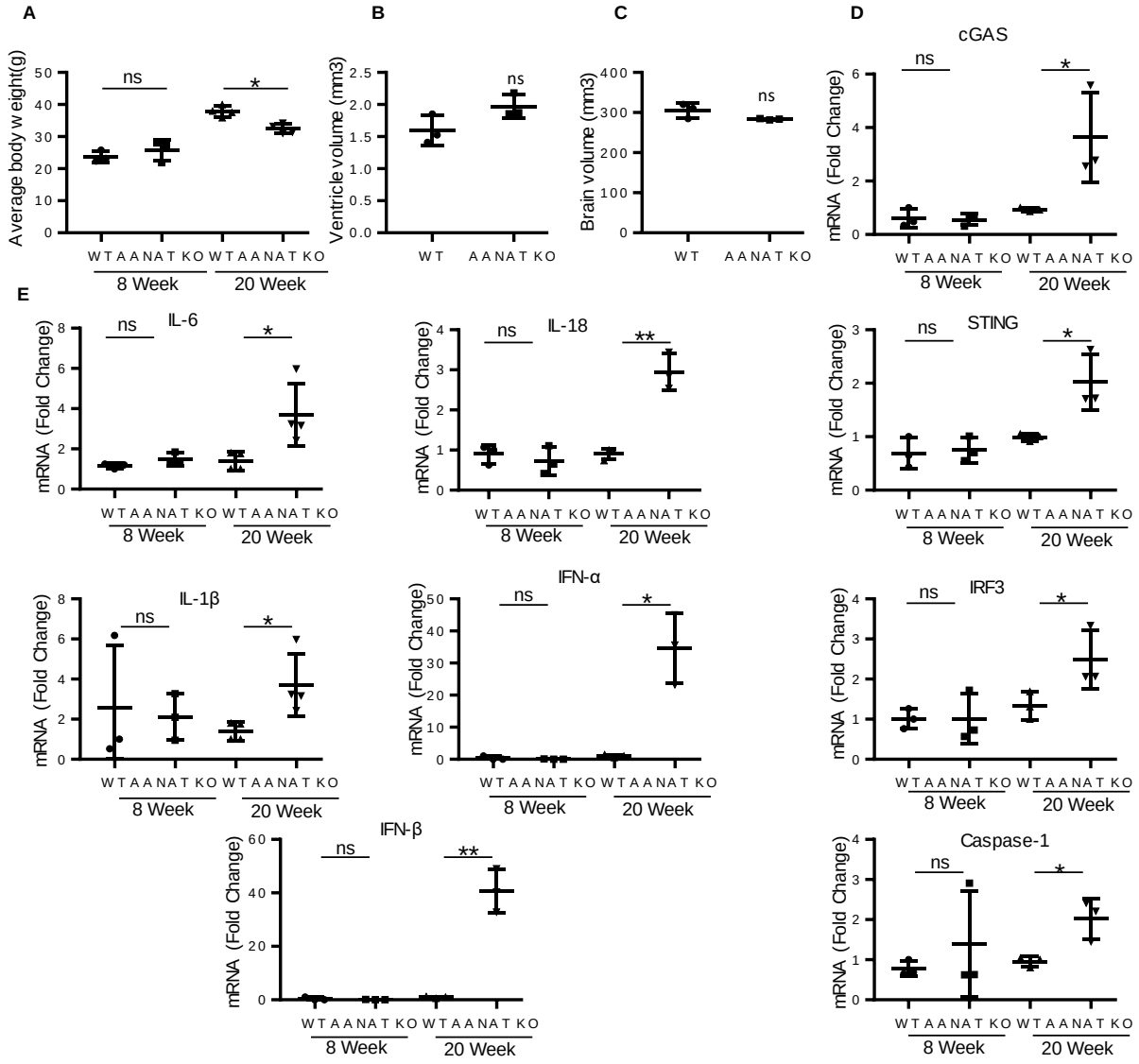
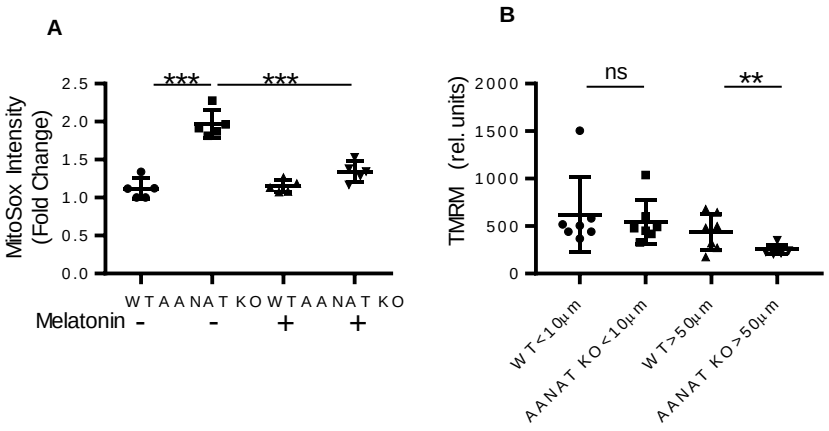
Supplementary Figures:
Supplemental Figure 1



