# Does (Pro)Renin Receptor Deserve Its Name?

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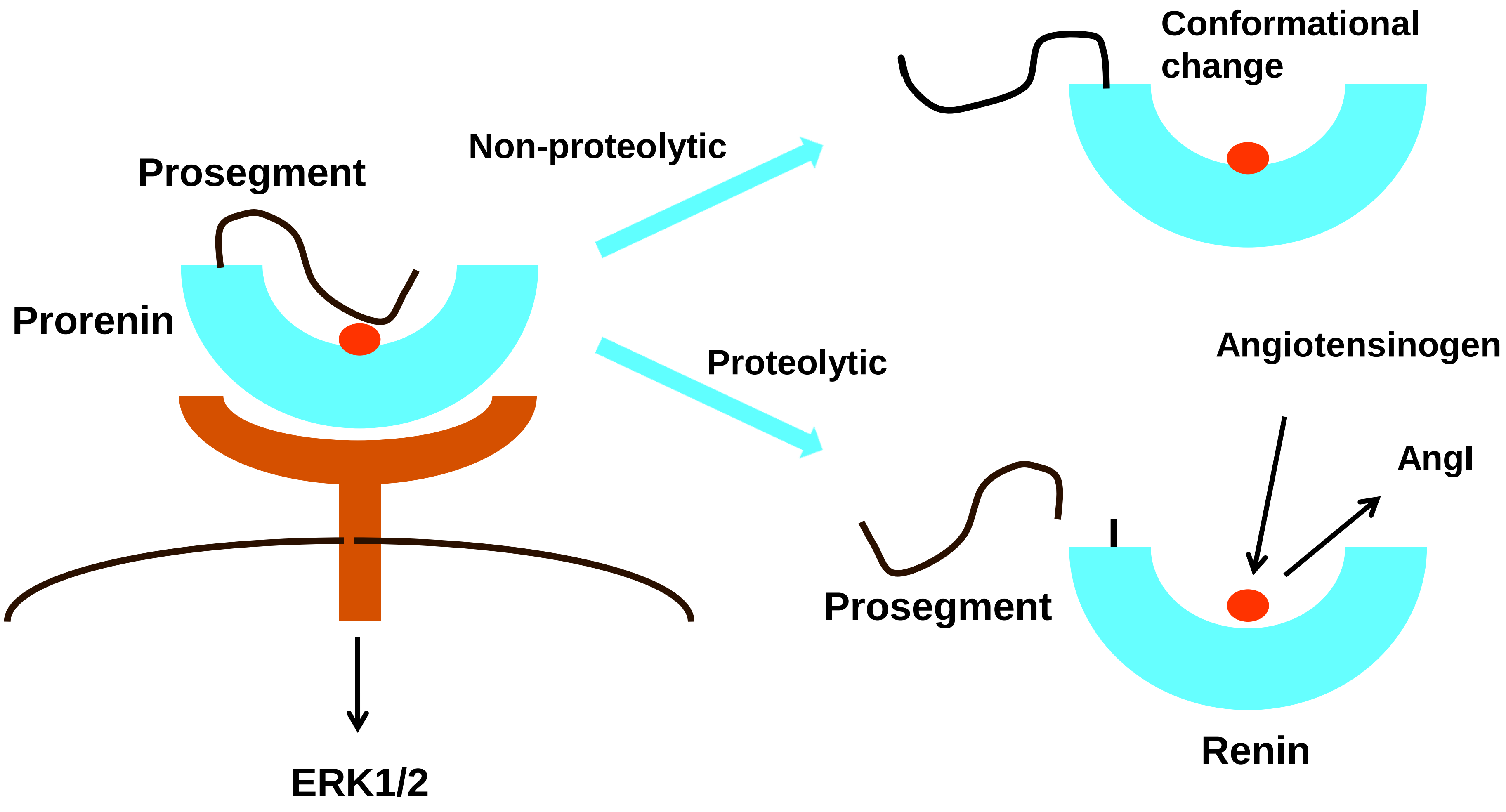
Institute of Hypertension, Sun Yat-sen University

# In Vitro Evidence for Renin Regulatory Property of PRR



# Lethal Phenotype in Various PRR KO Mice

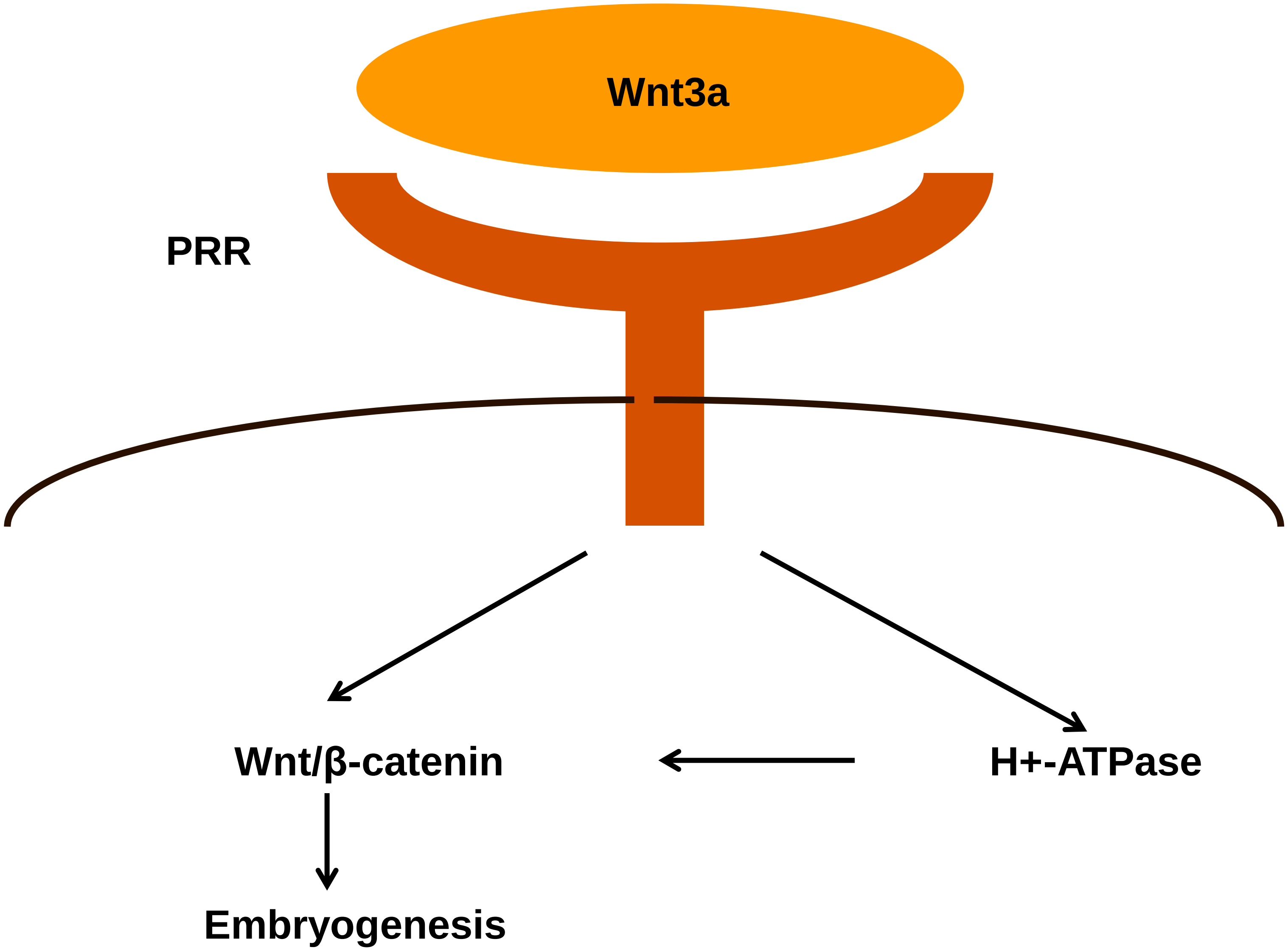
- Conventional PRR KO
- Cardiomyocyte PRR KO
- Podocyte PRR KO
- CD PRR KO (Hoxb7 Cre)

# Evidence Arguing against PRR as a Renin Regulator

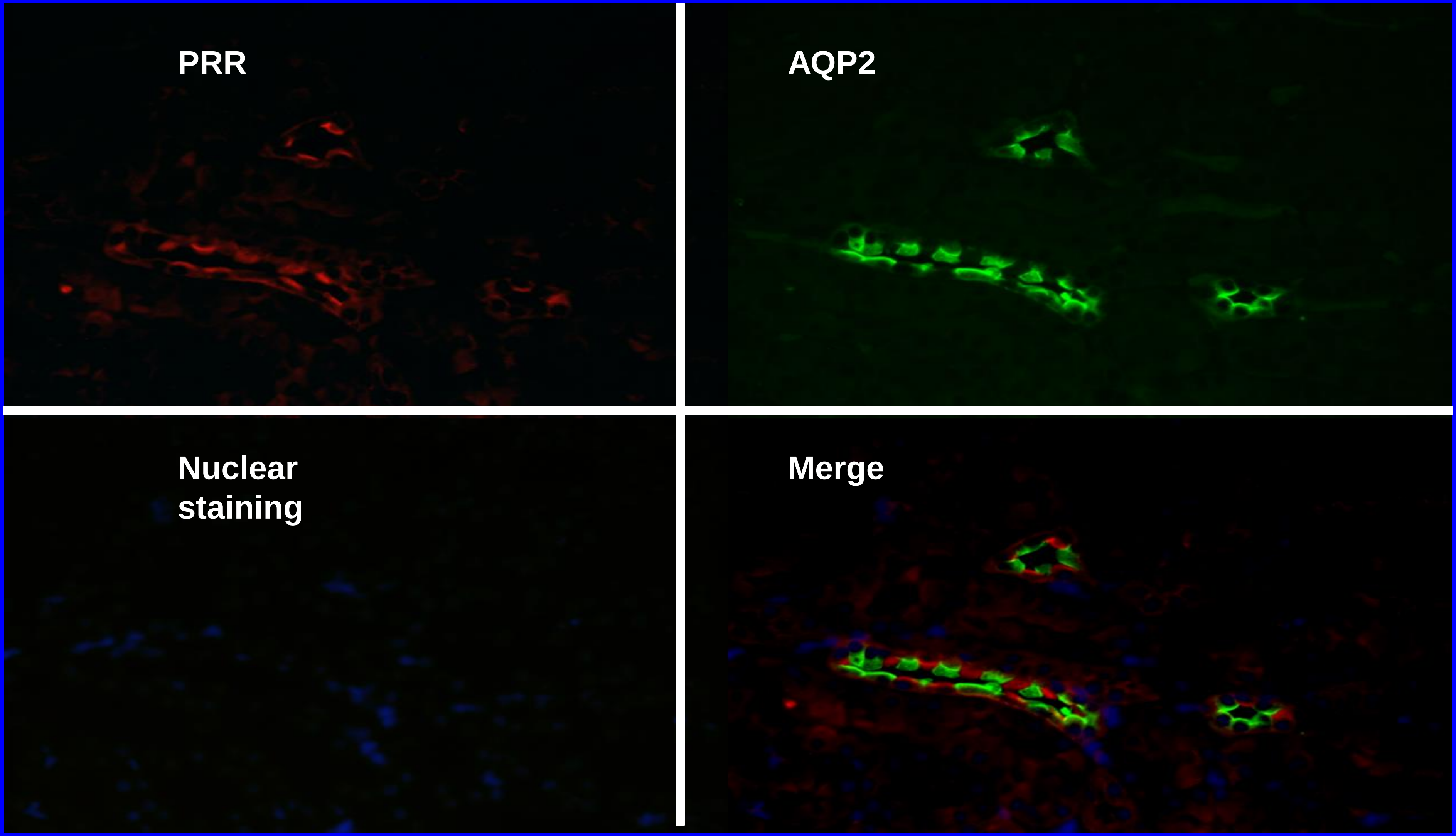
- Overexpression of human PRR in rats resulted in proteinuria and nephropathy but did not elevate BP or renal AngII levels (Kaneshiro Y 2007)

- Challenges: Lack of viable KO; controversal handle region peptide (HRP).

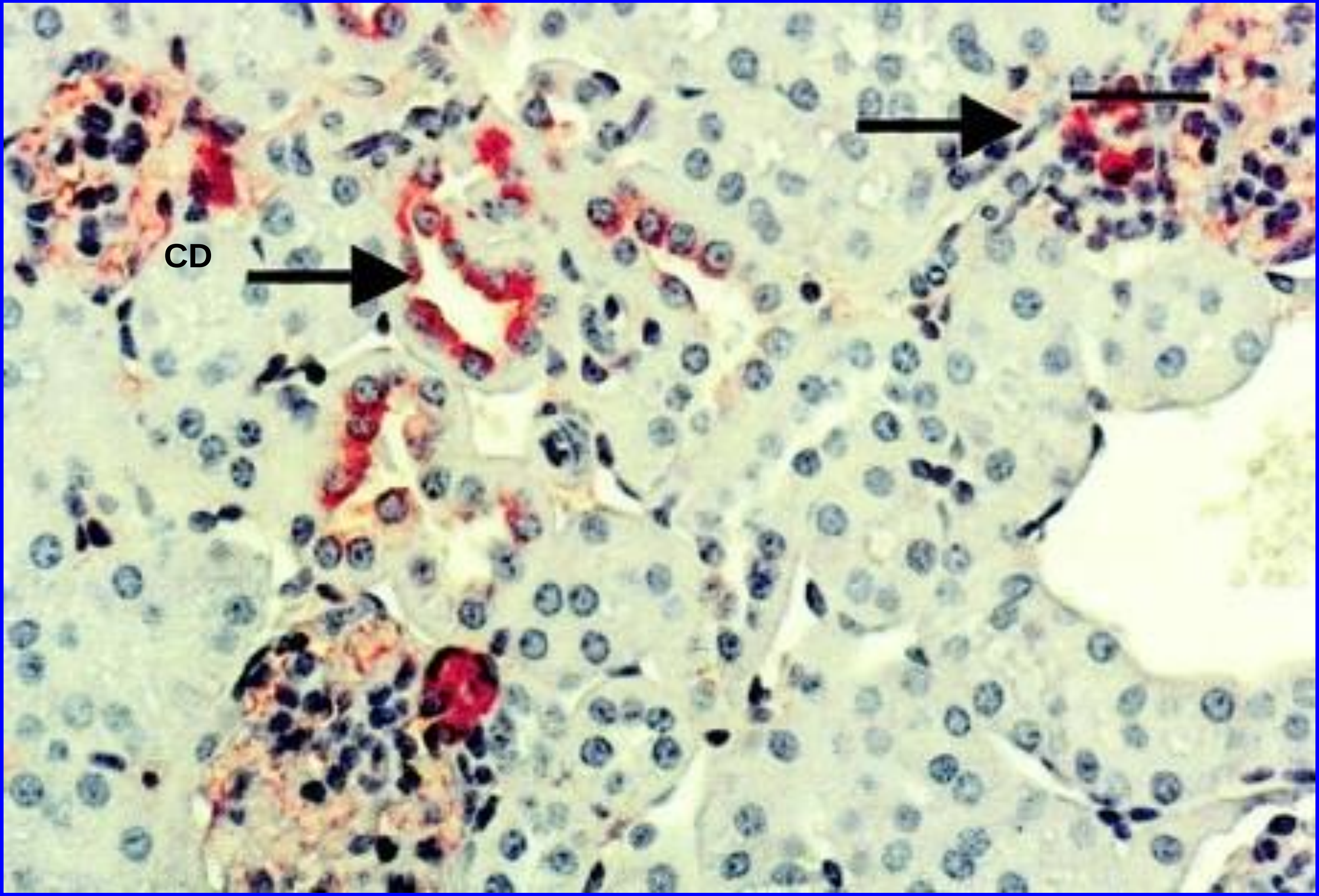
# (Pro)Renin Receptor (ATP6AP2) Signaling via Wnt/β-catenin in Low Vertebrates



# Localization of PRR in the Collecting Duct Intercalated Cells



# CD Expression of Renin




Rohrwasser A et al. *Hypertension* 1999

# COX-2/PGE2 Pathway Mediates AngII-Induced Renin Response and PRR Expression in CD Cells


Am J Physiol Renal Physiol 307: F25–F32, 2014.
First published April 16, 2014; doi:10.1152/ajprenal.00548.2013.

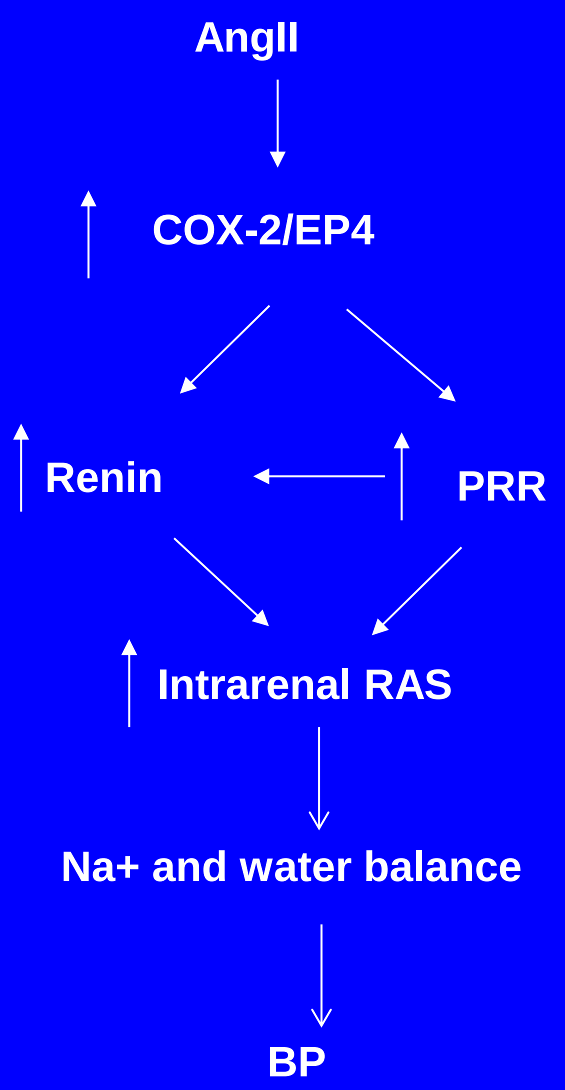
COX-2 mediates angiotensin II-induced (pro)renin receptor expression in the rat renal medulla

Fei Wang, Xiaohan Lu, Kexin Peng, Li Zhou, Chunling Li, Weidong Wang, Xueqing Yu, Donald E. Kohan, Shu-Feng Zhu, and Tianxin Yang

