Expanded View Figures

Figure EV1. Pedigrees, mtDNA haplotypes and variant counts in the 19 RIRCD families with homoplasmic m.14674T>C.

A Pedigrees and genetic variants. The * symbol indicates the individuals where DNA analysis was performed while circles represent female, square represents male and the filled-in square or circle represent individuals expressing mutations while deceased individuals were marked with a diagonal line.

B Exome mtDNA read depth and mtDNA haplogroups in the families homoplasmic for m.14674T>C.

C Variant Scatter/boxplot shows the significant difference between mean per group damaging allele counts (RIRCD affected vs unaffected vs 1,044 × 1000 Genomes control exomes) in the genes *EARS2*, *TRMU*, *QRSL1*, *GOT2*, *GLS*, *MSS51* and m.14674T>C. The counts are from individual patients and controls thus one point on the boxplot represents damaging allele counts for 1 person. The central band represents the median, the lower and upper hinges correspond to the first and third quartiles (25 and 75%) while the whiskers extend to the highest and lowest points within the data (1.5× the inter-quartile range).

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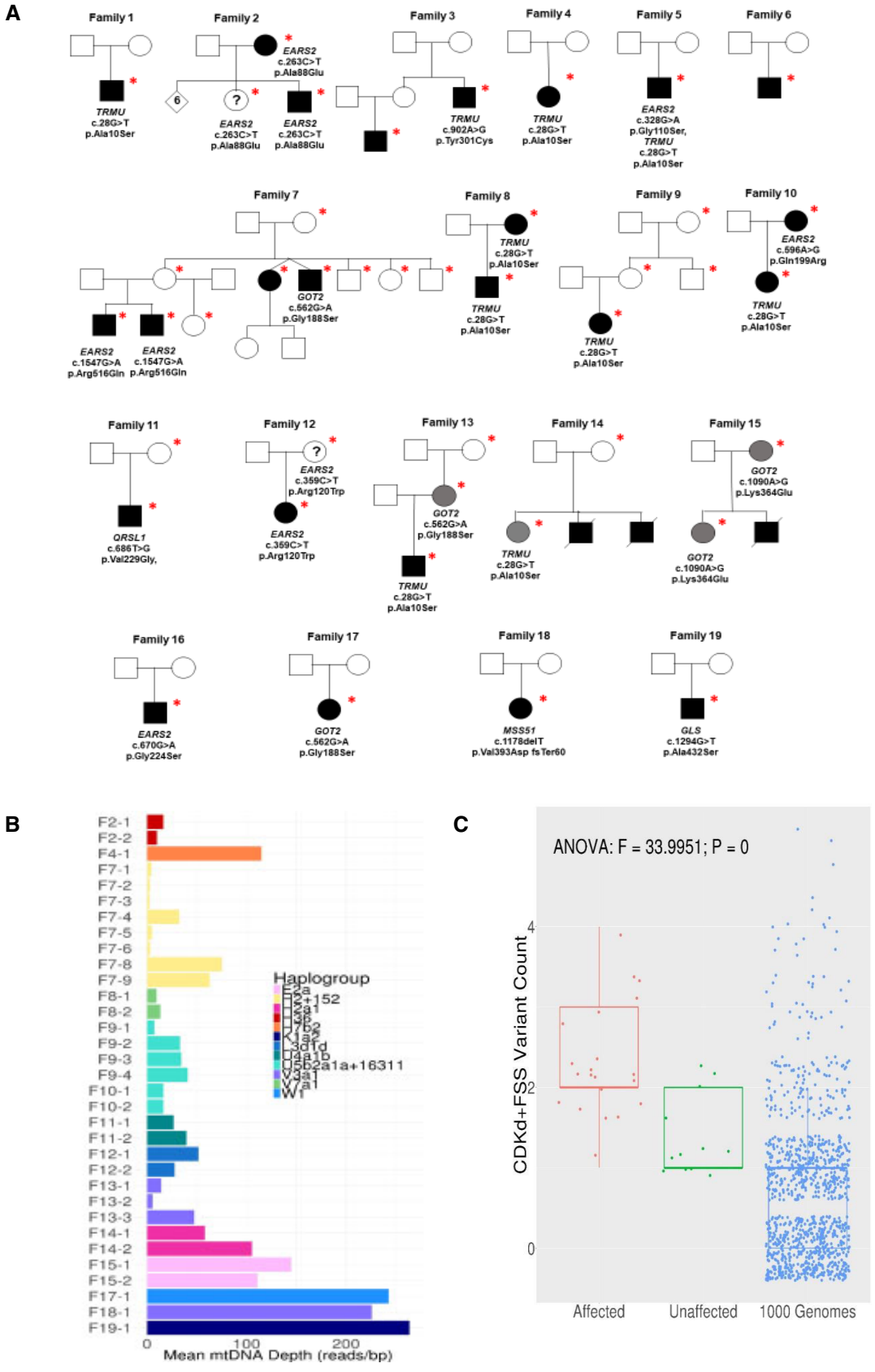
A
Family 1
TRMU c.28G>T p.Ala10Ser
Family 2
EARS2 c.263C>T p.Ala88Glu
6
?
EARS2 c.263C>T p.Ala88Glu
EARS2 c.263C>T p.Ala88Glu
Family 3
TRMU c.902A>G p.Tyr301Cys
Family 4
