

Q9BSI4	TINF2_HUMAN	370	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQGEK-----	407
G2HJU8	G2HJU8_PANTR	372	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQGEK-----	409
G1RZI8	G1RZI8_NOMLE	370	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQGEK-----	407
F7GSQ3	F7GSQ3_CALJA	370	PPRARK---PVCTPSLCSNVITIGDLVLDSD--EENGQGEK-----	407
A0A0D9RTU0	A0A0D9RTU0_CHLSB	370	PPRAKK---PVCPPSLCSSLVITIGDLVLDSD--EENGQGEK-----	407
A0A096NXA6	A0A096NXA6_PAPAN	369	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQGEK-----	406
F6ZRV3	F6ZRV3_HORSE	372	PPRARK---PVGPPSMCSSLVITIGDLVLDSD--EENGQREGR-----	409
M3W4C5	M3W4C5_FELCA	372	PPKARK---PVCPPSLCSSLVITIGDLVLDSD--EENDQREGR-----	409
I3M084	I3M084 ICTTR	368	PPRAKN---PVCSPSLCSSLVITIGDLVLDSD--EENSQREGK-----	405
F1SGN9	F1SGN9_PIG	371	PPRARK---PACPPSLCSSLVITIGDLVLDSEEEEEENGPREGK-----	410
M3Y318	M3Y318_MUSPF	371	PPRTRK---PLCPPSLCSSLVITIGDLVLDSD--EENGQKEGR-----	408
L8I0G5	L8I0G5_9CETA	372	PPRKS----VCPPSLYSSDITIGDLVLDSD--EEDGQRERR-----	407
A3KN17	A3KN17_BOVIN	372	PPRKS----VCPPSLYSSDITIGDLVLDSD--EEDGQRERR-----	407
A0A1U7U6L7	A0A1U7U6L7_TARSY	376	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQREGKVSQRKEQKARKGQGQNK	430
F1Q2J9	F1Q2J9_CANLF	385	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENVQKEGR-----	422
W5QCX6	W5QCX6_SHEEP	372	PPRKSALFPTVCPSSLYSSDITIGDLVLDSD--EEDGQREGR-----	412
K9J163	K9J163_DESRO	371	SPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQREGM-----	408
G1MCN1	G1MCN1_AILME	372	PPKARK---PLCPPSLCSSLVITIGDLVLDSD--EENGQKEGR-----	409
L5M199	L5M199_MYODS	371	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENGQRGGM-----	408
G5B284	G5B284_HETGA	368	PPKAKK---PACSPFLTTSVITIGDLVLDSEE--EENSQREGK-----	405
G1SIM2	G1SIM2_RABIT	366	PPRARK---PVCPPSLCSSLVITIGDLVLDSD--EENSQKDRQ-----	403
G3TC22	G3TC22_LOXAF	368	PPKTRK---PASSASLCSVAITIGDLVLDSD--EDNGQREGK-----	405
A0A091DIW8	A0A091DIW8_FUKDA	355	PPKAKK---PACSPFLTTSVITIGDLVLDSEE--EENSQREGK-----	392
A0A1S3FW91	A0A1S3FW91_DIPOR	394	PPRAKK---RVCSPSLCSSLVITIGDLVLDSD--EGNSQREGK-----	431
H0XYH6	H0XYH6_OTOGA	335	PPRARK---PVSPSLCSSLVITIGDLVLDSD--EENGQRKGM-----	372
A0A1S3WT25	A0A1S3WT25_ERIEU	367	PSRARI---PECPSPMCSSLVITIGDLVLDSEEEESSQ----GK-----	402
A0A1U7RC63	A0A1U7RC63_MESAU	364	PPRAKK---PVQSPSLCSSLVITIGDLVLDSD--EENQ-KEGE-----	400
L5KHQ5	L5KHQ5_PTEAL	311	PPRARK---PVCLPSLCSFVITIGDLVLDSD--EENGQREGR-----	348
G3HDD2	G3HDD2_CRIGR	363	PPGAKK---PMQSPSLCSSLVITIGDLVLDSD--EENS-QKEG-----	399
Q5XIB8	Q5XIB8_RAT	337	PSRVKK---PVLSPSLCSSLVITIGDLVLDSD--EENNQKEGK-----	374
Q8K1K3	Q8K1K3_MOUSE	354	PLTVKK---PVLSPTPCSSLVITIGDLVLDSD--EENNQKEGK-----	391
Q8CH11	Q8CH11_MUSSP	351	PLRVKK---PVLSPTPCSSLVITIGDLVLDSD--EENNQKEGK-----	388
G3VNG5	G3VNG5_SARHA	377	AVGARE---PVSSPSLCSFVATIGDLVLDSD--EGNGQRGGK-----	414
F6VXG0	F6VXG0_MONDO	377	AVGARE---PVHSPSLCSFVITIGDLVLDSD--EGNGQRGGK-----	414

: *****: . .

