Supplemental Materials for "NAD+ biosynthetic impairment and urinary metabolomic alterations observed in hospitalized adults with COVID-19-related acute kidney injury."

Supplement 1: Supplemental Methods

Urine Collection and Processing:

Urine specimens were collected a median of 6.5 days after the first positive SARS-CoV-2 test in the non-AKI group (12 d among Boston patients, 1 d among Birmingham patients) and 4 days after the first positive test in the AKI group (10 d among Boston patients, 2 d among Birmingham patients). Urine was collected either as a voided specimen or from a urinary catheter. Specimens were subsequently transferred to a biosafety level (BSL) 2+ Laboratory. Samples in Birmingham were centrifuged immediately following collections, with cell-free supernatant collected. 0.35 ml of supernatant from each sample was mixed with 1.4 ml of ice-cold methanol and then stored at -80^{o}C. Samples in Boston were stored at -80^{o}C prior to centrifugation, and subsequently defrosted on ice. Defrosted specimens were centrifuged and cell-free supernatant was mixed with methanol at the same ratio of 1 part supernatant to 4 parts methanol, then re-frozen at -80^{o}C and shipped on dry ice to UAB for analysis. Samples containing 80% aqueous methanol without urine were prepared to serve as a blank at each site.

Metabolomic Studies:

Samples containing urine supernatant with methanol were brought to 4^{o}C and then centrifuged at 10,000 x g for 10 min. Each resulting supernatant was transferred to a new tube and evaporated to dryness under N_2. Dry residues were redissolved in double-distilled water (100 µL). Equal volumes of each sample were combined to make a pooled sample. Aliquots (20 µL) of each sample and the pooled sample (the latter three times) were individually injected onto a 10 cm x 2.1 mm ID LunaOmega column (Phenomenex, CA) equilibrated with 0.1% formic acid at flow rate of at 0.5 ml/min. Bound metabolites were eluted using a 6 min linear gradient (2-98%) of acetonitrile in 0.1% formic acid – from 6-7 min the mobile phase was 99.9% acetonitrile/0.1% formic acid; from 7-10 min the column was re-equilibrated with 0.1% formic acid at a flow rate of 0.5 ml/min. Eluates were passed into an electrospray ionization interface of a SCIEX (San Jose, CA) TripleTOF 5600plus mass spectrometer operating at -3500 V (negative ion spectra) or 4500 V (positive ion spectra). Full scan, high resolution/high mass accurate TOF MS spectra were collected for all samples every 250 ms. The peak widths were 2.5-3 s, meaning that each peak was mapped with 10-12 data points to accurately estimate the area under each peak. A pooled sample was separately used to collect MSMS spectra using the following 0.5 s duty cycle: 100ms full scan, high resolution/high mass accurate TOF MS spectrum, followed by up to eight 50 ms high resolution/high mass accurate TOF product ion

MSMS spectra from precursor ions selected using the quadrupole filter. This was repeated three times. The order of sample analysis was randomized in each batch with the proviso that the repeats of the pooled sample for the batch were distributed evenly across the analysis. The data were recorded as .wiff and .wiffscan files.

Data analysis

LC-MSMS spectral data .wiff and .wiffscan files were analyzed using MS-DIAL, version 4.48 (http://prime.psc.riken.jp/compms/msdial/main.html) with version 15 of the public database of MSMS files (290,915 positive ion MSMS spectra representing 13,303 compounds and 36,848 negative ion MSMS spectra representing 12,879 compounds). Mass accuracies for MS and MSMS spectra were set at 10 ppm and 15 ppm, respectively, and variance of the retention time at 0.10 min. The matched MSMS features were filtered to remove those that exhibited a coefficient of variation in the replicate pooled samples that exceeded 10%. Using a minimum ratio of 10:1 between the areas of the matched metabolites and the 80% aqueous methanol blank, other compounds were removed from the dataset. Those whose pooled values were greater than 25% of the means of the AKI or non-AKI samples were also removed. Some were from both positive and negative libraries as they formed $[M+H]+$ and $[M-H]^-$ molecular ions. All metabolite peak AUC values included in analyses were determined by MSMS except 3-hydroxyanthranilate and nicotinamide mononucleotide, which were determined by MS with retention time.

Validation of the metabolic intermediates present in the urines was carried out using the Mass Spectrometry Metabolite Library of Standards (IROA Technologies, Sea Girt, NJ). Where possible, authentic specimens of drugs and their metabolites were obtained in order to validate their presence in the urines.

