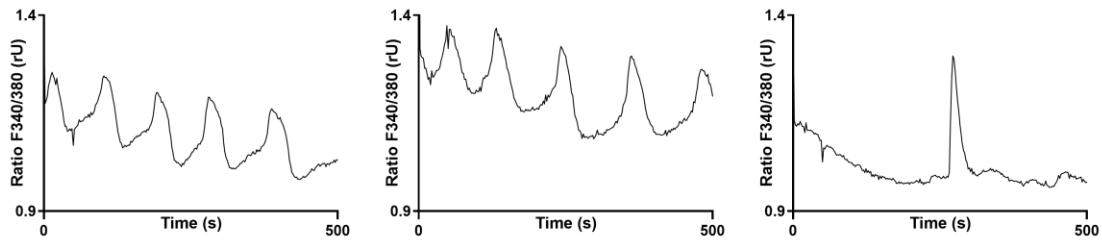


Figure S1. Significant correlations between depression severity and biological findings. Graphs represent Spearman correlation between respiratory data in the patients with MDD cohort ($n = 16$) and a simple linear regression. P-values are presented in Table S2.

Spontaneous Ca^{2+} transients



ATP-responsive Ca^{2+} transients

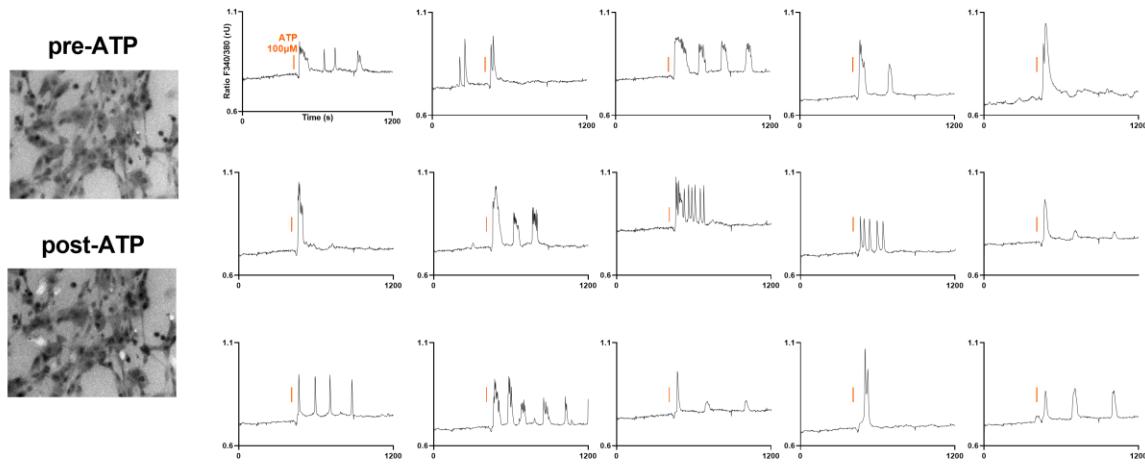


Figure S2. Calcium transients in astrocytes. Example spontaneous calcium transients in astrocytes loaded with Fura-2/AM. Curves show Fura ratio F340/380 nm in relative units (up). Example ATP-responsive calcium transients in astrocytes loaded with Fura-2/AM. ATP was added to a final concentration of 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)

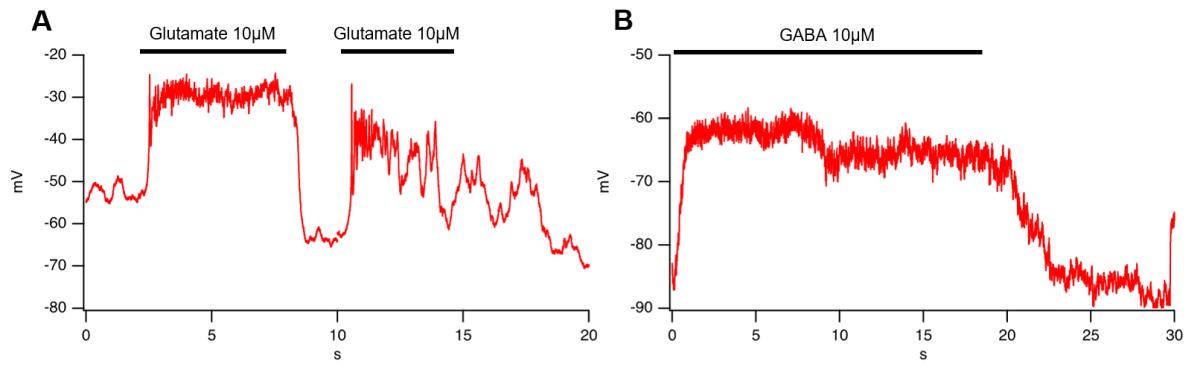


Figure S3. Glutamate and GABA response in current-clamp. Neurons were recorded in whole-cell patch clamp, in current clamp mode without injection of current. Membrane potential was measured while applying 10 μ M glutamate (A) or γ -Aminobutyric acid (GABA) (B) using a micro-perfusion system. Bars represent application duration. Note that the direction of GABA response results from a symmetrical chloride concentration in the bath and pipette solutions.