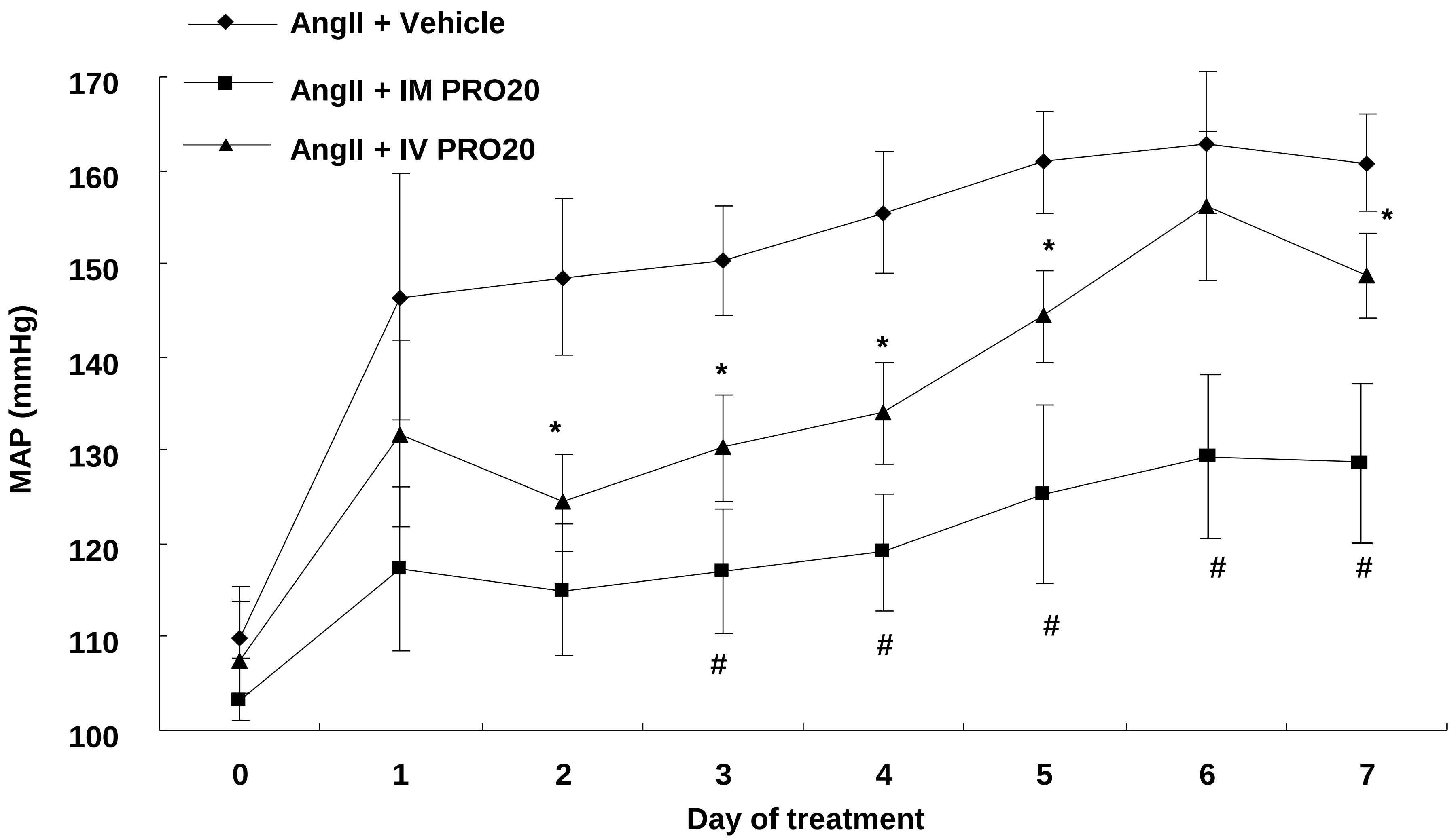

Renin–Angiotensin System

Prostaglandin E-Prostanoid$_4$ Receptor Mediates Angiotensin II–Induced (Pro)Renin Receptor Expression in the Rat Renal Medulla

Fei Wang, Xiaohan Lu, Kexin Peng, Yaomin Du, Shu-Feng Zhou, Aihua Zhang, Tianxin Yang

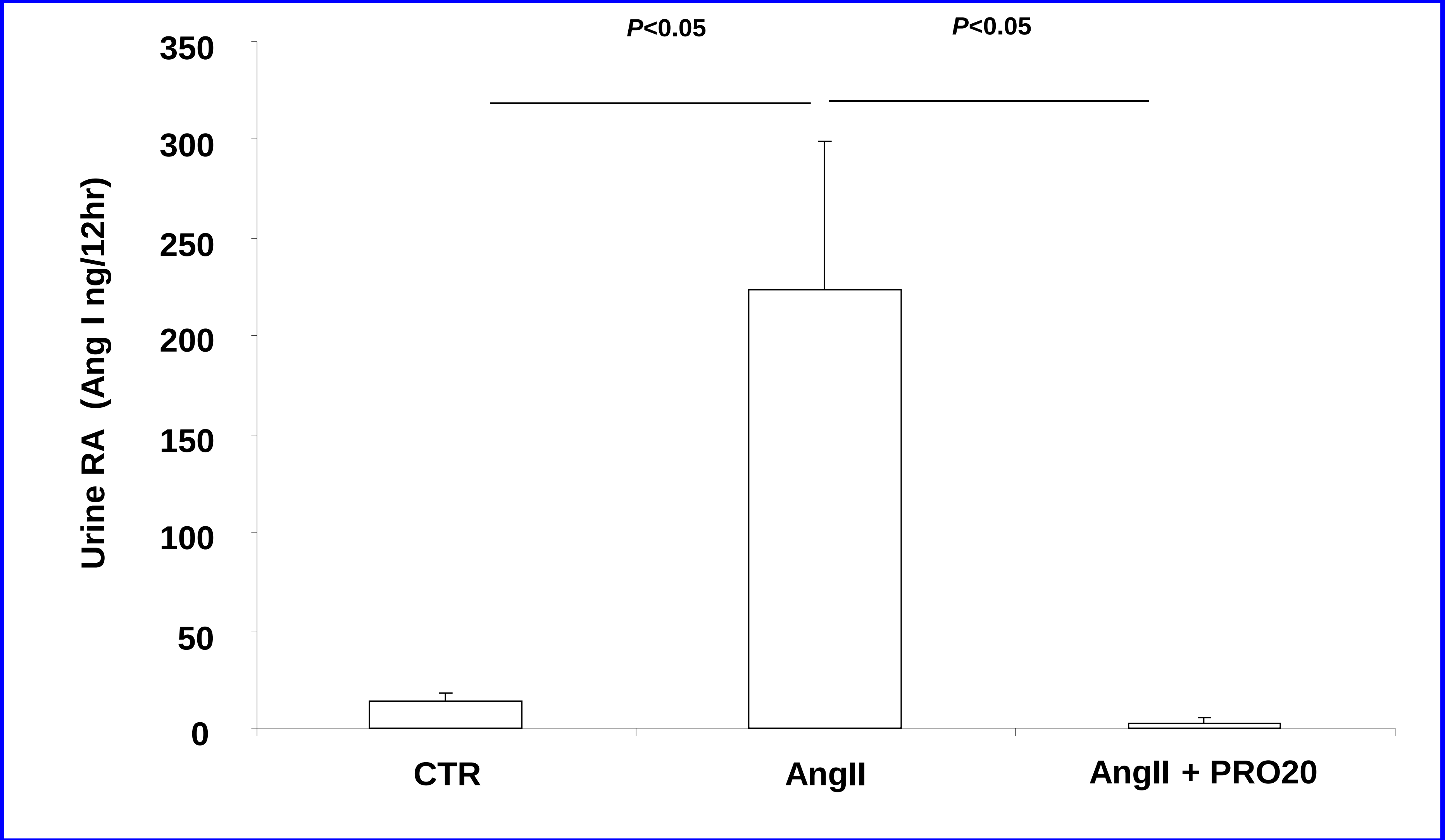


# MAP in Rats Receiving Intramedullary or Intravenous Infusion of PRO20



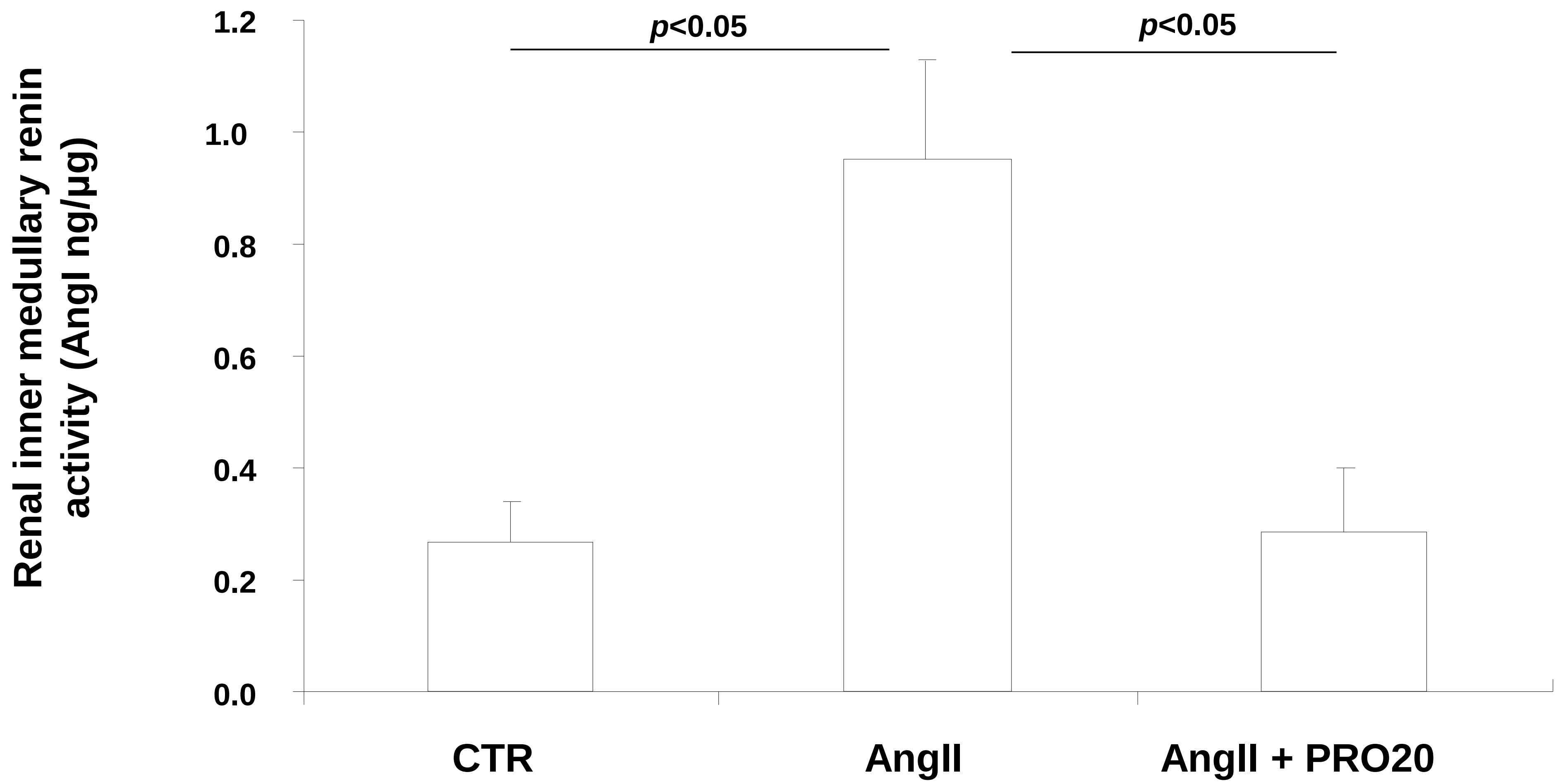

Wang F et al. *BMC Medicine* 2015

# Effect of Intramedullary Infusion of A PRR Blocker on AngII-Induced Urinary Renin Activity



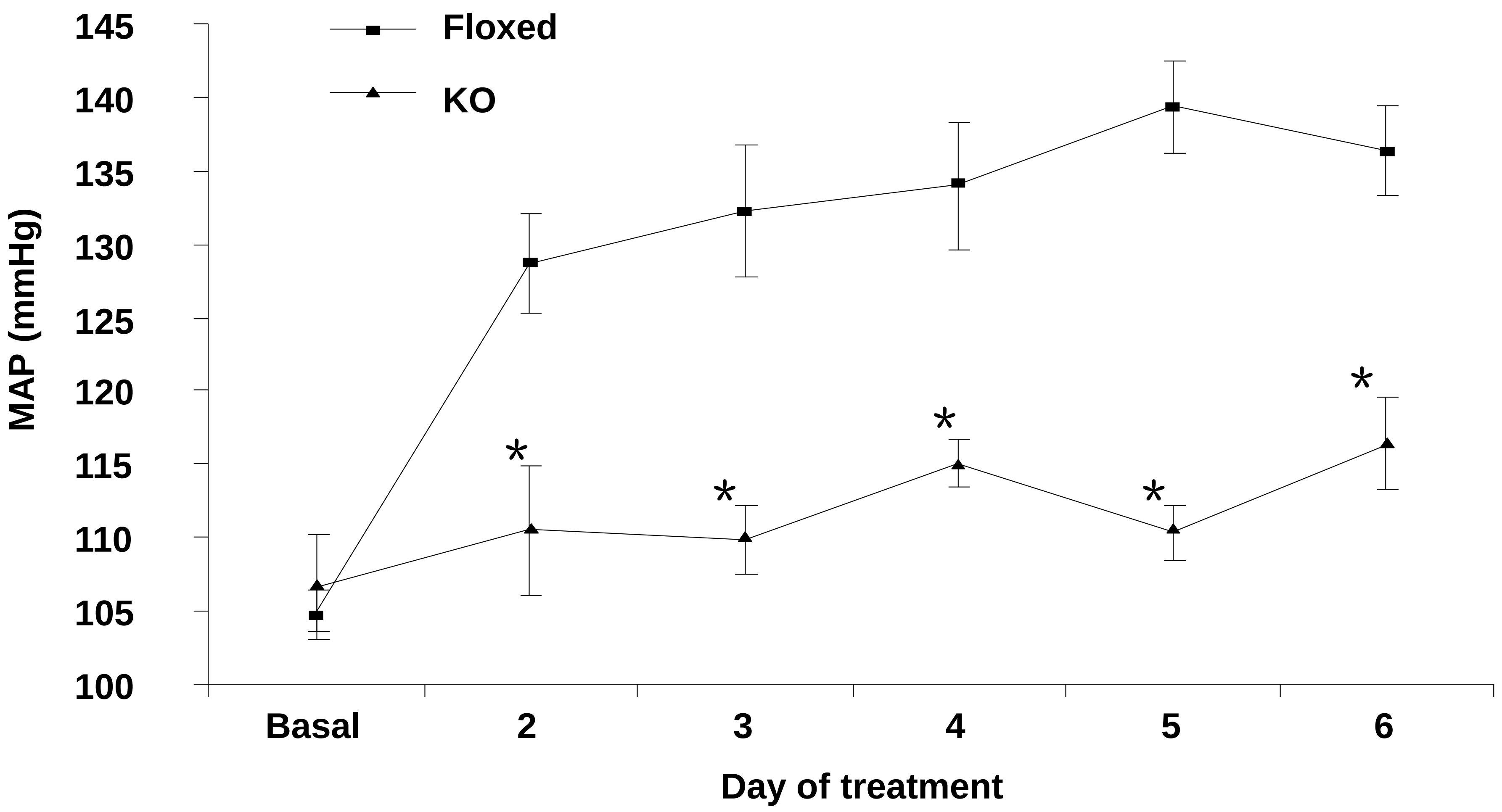

Wang F et al. *BMC Medicine* 2015

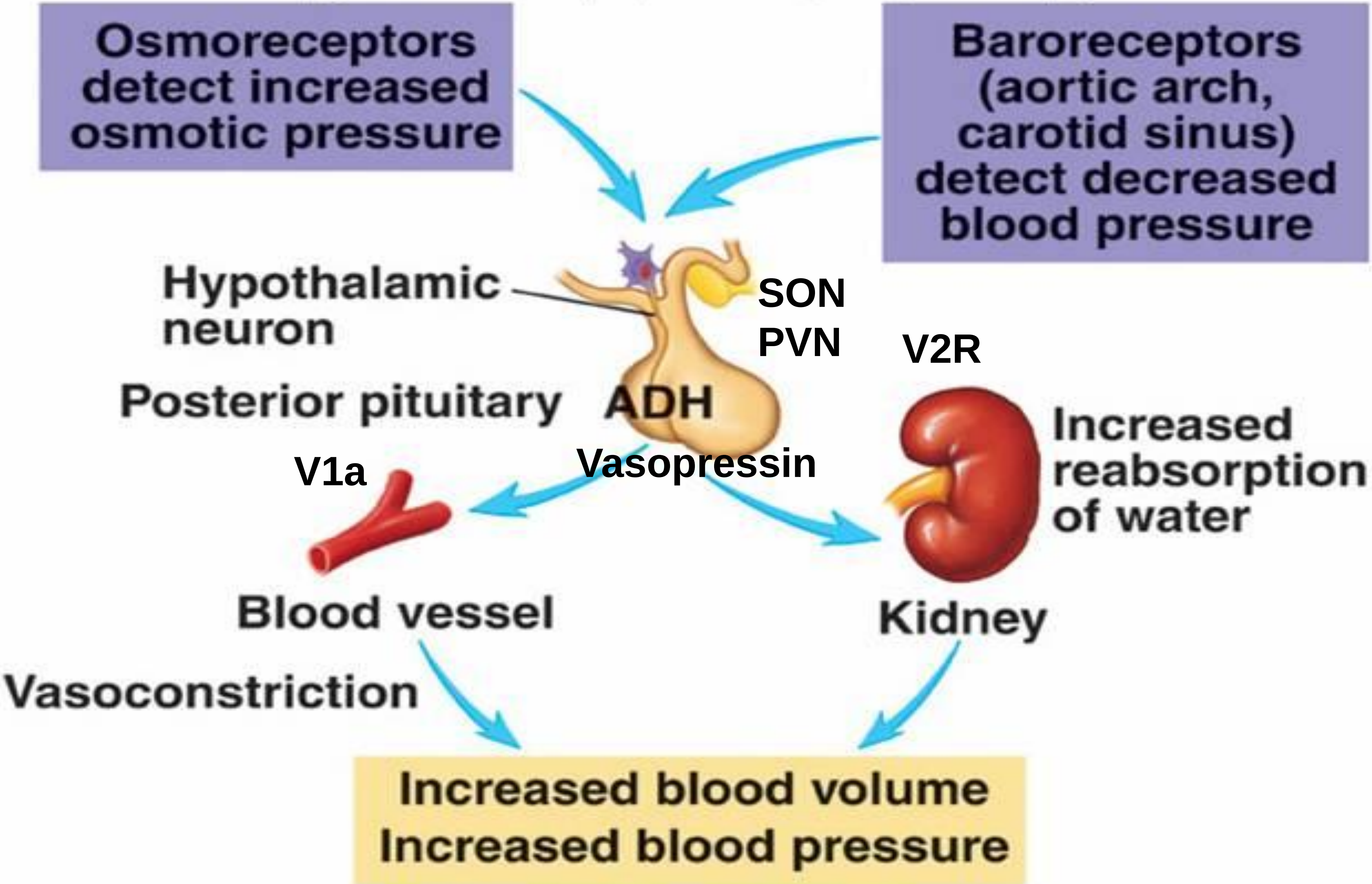
# Effect of Intramedullary Infusion of PRO20 on AngII-Induced Renal Medullary Renin Levels



