Comparison of region-specific metabolic changes in kidney of HFD/STZ-induced diabetic rats and db/db mice based on air-flow-assisted desorption electrospray ionization-mass spectrometry imaging

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Table S1. AFADESI-MSI parameter

AFADESI-MSI	Value
Spray voltage	±5KV
Transmission tube voltage	±3KV
Solvent composition	CAN/H_2O (8:2v/v)
Spray solvent flow rate	7uL/min; 5uL/min
Nebulizer pressure	0.6MPa; 0.5MPa
X-axis scan speed	0.2mm/s
Y-axis inter-row distance	0.2mm
Z vertical distance	0
Delay start time	6s
Scan range	100-1000 Da
Mass resolution	120,000
Capillary temperature	350 °C

Table S2. Discriminating metabolites obtained by negative AFADESI-MSI analysis of

renal sections from control and HFD/STZ-induced diabetic nephropathy (DN) group.

Species	Metabolite identification	Elemental composition	Adduct	Theoretical m/z	Measured m/z	Delta (ppm)	MS/MS	Distribution	FC(W)
Amino acid	Citrate	$C_6H_8O_7$	[M-H]⁻	191.0197	191.0204	3.72	129, 173, 191	OM	0.86
Amino- acid	Aspartate	$C_4H_7NO_4$	[M-H]⁻	132.0302	132.0300	-1.36	71, 88, 115, 132	W, OM, IM	0.67
Amino- acid	Glutamate	$C_5H_9NO_4$	[M-H]⁻	146.0459	146.0457	-1.64	102, 128, 146	OM	0.81
Amino acid	Histidylaspartic acid	$C_{10}H_{14}N_4O_5$	[M-H]⁻	269.0891	269.0887	-1.52	269	C	2.27
DG	DG (38:3) (OH)	$C_{41}H_{74}O_6$	[M-H]⁻	661.5413	661.5425	1.84	661	OM	1.28
FAHFA	FAHFA (34:0)	$C_{34}H_{66}O_4$	[M-H]⁻	537.4888	537.4896	1.44	255, 537	W, C, OM	2.70
FAHFA	FAHFA (34:1)	$C_{34}H_{64}O_4$	[M-H]⁻	535.4732	535.4741	1.60	253, 535	IM	0.94
Fatty acid	Linolenic acid	$C_{18}H_{30}O_2$	[M-H]⁻	277.2173	277.2178	1.70	59, 277	W, C, OM, IM	0.52
Fatty acid	Linoleic acid	$C_{18}H_{32}O_2$	[M-H]⁻	279.233	279.2334	1.40	59, 26, 1279	W, C, OM, IM	0.68
Fatty acid	Oleic acid	$C_{18}H_{34}O_2$	[M-H]⁻	281.2486	281.2489	1.11	59, 237, 281	W, C, OM	2.24
Fatty acid	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	[M-H]⁻	301.2173	301.2184	3.69	257, 283, 301	W, C, OM, IM	0.48
Fatty acid	Eicosenoic acid	$C_{20}H_{38}O_2$	[M-H]⁻	309.2799	309.2802	0.95	265, 309	W, C, OM, IM	1.99
Fatty acid	Docosahexaenoic acid	$C_{22}H_{32}O_2$	[M-H]⁻	327.2330	327.2328	-0.70	67,121, 229, 283, 309, 327	W, C, OM, IM	0.47
Fatty acid	Nervonic acid	$C_{24}H_{46}O_2$	[M-H]⁻	365.3425	365.3430	1.24	365	W, C, OM, IM	2.19
LysoPE	LysoPE (16:0)	$C_{21}H_{44}NO_7P$	[M-H]⁻	452.2783	452.2799	3.59	214, 255, 253, 452	W, C, OM	0.48
LysoPE	LysoPE (20:4)	$C_{25}H_{44}NO_7P$	[M-H]⁻	500.2783	500.2786	0.61	140, 214, 259, 303, 439, 500	W, C, OM	0.63
LysoPG	LysoPG (18:1)	$C_{24}H_{47}O_9P$	[M-H]⁻	509.2885	509.2896	2.15	152, 281,509	W, C, OM	3.97

