Neuron, Volume *111*

Supplemental information

Gasdermin-E mediates mitochondrial damage in axons and neurodegeneration

Dylan V. Neel, Himanish Basu, Georgia Gunner, Matthew D. Bergstresser, Richard M. Giadone, Haeji Chung, Rui Miao, Vicky Chou, Eliza Brody, Xin Jiang, Edward Lee, Michelle E. Watts, Christine Marques, Aaron Held, Brian Wainger, Clotilde Lagier-Tourenne, Yong-Jie Zhang, Leonard Petrucelli, Tracey L. Young-Pearse, Alice S. Chen-Plotkin, Lee L. Rubin, Judy Lieberman, and Isaac M. Chiu

Supplemental Figures and Tables:

Figure S1

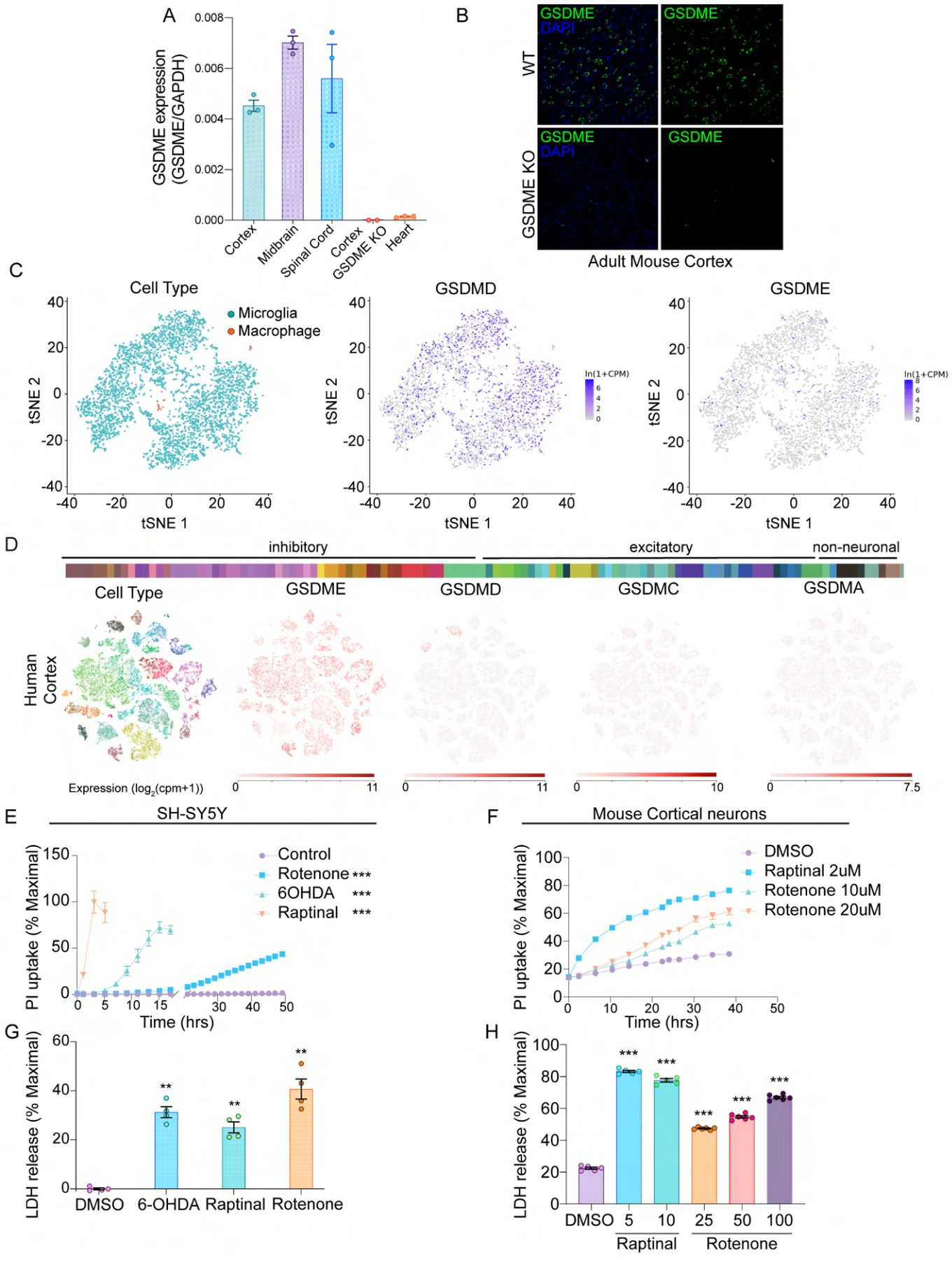


Figure S1: GSDME is expressed in mouse and human neurons (related to Figure 1 & 2)

(A) *Gsdme* RT-qPCR of several mouse brain regions from WT (n=3) and *Gsdme* knockout (n=2) mice

(B) Representative IHC images of cortical mouse brain sections from WT and *Gsdme* knockout animals stained with anti-GSDME and counterstained with DAPI.

(C) FACS-isolated brain myeloid cell transcriptomic data from the publicly available Tabula Muris database was mined for *Gsdme* and *Gsdmd* expression. TSNE plots showing cell types (left most plot) and expression of gasdermin genes (right) were generated using the CZI biohub tool (Available from: https://tabula-muris.ds.czbiohub.org/)

(D) Publicly available human cortical single-cell RNA-seq (10X Genomics) data was mined for gasdermin family expression levels. TSNE plots showing cell types (left most plot) and gasdermin genes were generated using the Allen Brain Atlas Transcriptomics Explorer tool (Allen Institute for Brain Science. Available from: human.brain-map.org)

(E)SH-SY5Y cells were incubated in propidium iodide (PI) containing media and treated with 5uM raptinal, 20uM rotenone or 30uM of 6-OHDA. Images were taken every 3h and quantified for PI uptake.

(F) primary neurons were incubated in propidium iodide (PI) containing media and treated with 5uM raptinal, 20uM rotenone and subsequently imaged to quantify PI uptake.

(G) SH-SY5Y were treated with 5uM raptinal, 20uM rotenone or 30uM of 6-OHDA and assessed for LDH release at 24h post-treatment.

(H) Primary neurons were incubated in media containing multiple doses of raptinal or rotenone and assessed for LDH release at 24h post-treatment.

Figure S2

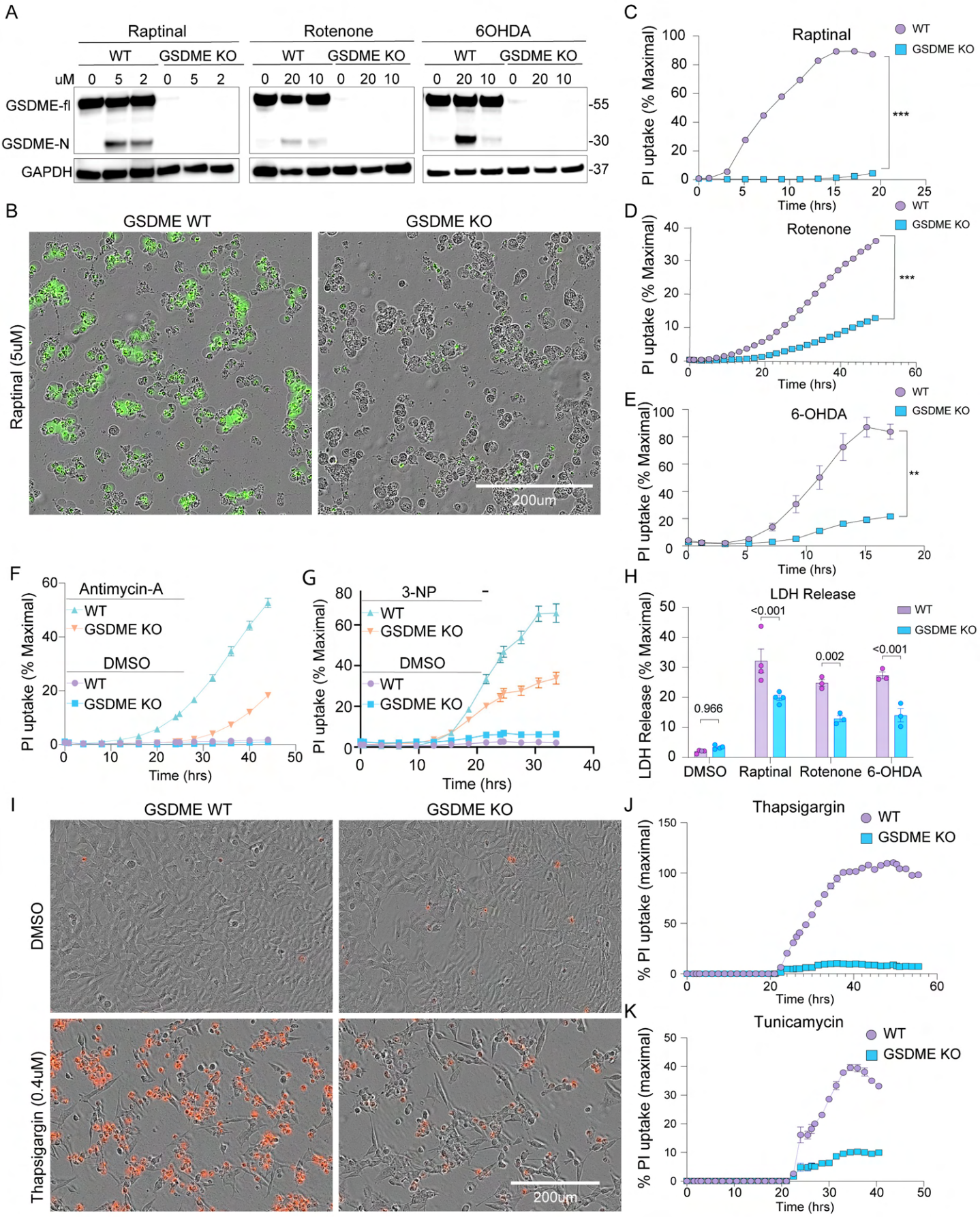

A
Raptinal
Rotenone
6OHDA
WT
GSDME KO
uM 0 5 2 0 5 2
0 20 10 0 20 10
0 20 10 0 20 10
GSDME-fl
GSDME-N
GAPDH
-55
-30
-37
B
GSDME WT
GSDME KO
Raptinal (5uM)
200um
C
Raptinal
PI uptake (% Maximal)
Time (hrs)
WT
GSDME KO
***
D
Rotenone
E
6-OHDA
**
F
Antimycin-A
DMSO
G
3-NP
H
LDH Release
LDH Release (% Maximal)
<0.001
0.002
0.966
DMSO
Raptinal
Rotenone
6-OHDA
I
GSDME WT
GSDME KO
DMSO
Thapsigargin (0.4uM)
200um
J
Thapsigargin
% PI uptake (maximal)
K
Tunicamycin

Figure S2: GSDME is activated by mitochondrial and ER toxins and mediates cell death in human SH-SY5Y cells (related to Figure 2).

(A) Immunoblots of WT and *GSDME* KO SH-SY5Y treated with DMSO, raptinal, rotenone or 6-OHDA. Cells treated with raptinal were harvested for lysates at 2h post-treatment, while cells treated with rotenone or 6-OHDA were collected at 12h. All concentrations are in micromolar units.

(B) Representative 20X Images of WT (left) or *GSDME* KO (right) SH-SY5Y cells at 2h following treatment with 5uM raptinal. Cells were incubated in *Sytox* Green-containing media, and green cells indicate dye uptake. Scale bar: 200 um

(C-G) WT SH-SY5Y and *GSDME* KO cells treated with (C) 5uM raptinal (D) 20uM rotenone (E) 30uM 6-OHDA (F) 1uM antimycin or (G) 1mM 3-nitropropionic acid were co-incubated with propidium iodide, imaged every 3h and quantified for PI uptake.

(H) WT or *GSDME* KO SH-SY5Y cells were incubated in media containing 5uM Raptinal, 20uM rotenone, or 30uM 6-OHDA and assessed for LDH release at 24h post-treatment.

(I) Representative 20X images of wild-type or *GSDME* knockout SH-SY5Y cells stained with propidium iodide (PI) and treated with DMSO or 0.4uM thapsiagargin (Tg) for 24h.

