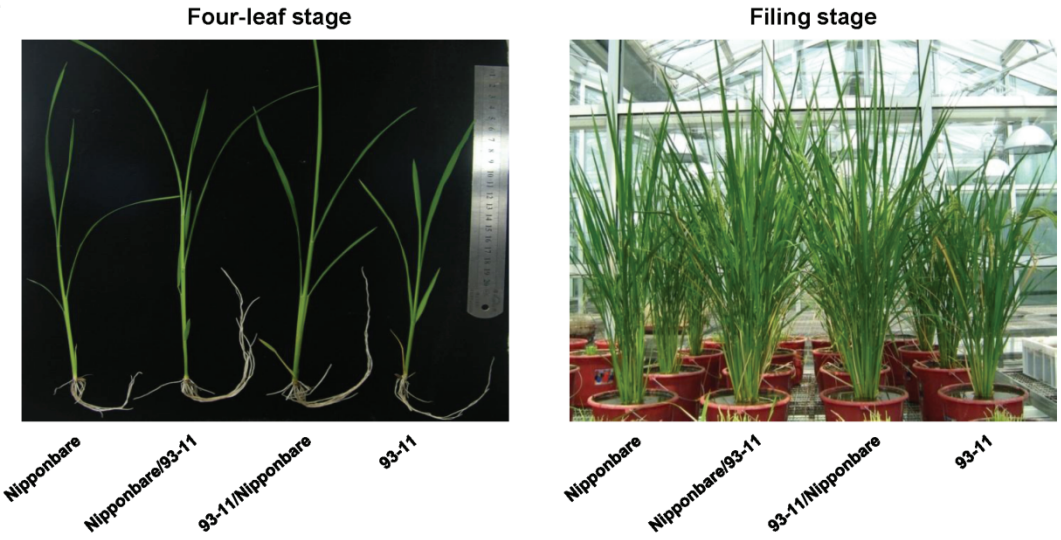
A



B

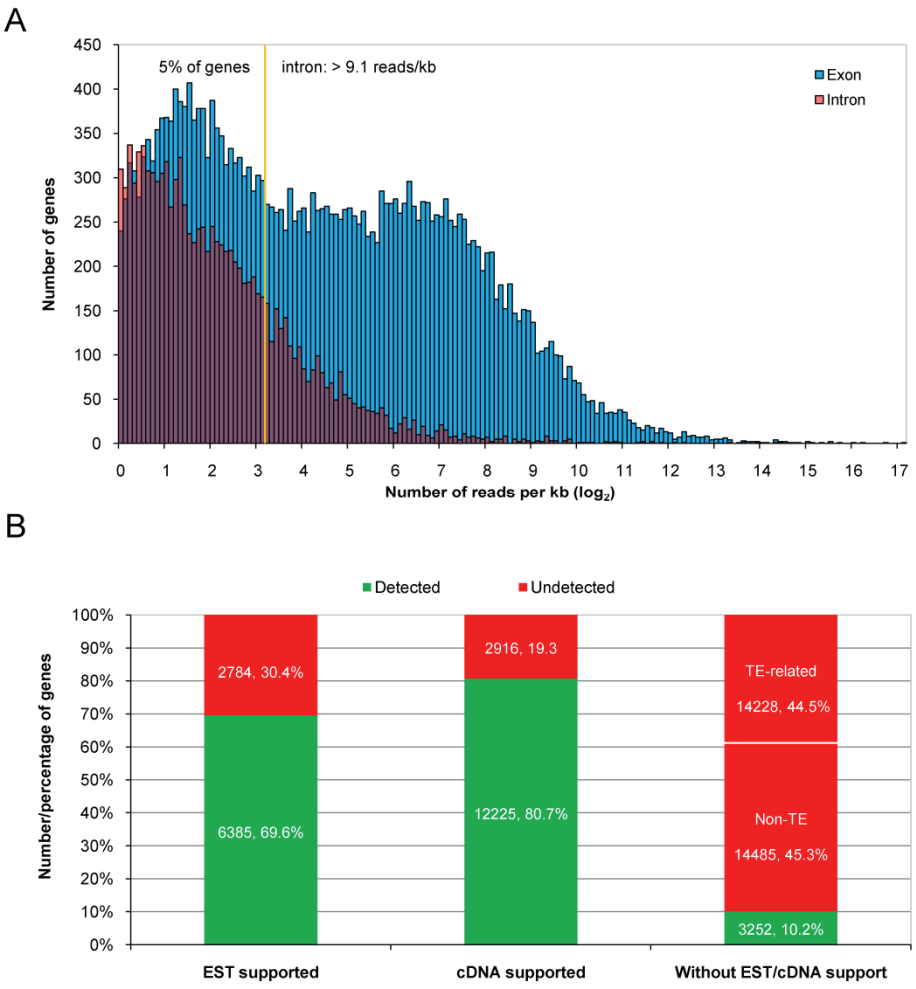
Tillering stage

Genotype	Seedling height (cm)	Number of tillers	Dry weight (g)
Nipponbare	42.5±0.50	2.2±0.00	0.540±0.03
93-11	39.3±2.52	2.60±0.58.	0.761±0.02
Nipponbare/93-11	48.8±2.11	4.1±0.77	1.008±0.11
93-11/Nipponbare	46.0±0.87	4.4±0.65	1.203±0.06

Supplemental Figure 1. Heterosis in reciprocal crosses between rice cultivars Nipponbare and 93-11.

(A) Growth vigor of reciprocal hybrids at four-leaf stage and filling stage.

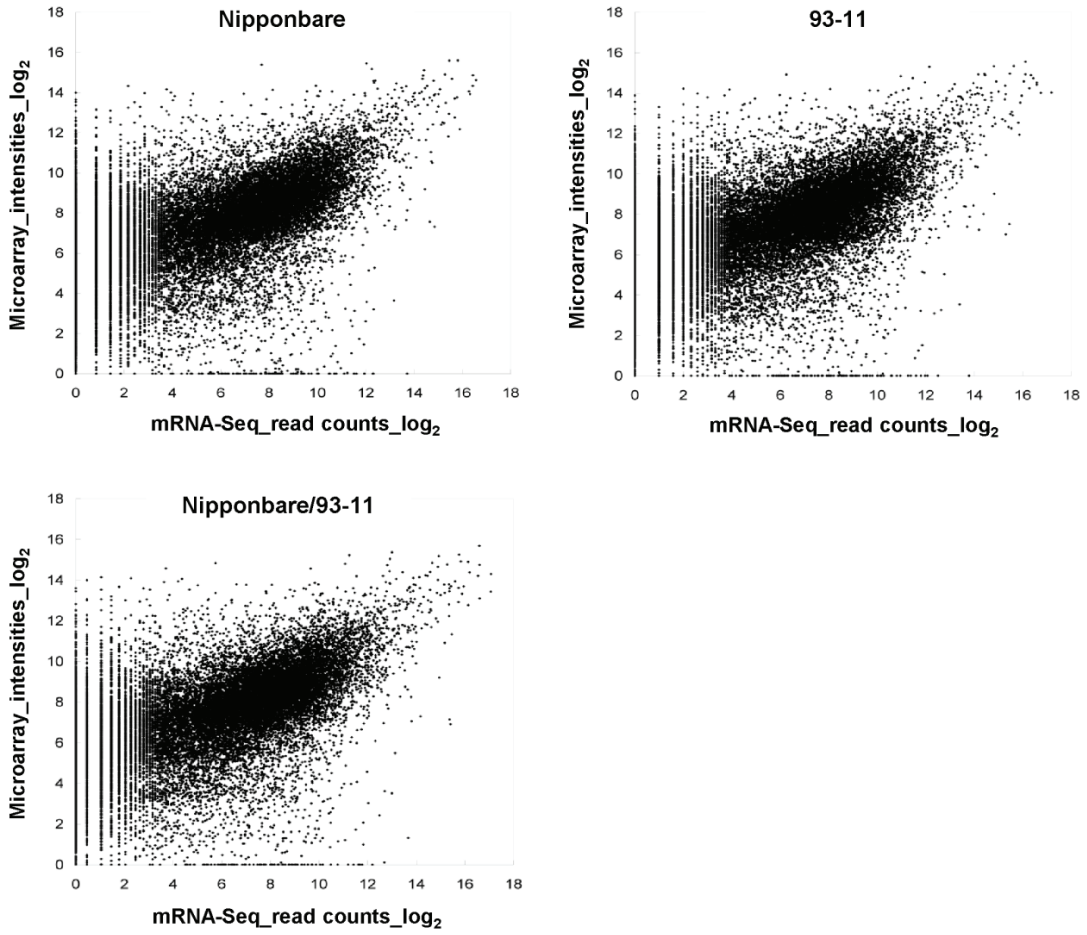
(B) Measurement of heterosis at the beginning of tillering.



