



## Supplementary Figure 1. Eroded telomeres are rearranged in quiescence.

a) Genomic DNA from quiescent *ter1*+ and *ter1*D cells was digested by *EcoR*I and analyzed by Southern blot with Telo/STE1 and chromosomal probes. 1, 4, 6, and 8 correspond to the number of days in G0 before harvesting. For *ter1* $\Delta$  cells, S1, S3, S5 and S7 indicate the number of days in senescence before cells were starved from nitrogen. STEEx are shown by asterisks

**b)** A senescence kinetics was performed from a strain expressing a catalytically dead version of the telomerase  $(trt1^{D743A})$  after the loss of a plasmid bearing trt1+. At days 1, 3 and 5 of senescence,  $trt1^{D743A}$  cells were starved from nitrogen and kept for 8 days in quiescence. Cells were collected at different time points of the senescence kinetics (S1-S5) and for S1, S3, and S5 cells were starved from nitrogen and collected after 1, 4, and 8 days in quiescence. A *ter1*D strain was used as control. Genomic DNA was extracted, digested with *EcoR*I and analyzed by Southern blot with the STE1/Telo probe. STEEx was detected in the  $trt1^{D743A}$  strain according to its *Nsi*I pattern (Supplementary Figure 3) and are shown by asterisks

>pNSU64 **TCAGATGCATAAAAAATTTGAAGTTGGTATGTATTGAGTGTGTGGAGTACGGTAAGTATAATAGGGGTAAATAA** Nsil AATGGGTAAAAAAAATTTTGAAATGTGTGGGAAGTTGAGTATGTTGGAGTACATTAAGTAGATTACAGTTGTGGAG pNsil Fw  ${\tt ATTAGGAGATATAATGAGATATGGTGAATAAAAAGTTGAAATGTGTGGGGCTTGAGTGCGGTTAGGGTGCAGTAAGT$ pNsil Rev AGAATAAAGGGGCG<mark>CAGTGTATTATGATAATTAAAATGG</mark>ATGAAAAATTTGAAGTTCACTCAGTCATAATTAATT pApaI Fw GGGTAACGGAGTAACAATATAGAATAAAGGGAATTTAGGAAGTGCGGTAAGTTGAATAAAGAAATAGAAATGAAA HRS ApaI pApaI Rev pSwal Fw ATAATTAAGCTGCGTTATTTATAAAAATTTAAAATTTACTTAAGTTTTTTCACATATACAATGCCCCCACTATTG SwaI pSwal Rev AGGTGTTGGAGTAGATTAAGTAGAATACGAGGATGTAGGGTAGTGCAATAGTGAAGATGGACAAAAAGTTGAAGT TCATGGAATTAGACTATGTTGGAATTCACTAATTGTAATACGGTGGTGTAGTGTGCATGGGTGAATAAAACGGAT EcoRI GAAAAATTTGAAGTTGATTTGAACTGAGTGTGTGTTAAAGTTCATTAAGTATAATACGGTGATGTAATGTACTATAG TATTTAGGATAGATAAAAAATTTGAAGTTGTATGAATTGAGTGTGCTGGAATACGTTAAGTATAATACAGTGATG TAGTGTACTATAATAATTAGGATGGGTAAAAAATTTGAAGTTGTATGAATTGAGTGTGTTATAGTTCATTAAGTA TAATACGGTGATGTAGTGTACTATAGGATTGGGATGGGTAAAAAATTGAAATGTGTGGGAATTGAGTATGTTGAA ATTCACTAAGTGTAATACTGTAGTGCAGCGTAATATGGTAATGGTAATGGATGAAAAATTTGAAGTTCACTCAGT CATAATTAATTGGGTTACAAAGTAACAATGTAGAATAAGGAGCAATTAGAACGTACGGTAATATAGATAAAGAAA GATAATTAAGTTGCGTTATTTATAAAAATTTAAATTTACTTAAGTTTTTTCACATATATAAAATGCCCCCACTA Swal AAAAGGTGTTGGAGTAGATTAAGTAGAATACGAGGATGTAGGGTAGTGCAATAGTGAAGATGGACAAAAAGTTGA AGTTCATGGAATTAGACTATGTTGGAATTCACTAATTGTAATAAGGTGGTGTAGTGTGTATGGGGTGAATAAAACG ECORI GATGAAAAATTTGAAGTTGATTTGAACTGAGTGTGTGTTAAAGTTCATTAAGTATAATACGGTGATGTAGTGTACTA TAGTATTTAGGATGGGTAAAAAATTGAAATGTGTGGGAATTGAGTGCTGGAGTACGTTAAGTATAATACGGTGA **GGTAGTGTACTATAATAATTAGGATGGTTAAAAAATTTGAAGTTGTATGAATTGAGTGTGTTAGAGTTCATTAAG** TATAATACGGTGATGTAGTGTACTATAGGATATTTAGGATAGGTAAAAAATTTGAAGTTGTATGAATTGAGTGTGTGT AGAGTTCATTAAGTATAATACGGTGAGGTAGTGTACTATAGTATTTAGGATGGGAAAAAATTTGAAGTTGTATGA **ATTGAGTGTGTTAGAGTTCATTAAGTATAATACGGTGAGGTAGTGTACTATAGTATTTAGGATGGGAAAAAATTG** AAATGTGTGGAATTGAGTATGGTGAAATTCACTAAGTGTAATACAGTAGTGCAGTGTATTATGATAATTAAAATG pApaI Fw GATGAAAAATTTGAAGTTCACTCAGTCATAATTAATTGGGTAACGGAGTAACAATATAGAATAAAGGGAATTTAG TERRA TSS HRS TTTCACAAATACAATGCCCCCACTATTGGGCCCCACCGTCAGCCGAGCCGTAAGGCGAGGCTGCGGGTTACAAG ApaI pApaI Rev GTTACGTGGTTACACGGTTACAGGTTACAGGGTTACAGGGGGGTTACGGGTTACAGGGGTTACGGTTAC CAGGGTTACAGGTTACACGGTTACAGGTTACAGGGTTACACGGTTACAGGTTACAGGGTTACAGGGTTACA GGGGTTACGGTTACAGGGTTACAGGGGGGGTTACAGGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGGTTACAGGGGTTACAGGGGGTTACAGGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGGTTACAGGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGGTTACAGGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGGTTACAGGTTACAGGGTTACAGGTTACAGGTTACAGGGTTACAGGGTTACAGGTTACAGGTTACAGGGTTACAGGGTTACAGGTTACAGGTTACAGGTTACAGGGTTACAGGGTTTACAGGGTTTACAGGGTTTACAGGGTTTACAGGGTTTACAGG

