Supplementary Information

Case Histories of mosaic depletion patients

Patient A

Male patient A has been previously described. He was hypotonic from birth and developed myoclonic epilepsy and respiratory insufficiency after general anaesthetic for a hernia repair. After an initial improvement he deteriorated progressively. CT scan detected substantial loss of grey matter in the cerebral cortex, although earlier scans were normal at admission. CSF lactate was mildly elevated (2.4mmol/l, normal range: 1.2-1.9). The patient died at 14 months of age. Post-mortem histology indicated neurological characteristics of Alper's syndrome, with foci of neuronal loss in hippocampi, thalami, cerebral cortex and cerebellum. Liver histology revealed mild centrilobular steatosis. Muscle histology showed 17% COX negative fibres. MtDNA: nDNA ratios were low in several tissues (7% liver, 23% muscle, 25% kidney, 37% brain, 100% heart). Sequence analysis revealed POLG1 mutations T914P and R1096C.

Patient B

Male patient B developed normally for 6 months and was referred with abnormal movements aged 9 months. He was floppy with poor head control, and seemed lethargic and unresponsive. He died three months later from an encephalopathic episode with seizures. The patient had been oedematous. CSF lactate was raised 5.7 mmol and plasma lactate was 4.2 mmol. Post-mortem biopsy revealed mitochondrial respiratory enzymes activities were normal in muscle, but indicated a large reduction in complex IV activity in liver (0.002 COX: Citrate synthase activity ratio (0.011-0.031). MtDNA: nDNA ratios were 32% in muscle, and <4% in liver. Fibroblast COX activity was normal at 65nmol/mg protein/min (normal range 30-90), but PDH activity was at the lower end of the normal range. Muscle histology showed proliferation of mitochondria on EM. Clinical presentation was considered suggestive of Alper's syndrome.

Patient C

Male patient C presented with liver failure, non-specific encephalopathy and blindness, and was the first-born child of consanguineous parents. Initial development was normal. At five months of age he presented with intermittent vomiting, jaundice without hepatosplenomegaly, and mild motor delay. At thirteen months he developed a severe coagulopathy, and hypoglycaemia, which was difficult to control, liver failure and seizures. An ultrasound revealed normal liver size, shape and echo pattern. Muscle biopsy showed Complex IV deficiency, with normal Complex I and II/III activities. Fibroblast cytochrome oxidase activity was reduced to 17 nmol/mg protein/min (normal range 30-90). EEG and auditory evoked responses were consistent with encephalopathy and visual evoked responses suggested a severe post-retinal dysfunction. Laboratory findings showed hyperbilirubinaemia, slightly raised liver enzymes, anaemia and thrombocytopenia. He died at age 15 months. His MRI was reported as having abnormal signal in the globus pallidus suggesting mitochondrial disease, even though his neurological examination was normal. A younger sibling presented with neonatal jaundice.

Patient D has been presented before [1]

References

[1] Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, et al. Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gammaA. Brain. 2005 Apr;128(Pt 4):723-31.

Figures

Supplimentary Figure 1 PicGreen labelling of early passage (< passage 6) MDS patient fibroblasts and controls

(A) PicoGreen labelling of control cells (B) PicoGreen labeling of patient A cells (C) PicoGreen labelling of patient B cells. (D) PicoGreen labeling of patient C cells. (E) PicoGreen labelling of patient D cells (F) PicoGreen labelling of cells from a patient with

autosomal dominant progressive external ophthalmoplegia (adPEO) due to a POLG mutation. Bar 20μM.