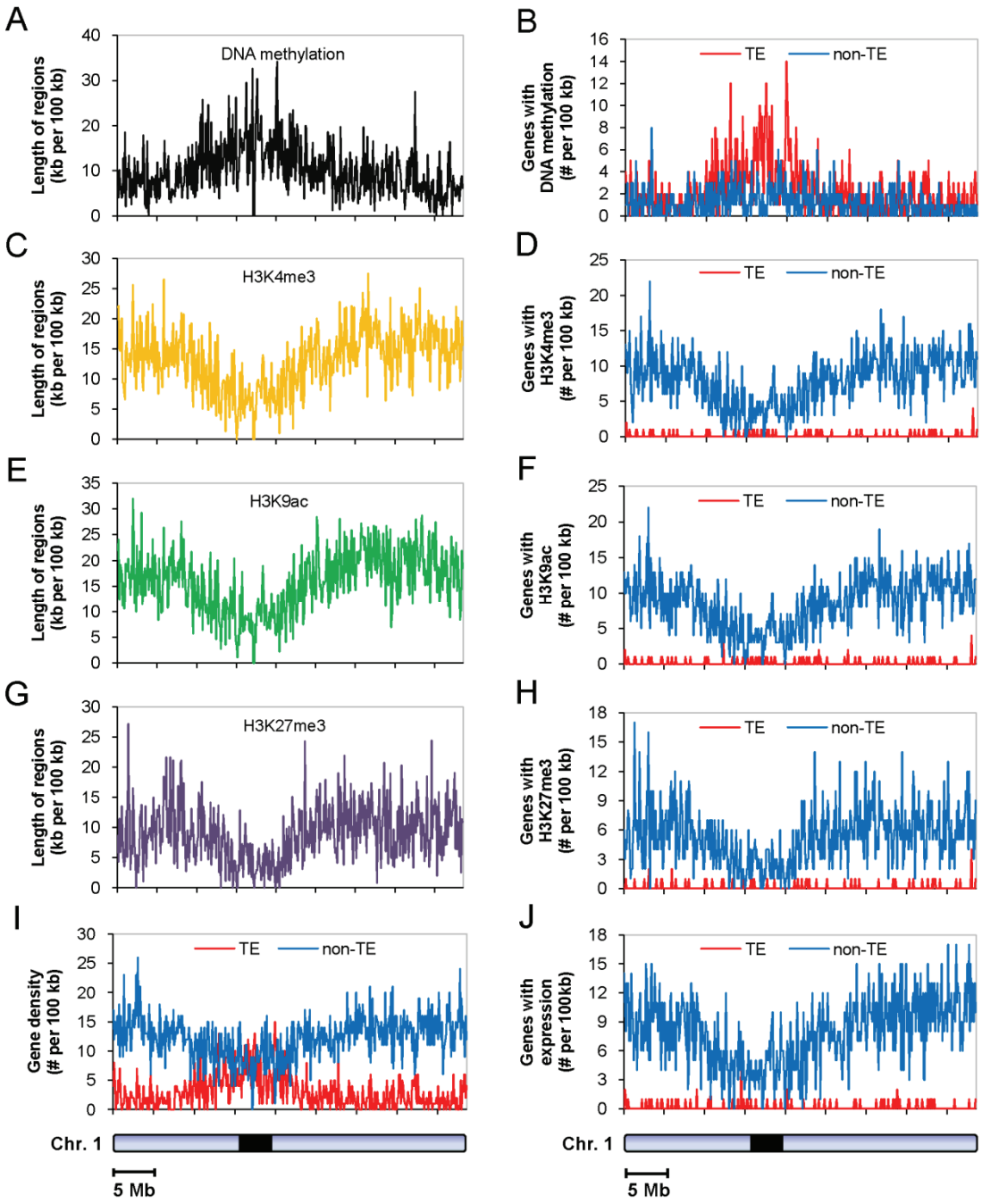
Supplemental Figure 2. Detection of gene expression in rice (cv. Nipponbare) by mRNA-Seq.

(A) Distribution of mRNA-Seq reads in exon and intron. Number of genes with serial reads density in exon or intron were plotted. A total of 49777 genes with only one annotated alternative splicing form were selected. About 5% of these genes contained > 9.1 reads per kb intron. Accordingly, 9.1 reads per kb exon was arbitrarily used as a cutoff for detection of gene expression in mRNA-Seq.

(B) mRNA-Seq detection of the expression of genes with or without EST or full-length cDNA support. Half of the genes without EST or full-length cDNA support undetected in mRNA-Seq are TE-related genes.



Supplemental Figure 3. Scatterplot comparing gene expression levels based on microarray hybridization signals (*y* axis; Zhang et al., 2008a) with gene expression levels based on mRNA sequencing (*x* axis; this study).



Supplemental Figure 4. Distribution of DNA methylation, H3K4me3, H3K9ac, H3K27me3 and annotated genes on rice chromosome 1.

(A), (C), (E), (G) Total length of genomic regions with DNA methylation or histone modifications per 100 kb sliding window over the entire rice chromosome 1.

(B), (D), (F), (H) Number of transposable element-related (TE-related) genes or non-transposable element protein coding (non-TE) genes with DNA methylation or

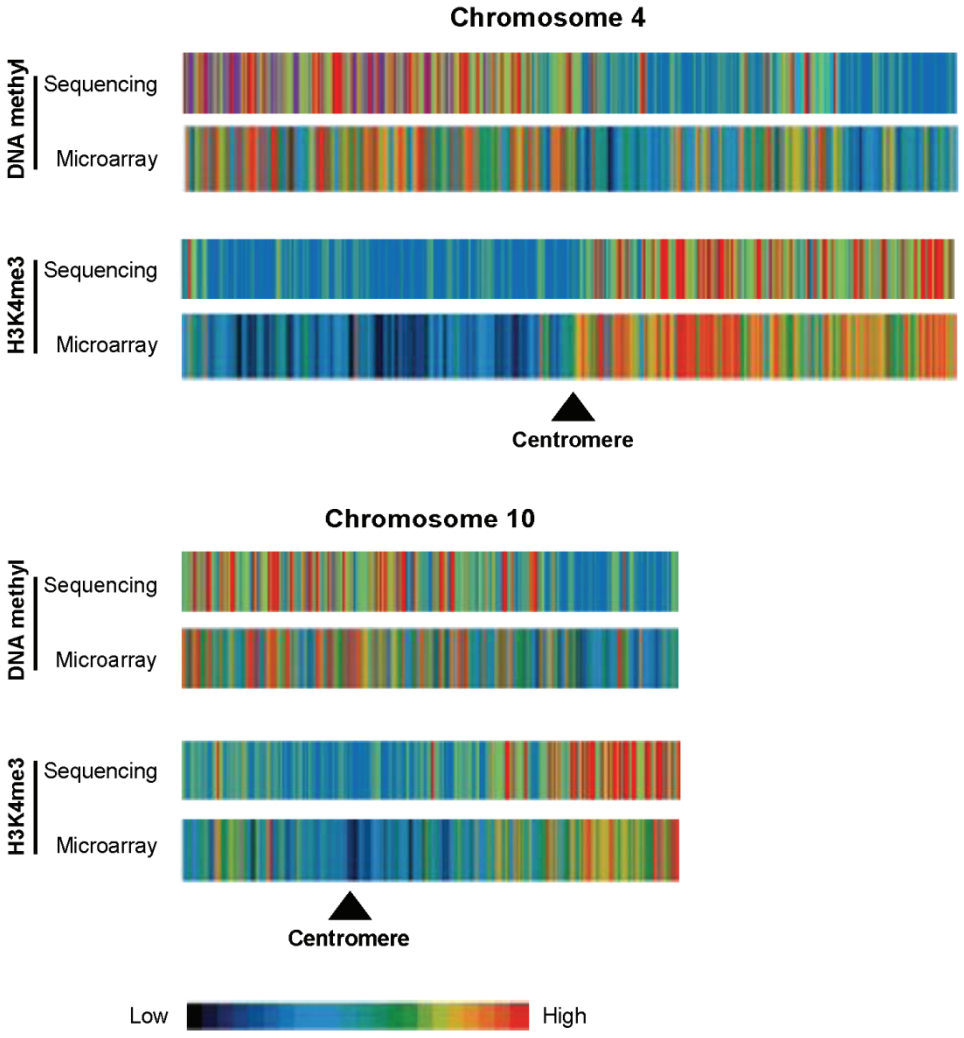
Supplemental Figure 4. (continued)

histone modifications per 100 kb sliding window over the entire rice chromosome 1.

(I) Number of TE-related or non-TE genes per 100 kb sliding window over the entire rice chromosome 1.

(J) Number of TE-related or non-TE genes with detected expression per 100 kb sliding window over the entire rice chromosome 1.

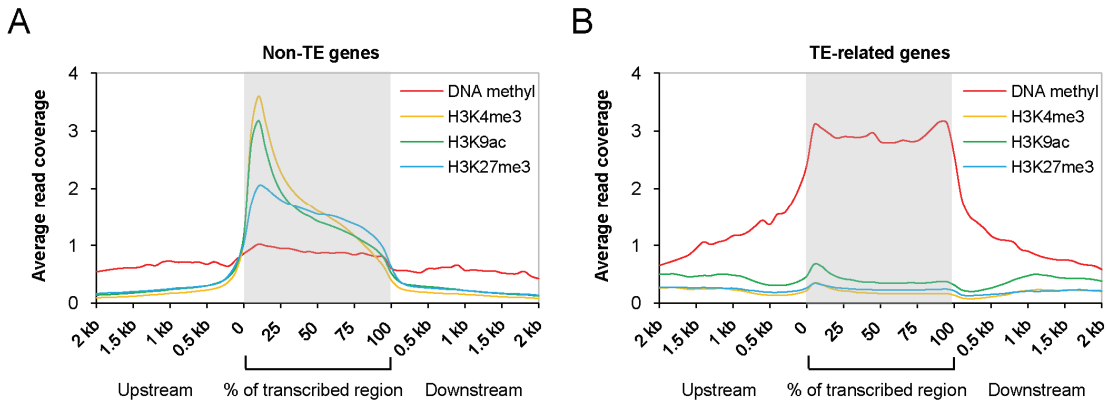
Black bar, pericentromeric region.



