SUPPLEMENTAL INFORMATION: Four tables (Table S1 – Table S4) plus a discussion of the approach taken to ascertain the reversibility of altered DNA methylation, "Reversibility of altered methylation: Calculations."

Table S1. Dissimilarity Between PB-Induced Methylation Changes and Control Methylation Patterns in B6C3F1 and C57BL/6 Mouse Liver

Dissimilarity Between:		**Total Regions[a]**	**Total RAM as Compared to Control[b]**	**Percent Dissimilarity To Control**	
B6C3F1					
Control	2 weeks 0.05% PB	153	69	69/153	**45%**
Control	4 weeks 0.05% PB	202	123	123/202	**61%**
C57BL/6					
Control	2 weeks 0.05% PB	147	98	98/147	**67%**
Control	4 weeks 0.05% PB	111	88	88/111	**79%**

[a] Total regions includes every PCR product size reporting between control and treated groups. Each PCR product represents a region of the genome.
[b] Total RAMs represents the number of regions exhibiting a statistically significant ($p<0.05$) change in methylation including complete hypomethylations (100%), partial hypomethylations, hypermethylations, or new methylations.

Table S2. Dissimilarity Between PB-Induced Methylation Changes in B6C3F1 and C57BL/6 Mouse Liver

Dissimilarity Between:		Total RAMs in B6[a]	Total Unique RAMs As Compared to C57BL/6[b]	Percent Dissimilarity To C57BL/6	
2 week 0.05% PB					
B6C3F1	C57BL/6	69	56	56/69	**81%**
4 week 0.05% PB					
B6C3F1	C57BL/6	98	83	83/98	**85%**

[a] Total RAMs in B6C3F1 is reported from Table 1
[b] Total Unique RAMs represents the number of regions exhibiting a statistically significant ($p<0.05$) change in methylation including complete hypomethylations (100%), partial hypomethylations, hypermethylations, or new methylations that were only observed in B6C3F1 mice.

Table S3. Dissimilarity Between Methylation Changes in the LIVER as compared to the KIDNEY following 4 wk of Treatment with PB

Dissimilarity Between:		Total RAMs in LIVER[a]	Total Unique RAMs As Compared to KIDNEY[b]	Percent Dissimilarity To KIDNEY	
B6C3F1					
LIVER	KIDNEY	98	92	92/98	**94%**
C57BL/6					
LIVER	KIDNEY	88	75	75/88	**85%**

[a] Total RAMs in liver is reported from Table 1
[b] Total Unique RAMs represents the number of regions exhibiting a statistically significant ($p<0.05$) change in methylation including complete hypomethylations (100%), partial hypomethylations, hypermethylations, or new methylations that were only observed in the liver.

Reversibility of altered methylation: Calculations

The extent to which altered methylation observed following specific periods of promotion was reversible was calculated by using each 2 or 4wk 0.05% PB promotion group as the working "control" pattern of methylation. The recovery data is calculated as a % of this "control". The 2wk, 0.05% PB promotion group served as the "control" for the 2wk 0.05% PB promotion, 2wk recovery group. The 4wk, 0.05% PB promotion group served as the "control" for the 4wk 0.05% PB promotion, 2wk recovery group.

A consensus, average, peak area for each PCR product reporting in 2 or 4wk, 0.05% PB promotion groups is prepared and designated as the "control" consensus. In addition a consensus, average, peak area for each PCR product reporting in 2 or 4wk 0.05% PB promotion, 2wk recovery is prepared and designated as the "reversal" consensus. "Control" consensus and "reversal" consensus peak areas at each specific PCR product are compared. The following equations are used to calculate the "reversal" consensus peak areas for each PCR product size as a percent of the corresponding "control" consensus peak areas.

% MspI "control" consensus = ((MspI "reversal" consensus – MspI "control" consensus)/MspI "control" consensus)) x 100

% HpaII "control" consensus = ((HpaII "reversal" consensus – HpaII "control" consensus)/HpaII "control" consensus)) x 100

As described previously, hypomethylations, hypermethylations and new methylations are tallied.

Steps to determining whether a PB-induced change in methylation reversed following the recovery period

1. A list of all the PCR product sizes exhibiting 2 or 4wk 0.05% PB-induced hypo-, hyper-, and new methylations are compiled.

2. A list of the PCR product sizes exhibiting changes in methylation that occurred during recovery as calculated above, are compiled.

3. The two lists are aligned by PCR product size so that changes in methylation due to promotion and changes in methylation which occur during recovery are directly comparable. An example of this appears in a Supplementary Table, Table S4, below.

Table S4. Example of Comparisons for Determining whether a PB-induced Change in Methylation Reversed Following the Recovery Period

4wk PB Promotion Group		4wk, PB Promotion, 2wk Recovery Group			
PCR product size (base pairs)	Change in Methylation	PCR product size	Change in Methylation	Changes in methylation are Opposite in Direction?	Promotion induced change in methylation is reversed?[a]
245	New Methylation	245	Hypomethylation	Yes	Yes
315	Hypermethylation	315	Hypermethylation	No	No
456	Hypomethylation	456	New Methylation	Yes	Yes
505	New Methylation	505	Hypomethylation	Yes	Yes
515	Hypermethylation	515	Hypomethylation	Yes	Yes

[a] Changes in methylation induced by PB promotion are only considered recoverable if a change in methylation with opposite direction (i.e. hypomethylations are opposite to hypermethylations and new methylations) is observed following recovery.