Department of Medical Science
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WORLD SALT SYMPOSIUM
June 19-21, 2018 Park City UT, USA

Novel Receptor Binding Protein ATRAP in the Regulation of Renal Sodium Reabsorption and Blood Pressure Response

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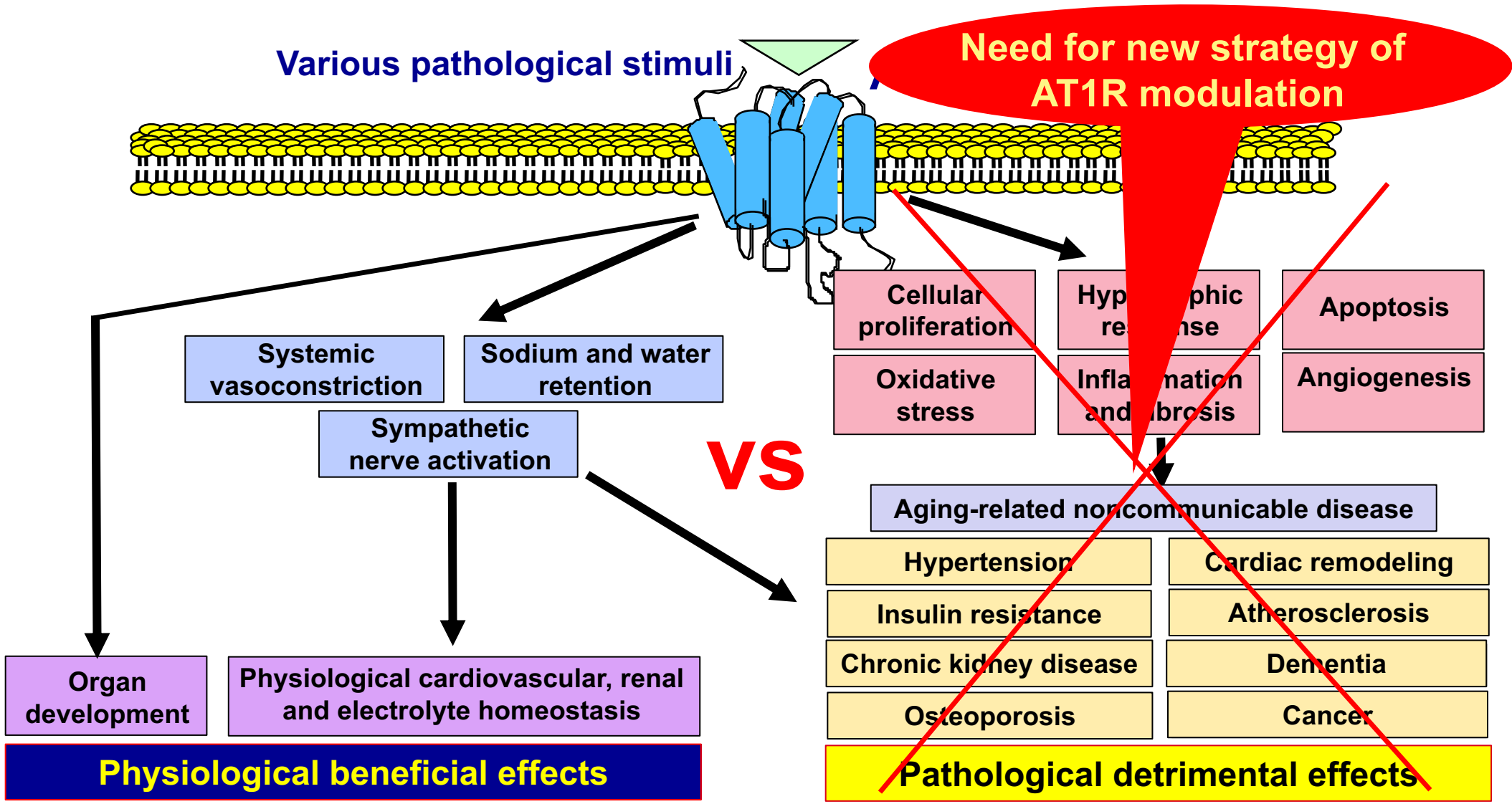
Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan

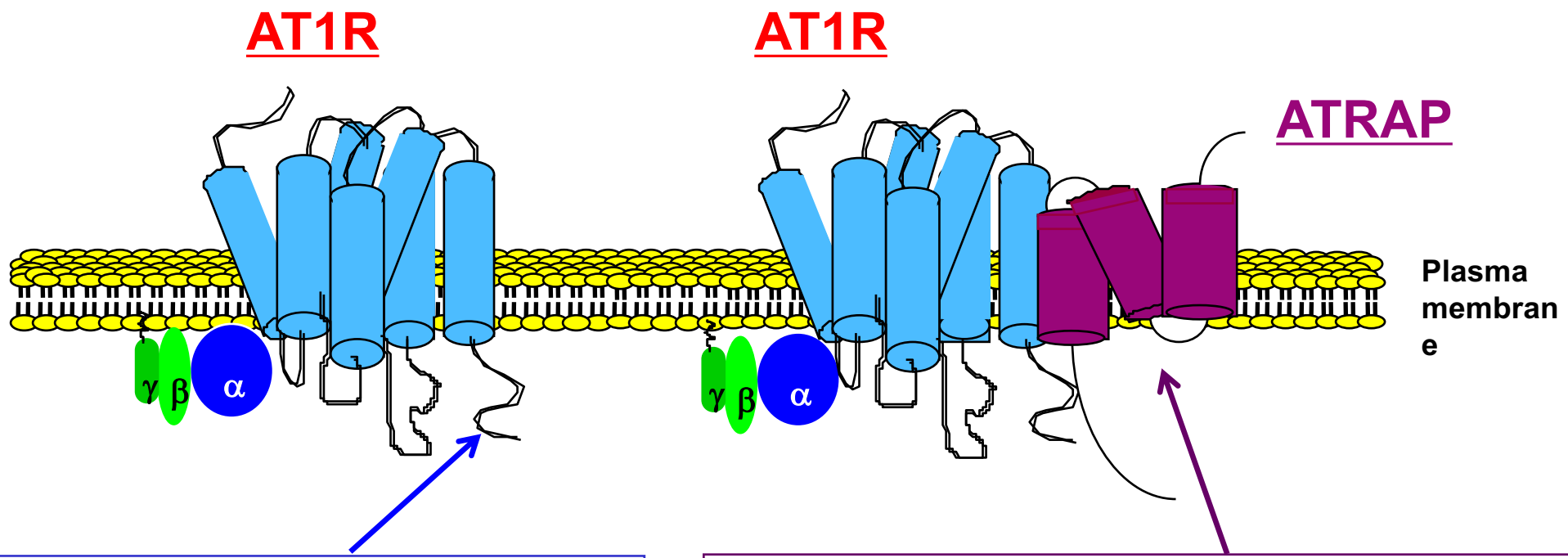
Pleiotropic effects of Angiotensin II type 1 receptor (AT1R) signaling: Physiological beneficial effects vs Pathophysiological detrimental effects



Tamura K, et al.

Modified from Kamo T, et al. Int Heart J. 2015 May 13;56(3):249-54.

In seeking for a new modulator of tissue AT1R signaling, AT1R-associated protein (ATRAP) was identified as a specific binding protein to C-domain of AT1R



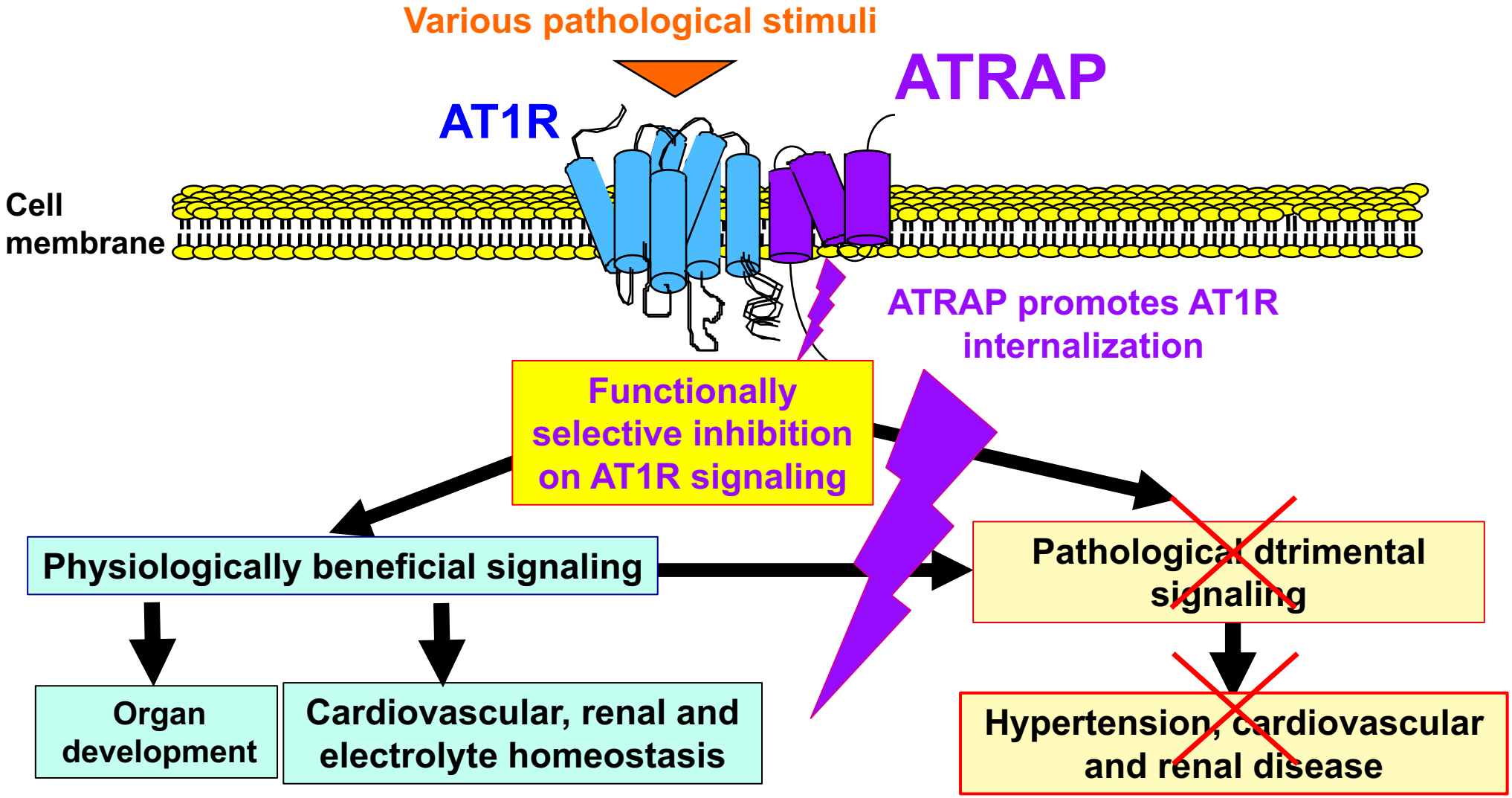
Carboxyl terminal domain of AT1R: Involved in receptor internalization and activation of signaling

