Supplementary Materials for

Beneficial effects of Procyanidin B2 on adriamycin-induced nephrotic syndrome mice: the multi-action mechanism for ameliorating glomerular permselectivity injury

Chuanping Zhao[1], Jiamei Tang[1], Xiaoya Li[1], Zihan Yan[1], Liangliang Zhao[2], Wenbo Lang[2], Chunmao Yuan[3], Chengyan Zhou[1*, 3]

1. Key Laboratory of Pharmaceutical Quality Control of Hebei Province, College of Pharmaceutical Sciences, Institute of Life Science and Green Development, Hebei University, 180 WuSi Road, Lianchi District, Baoding, 071002, China.

2. Department of Monitoring and Analysis, Baoding Environmental Monitoring Center of Hebei Province, 224 Dongfeng Road, Lianchi District, Baoding, 071000, China.

3. State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550014, China.

*Corresponding author at: Chengyan, Zhou, Key Laboratory of Pharmaceutical Quality Control of Hebei Province, College of Pharmaceutical Sciences, Institute of Life Science and Green Development, Hebei University, 180 WuSi Road, Lianchi District, Baoding, 071002, China. E-mail: xuefanone@163.com.

Tel/Fax: +86 312 5971107; E-mail address: xuefanone@163.com (C.Y. Zhou).

Table of contents

Supplementary Materials and Methods

2.3 Biochemical parameters analysis

The serum concentrations of creatinine (Cr, cat#.C011-2-1), uric acid (UA, cat#.C012-2-1), blood urea nitrogen (BUN, cat#.C013-1-1), albumin (ALB, cat#. A028-2-1), and total protein (TP, cat#. A045-4-2) were detected by using relevant assay kits. The concentrations of alanine aminotransferase (ALT, cat#.C009-2-1), aspartate aminotransferase (AST, cat#.C010-2-1), alkaline phosphatase (ALP, cat#.A059-2-2), total bilirubin (TBIL, cat#.C019-1-1), and direct bilirubin (DBIL, cat#.C019-2-1) were assessed by using relevant commercial assay kits. The activities of total superoxide dismutase (T-SOD, cat#.A001-1, Hydroxylamine method), catalase (CAT, cat#.A007-1-1, Visible light method), and glutathione peroxidase (GPx, cat#.A005-1-2) in kidney and serum were detected by using relevant activity assay kits. The amount of malonaldehyde (MDA, cat#. A003-1, TBA method) and Nitric Oxide (NO, cat#. A013-2-1, Microwell plate method) were measured by relevant commercial assay kits. All of the aforementioned kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The urinary levels of UP (cat#.C035-21) was measured by using urine protein test kit (CBB method). The serum levels of arginine vasopressin (AVP, cat#H353-1) were detected by using ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The formations of aldosterone (ALD, cat#.MB-3273B), cysteine proteinase inhibitor (Cys-C, cat#.MB-5786b) in serum were measured by using ELISA kits (Jiangsu Mei Biao Biological Technology Co., Ltd, Jiangsu, China). In addition, the examinations of reactive oxygen species (ROS, cat#.MB5886A), inducible nitric oxide synthase (iNOS, cat#.MB3190A), reduced glutathione (GSH, cat.#MB-5614B), and oxidized glutathione (GSSG, cat#.MB5614B) in serum and kidney, were performed by using ezyme-linked immunosorbent(ELISA) assay kits (Jiangsu Mei Biao Biological Technology Co., Ltd, Jiangsu, China). The levels of total cholesterol (T-CHO, cat#.A111-1-1, COD-PAP method), triglyceride (TG, cat#.A110-1-1, GPO-PAP method), high-density lipoprotein cholesterol (HDL-C, cat#.A112-1-1), and low-density lipoprotein cholesterol (LDL-C, cat#.A113-1-1) in serum, kidney, and liver, were detected by using relevant commercial assay kits, which were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The activity of lipoprotein lipase (LPL, cat#A067-1-2) in kidney were evaluated by using activity assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The levels of ATP-binding cassette transporter A1(ABCA1, cat#.YJ6c5782), ATP-binding cassette sub-family G member 1 (ABCG1, cat#.YJ79920), and Apolipoprotein CIII(APOCIII, cat#.YJ89877) in kidney, were assessed by using ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). The serum and renal levels of tumor necrosis factor alpha (TNF-α, cat#.MB-2868A), interferon gamma (IFNγ, cat#.MB-1739A), monocyte chemoattractant protein-1 (MCP-1, cat#.MB-2818A), heat shock protein 90 (Hsp90, cat#.MB-3193B), interleukin-1 beta (IL-1β, CAT#.MB-2776A), interleukin-6 (IL-6, cat#.MB-1731A), interleukin-17 (IL-17, cat#.MB-1629A), interleukin-5 (IL-5, cat#. MB-1635B), and nuclear transcription factor (NF-κB, cat#.MB-5996B) in serum and kidney, were detected by ELISA kits (Jiangsu Mei Biao Biological Technology Co., Ltd, Jiangsu, China). All the tests were performed under the instructions of the manufacture's protocols. Besides, the urinary and serum levels of Na were detected by using a NexION 1000 series inductively coupled plasma-mass spectrometry (ICP-MS, PerkinElmer, Inc., USA).

Table S1. The equation and the correlation coefficient of serum and renal FFAs.

ample	FFAs	Calibration equation	Regression coefficient

Serum	**C4:0**	Y = 231.9886X + 1142.191	0.999168
	C6:0	Y = 796.7527X + 22471.84	0.99971
	C8:0	Y = 1093.199X + 30765.63	0.999694
	C10:0	Y = 1121.834X + 38191.65	0.999074
	C11:0	Y = 1106.051X + 35247.64	0.999255
	C12:0	Y = 1098.118X + 36021.14	0.999095
	C13:0	Y = 1067.652X + 31197.79	0.999042
	C14:1	Y = 339.8378X + 5697.646	0.999528
	C14:0	Y = 1047.131X + 27457.05	0.999676
	C15:1	Y = 363.9882X + 8475.023	0.999787
	C15:0	Y = 1010.994X + 26165.28	0.999637
	C16:1	Y = 173.2855X + 5979.741	0.999619
	C16:0	Y = 1850.866X + 64132.3	0.999267
	C17:1	Y = 298.9931X + 6166.99	0.999827
	C17:0	Y = 920.7525X + 24140.94	0.999484
	C18:3n-6	Y = 285.2137X + 6128.049	0.999604
	C18:2n-6-cis	Y = 191.3951X + 8162.53	0.999394
	C18:1n-9-trans	Y = 37.35334X + 1032.889	0.999122
	C18:0	Y = 856.7376X + 30753.11	0.99902
	C20:4n-6	Y = 110.8932X + 5167.82	0.999223
	C20:5n	Y = 307.5555X + 1190.807	0.999925
	C20:3n-6	Y = 284.2601X + 5980.675	0.99962
	C20:2n-6	Y = 47.86521X + 3778.084	0.999159
	C20:1n-9cis	Y = 274.3791X + 1243.754	0.999358
	C20:0	Y = 792.1656X + 11811.08	0.999832
	C21:0	Y = 714.0643X + 2311.524	0.99994
	C22:6n-3	Y = 262.3222X - 1966.042	0.999851
	C22:2n-6	Y = 291.7868X + 766.1795	0.9999986
	C22:0	Y = 695.5308X - 856.9354	0.999921
	C23:0	Y = 651.3777X - 8646.585	0.999663
	C24:1	Y = 207.3918X + 474.0115	0.99976
	C24:0	Y = 562.3829X - 10564.72	0.999351
kidney	**C4:0**	Y = 231.9886X + 1142.191	0.999168
	C6:0	Y = 796.7527X + 22471.84	0.99971
	C8:0	Y = 1093.199X + 30765.63	0.999694
	C10:0	Y = 1121.834X + 38191.65	0.999074
	C11:0	Y = 1106.051X + 35247.64	0.999255
	C12:0	Y = 1098.118X + 36021.14	0.999095
	C13:0	Y = 1067.652X + 31197.79	0.999042
	C14:1	Y = 339.8378X + 5697.646	0.999528
	C14:0	Y = 1047.131X + 27457.05	0.999676
	C15:1	Y = 363.9882X + 8475.023	0.999787
	C15:0	Y = 1010.994X + 26165.28	0.999637
	C16:1	Y = 173.2855X + 5979.741	0.999619

