

Proximal tubule-specific expression of AT_{1a} receptors in the kidney mediates extracellular and intracellular angiotensin II-induced blood pressure responses in AT_{1a} receptor-knockout mice

¹ Xiao C. Li, ² Julia L. Cook, ¹Elisa Miguel-Qin, ¹Victor Gu, and ¹ Jia L. Zhuo
¹ Department of Pharmacology & Toxicology, The University of Mississippi Medical Center, Jackson, MS 39216-4505, USA
² Ochsner Clinic Foundation, New Orleans, LA 70121, USA

INTRODUCTION

- Angiotensin II (Ang II) plays an important role in maintaining blood pressure homeostasis in health and diseases.
- Recent studies on Kidney cross-transplantation between wild-type and AT_{1a} receptor-knockout (AT_{1a}-KO) mice demonstrated a key role of kidney AT_{1a} receptors in blood pressure control.
- However, Ang II receptors are widely expressed in intrarenal vasculature, proximal tubules, and renomedullary interstitial cells. The localization and mechanisms underlying the effect of kidney cross-transplantation on blood pressure has not been determined.
- The precise role of AT_{1a} receptors in proximal tubules (PTs) in the regulation of arterial blood pressure is not fully understood.

Construction of a proximal tubule-specific adenoviral GFP-tagged AT_{1a} receptor or ECFP-tagged Ang II fusion protein vector

- Construction of a wild-type GFP-tagged AT_{1a} receptor in a GFP-expressing vector (AT_{1a}R/GFP) by Origene., or Ang II fusion protein in an ECFP-expressing vector (Dr. Julia Cook, Ochsner Clinic).

- Subclone the AT_{1a}R/GFP gene or ECFP/AII into a vector encoding a proximal tubule-specific sodium and glucose co-transporter 2 promoter (sglt2-AT_{1a}R/GFP or sgl2-ECFP/AII) by Vector BioLabs.

- Construction of an adenoviral vector encoding sgl2-AT_{1a}R/GFP (Ad-sglt2-AT_{1a}R/GFP) or Ad-sglt2-ECFP/AII by Vector BioLabs (2.5 x 10¹¹ PFU/ml).

- | | I | II | III |
|------|---|----|-----|
| I: | Human Ad5-sequences (wt1-458); includes 5' L-ITR and packaging signal. | | |
| II: | transgene Sgl2-AT _{1a} R/GFP-PolyA. | | |
| III: | Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R-ITR. E3 deletion: nts 28587-30464. | | |

HYPOTHESIS

Proximal tubule-specific expression of AT_{1a} receptors mediates both extracellular and intracellular Ang II-induced blood pressure responses in AT_{1a}-KO mice.

RESULTS

The sgl2 promoter drives the expression of GFP-tagged wild-type AT_{1a} receptors selectively in proximal tubules of AT_{1a}-KO mice