Supplemental Figure 5. Comparisons of the distribution patterns of DNA methylation and H3K4me3 modification on chromosomes 4 and 10 obtained from sequencing data (this study) with that from previously published microarray data (Li et al., 2008b).

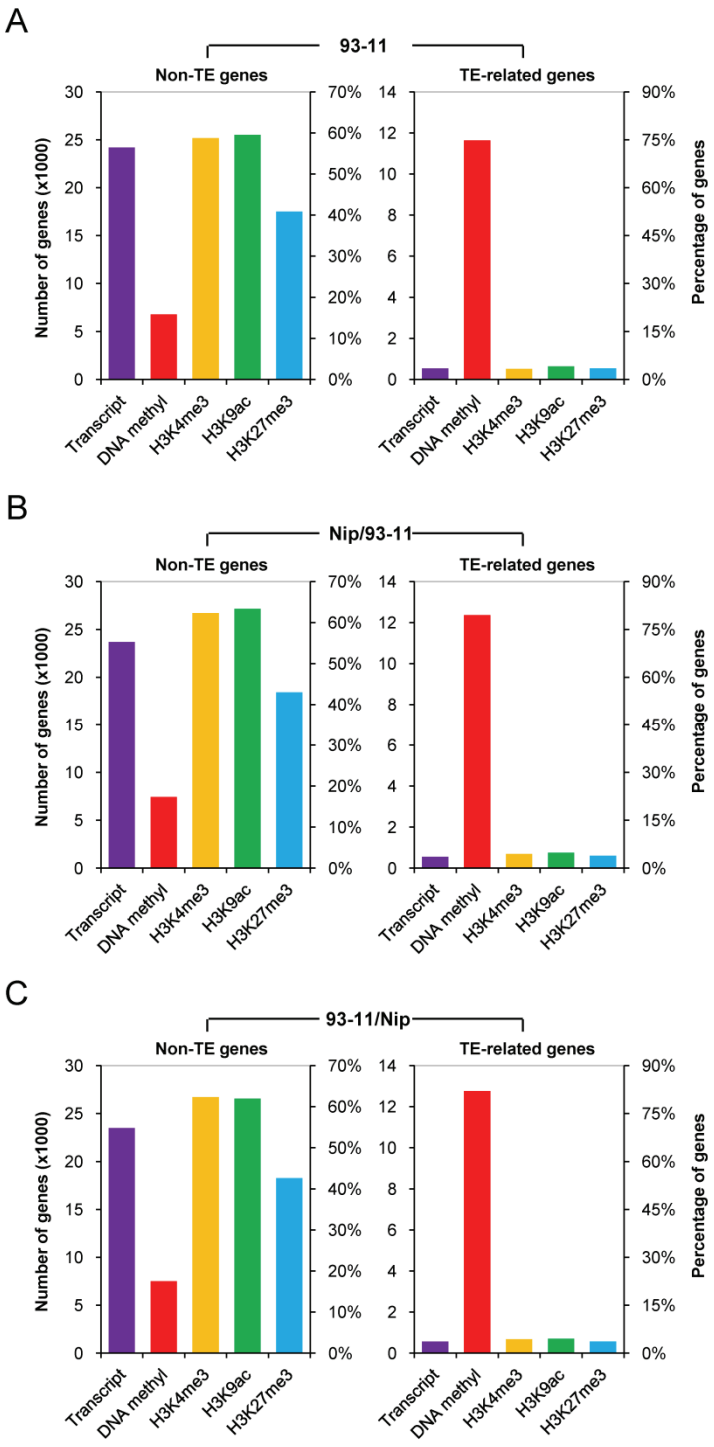
For sequencing data, color-coded bars represent total length of regions within each 100 kb bin that detected the indicated modification.

For microarray data, color-coded bars represent the percentage of probes within each 100 kb bin that detected the indicated modification.



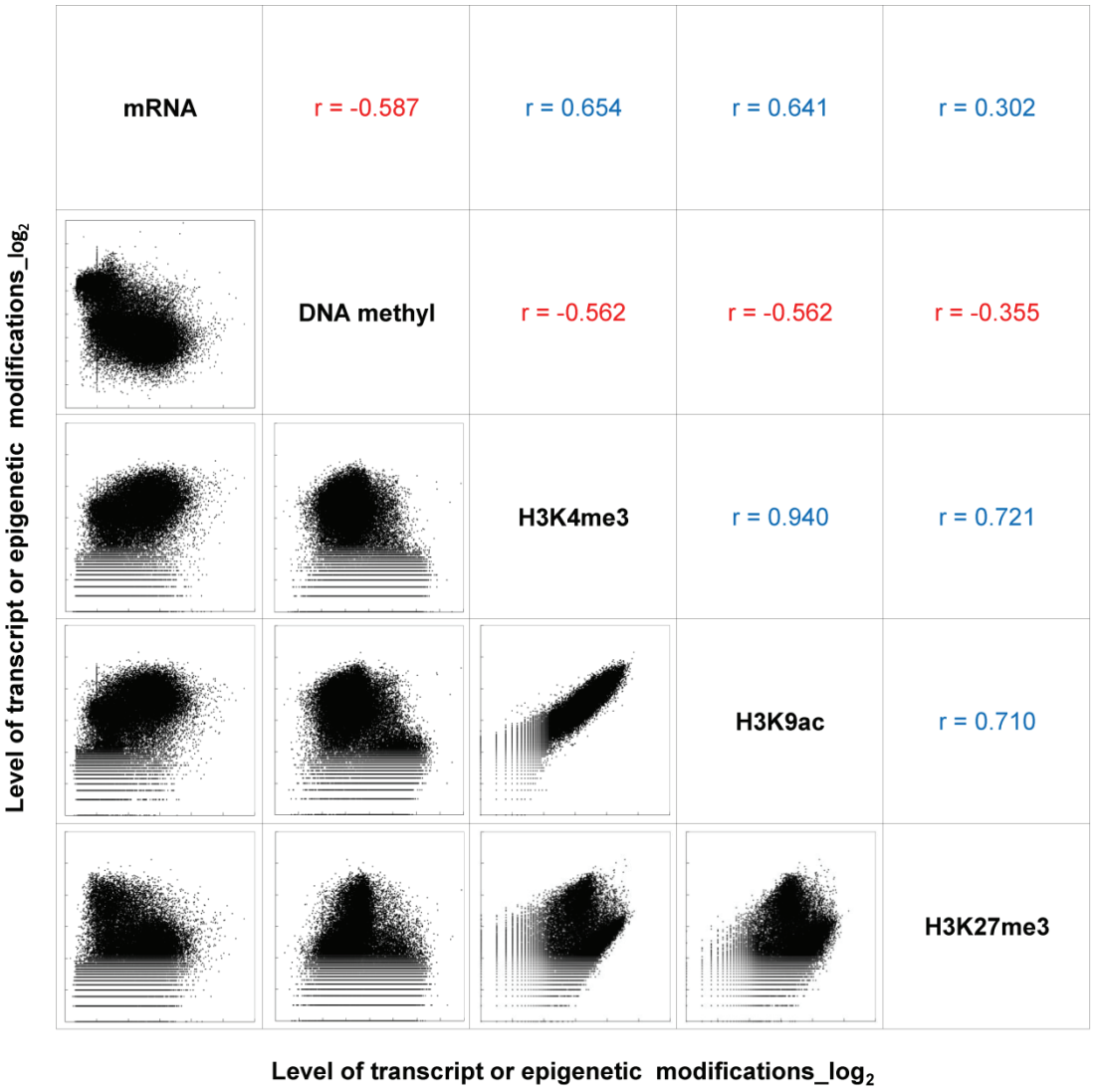
Supplemental Figure 6. Distribution of DNA methylation, H3K4me3, H3K9ac and H3K27me3 levels within non-TE genes (A) and TE-related genes (B).

Each gene was divided into 20 intervals (5% each interval), and the 2 kb regions upstream and downstream of each gene were divided into 100 bp intervals. The average read coverage of genes with epigenetic modifications in each interval was plotted (*y*-axis).



