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Supplementary Materials for

GABARAPs dysfunction by autophagy deficiency in adolescent brain impairs $GABA_A$ receptor trafficking and social behavior

Kelvin K. Hui, Noriko Takashima, Akiko Watanabe, Thomas E. Chater, Hiroshi Matsukawa, Yoko Nekooki-Machida, Per Nilsson, Ryo Endo, Yukiko Goda, Takaomi C. Saido, Takeo Yoshikawa, Motomasa Tanaka*

*Corresponding author. Email: motomasa.tanaka@riken.jp

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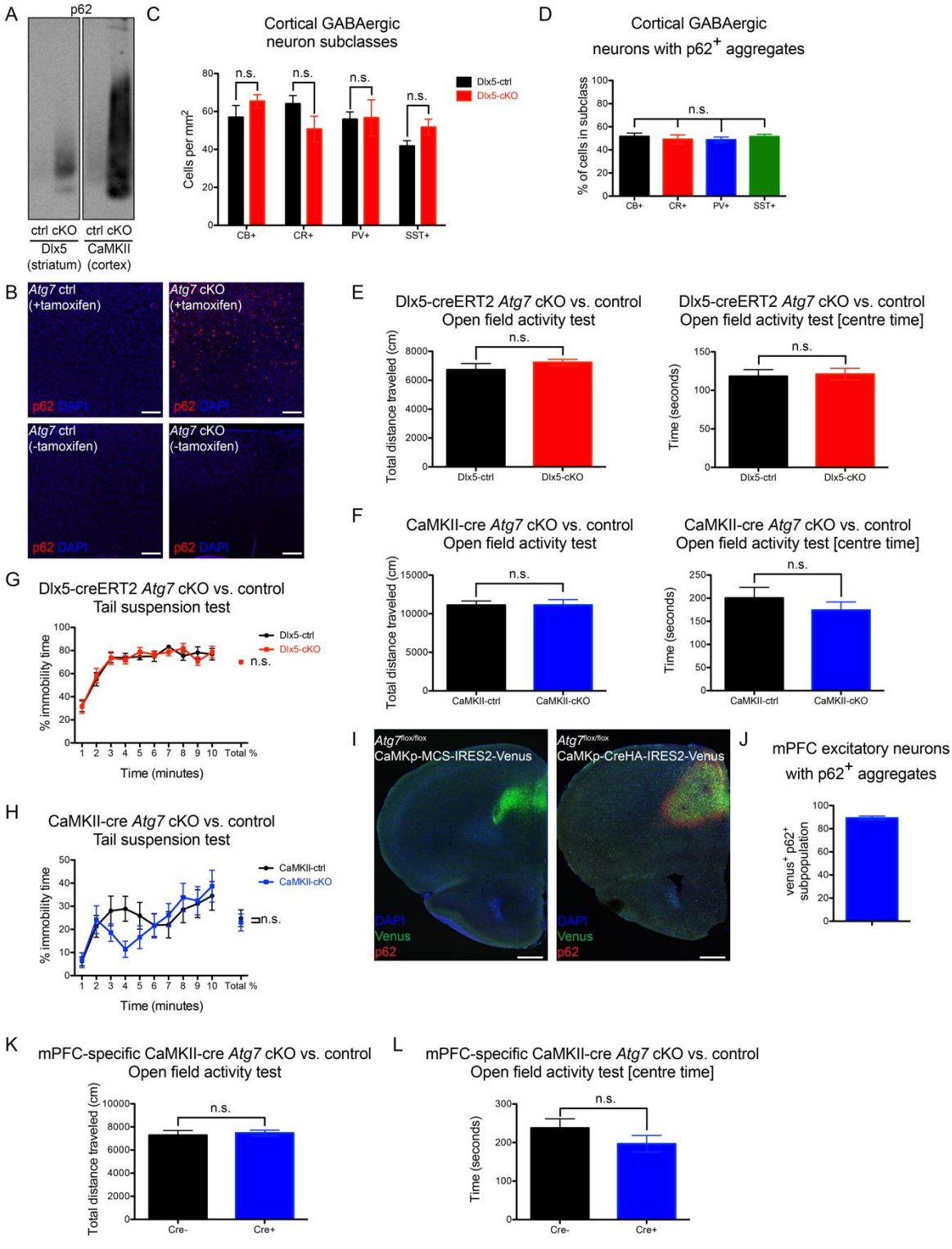
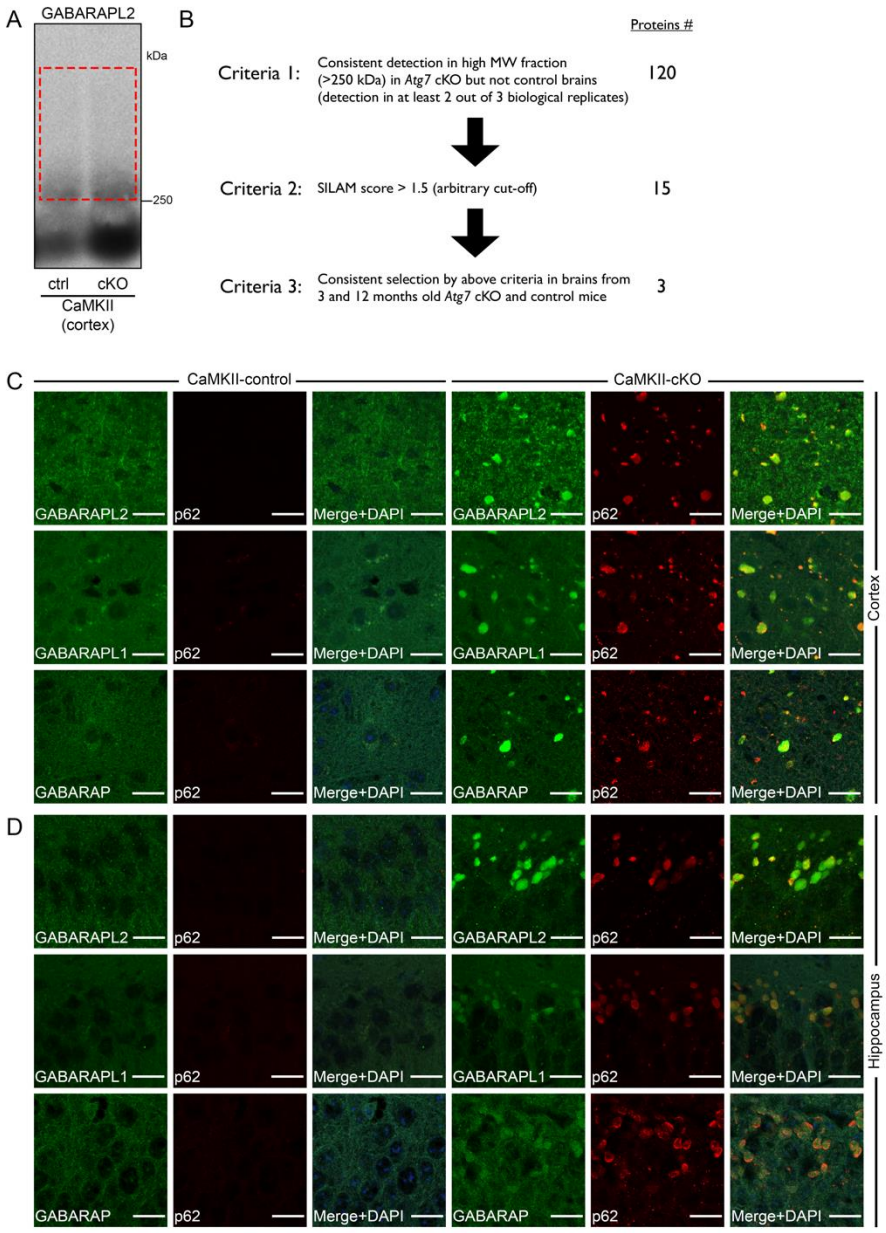