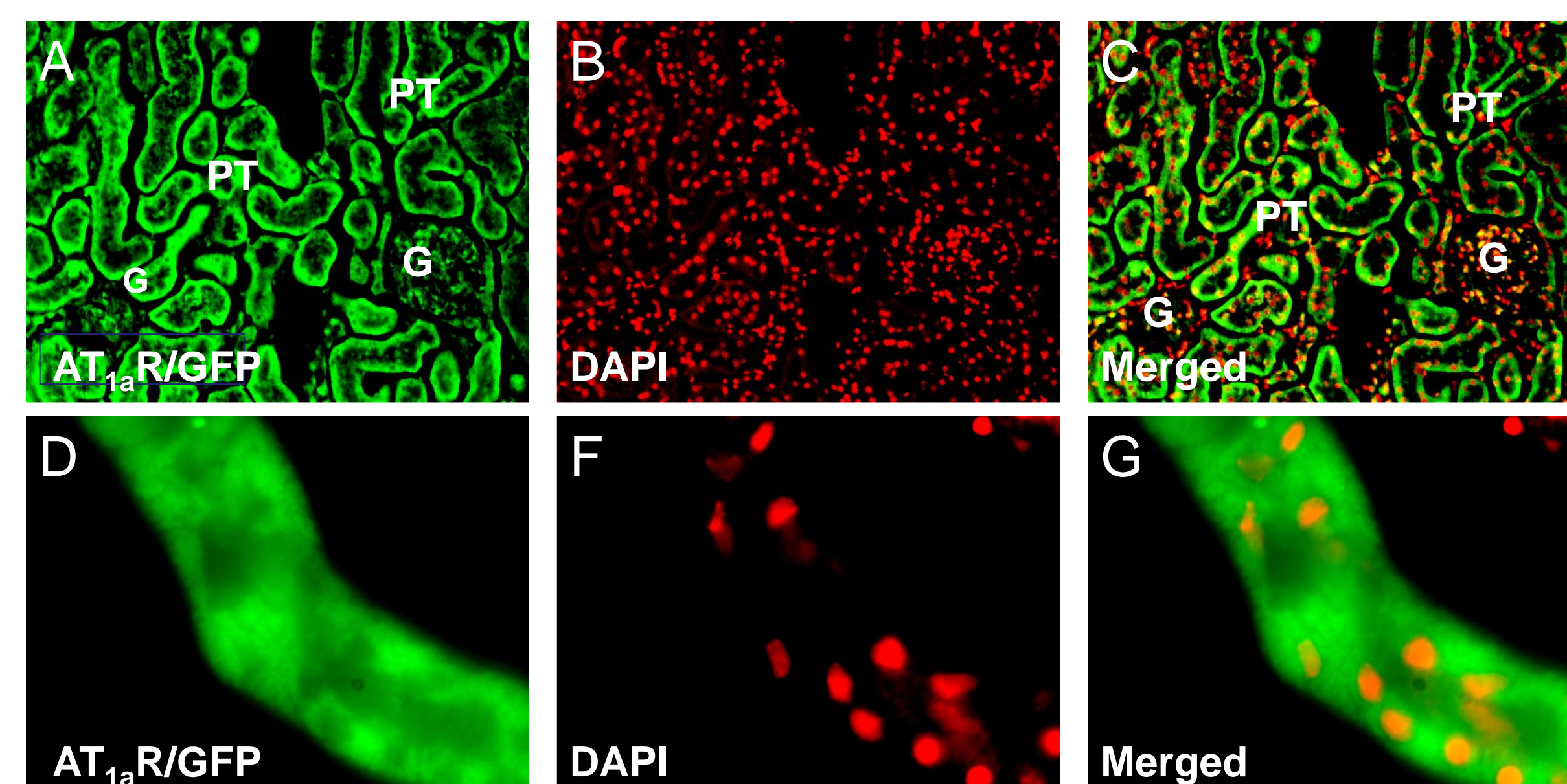


Figure 1: AT_{1a}R/GFP is expressed primarily in proximal tubules, and there is little AT_{1a}R/GFP expression in glomeruli. Magnification: 60 X (A-C); 200 X (D-G).

The sgl2 promoter drives expression of ECFP/AII selectively in proximal tubules of AT_{1a}-KO mice

