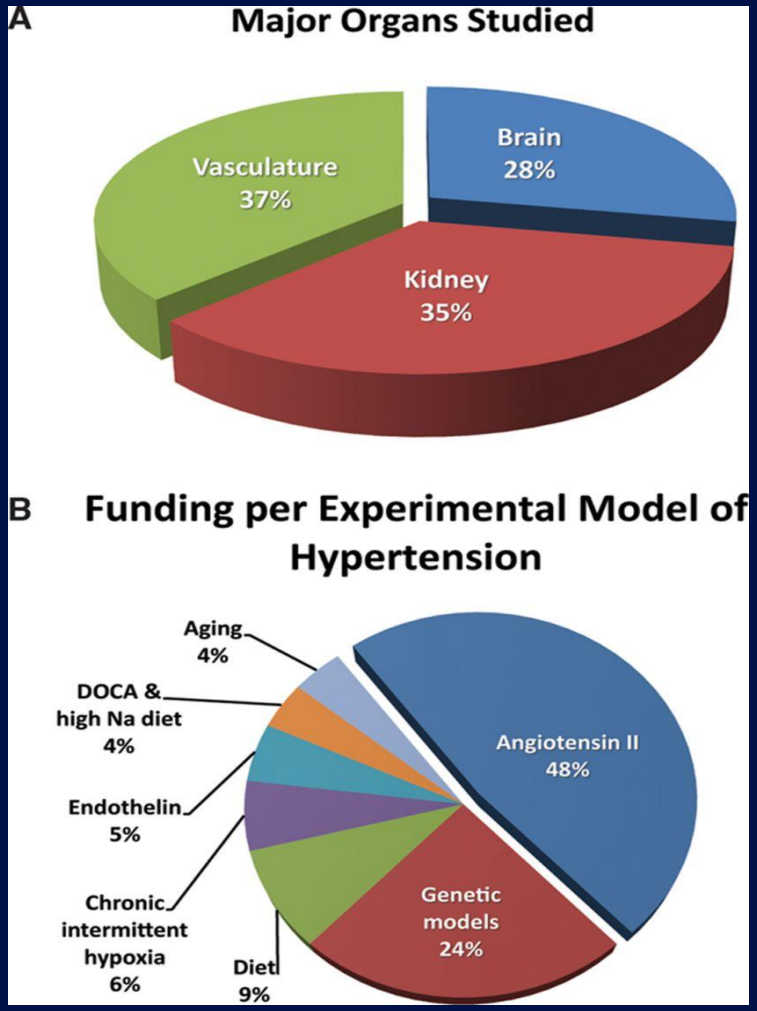
New Frontiers in The Intra-Renal Renin-Angiotensin System

Jia Long Zhuo (卓家隆), M.D., Ph.D., FAHA, FASN

Professor

Department of Pharmacology and Toxicology
Center of Excellence for Cardiovascular and Renal Research
Division of Nephrology and Hypertension
University of Mississippi Medical Center
Jackson, Mississippi, USA

Impact of RAS in cardiovascular and hypertension research in the USA



Analysis of Vascular Biology and Hypertension Branch (VBHB)-sponsored hypertension research by (A) major target organ studied and (B) major experimental model used.

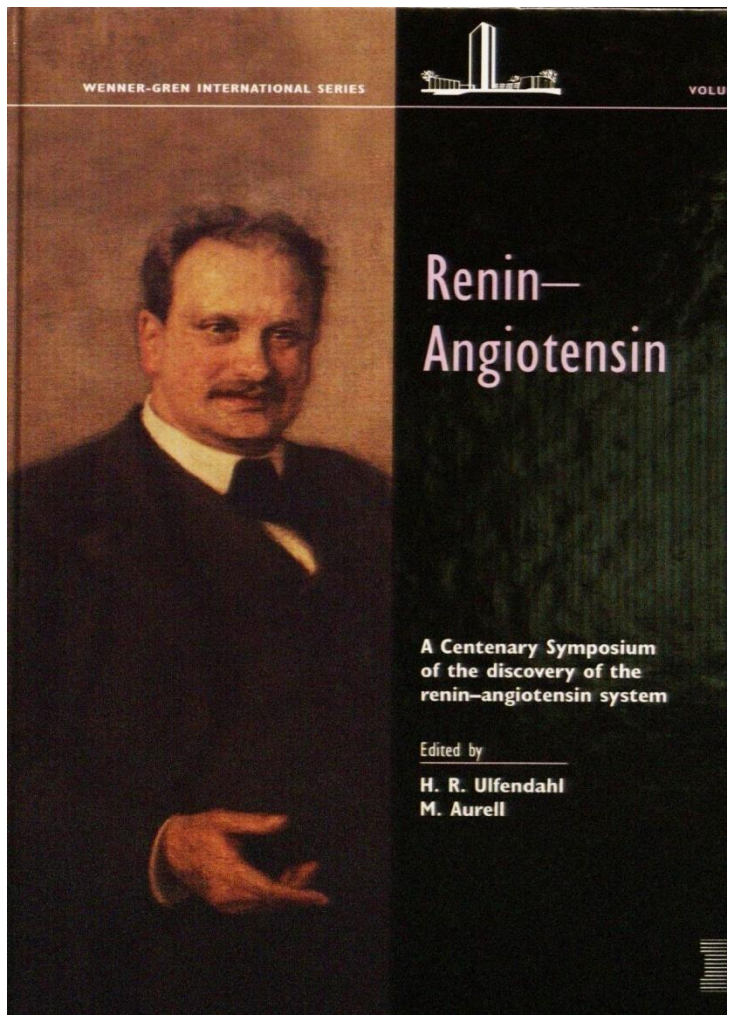
American Heart Association.

Galis Z S et al. Hypertension. 2013;61:757-761

Outline of the presentation

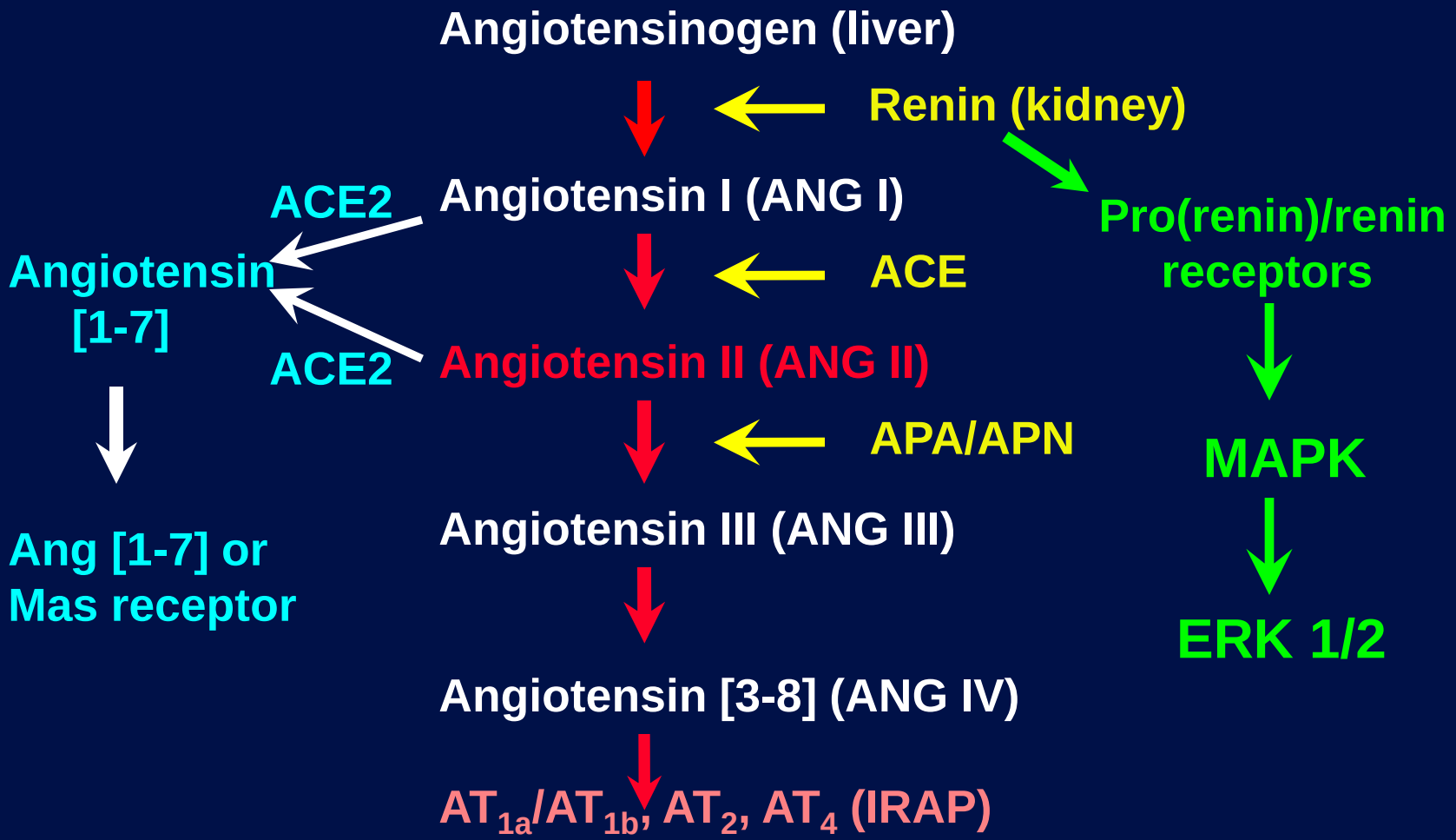
- Historical perspectives of the renin-angiotensin system (RAS)
- New frontiers in the RAS research field
- Anatomical localization of the intrarenal and/or intratubular RAS
- New insights into the roles of intracrine or intracellular RAS in the regulation of blood pressure

The RAS:
a centenary-old humoral system with evolving endocrine, paracrine, and intracrine roles



- Renin was discovered > a centenary ago by Robert Tigerstedt in 1898.
- The hypertensive role of the kidney renin was confirmed by Harry Goldblatt in his legendary studies on 2-kidney, 1-clip renal hypertension in 1934.
- Before 1980's, ANG II was considered a circulating or endocrine peptide that is secreted by the kidney and acts systemically in target tissues.
- ANG II was later recognized as both an endocrine and a local paracrine peptide.
- There is increasing evidence that ANG II may act as an intracrine or intracellular peptide.

The RAS:
Classical and nonclassical pathways

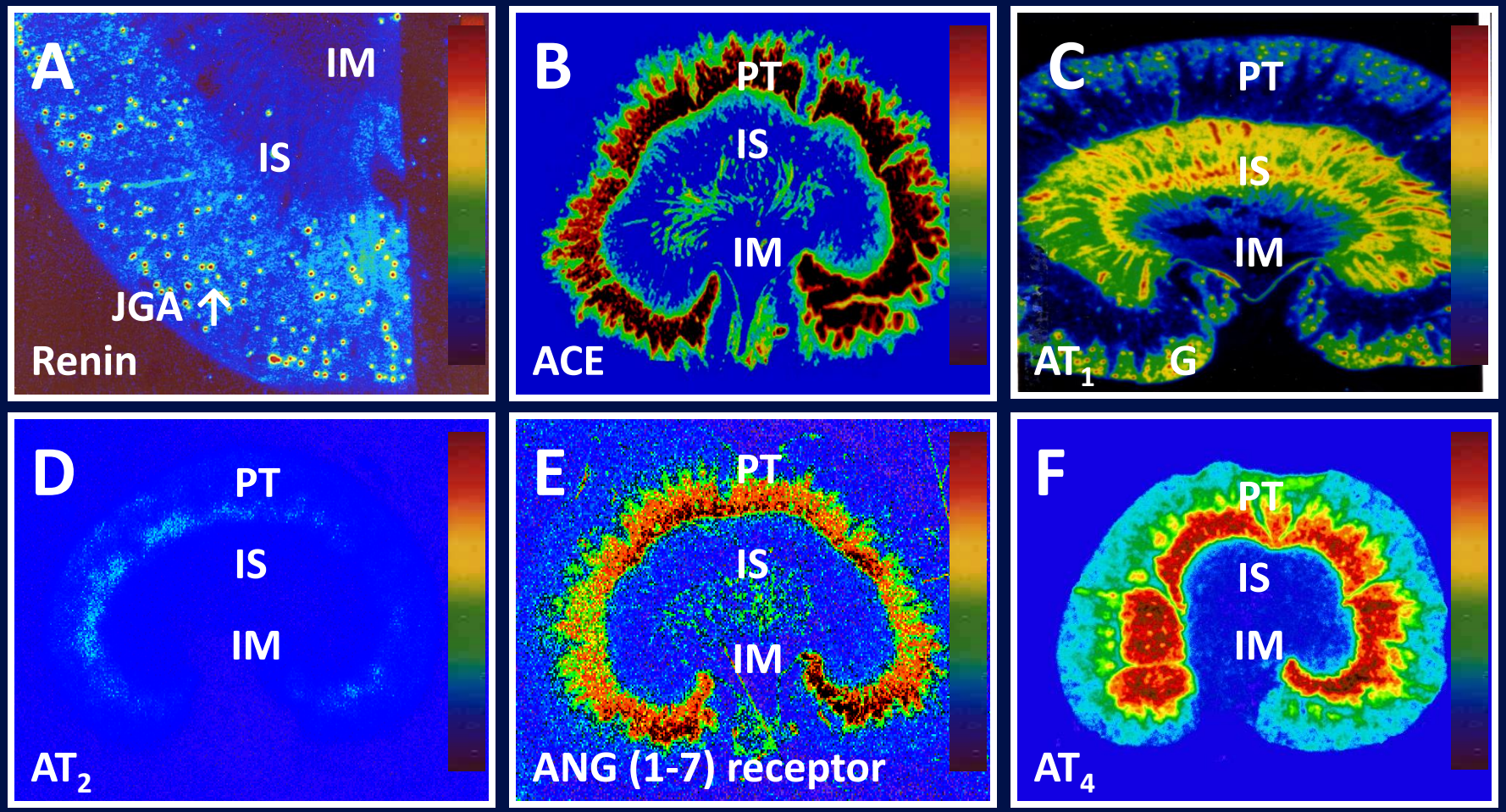


Zhuo & Li, Peptides 2011

Classical & nonclassical angiotensin receptor signaling

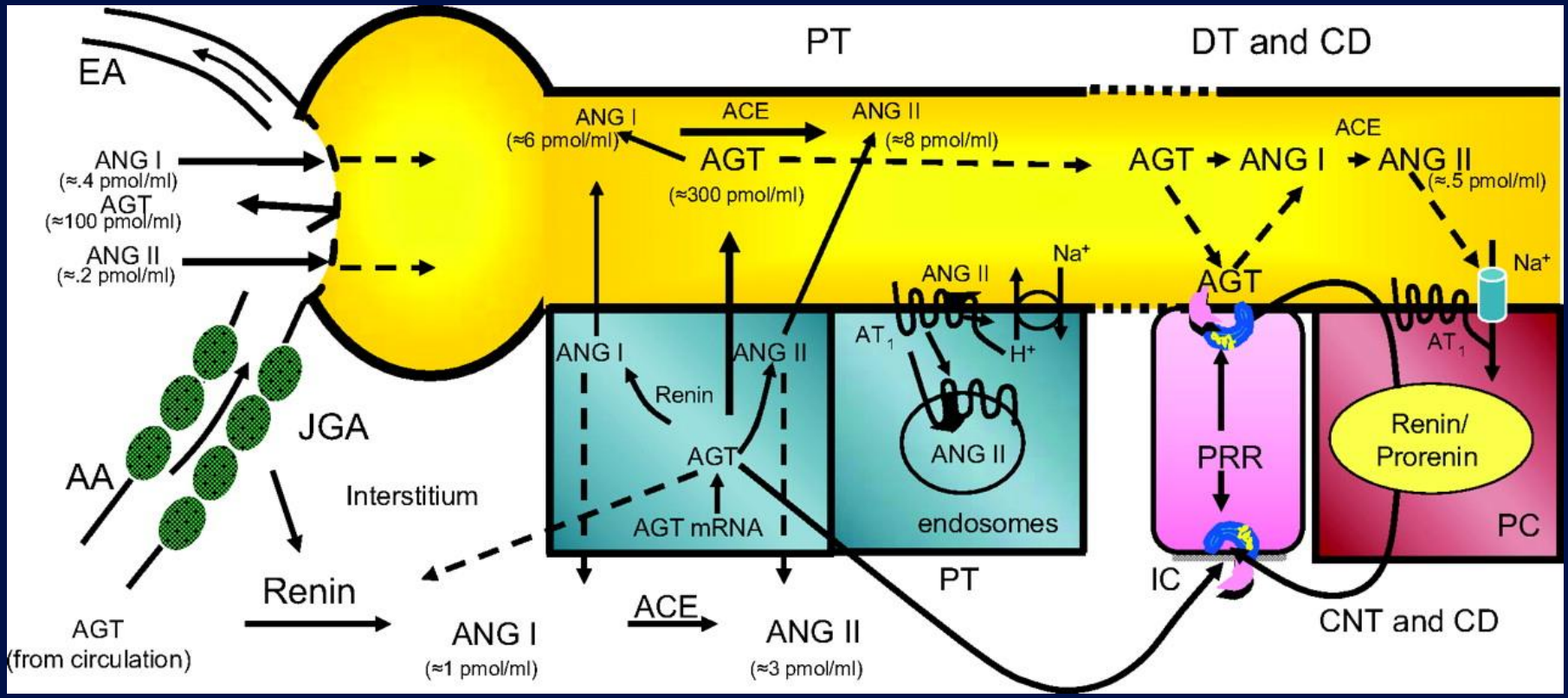
Receptor:	AT_1	AT_2	$AT_{(1-7)}$	AT_4
Subtype:	AT_{1a} & AT_{1b}	None	None	None
Structure:	7-TM 359 aa	7-TM 364 aa	GPCR Mas R?	IRAP
Signaling:	Gq/11 PLC-β IP_3, Ca^{2+} PKC MAPK JAK/STAT	G-proteins MAPK Protein tyrosine phosphatases NO ……	NO/BK /cGMP?	Glucose uptake Memory
Localization:	Kidney, liver, heart, vessel, adrenal and brain.			

