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Supplemental information

TCAB1 prevents nucleolar accumulation of the telomerase RNA to facilitate telomerase assembly

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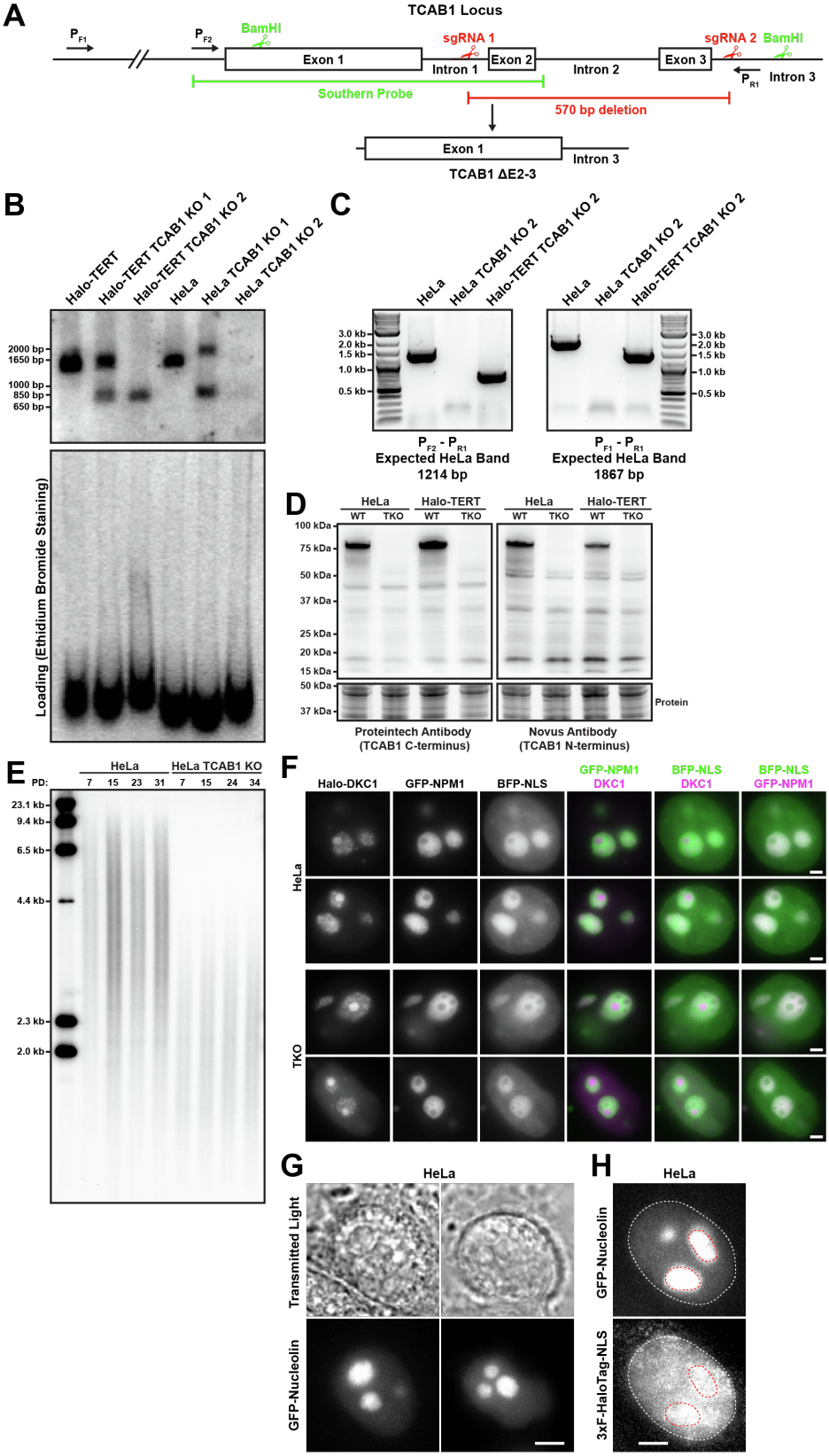
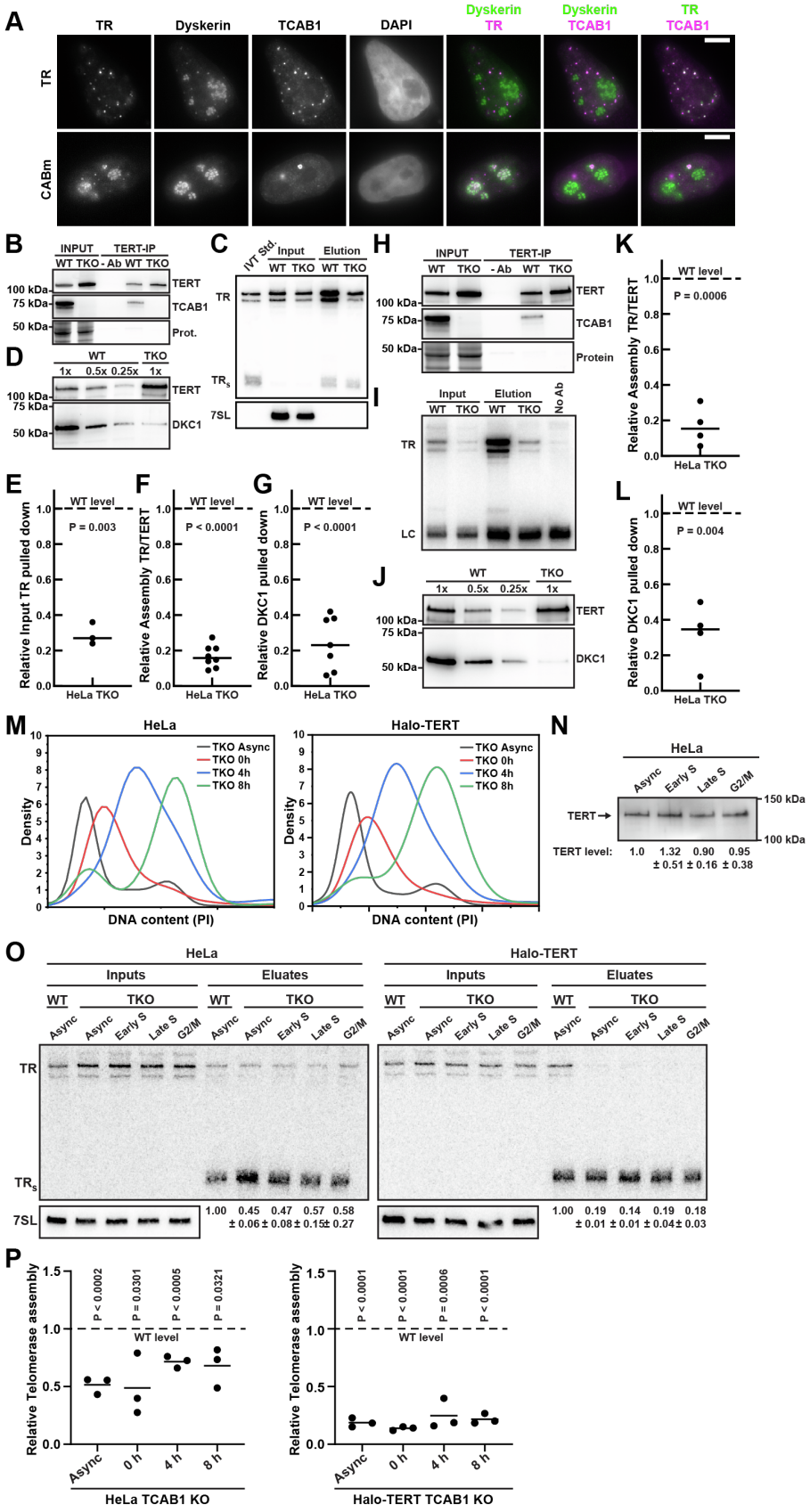
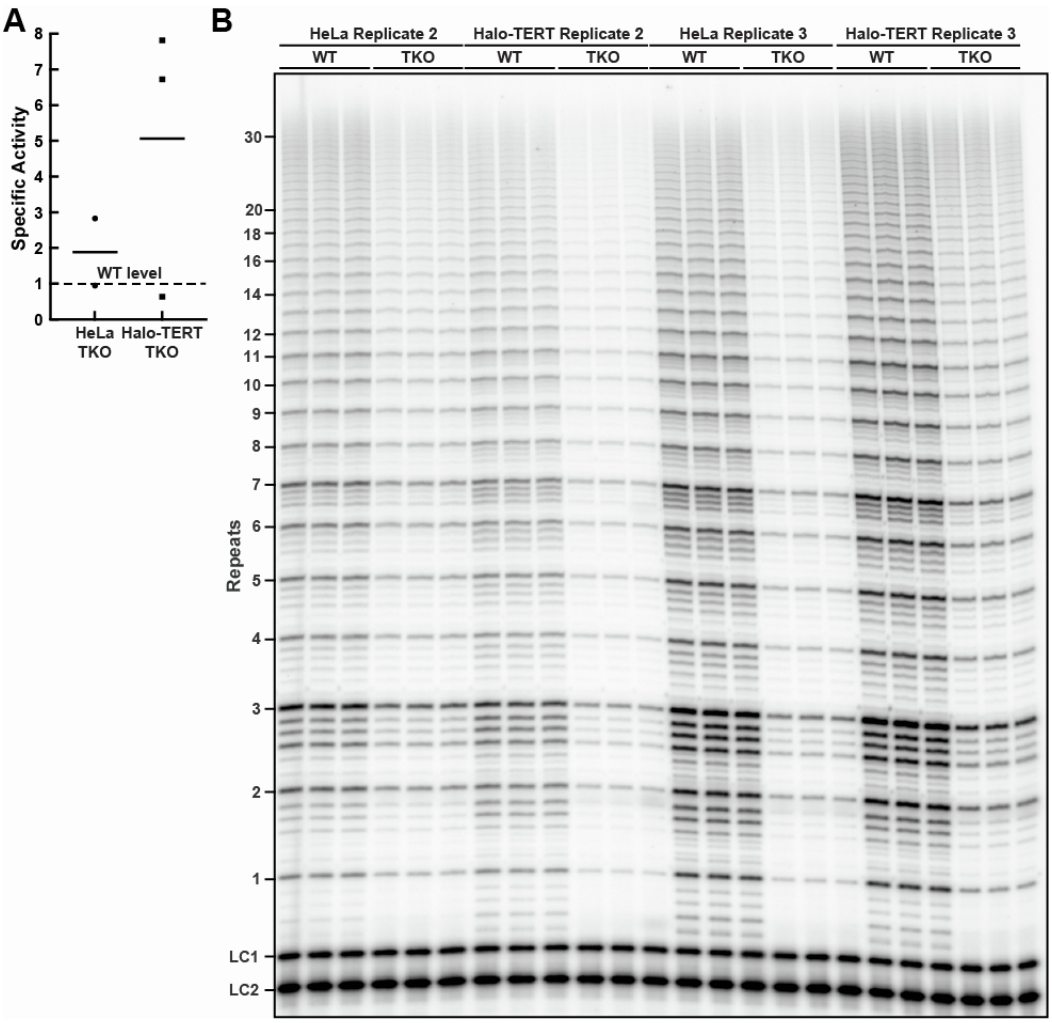
Supplementary Information



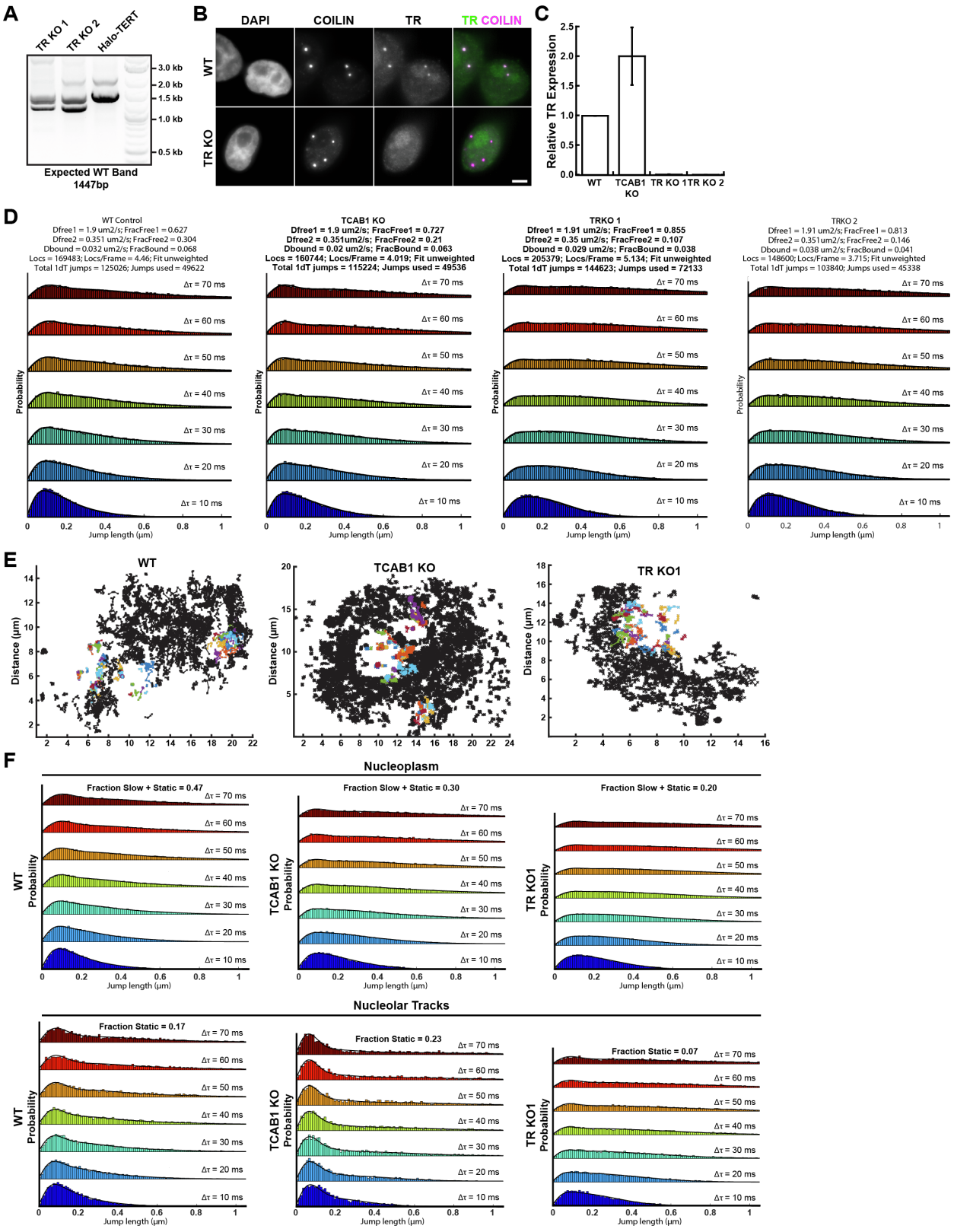
Supplementary Figure 1. TCAB1 knock-out strategy and characterization and analysis of nucleolar marker protein localization, Related to Figure 1. (A) Strategy to knock-out TCAB1 using Cas9 and two sgRNAs targeting introns 1 and 3. **(B)** Southern blot of genomic DNA digested with BamHI from parental cells and TCAB1 knock-out clones using a probes generated from a PCR product of the TCAB1 gene indicated in **(A)** demonstrating the expected truncation of the *TCAB1* gene in Halo-TERT TCAB1 KO 2. HeLa TCAB1 KO 2 carries larger deletions completely removing exons 1 and 2 from the *TCAB1* gene. **(C)** PCR using primers indicated in **(A)** of genomic DNA from parental cells and TCAB1 knock-out clones confirming the deletion of critical regions of the *TCAB1* gene show in **(B)**. **(D)** Western blots demonstrating the absence of TCAB1 protein in TCAB1 knock-out