LysoPI	LysoPI (16:0)	$C_{25}H_{49}O_{12}P$	[M-H]⁻	571.2889	571.2913	4.22	152, 255, 241, 391, 571	C	0.58
Nucleoside& Nucleotide& Nitrogenbase	AMP	$C_{10}H_{14}N_5O_7P$	[M-H]⁻	346.0558	346.0566	2.20	78, 96, 134, 211, 346	OM	0.89
Nucleoside& Nucleotide& Nitrogenbase	Inosine	$C_{10}H_{12}N_4O_5$	[M-H]⁻	267.0735	267.0746	4.01	107, 267	W, C, IM	0.54
Organic-acid	2(R)-hydroxydocosanoic acid	$C_{22}H_{44}O_3$	[M-H]⁻	355.3218	355.3221	0.71	309, 355	OM	3.88
Organic-acid	Taurine	$C_2H_7NO_3S$	[M-H]⁻	124.0074	124.0073	-0.48	80, 124	W, C, OM, IM	0.58
PA	PA (34:2)	$C_{37}H_{69}O_8P$	[M-H]⁻	671.4657	671.4663	0.92	671	W, C, OM	0.41
PE	PE (34:1)	$C_{39}H_{76}NO_8P$	[M-H]⁻	716.5236	716.5250	1.91	716	W, C, OM	0.52
PE	PE (36:2)	$C_{41}H_{78}NO_8P$	[M-H]⁻	742.5392	742.5402	1.38	253, 742	W, C	0.57
PE	PE (36:4)	$C_{41}H_{74}NO_8P$	[M-H]⁻	738.5079	738.5093	1.88	227, 738	W, C, OM	0.56
PE	PE (38:4)	$C_{43}H_{78}NO_8P$	[M-H]⁻	766.539	766.5401	1.39	196, 281, 766	W, C, OM	0.66
PE	PE(p-38:6)	$C_{43}H_{74}NO_7P$	[M-H]⁻	746.513	746.5113	-2.32	746	W, C, OM, IM	0.31
PE	PE (38:6)	$C_{43}H_{74}NO_8P$	[M-H]⁻	762.5079	762.5087	1.08	484, 762	W, C, OM, IM	0.33
PE	Phosphoryle thanolamine	$C_2H_8NO_4P$	[M-H]⁻	140.0118	140.0117	-0.71	78, 140	OM	0.78
PG	PG (32:0)	$C_{38}H_{75}O_{10}P$	[M-H]⁻	721.5025	721.5026	0.16	255, 721	W, C	0.46
PG	PG (34:2)	$C_{40}H_{75}O_{10}P$	[M-H]⁻	745.5025	745.5036	1.42	255, 279, 745	C	0.69
PG	PG (36:2)	$C_{42}H_{79}O_{10}P$	[M-H]⁻	773.5338	773.5347	1.21	279, 283, 773	W, C, OM	2.52
PG	PG (36:3)	$C_{42}H_{77}O_{10}P$	[M-H]⁻	771.5182	771.5189	0.93	283, 771	W, C, OM	3.36
PG	PG (36:4)	$C_{42}H_{75}O_{10}P$	[M-H]⁻	769.5025	769.5051	3.37	255, 303, 769	C	0.79
PG	PG (40:6)	$C_{46}H_{79}O_{10}P$	[M-H]⁻	821.5338	821.5354	1.97	303, 821	W, C, OM	3.04
PG	PG (40:8)	$C_{46}H_{75}O_{10}P$	[M-H]⁻	817.5025	817.5037	1.44	303, 817	W, C, OM, IM	0.43
PG	PG (42:10)	$C_{48}H_{75}O_{10}P$	[M-H]⁻	841.5025	841.5035	1.21	303, 841	W, OM, IM	0.53
PG	PG (44:12)	$C_{50}H_{75}O_{10}P$	[M-H]⁻	865.5025	865.5036	1.22	327, 865	W, C, OM, IM	0.22
PI	PI (34:2)	$C_{43}H_{79}O_{13}P$	[M-H]⁻	833.5186	833.5190	0.52	279, 833	W, C, OM	0.35
PI	PI (18:1(9Z)/18:1(9Z))	$C_{45}H_{83}O_{13}P$	[M-H]⁻	861.5499	861.5511	1.41	281, 861	W, C, OM	0.51
PI	PI (36:4)	$C_{45}H_{79}O_{13}P$	[M-H]⁻	857.5186	857.5193	0.79	303, 391, 553, 857	W, C, OM	0.50
PI	PI (38:5)	$C_{47}H_{81}O_{13}P$	[M-H]⁻	883.5342	883.5350	0.95	303, 417, 883	C	0.66
PI	PI (40:6)	$C_{49}H_{83}O_{13}P$	[M-H]⁻	909.5499	909.5499	-0.01	283, 909	W, C, OM, IM	0.33
PS	PS (34:1)	$C_{40}H_{76}NO_{10}P$	[M-H]⁻	760.5134	760.5139	0.66	227, 760	W, C, OM	0.63
PS	PS (36:1)	$C_{42}H_{80}NO_{10}P$	[M-H]⁻	788.5447	788.5456	1.12	152, 227, 701, 788	C	0.75
PS	PS (36:2)	$C_{42}H_{78}NO_{10}P$	[M-H]⁻	786.5291	786.5298	0.85	152, 255, 699, 786	W, C, OM	0.61
PS	PS (36:4)	$C_{42}H_{74}NO_{10}P$	[M-H]⁻	782.4978	782.4987	1.18	227, 695, 782	W, C	0.65
PS	PS (40:6)	$C_{46}H_{78}NO_{10}P$	[M-H]⁻	834.5291	834.5322	3.77	303, 834	W, C, OM, IM	0.39
Others	3-O-Sulfogalactosylceramide (d18:1/24:0)	$C_{48}H_{93}NO_{11}S$	[M-H]⁻	890.6397	890.6391	-0.67	890	OM	0.57
Others	Glucose	$C_6H_{12}O_6$	[M-H]⁻	179.0561	179.0563	1.06	161, 179	C, OM	1.27
Others	Chlorphenesin	$C_9H_{11}ClO_3$	[M-H]⁻	201.0324	201.0320	-1.94	59	W, C, OM, IM	0.31
Others	Ajoene	$C_9H_{14}OS_3$	[M-H]⁻	233.0134	233.0138	1.85	78, 233	OM, IM	0.57
Others	3-Hydroxysintaxanthin	$C_{31}H_{42}O_2$	[M-H]⁻	445.3112	445.3118	1.24	445	W, C, OM, IM	3.18
Others	Cholesterol sulfate	$C_{27}H_{46}O_4S$	[M-H]⁻	465.3044	465.3050	1.19	465	W, C, OM, IM	0.50

DG: diacylglycerol; FAHFA: fatty acid esters of hydroxy fatty acids; LysoPE: lysophosphatidyletethanolamine; PA: phosphatidic acid; LysoPG: lysophosphatidylglycerol; LysoPI: lysophosphatidylinositol; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PI: Phosphatidylinositol; PS: phosphatidylserine, AMP: adenosine monophosphate.

W: whole kidney; C: cortex; OM: outer medulla; IM: inner medulla.

Table S3. Discriminating metabolites obtained by positive AFADESI-MSI analysis of renal sections from control and HFD/STZ-induced diabetic nephropathy (DN) group.

Species	Metabolite identification	Elemental composition	Adduct	Theoretical m/z	Measured m/z	Delta (ppm)	MS/MS	Distribution	FC(W)
Choline	Choline	$C_5H_{13}NO$	$[M+H]^+$	104.1070	104.1071	0.76	58, 60, 104	W, C, OM, IM	0.54
Carnitine	L-Carnitine	$C_7H_{15}NO_3$	$[M+H]^+$	162.1122	162.1125	1.79	60, 85, 102, 103, 144, 162	W, C, IM	0.51
Carnitine	Stearoylcarnitine	$C_{25}H_{39}NO_4$	$[M+H]^+$	428.3734	428.3728	-1.50	85, 369, 428;	W, C, IM	3.39
PC	PC (34:1)	$C_{42}H_{82}NO_8P$	$[M+H]^+$	760.5851	760.5842	-1.19	86, 104, 124b, 184, 760	IM	1.47
PC	PC (34:2)	$C_{42}H_{80}NO_8P$	$[M+H]^+$	758.5694	758.5694	0.01	86, 124b, 184, 758	C	0.64
PC	PC (36:1)	$C_{44}H_{86}NO_8P$	$[M+Na]^+$	810.5983	810.5978	-0.56	184, 146	OM	1.64
PC	PC (36:2)	$C_{44}H_{84}NO_8P$	$[M+K]^+$	824.5566	824.5554	-1.48	104, 184	OM	1.29
PC	PC (36:4)	$C_{44}H_{80}NO_8P$	$[M+Na]^+$	804.5514	804.5478	-4.50	184	C	1.20
PE	PE (p-18:0/20:4 (5Z,8Z,11Z, 14Z))	$C_{43}H_{78}NO_7P$	$[M+Na]^+$	774.5408	774.5401	-0.87	-	W, C, OM	1.90
SM	SM (d18:1/16:0)	$C_{39}H_{79}N_2O_6P$	$[M+Na]^+$	725.5568	725.5556	-1.69	502, 542b, 666, 725	W, C	2.91
SM	SM (42:2)	$C_{47}H_{93}N_2O_6P$	$[M+H]^+$	813.6844	813.6828	-1.93	–	OM	0.89
Others	Creatine	$C_4H_9N_3O_2$	$[M+H]^+$	132.0768	132.0768	0.30	90, 115, 132	W, C	0.15

PC: phosphatidylcholine; PE: phosphatidylethanolamine; SM: sphingomyelin. W: whole kidney; C: cortex; OM: outer medulla; IM: inner medulla.

