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Supplemental Information

Modulation of Autophagy by BDNF Underlies Synaptic Plasticity

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SUPPLEMENTARY INFORMATION

Supplementary Figures & Legends

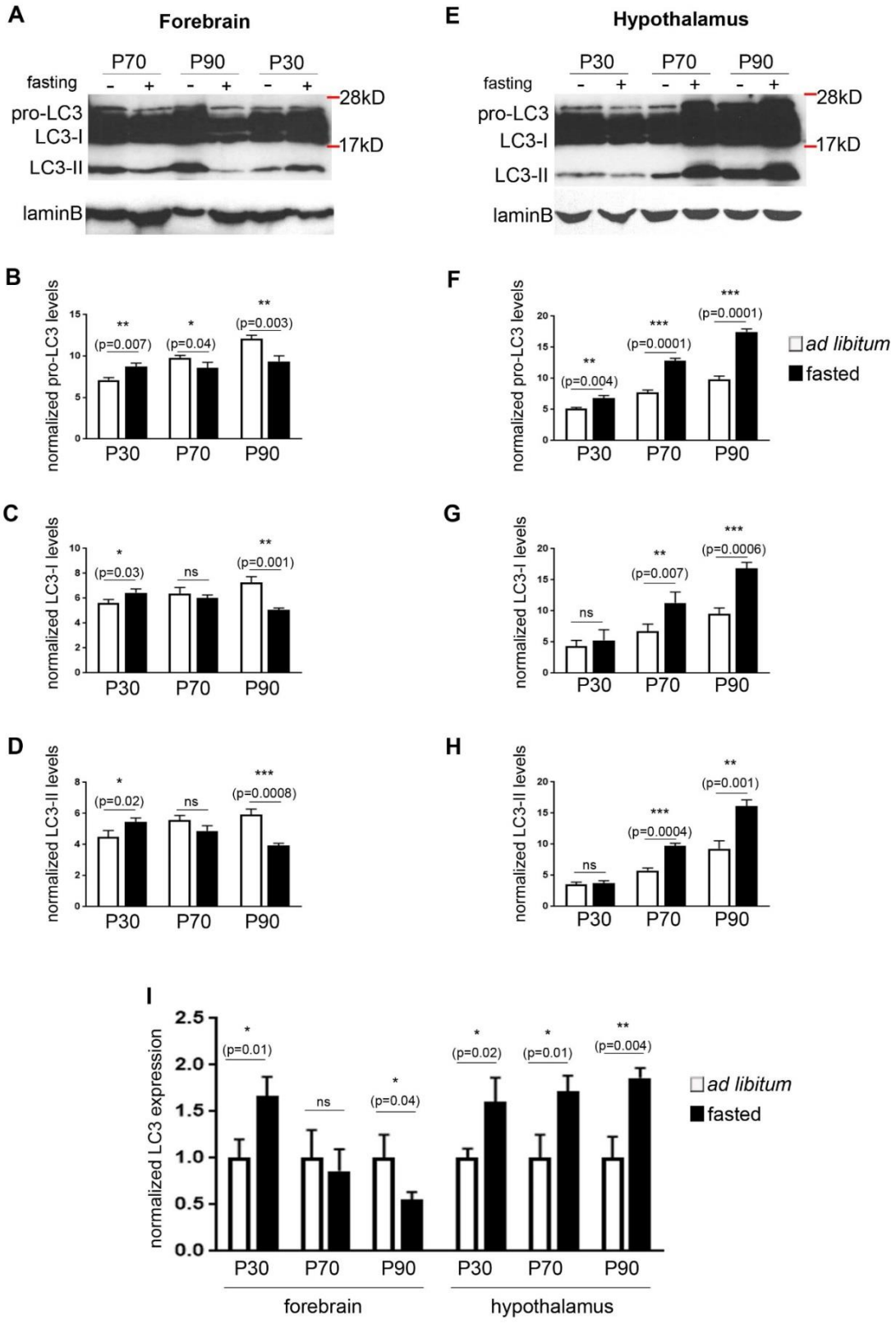

Figure S1 Related to Figure 1. Suppression of LC3 in the forebrain is a characteristic acquired with maturation

(A) Western blot analysis with an antibody against LC3 and Lamin B in forebrain lysates of male animals of the following ages, as indicated: P30, P70 and P90 (days post-natal). Animals were fed *ad libitum* or fasted for 12 hours.

