Supplementary Information

Understanding Metabolic Alterations in Advanced Stage Chronic Kidney Disease Patients by NMR-based metabolomics

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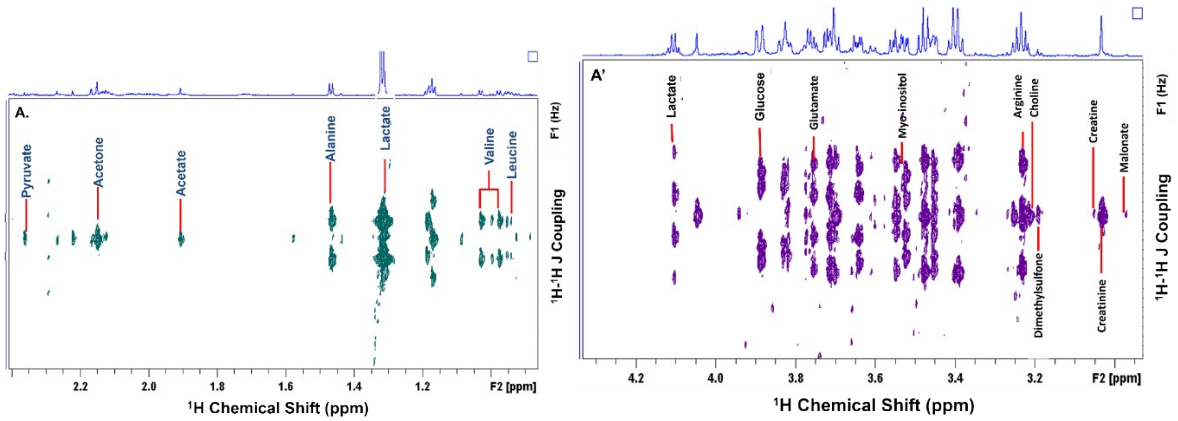
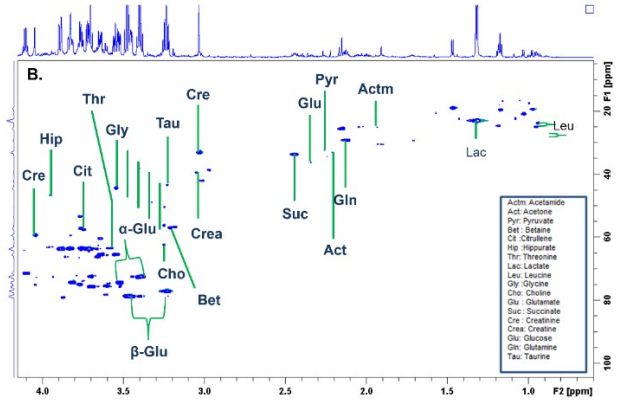
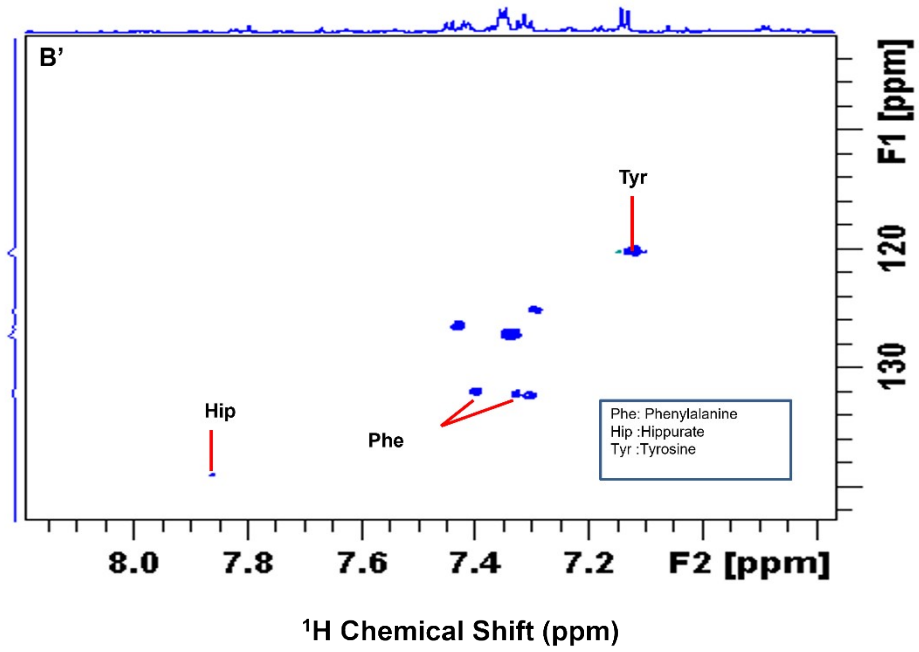
Supplementary Figures







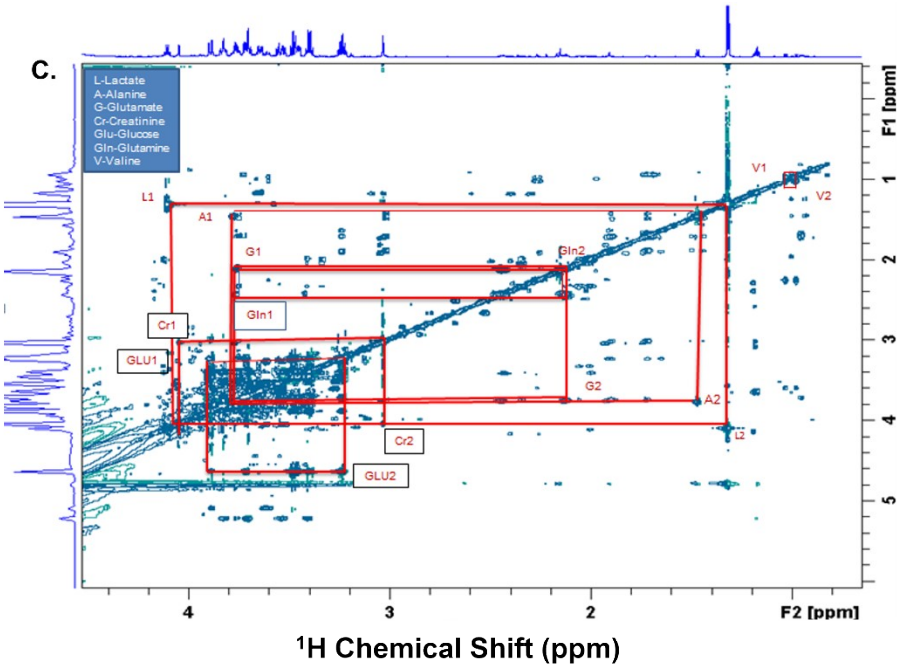
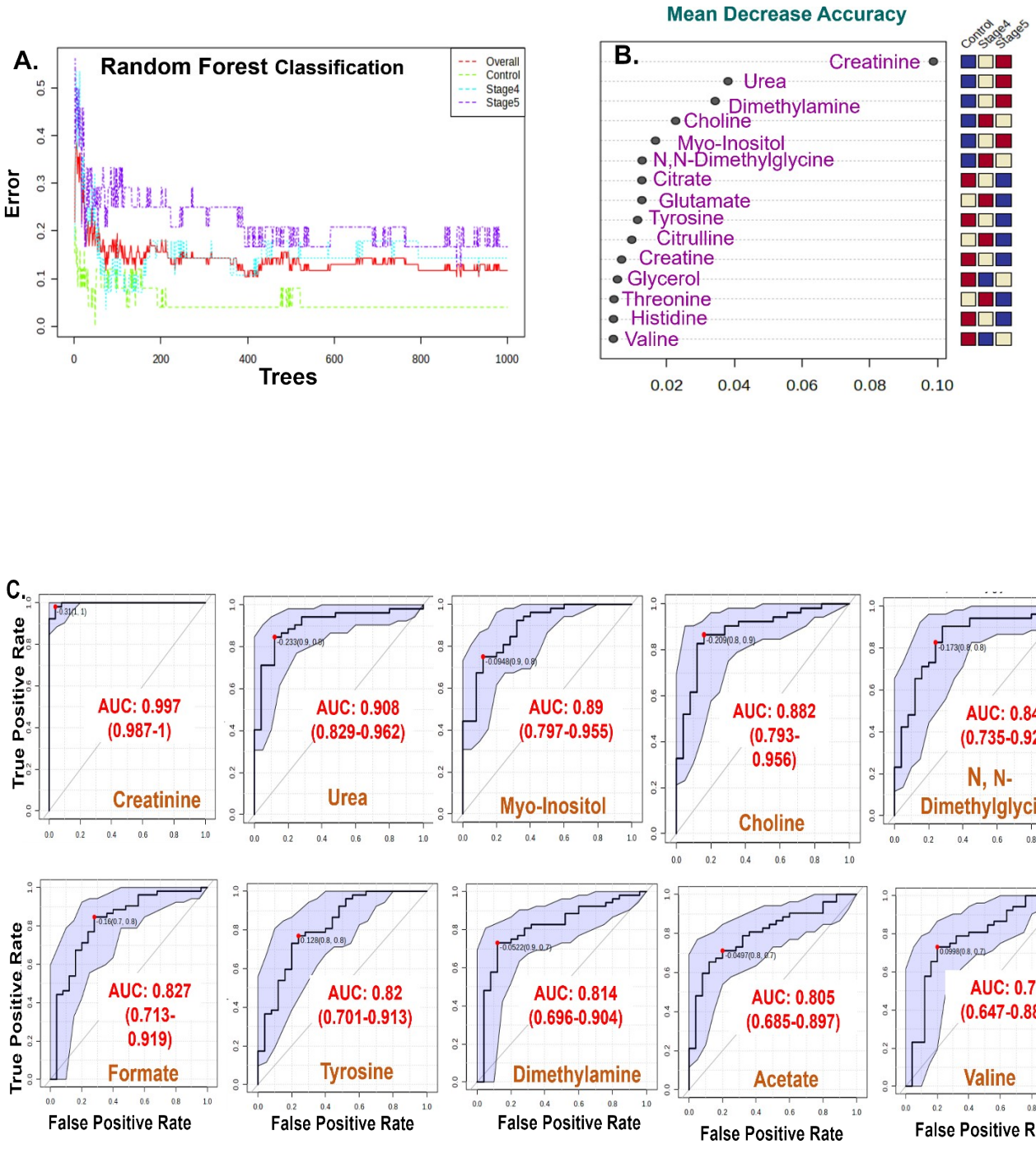


