Supplementary methods

Human subjects and serum collection

Uremic sera from patients of ESRD and control were pooled as described previously[8]. Briefly, the patients with ESRD on hemodialysis (HD) were recruited randomly from a pool of 150 patients at the DaVita Hemodialysis Center (Boston, MA). The protocol was approved both by Institutional Review Boards of Boston University Medical Center and Massachusetts Institute of Technology. Informed consents were obtained and 10 ml of blood collected prior to the next HD session. Patients with Hb <8 gm/dl were excluded. Control sera matched for age-, gender- and ethnicity-matched subjects were obtained from Research Blood Component Inc. (Boston, MA). The controls with creatinine more than 1.0 mg/dl were excluded.

LC/MS method validation

Linearity: IS standards in serum were linear in the range of 100 to 500,000 ng/ml during the LC-MS measurement. The linearity of IA and IS were 0.9998 and 0.9996, respectively (fig. S10B).

Lower Limit of Detectability (LLOQ**):** The concentration of IS in serum was diluted to 10, 20, 30, 40, 50, 100, 200, 300, 400, 500 ng/ml. Each sample was injected 6 times. The relative standard deviation was set at 15% for LLOQ. The LLOQ of serum IS is shown (Supplemental Figure 1).

Intra-assay and Inter-assay variation: Three spiked samples were used as quality controls. The value of 6 repeated injections in the same batch was used to calculate intra-assay variation. The samples analyzed in three different days were used to calculate inter-assay variation. The inter-assay and intra-assay variations were CV 5.4% and 0.9%, respectively (Supplemental table 2).

Recovery: Recovery describes the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps. 400, 2000, 8000 ng/ml (final concentration) of IS was added to human serum. The measured concentration was used to calculate recovery. The recovery was 97-100% for IS (Supplemental table 3).

Lysis, immunoblotting, immunoprecipitation and immunofluorescence.

Cell harvest and immunoblotting have been previously described[8]. Monoclonal antibodies specific for tissue factor (Thermoscientific, HTF1), GAPDH (Cell signaling) and AHR, (Pierce-Thermoscientific) were used. The TF bands were normalized to GAPDH and quantified using ImageJ (NIH). Immunoprecipitation using tissue factor antibody (Thermoscientific, HTF1) or AHR antibody followed by agarose (A+G plus) beads (Santa Cruz) were performed as described previously [8]. The vSMCs grown in chamber slides (Lab-Tek) were fixed with methanol and processed as described previously using Leica SP5 confocal microscopy [8]. The MDA-MB231cells were transfected using Lipofectamine 2000 (Life Technologies). For IHC, polyclonal AHR-specific (Sanatcruz) and monoclonal TF-specific (Abcam) antibodies were used at a 1:50 dilution.

TF stability assay, ubiquitination assay

vSMCs treated with 20 μM Emetine for different time intervals were harvested for stability assay. The ubiquitination of TF was examined by pretreating vSMCs with proteasome inhibitor MG132 (Boston Biochem) at 10 μM for 6 hours followed by immunoprecipitation as described above. vSMCs cells lysates treated with uremic serum or solutes for 24 hours using 50 mM Tris buffered saline (pH 8.0) with 1% Triton X-100 and centrifuged at 14,000 g for 20 minutes.

RT-PCR

RNeasy mini kit (Qiagen) was used to extract total RNA from VSMCs. 300ng of total RNA were converted to cDNA using Sensiscript RT kit® (Qiagen) followed by RT-PCR using human TF, *Cyp1a1*, *Cyp1b1* and *Ahrr* primers (Qiagen) and SYBRgreen (Applied Biosystems). *Beta actin* served as a loading control. Levels of mRNA were determined using comparative Ct method.

vSMC injury scratch model:

One million vSMCs seeded on culture plates with 9 mm molded grid were treated with uremic solutes for 24 hours. The cells were then injured with a pipette tip by dragging along the grid. Cells were washed and incubated without uremic solutes for 2 hours prior to harvest.

Immunohistochemistry.

Paraffin-embedded tissue blocks of de-identified patients were processed after IRB approval. Tissue section preparation: Tissue blocks were cut into 7 µm thick tissue sections using a rotary microtome. The sections were allowed to float in a 56° C water bath. Tissue sections were mounted on pre-coated glass slides and dried overnight on heating block.

Immunostaining: Rehydration of the sections involved immersing them in xylene (two times) for 10 minutes each, followed by 100% ethanol (two times) for 3 minutes each, 95% ethanol for 1 minute, and 75% ethanol for 1 minute. The slides were rinsed with water and followed by heat-induced epitope retrieval along with citrate antigen retrieval buffer (ADI-950-270-0500). The slides were washed with distilled water and wiped around the sections to remove water. Tissue sections were marked using a hydrophobic

pencil followed by hydrogen peroxide block for 10 minutes. Further steps were carried out according to the manufacturer's instructions (Abcam-ab94709). AHR- (Santa Cruz SC-5579 and Tissue Factor-specific (Abcam-ab48647) antibodies were used.

Bacloviral protein production and *in vitro* binding assay:

A viral stock of human full length AHR was used to infect Sf9 cells, which were grown in Grace's medium containing 10% fetal bovine serum and 50 ug/ml gentamycin at 28^{o} C for 72 hours. The cells were harvested using Triton lysis buffer (1M Tris HCl, pH=7.6, 0.5M EDTA, 5M NaCl, triton X 100 and glycerol). After homogenization, the lysates were spun at 16000xg for 15 minutes at 4^{0} C. The supernatant was purified for overexpressed AHR using profanity IMAC Ni-charged resin (Biorad Cat #156-0131). The resin was washed with triton lysis buffer with 0.15, 0.2 and 0.3 M NaCl. Purified AHR protein was used in an *in vitro* binding assay with 1500 pM recombinant Tissue factor (HTI- RTI-300). Immunoblotting was performed as described previously (12, 49).

AHR Activity assay

AHR activity was examined in vSMCs using a promoter- reporter plasmid. The time of treatment with different reagents was optimized to obtain the best luciferase signal. vSMCs stably expressing Cignal Lenti Reporter xenobiotic response element tethered to luciferase reporter (XRE-luc) (Qiagen, CLS-9045L-8) seeded at 1000/well in 48 well plate were serum starved using 0.5% CS for 16 hours and treated with IS with or without AHR antagonist for 24 hours (Figure 1C). The firefly luciferase measured using luciferase assay kit (Promega# E1501) was converted to AHR activity using a standard curve generated with a canonical AHR agonist, FICZ (Supplemental Figure 6A).

The AHR activity in human serum was examined as described previously[41]. The optimum concentration of serum used for AHR assay was determined by treating with different percentages of sera for 4 hours (Supplementary Figure 4). HepG2 cells were

lyzed with passive lysis buffer and the luciferase activity was measured using luciferase assay kit (Promega# E1501) according to manufacturer's instructions, and the signal was normalized to protein. Serum of 1% yielded a maximum difference between control and uremic groups. Therefore, one percent serum concentration was selected to examine the AHR activity in individual ESRD sera samples.