(B) Graph showing quantification of pro-LC3 levels, normalized to LaminB, in the forebrain of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test.

(C) Graph showing quantification of LC3-I levels, normalized to LaminB, in the forebrain of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test.

(D) Graph showing quantification of LC3-II levels, normalized to LaminB, in the forebrain of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test.

(E) Western blot analysis with an antibody against LC3 and Lamin B in hypothalamic lysates of male animals of the following ages, as indicated: P30, P70 and P90 (days post-natal). Animals were fed *ad libitum* or fasted for 12 hours.

(F) Graph showing quantification of pro-LC3 levels, normalized to LaminB, in the hypothalamus of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test.

(G) Graph showing quantification of LC3-I levels, normalized to LaminB, in the hypothalamus of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age

and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test.

(H) Graph showing quantification of LC3-II levels, normalized to LaminB, in the hypothalamus of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test.

(I) Graph showing normalized LC3 mRNA levels in the forebrain and hypothalamus of animals of the indicated ages that were fed *ad libitum* or fasted for 12 hours. (N=3 male animals per age and per condition). Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test to compare levels between *ad libitum* and fasted states at each age.

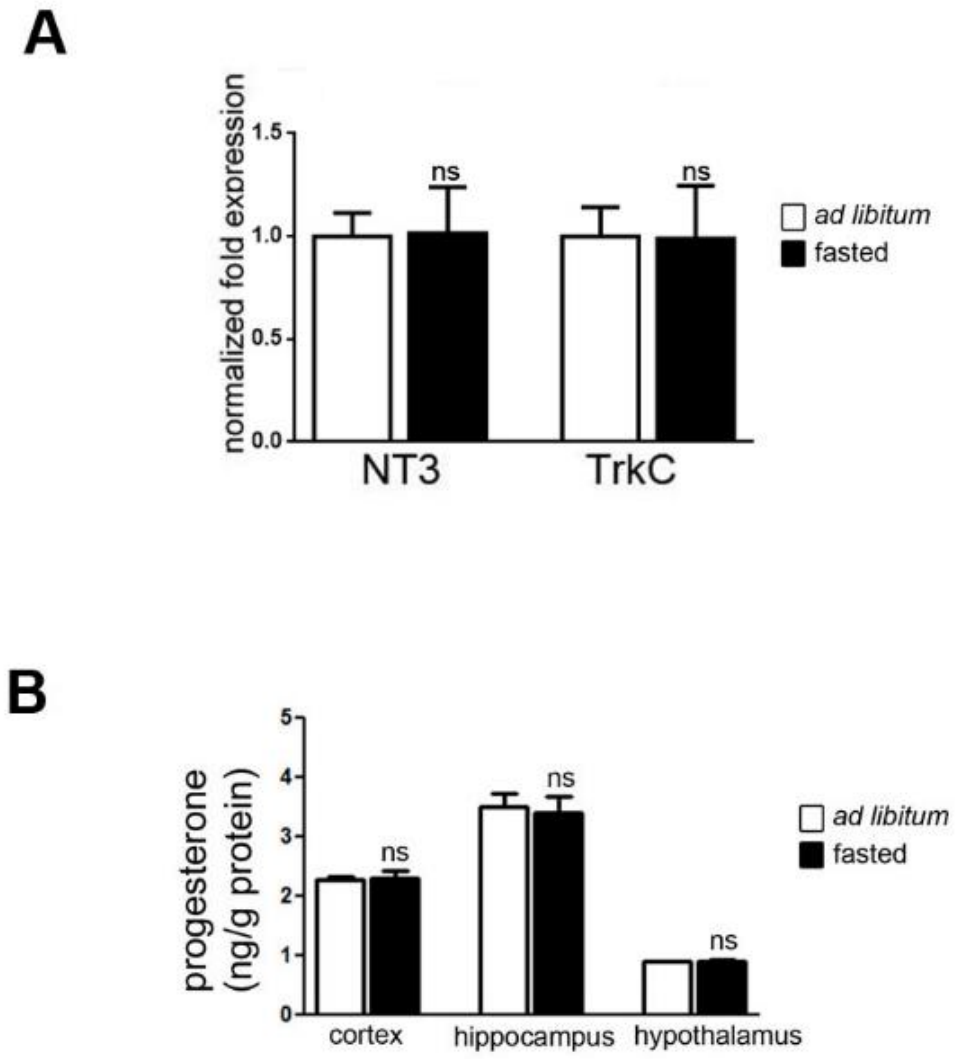

Figure S2. Related to Figure 2. Levels of NT3, TrkC and progesterone are not affected by fasting

(A) Graph showing normalized NT3 and TrkC mRNA levels in the forebrain of adult mice fed *ad libitum* or fasted for 24 hours. (N=6 adult male animals per condition). Note that the expression levels of NT3 and TrkC are not affected by fasting. Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test to compare levels between *ad libitum* and fasted states.

(B) Graph showing normalized progesterone levels as determined by ELISA immunoassay in the cortex, hippocampus and hypothalamus of adult animals fed ad libitum or fasted for 24 hours. (N=3 adult male animals per condition). Note that progesterone levels are not affected by fasting in any brain region. Bars represent mean values ± SEM. Statistical analyses were performed using Student's t-test to compare levels between *ad libitum* and fasted states in each brain region.

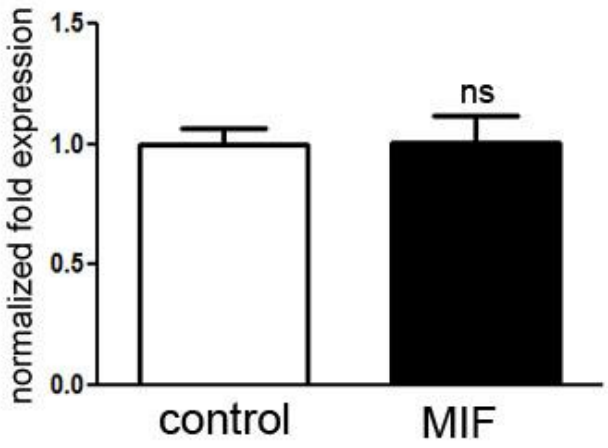

Figure S3. Related to Figure 2. p62 expression levels are not affected by treatment with Mifepristone

Graph showing the normalized p62 mRNA levels in the forebrain of animals treated with vehicle or with Mifepristone (MIF) for 24 hours. (N=3 animals per condition). Bars represent mean values± SEM. Statistical analyses were performed using Student's t-test.

