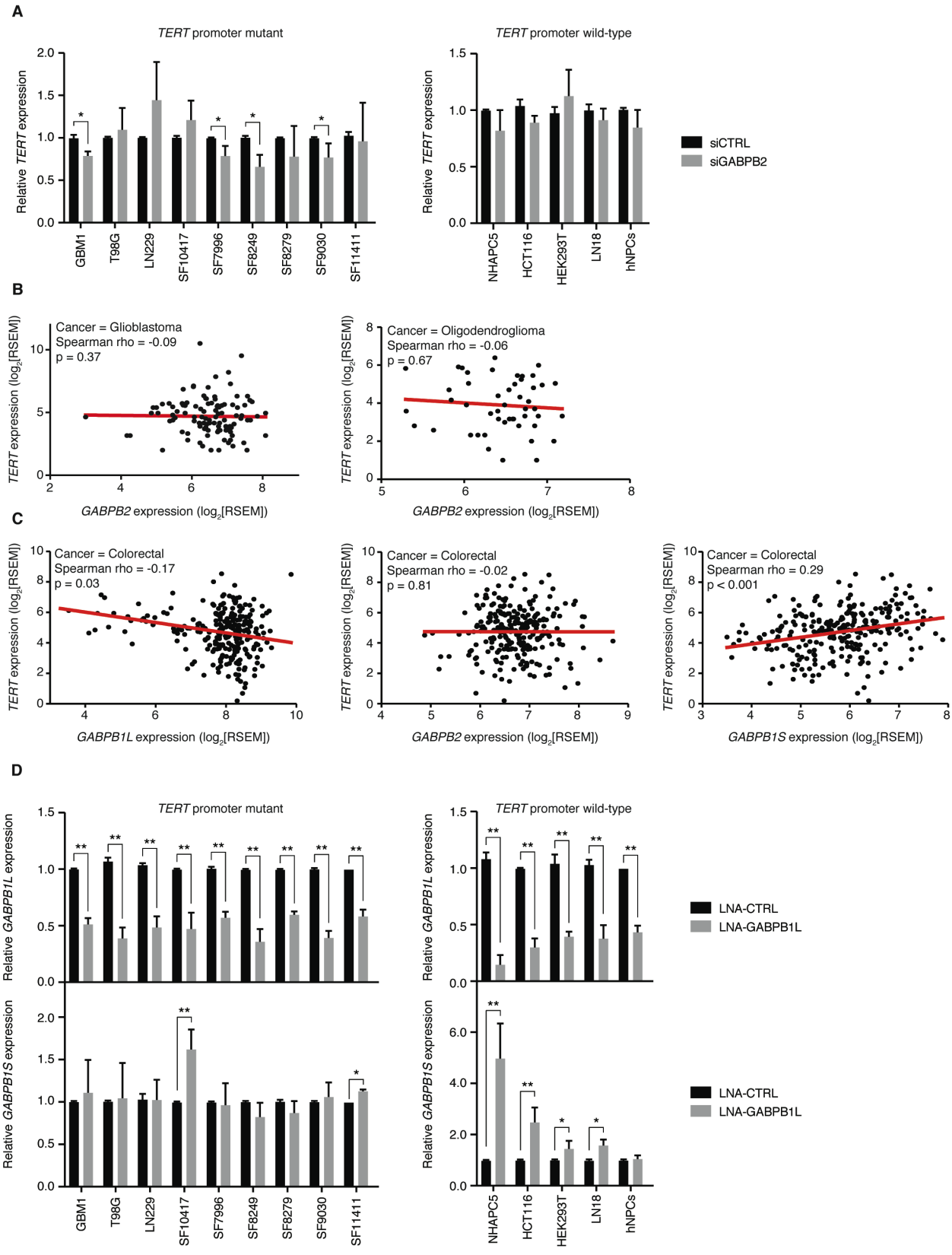
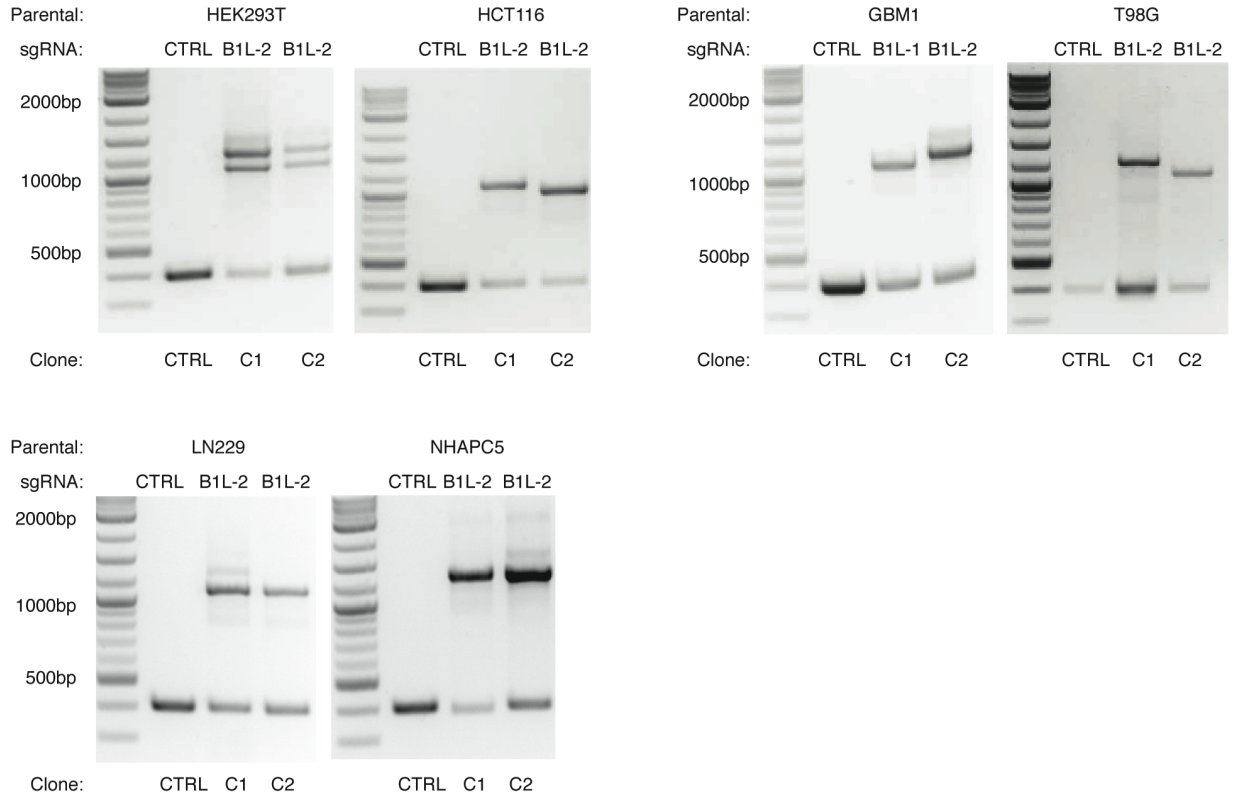

