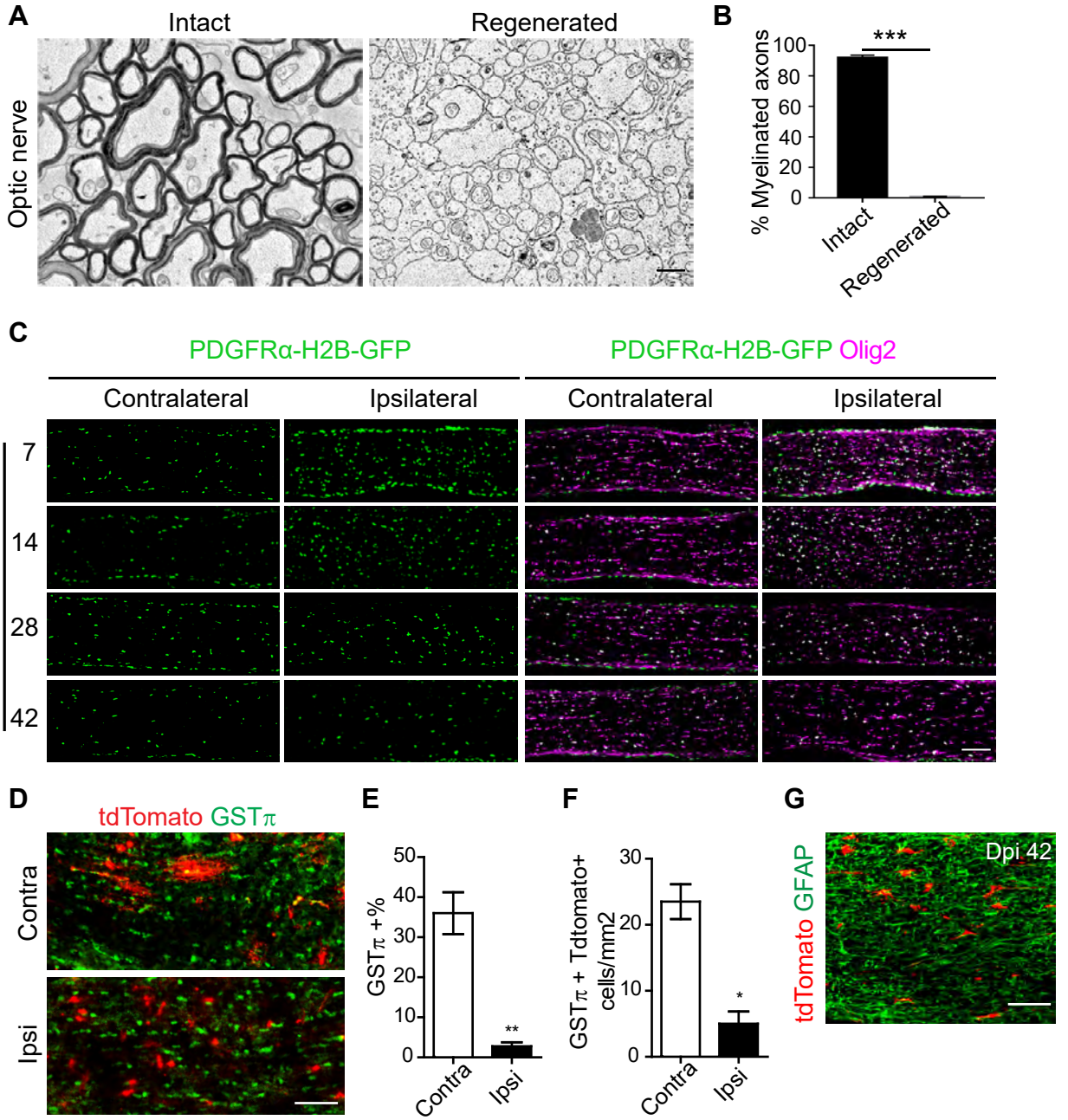
A
Intact
Regenerated
Optic nerve
B
% Myelinated axons
100
80
60
40
20
0
***
Intact
Regenerated
C
PDGFRα-H2B-GFP
PDGFRα-H2B-GFP Olig2
Contralateral
Ipsilateral
Contralateral
Ipsilateral
Days post injury
7
14
28
42
D
tdTomato GSTπ
Contra
Ipsi
E
GSTπ +%
50
40
30
20
10
0
**
Contra
Ipsi
F
GSTπ + Tdtomato+ cells/mm2
30
20
10
0
*
Contra
Ipsi
G
tdTomato GFAP
Dpi 42