Fig S1: The metabolite specific assignment of 1D 1H CPMG NMR peaks have been achieved making composite use of (A,A',B,C) 2D J-resolved two-dimensional(2D) J-resolved spectroscopy (JRES, coupling-resolved; the spectral regions from δ(0.8-2.2) and δ(3.0-4.2) ppm are expanded in (A), (A') respectively,(B) two-dimensional TOCSY (total proton-proton correlation spectroscopy), (C) and ,(C') HSQC(heteronuclear single quantum correlation)


A.
Random Forest Classification
Overall
Control
Stage4
Stage5
Error
Trees
B.
Mean Decrease Accuracy
Creatinine
Urea
Dimethylamine
Choline
Myo-Inositol
N,N-Dimethylglycine
Citrate
Glutamate
Tyrosine
Citrulline
Creatine
Glycerol
Threonine
Histidine
Valine
Control
Stage4
Stage5
C.
True Positive Rate
AUC: 0.997 (0.987-1)
Creatinine
AUC: 0.908 (0.829-0.962)
Urea
AUC: 0.89 (0.797-0.955)
Myo-Inositol
AUC: 0.882 (0.793-0.956)
Choline
AUC: 0.841 (0.735-0.925)
N, N-Dimethylglycine
AUC: 0.827 (0.713-0.919)
Formate
AUC: 0.82 (0.701-0.913)
Tyrosine
AUC: 0.814 (0.696-0.904)
Dimethylamine
AUC: 0.805 (0.685-0.897)
Acetate
AUC: 0.77 (0.647-0.887)
Valine
False Positive Rate

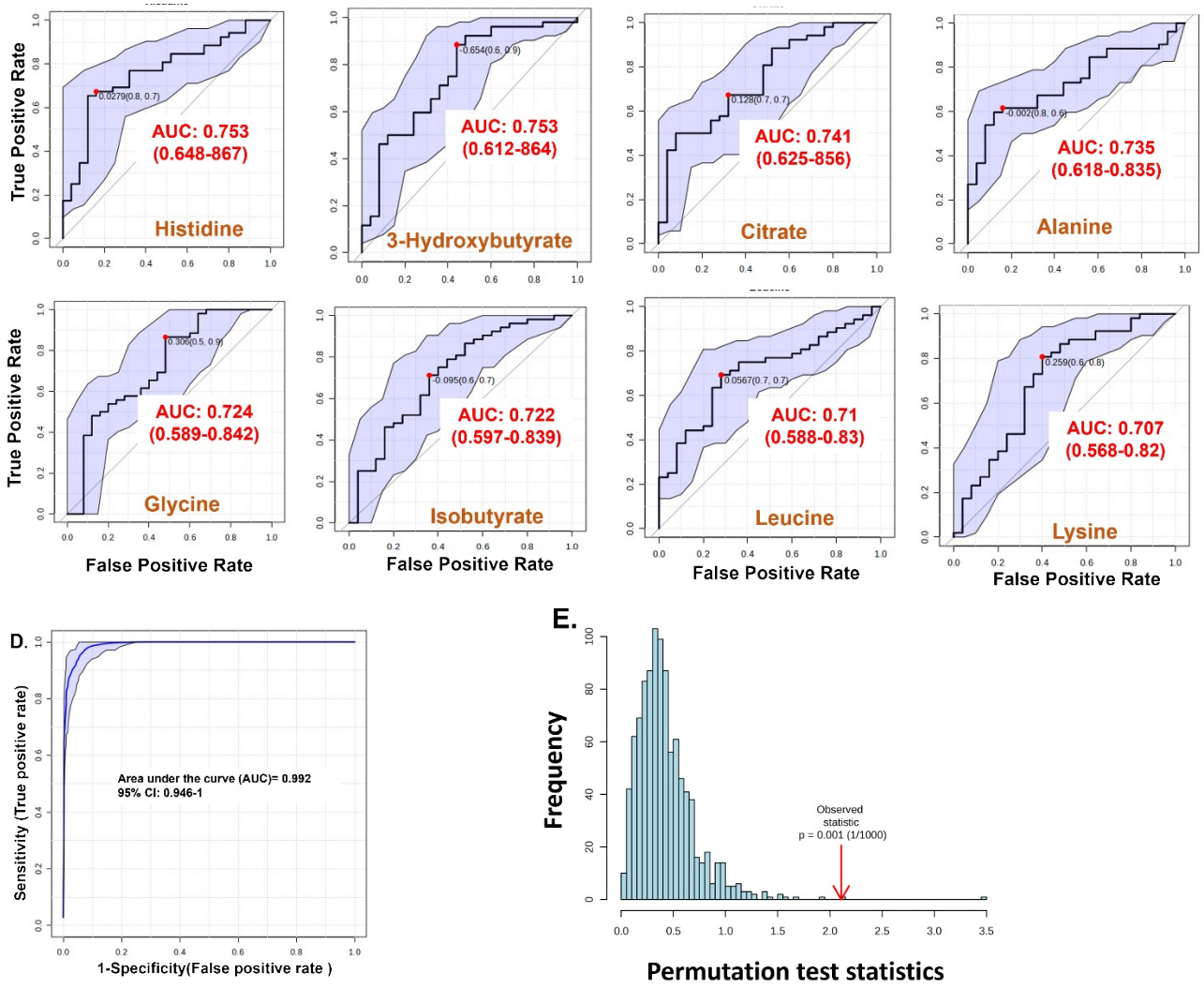


Fig S2: Random-forest (RF) classification model showing cumulative error rates measured for each class using RF machine learning algorithm. The overall error rate is shown as the red line and other color lines represent the error rates for each class as indicated. **(A)** The out-of-bag (OOB) error for the RF model was found to be 11.7%, suggesting moderate prediction accuracy of the RF model, that is, 88.3%. **(B)** The Mean Decrease Accuracy score reflects the impact of excluding a specific feature on the overall model's accuracy. **(C)** Univariate Receiver Operator Characteristics (ROC) curve plot in which Creatinine, Urea, Myo-inositol, Choline, N, N-dimethylglycine, Formate, Tyrosine, Dimethylamine, Acetate have an area under the curve (AUC) greater than 0.8, which shows high diagnostic potential. AUCs and computed confidence intervals have been shown for each biomarker in the figure. **(D)** The multivariate exploratory ROC curve analysis aims to assess the performance of biomarker models developed through automated feature selection. ROC curves are generated using Monte Carlo cross-validation (MCCV) with balanced subsampling. The figure displays the corresponding AUCs and confidence intervals for each model. It demonstrates a classification performance with an Area Under the Curve (AUC) of 0.999 and a 95% Confidence Interval (CI) of 0.946, highlighting the combined diagnostic accuracy of 16 biomarkers in the model. **(E.)** The performance of the PLS-DA model with (1000 permutation Iteration) is $p < 0.001$, indicating the significance of both models.

