

Supplementary Materials

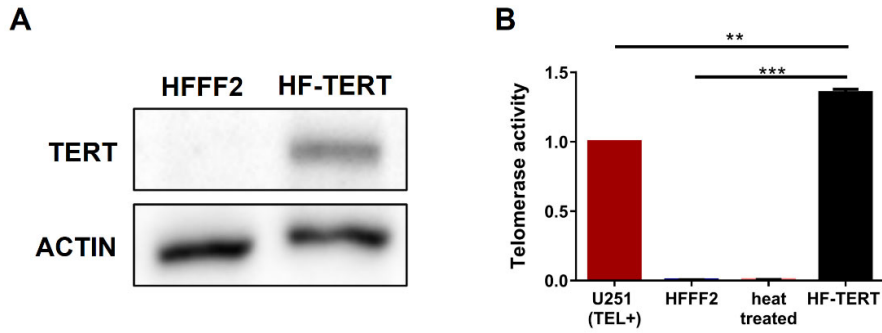


Figure S1. Efficiency of hTERT transduction in HFFF2 cell lines. (A) Western blot of total cellular proteins from HFFF2 and HF-TERT analyzed using anti-TERT antibody (upper panel) as well as anti-actin antibody (lower panel). (B) Telomerase activity measured by RQ-TRAP assay in HF-TERT fibroblasts compared to their normal counterparts and telomerase tumor cell lines (U251). U251 are used as positive control and heat-treated HF-TERT and HFFF2 cell lines are used as negative control. The error bars indicate the mean \pm SEM. Statistical analysis is performed both between normal and transduced cells and between U251 and transduced cells. ** $p < 0.01$; *** $p < 0.001$ by t -test.