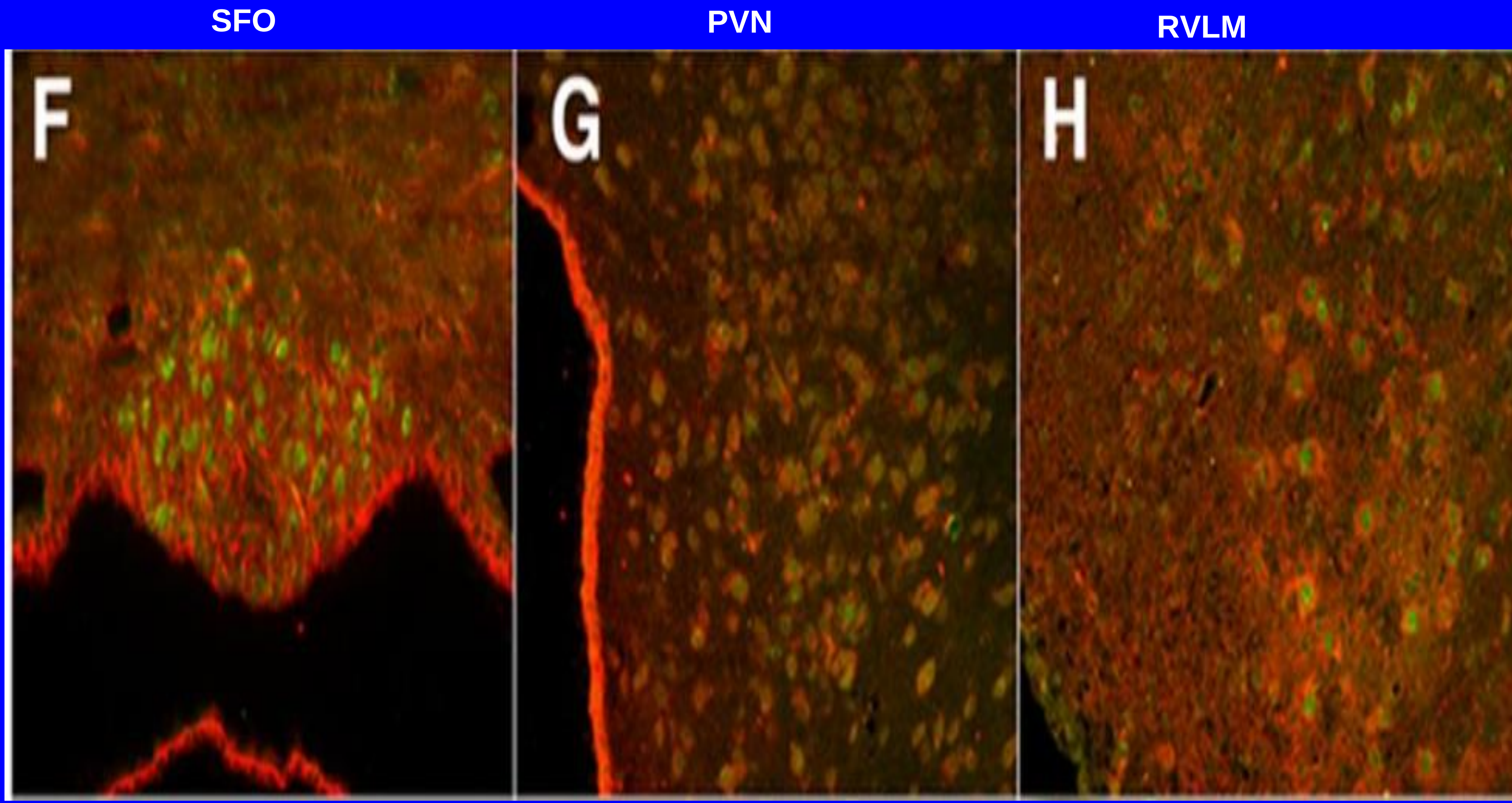

Wang F et al. *BMC Medicine* 2015

# MAP in CD PRR KO mice after AngII Infusion



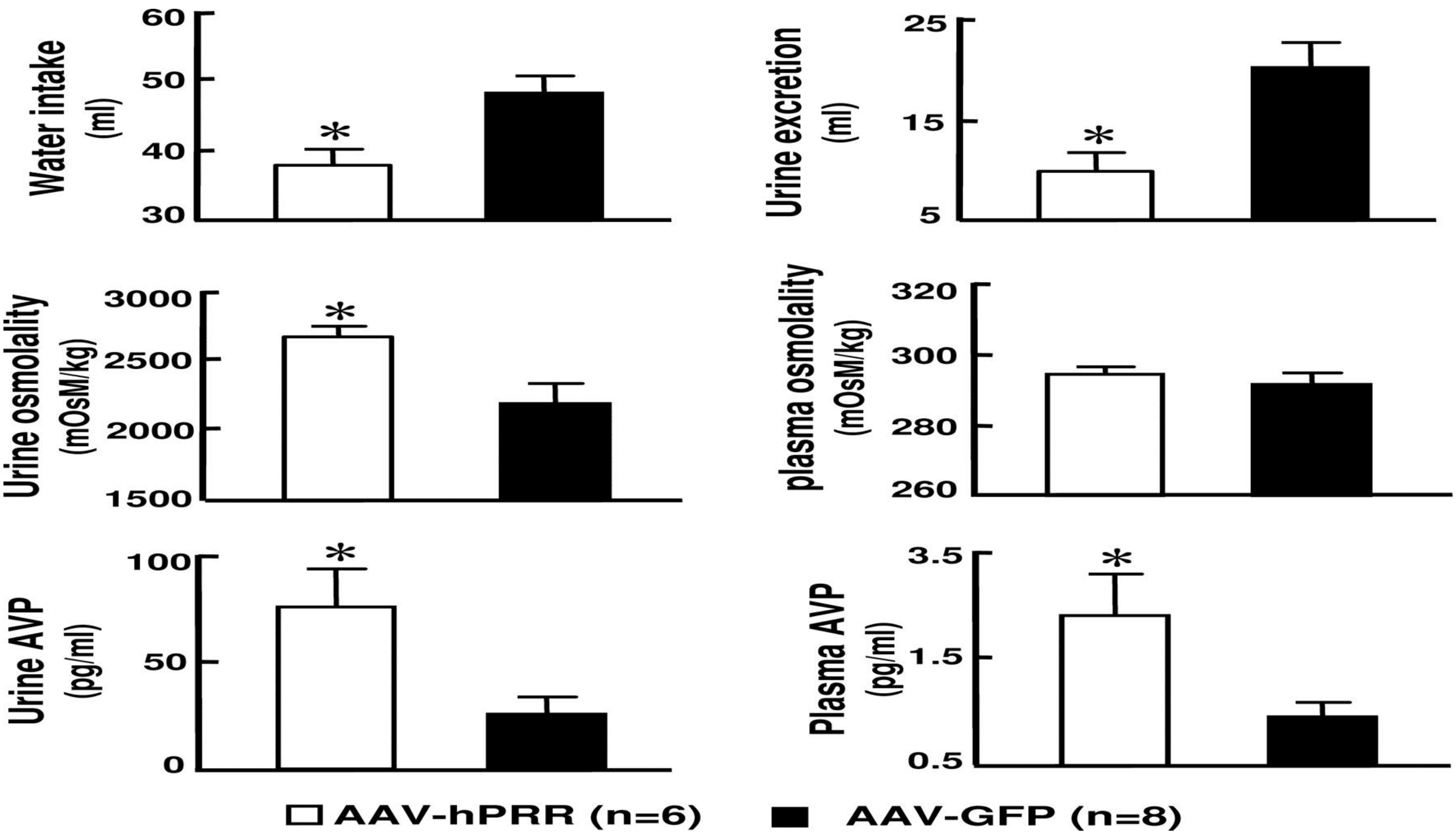

Osmoreceptors detect increased osmotic pressure
Baroreceptors (aortic arch, carotid sinus) detect decreased blood pressure
Hypothalamic neuron
SON
PVN
V2R
Posterior pituitary
ADH
Vasopressin
V1a
Increased reabsorption of water
Blood vessel
Kidney
Vasoconstriction
Increased blood volume
Increased blood pressure

# Immunostaining of PRR in Rat Brain



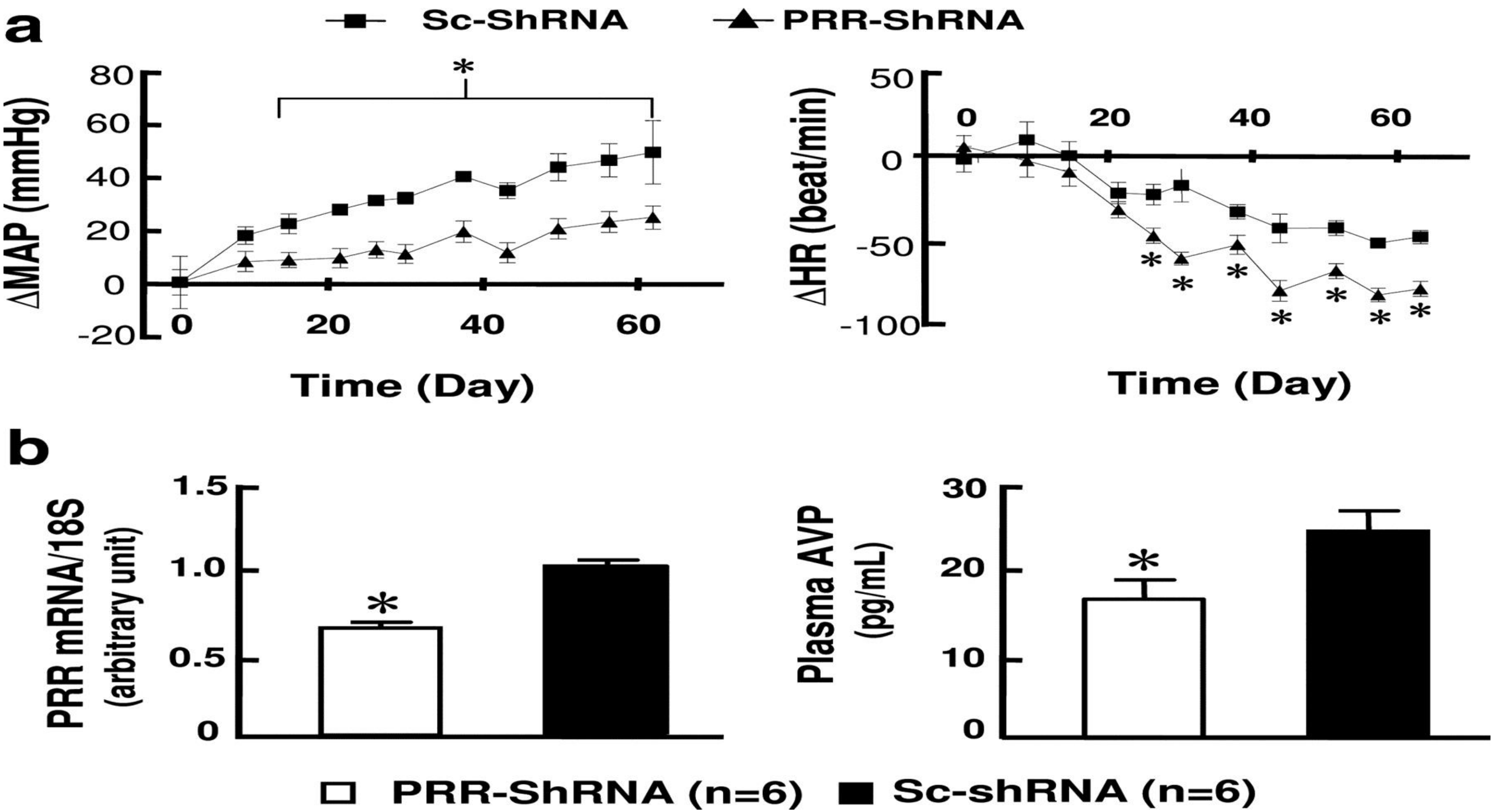

Wencheng Li et al. *Hypertension* 2014;63:316-323

Effect of PRR overexpression in the SON of SD rats on fluid homeostasis

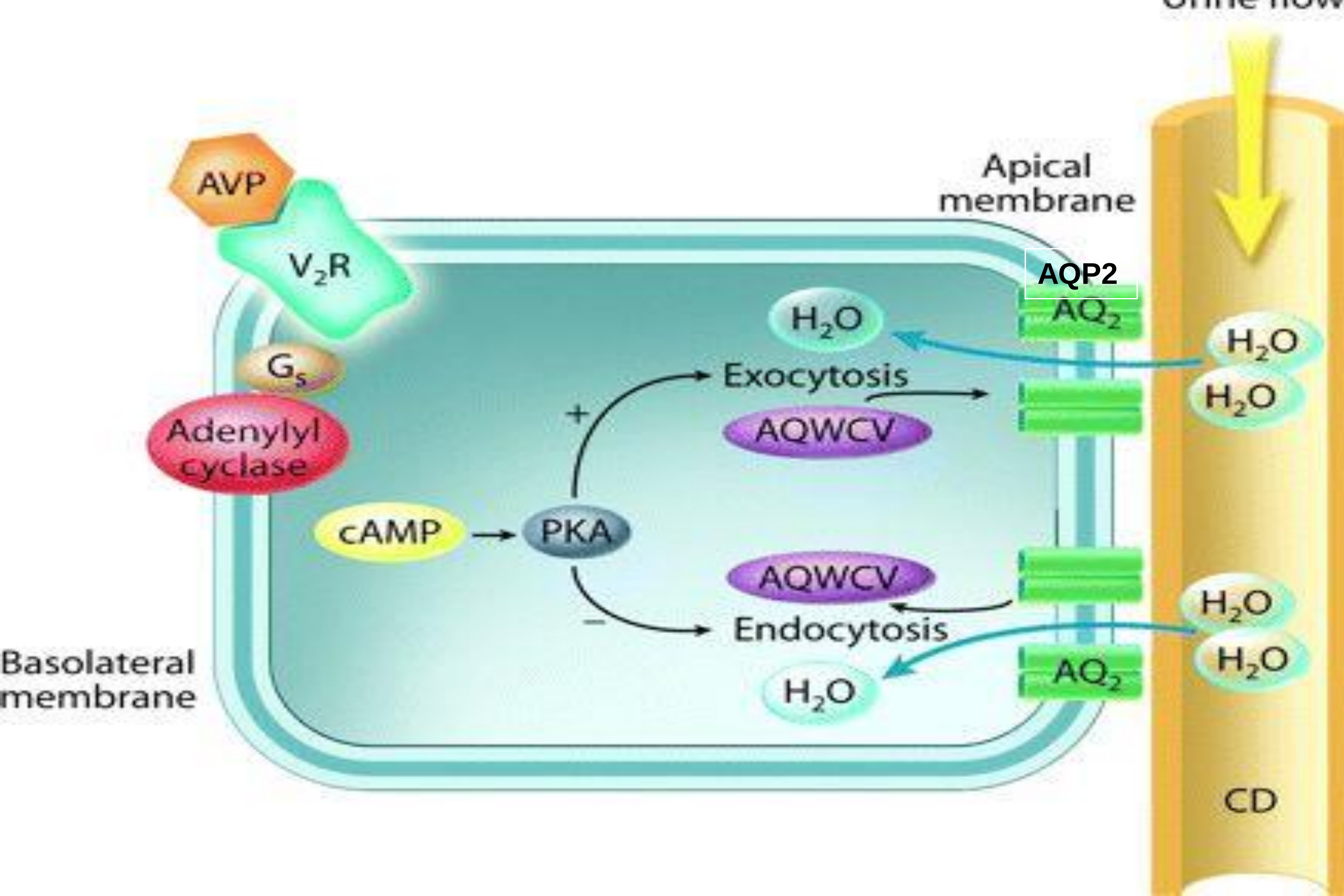



Shan et al. Circulation Research. 2010;107:934-938

# Effect of PRR knockdown in the SON of SHRs on cardiovascular function

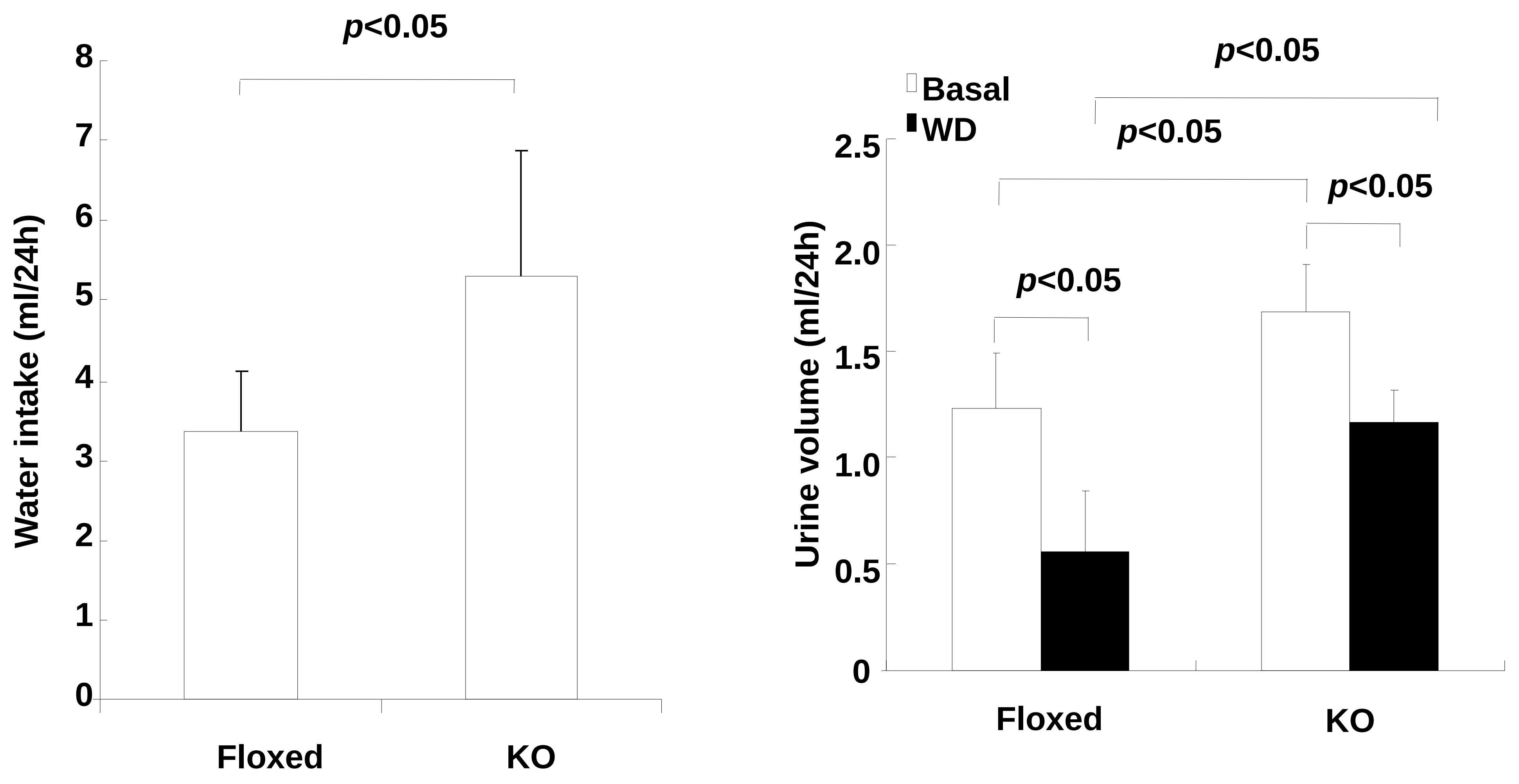



Shan et al. *Circulation Research* 2010;107:934-938

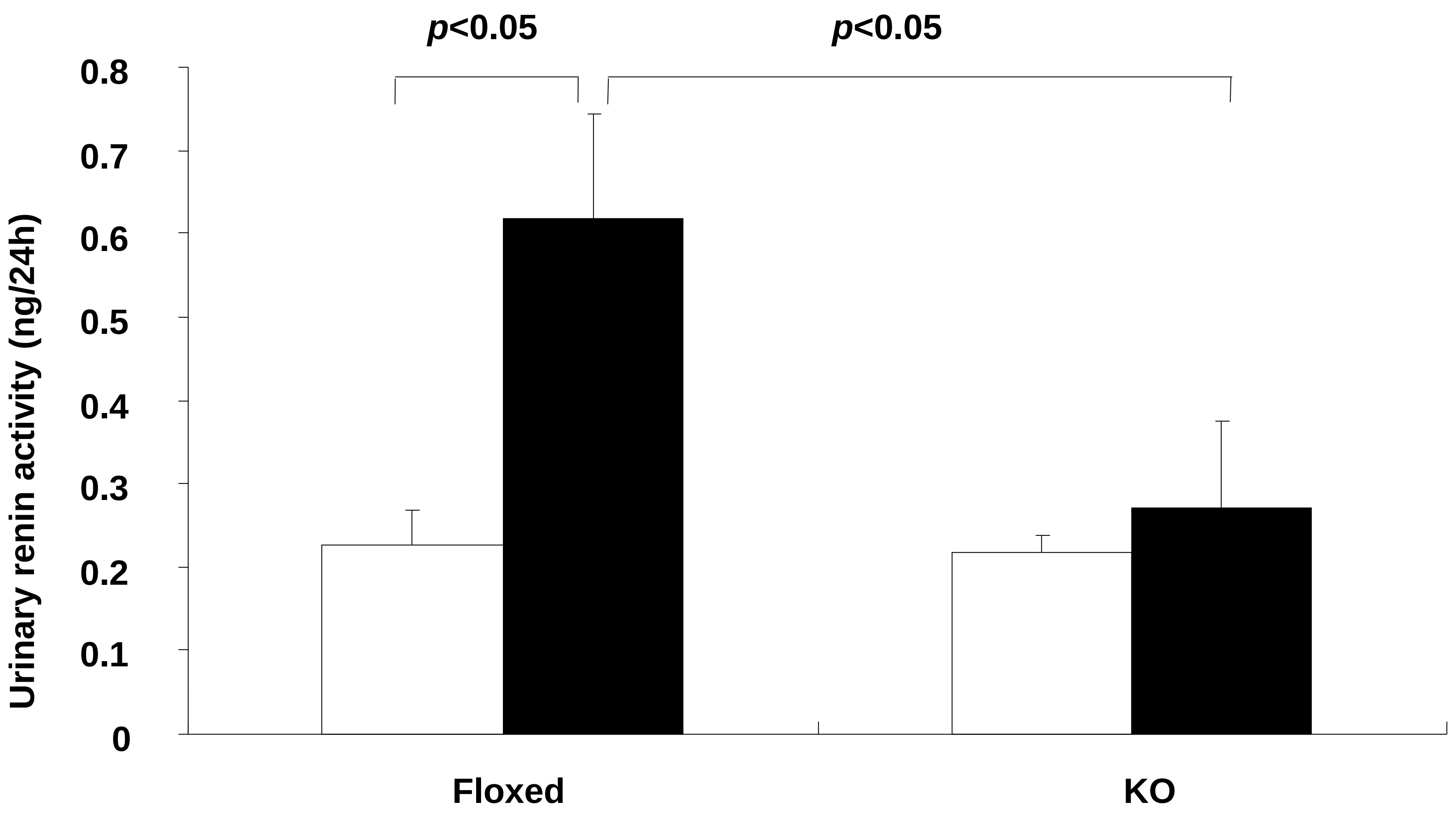


Urine flow
AVP
V2R
Gs
Adenylyl cyclase
cAMP
PKA
+
−
Apical membrane
AQP2
AQ2
H2O
Exocytosis
AQWCV
AQWCV
Endocytosis
H2O
H2O
H2O
H2O
H2O
H2O
AQ2
Basolateral membrane
CD