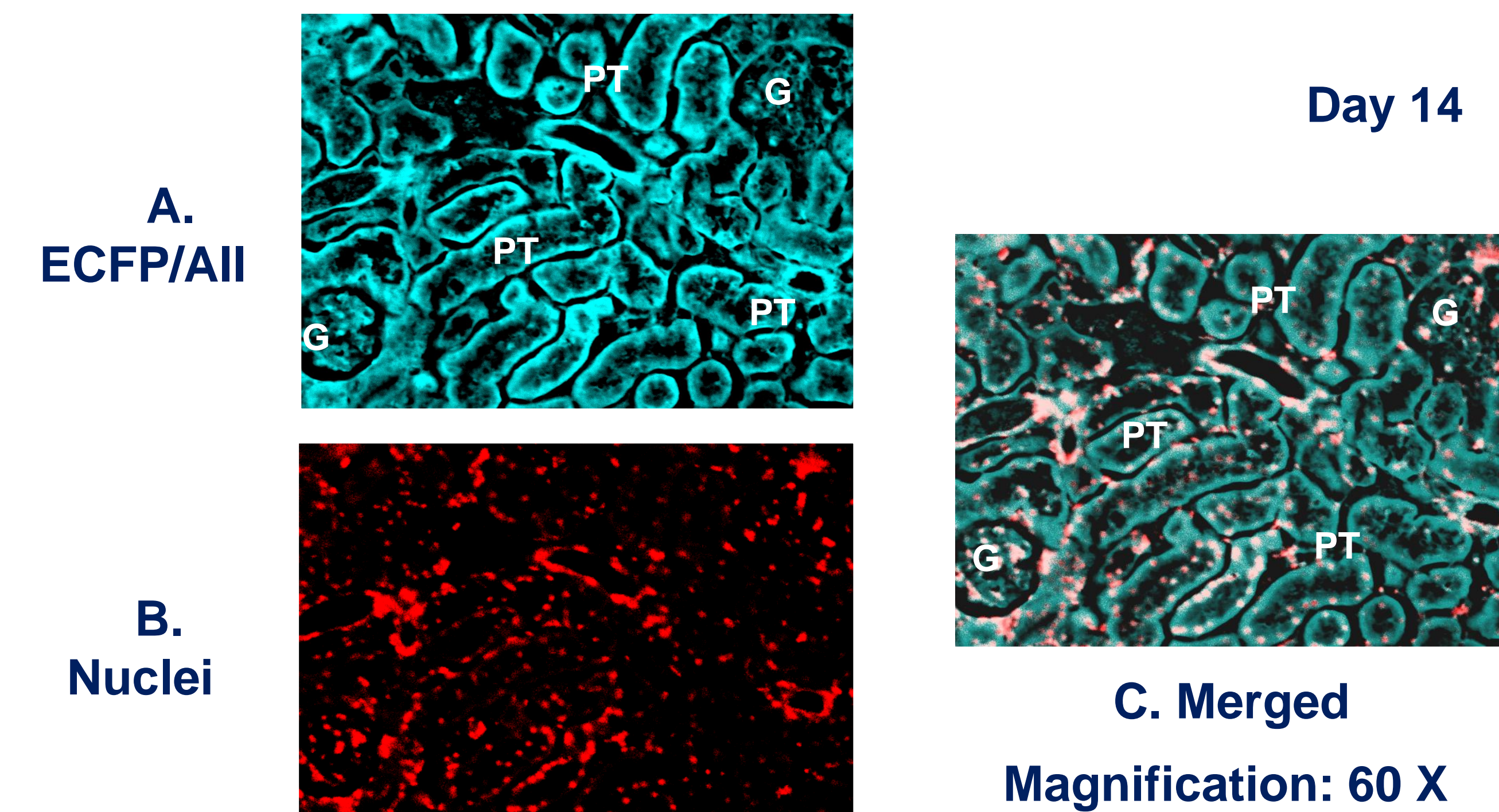
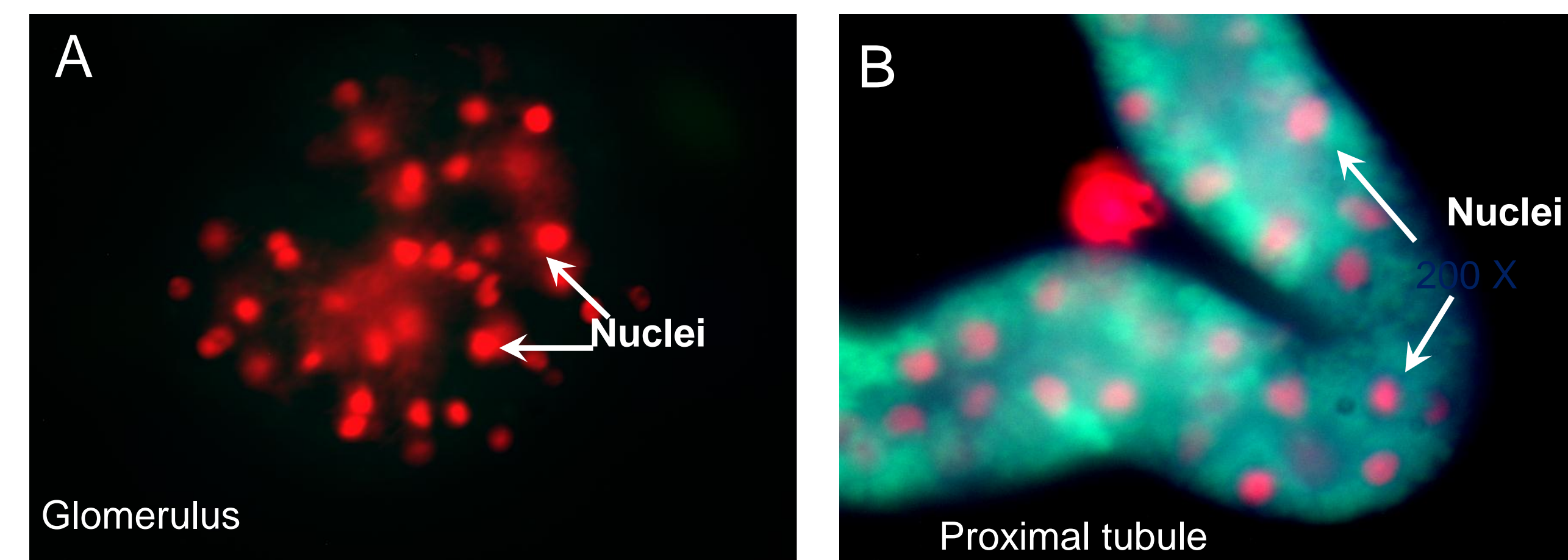


Figure 2: Panel A shows that ECFP/AII is expressed in proximal tubules (PT). Panel B is DAPI-stained nuclei. Panel C is the merged image of Panels A and B. G = glomeruli.

Proximal tubule-specific expression of ECFP/AII in AT_{1a}-KO mouse kidney



Magnification: 200 X

Day 14

Figure 3: Panel A shows that ECFP/AII is not expressed in freshly isolated glomeruli. Panel B shows that ECFP/AII is expressed in freshly isolated proximal tubule.

