Supplementary data for ***134965-JCI-RG-2***

PRICKLE3 linked to ATPase biogenesis manifested Leber's hereditary optic neuropathy

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The supplemental data included the following information:

1. Supplemental Methods
2. Supplemental Figure 1, 2, 3, 4, 5, 6 and 7
3. Supplemental Table 1, 2, 3, 4 and 5
4. Unedited gel image

1. Supplemental Methods

Quantification of mtDNA copy number (Related to supplemental Figure 4)

Total genomic DNAs were extracted with the MiniBEST Universal Genomic DNA Extraction Kit (TaKaRa, Tokyo) from mutant and control cell lines. Fifty ngs of genomic DNA were used in each QPCR reaction using Universal SYBR Green Master (Roche Applied Science, Mannheim) with two pairs of primers for mtDNA (300 nM each) in a 7900 HT Fast Real-time PCR System (Applied Biosystems, Forest City) with one pair of primers for *β*-actin for normalization. The following primers were used: MT-D-loop region: 5′-TCACCCTATTAACCACTCA-3′ (sense) and 5′-AGACAGATACTGCGACATA-3′ (anti-sense); MT-ND1: 5′-CACCCAAGAACAGGGTTTGT-3′ (sense) and 5′-TGGCCATGGGTATGTTGTTAA-3′ (anti-sense); *β*-Actin: 5′-TCACCCACACTGTGCCCATCTACGA-3′ (sense) and 5′-CAGCGGAACCGCTCATTGCCAATGG-3′ (antisense). mtDNA contents were calculated using the ΔΔCt method whereby all MT-D-loop and MT-ND1 (mtDNA target) Ct values were first normalized to *β*-actin. Data from multiple experiments were analyzed using the procedure as described elsewhere (61).

Assessment of mitochondrial membrane potential (Related to supplemental Figure 5)

Mitochondrial membrane potential was assessed with JC-10 Assay Kit-Microplate (Abcam, Cambridge, MA) following general manufacturer's recommendations with some modifications (68,73). In brief, cells were plated onto 96-well cell culture plate overnight in growth medium. JC-10 dye-loading solution was added for 30 min at 37°C in the presence of 5% CO_2. Alternatively, plated cells were preincubated with 10 μM of the protonophore uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP) for 30 min at 37°C, 5% CO_2 prior to staining with JC-10 dye. The fluorescent intensities for both J-aggregates and monomeric forms of JC-10 were measured at Ex/Em = 490/530 and 490/590 nm with a microplate reader (Syneregy H1, Bio-Tek, Winooski).

Measurement of mitochondrial ROS production (Related to supplemental Figure 5)

The levels of mitochondrial reactive oxygen species (ROS) generation were determined using MitoSOX assay as detailed previously (74-75). Briefly, approximate 2×10^6 cells of each cell line were harvested, resuspended in 5 μM MitoSOX reagent working solution and then incubated at 37◦C for 20 min. After washing with PBS twice, cells were resuspended in PBS in the presence of 2 mM freshly prepared H_2O_2 and 2% FBS and then incubated at room temperature for another 45 min. Cells were further washed with PBS and resuspended with 1 mL of PBS with 0.5%

paraformaldehyde. Samples with or without H_2O_2 stimulation were analyzed by BD-LSR II flow cytometer system (Beckton Dickson, Inc., Franklin Lakes), with an excitation at 488 nm and emission at 529 nm. Ten thousand events were analyzed in each sample.

Generation of *PRICKLE3* knocking down cells (Related to supplemental Figure 6)

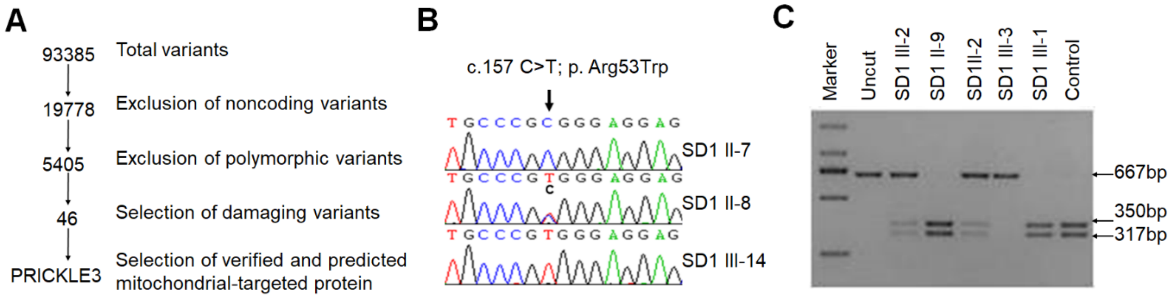
HeLa cells were cultured in DMEM, supplemented with 10% (FBS). The shRNA oligo primers targeting to PRICKLE3 were: Forward, 5' CCGG AA GCT TCG CGC CGT AGT CTT A CTCGAG T AAG ACT ACG GCG CGA AGC TT TTTTTG3', Reverse, 5' AATTCAAAAA AA GCT TCG CGC CGT AGT CTT A CTCGAG T AAG ACT ACG GCG CGA AGC TT 3'. Pairs of shRNA were cloned into PLKO.1 TRC (Addgene, Watertown). Lentiviral vectors, pLKO.1 with shRNA or scramble shRNA, were co-transfected into HeLa cells with psPAX2 and pMD2.G for lentivirus production. Complete medium was changed after 4-6 hours transfection. Virus was collected 48 hours after transfection and centrifuged at 12,000 g. Cells were cultured in medium containing virus for 48 hours. After selection with puromycin at 1μg/ml concentration for another 48 hours, the cells were subjected to the Western blot analysis to examine the level of PRICKL3 before using for various assays.

References:

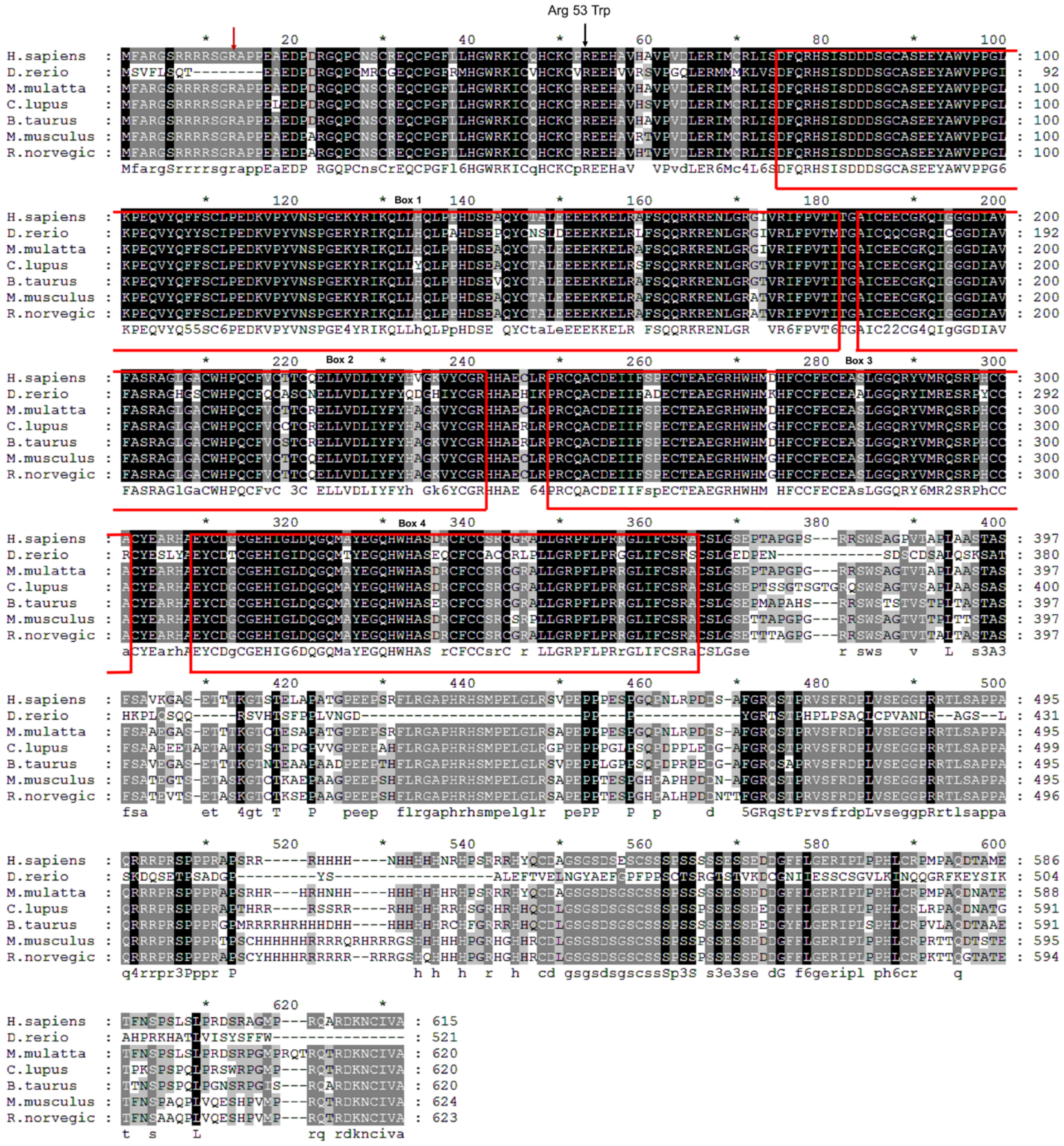
73. Reers M, Smiley ST, Mottola-Hartshorn C, Chen A, Lin M, Chen LB. Mitochondrial membrane potential monitored by JC-1 dye. *Methods Enzymol*. 1995; 260: 406–417

74. Mahfouz R, et al. Evaluation of chemiluminescence and flow cytometry as tools in assessing production of hydrogen peroxide and superoxide anion in human spermatozoa. *Fertil Steril*. 2009; 92(2): 819-827.

