

Supplementary Fig 1

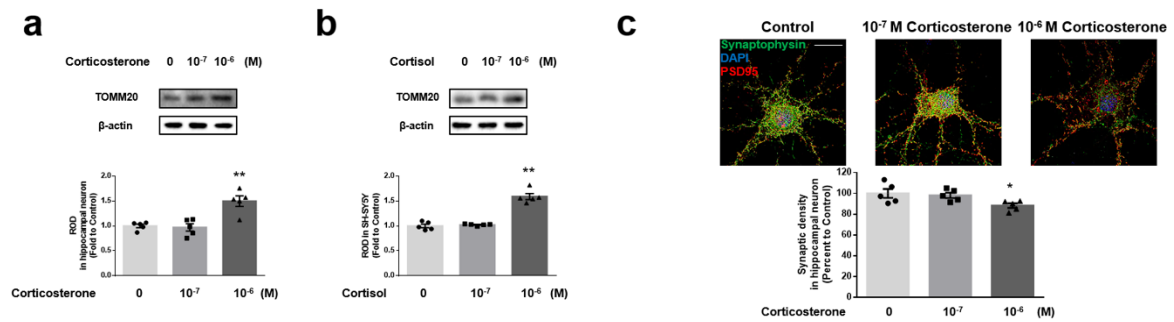


Fig 1. The dose-dependent effect of corticosterone and cortisol on mitophagy and synaptic density. (a – b) Hippocampal neurons and SH-SY5Y cells were treated with corticosterone and cortisol (0 – 1 μ M) for 24 h, respectively. TOMM20 level was detected by western blot. Loading control is β -actin. (c) Hippocampal neurons were treated with corticosterone (0 – 1 μ M) for 48 h. Hippocampal neurons were immunostained with synaptophysin (green), PSD95 (red), and DAPI (blue). Pearson's correlation coefficient was quantified for detecting synaptic density. Scale bars, 20 μ m (magnification, $\times$ 1,000). $n=5$. All blots and immunofluorescence images are representative. $n=5$ from independent experiments with two technical replicates each. Quantitative data are presented as a mean $\pm$ S.E.M. The representative images were acquired by SRRF imaging system. Two-sided one-way ANOVA was conducted. *, ** indicates $p<0.05$, $p<0.01$ versus control, respectively. Data are provided as a Source data file.

Supplementary Fig 2

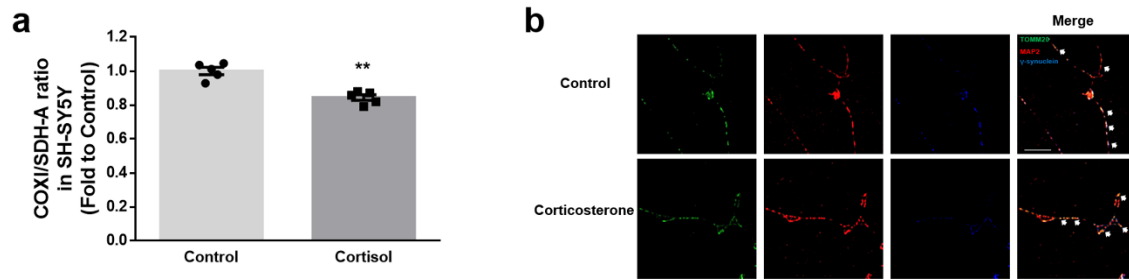


Fig 2. The effect of cortisol and corticosterone on mitochondrial biogenesis and transcellular mitophagy, respectively. (a) SH-SY5Y cells were treated with cortisol for 24 h. Subunit I of complex IV/70 kDa subunit of Complex II (COXI/SDH-A) ratio was measured to assess mitochondrial biogenesis. $n=5$ from independent experiments with two technical replicates each. Quantitative data are presented as a mean $\pm$ S.E.M. Two-sided unpaired student's t-test was conducted. ** indicates $p<0.01$ versus control. (b) Hippocampal neurons were treated with corticosterone for 24 h. Fixed coverslips were then immunostained with TOMM20 (green), MAP2 (red), and γ -synuclein (blue). Scale bars, 200 μ m (magnification, $\times 100$). $n=3$ from independent experiments with two technical replicates each. The representative images were acquired by SRRF imaging system. Data are provided as a Source data file.

