

## Supplementary Figure Legends

### **Figure S1.** Formalin-fixed tissues.

Formalin-fixed paraffin-embedded (FFPE) blocks were generated from tissues. Of note, the tissue numbers in Figure 1D–E and the FFPE block numbers in this figure are different; the blue numbers refer to the tissue numbers of the FFPE blocks, while the green numbers in parentheses correspond to the tissue numbers in Figure 1D–E.

### **Figure S2.** Sanger sequencing of the KCNJ5 gene in the index case and her parents

(a): Pedigree of the index case. Her father, mother, brother, and sister did not suffer from primary aldosteronism. (b–d): Sanger sequencing results for KCNJ5 in the adenoma (T1 in Figure 1E), normal adrenal (N1), and peripheral blood from the index case. (e–f): Sanger sequencing results for KCNJ5 in peripheral blood from the father and mother of the index case. Pink frames indicate the mutated portion (p. G151R) of the index case.

### **Figure S3.** Histology of frozen sections

Each image is labeled with the sample name (FB5, FB10, FB15-1, or FB15-2; labeled in Figure 1E), staining method (either immunohistochemistry for CYP11B1 [B1] and CYP11B2 [B2], or hematoxylin-and-eosin staining [HE]), and sample number.

**Figure S4.** Histology of FFPE sections

Each image is labeled with the sample name (1–2, 3–4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16; the block numbers are indicated as blue numbers in Figure S1), staining method (either double immunohistochemistry for CYP11B2 in blue and CYP11B1 in brown [B2B1], single immunohistochemistry in brown for CYP11B2 [B2], CYP11B1 [B1], CYP17A, and HSD3B2, or hematoxylin-and-eosin staining [HE]), and sample number.

**Figure S5.** Histology of the adrenal cortices of the previously removed left adrenal gland

The adrenal cortices was hyperplastic and had CYP11B2–positive cells with irregular arrangement.

**Figure S6.** Comparison of *CYP11B2* mRNA levels between this case and the archived cases of sporadic APA (n = 16)

*CYP11B2* expression levels were similar in non-tumor ( $816,286.5 \pm 428,233.7$ -fold) and tumor portions ( $539,737.3 \pm 336,381.8$ -fold) of the case ( $p=0.393$ , Unpaired Student's t-test  $\Delta\Delta C_t$  values). *CYP11B2* expression levels in the case (T1–T3 and N1–N3, 582,237 [interquartile range: 448,734–977,189] fold) and APA (574,401 [328,933–817,145] fold)

were significantly higher than that of the paired adjacent normal adrenals (966 [62–11,986] fold), and those in the case and APA were similar. \* $p < 0.05$

**Figure S7. Immunohistochemistry for KCNJ5**

Immunostaining of KCNJ5 in a normal adrenal gland from a renal cell carcinoma patient (SIMC–Uro#10635, left) and a frozen section from FB10 (right). Three portions in red frames in the subcapsular area of the normal adrenal, non-tumor portion of FB10, and tumor portion of FB10 are enlarged in panels B, C, and M, respectively.

**Figure S8. Immunohistochemistry for CTNNB1**

Immunostaining of CTNNB1 from frozen sections of FB10 (right). The two portions indicated by yellow arrowheads are enlarged in panels B and C. Blue and green arrowheads indicate positive and negative nuclear staining for CTNNB1, respectively.