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Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample sizes in this study were chosen according to published guidelines and to our previous experience. PNPT1 mutations represent a very rare cause of mitochondrial diseases. We have included in this manuscript the results obtained in fibroblasts of 4 unrelated patients. We did not choose the sample size but only used what we had in hand.

2. Data exclusions

Describe any data exclusions.

No data were excluded.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

The experimental findings were reliably reproduced through repeated experiments.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

The in vitro experiments were not randomized. The in vivo experiments were randomized.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were blinded to allocation during in vivo experiments (immunohistochemistry) and outcome assessment.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

GraphPad Prism, Microsoft Excel, FlowJo2, Imaris, OMERO, Multi Gauge V3.0, ScanR, ImageJ, Bowtie2, Bedtools, R, Python, Perl, Burrows-Wheeler Aligner (version 0.7.12), Genome Analysis Toolkit (GATK 3.7), SAMtools (version 1.4), Picard (version 2.9.0-1), GATK Unified Genotyper, Ensembl database (version 75), dbsnp (version 140), 1000 genome project (version 2013/05/02), Gnomad (version 2.0.2) and EVS (version ESP6500SI-V2), Polyweb software interface designed by the Bioinformatics platform of University Paris Descartes.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All unique materials used are available from standard commercial sources or from the authors upon request. hSUV3_WT/hSUV3_G207V 293 cells, PNPase_WT/PNPase_R445E-R446E HeLa cells, Fibroblasts of patients, PNPase HepKO mice liver samples and RIG-I +/-, RIG-I & MDA5 KO are unique and cannot be obtained by any commercial source.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Sources and usage details of all the antibodies used in the study are described in the methods section.

Antibodies used -

Antibody	Catalog No.	Dilution
rabbit anti-PNPT1	ab96176, abcam	1:1000
rabbit anti-PNPT1	sc-49315, Santa cruz	1:500
mouse anti-ADAR1	sc-73408, Santa cruz	1:1000
mouse anti-dsRNA	10010500, Scions	1:200, 2.5 µg/ml
anti-DNA	61014, Progen	0.5 µg/ml
rabbit anti-SUV3	A303-055A, Bethyl Laboratories	1:1000
rabbit anti-SUV3	(Szczesny et al, 2010)	1:3000
mouse anti-RIG-I	AG-20B-0009, AdipoGen	1:1000
rabbit anti-COX IV	3E11, Cell signaling	1:1000
rabbit anti-Cytochrome C	NB100-91732, Novus Biologicals	1:500
rabbit anti-Calnexin	2433, Cell signalling	1:500
mouse anti-Lamin A/C	4C11, Cell signalling	1:1000
anti-mouse IgG (H+L) Alexa Fluor 488	A-21202, Thermo Fisher Scientific	1:300, 2 µg/ml
mouse IgG2a	sc-3878, Santa cruz	2.5 µg/ml
mouse anti-MDA5	(in house from Jan Rehwinkel, Hertzog et al 2018)	1:500
rabbit anti-MAVS	ALX-210-929-C100, Enzo Life Sciences	1:500
rabbit anti-Bax	2772T, Cell signalling	1:500
rabbit anti-Bak	6947T, Cell signalling	1:200
mouse anti-α-Tubulin	T5168,sigma	1:2000
mouse anti-actin	ab8226,abcam	1:1000
mouse anti-actin	A5441, Sigma	1:3000
rabbit anti-FLAG	PA1-984B, Thermo Fisher Scientific	1:1000
goat anti-mouse IgM Alexa Fluor 555	A-21426, Thermo Fisher Scientific	2 µg/ml
anti-mouse IgG HRP	A9044,Sigma	1:5000
anti-mouse IgG HRP	ab6728, abcam	1:2000
anti-rabbit IgG HRP	A0545,Sigma	1:2000
anti-rabbit IgG HRP	ab6721, abcam	1:2000
rabbit anti-OXA1L	HPA003531,Sigma	1:100
anti-mouse IgG (20nm gold)	ab27242, abcam	1:10

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HeLa was obtained from ATCC and source of all the cell lines used is provided in the methods section. Cultured skin fibroblasts from 4 patients with PNPT1 mutations were obtained from skin biopsies of the patients who are managed by Prof Arnold Munnich and Dr Manuel Schiff. hSUV3_WT/hSUV3_G207V 293 cells were described in Szczesny et al. 2010, PNPase_WT/ PNPase_R445E-R446E HeLa cells were made in house by Szczesny et al. , 293 Flp-In T-Rex cells (Thermo Fisher Scientific), MEFs RIG-I +/-, RIG-I -/-, MDA5 -/- were described in Deddouche et al. 2014.

b. Describe the method of cell line authentication used.

We subjected parental HeLa Flp-In T-Rex cells to authentication. See methods section under plasmid transfection and establishing of stable cell lines

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines tested mycoplasma negative.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Hepatocytes were isolated from perfused livers of two PNPASE (Pnpt1) liver specific knockout C57BL/6J mice (AlbCRE/WTPnpt1neo-flox/neo-flox) designated HepKO; 1 male aged 12.9 weeks, 1 female aged 4.29 weeks, two independent experiments) and two sex-matched wildtype littermate mice (AlbWT/WTPnpt1neo-flox/neo-flox, designated WT) (Wang et al. Cell 2010 (PMID: 20691904)).
Mice are housed, bred and studied in accordance with an approved protocol consistent with the UCLA Chancellor's Animal Research Committee (ARC) policies and procedures, as stated in Laboratory Animals in Teaching and Research (rev. 1998), the provisions of the NIH Guide for the Care and Use of Laboratory Animals and all applicable state and federal regulations.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human research participants have mutations in PNPT1 gene. Informed consent for diagnostic and research studies was obtained for all subjects in accordance with the Declaration of Helsinki protocols and approved by local Institutional Review Boards in Paris.
Patient 1: male, Died aged 2 years, diagnosis of mitochondrial disorder
Patient 2: male, alive at age 1y ear, diagnosis of mitochondrial disorder
Patient 3: female, 7 years, diagnosis of mitochondrial disorder
Patient 4: male, 13 years, diagnosis of mitochondrial disorder