Supplemental Figure 1. Establishment of silencing at P1-pr.

The P1-pr allele originated as a spontaneous somatic silencing event from the P1-rr-4026 allele, which typically confers red ear pigmentation (see upper ear). Since kernel pericarp and the female gamete share a common cell lineage, epimutations of P1-rr, which are visible in the pericarp (notice the variegated kernel in the upper ear), may affect the female gamete as well. Therefore, silenced P1-rr alleles are potentially transmissible. The plant grown from the variegated kernel gave rise to a weakly pigmented ear (see lower ear). The repressed P1-rr allele, designated P1-pr, reveals a reduced p1 transcript level and is hypermethylated at regulatory sequences compared to P1-rr. P1-pr has a permanently condensed chromatin structure in p1-expressing and non-expressing tissues. Please note that the P1-pr-1 allele used in this study is not the P1-pr isolate shown in this figure.
Supplemental Figure 2. F1 ear pigmentation levels.

F1 ears were sorted according to genotype and pigmentation level. A dark homozygous $P1$-$rr$ ear was assigned a value of 100%, while a colorless homozygous $p1$-$ww$ ear was set as 0%. Numbers written underneath the columns correspond to the individual F1 plants that gave rise to the investigated ears (see Fig. 3A). Please note that ear #28 contained a large $P1$-$rr$ ear sector (not visible in Fig. 3A) that increased the average pigmentation of the ear. Numbers framed in red indicate F1 plants that were also utilized for $p1$ and $a1$ transcript analysis. TC specifies F1 plants 10, 33, and 32 that were used for progeny analysis. Plants 10 and 41 labeled with BSS were chosen for bisulfite conversion.
Supplemental Figure 3. P1-pr and P1-rr genotyping.

P1-pr contains 6 additional bp (red letters) in intron 2 compared to P1-rr (see Materials and Methods). PCR primers (sequence in blue color) that are flanking the indel were used to amplify DNA fragments of both genotypes. P1-pr produces a 160-bp fragment, while P1-rr gives rise to a 154-bp fragment. p1-ww, which lacks a coding region, does not produce a band derived from p1. The lower band present in all genotypes stems from the p2 gene, which renders p2 an internal PCR control.
Supplemental Figure 4. *P1-pr* is not reactivated in F1 ears.

The F1 ears shown were used for *p1* transcript analysis. RNA was isolated from developing pericarp 20 DAP. RT-PCR was carried out, employing the primer set described in supplemental Fig. 3. The indel-containing fragments, which were amplified from non-spliced *p1* transcript, were separated on an 8% polyacrylamide gel. Genomic DNA was amplified as marker. Since *p2*-derived bands were not detected, the RNA samples had to be free of DNA contamination. Fragments amplified from *P1-rr* transcripts and *P1-pr* transcripts are 154 bp and 160 bp in size, respectively.
Supplemental Figure 5. Cytosine methylation analysis of F1 plants.
Cytosine methylation of \textit{P1-pr/P1-rr} (A) and \textit{p1-ww/P1-rr} (B) heterozygotes were analyzed by Southern hybridization using the methylation-sensitive restriction enzyme \textit{SalI} combined with probe p15. Genomic DNA was isolated from leaves that were collected from the F1 plants used for pigment and transcript analysis (see Fig. 3A, supplemental Fig. 2, and Fig. 3B). DNA samples were sorted according to their corresponding ear phenotypes (from light ears on the left to darker ears on the right). Numbers on top of the lanes indicate the individual F1 plant that DNA was extracted from. Numbers framed in red point to F1 plants that were also used for transcript analysis. TC and BSS specify F1 plants that were used for testcross analysis and bisulfite sequencing, respectively. The parental genotypes for the F1 are shown in green letters. Control samples (blue letters) were loaded to the left. Sizes of hybridizing fragments are shown at left.
**Supplemental Figure 6. Cytosine methylation at the BSS7-8 fragment in F1 plants.**