(J-K) SH-SY5Y were incubated in propidium iodide containing media and treated with (J) 0.4uM thapsiagargin (Tg) or (K) 4uM tunicamycin. Images were taken every 4h in an IncuCyte ZOOM machine and propidium iodide uptake was quantified.

Figure S3

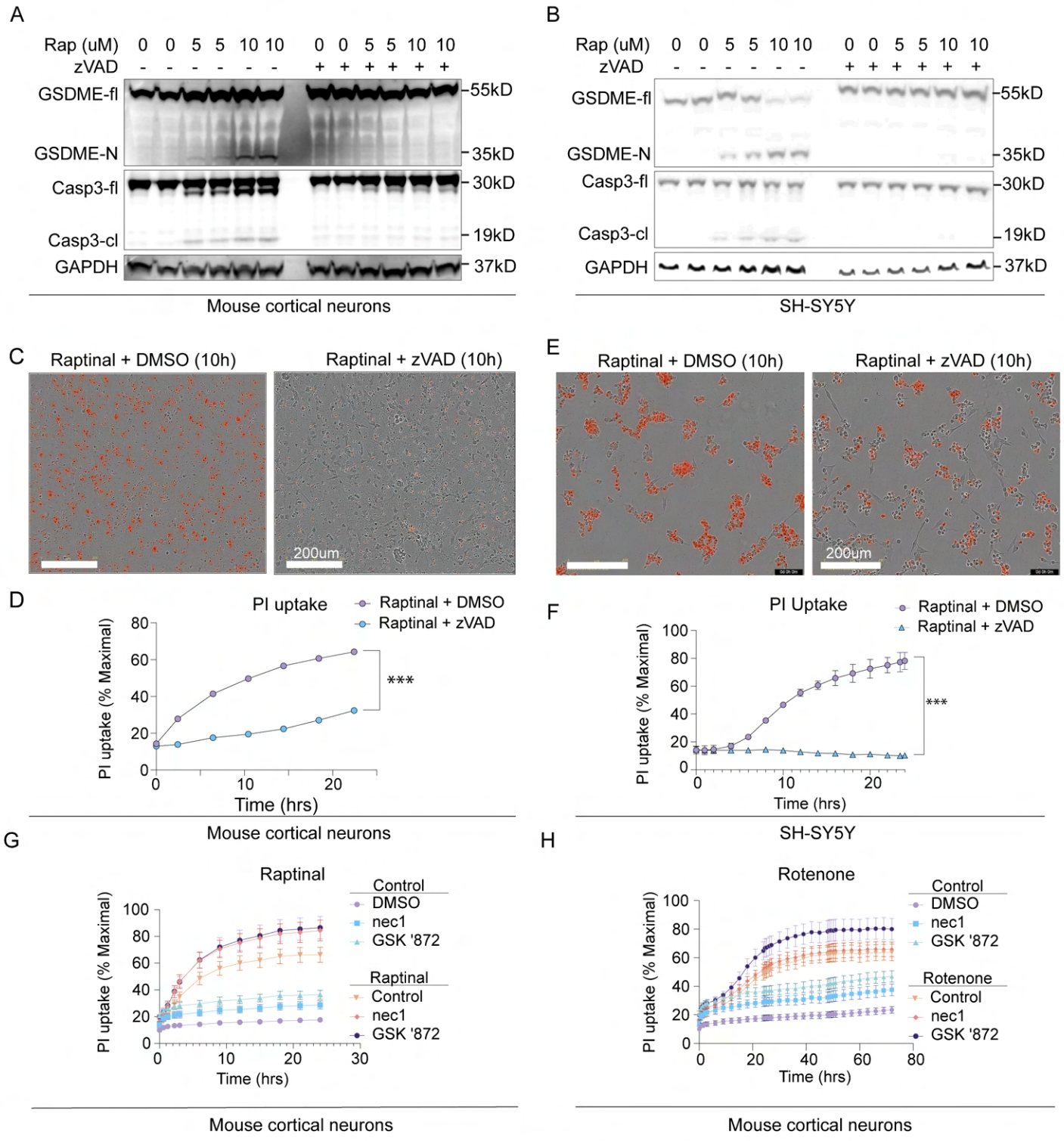

A
Rap (uM) 0 0 5 5 10 10 0 0 5 5 10 10
zVAD - - - - - - + + + + + +
GSDME-fl
GSDME-N
Casp3-fl
Casp3-cl
GAPDH
55kD
35kD
30kD
19kD
37kD
Mouse cortical neurons
B
Rap (uM) 0 0 5 5 10 10 0 0 5 5 10 10
zVAD - - - - - - + + + + + +
GSDME-fl
GSDME-N
Casp3-fl
Casp3-cl
GAPDH
55kD
35kD
30kD
19kD
37kD
SH-SY5Y
C
Raptinal + DMSO (10h)
Raptinal + zVAD (10h)
200um
E
Raptinal + DMSO (10h)
Raptinal + zVAD (10h)
200um
D
PI uptake
Raptinal + DMSO
Raptinal + zVAD
PI uptake (% Maximal)
Time (hrs)
***
Mouse cortical neurons
F
PI Uptake
Raptinal + DMSO
Raptinal + zVAD
PI uptake (% Maximal)
Time (hrs)
***
SH-SY5Y
G
Raptinal
Control
DMSO
nec1
GSK '872
Raptinal
Control
nec1
GSK '872
PI uptake (% Maximal)
Time (hrs)
Mouse cortical neurons
H
Rotenone
Control
DMSO
nec1
GSK '872
Rotenone
Control
nec1
GSK '872
PI uptake (% Maximal)
Time (hrs)
Mouse cortical neurons

Figure S3: Caspase-3 but not RIPK1 inhibition protects neurons from mitochondrial toxins (related to Figure 2)

(A-B) Immunoblots of (A) primary mouse neurons or (B) SH-SY5Y treated with DMSO or raptinal (5-10uM) in the presence or absence of 20uM zVAD-FMK.

(C-D) Representative 20X images of primary neurons stained with PI and treated with either 5uM raptinal or 5uM raptinal + 20uM zVAD-FMK (C). Images were taken every 3h by an IncuCyte ZOOM and PI uptake was quantified (D). Scale bar: 200um

(E-F) Representative 20X images of SH-SY5Y stained with PI and treated with either 5uM raptinal or 5uM raptinal + 20uM zVAD-FMK (E). Images were taken every 3h in an IncuCyte ZOOM and PI uptake was quantified (F). Scale bar: 200um

(G-H) Primary mouse neurons treated with (G) 5uM raptinal or (H) 30uM rotenone were co-incubated in media containing PI and either DMSO, nec1 or GSK ‘872 (RIPK1 inhibitors). Images were taken every 3h and quantified for PI uptake.

Figure S4

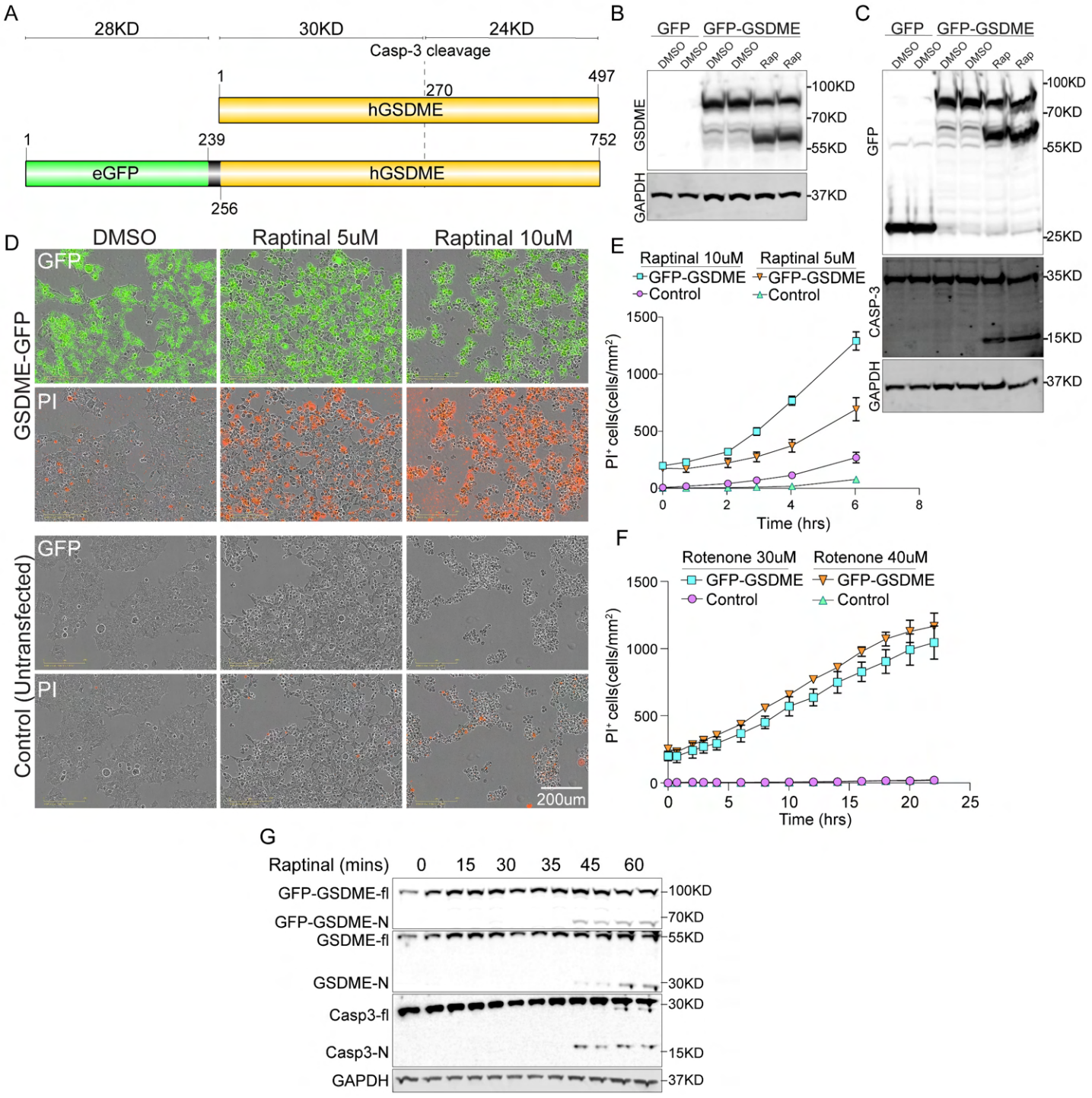

A
28KD
30KD
24KD
Casp-3 cleavage
1
270
497
hGSDME
1
239
752
eGFP
hGSDME
256
B
GFP
GFP-GSDME
DMSO
DMSO
DMSO
DMSO
Rap
Rap
GSDME
GAPDH
100KD
70KD
55KD
37KD
C
GFP
GFP-GSDME
DMSO
DMSO
DMSO
DMSO
Rap
Rap
GFP
CASP-3
GAPDH
100KD
70KD
55KD
25KD
35KD
15KD
37KD
D
DMSO
Raptinal 5uM
Raptinal 10uM
GSDME-GFP
Control (Untransfected)
GFP
PI
GFP
PI
200um
E
Raptinal 10uM
GFP-GSDME
Control
Raptinal 5uM
GFP-GSDME
Control
PI+ cells(cells/mm2)
1500
1000
500
0
0
2
4
6
8
Time (hrs)
F
Rotenone 30uM
GFP-GSDME
Control
Rotenone 40uM
GFP-GSDME
Control
PI+ cells(cells/mm2)
1500
1000
500
0
0
5
10
15
20
25
Time (hrs)
G
Raptinal (mins)
0
15
30
35
45
60
GFP-GSDME-fl
GFP-GSDME-N
GSDME-fl
GSDME-N
Casp3-fl
Casp3-N
GAPDH
100KD
70KD
55KD
30KD
30KD
15KD
37KD

Figure S4: GFP-GSDME cleavage by mitochondrial toxins and downstream caspase-3 causes enhanced cell death and puncta formation (related to Figures 2 & 3)

(A) Diagram showing the full-length human GSDME (yellow), and caspase-3 cleavage site (dotted line). An eGFP (green) sequence was placed on the N-terminal side of hGSDME.

(B-C) HEK 293T cells were transfected with GFP (control) or GFP-fl-GSDME and treated with either DMSO or raptinal (5uM). Immunoblots for (B) GSDME, (C) GFP or CASP3 show cleavage and activation of CASP3 and the GFP-GSDME construct.