cell lines generated in HeLa and Halo-TERT cells lines using two antibodies targeting the N-terminus and C-terminus of TCAB1. **(E)** Telomere length analysis by Southern blot of telomeric restriction fragments, indicating that telomeres in TCAB1 knock-out cells are short but stable in length. **(G)** Images of HeLa cells transiently expressing GFP-nucleolin to mark nucleoli. The GFP-nucleolin signal overlaps with circular shapes visible under transmitted light illumination (scale bar = 2 μm). **(H)** Images of HeLa cells transiently expressing GFP-nucleolin and 3xFLAG-HaloTag-NLS labeled with JF646. The 3xFLAG-HaloTag-NLS signal (maximum intensity projection of 1000 frames of a single-molecule imaging movie) clearly overlaps with the GFP-nucleolin signal (red dashed outline), demonstrating that 3xFLAG-HaloTag-NLS can enter the nucleolus (scale bar = 2 μm).


A
TR
Dyskerin
TCAB1
DAPI
Dyskerin TR
Dyskerin TCAB1
TR TCAB1
CABm
B
INPUT
TERT-IP
WT TKO - Ab WT TKO
100 kDa
75 kDa
50 kDa
TERT
TCAB1
Prot.
C
IVT Std.
Input
Elution
WT TKO WT TKO
TR
TRs
7SL
D
WT
TKO
1x 0.5x 0.25x 1x
TERT
DKC1
H
INPUT
TERT-IP
WT TKO - Ab WT TKO
TERT
TCAB1
Protein
I
Input
Elution
No Ab
WT TKO WT TKO
TR
LC
J
WT
TKO
1x 0.5x 0.25x 1x
TERT
DKC1
K
WT level
P = 0.0006
Relative Assembly TR/TERT
HeLa TKO