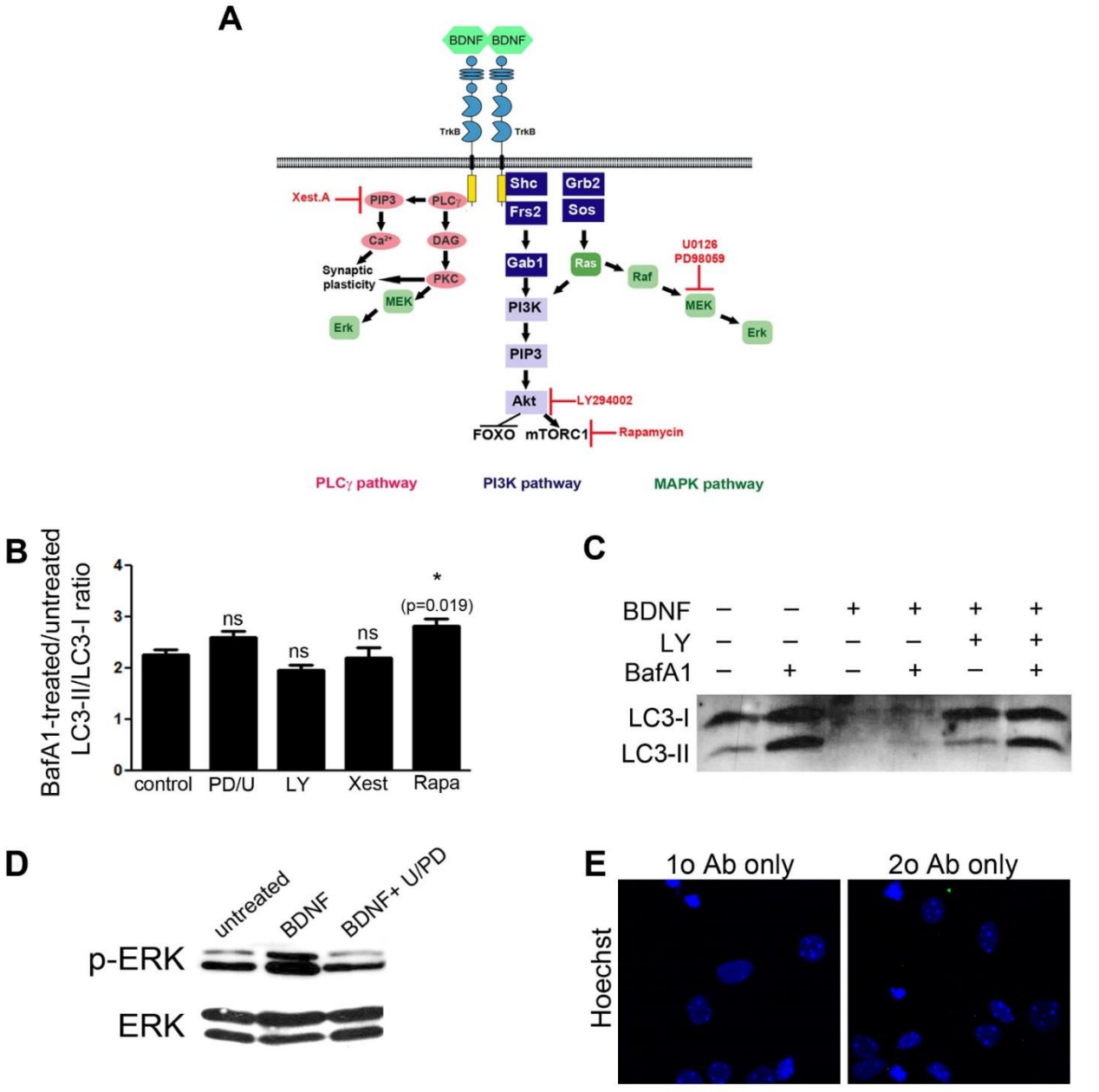
A
BDNF
BDNF
TrkB
TrkB
Xest.A
PIP3
PLCγ
Shc
Grb2
Frs2
Sos
Ca2+
DAG
Synaptic plasticity
PKC
MEK
Erk
Gab1
Ras
Raf
U0126
PD98059
MEK
Erk
PI3K
PIP3
Akt
LY294002
FOXO
mTORC1
Rapamycin
PLCγ pathway
PI3K pathway
MAPK pathway
B
BafA1-treated/untreated LC3-II/LC3-I ratio
ns
ns
ns
*
(p=0.019)
control
PD/U
LY
Xest
Rapa
C
BDNF
LY
BafA1
LC3-I
LC3-II
D
untreated
BDNF
BDNF+ U/PD
p-ERK
ERK
E
1o Ab only
2o Ab only
Hoechst

Figure S4. Related to Figure 3. Effects of different inhibitors on the autophagic flux of neurons.

