

1 **Supplementary Figures and Tables**

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3 **Title:**

4 CRISPR/Cas9-Mediated TERT Disruption in Cancer Cells

5 **Authors**

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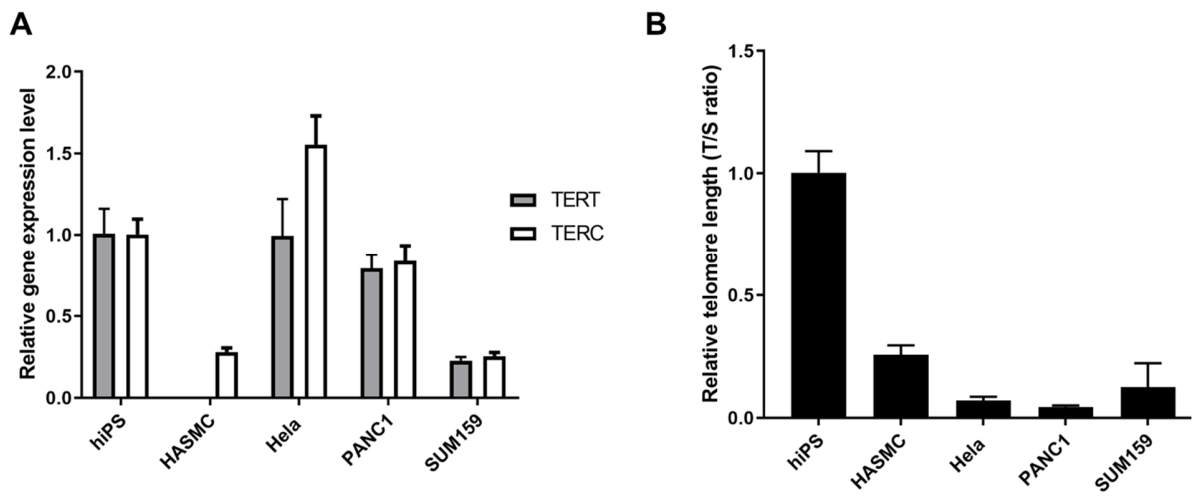
7 Eugene Chen ¹, Jifeng Zhang ¹, Peter X. Ma ³ and Jie Xu ^{1,*}

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10 **Supplementary Figure S1.** Expressions of telomerase genes and relative telomere contents in
11 different cell lines. (A) Relative expression levels of TERT and TERC in hiPS, HASMC, HeLa,
12 PANC1, and SUM159 cells. (B) Relative telomere content determined by T/S ratio in hiPS,
13 HASMC, HeLa, PANC1, and SUM159 cells.

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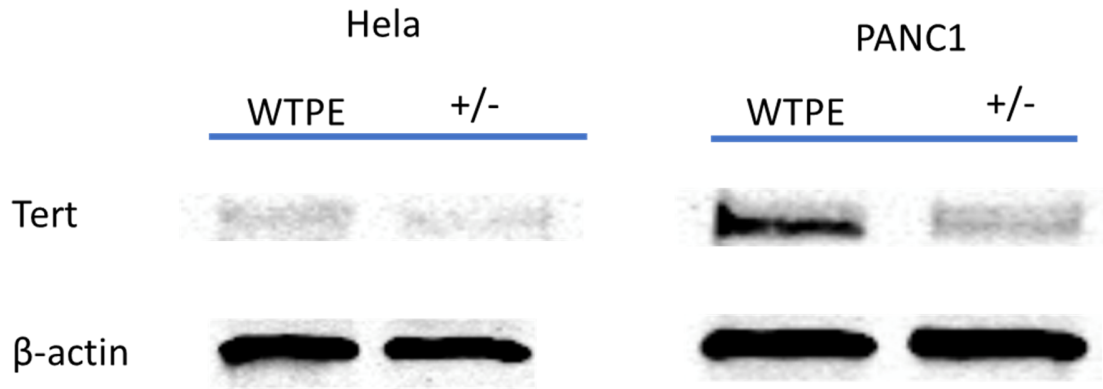