C16:0	Y = 1850.866X + 64132.3	0.999267
C17:1	Y = 298.9931X + 6166.99	0.999827
C17:0	Y = 920.7525X + 24140.94	0.999484
C18:3n-6	Y = 285.2137X + 6128.049	0.999604
C18:2n-6-cis	Y = 191.3951X + 8162.53	0.999394
C18:1n-9-trans	Y = 37.35334X + 1032.889	0.999122
C18:0	Y = 856.7376X + 30753.11	0.99902
C20:4n-6	Y = 110.8932X + 5167.82	0.999223
C20:5n	Y = 307.5555X + 1190.807	0.999925
C20:3n-6	Y = 284.2601X + 5980.675	0.99962
C20:2n-6	Y = 47.86521X + 3778.084	0.999159
C20:1n-9cis	Y = 274.3791X + 1243.754	0.999358
C20:0	Y = 792.1656X + 11811.08	0.999832
C21:0	Y = 714.0643X + 2311.524	0.99994
C22:6n-3	Y = 262.3222X - 1966.042	0.999851
C22:2n-6	Y = 291.7868X + 766.1795	0.9999986
C22:0	Y = 695.5308X - 856.9354	0.999921
C23:0	Y = 651.3777X - 8646.585	0.999663
C24:1	Y = 207.3918X + 474.0115	0.99976
C24:0	Y = 562.3829X - 10564.72	0.999351

*X: the quality of fatty acids; Y: peak area of fatty acid.

Table S2. Names, characteristic fragment ions and retention times of all 32 fatty acid methyl esters in the method.

Abbreviation	(Methyl)-name	MW	Fragment ions (m/z)			Rt (min)
C4:0	Butyrate	102	43	71	74	3.885
C6:0	Hexanoate	130	74	43	87	8.840
C8:0	Octanoate	158	74	43	87	12.575
C10:0	Decanoate	186	74	43	87	18.365
C11:0	Undecanoate	200	74	43	87	21.925
C12:0	Dodecanoate	214	74	43	87	25.675
C13:0	Tridecanoate	228	74	43	87	29.450
C14:1	9Z-Tetradecenoate	240	55	74	41	32.695
C14:0	Tetradecanoate	242	74	87	43	33.155
C15:1	10Z-Pentadecenoate	254	55	71	41	36.310
C15:0	Pentadecanoate	256	74	87	43	36.750
C16:1	9Z-Hexadecenoate	268	55	69	41	39.485
C16:0	Hexadecanoate	270	74	87	43	40.210
C17:1	10Z-Heptadecenoate	282	55	69	41	42.895
C17:0	Heptadecanoate	284	74	87	43	43.690
C18:3n-6	6Z,9Z,12Z-Octadecatrienoate	292	67	79	41	45.520
C18:2n-6-cis	9Z,12Z-Octadecadienoate	294	67	81	95	46.040
C18:1n-9-trans	9E-Octadecenoate	296	264	109	222	46.460
C18:0	Octadecanoate	298	74	87	43	47.050
C20:4n-6	5Z,8Z,11Z,14Z-Eicosatetraenoate	318	79	91	67	50.575
C20:5n	5Z,8Z,11Z,14Z,17Z-Eicosapentaenoate	316	79	91	67	50.740
C20:3n-6	8Z,11Z,14Z-Eicosatrienoate	320	67	79	93	51.010
C20:2n-6	11,14-Eicosadienoate	322	67	81	291	51.455
C20:1n-9cis	11Z-Eicosenoate	324	69	74	292	51.570
C20:0	Eicosanoate	326	74	87	283	52.150
C21:0	Heneicosanoate	340	74	87	297	54.245
C22:6n-3	4,7,10,13,16,19-Docosahexaenoate	350	79	91	67	54.760
C22:2n-6	13,16-Docosadienoate	350	67	55	319	55.625
C22:0	Docosanoate	354	74	87	311	56.145
C23:0	Tricosanoate	368	74	87	325	57.905
C24:1	15Z-Tetracosenoate	380	55	69	306	59.180
C24:0	Tetracosanoate	382	74	87	339	59.550

MW: Molecular weight; Rt: Retention time.

Table S3. The equation and the correlation coefficient of metallic elements in serum and urine.

Sample	Metallic element	Calibration equation	Regression coefficient	Limit of detection
serum	**Na**	Y=34.534X－4.1796	0.9995	0.001245
	Mg	Y=21.993X－1.6588	0.9996	0.000599
	K	Y=12.954X－1.5939	0.9996	0.001784
	Ca	Y=0.359X－0.0249	0.9994	0.009145
	Be	Y=0.0117X+0.0126	0.9993	0.000102
	B	Y=0.0033X+0.0041	0.9994	0.277655
	Al	Y=0.0095X+0.0039	0.9999	0.631010
	V	Y=0.1046X+0.0945	0.9994	0.009036
	Mn	Y=0.0628X+0.0356	0.9998	0.066127
	Fe	Y=0.0026X+0.0002	1.0000	0.329695
	Co	Y=0.2193X+0.0736	1.0000	0.004181
	Ni	Y=0.0648X+0.0397	0.9997	0.061710
	Cu	Y=0.4543X+0.6482	0.9992	0.016021
	Zn	Y=0.0648X+0.0815	0.9991	0.229393
	Mo	Y=0.0063X+0.0088	0.9992	0.030939
	Cd	Y=0.0035X+0.0026	0.9997	0.036909
	Sb	Y=0.0202X+0.0068	1.0000	0.007734
	Ba	Y=0.0058X+0.002	0.9999	0.065140
	Tl	Y=0.062X+0.0877	0.9993	0.000212
	Pb	Y=0.1118X+0.1338	0.9991	0.013341
Urine	**Na**	Y=59.019X－5.3456	0.9997	0.000441
	Mg	Y=36.049X－2.5413	0.9998	0.000049
	K	Y=14.848X－1.5168	0.9995	0.002589
	Ca	Y=0.3778X－0.0581	0.9991	0.001107

Be	Y=0.006X+0.0077	0.9993	0.013729
B	Y=0.0019X+0.0018	0.9990	0.447451
Al	Y=0.0086X+0.0037	1.0000	0.008101
V	Y=0.1078X+0.123	0.9976	0.009036
Mn	Y=0.0854X+0.0042	1.0000	0.003456
Fe	Y=0.0028X+0.0045	0.9993	0.394351
Co	Y=0.2535X−0.3931	0.9994	0.001551
Ni	Y=0.0556X+0.0523	0.9992	0.007469
Cu	Y=0.3378X+0.1673	0.9997	0.010768
Zn	Y=0.06X+0.0645	0.9997	0.030924
Mo	Y=0.007X+0.0012	1.0000	0.010857
Cd	Y=0.0045X+0.0028	0.9998	0.001090
Sb	Y=0.019X+0.0003	1.0000	0.007734
Ba	Y=0.0061X−0.0002	1.0000	0.007009
Tl	Y=0.0249X−0.0103	0.9999	0.001919
Pb	Y=0.0407X+0.0014	1.0000	0.003407

*X: the concentration of metallic element; Y: relative peak area = peak area of metallic element /peak area of internal standard.

