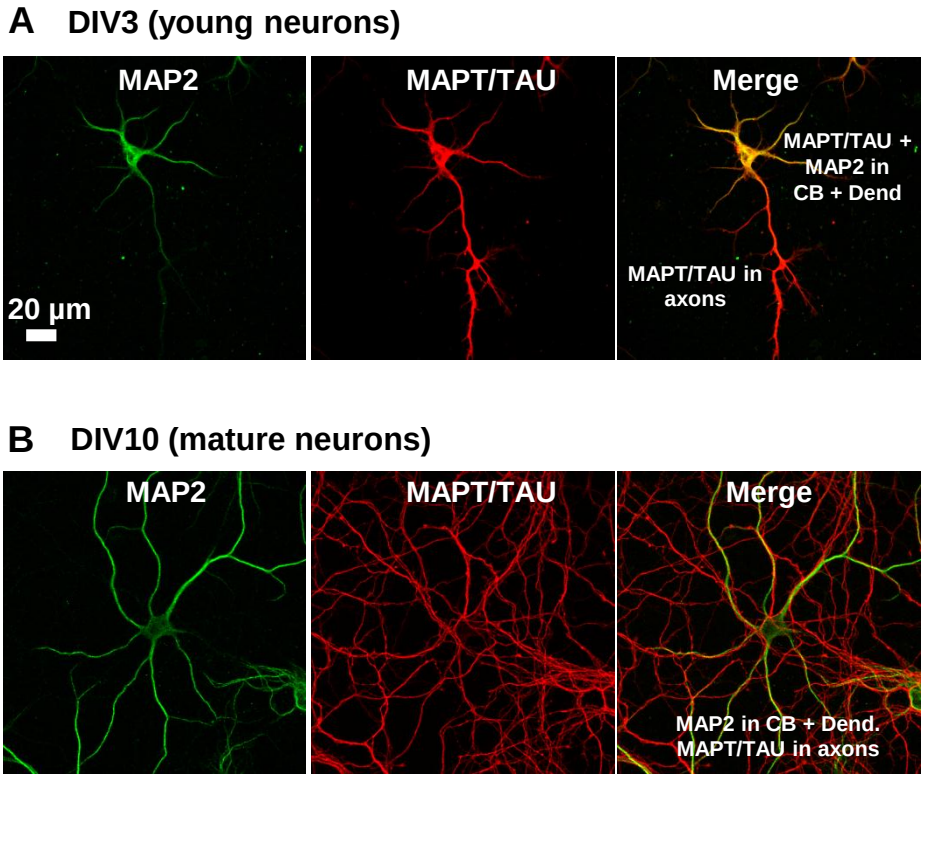
Fig. 1 A-C


A
DIV3 (young neurons)
MAP2
MAPT/TAU
Merge
MAPT/TAU + MAP2 in CB + Dend
MAPT/TAU in axons
20 µm
B
DIV10 (mature neurons)
MAP2
MAPT/TAU
Merge
MAP2 in CB + Dend.
MAPT/TAU in axons

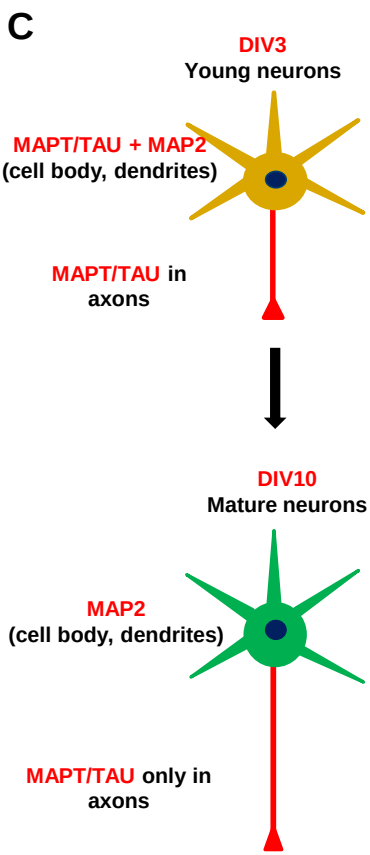

C
DIV3
Young neurons
MAPT/TAU + MAP2
(cell body, dendrites)
MAPT/TAU in axons
DIV10
Mature neurons
MAP2
(cell body, dendrites)
MAPT/TAU only in axons

Fig. 2 A-E

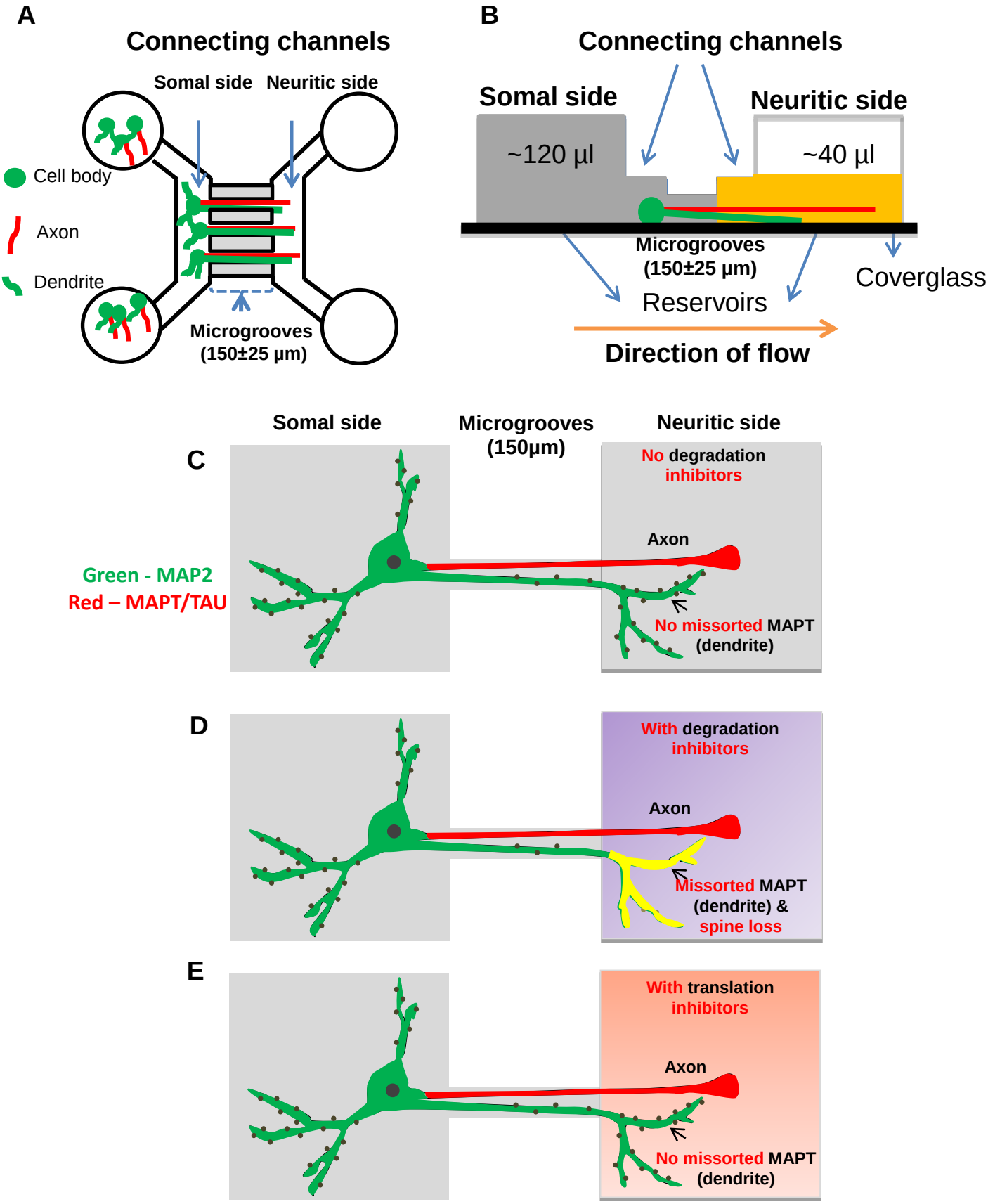

A
Connecting channels
Somal side
Neuritic side
Cell body
Axon
Dendrite
Microgrooves
(150±25 µm)
B
Connecting channels
Somal side
Neuritic side
~120 µl
~40 µl
Microgrooves
(150±25 µm)
Coverglass
Reservoirs
Direction of flow
Somal side
Microgrooves
(150µm)
Neuritic side
C
No degradation
inhibitors
Axon
Green - MAP2
Red – MAPT/TAU
No missorted MAPT
(dendrite)
D
With degradation
inhibitors
Axon
Missorted MAPT
(dendrite) &
spine loss
E
With translation
inhibitors
Axon
No missorted MAPT
(dendrite)