A
TERT promoter mutant
TERT promoter wild-type
Relative TERT expression
GBM1
T98G
LN229
SF10417
SF7996
SF8249
SF8279
SF9030
SF11411
NHAPC5
HCT116
HEK293T
LN18
hNPCs
siCTRL
siGABPB2
B
Cancer = Glioblastoma
Spearman rho = -0.09
p = 0.37
Cancer = Oligodendroglioma
Spearman rho = -0.06
p = 0.67
TERT expression (log2[RSEM])
GABPB2 expression (log2[RSEM])
C
Cancer = Colorectal
Spearman rho = -0.17
p = 0.03
Cancer = Colorectal
Spearman rho = -0.02
p = 0.81
Cancer = Colorectal
Spearman rho = 0.29
p < 0.001
GABPB1L expression (log2[RSEM])
GABPB2 expression (log2[RSEM])
GABPB1S expression (log2[RSEM])
D
TERT promoter mutant
TERT promoter wild-type
Relative GABPB1L expression
Relative GABPB1S expression
LNA-CTRL
LNA-GABPB1L

Figure S1, related to Figure 1. β1S and β2 do not robustly positively regulate *TERT* in *TERT* promoter mutant cancer with intact β1L. **(A)** *TERT* expression following siRNA-mediated knockdown of β2 (siGABPB2) in *TERT* promoter mutant (left) or wild-type (right) cell lines and primary cultures. *p value<0.05, two-sided Student's t-test compared to a non-targeting siRNA (siCTRL) in each respective line. **(B,C)** Correlation of *GABPB1L*, *GABPB1S*, or *GABPB2* mRNA expression (log_2[RSEM normalized counts]) versus *TERT* mRNA expression (log_2[RSEM normalized counts]) from 109 *TERT*-expressing GBMs and 49 *TERT* promoter-mutant oligodendrogliomas **(B)** and 262 *TERT*-expressing colorectal cancers **(C)**. Red line indicates trend line. Spearman's Rank-Order Correlation was used to generate Spearman's rho and p values for each monotonic correlation. **(D)** *GABPB1L* and *GABPB1S* expression following LNA-ASO knockdown of β1L (LNA-GABPB1L) in *TERT* promoter mutant (left) or wild-type (right) cell lines and primary cultures compared to an LNA-ASO control (LNA-CTRL). *p value<0.05, **p value<0.01, two-sided Student's t-test compared to LNA-CTRL in each respective line. All values are mean ± S.D. of at least three independent experiments (two independent experiments for SF10417 line).

A


Parental:
HEK293T
HCT116
GBM1
T98G
LN229
NHAPC5
sgRNA:
CTRL B1L-2 B1L-2
CTRL B1L-1 B1L-2
2000bp
1000bp
500bp
Clone:
CTRL C1 C2

