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Supplemental Information

MAP7D2 Localizes to the Proximal Axon and Locally Promotes Kinesin-1-Mediated Cargo Transport into the Axon

Xingxiu Pan, Yujie Cao, Riccardo Stucchi, Peter Jan Hooikaas, Sybren Portegies, Lena Will, Maud Martin, Anna Akhmanova, Martin Harterink, and Casper C. Hoogenraad

SUPPLEMENTAL INFORMATION INVENTORY

Supplemental Figures S1 - S6

Figure S1, related to Figure 1

Figure S2, related to Figure 2

Figure S3, related to Figure 3

Figure S4, related to Figure 3

Figure S5, related to Figure 3

Figure S6, related to Figure 4, 5

SUPPLEMENTARY FIGURES

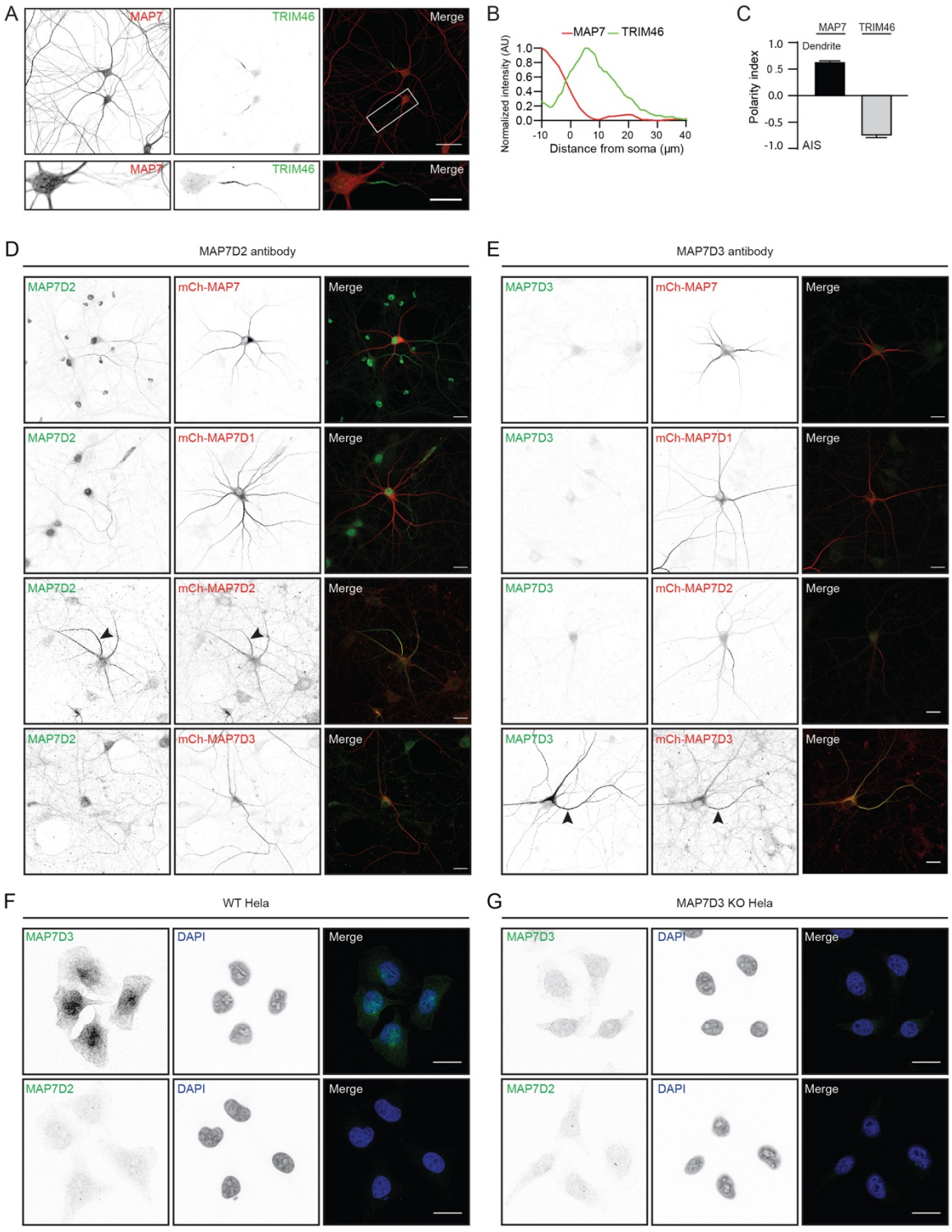


Figure S1, related to Figure 1. Characterization of the MAP7, MAP7D2 and MAP7D3 antibodies in neurons and cultured cells.