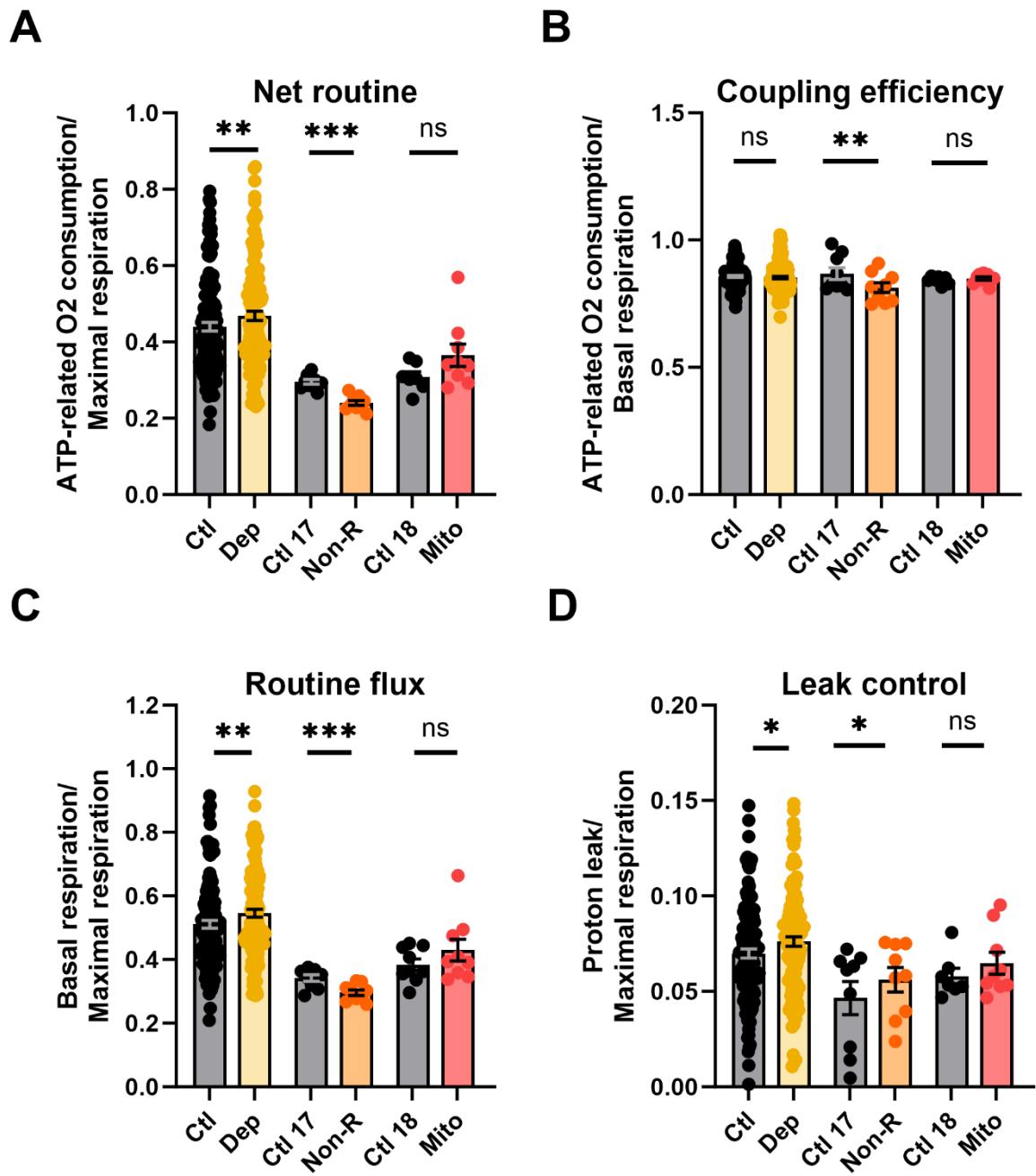


Figure S4. Mitochondrial flux ratios in fibroblasts. (A) **Net routine** is the ratio between ATP-related O₂ consumption and maximal respiration (B) **Coupling efficiency** is the ratio between ATP-related O₂ consumption and basal respiration (C) **Routine flux** is the ratio between basal and maximal respiration (D) **Leak control** is the ratio between proton leak and maximal respiration. Dot plots show the respective ratios \pm SEM Ctl: non-depressed controls cohort; MDD: major depressive disorder cohort; Ctl 17 and Ctl 18: non-depressed controls; Non-R: non-responder patient; Mito: mitochondriopathy patient. All data were analyzed with paired t-test, significant differences were indicated with * ($p<0.05$), ** ($p<0.005$), *** ($p<0.0005$)

