SUPPLEMENTAL FIGURES

Figure S1. Proliferating OPCs have Uniform Microtubule Polarity, Related to Figure 1

(A) Image of bipolar OPC proliferated for 2 days in culture in the presence of PDGF and expressing EB3-EGFP.
(B) Kymograph of proliferating OPC process expressing EB3-EGFP.
(C) Proliferating OPCs contain microtubules that mostly grow in the anterograde direction away from the cell body (97%) and few that grow in the retrograde direction (3%). n = 3 cells.
(D) EM images from P14 mouse spinal cord with the putative oligodendrocyte process pseudocolored in green. This process contains many ribosomes (small dots), which is characteristic of oligodendrocytes, and differs in appearance from a nearby axon in transverse section (lower right).

Figure S2. Motile Golgi Outposts Can Give Rise to Microtubules, Related to Figures 1 and 2

(A) Micrographs from dual-color live-cell imaging movies of cells co-expressing both EB3-EGFP and ManII–tdTomato.
(B) Kymographs and colocalization analysis.
(C) Quantification of the percentage of Golgi outposts that give rise to EB3-labeled growing microtubules at DIV3 (40.6 $\pm$ 6.5%) and DIV5 (32.0 $\pm$ 5.3%).
(D) Probability analysis comparing actual rates of EB3-positive puncta arising from ManII+ puncta versus probability based on chance. Based on the binomial distribution, the average probability of these outcomes is 0.133.
(E) ManII+ Golgi outposts binned by how many EB3 events originated from them and plotted by the net speeds of each Golgi outpost. ManII+ puncta that give rise to EB3 events have average speed of 0.013 $\pm$ 0.006 μm/s while those that do not have average speed of 0.056 $\pm$ 0.011 μm/s. n = 3 biological replicates, 12 cells, 94 ManII+ Golgi.
(F) Enlarged view of super-resolution image of a DIV4 rat oligodendrocyte (from Figure 2D) stained against tubulin.

Figure S3. γ-Tubulin Staining and Mass Spectrometry, Related to Figure 3

(A) Chart of antibodies used in immunostaining experiments.
(B) Max projection of GTU-88 staining of DIV3 rat oligodendrocyte. Arrowhead points to centrosome staining identified near the nucleus in separate z-stack from TPPP puncta.
(C) Max projection of TU-30 staining of DIV3 rat oligodendrocyte. Arrowhead points to centrosome staining identified near the nucleus in separate z-stack from TPPP puncta.
(D) Chart of spectral counts for all tubulins detected in mass spectrometry experiments in Figure 3.

Figure S4. EB3-Loaded Beads Do Not Nucleate Microtubules, Related to Figure 4

(A) On-bead microtubule nucleation assay with EB3-loaded beads with 1μM EB3, 15μM Tubulin, 1 mM GTP.
(B) TPPP-EGFP puncta from oligodendrocyte lysate concentrates rhodamine-labeled tubulin in the absence of GTP.

Figure S5. TPPP Knockdown in Spinal Cord Oligodendrocytes, Related to Figure 5

(A) Immunopanned rat oligodendrocytes were electroporated with siRNA directed against TPPP and lysed. Western blot of cell lysates cultured for 3 days or 5 days in differentiation media shows no immunoreactivity using TPPP antibody.
(B) Micrographs of rat spinal cord oligodendrocytes electroporated with TPPP siRNA or a sequence-specific scrambled control and differentiated DIV3, then stained with an antibody against tubulin.
(C) Micrographs of rat cortex oligodendrocytes electroporated with EB3-GFP and TPPP siRNA or a sequence-specific scrambled control and differentiated DIV3.
(D) Sholl analysis of rat spinal cord oligodendrocytes from Figure S5B. n = 3 biological replicates, 11–15 cells.
(E) Sholl analysis of rat cortex oligodendrocytes from Figure S5C. n = 2 biological replicates, 5 cells.
(F) Representative confocal micrographs of DIV4 oligodendrocytes cultured from WT or TPPP KO mice that are high expressers of MBP protein.
(G) Normalized MBP protein intensity for all DIV4 oligodendrocytes cultured from WT or TPPP KO mice. Data represents both high and low expressers of MBP protein. n = 19 – 23 cells, 3 biological replicates from 3 mice per genotype.

