**Supplementary Information for**

# Mutational Landscape of Non-functional Adrenocortical Adenomas

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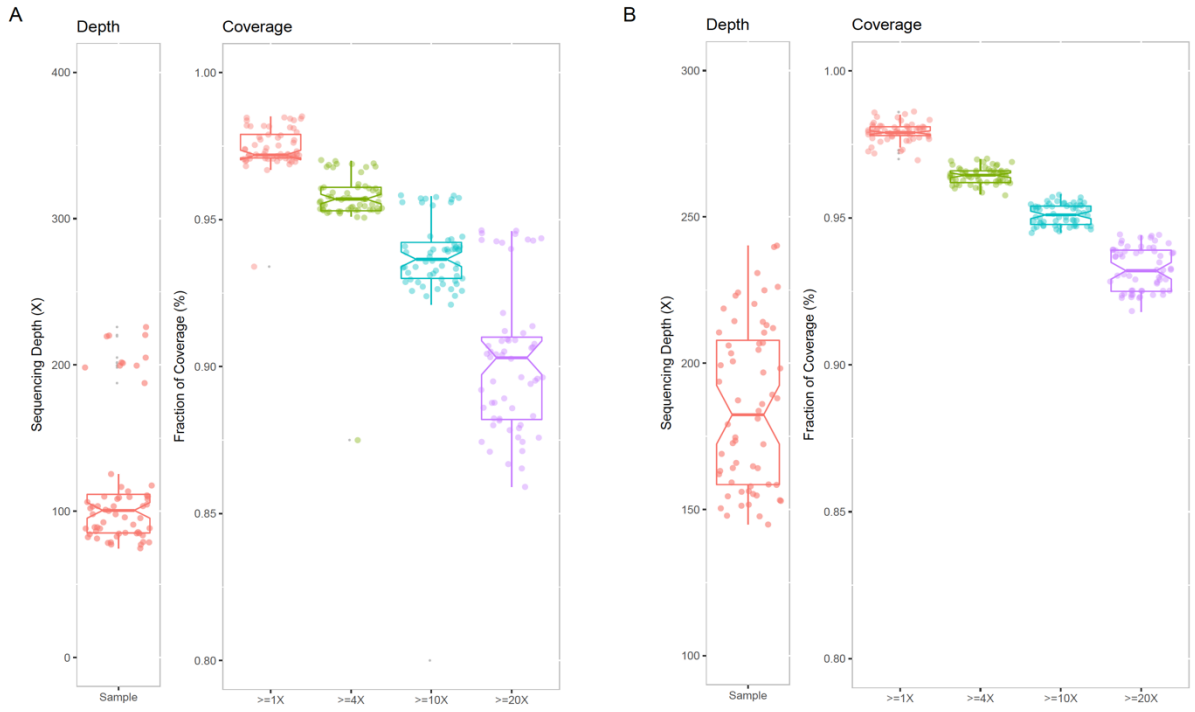
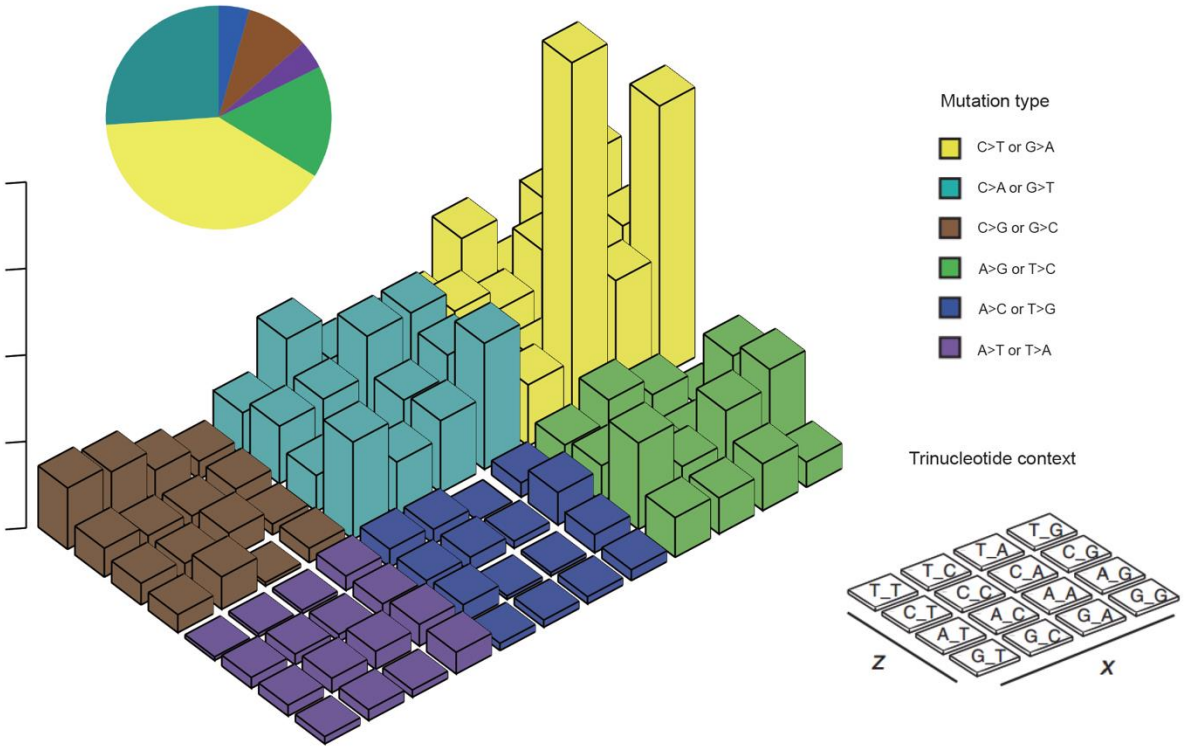
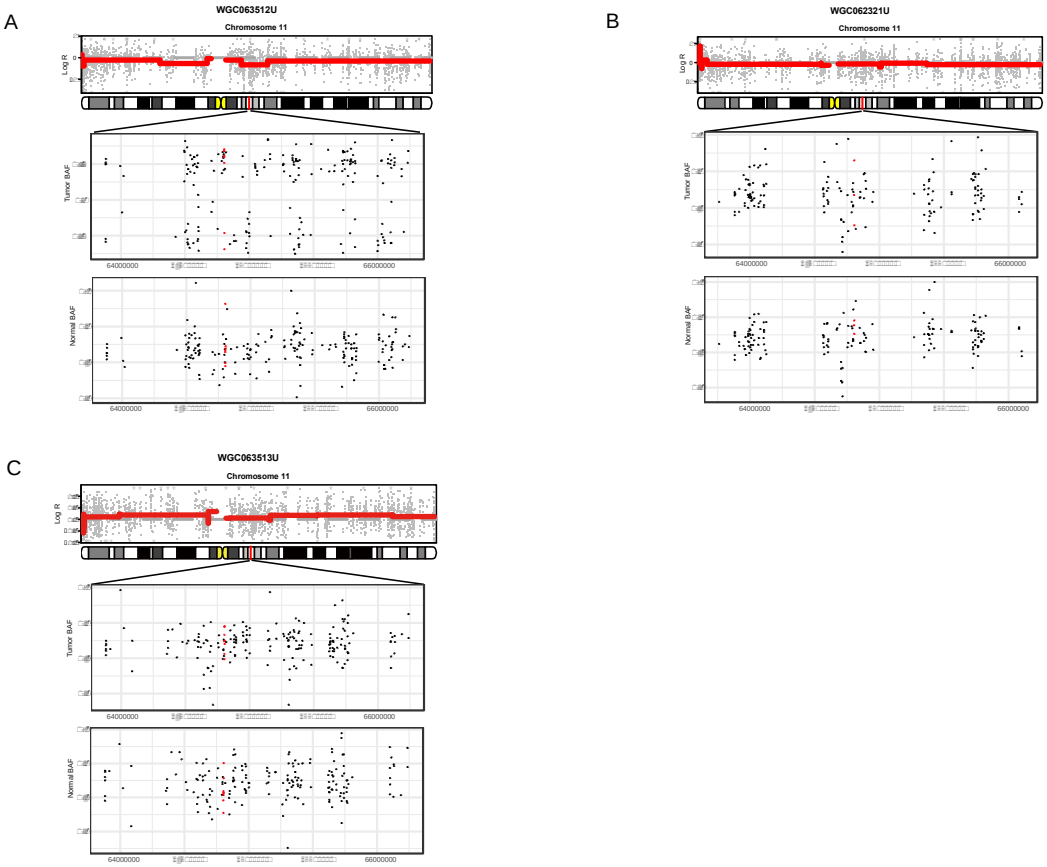
## Supplementary Figures



