Supplemental Information for

E4F1 and ZNF148 are transcriptional activators of the -57A>C and wild-type *TERT* promoter

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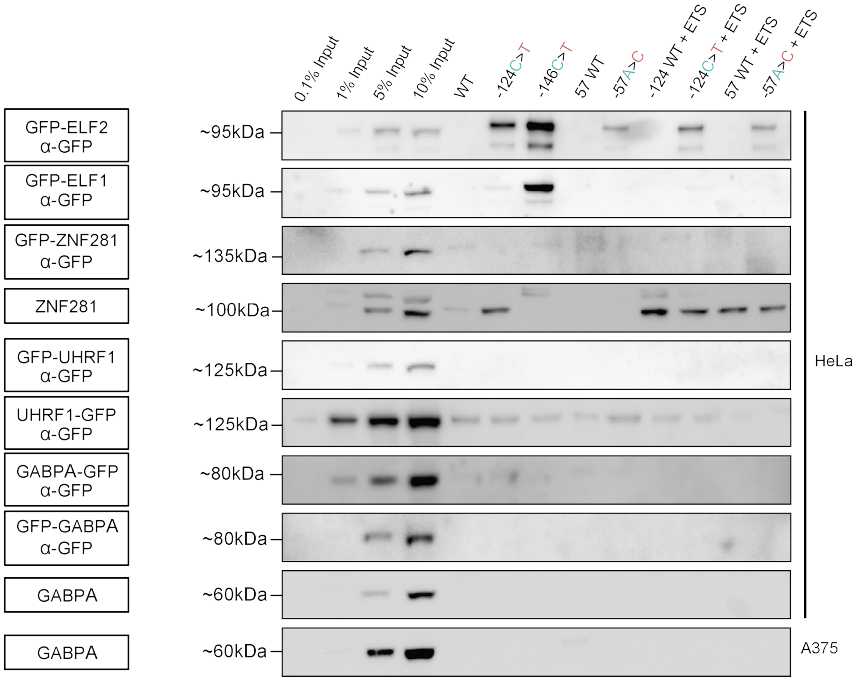
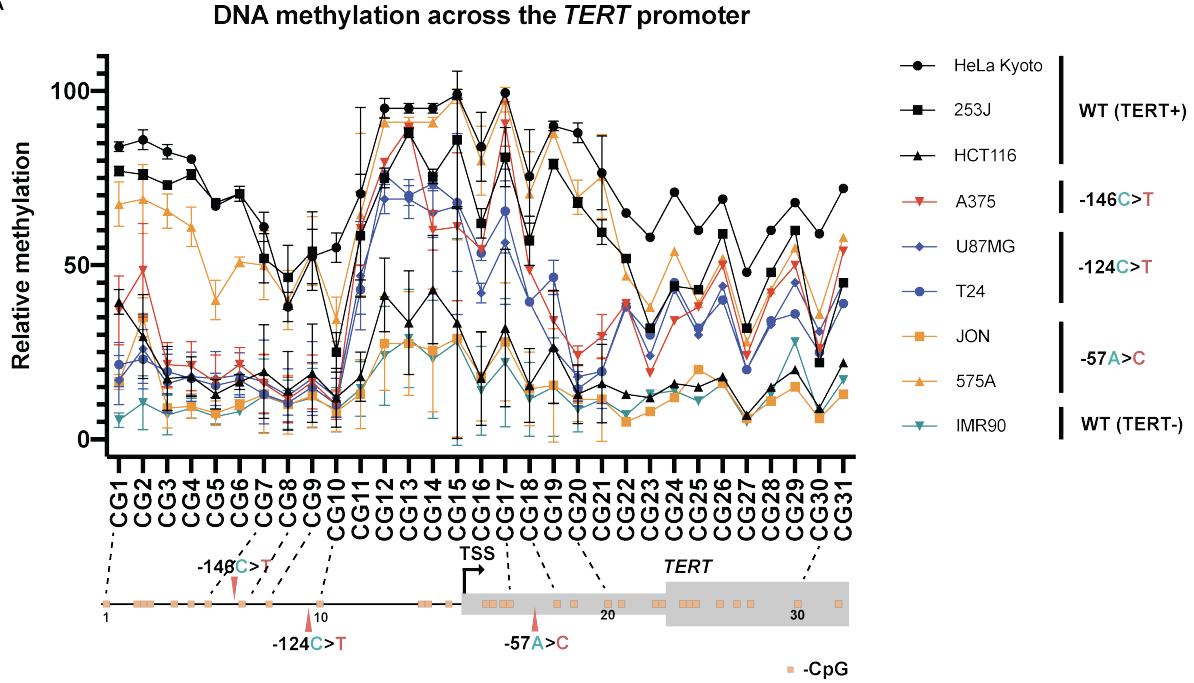
Content:

