Supplementary Material

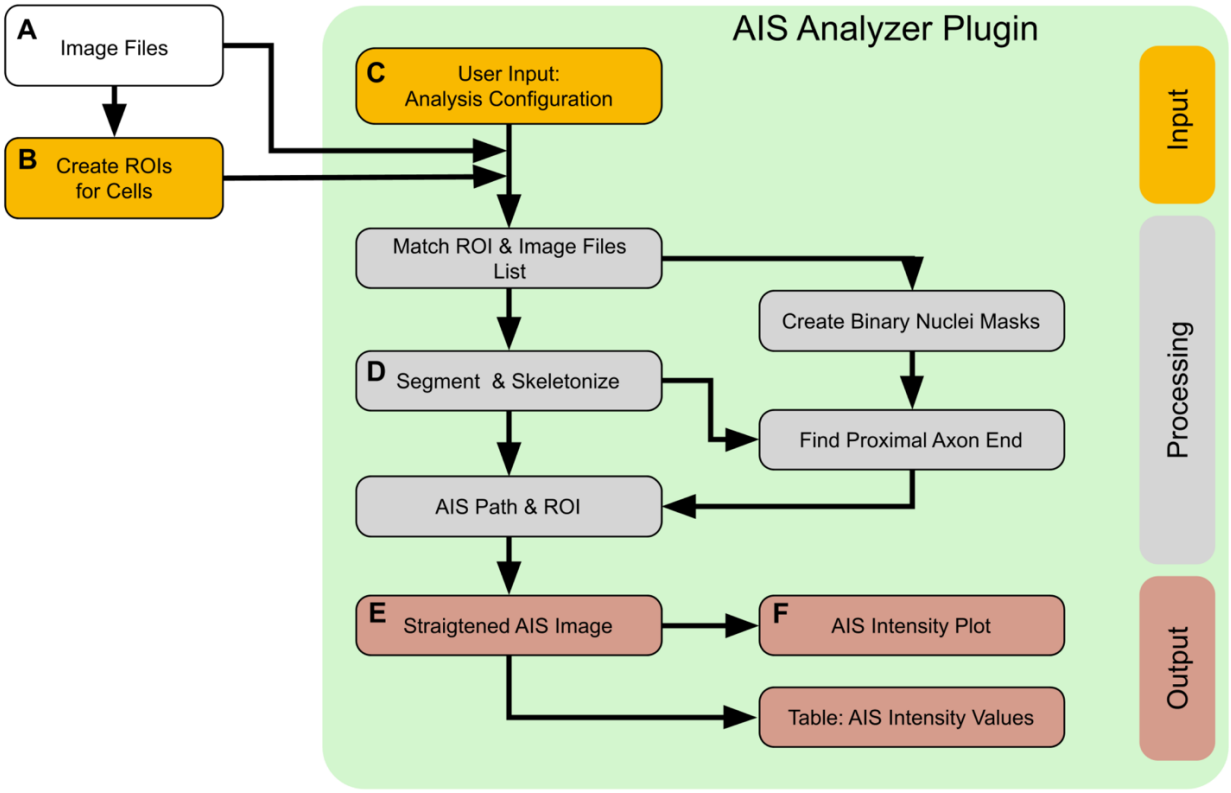
Extracellular Tau Oligomers Damage the Axon Initial Segment

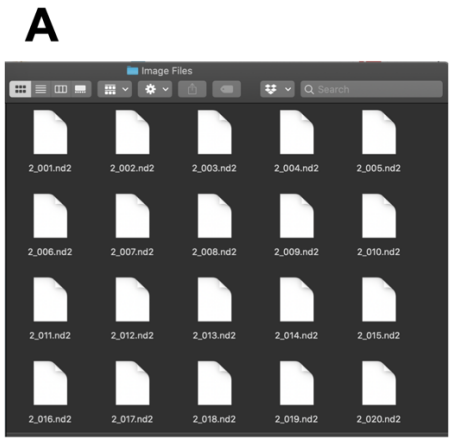
Supplementary Table 1. Primary and secondary antibodies used in this study.

