SUPPLEMENTARY MATERIAL

Figure S1. Mouse ovarian tissue cells labeled by the different cytogenetic techniques used. Oocytes I (left) and ovarian SYCP3(-) cells (right) showing TERRA (red) co-localization with TRF2 (green); TERT (green) co-localization with TRF2 (red); and telomeres marked with the telomeric PNA probe (green). Complexes are revealed by SYCP3 (light blue) and DNA is counterstained with DAPI (blue). All images were captured at 100x. Insets indicate 200% enlarged regions showing co-localizing signals.

Figure S2. Correlations between TERRA foci and TERRA/TRF2 co-localization in mouse and human germ cells. TERRA foci and TERRA-TRF2 co-localization were inversely correlated in mouse and human samples and in both cells types. In contrast, TERRA foci and TRF2-TERRA co-localization were positively correlated in mouse and human samples and in both cells types. Asterisks indicate significant correlations calculated by Pearson’s coefficient, (*) p < 0.05, (**) p < 0.001.

Figure S3. Mouse spermatogenic FACS populations. Example of a graph obtained after flow-sorting spermatogenic populations from mouse testes. Each point stands for a singular cell event. Red, yellow and blue zones indicate low, medium and high density of events, respectively. The Y-axis, FL 8 Lin (blue Hoechst fluorescence detector), measures DNA complexity whereas the X-axis, FL 9 Lin (red Hoechst fluorescence detector), measures DNA quantity. R5, R6, R7 and R9 populations correspond to spermatogonia, spermatocytes II, spermatocytes I and round spermatids populations, respectively. Images show cells stained by IF against H1t (red) and SYCP3 (green) proteins, and DNA counterstained with DAPI (blue) used to evaluate the enrichment achieved for each cell population. All images were captured at 100x. Numbers indicate the diploid number for each cell type and, between parentheses, the DNA content per cell.

Figure S4. Telomere structure of human spermatocytes I. (A) Profiles obtained by the analysis in spermatocytes I of the fluorescence intensity (measured as arbitrary units) of TRF2-TERT co-localizing signals, schematically illustrated in the pictures below. (B) Distances between TRF2 and TERT fluorescence signals obtained by the analysis of the profiles expressed in nm. The black line indicates the mean of TRF2-TERT distance. n = number of telomeres with TRF2-TERT co-localizing signals. (C) Proportion of totally or partially co-localizing TRF2 and TERT fluorescence profiles.