>sp|Q9BSI4|TINF2_HUMAN TERF1-interacting nuclear factor 2 OS=Homo sapiens
>tr|G2HJU8|G2HJU8_PANTR TERF1-interacting nuclear factor 2 OS=Pan troglodytes
>tr|G1RZI8|G1RZI8_NOMLE TERF1-interacting nuclear factor 2 OS=Nomascus leucogenys
>tr|F7GSQ3|F7GSQ3_CALJA TERF1-interacting nuclear factor 2 OS=Callithrix jacchus
>tr|A0A0D9RTU0|A0A0D9RTU0_CHLSB TERF1-interacting nuclear factor 2 OS=Chlorocebus sabaeus
>tr|A0A096NXA6|A0A096NXA6_PAPAN TERF1-interacting nuclear factor 2 OS=Papio anubis
>tr|F6ZRV3|F6ZRV3_HORSE TERF1-interacting nuclear factor 2 OS=Equus caballus
>tr|M3W4C5|M3W4C5_FELCA TERF1-interacting nuclear factor 2 OS=Felis catus
>tr|I3M084|I3M084 ICTTR TERF1-interacting nuclear factor 2 OS=Ictidomys tridecemlineatus
>tr|F1SGN9|F1SGN9_PIG TERF1-interacting nuclear factor 2 OS=Sus scrofa
>tr|M3Y318|M3Y318_MUSPF TERF1-interacting nuclear factor 2 OS=Mustela putorius furo
>tr|L8I0G5|L8I0G5_9CETA TERF1-interacting nuclear factor 2 OS=Bos mutus
>tr|A3KN17|A3KN17_BOVIN TERF1-interacting nuclear factor 2 OS=Bos taurus
>tr|A0A1U7U6L7|A0A1U7U6L7_TARSY TERF1-interacting nuclear factor 2 isoform X1 OS=Tarsius syrichta
>tr|F1Q2J9|F1Q2J9_CANLF TERF1-interacting nuclear factor 2 OS=Canis lupus familiaris
>tr|W5QCX6|W5QCX6_SHEEP TERF1-interacting nuclear factor 2 OS=Ovis aries
>tr|K9J163|K9J163_DESRO Putative terf1-interacting nuclear factor 2 OS=Desmodus rotundus
>tr|G1MCN1|G1MCN1_AILME TERF1-interacting nuclear factor 2 OS=Ailuropoda melanoleuca
>tr|L5M199|L5M199_MYODS TERF1-interacting nuclear factor 2 OS=Myotis davidii
>tr|G5B284|G5B284_HETGA TERF1-interacting nuclear factor 2 OS=Heterocephalus glaber
>tr|G1SIM2|G1SIM2_RABIT TERF1-interacting nuclear factor 2 OS=Oryctolagus cuniculus
>tr|G3TC22|G3TC22_LOXAF TERF1-interacting nuclear factor 2 OS=Loxodonta africana
>tr|A0A091DIW8|A0A091DIW8_FUKDA TERF1-interacting nuclear factor 2 OS=Fukomys damarensis
>tr|A0A1S3FW91|A0A1S3FW91_DIPOR TERF1-interacting nuclear factor 2 isoform X1 OS=Dipodomys ordii
>tr|H0XYH6|H0XYH6_OTOGA TERF1-interacting nuclear factor 2 OS>Otolemur garnettii
>tr|A0A1S3WT25|A0A1S3WT25_ERIEU TERF1-interacting nuclear factor 2 isoform X2 OS=Erinaceus europaeus
>tr|A0A1U7RC63|A0A1U7RC63_MESAU TERF1-interacting nuclear factor 2 isoform X1 OS=Mesocricetus auratus
>tr|L5KHQ5|L5KHQ5_PTEAL TERF1-interacting nuclear factor 2 OS=Pteropus alecto
>tr|G3HDD2|G3HDD2_CRIGR TERF1-interacting nuclear factor 2 OS=Cricetulus griseus
>tr|Q5XIB8|Q5XIB8_RAT TERF1 (TRF1)-interacting nuclear factor 2 OS=Rattus norvegicus
>tr|Q8K1K3|Q8K1K3_MOUSE TERF1-interacting nuclear factor 2 OS=Mus musculus
>tr|Q8CH11|Q8CH11_MUSSP TRF1-interacting protein TIN2 OS=Mus spretus
>tr|G3VNG5|G3VNG5_SARHA TERF1-interacting nuclear factor 2 OS=Sarcophilus harrisii
>tr|F6VXG0|F6VXG0_MONDO TERF1-interacting nuclear factor 2 OS=Monodelphis domestica