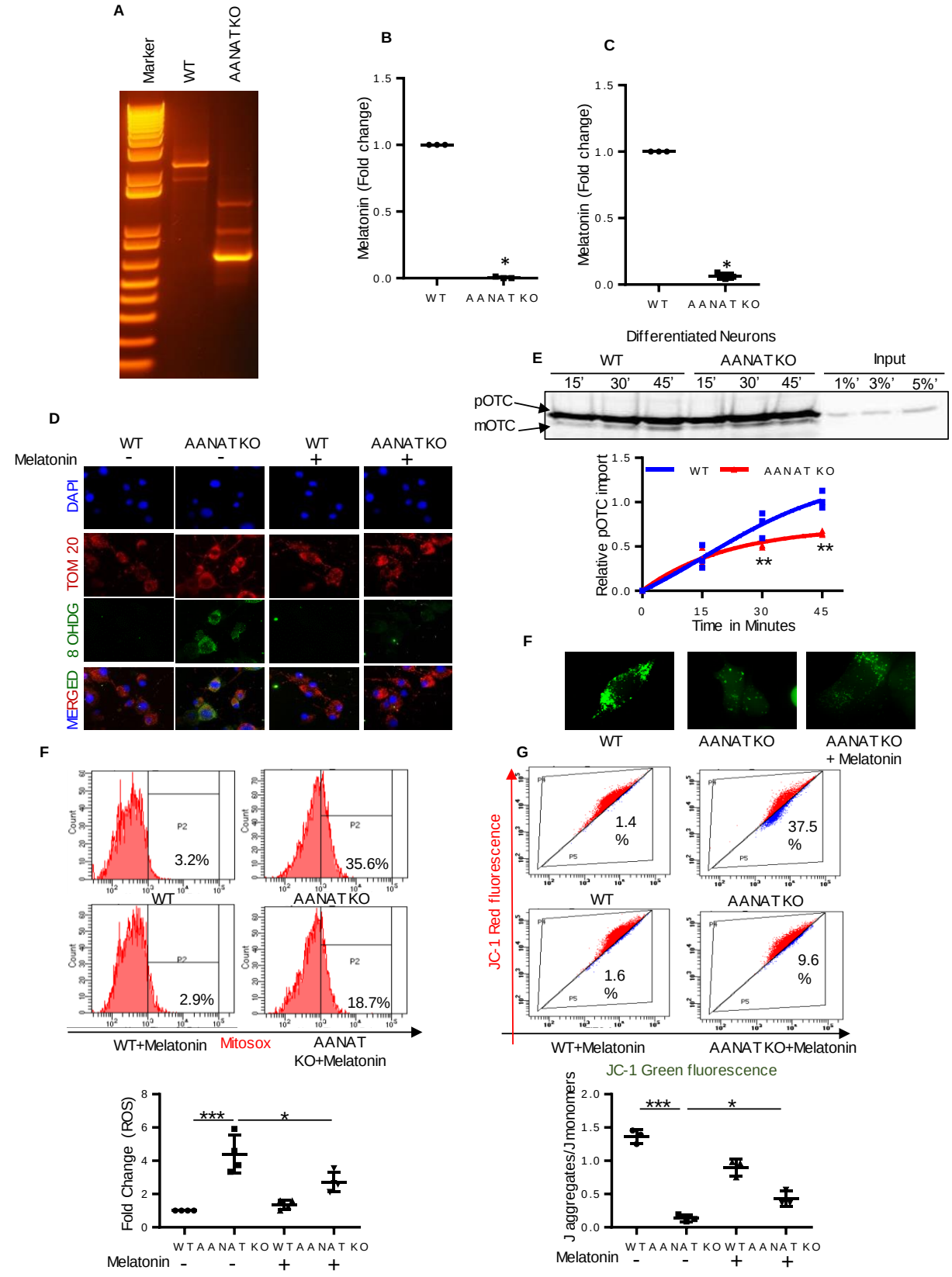
Supplemental Figure 1. AANAT-KO induces mtDNA mediates inflammation: (A) Average body weight in WT and AANAT-KO mice (age 8 and 20-week) N=4. (B) ventricle and (C) brain volume in 20-week WT and AANAT KO brain, N=3. (D) qPCR analysis of mRNA of cGAS, STING, IRF3 and caspase-1 in WT and AANAT-KO brain (age 8 and 20-week). MRNA expression is plotted relative to amount in WT brain, after normalization to β-Actin, N=3. (E) qPCR analysis of mRNA of proinflammatory cytokines and interferons in WT and AANAT-KO brain (age 8 and 20-week). MRNA expression is plotted relative to amount in age matched WT brain, after normalization to β-actin, N=3 for 8 week and N=4 for 20 week. Data are shown as mean+/−SD and analyzed by Student's T test (panel B&C) and one way ANOVA (panel A, D & E). *P<0.05, **P<0.01, ns; Non-significant.