Table S4. Discriminating metabolites obtained by negative AFADESI-MSI analysis of renal sections from control and db/db diabetic nephropathy (DN) group.

Species	Metabolite identification	Elemental composition	Adduct	Theoretical m/z	Measured m/z	Delta (ppm)	MS/MS	Distribution	FC(W)
Amino- acid	Glutamate	$C_5H_9NO_4$	$[M-H]^-$	146.0459	146.0452	-4.54	85, 100, 102, 128, 129, 146	W, C, OM, IM	0.72
Amino- acid	Histidylaspartic acid	$C_{10}H_{14}N_4O_5$	$[M-H]^-$	269.0891	269.0892	0.39	59b, 269	W, C, OM, IM	14.77
Fatty- acid	Palmitelaidic acid	$C_{16}H_{30}O_2$	$[M-H]^-$	253.2173	253.2178	1.78	253	OM, IM	0.79
Fatty- acid	Linolenic acid	$C_{18}H_{30}O_2$	$[M-H]^-$	277.2173	277.2181	3.01	59, 277	W, C	0.77
Fatty- acid	Eicosenoic acid	$C_{20}H_{38}O_2$	$[M-H]^-$	309.2799	309.2794	-1.46	59, 309	W, C, OM, IM	0.65
Fatty- acid	Arachidonic acid	$C_{20}H_{32}O_2$	$[M-H]^-$	303.233	303.2338	2.48	59, 259, 303	OM	0.90
Fatty- acid	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	$[M-H]^-$	301.2173	301.2183	3.38	59, 257, 301	OM	1.21
LysoPG	LysoPG (18:1)	$C_{24}H_{47}O_9P$	$[M-H]^-$	509.2885	509.2896	2.26	152, 227, 281, 509	W, OM, IM	2.09
LysoPG	LysoPG (18:2)	$C_{24}H_{45}O_9P$	$[M-H]^-$	507.2728	507.2745	3.38	152, 227, 245, 279, 415, 433, 507	W, C, OM, IM	1.73
Nucleoside& Nucleotide& Nitrogenbase	AMP	$C_{10}H_{14}N_5O_7P$	$[M-H]^-$	346.0558	346.0565	2.13	78, 96, 134, 211, 346	W, C, OM, IM	1.88
Nucleoside& Nucleotide& Nitrogenbase	GMP	$C_{10}H_{14}N_5O_8P$	$[M-H]^-$	362.0507	362.0515	2.19	78, 96, 150, 362	OM	1.27
Organic-acid	Succinate	$C_4H_6O_4$	$[M-H]^-$	117.0193	117.0195	1.47	71, 73, 117	W, C, OM	1.92
Organic-acid	Malate	$C_4H_6O_5$	$[M-H]^-$	133.0142	133.0147	3.97	71, 72, 89, 115, 133	OM	0.83
Organic-acid	dobesilic acid	$C_6H_6O_5S$	$[M-H]^-$	188.9863	188.9865	1.18	80, 188	W, C, OM, IM	7.46
PA	PA (16:0/18:1(9Z))	$C_{37}H_{71}O_8P$	$[M-H]^-$	673.4814	673.4801	-1.97	255, 673	W, C, OM, IM	3.32
PA	PA (38:4)	$C_{41}H_{72}O_8P$	$[M-H]^-$	723.49698	723.4943	-3.74	152, 281, 723	W, C, OM	1.39
PA	PA (41:5)	$C_{44}H_{75}O_9P$	$[M-H]^-$	777.5076	777.5060	-2.01	777	W, C, OM, IM	0.06

Species	Metabolite identification	Elemental composition	Adduct	Theoretical m/z	Measured m/z	Delta (ppm)	MS/MS	Distribution	FC(W)
PC	PC (36:4)	$C_{44}H_{80}NO_8P$	[M+Cl]⁻	816.5316	816.5328	1.43	209, 211	W, C, OM	1.54
PE	PE (p-34:1)	$C_{39}H_{76}NO_7P$	[M-H]⁻	700.5287	700.5292	0.73	700	OM	1.40
PE	PE (34:1)	$C_{39}H_{76}NO_8P$	[M-H]⁻	716.5235	716.5253	2.46	716	OM	1.04
PE	PE (36:4)	$C_{41}H_{74}NO_8P$	[M-H]⁻	738.5079	738.5103	3.27	738	C	1.29
PE	PE (36:6)	$C_{41}H_{70}NO_8P$	[M-H]⁻	734.4766	734.4763	-0.47	734	OM	0.98
PE	PE (p-38:4)	$C_{43}H_{78}NO_7P$	[M-H]⁻	750.5443	750.5451	1.08	750	C	1.30
PE	PE (40:1)	$C_{45}H_{86}NO_9P$	[M+Cl]⁻	850.5734	850.5738	0.48	-	IM	1.47
PE	PE (p-40:4)	$C_{45}H_{82}NO_7P$	[M-H]⁻	778.5756	778.5777	2.64	778	W, OM	0.53
PE	PE (p-40:7)	$C_{45}H_{76}NO_7P$	[M-H]⁻	772.5287	772.5294	0.85	327, 444, 772	W, C	0.74
PE	PE (42:1)	$C_{46}H_{89}NO_{12}S$	[M-H]⁻	878.6032	878.6052	2.23	-	W, C, IM	1.58
PG	PG (32:0)	$C_{38}H_{75}O_{10}P$	[M-H]⁻	721.5025	721.5041	2.28	152, 255, 721	W, C, OM	0.28
PG	PG (34:1)	$C_{40}H_{77}O_{10}P$	[M-H]⁻	747.5182	747.5187	0.62	152, 171, 255, 281, 465, 747	W, C, OM, IM	1.55
PG	PG (38:4)	$C_{44}H_{79}O_{10}P$	[M-H]⁻	797.5338	797.5342	0.45	279, 307, 507, 797	C	0.64
PG	PG (40:8)	$C_{46}H_{75}O_{10}P$	[M-H]⁻	817.5025	817.5056	3.77	817	IM	0.90
PI	PI (34:0)	$C_{43}H_{83}O_{13}P$	[M-H]⁻	837.5499	837.5508	2.20	283, 419, 837	OM	1.29
PI	PI (38:4)	$C_{47}H_{83}O_{13}P$	[M-H]⁻	885.5499	885.5519	2.22	419, 303, 439, 581, 599, 885	W, C, IM	1.40
PI	PI (38:5)	$C_{47}H_{81}O_{13}P$	[M-H]⁻	883.5342	883.5361	2.19	303, 883	W, C, IM	1.47
PI	PI (40:6)	$C_{49}H_{83}O_{13}P$	[M-H]⁻	909.5499	909.5522	2.48	283, 419, 625, 909	W, C, OM	0.60
PS	PS (36:1)	$C_{42}H_{80}NO_{10}P$	[M-H]⁻	788.5447	788.5417	-3.78	152, 701, 788	IM	1.34
PS	PS (36:4)	$C_{42}H_{74}NO_{10}P$	[M-H]⁻	782.4978	782.4998	2.58	695, 782	W, C	1.53
Others	Glucose	$C_6H_{12}O_6$	[M+Cl]⁻	215.0328	215.0325	-1.21	59, 71, 87, 119	W, C, OM, IM	5.97
Others	Prinomide	$C_{15}H_{13}N_3O_2$	[M+Cl]⁻	302.0702	302.0701	-0.22	-	W, C, OM, IM	0.51
Others	GSH	$C_{10}H_{17}N_3O_6S$	[M-H]⁻	306.0766	306.0763	-0.99	59, 127	W, C, OM	1.85
Others	Calycanthidine	$C_{23}H_{28}N_4$	[M-H]⁻	359.2241	359.2235	-1.54	359	W, C, OM	0.27
Others	3-O-Sulfogalactosylceramide (d18:1/24:0)	$C_{48}H_{93}NO_{11}S$	[M-H]⁻	890.6397	890.6414	1.85	890	C	0.94

