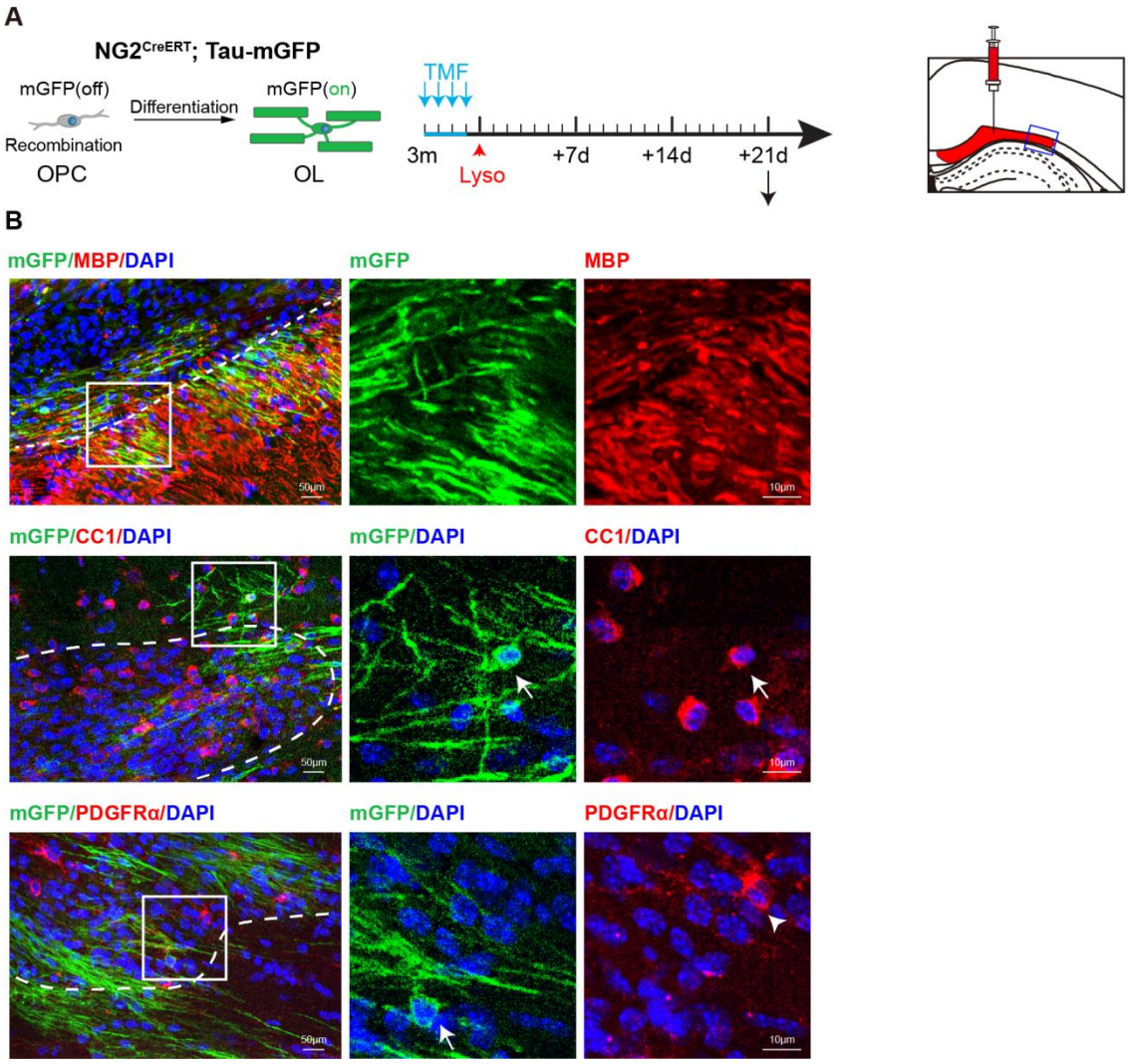
EXTENDED DATA FIGURES

Extended Data Figure 1



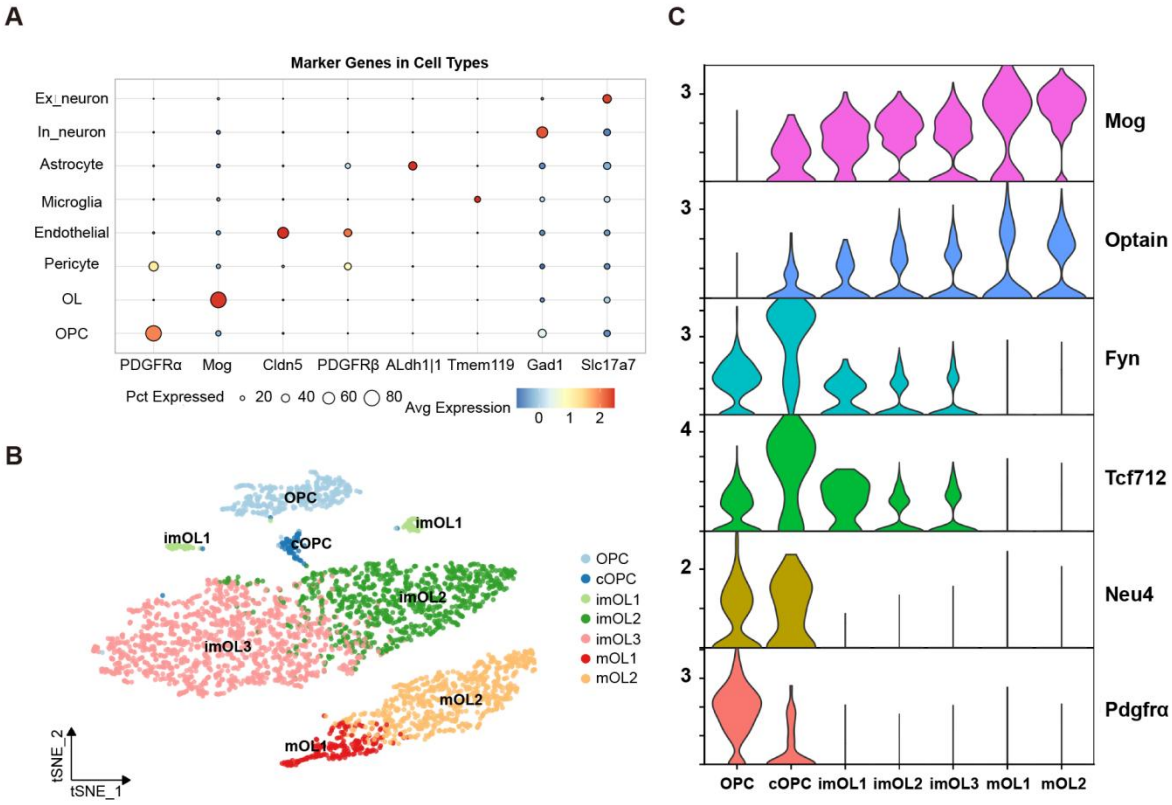
Extended Data Fig. 1: Labeling remyelination by cell-lineage labeling and tracing

(A) Schematic diagram displaying the time course for tamoxifen induction, Lyso injection, histology, and expression pattern of mGFP in NG2^{CreERT}; Tau-mGFP mice.

(B) Representative images for mGFP-positive signal (green) with MBP (red, upper panels), CC1-positive cells (red, middle panels), and PDGFRα-positive cells (red, lower panels), counterstained with DAPI, respectively. The dotted lines indicate lesion area. The middle and right panels are enlarged images of box area in left panels.

Scale bars: 50 μm (left panels of B); 10 μm (middle and right panels of B).