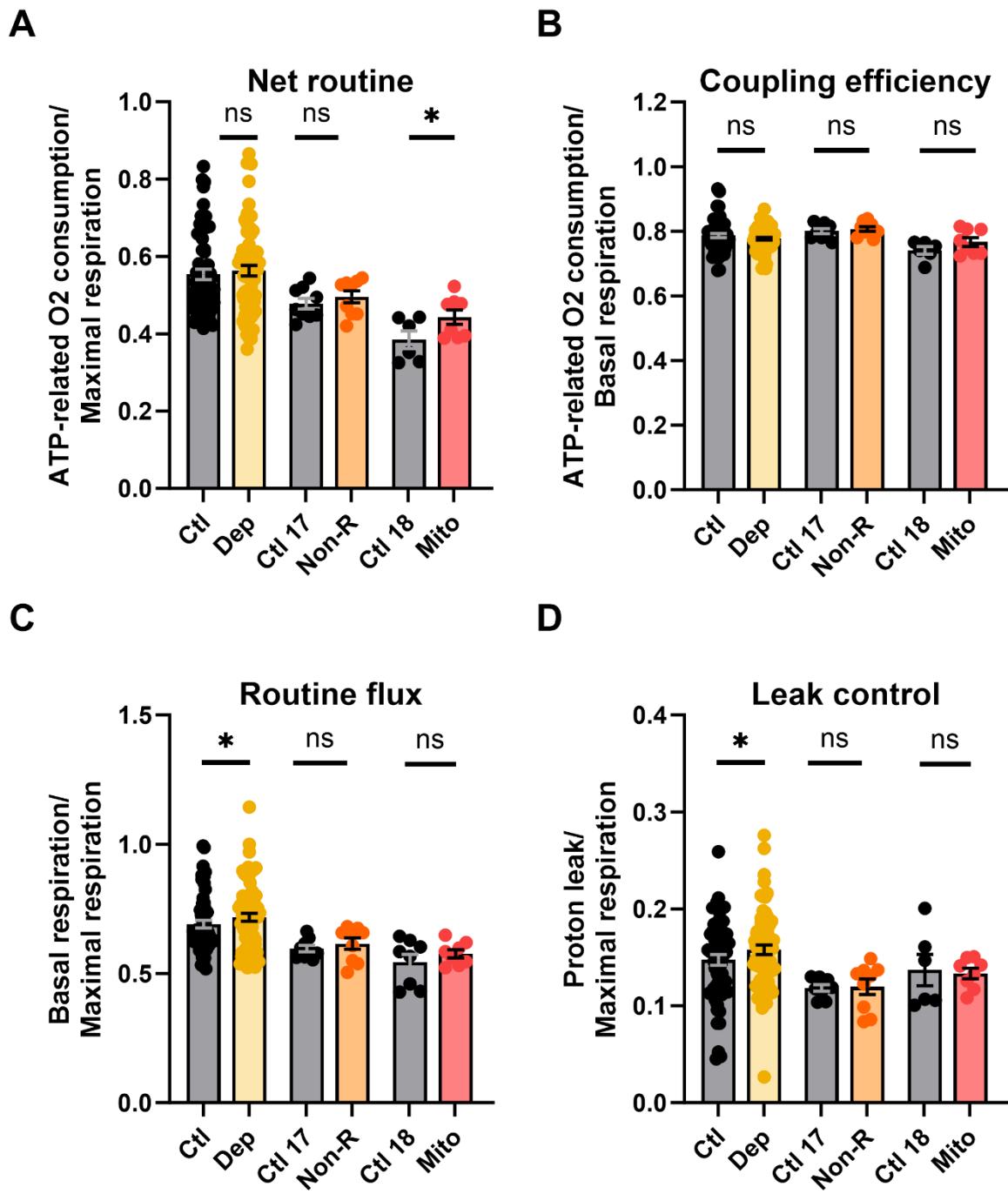


Figure S5. Mitochondrial flux ratios in NPCs. (A) **Net routine** is the ratio between ATP-related O₂ consumption and maximal respiration (B) **Coupling efficiency** is the ratio between ATP-related O₂ consumption and basal respiration (C) **Routine flux** is the ratio between basal and maximal respiration (D) **Leak control** is the ratio between proton leak and maximal respiration. Dot plots show the respective ratios \pm SEM Ctl: non-depressed controls cohort; MDD: major depressive disorder cohort; Ctl 17 and Ctl 18: non-depressed controls; Non-R: non-responder patient; Mito: mitochondriopathy patient. All data were analyzed with paired t-test, significant differences were indicated with * ($p<0.05$), ** ($p<0.005$), *** ($p<0.0005$)