LysoPG: lysophosphatidylglycerol; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PI: Phosphatidylinositol; PS: phosphatidylserine. W: whole kidney; C: cortex; OM: outer medulla; IM: inner medulla.

Table S5. Discriminating metabolites obtained by positive AFADESI-MSI analysis of renal sections from control and db/db diabetic nephropathy (DN) group.

Species	Metabolite identification	Elemental composition	Adduct	Theoretical m/z	Measured m/z	Delta (ppm)	MS/MS	Distribution	FC(W)
Choline	Choline	$C_5H_{13}NO$	[M+H]⁺	104.1070	104.1070	0.39	58, 60, 86, 104	W, OM, IM	0.62
Choline	GPC	$C_8H_{20}NO_6P$	[M+K]⁺	296.0660	296.0656	-1.48	84, 86, 104	C, IM	1.13
Carnitine	Palmitoylcarnitine	$C_{23}H_{45}NO_4$	[M+H]⁺	400.3421	400.3427	1.51	85, 341, 400	W, OM, IM	1.74
Carnitine	Linoleylcarnitine	$C_{25}H_{45}NO_4$	[M+H]⁺	424.3421	424.3434	3.17	85, 424	IM	3.19
Carnitine	Octadecenoylcarnitine	$C_{25}H_{47}NO_4$	[M+H]⁺	426.3578	426.3585	1.56	85, 426	W, OM, IM	3.28
LysoPC	LysoPC (18:0)	$C_{26}H_{54}NO_7P$	[M+H]⁺	524.3711	524.3708	-0.49	86, 104, 124, 184, 240, 258, 341, 506, 524	W, C, OM, IM	2.70
PC	PC (34:0)	$C_{42}H_{84}NO_8P$	[M+H]⁺	762.6007	762.6020	1.74	86, 184, 762	W, C, OM, IM	2.51
PC	PC (34:1)	$C_{42}H_{82}NO_8P$	[M+H]⁺	760.5851	760.5870	2.51	124, 184, 760	W, C, OM, IM	1.90
PC	PC (34:2)	$C_{42}H_{80}NO_8P$	[M+H]⁺	758.5694	758.5711	2.22	184	OM	1.16
PC	PC (36:2)	$C_{44}H_{84}NO_8P$	[M+H]⁺	786.6007	786.6018	1.36	124, 184, 603, 727, 786	W, OM, IM	1.57
PC	PC (36:3)	$C_{44}H_{82}NO_8P$	[M+Na]⁺	806.5670	806.5659	-1.41	184, 623, 747, 806	C, OM, IM	1.13

PC	PC (38:4)	$C_{46}H_{84}NO_8P$	$[M+Na]^+$	832.5827	832.5801	-3.09	184, 649, 773, 832	IM	1.01
PC	PC(p-38:5)	$C_{46}H_{82}NO_7P$	$[M+Na]^+$	814.5721	814.5718	-0.31	184	W, C, OM, IM	0.42
PE	PE(p-36:4)	$C_{41}H_{74}NO_7P$	$[M+H]^+$	724.5276	724.5274	-0.21	724	W, OM	1.99
SM	SM(d18:1/16:0)	$C_{39}H_{79}N_2O_6P$	$[M+K]^+$	741.5307	741.5313	0.83	184, 666	OM	1.07
SM	SM(d18:1/24:1(15Z))	$C_{47}H_{93}N_2O_6P$	$[M+K]^+$	851.6403	851.6398	-0.57	-	OM	1.14
Others	betaine	$C_5H_{11}NO_2$	$[M+H]^+$	118.0862	118.0860	-1.86	58, 59, 74, 100, 118	W, C	1.78

GPC: Glycerophosphocholine; LysoPC: lysophosphatidylcholine; PC: phosphatidylcholine; PE: phosphatidylethan-

olamine; SM: sphingomyelin. W: whole kidney; C: cortex; OM: outer medulla; IM: inner medulla.
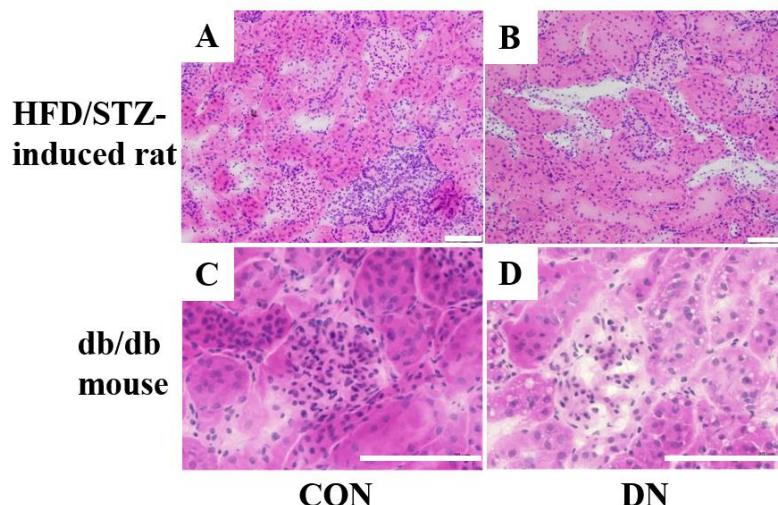
Figure S1. H&E staining in the HFD/STZ-induced diabetic rats (**A, B**) and db/db mice

