Table S1 Sequence information of primers and probes

Name	Type	Sequence	Length
GAPDH	Upper	GCATCCTGGGCTACACTGAG	163bp
	Lower	TCCACCACCCTGTTGCTGTA	
	Probe	TCCTCTGACTTCAACAGCGACACCC	
CDKAL1	Upper	GGAGAAGTCTCGGGTTTG	155bp
	Lower	ATCCTCAGCGCACAGTCT	
	Probe	CCACACCTCTGCTGCATCTCAGTG	
CDKN2A	Upper	CACCAGAGGCAGTAACCA	139bp
	Lower	AGTTGTGGCCCTGTAGGA	
	Probe	CCCGATTGAAAGAACCAGAGAGGC	
CDKAN2B	Upper	CGAGGAGAACAAGGGCAT	160bp
	Lower	CATCATGACCTGGATCGC	
	Probe	GGGACTAGTGGAGAAGGTGCGACA	
IGF2BP2	Upper	CACCGGAAGCCCAGTTCAA	145bp
	Lower	CCACCTTTGCCAATCACCC	
	Probe	TGAAGCTGGAAGCGCATATCAGAG	
AR	Upper	CTGTGACACCAGAACGCATT	141bp
	Lower	GGTAATCCTTGTGGGAGGTA	
	Probe	TGAACTGAGCAGCCAGGATATGACCACCTT	
AGER	Upper	ATCAGGGCTGGGAACTCTAG	16bp
	Lower	GATTCAGTTCTGCACGCTCC	
	Probe	GCTCATTGGGGTCATCTTGTGGCAAAGG	
GLUT1	Upper	TTCTCCAACTGGACCTCAA	147bp
	Lower	GCCTTTAGTCTCAGGAACTT	
	Probe	TGGAGCAACTGTGTGGTCCCTACGTCTTCA	
IGF2	Upper	GAAAACCAAAATCCATGCAAATG	88bp
	Lower	TGAGTGCTTTTTAGGATGGGAATT	
	Probe	AATGTAATTGGCACGACCCTCACCCC	
AGT	Upper	CCTGCAAACTTCGGTAAATGTG	81bp
	Lower	CTTAGTGATCGATGCAGAGTTTCAC	
	Probe	ACTCGACCCTGCACCGGCTCA	
ADRB3	Upper	GAGCTCCCCTGGTTCCATTC	70bp
	Lower	CCACAGAGCCTGGAGAACACTA	
	Probe	TTCTGCCACCCAAACCCTGATGAGACC	
AGTR2	Upper	CCACCCTTGCCACTACTAGCA	73bp
	Lower	ATTGTTGCCAGAGATGTTCACAA	
	Probe	AAACATTACCAGCGGTCTTCACTTCGGG	
ACE	Upper	CCGAAATACGTGGAACTCATCAA	67p
	Lower	CACGAGTCCCCTGCATCTACA	
	Probe	CAGGCTGCCCGGCTCAATGGC	
PRKCA	Upper	AACTGGAGAACAGGGAGATC	155bp
	Lower	CCCTTCAAAATCAGACTGGT	

	Probe	TTTGACAAGTTCTTCACACGAGGACAGCCC	
MTHFR	Upper	GGGCTATGTCTTCCAGAAGG	211bp
	Lower	TACGGTGGGCTGGATGATCT	
	Probe	GACAAGGTGGTAATTAACCCGGAGCTCG	

Table S2 Quantitative formulas of gene expression

Gene	Standard curve	R^2
GAPDH	CT=-3.72log(copy)+45.66	0.9992
CDKN2A	CT=-3.85log(copy)+45.76	0.9996
CDKN2B	CT=-3.56log(copy)+45.01	0.9991
IGF2BP2	CT=-3.92log(copy)+41.47	0.9916
CDKAL1	CT=-3.86log(copy)+43.04	0.9976
AR	CT=-3.01log(copy)+43.79	0.9972
AGER	CT=-3.76log(copy)+44.06	0.9983
GLUT1	CT=-3.37log(copy)+46.14	0.9944
IGF2	CT=-4.70log(copy)+58.08	0.9964
AGT	CT=-3.81log(copy)+48.08	0.9981
ADRB3	CT=-3.90log(copy)+49.64	0.9962
AGTR2	CT=-3.70log(copy)+48.68	0.9997
ACE	CT=-4.26log(copy)+52.44	0.9993
PRKCA	CT=-3.75log(copy)+46.55	0.9995
MTHFR	CT=-3.74log(copy)+46.43	0.9987