The RAS in the kidney: Anatomical and cellular localization as visualized by quantitative in vitro autoradiography



Zhuo & Li, Frontiers in Cell Endocrinol 2013

Intratubular RAS and its potential role in ANG II-dependent hypertension
肾小管内肾素血管紧张素II系统在高血压发病机制中的关键作用

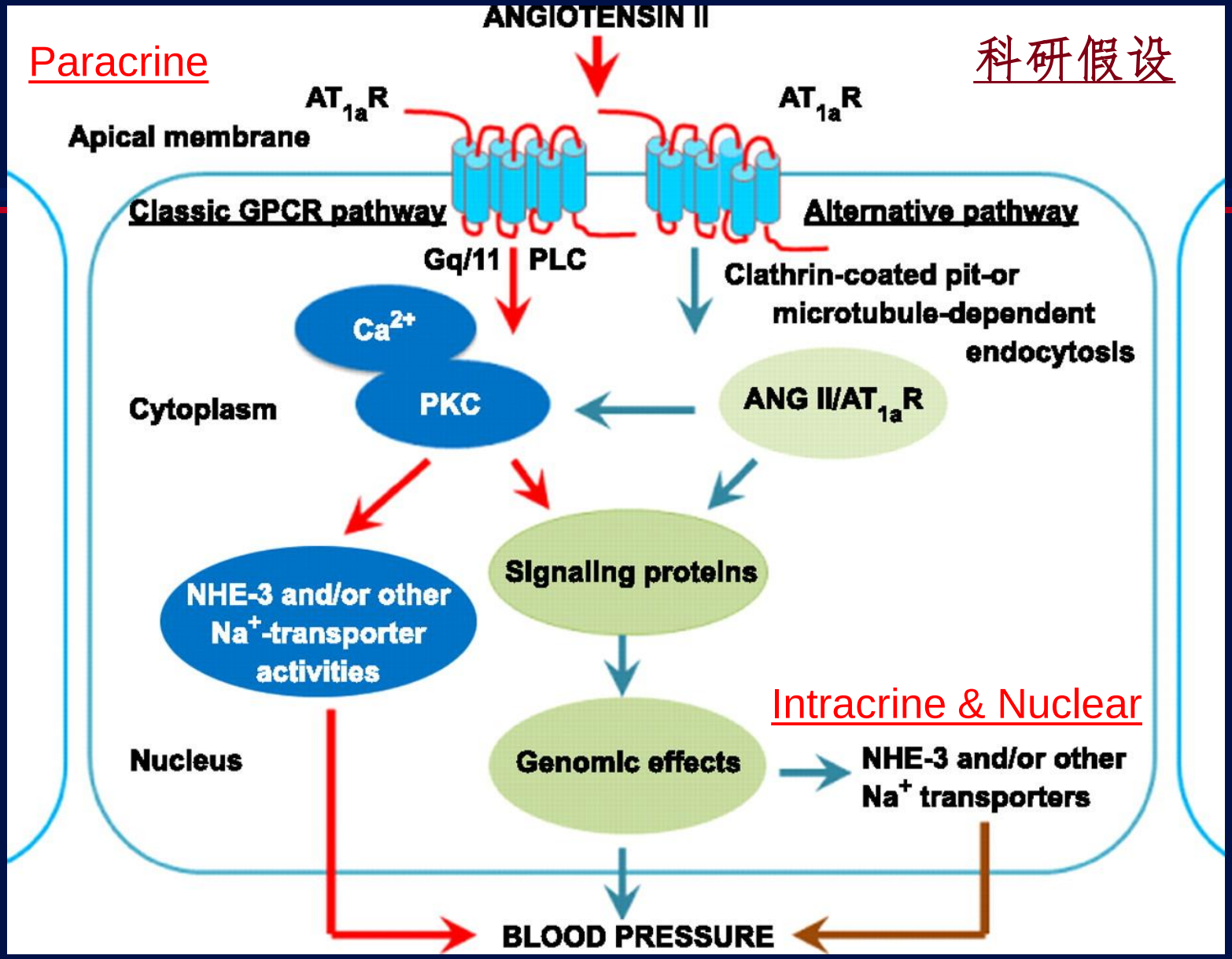


Navar L G et al. *Hypertension*. 2011;57:355-362

American Heart Association.

Intracrine or intracellular ANG II: A new player with physiological, pharmacological and clinical relevance

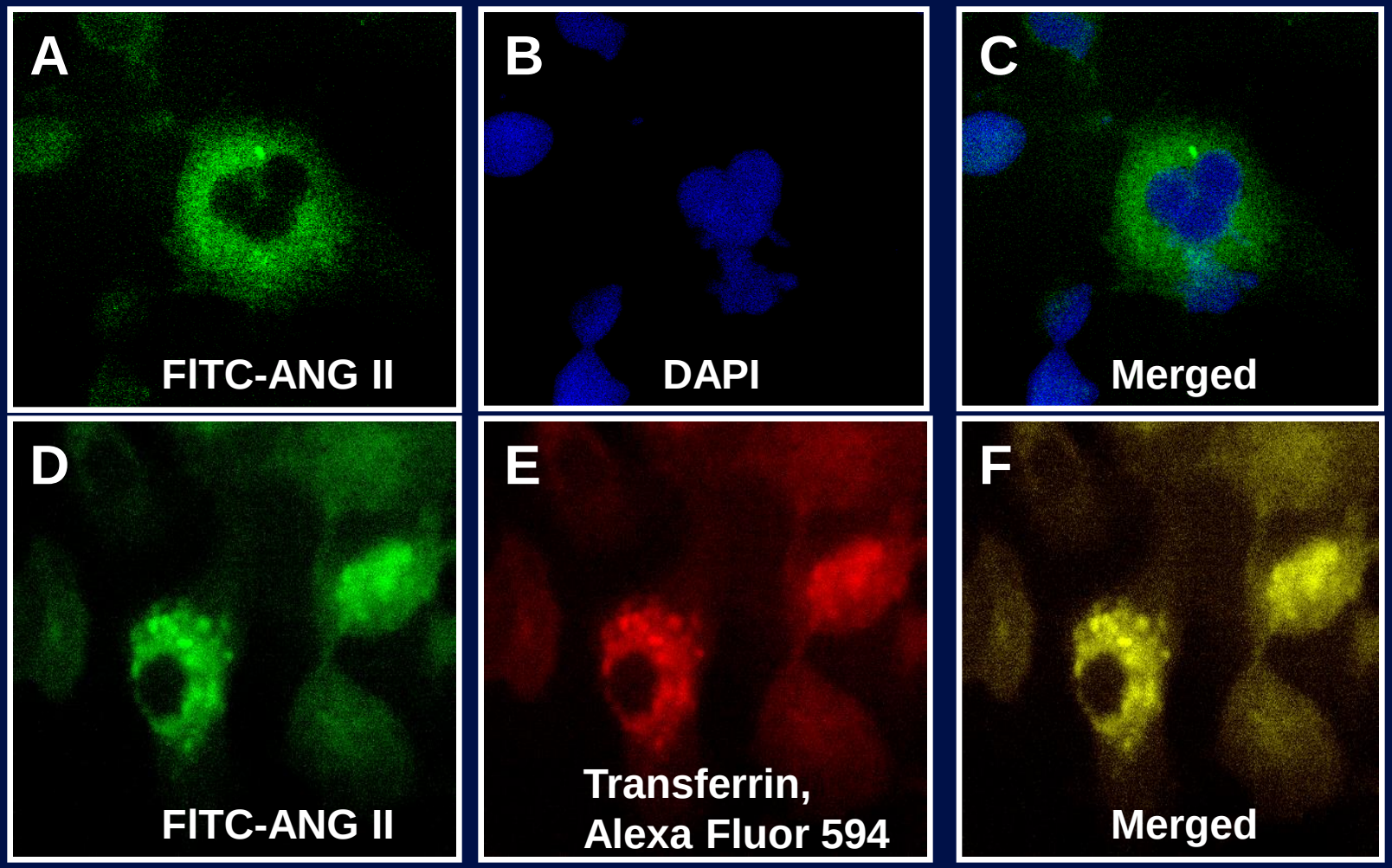
- ANG II exerts long-term genomic effects, which may not be induced entirely by activation of cell surface G protein-coupled receptors (GPCR).
- ANG II receptors are desensitized upon stimulation, with ANG II receptors internalized after ANG II binds and activates cell surface receptors.
- Long-term infusion of ANG II induces hypertension and target organ damage, suggesting that internalized ANG II/receptor may continue to transmit signals to induce intracellular and nuclear effects.
- Not all ANG II receptor blockers (ARB) are created equal to block the actions of circulating and intracellular ANG II due to their different lipophilic abilities.
- Clinically, only 15% to 30% of ARB-treated hypertensive patients may achieve blood pressure-reducing target of 140/90 mmHg.



Ellis J, Li XC, Zhuo JL. Am J Physiol Regul Integr Comp Physiol 2012;302:R494-R509

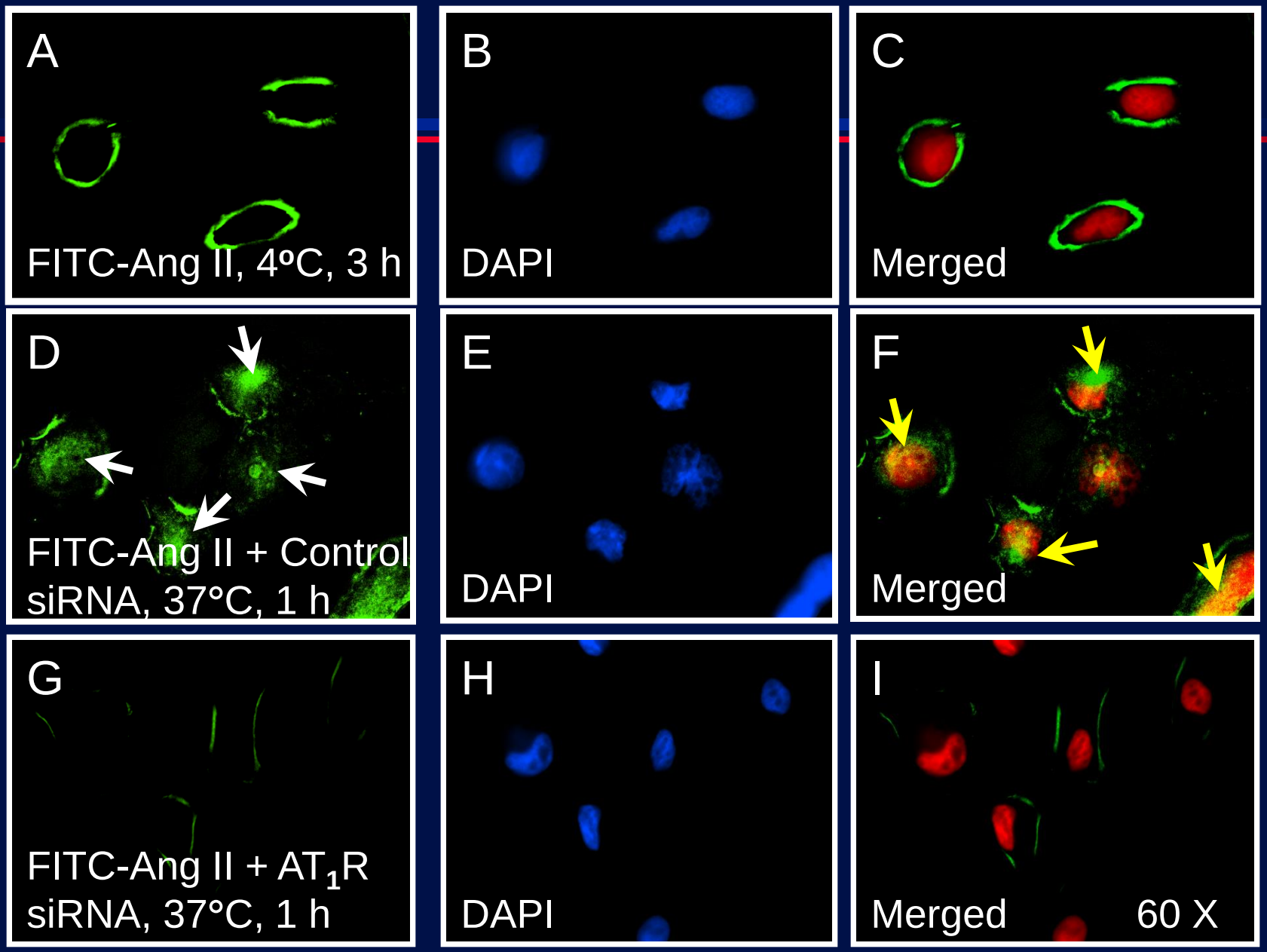
AMERICAN JOURNAL OF PHYSIOLOGY
Regulatory, Integrative and Comparative Physiology

In vitro evidence that FITC-labeled ANG II is taken up by proximal tubule (PT) cells (live cell fluorescent imaging)



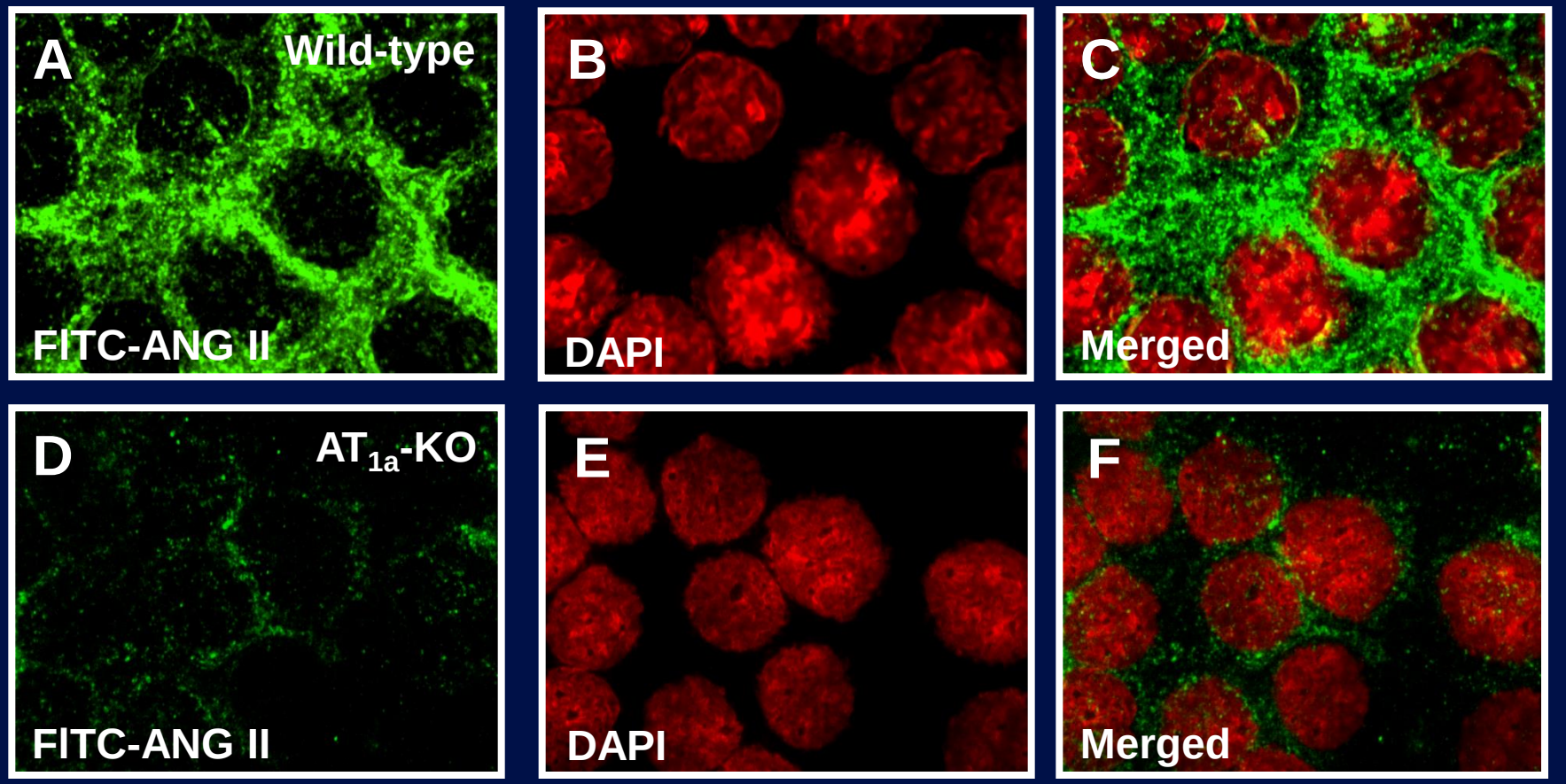
Am J Physiol Renal Physiol 291: F375-F383, 2006.

AT_{1a} receptor-mediated uptake of FITC-ANG II in PT cells



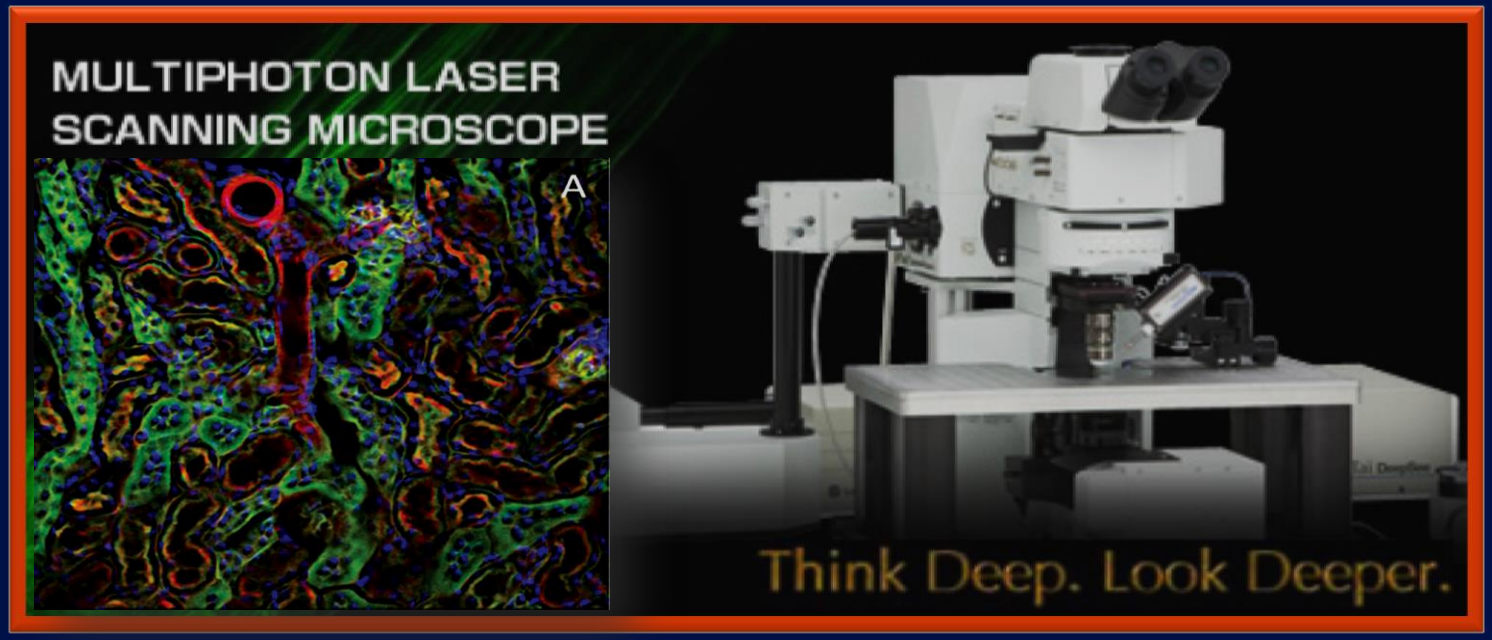
Li, X. C. et al. Am J Physiol Renal Physiol 297:F1342-F1352, 2009

AT_{1a} receptor-mediated ANG II uptake in wild-type, but not AT_{1a}-KO, mouse proximal tubule cells