(D-F) Cells were transfected with GFP-GSDME (top panels) or control vector (bottom panels) and co-incubated with PI and either raptinal or rotenone. Cells were imaged every 1h and quantified for PI uptake (E-F).

(G) Primary WT neurons transduced with GFP-GSDME were incubated with 5uM raptinal and immunoblots were performed with cells treated for different times to assess the temporal dynamics of caspase-3 and GSDME activation.

Figure S5

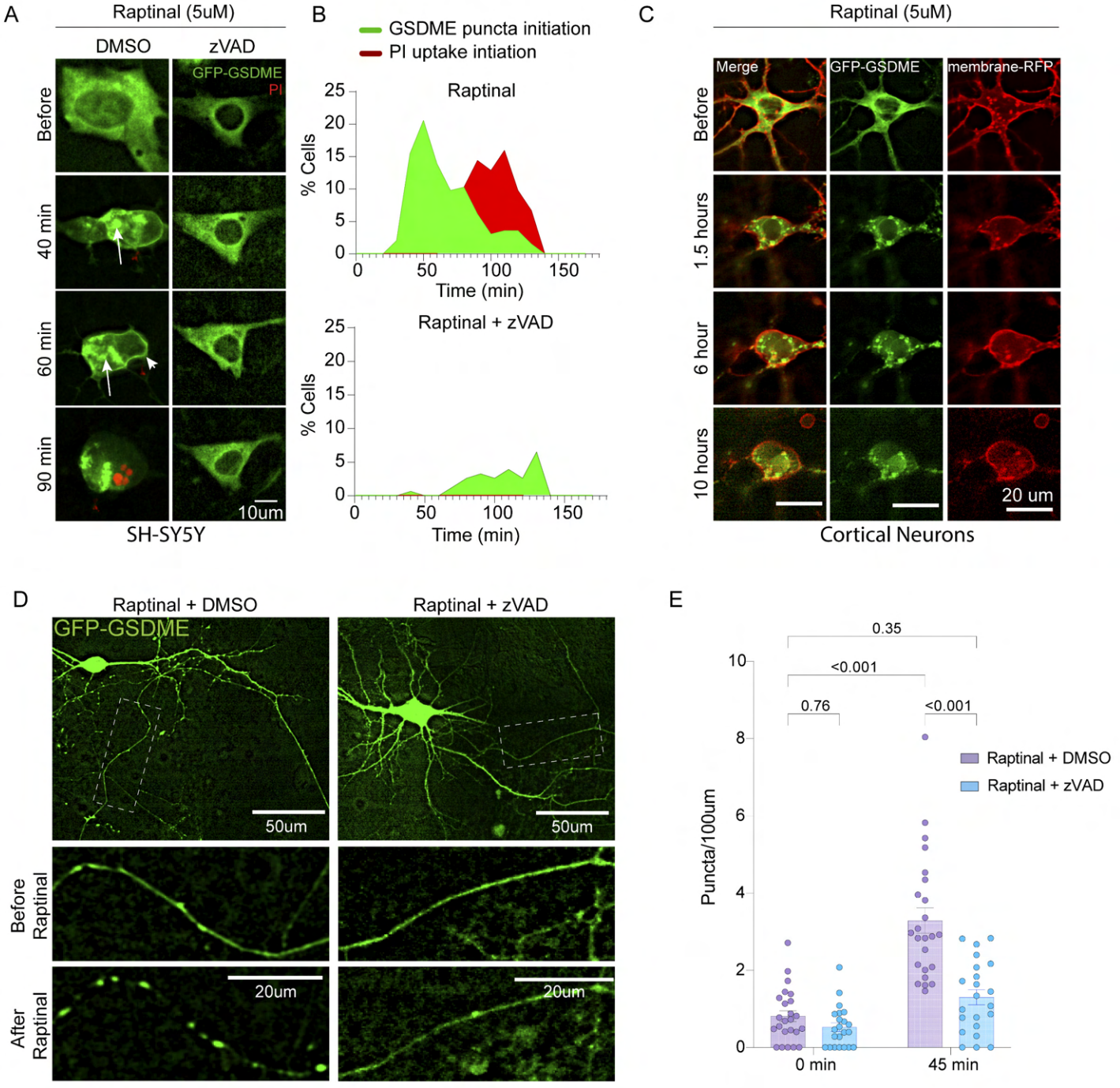

A
Raptinal (5uM)
DMSO
zVAD
GFP-GSDME
PI
Before
40 min
60 min
90 min
10um
SH-SY5Y
B
GSDME puncta initiation
PI uptake intiation
Raptinal
% Cells
Time (min)
Raptinal + zVAD
C
Raptinal (5uM)
Merge
GFP-GSDME
membrane-RFP
Before
1.5 hours
6 hour
10 hours
20 um
Cortical Neurons
D
Raptinal + DMSO
Raptinal + zVAD
GFP-GSDME
50um
Before Raptinal
After Raptinal
20um
E
0.35
<0.001
0.76
<0.001
Puncta/100um
Raptinal + DMSO
Raptinal + zVAD
0 min
45 min

Figure S5: Mitochondrial toxins induce GFP-tagged GSDME to form puncta in a caspase-dependent manner (related to Figures 3).

(A-B) SH-SY5Y cells treated with raptinal + DMSO, or raptinal + 20uM zVAD, and imaged over 90 minutes. White arrows represent intracellular GFP-GSDME puncta, while arrowheads denote plasma membrane enrichment (A). Images were captured every 10 min and the frequency of cells showing intracellular puncta, as well as positive PI staining were quantified (B). Scale bar: 10um

(C) Primary mouse neurons were co-transfected with mTagRFP-Membrane-1 (neuromodulin: aa 1-20) and GFP-GSDME and treated with 5uM raptinal. Following toxin treatment, images were taken every 30 min (for 10h total) to study the colocalization of these two markers. Scale bar: 20um

(D) Primary neurons transfected with GFP-GSDME were treated with 5uM raptinal or 5uM raptinal+ 20uM zVAD-FMK and imaged 45 minutes after drug treatment. Top (large) panels show representative single neurons prior to any drug treatment. Scale bars: 50 um. The bottom panels show magnified axonal segments (white boxes) before and after 45 min of drug treatment. Scale bars: 20 um

(E) Quantification of GFP-GSDME puncta treated with either 5uM raptinal or 5uM raptinal + 20uM zVAD-FMK. Each dot represents a single neuron, and 8-10 neurons from three independent experiments were used for quantification. Data was analyzed using paired Student's t-tests comparing before and after drug treatment. P-values were adjusted for multiple comparisons using the Tukey method.

Figure S6

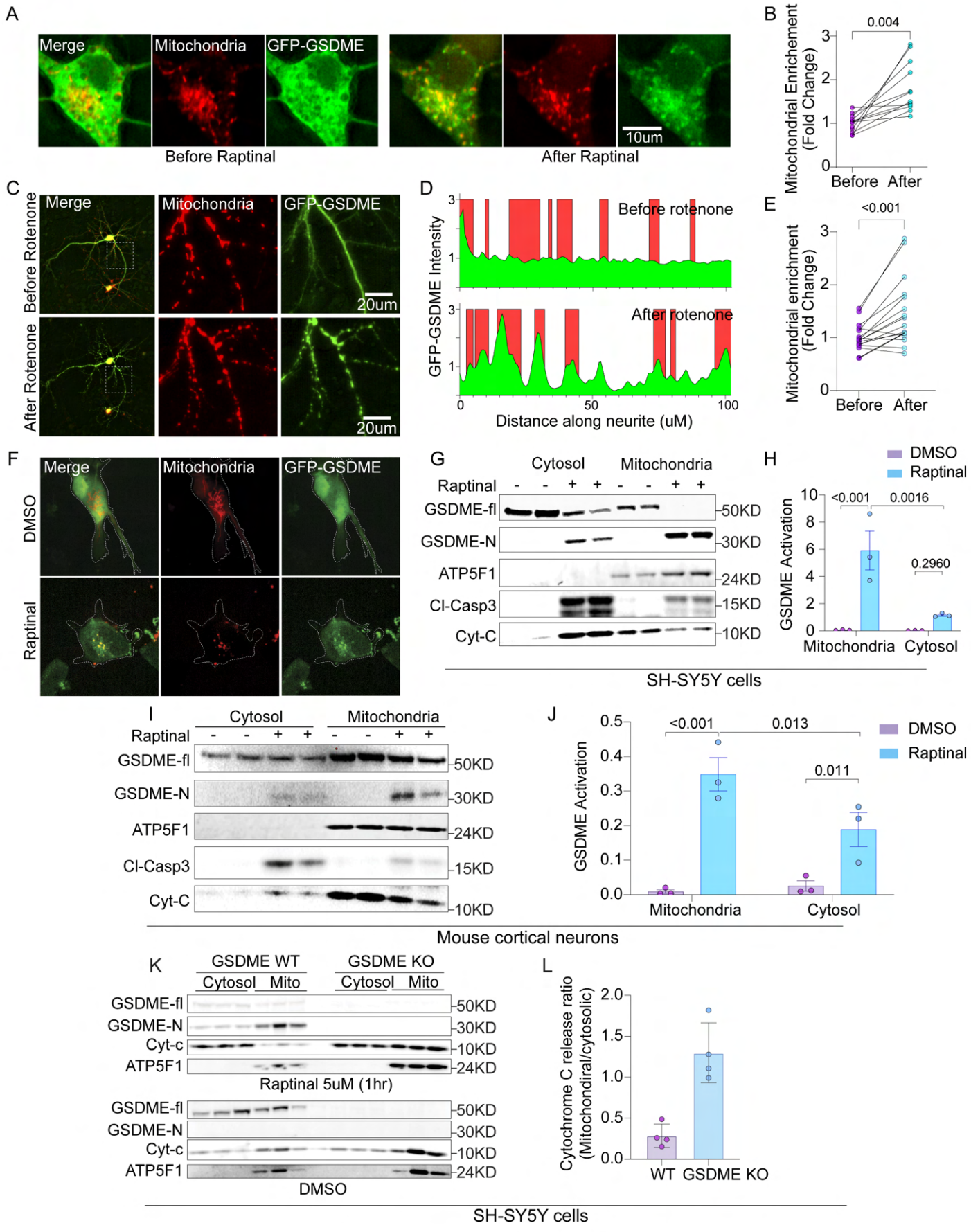

A
Merge
Mitochondria
GFP-GSDME
Before Raptinal
After Raptinal
10um
B
0.004
Mitochondrial Enrichement (Fold Change)
Before
After
C
Before Rotenone
After Rotenone
Merge
Mitochondria
GFP-GSDME
20um
D
Before rotenone
After rotenone
GFP-GSDME Intensity
Distance along neurite (uM)
E
<0.001
Mitochondrial enrichment (Fold Change)
F
DMSO
Raptinal
G
Cytosol
Mitochondria
Raptinal
GSDME-fl
GSDME-N
ATP5F1
Cl-Casp3
Cyt-C
50KD
30KD
24KD
15KD
10KD
H
DMSO
Raptinal
<0.001
0.0016
0.2960
GSDME Activation
Mitochondria
Cytosol
SH-SY5Y cells
I
Cytosol
Mitochondria
Raptinal
GSDME-fl
GSDME-N
ATP5F1
Cl-Casp3
Cyt-C
50KD
30KD
24KD
15KD
10KD
J
<0.001
0.013
0.011
DMSO
Raptinal
GSDME Activation
Mitochondria
Cytosol
Mouse cortical neurons
K
GSDME WT
GSDME KO
Cytosol
Mito
GSDME-fl
GSDME-N
Cyt-c
ATP5F1
50KD
30KD
10KD
24KD
Raptinal 5uM (1hr)
DMSO
L
Cytochrome C release ratio (Mitochondiral/cytosolic)
WT
GSDME KO
SH-SY5Y cells

Figure S6: Toxin treatment causes GSDME to colocalize with mitochondria (related to Figure 3)

(A-B) Representative cell bodies from (A) mouse neurons transfected with mitochondrial marker mKate-OMP25 (red) and GFP-GSDME (green) before and after 3h treatment with 5uM raptinal are shown. (B)The enrichment of the green signal on mitochondria over cytosol was quantified and compared before and after raptinal treatment (N=12 cell bodies representing neurons from 3 replicate experiments).

