Supporting Information

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SI Materials and Methods

Cell Culture, Transfection, and Drug Treatment. GH, NCCIT, H1299, and U2OS cells were maintained in Dulbecco's Modified Eagle medium and HCT116 cells in McCoy's medium, supplemented with 10% FBS. Cisplatin and Trichostatin A (dissolved in DMSO; Sigma) were added as indicated, with corresponding DMSO alone added to controls. Transfection of siRNAs was performed using HiPerFect (Qiagen). In experiments involving siRNA transfection and drug treatment, cells were first transfected, incubated for 36 h, and then treated with the drug.

Amplification of Genomic DNA. Genomic DNA isolated from human cells or diverse ape species was PCR-amplified with Taq polymerase (Fermentas; initial denaturation 3 min at 95 °C, 35 cycles of 15 s at 95 °C, 15 s at 59 °C, and 20 s at 72 °C). PCR products were analyzed by agarose gel electrophoresis. Primers are listed in Table S1.

Quantitative Analysis by Reverse-Transcription PCR. Total RNA was isolated using TRIzol (Invitrogen), followed by reverse transcription with Moloney Murine Leukemia Virus reverse transcriptase (New England Biolabs) and a mixture of oligo-dT and random nonamer primers. IQ SYBR Green Supermix (Bio-Rad) was used for real-time PCR. The primer sequences are listed in Table S1. PCR conditions were 2 min at 95 °C, followed by 40 cycles of 95 °C for 15 s, 59 °C for 20 s, and 72 °C for 40 s. Gene expression levels were normalized to GAPDH or 36B4 as reference genes and calculated using the $2^{-\Delta\Delta Ct}$ method.

RACE. The 5′ RACE experiments were performed using the Rapid cDNA Amplification Kit (Clontech). In brief, 1 μg of total RNA was transcribed by using the 5′ CDS primer and the SMART oligo (Clontech), resulting in cDNAs with the SMART oligo sequence at the 5′ end of the cDNA. Amplification of cDNAs was performed with the 5′-UPM forward primer (Clontech) and a p63-specific reverse primer (Table S1). To enrich for specific RACE fragments, the PCR products were reamplified using the UPM forward primer and the nested p63 specific reverse primer. Purified RACE fragments were cloned into the pCR4-TOPO vector (Invitrogen) and sequenced. Sequence alignments were done with the ClustalW algorithms (1) (www.ch.embnet.org/software/ClustalW.html).

Immunoblot Analysis. After separation by SDS/PAGE, proteins were transferred to nitrocellulose, followed by overnight incubation with antibodies diluted in PBS containing 5% dried milk and 0.1% Tween 20. Antibodies were mouse anti-p63 (4A4; Santa Cruz), mouse anti-β-actin (ab6225; Abcam), mouse anti-lamin B1 (33-2000, ZYMED), rabbit anti-Cox IV (ab16056; Abcam) and rabbit anti-GFP (A11122; Invitrogen). Primary antibodies were detected by peroxidase-coupled secondary antibodies (Jackson immunoresearch) and chemiluminescence (Pierce).

Clonogenic Assays. H1299 cells were transfected using Lipofectamine (Invitrogen), reseeded at equal cell numbers after 24 h, and maintained for 7 to 10 d with geneticin (800 μg/mL), followed by Crystal violet staining. Plates were scanned and the blue pixel rate was determined using Adobe Photoshop 9.