TF procoagulant activity

TF surface/procoagulant activity was measured using a two-step FXa generation assay. A standard curve was generated by incubating human, recombinant lipidated TF (Enzolifesciences, Cat# SE-537) ranging from 0-500 pM along with 5nM of human factor VIIa (Enzyme Research Laboratories Cat# HFVIIa) and 150nM of factor X (Enzyme Research Laboratories Cat# HFX 1010) and CaCl2- 5mM for 30 minutes at 37^0C. The reaction mixture was incubated with chromogenic substrate for factor Xa (Chromogenix Cat# S2765, 1mM-final concentration). The reaction was stopped after 5 minutes using 10ul of 50% glacial acetic acid and read at 405nm absorbance. In vSMCs, the TF procoagulant activity was examined in 96 well plate format with 1000 vSMCs seeded per well. The cells were serum starved for 16 hours and treated with 1% sera for 24 hours. Cells were washed with TBS (50mM Tris HCl, 120mM NaCl, 2.7mM KCl, 3mg/ml BSA, pH=7.4) and incubated with 55ul TBS containing 5nM of human factor VIIa and 150nM of factor X and CaCl2- 5mM for 30 minutes at 37^0C. 50ul of the supernatant was incubated with chromogenic substrate at 1mM-final concentration. The reaction was stopped after 5 minutes by adding 10ul of 50% glacial acetic acid and the absorbance was read at 405nm. TF activity levels for samples were calculated using the regression analysis. To confirm the specificity of the TF activity assay, the TF activity was measured in the cells pre-treated with anti-TF neutralizing antibody (50ug/ml) or control for an hour, as described previously[8] (Supplemental Figure 2B). Platelet derived growth factor

(PDGF, R & D systems- cat# 220-BB-050) served as positive control (Supplemental Figure 2A).

Generation of CB7993113

CB7993113 was identified as an AHR antagonist and characterized as described[17].

Reagents.

Commercial chemical libraries of test compounds were acquired from ChemBridge Corporation and Enamine Ltd. Dimethyl sulfoxide (DMSO), b-Naphthoflavone (b-NF), 7,12-dimethylbenz[*a*]anthracene (DMBA), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other chemical reagents were obtained from Sigma-Aldrich unless otherwise indicated. CB7993113 and CH223191 were synthesized as below. All compounds submitted for biological testing were deemed at least >98% pure by HPLC (with UV and mass spectral detection) and ^{1}HNMR. PDGF was obtained from R & D systems.

Chemical synthesis of 2-((2-(5-bromofuran-2-yl)-4-oxo-4H-chromen-3-yl)oxy)acetamide (CB7993113). 1-(2-hydroxyphenyl)ethanone (1.720 g, 14.29 mmol) and 5-bromofuran-2-carbaldehyde (2.5 g, 14.29 mmol) were dissolved in a round bottom flask in 10 mL of ethanol. Then, 1.5 mL of a solution of 17 M NaOH in water was added under vigorous stirring. Precipitate formed under addition of base and a thick paste was formed. The mixture was stirred for 24 hours at room temperature. Ethanol (50 mL) was added with 0.5 mL of 2.5 M NaOH. The mixture was cooled to 15° C and hydrogen peroxide was added (35% in water; 6.25 mL, 71.4 mmol). After 4 hours, dilute sulfuric acid was added to neutralize to pH 7.0 and the reaction was poured into 250 mL of water and stirred for 2 hours. The solid material was collected by filtration and dried under vacuum to give a yellow solid (1.01 g, 23% yield). ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 4.60 (s, 2 H) 6.98 (d, *J*=3.66 Hz, 1 H) 7.39 (br. s., 1 H) 7.50 (t, *J*=7.33 Hz, 1 H) 7.67 - 7.78 (m, 3 H) 7.81 (d, *J*=7.33 Hz, 1 H) 8.08 (d, *J*=8.06 Hz, 1 H).) A mixture of 2-(5-

bromofuran-2-yl)-3-hydroxy-4H-chromen-4-one, thus, obtained (910 mg, 2.96 mmol), 2-bromoacetamide (409 mg, 2.96 mmol), potassium carbonate (1229 mg, 8.89 mmol) and dimethylformamide (30 mL) were stirred for 6 hours at 80° C. The solution was cooled and extracted with ethyl acetate. The combined organic layers were washed with water, dried with sodium sulfate, filtered and concentrated. Toluene was added and evaporated repeatedly until dry crystals of crude product were formed. The resulting material was purified by column chromatography, first eluting impurities with 100% ethyl acetate, followed by 10% methanol in methylene chloride to provide CB7993113 as a yellow solid (900 mg, 83% yield). ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 4.60 (s, 2 H) 6.98 (d, *J*=3.66 Hz, 1 H) 7.39 (br. s., 1 H) 7.50 (t, *J*=7.33 Hz, 1 H) 7.67 - 7.78 (m, 3 H) 7.81 (d, *J*=7.33 Hz, 1 H) 8.08 (d, *J*=8.06 Hz, 1 H). (ESI) found 363.9 $[M + H]^{+}$.

Supplemental Figure 1. Development of LC/MS assay for IS measurement

A. Separation and detection of serum IS IS-d4. Separation was on Gemini C18 3µ column 50×2.0 mm Phenomenex. Analytes were eluted with a binary gradient consisting of solvent A (5 mmol/L ammonium acetate solution) and solvent B (methanol). The injection volume was 50 µL. (Insert) Fragmentation of IS. The fragment (M/Z 80.0) was the main fragment of IS. M/Z 212>80 was used for IS detection.

B. Linearity of IS on LC-MS/MS measurement. Known concentrations of IS were measured by above methods and correlation was performed using linear regression. Mean of two independent experiments is shown. Correlation coefficient was calculated using R^2.

C. Lower limit of quantitation (LLOQ). The concentration of IS in the spiked serum was diluted to 10,20,30,40,50,100,200,300,400 and 500 ng/ml. Each sample was injected 6 times and the relative standard deviation was calculated. Fifteen percent of relative standard deviation was set as LLOQ. The LLOQ of serum IS was 20 ng/ml.

Supplemental Figure 2. Development and validation of procoagulant TF activity assay in vSMCs.

A. Increase in procoagulant TF activity in vSMCs by PDGF. Dynamic range of the procoagulant TF activity assay was determined using PDGF, a known inducer of TF activity[9]. Serum starved vSMCs were incubated with various concentration PDGF for 24 hours. An average of three independent experiments is shown. Error bars = SD. Compared to control, p = 0.02 for PDGF of 10 ng/ml, p = 0.004 of 20 ng/ml, p = 0.001 of 30 ng/ml, p = 0.03 of 40 ng/ml.

B. Specificity of procoagulant TF activity assay. Serum starved vSMCs were treated with 5% pooled control or uremic serum control or anti-TF neutralizing antibody for 24 hours, as described previously[8] and the procoagulant TF activity assay was performed.

An average of three independent experiments is shown. Error bars = SD. Compared to control serum, p = 0.001 for uremic serum. In the both groups, the anti-TF neutralizing antibody significantly suppressed the TF activity, p = 0.01 in the control serum group, and p = 0.004 in the uremic serum group.

