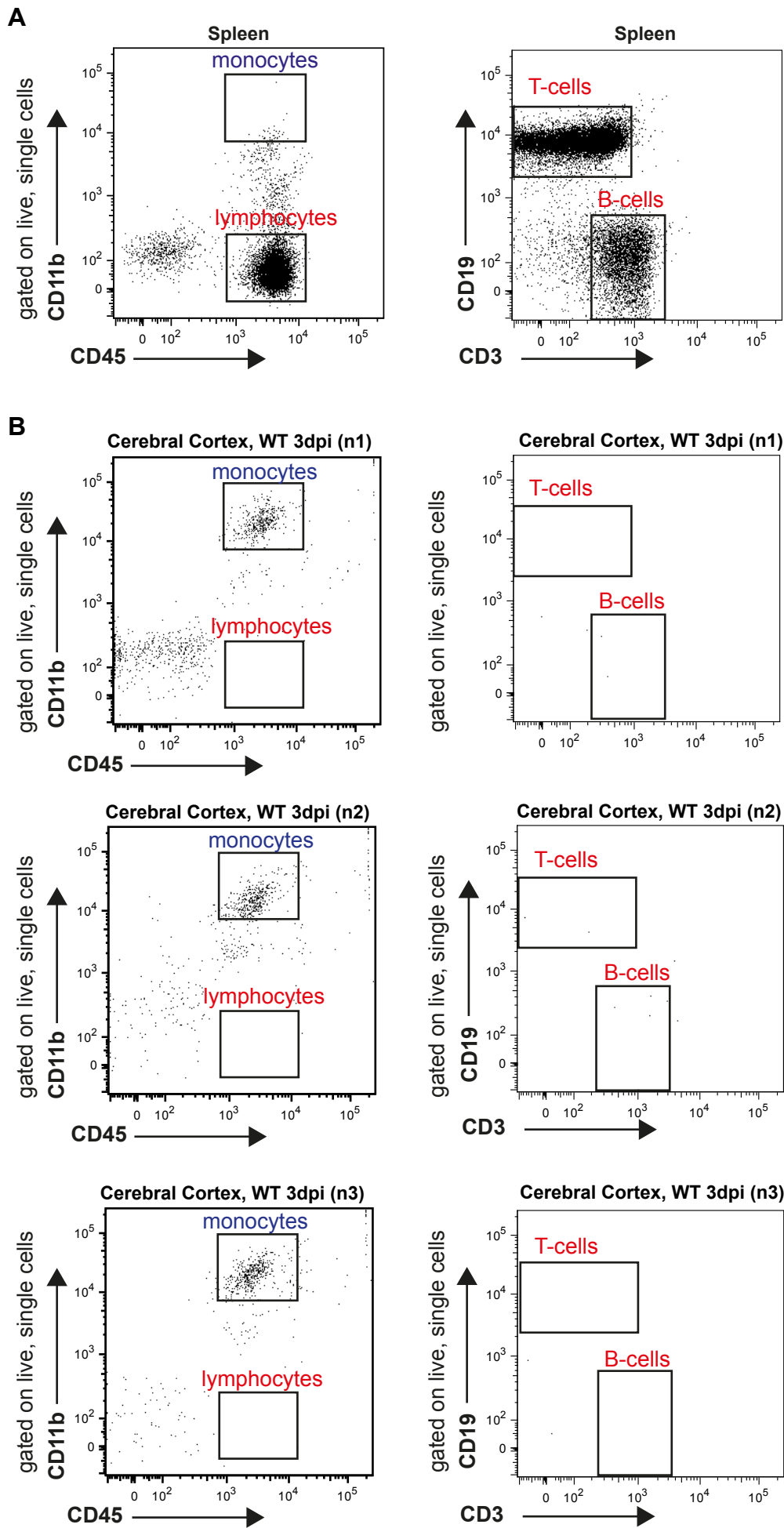
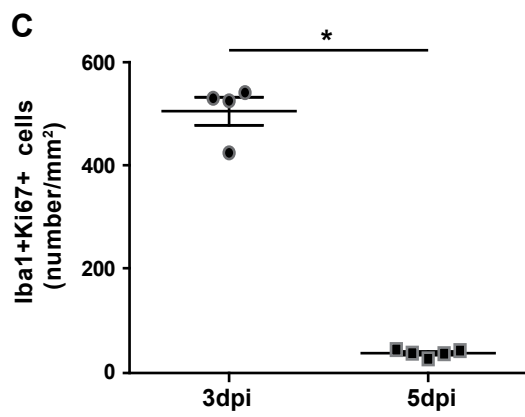