L
WT level
P = 0.004
Relative DKC1 pulled down
HeLa TKO
E
WT level
P = 0.003
Relative Input TR pulled down
HeLa TKO
F
WT level
P < 0.0001
Relative Assembly TR/TERT
HeLa TKO
G
WT level
P < 0.0001
Relative DKC1 pulled down
HeLa TKO
M
HeLa
Halo-TERT
TKO Async
TKO 0h
TKO 4h
TKO 8h
Density
DNA content (PI)
N
HeLa
Async
Early S
Late S
G2/M
TERT
150 kDa
100 kDa
TERT level:
1.0 1.32 0.90 0.95
± 0.51 ± 0.16 ± 0.38
O
HeLa
Halo-TERT
Inputs
Eluates
WT
TKO
Async
Early S
Late S
G2/M
TR
TRs
7SL
1.00 0.45 0.47 0.57 0.58
± 0.06 ± 0.08 ± 0.15 ± 0.27
1.00 0.19 0.14 0.19 0.18
± 0.01 ± 0.01 ± 0.04 ± 0.03
P
Relative Telomerase assembly
P < 0.0002
P = 0.0301
P < 0.0005
P = 0.0321
WT level
Async
0 h
4 h
8 h
HeLa TCAB1 KO
P < 0.0001
P < 0.0001
P = 0.0006
P < 0.0001
Halo-TERT TCAB1 KO

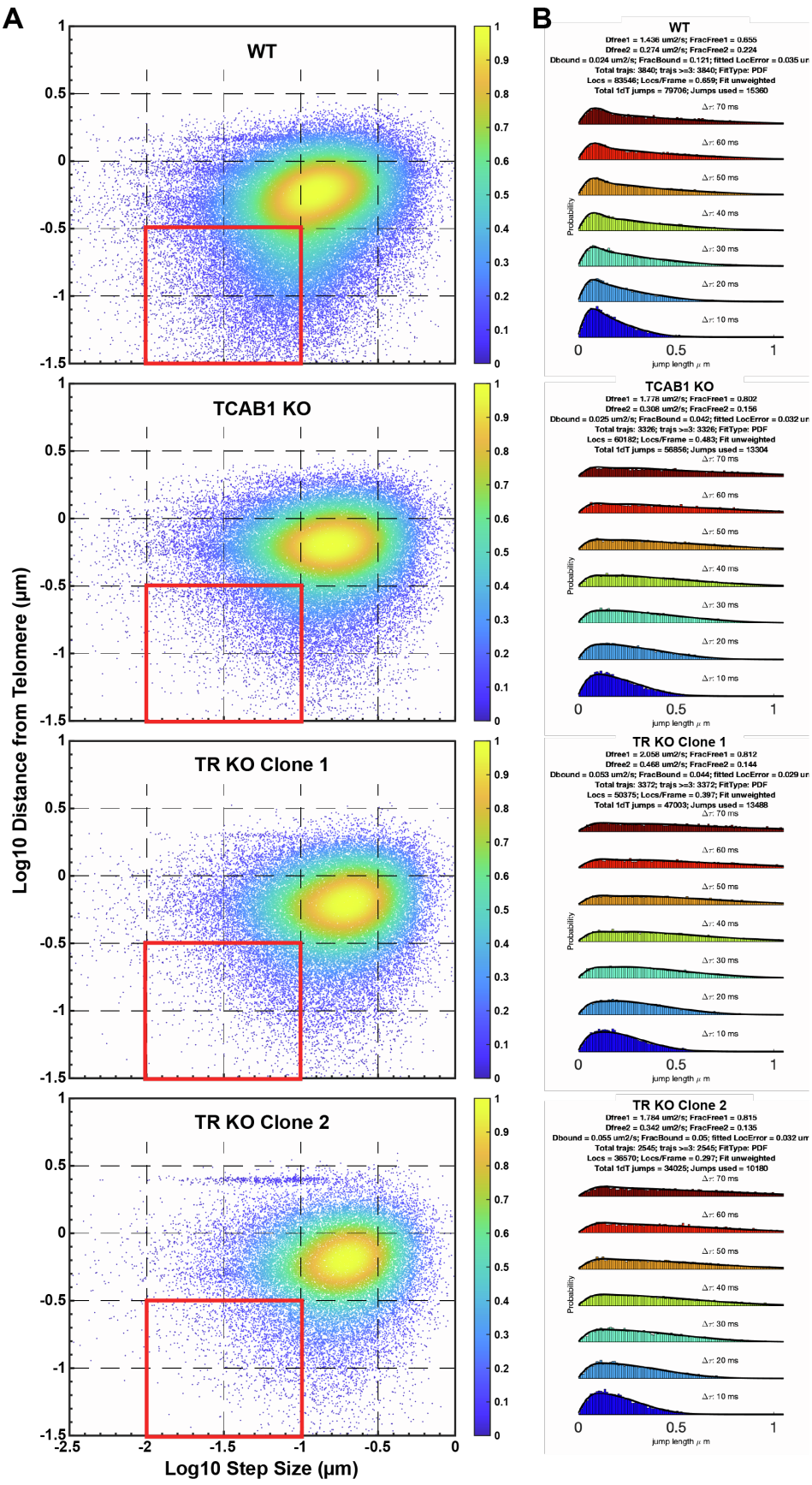
Supplementary Figure 2. Telomerase assembly is reduced in the absence TCAB1 after telomerase over-expression and throughout the cell-cycle, Related to Figure 2. (A) Immuno-fluorescence with anti-dyskerin and anti-TCAB1 antibodies coupled to fluorescence in-situ hybridization with probes against TR, demonstrating TR localization to nucleoli in cells expressing TERT and TR G414C (CABm) (scale bar = 5 μm). **(B-L)** TERT immuno-purification (using a sheep anti-TERT