Table S4. Effect of PB2 on organs relative weights in ADR mice.

	Groups	Body weight	liver	kidney
second week	NC	41.5±1.02	5.410±0.37	1.487±0.25
	ADR	35.9±4.20***	6.144±0.105***	1.954±0.20**
	SIR	39.5±3.75###	5.921±0.11	1.786±0.21
	PB2H	36.4±2.47	5.436±0.20###	1.611±0.01#
	PB2L	38.4±3.34#	5.541±0.09###	1.653±0.02
fourth week	NC	44.58±0.36	5.463±0.05	1.488±0.01
	ADR	34.4±2.53***	6.180±0.10***	2.223±0.03***
	SIR	42.9±3.26###	5.953±0.04###	1.821±0.02###
	PB2H	39.0±3.93##	5.623±0.07###	1.592±0.02###
	PB2L	42.3±3.22###	5.545±0.06###	1.671±0.01###
fifth week	NC	46.9±0.41	5.463±0.04	1.483±0.01
	ADR	31.5±0.83***	6.199±0.04***	2.468±0.03***
	SIR	38.1±1.25###	5.957±0.02###	1.842±0.03###
	PB2H	36.5±2.49###	5.616±0.03###	1.615±0.02###
	PB2L	35.0±3.45###	5.509±0.06###	1.670±0.01###

Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test.

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

Table S5. Effect of PB2 on renal function in serum of ADR mice.

	Groups	NC	ADR	SIR	PB2H	PB2L
second week	**ALB (g/L)**	6.78±1.23	3.21±0.21***	5.6±0.19$^{\#\#\#}$	4.25±0.04$^{\#}$	3.85±0.09
	TP (mg/mL)	80.4±4.59	61.11±1.65***	69.64±1.56$^{\#\#}$	72.37±0.74$^{\#\#\#}$	64.45±1.07
	Cr (μmol/L)	27.43±1.81	35.79±2.18***	28.25±2.12$^{\#\#\#}$	22.84±1.39$^{\#\#\#}$	31.68±1.75$^{\#}$
	BUN (mmol/L)	53.84±1.67	92.42±3.21***	83.7±1.3$^{\#\#}$	76.4±3.33$^{\#\#\#}$	83.91±3.66$^{\#\#}$
	UA (μmol/L)	41.79±2.23	56.26±2.1***	49.32±1.14$^{\#}$	52.2±1.55	55.08±0.69
	Cys-c (μg/L)	710.5±98.47	747.5±52.35	711.9±52.19	701.0±53.39	729.7±38.55
fourth week	**ALB (g/L)**	5.68±0.39	3.33±0.2***	4.68±0.13$^{\#\#\#}$	4.27±0.07$^{\#\#\#}$	4.23±0.04$^{\#\#\#}$
	TP (mg/mL)	82.4±2.13	55.26±1.88***	74.42±1.88$^{\#\#\#}$	75.29±1.61$^{\#\#\#}$	67.03±1.63$^{\#\#\#}$
	Cr (μmol/L)	28.62±1.52	38.17±2.68***	30.27±2.52$^{\#\#\#}$	24.46±2.04$^{\#\#\#}$	32.78±1.88$^{\#}$
	BUN (mmol/L)	53.24±2.11	108.25±2.52***	72.74±2.62$^{\#\#\#}$	61.44±2.15$^{\#\#\#}$	70.75±1.64$^{\#\#\#}$
	UA (μmol/L)	50.42±3.73	134.52±4.74***	81.27±5.64$^{\#\#\#}$	104.25±3.79$^{\#\#}$	115.4±2.74$^{\#}$
	Cys-c (μg/L)	734.4±81.42	713.2±88.06	805.3±121.2	635.4±94.08	748.1±55.64

The serum levels of ALB, TP, Cr, BUN, and UA were detected by relevant assay kits; the serum levels of Cys-c were measured by ELISA kit. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

Table S6. Effects of PB2 on serum SOD, NO, iNOS, ROS, MDA, GSH, CAT and GPx in ADR mice (fifth week).

Groups	NC	ADR	SIR	PB2H	PB2L
T-SOD (U/mL)	292.29±9.4	194.49±7.13***	267.22±8.1$^{###}$	256.07±10.95$^{###}$	234.94±6.49$^{###}$
NO (ng/mL)	35.54±4.74	72.42±1.64***	56.13±2.68$^{###}$	45.18±3.05$^{###}$	60.36±1.64$^{###}$
iNOS (μmol/L)	11.84±1.07	19.62±1.3***	13.86±1.3$^{###}$	12.3±1.07$^{###}$	14.58±0.9$^{###}$
ROS（nmol//mL）	692.39±59.34	862.86±91.32***	718.56±90.88$^{###}$	758.33±88.32$^{###}$	826.14±73.93$^{###}$
MDA (nmol/mL)	3.62±0.07	6.54±0.48***	3.64±0.27$^{###}$	4.01±0.15$^{###}$	5.42±0.1$^{###}$
GSH (μmol/L)	98.10±13.93	30.73±4.93***	69.30±9.32$^{###}$	78.32±12.34$^{###}$	57.39±7.00$^{###}$
CAT (U/mL)	31.89±3.48	21.04±1.73***	27.5±1.59$^{##}$	25.77±1.14$^{#}$	23.39±2.81
GPx (mU/ml)	226.07±3.89	124.44±3.74***	194.36±8.29$^{###}$	209.28±5.47$^{###}$	195.41±5.91$^{###}$

The activities of T-SOD, CAT, and Gpx in the serum were assessed by relevant activity assay kits; the productions of MDA and NO in the serum were detected by commercial assay kits; the concentrations of iNOS, GSH, and ROS were measured by ELISA kits. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{#}P < 0.05$, $^{##}P < 0.01$, $^{###}P < 0.001$ vs ADR.

Table S7. Effects of PB2 on serum TNF-α, NF-κB, IL-1β, IFNγ, IL-6, IL-17, MCP-1 and IL-5, Hsp90 in ADR mice (fifth week)

Groups	NC	ADR	SIR	PB2H	PB2L
TNF-α (ng/L)	573.63 ± 9.61	697.75 ± 15.4***	628.79 ± 10.95##	614.63 ± 14.96###	637.84 ± 13.17##
NF-κB (ng/L)	348.28 ± 16.59	745.38 ± 29.06***	635.62 ± 22.25###	607.47 ± 14.05###	613 ± 28.74###
IL-1β (ng/L)	28.5 ± 2.47	57.13 ± 2.95***	43.22 ± 4.36###	47.06 ± 3.67##	50.14 ± 3.29#
IFNγ (ng/L)	982.77 ± 44.81	554.58 ± 28.25***	697.99 ± 24.57###	715.07 ± 26.16###	706.78 ± 14.4###
IL-6 (pg/mL)	5.06±1.16	15.57±2.88***	11.43±2.09###	9.37±1.27###	11.59±1.61###
IL-17 (ng/L)	3.57 ± 0.44	6.67 ± 0.83***	5.85 ± 0.63###	4.87 ± 0.57###	5.59 ± 0.49###
MCP-1 (pg/mL)	95.62±6.67	163.3±20.01***	117.3±5.67##	109.8±4.66###	159.6±13.96
IL-5 (pg/ml)	2.43 ± 0.16	14.17 ± 2.05***	9.29 ± 2.67##	6.34 ± 0.83###	10.97 ± 1.21#
Hsp90 (g/ml)	32.43±0.36	52.66±0.16**	35.98±0.27###	32.01±0.21#	21.77±0.24##

