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Supplementary Materials and Methods:

Carotid artery injury model of vascular thrombosis:

For all animal models, following induction of CKD with an 0.25% adenine diet, thrombosis was analyzed using the carotid artery injury model of vascular thrombosis, as previously described[1]. Briefly, the right carotid artery was exposed following anesthesia with isoflurane. Baseline blood flow was recorded using a 0.5PSB S-Series flow probe connected to a TS420 perivascular transit-time flow meter (Transonic; Ithaca, NY.). The probe was removed and damage to the artery was performed by placing a piece of Whatman filter paper (1x3mm) soaked in 5% ferric chloride ($FeCl_3$) under it for 1 min. Following washing with physiological saline (0.9% sodium chloride, 37°C) blood flow was monitored and TTO was determined as the first measurement of blood flow <0.299 mL/min. If no clot developed, the assay was terminated after 15 min. Animals were sacrificed by cervical dislocation following blood collection from the carotid artery into heparin coated tubes. Animals were perfused with an excess of ice cold 1x PBS. Tissues were harvested and preserved in either 10% formalin or snap frozen on liquid nitrogen for subsequent analysis.

Animal tissue lysis and Immunoblot:

10-15mg of aorta was lyzed in 2% sodium dodecyl sulfate (SDS) with 5mM EDTA and 1mM sodium orthovanadate (Tissue lysis buffer). Tissue was mechanically homogenized and lysed using sonication with insoluble debris removed by centrifugation for 15min (13,000 RPM at 4°C). The supernatant was removed and protein concentration determined using a BCA protein assay (Pierce, cat# 23227) Lysates were resolved by electrophoresis on a 10% agarose gel before transferring to a 0.2µm nitrocellulose membrane (Bio-Rad, cat# 1620112) (1hr at 4°C). Membranes were cut and blocked in 5% non-fat milk for 1hr at room temperature (RT). Blots were incubated overnight at 4°C with primary antibodies (Table 1). GAPDH served as a loading control for all immunoblots. Following washing with 1x

TBS-T (3x, 10min), blots were incubated at RT for 1hr with an appropriately horseradish peroxidase (HRP) labeled secondary antibody and developed using Western HRP substrate (Millipore, cat# WBLUC0500). In the experiments with different groups and several samples in each group, all the samples could not be included on a single gel, which represents a limitation. However, all the bands were normalized to the loading control such as GAPDH or actin.

Immunohistochemistry:

Following sacrifice and perfusion of indoxyl sulfate treated animals, sections of the aorta from each mouse were fixed in 10% formalin, overnight, at 4°C before being processed and embedded in paraffin. 5 µM sections were deparaffinized in xylenes and rehydrated in decreasing concentrations of ethanol before antigen retrieval with a sodium citrate buffer with high heat for 20 mins. Following antigen retrieval, endogenous peroxidase was blocked and slides subsequently washed 2x in 1x TBS-T for 5mins. After blocking for 30mins with a serum free protein block, sections were incubated overnight with an anti-IDO antibody (Supplementary Table 1) at 4°C. Sections were washed 2x in 1x TBS-T before incubating with an appropriate horseradish peroxide (HRP) labeled secondary antibody. The color reaction was monitored under a microscope during incubation with 3, 3'-Diaminobenzidine (DAB), with the reaction stopped by incubating the sections in water. Sections were counterstained in hematoxylin, then dehydrated in increasing concentrations of ethanol and cleared in xylenes before mounting. Sections were imaged using a Nikon TE 2000 wide-field microscope at 200x magnification. Intensity of IDO-1 signal in the aorta was analyzed using ImageJ analysis by measuring the intensity along a line from the intima through the media layers of the aorta. Five line analyses were done per section for each of the control (n=4) and experimental animals that received indoxyl sulfate (n=5).

Immunofluorescence

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 4μM and subjected to a heat-induced antigen retrieval using 10mM sodium citrate, pH 6.0 for 20mins. Tissue sections were blocked with 1% BSA in 1x PBS containing 0.3% Triton X-100 for 30mins at room temperature. Tissue sections were subsequently incubated with primary antibodies or a corresponding isotype control overnight at 4°C. Following washes in 1x TBS-T, sections were incubated with corresponding Alexa Fluor 488 or 594 conjugated fluorescent secondary antibodies for 1hr at room temperature. Tissue sections were washed in 1x TBS-T before staining nucelli with DAPI, mounted with fluorescence mounting medium and cover slip. All sections were imaged using a Leica SP5 point-scan epifluorescence laser confocal microscope.