(C-E) Mouse neurons transfected with mitochondrial marker mKate-OMP25 or GFP-GSDME were imaged before and after treatment with 20uM rotenone. (C) Representative images of neurons at baseline and 4h post-rotenone treatment. (D) The location of mitochondria (red) and GFP-GSDME (green) peaks were identified along the neurites. (E) The position of the red signal overlapping with green GSDME fluorescence was quantified and compared before and after raptinal treatment.

(F) SH-SY5Y cells transfected with mitochondrial marker mKate-OMP25 or GFP-GSDME were imaged before and after treatment with 5uM raptinal. Images were captured 2.5h post-raptinal treatment.

(G-H) Immunoblot of enriched cytosolic and mitochondrial (ATP5F1 positive) fractions from SH-SY5Y treated with either DMSO or 10uM raptinal for 1h. (G) The top two panels represent full length and cleaved GSDME fragments. (H) Quantification of the intensity of cleaved over full length GSDME bands in immunoblots from cytosolic or mitochondrial SH-SY5Y fractions (n=3).

(I-J) Immunoblot of enriched cytosolic and mitochondrial (ATP5F1 positive) fractions from primary neurons treated with either DMSO or 10uM raptinal for 1h. The top two panels (I) represent full length and cleaved GSDME fragments. (J) Quantification of the intensity of cleaved over full length GSDME bands in immunoblots from cytosolic or mitochondrial primary neuron fractions (n=3)

(K-L) Immunoblots of WT or GSDME KO SH-SY5Y treated with DMSO or 5uM raptinal for 1h. (K) Mitochondrial (ATP5F1) and cytosolic fractions were blotted for GSDME, Cl-CASP3 and cytochrome-c (Cyt-c). The ratio of mitochondrial to cytosolic cytochrome-c signal by immunoblot was (L) quantified for WT and KO cells treated with 5uM raptinal (n=4).

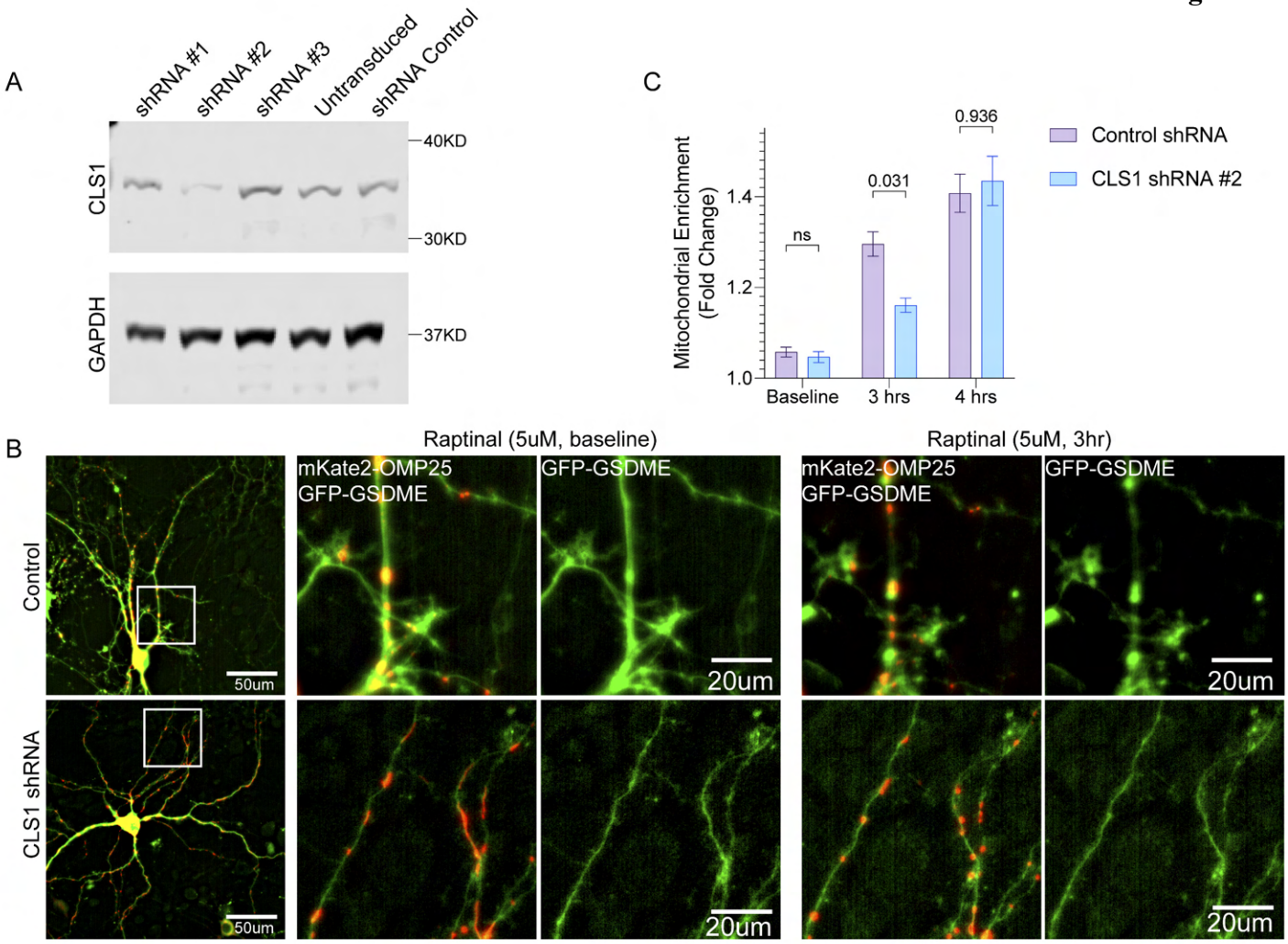


Figure S7: GSDME recruitment to mitochondria is cardiolipin dependent (related to Figure 3)

(A) Primary mouse cortical neurons were transduced with lentiviruses encoding 3 different shRNAs (#1-3) targeting mouse cardiolipin synthase 1 (CLS1) and scrambled control. Immunoblotting against CLS1 revealed efficient KD with shRNA#2

(B-C) Representative images of GSDME KO mouse neurons transfected with plasmids encoding CLS1-shRNA#2 or scrambled control and co-transfected with both GFP-GSDME and mKate-OMP25. 3d post-transfection, cells were treated with 5uM of raptinal and imaged at 0, 3h and 4h post treatment to quantify GSDME puncta co-localized with mitochondria (C).

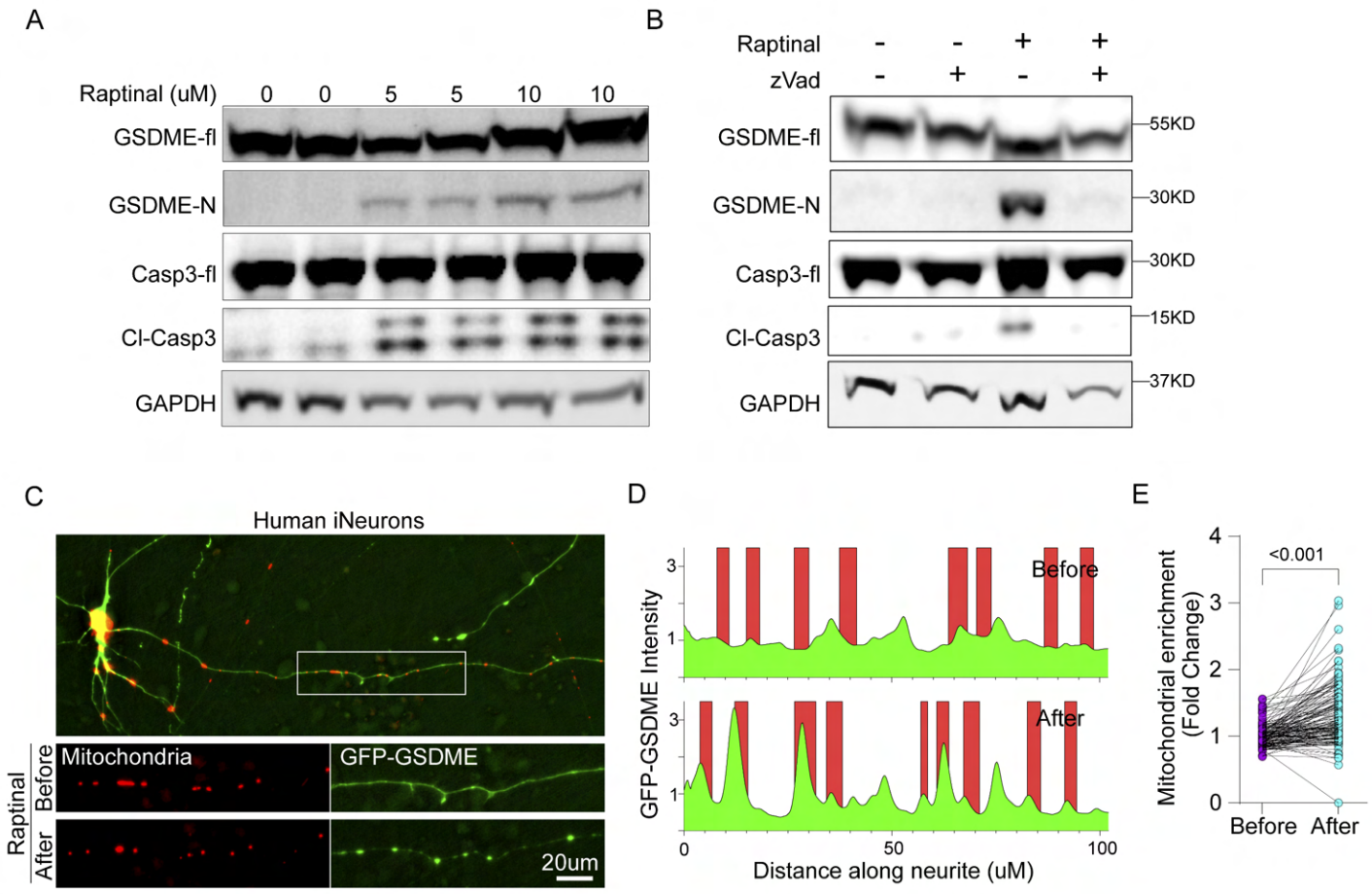


Figure S8: Toxins cause GSDME activation and mitochondrial colocalization in human iNeurons (related to Figure 3).

(A-B) Immunoblots of human iPSC-derived cortical neuron (iNeuron) cultures. (A) Immunoblot of human iNeurons treated with 5uM or 10uM raptinal for 1h. (B) Immunoblot of human iNeurons treated with DMSO, 20uM zVAD, 5uM raptinal or a combination of raptinal and zVAD for 1h.

(C-E) Human iPSC-derived cortical neurons transfected with mitochondrial marker mKate-OMP25 and GFP-GSDME were imaged before and after a 2.5h treatment with 5uM raptinal. A representative neuron (C, top panel), with magnified images of a neurite (indicated by dotted white box) before and after raptinal treatment are shown (C, bottom panel). (D) Locations of mitochondria and intensity of GFP-GSDME were measured as line scans along neurites. Line scans (as shown in panel D) were analyzed to calculate (E) GFP-GSDME enrichment on mitochondria before and after raptinal treatment. N >100 axon segments representing iNeurons originating from 3 independent differentiations.

