Supplemental Information for

Selective degradation of tRNA^{Ser}(AGY) is the primary driver for mitochondrial seryl-tRNA synthetase-related disease

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Supplemental Methods

Clinical manifestations

A patient of multisystem disorder was studied. Her prenatal history was unremarkable. She was full-term at birth and exhibited a normal weight and length. Delayed development was noticed in the first year of life. At 19 months, increased muscle tone in the lower limbs was noted during physical examination. Moderate primary pulmonary hypertension and right ventricular hypertrophy were diagnosed by echocardiography. At 21 months of age, the developmental quotient (DQ) was assessed using the Chinese version of the Gesell Development Diagnosis Scale. The patient displayed normal social-emotional responses. However, her DQ score for gross motor function was only 57, falling within the mild mental retardation range ($55 \leq DQ \leq 75$). The DQ scores for fine motor skills, language, and adaptability fell in the borderline mental retardation range ($76 \leq DQ \leq 85$).

At 3.5 years of age, she was admitted to the emergency department for vomiting, high-grade fever, and convulsions. Her overall clinical condition soon deteriorated. She fell into a coma and developed status epilepticus. Chest computed tomography imaging revealed double pheumonia. Cerebral MRI revealed diffuse brain edema, indicating infection of the CNS (Fig. 1b). Laboratory tests showed hyperlactacidemia (4.20 mmol/L [N, 0.7–2.1 mmol/L]), anemia (Hb 87 g/L [N, 110–160 g/L]), hypoproteinemia (total protein 45.6 g/L [N, 65–81 g/L], albumin 28.9 g/L [N, 35–50 g/L], prealbumin 0.06 g/L [N, 0.12–0.24 g/L] and globulin 16.7 g/L [N, 20–32 g/L]), and elevated blood creatine kinase (493 U/L [N, 30–135 U/L]). Blood electrolyte disturbance was indicated by hyponatremia (126.1 mmol/L [N, 137–145 mmol/L]), hypomagnesemia (0.41 mmol/L [N, 0.7–1.0 mmol/L]), and hypochloremia (84 mmol/L [N, 98–107 mmol/L]). Levels of lactate dehydrogenase (578 U/L [N, 100–252 U/L]), and total protein (> 3000 mg/L [N, 120–600 U/L]) in cerebrospinal fluid (CSF) were elevated. Masses of erythrocytes (2.5×1010/L [N, 0 /L]) and leukocytes (5.12×10^8/L [N, 0-15 /L], 70% multinucleate cells, and 30% mononuclear cells) were detected in the CSF. Blood and CSF cultures were negative, and (1,3)-β-D-Glucan was not detected in this patient. Pathogenic microorganisms such as *Legionella pneumophila*, *Mycoplasma pneumonia*, cytomegalovirus, enterovirus 71, adenovirus, and respiratory syncytial virus were excluded by laboratory tests. Liver and kidney function test results fell within normal limits. Symptoms improved after a treatment regimen including mechanical ventilation; anti-infection, anti-convulsion, and continuous renal replacement therapy; sedation; and analgesia.

After recovery from the acute episode, the patient presented with complete paralysis and mental deterioration accompanied by frequent refractory epilepsy. Isolated spasms and partial seizures were indicated by electroencephalogram examination at the age of 5.5 years, respectively. She developed renal insufficiency at 7 years and 11 months of age, characterized by high creatinine (195.4 μmol/L [N, 9–88 μmol/L]) and urea nitrogen (16.17 mmol/L [N, 2.5–6.4 mmol/L]) levels. Hyperuricemia (562 μmol/L [N, 115–428 μmol/L]) and scoliosis were also observed in the patient. No clinical abnormalities were noted in the family members.

RT-PCR

Total RNA was isolated from the peripheral leukocytes of the paternal parent and a healthy control using the QIAmp RNA Blood Kit (Qiagen). First-strand cDNA was synthesized using random primers, oligo primers, and PrimeScript Reverse Transcriptase (Takara, Dalian, China). The synthesized products were further amplified with primer P1, 5'- CCCAACCAGACCCACCCA -3', which was located in exon 4; and primer P2, 5'- TGTTTGTCTCTGCCCGGTAG -3', which was located in exon 11 of *SARS2*. The products were cloned into the pMD 18-T vector (Takara Biotechnology). A total of 126 paternal clones and 107 normal control clones were selected and sequenced.

Plasmids and cloning

The wild-type and mutant *SARS2* coding regions were synthesized and cloned into pcDNA3.1-Myc and pcDNA3.1-FLAG to generate C-terminal Myc-tagged and FLAG-tagged *SARS2*. The C-terminal FLAG-tagged *SARS2* coding region was further subcloned into the pLVX-EF1α-IRES-Puro lentiviral vector. All vectors were confirmed by DNA sequencing.

Construction of mouse model

Ins12 and dupC mice were generated using CRISPR/Cas9 technology (Cyagen Biosciences, Suzhou, China). Briefly, Cas9 protein, guide RNA, and a donor vector containing the desired mutation were injected into fertilized eggs of C57BL/6 mice. The embryos were transferred to recipient female mice to obtain F0 mice. F0 founders were bred with wild-type mice with a matching strain background to obtain F1 mice bearing the mutant allele. DNA sequence analysis and Southern blot were used to detect homologous recombination and single integration in F1 mice. F1 mice were intercrossed to generate F2 mice with homozygous or compound heterozygous *Sars2* mutations. The guide RNA used for Ins12 mice construction: 5'-GTAAAGCATCCTGCTCCCTC-3', and the guide RNA used for dupC mice construction: 5'-CATCTGGTGGCAGACTGGCC-3'.

Transfection, lentivirus production, and infection

Transfection was performed using jetPRIME transfection reagent (Polyplus Transfection, Illkirch, France) according to manufacturer instructions. The lentiviral constructs were transfected into HEK293T cells with the packaging plasmid psPAX2 and envelope plasmid pMD2.G. Virus-containing supernatants were harvested, passed through a 0.45 μm filter (Sigma-Aldrich, St. Louis, MO, USA), and concentrated using 100 kDa centrifugal ultrafiltration devices (Merck, Darmstadt, Germany). The iPSCs were virally transduced for 24 h, then the selection of iPSCs with lentivirus integration was initiated 48 h after infection by adding 0.625 μg/mL puromycin (Selleck, Houston, TX, USA). Selected iPSCs were verified by SARS2 expression by western blot.