(A) corresponds to figure 1B. Representative images of DIV21 neurons co-stained for MAP7 (red) and TRIM46 (green). Square box is magnified below. Scale bars: 50 μm (A: upper row), 20 μm (A: bottom row)
(B) Line scans indicate normalized intensity of MAP7 and TRIM46 from soma to axon.
(C) Polarity index of MAP7 and TRIM46 in DIV21 hippocampal neurons (n=15 neurons).
(D) DIV10 neurons were transfected with mCherry-MAP7, mCherry-MAP7D1, mCherry-MAP7D2 or mCherry-MAP7D3 for 2 days, and stained for MAP7D2 (green). Arrowheads correspond to the proximal axon where MAP7D2 antibody recognizes mCherry-MAP7D2.
(E) DIV10 neurons were transfected with mCherry-MAP7, mCherry-MAP7D1, mCherry-MAP7D2 or mCherry-MAP7D3 for 2 days, and stained for MAP7D3 (green). Arrowheads correspond to the proximal axon where MAP7D3 antibody recognizes mCherry-MAP7D3.
(F and G) MAP7D3 WT or KO Hela cells were stained for MAP7D3 (green) or MAP7D2 (green) respectively. DAPI (blue) was used as nucleus marker. The MAP7D2 antibody recognizes centrosomes in Hela cells.
Scale bars: 20 μm

