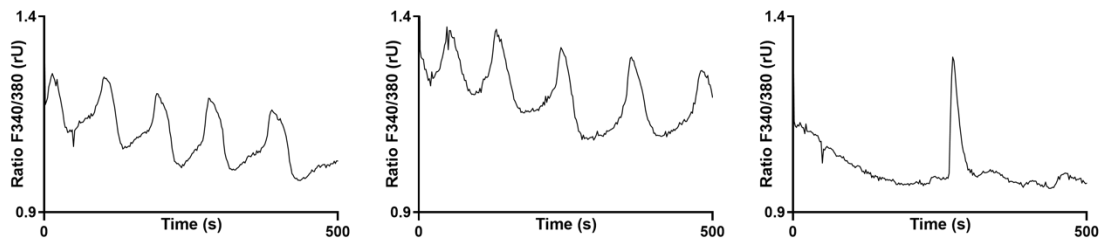


Spontaneous Ca^{2+} transients



ATP-responsive Ca^{2+} transients

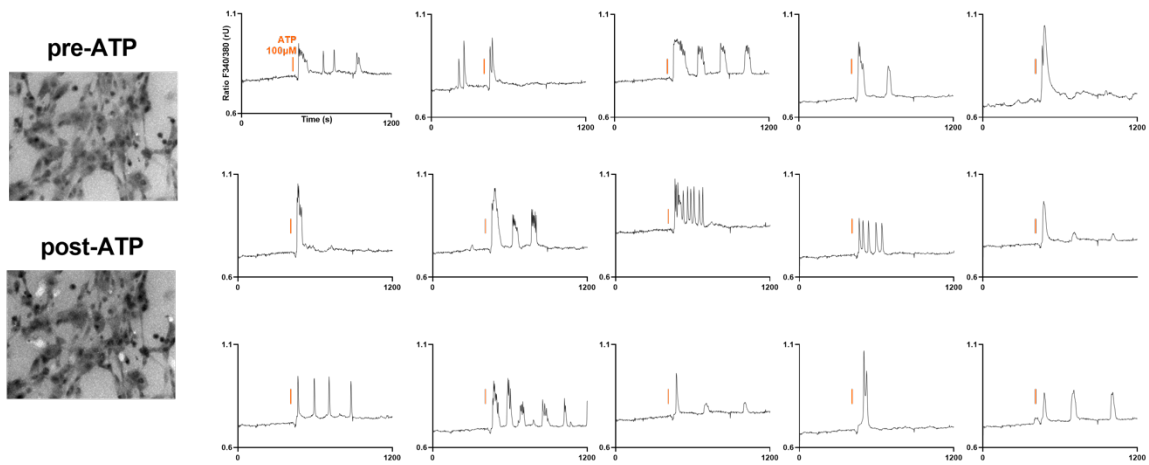


Figure S1. Calcium transients in astrocytes. Example spontaneous calcium transients in astrocytes loaded with Fura-2/AM. Curves show Fura ratio F340/380 nm in relative units (up). Example ATP-responsive calcium transients in in astrocytes loaded with Fura-2/AM. ATP was added to a final concentration of 100 μM . On the left, example Fura ratio images showing brighter cells post-ATP. Curves show Fura ratio F340/380 nm in relative units (down)