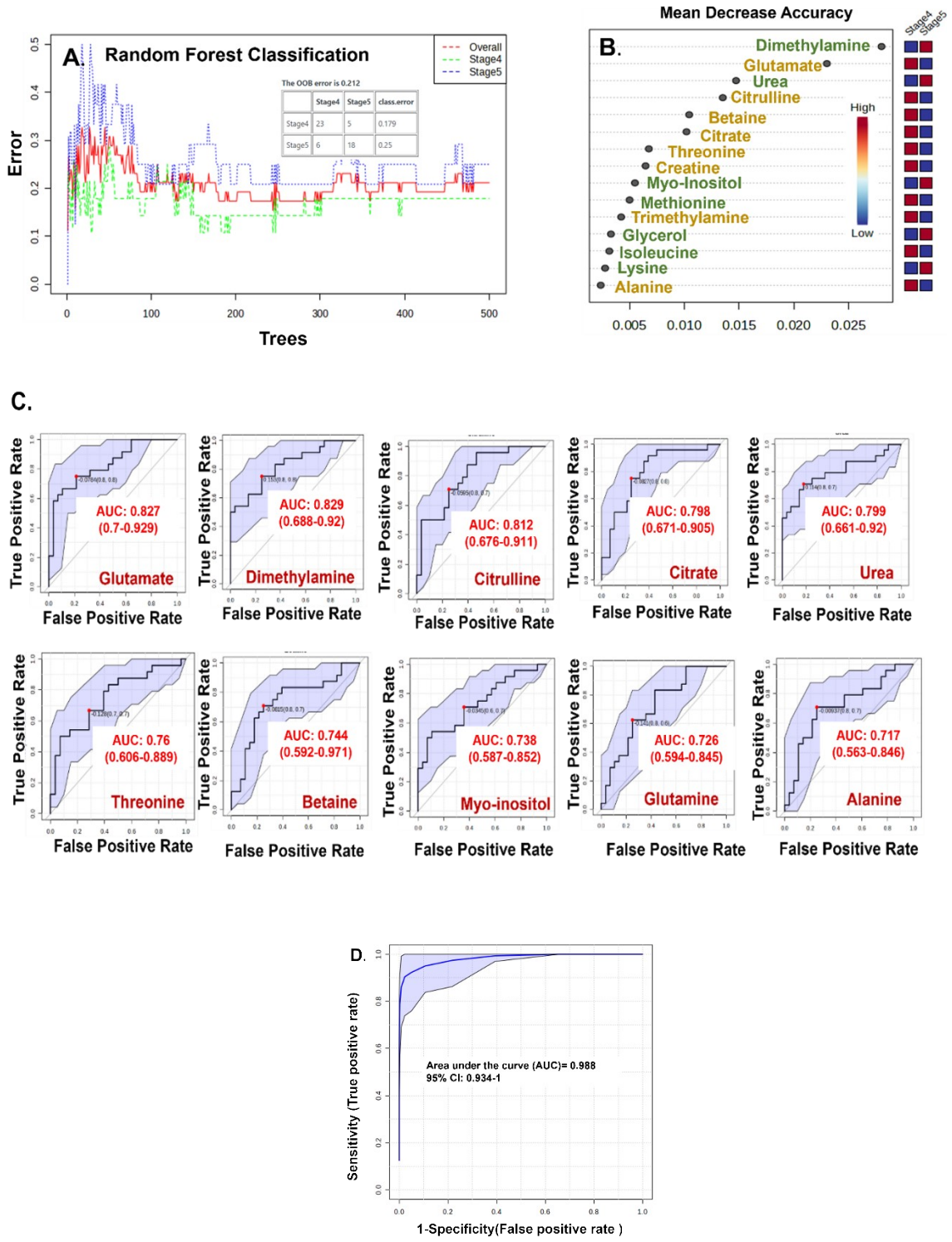


Fig S3: Serum metabolic profile of patients within advanced stage CKD S4 (n=32) & S5 (n=21) compared using Random-forest classification methods. **(A.)** Random Forest was generated using spectral features with an out-of-box error of 21.2%, suggesting a model accuracy of 79.8%. **(B.)** Discriminatory metabolites were identified from the MDA plot produced through RF classification analysis. **(C.)** Univariate ROC curve analysis for evaluating diagnostic potential between severe stage S4,and S5 CKD patients . (AUROC values with standard error and 95% CI are displayed in Fig.) Glutamate, Dimethylamine, and Citrulline show AUC >0.8. **(D.)** Multivariate ROC analysis using 10 Significant metabolites having AUC 0.988, C.I is 0.934-1.

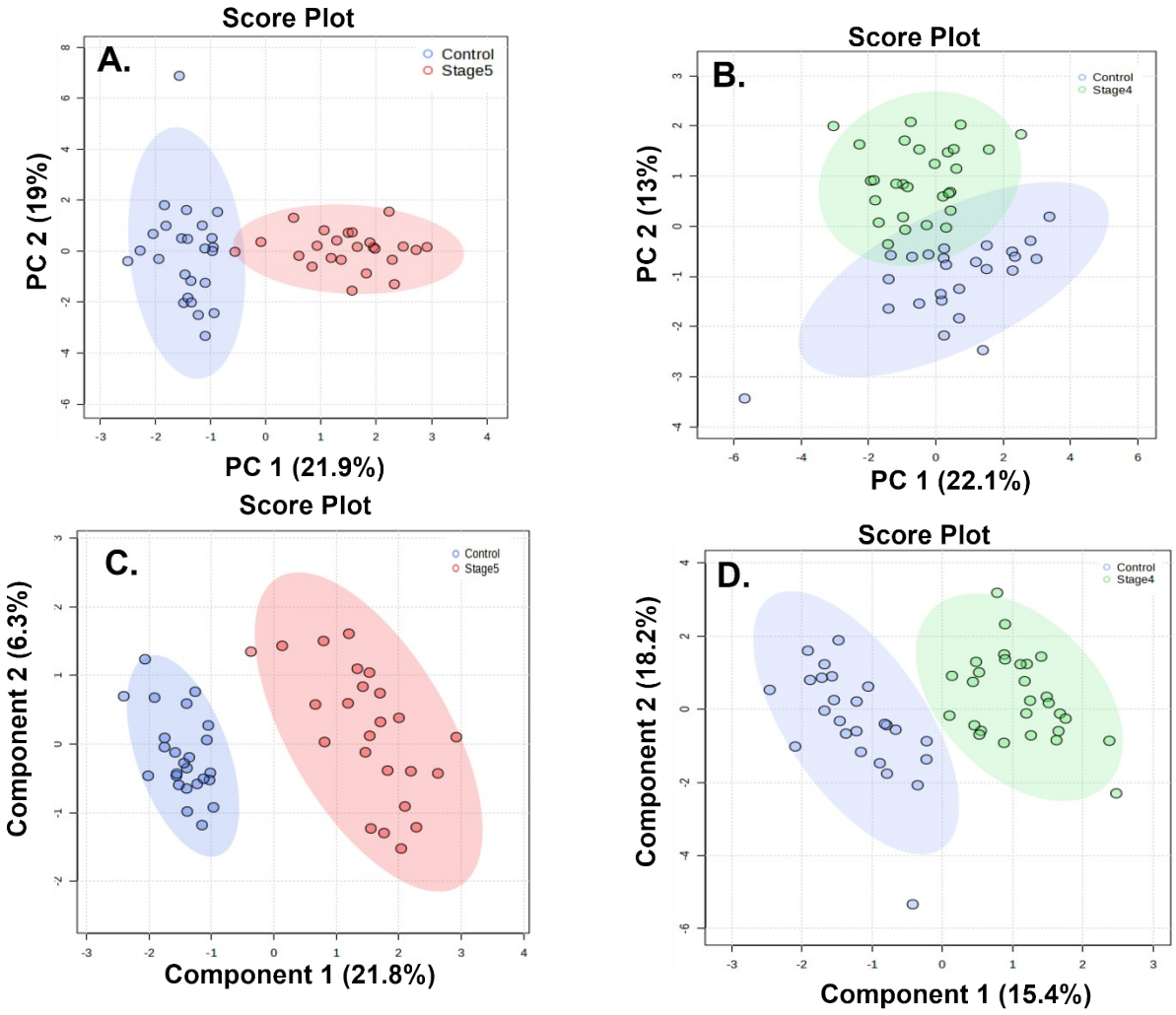


Fig S4: Multivariate analysis illustrating metabolic distinctions between healthy controls and CKD stages. (A, B) PCA score plots: (A) Control vs Stage 5, (B) Control vs Stage 4. (C, D) PLS-DA score plots: () Control vs Stage 5, (D) Control vs Stage 4.

