Fig 1-S1

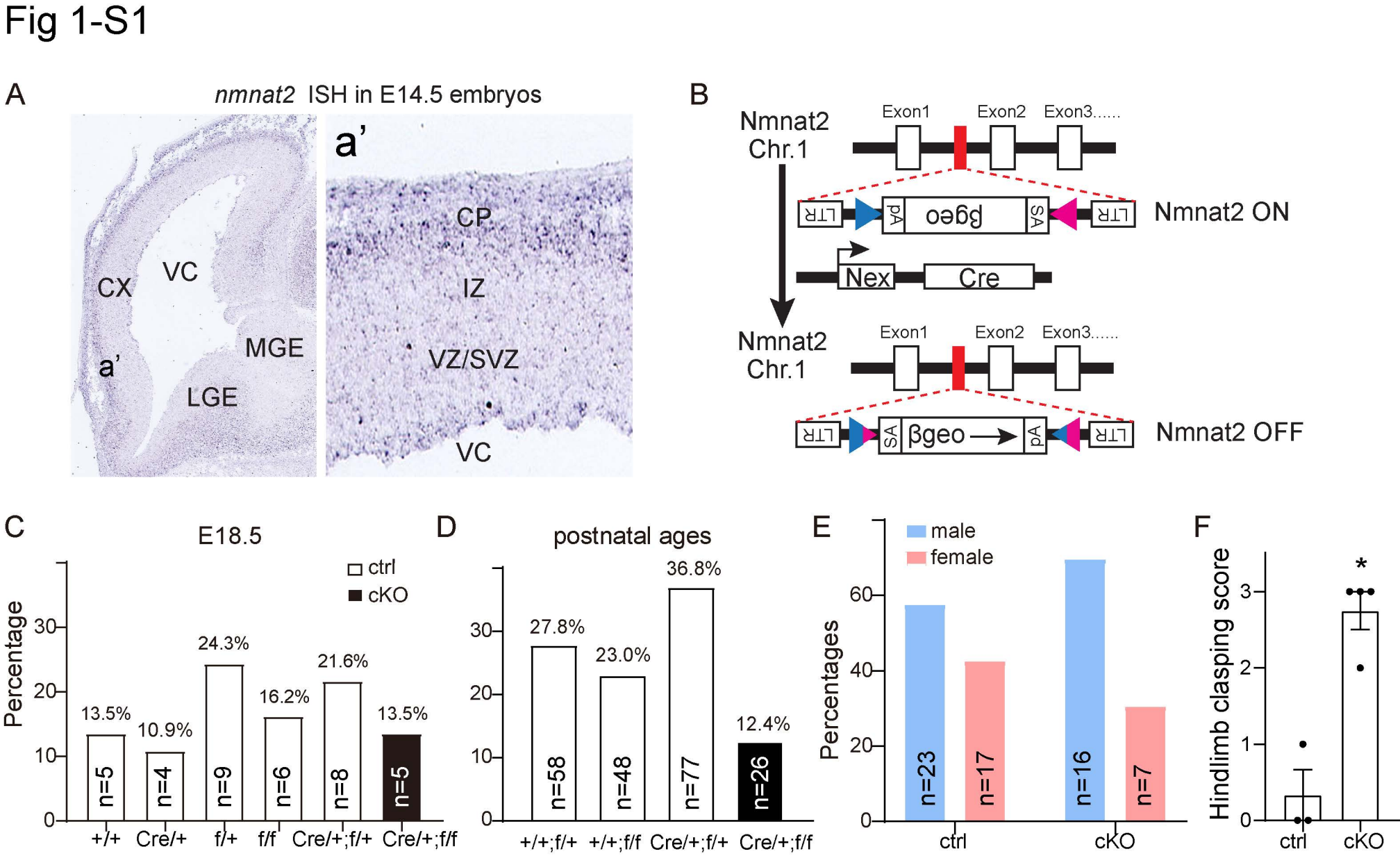

A
nmnat2 ISH in E14.5 embryos
CX
VC
MGE
LGE
a'
a'
CP
IZ
VZ/SVZ
VC
B
Nmnat2
Chr.1
Exon1
Exon2
Exon3.....
LTR
pA
βgeo
SA
LTR
Nmnat2 ON
Nex
Cre
Nmnat2
Chr.1
Exon1
Exon2
Exon3.....
LTR
SA
βgeo
pA
LTR
Nmnat2 OFF
C
E18.5
ctrl
cKO
Percentage
13.5%
10.9%
24.3%
16.2%
21.6%
13.5%
n=5
n=4
n=9
n=6
n=8
n=5
+/+
Cre/+
f/+
f/f
Cre/+;f/+
Cre/+;f/f
D
postnatal ages
27.8%
23.0%
36.8%
12.4%
n=58
n=48
n=77
n=26
+/+;f/+
+/+;f/f
Cre/+;f/+
Cre/+;f/f
E
male
female
Percentages
n=23
n=17
n=16
n=7
ctrl
cKO
F
Hindlimb clasping score
*
ctrl
cKO

Fig. 2-S1

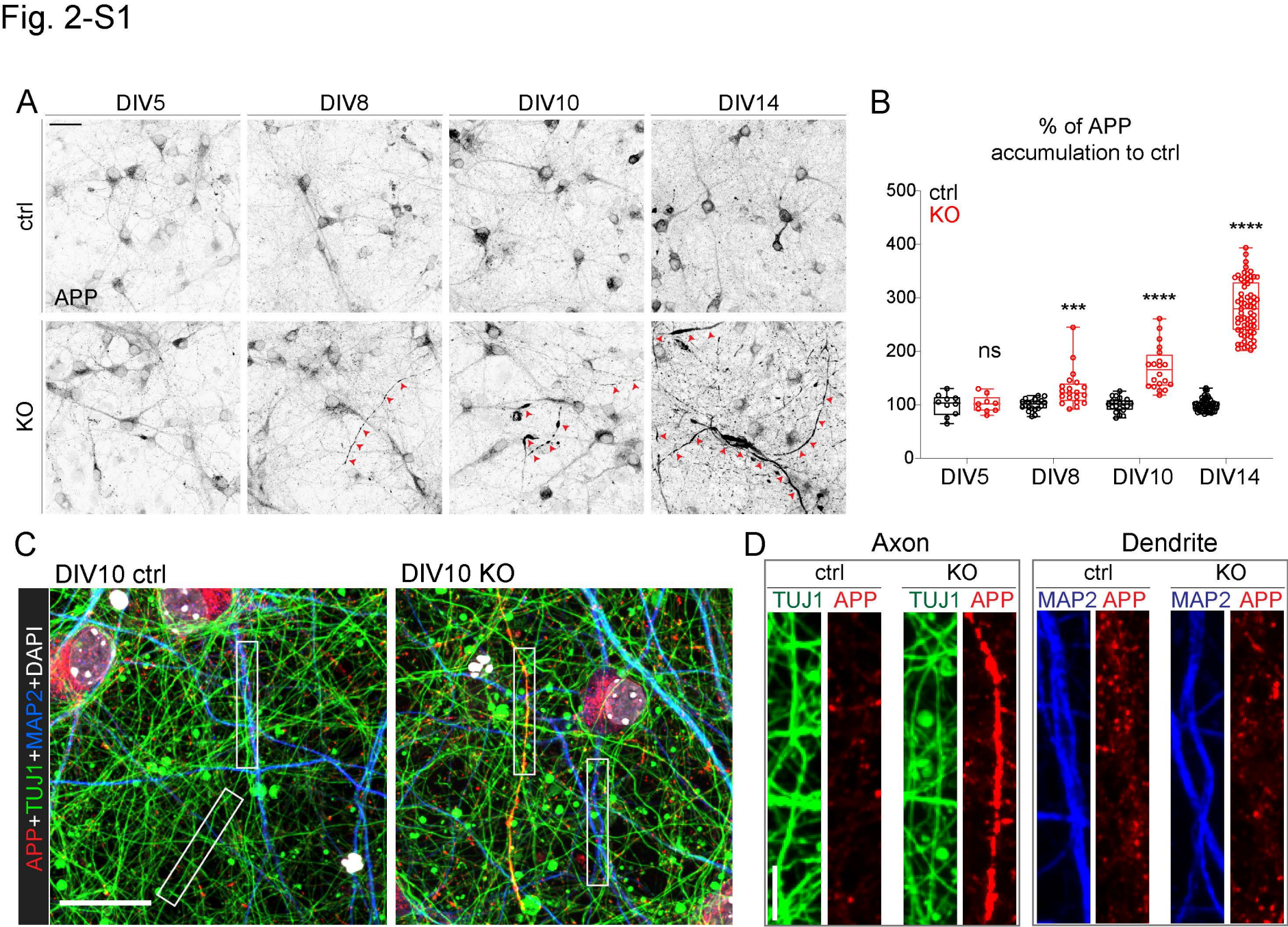

A
DIV5
DIV8
DIV10
DIV14
ctrl
KO
APP
B
% of APP
accumulation to ctrl
ctrl
KO
500
400
300
200
100
0
ns
***
****
****
DIV5
DIV8
DIV10
DIV14
C
DIV10 ctrl
DIV10 KO
APP+TUJ1+MAP2+DAPI
D
Axon
Dendrite
ctrl
KO
TUJ1 APP
TUJ1 APP
MAP2 APP
MAP2 APP

