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Supplemental information

Genetic variants affecting NQO1 protein levels impact the efficacy of idebenone treatment in Leber hereditary optic neuropathy

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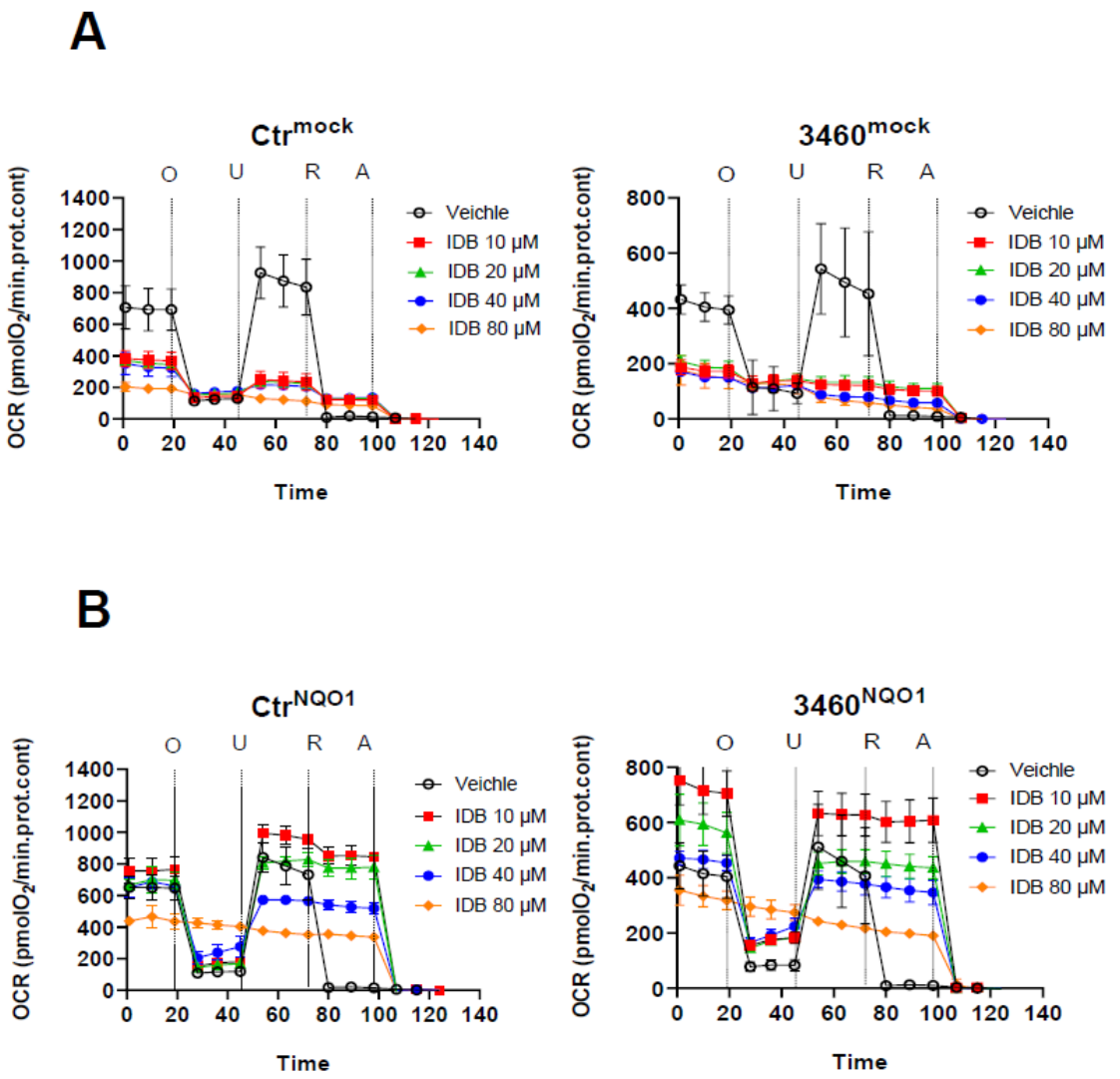
Supplementary Figures



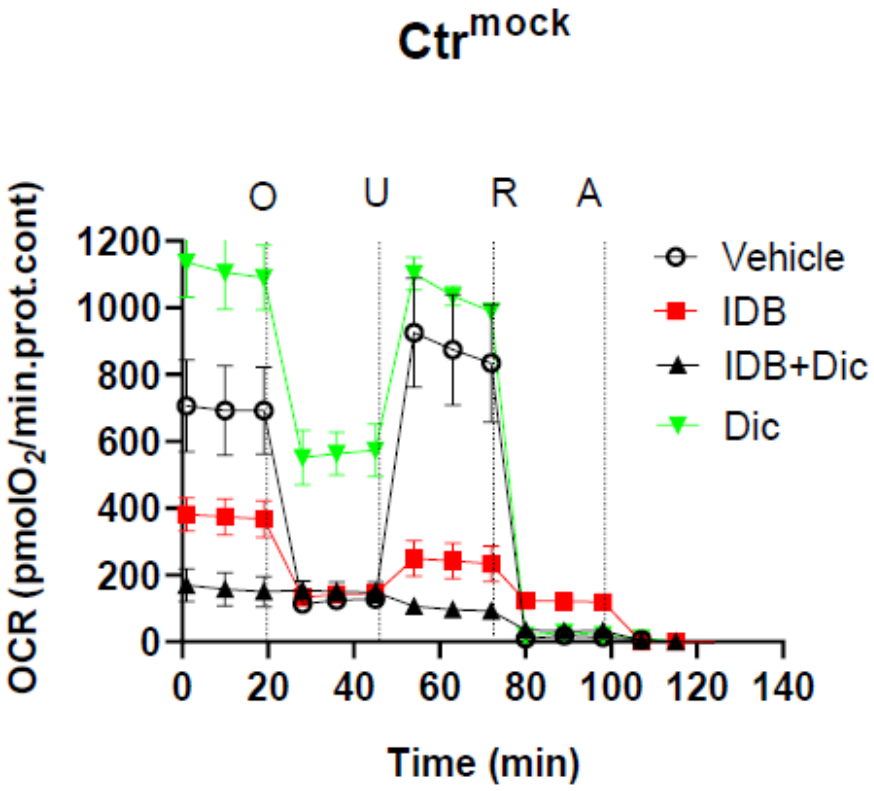
Supplementary Figure 1 related to Figure 1B and 1C

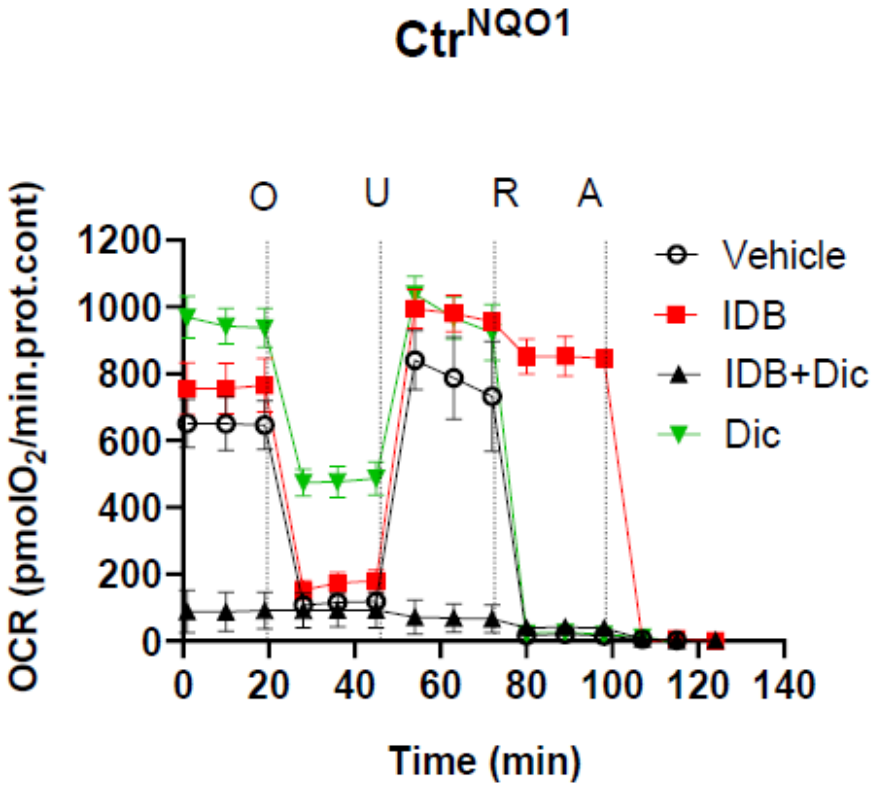
Idebenone dose-dependence of mitochondrial respiration.

