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Supplemental Information

TRF2 Mediates Replication Initiation within Human Telomeres to Prevent Telomere Dysfunction

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Figure S1

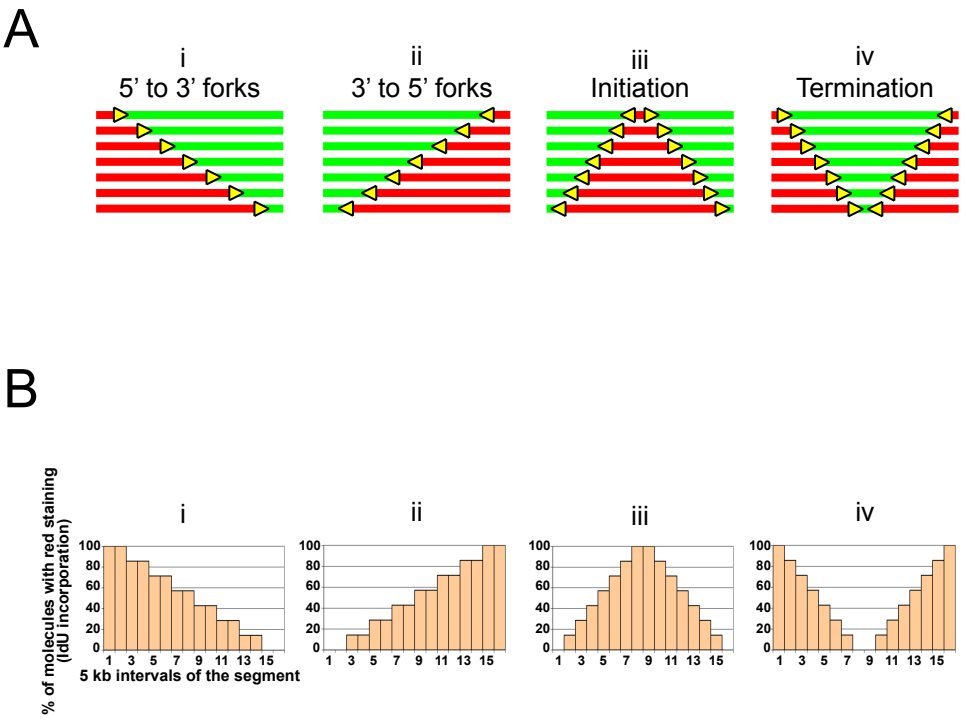


Figure S1 SMARD Replication Profiles related to Figures 1, 2, 3, 5 and 6. (A) Images of individual fully labeled molecules containing both red and green labeling are aligned then arranged by increasing content of DNA labeled during the first labeling pulse (red) to produce a composite profile of replication. Characteristic patterns in the aligned molecule images identify specific replication events. Alignments displaying molecules of increasing red stain from one end indicate replication forks progressing in a single direction through the segment (i and ii). Initiation events are observed as a red tract flanked on both sides by green (iii), while a green tract flanked on both sides by red (iv) indicates a termination event.
(B) Molecule alignments can be graphically represented as histograms of the percentage of molecules containing red staining (IdU incorporation) within each 5 kb of a segment. The 5 kb intervals that have the highest percentage of molecules stained in red are those that on average replicate first. Specific replication events produce characteristic features in these replication profiles. Replication progressing through the segment primarily in one direction (5' to 3' or 3' to 5' relative to the polarity of the segment upper strand) from an external origin is observed as a progressive decrease in the percentage of red staining from one end to the other across the segment (x-axis) (i and ii). Locations of the centers of initiation sites are indicated by peaks (iii) while termination events are seen as valleys (iv).