TRMU c.28G>T p.Ala10Ser
Family 5
EARS2 c.328G>A p.Gly110Ser, TRMU c.28G>T p.Ala10Ser
Family 6
Family 7
EARS2 c.1547G>A p.Arg516Gln
EARS2 c.1547G>A p.Arg516Gln
GOT2 c.562G>A p.Gly188Ser
Family 8
TRMU c.28G>T p.Ala10Ser
TRMU c.28G>T p.Ala10Ser
Family 9
TRMU c.28G>T p.Ala10Ser
Family 10
EARS2 c.596A>G p.Gln199Arg
TRMU c.28G>T p.Ala10Ser
Family 11
QRSL1 c.686T>G p.Val229Gly,
Family 12
?
EARS2 c.359C>T p.Arg120Trp
EARS2 c.359C>T p.Arg120Trp
Family 13
GOT2 c.562G>A p.Gly188Ser
TRMU c.28G>T p.Ala10Ser
Family 14
TRMU c.28G>T p.Ala10Ser
Family 15
GOT2 c.1090A>G p.Lys364Glu
GOT2 c.1090A>G p.Lys364Glu
Family 16
EARS2 c.670G>A p.Gly224Ser
Family 17
GOT2 c.562G>A p.Gly188Ser
Family 18
MSS51 c.1178delT p.Val393Asp fsTer60
Family 19
GLS c.1294G>T p.Ala432Ser
B
F2-1
F2-2
F4-1
F7-1
F7-2
F7-3
F7-4
F7-5
F7-6
F7-8
F7-9
F8-1
F8-2
F9-1
F9-2
F9-3
F9-4
F10-1
F10-2
F11-1
F11-2
F12-1
F12-2
F13-1
F13-2
F13-3
F14-1
F14-2
F15-1
F15-2
F17-1
F18-1
F19-1
Haplogroup
L3d1d
U4a1b
U5b2a1a+16311
0
100
200
Mean mtDNA Depth (reads/bp)
C
ANOVA: F = 33.9951; P = 0
CDKd+FSS Variant Count
4
2
0
Affected
Unaffected
1000 Genomes

Figure EV1.

Figure EV2. Summary of variants in our cohort.

A Mitochondrial haplogroups and digenic nuclear variants in affected RIRCD family members.
B Number of exome variants at each stage of filtering in total and for affected and unaffected individuals in RIRCD families with homoplasmic m.14674T>C.