IDO-1 inhibitor treatment:

Primary human aortic vascular smooth muscle cells (VSMCs) (P5) were plated at a confluence of 50-60% in 6 well culture plates**.** Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, cat# 11054-020) supplemented with 5% calf sera, glutamine, and penicillin/streptomycin (P/S). VSMCs were serum starved overnight in full media supplemented with 0.5% calf sera. Cells were co-treated with increasing concentrations of indoleamine 2,3-dioxygenase-1 (IDO-1) inhibitors (Table 2) for 24hr with 5% uremic sera. DMSO and 5% sera from control patients served as controls. Cells were lysed in 1x RIPA buffer (Boston Bioproducts, cat# BP-115). Protein concentrations were determined using a Bradford protein assay and insoluble debris pelleted as described above. Proteins were resolved on a 10% agarose gel and transferred to a nitrocellulose membrane as described above before immunoblotting with an antibody raised against human Tissue Factor as described above.

Indoxyl Sulfate-Solute Specific Model

Twelve week old C57/BL6J mice were administered indoxyl sulfate (IS), *ad libitum*, through drinking water (4mg/mL). In order to simulate an increase in the IS similar to seen in patients with CKD,

excretion of IS was inhibited by twice daily injection of the organic anion transporter (OAT) inhibitor, probenecid (150mg/kg, intraperitoneal injection)[2]. Mice were administered this protocol for 4 days before sacrifice and perfusion with 1x ice cold PBS. The aorta was removed and lysed to determine the direct effects of IS on IDO-1 protein expression in endothelial and vascular smooth muscle cells.

Metabolomics/Mass Spec:

Metabolites, indoxyl sulfate and kynurenine, were measured using liquid chromatography/mass spectrometry (LC/MS) as previously described[2]. Briefly, sera samples were diluted 1:8 (sample: solvent) in an 8:1:1 mix of Acetonitrile:Methanol:Acetone and kept on ice for 30 mins to precipitate proteins and lipids. Proteins and lipids were pelleted by centrifugation at 15,000 rcf for 10 mins at <10^{o}C, followed by drying in a speed vacuum and reconstituted in 40µl µL H_20 with 0.1% formic acid and vortexed. Supernatant was transferred to a labeled glass LC vial with glass insert and placed into an Agilent HPLC 1100 series auto sampler.

For negative polarity detection of IS and Kyn a gradient of 95% H2O and 0.1% formic acid (buffer A) and 5% methanol (buffer B) at 0-0.5min, 95% buffer B at 5min, 98% buffer B at 8.5min, and 5% buffer B at 9.0- 10.0min at a flow rate of 0.15ml/min. A LTQ XL (Thermo Scientific) with an ESI source was used in negative mode with first event a full MS scan at 55.0-250.0m/z, isolation width 1.0, mass range normal, scan rate normal and data type profile. Scan event two was set to fragment indoxyl sulfate parent ion 212.0, CID activation with normalized collision energy 50.0, isolation width 3.0, activation Q at 0.25 and activation time of 30.0 ms. For positive polarity detection a gradient of 95% buffer A at 0.00-1.00min, 15% buffer B at 4.00min, 95% buffer B at 7.00-8.00 min, and 5% buffer B at 8.50-10.0min. In positive polarity mode the first full MS scan was at 55.0-500.0 m/z, isolation width 1.0, mass range normal, scan rate normal and data type profile. Scan event two was set to target kynurenine parent ion 209.00 m/z with a scan range of 55.0-225.0 m/z, CID activation with normalized collision energy 50.0,

isolation width 3.0, activation Q at 0.25 and activation time of 30.0 ms. Wideband activation was checked.

Supplementary Figure Legend

Supplementary Figure 1. An adenine supplemented diet results in chronic kidney disease and increased incidence of thrombosis with elevated uremia in mice

A. Experimental design. A group of female C57Bl/6 mice were fed a normal or 0.25% adenine supplemented for 14 days with thrombosis determined using a $FeCl_3$ model of vascular injury on the carotid artery (n=5 mice per group).

