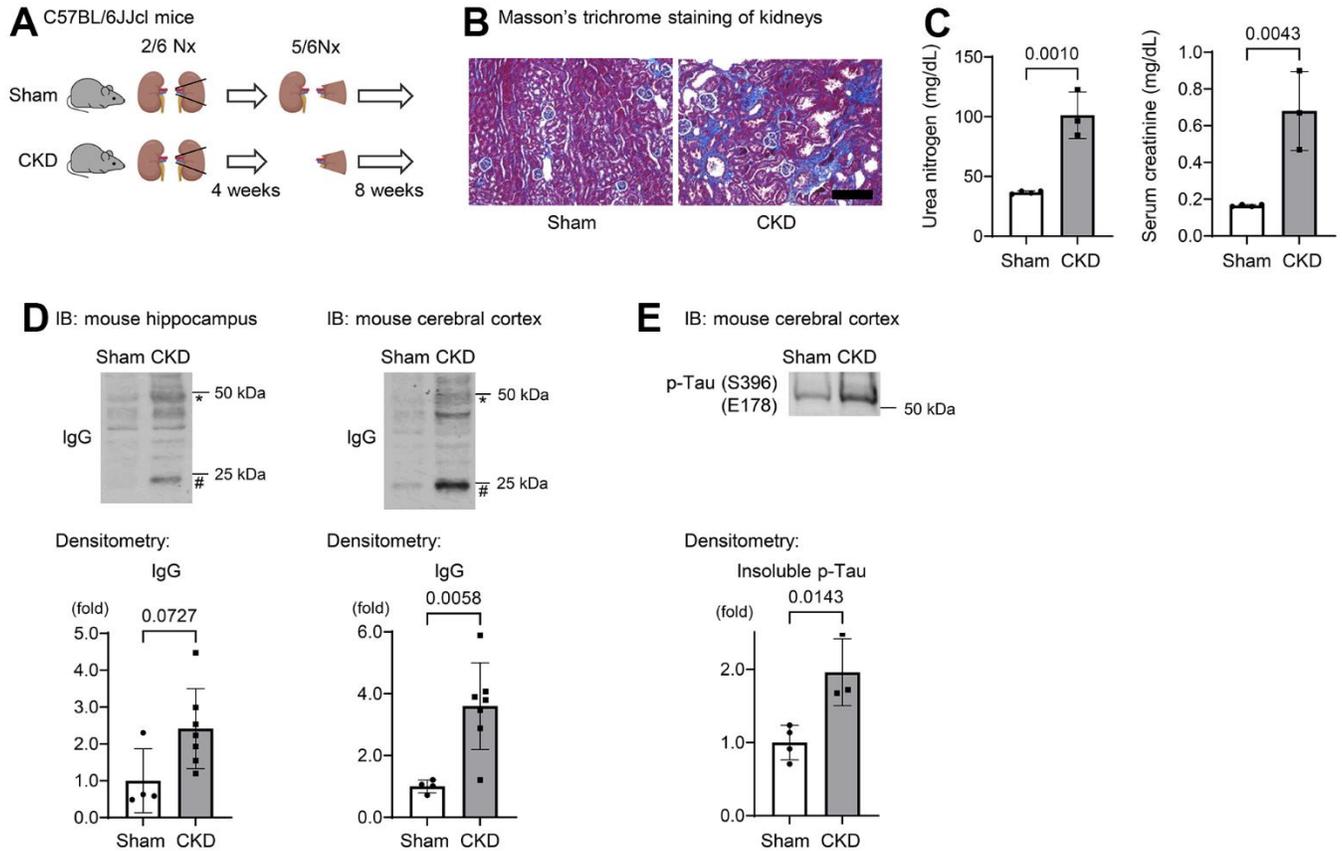
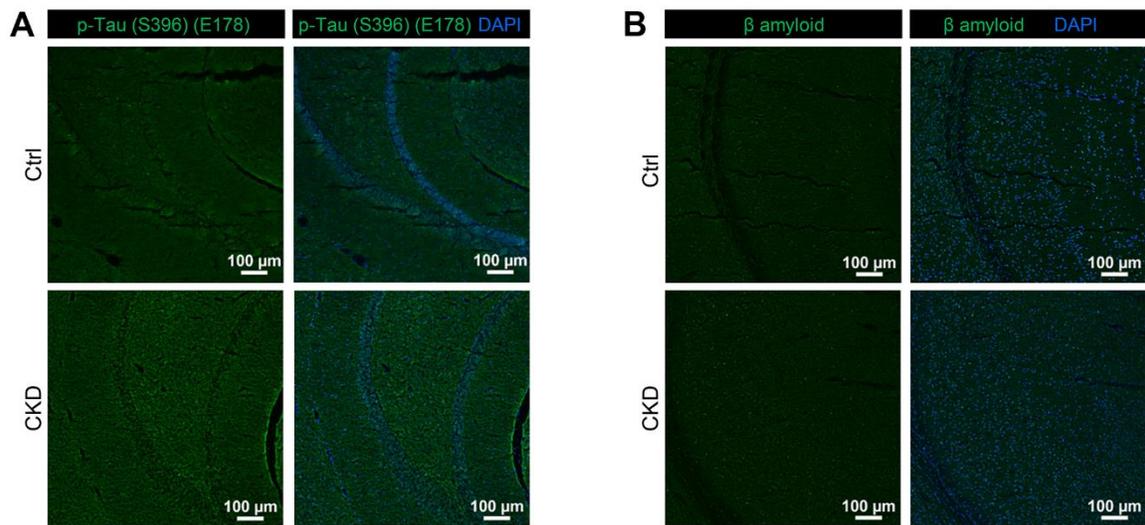


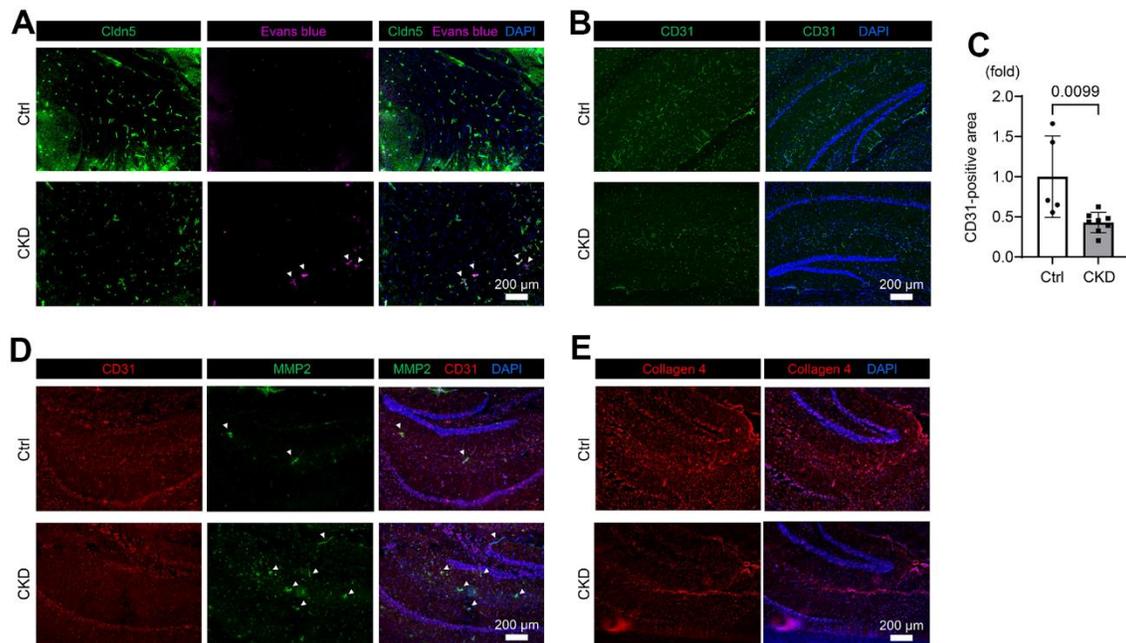
SUPPLEMENTARY FIGURES



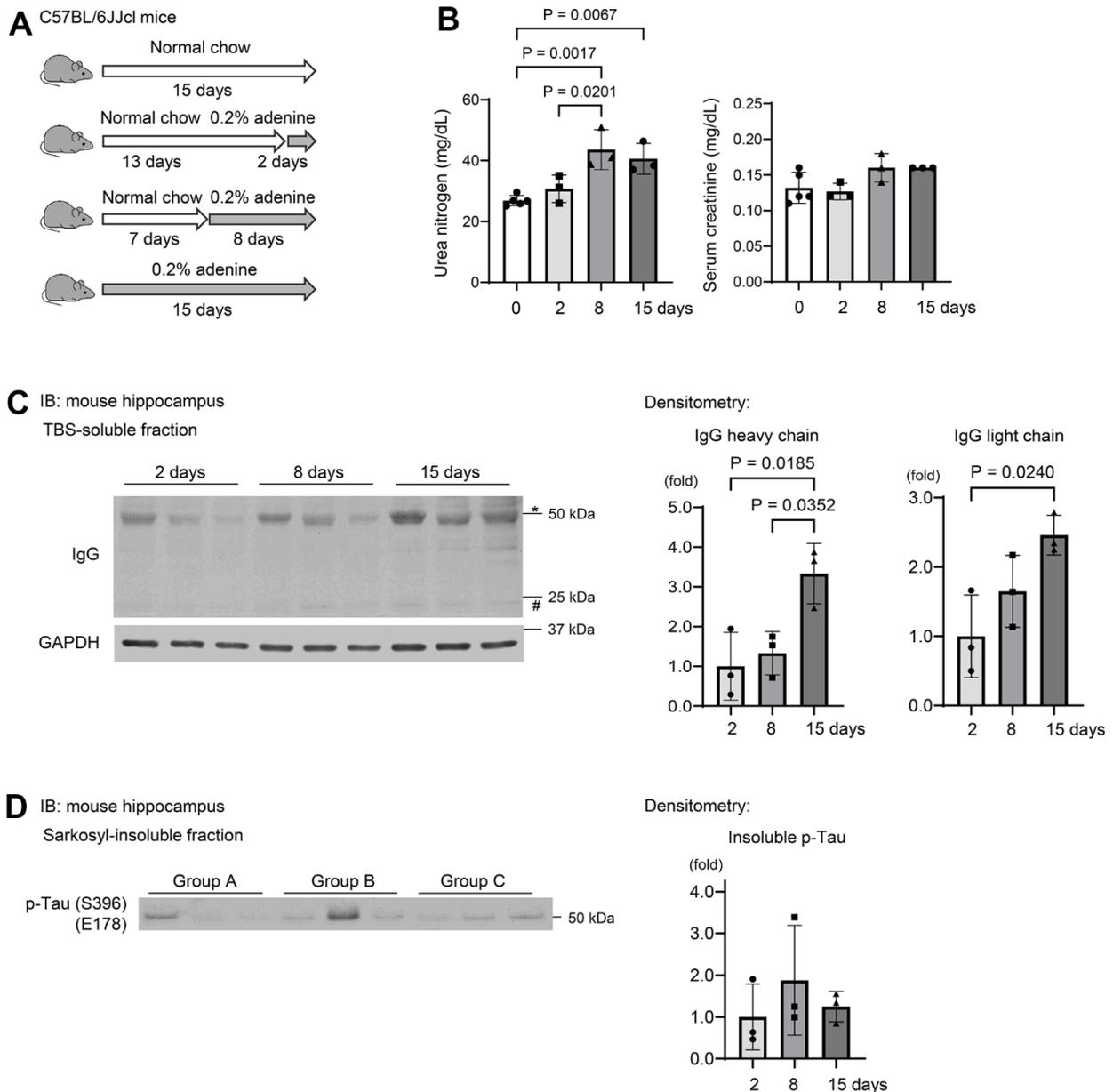
Supplementary Figure 1. A model of CKD established by a 5/6 nephrectomy causes a BBB breakdown and the accumulation of insoluble tau. (A) Establishment of a CKD model in mice by 5/6 nephrectomy in C57BL/6J mice. (B) Masson's Trichrome staining showed tubulointerstitial fibrosis in the kidneys after 5/6 nephrectomy. Scale bar = 100 μ m. (C) Serum urea nitrogen and creatinine, indicating uremic solutes, were elevated in CKD ($n = 4$ in the sham-operation group; $n = 3$ in the CKD group). (D) Western blotting showed CKD increased expressions of IgG in hippocampus and neocortex tissues of CKD mice ($n = 4$ in the sham-operation group; $n = 7$ in the CKD group). (E) Phosphorylated tau in the sarkosyl-insoluble (aggregated) fraction in CKD mice was elevated compared with the control group ($n = 4$ in the sham-operation group; $n = 3$ in the CKD group). Data are presented as