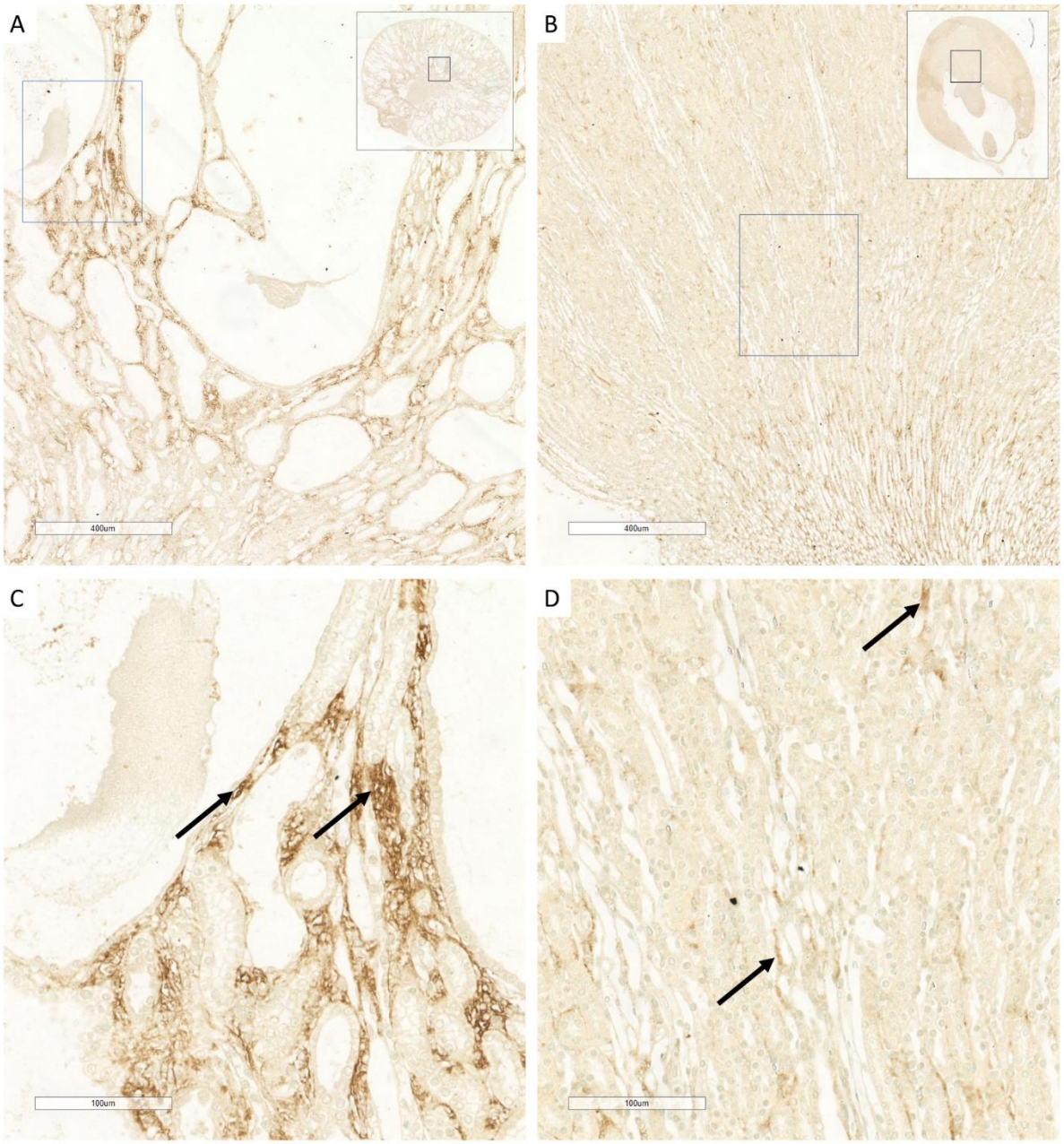
Supplementary Methods

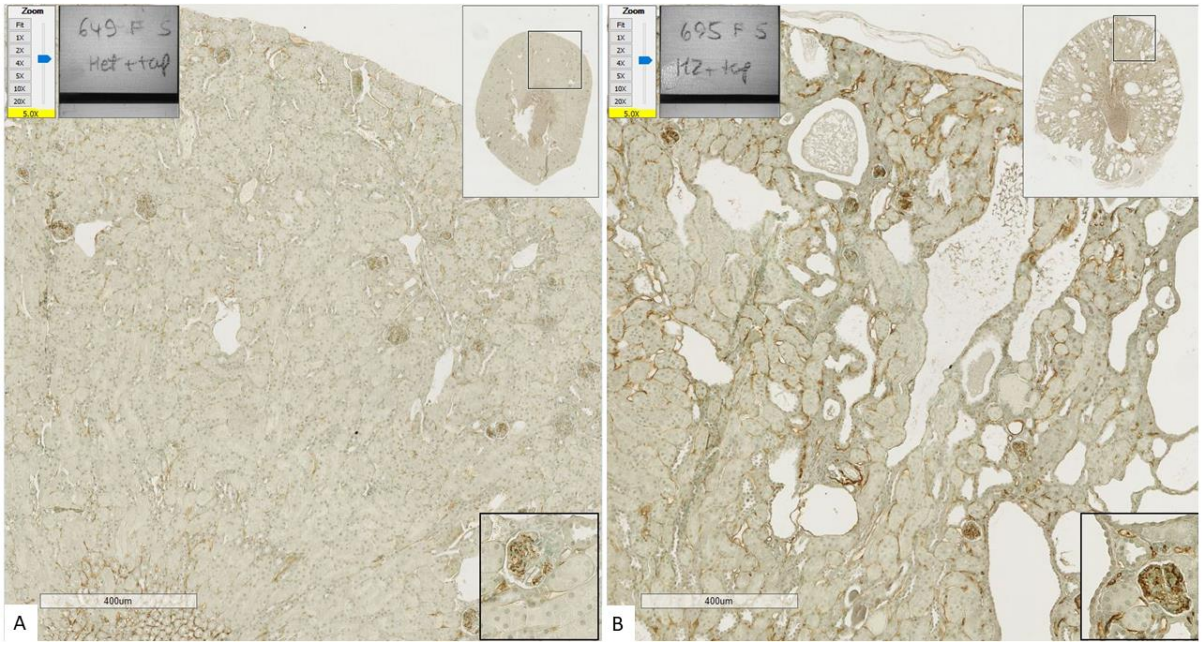
Histology and Immunohistochemistry

Kidneys obtained at the time of sacrifice were fixed in either 10% neutral buffered formalin or methyl Carnoy's (MC) solution and then embedded in paraffin blocks. Six micron thick formalin kidney sections obtained from the jck mouse studies were stained with Masson's trichrome to determine the extent of interstitial fibrosis. In order to visualize the renal microvasculature, primary antibodies targeted at antigens located in the vascular endothelium were used. For rat tissue, this was monoclonal mouse anti-rat RECA-1, IgG1 isotype (Serotec, UK); and for mouse tissue this was monoclonal rat anti-mouse CD34 [MEC 14.7], isotype IgG2a (Abcam). Six micron (jck mice) and four micron thick (LPK rats) sections were deparaffinised in Histoclear® (National Diagnostics, Atlanta, GA, USA), rehydrated and then immersed in 3% hydrogen peroxide in methanol to quench peroxidase activity. Non-specific binding was prevented by incubation with Background Buster® (Accurate Chemicals and Scientific Corporation, Westbury, NY, USA). Sections were incubated overnight at 4ºC with the chosen primary antibody and then incubated with a relevant secondary antibody for 30 minutes (listed in Table 2). Da Vinci Green® (Biocare Medical, Concord, CA, USA) was used for dilution of all primary and secondary antibodies. Immunoreactivity was