Effects of proximal tubule-specific expression of AT_{1a}R/GFP on blood pressure in AT_{1a}-KO mice

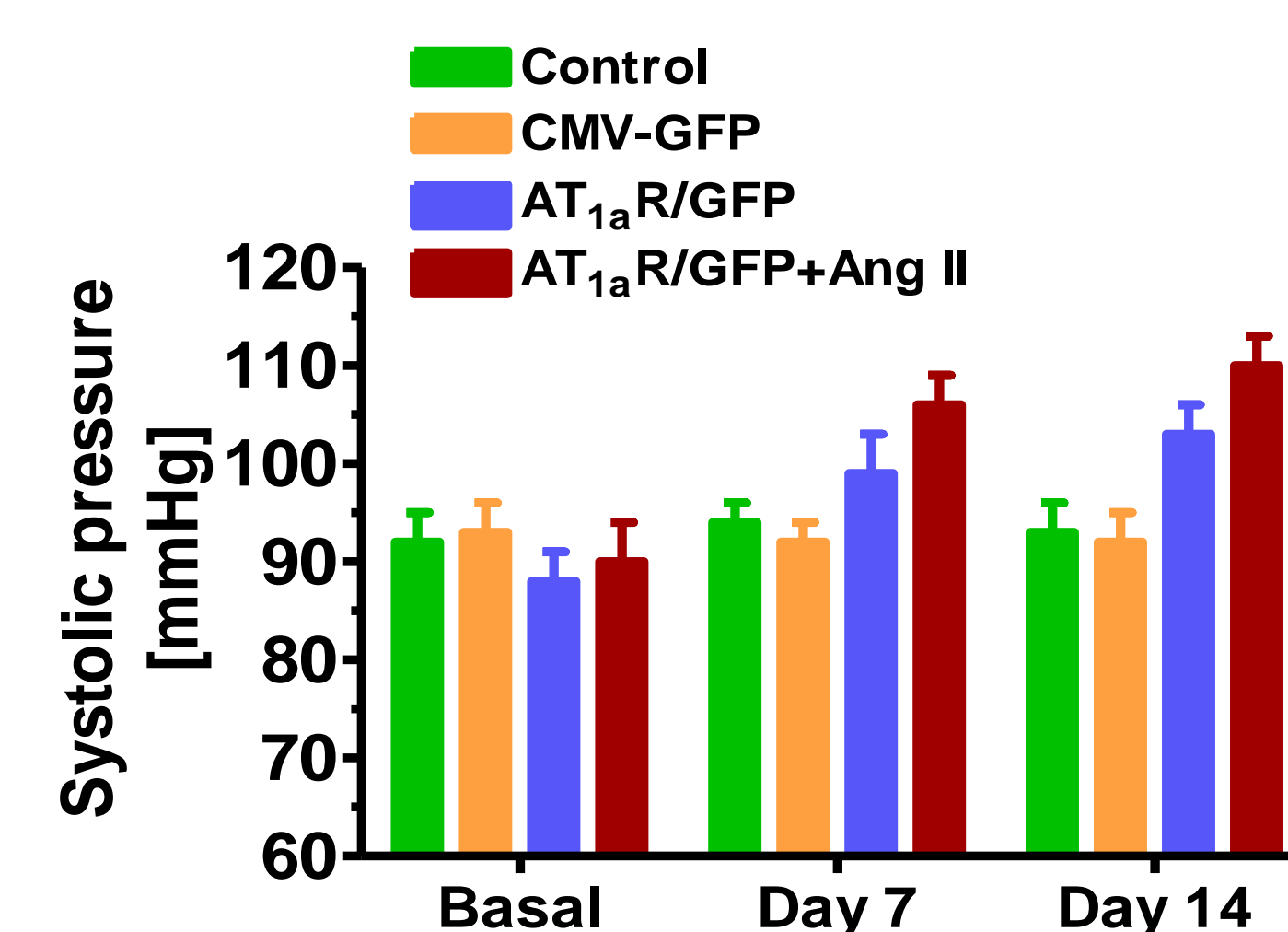


Figure 4: ** *p* < 0.05 vs. basal; + *p* < 0.05 vs. AT_{1a}R/GFP. N = 10-12

Effects of proximal tubule-specific co-expression of ECFP/AII and AT_{1a} receptors on blood pressure in AT_{1a}-KO mice