(**C, D**). CON: control group; DN: diabetic nephropathy group; HFD/STZ-induced rat:

High-Fat diet feeding combined with intraperitoneal injection of low dose of STZ. Scale bar:

100um.
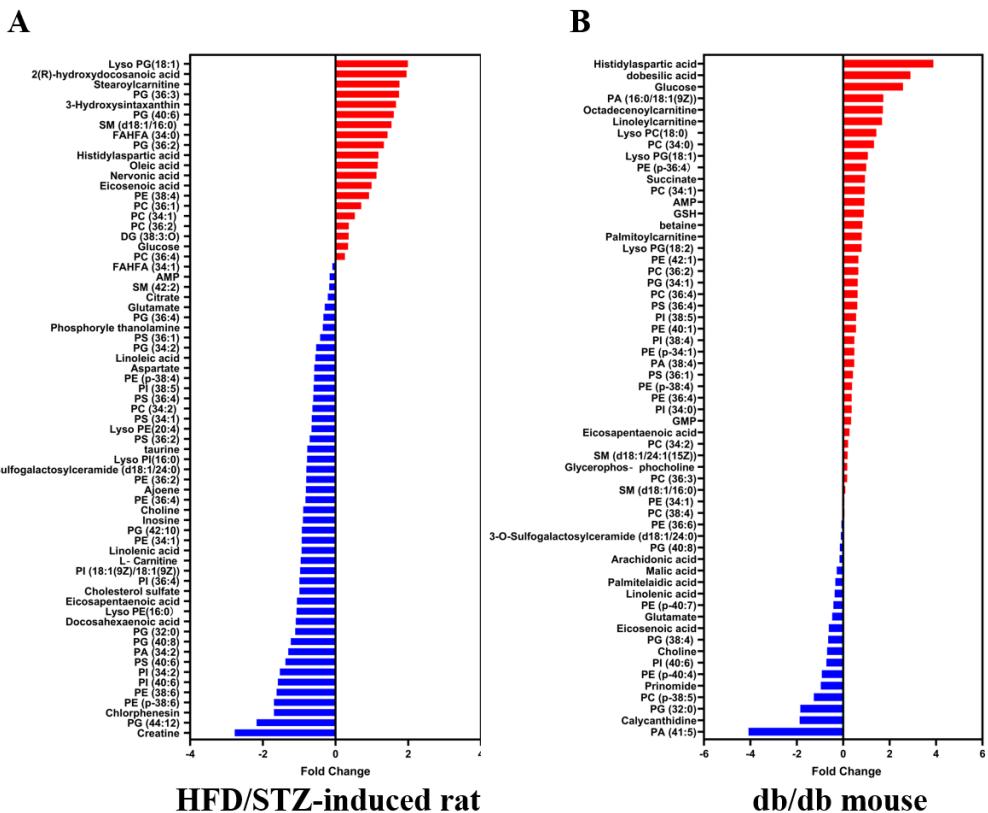
Figure S2. Fold change plots of discriminating metabolites in HFD/STZ-induced diabetic rats versus db/db mice. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PS: phosphatidylserine; PI: phosphatidylinositol; PG: phosphatidylglycerol; DG: diacylglycerol; SM: sphingomyelin; LysoPG: lysophosphatidylglycerol; LysoPI: lysophosphatidylinositol; PA: phosphatidic acid; LysoPC: Lysophosphatidylcholine; FAHFAs: fatty acid esters of hydroxy fatty acids.
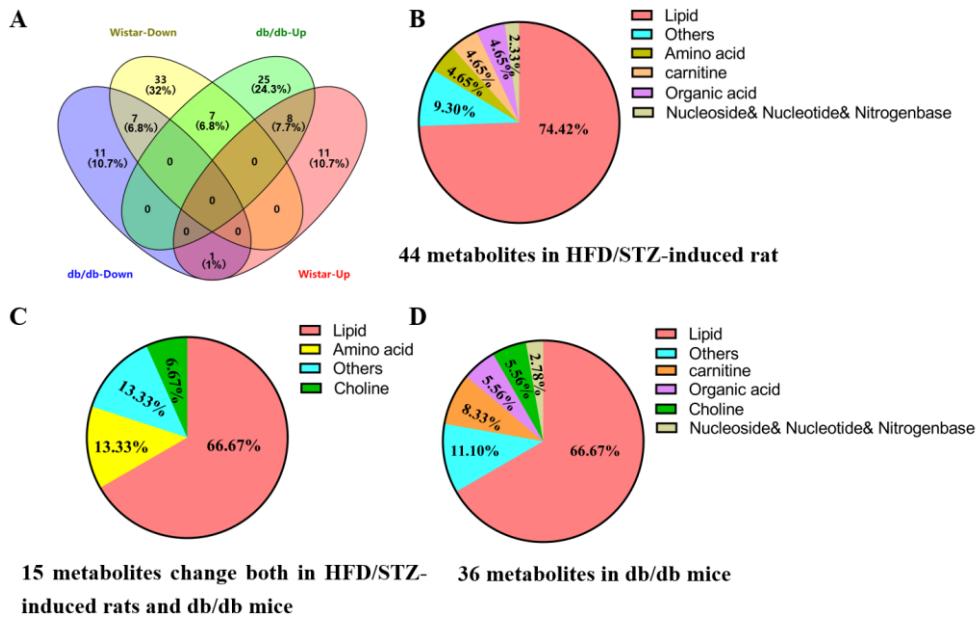
Figure S3. The comparison of discriminating metabolites associated DN between the two models. Wistar-down: Wistar rats discriminating metabolites were down-regulated in the HFD/STZ-induced diabetic rats compared to the control group. Wistar-up: Wistar rats discriminating metabolites were up-regulated in the HFD/STZ-induced diabetic rats compared to the control group. db/db-down: db/db mice discriminating metabolites were down-regulated compared to the control group. db/db-up: db/db mice discriminating metabolites were up-regulated compared to the control group.
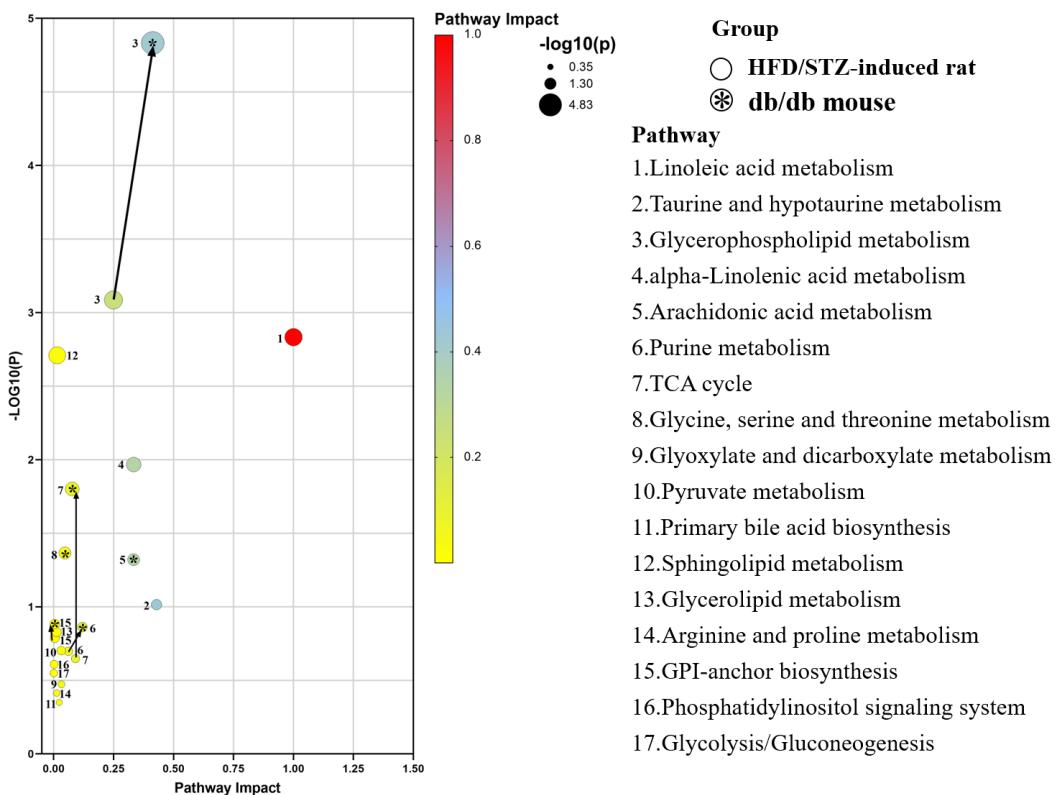
Figure S4. Regulation of discriminating metabolites in HFD/STZ-induced diabetic rats and db/db mice. HFD: High-Fat diet. STZ: Streptozotocin. TCA: tricarboxylic acid. GPI-anchor biosynthesis: Glycosylphosphatidylinositol-anchor biosynthesis metabolism.