Immunofluorescence

HEK293T cells were transfected with wild-type and mutant Myc-tagged *SARS2*

constructs. Twenty-four hours after transfection, cells were stained with 500 nM MitoTracker Deep Red FM (YEASEN, Shanghai, China) for 30 min, fixed in 4% paraformaldehyde, permeabilized in 0.25% Triton X-100, and then blocked in 1% BSA. Immunostaining was performed using mouse anti-Myc monoclonal antibody (Cell Signaling Technology, Danvers, MA, USA) followed by labeling with Alexa Fluor® 555 conjugated anti-rabbit IgG (Cell Signaling Technology). The cover slips were then mounted on microscope slides using a mounting medium with DAPI (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed using a Leica SP8 LIGHTNING confocal microscope (Leica Microsystems, Wetzlar, Germany).

Co-IP

The Myc- and FLAG-tagged *SARS2* vectors were co-transfected into HEK293T cells. Thirty-six hours after transfection, the cells were lysed and incubated with anti-Myc magnetic beads (Thermo Fisher Scientific) according to manufacturer instructions. Cell lysates and immune complexes were separated on 10% SDS-PAGE gels, transferred to polyvinylidene difluoride (PVDF) membranes (Merck), and probed with either anti-FLAG or anti-Myc antibodies (Sigma-Aldrich).

Western blot

Transfected cells, iPSCs, and ground mouse heart, brain, and skeletal muscle tissues were lysed in RIPA lysis buffer (Abcam, Cambridge, MA, USA), loaded onto SDS-PAGE gels, transferred to PVDF membranes, and incubated with antibodies. The membranes were incubated with Pierce ECL Western Blot Substrate (Thermo Fisher Scientific). Grayscale values were analyzed using ImageJ software.

Northern blot

Total RNA was extracted from iPSCs and ground mouse heart, brain, and skeletal muscle tissues using TRIzol reagent (Thermo Fisher Scientific) following manufacturer instructions. To assess steady-state levels, RNA samples in 0.1 M sodium acetate (pH 4.5) were separated on 10 % PAGE-8 M urea gel in 1× TBE buffer and transferred to nylon membrane (Merck). To determine aminoacylation level, RNA samples in 0.1 M sodium acetate (pH 4.5) were separated on 10 % PAGE-8 M urea-0.1 M sodium acetate (pH 4.5) gel in 0.1 M sodium acetate (pH 4.5) buffer and transferred to nylon membrane. The membrane was fixed using an UVP Crosslinker CL-1000 at 120000 μJ/cm^2 (UVP, Jena, Germany) and hybridized with probes for tRNAs or 5s rRNA at 55 °C overnight after pre-hybridization. The membrane was washed and incubated with blocking buffer, antibody solution, and CDP-Star® (Roche, Basel, Switzerland) at room temperature. After exposure using Amersham Imager 680 blot and gel imager (GE Healthcare, Fairfield, CT, USA), grayscale values were processed using MultiGauge software.

Degradation rate analysis of SARS2

HEK293T cells were transfected with the wild-type and mutant Myc-tagged *SARS2* constructs. After 24 h, 100 μM CHX was added to the culture medium. Whole cell lysates were collected after 0, 2, 4, 6, 8, and 10 h of treatment. The steady-state

abundance of SARS2 in the samples was detected using western blot. The degradation rate of SARS2 was calculated based on the steady-state level at different processing time points using GraphPad Prism software.

Degradation rate analysis of hmtRNA^{Ser}(AGY)

Total RNA was extracted from iPSCs cultured in PSCeasy medium containing 250 ng/μL EB for 0, 6, 12, and 24 h using TRIzol reagent following manufacturer instructions. RNA samples at different processing time points in 0.1 M sodium acetate (pH 4.5) were analyzed for steady-state levels of hmtRNA^{Ser}(AGY) via northern blot.

Detection of mitochondrial genome transcription

Total RNA was extracted from iPSCs and cDNA was obtained by reverse transcription. The genes encoding respiratory chain complexes in the mitochondrial genome were amplified using 2x TSINGKE Master qPCR Buffer (TSINGKE Biotechnology, Beijing, China) and the reactions were performed using a LightCycler® 96 fluorescence quantitative PCR instrument.

OCR determination

The OCR of the iPSCs was determined using a Seahorse XFe24 Analyzer (Agilent Technologies, Santa Clara, CA, USA). Cells were seeded in the detection plate in advance. Unbuffered DMEM (37°C, pH7.4, XF assay medium, 2 mM L-glutamin, 10 mM D-Glucose, 1 mM sodium pyruvate) was prepared and filtered to remove bacteria. Cells were cultured in unbuffered DMEM in a carbon dioxide–free incubator for 30–45 minutes, and then MRC inhibitors including oligomycin, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), antimycin, and rotenone were added according to the preset program when the machine was ready. Six parameters, including basal, ATP-coupled, proton leak, maximum, reserve capacity, and non-mitochondrial OCR values were generated. The cells were then stained with Hoechst and propidium iodide and counted using an EnSight™ Multimode Microplate Reader (PerkinElmer).

ECAR determination

The cells were prepared in the same manner described in "OCR determination**"**. Unbuffered DMEM (37 °C, pH7.4, XF assay medium, 2 mM L-glutamin) was prepared and filtered to remove bacteria. According to the preset program, glucose, oligomycin, and glycolysis inhibitor 2-DG were automatically added after loading the detection plate. The ECAR was monitored in real time to obtain the non-glycolytic acid value, glycolytic capacity, maximum glycolytic potential, and glycolytic reserve. The cell count was determined as for OCR.

MMP assessment

MMP was assessed with the JC-10 Mitochondrial Membrane Potential Assay Kit (Abcam) following the general recommendations of the manufacturer. Briefly, iPSCs were detached using Acctuase (Stemcell Technology, Vancouver, Canada), washed with PBS, and incubated with the JC-10 probe at 37 °C for 15 min in the dark. Green JC-10

monomeric and red JC-10 aggregates were measured using a BD FACS Canto Plus cytometer (BD Biosciences), analyzed following compensation for spectral overlap, and reported as the ratio between red and green fluorescence.