Identification of a novel interacting protein with carboxyl terminal domain of AT1R as ATRAP (=AngioTensin II Receptor-Associated Protein)

Tamura K, et al.

Daviet L, et al. J Biol Chem 1999; Lopez-Ilasaca M, et al. Mol Biol Cell 2003; Mogi M, et al. ATVB 2007; Tamura K, et al. Curr Hyp Rep 2007; Horiuchi M, et al. Clin Sci (Lond) 2012; Tamura K, et al. Curr Pharm Des 2013.

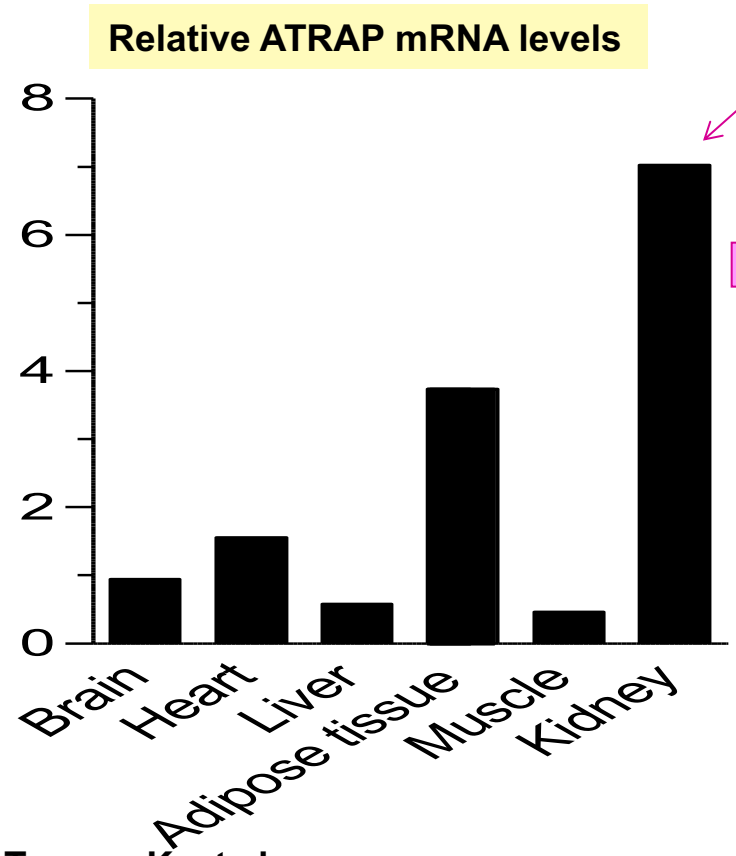
Hypothesis: ATRAP may exert a functionally selective inhibition on pathological detrimental AT1R signaling



Daviet L, et al. J Biol Chem 1999; Lopez-Ilasaca M, et al. Mol Biol Cell 2003; Mogi M, et al. ATVB 2007; Tamura K, et al. Curr Hyp Rep 2007; Tamura K, et al. Curr Pharm Des 2013; Wakui H, et al. Hypertension 2013; Ohsawa M, et al. Kid Int 2014; Wakui H, et al. JAHA 2015.

In normal human kidney, ATRAP is abundantly distributed in renal epithelial cells along renal tubules

A: Tissue distribution of ATRAP mRNA in normal human subjects (pooled donors)



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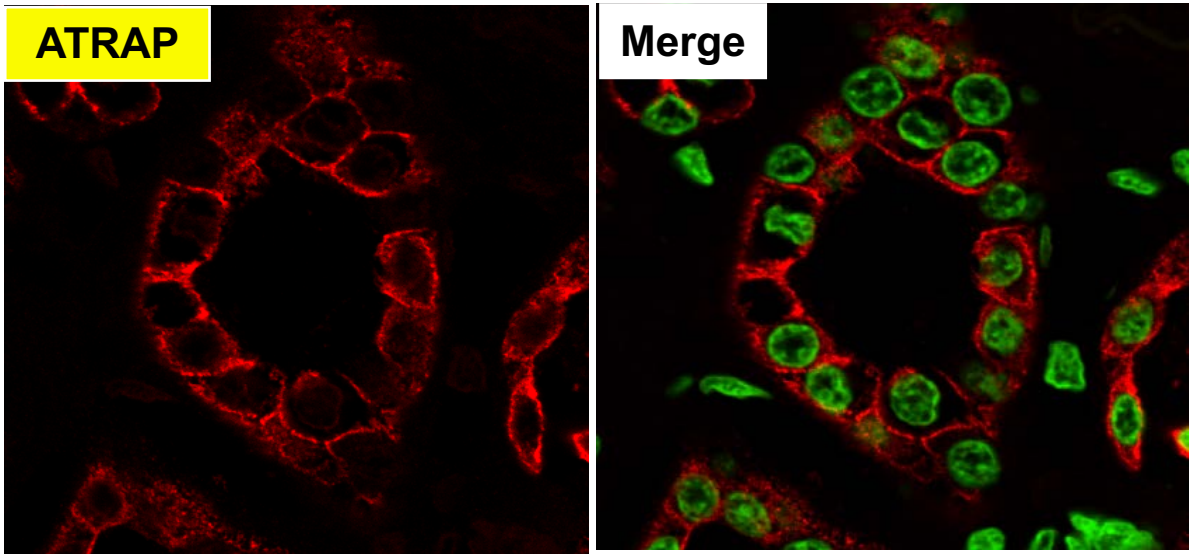
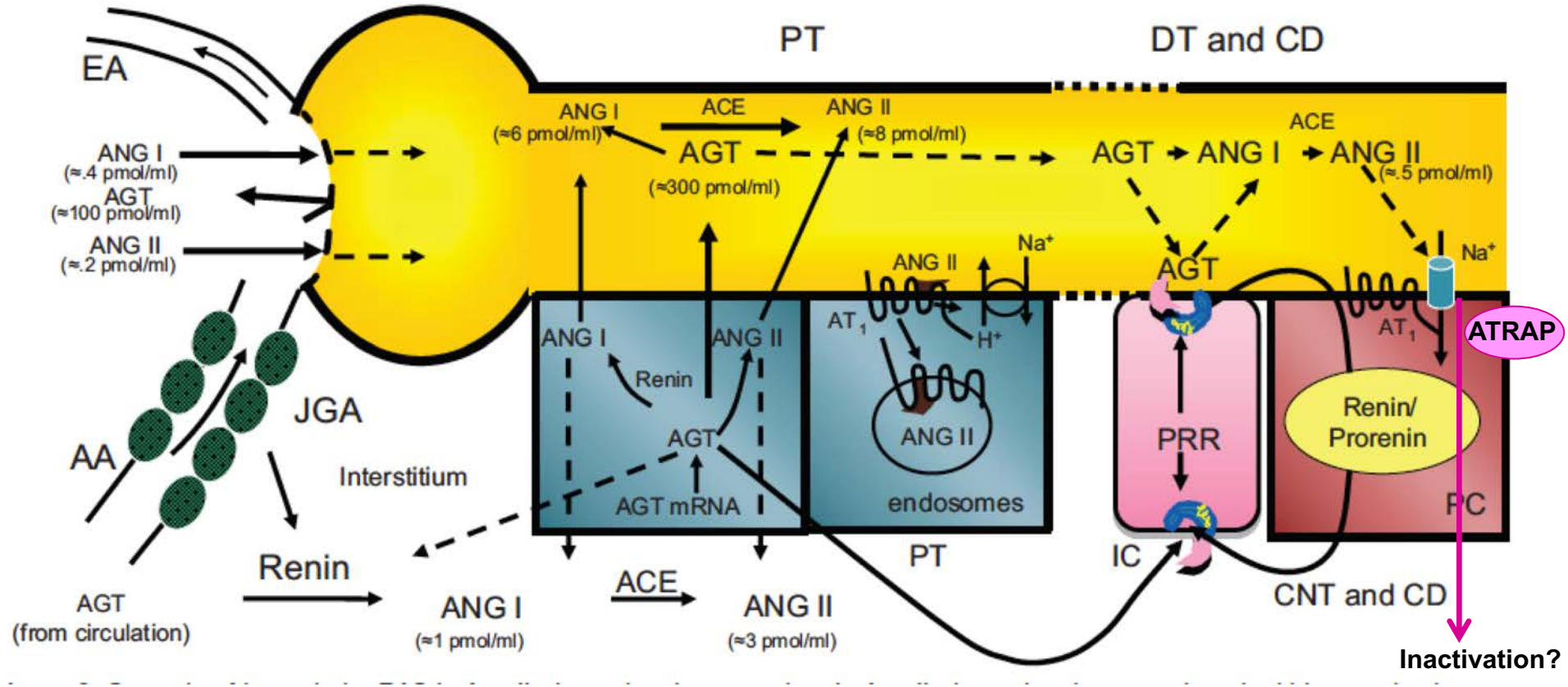
B: Representative confocal laser-scanning microscopy image of renal tubules of normal human kidney



Red, ATRAP; green, nuclei.

