APPENDIX

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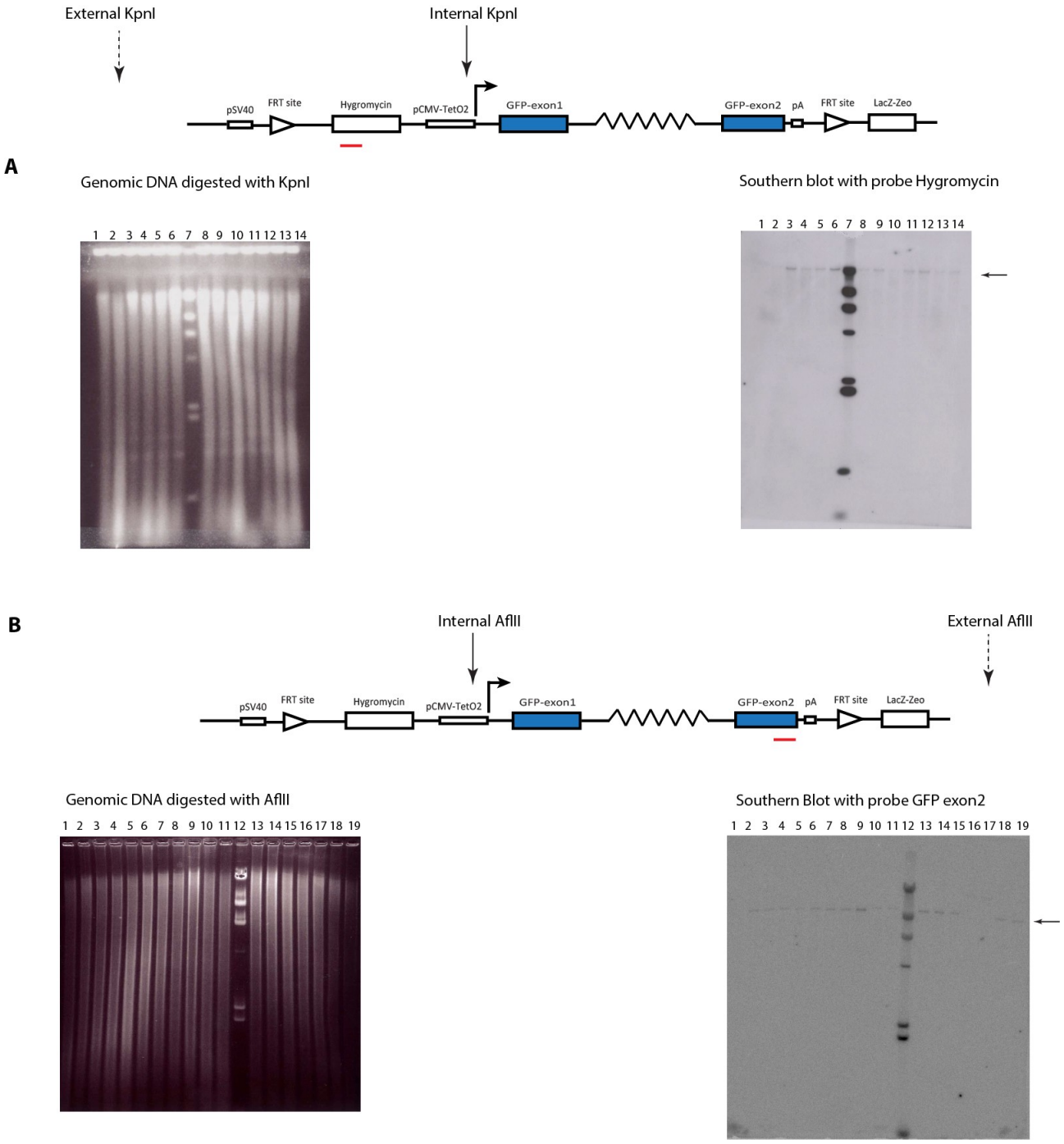
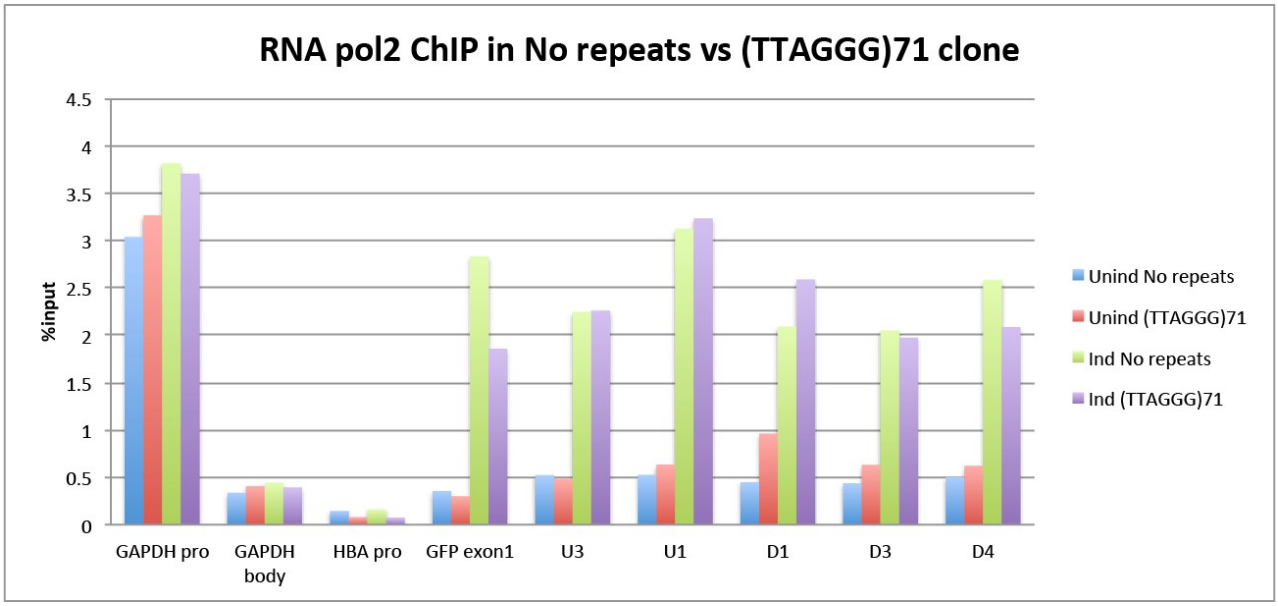