A
p62
ctrl cKO
Dlx5
(striatum)
ctrl cKO
CaMKII
(cortex)
C
Cortical GABAergic
neuron subclasses
Cells per mm2
n.s.
Dlx5-ctrl
Dlx5-cKO
CB+
CR+
PV+
SST+
D
Cortical GABAergic
neurons with p62+ aggregates
% of cells in subclass
n.s.
CB+
CR+
PV+
SST+
B
Atg7 ctrl
(+tamoxifen)
Atg7 cKO
(+tamoxifen)
Atg7 ctrl
(-tamoxifen)
Atg7 cKO
(-tamoxifen)
p62 DAPI
E
Dlx5-creERT2 Atg7 cKO vs. control
Open field activity test
Total distance traveled (cm)
n.s.
Dlx5-ctrl
Dlx5-cKO
Dlx5-creERT2 Atg7 cKO vs. control
Open field activity test [centre time]
Time (seconds)
n.s.
Dlx5-ctrl
Dlx5-cKO
F
CaMKII-cre Atg7 cKO vs. control
Open field activity test
Total distance traveled (cm)
n.s.
CaMKII-ctrl
CaMKII-cKO
CaMKII-cre Atg7 cKO vs. control
Open field activity test [centre time]
Time (seconds)
n.s.
CaMKII-ctrl
CaMKII-cKO
G
Dlx5-creERT2 Atg7 cKO vs. control
Tail suspension test
% immobility time
Dlx5-ctrl
Dlx5-cKO
n.s.
Total %
Time (minutes)
H
CaMKII-cre Atg7 cKO vs. control
Tail suspension test
% immobility time
CaMKII-ctrl
CaMKII-cKO
n.s.
Total %
Time (minutes)
I
Atg7flox/flox
CaMKp-MCS-IRES2-Venus
Atg7flox/flox
CaMKp-CreHA-IRES2-Venus
DAPI
Venus
p62
J
mPFC excitatory neurons
with p62+ aggregates
venus+ p62+
subpopulation
K
mPFC-specific CaMKII-cre Atg7 cKO vs. control
Open field activity test
Total distance traveled (cm)
n.s.
Cre-
Cre+
L
mPFC-specific CaMKII-cre Atg7 cKO vs. control
Open field activity test [centre time]
Time (seconds)
n.s.
Cre-
Cre+

Fig. S1. *Atg7* deletion in forebrain GABAergic neurons by Dlx5-creERT2. (**A**) p62 accumulation resulted in the formation of high molecular species as detected by SDD-AGE. (**B**) $p62^+$ aggregate formation was observed only in tamoxifen-treated Dlx5-creERT2 *Atg7* cKO mice (cortex shown), indicating little or no non-induced Cre-mediated recombination to delete *Atg7*. (**C**) Cell counts of major cortical GABAergic interneuron subclasses did not show any significant differences between Dlx5-creERT2 *Atg7* cKO and control mice. $n = 6$ animals per genotype. (**D**) Cell counts of major subclasses of cortical GABAergic interneurons of Dlx5-creERT2 *Atg7* cKO mice showed that approximately half (49-52%) of each subpopulation had formed clear somal $p62^+$ aggregates. $n = 4$ animals per genotype. (**E-F**) No significant differences were observed in open field activity between Dlx5-creERT2 or CaMKII-cre *Atg7* cKO mice and littermate controls. $n = 10$ (Dlx5-control), $n = 8$ (Dlx5-cKO), $n = 16$ (CaMKII-control), $n = 15$ (CaMKII-cKO). (**G-H**) No significant changes were observed in tail suspension test for Dlx5-creERT2 or CaMKII-cre *Atg7* cKO mice. $n = 10$ (Dlx5-control), $n = 8$ (Dlx5-cKO), $n = 16$ (CaMKII-control), $n = 16$ (CaMKII-cKO). (**I**) Representative immunofluorescence images showing the infected mPFC excitatory neurons ($Venus^+$) and $p62^+$ aggregate formation in cells also expressing Cre recombinase. (**J**) Cell counts of $Venus^+$ neurons showed that nearly all infected cells (90%) had accumulated and formed clear somal $p62^+$ aggregates. $n = 4$ animals per genotype. (**K-L**) $Atg7^{flox/flox}$ mice with mPFC-specific expression of Cre recombinase driven by CaMKII promoter showed no difference in movement and exploration compared to control mice. $n = 7$ animals per genotype. Data are presented as means ± SEM; n.s. indicates not statistically significant by unpaired, two-tailed Student's *t*-test (**C, E, F, G, H, K, L**) or one-way ANOVA (**D**). Scale bars: (**B**), 200 μm; (**I**), 500 μm.