Fig. 2-S2

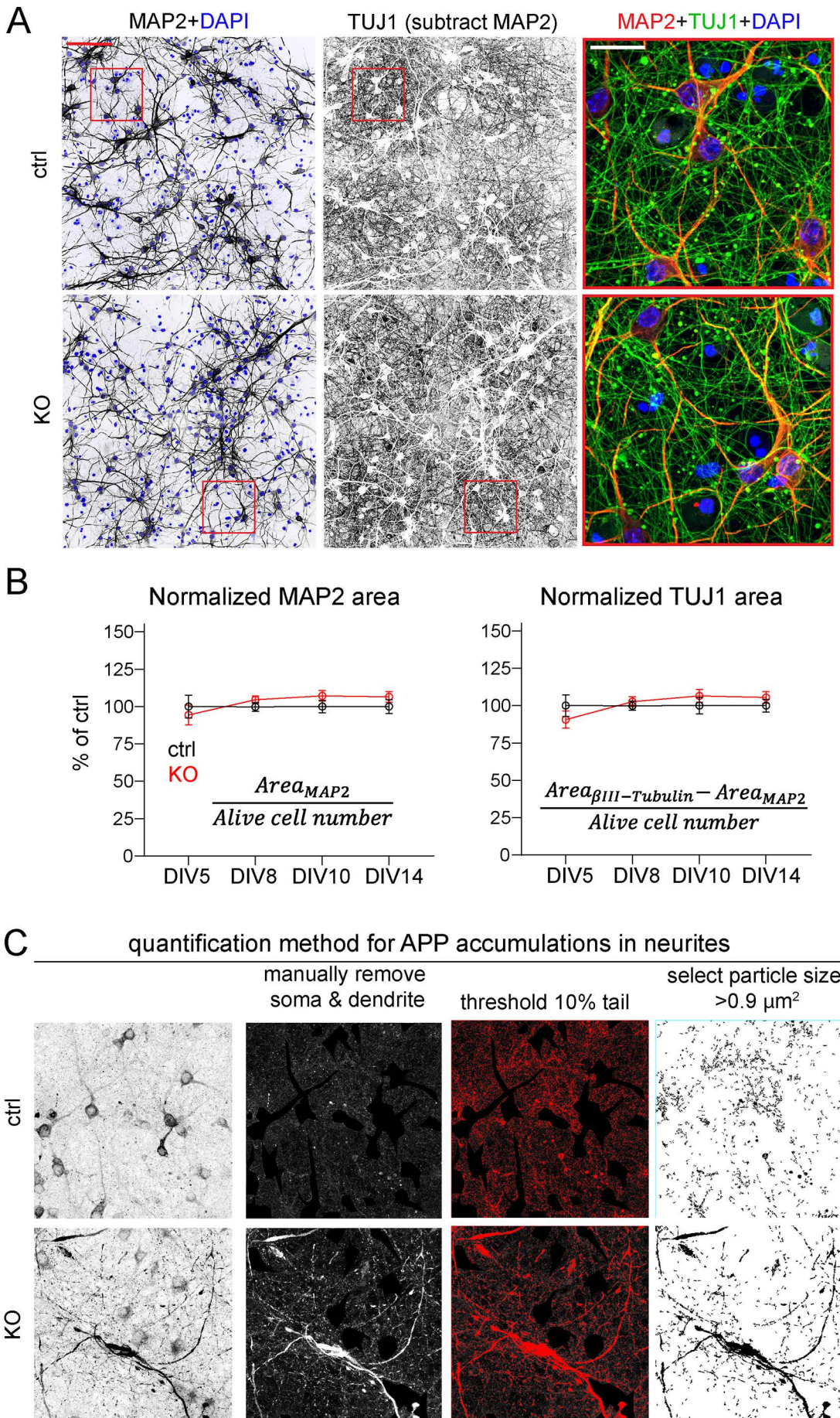

A
MAP2+DAPI
TUJ1 (subtract MAP2)
MAP2+TUJ1+DAPI
ctrl
KO
B
Normalized MAP2 area
Normalized TUJ1 area
% of ctrl
150
125
100
75
50
25
0
ctrl
KO
$\frac{Area_{MAP2}}{Alive\ cell\ number}$
$\frac{Area_{\beta III-Tubulin} - Area_{MAP2}}{Alive\ cell\ number}$
DIV5
DIV8
DIV10
DIV14
C
quantification method for APP accumulations in neurites
manually remove soma & dendrite
threshold 10% tail
select particle size >0.9 μm²
ctrl
KO

Fig. 2-S3

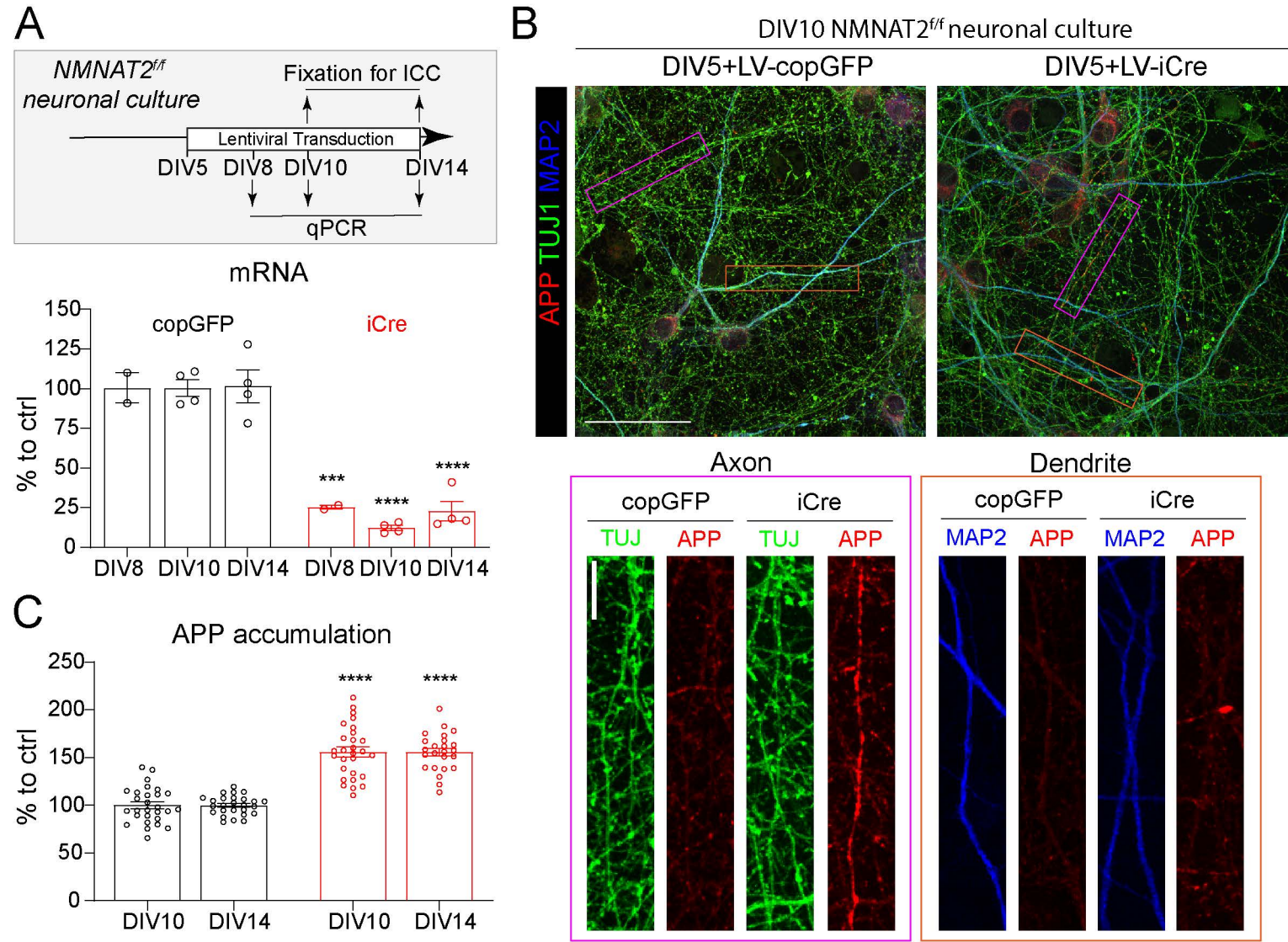

A
NMNAT2f/f neuronal culture
Fixation for ICC
Lentiviral Transduction
DIV5 DIV8 DIV10 DIV14
qPCR
mRNA
copGFP
iCre
% to ctrl
150
125
100
75
50
25
0
***
****
****
DIV8 DIV10 DIV14 DIV8 DIV10 DIV14
B
DIV10 NMNAT2f/f neuronal culture
DIV5+LV-copGFP
DIV5+LV-iCre
APP TUJ1 MAP2
Axon
Dendrite
copGFP
iCre
TUJ
APP
MAP2
C
APP accumulation
% to ctrl
250
200
150
100
50
0
****
****
DIV10 DIV14 DIV10 DIV14