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A

Family	Haplogroup	Relationship	Nuclear Risk Variants		
			Gene	Protein	Genotype
F1	n. d.	Proband	*TRMU*	p.Ala10Ser	0/1
F2	H36	Proband	*EARS2*	p.A88Glu	0/1
		Mother of F2/1	*EARS2*	p.Ala88Glu	0/1
F3	n. d.	Uncle of F3/2	*TRMU*	p.Tyr301Cy	0/1
F4	H7b2	Proband	*TRMU*	p.Ala10Ser	0/1
F5	V7a1	Proband	*EARS2*	p.Gly110Ser*	0/1
			TRMU	p.Ala10Ser	0/1
F7	H+152	Sibling of F7/2	*EARS2*	p.Arg516Gln*	0/1
		Sibling of F7/1	*EARS2*	p.Arg516Gln*	0/1
		Uncle of F7/1-2	*GOT2*	p.Gly188Ser	0/1
F8	U5b2a1a+16311	Proband	*TRMU*	p.Ala10Ser	0/1
F9	U5b2a1a+16311	Proband	*TRMU*	p.Ala10Ser	0/1
F10	U5b2a1a+16311	Proband	*TRMU*	p.Ala10Ser	0/1
		Mother of F8/1	*EARS2*	p.Gln199Arg	0/1
F11	U4a1b	Proband	*QRSL1*	p.Val229Gly	0/1
F12	L3d1d	Proband	*EARS2*	p.Arg120Trp	0/1
F13	V3a1	Proband	*TRMU*	p.Ala10Ser	0/1
		Mother of F13/1	*GOT2*	p.Gly188Ser	0/1
F14	H2a1	Sister of F14/1	*TRMU*	p.Ala10Ser	0/1
F15	E2a	Sister of F15/1	*GOT2*	p.Lys364Glu	0/1
		Mother of F15/1	*GOT2*	p.Lys364Glu	0/1
F17	W1	Proband	*GOT2*	p.Gly188Ser	0/1
F18	V3a1	Proband	*MSS51*	p.Val393AspfsTer60	0/1
F19	K1a2	Proband	*GLS*	p.Ala432Ser	0/1

B

Type of Variant	Total Number of Variants	Affected Only* (n=18)	Unaffected Only* (n=12)
Total Variants	4,907,159	1,847,621	1,106,024
Total Protein Altering	42,473	14,885	5,725
Conserved (2+)	15,775	6,051	2,344
Predicted Damaging (5+)	13,816	5,458	2,163
Conserved (2+), Damaging (5+)	10,991	4,340	1,705
Conserved (2+), Damaging (5+), Known Domain	5,013	2,022	779
Conserved, Damaging, Known Domain, Splicing, Stop-Loss/Gain, Frameshift	**6,998**	**2,698**	**1,067**

Figure EV2.

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Figure EV3. Functional characterisation of the p.Arg23Trp variant in *PDE12*.

A Western blot of total cell lysate of *PDE12* knockout cells expressing WT *PDE12*, p.Glu351Ala (catalytic mutant), Δ16 (coding for *PDE12* lacking 16 first aa), Δ23 mutants (coding for *PDE12* lacking 23 first aa) and p.Arg23Trp *PDE12* cDNA. B-actin was used as loading control. Our previous study (Pearce *et al*, 2017) showed that the steady-state levels of OXPHOS complexes are affected in the PDE12 KO as compared to the WT control cells.

B Radioactive MPAT assay for 16S mt-rRNA extracted from HEK293T (WT), *PDE12* knockout cells (KO) and for *PDE12* KO cells expressing WT *PDE12*, p.Glu351Ala, Δ16, Δ23 mutants and p.Arg23Trp *PDE12* cDNA for 24 hr. Experiments and cell lines as described in (Pearce *et al*, 2017).