Supplementary Fig 3

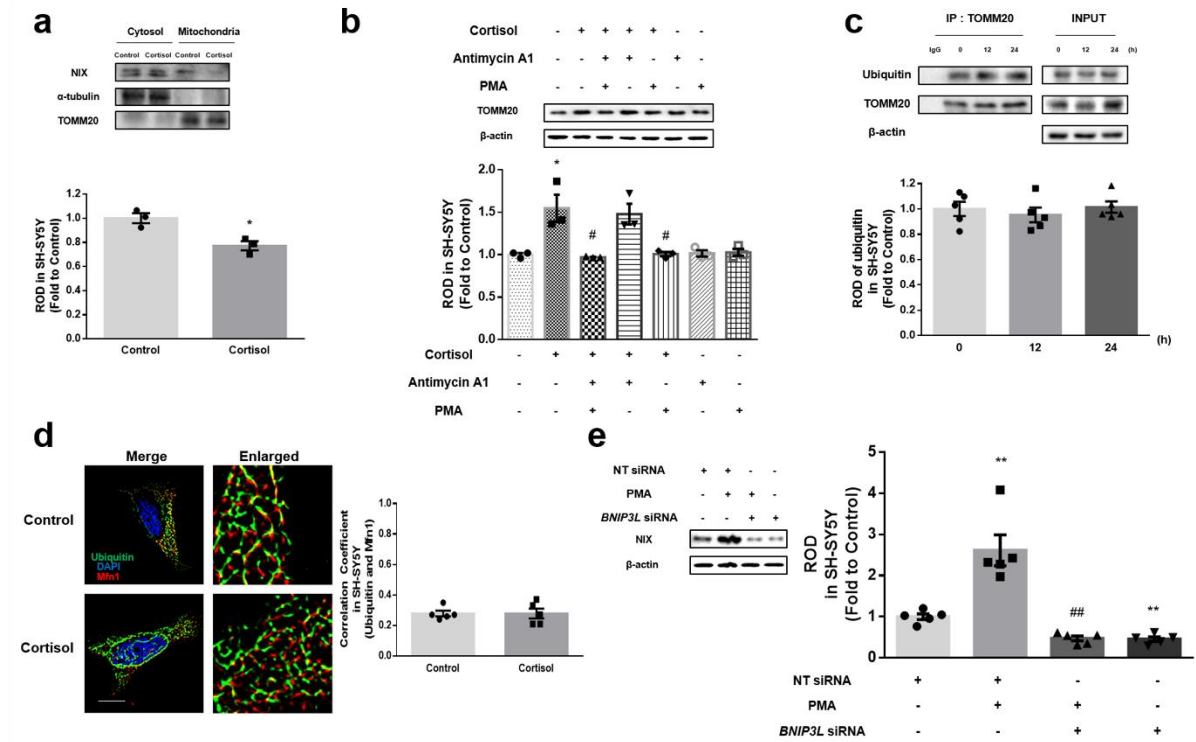


Fig 3. Decreases in mitochondrial NIX expression by cortisol inhibits mitophagy independently from the PINK1-parkin pathway. (a) SH-SY5Y cells were treated with cortisol for 24 h. NIX expressions in subcellular fraction samples were detected by western blotting. The α -tubulin and TOMM20 were used as cytosolic and mitochondrial loading control, respectively. The level of NIX in mitochondrial parts was quantified. $n=3$. Two-sided unpaired student's t-test was conducted. * indicates $p < 0.05$ versus control. (b) SH-SY5Y cells were pre-treated with phorbol 12-myristate 13-acetate (PMA, 10 nM) or antimycin A (10 μ M) for 30 min and incubated with cortisol for 24 h. TOMM20 levels were detected by western blot. Loading control is β -actin. $n=3$. * indicates $p < 0.05$ versus control and # indicates $p < 0.05$ versus cortisol in SH-SY5Y, respectively. Two-sided two-way ANOVA was conducted. (c) SH-SY5Y cells were treated with cortisol for various time (0 – 24 h). TOMM20 was co-immunoprecipitated with ubiquitin. The level of ubiquitin in immunoprecipitated samples was quantified. $n=5$. Two-sided one-way ANOVA was conducted. The p value of 12 h and 24 h treatment of cortisol versus control is 0.5765 and 0.846, respectively. (d) SH-SY5Y cells were incubated with cortisol for 24 h. Co-localization of ubiquitin (green) and Mfn1 (red) was visualized with SRRF imaging system. DAPI was used for nuclear counterstaining (blue). Scale bars represent 20 μ m (magnification, $\times 1,000$). $n=5$. Correlation coefficient analysis using Pearson's coefficient value was done. Two-sided unpaired student's t-test was conducted. The p value versus

control is 0.99. (e) NT or *BNIP3L* siRNA was transfected to SH-SY5Y cells for 24 h prior to PMA (10 nM) for 24 h. The expressions of NIX were detected with western blot where β -actin was used as loading control. $n=5$. Two-sided two-way ANOVA was conducted. ** indicates $p<0.01$ versus control. ## indicates $p<0.01$ versus PMA. All blots and immunofluorescence images are representative. $n=3$ or 5 from independent experiments with two technical replicates each. Quantitative data are presented as a mean $\pm$ S.E.M. The representative images were acquired by SRRF imaging system. Data are provided as a Source data file.