Antigen	*Host/ Label*	*Application(s)*	*Stock Concentration*	*Dilution(s)*	*Source/ Catalog #*
Primary Antibodies					
Ankyrin-G	Mouse (Monoclonal)	ICC	1 mg/mL	1:1000	NeuroMab/ N106/36
Ankyrin-G	Rabbit	WB	800 ug/ml	1:5000	P Jenkins/ University of Michigan
Tau (pS202/ pT205)	Rabbit (Monoclonal)	WB	Not Reported	1:1000	Cell Signaling/ 30505
α-tubulin	Mouse (Monoclonal)	WB	Not Reported	1:1000	Cedarlane/ CLT9002
βIII-tubulin (Clone Tuj1)	Mouse	WB	0.7 mg/mL	1:5000	T Spano and T Frankfurter/ University of Virginia
βIII-tubulin	Rabbit	WB	1 mg/mL	1:2500	Sigma/ T3952
Glial Fibrillary Acidic Protein (GFAP)	Mouse (Monoclonal)	ICC	0.2 mg/mL	1:500	Invitrogen/ MA5-12023
Tau	Mouse (Monoclonal, clone Tau-12)	WB, ICC, IHC	0.25 mg/mL	1:200, 1:500, 1:2500	LI Binder/ Northwestern University
Tau	Mouse (Monoclonal, clone Tau-5)	ICC	1 mg/mL	1:500	N Kanaan/ Michigan State University
TRIM46	Chicken	WB, ICC	1 mg/mL	1:1000, 1:2500	Synaptic Systems/ 377-006
TRIM46	Rabbit	WB, ICC, IHC	500 ug/mL	1:400, 1:500, 1:1000	Proteintech/ 21026-1-AP
MAP2	Chicken	ICC, IHC	Not Reported	1:2000	Abcam/ Ab92434
Neurofascin-186	Rabbit	ICC, WB	0.14 mg/mL	1:250, 1:2000	Invitrogen/ PA5-78668
Secondary Antibodies					
Chicken-IgG	Alexa Fluor Plus 680	WB	1 mg/mL	1:5000	LiCor/ A32934
Mouse-IgG	IRDye 680	WB	1 mg/mL	1:5000	LiCor/ 926-68070
Mouse-IgG	IRDye 800	WB	1 mg/mL	1:5000	LiCor/ 926-32210
Rabbit-IgG	IRDye 680	WB	1 mg/mL	1:5000	LiCor/ 926-68071
Rabbit-IgG	IRDye 800	WB	1 mg/mL	1:5000	LiCor/ 926-32211
Chicken-IgG	Alexa FluorTM 568	ICC, IHC	2 mg/mL	1:1000	Invitrogen/ A-11041
Mouse-IgG	Alexa FluorTM 488	ICC, IHC	2 mg/mL	1:1000	Invitrogen/ A-11029
Mouse-IgG2a	Alexa FluorTM 488	ICC	2 mg/mL	1:1000	Invitrogen/ A-21131
Rabbit-IgG	Alexa FluorTM 647	ICC, IHC	2 mg/mL	1:1000	Invitrogen/ A-21244

WB: Western Blot, ICC: Immunocytochemistry (Cultured Cells), IHC: Immunohistochemistry (Brain)

Supplementary Figure 1. Semi-automated image analysis with the AIS analyzer plugin. This plugin measures nucleus-AIS gap, immunofluorescence intensity for a protein of interest (TRIM46) within the AIS, and AIS length. The plugin requires the following inputs: A) a list of image files, B) ROIs defining individual cells and background regions, C) user input to configure analysis parameters. Automatic processing includes matching of ROIs and image files, creation of binary nuclei masks, D) segmentation and skeleton creation of the AIS, definition of proximal axon ends, calculation of AIS path and ROI. The created analysis output includes E) an image representation of the straightened AIS, F) creation of an intensity plot for the protein of interest along the axon path with highlighting of the AIS segment, and a CSV formatted table with measured fluorescence intensity values along the AIS path.