Figure S6. Oligodendrocyte Microfiber Cultures, Related to Figure 6

(A) Brightfield image of DIV9 rat oligodendrocytes cultured on 3D microfibers. Arrowheads point to cell bodies.
(B) Enlarged and rotated confocal micrographs acquired at 100x (from Figure 6B). WT DIV14 mouse oligodendrocyte cultured on 3D microfibers at 100x. Arrowhead point to cell body.
(C) Confocal micrographs acquired at 100x of DIV14 oligodendrocytes from WT or TPPP KO mice cultured on 3D microfibers. Arrowhead point to cell body. Arrows point to MBP bulbous hyperintensities.

Figure S7. TPPP Knockout Mouse and Single-Cell RNA-seq, Related to Figure 7

(A) Map of TPPP knockout mouse obtained from KOMP. We obtained heterozygous sperm for the VG12652 tm1.1 allele, in which the neomycin cassette has already been removed.
(B) Immunohistochemistry staining of TPPP knockout mouse brain using anti-TPPP antibody (green) and DAPI (blue).
(C) Neurofilament staining of TPPP knockout mouse brain.
(D) Single-cell RNA-seq tSNE graphs of TPPP expression in brain non-myeloid cells from adult mice, downloaded from the Tabula Muris database.
(E) Violin plots and table indicating TPPP expression levels in different cell types.

(F) Single-cell RNA-seq tSNE and bar graphs of TPPP expression in the dentate gyrus of the hippocampus from mice of different ages, downloaded from the Linnarson Lab database. In addition to expression in oligodendrocytes, TPPP is also expressed in a specific subpopulation of CA3 pyramidal neurons.