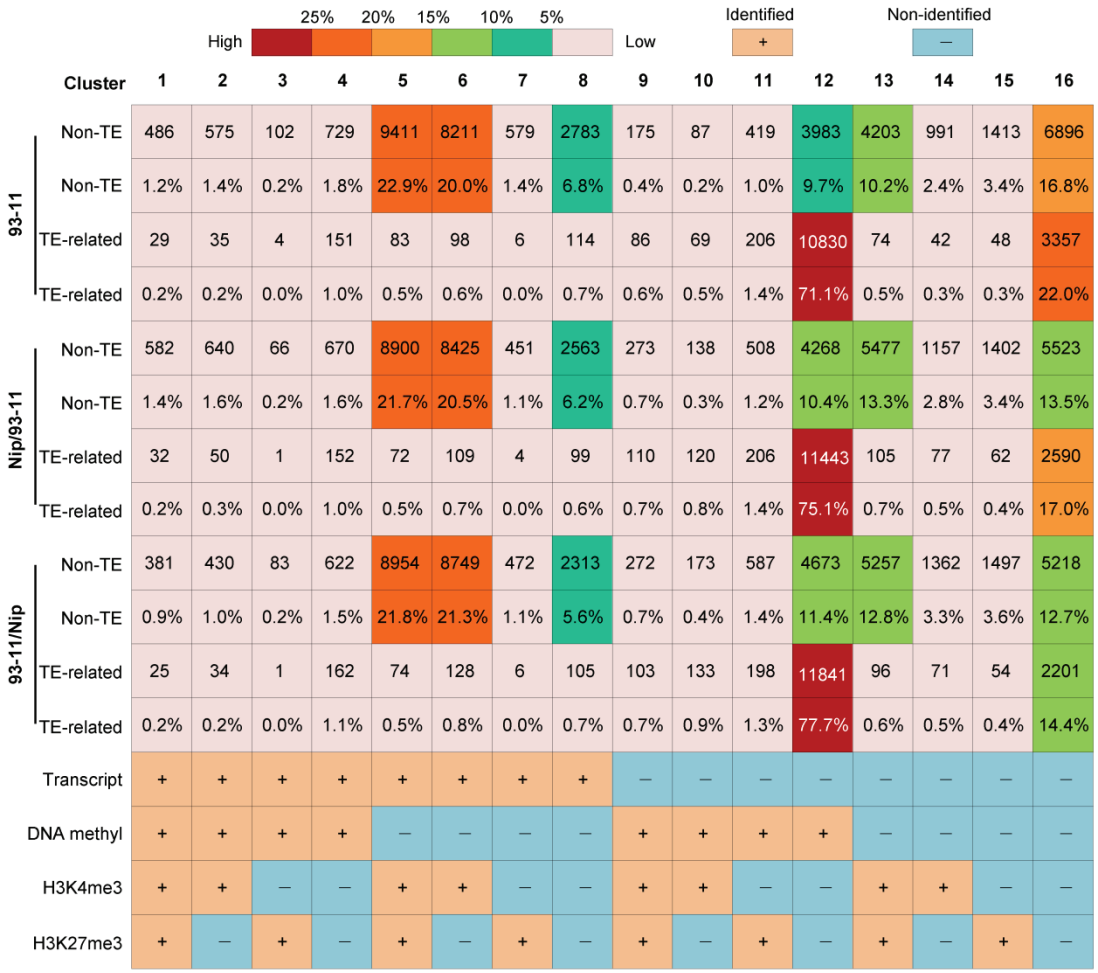
Supplemental Figure 7. Number and percentage of non-TE genes and TE-related genes identified with expression or epigenetic modifications in 93-11 (A), Nip/93-11 (B) and 93-11/Nip (C).

Nip, Nipponbare.

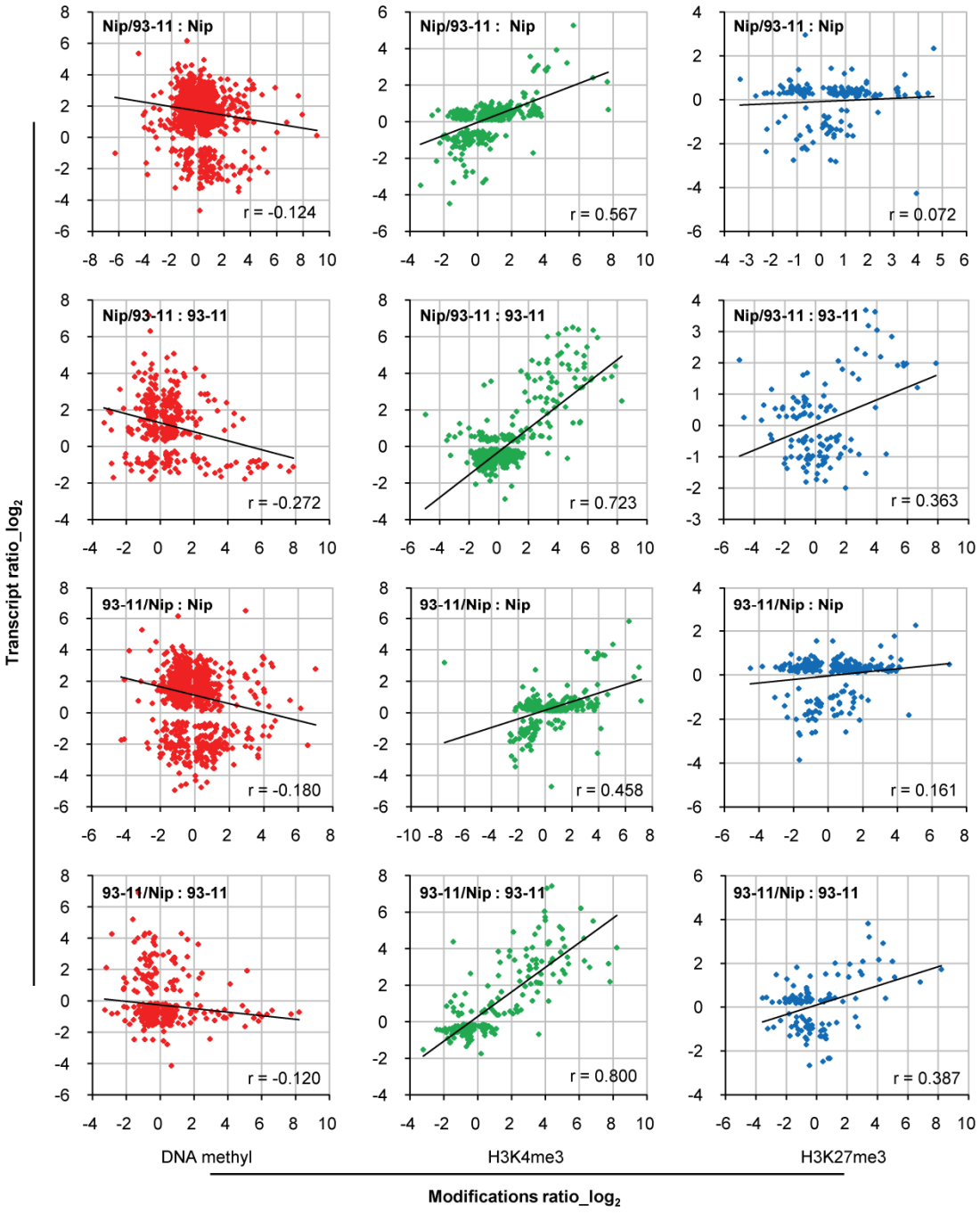


Supplemental Figure 8. Pairwise correlations among epigenetic modifications and gene expression.

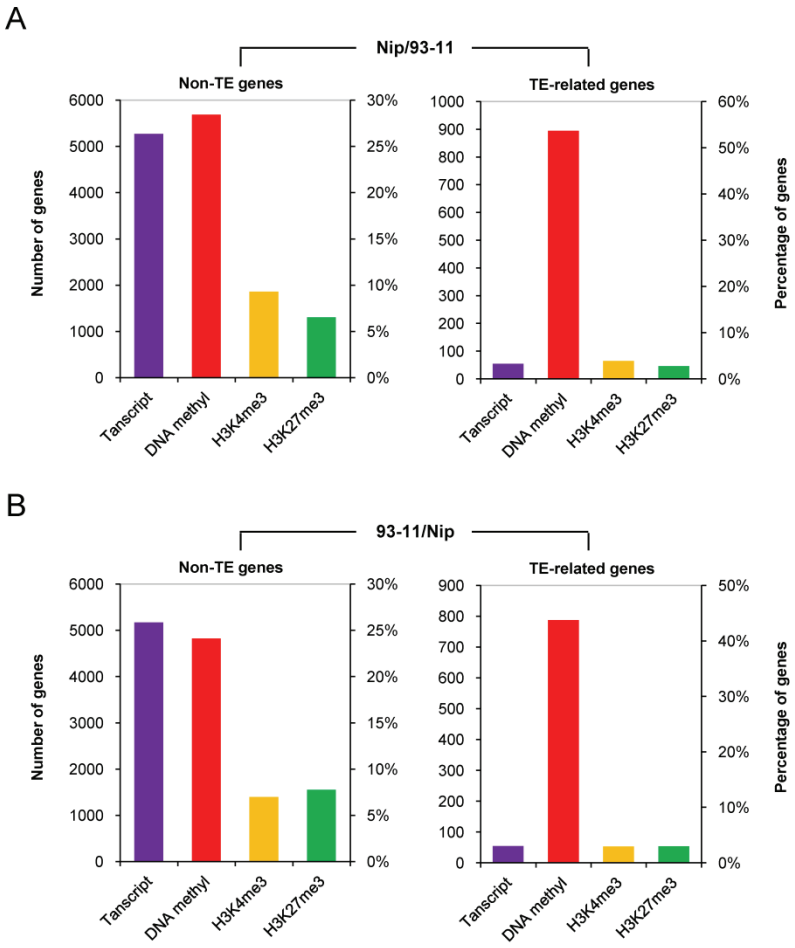
X and *y* axis represent the average read counts (log_2) for each gene. Pearson correlations are indicated above diagonal.