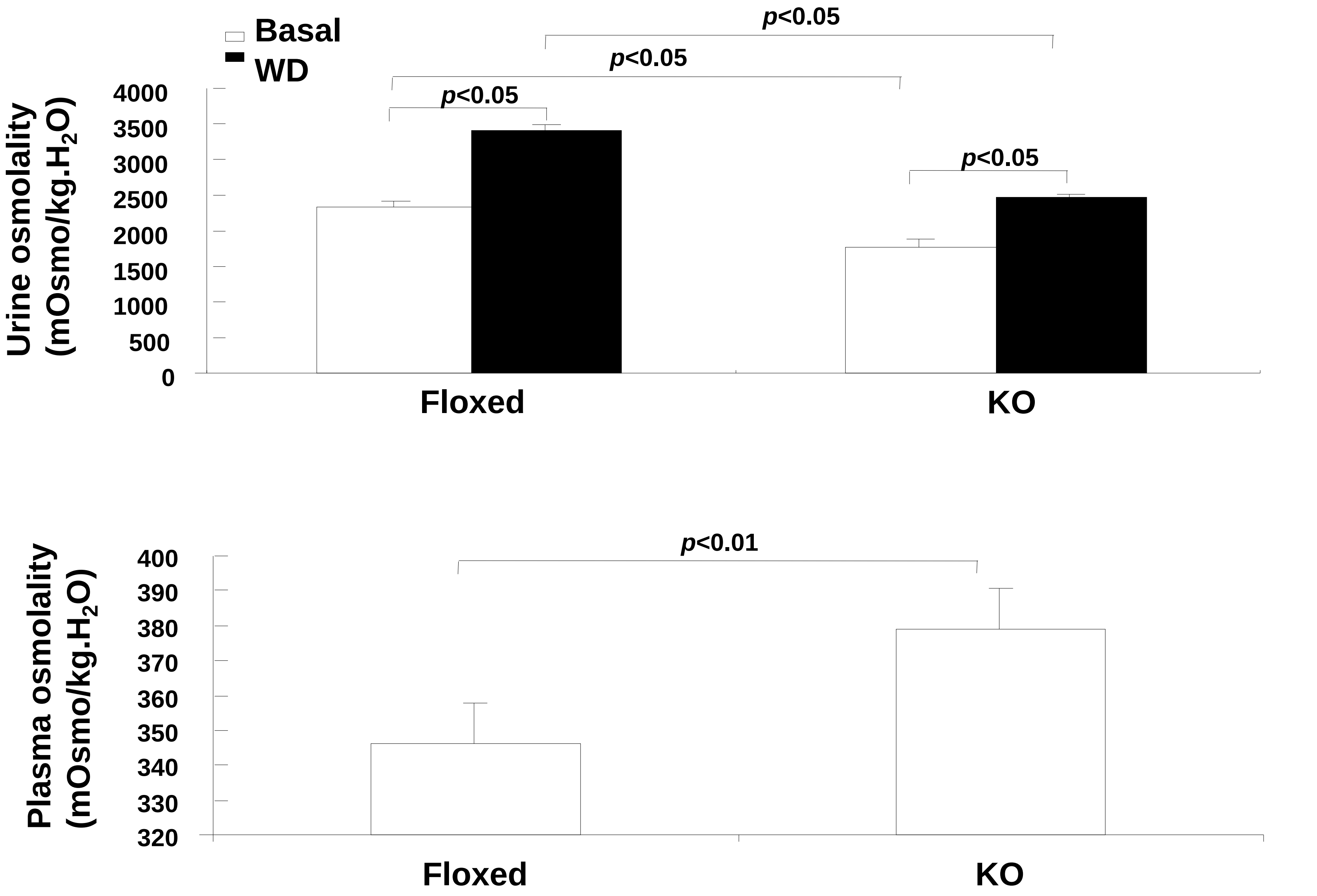
# Fluid Metabolism in CD PRR KO mice



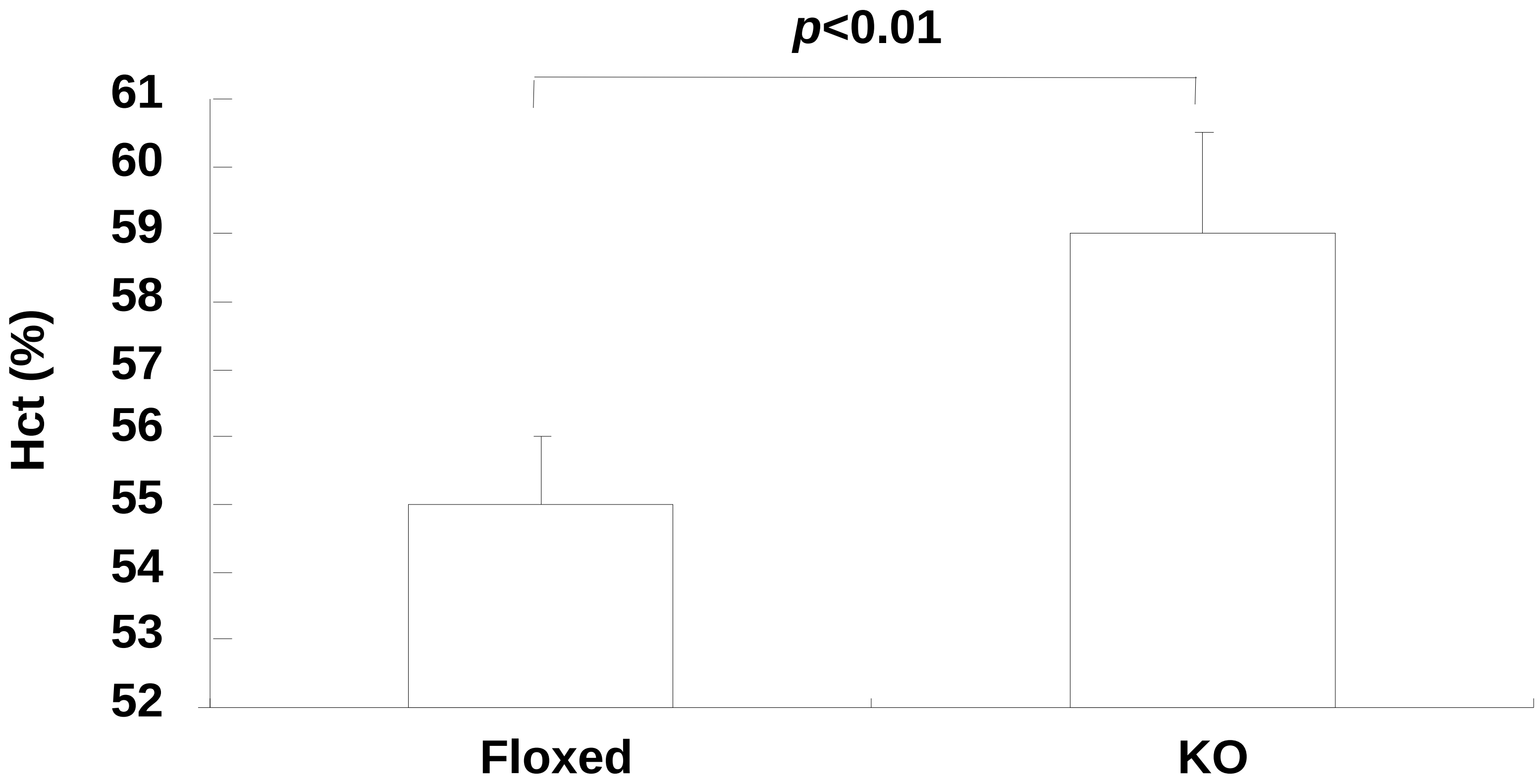
# Urinary Renin Activity in CD PRR KO mice



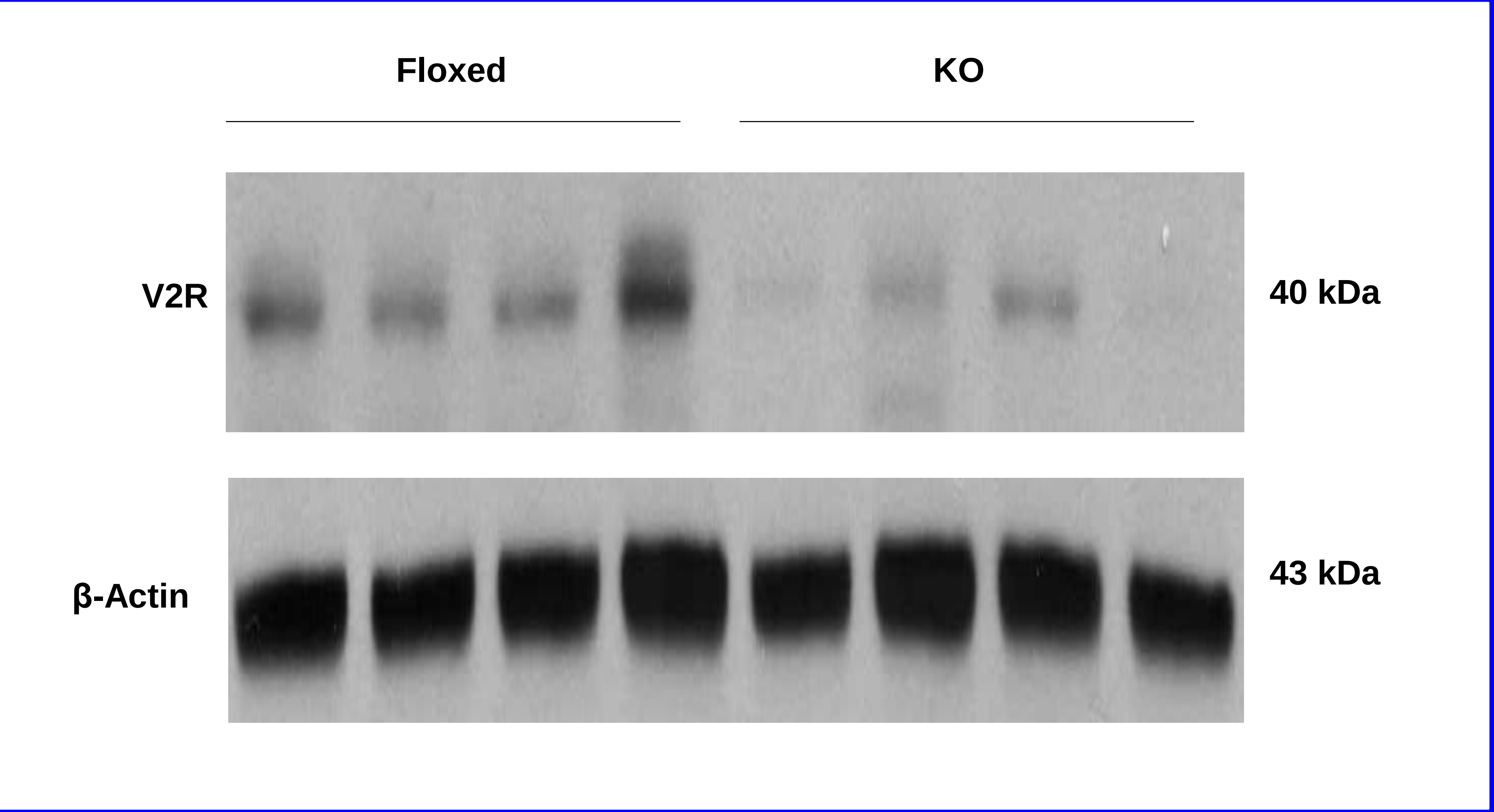
# Urine and Plasma Osmolality in CD PRR KO mice



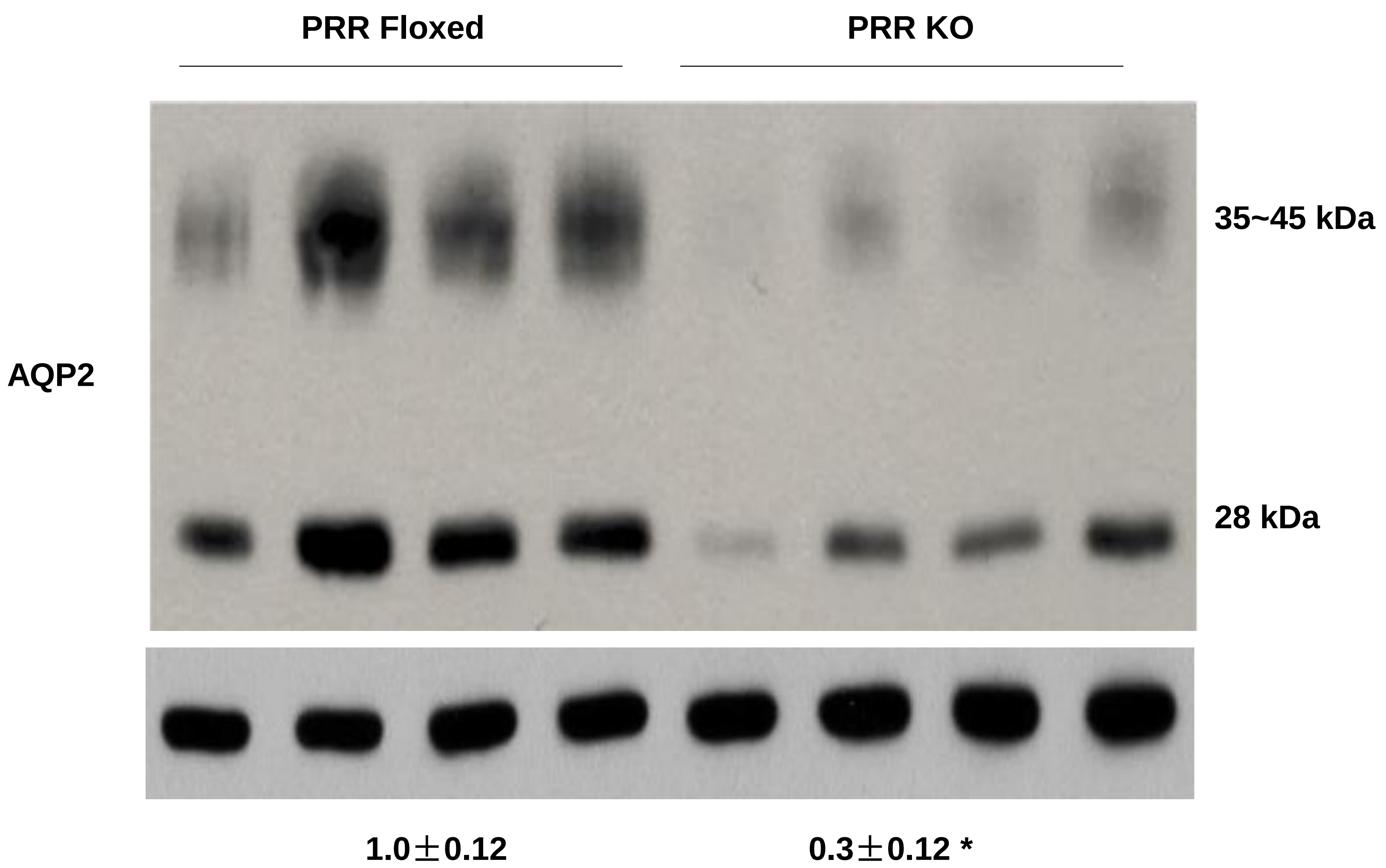
## Hct in CD PRR KO Mice after 24h Water Deprivation



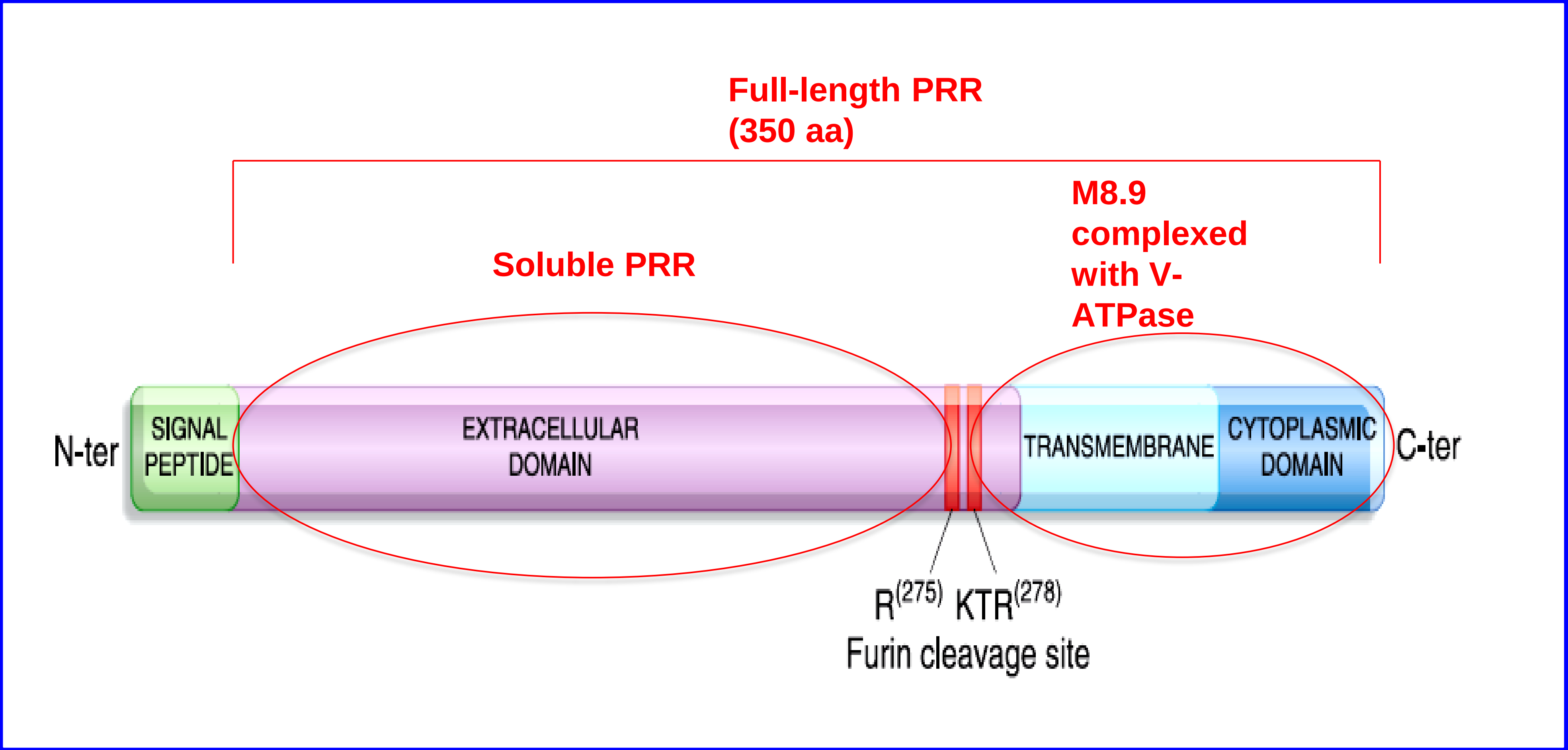
# Renal Medullary V2R Protein Expression in CD PRR KO Mice