A
GABARAPL2
kDa
250
ctrl
cKO
CaMKII
(cortex)
B
Proteins #
Criteria 1:
Consistent detection in high MW fraction
(>250 kDa) in Atg7 cKO but not control brains
(detection in at least 2 out of 3 biological replicates)
120
Criteria 2:
SILAM score > 1.5 (arbitrary cut-off)
15
Criteria 3:
Consistent selection by above criteria in brains from
3 and 12 months old Atg7 cKO and control mice
3
C
CaMKII-control
CaMKII-cKO
GABARAPL2
GABARAPL1
GABARAP
p62
Merge+DAPI
Cortex
D
Hippocampus

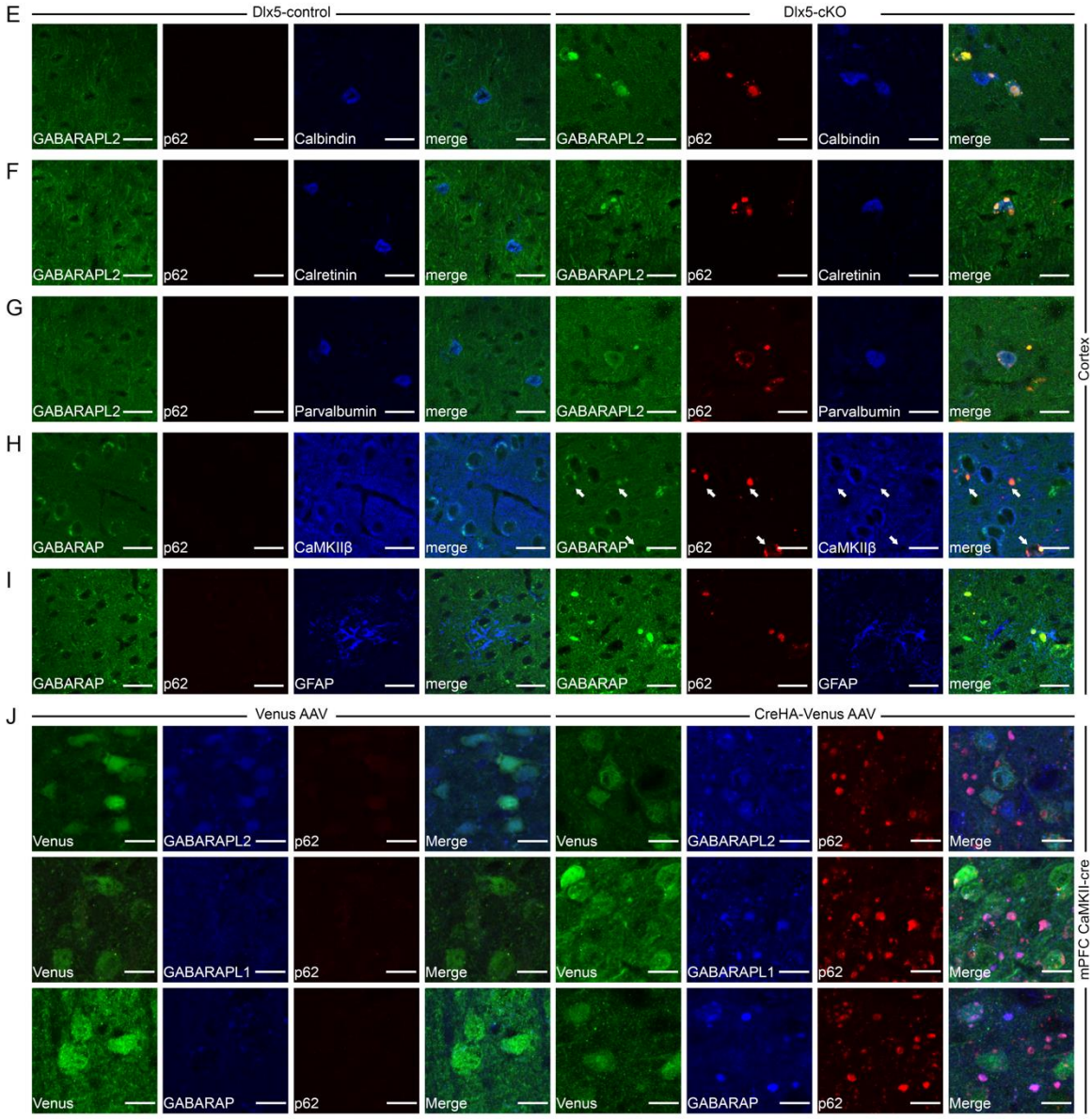

E
Dlx5-control
Dlx5-cKO
GABARAPL2
p62
Calbindin
merge
F
Calretinin
G
Parvalbumin
H
GABARAP
CaMKIIβ
I
GFAP
Cortex
J
Venus AAV
CreHA-Venus AAV
Venus
GABARAPL1
Merge
mPFC CaMKII-cre

Fig. S2. GABARAPs mislocalized to p62^{+} aggregates in affected neurons of Dlx5-creERT2 and CaMKII-cre *Atg7* cKO mice. (**A**) Detection of GABARAPL2 protein in high molecular weight fraction (>250 kDa) by SDD-AGE followed by immunoblotting. Indicated area closely corresponds to excised gel from which proteins were extracted for quantitative mass spectrometric analysis. (**B**) Schematic diagram of selection criteria applied and the number of selected proteins in the quantitative SDD-AGE/SILAM mass spectrometry experiment. (**C**-**D**) GABARAP family proteins accumulated and mislocalized to p62^{+} aggregates in cortex and hippocampi of CaMKII-cre *Atg7* cKO mice. (**E-I**) Mislocalization of GABARAPL2 or GABARAP to p62^{+} aggregates formed specifically in different cortical GABAergic interneuron subclasses of Dlx5-creERT2 *Atg7* cKO mice but not in CaMKII^{+} cortical excitatory neurons or GFAP^{+} astrocytes. (**J**) p62^{+} aggregates formed specifically in mPFC excitatory neurons with *Atg7* deletion and sequestered GABARAPs. Scale bars: (**C**), (**D**), (**E**), (**F**), (**G**), (**H**), (**I**), and (**J**), 20 μm.

