

Table S1. Methylome sequencing summary statistics

| Sample                               | Length (bp) | Mapped reads | Non-conversion (%) | Genome coverage | Coverage per-strand |
|--------------------------------------|-------------|--------------|--------------------|-----------------|---------------------|
| <i>Col-0 WT-1</i>                    | 150         | 25,858,836   | 0.30%              | 32.5            | 16.3                |
| <i>Col-0 WT-2</i>                    | 150         | 20,708,437   | 0.17%              | 26.0            | 13.0                |
| <i>35S:TET1-1</i>                    | 150         | 11,483,070   | 0.17%              | 14.4            | 7.2                 |
| <i>35S:TET1-2</i>                    | 150         | 25,300,675   | 0.12%              | 31.8            | 15.9                |
| <i>35S:TET1-3 rep1</i>               | 150         | 15,239,451   | 0.16%              | 19.2            | 9.6                 |
| <i>35S:TET1-3 rep2</i>               | 150         | 13,976,359   | 0.13%              | 17.6            | 8.8                 |
| <i>35S:TET1-2<sup>T5</sup></i>       | 150         | 10,149,189   | 0.19%              | 12.8            | 6.4                 |
| <i>35S:TET1-2<sup>T6</sup></i>       | 75          | 46,968,226   | 0.12%              | 29.5            | 14.8                |
| <i>35S:TET1-1.1<sup>+TET1</sup></i>  | 150         | 21,161,361   | 0.11%              | 26.6            | 13.3                |
| <i>35S:TET1-1.2<sup>+TET1</sup></i>  | 150         | 24,145,320   | 0.09%              | 30.4            | 15.2                |
| <i>35S:TET1-1.3<sup>-TET1</sup></i>  | 150         | 24,782,835   | 0.11%              | 31.2            | 15.6                |
| <i>35S:TET1-1.4<sup>-TET1</sup></i>  | 150         | 21,725,685   | 0.30%              | 27.3            | 13.7                |
| <i>35S:TET1-1.5<sup>-TET1</sup></i>  | 150         | 23,032,079   | 0.25%              | 29.0            | 14.5                |
| <i>ACT2:TET1-1.1<sup>+TET1</sup></i> | 150         | 25,564,705   | 0.16%              | 32.1            | 16.1                |
| <i>ACT2:TET1-1.2<sup>-TET1</sup></i> | 150         | 24,743,874   | 0.15%              | 31.1            | 15.6                |
| <i>ACT2:TET1-2</i>                   | 75          | 35,548,625   | 0.12%              | 22.3            | 11.2                |
| <i>ACT2:TET1-3</i>                   | 75          | 34,292,795   | 0.12%              | 21.6            | 10.8                |
| <i>ACT2:TET1-4</i>                   | 75          | 42,867,331   | 0.16%              | 26.9            | 13.5                |
| <i>ACT2:TET1-5</i>                   | 75          | 36,877,672   | 0.47%              | 23.2            | 11.6                |

