Supplemental Information

METHODS

Animals and domoic acid exposure. All studies involving animals were done in accordance with the institutional guidelines of the Medical University of South Carolina. For tissue distribution studies, adult 128Sv/Black Swiss mice were exposed to domoic acid by intraperitoneal injection at a dose of 2.5 mg/kg body weight (DA; 2.5 mg/kg; i.p.), and were euthanized 30, 60, or 120 min after exposure. Kidneys, liver, heart, hippocampus, and serum were harvested at each time point to measure DA concentration. For toxicity studies, mice were exposed to a single dose of DA for three consecutive days (0.1-2.5 mg/kg; ip) or an equal volume of diluents (isotonic saline), and were euthanized 24 h after the third dose. During toxicity studies, mice were housed in metabolic cages overnight following the initial dose and again following the third dose for urine collection and analysis. For immediate response gene expression studies, mice were exposed to a single dose of DA (0.0005-2.5 mg/kg; ip), and were euthanized 30 min after injection, and kidneys and hippocampus were harvested and flash frozen. In probenecid (PBN) pretreatment studies, mice were given PBN (600 mg/kg; i.p.) or vehicle (saline) five minutes prior to DA (2.5 mg/kg, i.p.). Mice were euthanized 30 min after DA injection and organs were collected for DA measurements. For kynurenic acid (KYNA) pretreatment studies, mice were injected with KYNA (12.5 mg/kg; i.p.) or vehicle (isotonic saline) five minutes prior to DA (2.5 mg/kg; i.p.). Mice were euthanized 30 min after DA injection, and kidneys were harvested and flash frozen for RNA processing.

Domoic acid measurements. DA concentration in tissues was measured by ELISA (Mercury Science, Durham, NC). Extraction of DA was performed using a modified version of a previously described protocol, which was adapted for use in mouse tissues [48]. Briefly, flash frozen tissue sections were lysed in 10X (w:v) 50% methanol using a pestle followed by sonication. The lysates were centrifuged for 20 min at 10,000 X g, and supernatant was taken for

analysis. The ELISA was performed according to the manufacturer's instructions.

RNA isolation and RT-PCR. RNA was obtained using an RNeasy RNA isolation kit (Qiagen) according to manufacturer's protocol. 1 ug of total RNA was converted to cDNA using an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). cDNA was diluted 10-fold with nuclease-free water prior to PCR reactions. For RT-PCR experiments, cDNA was combined with GoTaq Green PCR Master Mix (Promega, Madison, WI) and 400 nM each forward and reverse primers. The primer sequences for the receptor subtypes that were examined in this study have been previously reported [49], and were obtained from Integrated DNA Technologies (IDT, Coralville, IA). For qRT-PCR experiments, cDNA was combined with SsoAdvanced SYBR Green PCR Mix (Bio-Rad, Hercules, CA) and 400 nM each primer. The primer sequences for *c-fos*, *junb*, and *tubulin* were previously reported [33] and were obtained from IDT. For qRT-PCR experiments, reactions were subjected to 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 30 s at 55 °C, in an Eppendorf Mastercycler Realplex 4s (Hauppauge, NY).

Kidney function and injury measurements. Terminal blood was collected by venepuncture of the inferior vena cava at time of euthanasia. Serum was extracted from collected blood and used to measure creatinine (QuantiChrom Creatinine Assay Kit; BioAssay Systems, Hayward, CA) and cystatin c (Quantikine Cystatin C ELISA; R&D Systems, Indianapolis, IN). Urine collected overnight following the first and third injections was used to measure kidney injury molecule-1 (Quantikine KIM-1 ELISA; R&D Systems, Indianapolis, IN), neutrophil gelatinase-associated lipocalin (NGAL; Bioporto, Denmark), total protein (BCA assay), albumin (QuantiChrom Albumin Assay Kit; BioAssay Systems), and cystatin c.