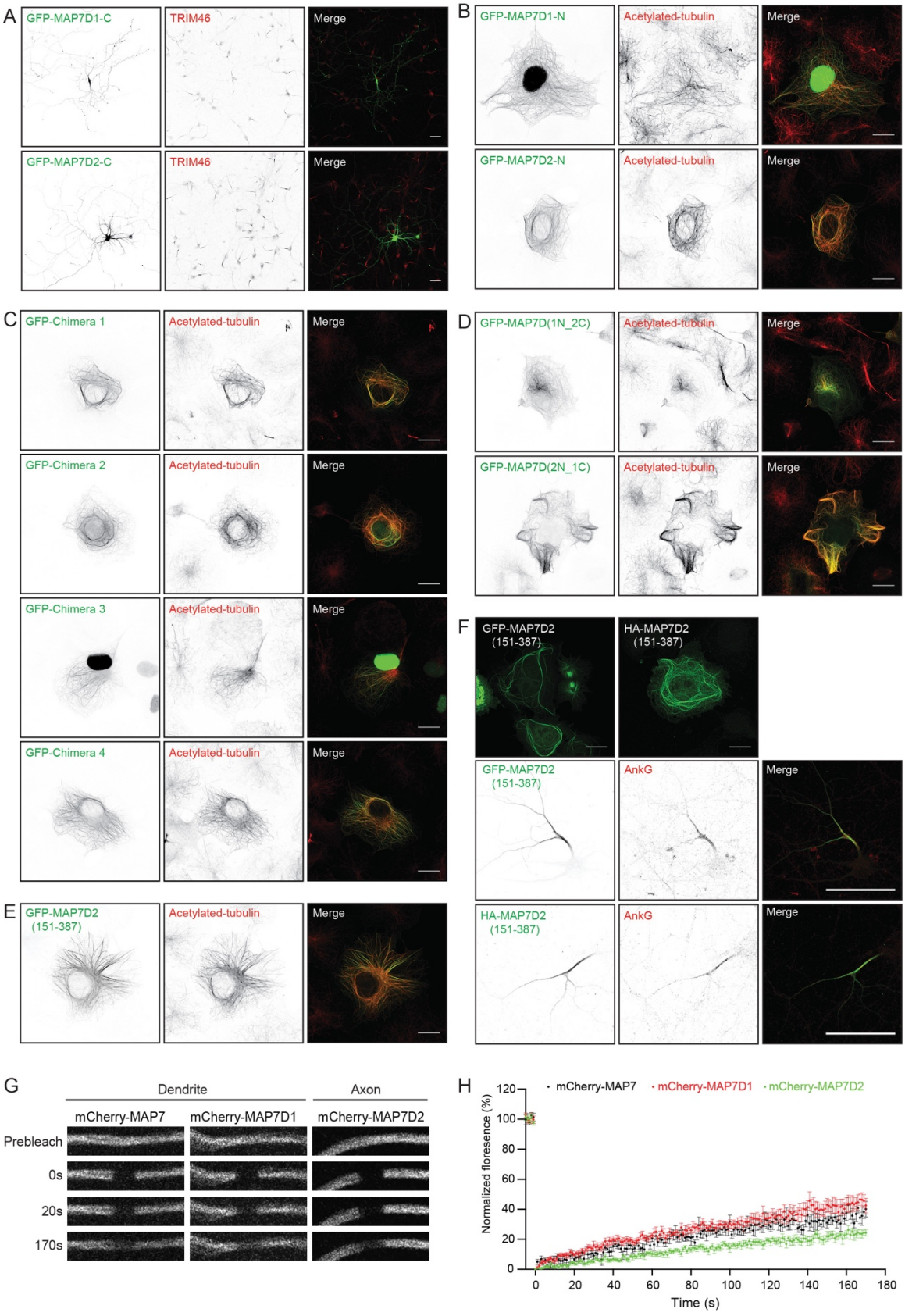

A
GFP-MAP7D1-C
TRIM46
Merge
GFP-MAP7D2-C
TRIM46
Merge
B
GFP-MAP7D1-N
Acetylated-tubulin
Merge
GFP-MAP7D2-N
Acetylated-tubulin
Merge
C
GFP-Chimera 1
Acetylated-tubulin
Merge
GFP-Chimera 2
Acetylated-tubulin
Merge
GFP-Chimera 3
Acetylated-tubulin
Merge
GFP-Chimera 4
Acetylated-tubulin
Merge
D
GFP-MAP7D(1N_2C)
Acetylated-tubulin
Merge
GFP-MAP7D(2N_1C)
Acetylated-tubulin
Merge
E
GFP-MAP7D2
(151-387)
Acetylated-tubulin
Merge
F
GFP-MAP7D2
(151-387)
HA-MAP7D2
(151-387)
GFP-MAP7D2
(151-387)
AnkG
Merge
HA-MAP7D2
(151-387)
AnkG
Merge
G
Dendrite
Axon
mCherry-MAP7
mCherry-MAP7D1
mCherry-MAP7D2
Prebleach
0s
20s
170s
H
mCherry-MAP7
mCherry-MAP7D1
mCherry-MAP7D2
Normalized floresence (%)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
Time (s)

Figure S2, related to Figure 2. MAP7 proteins associate with microtubules by their N-terminus and are largely immobile in neurons.

(A) corresponds to figure 2A and 2C. Representative images of GFP tagged C-terminus of MAP7D1 or MAP7D2 in DIV9 neurons, co-stained for TRIM46 for axons.

(B-E) correspond to figure 2A-F. Representative images of truncations and chimeras of MAP7D1 and MAP7D2 which are able to bind to microtubules in COS7 cells. Microtubule association is marked by antibody against acetylated-tubulin.

(F) corresponds to figure 2F. COS7 cells expressed with GFP tagged or HA tagged MAP7D2(151-387) fragments in first row. The second and third row are representative images of DIV9 neuron expressing GFP tagged or HA tagged MAP7D2(151-387) fragments, and co-stained for AnkG for axon.

(G and H) FRAP images of mCherry-MAP7, mCherry-MAP7D1 and mCherry-MAP7D2 in neurons. Averaged normalized intensity of FRAP graph of mCherry-MAP7 (n=9), mCherry-MAP7D1 (n=9) and mCherry-MAP7D2 (n=7). Error bars represents SEM.

N means numbers of FRAP regions.

Scale bars: 20 μm (A-D, F); 50 μm (E)

