Electronic Supplementary Material (ESI) for Toxicology Research.

Table S1. Details of the primers used in MeDIP and HmeDIP experiments.

Primer Name	Sequence (5'→3')	Amplified region
Primer-1	GTTTCCCAGTCACGGTC*T	-
Primer-2	**GACCGTGACTGGGAAAC	
Primer-3	GTTTCCCAGTCACGGTC	
Nega-Fw	GAGCATCTTCTCACATCCATCTGGCACACATTACT	chr18:8760558-8760642, strand=-
Nega-Rv	ATGTGGGAGATGTTTCTAGTGTGGGAGTTGGGAA	
Cyp2b10-Fw	AAATGATTCTGGTGCTGGGTGCCATGGTATATG	chr7:25890767-25890851 strand=+
Cyp2b10-Rv	CAAAGTTATAGCGATTCCCCTGCCTCTGCTTC	
Actb-Fw	GTCAAGATCCATGAAAACCTCTCCAACCAGAGTTC	chr5:142907278-142907365 strand=-
Actb-Rv	CCTTCTGCTGTGGTTCTAATTTGCTAGGTGTGTTG	
H19-Fw	GTGAGTCTCCTTCTTCTCTCTTGGCCTCGCTCCAC	chr7:142577685-142577764 strand=-
H19-Rv	TTCTTCTCACCAGGCCCCCTCTCTAAACCTTCGTT	
Tex19.1-Fw	CTCATGGGAGATATGTAAATGAGCTGGAGCATCG	chr11:121146077-121146166 strand=+
Tex19.1-Rv	GCTAAGCACTGAACACCAGGGAGGACGAAC	

* indicates a phosphothioate internucleoside linkage.
** indicates a 5'-phosphorylation site.

Table S2. Mortality, body and liver weight, food consumption, overall PB intake during treatment and plasma PB concentrationin in animals treated with PB for 27 weeks.

	C3H/HeNCrlCrlj mice			
			(n)	Fold
Mortality (number of death)				
PB 0 ppm	0		(20)	
PB 500ppm	1		(20)	
Body weight at commencement of treatment (g)				
Control 0 ppm	21.0 ± 1.1		(20)	
PB 500ppm	21.0 ± 1.1		(19)	
Body weight at termination of treatment (g)				
Control 0 ppm	31.1 ± 2.5		(20)	
PB 500ppm	31.8 ± 1.8		(19)	
Total body weight gain (g)				
Control 0 ppm	13.6 ± 2.3		(20)	
PB 500ppm	13.5 ± 1.6		(19)	
Absolute liver weight (g)				
Control 0 ppm	1.59 ± 0.11		(20)	1.0
PB 500ppm	2.33 ± 0.60	**	(19)	1.5
Relative liver weight (g/100 g)				
Control 0 ppm	5.06 ± 0.20		(20)	1.0
PB 500ppm	7.23 ± 1.71	**	(19)	1.4
Food consumption at termination of treatment (g)				
Control 0 ppm	4.0 ± 0.3		(18)	
PB 500ppm	3.9 ± 1.1		(19)	
Test article intake (mg/kg/day, average)				
PB 500ppm	69.9		(5)	
Plasma concentration levels of PB (µg/mL)				
	15.4 ± 7.1		(19)	

Results are presented as mean±SD.

Values significantly different from control (0 ppm) are: *p<0.05 and **p<0.01.

Table S3. Summary of necropsy and histopathological findings of liver in animals treated with PB for 27 weeks.