Extended Data Figure 2

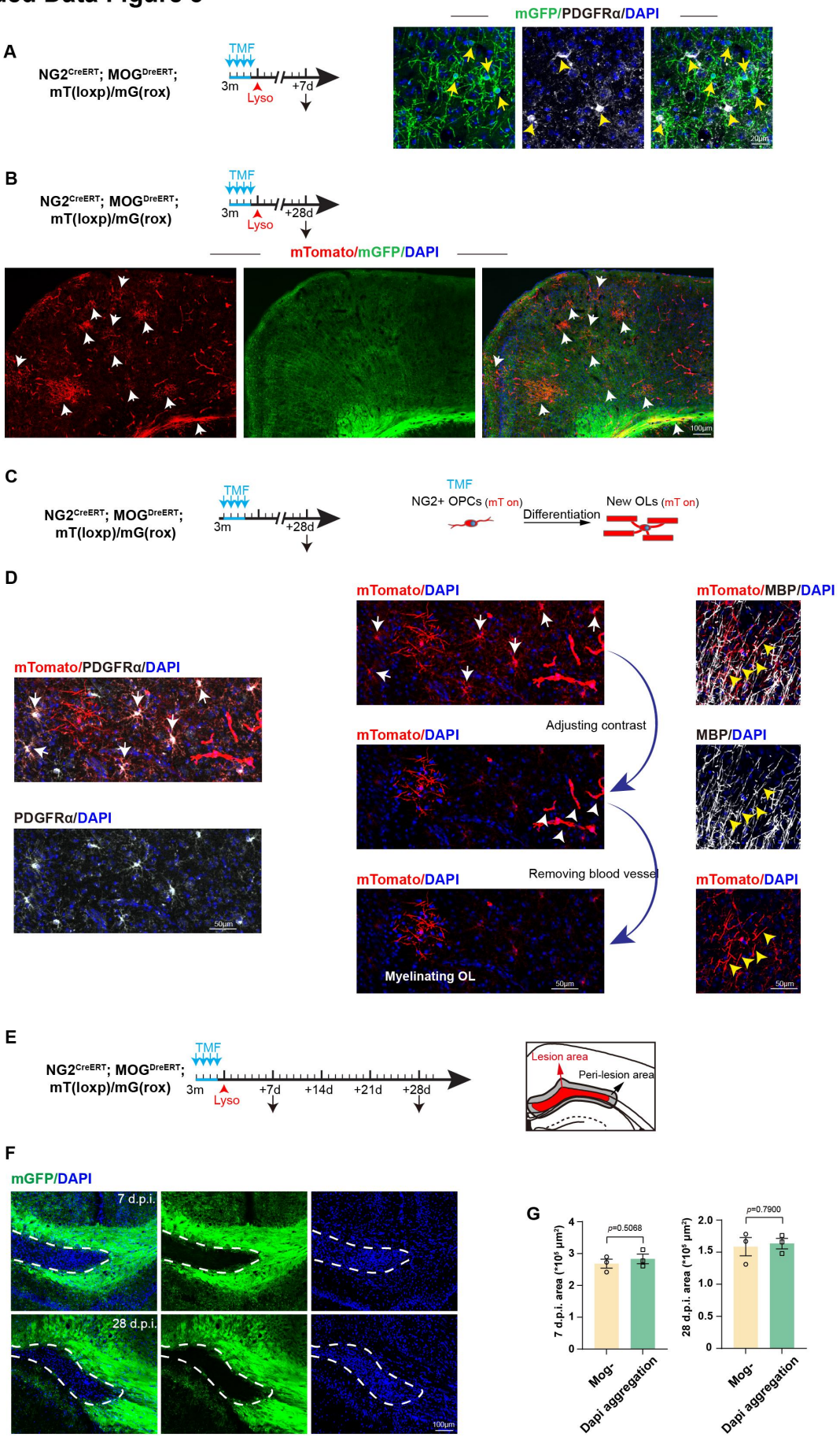


Extended Data Fig. 2: snRNA-seq data of brains after demyelination

(A) Dot plots showing the average expression of the known cell type markers in each cluster. **(B)** tSNE plots identifying OL lineage cells, including OPC, cOPC, imOL1, imOL2, imOL3, mOL1, and mOL2.

(C) Violin plots showing the expression level of known cell type markers for oligodendrocyte lineage cells.

Extended Data Figure 3



Extended Data Fig. 3: Validating the dual inducible mouse line for revealing myelin dynamics and workflow of segregating mTomato-positive newly-formed OLs

(A) Schematic diagram displaying tamoxifen induction, Lyso injection and histology (left panel), and representative images (right panels) displaying mGFP-positive signal (green), which were not co-labeling with PDGFRα (white). Yellow arrow: mGFP-positive cell. Yellow arrowhead: PDGFRα-positive cell.