Table S3 Quantification results of the potential biomarkers from metabolomics

Compound	Control	DM	DN stage 3	DN stage 4	DN stage 5
Lyso-PC(16:0)	995.3 ± 97.3	1444 ± 163	1399 ± 183	1359 ± 193	794.5 ± 154.9
Deoxycorticosterone	3.38 ± 2.80	14.08 ± 13.38	15.08 ± 15.12	185.2 ± 133.9	326.5 ± 107.2
Lyso-PC(18:2)	739.4 ± 63.8	1027 ± 103	966.9 ± 159.3	892.5 ± 113.3	620.6 ± 118.2
Lyso-PC(18:0)	623.4 ± 89.4	674.0 ± 183.7	958.0 ± 198.2	797.1 ± 170.0	369.3 ± 108.4
Lyso-PC(18:1)	422.2 ± 50.3	550.8 ± 114.4	615.0 ± 91.3	583.2 ± 101.5	332.4 ± 68.3
Corticosterone	0.71 ± 0.87	3.23 ± 3.96	4.19 ± 4.63	53.55 ± 46.04	118.1 ± 46.2
Pidolic acid	2.50 ± 1.27	9.12 ± 3.47	9.80 ± 3.22	61.39 ± 49.61	209.4 ± 62.2
L-kynurenine	72.00 ± 15.67	82.54 ± 21.73	68.60 ± 12.63	100.6 ± 49.6	156.2 ± 37.1
Lyso-PC(20:4)	100.6 ± 10.51	178.6 ± 25.7	152.3± 29.6	199.4 ± 30.6	125.7 ± 28.1
Lyso-PC(20:3)	68.53 ± 7.98	103.1 ±19.5	88.84 ± 40.37	75.11 ± 13.51	37.82 ± 7.13
Lyso-PC(22:6)	75.36 ± 12.76	87.38 ± 31.38	87.08 ± 28.45	66.59 ± 8.81	34.03 ± 10.32
L-valine	230.1 ± 21.2	272.5 ± 43.6	236.8 ± 42.2	213.6 ± 62.5	79.72 ± 13.32
Leucine/Isoleucine	63.23 ± 4.56	74.96 ± 7.96	70.08 ± 7.57	71.67 ± 15.83	29.47 ± 4.43
L-tryptophan	22.8 ± 2.66	21.94 ± 3.28	22.99 ± 4.30	22.29 ± 8.23	13.36 ± 3.17

Table S4 Quantification results of the potential biomarkers from absolute quantitative experiments