A

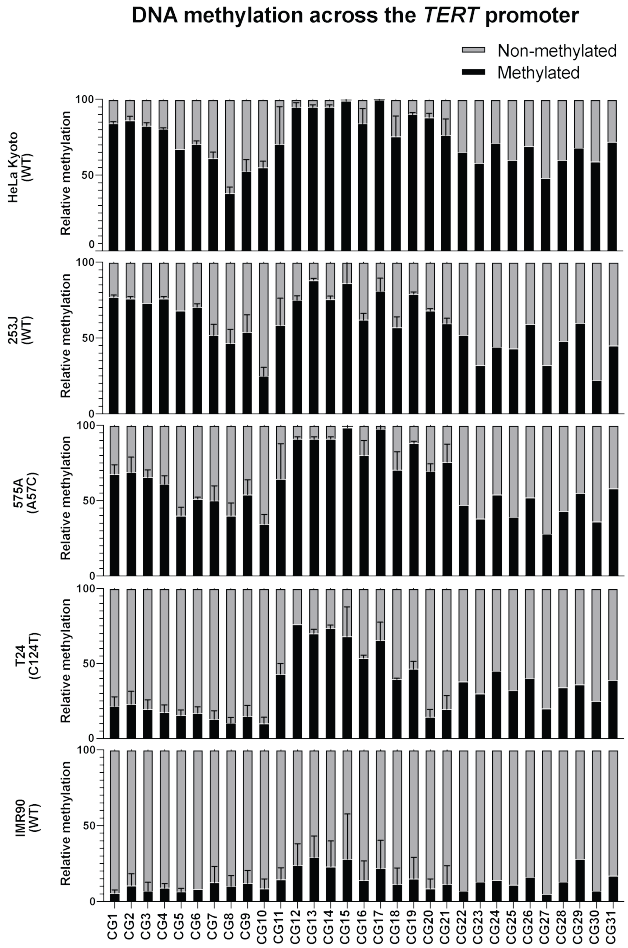


Supplemental Figure S1. (A) Sequence specific pull-down of endogenous and/or recombinant GFP-tagged ELF1, ELF2, ZNF281, UHRF1 and GABPA with HeLa and/or A375 nuclear extracts using the 9 probes shown in Fig. 1A.