Supplementary Fig 4

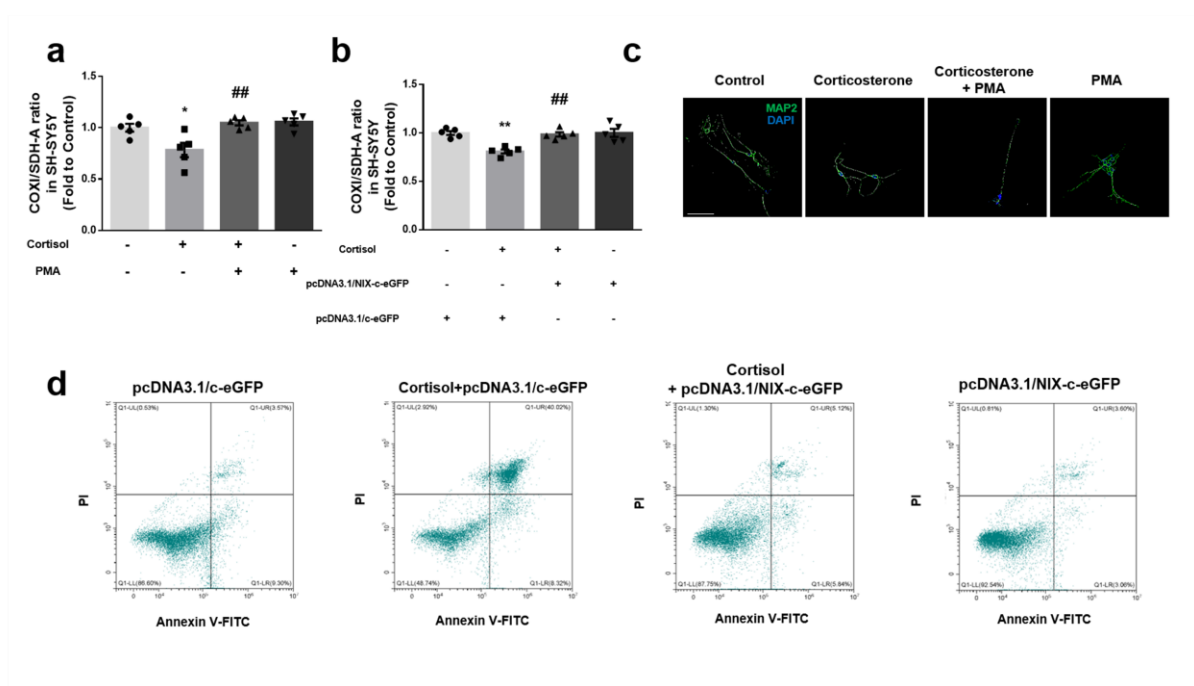


Fig 4. Glucocorticoid decreases mitochondrial biogenesis, synaptic function, and cell viability via inhibiting NIX expression. (a) SH-SY5Y cells were pretreated with phorbol 12-myristate 13-acetate (PMA, 10 nM) for 30 min prior to cortisol for 24 h. Subunit I of complex IV/70 kDa subunit of Complex II (COXI/SDH-A) ratio was measured to assess mitochondrial biogenesis. $n=5$. Two-sided two-way ANOVA was conducted. (b) SH-SY5Y cells were transfected with pcDNA3.1/c-eGFP or pcDNA3.1/NIX-c-eGFP vector for 24 h prior to cortisol treatment for 24 h. COXI/SDH-A ratio was measured to assess mitochondrial biogenesis. $n=5$. Two-sided two-way ANOVA was conducted. (c) Hippocampal neurons were treated with corticosterone for 48 h prior to PMA pretreatment (10 nM) for 30 min. Hippocampal neurons immunostained with MAP2 (green) and DAPI (blue) for measuring lengths of dendrites are visualized using SRRF imaging system. Scale bars, 100 μ m (magnification, $\times 200$). $n=5$. (d) SH-SY5Y cells were transfected with pcDNA3.1/c-eGFP or pcDNA3.1/NIX-c-eGFP vector for 24 h prior to cortisol treatment for 72 h. The percentages of apoptotic cells were analyzed by Annexin V/PI analysis, measured by flowcytometer. Annexin V-FITC only positive cells undergo early apoptosis whereas PI positive only cells undergo necrosis. Both Annexin V-FITC and PI positive cells undergo late apoptosis Annexin V positive cells were considered as apoptotic cells. $n=5$. $n=5$ from independent experiments with two technical replicates each. Quantitative data are presented as a mean $\pm$ S.E.M. *, ** indicates $p<0.05$, $p<0.01$ versus control, respectively. ## indicates $p<0.01$ versus cortisol, respectively. Data are provided as a Source data file.

