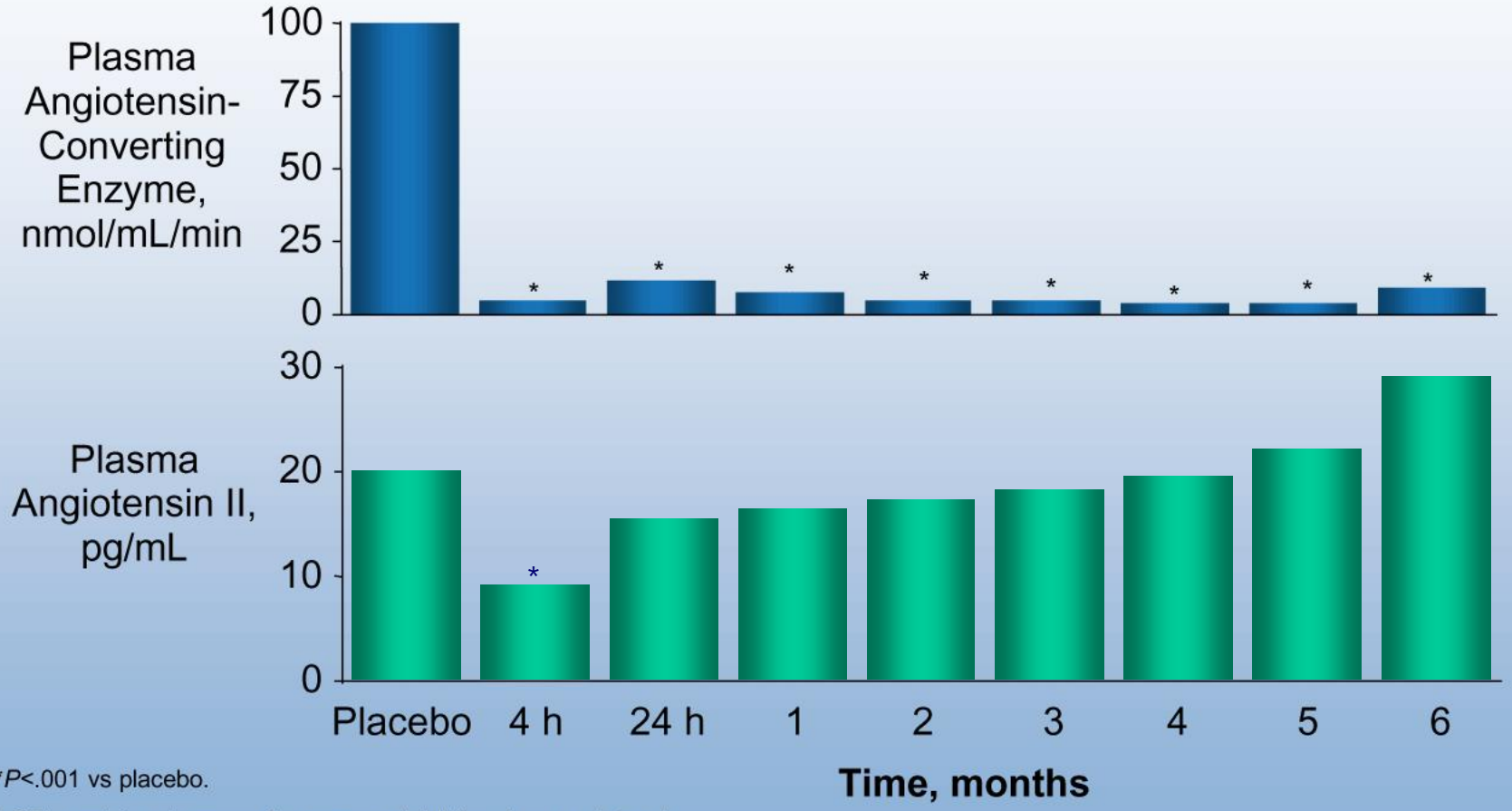
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AT_1 Receptor, angiotensin I receptor; Ang, angiotensin; ACE, angiotensin-converting enzyme.

Adapted from:

1. Stanton A. *J Renin Angiotensin Aldosterone Syst*. 2003;4:6-10.
2. Kelly DJ et al. *Hypertension*. 2005;46:471-472.
3. Fisher NDL et al. *J Am Soc Nephrol*. 2005;16:592-599.

ACE Escape With Long-Term ACEI Treatment

Ang II Returns to Baseline Levels



*P<.001 vs placebo.

ACEI, angiotensin-converting enzyme inhibitor; Ang, angiotensin.

Biollaz J et al. *J Cardiovasc Pharmacol.* 1982;4:966-972.

Potential ACE2 targets: a large repertoire of disease entities

- Acute Lung Injury
- Pulmonary Hypertension
- Congestive Heart Failure
- Diabetic Retinopathy
- Diabetic Kidney Disease
- Brain injury – Stoke
- Renin Dependent Hypertension
- Any Angiotensin II excess Syndrome

Methods of ACE 2 amplification

- Human Recombinant ACE2 (IP, minipumps, i.v)
- Murine Recombinant ACE2 (IP, minipumps, i.v)
- Lentivirus DNA delivery/Adeno-associated Virus delivery
- ACE2 transgenic models
- ACE2 Activators (XNT /DIZE/NCP-2454)
- Minicircle DNA delivery
- Bioencapsulation of ACE2
- Modified rACE2 with enhanced duration of action

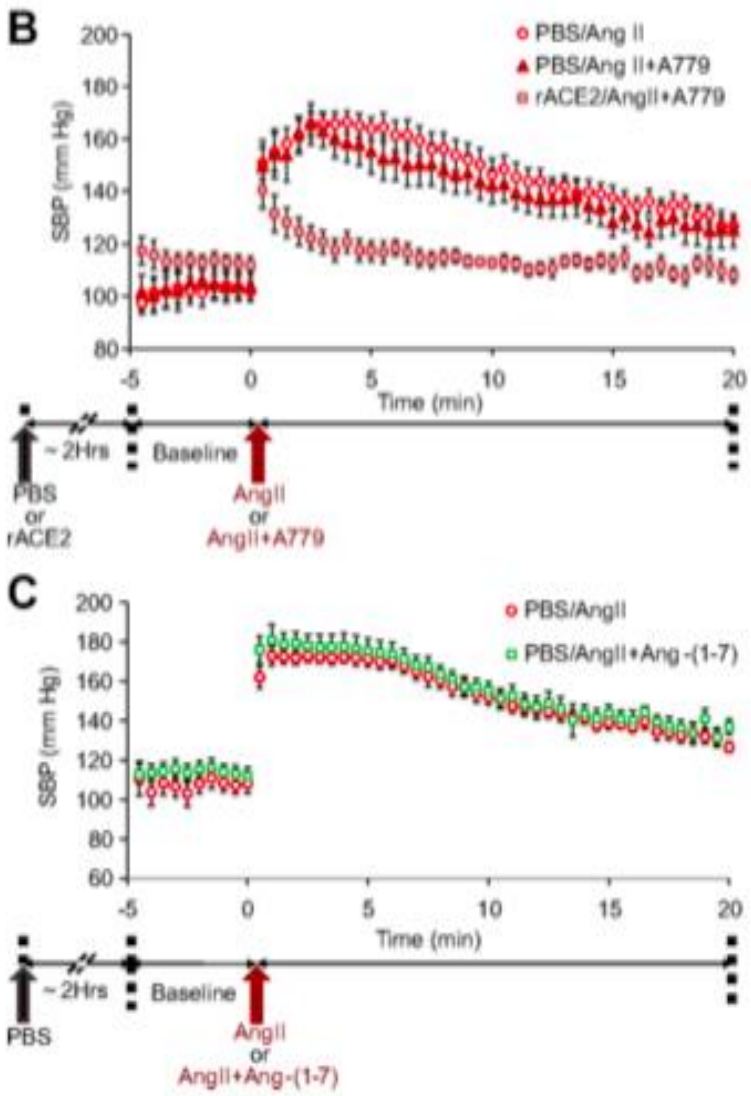
What is the target of ACE 2 amplification

- ACE2 deficiency (relative if inadequate compensation)
- Excess Angiotensin II (1-8)
- Deficiency of Ang (1-7)

All of the above ?

None of the above ?

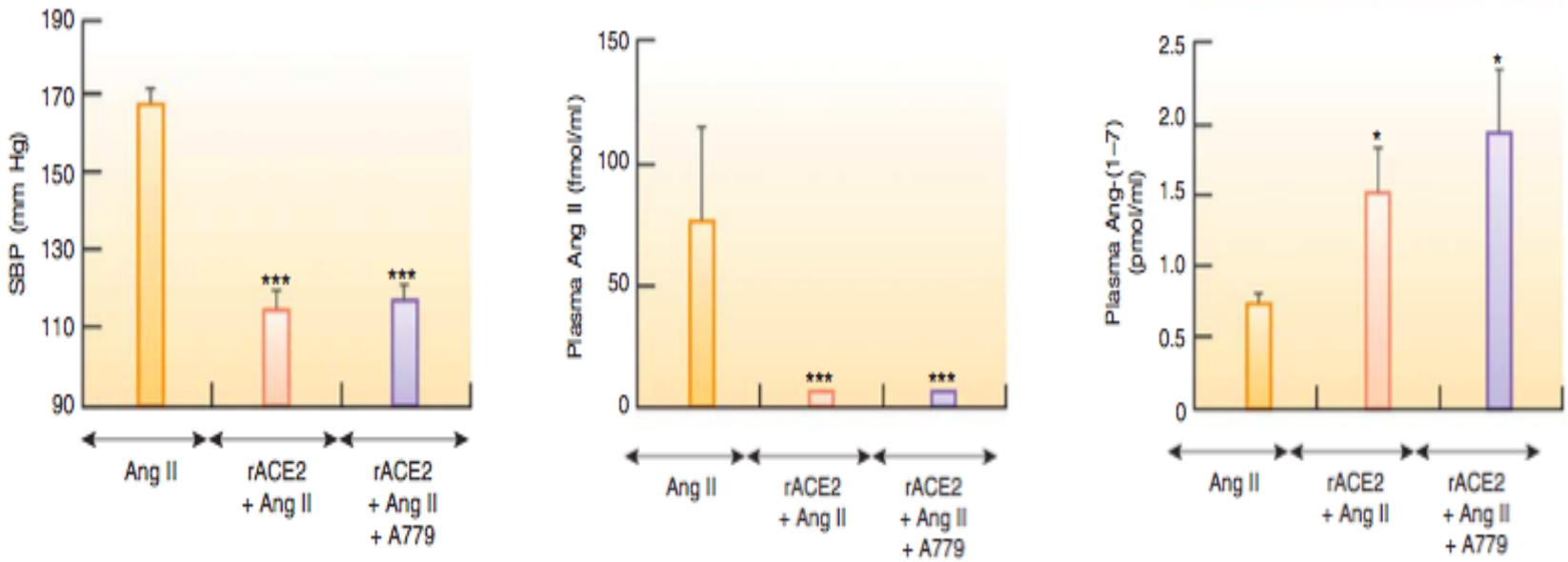
Less Ang II or more Ang 1-7? Evidence for the model of Ang II-induced hypertension