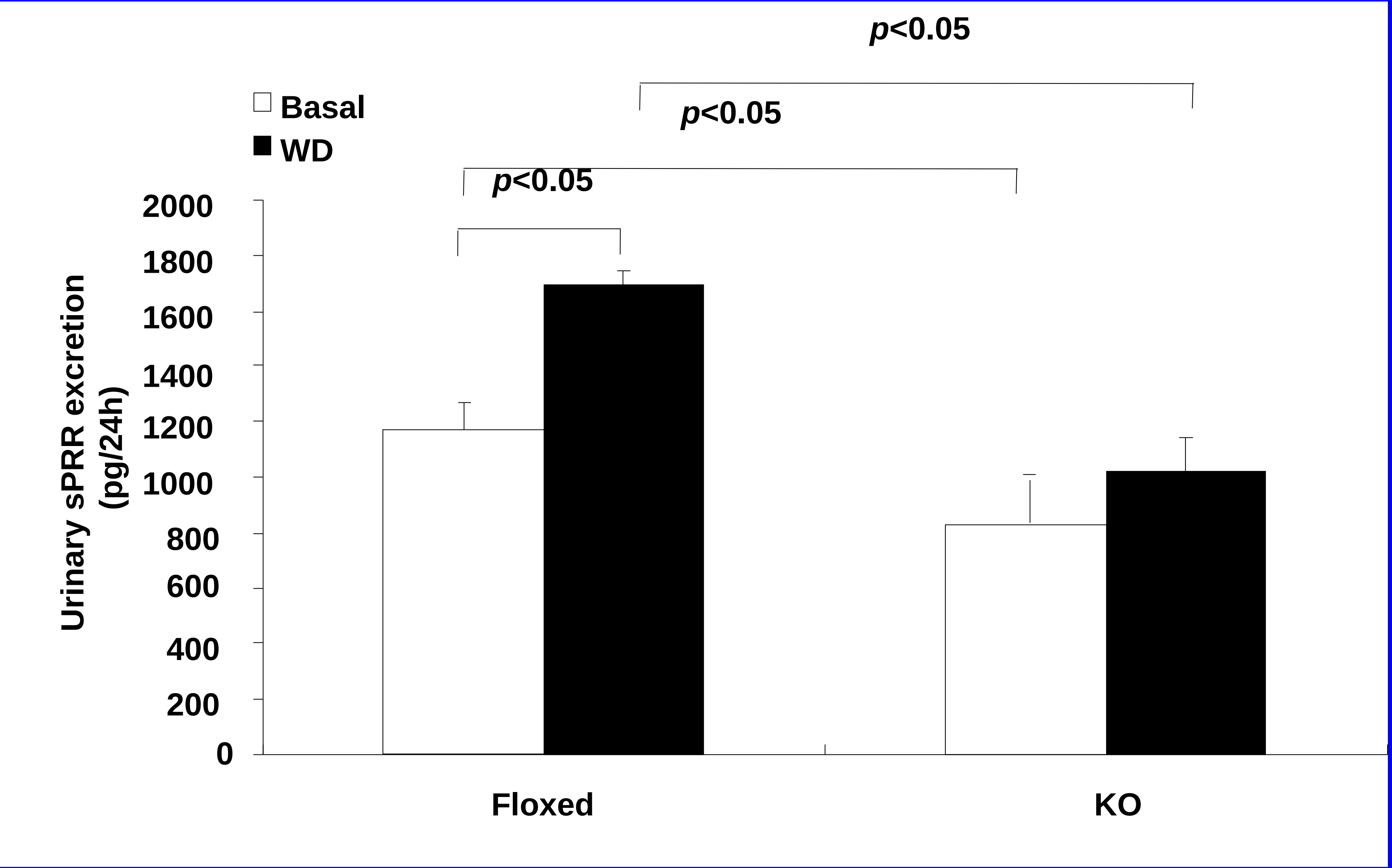
# Renal Medullary AQP2 Protein Expression in CD PRR KO Mice



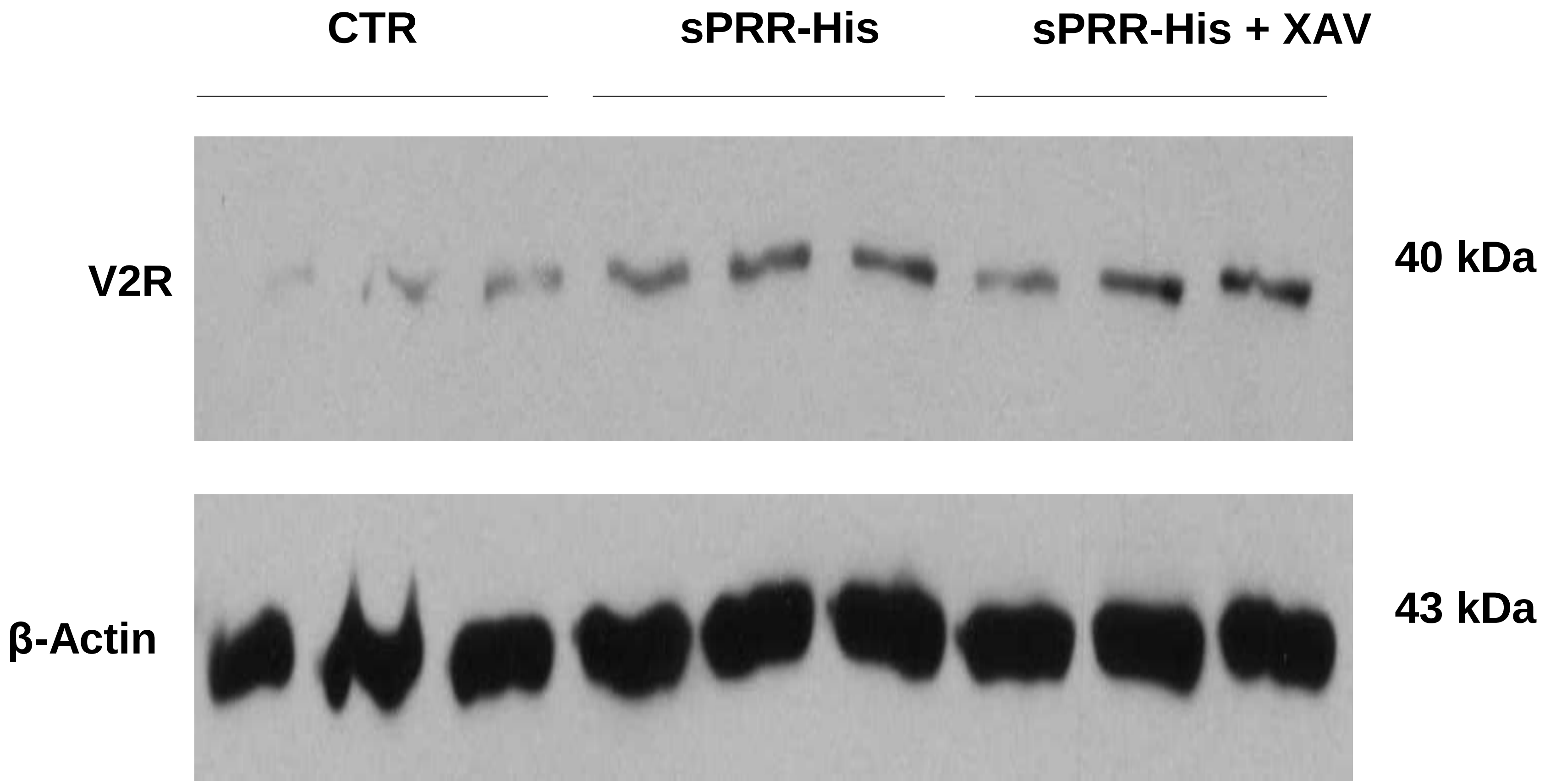
# Structure of (Pro)Renin Receptor (PRR) (ATP6AP2)



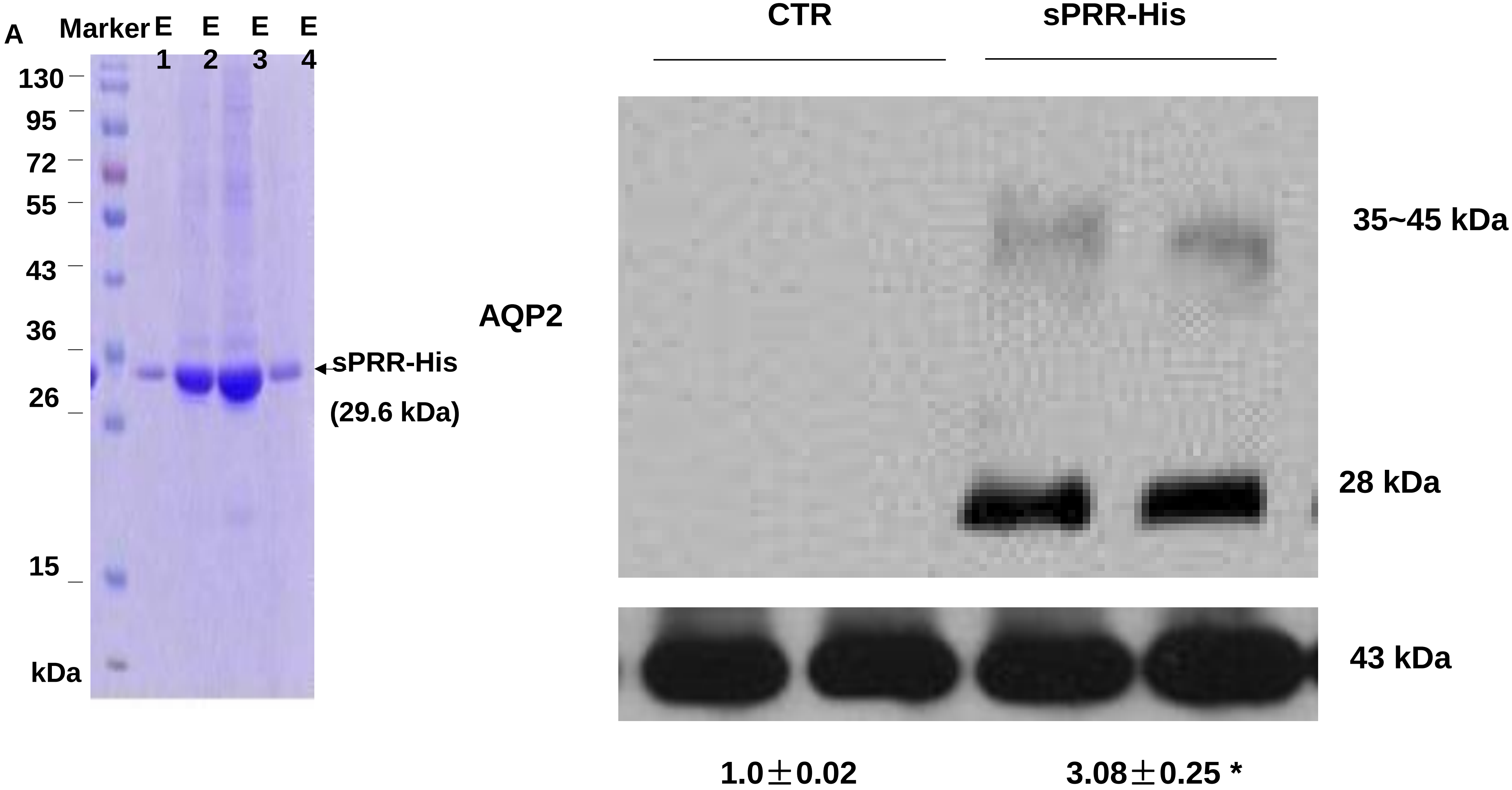
# Urinary sPRR Excretion in CD PRR KO Mice



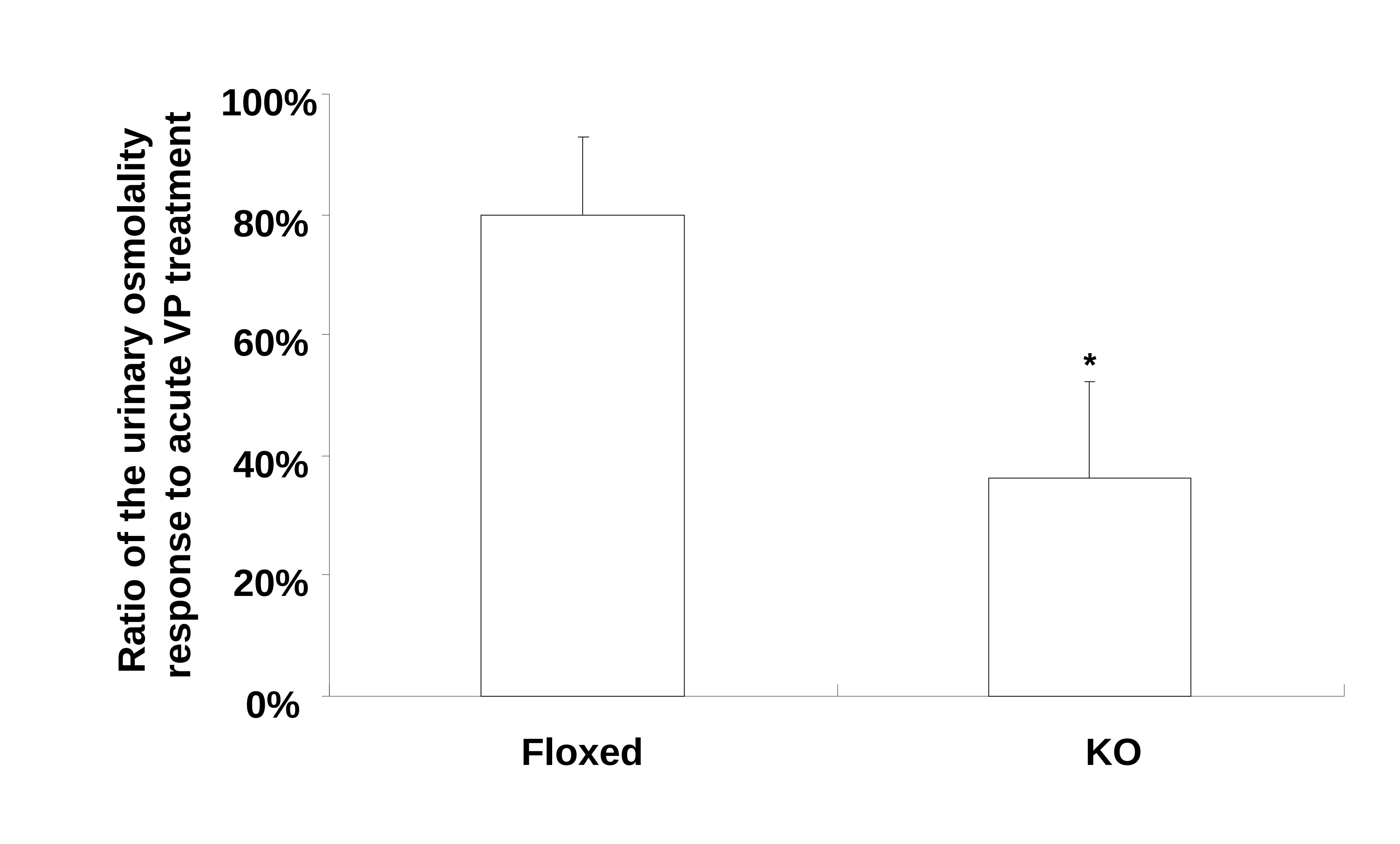
# sPRR Stimulated V2R Expression in Cultured IMCD Cells



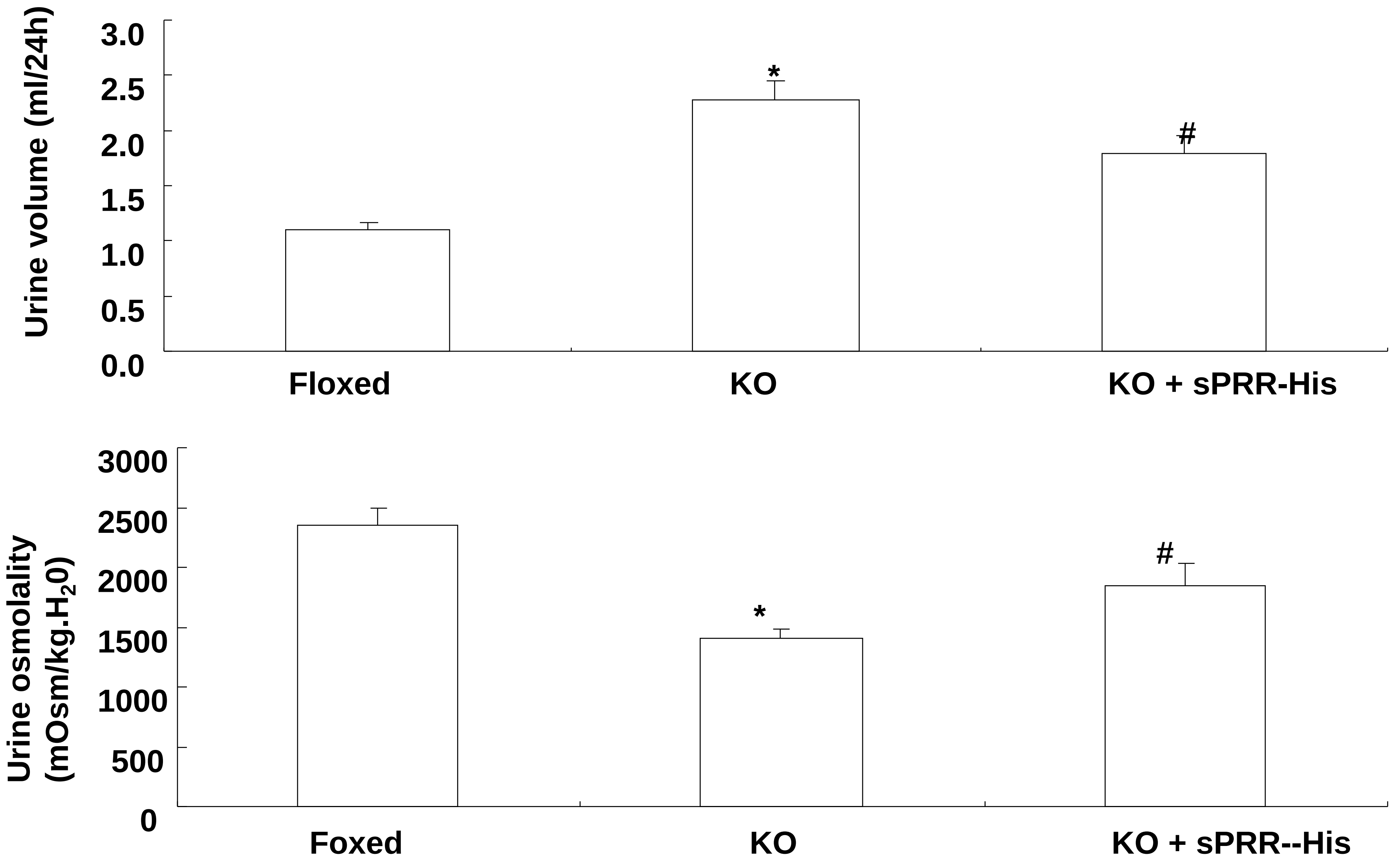
# Effect of sPRR-His on AQP2 Expression in Cultured IMCD Cells