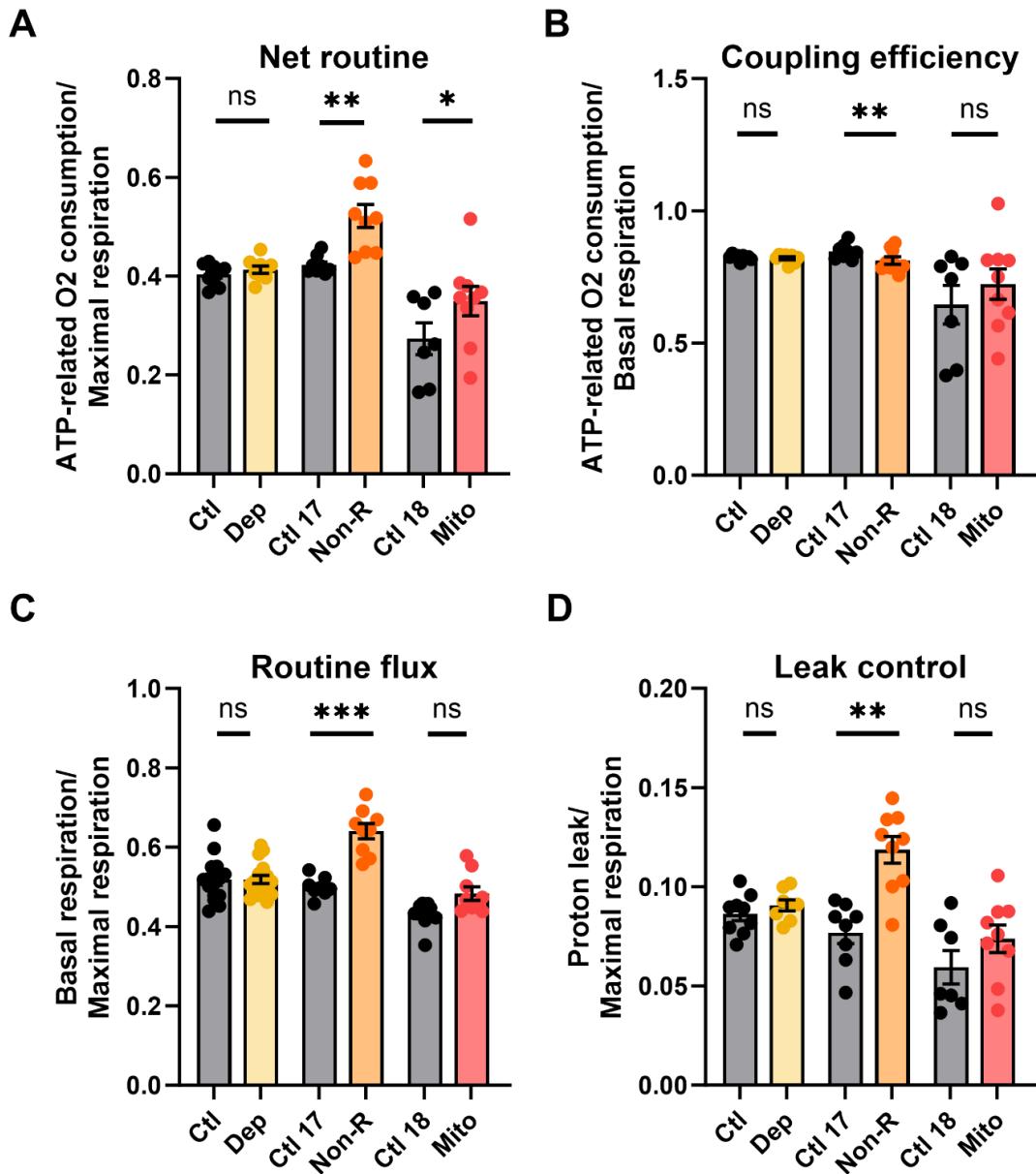


Figure S6. Mitochondrial flux ratios in astrocytes. (A) **Net routine** is the ratio between ATP-related O₂ consumption and maximal respiration (B) **Coupling efficiency** is the ratio between ATP-related O₂ consumption and basal respiration (C) **Routine flux** is the ratio between basal and maximal respiration (D) **Leak control** is the ratio between proton leak and maximal respiration. Dot plots show the respective ratios \pm SEM Ctl: non-depressed controls cohort; MDD: major depressive disorder cohort; Ctl 17 and Ctl 18: non-depressed controls; Non-R: non-responder patient; Mito: mitochondriopathy patient. All data were analyzed with paired t-test, significant differences were indicated with * ($p<0.05$), ** ($p<0.005$), *** ($p<0.0005$)