B. Histological examination of hematoxylin and eosin (H&E) stained kidneys showed a significant decrease in tubular mass (asterisk) as well as a glomeruli degeneration (arrow) compared to mice on a normal diet (200x magnification, scale bar=50 microns).

C. H&E stained sections from heart and liver were examined from mice after 14 days of a normal or 0.25% adenine supplemented diet. Gross changes to the kidney were observed in mice on an adenine supplemented diet with major findings being tubular degeneration, inflammation, and interstitial fibrosis. There were no gross differences between the heart and liver in mice on a normal diet compared to those on an adenine supplemented diet.

Supplementary Figure 2. Gross pathology of major organs with and without 0.25% adenine supplemented diet

A. Aortic lysates from mice on a normal diet or a 0.25% adenine diet for 14 days were probed with TF. GAPDH served as a loading control.

B. Quantification of TF band normalized to GAPDH was performed using ImageJ. There was a significant 1.65±0.14-fold increase in TF expression in the aorta of mice on an adenine diet for 14 days compared to mice on a normal diet (left, p=0.0301).

C. In mice on a 0.25% adenine diet, time to occlusion (TTO) was significantly decreased (p=0.0411) compared to mice on a normal diet, suggesting that with CKD there is an increase in thrombus formation with vascular injury**.**

Supplementary Figure 3. IDO-1 inhibitors did not affect Tissue Factor Expression in Smooth Muscle Treated with Control Serum

A-B. Primary human vascular smooth muscle cells were treated pooled control human serum obtained from subjected with normal renal function. Cells were treated with 5% control sera along with increasing concentrations of IDO-1 inhibitors. The lysates were probed for TF and HSP-90 served as loading controls. Representative images from three independent experiments are shown.

C-F. Quantification of TF expression was performed using ImageJ and normalized with HSP-90. Data is presented as an average and error bars = SEM.

Supplementary Table 1: Antibodies used and applications

Antibody	Company (Catalog Number)	Species Reactivity	Application	Dilution
GAPDH	Cell Signaling (#2118)	Mouse/Human	WB	1:1000
IDO, Clone 1F8.2	Millipore (#MAB10009)	Human	WB IHC	1:100 1:100
IDO, Clone 4B7	Millipore (#MABF850)	Mouse	WB IHC	1:100 1:100
CD142 (Tissue Factor)	BD Pharmigen (#550252)	Human	WB	1:2500
DYKDDDDK (FLAG)	Cell Signaling (#8146)	Mouse/Human	WB IP	1:1000 1µg/mL
Ubiquitin (P4D1)	Santa Cruz Biotechnology (sc-8017)	Human	WB	1:500

Supplementary Table 2: Competitive inhibitors of Indoleamine 2,3-Dioxygenase-1 (will have to get the references populating these numbers)

Compound	**Company (Catalog Number)**	**IC50**
NLG919	Medchem Express (HY-13983)	7nM
Indoximod	Medchem Express (HY-16724)	7µM
IDO5L	Medchem Express (HY-15683)	67nM
1-Methyl-L-Tryptophan	Enzo Life Sciences (ALX-106-040-M050)	7µM
INCB24360	Axon Medchem (2215)	10nM

Supplementary table 3. Comparison of TtO in mice in different group using Tukey's multiple pairwise comparisons