Figure S1. The CK2 consensus motif is conserved across mammalian TIN2 homologs. Alignment generated using Clustal Omega. Identity of species are shown below.

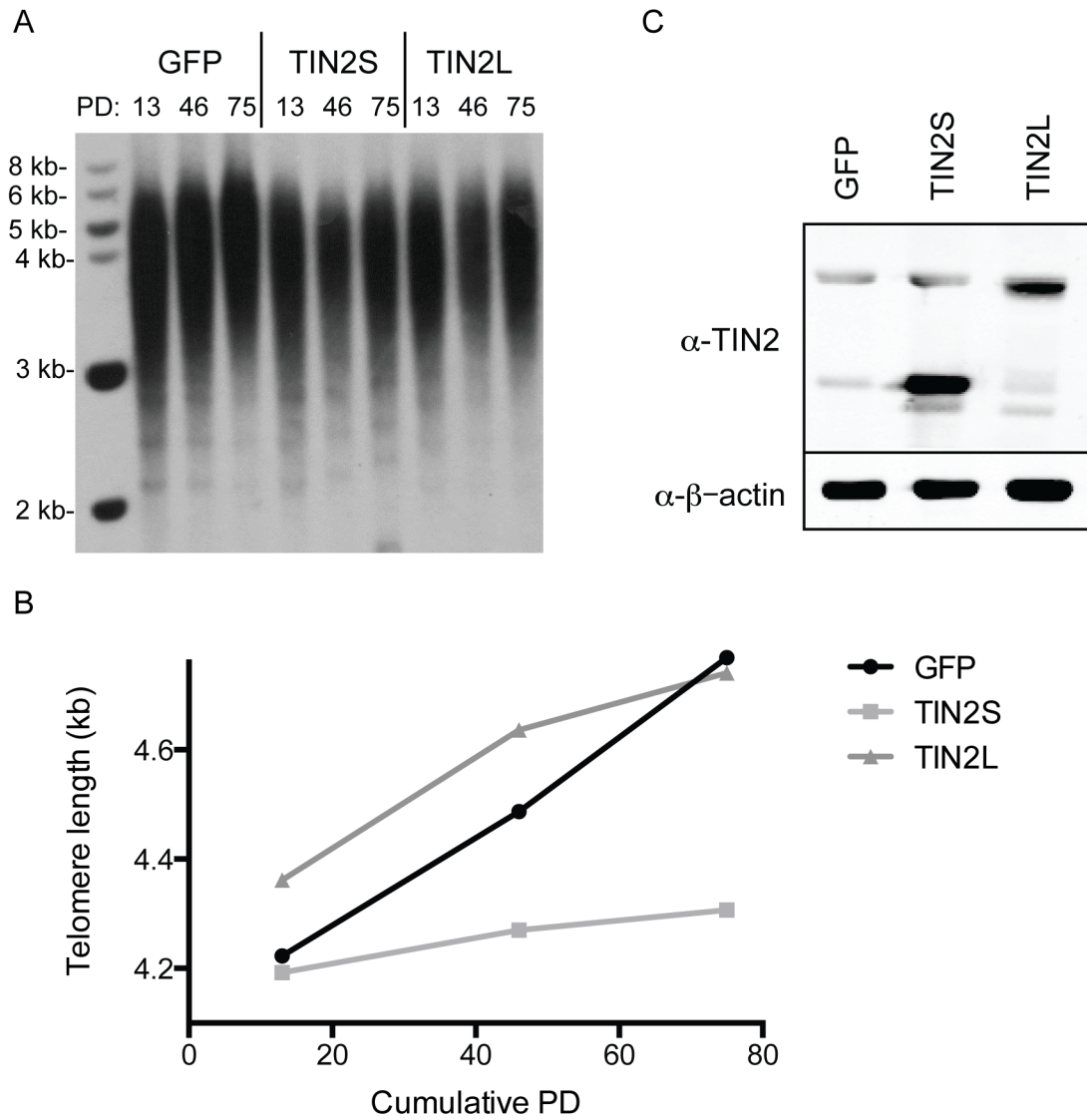


Figure S2. Telomeres in cells overexpressing TIN2L progressively elongated while telomere length remained static in cells overexpressing TIN2S. These cell lines were generated and assayed at a different time from those detailed in Fig. 7. (A) Representative telomere Southern blot analysis of telomere length in kilobasepairs (kb) over time in HT1080 cells overexpressing GFP, TIN2S, or TIN2L. HT1080 cells overexpressing the indicated TIN2 proteins were collected at various times following induction with lentivirus and blasticidin selection and analyzed by the terminal restriction fragment assay. (B) Quantification of (A) using densitometry analysis. (C) Western blot showing TIN2 expression levels in each cell line. Total cell protein lysates were prepared using 2X Laemmli buffer.

