Timashev, Babcock et al. SUPPLEMENTAL FIGURES AND LEGENDS

Figure S1. Timashev, Babcock et al.

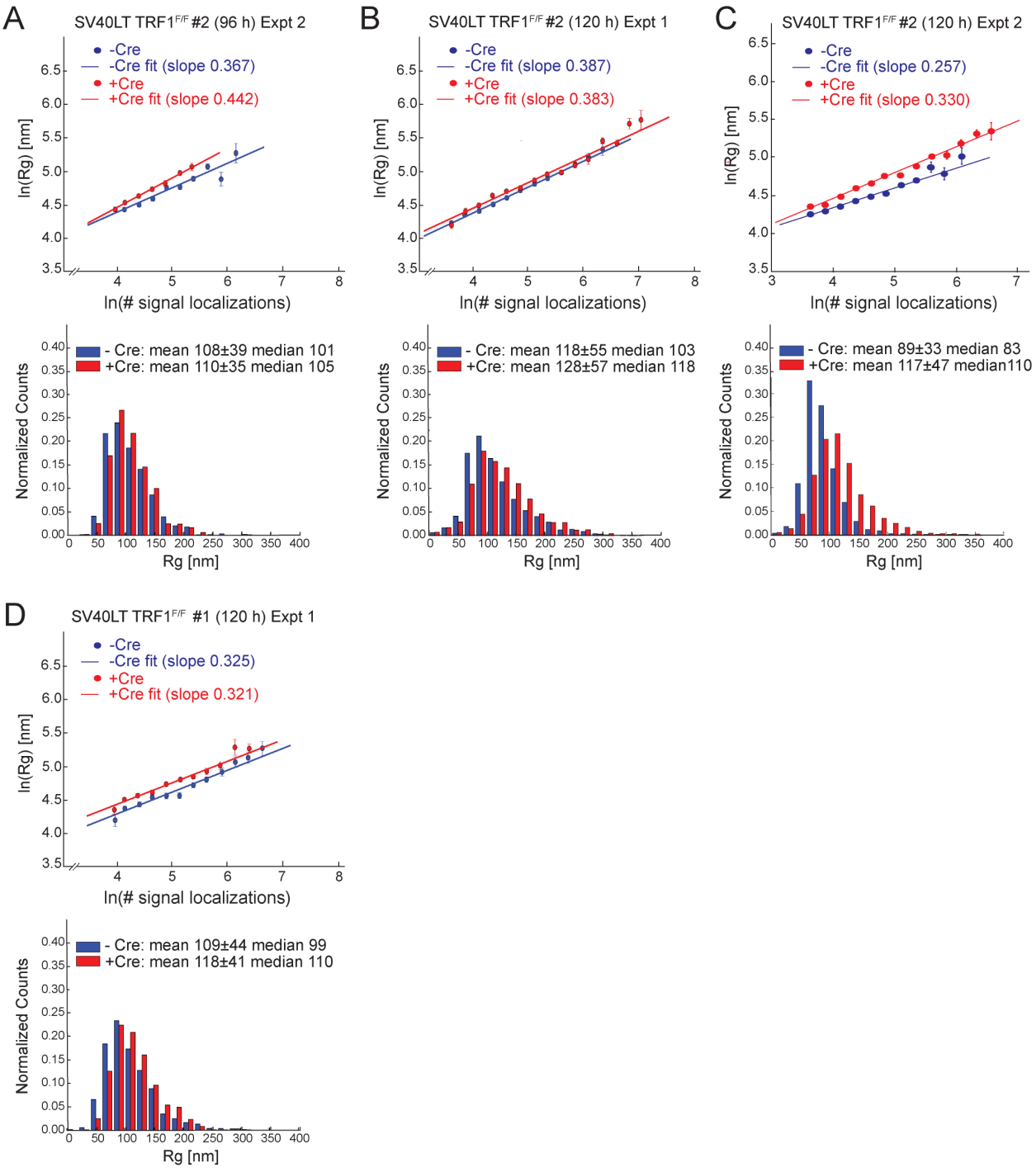

Figure S1. Effect of TRF1 deletion on telomere volume

(*A*-*D*) Measurements of telomere Rg in four additional independent TRF1 deletion experiments as in Figure 1. Graphs showing the natural log of Rg plotted vs the natural log of the number of telomere signal localizations obtained as in Fig. 1C from the indicated cells with and without Cre processed in parallel (n ≥ 10 cells for each condition in each independent experiment). Accompanying histograms show the distribution of Rg values with the means ± SDs and median values given.