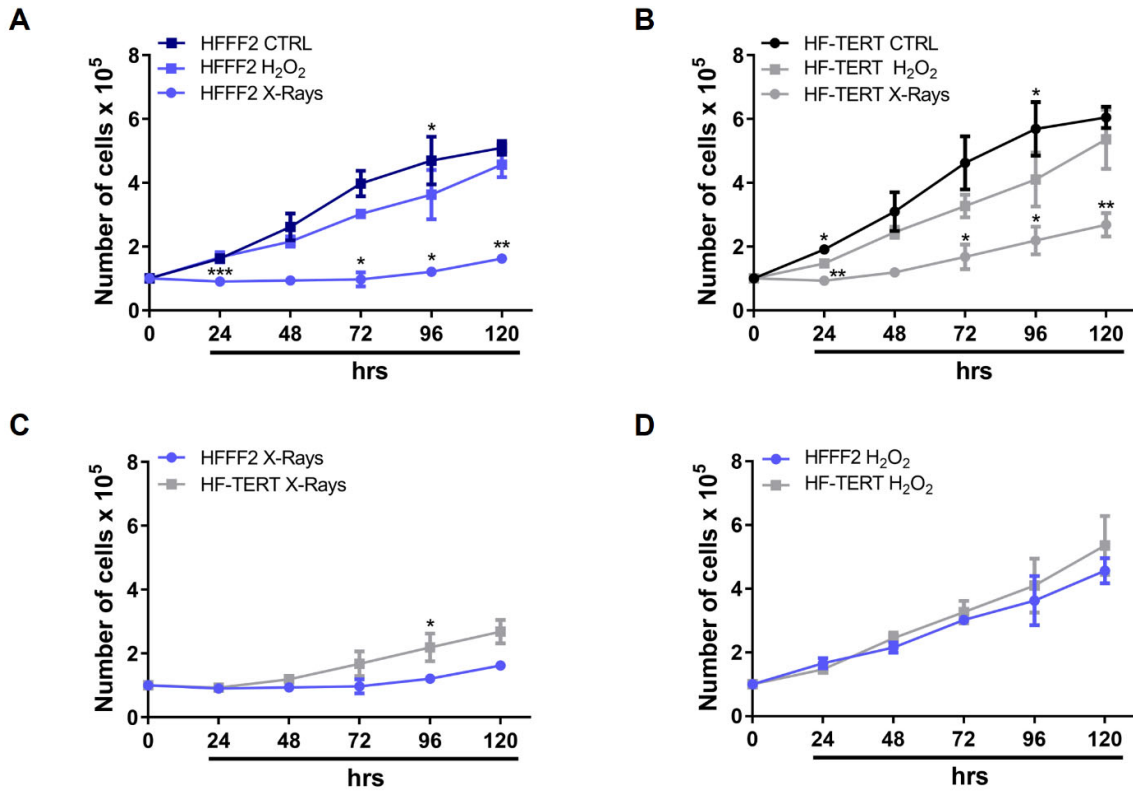


Figure S2. Growth curves after X-rays and H₂O₂ treatment. Cells were immediately seeded after treatment and were counted every 24 h until 120 h. (A,B) The graphs display the growth curves for HFFF2 and HF-TERT cells after 4 Gy of irradiation and 1 hour of hydrogen peroxide treatment. The error bars indicate the mean \pm standard error of the mean (SEM). Statistical analysis is performed between treated and untreated cells. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by t -test. (C,D) Growth curves of treated cells after X-ray irradiation and H₂O₂ treatment. The error bars indicate the mean \pm SEM. Statistical analysis is performed between normal and transduced cells. * $p < 0.05$; by t -test.