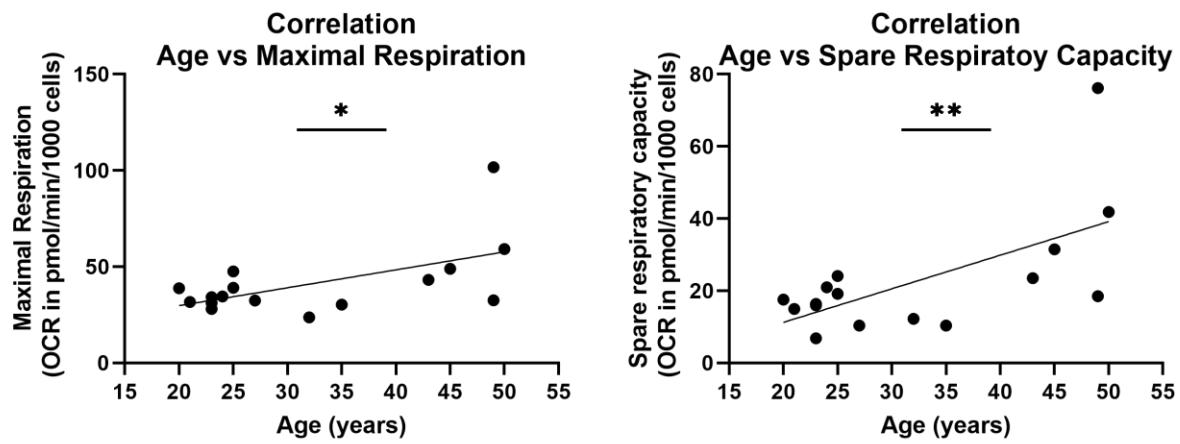


Figure S7. Correlation between age and mitochondrial respiration. Graphs represent Pearson correlation between respiratory data in the non-depressed control cohort ($n = 16$) and a simple linear regression. Maximal respiration vs age $p = 0.0223$, Spare Respiratory Capacity vs age $p = 0.0093$.

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

Table S1. Mitochondrial bioenergetics in fibroblasts

	Ctl	MDD	Ctl 17	Non-R	Ctl 18	Mito
Basal respiration (pmol/min/1000 cells)	18.45±0.47 n=140	15.81±0.41 n=140	15.17±0.54 n=9	19.81±1.04 n=9	15.20±0.58 n=9	13.69±0.75 n=9
	p<0.0001		p=0.039		p=0.1393	
Maximal respiration (pmol/min/1000 cells)	36.28±1.11 n=140	29.57±0.82 n=140	44.46±1.26 n=9	67.34±3.77 n=9	40.20±2.17 n=9	32.36±1.17 n=9
	p<0.0001		p=0.0006		p=0.0053	
Proton leak (pmol/min/1000 cells)	2.65±0.10 n=140	2.34±0.09 n=140	2.07±0.39 n=9	3.67±0.38 n=9	2.43±0.14 n=9	2.07±0.15 n=9
	p=0.0014		p=0.0004		p=0.1125	
ATP-related O₂ consumption (pmol/min/1000 cells)	15.77±0.41 n=140	13.25±0.31 n=140	13.10±0.31 n=9	16.14±0.98 n=9	12.98±0.60 n=9	11.62±0.63 n=9
	p<0.0001		p=0.0178		p=0.0486	
Spare respiratory capacity (pmol/min/1000 cells)	19.09±0.94 n=140	13.93±0.65 n=140	29.29±1.05 n=9	47.52±2.96 n=9	25.00±1.91 n=9	18.67±1.55 n=9
	p<0.0001		p=0.0004		p=0.0035	
Non-mito. O₂ consumption (pmol/min/1000 cells)	9.54±0.38 n=140	7.93±0.28 n=140	7.06±0.21 n=9	8.61±0.99 n=9	7.46±0.24 n=9	6.57±0.26 n=9
	p<0.0001		p=0.1418		p=0.0315	
ATP content (nM/μg/mL)			29.98±2.78 n=8	26.44±4.34 n=8	21.82±1.03 n=6	17.95±1.81 n=6
			p=0.5998		p=0.0895	
NAD/NADH (ratio)			7.89±0.80 n=9	7.15±0.95 n=8	7.37±0.79 n=8	6.80±0.65 n=9
			p=0.7491		p=0.4185	
Mitochondrial content (mean fluo.)			5942±348 n=4	8572±1212 n=4	5990±965 n=4	6808±1312 n=4
			p=0.0792		p=0.1948	
MMP (relative units)	1.55 ±0.01 n=3971	1.37±0.01 n=3718	1.45±0.07 n=92	1.39±0.10 n=93	2.25±0.07 n=148	2.01±0.05 n=142
	p<0.0001		p=0.2982		p<0.0001	
Cytosolic Ca²⁺ (relative units)	0.59±0.01 n=1020	0.59±0.01 n=1114	0.62±0.01 n=86	0.63±0.01 n=93	0.62±0.01 n=108	0.60±0.01 n=87
	p=0.1897		p=0.1037		p=0.3228	

Mito. Ca²⁺ (relative units)			257.4±5.1 n=93	337.2±5.8 n=100	253.6±5.2 n=99	241.1±3.8 n=93
	p<0.0001				p=0.3904	
Cell size (pixels)	2318±27 n=1063	1975±22 n=1105	2806±101 n=80	2124±102 n=87	2660±94 n=94	2301±82 n=83
	p<0.0001		p<0.0001		p=0.0238	
Cytosolic ROS (mean fluo.)			21111±4644 n=3	17900±4366 n=3	13811±2288 n=3	16434±4417 n=3
	p=0.5304				p=0.1791	
Mitochondrial ROS (mean fluo.)			2063±401 n=5	3321±215 n=5	2107±252 n=5	1993±247 n=5
	p=0.0074				p=0.7043	
Lipid peroxidation (pg/mL 8- isoprostanate/100 0 cells)			0.30±0.04 n=9	0.23±0.03 n=9	0.18±0.01 n=9	0.27±0.03 n=9
	p=0.1037				p=0.0033	
Glutathione (GSH/GSSG ratio)			22.9±3.31 n=3	24.5±3.78 n=3	22.1±1.98 n=3	13.1±1.21 n=3
	p=0.7644				p=0.0285	