The drugs used in the treatment of these two groups of patients that were detected by LC-MS were: acetaminophen, amitriptyline, amlopidine, atenolol, bumetanide, buspirone, citalopram, clindamycin, codeine, cortisone, descladinose-azithromycin, dextromethorphan, diphenhydramine, fluconazole, gabapentin, hydrocodone, iohexol, iopamidol, ketamine, lidocaine, metformin, metoclopramide, metoprolol, ondansetron, piperacillin, sertraline, and trimethoprim. These were excluded from subsequent analyses comparing metabolites between populations.

Peak AUC values for each metabolite were corrected for urine creatinine prior to analysis by dividing each peak AUC value by the corresponding urinary creatinine peak AUC value for each participant. Peak AUC values were log-transformed, mean centered, and divided by respective standard deviations as part of processing for analysis done in Metaboanalyst.

Supplement 2: Supplemental acknowledgments

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Supplement 3:

Supplemental Table S1. List of metabolites identified in patient urine using liquid chromatography - tandem mass spectrometry.

Boston +	Boston -	Alabama +	Alabama -
1-METHYL-L-HISTIDINE		1-METHYL-L-HISTIDINE	
1-METHYLNICOTINAMIDE		1-METHYLNICOTINAMIDE	
			2-OH-BUTYRATE
2,5-DIMETHYLPYRAZINE			
		3-(2-HYDROXYPHENYL) PROPANOATE	
		3-HYDROXYANTHRANILATE	
4-ACETAMIDOBUTANOATE		4-ACETAMIDOBUTANOATE	
			4-OH-BENZOATE
4-PYRIDOXATE	4-PYRIDOXATE	4-PYRIDOXATE	4-PYRIDOXATE
		5-METHYLCYTOSINE	
	6-CARBOXY HEXANOATE		6-CARBOXY HEXANOATE
ADENOSINE		ADENOSINE	
	ASCORBATE		ASCORBATE
BETAINE		BETAINE	
CHOLINE		CHOLINE	
	CITRAMALATE		CITRAMALATE
CREATININE		CREATININE	
	CYCLIC AMP	CYCLIC AMP	CYCLIC AMP
DEOXYCARNITINE			
		DIHYDROBIOPTERIN	
DIHYDROURACIL		DIHYDROURACIL	
			ETHYLMALONATE
	GLUCONOLACTONE		GLUCONOLACTONE
	GLUCURONATE		GLUCURONATE

GLUTAMINE			
	GLUTARATE		
GLUTARYLCARNITINE		GLUTARYLCARNITINE	
		GLYCOCHENODEOXY CHOLATE	
GLYCHOLATE		GLYCHOLATE	
		GUANIDINOSUCCINATE	
			GULOSE
HEXANOATE	HEXANOATE		HEXANOATE
HIPPURATE	HIPPURATE	HIPPURATE	HIPPURATE
HISTIDINE		HISTIDINE	HISTIDINE
HYPOXANTHINE	HYPOXANTHINE	HYPOXANTHINE	HYPOXANTHINE
	INOSINE		INOSINE
	ISOCITRATE		ISOCITRATE
		ISOLEUCINE	
KYNURENATE	KYNURENATE	KYNURENATE	KYNURENATE
KYNURENINE			
L-CARNITINE		L-CARNITINE	
LACTOSE	LACTOSE		
		LAUROYL CARNITINE	
LEUCINE (+)			
		LYSINE	
		MALATE	
METHIONINE		METHIONINE	
	METHYLGLUTARATE		METHYLGLUTARATE
METHYLTHIOADENOSINE			
			N-ACETYLALANINE
		N-ACETYL ASPARAGINE	
	N-ACETYL ASPARTATE		N-ACETYL ASPARTATE
	N-ACETYLCYSTEINE		