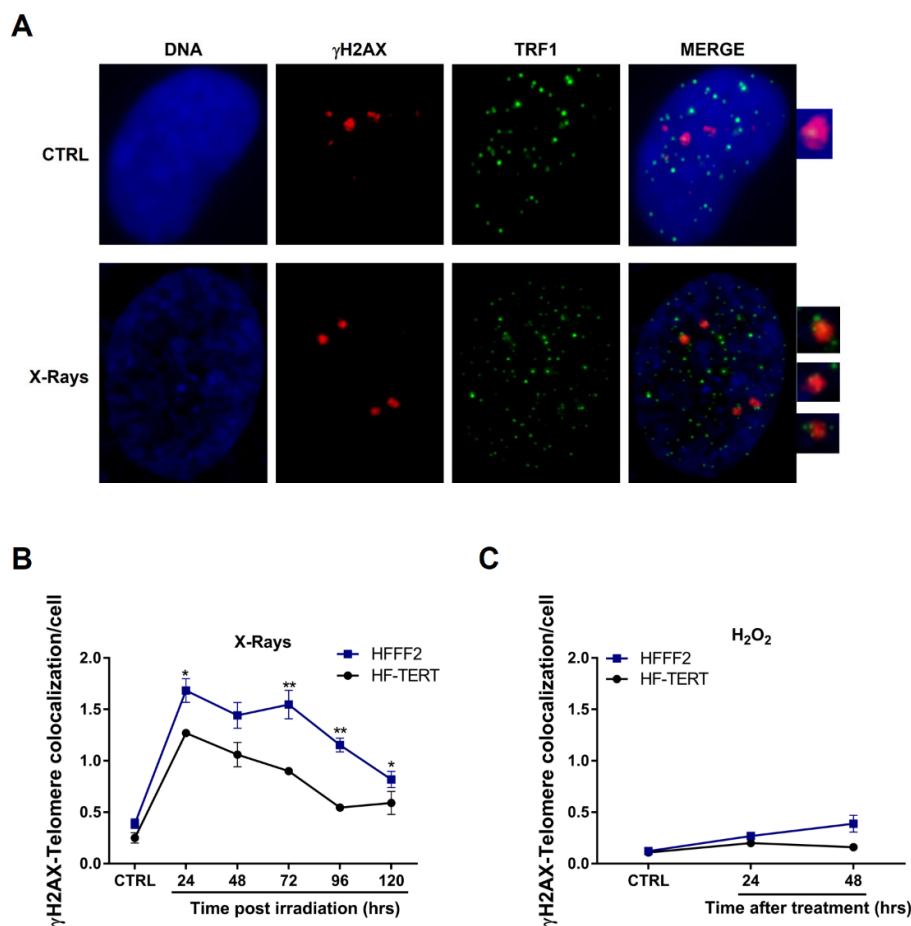


Figure S3. Immunofluorescence of telomere dysfunction-induced foci (TIF) between γ H2AX foci (red spot) and telomeric protein TRF1 (green spot). **(A)** The enlarged images show additional foci at telomeres from this and other nuclei. **(B,C)** Comparisons of γ -H2AX/TRF1 colocalization in both cell lines after both treatments. Bars represent the mean percentage \pm SEM. Statistical analysis is performed between HFFF2 and H-TERT treated samples. * $p < 0.05$; ** $p < 0.01$; by t-test.