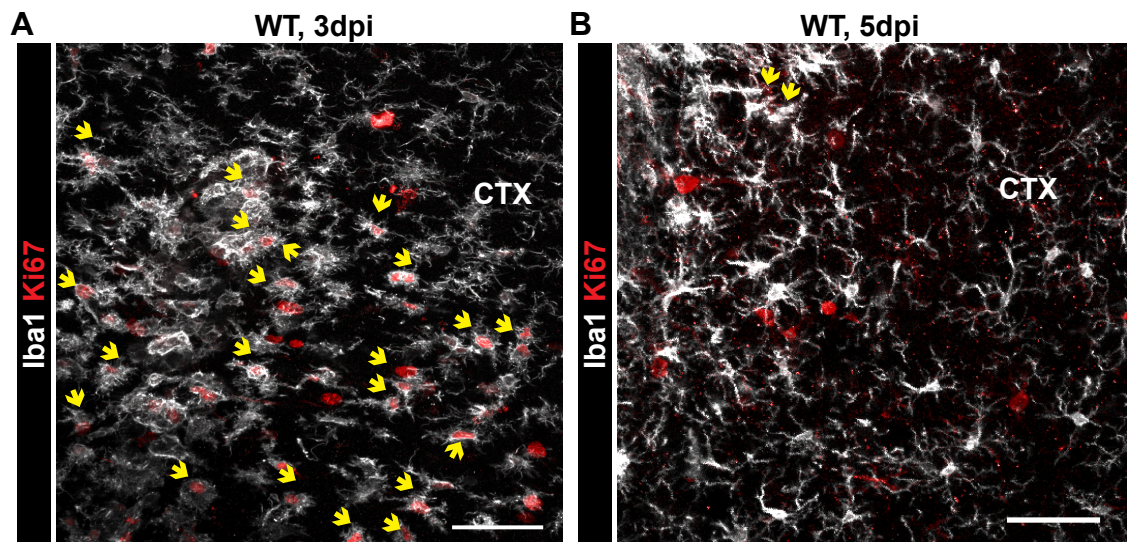


