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Figure S1. (A) E2F1 transcript levels determined by q-PCR to confirm expression microarray results, using GAPDH as control. The values plotted represent mean ± standard deviation (*n*=3). T-test p-values are indicated. (B) Quantification of protein levels in the western presented in Figure 2A. The values plotted represent mean ± standard deviation. T-test p-values are indicated. (C) Quantification of protein levels in the western presented in Figure 2B. The values plotted represent mean ± standard deviation. T-test p-values are indicated. (D) Quantification of protein levels in the western presented in Figure 2E. The values plotted represent mean ± standard deviation of the ratio E2F1/tubulin in the cell lines prior to apoptosis induction (white bars) and the ratio cleaved PARP/full-length PARP upon apoptosis induction (black bars).

Figure S2 – Tissue-specificity of AMPK-E2F1 mitochondrial stress pathway. (A) Western blot of phospho-ACC, phospho-AMPKα and E2F1 in whole tissue homogenates of three different tissues of 1-year old female wild-type and Tg-mtTFB1 mice. (B) Quantification of E2F1 mRNA in the organ of Corti, stria vascularis and brain of wild-type and Tg-mtTFB1 mice by q-PCR, using GAPDH RNA as control. The values plotted represent mean ± standard deviation (*n*=6), with t-test p-values indicated. (C) Immunohistochemistry for phospho-AMPKα in representative sections of cochlea of 1-year old wild-type and Tg-mtTFB1 mice that exhibit significant hearing loss. Arrows show regions in the stria vascularis where significant changes in the brown AMPK

signal are observed. (D) E2F1 transcript levels in the cochlea and in skeletal muscle of the mtDNA-mutator mouse. Publicly-available microarray data from cochlea (GSE4866) and from skeletal muscle (GSE21189) were used to determine if E2F1 transcriptional levels are affected in mtDNA-mutator mouse tissues, compared to wt mice. The values represented correspond to the RMA-normalized transcript levels of E2F1 (see *Methods*). A significant increase of E2F1 is observed in the mtDNA-mutator cochlea (black) compared to the wt cochlea (white), while no change is observed in the skeletal muscle. T-test p-values indicated.

Supplementary Table 2 – Correlation between 12S hypermethylation transcriptome and other microarray datasets and TF targets

Manipulation	**Dataset (GEO database)**	**p-value (Fisher's exact test)**
E2F1 overexpression	GSE498	0.0166
Rb KO	GSE9562	0.3845
TF targets	**Reference**	**p-value (Fisher's exact test)**
E2F1	Genomatix Model Inspector	0.0242

Supplementary Table 3 – Overlap between E2F1 targets induced in 12S hypermethylation and mutator tissues