C Immunofluorescence to confirm mitochondrial localisation of the p.Arg23Trp *PDE12* variant in human cells. TOM20, translocator of the outer mitochondrial membrane 20. Scale bar = 20 μm.

Source data are available online for this figure.

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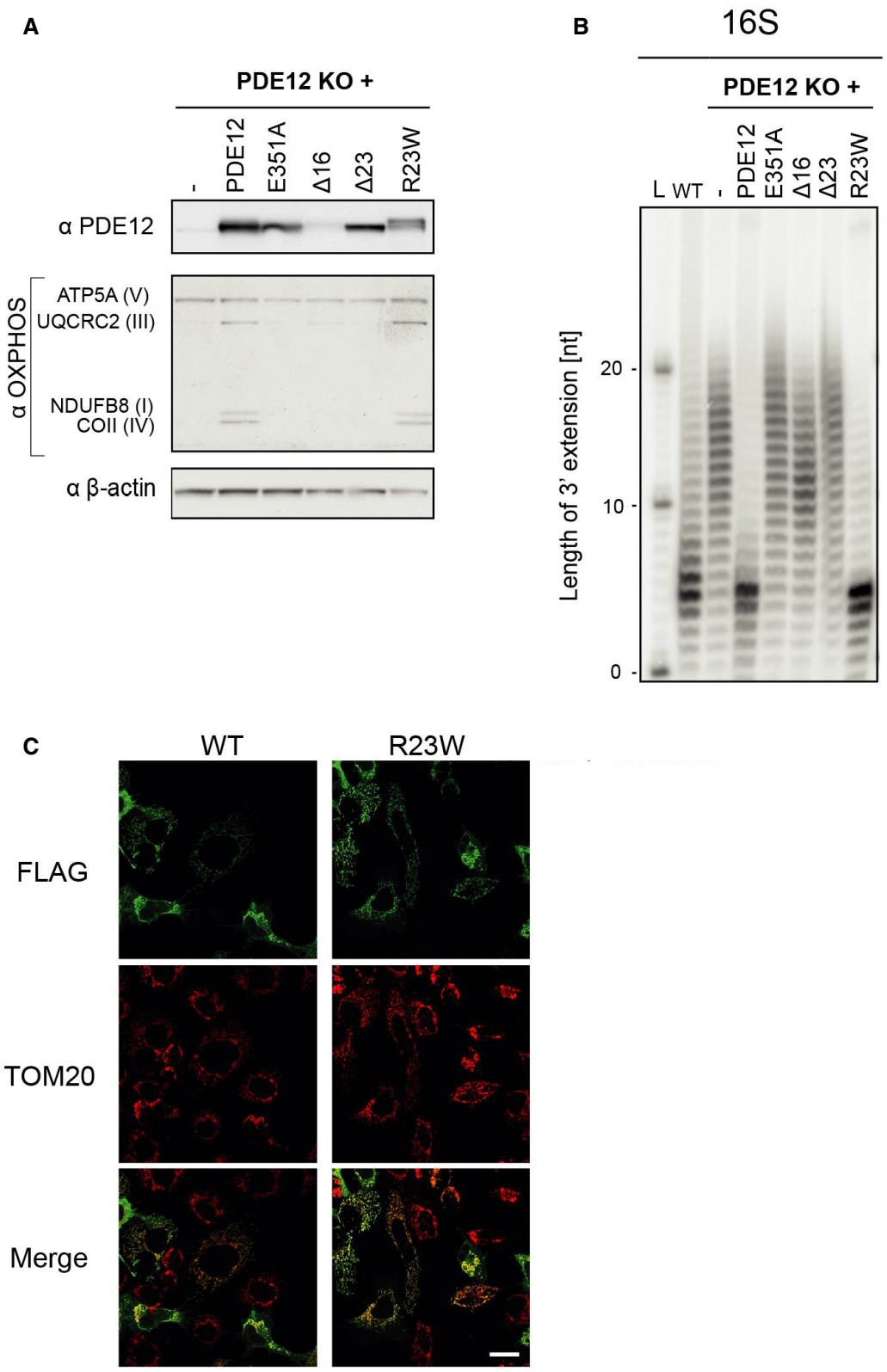
A
PDE12 KO +
-
PDE12
E351A
Δ16
Δ23
R23W
α PDE12
α OXPHOS
ATP5A (V)
UQCRC2 (III)
NDUFB8 (I)
COII (IV)
α β-actin
B
16S
PDE12 KO +
L WT
-
PDE12
E351A
Δ16
Δ23
R23W
Length of 3' extension [nt]
20
10
0
C
WT
R23W
FLAG
TOM20
Merge