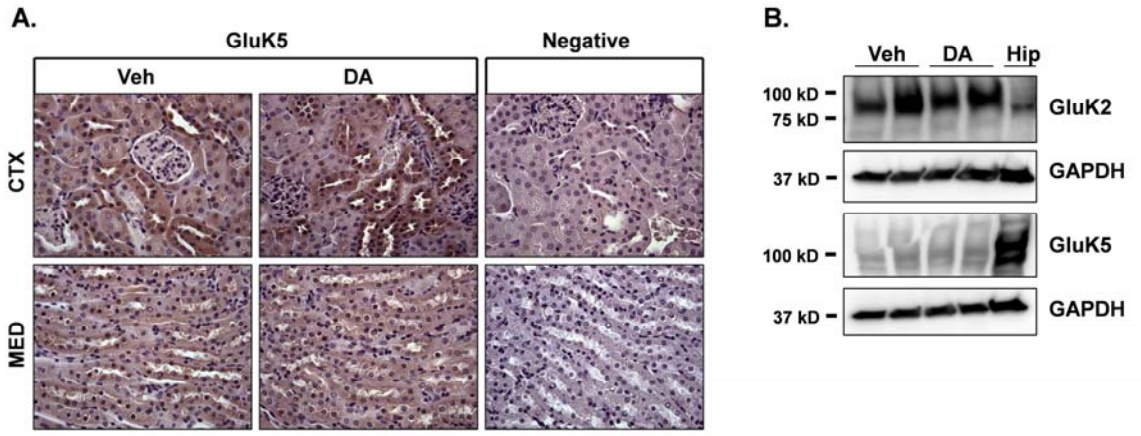
Histology and immunohistochemistry (IHC). Mice were exposed to DA for three days. At time of euthanasia, organs were immersion-fixed in 10% formalin and paraffin-embedded.

Paraffin sections were then processed and stained with hematoxylin and eosin or Masson trichrome stain for histological analysis. GluK2 and GluK5 IHC was performed by dewaxing the sections in xylenes and rehydration in a graded ethanol series. The sections were subjected to heat-mediated antigen retrieval in citrate buffer, followed by blocking in 5% normal goat serum, and incubation in primary antibody (Rabbit anti-GluK5, Abcam; Rabbit anti-GluK2, Aviva Biosystems; Rabbit anti-NGAL, Abcam) overnight at 4 °C. Following antibody incubation, the sections were incubated in biotinylated secondary antibody, HRP-linked avidin-biotin complex, and developed with diaminobenzidine (DAB) exposure using a Vectastain ABC kit (Vector Labs). The sections were counterstained with hematoxylin, dehydrated in ethanol and xylenes, and mounted with permount (Vector Labs).

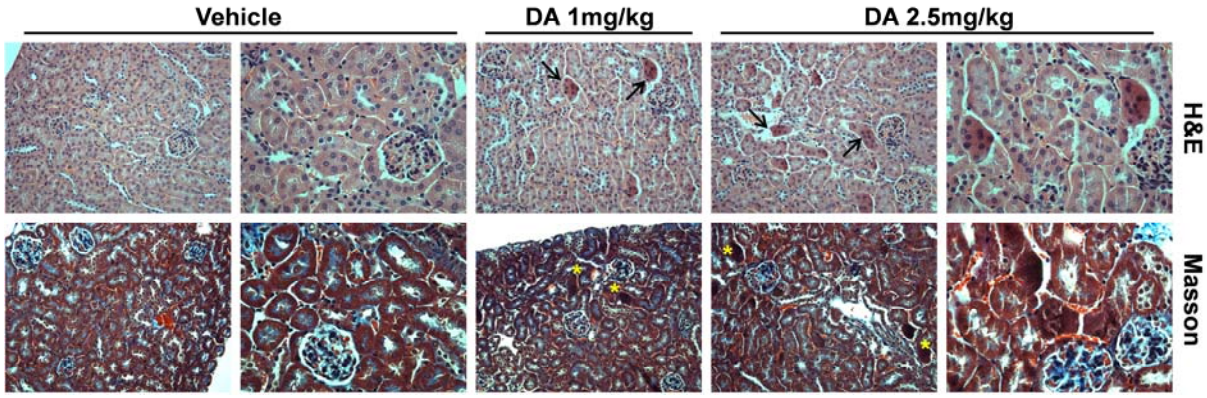
Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Parrafin-embedded kidney sections from mice treated for 72 h with 1.0 or 2.5 mg/kg DA were used for detection of apoptosis by TUNEL assay. The assay was performed using an ApopTag® Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, MA) according to the manufacturer's instructions, and developed with diaminobenzidine (DAB). Negative control sections were incubated in buffer without TdT enzyme. Positive control sections were incubated with DNase I at the beginning of the protocol.