Figure S2

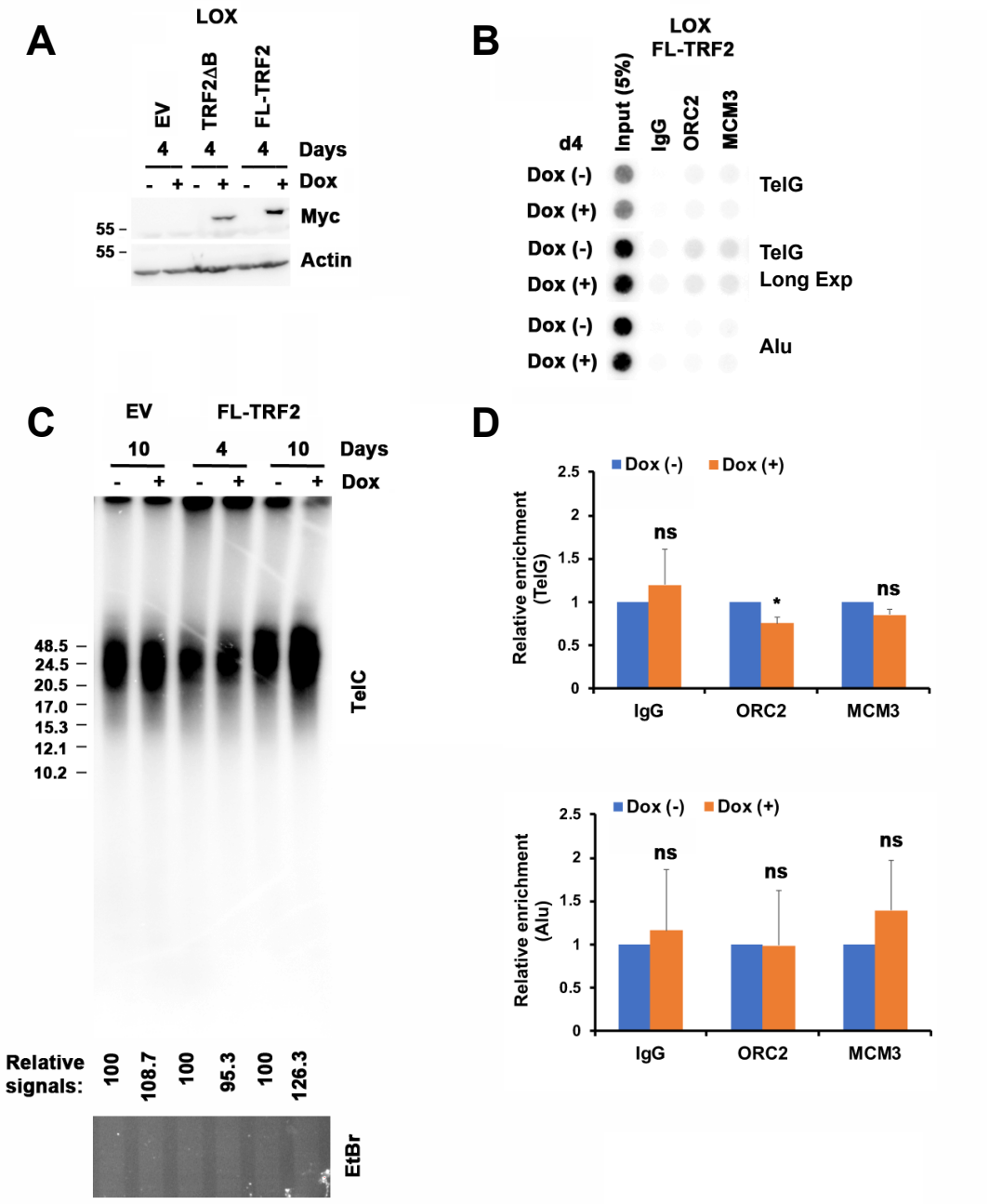
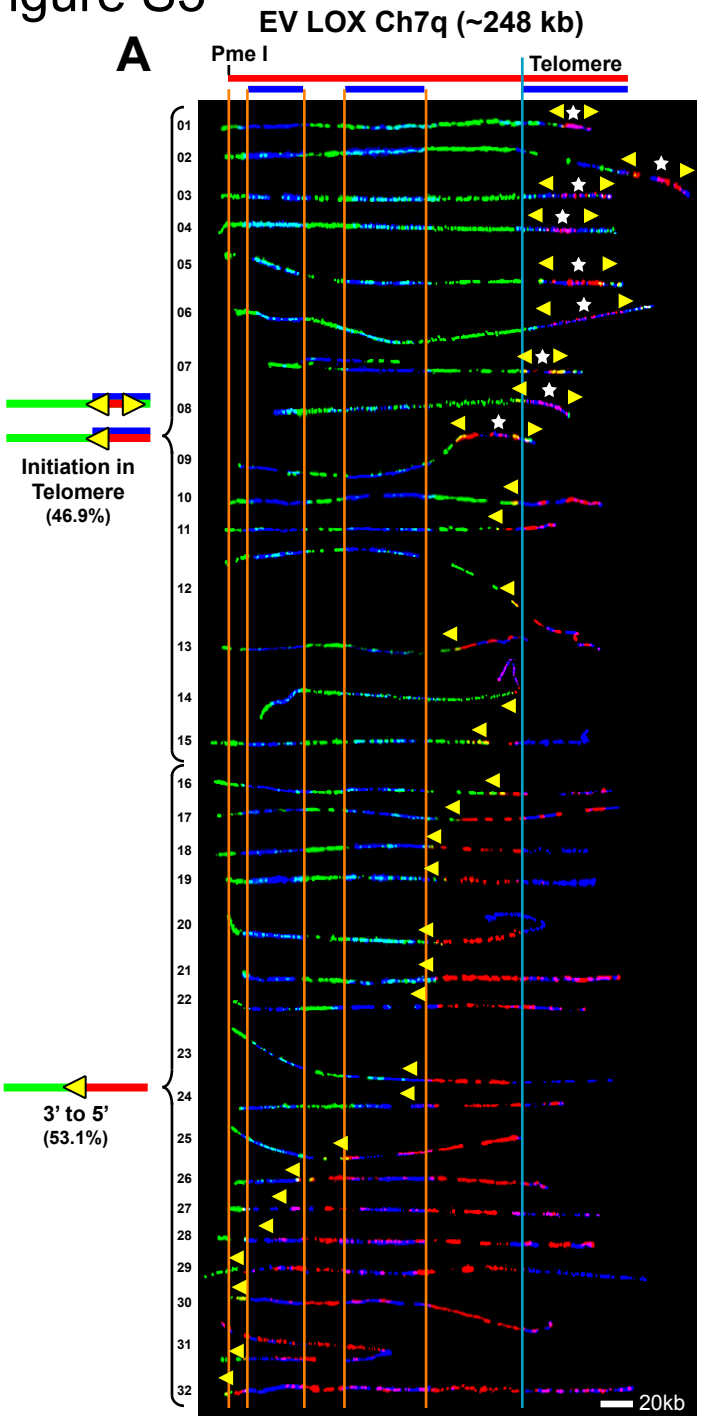