Supplemental Figure 3. AHR and TF expression in human blood vessels

A. Expression of AHR in vascular smooth muscle (vSMCs) in human interlobular renal artery. Paraffin-embedded sections of renal artery of a 51-year-old female were stained for isotype control IgG or AHR antibodies. A representative image from five independent patient samples is shown. Images were captured at 20X magnification. Atherosclerotic plaque with intimal vSMC is shown. EC= endothelial cells, vSMCs = vascular smooth muscle cells. The yellow arrow shows the fragmented internal elastic lamina. Scale bar = 100 µM

B. Expressions of TF and AHR in vSMCs in subcutaneous arterioles obtained from below knee-amputated limb. Paraffin-embedded sections from the amputated limb of a 79-year-old diabetic and hypertensive patient were stained for TF or AHR or isotype control antibodies. A representative image from three independent patient samples is shown. Images were captured at 20X magnification. The yellow arrow shows an integrated thrombus within the vessel wall. Scale bar = 100 µM

Supplemental Figure 4. AHR activity assay for human serum

We first determined the optimum concentration of human serum to be used for AHR activity by exposing serum starved cells stably expressing Cignal Lenti Reporter (XRE-luc) to different concentrations of pooled control or uremic sera for 4 hours [41]. AHR antagonist CH223191- 10 µM served as a negative control. The luciferase reading was normalized for protein content. An average of three different experiments performed in

duplicate is shown. One percent serum yielded highest difference between two groups (5.7 relative luciferase unit per µg protein, p = 0.001). Therefore, one percent serum concentration was selected to examine the AHR activity in individual ESRD sera samples as shown in Table 2. Error bars = SD. Compared to the control group, uremic group had following p values. p = 0.002 0.001% serum, p = 0.002 for 0.1% serum and p = 0.001 for 1% serum. Compared to vehicle treated cells, p = 0.01 in control serum and p = 0.0001 in uremic serum treated with 10 µM of AHR antagonist.

Supplemental Figure 5. Molecular structure of AHR antagonist

A. Structures of CB7993113 2-{[2-(5-bromo-2-furyl)-4-oxo-4H-chromen-3-yl]oxy}acetamide

B. Structures of CH223191 (E)-1-Methyl-N-(2-methyl-4-(o-tolyldiazenyl)phenyl)-1H-pyrazole-5-carboxamide

Supplemental Figure 6. AHR unit determination and bound form of IS induces AHR activity in an AHR-dependent manner

A. AHR activity unit determination. Primary aortic vSMCs stably expressing a dioxin responsive element (DRE) promoter-luciferase reporter construct were treated with indicated concentrations of FICZ for 24 hours in serum free medium. AHR activity was quantified by firefly luciferase units measured using luciferase assay kit (Promega# E1501) normalized to protein concentration. An average of three independent experiments is shown. Compared to control, p values for different FICZ concentrations were p = 0.042 for 1 ng/ml, p = 0.004 for 10 and 20 and 40 ng/ml of FICZ, and 0.001 for more than 60 ng/ml FICZ. Error bars = SD. The linear regression was applied to obtain the equation and R^2 value.

B. Bound form of IS corresponding to the levels in different stages of CKD induces AHR activity. vSMCs stably expressing xenobiotic responsive element (XRE) promoter-luciferase reporter construct were treated with different concentrations of IS or human serum albumin (Ctr) for 24 hours and the firefly luciferase measured as above was converted to AHR activity units using the standard curve generated with FICZ, a canonical AHR agonist (Supplemental Figure 6A). Mean result of three experiments is shown. Error bars = SD. Compared to the control (HSA) IS μg/ml, $p = 0.01$ for IS 5 μg/ml, $p = 0.001$ for IS 10, 15, 20 μg/ml and $p = 0.008$ for IS 40 μg/ml.

C. AHR antagonist inhibits AHR activity induced by bound form of IS. vSMCs stably expressing xenobiotic responsive element (XRE) promoter-luciferase reporter construct were treated with IS or human serum albumin (HSA) and different concentration of CB7993113 for 24 hours and the firefly luciferase was converted to AHR activity units as mentioned above. Mean result of three experiments is shown. Error bars = SD. Compared to IS alone, the IS + CB7993113 treated cells, $p = 0.05$ for 5 μM, $p = 0.002$ for 10, 20 μM and $p = 0.001$ for 30 and 40 μM.

Supplemental Figure 7. Wild-type mouse embryonic fibroblasts (MEFs) have higher TF compared to AHR KO MEFs; and modulation of TF by known AHR ligands

A. AHR KO MEFs have lower TF compared to wild-type MEFs. MEFs from C57BL/6 animals and AHR KO animal on the same background were lyzed and probed for TF. GAPDH served as loading control. A representative of 2 independent experiments is shown.

B. Regulation of TF by known AHR ligands. Primary aortic vSMCs at passage 6 were treated with AHR ligands (**B**) AHR agonists- 6-formylindole[3,2-b]carbazole (FICZ) (**C**) and β-naphtoflavone (β-NF) and (**D**) an AHR antagonist, 3'methoxy-4'nitroflavone (MNF)

in the concentration as shown for 24 hours. The lysates were probed for TF and GAPDH, where served as a loading control. TF bands were normalized by GAPDH. Representative figure from three independent experiments is shown.

Supplemental Figure 8. Rise in TF mRNA follows the effect on protein levels with IS treatment

TF mRNA increases after 4 hours of IS treatment. vSMCs treated with IS (25 µg/ml) or control were harvested at indicated time intervals. Quantitative RT-PCR in two steps was performed after extracting RNA and synthesizing cDNA using random hexamers and oligo dT primers. qRT-PCR reactions were processed in duplicate for each sample and quantified using real time PCR for detecting TF mRNA. The Ct values were utilized to generate delta-delta ct values normalized by GAPDH levels. An average from three independent experiments is shown. Error bar = SD. p = 0.008 for TF mRNA after 4 hours.

Supplemental Figure 9. TF half-life reduced with AHR antagonist

A. AHR antagonist destabilizes TF at baseline. vSMCs treated with CB7993113 10 µM for 24 hours were treated with Emetine 20 µM and harvested at indicated time for TF expression. A representative blot from three experiments is shown. For all the blots, the values below the blot represents the normalized bands against GAPDH using ImageJ.

B. Densitometry was performed on half-life study using Image J and GAPDH was used to normalize the TF signal. Average of three experiments is shown. Error bars = SD. Compared to TF in control, p = 0.05 at 0.5 hours, p = 0.01 at 1 hour and p = 0.002 at 4 hours in CB7993113 treated cells.

Supplemental Figure 10. IS corresponding to the levels seen in early stages of CKD increase TF expression and activity in vSMCs

A. IS levels corresponding to different stages of CKD increase TF expression. Lysates of vSMCs treated with IS or control at different concentrations were resolved on SDS page gel and immunoblotted with TF antibody. GAPDH served as loading control. Average IS levels found in the patients with different stages of CKD is shown in Supplementary table S3. A representative of three independent experiments is shown.

B. IS levels corresponding to different stages of CKD increase procoagulant TF activity. TF activity was examined in vSMCs exposed to IS levels corresponding to different stages of CKD as shown in supplementary table S3. An average of three experiments is shown. Error bars = SD. Compared to control, $p = 0.02$ for IS 1µM, $p = 0.001$ for IS 10µM, $p = 0.003$ for IS 10µM.