Supplementary Fig 5

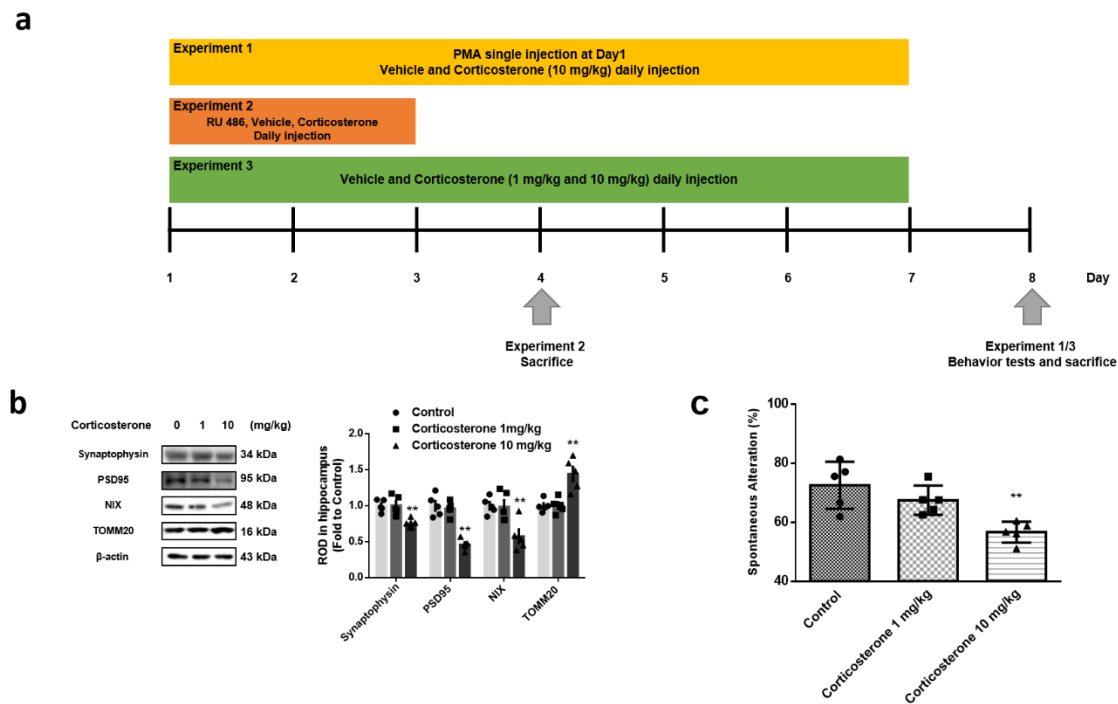


Fig 5. Stress-induced levels of corticosterone impairs mitophagy and spatial memory *in vivo*. (a) Brief illustration of drug injection schedule (b-c) Mice were exposed to vehicle and corticosterone (1 mg/kg and 10 mg/kg) for 7 days. (b) Expression of synaptophysin, PSD95, NIX, and TOMM20 were detected via western blot. Loading control is β -actin. $n=5$ from each animal with two technical replicates. (c) The mice were subjected to Y-maze test to evaluate spatial memory function. $n=5$. All blots and immunofluorescence images are representative. Quantitative data are presented as a mean $\pm$ S.E.M. Two-sided one-way ANOVA was conducted. ** indicates $p<0.01$ versus control and ## indicates $p<0.01$ versus corticosterone. Data are provided as a Source data file.