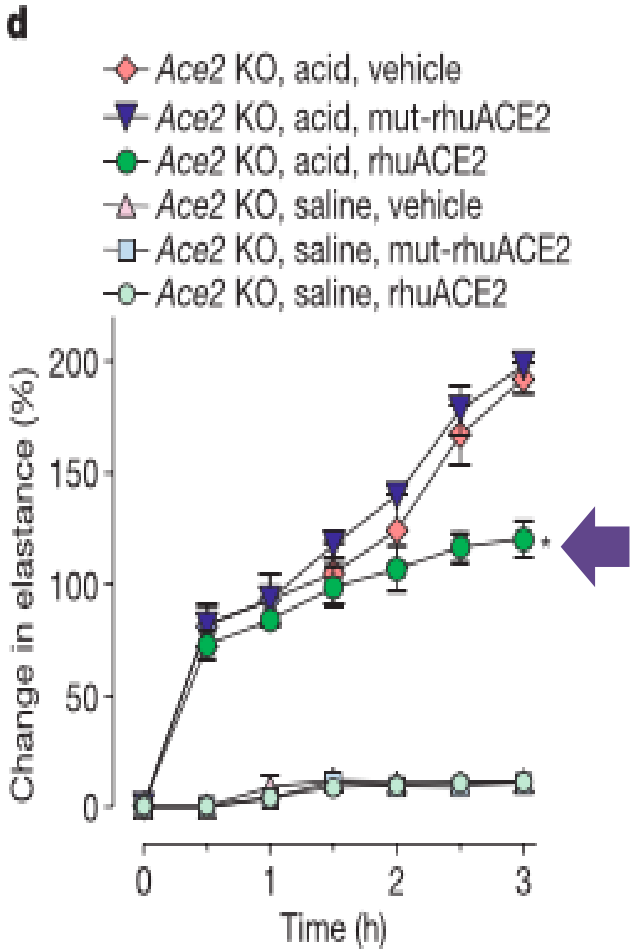
Jan Wysocki PhD

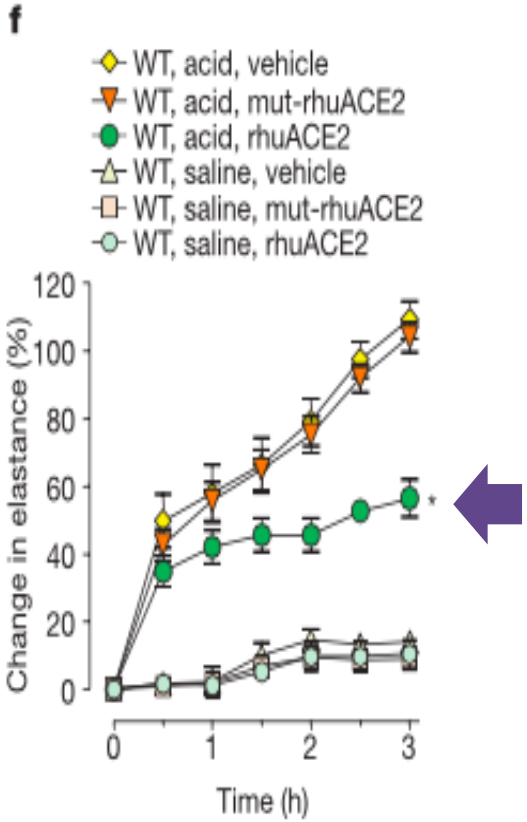
Northwestern Medicine

Ang 1-7 independent action of ACE2 on blood pressure



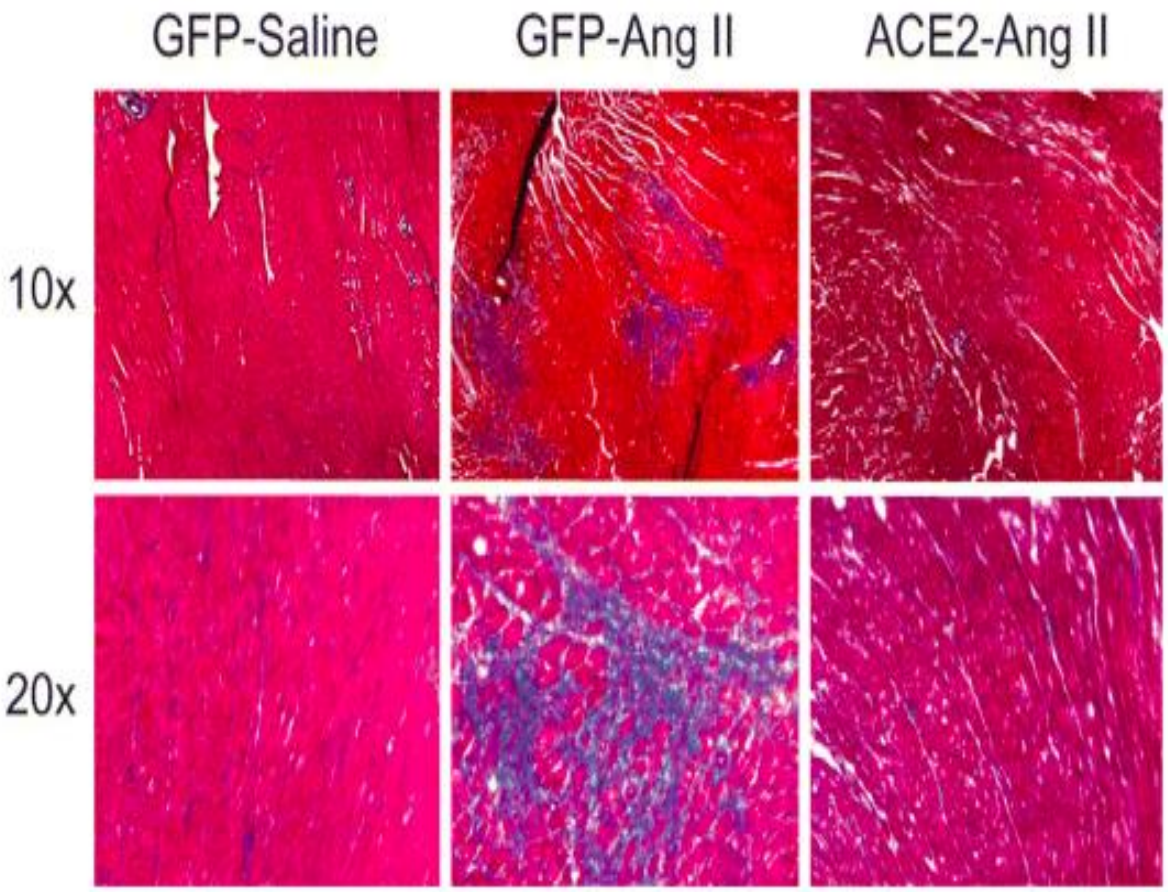
Acute Lungs Injury- Human Recombinant ACE2





Imai et al, Nature,2005

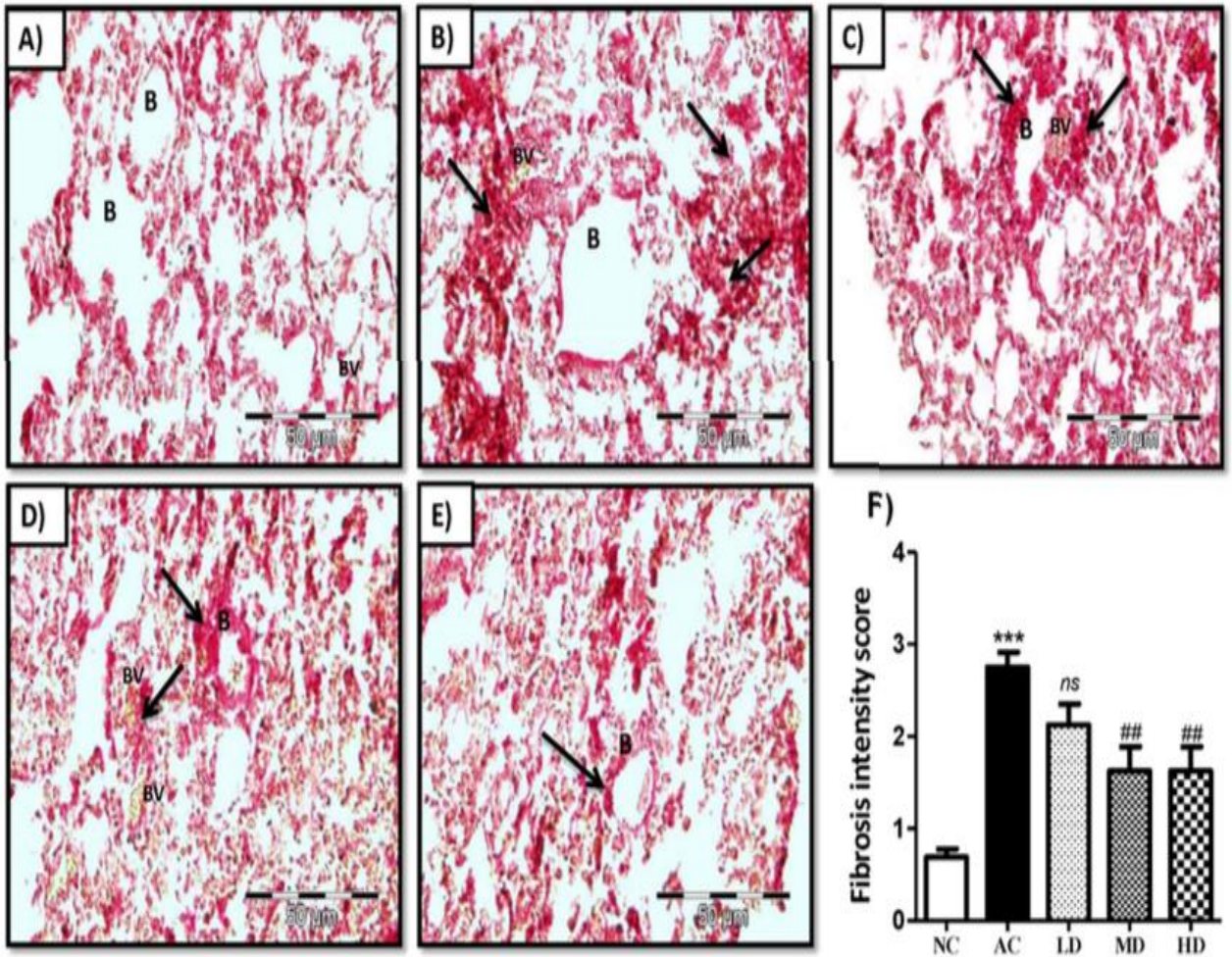
Ang II-Induced Cardiac Fibrosis - Studies with Lentivirus ACE2