Supplemental Figure 2



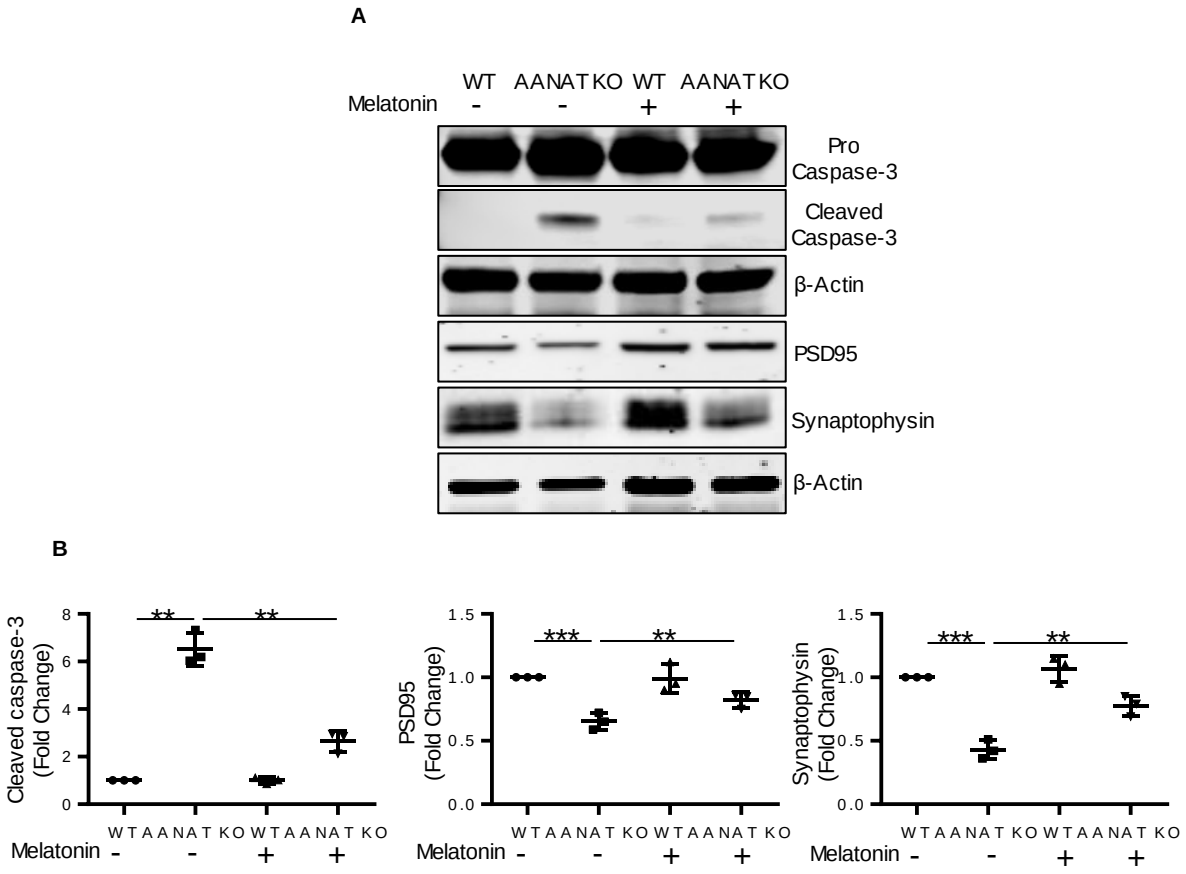
Supplemental Figure 2. Melatonin regulates mitochondrial dysfunction in PCNs: (A) Analysis of mitochondrial ROS by Mitosox in WT and AANAT-KO PCN at DIV21 with or without melatonin (5 µM) in culture medium. Mitosox fluorescence plotted relative to WT PCN after normalization to nuclear stain N=5. (B) TMRM fluorescence intensity (MMP) dependence on distance between mitochondria and nuclei for WT and AANAT PCN. Data normalized to mtGFP fluorescence intensity expressed as relative units as described (*52*), N=7. Data are shown as mean+/−SD analyzed by one way ANOVA (Panel A) and student t test (Panel B), *P<0.05, **P<0.01, ***P<0.001