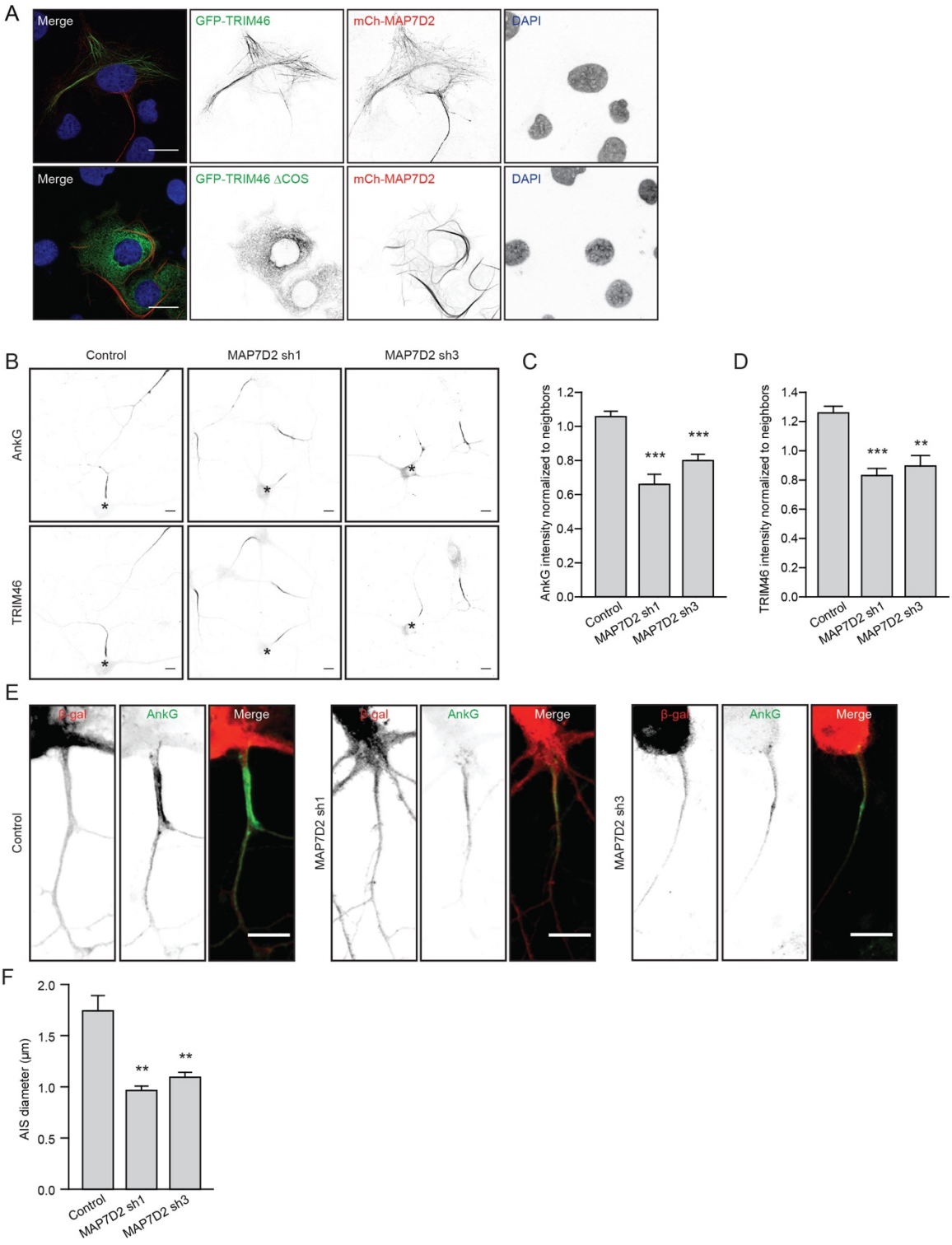


Figure S3, related to Figure 3. MAP7D2 does not interact with TRIM46, and is not essential for AIS assembly.

(A) Representative images of COS7 cells expressing mCherry-MAP7D2 and GFP-TRIM46 or GFP-TRIM46 ΔCOS. DAPI (blue) was used as nucleus marker. COS domain is essential for TRIM46 binding to microtubules.

(B) DIV11 neurons were transfected with control or MAP7D2 shRNAs together with a β-gal (red) fill for 3 days and co-stained for TRIM46 and AnkG. Asterisks pinpoint transfected neurons in each condition.

(C) Bar graph shows the normalized intensity of AnkG in control (n=30 neurons), or MAP7D2 KD (sh1 (n=33) and sh3 (n=33)) compared to neighbouring non-transfected neurons.

(D) Bar graph shows the normalized intensity of TRIM46 in control (n=57 neuron), or MAP7D2 KD (sh1 (n=60) and sh3 (n=62)) compared to neighbouring non-transfected neurons.

(E) Representative images of DIV11 neurons transfected with control or MAP7D2 shRNAs together with a β-gal fill (red) for 3 days and co-stained for AnkG (green).

(F) Bar graph shows the average AIS diameters measured by AnkG staining in control (n=41), or MAP7D2 KD (sh1 (n=46) and sh3 (n=47)).

$P<0.05$ *; $P<0.01$ **; $P<0.001$ ***. Unpaired t-test. Error bars represents SEM.

Scale bars: 20 μm (A); 10 μm (B,E)