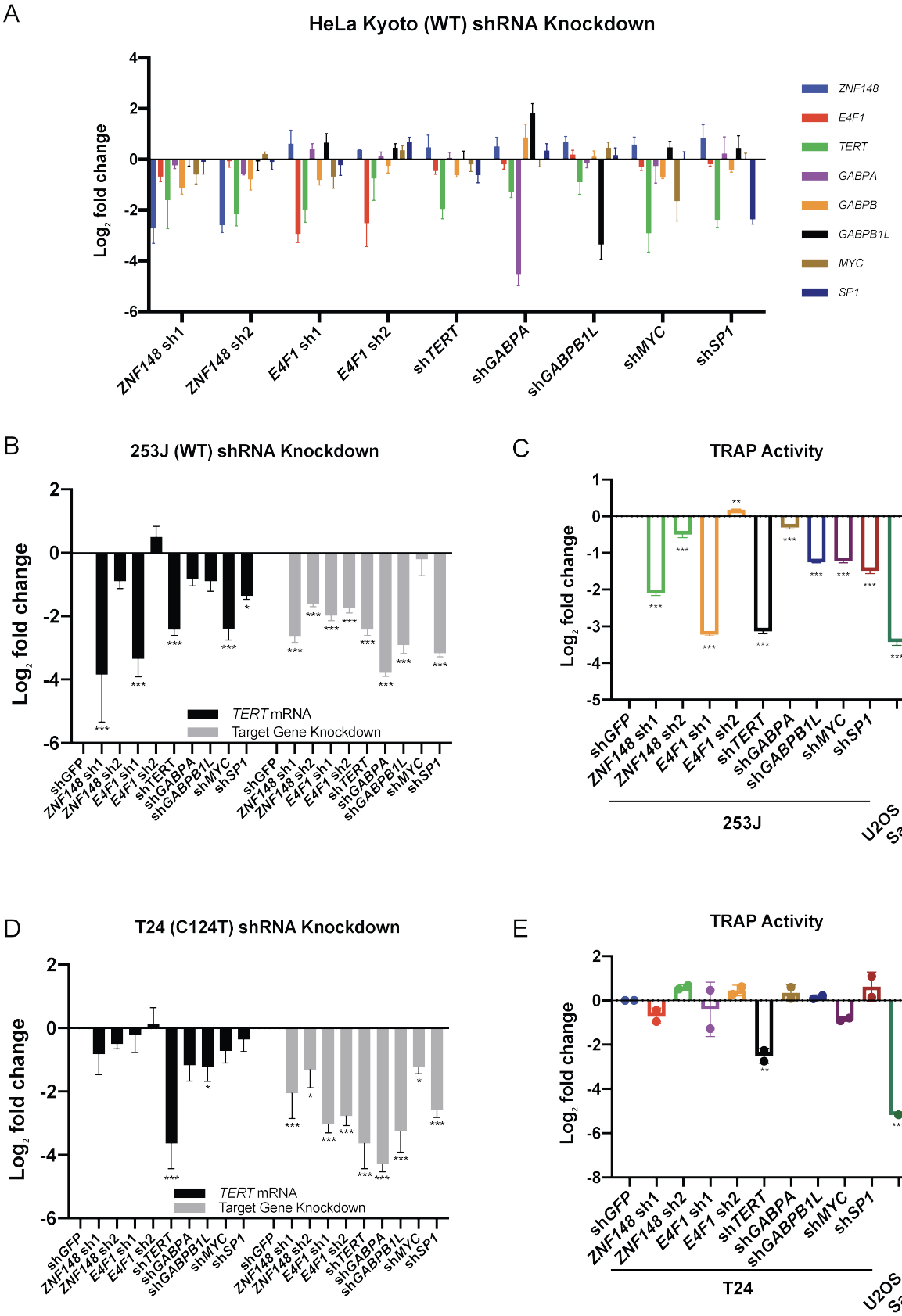
A


DNA methylation across the TERT promoter
Relative methylation
100
50
0
CG1 CG2 CG3 CG4 CG5 CG6 CG7 CG8 CG9 CG10 CG11 CG12 CG13 CG14 CG15 CG16 CG17 CG18 CG19 CG20 CG21 CG22 CG23 CG24 CG25 CG26 CG27 CG28 CG29 CG30 CG31
-146C>T
TSS
TERT
1
10
20
30
-124C>T
-57A>C
-CpG
HeLa Kyoto
253J
HCT116
A375
U87MG
T24
JON
575A
IMR90
WT (TERT+)
-146C>T
-124C>T
-57A>C
WT (TERT-)