Supplemental Figure 3



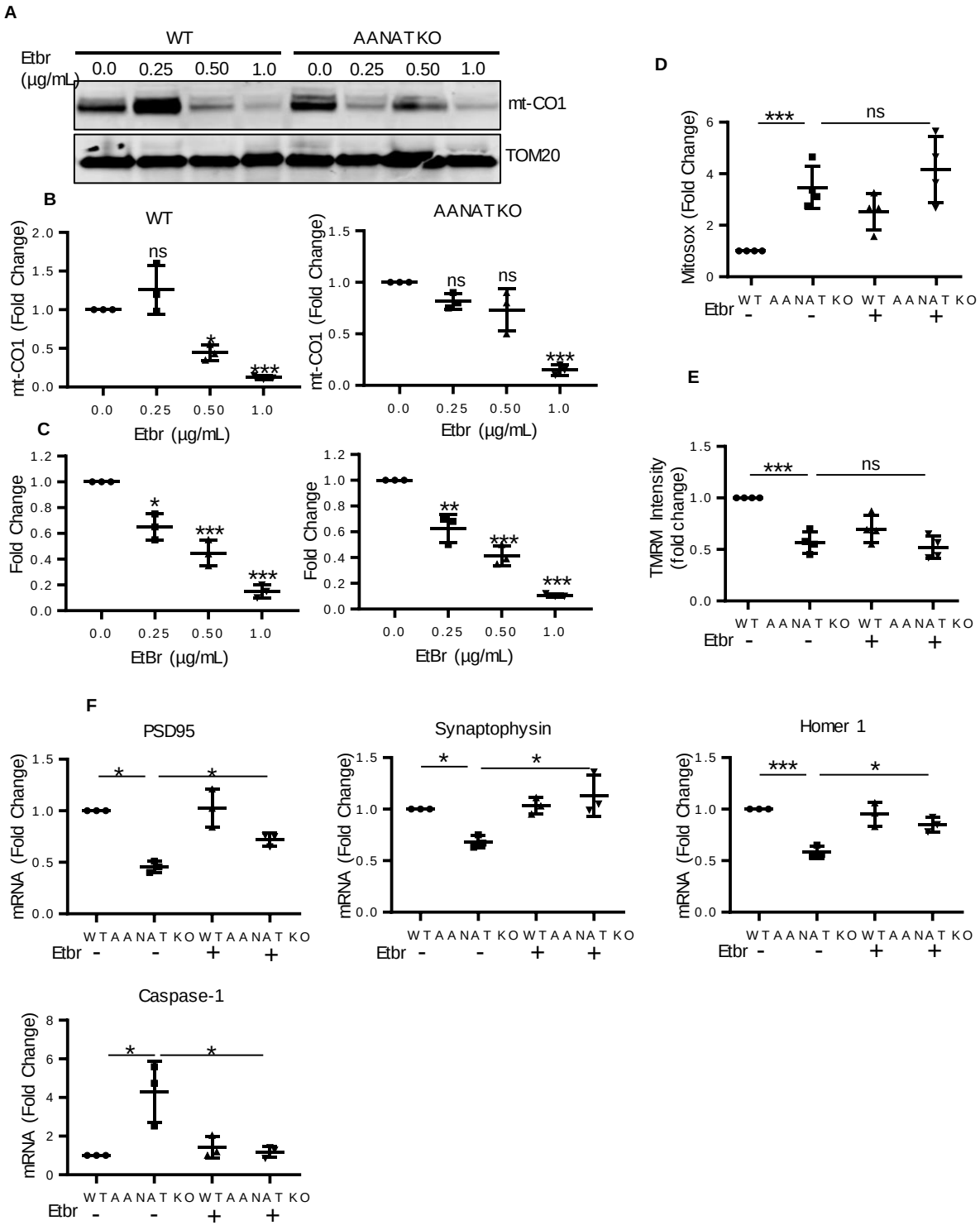
Supplemental Figure 3. Inhibition of melatonin synthesis alters mitochondrial homeostasis: (A) Agarose gel electrophoreses of PCR product of AANAT gene in WT and AANAT-KO N2a cells. AANAT-KO N2a cells were created using CRISPR/Cas-9 system as described in our previous study (*35*). (B) LC-MS quantification of melatonin in cell lysate and (C) mitochondria of WT and AANAT-KO N2a cells, N=3. (D) Representative immunofluorescence image of 8-OHDG, marker of DNA oxidation and TOM20, mitochondrial marker, in differentiated WT and AANAT-KO N2a cells with or without melatonin (5 μM) in culture medium, N=5, Scale bar 25μm. (E) Representative image and quantified pre and mature-orthithine transcarbamilase (pOTC and mOTC) protein import assay in mitochondria of differentiated WT and AANAT-KO N2a cells, N=3. Cleavage of pOTC into mOTC occurs in the mitochondrial matrix following import (*60*). (F) Flow cytometry analysis of Mitosox fluorescence in WT and AANAT-KO differentiated N2a cells with or without melatonin (5 μM) in culture medium N=4. (G) Flow cytometry analysis of JC-1 fluorescence in WT and AANAT-KO differentiated N2a cells with or without melatonin (5 μM) in culture medium N=3. (H) Representative image of mitochondrial permeability transition pore opening assay in WT and AANAT-KO differentiated N2a cells with or without melatonin (5 μM) in culture medium N=3. Data are shown as mean+/−SD analyzed by one sample t test (panel B&C) and by one way ANOVA (panel E-G). *P<0.05, **P<0.01, ***P<0.001