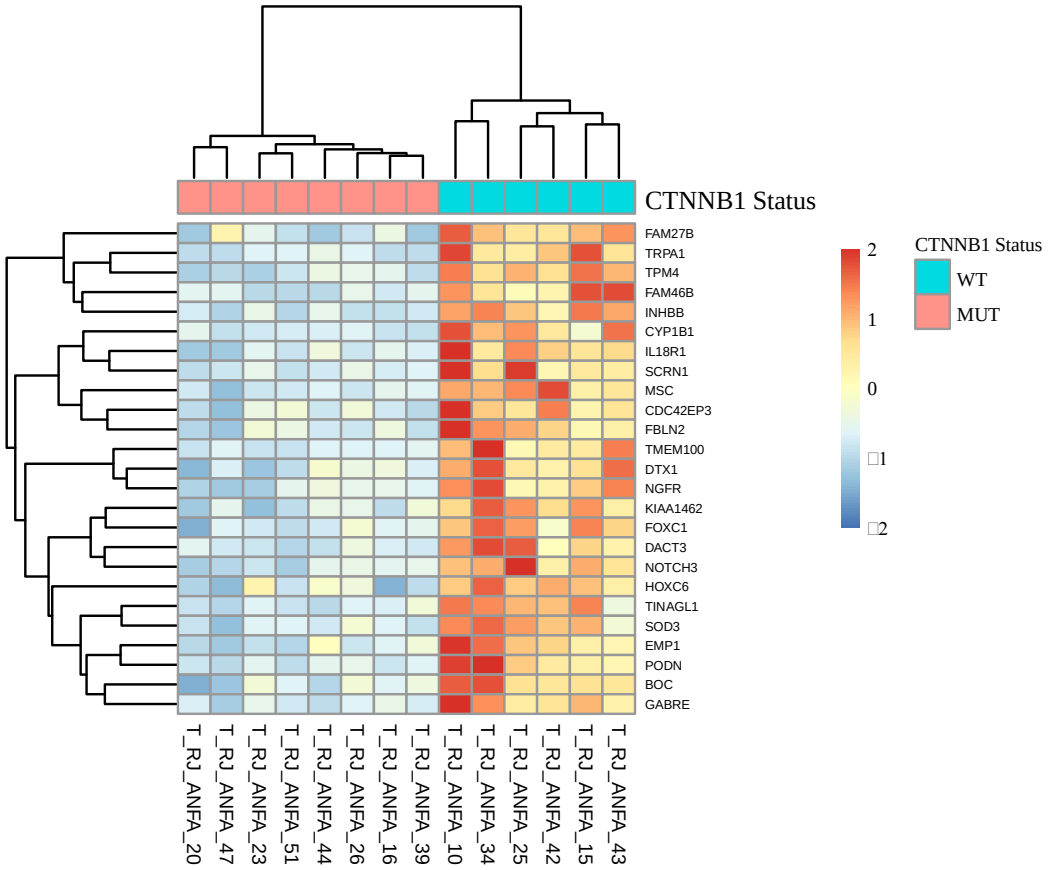

**Supplementary Figure 1. Fold coverage of exome regions in normal (A) and tumor (B) samples from NFACA patients.** Left, the distribution of average sequencing depth of samples. Right, the distribution of the fraction of exome bases covered by at least 1 reads, 4 reads, 10 reads and 20 reads across NFACA samples. Lines in the box show the medians and lines outside the box show the first or third quartiles of bases covered by reads.