B


DNA methylation across the TERT promoter
Non-methylated
Methylated
HeLa Kyoto (WT)
253J (WT)
575A (A57C)
T24 (C124T)
IMR90 (WT)
Relative methylation
100
50
0
CG1 CG2 CG3 CG4 CG5 CG6 CG7 CG8 CG9 CG10 CG11 CG12 CG13 CG14 CG15 CG16 CG17 CG18 CG19 CG20 CG21 CG22 CG23 CG24 CG25 CG26 CG27 CG28 CG29 CG30 CG31

Supplemental Figure S2. (A) Relative DNA methylation frequency of CpGs across the *TERT* promoter in telomerase-positive cell lines with the WT promoter (HeLa Kyoto, 253J, HCT116; black), the -146C>T mutation (A375; red), the -124C>T mutation (U87MG & T24; blue), the -57A>C mutation (575A & JON; orange) or telomerase-negative, non-cancerous cells (IMR-90; green). **(B)** DNA methylation on CpGs across the *TERT* promoter in selected cell lines. Generally, telomerase-positive cell lines exhibit higher methylation levels compared to the primary IMR-90 fibroblasts.


A
HeLa Kyoto (WT) shRNA Knockdown
Log2 fold change
ZNF148
E4F1
TERT
GABPA
GABPB
GABPB1L
MYC
SP1
ZNF148 sh1
ZNF148 sh2
E4F1 sh1
E4F1 sh2
shTERT
shGABPA
shGABPB1L
shMYC
shSP1
B
253J (WT) shRNA Knockdown
TERT mRNA
Target Gene Knockdown
C
TRAP Activity
253J
U2OS
Saos2
D
T24 (C124T) shRNA Knockdown
E
TRAP Activity
T24

Supplemental Figure S3. (A) mRNA expression data of *ZNF148*, *E4F1*, *TERT*, *GABPA*, *GABPB1L*, *MYC* and *SP1* following 48+72 h post-shRNA knockdown in HeLa (48h virus transduction, 72 hours puromycin selection), with sh*GFP* as control. Data shown as mean of values from three biological replicates. **(B)** mRNA expression data of *TERT* and target gene following 48+72 h post-shRNA knockdown in 253J, with sh*GFP* as control. **(C)** TRAP assay measuring telomerase activity following 48+72 h post-shRNA knockdown in 253J (48h virus transduction, 72 hours puromycin selection), with sh*GFP* as control. Telomerase-negative U2OS and Saos2 were used as negative controls. **(D)** mRNA expression data of *TERT* and target genes following 48+72 h post-shRNA knockdown in T24 (48h virus transduction, 72 hours puromycin selection), with sh*GFP* as control. Data shown as mean of values from three biological replicates. **(E)** ddPCR-TRAP assay measuring telomerase activity following 48+72 h post-shRNA knockdown in T24, with sh*GFP* as control. Telomerase-negative U2OS and Saos2 were used as negative controls. Data shown as mean of values from two biological replicates. All statistical significance was calculated using a two-sampled t-test, and the degree of significance is indicated as: * for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$.

Supplemental Tables

Supplemental Table S1. List of oligonucleotides used for DNA pulldown.

