

Supplemental Tables

Table S2. List of parameters employed for the computational mitochondrial model, Related to Figure 4 and Figure S9.

Parameter	Description	Value (Unit)	References
C_{mito}	Mitochondrial inner membrane divided by Faraday constant	1.8 ($\mu\text{M} \cdot \text{mV}^{-1}$)	[S156]
f_m	Fraction of free over buffer-bound $[\text{Ca}^{2+}]$ in mitochondria	0.00025	[S70]
L	Allosteric equilibrium constant for uniporter conformations	50	[S157]
α_c	Cytosolic ADP and ATP buffering coefficient	0.111	[S158]
α_m	Mitochondrial ADP and ATP buffering coefficient	0.139	[S158]
V_{AGC}	Maximum rate of NADH production via malate-aspartate shuttle	25 (μMs^{-1})	[S70]
V_{MCU}	Maximum rate of MCU calcium flux	0.00001 (μMs^{-1})	[S70]
V_{NCX}	Maximum rate of NCX calcium flux	0.00035 (μMs^{-1})	[S70]
V_{F1F0}	Maximum rate of F1F0 ATPase	35000, (varies) (μMs^{-1})	[S70]·This work
V_{ANT}	Maximum rate of Adenine Nucleotide Translocator	5000 (μMs^{-1})	[S156][S159]
V_p	Maximum rate of SERCA pump	144 (varies) (μMs^{-1})	This work
A_c^{TOT}	Total concentration of cytosolic adenine nucleotides	4500 (μM)	This work
A_m^{TOT}	Total concentration of mitochondrial adenine nucleotides	15000 (μM)	[S156]
NAD_m^{TOT}	Total concentration of mitochondrial pyridine nucleotides	250 (μM)	[S70]
K_1	Dissociation constant for $[\text{Ca}^{2+}]$ translocation by MCU	6 (μM)	[S70]
K_2	Dissociation constant for MCU activation by $[\text{Ca}^{2+}]$	0.38 (μM)	[S157]
K_p	Dissociation constant of $[\text{Ca}^{2+}]$ from SERCA	0.35 (μM)	[S160]
k_h	Michaelis-Menten constant for ATP hydrolysis	1000 (μM)	[S70]
k_{AGC}	Dissociation constant of $[\text{Ca}^{2+}]$ from AGC	0.14 (μM)	[S161]
k_e	Dissociation constant of ATP from SERCA pumps	0.05 (μM)	[S70][S162]
v_o	Rate of NADH oxidation by ETC	600 (varies) (μMs^{-1})	[S70]·This work
V_{GLY}	Rate of glycolysis	450 (varies) (μMs^{-1})	[S70]·This work
V_{HYD}	Maximum rate of ATP hydrolysis	100 (μMs^{-1})	[S70]
v_x	Maximum rate of bidirectional $[\text{Ca}^{2+}]$ leak from mitochondria	0.008 (s^{-1})	[S70]
b_1	Scaling factor between NADH consumption and change in membrane voltage	20	[S70]
b_2	Scaling factor between ATP production by ATPase and change in membrane voltage	3.43	[S156]
δ	Ratio between mitochondrial volume to cytosolic volume	0.0733	[S156]
p_1	Voltage dependence coefficient of MCU activity	0.1 (mV^{-1})	[S70]