Figure S2. Timashev, Babcock et al.

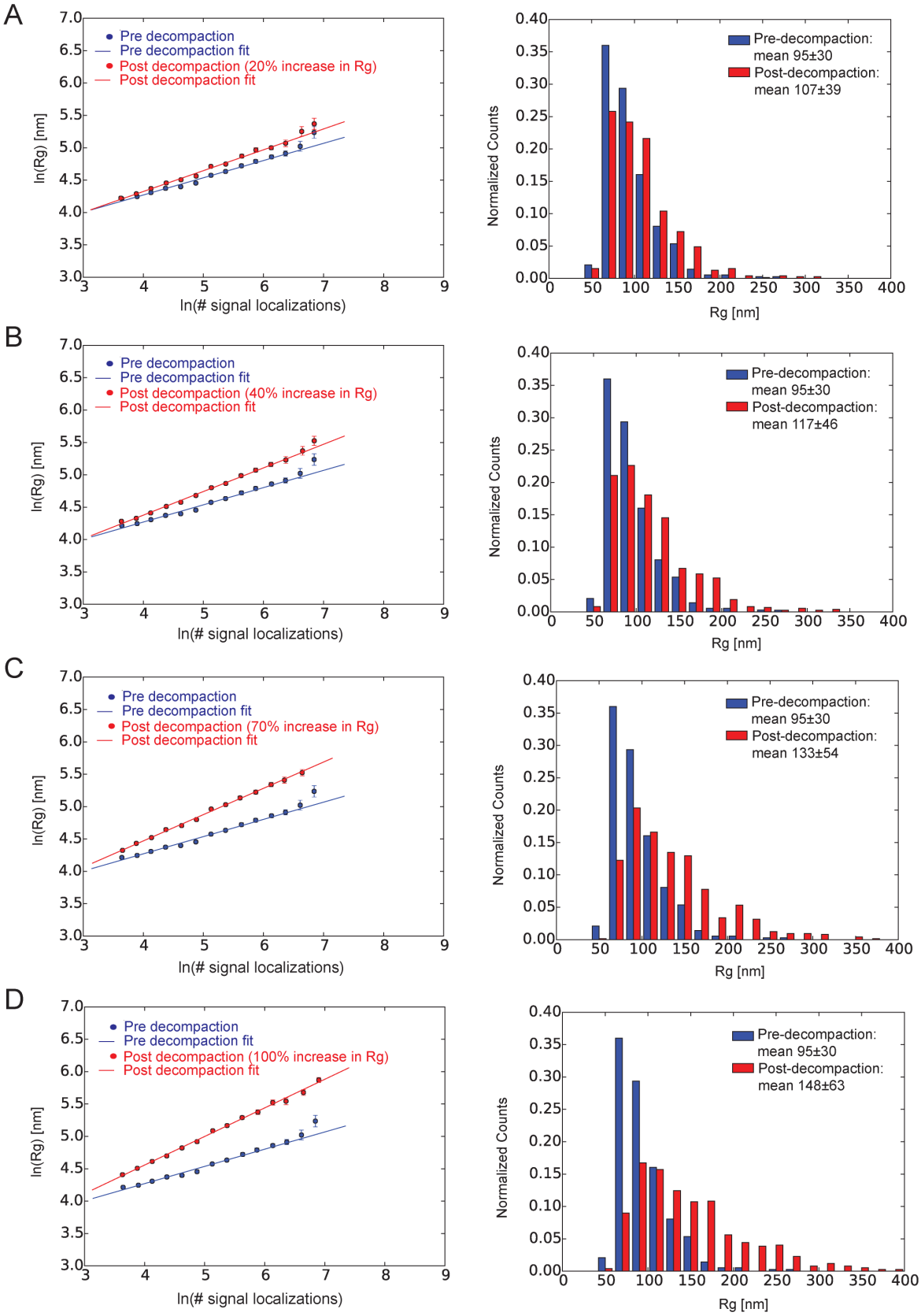

Figure S2. Simulation of telomere expansion

(*A*-*D*) Simulations using the experimentally measured Rg distribution, average localization number per telomeric focus, and background localization density to simulation STORM images of telomeres in the pre-decompaction condition and similar parameters to simulate STORM images of telomeres in the post-decompaction condition except that the average Rg values are increased by 20% (A), 40% (B), 70% (C), and 100% (D), respectively. Left panels: Plots of Rg values as a function of the localization number for the pre-decompaction condition (blue) and post decompaction conditions (red). Right panels: Bar graphs of distributions of Rg values for the pre-decompaction condition (blue) and post decompaction conditions (red). The means ± SDs are given in each case.

Figure S3. Timashev, Babcock et al.

