Supplemental materials

No Overt Nucleosome Eviction at Deprotected Telomeres

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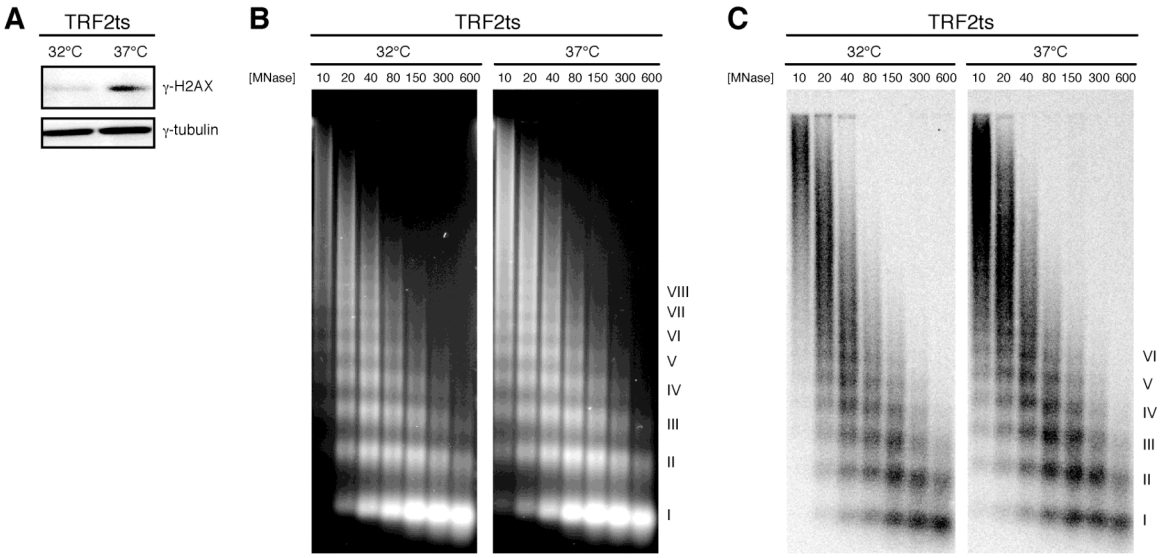
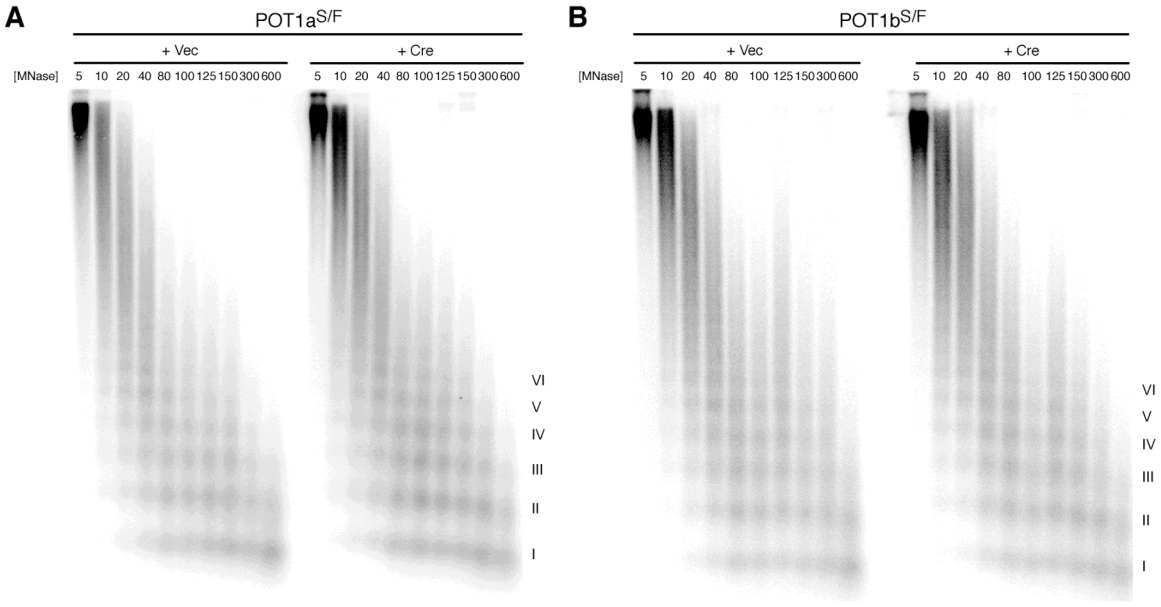
Supplemental Figures 1-5

