

### Supplemental Table S1

**Composition of prenatal diet.** \* AIN-76 prescription (Oriental Yeast Co., Ltd., Tokyo, Japan).  
mCN: maternal control diet; mLP: maternal low-protein diet.

Component (% w/w)	mCN (20% Casein)	mLP (9% Casein)
Casein	20.0	9.0
Corn starch	66.8	77.8
DL-Methionine	0.2	0.2
Soybean oil	5.0	5.0
Vitamin mixture*	1.0	1.0
Mineral mixture*	4.0	4.0
Cellulose powder	3.0	3.0

**Supplemental Table S2**

**Composition of low and high protein diets in offspring.** \* AIN-76 prescription (Oriental Yeast Co., Ltd., Tokyo, Japan). CN: control diet; LP: low-protein diet; HP: high-protein diet.

<b>Component (% w/w)</b>	<b>CN (20% Casein)</b>	<b>LP (9% Casein)</b>	<b>HP (40% Casein)</b>
Casein	20.0	9.0	40.0
Corn starch	66.8	77.8	46.8
DL-Methionine	0.2	0.2	0.2
Soybean oil	5.0	5.0	5.0
Vitamin mixture*	1.0	1.0	1.0
Mineral mixture*	4.0	4.0	4.0
Cellulose powder	3.0	3.0	3.0

### Supplemental Table S3

Statistical summary of methylome sequencing and the average methylation level of CN/LP group.

	CN	LP
<b>Read Number</b>	<b>754.6M</b>	<b>525.5M</b>
<b>Rate of Uniquely Mapped Reads</b>	<b>80.1%</b>	<b>80.7%</b>
<b>Rate of Reads Mapped on Multiple Sites</b>	<b>6.5%</b>	<b>6.6%</b>
<b>Rate of Reads Unmapped</b>	<b>13.4%</b>	<b>12.8%</b>
<b>Average Read Depth</b>	<b>CN</b>	<b>LP</b>
<b>All Nucleotides</b>	<b>24.9x</b>	<b>22.9x</b>
<b>All Cytosines</b>	<b>27.0x</b>	<b>25.0x</b>
<b>CpG sites</b>	<b>29.6x</b>	<b>26.6x</b>
<b>Average Methylation Level</b>	<b>CN</b>	<b>LP</b>
<b>All Cytosines</b>	<b>4.6%</b>	<b>4.5%</b>
<b>CpG sites</b>	<b>70.0%</b>	<b>70.0%</b>
<b>CHG sites</b>	<b>1.3%</b>	<b>1.2%</b>
<b>CHH sites</b>	<b>1.3%</b>	<b>1.2%</b>

## Supplemental Table S4

**Primer sequences for each gene. a-b** Primer sequences used for real-time PCR of (a) candidate genes narrowed by integrating data from both methylome and transcriptome profiling and (b) The seven genes closely associated with kidney disease that fluctuated only in the methylome analysis. (c) Primer sequences used for the bisulfite sequencing. The number of CpGs evaluated as well as the regions that were assessed when the transcription start site (TSS) was set to +1.

**a**

Target gene	Primer Sequence (5' to 3')
<i>Gnas</i> (GNAS complex locus)	Forward : AGCAGGGCAAACGAGAAGA Reverse : ATGCACACCGAGAGAGATGG
<i>Ptger1</i> (prostaglandin E receptor 1 subtype EP1)	Forward : AGTAGCTGGAGTGGGAGCA Reverse : GCTCATATCAGTGGCCAAGAGG
<i>Runx1</i> (runt-related transcription factor 1)	Forward : GAGGCCAACAGGAGGAAAC Reverse : CACCCGGAACCGAAAACA
<i>Tbx3</i> (T-box 3)	Forward : GGAGGAAGTGGGGTGAAAGG Reverse : GTCCAACAGGCACACGAAAG
<i>Actb</i> (beta-actin)	Rat Housekeeping Gene Primer Set, Rat Actb primer