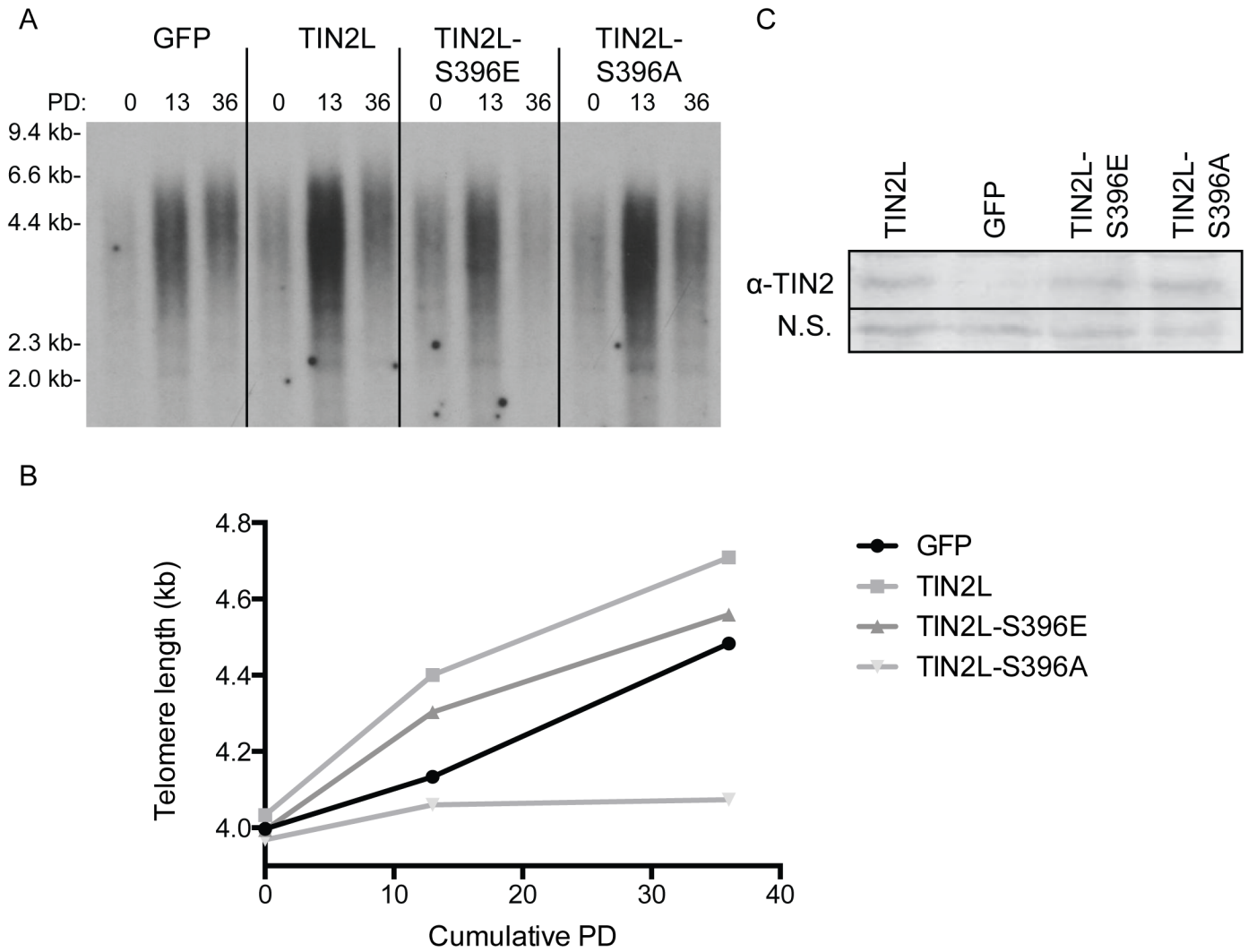


Figure S3. Loss of TIN2L phosphorylation inhibits progressive telomere elongation in telomerase positive HT1080 cells. These cell lines were generated and assayed at a different time from those detailed in Fig. 7. (A) Representative telomere Southern blot analysis of telomere length in kb over time in HT1080 cells overexpressing GFP, TIN2L, TIN2L-S396E, or TIN2L-S396A. HT1080 cells overexpressing the indicated TIN2 proteins were collected at various times following induction