Table S2. Transcriptome sequencing summary statistics

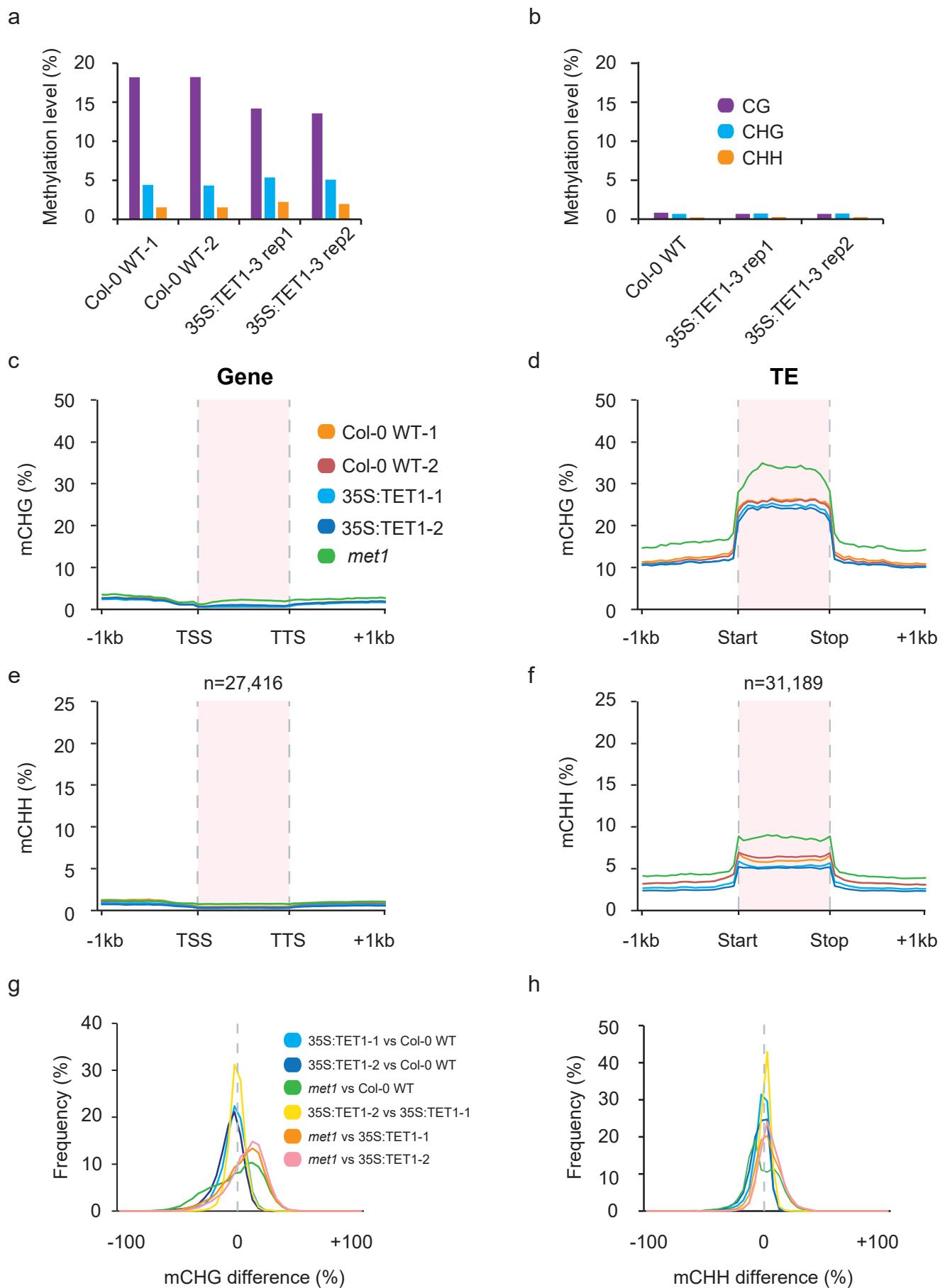
| Sample               | Mapped reads | Percent Mapped |
|----------------------|--------------|----------------|
| <i>Col-0 WT rep1</i> | 18,065,032   | 95.39%         |
| <i>Col-0 WT rep2</i> | 24,156,251   | 96.63%         |
| <i>Col-0 WT rep3</i> | 20,987,335   | 96.48%         |
| <i>35S:TET1-1</i>    | 19,919,035   | 95.38%         |
| <i>35S:TET1-2</i>    | 15,977,645   | 96.51%         |
| <i>met1</i>          | 45,615,533   | 94.61%         |
| <i>ibm1 rep1</i>     | 20,810,937   | 94.16%         |
| <i>ibm1 rep2</i>     | 22,011,997   | 96.17%         |
| <i>ibm1 rep3</i>     | 25,241,389   | 97.05%         |