A) Mitochondrial respiration rates measured with Mito-stress protocol of control (Ctr^{mock}) and m.3460G>A/*MT-ND1* LHON cybrids (3460^{mock}) with different concentrations of idebenone. Basal respiration (OCR basal), oligomycin (O) inhibited respiration (proton leak) and maximal respiration rate (OCR FCCP, U) were calculated subtracting the OCR in the presence of rotenone (R) and antimycin A (A). All values are normalized for protein cellular content measured by SRB assay. Empty circles correspond to OCR values measured in cells treated with the vehicle DMSO (ten and seven independent experiments for Ctr^{mock} and 3460^{mock}, respectively). Red squares correspond to OCR values measured in cells treated with 10

μM idebenone (six and five independent experiments for Ctr^{mock} and 3460^{mock}, respectively). Green triangles, blue circles and orange diamonds correspond to OCR values measured in cells treated with 20, 40, 80 μM idebenone, respectively (two independent experiments for each concentration). Data are reported as mean ± SD.

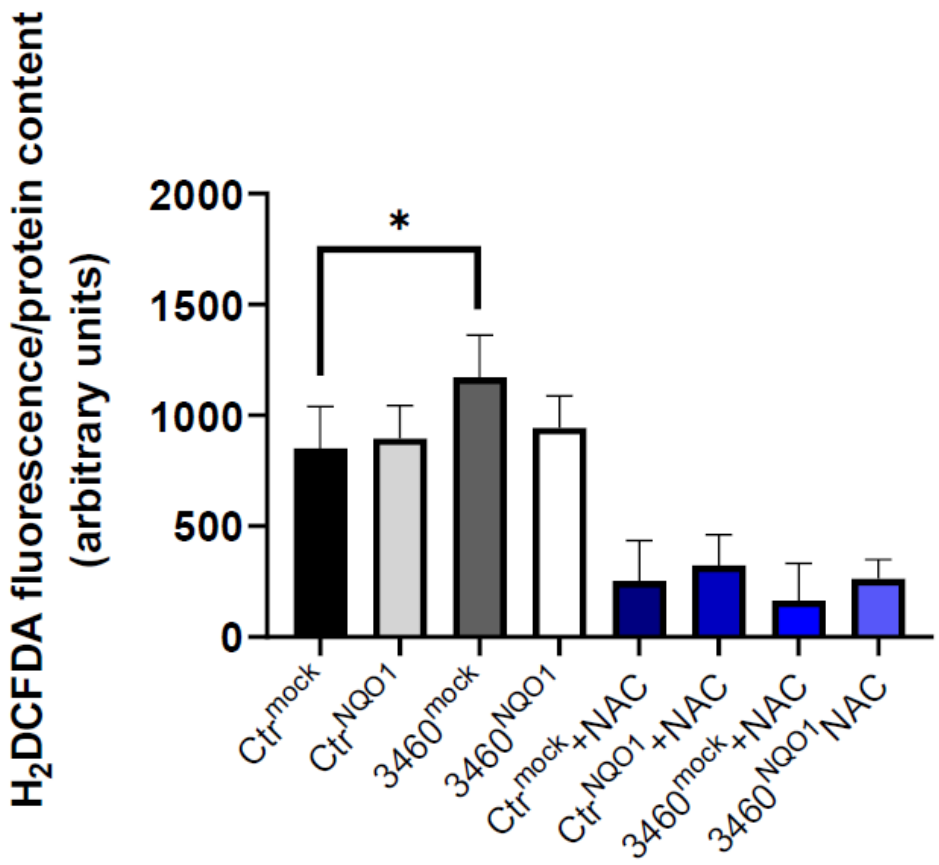
B) Mitochondrial respiration rates measured with Mito-stress protocol of control (Ctr^{NQO1}) and m.3460G>A/*MT-ND1* LHON cybrids (3460^{NQO1}) with different concentrations of idebenone. The experimental conditions are the same described in panel A. Empty circles correspond to OCR values measured in cells treated with the vehicle DMSO (eight and five experiments for Ctr^{NQO1} and 3460^{NQO1}, respectively). Red squares correspond to OCR values measured in cells treated with 10 μM idebenone (six and five experiments for Ctr^{NQO1} and 3460^{NQO1}, respectively). Green triangles, blue circles and orange diamonds correspond to OCR values measured in cells treated with 20, 40, 80 μM idebenone, respectively (two independent experiments for each concentration). Data are reported as mean ± SD.


Ctr^mock
O
U
R
A
OCR (pmolO2/min.prot.cont)
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
Time (min)
Vehicle
IDB
IDB+Dic
Dic


Ctr^NQO1
O
U
R
A
OCR (pmolO2/min.prot.cont)
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
Time (min)
Vehicle
IDB
IDB+Dic
Dic

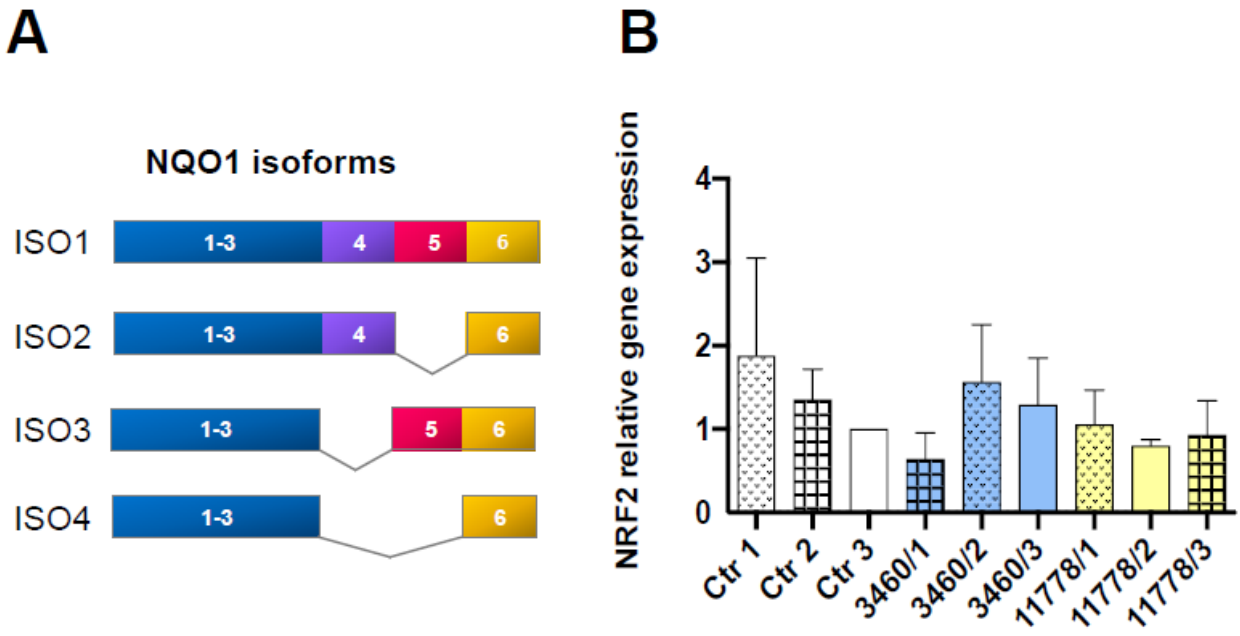
Supplementary Figure 2 related to Figure 1B and 1C