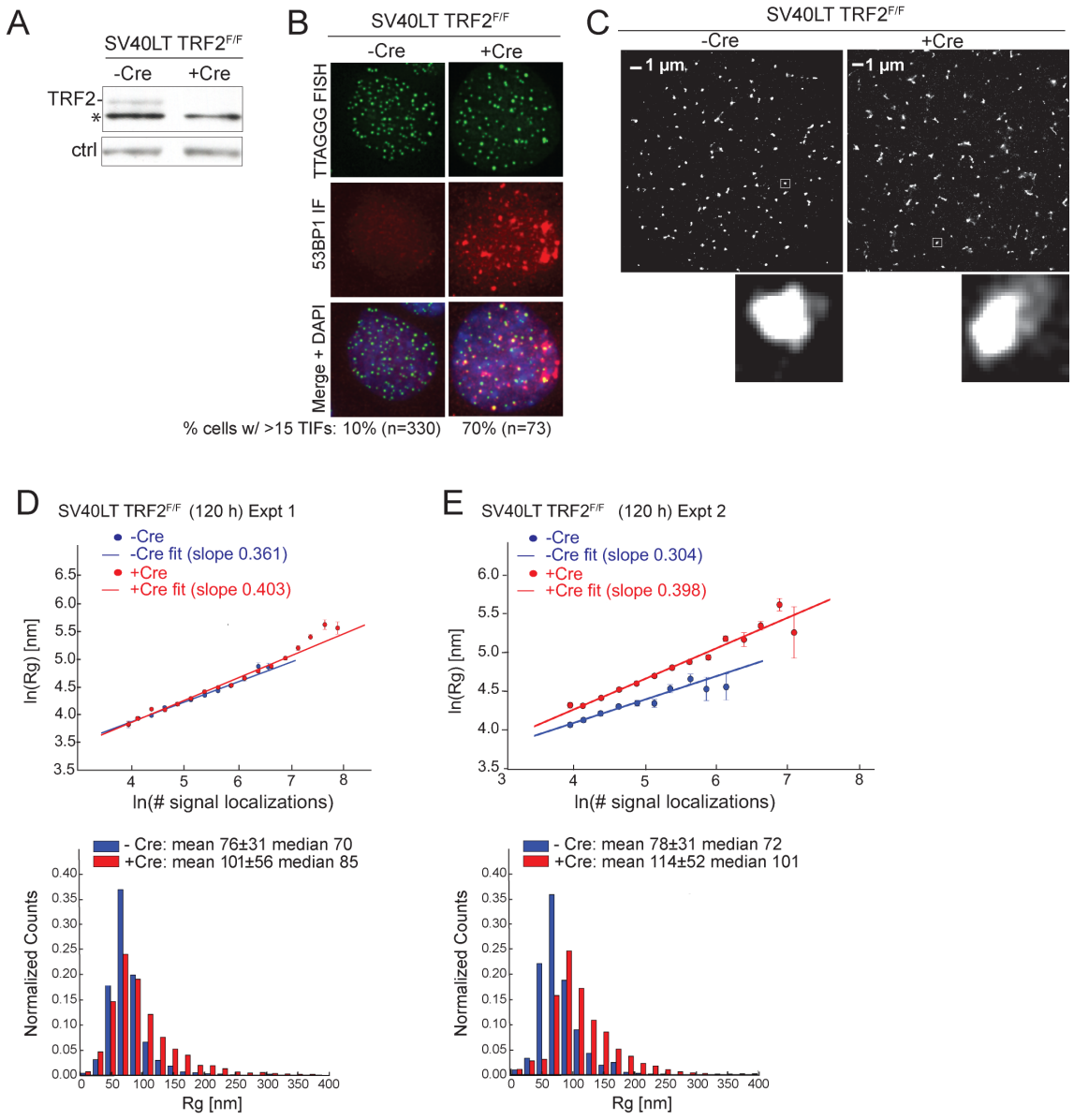

Figure S3. Telomere measurements after deletion of TRF2 from Lig4-proficient MEFs

(*A*) Representative immunoblot showing deletion of TRF2 96 h after treatment with tamoxifen from TRF2$^{F/F}$ Rosa-CreERT^{2} MEFs. Ctrl: non-specific band. Asterisk: non-specific band. (*B*) Projected z-stack immunofluorescence images showing the presence of TIFs 96 h after treatment with tamoxifen. Green, telomeric FISH with TelG-A647; red, IF for 53BP1. Merge,

green and red channels merged with DAPI (Blue). Percentage of cells with >15 TIFs is shown below. (*C*) Representative STORM images showing telomeric foci in cells with and without TRF2 as in (A) and enlarged images of selected foci shown below. (*D,E*) Graphs showing the natural log of Rg plotted vs the natural log of the number of telomere signal localizations in the indicated cells with and without Cre as in (A) obtained as in (C) and processed in parallel (n = 8 cells for -Cre; n = 6 cells for +Cre in each independent experiment). Each graph is paired with the accompanying histograms of distribution of Rg values with the means ± SDs and median values given. Cells were treated with Cre for 96 h. D and E represent two independent experiments.

Figure S4. Timashev, Babcock et al.