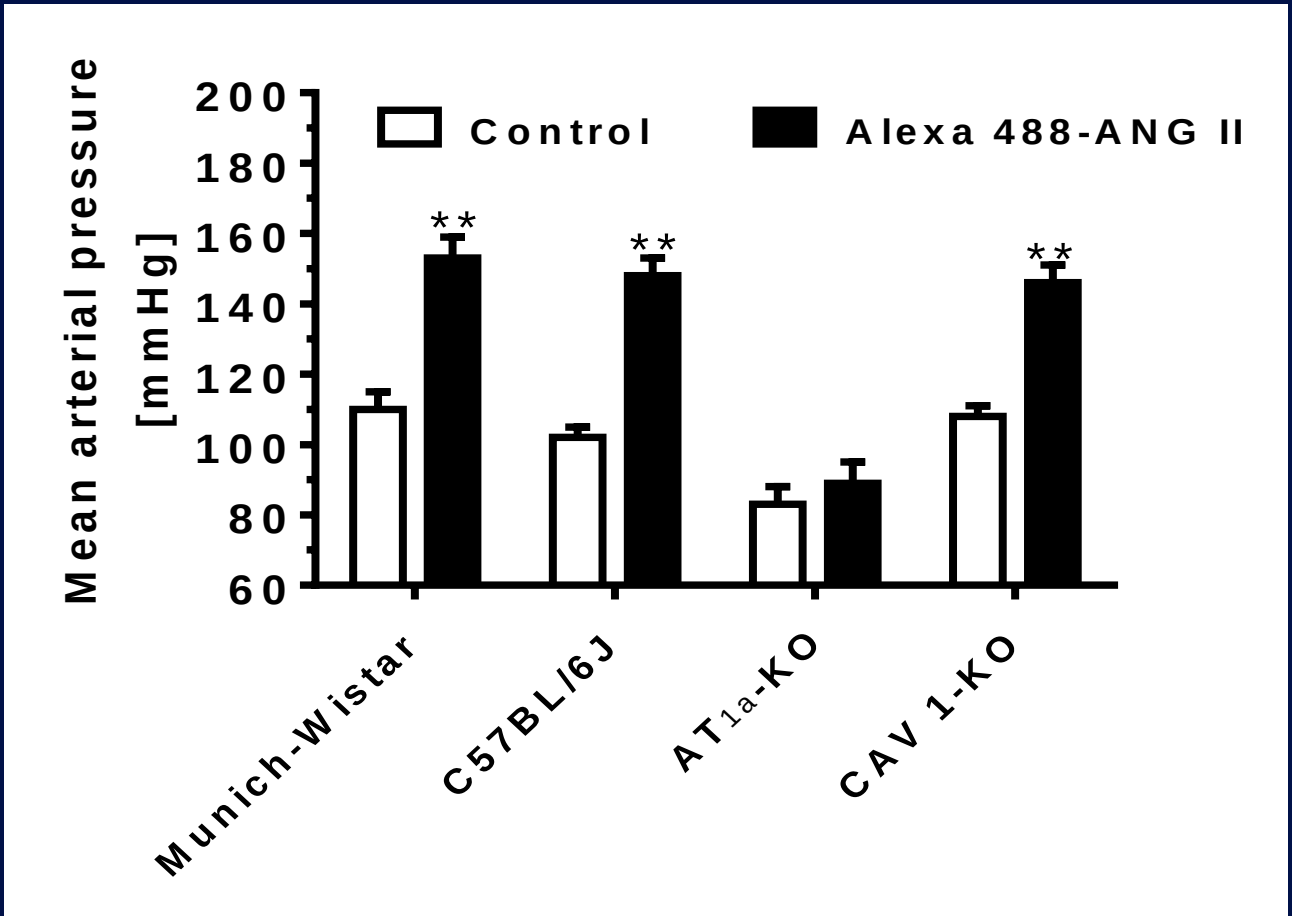
Am J Physiol Renal Physiol 297:F1342-F1352, 2009

In vivo evidence of AT_1 (AT_{1a}) receptor-mediated uptake of ANG II by the proximal tubule of the kidney, as revealed by intravital multiphoton imaging



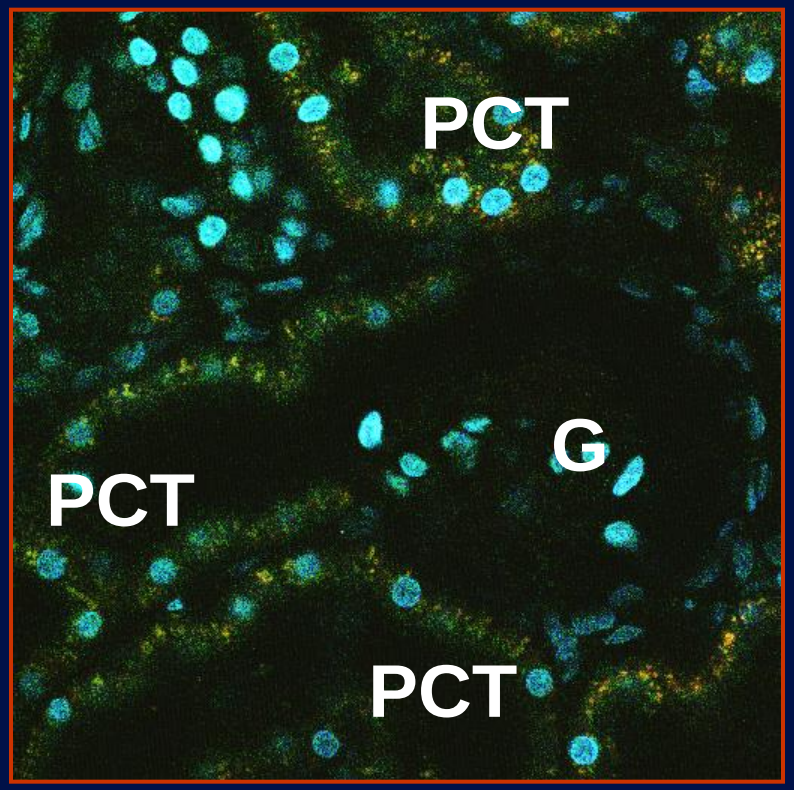
Intravital multiphoton fluorescence microscope system
George M. O'Brien Center, Indiana University

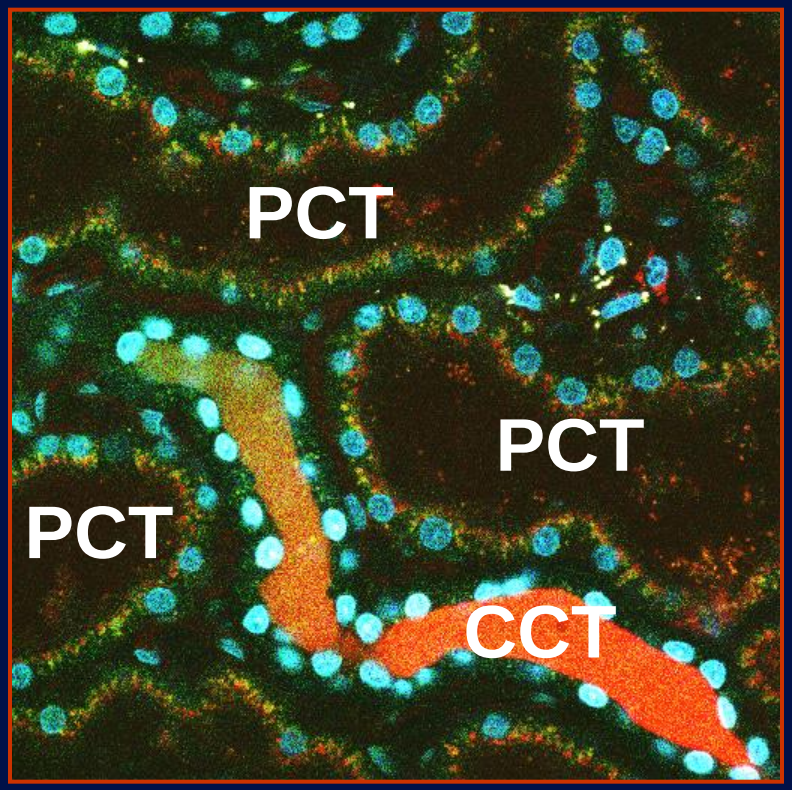
Effect of Alexa Fluor® 488-ANG II on arterial blood pressure in anesthetized rats or mice



Li XC et al. Am J Physiol Renal Physiol 2014

Intravital multiphoton imaging of Alexa Fluor® 488-ANG II uptake in the proximal tubule of the rat kidney





Green: Alexa 488-ANG II

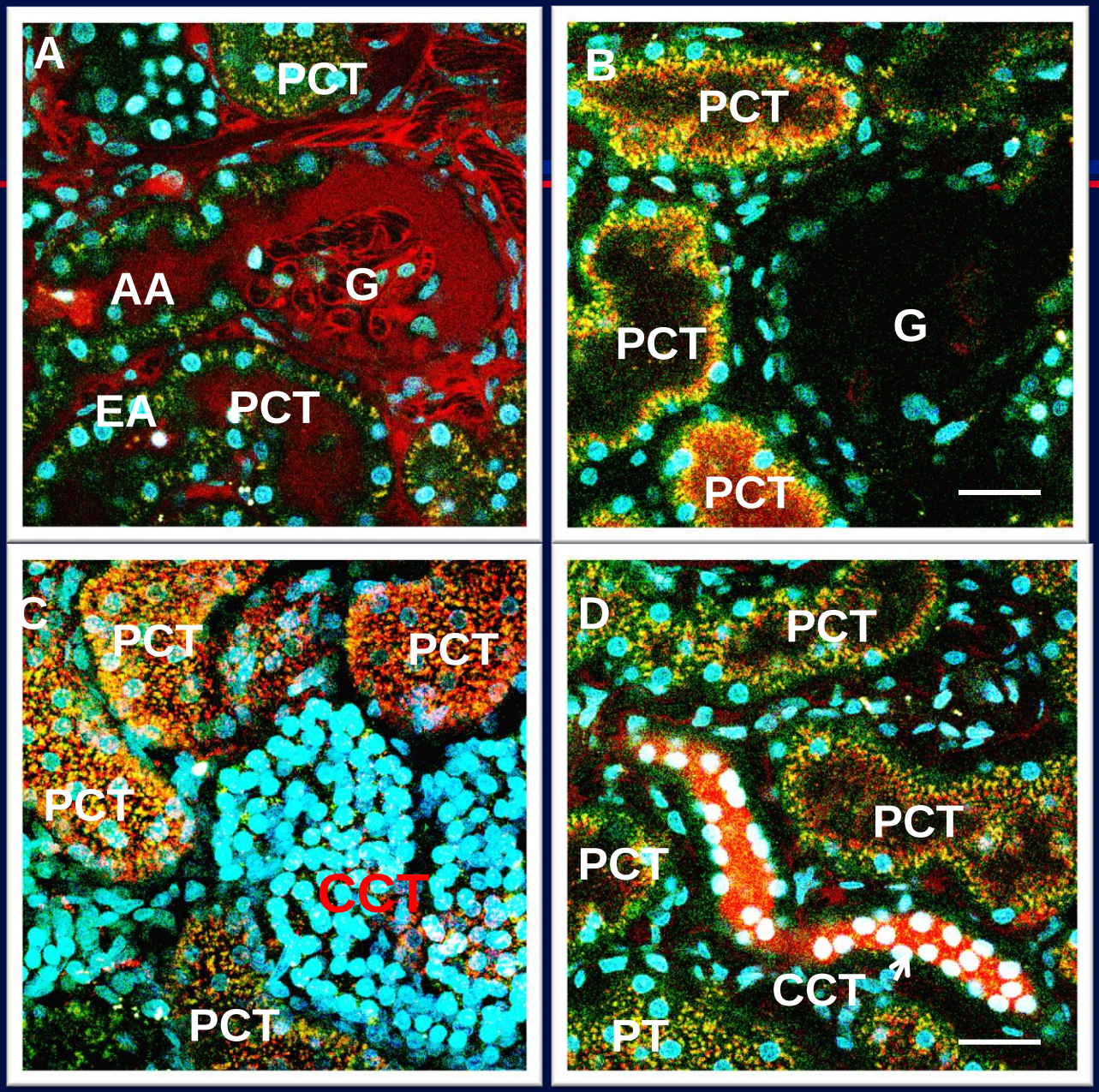
Red: Texas Red-labeled dextran

Blue = Hoechst 33342-labeled nuclei

G = glomerulus PCT = proximal convoluted tubule

CCT = cortical collecting tubule

Intravital Multiphoton imaging of Alexa 488-ANG II in rat kidney



Green = Alexa 488-ANG II uptake

Red = Texas Red-labeled dextran as proximal tubule marker

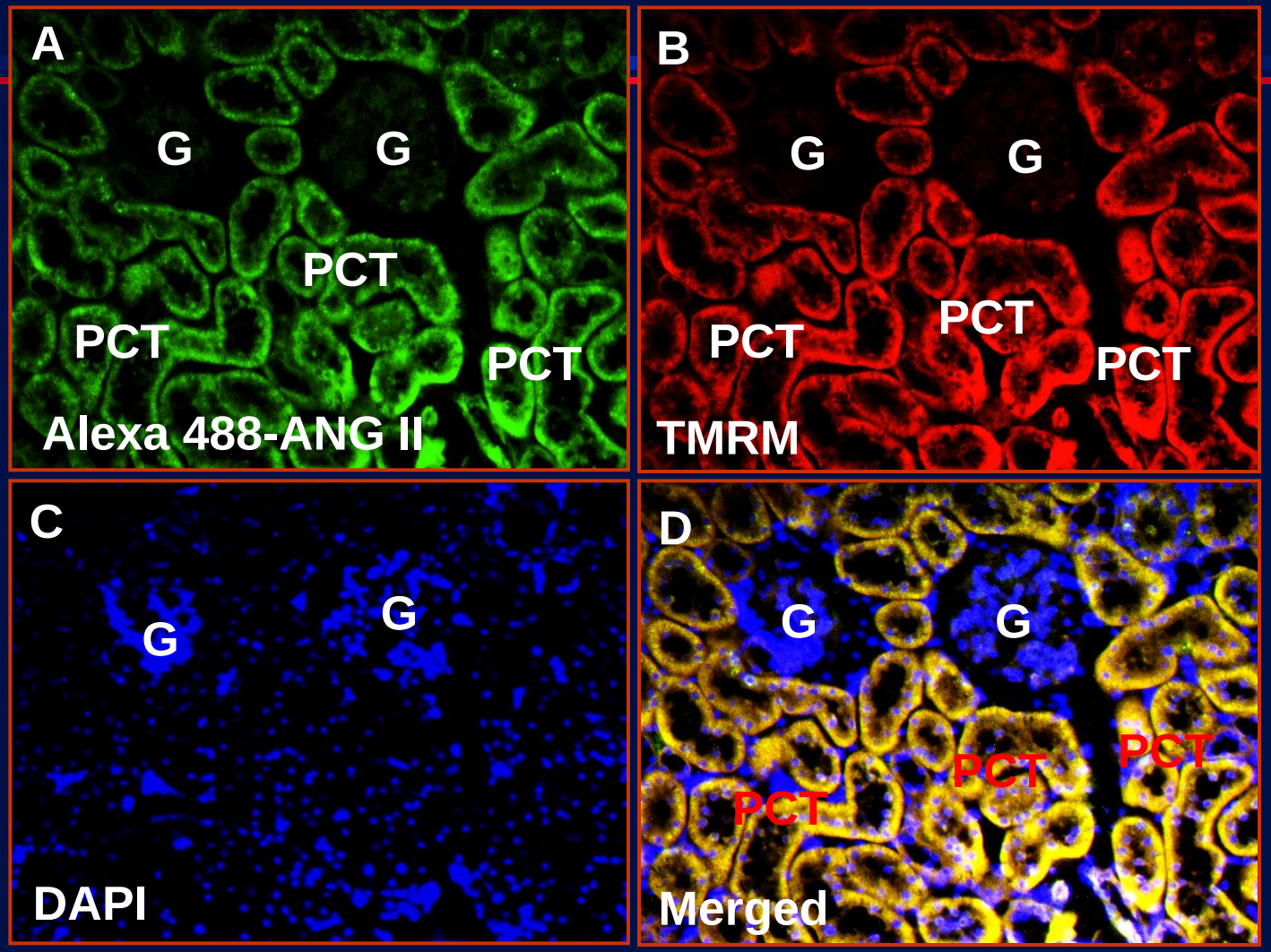
Blue = Hoechst 33342-labeled nuclei

G = glomerulus

PCT = proximal convoluted tubule

CCT = cortical collecting tubule

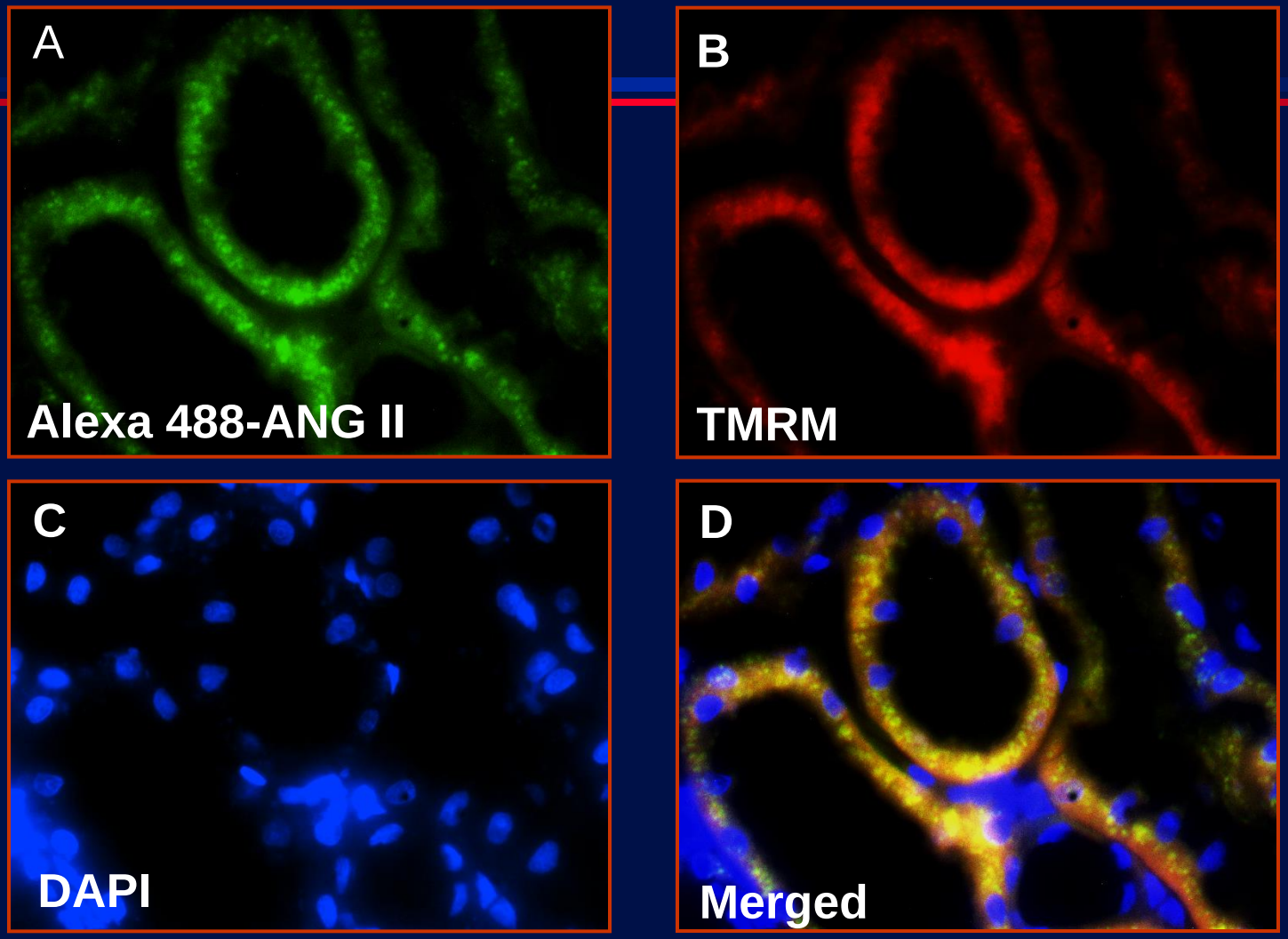
Colocalization of internalized Alexa Fluor® 488-ANG II and mitochondrial membrane potential-dependent dye TMRM in the proximal tubule of the rat kidney



