APPENDIX

for

Telomeric epigenetic response mediated by Gadd45a regulates stem cell aging and lifespan

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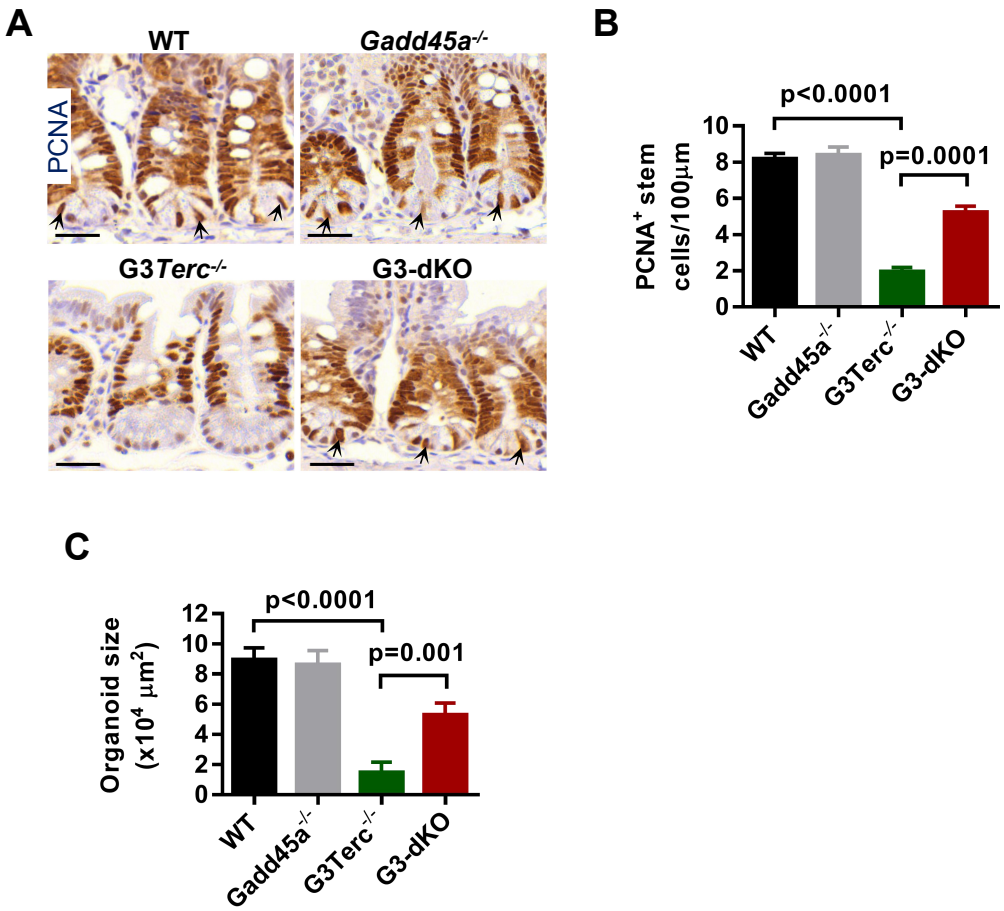
Table of Contents