**b**

Target gene	Primer Sequence (5' to 3')
<i>Aggf1</i> (angiogenic factor with G patch and FHA domains 1)	Forward : GACTCGCAGAGTGAGTGAG Reverse : GCACAGTGTGTCCATGTCC
<i>Cyp24a1</i> (cytochrome P450 family 24 subfamily a member 1)	Forward : CAAACCTGCATCGACAACC Reverse : CCGAATGGGAGATGAGCGAA
<i>Fat4</i> (FAT atypical cadherin 4)	Forward : TGTACAGCCAAACCGAGTCA Reverse : TGACCAGAAGTCCACACAGG
<i>Lhx1</i> (LIM homeobox 1)	Forward : ACTGGAGACGTTGAAGGCAG Reverse : AAAGACCTCCGACACATGG
<i>Pdgfra</i> (platelet-derived growth factor alpha polypeptide)	Forward : ACAAACCTGAGAGCCCATGG Reverse : CGCACATTGGCAATGAAGCA
<i>Ryr1</i> (ryanodine receptor 1)	Forward : TGAATACAACGCTGCTCTG Reverse : GCATAGGCAGTGTGATCTCG
<i>Sema5b</i> (semaphorin 5B)	Forward : AGGTGCTCGGCTACCTTAG Reverse : CAAGAACCCGAGTTGTCTCC

**c**

Name	Position	Number of CpGs analyzed	Primer Sequence (5' to 3')
<i>Ptger1</i> CpG island ①	+ 654 ~ + 953 (300 bp)	23 CpGs	Sense primer: AATATATTTGGTGTGTTAATAGG Antisense primer: ACCAAAAAACCATACAACC
<i>Ptger1</i> CpG island ②	+ 939 ~ + 1153 (215 bp)	18 CpGs	Sense primer: ATGGTTTTTTTGGTTTGTGTTAT Antisense primer: CACCAATACCAAAATACTATAACTC
<i>Ptger1</i> CpG island ③	+ 1158 ~ + 1420 (263 bp)	28 CpGs	Sense primer: ATTAGTTTTGGGTTTTTGGAGG Antisense primer: AAACAATAATAATAATAATAAC
<i>Ptger1</i> promoter	-318 ~ + 243 (561 bp)	10 CpGs	Sense primer: GTTTATGGTTTTTGTGTTAAGTTGTTA Antisense primer: CTTTCAAAAATCTCTTAACCTACC

## Supplemental Table S5

Primer sequences for the genes (*Slc14a2*, *Il1b*, *Corin*, *Hmox1*, *Gucy1b3*, and *Gdnf*) extracted in transcriptome analysis.

Target gene	Primer Sequence (5' to 3')
<b>Slc14a2</b> (solute carrier family 14 (urea transporter), member 2)	Forward : TGAATGGCTCTCAGAGGAG Reverse : ACGTTTGCTTACACTATGATTGGA
<b>Il1b</b> (interleukin 1 beta)	Forward : TCAAGCAGAGCACAGACCTG Reverse : AGAACTCAGTGCTGGCTGTG
<b>Corin</b> (corin, serine peptidase)	Forward : CCTAATTTAATTAGCCAGACATCAACG Reverse : CAAAACCAACCGACGGTCTTAA
<b>Hmox1</b> (heme oxygenase 1)	Forward : CAGGGGAGAATCTTGCCTGG Reverse : TACCACCCACCCCTCAAAG
<b>Gucy1b3</b> (guanylate cyclase 1, soluble, beta 3)	Forward : GTGGGCTTCAACGCTTTCTG Reverse : CCACAAAGGCTGCCATTGAG
<b>Gdnf</b> (glial cell derived neurotrophic factor)	Forward : ATGTAGTCCCAGACCCTCCG Reverse : CTTACTGTGGCAGACGCTCA

# Supplemental Table S6

**Pregnancy-related changes in maternal body weight and feed intake, and the body weight and kidney weight of pups. a** Changes in maternal body weight, weight gain, and feed intake during pregnancy. **b** Body weight and kidney weight at D5, D10, and D28 of postnatal rat. Data are expressed as means  $\pm$  standard error. Sample sizes for (a) were  $n = 6-7$ , and for (b) were  $n = 5$  rats per group.  $*p < 0.05$  vs. the D10-CN group according to a Student's  $t$ -test.