Tsurumi Y, et al. Kidney Int 2006; Wakui H, et al. Am J Physiolo Renal Physiol 2010; Masuda S, et al. Am J Physiol Renal Physiol 2010; Tamura K, et al. Curr Med Chem 2015.

Activation of cascade of intratubular AT1R signaling in the kidney promotes sodium reabsortption and is involved in hypertension

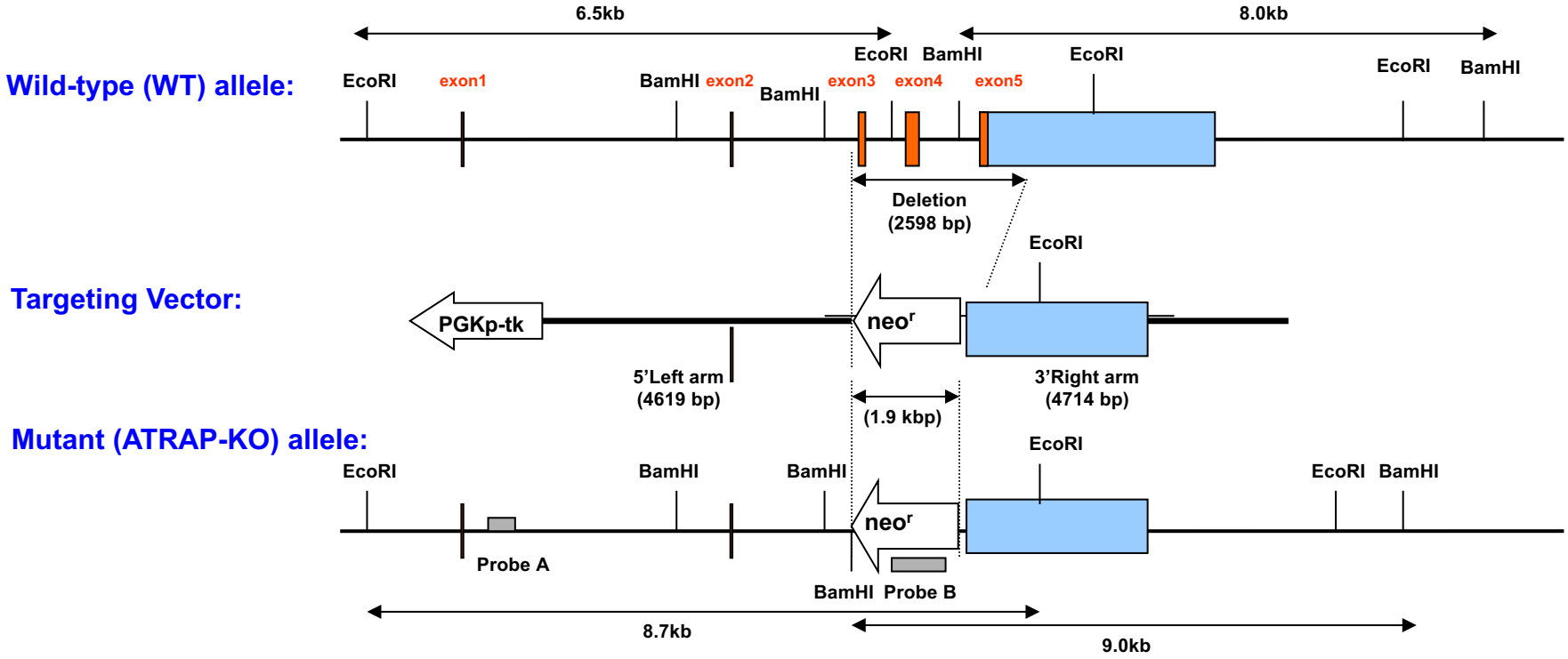


Tamura K, et al.

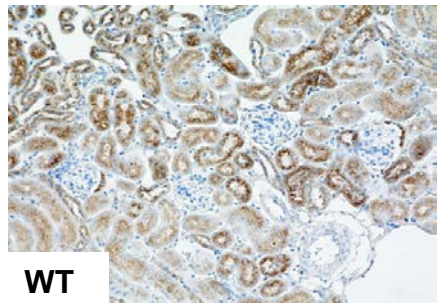
Modified from Navar LG, et al. Hypertension 2011; 57: 355-362.

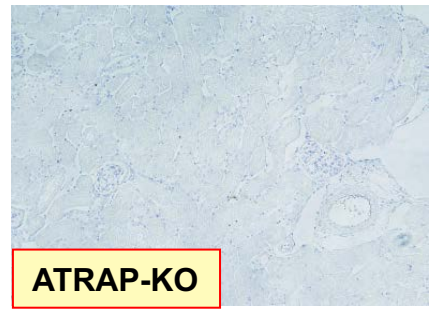
Effects of ATRAP-down: Targeted disruption of the gene encoding ATRAP to produce ATRAP-knockout (ATRAP-KO) mice

A: Schematic representation of the gene-targeting strategy to produce ATRAP-KO mice



B: Immunohistochemical analysis of ATRAP expression in the kidney of WT and ATRAP-KO mice (ATRAP staining, brown areas)





Ab: polyclonal anti-ATRAP

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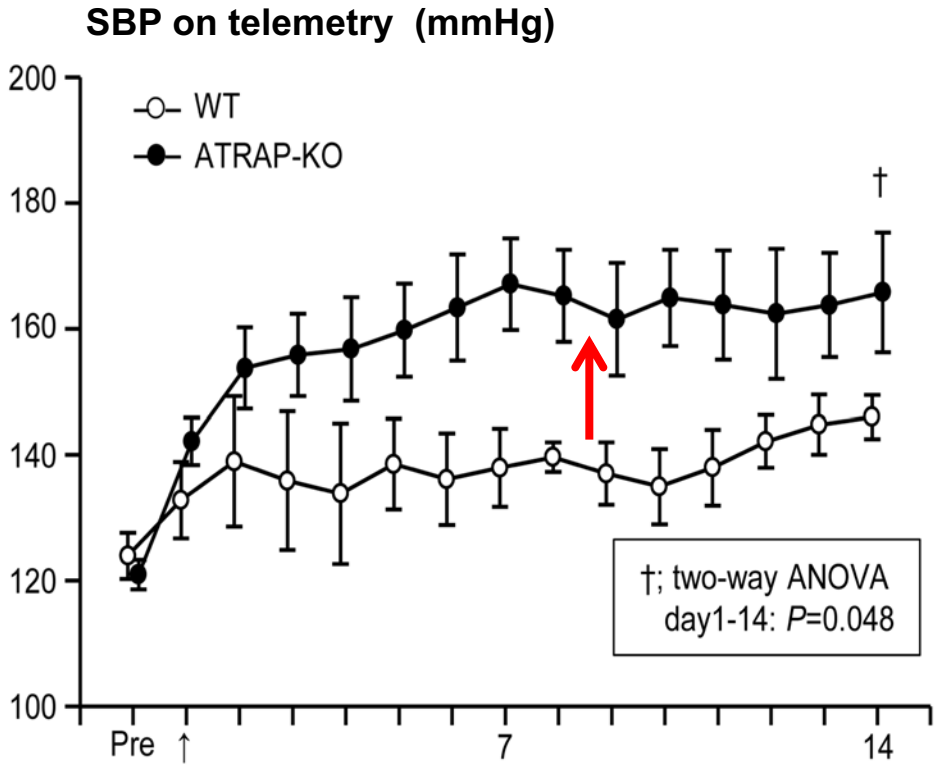
Genetic alteration of ATRAP expression exerts no evident effects on BP and kidney in mice under normal condition

Disrupted gene	Viability	Blood pressure vs wild type	Abnormality in kidney function and morphology	Ref.
Angiotensinogen -KO	Viable	-33 mmHg (SBP)	+	Tanimoto K, et al. J Biol Chem 1994; Kihara M, et al. JASN 1998
Renin-KO	Viable	-36 mmHg (SBP)	+	Yanai K, et al. J Biol Chem 2000
ACE-KO	Viable	-35 mmHg (SBP)	+	Krege JH, et al. Nature 1995
AT1a-KO	Viable	-22 mmHg (SBP)	+/-	Sugaya T, et al. J Biol Chem 1995
AT1a+AT1b-KO	Viable	-34 mmHg (MBP)	+	Tsuchida S, et al. J Clin Invest 1998
ATRAP-KO/ ATRAP-TGM	Viable	No change	-	Wakui H, et al. Hypertens 2010, 2013; Ohsawa M, et al. Kid Int 2014