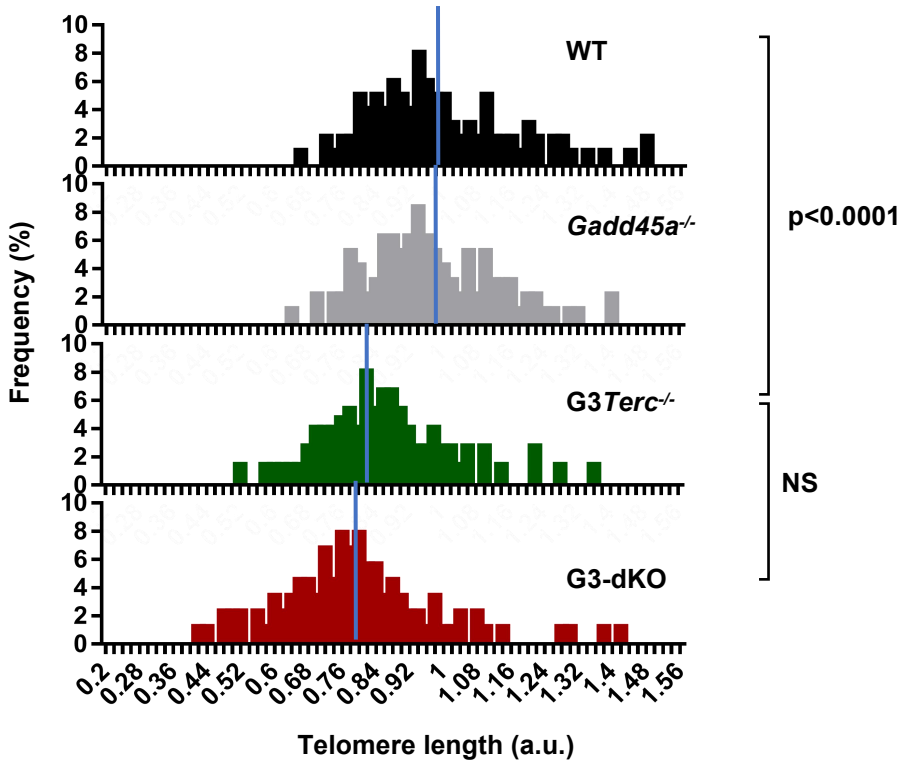


Appendix Figure S1. Gadd45a deletion improves intestinal stem cell maintenance and function in G3Terc$^{-/-}$ mice.

(A) Representative images of PCNA antibody staining indicating the intestine stem cells (ISC, arrows) at the bottom of small intestine crypts.

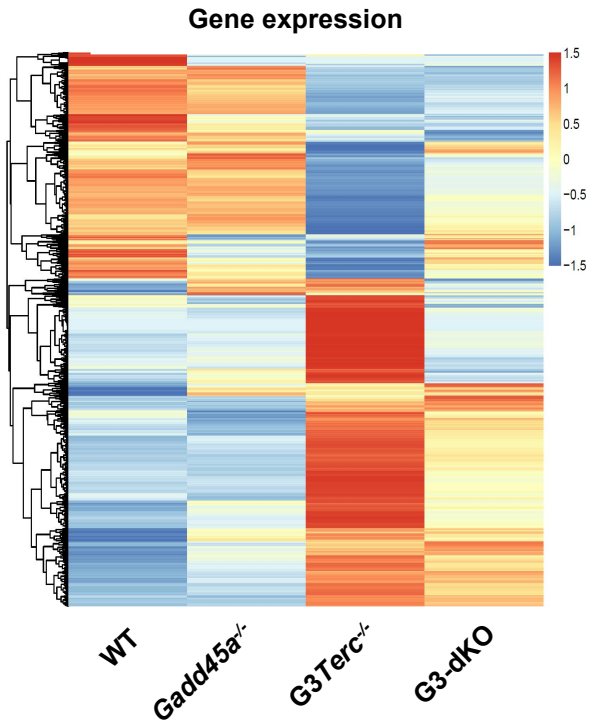
(B) Quantification of PCNA positive ISCs per 100 µm of small intestines (n=4-5 mice/group).

(C) The size of organoids on day 8 of culture (n=4-5 mice per genotype). Note: Scale bars represent 25 µm for Appendix Fig. S1A. Data are presented as mean±s.e.m.