Supplemental Table 1. ESRD patient characteristics

No	Age	Sex	Ethnicity	Cause	DM	HTN	BMI	SBP	DBP	Hb	Platelet	INR	Chol	LDL	Epogen	sp KT/V
1	60	M	AA	DM+HTN	1	1	38	182	89	8.9	246	0.87	100	54	9900	1.36
2	27	F	AA	HTN	0	1	30.6	156	82	8.6	295	1.00	245	161	12000	1.23
3	40	F	AA	Collapsing GN	0	1	40.58	174	93	9.2	232	0.99	170	74	8525	1.95
4	51	M	AA	Collapsing GN	0	1	24	182	86	11	257	1.00	283	183	39600	1.1
5	73	M	AA	DM+HTN	1	1	33.25	187	100	10.8	85	2.60	106	56	15400	1.6
6	41	M	AA	HTN	0	1	22.9	132	84	9.2	242	1.30	84	38	6600	1.05
8	52	F	HIPS	HTN	0	1	41.46	162	94	12	305	0.97	232	141	23870	1.25
9	33	M	AA	HTN	0	1	25.2	158	86	13.2	208	0.96	141	71	57490	1.7
10	57	M	AA	DM	1	1	17.6	152	90	10.7	172	1.20	88	52	20500	1.73
11	56	M	HISP	DM	1	1	28.7	162	96	10.8	143	0.97	195	132	18000	1.39
12	70	M	AA	DM+HTN	1	1	24.1	150	88	12	232	0.95	116	64	15000	1.05
13	36	F	HISP	SLE	0	0	18.7	93	68	12.1	120	1.70	176	115	NA	1.99
14	50	M	AA	HTN	0	1	21.8	150	85	11.6	188	0.96	170	85	NA	1.4
15	55	M	AA	DM+HTN	1	1	32.61	140	96	8.6	207	1.18	181	114	19800	1.66
16	52	M	ASIAN	DM	1	1	22.3	139	88	9.3	198	0.96	161	78	6600	1.76
17	46	M	AA	DM+HTN	1	1	27.09	152	92	9.9	178	0.96	145	87	NA	1.67
18	28	M	AA	HTN	0	1	26.27	142	96	10.8	247	0.88	231	146	39600	1.79
19	29	M	AA	Reflux Neph	0	1	24	178	102	12.3	220	0.88	NA	NA	NA	1.67
20	47	F	AA	FSGS	0	1	26	156	110	9.6	242	1.05	86	20	19800	1.25
21	48	M	AA	DM+HTN	1	1	31.3	128	86	9.2	210	0.96	195	113	NA	1.11

AA= African American, Hispan = Hispanic, DM= diabetes, HTN= hypertension, Reflux Neph = reflux nephropathy, SLE = systemic lupus erythematosus, GN = glomerulonephritis, 0 = absent, 1= present, BMI= kg/m2, SBP= systolic blood pressure mm Hg, DBP= diastolic blood pressure mm Hg, Platelet is K/ul, Chol = total cholesterol mg/dl, LDL = LDL cholesterol mg/dl, Epogen = unit/week, spKt/v= single pool Kt/V and NA= not available.

Supplemental Table 2. Inter assay and intra-assay variability of estimation of IS

Intra-assay Variation			
	Average	SD	CV (%)
QC1	236	14	5.8
QC2	377	29	7.7
QC3	4939	279	6
Inter-assay Variation			
	QC1	QC2	QC3
Assay 1	236	377	4939
Assay 2	259	424	4977
Assay 3	220	376	4889
Average	238	392	4935
SD	20	27	44
CV(%)	8.2	7.0	0.9

Supplemental Table 3. Recovery of IS sample

	Orogonal		Add 400 ng/ml			Add 2000 ng/ml			Add 80000 ng/ml	
	Conc ng/ml		Conc ng/ml	Recovery (%)		Conc ng/ml	Recovery (%)		Conc	Recovery (%)
Serum	376±29		831±35	113.7		2631±102	112.7		8988±402	107.6

Supplemental Table 4. Levels of IS in different stages of CKD[24, 37-39]

	eGFR ml/min	Average total IS µg/ml	Average total IS µM*	**Expected Free IS (µg/ml)
Normal	>90	0.243	0.97	0.02
Stage I**	>90	0.243	0.97	0.02
Stage II	60-89	0.5	1.98	0.05
Stage III	30-59	3.2	12.73	0.32
Stage IV	15-29	5.4	21.48	0.54
Stage V	<15	19.8	78.79	1.98
ESRD	<15HD	44.86	169.12	4.25

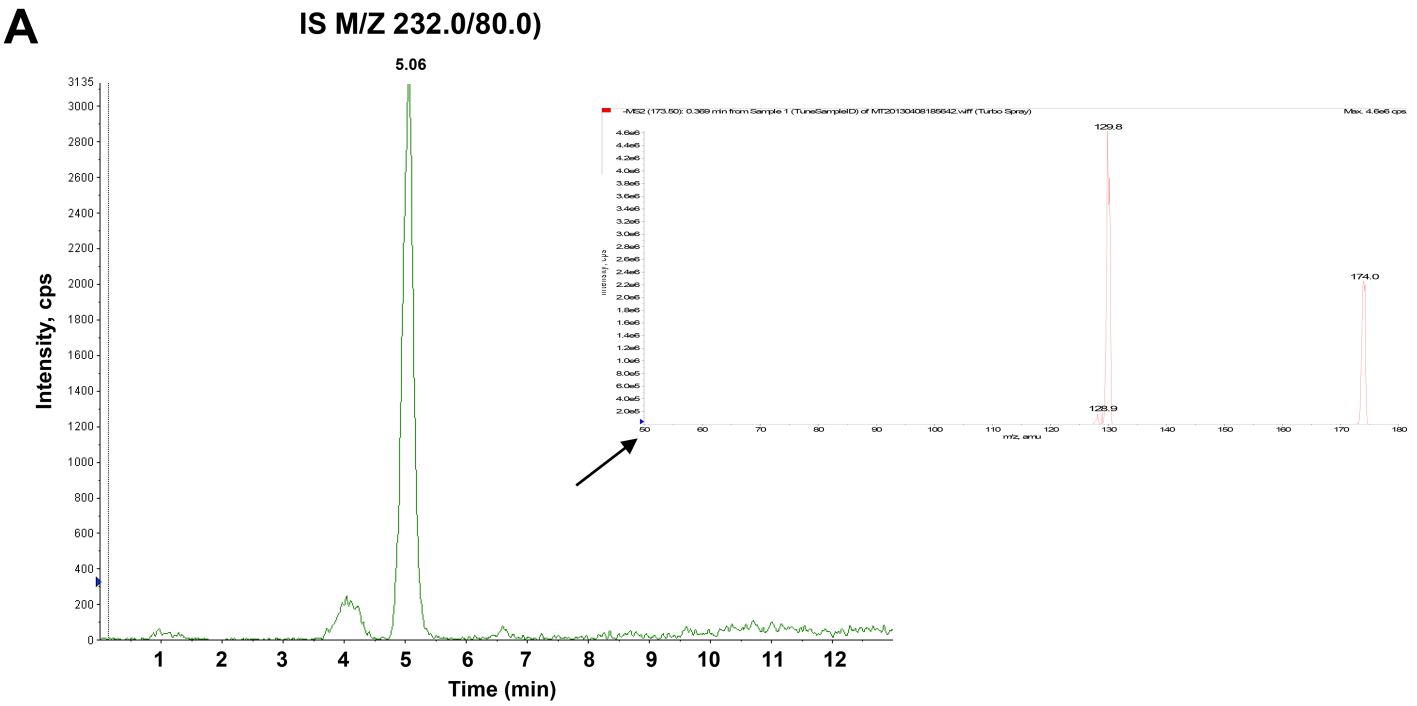
eGFR = estimated Glomerular filtration rate, mg/dL = milligram / 100 ml
*Calculated based on formula weight of IS = 251.30
**Free form of IS calculated as 10% of total IS