The levels of TNF-α, NF-κB, IL-1β, IFNγ, IL-6, IL-17, MCP-1, IL-5, and Hsp90 in the serum were assessed by ELISA kits. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

Table S8. Effect of PB2 on liver function in ADR mice

	Groups	NC	ADR	SIR	PB2H	PB2L
Serum	ALT (U/L)	8.34±0.47	20.71±2.68***	15.13±0.27###	14.62±0.69###	16.54±0.59##
	AST (U/L)	23.53±1.99	41.79±5.8**	31.61±4.34	28.85±6.59#	33.98±4.92
	ALP (U/L)	8.77±0.33	19.22±0.15***	12.99±1.20##	11.87±0.99##	13.76±0.55#
	TBLL (μ mol/L)	2.77±0.73	9.56±1.26***	6.99±0.26###	6.37±0.77###	9.02±1.87#
	DBIL (μ mol/L)	1.66±0.36	6.33±0.11***	4.62±0.64#	3.98±0.27##	5.20±0.65
Liver	ALT (U/ mg prot)	10.76±0.76	23.92±2.47***	16.58±1.61###	12.04±1.09###	18.38±1.35##
	AST (U/ mg prot)	7.14±1.39	13.48±3.25**	8.6±2.13#	7.82±1.29#	11.13±2.83
	ALP (U/ mg prot)	8.87±0.23	19.43±0.71**	12.99±0.62#	9.32±0.09###	14.98±0.23#
	TBLL (μ mol/ mg prot)	5.55±0.36	3.32±0.27**	4.67±0.37	4.66±0.28#	4.52±0.19##
	DBIL (μmol/ mg prot)	2.65±0.15	1.01±0.04*	2.12±0.06#	2.28±0.11#	1.87±0.21#

The activities of ALT, AST, and ALP in the serum and liver, were evaluated by relevant activity assay kits; the levels of TBIL and DBIL in the serum and liver, were measured by ELISA kits. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

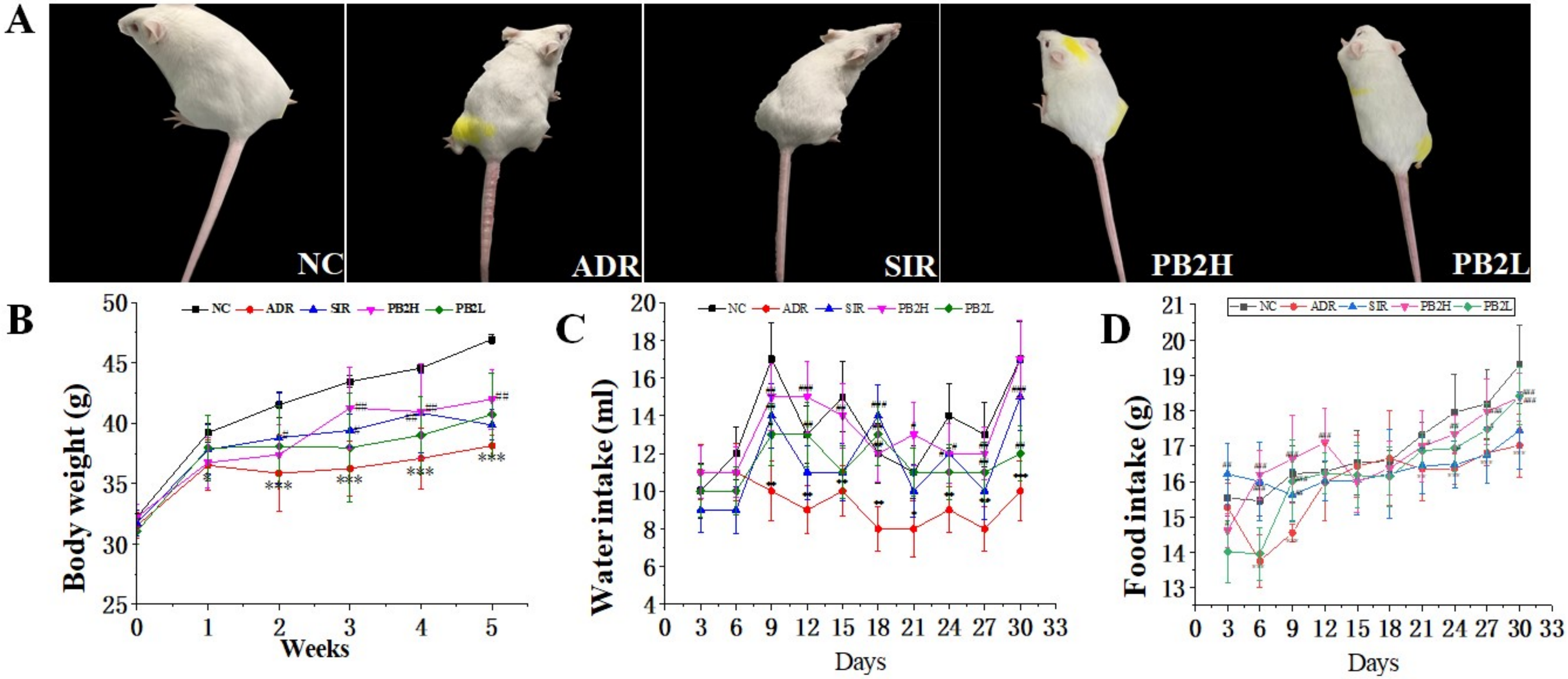

Figure S1. Effects of PB2 on general conditions of ADR-induced NS mice. Macroscopic observations of all groups (A); Body weight (B); water intake (C); food intake (D). Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