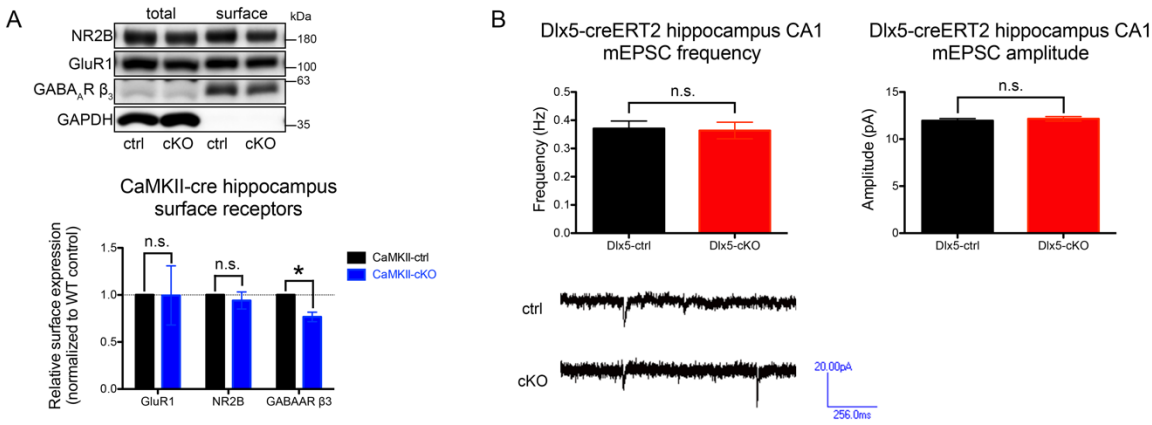

A
total
surface
kDa
NR2B
GluR1
GABA$_A$R β$_3$
GAPDH
180
100
63
35
ctrl
cKO
ctrl
cKO
CaMKII-cre hippocampus
surface receptors
Relative surface expression
(normalized to WT control)
n.s.
n.s.
*
CaMKII-ctrl
CaMKII-cKO
GluR1
NR2B
GABAAR β3
B
Dlx5-creERT2 hippocampus CA1
mEPSC frequency
Frequency (Hz)
n.s.
Dlx5-ctrl
Dlx5-cKO
Dlx5-creERT2 hippocampus CA1
mEPSC amplitude
Amplitude (pA)
n.s.
Dlx5-ctrl
Dlx5-cKO
ctrl
cKO
20.00pA
256.0ms

Fig. S3. Autophagy deficiency led to a reduction of surface $GABA_A$ receptors but no change in excitatory signaling. (**A**) A significant reduction in surface expression of $GABA_A$ receptors was observed in hippocampi of CaMKII-cre *Atg7* cKO mice. n = 8 animals per genotype**.** (**B**) No differences were observed in both frequency (left) or amplitude (right) of miniature excitatory postsynaptic currents (mEPSC) recorded from CA1 pyramidal cells between Dlx5-creERT2 *Atg7* cKO and control hippocampi. Representative traces are shown on the bottom. n = 40 cells recorded from 6 different animals (Dlx5-control) and 34 cells recorded from 5 different animals (Dlx5-cKO)**.** Data are presented as means ± SEM; * P < 0.05, paired, two-tailed Student's t-test. n.s. indicates not statistically significant.