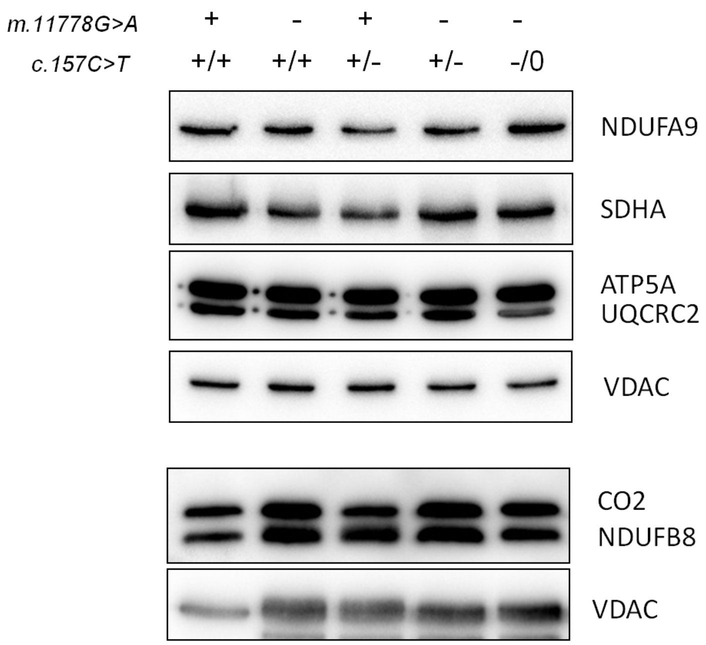
75. Jia Z, et al. A coronary artery disease-associated tRNA^{Thr} mutation altered mitochondrial function, apoptosis and angiogenesis. *Nucleic Acids Res*. 2019;47(4):2056-2074.



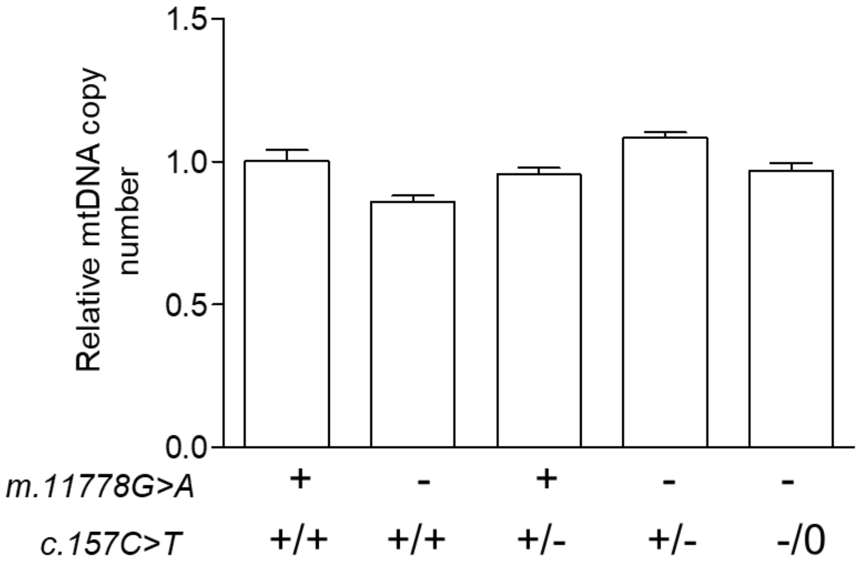
Supplemental Figure 1. Identification of c.157C>T (p.Arg53Trp) mutation in *PRICKLE3* gene. (**A**) Summary of whole exome sequencing of the proband (SD1 III-14) bearing the m.11778G>A mutation. The identified single nucleotide variant (SNV) c.157C>T (p.Arg53Trp) is located in *PRICKLE3*, a gene encoding a mitochondrial protein linked to biogenesis of ATPase. (**B**) Partial sequence chromatograms of *PRICKLE3* gene. Sanger sequencing of affected individuals II-8, III-14 and a married-in-control individual (II-7) of the SD1 family. The arrow indicates the location of the nucleotide changes at position 157. (**C**) RFLP analysis for the c.157C>T mutation in some members of SD1 family. Genotyping for the c.157C>T mutation in other subjects was PCR amplified for exon 3 of *PRICKLE3* and followed by digestion of the 667-bp segment with the restriction enzyme *Sac*II.



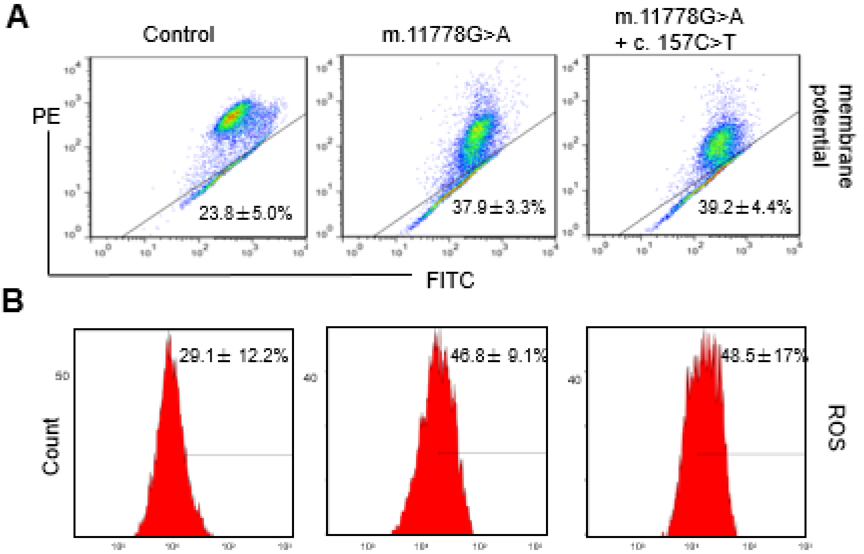
Supplemental Figure 2. Alignments of the amino acid sequences of PRICKLE3 family among different species. Residues shaded in black are completely conserved across all species, and residues shaded in gray are similar with respect to side chains. The dashes in the amino acid sequences indicate gaps introduced to maximize alignment. The red arrow indicates the potential cleavage site of mitochondrial targeting sequence (MTS), the position of p.Arg53Trp mutation is marked with a dark arrow. Protein accession numbers: *Homo sapiens* (A0A0A0MRT7); *Danio rerio* (F1Q568); *Macaca mulatta* (F6VGC9); *Canis lupus* (F1Q4H0); *Bos Taurus* (F6QKQ1); *Mos musculus* (Q8BNH2); *Rattus norvegic* (Q5U2Q0).



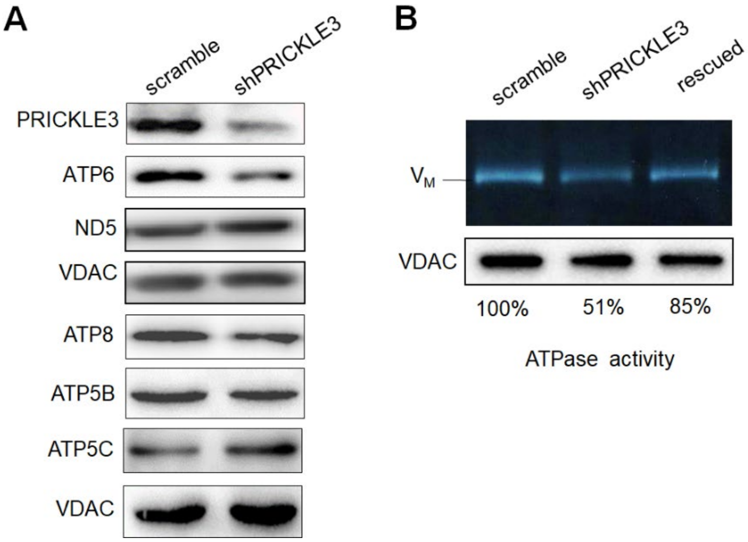
Supplemental Figure 3: Western blot analysis of mitochondrial proteins. Five micrograms of mitochondrial proteins from various cell lines were electrophoresed through a denaturing polyacrylamide gel, electroblotted and hybridized with the following antibodies: NDUFA9 and NDUFB8, subunits of complex I; SDHA, subunit of complex II; UQCRC2, subunit of complex III; CO2, subunit of complex IV; ATP5A, subunits of complex V and with VDAC as a loading control, respectively.



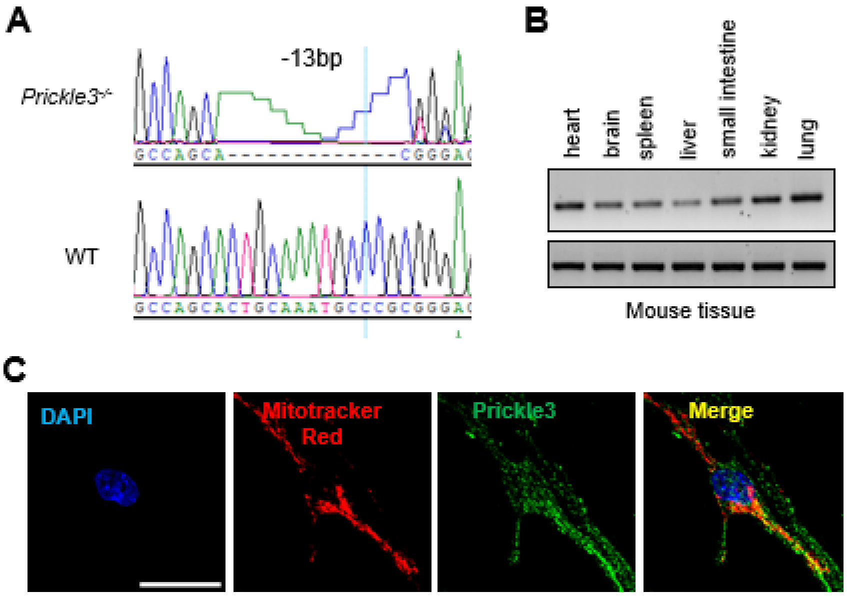
Supplemental Figure 4: Measurement of mtDNA copy numbers from various cell lines by real-time PCR (Supplemental methods). Mitochondrial DNAs were normalized to β-actin encoded by nuclear gene. n=6. Data were presented as the mean ± SEM of duplicates.



Supplemental Figure 5 (related to Figure 2): Mitochondrial membrane potential and ROS production assays from mutant and control cell lines**.** (**A**) Mitochondrial membrane potential analysis. The mitochondrial membrane potential (ΔΨm) was measured in various cell lines using a fluorescence probe JC-10 assay system. The percentage of cells count in different gate was presented. (**B**) Mitochondrial reactive oxygen species production. The rates of production in ROS from different cell lines were stained with mitochondrial ROS specific dye MitoSOX and then analyzed by BD-LSR flow cytometer system.