TABLE OF CONTENT

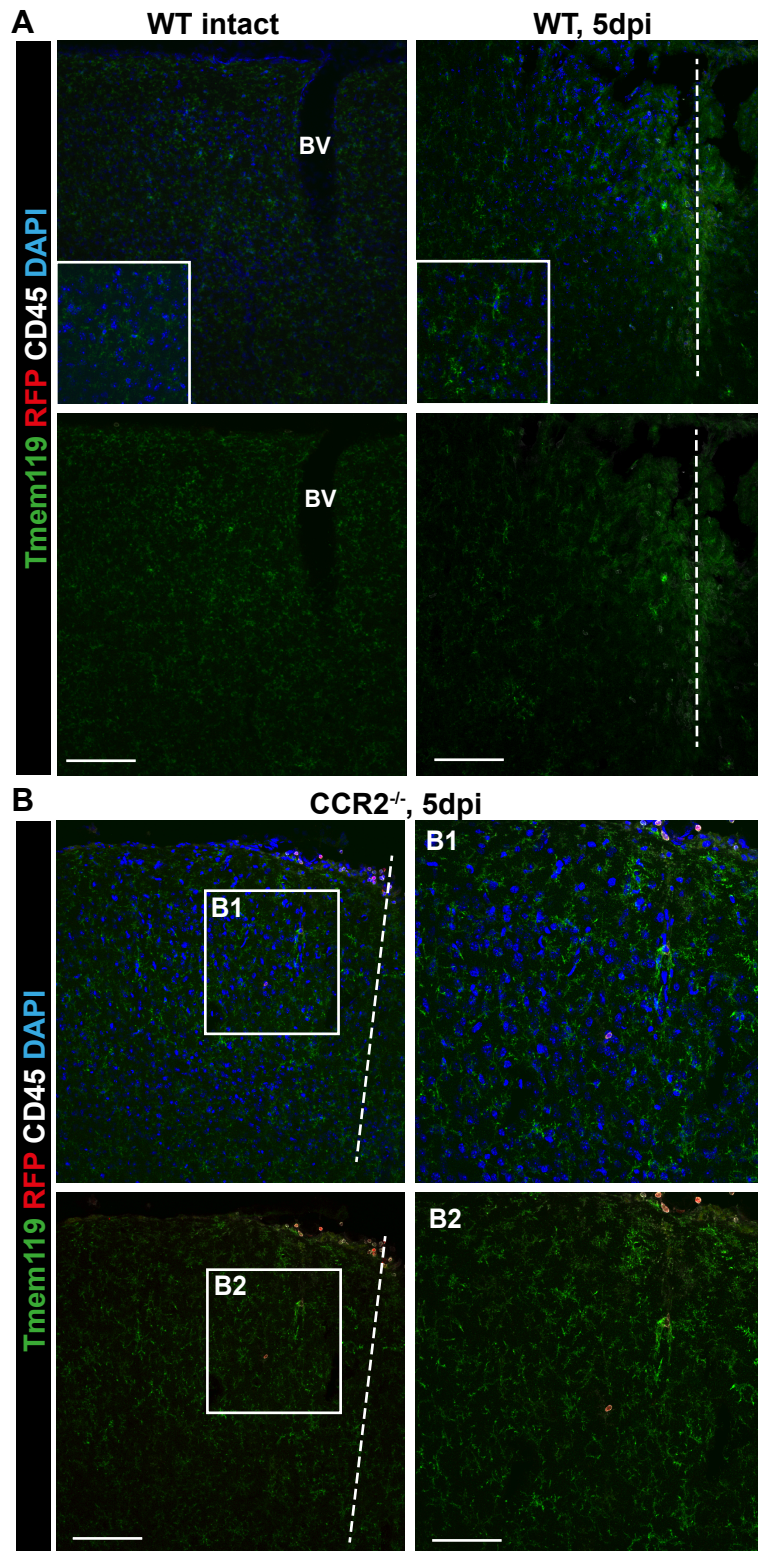
Appendix Figure S1. Absence of lymphocytes and predominance of CD45+/CD11b+ CD45+/CD11b+ monocytes/macrophages in the injured GM parenchyma at 3dpi	1
Appendix Figure S2. Microglia proliferation between 3 and 5 days after injury	2
Appendix Figure S3. Tmem119+ microglia around the stab- wound injury site of WT and CCR2 ^{-/-} mice at 5dpi	3
Appendix Figure S4. Faster resealing of the Blood-Brain Barrier (BBB) in the absence of monocyte invasion	4
Appendix Figure S5. Collagen I immunostaining in WT and CCR2 ^{-/-} mice at 28dpi	5
Appendix Figure S6. Reduced astrogliotic scar in CCR2 ^{-/-} mice at 90dpi	6
Appendix Figure Legends	7-9
Appendix Table S1. Significantly regulated proteins in the biopsy of the injury site isolated at 5dpi from WT compared to CCR2 ^{-/-} mice	10
Appendix Table S2. Biological processes overrepresented with proteins more abundant in WT	11
Appendix Table S3. Biological processes overrepresented with proteins more abundant in CCR2	12
Appendix Table S4. Primary and secondary antibodies used for immunohistological analysis	13