Supplemental Table 5. Levels of IS and AHR and TF activities in non-diabetic ESRD patients matched with controls

Pairs	IS µg/ml		AHR activity RLU/ug		TF activity pM	
	Control	Uremic	Control	Uremic	Control	Uremic
1.00	0.65	21.85	1.82	4.21	206.62	271.90
2.00	1.30	48.85	2.00	5.48	149.75	297.93
3.00	1.33	24.75	1.39	3.50	247.96	284.50
4.00	0.08	70.50	2.30	7.60	185.93	319.83
5.00	0.33	26.75	1.98	5.52	234.44	284.79
6.00	2.40	52.65	1.91	3.02	316.00	426.32
7.00	0.43	11.10	1.49	3.62	286.43	276.63
8.00	1.39	58.40	2.38	5.99	192.66	330.10
9.00	0.85	73.25	1.91	5.76	128.19	428.42
10.00	1.22	44.65	1.88	5.31	218.04	276.63
11.00	1.47	13.95	1.91	4.38	187.73	274.24
Mean	1.04	40.61	1.91	4.94	213.98	315.57
Median	1.22	44.65	1.91	5.31	206.62	284.79
SD	0.66	22.09	0.29	1.34	55.42	58.40

Supplemental Table 6. Comparision of controls and non-diabetic ESRD patients

	Paired T	Wilcoxon Signed-Rank
IS levels µg/ml	0.0001	0.001
AHR activity RLU/ug	<0.0001	0.001
TF activity pM	0.0021	0.002