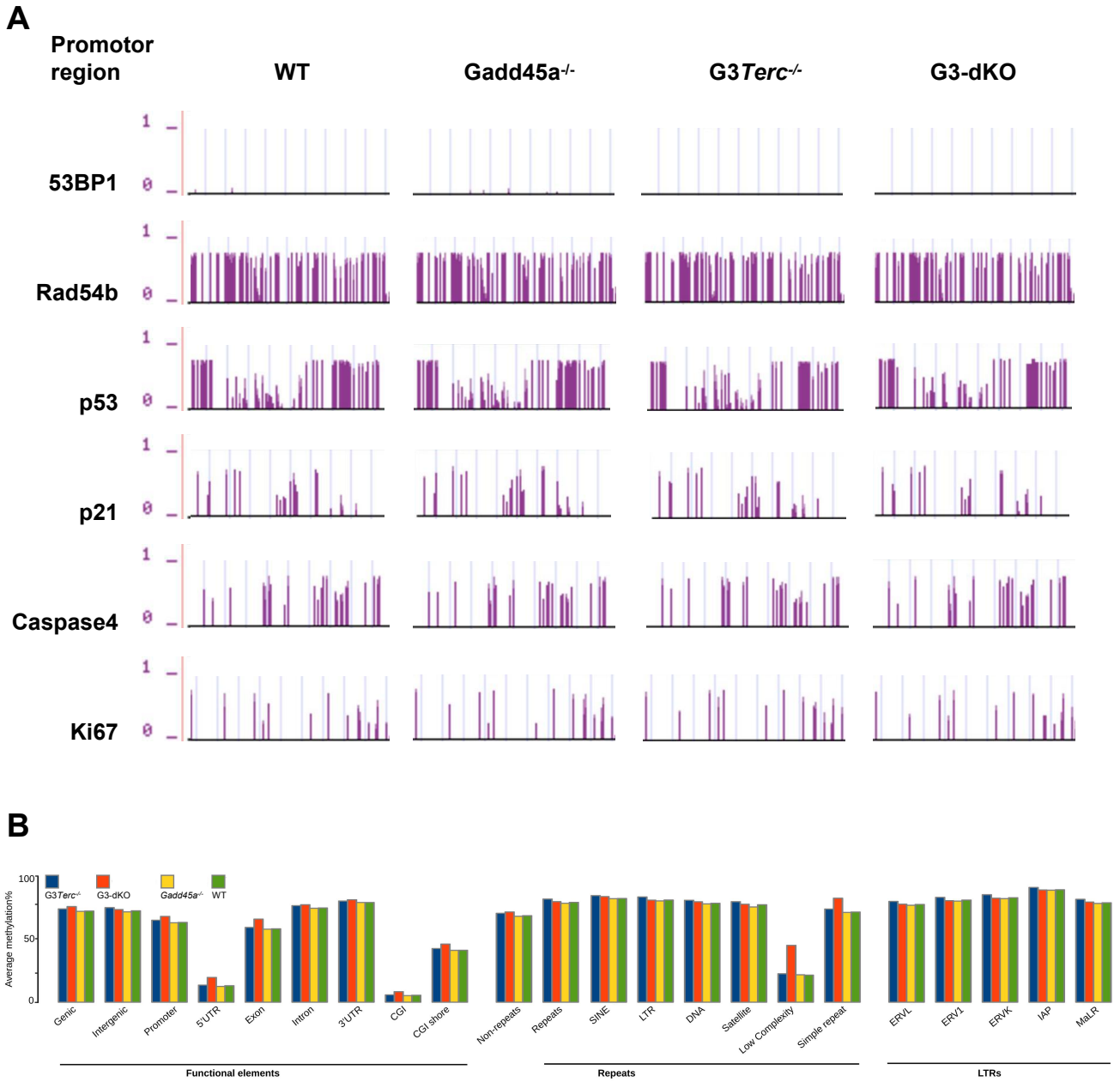
Appendix Figure S2. Gadd45a deficiency does not elongate telomere length in G3Terc$^{-/-}$ mice.

Telomere length profile represented by telomere signal intensity (200 cells per genotype were counted, n=4 mice per genotype; a.u., arbitrary units).



Appendix Figure S3. Gadd45a deletion partially reverses telomere dysfunction induced transcriptional changes.