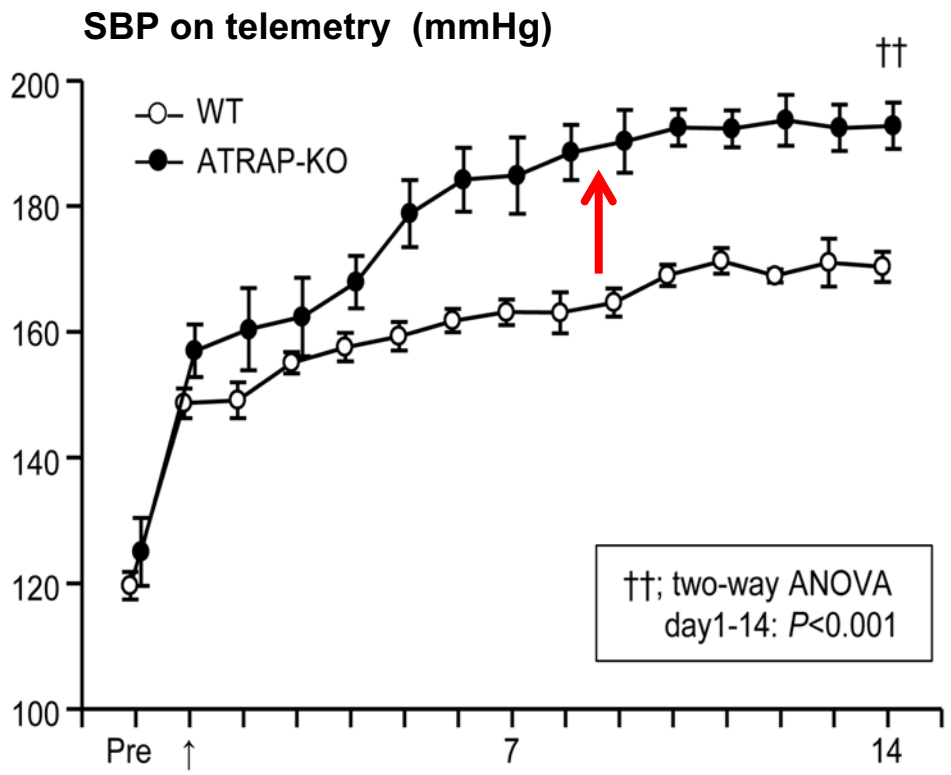
However, ATRAP-KO mice exhibit exacerbation of Ang II-mediated hypertension

Dr. Ohsawa

A: Daily and 24-h SBP (radiotelemetry) in WT and ATRAP-KO mice before (”pre”) and during Ang II (500 ng/kg/min) infusion



B: Daily and 24-h SBP (radiotelemetry) in WT and ATRAP-KO mice before (”pre”) and during Ang II (2000 ng/kg/min) infusion



Values are expressed as the mean±SE.

† *P*<0.05, †† *P*<0.01 versus WT mice.

Tamura K, et al.

Ohsawa M, et al. Kidney Int. 2014 Sep;86(3):570-81.

ATRAP-KO mice also exhibit exacerbation of target organ damage by Ang II

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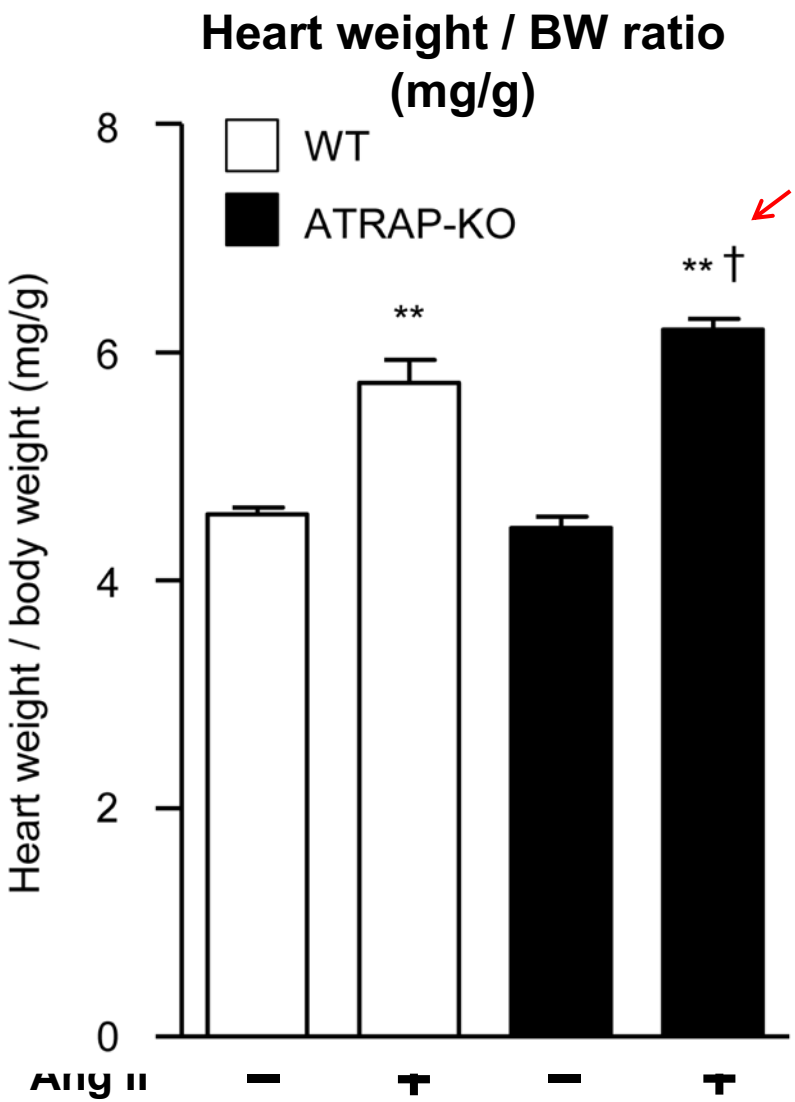
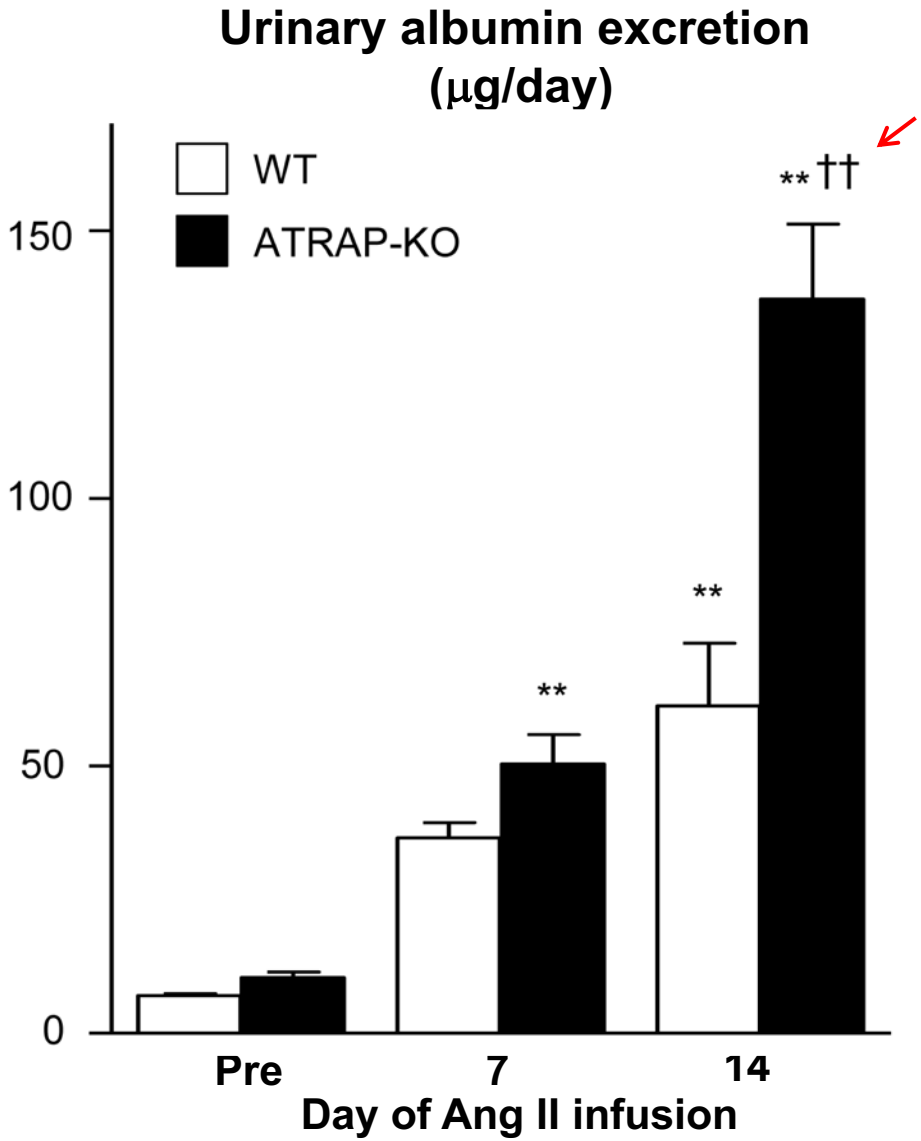




Values are expressed as the mean±SE.