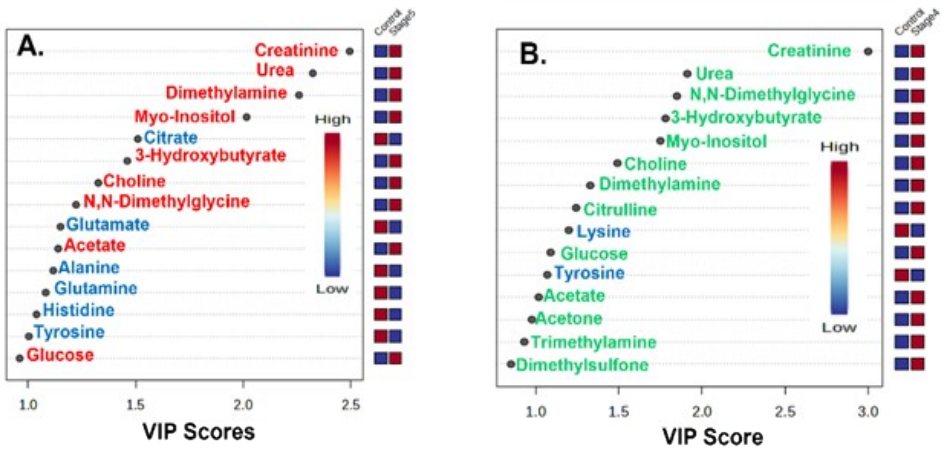


Fig S5: VIP score plots identifying key metabolites contributing to group separation. (A) Control vs Stage 5, (B) Control vs Stage 4. Colour scale represents relative metabolite concentration from low (blue) to high (red)

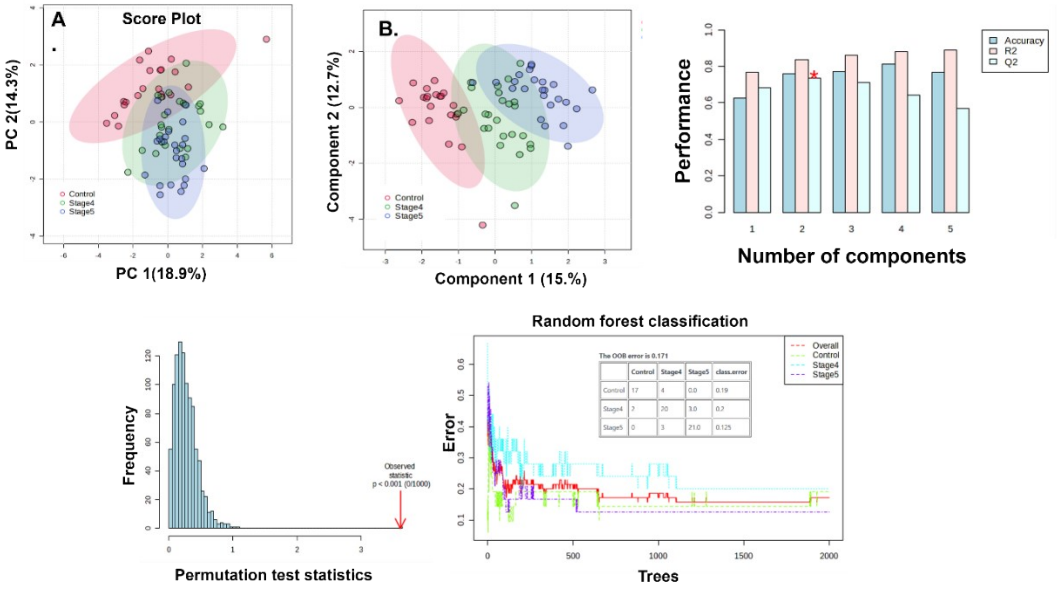


Fig S6: Multivariate and machine learning analysis of serum metabolic profiles across Control, Stage 4 (S4), and Stage 5 (S5) groups, without creatine metabolite

(A) PCA score plot shows partial separation between groups. (B) PLS-DA score plot demonstrates improved class discrimination. (C) 5-fold cross-validation of PLS-DA model with accuracy, R^2, and Q^2 metrics. (D) Permutation test (n = 1000) confirms model significance ($p < 0.001$). (E) Random forest classification error plot with an out-of-bag (OOB) error rate of 0.171, indicating reliable group prediction. Class-wise errors: Control = 0.10, S4 = 0.19, S5 = 0.123.

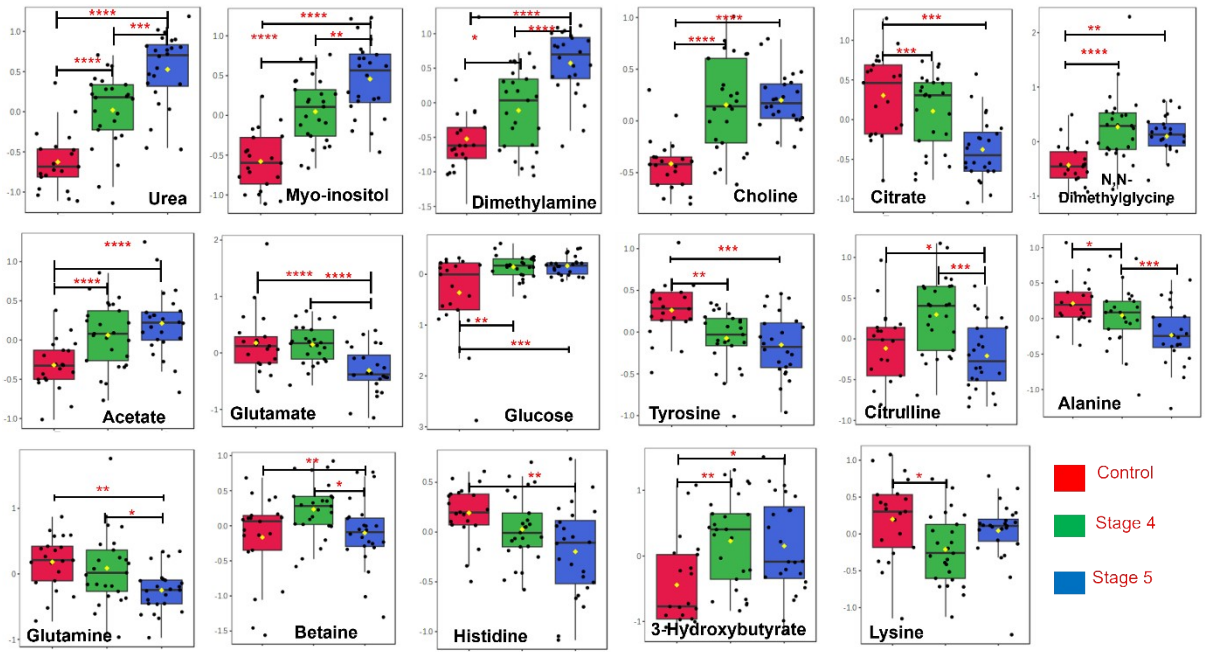


Fig S7. Differential serum metabolite levels across Control, Stage 4 (S4), and Stage 5 (S5) CKD groups after removing creatinine. Box-plots represent normalized intensities of 17 metabolites showing significant variation among the three groups ($p < 0.05$, one-way ANOVA with post-hoc correction). Urea, myo-inositol, dimethylamine, choline, and N, N-dimethylglycine displayed progressive elevation from Control to S5, reflecting metabolic dysregulation with disease severity. In contrast, citrate, glutamate, and histidine showed a declining trend. Notably, glucose and betaine remained relatively stable across stages. Colour codes: Control (red), S4 (green), S5 (blue).Asterisks denote statistically significant differences between groups: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***),$p < 0.0001$ (****).