Supplementary Table

Figure	<i>p</i> value	Figure	<i>p</i> value	Figure	<i>p</i> value
Fig 1a	MAP2 and MTR: <0.0001 Tau and MTR: 0.0026	Fig 4a	Versus control: 0.0176 Versus corticosterone: 0.0134	Fig 6a	Versus control: 0.0001 Versus corticosterone: 0.002
Fig 1b	Mitochondrial intensity: <0.0001 Pearson's correlation coefficient: <0.0001	Fig 4b	Versus control: 0.0038 Versus cortisol: 0.003	Fig 6b	Versus control: <0.0001 Versus cortisol: 0.0002
Fig 1c	Perinuclear region: <0.0001 Distal region: 0.0125	Fig 4c	Versus control: 0.0153 Versus corticosterone: 0.0003	Fig 6e	0.0045
Fig 1d	<0.0001	Fig 4d	Versus control: <0.0001 Versus cortisol: 0.0003	Fig 7a	Versus control: 0.0008 Versus corticosterone: 0.0011
Fig 1e	<0.0001	Fig 4f	Basal respiration: versus control (0.0005) versus corticosterone (<0.0001) Maximal respiration: versus control (0.0003) versus corticosterone (<0.0001) ATP production: versus control (0.0007) versus corticosterone (<0.0001) Proton leak: versus control (0.0003) versus corticosterone (<0.0001)	Fig 7b	Versus control: 0.001 Versus cortisol: 0.0134
Fig 1f	Synaptophysin: <0.0001 PSD95: 0.0004	Fig 4h	Basal respiration: versus control (0.0018) versus cortisol (0.0098) Maximal respiration: versus control (0.0004) versus cortisol (<0.0001) ATP production: versus control (0.0004) versus cortisol (0.0005) Proton leak: versus control (<0.0001) versus cortisol (0.0003)	Fig 7c	Versus control: 0.001 Versus corticosterone: 0.0054
Fig 1g	<0.0001	Fig 4i	Synaptophysin: versus control (<0.0001) versus corticosterone (0.0008) PSD95: versus control (0.0003) versus corticosterone (<0.0001)	Fig 7d	Versus control: <0.0001 Versus cortisol: <0.0001
Fig 1i	<0.0001	Fig 4j	Versus control: <0.0001 Versus corticosterone: 0.0001	Fig 7e	Versus control: 0.0001 Versus cortisol: 0.0004
Fig 2a	CCCP+Antimycin A1: <0.0001 Corticosterone: <0.0001	Fig 5a	Versus control: <0.0001 Versus corticosterone: <0.0001	Fig 7f	Versus control: 0.0005 Versus cortisol: 0.0454