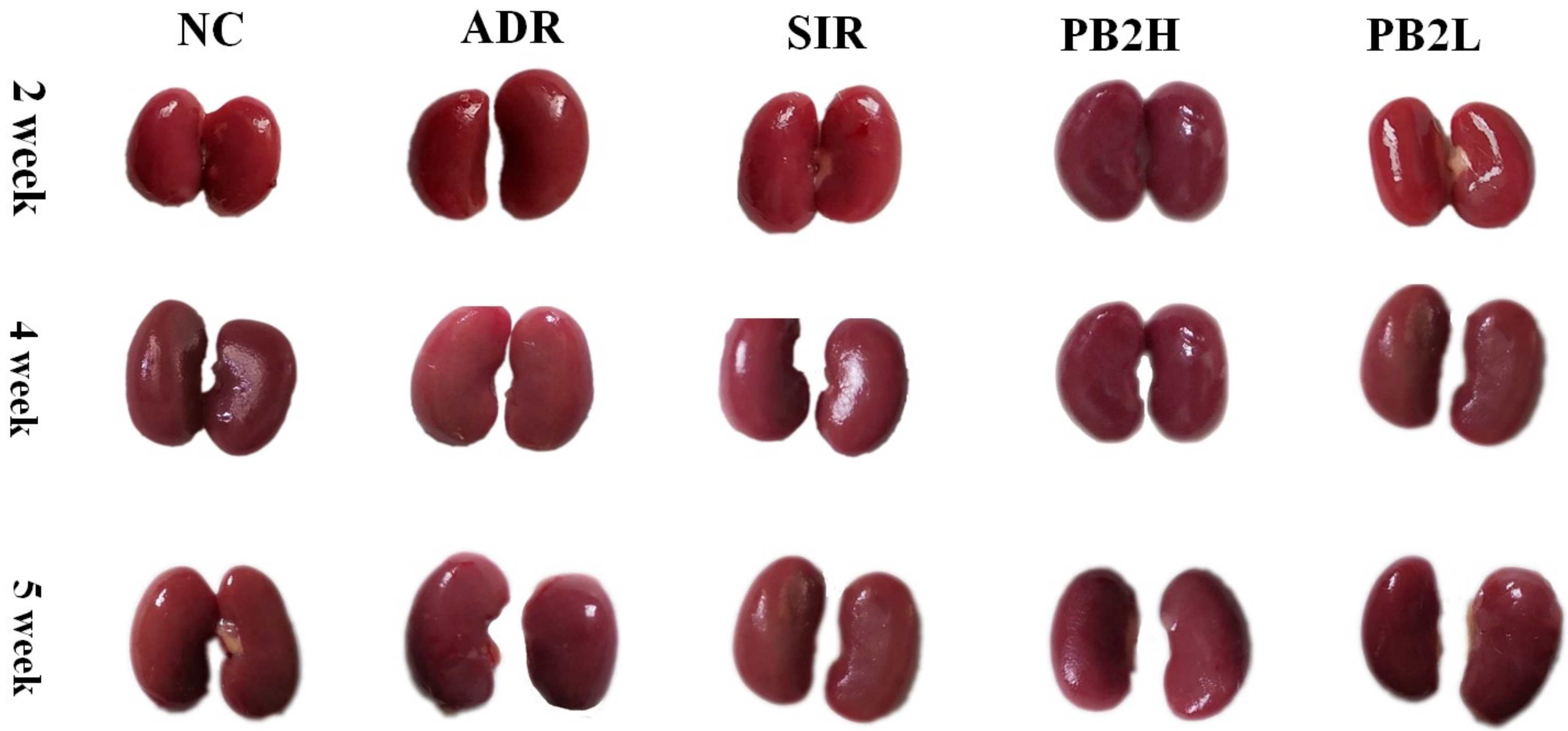

Figure S2. The general observation of kidney in each group at different periods. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

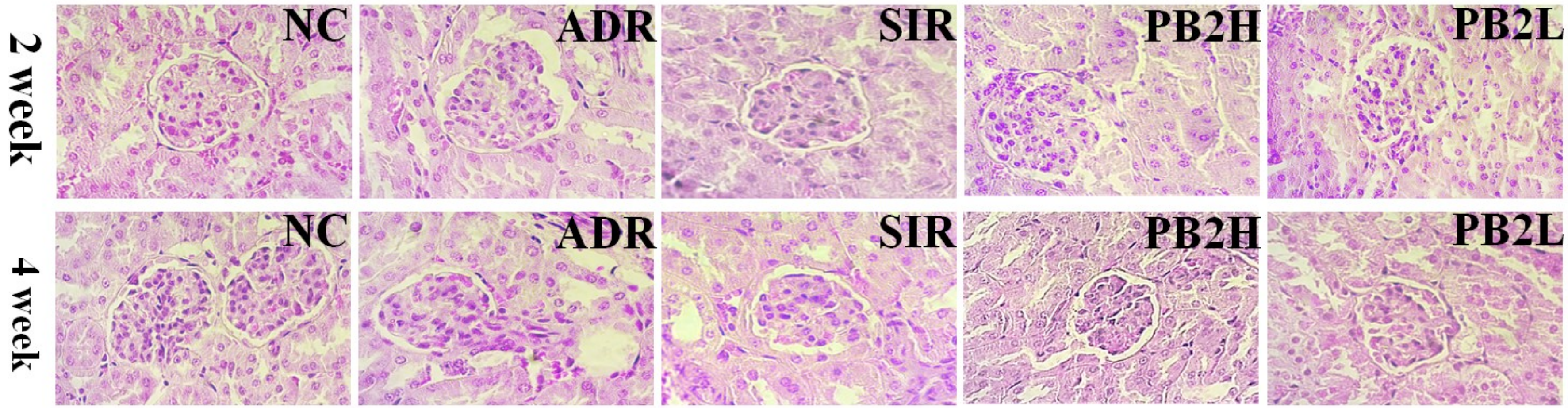

Figure S3. The kidney sections were stained with haematoxylin and eosin (H&E) to demonstrate the histopathological morphology at 2 and 4 weeks. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

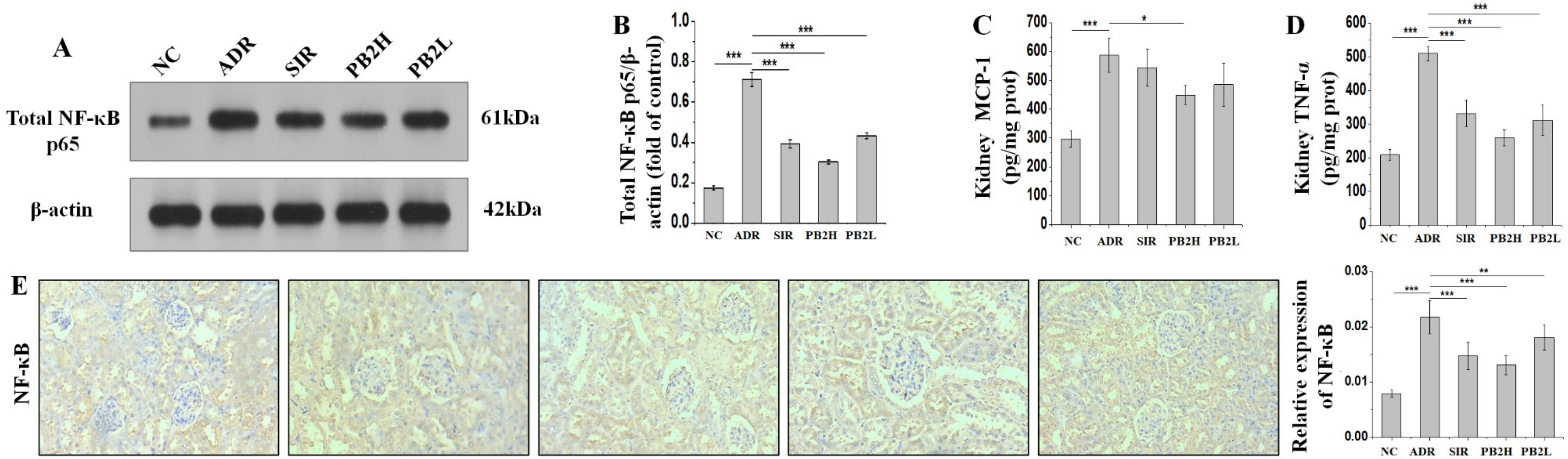

Figure S4. The effects of PB2 on renal inflammation. (A) Representative immunoblot for detection of total NF-κB p65; (B) Fold change in relative density of total NF-κB p65 protein bands. The relative density is presented as total NF-κB p65/β-actin; (C, D) The levels of MCP-1 and TNF-α in the kidney; (E) The immunohistochemical analyses and its quantifications of NF-κB in the kidney. The levels of total NF-κB P65 in the kidney were detected by Western blotting; the levels of MCP-1 and TNF-α in the kidney were measured using ELISA kits. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