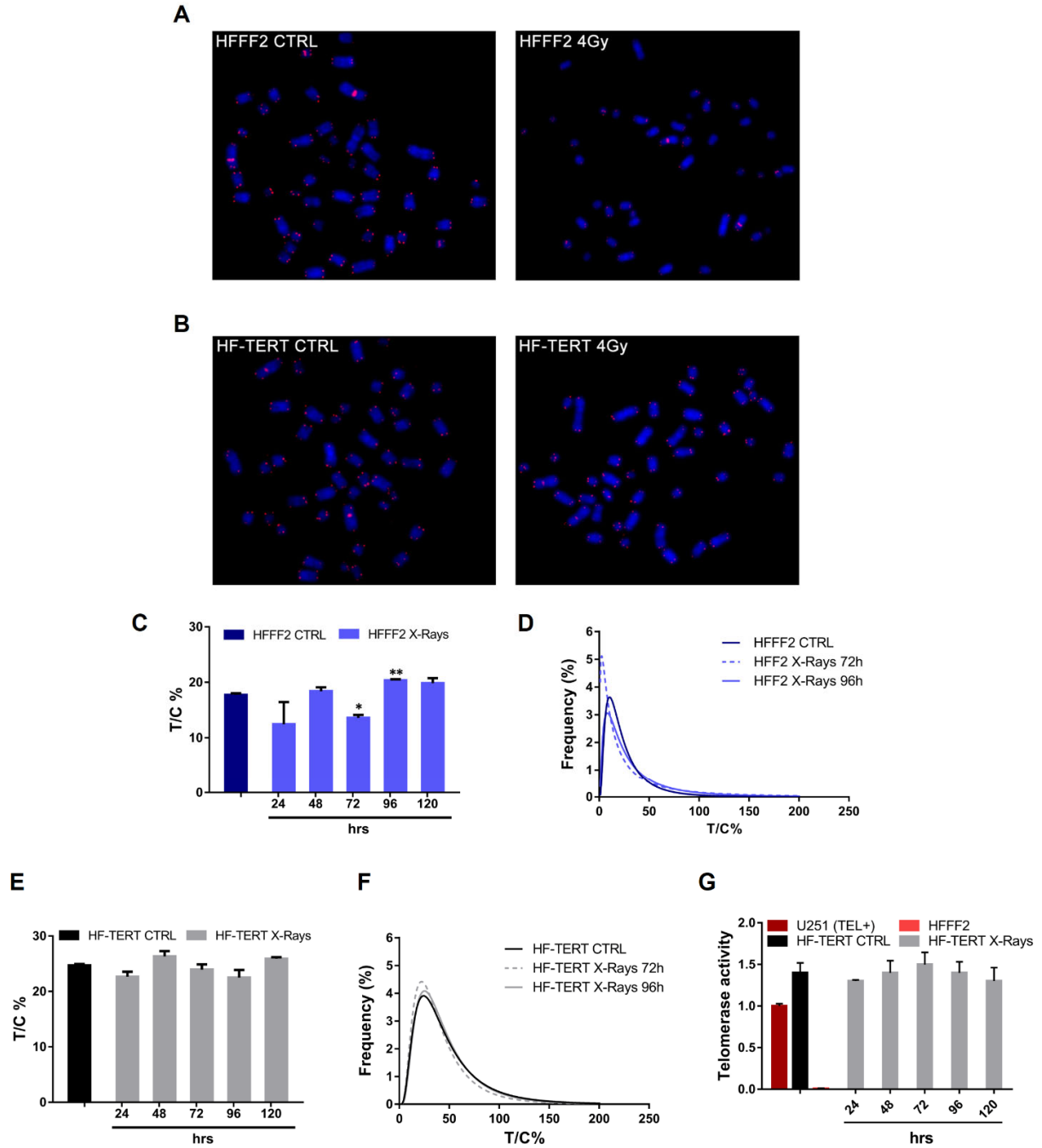


Figure S4. Analysis of telomere length in all samples after X-rays. **(A,B)** Example of metaphases of untreated and treated HFFF2 cells **(A)** and untreated and treated and HF-TERT cells **(B)**. **(C)** Results of telomere length after X-rays treatment in HFFF2 cells. **(D)** Log-Gaussian distributions of control and irradiated HFFF2 samples at 72 and 96 h since treatment. **(E)** The histogram represents the telomere length after X-irradiation in HF-TERT cells. **(F)** Log-Gaussian distributions of control and irradiated samples of HF-TERT at 72 and 96 h since X-rays. **(G)** Telomerase activity in control and X-rays treated HF-TERT cells. Telomerase-positive cell line (U251MG) is used as positive control and HFFF2 cells are used as negative control. Values are expressed as mean values \pm SEM. Statistical analysis is performed between treated and control samples. * $p < 0.05$; ** $p < 0.01$; by t Student test.

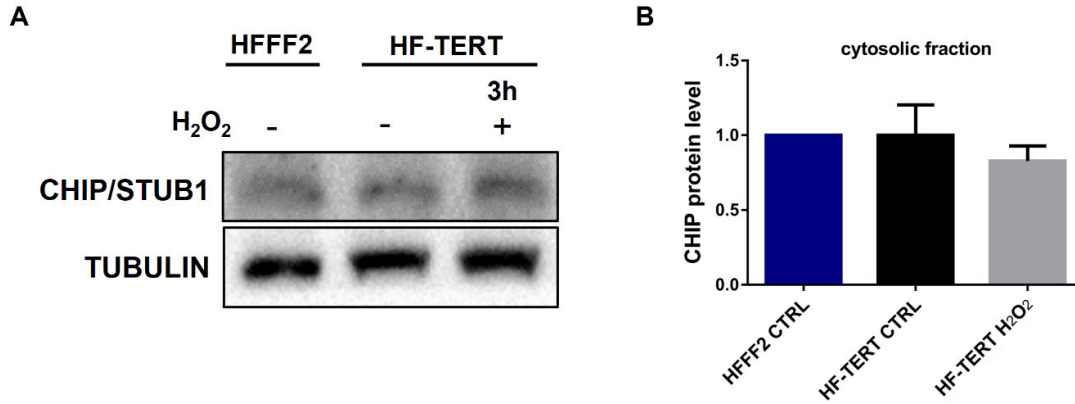


Figure S5. Western blot of CHIP. (A) Western blot shows specific bands for cytosol level of CHIP and tubulin, used as control protein in untreated HFFF2 and in untreated and treated HF-TERT cells. (B) Cytosolic level of CHIP protein after 3 h since treatment. Data represent mean \pm SEM.