Green: Alexa 488-ANG II Red: TMRM – Mitochondrial dye

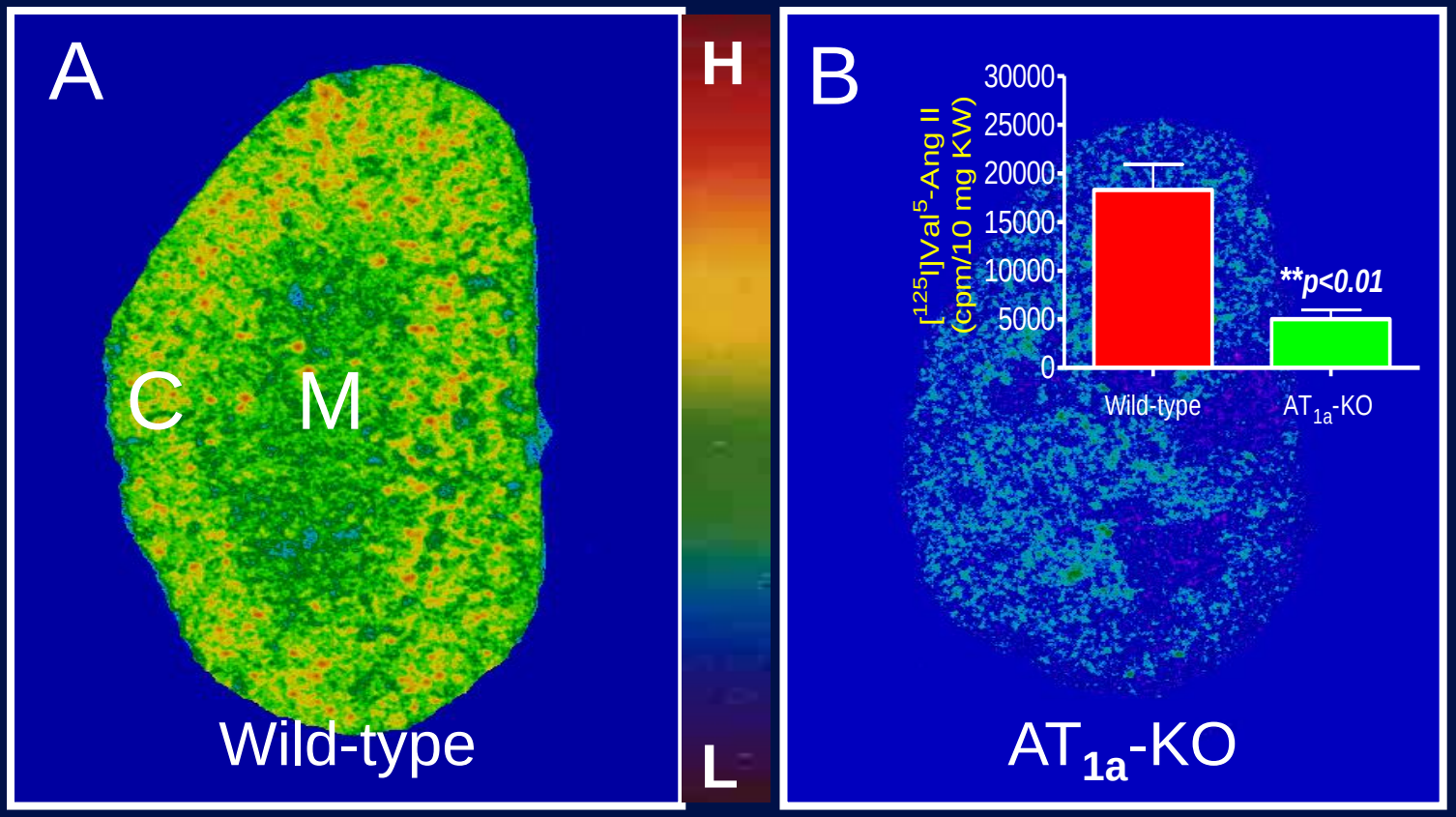
Blue: nuclei

Colocalization of internalized Alexa Fluor® 488-ANG II and mitochondrial membrane potential-dependent dye TMRM in the proximal tubule of the rat kidney



Green: Alexa 488-ANG II Red: TMRM – Mitochondrial dye
Blue: nuclei

In vivo evidence:
Circulating [^{125}I]Val^{5}-ANG II was taken up in kidneys of wild-type but not AT$_{1a}$-KO mice, as visualized by quantitative in vivo autoradiography

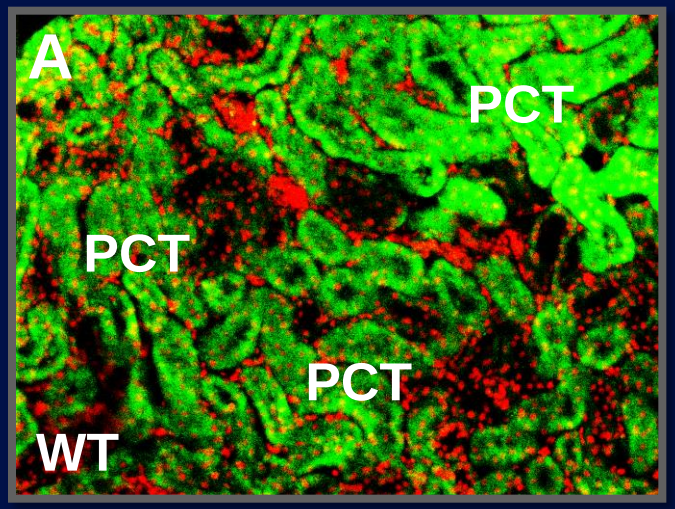


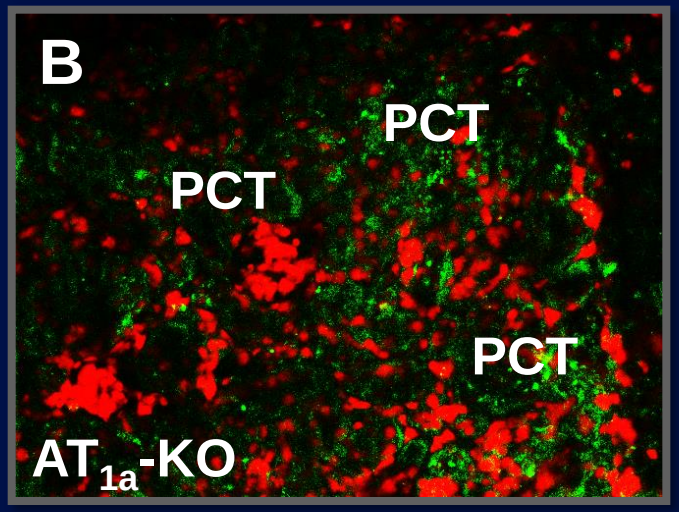
C: cortex; M: medulla

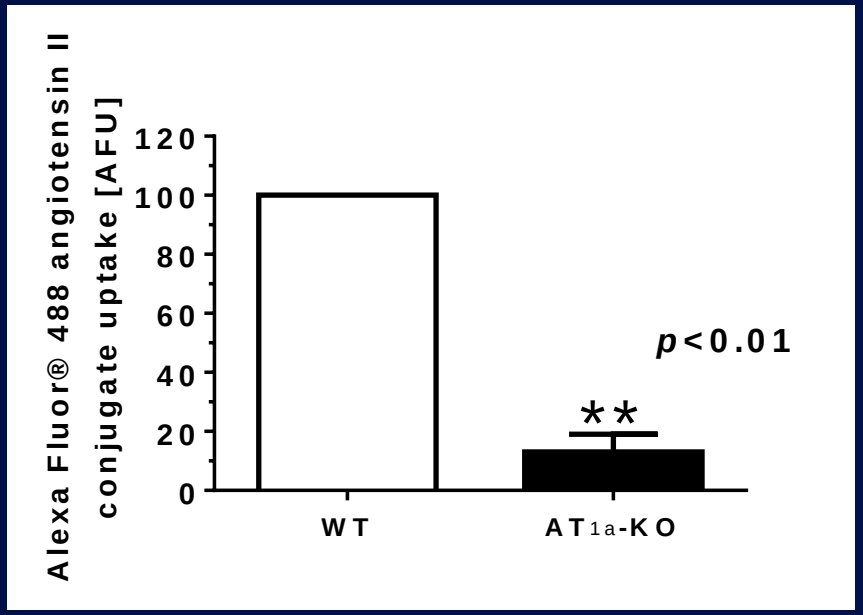
Am J Physiol Renal Physiol. 294(2):F293-302, 2008.

AJP - Renal Physiology

AT_{1a} receptor-mediated uptake of Alexa Fluor® 488-ANG II in the kidneys of wild-type and AT_{1a}-KO mice





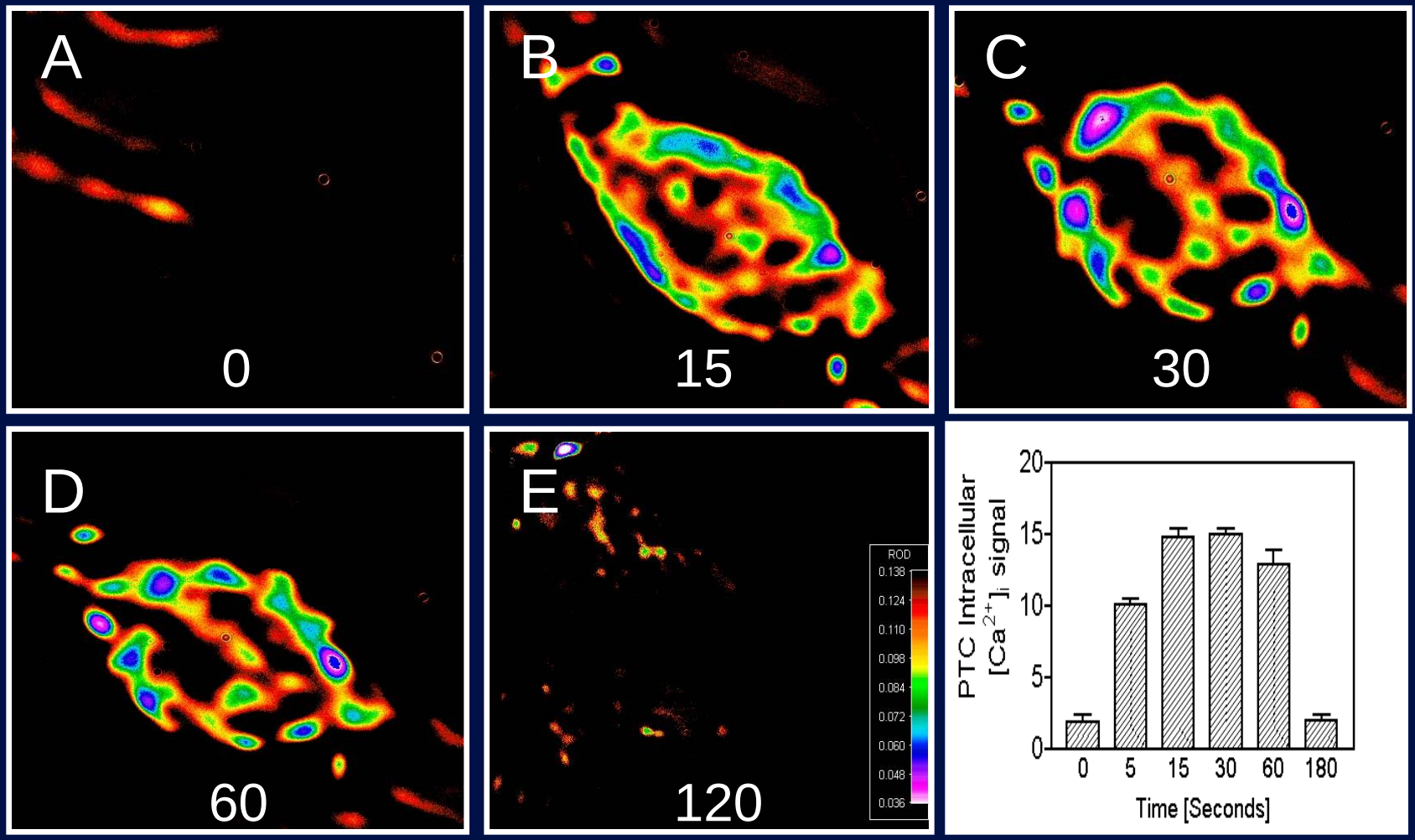


Li et al., Am J Physiol Renal Physiol 2014

Summary 1

- High levels of Alexa Fluor® 488-ANG II uptake were primarily localized in the proximal tubule of the kidney 2 h after i.v. infusion.
- The uptake of Alexa Fluor® 488-ANG II in the proximal tubule was largely blocked in the kidney of AT_{1a}-KO mice.
- Internalized Alexa Fluor® 488-ANG II and the mitochondrial membrane potential-dependent dye TMRM were colocalized in the proximal tubule.
- Little Alexa Fluor® 488-ANG II uptake was visualized in the glomeruli and cortical collecting tubules (CCT).

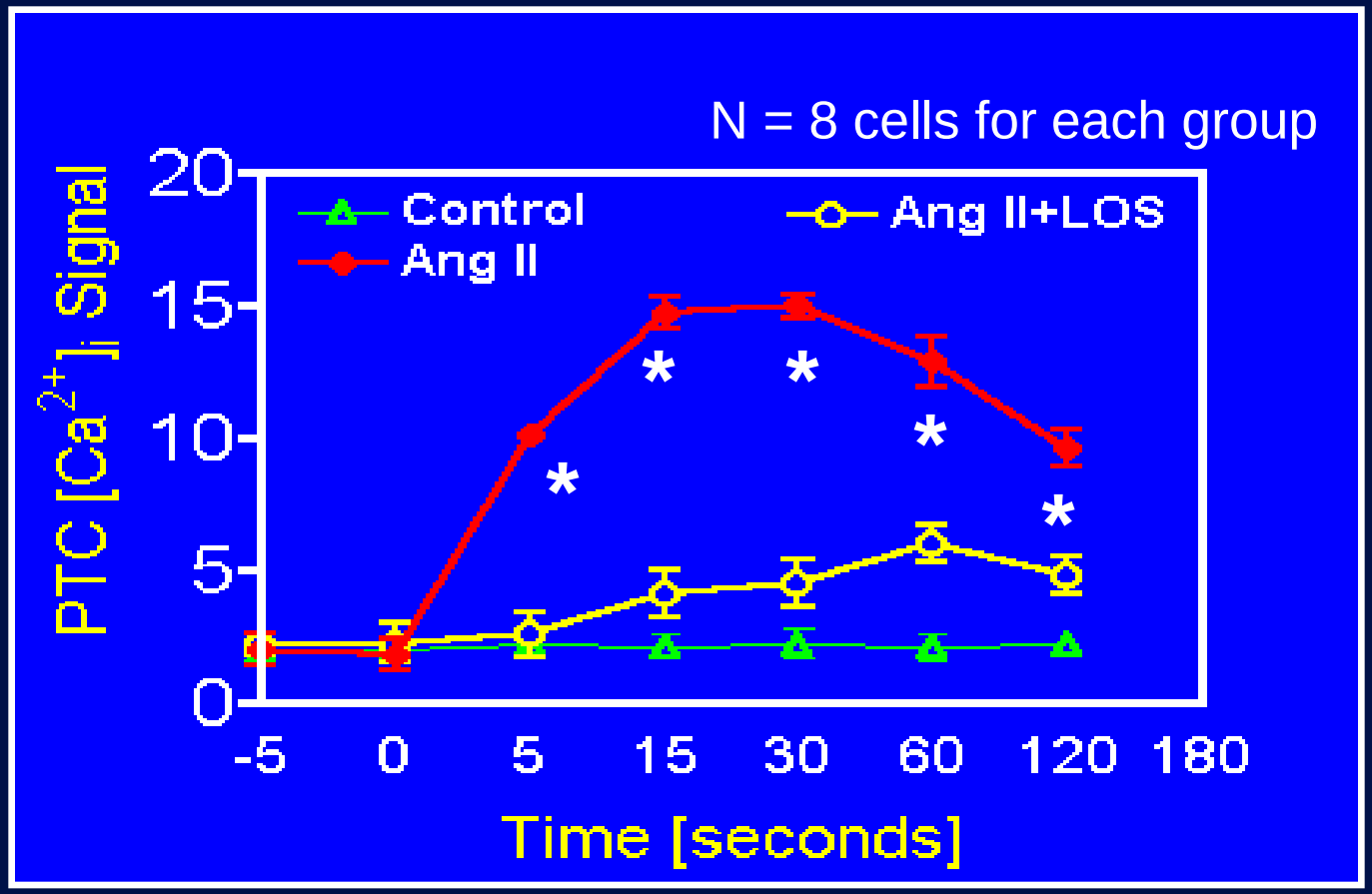
In vitro evidence that intracellular microinjection of ANG II on intracellular calcium responses in single proximal tubule cells



Zhuo et al. Am J Physiol Renal Physiol 290: F1382-F1390, 2006

AJP - Renal Physiology

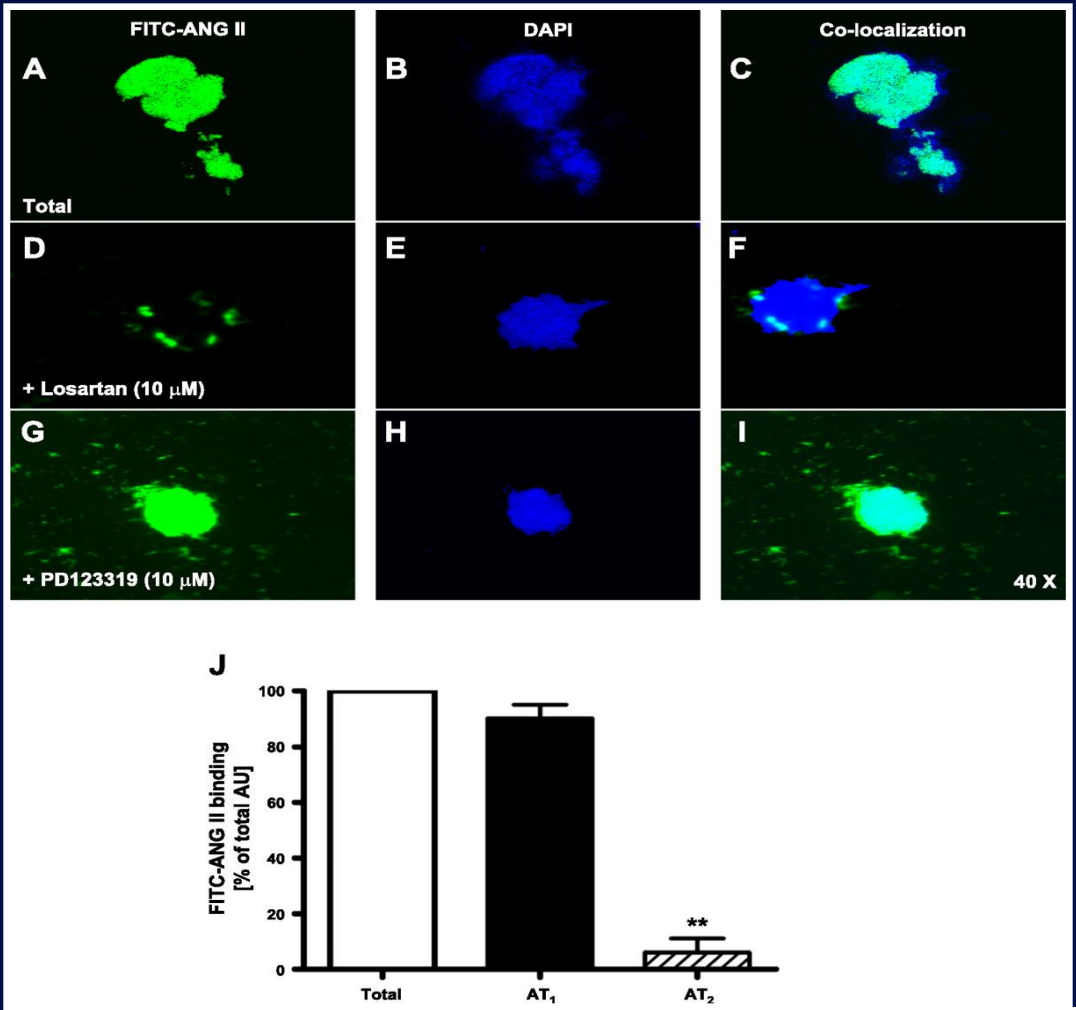
Losartan blocked intracellular Ca^{2+} response to microinjected ANG II in single proximal tubule cells