Figure S1
Supplemental Figure 1

Click here to access/download;Supplemental Figure;Figure S1 OPCs and EMs.pdf

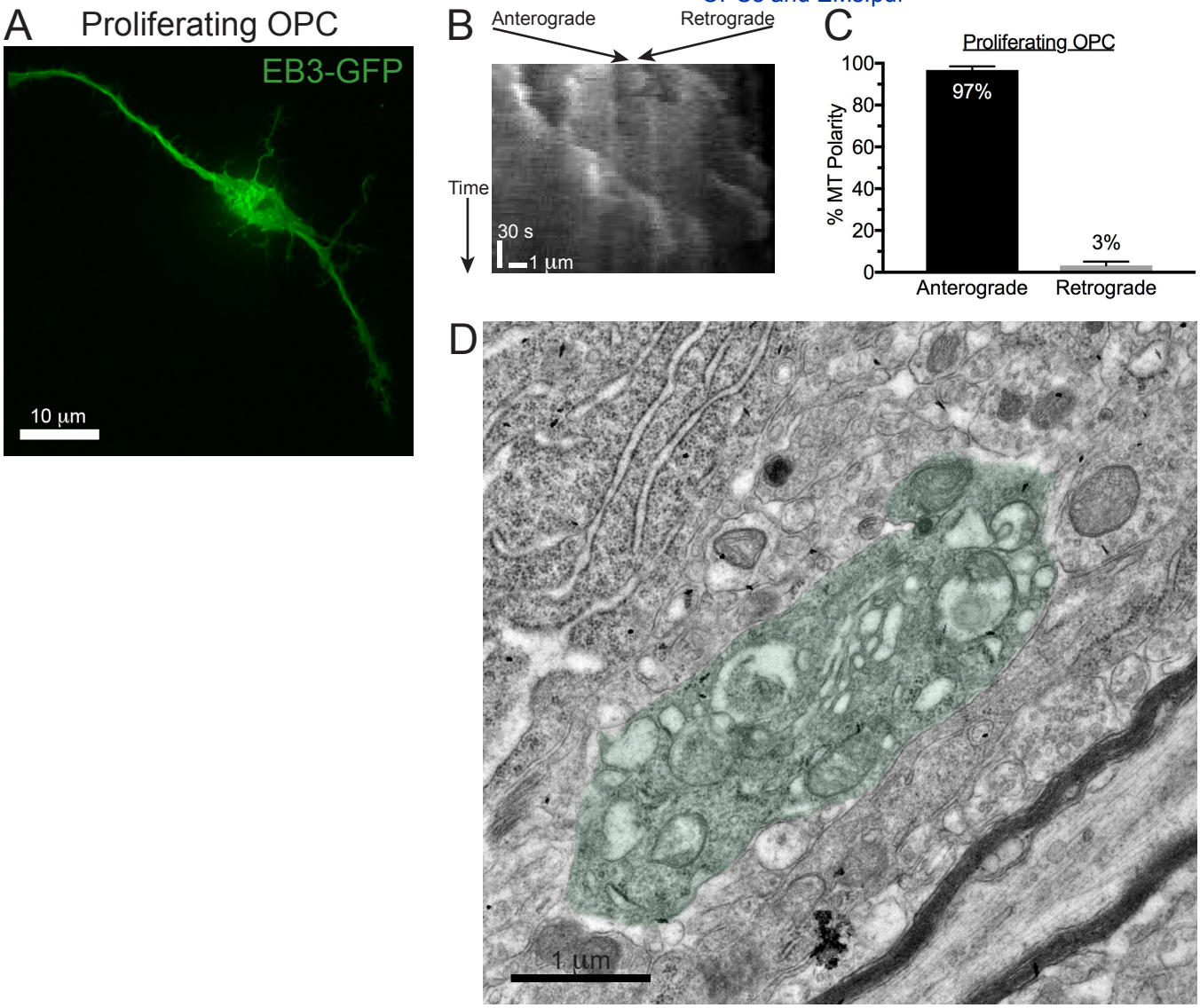


Figure S2

Supplemental Figure 2

Click here to access/download;Supplemental Figure;Figure S2 ManII 201906.pdf

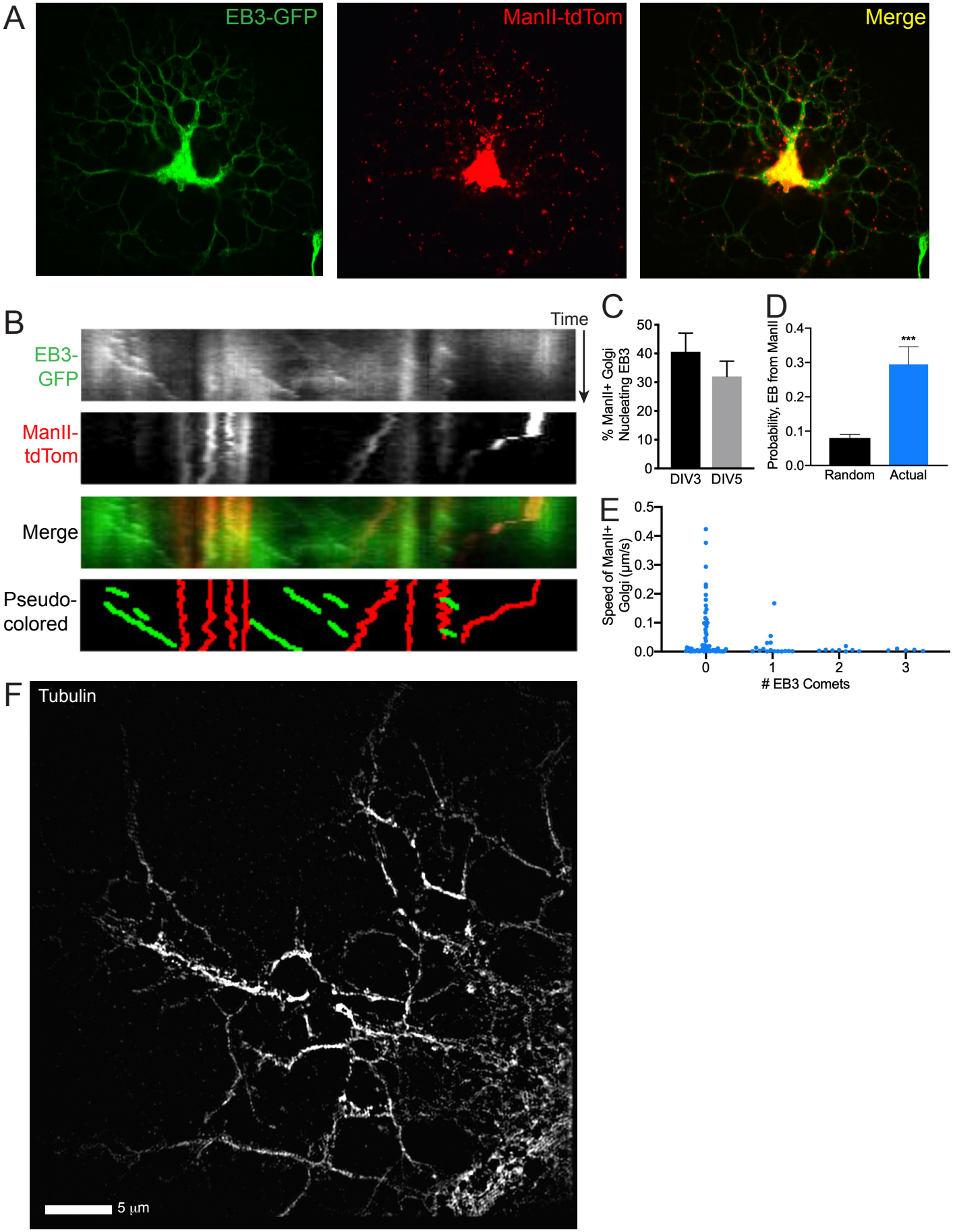


Figure S3

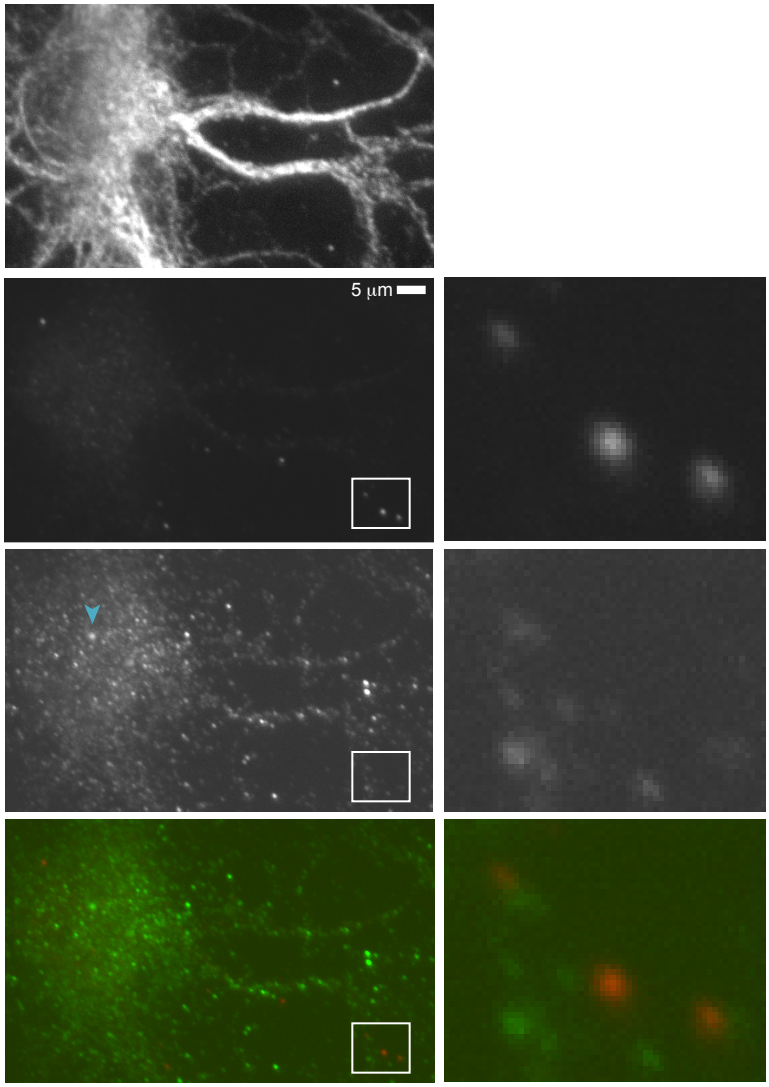
Supplemental Figure 3

Click here to access/download;Supplemental Figure;Figure S3 GTub.pdf

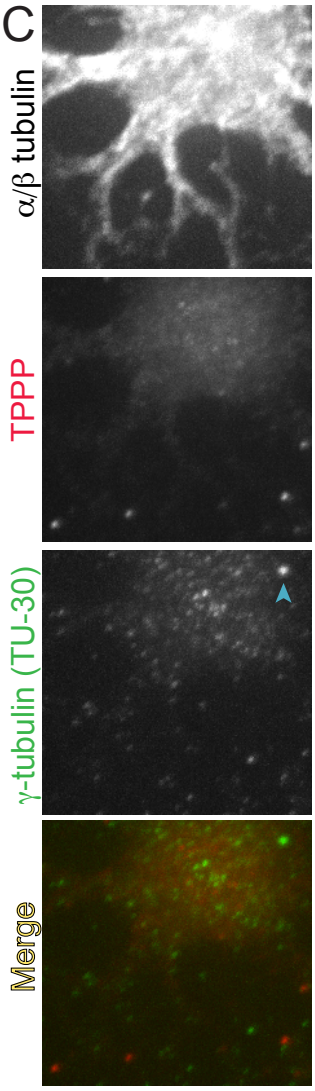
A

Antibody Name	Centrosome Staining?	Golgi Outpost Staining?	Antibody Type	Species Reactivity	Dilution