Fig 3-S1

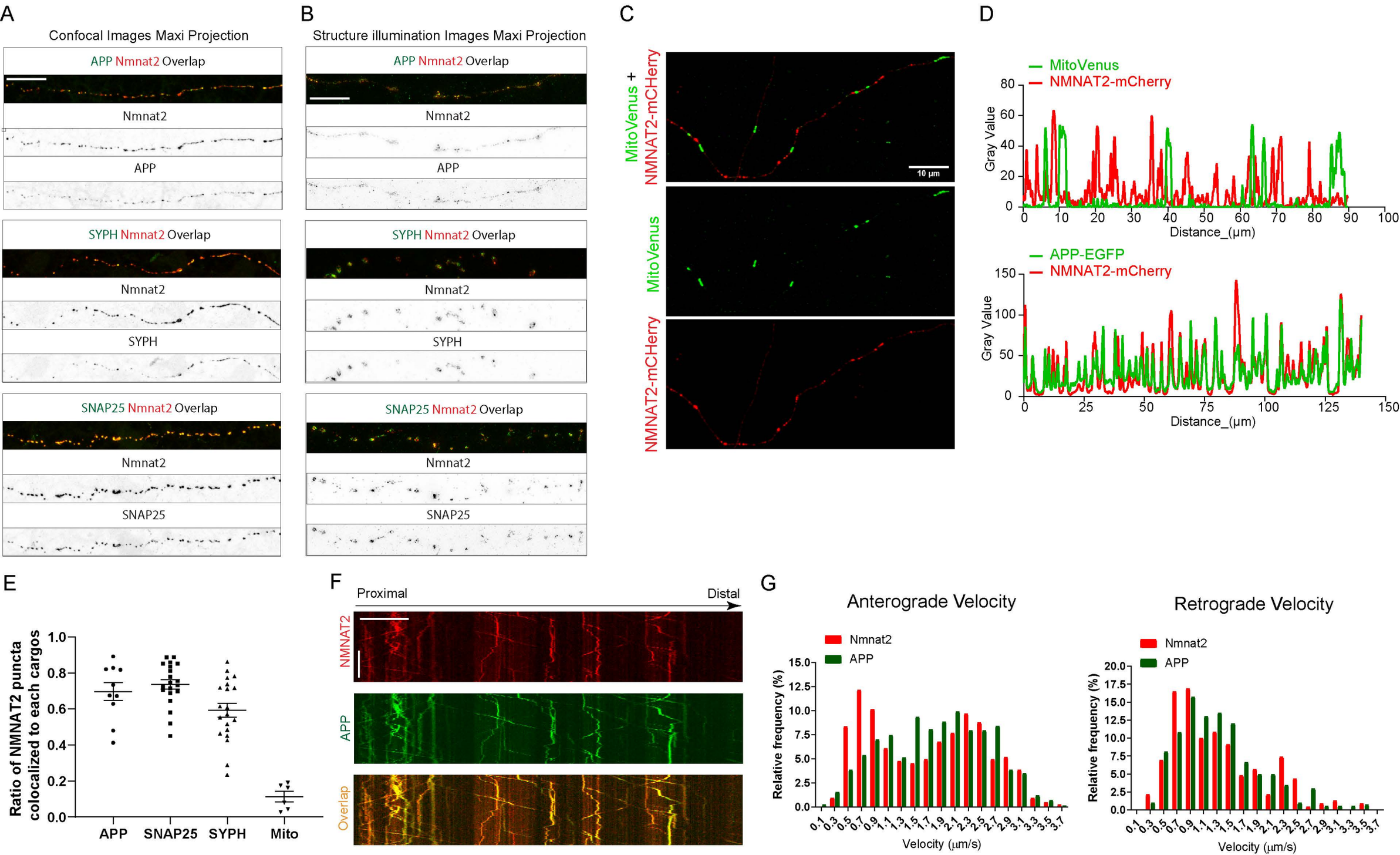

A
Confocal Images Maxi Projection
APP Nmnat2 Overlap
Nmnat2
APP
SYPH Nmnat2 Overlap
Nmnat2
SYPH
SNAP25 Nmnat2 Overlap
Nmnat2
SNAP25
B
Structure illumination Images Maxi Projection
APP Nmnat2 Overlap
Nmnat2
APP
SYPH Nmnat2 Overlap
Nmnat2
SYPH
SNAP25 Nmnat2 Overlap
Nmnat2
SNAP25
C
MitoVenus + NMNAT2-mCherry
MitoVenus
NMNAT2-mCherry
10 μm
D
MitoVenus
NMNAT2-mCherry
Gray Value
Distance_(μm)
APP-EGFP
NMNAT2-mCherry
Gray Value
Distance_(μm)
E
Ratio of NMNAT2 puncta colocalized to each cargos
APP
SNAP25
SYPH
Mito
F
Proximal
Distal
NMNAT2
APP
Overlap
G
Anterograde Velocity
Nmnat2
APP
Relative frequency (%)
Velocity (μm/s)
Retrograde Velocity
Nmnat2
APP
Relative frequency (%)
Velocity (μm/s)

Fig. 3-S2

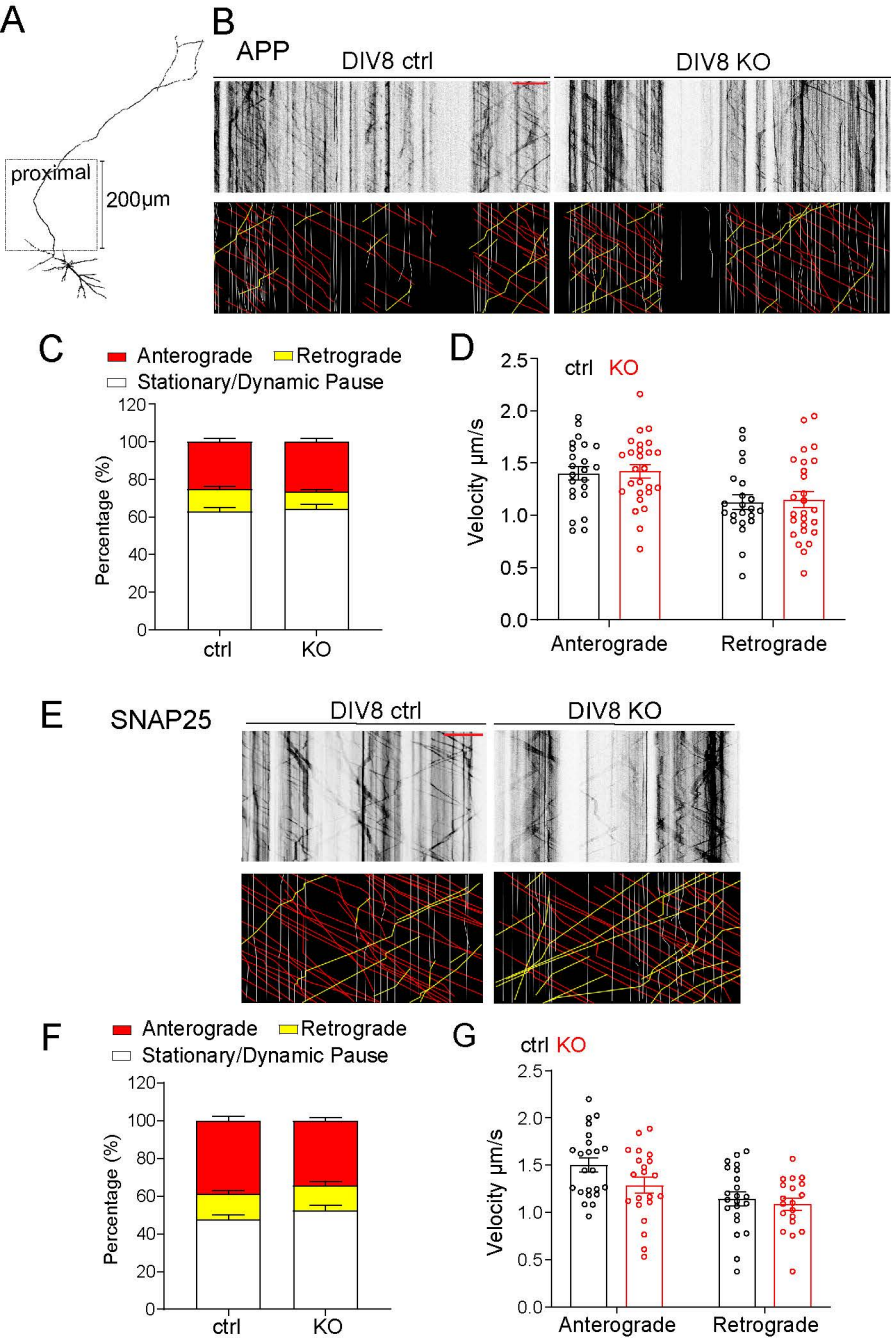

A
proximal
200μm
B
APP
DIV8 ctrl
DIV8 KO
C
Anterograde
Retrograde
Stationary/Dynamic Pause
Percentage (%)
ctrl
KO
D
ctrl KO
Velocity μm/s
Anterograde
Retrograde
E
SNAP25
DIV8 ctrl
DIV8 KO
F
Anterograde
Retrograde
Stationary/Dynamic Pause
Percentage (%)
ctrl
KO
G
ctrl KO
Velocity μm/s
Anterograde
Retrograde