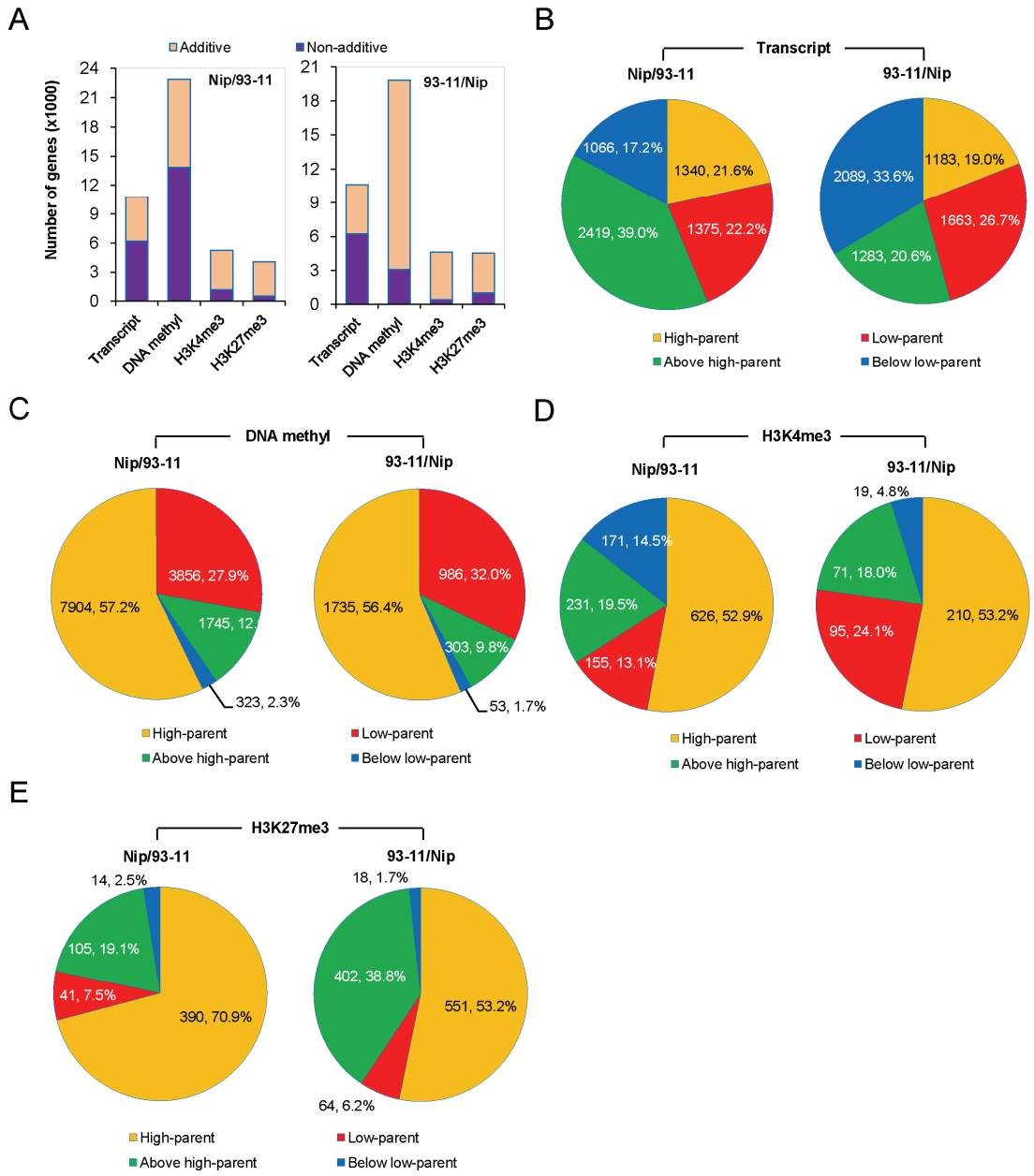
Supplemental Figure 9. Permutation tables for all combinations of DNA methylation, H3K4me3 and H3K27me3 modifications, and gene expression in 93-11, Nip/93-11 and 93-11/Nip.



Supplemental Figure 10. Correlations between variation of epigenetic modifications and variation of gene expression in reciprocal hybrids.



Supplemental Figure 11. Number of non-TE genes and TE-related genes exhibiting variation in gene expression or epigenetic modifications in Nip/93-11 (A) and 93-11/Nip (B).



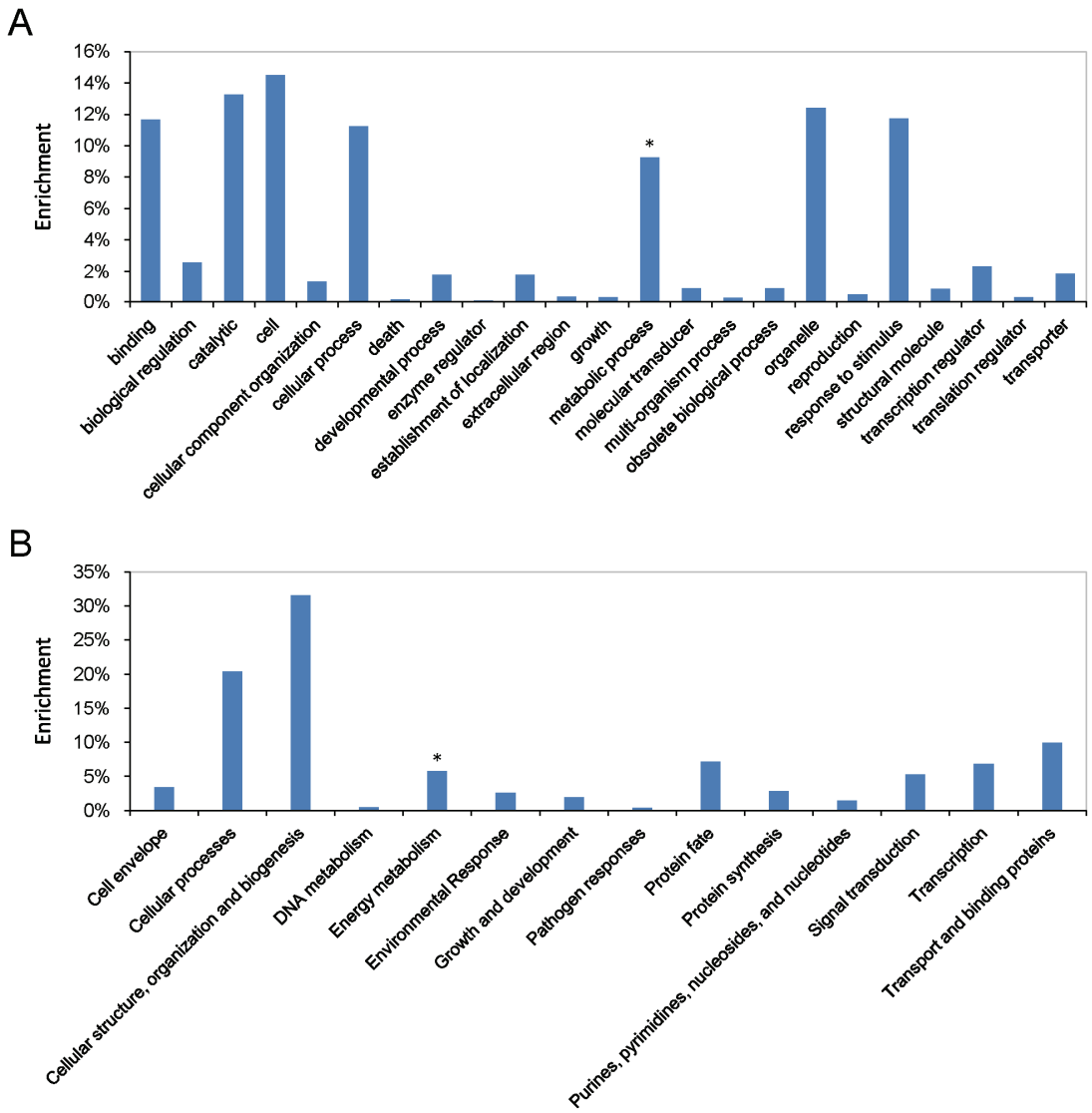
Supplemental Figure 12. Variation patterns of gene expression and epigenetic modifications in reciprocal hybrids investigated from all annotated rice genes (56278 genes).

(**A**) Additive and non-additive variation in gene expression and epigenetic modifications in hybrids.

(**B**) Subdivided patterns of non-additive variation in gene expression (high-parent, above high-parent, low-parent and below low-parent) in reciprocal hybrids.

Supplemental Figure 12. (continued)

(**C-E**) Subdivided patterns of non-additive variation in DNA methylation and histone modifications in hybrids.