Figure S9

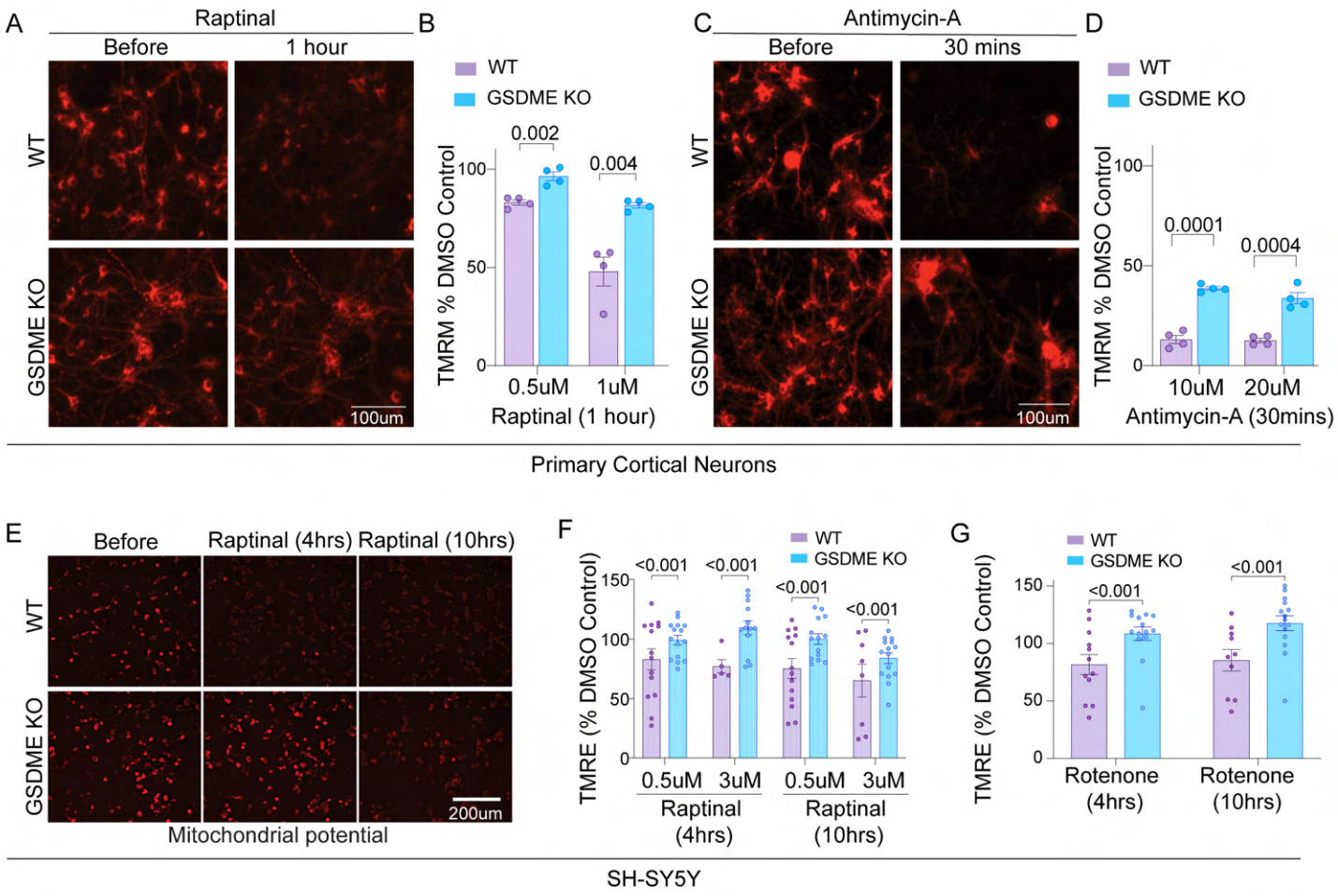


Figure S9: GSDME deficiency protects from TMRM loss in primary neurons and SH-SY5Y (related to Figure 4)

(A-D) Wild-type and *Gsdme* KO primary cortical neurons were stained with TMRM and incubated with (A-B) 0.5uM and 1uM raptinal or (C-D) 10uM and 20uM antimycin-A. Representative images of TMRM uptake are shown (A and C). TMRM intensity was quantified at 30 min and 1h post-toxin treatment, respectively (B and D). (E-G) Wild-type and *GSDME* KO SH-SY5Y were stained with TMRM and incubated with (E-F) 0.5uM or 3uM raptinal or (G) 10uM rotenone. Images captured at 4 and 10h post-treatment were quantified for TMRM intensity (F-G).

All TMRM intensities are represented as intensities to respective DMSO controls.

Figure S10

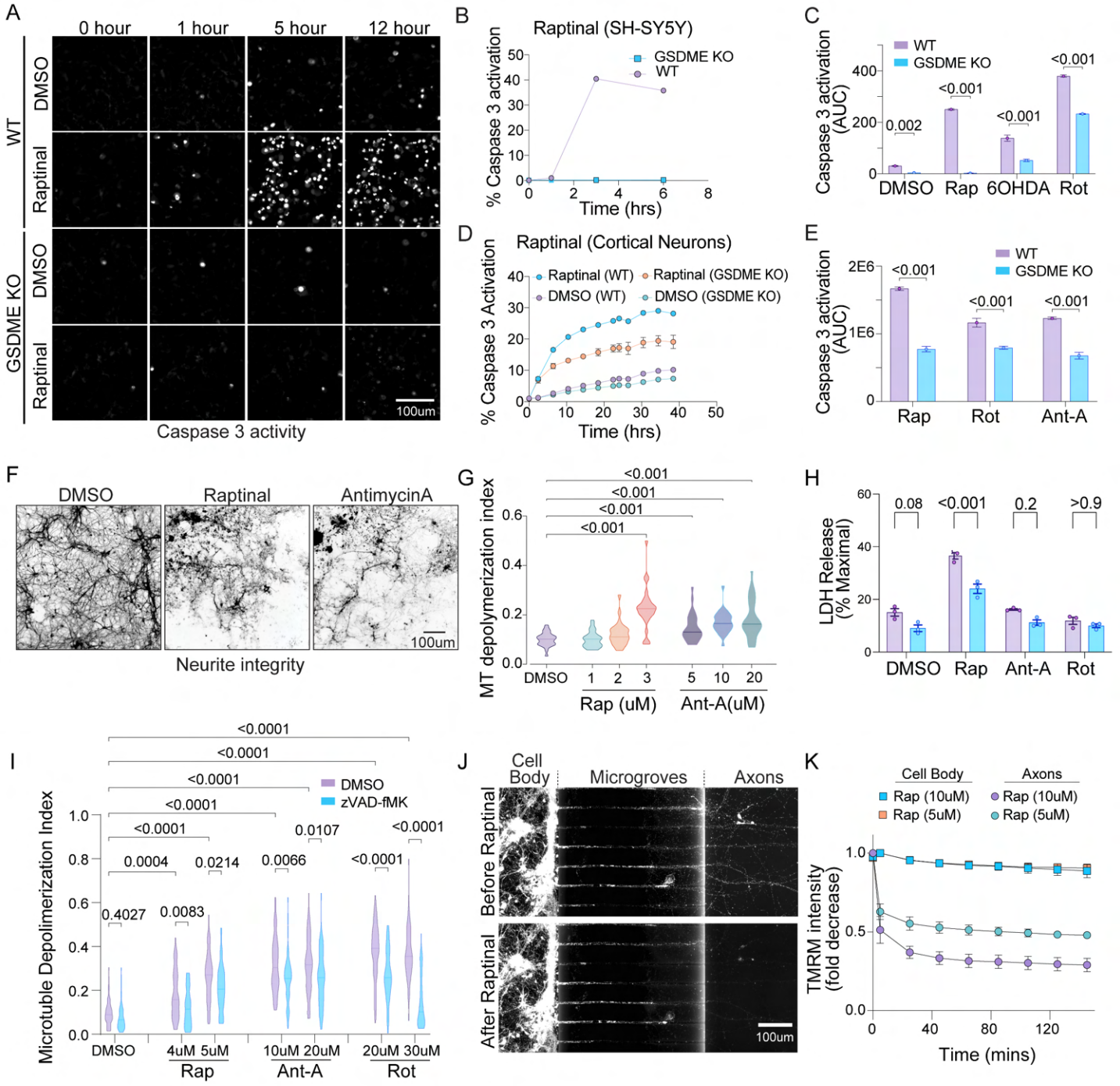

A
0 hour
1 hour
5 hour
12 hour
WT
DMSO
Raptinal
GSDME KO
DMSO
Raptinal
100um
Caspase 3 activity
B
Raptinal (SH-SY5Y)
GSDME KO
WT
% Caspase 3 activation
Time (hrs)
C
WT
GSDME KO
Caspase 3 activation (AUC)
0.002
<0.001
<0.001
<0.001
DMSO
Rap
6OHDA
Rot
D
Raptinal (Cortical Neurons)
Raptinal (WT)
Raptinal (GSDME KO)
DMSO (WT)
DMSO (GSDME KO)
% Caspase 3 Activation
Time (hrs)
E
WT
GSDME KO
Caspase 3 activation (AUC)
<0.001
<0.001
<0.001
Rap
Rot
Ant-A
F
DMSO
Raptinal
AntimycinA
100um
Neurite integrity
G
<0.001
<0.001
<0.001
<0.001
MT depolymerization index
DMSO
Rap (uM)
Ant-A(uM)
H
0.08
<0.001
0.2
>0.9
LDH Release (% Maximal)
DMSO
Rap
Ant-A
Rot
I
<0.0001
<0.0001
<0.0001
<0.0001
<0.0001
<0.0001
0.0107
<0.0001
0.0004
0.0214
0.0066
<0.0001
0.4027
0.0083
DMSO
zVAD-fMK
Microtuble Depolimerization Index
DMSO
4uM 5uM
Rap
10uM 20uM
Ant-A
20uM 30uM
Rot
J
Cell Body
Microgroves
Axons
Before Raptinal
After Raptinal
100um
K
Cell Body
Axons
Rap (10uM)
Rap (5uM)
Rap (10uM)
Rap (5uM)
TMRM intensity (fold decrease)
Time (mins)

Figure S10: GSDME deficiency protects from caspase-3 activation and neurite loss (related to Figures 4 & 5).

(A) Representative images at several timepoints of wild-type and *GSDME* KO SH-SY5Y cells treated with 2uM raptinal and incubated in media containing Incucyte Caspase-3/7 dye.

(B-C) Wild-type and *GSDME* KO SH-SY5Y incubated in Caspase-3/7 dye were treated with (B) 5uM raptinal and imaged every 3h post-treatment. (C) Area under the curve (AUC) measurements from 5uM raptinal (AUC_{6h}), 20uM rotenone (AUC_{24h}) and 30uM 6-OHDA (AUC_{12h}) treatment were generated and compared between WT and KO cells.

(D-E) Wild-type and *Gsdme* KO primary neurons incubated in Caspase-3/7 dye were treated with (D) 5uM raptinal and imaged every 2-3h post-treatment. (E) Area under the curve (AUC_{24h}) measurements from 5uM raptinal, 20uM rotenone and 20uM antimycin-A treatment were generated and compared between wild-type and *Gsdme* KO cells.

(F) Representative images of wild-type neurons treated with DMSO, 3uM raptinal and 20uM antimycin-A and stained for Tuj1 at 8h post-toxin treatment.

(G) Microtubule depolymerization index was calculated for WT neurons treated with several doses of raptinal and antimycin-A. Violin plots display the combined median and interquartile ranges for depolymerization index.

(H) Wild-type and *Gsdme* KO mouse cortical neurons were treated with 4uM raptinal, 20uM rotenone or 20uM antimycin-A and were assessed for LDH release at 8h post-treatment.

(I) Microtubule depolymerization index was calculated for WT neurons co-incubated with either DMSO or 20uM of zVAD-FMK and were then treated with several doses of raptinal, antimycin-A or rotenone (all concentrations are mentioned in micromolar units). Violin plots display the combined median and interquartile ranges for depolymerization indices taken from two independent experiments.

(J) Representative image of a microfluidic chamber plated with wild-type mouse cortical neurons stained with TMRM. The cell body, microgrooves and axonal compartments are labeled. The top panels represent the chamber prior to addition of 5uM raptinal, while the bottom panels show 1h post-toxin treatment.

(K) Quantification of TMRM intensity relative to baseline (time = 0) from the cell body and axonal chambers of plated WT neurons treated with 5 or 10uM raptinal.

For all datasets two-way ANOVA (row factor = toxin , column factor = genotype) was performed, followed by multiple comparisons for each group (p-values adjusted by the Tukey method are mentioned over respective comparisons). Data represents an average of at least 2 independent experiments.