(B) Schematic diagram displaying the time course for tamoxifen induction, Lyso injection and histology, and representative images of mTomato- and mGFP-positive signal in the $NG2^{CreERT}$; MOG^{DreERT}; mT(loxp)/mG(rox) mice. White arrow: mTomato-positive new myelin.

(C) Schematic diagram displaying the time course for tamoxifen induction and histology (left panel), and expression pattern of mTomato in $NG2^{CreERT}$; MOG^{DreERT}; mT(loxp)/mG(rox) mice (right panel).

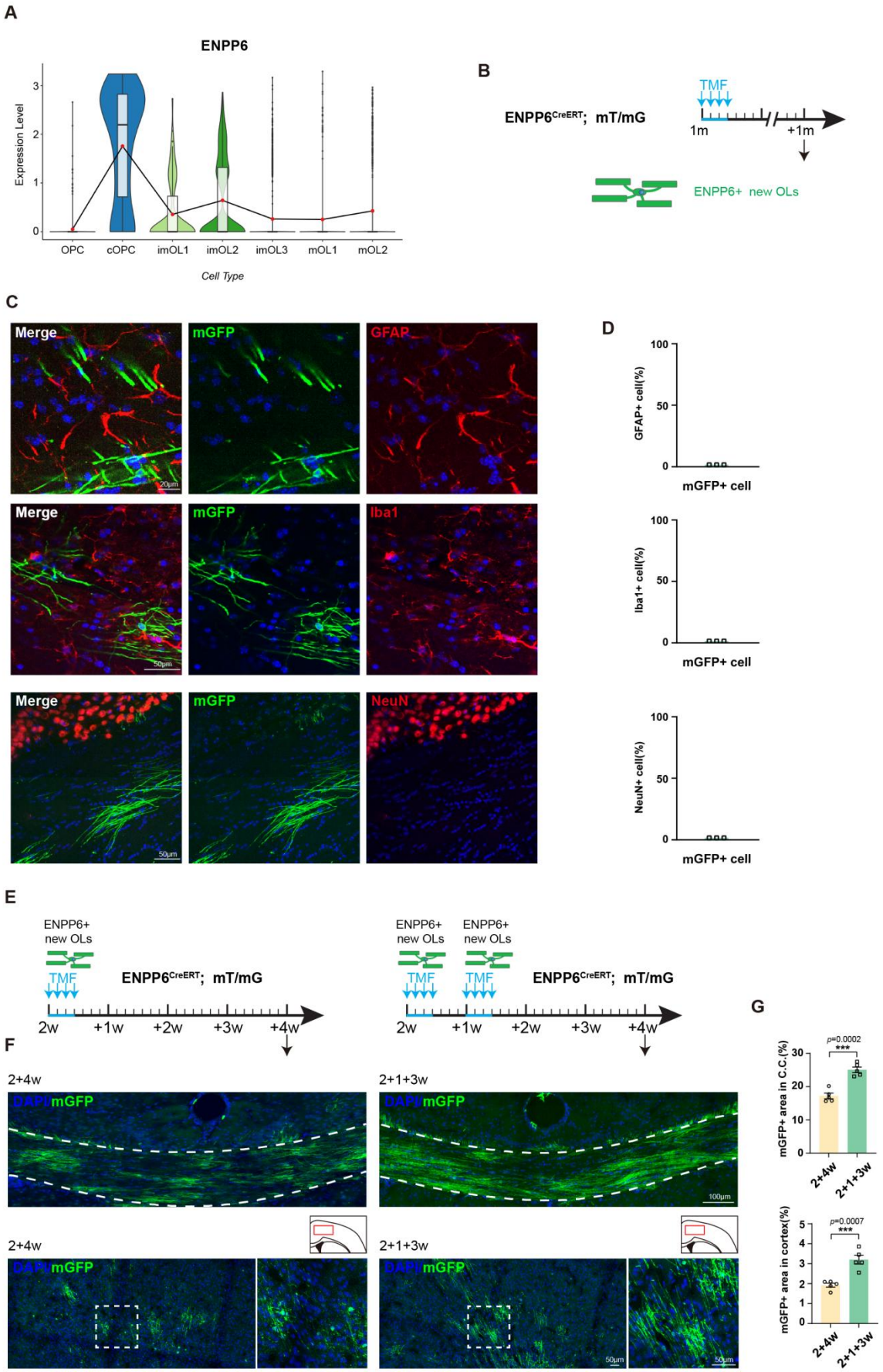
(D) Working flows segregating mTomato-positive OPCs and pericytes from mTomato-positive OLs in $NG2^{CreERT}$; MOG^{DreERT}; mT(loxp)/mG(rox) brains. White arrow: mTomato/PDGFRα double-positive OPCs. Yellow arrowhead: mTomato-positive new myelin. White arrowhead: mTomato-positive pericytes.