Supplemental Figure 6 (related to Figure 3). ***PRICKLE3*-silencing Hela cells exhibited the ATP synthase deficiency**. (A). Western blot analysis of various subunits of oxidative phosphorylation complexes, with VDAC serving as a loading control. (B) In-gel activity of ATPase. Hela cells were transfected by shRNA of *PRICKLE3* and scrambled shRNA as control. WT-PRICKLE3 was overexpressed in sh-PRICKLE3 cell line.



Supplemental Figure 7 (related to Figure 4): *Prickle3* expression and subcellular locations in mouse tissues and cells. (A) Mouse genotyping by Sanger sequencing: 13 bp deletion in *Prickle3* knock-out mice. **(B)** Expression profile of *Prickle3* in various tissues of mice with 8 week old by RT-PCR. **(C)** Distribution of Prickle3 in mouse embryonic fibroblast cells (MEF). Cells are stained with Mitotracker-Red before fixation. Mitotracker-Red, red; Prickle3, green; DAPI, blue. Bar, 30 μm.

Supplemental Table 1 (related to Figure 1): Summary of clinical data for members of 3 Chinese LHON families carrying the m.11778G>A mutation

Subject	Gender	Age of test (year)	Age-of-onset (year)	Vision acuity (Right/Left eyes)	Level of vision impairment	m.11778G>A mutation	PRICKLE3 c.157C>T mutation
SD1-I-2	F	75	18	0.03/0.05	Severe	-	+/-
SD1-II-1	M	78		0.9/0.8	Normal	+	+/0
SD1-II-2	F	71	8	0.01/0.01	Profound	-	+/-
SD1-II-3	M	58		0.7/0.65	Normal	+	+/0
SD1-II-4	F	72	7	0.01/0.01	Profound	-	+/-
SD1-II-5	M	68		0.63/0.67	Normal	+	+/0
SD1-II-6	F	24	23	0.07/0.1	Moderate	-	+/-
SD1-II-7	M	56		1.1/1.2	Normal	+	+/0
SD1-II-8	F	53	9	0.08/0.05	Moderate	-	+/-
SD1-II-9	M	49		0.7/0.6	Normal	+	+/0
SD1-II-10	F	45		0.96/1.0	Normal	+	+/+
SD1-III-1	M	50		0.85/0.9	Normal	+	+/0
SD1-III-2	F	51	18	0.02/0.01	Profound	-	+/-
SD1-III-3	M	42	24	0.01/0.01	Profound	-	-/0
SD1-III-4	M	39	12	0.2/0.2	Mild	-	-/0
SD1-III-5	M	14	12	0.03/0.02	Severe	-	-/0
SD1-III-6	F	40		1.1/1.2	Normal	+	+/+
SD1-III-7	M	42		1.3/1.3	Normal	+	+/0
SD1-III-8	F	25	17	0.05/0.07	Moderate	-	+/-
SD1-III-9	M	41		0.85/1.1	Normal	+	+/0
SD1-III-10	F	20	20	0.05/0.10	Moderate	-	+/-
SD1-III-11	M	35		1.1/1.2	Normal	+	+/0
SD1-III-12	F	19	16	0.01/0.02	Profound	-	+/-
SD1-III-13	M	24	22	0.08/0.09	Moderate	-	-/0
SD1-III-14	M	22	15	0.02/0.03	Severe	-	-/0
SD1-III-15	F	29		0.8/0.7	Normal	+	+/+
SD1-III-16	M	25		1.1/1.1	Normal	+	+/0

SD1-III-17	M	24		1.2/1.0	Normal	+	+/0
SD1-IV-1	M	18		0.7/0.8	Normal	+	+/0
SD1-IV-2	F	16		0.7/0.7	Normal	+	+/+
SD1-IV-3	F	14		1.1/1.1	Normal	+	+/-
SD1-IV-4	M	13		1.1/1.1	Normal	+	+/0
SD1-IV-5	M	16		0.9/0.7	Normal	+	+/0
SD1-IV-6	F	12		0.6/0.8	Normal	+	+/+
SD1-IV-7	F	9		0.9/0.7	Normal	+	+/+
XT-I-1	F	49	18	0.08/0.09	Moderate	-	+/-
XT-II-1	M	49		1.1/1.2	Normal	+	+/0
XT-II-2	F	48	18	0.02/0.04	Severe	-	+/-
XT-II-3	M	42		1.1/1.2	Normal	+	-/0
XT-II-4	F	40		0.6/0.6	Normal	+	+/+
XT-II-5	M	39	16	0.04/0.03	Severe	-	-/0
XT-II-6	F	37		1.0/0.8	Normal	+	+/+
XT-III-1	M	24	15	0.03/0.03	Severe	-	-/0
XT-III-2	F	22	14	0.05/0.03	Severe	-	+/-
XT-III-3	M	20	18	0.08/0.08	Moderate	-	-/0
XT-III-4	M	18		0.6/0.7	Normal		+/0
XT-III-5	F	16	15	0.03/0.05	Severe	-	+/-
XT-III-6	F	16		1.1/1.1	Normal		+/-
XT-III-7	M	14		1.2/1.3	Normal		+/0
AH-I-1	M	60		0.7/0.6	Normal		-/0
AH-I-2	F	60		0.5/0.5	Normal		+/+
AH-II-1	M	48		0.7/0.7	Normal		+/0
AH-II-2	F	46	20	0.03/0.04	Severe	-	+/-
AH-II-3	M	40		1.5/1.5	Normal		+/0
AH-II-4	F	38		0.5/0.7	Normal		+/+
AH-III-1	M	20		0.5/0.8	Normal		+/0
AH-III-2	M	18	16	0.05/0.03	Severe	-	-/0
AH-III-3	M	16		0.6/0.6	Normal		+/0

F= female; M= male; The degree of visual impairment was defined according to the visual acuity as follows: normal > 0.3, mild=0.3-0.1; moderate = 0.1-0.05; severe = 0.05-0.02; and profound <0.02

Supplemental Table 2 (related to Figure 1): Summary of whole exome sequencing data in four members of the Chinese pedigree SD1 with LHON.

Categories	III-14	III-15	II-7	II-8
Number of genomic positions for calling SNPs	138,625,532	139,194,661	139,194,661	139,194,661
Number of high-confidence genotypes	120,046,406	121,69,859	120,950,249	119,965,391
Number of high confidence genotypes in TR	49,122,298	49,258,873	49,250,087	49,311,694
Total number of SNPs	93,385	92,360	92,319	92,255
Synonymous –coding	5,795	5,740	5,674	5,745
Missense	11,200	11,000	11,129	11,257
Nonsense	130	132	139	120
Readthrough	47	41	45	42
Splice site	2,606	2,580	2,554	2,628
Intron	51,974	51,506	51,440	50,608
5’ UTR	2,139	2,064	2,096	2,211
3’ UTR	4,982	4,984	4,895	5,049
Intergenic	3,227	3,099	3,188	3,144
Homozygous	36,420	36,882	33,364	35,900
Heterozygous	56,965	55,478	58,955	56,355
Frame error	0	0	0	0

Supplemental Table 3. Oligonucleotides for *PRICKLE3* analysis

Primer names	**Sequence (5'-3')**	**Description**
exon primers		
PK3-EXON1F	AAAACGTCAACACAAGCACCCT	Sequencing
PK3-EXON1R	CACCTATTCGCTATGATGCCTATCC	Sequencing
PK3-EXON2F	AGTAGGGGTTCTGTGCTCTAGTG	Sequencing
PKE-EXON2R	GCTACGGGAGAACACTTCTGG	Sequencing
PK3-EXON3F	AGAGCCTCACCACAGGTTAGATG	Sequencing
PK3-EXON3R	TAATAGGATTGGGGTGAACAGGC	Sequencing
PK3-EXON45F	TGAGGTCAATACATGCAGGAACC	Sequencing
PK3-EXON45R	TCCACCAGGGGTTGCCTTAGAG	Sequencing
PK3-EXON67F	GAGCAGGGTCAGTCTCTAAGGTAC	Sequencing
PK3-EXON67R	TAAAGGCTAGGAGTAGGGCGAG	Sequencing
PK3-EXON8F	GGATTCCCCACCTCTTCTTGAG	Sequencing
PK3-EXON8R	TGTATTGACAGGGTGGAACCAG	Sequencing
PK3-EXON9F	TCCTTCCAACCCTCTCAACACCCA	Sequencing
PK3-EXON9R	GGAACGCTGACACGAGCCTGGAGT	Sequencing
RT- PCR		
PRICKLE3F	GGGCCATCTGTGAGGAGTG	mRNA
PRICKLE3R	CGCAGTAGACCTTGCCAACAT	mRNA
18SF	AGTCCCTGCCCTTTGTACACA	
18SR	CGATCCGAGGGCCTCATA	
c.157C>T mutation screening		
PK3-157-F	AGCCCACAAGTTCAGAGCCTCAC	mutational screening
PK3-157-R	GGAACTGGTCCCTCAGTCACCCAAG	

cDNA cloning	
PRICKLE3- cDNA -F	ATGTTCGCGCGTGGGT
PRICKLE3- cDNA -R	TCAAGCCACGATGCAGTT
PRICKLE3 HA plasmid construction	
PRICKLE3-HA-F	CGGAATTCCGATGTTCGCGCGTGGGT
PRICKLE3-HA-R	CGCGGATCCGCGAGCCACGATGCAGTT
Mice *Prickle3* genotyping	
gRNA	GATCACTAATAGGACTCACTATAGGACAGCATGCTCCTCCCGCGTTTTAGAGCTAGAAAT
Donor oligo	CTCCTATCCTGGCTCTCACAGAAAGATCTGCCAGCACTGCAAATG**T**CC**AT**GGGAGGAGCATGCTGTACGCACTGTACCTGTGGACCTAGAACGCATCAT
mPk3-Sacii-F	TGGTCACAAGGCAAAGTCA
mPk3-Sacii-R	TCCAACAAGTGGCGAATG
ShRNA primers for *PRICKLE3*	
PLKO.1 Scramble-F	CCGG AA GCT TCG CGC CGT AGT CTT A CTCGAG T AAG ACT ACG GCG CGA AGC TT TTTTTG
PLKO.1 Scramble-R	AATTCAAAAA AA GCT TCG CGC CGT AGT CTT A CTCGAG T AAG ACT ACG GCG CGA AGC TT
PLKO.1 seq-F	CCGG AA GCC TGT GAC GAG ATC ATC TTC CTCGAG GAA GAT GAT CTC GTC ACA GGC TT TTTTTG
PLKO.1 seq-R	AATTCAAAAA AA GCC TGT GAC GAG ATC ATC TTC CTCGAG GAA GAT GAT CTC GTC ACA GGC TT