Group Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		Significance
IDO-$1^{-/-}$ normal diet – IDO-$1^{-/-}$ Adenine	184.54	-46.07	415.16	
IDO-$1^{-/-}$ normal diet – IDO-$1^{+/+}$ normal diet	432.86	202.24	663.47	***
IDO-$1^{-/-}$ normal diet – IDO-$1^{+/+}$ Adenine	691.37	460.75	921.99	***
IDO-$1^{-/-}$ Adenine – IDO-$1^{-/-}$ normal diet	-184.54	-415.16	46.07	
IDO-$1^{-/-}$ Adenine – IDO-$1^{+/+}$ normal diet	248.31	17.70	478.93	***
IDO-$1^{-/-}$ Adenine – IDO-$1^{+/+}$ Adenine	506.83	276.21	737.45	***
IDO-$1^{+/+}$ normal diet – IDO-$1^{-/-}$ normal diet	-432.86	-663.47	-202.24	***
IDO-$1^{+/+}$ normal diet – IDO-$1^{-/-}$ Adenine	-248.31	-478.93	-17.70	***
IDO-$1^{+/+}$ normal diet – IDO-1 WT Adenine	258.51	27.90	489.13	***
IDO-$1^{+/+}$ Adenine – IDO-$1^{-/-}$ normal diet	-691.37	-921.99	-460.75	***
IDO-$1^{+/+}$ Adenine – IDO-$1^{-/-}$ Adenine	-506.83	-737.45	-276.21	***
IDO-$1^{+/+}$ Adenine - IDO-$1^{+/+}$ normal diet	-258.51	-489.13	-27.90	***

*** indicate significant p value.

Supplementary References

1. Matsuura S, Mi R, Koupenova M, et al. Lysyl oxidase is associated with increased thrombosis and platelet reactivity. *Blood*. 2016;127(11):1493-1501.

2. Walker JA, Richards S, Belghasem ME, et al. Temporal and tissue-specific activation of aryl hydrocarbon receptor in discrete mouse models of kidney disease. *Kidney Int*. 2020;97(3):538-550.