AIS Analyzer Plugin
A
Image Files
B
Create ROIs for Cells
C
User Input: Analysis Configuration
Match ROI & Image Files List
Create Binary Nuclei Masks
D
Segment & Skeletonize
Find Proximal Axon End
AIS Path & ROI
E
Straigtened AIS Image
F
AIS Intensity Plot
Table: AIS Intensity Values
Input
Processing
Output


A
Image Files
2_001.nd2
2_002.nd2
2_003.nd2
2_004.nd2
2_005.nd2
2_006.nd2
2_007.nd2
2_008.nd2
2_009.nd2
2_010.nd2
2_011.nd2
2_012.nd2
2_013.nd2
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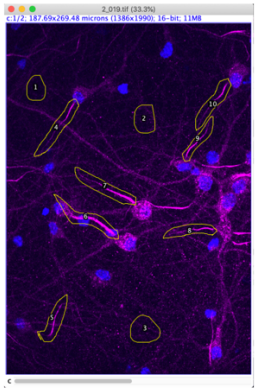
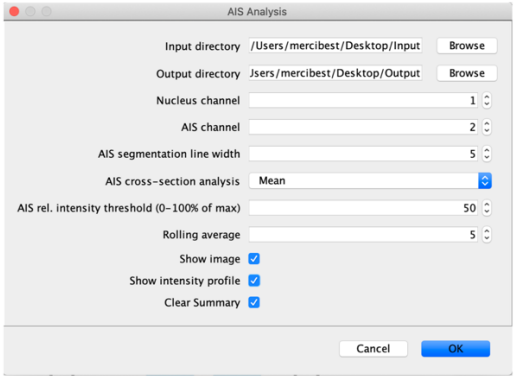
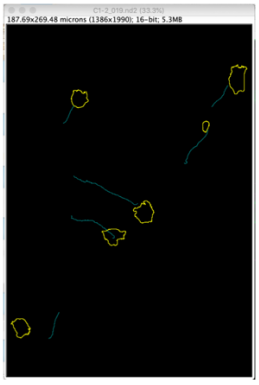
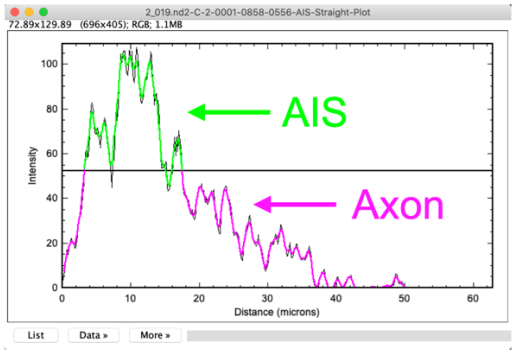
2_018.nd2
2_019.nd2
2_020.nd2


B

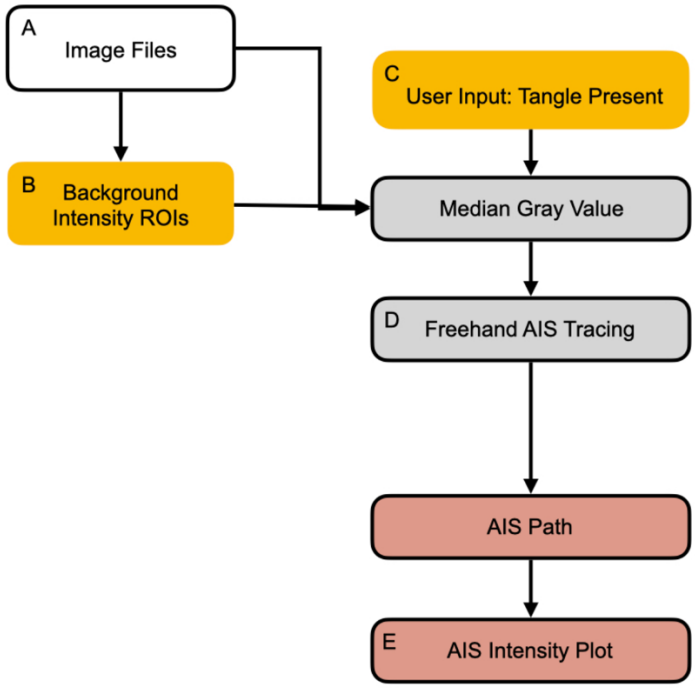

C
AIS Analysis
Input directory
/Users/mercibest/Desktop/Input
Browse
Output directory
/sers/mercibest/Desktop/Output
Browse
Nucleus channel
1
AIS channel
2
AIS segmentation line width
5
AIS cross-section analysis
Mean
AIS rel. intensity threshold (0-100% of max)
50
Rolling average
5
Show image
Show intensity profile
Clear Summary
Cancel
OK