mean \pm standard deviation of the mean. Normality was assessed with the Shapiro–Wilk test. Statistical significance between the two groups was evaluated using an unpaired t test or Wilcoxon signed-rank test. When variables were nonparametric, we used the Wilcoxon signed-rank test. $P < 0.05$ was considered statistically significant. CKD, chronic kidney disease.



Supplementary Figure 2. Phosphorylated tau and β amyloid in the hippocampus tissue of CKD mice and control mice. (A) Immunofluorescence staining revealed no obvious phosphorylated tau deposits in the hippocampus tissue of CKD model mice and control mice. (B) Immunofluorescence staining revealed no depositions of β amyloid in the hippocampus of CKD mice or control mice. CKD, chronic kidney disease.



Supplementary Figure 3. The downregulations of tight junction, adherens-junction, and basement membrane proteins in the CKD mouse brain. (A) Immunofluorescence indicating the staining pattern of claudin-5 in the hippocampus tissue of CKD mice compared to the control group. Leakage of Evans blue fluorescence was only seen in CKD mice (white arrowhead). (B) Immunofluorescence study showing that the adherens-junction protein platelet/endothelial cell adhesion molecule-1 (PECAM-1)/CD31 expression was decreased in the hippocampus of CKD mice compared to the control group. (C) CD-31-positive area was quantified and was found decreased in CKD ($n = 5$ in the control group; $n = 8$ in the CKD group). (D) Immunofluorescence study showing that the protein expression of MMP2 (white arrowhead) was increased in the hippocampus tissue of CKD mice, in contrast to the decreased CD31 expression. (E) Immunofluorescence study showing that the expression of collagen IV, a substrate of MMP2, a known collagenase that is a key protein of the basement membrane, was suppressed in the CKD hippocampus tissues. Data are presented as mean \pm standard deviation of the mean. Normality was assessed with the Shapiro–Wilk test. Statistical significance between the two groups was evaluated using an unpaired t test. $P < 0.05$ was considered statistically significant. CKD, chronic kidney disease; MMP2, matrix metalloproteinase-2.



Supplementary Figure 4. A leakage of IgG to the mouse hippocampus following elevated serum urea preceded the accumulation of insoluble tau in kidney impairment. (A) Short-term kidney impairment models in mice by providing wild-type C57BL/6J mice a diet containing 0.20% adenine with different allocation periods (2 days, 8 days, and 15 days). (B) Serum urea nitrogen and serum creatinine levels in short-term kidney impairment models ($n = 3$ per group). (C) Western blotting showed the increased soluble IgG heavy and light chains of mouse hippocampus 15 days after initiating 0.20%-adenine diet ($n = 3$ per group). * represents IgG heavy chain, and # represents the IgG light chain. (D) Insoluble phosphorylated tau levels were not increased during the time course ($n = 3$ per group). Data are presented as mean \pm standard deviation of the mean. Statistical analyses among multiple groups were performed using one-way ANOVA, followed by Turkey's post-hoc test. $P < 0.05$ was considered statistically significant.