Fig 3-S3

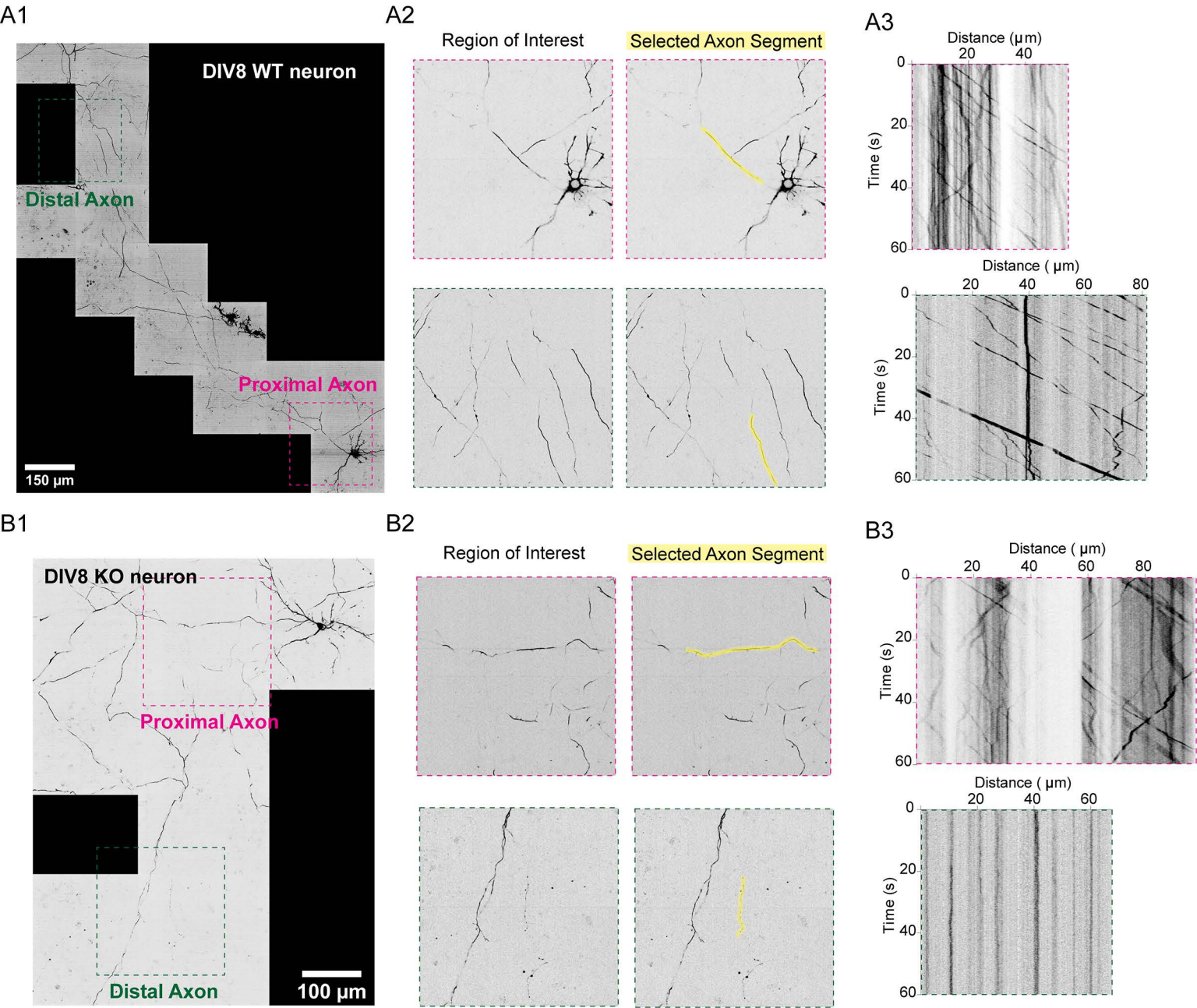

A1
DIV8 WT neuron
Distal Axon
Proximal Axon
150 µm
A2
Region of Interest
Selected Axon Segment
A3
Distance (µm)
20
40
0
20
40
60
Time (s)
Distance (µm)
20
40
60
80
0
20
40
60
Time (s)
B1
DIV8 KO neuron
Proximal Axon
Distal Axon
100 µm
B2
Region of Interest
Selected Axon Segment
B3
Distance (µm)
20
40
60
80
0
20
40
60
Time (s)
Distance (µm)
20
40
60
0
20
40
60
Time (s)

Fig 3-S4

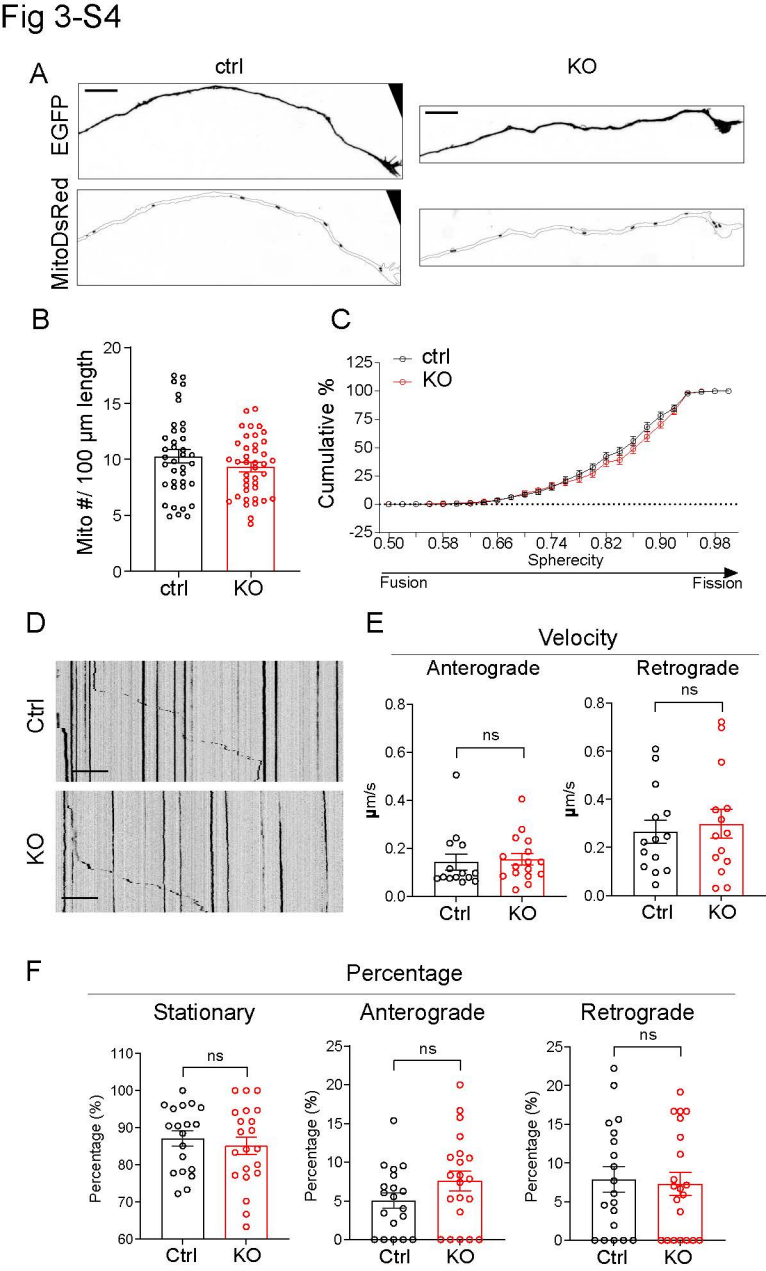

A
ctrl
KO
EGFP
MitoDsRed
B
Mito #/ 100 µm length
ctrl
KO
C
ctrl
KO
Cumulative %
Spherecity
Fusion
Fission
D
Ctrl
KO
E
Velocity
Anterograde
Retrograde
µm/s
ns
Ctrl
KO
F
Percentage
Stationary
Anterograde
Retrograde
Percentage (%)
ns
Ctrl
KO

Fig 5-S1

A

CytopHluorin imaging paradigm

pH calculation

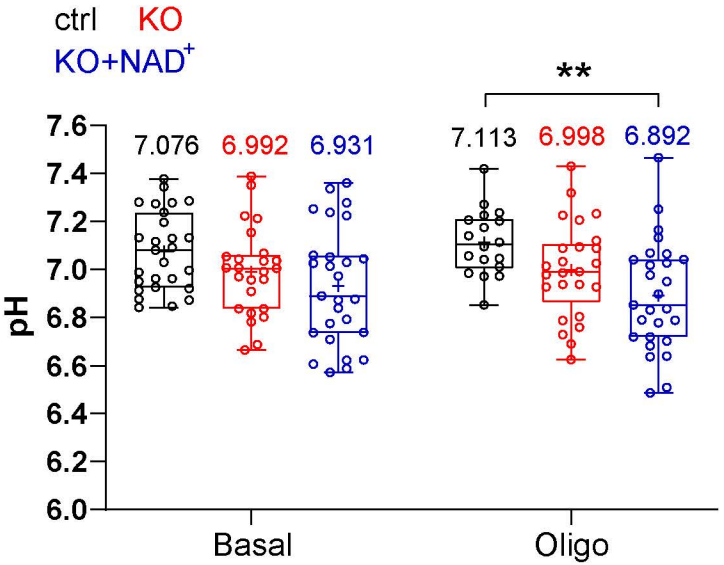
$$pH_{Basal} = \text{pKa} - \log[(\frac{1 + 10^{pKa-7.4}}{\frac{F_{Basal}}{F_{Max}}}) - 1]$$

$$pH_{Oligo} = \text{pKa} - \log[(\frac{1 + 10^{pKa-7.4}}{\frac{F_{Oligo}}{F_{Max}}}) - 1]$$

$pKa = 7.1\ for\ CytopHluorin$

B

pH measured by CytopHluorin



C

pH correction of L/F

$L_1 \sim Luminescence\ under\ pH_1$
$L_0 \sim Luminescence\ under\ pH_0$

$$pH_1 = \text{pKa} - \log[(\frac{1 + 10^{pKa-pH_0}}{\frac{L_1}{L_0}}) - 1]$$

$$L_0 = L_1 * (\frac{1 + 10^{pKa-pH_1}}{1 + 10^{pKa-pH_0}})$$

$pKa = 7.03\ for\ SynATP$

eg. Convert L in KO+NAD+ Oligo group (average pH=6.892) to ctrl Oligo condition (average pH=7.113)

$$L_{(pH=7.113)} = L_{(pH=6.892)} * (\frac{1 + 10^{7.03-6.892}}{1 + 10^{7.03-7.113}})$$