Fig 2b	CCCP+Antimycin A1: <0.0001 Cortisol: 0.0048	Fig 5b	Versus control: 0.0011 Versus cortisol: 0.004	Fig 7g	Versus control: 0.0002 Versus cortisol: 0.0004
Fig 2c	<0.0001	Fig 5c	Versus control: 0.0128 Versus corticosterone: 0.0048	Fig 8a	Versus control: 0.0008 Versus corticosterone: 0.0037
Fig 2d	0.0026	Fig 5d	Versus control: 0.0035 Versus cortisol: 0.0103	Fig 8b	NIX: 0.0014
Fig 2e	<0.0001			Fig 8c	Versus control: 0.001 Versus corticosterone: 0.0054
Fig 2f	<0.0001			Fig 8d	Versus control: 0.001 Versus corticosterone: 0.0054
Fig 2g	<0.0001			Fig 8e	Versus control: 0.001 Versus corticosterone: 0.0054
Fig 2h	<0.0001			Fig 8g	Versus control: 0.001 Versus corticosterone: 0.0054
Fig 2i	Cortisol: TOMM20 (0.0004), LC3II/I (0.0059) Bafilomycin: TOMM20 (0.002), LC3II/I (0.0058)			Supplementary figure <i>p</i> value	
Fig 3a	NIX: 0.0016			Fig 1a	1 μ M corticosterone (0.002)
Fig 3b	NIX: <0.0001			Fig 1b	1 μ M cortisol (<0.0001)
Fig 3c	NIX 24 h: 0.001			Fig 1c	1 μ M corticosterone (0.0391)
Fig 3d	0.0072			Fig 2a	0.0004
Fig 3e	0.0003			Fig 3a	0.0154
Fig 3f	Versus control: 0.0002 Versus cortisol: <0.0001			Fig 3b	Versus control: 0.0277 Versus cortisol: Cortisol+Antimycin A1+PMA (0.023) Cortisol+PMA (0.0295)
Fig 3g	Versus control: <0.0001			Fig 3e	Versus control: PMA (0.0028) BNI3L siRNA (0.0003) Versus PMA: 0.0005
Fig 3h	Versus control: 0.0002			Fig 4a	Versus control: 0.0259 Versus cortisol: 0.0072
Fig 3i	Versus control: <0.0001 Versus corticosterone: <0.0001			Fig 4b	Versus control: 0.0002 Versus cortisol: 0.0004
Fig 3j	Versus control: 0.0067 Versus cortisol: 0.0021			Fig 5b	10 mg corticosterone: Synaptophysin (0.0007) PSD95 (<0.0001) NIX (0.0043) TOMM20 (0.0031)
Fig 3k	Versus control: 0.0002 Versus corticosterone: 0.0009			Fig 5c	10 mg corticosterone (0.0036)
Fig 3l	Versus control: <0.0001 Versus cortisol: <0.0001				
Fig 3m	Versus control: 0.0001 Versus cortisol: 0.0017				

Table. The exact *p* value for each figure.