Figure EV3.

Figure EV4. Heat map of the top 100 transcripts altered in RNA-seq data resulted from muscle biopsies of RIRCD compared to control muscle resulted from DESeq2 analysis.
Gene expression is showed in normalized $\log_2$ counts. Differentially expressed genes were selected based on the $\log_2$ counts and *P*adj < 0.05.

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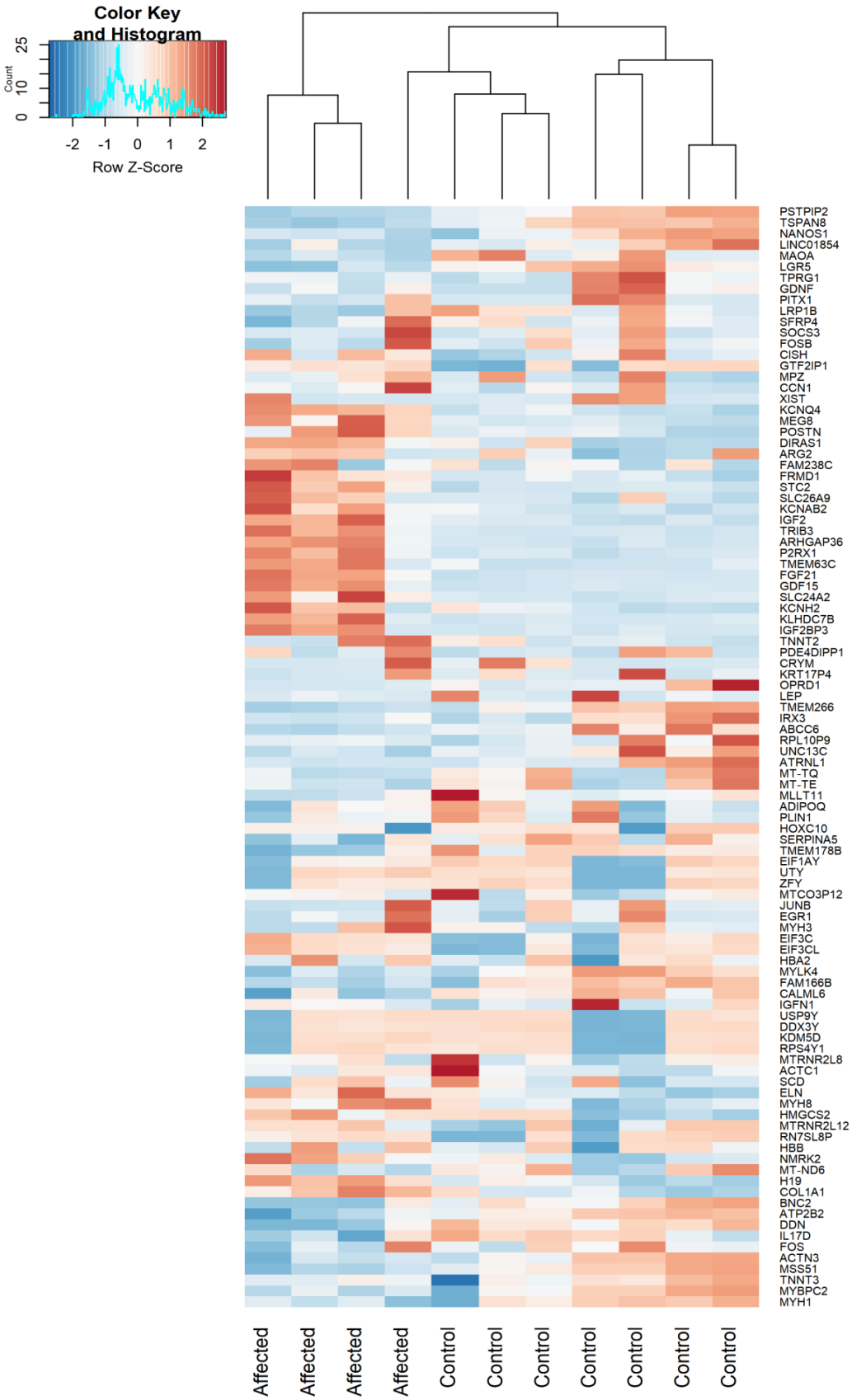