Figure S1

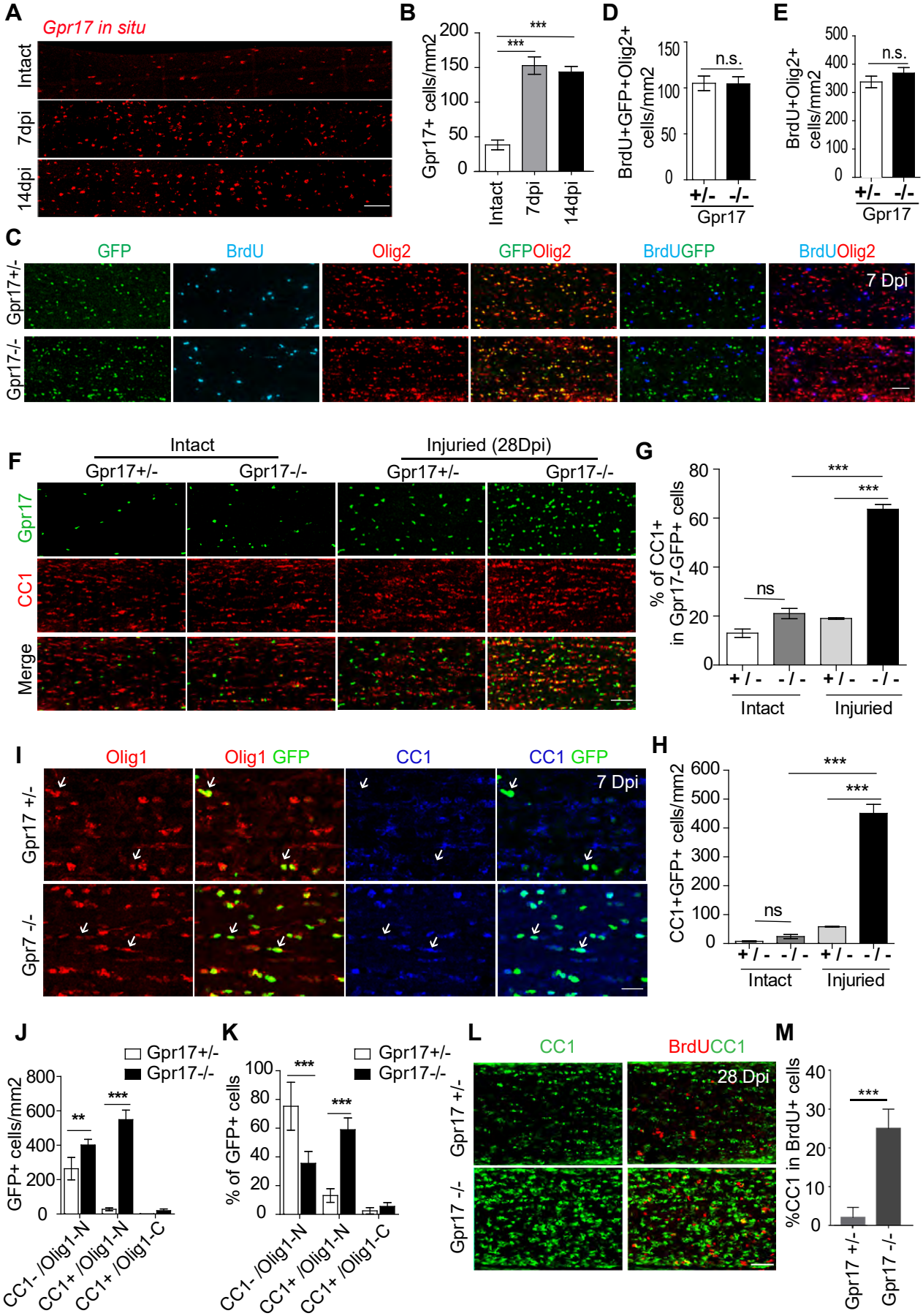
A
Gpr17 in situ
Intact
7dpi
14dpi
B
Gpr17+ cells/mm2
200
150
100
50
0
***
***
Intact
7dpi
14dpi
D
BrdU+GFP+Olig2+ cells/mm2
150
100
50
0
n.s.
+/-
-/-
Gpr17
E
BrdU+Olig2+ cells/mm2
500
400
300
200
100
0
n.s.
+/-
-/-
Gpr17
C
GFP
BrdU
Olig2
GFPOlig2
BrdUGFP
BrdUOlig2
Gpr17+/-
Gpr17-/-
7 Dpi
F
Intact
Injuried (28Dpi)
Gpr17+/-
Gpr17-/-
Gpr17+/-
Gpr17-/-
Gpr17
CC1
Merge
G
% of CC1+ in Gpr17-GFP+ cells
80
60
40
20
0
***
***
ns
+/-
-/-
+/-
-/-
Intact
Injuried
I
Olig1
Olig1 GFP
CC1
CC1 GFP
7 Dpi
Gpr17 +/-
Gpr7 -/-
H
CC1+GFP+ cells/mm2
600
500
400
300
200
100
0
***
***
ns
+/-
-/-
+/-
-/-
Intact
Injuried
J
Gpr17+/-
Gpr17-/-
GFP+ cells/mm2
800
600
400
200
0
**
***
CC1- /Olig1-N
CC1+ /Olig1-N
CC1+ /Olig1-C
K
Gpr17+/-
Gpr17-/-
% of GFP+ cells
100
80
60
40
20
0
***
***
CC1- /Olig1-N
CC1+ /Olig1-N
CC1+ /Olig1-C
L
CC1
BrdUCC1
28 Dpi
Gpr17 +/-
Gpr17 -/-
M
%CC1 in BrdU+ cells
40
30
20
10
0
***
Gpr17 +/-
Gpr17 -/-