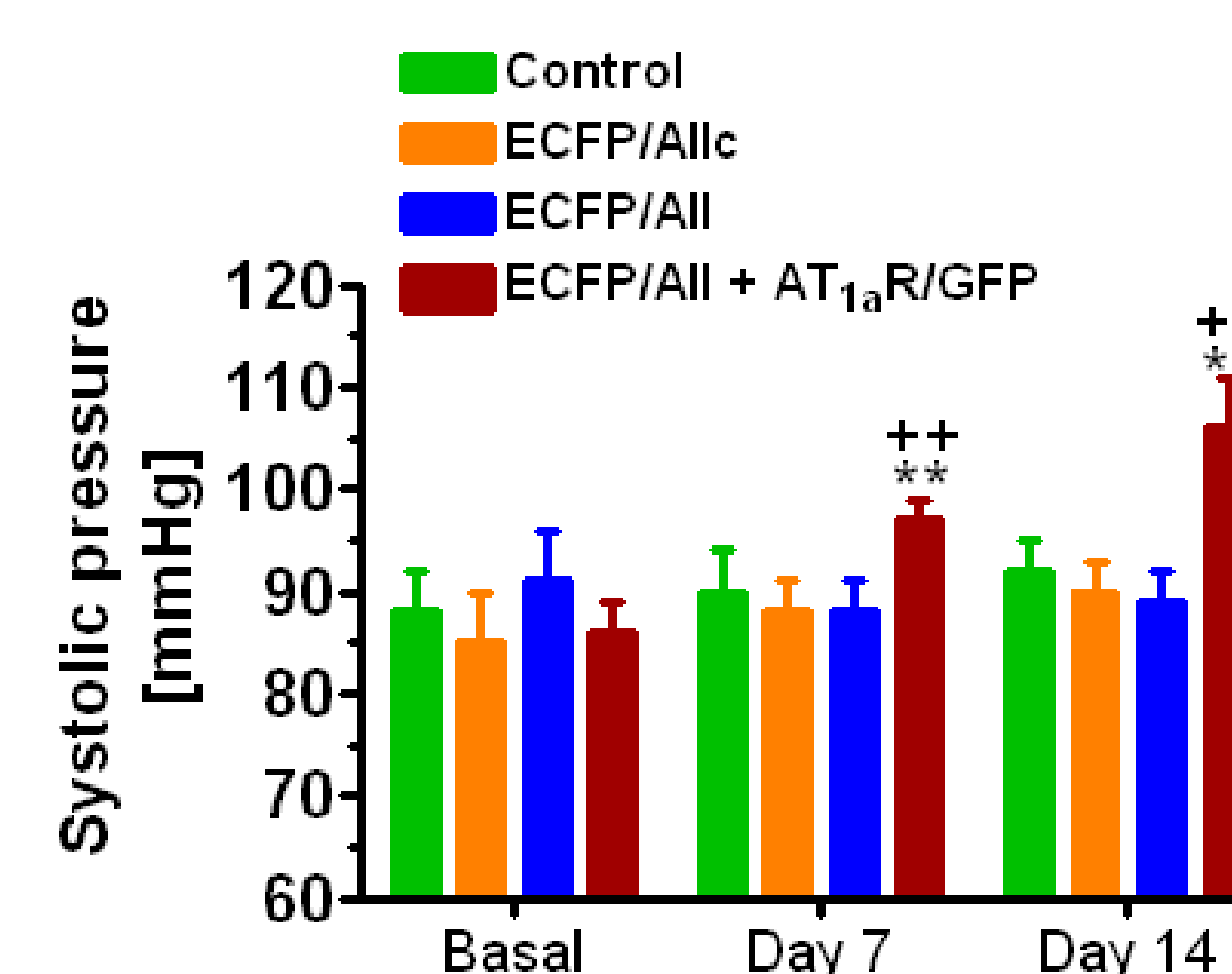


Figure 5: ** *p* < 0.01 vs. basal; ** *p* < 0.01 vs. control, ECFP/AII or ECFP/AII alone.

RESULTS (Cont.)

Effects of proximal tubule-specific expression of AT_{1a} receptors on urinary sodium excretion in AT_{1a}-KO mice