B

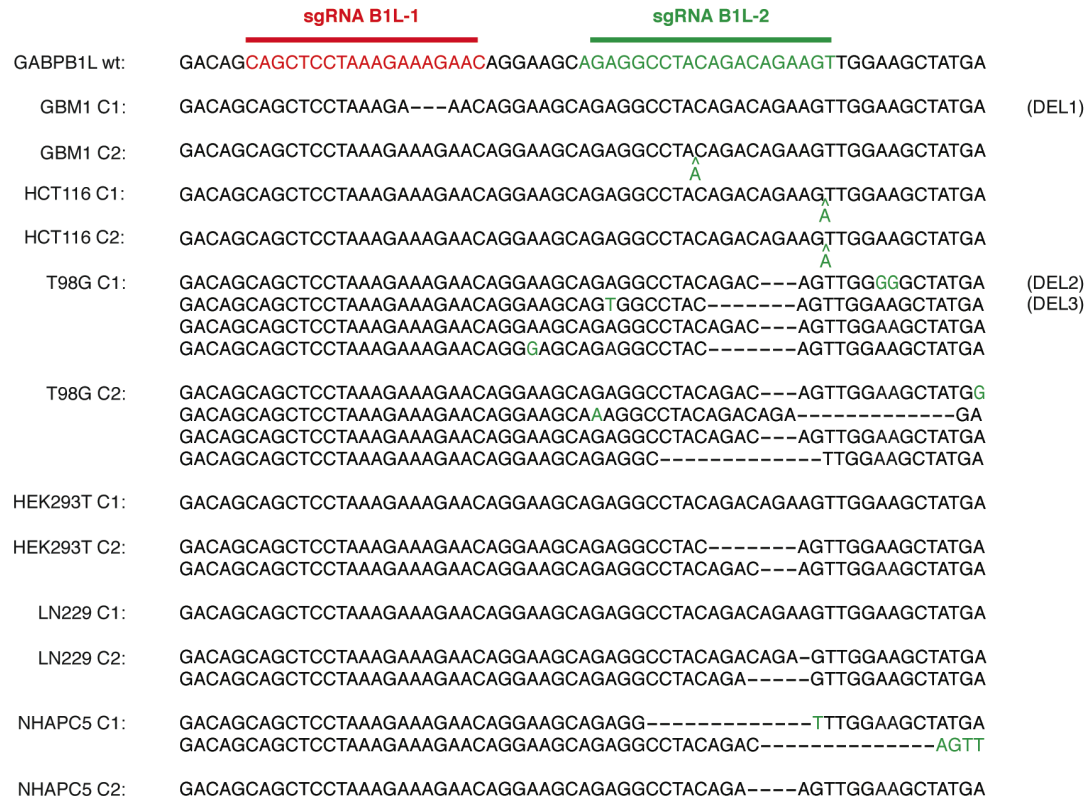

sgRNA B1L-1
sgRNA B1L-2
GABPB1L wt: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA
GBM1 C1: GACAGCAGCTCCTAAAGA---AACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA (DEL1)
GBM1 C2: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA
HCT116 C1: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA
HCT116 C2: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA
T98G C1: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGAC---AGTTGGGGGCTATGA (DEL2)
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGTGGCCTAC-------AGTTGGAAGCTATGA (DEL3)
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGAC---AGTTGGAAGCTATGA
GACAGCAGCTCCTAAAGAAAGAACAGGGAGCAGAGGCCTAC-------AGTTGGAAGCTATGA
T98G C2: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGAC---AGTTGGAAGCTATGG
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAAAGGCCTACAGACAGA----------GA
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGAC---AGTTGGAAGCTATGA
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGC----------TTGGAAGCTATGA
HEK293T C1: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA
HEK293T C2: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTAC-------AGTTGGAAGCTATGA
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGAC---AGTTGGAAGCTATGA
LN229 C1: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGAAGTTGGAAGCTATGA
LN229 C2: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGACAGA-GTTGGAAGCTATGA
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGA-----GTTGGAAGCTATGA
NHAPC5 C1: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGG----------TTTGGAAGCTATGA
GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGAC----------AGTT
NHAPC5 C2: GACAGCAGCTCCTAAAGAAAGAACAGGAAGCAGAGGCCTACAGA----AGTTGGAAGCTATGA

Figure S2, related to Figure 2. Validation of CRISPR-Cas9 editing. **(A)** UV images of successful integration of the targeting vector (~1.1kb for forward integration, ~1.3kb for reverse integration) into exon 9 of *GABPB1* for the lines used in this study. **(B)** Sanger sequencing of *GABPB1* exon 9 showing indels in alleles without targeting vector integration for each β1L-reduced clone. DEL1, DEL2, and DEL3 denote deletions induced in Figure 2B.