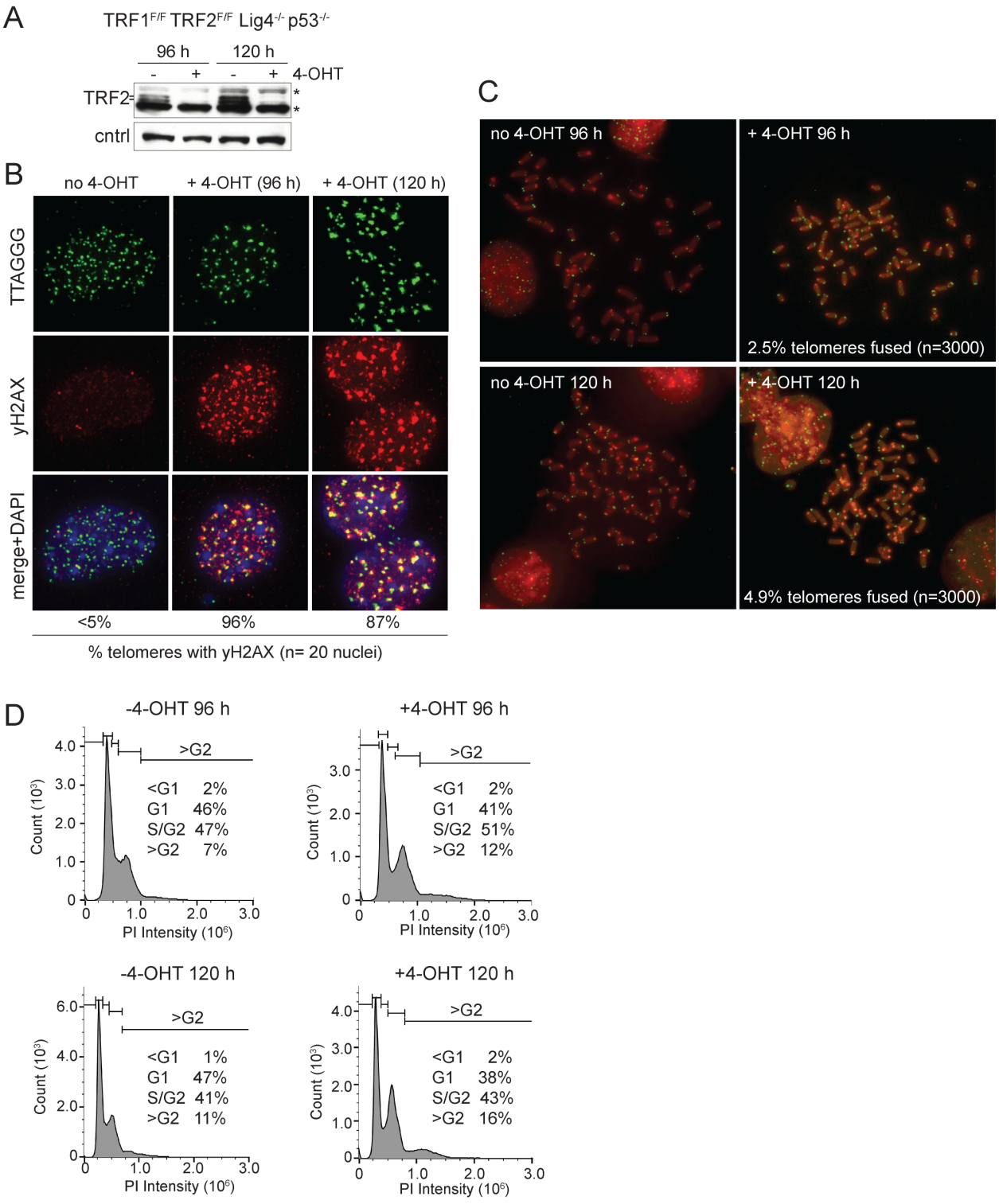

Figure S4. Quantification of telomere fusions and endoreduplication upon shelterin removal

(*A*) TRF2 immunoblot to verify the efficacy of Cre treatment in TRF1$^{F/F}$ TRF2$^{F/F}$ Lig4$^{-/-}$ p53$^{-/-}$ Rosa-Cre-ERT^{2} MEFs at 96 and 120 h after addition of tamoxifen. Cntrl: non-specific band used as loading control. Asterisks, non-specific bands. (*B*) Determination of the TIF response in the cells at the indicated time points. Green, telomeric FISH with TelG-A647; red, IF for γ-H2AX. Merged: green and red channels merged with DAPI DNA stain (blue). Quantification is given below the images. (*C*) Metaphases showing a low level of telomere fusions at 96 h and 120 h after induction of Cre. Quantification of the % of telomeres that are fused is indicated in the micrographs. (*D*) FACS analysis of the indicated cells to determine endoreduplication. Frequency of endoreduplication is approximated based on the % of cells with a DNA content >G2.

Figure S5. Timashev, Babcock et al.