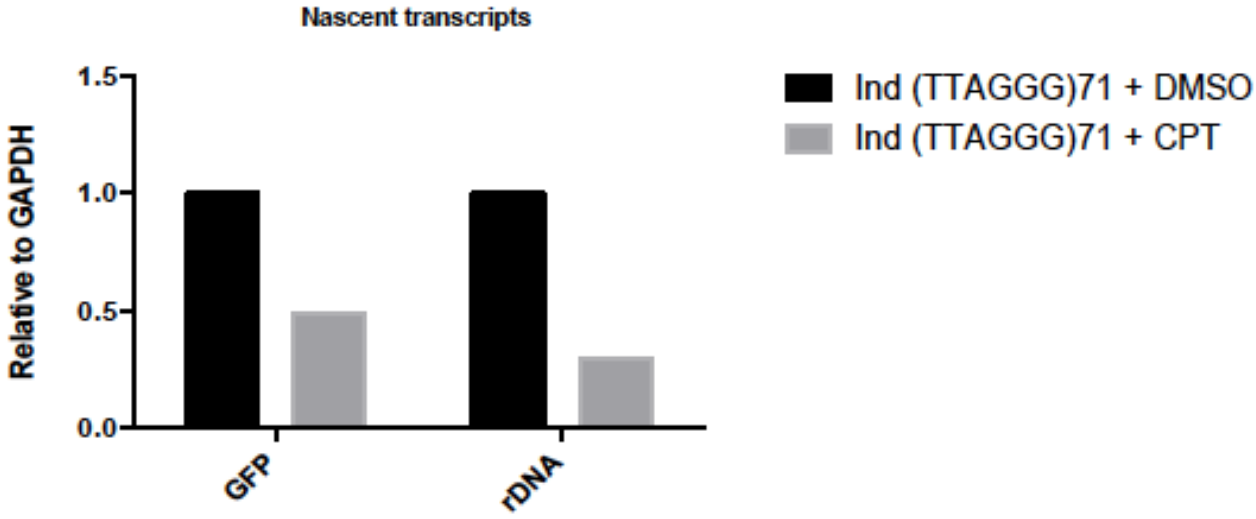
Appendix Figure S1. Southern blot showing single integration of the ectopic locus in 293 T-Rex. A) Verification of single integration of the ectopic ΨζVNTR constructs. Genomic DNA from the transfected clones were digested with KpnI, which cuts once in the