Fig. 3 A-D

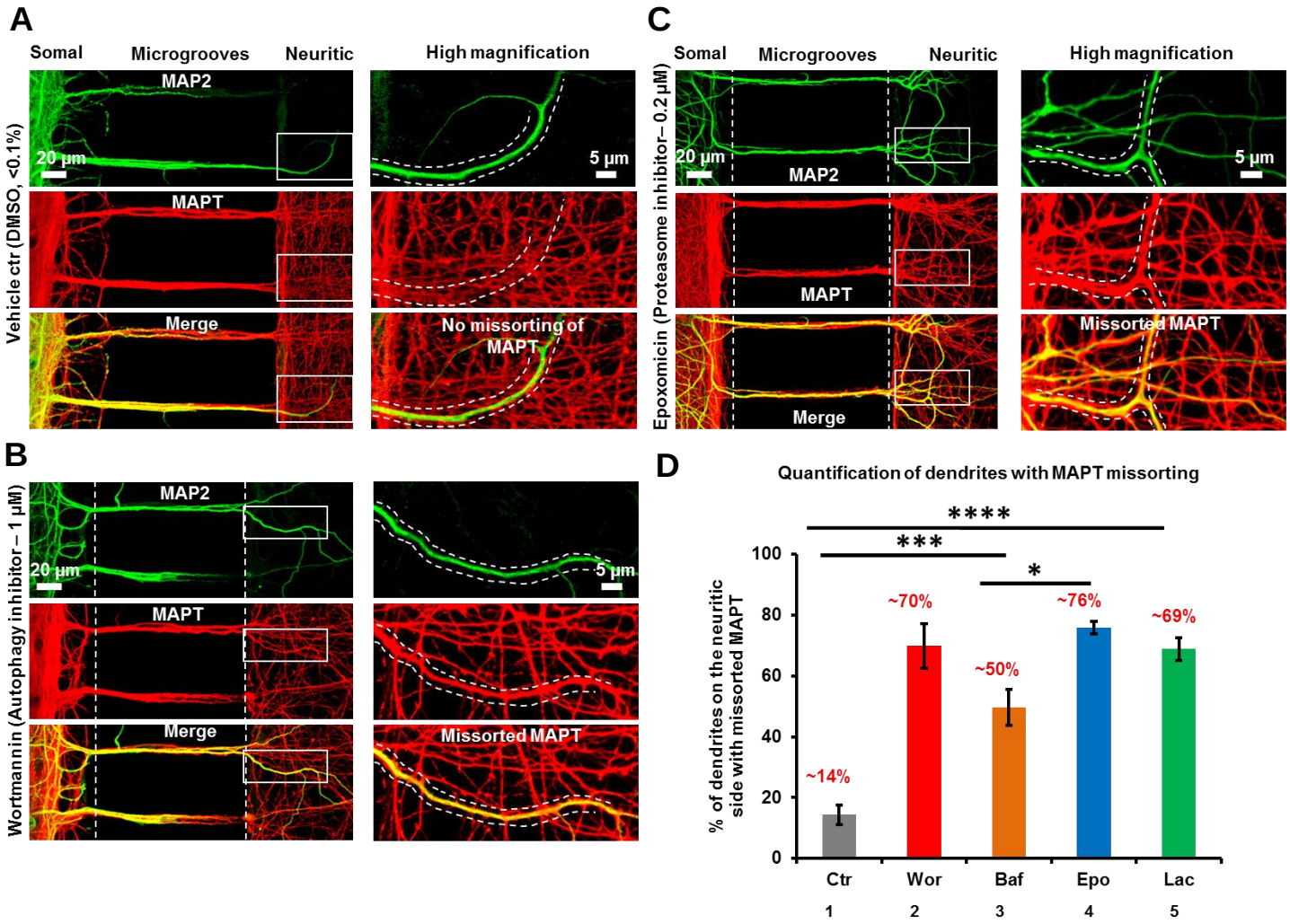
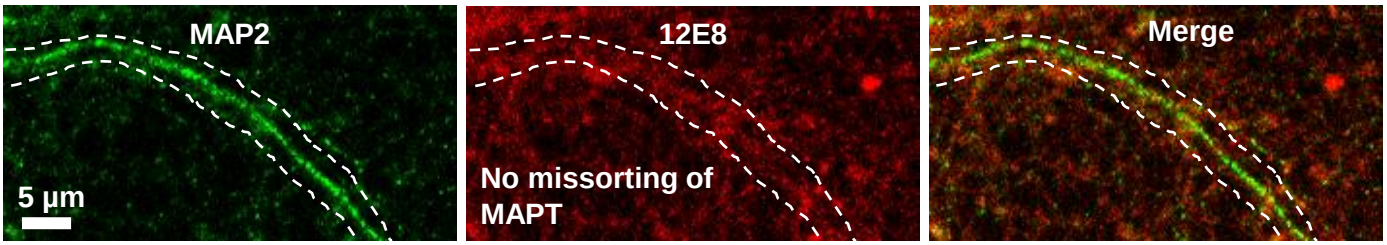
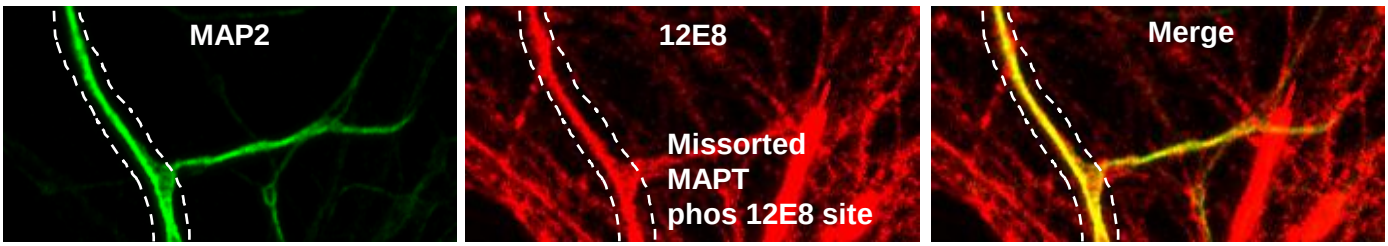

A
Somal
Microgrooves
Neuritic
High magnification
MAP2
MAPT
Merge
20 µm
5 µm
Vehicle ctr (DMSO, <0.1%)
No missorting of MAPT
C
Epoxomicin (Proteasome inhibitor– 0.2 µM)
Missorted MAPT
B
Wortmannin (Autophagy inhibitor – 1 µM)
Missorted MAPT
D
Quantification of dendrites with MAPT missorting
% of dendrites on the neuritic side with missorted MAPT
****
***
*
~14%
~70%
~50%
~76%
~69%
Ctr
Wor
Baf
Epo
Lac
1
2
3
4
5

Fig. 4 A-D

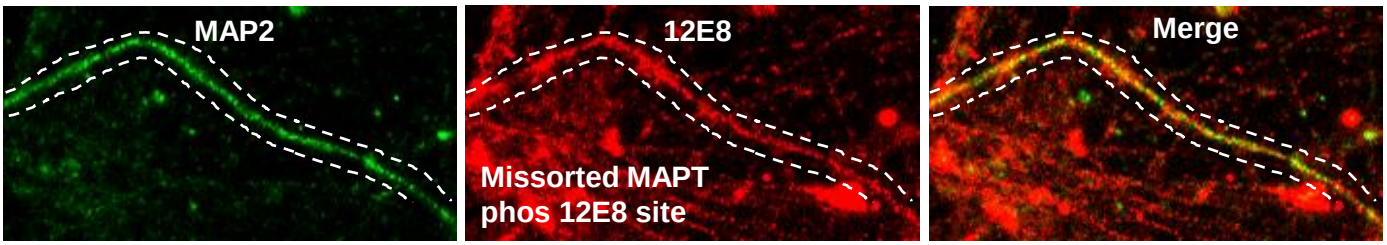
A Vehicle ctr (DMSO, <0.1%), 24 h, neuritic side



B Wortmannin (Autophagy inhibitor – 1 µM), 24 h, neuritic side



C Epoxomicin (Proteasome inhibitor – 0.2 µM), 24 h, neuritic side



D Quantification of dendrites with missorted phosphorylated MAPT after treatment

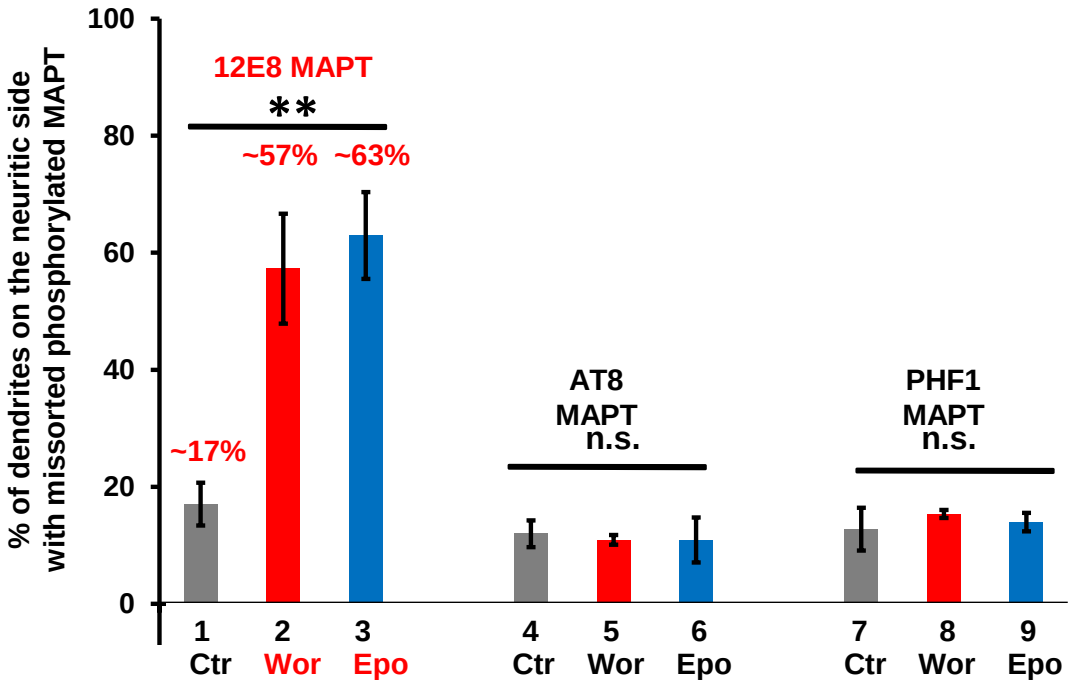
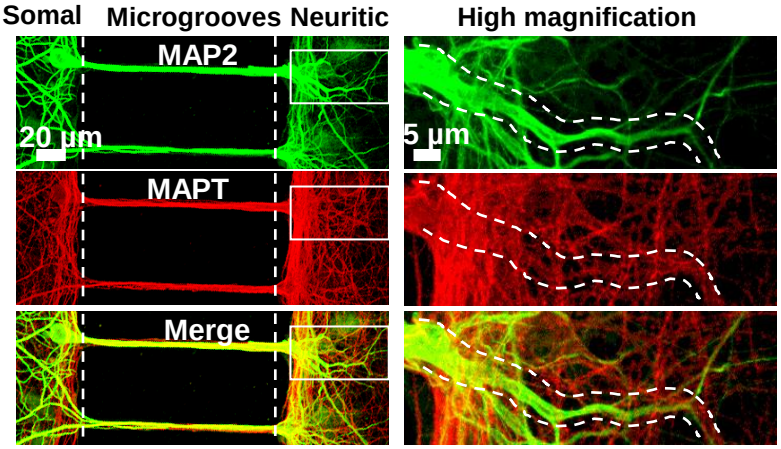