Measurement of apoptosis

Apoptosis was measured in iPSCs by flow cytometry using the FITC Annexin V Apoptosis Detection Kit (BioLegend, San Diego, CA, USA) following manufacturer protocol. Briefly, adherent and non-adherent cells were collected, resuspended in staining buffer, stained with FITC-annexin V and propidium iodide for 15 min at room temperature, and subsequently analyzed by flow cytometry using the BD FACS Canto Plus cytometer.

ROS Determination

The levels of ROS generated by mitochondria in viable cells were determined using the MitoSOX Red Mitochondrial Superoxide Indicator (Invitrogen) under normal conditions and H_2O_2 stimulation. Briefly, iPSCs were detached using Acctuase, incubated with 5 μM MitoSOX at 37 °C for 10 min in the dark, and then washed with PBS. Fluorescence was measured using the BD FACSCanto Plus cytometer. For H_2O_2 stimulation, iPSCs were cultured in PSCeasy medium with 0.3% freshly prepared H_2O_2 for 30 min before ROS measurement. Cells were incubated in 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA)-containing medium (Beyotime) at 37 °C for 20 min, washed three times with serum-free medium, and observed and photographed using a fluorescence microscope IX73 (Olympus, Tokyo, Japan).

Analysis of protein carbonylation level

Protein carbonyl groups generated from the oxidation of proteins in iPSCs were measured using a Protein Carbonyl Assay Kit (Abcam). The samples were processed according to manufacturer instructions and analyzed by western blot.

Analysis of autophagy level

iPSC protein lysates were collected for western blot and protein levels were detected using LC3 I/II and p62 antibodies and corresponding secondary antibodies. For the autophagy flow experiment, iPSCs were treated with a final concentration of 100 nM Baf A1 for 12 h, protein lysates of iPSCs were collected for western blot, and protein levels were detected with LC3 I/II and p62 antibodies and corresponding secondary antibodies.

Crystallization

Crystals of SARS2 and its mutants (Ins12 and dupC) were grown at 18 °C using the hanging-drop vapor-diffusion method. Briefly, 15 mg/mL of protein was pre-mixed with 5 mM serine, 10 mM ATP, and 10 mM $MnCl_2$ at 4 °C. Each crystal drop comprised 0.25 μL protein solution with equal volumes of reservoir solution: 0.25 M sodium acetate tetrahydrate, 0.1 M sodium citrate (pH 5.5), and 5% (w/v) PEG 4000. After 7–14 days of incubation, crystals were flash-frozen in liquid nitrogen with 75% reservoir

solution and 25% glycerol for data collection.

K_D determination for hmtRNA^{Ser} of SARS2, Ins12, and dupC

The interaction between the biotin-labeled hmtRNA^{Ser} and wild-type or mutant SARS2 was detected using the Octet molecular interaction technology platform (ForteBio, Ann Arbor, MI, USA) based on the optical interference technology of the biofilm layer. Streptavidin probes were used to obtain K_D values, reflecting whether the mutations affected the interaction between SARS2 and hmtRNA^{Ser}s.

Enzymatic assays

Amino acid activation kinetics were analyzed by the ATP-PPi exchange reaction in a mixture containing 60 mM Tris-HCl (pH 7.5), 60 mM KCl, 15 mM $MgCl_2$, 2.5 mM ATP, 5 mM DTT, 0–10 mM Ser, and 2 mM [^{32}P]-Na_4PPi. An aliquot of the reaction mixture was added to 200 μL quenching solution containing 2% activated charcoal, 3.5% $HClO_4$, and 50 mM tetrasodium pyrophosphate, and then vortexed for 20 s. The solution was filtered through a Whatman GF/C filter, then washed with 20 mL 10 mM tetrasodium pyrophosphate solution and 10 mL 100% ethanol. The filters were dried and [^{32}P]-ATP was counted using a scintillation counter (PerkinElmer). The aminoacylation kinetics were assayed in a reaction mixture containing 60 mM Tris-HCl (pH 7.5), 60 mM KCl, 15 mM $MgCl_2$, 2.5 mM ATP, 5 mM DTT, 0–11.5 μM hmtRNA^{Ser}(UCN), and 62.1 μM [^{14}C]-Ser. After incubation at 37 °C, the reaction solution was aspirated at specific intervals and quenched. The reaction was terminated in 5% trichloroacetic acid, washed with cold 5% trichloroacetic acid and 100% ethanol, and analyzed using a scintillation counter.

Observation of mitochondria by TEM

The iPSCs were collected in a 1.5 mL centrifuge tube. Heart, brain, and tibialis anterior muscles of one-month-old female mice were collected after anesthesia with 1.25% avertin, heart perfusion fixation with 4% paraformaldehyde, and fixation in 4% polyformaldehyde at 4 °C overnight. The right ventricular wall, primary motor cortex, and tibialis anterior muscle were cut to 1 mm^3 pieces for subsequent analysis. Samples were fixed with 2.5% glutaraldehyde at 4 °C overnight, washed with 0.1 M PBS, and fixed with 1% osmic acid at room temperature. An ethanol gradient was then used for gradual dehydration. After replacing ethanol with acetone, the samples were infiltrated in Epon812 epoxy overnight, transferred to the embedding tank, and polymerized in Epon812 at 60 °C for 48 h. The samples were then sliced, collected with copper mesh, and stained with 2% uranyl acetate aqueous solution and lead citrate at room temperature. Observations were performed using a transmission electron microscope Tecnai G2 Spirit (FEI, Hillsboro, OR, USA).

Histological assessment of skeletal muscle

The rectus femoris muscle was collected from 13–16-week-old male mice immediately after euthanasia. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Samples were sectioned at 3 μm and stained with hematoxylin and eosin. Four

independent visual fields were randomly selected in each sample. The myofiber cross-sectional area was measured using ImageJ software.