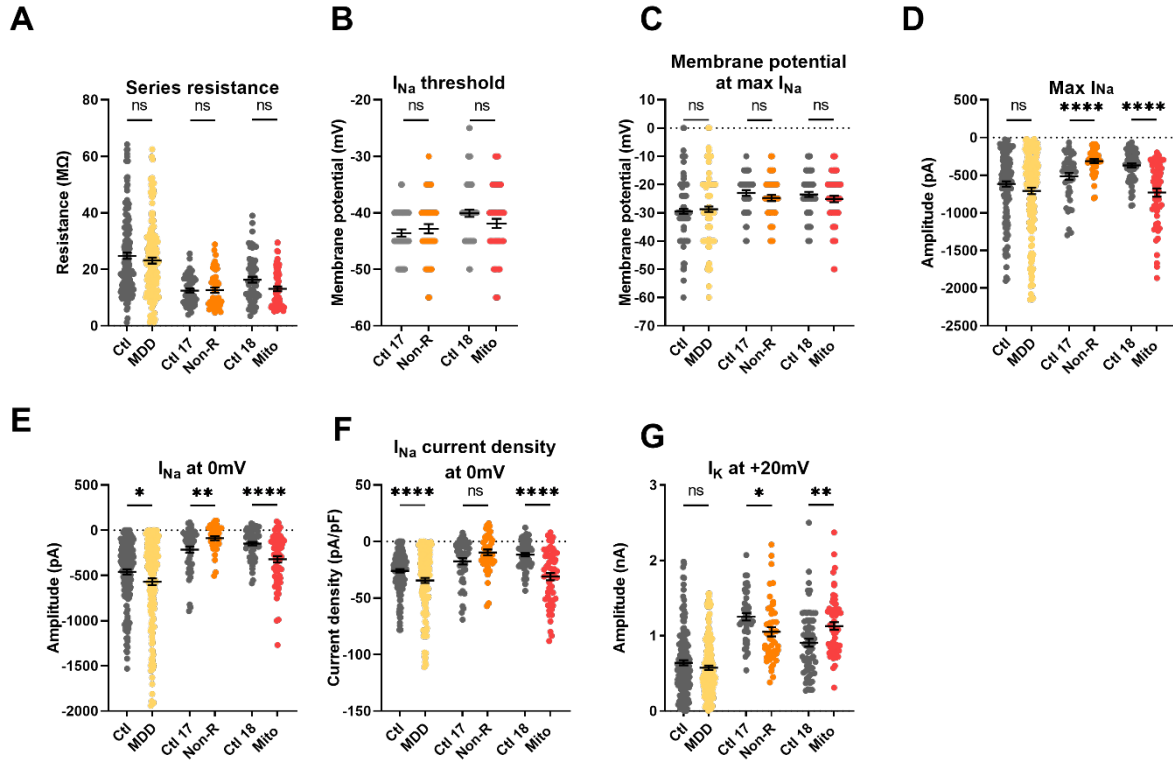


Figure S2. Electrophysiological recordings in iPS-neurons. (A) **Series resistance** was recorded immediately after reaching the whole-cell configuration, but was not compensated. Dot plot shows mean resistance in $M \Omega \pm SEM$. (B) **Sodium current threshold** represents the membrane potential at which the sodium inward current appeared. Dot plot shows mean membrane potential in $mV \pm SEM$. (C) **Membrane potential at maximum sodium current.** Dot plot shows mean membrane potential in $mV \pm SEM$. (D) **Maximum sodium current.** Dot plot shows mean maximal amplitude in $pA \pm SEM$. (E) **Sodium current at 0 mV.** Dot plot shows mean amplitude in $pA \pm SEM$. (F) **Sodium current density at 0 mV.** Dot plot shows mean current density in $pA/pF \pm SEM$. (G) **Potassium current at +20 mV.** Dot plot shows mean amplitude in $pA \pm SEM$.

Each dot on the graphs represents a replicate. All data were analyzed with paired t-test, except spontaneous activity which was analyzed with Fisher's exact test. Significant differences are indicated with * ($p < 0.05$), ** ($p < 0.005$), *** ($p < 0.0005$) and **** ($p < 0.0001$).

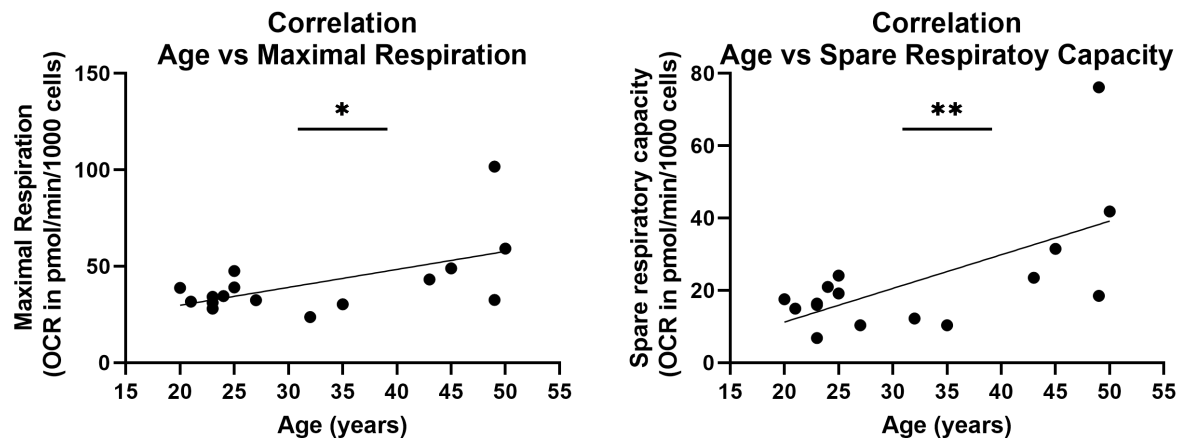


Figure S3. Correlation between age and mitochondrial respiration. Graphs represent Pearson correlation between respiratory data in the non-depressed control cohort ($n = 16$) and a simple linear regression. Maximal respiration vs age $p = 0.0223$, Spare Respiratory Capacity vs age $p = 0.0093$

Each dot on the graphs represents an individual. Significant differences are indicated with * ($p < 0.05$) and ** ($p < 0.005$).