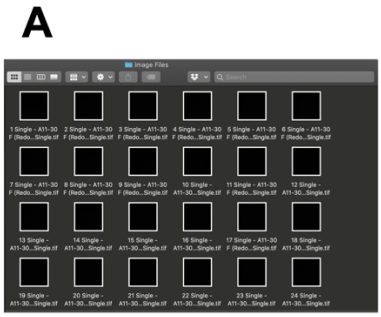

D

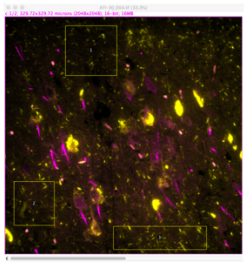

E
2_019.nd2-0001-0858-0556-AIS-straight.tif
62.97x0.68 microns (465x5); 32-bit; 9K

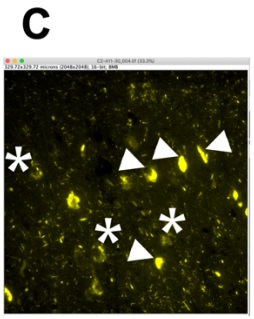

F
2_019.nd2-C-2-0001-0858-0556-AIS-Straight-Plot
72.89x129.89 (696x405); RGB; 1.1MB
AIS
Axon
Intensity
Distance (microns)
List
Data »
More »

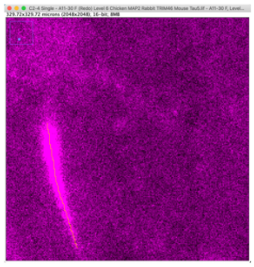
Supplementary Figure 2. Manual image analysis for processing of AD and non-AD datasets in Fig. 5. A) Image files. B) Background intensity ROIs. C) User input: tangle present. Median gray value. D) Freehand AIS tracing; AIS path. E) AIS intensity plot.