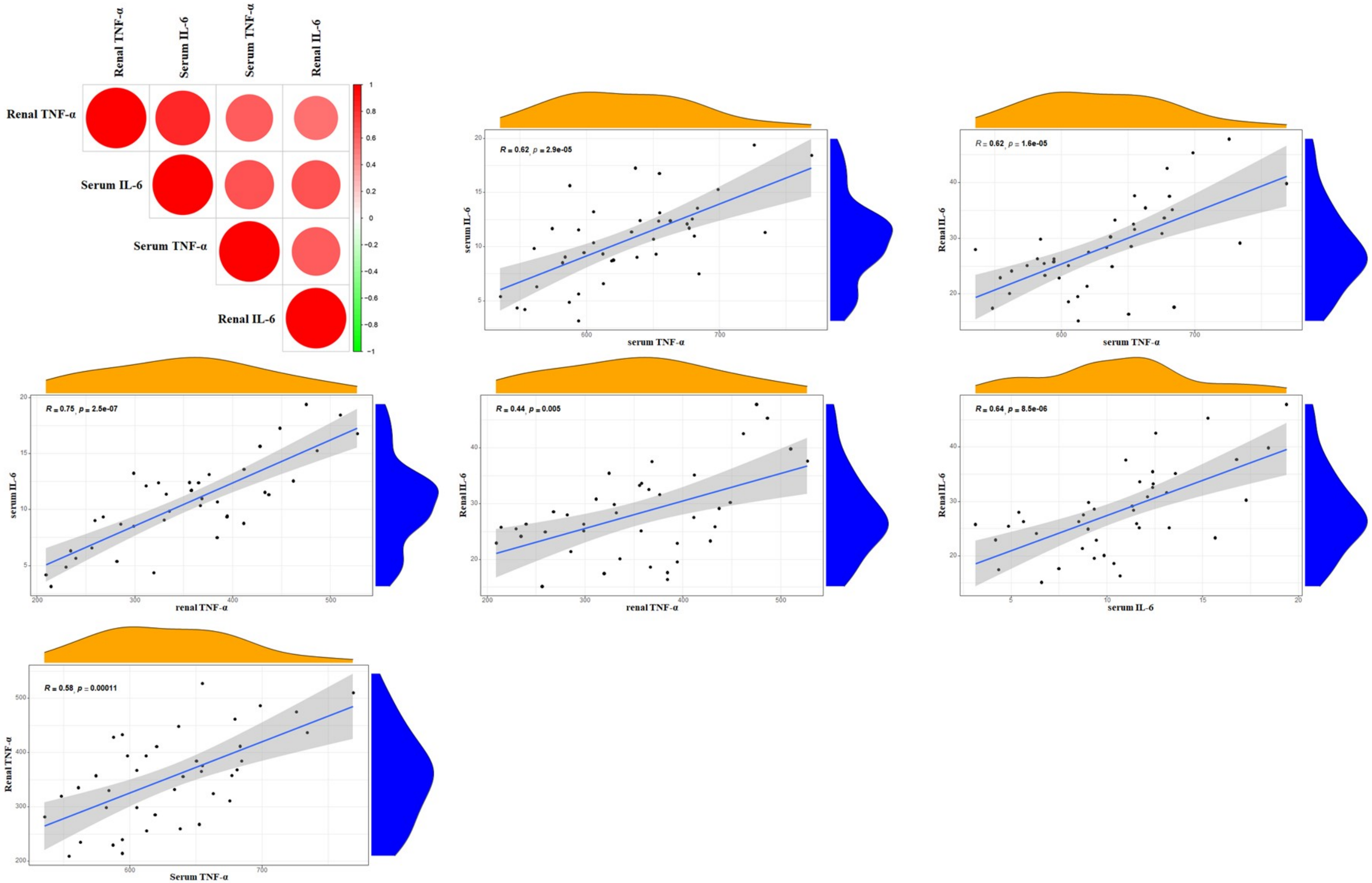

Figure S5. The statistical study of correlation between serum and renal levels of TNF-α and IL-6.

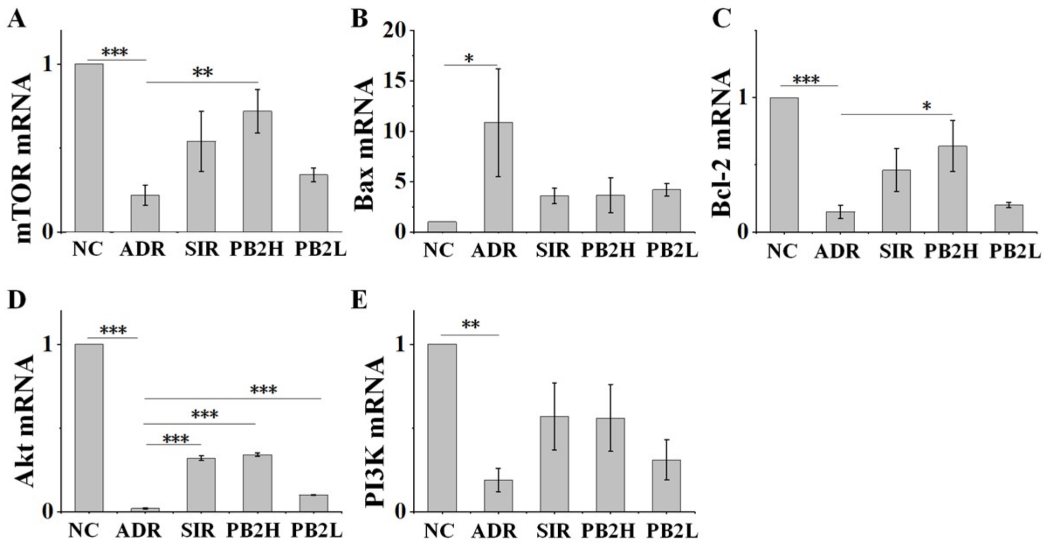

Figure S6. The mRNA expressions of mTOR(A), Bax(B), Bcl-2(C), Akt(D) and PI3K(E) in kidney were evaluated by qRT-PCR analysis. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

