Supplementary Information

Mitochondria DNA copy number, mitochondria DNA total somatic deletions, Complex I activity, synapse number, and synaptic mitochondria number are altered in schizophrenia and bipolar disorder

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Table ST1A: Demographics of subjects

A. Demographics of three groups analyzed for complex I activity, mtDNA common deletion level, mtDNA copy number, and mtDNA total deletion in DLPFC, STG, and V1 cortex.

Sex		BD	CON	SZ
Female (N)		13	6	3
Male (N)		13	35	24
	F : M	1:1	1:5.8	1:8 #
pH	Mean	6.68*	6.86	6.64*
	Std Dev	0.15	0.22	0.2
Age	Mean	46.0*	57.9	41.2*
	Std Dev	12.5	12.7	10.1
PMI	Mean	20.5	18.8	21
	Std Dev	6.61	7.12	7.39
# of Meds	Mean	3.7*	0.9	2.1*
	Std Dev	3.2	1.7	1.7
	Min	0	0	0
	Max	10	10	5

Number of male and female subjects is different between groups

* Significantly different from control group ($p < 0.05$).

B. Subset of samples depicted in (A) analyzed for complex I activity, mtDNA common deletion level, mtDNA copy number, and mtDNA total deletion in NAc.

Gender		BD	CON	SZ
Female (N)		7	3	1
Male (N)		9	18	10
All		16	21	11
	F:M	**1:1.3**	**1:6**	**1:10**
Age	Mean	43.81***	60.76	43.00***
Age	Std Dev	10.51	13.91	11.8
pH	Mean	6.71*	6.87	6.63*
pH	Std Dev	0.14	0.28	0.28
PMI	Mean	20.82	17.72	20.96
PMI	Std Dev	6.84	8.53	6.94

*** $p < 0.0005$, ** $p < 0.005$, * $p < 0.05$

C. Demographics of three groups analyzed for colocalization of TOMM40 and PSD95 in DLPFC.

Sex		BD	CON	SZ
Female (N)		8	7	5
Male (N)		7	12	12
	F : M[#]	1:0.88	1:1.7	1:2.4
pH	Mean	6.62**	6.85	6.67*
	Std Dev	0.12	0.23	0.21
Age	Mean	43.92***	64.1	43.5***
	Std Dev	11.92	14.32	11.97
PMI	Mean	19.69	18.98	22.59
	Std Dev	6.15	6.51	6.11

Chi-square statistic 2.99 (N = 51, *p*-value = 0.22) for sex distribution by group.
* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, compared to control.

Table ST2: mtDNA copy number in four brain regions among control, schizophrenia, and bipolar disorder. Repeated measure analysis was conducted based on subject, region, and diagnosis. The DLPFC and STG data were analyzed together. The V1 and NAc data were analyzed individually.

DLPFC mtDNA Copy Number--qPCR					**V1 mtDNA Copy Number--ddPCR**			
	Least Square Mean	Std Error	p-Value			Least Square Mean	Std Error	p-Value
CON	1394.3	79			CON	1697.8	127.9	
SZ	1799.5	110.4	0.003		SZ	1541.7	167.1	0.46
BD	1702.9	91.9	0.011		BD	1684.4	140.5	0.94
STG mtDNA Copy Number--qPCR					**NAc mtDNA Copy Number--qPCR**			
	Least Square Mean	Std Error	p-Value			Least Square Mean	Std Error	p-Value
CON	1661.9	79			CON	6016.3	1008.2	
SZ	1492.1	106.4	0.202		SZ	6369.1	1265.5	0.82
BD	1406.2	91.9	0.036		BD	7700.4	722.1	0.17

Table ST3: mtDNA common deletion in four brain regions among control, schizophrenia, and bipolar disorder. DLPFC, V1, and STG data were analyzed together, whereas NAc was analyzed separately.

DLPFC mtDNA Common Deletion--qPCR					**V1 mtDNA Common Deletion--qPCR**			
	Least Square Mean	Std Error	p-Value			Least Square Mean	Std Error	p-Value
CON	0.082	0.01			CON	0.021	0.01	
SZ	0.065	0.015	0.36		SZ	0.037	0.015	0.39
BD	0.128	0.012	0.004		BD	0.04	0.013	0.24
STG mtDNA Common Deletion--qPCR					**NAc mtDNA Common Deletion--qPCR**			
	Least Square Mean	Std Error	p-Value			Least Square Mean	Std Error	p-Value
CON	0.006	0.01			CON	0.074	0.013	
SZ	0.007	0.014	0.96		SZ	0.066	0.017	0.73
BD	0.011	0.012	0.72		BD	0.06	0.014	0.54

Table ST4: mtDNA deletions per 10K in four brain regions among control, schizophrenia, and bipolar disorder. The DLPFC, V1, and STG data were analyzed together, whereas the NAc was analyzed separately.

Deletions per 10K-DLPFC				Deletions per 10K-V1			
	Least Square Mean	Std Error	p-Value		Least Square Mean	Std Error	p-Value
CON	0.35	0.17		CON	-0.49	0.20	
SZ	0.87	0.16	0.036	SZ	-0.53	0.19	0.867
BD	1.27	0.16	0.000055	BD	-0.40	0.19	0.713
Deletions per 10K-STG				**Deletions per 10K-Nac**			
	Least Square Mean	Std Error	p-Value		Least Square Mean	Std Error	p-Value
CON	-0.30	0.15		CON	-0.122	0.23	
SZ	-0.23	0.14	0.781	SZ	-0.018	0.27	0.77
BD	-0.21	0.14	0.683	BD	0.172	0.26	0.40

Table ST5: Complex I activity in four brain regions among control, schizophrenia, and bipolar disorder. The DLPFC, V1, and STG data were analyzed together, whereas the NAc was analyzed separately.

Complex I activity-DLPFC				Complex I activity-V1			
	Least Square Mean	Std Error	p-Value		Least Square Mean	Std Error	p-Value
CON	0.00046	0.00007		CON	0.0010	0.0002	
SZ	0.00020	0.00008	0.03	SZ	0.0008	0.0003	0.53
BD	0.00018	0.00006	0.01	BD	0.0012	0.0002	0.61
Complex I activity-STG				**Complex I activity-Nac**			
	Least Square Mean	Std Error	p-Value		Least Square Mean	Std Error	p-Value
CON	0.00029	0.00005		CON	0.0006	0.0003	
SZ	0.00018	0.00006	0.16	SZ	0.0004	0.0004	0.77
BD	0.00023	0.00004	0.43	BD	0.0006	0.0003	0.98

Table ST6: The overall mitochondria number, synapse number, and synaptic mitochondria number in DLPFC of CON, SZ, and BD subjects determined by IHC.