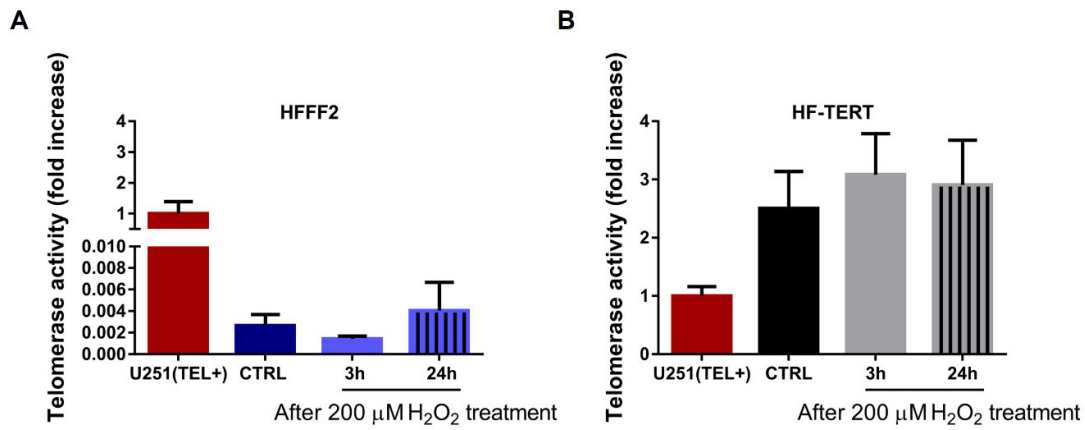


Figure S6. RQ-TRAP assay. (A) The data on telomerase activity in untreated and treated fibroblasts after 3 and 24 h post treatment. (B) Telomerase activity in untreated and treated hTERT overexpressing fibroblasts after 3 and 24 h since H₂O₂ treatment. Data are normalized to the control value represented by U251MG used as positive control. The error bars indicate the mean \pm SEM.

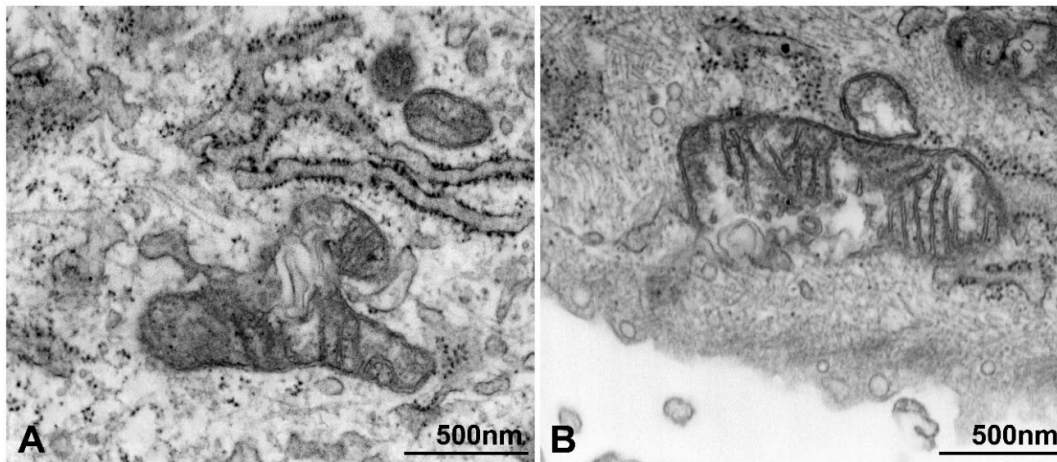


Figure S7. Close up of mitochondria showing outer membrane rupture in HFFF2 cell (A) and HF-TERT (B) cells following H₂O₂ treatment. Note the severe structural alteration of the organelles, which may assume a branched appearance.

