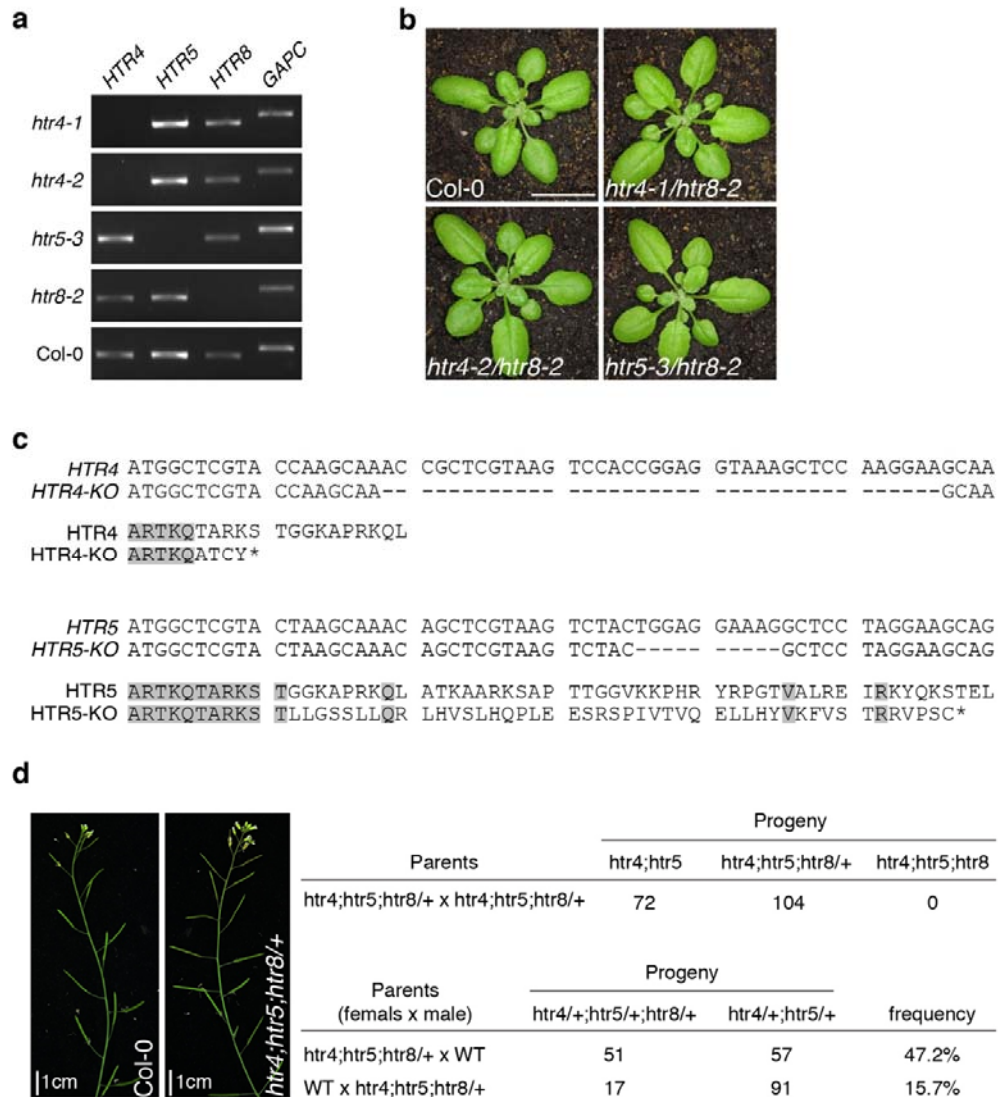


The histone H3 variant H3.3 regulates gene body DNA methylation in *Arabidopsis thaliana*