No	Primers	Sequence (5' → 3')
1a	*TERT* promoter WT forward	CTGGGAGGGCCCGGA**G**GGGGCTGGGCCGGGGACCCGG**G**AGGGGTCGGGACGGGGCG
1b	*TERT* promoter WT reverse	AGCGCCCCGTCCCGACCCCT**C**CCGGGTCCCCGGCCCAGCCCC**C**TCCGGGCCCTCCC
2a	*TERT* promoter -124C>T forward	CTGGGAGGGCCCGGA**A**GGGGCTGGGCCGGGGACCCGG**G**AGGGGTCGGGACGGGGCG
2b	*TERT* promoter -124C>T reverse	AGCGCCCCGTCCCGACCCCT**C**CCGGGTCCCCGGCCCAGCCCC**T**TCCGGGCCCTCCC
3a	*TERT* promoter -146C>T forward	CTGGGAGGGCCCGGA**G**GGGGCTGGGCCGGGGACCCGG**A**AGGGGTCGGGACGGGGCG
3b	*TERT* promoter -146C>T reverse	AGCGCCCCGTCCCGACCCCT**T**CCGGGTCCCCGGCCCAGCCCC**C**TCCGGGCCCTCCC
4a	*TERT* promoter -57WT forward	CTCCTCGCGGCGCGAGTTTC**A**GGCAGCGCTGCGTCCTGCTGCGCACGTGGGA
4b	*TERT* promoter -57WT reverse	AGTCCCACGTGCGCAGCAGGACGCAGCGCTGCC**T**GAAACTCGCGCCGCGAGG
5a	*TERT* promoter -57A>C forward	CTCCTCGCGGCGCGAGTTTC**C**GGCAGCGCTGCGTCCTGCTGCGCACGTGGGA
5b	*TERT* promoter -57A>C reverse	AGTCCCACGTGCGCAGCAGGACGCAGCGCTGCC**G**GAAACTCGCGCCGCGAGG
6a	*TERT* promoter wt(124)+ETS96+ETS91 forward	ACCGGGGCCGCGGAAAGGAAGGGGAGGGGCTGGGAGGGCCCGGA**G**GGGGCTGGGCCGGGG
6b	*TERT* promoter wt(124)+ETS96+ETS91 reverse	GTCCCCGGCCCAGCCCC**C**TCCGGGCCCTCCCAGCCCCTCCCCTTCCTTTCCGCGGCCCCG
7a	*TERT* promoter mt(-124C>T) ETS96+ETS91 forward	ACCGGGGCCGCGGAAAGGAAGGGGAGGGGCTGGGAGGGCCCGGA**A**GGGGCTGGGCCGGGG
7b	*TERT* promoter mt(-124C>T)+ETS96+ETS91 reverse	GTCCCCGGCCCAGCCCC**T**TCCGGGCCCTCCCAGCCCCTCCCCTTCCTTTCCGCGGCCCCG
8a	*TERT* promoter wt(57)+ETS96+ETS91 forward	AGGCAGCGCTGCC**T**GAAACTCGCGCCGCGAGGAGAGGGCGGGGCCGCGGAAAGGAAGGGG
8b	*TERT* promoter wt(57)+ETS96+ETS91 reverse	CTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTC**A**GGCAGCGCTGC
9a	*TERT* promoter mt(-57A>C)+ETS96+ETS91 forward	AGGCAGCGCTGCC**G**GAAACTCGCGCCGCGAGGAGAGGGCGGGGCCGCGGAAAGGAAGGGG
9b	*TERT* promoter mt(-57A>C)+ETS96+ETS91 reverse	CTCCCCTTCCTTTCCGCGGCCCCGCCCTCTCCTCGCGGCGCGAGTTTC**C**GGCAGCGCTGC
10a	rs36115365 major SNP forward	AGACAGGAGGAAATGGTCTCAGCCTCACC**G**TCCGTGGCCACGGCAGCTTCAGTGAGCCAG
10b	rs36115365 major SNP reverse	CTCTGGCTCACTGAAGCTGCCGTGGCCACGGA**C**GGTGAGGCTGAGACCATTTCCTCCTGT
11a	rs36115365 minor SNP forward	AGACAGGAGGAAATGGTCTCAGCCTCACC**C**TCCGTGGCCACGGCAGCTTCAGTGAGCCAG
11b	rs36115365 minor SNP reverse	CTCTGGCTCACTGAAGCTGCCGTGGCCACGGA**G**GGTGAGGCTGAGACCATTTCCTCCTGT
12a	ZNF148 binding motif forward	GTCCAGCGCACCAACGCAGGCGAGGGACTGGGGGAGGAGGGAAGTGCCCTCCTGCAGCAC
12b	ZNF148 binding motif reverse	ACGTGCTGCAGGAGGGCACTTCCCTCCTCCCCCAGTCCCTCGCCTGCGTTGGTGCGCTGG
13a	rs509813 major SNP forward	CTCACAAAGGCACACTGTTTCCTTGGGCTCCTCCC**C**CCACCCAACCTTAGAGCCCCCAGC
13b	rs509813 major SNP reverse	AGGCTGGGGGCTCTAAGGTTGGGTGG**G**GGGAGGAGCCCAAGGAAACAGTGTGCCTTTGTG
14a	rs509813 minor SNP forward	CTCACAAAGGCACACTGTTTCCTTGGGCTCCTCCC**G**CCACCCAACCTTAGAGCCCCCAGC
14b	rs509813 minor SNP reverse	AGGCTGGGGGCTCTAAGGTTGGGTGG**C**GGGAGGAGCCCAAGGAAACAGTGTGCCTTTGTG