Compound	Control	DM	DN stage 3	DN stage 4	DN stage 5
Adenosine, mg/L	0.136 ± 0.030	0.138 ± 0.029	0.295 ± 0.071	0.540 ± 0.080	1.870 ± 0.407
Uric acid, mg/L	46.53 ± 3.06	58.62 ± 5.66	60.46 ± 8.60	64.87 ± 7.85	76.45 ± 5.65
Xanthine, mg/L	0.477 ± 0.062	0.554 ± 0.159	0.663 ± 0.131	0.839 ± 0.179	2.034 ± 0.657
Cytidine, mg/L	0.046 ± 0.012	0.051 ± 0.007	0.056 ± 0.014	0.091 ± 0.021	0.296 ± 0.069
Cytosine, mg/L	0.115 ± 0.015	0.194 ± 0.038	0.293 ± 0.076	0.305 ± 0.069	0.520 ± 0.167
Inosine, mg/L	0.077 ± 0.012	0.080 ± 0.015	0.284 ± 0.021	0.319 ± 0.079	0.947 ± 0.346
Thymidine, mg/L	0.028 ± 0.005	0.039 ± 0.012	0.081 ± 0.030	0.122 ± 0.054	0.339 ± 0.093
Homocystein, μg/L	704.3 ± 265.3	729.5 ± 370.2	764.6 ± 349.9	1069 ± 398	1773 ± 842
S-adenosylmethionine, μg/L	13.01 ± 4.17	14.13 ± 4.08	14.71 ± 5.70	27.82 ± 11.72	30.91 ± 18.36
S-adenosylhomocysteine, μg/L	6.00 ± 4.18	6.75 ± 2.75	10.26 ± 5.62	26.35 ± 10.70	116.4 ± 68.7
Glutathione, μg/L	915.0 ± 352.6	762.7 ± 296.7	644.2 ± 237.2	618.7 ± 244.5	596.7 ± 181.3
PC (C16:0/C18:2), mg/L	639.0 ± 34.2	549.5 ± 44.7	591.3 ± 62.2	534.7 ± 108.8	508.7 ± 44.3
PC (C16:0/C17:0), mg/L	402.4 ± 74.9	292.4 ± 107.3	357.4 ± 72.0	320.0 ± 100.3	313.4 ± 77.5
PC (C16:0/C20:4, C18:2/C18:2), mg/L	191.8 ± 49.9	172.8 ± 50.2	169.7 ± 49.4	159.6 ± 39.8	171.4 ± 53.3
PC (C18:0/C18:2, C18:1/C18:1), mg/L	286.9 ± 58.9	227.5 ± 69.5	247.1 ± 52.5	234.1 ± 73.1	223.7 ± 62.0
PC (C16:0/C22:4, C18:0/C20:4), mg/L	132.7 ± 10.5	114.9 ± 11.9	113.7 ± 13.7	109.4 ± 19.4	105.9 ± 12.8
SM (dC18:1/C16:2),	190.7 ± 10.8	173.9 ± 22.2	209.3 ± 38.0	213.5 ± 38.9	93.37 ± 19.94

mg/L					
PE (pC18:0/C20:4, pC16:0/C22:4), mg/L	55.18 ± 8.52	45.36 ± 11.82	42.05 ± 13.14	30.48 ± 10.02	29.23 ± 5.38
PI (C18:0/C20:4, C18:1/C20:3), mg/L	37.32 ± 8.21	31.84 ± 7.67	40.32 ± 8.82	38.02 ± 10.73	27.82 ± 6.40
LysoPC C16:0, mg/L	30.18 ± 3.08	35.89 ± 7.23	33.11 ± 7.82	36.65 ± 9.03	23.33 ± 2.80
C12:0, mg/L	1.51 ± 0.23	1.06 ± 0.17	1.60 ± 0.61	1.11 ± 0.51	1.20 ± 0.26
C18:0, mg/L	42.08 ± 23.34	56.97 ± 18.84	59.32 ± 14.39	27.45 ± 9.61	47.37 ± 13.01
C18:2, mg/L	204.8 ± 115.8	293.9 ± 108.3	277.4 ± 75.3	126.1 ± 57.0	215.6 ± 98.1
C20:2, mg/L	2.18 ± 0.49	3.50 ± 1.70	3.29 ± 0.97	2.13 ± 0.68	2.45 ± 0.35
C20:3, mg/L	12.18 ± 7.37	23.32 ± 14.91	21.34 ± 8.78	10.47 ± 4.42	12.87 ± 3.69
C20:4, mg/L	44.78 ± 29.15	93.62 ± 36.82	88.38 ± 24.61	42.95 ± 27.48	67.30 ± 21.05
C20:5, mg/L	5.29 ± 4.07	6.07 ± 3.41	5.26 ± 1.65	2.75 ± 1.24	3.89 ± 1.24
C22:6, mg/L	21.10 ± 18.45	61.51 ± 35.71	68.92 ± 37.56	24.67 ± 26.52	29.35 ± 13.39

Table S5 Critical concentrations and corresponding sensitivity and specificity of the five metabolites

Metabolite	Critical concentration	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Lyso-PC(18:0)	881.0	0.722	0.750	0.743	0.730
Inosine	0.162 mg/L	0.889	1.000	1.000	0.900
Cytosine	0.245 mg/L	0.667	0.917	0.889	0.734
Thymidine	0.062 mg/L	0.833	0.917	0.909	0.846
SAH	7.133 μg/L	0.722	0.750	0.743	0.730