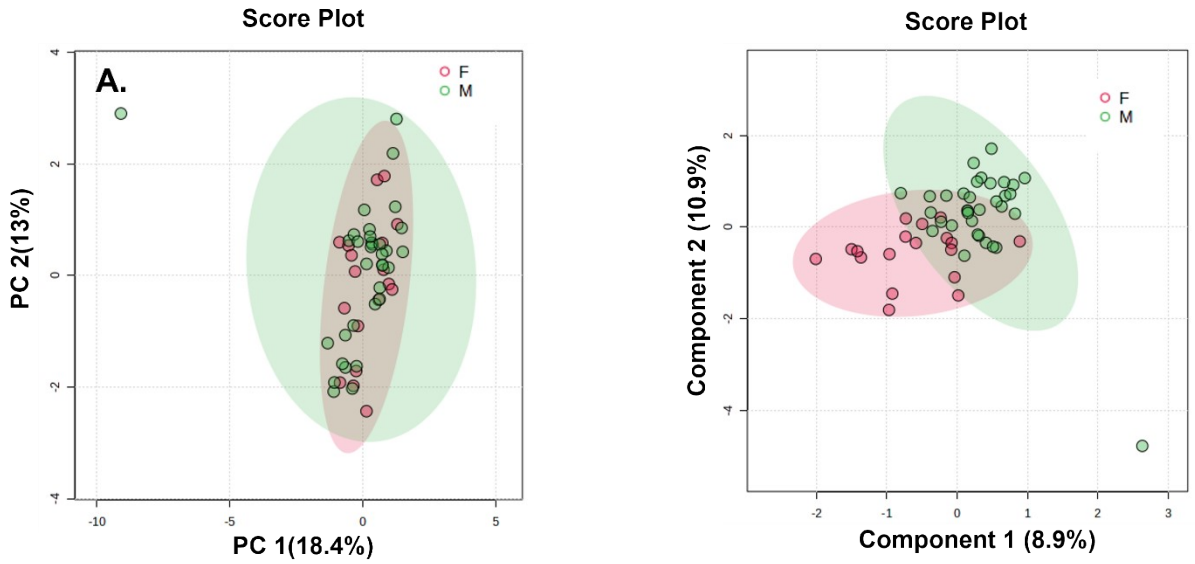


Fig S8: A) PCA (left) and B) PLS-DA (right) score plots evaluating the influence of sex on serum metabolomic profiles. Samples were grouped as male (green) and female (pink). No distinct clustering by sex was observed, indicating minimal sex-related variation in the dataset.

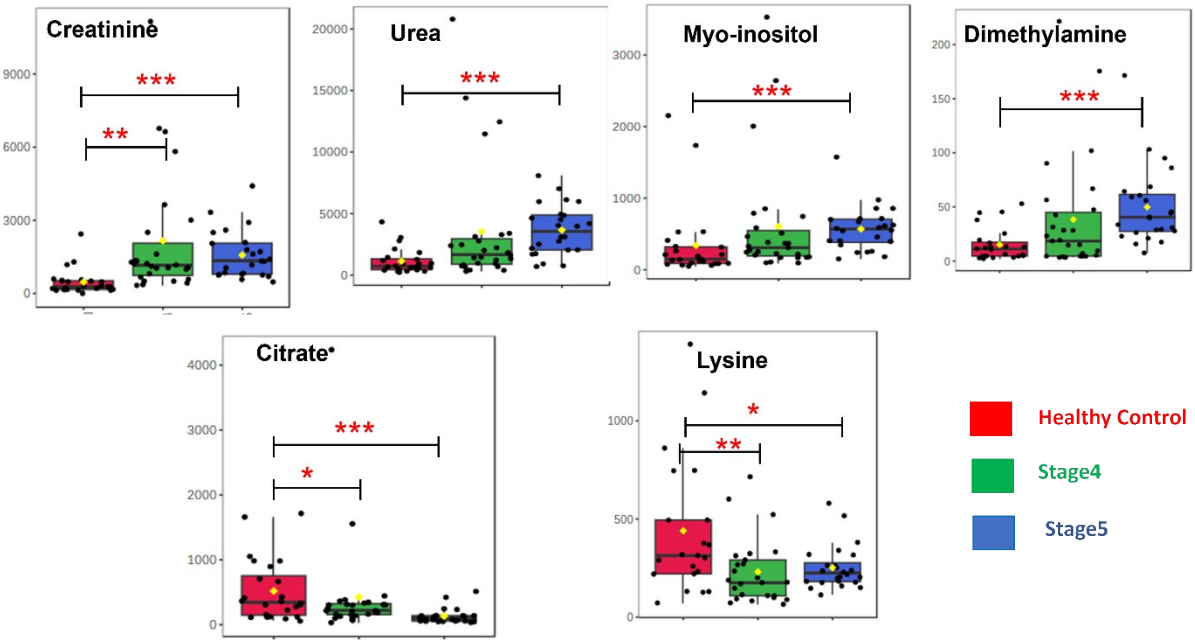
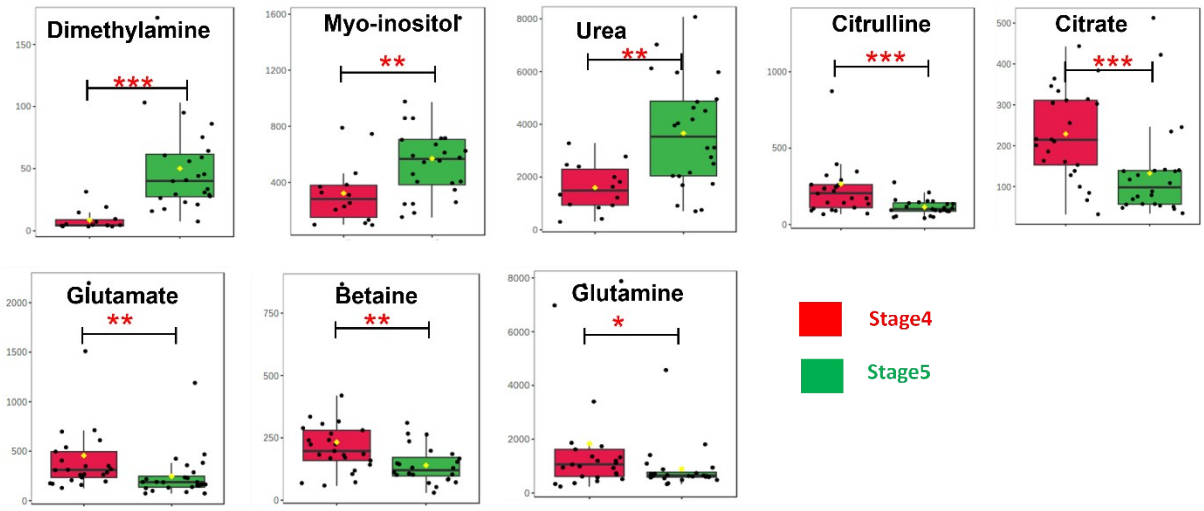


Fig. S9: Boxplots showing the original concentrations of selected serum metabolites in healthy controls (red), CKD stage 4 (green), and CKD stage 5 (blue) groups. Asterisks denote statistically significant differences between groups based on Tukey's HSD test: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).