Group Effect: PSD95

Group	LSM	Std Error	p-Value
CON	8.2	0.62	
SZ	6	0.62	0.02
BD	5.01	0.57	0.0008

Group Effect: Tom40

Group	LSM	Std Error	p-Value
CON	18.02	1.92	
SZ	15.61	1.91	0.39
BD	12.88	1.78	0.06

Group Effect: Coloc

Group	LSM	Std Error	p-Value
CON	2.22	0.24	
SZ	1.49	0.24	0.04
BD	1.98	0.22	0.48

Sex x Group Effect: PSD95

Sex	Group	LSM	Std Error	p-Value
M	CON	1.67	0.32	
F	CON	2.78	0.31	
M	SZ	1.76	0.31	0.54
F	SZ	1.21	0.35	0.006
M	BD	2.52	0.32	0.07
F	BD	1.45	0.27	0.0008

Sex x Group Effect: Tom40

Sex	Group	LSM	Std Error	p-Value
M	CON	7.89	0.84	
F	CON	8.52	0.81	
M	SZ	7.1	0.81	0.96
F	SZ	4.9	0.92	0.28
M	BD	5.49	0.84	0.21
F	BD	4.53	0.7	0.004

Sex x Group Effect: Coloc

Sex	Group	LSM	Std Error	p-Value
M	CON	1.67	0.32	
F	CON	2.78	0.31	0.013
M	SZ	1.76	0.31	0.85
F	SZ	1.21	0.35	0.002
M	BD	2.52	0.32	0.1
F	BD	1.45	0.27	0.003

Table ST7: Correlation of age on mtDNA copy number in DLPFC, STG, V1, and NAc.

	DLPFC	STG	V1
Pearson r	-0.18009	-0.20203	-0.00118
p-Value	0.087761	0.050887	0.992
df	93	94	86

Table ST8: Effect of Medications on mtDNA copy number in DLPFC, STG, V1, and NAc.

Group 1 = Pooled BD and SZ subjects positive for neuroactive medications by toxicology screen
Group 2= Pooled BD and SZ subjects negative for neuroactive medications by toxicology screen
Group C = Controls

	Group					Group			
Region	**1**	**2**	**Controls**	**t-test (p-value)**	**Region**	**1**	**2**	**Controls**	**t-test (p-value)**
DLPFC				**1,2**	**STG**				**1,2**
Mean	1691.461	1992.11	1413.26	0.05868	Mean	1495.799	1430.379	1538.43	0.521886
Stdev	457.7294	624.761	489.919		Stdev	326.4067	356.95	421.657	
Fold Change	1.196852	1.40958		1, C	Fold Change	0.97229	0.929767		1, C
Group	1/C	2/C		0.012616	Group	1/C	2/C		0.621633
				2, C					2, C
				0.000461					0.363372
	Group					**Group**			
Region	**1**	**2**	**Controls**	**t-test (p-value)**	**Region**	**1**	**2**	**Controls**	**t-test (p-value)**
V1				1,2	**NAc**				1,2
Mean	1611.813	1567.75	1665.46	0.832819	Mean	8062.819	8644.295	7099.24	0.642232
Stdev	737.8149	471.97	605.694		Stdev	2156.662	4202.303	2639.04	
Fold Change	0.967787	0.94133		1, C	Fold Change	1.13573	1.217637		1, C
Group	1/C	2/C		0.739918	Group	1/C	2/C		0.273168
				2, C					2, C
				0.579958					0.230739

Table ST9: Inter-region correlation and age-effect on mtDNA common deletion in DLPFC, STG, V1, and NAc.

Correlation	**CD%-STG**	**CD%-DLPFC**	**CD%-NAc**	**CD %-V1**	**Age**
CD%-STG	1	0.758212	0.462624	0.821403	0.273021
CD%-DLPFC	0.758212	1	0.531127	0.718525	0.344293
CD%-NAc	0.462624	0.531127	1	0.369471	0.625985
CD %-V1	0.821403	0.718525	0.369471	1	0.22768
Age	0.273021	0.344293	0.625985	0.22768	1
p-Value	**CD%-STG**	**CD%-DLPFC**	**CD%-NAc**	**CD %-V1**	**Age**
CD%-STG	0	3.25E-18	0.00106	1.11E-22	0.008101
CD%-DLPFC	3.25E-18	0	0.000123	3.24E-15	0.000833
CD%-NAc	0.00106	0.000123	0	0.010591	2.53E-06
CD %-V1	1.11E-22	3.24E-15	0.010591	0	0.032892
Age	0.008101	0.000833	2.53E-06	0.032892	0

Table ST10: Inter-region correlation and age-effect on deletions per 10K across DLPFC, STG, V1, and NAc.

Correlation	**Age**	**DLPFC**	**NAc**	**STG**	**V1**
Age	1	0.078035	-0.00125	-0.06097	0.008678
DLPFC	0.078035	1	-0.01485	0.050401	0.146984
NACC	-0.00125	-0.01485	1	-0.1166	-0.29514
STG	-0.06097	0.050401	-0.1166	1	0.00112
V1	0.008678	0.146984	-0.29514	0.00112	1
p-Value	**Age**	**DLPFC**	**NACC**	**STG**	**V1**
Age	0	0.454702	0.993438	0.563717	0.93457
DLPFC	0.454702	0	0.921972	0.633274	0.162066
NAc	0.993438	0.921972	0	0.440285	0.046454
STG	0.563717	0.633274	0.440285	0	0.991544
V1	0.93457	0.162066	**0.046454**	0.991544	0

Table ST11: Inter-region correlation and age-effect on complex I activity across DLPFC, STG, V1, and NAc.

