SUPPLEMENTAL DIGITAL CONTENT

SUPPLEMENTAL METHODS

Rat kidney transplant procedure

Donor nephrectomy was performed via midline incision and an operating microscope was used to visualize and ligate the ureter of the left kidney, as described previously.[1,2] The distal abdominal aorta was cannulated and the donor kidney was perfused in situ with 3 to 5 mL of 4ºC heparinized saline (1%). The renal artery and vein were ligated distal to the junction of the aorta and vena cava and the kidney was removed en block. The donor kidney was preserved in 4ºC heparinized saline solution (1%) during recipient preparation. Average cold ischemia time for donor surgery was 20 to 30 minutes.

Transplant recipients underwent midline laparotomy for kidney transplantation. Renal vessels were occluded with microvascular clamps and a left native nephrectomy was performed at the time of transplant. The donor kidney was placed in the posterior abdominal cavity of the recipient and anastomosis of donor and recipient renal vasculature and ureters was performed. Average operative time for recipient surgery was 45 to 60 minutes. To avoid graft loss from acute T cell mediated rejection, all recipients were given cyclosporine (1.5 mg/kg/day) via intraperitoneal injection for 10 days. For the remainder of the experimental duration recipients were free of immunosuppression.

Right native nephrectomy was performed 10 days following the kidney transplant and the allograft was visually inspected to exclude hydronephrosis. Specimens from recipients were procured at 3- or 6-month time points after date of transplantation.

Biochemical assays

Blood was collected at 3- and 6-month time points for analysis of serum creatinine and BUN levels. Blood urea nitrogen (BUN) and serum creatinine measurements were determined by automated analysis (VetTest Analyzer Technology).

REFERENCES

1. Panzer SE, Wilson NA, Verhoven BM, et al. Complete B cell deficiency reduces allograft inflammation and intragraft macrophages in a rat kidney transplant model. *Transplantation.* 2018;102(3):396–405.

2. Djamali A, Reese S, Oberley T, et al. Heat shock protein 27 in chronic allograft nephropathy: a local stress response. *Transplantation.* 2005;79(12):1645–1657.

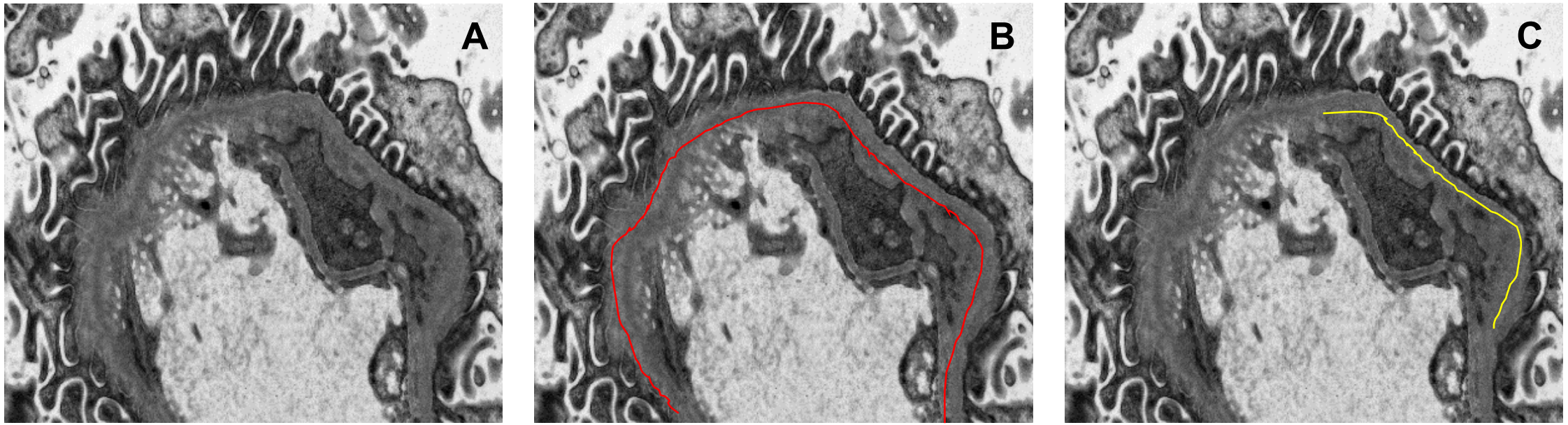

Figure S1

FIGURE S1. Method for determination of the portion of glomerular basement membrane with double contours. A, Representative electron micrograph demonstrating the glomerular capillary loop for assessment of 1) total length of glomerular basement membrane and 2) areas of duplication. B, An electron micrograph showing measurement of the total length of glomerular basement membrane (red line). C, Segment of glomerular basement membrane measured with duplication (yellow line).