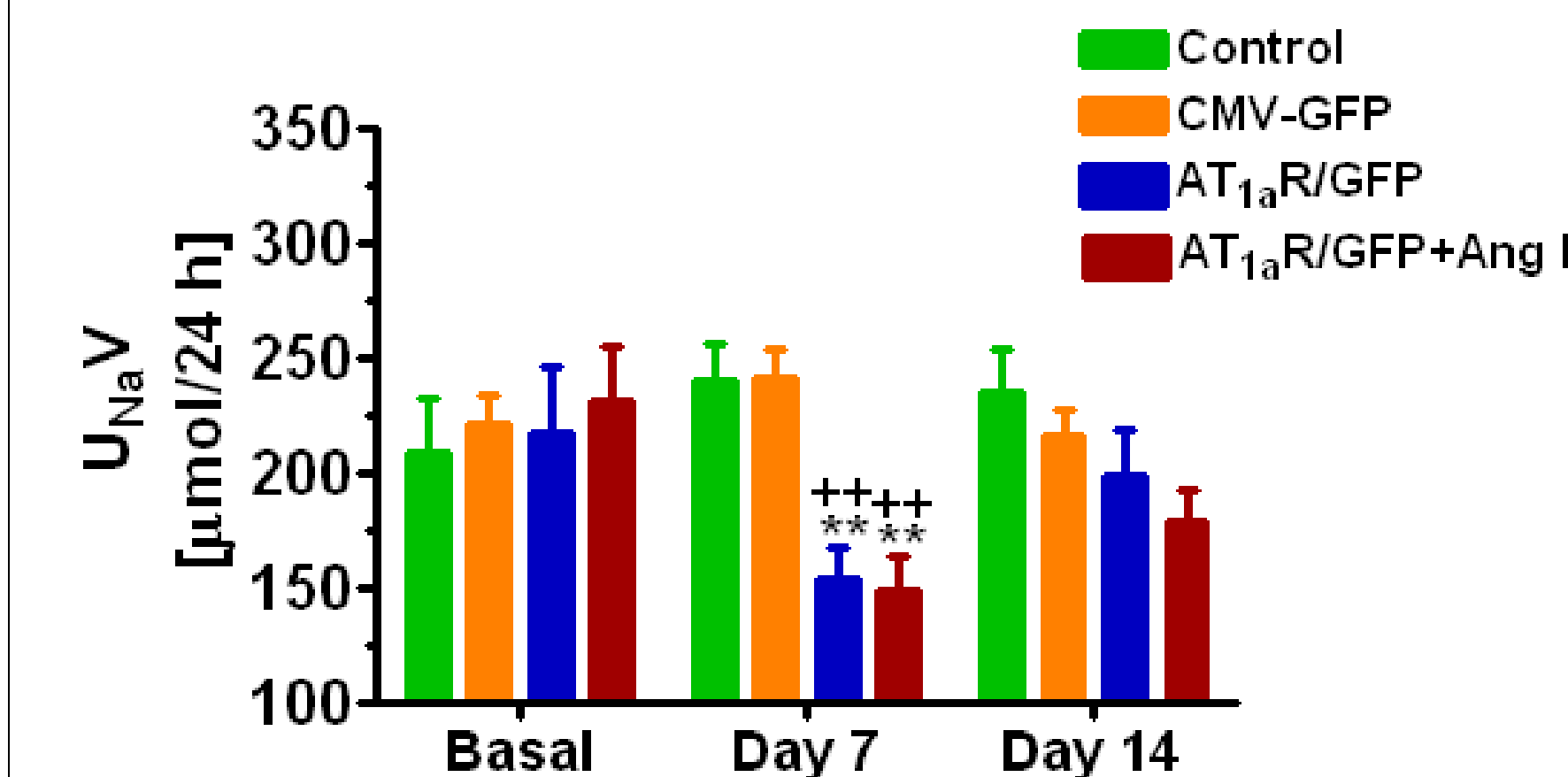


Figure 6: ** *p* < 0.01 vs. basal; * *p* < 0.01 vs. control or CMV-GFP. N = 10-12

Effects of proximal tubule-specific co-expression of ECFP/AII and AT_{1a} receptors on urinary sodium excretion in AT_{1a}-KO mice