Huentelman, M. J.,et al Experimental Physiology2005

Northwestern Medicine

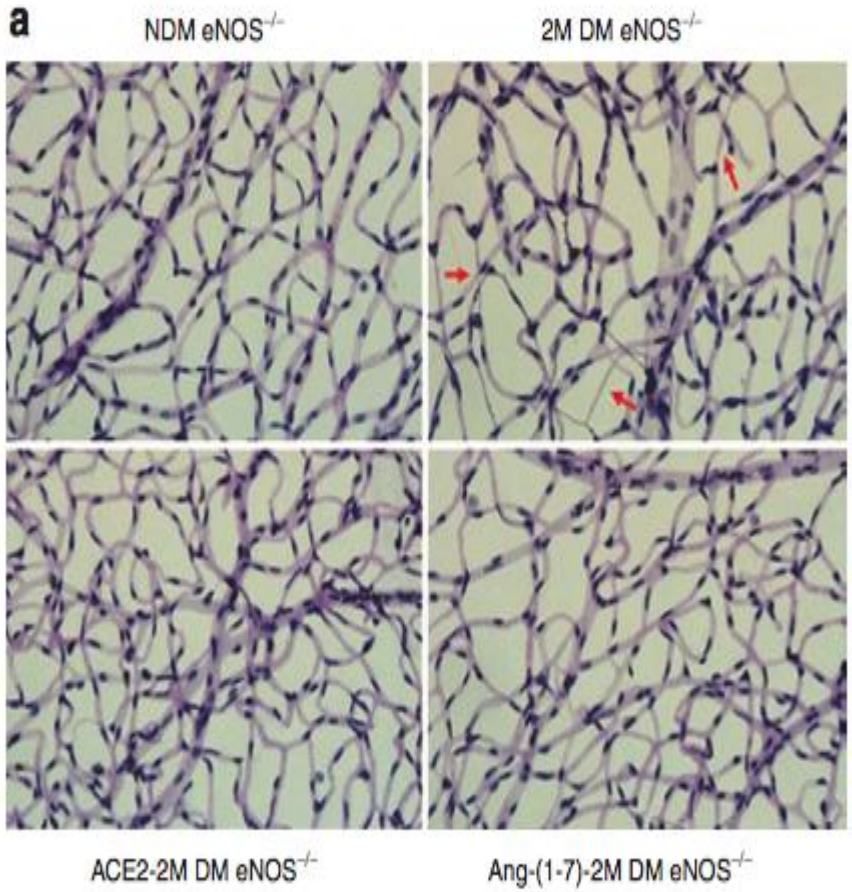
Lung Fibrosis-Asthma model- ACE2 activator (DIZE)

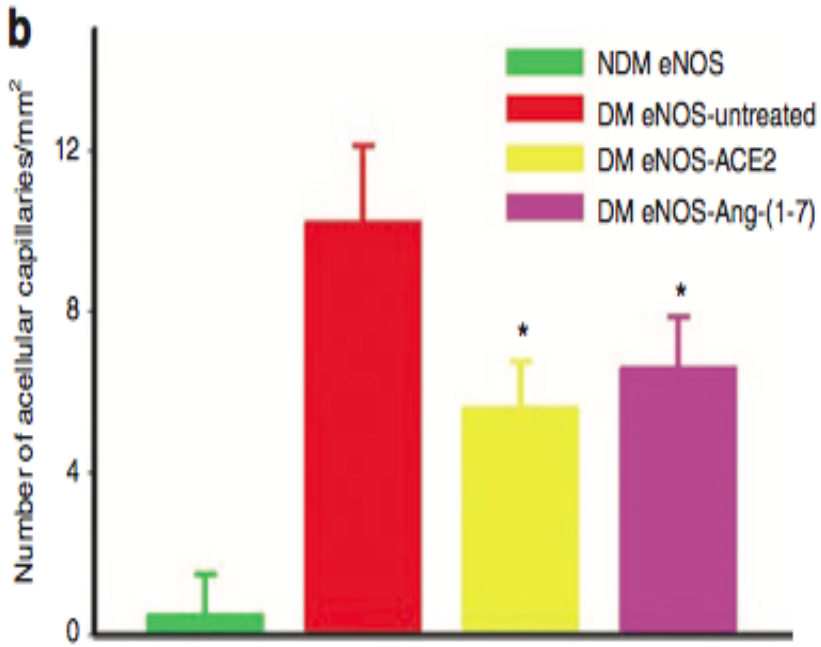


Northwestern Medicine

Vaibhav Shrirang Dhawale et al, In Toxicology and Applied Pharmacology, 2016

Diabetic Retinopathy- Studies with AAV ACE2

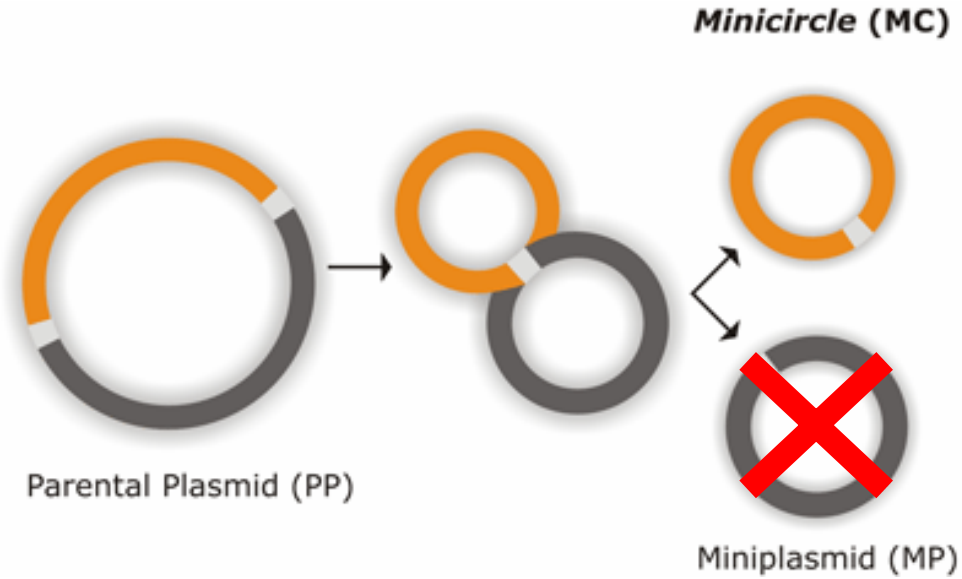


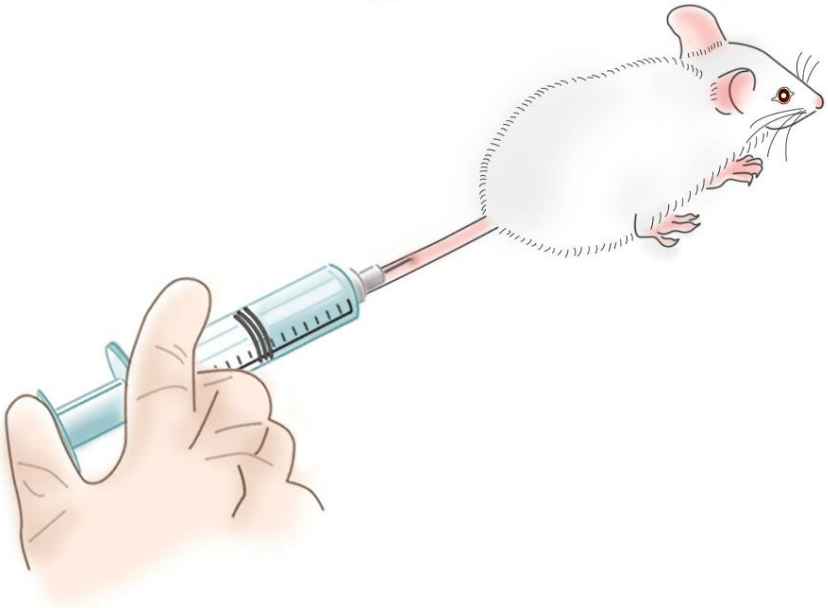


Northwestern Medicine

Amrisha Verma et al ACE2 and Ang 1-7 diabetic retinopathy, 2012

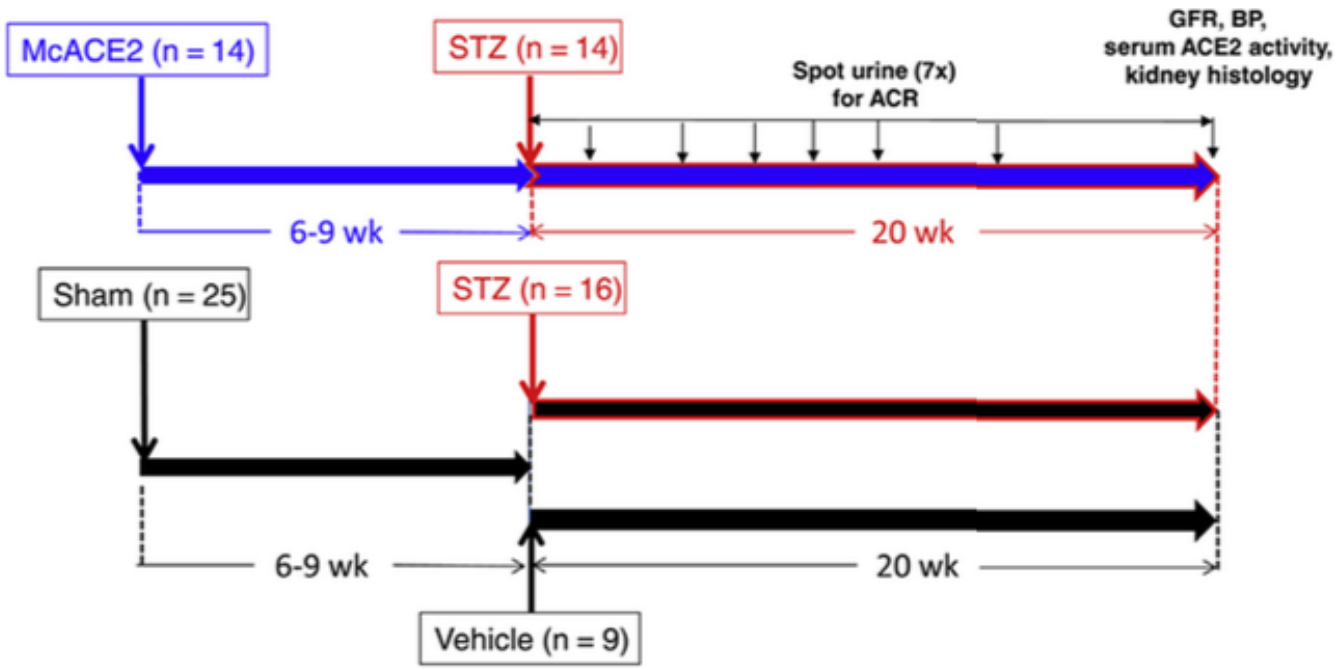
Mini-circles ACE2 DNA delivery by hydrodynamic injection





