Fig. S1. Controls of antibody specificity and mitochondrial purity. (A) hTERT was immunoprecipitated from GM847 hTERT (lane 1), hTERT-HA (lane 2) or empty vector (lane 3). Samples were submitted to Western blots using the anti-hTERT antibody from Rockland (left panel) or an anti-HA antibody (right panel). (B) Upper panel: GM847 hTERT-HA cells and the telomerase-positive prostate cancer cell line LNCaP were stably transfected with control or short hairpin RNA against hTERT (7). hTERT was probed by Western blots using the Rockland antibody in 100 µg of total cell lysate. Tubulin was used as loading control. Lower panel: total cell lysate of GM847 hTERT-HA (50 µg) or MEFs derived from WT or KO TERT animals (150 µg) were probed for TERT; actin was used as loading control. (C) Increased mitochondria purity in different steps of fractionation using SQ20B and SCC61 cells. Subcellular fractions submitted to different isolation protocols were probed for mitochondrial (HSP70), cytoplasmic (tubulin) and nuclear (Ku80) markers. The latter has two forms, a higher molecular weight that is nuclear and a C-terminally truncated version that is mitochondrial (1). The arrows indicate the two forms of the protein. Mitoplasts were generated by stripping the outer mitochondrial membrane using digitonin. (D) Aconitase activity was determined in SQ20B cells using an in-gel assay; M: mitochondrial, C: cytosolic version of aconitase. Aconitase is active in both mitochondria and cytoplasm (2).

Fig. S2. hTERT interacts with mitochondrial tRNAs. GM847 fibroblasts expressing either empty vector (EV) or hTERT-HA were crosslinked with formaldehyde and hTERT pulled down using an anti-HA antibody. RNAs bound to it were identified using RT-PCR. (A) Panel shows results obtained when RNA was used as template for the PCR prior to (lanes 2 and 3) and after DNase I treatment (lane 4). RNA were submitted to two consecutive DNase I treatment. Lane 5 shows that cDNA was amplified after treatment of RNA with DNase I. (B) Panel shows negative results obtained with EV control. (C) Crosslinking and IPs were performed *in organello* and the RNA of the RMRP and the 5.8S rRNA amplified using specific primers (see Table S1). Arrow indicates expected band size.

Fig. S3. hTERT drives first strand cDNA synthesis in vitro at 37°C and at 50°C. RRL-translated hTERT was used to perform first strand cDNA synthesis using a commercially available kit from Invitrogen. The RT provided in the kit was used as positive control (superscriptase) and cDNA amplified using PCR. Upper panel: First strand synthesis was performed at 37°C and at 50°C as indicated. cDNA and genomic DNA obtained from HeLa cells were included as controls (lanes 1 and 2, respectively). The RNA template for the other reactions was obtained from VA13 cells, which lack endogenous hTR. Lower panel shows reactions performed at 50°C and included the DN hTERT and RRL-only as controls.

Supplementary References

1. Coffey,G., and Campbell,C. (2000) An alternate form of Ku80 is required for DNA end-binding activity in mammalian mitochondria. Nucleic Acids Res.28, 3793-3800.
2. Regev-Rudzki,N., Yogev,O., and Pines,O. (2008) The mitochondrial targeting sequence tilts the balance between mitochondrial and cytosolic dual localization. J Cell Sci. 121, 2423-2431.