Treadmill experiment

The treadmill test was divided into adaptation and testing phases. In the adaptation phase, the speed of the conveyor belt was set to 5 m/min and the inclination angle was set to 0 °. The mice were placed on a track to adapt to the exercise for 5 min. In the testing phase, the speed of the treadmill and the inclination angle of the conveyor belt slowly increased from 5 m/min to 20 m/min and from 0 ° to 12 °, respectively, in a step-up manner at the same time. The experiment ended when the exercise time on the conveyor belt reached 80 min or the animal reached its exercise capacity limit. The exercise time and speed at exhaustion were recorded.

Table S1. Candidate variants identified by WES

No.	Genomic coordinates	Gene	description	Transcript variant	Protein variant	genotype
1	19:39406283	*SARS2*	Seryl-tRNA Synthetase 2, Mitochondrial	c.1519dupC	p.R507Pfs*41	Het
2	19:39410521	*SARS2*	Seryl-tRNA Synthetase 2, Mitochondrial	c.654-14T>A	-	Het
3	8:68423789	*CPA6*	Carboxypeptidase A6	c.419A>G	p.H140R	Het
4	X:53280098	*IQSEC2*	IQ Motif And Sec7 Domain ArfGEF 2	c.1045G>T	p.V349F	Het
5	16:89357426	*ANKRD11*	Ankyrin Repeat Domain Containing 11	c.392G>A	p.S131N	Het
6	18:29121228	*DSG2*	Desmoglein 2	c.1952T>C	p.I651T	Het
7	2:128186354	*PROC*	Protein C, Inactivator Of Coagulation Factors Va And VIIIa	c.1218G>A	p.M406I	Het
8	10:43604499	*RET*	Ret Proto-Oncogene	c.1084C>A	p.L362I	Het
9	10:71151976	*HK1*	Hexokinase 1	c.2131G>C	p.A711P	Het
10	1:2235475	*SKI*	SKI Proto-Oncogene	c.1408C>T	p.P470S	Het
11	3:57130461	*IL17RD*	Interleukin 17 Receptor D	c.2180G>A	p.R727H	Het
12	3:57131694	*IL17RD*	Interleukin 17 Receptor D	c.2037G>A	p.M679I	Het
13	19:50101005	*PRR12*	Proline Rich 12	c.3413G>A	p.G1138E	Het
14	12:6690519	*CHD4*	Chromodomain Helicase DNA Binding Protein 4	c.4714_4716delAAA	p.K1572del	Het
15	20:62641584	*PRPF6*	Pre-MRNA Processing Factor 6	c.1218G>C	p.L406F	Het
16	2:86259439	*POLR1A*	RNA Polymerase I Subunit A	c.4228G>A	p.A1410T	Het

Table S2. Data collection and refinement statistics

	SARS2	Ins12
Data collection		
Space group	*P3$_1$12*	*P4$_3$2$_1$2*
Cell dimensions		
a, *b*, *c* (Å)	89.85, 89.85, 86.22	144.18, 144.18, 245.47
α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 90.00
Resolution (Å)	44.92–2.80 (2.95–2.80)*	49.18–3.20 (3.32–3.20)
Total reflections	94001 (14262)	853392 (92999)
Unique reflections	9757 (1446)	42890 (4415)
Wilson B factor	79. 014	72. 982
CC(1/2)	0.997 (0.354)	0.997 (0.490)
I / s*I*	8.3 (1.0)	9.5 (1.0)
Completeness (%)	97.6 (100.0)	100.0 (100.0)
Redundancy	9.6 (9.9)	19.9 (21.1)
Refinement		
R_{work} / R_{free} (%)	28.9/33.3	20.3/22.8
No. atoms		
Protein	1988	7952
Ligand or solvent	0	0
B-factors		
Protein	98.509	122.672
R.m.s. deviations		
Bond lengths (Å)	0.007	0.006
Bond angles (°)	0.974	1.056
Ramachandran plot		
Most favored [%]	96.3	96.3
Additional allowed [%]	2.9	2.8

*Values in the parentheses are for the highest resolution shell.

Table S3. Genotype of mice offsprings

Genotype	Amount (*Sars2*$^{dupC/+}$×*Sars2*$^{Ins12/+}$)	Amount (*Sars2*$^{dupC/+}$×*Sars2*$^{dupC/+}$)	Amount (*Sars2*$^{Ins12/+}$×*Sars2*$^{Ins12/+}$)
WT	43	30	30
Sars2$^{dupC/+}$	22	17	-
Sars2$^{Ins12/+}$	19	-	29
Sars2$^{dupC/Ins12}$	0	-	-
Sars2$^{dupC/dupC}$	-	0	-
Sars2$^{Ins12/Ins12}$	-	-	0
Total	84	47	59

Table S4. Phenotypes and genotypes of patients with *SARS2* variantions

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Gender	M	F	F	F	M	F	M	M	M	F
Ethnicity	Palestinians	Palestinians	Palestinians	Spanish	Spanish	Chinese	NA	Indian	Brazilian	Chinese
Genotype	Homo c.1169A>G, p.D390G	Homo c.1169A>G, p.D390G	Homo c.1169A>G, p.D390G	Homo c.1205G>A, p.R402H	Homo c.1205G>A, p.R402H	c.667G>A, p.V223M / c.1205G>A, p.R402H	c.1031G>A, p.R344Q / c.1205G>A p.R402H	Homo c.1347G>A, splicing defect	Homo c.1343A>T, p.H448L	c.654-14T>A / c.1519dupC, p.R507Pfs*41
Prematurity	+	+	+	-	+	+	NA	-	NA	-
Developmental delay	+	+	+	+	+	+	+	+	NA	+
Hyperuricemia	+	+	+	+	+	+	+	-	NA	+
Metabolic alkalosis	+	+	+	+	+	-	NA	-	NA	-
Pulmonary hypertension	+	+	+	NA *	+	-	+	-	NA	+
Progressive renal failure	+	+	+	+	+	+	+	-	NA	+
anemia	+	+	NA	+	+	+	+	NA	NA	+
epilepsy	-	-	-	-	-	-	-	-	-	+
cerebral atrophy	-	NA	-	NA	NA	NA	+	+	NA	+
muscle tone	decreased	NA	NA	decreased	NA	Normal	NA	increased	increased	increased
paralysis	-	-	-	-	-	-	-	+	+	+
Elevated serum lactate	+	+	+	-	-	+	+	-	NA	+
Electrolyte imbalance	+	+	+	+	+	-	NA	-	NA	+
Outcome	Died at 14 m	Died at 10 m	Died at 13 m	Died at 26 m	Died at 21 m	Died at 5.8 Y	Died at 5 Y	Alive at 10 y	Alive at 19 y	Alive at 8.5 y