Table S1. Patients information

	Ctl 17	Non-R	Ctl 18	Mito
Age (years)	43	43	20	18
Sex	Male	Male	Female	Female
Clinical findings	Absence of past or present depressive or mental disorders Absence of documented health conditions	Depression ratings - HAM-D t0: 34 (very severe) - HAM-D t0+2months: 22 (severe)	Absence of past or present depressive or mental disorders Absence of documented health conditions	Symptoms - Neurological deficits (paresthesia in arms) - Weakness - Joint pain - Headaches - Gastrointestinal symptoms - Loss of visual acuity - Hypersomnia - Exhaustion Blood test - Elevated blood lactate - Low LDH subunit 3h1m - High LDH subunit 1h3m - High M2 pyruvate kinase - Low Coenzyme Q10
Treatment	None	Antidepressants - Duloxetine - Lithium + venlafaxine Others - Enalapril (hypertension)	None	None

Table S2. Neural progenitor cells markers PAX6 and SOX2

	DAPI	SOX2	% SOX2	PAX6	% PAX6
Ctl 17 #1	112	97	86.6	106	94.6
Ctl 17 #2	88	82	93.2	83	94.3
Ctl 17 #3	101	93	92.1	97	96.0
Ctl 17 #4	301	272	90.4	286	95.0
Total	602	544	90.4	572	95.0
Non-R #1	88	78	88.6	81	92.0
Non-R #2	101	94	93.1	94	93.1
Non-R #3	87	79	90.8	84	96.6
Total	878	795	90.5	831	94.6
Ctl 18 #1	88	77	87.5	84	95.5
Ctl 18 #2	96	88	91.7	91	94.8
Ctl 18 #3	101	94	93.1	97	96.0
Ctl 18 #4	285	259	90.9	272	95.4
Total	570	518	90.9	544	95.4
Mito #1	81	74	91.4	76	93.8
Mito #2	89	85	95.5	88	98.9
Mito #3	80	75	93.8	77	96.3
Mito #4	101	94	93.1	96	95.0
Total	351	328	93.4	337	96.0

Table S3. Patients and controls information – Astrocytes cohort

	Ctl 5	MDD 5	Ctl 10	MDD 10	Ctl 11	MDD 11
Age (years)	24	18	20	21	23	23
Sex	Male	Male	Male	Male	Male	Male
Clinical findings	Absence of past or present depressive or mental disorders Absence of documented health conditions	Recurrent depressive disorder, current episode severe with psychotic symptoms (F33.3)	Absence of past or present depressive or mental disorders Absence of documented health conditions	Severe depressive episode without psychotic symptoms (F32.2)	Absence of past or present depressive or mental disorders Absence of documented health conditions	Severe depressive episode without psychotic symptoms (F32.2)
Treatment	None	Bupropion, Mirtazapin	None	Valdoxan	None	Cipralex

Genetic testing

Total genomic DNA was extracted from primary fibroblasts using DNA MiniKit (QUIAGEN, Hilden, Germany). Exome capture was conducted using Twist Human Core Exome (Twist Bioscience HQ, CA, USA) according to the manufacturer's instructions. Sequencing was performed using an Illumina NextSeq platform. Exome data alignment to the GRCh38/hg38 reference genome, variant calling and variant annotation was done using the CLC Main Workbench (Qiagen, Hilden, Germany). Variant filtering was performed by an in-house bioinformatics pipeline. Variant classification was done according to ACMG guidelines. A targeted virtual panel including 387 nuclear genes known to cause mitochondrialopathies (AARS2, ABCB7, ABCD1, ABHD5, ACAD9, ACADM, ACADS, ACADVL, ACO2, ACOX1, ACTG2, ADGRV1, AFG2A, AFG3L2, AGK, AGL, AGRN, AIFM1, ALAS2, ALDH3A2, ALDH7A1, ALG14, ALG2, ANO10, APTX, ARG1, ARHGEF9, ARSA, ARX, ASPA, ATAD3A, ATP13A2, ATP1A2, ATP1A3, ATP5F1A, ATP5F1D, ATP5F1E, ATP5MK, ATP7B, ATPAF2, AUH, BCS1L, BOLA3, BTBD, C19orf12, C1QBP, C2orf69, CA5A, CACNA1A, CACNA1H, CACNB4, CAD, CARS2, CASR, CCDC115, CDKL5, CHAT, CHCHD10, CHD2, CHD8, CHKB, CHRNA1, CHRNA2, CHRNA4, CHRNA1, CHRNA2, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA8, CHRNA9, CHRNA10, CHRNA11, CHRNA12, CHRNA13, CHRNA14, CHRNA15, CHRNA16, CHRNA17, CHRNA18, CHRNA19, CHRNA20, CHRNA21, CHRNA22, CHRNA23, CHRNA24, CHRNA25, CHRNA26, CHRNA27, CHRNA28, CHRNA29, CHRNA30, CHRNA31, CHRNA32, CHRNA33, CHRNA34, CHRNA35, CHRNA36, CHRNA37, CHRNA38, CHRNA39, CHRNA40, CHRNA41, CHRNA42, CHRNA43, CHRNA44, CHRNA45, CHRNA46, CHRNA47, CHRNA48, CHRNA49, CHRNA50, CHRNA51, 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Lipid extraction