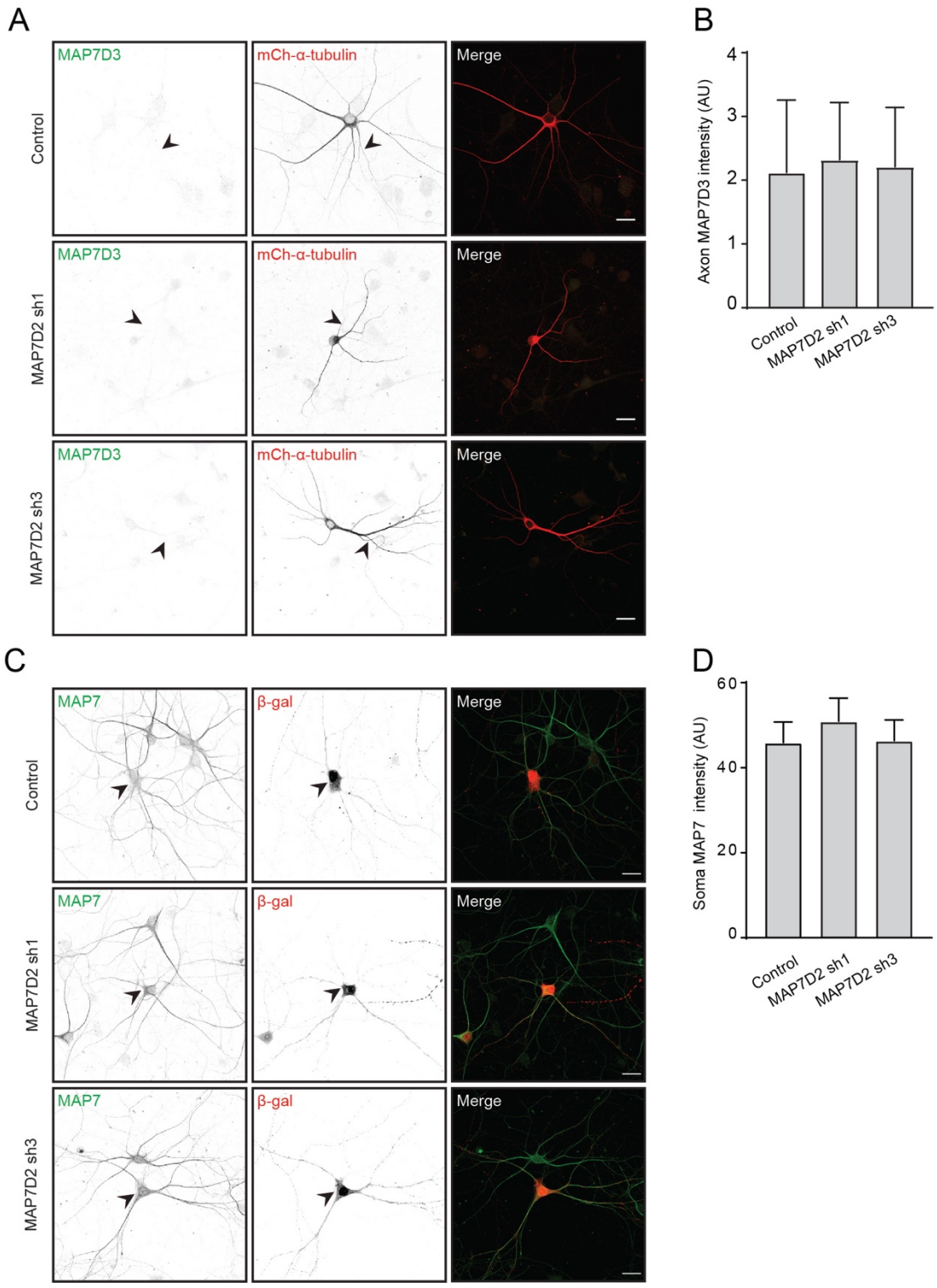


Figure S4, related to Figure 3. MAP7D2 knockdown does not lead to changes in MAP7 nor does it lead to expression of MAP7D3.

(A) DIV11 neurons were transfected with control or MAP7D2 shRNAs together with a mCherry-α-tubulin fill for 3 days and stained for MAP7D3 (green). Arrowheads correspond to the proximal axon.

(B) Bar graph shows the average intensity of MAP7D3 in the proximal axon under Control (n=8), or MAP7D2 KD (sh1 (n=8) and sh3 (n=7)), which is probably background fluorescence.

(C) DIV11 neurons were transfected with control or MAP7D2 shRNAs together with a β-gal (red) fill for 3 days and stained for MAP7 (green). Arrow heads correspond to the soma of transfected neuron.

(D) Bar graph shows the average intensity of MAP7 in soma under Control (n=12), or MAP7D2 KD (sh1 (n=12) and sh3 (n=15)).

$P<0.05$ *; $P<0.01$ **; $P<0.001$ ***. Unpaired t-test. Error bars represents SEM.