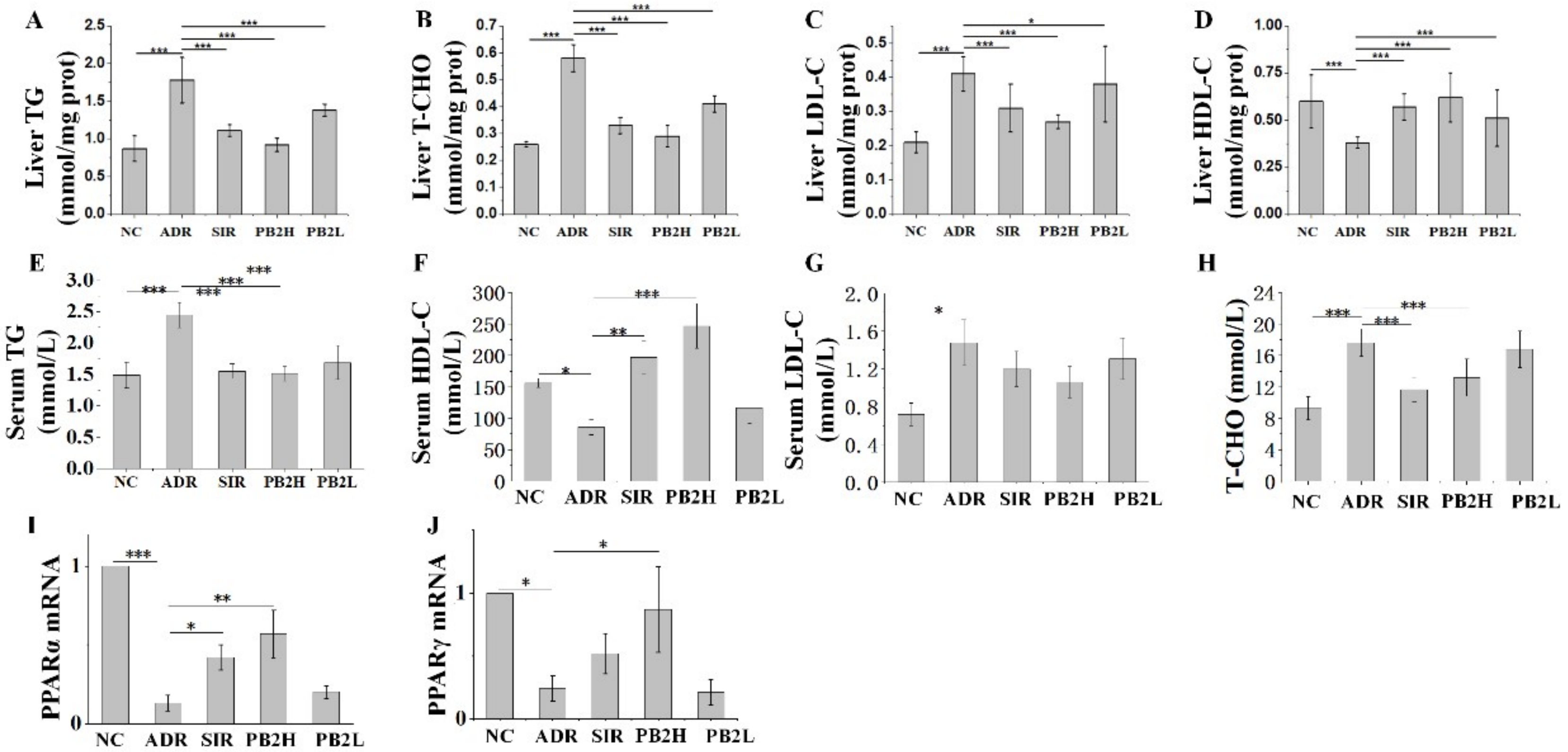

Figure S7. Effects of PB2 on lipid dysmetabolism. (A-D) The levels of TG, T-CHO, LDL-C, HDL-L in liver; (E-H) The levels of TG, T-CHO, LDL-C, HDL-L in serum; (I-J) The mRNA expressions of PPARα and PPARγ in the kidney. The levels of T-CHO, TG, LDL-C, and HDL-C in the liver and serum, were detected by assay kits; the mRNA expressions of PPARα and PPARγ in the kidney were evaluated by qRT-PCR analysis. Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

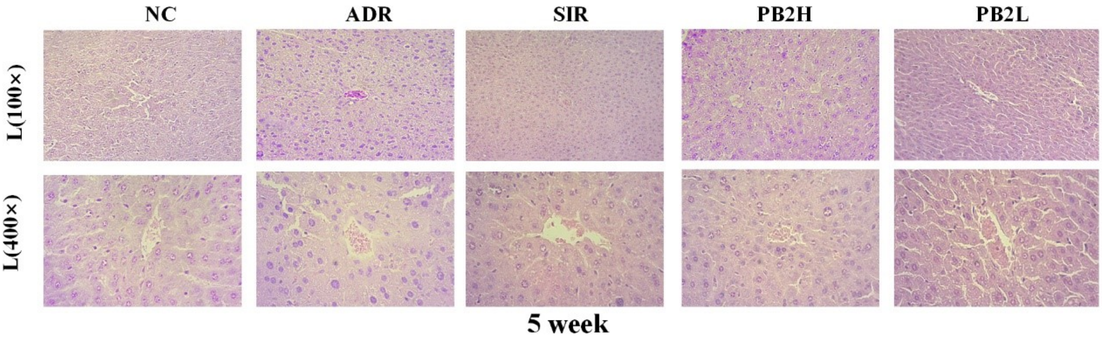

Figure S8. The liver sections were stained with haematoxylin and eosin (H&E) to demonstrate the histopathological morphology (100×，400×) at fifth week; Liver sections stained with H&E (B). Values are presented as the mean ± SEM (n=8). Data analyzed by one-factor analysis of variance (ANOVA) followed by a Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs ADR.

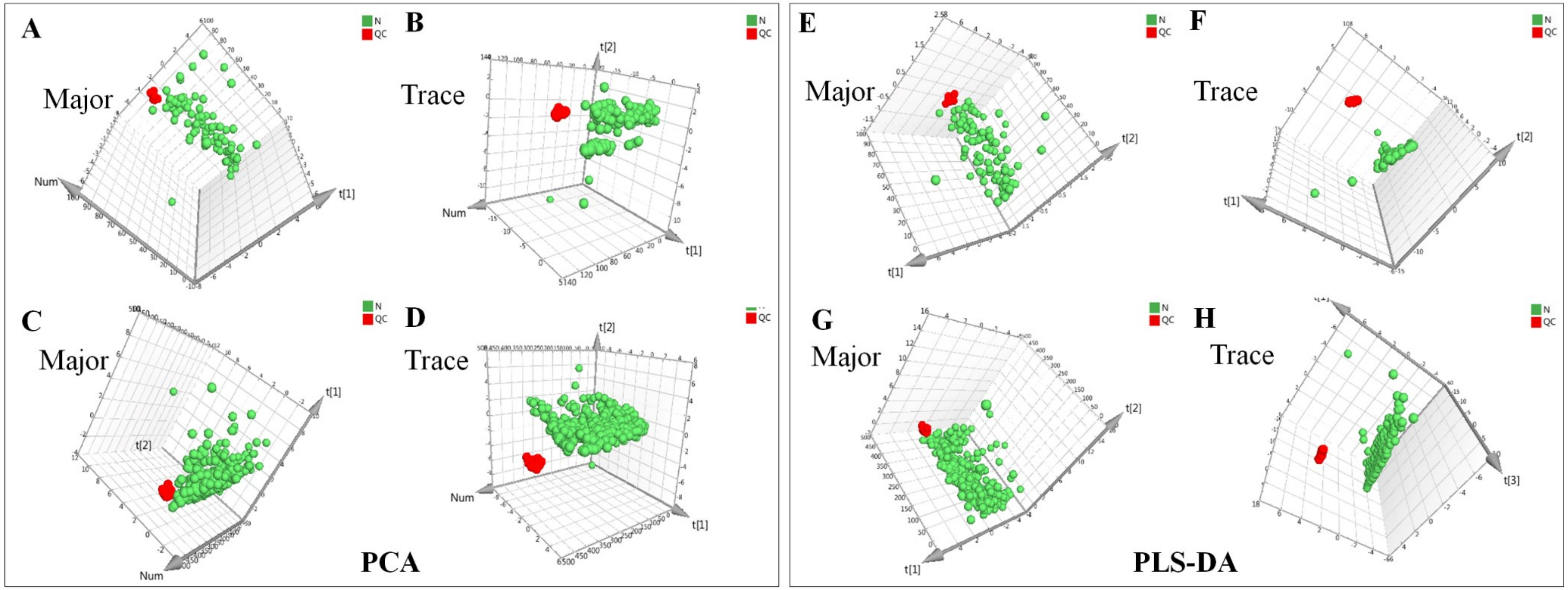

Figure S9. The QC of PCA and PLS-DA. (A-D) PCA score plots: (A) serum major elements, (B) serum trace elements, (C) urine major elements and (D) urine trace elements. (E-H) PLS-DA score plots: (E) serum major elements, (F) serum trace elements, (G) urine major elements and (H) urine trace elements.