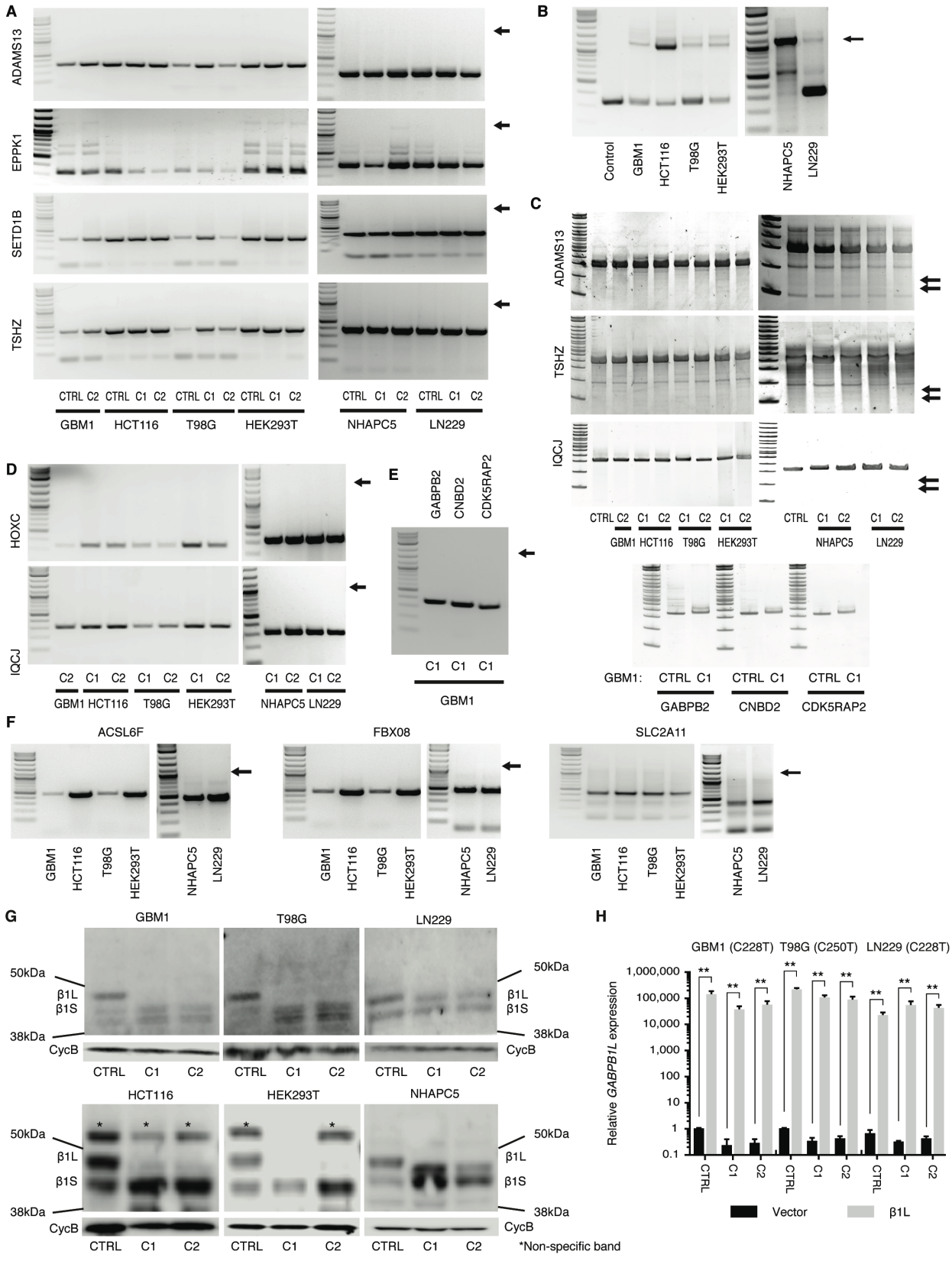

A
ADAMS13
EPPK1
SETD1B
TSHZ
CTRL C2 CTRL C1 C2 CTRL C1 C2 CTRL C1 C2
CTRL C1 C2 CTRL C1 C2
GBM1 HCT116 T98G HEK293T
NHAPC5 LN229
B
Control GBM1 HCT116 T98G HEK293T
NHAPC5 LN229
C
ADAMS13
TSHZ
IQCJ
CTRL C2 C1 C2 C1 C2 C1 C2
CTRL C1 C2 C1 C2
GBM1 HCT116 T98G HEK293T
NHAPC5 LN229
D
HOXC
IQCJ
C2 C1 C2 C1 C2 C1 C2
C1 C2 C1 C2
GBM1 HCT116 T98G HEK293T
NHAPC5 LN229
E
GABPB2 CNBD2 CDK5RAP2
C1 C1 C1
GBM1
GBM1: CTRL C1 CTRL C1 CTRL C1
GABPB2 CNBD2 CDK5RAP2
F
ACSL6F
FBX08
SLC2A11
GBM1 HCT116 T98G HEK293T NHAPC5 LN229
G
GBM1
T98G
LN229
50kDa
β1L
β1S
38kDa
CycB
CTRL C1 C2
HCT116
HEK293T
NHAPC5
*Non-specific band
H
GBM1 (C228T) T98G (C250T) LN229 (C228T)
Relative GABPB1L expression
1,000,000
100,000
10,000
1,000
100
10
1
0.1
**
CTRL C1 C2
Vector
β1L

Figure S3, related to Figure 2. Validation of β1L protein reduction and analysis of potential off-target mutations introduced by CRISPR-Cas9 editing. **(A)** PCR analysis for potential non-specific integration of the targeting vector at off-target coding regions for the universal sgRNA. **(B)** On-target integration of the targeting vector at the negative control locus. **(C)** Surveyor analysis to detect potential mutations introduced by CRISPR-Cas9 at coding sequences for all sgRNAs. Arrows indicate expected size of fragments from Surveyor assay if mutations are present. **(D-F)** PCR analysis for potential non-specific integration of the targeting vector at off-target coding regions for *GABPB1L* sgRNA-2 **(D)**, *GABPB1L* sgRNA-1 **(E)** and control sgRNA **(F)**. **(G)** Immunoblotting for β1 with β1L isoform (top, upper marked band) and β1S isoforms (top, lower marked bands) compared to a Cyclophilin B loading control (bottom) in all CRISPR-Cas9-edited cell lines. Asterisk (*) designates non-specific bands. **(H)** *GABPB1L* expression in β1L-reduced clones relative to CTRL 48 hr following transfection with empty vector (VECTOR) or β1L expression vector. **p value<0.01, two-sided Student's t-test compared to respective VECTOR control. Values are mean ± S.D. of three independent experiments.

Table S2, related to Figure 2. Summary of clones generated by CRISPR-Cas9 editing.

Parental	sgRNA	GABPB1L copy number	Clone #	GABPB1L allele 1	GABPB1L allele 2	GABPB1L allele 3	GABPB1L allele 4	GABPB1L allele 5
GBM1	CTRL							
GBM1	GABPB1L-1	2	1	Puro cassette insertion	3bp in-frame deletion			
GBM1	GABPB1L-2	2	2	Puro cassette insertion	A frameshift insertion			
T98G	CTRL							
T98G	GABPB1L-2	5	1	Puro cassette insertion	3bp in-frame deletion + AA>GG dinucleotide sub	7bp frameshift deletion + A>T sub	3bp in-frame deletion	7bp frameshift deletion + A>G sub
T98G	GABPB1L-2	5	2	Puro cassette insertion	3bp in-frame deletion + A>G sub	13bp frameshift deletion + G>A sub	3bp in-frame deletion	13bp frameshift deletion
LN229	CTRL							
LN229	GABPB1L-2	3	1	Puro cassette insertion	Puro cassette insertion	Widl-type		
LN229	GABPB1L-2	3	2	Puro cassette insertion	1bp frameshift deletion	5bp frameshift deletion		
HCT116	CTRL							
HCT116	GABPB1L-2	2	1	Puro cassette insertion	A frameshift insertion			
HCT116	GABPB1L-2	2	2	Puro cassette insertion	A frameshift insertion			

HEK293T	CTRL						
HEK293T	GABPB1L-2	3	1	Puro cassette insertion	Puro cassette insertion	Wild-type	
HEK293T	GABPB1L-2	4	2	Puro cassette insertion	Puro cassette insertion	7bp frameshift deletion	3 bp-inframe deletion
NHAPC5	CTRL						
NHAPC5	GABPB1L-2	3	1	Puro cassette insertion	13bp frameshift deletion + G>T sub	14bp frameshift deletion + TGA>GTT trinucleotide sub	
NHAPC5	GABPB1L-2	2	2	Puro cassette insertion	4bp frameshift deletion		

Table S3, related to Figure 2. Deletions induced by site-directed mutagenesis for the NanoBiT Protein-Protein Interaction assay.