Supplemental Figure 4



Supplemental Figure 4. Melatonin regulates mtDNA release and caspase activation in differentiated neurons: (A) Representative Immunoblots and (B) quantitation for caspase-3, PSD95, synaptophysin and β-actin in total cell lysate of WT and AANAT-KO differentiated N2a cells growing with or without melatonin (5 μM) in culture medium. β-Actin was used as a loading control and protein levels are expressed as fold change compared with untreated WT, N=3. Data are shown as mean+/−SD analyzed by one way ANOVA. *P<0.05, **P<0.01, ***P<0.001.

Supplemental Figure 5

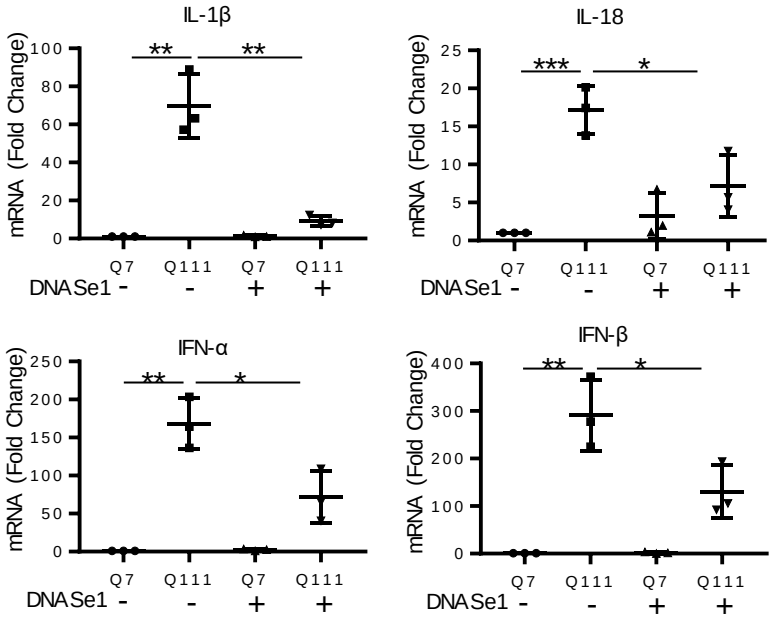


Supplemental Figure 5. EtBr treatment depletes mtDNA and its encoded proteins in WT and AANAT-KO N2a Cells:

