SUPPLEMENTARY DATA:

SUPPLEMENTARY METHODS:

Analysis of DNA methylation in centromeric repeats

500 ng of genomic DNA was digested overnight at 37 ºC by the methylation sensitive restriction endonuclease *Hpa*II (NEB, 10 U) and separated in a 1% (w/v) agarose gel. DNA was blotted onto HybondTM–XL membrane (GE Healthcare) and hybridised with a radioactively labelled pericentromeric 180–bp repeat as previously described (48). Hybridisation patterns were visualised using a phosphoimager FLA 7000 (FujiFilm).

Quantitative analysis of transcription

Total RNA was isolated from *A. thaliana* tissues using the RNeasy Plant Mini Kit (Qiagen) followed by DNaseI treatment (TURBO DNA-free, Applied Biosystems/Ambion). cDNA was prepared by reverse transcription of 1 μg of RNA using M-MuLV reverse transcriptase (NEB) and Random Nonamers (Sigma). Quantification of the *AtTERT, AtTER1, AtTER2, AtCTC1, AtTRB1*, *AtPOT1a*, *AtPOT1b* transcripts relative to the ubiquitin reference gene was done using FastStart SYBR Green Master (Roche) by the Rotorgene6000 (Qiagen) machine. Sequences of primers are shown in Table S1. Reactions were carried out in triplicate; the PCR cycle consisted of 15 min of initial denaturation followed by 40 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C. Analyses were performed on two technical replicates using at least three biological replicates (buds collected from 3 plants of respective genotype). Transcription was calculated as the fold increase/decrease relative to the wild type/control tissue (ΔΔCt method (73)).

Quantitative analysis of telomerase activity by the *in vitro* TRAP (Telomere Repeat Amplification Protocol) assay

Telomerase extracts from Arabidopsis tissues were prepared as described (58,74). The quantitative version of the TRAP assay was performed as described in (75) using FastStart SYBR Green Master (Roche) and TS21 and TELPR primers (Table S1). Samples were analysed in triplicate in 20-μl reactions, in two technical replicates using at least three biological replicates. Ct values were determined using the Rotorgene6000 (Qiagen) software and relative telomerase activity was calculated by the ΔCt method (73).

Analysis of TERRA transcripts

Telomeric transcripts were analysed according to the protocol, using specific primers for 2R, 3L and 5R chromosomal arms (Table S1) as described in (8).

SUPPLEMENTARY FUGURE LEGENDS:

Figure S1: Analysis of cytosine methylation in centromeric repeats in *met1-3* and *ddm1-8* mutant plants (A) and in plants germinated in the presence of hypomethylation drugs (B). DNA isolated from plant tissues was digested by methylation sensitive *Hpa*II and analysed by Southern hybridisation with a radioactively labelled centromeric repeat probe as previously described (48). A: DNA isolated from leaves of *met1-3* and *ddm1-8* mutant plants of G2 and G4 generations was subjected to analysis. Seven *ddm1-8* mutant plants of G2, two *met1-3* G2, three *ddm1-8* G4 and three *met1-3* G4 representatives were analysed. Restriction profiles of methylation mutants showed higher *Hpa*II digestibility as compared to the Columbia (Col; two plants analysed) wt plants, i.e. a lower level of methylated cytosines in CCGG sequences. he, plants heterozygous for T-DNA insertion; ho, plants homozygous for T-DNA insertion. In wild type plants segregated from the *met1-3* G3 mutant (seg wt; seven plants analysed) the methylation pattern partially or totally returned to the level in Columbia wt. B: DNAs isolated from 7-day-old seedlings and mature leaves of non-treated *A. thaliana* (CTR; leaves collected from four plants), *A. thaliana* germinated in the presence of 100 μM DHPA (D100; three plants), 250 μM DHPA (D250; four plants), 100 μM zebularine (Z100; four plants), 250 μM zebularine (Z250; four plants) and in the progenies of plants treated with 250 μM zebularine (Z250 G2; four plants) were analysed. In seedlings, methylation in treated samples was significantly lower as compared to the control; in mature leaves, restoration of the methylation pattern was observed in DHPA-treated samples while in zebularine-affected plants inter-individual variability was observed; some plants maintained hypomethylated cytosines even in progenies of zebularine treated specimens (Z250 G2).

