

## Supplemental Data

### **Aldosterone is essential for angiotensin II-induced upregulation of pendrin**

Daigoro Hirohama<sup>1</sup>, Nobuhiro Ayuzawa<sup>1</sup>, Kohei Ueda<sup>1</sup>, Mitsuhiro Nishimoto<sup>1</sup>, Wakako Kawarazaki<sup>1</sup>, Atsushi Watanabe<sup>1</sup>, Tatsuo Shimosawa<sup>2</sup>, Takeshi Marumo<sup>1</sup>, Shigeru Shibata<sup>1,3</sup>, Toshiro Fujita<sup>1,4</sup>

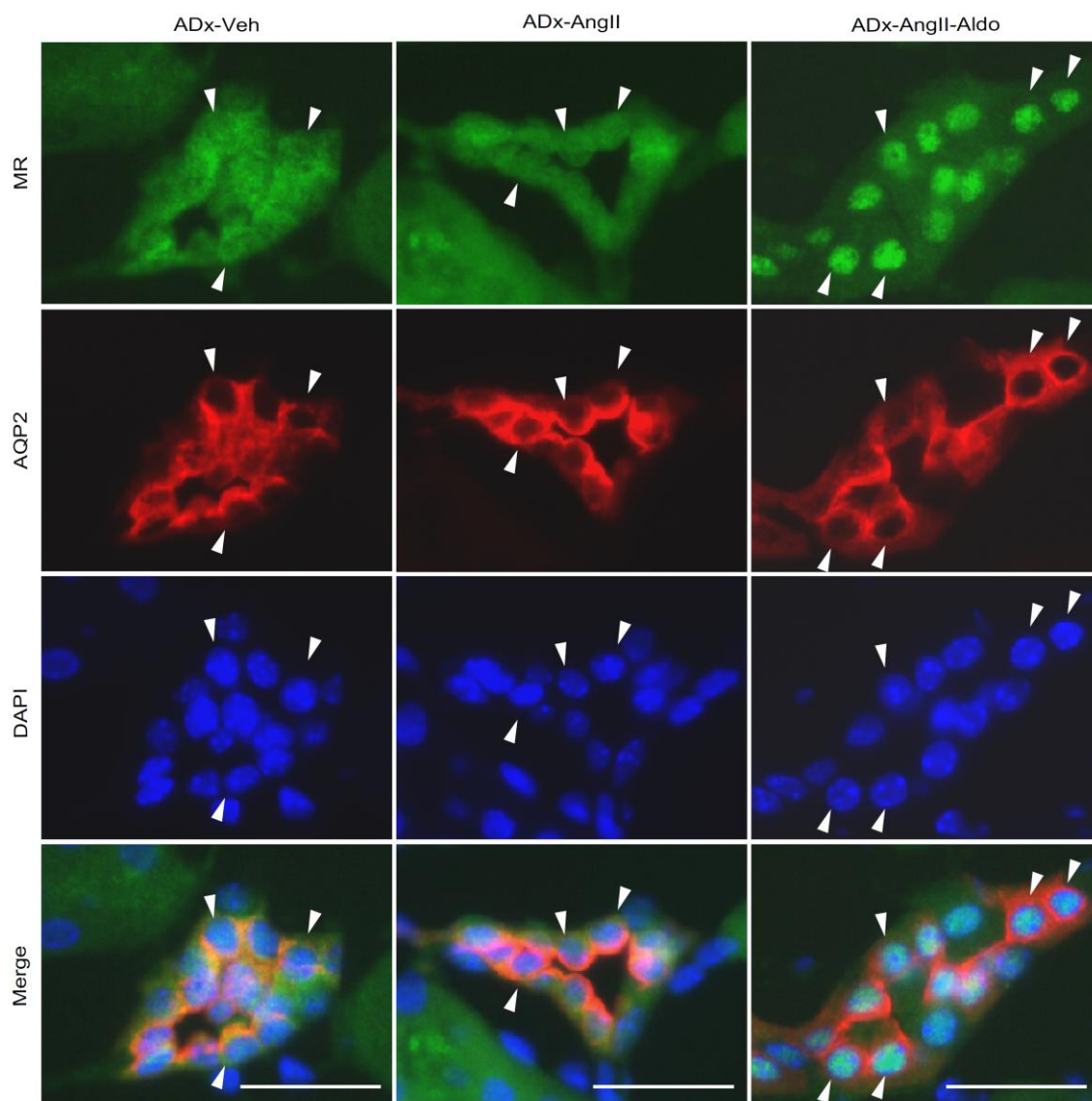
<sup>1</sup> Division of Clinical Epigenetics, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