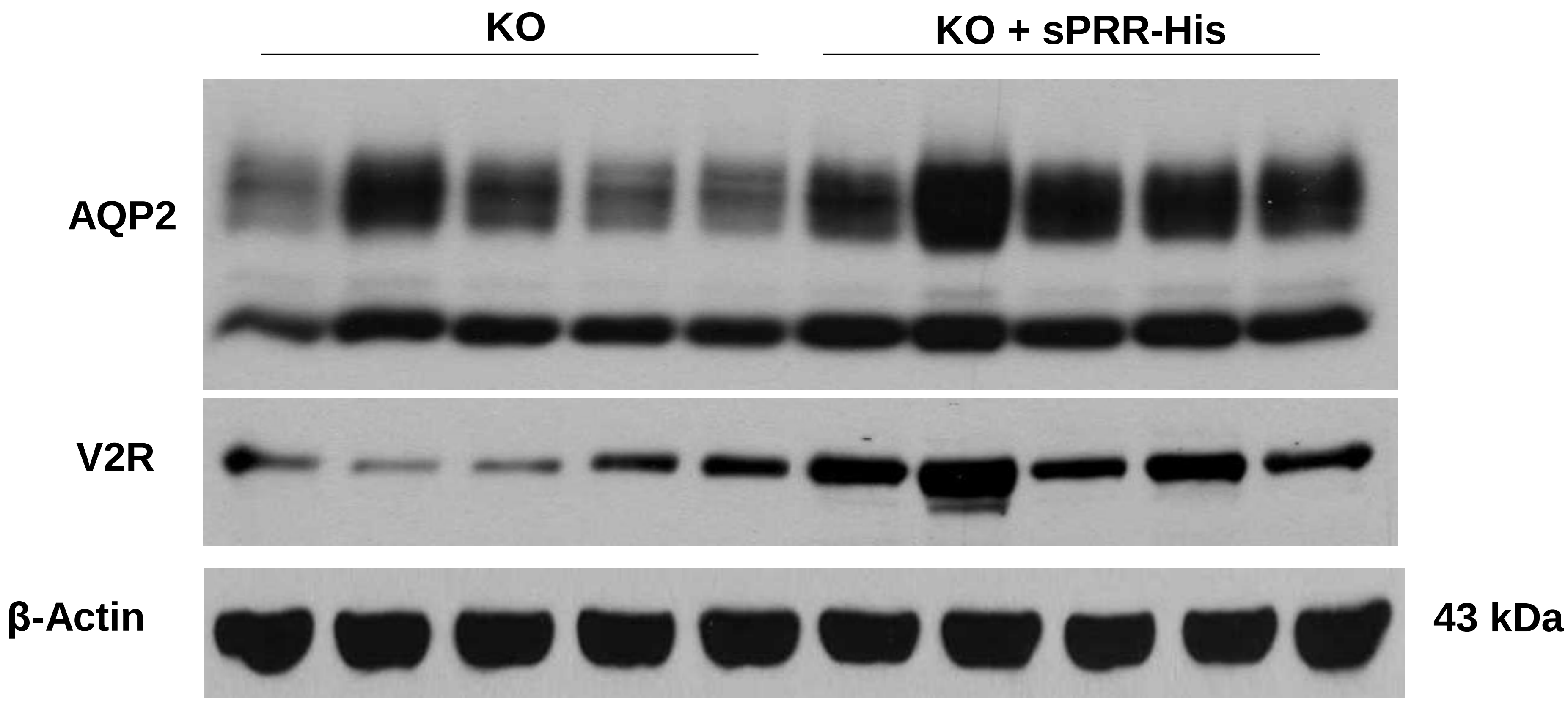
# Response of Urine Osmolality to AVP



# IV Infusion of sPRR-His Partially Rescues the Polyuria Phenotype of CD PRR KO Mice

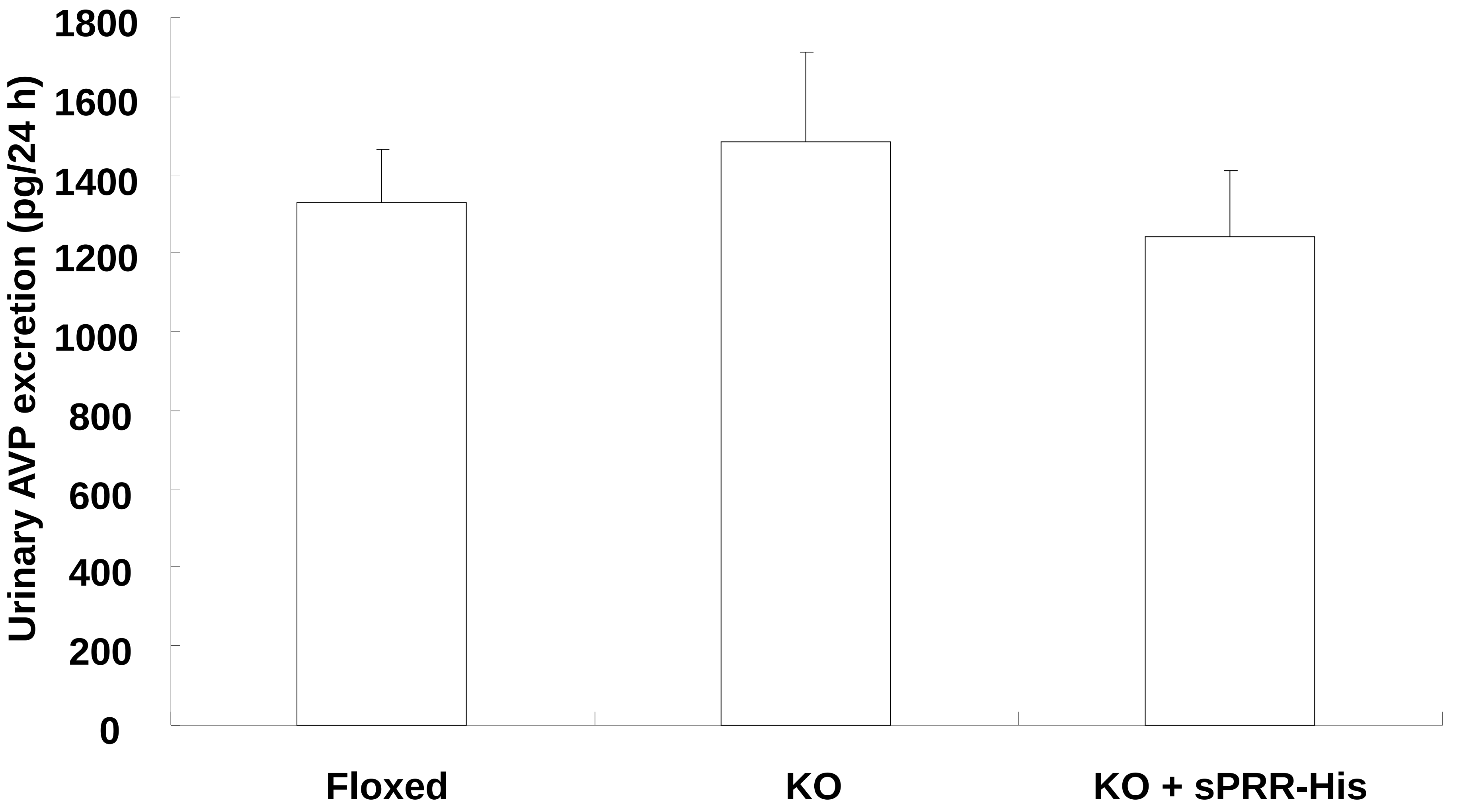


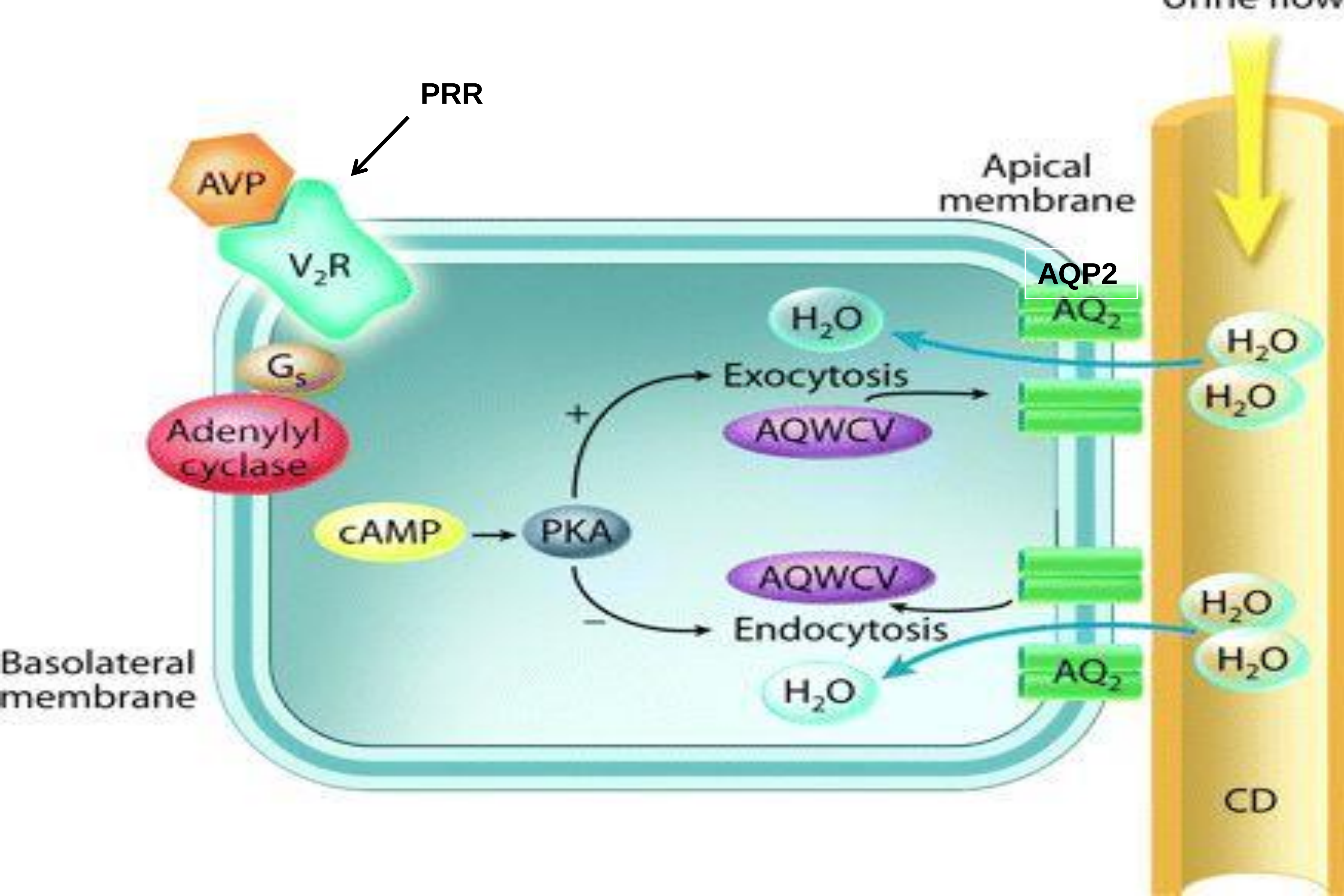
# IV Infusion of sPRR-His Elevated Renal V2R and AQP2 Expression in CD PRR KO Mice




| | KO | KO + sPRR-His |
|---|---|---|
| AQP2 | 1.0±0.12 | 1.59±0.09 # |
| V2R | 1.0±0.20 | 3.41±0.52 # |

# No Change in AVP Production





Urine flow
PRR
AVP
$V_2R$
$G_s$
Adenylyl cyclase
cAMP
PKA
+
−
Exocytosis
Endocytosis
AQWCV
AQWCV
$H_2O$
$H_2O$
$H_2O$
$H_2O$
$H_2O$
$H_2O$
AQP2
$AQ_2$
$AQ_2$
Apical membrane
Basolateral membrane
CD

# Summary/Conclusion

- We provide in vivo evidence for PRR as a regulator of local renin in the the kidney.
- Activation of CD PRR mediates local renin response and determines AngII-induced hypertension.
- In addition, CD PRR regulates BP and fluid balance via simultaneously controlling central AVP production and peripheral AVP sensitivity.
- The action of CD PRR is mediated by sPRR .

# ACKNOWLEDGEMENTS

**Yang Lab**

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**Collaborators**

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Tokyo Women's

Medical Univ.

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UCLA

Russell S. Richardson
Univ. of Utah

OncoMed Pharmaceuticals

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VA

NSFC

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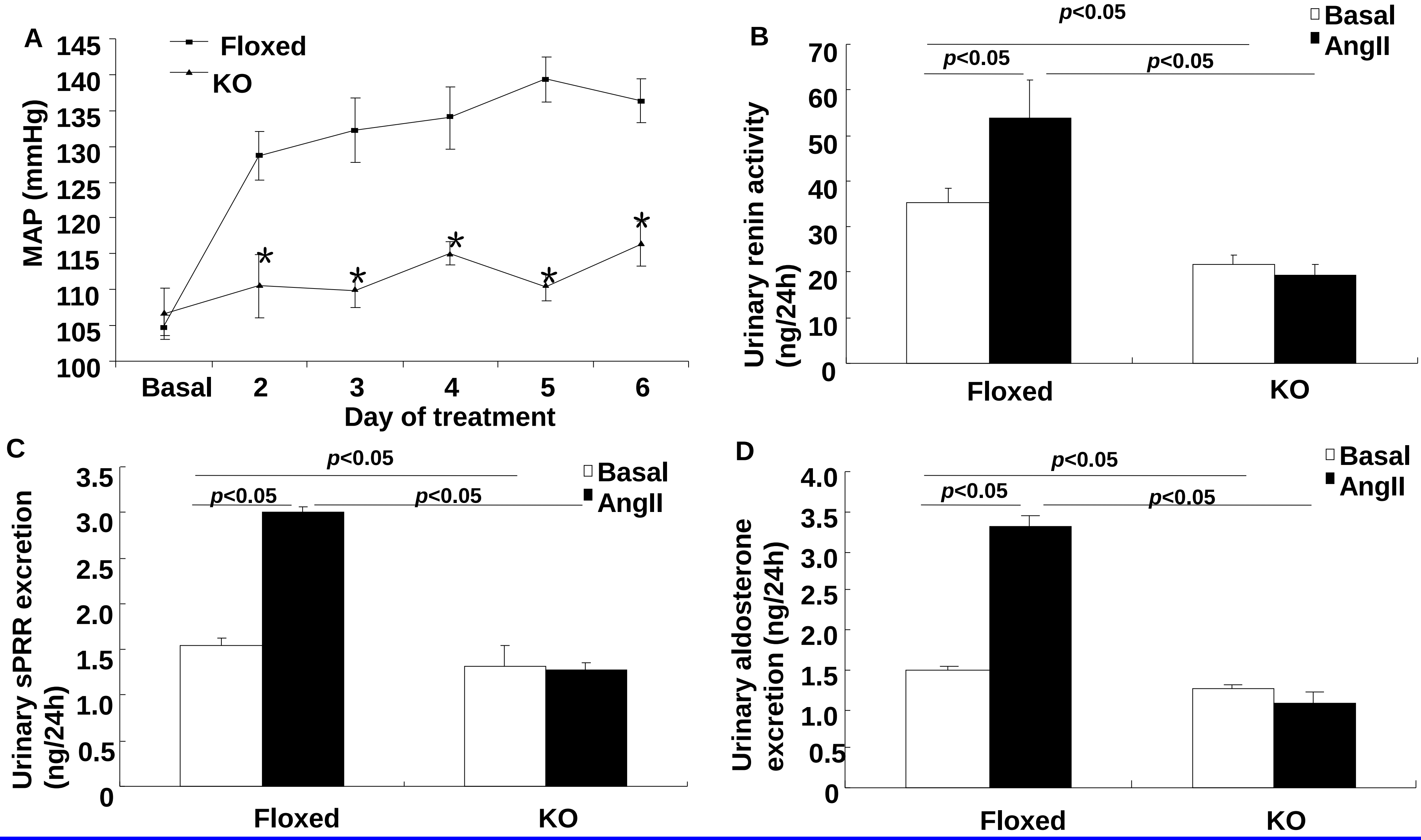
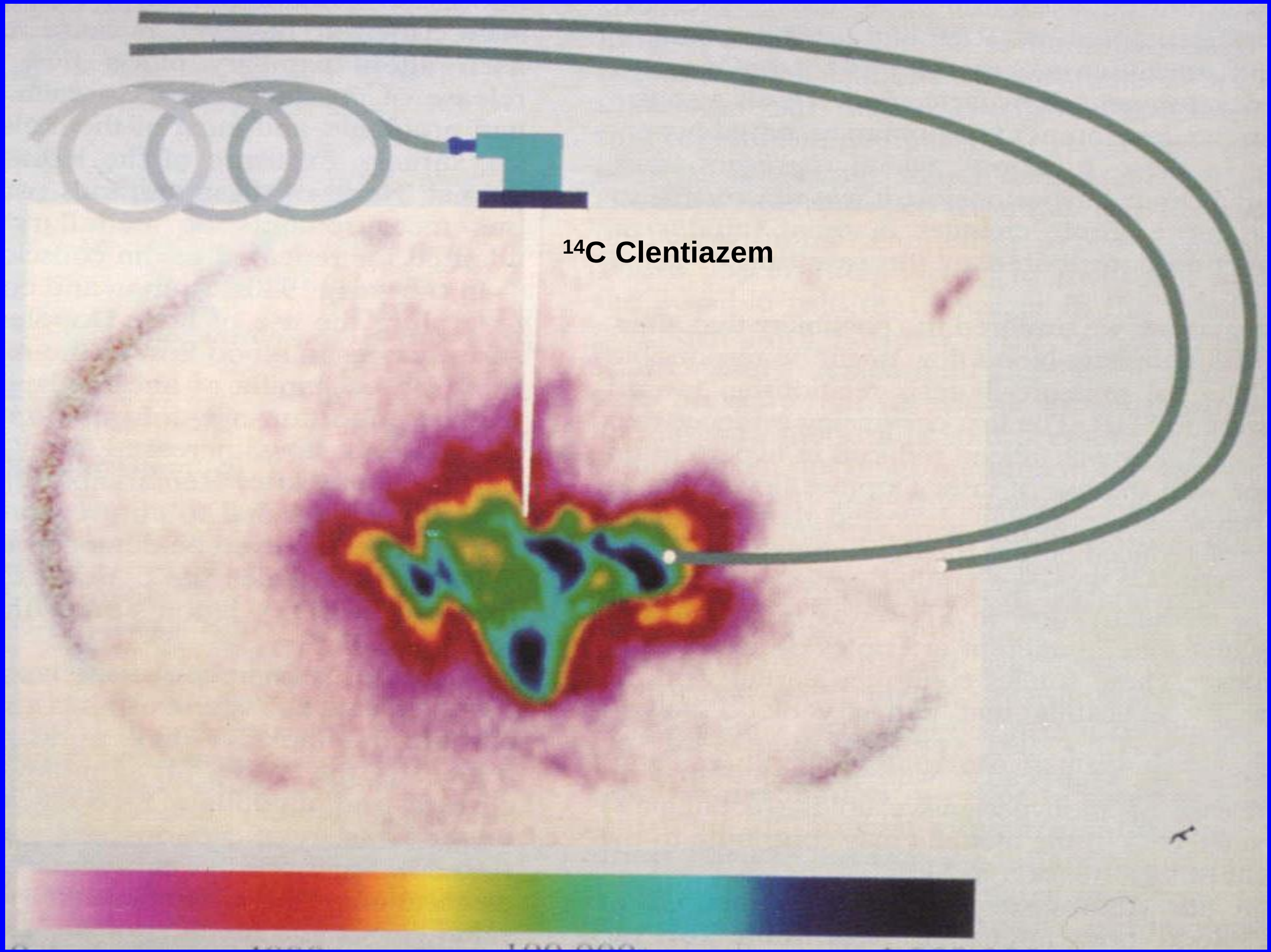
# Phenotypical Analysis of CD PRR KO mice after AngII Infusion