(A) Representative Immunoblots and (B) quantitation for mt-CO1 and TOM20 in total cell lysate of WT and AANAT-

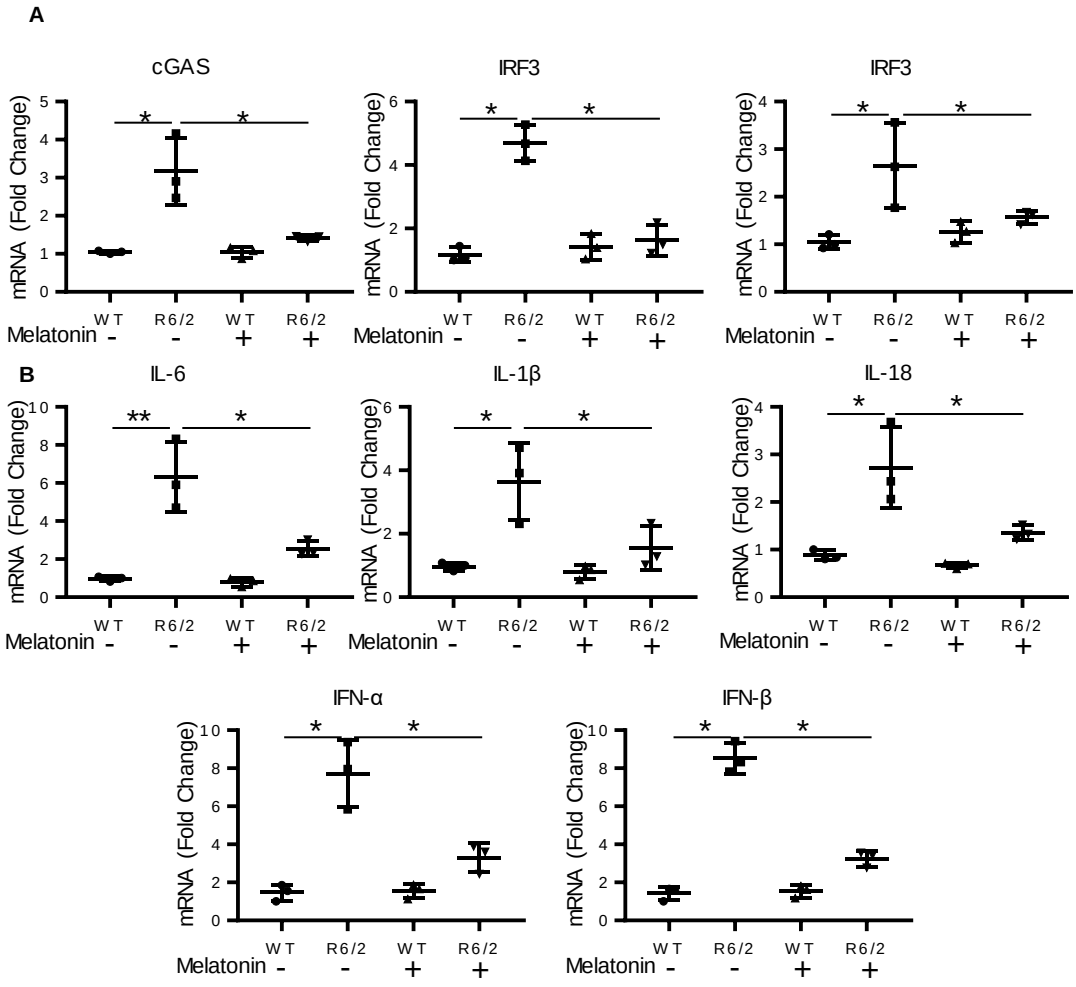
KO N2a cells exposed with different concentration of EtBr. TOM20 was used as a loading control and protein levels are shown as fold change as compared with untreated for each genotype N=3. (C) qPCR analysis of mitochondrial DNA copy number in WT and AANAT-KO N2a cells exposed with EtBr, β-Actin was used as endogenous control, mtDNA is plotted relative to amount in WT N2a cells after normalization to β-actin N=3. (D) Analysis of mitochondrial ROS by Mitosox in WT and AANAT-KO differentiated N2a cells with or without EtBr (1 µg/mL) treatment. Mitosox fluorescence is plotted relative to amount in WT differentiated N2a cells after normalization to nuclear stain, N=3. (E) Analysis of MMP by TMRM in WT and AANAT-KO N2a cells exposed with or without EtBr (1 µg/mL). TMRM fluorescence is plotted relative to the amount in WT differentiated N2a cells after normalization to nuclear stain N=3. (F) qPCR analysis for mRNA of PSD95, synaptophysin, homer 1 and caspase-1 in WT and AANAT-KO differentiated N2a cells with or without EtBr treatment (1 µg/mL). β-actin was used as endogenous control, and data is plotted relative to amount in WT N2a cells after normalization to β-Actin for each gene, N=3. Data are shown as mean+/−SD analyzed by one way ANOVA, ns; non-significant*P<0.05, ***P<0.001.