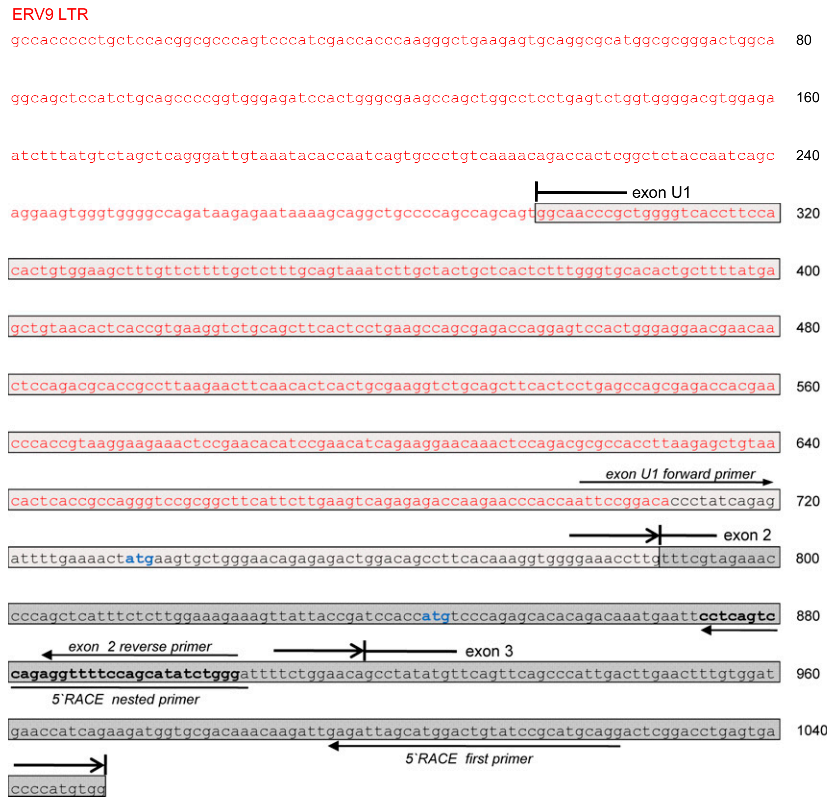
Isolation of Mitochondria. Twenty-four hours after treatment of H1299 cells with 30 μM cisplatin, cells were harvested by scraping. Cell pellets were washed in TD buffer (135 mM NaCl, 5 mM KCl, 25 mM Tris-HCl, pH 7.6). Cell membranes were disrupted by swelling in CaRSB buffer (10 mM NaCl, 1.5 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.5) and dounce homogenization, followed by resuspension in MS buffer (210 mM mannitol, 70 mM sucrose, 5 mM EDTA, 5 mM Tris-HCl, pH 7.6). After centrifugation, the supernatant was loaded on a discontinuous sucrose gradient (1–1.5 M sucrose). Upon ultracentrifugation at 86,000 × g, the mitochondria fraction was aspirated from the interphase of the sucrose gradient. Washing of mitochondria in MS buffer was followed by pelleting at 16,000 × g for 15 min and resuspension in MS buffer. Purified mitochondria together with control crude cell lysates were analyzed by immunoblots.

Cell Cycle Analysis. Attached cells were harvested and combined with floating cells. Cells were fixed overnight in 70% ethanol at −20 °C, and resuspended in PBS containing 1 mg/mL RNase A. Propidium iodide was added to the samples at a final concentration of 30 μg/mL, followed by analysis of fluorescence signals with the Guava EasyCyte flow cytometry system (Millipore). Cell cycle analysis was carried out by the ModFit software. Detection of active caspase 3 and 7 in apoptotic cells was performed by staining with sulforhodamine-coupled caspase inhibitor DEVD (Guava caspase 3/7 SR kit; Millipore) and analysis of fluorescence by Guava EasyCyte; 7-AAD was used to stain cells that had undergone late apoptosis or necrosis and therefore had disrupted membranes.

Immunohistochemistry of Testicular Tissue. Paraffin-embedded tissue sections (5 μm) of normal human testis and testicular cancers were obtained from the archives of the Department of Pathology of Stony Brook University Hospital in accordance with all Internal Review Board and federal regulations and subjected to standard immunohistochemistry. For antigen retrieval, sections were boiled for 5 min in 10 mM citrate buffer, followed by blocking of endogenous peroxidase with 10% goat serum. Sections were incubated overnight at 4 °C with different p63 antibodies (polyclonal 9424 raised against human p63, from ThermoFisher Sci. and monoclonal 4A4 raised against mouse p63 from SantaCruz Biotechnolgoy), followed by secondary biotinylated anti-mouse or anti-rabbit antibodies and streptavidin-peroxidase/3,3′-diaminodbenzidine detection.

1. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.

a Sequence GTAp63



Sequence exon U2

aaattgtggcttttatgacttcctgatgatcagtaccaaatcaacaaggcacaaatttgtgaatctatgtctgaaagaga 80
ggttcagcaacagctgtcttatgggctcaaactaaccaagggaag

Sequence exon U3

gcatccaatcacgacagagatcagaagttcagagatgcctccagctccaaattgccaacaacaagtgtggctactatacgt 80
caaggactctgaagccgtgagagaggggggaagaacaacagtagagaggatgcccagctg

Fig. S1. (Continued)

Fig. S1. (Continued)

Fig. S1. (Continued)

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Homo sapiens       TTGGGCATAT CCAGGACTAG AAATCCATAA GAAAACAGTA GATTGGAAAC TGAAAACACT 60
Hylobates lar      TTGGGCATAT CCAGGACTAG AAATCCGTAA GAAAACAGTA GATTGGAAAC TGAAAACACT
Macaca mulatta     TTGGGCATAT CCAGGACTAG AAATCCTTAA GAAAACAGTA GATTGGAAAC TGGAAACACT

Homo sapiens       ATTGAGAGGT GACAGCGTGC TGGCAGTCCT CACAGCCCTC GCTCGCTCTG GGCGCCTCCT 120
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       CTGCCTGGGC TCCCACTTTG GTGGCACTTG AGGAGCCCTT CAGCCCACTG CTGCACTGTG 180
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       GGAGCCTCTT CTGGGCTGGC CAAGGCCGGA GCCCGCTCCC TCAGCTTGCA GGGAGGTGTG 240
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       GAAAGAGAGG TGGGAGCGGG AACTGGGGCT GCGCGCGGCG CTTCCGGGCC AGCTGGAGTT 300
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       CCGGGTGGGC GTGGGCTTGG CGGGCTCTGC ACTCGGAACA GCCGGCCGGC CCCGCCGGCC 360
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       CCAGGCAATG AGGGGCTTAG CACCCGGGCC AGCAGCTGTG GAGGGTGTAC TGGGTCCCCC 420
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       AGCAGTGCCA GCCCACCAGC GCTGCGCTCG ATTTCTCACC GGGCCTTAGC TGCCTTCCCG 480
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       TGGGGCAGGG CTCGGGACCT GCAGCCCGCC ATGCCTGAGC CTCCCACCCG CTCTGTGGGC 540
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       TCCTGTGTGG CCGGAGCCTC CTCGACAAGC GCCACCCCCT GCTCCACGGC GCCCAGTCCC 600
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       ATCGACCACC CAAGGGCTGA AGAGTGCAGG CGCATGGCGC GGGACTGGCA GGCAGCTCCA 660
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       TCTGCAGCCC CGGTGGGAGA TCCACTGGGC GAAGCCAGCT GGCCTCCTGA GTCTGGTGGG 720
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       GACGTGGAGA ATCTTTATGT CTAGCTCAGG GATTGTAAAT ACACCAATCA GTGCCCTGTC 780
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       AAAACAGACC ACTCGGCTCT ACCAATCAGC AGGAAGTGGG TGGGGCCAGA TAAGAGAATA 840
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       AAAGCAGGCT GCCCCAGCCA GCAGTGGCAA CCCGCTGGGG TCACCTTCCA CACTGTGGAA 900
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       GCTTTGTTCT TTTGCTCTTT GCAGTAAATC TTGCTACTGC TCACTCTTTG GGTGCACACT 960
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens       GCTTTTATGA GCTGTAACAC TCACCGTGAA GGTCTGCAGC TTCACTCCTG AAGCCAGCGA 1020
Hylobates lar      ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta     ---------- ---------- ---------- ---------- ---------- ----------
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Fig. S1. (Continued)

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Homo sapiens     GACCAGGAGT CCACTGGGAG GAACGAACAA CTCCAGACGC ACCGCCTTAA GAACTTCAAC 1080
Hylobates lar    ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta   ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens     ACTCACTGCG AAGGTCTGCA GCTTCACTCC TGAGCCAGCG AGACCACGAA CCCACCGTAA 1140
Hylobates lar    ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta   ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens     GGAAGAAACT CCGAACACAT CCGAACATCA GAAGGAACAA ACTCCAGACG CGCCACCTTA 1200
Hylobates lar    ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta   ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens     AGAGCTGTAA CACTCACCGC CAGGGTCCGC GGCTTCATTC TTGAAGTCAG AGAGACCAAG 1260
Hylobates lar    ---------- ---------- ---------- ---------- ---------- ----------
Macaca mulatta   ---------- ---------- ---------- ---------- ---------- ----------