**a**

Type of record	Groups	
Gestational period	CN	LP
1st day pregnancy weight (g)	175.13 $\pm$ 2.62	168.42 $\pm$ 3.60
21st day pregnancy weight (g)	261.50 $\pm$ 4.87	251.40 $\pm$ 7.08
Weight gain during pregnancy (g)	86.38 $\pm$ 3.80	82.98 $\pm$ 4.06
Feed intake (g/day)	14.73 $\pm$ 0.21	14.96 $\pm$ 0.26

**b**

Type of record	Groups	
5 day old rat pups	D5-CN	D5-LP
Final weight (g)	7.48 $\pm$ 0.10	7.30 $\pm$ 0.15
Kidney weight (mg)	83.32 $\pm$ 3.32	88.00 $\pm$ 5.82
10 day old rat pups	D10-CN	D10-LP
Final weight (g)	13.59 $\pm$ 0.46	14.15 $\pm$ 0.27
Final weight (mg)	159.28 $\pm$ 3.50	173.62 $\pm$ 2.54 *
28 day old rat pups	D28-CN	D28-LP
Final weight (g)	55.51 $\pm$ 0.23	57.33 $\pm$ 0.72
Final weight (mg)	569.66 $\pm$ 29.99	611.98 $\pm$ 18.85