cause of death	multiorgan failure	respiratory insufficiency	refractory pulmonary hypertension	multiorgan failure	pulmonary hemorrhage, refractory pulmonary hypertension and cardiac failure	uremia	pulmonary hypertension	/	/	/
diagnosis	HUPRA syndrome	HUPRA syndrome	HUPRA syndrome	HUPRA syndrome	HUPRA syndrome	HUPRA syndrome	HUPRA syndrome	progressive spastic paresis	hereditary spastic paraplegia	Novel HUPRA syndrome
Ref. in main text	21	21	21	22	22	23	24	25	26	this study

P1, P2 are distant cousins of the same family; P4 and P5 are siblings of the same parents; P9 is from a consanguineous kindred.

* hypertrophic cardiomyopathy found in the necropsy; NA, not available; Ref., references.

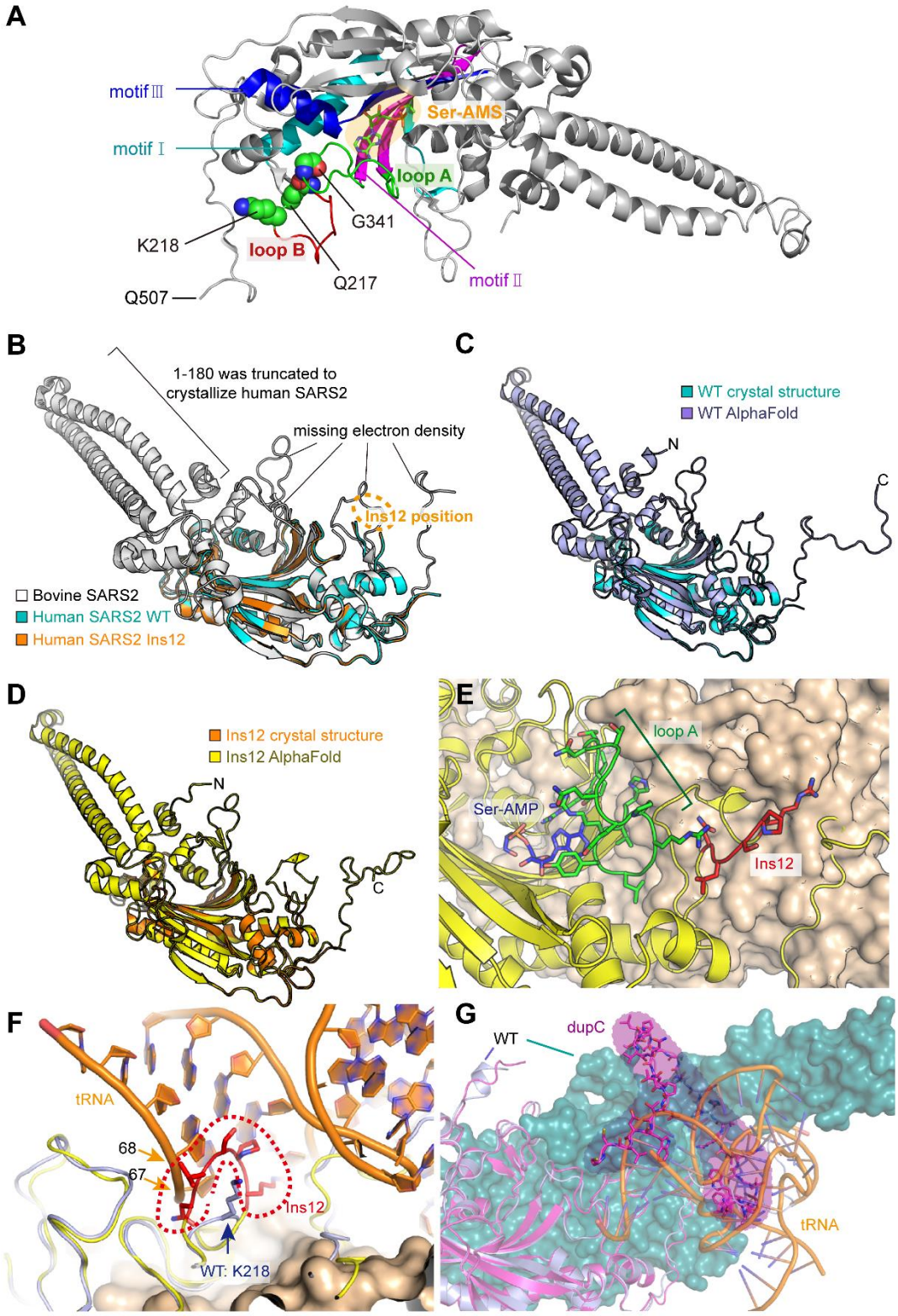

Fig. S1

Figure S1. Structural analyses of SARS2 and mutants

A, Structure of bovine SARS2 (PDB No. 1WLE), showing the accommodation of Ser-AMS by loop A (green line), which is stabilized by loop B (red line). The side chains

