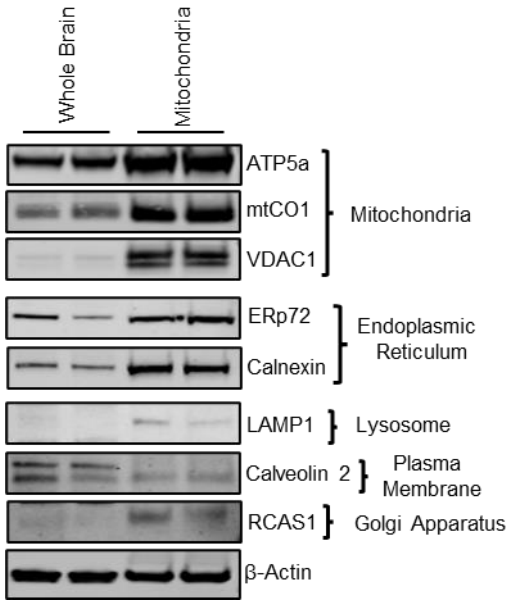
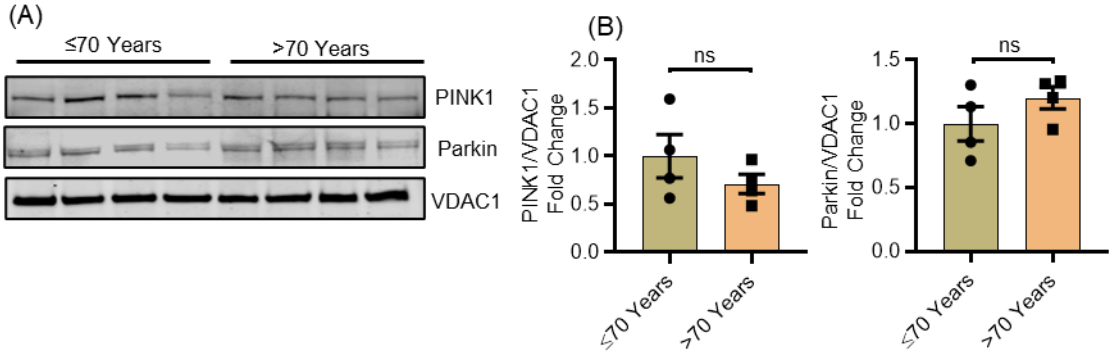
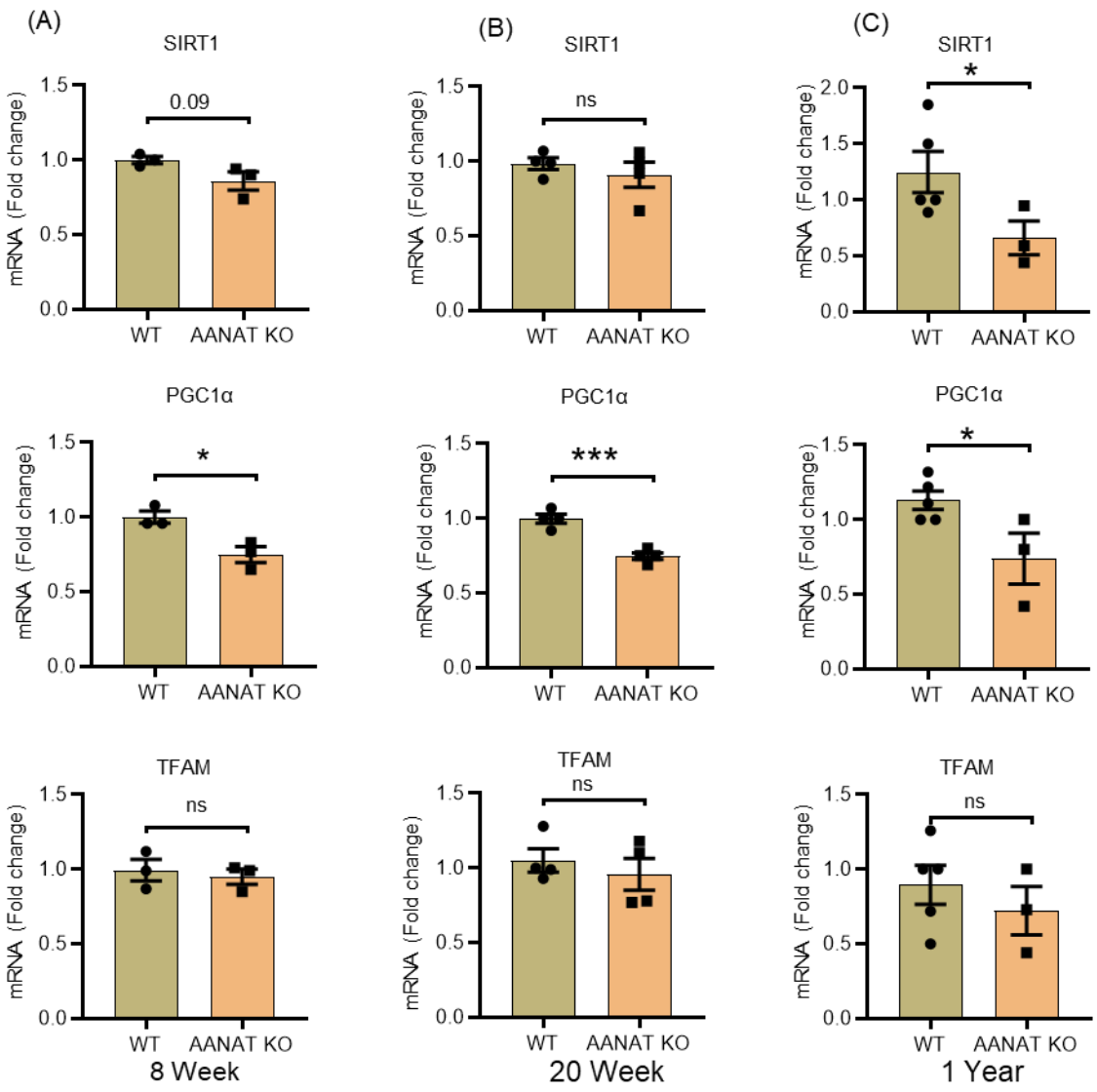