Homo sapiens     AACCCACCAA TTCCGGACAC CCTATCAGAG ATTTTGAAAA CTATGAAGTG CTGGGAACAG 1320
Hylobates lar    ---------- ---------- ---ATCAGAG ATTTTAAAAA CTGTGAGGTG CTGGGAACAG
Macaca mulatta   ---------- ---------- ---GTCAGAG ATTTTAAAAA CTATGAAGTG CTAGAAACAG

Homo sapiens     AGAGACTGGA C---AGCCTT CACAAAGGTG GGGAAACCTT GGTAAGTACT CAG--TTTTC 1380
Hylobates lar    AGAGACTGGA GTTGAAGCTT CACAAAGGTG GGGAAACCTT GGTAAATACT CAGAGTTTTC
Macaca mulatta   AGAAATTGGA GTTGAAGCTT CACAAAGGCG GGGAAACCTT GGTAAATACT CAGAGTTTTC

Homo sapiens     AGTTGAAACC CAAGAAGATA AAAATCTAAA GGACCATGCA TTAAGAGTAA G
Hylobates lar    AGTTGAGATC CAAGAGGATA AAAATCTAAA GGACCATGCA TTAAGAGTAA G
Macaca mulatta   AGCTGAGACC CAAGAGGATA AAAATCTAAA GGACCATGCA TTAAGAGTAA G
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Fig. S1. Sequence and expression of GTAp63. (*A*) (*Upper*) The recently identified p63 exon U1 together with known exons 2 and 3 constitutes the major 5′ end of GTAp63 mRNA. Primer binding sites for RACE and subsequent PCR analysis are indicated. The sequence corresponding to the endogenous retrovirus 9 (ERV9) LTR, as well as the translational start codons (ATG), are shown in red. Sequences corresponding to exons are framed. (*Lower*) Transcripts of minor abundance contain either exons U2 or U3 (mutually exclusive), inserted between exons U1 and exon 2. (*B*) Translation of GTAp63 mRNA predominantly starts at the first ATG initiation codon. Full-length mRNA of GTAp63α, including all known 5′ and 3′ UTRs, was cloned into pcDNA3 and expressed in H1299 cells, followed by p63 immunoblot analysis with the monoclonal antibody 4A4. The long GTAp63 is the predominant product (lane 2). Within this wild-type GTAp63, each ATG was separately mutated to CTG. Mutating the first ATG eliminated the normal pattern, generating instead an aberrant "escape" TAp63 product in abundance (lane 3); its size is easier to estimate on the shorter exposure. In contrast, mutating the second ATG retained the pattern of a predominant upper GTAp63 band (lane 4). Thus, the first ATG codon is predominantly used to generate the GTAp63 protein. A minor translation product results from the use of the second ATG, generating TAp63 (*C*). The pcDNA3 containing conventional p63 starting with exon 1 was used as control. It generates TA*p63 and TAp63 (lane 5). Coexpressed GFP and Ponceau S staining were used as loading controls. (*C*) Comparative mRNA expression of conventional TAp63 and GTAp63. H1299 cells were transiently transfected with the same plasmid backbone encoding one of the indicated p63 full-length mRNAs under the same conditions. RT-PCR analysis of mRNA levels, normalized to GAPDH. Mean and SE from three independent experiments are shown for both. The level of conventional TAp63α was set to 1. *P* values were calculated using the two-sided Student`s *t* test. (*D*) Similar stability of conventional TAp63 and GTAp63 