C3H/HeNCrlCrlj mice /male

Organs, Tissues		Findings	Test article Dose Number of Animals	Control (20)	PB 500ppm (19)
Liver				(20)	(19)
		Within normal limits		[3]	[0]
	Necropsy	Dark red focus		[1]	[11]
			P	1	11
		Enlarged		[0]	[1]
			P	0	1
		Grayish-white focus		[0]	[1]
			P	0	1
		Mass		[3]	[16]
			P	3	16
		White focus		[16]	[19]
			P	16	19
	Histopathology	Adenocarcinoma		[0]	[3]
			U	0	3
		Adenoma, hepatocellular		[7]	[19]
			U	7	19
		Focus of cellular alteration, basophilic		[8]	[13]
			±	7	12
			+	1	1
		Focus of cellular alteration, clear cell		[10]	[15]
			±	9	15
			+	1	0
		Focus of cellular alteration, eosinophilic		[1]	[19]
			±	1	14
			+	0	5
		Focus of cellular alteration, mixed cell		[1]	[0]
			±	1	0
		Hypertrophy, hepatocyte, centrilobular		[3]	[19]
			±	3	0
			+	0	19
		Infiltration, mononuclear		[1]	[0]
			±	1	0
		Mineralization		[3]	[0]
			±	3	0
		Necrosis, single cell		[0]	[1]
			±	0	1

P: Present, ±: Slight, +:Mild, U: Undetermined

Table S4. Summary of individual histopathological findings of liver in animals used in DNA modification and gene expression analyses.

C3H/HeNCrlCrlj mice /male

Organs, Tissues	Test article	Control			PB		
Findings	Dose	0 ppm			500 ppm		
	Animal No.	1	2	3	1	2	3
Liver		N	N	N	Y	Y	Y
	Adenoma, hepatocellular	-	-	-	P	P	P
	Focus of cellular alteration, basophilic	-	-	-	±	±	±
	Focus of cellular alteration, clear cell	-	-	-	±	±	±
	Focus of cellular alteration, eosinophilic	-	-	-	±	±	±
	Hypertrophy, hepatocyte, centrilobular	-	-	-	+	+	+

Y: Findings present, N: Within normal limits

-: Within normal limits, ±: Slight, +: Mild, P: Present

Table S5. Overlap between hypo-5mC & up-regulated genes of hepatocellular adenoma induced by DEN/PB in C3H mice, up-regulated genes of liver induced by PB in CD-1 mice and/or in chimeric mice with human hepatocytes (Yamada et al., 2014).

[Overlapped between hepatocellular adenoma in C3H mice and liver in CD-1 mice but not in chimeric mice with human hepatocytes]

Gene Symbol	C3H mice (Hypo-5mC)		C3H mice (Upregulated)		CD-1 mice		Agilent GO
(mouse)	p-value(region)	MAT-score[†]	p-value	Fold-Change	p-value	Fold-Change	
Amot	1.02E-03	-4.016	5.01E-03	1.756	3.56E-02	2.532	n
Cblb	9.52E-03	-3.089	4.21E-02	2.155	3.80E-02	2.015	b
Ccdc85b	6.52E-03	-3.257	7.77E-03	1.617	8.09E-03	3.326	d
Cdca8	6.31E-03	-3.275	1.13E-03	7.887	3.46E-02	2.667	n
Gstt3	8.18E-03	-3.161	3.25E-03	2.305	4.35E-02	6.897	n
Mctp2	7.54E-03	-3.192	1.74E-02	1.403	4.15E-02	2.435	n
Mical1	4.22E-03	-3.454	4.50E-02	2.783	4.87E-02	37.038	n
Prg4	6.86E-03	-3.238	1.09E-02	1.771	4.99E-03	3.204	a, b
Racgap1	4.59E-04	-4.336	3.67E-03	11.474	1.75E-02	2.591	n
Rbp1	8.20E-03	-3.158	5.20E-04	4.768	1.59E-02	3.272	n
Slco1a4	3.30E-03	-3.559	1.92E-03	2.498	1.18E-02	2.418	n

[Overlapped between hepatocellular adenoma in C3H mice and liver in chimeric mice with human hepatocytes but not in CD-1 mice]

Gene Symbol	C3H mice (Hypo-5mC)		C3H mice (Upregulated)		Gene Symbol	Chimeric mice		Agilent GO
(mouse)	p-value(region)	MAT-score[†]	p-value	Fold-Change	(human)	p-value	Fold-Change	
Abcc2	9.90E-03	-3.069	7.55E-05	2.234	ABCC2	3.02E-04	2.066	n
Fbxo15	8.30E-03	-3.151	6.46E-03	6.320	FBXO15	1.60E-02	4.164	n
Fmo1	5.27E-03	-3.353	4.36E-03	2.103	FMO1	3.05E-02	2.224	n
Gpx2	7.62E-03	-3.189	1.51E-02	33.192	GPX2	2.89E-04	6.151	n
Nqo1	9.25E-03	-3.101	3.39E-04	6.660	NQO1	7.54E-03	19.218	n
Sgk2	8.75E-03	-3.126	6.66E-03	2.319	SGK2	1.62E-02	2.386	n