Table S2. Correlations between depression severity and biological findings

	Non-Mito. O ₂ Consumpti on	Basal Respiration	Maximal Respiration	Proton Leak	ATP - related O ₂ Consumpti on	Spare Respiratory Capacity	MMP	Cytosolic Ca ²⁺	Cell size
Spearman r	-0.422	-0.321	-0.640	-0.309	-0.33	-0.553	0.222	0.416	-0.0771
95% confidence interval	-0,775 to 0,132	-0,724 to 0,245	-0,872 to - 0,175	-0,717 to 0,258	-0,728 to 0,235	-0,835 to - 0,0394	-0,342 to 0,669	-0,139 to 0,772	-0,567 to 0,453
P (two-tailed)	0.1184	0.2416	0.0118	0.2613	0.2282	0.0349	0.4226	0.1235	0.7848
P value summary	ns	ns	*	ns	ns	*	ns	ns	ns
Significant? (alpha = 0.05)	No	No	Yes	No	No	Yes	No	No	No

Table S3. 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 S4. Mitochondrial bioenergetics in neural progenitor cells (NPCs)

	Ctl	MDD	Ctl 17	Non-R	Ctl 18	Mito
Basal respiration (pmol/min/1000cells)	7.61±0.28 n=63	6.76±0.23 n=70	5.75±0.28 n=9	7.63±0.47 n=9	5.64±0.35 n=8	5.13±0.12 n=8
		p=0.0008		p=0.0143		p=0.1133
Maximal respiration (pmol/min/1000cells)	11.1±0.44 n=63	9.71±0.39 n=70	9.67±0.49 n=9	12.6±0.92 n=9	10.7±0.92 n=8	8.96±0.39 n=8
		p=0.0001		p=0.0197		p=0.0354
Proton leak (pmol/min/1000cells)	1.77±0.08 n=63	1.48±0.05 n=70	1.14±0.06 n=9	1.48±0.13 n=9	1.33±0.06 n=8	1.20±0.08 n=8
		p=0.0007		p=0.0329		p=0.3496
ATP-related O₂ consumption (pmol/min/1000cells)	6.67±0.33 n=63	5.31±0.19 n=70	4.61±0.24 n=9	6.14±0.35 n=9	4.31±0.33 n=8	3.93±0.11 n=8
		p<0.0001		p=0.0143		p=0.0903
Spare respiratory capacity (pmol/min/1000cells)	3.73±0.27 n=63	3.00±0.22 n=70	3.92±0.25 n=9	4.93±0.59 n=9	5.02±0.73 n=8	3.84±0.30 n=8
		p=0.0055		p=0.1210		p=0.0403
Non-mito. O₂ consumption (pmol/min/1000cells)	3.45±0.13 n=63	3.01±0.11 n=70	2.19±0.13 n=9	2.36±0.16 n=9	2.32±0.12 n=8	2.00±0.15 n=8
		p=0.0007		p=0.2530		p=0.0619
ATP content (nM/μg/mL)			25.7±1.54 n=6	24.1±2.11 n=6	22.6±2.15 n=6	24.9±1.61 n=6
			p=0.5912		p=0.1331	
NAD/NADH (ratio)			4.67±0.25 n=3	5.33±0.29 n=3	4.64±0.24 n=3	5.65±0.16 n=3
			p=0.2374		p=0.0288	
Mitochondrial content (mean fluo.)			21792±3230 n=9	18292±3811 n=9	20847±3802 n=6	22812±4514 n=6
			p=0.3030		p=0.2756	
MMP (relative units)	2.42±0.01 n=7118	2.17±0.01 n=7120	3.22±0.09 n=300	3.22±0.08 n=296	2.38±0.08 n=300	3.33±0.09 n=298
		p=0.148		p=0.9207		p<0.0001
Cytosolic Ca²⁺ (relative units)	0.56±0.01 n=4700	0.57±0.01 n=4706	0.63±0.01 n=492	0.62±0.02 n=457	0.64±0.02 n=501	0.65±0.02 n=513
		p=0.041		p=0.2137		p=0.0001
Mito. Ca²⁺ (relative units)			372.4±4.2 n=420	487.3±5.6 n=452	512.3±6.3 n=551	444.2±4.3 n=534
			p<0.0001		p<0.0001	
Cell size (pixels)	190±1.2 n=2866	165±1.1 n=2844	274±4.8 n=491	243±3.4 n=455	261±3.5 n=500	221±3.0 n=523
		p<0.0001		p<0.0001		p<0.0001