visualized with Vectastain Elite ABC® reagent (Vector Laboratories, Bulingame, CA, USA) and the chromogen 3,3'-diaminobenzidine (DAB; Sigma-Aldrich, Saint Louis, MO, USA). Counterstaining by methyl green (DakoCytomation, Carpentaria, CA, USA) was followed by dehydration and the application of coverslips using Vectamount® (Vector Lab, Burlingame, CA, USA).

Supplementary Figures



Supplementary Figure 1. Representative photomicrograph showing HIF1α staining (shown with arrows) of LPK and Lewis kidney sections at 100X (A, B) and 200X (C, D) respectively.



Supplementary Figure 2. Representative sections of WT (A) and *jck* mice (B) kidneys used for CD34 scoring. Two methods of analysis were used to quantify cortical peritubular capillary density in *jck* and wild type mice. First, quantitative analysis measured the percent area of positive CD34 immunostaining. Ten fields of view (400x) were taken per section and the region of interest (ROI) selected manually using image analysis software to exclude glomerular capillaries and the area occupied by cysts in *jck* mice. The mean percent area per section was then used to calculate the mean percent area per group. Second, cortical peritubular capillary density was measured by blinded, semi-quantitative analysis. Fields of view used in quantitative analysis were graded from 0-4 according the degree of CD34 immunostaining (Table 3). The mean score per section was used to calculate the mean score per group.