Northwestern Medicine®

Minicircle ACE2 delivery in the STZ model

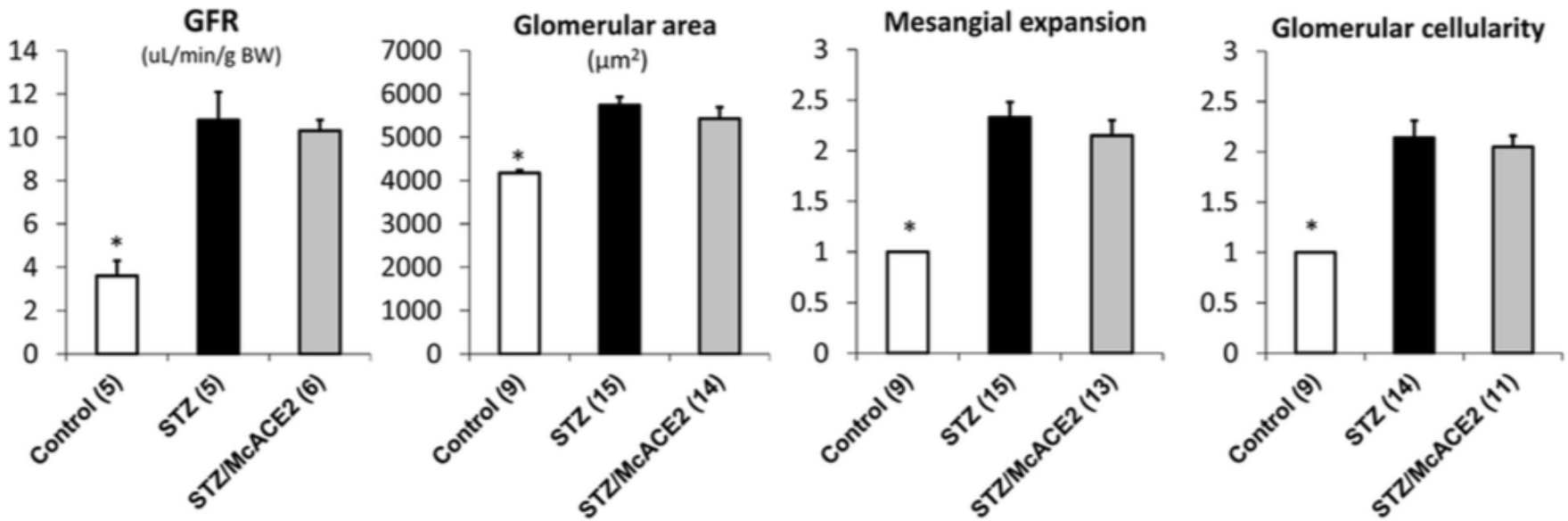


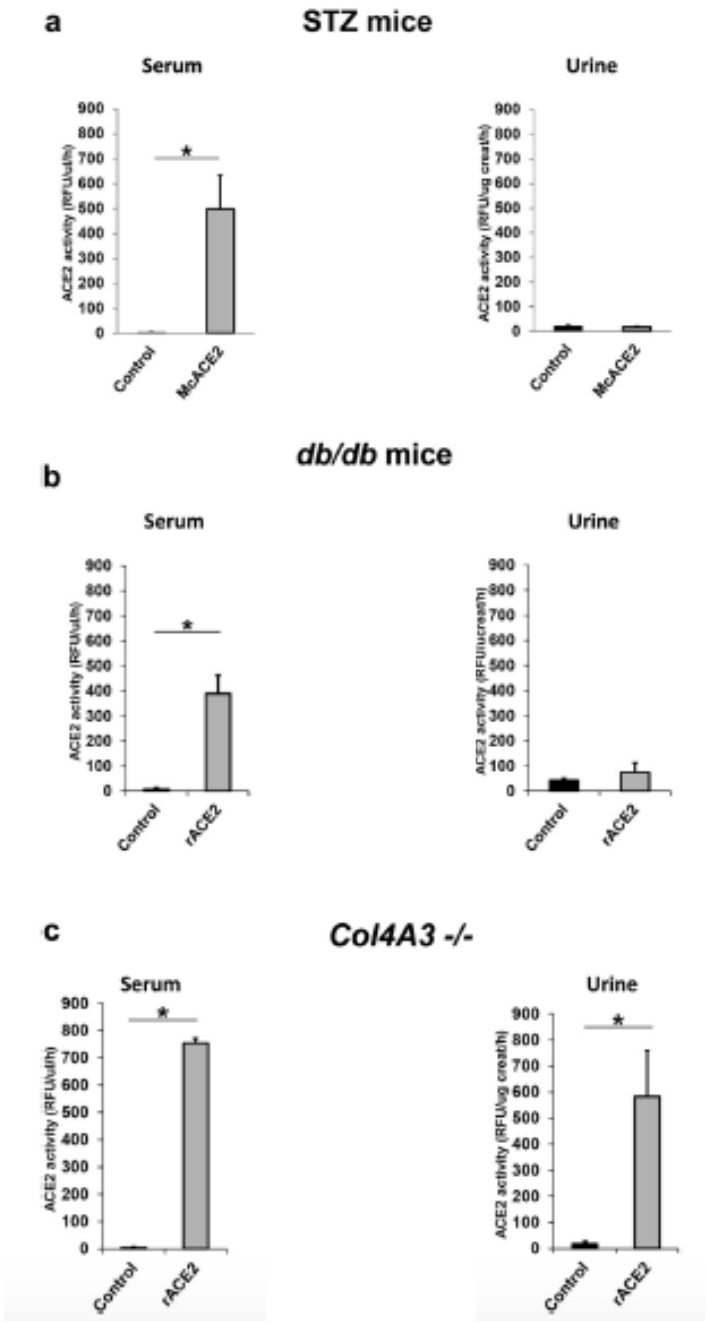
Minicircle ACE2 delivery in the STZ model

Table 1 | General parameters in mice 20 weeks after diabetes induction with STZ and in vehicle-treated mice that served as a nondiabetic control group

Parameter	Controls	STZ	STZ/McACE2
Blood glucose, mg/dl	177 ± 16[a]	457 ± 36	449 ± 32
Body weight, g	24.3 ± 0.4	25.9 ± 0.6	26.1 ± 0.6
Serum ACE2 activity, RFU/ul/h	1.4 ± 0.3[a]	2.4 ± 0.3	497 ± 135[b]
L + R kidney weight, g	0.291 ± 0.0087[a]	0.437 ± 0.0138	0.415 ± 0.0204
Kidney/body weight ratio, mg/g	12.2 ± 0.4[a]	17.2 ± 0.5	16.3 ± 1.0
Heart weight, g	0.093 ± 0.004	0.097 ± 0.004	0.094 ± 0.003
Heart/body weight, mg/g	3.89 ± 0.18	3.79 ± 0.11	3.65 ± 0.11
SBP, mm Hg	125 ± 8	106 ± 6	109 ± 5