p ₂	Voltage dependence coefficient of NCX activity	0.016 (mV ⁻¹)	[S70]
p ₃	Voltage dependence coefficient of [Ca ²⁺] leak	0.05 (mV ⁻¹)	[S70]
p ₄	Voltage dependence coefficient of AGC activity	0.01 (mV ⁻¹)	[S70]
F	Faraday's constant	96480 (Cmol ⁻¹)	
T	Temperature	310.16 (K)	
R	Ideal gas constant	8315 (mJ.mol ⁻¹ K ⁻¹)	
q ₁	Michaelis-Menten-like constant for NAD ⁺ consumption by the TCA cycle	1 (μM)	[S156]
q ₂	Half-maximal activating of the TCA cycle by mitochondrial [Ca ²⁺]	0.1 (μM)	[S70]
	Half-maximal activating for indirect inhibition of the AGC by cytosolic [Ca ²⁺]	0.1 (μM)	[S70]
q ₃	Michaelis-Menten constant for NADH consumption by the ETC	100 (μM)	[S156]
q ₄	Voltage dependence coefficient 1 of ETC activity	177 (mV)	[S156]
q ₅	Voltage dependence coefficient 2 of ETC activity	5 (mV)	[S156]
q ₆	Inhibition constant of ATPase activity by ATP	10000 (μM)	[S156]
q ₇	Voltage dependence coefficient of ATPase activity	190 (mV)	[S156]
q ₈	Voltage dependence coefficient of ATPase activity	8.5 (mV)	[S156]
q ₉	Voltage dependence of the proton leak	2 (μMs ⁻¹ mV ⁻¹)	[S156]
q ₁₀	Rate constant of voltage-independent proton leak	-30 (μMs ⁻¹)	[S156]
K _i	Half-max for IP ₃ activation of IP ₃ R	1.0 (uM)	[S156]
f _{ER}	Buffering coefficient in the ER	0.01	[S70]
na	Cooperativity of Ca ²⁺ on IP ₃ R	3.0	[S70]
α	Volumic ratio between the endoplasmic reticulum and the cytosol	0.1	[S70]
C _{tot}	Total calcium in the system	3240 (μM)	[S70]
v _{SOCC}	Maximum rate of Ca ²⁺ entry via SOCC	1.6 (μMs ⁻¹)	[S156]
v _{PMCA}	Maximum rate of Ca ²⁺ flux through PMCA	20 (μMs ⁻¹)	This work
K _{SOCC}	Half-saturation constant for SOCC	100.0 (uM)	This work
K _a	Half-max for Ca ²⁺ activation of IP ₃ R	0.3 (uM)	[S70]
v _{IP3}	Maximum flux through IP ₃ receptors	95.0 (μMs ⁻¹)	[S70]
n _i	Hill coefficient for IP ₃ binding	4	[S70]
b _{ip3}	Baseline IP ₃ receptor open probability	0.01	[S70]
f _c	Buffering coefficient in cytosol	0.01	[S70]
k ⁺	Calcium-dependent activation rate constant for receptor gating variable R _i	20 (μM ^{-n_i} s ⁻¹)	[S70]
k ⁻	Inactivation rate constant	0.02 (s ⁻¹)	[S70]
t _{stim}	Time of stimulation	200 (s)	This work
a _{o1}	IP ₃ pulse rate	500 (uM/s)	This work

Table S3. Clinical outcomes in six LS patients carrying *MT-ATP6* mutations upon treatment with sildenafil, Related to Table 1.