[Overlapped between liver in CD-1 mice and chimeric mice with human hepatocytes but not hepatocellular adenoma in C3H mice]

Gene Symbol	CD-1 mice		Gene Symbol	Chimeric mice		Agilent GO
(mouse)	p-value	Fold-Change	(human)	p-value	Fold-Change	
Cyp2a4	4.08E-02	17.942	CYP2A7	8.58E-04	5.251	n
Cyp2b10	3.49E-02	118.828	CYP2B6	1.57E-04	7.148	n
Cyp2c54	1.26E-02	7.972	CYP2C19	4.64E-04	2.225	n
Cyp3a16	1.14E-02	3.354	CYP3A4	2.37E-04	3.169	n
Ephx1	3.06E-02	2.985	EPHX1	1.04E-04	2.475	n
Gstm2	1.68E-02	10.352	GSTM1	1.88E-02	3.951	n
Gtdc1	1.21E-02	2.440	GTDC1	1.99E-04	6.582	n
Mpv17l	4.07E-02	2.422	MPV17L	2.72E-02	2.000	n
Tfrc	4.88E-03	2.333	TFRC	1.07E-03	2.949	n

[†] The relative methylation level calculated by the MAT (Model based Analysis of Tiling-arrays) algorithm

a: With the Gene Ontology (GO) term "cell proliferation" and/or its lower tiers

b: With the GO term "regulation of cell proliferation" and/or its lower tiers

c: With the GO term "cell growth" and/or its lower tiers

d: With the GO term "regulation of cell growth" and/or its lower tiers

n: Without any of the GO term above

Table S6. Overlap between hypo/hyper-5hmC & up-regulated genes of hepatocellular adenoma induced by DEN/PB in C3H mice, up-regulated genes of liver induced by PB in CD-1 mice and/or in chimeric mice with human hepatocytes (Yamada et al., 2014).

[Overlapped between hepatocellular adenoma in C3H mice and liver in CD-1 mice but not in chimeric mice with human hepatocytes]