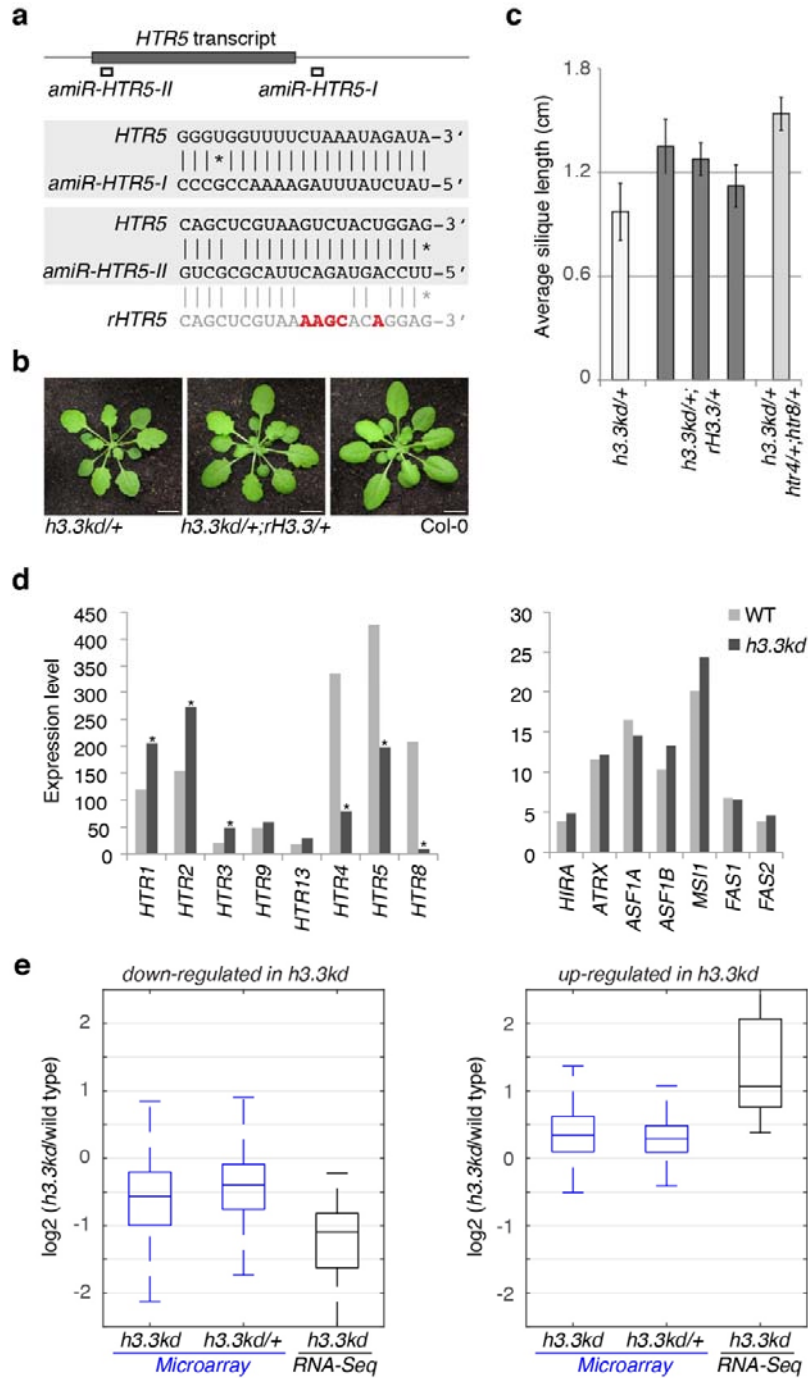
Additional file2: Supplemental Figures



Additional file 2 Figure S1: Generation of *h3.3KO*.

(a) RT-PCR analysis of full-length transcript (5' to 3' UTR) in *H3.3* T-DNA insertion lines. (b) Phenotypes of three different *H3.3* double mutant combinations compared to WT. (c) Partial gene and protein sequence of HTR4 and HTR5 in wildtype and *htr4;htr5* CRISPR/Cas9 double mutant. (d) Phenotype of Col-0 wildtype and *htr4;htr5;htr8/+* siliques and frequencies of progenies obtained from crosses.