FigS10: Box plots showing the original concentrations of selected serum metabolites in Stage 4 (red) and Stage 5 (green) CKD groups. Asterisks denote statistically significant differences between groups: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

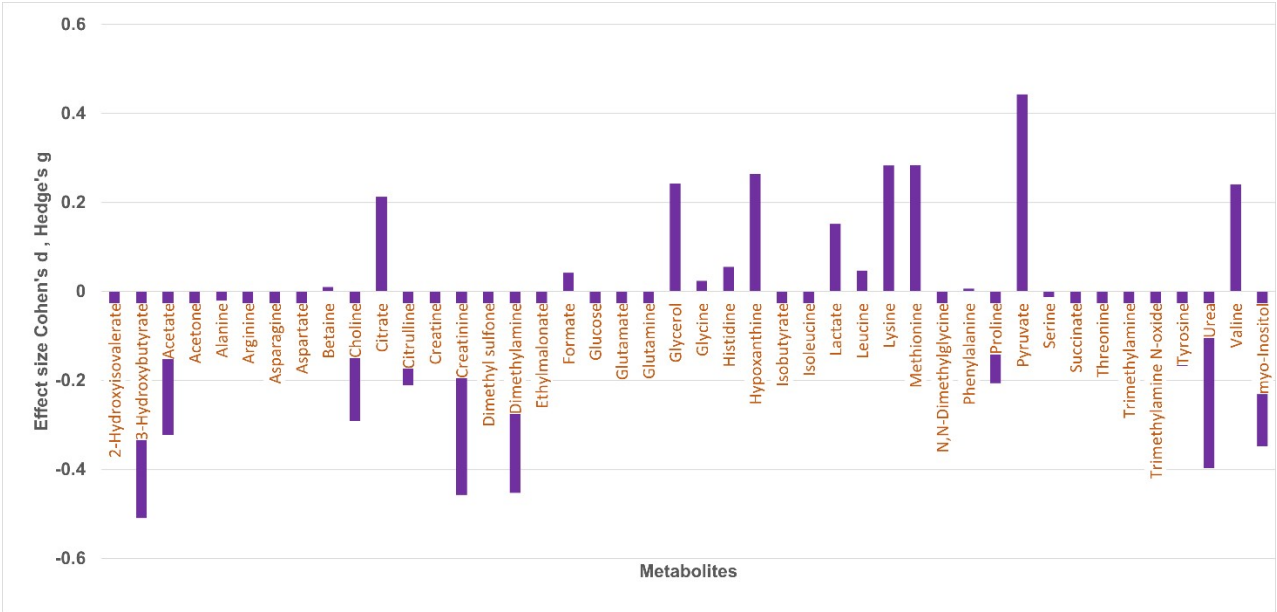


Fig. S11: Bar graph shows Cohen's d effect sizes for metabolites distinguishing control and disease groups. Positive values indicate higher levels in controls, while negative values indicate elevation in disease. Metabolites with (d) > 0.6 reflect strong group separation and potential biomarker relevance.

Supplementary Tables

Table S1: Metabolites identified in serum is listed here are confirmed by 1D ^{1}H NMR, 2D *J*-RES, HSQC, and TOSCY with the help of HMDB, CHENOMX

S. No	Metabolites	Multiplicity (^{1}H)	^{13}C	JRES	Confirmation
1.	3-Hydroxybutyrate	1.19(d) 2.30(dd)	24.4 49.12	6.26 14.36 , 6.29	1D , HSQC,JRES , TOSCY
2.	2-Hydroxyisovalerate	0.83(d)			1D , HSQC,JRES
3.	Acetate	1.90 (s)	26.28	-	1D , HSQC,JRES
4.	Acetone	2.21(s)	33.18		1D , HSQC,JRES
5.	Alanine	1.46(d) 3.76(q)	19.12 53.52	7.2	1D , HSQC,JRES , TOSCY
6.	Arginine	3.76(t) 3.23(t) 1.89(m) 1.67(m)	57.26 43.32 30,49 26.46	6.93 6.11	1D , HSQC,JRES , TOSCY
7.	Aspartate	2.68(dd) 2.82(dd) 3.91(dd)	39.3 39.4 55.1	17.45, 8.85 3.72 3.75	1D , HSQC,JRES , TOSCY
8.	Asparagine	2.86(dd) 2.96(dd) 4.0(dd)	37.3 37.4 54.1	7.89, 4.20	1D , HSQC,JRES , TOSCY
9.	Betaine	3.28(s)	56.16		1D , HSQC,JRES
10.	Citrate	2.55(d) 2.70(d)	40.6 40.6		1D , HSQC,JRES , TOSCY

11.	Creatine	3.04(s) 3.94(s)	39.55 54.16		1D , HSQC,JRES
12.	Creatinine	3.03(s) 4.04(s)	30.5 56.8		1D , HSQC,JRES
13.	Choline	3.19(s)	59.34		1D , HSQC,JRES
14.	Dimethylamine	2.72(s)	37.3		1D , HSQC,JRES
15.	DimethylSuflone	3.14(s)	44.23		1D , HSQC,JRES
16.	Ethylmalonate	2.99 1.71 0.89	62.30 26.13 14.54		1D , HSQC,JRES
17.	Formate	8.44(s)	174		1D , HSQC,JRES
18.	Glucose	3.40(m) 3.52(dd) 3.70(t) 35.82(m) 5.22(d)	72.39 74.52 75.6 63.32 94.88		1D , HSQC,JRES , TOSCY
19.	Glutamate	3.74(dd) 2.34(t) 2.08 1.84(td)	57.64 36.35 29.81	6.2 6.8 6.8, 6.2	1D , HSQC,JRES , TOSCY
20.	Glutamine	3.76(t) 2.43(m) 2.12(m)	57.23 33.92 29.28	6.21 - -	1D , HSQC,JRES , TOSCY 1D , HSQC,JRES
21.	Glycine	3.54(s)	44.30		1D , HSQC,JRES
22.	Histidine	7.05 7.79	120.23 140.34		1D , HSQC,JRES
23.	Hippurate	7.82(dd) 7.62(tt) 7.54 3.95(d)	129.93 134.91 131.54 46.75	8.41 , 1.24 7.47, 1.52 5.84	1D , HSQC,JRES , TOSCY
24.	Hypoxanthine	8.20(s) 8.21(s)			1D
25.	Isobutyrate	1.04(d) 2.37(m)	22.00 39.57	7.12	1D , HSQC,JRES , TOSCY
26.	Isoleucine	0.94(t) 1.02(d)	14.2 17.36	7.5 6.5	1D , HSQC,JRES , TOSCY
27.	Lactate	1.31(d) 4.11(q)	23.01 71.46	7.00 6.91	1D , HSQC,JRES , TOSCY
28.	Leucine	0.93(d) 0.94(d)	23.78 24.93	7.30 6.40	1D , HSQC,JRES

					, TOSCY
29.	Lysine	1.46(m) 1.71(m) 1.84(m) 3.01(t)	22.7 29.6 32.2 42.2		1D , HSQC,JRES
30.	Methionine	3.84(s)	56.84		
31.	Myo-inositol	3.37(t) 3.53(dd) 3.63(t) 4.06(t)	77.15 73.95 75.13 74.93	9.57 9.94 , 3.80	1D , HSQC,JRES , TOSCY
32.	N, N-Dimethylglycine	2.91(s) 3.70(s)	46.34 62.66		1D , HSQC,JRES
33.	Proline	1.99(m) 2.06(m) 2.34(m) 3.33(dt) 3.41(dt) 4.12(dd)	26.45 31.84 31.72 48.95 48.95 64.0	- - 14.02,7.1 11.65,7.0 8.63 , 6.42	1D , HSQC,JRES , TOSCY
34.	Pyruvate	2.35(s)	29.3		1D , HSQC, TOSCY
35.	Phenylalanine	3.12(dd) 3.26(dd) 7.33(m) 7.35(m) 7.40(m)	39.10 39.11 132.11 130.37 131.84		1D , HSQC, TOSCY
36.	Succinate	2.42(s)	36.72		1D , HSQC,JRES
37.	Trimethylamine	2.86(s)	48.0		1D , HSQC,JRES
38.	Trimethylamine N-oxide	3.25(s)	62.19		1D , HSQC,JRES
39.	Threonine	3.58(d) 1.31(d) 4.25(m)	63.46 6.5 68.91	4.81 6.76	1D , HSQC,JRES , TOSCY
40.	Tyrosine	7.18(d) 6.89(d) 3.93(t)	132.01 119.10 58.98	6.3	1D , HSQC,JRES , TOSCY
41.	Urea	5.78(s)			
42.	Valine	0.99(d) 1.02(d)	19.50 20.85	7.03 7.08	

Table S2: Serum metabolic entities exhibiting statistically significant differences between the three study groups (HC vs S4 and S5) evaluated using ANOVA statistics based on Fisher's least significant difference (LSD, a commonly used post-hoc test). The analysis has been performed using the ANOVA Statistical analysis module of Metaboanalyst. subjects)