Figure S2

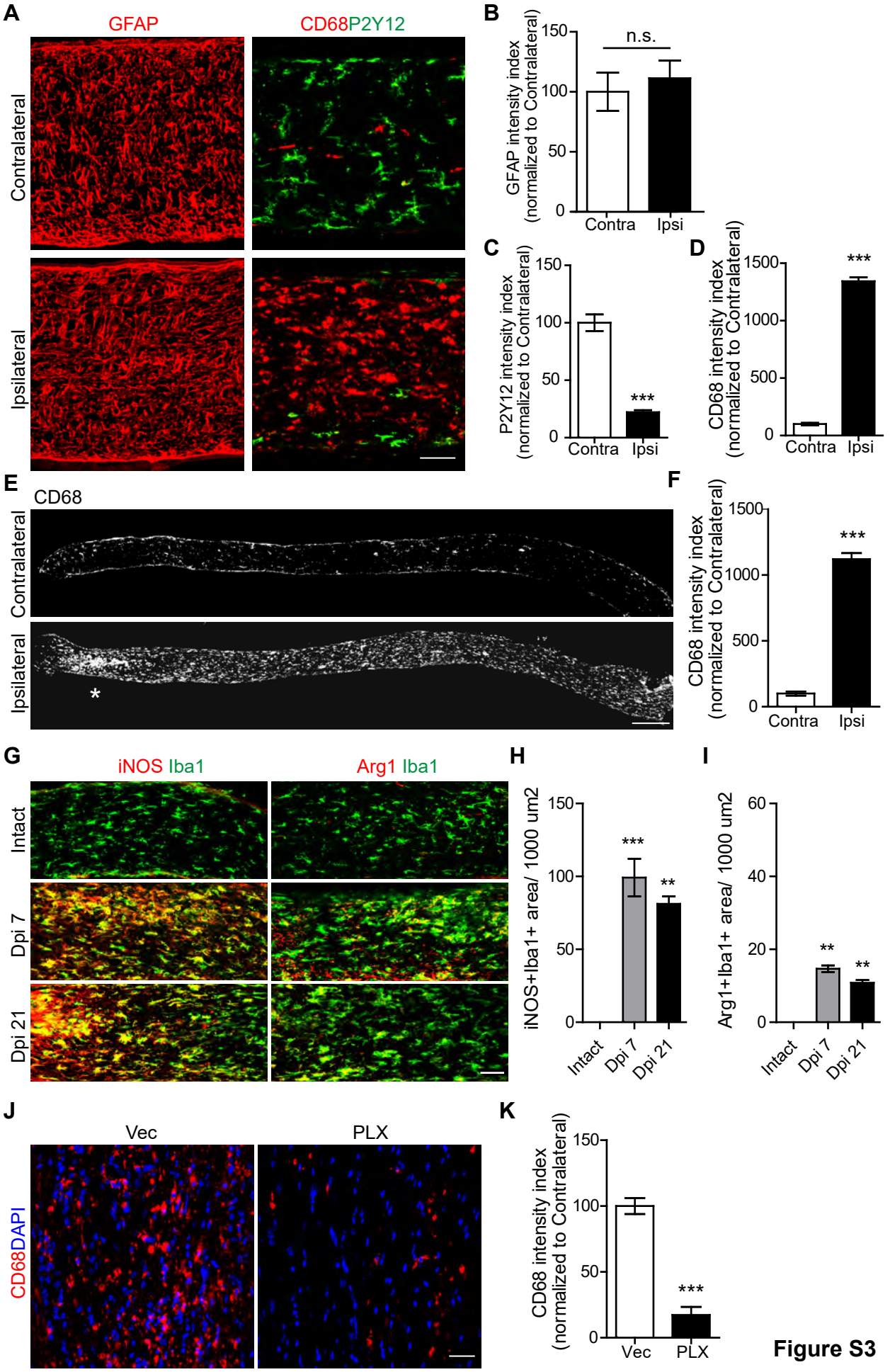
A
GFAP
CD68P2Y12
Contralateral
Ipsilateral
B
GFAP intensity index (normalized to Contralateral)
n.s.
Contra
Ipsi
C
P2Y12 intensity index (normalized to Contralateral)
***
D
CD68 intensity index (normalized to Contralateral)
E
CD68
F
G
iNOS Iba1
Arg1 Iba1
Intact
Dpi 7
Dpi 21
H
iNOS+Iba1+ area/ 1000 um2
I
Arg1+Iba1+ area/ 1000 um2
J
Vec
PLX
CD68DAPI
K