Cytosolic ROS (mean fluo.)		1223±184 n=8	1559±283 n=8	1220±192 n=5	1414±140 n=5
p=0.3286			p=0.1765		
Mitochondrial ROS (mean fluo.)		301±34 n=8	267±31 n=8	327±31 n=5	554±51 n=5
p=0.4287			p=0.0036		
Lipid peroxidation (pg/mL 8-isoprostanate/1000 cells)		0.00233±0.00012 n=6	0.000942±8.5 0e-005 n=6	0.00107±0.0001 1 n=6	0.00180±0.000 27 n=6
p=0.0002			p=0.0186		

Table S5. 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	Recurrent depressive disorder, current episode severe with psychotic symptoms (F33.3)	Absence of past or present depressive or mental disorders	Severe depressive episode without psychotic symptoms (F32.2)	Absence of past or present depressive or mental disorders	Severe depressive episode without psychotic symptoms (F32.2)
	Absence of documented health conditions		Absence of documented health conditions		Absence of documented health conditions	
Treatment	None	Bupropion, Mirtazapin	None	Valdoxan	None	Cipralex

Table S6. Mitochondrial bioenergetics in astrocytes

	Ctl	MDD	Ctl 17	Non-R	Ctl 18	Mito
Basal respiration (pmol/min/1000cells)	21.8±0.9 n=22	19.5±0.8 n=24	19.8±1.6 n=9	16.7±1.1 n=9	19.4±0.5 n=9	20.9±1.0 n=9
	p=0.0024		p=0.0207		p=0.2087	
Maximal respiration (pmol/min/1000cells)	39.6±1.9 n=19	37.4±1.8 n=22	41.2±4.3 n=9	29.9±2.8 n=9	45.3±2.4 n=9	44.1±1.8 n=9
	p=0.0190		p=0.0018		p=0.6975	
Proton leak (pmol/min/1000cells)	3.73±0.19 n=22	3.07±0.23 n=24	3.27±0.29 n=9	2.82±0.29 n=9	3.42±0.12 n=9	3.61±0.16 n=9
	p=0.0119		p=0.0903		p=0.2257	
ATP-related O₂ consumption (pmol/min/1000cells)	18.8±0.8 n=24	16.2±0.6 n=24	16.6±1.3 n=9	13.9±0.9 n=9	16.0±0.5 n=9	17.3±0.9 n=9
	p=0.0033		p=0.0259		p=0.2153	
Spare respiratory capacity (pmol/min/1000cells)	18.22±1.2 n=20	18.6±1.0 n=24	21.4±2.9 n=9	13.2±1.9 n=9	24.1±1.0 n=8	23.2±1.5 n=9
	p=0.5654		p=0.0007		p=0.3528	
Non-mito. O₂ consumption (pmol/min/1000cells)	7.78±0.43 n=22	6.28±0.33 n=24	7.72±0.62 n=9	6.80±0.16 n=9	6.17±0.33 n=9	5.86±0.49 n=9
	p=0.0067		p=0.2284		p=0.4339	
ATP content (nM/μg/mL)	36.7±3.03 n=14	39.1±2.83 n=14	33.9±5.21 n=7	29.3±5.80 n=4	34.1±5.19 n=6	23.9±4.62 n=8
	p=0.2442		p=0.1309		p=0.0486	
Mitochondrial content (mean fluo.)			20454±3682 n=5	15794±1731 n=5	31306±5570 n=5	20573±4766 n=5
			p=0.1248		p=0.1168	
MMP (relative units)	4.14±0.07 n=888	3.23±0.05 n=1196	2.59±0.05 n=382	3.69±0.07 n=382	1.97±0.07 n=240	1.29±0.03 n=240
	p<0.0001		p<0.0001		p<0.0001	
Cytosolic Ca²⁺ (relative units)	0.54±0.00 n=575	0.5324±0.00 n=709	0.60±0.00 n=343	0.67±0.00 n=378	0.57±0.00 n=223	0.58±0.00 n=235
	p=0.0009		p<0.0001		p=0.0018	
Mito. Ca²⁺ (relative units)	201±2 n=550	177±1 n=693	361±7 n=341	316±5 n=373	268±3 n=228	246±2 n=235
	p<0.0001		p<0.0001		p<0.0001	
Cell size (pixels)	1110±27 n=448	945±21 n=469	1288±39 n=223	1130±30 n=270	1377±48 n=159	1538±48 n=160
	p<0.0001		p<0.0001		p=0.0061	

Cytosolic ROS (mean fluo.)		4027±518 n=5	2496±100 n=5	2622±75 n=5	3561±340 n=5
p=0.0359			p=0.0120		
Mitochondrial ROS (mean fluo.)		1697±285 n=5	1378±176 n=5	1714±367 n=5	1729±371 n=5
p=0.3620			p=0.7621		