** *P*<0.01 versus vehicle. † *P*<0.05, †† *P*<0.01 versus WT mice.

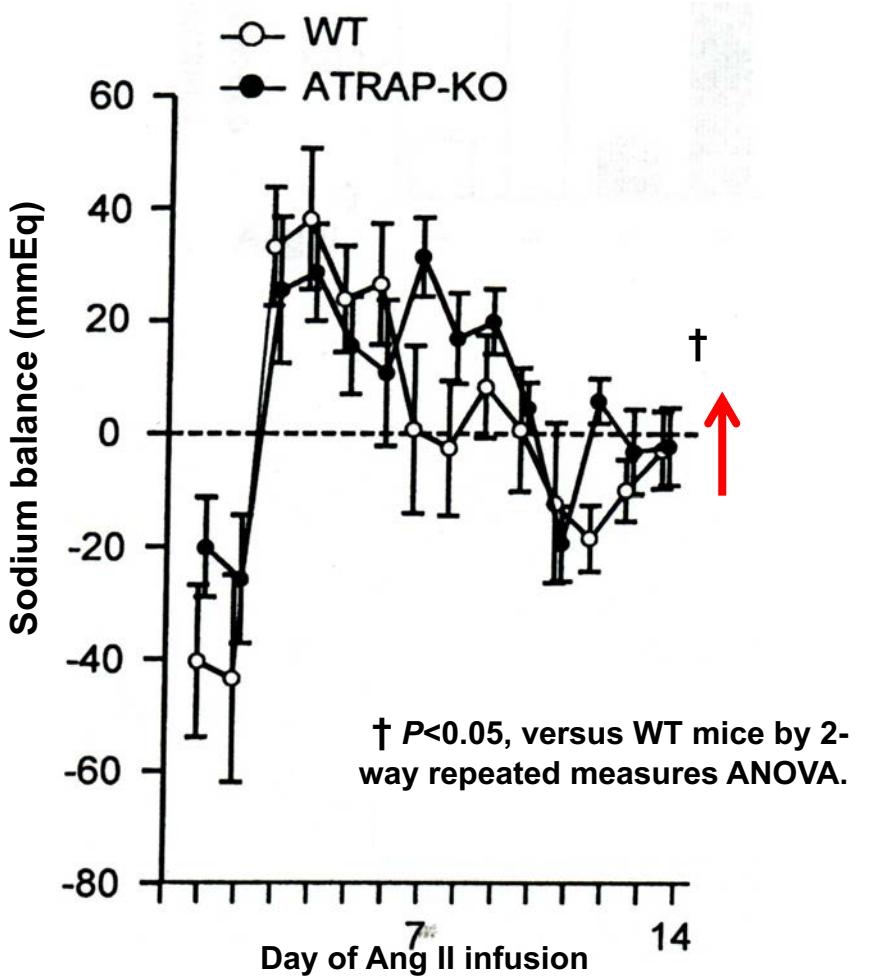
Tamura K, et al.

Ohsawa M, et al. Kidney Int. 2014 Sep;86(3):570-81.

Metabolic cage analysis indicates blunted natriuresis as a mechanism for exacerbation of Ang II-mediated hypertension in ATRAP-KO mice

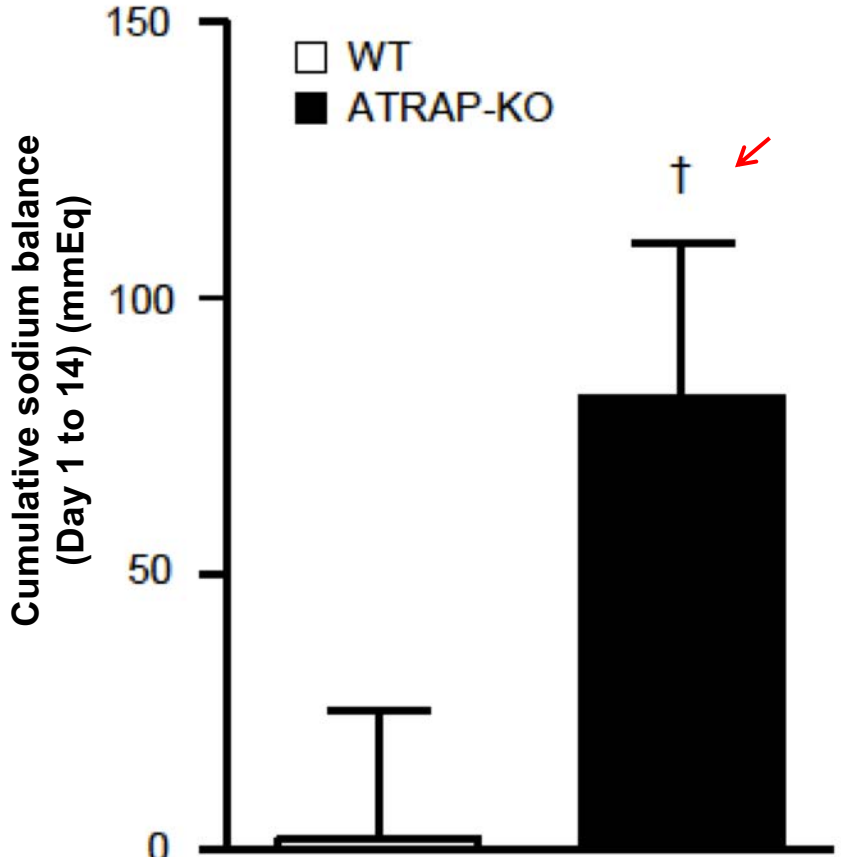
Dr. Ohsawa

A: The extent of daily positive sodium balance was elevated in ATRAP-KO mice compared to WT mice during Ang II infusion



† *P*<0.05, versus WT mice by 2-way repeated measures ANOVA.

B: The extent of cumulative positive sodium balance during Ang II infusion (Day 1 to Day 14) was significantly increased in ATRAP-KO mice compared to WT mice



† *P*<0.05, versus WT mice by unpaired *t*-test.

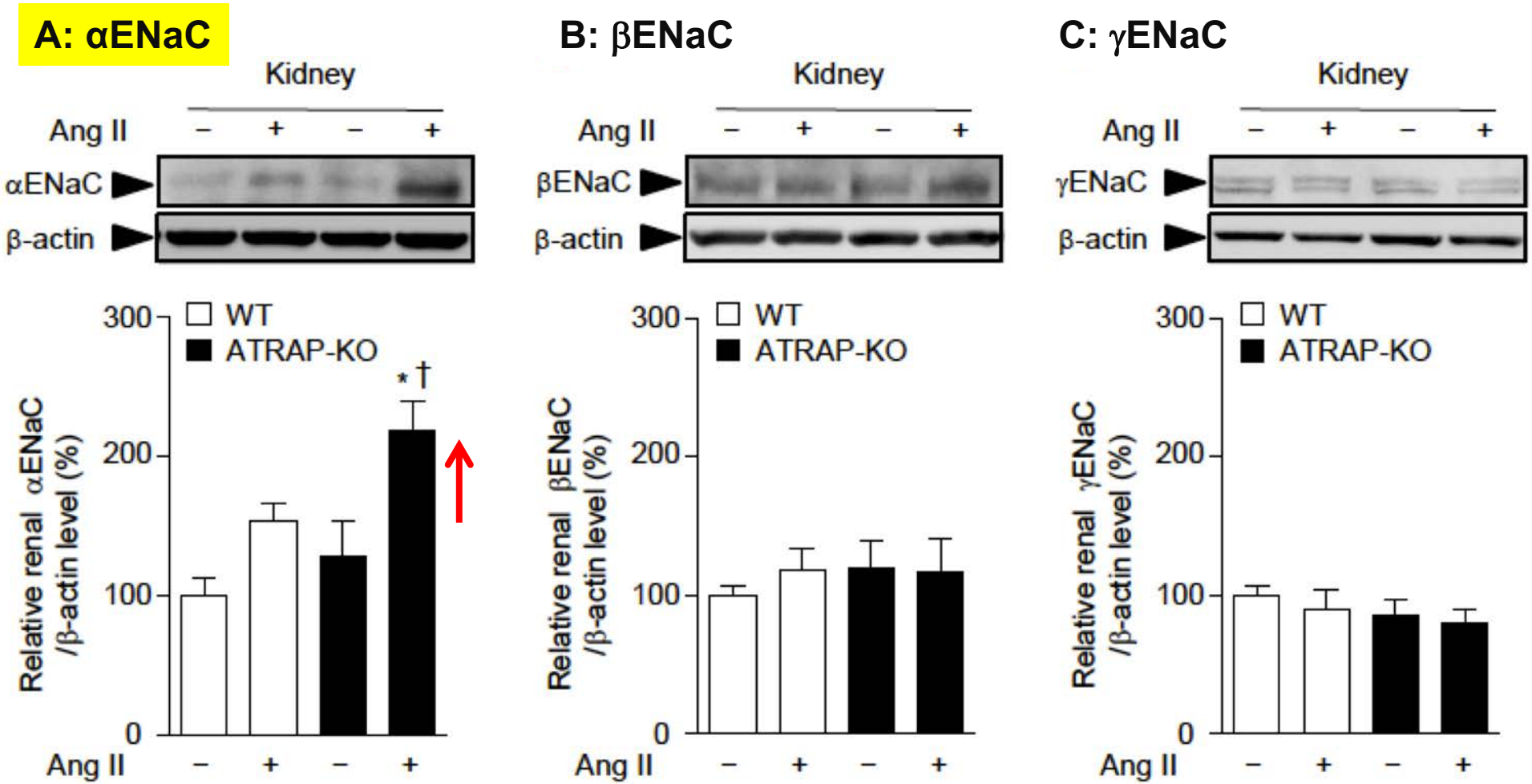
Values are expressed as the mean±SE.

Tamura K, et al.

Ohsawa M, et al. Kidney Int. 2014 Sep;86(3):570-81.

ATRAP-KO mice exhibit a significant enhancement of Ang II-mediated up-regulation of αENaC in the kidney

Dr. Ohsawa



Values are expressed as the mean±SE.

* *P*<0.05, versus vehicle. † *P*<0.05, versus WT mice.