The unique BSS7-8 sequence consists of the 3’ end of the hAT transposable element and the 5’ end of the adjacent p1-repeat. This fragment was amplified after bisulfite conversion in P1-rr/P1-rr (panels A and E), P1-pr/P1-pr (panels B and F), P1-pr/P1-rr (14.3%) (panels C and G) and P1-pr/P1-rr (60.9%) (panels D and H). The methylation status of all cytosines within CG and CHG contexts are listed for each individual clone. The clone number can be found on the right of the sequence. The bisulfite treatment converts unmethylated cytosines into uracils, which are amplified as thymines at the PCR step (shown as T on a red background). Cytosines that are methylated remain unchanged (shown as C). The position of cytosine is measured from the transcription start site and shown on top of the sequences. The numbers below the clones refer to the position in the BSS7-8 fragment. Numbers on a green background indicate cytosines that are in a CCG context. Please notice that the first cytosine within CCG is barely methylated whereas methylation of the second cytosine does not appear to be influenced by the first one.
A  testcross 1: P1-pr/P1-rr: 14.3% x p1-ww/p1-ww

B  testcross 2: P1-pr/P1-rr: 25.8% x p1-ww/p1-ww

C  testcross 3: P1-pr/P1-rr: 56.5% x p1-ww/p1-ww
Supplemental Figure 7. Pigmentation levels of ears derived from three representative testcrosses.

Pigmentation levels of ears derived from three testcrosses were determined and arranged based on genotype and pigmentation intensity (A-C). A dark homozygous $P1$-$rr$ ear and a colorless homozygous $p1$-$ww$ ear were given the values of 100% and 0%, respectively. Numbers distinguished by a red frame indicate plants that were utilized for $p1$ and $a1$ transcript analysis. Plants that were used for bisulfite sequencing are labeled with BSS.
A. Testcross 1: P1·pr/P1·rr:14.3% x p1·ww/p1·ww

[Image of gel with band patterns and labels indicating bands 11.4 kb, 12.8 kb, 4.6 kb, 4.0 kb, 3.3 kb, 2.7 kb, 1.3 kb, and 1.1 kb.]

SalI (G/C/GAC) digest

B. Testcross 2: P1·pr/P1·rr:25.8% x p1·ww/p1·ww

[Image of gel with band patterns and labels indicating bands 11.4 kb, 12.8 kb, 4.6 kb, 4.0 kb, 3.3 kb, 2.7 kb, 1.3 kb, and 1.1 kb.]

SalI (G/C/GAC) digest

C. Testcross 3: P1·pr/P1·rr:56.5% x p1·ww/p1·ww

[Image of gel with band patterns and labels indicating bands 11.4 kb, 12.8 kb, 4.6 kb, 4.0 kb, 3.3 kb, 2.7 kb, 1.3 kb, and 1.1 kb.]

SalI (G/C/GAC) digest
Supplemental Figure 8. Cytosine methylation analysis of *P1-pr/p1-ww* and *P1-rr'/p1-ww* plants derived from three testcrosses.

DNA methylation patterns of plants that resulted from three testcrosses were evaluated by Southern hybridization using the methylation-sensitive restriction enzyme *SalI* and probe p15 (A-C). These testcross plants were also used for transcript (plant number surrounded by red frame) and pigment analysis. Genomic DNA samples, which were extracted from leaves, were sorted according to their corresponding genotype and increasing ear pigmentation. The parental genotypes and control samples for the testcrosses were loaded to the left and are labeled in green and blue letters, respectively. Fragment sizes of hybridizing bands are shown at left. Four plants (indicated by BSS) were chosen for a detailed methylation analysis by bisulfite sequencing (see Figs. 7 and 8).
Supplemental Figure 9. Phlobaphene pigmentation is inversely correlated with CG and CHG methylation in four testcross plants.