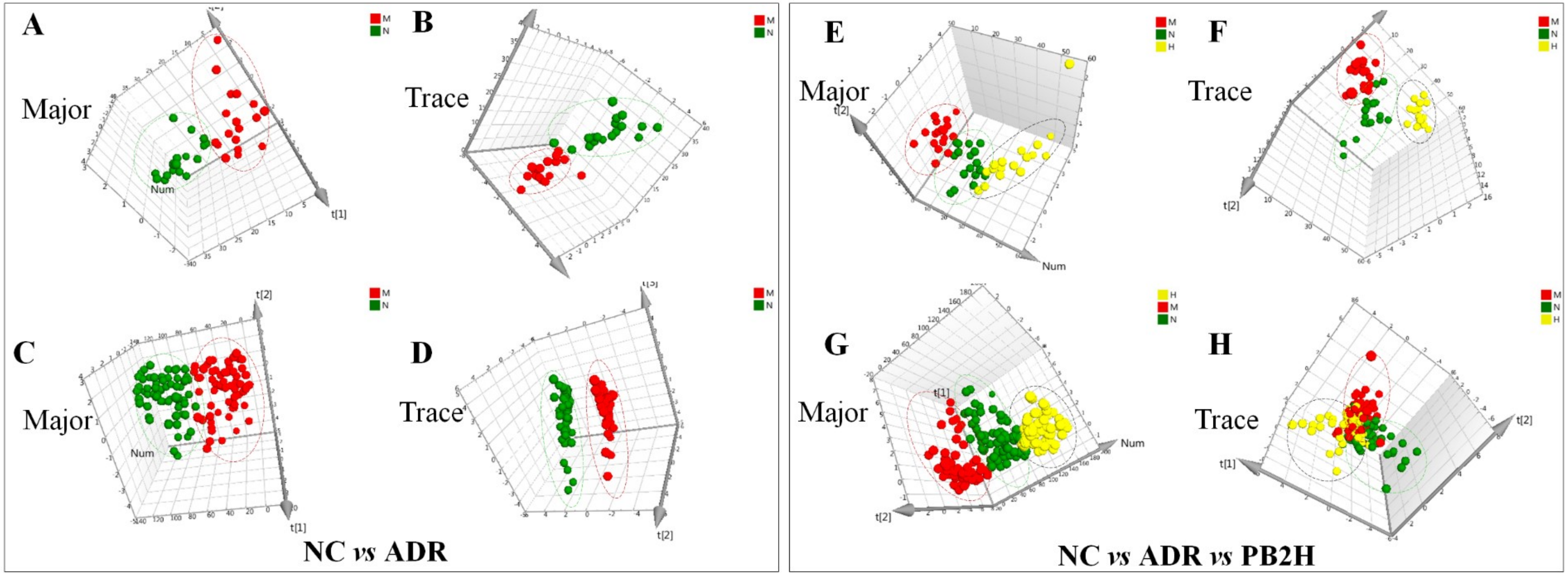

Figure S10. PLS-DA score plots model of elements derived from the serum and urine of mice. (A) serum major element from the NC group and ADR group; (B) serum trace element from the NC group and ADR group; (C) urine major element from the NC group and ADR group; (D) urine trace element from the NC group and ADR group; (E) serum major element from the NC group, ADR group and PB2H group; (F) serum trace element from the NC group, ADR group and PB2H group; (G) urine major element from the NC group, ADR group and PB2H group; (H) urine trace element from the NC group, ADR group and PB2H group.

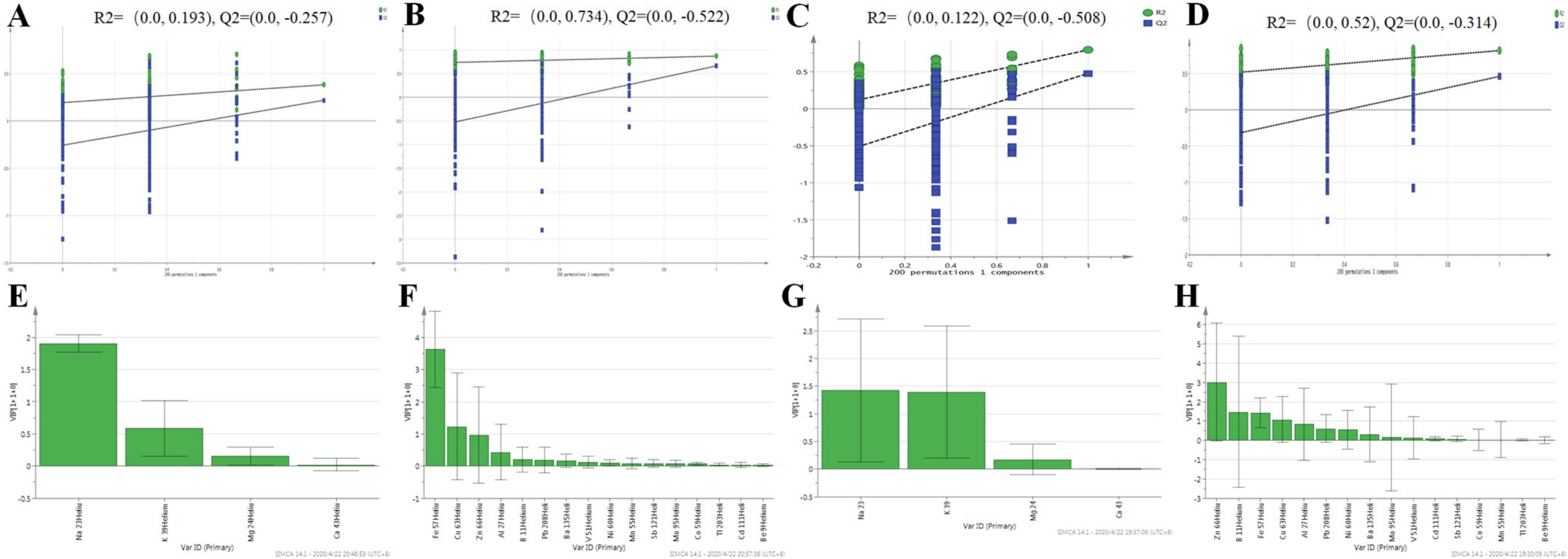

Figure S11. The result on permutation test of OPLS-DA model and VIP plot of NC group vs ADR group. (A-D) The result on permutation test of OPLS-DA model in the (A) serum major elements R2(0.0, 0.193), Q2(0.0, -0.257), (B) serum trace elements R2(0.0, 0.734), Q2(0.0, -0.522), (C) urine major elements R2(0.0, 0.122), Q2(0.0, -0.508) and (D) urine trace elements R2(0.0, 0.52), Q2(0.0, -0.314) from the ICP-MS spectra of NC and ADR at the 5 weeks. (E-H) VIP plot of NC vs ADR in the (E) serum major elements, (F) serum trace elements, (G) urine major elements and (H) urine trace elements at the 5 weeks