

**Figure S1.** Phenotypic variation of soybean mutations. The variations of plant architecture (a) and seed (b) in soybean M3 mutants

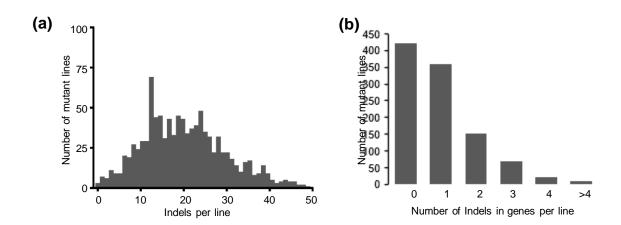
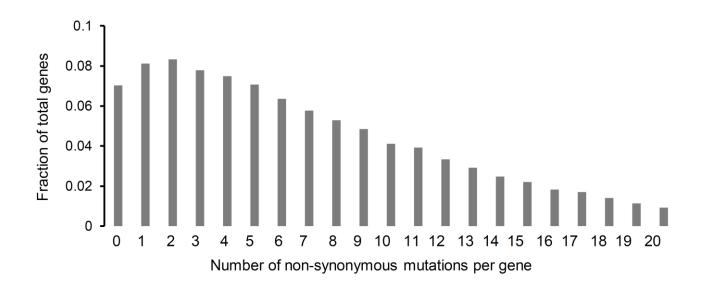
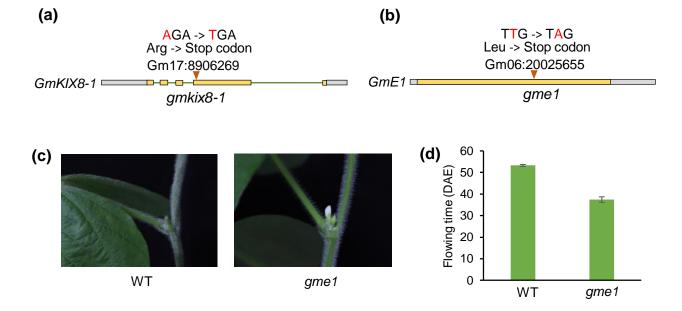


Figure S2. Distribution of small Indels in soybean mutant population.

- (a) Distribution of small Indels per mutant line in mutant population.
- (b) Frequency of mutant lines carrying different number of small Indels in gene region.

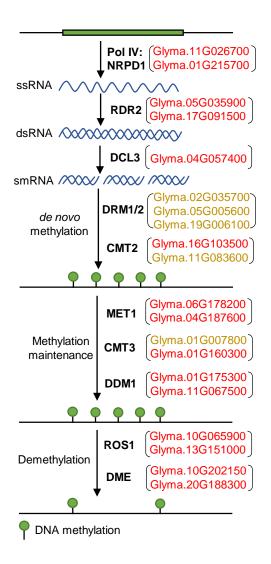


**Figure S3.** Frequency of genes carrying different number of non-synonymous mutations, including stop gained, mis-splicing, start lost, and missense mutations.

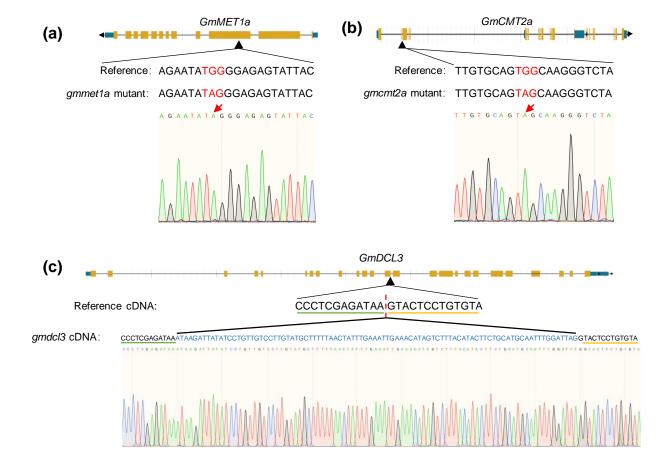


**Figure S4.** Knockout of *GmKIX8-1* and *GmE1* genes in mutant population.

- (a) Genomic location and amino acid change of homozygous EMS-induced mutation in *gmkix8-1* mutant (NJAU1840).
- **(b)** Genomic location and amino acid change of homozygous EMS-induced mutation in *gme1* mutant (NJAU0143).
- **(c)** Phenotype of wild type (WT) and *gme1* mutant. WT did not bloom when *gme1* mutant was flowering in long-day condition (16 h/8 h, light/dark).
- **(d)** Flowering time of WT and *gme1* mutant in long-day condition (16 h/8 h, light/dark). DAE: days after emergence.



**Figure S5.** Mutations identified in genes involved in DNA methylation and demethylation. Genes in Red indicate genes carrying truncation mutations, including stop gained, missplicing, start lost. Genes in brown indicates genes carrying missense mutations.



**Figure S6.** Validation of truncation mutations in DNA methylation related genes. **(a-c)** Sanger sequencing validation of truncation mutations in *GmMET1a* **(a)**, *GmCMT2a* **(b)** and *GmDCL3* **(c)**, respectively. Black triangles indicate positions of mutations on genes. Retained intron in mature mRNA in *gmdcl3* mutant is indicated in blue.