Supplemental Table 4. mtDNA variants in 3 Han Chinese probands with LHON

Gene	Position	Replacement	AA change	[a]Conservation (H/B/M/X)	[b]CRS	SD1	AH1	XT1	[c]Previously reported
D-loop	73	A-G			A	G	G	G	Yes
	189	A-G			A			G	Yes
	195	T-C			T	C			Yes
	249	A-del			A	A-del			Yes
	263	A-G			A		G	G	Yes
	309	C-CC			C		CC		Yes
	310	T-C						C	Yes
	310	T-TC			T	TC			Yes
	310	T-CTC			T		CTC		Yes
	489	T-C			T	C		C	Yes
	522	C-del			C	C-del			Yes
	523	A-del			A	A-del			Yes
	16093	T-C			T	C			Yes
	16129	G-A			G	A			Yes
	16183	A-C			A		C		Yes
	16184	C-T			C			T	Yes
	16189	T-C			T	C	C		Yes
	16217	T-C			T		C		Yes
	16223	C-T			C			T	Yes
	16298	T-C			T			C	Yes
	16319	G-A			G			A	Yes
	16519	T-C			T	C	C		Yes
12S rRNA	750	A-G			A	G	G	G	Yes
	827	A-G			A		G		Yes
	1438	A-G			A	G	G	G	Yes
16S rRNA	1715	C-T			C	T			Yes
	2706	A-G			A	G	G		Yes
	2835	C-T			C			T	Yes
ND1	3552	T-A			T	A			Yes
ND2	4715	A-G			A	G		G	Yes
	4769	A-G			A		G	G	Yes
CO1	6026	G-A			G	A			Yes
	6179	G-A			G			A	Yes
	7001	A-G			A	G			Yes
	7028	C-T			C	T	T	T	Yes
CO2	7196	C-A			C	A		A	Yes
	7999	T-C			T	C			Yes
NC7	8271_9	9bp Del					9bp Del		Yes
ATP6	8530	A-G	Asn-Asp	N/N/N/N	A		G		Yes
	8584	G-A	Ala-Thr	A/V/V/I	G	A		A	Yes
	8684	C-T	Thr-Ile	T/V/I/F	C			T	Yes
	8701	A-G	Thr-Ala	T/S/L/Q	A	G		G	Yes
	8860	A-G	Thr-Ala	T/A/A/T	A	G	G	G	Yes
CO3	9540	T-C			T	C		C	Yes
	9545	A-G			A	G			Yes

ND3	10398	A-G	Thr-Ala	T/T/T/A	A	G		G	Yes
	10400	C-T			C	T		T	Yes
	10668	G-A			G	A			Yes
	10873	T-C			T	C		C	Yes
ND4	11719	G-A			G	A	A	A	Yes
	11778	**G-A**	**Arg-His**	**R/R/R/R**	**G**	**A**	**A**	**A**	**Yes**
	11914	G-A			G	A	A		Yes
	11969	G-A	Ala-Thr	A/A/G/A	G	A			Yes
ND5	12705	C-T			C			T	Yes
	12672	A-G			A	G			Yes
	12705	C-T			C	T			Yes
ND5	12732	T-C			T		C		Yes
	13263	A-G			A	G			Yes
	13942	A-G	Thr-Ala	T/L/I/M	A		G		Yes
	14318	T-C	Asn-Ser	N/N/D/S	T	C			Yes
ND6	14470	T-C			T			C	Yes
Cytb	14766	C-T	Thr-Ile	T/S/T/S	C	T	T	T	Yes
	14783	T-C			T	C		C	Yes
	15038	A-G	Ile-Val	I/V/V/I	A		G		Yes
	15043	G-A			G			A	Yes
	15301	G-A			G			A	Yes
	15326	A-G	Thr-Ala	T/M/I/I	A		G	G	Yes
	15487	A-T			A			T	Yes
	15535	C-T			C		T		Yes
tRNA^{Thr}	15930	G>A		G/G/A/G	G		A		Yes

[a] Conservation of amino acid for polypeptides in human (H), bovine (B), mouse (M), and Xenopus (X).

[b] CRS: Cambridge reference sequence.

[c] See the online mitochondrial genome database MITOMAP

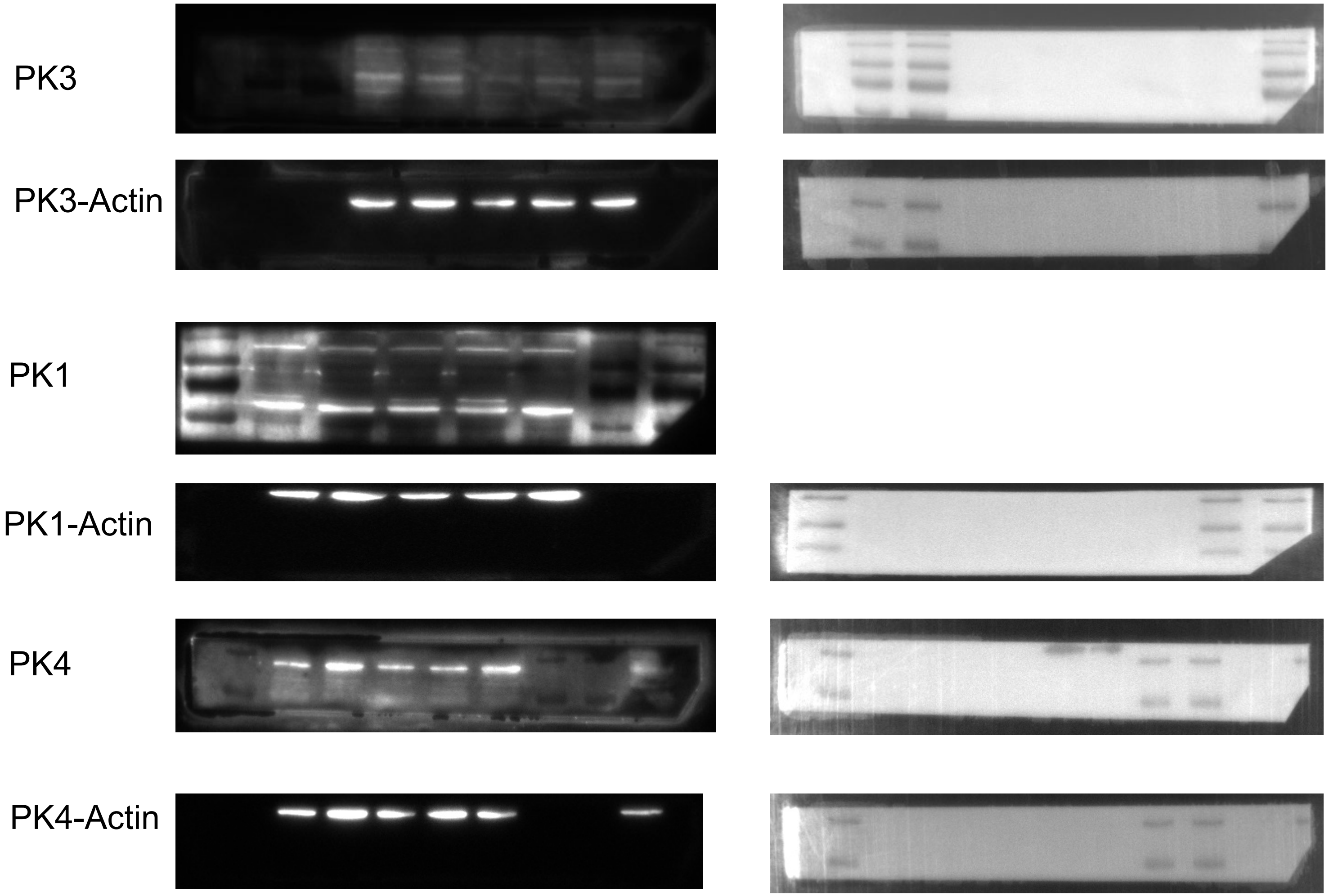
Supplemental Table 5. KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
TOMM20	Abcam	Cat# ab56783
UQCRC2	Abcam	Cat# ab14745
PRICKLE1	Proteintech	Cat# 22589-1-AP
PRICKLE3	Abcam	Cat# ab183522
PRICKLE3	Proteintech	Cat# 19098-1-AP
PRICKLE4	Abcam	Cat# ab119773
β-actin	Abcam	Cat# ab8226
ATP6	Proteintech	Cat# 55313-1-AP
ATP8	Proteintech	Cat# 26723-1-AP
ATP5A	Proteintech	Cat# 14676-1-AP
ATP5B	Proteintech	Cat# 17247-1-AP
ATP5C	Proteintech	Cat# 60284-1-IG
ATP5F	Proteintech	Cat# 15999-1-AP
ATPAF1	Proteintech	Cat# 18016-1-AP
GAPDH	Proteintech	Cat# 60004-1-Ig
VDAC	Proteintech	Cat# 55259-1-AP
NF-H antibody	Cell Signaling Technology	Cat# 2836
HA-tagged monoclonal antibody	Thermo fisher scientific	Cat# 26183
ATP Synthase Immunocapture Kit	Abcam	Cat# ab109715
Goat anti mouse IgG(H+L) (HRP)	Beyotime	Cat# A0216
Goat anti rabbit IgG(H+L) (HRP)	Beyotime	Cat# A0208
Goat anti-mouse IgG H+L (Alexa Fluor 488)	Abcam	Cat# ab150113
Goat anti-rabbit IgG H+L (Alexa Fluor 594)	Abcam	Cat# ab150080
Donkey anti-rabbit IgG H+L (Alexa Fluor 647)	Abcam	Cat# ab150075
Critical Commercial Assays		
CellTiter-Glo® Luminescent Cell Viability Assay kit	Promega	Cat# G7571
MitoSOX™ Red Mitochondrial Superoxide Indicator, for live-cell imaging	Invitrogen	Cat# M36008
JC-10 Mitochondrial Membrane Potential Assay Kit (Flow Cytometry)	Abcam	Cat# ab112133
TRIzol reagent	Invitrogen	Cat# 15596018
PrimeScript II 1st strand cDNA synthesis Kit	TaKaRa	Cat# 6210A
Chemicals and plasmids		
Antimycin A	Sigma	Cat# A8674-50MG