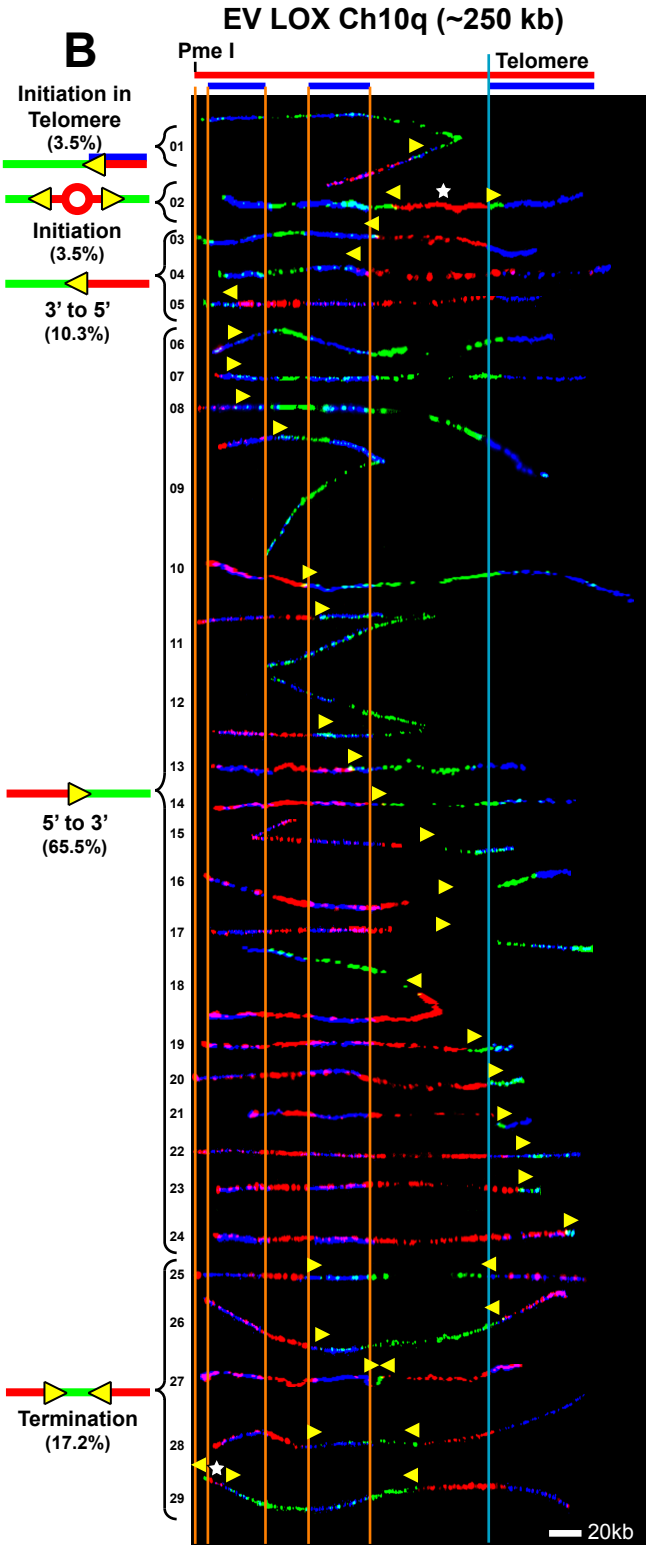
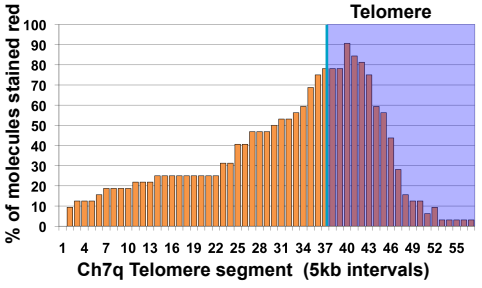