Figure S11

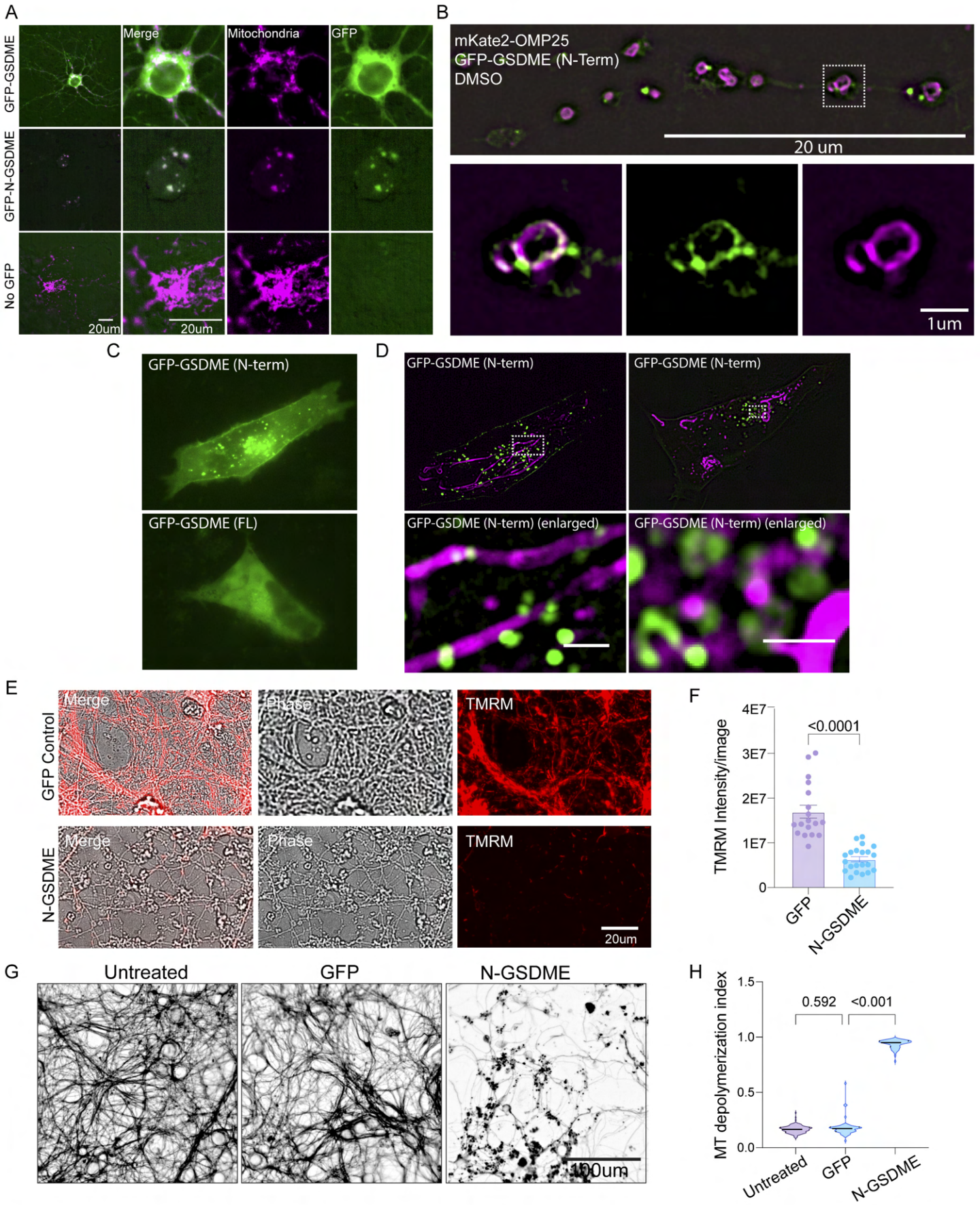

A
GFP-GSDME
GFP-N-GSDME
No GFP
Merge
Mitochondria
GFP
20um
20um
B
mKate2-OMP25
GFP-GSDME (N-Term)
DMSO
20 um
1um
C
GFP-GSDME (N-term)
GFP-GSDME (FL)
D
GFP-GSDME (N-term)
GFP-GSDME (N-term)
GFP-GSDME (N-term) (enlarged)
GFP-GSDME (N-term) (enlarged)
E
GFP Control
N-GSDME
Merge
Phase
TMRM
Merge
Phase
TMRM
20um
F
TMRM Intensity/image
4E7
3E7
2E7
1E7
0
<0.0001
GFP
N-GSDME
G
Untreated
GFP
N-GSDME
100um
H
MT depolymerization index
1.5
1.0
0.5
0.0
0.592
<0.001
Untreated
GFP
N-GSDME

Figure S11: Expression of N-GSDME in neurons is sufficient to drive mitochondrial localization, mitochondrial damage and neurite loss (related to Figure 5)

(A) Representative widefield images of mouse neurons transfected with mKate2-OMP25 and either full-length GFP-GSDME (top row), N-terminal GSDME (middle row) or empty vector control (bottom row). Cells were imaged 16h post-transfection.
(B) Structured illumination microscopy (SIM) images of mitochondria (OMP25-mKate) in axons of mouse neurons transfected with GFP-N-GSDME to assess colocalization.
(C) Representative widefield images of SH-SY5Y transfected with mKate2-OMP25 and either full-length GFP-GFP-N-GSDME (top image) or GSDME (bottom image).
(D) Structured illumination microscopy (SIM) images of SH-SY5Y mitochondria (mKate-OMP25) transfected with GFP-N-GSDME to assess colocalization.
(E-F) Primary mouse neurons (DIV3) were transduced with lentivirus encoding either N-GSDME or GFP-control. (E) 4d post-transduction (DIV7) neurons were stained with TMRM. (F) Images from 6 wells per condition across 2 independent experiments were quantified by measuring TMRM intensity along the length of neurites.
(G-H) Primary mouse neurons (DIV3) were transduced with lentivirus encoding either N-GSDME or GFP-control. (G) 4d post-transduction, neurons were fixed and stained for Tuj1+ signal. (H) Images from 6 wells per condition across 2 independent experiments were used to calculate microtubule depolymerization index for each condition.

For datasets F, H one-way ANOVA was performed, followed by multiple comparisons for each group (adj p-values, Tukey method). For F,H data represents an average of 2 independent experiments (3 technical replicates/experiment) $\pm$ SEM.

Figure S12

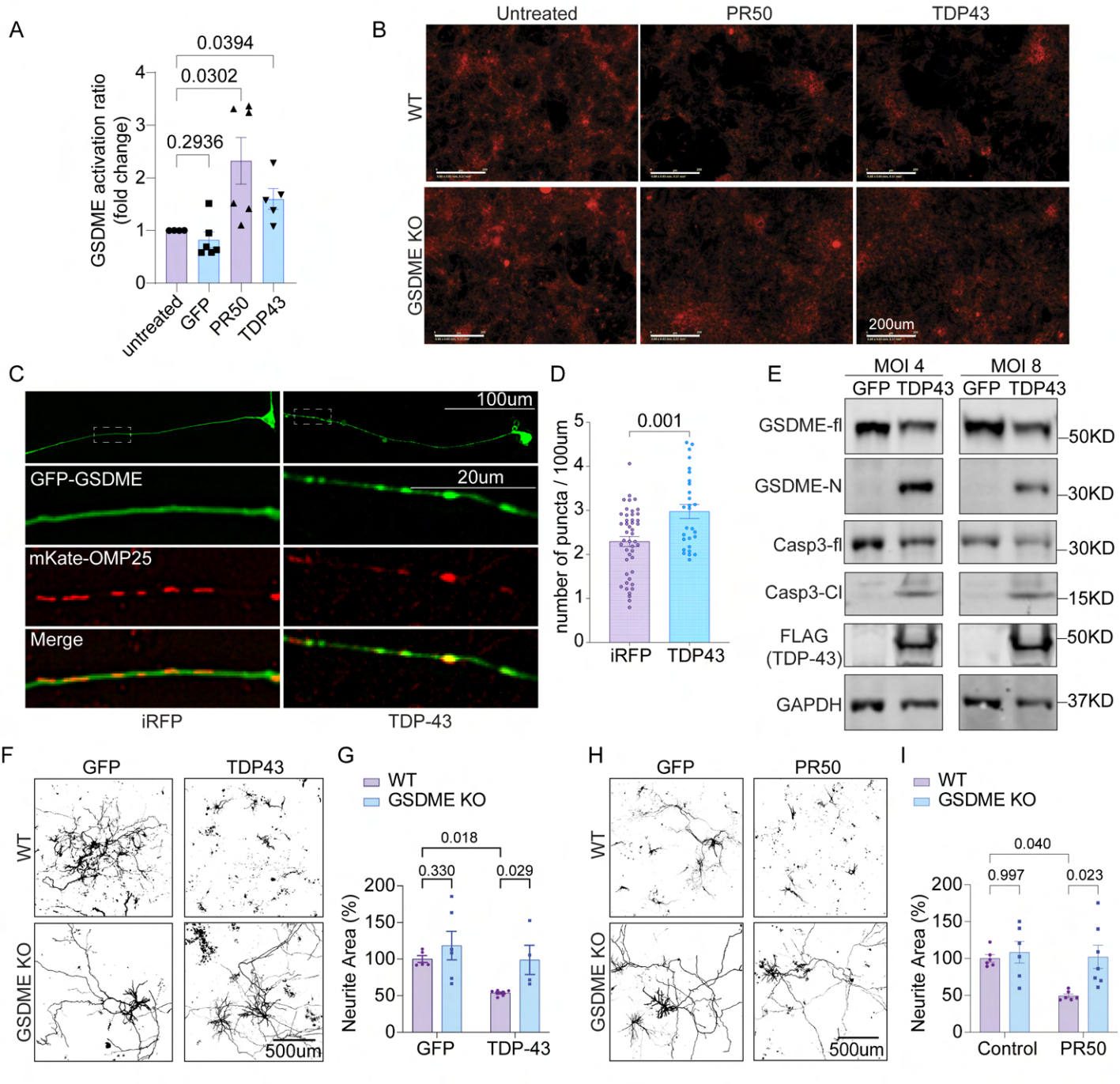

A
GSDME activation ratio (fold change)
0.0394
0.0302
0.2936
untreated
GFP
PR50
TDP43
B
Untreated
PR50
TDP43
WT
GSDME KO
200um
C
100um
20um
GFP-GSDME
mKate-OMP25
Merge
iRFP
TDP-43
D
number of puncta / 100um
0.001
iRFP
TDP43
E
MOI 4
MOI 8
GFP TDP43 GFP TDP43
GSDME-fl
GSDME-N
Casp3-fl
Casp3-Cl
FLAG (TDP-43)
GAPDH
50KD
30KD
30KD
15KD
50KD
37KD
F
GFP
TDP43
WT
GSDME KO
500um
G
WT
GSDME KO
0.018
0.330
0.029
Neurite Area (%)
GFP
TDP-43
H
GFP
PR50
WT
GSDME KO
500um
I
WT
GSDME KO
0.040
0.997
0.023
Neurite Area (%)
Control
PR50

Figure S12: GSDME is activated in iNeurons and mediates neurite loss caused by FTD/ALS proteins (related to Figure 6)

(A) GSDME immunoblot quantification (n=5-6) of primary cortical neurons transduced with lentivirus encoding GFP, PR-50, TD-43 or un-transduced control. Four days following transduction with lentiviruses, samples were lysed and probed with anti-GSDME and GAPDH (loading control). The ratio of N-terminal GSDME to full-length was calculated and plotted relative to untreated control.

(B) Representative images of WT and *Gsdme* KO mouse cortical neurons stained with TMRM (red) and transduced with lentiviruses encoding PR-50 or TDP-43 (4d post-transduction).

(C-D) Human iNeurons were transfected with mKate-OMP-25, GFP-GSDME and TDP-43 or iRFP (control). (C) Cells were imaged 72h post-transfection (D) Quantification of the number of GFP-GSDME puncta per 100um in neurites.

(E) Human iNeurons were transduced with control virus (GFP) or lentivirus encoding FLAG-TDP43 at multiplicity of infection (MOI) of 4 or 8. 5d post-transduction, cells were lysed for immunoblot analysis.

(F-I) Primary wild-type and *Gsdme* KO mouse neurons were co-transfected with RFP (shown in black) and either GFP, (F-G) TDP-43 or (H-I) PR-50 and imaged for neurite (RFP signal) area. Representative images of WT and KO neurons transfected with GFP and either TDP-43 (F) or PR-50 (H) are shown along with the quantifications of neurite area (G and I). Each dot represents an average of 10-12 transfected neurons imaged in an independent well. Every condition was imaged across two independent experiments.

For datasets A, G, I two-way ANOVA (row factor = lentivirus treatment, column factor = genotype) was performed, followed by multiple comparisons for each group (adj p-values, Tukey method). For F-I, data represents an average of 2 independent experiments $\pm$ SEM.

