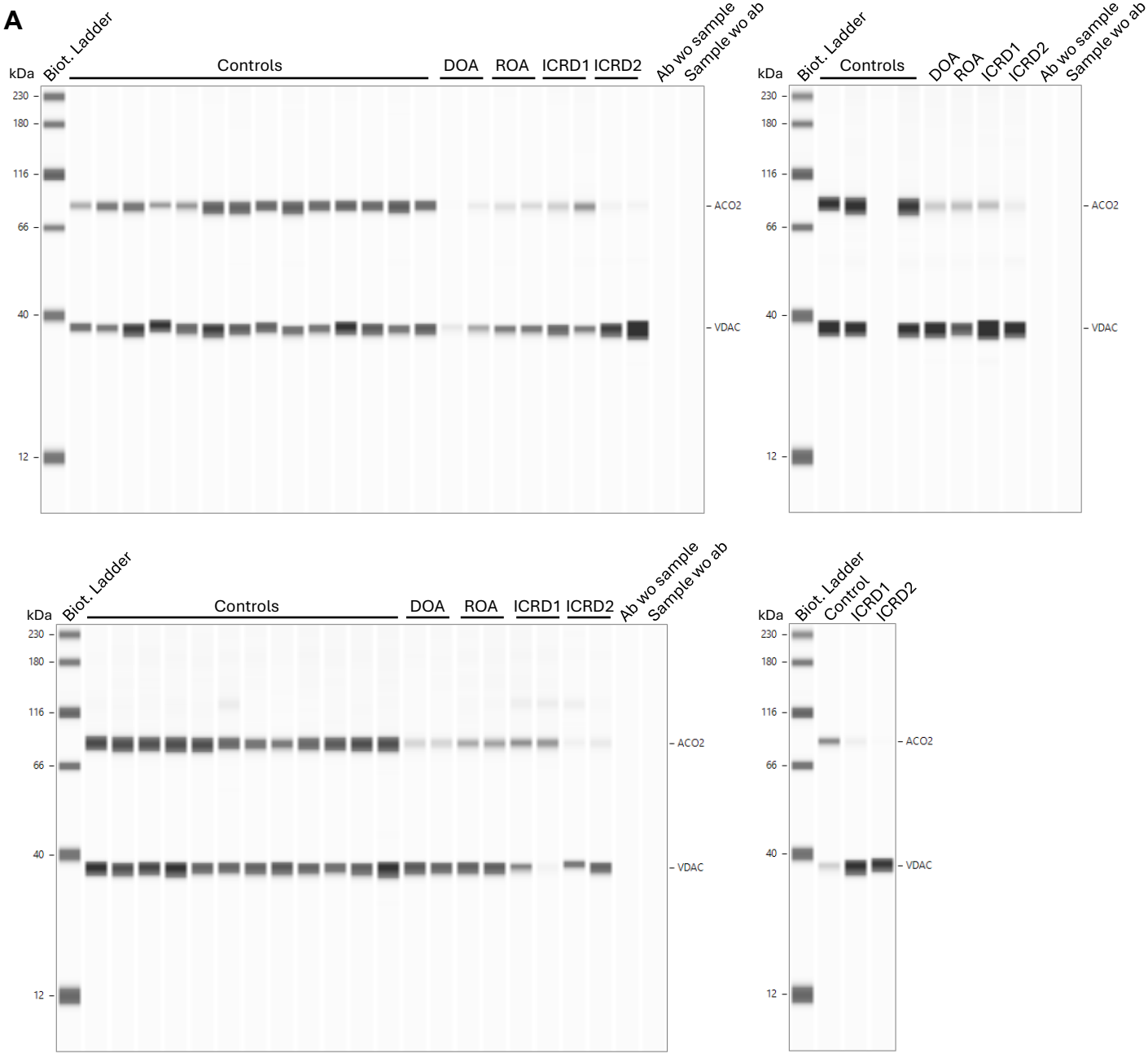
Supplementary Table 1. Clinical and molecular characteristics summary of the *ACO2* patient fibroblasts							
ID	**DOA**	**ROA**		**ICRD1**		**ICRD2**	
Phenotype	Non-syndromic	Non-syndromic		Syndromic		Syndromic	
Sex	F	M		F		F	
Age at onset	9 years	4 years		<1 year		5 months	
Age at sampling (years)	19	28		2		20	
Genotype	HT	Compound HT		Compound HT		Compound HT	
cDNA variant	c.1254dupA	c.36+5delG	c.719G>C	c.487G>A	c.2048G>A	c.2208G>C	c.2328_2331delGAAG
Location	Exon 10	Intron 1	Exon 6	Exon 4	Exon 16	Exon 17	Exon 18
Protein variant	p.Gly419Argfs*10	/	p.Gly240Ala	p.Val163Met	p.Gly683Asp	p.Lys736Asn	p.Lys776Asnfs*49
Variant type	Frameshift	Splice	Missense	Missense	Missense	Missense	Frameshift
ACMG	P	VUS	LB	VUS	VUS	LP	LP
GnomAD frequency	/	/	8.3E-04	3.72E-06	1.24E-06	/	/
ClinVar	P	/	VUS	VUS	VUS	P	P
LOVD	P	P	Conflicting (VUS, LP, P)	LP	P	LP	LP