Figure S2: Analysis of cytosine methylation in the proximal part of the 1L telomere arm in seedlings germinated in the presence of hypomethylation drugs. A: Distribution of methylated cytosines along the 389-bp region, which is delimited by primers derived from the subtelomeric region and a specific insertion in the 1L telomere (8). 5 clones from seedlings

germinated on the control media (CTR), 5 clones from seedlings germinated in the presence of 250 μM DHPA (D250) and 5 clones from seedlings germinated in the presence of 250 μM zebularine (Z250) were analysed. Red circles, CG methylation; blue squares, CHG methylation; green triangles, CHH methylation; filled symbols, methylated cytosine; empty symbols, non-methylated cytosine. Positions of cytosines located in perfect telomeric repeats are delimited by the black lines below the figure. The arrowhead determines the direction to the perfect telomere. B: Graphical representation of the level of all methylated cytosines located in non-symmetrical sequences (all CHH), in perfect telomeric repeats (tel CHH) and outside of these repeats (non tel CHH). Standard deviations reflect the variability between clones. Essentially the same results were obtained in three independent experiments.

Figure S3: Transcription of the *AtTERT* gene (A) and telomerase activity (B) in buds of *met1-3* and *ddm1-8* mutant plants. Transcription of the gene encoding the catalytic subunit of telomerase, and telomerase activity, were determined by quantitative RT-PCR and TRAP assays, respectively. Buds from three representatives of each group were collected for analysis and results were expressed relative to the Columbia wt individuals. Col, Columbia wt plant; G2 and G4, the second and the fourth generation of mutant plants.

Figure S4: TERRA transcripts in plants germinated in the presence of hypomethylation drugs. Levels of TERRA transcripts were analysed in buds of plants germinated on the control medium (CTR), in the presence of 250 μM DHPA (D250) and in the presence of 250 μM zebularine (Z250), using RT-PCR and specific primers for 2R, 3L and 5L chromosome arms (8). Analysis of the ubiquitine transcript is presented as a control of the template cDNA loading. DNA, positive control, PCR with genomic DNA from *A. thaliana* leaves; -, negative control, no-template reaction.

REFERENCES IN SUPPLEMENTS

8. Vrbsky, J., Akimcheva, S., Watson, J.M., Turner, T.L., Daxinger, L., Vyskot, B., Aufsatz, W. and Riha, K. (2010) siRNA-mediated methylation of Arabidopsis telomeres. *PLoS Genet.*, 6, e1000986.
48. Saze, H., Mittelsten Scheid, O. and Paszkowski, J. (2003) Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nat. Genet.*, 34, 65-69.

58. Fitzgerald, M.S., McKnight, T.D. and Shippen, D.E. (1996) Characterization and developmental patterns of telomerase expression in plants. *Proc. Natl. Acad. Sci. U S A*, 93, 14422-14427.
59. Cifuentes-Rojas, C., Kannan, K., Tseng, L. and Shippen, D.E. (2011) Two RNA subunits and POT1a are components of Arabidopsis telomerase. *Proc. Natl. Acad. Sci. U S A*, 108, 73-78.
73. Pfaffl, M.W. (2004) In Bustin, S. A. (ed.), *A-Z of quantitative PCR*. International University Line, La Jolla, CA, USA, pp. 87-112.
74. Sykorova, E., Lim, K.Y., Chase, M.W., Knapp, S., Leitch, I.J., Leitch, A.R. and Fajkus, J. (2003) The absence of Arabidopsis-type telomeres in Cestrum and closely related genera Vestia and Sessea (Solanaceae): first evidence from eudicots. *Plant J.*, 34, 283-291.
75. Herbert, B.S., Hochreiter, A.E., Wright, W.E. and Shay, J.W. (2006) Nonradioactive detection of telomerase activity using the telomeric repeat amplification protocol. *Nat. Protoc.*, 1, 1583-1590.
76. Fojtova, M. Peska, V., Dobsakova, Z., Mozgova, I., Fajkus, J. and Sykorova, E., (2011) Molecular analysis of T-DNA insertion mutants identified putativeregulatory elements in the AtTERT gene. *J. Exp Bot.*, 62, 25531-5545.
77. Dvorackova, M., Rossignol, P., Shaw, P.J., Koroleva, O.A., Doonan, J.H. and Fajkus, J. (2010) AtTRB, a telomeric DNA-binding protein from Arabidopsis, is concentrated in the nucleolus and shows highly dynamic association with chromatin. *Plant J.*, 61, 637-649.