antibody) from **(B-G)** Hela cells and the corresponding TCAB1 knock-out cells and **(H-L)** Halo-TERT cells and the corresponding TCAB1 knock-out cells, overexpressing TERT and TR. **(B, H)** Western blots analyzing TERT immuno-purification (using a sheep anti-TERT antibody) probed with a rabbit anti-TERT antibody (Abcam) and a TCAB1 antibody. **(C, I)** Northern blot of RNA extracted from input and purified TERT samples from probed with radiolabeled DNA oligonucleotides complementary to TR. Standards are *in vitro* transcribed full-length TR and truncated TR_S. TR_S was added to samples prior to RNA extraction as loading and recovery control. **(D, J)** Western blots to analyze immuno-purified telomerase RNP composition. A single membrane was cut into two pieces that were probed with TERT and dyskerin antibodies, respectively. (**E**) Quantification of the amount of TR purified relative to input TR in TERT purifications from TCAB1 knock-out cells overexpressing TERT and TR compared to parental controls (n = 3, mean, T-Test). **(F, K)** Quantification of the ratio of TR to TERT (n = 8, n = 4) in TERT purifications from TCAB1 knock-out cells overexpressing TERT and TR compared to parental controls (mean, T-Test). **(G, L)** Quantification of the amount of dyskerin (n = 7, n = 4) in TERT purifications from TCAB1 knock-out cells overexpressing TERT and TR compared to parental controls (mean, T-Test). The dashed lines indicate the level in telomerase purified from wild-type TCAB1 control cells which was normalized to 1.0. **(M)** DNA content analysis (PI staining) by flow cytometry of synchronized cell populations used for telomerase purifications. **(N)** Western blots analyzing endogenous TERT immuno-purifications (using a sheep anti-TERT antibody) from synchronized control HeLa and TCAB1 knock-out cells probed with a rabbit anti-TERT antibody (n = 3, mean ± SD). **(O)** Northern blot of RNA extracted from input and purified TERT samples from asynchronous control HeLa cells and asynchronous and synchronized TCAB1 knock-out cells probed with radiolabeled DNA oligonucleotides complementary to TR. Standards are *in vitro* transcribed full-length TR and truncated TR_S. TR_S was added to samples prior to RNA extraction as loading and recovery control. Input samples were also probed for 7SL RNA as loading control (n = 3, mean ± SD). **(P)** Quantification (n = 3, mean, T-Test) of fraction of input TR (normalized to 7SL) co-purified with TERT or HaloTag-TERT (normalized to TR_S) from TCAB1 knock-out cells relative to control cells (dashed line).