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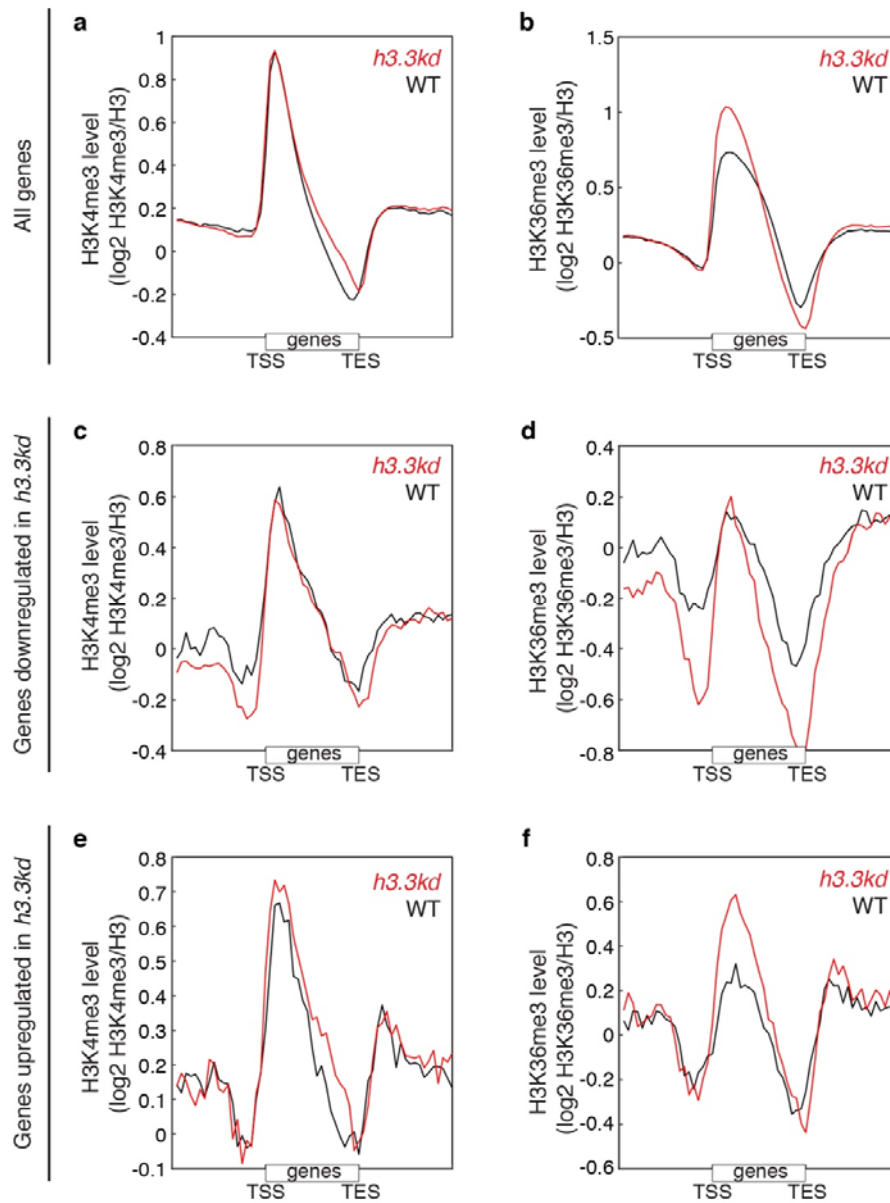
Additional file 2 Figure S2: Generation of *h3.3kd*.

(a) Sequences of the two artificial miRNAs *amiR-HTR5-I* and *amiR-HTR5-II* designed to target two different regions of the *HTR5* transcript (shown schematically on top).

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Design of *rHTR5* is shown at the bottom. Silent mutations within the *amiR-HTR5-II* target site ensure limited complementarity to the amiRNA. **(b, c)** Phenotypic rescue of *h3.3kd*-specific serrated leaf shape and smaller rosette size. Compared with *h3.3kd/+*, i.e. *htr4-2:htr8-2* double mutants with only one copy of the *amiR-HTR5-II* transgene (*htr4;htr8;amiR-HTR5-II/+*, left panel), a partial rescue of the reduced growth and leaf shape **(b)** and sterility measure by silique elongation **(c)** is observed in *h3.3kd/+;rH3.3/+*, i.e. *htr4-2:htr8-2* double mutants with one copy of the *amiR-HTR5-II* transgene and one copy of the *rHTR5* amiR-resistant *HTR5* transgene (*htr4;htr8;amiR-HTR5-II/+;rHTR5/+*, middle panel); three independent transgenic *rH3.3* lines. Average values reflect 10 siliques per plant from three or more plants for each genotype. **(d)** Expression levels of histone H3 variants and putative H3 chaperones from RNA-seq analysis of WT and *h3.3kd*. **(e)** Misexpression of genes in *h3.3kd* versus WT Col, downregulated genes (left panel) and upregulated genes (right panel). Trends from genes misexpressed in RNA-Seq profiles (black) are similar to microarray-based profiles (blue).

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Additional file 2 Figure S3: Impact of *h3.3kd* on active chromatin modifications.

(a) ChIP-seq profiles depicting the enrichment of H3K4me3 in WT and *h3.3kd* over all genes. (b) ChIP-seq profiles depicting the enrichment of H3K36me3 in WT and *h3.3kd* over all genes. (c, d) ChIP-seq profiles depicting the enrichment of H3K4me3 (c) and H3K36me3 (d) in WT and *h3.3kd* over genes downregulated in *h3.3kd*. (e, f) ChIP-seq profiles depicting the enrichment of H3K4me3 (e) and H3K36me3 (f) in WT and *h3.3kd* over genes upregulated in *h3.3kd*.