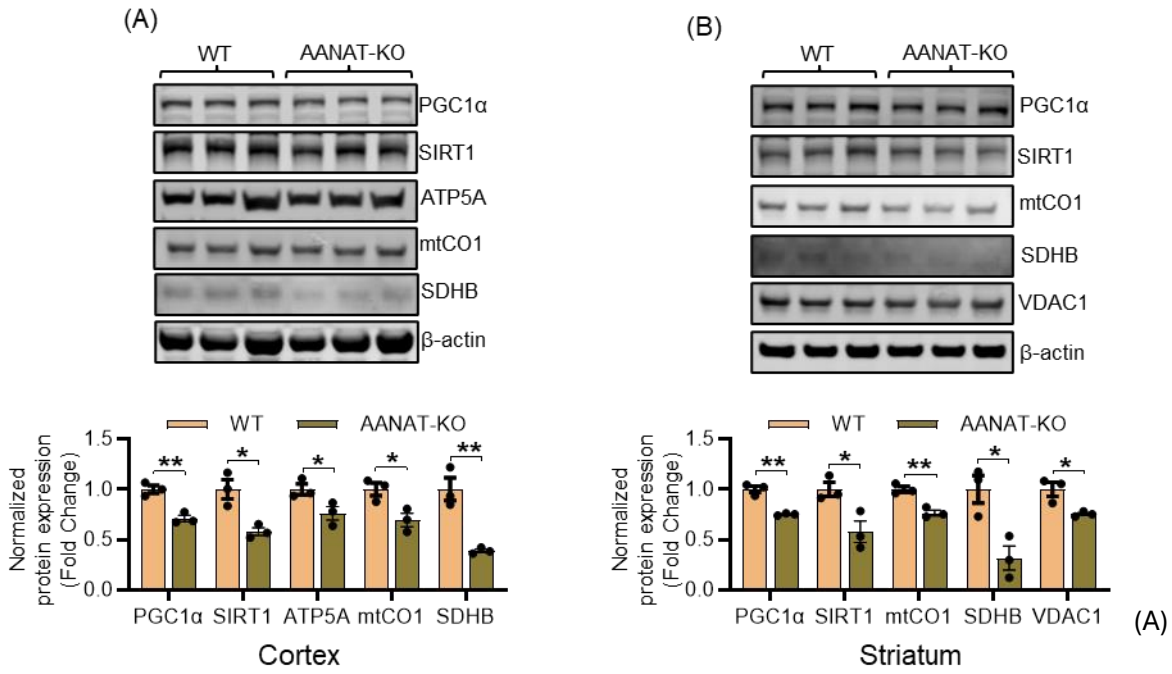
Supplementary Figure 1. Characterization of Mitochondria isolated from frozen Human brain: Immunoblots of mitochondrial markers, endoplasmic reticulum marker, lysosomal markers, plasma membrane marker, golgi apparatus markers in whole brain lysate and isolated mitochondria to characterize the quality and possible contaminant in isolated mitochondria.