Mitochondrial respiration rates measured with Mito-stress protocol of control ($Ctr^{mock}$and Ctr^{NQO1}). Basal respiration (OCR basal), oligomycin (O) inhibited respiration (proton leak) and maximal respiration rate (OCR FCCP, U) were calculated subtracting the OCR in the presence of rotenone (R) and antimycin A (A). All values are normalized for protein cellular content measured by SRB assay. Empty circles correspond to OCR values measured in cells treated with the vehicle DMSO (ten and seven independent experiments for Ctr^{mock} and 3460^{mock}, respectively). Red squares correspond to OCR values measured in cells treated with 10 µM idebenone (six and five independent experiments for Ctr^{mock} and 3460^{mock}, respectively). Green triangles correspond to OCR values measured in presence of 10µM dicoumarol, black triangles correspond to OCR values measured in presence of 10 µM idebenone and 10µM dicoumarol (IDB+Dic) (two independent experiments for each concentration). Data are reported as mean ± SD.



Supplementary Figure 3 related to Figure 1E and 1F

Rate of H_2O_2 production in cells grown in 10 mM glucose, 5 mM pyruvate and 1 mM glutamine DMEM in presence or absence of 10 mM N-acetylcysteine (NAC). The H_2O_2 production amount measured in the first 6h of the experiment is expressed as arbitrary unit of fluorescence corrected for the cellular protein content (SRB assay) as detailed in Materials and Methods. Data are reported as mean ± SEM of four to six independent experiments.

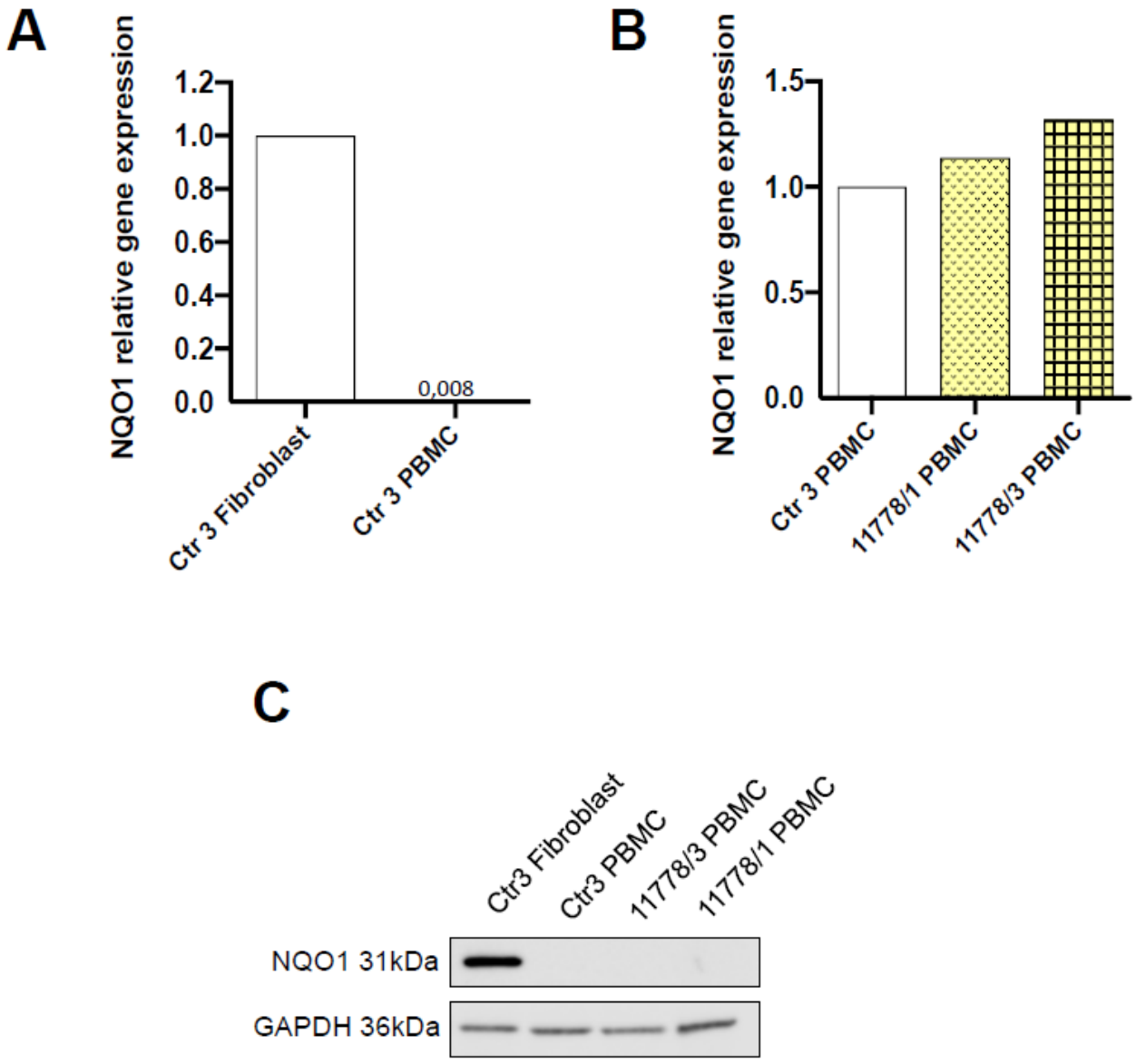


Supplementary Figure 4 related to Figure 3

NOQ1 isoforms and NFR2 gene expression.

A) Schematic representation of the NQO1 transcript variants. Alternative splicing of exon 4 and 5 generate four NQO1 variants (isoforms 1–4).

B) NRF2 gene expression evaluated by qPCR, with GAPDH was used as reference gene. Fold-changes are normalized to Ctr3 and are expressed as means ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA test with a Dunnett's *post-hoc* test.