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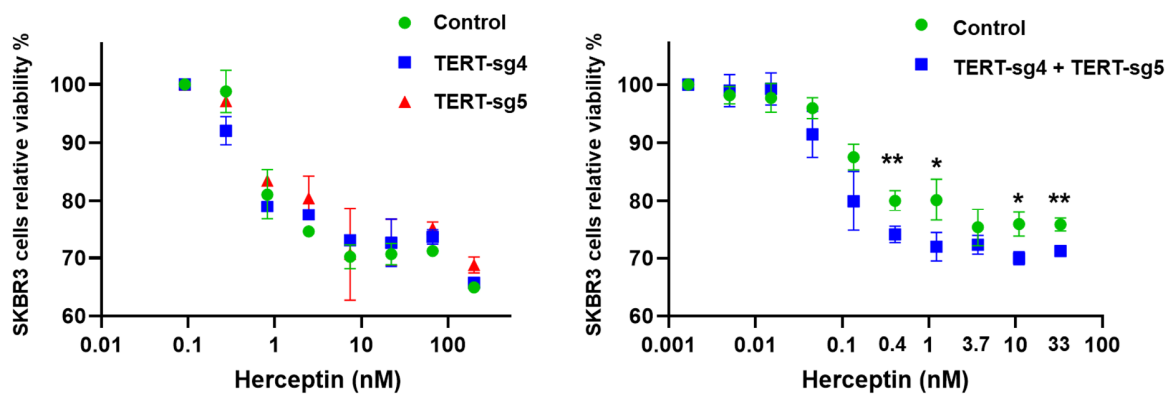
17 **Supplementary S2.** Western blot analysis of TERT protein expression in WTPE and Tert^{+/-} HeLa and
18 PANC1 cells reveal lower signals in Tert^{+/-} than those in WTPE in HeLa (left panel) and PANC1 (right
19 panel) cells.

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22 **Supplementary Figure S3.** Efficacy of TERT disruption in suppressing growth of breast cancer
 23 line SKBR3 cells in vitro. (A) Cas9 mediated TERT disruption by sg4 (blue dots) or sg5 (red dots)
 24 alone has no additive growth inhibiting effects on SKBR3 cells in the presence of Herceptin (green
 25 dots) of concentrations ranging from 0.015 to 33 $\mu\text{g/mL}$. (B) Cas9 mediated TERT disruption by
 26 the exon removal strategy (using both sg4 and sg5, red dots) enhances the efficacy of Herceptin
 27 (green dots) of concentrations ranging from 0.4 to 33 $\mu\text{g/mL}$ in inhibiting the growth of breast
 28 cancer line SKBR3 cells. * $P < 0.05$. ** $P < 0.01$.



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31 **Supplementary Table S1.** Off-target analysis of sg4 and sg5 in HeLa cells. Blue colored letters
 32 indicate the PAM sequence. Red highlighted letters marked the differences of the gRNA with the
 33 target sequence. Indel column shows the detected off target results. No off-target edits were
 34 detected in all the loci tested for sg4 (n=9) and sg5 (n=9).

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Genomic location	Locus details	Sequence	Indel
sg4			
on target	Intron: Tert	TCAGCCAGACAACAGACTAG GGG	
off-targets			
Chr9: 110141708-110141730	intron: PALM2-AKAP2/AKAP2	TGAGTCACACAACA AACTAG TGG	no
Chr4: 162701165-162701187	intergenic: FSTL5-AC005150.3	TCAGCAAGACGGCTGACTAG AGG	no
Chr5: 171194393-171194415	intron: RANBP17	ACAGACAGAAAACAGATTAG TGG	no
Chr4: 113606577-113606599	intron: CAMK2D	ACAGAAAGACAACAGACTAC GGG	no
Chr2: 235792702-235792724	intergenic: AC064874.1-AGAP1	TGAGCTAGAAAACAGACGAG AGG	no
Chr11: 102361958-102361980	intron: BIRC2	TCAGCAAGACAGCAGACTAG GAG	no
Chr1: 89114677-89114699	intron: GBP2	TAAGCCAGACACCAGACTAG TGA	no
Chr14: 74532057-74532079	intron: LTBP2	TCAGCCAGACATCAGAGCAG AGG	no
Chr7: 96705111-96705133	intron: SHFM1	TGAGGCAGACAACAGACTGG TGA	no
sg5			
on target	Intron: Tert	CCTCCAGAAAAGCAGCGTGG GGG	no
off-targets			
Chr5: 61144587-61144609	intergenic: CTC-436P18.4-NDUFAF2	GCTCCAGAGAAGCAGAAATGG TGG	no
Chr11: 127252553-127252575	intergenic: CTD-2234N14.1-RP11-480C22	CCTCCCTGGAAAAGCAAAGTGG GGG	no
Chr4: 156257157-156257179	intergenic: CTSO-AC108211.1	GCTCCAGAAAGAGCAGCGTGG TGG	no
Chr10: 104560185-104560207	intergenic: RP11-127O4.3-RP11-127O4.2	CCTCCAGCATAGCAGCGTGG GGG	no
ChrX: 4912396-4912418	intergenic: AC074035.1-SNORA20	TCTCCAGAACAGCAGCATGG GGG	no
Chr11: 16481443-16481465	intergenic: SOX6-RN7SL188P	CTTCCAGAACAGCAGAGTGG TGG	no
Chr17: 18017527-18017549	intergenic: LRRC48-ATPAF2	CCTCCAGAAAGAGCAGAGTGC AGG	no
Chr8: 89901981-89902003	exon: OSGIN2	CTTCCATAAAAAGAAGCGTGG TGG	no
Chr17: 3515883-3515905	intron: TRPV3	CCTCCA AAAAAGCAGAGAGA GGG	no

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39 **Supplementary Table S2.** Primers used in the present work.