Correlation	Complex I activity STG	Complex I Activity DLPFC	Complex I activity V1	Complex I Activity NAc	Age
Complex I activity-STG	**1.0000**	0.1768	0.0611	0.0417	0.2694
Complex I activity-DLPFC	0.1768	**1.0000**	-0.0927	0.0328	0.1513
Complex I activity-V1	0.0611	-0.0927	**1.0000**	0.1305	-0.0171
Complex I activity-NAc	0.0417	0.0328	0.1305	**1.0000**	-0.1775
Age	0.2694	0.1513	-0.0171	-0.1775	**1.0000**

P-Value	Complex I activity STG	Complex I Activity DLPFC	Complex I activity V1	Complex I Activity NAc	Age
Complex I activity-STG	**<.0001**	0.1122	0.5857	0.7856	**0.0144**
Complex I activity-DLPFC	0.1122	**<.0001**	0.3988	0.8306	0.1668
Complex I activity-V1	0.5857	0.3988	**<.0001**	0.3930	0.8705
Complex I activity-NAc	0.7856	0.8306	0.3930	**<.0001**	0.2435
Age	**0.0144**	0.1668	0.8705	0.2435	**<.0001**

Table ST12: Effect of Medication/Drugs on complex I activity in DLPFC, STG, V1, and NAc using pooled SZ/BD subjects.

P-value	Complex I activity STG	Complex I Activity DLPFC	Neuroactive Drugs	Complex I activity V1	Complex I Activity NAc
Complex I activity-STG	**<.0001**	0.1093	0.4905	0.3879	0.5834
Complex I activity-DLPFC	0.1093	**<.0001**	**0.0073**	0.3890	0.5848
Neuroactive Drugs	0.4905	**0.0073**	**<.0001**	0.1461	0.8634
Complex I activity-V1	0.3879	0.3890	0.1461	**<.0001**	0.5101
Complex I activity-NAc	0.5834	0.5848	0.8634	0.5101	**<.0001**

Table ST13: Effect of Age on number of TOM40, PSD95, and coloc in DLPFC.

Correlation	Age	PSD95/100µm³	TOM40/100µm³	PSD95coloc w/ Tomm40
Age	**1.0000**	-0.2159	-0.0603	-0.1068
PSD95/100µm³	-0.2159	**1.0000**	**0.4116**	**0.5132**
TOM40/100µm³	-0.0603	**0.4116**	**1.0000**	**0.6865**
PSD95coloc w/ Tomm40	-0.1068	**0.5132**	**0.6865**	**1.0000**

P-Value	Age	PSD95/100µm³	TOM40/100µm³	PSD95coloc w/ Tomm40
Age	**<.0001**	0.2794	0.7653	0.5959
PSD95/100µm³	0.2794	**<.0001**	**0.0329**	**0.0062**
TOM40/100µm³	0.7653	**0.0329**	**<.0001**	**<.0001**
PSD95coloc w/ Tomm40	0.5959	**0.0062**	**<.0001**	**<.0001**

Table ST14: Effect of Medication/Drugs on TOM40, PSD95, and Coloc in DLPFC.

Correlation	PSD95/100µm³	TOM40/100µm³	PSD95coloc w/ TOM40	Neuroactive Drugs
PSD95/100µm³	**1.0000**	**0.4116**	**0.5132**	-0.3133
TOM40/100µm³	**0.4116**	**1.0000**	**0.6865**	-0.1724
PSD95coloc w/ TOM40	**0.5132**	**0.6865**	**1.0000**	-0.2477
Neuroactive Drugs	-0.3133	-0.1724	-0.2477	**1.0000**

p-Value	PSD95/100µm³	TOM40/100µm³	PSD95coloc w/ TOM40	Neuroactive Drugs
PSD95/100µm³	**<.0001**	**0.0329**	**0.0062**	0.1116
TOM40/100µm³	**0.0329**	**<.0001**	**<.0001**	0.3898
PSD95coloc w/ TOM40	**0.0062**	**<.0001**	**<.0001**	0.2130
Neuroactive Drugs	0.1116	0.3898	0.2130	**<.0001**

Figure SF1: mtDNA copy number correlation across DLPFC, V1, and STG.