Am J Physiol Renal Physiol 290: F1382-F1390, 2006

AJP - Renal Physiology

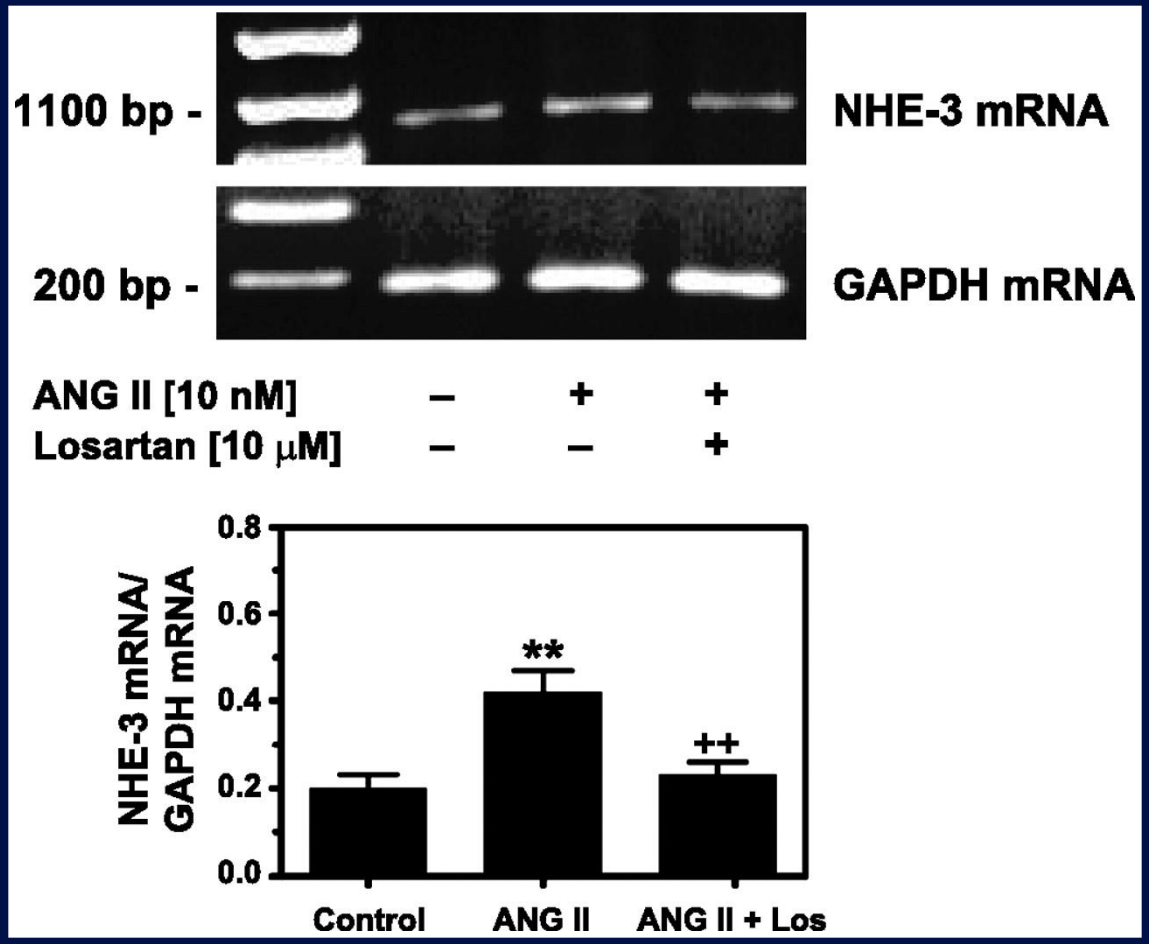
In vitro evidence that AT_1 receptors are present in the nuclei of of freshly isolated rat renal cortical cells



Li, X. C. et al. Am J Physiol Cell Physiol 294: C1034-C1045, 2008

AJP - Cell Physiology

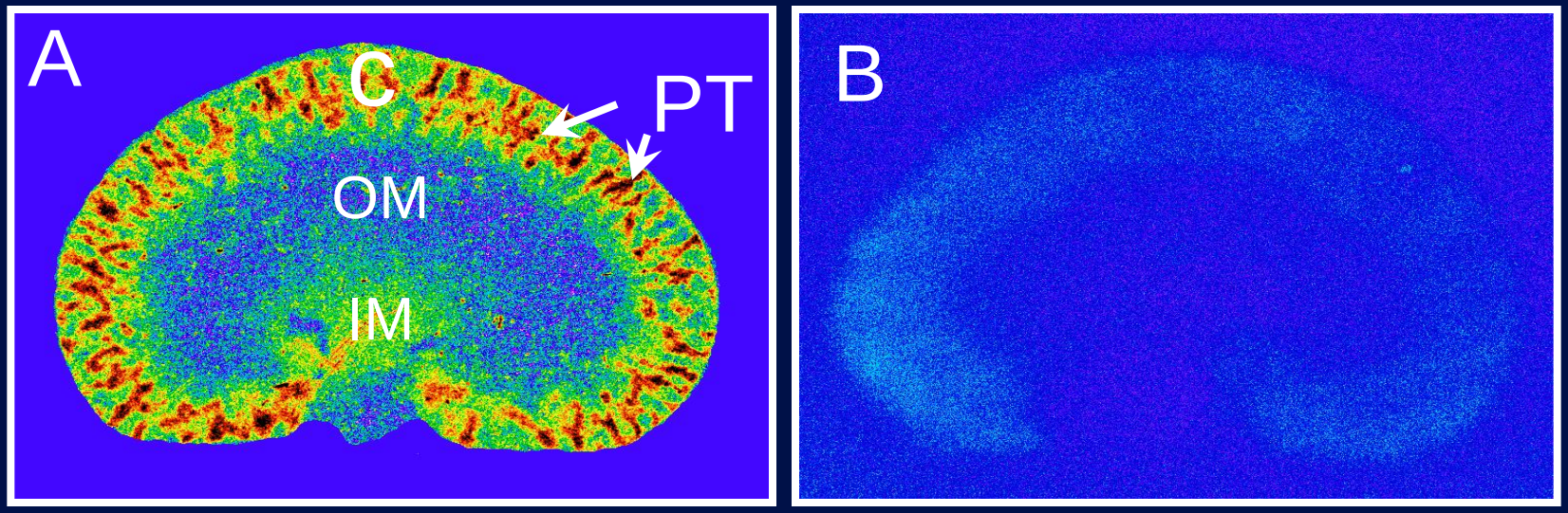
In vitro transcriptional effect of intracellular ANG II on the sodium and hydrogen exchanger-3 (NHE-3) mRNA in freshly isolated rat renal cortical nuclei



Li, X. C. et al. Am J Physiol Cell Physiol 294: C1034-C1045, 2008

AJP - Cell Physiology

In vivo evidence that losartan is internalized via the AT_1-mediated mechanism in the rat renal cortex



Control

Losartan-pretreated
10 mg/kg, i.v.

[^{3}H]-losartan, 1 nmol/min, i.v., 60 min (Merck Inc.)

Does internalized or intracellular ANG II play a physiological role in the regulation of proximal tubule function and blood pressure?

- Intra-cardiac adenoviral transfer of an intracellular ANG II peptide induced cardiac hypertrophy without altering blood pressure (Baker et al., 2004).
- Global overexpression of an intracellular fluorescent fusion of ANG II protein increased blood pressure and induced microangiopathy in the kidney (Redding et al., 2010).
- It is not known whether intracellular ANG II plays a physiological role in the regulation of proximal tubule reabsorption and blood pressure.

Hypothesis

- Intrarenal adenoviral transfer of an intracellular cyan fluorescent fusion of ANG II (ECFP/ANG II) selectively in the proximal tubule increases blood pressure in rats and mice.
- The blood pressure-increasing effect of ECFP/ANG II in the proximal tubule is mediated by AT_1 (AT_{1a}) receptors.
- 运用腺病毒转基因的技术在肾脏进曲小管细胞内表达细胞内血管紧张素II研究血压的调节机制.

Construction of a proximal tubule cell-specific adenoviral vector encoding an intracellular ANG II fusion protein (ECFP/ANG II)

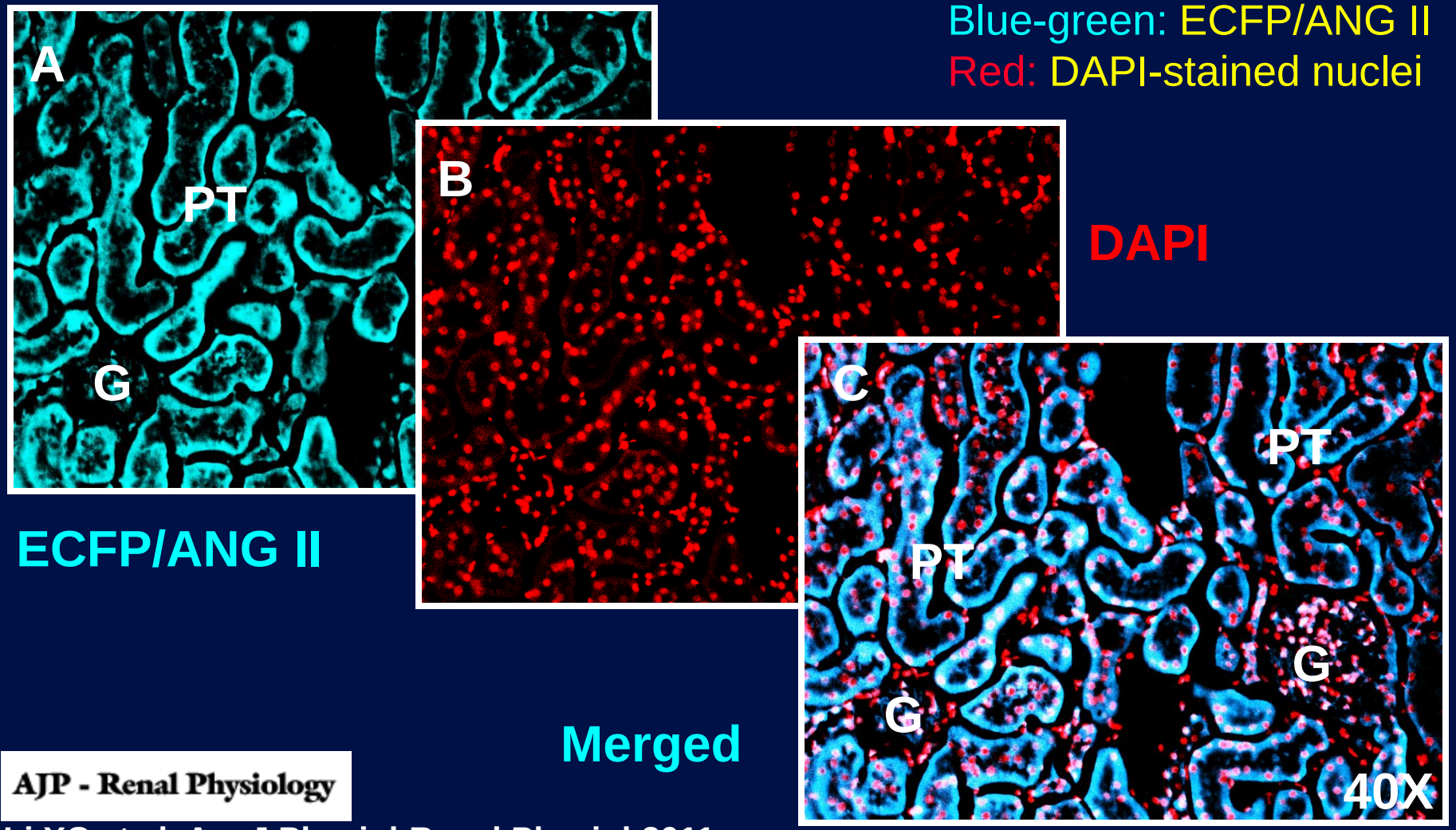
A. Construction of pECFP/ANG II plasmid by Dr. Julie Cook of Ochsner Clinic.

B. Subclone the gene of interest, ECFP/ANG II, into a proximal tubule-specific promoter sglt2 vector, constructed by Drs. Rubera and Tauc of France.

C. Construction of an adenoviral vector encoding recombinant human Ad-sglt2-ECFP/ANG II by Vector BioLabs (2.5 x 10^{11} PFU/ml).

- **I:** Human Ad5-sequences (wt1-458); includes 5' L-ITR and packaging signal.
- **II:** transgene Sglt2-ECFP/ANG II-PolyA.
- **III:** Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R-ITR. E3 deletion: nts 28587-30464.

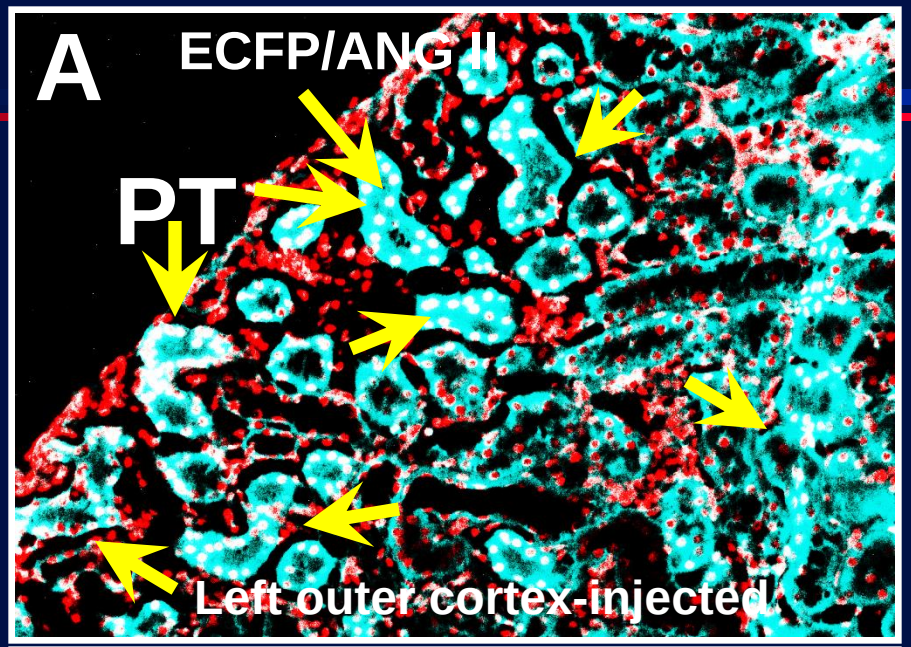
The sglt2 promoter drives ECFP/ANG II expression selectively in the proximal tubule of the kidney