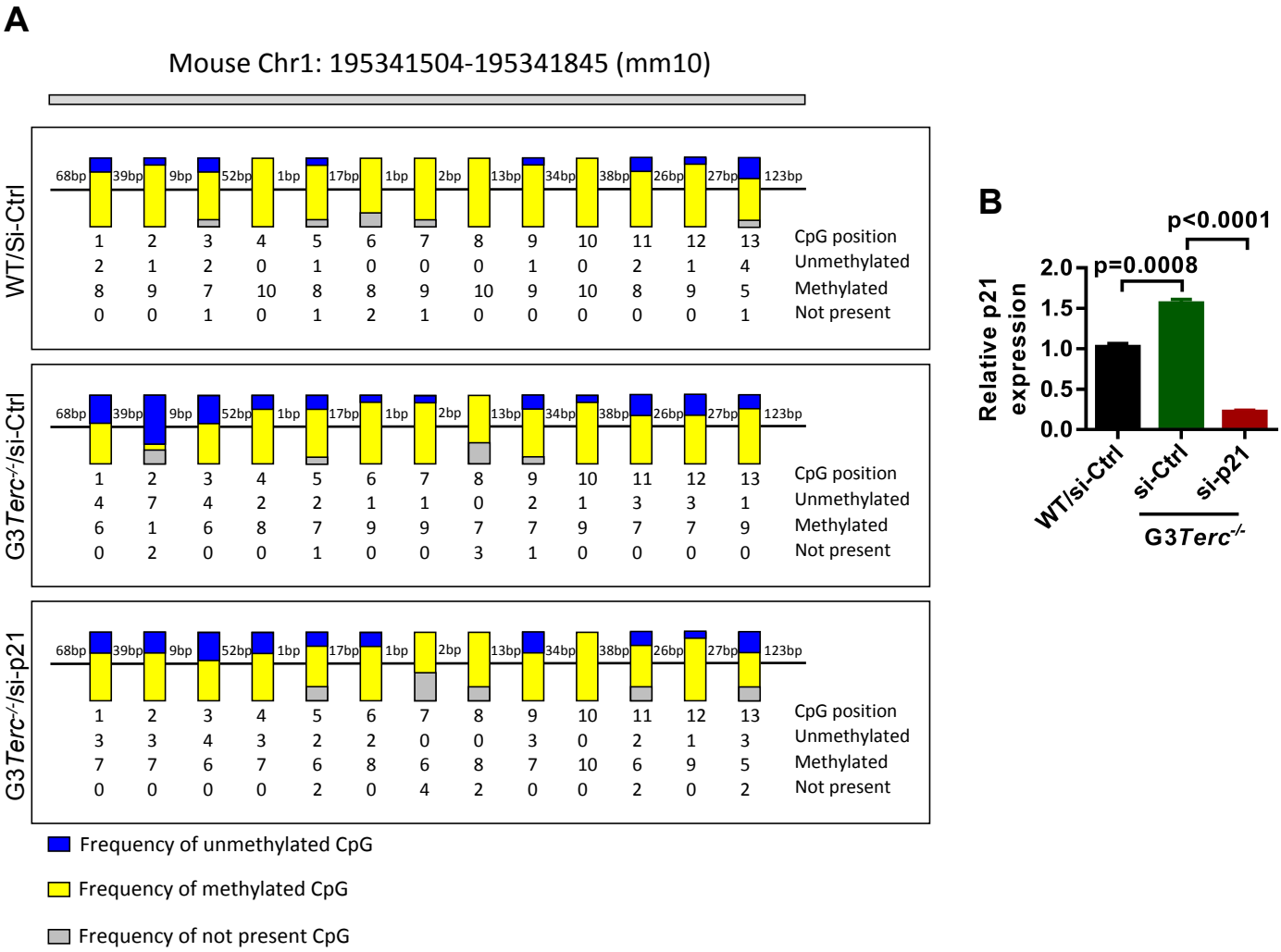
Heatmap shows gene expression profiles from MEFs with indicated genotypes. Red and blue colours depict high and low gene expression levels, respectively, based on the log2 P-values of their RPKMs.



Appendix Figure S4. Gadd45a deficiency does not change DNA methylation in the promotor region of DDR related genes.

(A) The DNA methylation level in the promotor region of DDR related genes in MEF cells with indicated genotypes.

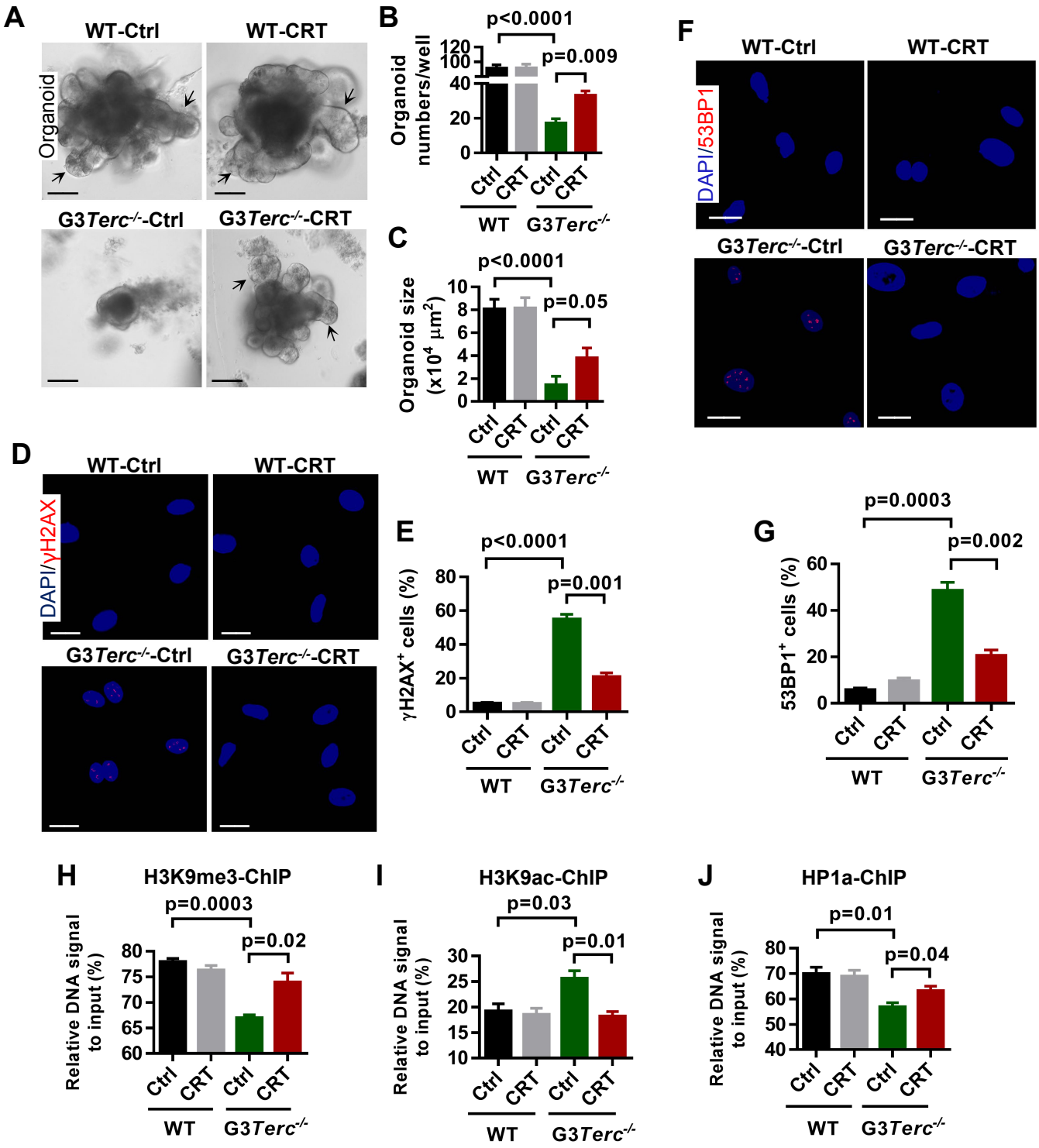
(B) The DNA methylation status of non-telomeric repetitive elements.