Patient 1	
Age, sex assigned at birth	16 years, male
Variant	<i>MT-ATP6</i> m.9176T>G (99.4 % heteroplasmy)
Past medical history	Mildly globally delayed early childhood development, tendency to walk insecurely (ataxia), delayed speech development and dysarthria and at 14 years of age diagnosis of a combined axonal and demyelinating neuropathy and increasing lower limb weakness, thigh atrophy, and cavus foot deformity.
Symptoms	At the age of 16 years, several days after an upper respiratory tract infection a metabolic crisis developed with encephalopathy, cerebral seizures, severe motor-sensory neuropathy predominantly in the legs, mechanical ventilation due to respiratory insufficiency, hardly any spontaneous movements possible, cardiomyopathy (LVEF around 45 %, histology: chronic myocardial damage, macrophage-dominated inflammatory reaction), nutrition only possible via PEG feeding tube. The motor-sensory neuropathy had been preexistent and had impeded his walking ability, which was, however, possible with gait ataxia. Episodes of movement difficulties occurred frequently since the age of 8-10 years and each one lasted for a few hours. In these episodes, the patient and his parents report that he had been completely unable to move his legs for half a day, which later gradually improved without intervention. There was no trigger, infection, or fever.
Sildenafil Doses	3 x 40 mg (2 mg/kg body weight (BW)/day in 3 single doses)
Treatment progress: treatment started in December 2018 and is ongoing	
After 1 month	Extubation possible, can breathe independently
After 3 months	Sitting stably, movements against gravity possible, stable respiration, ECHO cardiography left ventricle ejection fraction (LVEF) increase to 65 %
After 5 months	Tracheostomy closure and percutaneous gastrostomy (PEG) feeding tube removal, EEG normal, discontinuation of antiepileptic drugs, after 10 months back to regular school attendance
After 18 months	Independently moves while sitting in the wheelchair, even manages to climb curbstones with the wheelchair and maneuver on ascending terrain
After 30 months	Lifts himself into a wheelchair, walks at times with a walker with forearm supports, medication was very well tolerated throughout the period, echocardiographic investigations have been normal the entire time, cerebral seizures did not recur. Peripheral neuropathy did not improve, but did not progress either.
After 3 years	The movement episodes, that occurred approximately every 2 months before the start of the sildenafil therapy, disappeared after the initiation of sildenafil therapy. As the patient had increased in body weight, we re-adapted the dosage to 2 mg/kg BW/day.
Last assessment after 6 years	The patient has remained stable and has started professional training as a clerk, no further metabolic crises despite two COVID19 infections. He is able to move independently in his wheelchair, and he can use a walker with arm support indoors. He can eat a normal diet. Sometimes he complained about palpitations, but echocardiography and ECG-monitoring over 24 hours was normal in December 2024. Neuropathy and dysarthria have not improved.
Patient 2	
Age, sex assigned at birth	15 years, male
Variant	<i>MT-ATP6</i> m.8993T>G (87.7 % heteroplasmy)
Past medical history	The motor development of the patient was delayed with sitting and crawling at one year of age, free walking at 2.5 years of age. The boy was always ataxic and fell frequently. Parents noted an insensitivity to pain. Heart, eye, and hearing examinations were always normal.
Symptoms	Parents report that about twice a year there were situations in which he acutely decompensated during febrile illnesses. This was accompanied by severe

	vomiting and deterioration of speech with slurred articulation (transient aphasia). At the end of the infections, he was "not like before" and it took him at least two months before he fully recovered.
Sildenafil Doses	3 x 20 mg (1.2 mg/kg BW/day in 3 single doses)
Treatment progress: treatment started in October 2019 and is ongoing	
After 2 months	Increase in free walking distance from 500 m to >2000 m, significantly less exhaustible, increase in speed and safety in the Nine-Hole-Peg Test. Medication is well tolerated, normal cardiac examination findings
Last assessment after 5 years	No more metabolic crisis recorded to date. Parents describe their child as having considerably more energy over the day in comparison to his state before initiation of sildenafil treatment. He can now stay up longer and does not become sleepy over the day as before. He has taken up sports (tennis and swimming). His ataxia has improved and his tendency to stumble and fall is greatly diminished. He tolerates the medication well without unwanted side effects. He can now walk up to 5 km without longer pausing. Normal cardiologic function as assessed by echocardiography and ECG monitoring in June 2024. His SARA score improved from 15.5 (year 2018) to 11 (year 2024).
Patient 3	
Age, sex assigned at birth	21 years, female
Variant	<i>MT-ATP6</i> m.9185T>C (100 % homoplasmic)
Past medical history	Mildly delayed motor development, first metabolic crisis at the age of 5 years during a viral infection with the loss of ambulation. These incidences increased in frequency starting at the onset of puberty. The episodes lasted from 3 hours to 3 days. At the same time, horizontal ophthalmoplegia and progressive dependence from a wheelchair. Axonal neuropathy was noted from 12 years of age with a cavus foot deformity and absent lower limb reflexes.
Symptoms	Relapsing episodes of muscle weakness and paralysis (4-5 times per week), severe demyelinating neuropathy, urinary incontinence, chronic metabolic acidosis, exercise insufficiency.
Sildenafil Doses	3 x 25 mg (1.4 mg/kg BW/day in 3 single doses) slow increase up to 2.9 mg/kg BW/day
Treatment progress: treatment started in May 2019 and has been discontinued in November 2020	
After 6 months	Significant decrease in paralytic episodes and absence of muscle cramps. The medication is well tolerated. The motor neuropathy did not improve.
After 12 months	The patient has experienced an itchy rash predominantly in the face and trunk possibly as an allergic reaction against sildenafil and it was decided to discontinue the sildenafil medication. However, the symptoms of muscle cramps and paralytic episodes returned upon discontinuing sildenafil .
Last assessment after 18 months	The sildenafil medication had been reinitiated and the symptoms of the intermittent paraplegia improved again. However, also the drug rash reappeared. The sildenafil medication was discontinued.
Patient 4	
Age, sex assigned at birth	38 years, male
Variant	<i>MT-ATP6</i> m.8993T>G (96.7 % heteroplasmy)
Past medical history	Psychomotor developmental delay (started walking at 2 years, started speaking at 3 years), progressive ataxia, loss of independent walking at 15 years, loss of hearing at 28 years, considerable exercise intolerance starting at 31 years, massive myoclonic seizures, loss of the capability to sit independently at 35 years, loss of head control.
Symptoms	Chronic condition with progressive worsening of motor function
Sildenafil Doses	May 2022, starting with 3 x 10 mg/day, increased dosage to actual 3 x 30 mg/d (1.5 mg/kg BW/day)
Treatment progress: treatment started in May 2022 and is ongoing	
After 8 months	He experienced quite sudden improvement after 2 days at the lower dosage (3 x 10 mg/day, in terms of muscle strength. He was able to move and raise the legs from the bed and his head control improved. The dysphagia also improved. This initial improvement was maintained but no further improvements were noticed