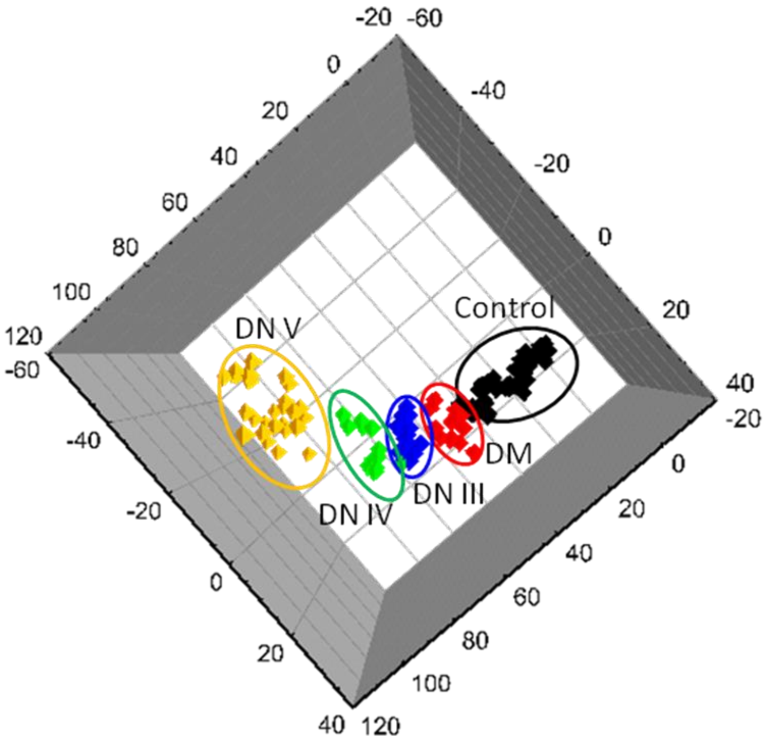

Figure S1 Score plot of the PCA analysis in metabolomics

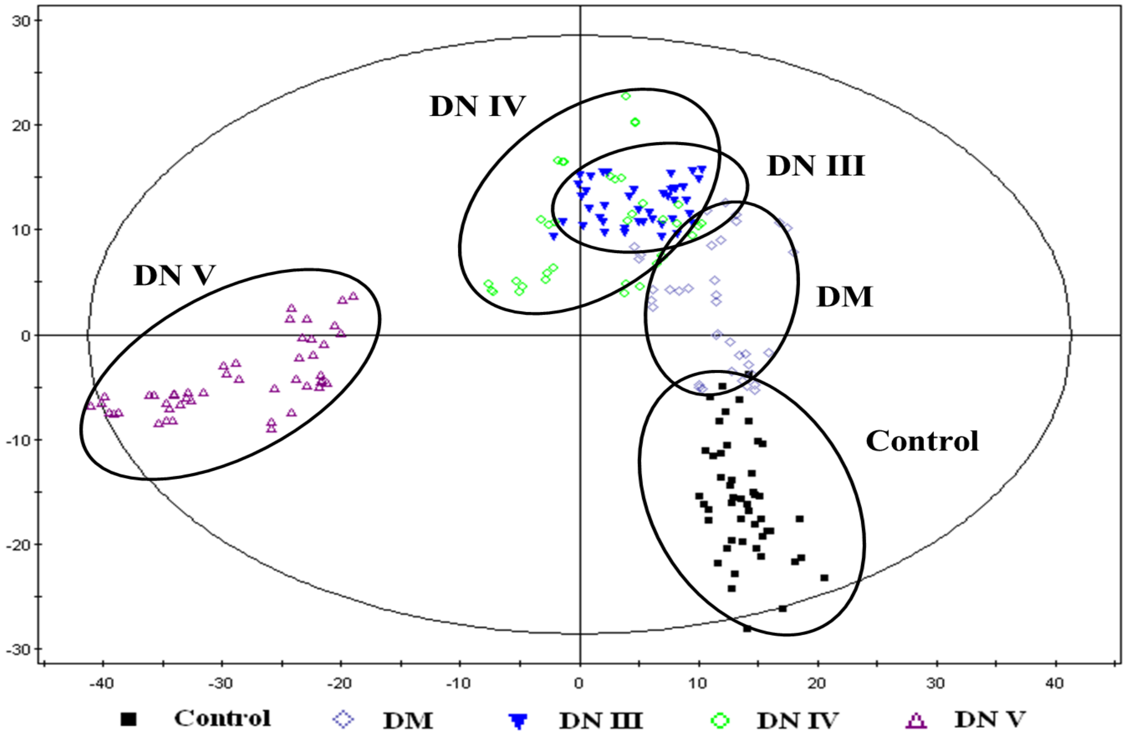

Figure S2 Score plot of the PLS-DA analysis in metabolomics