Lipid extraction was performed according to the method of Bligh and Dyer 1 in the presence of not naturally occurring lipid species as internal standards. The following lipid species were added as internal standards: CE 17:0, CE 22:0, CL 14:0/14:0/14:0/14:0, Cer 18:1;O2/14:0, Cer 18:1;O2[D7]/18:0, DG 14:0/14:0, DG 20:0/20:0, FC[D7], GlcCer 18:1;O2/12:0, GlcCer 18:1;O2[D5]/18:0, LPC 13:0, LPC 19:0, LPE 13:0, LPE 18:1[D7], PC 14:0/14:0, PC 22:0/22:0, PE 14:0/14:0, PE 20:0/20:0 (di-phytanoyl), PG 14:0/14:0, PG 20:0/20:0, PI 18:1[D7]/15:0, PS 14:0/14:0, PS 20:0/20:0 (di-phytanoyl), SM 18:1;O2/12:0, SM 18:1;O2/18:1[D9], TG 17:0/17:0/17:0, and TG 19:0/19:0/19:0.

A sample amount corresponding to 100 µg protein was extracted. Chloroform phase was recovered by a pipetting robot (Tecan Genesis RSP 150) and vacuum dried. The residues were dissolved in chloroform/methanol/2-propanol (1:2:4 v/v/v) with 7.5 mM ammonium formate.

Quantitative lipidomics

The analysis of lipids was performed by direct flow injection analysis (FIA) using a hybrid quadrupole-Orbitrap mass spectrometer (FIA-FTMS; high mass resolution).

The setup is described in detail in Høring et al. 2. Triglycerides (TG), diglycerides (DG) and cholesteryl ester (CE) were recorded in positive ion mode FTMS in m/z range 500 - 1000 for 1 min with a maximum injection time (IT) of 200 ms, an automated gain control (AGC) of 1*10⁶, three microscans and a target resolution of 140,000 (at 200 m/z). The mass range of negative ion mode was split into two parts. Lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) were analyzed in the range 400 - 650 m/z. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin (SM) and ceramide were measured in m/z range 520 - 960. Multiplexed acquisition (MSX) was used for, the [M +NH₄]⁺ of free cholesterol (FC) (404.39 m/z) and FC[7D] (411.43 m/z) 0.5 min acquisition time, with a normalized collision energy of 10 %, an IT of 100 ms, AGC of 1*10⁵, isolation window of 1 m/z, and a target resolution of 140,000 (see 3). Data processing details were described in Høring et al. 2 using the ALEX software 4 for peak assignment and intensity picking. The quantification was performed by multiplication of the spiked IS amount with analyte-to-IS ratio.

Lipid species were annotated according to the latest proposal for shorthand notation of lipid structures that are derived from mass spectrometry 5.

1. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; **37**(8): 911-917.
2. Horing M, Ejlsing CS, Krautbauer S, Ertl VM, Burkhardt R, Liebisch G. Accurate quantification of lipid species affected by isobaric overlap in Fourier-transform mass spectrometry. *J Lipid Res* 2021; **62**: 100050.
3. Horing M, Ejlsing CS, Hermansson M, Liebisch G. Quantification of Cholesterol and Cholesteryl Ester by Direct Flow Injection High-Resolution Fourier Transform Mass Spectrometry Utilizing Species-Specific Response Factors. *Anal Chem* 2019; **91**(5): 3459-3466.
4. Husen P, Tarasov K, Katafiasz M, Sokol E, Vogt J, Baumgart J *et al.* Analysis of lipid experiments (ALEX): a software framework for analysis of high-resolution shotgun lipidomics data. *PLoS One* 2013; **8**(11): e79736.

5. Liebisch G, Fahy E, Aoki J, Dennis EA, Durand T, Ejsing CS *et al.* Update on LIPID MAPS classification, nomenclature, and shorthand notation for MS-derived lipid structures. *J Lipid Res* 2020; **61**(12): 1539-1555.