A
IS M/Z 232.0/80.0)
5.06
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Time (min)
129.8
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0618 validation 1 .rdb (IS): "Linear" Regression ("NO" weighting): y=0.00347 x + 0.0533 (r=0.9996)
Analyte Area / IS Area
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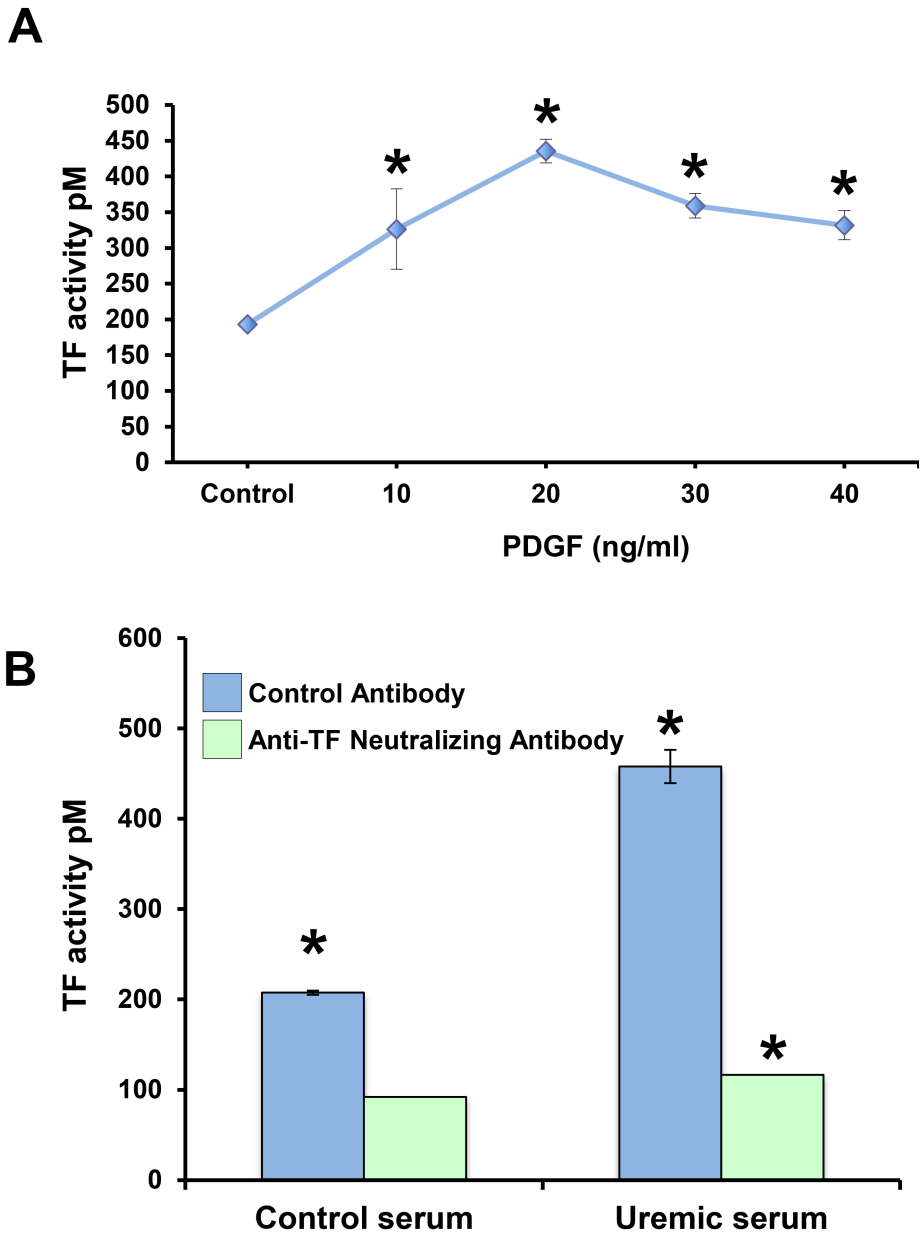
C
Lower Limit of Quantitation of Plasma Indoxyl Sulfate
Relative Standard Deviation (%)
Concentration of Plasma Sulfate (ng/ml)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TF activity pM
500
450