with lentivirus and blasticidin selection and analyzed by the terminal restriction fragment assay. (B) Quantification of a using densitometry analysis. (C) Western blot showing TIN2 expression levels in each cell line. Total cell protein lysates were prepared using 2X Laemmli buffer.

Nelson Figure S4

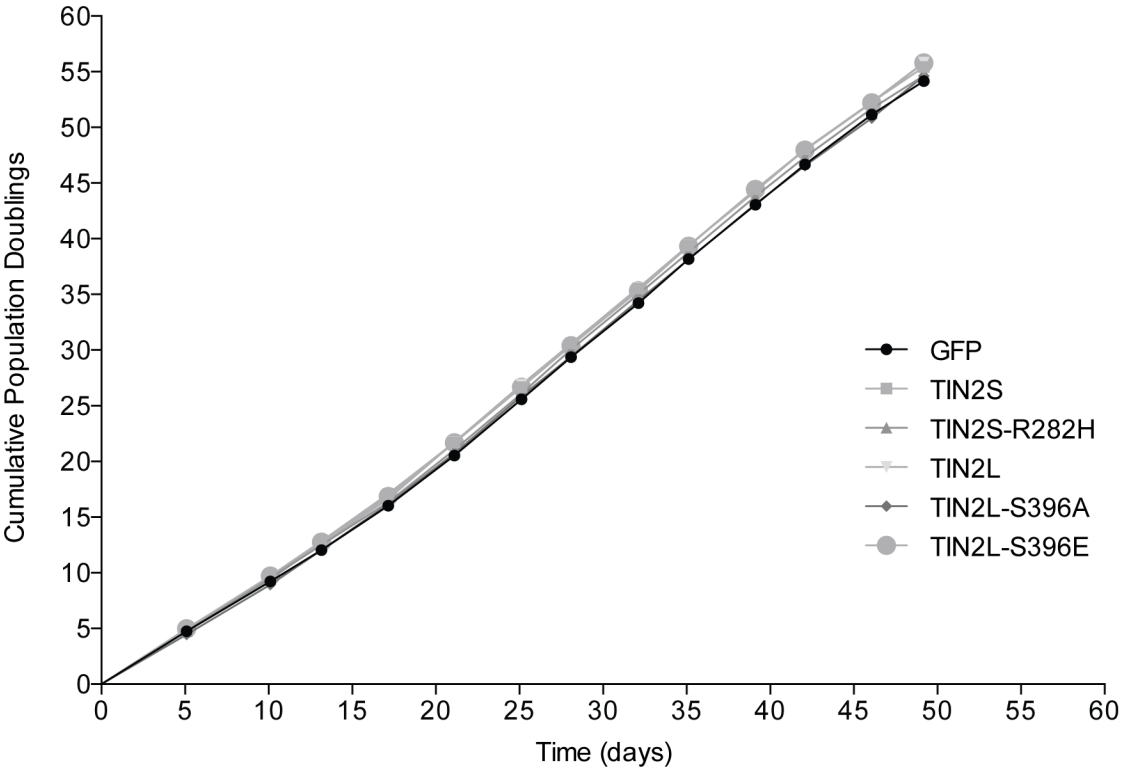
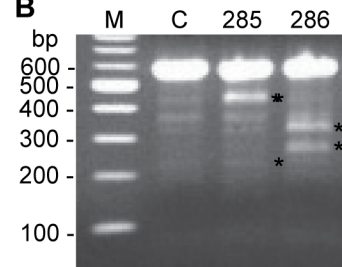


Figure S4. Overexpression of TIN2S, TIN2S-R282H, TIN2L, TIN2L-S396A, and TIN2L-S396E had no effect on HT1080 population doubling time.