Gene	C3H mice (Hyper-5hmC)		C3H mice (Hypo-5hmC)		C3H mice (Upregulated)		CD-1 mice		Agilent GO
Symbol	p-value(region)	MAT-score[†]	p-value(region)	MAT-score[†]	p-value	Fold-Change	p-value	Fold-Change	
1700016H13Ril	—	—	4.77E-03	-3.775	5.13E-03	4.096	2.08E-02	2.020	n
2010012O05Ril	—	—	2.38E-04	-5.035	4.79E-02	1.433	1.21E-03	2.005	n
Abcc3	—	—	8.83E-06	-7.846	1.28E-02	1.537	3.95E-02	2.337	n
Abcc4	2.33E-03	4.113	—	—	8.93E-05	104.220	4.70E-02	7.349	b
Akr1b7	—	—	5.95E-03	-3.679	1.29E-03	90.527	2.66E-02	52.057	n
Amot	5.21E-03	3.688	8.83E-06	-7.297	5.01E-03	1.756	3.56E-02	2.532	n
BC021614	—	—	8.83E-06	-11.135	3.51E-02	1.460	1.50E-04	2.214	n
Btg3	1.96E-03	4.183	—	—	1.38E-03	3.845	1.22E-02	2.193	n
Cblb	1.27E-03	4.403	—	—	4.21E-02	2.155	3.80E-02	2.015	b
Cbr3	—	—	8.29E-03	-3.507	2.83E-05	13.245	4.86E-02	7.296	n
Cdca8	—	—	9.71E-05	-5.307	1.13E-03	7.887	3.46E-02	2.667	n
Ces2c	—	—	7.42E-03	-3.569	1.41E-03	5.397	4.81E-03	5.664	n
Cidec	—	—	8.83E-06	-7.176	2.45E-02	4.934	9.39E-03	3.520	n
Cks1b	2.82E-04	5.052	—	—	1.60E-02	2.038	6.63E-03	2.177	n
Gm3448	3.23E-03	3.947	8.83E-06	-8.073	1.56E-02	1.855	2.29E-03	2.712	n
Gsta2	—	—	2.30E-04	-5.076	1.43E-03	2.670	1.97E-02	17.260	n
Gstm1	—	—	1.06E-03	-4.510	8.75E-05	2.500	9.99E-03	7.746	n
Gstm3	3.06E-03	3.974	1.59E-04	-5.197	1.42E-05	4.705	2.02E-02	44.622	n
Gstm4	—	—	8.83E-06	-6.002	8.75E-04	4.710	1.69E-02	5.521	n
Gstm5	—	—	1.88E-03	-4.223	1.88E-02	2.223	4.24E-03	5.503	n
Gstt3	—	—	8.83E-06	-12.018	3.25E-03	2.305	4.35E-02	6.897	n
Gyg	2.96E-03	3.992	—	—	1.19E-02	2.081	1.73E-03	2.314	n
Krt31	5.38E-04	4.794	—	—	2.56E-04	12.291	1.12E-02	3.758	n
Morc4	4.64E-03	3.750	—	—	9.61E-03	2.845	5.92E-03	3.465	n
Orm3	—	—	8.83E-06	-6.140	8.70E-05	6.412	1.13E-03	8.062	n
Pnliprp1	8.60E-03	3.470	—	—	4.11E-04	2148.926	1.07E-02	6.728	n
Prg4	—	—	6.05E-03	-3.669	1.09E-02	1.771	4.99E-03	3.204	a, b
Prss8	—	—	8.83E-06	-6.285	1.74E-06	18.729	3.77E-02	3.359	n
Serpina6	1.97E-03	4.182	—	—	2.09E-03	3.532	4.74E-02	5.072	n
Slc20a1	—	—	2.03E-03	-4.179	8.09E-04	3.754	2.85E-02	2.204	n
Smpd3	9.24E-03	3.429	—	—	5.95E-03	8.679	3.38E-02	3.410	n
Sort1	8.67E-03	3.465	—	—	4.96E-02	2.202	6.69E-04	2.667	n
Syngr4	4.19E-03	3.797	1.94E-03	-4.206	3.43E-02	1.912	1.99E-03	2.322	n
Tff1	2.22E-03	4.133	8.83E-06	-7.305	1.97E-04	16.284	4.26E-03	7.240	b
Tnfrsf11a	8.22E-03	3.492	—	—	4.40E-02	1.158	1.96E-02	6.298	n
Tnfrsf19	9.98E-03	3.396	6.45E-03	-3.637	4.54E-04	13.815	1.10E-02	6.560	n

[Overlapped between hepatocellular adenoma in C3H mice and liver in chimeric mice with human hepatocytes but not in CD-1 mice]