(A) Schematic showing the three signaling pathways activated downstream of TrkB upon ligation of BDNF. The inhibitors used to pharmacologically block each pathway are outlined.

(B) Graph showing the effect of each inhibitor on the autophagic flux of cultured hippocampal neurons. Autophagic flux was calculated as the ratio of the LC3-II band in neurons cultured for 6h in the presence over absence of BafA1. BafA1 is an agent that inhibits the fusion of autophagosomes with lysosomes, hence resulting in their accumulation, which is reflected by increased levels of LC3-II. Bars represent mean values of densitometric analyses of LC3-II from western blots ±SEM. N=6 independent cultures per condition. Statistical analysis was performed by t-test comparing each inhibitor with the control.

(C) Overexposed western blot from figure 3G that allows the visualization of the LC3-II band for quantification purposes.

(D) Western blot analysis with antibodies against p-ERK and total ERK in lysates of hippocampal neurons that were either untreated, treated with recombinant BDNF for 1h, or with PD98059 and U0126 (U/PD), 2 potent inhibitors of the MEK/ERK pathway, and recombinant BDNF for 1h. Note that the inhibitors prevent the BDNF-induced activation of ERK, without affecting total ERK levels.

(E) Confocal images of hippocampal neurons stained only with primary antibodies against LC3, or only with a secondary antibody (anti-mouse Alexa 488). Note that no staining is observed under these conditions.

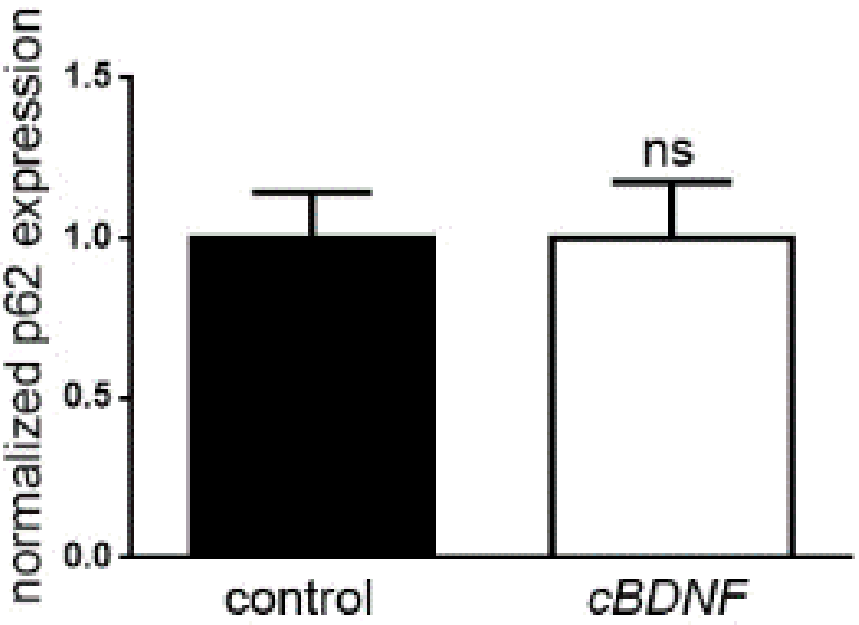

Figure S5. Related to Figure 4. p62 mRNA levels are not altered in *cBDNF* animals

Graph showing the normalized p62 mRNA levels in the forebrain of control (*BDNF$^{f/f}$*) and *cBDNF* (*Nestin-Cre; BDNF$^{f/f}$*) animals. (N=6 animals per genotype). Bars represent mean values± SEM. Statistical analyses were performed using Student's t-test.