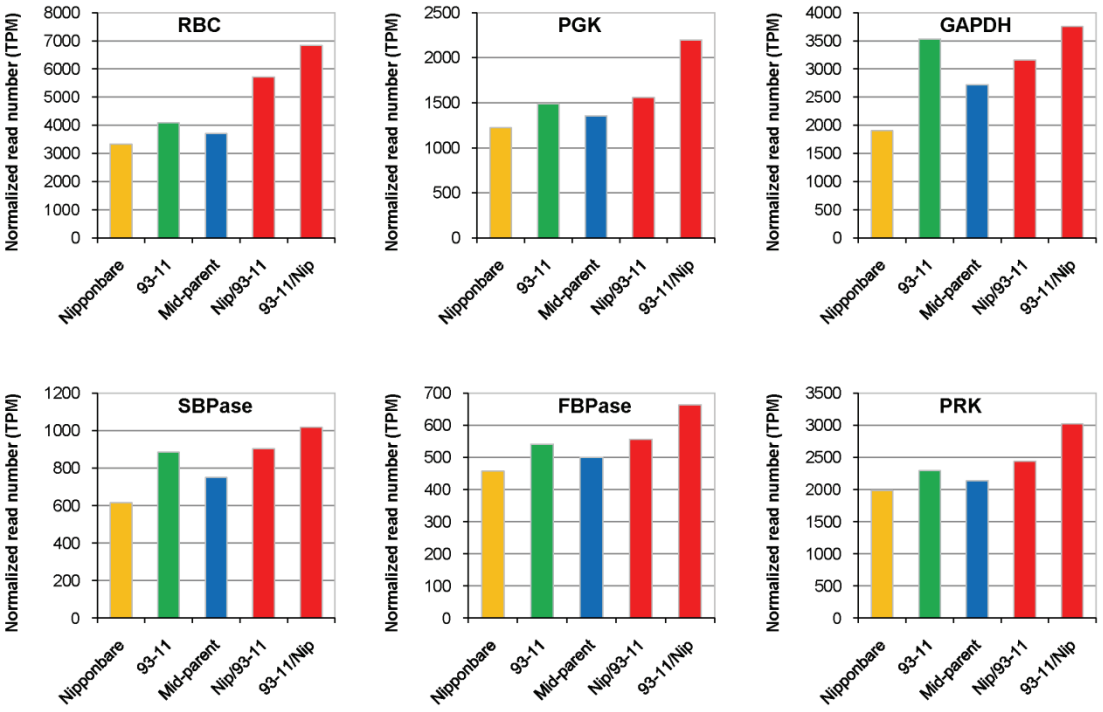


Supplemental Figure 13. Functional categories of 2800 genes showing the same direction of non-additive expression in both reciprocal identified from all annotated rice genes.

(A) Gene ontology classification using the Web Gene Ontology Annotation Plotting tool WEGO (http://wego.genomics.org.cn) (Ye et al., 2006).

(B) Gene ontology classification provided using Gene Ontology Consortium databases (http://www.geneontology.org/external2go/tigr2go).

* Denotes significant enrichment of a function category with p-value < 0.05.



Supplemental Figure 14. Up-regulation of six key components involved in Calvin cycle (carbon metabolism) relative to mid-parent in both reciprocal hybrids.

RBC, Ribulose bisphosphate carboxylase

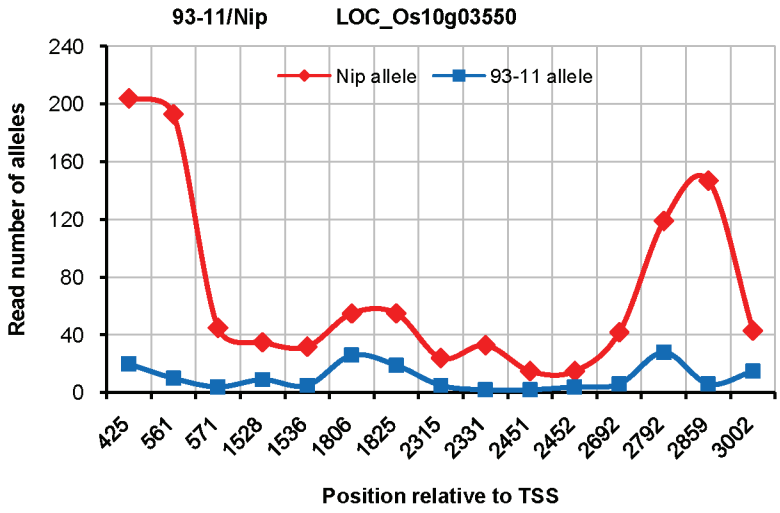
PGK, Phosphoglycerate kinase

GAPDH, Glyceraldehyde 3-phosphate dehydrogenase

SBPase, Sedoheptulose-1,7-bisphosphatase

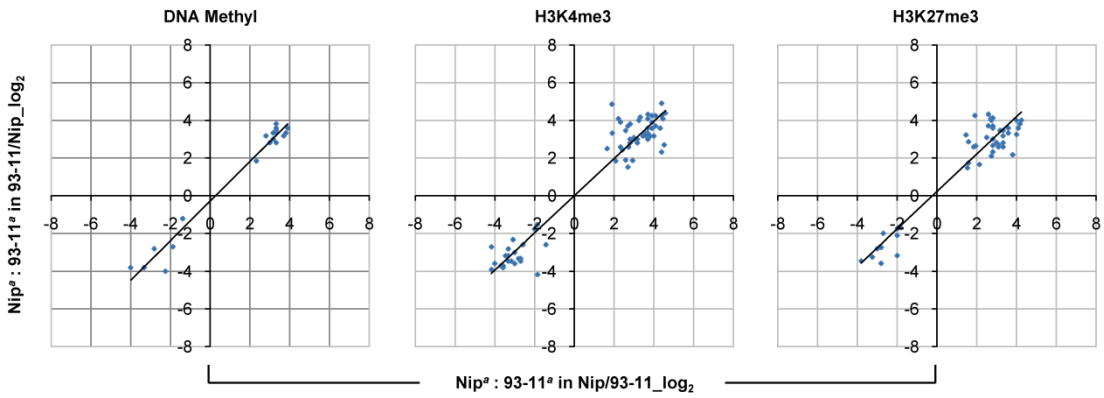
FBPase, Fructose-1,6-bisphosphatase

PRK, Phosphoribulokinase



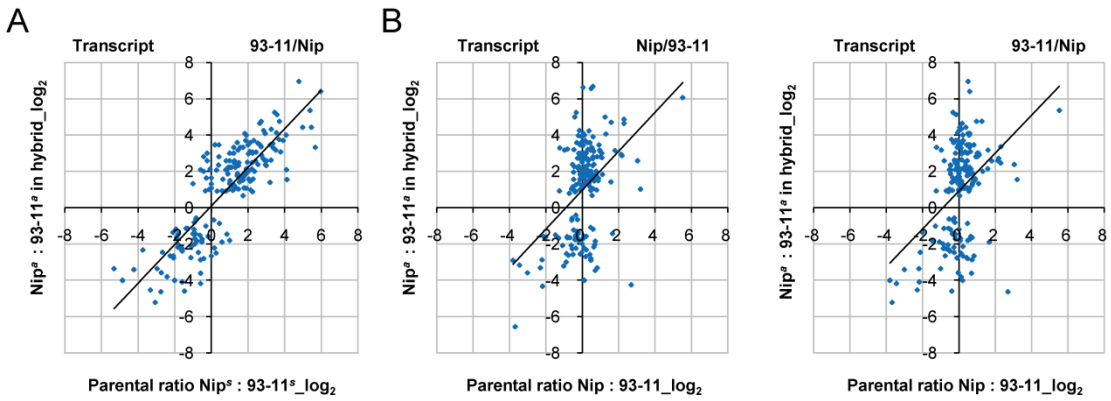
Supplemental Figure 15. An example of a gene exhibiting allelic expression bias in 93-11/Nip hybrid.

Read number of alleles detected by SNPs at each position was plotted.



Supplemental Figure 16. Correlation of allelic bias in epigenetic modifications between reciprocal hybrids.

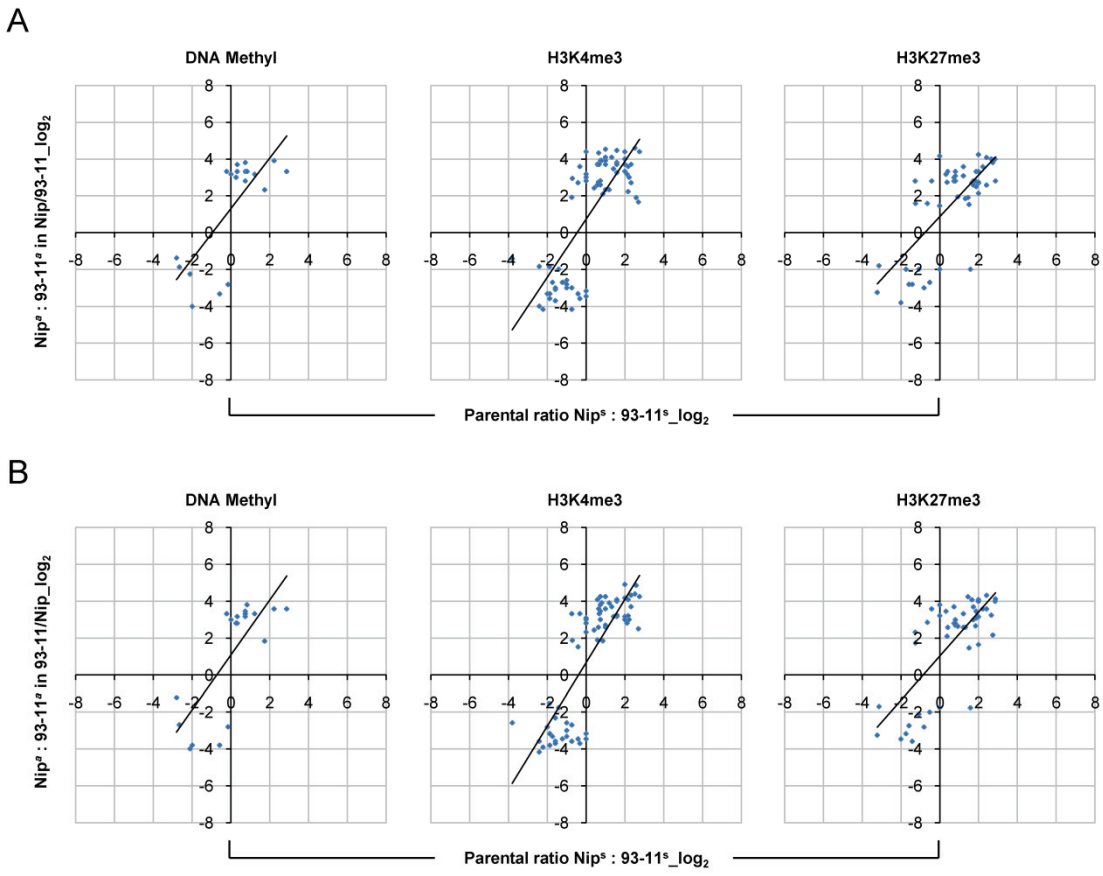
Nip^a, Nipponbare allele; 93-11^a, 93-11 allele.