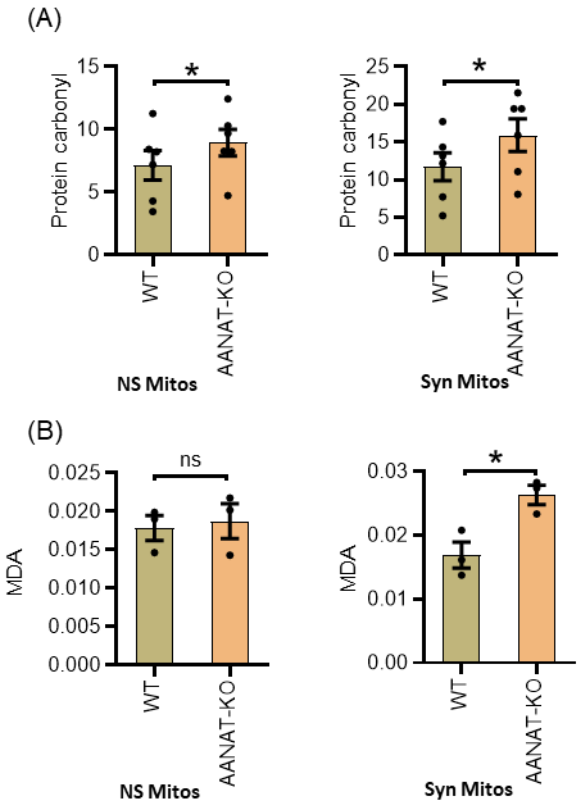
Supplementary Figure 2. Estimation of autophagy/mitophagy in Human ageing brain: (A) Immunoblots of PINK1, Parkin and VDAC1 and their (B) normalized quantification in isolated mitochondria from human postmortem brain samples ≤70 years and ≥70 yearsn=4. Normalized fold change was calculated by using VDAC1 as a loading control and ≤70 years as a reference. All data are expressed as mean ± SEM and analyzed by student-t-test. *P < 0.05; **P < 0.01; ***P < 0.001 ns: non-significant.