Supplemental Figure 6



Supplemental Figure 6. DNAse1 degrades mtDNA in HD cells: qPCR analysis of mRNA of IL-1β, IL-18, IFN-α and IFN-β in Q7 and Q111 differentiated cells transfected with or without DNAse1. β-actin was used as endogenous control, mRNA expression is plotted relative to amount in LDH-treated Q7 differentiated cells after normalization to β-actin, N=3. Data are shown as mean+/−SD analyzed by one way ANOVA *P<0.05, **P<0.01, ***P<0.001.

Supplemental Figure 7



Supplemental Figure 7. Melatonin regulates mtDNA-mediated inflammation in HD Striatum: (A) qPCR analysis of mRNA of cGAS, STING, IRF3 in WT and R6/2 striatum with or without melatonin (30 mg/kg) treatment. β-actin was used as endogenous control, the amount of mRNA is presented relative to the amount in vehicle-treated WT striatum after normalization to β-actin, N=3. (B) qPCR analysis of mRNA of IL-6, IL-1β, IL-18, IFN-α and IFN-β in WT and R6/2 striatum with or without melatonin (30 mg/kg) treatment. β-actin was used as endogenous control, the amount of mRNA is presented relative to the amount in vehicle-treated WT striatum after normalization to β-Actin N=3. Data are shown as mean+/− SD analyzed by one way ANOVA *P<0.05, **P<0.01, ***P<0.001.

Supplemental Table 1

Type	Grade	Specimen	Age	Gender	CAG repeats	Standard brain block (SBB)(64)	Postmortem interval before frozen
Control	n.a.	T-110	62	M	N.E.	SBB7.1	N.E.
Control	n.a.	T-133	33	F	N.E.	SBB7.1	11:25
Control	n.a.	T-169	69	M	N.E.	SBB6.2	49:20
Control	n.a.	T-180	52	F	N.E.	SBB7.2	6:07
Control	n.a.	T-638	78	M	N.E.	SBB7.1	8:00
Control	n.a.	T-3925	79	F	N.E.	SBB6.1	18:50
Control	n.a.	T-5404	54	F	N.E.	SBB7.1	16:36
HD2	2	T-461	77	M	41/15	SBB7.2	19:28
HD2	2	T-3221	58	M	42/15	SBB7.2	85:11
HD2	2	T-4394	75	F	41/17	SBB7.2	22:50
HD2	2	T-4498	59	M	43/17	SBB6.2	32:45
HD2	2	T-4964	89	M	40/17	SBB6.0	10:35
HD2	2	T-4989	75	F	42/15	SBB6.1	37:35
HD2	2	T-5263	55	F	54/30	SBB6.2	30:50

Note: Grade - diagnosed HD grade, Specimen - frozen tissue samples, Age - years at death, F - female, M – male, N.E. - not estimated, n.a. - not applicable, CAG repeats - number of CAG repeats of both alleles.