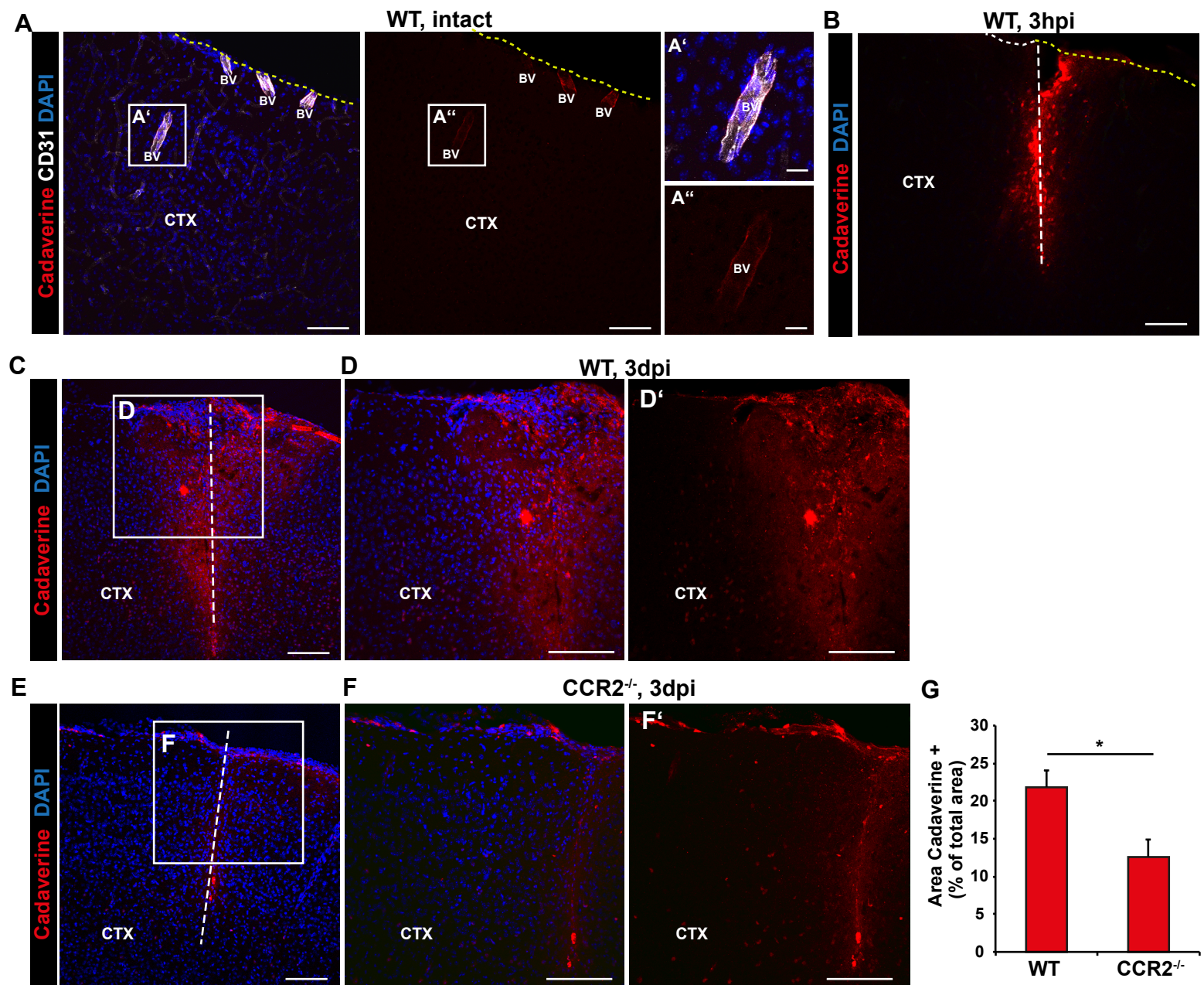
Appendix Figure S1



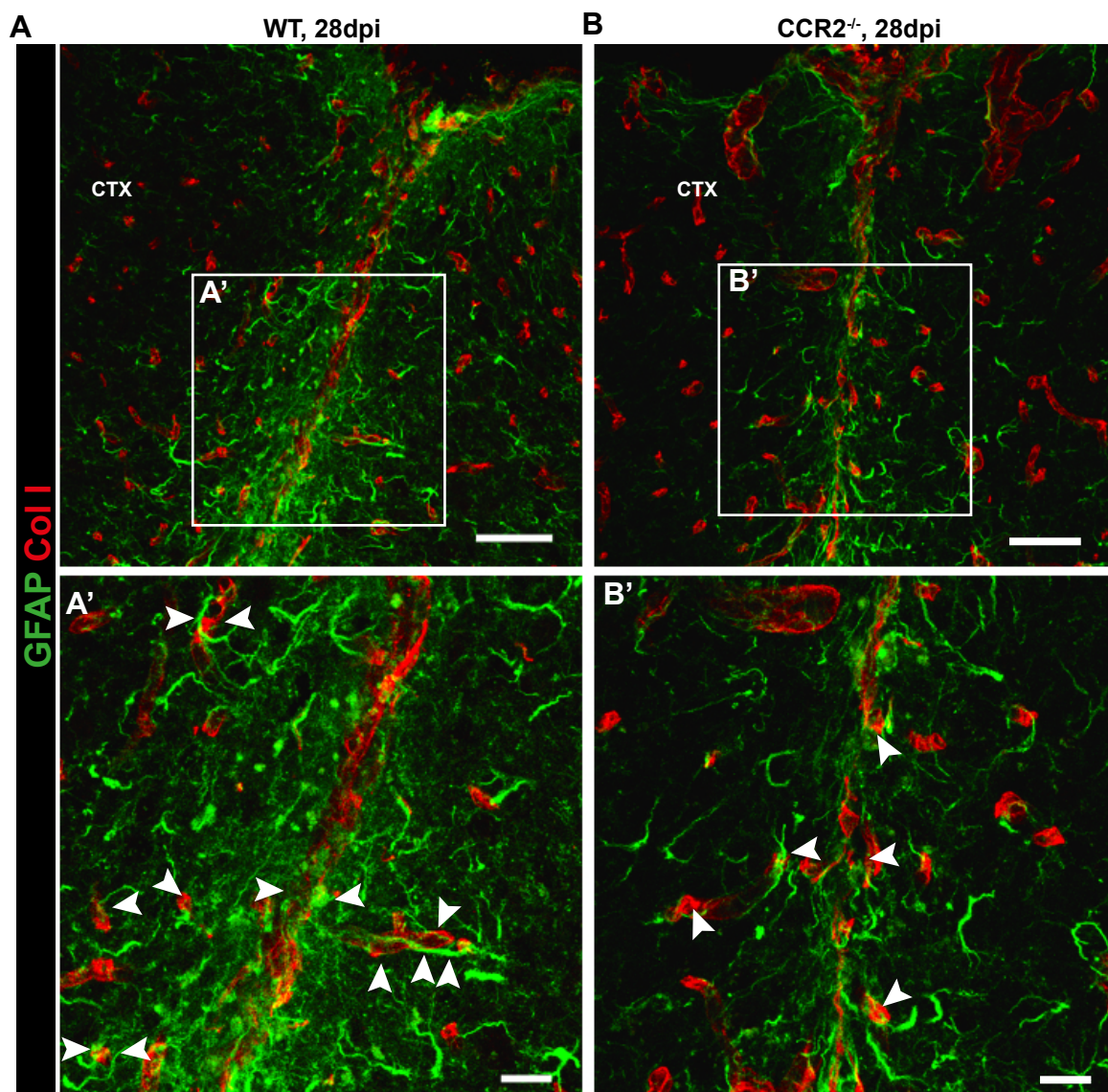
Appendix Figure S2