Figure S2: Overexpression of full length WT TRF2 slightly reduces ORC2 recruitment to telomeres and has a minimal effect on telomeric DNA signal in LOX cells, related to Figure 3. (A) Immunoblot of cell lysates of LOX cell lines stably expressing doxycycline inducible Myc-tagged full length (FL) TRF2, TRF2ΔB or control empty vector (EV). (B) ChIP analysis of LOX full length TRF2 cells grown in the absence (-) or presence (+) of 1µg/ml doxycycline (Dox) for 4 days (d4) using antibodies against ORC2, MCM3 or IgG (control). Blots were probed by hybridization with 32P-labeled telomeric TTAGGG (TelG) or Alu repeat (Alu) probes. (Long Exp = Long exposure). (C) Telomere length analysis of full length WT TRF2 expressing LOX cells grown in the absence (-) or presence (+) of 1µg/ml Dox for 4 or 10 days. Telomere length and relative amount of telomeric DNA was determined by restriction digestion of genomic DNA with AluI/MboI, followed by PFGE and Southern hybridization with a 32P-labeled (CCCTAA)4 probe. Ethidium bromide staining of total DNA digest was used to normalize for DNA loading (bottom). Fragment size (in kb) is indicated on left of blot. Relative intensity of telomeric DNA signals (- Dox vs. + Dox) is indicated below blot. (D) Quantification of at least three independent experiments represented in (B). The ChIP data was first normalized to input, and the % input for dox (+) was shown as relative to dox (-) which was set as 1. Student's t-test was used for statistical analysis. Error bars indicate SD. * = p-value of <0.02, ns = no statistical significance (p-value > 0.05).

Figure S3


A
EV LOX Ch7q (~248 kb)
Pme I
Telomere
Initiation in Telomere (46.9%)
3' to 5' (53.1%)
20kb


B
EV LOX Ch10q (~250 kb)
Pme I
Telomere
Initiation in Telomere (3.5%)
Initiation (3.5%)
3' to 5' (10.3%)
5' to 3' (65.5%)
Termination (17.2%)
20kb


Telomere
% of molecules stained red
Ch7q Telomere segment (5kb intervals)