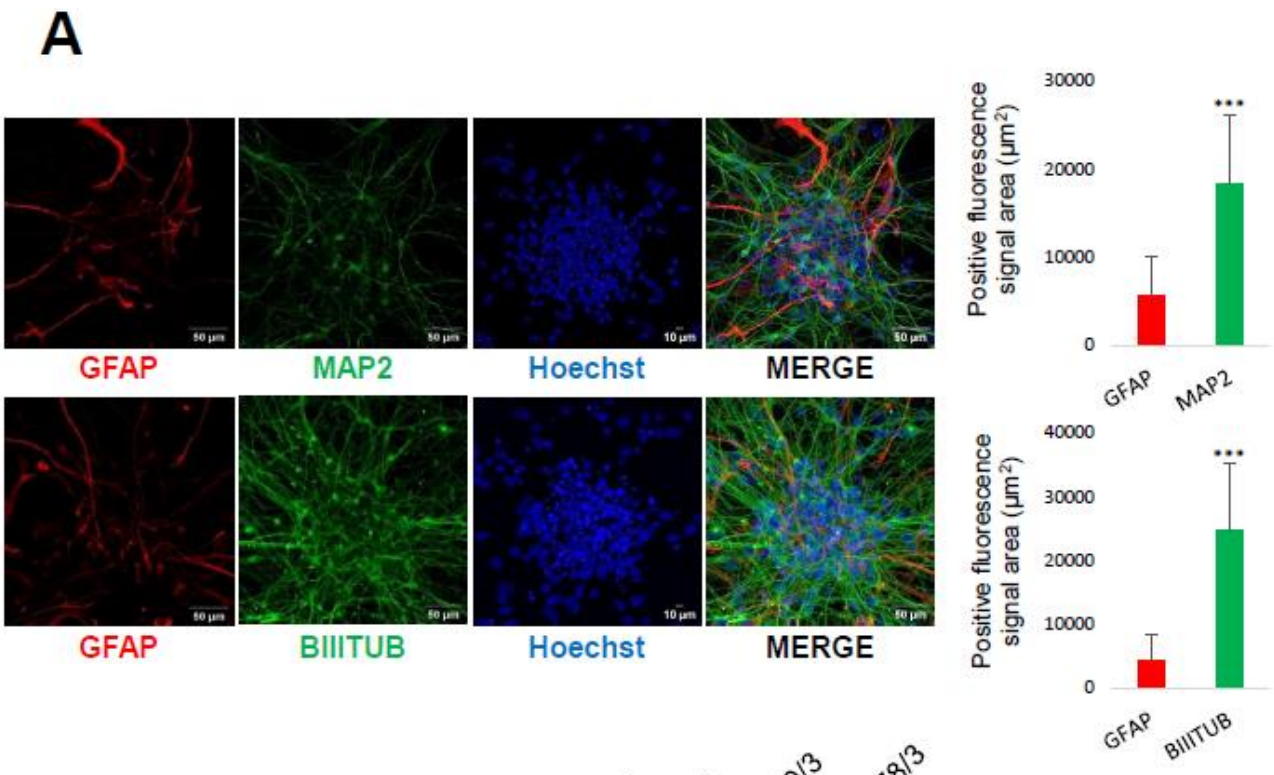
Supplementary Figure 5 related to Figure 3

NQO1 gene expression and protein levels in PBMC.

A) *NQO1* gene expression evaluated by qPCR in fibroblasts and PBMC of Ctr3. GAPDH was used as reference gene.

B) *NQO1* gene expression evaluated by qPCR in PBMC of Ctr3, 11778/1 and 11778/3. Fold-changes are normalized to Ctr3 and GAPDH was used as reference gene.

C) Western blot analysis of NQO1 level in lysates from Ctr3 fibroblast and PBMC, and PBMC of 11778/1 and 11778/3. GAPDH was used as loading control.


A
GFAP
MAP2
Hoechst
MERGE
GFAP
BIIITUB
Hoechst
MERGE
Positive fluorescence signal area (µm²)
30000
20000
10000
0
***
GFAP
MAP2
Positive fluorescence signal area (µm²)
40000
30000
20000
10000
0
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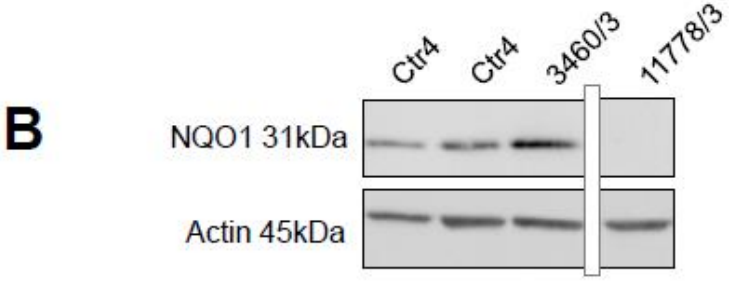
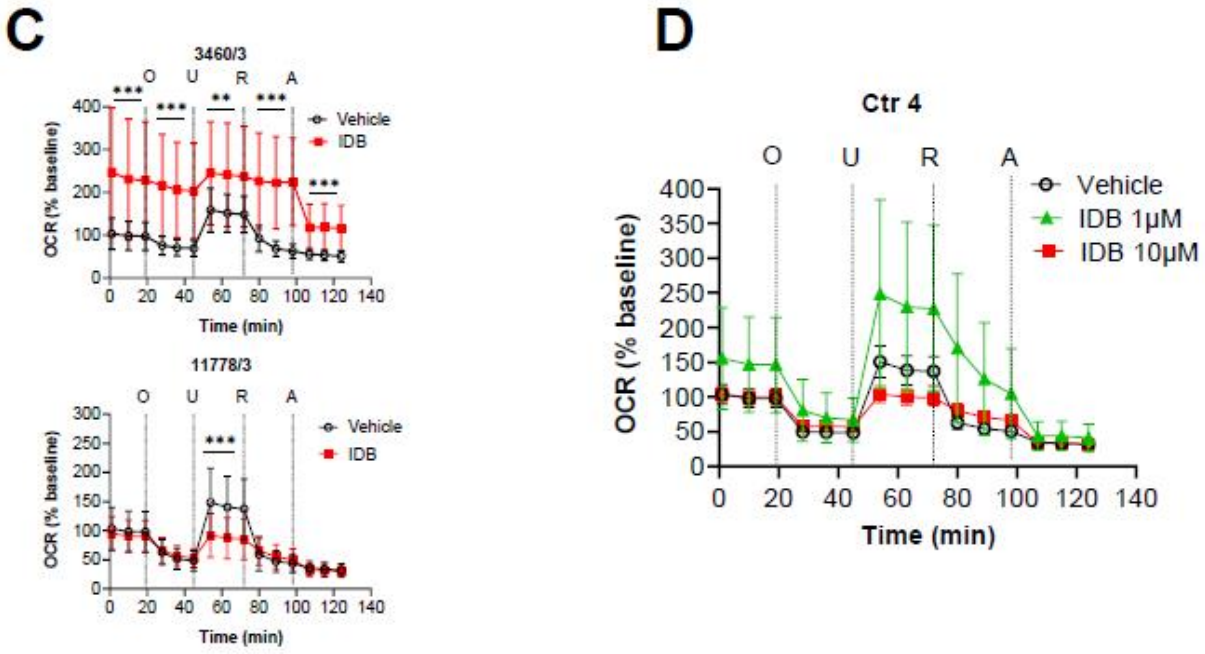
GFAP
BIIITUB


B
Ctr4
Ctr4
3460/3
11778/3
NQO1 31kDa
Actin 45kDa


C
3460/3
O U R A
Vehicle
IDB
OCR (% baseline)
Time (min)
11778/3
O U R A
Vehicle
IDB
OCR (% baseline)
Time (min)
D
Ctr 4
O U R A
Vehicle
IDB 1µM
IDB 10µM
OCR (% baseline)
Time (min)

Supplementary Figure 6 related to Figure 4C

Analysis of NQO1 expression levels and effect of idebenone on OCR of iPSCs-derived neurons.