Minicircle ACE2 delivery in the STZ model

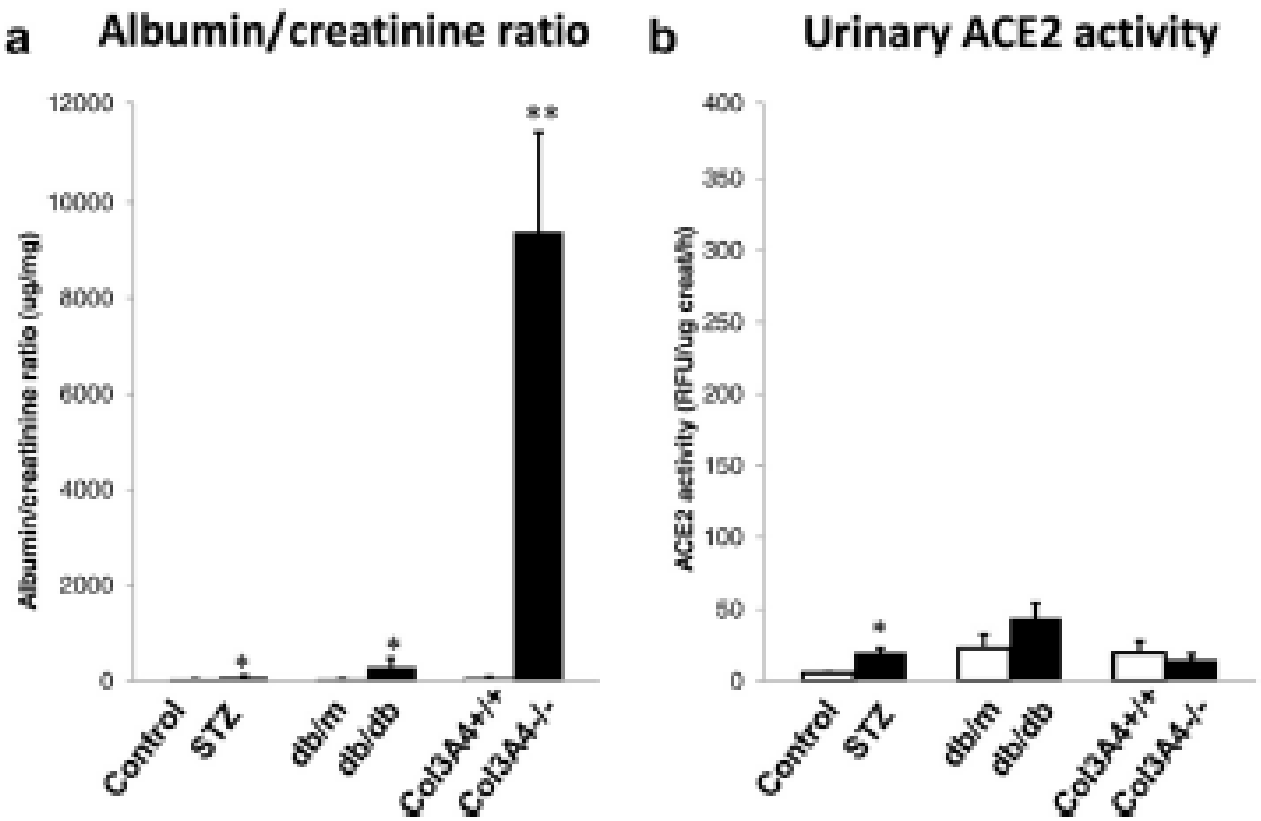




Wysocki et al, KI, 2017

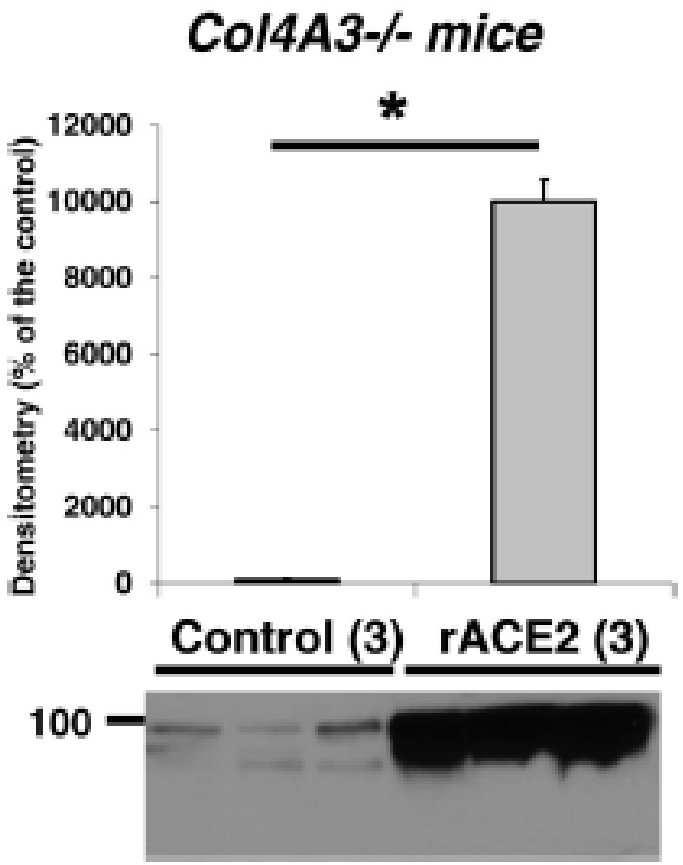
Northwestern Medicine®

Albumin excretion Rates in different model of Kidney Diseases

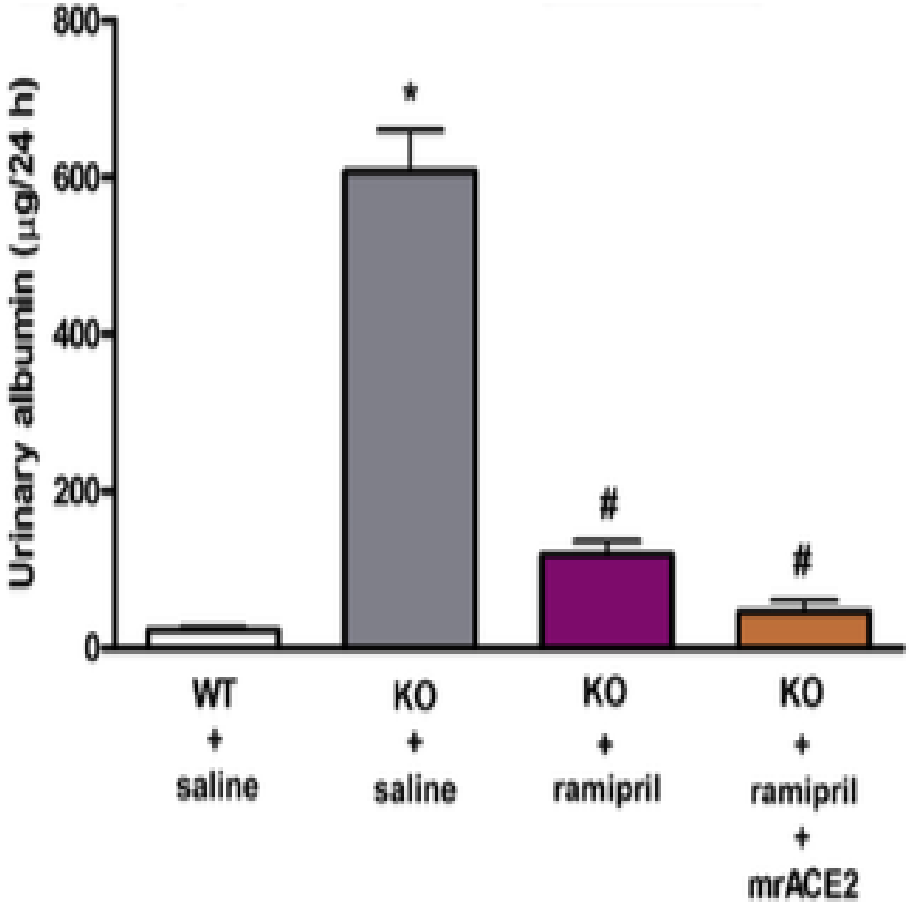


Northwestern Medicine

Recovery of ACE2 protein in the urine in the Alport Mice Model



Alport Syndrome- Murine Recombinant ACE2



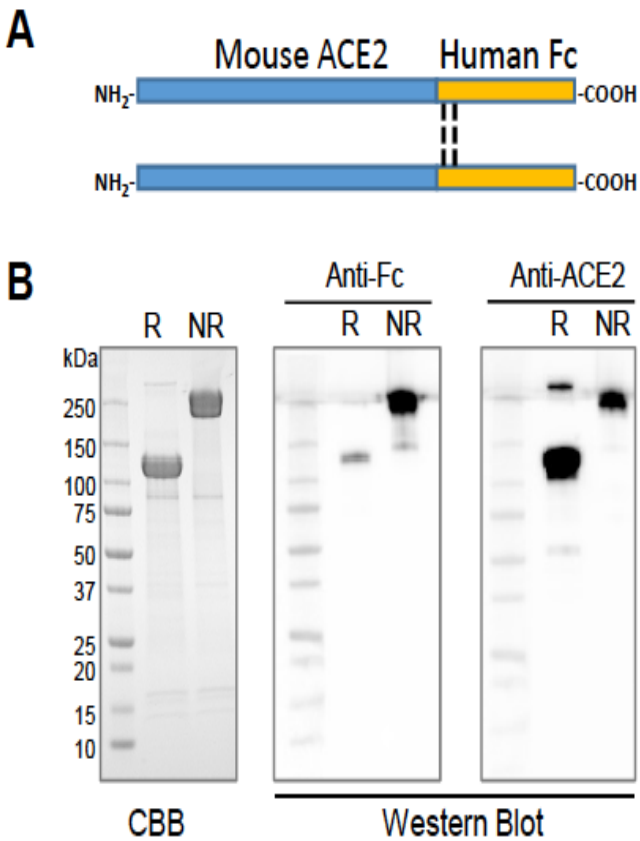
Bae et al, Kidney International,

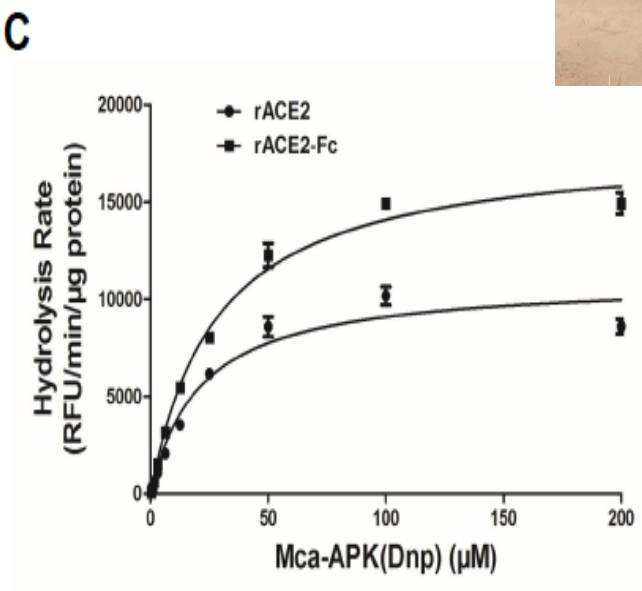
www.kidney-international.org

ACE2 as therapy for glomerular disease: the devil is in the detail

Michael J. Ross[1] and Masaomi Nangaku[2]