of Q217 and K218 are spherical. Q217 interacts directly with G341 in loop A. The structure terminates at Q507 with an invisible C-terminus (508 KPRLPGQPASS518). **B,** Superimposition of the crystal structures of human SARS2 (cyan), Ins12 (orange), and bovine SARS2 (PDB No. 1WLE, grey). **C,** Superimposition of the crystal structures of human SARS2 (cyan) and the AlphaFold-calculated SARS2 model (light blue). **D,** Superimposition of the crystal structures of Ins12 (orange) and the AlphaFold-calculated Ins12 model (yellow). **E,** AlphaFold-calculated Ins12 model, shown in the dimer form. One chain is shown as yellow cartoons, and the other chain is shown as the wheat surface. The side chains of loop A residues are shown as green sticks, the side chains of Ins12 involved residues are shown as red sticks, and the reaction intermedia Ser-AMP is shown as blue sticks. **F,** Structural models of human SARS2 and Ins12 are superimposed on the *T. thermophilus* SerRS-tRNA structure (PDB No. 1SER). WT and mutant AlphaFold models are shown as light blue and yellow cartoons, respectively. The tRNA molecule is shown in orange. The side chain of the WT K218 residue is shown as blue sticks, and the side chains of Ins12 residues are shown as red sticks and circled with red dashed lines. The positions of tRNA bases 67 and 68 are indicated by orange arrows. **G,** The AlphaFold SARS2 model is shown in the dimer form. One chain is shown as light-blue cartoons, and the other chain is shown as a slate surface. The dupC model and *Thermus thermophilus* SerRS-tRNA structure (PDB No. 1SER) were superimposed onto the first chain of the wild-type SARS2 model. The tRNA molecule is shown in orange. The dupC-containing residues are shown as purple sticks and are highlighted in purple.

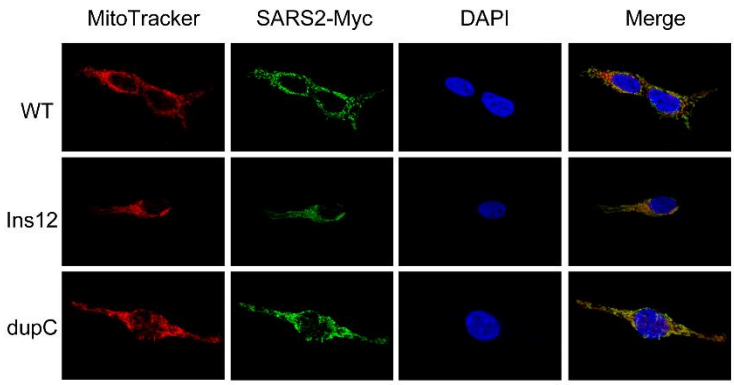

Fig. S2

Figure S2. Detection of mitochondrial localization of WT and mutant *SARS2* by immunofluorescence

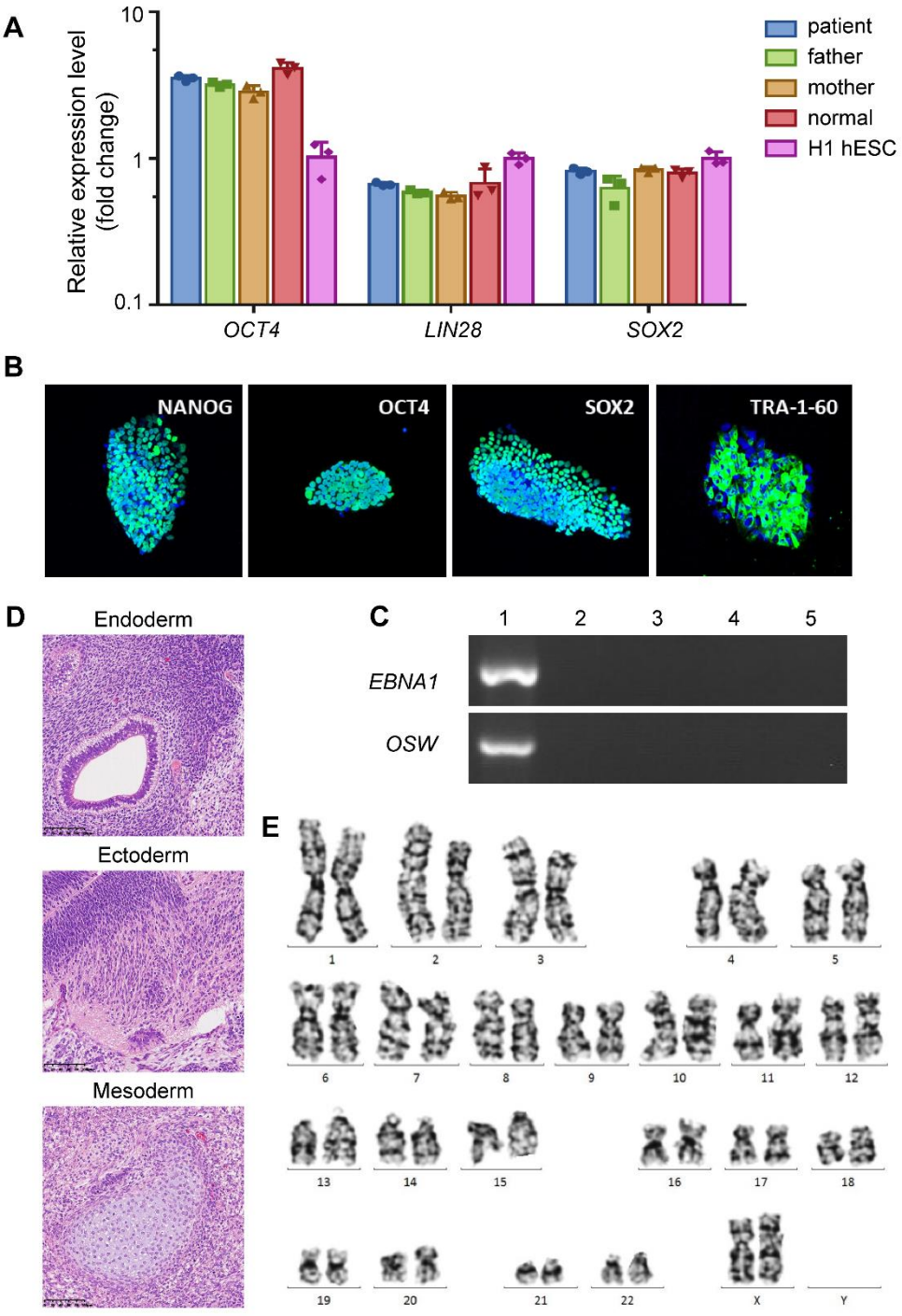

Fig. S3

Figure S3. Characterization of iPSCs generated from human peripheral blood cells

A, Expression level of stem cell markers in iPSCs compared with those in the human embryonic stem cell (hESC) line H1, determined by qPCR. Values are expressed as mean ± SD (*n* = 3). **B,** Immunofluorescence staining for stem cell markers NANOG,

OCT4, SOX2, and TRA-1-60 (green). DAPI was used for nuclear counterstaining (blue). Representative images were captured using a confocal microscope. **C,** Vector sequences (*EBNA1* and *OSW*) were not identifed in expanded iPSCs based on PCR-based detection. Lane 1: vector control; lane 2: patient iPSCs; lane 3: paternal iPSCs; lane 4: maternal iPSCs; lane 5: control iPSCs. **D,** Teratomas formed from iPSCs in immunodeficient mice. Hematoxylin and eosin staining of representative teratomas with derivatives of three embryonic germ layers: glands (endoderm), glial cells (ectoderm), and cartilage (mesoderm). Scale bars represent 100 μm. **E,** Representative karyotype of patient-derived iPSCs. A normal karyotype was detected for all iPSCs derived from the patient pedigree and a healthy control.