ectopic locus by an internal KpnI site (solid line arrow) and presumably also cuts outside the locus by an external site (a dashed arrow). The red line indicates the position of the probe used for Southern Blot analysis. The agarose gel image shows KpnI digest of genomic DNA from the indicated clones. Lane 1: untransfected 293TR-Ex (negative control), lane 2-3: clones containing No VNTR (clone N2S-4 and N2S-9), lane 4-6: clones containing 140bp ΨζVNTR (clone N1-2, N1-4 and N1-5), lane 7: Lamda DNA HindIII digest ladder, lane 8-9: clones containing 240bp ΨζVNTR (clone N2-1 and N2-2), lane 10-11: clones containing 390bp ΨζVNTR (clone N3-1 and N3-2), and lane 12-14: clones containing 490bp ΨζVNTR (clone LB8-7, LB8-8 and LB8-9). Southern Blot assay of the KpnI digest using P^{32} labeled probe Hygromycin (red line in the diagram) shows single bands, suggesting single integration events, in the indicated clones except for the untransfected 293T-REx.

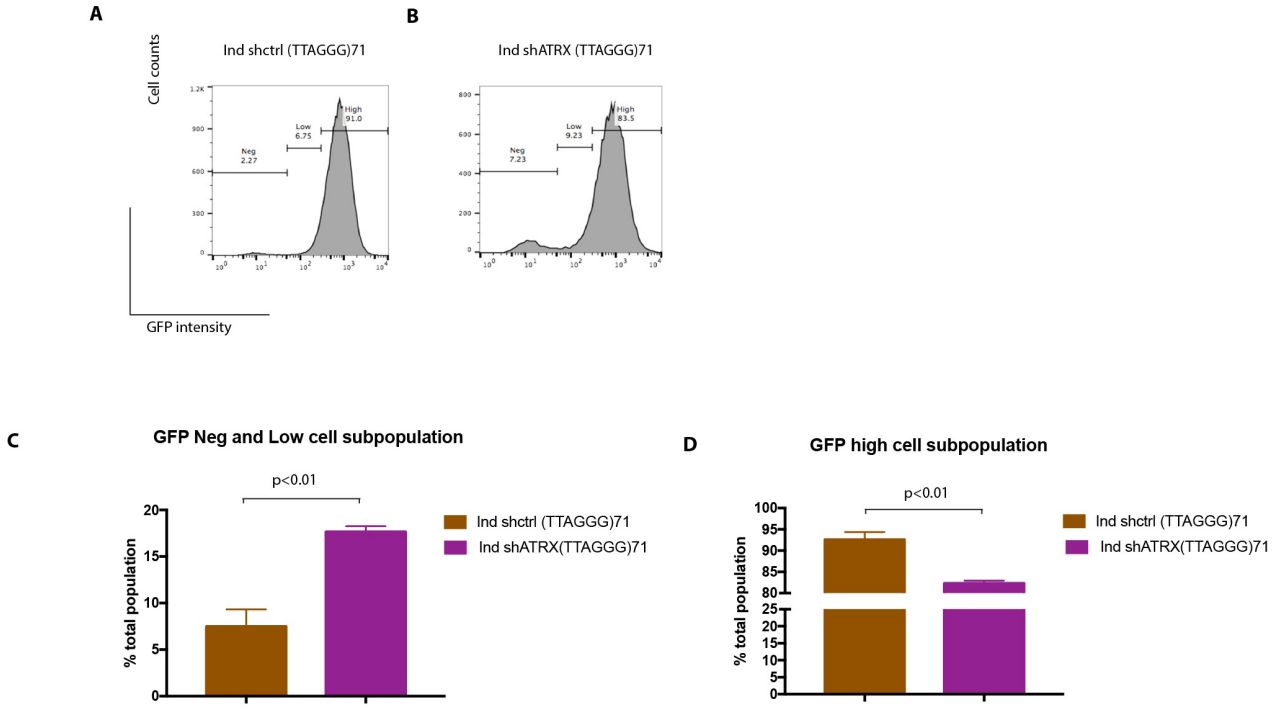
B) Verification of single integration of the ectopic telomere constructs. Genomic DNA from the transfected clones were digested with AflII, which cuts once in the ectopic locus by an internal AflII site (solid line arrow) and presumably also cuts outside the locus by an external site (a dashed arrow). The red line indicates the position of the probe used for Southern Blot analysis. The agarose gel image shows AflII digest of genomic DNA from the indicated clones. Lane 1: untransfected 293TR-Ex (negative control), lane 2: a clone containing 490bp ΨζVNTR (LB8-7, positive control), lane 3-6: clones containing GAA repeat, lane 7-11: Clones containing $(TTAGGG)_{42}$ (clone 7, 6, 5, 3 and 1, respectively), lane 12 marker, lane 13-19: clones containing $(TTAGGG)_{71}$ (clone 7, 6, 5, 4, 3, 2 and 1, respectively). Southern blot assay of the AflII digest using P^{32} labeled probe GFP exon2 (red line in the diagram) shows single bands, suggesting single integration events, in the indicated clones except for the untransfected 293T-REx.