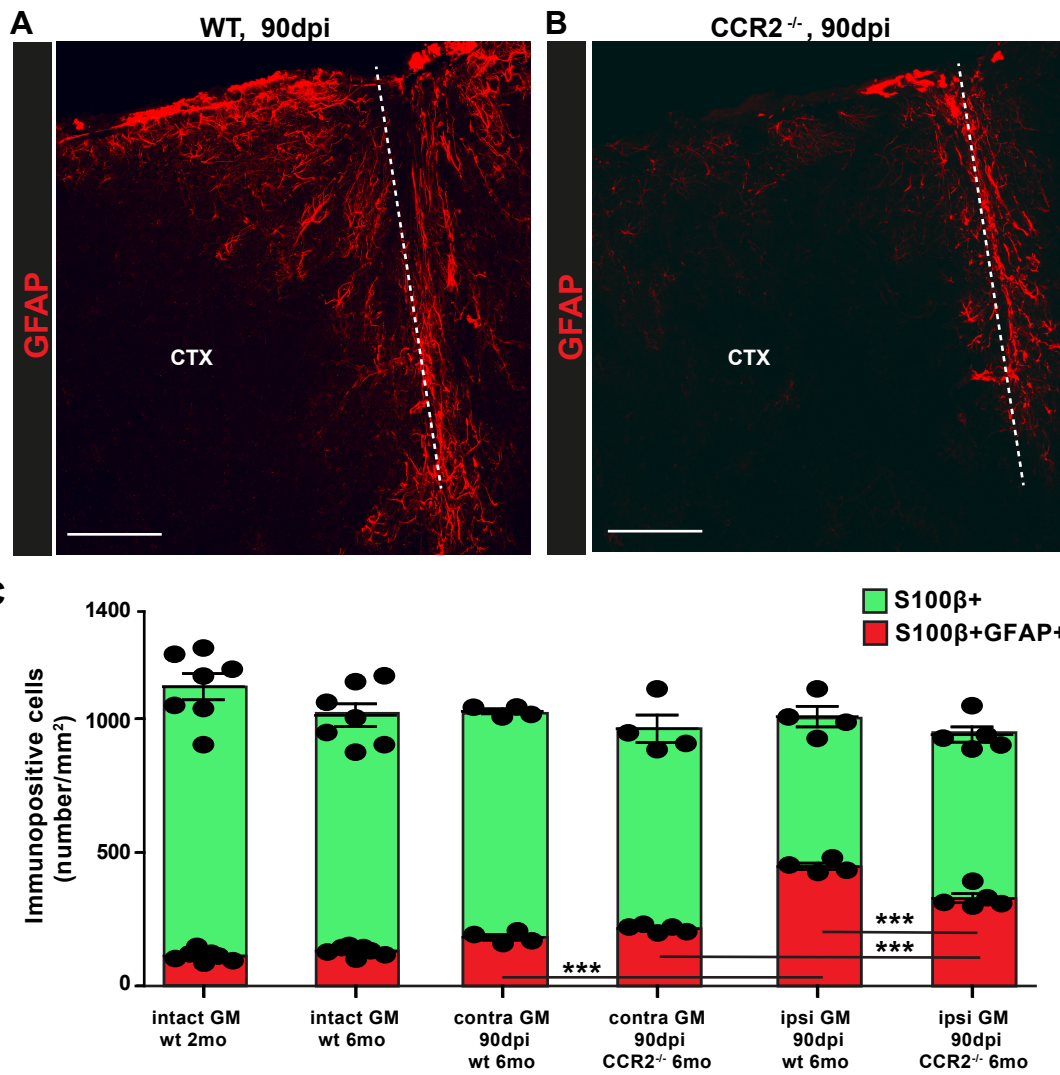
Appendix Figure S3



Appendix Figure S4



Appendix Figure S5



Appendix Figure S6

Appendix Figure Legends

Appendix Figure S1: Absence of lymphocytes and predominance of CD45⁺/CD11b⁺ monocytes/macrophages in the injured GM parenchyma at 3 dpi.