Table S3. TAB-seq summary statistics

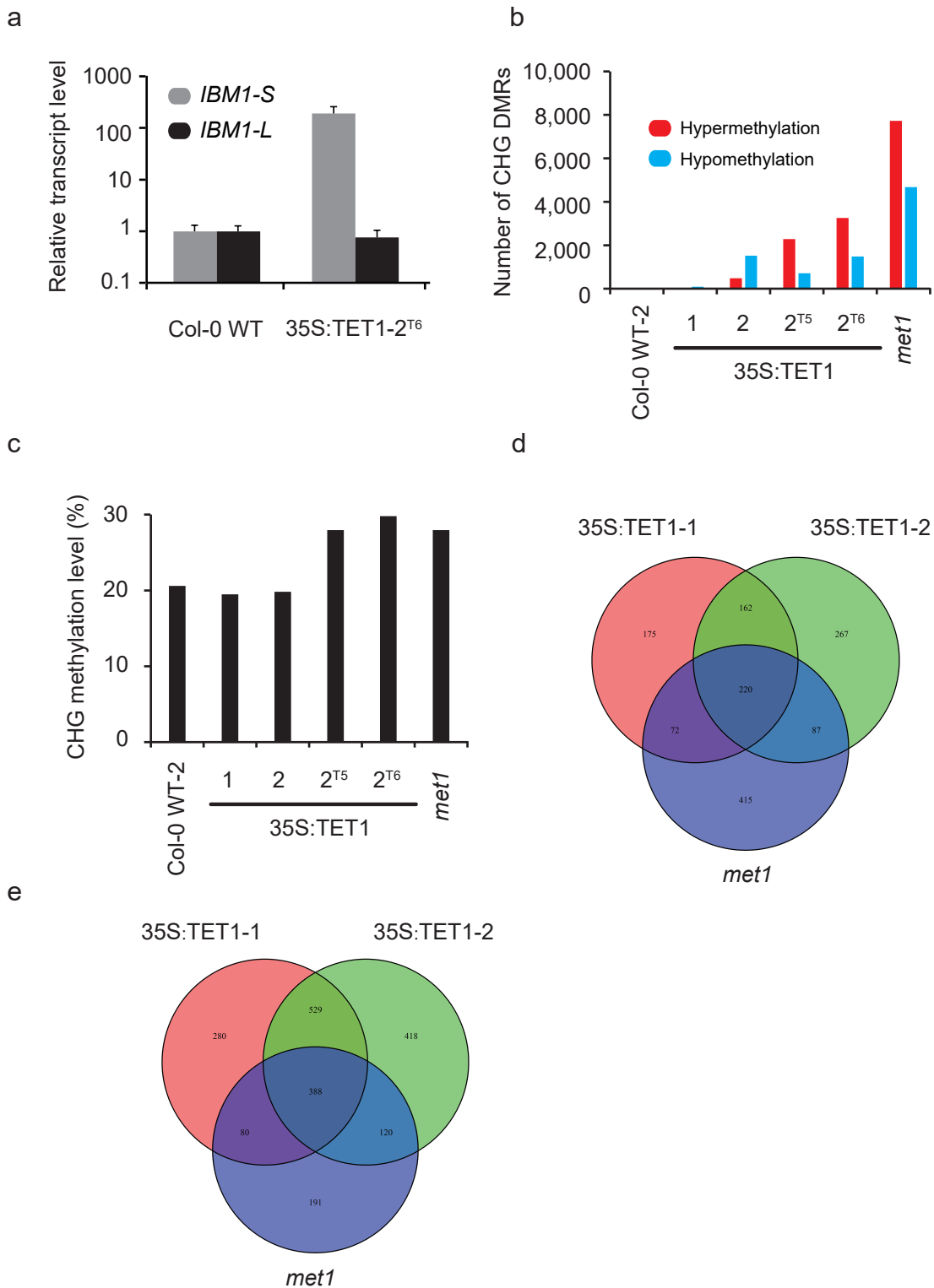
|                        | Mapped reads | 5mC non-conversion (%) | Non-conversion (%) | Genome coverage |
|------------------------|--------------|------------------------|--------------------|-----------------|
| <i>Col-0 WT</i>        | 15,044,614   | 3.97%                  | 0.28%              | 18.9            |
| <i>35S:TET1-3 rep1</i> | 14,293,516   | 4.69%                  | 0.28%              | 18.0            |
| <i>35S:TET1-3 rep2</i> | 16,145,140   | 4.68%                  | 0.29%              | 20.3            |