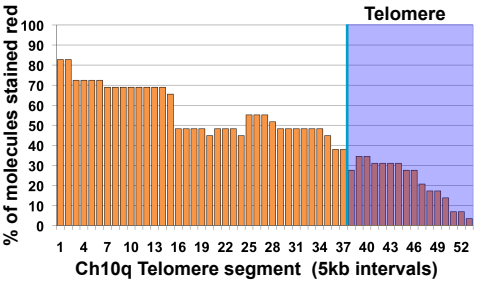
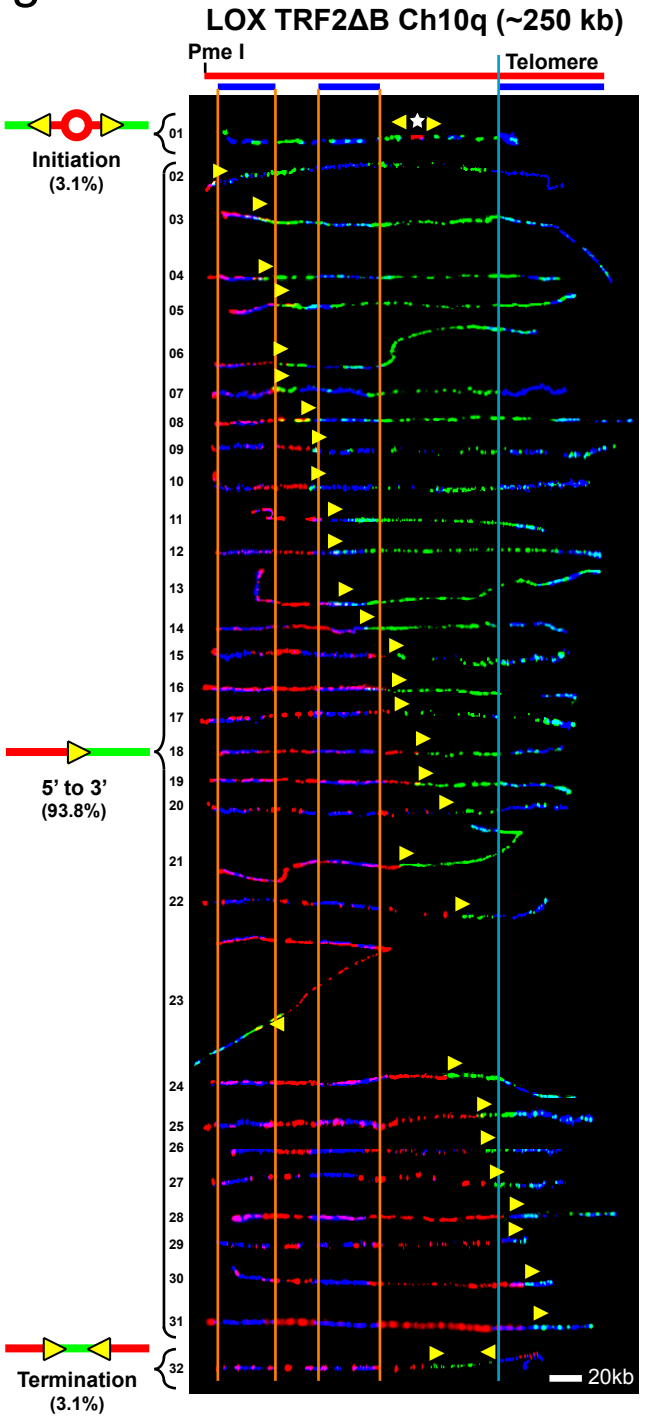

Telomere
% of molecules stained red
Ch10q Telomere segment (5kb intervals)

Figure S3: Expression of empty vector control in doxycycline-induced LOX cells does not alter the replication programs of Ch7q and Ch10q telomeres, related to Figures 1 and 2 (A) SMARD analysis of the Ch7q telomere segment from LOX cells expressing empty vector (EV) (=pInducer10L) control. Cells were grown for 3 days in the presence of 1μg/ml doxycycline. Alignments of replicated molecules fully labeled with both IdU (red) and CldU (green) are shown, collected from three independent samples stretched on slides (149 fully red and 141 fully green labeled molecules were also collected). (B) SMARD analysis of the Ch10q telomere segment from LOX cells expressing empty vector (EV) (=pInducer10L) control as in (A). Alignments of replicated molecules fully labeled with both IdU (red) and CldU (green) are shown, collected from two independent samples stretched on slides (163 fully red and 139 fully green labeled molecules were also collected). Vertical lines (orange and blue) demarcate the boundaries where FISH probes bind, as described in Figure 1. Symbols as in Figure 1. Replication profile histograms shown under the molecule alignments.

Figure S4


LOX TRF2ΔB Ch10q (~250 kb)
Pme I
Telomere
Initiation
(3.1%)
5' to 3'
(93.8%)
Termination
(3.1%)
01
02
03
04
05
06
07
08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
20kb