AGGGTTACACGGTTACAGGGTTAC

CACGGTTACAGGGTTAC

>pNSU70

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ATTAGGAGATATAATGAGATATGGTGGAATAAAAAGTTGAAATGGTGG
pNsil Rev
AGAATAAAGGGGGCG <mark>CAGTGTATTATGATAATTAAAATGG</mark> ATGAAAAATTTGAAGTTCACTCAGTCATAATTAATT
pApaI Fw
GGGTAACGGAGTAACAATATAGAATAAAGGGAATTTAGGAAGTGCGGTAAGTTGAATAAAGAAATAGAAATGAAA
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pSwal Fw
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SwaT
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GAAAAATTTGAAGTTGATTTGAACTGAGTGTGTGTTAAAGTTCATTAAGTATAATACGGTGATGTAGTGTACTATAG
TATTTAGGATGGGTAAAAAATTGAAATGTGTGGGAATTGAGTGTGCTGGAGTACGTTAAGTATAATACGGTGAGGT
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GGTTACAGGTTACACGGTTACAGTTTACGGTTACAGGGTTACACGGTTACAGGGTTACAGGGGTTACGGTTACAC ACGGTTACAGGGGTTACAGG

Supplementary Figure 2. Subtelomeric region sequences of pNSU70 and pNSU64. The homologous repeated sequence (HRS, grey box), colored oliogonucleotides used for RT-qPCR, and restriction sites are annotated in subtelomeric sequences of pNSU64 and pNSU70.



Supplementary Figure 3. Subtelomeric patterns of strains used in this study. Genomic DNA of the indicated strains was digested by *Nsi*l and analyzed by Southern blot with a STE1 probe.



## Supplementary Figure 4. Cell viability at G0 exit.

a) EcoRI-digested genomic DNAs from senescent and nitrogen-starved ter1D cells were analyzed by Southern blot.
b) Senescent (Sen) and quiescent ter1D cells after 1, 4, or 8 days in G0 were micromanipulated and plated onto YES plate. The percentage of cells that were not able to form a colony was plotted. Pictures of agar plates are shown
c) Fifteen clones that were able to exit G0 after 8 days of quiescence were grown for approximately 32 pds. Their genomic DNA was then extracted, digested by EcoRI and analyzed by Southern blot with a Telo/STE1 probe. STEEx are shown by asterisks.