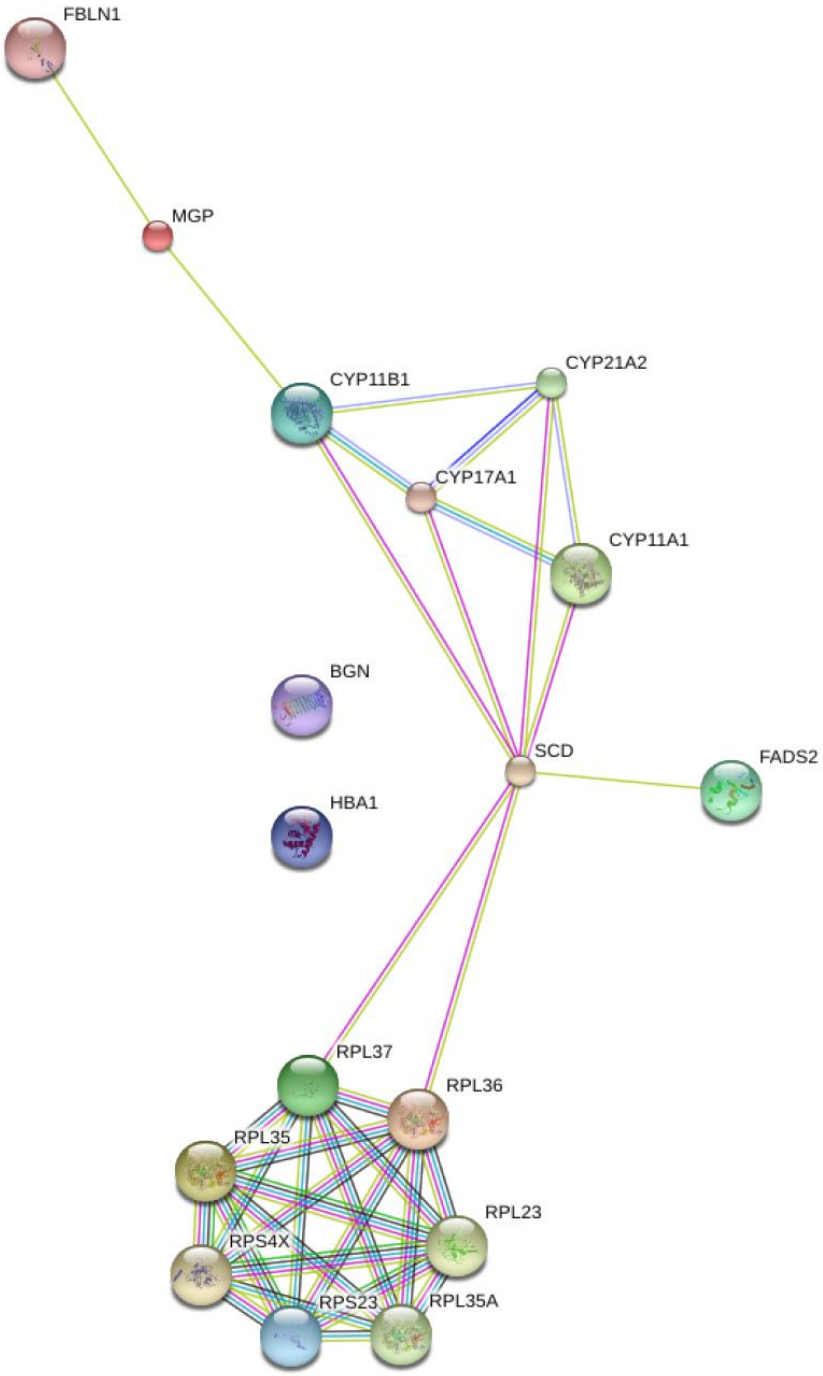

**Supplementary Figure 2. Lego plots of mutation frequencies for coding regions in NFACA.** Base substitutions were classified into six subtypes and each category was represented by different colors. Pie charts represent the distribution of the six subtypes. Base substitutions were further divided into 96 possible mutation types according to the flanking nucleotides surrounding the mutated base.