	after he reached the high dose (3 x 30 mg/day) from June 2022. He tolerated well the drug (no hypotension, only occasional priapism).
Last assessment after 2.5 years	The parents are satisfied with the outcome of the treatment. Already after few days with dosages of 3 x 30 mg he started moving the legs again. These improvements are maintained, and the patient keeps making small progresses. The parents found a significant improvement in speaking, eating, and in the movement of the arms. He can now even almost stay in the sitting position by himself, which was not possible before the therapy. Parents noted that when he had to discontinue his sildenafil medication in the course of cataract surgery, he immediately became more flaccid and could not hold his head anymore. This improved as soon as sildenafil therapy could be re-initiated after surgery.
Patient 5	
Age, sex assigned at birth	9 months, male
Variant	<i>MT-ATP6</i> m.8570T>C (98.5 % heteroplasmy)
Past medical history	Immediately after birth, the patient was diagnosed as floppy infant (severe muscle hypotonia). He was too weak for feeding and had to be fed with a gastroenteral tube, anemia with Hb of 5 g/dl, serum lactate elevation to 5.48 mmol/l, hypertrophic cardiomyopathy. By 9 months of age, he had to be admitted to hospital 10 times due to metabolic crises associated with febrile infections.
Symptoms	Due to the severe state of health, palliative care had been initiated shortly before the start of the sildenafil therapy.
Sildenafil Doses	3 x 4 mg (1.5 mg/kg BW/day in 3 single doses) has then been adapted to the increasing body weight.
Treatment progress: treatment started in September 2019 and is ongoing	
After 12 months	No more palliative care was required. No metabolic crisis observed since starting treatment. The child has progressed in his motor development: can turn, can swallow soft foods, can sit stably at table, head control exists, has gained weight well. The medication is well tolerated.
Last assessment after 5 years	No further metabolic crises despite several febrile infections. In terms of his development, he has made significant progress. He understands and responds to speech, laughs, listens to the phone, reaches for objects that interest him. No seizures. Normal night sleep. He likes to move around the home in his walker and takes his own steps. When he has his ankle clipers on, he can briefly support his body weight. No unwanted side effects of sildenafil have been observed.
Patient 6	
Age, sex assigned at birth	7 years, female
Variant	<i>MT-ATP6</i> m.8993T>C (100 % homoplasmy)
Past medical history	Normal psychomotor development, reaching all developmental milestones until the age of 7 years
Symptoms	At the age of 7 years and 4 months, during febrile upper respiratory tract infection with vomiting for 6 days she experienced a metabolic crisis with genialized muscular hypotonia, loss of head control, declining cognitive capabilities, loss of speech, and dysphagia. CK levels increased to >3000 U/L (N 29-168) and increased liquor lactate levels of 2.8–2.9 mmol/l (N < 2.2). MRI showed bilateral regions in the basal ganglia with increased T ₂ signal intensity. Subsequently, she developed progressive distal spastic paraplegia and dystonia of upper limbs and face.
Sildenafil Doses	1.8 mg/kg BW/day in 3 single doses
Treatment progress: treatment started in June 2024 and is ongoing.	
After 3 months	The girl showed significant improvements in both motor and cognitive abilities. She regained full head control, was able to sit unsupported, and could stand with assistance. Her facial expressions improved, and her consciousness normalized. While she remained nonverbal, her vocalizations and emotional expression showed notable improvement.
After 8 months	Verbal communication capabilities improved, she gained muscle strength, the ataxia subsided, however, she suffers from progressive dystonia. Intentional motor movements are now possible.