Transmission electron microscopy (TEM). Following 72 h DA exposure, kidneys were harvested and immediately placed in a dish containing a modified Karnovsky's buffer (2.5% glutaraldehyde/2.5% formalin in PBS). After fixation the specimens were rinsed several times with sodium cacodylate buffer pH 7.4 0.1M followed by post fixation with 1% osmium tetroxide in cacodylate buffer for one hour in dark. After rinsing again with Cacodylate buffer, the specimens were dehydrated through a series of graded ethyl alcohols from 50 to 100%. After dehydration the infiltration process requires steps through an intermediate solvent, 2 changes of

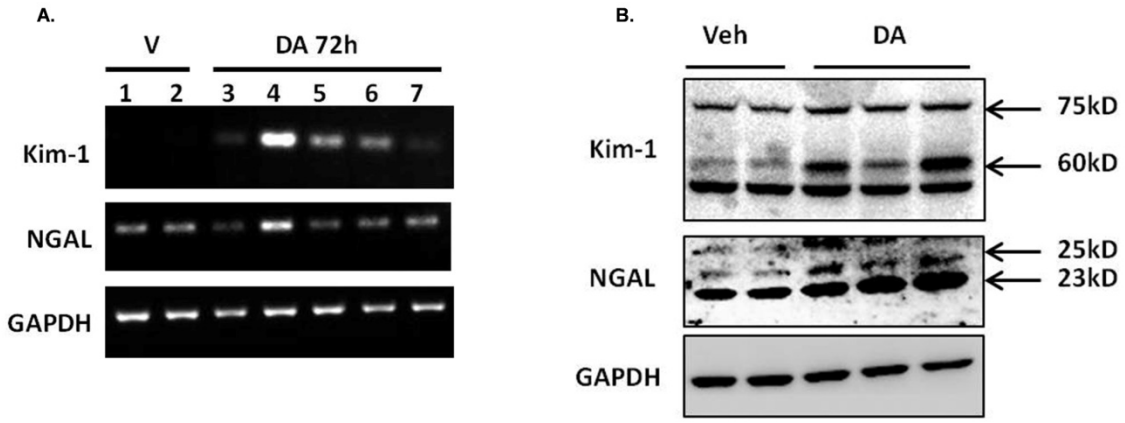
100% propylene oxide (P.O.) for 10 minutes each and finally into a 50:50 mixture of P.O. and the embedding resin (Embed 812, Electron Microscopy Sciences, Hatfield, PA) for 12-18 hours. The specimen is transferred to fresh 100% embedding media. The following day the specimen is then embedded in a fresh change of 100% embedding media. Blocks polymerize overnight in a 60 degree C embedding oven and are then ready to section. The resin blocks are first thick sectioned at 1 micron with a histo diamond knife using an Ultracut UCT 7 (Leica, Bannockburn, IL) sections are collected on slides and stained with Toluidine Blue. These sections are used as a reference to trim blocks for thin sectioning. The appropriate blocks are then thin sectioned using a diamond knife (Diatome, Electron Microscopy Sciences, Hatfield, PA) at 70-90nm (silver to pale gold using color interference) and sections are then placed on copper grids. After drying, the sections are stained with the heavy metals uranyl acetate and lead citrate for contrast. After drying the grids are then viewed on a Tecnai Spirit 120kv TEM (FEI, Hillsboro, OR). Digital images are taken with an AMT CCD camera.



Supplemental Figure 1. Kainate receptor expression in the kidney. (A) GluK5 expression was examined by IHC in the cortex and medulla of mice treated with DA (2.5 mg/kg, 72 h) or vehicle. (B) GluK2 and GluK5 protein expression was examined by immunoblot analysis in kidneys from vehicle- and DA-treated mice at 72 h. Hippocampal lysates (Hip) were used for positive control.



Supplemental Figure 2. Renal histopathology after DA exposure. Renal histopathology was examined by H&E and Masson trichrome stains at 72 h after exposure to either 1.0 or 2.5 mg/kg DA. Note intense hypereosinophilic tubules after either dose (arrows) in the H&E sections. Similar structures were also noticeable in Masson trichrome sections at both doses (asterisks).



Supplemental Figure 3. KIM-1 and NGAL mRNA and protein expression in kidneys after DA. (A) KIM-1 and NGAL mRNA abundance was examined by RT-PCR in kidneys from vehicle- and DA-treated mice 72 h after DA administration. (B) KIM-1 and NGAL protein expression was examined by immunoblot analysis in kidneys from vehicle- and DA-treated mice 72 h after DA administration. Several bands were detected in the KIM-1 blots corresponding to different glycosylated forms of the protein. In NGAL blots, the 25 kD band corresponds to the form that is upregulated after ischemia-reperfusion injury.