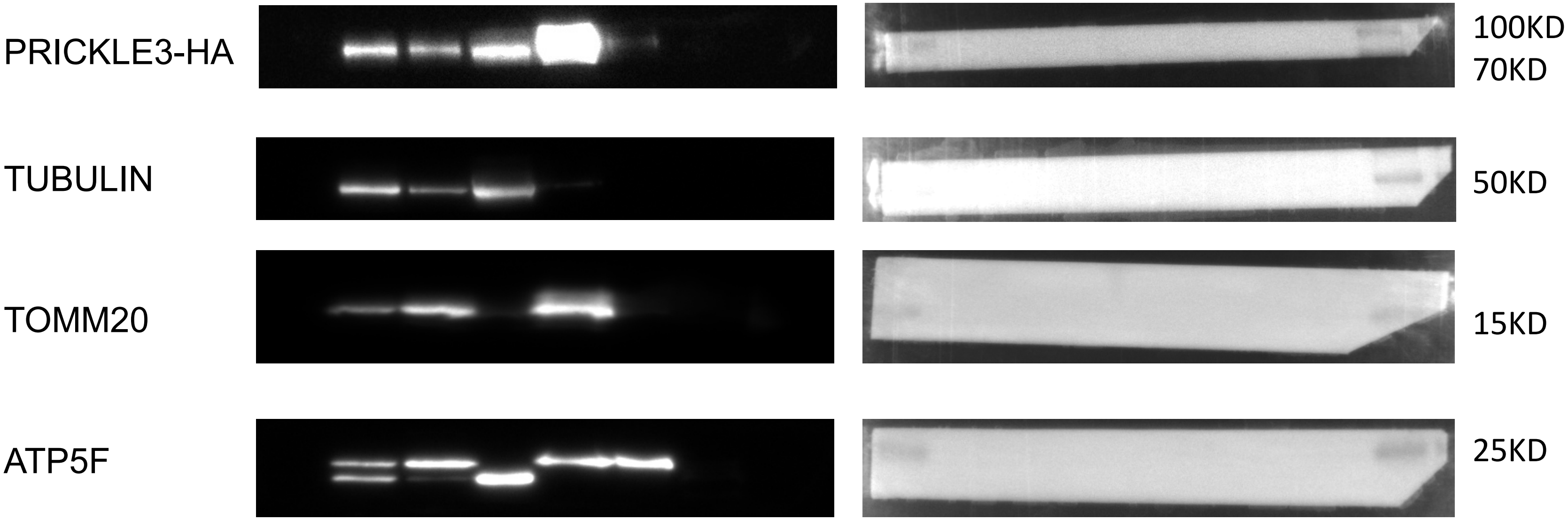
Oligomycin	Sigma	Cat# 495455-10MG
FCCP	Sigma	Cat# C2920-10MG
Rotenone	Sigma	Cat# 45656-250MG
pGEM-TEASY vector	Promega	Cat# A1360
2-DG	Sigma	Cat# D8375-1G
Software		
Annotation Variants annotation tools		http://wannovar.usc.edu/
1000 Genomes		http://www.1000genomes.org/
ExAC (Exome Aggregation Consortium)		http://exac.broadinstitute.org/
ESP (Exome Sequencing Project)		http://evs.gs.washington.edu/EVS/
SIFT		http://sift.jcvi.org/
PolyPhen2		http://genetics.bwh.harvard.edu/pph2/
CADD		http://cadd.gs.washington.edu/
dbNSFP		http://varianttools.sourceforge.net/Annotation/DbNSFP
BWA (Burrows-Wheeler Aligner)		https://github.com/lh3/bwa
GATK (Genome Analysis Toolkit)		https://www.broadinstitute.org/gatk/
Picard		http://broadinstitute.github.io/picard/
SOAPSNP (Short Oligonucleotide Analysis Package SNP)		http://soap.genomics.org.cn/.
Mitoprot		https://ihg.gsf.de/ihg/mitoprot.html
GraphPad Prism7	GraphPad Software	
Microsoft-Excel	Microsoft	N/A
7900 Real-Time PCR Software	Applied Biosystems	N/A
Olympus Fluoview Ver.4.0a Viewer	Olympus	N/A
Oligonucleotides		
Primers	This paper	See Table S3
Other		
Synergy H1 hybrid Reader	Biotek	N/A
Seahorse XF96 Extracellular Flux Analyzer	Angilent	N/A
ERG		N/A
Phoenix Image-Guide OCT2	Phoenix	N/A
Phoenix Micron Light Source	Phoenix	N/A