Table S4. Questionnaire on side effects of continuous sildenafil use in six LS patients treated with sildenafil for longer than six months, Related to Table 1.

Queries refer to whether (i) the symptom is absent (N), (ii) the severity of the symptom (mild 1 to severe 5), (iii) no information provided (-).

	patient 1	patient 2	patient 3	patient 4	patient 5	patient 6
Cardiovascular system						
arterial hypotension	N	N	N	N	N	N
postural syncope (dizziness when getting up)	N	N	N	-	-	N
worsening of existing heart failure	N	N	N	N	N	N
palpitations	3 ^a	N	N	N	N	N
Chest						
chest pain	N	N	N	-	-	N
difficulties breathing	N	N	1	N	N	N
burning feeling in the chest	N	N	N	-	-	N
tightness of the chest	N	N	-	-	-	N
Gastrointestinal system						
difficulty in swallowing	N	N	N	N	N	4 ^d
excessive hunger	5 ^b	N	N	N	-	N
nausea	N	N	N	N	-	N
vomiting	N	N	N	N	-	N
stomach upset	N	N	N	N	-	N
burning feeling in the stomach	3	N	N	-	-	N
diarrhea	N	N	1	N	N	N
indigestion	N	N	4	N	-	N
Genitourinary system						
priapism (prolonged, painful erection of penis)	N	N	-	N	N	-
bladder pain (increased urinary frequency)	N	N	N	N	-	N
pain on urination	N	N	N	N	-	N
urinary urgency	N	N	3	N	N	N
Brain, head, and sensory system						
headaches, migraine	N	N	N	N	-	N
convulsions (seizures)	N	N	-	N	N	N
slurred speech	N	N	N	N	-	? ^e
trembling and shaking	N	N	1	N	N	N
lack of coordination	N	N	N	N	-	? ^f
nosebleeds	N	N	N	N	N	N
facial flushing	N	N	5 ^c	N	-	N
transient visual disturbances	N	N	N	N	-	N
double vision	N	N	N	N	-	N
eye pain	N	N	N	N	-	N
deafness or hearing loss	N	N	N	N	N	N
tinnitus	N	N	N	N	-	N

Psychiatric symptoms						
anxiety	N	N	N	N	N	N
confusion	N	N	N	N	N	N
unusual tiredness or weakness	N	N	N	N	N	N
difficulty in concentrating	N	N	N	N	N	N
abnormal dreams	N	N	N	-	-	N
sleepiness	N	N	N	N	N	N
sleeplessness	N	N	N	N	-	N
mental depression	N	N	N	N	-	N

Notes: **(a)** The palpitations were investigated by a cardiologist, but no arrhythmia was detected. **(b)** The hyperphagia was related to a transition period from school to vocational training (3 years after the start of sildenafil treatment), had since improved. **(c)** The patient suffered from an itchy rash and had to discontinue the drug, whereupon the symptoms recurred. **(d)** The dysphagia is due to Leigh syndrome (was present before sildenafil therapy). Cannot be evaluated due to **(e)** dysarthria and **(f)** incoordination due to Leigh syndrome.

Table S5. List of oligonucleotides, Related to STAR Methods.