Appendix Figure S5. p21 knockdown does not change DNA methylation in the subtelomeric region of G3Terc$^{-/-}$ mice.

(A) The methylation status of CpG island in subtelomeric regions on chromosome 1q. gDNAs from MEF cells treated with and without p21 siRNA were PCR amplified and analysed by bisulfite sequencing. Yellow and blue bars indicate the frequencies of methylated and unmethylated CpG islands, respectively, at each position.

(B) Efficiency of p21 silence by siRNA in G3*Terc*$^{-/-}$ MEF cells.



Appendix Figure S6. APE1 inhibition rescues intestine stem cell impairment in G3Terc$^{-/-}$ mice.

(A) Representative images of small intestine organoids from WT and G3Terc-/- mice incubated with or without APE1 inhibitor (CRT0044876, 100μM) treatment. Arrows indicate the crypt-like structure in organoid cultures.

(B-C) Quantification of organoid number (per well) and size (in 10^4 μm^2) from crypt cultures (n=3-4 mice per genotype/treatment).
(D) Representative images of γH2AX antibody staining on dissociated organoid cells from WT and G3*Terc*$^{-/-}$ organoid cultures with or without APE1 inhibitor (CRT0044876, 100μM) (DNAs are stained with DAPI).
(E) Quantification of γH2AX positive cells (n=3-4 mice per group).
(F) Representative images of 53BP1 antibody staining on dissociated organoid cells from WT and G3*Terc*$^{-/-}$ crypt cultures with or without APE1 inhibitor (CRT0044876, 100μM) (DNAs were stained with DAPI).
(G) Quantification of 53BP1 positive cells (n=3-4 mice per group).
(H-J) Quantification of H3K9me3-ChIP (panel H), H3K9ac-ChIP (panel I), and HP1a-ChIP (panel J) values as percentage of the telomeric DNA levels, MEF cells were treated with or without APE1 inhibitor (CRT0044876, 100μM) before CHIP assay.
Note: Scale bars represent 60 μm for Appendix Fig. S6A and 5 μm for Appendix Fig. S6D and F. Data are presented as mean±s.e.m.

	Paraffin block No.	Patient No.	Gender	Age (y/o)
Young	2892	3247171	male	30
	2720	3142790	male	45
	2865	3220174	male	45
	2870	3231739	male	50
	2783	3178899	female	33
	2653	3099630	female	41
	2893	3242481	female	44
	2695	3131473	female	44
Old	2708	2023972	male	85
	2697	3110981	male	84
	2639	2021719	male	82
	2633	3073271	male	82
	2730	3147062	female	82
	2869	3223800	female	83
	2657	3085289	female	84
	2880	3214092	female	80

Appendix Table S1. Source of human colon samples.
Human colon samples were separated into young and old groups, each group contains 8 person which incudes 4 male and 4 female.