4. Unedited gel images

Full unedited gel for Figure 1C

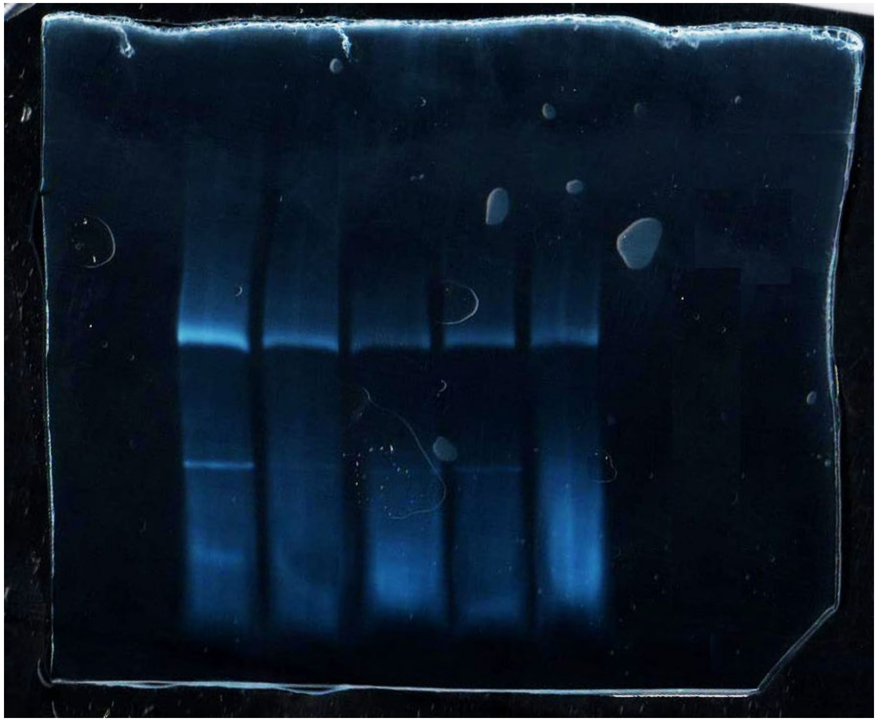


Full unedited gel for Figure 1D

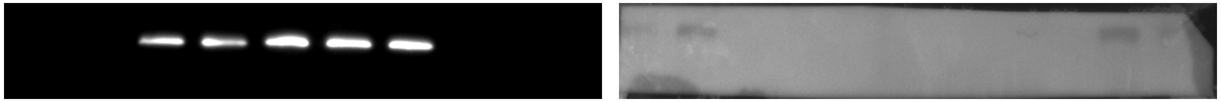


Full unedited gel for Figure 2B

In-gel activity

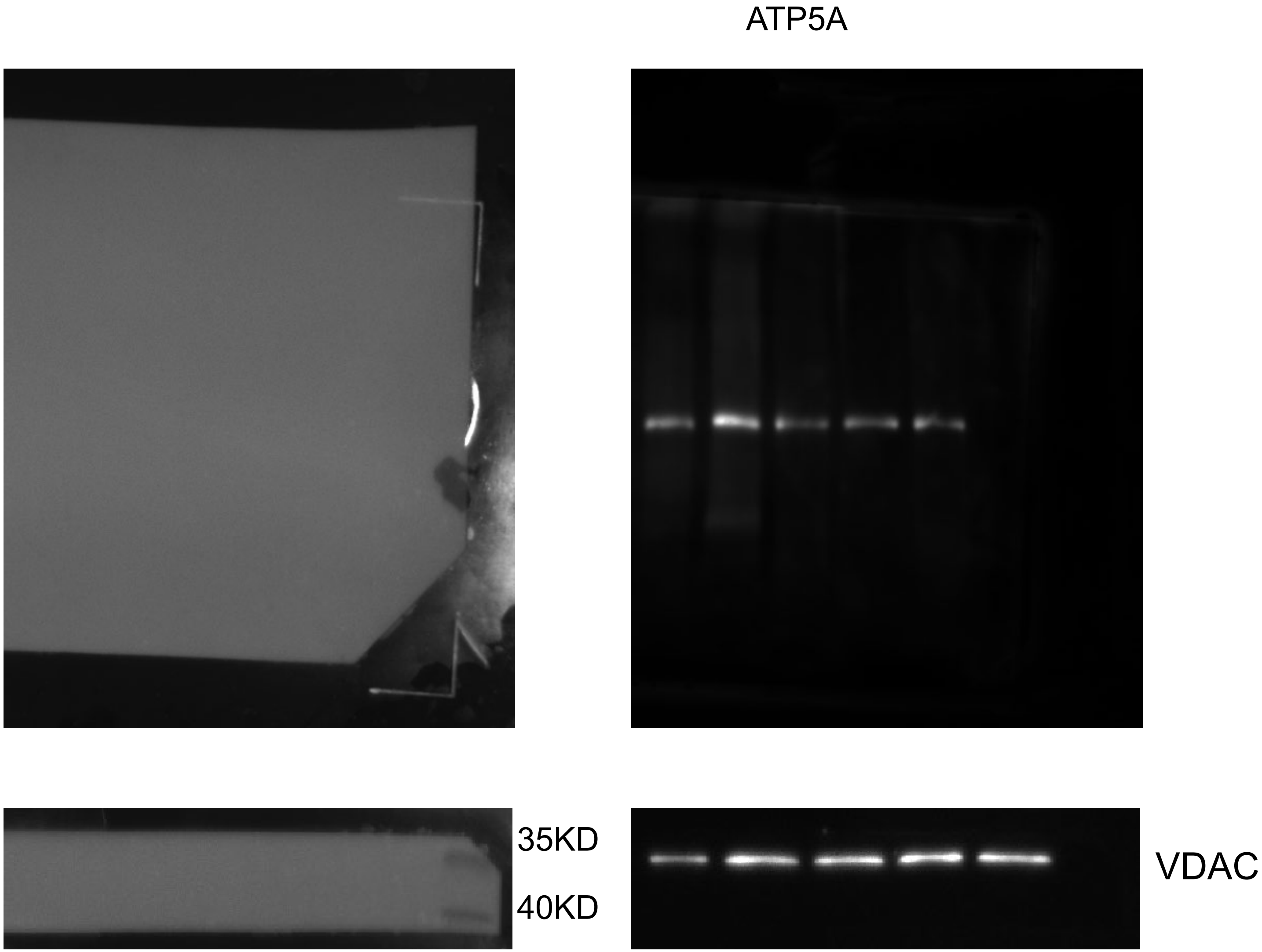


VDAC

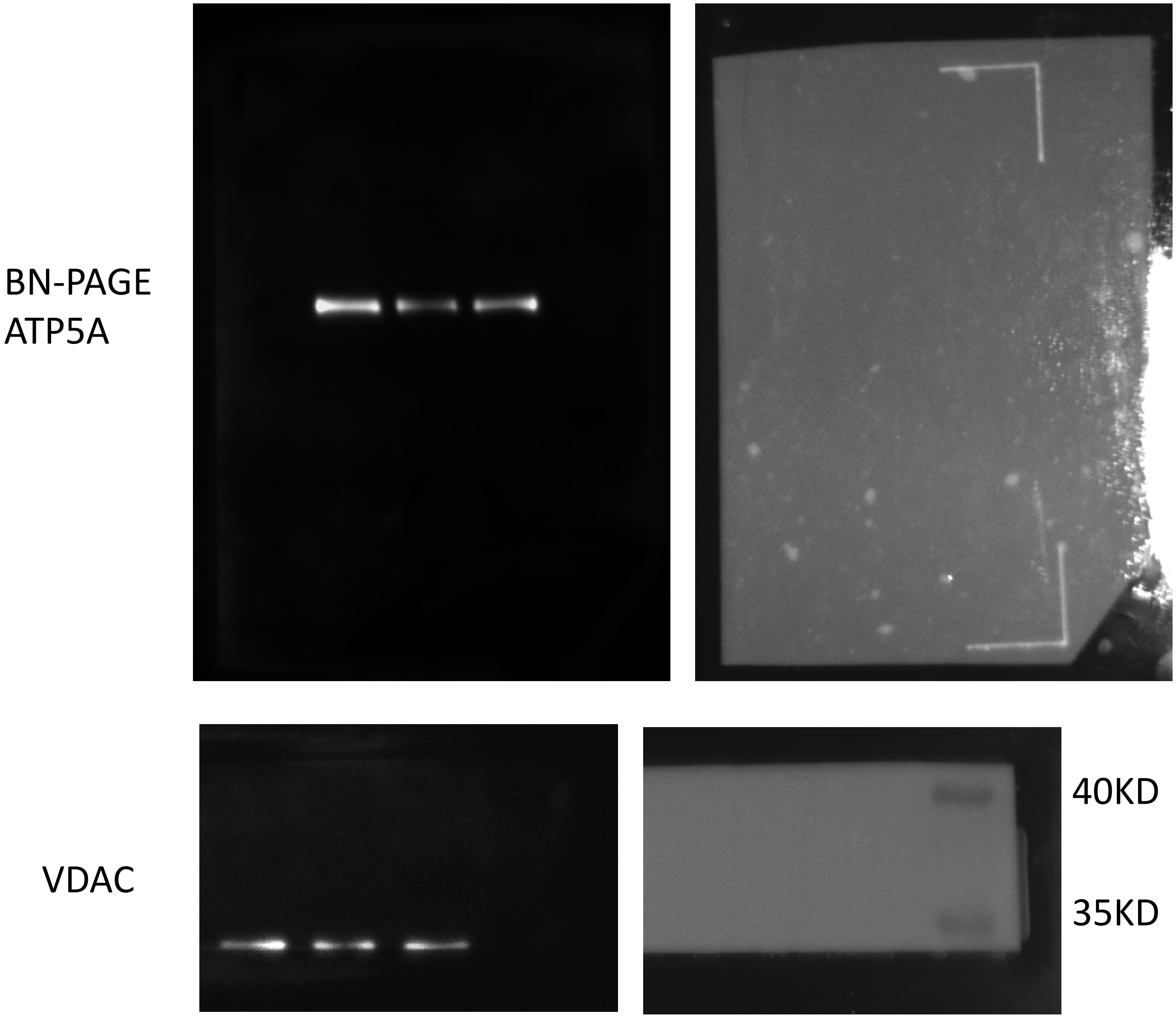


40KD

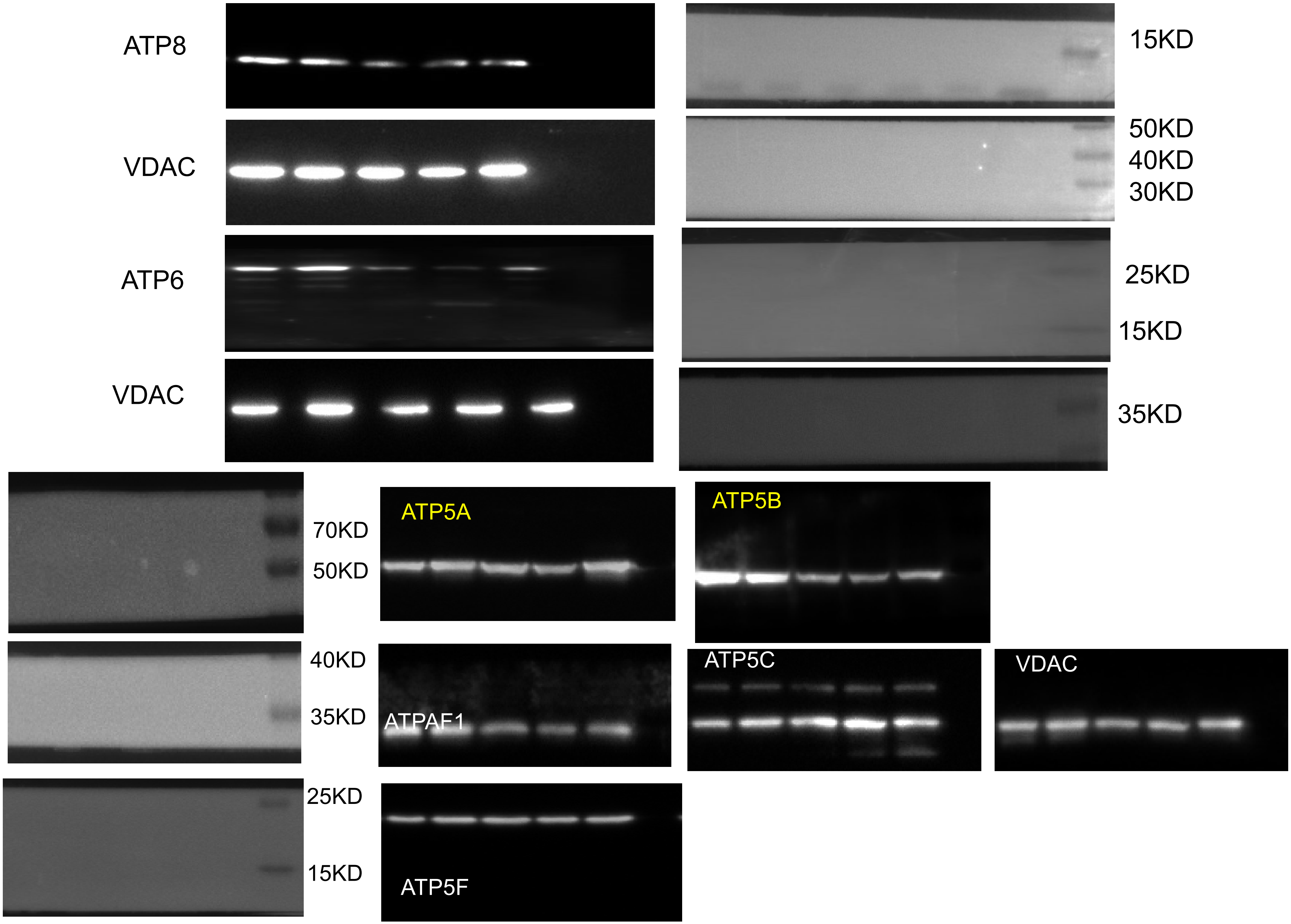
Full unedited gel for Figure 3A



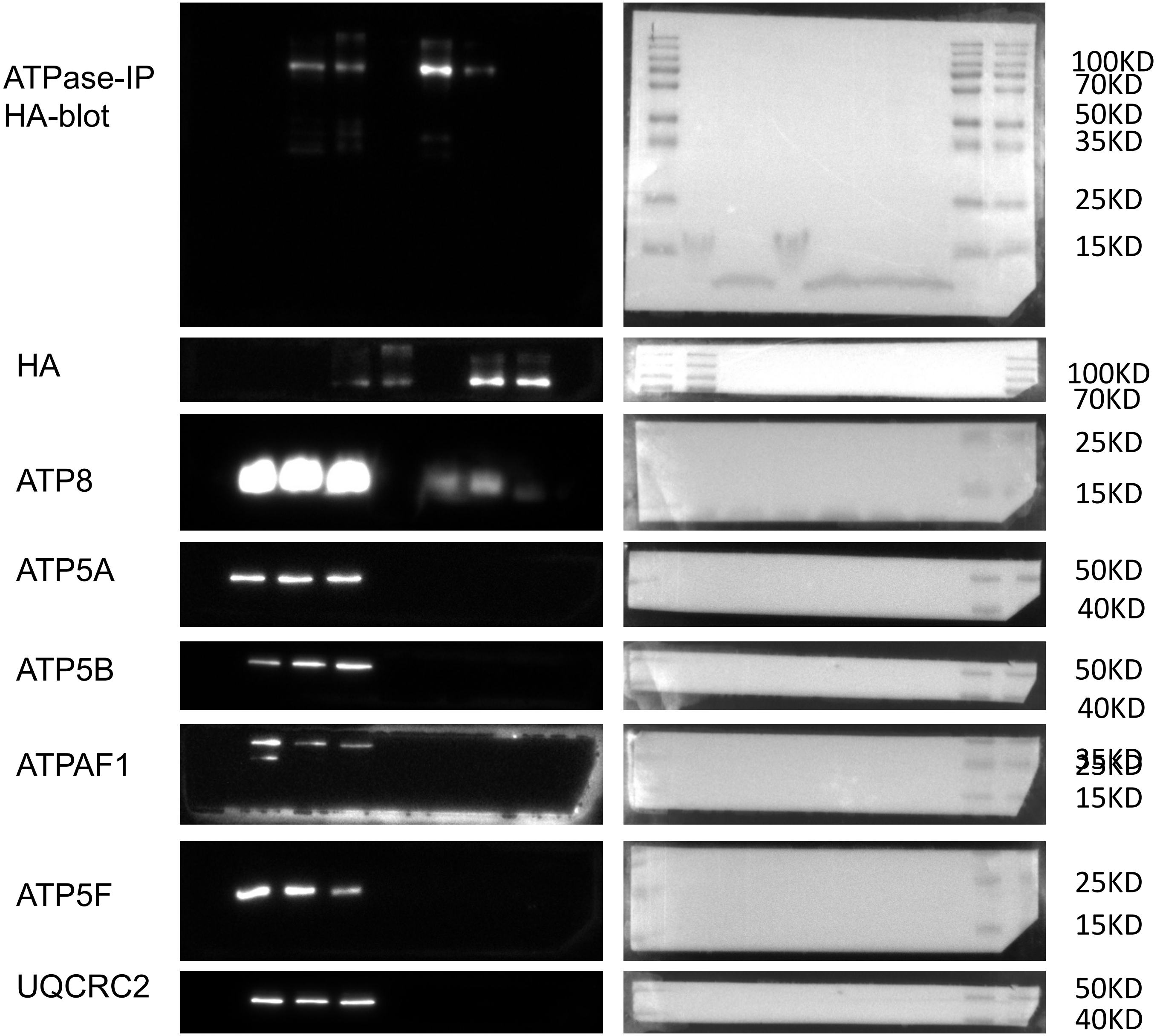
Full unedited gel for Figure 3B