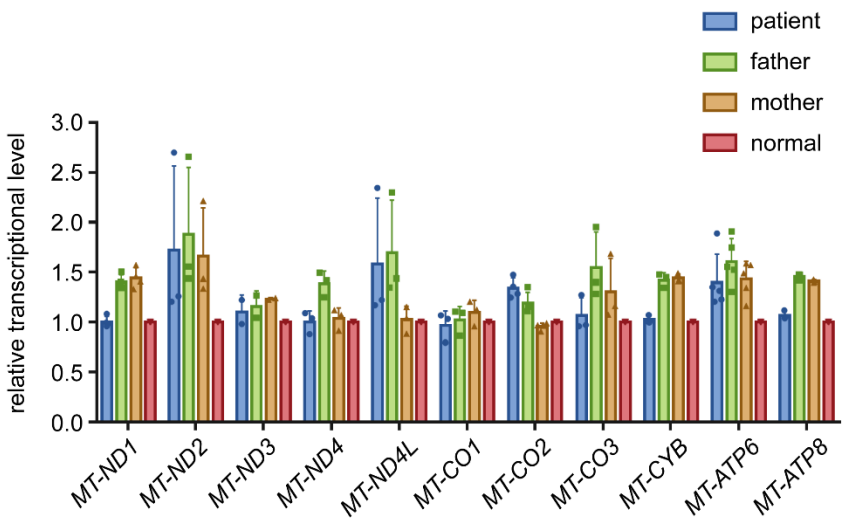

Fig. S4

Figure S4. Transcript levels of mtDNA-encoded mRNAs detected by RT-PCR
Values are expressed as mean ± SD (*n* = 2-5).

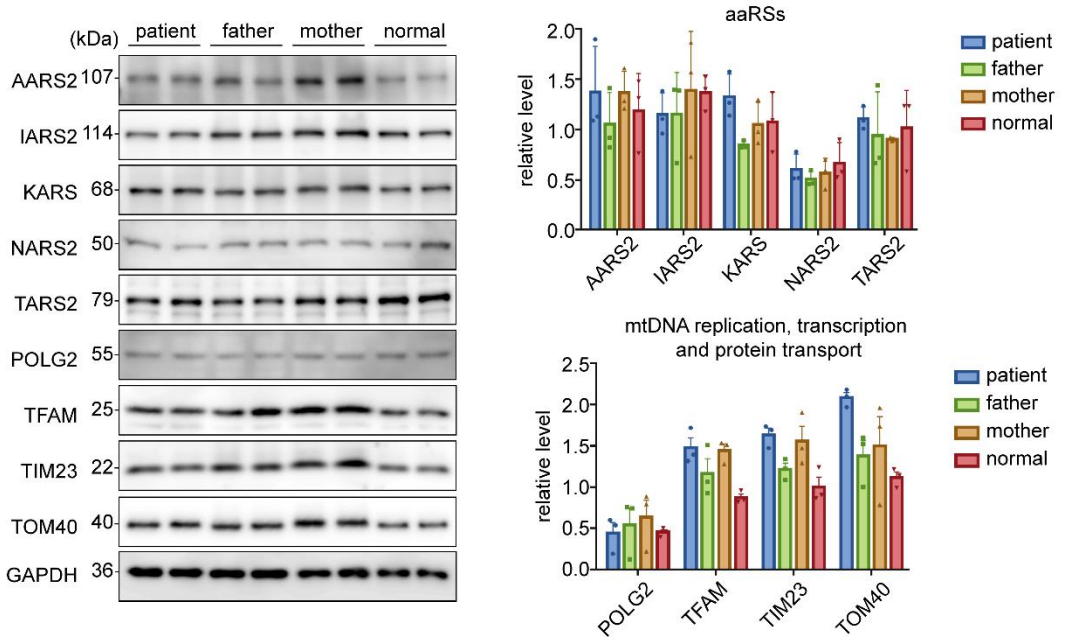

Fig. S5

Figure S5. Mitochondrial biogenesis protein levels

Band intensities were measured and normalized to GAPDH (right panels). Values are expressed as mean ± SD (n = 3).