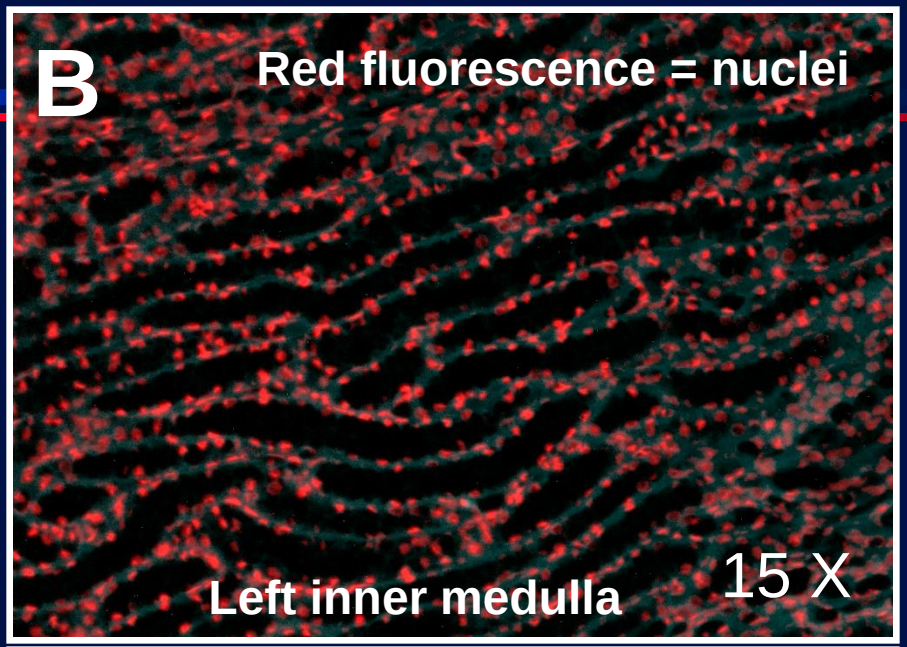


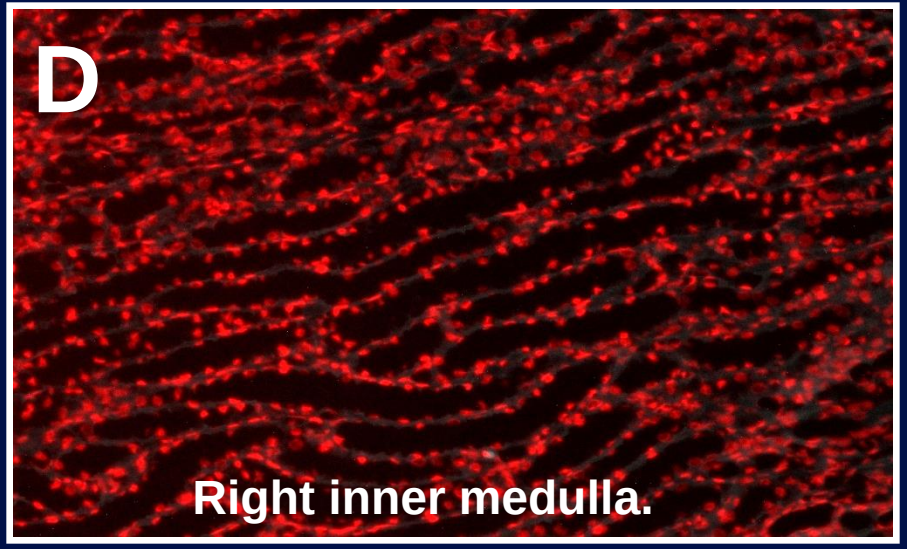
AJP - Renal Physiology

Li XC et al Am J Physiol Renal Physiol 2011

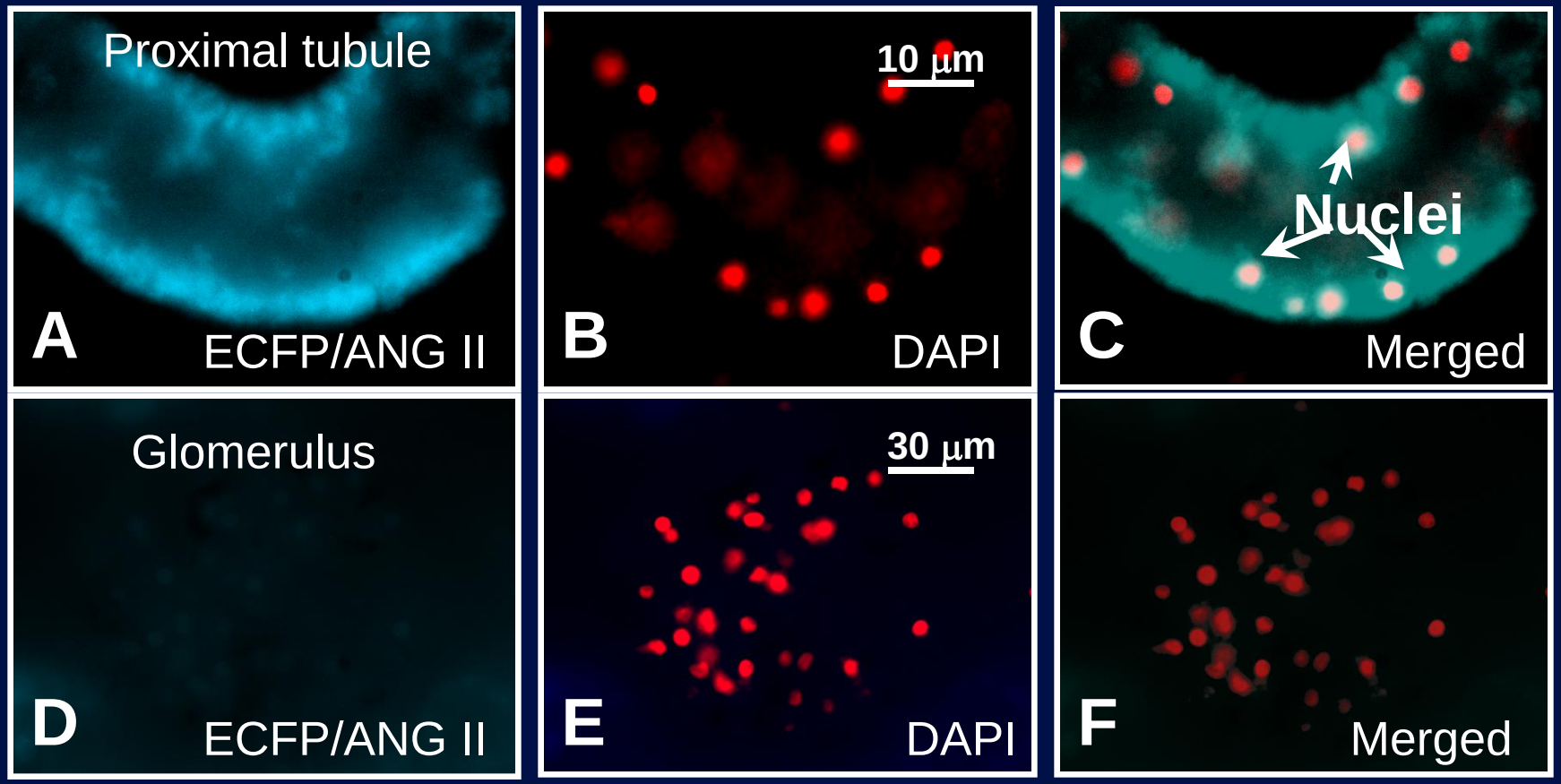
The sglt2 promoter drives ECFP/ANG II expression selectively in the proximal tubule of the kidney







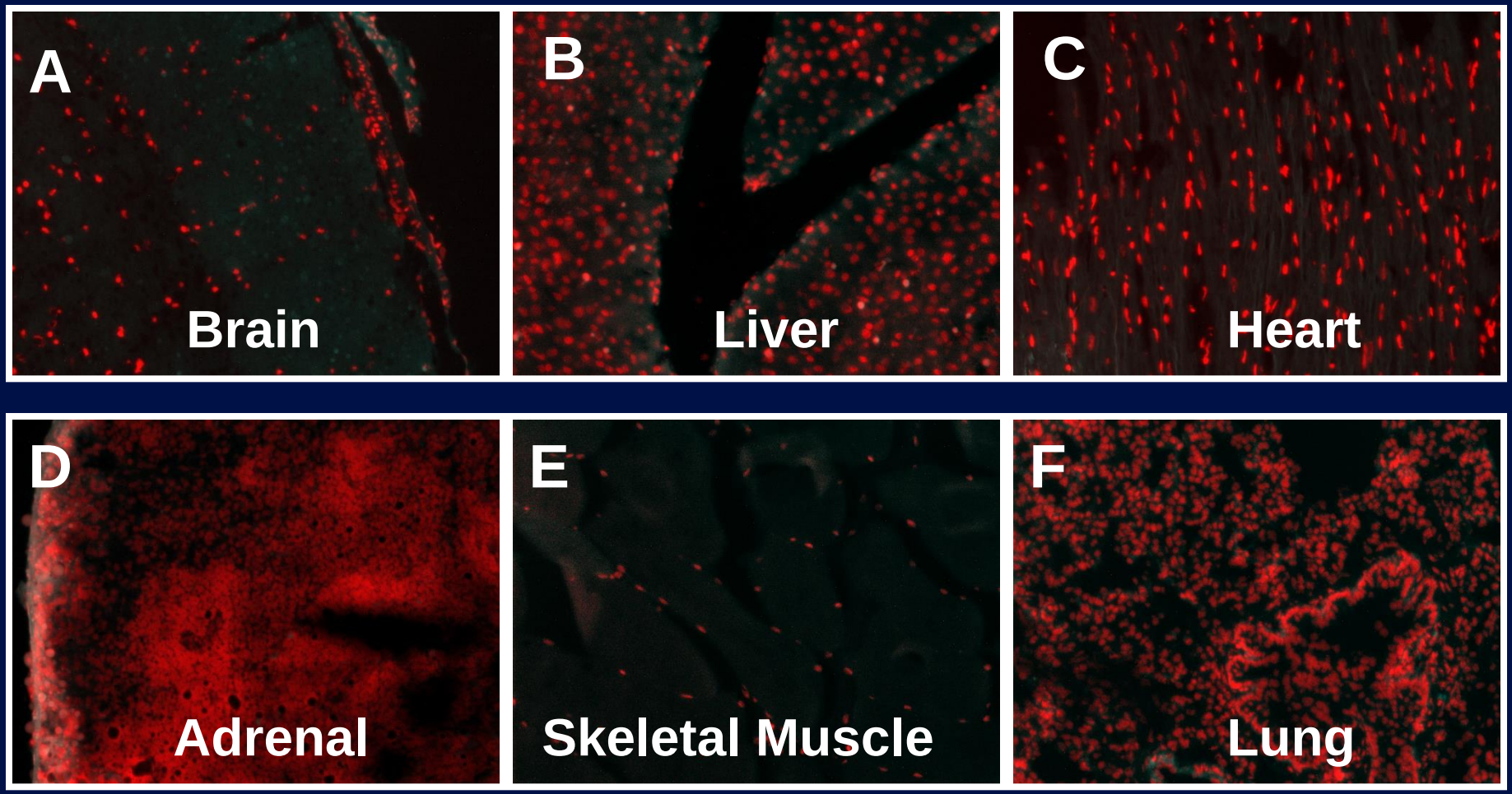
The sglt2 promoter drives ECFP/ANG II expression selectively in the proximal tubule of the kidney



Li XC et al Am J Physiol Renal Physiol 2011

AJP - Renal Physiology

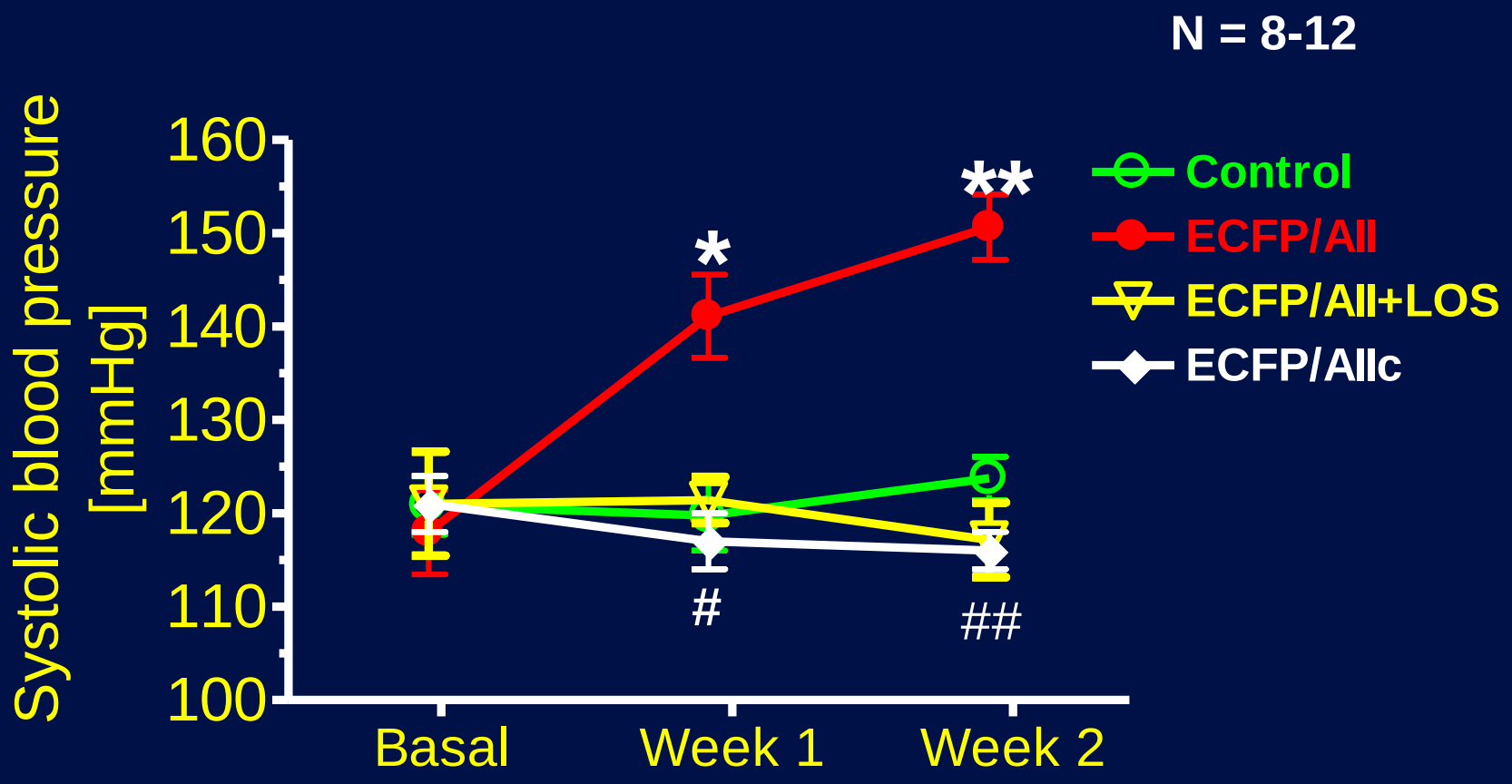
Effects of proximal tubule-specific transfer of ECFP/ANG II on ectopic ECFP/ANG II expression in extra-renal tissues



Li XC et al Am J Physiol Renal Physiol 2011

AJP - Renal Physiology

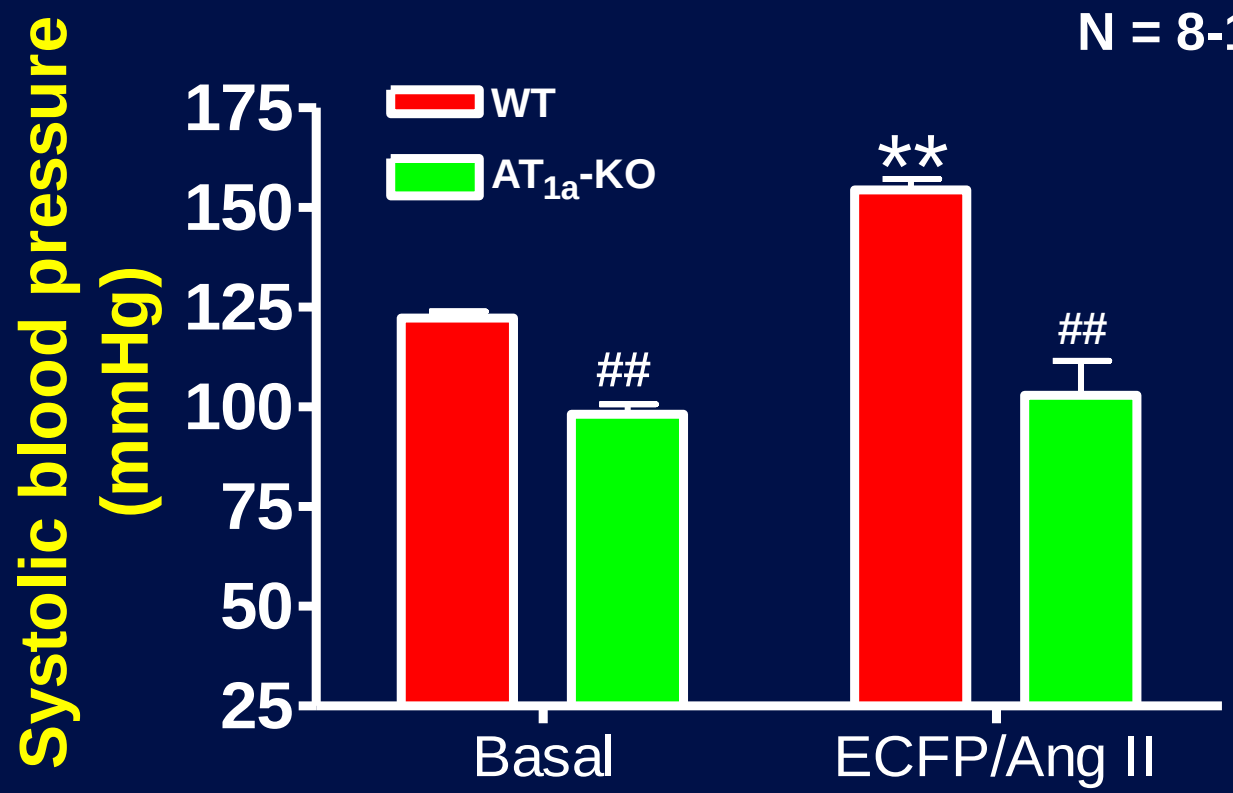
Proximal tubule-specific transfer of ECFP/ANG II in the kidney increases systolic blood pressure in rats: blockade by losartan



Li XC et al Am J Physiol Renal Physiol 2011

AJP - Renal Physiology

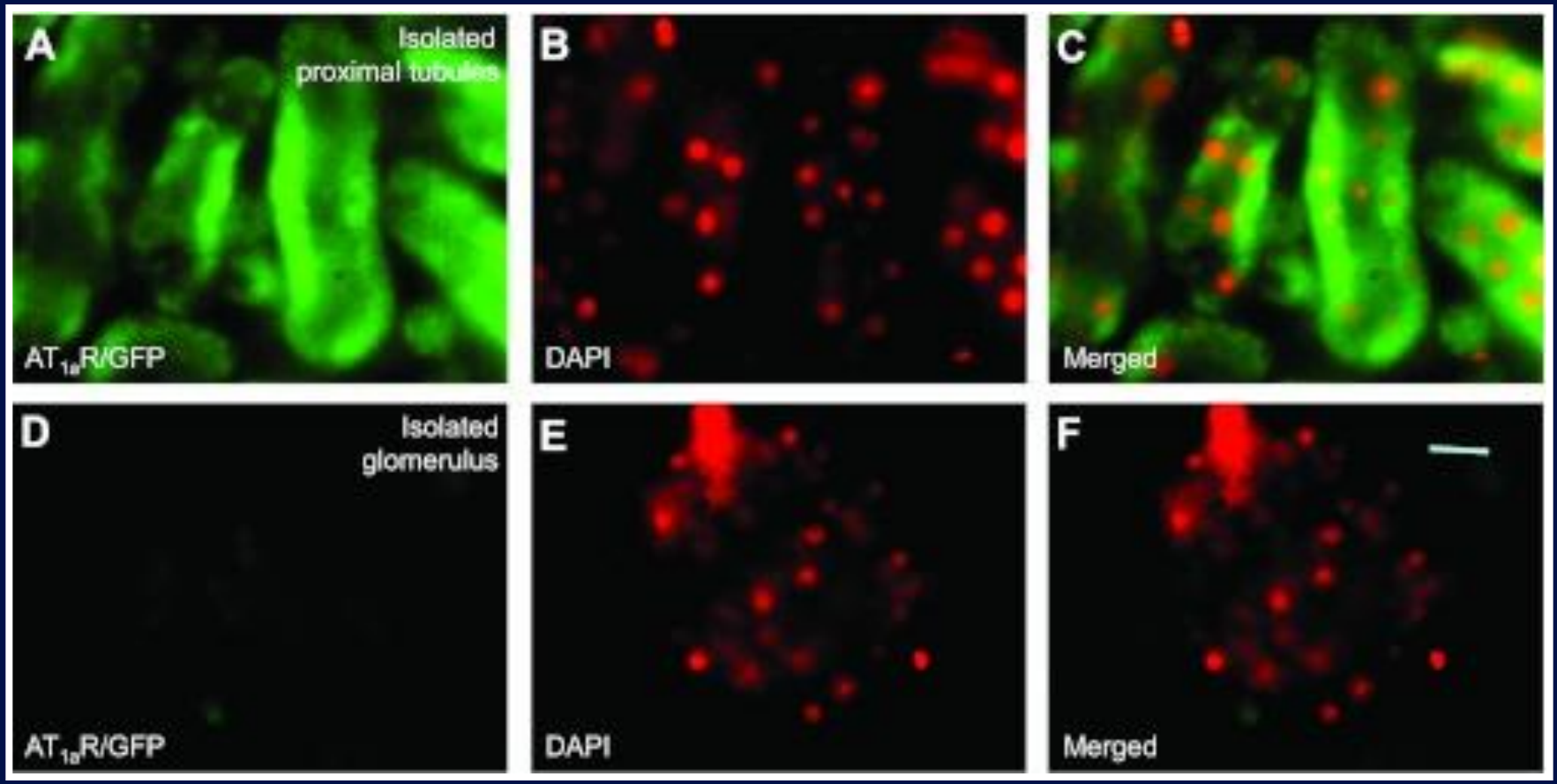
Proximal tubule-specific transfer of ECFP/ANG II in the kidney increases systolic blood pressure in wild type, but not AT_{1a}-KO mice