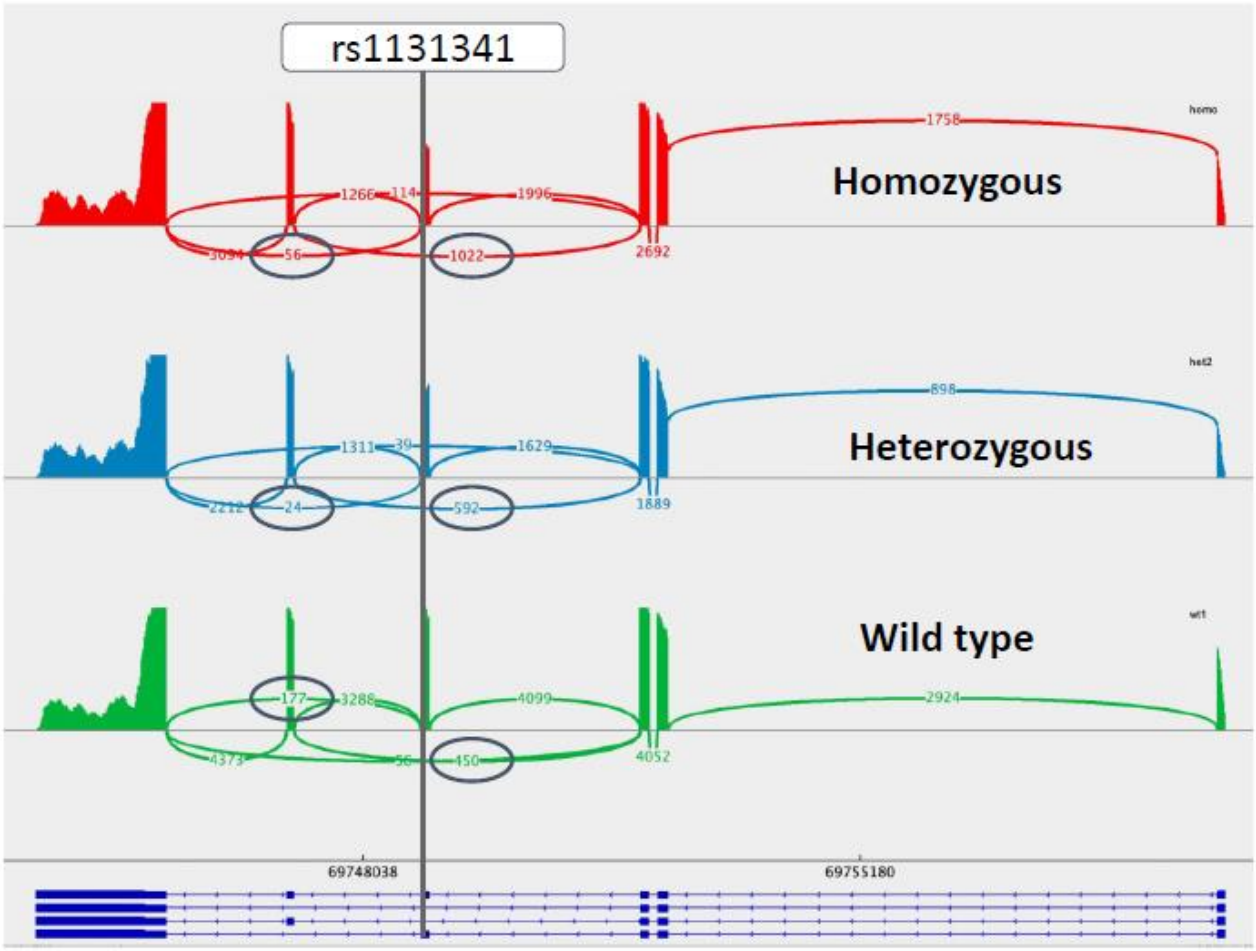
A) Immunofluorescence analysis of iPSCs-derived neuronal population differentiated from Ctr4. Representative images of the result obtained using two different antibodies specifically directed against neurons (MAP2 and IIITUBULIN shown in green) and one antibody against astrocytes (GFAP shown in red). Nuclei are stained with Hoechst (shown in blue).

A histogram reporting fluorescence quantification is presented on the right. Images were taken at 40X.

B) Western blot analysis of NQO1 level in cellular lysates from a control (two biological replicates of Ctr4), 3460/3 and 11778/3 iPSC-derived neurons. Actin was used as loading control. Due to the very low number of iPSC-derived neurons the western blot was performed only once.

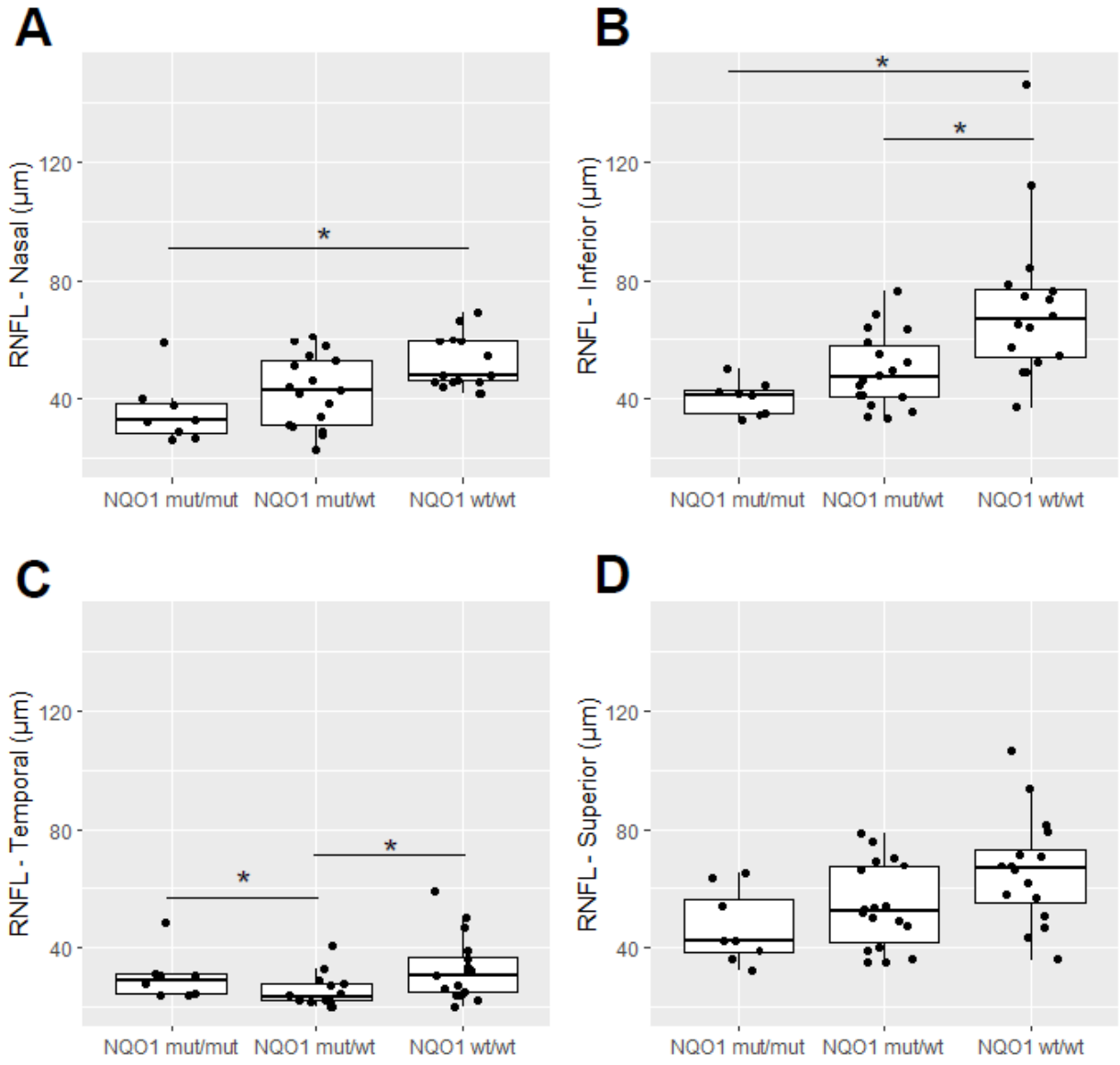
C) OCR measurement in LHON (3460/3, 11778/3) iPSCs-derived neurons in presence or absence of idebenone. OCR measurements were obtained before and after addition of oligomycin (O), FCCP (U), rotenone (R) and antimycin A (A). OCR data were normalized on cell counts and expressed as a percentage of the baseline measurement of untreated lines. Open circles correspond to OCR values measured in cells treated with the vehicle DMSO, red squares to OCR values measured in cells treated with 10 μM idebenone (IDB). Data are reported as mean ± SD of 20 replicates for the untreated and 16 replicates for the idebenone-treated condition for 3460/3 neurons (derived from 1 experiment being 3460/3 NPCs particularly refractory to terminally differentiate into neurons); 66 replicates for the untreated condition and 54 replicates for the idebenone-treated condition for 11778/3 (derived from 4 independent experiments). Statistical analysis is the same reported in Figure 1B and Methods section. Asterisks denote values significantly different $^{**}p < 0.01$; $^{***}p < 0.001$.