## Supplemental Table S7

**Statistical data for six genes that fluctuate in both methylome and transcriptome analyses.**

**a** The location and number of fluctuating CpG sites extracted in methylome analysis. **b** Fold changes in gene expression obtained from transcriptome analysis.

**a**

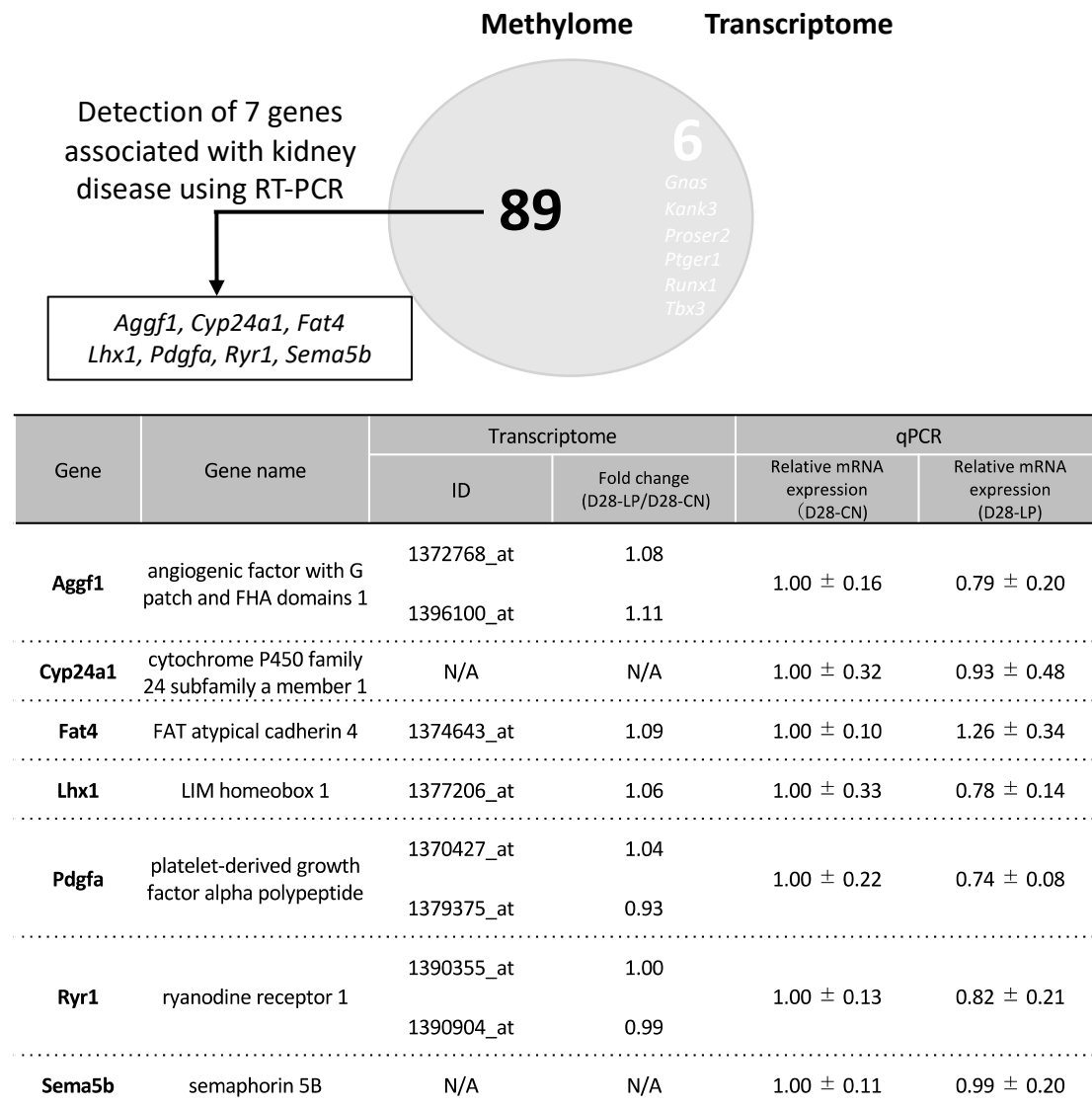
Chr	Gene	Gene name	Position of CpG analyzed	Number of CpGs analyzed	Number of hypermethylated CpGs in D28-LP	Number of hypomethylated CpGs in D28-LP
Chr. 3	Gnas	GNAS complex locus	intragenic	148	36	112
Chr. 7	Kank3	KN motif and ankyrin repeat domains 3	intragenic	147	46	101
Chr. 11	Runx1	runt-related transcription factor 1	intragenic	98	45	53
Chr. 12	Tbx3	T-box 3	intragenic	88	31	57
Chr. 17	Proser2	proline and serine rich 2	intragenic	118	38	80
Chr. 19	Ptger1	prostaglandin E receptor 1 (subtype EP1)	intragenic	156	114	42