<sup>2</sup> Department of Clinical Laboratory, School of Medicine, International University of Health and Welfare, Chiba, Japan

<sup>3</sup> Division of Nephrology, Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan

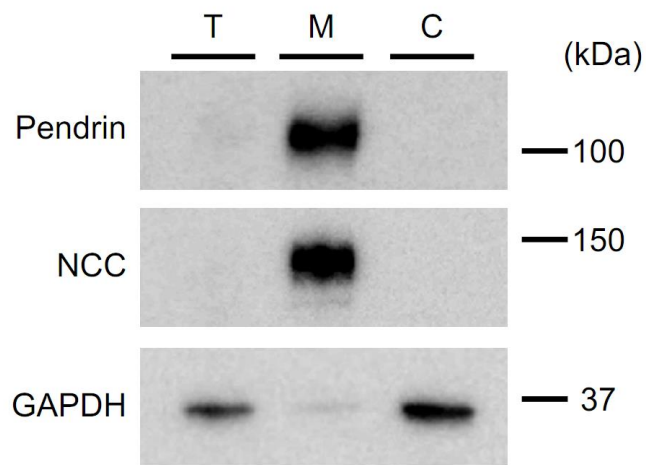
<sup>4</sup> CREST, Japan Science and Technology Agency, Tokyo, Japan

## Supplemental Figures



**Supplemental Figure S1. Nuclear translocation of MR in principal cells of kidneys from adrenalectomized mice treated with either vehicle, AngII or AngII and aldosterone co-administration.**

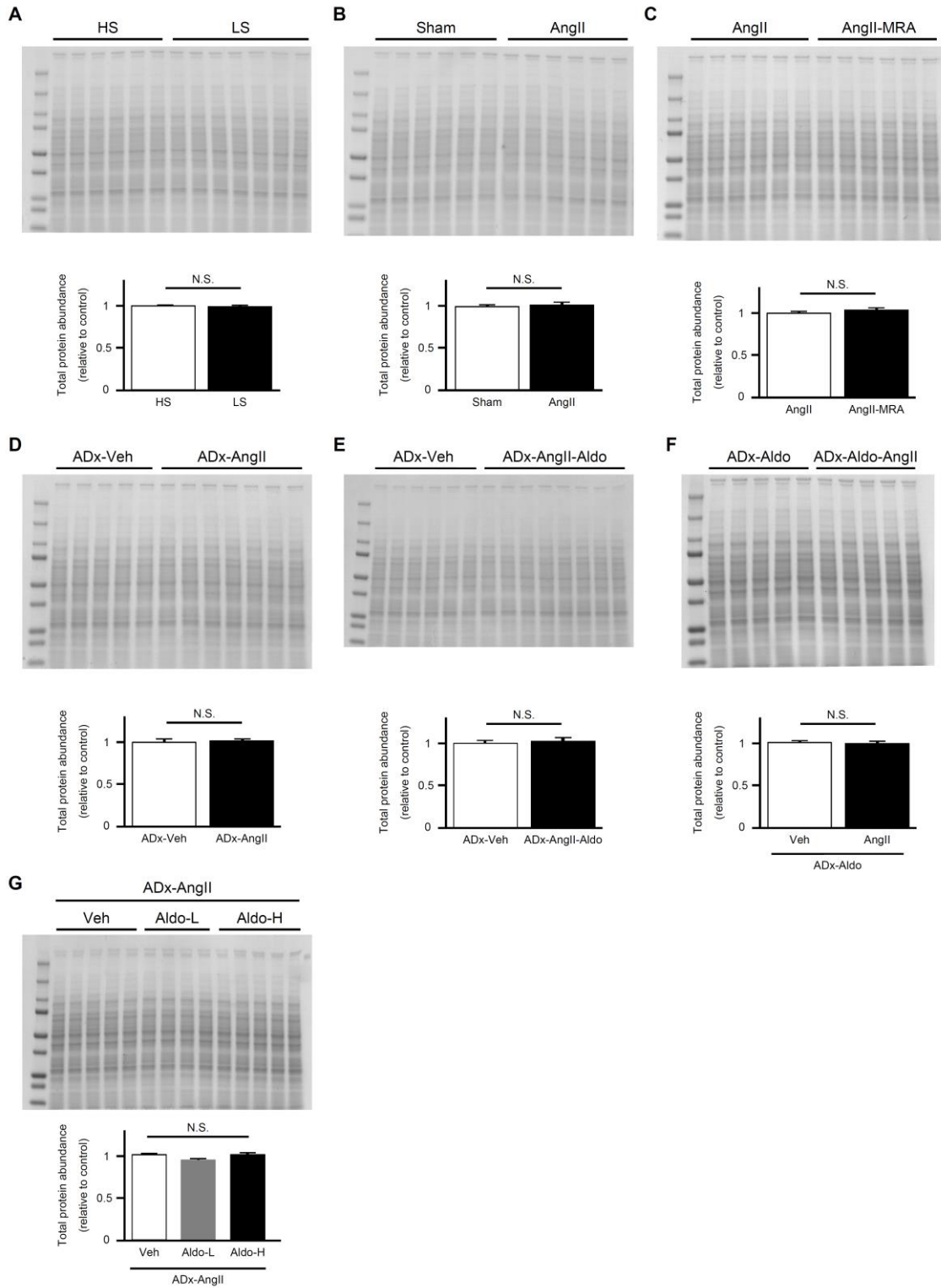
Immunofluorescence analyses of MR (green), AQP2 (red), nuclei (DAPI; blue), and a merged image in kidneys from adrenalectomized C57BL/6J mice treated with either vehicle (ADx-Veh), AngII (ADx-AngII) or AngII and aldosterone (1.0  $\mu\text{g}/\text{day}$ ) co-administration (ADx-AngII-Aldo). Arrowheads indicate principal cells. Scale bars represent 25  $\mu\text{m}$ . DAPI, 4',6-diamidino-2-phenylindole.