PRIMER	SEQUENCE (5´→ 3´direction)	METHOD	NOTE
ddm1Fw[1]	TTCGAAAGAACTGCAACCATC	*ddm1-8* genotyping	
ddm1Rev[1]	AGCTTCAGCAGCTTGTGTCTC	*ddm1-8* genotyping	
SALK[1,3]	CAACACTCAACCCTATCTCGG	*ddm1-8*, *tert-/-* genotyping	
met1Fw[2]	GATTGTGTCTCTACTACAGAGGC	*met1-3* genotyping	
met1Rev[2]	GTTAAGCTCATTCATAGCCTTGC	*met1-3* genotyping	
barbiG[2]	GGTTCTTATAGGGTTTCGCTC	*met1-3* genotyping	
9exFw[3]	CTTTATTTGGGCCATCTGTAGGTAC	*tert-/-* genotyping	(76)
9exRev[3]	TGATTCACTGTGCATTTCTCATTTC	*tert-/-* genotyping	(76)
TS21	GACAATCCGTCGAGCAGAGTT	qTRAP	
TELPR	CCGAATTCAACCCTAAACCCTAAACCCTAAACCC	qTRAP	
AtTERTex1Fw	CCGATGATCCCATTCACTACCGTAAACT	*AtTERT* transcription	(76)
AtTERTex1Rev	TCTCTGTGACCACCAAGATGTTGGAGA	*AtTERT* transcription	(76)
AtTER1Fw	CCCATTTCGTGCCTATCAGACGAC	*AtTER1* transcription	(59)
AtTER1Rev	TCTCCGACGACCATTCTCTCGATA	*AtTER1* transcription	(59)
AtTER2Fw	GACGACAACTAAACCCTACGCTTACA	*AtTER2* transcription	(59)
AtTER2Rev	CAGGATCAATCGGAGAGTTCAATCTC	*AtTER2* transcription	(59)
AtPOT1aFw	TGTCGCAACAATGATTGGAT	*AtPOT1a* transcription	
AtPOT1aRev	TCGTCGTGGTTCTTGATTTG	*AtPOT1a* transcription	
AtPOT1bFw	ATGGGTCATGCTGTTTCACA	*AtPOT1b* transcription	
AtPOT1bRev	TTCCATCCCAAACAAAGAGG	*AtPOT1b* transcription	

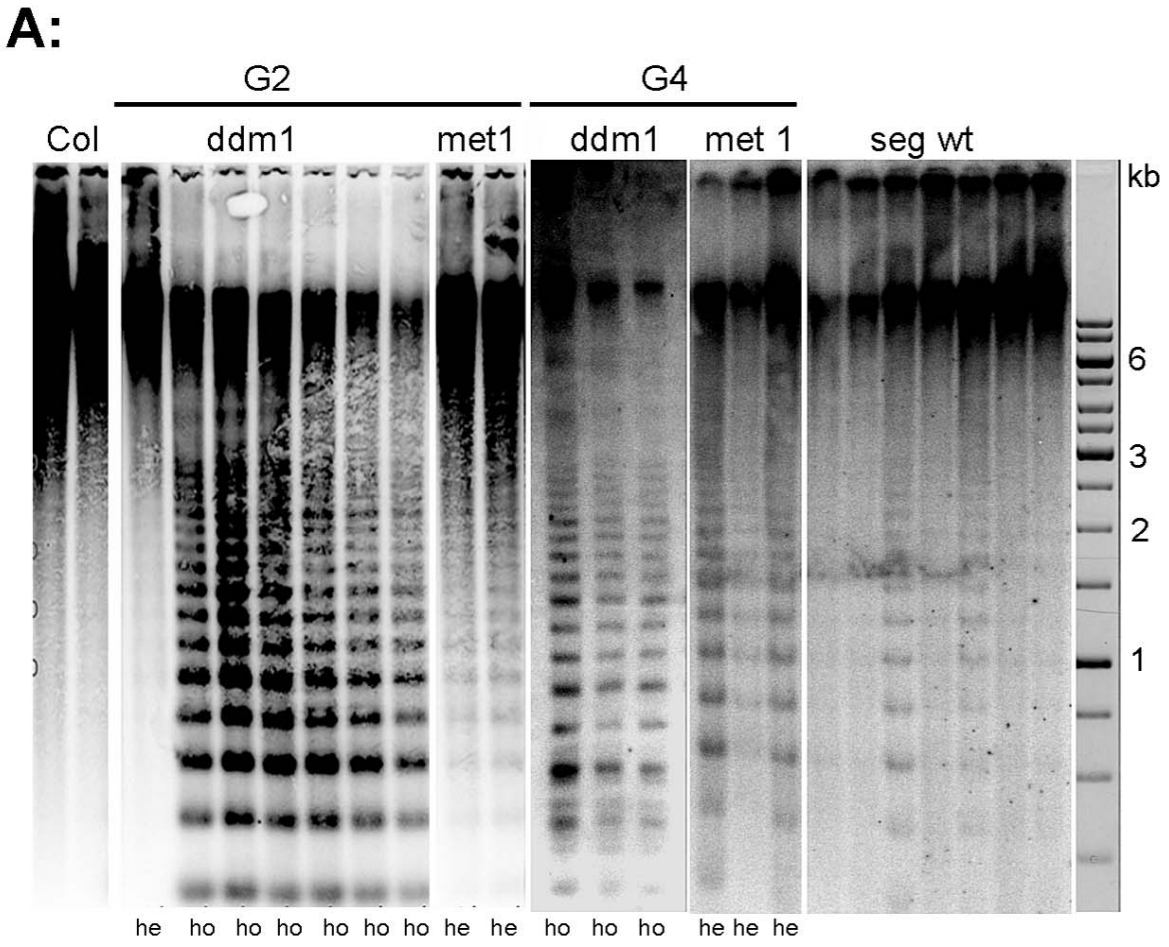
AtCTC1Fw	GCCCTGTGTCTGTTGTTCCT	*AtCTC1* transcription	
AtCTC1Rev	CAACTCGCAGCCTCTAAACC	*AtCTC1* transcription	
AtTRB1 A Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATG GGTGCTCCTAAGCAGAAA	*AtTRB1* transcription	(77)
AtTRB1 B Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTTAG GAAGGGAGAACGTCCTTTT	*AtTRB1* transcription	(77)
Tel21	ACCCTAAACCCTAAACCCTAA	TERRA reverse transcription	
2R-2	CTCCTAAAATATTTGCTAAGTAAATTGTCCAAC	TERRA transcription	(8)
2R-turn	TCTAGACTCATACAAAATTTTATGGTTA	TERRA transcription	(8)
3L-5	CCCTAATCTTTAGTTCCTAGACCCTAAATC	TERRA transcription	(8)
3L-6	CAACACAGAGAAGAAACAAGAGAAAG	TERRA transcription	(8)
5L-2	TTGCATAAAGCGTCACGTATAA	TERRA transcription	(8)
5L-10	CTGCCGCAAGCATGGGCTTG	TERRA transcription	(8)
ubqFw	AACGGGAAAGACGATTAC	ubiquitine transcription	
ubqRev	ACAAGATGAAGGGTGGAC	ubiquitine transcription	
1L-0 BISFw	TCCAAACATAAACARTCCAAACAA	methylation of telomeric cytosines	(8)
1L-0 BISRev	ATTTTTYYATAGGATAGATAGGG	methylation of telomeric cytosines	(8)