Deletion	Deletion	Forward Primer	Reverse Primer
Reference	CAGCTCCTAAAGAAAGAACAGGAAGC AGAGGCCTACAGACAGAAGT	N/A	N/A
DEL1	CAGCTCCTAAAGA---AACAGGAAGCA GAGGCCTACAGACAGAAG	GCCTCTGCTTCCTGTTTCTTTAGGAGC TGCTGT	ACAGCAGCTCCTAAAGAAACAGGAAG CAGAGGC
DEL2	CAGCTCCTAAAGAAAGAACAGGAAGC AGAGGCCTACAGAC---AG	GCAGAGGCCTACAGACAGTTGGAAGC TATGAC	GTCATAGCTTCCAACTGTCTGTAGGCC TCTGC
DEL3	CAGCTCCTAAAGAAAGAACAGGAAGC AGTGGCCTAC-------AG	GTCATAGCTTCCAACTGTAGGCCTCTG CTTCC	GGAAGCAGAGGCCTACAGTTGGAAGC TATGAC

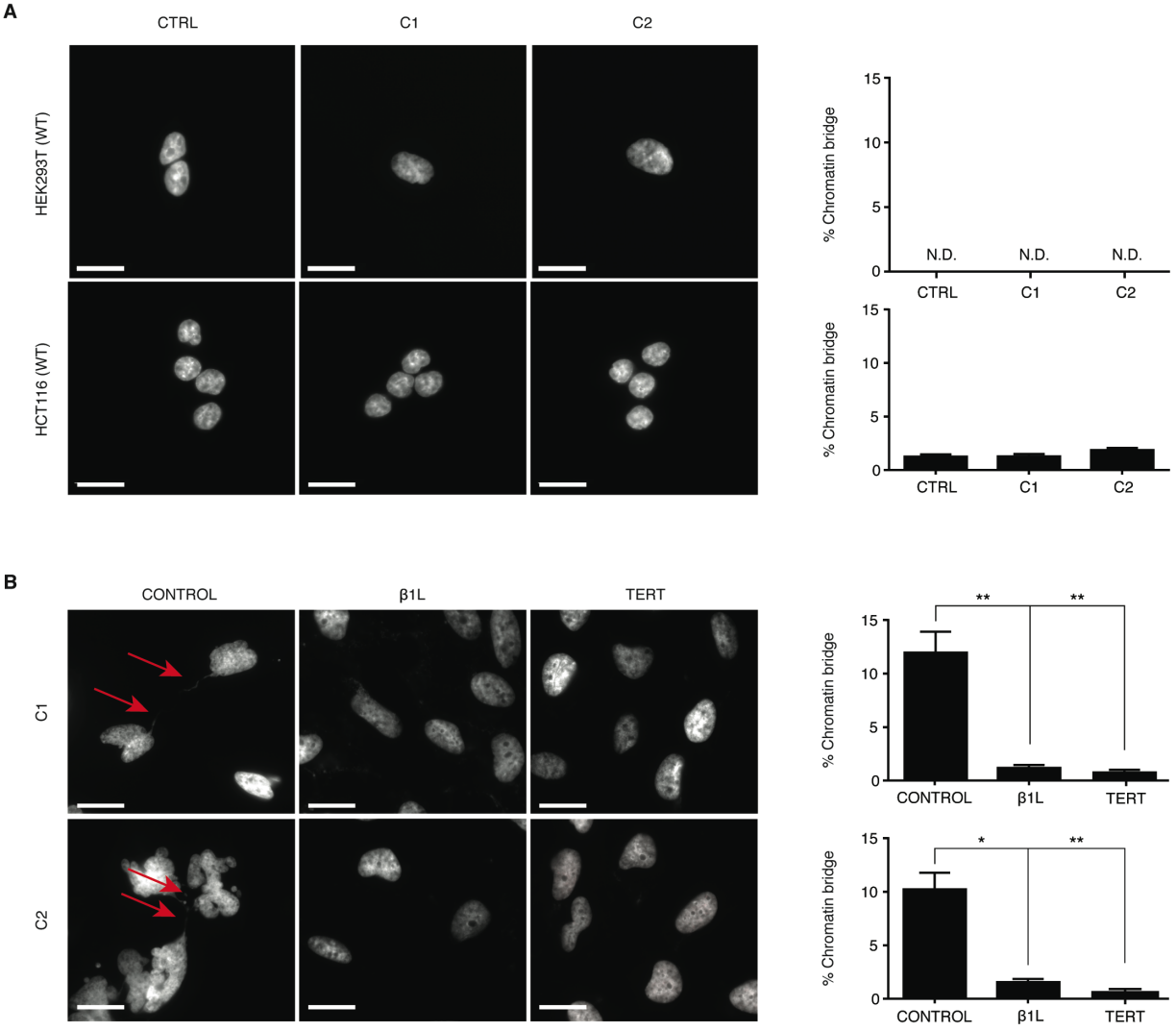

A
CTRL
C1
C2
HEK293T (WT)
HCT116 (WT)
% Chromatin bridge
N.D.
N.D.
N.D.
CTRL
C1
C2
B
CONTROL
β1L
TERT
C1
C2
**
**
*
**
CONTROL
β1L
TERT
C
C1
C2
Relative TERT expression
1000000
100000
10000
1000
100
10
1
0.1
CONTROL
TERT
CONTROL
TERT

Figure S4, related to Figure 3. Expression of exogenous β1L or TERT is sufficient to rescue telomere dysfunction in β1L-reduced LN229 lines. **(A)** Representative DAPI images (left images) and quantification (right graphs) of chromatin bridges in CTRL or β1L-reduced clones derived from HCT116 or HEK293T *TERT* promoter wild-type lines. Scale bar = 20 μm. N.D. = Not detected. Quantification values are weighted mean ± S.D. of at least ten independent fields of view. **(B)** Representative DAPI images (left images) and quantification (right graphs) of chromatin bridges in β1L-reduced LN229 clones transfected with a CONTROL, β1L, or TERT expression vector. Scale bar = 20 μm. *p value<0.05, **p value<0.01, two-sided Student's t-test relative to CONTROL. Quantification values are weighted mean ± S.D. of at least ten independent fields of view. **(C)** *TERT* expression measured by RT-qPCR 7 days post-transfection of β1L-reduced LN229 clones (58 days post-editing) with either a CONTROL or TERT expression vector. **p value<0.01, two-sided Student's t-test relative to CONTROL. Values are mean ± S.D. of three independent experiments.

