Supplementary Figures

Supplementary Figure 1 | Experimental design. Strains were derived from a single parent. The seeds were propagated by single-seed descent, with separate lineages for the 3rd and 31st generation individuals. Strains 39 and 49 were propagated for one more generation from siblings of the plants analyzed by sequencing (grey outlines). Black outlines indicate individuals analyzed by bisulphite sequencing. Red outlines indicate individuals analyzed by RNA-seq.

Supplementary Figure 2 | Effect of read coverage and methylation rate on power to detect methylation gain in pairwise comparisons. Each panel represents a different local methylation rate in the reference strain. The x-axis indicates the read coverage at a specific position in the reference strain, and the y-axis the read coverage in the strain that is being compared to the reference strain. Inset indicates methylation rate in reference strain. Colour indicates the number of methylation-supporting reads required to ascertain significantly increased methylation (p < 0.05). White areas represent coverage/methylation rate combinations for which there is no statistical power to determine differential methylation.

Supplementary Figure 3 | Effect of coverage and methylation rate on power to detect of methylation loss in pairwise comparisons. See legend of Supplementary Fig. 2 for details.

Supplementary Figure 4 | Frequency distribution of read coverages and methylation rates. a, Relative frequency of per-strand read coverage per site for the 3,067,017 cytosine positions analyzed for differential methylation. b, Relative frequency of methylation rates by sequence context in the 3rd-generation strains for sites methylated in at least one sibling. c, Relative frequency of methylation rates by
sequence context in the 3rd-generation strains for the 3,067,017 cytosine positions analyzed for differential methylation. As these sites were required to be methylated in at least one strain, the fraction of weakly methylated sites is higher than in (b).

**Supplementary Figure 5 | Frequency distribution of DMPs and N-DMPs.**

a, Frequency along genes as shown in Fig. 1e, limited to genes of up to 2,000, 1,000 and 500 bp in length, respectively. Note that relative gene body methylation decreases with gene size. b, Frequency along exons and introns. c, Frequency along TEs. Data were normalized to the highest value for each sequence context and class. Statistical significance for observed changes in distributions along TEs could not be ascertained due to the small sample size of DMPs. CHH methylation is not shown due to the reduced statistical power in detecting differential methylation.

**Supplementary Figure 6 | Distance to transposable elements and siRNAs.**

a, Frequency distribution of the distance between DMPs and N-DMPs to the closest 24-nt siRNA mapping upstream and downstream of the focal position. b, Frequency distribution of the distance between DMPs and N-DMPs to the closest TE upstream and downstream of the focal position. c, Same analysis as in (b), but excluding TE-rich pericentromeric regions.

**Supplementary Figure 7 | Mutation of the MEE57 gene (AT4G13610) in line 69.** Line 69 has a G→T mutation, leading to an amino acid change in a highly conserved residue (highlighted in yellow). The alignment shown is restricted to the C-terminal part of the protein.

**Supplementary Figure 8 | DMR annotation.** Top, frequency of methylated positions in DMRs along genic elements. Bottom, frequency of methylated positions along exons.
and introns. Data were normalized to the highest value for each sequence context and class.

Supplementary Figure 9 | DMR identified between the 31st and 32nd generation of strain 39. Compared to the 3rd generation, this 150 bp region (Chr3:7,093,900..7,094,050) showed a loss of methylation in the 31st generation, but a methylation pattern similar to the 3rd had been re-established in the 32nd generation. Line 49 showed no change.

Supplementary Figure 10 | Correlation between RNA expression and DNA methylation. The plot shows the distribution of methylated cytosines (blue) and annotated transcripts without RNA-seq expression support (green) along the five chromosomes.

Supplementary Figure 11 | Differentially methylated regions overlapping with genic regions. Regions that overlap with the differentially expressed genes AT1G53480 and AT1G53490 (Chr1:19,962,980..19,964,439), AT3G09450 (Chr3:2,909,299..2,909,368), AT3G47420 (Chr3:17,472,697..17,472,775), AT4G38550 (Chr4:18,027,017..18,027,146) and AT5G25930 (Chr5:9,052,699..9,052,787) are shown. For simplicity, only one sibling is shown per strain.

Supplementary Figure 12 | False positives in methylation detection. a, Scatter plot illustrating the relationship of total read coverage and per-site estimates of the false positive methylation rate from reads mapping to the chloroplasts. In red: false methylation rate estimated from groups of sites binned by multiples of 5-fold coverage. b, False methylation rate estimates for individual libraries and coverage bins from reads mapping to the chloroplasts.
Supplementary Figure 13 | Detection of DMPs at different coverages. Number of identified DMPs when the dataset was subsampled at different coverages.
Supplementary Figure 1

Premutation genotype

Generation 0 (Founders)

Generation 3

Generation 31

Generation 32
Supplemental Figure 5

a

![Graph a](image1)

b

![Graph b](image2)

c

![Graph c](image3)
Supplemental Figure 8

(a) Relative methylation across the genome:
- **mC** (black line)
- **DMRs** (green line)

(b) Relative methylation across transcript:
- **mC** (black line)
- **DMRs** (green line)
Supplementary Figure 10
Supplementary Tables (separate files)

Supplementary Table 1 | Sequencing depth, covered cytosine positions and methylated sites. Listed are the average coverage, the cytosine positions covered by one and three independent reads, respectively, and the positions identified as methylated in the different sequence contexts per strain and replicate.

Supplementary Table 2 | Differentially methylated regions (FDR < 0.05). Indicated are the start and end positions on the chromosome, the length in base pairs, the number of contained N-DMPs and DMPs and, if applicable, overlapping gene models. Gain or loss of methylation is in comparison to the 3rd generation strains.

Supplementary Table 3 | Differentially expressed genes. Genes identified as differentially expressed between strains 4, 8, 69 and 109 with their respective log2-fold changes.

Supplementary Table 4 | Scoring matrix for the assessment of the alignment quality at single sites. Matrix indicating the penalties applied during the assessment of the quality of the read and alignment data for every cytosine position using the SHORE pipeline.