**b**

Gene	Gene name	ID	Fold change (LP/CN)
Gnas	GNAS complex locus	1387906_a_at	1.24
		1390263_at	1.29
Kank3	KN motif and ankyrin repeat domains 3	1373483_at	1.25
Runx1	runt-related transcription factor 1	1368914_at	1.24
Tbx3	T-box 3	1390627_a_at	1.25
		1393160_at	1.68
		1379057_at	1.38
Proser2	proline and serine rich 2	1385668_at	1.33
		1379057_at	1.38
		1385668_at	1.33
Ptger1	prostaglandin E receptor 1 (subtype EP1)	1369254_a_at	1.49

## Supplemental Table S8

The genes that fluctuate in the kidneys of SHRSP pups (D28) exposed to a fetal low-protein diet. The expression of seven genes (*Aggf1*, *Cyp24a1*, *Fat4*, *Lhx1*, *Pdgfa*, *Ryr1*, and *Sema5b*) closely associated with kidney disease, which fluctuated only in methylome analysis, were analyzed by transcriptome analysis and real-time PCR. Data are expressed as means  $\pm$  standard error ( $n = 5$ ). N/A, Not Applicable. CN and LP, offspring CN/LP diet.

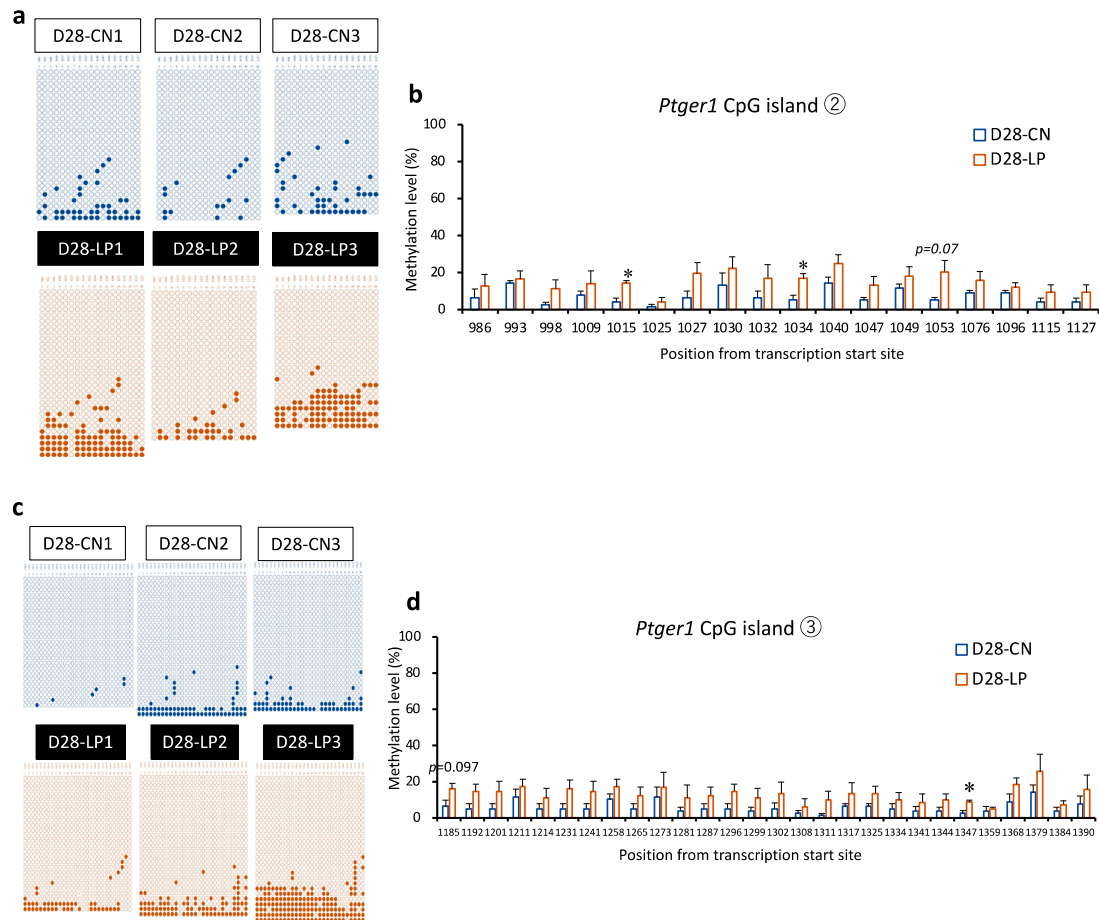




# Supplemental Table S9

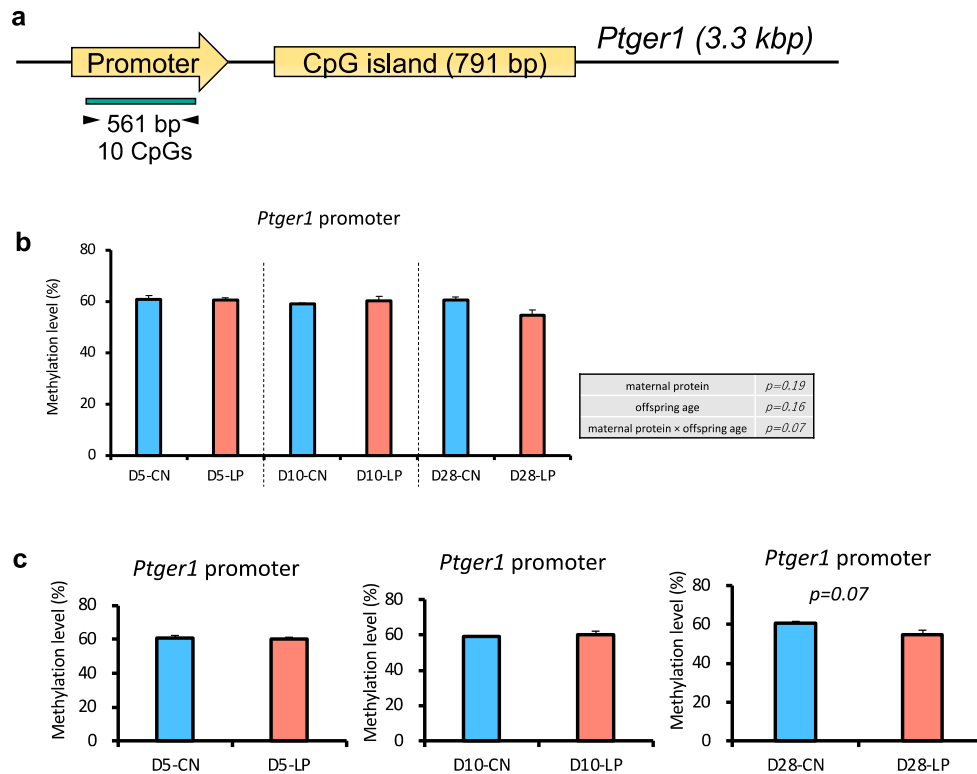