Table S4. ChIP-seq summary statistics

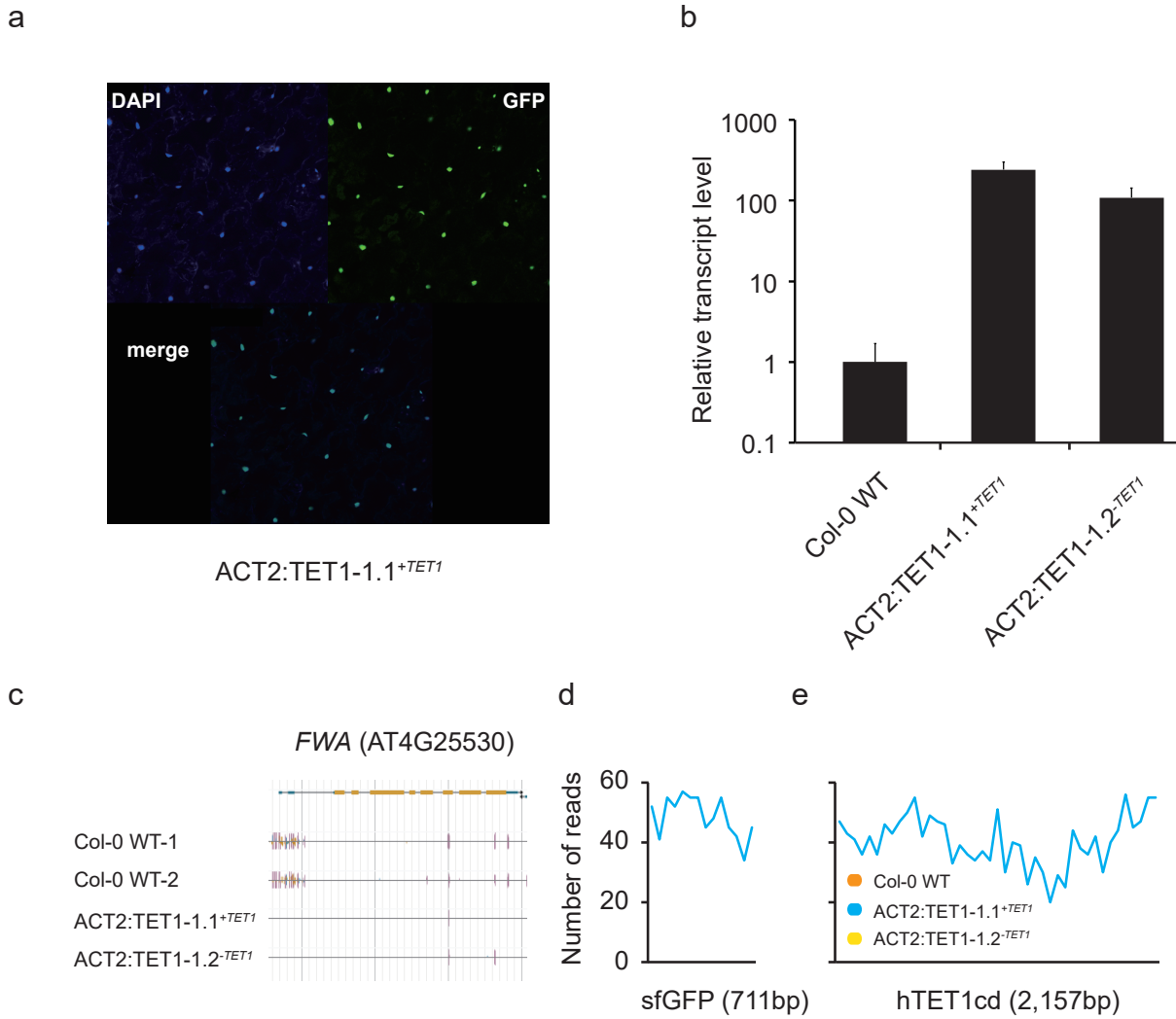
|                                 | Uniquely mapped reads | Non-clonal reads |
|---------------------------------|-----------------------|------------------|
| <i>35S:TET1-2</i> <sup>T6</sup> | 6,754,041             | 4,639,956        |