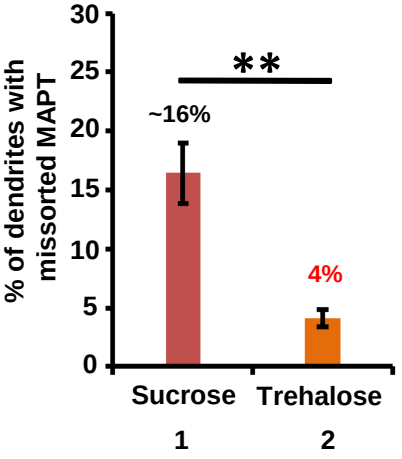
Fig. 5 A-D

A

Trehalose (Autophagy enhancer -150 mM), 24 h, neuritic side

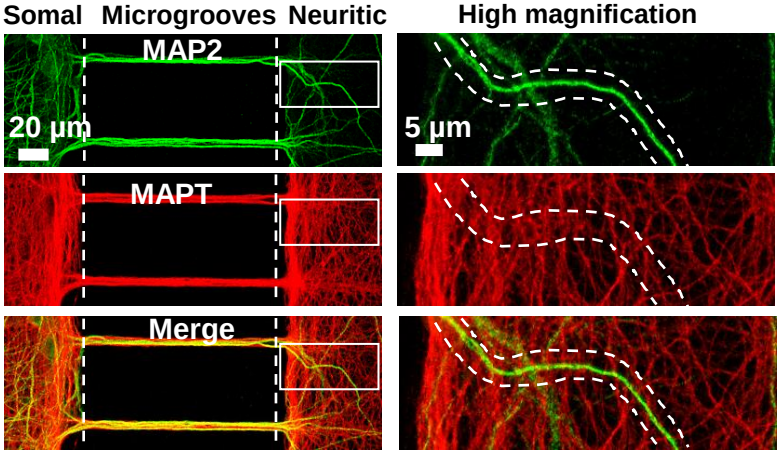


B



C

Rolipram (Proteasome enhancer – 10 µM), 24 h, neuritic side



D

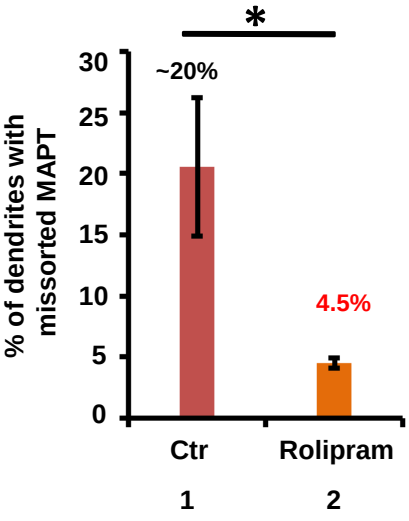
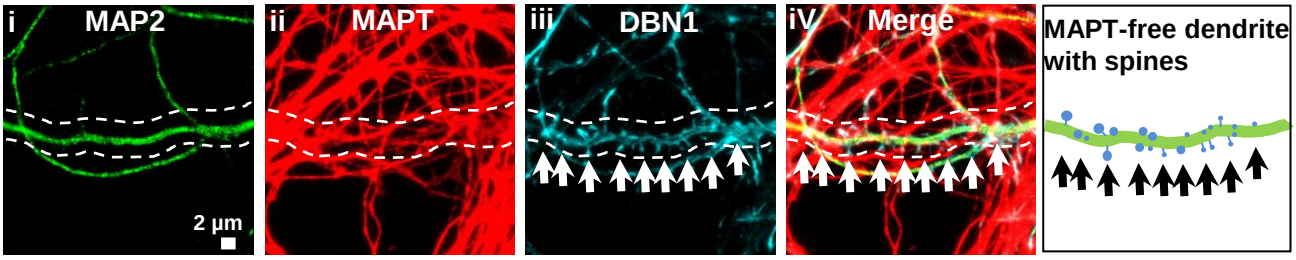
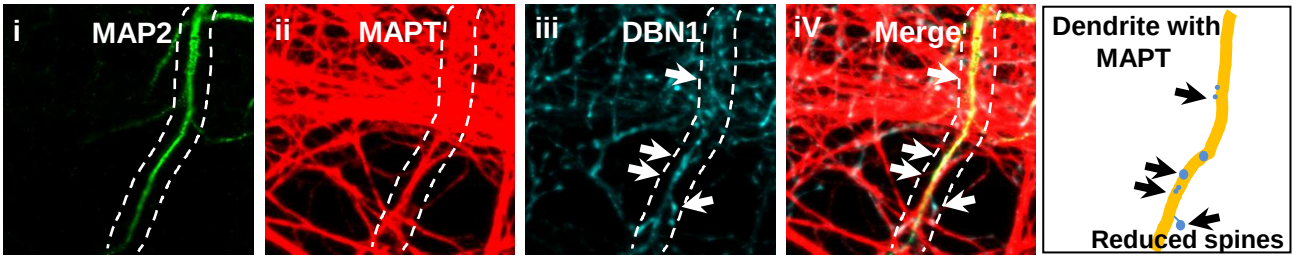


Fig. 6 A-E

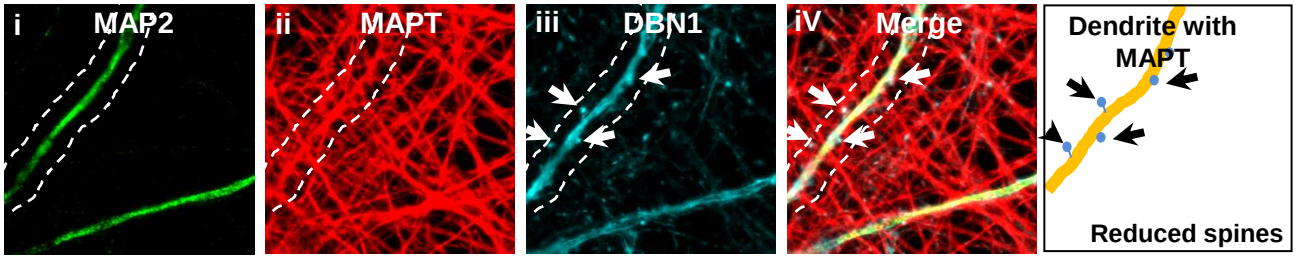
A Vehicle ctr (DMSO, <0.1%), 24 h, neuritic side



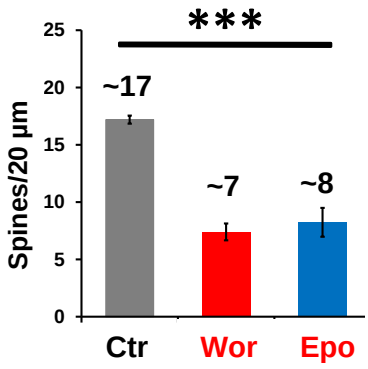
B Wortmannin (Autophagy inhibitor – 1 µM), 24 h, neuritic side



C Epoxomicin (Proteasome inhibitor – 0.2 µM), 24 h, neuritic side



D Quantification of spine density



E Enlarged version of fig.6Aiii

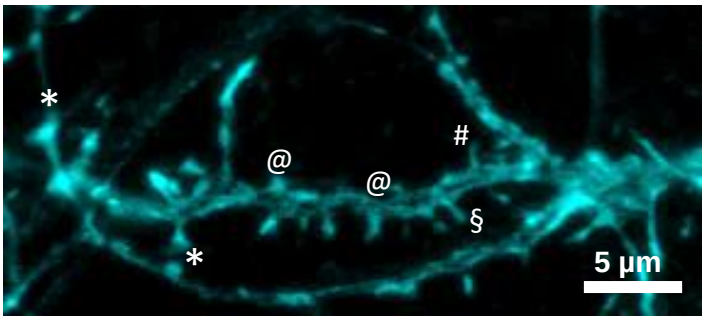
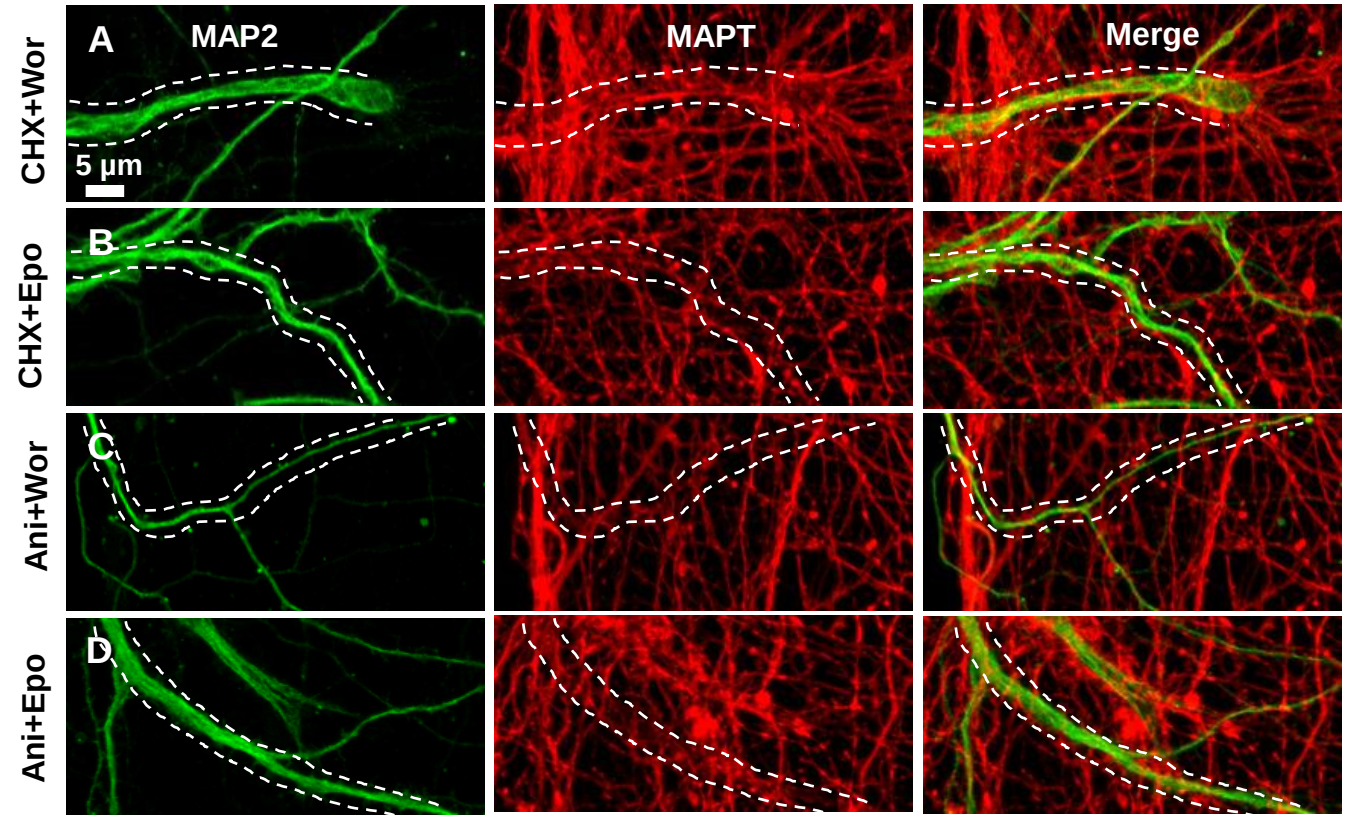
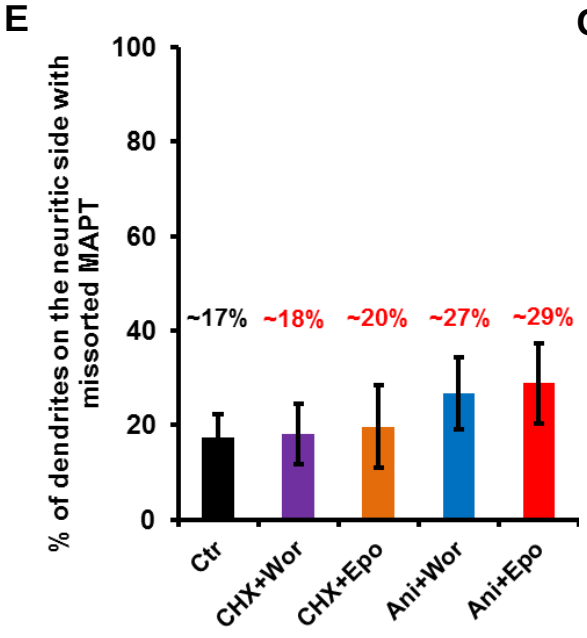
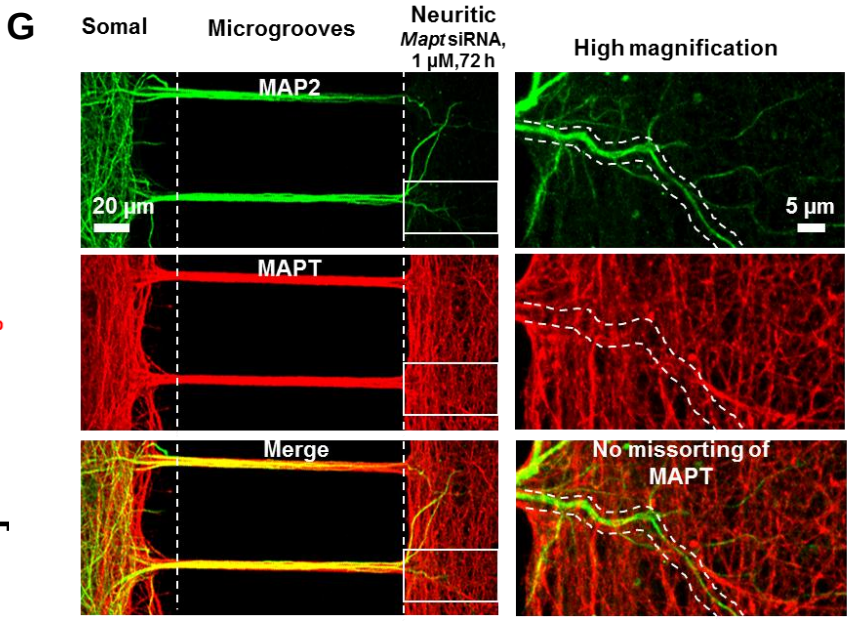
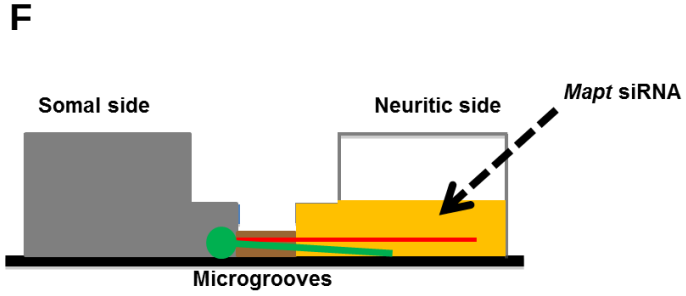