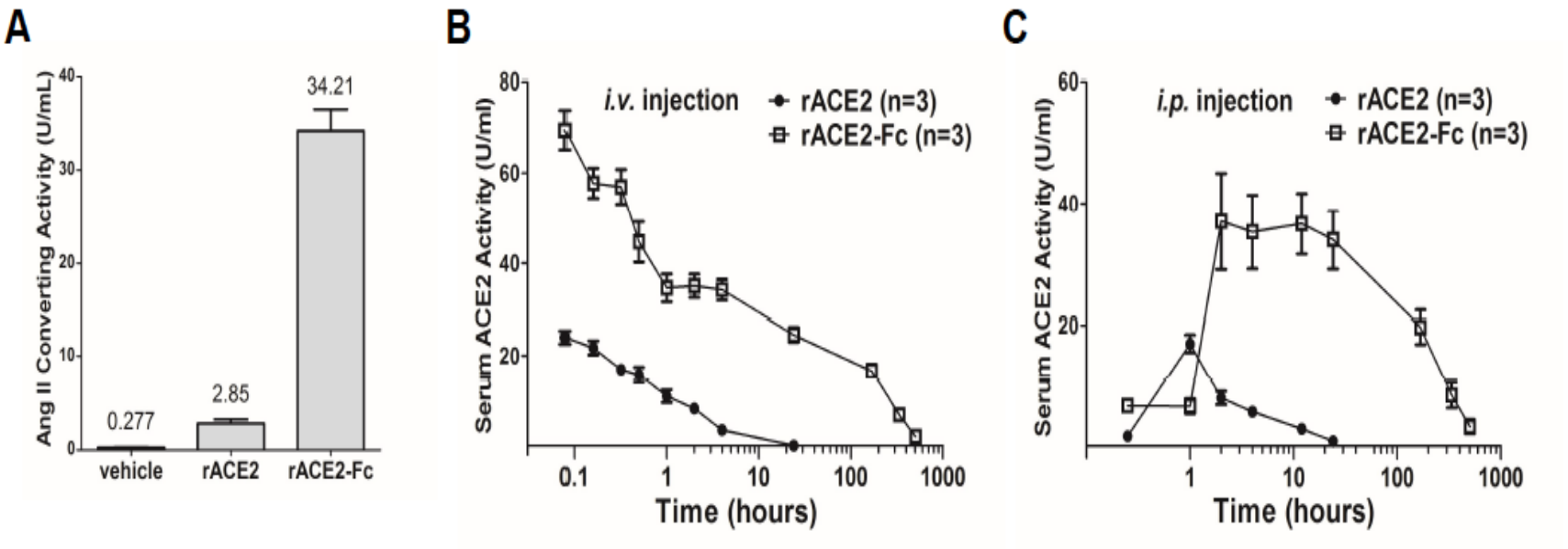
Extending ACE2 half life by Fc-fusion



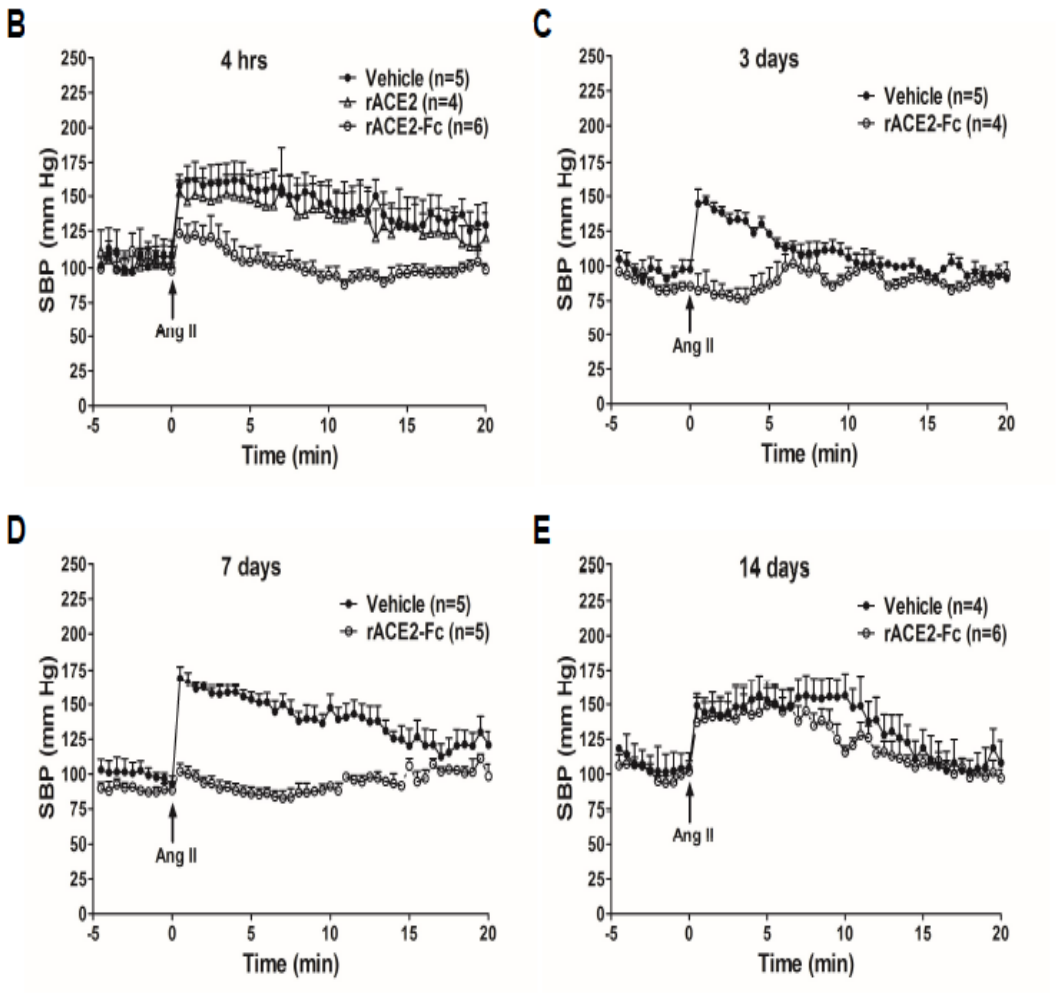


Enzyme	K_m (mM)	K_{cat} (min^{-1})	K_{cat}/K_m
rACE2	21.07 ± 2.92	4661 ± 198.9	221.21
rACE2-Fc	27.98 ± 2.18	7632 ± 196.5	272.77

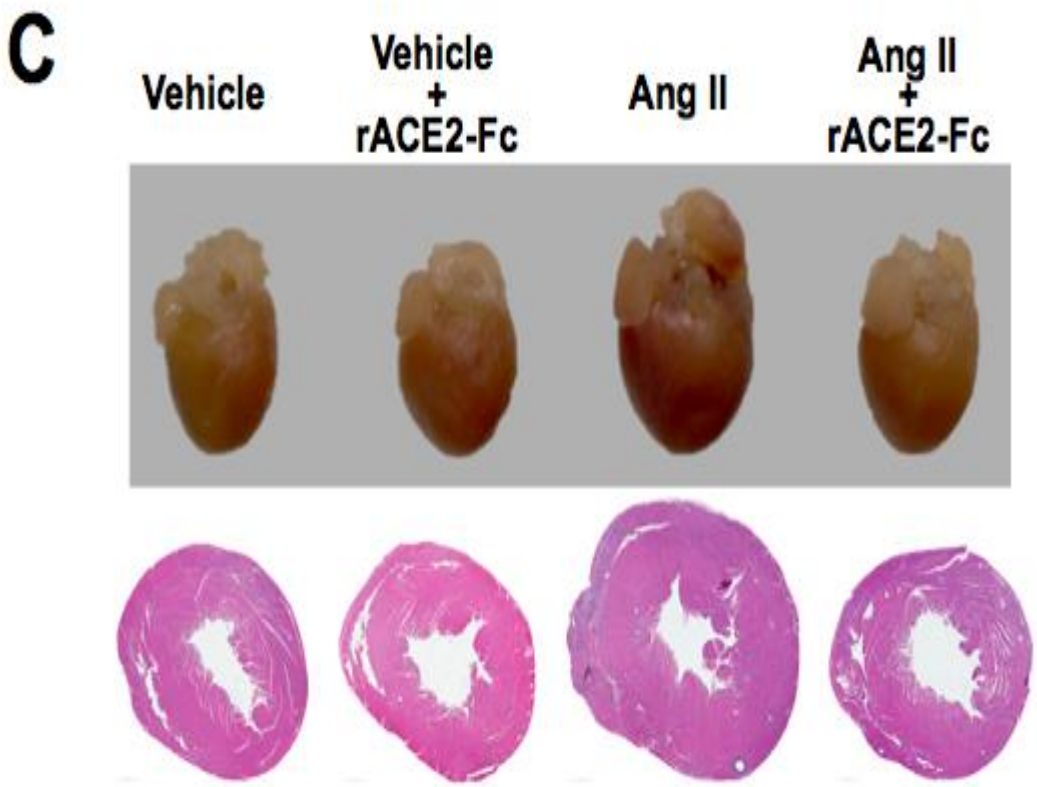
Extending ACE2 half life by Fc-fusion

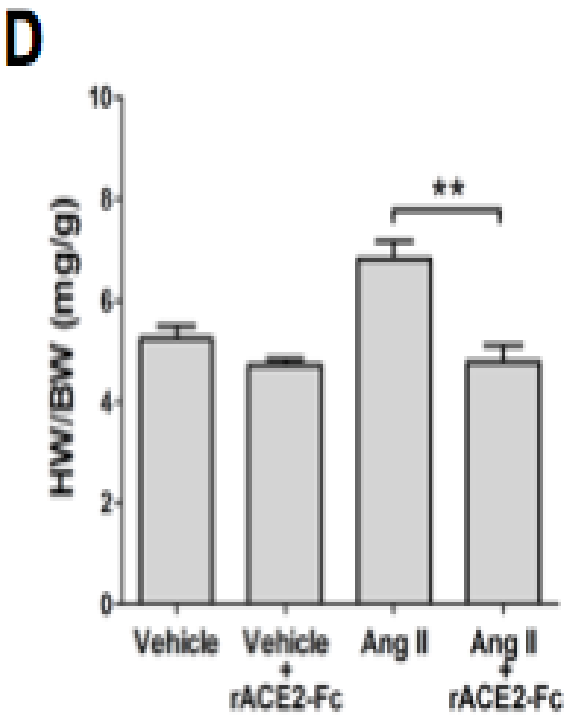


Extending ACE2 half life by Fc-fusion



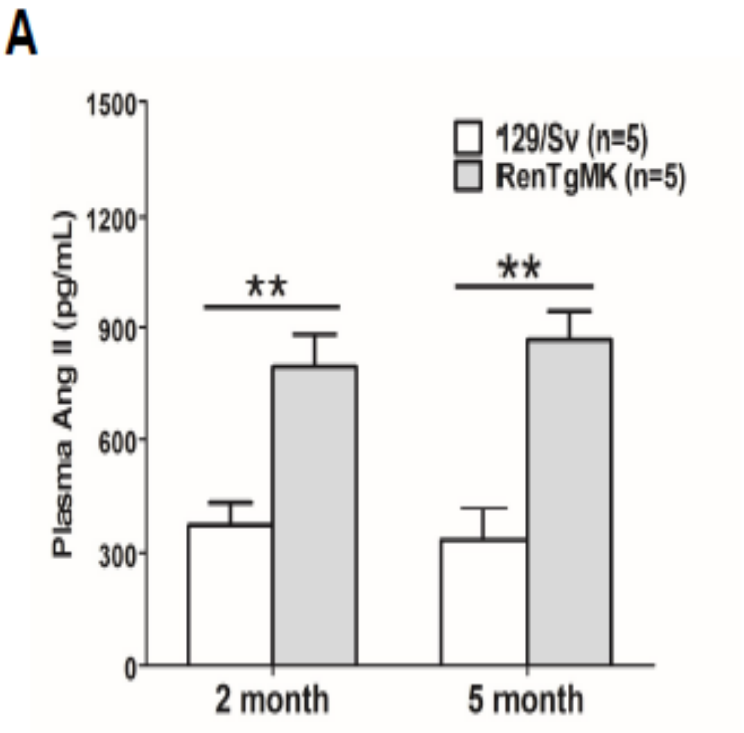
Effect of rACE2-Fc on Ang II-induced cardiac hypertrophy