proteins. H1299 cells were transfected as in *C*, followed by treatment for the indicated periods of time with a general inhibitor of translation, cycloheximide. The p63 was detected by immunoblot analysis (mAb 4A4). No gross difference in protein stability was observed between conventional TAp63 and GTAp63. Note that longer exposures were used for conventional TAp63 to compensate for its lower expression levels, but this did not affect the relative signal intensities obtained after cycloheximide chase. (*E*) The mRNA levels of GTAp63 and conventional TAp63 in testes and ovaries from human donors of various ages were determined by RT-PCR, as in *D*. Although some GTAp63 was detected in ovaries from a young donor, GTAp63 levels were far lower in ovaries than in testes. In contrast, the levels of conventional TAp63 were comparable. Because the preparation of isolated human primordial and primary oocytes (which are the ones selectively expressing TAp63) in amounts sufficient for RNA analysis is incompatible with ethical standards, we had to restrict our further analysis to total human ovaries. Whereas conventional TAp63 mRNA levels were comparable between testis and ovary, GTAp63 mRNA levels were far lower than in testis, at least suggesting that the expression of GTAp63 is biased toward the male germ line. (*F*) The ERV9 LTR upstream of human *TP63* in comparison with other ERV9 LTRs. ERV9 left a trace of > 100 provirus-like copies and at least 4,000 solitary LTRs in the the human genome (1). Sequence comparison by the ClustalW algorithm reveals that the TP63-associated ERV9 LTR belongs to the subfamily IX of ERV9 (2), strongly suggesting that its insertion occurred less than 18.7 million years ago. (*Left*) A depiction of the evolutionary distances of ERV9 subfamilies. (*Right*) Association of individual members of subfamily IX, including the LTR upstream of *TP63*. The sequence names within refer to a previous assembly of ERV9 LTR sequences (2). The sequence alignments are provided in Table S2. (*G*) (*Left*) PCR amplification from genomic DNA of exon U1, comprising the ERV9 LTR and GTAp63 sequences, yields a specific product in *Hominidae* but not in other primates. Primers "LTR forward" and "LTR reverse" were used (Table S1). In contrast, exon 2 was successfully amplified from all primates. (*Right*) Primers spanning the LTR insertion site ("upstream LTR forward" and "downstream LTR reverse") amplified the entire LTR with flanking sequences from genomic DNA of *Homo sapiens* (lane 1). In contrast, they amplified only the acceptor region without the LTR from nonhominid primates (lanes 2 and 3). Sequence alignments of the PCR products are shown in *H*. (*H*) PCR-amplification revealed the presence or absence of a LTR upstream of *TP63* (Fig. 2*B*). The sequences of the PCR products amplified from different primates are shown. The LTR sequence is marked in red.