Fig. 7 A-H


A
MAP2
MAPT
Merge
5 µm
CHX+Wor
B
CHX+Epo
C
Ani+Wor
D
Ani+Epo


E
% of dendrites on the neuritic side with missorted MAPT
100
80
60
40
20
0
~17%
~18%
~20%
~27%
~29%
Ctr
CHX+Wor
CHX+Epo
Ani+Wor
Ani+Epo


G
Somal
Microgrooves
Neuritic
Mapt siRNA,
1 µM,72 h
High magnification
MAP2
20 µm
5 µm
MAPT
Merge
No missorting of
MAPT


F
Somal side
Neuritic side
Mapt siRNA
Microgrooves

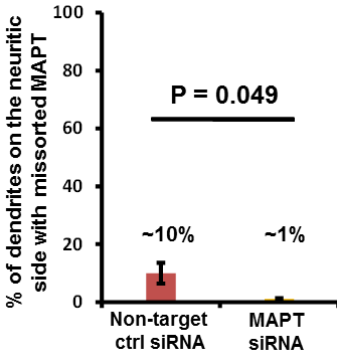
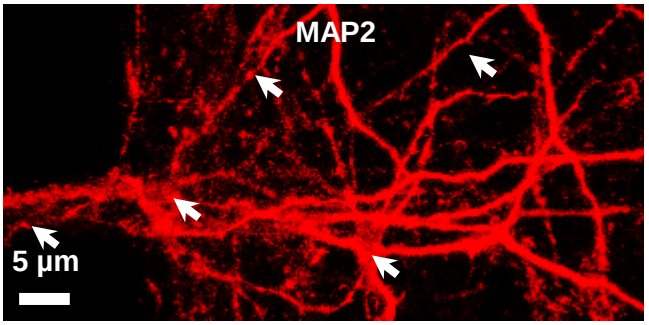

H
% of dendrites on the neuritic side with missorted MAPT
100
80
60
40
20
0
P = 0.049
~10%
~1%
Non-target ctrl siRNA
MAPT siRNA

Fig. 8 A-E

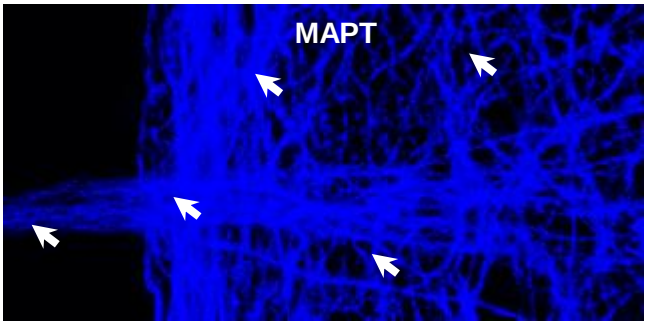
Fluorescence *in situ* hybridization of *Mapt/Tau* mRNA in rat hippocampal neurons cultured in microfluidic chambers

***Mapt/Tau* mRNA in dendrites on the neuritic side**

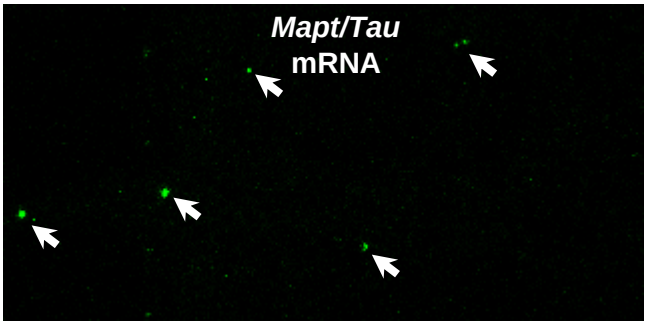
A



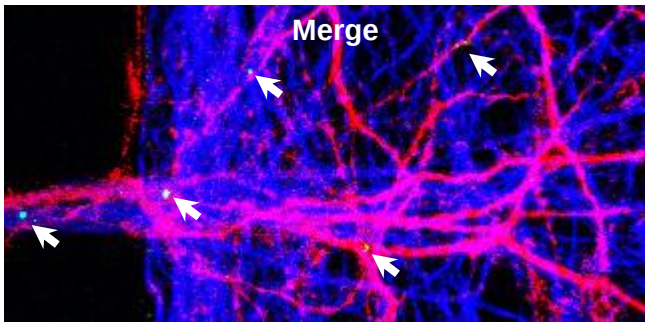
B



C



D



E Quantification of the average number of *Mapt/Tau* mRNA

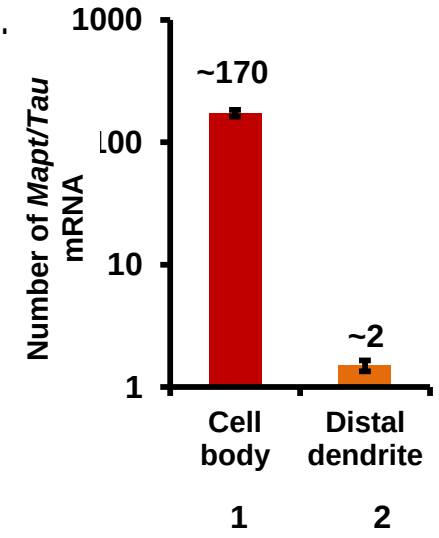
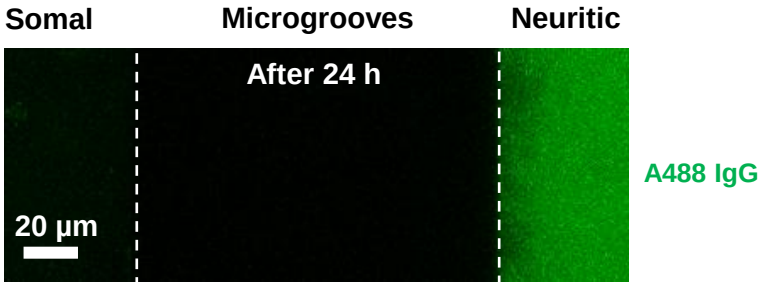
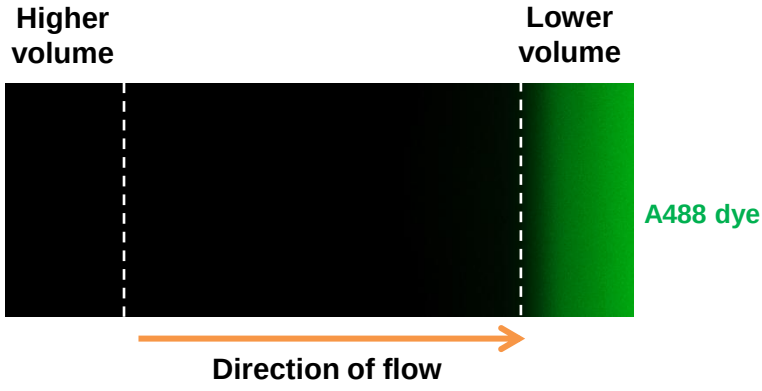