Table S1: Sequences of primers used in genotyping the mutant lines, telomerase activity assay, analyses of transcription and analysis of telomeric cytosines methylation by bisulfite genomic sequencing.

[1], wild type – PCR product using ddm1Fw + ddm1Rev primers; mutant – PCR product using SALK + ddm1Rev primers.

[2] , wild type – PCR product using met1Fw + met1Rev primers; mutant – PCR product using met1Fw + barbiG primers.

[3], wild type – PCR product using 9exFw + 9exRev primers; mutant – PCR product using 9exFw + SALK primers.


A:
G2
G4
Col
ddm1
met1
ddm1
met 1
seg wt
kb
6
3
2
1
he ho ho ho ho ho ho he he ho ho ho he he he

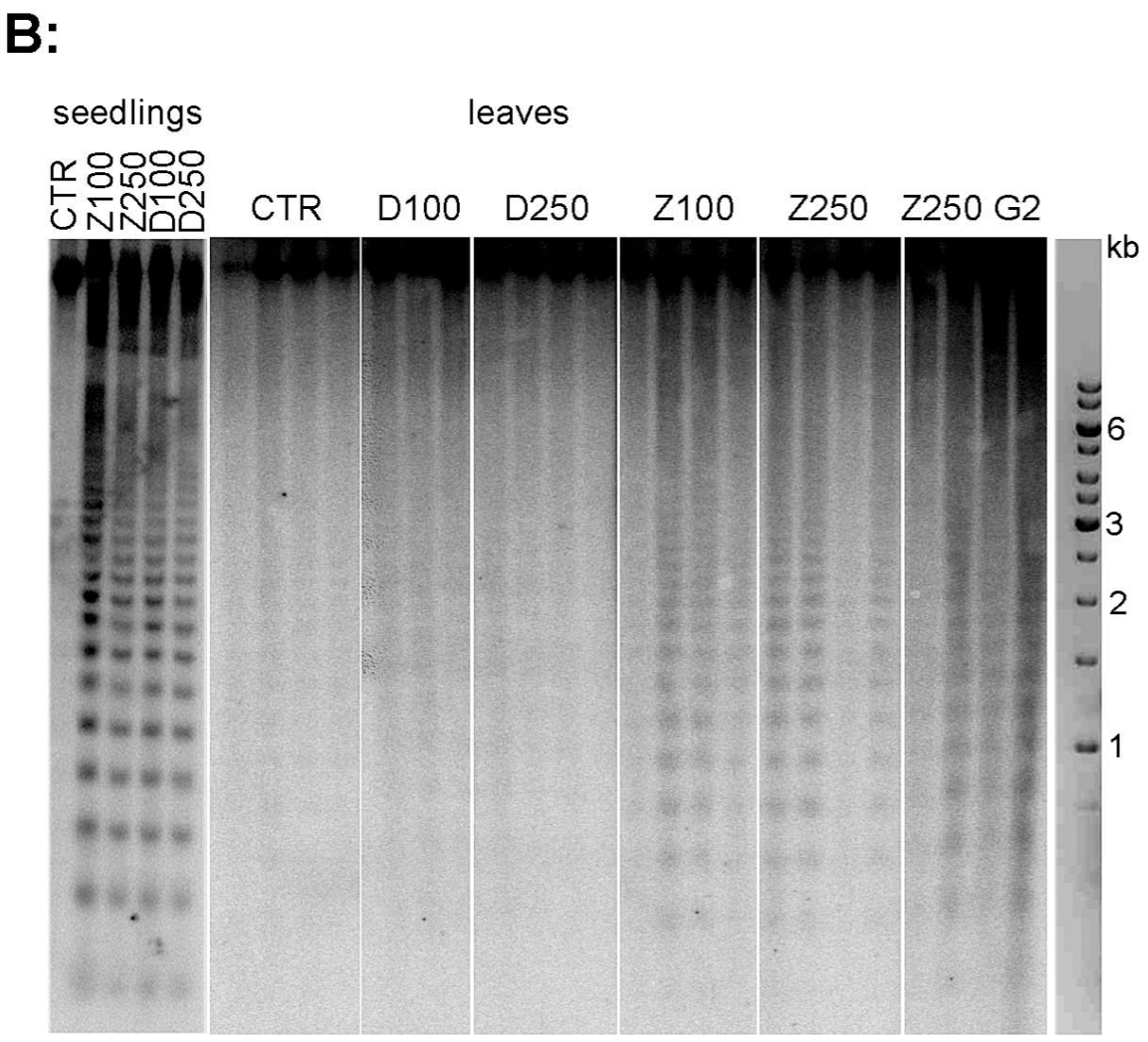
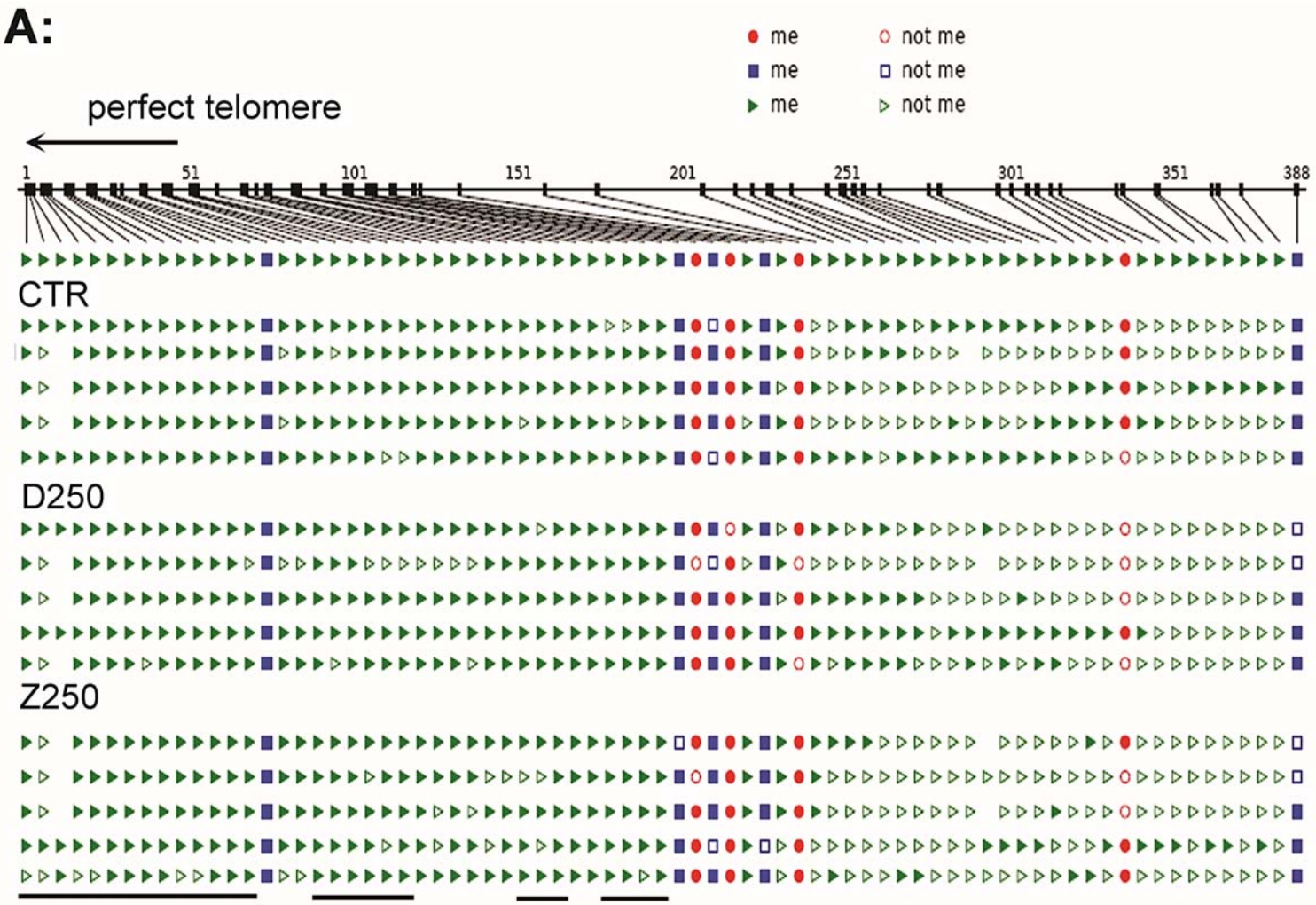