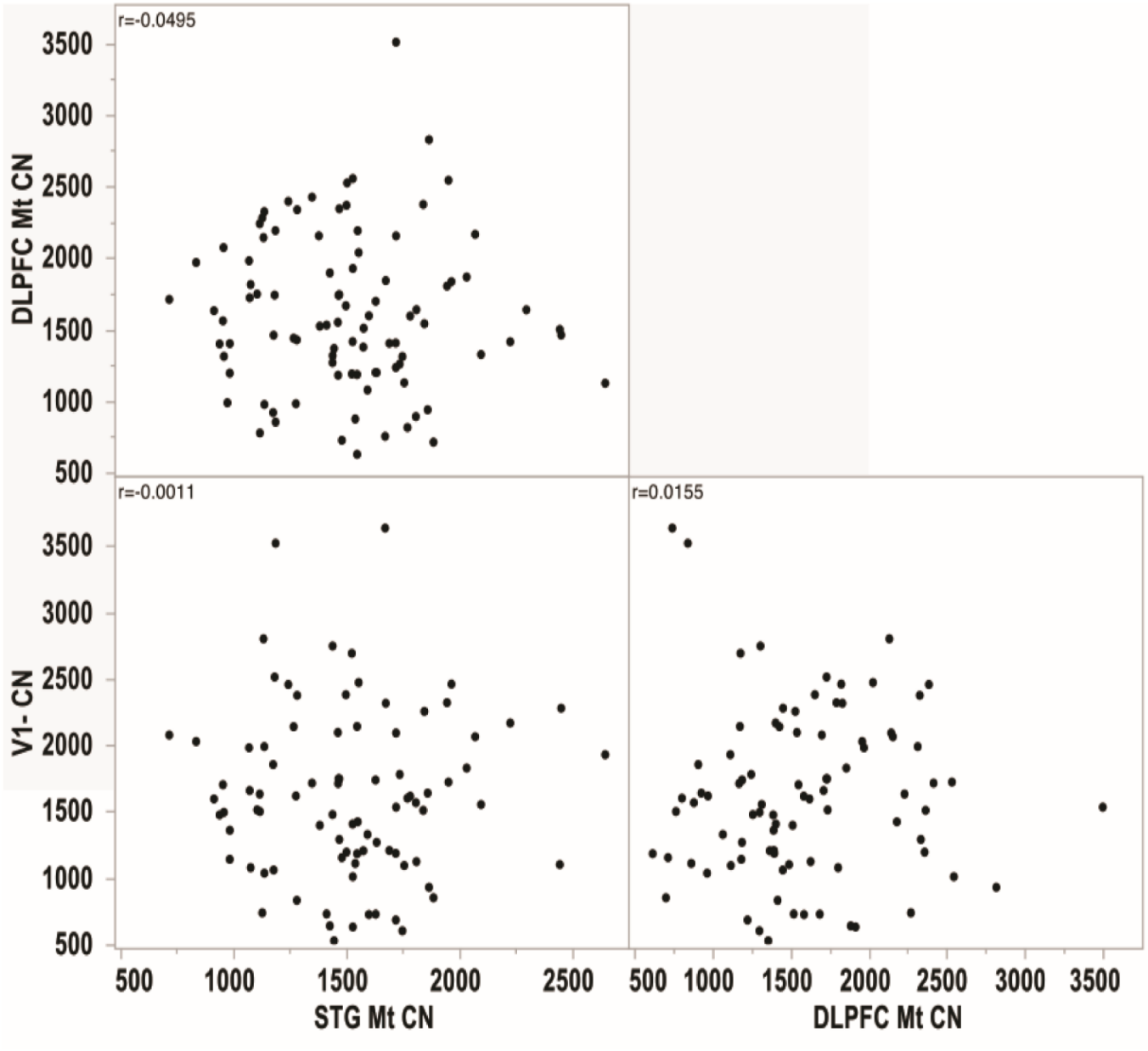


Figure SF2: mtDNA common deletion correlation across DLPFC, STG, V1, and NAc.

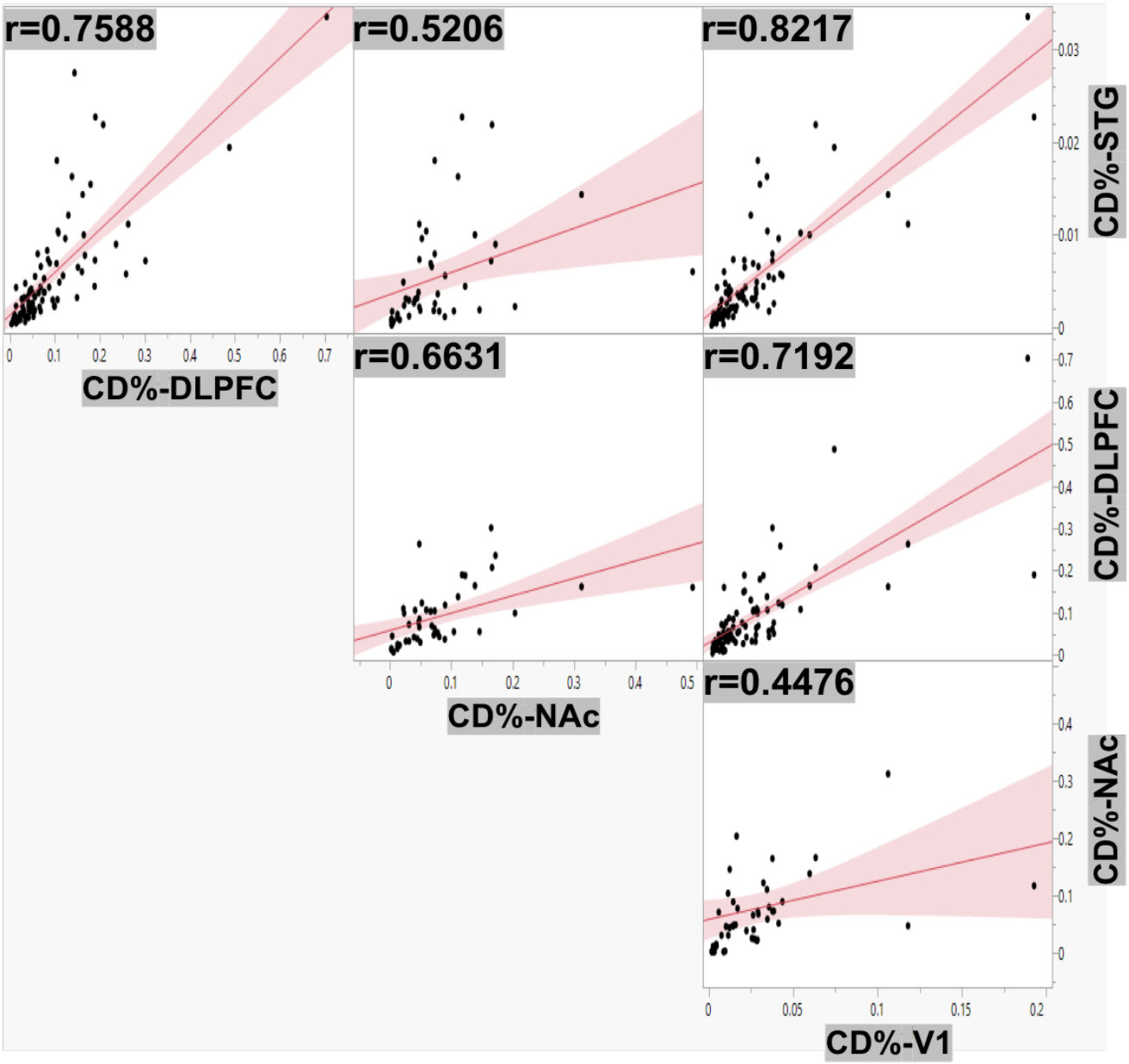


Figure SF3: Effect of age on common deletions (%) in DLPFC, STG, V1, and NAc.