**Supplemental Figure S2. Purification of total membrane proteins.**

Total proteins from the membrane and cytoplasmic fractions were purified from wild-type mouse kidneys using a commercial kit (Invent Biotechnologies, Eden Prairie, MN, USA). Each fraction was analyzed by western blot using the indicated antibodies. Total cell lysates were extracted using lysis buffer containing 1% NP-40, and the same masses of protein were loaded into each well. The results show that membrane proteins, but not cytoplasmic proteins, were highly enriched in the total membrane fraction.

C, cytoplasmic fraction; M, membrane fraction; T, total cell lysate.



**Supplemental Figure S3. Coomassie blue-stained polyacrylamide gels used to control protein loading.**

Protein samples loaded onto gels are the same as those used for subsequent western blot experiments. (A) Total membrane proteins from mice fed either a high-salt (HS) or low-salt (LS) diet. (B) Total membrane proteins from mice undergoing either a sham operation (Sham) or AngII infusion (AngII). (C) Total membrane proteins from mice treated with either AngII infusion or AngII infusion along with mineralocorticoid receptor antagonist (AngII-MRA). (D) Total membrane proteins from adrenalectomized mice treated with either vehicle (ADx-Veh) or AngII infusion (ADx-AngII). (E) Total membrane proteins from adrenalectomized mice treated with either vehicle (ADx-Veh) or AngII and aldosterone co-administration (ADx-AngII-Aldo). (F) Total membrane proteins from aldosterone-treated adrenalectomized mice received either vehicle (ADx-Aldo) or AngII infusion (ADx-Aldo-AngII). (G) Total membrane proteins from AngII-treated adrenalectomized mice with the treatment of vehicle (ADx-AngII), low-dose aldosterone (ADx-AngII-Aldo-L), and high-dose aldosterone (ADx-AngII-Aldo-H). Proteins (15 µg/lane) were loaded onto all gels. Bands from these gels were analyzed using densitometry to provide quantitative assessment of loading. Density values were normalized by dividing by the mean value for the control group (the mean for the control group was defined as 1). Bar graphs show the summary of densitometric analyses. For (A)–(F), normalized band densities were compared with those of controls using an unpaired *t* test. For (G), normalized band densities were compared with those of controls using a two-way ANOVA, followed by Tukey-Kramer multiple-comparison tests. Values represent the mean ± SEM. In all gels, no difference was noted between groups, confirming that subsequent immunoblots were uniformly loaded.