Scale bars: 20 μm

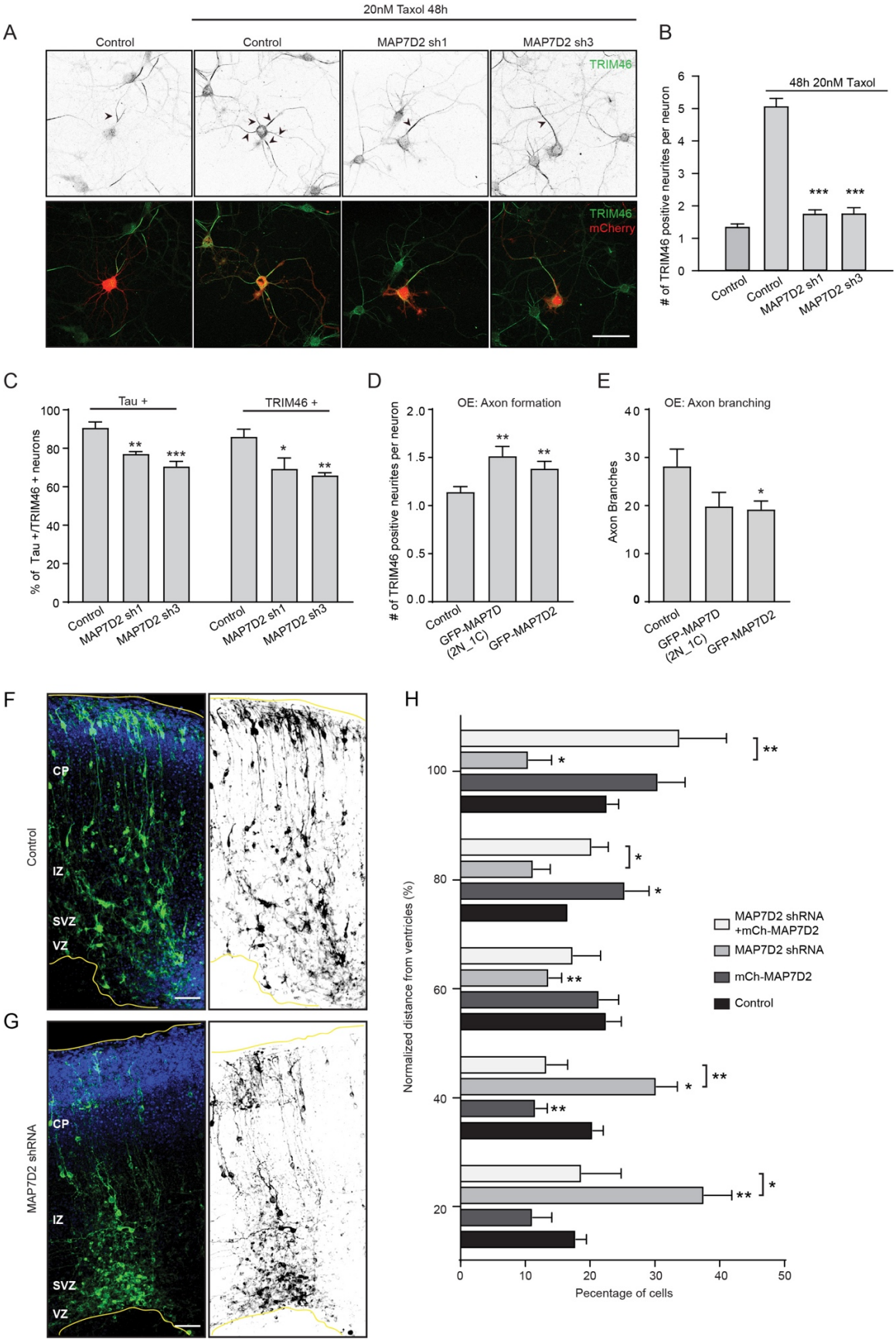

A
20nM Taxol 48h
Control
Control
MAP7D2 sh1
MAP7D2 sh3
TRIM46
TRIM46
mCherry
B
48h 20nM Taxol
# of TRIM46 positive neurites per neuron
***
***
Control
Control
MAP7D2 sh1
MAP7D2 sh3
C
Tau +
TRIM46 +
% of Tau +/TRIM46 + neurons
**
***
*
**
Control
MAP7D2 sh1
MAP7D2 sh3
Control
MAP7D2 sh1
MAP7D2 sh3
D
OE: Axon formation
# of TRIM46 positive neurites per neuron
**
**
Control
GFP-MAP7D (2N_1C)
GFP-MAP7D2
E
OE: Axon branching
Axon Branches
*
Control
GFP-MAP7D (2N_1C)
GFP-MAP7D2
F
Control
CP
IZ
SVZ
VZ
G
MAP7D2 shRNA
CP
IZ
SVZ
VZ
H
Normalized distance from ventricles (%)
MAP7D2 shRNA +mCh-MAP7D2
MAP7D2 shRNA
mCh-MAP7D2
Control
Pecentage of cells

Figure S5, related to Figure 3. MAP7D2 is important for axon formation and mouse cortical neuron migration.

