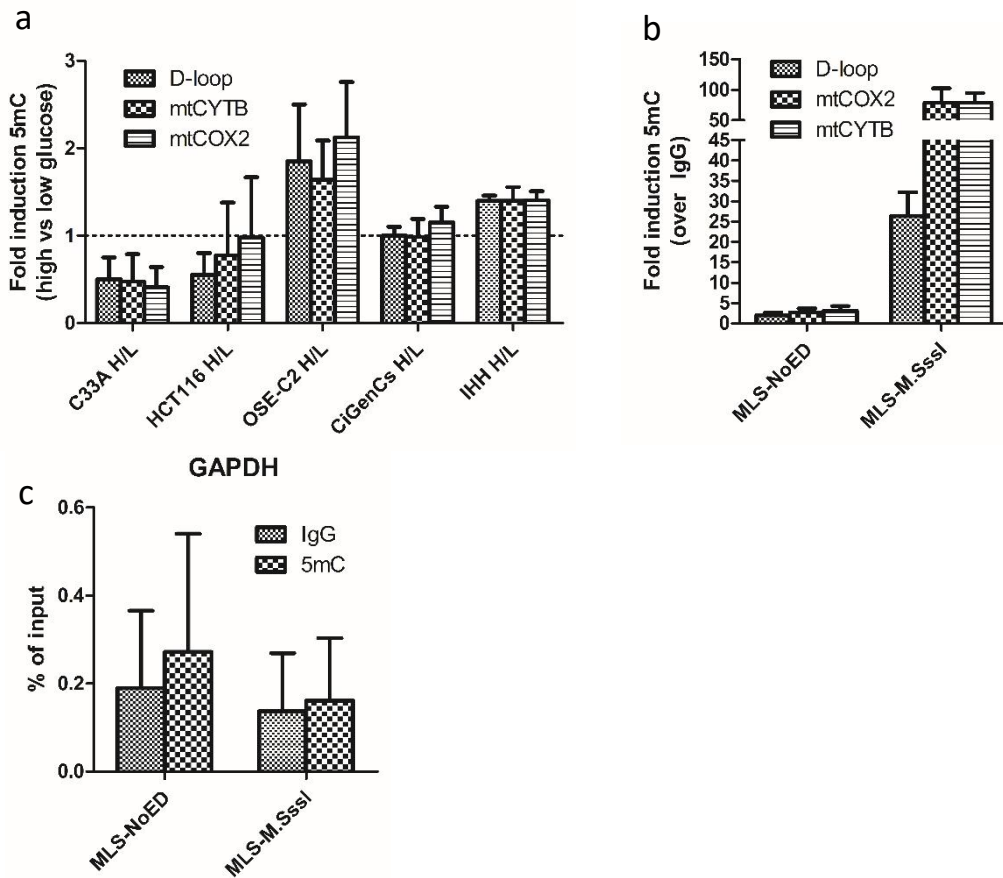


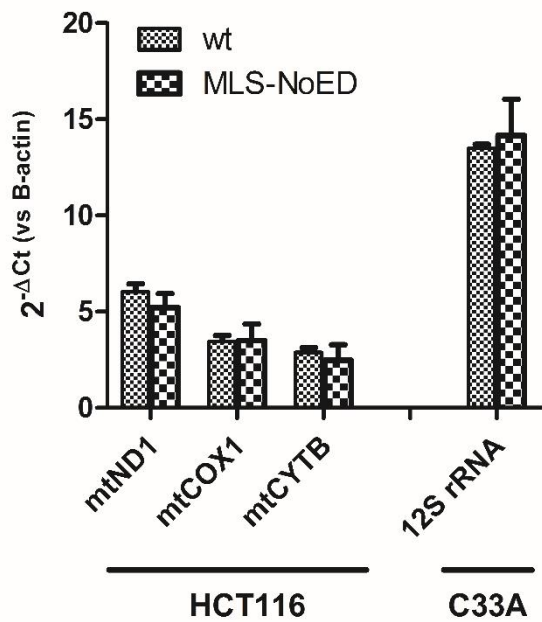
## **Supplementary information**

**Experimental mitochondria-targeted DNA methylation identifies GpC methylation, not CpG methylation, as potential regulator of mitochondrial gene expression.**

Monique G.P. van der Wijst, Amanda Y. van Tilburg, Marcel H.J. Ruiters, Marianne G. Rots