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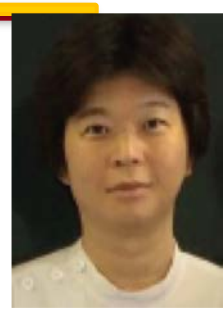
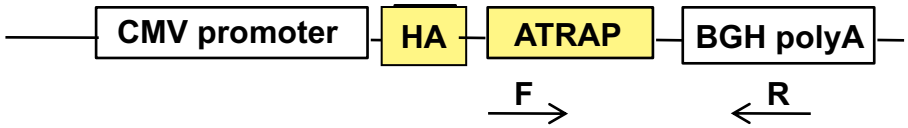
Ohsawa M, et al. Kidney Int. 2014 Sep;86(3):570-81.

Effects of ATRAP-up: Generation of renal ATRAP-transgenic mice (renal ATRAP-Tg, strain 12)

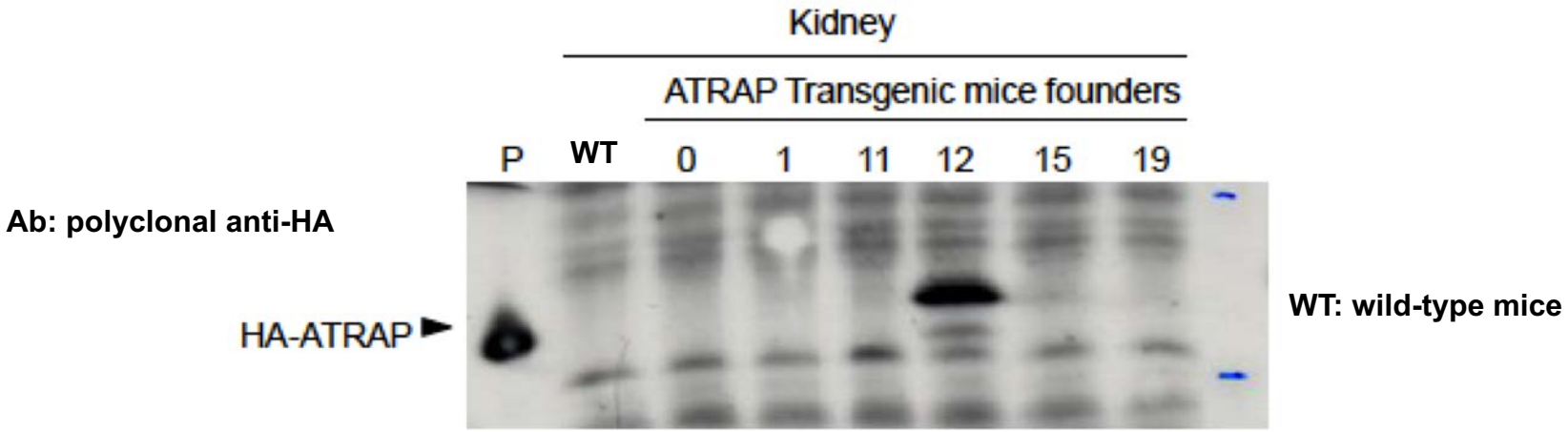


Dr. Wakui

A: Schematic representation of Tg vector

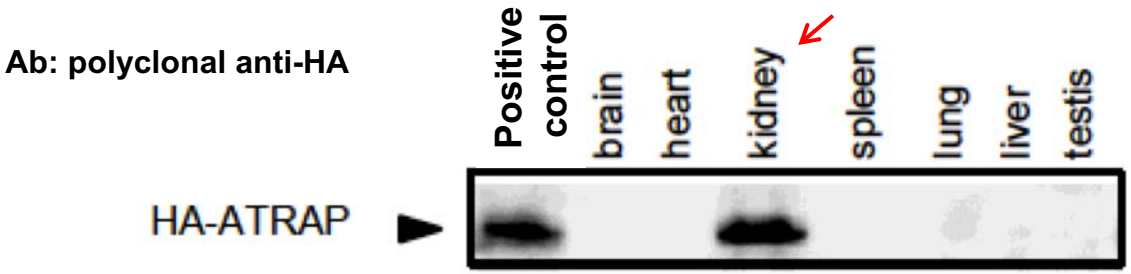


B: Western blot analysis in the kidney of WT and renal ATRAP Tg mice founders



WT: wild-type mice

C: Western blot analysis of the HA-ATRAP transgene in the renal ATRAP-Tg mice (Tg, strain 12)



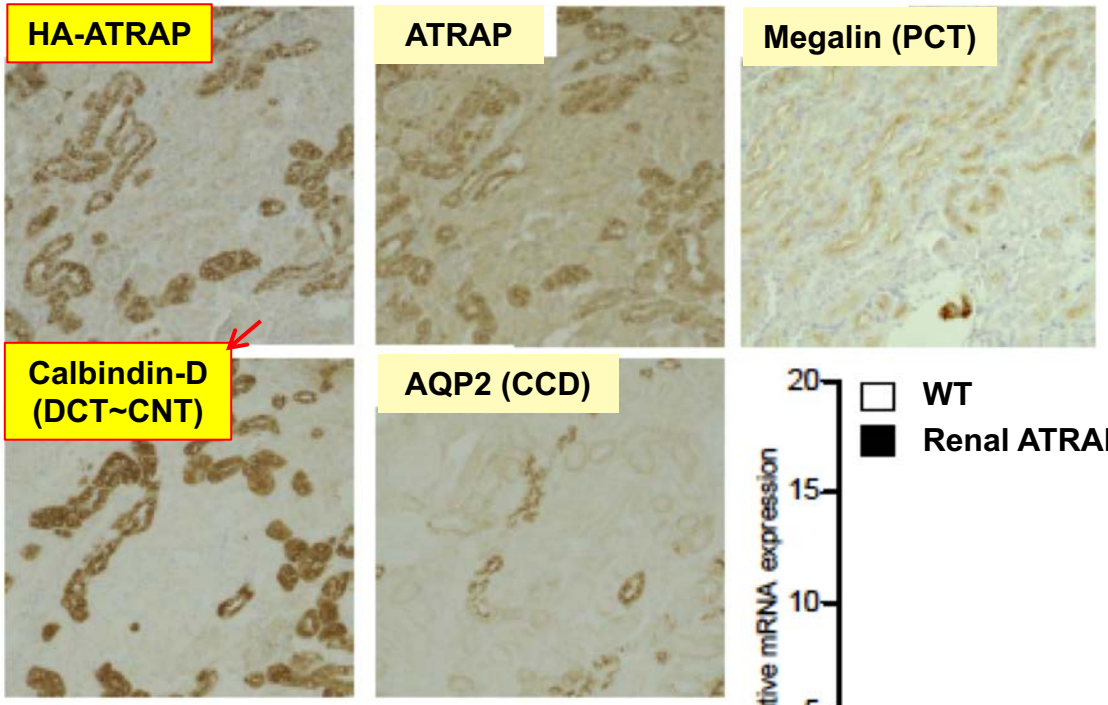
Tamura K, et al.

Wakui H, et al. Hypertension. 2013 Jun;61(6):1203-10.

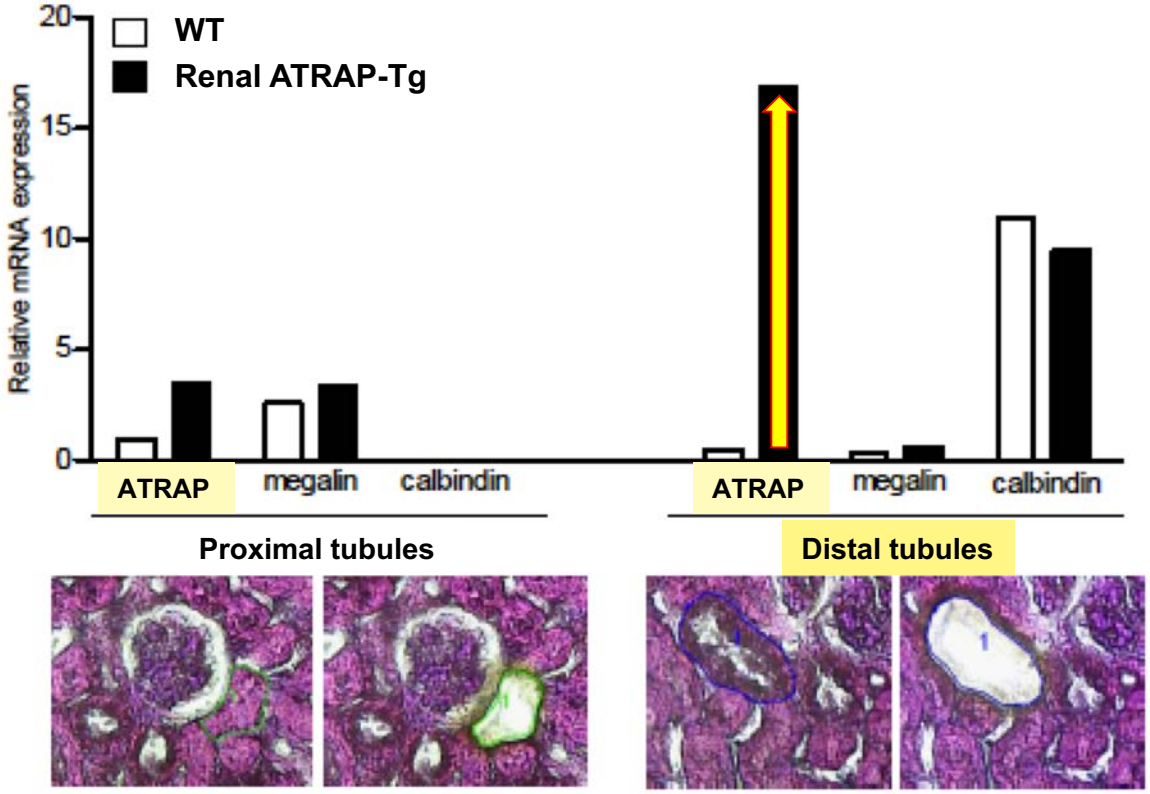
Renal ATRAP-Tg mice exhibit distal tubule (from DCT to CNT)-dominant ATRAP enhancement in the kidney