Figure S13

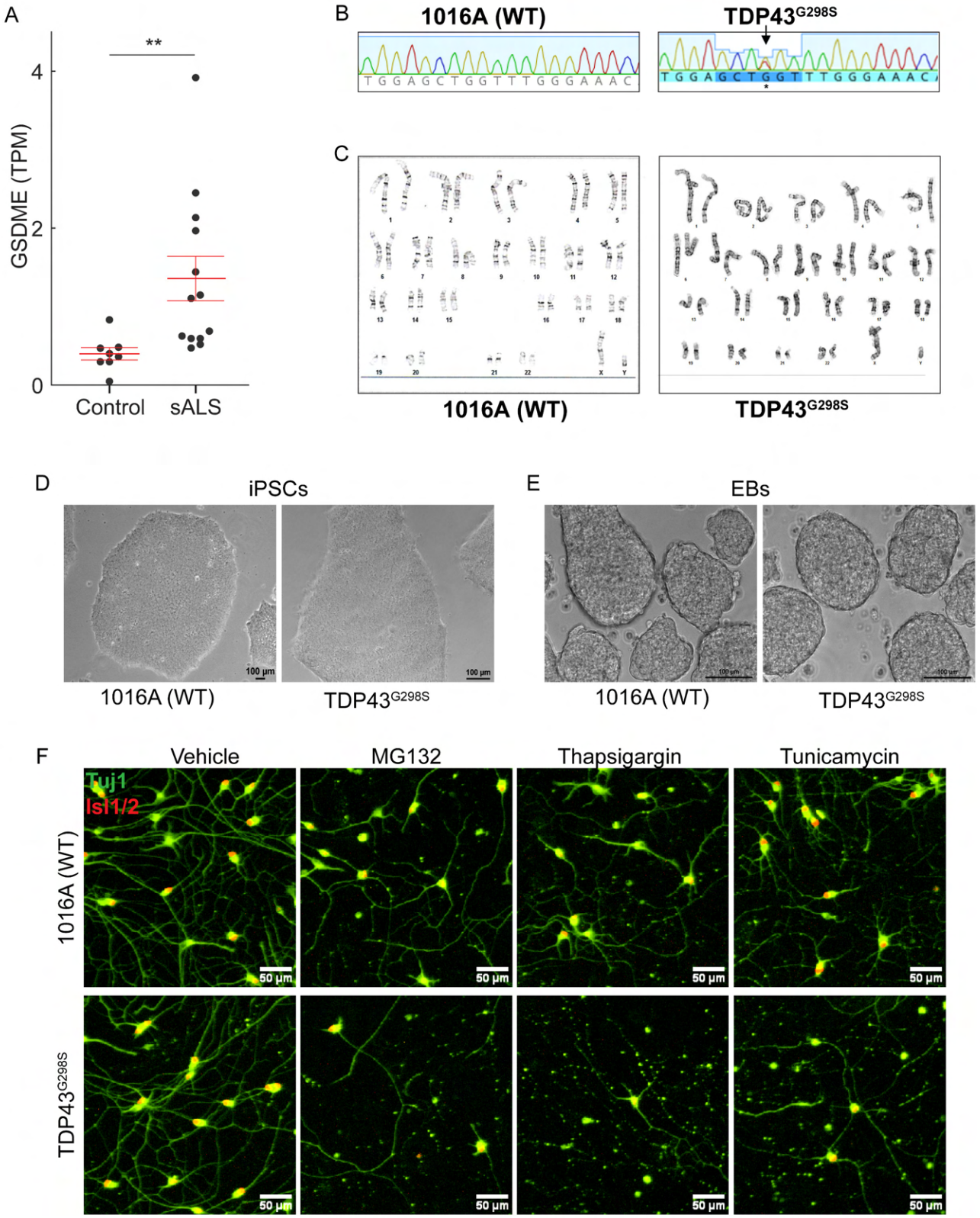

A
**
GSDME (TPM)
4
2
0
Control
sALS
B
1016A (WT)
TDP43G298S
T G G A G C T G G T T T G G G A A A C
T G G A G C T G G T T T G G G A A A C
C
1016A (WT)
TDP43G298S
D
iPSCs
100 µm
1016A (WT)
TDP43G298S
E
EBs
1016A (WT)
TDP43G298S
F
Vehicle
MG132
Thapsigargin
Tunicamycin
Tuj1
Isl1/2
1016A (WT)
TDP43G298S
50 µm

Figure S13: Transcriptomic analysis of human spinal cord, and validation of iPSC-derived motor neurons (related to Figure 7)

(A) RNA-seq transcriptomic data from laser capture micro-dissected motor neurons from patients with sporadic ALS and age-matched controls[1].

(B) Sanger sequencing chromatograms of 1016A (WT) and *TDP43*^{G298S} iPSC-derived motor neurons at the TDP-43 locus.

(C) G-banded karyotypes of 1016A (WT) and *TDP43*^{G298S} iPSC-derived motor neurons

(D) Phase contrast imaging of iPSC morphology from 1016A (WT) and *TDP43*^{G298S} cell lines.

(E) Phase contrast imaging of embryoid body morphology from 1016A (WT) and *TDP43*^{G298S} cell lines.

(F) Immunostaining of 1016A (WT) and *TDP43*^{G298S} iPSC-derived motor neurons positive for Islet 1/2 (motor neuron marker), co-stained with Tuj1, and then treated with MG-132, thapsigargin and tunicamycin for 48h.

Figure S14

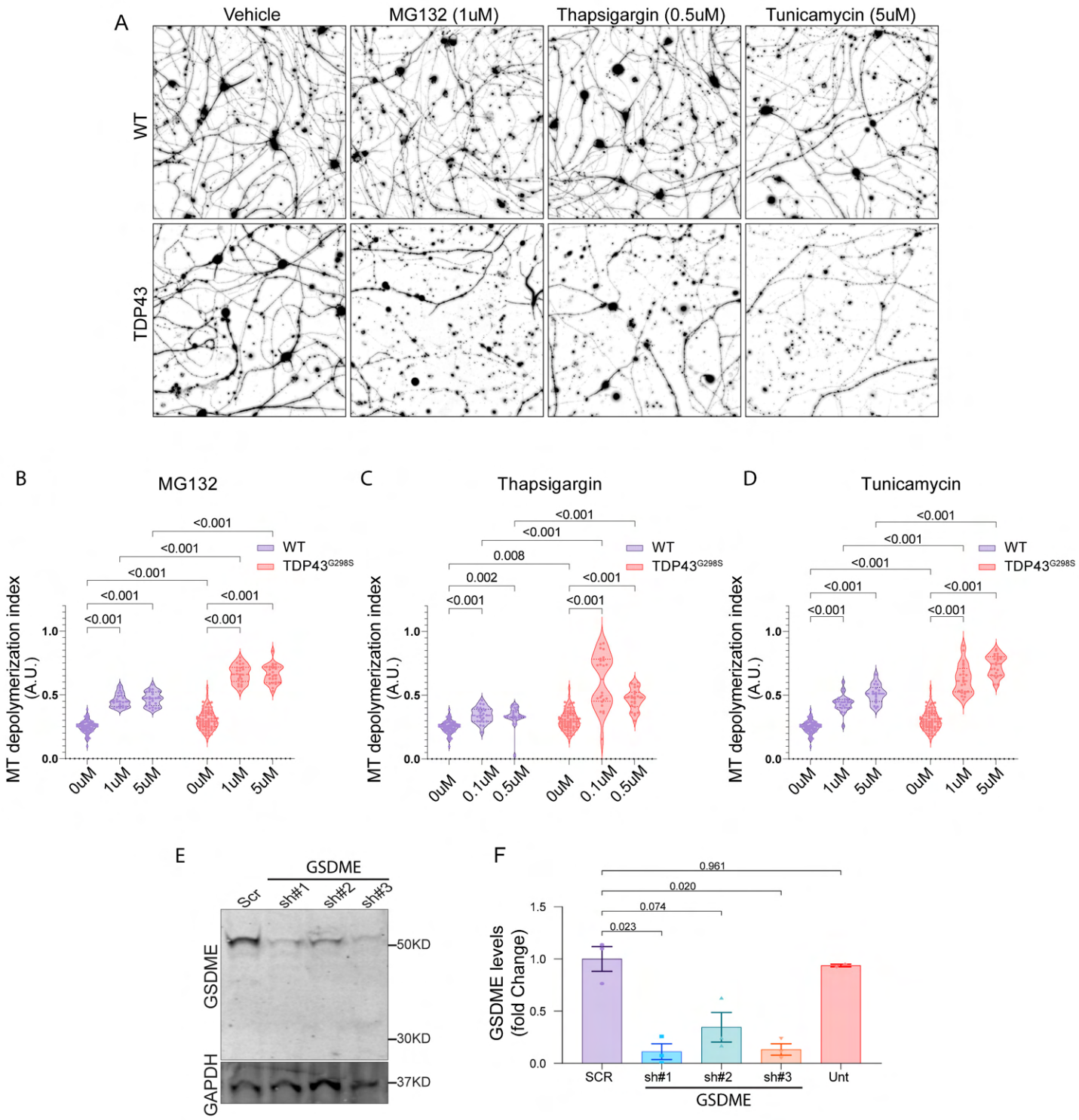
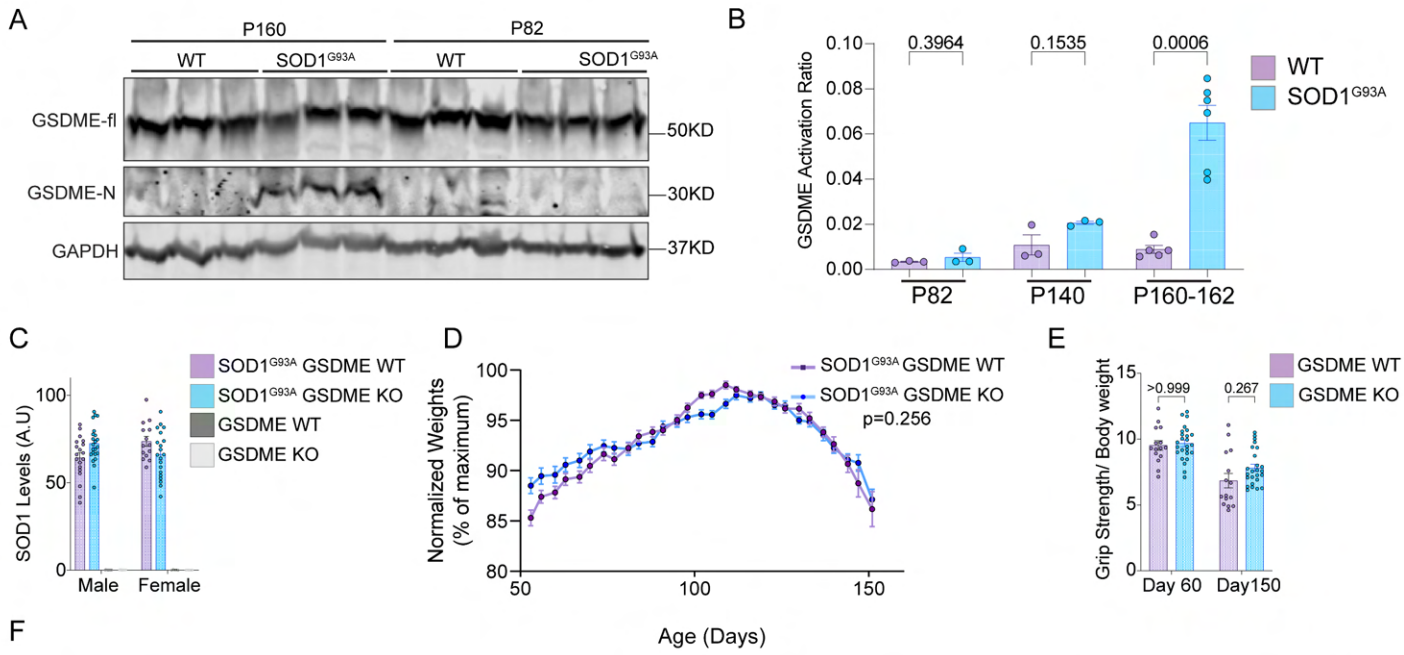

A
Vehicle
MG132 (1uM)
Thapsigargin (0.5uM)
Tunicamycin (5uM)
WT
TDP43
B
MG132
C
Thapsigargin
D
Tunicamycin
WT
TDP43G298S
MT depolymerization index (A.U.)
0uM
1uM
5uM
0.1uM
0.5uM
<0.001
0.008
0.002
E
GSDME
Scr
sh#1
sh#2
sh#3
50KD
30KD
37KD
GAPDH
F
GSDME levels (fold Change)
0.961
0.020
0.074
0.023
SCR
Unt

Figure S14: Endoplasmic reticulum stressors and proteasome inhibition cause neurite loss in susceptible TDP43 G298S iPSC-derived motor neurons (related to Figure 7)

(A) Representative images of control 1016A (WT) or *TDP43*^{G298S} iPSC-derived motor neurons treated with DMSO, MG132, tunicamycin or thapsigargin, and stained for Tuj1 at 48h post-toxin treatment.