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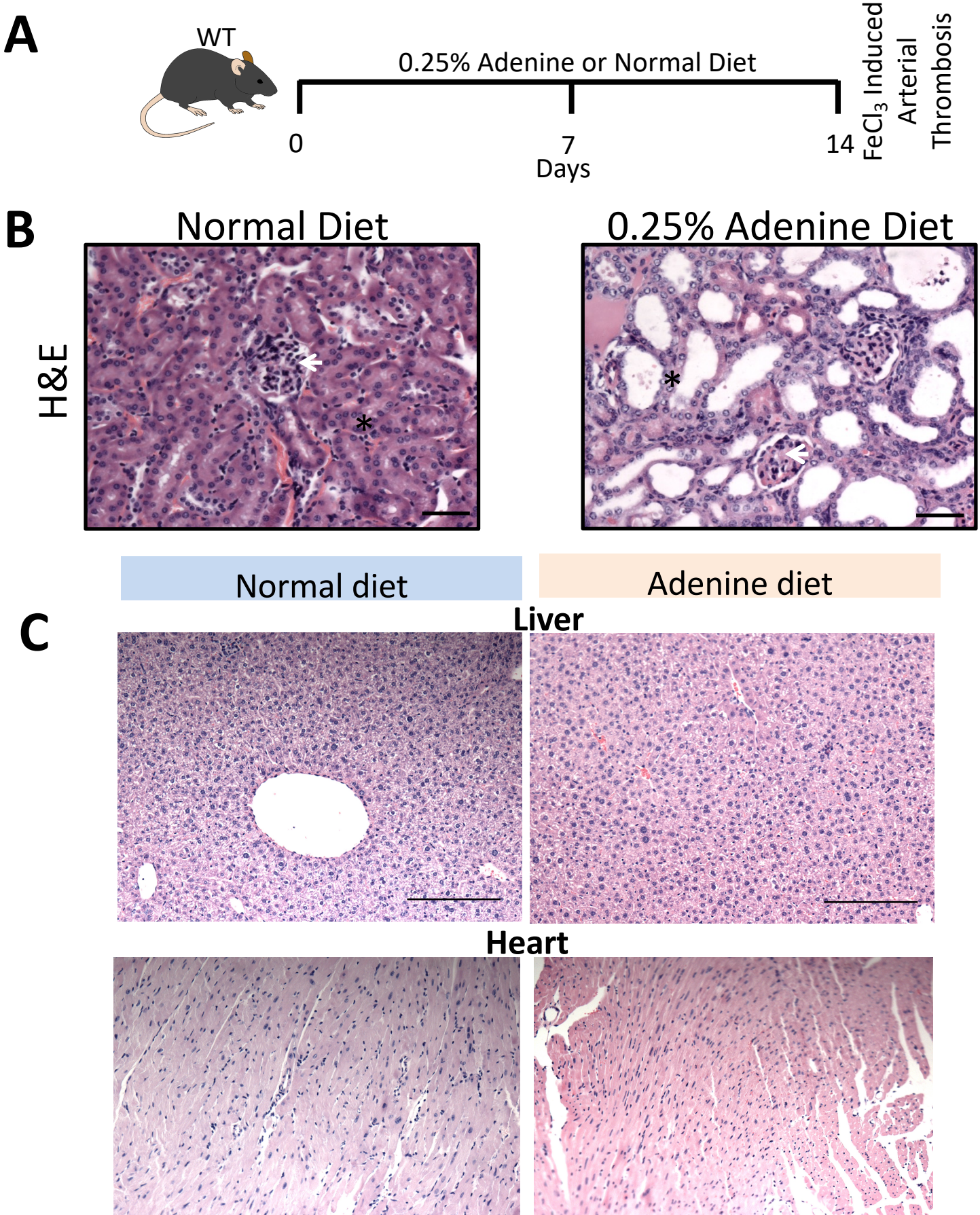
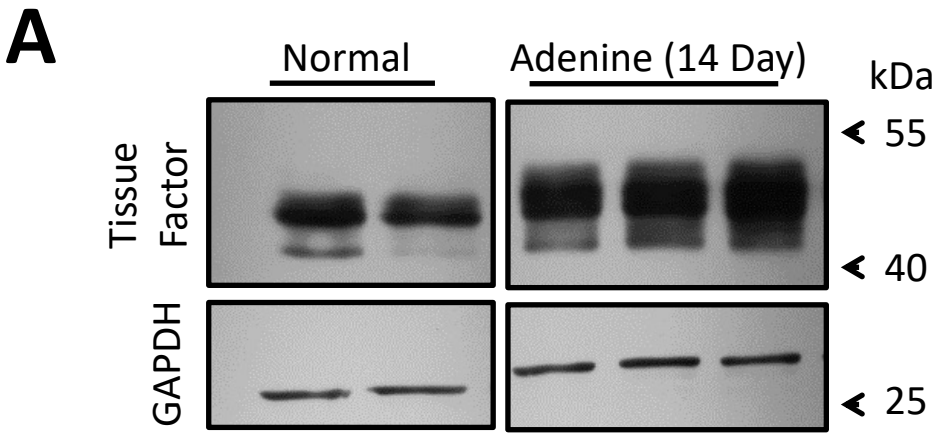
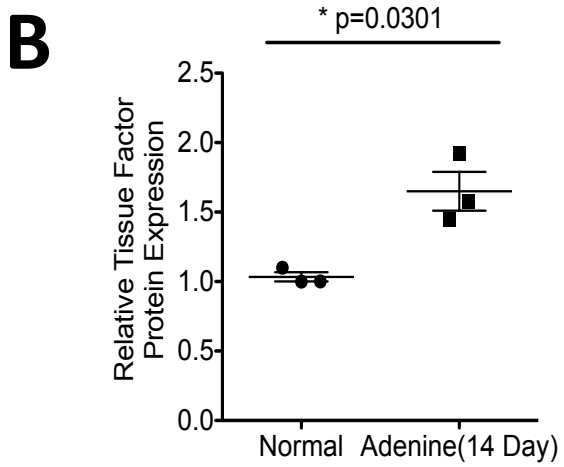
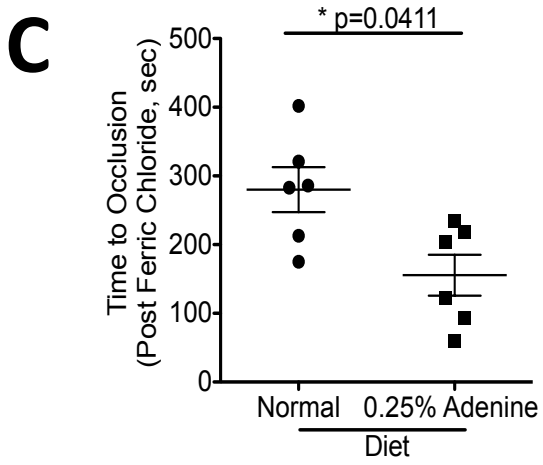
Supplementary Figure 1



Supplementary Figure 2







Supplementary Figure 3