Figure EV4.

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Figure EV5. Aminoacylation assay in fibroblasts.

Aminoacylation assay of mt-tRNA^{Glu} and mt-tRNA^{Gln} in fibroblasts carrying digenic mutations in mtDNA (m.14674T>C) and in *EARS2* (p.Arg516Gln; F7/1M, F7/2M), carrying only m.14674T>C (F7/5F, F7/7F) and healthy controls. The cells were cultures in low (low aa) and high amino acid (aa) concentrations. "dAc" indicated deacylated samples while "Ac" represents the aminoacylated tRNA. There is no relevant difference in the aminoacylation of fibroblasts of patients with digenic mutations, carriers of m.14674T>C and healthy controls.

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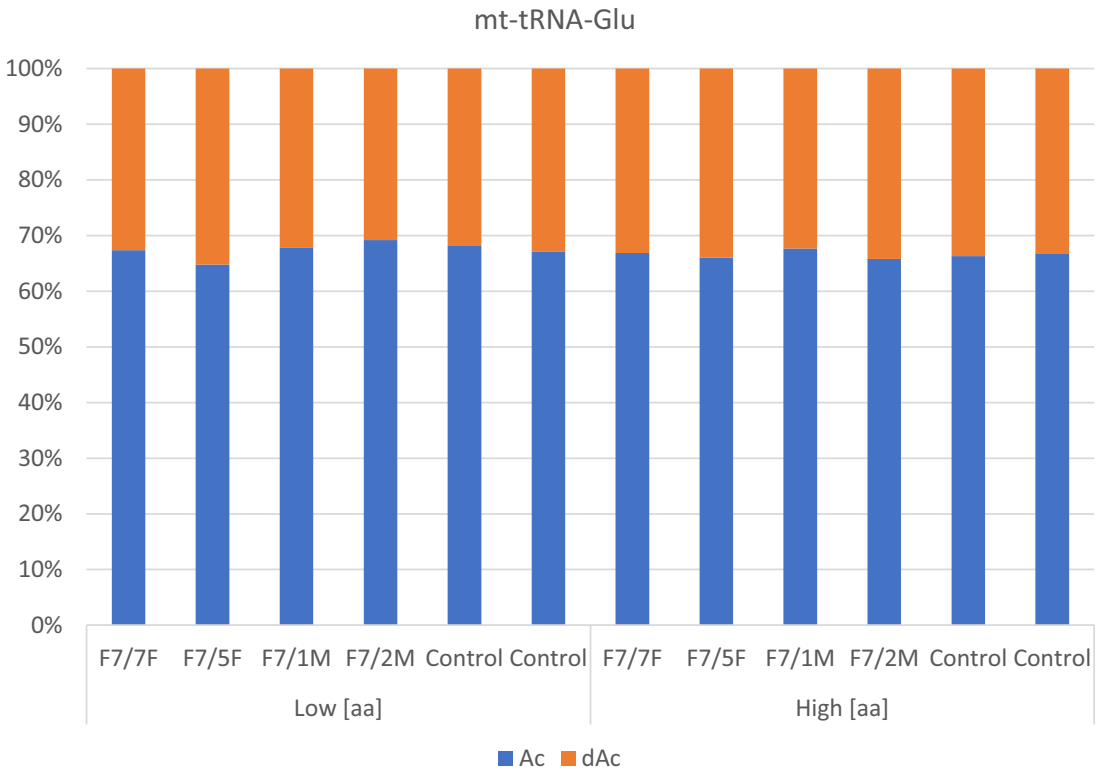