Dr. Wakui

A: Immunohistochemical analysis in the renal cortex of renal ATRAP-Tg mice（staining, brown areas）



B: Quantitative analysis of ATRAP mRNA expression in the proximal and distal tubules of the renal cortex by laser microdissection



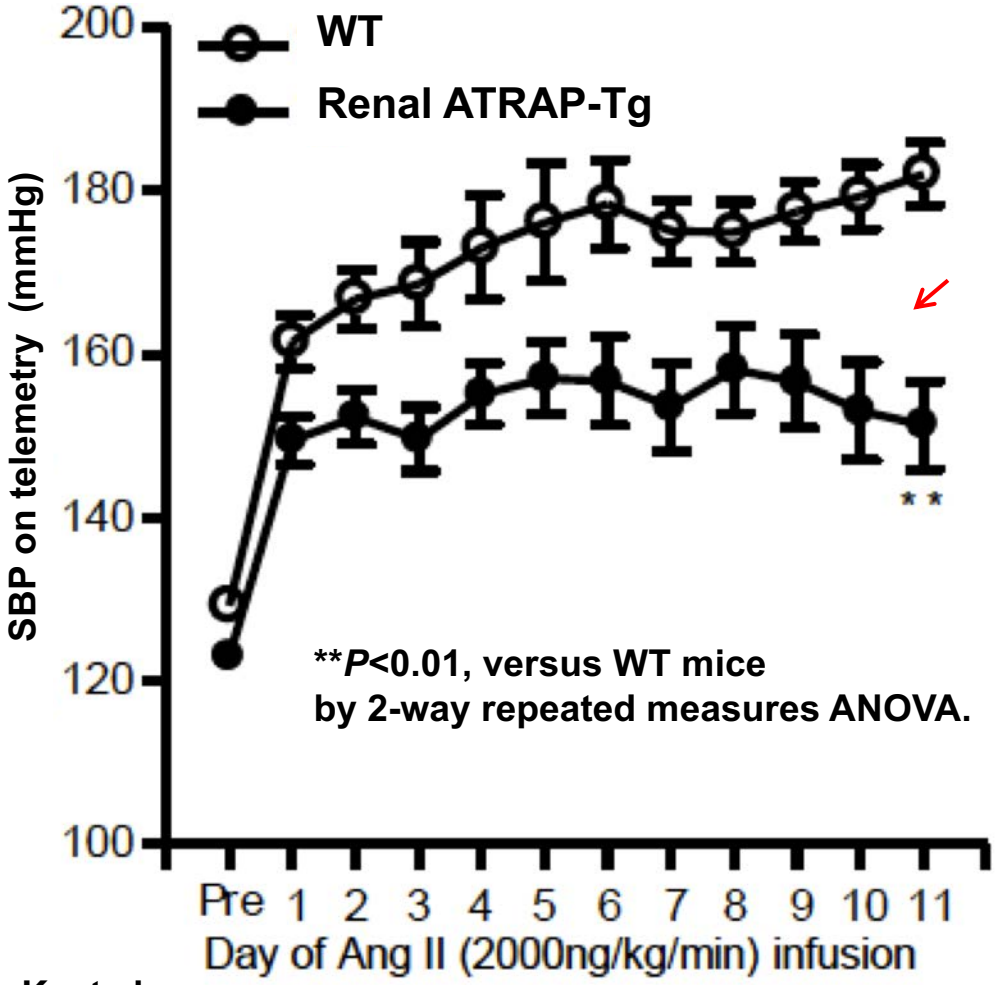
Wakui H, et al. Hypertension. 2013 Jun;61(6):1203-10.

Tamura K, et al.

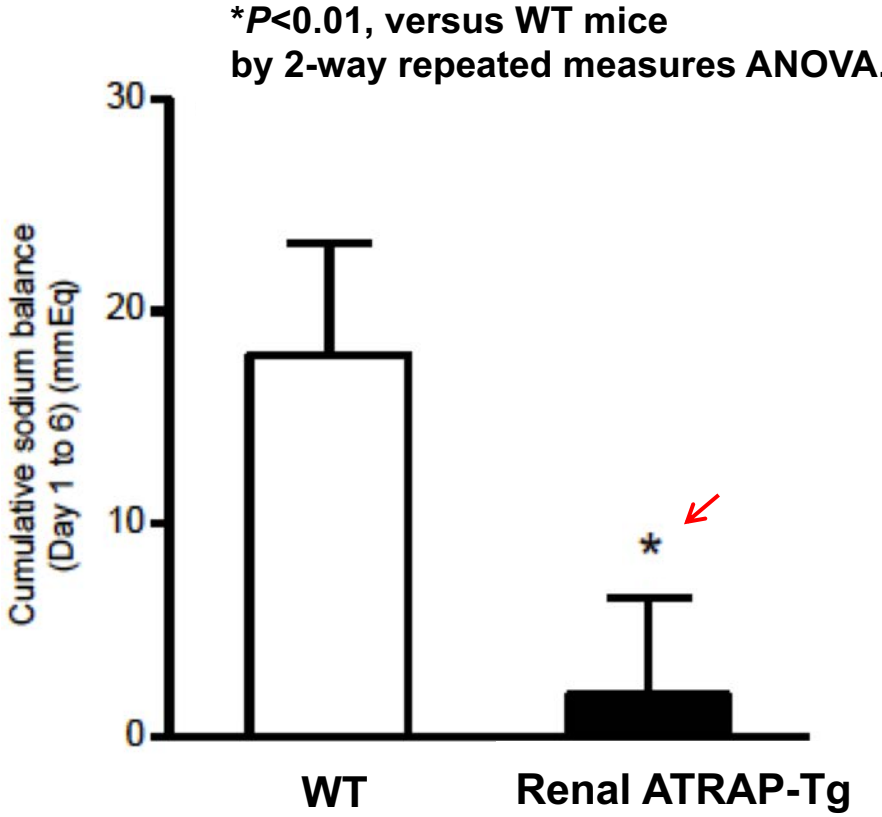
Renal ATRAP-Tg mice exhibit suppression of Ang II-mediated hypertension with enhanced natriuresis

Dr. Wakui

A: Daily and 24-h SBP in WT and renal ATRAP-Tg mice before (”pre”) and during Ang II (2000 ng/kg/min) infusion



B: The extent of cumulative positive Na^+ balance during the early phase (Day 1 to Day 6) was significantly decreased in renal ATRAP-Tg mice compared to WT mice



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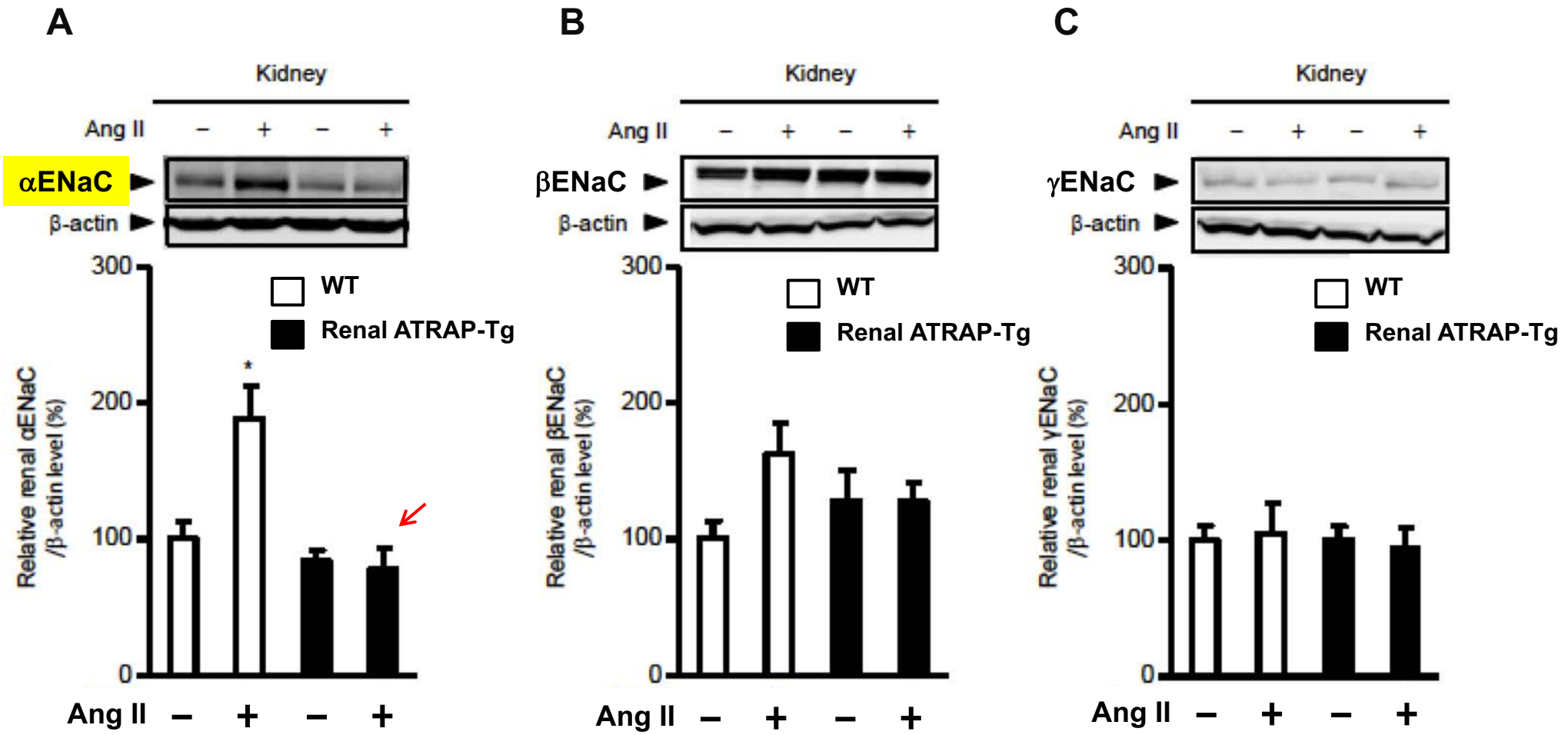
Renal ATRAP-Tg mice exhibit a significant suppression of Ang II-mediated up-regulation of αENaC in the kidney

Dr. Wakui



Values are expressed as the mean±SE.