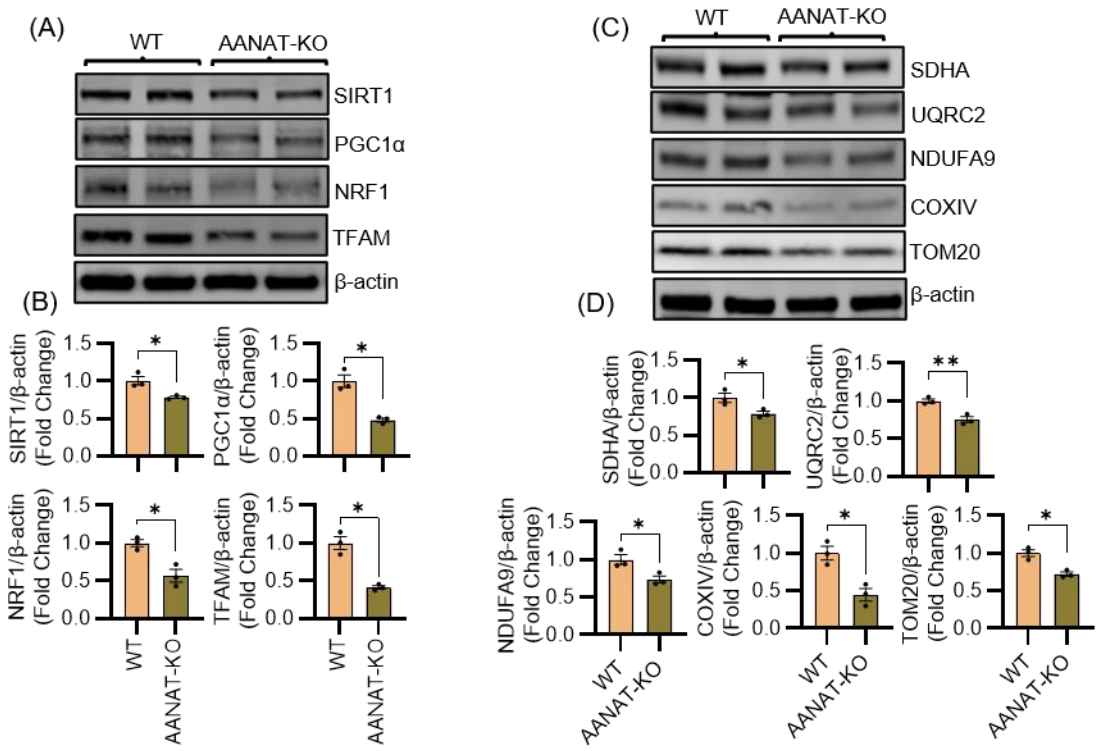
Supplementary Figure 3. Melatonin deficiency modulates mRNA expression of SIRT1, PGC1α and TFAM in AANAT KO brain: (A) Relative mRNA expression of SIRT1, PGC1α and TFAM in 8-week, n=3 (B) 20-week n=4 and (C) 1-year old WT, n=5 and AANAT-KO, n=3 brain. Normalized fold change was calculated by using β-Actin as loading control and WT as the reference. All data are expressed as mean ± SEM and analyzed by student-t-test. *P < 0.05; **P < 0.01; ***P < 0.001 ns: non-significant.



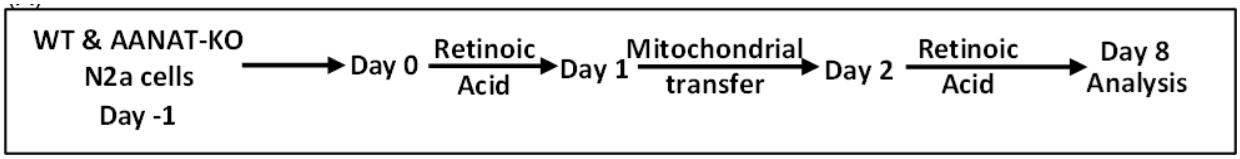
Supplementary Figure 4. AANAT deficiency impairs mitochondrial biogenesis in C3H mice: (A) Representative immunoblots and densitometric quantification of mitochondrial biogenesis markers PGC-1α, SIRT1, ATP5A, mtCO1, and SDHB in the cortex of WT and AANAT-KO C3H mice. β-Actin was used as a loading control. Protein expression levels were normalized to β-Actin and quantified relative to WT controls. (B) Representative immunoblots and densitometric quantification of PGC-1α, SIRT1, ATP5A, mtCO1, and SDHB in the striatum of WT and AANAT-KO C3H mice. β-Actin was used as a loading control. Protein expression levels were normalized to β-Actin and quantified relative to WT controls. Data are presented as mean ± SEM from independent biological replicates (n=3 mice per group). Statistical significance was determined using an unpaired two-tailed Student's *t*-test. *P < 0.05; **P < 0.01; ***P < 0.001.