	N-ACETYL GALACTOSAMINE		
	N-ACETYL-GLUTAMATE	N-ACETYLGLUTAMATE	N-ACETYL-GLUTAMATE
N-ACETYL NEURAMINATE	N-ACETYL NEURAMINATE		N-ACETYL NEURAMINATE
			N-ACETYL PHENYLALANINE
			N-ACETYLSERINE
N-ACETYLTRYPTOPHAN		N-ACETYLTRYPTOPHAN	N-ACETYL TRYPTOPHAN
N-ALPHA-ACETYLLYSINE		N-ALPHA-ACETYLLYSINE	
N,N-DIMETHYLARGININE		N,N-DIMETHYLARGININE	
N,N,N-TRIMETHYLLYSINE		N,N,N-TRIMETHYLLYSINE	
NICOTINATE		NICOTINATE	NICOTINATE
	NICOTINAMIDE		
O-ACETYLCARNITINE		O-ACETYLCARNITINE	
			OROTATE
	P-HYDROXY PHENYLACETATE		P-HYDROXY PHENYLACETATE
PANTOTHENATE		PANTOTHENATE	PANTOTHENATE
PHENYLALANINE		PHENYLALANINE	
PYROGLUTAMATE		PYROGLUTAMATE	
QUINOLINATE	QUINOLINATE	QUINOLINATE	QUINOLINATE
RIBOFLAVIN	RIBOFLAVIN	RIBOFLAVIN	
		S-ADENOSYL HOMOCYSTEINE	
S-ADENOSYLMETHIONINE			
	SACCHARATE		
	TARTRATE		
		THEOBROMINE	
	TRANS-ACONITATE		TRANS-ACONITATE
	TREHALOSE		TREHALOSE

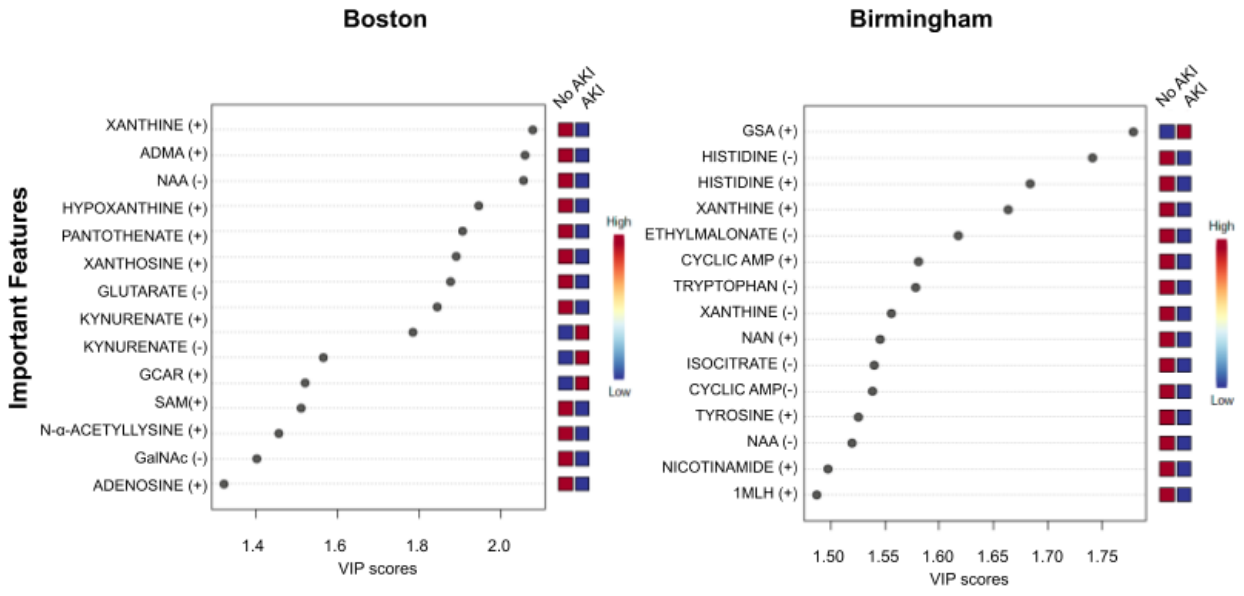
		TRIGONELLINE	
TRYPTOPHAN	TRYPTOPHAN	TRYPTOPHAN	TRYPTOPHAN
TYROSINE	TYROSINE	TYROSINE	TYROSINE
URATE	URATE	URATE	URATE
UROCANATE		UROCANATE	
VALINE		VALINE	
XANTHINE	XANTHINE	XANTHINE	XANTHINE
XANTHOSINE	XANTHOSINE	XANTHOSINE	XANTHOSINE
XANTHURENATE	XANTHURENATE	XANTHURENATE	XANTHURENATE

Supplement 4:

Supplemental Table S2. Differences in metabolites related to NAD+ synthesis between groups of hospitalized COVID-19 patients with and without AKI. Values are peak area under curve (AUC) of metabolites by liquid chromatography - tandem mass spectrometry LC-MSMS divided by peak AUC of urinary creatinine for each respective patient. Metabolites are cationic forms only where both anion and cation forms are identifiable by LC-MSMS, and are presented as either mean ($\bar{x}$) or median (M) depending on whether their distribution is Gaussian or not. P-values are calculated using Student's t-test for variables with Gaussian distribution, and are otherwise calculated using the Mann-Whitney test. Quin, quinolinate; NaMN, nicotinic acid mononucleotide; NAD+, nicotinamide adenine dinucleotide; MNA, 1-methyl nicotinamide; NAM, nicotinamide; NMN, nicotinamide mononucleotide.