DN model of HFD/STZ-induced rat   **DN model of db/db mouse**
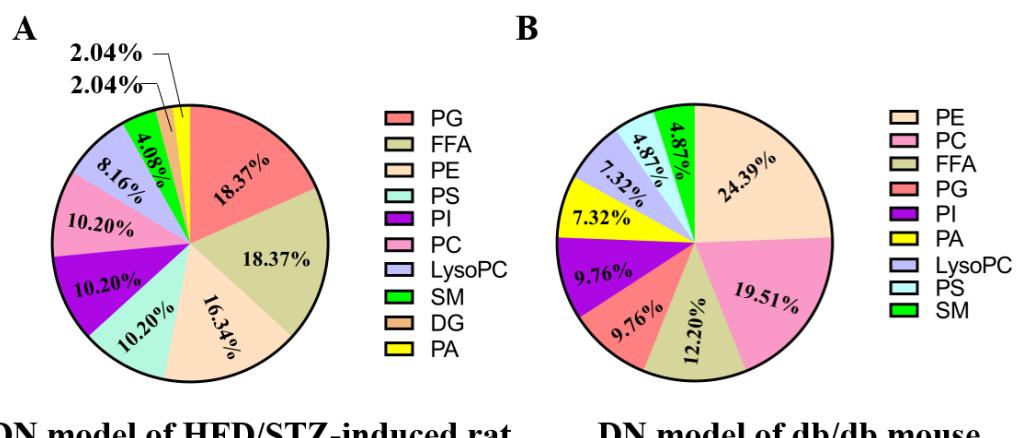
Figure S5. Fan chart of discriminating lipid species in HFD/STZ-induced diabetic rats