Supplemental Table S2. *TERT* promoter mutation status at different positions of various cell lines.

No	Primers	-146	-124	-57
1	HeLa Kyoto	100% C	100% C	100% A
2	HCT116	100% C	100% C	100% A
3	253J	100% C	100% C	100% A
4	A375	20% C, 80% T	100% C	100% A
5	U87MG	100% C	15% C, 85% T	100% A
6	T24	100% C	100% T	100% A
7	JON	100% C	100% C	40% A, 60% C
8	575A	100% C	100% C	80% A, 20% C

Supplemental Table S3. MS data for SILAC-based in-vitro DNA reconstitution pull-downs using U87MG nuclear extract with -124C>T vs. WT *TERT* promoter probes.

Supplemental Table S4. MS data for SILAC-based in-vitro DNA reconstitution pull-downs using U87MG nuclear extract with -146C>T vs. WT *TERT* promoter probes.

Supplemental Table S5. MS data for SILAC-based in-vitro DNA reconstitution pull-downs using U87MG nuclear extract with -57A>C vs. WT *TERT* promoter probes.

Supplemental Table S6. List of oligonucleotides used for cloning and sequencing of cDNA and gDNA sequences.

Primer	Primer sequence (5' → 3')	Size	Anneal	Extend
EGFP pcDNA4 forward (Colony PCR and sequencing)	CATGGTCCTGCTGGAGTTCGTG	-	~65°C	30 sec/ kb
E4F1 cloning forward	ATGGAGGGCGCGATGGCA	2355 bp	72°C	75 sec
E4F1 cloning reverse	CTAGACGATGACCGTCTGCACCT			
E4F1 cloning forward	ATGGAGGGCGCGATGGCA	2352 bp	72°C	75 sec
E4F1 cloning reverse 2	GACGATGACCGTCTGCACCTCC			
GABPA cloning forward	ATGACTAAAAGAGAAGCAGAGGAG	1365 bp	64°C	46 sec
GABPA cloning reverse	TCAATTATCCTTTTCCGTTTGCAG			
GABPA cloning forward	ATGACTAAAAGAGAAGCAGAGGAG	1362 bp	64°C	45 sec
GABPA cloning reverse 2	ATTATCCTTTTCCGTTTGCAGAGA			
UHRF1 cloning forward	ATGTGGATCCAGGTTCGGACC	2379 bp	70°C	76 sec
UHRF1 cloning reverse 2	CCGGCCATTGCCGTAGCC			
ZNF148 cloning forward	ATGAACATTGACGACAAACTG	2385 bp	62°C	75 sec
ZNF148 cloning reverse	TTAGCCAAAAGTCTGGCCAG			
ZNF281 cloning forward	ATGAAAATCGGCAGTGGGTT	2688 bp	66°C	85 sec
ZNF281 cloning reverse	TTACCTGTAACTCTGGCTGGTG			
ZNF281 cloning forward	ATGAAAATCGGCAGTGGGTT	2685 bp	66°C	85 sec
ZNF281 cloning reverse 2	CCTGTAACTCTGGCTGGTGG			
PCR8 381bp forward (Colony PCR and sequencing)	ACAACGTTCAAATCCGCTCC	-	~61°C	30 sec/ kb
TERT promoter sequencing forward primer	GATTCGACCTCTCTCCGCTG	1151 bp	61°C	40 sec
TERT promoter sequencing reverse primer	CTCCCTGACGCTATGGTTCC			

Supplemental Table S7. List of oligonucleotides used for shRNA generation.

