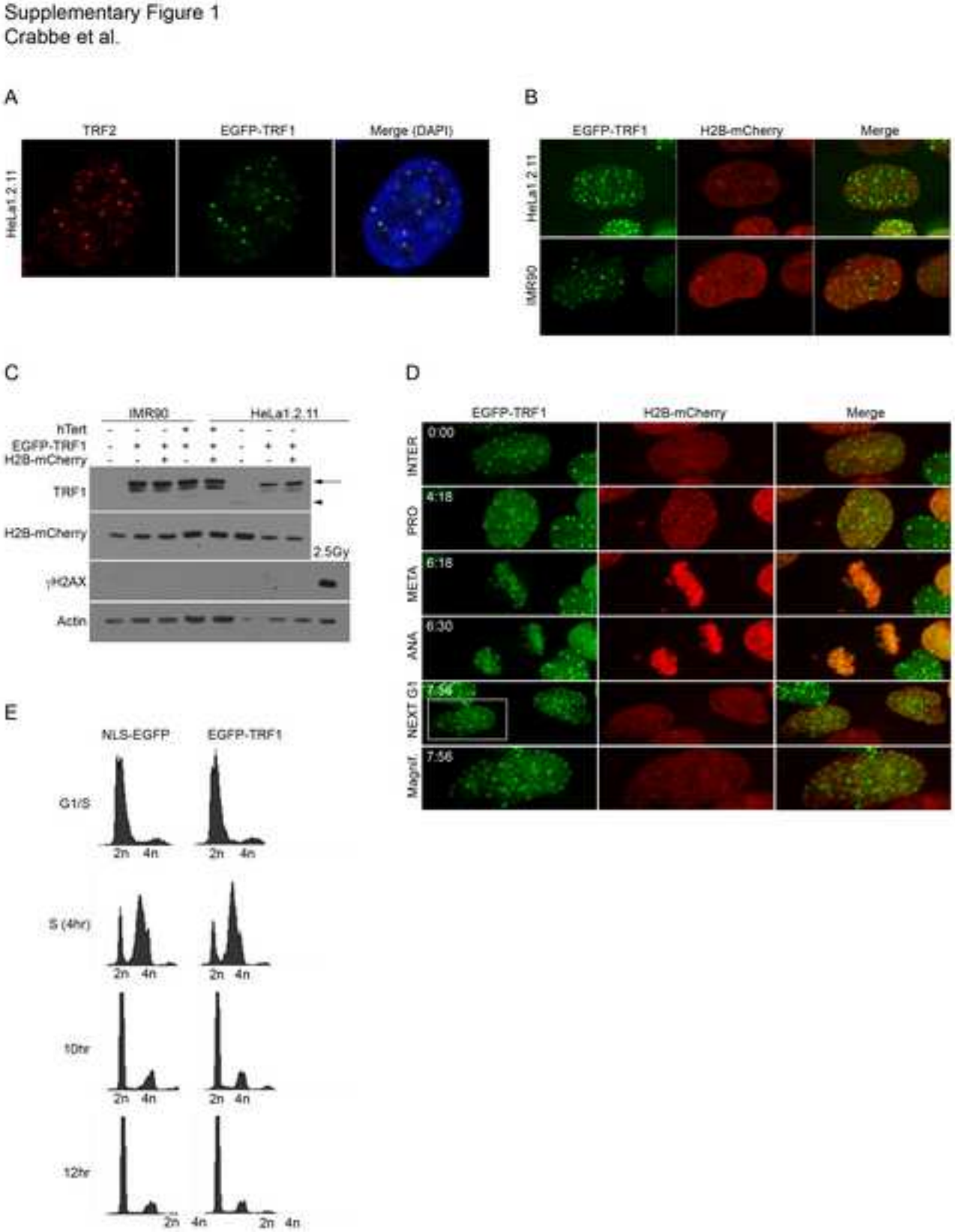
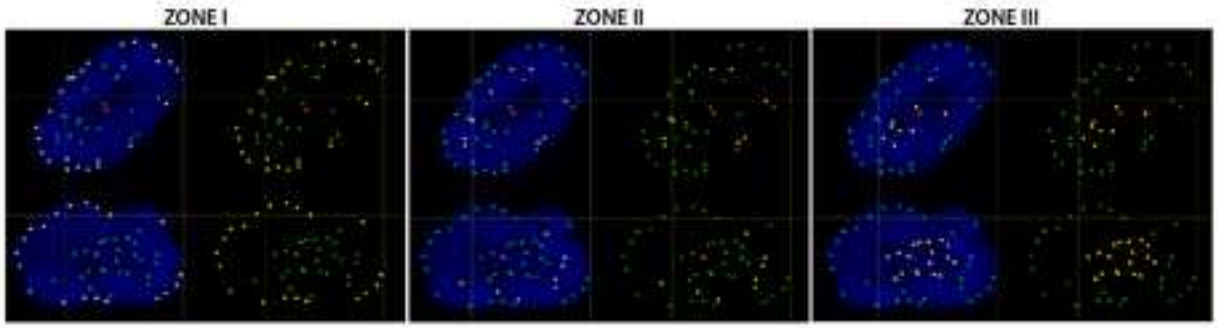
Supplemental Figure 1