Table legends

Table S1. Sequence of primers used for RIP analysis

Figure S1

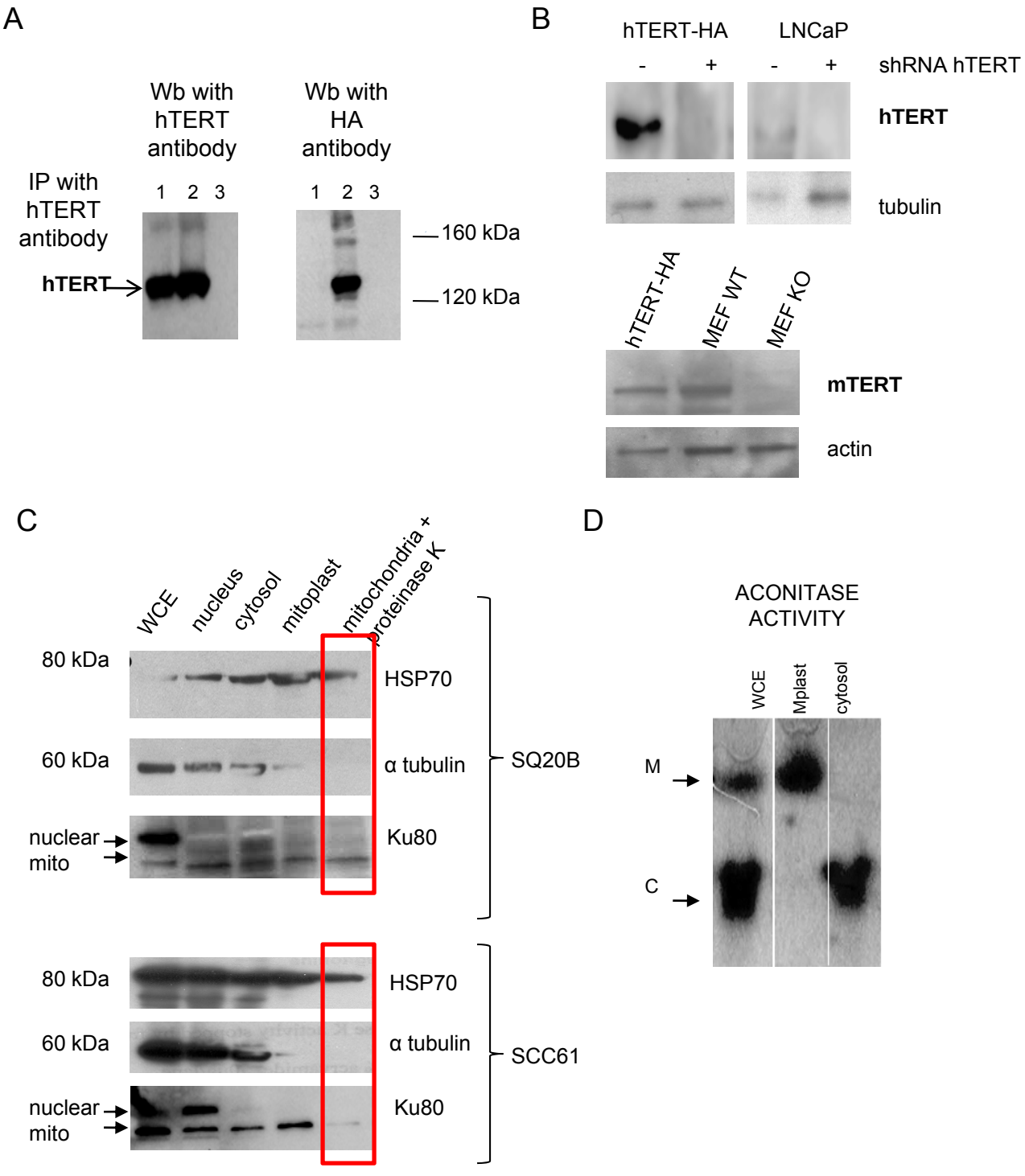
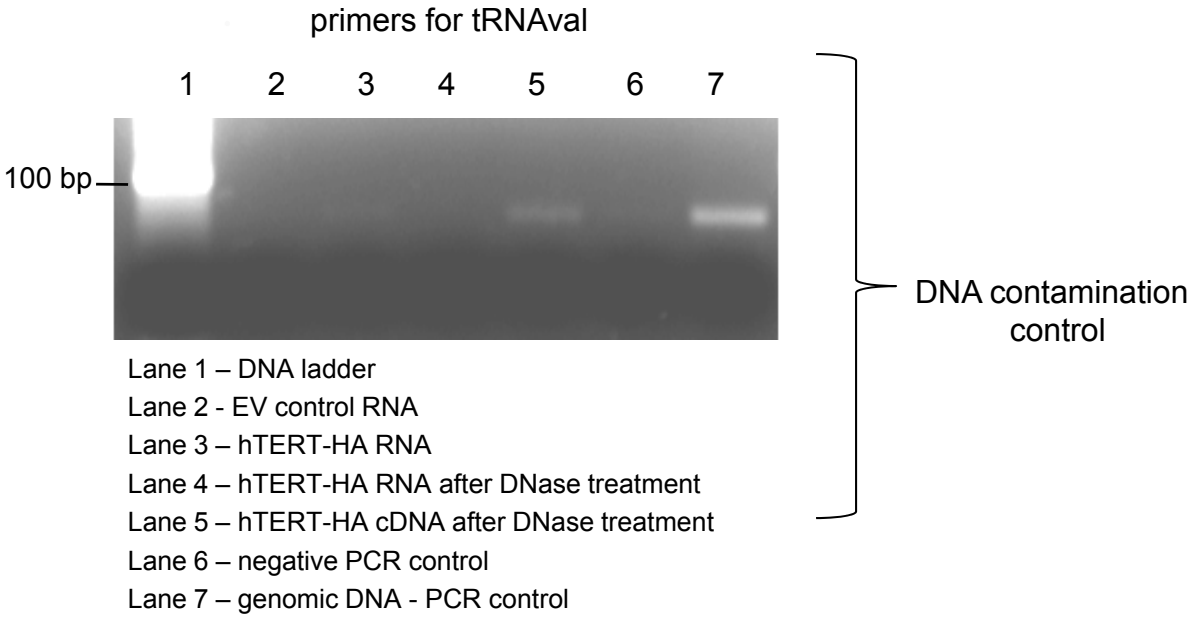