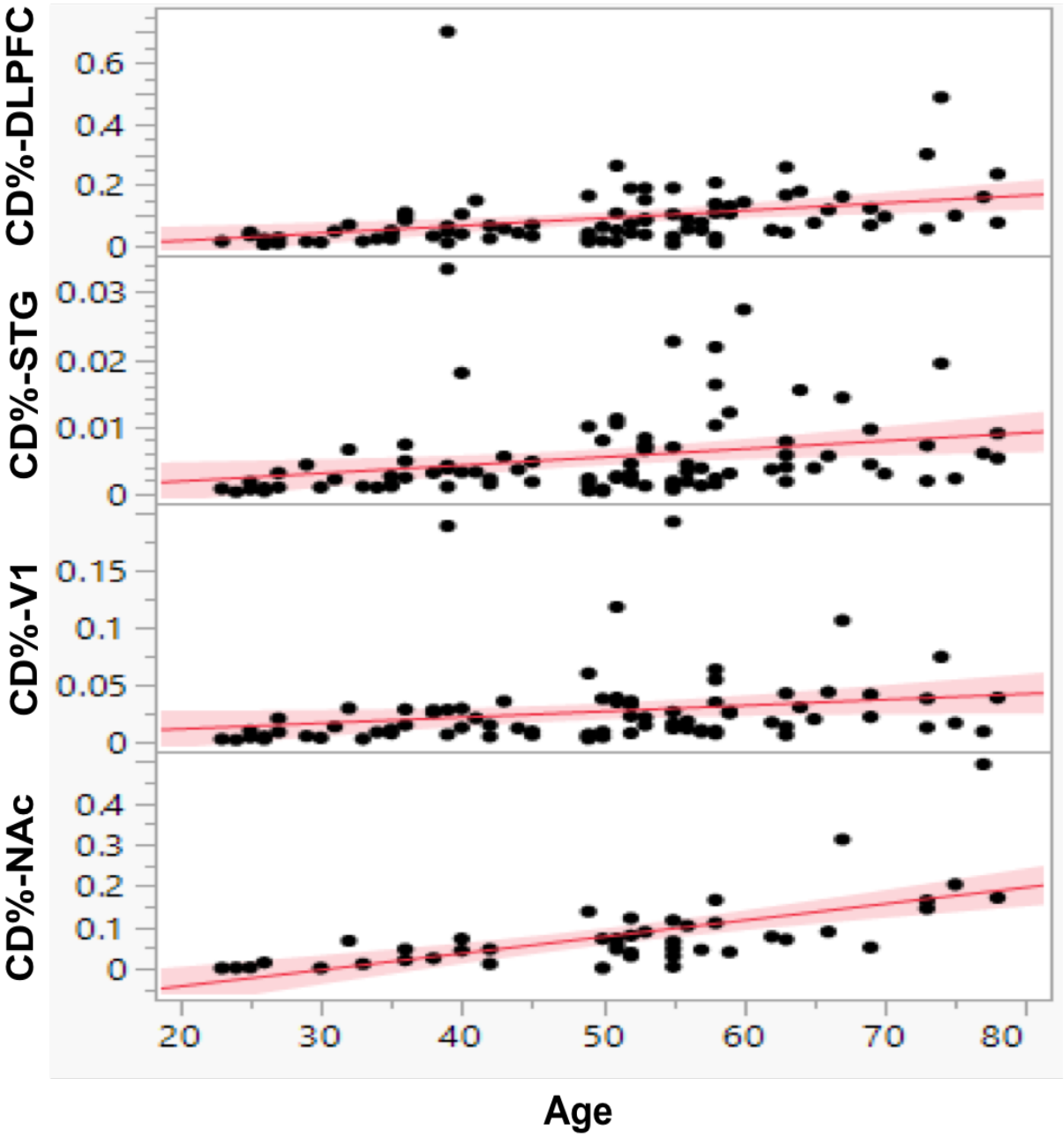


Figure SF4: Effect of Medication/Drugs on deletions per 10K in DLPFC, STG, and V1.

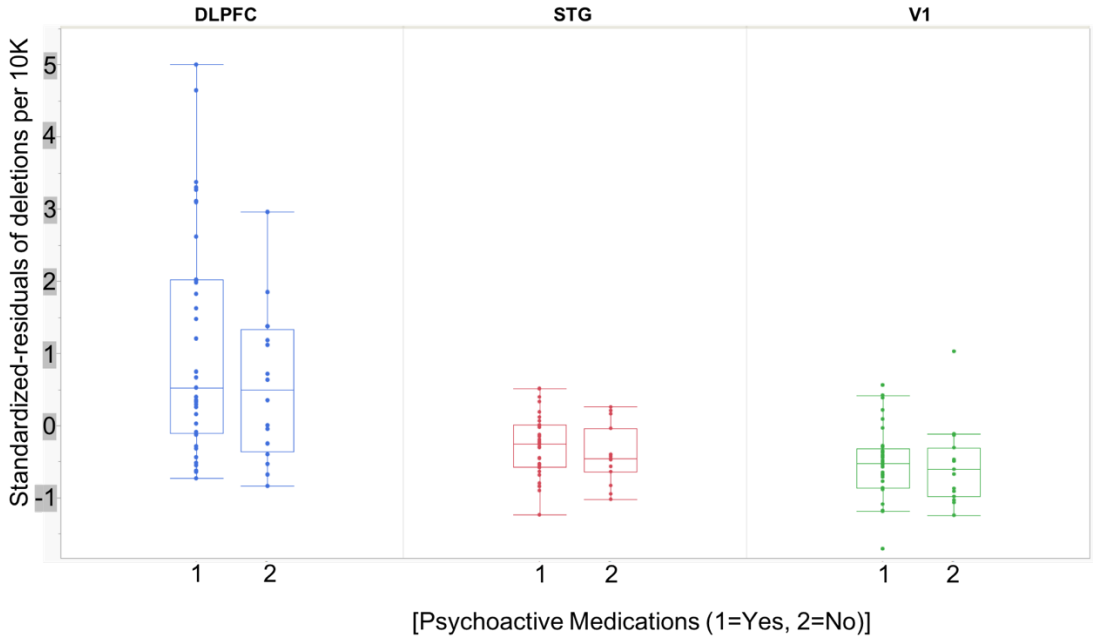


Figure SF5: Correlation of standardized residuals of deletions per 10K coverage with complex I activity. The correlation was not statistically significant (p=0.3081) across the three cortical brain regions (DLPFC, STG and V1). Individual brain region correlation were also not significant.