Supplemental Figure 1. Wu and de Lange



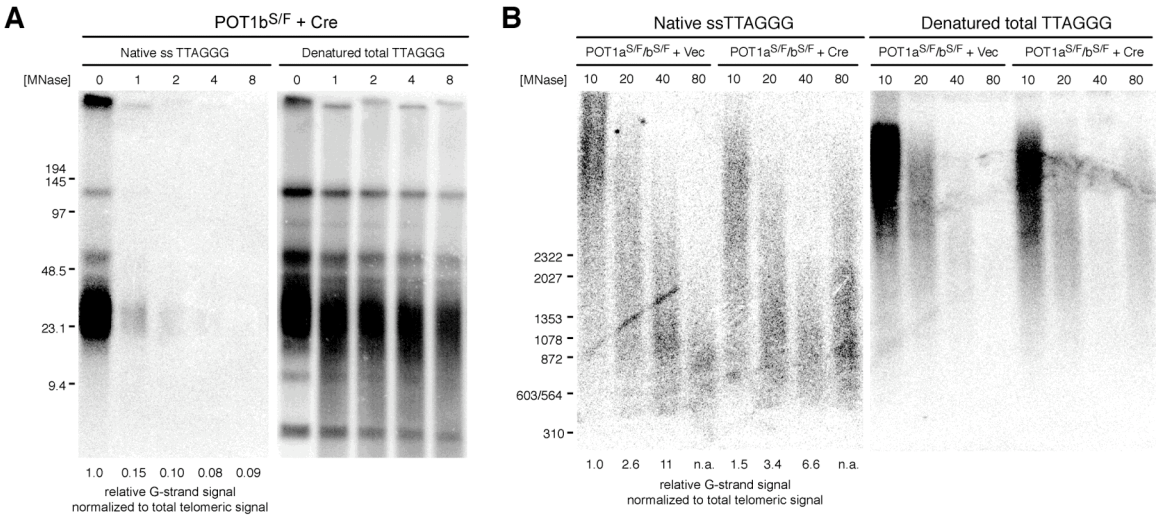
Supplemental Figure 1. No disruption of telomeric chromatin at 6 h after inactivation of TRF2ts. (A) Immunoblot for γ-H2AX, confirming activation of DNA damage signaling after 6 h shift of TRF2ts cells from the permissive temperature (32°C) to the non-permissive temperature (37°C). γ-tubulin is shown as a loading control. (B) Bulk nucleosomes in cells with and without TRF2 detected by ethidium bromide staining of DNA from nuclei digested with MNase and electrophoresed on a 1% agarose gel. (C) Telomeric nucleosomes detected by Southern blot hybridization with a $[^{32}P](CCCTAA)_4$ probe. MNase concentrations are given as U/ml. Roman numerals represent oligonucleosomes formed by partial digestion.

Supplemental Figure 2. Wu and de Lange



Supplemental Figure 2. No disruption of bulk telomeric chromatin following deletion of POT1a or POT1b alone. Southern blot detection of telomeric nucleosomes by hybridization of a [^{32}P](CCCTAA)$_4$ probe to DNA fragments isolated and fractionated following MNase digestion of nuclei in (A) POT1a$^{S/F}$ cells infected with pWzl vector or pWzl-Cre at 5 days post-selection with hygromycin and (B) POT1b$^{S/F}$ cells infected with pWzl vector or pWzl-Cre at 5 days post-selection with hygromycin. MNase concentrations are given as U/ml. Roman numerals represent oligonucleosomes formed by partial digestion.