D) OCR measurement in Ctr4 iPSCs-derived neurons in presence or absence of different idebenone concentrations (1 μM in green and 10 μM in red). 49 replicates for the untreated condition, 48 for the 10 μM idebenone-treated condition (derived from 2 independent experiments), and 8 for the 1 μM idebenone-treated condition (one experiment).



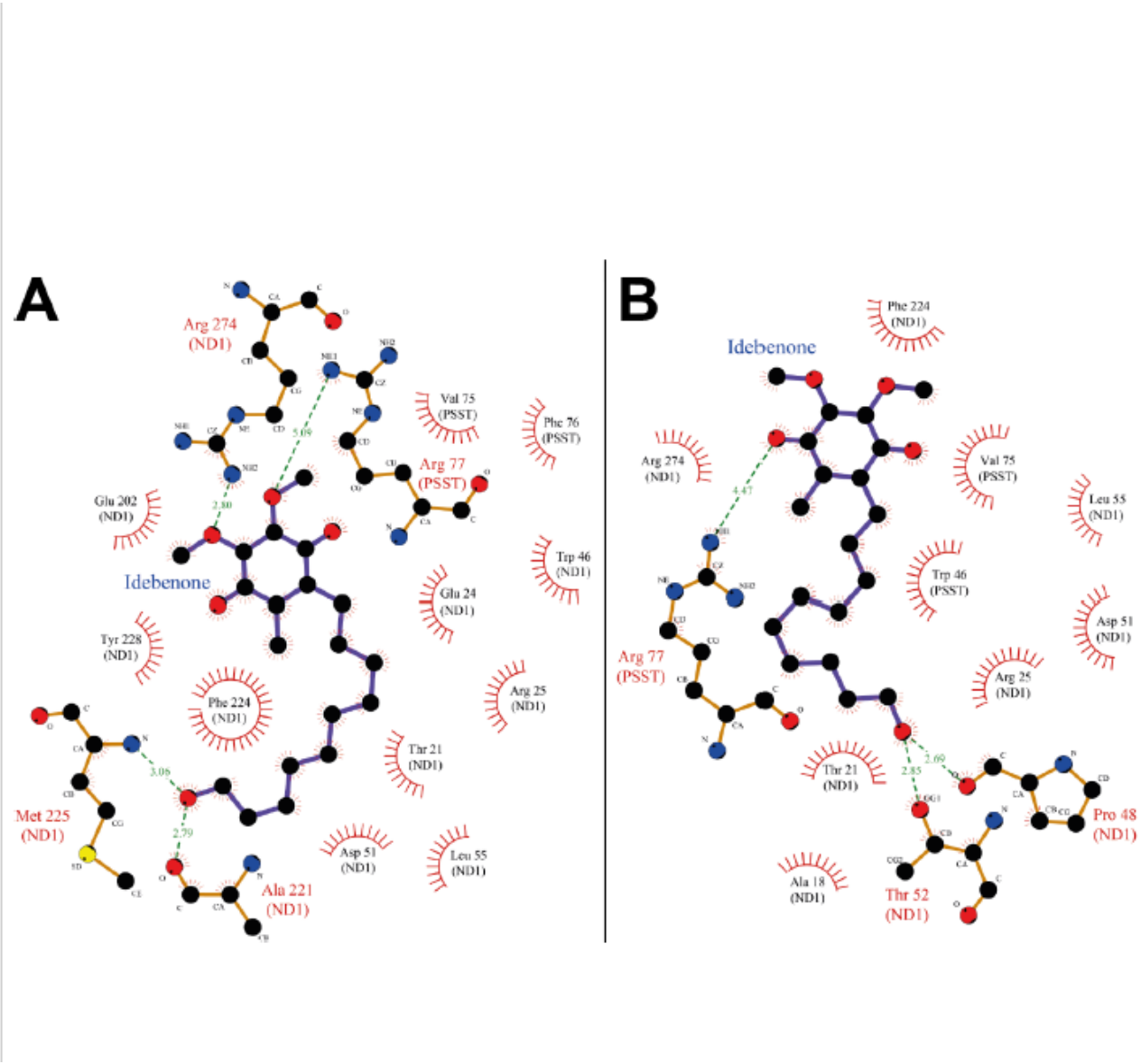
Supplementary Figure 7 related to Figure 5

NQO1 Sashimi plot of exon skipping in homozygous rs1131341 carriers (red) and heterozygous carriers (blue) and unaffected fibroblasts (green). The RNA coverage is given as the log10 RPKM-value and the number of split reads spanning the given intron is indicated on the exon-connecting lines. The gene model of the RefSeq annotation is depicted at the bottom.



Supplementary Figure 8 related to Figure 6

Panels **A – D** show box plots of sectorial RNFL thickness at last visit in LHON patients referring to the clinical site IRCCS Istituto delle Scienze Neurologiche di Bologna, Bellaria Hospital (Bologna, Italy), with solid lines representing median value for *NQO1* mut/mut, *NQO1* mut/wt and *NQO1* wt/wt genotypes. All these patients were evaluated with swept-source OCT (DRI Triton OCT, *Topcon*, Tokyo, Japan). *$p < 0.05$



Supplementary Figure 9 related to Figure 7

Detail of the intermolecular interactions of the best docking poses for idebenone in the WT (**A**) and A52T (ND1) mutated (**B**) CI. In the figure are represented H-bonds (green dashed lines) and van der Waals (red spiked arcs) interactions. The inhibitor, as well as CI residues H-bonded to them, are in ball-and-stick coloured according to the atom type.

Supplementary Table 1 related to Figure 2B. Demographic and clinical information of the six LHON patients from which fibroblast cell lines was derived

Cell line	**LHON mt.DNA mutation**	**Age at onset Right eye**	**Age at onset Left eye**	**Therapy (total daily dose)**	**Outcome**
3460/1	m.3460G>A	17 years	16 years	Idebenone 405 mg	Non-Responder
3460/2	m.3460G>A	6 years	20 years	Idebenone 675 mg	Responder on the left eye treated
3460/3	m.3460G>A	16 years	16 years	EPI-743 400 mg	Non-Responder
11778/1	m.11778G>A	26 years	26 years	Idebenone 405 mg	Non-Responder
11778/2	m.11778G>A	26 years	25 years	Idebenone 405 mg	Responder
11778/3	m.11778G>A	25 years	25 years	Idebenone 900 mg	Non-Responder

We defined the responder/non-responder status as assessed between nadir and last visit: responders were those eyes that experienced a VA improvement from off-chart to on-chart of at least 1 full line (5 letters), or an on-chart VA improvement of at least 2 lines (10 letters=0.2 logMar). Idebenone dosages differ from patient to patient as in most cases these patients were treated off label before EMA approval and current guidelines for treatments, which are set at 900 mg/day (300 mg x 3 with meals).