D

Raw SynATP L/F

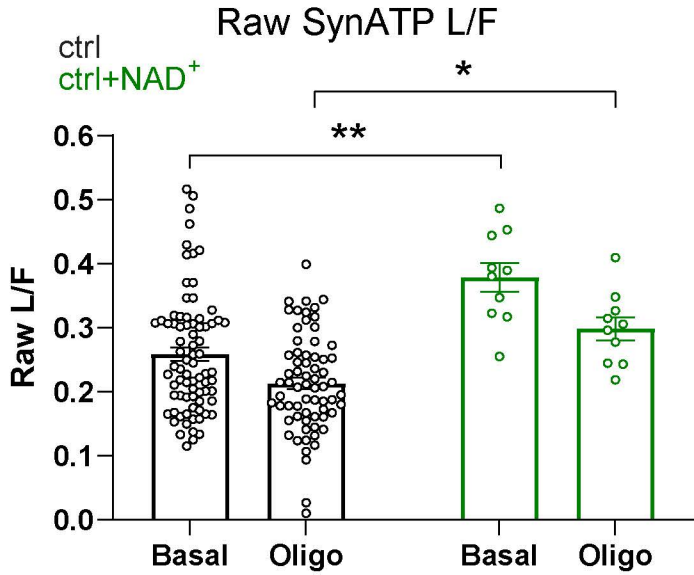


Fig 5-S2

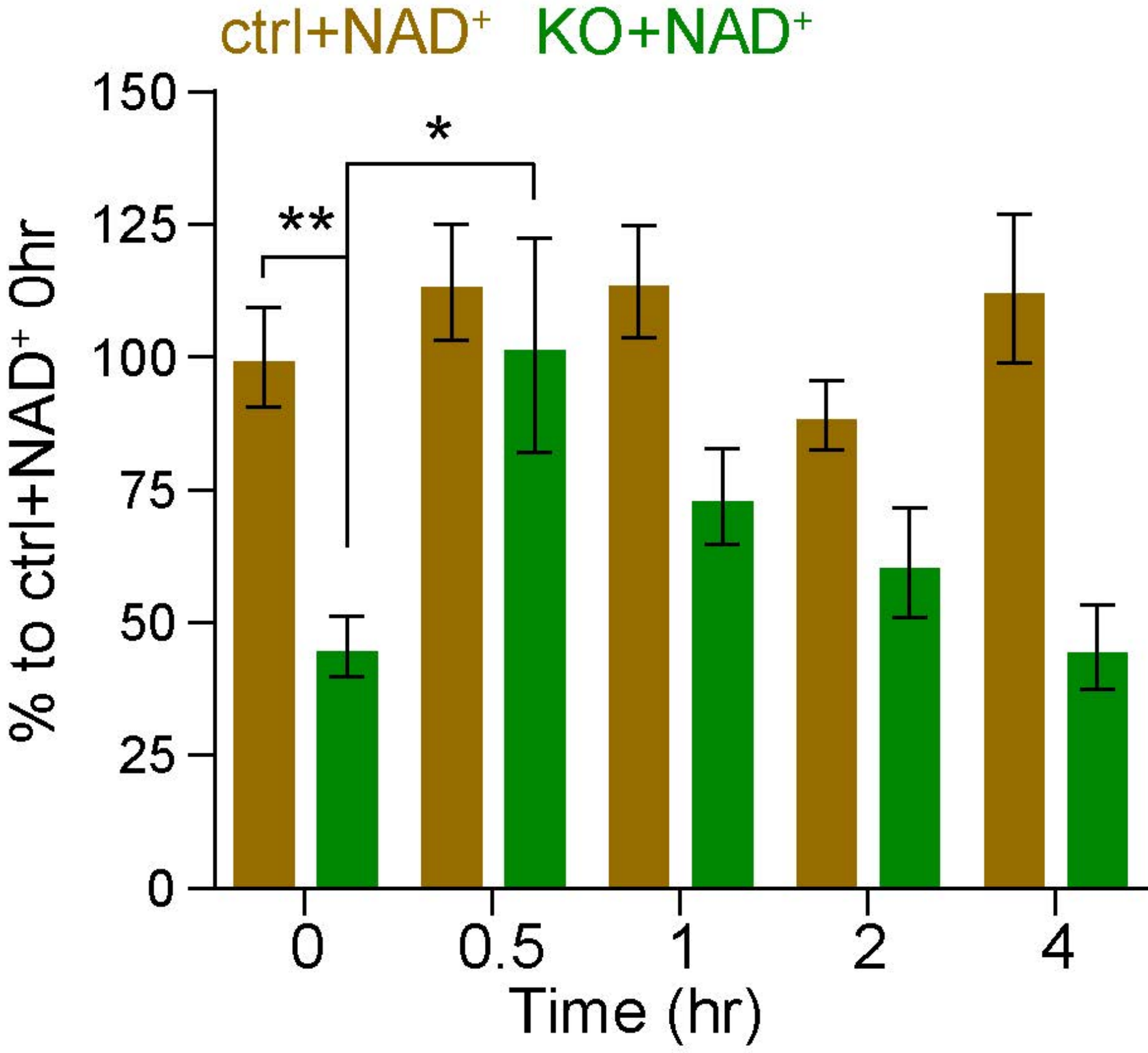
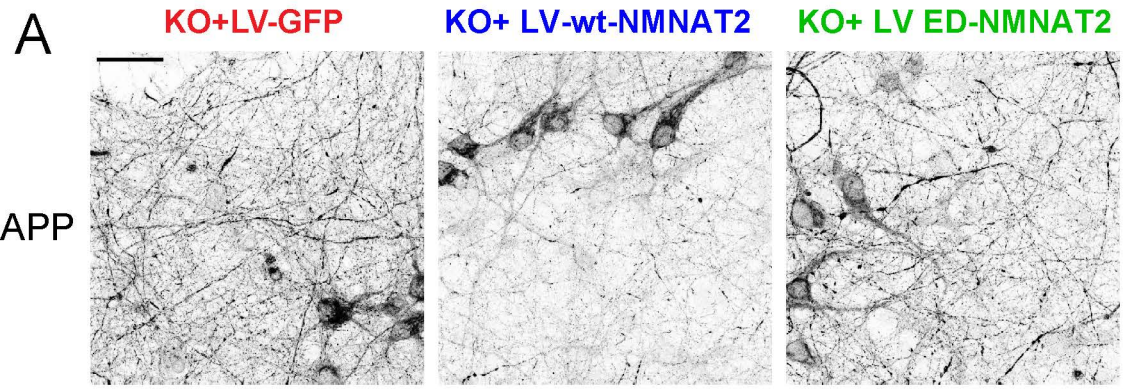
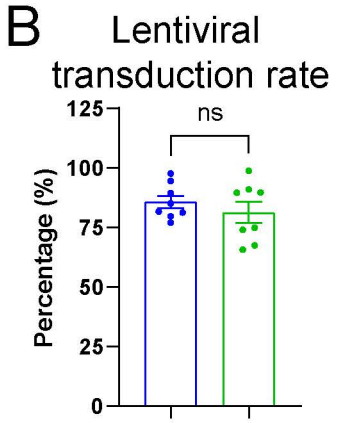

ctrl+NAD+
KO+NAD+
150
125
100
75
50
25
0
% to ctrl+NAD+ 0hr
**
*
0
0.5
1
2
4
Time (hr)