A
Wb with hTERT antibody
Wb with HA antibody
IP with hTERT antibody
1 2 3
1 2 3
hTERT
160 kDa
120 kDa
B
hTERT-HA
LNCaP
- + - +
shRNA hTERT
hTERT
tubulin
hTERT-HA
MEF WT
MEF KO
mTERT
actin
C
WCE
nucleus
cytosol
mitoplast
mitochondria + proteinase K
80 kDa
HSP70
60 kDa
α tubulin
nuclear
mito
Ku80
SQ20B
80 kDa
HSP70
60 kDa
α tubulin
nuclear
mito
Ku80
SCC61
D
ACONITASE ACTIVITY
WCE
Mplast
cytosol
M
C

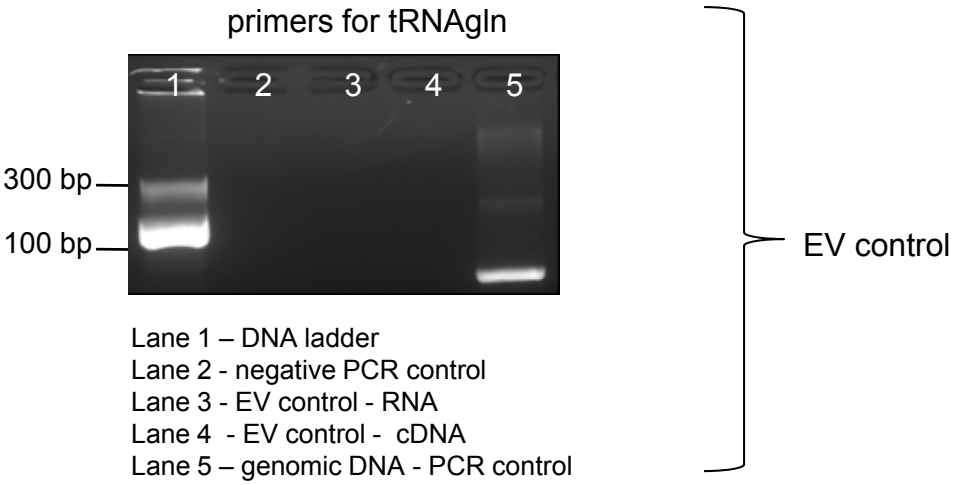
Figure S2

A



Lane 1 – DNA ladder
Lane 2 - EV control RNA
Lane 3 – hTERT-HA RNA
Lane 4 – hTERT-HA RNA after DNase treatment
Lane 5 – hTERT-HA cDNA after DNase treatment
Lane 6 – negative PCR control
Lane 7 – genomic DNA - PCR control

B



Lane 1 – DNA ladder
Lane 2 - negative PCR control
Lane 3 - EV control - RNA
Lane 4 - EV control - cDNA
Lane 5 – genomic DNA - PCR control