Supplemental Table 1: Characteristics of control human samples

Supplemental Table 2

Antibodies	Vendor	Detail
Mouse monoclonal anti-β-Actin	Millipore-Sigma	Cat#A5441 RRID:AB_476744
Rabbit monoclonal anti-cGAS	Cell signaling Technology	Cat#31659 RRID:AB_2799008
Rabbit monoclonal anti-STING	Cell signaling Technology	Cat#50494 RRID:AB_2799375
Rabbit monoclonal anti-caspase-3	Cell signaling Technology	Cat#9662S RRID: AB_331439
Mouse monoclonal anti-caspase-9	Cell signaling Technology	Cat#9508S RRID: AB_2068620
Rabbit monoclonal anti-pNF-kB	Cell signaling Technology	Cat#3033S RRID: AB_331284
Rabbit polyclonal anti-IRF3	Proteintech	Cat#11312-1-AP RRID: AB_2127004
Rabbit polyclonal anti-TOM20	Proteintech	Cat#11802-1-AP RRID: AB_2207530
Rabbit polyclonal anti cGAS antibody	Proteintech	Cat#26416-1-AP RRID: Not Available
Rabbit polyclonal anti-caspase-1	Santacruz biotechnology	SC-1218R RRID: AB_630973
Rabbit polyclonal anti-PSD95	Abcam	Cat# ab18258 RRID: AB_444362
Mouse monoclonal anti-Cytochrome C	Abcam	Cat#ab110325 RRID:AB_10864775
Mouse monoclonal anti-mt-CO1	Abcam	Cat# ab14705 RRID:AB_2084810
Mouse monoclonal anti-Synaptophysin	Santacruz biotechnology	Cat# sc-17750 RRID: AB_628311
Goat polyclonal anti-BID	R&D Systems	Cat# AF860-SP RRID:AB_2065622
Mouse monoclonal anti-8'-OHDG	Stressmarq Biosciences	Cat# SMC-155 RRID: AB_889490
Mouse monoclonal anti-βIII-tubulin	Millipore-Sigma	Cat#T8660 RRID: AB_477590
Rabbit polyclonal anti-Map2	Santacruz Biotechnology	Cat#sc-20172 RRID:AB_2250101
IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody	LI-COR	Cat#925-32211 RRID: AB_2651127
IRDye® 680RD Goat anti-Mouse IgG Secondary Antibody	LI-COR	Cat#925-68070 RRID: AB_2651128
IRDye® 800CW Donkey anti-Goat IgG Secondary Antibody	LI-COR	Cat#925-32214: RRID: AB_2687553
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594		Cat#A-21203 RRID: AB_2535789
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488		Cat#A-21206 RRID: AB_2535792

Supplemental Table 2: Information of Antibody used in the study.

Supplemental Table 3

Oligonucleotide/Primers sequence	
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'
	RP; 5'-AGCGAGAAGAGGGGCATT-3'
cGAS	FP; 5'-ACCGGACAAGCTAAAGAAGGTGCT-3'
	RP; 5'-GCAGCAGGCGTTCCACAACTTTAT-3'
STING	FP; 5'-GTCCTCTATAAGTCCCTAAGCATG-3'
	RP; 5'-AAGATCAACCGCAAGTACCC-3'
IRF3	FP; 5'-CACAAGGACAAGGACGGAG-3'
	RP; 5'-ATGCAGAACCACAGAGTGTAG-3'
Caspase-1	FP; 5'-TCTGTATTCACGCCCTGTTG-3'
	RP; 5'-GATAAATTGCTTCCTCTTTGCCC-3'
IL-6	FP; 5'-CCACTCACCTCTTCAGAACG-3'
	RP; 5'-CATCTTTGGAAGGTTCAGGTTG-3'
IL-1β	FP; 5'-ACGGACCCCAAAAGATGAAG-3'
	RP; 5'-TTCTCCACAGCCACAATGAG-3'
IL-18	FP; 5'-GCCTCAAACCTTCCAAATCAC-3
	RP; 5'-GTTGTCTGATTCCAGGTCTCC-3'
IFN-α	FP; 5'-TCTGTGCTTTCCTGATGGTC-3'
	RP; 5'-GGTTATGAGTCTGAGGAAGGTC-3'
IFN-β	FP; 5'-CAGCCCTCTCCATCAACTATAAG-3'
	RP; 5'-TCTCCGTCATCTCCATAGGG-3'
PSD95	FP; 5'-GTGACAACCAAGAAATACCGC-3'
	RF; 5'-TTCACCTGCAACTCATATCCTG-3'
Synaptophysin	FP; 5'-AGTGCCCTCAACATCGAAG-3'
	RP; 5'-GCCACGGTGACAAAGAATTC-3'
Homer-1	FP; 5'-CAGAGCAAGTTTTCATTGGGC-3'
	RP; 5'- TGTGTTCGGGTCAATCTGG-3'
β-Actin	FP; 5'-ACCTTCTACAATGAGCTGCG-3'
	RP; 5'-CTGGATGGCTACGTACATGG-3'

Supplemental Table 3: Sequence of primers used in qPCR

FP: Forward Primer, RP: Reverse Primer