(E) Schematic diagram displaying the time course for tamoxifen induction, Lyso injection and histology in the $NG2^{CreERT}$; MOG^{DreERT}; mT(loxp)/mG(rox) mice.

(F, G) Representative images (F) and quantification (G) of mGFP-negative demyelinated lesion area and DAPI-aggregating area (blue) at 7 d.p.i. and 28 d.p.i.. The dotted lines indicate lesion area (F).

Scale bars: 20 μm (A); 100 μm (B and F); 50 μm (D).

Extended Data Figure 4



Extended Data Fig. 4: Validating the ENPP6^{CreERT} line for labeling newly-formed OLs

(A) Violin plots showing the expression level of *Enpp6* in cluster of oligodendrocyte lineage cells.

(B) Schematic diagram displaying the time course for tamoxifen induction and histology in ENPP6^{CreERT}; mT/mG mice.

(C) Representative images for mGFP-positive signal (green) with GFAP (red, upper panels), Iba1 (red, middle panels), and NeuN (red, lower panels), respectively.

(D) Quantification of GFAP-positive cells, Iba1-positive cells, and NeuN-positive cells in mGFP-positive cells in ENPP6^{CreERT}; mT/mG mice, respectively.

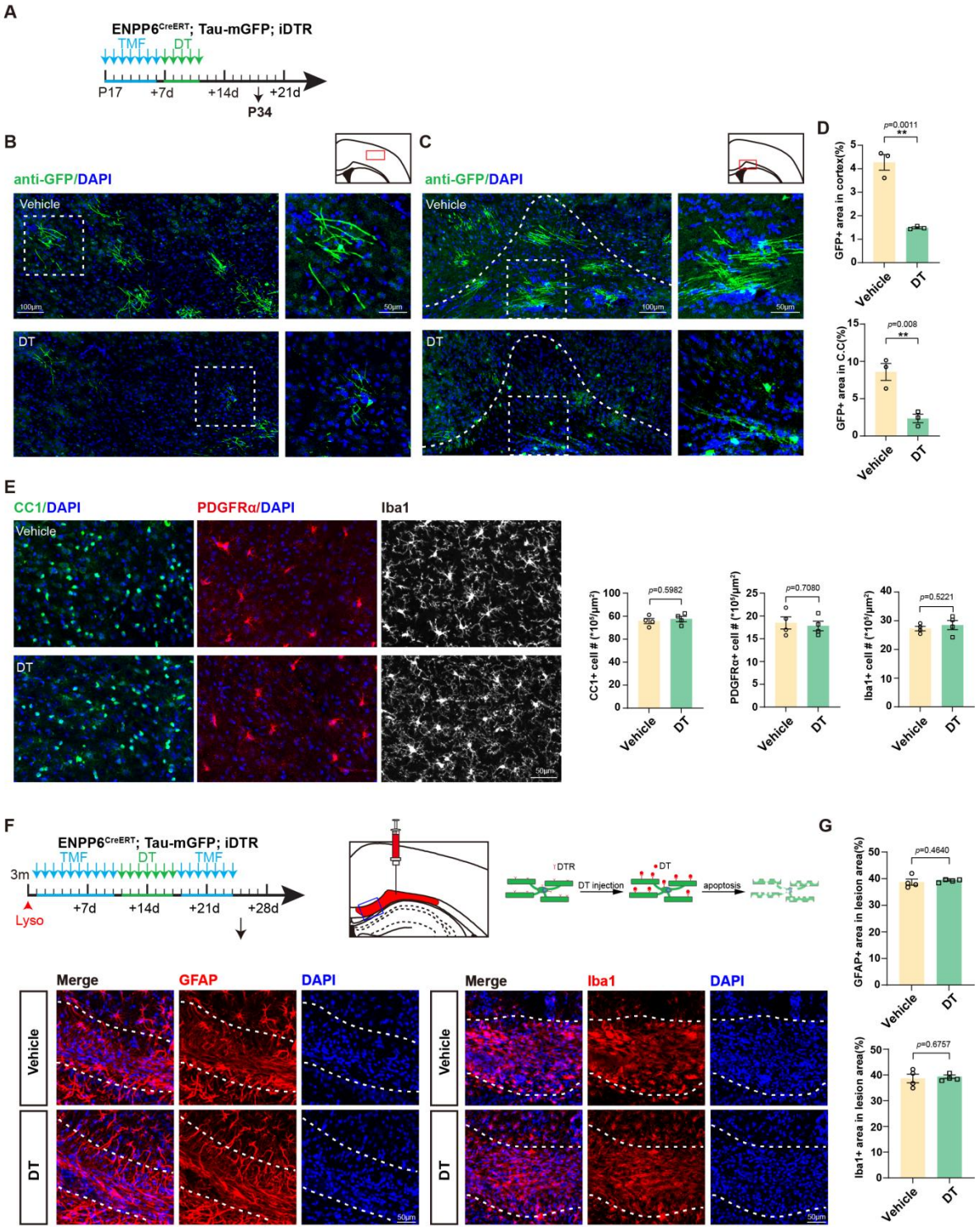
(E) Schematic diagram displaying the time course for the pattern of tamoxifen induction and histology in ENPP6^{CreERT}; mT/mG mice.

(F) Representative images of mGFP-positive signal in corpus callosum (upper panels) and cortex (lower panels) in 6-week-old brains, which experienced different frequencies of tamoxifen induction. The dotted lines indicate corpus callosum (upper panels). The right panels are enlarged images of dotted boxes in the left panels (lower panels).