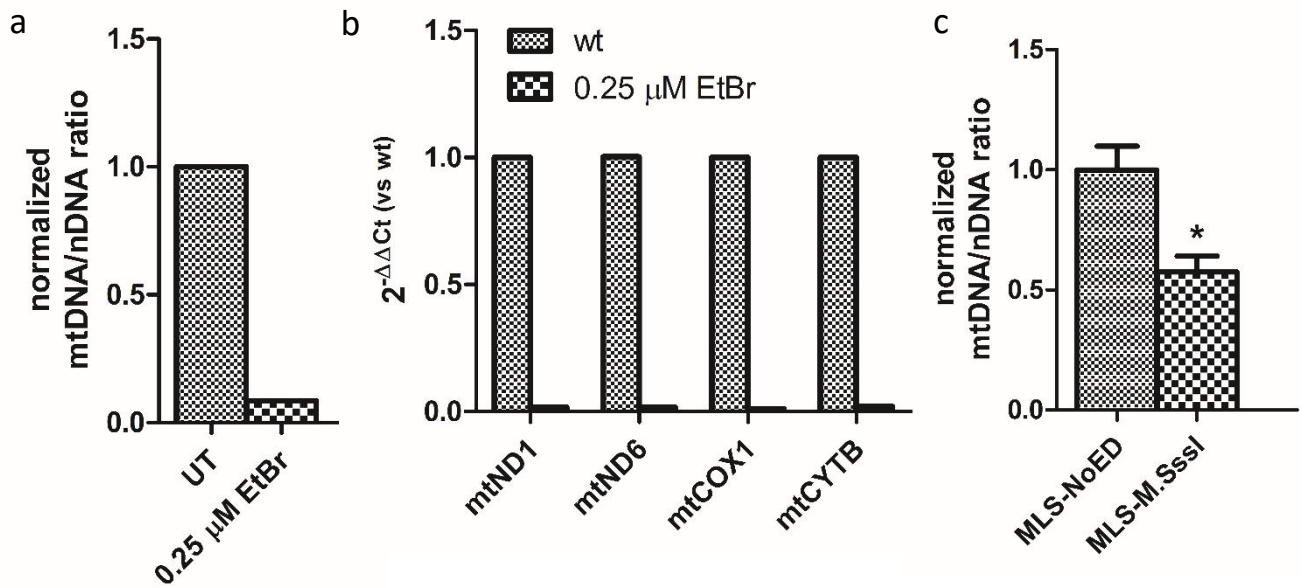
**Supplementary Figure 1.** Methylated DNA immunoprecipitation (MeDIP) of three mtDNA regions. **(a)** Cancerous (C33A, HCT116) and normal (OSE-C2, CiGenCs, immortalized human hepatocytes (IHH)) cell lines were exposed to high (H, 25 mM) versus low (L, 5 mM) glucose for 4 days. Thereafter, their mtDNA methylation level was determined in three mtDNA regions (D-loop, mtCYTB, mtCOX2). **(b)** The mtDNA methylation level of HCT116 cells stably expressing mitochondria-targeted M.SssI (MLS-M.SssI) or empty vector (MLS-NoED) was determined in three mtDNA regions (D-loop, mtCYTB, mtCOX2) and a **(c)** hypomethylated nuclear DNA region (GAPDH, N=2) using a MeDIP approach.



**Supplementary Figure 2.** Gene expression relative to  $\beta$ -actin. The effect of our mitochondria-targeting construct (MLS-NoED) on mitochondrial gene expression was determined compared to wild-type (wt) HCT116 and C33A cells. Note that the MLS-NoED expressing cells were processed in a different set of experiments (M.Sssl experiments) compared to the wt cells (M.CviPI experiments). Only those genes showing an effect on mitochondrial gene expression in Figure 5 and those taken along in both M.Sssl and M.CviPI experiments, were compared in our analysis.

### Supplementary Figure 3

van der Wijst et al



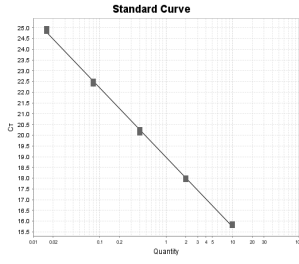
**Supplementary Figure 3.** Mitochondrial DNA copy number and gene expression. (a, b) As a positive control for mtDNA depletion, HeLa cells were treated for 2 days with 0.25  $\mu$ M EtBr. The effect on mtDNA copy number (a) and mtDNA gene expression of four mitochondrial genes (*mtND1*, *mtND6*, *mtCOX1* and *mtCYTB*) (b) was determined (N=1). (c) Validation of decrease in mtDNA copy number in HCT116 cells expressing a mitochondria-targeted M.SssI (MLS-M.SssI) using an independent primer pair (mtCOX1 region).