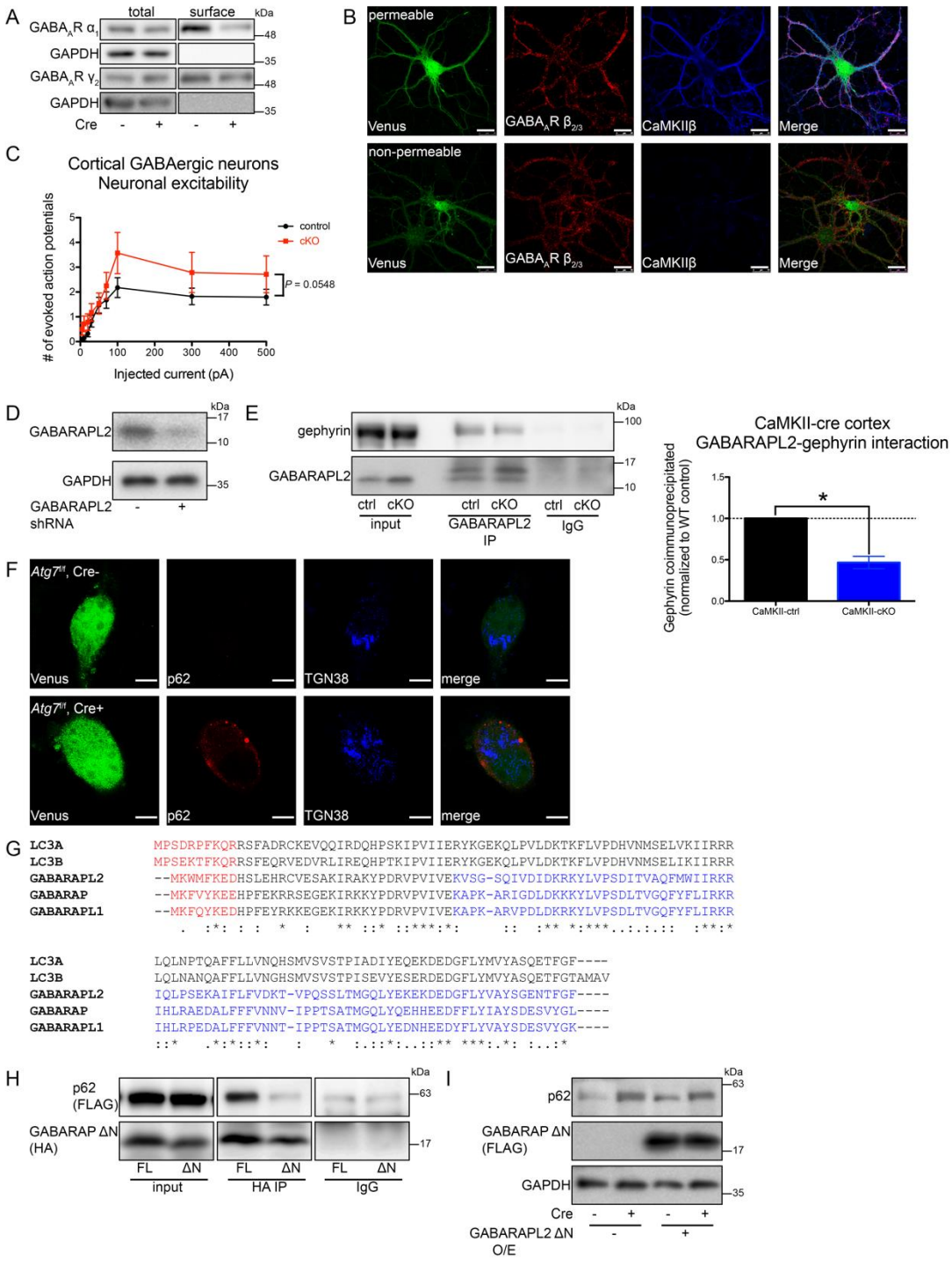

A
total
surface
kDa
GABA$_A$R α$_1$
GAPDH
GABA$_A$R γ$_2$
GAPDH
48
35
48
35
Cre - + - +
B
permeable
Venus
GABA$_A$R β$_{2/3}$
CaMKIIβ
Merge
non-permeable
Venus
GABA$_A$R β$_{2/3}$
CaMKIIβ
Merge
C
Cortical GABAergic neurons
Neuronal excitability
# of evoked action potentials
Injected current (pA)
control
cKO
P = 0.0548
D
kDa
GABARAPL2
GAPDH
17
10
35
GABARAPL2 shRNA - +
E
kDa
gephyrin
GABARAPL2
100
17
10
ctrl cKO ctrl cKO ctrl cKO
input
GABARAPL2 IP
IgG
CaMKII-cre cortex
GABARAPL2-gephyrin interaction
Gephyrin coimmunoprecipitated (normalized to WT control)
CaMKII-ctrl
CaMKII-cKO
F
Atg7f/f, Cre-
Venus
p62
TGN38
merge
Atg7f/f, Cre+
Venus
p62
TGN38
merge
G
LC3A MPSDRPFKQRRSFADRCKEVQQIRDQHPSKIPVIIERYKGEKQLPVLDKTKFLVPDHVNMSELVKIIRRR
LC3B MPSEKTFKQRRSFEQRVEDVRLIREQHPTKIPVIIERYKGEKQLPVLDKTKFLVPDHVNMSELIKIIRRR
GABARAPL2 --MKWMFKEDHSLEHRCVESAKIRAKYPDRVPVIVEKVSG-SQIVDIDKRKYLVPSDITVAQFMWIIRKR
GABARAP --MKFVYKEEHPFEKRRSEGEKIRKKYPDRVPVIVEKAPK-ARIGDLDKKKYLVPSDLTVGQFYFLIRKR
GABARAPL1 --MKFQYKEDHPFEYRKKEGEKIRKKYPDRVPVIVEKAPK-ARVPDLDKRKYLVPSDLTVGQFYFLIRKR
LC3A LQLNPTQAFFLLVNQHSMVSVSTPIADIYEQEKDEDGFLYMVYASQETFGF----
LC3B LQLNANQAFFLLVNGHSMVSVSTPISEVYESERDEDGFLYMVYASQETFGTAMAV
GABARAPL2 IQLPSEKAIFLFVDKT-VPQSSLTMGQLYEKEKDEDGFLYVAYSGENTFGF----
GABARAP IHLRAEDALFFFVNNV-IPPTSATMGQLYQEHHEEDFFLYIAYSDESVYGL----
GABARAPL1 IHLRPEDALFFFVNNT-IPPTSATMGQLYEDNHEEDYFLYVAYSDESVYGK----
H
p62 (FLAG)
GABARAP ΔN (HA)
kDa
63
17
FL ΔN FL ΔN FL ΔN
input
HA IP
IgG
I
p62
GABARAP ΔN (FLAG)
GAPDH
kDa
63
17
35
Cre - + - +
GABARAPL2 ΔN O/E - +

Fig. S4. Reduction of $GABA_A$ receptors in cortical GABAergic interneurons due to compromised functions of GABARAPL2 by autophagy deficiency. (**A**) Significant reductions of other $GABA_A$ receptor subunits were also observed in *Atg7* cKO cultured cortical GABAergic interneurons. (**B**) Verification of non-permeable condition for immunofluorescence analysis of surface $GABA_A$ receptors ($\beta_{2/3}$ subunit) in WT cultured cortical excitatory neurons using CaMKIIβ as an intracellular protein control. Note that CaMKIIβ, a highly abundant intracellular protein, was not detected under the non-permeable condition. (**C**) The number of action potentials evoked over a range of different current injections was increased in *Atg7* cKO cultured cortical GABAergic interneurons ($P = 0.0548$ by two-way ANOVA). $n > 25$ cells per genotype from three distinct preparations of primary cultured neurons**.** (**D**) *Gabarapl2* knocked down by gene-specific shRNA in WT cultured cortical GABAergic interneurons. (**E**) GABARAPL2-gephyrin interaction was significantly reduced in the brains of CaMKII-creERT2 (cortex) *Atg7* cKO mice. Quantifications shown represent the amount of gephyrin co-immunoprecipitated normalized by total gephyrin and amount of GABARAPL2 immunoprecipitated from individual samples. $n = 4$ independent experiments using tissue homogenates from distinct animals**.** (**F**) $p62^+$ aggregates formed in *Atg7* cKO cultured cortical GABAergic interneurons do not reside within the trans-golgi network as marked by TGN38. (**G**) Amino acid-level sequence alignment of LC3 and GABARAPs. Red lettering indicates region required for interacting with p62 and blue lettering indicates GABARAP fragment previously shown to interact with gephyrin. (**H**) Interaction between GABARAPL2 and p62 was disrupted by the removal of the first 8 amino acids (GABARAPL2 ΔN). (**I**) Overexpression of GABARAPL2 ΔN in *Atg7* cKO cultured cortical GABAergic interneurons and controls. Data are presented as means ± SEM; * $P < 0.05$, paired, two-tailed Student's *t*-test. Scale bars: (**B**), 25 μm; (**E**), 5 μm.

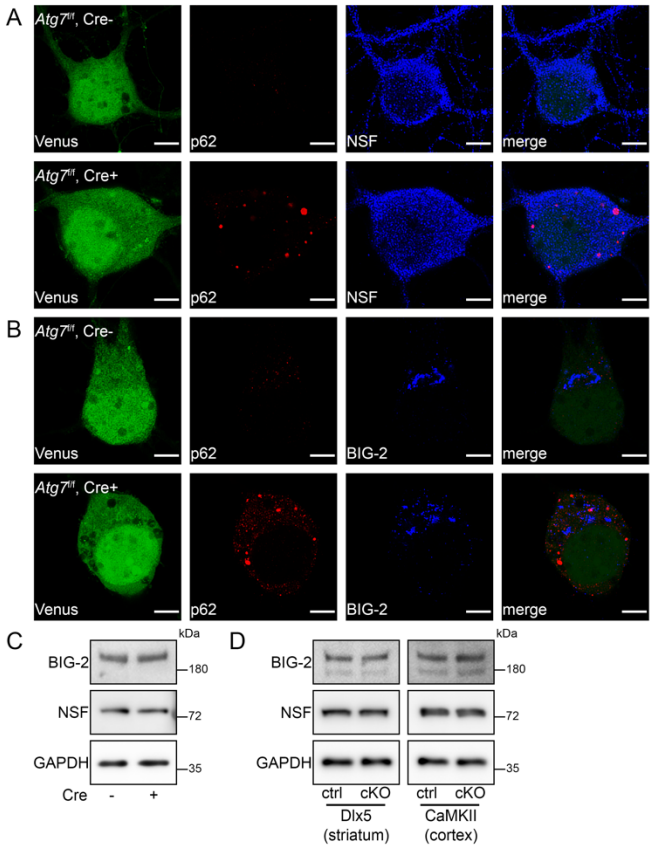
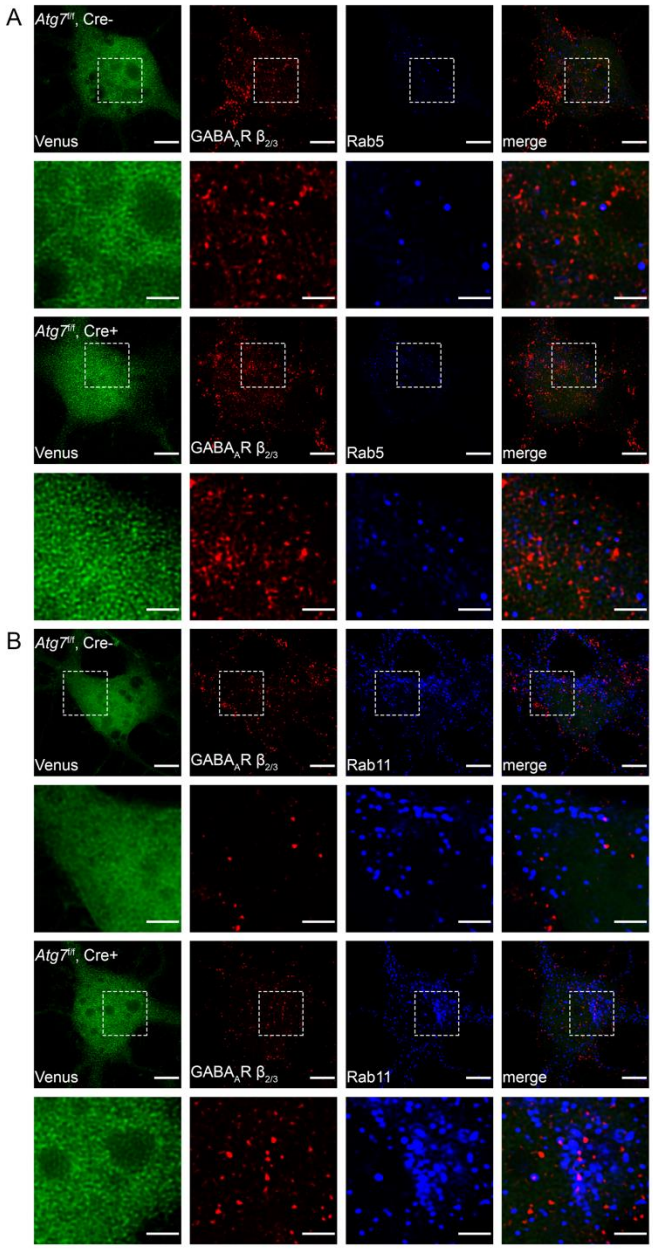