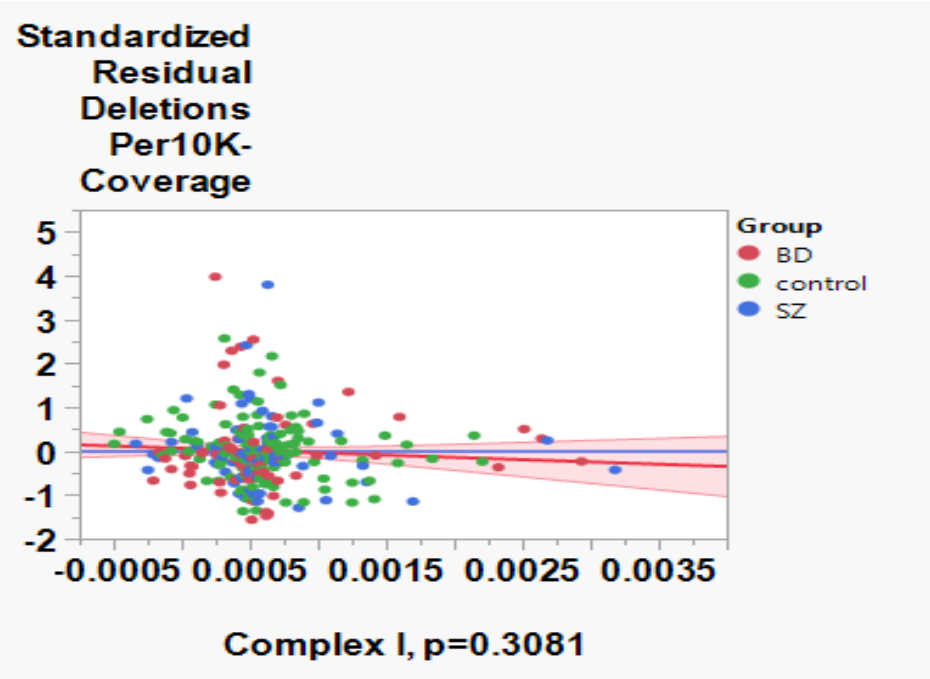


Figure SF6: Complex I protein concentration in CON, SZ and BD groups. The samples with low protein concentrations will require higher volumes to obtain equal amounts of proteins. Thus, significantly higher sample volume indicates significantly lower protein concentration. Both SZ and BD groups had significantly lower complex I protein concentration (higher volumes), compared to CON group, across the three cortical brain regions (DLPFC, STG and V1). Data are represented as least square mean (LSM) ± Std. error. $p<0.01$ is denoted by **, otherwise $p>0.05$.

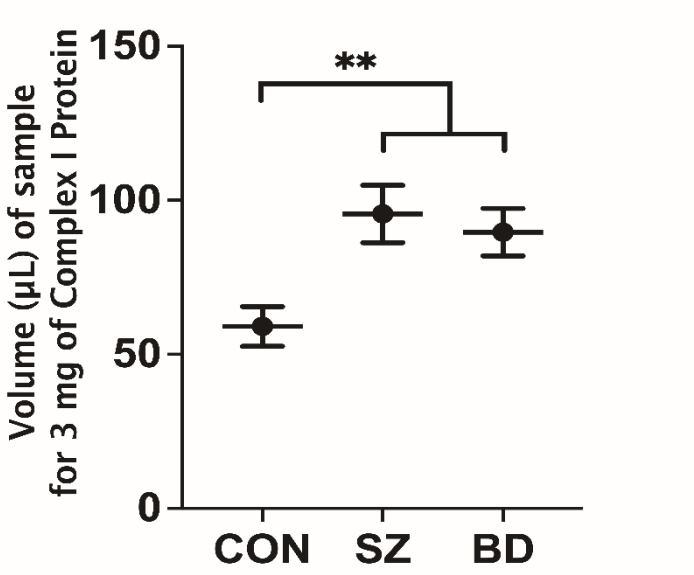
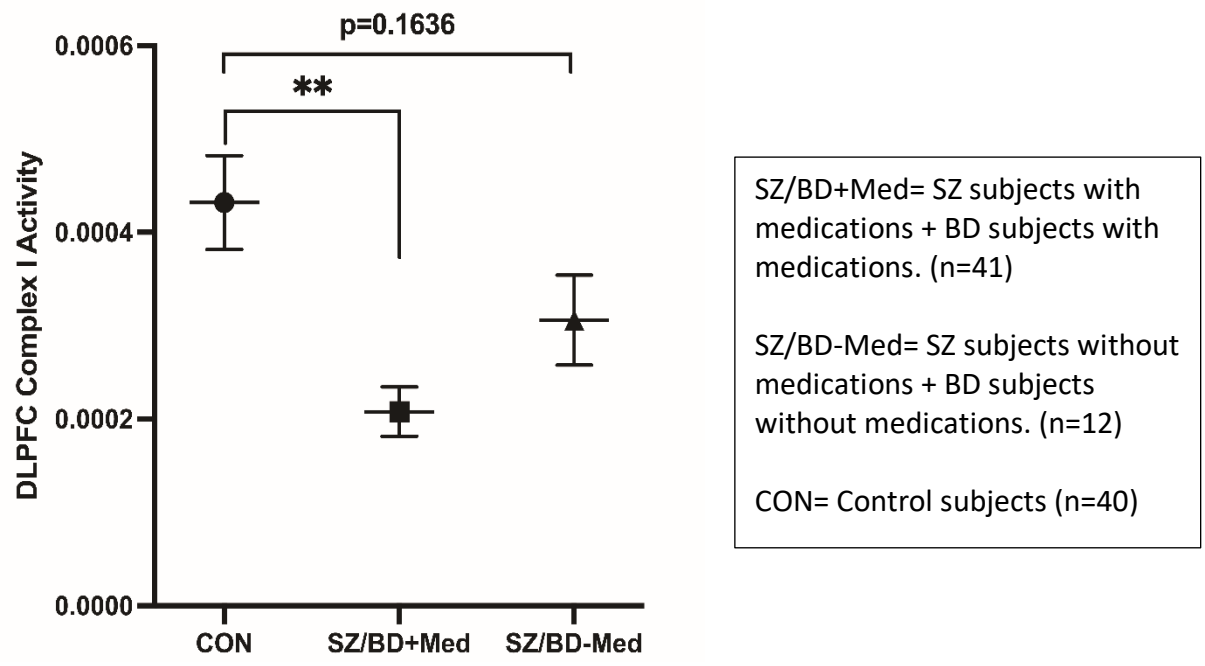


Figure SF7: Role of medications on DLPFC complex I activity. The SZ/BD+Med had significantly lower DLPFC complex I activity compared to CON. The DLPFC complex I activity in SZ/BD-Med groups was not significantly different from CON group. Data are represented as least square mean (LSM) ± Std. error. $p<0.01$ is denoted by **, otherwise $p>0.05$.