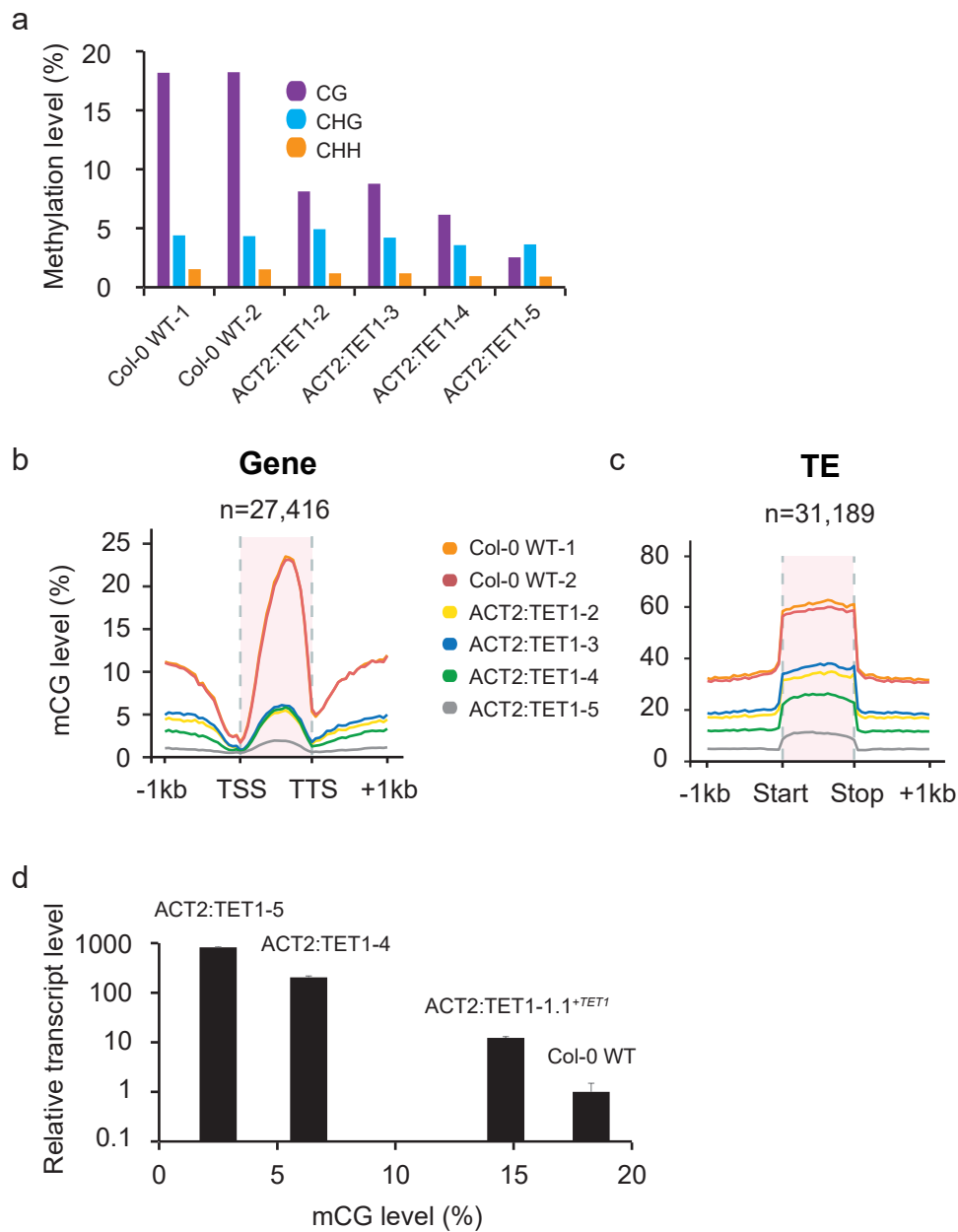
**Figure S1.** Global methylation (a) and 5hmC (b) levels of Col-0 WT plants and 35S:TET1 transgenic plants. Metagene plots of CHG (c and d) and CHH (e and f) methylation level across gene bodies and transposable elements. Frequency plots of mCHG (g) and mCHH (h) differences in CG DMRs that possess non-CG methylation in wild type. The average value of two Col-0 WT plants was defined as the Col-0 WT.



**Figure S2.** (a) Relative mRNA expression of *IBM1-L* and *IBM1-S* in Col-0 WT and 35S:TET1-2<sup>T6</sup> examined by RT-qPCR. Error bars indicate s.d. (b) Number of identified CHG DMRs in 35S:TET1 individuals. These DMRs were defined relative to Col-0 WT-1, as Col-0 WT-2 DMRs were used to assess background interference. (c) Bar plot of CHG methylation levels in identified CHG DMRs. Venn diagrams of upregulated genes (d) and downregulated genes (e) in two 35S:TET1 transgenic individuals and *met1*.



**Figure S3.** (a) Confocal microscopy of sfGFP-TET1cd fusion protein in young cotyledons. DAPI and GFP stains show the locations of nuclei and GFP signal, respectively. (b) Relative expression of *FWA* examined by RT-qPCR. Error bars indicate s.d. (c) Genome browser view of *FWA* (AT4G25530). Read distribution (50bp windows) in (d) sfGFP and (e) hTET1cd sequences indicate the absence of sfGFP-hTET1cd in Col-0 WT and ACT2:TET1-1.2<sup>-TET1</sup>.



**Figure S4.** (a) Bar plot of global methylation levels in two Col-0 WT replicates and four ACT2:TET1 T1 plants. Metagene plots of CG methylation level across (b) gene bodies and (c) transposable elements. (d) Relative expression of sfGFP-hTET1cd examined by RT-qPCR and corresponding mCG level indicate a relationship between transgene expression level and mCG loss. Error bars indicate s.d.