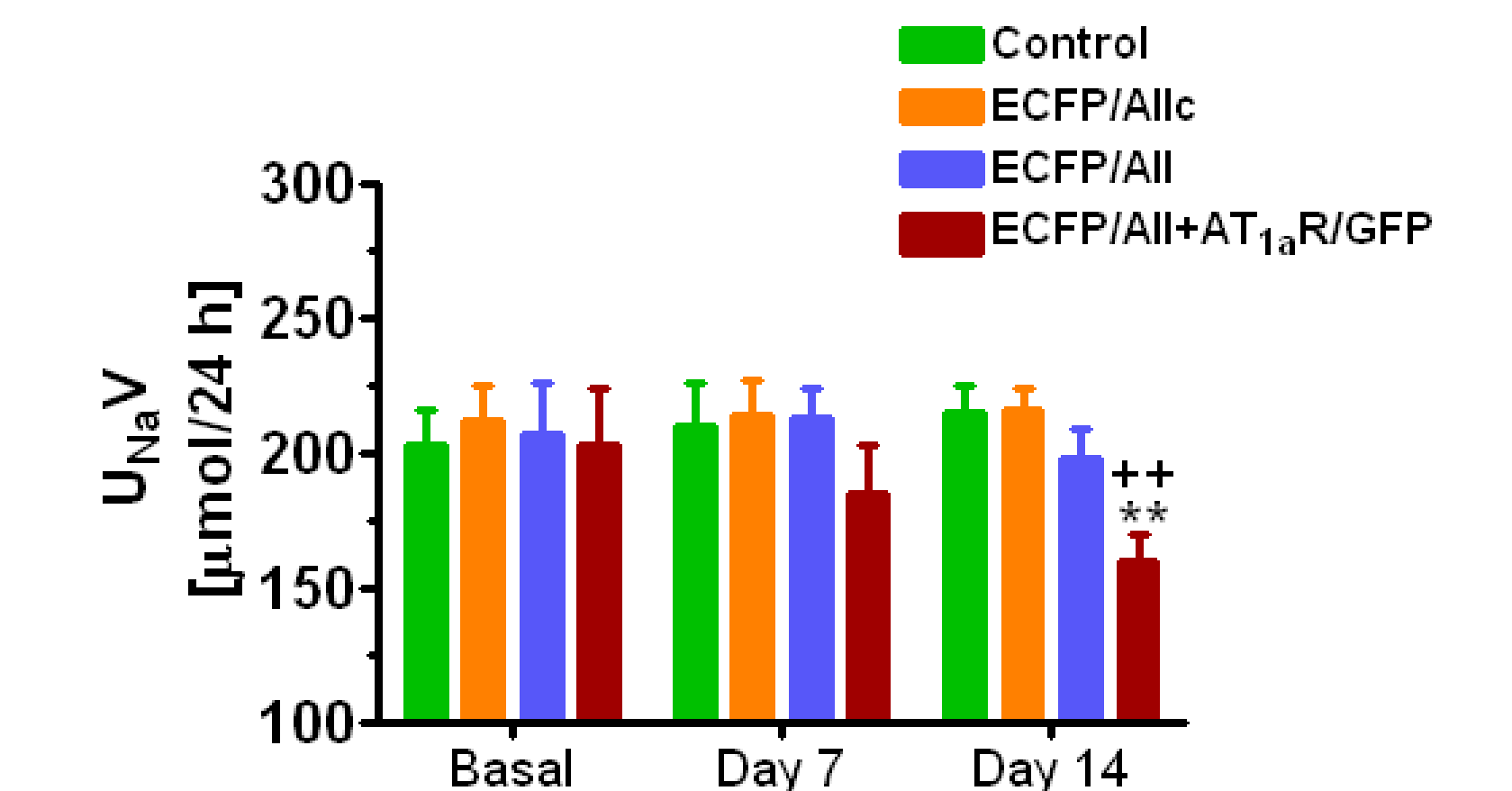


Figure 7: ** *p* < 0.01 vs. basal; ** *p* < 0.01 vs. control, ECFP/AII or ECFP/AII alone.

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

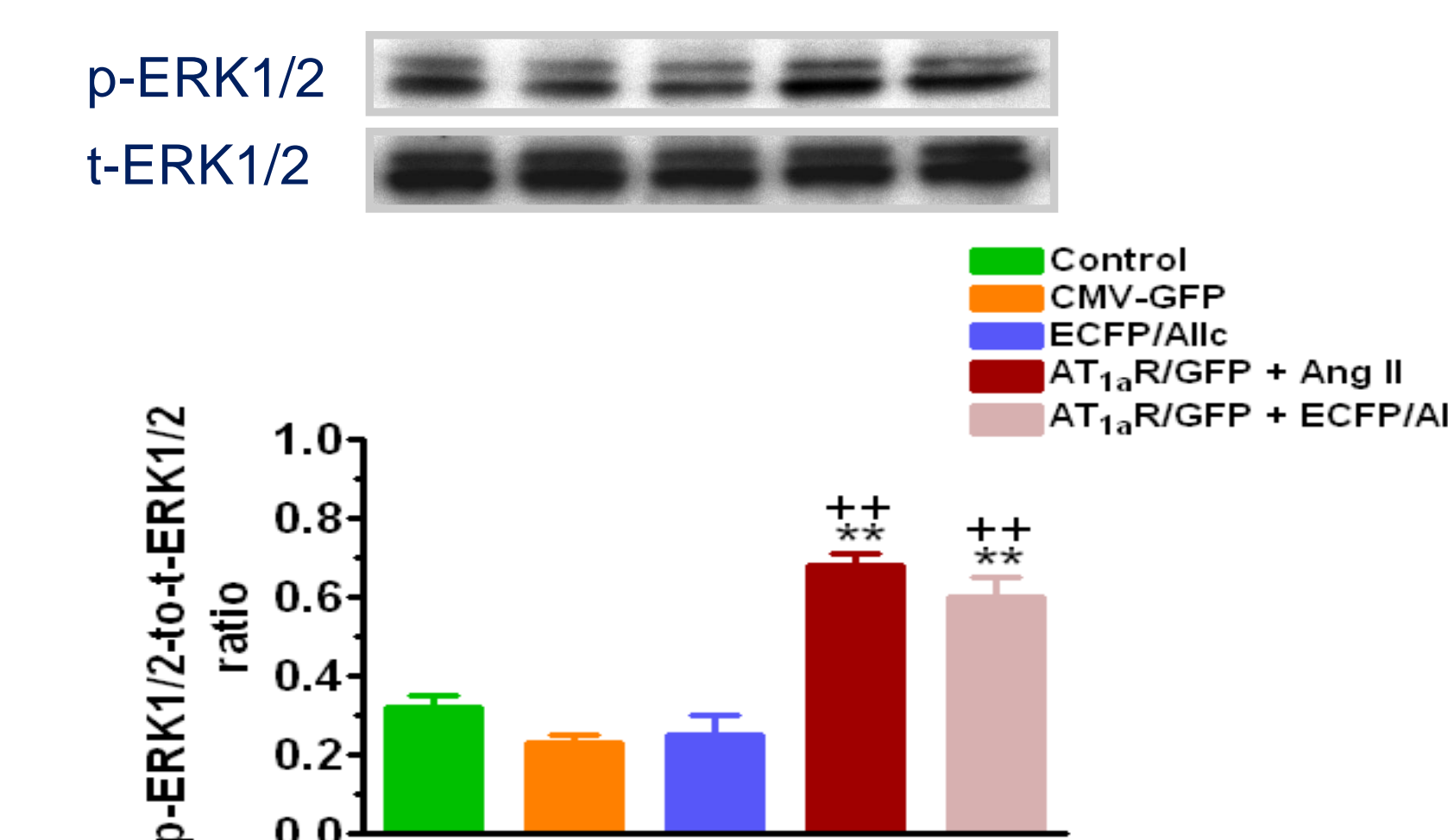


Figure 8: ** *p* < 0.01 vs. basal; ** *p* < 0.01 vs. CMV-GFP or ECFP/AII alone.