No	Gene shRNA primer	Clone ID	Primer sequence (5' → 3')
1a	sh*E4F1* #1 forward	TRCN0000419634	CCGGAGGACGTGGTTGTCAGCAAAGCTCGAGCTTTGCTGACAACCACGTCCTTTTTTG
1b	sh*E4F1* #1 reverse		AATTCAAAAAAGGACGTGGTTGTCAGCAAAGCTCGAGCTTTGCTGACAACCACGTCCT
2a	sh*E4F1* #2 forward	TRCN0000013826	CCGGTGTTCAGCACAAGATTCAGAACTCGAGTTCTGAATCTTGTGCTGAACATTTTTG
2b	sh*E4F1* #2 reverse		AATTCAAAAATGTTCAGCACAAGATTCAGAACTCGAGTTCTGAATCTTGTGCTGAACA
3a	sh*GABPA* forward	TRCN0000018290	CCGGGCTAGAACTTCTTACTGATAACTCGAGTTATCAGTAAGAAGTTCTAGCTTTTTG
3b	sh*GABPA* reverse		AATTCAAAAAGCTAGAACTTCTTACTGATAACTCGAGTTATCAGTAAGAAGTTCTAGC
4a	sh*GABPB1L* forward	-	CCGGGAGAGAAGCTCTTCAGAAACACTCGAGTGTTTCTGAAGAGCTTCTCTCTTTTTG
4b	sh*GABPB1L* reverse		AATTCAAAAAGAGAGAAGCTCTTCAGAAACACTCGAGTGTTTCTGAAGAGCTTCTCTC
5a	sh*MYC* forward	TRCN0000039639	CCGGCCCAAGGTAGTTATCCTTAAACTCGAGTTTAAGGATAACTACCTTGGGTTTTTG
5b	sh*MYC* reverse		AATTCAAAAACCCAAGGTAGTTATCCTTAAACTCGAGTTTAAGGATAACTACCTTGGG
6a	sh*SP1* forward	TRCN0000360587	CCGGAGCCATCATGCCTTGATAAATCTCGAGATTTATCAAGGCATGATGGCTTTTTTG
6b	sh*SP1* reverse		AATTCAAAAAAGCCATCATGCCTTGATAAATCTCGAGATTTATCAAGGCATGATGGCT
7a	sh*TERT* forward	TRCN0000240466	CCGGGACGGTGTGCACCAACATCTACTCGAGTAGATGTTGGTGCACACCGTCTTTTTG
7b	sh*TERT* reverse		AATTCAAAAAGACGGTGTGCACCAACATCTACTCGAGTAGATGTTGGTGCACACCGTC
8a	sh*ZNF148* #1 forward	TRCN0000230462	CCGGAGTACCACGGCATCCATATTACTCGAGTAATATGGATGCCGTGGTACTTTTTTG
8b	sh*ZNF148* #1 reverse		AATTCAAAAAAGTACCACGGCATCCATATTACTCGAGTAATATGGATGCCGTGGTACT
9a	sh*ZNF148* #2 forward	TRCN0000218261	CCGGCCTGTGCATAGTAGTACTAATCTCGAGATTAGTACTACTATGCACAGGTTTTTG
9b	sh*ZNF148* #2 reverse		AATTCAAAAACCTGTGCATAGTAGTACTAATCTCGAGATTAGTACTACTATGCACAGG
10a	sh*GFP* forward	-	CCGGACAACAGCCACAACGTCTATACTCGAGTATAGACGTTGTGGCTGTTGTTTTTTG
10b	sh*GFP* reverse		AATTCAAAAAACAACAGCCACAACGTCTATACTCGAGTATAGACGTTGTGGCTGTTGT

Supplemental Table S8. List of oligonucleotides used for quantiative PCR.