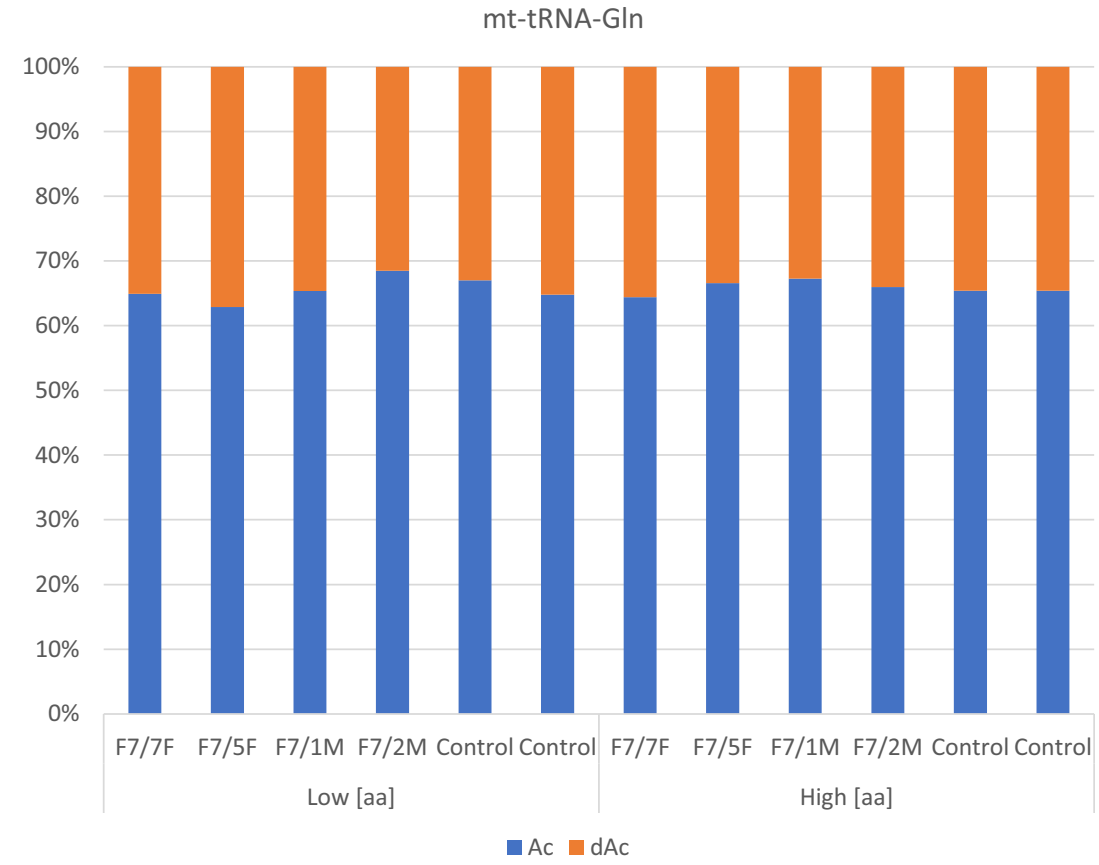

Figure EV5.

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