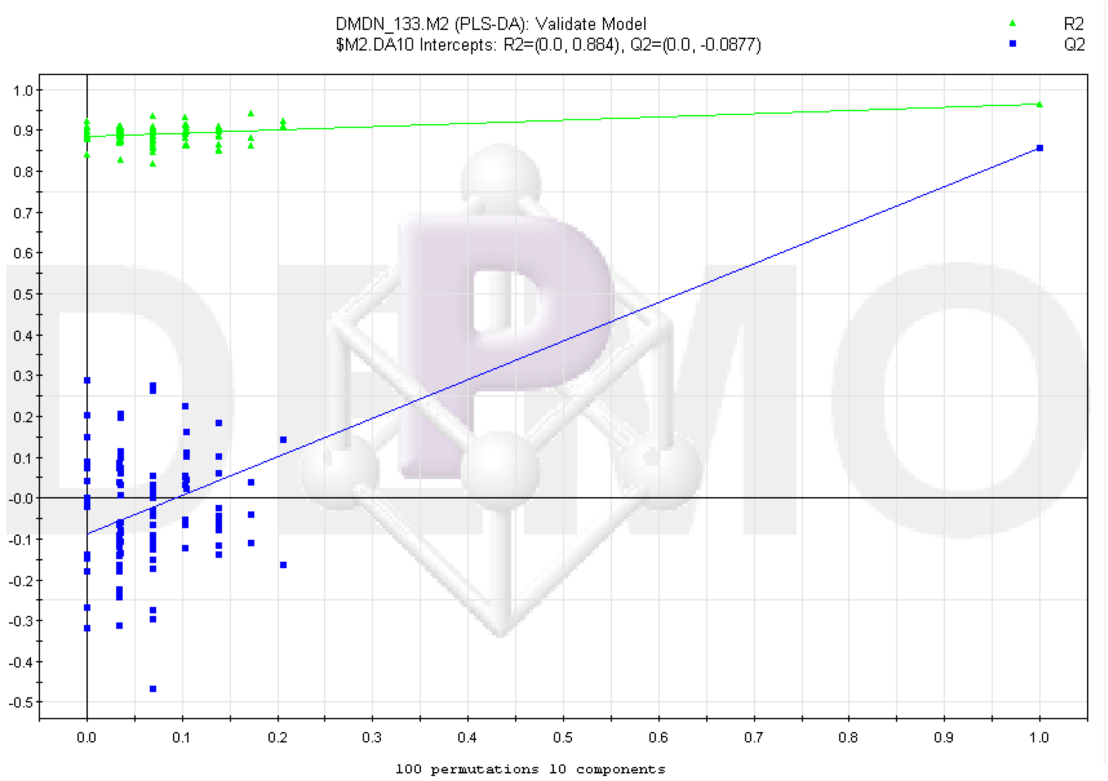

Figure S3 Validation models of a 100 random permutation test for the PLS-DA model, Y-axis intercepts: R^2= (0, 0.884), Q^2= (0, -0.0887)