(A) DIV3 neurons were transfected with control or MAP7D2 shRNAs together with a mCherry (red) fill, treated with 20 nM Taxol for 48 hours, and stained for TRIM46 (green). Arrow heads mark the axons. Scale bars: 20 μm

(B) Bar graph shows the numbers of TRIM46 positive neurites per neuron in control before (n=27) and after (n=59) Taxol treatment or MAP7D2 KD (sh1 (n=57) or sh3 (n=27)) after Taxol treatment.

(C) Percentage of DIV3 neurons electroporated with control, MAP7D2 sh1 and 3 constructs and quantified neurites positive for Tau or TRIM46, n>500 neurons for each condition.

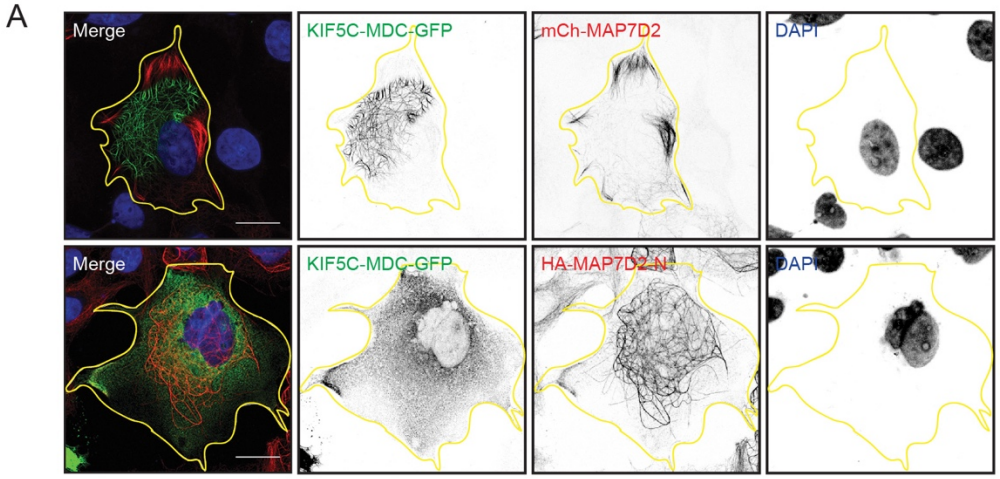
(D) Quantifications of overexpression effect of Control (GFP empty) (n=63), GFP-MAP7D(2N_1C) (n=60) or GFP-MAP7D2 (n=57) on axon formation, TRIM46 antibody was used for marking the axon.

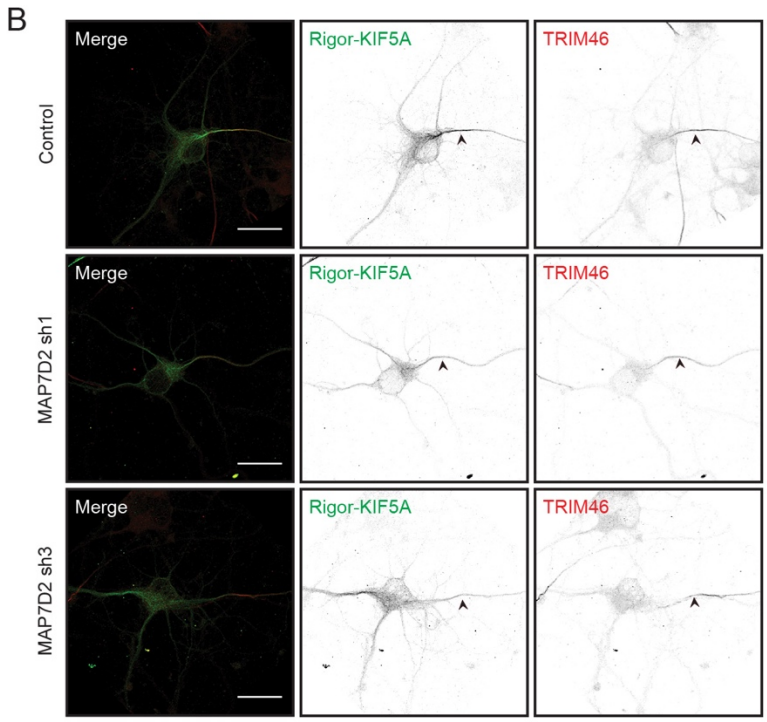
(E) Quantifications of overexpression effect of Control (GFP empty) (n=16), GFP-MAP7D(2N_1C) (n=12) or GFP-MAP7D2 (n=15) on axon branching, a β-galactosidase fill was used for tracing the axon morphology.