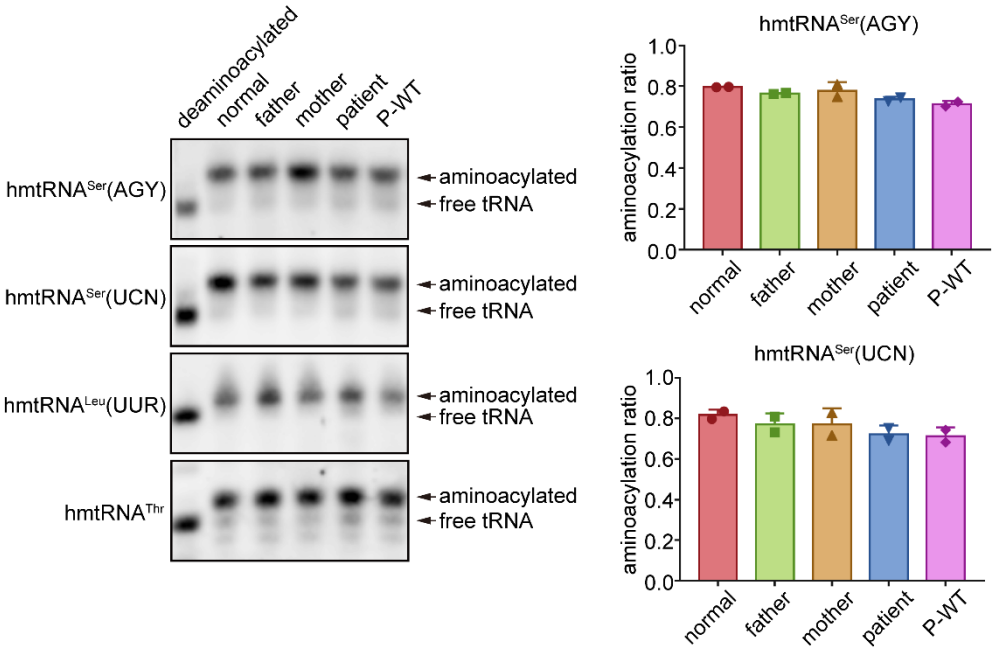

Fig. S6

Figure S6. Aminoacylation levels of mitochondrial tRNAs in P-WT cells determined by acid northern blot

The proportion of aminoacylated hmtRNA^{Ser}s was quantified (right panel). Values are expressed as mean ± SD (n = 2).

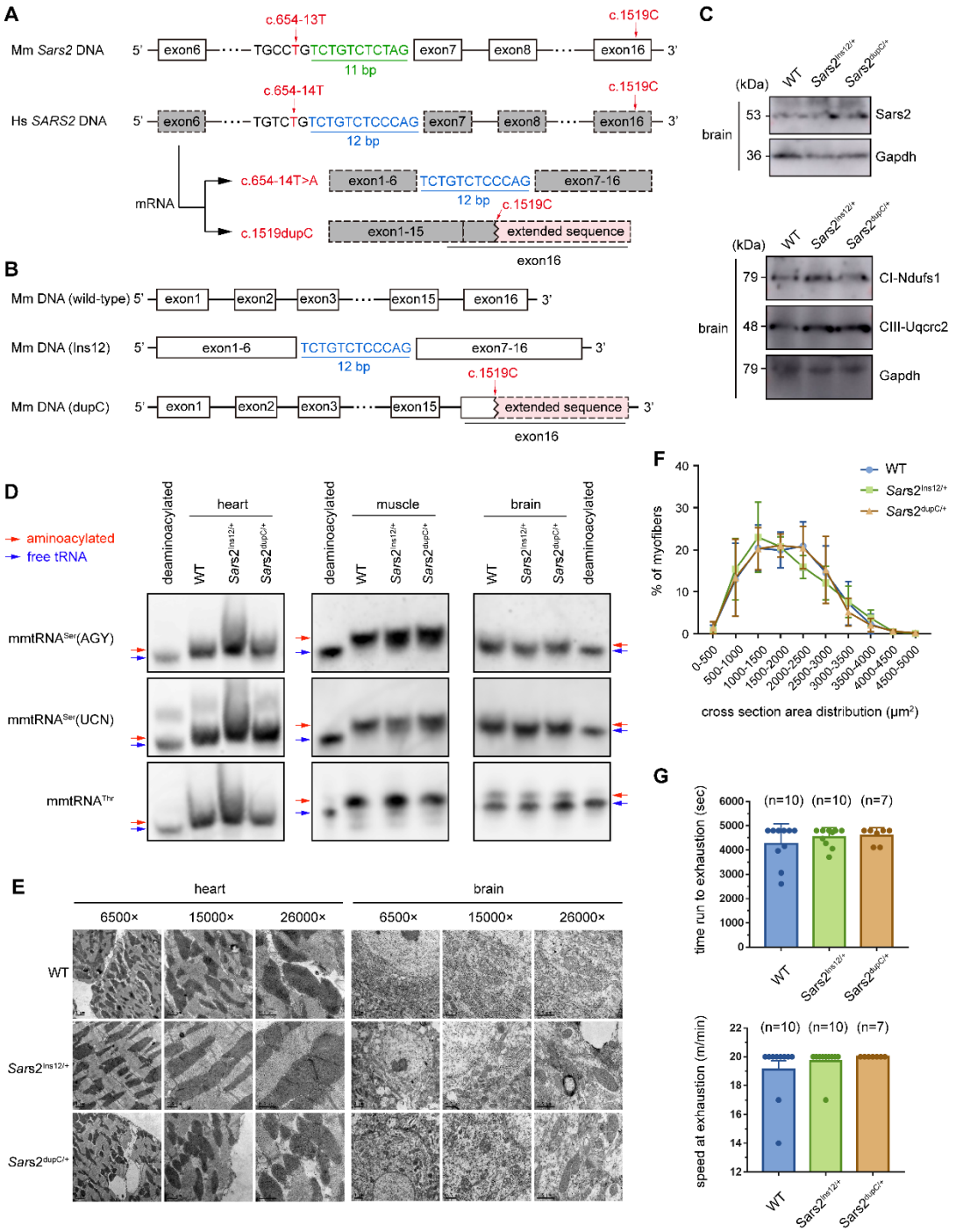

Fig. S7

Figure S7. Construction of *Sars2* mutant knock-in mice, protein levels, mitochondrial morphology, myofiber cross-sectional area, and treadmill test

A, *SARS2* sequence comparison between humans (exons in grey) and mice (exons in white). **B,** *Sars2* sequences of wild-type, Ins12, and dupC mouse models. **C,** Expression of Sars2, Ndufs1, and Uqcrc2 detected in brain tissue by western blot. Gapdh was used as a loading control. **D**, Aminoacylation levels of mmtRNA^{Ser}(AGY) and mmtRNA^{Ser}(UCN) in various tissues determined by acidic northern blot. **E,**

Mitochondria in the heart and brain tissues observed by TEM. **F,** Percentage distribution of myofiber cross-sectional area. Four independent visual fields with approximately 850 myofibers were examined in each rectus femoris muscle. Four to six male mice aged 13–16 weeks were included in each group. Values represent means ± SD. **G,** Motor function was assessed using a treadmill test in three-month-old male mice. Seven to ten mice were included in each group. The total running time and speed at exhaustion were recorded. Values are expressed as means ± SD.