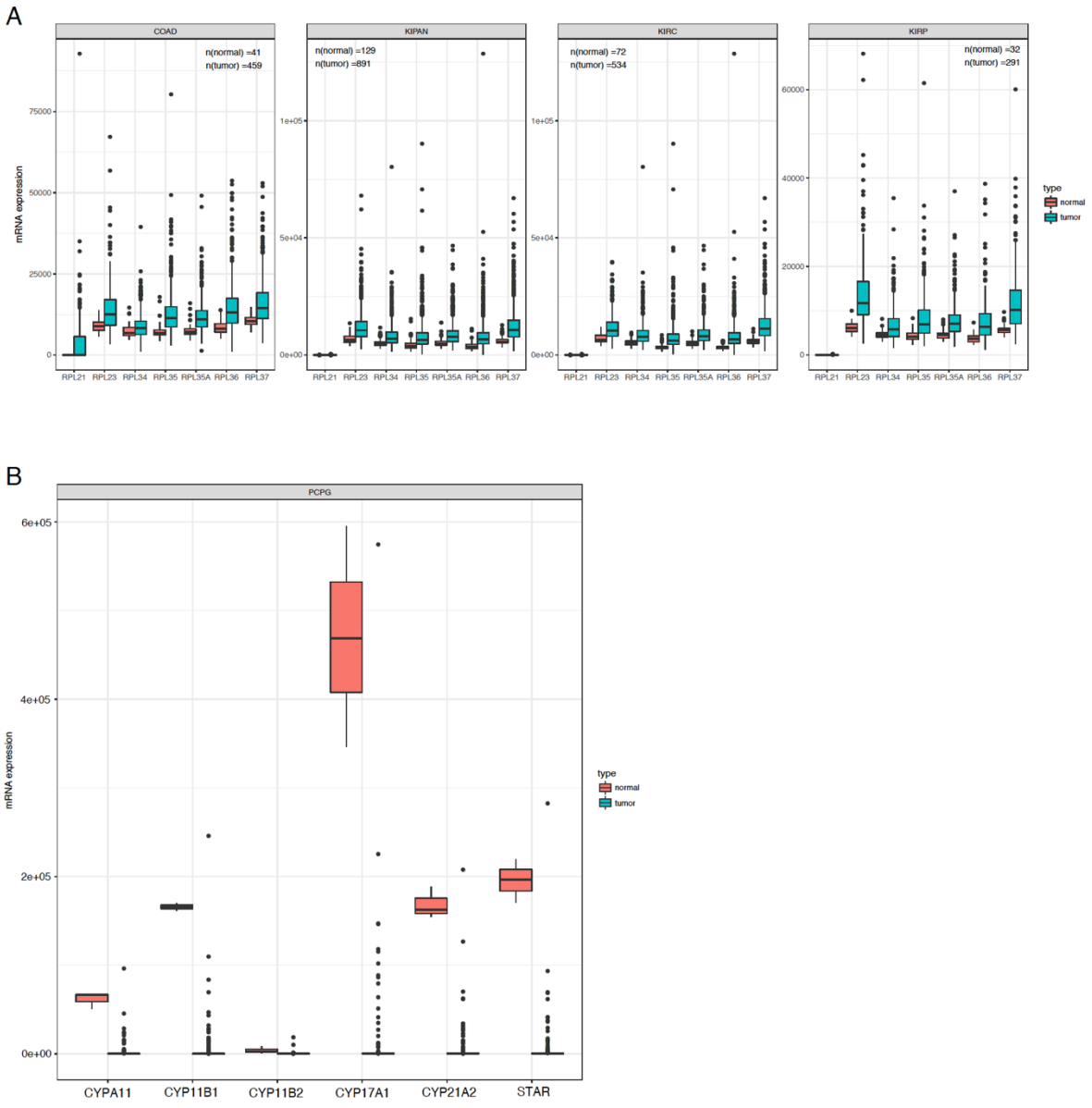

**Supplementary Figure 3. Loss of heterozygosity (LOH) at chromosome 11 analysis in one of the tumors.**



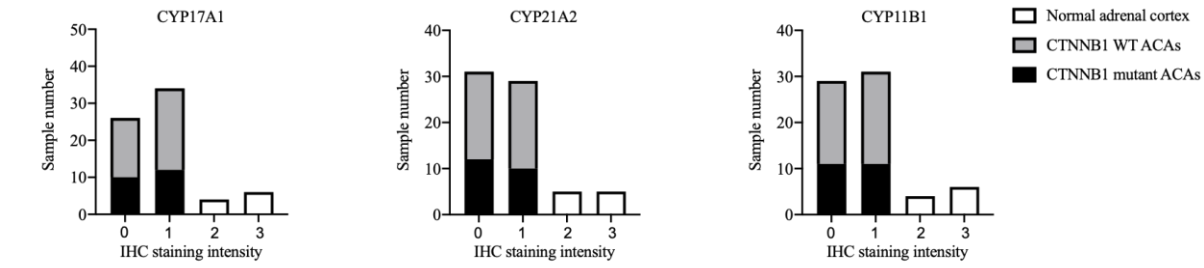

**Supplementary Figure 4. Molecular characterization analysis of mRNA expression in NFACAs with and without CTNNB1 mutations.**




**Supplementary Figure 5. Protein-protein interaction network based on genes that significantly enriched on terms of molecular function.** Colored lines between genes indicate the various types of interaction evidence.




**Supplementary Figure 6. Expression distribution of *RPL*- (A) and *CYP*- (B) genes between tumor and normal in TCGA cancer types.** COAD: Colon adenocarcinoma. KIPAN: Pan-kidney cohort, including Kidney Chromophobe, Kidney renal clear cell carcinoma and Kidney renal papillary cell carcinoma. KIRC: Kidney renal clear cell carcinoma. KIRP: Kidney renal papillary cell carcinoma. PCPG: Pheochromocytoma and Paraganglioma.




| IHC staining intensity | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| CTNNB1 mutant ACAs | 10 | 12 | 0 | 0 |
| CTNNB1 WT ACAs | 16 | 22 | 0 | 0 |
| Normal adrenal cortex | 0 | 0 | 4 | 6 |

| IHC staining intensity | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| CTNNB1 mutant ACAs | 12 | 10 | 0 | 0 |
| CTNNB1 WT ACAs | 19 | 19 | 0 | 0 |
| Normal adrenal cortex | 0 | 0 | 5 | 5 |

| IHC staining intensity | 0 | 1 | 2 | 3 |
|---|---|---|---|---|
| CTNNB1 mutant ACAs | 11 | 11 | 0 | 0 |
| CTNNB1 WT ACAs | 18 | 20 | 0 | 0 |
| Normal adrenal cortex | 0 | 0 | 4 | 6 |

**Supplementary Figure 7. Immunohistochemical staining intensity of *CYP17A1*, *CYP21A2*, *CYP11B1* in normal adrenal cortex and NFACAs with different genotypes by QuPath software.**