1. López-Sánchez P, Costas JC, Naveira HF (2005) Paleogenomic record of the extinction of human endogenous retrovirus ERV9. *J Virol* 79:6997–7004.
2. Costas J, Naveira H (2000) Evolutionary history of the human endogenous retrovirus family ERV9. *Mol Biol Evol* 17:320–330.

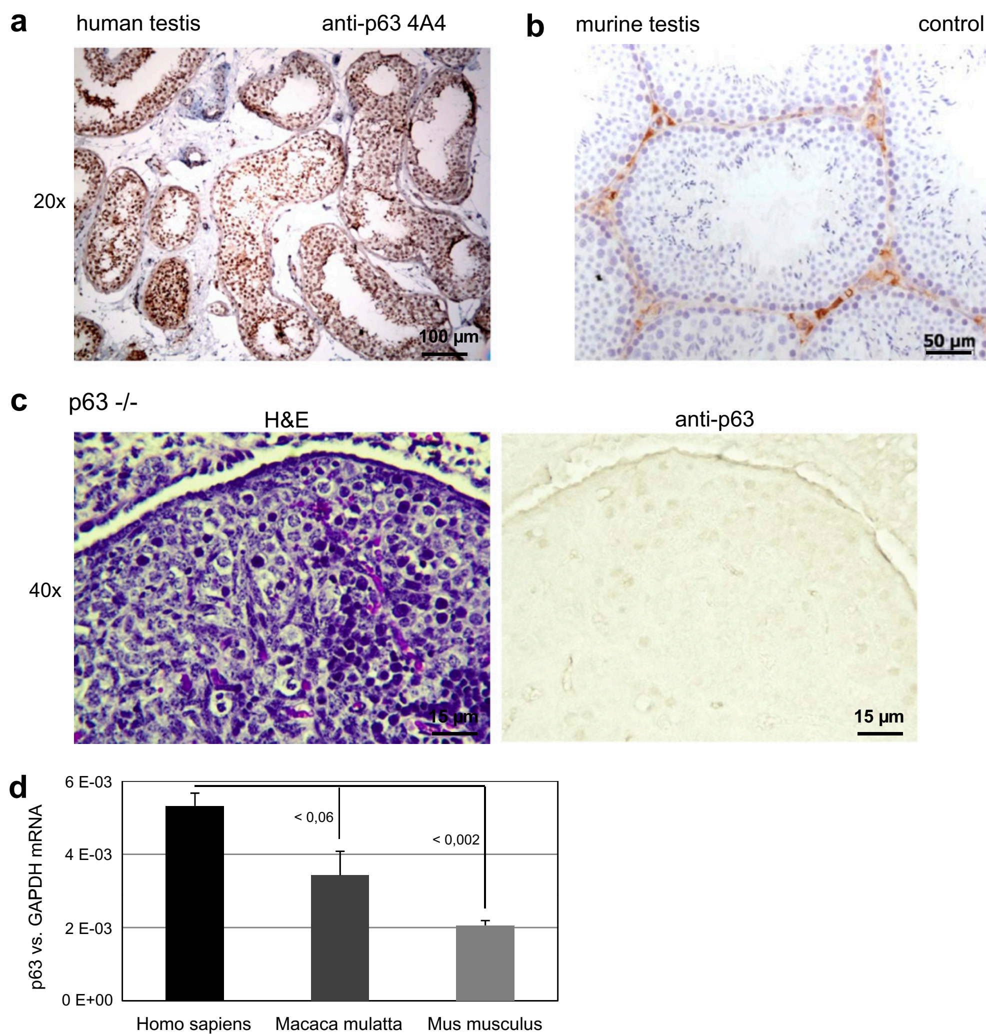

Fig. S2. Immunostaining of p63 in testis. (*A*) Expression of p63 in human testis, detected by monoclonal antibody 4A4. Expression of p63 in the basal and suprabasal layers of germ-cell precursors within seminiferous tubules. This pattern was seen in more detail on a higher magnification (Fig. 2*C*). It is identical to the pattern detected with the polyclonal p63 antibody 9424, shown in Fig. 2*B*. (*B*) No staining is seen when the first antibody is omitted. (*C*) Specificity control. Staining is not detected with 4A4 antibody in the testis of E17 mouse embryos carrying a targeted deletion of the *Trp63* gene. (*Left*) Corresponding H&E. (*D*) The mRNA levels encoding transactivating p63 (corresponding to exon 2 and exon 3) in the testis of humans, mice, and Rhesus macaques, as determined by qRT-PCR. The p63 mRNA was increased two- to threefold in human testis relative to murine or simian testis. Note that in addition to this moderate global increase, there is also a difference in relative cellular distribution within the spermatogenic epithelium between human and mouse, as shown in Fig. 2 *B*–*D*.

Fig. S3. (Continued)