# Supplementary Figure 4

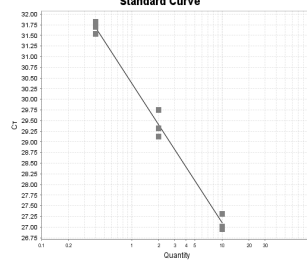
van der Wijst et al

## qPCR DNA

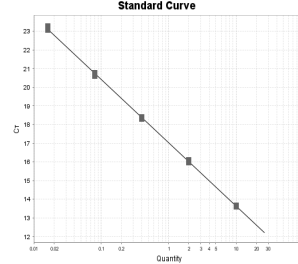
### mtDNA ratio D-loop



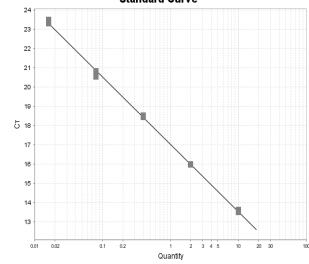
### nDNA ratio $\beta$ -actin



### 7S DNA A+B1

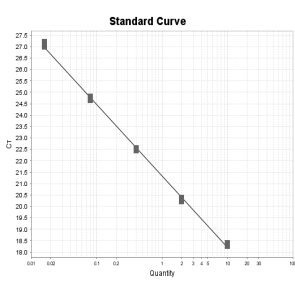


### 7S DNA A+B2

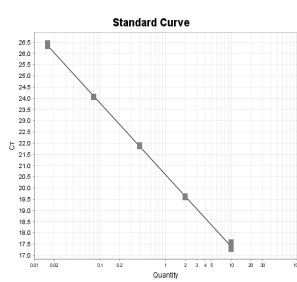


## qRT-PCR mitochondrial-encoded genes

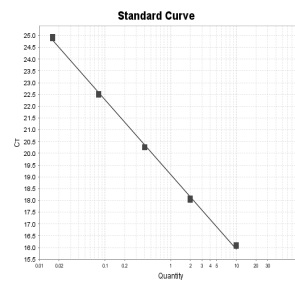
### mtND1



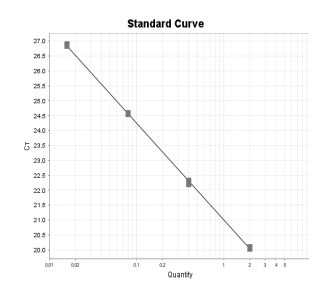
### mtND6



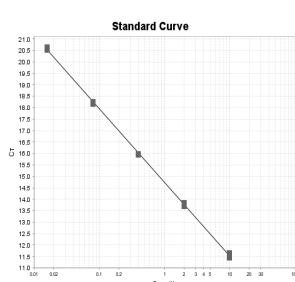
### mtCOX1



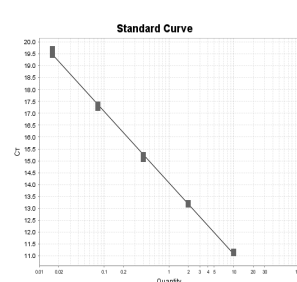
### mtCYTB



### 12S rRNA

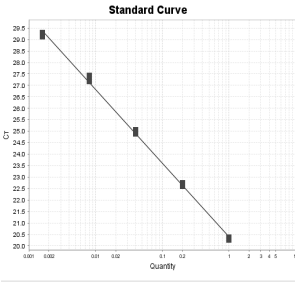


### 16S rRNA

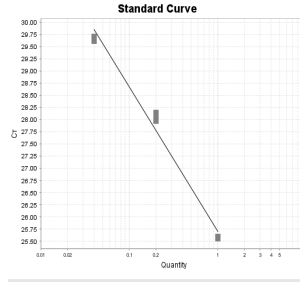


## qRT-PCR nuclear-encoded genes

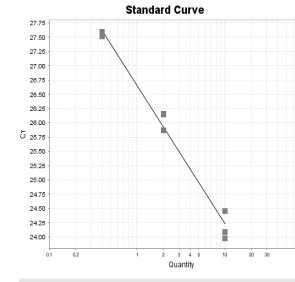
### TFAM



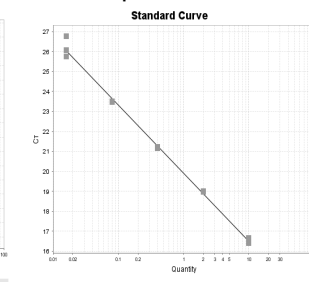
### NRF1



### PGC1 $\alpha$

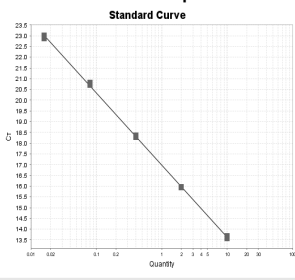


### $\beta$ -actin

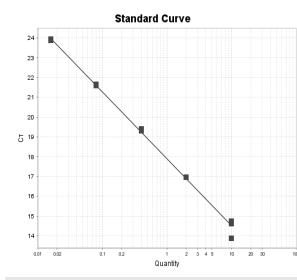


## qPCR MeDIP

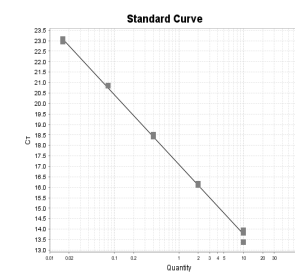
### D-loop



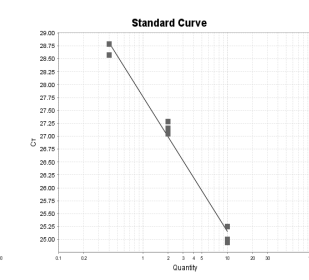
### mtCOX2



### mtCYTB



### GAPDH



Supplementary Figure 4. Standard curves for all primers used in q(RT)-PCR.

## Supplementary Table 1

van der Wijst et al

Locus (position in mtDNA)	# of Cs	Cell line	Unconverted Cs at CpG sites	Unconverted Cs at non-CpG sites	
<b>D-loop</b> H: 357–621 bp  L: 357–608 bp	H: 99 (95x non-CpG, 4x CpG)	C33A	H: 6/109 (5.5%) H: 0/108 (0%) H: 6/86 (8.8%) L: 0/76 (0%)	H: 8/1615 (0.5%) H: 0/2565 (0%)  L: 6/1598 (0.4%)	