Fig 6-S1


A
KO+LV-GFP
KO+ LV-wt-NMNAT2
KO+ LV ED-NMNAT2
APP


B
Lentiviral transduction rate
ns
Percentage (%)
125
100
75
50
25
0

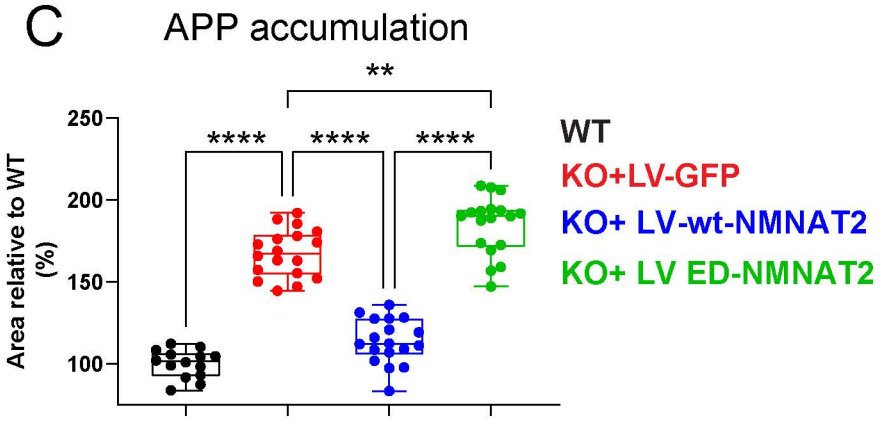
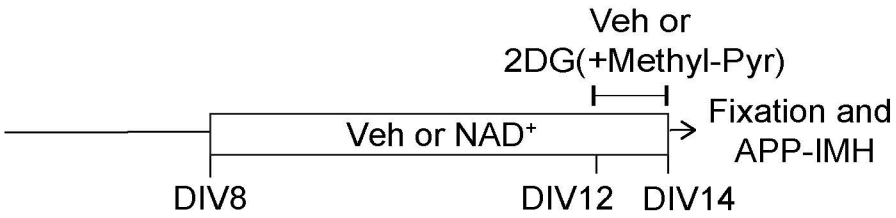
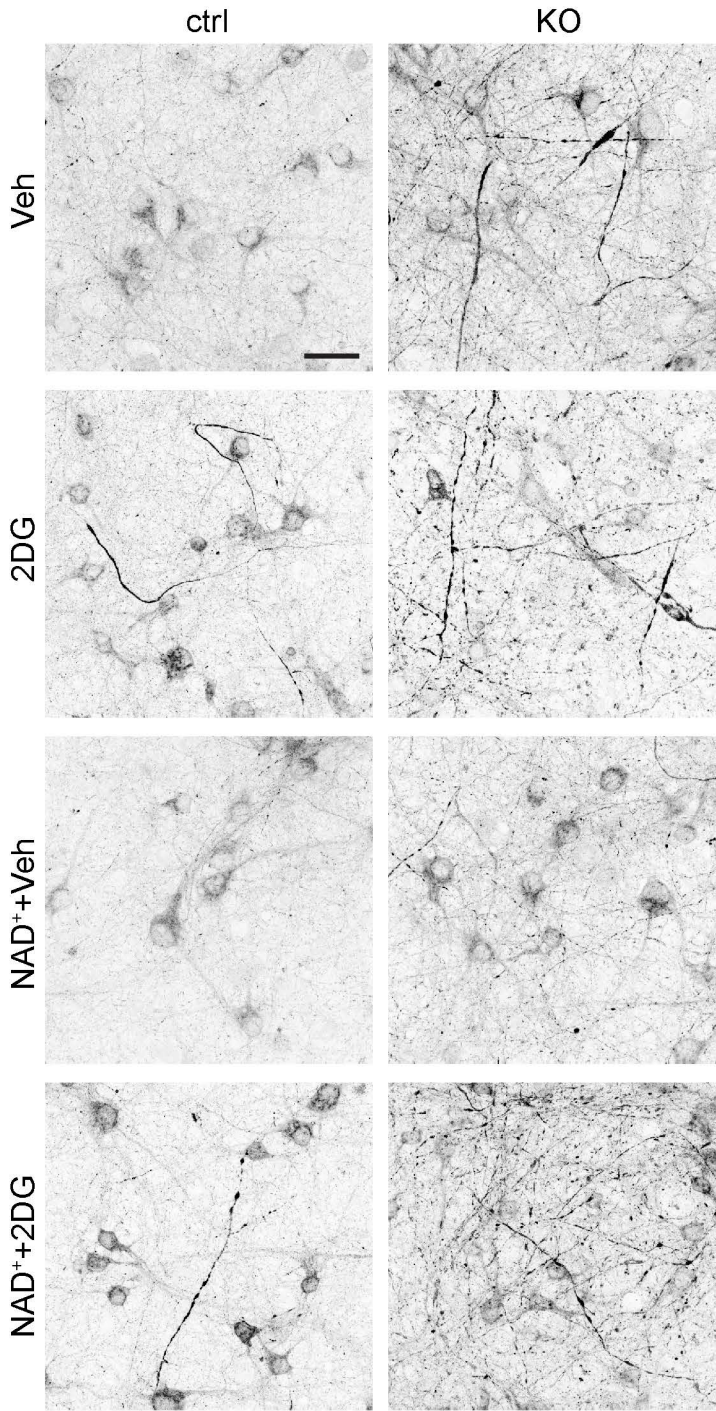

C
APP accumulation
**
****
****
****
Area relative to WT (%)
250
200
150
100
WT
KO+LV-GFP
KO+ LV-wt-NMNAT2
KO+ LV ED-NMNAT2

Fig 6-S2

A


Veh or
2DG(+Methyl-Pyr)
Veh or NAD+
Fixation and
APP-IMH
DIV8
DIV12
DIV14

B


ctrl
KO
Veh
2DG
NAD+Veh
NAD+2DG

C

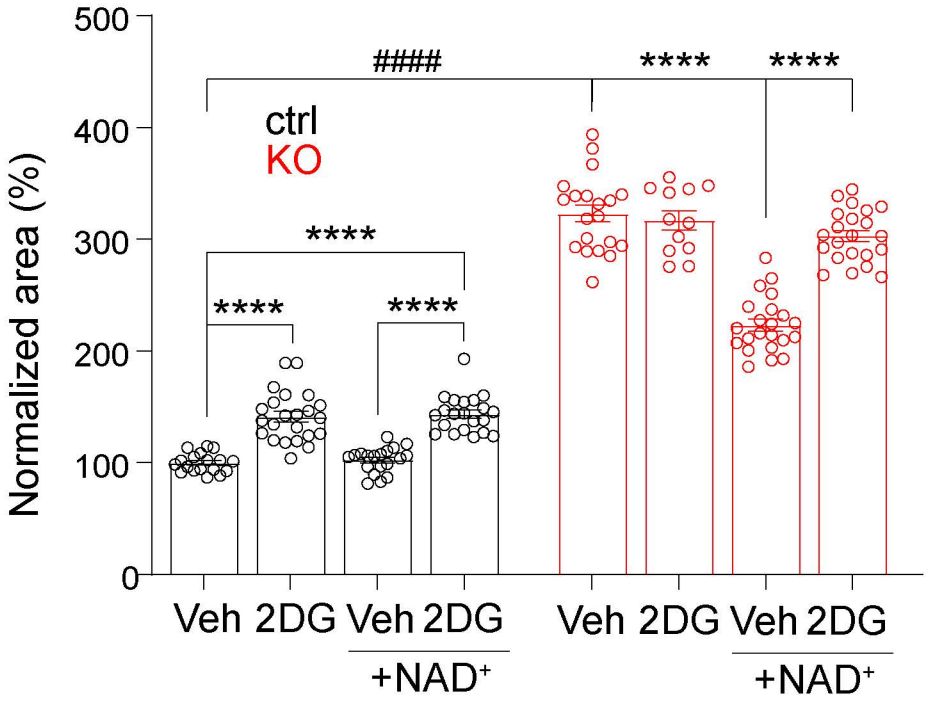
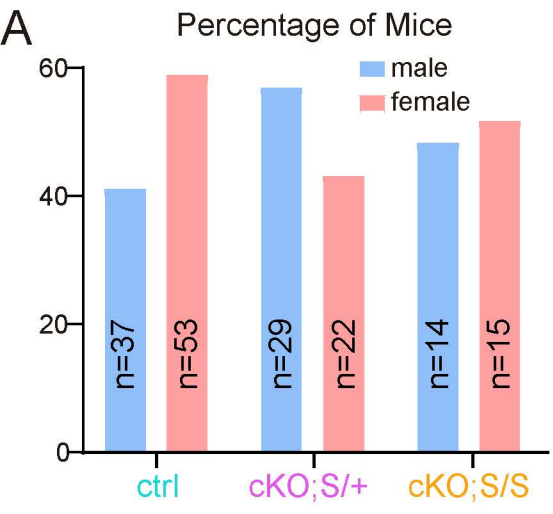
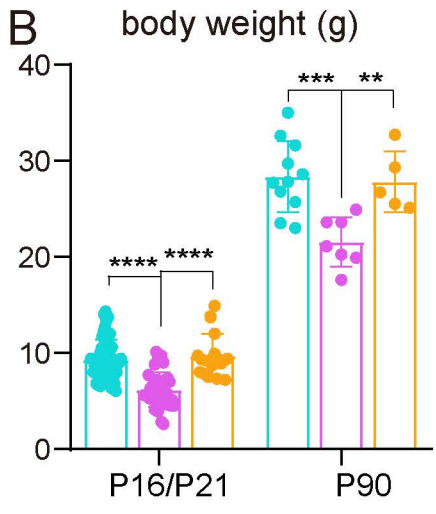

Normalized area (%)
500
400
300
200
100
0
ctrl
KO
####
****
****
****
****
Veh
2DG
Veh
2DG
+NAD+
Veh
2DG
Veh
2DG
+NAD+

Fig 7-S1


A
Percentage of Mice
male
female
60
40
20
0
n=37
n=53
n=29
n=22
n=14
n=15
ctrl
cKO;S/+
cKO;S/S