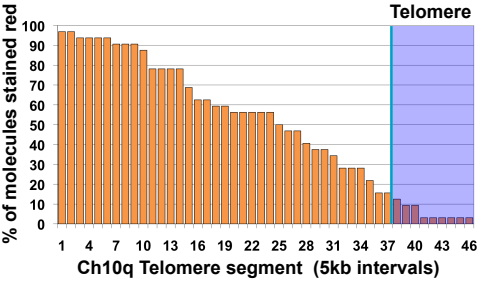
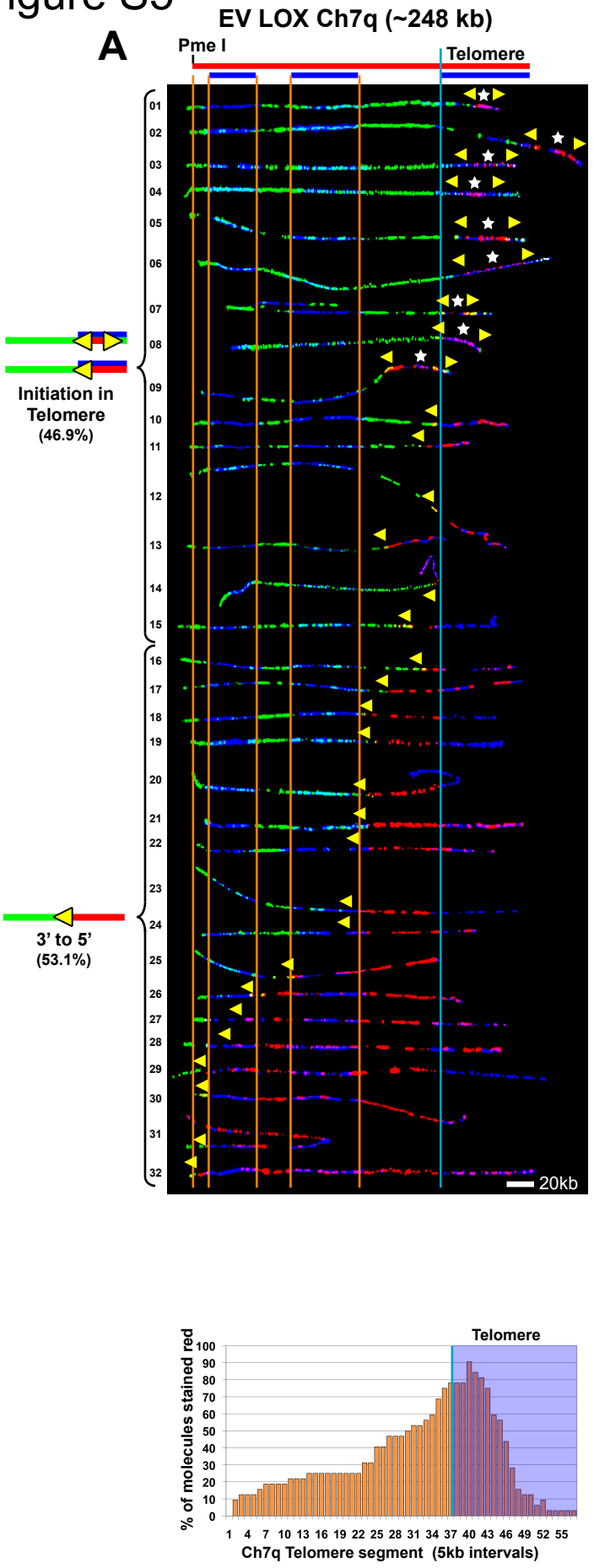

Telomere
% of molecules stained red
100
90
80
70
60
50
40
30
20
10
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
Ch10q Telomere segment (5kb intervals)

Figure S4: Expression of TRF2ΔB decreases replication initiation within the Ch10q telomere in LOX cells, related to Figure 3. SMARD analysis of the Ch10q telomere segment from LOX TRF2ΔB cells grown for 3 days in the presence of 1μg/ml doxycycline. Alignments of replicated molecules fully labeled with both IdU (red) and CldU (green) are shown, collected from three independent samples stretched on slides (56 fully red and 51 fully green labeled molecules were also collected). Vertical lines (orange and blue) demarcate the boundaries where FISH probes bind, as described in Figure 1. Symbols as in Figure 1. Replication profile histogram shown to the right of molecule alignment.

Figure S5


A
EV LOX Ch7q (~248 kb)
Pme I
Telomere
Initiation in Telomere (46.9%)
3' to 5' (53.1%)
20kb
% of molecules stained red
Telomere
Ch7q Telomere segment (5kb intervals)