Relative ratios (Fold-change) of kidney disease-related genes extracted in transcriptome analysis from D5 to D28 over time in pup kidneys.

Gene	Gene name	ID	Fold-change(D5)	Fold-change(D10)	Fold-change(D28)
Increase (Continuation)					
Slc14a2	solute carrier family 14 (urea transporter), member 2	1387550_a_at	1.24	1.45	1.33
Decrease (Continuation)					
Il1b	interleukin 1 beta	1398256_at	0.68	0.68	0.78
Increase (Cancel)					
Bmpr2	bone morphogenetic protein receptor, type II (serine/threonine kinase)	1376843_at	1.25	1.03	1.10
Cdkn1c	cyclin-dependent kinase inhibitor 1C	1372299_at	1.27	1.00	1.19
Corin	corin, serine peptidase	1382954_at	1.53	0.97	1.02
Cryab	crystallin, alpha B	1370026_at	1.35	1.02	1.14
Hba1 /// Hba2	hemoglobin, alpha 1 /// hemoglobin, alpha 2	1388608_x_at	1.27	1.20	1.03
Hmox1	heme oxygenase 1	1370080_at	1.34	0.85	1.09
Myh11	myosin, heavy chain 11, smooth muscle	1370896_a_at	1.29	1.45	1.02
Decrease (Cancel)					
Apob	apolipoprotein B	1388190_at	0.63	1.13	0.90
Gdnf	glial cell derived neurotrophic factor	1385180_at	0.76	0.95	0.86
Gucy1b3	guanylate cyclase 1, soluble, beta 3	1374389_at	0.73	0.86	0.89