Li XC et al Am J Physiol Renal Physiol 2011

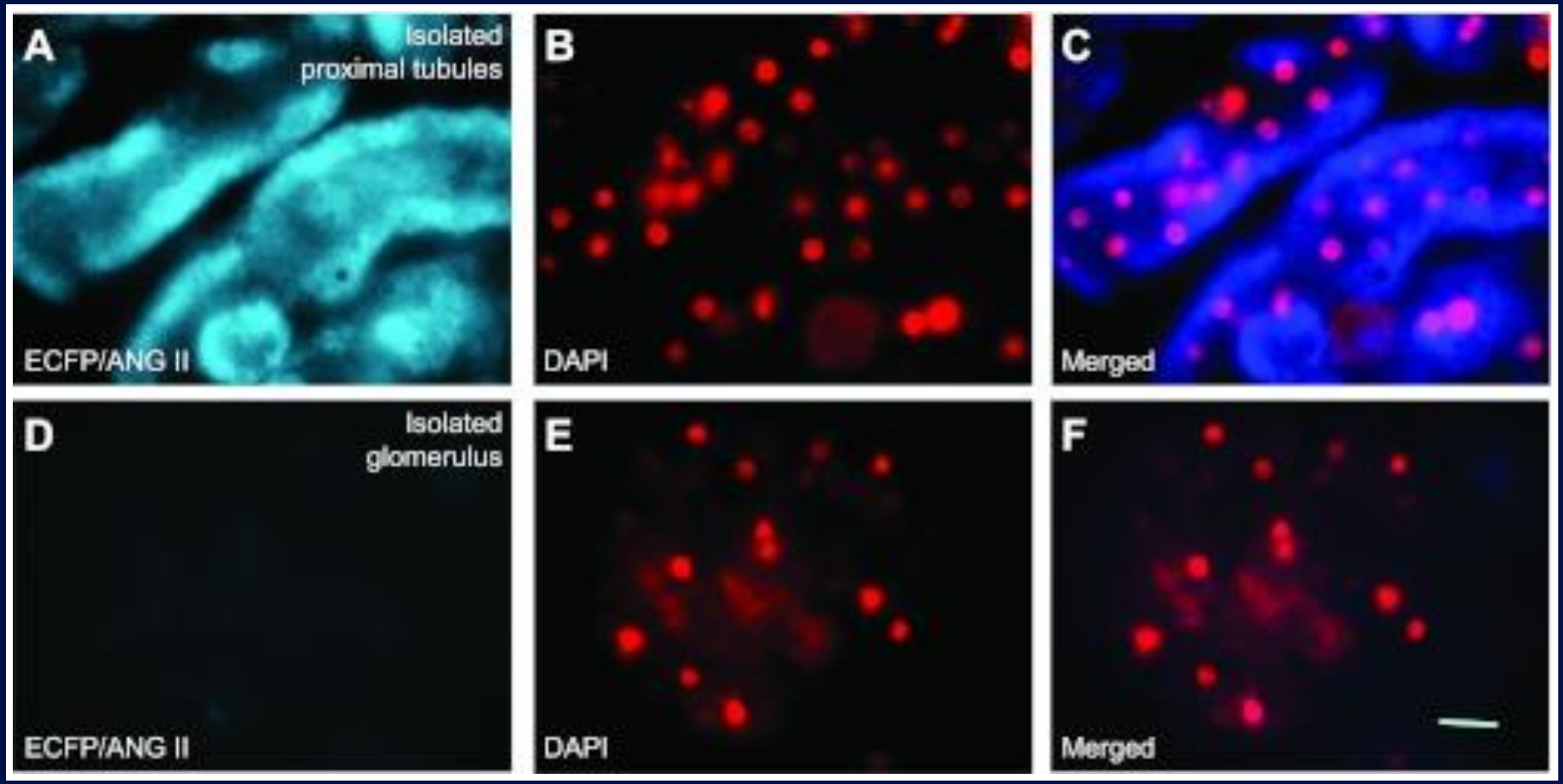
AJP - Renal Physiology

AT_{1a}R/GFP expression in freshly isolated proximal tubules or glomerulus of a representative AT_{1a}-KO mouse kidney 2 wk after intrarenal adenoviral AT_{1a}R/GFP transfer



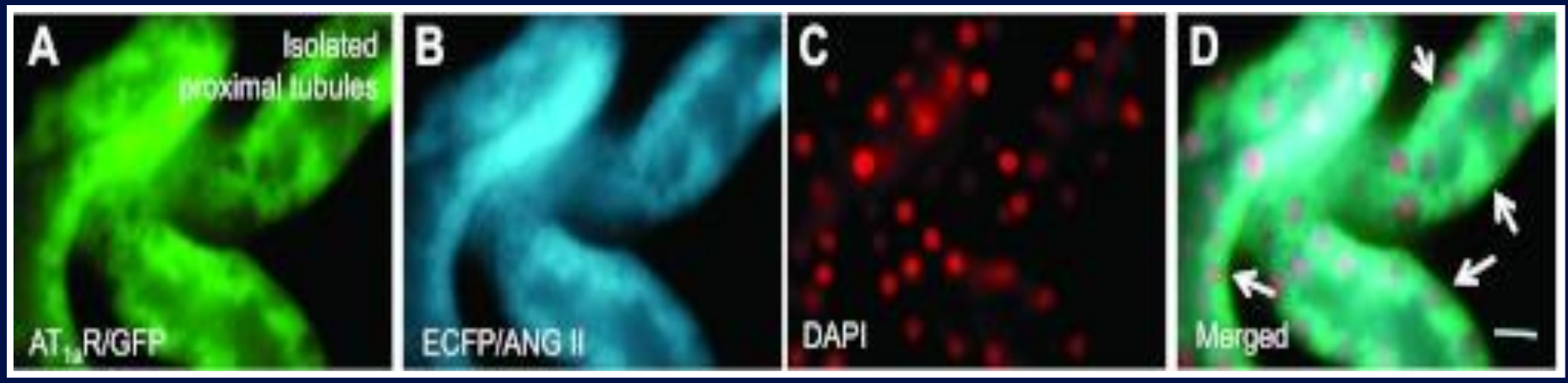
Am J Physiol Regul Integr Comp Physiol. 304(8): R588–R598, 2013

ECFP/ANG II expression in freshly isolated proximal tubules or glomerulus of a representative AT_{1a}-KO mouse kidney 2 wk after intrarenal adenoviral ECFP/ANG II transfer



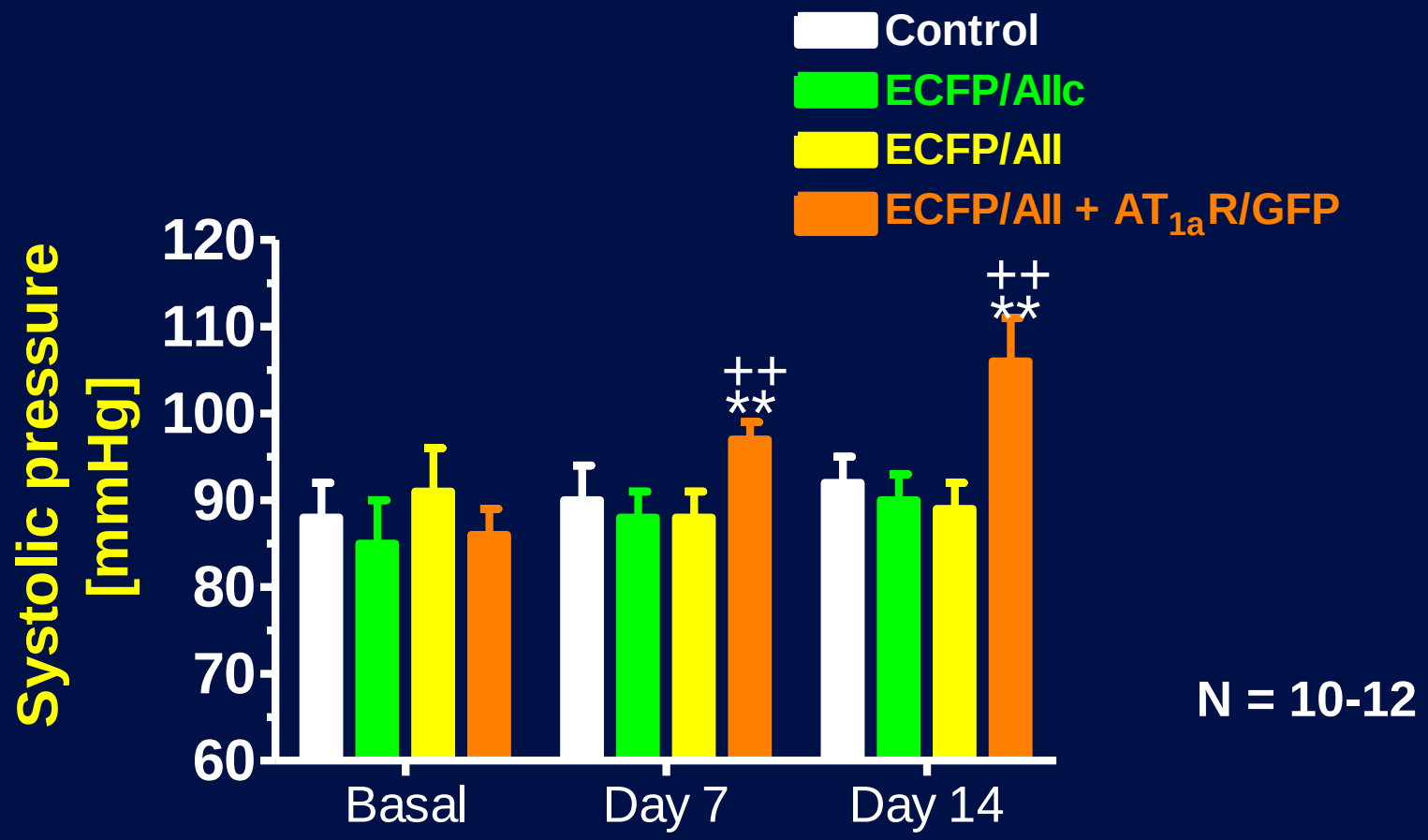
Am J Physiol Regul Integr Comp Physiol. 304(8): R588–R598, 2013

Proximal tubule-dominant expression of ECFP/ANG II in a representative AT_{1a}-KO mouse kidney 2 wk after intrarenal adenoviral transfer



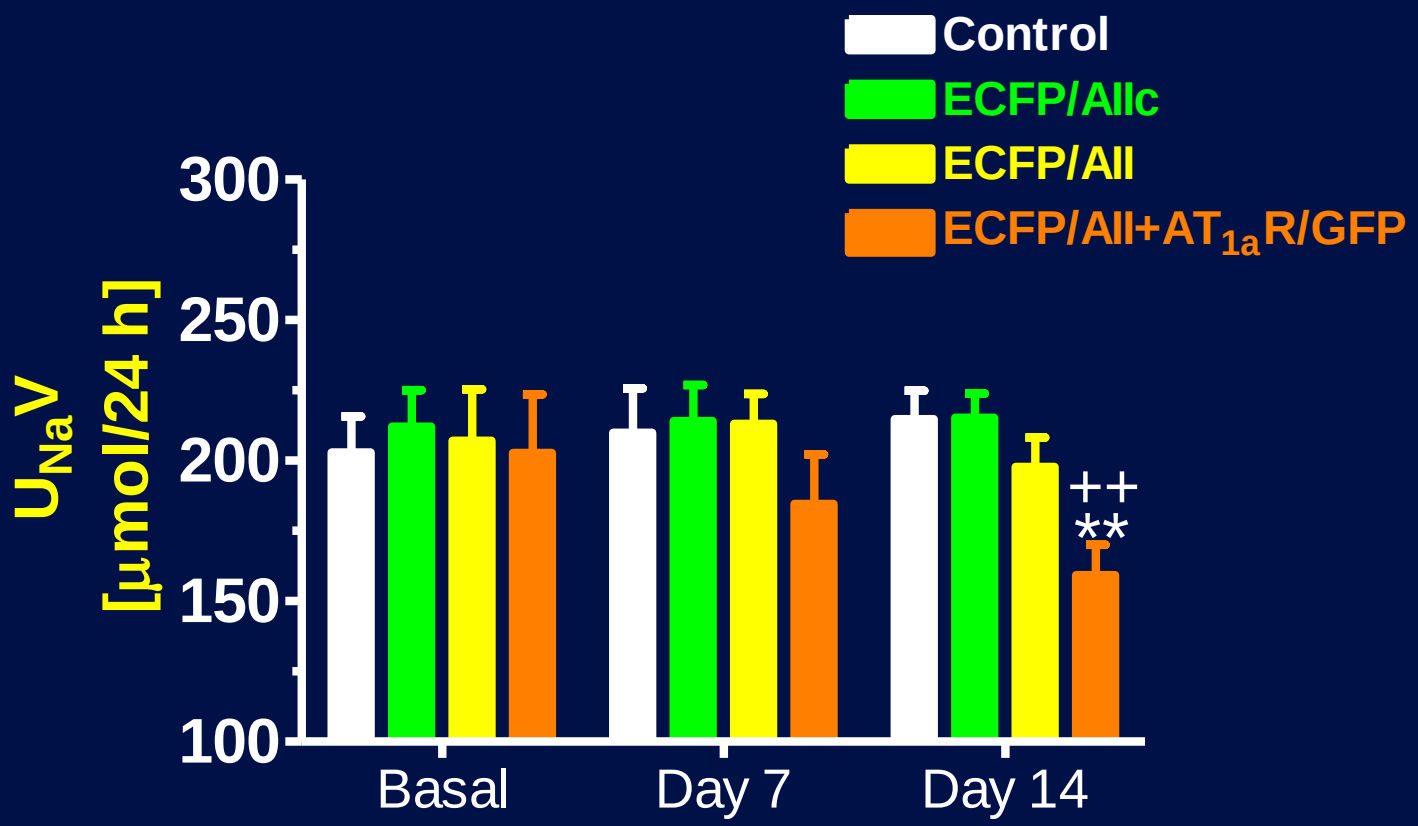
Am J Physiol Regul Integr Comp Physiol. 304(8): R588–R598., 2013

Effects of proximal tubule-specific co-expression of ECFP/Ang II and $AT_{1a}R$/GFP on blood pressure in AT_{1a}-KO mice



**p<0.01 vs. basal; ^{++}p<0.01 vs. control, ECFP/AIIc or ECFP/AII alone.

Effects of proximal tubule-specific co-expression of ECFP/AII and $AT_{1a}R$/GFP on urinary sodium excretion ($U_{Na}V$) in AT_{1a}-KO mice



****p<0.01 vs. basal; ^{++}p<0.01 vs. control, ECFP/AIIc or ECFP/AII alone**

Summary and Conclusions

- Proximal tubule -specific transfer of ECFP/ANG II in rats and mice induces the expression of ECFP/ANG II selectively in the proximal tubule.
- No significant ectopic expression of the transgene in extra-renal tissues.
- ECFP/ANG II transfer increases blood pressure, that is blocked by losartan treatment in rats and in AT_{1a}-KO mice.
- Proximal tubule-specific expression of ECFP/ANG II increase arterial pressure via AT_{1a} receptors.
- Intracellular ANG II may play a physiological role in the regulation of proximal tubule transport and blood pressure.

Acknowledgements

Fundings:

- NIDDK (2R01DK067299-12)
- NIGMS (1R01DK102429-01)
- ASN
- Hearin Foundation

Collaborators:

- L. Gabriel Navar, Ph.D.
  Tulane University
- Ulrich Hopfer, Ph.D.
  Case Western Reserve University
- Bruce Molitoris, M.D.
  Indiana University
- Julia Cook, Ph.D.
  Ochsner Clinic Foundation
- Isabel Rubera, Ph.D.
  France

Construction of a proximal tubule cell-specific adenoviral vector encoding a GFP-tagged wild-type AT_{1a} receptor (AT_{1a}R/GFP)

I: **Human Ad5-sequences (wt1-458); includes 5' L-ITR and packaging signal.**

II: **transgene Sglt2-EGFP/AT_{1a}R-PolyA.**

III: **Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R- ITR. E3 deletion: nts 28587-30464.**

Am J Physiol Regul Integr Comp Physiol. 304(8): R588–R598, 2013

Acknowledgements

Funding Sources:

- NIDDK
- NIDDK/NIGMS

Collaborators:

- Ruben M. Sandoval, M.Sc.
  Indiana University
- Bruce Molitoris, M.D.
  Indiana University

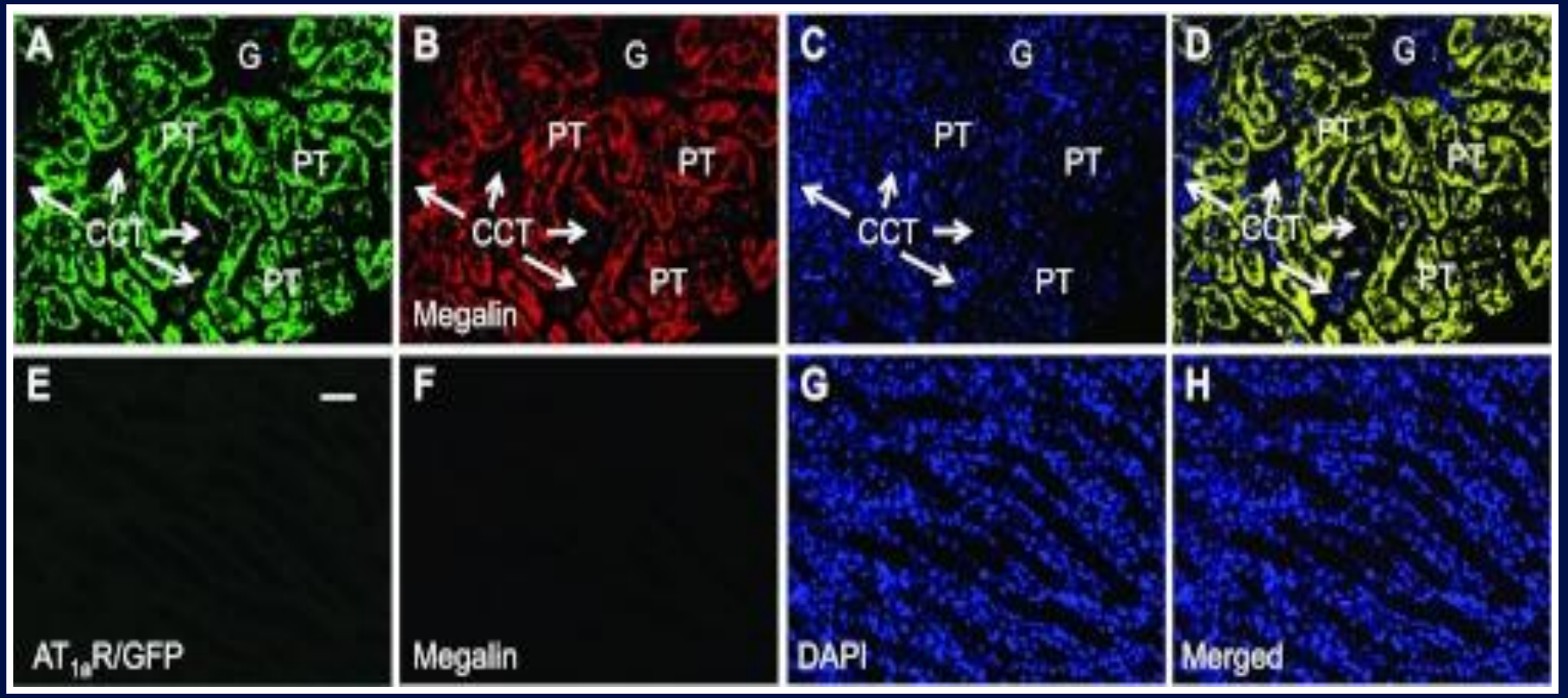
In vitro evidence that extracellular ANG II is internalized in cardiovascular and renal cells and acts as an intracellular peptide

- Circulating ANG II is taken up by the kidney via AT_1 (AT_{1a}) receptor-mediated mechanisms (von Thun et al., 1994; Zou et al., 1998; van Kats et al., 2001; Zhuo et al., 2002; Li et al., 2006).