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Control
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PDGF (ng/ml)
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B
TF activity pM
600
500
400
300
200
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Control Antibody
Anti-TF Neutralizing Antibody
Control serum
Uremic serum
*
*
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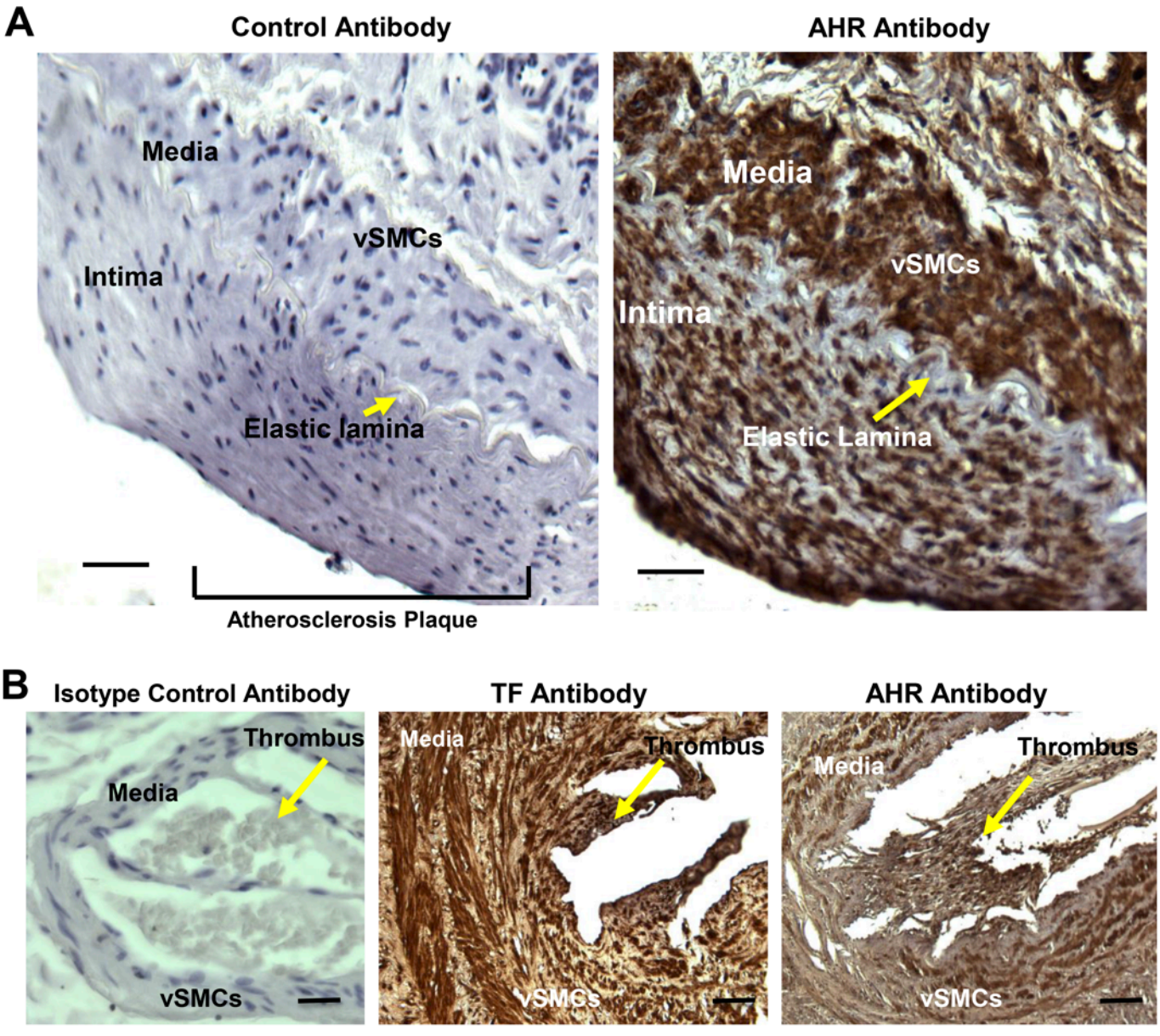
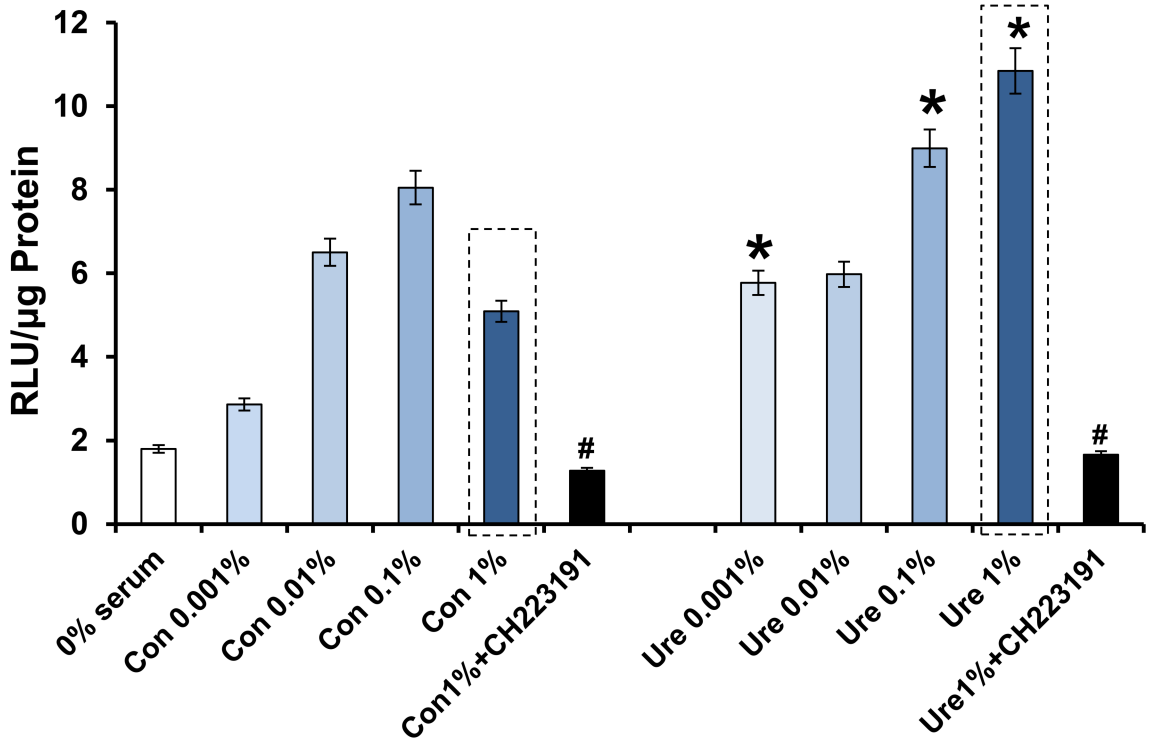
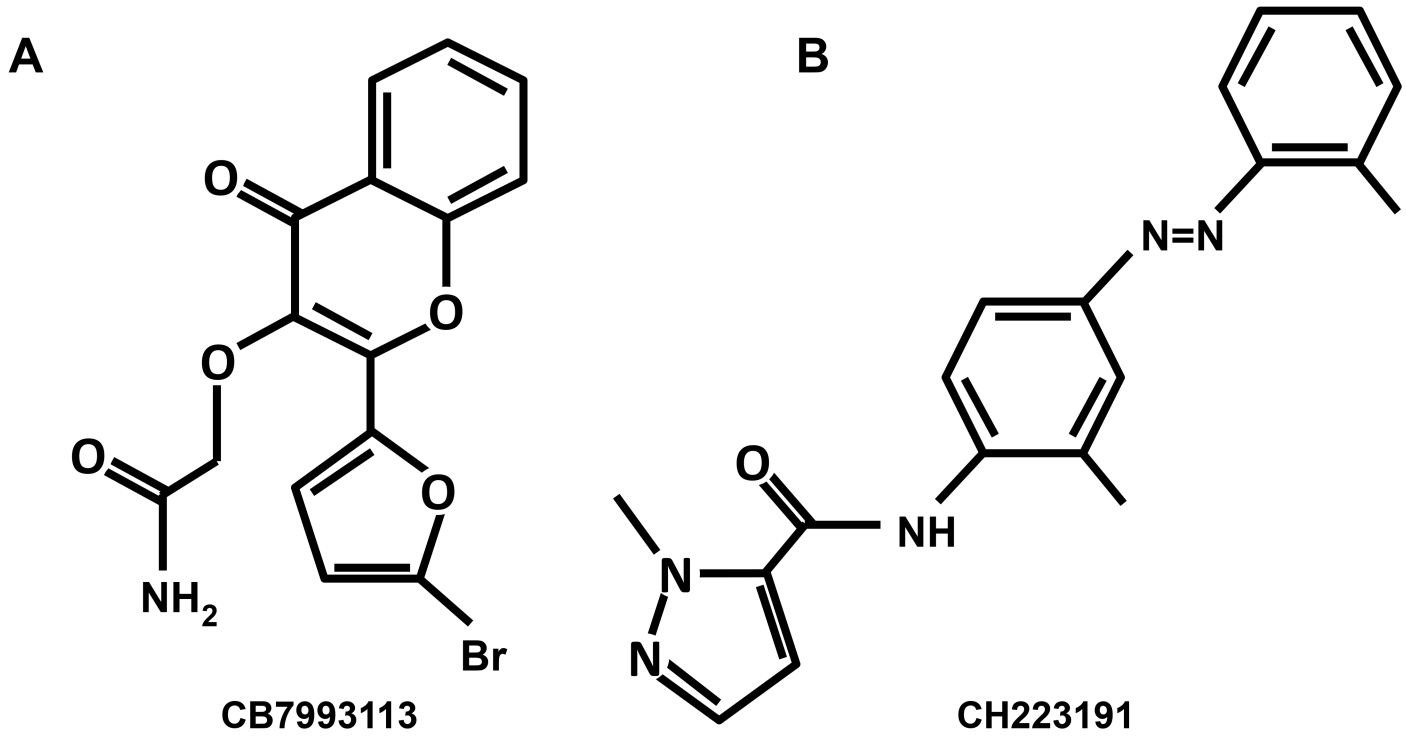
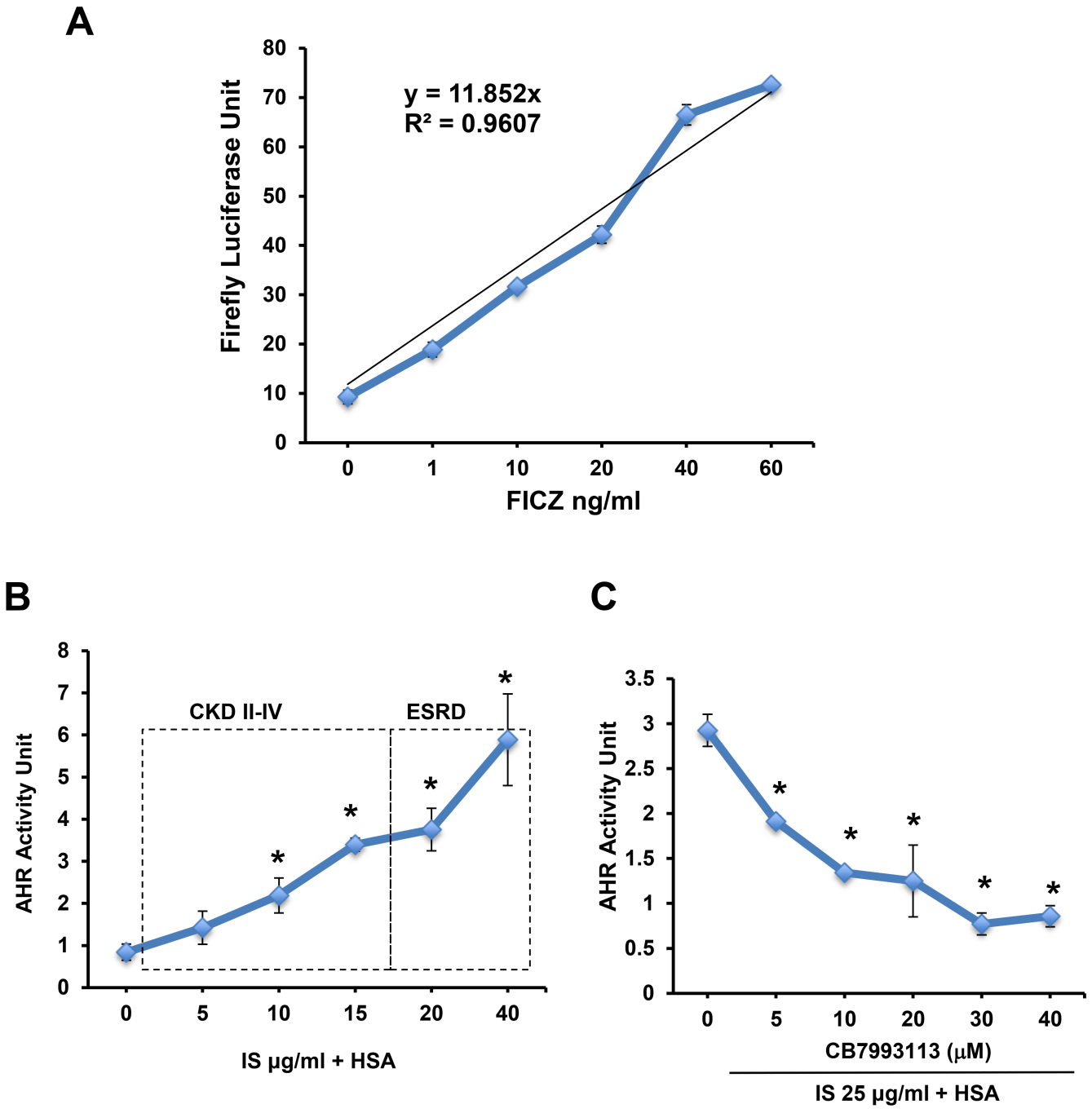
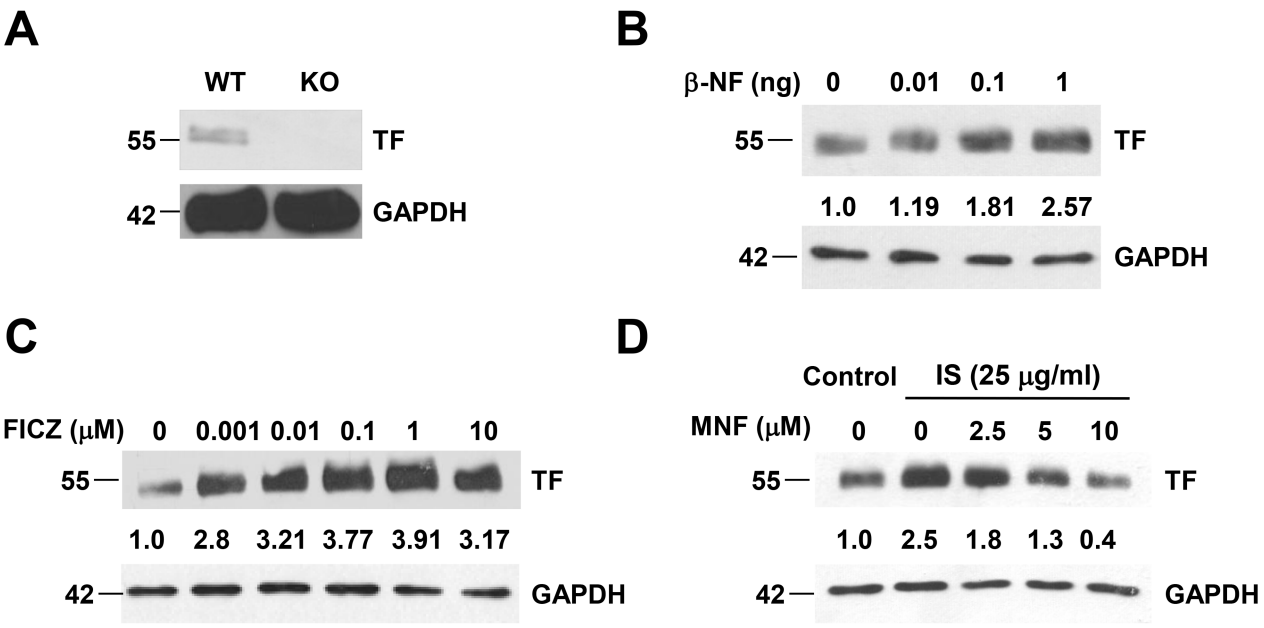