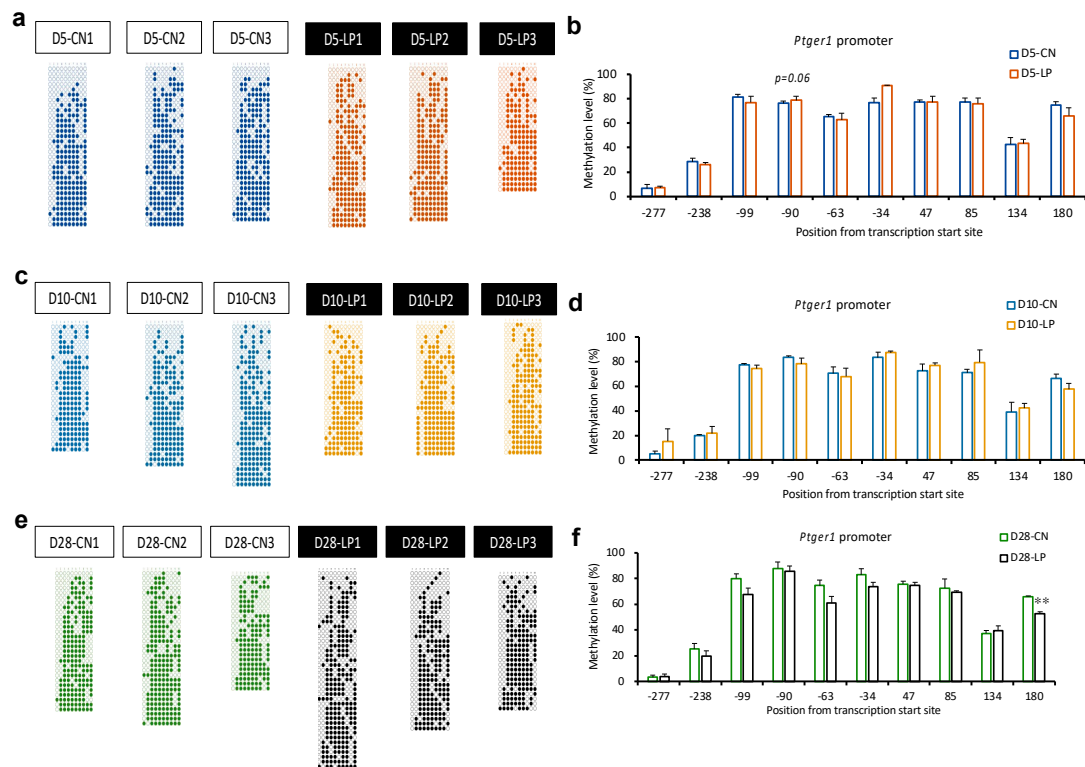
**Supplemental Figure S1**

***Ptger1* CpG island (CpG② and CpG③) DNA methylation levels in pup (D28) kidneys of fetal exposure to maternal low protein diet.** **a** Bisulfite sequencing analysis of the *Ptger1* CpG island ②. **b** DNA methylation levels of each CpG site in the *Ptger1* CpG island ② were calculated using the methylation data shown in (a). **c** Bisulfite sequencing analysis of the *Ptger1* CpG island ③. **d** DNA methylation levels of each CpG site in the *Ptger1* CpG island ③ were calculated using the methylation data shown in (c). The horizontal axis shows each evaluated CpG site. ○, unmethylated cytosine; ●, methylated cytosine. Data are expressed as means ± standard error ( $n = 3$ ). \* $p < 0.05$  vs. the D28-CN group according to a Student's t-test. *Ptger1*, Prostaglandin E Receptor 1. CpG, 5'-C-phosphate-G-3'.