Supplementary Materials and Methods

Brain Tissue Dissection:

All the dissections were performed using frozen coronal 1 cm brain sections on dry ice blocks using cooled tools to prevent melting and tissue quality degradation. The PI was not blinded during the experiment, and the co-investigators were not blinded to the group assignment. However, all groups were run simultaneously to avoid batch effect, and to avoid biasing any assay towards a specific group. The primary visual cortex was identified from occipital pole coronal sections using the striate cortex gyrus as a landmark characterized by the presence of the Line of Gennari. The STG was located in frozen sections between the lateral sulcus above and the superior temporal sulcus in the anterior temporal lobe. The NAc was dissected from the ventral portion of the anterior striatum under the internal capsule before the presence of the globus pallidus for consistency. Postmortem subjects went through an extensive review of multiple sources of information including the medical examiner's conclusions, coroner's investigation, medical and psychiatric records, and interviews of the decedents' next-of-kin, in order to rule out subjects with neurological disorders or cancer. Additionally, a neuropathological examination of each brain was conducted in order to exclude cases with cerebrovascular disease (infarcts or hemorrhages), subdural hematoma, brain cancer, or other significant pathological features using high-definition pictures taken every 1 cm before freezing the brain. The subjects were previously diagnosed, and postmortem brain, hence no randomization of treatment was applied. The samples were preselected based upon availability and grant funding. A post hoc power analysis, shows that the experiment with the current sample size and effect size, could be replicated with 70% power to detect significant difference in Complex I in DLPFC between groups. For the colocalization of TOM40 and PSD95, there is a 95% power to replicate the reported effect with the sample size used (n = 47). For Complex I ELISA protein difference in the STG, there is a 62% power to replicate the experiment with the current sample sizes.

mtDNA Copy Number and Common Deletion:

The total DNA (nDNA+mtDNA) was extracted from DLPFC, STG, V1 and NAc samples using a phenol-chloroform extraction method. The mtDNA CN and mtDNA CD in DLPFC, STG and NAc samples was determined with quantitative real-time PCR (qPCR), whereas mtDNA CN and mtDNA CD in V1 samples were determined using the QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA). Quantitative real-time PCR was run using the Applied Biosystems 7900HT real-time instrument in 12-µL reaction volumes using 2 ng gDNA and 2 µl of each standard with published conditions in triplicate. For mtDNA CN, a 200-bp gBlock oligonucleotide (Integrated DNA Technologies, Coralville, IA, USA) representing the human albumin gene (ALB) on chromosome 4 is the reference DNA standard. An 11- point standard curve created with this gBlock has copies ranging from 50 - 50,000 copies per µl of the albumin gene. A 326-bp gBlock Gene Fragment (Integrated DNA Technologies, Coralville, IA) for the mitochondrial wild-type sequence (mt13176-13501) is used as a copy number standard for mtDNA. An 11-point standard curve is created with copies ranging from 20,000-10,000,000 per µL of mitochondria wild type product. Mitochondrial DNA CN for the samples is determined using the ratio of mtDNA CN/ALB CN. For the mtDNA CD, a 301-bp gBlock Gene Fragment (Integrated DNA Technologies, Coralville, IA, USA) encompassing the common deletion breakpoints (mt8224–8469 and

mt13447–13501) is used as a standard for the deletion. An 11-point standard curve is created with copies ranging from 50-50,000-copies/µL. CD% is calculated using the following equation: CD% = (mtDNA deletion copies)/ (mtDNA wild type copies + deletion copies) × 100.
The following primers were used:

mtDNA CN/CD based on SYBR Green chemistry:
ALB_Primer_F: 5′-TGCATGAGAAAACGCCAGTA-3′
ALB_Primer_R: 5′-TCTGCATGGAAGGTGAATGT-3′
mtWT_Primer_F: 5′-TTACAATCGGCATCAACCAA-3′
mtWT_Primer_R: 5′-GCTAATGCTAGGCTGCCAAT-3′
mtDel_Primer_F: 5′-AGGGCCCGTATTTACCCTAT-3′
mtDel_Primer_R: 5′-GCTAATGCTAGGCTGCCAAT-3′

mtDNA CN and mtDNA CD in V1 samples were determined using the QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA). For each assay, a mixture of both the forward and reverse primers and probes were combined. Stock concentrations of each primer and probe were 100 µM, and to make 100 reactions worth of a 20x assay, we used 18 µL of the forward primer stock at 100 µM, 18 µL of reverse primer stock at 100 µM, 5 ul of probe stock at 100 µM and 59 µL of water. The final 1x concentration was 900 nM for each primer and 250 nM for each probe. Each 20 µL reaction consisted of 10 µL 2x ddPCR Supermix (Bio-Rad, Hercules, CA, USA), 1 µL of the above 20x assay consisting of the primers and probe, and the appropriate amount of gDNA and water up to the final 20 µL volume. To run the mitochondria wild-type assay, 0.01 ng of gDNA was used. To run the ALB and common deletion assays 10 ng of gDNA were used. Samples were placed in the QX200 Droplet Generator to partition each sample into 20,000 nanoliter-sized droplets. Droplets were then run on the thermal cycler with the following program: 95 x 10 min, followed by 40 cycles of 94 x 30 sec, then 60 x 1 min. A final enzyme deactivation for 98 x 10 min was followed by a hold at 4. Following PCR amplification, the droplets were analyzed in the QX200 Droplet Reader. QuantaSoft Software (Bio-Rad, Hercules, CA, USA) was used to measure the number of positive and negative droplets for each fluorophore.
The following primers and probe Integrated DNA Technologies, Coralville, IA, USA) were used for ddPCR (all as listed 5'-3'):
Primers for ALB: F-TGCATGAGAAAACGCCAGTA, R- TCTGCATGGAAGGTGAATGT
Probe for ALB: /5HEX/TGGTGAACA/ZEN/GGCGACCATGCT/3IABkFQ/.
For the mito wildtype (WT) assay, the primers and probe were
Primers for WT: F- GGAAGCCTATTCGCAGGATT, R- CTAGGAAAGTGACAGCGAGG
Probe for WT: /56-FAM/CCCCTTCCA/ZEN/AACAACAATCCCC/3IABkFQ/.
For the common deletion (CD) assay the primers and probe were-
Primers for CD: F-AGGGCCCGTATTTACCCTAT, R-GCTAATGCTAGGCTGCCAAT
Probe for CD: /5HEX/CCCCTCTAG/ZEN/AGCCCACTGTAAAGC/3IABkFQ/.