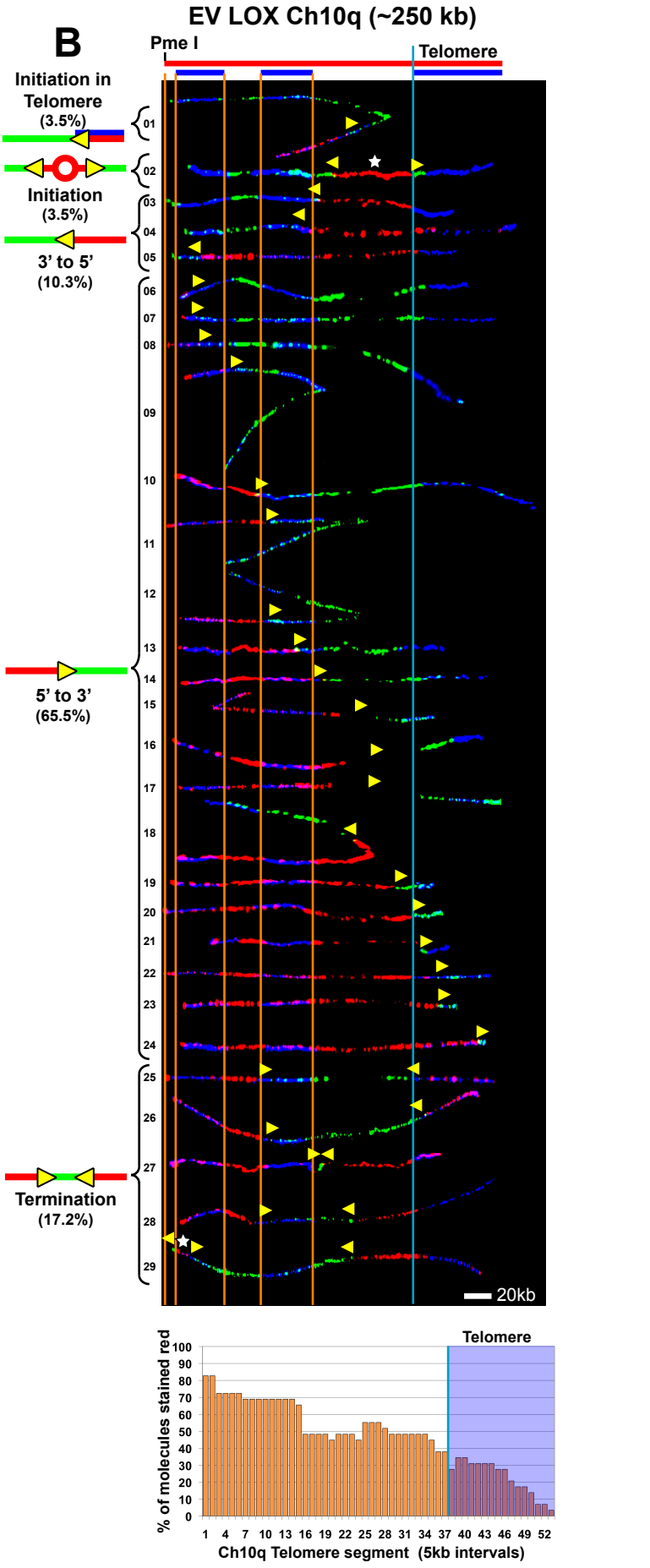

B
EV LOX Ch10q (~250 kb)
Pme I
Telomere
Initiation in Telomere (3.5%)
Initiation (3.5%)
3' to 5' (10.3%)
5' to 3' (65.5%)
Termination (17.2%)
20kb
% of molecules stained red
Telomere
Ch10q Telomere segment (5kb intervals)

Figure S5: Luc control shRNA knockdown in LOX cells related to Figure 6 (A) SMARD analysis of the Ch7q telomere segment from LOX cells inducibly expressing shRNA against Luciferase (Luc) control. Cells were grown in the presence of 1μg/ml doxycycline for 48 h prior to pulse-labeling with IdU followed by labeling with CldU in the absence of doxycycline. Alignments of replicated molecules fully labeled with both IdU (red) and CldU (green) are shown, collected from four independent samples stretched on slides (133 fully red and 119 fully green labeled molecules were also collected). (B) SMARD analysis of the Ch10q telomere segment from LOX cells inducibly expressing shRNA against Luc control as in (A). Alignments of replicated molecules fully labeled with both IdU (red) and CldU (green) are shown, collected from two independent samples stretched on slides (162 fully red and 149 fully green labeled molecules were also collected). Vertical lines (orange and blue) demarcate the boundaries where FISH probes bind, as described in Figure 1. Symbols as in Figure 1. Replication profile histograms shown under the molecule alignments.