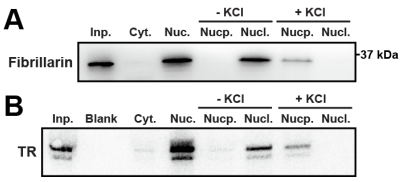
Supplementary Figure 3. The specific activity of telomerase is not reduced in the absence of TCAB1, Related to Figure 3. (A) Specific activity of endogenous telomerase purified from TCAB1 knock-out cells relative to parental controls. Specific activity was calculated by dividing the relative activity (see Fig. 3A,B) by the relative amount of TR present in immuno-purified TERT samples (Fig. 2B). The dashed lines indicate the activity level in telomerase purified from wild-type TCAB1 control cells which was normalized to 1.0 (n = 2-3, mean). **(B)** Direct telomerase extension assay of overexpressed telomerase immuno-purified from parental (WT) and TCAB1 knock-out (TKO) HeLa and Halo-TERT cell lines. LC1 and LC2, radiolabeled DNA oligonucleotide loading controls.


A
TR KO 1
TR KO 2
Halo-TERT
3.0 kb
2.0 kb
1.5 kb
1.0 kb
0.5 kb
Expected WT Band
1447bp
B
DAPI
COILIN
TR
TR COILIN
WT
TR KO
C
Relative TR Expression
WT
TCAB1 KO
TR KO 1
TR KO 2
D
WT Control
Dfree1 = 1.9 um2/s; FracFree1 = 0.627
Dfree2 = 0.351 um2/s; FracFree2 = 0.304
Dbound = 0.032 um2/s; FracBound = 0.068
Locs = 169483; Locs/Frame = 4.46; Fit unweighted
Total 1dT jumps = 125026; Jumps used = 49622
TCAB1 KO
Dfree1 = 1.9 um2/s; FracFree1 = 0.727
Dfree2 = 0.351um2/s; FracFree2 = 0.21
Dbound = 0.02 um2/s; FracBound = 0.063
Locs = 160744; Locs/Frame = 4.019; Fit unweighted
Total 1dT jumps = 115224; Jumps used = 49536
TRKO 1
Dfree1 = 1.91 um2/s; FracFree1 = 0.855
Dfree2 = 0.35 um2/s; FracFree2 = 0.107
Dbound = 0.029 um2/s; FracBound = 0.038
Locs = 205379; Locs/Frame = 5.134; Fit unweighted
Total 1dT jumps = 144623; Jumps used = 72133
TRKO 2
Dfree1 = 1.91 um2/s; FracFree1 = 0.813
Dfree2 = 0.351 um2/s; FracFree2 = 0.146
Dbound = 0.038 um2/s; FracBound = 0.041
Locs = 148600; Locs/Frame = 3.715; Fit unweighted
Total 1dT jumps = 103840; Jumps used = 45338
Δτ = 70 ms
Δτ = 60 ms
Δτ = 50 ms