Pooled Effect of Total Deletion of mtDNA by High-resolution Pipeline "Splice-Break":

In this manuscript, we present the results of the number of deletion species per 10K coverage as this showed a significant effect across diagnoses. The number of deletions per 10K was calculated by counting the total number of deletion species (a unique set of breakpoints) in a particular

sample, then dividing by the benchmark coverage and multiplying by 10000. The Splice-Break pipeline included long-range (LR) PCR amplification of mtDNA, next-generation sequencing (NGS), and MapSplice (v.2.1.18) algorithm (which detects putative mtDNA deletion breakpoint "junction reads"). Before NGS library preparation, the mtDNA was enriched for each sample using a long-range (LR) PCR utilizing back-to-back primers that hybridize to the control region of the mitochondrial genome. Primer sequences used for most samples were F 5'-CCGCACAAGAGTGCTACTCTCCTC-3' and R 5'-GATATTGATTTCACGGAGGATGGTG-3' (Integrated DNA Technologies). In cases where back-to-back primers failed to amplify the WT mitochondrial genome, the following alternate primers were used: 5'-AAGCCTAAATAGCCCACACG-3' and 5'-GTGGCTTTGGAGTTGCAGTT-3'. For NGS, libraries were prepared using TrueSeq Kit for DLPFC, STG, and V1 samples. Library preparation for NAc samples was performed using the 384 Seq-Well kit (San Diego, CA). NAc Libraries were sequenced as 150-mer paired-end reads on a patterned flow cell using the Illumina NovaSeq6000 at the UCI Genomics High Throughput Facility. DLPFC, STG, and V1 libraries were sequenced as 150-mer paired-end reads on Illumina HiSeq2500 at the UCI Genomics High Throughput Facility. We used our custom bash script Splice-Break.sh (https://github.com/brookehjelm/Splice-Break/) to perform automated filtering, normalization, and annotation of mtDNA deletion breakpoints and performed manual calculations of the cumulative deletion metrics.

Complex I Activity (activity/unit of complex I protein):

Briefly, brain tissue (60–100 mg) was homogenized in PBS at 4°C and centrifuged at 3000 rpm for 5 min. After centrifugation, the first supernatant is collected and measured for protein concentration to ensure adequate protein for both ELISA and activity assays. Equivalent amounts of supernatant protein are extracted (Part 8201086, Abcam, Cambridge, UK), the second supernatants are collected (16000xg, 4°C, 20 min) and stored at –80°C for complex I ELISA and activity assays. The complex I ELISA assay (AB178011, Abcam, Cambridge, UK) quantitatively measures NADH dehydrogenase protein. Equal protein amounts are used with a specific monoclonal capture and detection antibodies reactive to CI isolates. All standards, samples, and controls are run in duplicate in 96-well plates pre-coated with a capture antibody provided in the Abcam kit. A standard curve prepared from the HeLa lyophilized cell extract and additional standards prepared from a postmortem human PFC brain extract are used. The standard CI ELISA protein curve is calculated from the regression of the average OD 450 nm reading of each HeLa standard against their concentrations. The second part of the assay is the CI activity assay. Each brain sample is diluted to the same CI protein (amount determined above by ELISA) to determine the rate of CI activity. All samples and controls were run in duplicate according to the manufacturer's protocol (AB109721, Abcam, Cambridge, UK). The activity rate is expressed as the change in absorbance per minute using this equation: complex I activity for each sample (milli-optical density units, mOD/min) = (1,000 × ΔOD450/30 min) where Δ represents the final minus initial OD450.

Immunohistochemistry (IHC) for Colocalization of Mitochondria and Synaptic Marker:

Fresh frozen DLPFC tissue blocks (~1 cm^3) were immersion-fixed in freshly made 4% PFA (in PBS) for 24 hours at 4°C. Post-fixation, tissue blocks were cryopreserved in 20% sucrose and 0.1% Na-Azide in PBS for 3 days, then placed in 30% sucrose for 3 days at 4°C. After cryopreservation, blocks are stored frozen at -80°C until cryo-sectioning. Free-floating 10 µm tissue cryostat sections are collected in 0.1% sodium azide in PBS. Antigen-retrieval was performed by incubating free-floating tissue sections in 1 mM EDTA buffer (pH 8) at 100°C and high pressure for 5 min. After antigen retrieval, slices were incubated in PBS-Triton (0.3%) for 1 hour. Slices were blocked with 10% goat serum. Primary antibodies for excitatory postsynaptic markers (PSD95) (MA1-045, Thermofisher) and mitochondria (TOM40) (ab185543, Abcam) were added and incubated for 24 hours at 4°C. The next day, slices were washed with PBS three times, followed by respective secondary antibodies incubation for 24 hours at 4°C. After washing secondary antibodies, slices were incubated with DAPI as a nuclear counterstain for 10 min. Next, slices were incubated in 70% ethanol for 5 min followed by incubation with an auto-fluorescence eliminator reagent (Sigma Aldrich, Cat#2160) for 1 min. Slides were mounted in Prolong diamond antifade solution (Thermofisher, P36970). Digital z-stack images are collected using a Zeiss LSM 780 confocal microscope with oil X 63 objective (NA=1.4). Acquired image parameters: voxel size 0.07 x 0.07 x 0.32 $micron^3$, 1024 x 1024 pixels with a depth of 12 bit. Colocalization analysis was performed through IMARIS software (Bitplane). The spots of TOM40 and PSD95 with a diameter of greater than 1.2 µm were excluded from the analysis. The colocalization was defined as the number of TOM40 spots within the distance of 0.25 µm from PSD95 spots. 5-8 images from 2-3 sections per subject were captured. The total number of subjects evaluated for IHC was 51.