DQ-19 (Sigma)	Yes	No	Rabbit Polyclonal IgG	Human, rat, Xenopus	1:250
GTU-88 (Sigma)	Yes	No	Mouse Monoclonal IgG1	Chicken, cow, dog, hamster, human, mouse, rat, Xenopus	1:500
T3559 (Sigma)	Yes	No	Rabbit Polyclonal IgG	Chicken, human	1:5000
T5192 (Sigma)	No	No	Rabbit Polyclonal	Chicken, human	1:1000
TU-30 (Santa Cruz)	Yes	No	Mouse Monoclonal IgG1	Human, mouse, pig, rat	1:50

B



C



D

Protein Name	Gene	TRIAL #1 GFP IP (Control)	TRIAL #1 TPPP IP	TRIAL #1 Golgi Input	TRIAL #2 GFP IP (Control)	TRIAL #2 TPPP IP	TRIAL #2 Golgi Input	TRIAL #3 GFP IP (Control)	TRIAL #3 TPPP IP	TRIAL #3 Golgi Input
Tubulin alpha-1A	*Tuba1a*	9	5	79	5	111	214	5	56	251
Tubulin alpha-1B	*Tuba1b*	0	67	6	5	107	208	5	53	238
Tubulin alpha-1C	*Tuba1c*	0	0	0	5	102	194	5	52	230
Tubulin alpha-4A	*Tuba4a*	0	2	0	2	74	143	2	35	143
Tubulin alpha-L3	*Tubal3*	0	0	0	1	15	29	0	0	0
Tubulin beta-2A	*Tubb2a*	0	85	6	5	133	231	5	100	435
Tubulin beta-2B	*Tubb2b*	13	3	91	5	132	231	0	0	0
Tubulin beta-3	*Tubb3*	0	28	12	0	111	204	3	76	319
Tubulin beta-4A	*Tubb4a*	0	2	0	2	99	211	0	60	276
Tubulin beta-4B	*Tubb4b*	0	12	0	3	107	236	0	72	337
Tubulin beta-5	*Tubb5*	0	7	2	4	125	259	6	93	359
Tubulin beta-6	*Tubb6*	0	2	0	0	64	127	0	44	190
Tubulin gamma-1	*Tubg1*	0	0	0	0	0	0	0	0	2