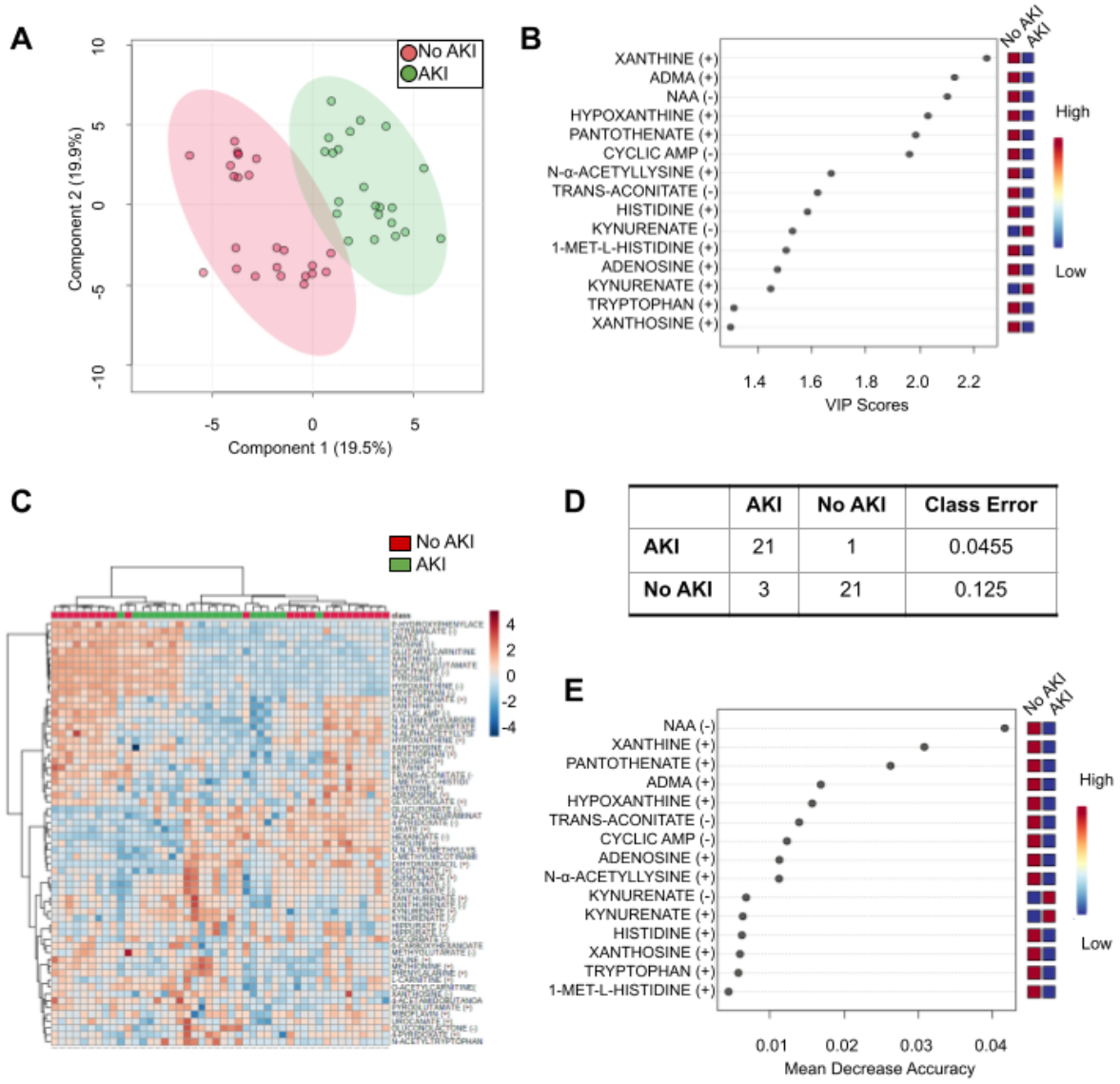
Metabolite	Boston			Alabama			Pooled		
	AKI	No AKI	p	AKI	No AKI	p	AKI	No AKI	p
Tryptophan ($\bar{x}$)	0.016	0.046	0.09	0.023	0.076	0.005	0.019	0.057	0.002
Kynurenine (M)	0.006	0.022	0.03	0.008	0.008	0.99	0.006	0.015	0.07
Kynurenate ($\bar{x}$)	0.082	0.025	0.004	0.070	0.049	0.11	0.078	0.035	0.001
3-Hydroxyanthranilate (M)	0.001	0.003	0.04	0.001	0.004	0.006	0.001	0.004	0.001
Quinolinate ($\bar{x}$)	0.037	0.022	0.21	0.016	0.014	0.60	0.028	0.018	0.16
Nicotinate ($\bar{x}$)	0.033	0.019	0.20	0.017	0.009	0.03	0.027	0.015	0.08
1-Methyl nicotinamide ($\bar{x}$)	0.037	0.24	0.10	0.009	0.017	0.11	0.027	0.021	0.32
Nicotinamide ($\bar{x}$)				0.0009	0.003	0.003			
Nicotinamide mononucleotide (M)	0.001	0.0007	0.23	0.0004	0.0004	0.15	0.0005	0.0005	0.75