(B-D) Microtubule depolymerization index was calculated for 1016A (WT) and *TDP43*^{G298S} motor neurons treated with DMSO (vehicle) or several doses (C) MG132 (D) thapsigargin or (E) tunicamycin (uM). Violin plots display the combined median and interquartile ranges for depolymerization index.

(E-F) Immunoblots of iPSC-derived cortical neurons 4d post-transdution with scrambled or GSDME-targeting shRNAs (E), which was quantified in (F).



F

	SOD1^{G93A} GSDME WT			SOD1^{G93A} GSDME KO		
	Mixed Gender	Males	Females	Mixed Gender	Males	Females
Survival (Days) (Mean ± SEM)	159.2 ± 1.9	158.6 ± 2.6	160.0 ± 2.8	171.0 ± 2.5	164.9 ± 1.9	177.6 ± 4.5
Disease Progression (Days) (Mean ± SEM)	48.1 ± 2.6	49.2 ± 4.5	47.0± 2.6	57.0 ± 2.5	53.0 ± 3.9	59.44 ± 3.1
Grip strength at day 150 (Mean ± SEM)	56.02 ± 3.90	55.98 ± 3.66	56.05 ± 6.66	85.99 ± 6.77	66.83 ± 5.61	95.11 ± 9.04

Figure S15: GSDME is activated in the SOD1 G93A mouse model and plays a role in disease progression (related to Figure 8)

(A-B) GSDME and GAPDH immunoblots of spinal cord lysates from (A) pre-symptomatic stage (P82) and end-stage (P160) SOD1^{G93A} mice. (B) The activation ratio of GSDME is quantified as the levels of GSDME N-terminal compared to total GSDME (full length + cleaved fractions).

(C) Expression of the SOD1^{G93A} transgene, as assessed by RT-qPCR, was quantified for each group and genotype. This was done to confirm that the copies of this ALS-causing transgene were comparable between experimental groups.

(D) Weights of transgenic SOD1^{G93A} *Gsdme* WT or SOD1^{G93A} *Gsdme* KO mice were tracked over time starting at week 7 until week 21.

(E) Grip strength measures of nTg WT and nTg *Gsdme* KO mice (lacking SOD1^{G93A}) were recorded at P60 and P150 and normalized by body weight.

(F) Table of summary values for male and female transgenic mice displaying key measures of survival, disease progression and motor function.

Figure S16

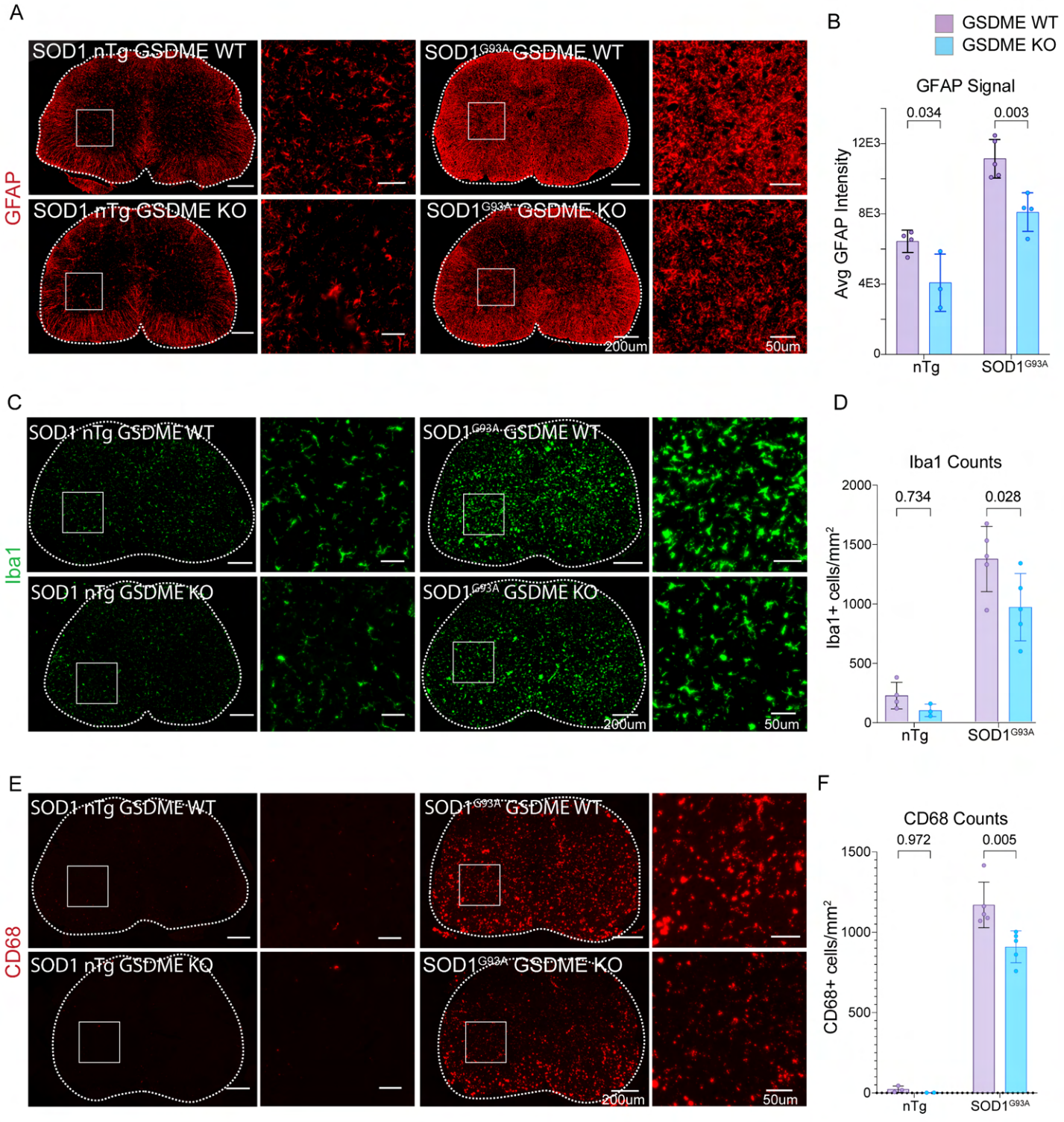

A
SOD1 nTg GSDME WT
SOD1G93A GSDME WT
SOD1 nTg GSDME KO
SOD1G93A GSDME KO
GFAP
200um
50um
B
GSDME WT
GSDME KO
GFAP Signal
0.034
0.003
Avg GFAP Intensity
12E3
8E3
4E3
0
nTg
SOD1G93A
C
SOD1 nTg GSDME WT
SOD1G93A GSDME WT
SOD1 nTg GSDME KO
SOD1G93A GSDME KO
Iba1
200um
50um
D
Iba1 Counts
0.734
0.028
Iba1+ cells/mm2
2000
1500
1000
500
0
nTg
SOD1G93A
E
SOD1 nTg GSDME WT
SOD1G93A GSDME WT
SOD1 nTg GSDME KO
SOD1G93A GSDME KO
CD68
200um
50um
F
CD68 Counts
0.972
0.005
CD68+ cells/mm2
1500
1000
500
0
nTg
SOD1G93A

Figure S16: GSDME knockout reduces histological markers of gliosis in the SOD1^{G93A} mouse model of ALS (related to Figure 8)

(A-B) Representative images of lumbar spinal cord sections from SOD1^{G93A} *Gsdme* WT and SOD1^{G93A} *Gsdme* KO animals at P150 (A) stained with anti-GFAP to mark astrocytes. (B) The intensity of GFAP in the ventral horn of P150 transgenic mice normalized by the ventral horn area was quantified. Each dot represents the average value of 6-8 stained lumbar spinal cord sections taken from n=3-5 mice. Magnified regions (white box) delineate a ventral horn area of a spinal cord section.

(C-D) Representative images of lumbar spinal cord sections from SOD1^{G93A} *Gsdme* WT and SOD1^{G93A} *Gsdme* KO animals at P150, stained with anti-Iba1(C). Magnified regions represent the ventral horns of the spinal cord used for (D) quantification of mean Iba1 counts per um^2. Each dot represents the average value of 6-8 stained lumbar spinal cord sections taken from n=3-5 mice.

(E-F) Representative images of lumbar spinal cord sections from SOD1^{G93A} *Gsdme* WT and SOD1^{G93A} *Gsdme* KO animals at P150, stained with anti-CD68 (a lysosomal activation marker), to assess disease associated inflammation (E). Magnified regions represent the ventral horns of the spinal cord used for (F) quantification of mean CD68 counts per um^2. Each dot represents the average value of 6-8 stained lumbar spinal cord sections taken from n=3-5 mice.

Patient	Age at death	Sex	Neuropathological Diagnosis	Clinical Diagnosis	Disease Duration (years)	Amyloid Pathology (full brain)	Tau Pathology	Synuclein Pathology
NC 1	66	M	Unremarkable adult brain	Normal	N/A	0	0	0
NC 2	60	F	Unremarkable adult brain	Normal	N/A	0	0	0

Table S1: Patient characteristics of human temporal lobe cortical tissue sections. NC = normal controls, with patient IDs and represented in images shown in the manuscript (Related to Fig 1).

Agent	Source / Sequence
Lenti-Syn-PR50	Gift from Gitler lab [2]
Lenti-Syn-eGFP	Gift from Gitler lab [2]
Lenti-Syn-TDP43	Gift from Gitler lab [2]
Mouse GSDME forward Primer	5'- TGCAACTTCTAAGTCTGGTGACC-3'
Mouse GSDME reverse Primer	5'- CTCCACAACCACTGGACTGAG-3'
Mouse GAPDH forward Primer	5'- GGGTGTGAACCACGAGAAATATG-3'
Mouse GAPDH reverse Primer	5'- TGTGAGGGAGATGCTCAGTGTTG-3'
Human TDP43^{G298S} forward Primer	5'- CGACTGAAATATCACTGCTGCTG-3'
Human TDP43^{G298S} reverse Primer	5'- GGATGCTGATCCCCAACCAA-3'
Cardiolipin shRNA 1	5'- GCATCAGCATACAGTTATTAT-3'
Cardiolipin shRNA 2	5'- CGGATTTGTTGGATGGATTTA-3'
Cardiolipin shRNA 3	5'- CCCACTCACTTACATGATAAT-3'
Scrambled shRNA	5'- CCTAAGGTTAAGTCGCCCTCG-3'
GSDME shRNA 1	5'- GATGATGGAGTATCTGATCTT-3'
GSDME shRNA 2	5'- GCGGTCCTATTTGATGATGAA-3'
GSDME shRNA 3	5'- GCATGATGAATGACCTGACTT-3'

Table S2: Oligonucleotides and virus details (Related to STAR Methods Key Resources Table)

Line ID	Diagnosis	Mutation	Age of Onset	Age of Biopsy	Gender	Lab	Publication
1016A	Healthy Control	/		20s	M	Melton	Pagliuca et al., 2014
47d	ALS	TDP43^{G298S}	43	43	M	Eggan	Alami et al., 2014

Table S3: Donor information for iPSC derived motor neurons (Related to Fig 7).

Supplementary References

1. Krach, F., Batra, R., Wheeler, E.C., Vu, A.Q., Wang, R., Hutt, K., Rabin, S.J., Baughn, M.W., Libby, R.T., Diaz-Garcia, S., et al. (2018). Transcriptome–pathology correlation identifies interplay between TDP-43 and the expression of its kinase CK1E in sporadic ALS. Acta Neuropathol *136*, 405–423. 10.1007/s00401-018-1870-7.
2. Maor-Nof, M., Shipony, Z., Lopez-Gonzalez, R., Nakayama, L., Zhang, Y.J., Couthouis, J., Blum, J.A., Castruita, P.A., Linares, G.R., Ruan, K., et al. (2021). p53 is a central regulator driving neurodegeneration caused by C9orf72 poly(PR). Cell *184*, 689-708.e20. 10.1016/j.cell.2020.12.025.