Metabolites	f.value	p.value	-log10(P)	FDR	Fisher's LSD
Creatinine	79.368	3.87E-19	18.412	1.70E-17	Stage4 - Control; Stage5 - Control
Urea	42.155	6.02E-13	12.22	1.33E-11	Stage4 - Control; Stage5 - Control; Stage5 - Stage4
myo-Inositol	34.51	2.58E-11	10.588	3.79E-10	Stage4 - Control; Stage5 - Control; Stage5 - Stage4
Dimethylamine	26.189	2.51E-09	8.6004	2.76E-08	Stage4 - Control; Stage5 - Control; Stage5 - Stage4
Choline	23.293	1.42E-08	7.8464	1.25E-07	Stage4 - Control; Stage5 - Control
Citrate	16.687	1.04E-06	5.9818	7.65E-06	Control - Stage5; Stage4 - Stage5
N,N-Dimethylglycine	14.354	5.40E-06	5.2677	3.39E-05	Stage4 - Control; Stage5 - Control
Tyrosine	13.259	1.20E-05	4.9214	6.59E-05	Control - Stage4; Control - Stage5
Acetate	12.325	2.40E-05	4.6201	0.000106	Stage4 - Control; Stage5 - Control
Glutamate	12.316	2.42E-05	4.6171	0.000106	Control - Stage5; Stage4 - Stage5
Histidine	11.215	5.57E-05	4.2543	0.000223	Control - Stage4; Control - Stage5; Stage4 - Stage5
Citrulline	10.71	8.22E-05	4.0852	0.000301	Stage4 - Control; Stage4 - Stage5
Alanine	10.575	9.13E-05	4.0396	0.000309	Control - Stage5; Stage4 - Stage5
Glucose	7.9025	0.000775	3.1105	0.002413	Stage4 - Control; Stage5 - Control
Glutamine	7.8304	0.000823	3.0847	0.002413	Control - Stage5; Stage4 - Stage5
3-Hydroxybutyrate	6.8511	0.001863	2.7298	0.005123	Stage4 - Control; Stage5 - Control
Lysine	5.5621	0.005619	2.2504	0.014542	Control - Stage4
Threonine	5.4398	0.00625	2.2041	0.015277	Control - Stage5; Stage4 - Stage5
Methionine	5.3142	0.006974	2.1565	0.01615	Control - Stage5
Leucine	4.5744	0.013393	1.8731	0.029465	Control - Stage4; Control - Stage5

Table S3: Diagnostic potential features identified by Univariate ROC plot in study groups CKD advanced stage (S4, S5) patients and HC subjects.

Metabolites	AUC	P-value	FC
Creatinine	0.996154	5.72E-20	-1.4163
Urea	0.905385	1.19E-10	-

			0.65577
myo-Inositol	0.887692	7.34E-10	-0.61312
Choline	0.882308	1.62E-09	0.06587
N,N-Dimethylglycine	0.840769	2.77E-06	-1.1709
Formate	0.819231	0.028525	0.49594
Tyrosine	0.817692	3.26E-06	1.3973
Dimethylamine	0.811538	2.81E-06	-0.24792
Acetate	0.801538	1.44E-05	0.21652
Valine	0.771538	0.119439	1.588
Histidine	0.76	0.000266	1.2103
3-Hydroxybutyrate	0.755385	0.000374	-0.43998
Citrate	0.745385	0.000332	1.3365
Alanine	0.733077	0.001362	0.94434
Glycine	0.721538	0.076353	0.6437
Isobutyrate	0.713846	0.03059	0.25314
Leucine	0.708462	0.003773	1.025
Lysine	0.703077	0.004506	1.2306

Table S4: The Pearson r correlation of creatinine with other NMR-based serum metabolic features. The correlation analysis was performed using the Pattern Hunter feature of the Statistical analysis module of Metaboanalyst v 6.0. (Control vs disease)

Metabolites	correlation	t-stat	p-value	FDR
Creatinine	1	Inf	0	0
Dimethylamine	0.62641	5.6824	6.79E-07	1.43E-05
Urea	0.36918	2.8089	0.007074	0.099037
myo-Inositol	0.33462	2.5109	0.015323	0.16089
Lactate	-0.3113	-2.3163	0.02468	0.20731
Tyrosine	-0.28045	-2.066	0.044031	0.30822
Alanine	-0.26182	-1.9183	0.060795	0.36477
Glutamate	-0.21544	-1.5601	0.12506	0.65654
Creatine	0.16992	1.2192	0.22848	0.78543
Citrate	-0.16632	-1.1926	0.23864	0.78543
Lysine	0.16567	1.1879	0.24049	0.78543
Choline	0.15595	1.1164	0.26958	0.78543
Hypoxanthine	-0.14667	-1.0485	0.29946	0.78543
Leucine	-0.1429	-1.0209	0.31221	0.78543
Pyruvate	0.13849	0.98879	0.32753	0.78543
Glucose	-0.12498	-	0.37736	0.78543

		0.89069		
Trimethylamine	0.12495	0.89048	0.37747	0.78543
Threonine	-0.12169	-0.86693	0.39012	0.78543
3-Hydroxybutyrate	0.11894	0.84707	0.40099	0.78543
Methionine	-0.11678	-0.83143	0.40969	0.78543
Succinate	0.11493	0.81806	0.4172	0.78543
Proline	0.11489	0.81783	0.41733	0.78543
Glycerol	0.10125	0.71962	0.47511	0.78543
Betaine	0.098376	0.69901	0.48778	0.78543
Valine	0.09663	0.68649	0.49558	0.78543
Acetone	0.092406	0.65622	0.51469	0.78543
Aspartate	0.09099	0.64608	0.52118	0.78543
Glycine	-0.09046	-0.64229	0.52362	0.78543
Trimethylamine N-oxide	0.052459	0.37146	0.71187	0.93511
Isobutyrate	-0.04972	-0.35198	0.72633	0.93511
Acetate	0.045793	0.32415	0.74718	0.93511
Isoleucine	-0.0438	-0.31001	0.75784	0.93511
2-Hydroxyisovalerate	0.040839	0.28901	0.77377	0.93511
Dimethyl sulfone	0.040172	0.28429	0.77736	0.93511
Ethylmalonate	-0.03981	-0.28172	0.77932	0.93511
Glutamine	-0.03572	-0.25272	0.80152	0.93511
N,N-Dimethylglycine	0.022124	0.15648	0.87629	0.94905
Histidine	-0.01841	-0.13023	0.89691	0.94905
Formate	0.016729	0.11831	0.9063	0.94905
Asparagine	-0.01354	-0.09576	0.92409	0.94905
Phenylalanine	-0.01312	-0.09277	0.92646	0.94905
Arginine	-0.00036	-0.00255	0.99798	0.99798

Table S5: The top 10 significant metabolites were identified by using t-test between the serum sample of S4 and S5.

Metabolites	t.stat	p.value	-log 10(p)	FDR
Glutamate	4.9207	9.77E-06	5.0101	0.00041
Dimethylamine	-4.5413	3.55E-05	4.4503	0.00075

Citrate	4.2413	9.59E-05	4.0184	0.00134
Urea	-3.7783	0.000421	3.3755	0.00442
myo-Inositol	-3.3556	0.001519	2.8186	0.01276
Threonine	3.1166	0.003029	2.5187	0.02121
Betaine	2.9282	0.005121	2.2906	0.029
Glutamine	2.9005	0.005524	2.2577	0.029
Alanine	2.8001	0.007242	2.1401	0.0338
Citrulline	2.6236	0.011505	1.9391	0.04832

Table S6: Diagnostic potential of biomarker identified by univariate ROC analysis between S4 and S5.