A

<i>TINF2</i> exon	Name/plasmid	Guide sequence	Quality Score	BLAST results
7	TINF2ex7-1 Ap285	GAGGTAATAATGATAGTCTC	76	15/20 nucleotides match <i>KCNC2</i>
8	TINF2ex8-1 Ap286	GTCTAAAACCAAGTCCCCTA	78	18/20 nucleotides match <i>MED1</i>

B



C

T-REx-293 285-A 285-F4 286-A	285-F10	g.2370_2379del p.(R365SfsTer24)	g.2378del p.(L364HfsTer22)
	285-F3	g.2378_2379insA p.(L366TfsTer6)	g.2378del p.(L364HfsTer22)
	286-R	g.2525_2568del p.(P379FfsTer3)	g.2560_2567del p.(D391FfsTer3)
HT1080 285-1 286-29	286-6A 286-6B 286-6E	g.2551_2565del p.(I387_L392delinsM)	g.2561del p.(D391TfsTer3)

Figure S5. CRISPR/Cas9 targeting of *TIN2L* expression. A. Guides utilized. Quality scores determined using the CRISPR Design Tool. B. Efficiency of site specific targeting as determined using the Guide-It Mutation Detection Kit (Clontech). Predicted fragments indicated by asterisks. C. Mutants generated with genomic changes on each allele as determined by Sanger sequencing of amplicons encompassing the targeted region and corresponding protein changes. Altered sequences indicated in the schematics as colored bars. Individual wild type and mutant clone IDs indicated. 286-6A, 6B, and 6E are individual subclones.