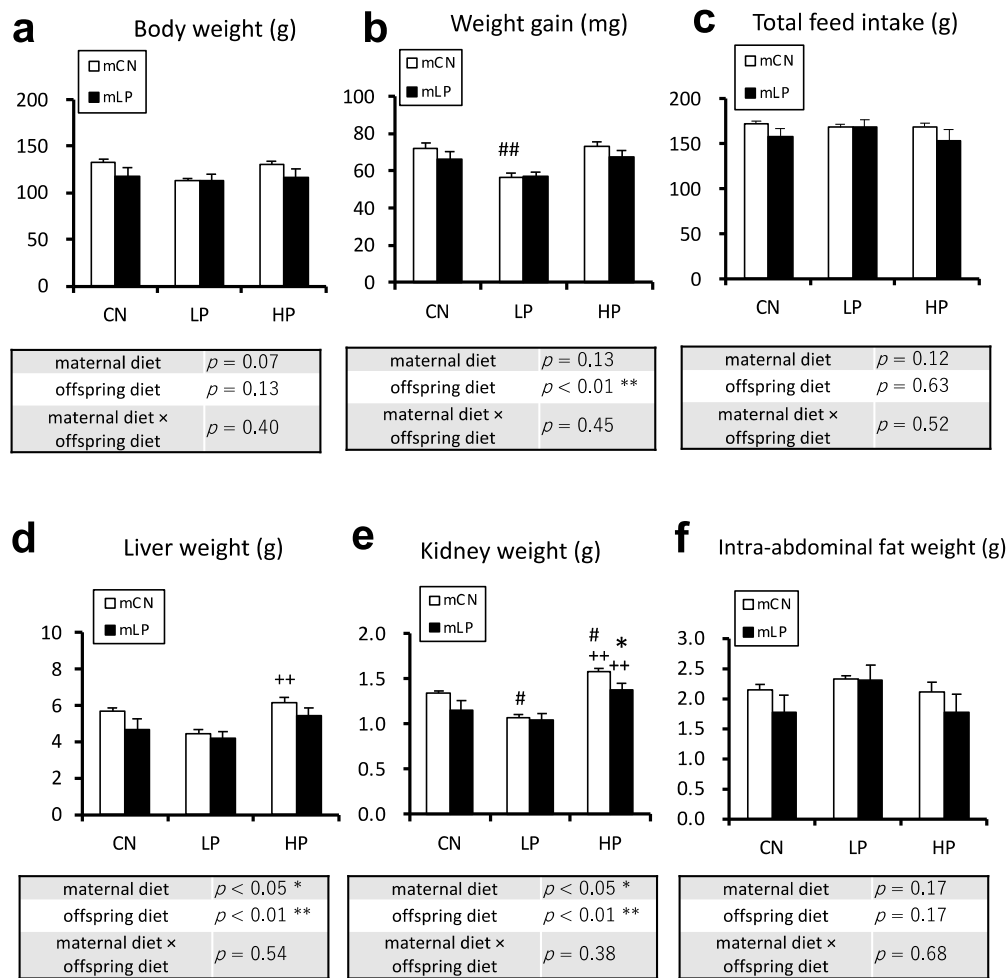
**Supplemental Figure S2**

***Ptger1* promoter DNA methylation levels from D5 to D28 in pup kidneys exposed to a fetal low-protein diet.** **a** Cartoon showing the evaluated *Ptger1* promoter region. Primers were designed in the region around the TSS (-318 bp to + 243 bp). **b** DNA methylation levels of the *Ptger1* promoter over time in pup kidneys from D5 to D28 according to maternal diet and offspring age. **c** DNA methylation levels of the *Ptger1* promoter at D5, D10, and D28 according to maternal diet. Data are expressed as means  $\pm$  standard error ( $n = 5$ ). Statistical analysis was performed using Tukey's test (**b**) and Student's *t*-test (**c**).



### Supplemental Figure S3

***Ptger1* promoter DNA methylation levels from D5 to D28 in pup kidneys exposed to a fetal low-protein diet. a, c, and e** Bisulfite sequencing analysis of *Ptger1* promoter in D5, D10 and D28. The horizontal axis shows each evaluated CpG site. ○, Unmethylated cytosine; ●, methylated cytosine. **b, d, and f** DNA methylation levels at each CpG site in *Ptger1* promoter were calculated from the bisulfite sequencing data shown in (a), (c) and (e), respectively. Data are expressed as means  $\pm$  standard error ( $n = 5$ ). \*\* $p < 0.01$  vs. the D28-CN group according to a Student's *t*-test.



**Supplemental Figure S4**

**Body weight, feed intake, and tissue weight either on a low-protein diet or a high-protein diet for 2 weeks after weaning (at week 4).** **a** Body weight of adult offspring (D42) in each group. **b-c** The graphs illustrate the weight gain (**b**) or total feed intake (**c**) from the age of 4 to 6 weeks during the low-protein or high-protein diet trials. **d-f** The graphs illustrate the liver weight (**d**), kidney weight (**e**) and intra-abdominal fat weight (**f**) (total weight of mesenteric fat, retroperitoneal fat, and peritesticular fat) in 6-week-old offspring of each group. Data are expressed as means  $\pm$  standard error ( $n = 5$ ).  $^{*}p < 0.05$  vs. corresponding mCN group;  $^{**}p < 0.01$ ,  $^{#}p < 0.05$  vs. corresponding CN group;  $^{++}p < 0.01$  vs. corresponding LP group. Statistical analysis was performed using two-way analysis of variance followed by Tukey's test. mCN/mLP: maternal control diet/maternal low protein diet; CN/LP/HP: offspring CN/LP/HP diet.