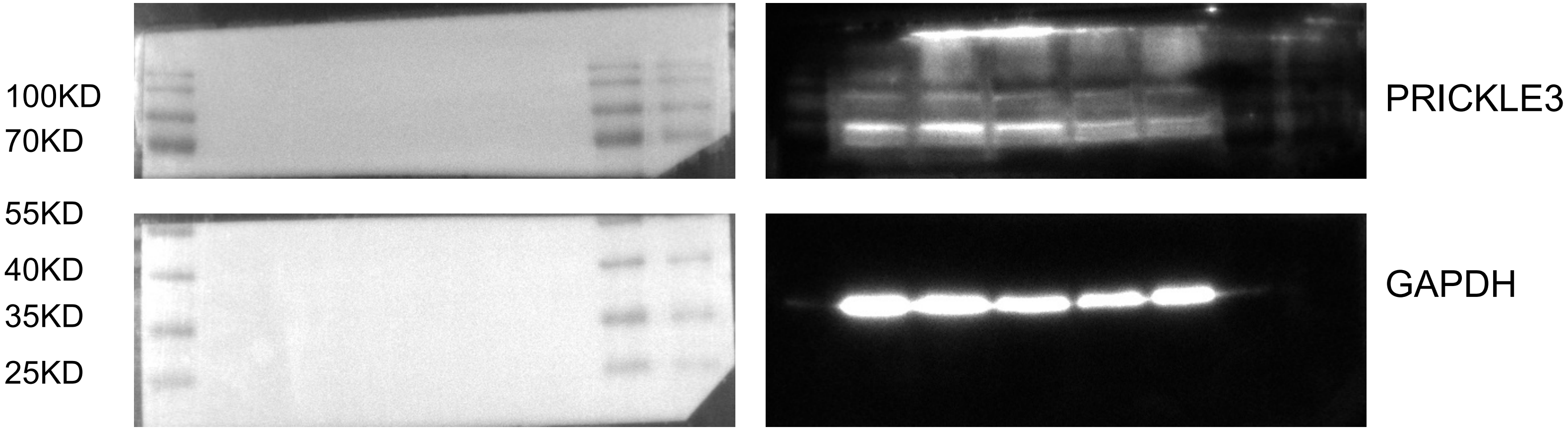
Full unedited gel for Figure3C



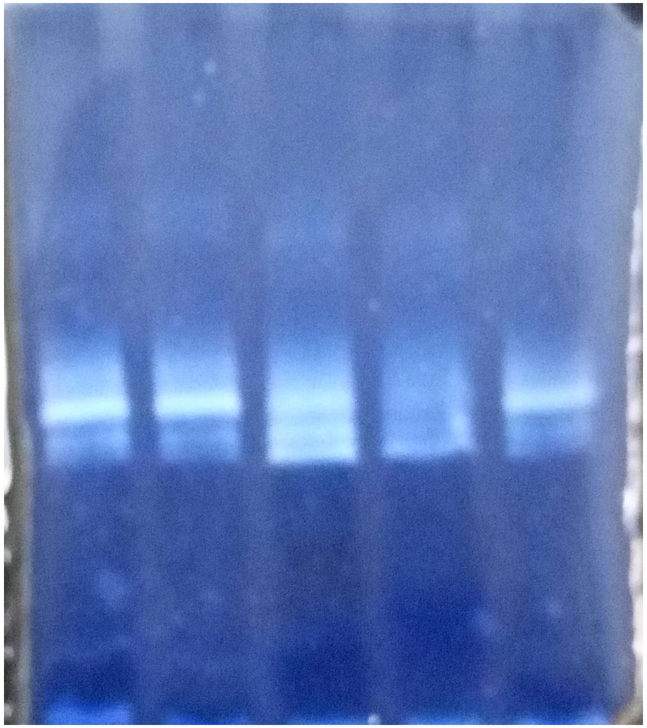
Full unedited gel for Figure 3D

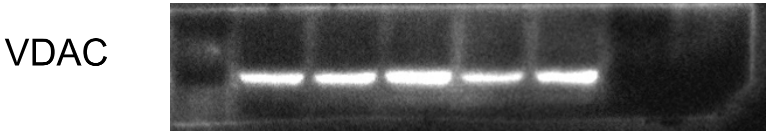


Full uneditied gel for Figure 4B

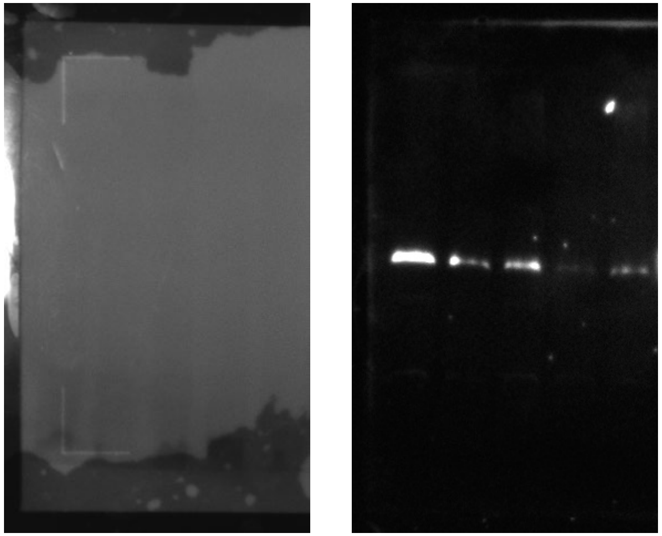


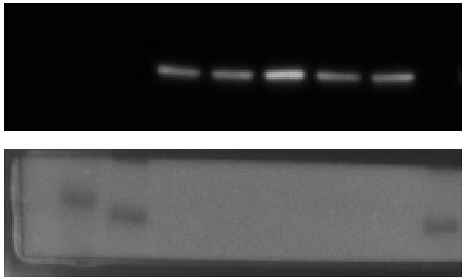
Full unedited gel for Figure 4E



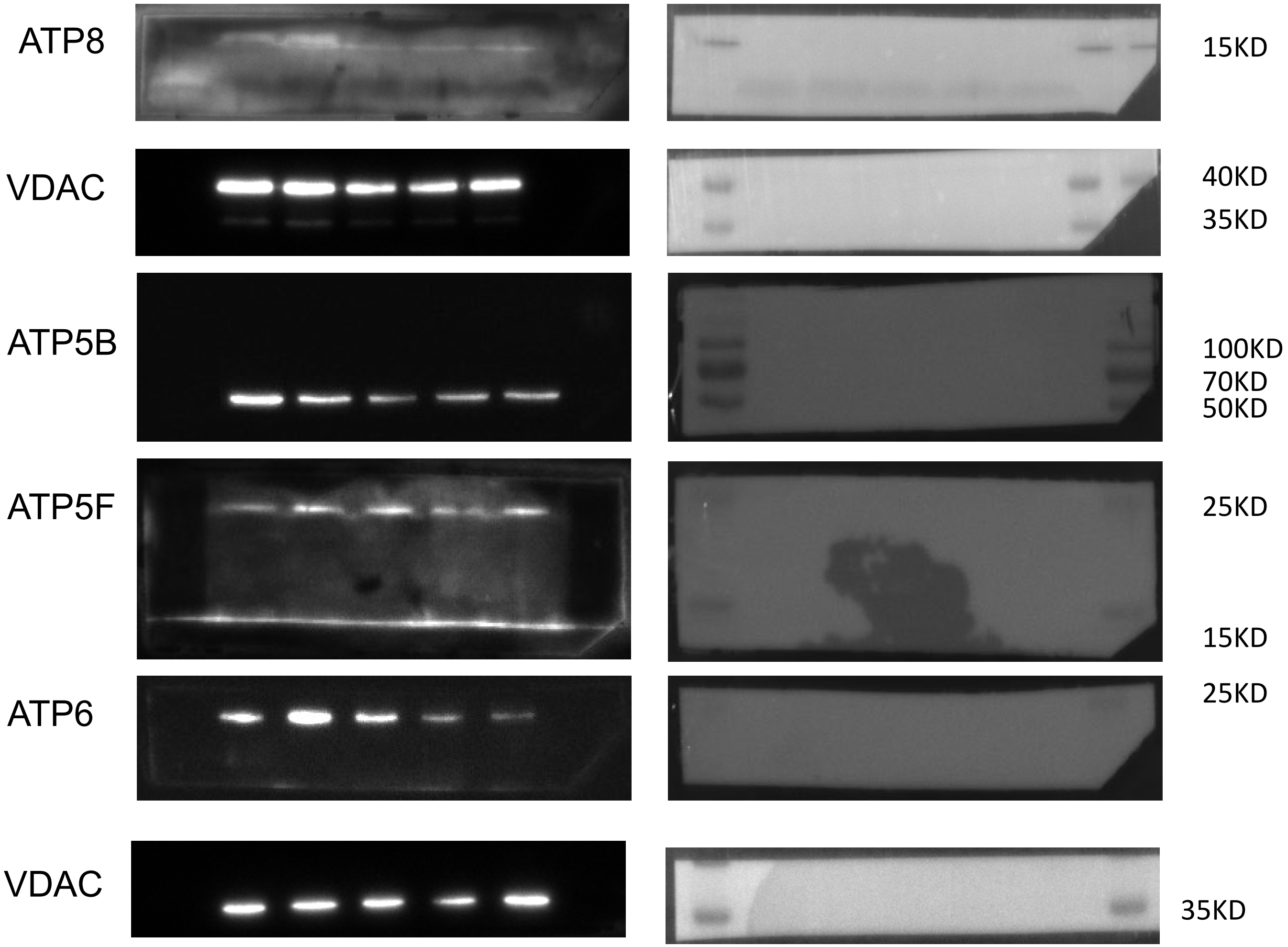


Full unedited gel for Figure 4F



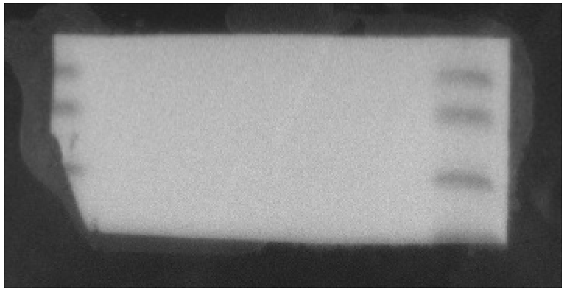


Full unedited gel for Figure 4G