Gene	C3H mice (Hyper-5hmC)		C3H mice (Hypo-5mC)		C3H mice (Upregulated)		Gene	Chimeric mice		Agilent GO
Symbol	p-value(region)	MAT-score[†]	p-value(region)	MAT-score[†]	p-value	Fold-Change	Symbol	p-value	Fold-Change	
9630033F20Ril	2.99E-03	3.986	—	—	2.24E-02	2.415	C12orf5	3.12E-02	2.712	n
Akr1b10	4.63E-03	3.751	—	—	1.40E-02	1.734	AKR1B10	2.20E-02	26.439	n
Aox1	—	—	8.80E-03	-3.474	1.92E-04	2.143	AOX1	5.00E-04	2.520	n
Cyp1a1	—	—	8.83E-06	-6.137	6.51E-03	3.414	CYP1A1	3.35E-02	2.493	n
D1Ertd622e	5.67E-03	3.654	8.83E-06	-6.950	8.50E-03	1.518	C5orf30	3.47E-03	2.069	n
Dennd2d	8.92E-03	3.449	1.41E-04	-5.222	3.00E-03	1.597	DENND2D	2.87E-03	2.113	n
Hsp90aa1	—	—	9.59E-03	-3.433	3.51E-02	1.690	HSP90AA1	4.81E-03	2.186	n
Htatip2	—	—	1.80E-03	-4.237	1.07E-02	2.426	HTATIP2	1.41E-03	2.289	n
Mras	—	—	1.94E-04	-5.120	5.66E-04	3.114	MRAS	3.48E-02	2.520	n
Pir	6.64E-03	3.581	—	—	7.38E-03	2.223	PIR	1.43E-02	2.110	n
Sgk2	—	—	8.83E-06	-8.163	6.66E-03	2.319	SGK2	1.62E-02	2.386	n
Srxn1	—	—	4.88E-03	-3.764	4.10E-02	3.368	SRXN1	1.39E-02	2.360	n
Tkt	—	—	4.47E-03	-3.803	3.65E-04	1.616	TKT	6.05E-03	2.841	n
Tmem164	2.39E-03	4.098	—	—	3.01E-02	1.567	TMEM164	1.72E-02	2.076	n
Ugdh	2.73E-03	4.034	5.91E-04	-4.719	1.37E-03	2.105	UGDH	2.29E-03	3.302	n
Ugt1a6b	—	—	8.83E-06	-7.457	5.42E-03	1.679	UGT1A6	5.00E-04	4.186	n

[Overlapped between hepatocellular adenoma in C3H mice, liver in CD-1 mice and in chimeric mice with human hepatocytes]

Gene	C3H mice (Hyper-5hmC)		C3H mice (Hypo-5mC)		C3H mice (Upregulated)		CD-1 mice		Gene	Chimeric mice		Agilent GO
Symbol	p-value(region)	MAT-score[†]	p-value(region)	MAT-score[†]	p-value	Fold-Change	p-value	Fold-Change	Symbol	p-value	Fold-Change	
Cyp2a4	—	—	4.93E-03	-3.758	3.06E-05	3.940	4.08E-02	17.942	CYP2A7	8.58E-04	5.251	n
Cyp2b10	—	—	8.83E-06	-9.137	3.58E-05	53.304	3.49E-02	118.828	CYP2B6	1.57E-04	7.148	n
Ephx1	—	—	1.65E-03	-4.273	4.95E-04	2.227	3.06E-02	2.985	EPHX1	1.04E-04	2.475	n
Gstm2	—	—	2.07E-03	-4.167	5.48E-04	8.472	1.68E-02	10.352	GSTM1	1.88E-02	3.951	n

[Overlapped between liver in CD-1 mice and chimeric mice with human hepatocytes but not hepatocellular adenoma in C3H mice]

Gene	CD-1 mice		Gene Symbol	Chimeric mice		Agilent GO
Symbol	p-value	Fold-Change	(human)	p-value	Fold-Change	
Cyp2c54	1.26E-02	7.972	CYP2C19	4.64E-04	2.225	n
Cyp3a16	1.14E-02	3.354	CYP3A4	2.37E-04	3.169	n
Gtdc1	1.21E-02	2.440	GTDC1	1.99E-04	6.582	n
Mpv17l	4.07E-02	2.422	MPV17L	2.72E-02	2.000	n
Tfrc	4.88E-03	2.333	TFRC	1.07E-03	2.949	n

[†] The relative methylation level calculated by the MAT (Model based Analysis of Tiling-arrays) algorithm

—: No change

a: With the Gene Ontology (GO) term "cell proliferation" and/or its lower tiers

b: With the GO term "regulation of cell proliferation" and/or its lower tiers

c: With the GO term "cell growth" and/or its lower tiers

d: With the GO term "regulation of cell growth" and/or its lower tiers

n: Without any of the GO term above

Table S7. Overlap between hyper-5mC & down-regulated genes of hepatocellular adenoma induced by DEN/PB in C3H mice, down-regulated genes of liver induced by PB in CD-1 mice and/or in chimeric mice with human hepatocytes (Yamada et al., 2014).

