Supplementary information, Figure S3

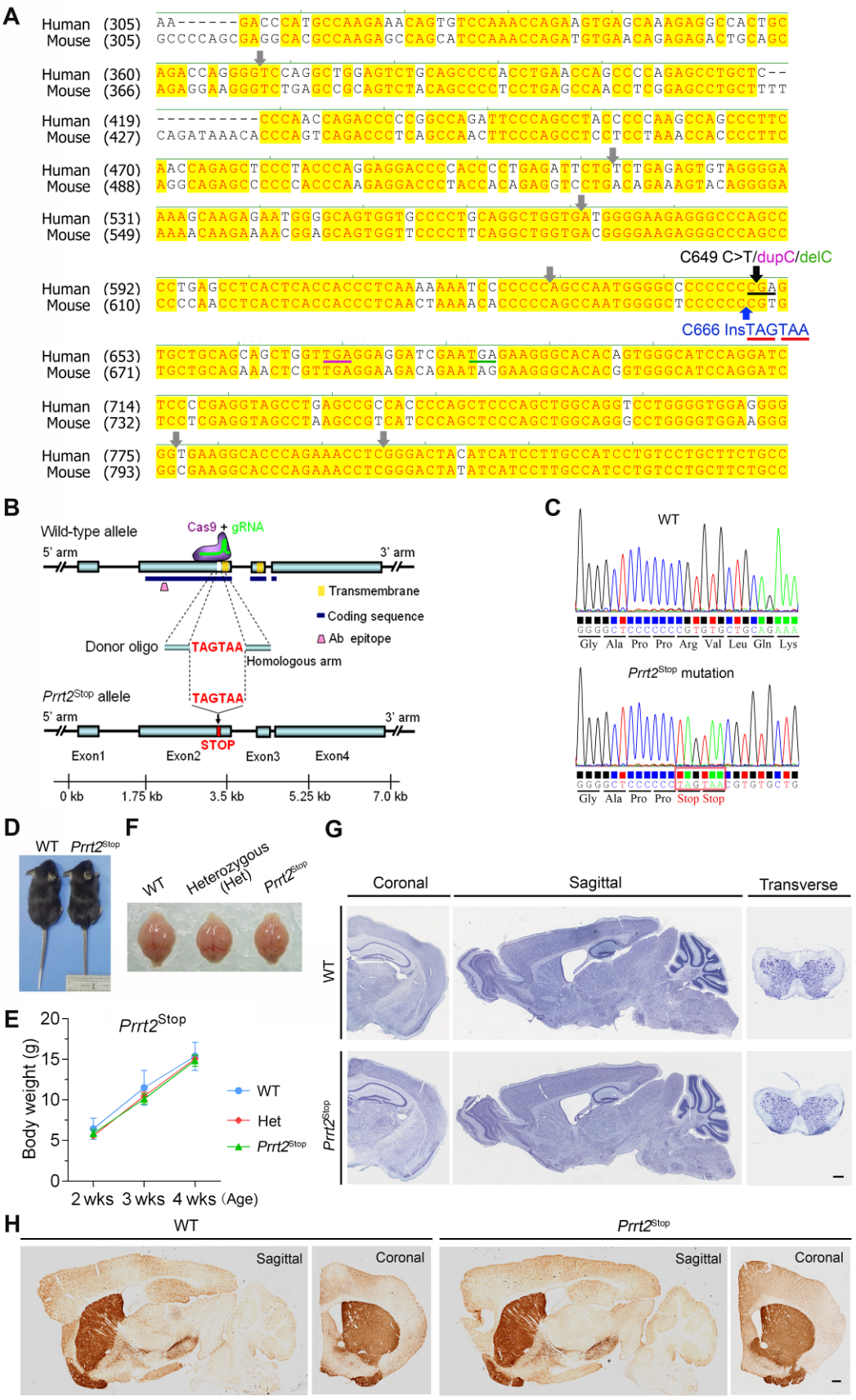

Figure S3 Generation of mutant mice to mimic the hot-spot mutation of human *PRRT2* in patients with PKD. (**A**) Shown is partial sequence alignment of human *PRRT2* (NM_145239.2) and mouse *Prrt2* (NM_001102563.1), indicating their identical overall organization and strong sequence conservation. Identical nucleotide sequences are shaded in yellow. A mutational hotspot (black arrow) of nine cytosines (c.649) in human *PRRT2* was responsible for most PKD cases, introducing a stop codon (underlined by black, pink, green bars, respectively) in advance prior to the sequences coding two transmembrane domains (807-867 and 954-1014, respectively). We thus planned to generate *Prrt2*-mutant mice with insertion of two stop codons (red bars) into C666 sites (blue arrow) of murine *Prrt2* to mimic the hot-spot nonsense mutation at the C649 site of human *PRRT2* gene in PKD patients. Some of other mutated sites found in PKD patients are indicated by gray arrows. (**B**) Schematic diagrams of generation of mutant mouse with incorporation of two stop codons (TAGTAA) into its endogenous *Prrt2* gene (referred to as $Prrt2^{Stop}$). Cas9/Crispa-mediated gene-targeting strategy was used to insert stop codons into murine *Prrt2*. Nucleotide sequence coding the epitope of specific PRRT2 antibody used in this study is indicated by pink trapezoid box. (**C**) DNA sequencing confirmed the correct incorporation of exogenous nucleotides into the target site of $Prrt2^{Stop}$ mice. (**D**) No obvious changes in gross morphology of the mutant mice. (**E**) No significant changes in body weight (n=7, 14, 8 for WT, heterozygous and homozygous mice, respectively) of the mutant mice. Error bars, mean ± SEM. (**F**) Comparison of brains of mutant mice and their control littermates at 1 month of age. WT, wild-type; Het, heterozygous. (**G**) Nissl staining of sections from the mouse brains (left panel, coronal; middle panel, sagittal) and spinal cord (right panel) with the indicated genotypes showed that the gross anatomic structure of central nervous system was not apparently altered in $Prrt2^{Stop}$ mutant mice. Scale bars: 600 μm, for brain; 300 μm, for spinal cord. (**H**) Tyrosine hydroxylase (TH) staining of dopaminergic neurons in the substantia nigra and terminals in the striatum showed comparable patterns in both WT and $Prrt2^{Stop}$ mice. Scale bar, 400 μm.