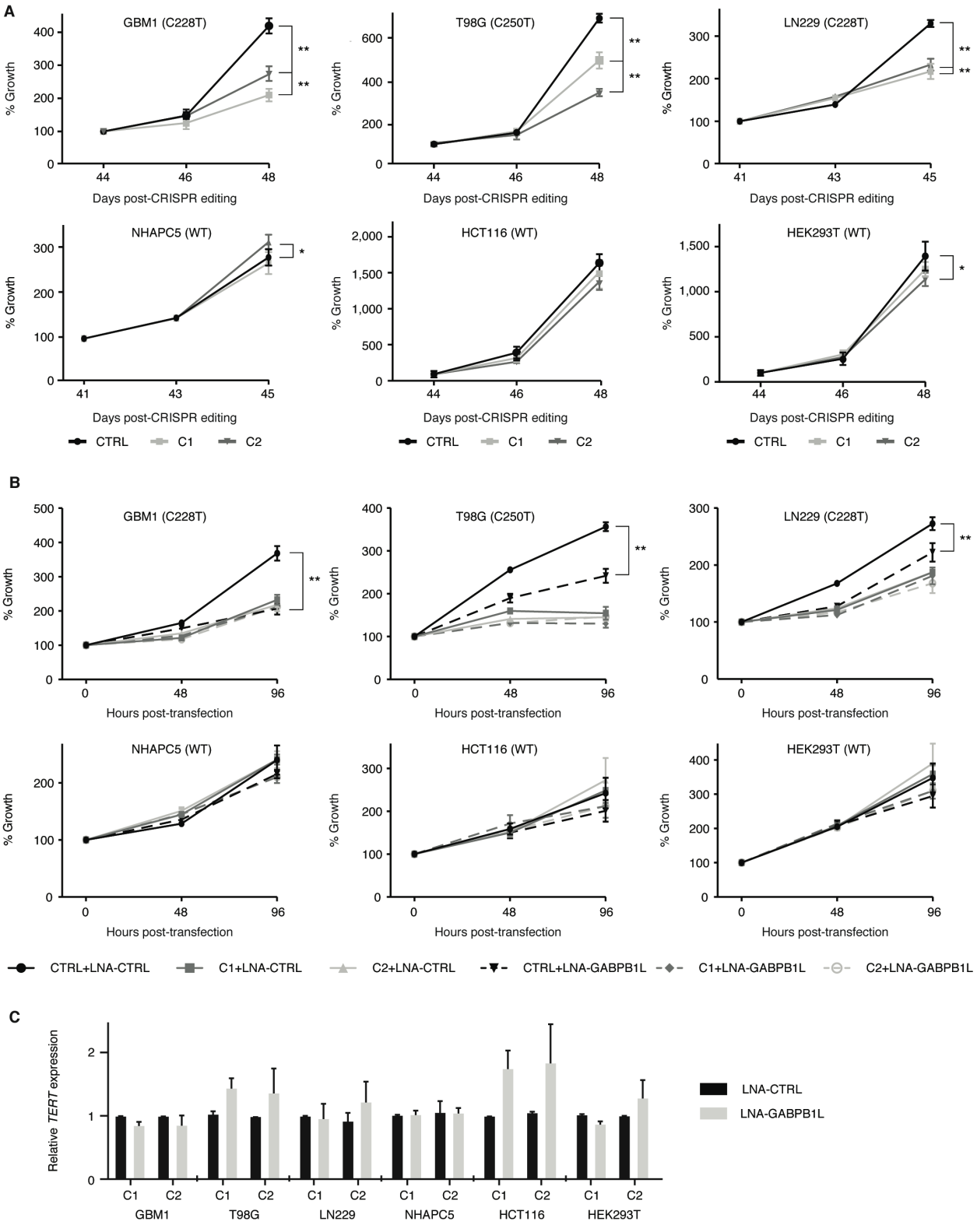


Figure S5, related to Figure 4. β1L reduction induces growth defects in *TERT* promoter mutant lines. **(A)** Percent growth of *TERT* promoter mutant (top graphs) and wild-type (bottom graphs)

CRISPR control (CTRL) or β1L-reduced clones (C1 and C2) relative to the initial time point. *p value<0.05, **p value<0.01, two-sided Student's t-test compared to CTRL (final time point). **(B)** Percent growth of *TERT* promoter mutant (top graphs) and wild-type (bottom graphs) CRISPR control (CTRL) or β1L-reduced clones (C1 and C2) relative to the initial time point following transfection with a scrambled control (LNA-CTRL) or *GABPB1L*-targeting (LNA-GABPB1L) LNA-ASO. Growth was measured 0, 48, and 96 hr post-transfection. *p value<0.05, **p value<0.01, two-sided Student's t-test compared to a control LNA-ASO (LNA-CTRL) at the final time point for each clone (CTRL or β1L-reduced). **(C)** Relative *TERT* expression of β1L-reduced clones following transfection with a scrambled control (LNA-CTRL) or *GABPB1L*-targeting (LNA-GABPB1L) LNA-ASO. All values are mean ± S.D. of three independent experiments.

Table S5, related to Figure 5. GO-enrichment for genes differentially expressed between control and β1L-reduced *TERT* promoter mutant lines.