Pigmentation data of four testcross plants were plotted against their cytosine methylation levels. The final data point (100% pigmentation, 2.6% methylation) corresponds to the $P1$-$rr$ reference allele. The plot does not show a linear relationship between both variables.
**F1 cytosine methylation analysis**

Previously, DNA methylation at \textit{P1-pr} was found to be identical in \textit{p1} expressing and non-expressing tissues (Das and Messing 1994). Genomic DNA was isolated from leaves (tissue that does not express \textit{p1}) and digested with various methylation-sensitive restriction enzymes. After separation by gel electrophoresis, DNA fragments were blotted on membranes that were hybridized with probe p15 (Lechelt et al. 1989). Because \textit{SalI} digested \textit{P1-pr} DNA gives rise to higher molecular weight fragments compared to \textit{P1-rr}, \textit{P1-pr} is more densely methylated than \textit{P1-rr}. Whereas the p15 probe detected \textit{P1-pr}-derived bands of 12.8 kb, 11.4 kb, 4.6 kb and 4.0 kb, p15 hybridized to \textit{P1-rr} fragments of 3.3 kb, 2.7 kb and 1.3 kb (Fig. 1). Heterozygous \textit{P1-pr/P1-rr} plants produced a \textit{P1-pr} banding pattern superimposed on a \textit{P1-rr} pattern (supplemental Fig. 5A). F1 DNA samples were sorted according to ear phenotype, i.e. the \textit{P1-pr/P1-rr} DNA sample loaded to the left was isolated from plant #2, which produced a nearly colorless ear, whereas the DNA sample on the right corresponded to plant #28, which generated a dark red ear. Despite large differences among F1 plants regarding pigmentation and transcript levels in pericarp, the hybridization patterns of F1 plants were nearly identical. However, band intensities might slightly vary, as could be noticed best at the 1.3-kb band: the stronger the 1.3-kb band, the darker the F1 ear phenotype. \textit{p1-ww/P1-rr} plants, which homogenously yielded fully pigmented kernels, gave rise to a typical \textit{P1-rr} banding pattern with similar intensities (supplemental Fig. 5B). The additional \textit{p1-ww}-derived band of 1.1 kb was used for genotyping F1 plants.

**Cytosine methylation analysis of testcross plants**

Cytosine methylation of \textit{P1-pr/p1-ww} plants, which were derived from three testcrosses, always resembled the parental \textit{P1-pr} allele in Southern blots (supplemental Figs. 8A-C). The presence of 12.8 kb and 11.4 kb bands indicated that \textit{P1-pr} copies had fully methylated \textit{SalI} sites as shown in Fig. 1. In addition, partial DNA methylation was detectable as 4.0 kb and 4.6 kb bands of variable intensities. The \textit{P1-rr}-hybridizing bands of 1.3 kb, 2.7 kb, and 3.3 kb were largely missing. The testcrosses revealed that cytosine methylation of the paramutant \textit{P1-rr'} allele was mostly increased compared to the \textit{P1-rr}
parent (supplemental Figs. 8A-C). However, $P1-rr'/p1-ww$ plants were clearly less methylated than their $P1-pr/p1-ww$ siblings because they retained their $P1-rr$ banding pattern of 1.3 kb, 2.7 kb and 3.3 kb in all testcrosses. $P1-rr'$ testcross plants derived from the lightest pigmented F1 ear ($P1-pr/P1-rr$ (14.3%) showed a wide range of methylation patterns based on the full band set in the Southern blot (supplemental Fig. 8A). Accordingly, $P1-rr'$ methylation varied from cell to cell (assuming a complete $SalI$ digest). $P1-rr'$ plants that originated from $P1-pr/P1-rr$ (25.8%) in the second testcross were less methylated and therefore did not produce the strong high-molecular weight bands of 12.8 kb and 11.4 kb (supplemental Fig. 8B). Cytosine methylation of the $P1-pr/P1-rr$ (56.5%) progeny from the third testcross was even more reduced leading to only faint 4.6 kb and 4.0 kb bands (supplemental Fig. 8C).

**Additional Reference**