Table S7. Mitochondrial membrane potential (MMP), calcium homeostasis and dynamics in neurons

	Ctl 17	Non-R	Ctl 18	Mito
MMP somas (relative units)	1.20±0.03 n=342	0.895±0.01 n=771	1.08±0.01 n=865	1.15±0.02 n=1075
		p<0.0001		p=0.0002
MMP neurites (relative units)	6.89±0.32 n=166	6.31±0.31 n=156	5.35±0.19 n=155	8.87±0.22 n=201
		p=0.3834		p<0.0001
Cytosolic calcium (relative units)	0.928±0.01 n=372	0.763±0.01 n=302	0.797±0.01 n=837	0.736±0.01 n=661
		p<0.0001		p<0.0001
Mitochondrial calcium (relative units)	344±6 n=453	354±7 n=359	449±6 n=449	316±4 n=493
		p=0.2800		p<0.0001
Cell size (pixels)	131±2 n=374	132±3 n=291	109±1 n=938	107±1 n=670
		p=0.5262		p=0.0117
Calcium transients amplitude (relative units)	0.26±0.00 n=952	0.26±0.01 n=156	0.24±0.00 n=1189	0.23±0.00 n=1255
		p=0.0015		p=0.0843
Calcium transients rise time (ms)	3.02±0.05 n=948	3.25±0.15 n=148	2.39±0.04 n=1056	3.03±0.05 n=1252
		p=0.0048		p<0.0001
Calcium transients time constant of decay (ms)	6.74±0.14 n=736	9.95±0.90 n=115	5.49±0.08 n=961	12.0±0.26 n=994
		p<0.0001		p<0.0001

Table S8. Electrophysiological properties of patients-derived neurons

	Ctl	MDD	Ctl 17	Non-R	Ctl 18	Mito
RMP (mV)	-26.7±1.02 n=161	-23.7±0.84 n=170	-47.9±2.14 n=55	-56.0±1.71 n=48	-53.1±1.36 n=74	-48.4±1.68 n=68
	p=0.0278		p=0.0024		p=0.0428	
Capacitance (pF)	18.1±0.9 n=161	15.8±0.8 n=169	12.8±0.4 n=56	9.2±0.4 n=49	11.4±0.4 n=75	9.7±0.3 n=67
	p=0.0330		p<0.0001		p=0.0065	
INa 0mV current density (pA/pF)	-33.0±2.5 n=157	-42.6±3.2 n=163	-17.6±3.1 n=47	-9.7±2.5 n=43	-11.7±1.6 n=60	-31.2±3.1 n=58
	p=0.0116		p=0.4348		p<0.0001	
IK +20mV current density (pA/pF)	41.4±1.9 n=226	38.2±1.6 n=245	102±5.4 n=50	123±7.3 n=43	91.2±5.7 n=65	118±5.9 n=61
	p=0.2956		p=0.0254		p=0.0015	
Spontaneous PSCs (act./not act.)			26/32=81.25%	6/35=17.14%	23/41=56.10%	33/46=71.74%
			p<0.0001		p=0.1786	
# of PSCs (mean number)			106±17.7 n=26	8.5±2.48 n=6	23.0±6.2 n=23	30.5±5.7 n=33
			p<0.0001		p=0.4214	
Spontaneous AP -50mV (act./not act.)	18/162=11.11%	35/163=21.74%	19/24=79.14%	16/32=50%	17/35=77.14%	29/38=76.32%
	p=0.016		p=0.0301		p=0.0170	
# of AP -50mV (mean number)			63.2±15.9 n=19	12.9±2.09 n=16	34.4±9.18 n=17	63.2±13.8 n=29
			p=0.0240		p=0.0803	
Spontaneous AP -80mV (act./not act.)			21/24=87.50%	20/34=58.82%	19/36=52.78%	27/41=65.85%
			p=0.0216		p=0.2563	
# of AP -80mV (mean number)			20.1±5.58 n=21	12.4±2.4 n=20	23.8±6.83 n=19	34.5±8.92 n=27
			p=0.4184		p=0.7273	
Max amplitude spont. AP (mV)			-8.09±0.58 n=314	-9.54±0.77 n=127	-10.3±0.48 n=283	-4.11±0.38 n=779
			p<0.0001		p<0.0001	

Spont. AP FWHM (ms)	5.94±0.14 n=247	5.22±0.14 n=124	6.09±0.13 n=234	5.25±0.07 n=738
p<0.0001		p<0.0001		
PSC amplitude (pA)	-59.4±0.54 n=2045	-121.2±16.5 n=54	-55.9±0.9 n=634	-39.5±0.6 n=353
p=0.006		p<0.0001		
PSC rise time (ms)	0.432±0.00 n=1994	0.469±0.01 n=50	0.462±0.01 n=691	0.765±0.02 n=372
p=0.025		p<0.0001		
PSC time constant of decay (ms)	1.23±0.01 n=1866	1.07±0.07 n=49	1.22±0.02 n=630	2.27±0.07 n=337
p=0.7987		p<0.0001		