B:
seedlings
leaves
CTR
Z100
Z250
D100
D250
CTR
D100
D250
Z100
Z250
Z250 G2
kb
6
3
2
1

Figure S1


A:
me
not me
me
not me
me
not me
perfect telomere
1
51
101
151
201
251
301
351
388
CTR
D250
Z250

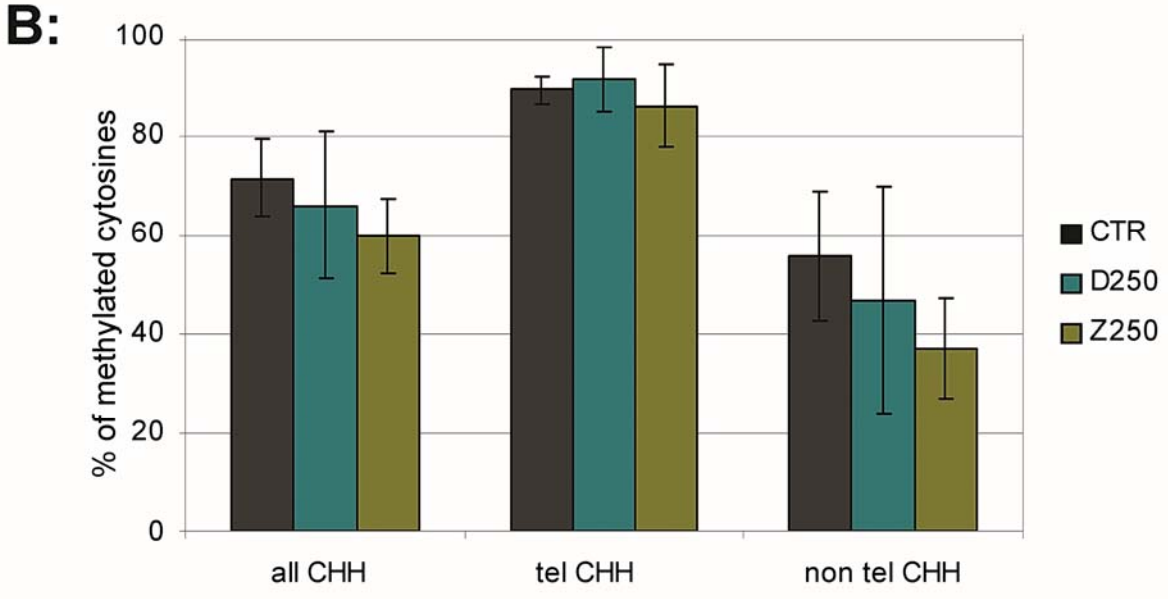
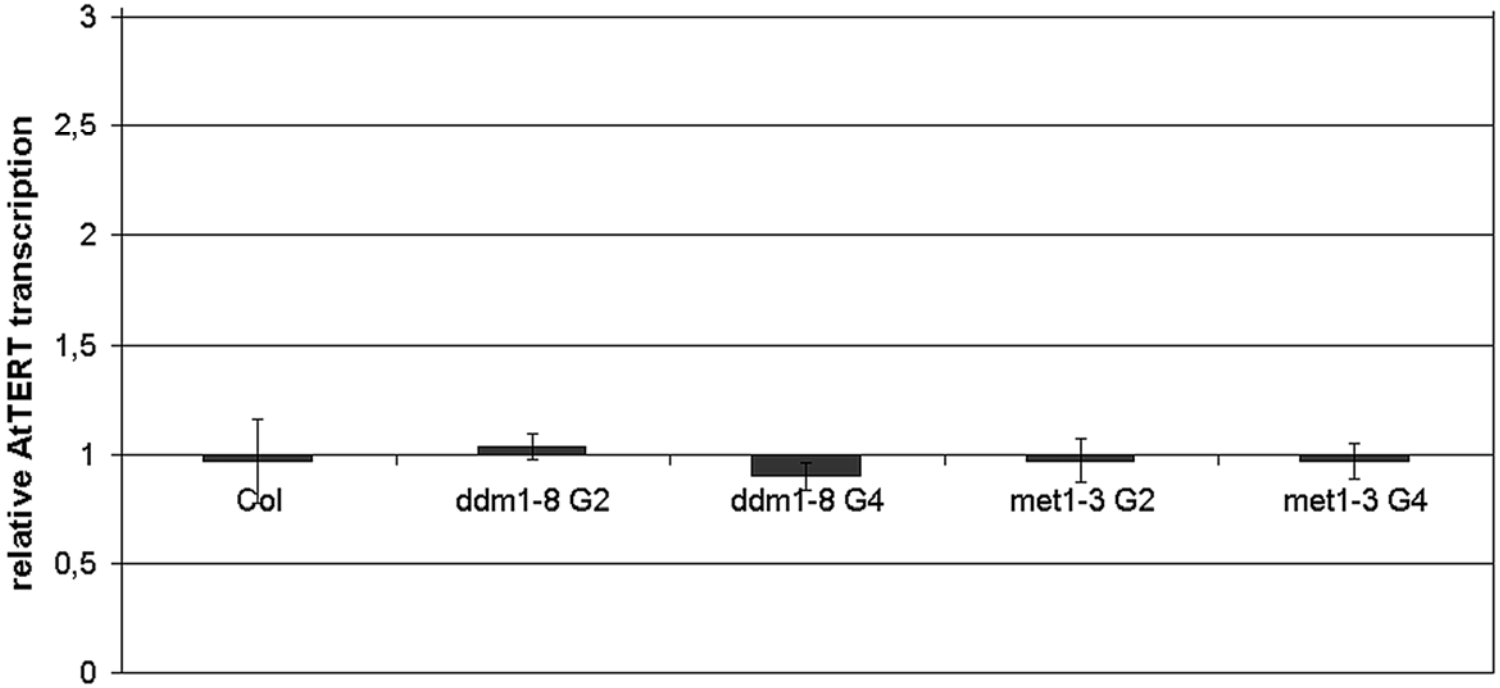