A
Atg7f/f, Cre-
Venus
p62
NSF
merge
Atg7f/f, Cre+
Venus
p62
NSF
merge
B
Atg7f/f, Cre-
Venus
p62
BIG-2
merge
Atg7f/f, Cre+
Venus
p62
BIG-2
merge
C
kDa
BIG-2
NSF
GAPDH
180
72
35
Cre - +
D
kDa
BIG-2
NSF
GAPDH
180
72
35
ctrl cKO
Dlx5
(striatum)
ctrl cKO
CaMKII
(cortex)

Fig. S5. Levels and localizations of BIG-2 and NSF were not affected by autophagy deficiency. (**A-B**) Immunofluorescence analysis revealed that BIG-2 and NSF did not mislocalize to $p62^{+}$ aggregates formed in *Atg7* cKO cultured cortical GABAergic interneurons. (**C**) BIG-2 and NSF did not accumulate in *Atg7* cKO cultured cortical GABAergic interneurons. (**D**) BIG-2 and NSF levels were not altered by autophagy deficiency in Dlx5-creERT2 and CaMKII-cre *Atg7* cKO mice. Scale bars: (**A**) and (**B**), 5 μm; insets, 2 μm.


A
Atg7f/f, Cre-
Venus
GABAAR β2/3
Rab5
merge
Atg7f/f, Cre+
Venus
GABAAR β2/3
Rab5
merge
B
Atg7f/f, Cre-
Venus
GABAAR β2/3
Rab11
merge
Atg7f/f, Cre+
Venus
GABAAR β2/3
Rab11
merge

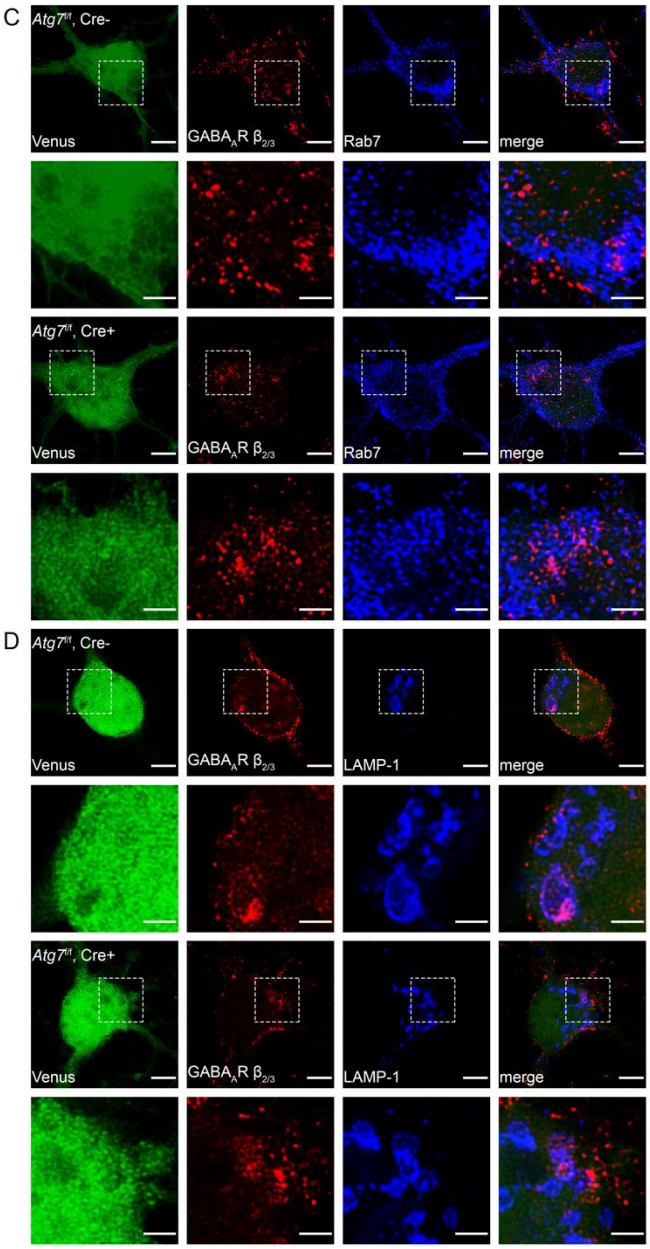

C
Atg7f/f, Cre-
Venus
GABAAR β2/3
Rab7
merge
Atg7f/f, Cre+
Venus
GABAAR β2/3
Rab7
merge
D
Atg7f/f, Cre-
Venus
GABAAR β2/3
LAMP-1
merge
Atg7f/f, Cre+
Venus
GABAAR β2/3
LAMP-1
merge