Supplementary Table 2 related to Figure 6A-D. Demographic and clinical features of LHON patients

	All	NQO1 mut/mut	NQO1 mut/wt	NQO1 wt/wt
N, subjects	118 (100%)	13 (11%)	34 (28.8%)	71 (60.2%)
Gender				
Male	95 (80%)	13 (100%)	25 (74%)	57 (80%)
Female	23 (20%)	-	9 (26%)	14 (20%)
Mutation				
m.3460G>A/*MTND1*	23 (20%)	7 (54%)	7 (21%)	9 (13%)
m.11778G>A/*MTND4*	95 (80%)	6 (46%)	27 (79%)	62 (87%)
Interval of onset between eyes[#] (d)	80 ± 151 (0 - 91)	115 ± 162 (46 - 122)	51 ± 89 (0 - 62)	86 ± 171 (0 - 91)
N, eyes	233 (100%)	26 (11.1%)	67 (28.8%)	140 (60.1%)
Age at onset (y)	34 ± 17 (18 - 48)	27 ± 13 (17 - 33)	34 ± 18 (18 - 48)	35 ± 17 (19 - 49)
Time since onset at idebenone start[¥] (mo)	8 ± 12 (2 - 8)	9 ± 15 (1 - 9)	8 ± 11 (2 - 9)	8 ± 11 (2 - 8)
BCVA at nadir[†] (logMar)	1.8 ± 0.5 (1.5 – 2.2)	1.8 ± 0.5 (1.6 – 2.2)	1.8 ± 0.4 (1.5 – 2.2)	1.7 ± 0.5 (1.4 – 2.1)
BCVA off-chart[*] at nadir[†]	131 (56%)	14 (54%)	43 (64%)	74 (53%)
Time since nadir[†] at last visit[††] (mo)	48 ± 35 (20 - 69)	48 ± 39 (13 - 81)	56 ± 40 (23 - 83)	44 ± 31 (17 - 59)
Primary visual outcome[**]				
Responder	122 (52%)	7 (27%)	35 (52%)	80 (57%)
Non-responder	111 (48%)	19 (73%)	32 (48%)	60 (43%)
BCVA at last visit[††] (logMar)	1.4 ± 0.6 (1 – 2.1)	1.7 ± 0.5 (1.4 – 2.1)	1.4 ± 0.6 (1.2 – 2.1)	1.4 ± 0.6 (1 – 2.1)
RNFL – AVG, at last visit[††] (µm)				
Italians	n = 85 55 ± 16 (42 - 66)	n = 10 42 ± 11 (33 - 49)	n = 34 54 ± 18 (40 - 63)	n = 41 59 ± 15 (48 - 68)
Germans	n = 96 49 ± 15 (38 - 55)	n = 11 41 ± 11 (35 - 47)	n = 19 49 ± 16 (35 - 57)	n = 66 49 ± 15 (39 - 55)
Sectorial RNFL[&] - at last visit[††] (µm)	n = 42	n = 8	n = 18	n = 16
Nasal	43 ± 13 (33 - 54)	35 ± 10 (28 - 38)	41 ± 13 (31 - 52)	50 ± 12 (46 - 59)
Inferior	56 ± 22 (41 - 65)	44 ± 6 (35 - 43)	50 ± 13 (41 - 58)	72 ± 27 (54 - 78)
Temporal	28 ± 9 (22 - 31)	31 ± 8 (24 - 31)	24 ± 6 (20 - 26)	33 ± 11 (25 - 37)
Superior	57 ± 17 (42 - 68)	47 ± 12 (38 - 56)	54 ± 14 (41 - 67)	66 ± 19 (55 - 73)

Values are given as n (%) or mean ± SD and (Q1-Q3).

Based on the studied NQO1 variants: NQO1 mut/mut stands for heterozygous at both polymorphic sites, meaning compound heterozygote, or homozygous either at one or the other polymorphic site; NQO1 mut/wt stands for heterozygous at only one of the two polymorphic sites; NQO1 wt/wt stands for wildtype at both polymorphic sites.

[#] Time between onset of first and second affected eye.

[¥] Time from LHON symptom onset to start of idebenone-treatment.
[†] Nadir is defined as the value when BCVA reached its worst point (= highest logMar value).
[*] If BCVA was > 1.68 logMar or off-chart [regardless of being assessed as counting fingers (2.1 logMar), hand motion (2.2 logMar), light perception (2.3 logMar) or no-light perception (2.8 logMar)].
[† †] Last visit (LV) corresponds to the last observation before stopping the therapy or, for patients still receiving idebenone, is the last visit available.
[&]Sectorial RNFL thicknesses at last visit are related to subgroup of LHON patients referring to the clinical site IRCCS Istituto delle Scienze Neurologiche di Bologna, Bellaria Hospital (Bologna, Italy).
[**] Responder outcome is defined as a VA improvement: from "off-chart" (the equivalent of counting fingers, CF/ hand motion, HM/light perception, LP or no-light perception, NLP) to at least 1.6 logMar value or of at least 0.2 logMar value within "on-chart".
d, days; y, years; mo, months; BCVA, best-corrected visual acuity; logMar, logarithm of the minimal angle of resolution; RNFL, peripapillary retinal nerve fiber; AVG, average thickness.

Supplementary Table 3 related to Figure 6E-H. Demographic and clinical features of m.11778G>A/*MTND4* LHON patients

	All	NQO1 mut/mut	NQO1 mut/wt	NQO1 wt/wt
N, subjects	95 (100%)	6 (6%)	27 (28%)	62 (66%)
Gender				
Male	78 (82%)	6 (100%)	21 (78%)	51 (82%)
Female	17 (18%)	-	6 (22%)	11 (18%)
Interval of onset between eyes[#] (d)	84 ± 161 (0 - 94)	158 ± 226 (61 -130)	58 ± 98 (0 - 84)	87 ± 175 (0 - 92)
N, eyes	187 (100%)	12 (6.4%)	53 (28.4%)	122 (65.2%)
Age at onset (y)	33 ± 17 (18 - 45)	26 ± 9 (17 - 33)	31 ± 17 (18 - 45)	35 ± 17 34 (18 - 49)
Time since onset at idebenone start[¥] (mo)	8 ± 12 (2 - 8)	14 ± 21 (1 - 16)	8 ± 12 (3 - 9)	7 ± 11 (2 - 8)
BCVA at nadir[†] (logMar)	1.8 ± 0.5 (1.5 – 2.1)	1.8 ± 0.3 (1.6 – 2.1)	1.8 ± 0.5 (1.5 – 2.2)	1.7 ± 0.5 (1.4 – 2.1)
BCVA off-chart[*] at nadir[†]	103 (55%)	5 (42 %)	33 (62%)	65 (53%)
Time since nadir[†] at last visit[††] (mo)	47 ± 34 (17 - 67)	56 ± 50 (14 - 91)	59 ± 42 (24 - 83)	42 ± 27 (16 - 57)
Primary visual outcome[**]				
Responder				
Non-responder	104 (56%) 83 (44%)	5 (42%) 7 (58%)	29 (55%) 24 (45%)	70 (57%) 52 (43%)
BCVA at last visit[††] (logMar)	1.4 ± 0.6 (1 - 2)	1.4 ± 0.5 (1.3 – 1.7)	1.4 ± 0.6 (1.1 – 1.7)	1.4 ± 0.6 (1 - 2)
RNFL – AVG, at last visit[††] (µm)				
Italians	n = 69 58 ± 16 (48 – 68)	n = 8 45 ± 9 (39 – 51)	n = 26 58 ± 17 (44 – 66)	n = 35 62 ± 14 (52 – 69)
Germans	n = 71 50 ± 15 (40 – 57)	n = 2 45 ± 3 (44 – 46)	n = 13 51 ± 13 (42 – 57)	n = 56 50 ± 16 (39 – 56)