Figure S3

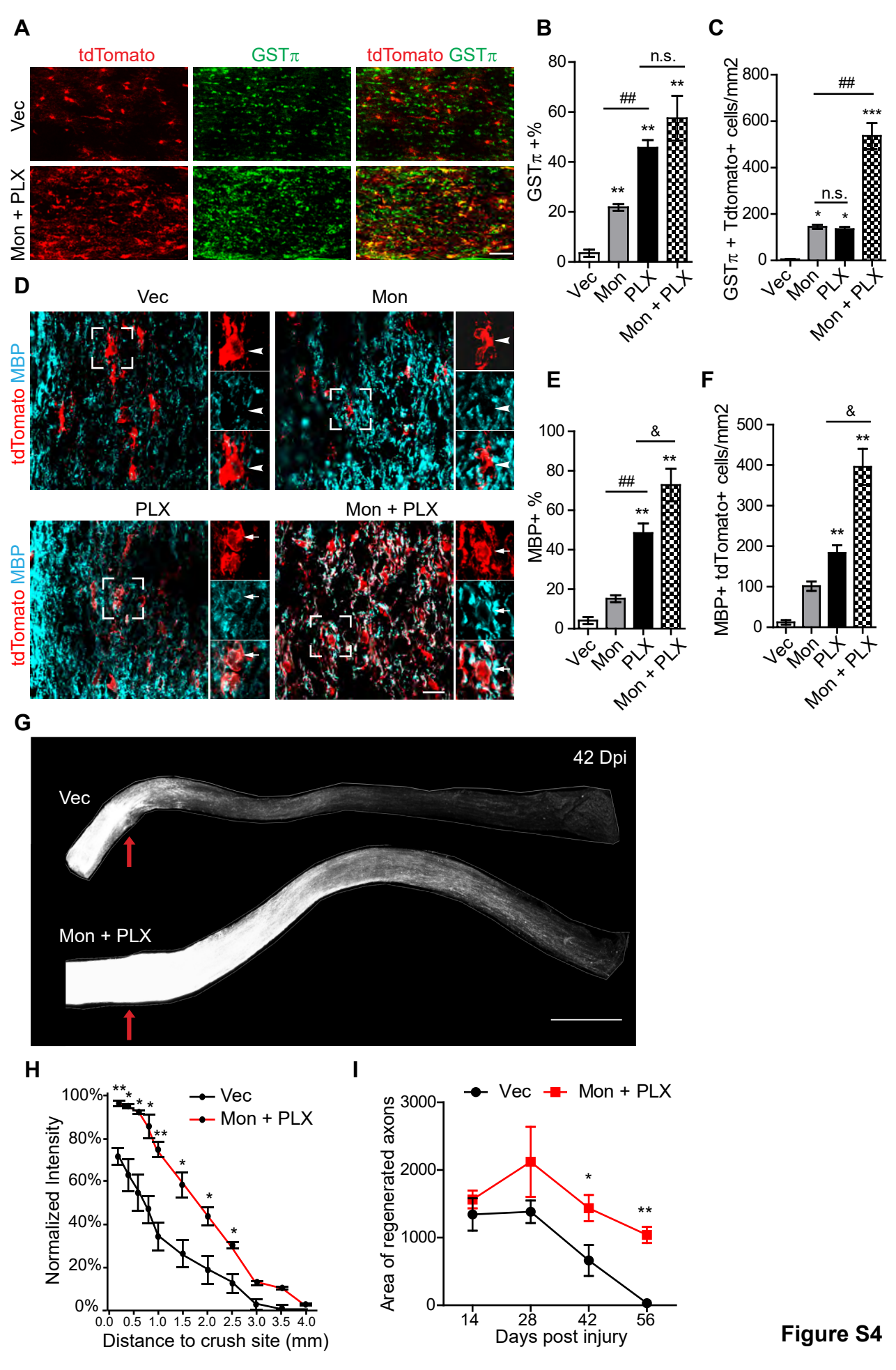
A
tdTomato
GSTπ
tdTomato GSTπ
Vec
Mon + PLX
B
GSTπ +%
n.s.
##
**
Vec
Mon
PLX
Mon + PLX
C
GSTπ + Tdtomato+ cells/mm2
##
n.s.
*
***
D
Vec
Mon
PLX
Mon + PLX
tdTomato MBP
E
MBP+ %
&
F
MBP+ tdTomato+ cells/mm2
G
42 Dpi
Vec
Mon + PLX
H
Normalized Intensity
Distance to crush site (mm)
I
Area of regenerated axons
Days post injury

Figure S4

Figure S1. OPC proliferation and differentiation after optic nerve injury, Related to Figure 1. **(A-B)** Representative electron microscopy images and quantification of myelination percentage of axons in intact or injured optic nerves with regenerated axons. Most axons are myelinated in intact optic nerves while regenerated axons very rarely show spontaneous myelination post optic nerve crush injury in adult mice. N = 6 mice per group. **(C)** Representative images of Olig2/GFP immunostaining after unilateral optic nerve crush injury using PDGFRα-H2B-GPP reporter mice, showing dynamic change of total OPC numbers at different time points. See quantification results in Figure 1C. **(D-F)** Representative images (D) and quantification (proportion in E and cell number in F) of GST-π+ and tdTomato+ cells in injured and intact optic nerves. N = 6 mice per group. **(G)** Representative images of injured optic nerves from PDGFRα-CreER/tdTomato mice, showing very few overlapping between tdTomato and GFAP. Scale bar: 600 nm (A), 100 μm (C, D), 150 μm (G). *, **, *** P < 0.05, 0.01, 0.001, respectively.