Supplemental Figure 17. Correlation between differential parental expression and allelic expression bias in hybrids.

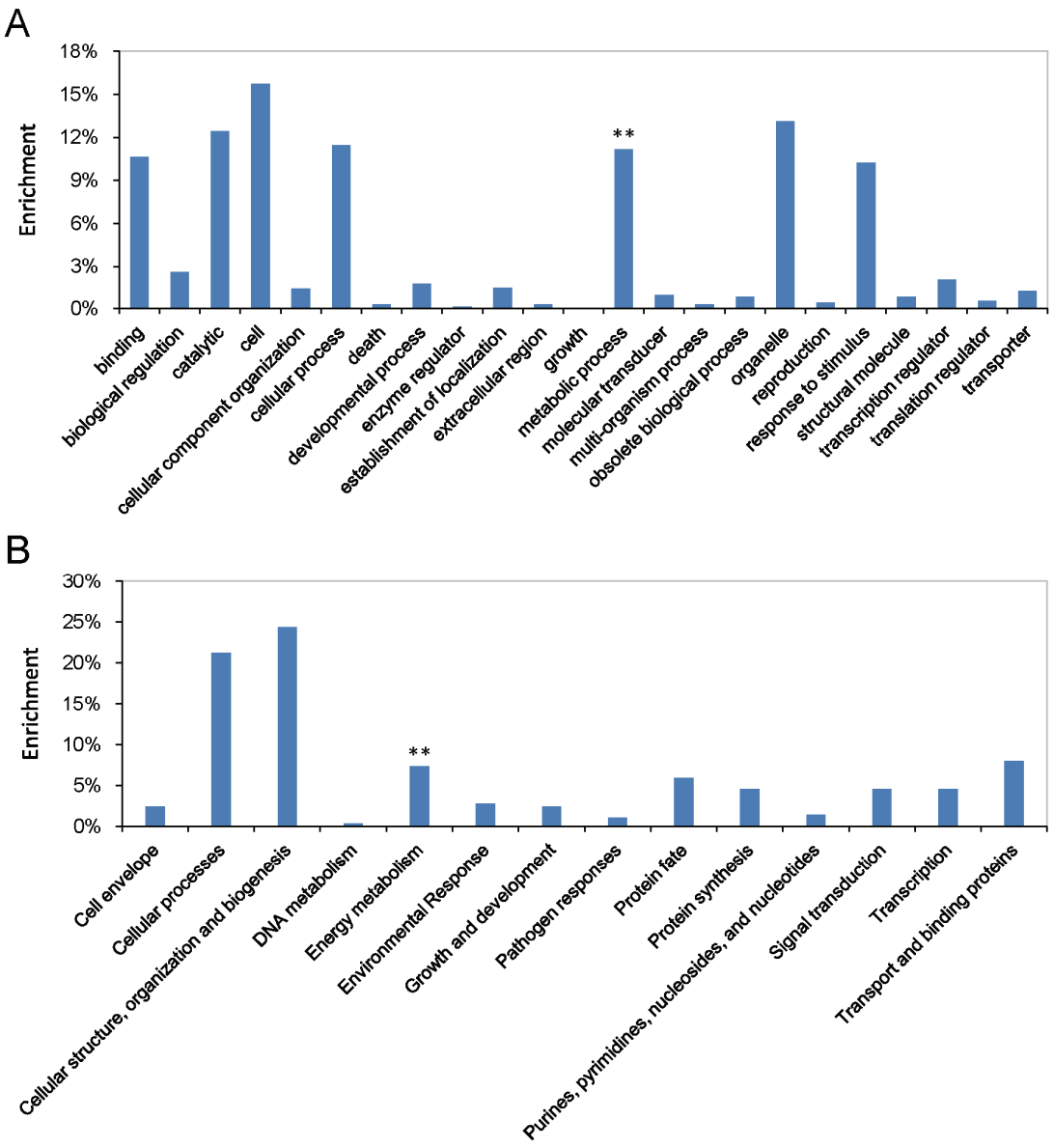
(A) Correlation between the differential parental expression (represented by the number of reads covering a SNP) and allelic expression bias in 93-11/Nip. Nip^a, Nipponbare allele; 93-11^a, 93-11 allele. Nip^s, Nipponbare transcripts represented by the number of reads covering a SNP; 93-11^s, 93-11 transcripts represented by the number of reads covering a SNP.

(B) Correlation between the differential parental expression (represented by the reads number per kb predicted mRNA) and allelic expression bias in reciprocal hybrids.



Supplemental Figure 18. Correlation between differential parental epigenetic modifications (represented by the number of reads covering a SNP) and allelic bias of epigenetic modifications in Nip/93-11 (A) and 93-11/Nip (B).

Nip^a, Nipponbare allele; 93-11^a, 93-11 allele. Nip^s, Nipponbare transcripts represented by the number of reads covering a SNP; 93-11^s, 93-11 transcripts represented by the number of reads covering a SNP.

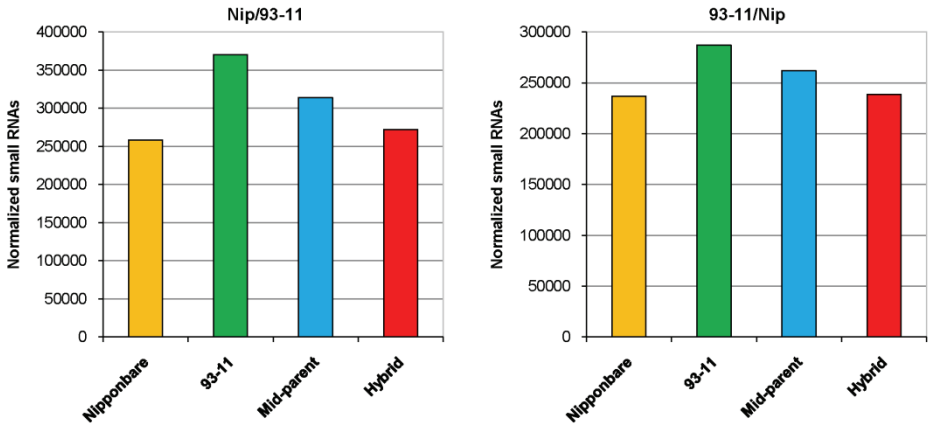


Supplemental Figure 19. Functional categories of 188 genes exhibiting allelic expression bias in both reciprocal hybrids.

(A) Gene ontology classification using the Web Gene Ontology Annotation Plotting tool WEGO (http://wego.genomics.org.cn) (Ye et al., 2006).