Values are given as n (%) or mean ± SD and (Q1-Q3).
Based on the studied NQO1 variants: NQO1 mut/mut stands for heterozygous at both polymorphic sites, meaning compound heterozygote, or homozygous either at one or the other polymorphic site; NQO1 mut/wt stands for heterozygous at only one of the two polymorphic sites; NQO1 wt/wt stands for wildtype at both polymorphic sites.
[#] Time between onset of first and second affected eye.
[¥] Time from LHON symptom onset to start of idebenone-treatment.
[†] Nadir is defined as the value when BCVA reached its worst point (= highest logMar value).
[*] If BCVA was > 1.68 logMar or off-chart [regardless of being assessed as counting fingers (2.1 logMar), hand motion (2.2 logMar), light perception (2.3 logMar) or no-light perception (2.8 logMar)].
[††] Last visit (LV) corresponds to the last observation before stopping the therapy or, for patients still receiving idebenone, is the last visit available.
[**] Responder outcome is defined as a VA improvement: from "off-chart" (the equivalent of counting fingers, CF/ hand motion, HM/light perception, LP or no-light perception, NLP) to at least 1.6 logMar value or of at least 0.2 logMar value within "on-chart".

d, days; y, years; mo, months; BCVA, best-corrected visual acuity; logMar, logarithm of the minimal angle of resolution; RNFL, peripapillary retinal nerve fiber; AVG, average thickness.

Supplementary Table 4 Related to Figure 6I-L. Demographic and clinical features of m.3460G>A/*MTND1* LHON patients

	All	NQO1 mut/mut	NQO1 mut/wt	NQO1 wt/wt
N, subjects	23 (100 %)	7 (30.5%)	7 (30.5%)	9 (39%)
Gender				
Male	17 (74%)	7 (100%)	4 (57%)	6 (67%)
Female	6 (26%)	-	3 (43%)	3 (33%)
Interval of onset between eyes[#] (d)	64 ± 104 (0 - 76)	79 ± 78 (26 - 106)	24 ± 30 (1 - 43)	83 ± 149 (0 - 76)
N, eyes	46 (100%)	14 (30.4%)	14 (30.4%)	18 (39.2%)
Age at onset (y)	37 ± 19 (18 - 56)	27 ± 17 (17 - 41)	45 ± 19 (29 - 58)	37 ± 17 (19 - 44)
Time since onset at idebenone start[¥] (mo)	7 ± 8 (2 - 9)	5 ± 4 (2 - 5)	7 ± 6 (2 - 10)	9 ± 12 (3 - 7)
BCVA at nadir[†] (logMar)	1.9 ± 0.4 (1.6 – 2.2)	1.8 ± 0.7 (1.6 – 2.2)	1.9 ± 0.3 (1.7 – 2.2)	1.8 ± 0.4 (1.4 – 2.2)
BCVA off-chart[*] at nadir[†]	28 (61%)	9 (64%)	10 (71%)	9 (50%)
Time since nadir[†] at last visit[††] (mo)	53 ± 37 (26 - 81)	43 ± 30 (15 - 59)	46 ± 31 (21 - 74)	63 ± 44 (30 - 80)
Primary visual outcome[**]				
Responder				
Non-responder	18 (39%) 28 (61%)	2 (14%) 12 (86%)	6 (43%) 8 (57%)	10 (56%) 8 (44%)
BCVA at last visit[††] (logMar)	1.6 ± 0.6 (1.2 – 2.1)	1.9 ± 0.4 (1.6 – 2.1)	1.6 ± 0.5 (1.3 – 2.1)	1.4 ± 0.6 (0.9 – 2.1)
RNFL – AVG, at last visit[††] (µm)				
Italians	n = 16 41 ± 11 (30 - 46)	n = 2 29 ± 0.7 (29 - 30)	n = 8 40 ± 12 (30 - 46)	n = 6 46 ± 8 (41 - 45)
Germans	n = 25 45 ± 14 (35 - 53)	n = 9 41 ± 12 (35 - 48)	n = 6 46 ± 22 (32 - 63)	n = 10 47 ± 8 (40 - 53)

Values are given as n (%) or mean ± SD and (Q1-Q3).
Based on the studied NQO1 variants: NQO1 mut/mut stands for heterozygous at both polymorphic sites, meaning compound heterozygote, or homozygous either at one or the other polymorphic site; NQO1 mut/wt stands for heterozygous at only one of the two polymorphic sites; NQO1 wt/wt stands for wildtype at both polymorphic sites.
[#] Time between onset of first and second affected eye.
[¥] Time from LHON symptom onset to start of idebenone-treatment.
[†] Nadir is defined as the value when BCVA reached its worst point (= highest logMar value).
[*] If BCVA was > 1.68 logMar or off-chart [regardless of being assessed as counting fingers (2.1 logMar), hand motion (2.2 logMar), light perception (2.3 logMar) or no-light perception (2.8 logMar)].
[††] Last visit (LV) corresponds to the last observation before stopping the therapy or, for patients still receiving idebenone, is the last visit available.

** Responder outcome is defined as a VA improvement: from “off-chart” (the equivalent of counting fingers, CF/ hand motion, HM/light perception, LP or no-light perception, NLP) to at least 1.6 logMar value or of at least 0.2 logMar value within “on-chart”.

d, days; y, years; mo, months; BCVA, best-corrected visual acuity; logMar, logarithm of the minimal angle of resolution; RNFL, peripapillary retinal nerve fiber; AVG, average thickness.

Supplementary Table 5. Primers list for *NQO1* isoforms and NRF2 expression. Related to STAR Methods

Primer	**Sequence**
GAPDH Fw	AGCCACATCGCTCAGACAC
GAPDH Rv	GCCCAATACGACCAAATCC
NQO1 Fw	CTCACCGAGAGCCTAGTTCC
NQO1 Rv	TCAGTGCTCTTCTGCCGAC
NQO1-ISO1 Fw	GCTGGTTTGAGCGAGTGTTC
NQO1-ISO1 Rv	CTGCCTTCTTACTCCGGAAGG
NQO1-ISO2 Fw	GACCCTTCCGGAGTGGCA
NQO1-ISO2 Rv	TGGAGTGTGCCCAATGCTAT
NQO1-ISO3 Fw	ACCTTGTGATATTCCAGAGTAAGAA
NQO1-ISO3 Rv	CTGGAGTGTGCCCAATGCTA
NQO1-ISO4 Fw	CCTTGTGATATTCCAGAGTGGC
NQO1-ISO4 Rv	CTGGAGTGTGCCCAATGCTA
NRF (NFE2L2) Fw	ATCCATTCCTGAGTTACAGTGTCT
NRF (NFE2L2) Rv	AGTTTGGCTTCTGGACTTGGA

Supplementary Table 6. Primer list for NQO1 sequencing. Related to STAR Methods

Primer	**Sequence**
NQO1_1F	GAAGTGTGTTGTATGGGCCC
NQO1_1R	GCTCATCCCAGGTCCCTAAT
NQO1_2-3F	CACAGAGCCCAGTTGTCAAC
NQO1_2-3R	ACATGCAACAGTAATACAGCCA
NQO1_4-6F	TGTTGATACCACACCTACTAAAGCA
NQO1_4-6R	GATGACTGCAGCAAAGAAGAGATTT

Supplementary Table 7. Primers list for NQO1 SNaPshot assay. Related to STAR Methods

Primer	**Sequence**
NQO1_R139W_F	CCTTCTGGGCTTGGGGAG
NQO1_R139W_R	AACAAACACCCCTGCATCAG
NQO1_R139W_SS	GCTGCCATGTATGACAAAGGACCCTTC
NQO1_P187S_F	CTTACCTCTCTGTGCTTTCTGTA
NQO1_P187S_R	GGAAGCCTGGAAAGATACCC
NQO1_P187S_SS	GACTGACTCTGCATTTCTGTGGCTTCCAAGTCTTAGAA