Supplement 5:



Supplemental Figure S1. Important features identified by variable importance in projection (VIP) scores in supervised partial least squares analysis. Urine metabolomic features are compared between patients with COVID-19 with and without AKI in a critically ill population in Boston and a general hospital population in Alabama. ADMA, N,N dimethylarginine; NAA, n-acetylaspartate; GCAR, glutarylcarnitine; SAM, s-adenosylmethionine; GalNAc, n-acetylgalactosamine; GSA, guanidinosuccinate; NAN, n-acetylasparagine; 1MLH, 1-methyl-L-histidine.

Supplement 6:



	AKI	No AKI	Class Error
AKI	21	1	0.0455
No AKI	3	21	0.125

Supplemental Figure S2. Urine metabolomic features distinguishing acute kidney injury (AKI) among COVID-19 patients pooled across Boston and Birmingham cohorts. A) Supervised Partial Least Squares-Discriminant Analysis (PLS-DA) plot comparing patients with (green) and without (red) AKI. B) Important features identified by variable importance in projection (VIP) scores in PLS-DA. C) Unsupervised hierarchical clustering analysis comparing urine metabolomic features in patients with (green) and without (red) AKI and relative increases (red) or decreases (blue) in metabolite levels. D) Supervised Random Forest predictions and class error comparing AKI and No AKI groups. E) Key features identified by Random Forest comparing patients with and without AKI; red and blue boxes respectively denote relatively high

or low concentrations of metabolites important in classification. NAA, n-acetylaspartate; ADMA, N,N dimethylarginine.

Supplement 7: Modified STROBE Statement

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract "Observed" included in title
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found ✓ pg 2-3
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✓ pg 4-5
Objectives	3	State specific objectives, including any prespecified hypotheses ✓ final paragraph of introduction (pg 5)
Methods		
Study design	4	Present key elements of study design early in the paper ✓ pg 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✓ pg 5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls ✓ pg 6, fig 1

		Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✓ pg 6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). ✓ pg 6-7, supplemental methods
Bias	9	Describe any efforts to address potential sources of bias ✓ pg 7
Study size	10	Explain how the study size was arrived at (if applicable) ✓ pg 6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✓ pg 7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✓ pg 7-8
		(*b*) Describe any methods used to examine subgroups and interactions ✓ pg 7-8
		(*c*) Explain how missing data were addressed N/a

		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed ✓ Participants section of methods *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy ✓ pg 7-8
		(e) Describe any sensitivity analyses ✓ supplement 6
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed ✓ Figure 1
		(c) Use of a flow diagram ✓ Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✓ Table 1
		(b) Indicate number of participants with missing data for each variable of interest ✓ Only area of missing data was with baseline creatinine – methodology used to determine this is described on page 6
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time

		Case-control study—Report numbers in each exposure category, or summary measures of exposure ✓ Figure 1
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ✓ pg 8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses ✓ pg 9
Discussion		
Key results	18	Summarise key results with reference to study objectives ✓ pg 9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✓ pg 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✓ pg 10-13
Generalisability	21	Discuss the generalisability (external validity) of the study results ✓ pg 12-13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.