No	Primers	Sequence (5' → 3')	Product size
1a	*E4F1* forward	GCACAGAGAAAATCCGCTTC	139 bp
1b	*E4F1* reverse	GGTGAAGTCTCTATAGGCTCG	
2a	*GABPA* forward	GAGAAACTCAGTCGTGCATTAAG	149 bp
2b	*GABPA* reverse	CATTCTGTGACCAAACGGTTC	
3a	*GABPB* forward	TCCACTTCATCTAGCAGCACA	107 bp
3b	*GABPB* reverse	GTAATGGTGTTCGGTCCACTT	
4a	*GABPB1L* forward	ATTGAAAACCGGGTGGAATC	134 bp
4b	*GABPB1L* reverse	CTGTAGGCCTCTGCTTCCTG	
5a	*MYC* forward	TTCGGGTAGTGGAAAACCAG	108 bp
5b	*MYC* reverse	AGTAGAAATACGGCTGCACC	
6a	*SP1* forward	CTCCAGACCATTAACCTCAGTG	143 bp
6b	*SP1* reverse	TGTATTCCATCACCACCAGC	
7a	*TBP* forward	TTCGGAGAGTTCTGGGATTG	144 bp
7b	*TBP* reverse	CTCATGATTACCGCAGCAAA	
8a	*TERT* forward	TCACGGAGACCACGTTTCAAA	94 bp
8b	*TERT* reverse	TTCAAGTGCTGTCTGATTCCAAT	
9a	*ZNF148* forward	GCTGCCTTTAGAACGAACTATCA	167 bp
9b	*ZNF148* reverse	CCACATTCATCACAGCGAAAT	

Supplemental Table S9. List of antibodies used for Western blot.

Antibody	Company	Catalogue No.	Origin	Dilution used for WB
GFP	Roche	11814460001	mouse monoclonal	1:4,000
MYC	Santa-Cruz	sc-40	mouse monoclonal	1:250
Tubulin	MPI-CPG		mouse monoclonal	1:10,000
E4F1	Santa-Cruz	sc-514718	mouse monoclonal	1:500
GABPA	Santa-Cruz	sc-28312	mouse monoclonal	1:250
GABPB1/2	Santa-Cruz	sc-271571	mouse monoclonal	1:250
GAPDH	Novus Biologicals	NB300-221	mouse monoclonal	1:1,000
SP1	Santa-Cruz	sc-17824	mouse monoclonal	1:250
ZNF148	Santa-Cruz	sc-137171	mouse monoclonal	1:500

Supplemental Table S10. List of oligonucleotides used for pyrosequencing.

No	Primers	Sequence (5' → 3')	Location
1a	Region 1 forward (biotinylated)	GGTGGTAGGGGTTAGGGTTTTTTA	CpGs 1-10 in the *TERT* promoter
1b	Region 1 reverse	TCCTACCCCTTCACCTTCCAA	
1c	Region 1 sequencing primer	CTTCACCTTCCAACT	
2a	Region 2 forward	GGGGTGGTAGGGGTTAGG	CpGs 11-21 in the *TERT* promoter
2b	Region 2 reverse (biotinylated)	TCCTACCCCTTCACCTTCCAA	
2c	Region 2 sequencing primer	GGGGTTAGGGTTTTT	
3a	Region 3 forward	AGGGTAAGTATATTAGGTATTGGGTTATT	CpGs 22-31 in the *TERT* promoter
3b	Region 3 reverse (biotinylated)	AACCCCTCCCCTTCCTTT	
3c	Region 3 sequencing primer	GTGGTTGAGTAGTAGGGA	

Supplemental Code. Python script for plotting of SILAC DNA pull-down data in Fig. 1C-E (also deposited to GitHub, github.com/Kappei-Lab/SILAC-Data-Plotting)