Fig. S1 A-C

A Test with Alexa Fluor 488 IgG



B Test with Alexa Fluor 488 dye



C Orthogonal view of axons and dendrites in microgrooves

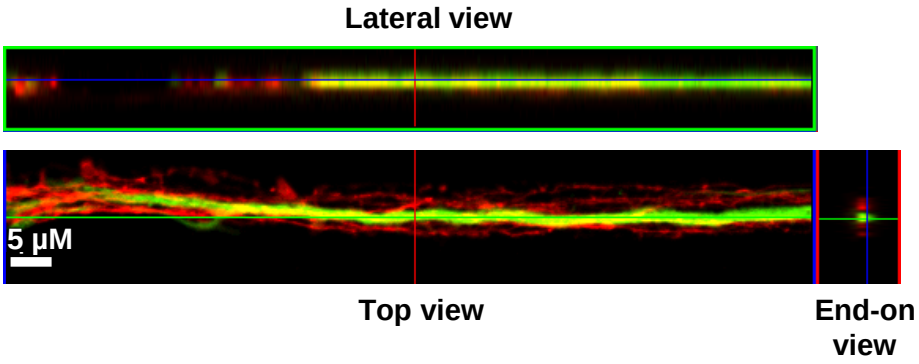


Fig. S2

on neuritic side

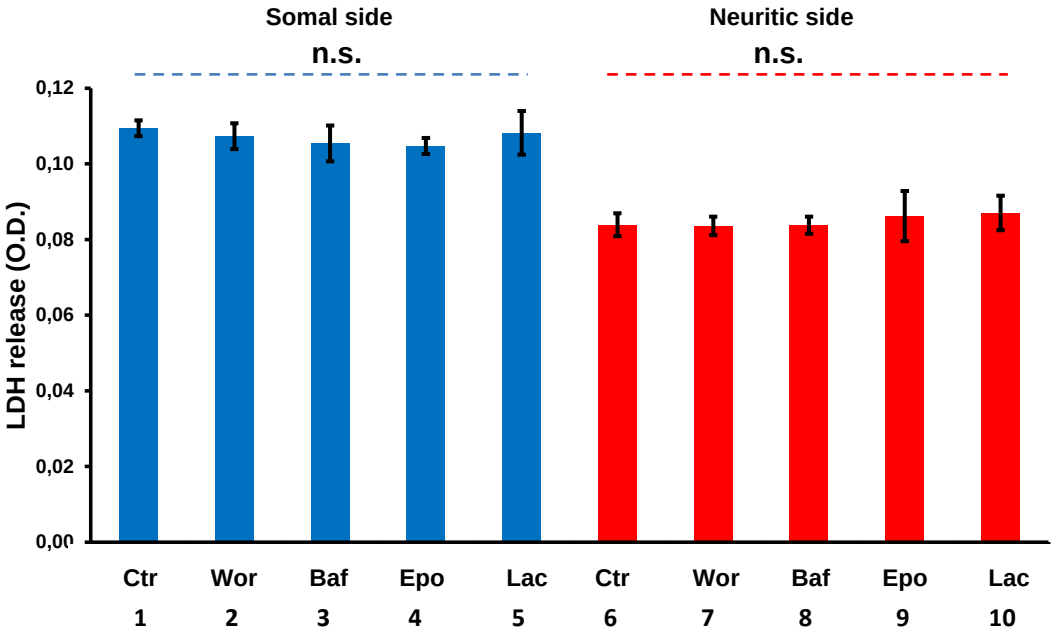
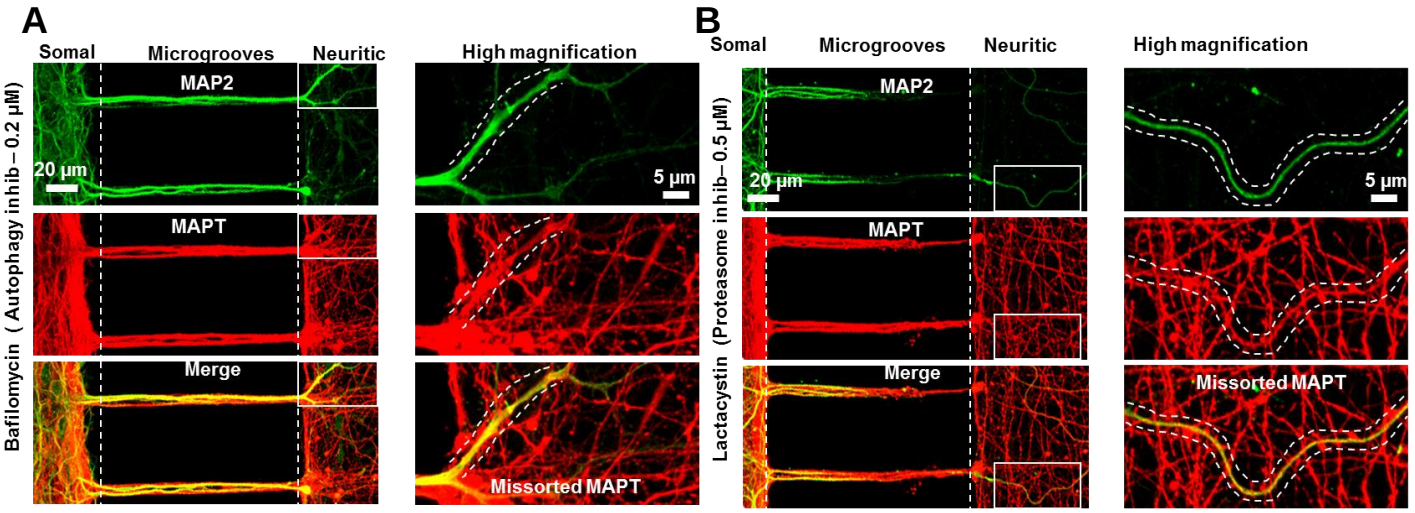


Fig. S3 A-C


A
Somal
Microgrooves
Neuritic
High magnification
MAP2
MAPT
Merge
20 μm
5 μm
Missorted MAPT
Bafilomycin (Autophagy inhib– 0.2 μM)
B
Somal
Microgrooves
Neuritic
High magnification
MAP2
MAPT
Merge
20 μm
5 μm
Missorted MAPT
Lactacystin (Proteasome inhib– 0.5 μM)

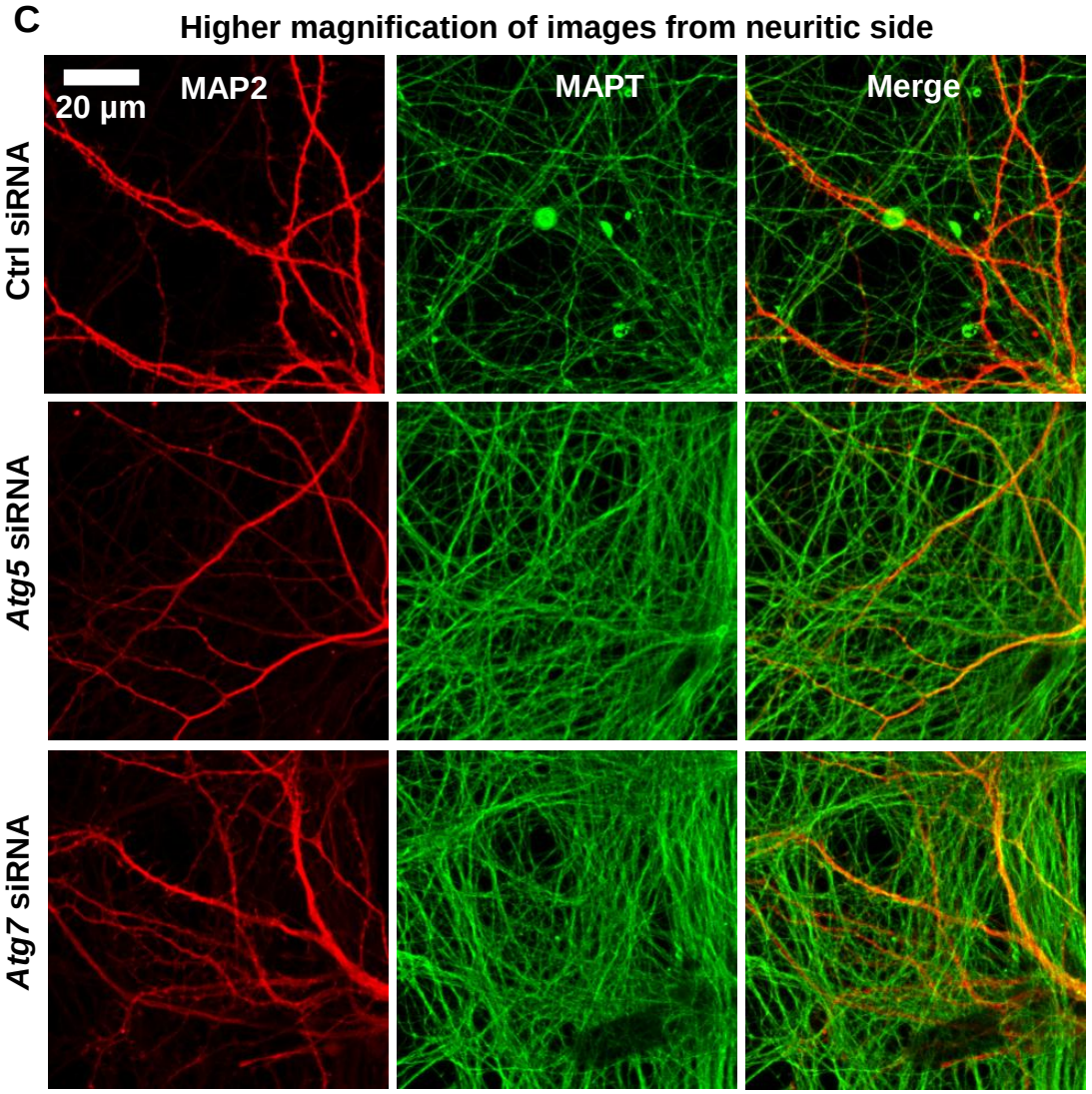
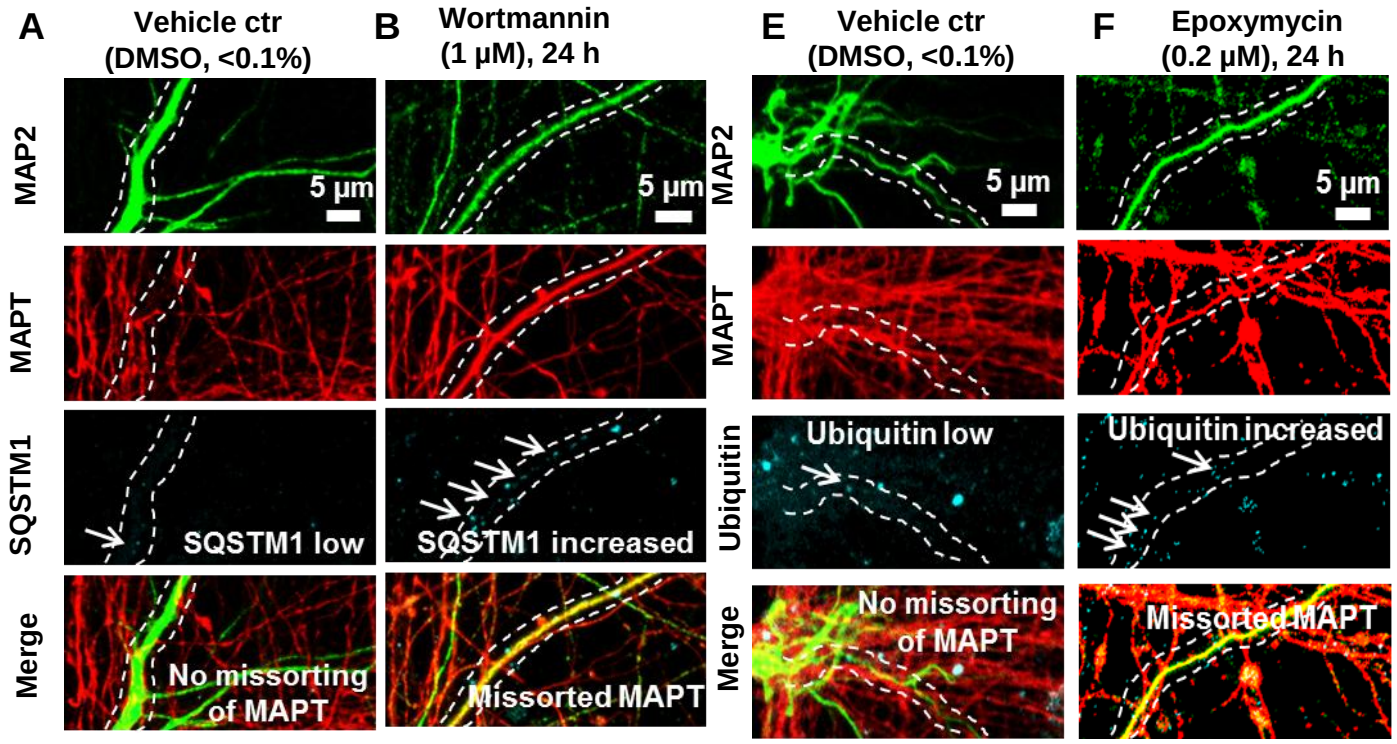
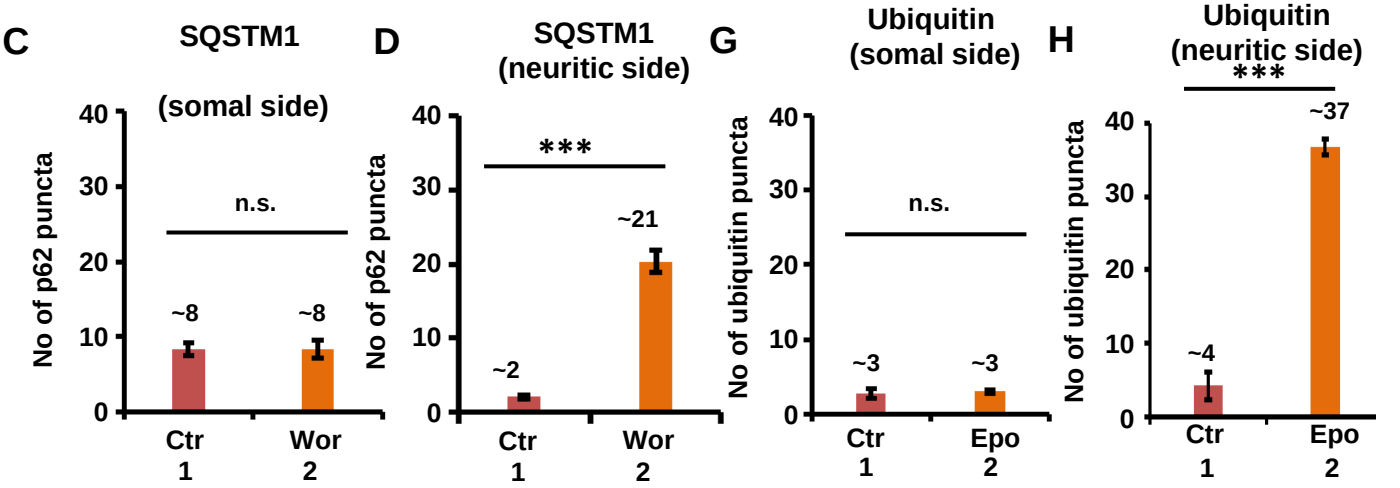