Figure S2. Injury-induced GPR17 expression and their inhibitory effects on OPC differentiation, Related to Figure 2. **(A, B)** Representative *in situ* hybridization images (A) of optic nerves showing injury-induced GPR17 expression and their quantification results (B). N = 6 mice per group. **(C-E)** Representative images of cell proliferation in GPR17 KO/GFP KI mice after optic nerve injury. Mice received BrdU injection 3 hours before sample collection, stained with GFP (GPR17), BrdU and/or Olig2 (C). Quantification shows that GPR17 knockout did not affect OPC proliferation (D, E). **(F-H)** Representative images of OPC differentiation in GPR17 KO/GFP KI mice after optic nerve injury. Samples were stained with GFP (GPR17) and CC1 (F), and quantification of the density (H) and the proportion of CC1+ cells among GFP+ cells (G). GPR17 knockout significantly increased CC1+ cells in injured but not intact optic nerves. N = 6 mice per group. **(I-K)** Representative images (I) and quantifications (J-K) of immature and mature oligodendrocytes in GPR17 KO/GFP KI mice after optic nerve injury. Cell populations: CC1-/Olig1-N for un-differentiated OPCs, CC1+/Olig1-N for pre-myelinating oligodendrocytes, and CC1+/Olig1-C for mature myelinating oligodendrocytes. N = 6 mice per group. **(L, M)** Representative images of BrdU-traced OPC differentiation in GPR17 KO/GFP KI mice after optic nerve injury. Mice received daily BrdU injection from dpi 4-10, and samples were stained with GFP (GPR17) and CC1 (L). Quantification results of the proportion of CC1+ cells among BrdU+ cells (M).. N = 6 mice per group. Scale bar: 100 μm (A, C, F, L), 50 μm (I). *, **, *** P < 0.05, 0.01, 0.001, respectively.

Figure S3. Activated microglia in injured optic nerves were depleted by PLX3397 treatment, Related to Figure 3. **(A-D)** Representative images of glial activation from adult mice 4 weeks after unilateral optic nerve crush injury, stained with antibodies against GFAP, CD68 or P2Y12 (A), and quantification of respective immunoreactivity signals (B-D). N =10 mice per group. **(E, F)** Sustained activated microglia in the entire optic nerves distal to the lesion, taken from adult mice at 6 weeks post injury. N = 10 mice per group. **(G-I)** Representative images (G) and quantification (H, I) of optic nerves stained with M1/M2 markers, iNOS, Arginase 1 (Arg1) and Iba1. N = 4 mice per group. **(J-K)** Representative images of the efficiency of PLX 3397 *in vivo*. Adult optic nerves were collected 2 weeks post crush injury, with or without PLX3397 treatment, stained with antibodies against CD68 (J) and quantification of respective immunoreactivity signals (K). N = 6-10 mice per group. Scale bar: 50 μm (A, G), 500 μm (E), 40 μm (J). *, **, *** $P < 0.05$, 0.01, 0.001, respectively.

Figure S4. Better preserved regenerated axons in the mice with the combinatorial treatment of Montelukast and PLX3397, Related to Figure 4. **(A-C)** Representative images (A) and quantification (proportion of GST-π over total tdTomato+ cells in B and cell number in C) of GST-π+ and tdTomato+ cells in optic nerves of different groups. N = 6 mice per group. **(D-F)** Representative images (D) and quantification (proportion in E and cell number in F) of MBP+ and tdTomato+ cells in optic nerves of different groups. D, arrowheads indicate MBP- cells in Vec and Mon groups, while arrows indicate MBP+ cells in PLX and combined treatment groups. N = 6 mice per group. **(G)** Representative images of CTB-labeled regenerating axons in injured optic nerves (42 dpi) from wild-type mice that received intravitreal injection with AAV2/2-CNTF/IGF/OPN, followed by optic nerve crush and with or without combined treatment. **(H, I)** Quantification of regenerating axons in each group. N = 4 mice per group. Scale bar: 750 μm.Scale bar: 60 μm (A), 25 μm (D), 750 μm (H). *, **, *** $P < 0.05$, 0.01, 0.001, respectively compared with Vec. ##, $P < 0.01$ compared with Mon, &, $P < 0.05$ compared with PLX.