OLIGONUCLEOTIDE		SOURCE	IDENTIFIER
<i>MT-ATP6</i> CAACCGACTAATCACCACCC	Forward:	IDT	N/A
<i>MT-ATP6</i> GTTGAGCCGTAGATGCCGTC	Reverse:	IDT	N/A
<i>ATP6_2</i> AACCAATAGCCCTGGCCGTA	Forward:	IDT	N/A
<i>ATP6_2</i> AGGGCTCATGGTAGGGGTAAA	Reverse:	IDT	N/A
<i>GAPDH</i> CTGGTAAAGTGGATATTGTTGCCAT	Forward:	IDT	N/A
<i>GAPDH</i> TGGAATCATATTGGAACATGTAAACC	Reverse:	IDT	N/A
<i>OAZ1</i> Forward: GGATCCTCAATAGCCACTGC		IDT	N/A
<i>OAZ1</i> Reverse: TACAGCAGTGGAGGGAGACC		IDT	N/A
<i>NESTIN</i> Forward: TTCCCTCAGCTTTCAGGAC		IDT	N/A
<i>NESTIN</i> Reverse: GAGCAAAGATCCAAGACGC		IDT	N/A
<i>PAX6</i> Forward: CCAGGGCAATCGGTGGTAGT		IDT	N/A
<i>PAX6</i> Reverse: ACGGGCACTCCCGCTTATAC		IDT	N/A
<i>CTIP</i> Forward: TGGGTGCCTGCTATGACAAG		IDT	N/A
<i>CTIP</i> Reverse: GATGCCTTTCGTGGGTGAGA		IDT	N/A
<i>SOX2</i> GTATCAGGAGTTGTCAAGGCAGAG	Forward:	IDT	N/A
<i>SOX2</i> TCCTAGTCTTAAAGAGGCAGCAAAC	Reverse:	IDT	N/A
<i>PRKG1</i> ACAACTGTACCCGGACAGCGA	Forward:	IDT	N/A
<i>PRKG1</i> TCCTCTTGACACCCTGCCTGAT	Reverse:	IDT	N/A
<i>OCT4</i> Forward: GTGGAGGAAGCTGACAACAA,		IDT	N/A
<i>OCT4</i> Reverse: ATTCTCCAGGTTGCCTCTCA		IDT	N/A
<i>NANOG</i> Forward: CCTGTGATTTGTGGGCCTG		IDT	N/A
<i>NANOG</i> GACAGTCTCCGTGTGAGGCAT	Reverse:	IDT	N/A
<i>DBX1</i> Forward: CTGGGCCTGAAAGACTCGC,		IDT	N/A
<i>DBX1</i> Reverse: CCGCTAGACAGGAGTTCGC		IDT	N/A
<i>Myco-f1</i> : CGCCTGAGTAGTACGTTTCGC	Forward:	IDT	N/A
<i>Myco-f2</i> : GCGGTGTGTACAAACCCCGA	Forward:	IDT	N/A
<i>Myco-f3</i> : TGCCTGAGTAGTCACTTCGC	Forward:	IDT	N/A
<i>Myco-f4</i> : CGCCTGGGTAGTACATTTCGC	Forward:	IDT	N/A

Myco-f5: CGCCTGAGTAGTAGTCTCGC	Forward:	IDT	N/A
Myco-f6: TGCCTGGGTAGTACATTCGC	Forward:	IDT	N/A
Myco-r1: GCGGTGTGTACAAGACCCGA	Reverse:	IDT	N/A
Silencer™ select siRNA <i>PRKG1</i> Forward: GGAUAGAGGUUCGUUUGAATT	Ambion	Cat#4392420; assay ID s11131	
Silencer™ select siRNA <i>PRKG1</i> Reverse: UUCAAACGAACCUCUAUCCCT	Ambion	Cat#4392420; assay ID s11131	
Silencer™ select SiRNA negative Control No. 1	Ambion	Cat#4390843	
<i>MT-ND1</i> Forward: ccctaaaacccgccacatct	IDT	N/A	
<i>MT-ND1</i> Reverse: ggcctaggttgaggttgacc	IDT	N/A	
<i>NDUVF1</i> Forward: tgatcctcacaagctgctgg	IDT	N/A	
<i>NDUVF1</i> Reverse: ctgcagattggaggcctcat	IDT	N/A	