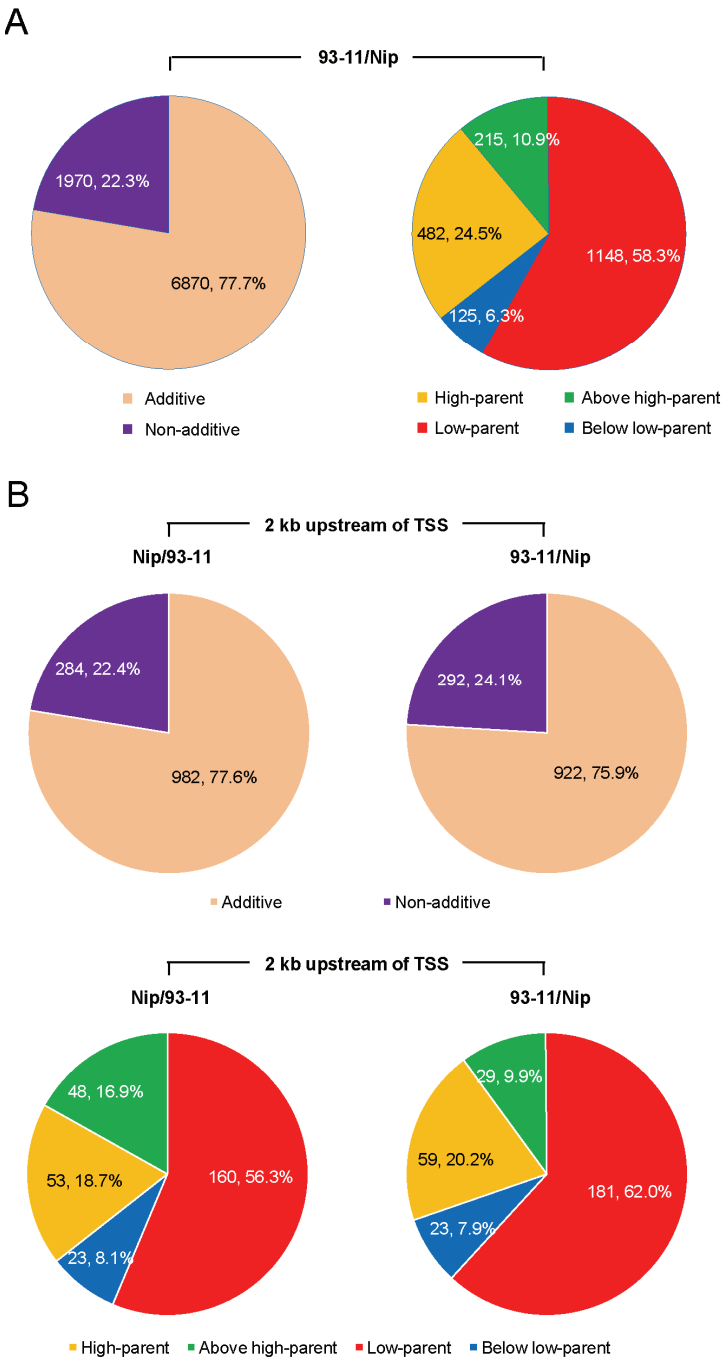
(B) Gene ontology classification provided using Gene Ontology Consortium databases (http://www.geneontology.org/external2go/tigr2go).

** Denotes significant enrichment of a functional category with p-value < 0.01.



Supplemental Figure 20. Suppression of small RNAs in hybrids.

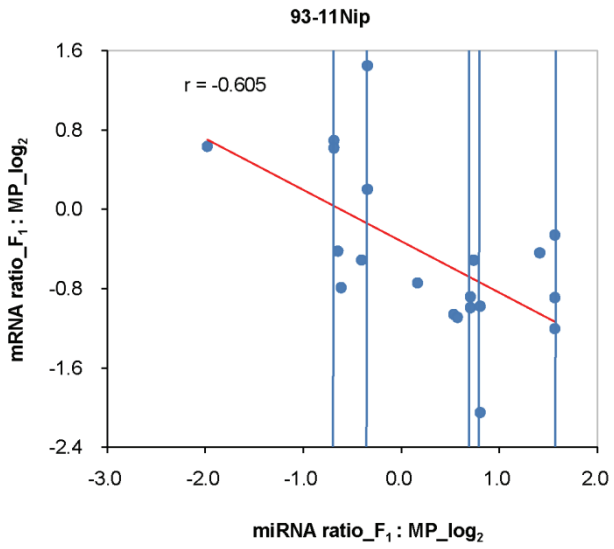
Sum of small RNA reads involved in all siRNA clusters that showed differential expression relative to mid-parent.



Supplemental Figure 21. Expression patterns of small RNAs in hybrids.

(A) Expression patterns of siRNA clusters in 93-11/Nip hybrid.

(B) Expression patterns of small RNAs in reciprocal hybrids, in which small RNAs in 2 kb upstream of TSS of 20638 homologous genes between Nipponbare and 93-11 were used for analysis.



Supplemental Figure 22. Negative correlation between the expression of 14 miRNAs and their 20 targets that are both exhibiting non-additive variation in both reciprocal hybrids. Multiple targets of a single miRNA are indicated by a vertical line.

Supplemental Table 1. Summary statistics for Illumina 1G sequencing libraries

	Nipponbare	93-11	Nip/93-11	93-11/Nip
Transcript				
Total reads	21,249,009	19,646,721	28,106,273	31,707,464
Mapped reads[1]	19,144,327	17,309,411	25,146,581	28,399,203
DNA methylation				
Total reads	13,395,883	13,778,550	22,556,971	21,565,682
Mapped reads	12,896,603	12,659,862	21,204,765	20,344,539
H3K4me3				
Total reads	7,344,605	11,121,581	14,558,730	15,163,166
Mapped reads	6,082,405	8,488,207	12,272,582	12,166,315
H3K9ac				
Total reads	14,066,257	15,702,340	18,519,649	17,770,148
Mapped reads	10,539,049	11,089,290	13,721,506	12,084,755
H3K27me3				
Total reads	7,876,647	10,778,973	14,597,882	14,337,775
Mapped reads	5,857,962	7,406,270	10,223,625	10,629,730
Small RNA				
Total reads	5,191,142	5,649,145	10,756,462	10,708,521
Mapped reads	4,829,223	5,247,449	9,994,469	9,985,054
Filtered reads[2]	3,379,279	3,284,035	6,845,274	6,779,118

[1]Reads that match to the genome of *Oryza sativa* ssp. *japonica* cv. Nipponbare.

[2]Reads after the removal of rRNAs, tRNAs, snRNA and snoRNA.

Nip, Nipponbare.

SUPPLEMENTAL METHODS

Data Processing and Analysis

Sequencing reads from all libraries were mapped to the reference genome of rice (*Oryza sativa* L. ssp. *japonica* cv. Nipponbare) using MAQ software (Li et al., 2008a). The output of the analysis pipeline was converted to browser extensible data (BED) files for viewing the data in the UCSC genome browser (Kent et al., 2002b). Reads which could be mapped equally well to multiple locations without mismatch or with identical mismatches were assigned to one position at random and were retained for further analyses as described previously (Wang et al., 2009). Genomic regions associated with DNA methylation and histone modifications were identified using MACS software (Zhang et al., 2008b), in which default parameters (bandwidth, 300 bp; mfold, 32; p-value of 1.00e-05) were set up to call peaks representing enriched epigenetic marks as described previously (Wang et al., 2009). The small RNA reads that corresponded to the precursor of all known rice miRNAs from miRBase (http://www.mirbase.org/index.shtml) were used to estimate the expression level of each rice miRNA (Nobuta et al., 2009). miRNA targets were predicted by the web tool psRNATarget (Zhang, 2005) at http://bioinfo3.noble.org/psRNATarget, using default parameters. A siRNA cluster was defined to contain a minimum of four small RNA reads, each separated from the nearest neighbor by a maximum of 200 nt, using the pooled small RNA reads from four genotypes.

The complete genomic sequences of Nipponbare and 93-11 were aligned by

BLAT (Kent, 2002a) to identify orthologous genomic regions between both rice subspecies. The coordinates of all annotated rice genes (TIGR version 5.0) from orthologous genomic regions in Nipponbare were converted to the 93-11 genome using the UCSC LiftOver tool (http://genome.ucsc.edu/cgi-bin/hgLiftOver). In this manner, a total of 20638 homologous genes, in which each exon had a counterpart between two rice subspecies, were identified as gold standard for differential gene analysis.

SNPs were identified by comparing sequencing reads of all homologous genes between Nipponbare and 93-11 with the following criteria: there are at least three sequencing reads covering a SNP position in each parent; all reads from one parent contain the same nucleotide at the SNP position and are different from that of another parent at the same position. Allelic bias in hybrids was identified by determining if there is significant deviation from binomial distribution (i.e., the allele ratio in hybrids deviated from 1.0) of parental alleles defined by SNPs.

For all comparisons, read counts for a gene or genomic region from each library were normalized to TPM (Tags Per Million), which divided the read number of each gene or region by total read number in each library, and multiplied by 10^6. Significant differential gene expression, small RNA expression and epigenetic modifications were determined using a published statistical model, in which a Bayesian approach was applied to calculate the p-value (P) of differential according to observing a given number of tags (Audic and Claverie, 1997; Man et al., 2000):

$$p(y|x) = \left(\frac{N_2}{N_1}\right)^y \frac{(x+y)!}{x!\,y!\left(1+\frac{N_2}{N_1}\right)^{(x+y+1)}}$$

$$\mathrm{P} = \min\left\{\sum_{\mathrm{k}=0}^{\mathrm{k}\le\mathrm{y}} \mathrm{p(k|x)}\,,\ \sum_{\mathrm{k}=\mathrm{y}}^{\infty} \mathrm{p(k|x)}\right\}$$

N_1: the total number of reads in sequencing library #1.

N_2: the total number of reads in sequencing library #2.

x: number of reads for a gene or genomic region in library #1.

y: number of reads for a gene or genomic region in library #2.

The FDR (false discovery rate) was estimated by dividing the total number of genes (or allelic pairs, miRNA, siRNA cluster) investigated and the significance level, by the number of genes (or allelic pairs, miRNA, siRNA cluster) with p-values below the significance level.

Gene ontology classification provided in TIGR Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) was used to assign genes to a hierarchical biological process following the criteria of the Gene Ontology Consortium databases (http://www.geneontology.org/external2go/tigr2go), or using the Web Gene Ontology Annotation Plotting tool WEGO (http://wego.genomics.org.cn) (Ye et al., 2006). The p-value of a particular pathway that corresponds to a test statistic was evaluated using a previously reported method (Mao et al., 2005), with a p-value cutoff of 0.05 as the significance threshold.

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