Supplemental Figure 4

Click here to access/download;Supplemental Figure;Figure S4 Biophysics EB.pdf

Figure S4

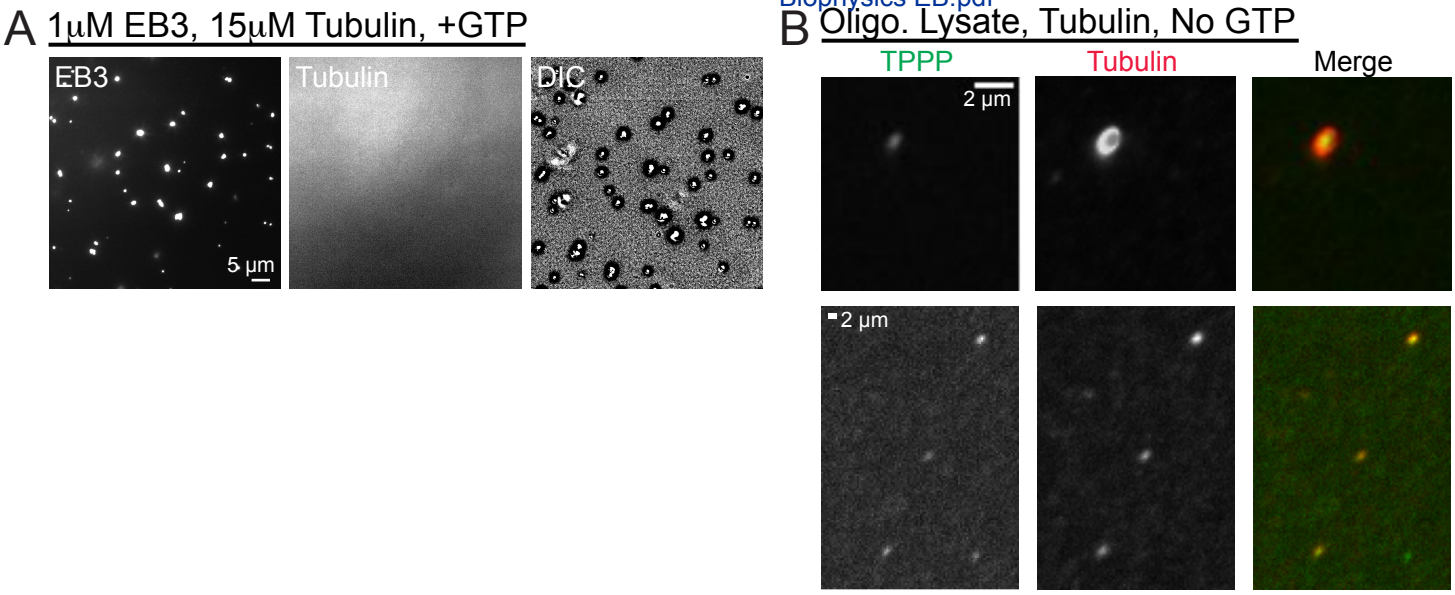


Figure S5
Supplemental Figure 5
Click here to access/download;Supplemental Figure;Figure S5 SpinalCord siRNA 201906.pdf