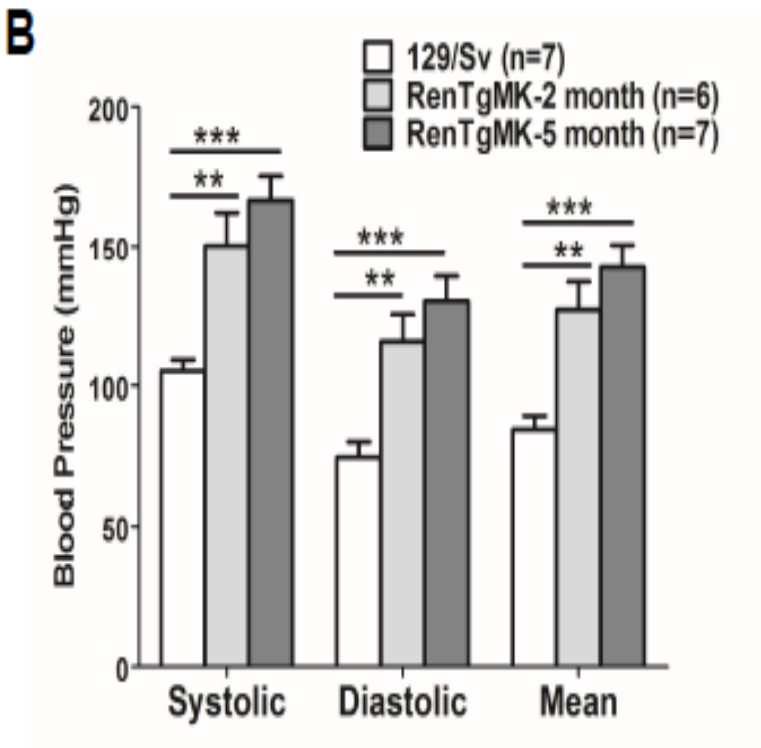




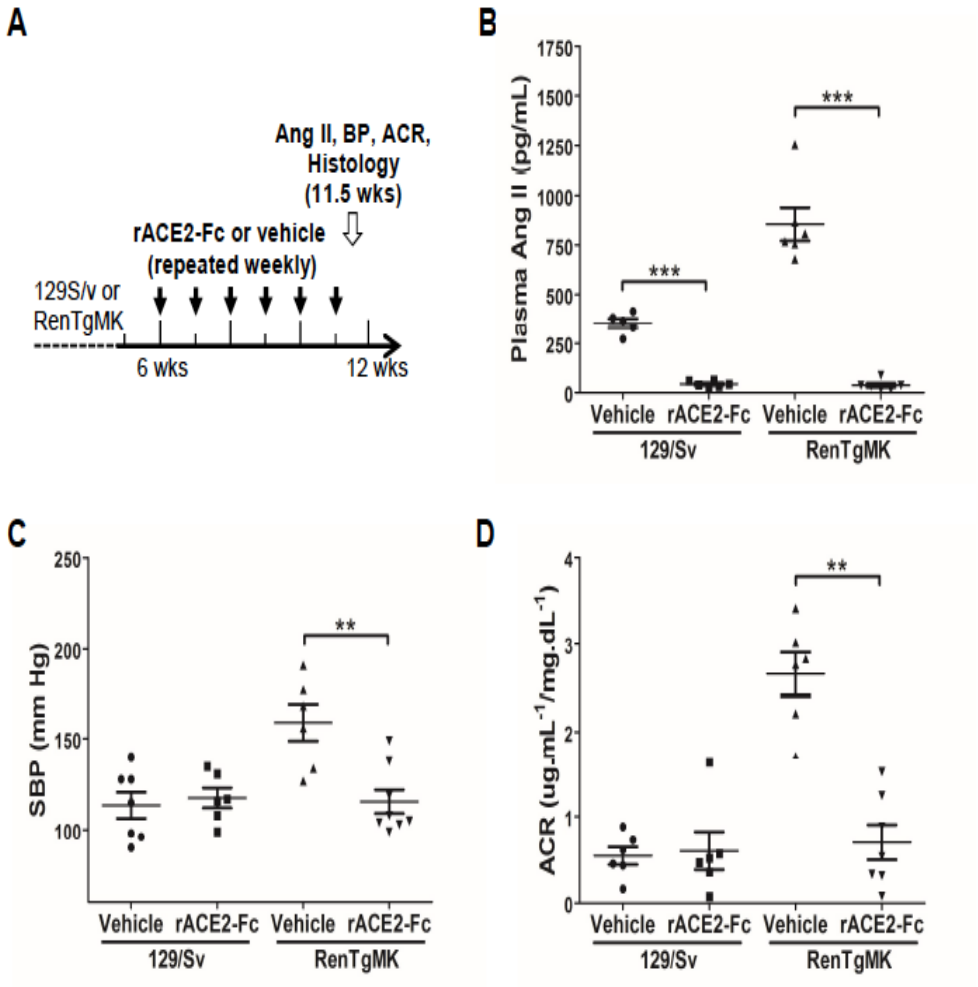
Pan liu et al, unpublished

Renin Transgenic Mice



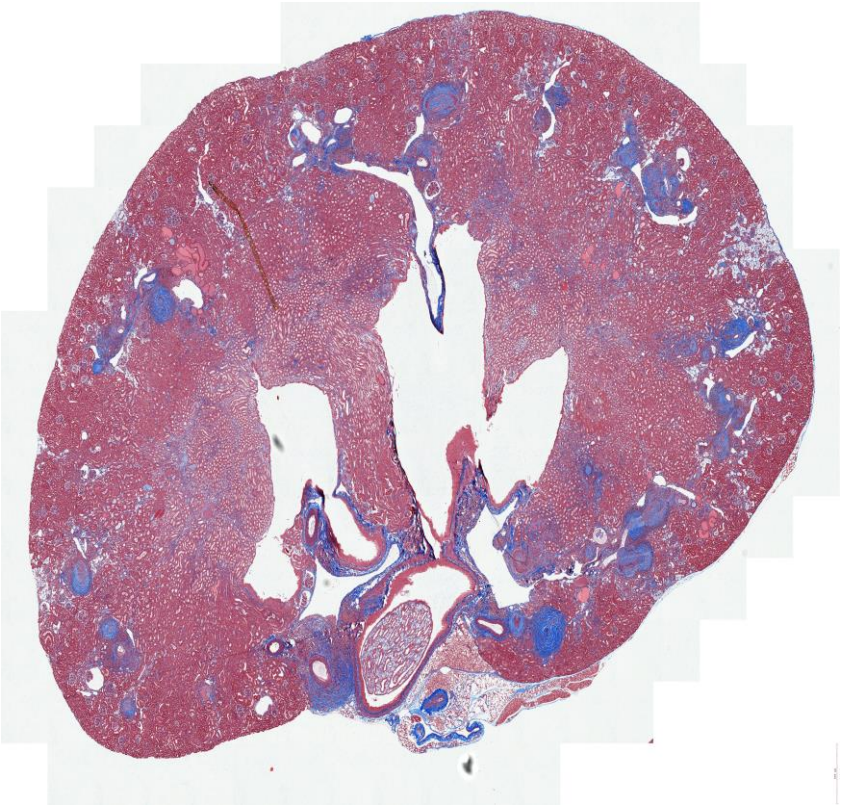


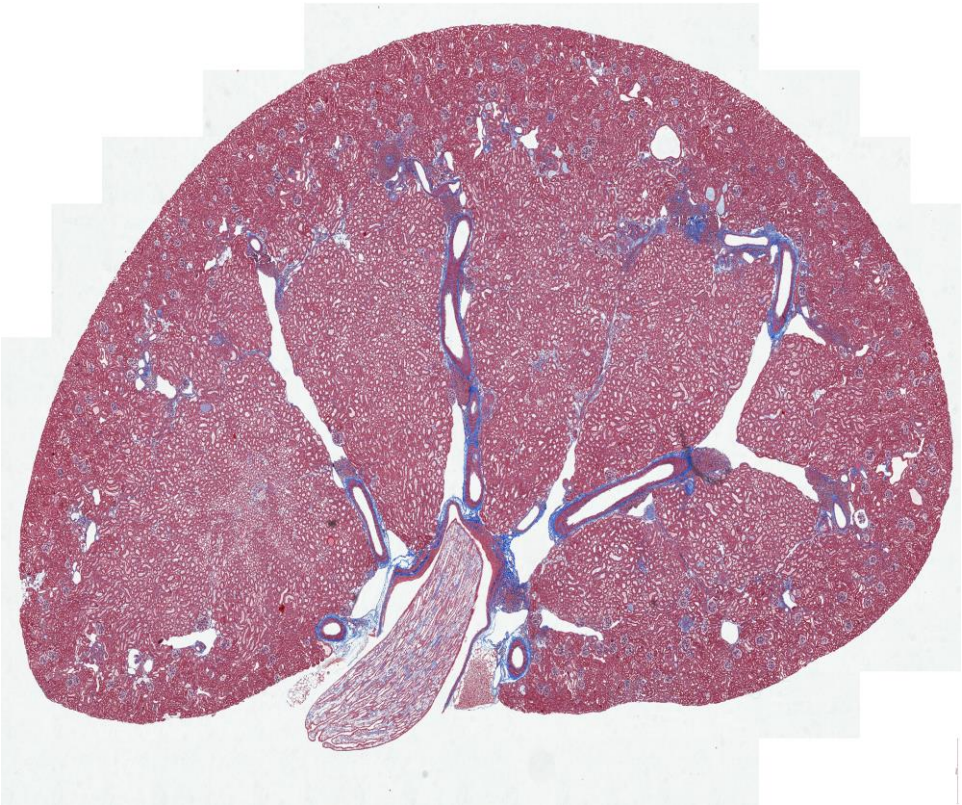
Effect of rACE2-Fc in the Renin transgenic mice (RnTgMK)