A
Control Antibody
AHR Antibody
Media
vSMCs
Intima
Elastic lamina
Media
vSMCs
Intima
Elastic Lamina
Atherosclerosis Plaque
B
Isotype Control Antibody
TF Antibody
AHR Antibody
Thrombus
Media
vSMCs
Media
Thrombus
vSMCs
Media
Thrombus
vSMCs

Supplementary Figure 4

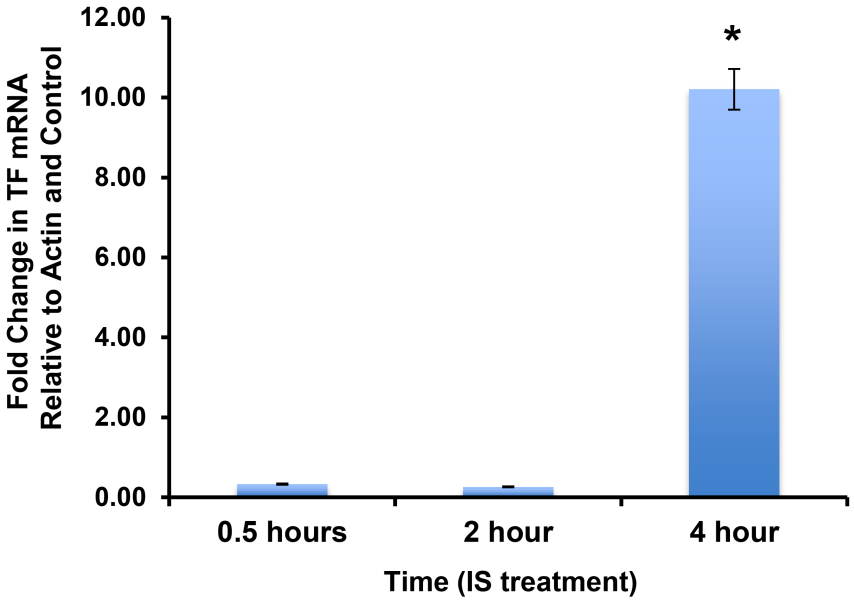


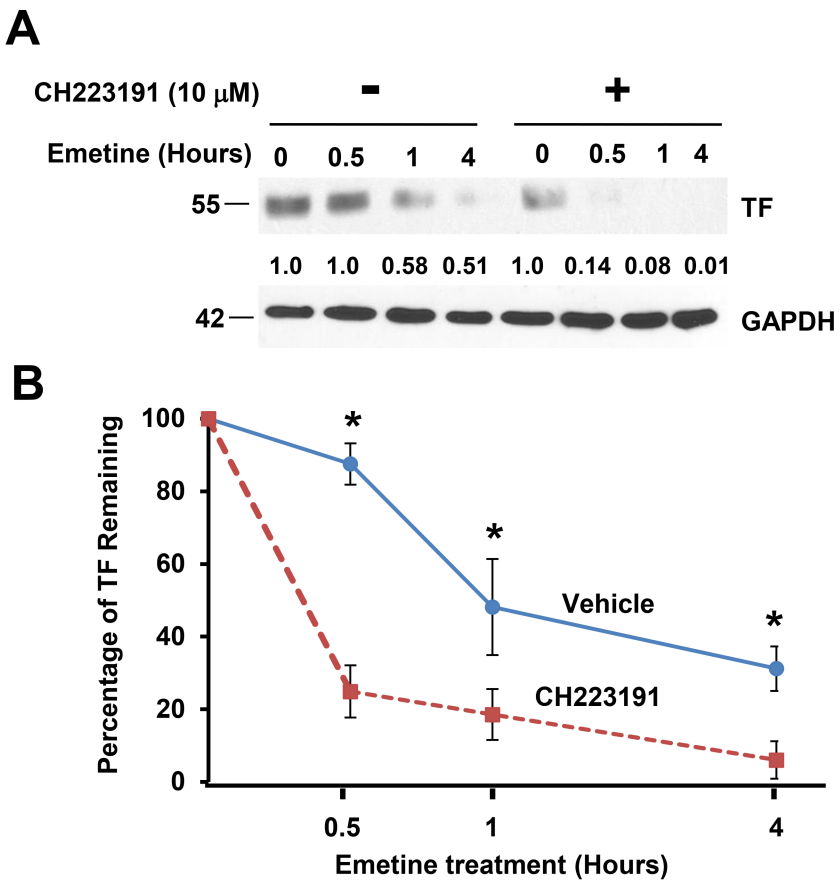

A
O
O
O
O
O
NH2
Br
CB7993113
B
N=N
O
NH
N
N
CH223191


A
y = 11.852x
R² = 0.9607
Firefly Luciferase Unit
FICZ ng/ml
B
CKD II-IV
ESRD
AHR Activity Unit
IS μg/ml + HSA
C
AHR Activity Unit
CB7993113 (μM)
IS 25 μg/ml + HSA


A
WT
KO
55
TF
42
GAPDH
B
β-NF (ng) 0 0.01 0.1 1
55
TF
1.0 1.19 1.81 2.57
42
GAPDH
C
FICZ (μM) 0 0.001 0.01 0.1 1 10
55
TF
1.0 2.8 3.21 3.77 3.91 3.17
42
GAPDH
D
Control
IS (25 μg/ml)
MNF (μM) 0 0 2.5 5 10
55
TF
1.0 2.5 1.8 1.3 0.4
42
GAPDH

Supplementary Figure 8




A
CH223191 (10 μM)
-
+
Emetine (Hours)
0
0.5
1
4
0
0.5
1
4
55
TF
1.0
1.0
0.58
0.51
1.0
0.14
0.08
0.01
42
GAPDH
B
Percentage of TF Remaining
100
80
60
40
20
0
*
*
*
Vehicle
CH223191
0.5
1
4
Emetine treatment (Hours)

Supplementary Figure 10