ID: cell line identifier; DOA: Dominant Optic Atrophy; ROA: isolated Recessive Optic Atrophy; ICRD: Infantile Cerebellar-Retinal Degeneration; M: male; F: female; HT: Heterozygous; ACMG: American College of Medical Genetics and Genomics; Leiden Open Variation Database; P: pathogenic; LP: likely pathogenic; VUS: variant of uncertain significance; LB: likely benign



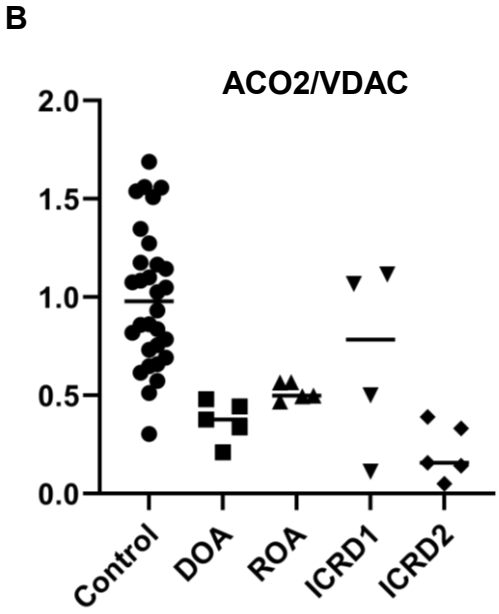


Figure S1. ACO2 protein levels blot transparency.
ACO2 and VDAC protein levels were determined by capillary electrophoresis immunoassays (Jess Simple Western) and normalized to total protein content. DOA: Dominant Optic Atrophy; ROA: Recessive Optic Atrophy; ICRD: Infantile Cerebellar-Retinal Degeneration.

(A) Blot-like view of ACO2 (85 kDa) and VDAC (37 kDa) protein intensities. Biot. Ladder: biotinylated ladder. Negative controls were used to verify the absence of experimental artifacts: antibody, no sample (ab wo sample); control sample, no antibody (sample wo ab)

(B) ACO2/VDAC ratio. Individual values are plotted for each cell line, after removal of outliers (ROUT, Q = 1%).