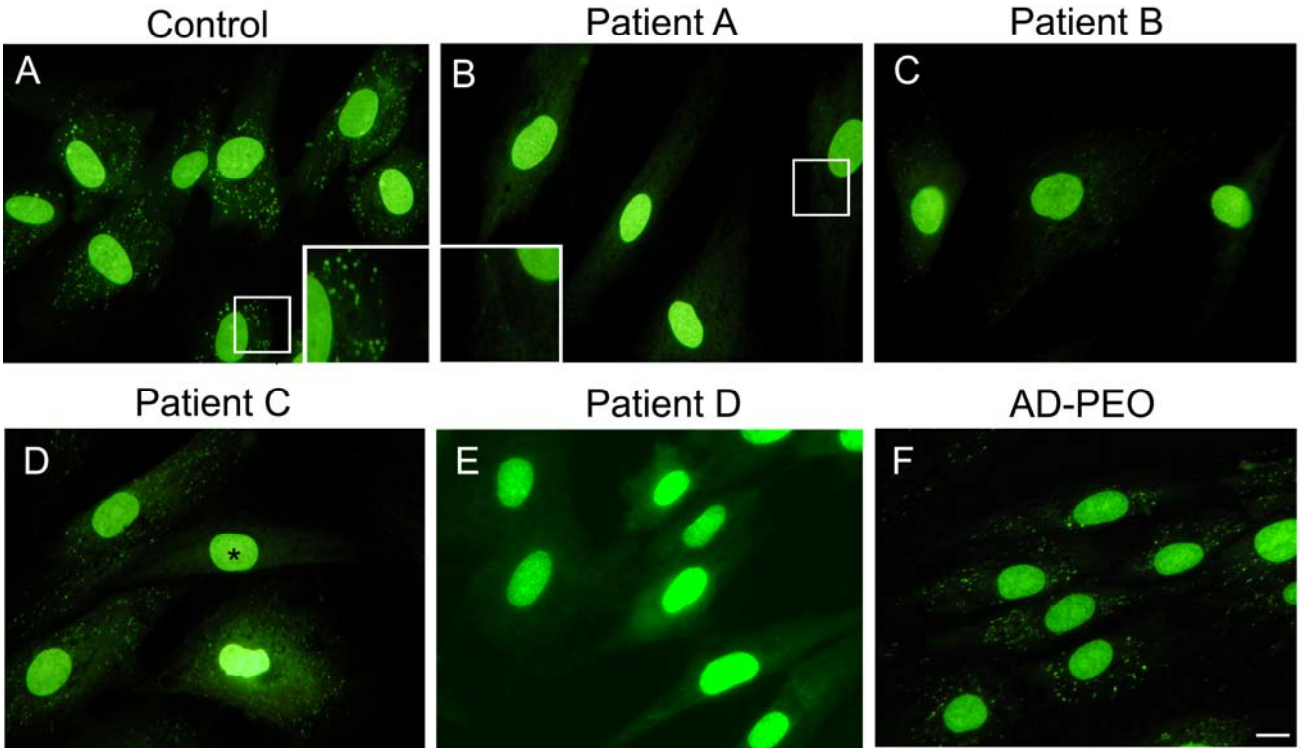
Supplementary figure 2 Quantification of PicoGreen staining of mosaic MDS cell cultures A-C and controls (same experiment as 2c and 2d)
Digital image analysis of PicoGreen fluorescence microscopy shows that mtDNA content of patient fibroblast cultures (top two panels) is lower than controls (bottom panel) at late time points. In patients B and C comparison of baseline (top panel) with late time points shows that mtDNA content progressively declines.

Supplementary figure 3 Residual TFAM and COXI labelling within mtDNA depleted cells

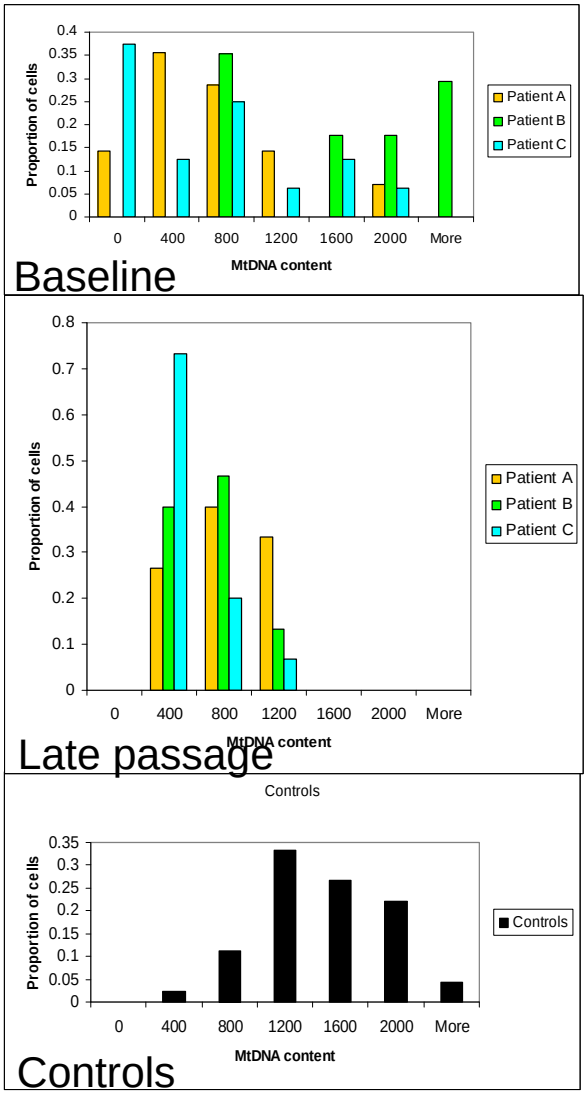
(A) Anti-TFAM/Mitotracker co-labelling of cells from controls and MDS patient A. A TFAM depleted cell with reduced Mitotracker labelling is marked with an asterisk. The arrows highlight a region of residual TFAM expression which co-localizes with residual Mitotracker labelling. (B) Anti-COXI/Mitotracker co-labelling of cells from controls and MDS patient A. A COXI depleted cell with reduced Mitotracker labelling is marked with an asterisk. The arrows highlight a region of residual COXI expression which co-localizes with residual Mitotracker labelling. Bars 20μM

Supplementary figure 4 A cellular model of mtDNA depletion
Likely chain of events underlying the cellular changes observed in fibroblast cultures from patients A-D.