C
Higher magnification of images from neuritic side
20 μm
MAP2
MAPT
Merge
Ctrl siRNA
Atg5 siRNA
Atg7 siRNA

Fig. S4 A-J


A
Vehicle ctr
(DMSO, <0.1%)
B
Wortmannin
(1 μM), 24 h
E
Vehicle ctr
(DMSO, <0.1%)
F
Epoxymycin
(0.2 μM), 24 h
MAP2
MAPT
SQSTM1
Ubiquitin
Merge
5 μm
SQSTM1 low
SQSTM1 increased
Ubiquitin low
Ubiquitin increased
No missorting
of MAPT
Missorted MAPT


C
SQSTM1
(somal side)
D
SQSTM1
(neuritic side)
G
Ubiquitin
(somal side)
H
Ubiquitin
(neuritic side)
No of p62 puncta
No of ubiquitin puncta
n.s.
***
~8
~8
~2
~21
~3
~3
~4
~37
Ctr
1
Wor
2
Epo
2

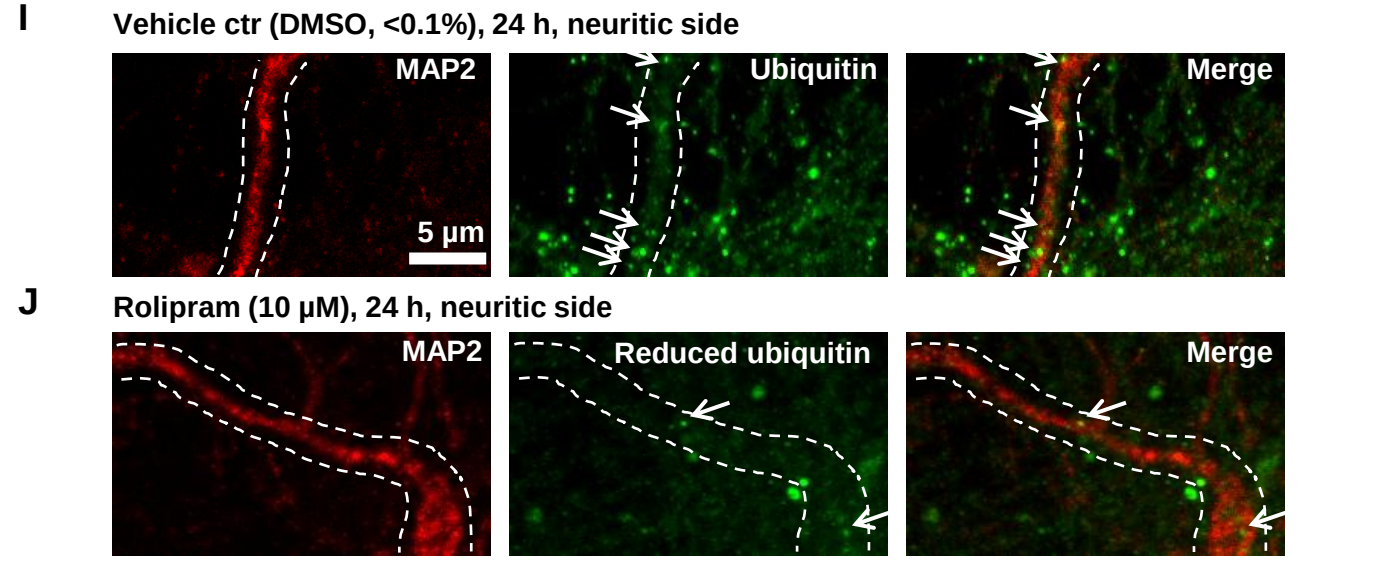

I
Vehicle ctr (DMSO, <0.1%), 24 h, neuritic side
MAP2
Ubiquitin
Merge
5 μm
J
Rolipram (10 μM), 24 h, neuritic side
MAP2
Reduced ubiquitin
Merge