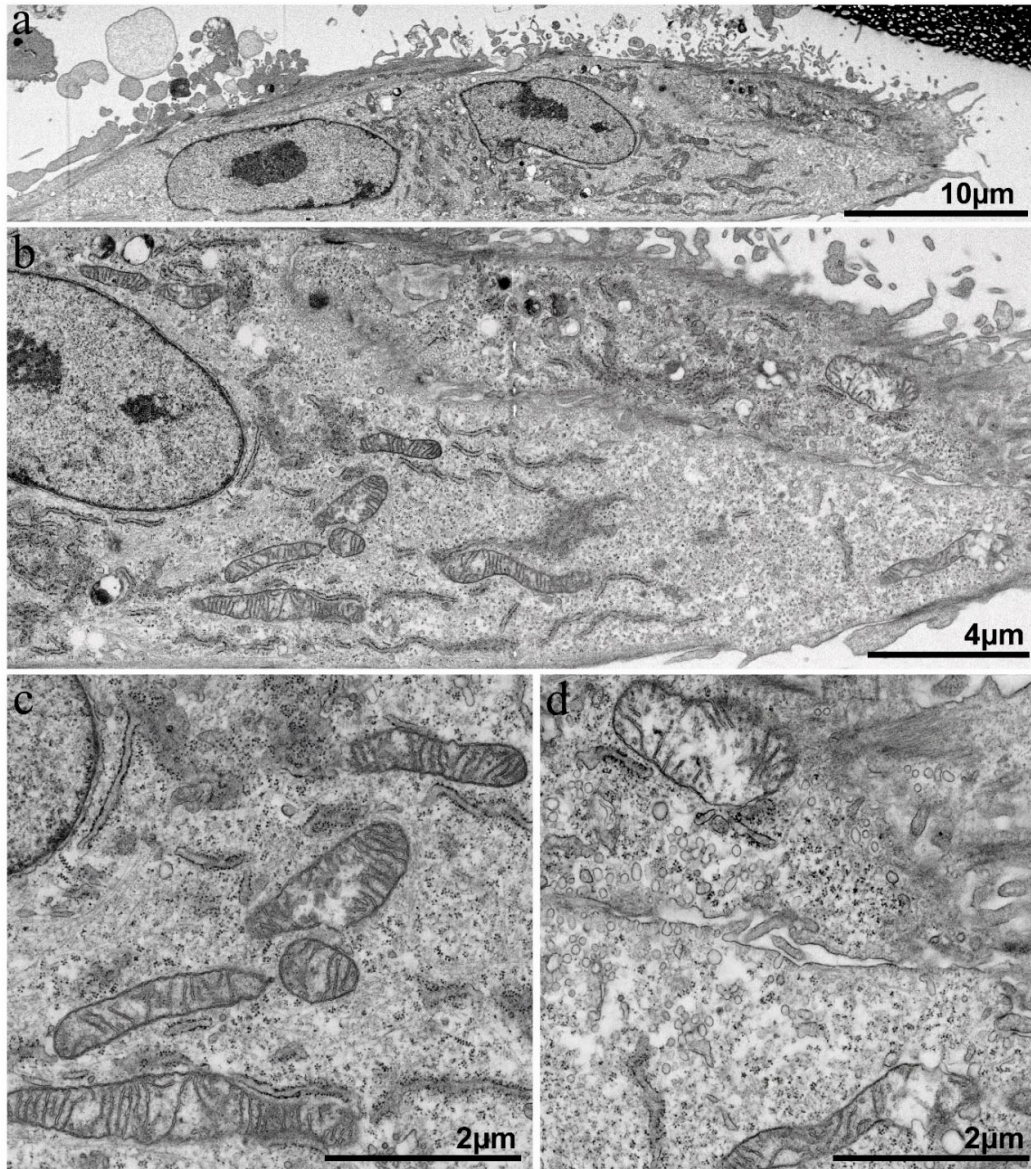


Figure S8. (A,B) Micrographs with incremental magnification of a HFFF2 cells after hydrogen peroxide treatment. Note in figure (C) swelling of the mitochondria and in figure (D) the extensive morphological damage in the form of outer membrane rupture and severe loss and fragmentation of the *cristae*.