[Overlapped between hepatocellular adenoma in C3H mice and liver in CD-1 mice but not in chimeric mice with human hepatocytes]

Gene Symbol	C3H mice (Hyper-5mC)		C3H mice (Downregulated)		CD-1 mice		Agilent GO
(mouse)	p-value(region)	MAT-score[†]	p-value	Fold-Change	p-value	Fold-Change	
9330151L19R	5.82E-03	3.234	4.54E-03	-2.487	2.29E-02	-2.524	n
9930021J03R	6.00E-04	4.124	2.02E-02	-1.452	2.38E-02	-2.255	n
Acat1	4.68E-04	4.220	2.51E-03	-1.576	7.97E-03	-2.149	n
Adrb3	5.65E-04	4.157	4.23E-02	-2.049	2.01E-02	-2.788	n
Aox3	5.23E-03	3.283	1.28E-02	-1.625	1.14E-03	-3.852	n
Apoa1	3.91E-03	3.404	2.36E-03	-1.483	2.67E-02	-2.126	a
C8a	3.34E-03	3.453	4.36E-02	-3.422	2.26E-03	-2.093	n
Cacna2d2	7.71E-03	3.120	6.31E-03	-1.448	1.75E-02	-2.946	n
Cdk5r1	2.56E-04	4.551	2.43E-02	-1.214	1.34E-02	-2.210	d
Ctrl	5.22E-03	3.283	3.42E-02	-1.469	2.30E-03	-2.130	n
Dlg1	5.39E-03	3.265	9.79E-03	-1.560	4.02E-02	-2.026	a, b
Egfr	2.65E-04	4.492	3.18E-02	-3.202	4.54E-03	-2.848	a, b
Enho	3.71E-04	4.335	3.31E-02	-12.837	2.28E-02	-2.996	n
Fat2	7.43E-03	3.130	4.35E-02	-1.478	2.19E-03	-2.437	n
Gpatch8	7.87E-03	3.112	5.35E-03	-1.458	3.63E-02	-2.197	n
Hectd2	5.31E-03	3.277	4.14E-02	-1.587	1.90E-05	-4.577	n
Hsd17b6	5.71E-03	3.241	4.91E-02	-9.256	1.48E-02	-2.865	n
Nsa2	8.85E-03	3.058	3.07E-02	-1.213	3.57E-03	-2.164	n
Olfr638	6.61E-03	3.176	2.72E-02	-1.451	1.26E-02	-3.987	n
Siglech	1.16E-03	3.862	3.65E-02	-3.400	1.30E-02	-4.184	n
Slc22a7	9.54E-03	3.025	2.06E-02	-7.142	7.04E-03	-4.292	n
Snora47	6.29E-03	3.202	9.11E-03	-3.444	2.70E-03	-2.503	n
Tmem116	8.90E-03	3.056	1.11E-06	-1.408	8.04E-03	-2.086	n

[Overlapped between hepatocellular adenoma in C3H mice and liver in chimeric mice with human hepatocytes but not in CD-1 mice]

Gene Symbol	C3H mice (Hyper-5mC)		C3H mice (Downregulated)		Gene Symbol	Chimeric mice		Agilent GO
(mouse)	p-value(region)	MAT-score[†]	p-value	Fold-Change	(human)	p-value	Fold-Change	
Pnmt	6.51E-03	3.183	6.65E-04	-2.978	PNMT	3.97E-02	-6.414	n

[Overlapped between liver in CD-1 mice and chimeric mice with human hepatocytes but not hepatocellular adenoma in C3H mice]

Gene Symbol	CD-1 mice		Gene Symbol	Chimeric mice		Agilent GO
(mouse)	p-value	Fold-Change	(human)	p-value	Fold-Change	
Col5a3	3.09E-02	-2.383	COL5A3	3.15E-02	-3.124	n

[†] The relative methylation level calculated by the MAT (Model based Analysis of Tiling-arrays) algorithm

a: With the Gene Ontology (GO) term "cell proliferation" and/or its lower tiers

b: With the GO term "regulation of cell proliferation" and/or its lower tiers

c: With the GO term "cell growth" and/or its lower tiers

d: With the GO term "regulation of cell growth" and/or its lower tiers

n: Without any of the GO term above