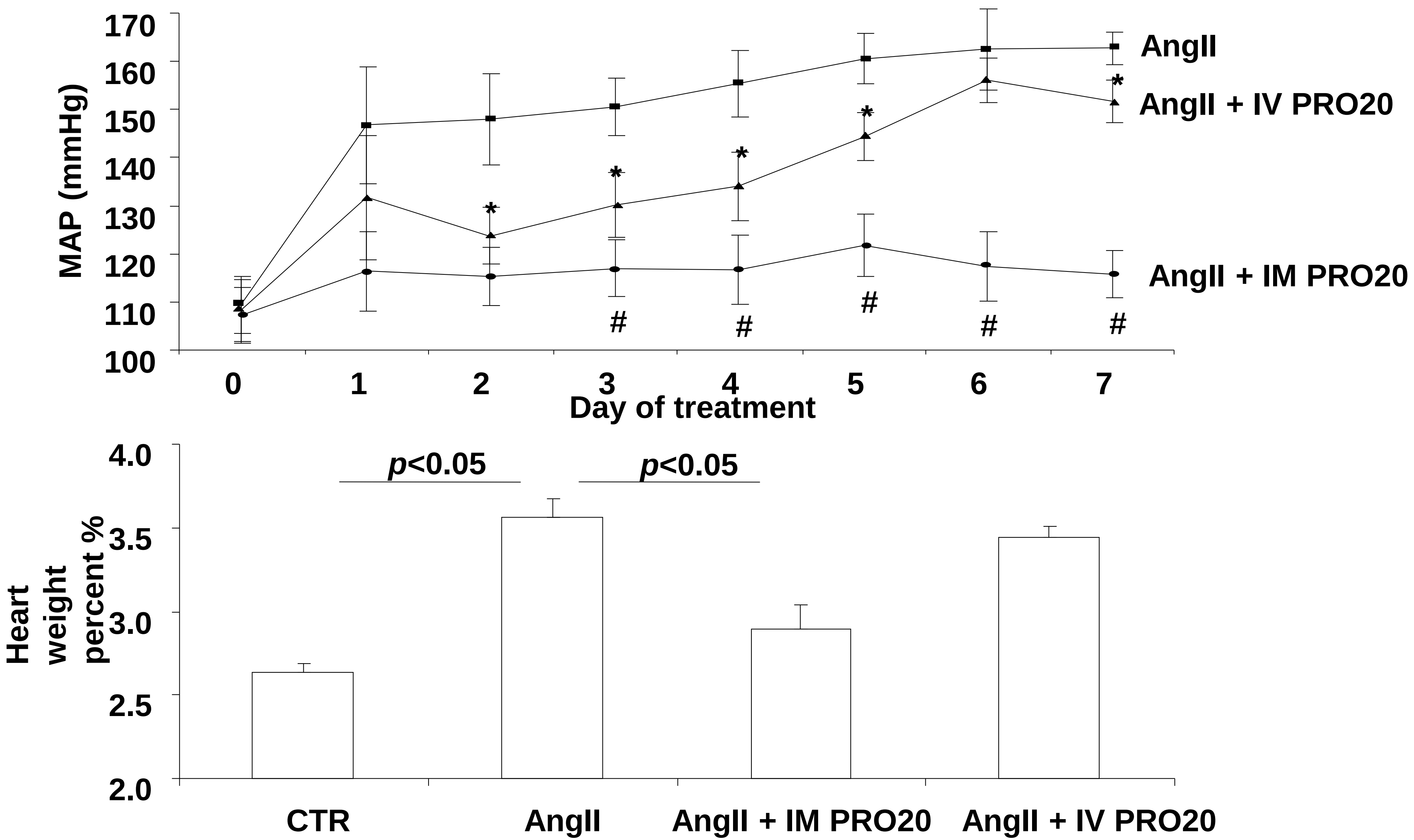
# Intramedullary Infusion Technique

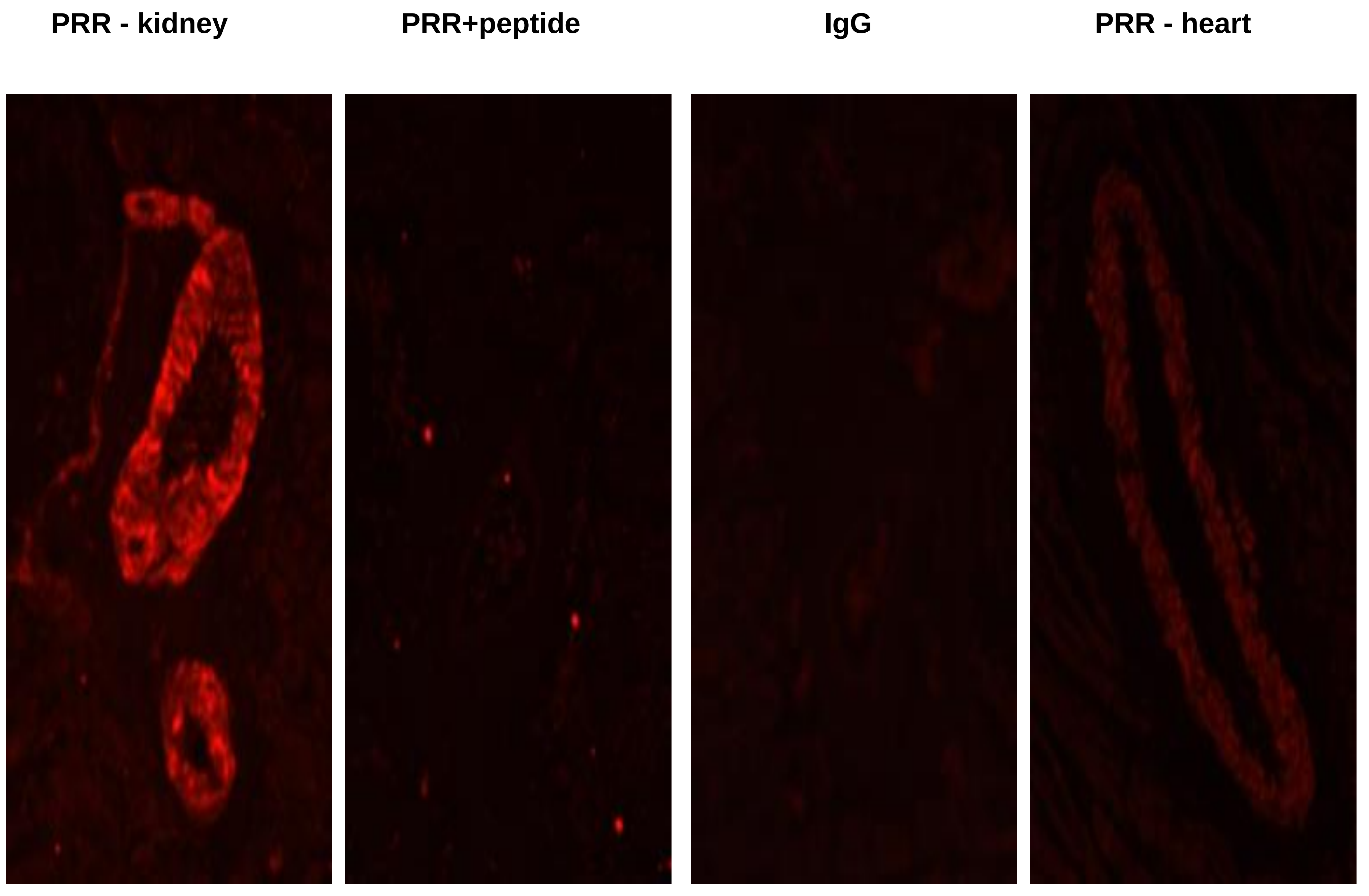



4000 10,000 100,000

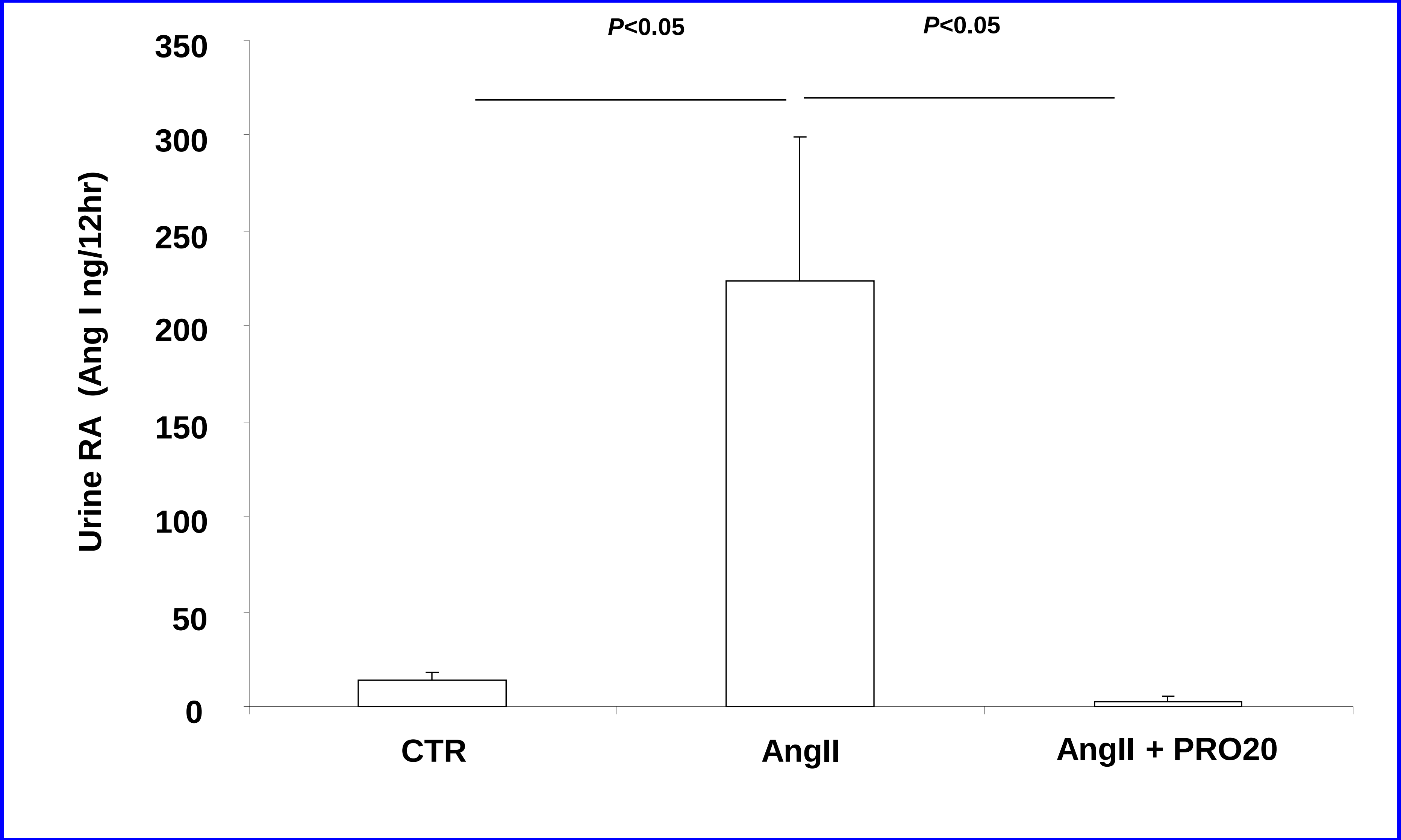
Cowley et al. *Hypertension* 1995

# MAP in Rats Receiving Intramedullary or Intravenous Infusion of PRO20




PRR - kidney
PRR+peptide
IgG
PRR - heart

# Effect of Intramedullary Infusion of A PRR Blocker on AngII-Induced Urinary Renin Activity



# Central PRR

- Within the CNS, PRR is predominantly expressed in cardiovascular-relevant neurons including the paraventricular (PVN) and supraoptic nuclei (SON) and the subfornical organ (SFO) (Shan Z et. al 2010; Takahashi K et al. 2010)

- PRR is co-localized with vasopressin (AVP) and oxytocin in SON and PVN in humans (Takahashi K et al. 2010)

- PRR knockdown in the SON of spontaneously hypertensive rats (SHR) decreases MAP and heart rate and plasma AVP level (Shan Z et. al 2010)

- Conversely, in the same study, targeted overexpression of human PRR in the SON of Wistar-Kyoto (WKY) rats induced a 2-fold increase in plasma AVP and a 3-fold increase in urinary AVP, in parallel with enhancement of urine concentrating capability (Shan Z et al. 2010)

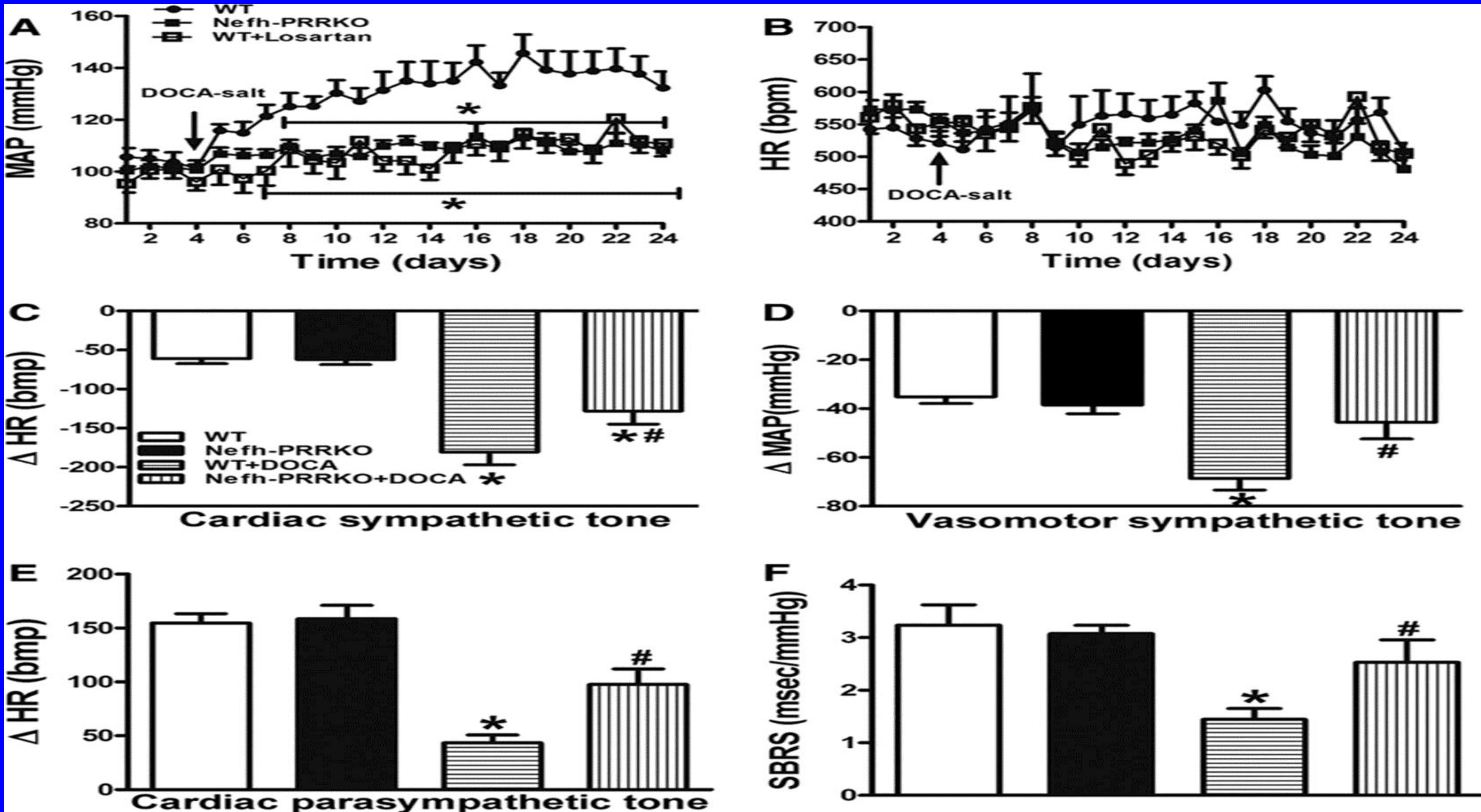
- Neuron-specific deletion of PRR or intracerebroventricular (ICV) infusion of a PRR decoy inhibitor PRO20 attenuates the hypertension induced by AngII or DOCA-salt (Li W et al. 2012; Li W et al. 2014; Li W et al. 2014).

# Evidence Arguing against PRR as a Renin Regulator

- Overexpression of human PRR in rats resulted in proteinuria and nephropathy but did not elevate BP or renal AngII levels (Kaneshiro Y 2007)

- Challenges: Lack of viable KO; controversal handle region peptide (HRP).

# Neuron-Specific PRR Deletion Attenuates DOCA-Salt-Induced hypertension in Mice




Wencheng Li et al. Hypertension. 2014;63:316-323

**Activation of the (pro)renin receptor in the paraventricular nucleus increases sympathetic outflow in anesthetized rats**

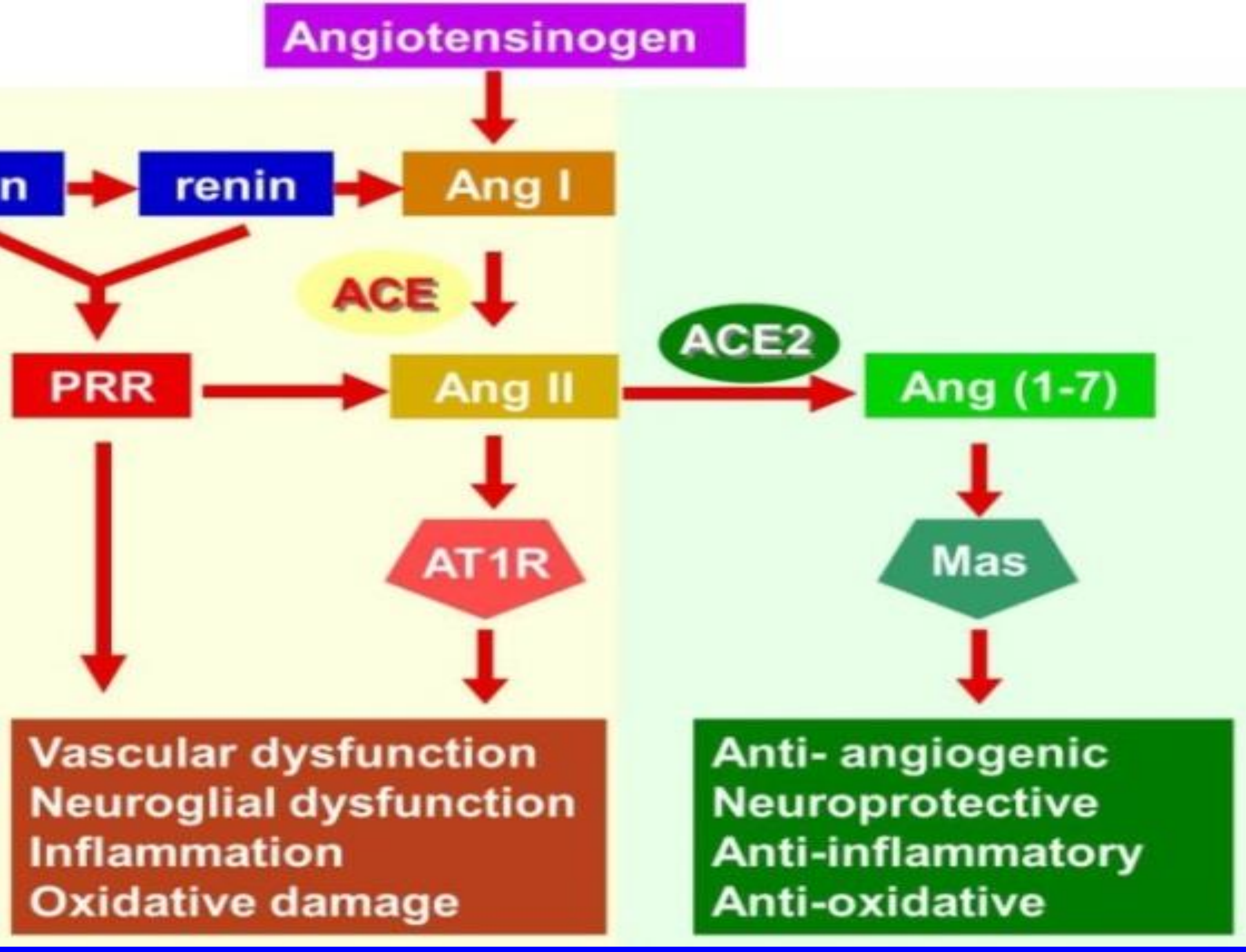
**Michael J. Huber, Rupsa Basu, Cassie Cecchettini, Adolfo E. Cuadra, Qing-Hui Chen, Zhiying Shan**

**American Journal of Physiology - Heart and Circulatory Physiology Published 1 September 2015 Vol. 309 no. 5, H880-H887 DOI: 10.1152/ajpheart.00095.2015**

**Abstract**

**Previous studies have indicated that hyperactivity of brain prorenin receptors (PRR) is implicated in neurogenic hypertension. However, the role of brain PRR in regulating arterial blood pressure (ABP) is not well understood. Here, we test the hypothesis that PRR activation in the hypothalamic paraventricular nucleus (PVN) contributes to increased sympathetic nerve activity (SNA). In anaesthetized adult Sprague-Dawley (SD) rats, bilateral PVN microinjection of human prorenin (2 pmol/side) significantly increased splanchnic SNA (SSNA; 71 ± 15%, $n$ = 7). Preinjection of either prorenin handle region peptide, the PRR binding blocker (PRRB), or tiron (2 nmol/side), the scavenger of reactive oxygen species (ROS), significantly attenuated the increase in SSNA (PRRB: 32 ± 5% vs. control, $n$ = 6; tiron: 8 ± 10% vs. control, $n$ = 5; $P < 0.05$) evoked by prorenin injection. We further investigated the effects of PRR activation on ROS production as well as downstream gene expression using cultured hypothalamus neurons from newborn SD rats. Incubation of brain neurons with human prorenin (100 nM) dramatically enhanced ROS production and induced a time-dependent increase in mRNA levels of inducible nitric oxide synthase (iNOS), NAPDH oxidase 2 subunit cybb, and FOS-like antigen 1 (fosl1), a marker for neuronal activation and a component of transcription factor activator protein-1 (AP-1). The maximum mRNA increase in these genes occurred 6 h following incubation (iNOS: 201-fold; cybb: 2 -fold; Ffosl1: 11-fold). The increases in iNOS and cybb mRNA were not attenuated by the $AT_1$ receptor antagonist losartan but abolished by the AP-1 blocker curcumin. Our results suggest that PVN PRR activation induces sympathoexcitation possibly through stimulation of an ANG II-independent, ROS-AP-1-iNOS signaling pathway**