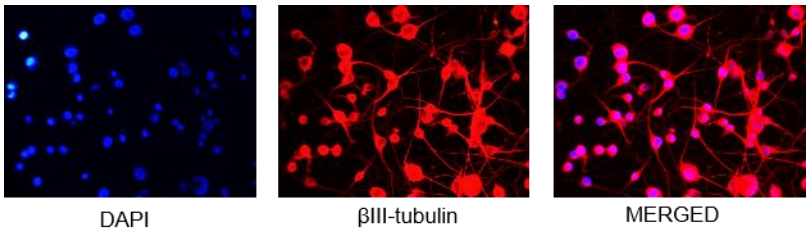
Supplementary Figure 5: Melatonin deficiency induced mitochondrial dysfunction in AANAT-KO mitochondria. (A) Protein carbonylation levels were measured in non-synaptosomal and synaptosomal mitochondria isolated from 6-month-old WT and AANAT-KO mouse brains. (B) Lipid peroxidation was assessed in the same non-synaptosomal and synaptosomal mitochondrial fractions from WT and AANAT-KO brains. Data are presented as mean ± SEM and analyzed by student-t-test. *P < 0.05; **P < 0.01; ***P < 0.001 versus WT.



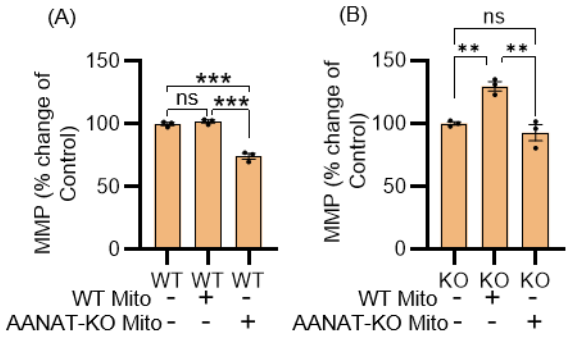
Supplementary Figure 6. Melatonin deficiency reduces mitochondrial biogenesis and mitochondrial protein abundance in differentiated neurons. (A) Representative immunoblots of SIRT1, PGC-1α, NRF1, TFAM, and β-actin and (B) corresponding normalized quantification in WT and AANAT-KO differentiated neurons (n=3). AANAT-KO differentiated neurons exhibited reduced expression of SIRT1, PGC-1α, NRF1, and TFAM compared with WT neurons. (C) Representative immunoblots of SDHA, UQCR2, NDUFA9, COX IV, TOM20, and β-actin and (D) corresponding normalized quantification in WT and AANAT-KO differentiated neurons (n=3 independent experiments). AANAT-KO neurons exhibited reduced abundance of mitochondrial proteins compared with WT neurons. Data are presented as mean ± SEM and were analyzed using an unpaired, 2-tailed Student's *t* test. $P < 0.05$, $^{*}P < 0.01$, $^{**}P < 0.001$ versus WT.



Supplementary Figure 7. Experimental scheme for mitochondrial transfer in differentiated neurons.



Supplementary Figure 8. Experimental scheme for mitochondrial transfer in differentiated neurons



Supplemental Figure 9. Mitochondrial transplantation modulates mitochondrial membrane potential in differentiated neurons. (A) Mitochondrial membrane potential (MMP) in WT N2a differentiated neurons following transplantation with WT or AANAT-KO mitochondria (n = 3). (B) MMP in AANAT-KO N2a differentiated neurons following transplantation with WT or AANAT-KO mitochondria (n=3 independent experiments). MMP was assessed by TMRE fluorescence using a microplate reader and normalized to nuclear staining with NucBlue to account for differences in cell number. Data are presented as mean ± SEM. Statistical significance was determined using 1-way ANOVA followed by Tukey's multiple-comparisons post hoc test. $P < 0.05$, *$P < 0.01$, **$P < 0.001$; ns, not significant.