and db/db mice. PG: phosphatidylglycerol; FFA: free fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PS: phosphatidylserine; PI: phosphatidylinositol; DG: diacylglycerol; SM: sphingomyelin; LysoPG: lysophosphatidylglycerol; LysoPI: lysophosphatidylinositol; PA:phosphatidic acid; LysoPC: Lysophosphatidylcholine; FAHFAs: fatty acid esters of hydroxy fatty acids.
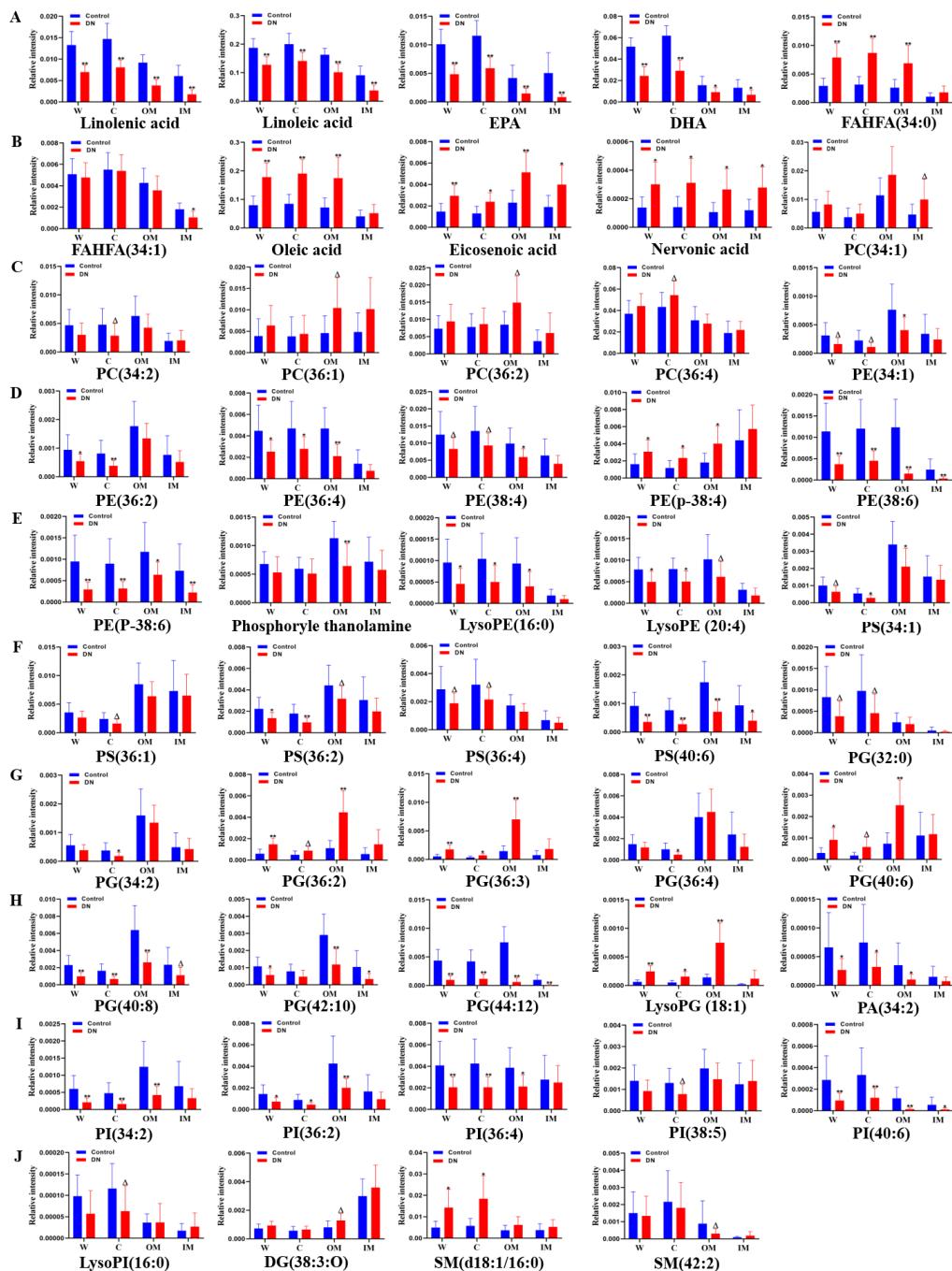
Figure S6. Histogram of metabolites involved in lipid metabolism in HFD/STZ-induced diabetic rats. W: whole; C: cortex; OM: outer medullar; IM: inner medullar. Control: control group; DN: diabetic nephropathy groups; EPA: eicosapentaenoic acid; DHA: do-cosahexaenoic acid; FAHFAs: fatty acid esters of hydroxy fatty acids; PC: phosphatidylcholine; PE:phosphatidylethanolamine; PE(38:4):PE(P18:0/20:4(5Z,8Z,11Z,14Z)); LysoPE: lysophosphatidylethanolamine; PS: phosphatidylserine; PG: phosphatidylglycerol; LysoPG: lysophosphatidylglycerol; PA: phosphatidic acid; PI: phosphatidylinositol; LysoPI: lysophosphatidylinositol; PI(36:2): PI(18:1(9Z):18:1(9Z)); DG: diacylglycerol; SM: sphingomyelin. Scale bar: 3mm, $^{\triangle}P < 0.1$, $*P < 0.05$，$**P < 0.01$.
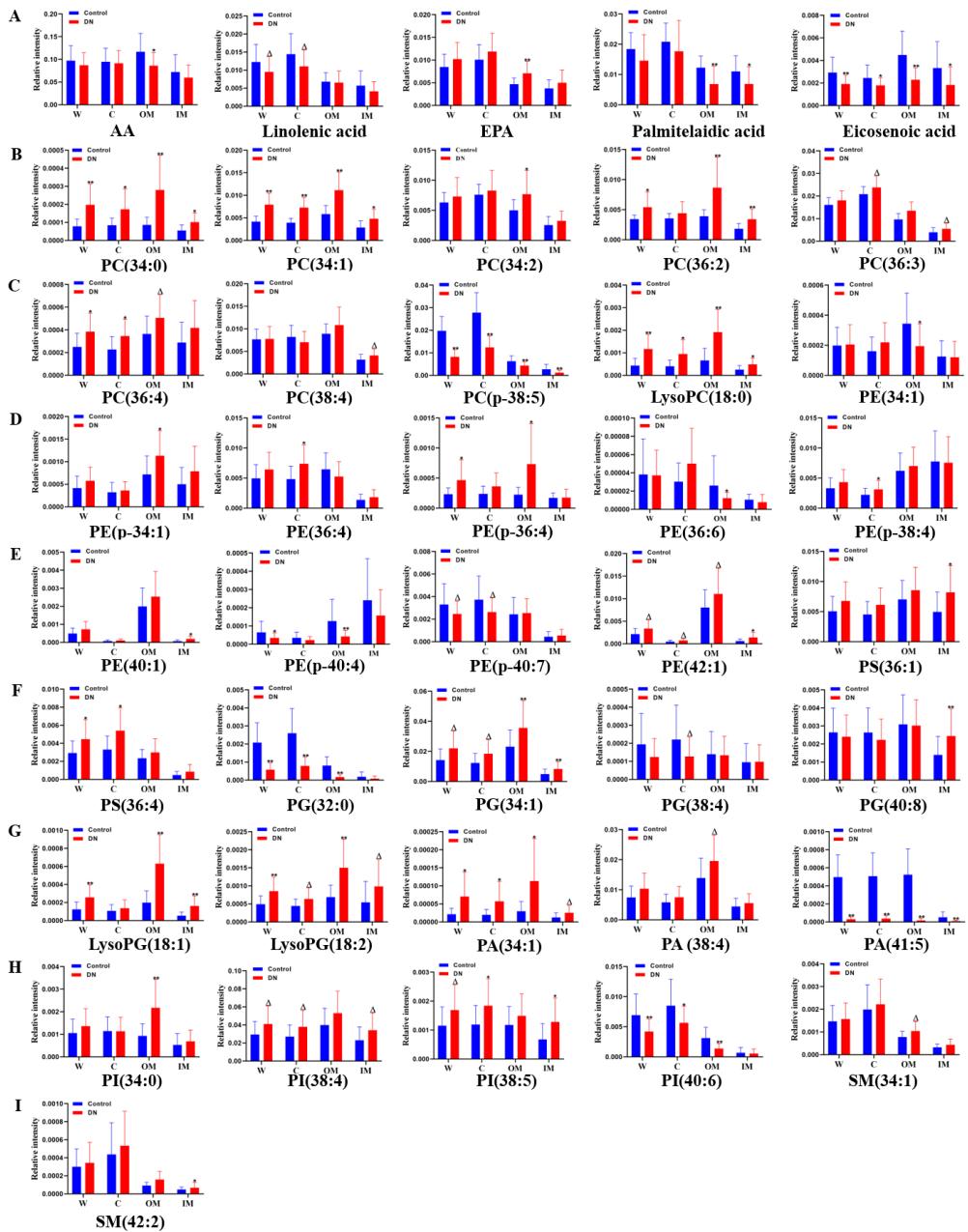
Figure S7. Histogram of metabolites involved in lipid metabolism in db/db mice. W: whole; C: cortex; OM: outer medullar; IM: inner medullar. Control: control group; DN: diabetic nephropathy groups; AA: Arachidonic acid; EPA: eicosapentaenoic acid; PC: phosphatidylcholine; LysoPC: Lysophosphatidylcholine; PE: phosphatidylethanolamine; PS: phosphatidylserine; PG: phosphatidylglycerol; LysoPG: lysophosphatidylgly-