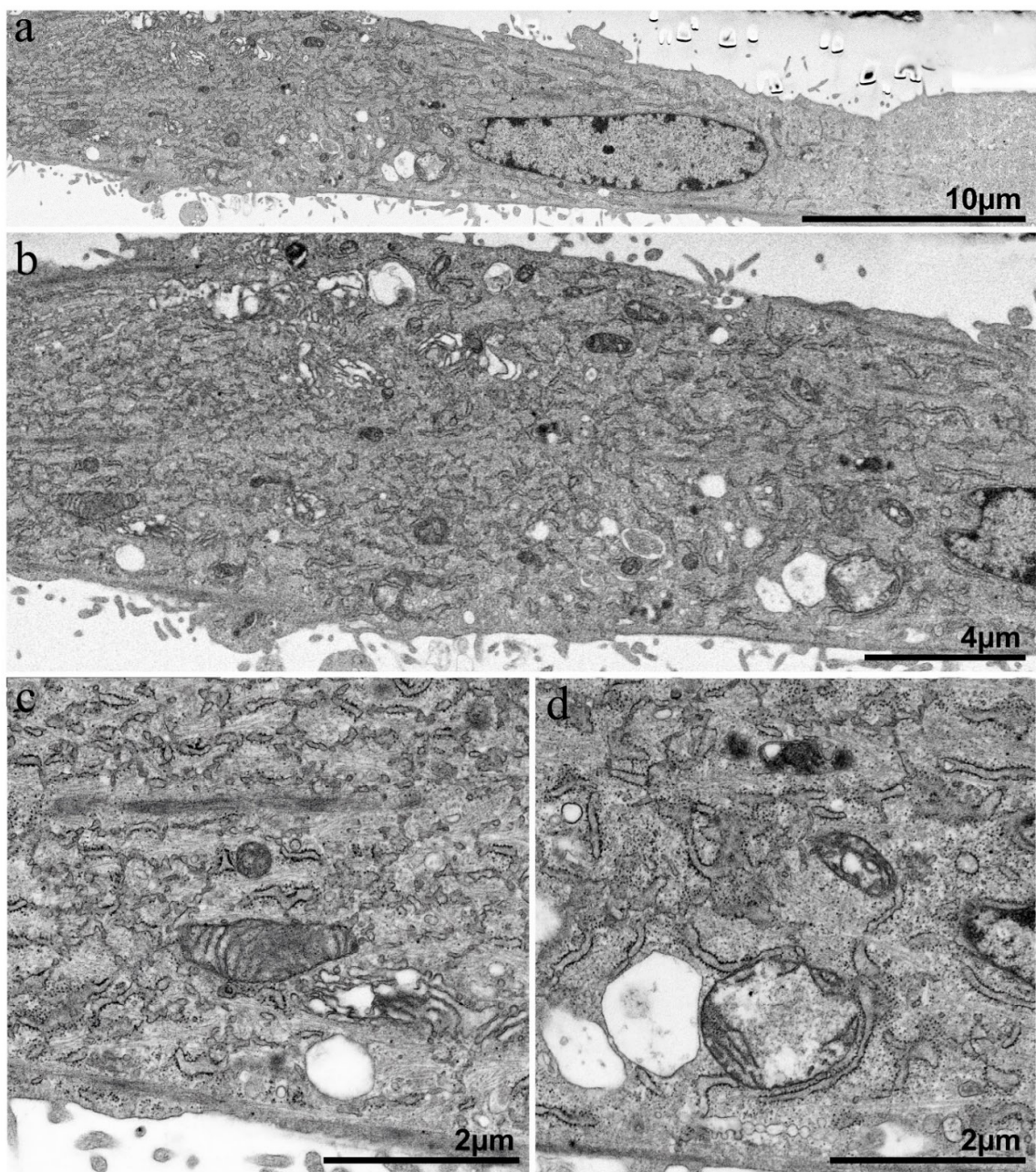


Figure S9. (A,B) Micrographs with incremental magnification of HF-TERT cells after hydrogen peroxide treatment. Figure (D) depicts a strongly swollen mitochondrion, while figure (C) shows an organelle with a well-preserved morphology that is similar to those observed in the control cells.