B:
100
80
60
40
20
0
% of methylated cytosines
all CHH
tel CHH
non tel CHH
CTR
D250
Z250

Figure S2

A:


relative AtTERT transcription
3
2,5
2
1,5
1
0,5
0
Col
ddm1-8 G2
ddm1-8 G4
met1-3 G2
met1-3 G4

B:

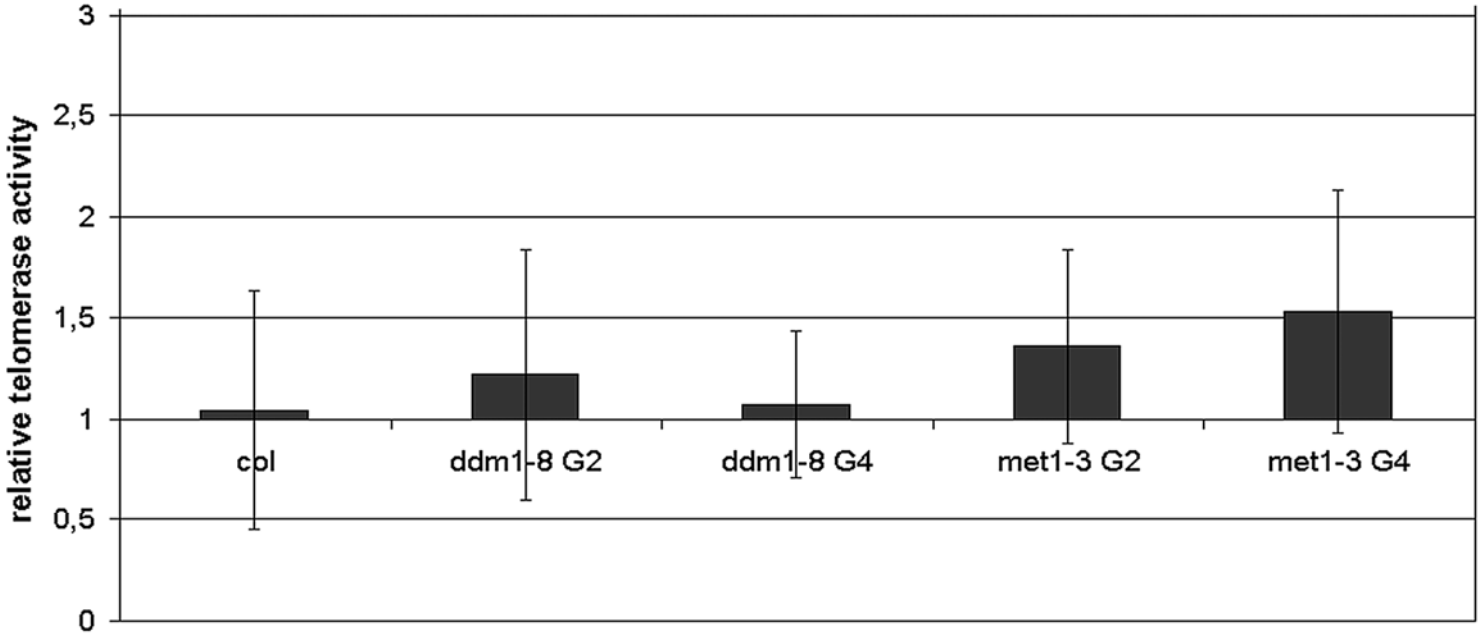

relative telomerase activity
3
2,5
2
1,5
1
0,5
0
col
ddm1-8 G2
ddm1-8 G4
met1-3 G2
met1-3 G4

Figure S3

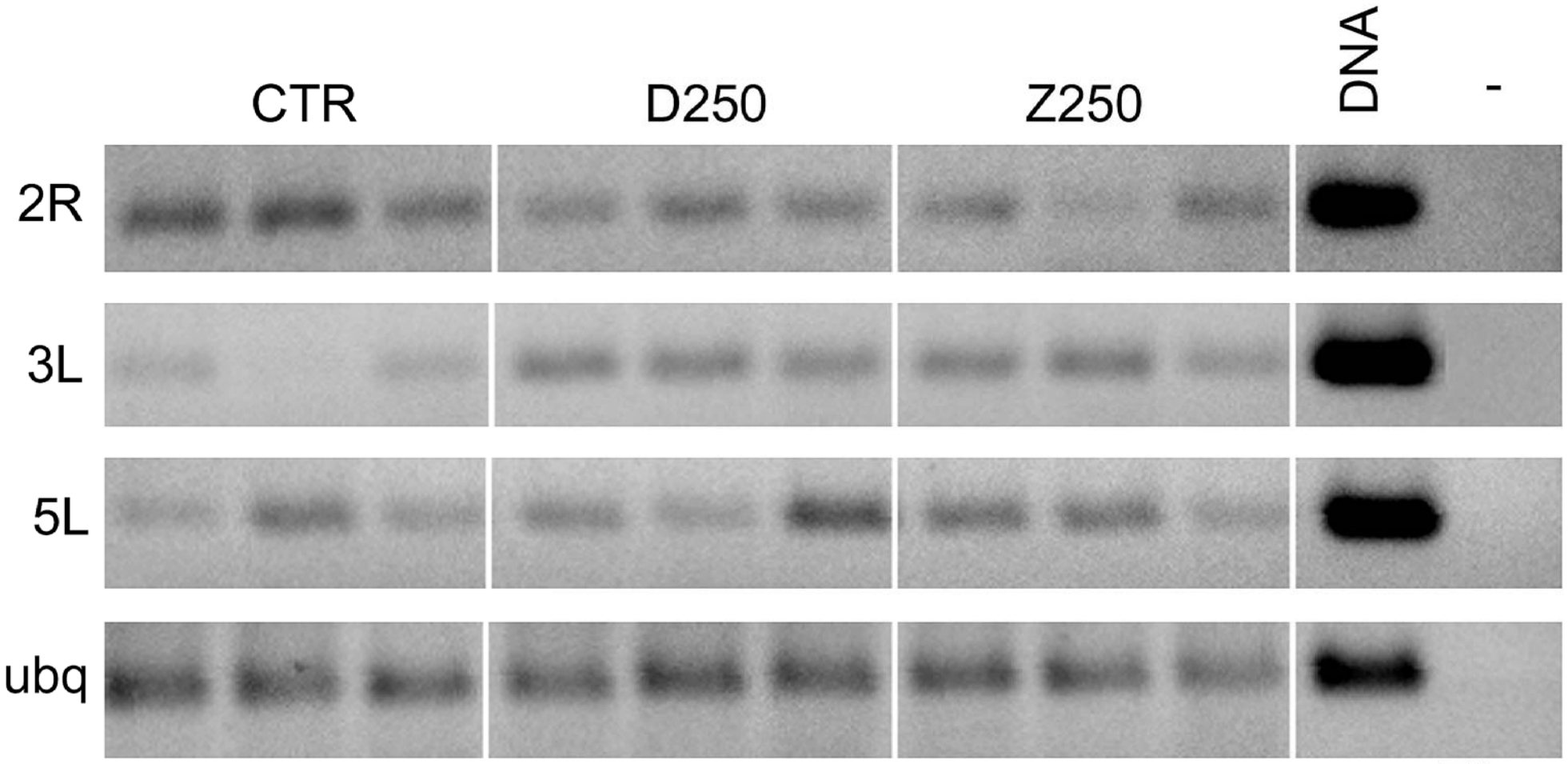


Figure S4