**SUPPLEMENTAL FILE – DETAILED METHODS**

*Animals, Cells, Reagents.* Female C57BL/6 mice were purchased from The Jackson Laboratory. Experiments were performed in compliance with federal laws and institutional guidelines. The animal protocol was approved by the Augusta University Institutional Animal Care and Use Committee (no. A3307-01). 12-week-old mice (18–20 g) were used for all experiments. Human glomerular endothelial cells were purchased from Cell Systems, Kirkland, WA and were used up to passage 8. Murine glomerular endothelial cells were isolated from C57BL6 mice, as described[32]. SB203580 was purchased from Sigma-Aldrich. Circular 17-residue TIP peptide, with sequence CGQRETPEGAEAKPWYC[17,20] and mutant TIP peptide, with sequence CGQREAPAGAAAKPWYC[17,20] were purchased from Ambiopharm, North Augusta, SC. Primary antibodies for use in Western blotting were either made in house (anti-ENaC-$\alpha$ and ENaC-$\beta$[20,22]) or were purchased (anti-ENaC-$\gamma$, Abcam; phospho and total p38, NF-$\kappa$B, Cell Signaling). HRP-conjugated secondary antibodies were from Cell Signaling.

*Nephrotoxic Nephritis*. Sheep nephrotoxic serum was prepared and administered as described previously[32,33] using a single dose i.p. (8 $\mu$l/g to allow for progression to autologous phase, 13.5 $\mu$l/g for moderate NTN and 14.5 $\mu$l/g for severe NTN). Mice were injected on days 2, 4 and 6 of NTN with either native TIP peptide (2.5 mg/kg), mutant TIP peptide (2.5 mg/kg) or F1.1-TIP conjugate (10 mg/kg). Mice were then followed with measurement of blood urea nitrogen levels, and kidneys were removed, fixed, processed for microscopy and scored

as described[36,37]. Clinical scores of glomerular injury were graded into five grades: 0 (normal), 1 (mild increase in cellularity), 2 (focal hypercellularity with increase of matrix), 3 (focal hypercellularity and proliferation in >50% of glomeruli), and 4 (diffuse proliferative change with crescents and sclerosis in >50% of glomeruli). Tubulo-interstitial lesions were also graded from 0 to 4 according to the severity of inflammatory cell infiltration.

*Telemetry studies.* Under isoflurane anesthesia, an incision was made in the ventral neck region of mouse. The left carotid artery was isolated, and a subcutaneous tunnel was made on the right side to form a pocket in the mid-scapular region. The catheter tip from a sterile, Data Sciences (PA-C10) transmitter unit was inserted into the carotid artery of mouse, and secured in place with 6-0 silk ties. The transmitter was routed in the subcutaneous pocket. The neck incision was closed with sterile, absorbable 6-0 suture. Mice were transferred to a light- and temperature-controlled room, and were housed individually in standard mouse cages with tap water and rodent chow available ad libitum. Animals recovered from surgery for 5–7 days before baseline control measurements were made. After three days of recordings, animals in the standard chow groups were injected i.p. with NTS or saline. TIP peptide (2.5 mg/kg) or vehicle were administered i.p. on days 1, 3 and 5 of NTN. In the studies under high salt conditions, MAP was recorded in mice under standard chow diet (0.4% sodium, Harlan-for 3 days, and then diet was switched to 4% high salt (Harlan, n=3 per group). In these mice, TIP peptide was given on days

10, 12, 14 and 16 of high salt diet. NTS was given on day 10 of salt diet, followed by saline or TIP peptide injections on days 1, 3, 5 and 7 post NTS. Blood pressure was recorded for two weeks before NTS treatment, and two weeks after NTS treatment (provided mice died before), for a maximum of four weeks.

*Immunohistochemistry and Immunofluorescence. F4/80, CD11b, GR-1 and synaptopodin staining.* Frozen kidney sections were thawed at RT, fixed with ice-cold acetone for 6 min, air dried for 15 min and twice re-hydrated for 5 min in PBS. Subsequently, sections were blocked with 1% BSA for 35 min, stained with anti-mouse F4/80 FITC (eBioscience), CD11b FITC and anti-GR-1 PE (Miltenyi Biotec) for 2h at RT. Counterstaining was done with DAPI followed by mounting with Vectashield (Vector). Images were acquired using a Zeiss 780 Confocal Microscope. Positive cells were counted manually at randomly selected non-overlapping fields at 20x magnification for all samples. Synaptopodin expression in isolated glomeruli was assessed using antibodies from Santa Cruz Biotechnology[32]. *CD3 and IL-17A staining.* Mouse kidneys were dissected, fixed in 4% paraformaldehyde, dehydrated through a graded series of ethanol, embedded in paraffin, sectioned (5 mm), and mounted on glass slides, which were then de-paraffinized and rehydrated. Rehydrated kidney sections were subjected to antigen retrieval followed by blocking with 2% BSA in phosphate-buffered saline. Sections were incubated overnight with primary antibodies specifically against CD3 (1:200) and IL-17A (1:20). Subsequently, sections were washed three times in PBS, incubated with appropriate secondary antibodies,

and washed five times with PBS again. For immunohistochemistry, signals were visualized using VECTASTAIN ABC kits (Vector), followed by counterstaining with hematoxylin and capturing images using a CX31 microscope with a DP73-1-51 digital camera (Olympus). Frequency of CD3+ cells was quantified by counting 10 high-power fields per kidney (magnification, x400), which were randomly selected. Since the mouse number for each group was 4, in total 40 images from each group were compared between 3 groups of mice. For immunofluorescence staining, nuclei were counterstained with DAPI (4,6-diamidino-2-phenylindole). Images were captured using an Olympus D73 fluorescence microscope (Tokyo, Japan) and Exi-blue digital camera.

*Depletion of ENaC-$\alpha$ in HGEC.* HGEC (Cell Systems, Kirkland, WA) were transfected at 70-80 % confluence with 25-50 nM final concentration of Silencer® Select Pre-Designed 21 nt siRNAs (Ambion, Grand Island, NY), using Lipofectamine® RNAiMAX transfection reagent in order to knock down ENaC-$\alpha$ gene expression. A non-specific, non-targeting siRNA from the same manufacturer was used as control. Cells were used for further experiments at 48h post transfection.

*$PGE_2$ measurements*. hGECs (Cell Systems, Kirkland, WA) (passage 5 - 8) were seeded into 24-well plates until confluent, washed twice with PBS and equilibrated for 2h in CSC medium (Cell Systems). Medium was subsequently replaced with 0.5 ml fresh $CO_2$-equilibrated serum-free CSC medium. Pre-

treatment was performed by adding TIP peptide (50 µg/ml) or the p38 inhibitor SB (15 min), followed by hTNF treatment (1 ng/ml) for 30 min. 200 µl of supernatant was collected and centrifuged (1,500 rpm x 10', 4$^{o}$C). $PGE_2$ levels were measured using an ELISA kit (Enzo Life Sciences, Farmingdale, NY).

*Nitric oxide (NO) measurements. W*e analyzed nitrite ($NO^{-}_{2}$) in culture medium supernatants using NO-specific chemiluminescence. In brief, samples containing $NO^{-}_{2}$ were refluxed in glacial acetic acid containing sodium iodide. $NO^{-}_{2}$ is quantitatively reduced to NO under these conditions, and then quantified by a chemiluminescence detector after reaction with ozone in a NO analyzer (Sievers)[19].