Fig. S6. $GABA_A$ receptors are not substantially localized to endosomal and lysosomal compartments in cortical GABAergic interneurons and are not altered by autophagy deficiency. (**A**) Immunofluorescence images showing that few $GABA_A$ receptors (<1%) were localized to early endosomes ($Rab5^+$) in *Atg7* cKO cultured cortical GABAergic interneurons and controls. (**B**) Immunofluorescence images showing that few $GABA_A$ receptors (<1%) were localized to recycling endosomes ($Rab11^+$) in *Atg7* cKO cultured cortical GABAergic interneurons and controls. (**C**) Few $GABA_A$ receptors (<5 %) were observed by immunofluorescence to be localized in late endosomes ($Rab7^+$) in *Atg7* cKO cultured cortical GABAergic interneurons and controls. (**D**) Few $GABA_A$ receptors (<5 %) were observed by immunofluorescence to be localized in LAMP-1^+ lysosomes in *Atg7* cKO cultured cortical GABAergic interneurons and controls. Scale bars: (**A**), (**B**), (**C**), and (**D**), 5 μm; insets, 2 μm.

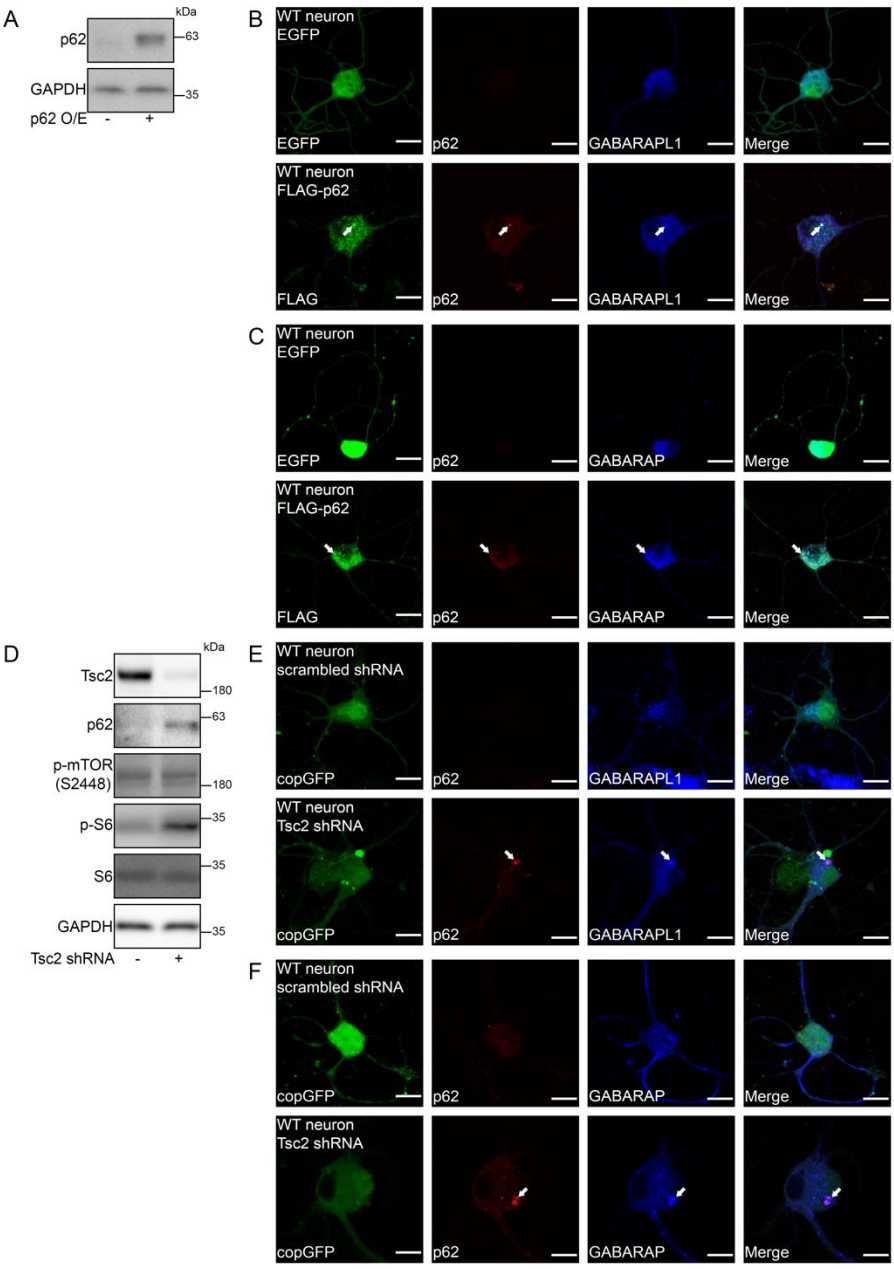

A
kDa
p62
63
GAPDH
35
p62 O/E - +
B
WT neuron
EGFP
EGFP
p62
GABARAPL1
Merge
WT neuron
FLAG-p62
FLAG
p62
GABARAPL1
Merge
C
WT neuron
EGFP
EGFP
p62
GABARAP
Merge
WT neuron
FLAG-p62
FLAG
p62
GABARAP
Merge
D
kDa
Tsc2
180
p62
63
p-mTOR
(S2448)
180
p-S6
35
S6
35
GAPDH
35
Tsc2 shRNA - +
E
WT neuron
scrambled shRNA
copGFP
p62
GABARAPL1
Merge
WT neuron
Tsc2 shRNA
copGFP
p62
GABARAPL1
Merge
F
WT neuron
scrambled shRNA
copGFP
p62
GABARAP
Merge
WT neuron
Tsc2 shRNA
copGFP
p62
GABARAP
Merge

Fig. S7. Reduced surface $GABA_A$ receptor expression by manipulation of p62 levels. (**A**) p62 overexpression in WT cultured cortical GABAergic interneurons. (**B-C**) $p62^+$ aggregates formed in WT cultured cortical GABAergic interneurons due to overexpression caused mislocalization of GABARAPL1 and GABARAP, similar to GABARAPL2 shown in Fig. 5A. (**D**) *Tsc2* knockdown in WT cultured cortical excitatory neurons led to mTOR hyperactivation as indicated by increased phosphorylation of ribosomal protein S6 and subsequent p62 accumulation due to autophagy suppression by enhanced mTOR signalling. S2448 phosphorylation of mTOR was unchanged, consistent with allosteric activation of mTOR via Tsc1/2-Rheb signalling, rather than phosphorylation via the PI3K-AKT-S6K1 pathway. (**E-F**) p62 accumulation and aggregate formation was observed due to autophagy suppression by hyperactivated mTOR in WT cultured cortical excitatory neurons with *Tsc2* knockdown, and in turn sequestered GABARAPL1 and GABARAP, in addition to GABARAPL2 shown in Fig. 5D. Scale bars: (**B**), (**C**), (**E**), and (**F**), 15 μm.