Nguyen 2002


Angiotensinogen
prorenin
renin
Ang I
ACE
PRR
Ang II
ACE2
Ang (1-7)
AT1R
Mas
Vascular dysfunction
Neuroglial dysfunction
Inflammation
Oxidative damage
Anti- angiogenic
Neuroprotective
Anti-inflammatory
Anti-oxidative

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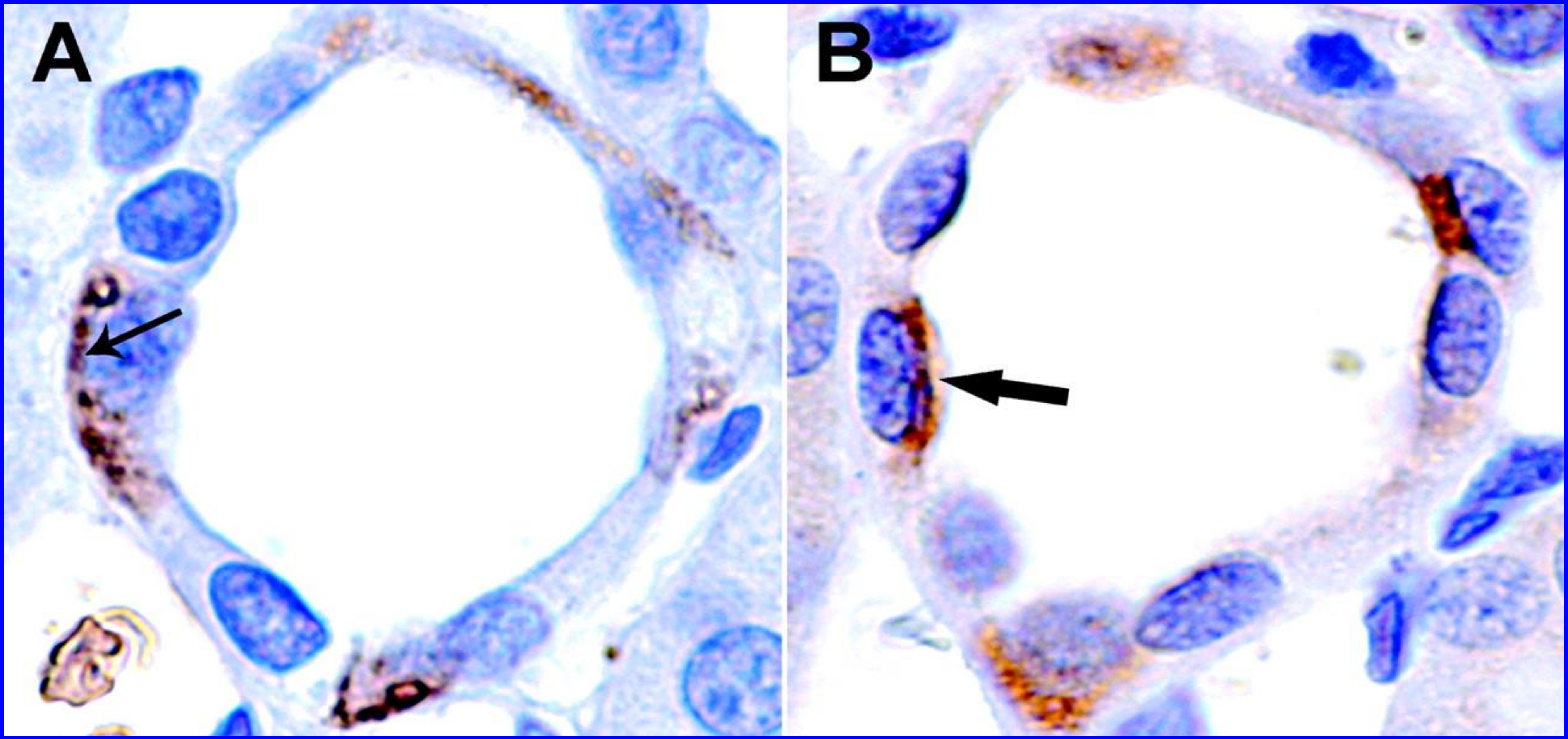
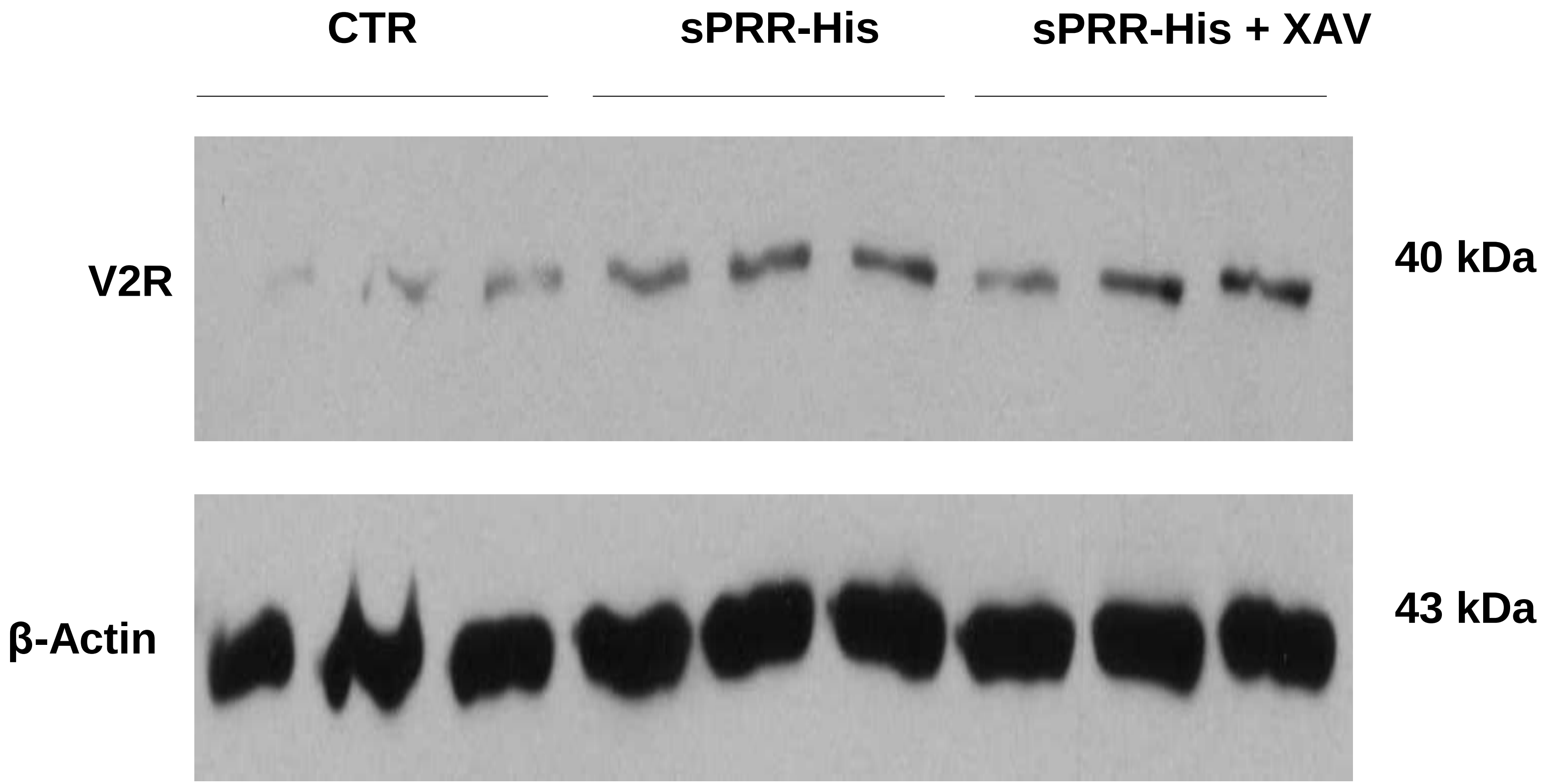
Consecutive rat kidney sections stained for either anion exchanger 1 (AE1), which is expressed on the basolateral border of A-ICs (A, thin arrow) or (P)RR (B).

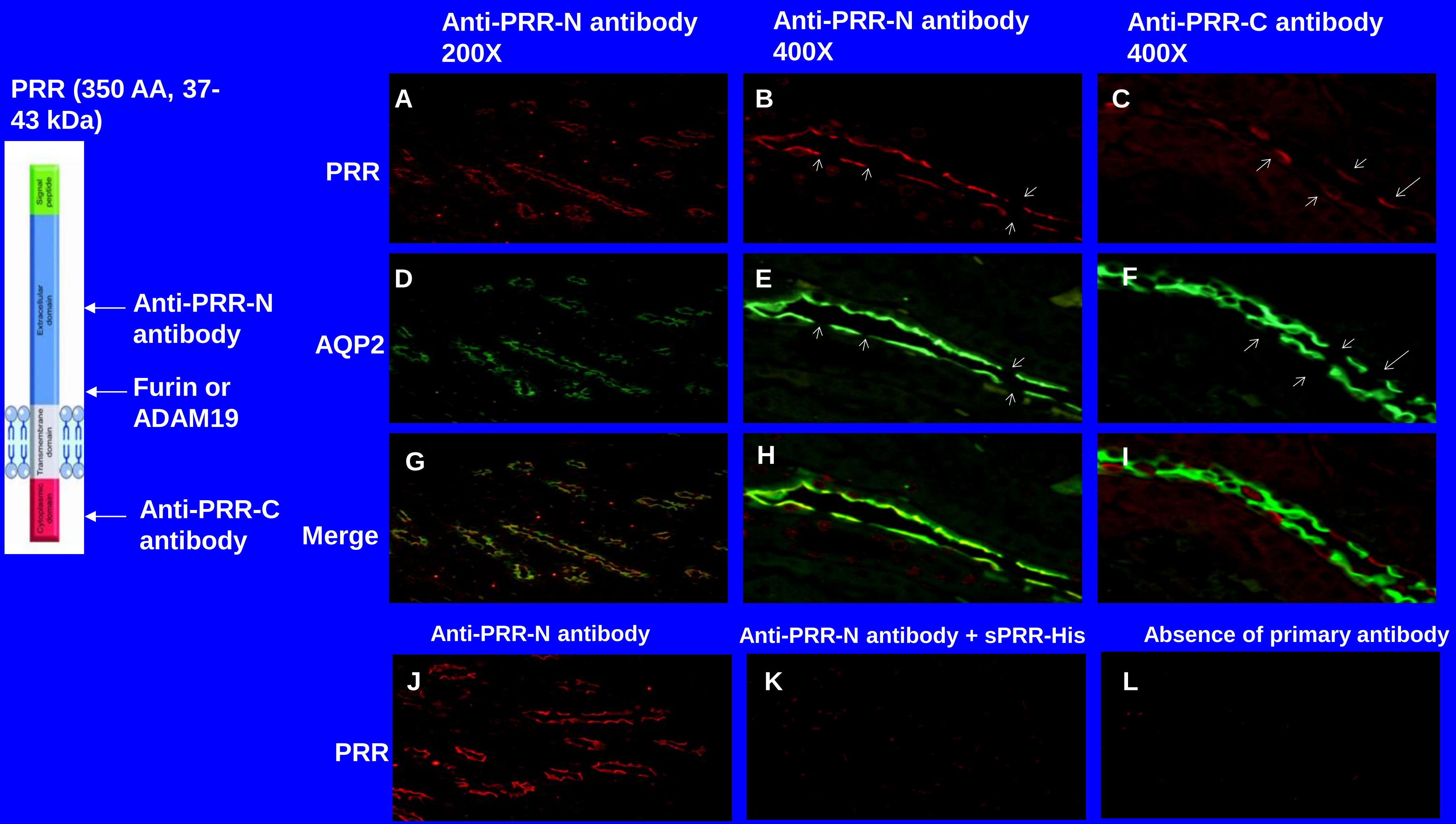



Andrew Advani et al. Hypertension. 2009;54:261-269

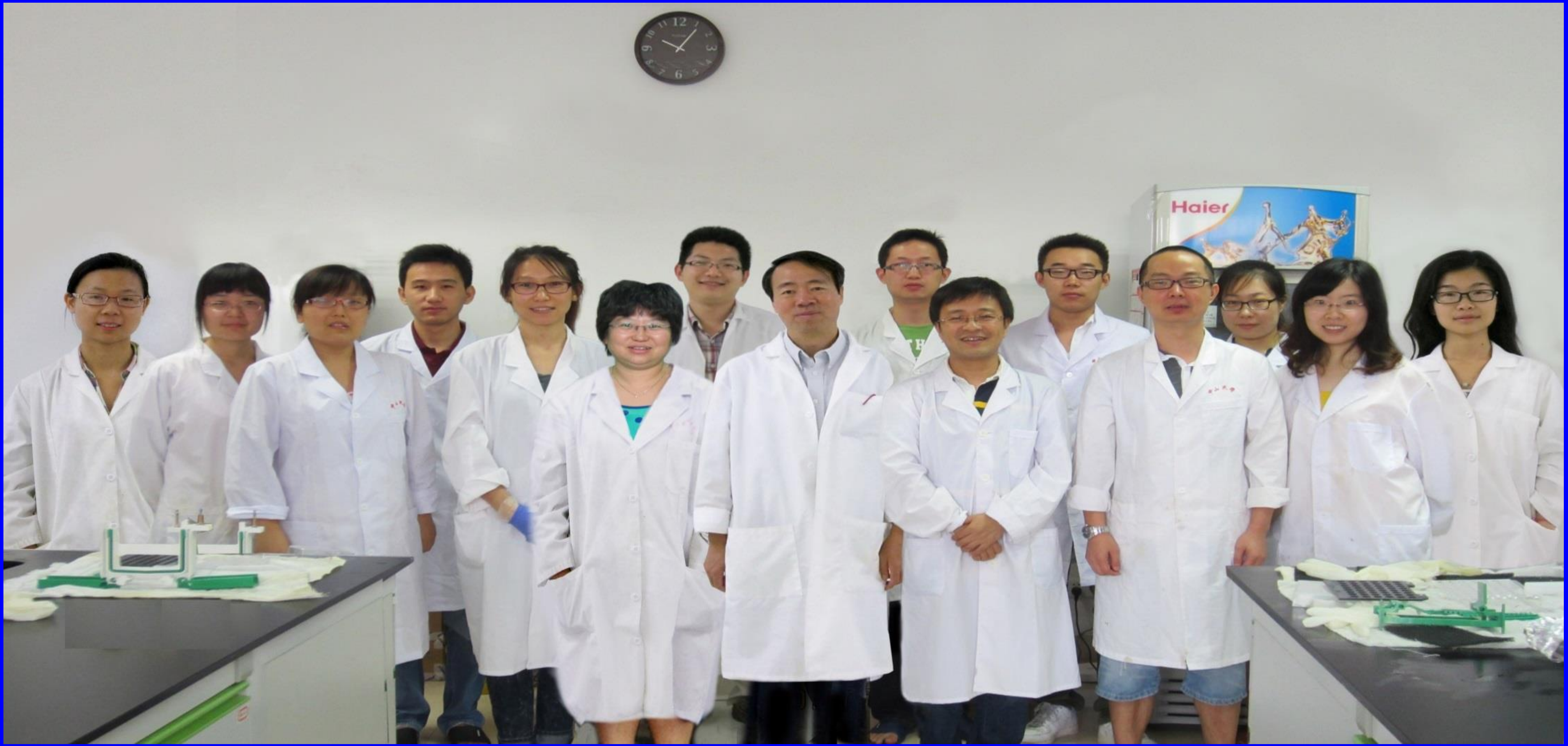
# sPRR Stimulated V2R Expression in Cultured IMCD Cells

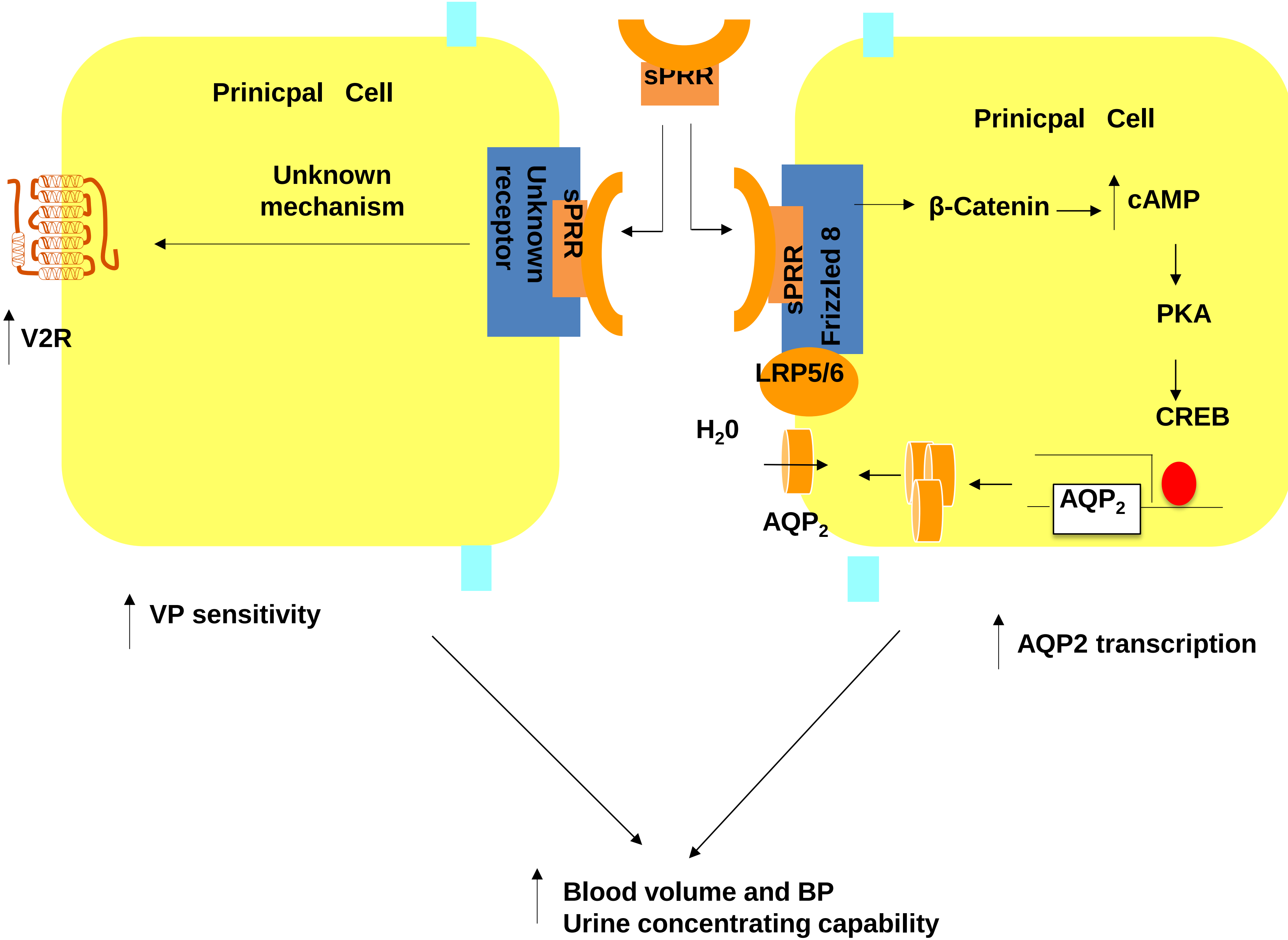


# Immunostaing with Antibodies Recognizing Different PRR Domains



# Institute of Hypertension, Sun Yat-sen University School of Medicine, Guangzhou, China




sPRR
Prinicpal Cell
Unknown mechanism
Unknown receptor
sPRR
V2R
Prinicpal Cell
sPRR
Frizzled 8
β-Catenin
cAMP
PKA
CREB
LRP5/6
H2O
AQP2
AQP2
VP sensitivity
AQP2 transcription
Blood volume and BP
Urine concentrating capability