Δτ = 40 ms
Δτ = 30 ms
Δτ = 20 ms
Δτ = 10 ms
Probability
Jump length (μm)
E
WT
TCAB1 KO
TR KO1
Distance (μm)
F
Nucleoplasm
Fraction Slow + Static = 0.47
Fraction Slow + Static = 0.30
Fraction Slow + Static = 0.20
Nucleolar Tracks
Fraction Static = 0.17
Fraction Static = 0.23
Fraction Static = 0.07

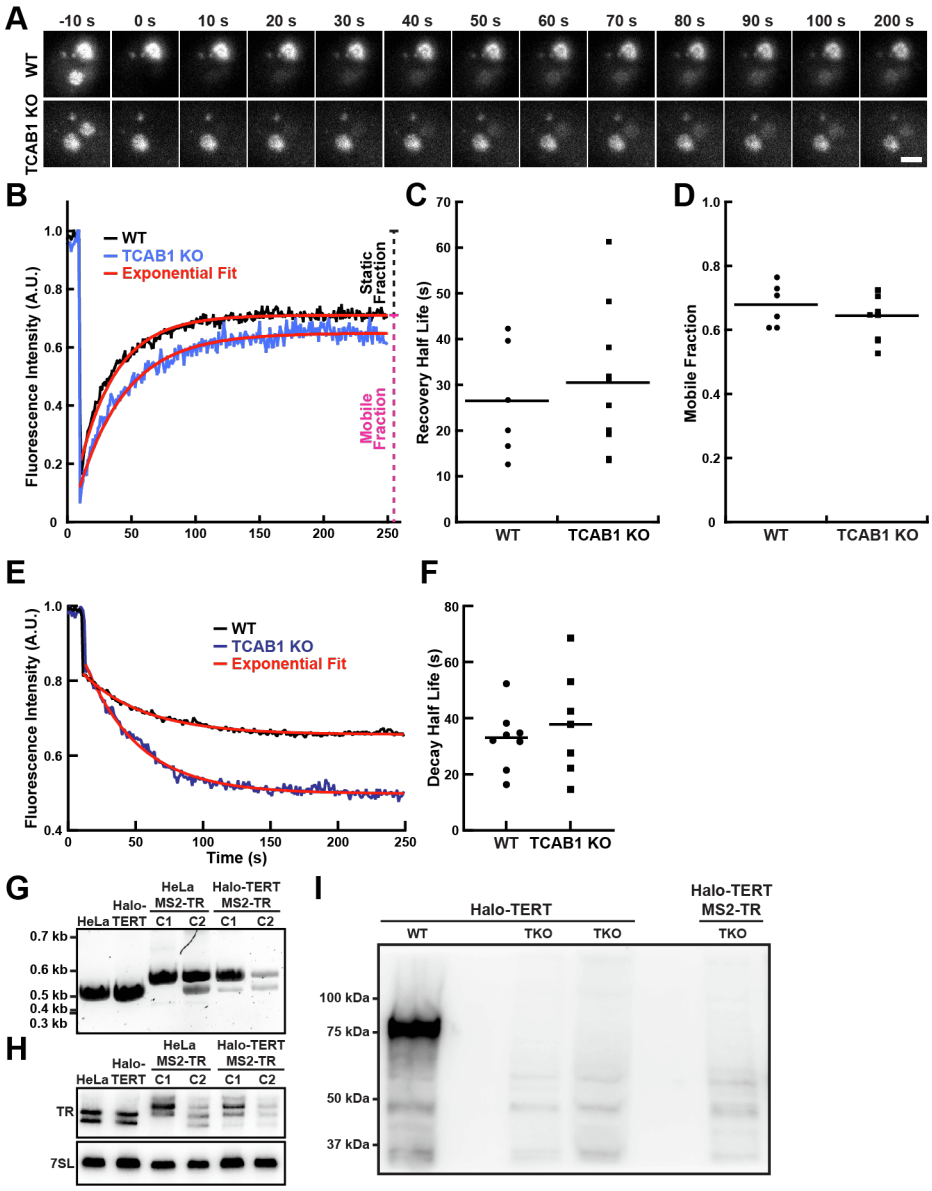
Supplementary Figure 4. Analysis of TERT diffusion dynamics in the absence of TCAB1 and TR in the nucleus and the nucleolus, Related to Figure 4. (A) PCR analysis of the TR locus in parental and TR knock-out clones. Both TR knock-out clones show PCR products with reduced length that were confirmed to be knock-outs by Sanger sequencing. **(B)** Images of control and TR knock-out cells probed with an antibody against coilin and FISH probes specific for TR, demonstrating the lack of TR signal in TR knock-out cells (scale bar = 5 μm). **(C)** Determination of TR levels in control, TCAB1 knock-out, and TR knock-out cells, using RT-qPCR with primers specific to TR normalized to GAPDH (3 independent biological replicates, 3 technical replicates for each biological replicate, mean ± SD). **(D)** Probability density function of step sizes of HaloTag-TERT molecules from control, TCAB1 knock-out, and TR knock-out cells and the corresponding 3-state model fit using the spot on tool (Data from one of three biological replicates, >15 cells per cell line) (Fig. 4A-B) **(E)** Graphs of HaloTag-TERT tracks that are nucleoplasmic (black) or overlap with the nucleolus for at least one frame (color) in control (WT), TCAB1 knock-out, and TR knock-out cells. **(F)** Probability density functions of the step-sizes derived from HaloTag-TERT molecules that overlap with the nucleoplasm or the nucleolus for at least one frame and the corresponding 3-state model fit using the SpotOn tool (pooled data from 3 independent experiments, >18 cells total per cell line).