*P<0.05 versus vehicle. †P<0.05 versus WT mice.

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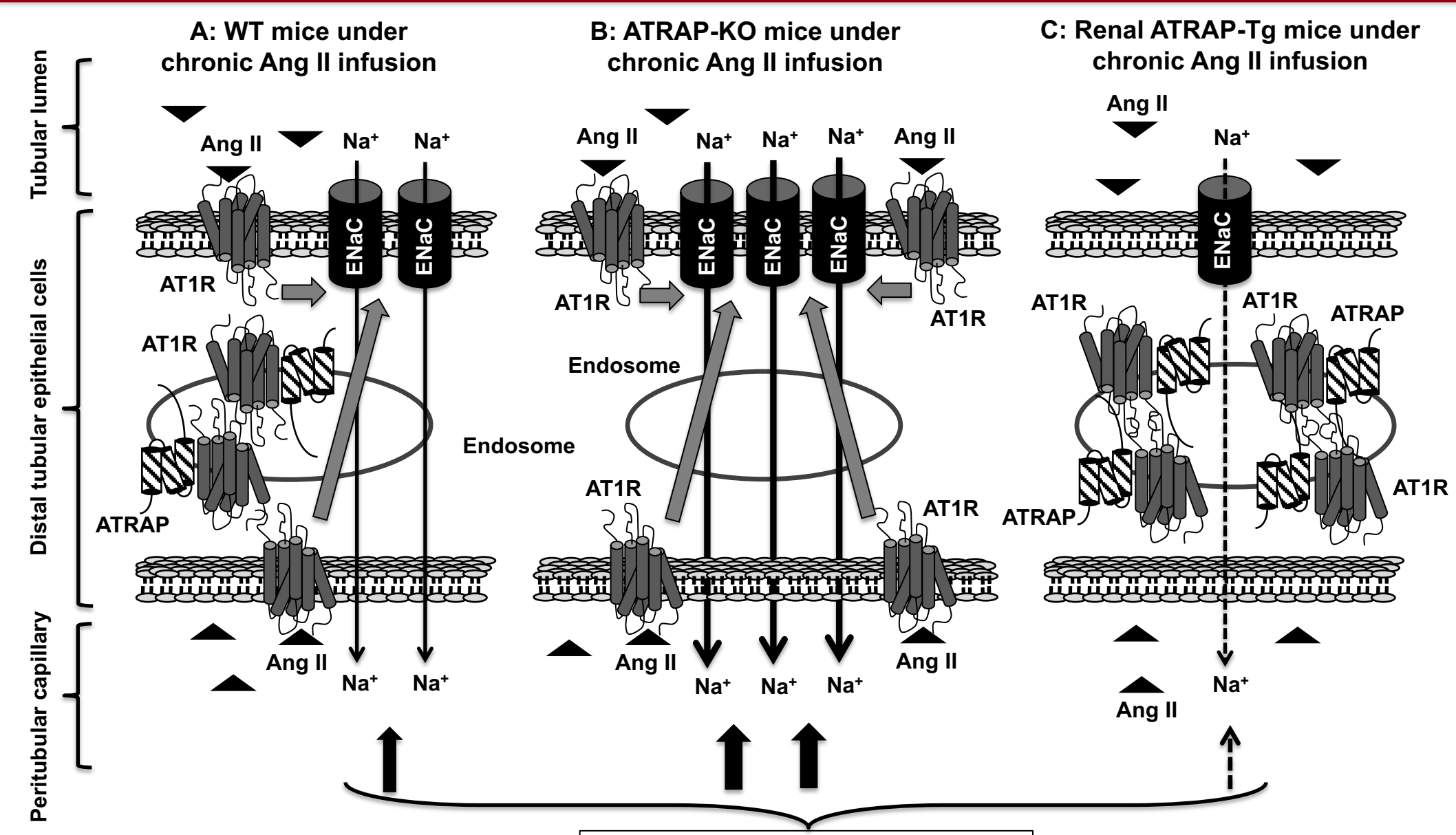
Wakui H, et al. Hypertension. 2013 Jun;61(6):1203-10.

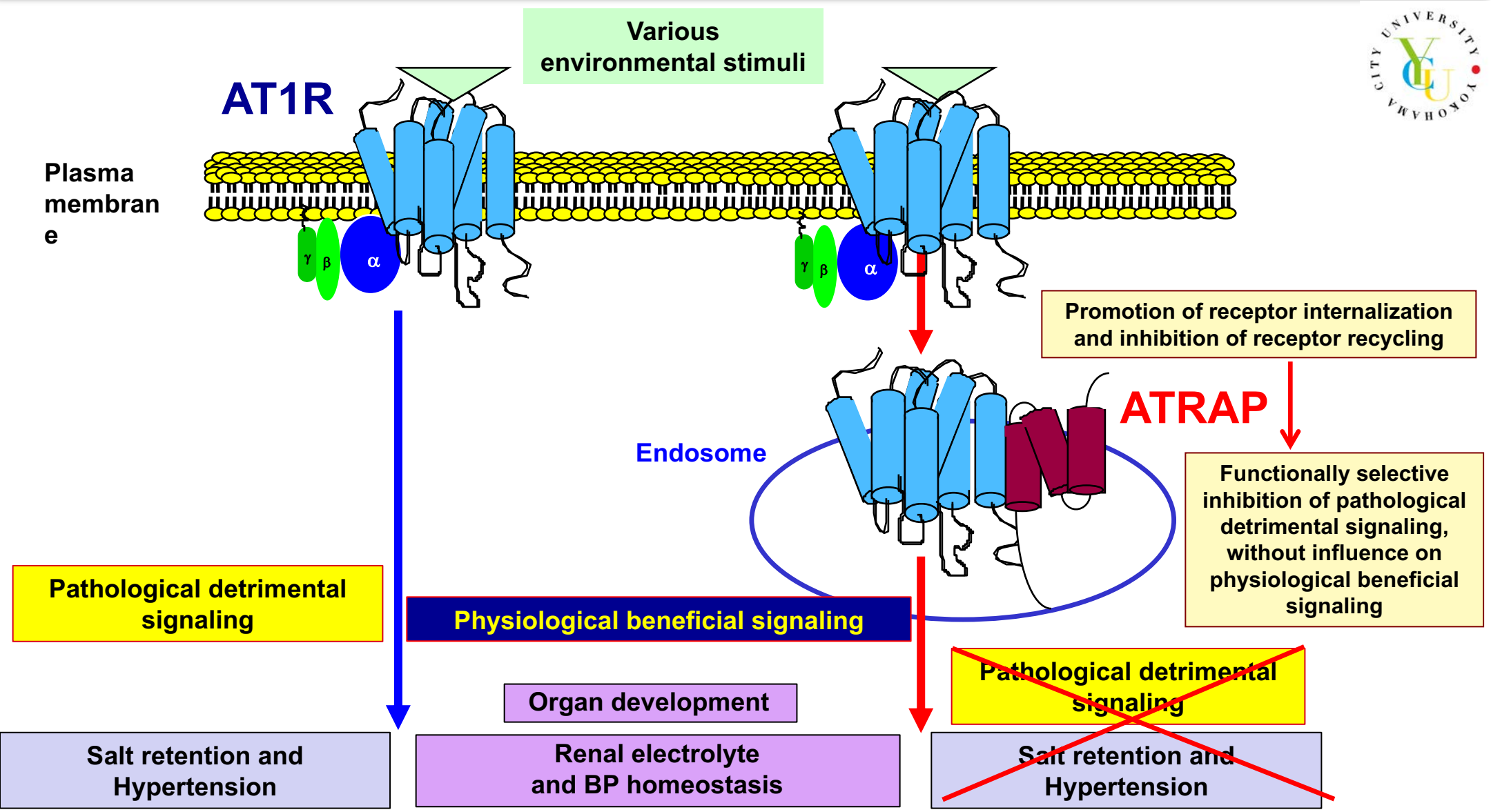
Schema illustrating effects of changes in renal tubular ATRAP expression on sodium handling and BP regulation in response to Ang II



Tamura K, et al.

Tamura K, et al. Curr Med Chem. 2015.

ATRAP exerts beneficial modulatory effects on renal sodium handling and BP regulation in response to pathological stimuli, without effects on baseline renal sodium handling and BP. Therefore, ATRAP is a potential novel target of interest in salt-sensitive hypertension



Wakui H, Dejima T, et al. Cardiovasc Res 2013; Tamura K, et al. Curr Pharma Des 2013; Tamura K, et al. Curr Med Chem 2015.

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The presented work was supported by the Salt Science Research Foundation, et al.
Thank you very much!

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