	p-value
mutator cochlea	0.0015
mutator skeletal muscle	0.7600

Experimental Procedures

Auditory Brainstem Response Protocol Details

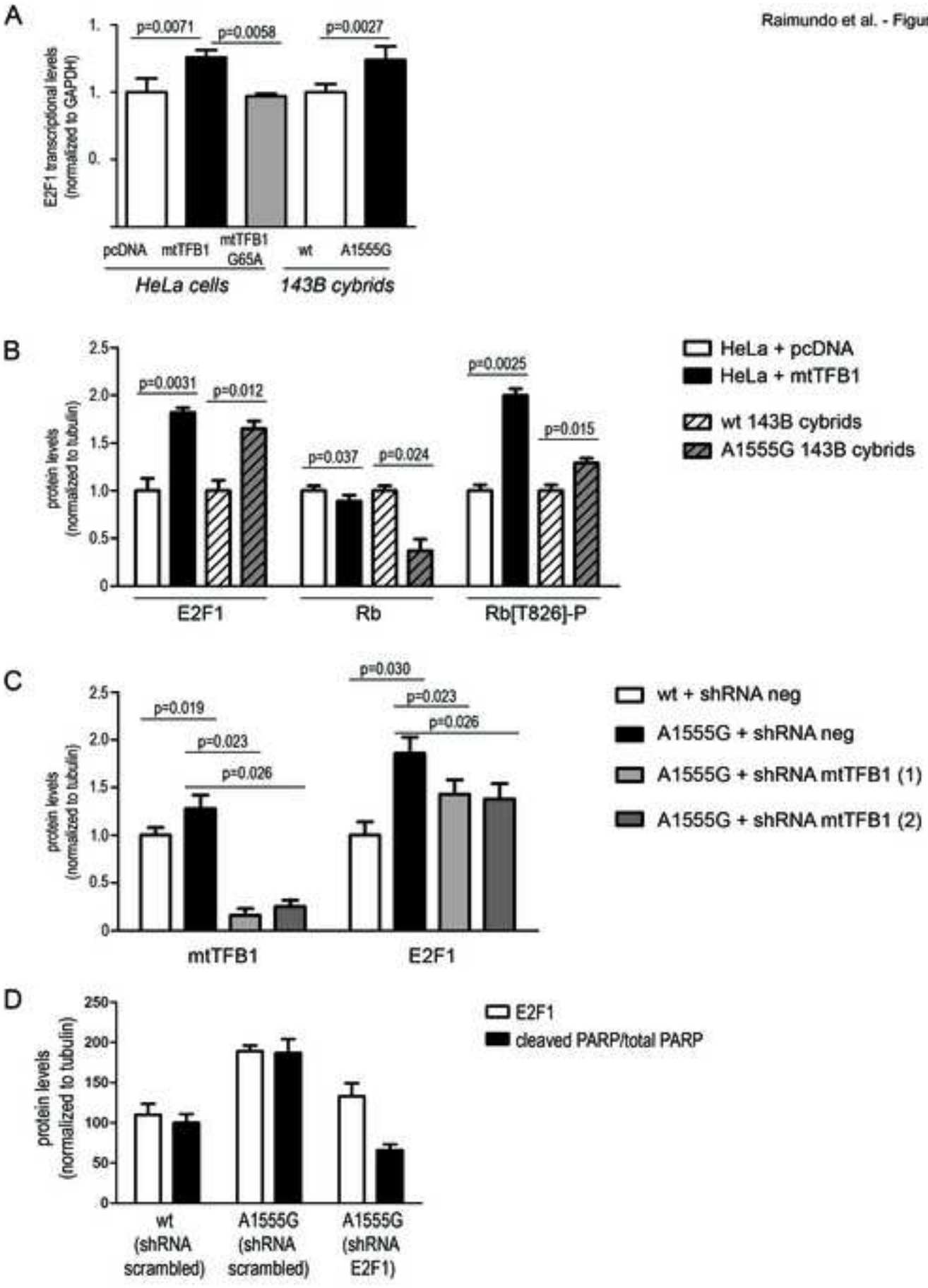
Animals were anesthetized with chloral hydrate (480 mg/kg IP) and all recordings were conducted in a sound-attenuating chamber (Industrial Acoustics Corp). A customized TDT3 system (Tucker-Davis Technologies, Inc.) was used for ABR recordings. Subdermal needle electrodes (Rochester Electro-Medical, Inc.) were positioned at the vertex (active, non-inverting), the infra-auricular mastoid region (reference, inverting), and in the neck region (ground). Differentially recorded scalp potentials were amplified 50,000 times, and bandpass filtered between 0.05 and 3 kHz over a 15-ms epoch. A total of 400 trials were averaged for each waveform for each stimulus condition.

Symmetrically shaped tone bursts were 3 ms long (1-ms raised cosine on/off ramps and 1-ms plateau). All acoustic stimuli were delivered free field via a speaker (TDT Part FF1 2021) positioned 10 cm from the vertex. Stimulus levels were calibrated using a 0.5-in condenser microphone (Model 4016, ACO Pacific) positioned at the approximate location of the animal's head during recording sessions and are reported in decibels sound pressure level (dB SPL: referenced to 20 µPa). Stimuli of alternating polarity were delivered at a rate of approximately 20/s.

Tone burst responses were collected in half octave steps ranging from 32 to 2.0 kHz. The effects of level were determined by decreasing stimulus intensity in 10-dB steps. Near threshold, levels were adjusted in 5-dB steps. A maximum stimulus level of 90 dB SPL was used. ABR thresholds were determined visually by noting the

disappearance of response waveforms into the noise floor. A T-test was used to determine the statistical significance of the thresholds at each frequency between wt and Tg-mtTFB1 mice.

Supplemental Figure 1



Supplemental Figure 2