(G) Quantification of mGFP-positive area in the corpus callosum and cortex in 6-week-old brains, experiencing different frequencies of tamoxifen induction.

Scale bars: 20 μm (upper panels of C); 50 μm (middle and lower panels of C, and lower panels of F); 100 μm (upper panels of F). Error bars represent mean ± s.e.m. ***$p < 0.001$.

Extended Data Figure 5



Extended Data Fig. 5: Validation of DT-induced ENPP6-positive cell apoptosis

(A) Schematic diagram displaying the time course for tamoxifen induction, DT injection, and histology in ENPP6^{CreERT}; Tau-mGFP; iDTR mice.

(B, C) Representative images of GFP expression in the cortex (B) and corpus callosum (C) of vehicle- and DT-treated brains. The right panels are enlarged images of dotted boxes in the left panels. The dotted lines indicate the corpus callosum (C).

(D) Quantification of GFP expression in corpus callosum and cortex of vehicle- and DT-treated mice.
(E) Representative images and quantification of CC1-positive (green), PDGFRα-positive (red), and Iba1-positive (white) cells in the cortex after DT or vehicle injection.
(F) Schematic diagram displaying the time course for Lyso injection, tamoxifen induction, DT injection, and histology (upper left panels), and schematic pattern of ENPP6^{CreERT}; Tau-mGFP; iDTR mice (upper right panel). Representative images of GFAP-positive and Iba1-positive cells in the lesion area (lower panels). Dotted lines indicate the lesion area.
(G) Quantification of GFAP-positive and Iba1-positive area in lesions of vehicle- and DT-treated mice.
Scale bars: 50 μm (right panels of B and C, E and F), 100μm (left panels of B and C). Error bars represent mean ± s.e.m. $^{**}p < 0.01$.