B
body weight (g)
40
30
20
10
0
****
****
***
**
P16/P21
P90

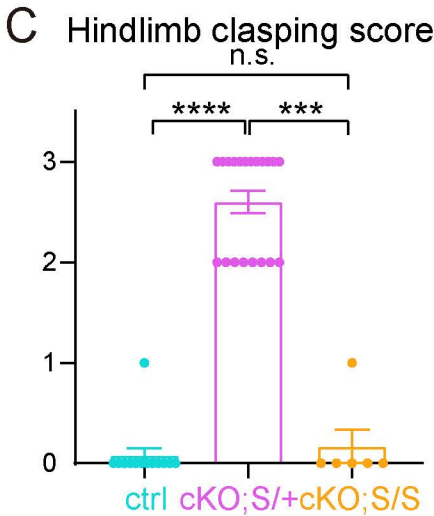
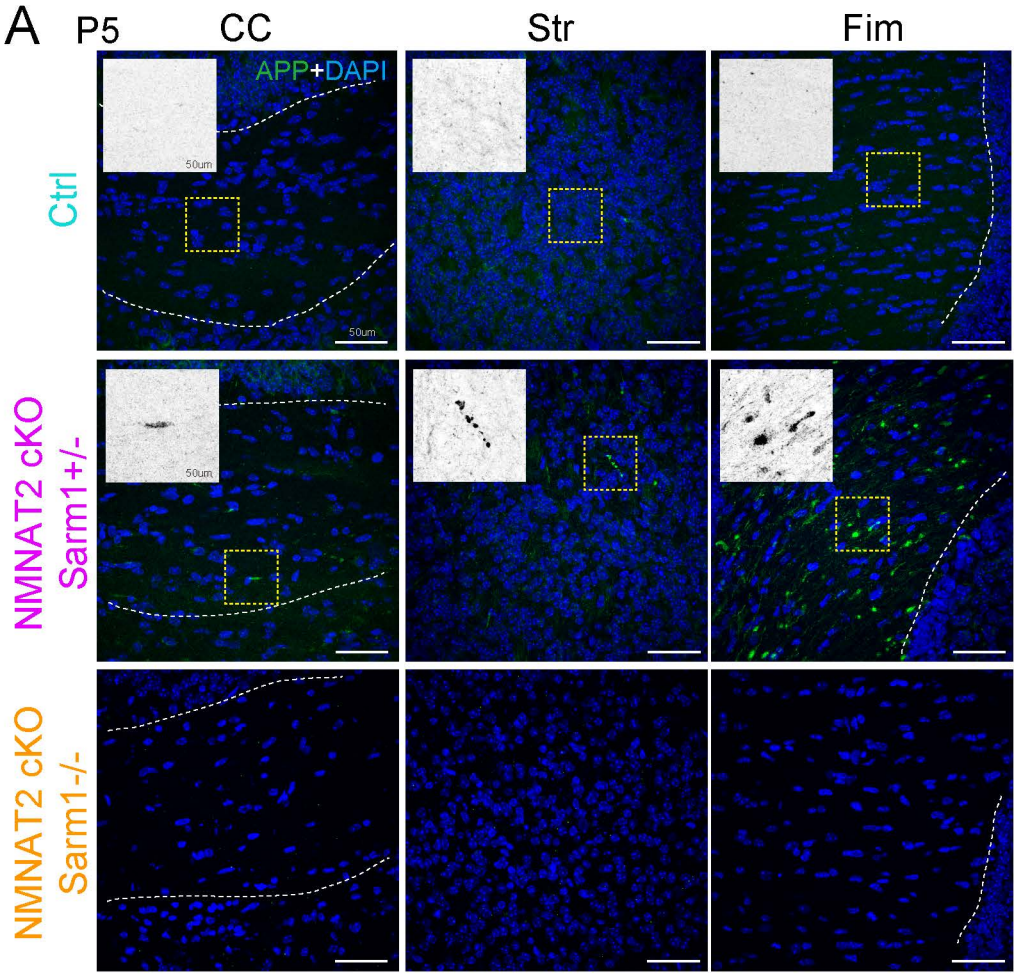

C
Hindlimb clasping score
n.s.
****
***
3
2
1
0
ctrl
cKO;S/+
cKO;S/S

Fig 7-S2


A
P5
CC
Str
Fim
APP+DAPI
Ctrl
NMNAT2 cKO
Sarm1+/-
NMNAT2 cKO
Sarm1-/-
50um

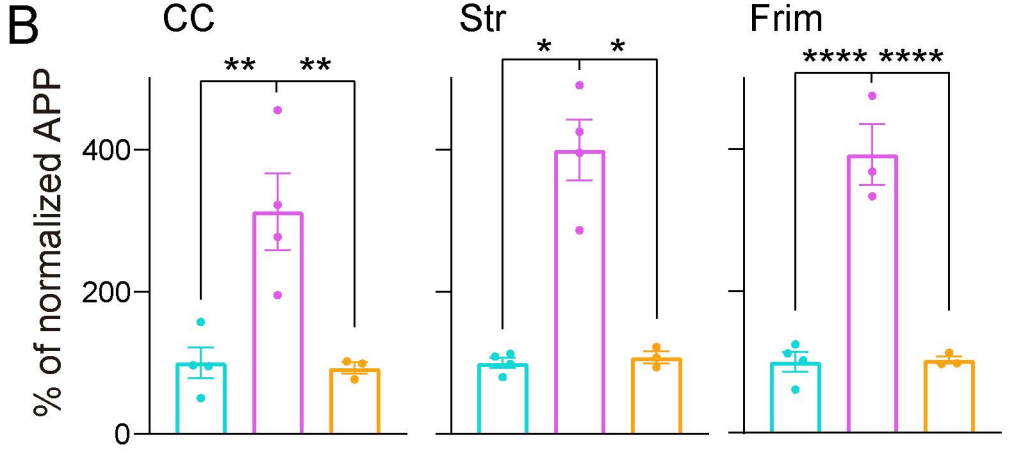
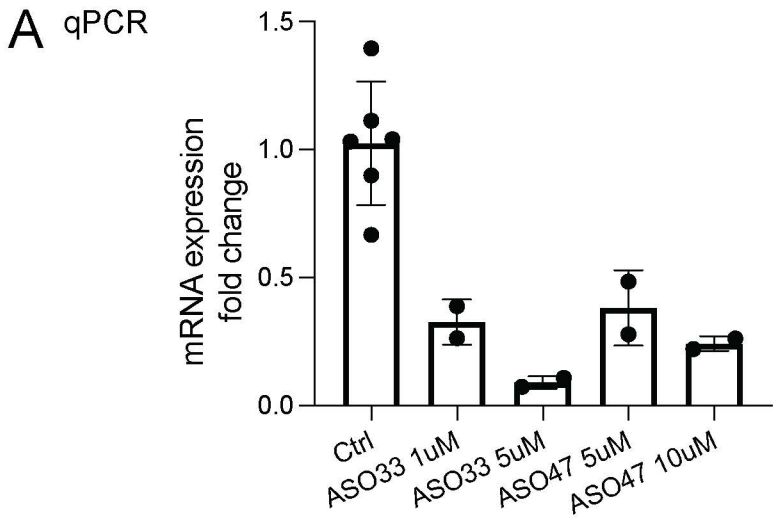
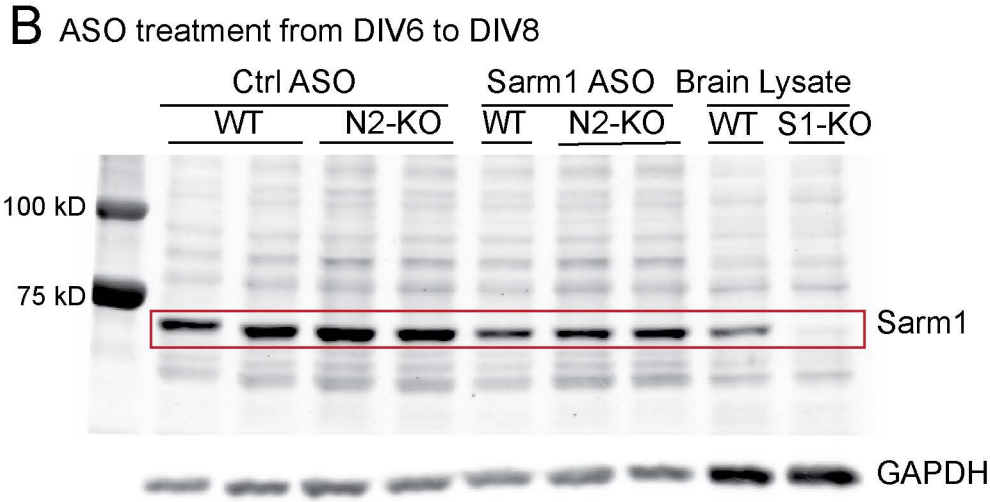

B
CC
Str
Frim
% of normalized APP
400
200
0
**
**
*
*
****
****

Fig 8-S1


A
qPCR
mRNA expression
fold change
1.5
1.0
0.5
0.0
Ctrl
ASO33 1uM
ASO33 5uM
ASO47 5uM
ASO47 10uM


B
ASO treatment from DIV6 to DIV8
Ctrl ASO
Sarm1 ASO
Brain Lysate
WT
N2-KO
WT
N2-KO
WT
S1-KO
100 kD
75 kD
Sarm1
GAPDH

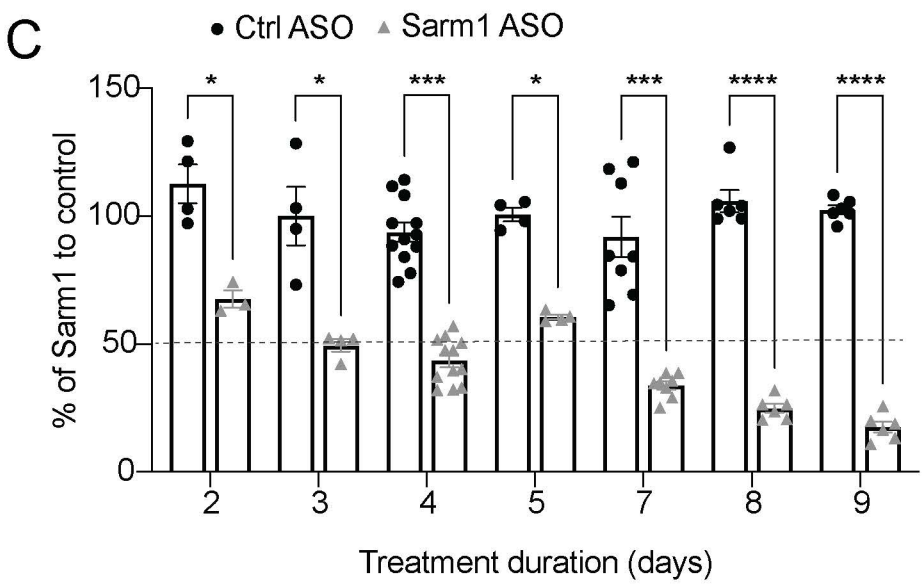

C
Ctrl ASO
Sarm1 ASO
% of Sarm1 to control
150
100
50
0
*
*
***
*
***
****
****
2
3
4
5
7
8
9
Treatment duration (days)