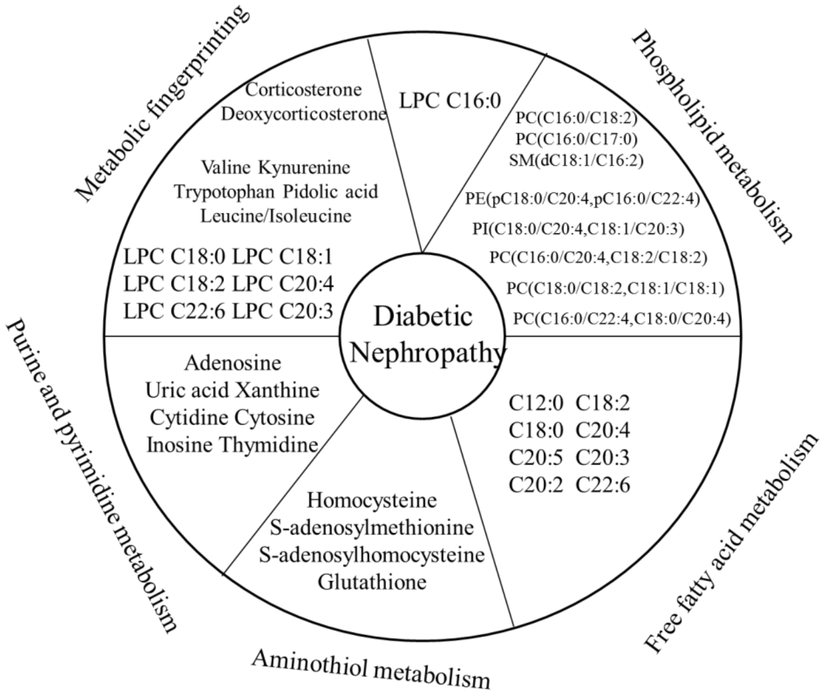

Figure S4 Forty potential biomarkers in metabolomics and their related metabolic pathways

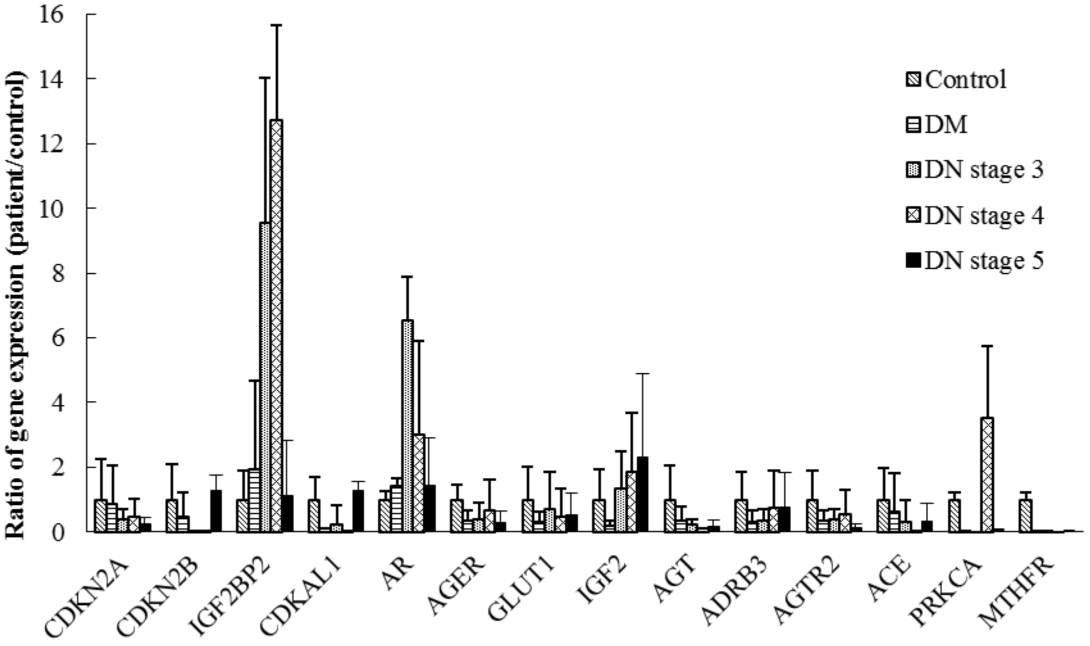

Figure S5 Quantitative results of fourteen key genes (expression ratio of patients to control)