Metabolites	AUC	P-val	FC
Glutamate	0.831845	9.77E-06	2.6538
Dimethylamine	0.821429	3.55E-05	0.38694
Citrate	0.797619	9.59E-05	2.0873
Urea	0.791667	0.000421	0.84614
Threonine	0.754464	0.003029	2.2781
Betaine	0.738095	0.005121	1.9177
myo-Inositol	0.732143	0.001518	0.72007
Glutamine	0.723214	0.005524	2.1822
Alanine	0.720238	0.007242	1.9688
Citrulline	0.712798	0.03588	1.9875

Table S7: The table below shows the detailed results from the pathway analysis. In particular, the Total is the total number of compounds in the pathway; the Hits is the actual matched number from the user-uploaded data; the Raw p is the original p value calculated from the enrichment analysis; the Holm p is the p-value adjusted by Holm-Bonferroni method; the FDR p is the p value adjusted using False Discovery Rate; the Impact is the pathway impact value calculated from pathway topology analysis

Metabolic Pathway	Total	Hits	Raw P	Impact	Holm adjust	FDR
Phenylalanine, Tyrosine, Tryptophan Biosynthesis	4	2	4.46E-44	1	1.01E-02	8.92E-04
Glycine, Serine, Threonine metabolism	33	8	1.43E-04	0.6	4.29E-03	4.86E-04
Alanine, Aspartate, Glutamate metabolism	28	8	6.50E-03	0.53	8.45E-02	9.29E-03
Arginine Biosynthesis	14	6	5.43E-07	0.42	2.12E-05	8.43E-06
Starch and Sucrose metabolism	18	1	4.06E-04	0.42	1.01E-02	8.29E-04

Table S8: Serum metabolic entities exhibiting statistically significant differences between the three study groups after removing Creatinine metabolite (HC vs S4 and S5) evaluated using ANOVA statistics based on Fisher's least significant difference (LSD, a commonly used post-hoc test). The analysis has been performed using the ANOVA Statistical analysis module of Metaboanalyst. subjects)

Metabolies	f.value	p.value	-log10(P)	FDR	Fisher's LSD
Urea	39.422	4.82E-12	11.317	2.07E-10	Stage4 - Control; Stage5 - Control; Stage5 - Stage4
myo-Inositol	34.905	4.11E-11	10.386	8.83E-10	Stage4 - Control; Stage5 - Control; Stage5 - Stage4
Dimethylamine	24.25	1.19E-08	7.923	1.71E-07	Stage4 - Control; Stage5 - Control; Stage5 - Stage4
Choline	22.266	3.85E-08	7.4145	4.14E-07	Stage4 - Control; Stage5 - Control
Citrate	13.32	1.35E-05	4.8705	0.00011589	Control - Stage5; Stage4 - Stage5
N,N-Dimethylglycine	12.109	3.24E-05	4.4893	0.0002323	Stage4 - Control; Stage5 - Control
Acetate	11.058	7.08E-05	4.1499	0.00043496	Stage4 - Control; Stage5 - Control
Glutamate	9.3559	0.000261	3.5834	0.0014029	Control - Stage5; Stage4 - Stage5
Glucose	9.1025	0.00031835	3.4971	0.001521	Stage4 - Control; Stage5 - Control
Tyrosine	8.8492	0.00038877	3.4103	0.0016717	Control - Stage4; Control - Stage5
Citrulline	8.5865	0.00047885	3.3198	0.0018719	Stage4 - Control; Stage4 - Stage5
Alanine	7.6024	0.0010579	2.9755	0.0037909	Control - Stage5; Stage4 - Stage5
Histidine	6.3627	0.0029514	2.53	0.0097623	Control - Stage5; Stage4 - Stage5
3-Hydroxybutyrate	6.1372	0.003569	2.4475	0.010962	Stage4 - Control; Stage5 - Control
Glutamine	5.8636	0.0045013	2.3467	0.012904	Control - Stage5; Stage4 - Stage5
Betaine	4.4815	0.014904	1.8267	0.040056	Stage4 - Control; Stage4 - Stage5
Lysine	4.283	0.017765	1.7504	0.044935	Control - Stage4

Table S9: The top 7 significant metabolites were identified by using an ANOVA between the serum samples of S4, S5, and Healthy Control using original concentrations.

Metabolites	f.value	p.value	-log10(P)	FDR	Tukey's HSD
Dimethylamine	10.457	0.00011155	3.9525	0.0033139	Stage5-Control; Stage5-Stage4
Citrate	9.9589	0.00016344	3.7866	0.0033139	Stage4-Control; Stage5-Control
Creatinine	9.5408	0.00022595	3.646	0.0033139	Stage4-Control; Stage5-Control
myo-Inositol	8.5967	0.000475	3.3233	0.005225	Stage5-Control
Urea	7.8434	0.00086973	3.0606	0.0076536	Stage5-Control
Lysine	5.7459	0.0049761	2.3031	0.031278	Stage4-Control; Stage5-Control

Table S10: The top 8 significant metabolites were identified by using a t-test between the serum samples of S4 and S5 using original concentrations.

Metabolies	f.value	p.value	-log10(P)	FDR
Citrate	465	0.000721	3.1421	0.018629
Citrulline	463	0.000847	3.0722	0.018629
Dimethylamine	144	0.00187	2.7282	0.02314
Glutamate	453	0.002288	2.6406	0.02314
Urea	152	0.00263	2.5801	0.02314
Betaine	438	0.005229	2.2816	0.038343
myo-Inositol	167	0.007233	2.1407	0.045462
Glutamine	401	0.043718	1.3593	0.22253

Table S11: Metabolites showing effect sizes of all metabolites (|Cohen's d|) between control and disease groups with 95% Confidence interval (C.I), . Positive values indicate higher abundance in controls, while negative values reflect elevation in the disease group.

Metabolite	Effect size Cohen's d , Hedge's g)	C.I	Confidence cofficient
2-Hydroxyisovalerate	-0.162	[-0.647]-[-0.322]	95%
3-Hydroxybutyrate	-0.509	[-1]-[-0.019]	95%
Acetate	-0.322	[-0.809] - [0.164]	95%
Acetone	-0.09	[-0.574] - [0.394]	95%
Alanine	-0.02	[-0.503] - [0.464]	95%
Arginine	-0.051	[-0.535] - [0.433]	95%

Asparagine	-0.099	[-0.583] - [0.385]	95%
Aspartate	-0.089	[-0.573] - [0.395]	95%
Betaine	0.01	[-0.474] - [0.493]	95%
Choline	-0.291	[-0.776] - [0.195]	95%
Citrate	0.213	[-0.271] - [0.698]	95%
Citrulline	-0.211	[-0.696] - [0.274]	95%
Creatine	-0.056	[-0.54] - [0.428]	95%
Creatinine	-0.457	[-0.946] - [0.032]	95%
Dimethyl sulfone	-0.289	[-0.775] -[0.197]	95%
Dimethylamine	-0.453	[-0.942] - [0.036]	95%
Ethylmalonate	-0.249	[-0.734] - [0.236]	95%
Formate	0.042	[-0.442] - [0.526]	95%
Glucose	-0.148	[-0.632] -[0.336]	95%
Glutamate	-0.046	[-0.53] - [0.438]	95%
Glutamine	-0.046	[-0.53] - [0.438]	95%
Glycerol	0.243	[-0.243] - [0.728]	95%
Glycine	0.024	[-0.46] -[0.508]	95%
Histidine	0.055	[-0.429] -[0.539]	95%
Hypoxanthine	0.264	[-0.222] - [0.749]	95%
Isobutyrate	-0.161	[-0.645] -[0.323]	95%
Isoleucine	-0.074	[-0.558] -[0.41]	95%
Lactate	0.152	[-0.332] -[0.637]	95%
Leucine	0.047	[-0.437] -[0.53]	95%
Lysine	0.283	[-0.203] - [0.769]	95%
Methionine	0.284	[-0.202] - [0.77]	95%
N,N-Dimethylglycine	-0.352	[-0.839] -[0.135]	95%
Phenylalanine	0.006	[-0.478] - [0.49]	95%
Proline	-0.206	[-0.69] - [0.279]	95%
Pyruvate	0.443	[-0.046] - [0.932]	95%
Serine	-0.012	[-0.495] - [0.472]	95%
Succinate	-0.176	[-0.66] -[0.309]	95%
Threonine	-0.115	[-0.599] -[0.369]	95%
Trimethylamine	-0.115	[-0.599] -[0.369]	95%
Trimethylamine N-oxide	-0.167	[-0.651] - [0.317]	95%
Tyrosine	-0.167	[-0.318] -[0.651]	95%
Urea	-0.397	[-0.885] - [0.09]	95%
Valine	0.241	[-0.244] -[0.726]	95%
myo-Inositol	-0.348	[-0.835] -[0.139]	95%