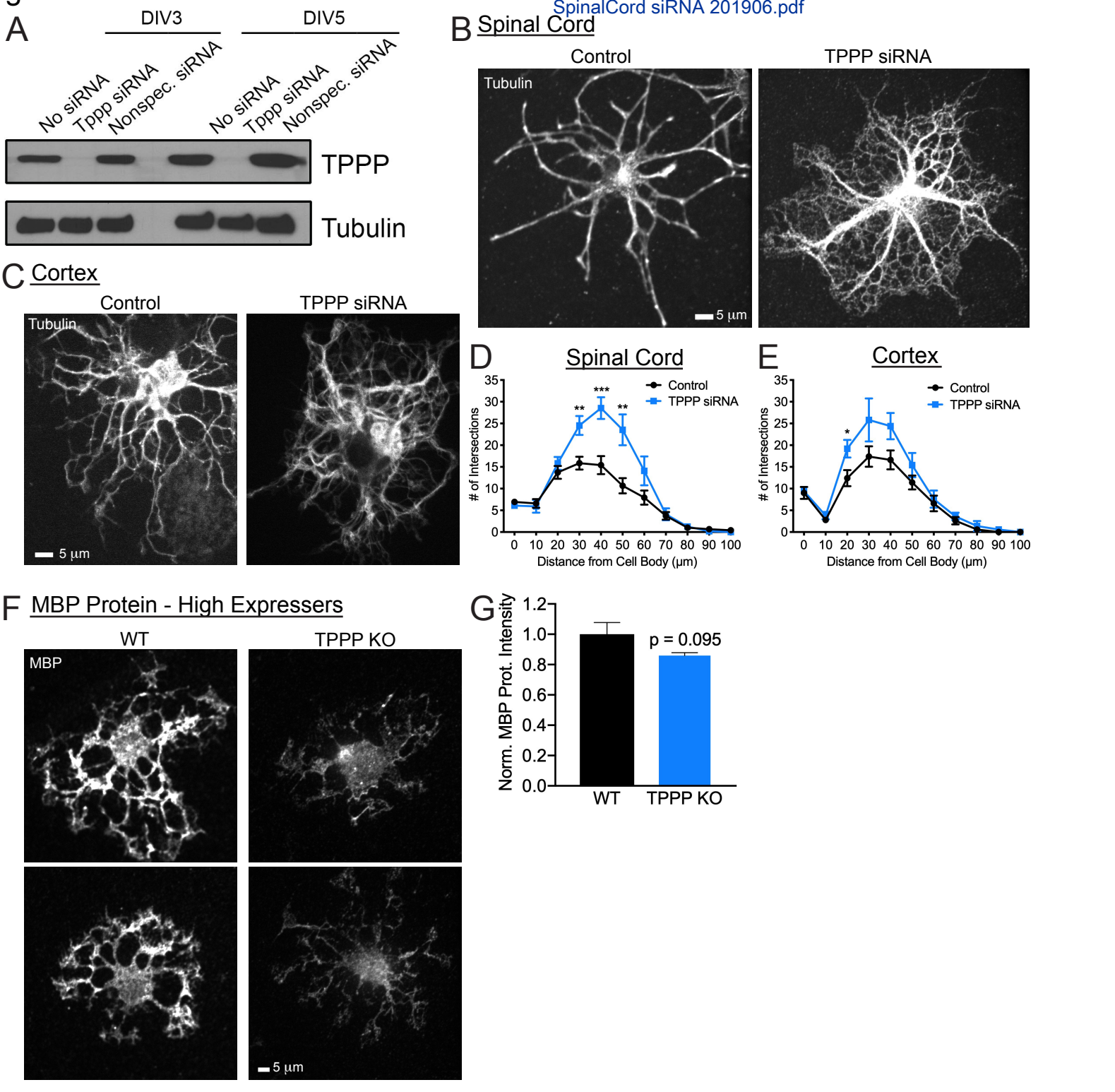


Figure S6

Supplemental Figure 6

Click here to access/download;Supplemental Figure;Figure S6 TPPPKO Histology copy.pdf