Process	GOTerm Accession	Gene Determinants	FDR
Glucuronidation	0052697 1904223 1904224 2001030 2001029 0052696 0052695 0006063 0019585 0044707	UGT1A10, UGT1A8, UGT1A1, UGT1A4, UGT1A5, UGT1A3, UGT1A9	1.44E-11
Cellular development	0044767 0032502 0044763 0048856 0048869	HOXC12, RHOU, FST, COL9A1, TMEM176B, SHANK1, UGT1A1, LRP5, EDIL3, CXCR4, NTF3, NPY, OGN, DKK1, GPR183, ZDHHC15, LGI4, RPS6KA6, ICK, FRK, MGP, LPAR3, ADAMTS2, KCNJ10, FERMT3, SP7, TERT, S100A9, VNN1, NTRK2, GABRA4, STC1, S100A8, PCOLCE, CD1D, PTGS2, KCNA1, ANKRD1, HGF	3.0903E-08
Cell-to-cell signaling	007275 0007267 0099536 0099537	STX11, DKK1, LPAR3, TACR1, HCN4, KCNJ10, NPBWR1, SHANK1, TERT, S100A9, NTRK2, CXCL5, GABRA4, LRP5, CCL8, PTGS2, KCNA1, NTF3, NPY, HGF	8.26038E-08
Fatty acid metabolism	0045922 0019217 0045833	UGT1A10, UGT1A8, UGT1A1, UGT1A4, UGT1A3, UGT1A9	8.31764E-07

Cell differentiation	0030154	DKK1, FST, GPR183, LGI4, FRK, MGP, COL9A1, LPAR3, TMEM176B, KCNJ10, FERMT3, SP7, SHANK1, VNN1, S100A9, NTRK2, STC1, LRP5, CXCR4, S100A8, CD1D, PTGS2, KCNA1, NTF3, NPY, OGN, HGF, ANKRD1	2.58226E-07
Cell proliferation	0008283	GPR183, LGI4, FRK, TACR1, ABCB1, ZP4, TERT, NTRK2, CXCL5, STC1, LRP5, CCL8, CD1D, PTGS2, KCNA1, NPY, NTF3, HGF, OGN	8.29851E-06

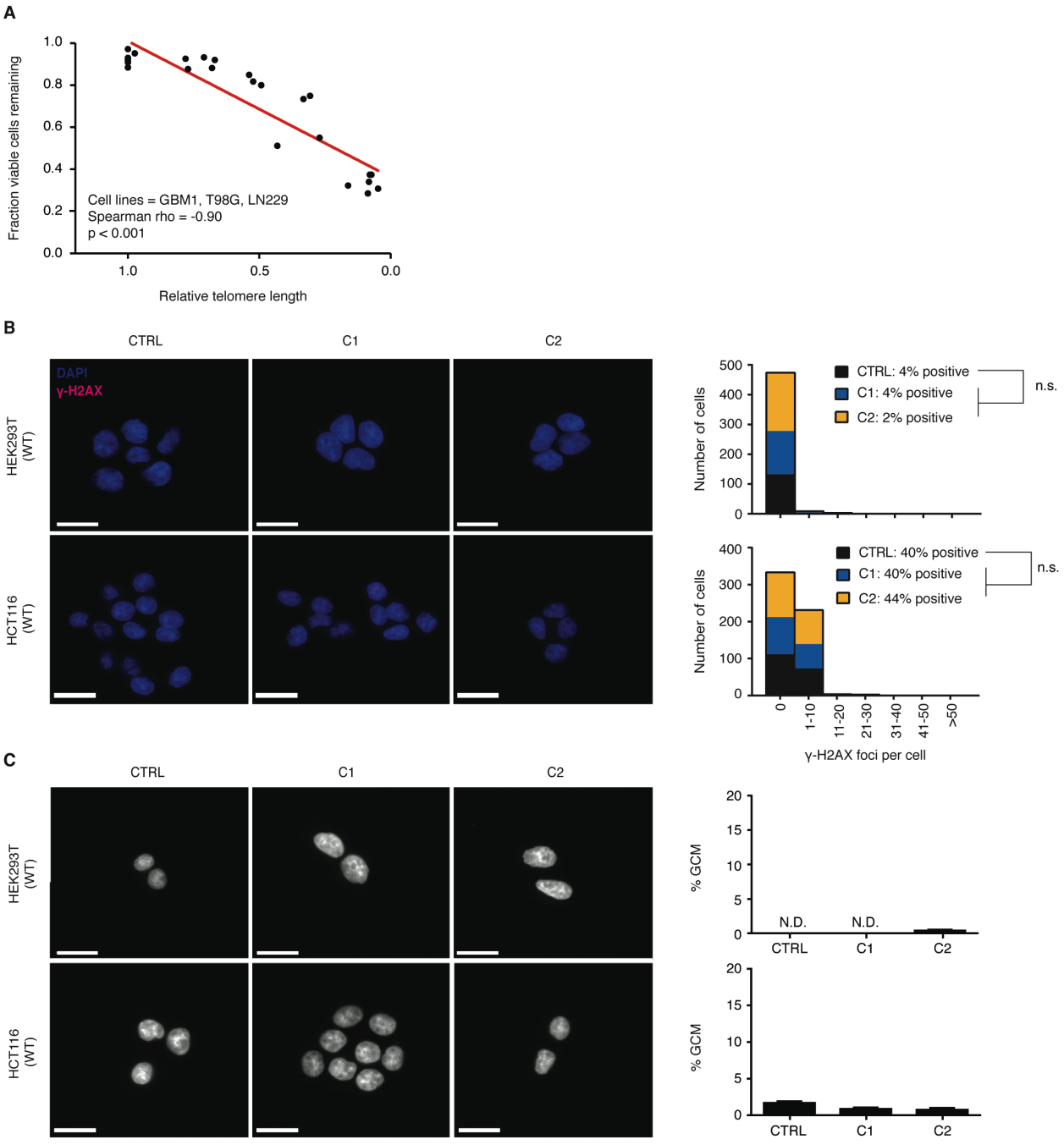


Figure S6, related to Figure 6. β1L reduction does not induce DNA damage and mitotic cell death in *TERT* promoter wild-type cell lines. **(A)** Correlation of relative telomere length and cellular viability across β1L-reduced clones from all *TERT* promoter-mutant cell lines (GBM1, T98G, and LN229). Spearman rho=-0.90, p value<0.001. **(B)** Representative images (left

images) and quantification (right graphs) of γ-H2AX staining in CTRL or β1L-reduced HCT116 and HEK293T clones. Scale bar = 20 μm. n.s. = not significant, two-sided Student's t-test compared to CTRL. Quantification values are sums of at least ten independent fields of view. **(C)** Representative DAPI images (left images) and quantification (right graphs) of giant cell micronucleation (GCM) in CTRL or β1L-reduced HCT116 and HEK293T clones. Scale bar = 20 μm. N.D = Not detected. Quantification values are weighted mean ± S.D. of at least ten independent fields of view.