C

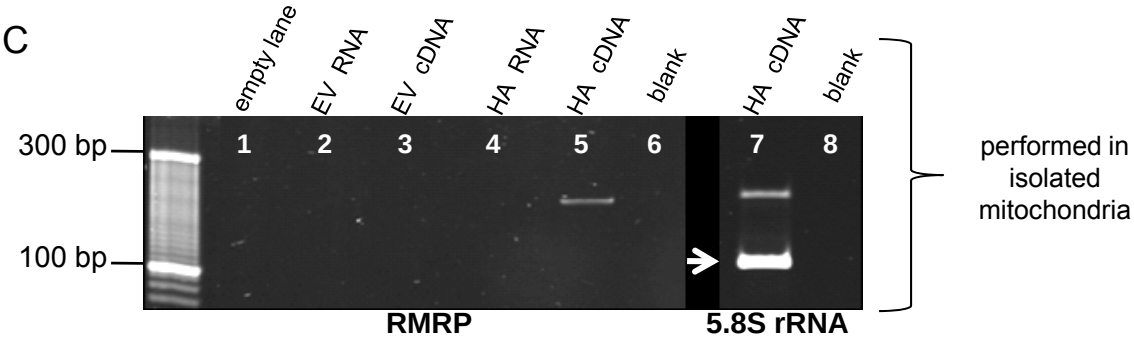
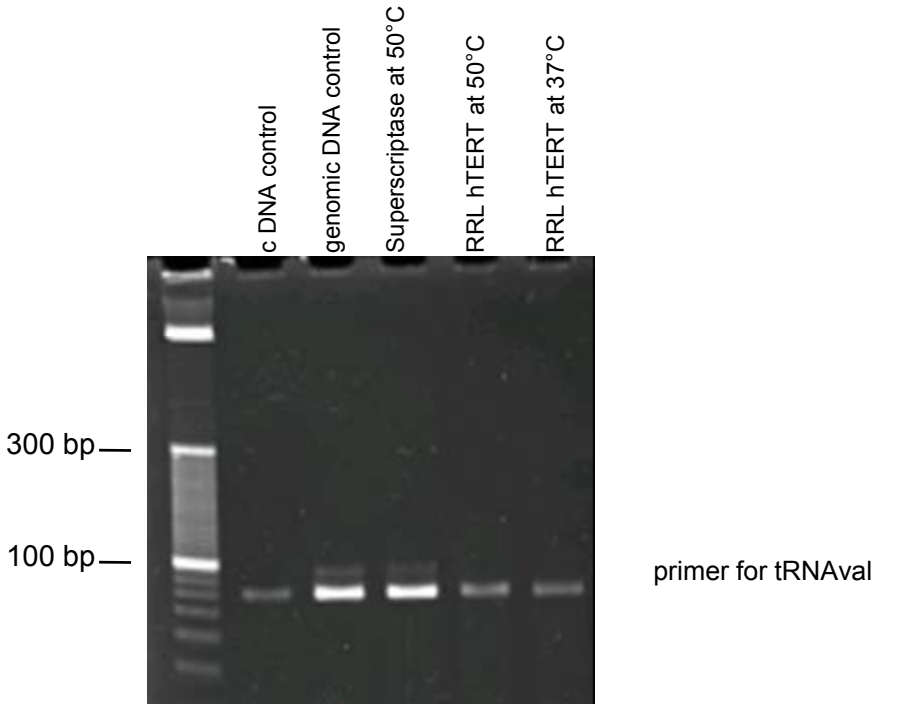
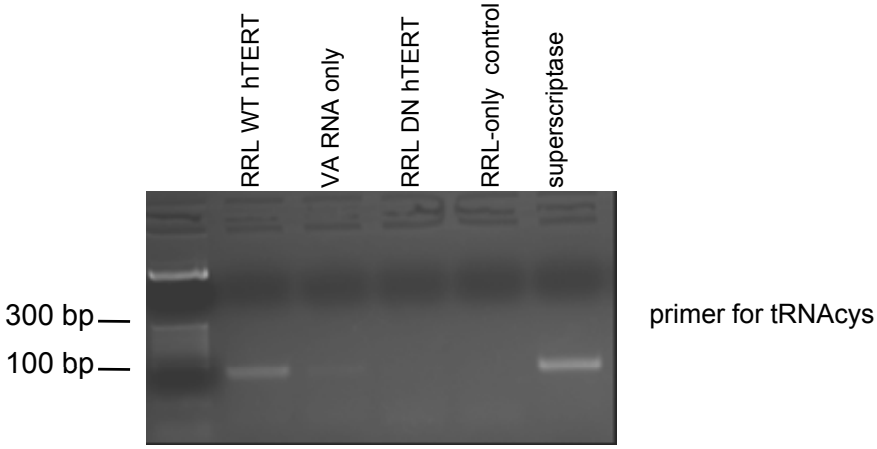


Figure S3


c DNA control
genomic DNA control
Superscriptase at 50°C
RRL hTERT at 50°C
RRL hTERT at 37°C
300 bp
100 bp
primer for tRNAval


RRL WT hTERT
VA RNA only
RRL DN hTERT
RRL-only control
superscriptase
300 bp
100 bp
primer for tRNAcys