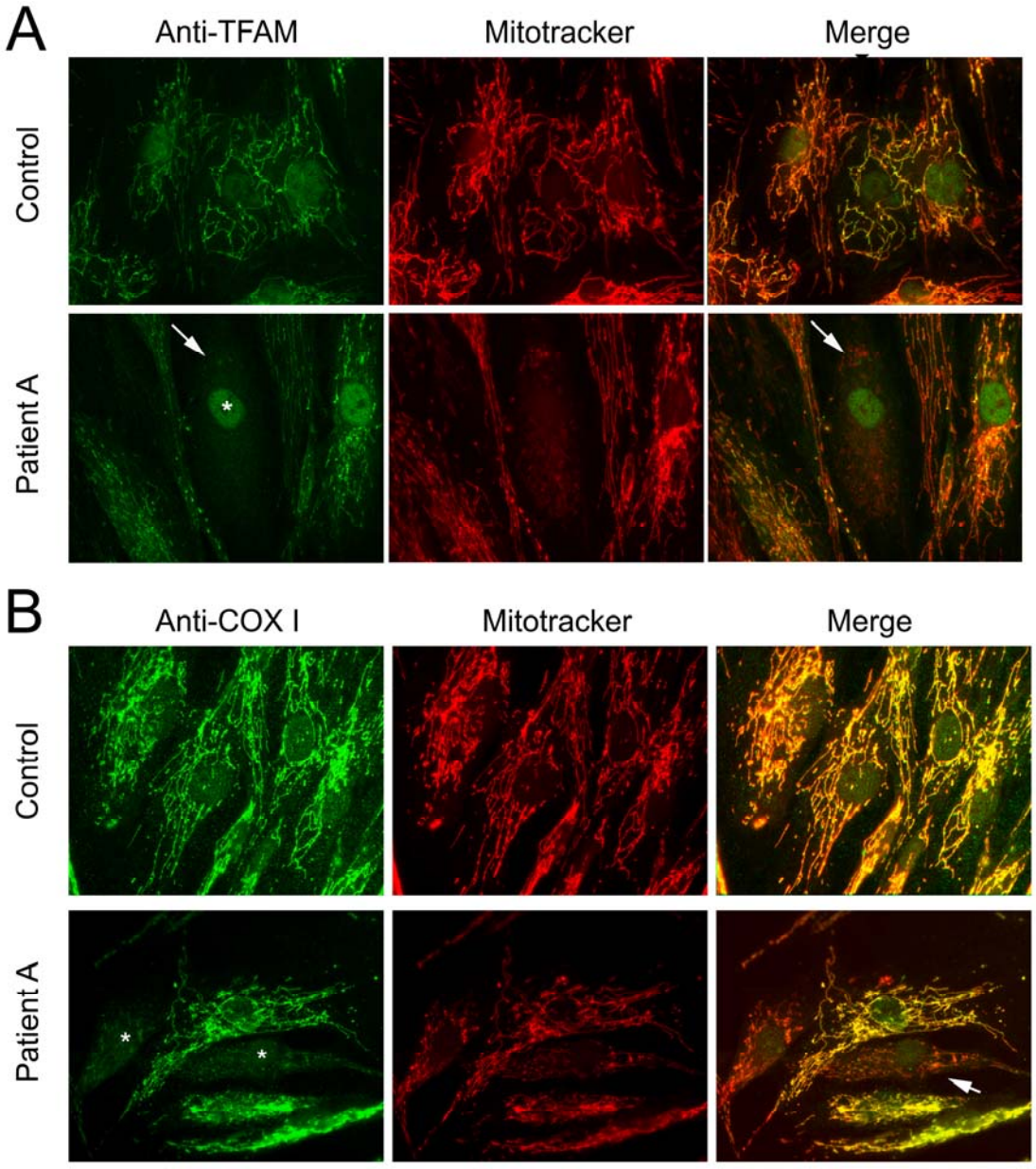
Supplementary Figure 1



Supplememtory figure 2 Digital image analysis of PicoGreen fluorescence microscopy shows that mtDNA content of patient fibroblast cultures (top two panels) is lower than controls (bottom panel) at late time points.
In patients B and C comparison of baseline (top panel) with late time points shows that mtDNA content progressively declines.



Supplementary Figure 3



Supplementary Figure 4