Table S1. shRNA sequences used in this study.

Gene	**Sequence**
Gabarapl2	Targeted: ATGAAGATGGATTCTTGTATG Scrambled: GGATTTATGACGTGATATGAT
Sqstm1 (p62)	Targeted: CCGCATCTACATTAAAGAGAA Scrambled: GACCCGACATAATTGCATAAA
Tsc2	Targeted: GGTGAAGAGAGCCGTATCACA Scrambled: GGCGAATGGGCTCGAATACAA

Table S2. Antibodies used in this study.

Antigen	Source	Catalogue number	Application details
p62	Progen Biotechnik	GP62-C	IB 1:2000 IF 1:4000
Atg7	Cell Signaling Technologies	2631	IB 1:2000
LC3	Novus Biologicals	NB100-2331	IB 1:1000
GAPDH	Santa Cruz Biotechnology, Inc.	sc-32233	IB 1:10000
Tsc2	Cell Signaling Technologies	4308	IB 1:2000 IF 1:200
GABARAP	Santa Cruz Biotechnology, Inc.	sc-377300	IB 1:500 IF 1:100
GABARAPL1	Proteintech	11010-1-AP	IB 1: 1000 IF 1:300
GABARAPL2	Proteintech	18724-1-AP	IB 1:1000 IF 1:300
NR2B	NeuroMab	75-097	IB 1:2000
GluR1	EMD Millipore Co.	AB1504	IB 1:1000
GluR1	NeuroMab	75-327	IB 1:1000
$GABA_A$ receptor α_1	NeuroMab	75-136	IB 1:1000
$GABA_A$ receptor β_3	NeuroMab	75-149	IB 1:1000
$GABA_A$ receptor $\beta_{2/3}$	EMD Millipore Co.	05-474	IB 1:1000 IF 1:200
$GABA_A$ receptor γ_2	NeuroMab	75-442	IB 1:1000
gephyrin	Synaptic Systems	147-111	IB 1:4000 IF 1:600
GAD67	EMD Millipore Co.	MAB5406	IB 1:1000 IF 1:4000
Calbindin	Sigma-Aldrich Co.	C9848	IB 1:1000 IF 1:200
Calretinin	EMD Millipore Co.	MAB1568	IB 1:1000 IF 1:100

Parvalbumin	Swant	235	IB 1:1000 IF 1:400
Somatostatin	EMD Millipore Co.	AB5494	IB 1:1000 IF 1:200
CaMKII-β	abcam	ab34703	IF 1:200
GFAP	Dako	Z0334	IF: 800
phospho-mTOR (S2448)	EMD Millipore Co.	09-213SP	IB 1:1000
S6	Cell Signaling Technologies	2217	IB 1:1000
phospho-S6	Cell Signaling Technologies	4858	IB 1:2000
Rab5	Cell Signaling Technologies	3547	IF 1:200
Rab7	Cell Signaling Technologies	9367	IF 1:200
Rab11	Cell Signaling Technologies	5589	IF 1:200
LAMP-1	Developmental Studies Hybridoma Bank	1D4B	IF 5 µg/mL
TGN38	Santa Cruz Biotechnology, Inc.	sc-271624	IF 1:100
BIG-2	Atlas Antibodies	HPA026078	IB 1:250 IF 1:50
NSF	Synaptic Systems	123-002	IB 1:1000 IF 1:200

Table S3. Patient information for frozen postmortem human brain samples.

#	A=Autism C=Control	Age	Ethnicity	Sex	PMI Group (hours)	RIN
1407	C	9y46d	African American	female	18 - 23	
1182	A	9y354d	African American	female	24 - 29	3.7
1846	C	20y221d	Caucasian	female	6 - 11	
1638	A	20y277d	Caucasian	female	> 48	6.4
4337	C	8y90d	African American	male	12- 17	8.4
4721	A	8y304d	African American	male	12 - 17	6.1
5408	C	6y309d	African American	male	12- 17	7.6
4849	A	7y171d	African American	male	18 - 23	6.7
5163	C	14y315d	Caucasian	male	12- 17	7
4899	A	14y126d	Caucasian	male	6 - 11	6.5
1376	C	37y140d	African American	male	12- 17	
5027	A	37y353d	African American	male	24 - 29	6.8
4590	C	20y179d	Caucasian	male	18 - 23	
4999	A	20y274d	Caucasian	male	12- 17	7.5
4781	C	45y364d	Caucasian	male	12- 17	7.4
5115	A	46y135d	Caucasian	male	24 - 29	7.3
5391	C	8y286d	Caucasian	male	12- 17	6.9
5144	A	7y55d	Caucasian	male	0 - 5	7.7
5342	C	22y355d	African American	male	12- 17	8.5
5176	A	22y199d	African American	male	18 - 23	6.8
5168	C	15y361d	Caucasian	female	6 - 11	3.9
5278	A	15y324d	Caucasian	female	12- 17	6.9
5334	C	12y249d	unknown	male	12- 17	2.4
4334	A	11y17d	unknown	male	24 - 29	7.1
5079	C	33y64d	Caucasian	male	12- 17	7.4
5297	A	33y83d	Caucasian	male	> 48	6.4
4848	C	16y271d	Caucasian	male	12- 17	
5302	A	16y119d	Caucasian	male	18 - 23	3.5
4669	C	16y125d	Caucasian	male	12- 17	
5403	A	16y266d	Caucasian	male	30 - 35	5.5
1029	C	29y300d	unknown	male	12- 17	
M3663M	A	27y109d	unknown	male	18 - 23	6.8

1612	C	19y0d	Caucasian	female	24 - 29	
5419	A	19y350d	Caucasian	female	18 - 23	8.4
1714	C	12y164d	African American	male	18 - 23	
5565	A	12y89d	African American	male	18 - 23	8.3
1347	C	19y76d	Caucasian	female	12- 17	
5561	A	19y218d	Caucasian	female	30 - 35	3.2
4599	C	23y320d	African American	male	18 - 23	
5574	A	23y252d	African American	male	12- 17	3.1
4670	C	4y237d	Caucasian	male	12 - 17	7.7
5308	A	4y182d	Caucasian	male	18 - 23	6
1578	C	53y112d	Caucasian	male	12- 17	8.4
5340	A	53y177d	Caucasian	male	24 - 29	7.8