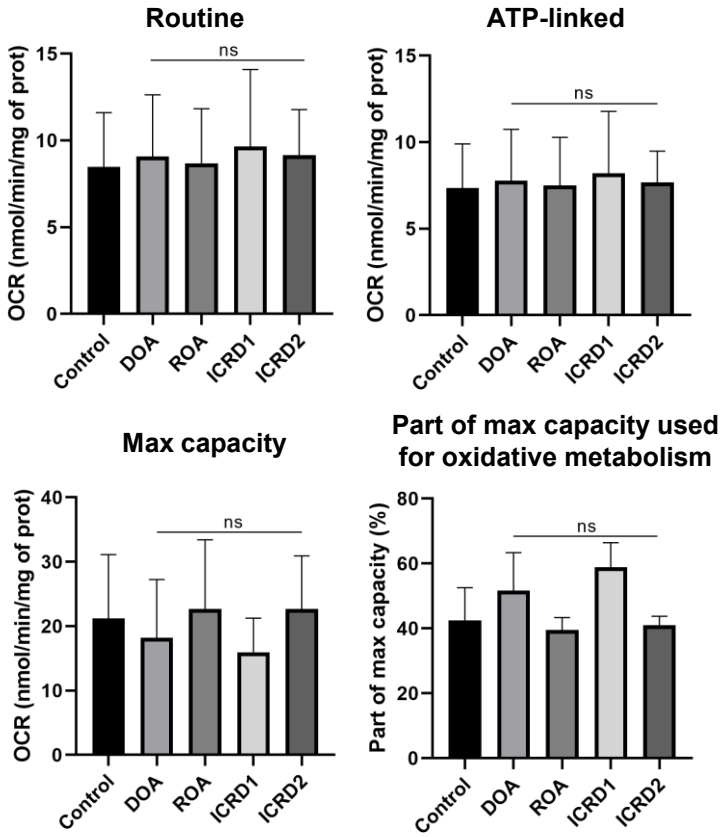


Figure S2. Cellular respiration assessment in ACO2 patient fibroblasts. Oxygen consumption rates were measured on intact cells by high-resolution respirometry. **Top right panel:** Routine respiration; **Top left:** ATP-linked respiration; **Bottom right:** maximum capacity of the respiratory chain; **Bottom left:** fraction of the maximum capacity used for oxidative metabolism. Data are presented as mean ± SD of independent biological triplicates. ns: not significant (Normally distributed variables: One-way ANOVA + Dunnett's multiple comparisons test; non-normally distributed variables: Kruskal-Wallis + Dunn's multiple comparisons test).

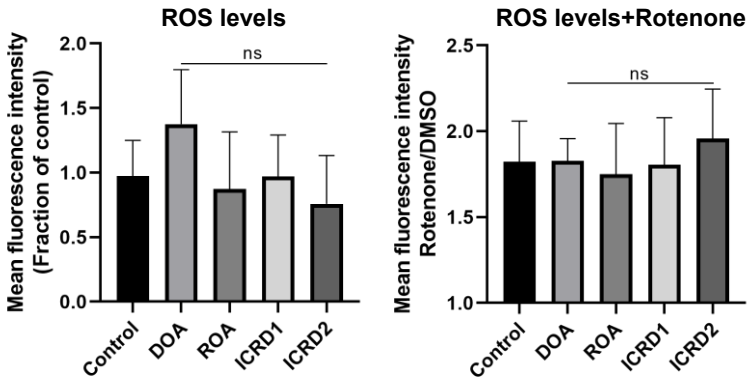


Figure S3. Mitochondrial reactive oxygen species (ROS) production measurement. Mitochondrial superoxide levels were quantified by flow cytometry using MitoSOX red. **Left panel:** Basal ROS production reflected by MitoSOX mean fluorescence intensity (MFI), with results normalized to the control group. **Right panel:** ROS production under rotenone-induced stress. Results are expressed as $MFI_{(rotenone\text{-}treated\ cells)}/MFI_{(DMSO\text{-}treated\ cells)}$ ratio. Data are presented as mean ± SD of independent biological triplicates. ns: not significant (One-way ANOVA + Dunnett's multiple comparisons test).