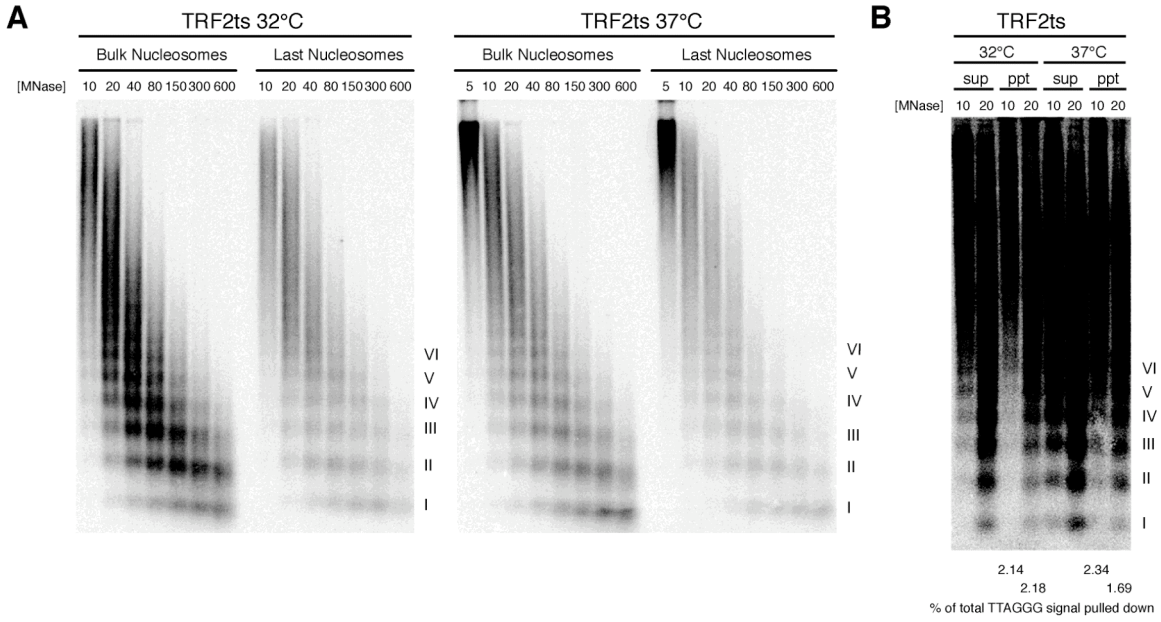
Supplemental Figure 3. Wu and de Lange



Supplemental Figure 3. MNase protection of the single-stranded overhang in chromatin. In gel overhang assay of DNA fragments recovered from MNase digestion of (A) agarose-embedded genomic DNA and (B) nuclei from POT1a$^{S/F}$ POT1b$^{S/F}$ cells