Figure S6

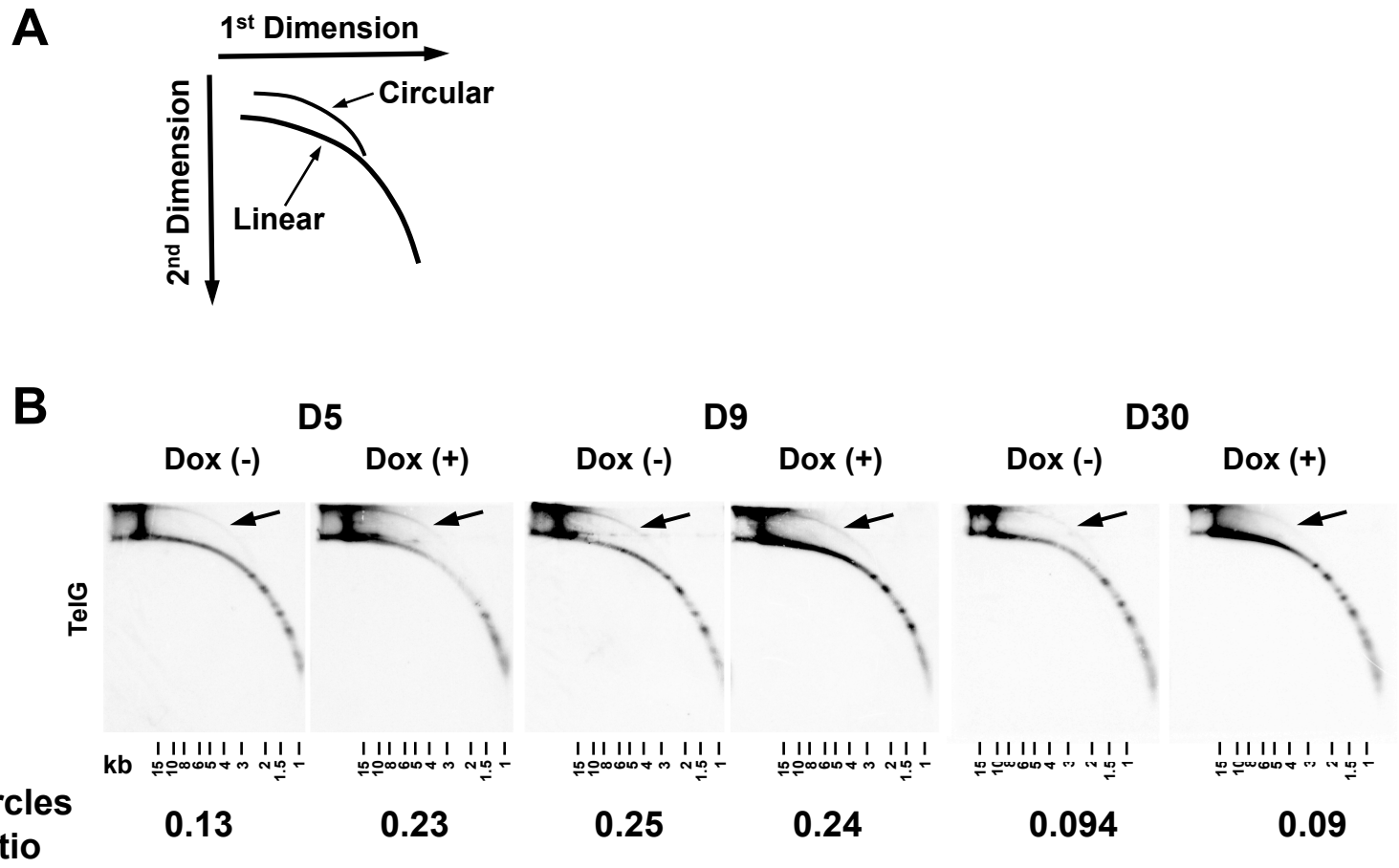


Figure S6: Expression of TRF2ΔB results in only slight and transient increases in telomeric circle formation in LOX cells, related to Figure 4. (A) Schematic representation of the migration of linear and circular telomeric DNA in 2D neutral/neutral (N/N) agarose gels. (B) Telomeric circle analysis of LOX TRF2Δ B cells grown in the absence (-) or presence (+) of 1µg/ml doxycycline (Dox) for 5 (D5), 9 (D9), or 30 (D30) days. Telomere-repeat restriction digest (AluI/MboI) fragments were analyzed by Southern blotting of 2D N/N agarose gels probed with 32P-labeled (TTAGGG)4 repeat (Tel G) sequences. Telomere circles (T circles) are indicated by arrows. A quantification of circular relative to linear telomeric DNA (T circles ratio) is indicated below. Positions of size standards (in kb) after the 1st dimension of electrophoresis are also shown below blots.