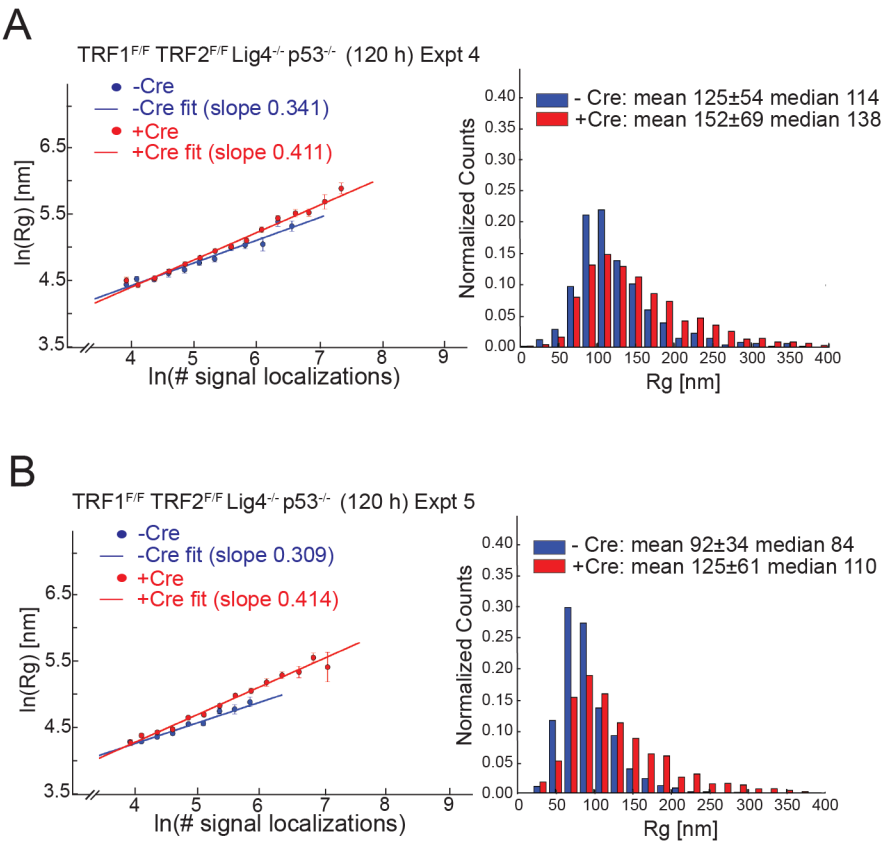

Figure S5. Effect of shelterin removal on telomere volume

(*A-D*) Measurements of telomere Rg in two additional independent TRF1/TRF2 co-deletion experiments as in Figure 3. Graphs showing the natural log of Rg plotted vs the natural log of the number of telomere signal localizations obtained as in Fig. 3C from the indicated cells with and without Cre and processed in parallel (n ~ 10 or more cells for each condition in each independent experiment). Accompanying histograms show the distribution of Rg values with the means ± SDs and median values given.

Figure S6. Timashev, Babcock et al.