Group	CW#	Age	Race	Sex	PMI (hours)
Control (>75 years)	CW02-010	89	W	F	9
	CW03-012	90	W	M	4
	CW05-040	76	W	M	14
	CW14-025	92	W	F	12
	CW16-028	90	W	M	9
Control (<70 years)	CW02-008	54	W	F	6
	CW02-014	65	W	M	5
	CW16-099	63	W	F	15
	CW97-043	70	W	M	9
	CW99-100	54	W	M	6

Supplementary Table 1: Information of Human postmortem brain samples. Summary of key details for each human hippocampal sample used in this study, including age at death, sex, and race.

Antibody name	Vendor	Catalogue No.
SIRT1	Proteintech	13161-1-AP
PGC1α	Proteintech	66369-1
NRF1	Cell Signaling Technology	46743
TFAM	Proteintech	22586-1-AP
Total OXPHOS Rodent WB Antibody Cocktail	Abcam	ab110413
COX-IV	Abcam	ab16056
β-Actin	Sigma	L5441
VDAC1	Proteintech	55259-1-AP
Cytochrome C	Proteintech	10993-1-AP
ATP5a	Abcam	ab14748
mtCO1	Abcam	ab14705
ERp72	Abcam	ab155800
Calnexin	Abcam	ab22595
LAMP1	Abcam	ab25630
Calveolin2	Abcam	ab32577
RCAS1	Abcam	ab251310

Supplementary Table 2: Information of antibodies used in study. Details of all primary and secondary antibodies used for immunoblotting, immunofluorescence, and other assays, including target protein, host species, supplier, and catalog numbers.

Primer sequences	
mt-CO1	FP; 5’-GCCCCAGATATAGCATTCCC-3’
	RP; 5’-GTTCATCCTGTTCCTGCTCC-3’
mt-Dloop1	FP; 5’-AATCTACCATCCTCCGTGAAACC-3’
	RP; 5’-TCAGTTTAGCTACCCCCAAGTTTAA-3’
mt-Dloop3	FP; 5’-TCCTCCGTGAAACCAACAA-3’
	5’-AGCGAGAAGAGGGGCATT-3’
SIRT1	FP; 5’-CTCTGAAAGTGAGACCAGTAGC-3’
	RP; 5’-TGTAGATGAGGCAAAGGTTCC-3’
PGC1α	FP; 5’-CACCAAACCCACAGAAAACAG-3
	RP; 5’-GGGTCAGAGGAAGAGATAAAGTTG-3’
TFAM	FP; 5’-CACCCAGATGCAAAACTTTCAG-3’
	RP; 5’-CTGCTCTTTATACTTGCTCACAG-3’
ND1	FP; 5’-TGCACCTACCCTATCACTCA-3’
	RP; 5’-GGCTCATCCTGATCATAGAATGG-3’
β-Actin	FP; 5’-ACCTTCTACAATGAGCTGCG-3’
	RP; 5’-CTGGATGGCTACGTACATGG-3’
ND2	FP; 5’-ATACTAGCAATTACTTCTATTTTCATAGGG-3’
	RP; 5’-GAGGGATGGGTTGTAAGGAAG-3’
ND4	FP;-5’-CATCACTCCTATTCTGCCTAGC-3’
	RP;-5’-GTGGCTATAAGTGGGAAGACC-3’
ND6	FP;-5’-TGTTGGAGTTATGTTGGAAGGAG-3’
	RP;-5’-CAAAGATCACCCAGCTACTACC-3’
CytB	FP;-5’-ACCCCAATACGCAAAATTAACC-3’
	FP;-5-TACGGATGCTACTTGTCCAAT G-3’
mtCO2	FP;-5’-AGTTGATAACCGAGTCGTTCTG-3’
	RP;-5’-CTGTTGCTTGATTTAGTCGGC-3’
ATPase6	FP;-5’-GCCACAACTAGATACATCAACATG-3’
	RP;-5’-TGGTTGTTAGTGATTTTGGTGAAG-3’
ATPase8	FP;-5’-TCCTATTCCCATCCTCAAAACG-3’
	RP;-5’-CATGTTCGTCCTTTTGGTGTG-3’

Supplementary Table 3: Primer sequences used in study. List of all forward and reverse primers used for qPCR, including target gene, primer sequence (5′→3′)**.**