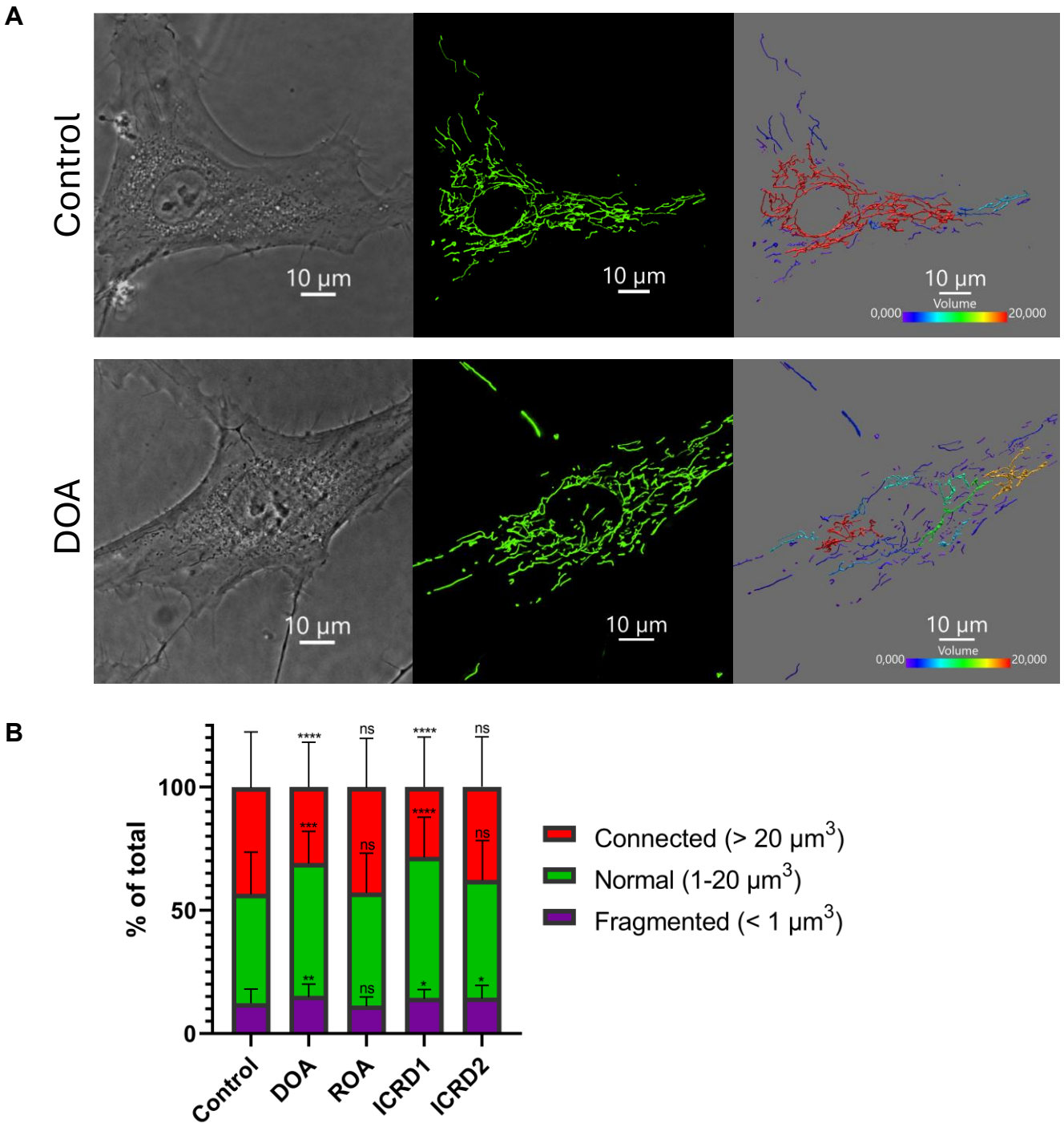


Figure S4. Mitochondrial network imaging and analysis in ACO2 patient fibroblasts.

(A) Mitochondrial network imaging. Cells were stained with MitoTracker Green FM and imaged by inverted wide-field fluorescence microscopy. Scale bars: 10 µm. Left panels: 2D images in trans; middle panels: mitochondrial network visualization; right panels: volume-coded coloring of the mitochondrial network (colored scale bar indicates volumes in µm3).Top panels: images from one control cell line; bottom panels: images from the DOA cell line, representing a cell with a fragmented mitochondrial network to illustrate the volumes quantified in (B).

(**B) Mitochondrial network volume distribution.** For each cell, the proportions of fragmented, normal and connected network were quantified using the Imaris v11.0 software 3D surface rendering. Results display the mean percentage of each volume category across 50-60 images per cell line ± SD. ns: not statistically significant; *: P <0.05; **: P <0.01; ***: P <0.001; ****: P <0.0001 (Two-way ANOVA followed by Dunnett's multiple comparisons).