Fig. S5 A-F

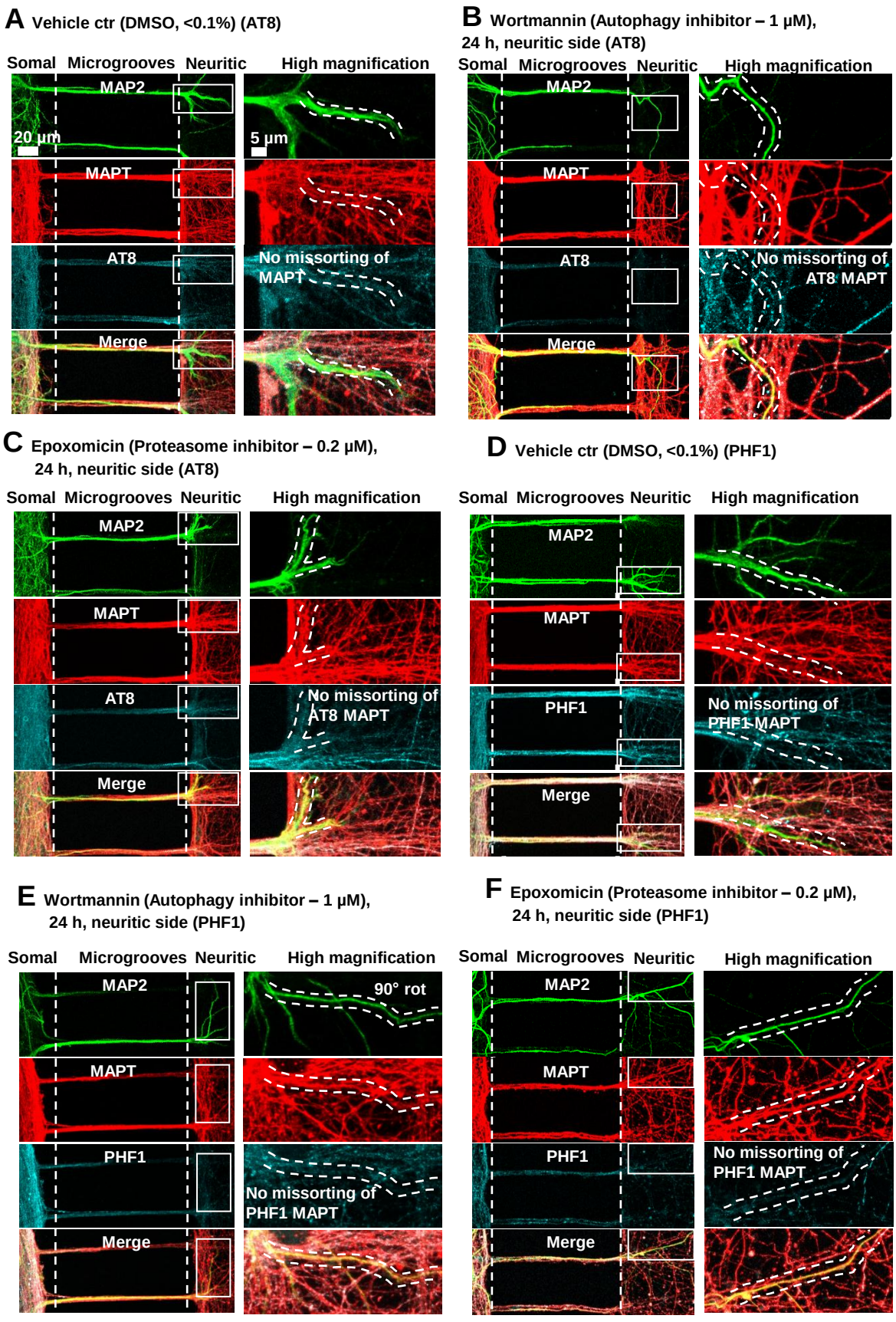
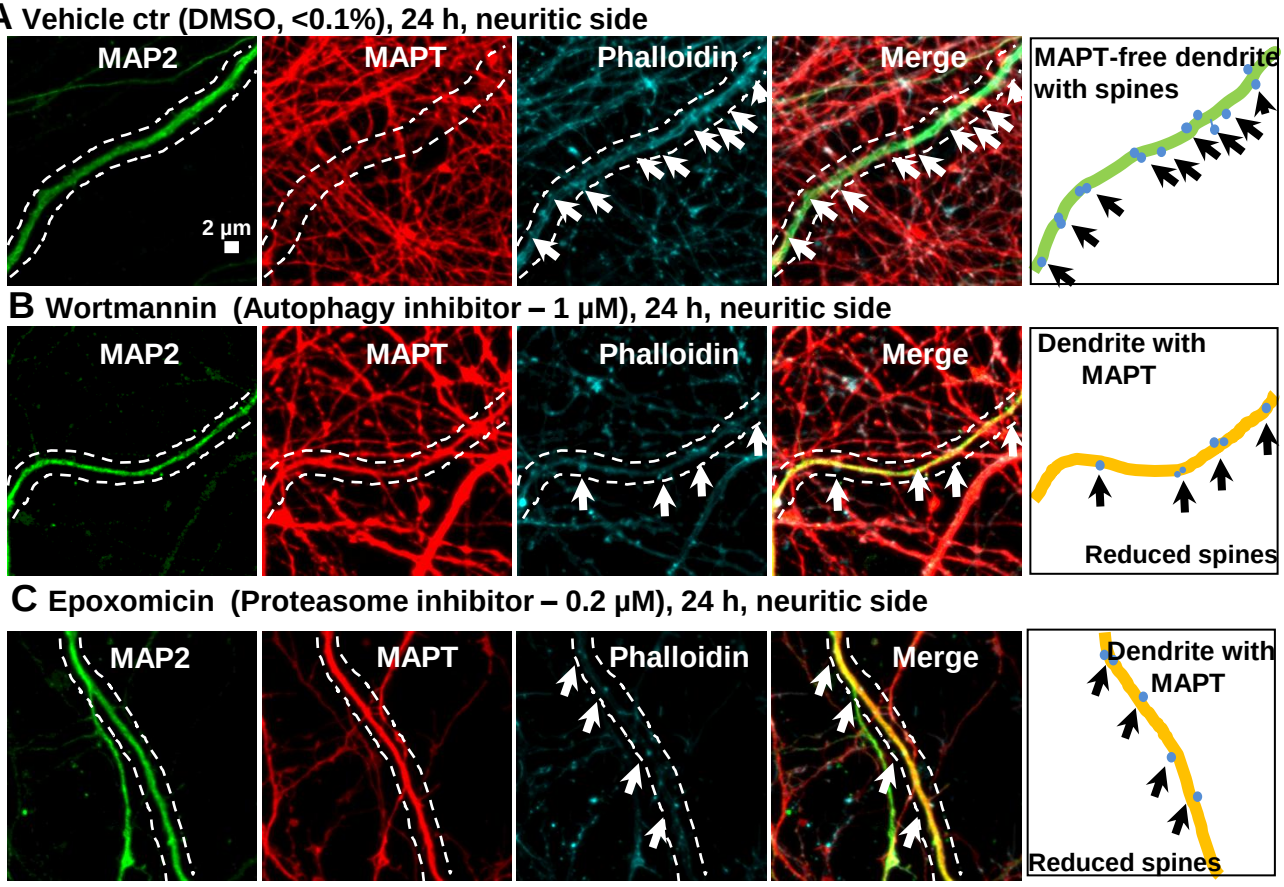
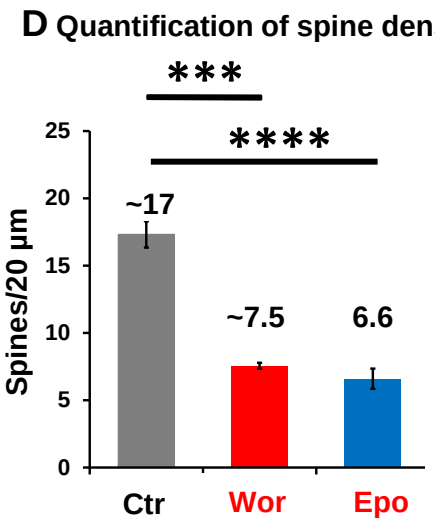
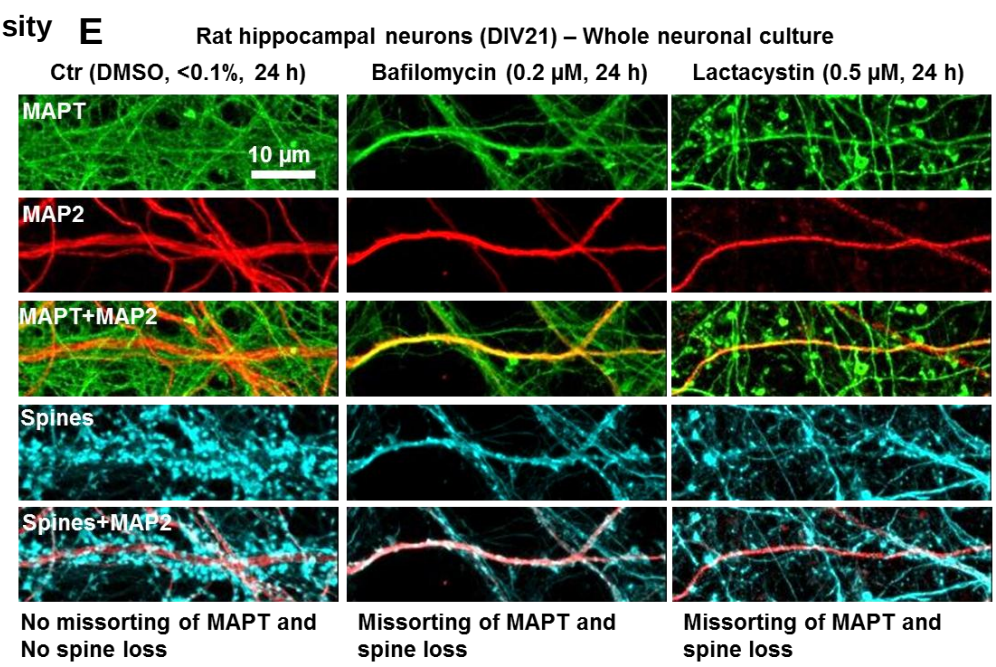

A Vehicle ctr (DMSO, <0.1%) (AT8)
Somal
Microgrooves
Neuritic
High magnification
MAP2
MAPT
AT8
Merge
20 µm
5 µm
No missorting of MAPT
B Wortmannin (Autophagy inhibitor – 1 µM), 24 h, neuritic side (AT8)
No missorting of AT8 MAPT
C Epoxomicin (Proteasome inhibitor – 0.2 µM), 24 h, neuritic side (AT8)
No missorting of AT8 MAPT
D Vehicle ctr (DMSO, <0.1%) (PHF1)
PHF1
No missorting of PHF1 MAPT
E Wortmannin (Autophagy inhibitor – 1 µM), 24 h, neuritic side (PHF1)
90° rot
No missorting of PHF1 MAPT
F Epoxomicin (Proteasome inhibitor – 0.2 µM), 24 h, neuritic side (PHF1)
No missorting of PHF1 MAPT

Fig. S6 A-F


A Vehicle ctr (DMSO, <0.1%), 24 h, neuritic side
MAP2
MAPT
Phalloidin
Merge
2 µm
MAPT-free dendrite with spines
B Wortmannin (Autophagy inhibitor – 1 µM), 24 h, neuritic side
MAP2
MAPT
Phalloidin
Merge
Dendrite with MAPT
Reduced spines
C Epoxomicin (Proteasome inhibitor – 0.2 µM), 24 h, neuritic side
MAP2
MAPT
Phalloidin
Merge
Dendrite with MAPT
Reduced spines


D Quantification of spine density
***
****
Spines/20 µm
25
20
15
10
5
0
~17
~7.5
6.6
Ctr
Wor
Epo


E
Rat hippocampal neurons (DIV21) – Whole neuronal culture
Ctr (DMSO, <0.1%, 24 h)
Bafilomycin (0.2 µM, 24 h)
Lactacystin (0.5 µM, 24 h)
MAPT
10 µm
MAP2
MAPT+MAP2
Spines
Spines+MAP2
No missorting of MAPT and No spine loss
Missorting of MAPT and spine loss
Missorting of MAPT and spine loss

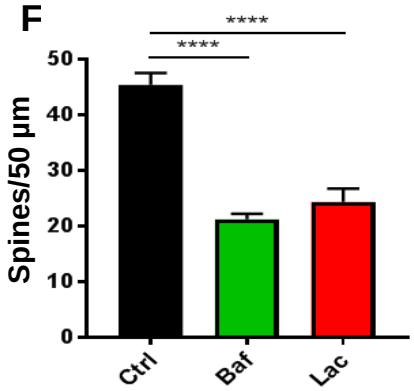
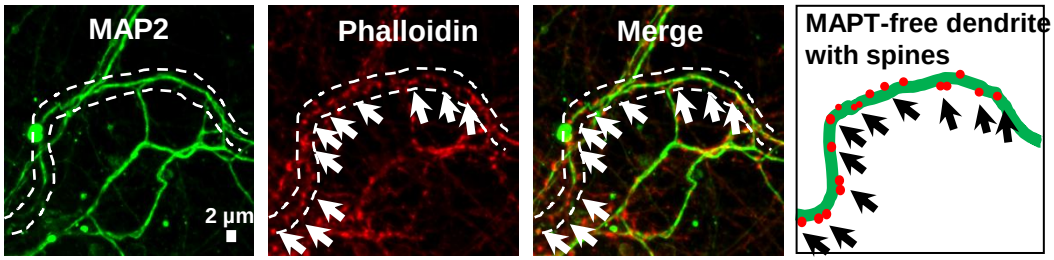