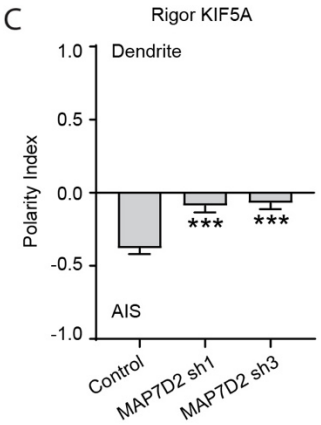
(F and G) Representative images of mouse cortex after 4 days of ex vivo electroporated with GFP and control or MAP7D2 shRNA. DAPI is in blue. Pail surface at the top and ventricle at the bottom are outlined in yellow. CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VA, ventricular zone. Right panels represent GFP only channel. Scale bars: 100 μm

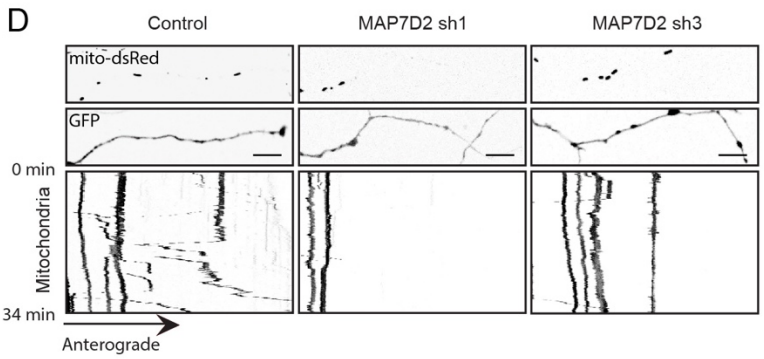
(H) Quantifications of the normalized neuronal distribution along the radial axis from ventricle to the pail surface under control (N=5 embryos, n=8 slices), mCherry-MAP7D2 (N=3 embryos, n=7 slices), MAP7D2 shRNA (N=7 embryos, n=13 slices) and MAP7D2 shRNA plus mCherry-MAP7D2 (N=4 embryos, n=7 slices) for rescue.

P<0.05 *; P<0.01 **; P<0.001 ***. Unpaired t-test. Error bars represents SEM.


A
Merge
KIF5C-MDC-GFP
mCh-MAP7D2
DAPI
Merge
KIF5C-MDC-GFP
HA-MAP7D2-N
DAPI


B
Control
MAP7D2 sh1
MAP7D2 sh3
Merge
Rigor-KIF5A
TRIM46


C
Rigor KIF5A
Dendrite
AIS
Polarity Index
1.0
0.5
0.0
-0.5
-1.0
***
***
Control
MAP7D2 sh1
MAP7D2 sh3


D
Control
MAP7D2 sh1
MAP7D2 sh3
mito-dsRed
GFP
0 min
Mitochondria
34 min
Anterograde

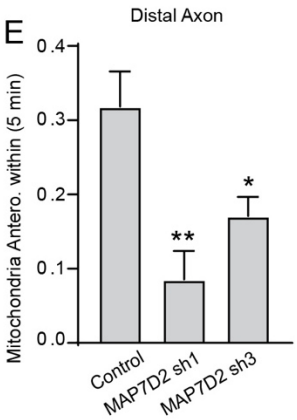

E
Distal Axon
Mitochondria Antero. within (5 min)
0.4
0.3
0.2
0.1
0.0
**
*
Control
MAP7D2 sh1
MAP7D2 sh3

Figure S6, related to Figure 4, 5. MAP7D2 is important for recruitment of Kinesin-1 on microtubules, and affects mitochondria transport at distal axon.

(A) Representative images of COS7 cells co-transfected with KIF5C-MDC-GFP and mCherry-MAP7D2 or HA-MAP7D2-N in red. Cell borders are outlined in yellow. HA-MAP7D2-N lacks the C-terminal kinesin-1 binding domain.

(B) Representative images of neurons co-transfected with Rigor-KIF5A-GFP and Control or MAP7D2 shRNAs, and co-stained with TRIM46 for axon. Arrow heads indicate AIS.

(C) Polarity index of Rigor KIF5A-GFP in Control (n=17), MAP7D2 sh1 (n=15) and sh3 (n=20).

(D) DIV1 neurons were transfected with control or MAP7D2 shRNAs together with a GFP fill and mito-dsRed respectively for 3 days, and Representative images at the upper two panels shows mito-dsRed and GFP fill in the distal axon, kymographs at the bottom showing live cell imaging of mitochondria. Scale bars: 10 μm

(E) Quantifications for mitochondria anterograde movement along distal axon within 5 minutes under control (n=36), MAP7D2 sh1 (n=35) and MAP7D2 sh3 (n=36).

$P<0.05$ *; $P<0.01$ **; $P<0.001$ ***. Unpaired t-test. Error bars represents SEM.

Scale bars: 20 μm