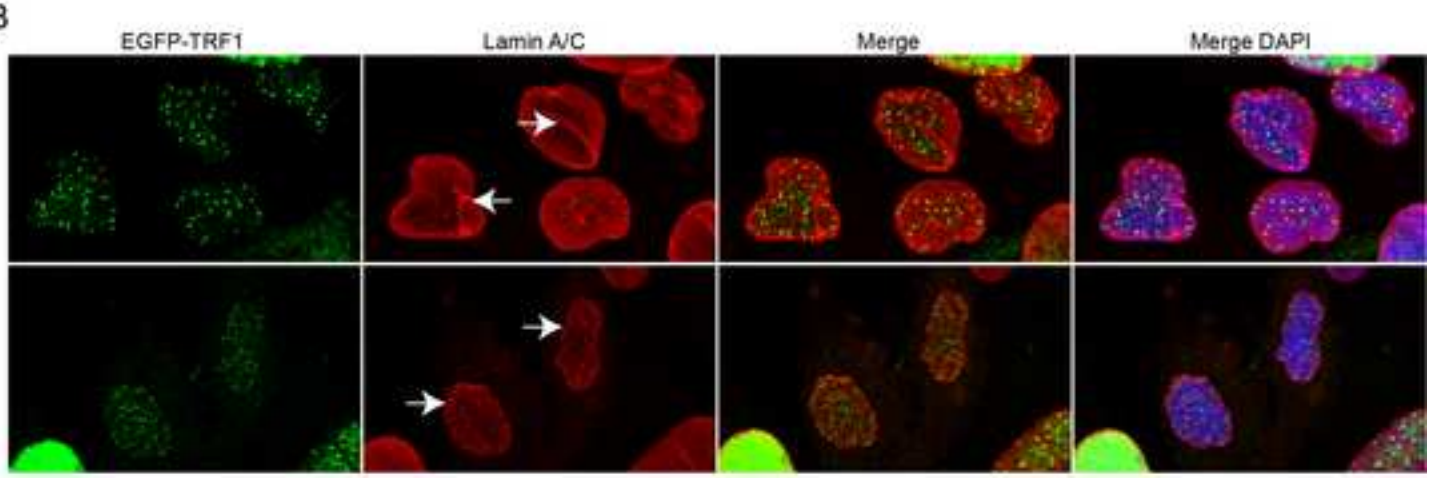
Supplemental Figure 2

Supplementary Figure 2
Crabbe et al.

