Supplemental Methods

Protein isolation and western analysis

Total protein from each well was isolated by lysing cells in MPER® Mammalian Lysis buffer (Thermo Pierce) by repeated pipetting for homogenization. At the termination of treatments, nuclear protein isolation was accomplished by rinsing cells twice with PBS containing Halt™ Protease Inhibitor Cocktail (Thermo Scientific) and PhosSTOP EASYpack (phosphatase inhibitor cocktail, Roche). Nuclear protein was isolated using the NE-PER Nuclear and Cytoplasmic Extraction reagents (Thermo Scientific). Protein was estimated using the Micro BCA Kit (Thermo Pierce). 20 µg aliquots of cell protein were stored at -80ºC until use for western analysis. Protein samples were reduced by boiling at 95ºC for 5 min in sample buffer containing MPER® Mammalian Lysis buffer, NuPAGE® LDS sample buffer (Life Technologies) and NuPAGE® sample reducing agent (Life Technologies), and separated on a 10% BisTris gel by electrophoresis (200 V, 50 min) using the XCell SureLock system (Life Technologies). Protein bands were then transferred to an activated PVDF membrane (1.5 h, 40 V). After transfer, the membrane was incubated for 1 h at room temperature with either 5 % BSA or non-fat dry milk in Tris-buffered saline-0.1% Tween (TBST) blocking solutions. Primary and secondary antibodies were diluted using the blocking solution as the diluent. The regular ECL Kit or the ECL SuperSignal West Pico Kit (Thermo Pierce) was used for signal development. Immunodetection of β-actin (Sigma Aldrich), GAPDH (Thermo Pierce) or Lamin A/C (Santa Cruz) was used for protein normalization, as indicated. The details of the antibodies are listed in Supplementary Table 1.

Aldosterone enzyme immunoassay

The aldosterone immunoassay (Gomez-Sanchez, et al. 1987; Manolopoulou, et al. 2008) was used to measure aldosterone in time course, verapamil dose response experiments and primary human adrenal cell culture experiments. For all other experiments, all steroids including aldosterone were measured by the LC-MS/MS. Briefly, 96-well costar plates were coated overnight in goat anti-mouse IgG. After three washes in 1X PBS with 0.1% Tween, the plate was incubated in aldosterone antibody for 1 h. Aldosterone standards or media samples (50 µL) were added to the plate, followed by biotin-linked competitive aldosterone, and incubated for 1 h. At the end of the incubation, the plate was washed and incubated in horseradish peroxidase for 30 min. Chromogen was developed by incubation with TMB substrate for 30 min. The reaction was stopped by the addition of 1N sulfuric acid and read on a colorimeter at 450 nM.

Statistical analysis

Results were calculated as means ± SEM from at least three independent experiments. One-way ANOVA was used for statistical analyses using SigmaPlot 12.5 software (Systat Software, Inc). Significance was accepted for $p \leq 0.05$.

Supplementary figure legends

Supplemental Figure S1. Doxycycline treatment does not effect wild-type HAC15 cell gene expression of (A) *KCNJ5*, (B) *CYP11B2* or (C) *NURR1*. Control wildtype were incubated with 1 µg/ml doxycycline and analyzed by real time qPCR. Doxycycline itself did not have any effect on endogenous *KCNJ5 (Panel A)*, *CYP11B2 (Panel B)* or *NURR1 (Panel C)* mRNA expression. CYP111B2 agonist K^+ was used as a positive control. Results represent the means ± S.E.M. of three independent experiments. Statistical analyses were performed using one-way ANOVA (*p<0.05 versus basal).

Supplemental Figure S2. Effect of *KCNJ5*^{T158A} expression in primary cultures of human adrenal ZF/ZR cells. Primary cultures of ZF/ZR cells were transduced with lentiviruses constitutively expressing GFP (control, multiplicity of infection or MOI 10) or *KCNJ5*^{T158A} (at indicated MOI doses). The effect of *KCNJ5*^{T158A} on gene expression levels for m*KCNJ5* (*Panel A), CYP11B2 (Panel B)* and aldosterone production (*Panel C)* were analyzed. Results represent the means ± S.E.M. of three independent experiments. Statistical analyses were performed using one-way ANOVA (*p<0.05 versus basal).

References

Gomez-Sanchez CE, Foecking MF, Ferris MW, Chavarri MR, Uribe L & Gomez-Sanchez EP 1987 The production of monoclonal antibodies against aldosterone. *Steroids* **49** 581-587.

Manolopoulou J, Bielohuby M, Caton SJ, Gomez-Sanchez CE, Renner-Mueller I, Wolf E, Lichtenauer UD, Beuschlein F, Hoeflich A & Bidlingmaier M 2008 A highly sensitive

immunofluorometric assay for the measurement of aldosterone in small sample volumes: validation in mouse serum. *J Endocrinol* **196** 215-224.