		HCT116	H: 1/40 (2.5%)	H: 1/950 (0.1%)	
		HeLa	H: 0/28 (0%) L: 0/24 (0%)	H: 3/376 (0.8%) L: 2/564 (0.4%)	
		SKOV3	H: 1/36 (2.8%)	H: 1/846 (0.1%)	
	<b>mtCOX2</b> L: 7909–8165 bp	L: 81 (64x non-CpG, 17x CpG)	C33A	1/204 (0.5%)	1/768 (0.1%)
			HCT116	0/119 (0%) 0/323 (0%)	2/448 (0.4%) 6/704 (0.9%)
HeLa			1/85 (1.2%)	2/256 (0.8%)	

**Supplementary Table 1.** Overview of CpG and non-CpG methylation in the mtDNA in several cancer cell lines (analyzed by bisulfite sequencing). Each row represents an independent bisulfite sequencing batch. H, heavy strand; L, light strand.

## Supplementary Table 2

van der Wijst et al

Category	Gene/Transcript	C33A ( $2^{-\Delta Ct}$ )	HCT116 ( $2^{-\Delta Ct}$ )	Difference (HCT116/C33A)
mtDNA	mtND1 (H)	4.889	6.028	1.2x
OXPHOS genes	mtND6 (L)	2.511	4.260	1.7x
	mtCOX2 (H)	3.162	3.434	1.1x
	mtCYTB (H)	2.144	2.878	1.3x
mtDNA rRNA genes	12S rRNA	13.486	47.229	3.5x
	16S rRNA	25.865	40.303	1.6x
Nuclear-encoded mtDNA biogenesis genes	PGC1 $\alpha$	0.0011	0.0034	3.1x
	NRF1	0.012	0.021	1.8x
	TFAM	0.040	0.064	1.6x
	TFAM/mtDNA	0.0000232	0.0000347	1.5x
DNA replication	7S DNA primer	0.83	0.70	0.8x
	mtDNA copy number	1722	1840	1.1x

**Supplementary Table 2.** Overview of the differences between C33A and HCT116 cells regarding mtDNA transcription and replication.  $2^{-\Delta Ct}$ , relative expression to  $\beta$ -actin (genes), to mtDNA (7S DNA), or to nDNA (mtDNA copy number).