A
B
WT
TCAB1 KO
TR KO Clone 1
TR KO Clone 2
Log10 Distance from Telomere (μm)
Log10 Step Size (μm)
Probability
jump length μ m
Dfree1 = 1.436 um2/s; FracFree1 = 0.855
Dfree2 = 0.274 um2/s; FracFree2 = 0.224
Dbound = 0.024 um2/s; FracBound = 0.121; fitted LocError = 0.035 um
Total trajs: 3840; trajs >=3: 3840; FitType: PDF
Locs = 83546; Locs/Frame = 0.659; Fit unweighted
Total 1dT jumps = 79706; Jumps used = 15360
Dfree1 = 1.778 um2/s; FracFree1 = 0.802
Dfree2 = 0.308 um2/s; FracFree2 = 0.156
Dbound = 0.025 um2/s; FracBound = 0.042; fitted LocError = 0.032 um
Total trajs: 3326; trajs >=3: 3326; FitType: PDF
Locs = 60182; Locs/Frame = 0.483; Fit unweighted
Total 1dT jumps = 56856; Jumps used = 13304
Dfree1 = 2.058 um2/s; FracFree1 = 0.812
Dfree2 = 0.468 um2/s; FracFree2 = 0.144
Dbound = 0.053 um2/s; FracBound = 0.044; fitted LocError = 0.029 um
Total trajs: 3372; trajs >=3: 3372; FitType: PDF
Locs = 50375; Locs/Frame = 0.397; Fit unweighted
Total 1dT jumps = 47003; Jumps used = 13488
Dfree1 = 1.784 um2/s; FracFree1 = 0.815
Dfree2 = 0.342 um2/s; FracFree2 = 0.135
Dbound = 0.055 um2/s; FracBound = 0.05; fitted LocError = 0.032 um
Total trajs: 2545; trajs >=3: 2545; FitType: PDF
Locs = 36570; Locs/Frame = 0.297; Fit unweighted
Total 1dT jumps = 34025; Jumps used = 10180
Δτ: 70 ms
Δτ: 60 ms
Δτ: 50 ms
Δτ: 40 ms
Δτ: 30 ms
Δτ: 20 ms
Δτ: 10 ms

Supplementary Figure 5. TERT recruitment to telomeres is reduced in the absence of TCAB1 and TR, Related to Figure 4. (A) Analysis if the step size of telomeric TERT particles relative to the distance of the particle to the closest telomere (pooled results from 3 independent biological replicates with 19-30 cells analyzed per replicate). TERT molecules bound to the telomere are expected to have small step sizes and a short distance to the closest telomere, which is apparent in the enrichment of events in the lower left quadrants in the WT control (red box). This enrichment is not observed in TCAB1 and TR knock-out cells (red box). **(B)** Spot-On analysis of telomeric TERT particles (pooled results from 3 independent biological replicates with 19-30 cells analyzed per replicate). The fraction of bound TERT particles in TCAB1 and TR knock-out cells is 4-5%, compared to 12% in the WT control cells.



Supplementary Figure 6. High salt concentrations release fibrillarin and TR from the nucleolus into the nucleoplasm, Related to Figure 6. (A) Western blot and **(B)** Northern blot of cellular fractions from TCAB1 knock-out cells probed with an antibody against fibrillarin and probes for TR, respectively. Ruptured nuclei were either maintained at a low salt concentration or exposed to 357.5 mM KCl. The results demonstrate that nucleoli are dissolved in the presence of a high salt concentration, releasing fibrillarin and TR into the nucleoplasmic fraction.



Supplementary Figure 7. Analysis of nucleolar dyskerin dynamics and characterization of 3xMS2 TR expression and TCAB1 knock-out in HeLa cells, Related to Figure 7. (A) Images of control and TCAB1 knock-out cells expressing 3xFLAG-HaloTag-dyskerin before and after photobleaching of nucleolar dyskerin (JFX650, scale bar = 5 µm). **(B)** Fluorescence recovery curves of nucleolar dyskerin in control and TCAB1 knock-out cells. Data was fit with a single exponential function. **(C)** Quantification of half-life of fluorescence recovery, calculated from the rate constant of the single exponential fit of the data shown in (B) (n = 6 and 9, mean). **(D)** Quantification of the mobile fraction of nucleolar dyskerin based on the single exponential fit of the data shown in (B) (n = 6 and 9, mean). **(E)** Fluorescence decay curves of unbleached nucleolar dyskerin in control and TCAB1 knock-out cells. Data was fit with a single exponential function. **(F)** Quantification of half-life of fluorescence decay, calculated from the rate constant of the single exponential fit of the data shown in **(E)** (n = 8 and 7, mean). **(G)** Western blot probed with an antibody against TCAB1, demonstrating the knock-out of TCAB1 in Halo-TERT MS2-TR HeLa cells. **(H)** PCR analysis of the endogenous TR locus after insertion of the 3xMS2 sequence. **(I)** Northern blot probed with radioactively labeled anti-TR oligonucleotides of control cells and genome edited clones expressing 3xMS2-TR.