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cerol; PA: phosphatidic acid; PA(34:1): 16:0/18:1(9Z)); PI: phosphatidylinositol; SM:

sphingomyelin; SM (34:1): SM(d18:1/16:0); SM(42:2): SM(d18:1/24:1(15Z)); Scale

bar: 3mm,$^{\triangle}P < 0.1$, $*P < 0.05$, $**P < 0.01$.
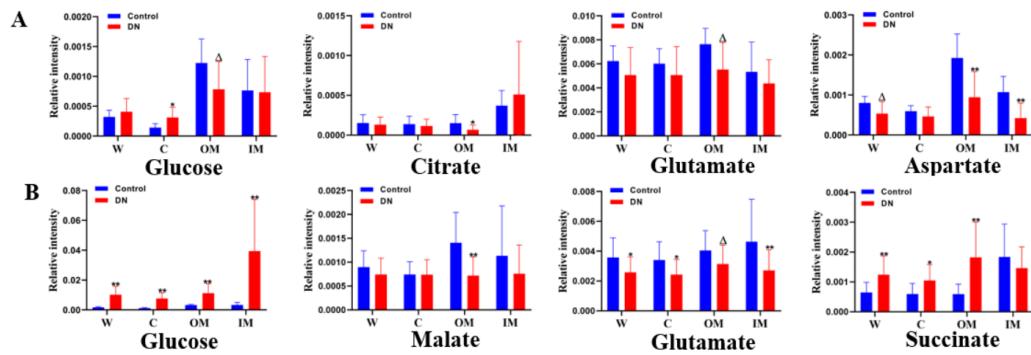
Figure S8. Histogram of metabolites involved in glycolysis and TCA cycle in

HFD/STZ-induced diabetic rats (A) and db/db mice(B). W: whole; C: cortex; OM: outer

medullar; IM: inner medullar. Control: control group; DN: diabetic nephropathy groups;

Scale bar: 3mm, $^{\triangle}P < 0.1$, $*P < 0.05$, $**P < 0.01$.
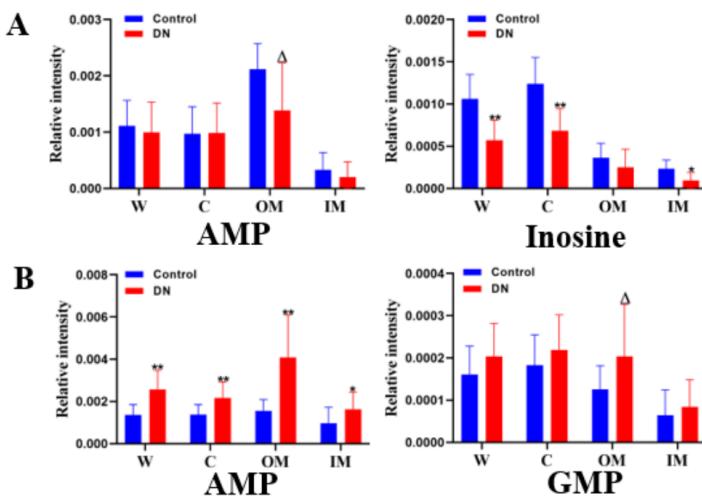
Figure S9. Histogram of metabolites involved in purine metabolism in HFD/STZ-

induced diabetic rats (A) and db/db mice(B). W: whole; C: cortex; OM: outer medullar;

IM: inner medullar. Control: control group; DN: diabetic nephropathy groups; AMP: adenosine monophosphate; GMP: guanine monophosphate. Scale bar: 3mm, $^{\triangle}P < 0.1$, $*P < 0.05$, $**P < 0.01$.
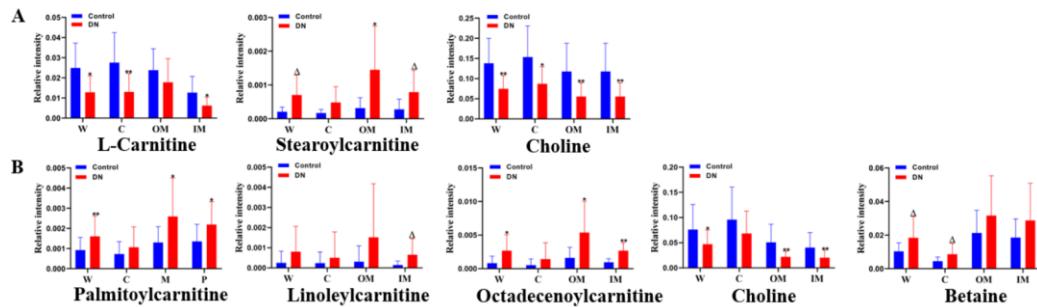

Figure S10. Histogram of metabolites involved in carnitine and choline metabolism in HFD/STZ-induced diabetic rats (A) and db/db mice(B). W: whole; C: cortex; OM: outer medullar; IM: inner medullar. Control: control group; DN: diabetic nephropathy groups; Scale bar: 3mm, $^{\triangle}P < 0.1$, $*P < 0.05$, $**P < 0.01$.

Supplementary Methods

1. LC-MS/MS analysis

LC-MS/MS experiments were performed in positive and negative ion mode on a Q-OT-qIT hybrid mass spectrometer (Orbitrap Fusion Lumos, Thermo Fisher Scientific, USA). Renal tissue samples were weighed at approximately 100 mg and placed into 2 ml centrifuge tubes, homogenized in a ratio of 1 g: 4 mL in 0.4 mL methanol/water (8:2 v/v). Sonicate the sample for 10 minutes, the supernatant was obtained by centrifugation at 12,000 R/min for 10 min at 4 °C. The supernatant was then concentrated in a centrifugal vacuum concentrator (CHRIST RVC 2-25 CD plus,

CHRIST, Osterode, Germany) for 4 h. The residues were dissolved in 200 uL of 1% acetonitrile and centrifugation was continued for 10 min under the same conditions. Then the supernatant was transfered to a sample vial for LC-MS/MS analysis. For the ions of interest, targeting analysis was performed in full scan and $DDMS^2$ scan modes with normalized HCD collision energy values set to 15%, 30% and 45%. For MS/MS acquisition, the mass resolving was set to 15000, with a maximum injection time of 100 ms and a range of 67–1000 Da.