Figure S1 (A) iPSCs derived from healthy or mutated somatic cells stain positive for common pluripotency markers (magnification 100x). (B) Representative histology of teratomas derived from telomerase mutant iPSCs (magnification 200x). (C) Dendrogram from the hierarchical cluster analysis using squared Pearson correlation as distance matrix. Two classes were found, one contains all the fibroblast control cell line samples and the other one includes all iPSC samples and ESc H1.



В



**Figure S2** Confirmation of mutations TERT [R1084P] (A), TERT [R889X]f (B), TERT [R889X]m (C) and TERC[-58C>G](D) in the parental somatic cells and the derived iPSCs (wt= wild type control)



**Figure S3** Southern blot confirming successful excision of reprogramming transgenes. Genomic DNA was digested with BamHI and the remaining proviral/genomic DNA fragment was detected with a p32 labeled probe directed against the wpr-element. Letters in bold and italic indicate the parental, non excised iPSC clone followed by their excised counterparts.



**Figure S4** TERC (A) and TERT (B) expression in cre-excised clones in comparison to their non-excised parental iPSC. Shown are transgene free clones 20-6, 20-7, 20-12 derived from clone TERT[R889X]f c20; 20-8, 20-9 derived from TERT[R889X]m c20 and 18-1, 18-8, 18-11 derived from TERC[-58C>G]c18







Cell type and passage

**Figure S5** Telomerase activity was measured using the TRAPeze XL kit and normalized to activity of HeLa cells. Reactions were performed in triplicates and error bars represent s.e.m. Shown are the relative activity levels for individual clones at a particular passage point. (Fib = parental fibroblast)



Cell type and passage

**Figure S6** Southern blot analysis of telomere length. Telomere attrition in clone TERT[R889X]f c4.1 after initial elongation.



## Supplementary Table I Overview of performed iPSC characterization studies (EB= embryoid

body, IHC= Immunohistochemistry)

iPS clone	Marker expression		Pluripotency		Confirmed	
					mutation in the	Karyotyping
	mRNN-array	IHC	EB	Teratoma	iPSC	
CTRL-1 c2		$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$
CTRL-1 c3	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$
CTRL-1 c26	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$
CTRL-2 c1		$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$
CTRL-2 c4		$\checkmark$	$\checkmark$	$\checkmark$		$\checkmark$
TERT[R889X]f c2	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERT[R889X]f c4.1	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERT[R889X]f c20	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERT[R889X]m c20	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERT[R10084P] c5.1	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERT[R10084P] c7	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERT[R10084P] c13	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
TERC[-58C>G] c13	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$		
TERC[-58C>G] c14	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$		
TERC[-58C>G] c18	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$

## Supplementary Table II Primers used for q-PCR and mutational analysis

Assay	Primer Name	Sequence (5'-3')
Telomere q-PCR:		
Telomeric repeats (T)	Tfwd	ACACTAAGGTTTGGGTTTGGGTTTGGGTTAGTGT
	Trev	TGTTAGGTATCCCTATCCCTATCCCTATCCCTAACA
<i>36B4</i> (S)	Sfwd	CAGCAAGTGGGAAGGTGTAATCC
	Srev	CCCATTCTATCATCAACGGGTACAA
TERT/TERC-RT-PCR	TaqMan® Gene Express	ion Assays for telomerase reverse transcriptase (TERT) labeled with 6FAM, Applied
Biosystems, catalog number 4331182.		ber 4331182.
TERT		
	RTC_TERC R	AAGAGTTGGGCTCTGTCAGC
	RTC_TERC L	ATGTGTGAGCCGAGTCCTG
TERC	RTC_TERC probe_Tye	TCCGTTCCTCTTCCTGCGGC
	B-actin F 1021-1039	GGCACCCAGCACAATGAAG
	B-actin R 1068-1087	GCCGATCCACACGGAGTACT
	Actin RT probe 1041-	
Actin	1066_MAX	TCAAGATCATTGCTCCTCGAGCGC
Sequencing primers	E10 TERTF10	GCACAGAATTGCACAAGCTGATG
TERT[R1084P]	TERTseqR10	CTGCTCTTGCGGATCCAGCACC
	TERTF9	GGCTGAATGGTAGACGTGTCG
TERT[R889X]	TERTR9	GAGCAGTCATGGTCTCCAGAGC
	TERCR4	GGTGACGGATGCGCACGAT
TERC[58C>G]	TERCF4	TCATGGCCGGAAATGGAACT
Probe for transgene Southern		
blot	for	GACTGGTATTCTTAACTATGTTGCTCC
	rev	CCAAGGGAGATCCGACTCGTCT

Marker	Vendor	Primary antibody (1:100)	Secondary antibody (1:500)
SSEA4	BD 560218	Mouse IgG3k AlexaFluor555 (red)	
Nanog	Abcam ab21624	Rabbit IgG	AlexaFluor555: Donkey anti-rblgG (Invitrogen: A-31572)
Tra 1-81	BD 560174	Mouse IgMk AlexaFluor488 (green)	
Oct4	Abcam ab 19857	Rabbit IgG	AlexaFluor555: Donkey anti-rblgG (Invitrogen: A-31572)
Tra 1-60	BD 560173	Mouse IgMk AlexaFluor488 (green)	

## Supplementary Table III Antibodies used for immunohistochemical studies

## Supplementary Table IV Composite karyotype by SKY-analysis\*

iPS clone	Clonal	Non clonal aberrations		Karyotype
	aberrations	Structural	Numerical	
CTRL-1 c2, p10	0/15	0/15	8/15	46, XY, normal
CTRL-1 c3, p24	3/11	1/11	7/11	46, XY, normal (4cells), 44, XY, -8,-
				?(3cells), instable culture
CTRL-1 c26, p24	0/16	1/16	5/16	46, XY, normal
CTRL-2 c1, p11	0/20	n.d.	n.d.	46, XY, normal, *conventional G-
				banding no sky performed
CTRL-2 c4, p17	8/10	0/10	6/10	46, XY, inv(1)(q22p21)
TERT[R889X]f c2, p31	0/8	0/8	4/8	46, XX, normal
TERT[R889X]f c4.1, p26	11/11	0/11	2/11	46,XX,der(X)t(X;2)(p22.3:p23) in 11/11
TERT[R889X]f c20, p15	0/7	0/7	0/7	46, XX, normal
TERT[R889X]m c20, p12	0/10	0/10	0/10	46, XY normal
TERT[R10084P] c5.1, p50	0/10	1/10	3/10	46, XY, nomal
TERT[R10084P] c7, p47	0/12	1/12	4/12	46, XY, normal
TERT[R10084P] c13	0/11	0/11	4/11	46, XY, normal
TERC[-58C>G] c13, p19	6/8	1/8	2/8	46 XX,t(9;10)(q22;q24)
TERC[-58C>G] c14, p25	0/14	2/14	15/14	46, XX, normal
TERC[-58C>G] c18, p12	0/16	0/16	5/14	46, XX, normal