Fig. S3. GTAp63 induces apoptosis and associates with mitochondria in cisplatin-treated cells. (*A*) Cleavage of GTAp63α by caspases in response to genotoxic stress. H1299 cells were stably transfected to express wild-type GTAp63alpha or the indicated mutants. Upon treatment with cisplatin for 24 h, p63 was detected by immunoblot analysis. Upon cisplatin treatment, a cleaved fragment of p63 is detected, but not when the known caspase cleavage site (1) is removed by a D497A point mutation. (*B*) H1299 cells stably expressing GTAp63 or the indicated mutants were treated as described in Fig. 3*D*, followed by flow cytometry to quantify the apoptotic cell fraction. Staining with DEVD (binding to active caspase 3 and 7) was performed to detect mid- and late-stage apoptosis, and simultaneous incubation with 7-amino actinomycin (7-AAD) revealed plasma membrane desintegration typical for necrosis or late apoptosis. (*C*) Decreased clonogenic survival upon expression of a truncated GTAp63 protein that mimics the caspase cleavage product normally generated by DNA damage. Bicistronic expression plasmids (pIRESneo) for GTAp63 and neomycin resistence were transfected into H1299 cells, followed by G418 selection, Crystal violet staining, and quantitation of the area covered by cell clones. The ratio of areas covered by cells upon transfection of GTAp63 vs. vector control is displayed as the mean and SD from three independent experiments. A stop codon was introduced into the GTAp63 coding region after the caspase cleavage site to mimic the cleavage fragment (Fig. 3*A*), which resulted in strong suppression of clonogenic survival. (*D*) GTAp63 augments cisplatin-induced apoptosis even when transcription is blocked by actinomycin *E*. H1299 cells stably expressing GTAp63α or transfected with empty vector (control) were treated with 30 μM cisplatin or an inhibitor of transcription, actinomycin D (4 μM). Cell death was assessed as the percentage of cells in the SubG1 shoulder of propidium iodide stained cells using flow cytometry. The raw data are shown in *E*. Means and SD from three independent experiments are shown. Indeed, wild-type GTAp63 enhanced cell death even when transcription was abolished by actinomycin D. Thus, GTAp63-mediated cell death does not solely rely on the induction of proapoptotic genes. (*D*) Raw data corresponding to *C*. H1299 cells stably transfected to express wild-type or mutant GTAp63 were treated with cisplatin and transcriptional inhibitor actinomycin D, followed by propidium iodide staining and flow cytometry to determine the DNA content, as described in *C*. Sub-G1 DNA content (blue) is indicative for cell death. The percentage of cells with a sub-G1 DNA content is indicated. (*F*) GTAp63 and its caspase cleavage fragment associate with mitochondria in response to DNA damage. H1299 cells stably expressing GTAp63alpha or the C306R DNA binding mutant were treated with 30 μM cisplatin or DMSO alone for 24 h, followed by cell fractionation and isolation of mitochondria. Total cell lysates and mitochondrial fractions (verified by immunoblot detection of mitochondrial Cox IV; contamination ruled out by staining of nuclear Lamin B1) were subjected to immunoblot analysis. GTAp63 associates with

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mitochondria in unstressed and stressed cells, with enrichment of its caspase cleavage fragment upon stress (long exposures). (*G*) Quantification of the cleaved GTAp63 fragment associated with mitochondria. The signal intensities from the blot in *B* were quantified, and the ratio of cleaved vs. full length GTAp63 was calculated for total lysates and mitochondria. Upon cisplatin treatment, a twofold increase in caspase-cleaved vs. full-length GTAp63 was found at the mitochondria, compared with crude lysate. Note that cleaved GTAp63 is at or near zero in DMSO-only treated cells (black bar) because of lack of caspase activity. Taken together, these results strongly suggest that GTAp63, in particular when cleaved by caspases, enhances apoptosis not only through gene activation, but also by directly activating the intrinsic apoptotic pathway at the mitochondria. These mechanisms appear to provide a positive feedback for GTAp63-mediated apoptosis upon DNA damage.

1. Sayan BS, et al. (2007) Cleavage of the transactivation-inhibitory domain of p63 by caspases enhances apoptosis. *Proc Natl Acad Sci USA* 104:10871–10876.

Fig. S4. Loss of GTAp63 in testicular cancer is rescued by histone deacetylase inhibition but not by DNA methyltransferase inhibition. (*A*) In most, albeit not all seminomas, p63 is undetectable. (*Lower*) Loss of p63 in an ovarian germ-cell tumor (called dysgerminoma, the rare female counterpart of seminoma). (*Left*) H&E staining reveals their characteristic histologic pattern. (*Right*) Staining with p63-specific polyclonal antibody 9424 reveals no specific signal in cases I and II, but does detect retained nuclear p63 in case III. (*B*) The mRNA levels of conventional TAp63 and TAp73 in human testicular cancer cells. GH cells were treated with trichostatin A (TSA) followed by RT-PCR analysis, as in Fig. 4*C*. In contrast to GTAp63 (>3,000-fold induction; see Fig. 4*C*), conventional TAp63 was induced less than 20-fold and TAp73 remained unchanged in response to TSA. (*C*) TSA but not 5-azacytidine (5-aza) induced re-expression of p63 in GH cells. Treatment for 24 h at the indicated concentrations.

Table S1. Primers used for PCR

Table S1

Table S2. Sequence comparison of ERV9 LTRs with exon U1 of TP63

Table S2

Table S3. Alignment of TP63 exon U1 from different species within the family of *Hominidae* (humans and great apes)

Table S3