Primers for amplification of gRNA edited sequences	
Primers for gRNA1 edited products:	Forward 5' -AAGAACGTGCTGGCCTTCG-3' Reverse 5' -AGCCACCAGCACAAAGAGC-3'
Primers for gRNA2 edited products	Forward 5' -GGGGCTCAAACGCACTTCT-3' Reverse 5' -ACGTCCAGACTCCGCTTCAT-3'
Primers for gRNA3 edited products	Forward 5' -CGTGAACCTTACGTGGCTCTT-3' Reverse 5' -ACTCACACAGGTGGATGTGAC-3'
Primers for gRNA4 edited products	Forward 5' -AGCTGATTCTGCTCCTTGGC-3' Reverse 5' -TCCCACGACGTAGTCCATGT-3'
Primers for gRNA5 edited products	Forward 5' -GTCTGTTGTCTGGCTGAGCA-3' Reverse 5' -GTTTGAAACGGGTTCCCTGGC-3'
Primers for detection of E4 removal	
	Forward 5' -GAAGGGATGTGGCTCTTTCTACCTG-3' Reverse 5' -CTCACCCACACTCTCCTCAGATG-3'
Primers for T/S ratio analysis	
Primers for T/S ratio	Forward 5' -GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3' Reverse 5' -TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3'
Primers for internal control 36B4	Forward 5' -CAGCAAGTGGGAAGGTGTAATCC-3' Reverse 5' -CCCATTCTATCATCAACGGGTACAA-3'
Primers for off-target (OT) analysis	
Sg4-OT1	Forward 5' -TGTTGTCCATCAGGCTTCCC-3' Reverse 5' -CATTTCCACACCAGGGGGAT-3'
Sg4-OT2	Forward 5' -GATAGCTTGGGGCACTGTGA-3' Reverse 5' -GATAGCTTGGGGCACTGTGA-3'
Sg4-OT3	Forward 5' -AGCTAATCACCTCCGCTGTG-3' Reverse 5' -TTCCCCCAACTCCCCAGTAA-3'
Sg4-OT4	Forward 5' -TCCCTGCGTTCCAATAACCTC-3' Reverse 5' -TTGCTCATGTGGCCGTAGTT-3'
Sg4-OT5	Forward 5' -CTGTGGTGTGTTAGGGGCAT-3' Reverse 5' -CATGGTCACTGGGAGTGGTC-3'
Sg4-OT6	Forward 5' -AAGCTGTCAGTGTCTCTGC-3' Reverse 5' -AGCGTCTGCACCTACAAAGG-3'
Sg4-OT7	Forward 5' -CTGTCTCCAGCGTTTCGAGT-3'

	Reverse 5' -CACTGCTGATGGCATTGACG-3'
Sg4-OT8	Forward 5' -GGTGGACAGTGGGAGACAAG-3' Reverse 5' -CCCTGGAAACCACAGAGGTC-3'
Sg4-OT9	Forward 5' -GCAAAAGCCCTTTTTGGCAC-3' Reverse 5' -CATACGGTCAGGTTGGTGGT-3'
Sg5-OT1	Forward 5' -TGGGCCTAAGACTGACTCCT-3' Reverse 5' -CTGGGCTTTGGAAATGCACTC-3'
Sg5-OT2	Forward 5' -AGAGGTTGGAGATGCCTTGG-3' Reverse 5' -CTGAACTAGGCCAGGAGGTTG-3'
Sg5-OT3	Forward 5' -ATATGCACCTGCATGCCCTG-3' Reverse 5' -GGACCACCCTGTCACCTCTA-3'
Sg5-OT4	Forward 5' -GCCACTTGCTCTACGTCACA-3' Reverse 5' -GGAGTCCATCCTCTGGTCCT-3'
Sg5-OT5	Forward 5' -GCTGGTCTAGGCTTTGCTTG-3' Reverse 5' -GGGAGGCAGAAAAGAGTGTG-3'
Sg5-OT6	Forward 5' -TCAAGTGGGTTTTGCAGGAGA-3' Reverse 5' -CCAAGTTCATTCCAACCACCATC-3'
Sg5-OT7	Forward 5' -TGGCCAGCAGTGAATGTAGG-3' Reverse 5' -TTGAGATGAACAGCCGGTCC-3'
Sg5-OT8	Forward 5' -TGCATAACTCTGGGGCAGTC-3' Reverse 5' -CGGCAATCGAGACCTAGACC-3'
Sg5-OT9	Forward 5' -GATTCCGGATGGGAGAGCTG-3' Reverse 5' -GGTAGGAGGAGGTGGGCTTA-3'

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