Appendix Figure S2. RNA Pol II is recruited to the transcribed gene regardless of the presence of telomeric repeats. RNA Pol II ChIP analysis in stable clones containing either no repeat or the $(TTAGGG)_{71}$ repeat uninduced or after induction of transcription by the addition of 1µg/mL doxycycline for 24 hours. The enrichment of RNA Pol II is represented as %input the GAPDH promoter was used as a positive control and the HBA promoter as a negative control.



Appendix Figure S3. **Camptothecin treatment alters transcription.** Real time-quantitative PCR analysis of nascent transcripts over the indicated region from cells with the ectopic telomeric sequence $(TTAGGG)_{71}$ grown in medium containing 1μg/mL doxycycline for 24 hours, then treated with 10μM camptothecin or DMSO solvent for 30 minutes. Values are relative to GAPDH. Data bars are represented as means from two independent experiments.



Appendix Figure S4. FACS analysis of induced cells. **A-B)** Representative graph showing FACS analysis of GFP expression upon transcription induction by 1µg/mL Doxycycline for 24 hours in **A)** cells with the ectopic sequence $(TTAGGG)_{71}$ treated with shRNA control. **B)** cells with the ectopic sequence $(TTAGGG)_{71}$ treated with shRNA against ATRX (shATRX-2). Each cell population is divided into 3 sub-population: Neg which are cells that do not express GFP, Low which are cells that express a low level of GFP and High which are cells that express a high level of GFP. The numbers indicate percentage of the whole population. **C-D)** Graphs showing average quantitation of GFP negative and GFP low expressing cells, cells expressing a high level of GFP by FACS analysis in the graph A-B from three independent experiments in each case (+/- S.E.M.).