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

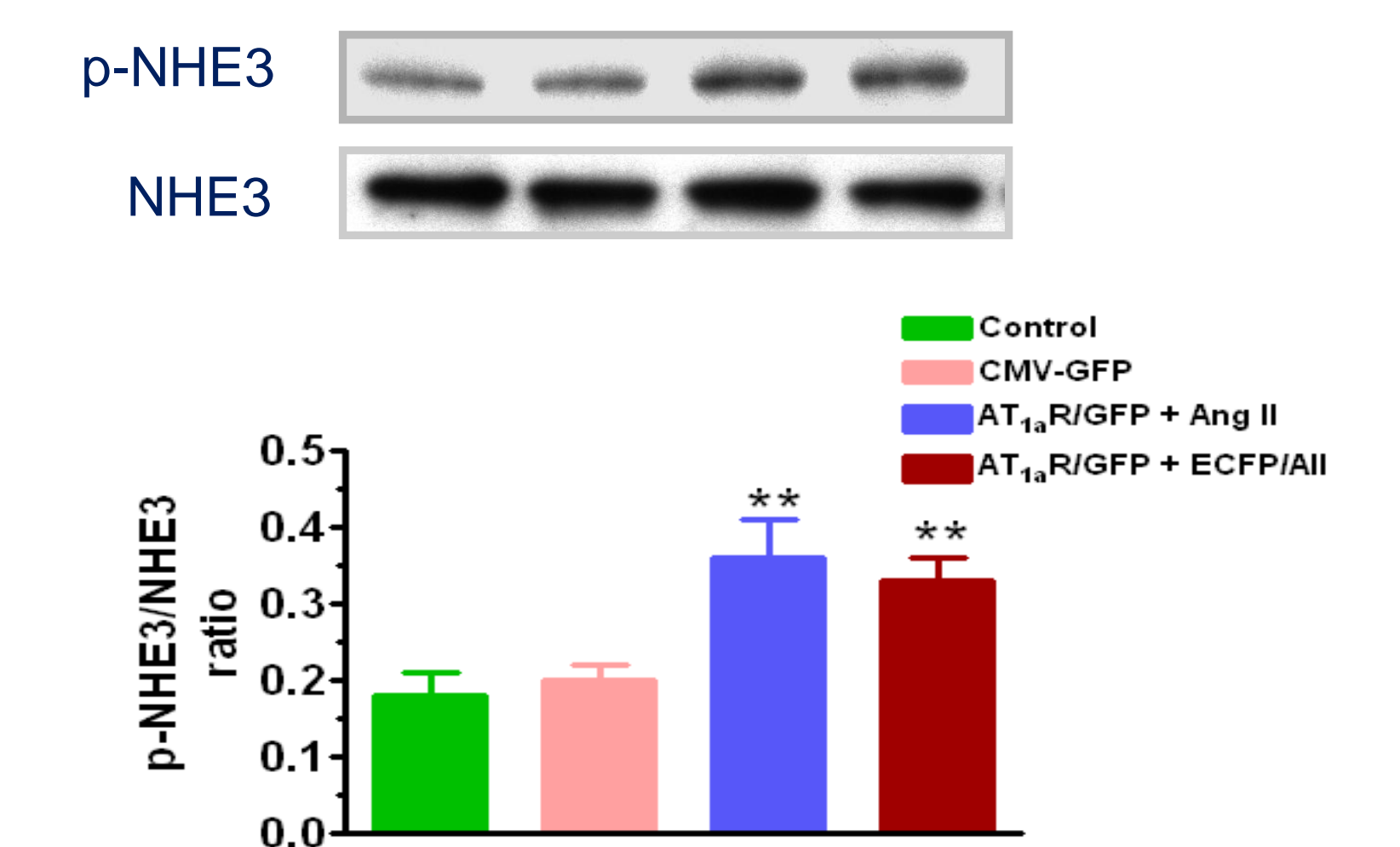


Figure 9: ** *p* < 0.01 vs. Control or CMV-GFP, n = 6-8

SUMMARY

The sgl2 promoter selectively drove AT_{1a}R/GFP or ECFP/AII expression in proximal tubules (PT) of AT_{1a}-KO mice.

PT-specific expression of AT_{1a}R/GFP induced moderate increases in blood pressure.

PT-specific expression of ECFP/AII increased blood pressure moderately only in the presence of AT_{1a}R/GFP receptors.

The blood pressure responses to AT_{1a}R/GFP receptor or ECFP/AII expression were associated with moderate decreases in U_{Na}V and increases in p-ERK1/2 and p-NHE3 proteins.

CONCLUSION

Proximal tubule-specific expression of AT_{1a} receptors in the kidney mediates extracellular and intracellular Ang II-induced blood pressure responses in AT_{1a} receptor-knockout mice.

ACKNOWLEDGEMENTS

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