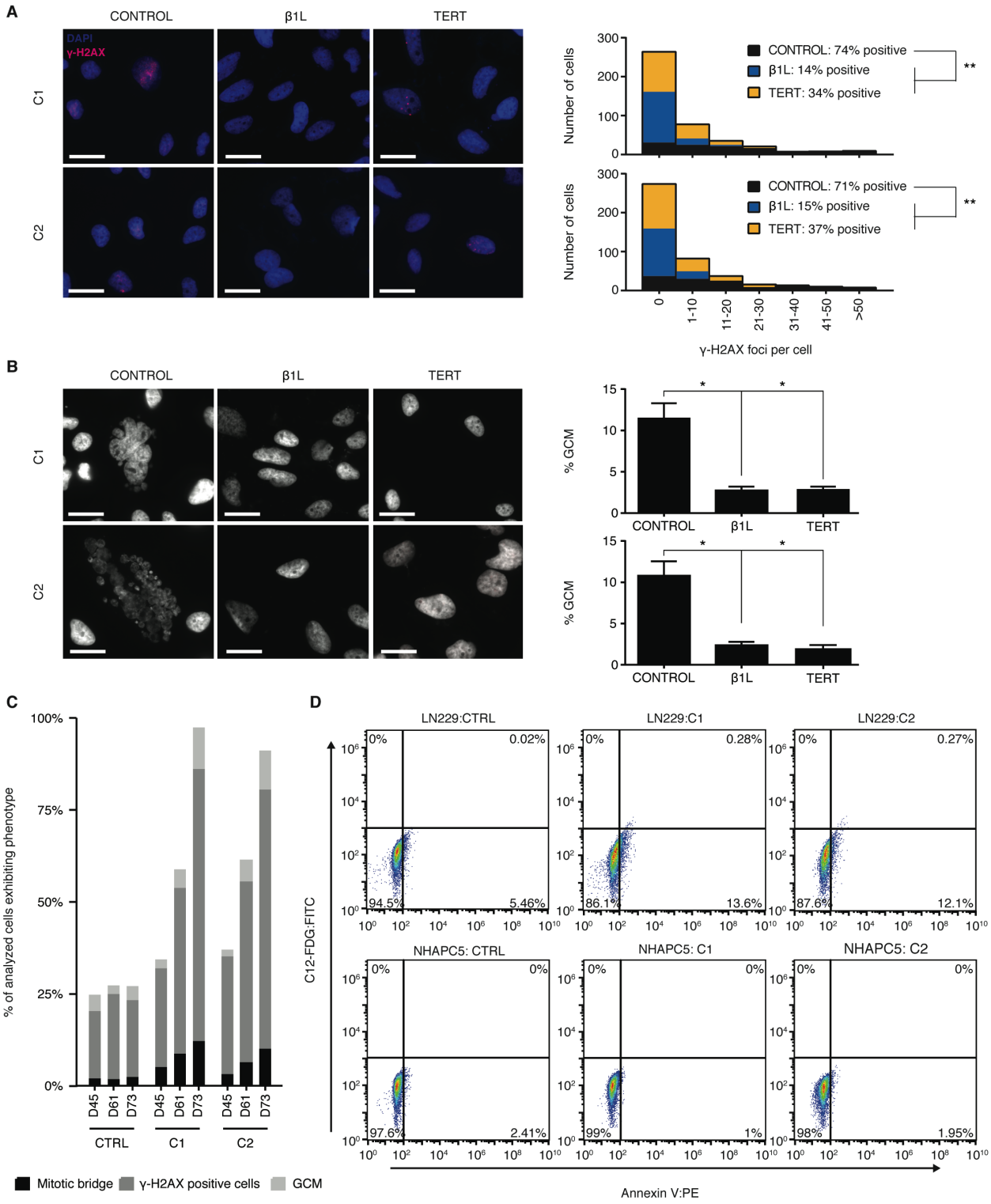


Figure S7, related to Figure 6. Expression of exogenous β1L or TERT is sufficient to rescue DNA damage and mitotic cell death in β1L-reduced LN229 clones. **(A)** Representative images

(left images) and quantification (right graphs) of γ-H2AX staining in β1L-reduced LN229 clones transfected with a CONTROL, β1L, or TERT expression vector at 73 days post-editing. Scale bar = 20 μm. *p value<0.05, **p value<0.01, Student's t-test relative to CONTROL. Quantification values are sums of at least ten independent fields of view. **(B)** Representative DAPI images (left images) and quantification (right graphs) of giant cell micronucleation (GCM) in β1L-reduced LN229 clones transfected with a CONTROL, β1L, or TERT expression vector at 73 days post-editing. Scale bar = 20 μm. *p value<0.05, Student's t-test relative to CONTROL. Quantification values are weighted mean ± S.D. of at least ten independent fields of view. **(C)** Quantification of chromatin bridge formation, γ-H2AX staining (% positive cells), and giant cell micronucleation (GCM) in LN229 CTRL and β1L-reduced lines at days 45, 61, and 73 post-editing. Values are mean of at least five independent fields of view. **(D)** Dot plots quantifying expression of the apoptosis/necrosis marker annexin-V (PE; x-axis) and the senescence marker SA-β-Gal (C-12FDG [FITC]; y-axis) as determined by flow cytometry at day 75 post-editing.