Fig 8-S2

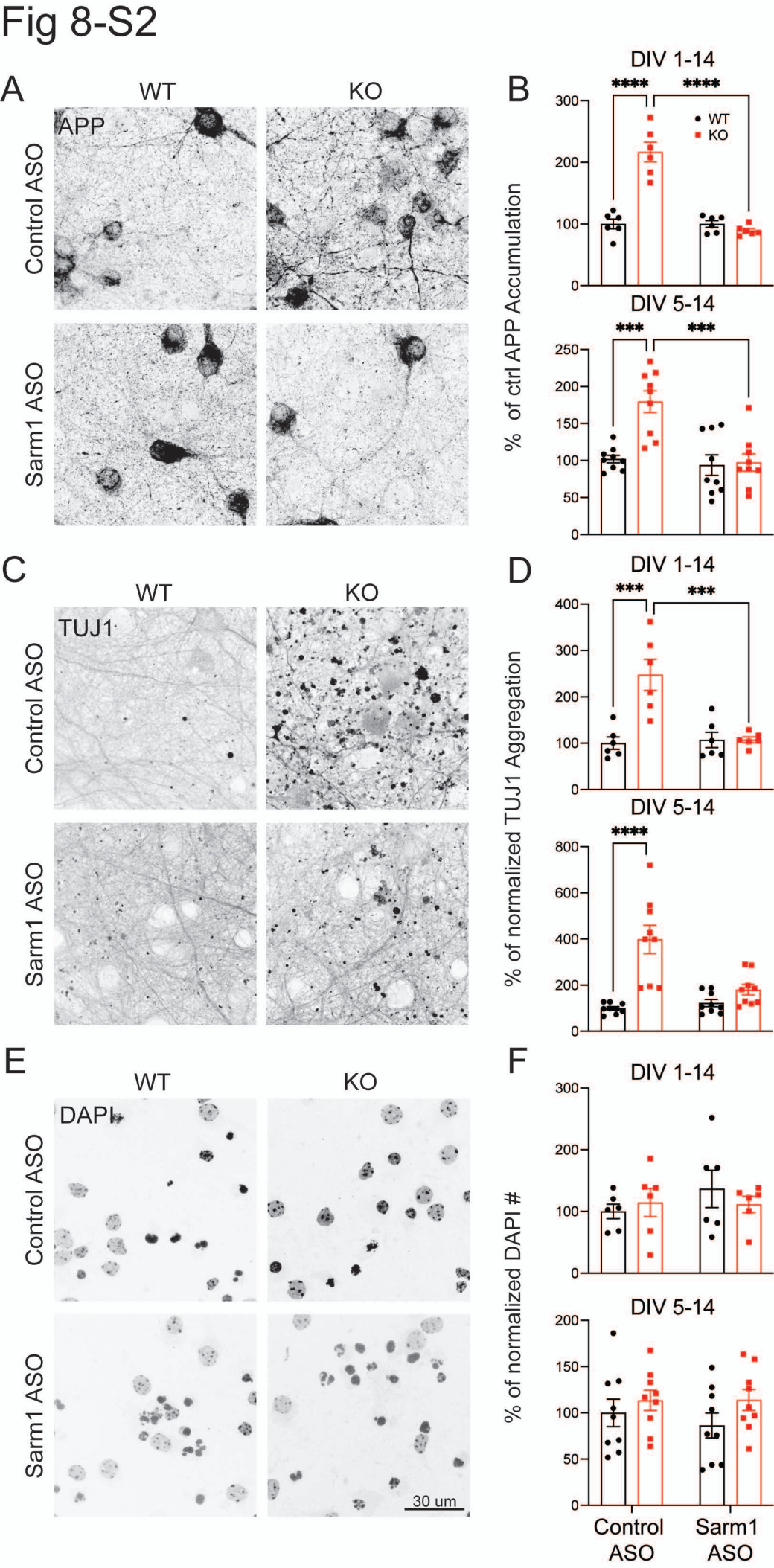
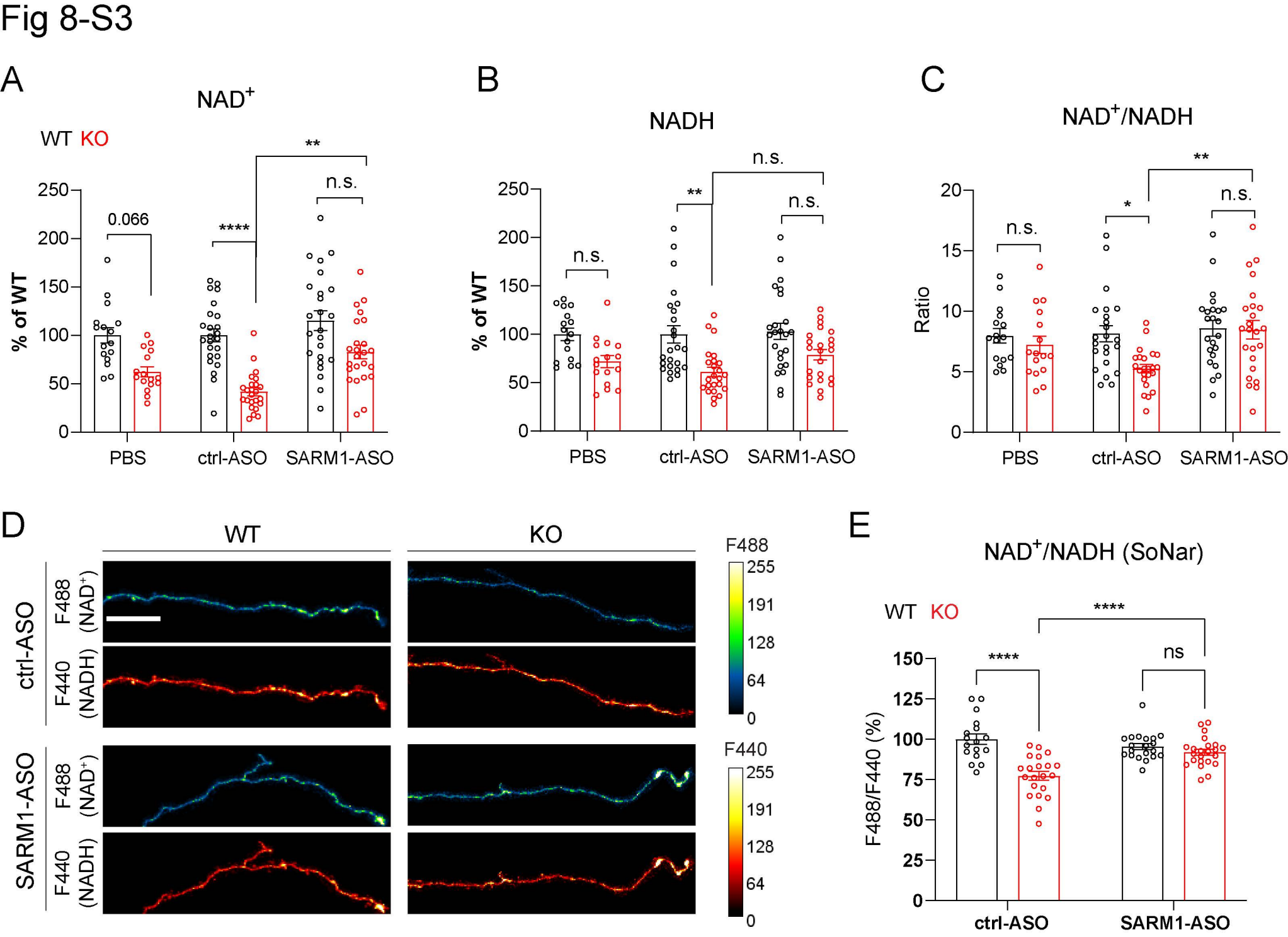

A
WT
KO
APP
Control ASO
Sarm1 ASO
B
DIV 1-14
****
****
WT
KO
% of ctrl APP Accumulation
DIV 5-14
***
***
C
TUJ1
D
DIV 1-14
***
***
% of normalized TUJ1 Aggregation
DIV 5-14
****
E
DAPI
30 um
F
DIV 1-14
% of normalized DAPI #
DIV 5-14
Control ASO
Sarm1 ASO

Fig 8-S3


A
NAD+
WT KO
% of WT
0.066
****
**
n.s.
PBS
ctrl-ASO
SARM1-ASO
B
NADH
% of WT
n.s.
**
n.s.
n.s.
PBS
ctrl-ASO
SARM1-ASO
C
NAD+/NADH
Ratio
n.s.
*
**
n.s.
PBS
ctrl-ASO
SARM1-ASO
D
WT
KO
ctrl-ASO
SARM1-ASO
F488 (NAD+)
F440 (NADH)
F488
255
191
128
64
0
F440
255
191
128
64
0
E
NAD+/NADH (SoNar)
WT KO
F488/F440 (%)
****
****
ns
ctrl-ASO
SARM1-ASO