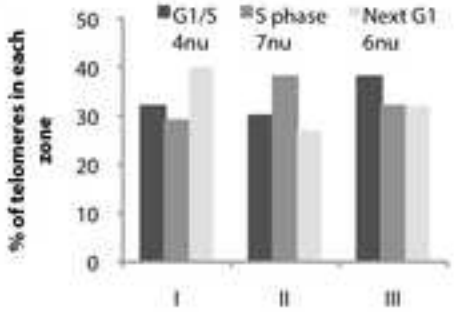
A



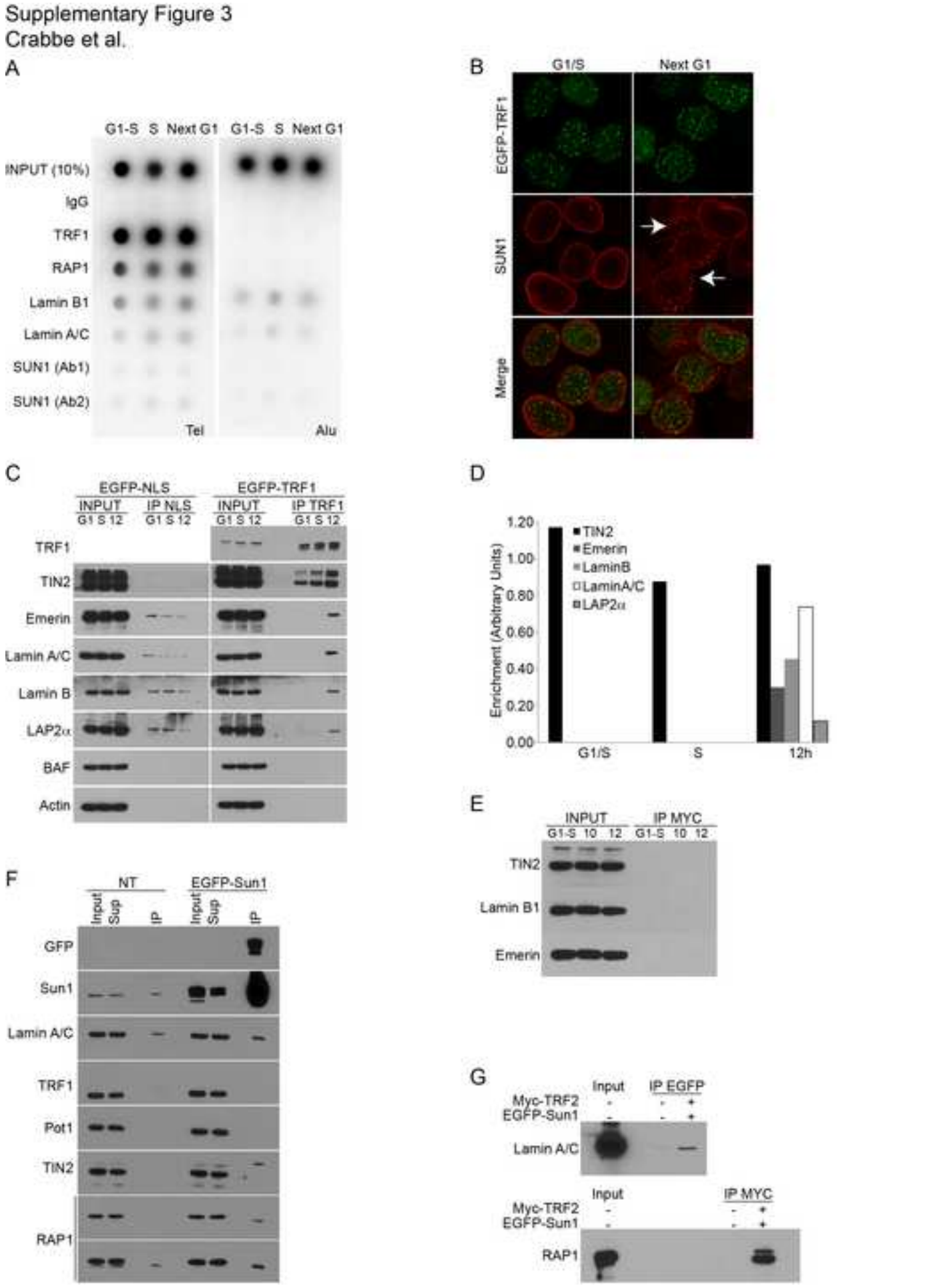
B



C



Supplemental Figure 3



Supplementary information

Extended Experimental Procedures:

Plasmids cloning strategies:

pLPC-EGFP-TRF1: EGFP sequence was cloned into pLPC-TRF1 by PCR using primers BamHI-EGFP-For and BamHI-EGFP-Rev. pLPC-NLS-EGFP: NLS-EGFP sequence was cloned into pLPC vector by PCR using primers EcoRI-NLS-EGFP-For and XhoI-EGFP-Rev. pLPC-Myc-RAP1: RAP1 cDNA was cloned into pLPC using BamHI and EcoRI by restriction digest from p-NTAP-RAP1 vector. pLPC-Flag-TRF1 and pLPC-Myc-TRF2. pLXSN-H2B-mCherry: H2B-mCherry cDNA was cloned by PCR into pLXSN vector with primers XhoI-H2B-For and BamHI-mCherry-Rev. pDEST-Sun1-EGFP (gift from Hetzer lab). pDEST-Sun1-mCherry: Gateway cloning (Invitrogen) from pDONR-Sun1 (gift from Hetzer lab) into pDEST-mCherry (Cter tagging, gift from Hetzer lab). pDEST-Sun1-NΔ200: sun1-NΔ200 was cloned by PCR into pDONR221 with primers attB1-Sun1Δ200-For and attB2-Sun1-Rev using Gateway (Invitrogen).