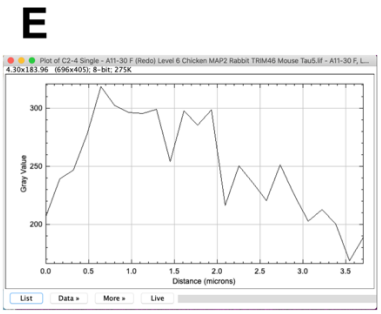




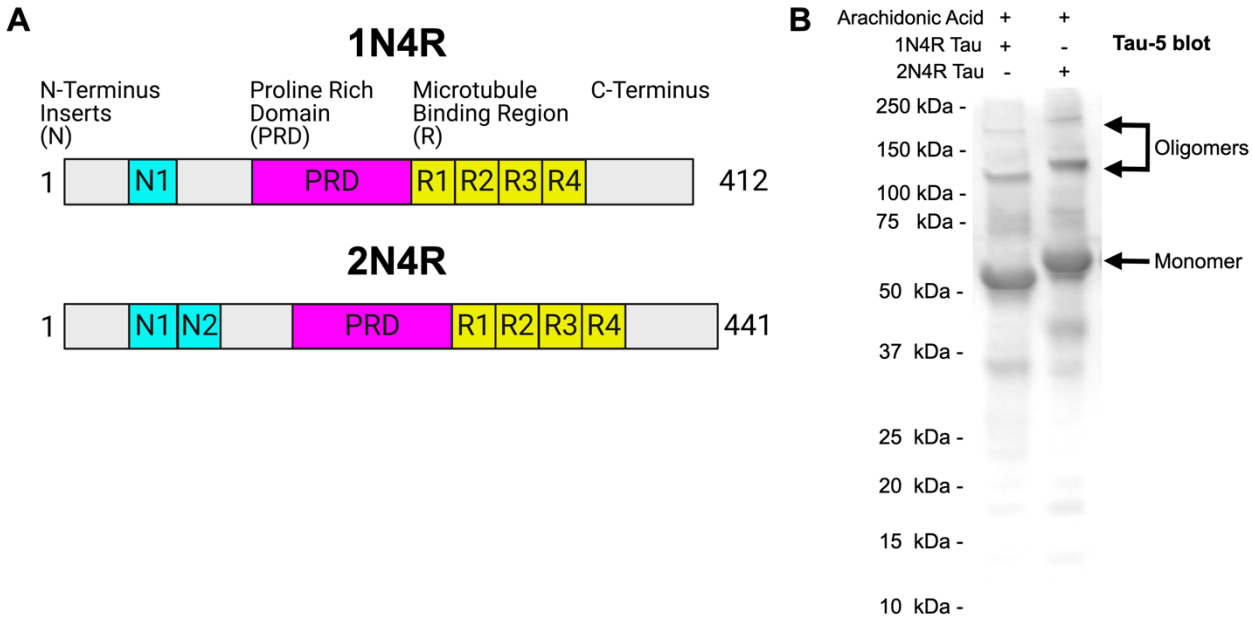




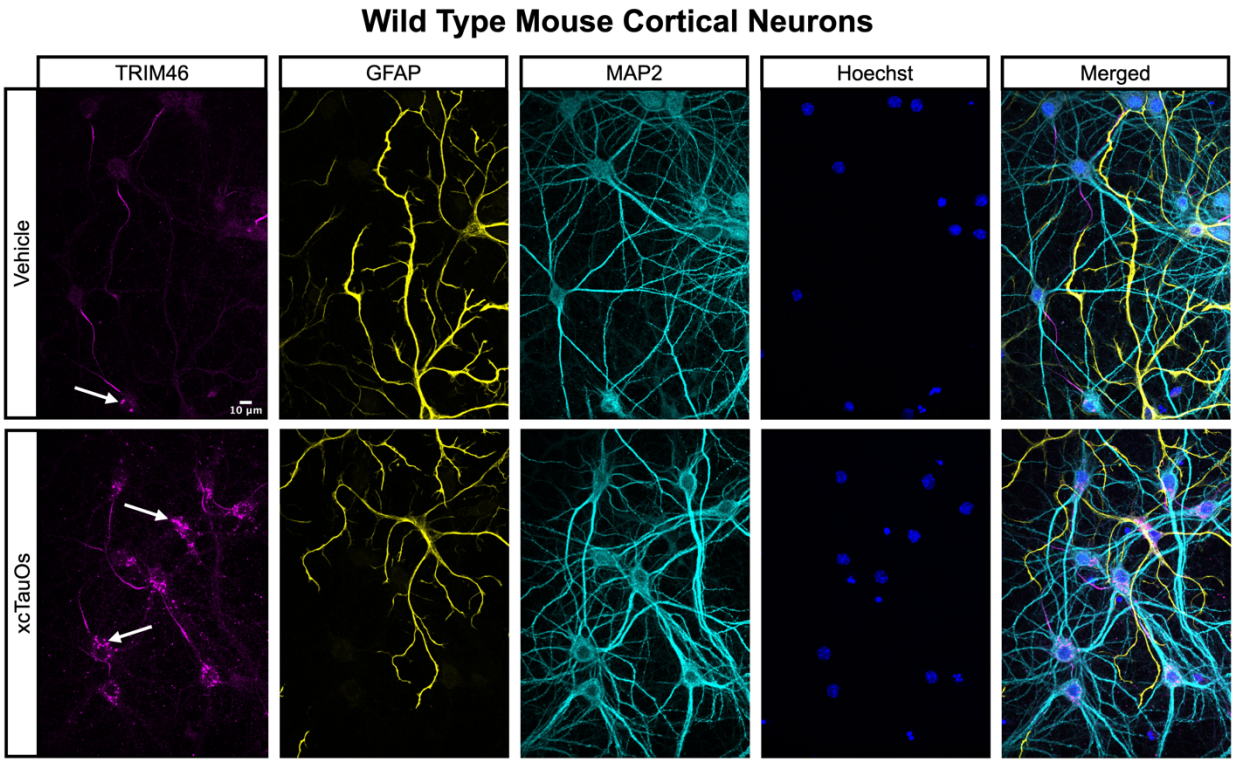




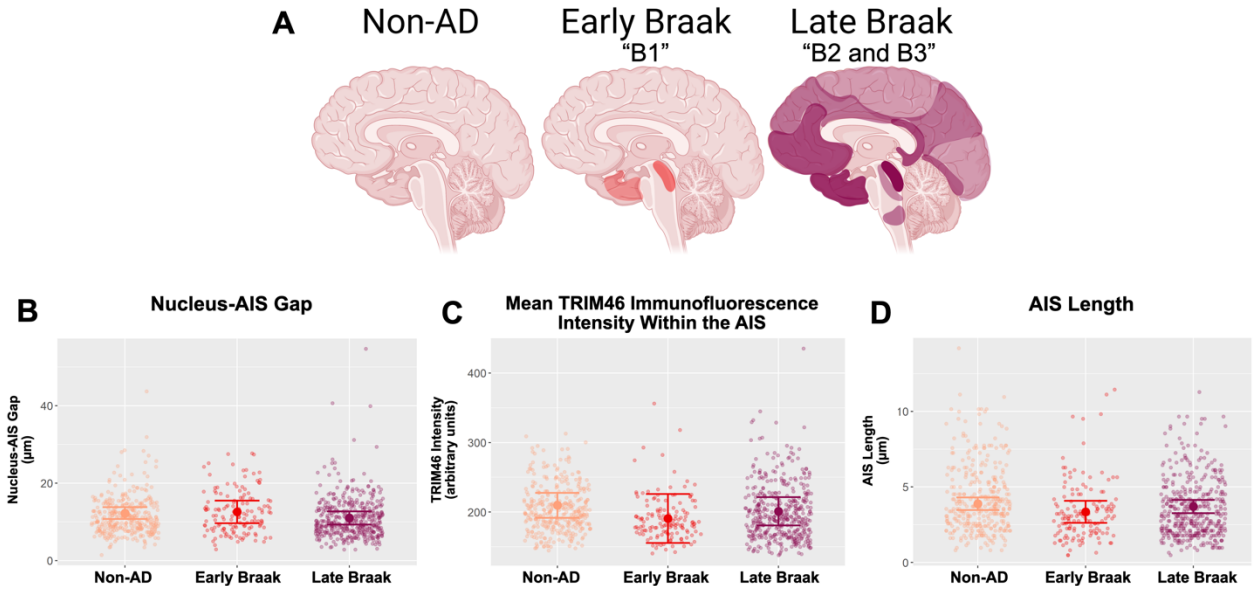
Supplementary Figure 3. Characterization of Tau Oligomers. A) Diagrams of the recombinant human 1N4R and 2N4R tau that were expressed in *E. coli*, purified, and used for making xcTauOs. B) Western blotting of xcTauOs with the pan-tau antibody, Tau-5.



Supplementary Figure 4. xcTauOs cause accumulation of TRIM46 puncta in the somatodendritic compartment of neurons and in astrocytes. Localization of TRIM46 puncta (white arrows), astrocytes (GFAP), neuronal somatodendritic compartment (MAP2), and nuclei (Hoechst) in primary WT mouse cortical neuron cultures.



Supplementary Figure 5. Braak stages do not correlate with changes in the AIS on a tissue wide basis. A) Schematic of non-AD, early Braak (B1), and late Braak (B2-B3) stages. Plots of (B) Nucleus-AIS gap, (C) mean TRIM46 immunofluorescence intensity within the AIS, a surrogate measure of TRIM46 concentration, and (D) AIS length. Graphs were generated from 8 biological replicates (independent tissue samples), which accounted for a total of ~387 neurons per group (non-AD, early Braak, or late Braak). p-values from mixed model linear regression are indicated. Large dot indicates the predicted mean and error bars represent the 95% confidence interval.



Supplementary Figure 6. Co-localization of multiple AIS proteins and their sensitivity to xcTauOs. Localization of TRIM46 (arrows), ankyrin-G (asterisks), neurofascin-186 (arrowhead), and nuclei (Hoechst) in primary WT mouse cortical neuron cultures.