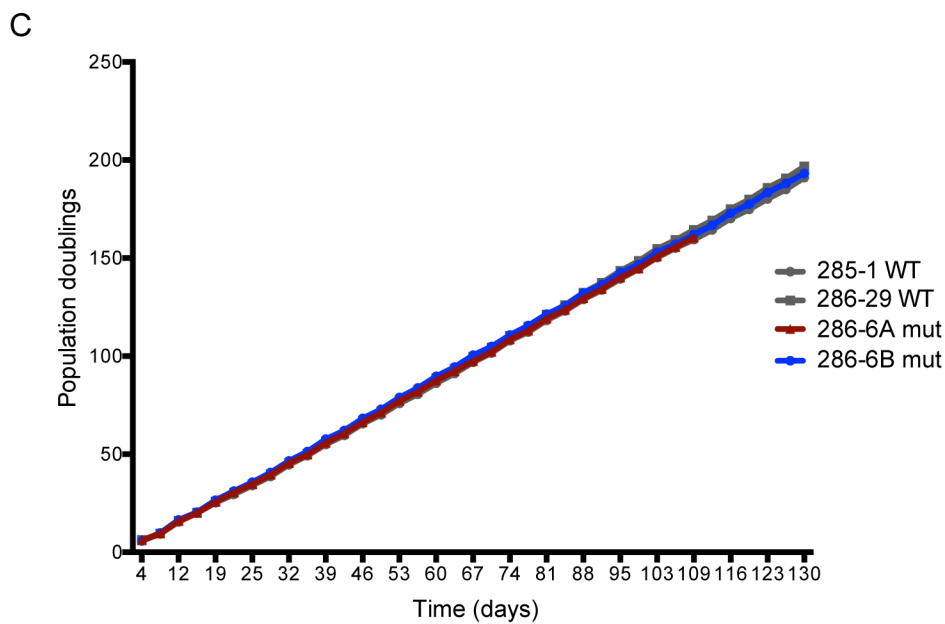
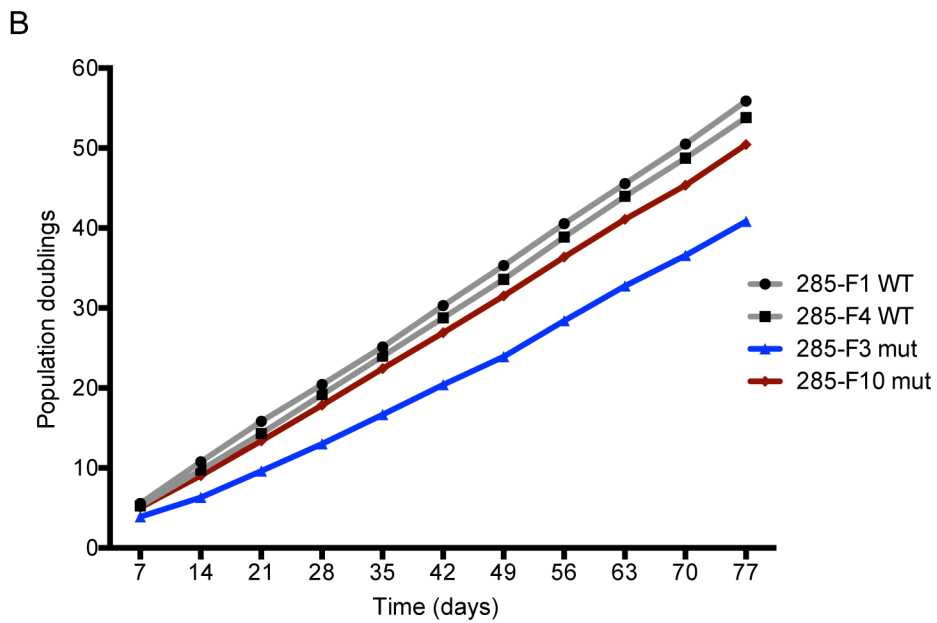
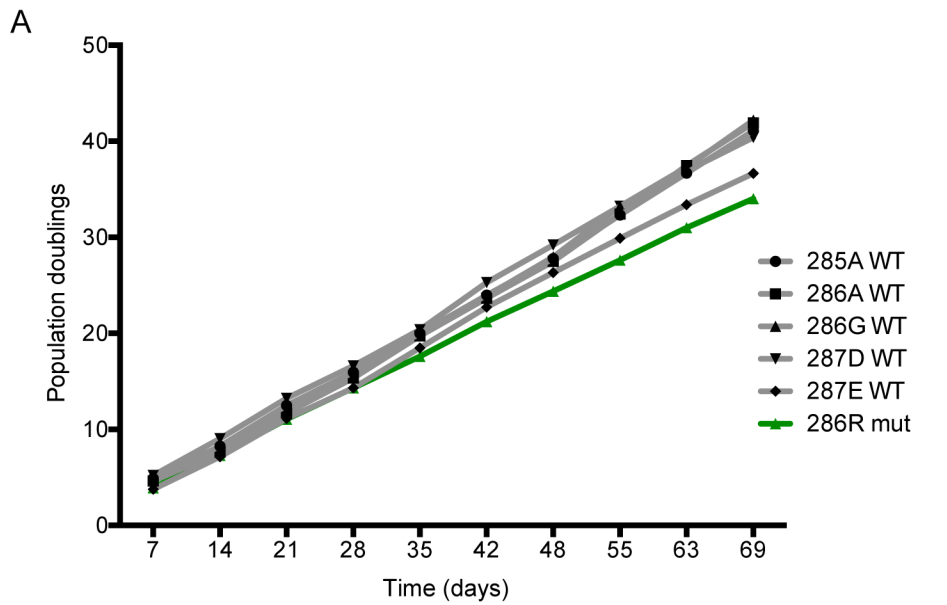


Figure S6. Loss of TIN2L had variable impact on growth. (A) and (B) HT1080 WT and mutant cell lines. (C) T-REx 293 WT and mutant cell lines.