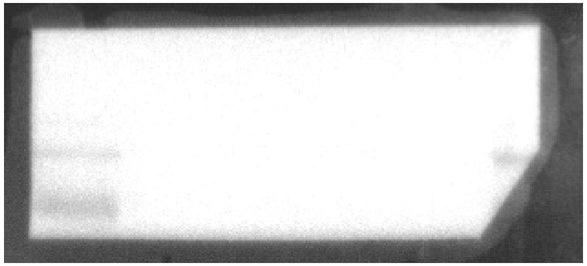


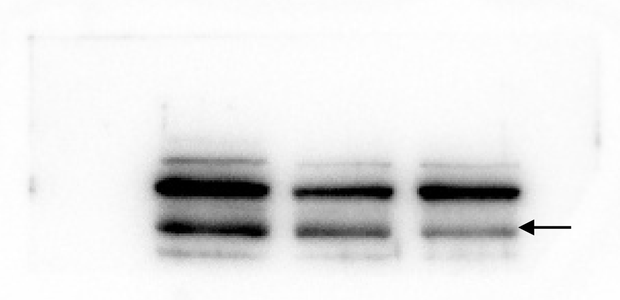
Full unedited gel for Figure 5E

Gapdh

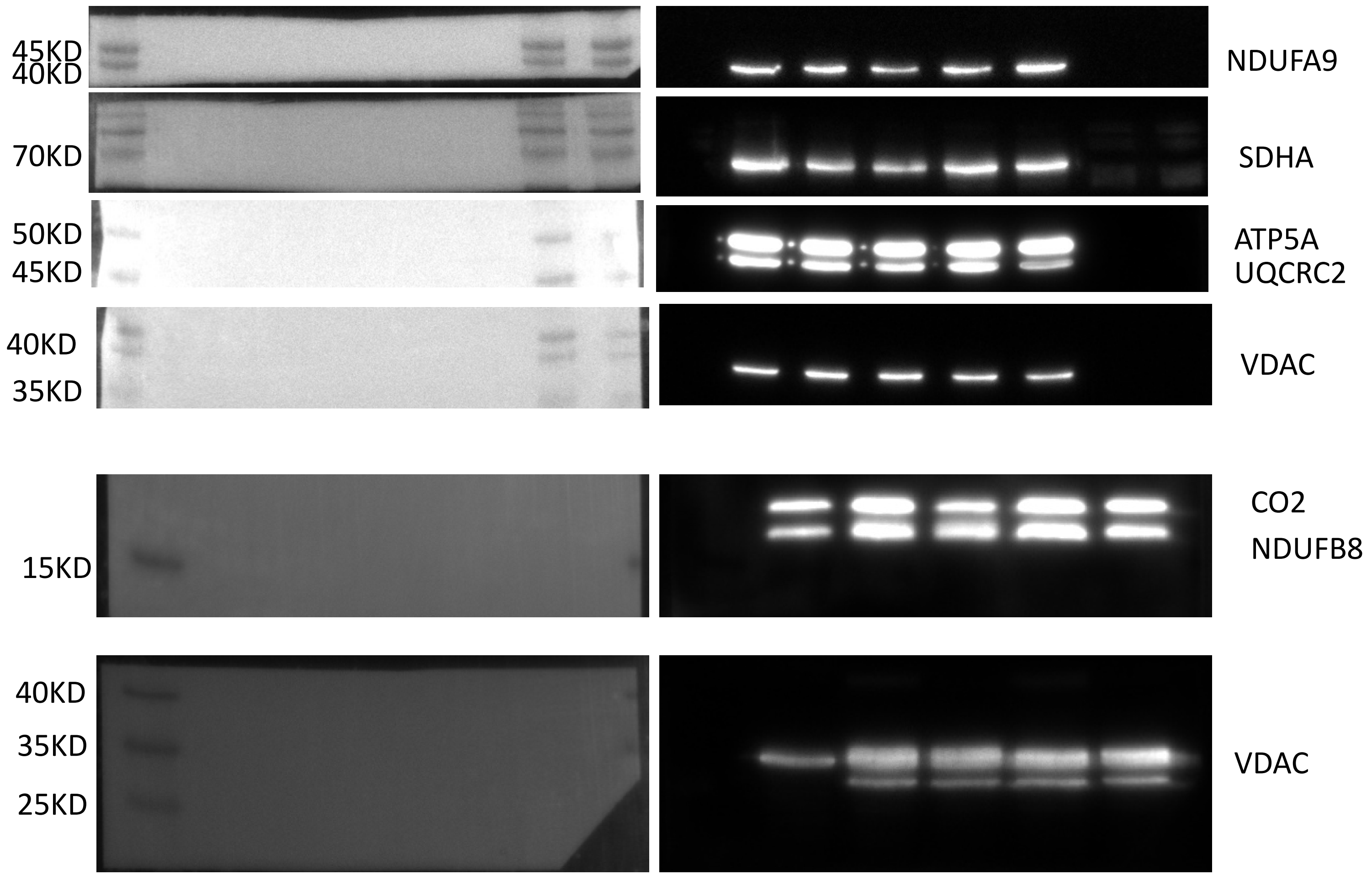


Psd95



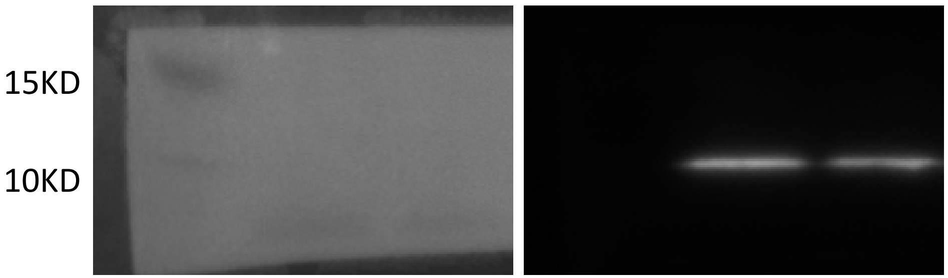


Full unedited gel for Figure S 3

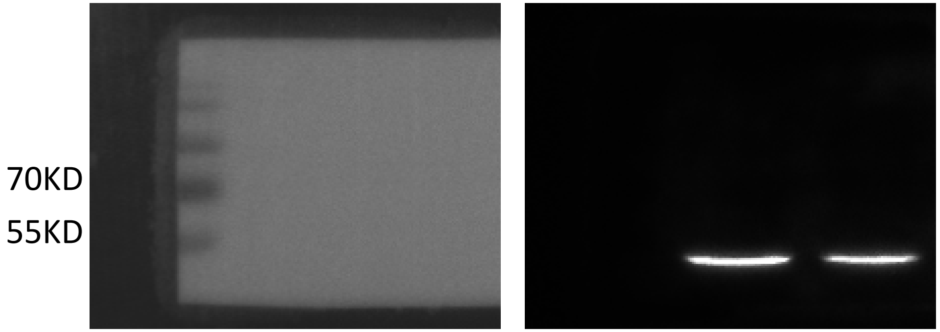


Full unedited gel for Figure S 6A

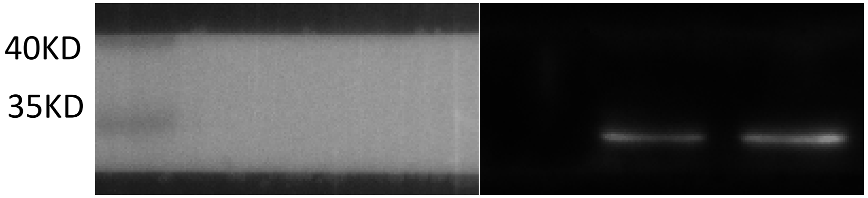
ATP8



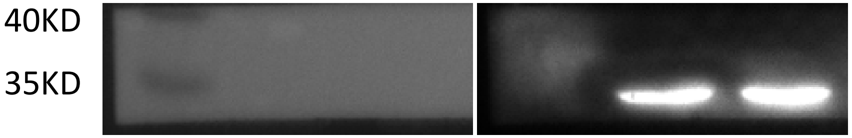
ATP5B



ATP5C



VDAC



Full unedited gel for Figure S 6B