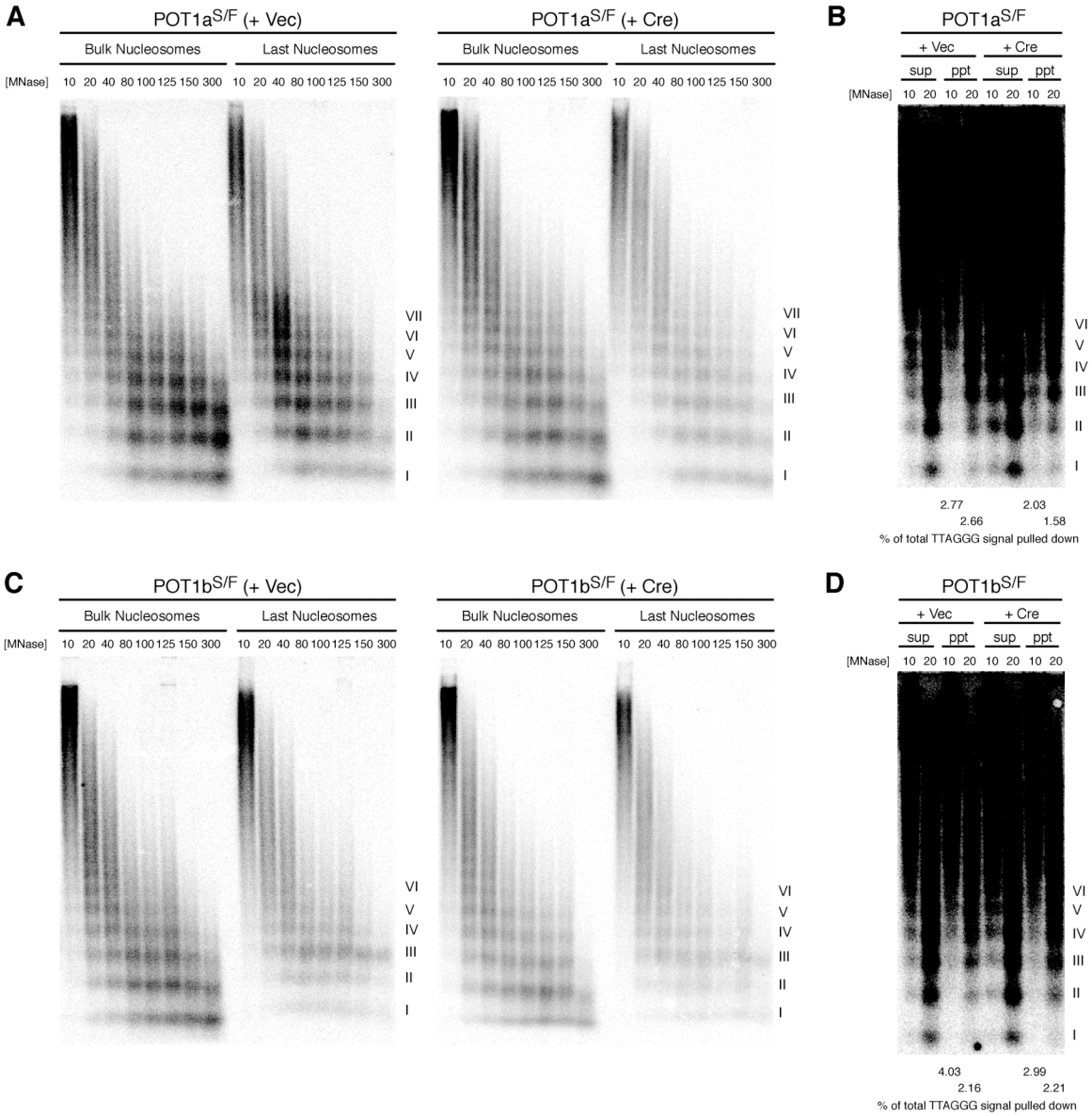
infected with pWzl vector (left half) or pWzl-Cre (right half) at 5 days post-selection with hygromycin. MNase concentrations are given as U/ml. Agarose plugs were digested overnight with MboI and fractionated on a 1% agarose gel in 0.5x TBE by by pulse field electrophoresis. Fragments in solution were treated with *Alu*I/*Mbo*I and fractionated on a 0.7% agarose gel. Gels were dried by vacuum suction and hybridized with $[^{32}P](CCCTAA)_4$. The gel was denatured *in situ* and rehybridized with the same probe. The relative G-strand signal in the native gel was normalized to the total telomeric signal detected in the denatured gel. The numbers below each lane correspond to the ratio relative to the value in the first lane of that panel. n.a.: not applicable. The relative overhang signal at [MNase] = 80 U/ml in (B) was not quantified because the duplex signal was at background level making the ratio between ss overhang signal and duplex TTAGGG repeats unreliable.

Supplemental Figure 4. Wu and de Lange



Supplemental Figure 4. No nucleosome eviction from the telomere terminus after temperature shift of TRF2ts cells. MNase sensitivity of bulk and last telomeric nucleosomes assessed by the last nucleosomal assay in (A) TRF2ts cells at 32°C (left) or after 6 hours of incubation at 37°C (right). MNase concentrations are given as U/ml. Roman numerals represent oligonucleosomes formed by partial digestion. (B) Longer exposure of the telomere blot for the last nucleosome assay in TRF2ts cells at 32°C and 37°C, with 4% of total supernatant and 100% of total pull down loaded onto the gel. Quantitation of the percentage of total TTAGGG signal pulled down is provided below the blot.

Supplemental Figure 5. Wu and de Lange



Supplemental Figure 5. No overt nucleosome remodeling at the telomere terminus following deletion of POT1a or POT1b alone. MNase sensitivity of bulk and last telomeric nucleosomes assessed by the last nucleosomal assay in (A) POT1a$^{S/F}$ cells infected with pWzl vector (left) or pWzl-Cre (right) at 5 days post-selection with hygromycin and C) POT1b$^{S/F}$ cells infected with pWzl vector (left) or pWzl-Cre (right) at 5 days post-selection with hygromycin. MNase concentrations are given as U/ml. Roman numerals represent oligonucleosomes formed by partial digestion. (B) and (D) Longer exposure of the telomere blots for the last nucleosome assay in POT1a and POT1b deleted cells, respectively, with 4% of total supernatant and 100% of total pull down loaded onto the gel. Quantitation of the percentage of total TTAGGG signal pulled down is provided below the blot.