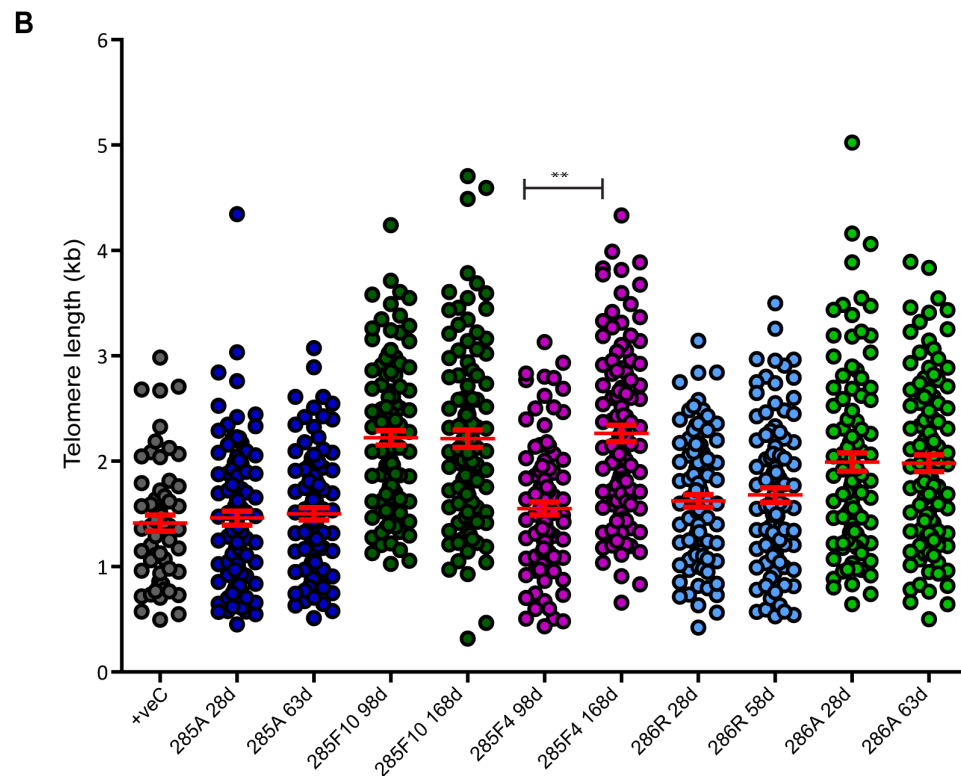
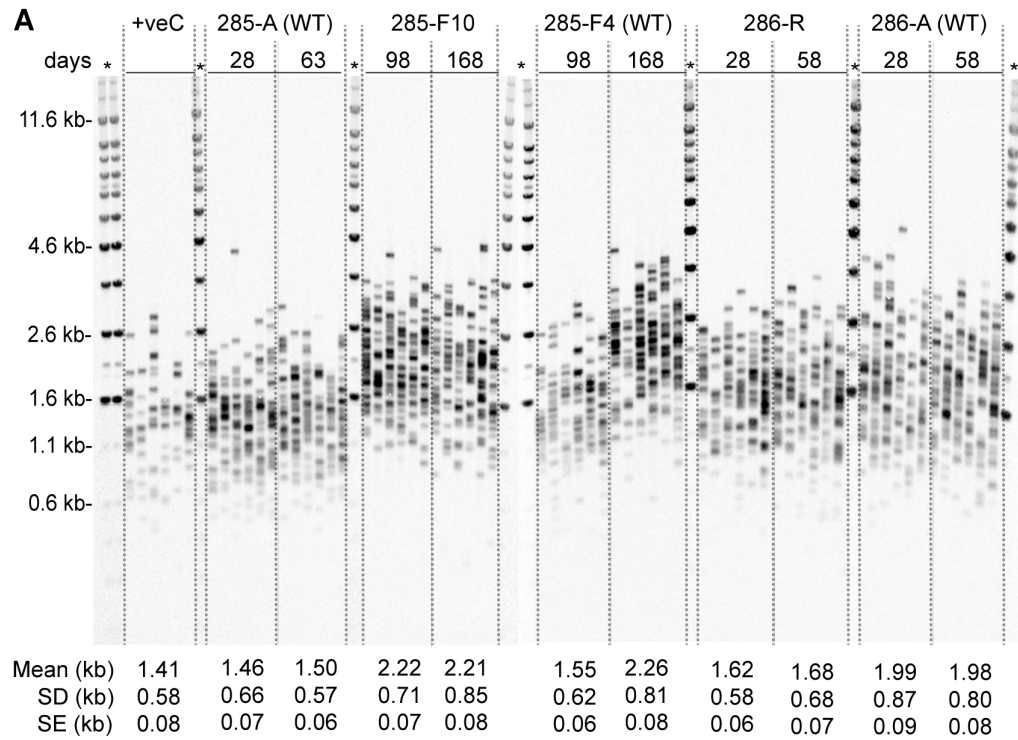


Figure S7. Telomere lengths of TIN2L mutant Flp-In T-REx lines are indistinguishable from TIN2L wildtype lines. (A) 17p STELA analysis of DNA isolated at the indicated numbers of days from the point of clonal line derivation. Mean lengths, standard deviation (SD), and standard error (SE) in kb are indicated. (B) Individual telomere lengths in the designated lines at the designated days.