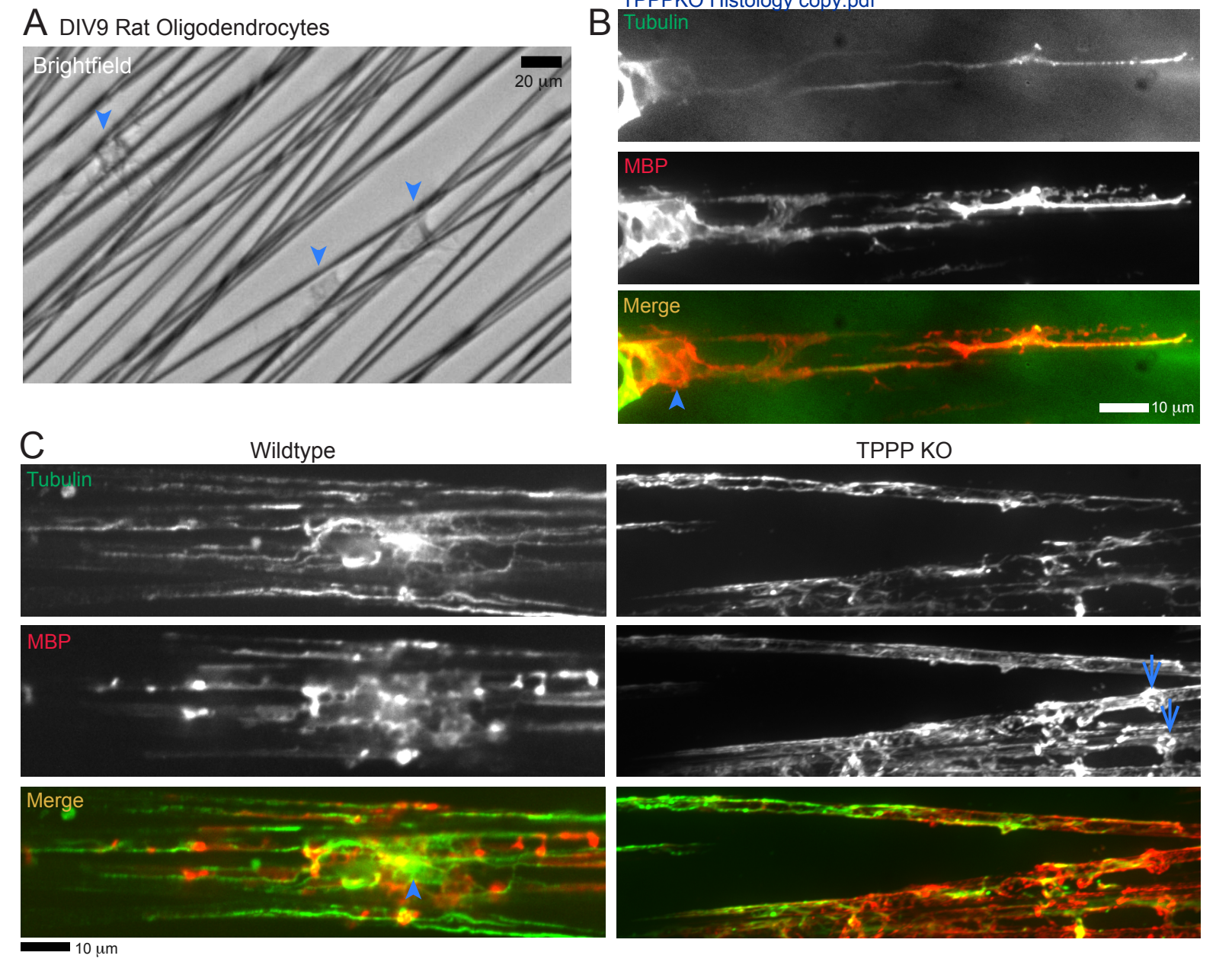
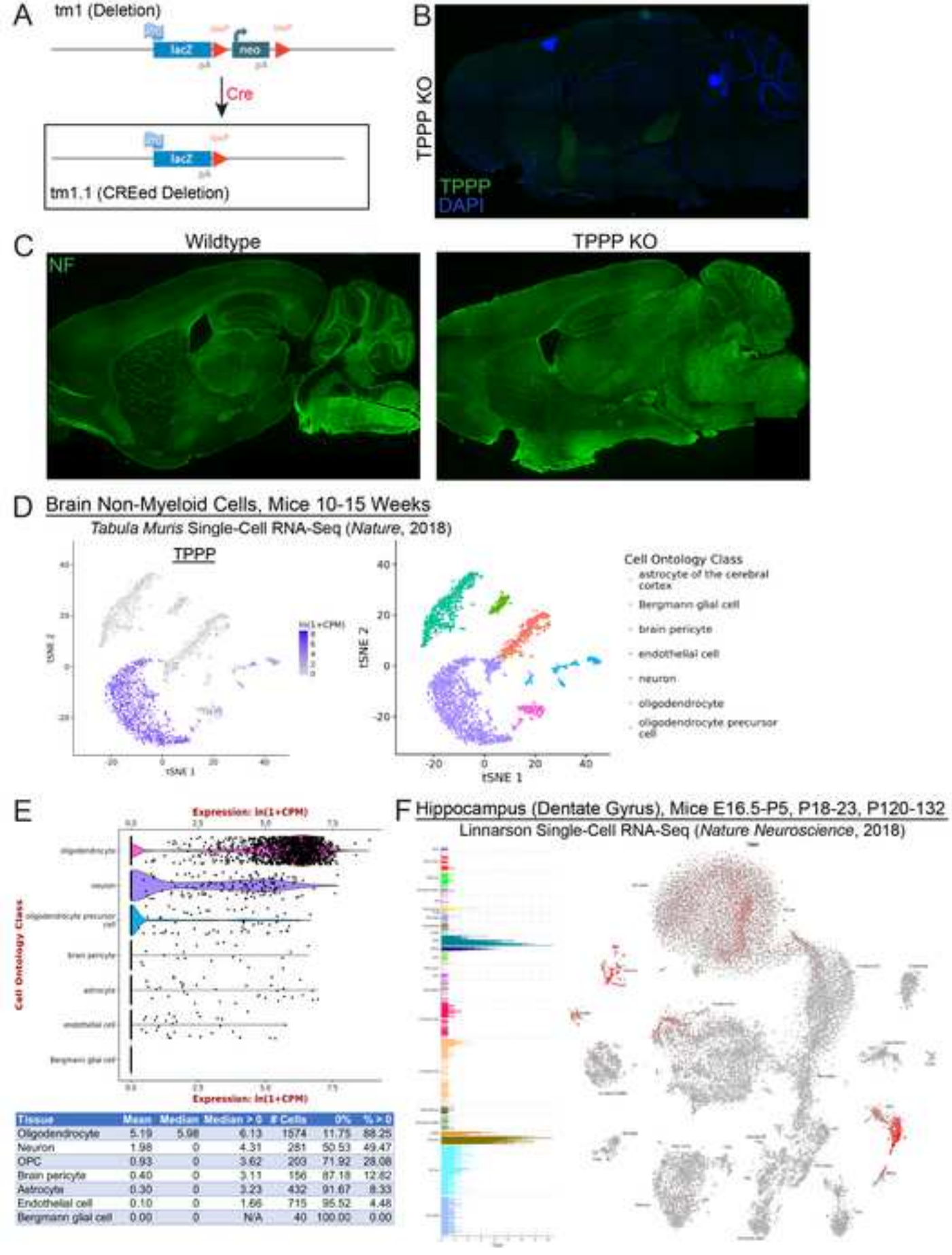


Figure S7



Tissue	Mean	Median	Median > 0	# Cells	0%	% > 0
Oligodendrocyte	5.19	5.98	6.13	1574	11.75	88.25
Neuron	1.98	0	4.31	281	50.53	49.47
OPC	0.93	0	3.62	203	71.92	28.08
Brain pericyte	0.40	0	3.11	156	87.18	12.82
Astrocyte	0.30	0	3.23	432	91.67	8.33
Endothelial cell	0.10	0	1.66	715	95.52	4.48
Bergmann glial cell	0.00	0	N/A	40	100.00	0.00