Supplementary Figure 1.

Expression of tagged telomeric proteins in HeLa and IMR90 cells, Related to Figure 1. (A) Immunofluorescence staining of HeLa1.2.11 cells expressing EGFP-TRF1 (green). TRF2 has been labeled in red and the DNA in blue. (B) Time-lapse images from movies of HeLa1.2.11 or IMR90 cells stably expressing

EGFP-TRF1 (green) and H2B-mCherry (red). A single focal plane is shown. (C) Western Blotting of IMR90 or HeLa1.2.11 cells expressing EGFP-TRF1 or H2B-mCherry as indicated. HeLa1.2.11 were irradiated with 2.5 Gy γ-irradiation when indicated. Antibodies used are indicated on the left. The arrow points to the band for EGFP-TRF1. The arrowhead points to the band for endogenous TRF1. (D) Time-lapse images from movies of IMR90 cells stably expressing EGFP-TRF1 (green) and H2B-mCherry (red). A single focal plane is presented per time-lapse (indicated in hr:min). Cell cycle phases have been indicated on the left. (E) FACS profiles of synchronized HeLa1.2.11 cells stably expressing NLS-EGFP or EGFP-TRF1.

Supplementary Figure 2.

Telomere localization in primary cells, Related to Figures 1, 2.

(A) Representative example of a 2D reconstruction of telomeres from IMR90 (green/yellow dots) of two nuclei after mitosis (blue), and their classification into one of the three zones as indicated. Telomeres that belong to each specific zone are displayed in yellow. The center of the nuclei are displayed in red. (B) Immunofluorescence staining of IMR90 cells expressing EGFP-TRF1 (green). Lamin A/C has been labeled in red and the DNA in blue. The arrows point to invagination of the nuclear envelope membrane into the nucleus. (C) Quantification of telomere position in the nucleus of IMR90 cells derived from immunofluorescence confocal images. For each stage of the cell cycle (G1, S phase and Next G1), data are represented as a percentage of spots (y axis) per

zone (x axis); as mean. The number of nuclei analyzed is indicated for each cell cycle phase.

Supplementary Figure 3.

Biochemical interactions of telomeric proteins with factors of the nuclear envelope, Related to Figure 3. (A) ChIP experiments from synchronized HeLa1.2.11 in G1-S, S phase, or 12 hours after release (Next G1). Antibodies used are indicated on the left, and 10% of the input is shown. DNA was subjected to Southern blot analysis using telomeric (left panel) or ALU repetitive probes (right panel). (B) Immunofluorescence staining of HeLa1.2.11 cells in G1/S or after mitosis (Next G1) expressing EGFP-TRF1 (green). Sun1 has been labeled in red. Arrows point to aggregates of Sun1 stored in the endoplasmic reticulum (C) Telomere association assay of HeLa1.2.11 cells stably expressing NLS-EGFP (panel IP NLS) or EGFP-TRF1 (panel IP TRF1). GFP beads were used for immunoprecipitation from lysates of cells collected at the G1/S block, in Sphase, or 12 hours after release. Antibodies used are indicated on the left. (D) Quantification of the telomere signal from panel B. (E) Telomere association assay of HeLa1.2.11 cells stably expressing EGFP-TRF1. MYC beads were used for immunoprecipitation in lysates from cells collected at the G1/S block, in Sphase, or 12 hours after release. Antibodies used are indicated on the left. (F) Co-immunoprecipitation of HeLa1.2.11 cells not transfected (NT) or expressing EGFP-Sun1. GFP beads were used for immunoprecipitation. Antibodies used are indicated on the left. (G) Co-immunoprecipitation of HeLa1.2.11 cells expressing

Myc-TRF2 and EGFP-Sun1. Myc beads or GFP beads were used for immunoprecipitation as indicated (IP MYC, IP EGFP). Antibodies used are indicated on the left.

Supplementary Movie 1

HeLa1.2.11 cells expressing EGFP-TRF1 (green) and H2B-mCherry (red), Related to Figure 1.

Supplementary Movie 2

IMR90 cells expressing EGFP-TRF1 (green) and H2B-mCherry (red), Related to Figures 1, 2.

Supplementary Movie 3

HeLa1.2.11 cells expressing EGFP-TRF1 (green) and Sun1-NΔ200-mCherry (red), Related to Figure 4.

Supplementary Table 1
Number of nuclei, telomeres and centromeres used for quantification, Related to Figures 2C and 4F.

Supplementary Table 2
List of primers used in this study, Related to Experimental Procedures.