F
****
****
Spines/50 µm
50
40
30
20
10
0
Ctrl
Baf
Lac

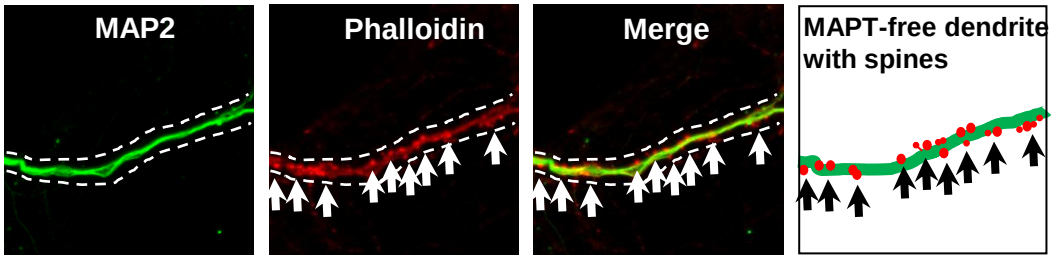
Fig. S7 A-D

MAPT knock-out neurons

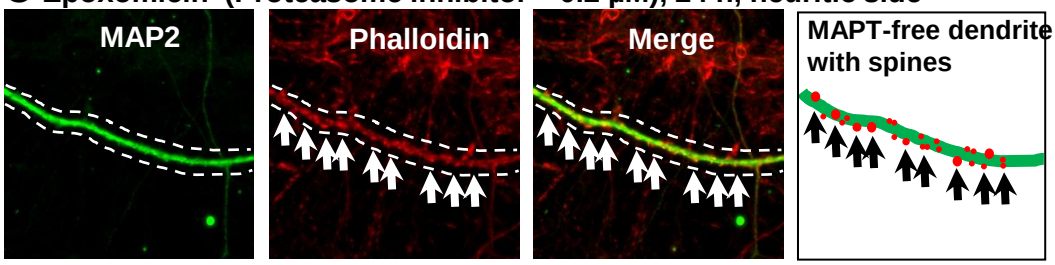
A Vehicle ctr (DMSO, <0.1%), 24 h, neuritic side



B Wortmannin (Autophagy inhibitor – 1 µM), 24 h, neuritic side



C Epoxomicin (Proteasome inhibitor – 0.2 µM), 24 h, neuritic side



D Quantification of spine density

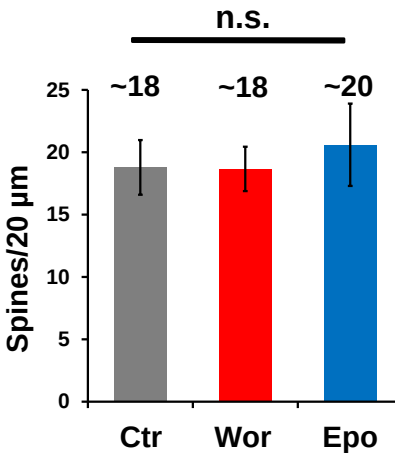
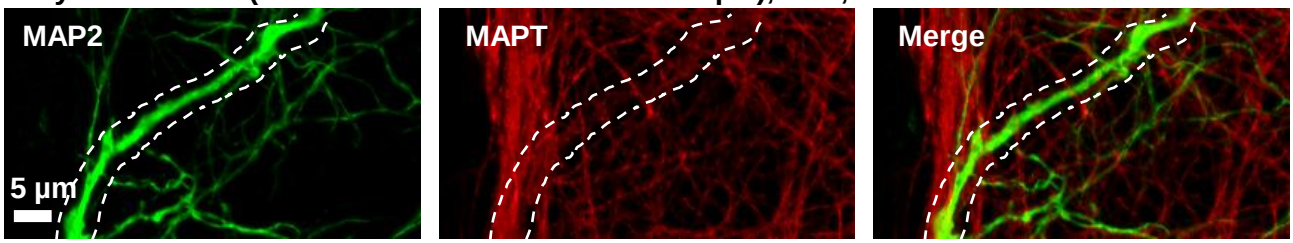


Fig. S8 A and B

A Cycloheximide (Protein translation inhibitor – 10 µM), 24 h, neuritic side



B Anisomycin (Protein translation inhibitor – 10 µM), 24 h, neuritic side

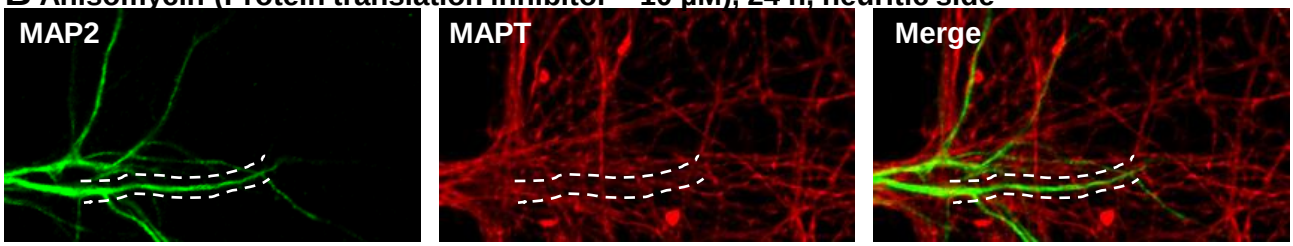


Fig. S9 A and B

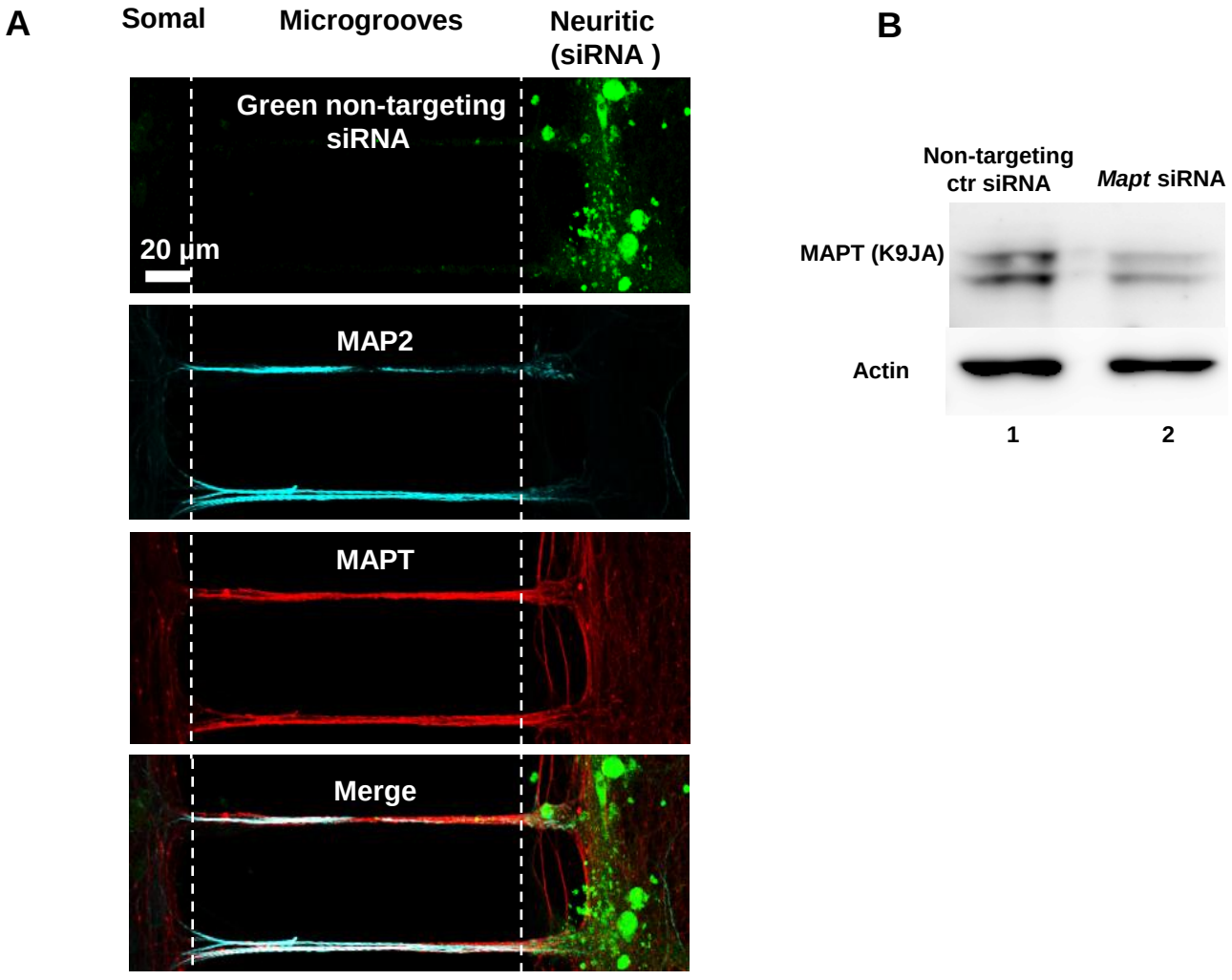
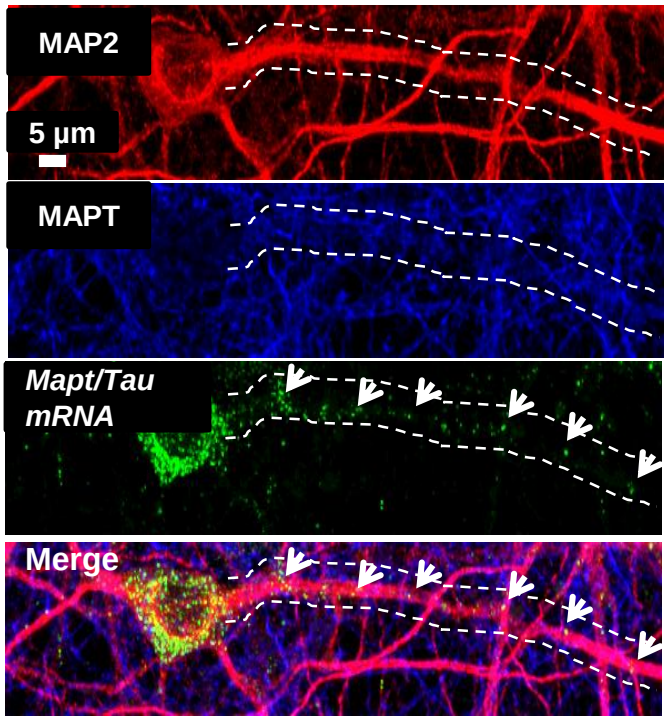

A
Somal
Microgrooves
Neuritic (siRNA)
Green non-targeting siRNA
20 µm
MAP2
MAPT
Merge
B
Non-targeting ctr siRNA
Mapt siRNA
MAPT (K9JA)
Actin
1
2

Fig. S10 A and B

A



B