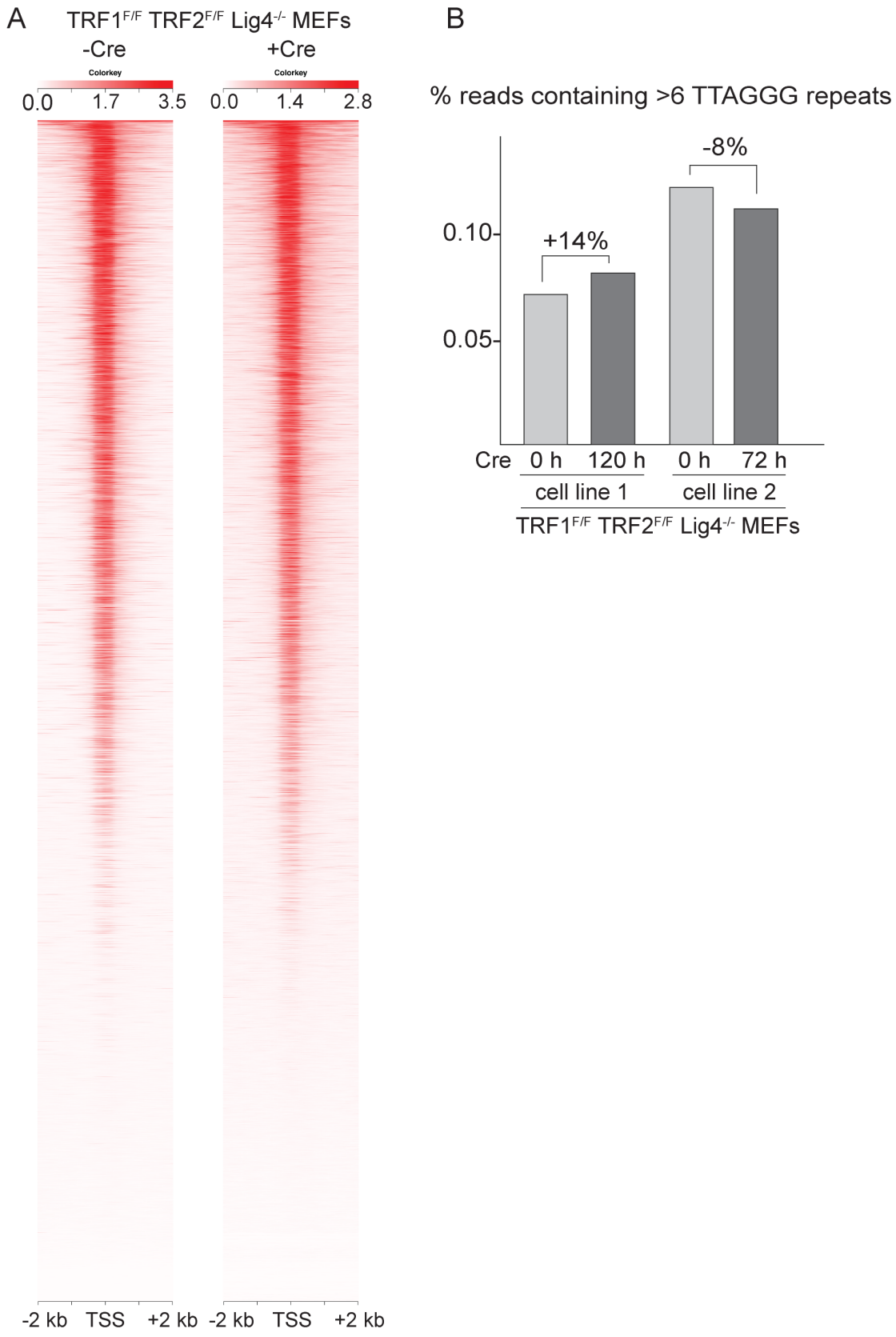

Figure S6. ATAC-seq to determine accessibility of telomeric chromatin

(*A*) ATAC-seq on TRF1$^{F/F}$ TRF2$^{F/F}$ Lig4$^{-/-}$ p53$^{-/-}$ Rosa-Cre-ERT^{2} MEFs before and after (120 h) treatment with tamoxifen to induce removal of shelterin. The expected preferential integration of Tn5 into regions surrounding the transcription start site (TSS) serves as a positive control for the efficacy of the ATAC-seq. (*B*) Bar graph representing the percentage of total paired-end reads (50-100 million) that contained at least 7 tandem TTAGGG (or CCCTAA) repeats in the indicated cells with and without Cre treatment for the indicated time. Cell lines 1 and 2 are independent MEFs lines with the same genotype. Between 50 and 100 million reads were obtained in each ATAC-seq experiment.

Figure S7. Timashev, Babcock et al.