Northwestern Medicine®

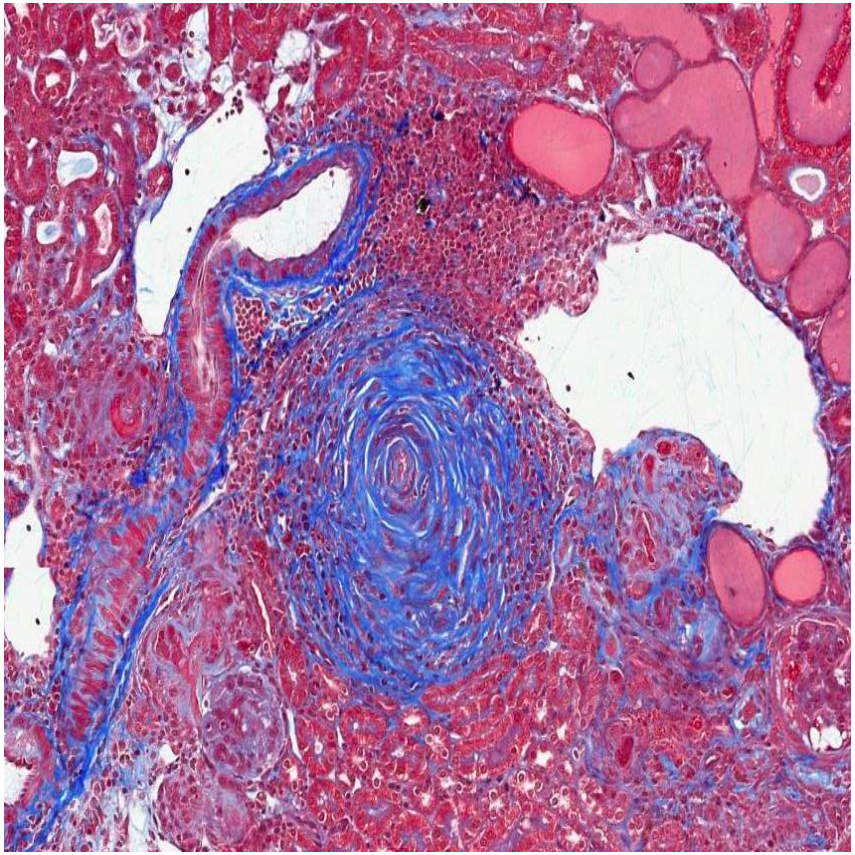
Renin Transgenic Model Untreated and Treated with rACE2-Fc

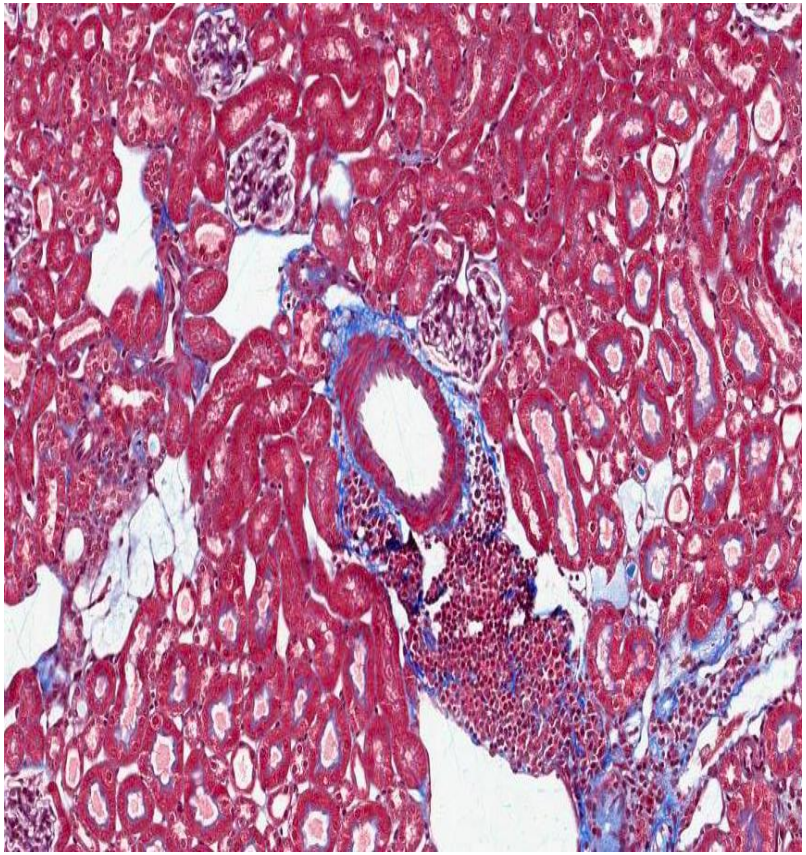




Northwestern Medicine®

Renin Transgenic Model Untreated and Treated with rACE2-Fc





Northwestern Medicine®

Ongoing Clinical Trials

- **Hormonal, Metabolic, and Signaling Interactions in PAH**

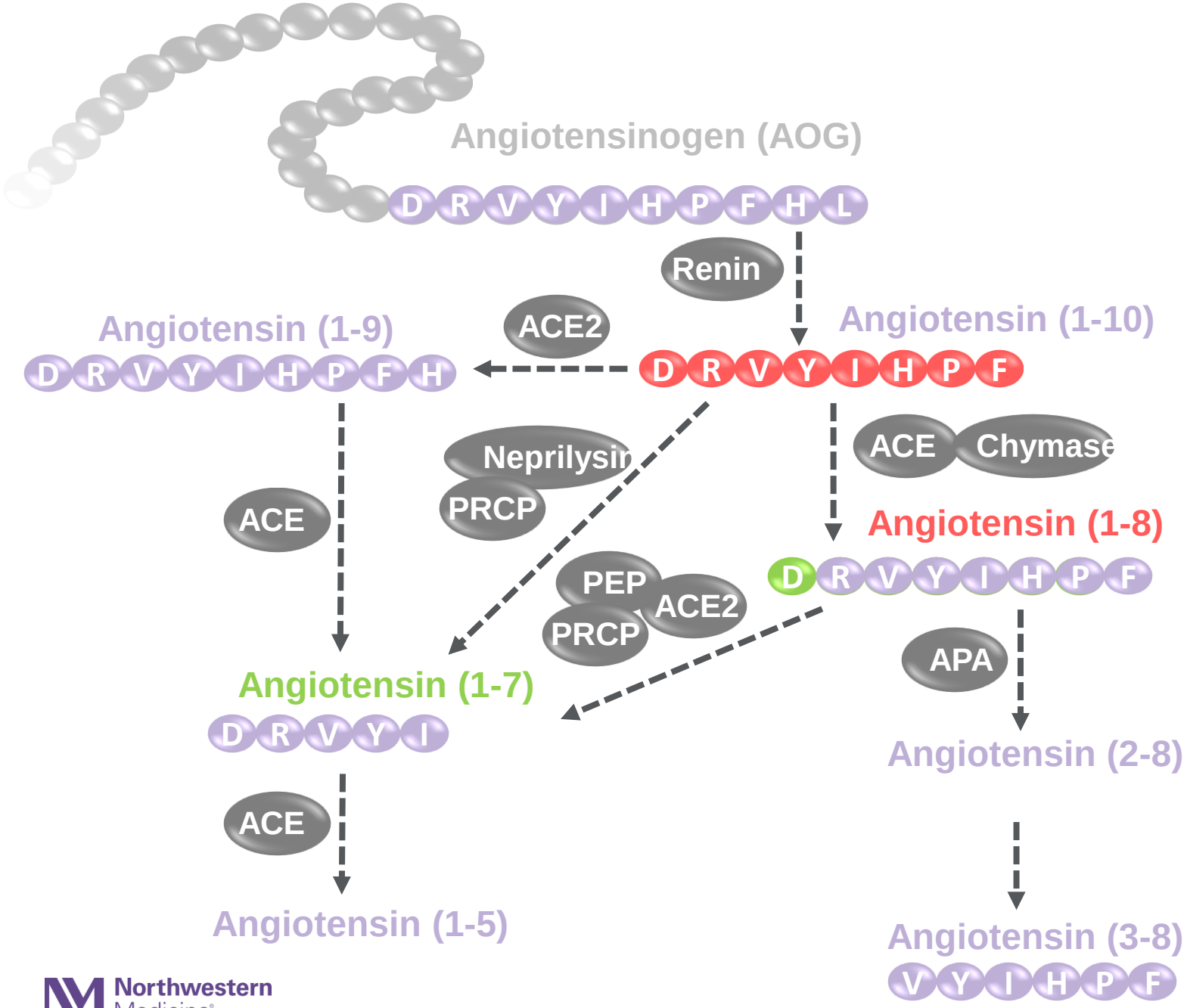
- **Study of GSK2586881 on Acute Hypoxia and Exercise**

- **Safety and Tolerability Study of APN01 (Recombinant Human**
- **Angiotensin Converting Enzyme 2)**

  COMPLETED

Summary

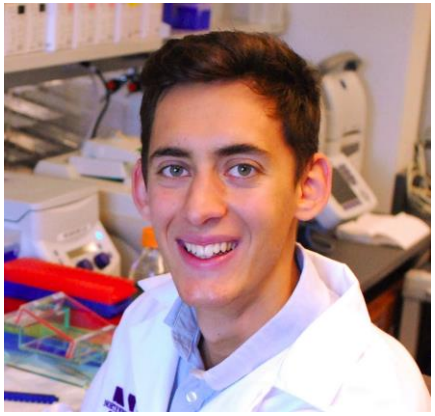
- Ace 2 amplification offers spectrum of potential therapeutic applications including:
  - Acute Lung Injury
  - Pulmonary Hypertension
  - Heart Failure and Cardiac Fibrosis
  - Diabetic Retinopathy
  - Certain types of diabetic and non diabetic kidney disease .
- While available presumed ACE2 activators provide therapeutic benefit the mechanism of action is unclear.
- Recombinant ACE2 protein with prolonged duration of action provides a therapeutically attractive strategy for ACE 2 amplification and down regulation of hyperactive RAS systemically
- Effective kidney delivery remains a challenge for early diabetic kidney disease



Northwestern Medicine®

Jan Wysocki MD PHD

Tilman Muller Alonso Marquez



Peter Daniel Serfozo

Jeannette Tang

Alejandro Sanchez

Sheeba Habeed

Jing Jin

Pan Liu

Northwestern Medicine

Northwestern Medicine®

Batlle's Laboratory

Feinberg School of Medicine Chicago IL
Division of Nephrology and Hypertension

Jan Wysocki
Minghao Ye
Sheeba Habeeb
Jeannette Tang
Tilman Mueller
Alejandro Sanchez
Alonso Marquez