- AT_1 (AT_{1a}) receptor-mediated endocytosis or uptake of ANG II is associated with phospholipase C/PKC activation (Schelling et al., 1992), cAMP signaling (Thekkumkara & Linas, 2002; Li et at., 2006), and NHE-3 expression (Li et al., 2007; Li et al., 2008).

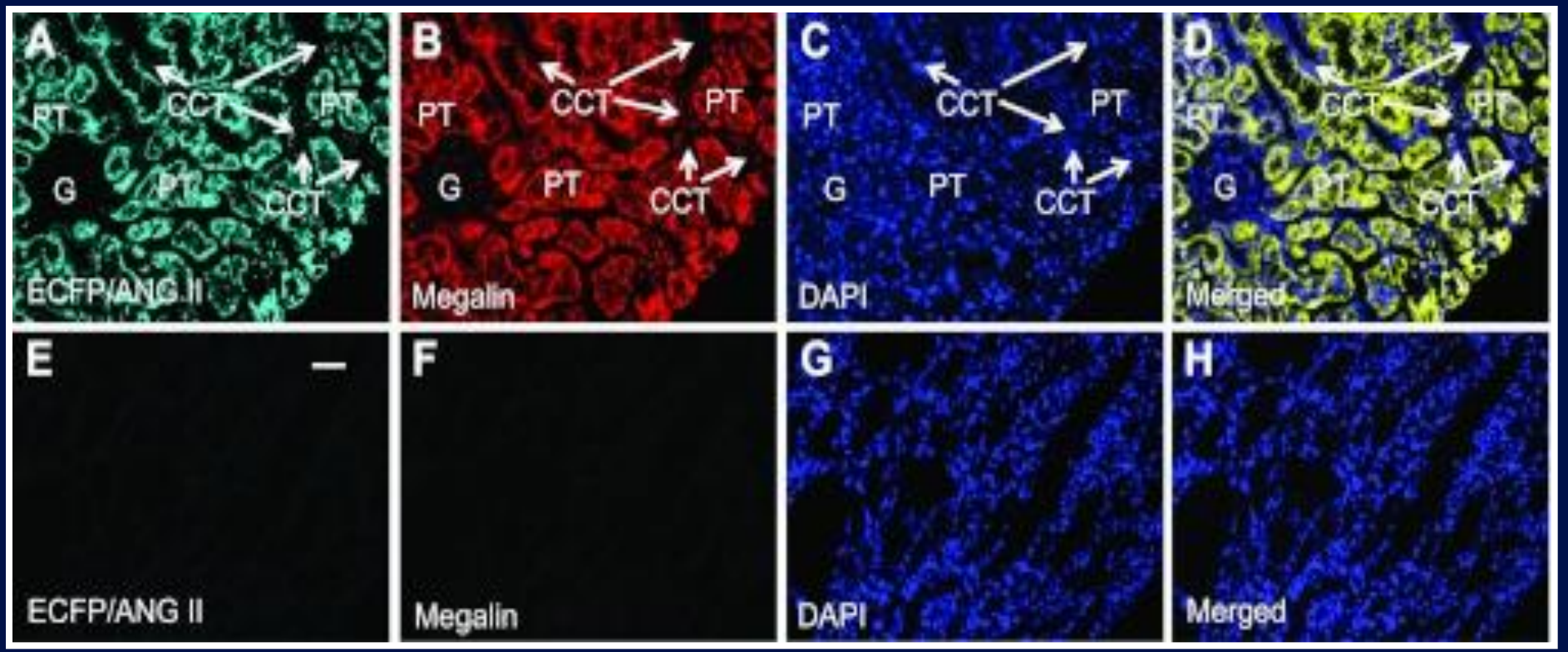
- In vitro, intracellular ANG II induced intracellular calcium responses (Haller et al., 1996; Zhuo et al., 2006), CREB activation (Cook et al., 2004), increase in cardiac inward calcium currents (De Mello 1998 & 2006), cardiac hypertrophy (Baker et al 2006), nuclear NO /O_2^- production (Gwathmey et al., 2009 & 2010), and transcriptional responses (Re & Parab 1984; Eggena et al., 1993; Li & Zhuo, 2008)

Proximal tubule-dominant expression of $AT_{1a}R$/GFP in the cortex and medulla of a representative AT_{1a}-KO mouse kidney 2 wk after intrarenal adenoviral transfer



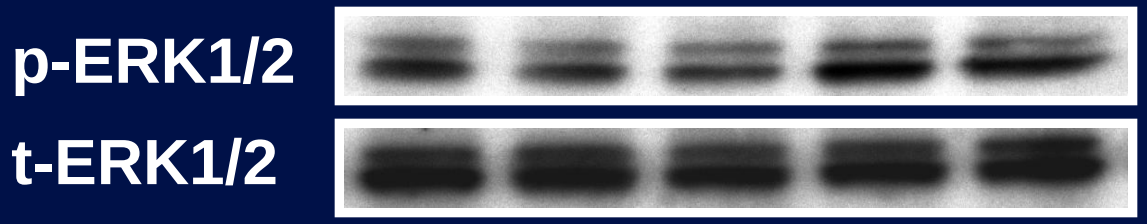
Am J Physiol Regul Integr Comp Physiol. 304(8): R588–R598, 2013

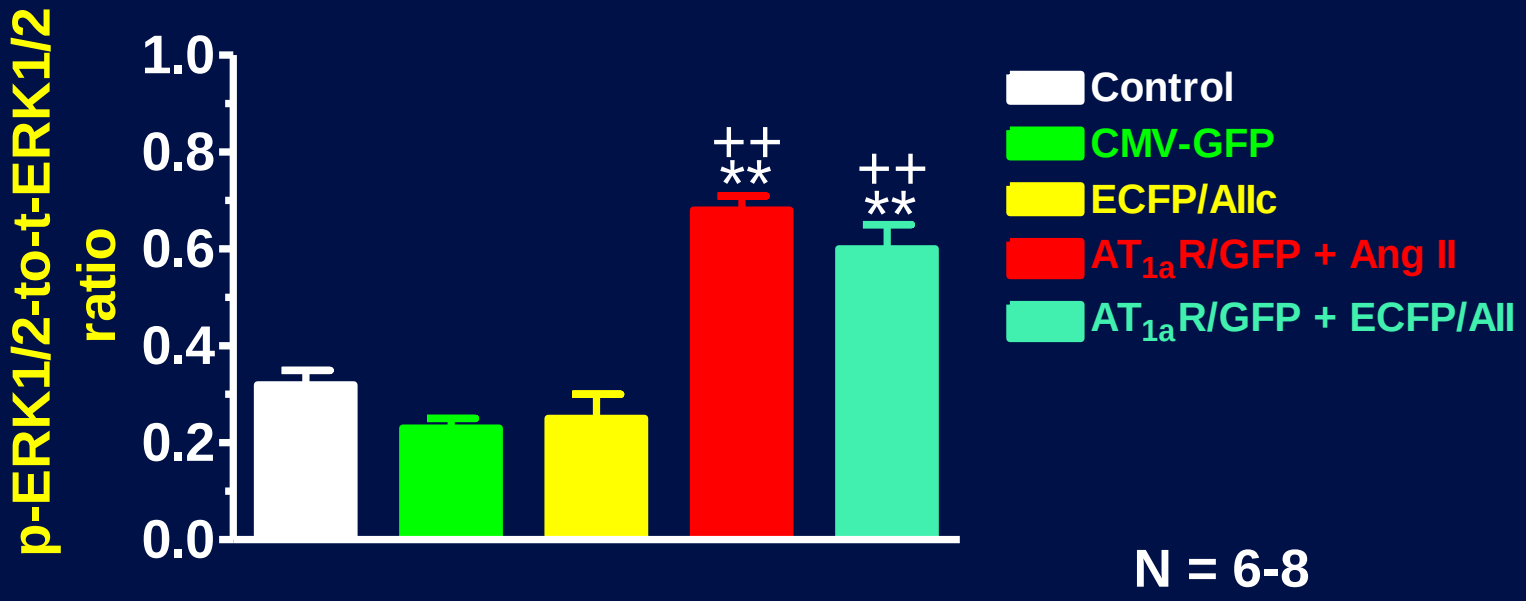
Proximal tubule-dominant expression of ECFP/ANG II in a representative AT_{1a}-KO mouse kidney 2 wk after intrarenal adenoviral transfer



Am J Physiol Regul Integr Comp Physiol. 304(8): R588–R598, 2013

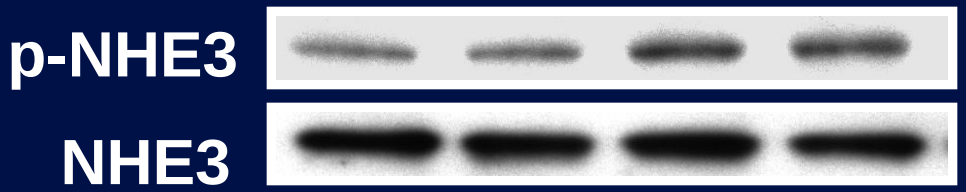
Effects of proximal tubule-specific co-expression of AT_{1a} receptors and ECFP/AII on phosphorylated ERK1/2 proteins in AT_{1a}-KO mice

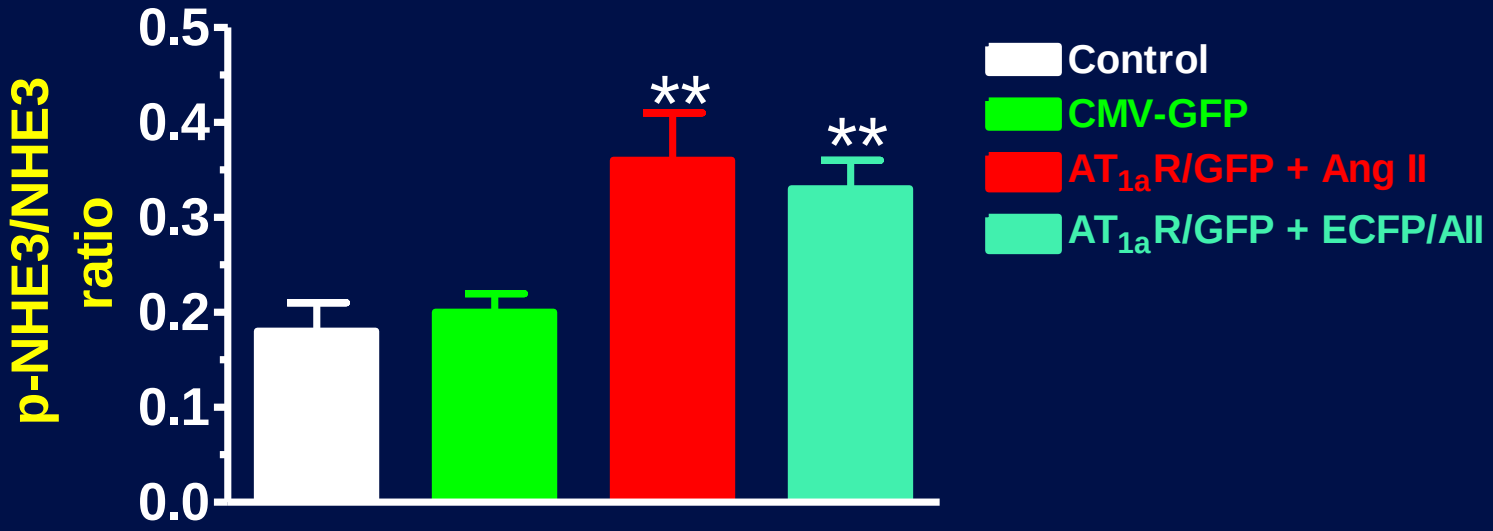




**p<0.01 vs. basal; ^{++}p<0.01 vs. CMV-GFP or ECFP/AIIc alone.

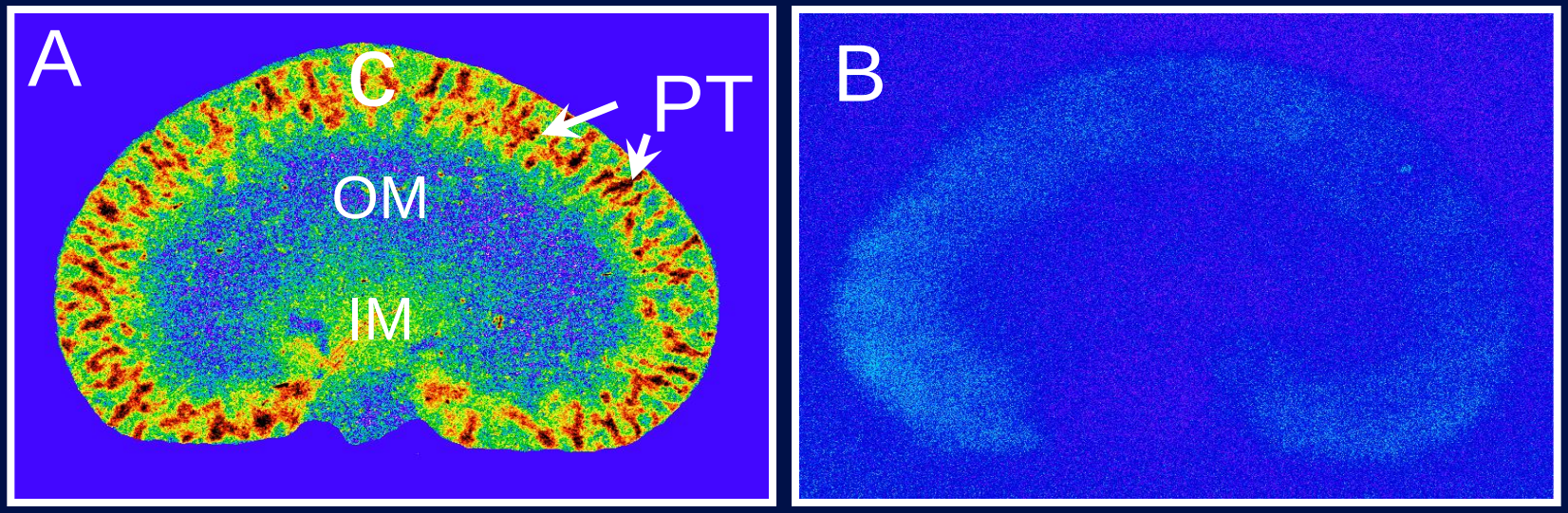
Effects of proximal tubule-specific co-expression of $AT_{1a}R$/GFP and ECFP/AII on phosphorylated NHE3 proteins in AT_{1a}-KO mice





**p<0.01 vs. Control or CMV-GFP, n = 6-8

AT_1-mediated uptake of [^{3}H]-losartan in the renal cortex of control and losartan-treated rats



Control

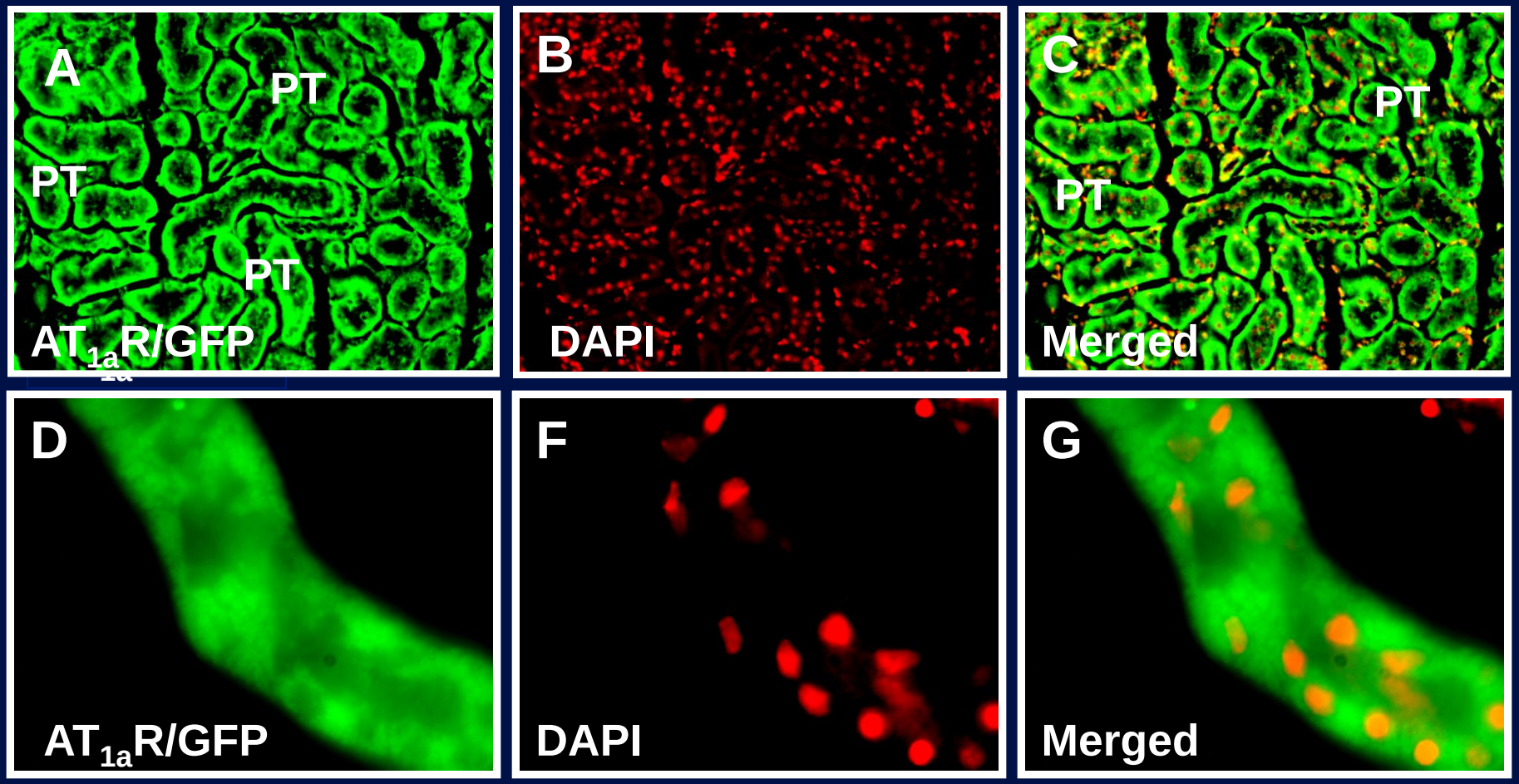
Losartan-pretreated
10 mg/kg, i.v.

[^{3}H]-losartan, 1 nmol/min, i.v., 60 min (Merck Inc.)

The 1st Asian and Pacific Congress of Physiological Sciences, Bangkok, Thailand, 1986

Dr. Navar Received 2012 AHA Excellence Award for High Blood Pressure Research

The sglt2 promoter drives the expression of GFP-tagged wild-type AT_{1a} receptors (AT_{1a}R/GFP) selectively in proximal tubules of AT_{1a}-KO mice



Magnification: 60 X (A-C); 200 X (D-G).

Am J Physiol Regul Integr Comp Physiol. Apr 15, 2013; 304(8): R588–R598.