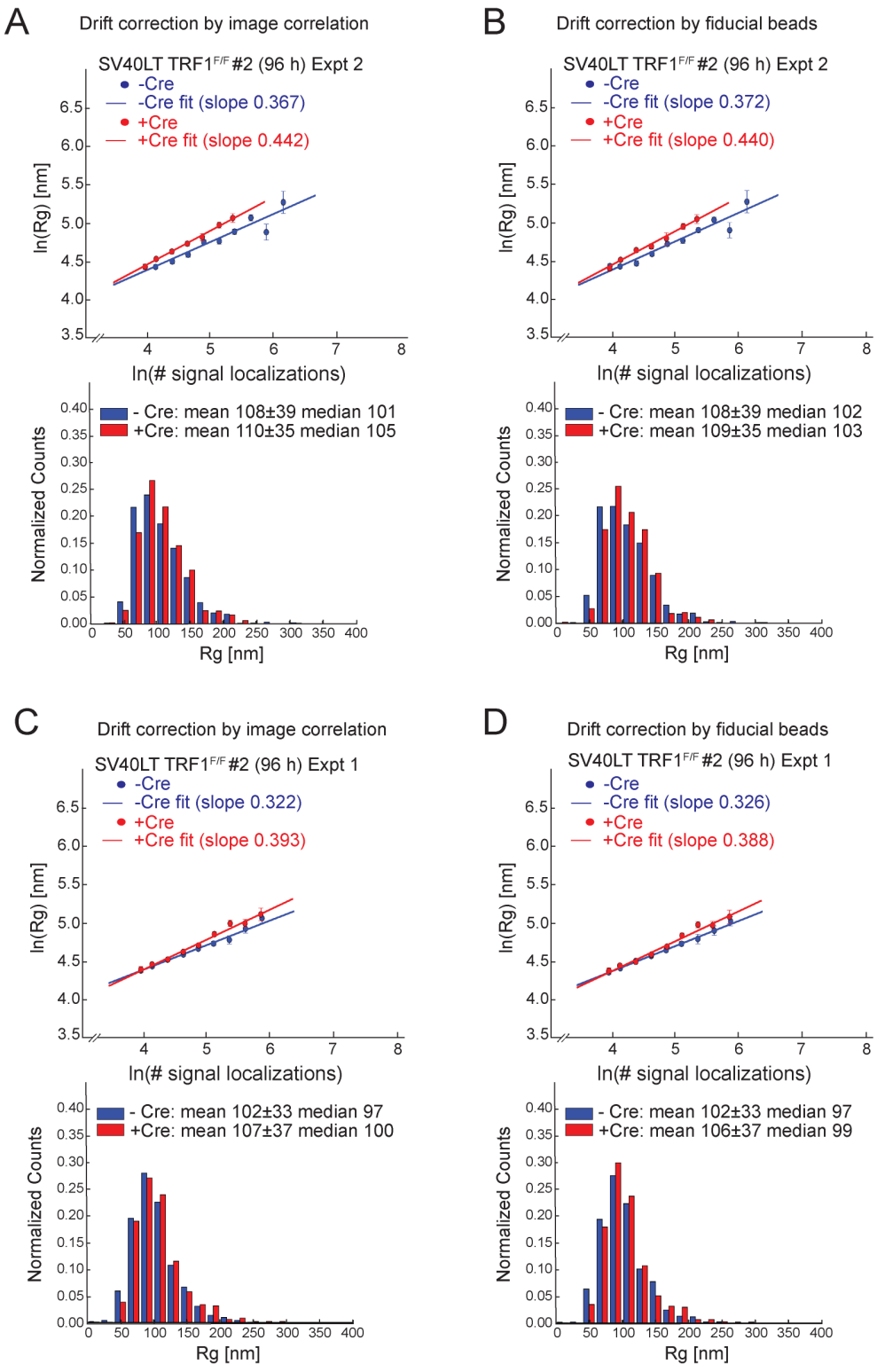

Figure S7. Comparison of STORM results obtained using drift correction by image correlation or by fiducial beads.

(A, B) Measurements of telomere Rg in one experiment using two different drift correction methods: (A) image correlation; (B) fiducial beads. Graphs showing the natural log of Rg plotted vs the natural log of the number of telomere signal localizations obtained from the indicated ±Cre cells and processed in parallel (n > 10 cells for each condition in each independent experiment). Accompanying histograms show the distribution of Rg values with the means ± SDs and median values given. (A) is a reproduction of Fig. S1A.

(C, D) Same as (A, B) but for another independent experiment. (C) is a reproduction of Fig. 1F.