Fluorescence-Activated Cell Sorting (FACS) of CD45⁺ cells (isolated previously by magnetic activated cell sorting) was performed on spleen **(A)** and CD45⁺ cells from the GM tissue at 3dpi isolated by MACS **(B)**. The identity of CD45⁺ cells was determined by staining with CD11b (high: monocytes/macrophages, see B, n1, left panel; low: lymphocytes, see A), CD19 (T-cells, see A, right panel) and CD3 (B-cells, see A right panel). While lymphocytes were readily detectable in samples taken from spleen **(A)**, they were absent in brain tissue collected from the lesion site where CD45⁺ cells were exclusively CD11b high monocytes/macrophages.

Appendix Figure S2: Microglia proliferation between 3 and 5 days after injury.

Confocal images of Iba1 and Ki67 double-immunostaining in WT GM at 3 dpi **(A)** and 5 dpi **(B)**. Arrows point to double-positive cells, the proliferating microglia at the injury site. **(C)** Histogram depicting the number of Iba1⁺Ki67⁺ cells in the injured cortical GM of WT mice at 3 dpi and 5 dpi. Of note, there was the steep decline in microglia proliferation at the injury site between 3-5 dpi. Data are represented as mean \pm SEM per independent experiments ($p=0.0159$, $n=4$ for 3 dpi and $n=5$ for 5 dpi). Marks represent individual data points. Significance of differences between means was analyzed using Mann-Whitney test. Scale bars: 50 μ m.

Appendix Figure S3: Tmem119+ microglia around the stab- wound injury site of WT and CCR2^{-/-} mice at 5 dpi. Representative confocal images of Tmem119 immunostaining in the intact (left panel) or injured (right panel) GM of WT **(A)** or CCR2^{-/-} mice **(B)** shown with DAPI staining to visualize nuclei in the upper row and without DAPI in the lower row of panels. Higher magnification views are shown as boxes in **(A)** and as separate panels in B1 and B2 (location is indicated by boxed area in the left panels of B). The dashed lines indicate the injury site. Note the increase in Tmem119 immunostaining at the lesion site. Scale bars: 125µm (A, B), 50µm (B1, B2).

Appendix Figure S4: Faster resealing of the Blood-Brain Barrier (BBB) in the absence of monocyte invasion. Confocal images of the intact GM parenchyma showing restriction of 1-kDa Alexa Fluor 555–Cadaverine exclusively to the cerebral vasculature **(A)**. Higher magnification view of the boxed areas **(A'** and **A''** in **A)** shows Cadaverine within the lumen of blood vessels (BV). Representative images displaying the injury-related extravasation of Cadaverine-Alexa-555 into the GM parenchyma at 3 hours (3hpi) **(B)** and 3 days post stab wound in WT **(C, D)** and CCR2^{-/-} **(E, F)** mice. The dashed lines indicate the site of injury. The cell nuclei were counterstained with DAPI and this staining is omitted in the panels **D'** and **F'**. **(G)** Quantification of Cadaverine labelled area within the injured GM at 3 dpi showed a significant reduction in CCR2^{-/-} stab wound injured brains (Mann-Whitney test, **p*=0.0376, n=6), suggesting faster resealing of the BBB in the absence of monocyte invasion. Data are represented as mean ± SEM per independent experiments. Scale bars: 100µm (A, B, C, D), 30µm (A', A'').

Appendix Figure S5: Collagen I immunostaining in WT and CCR2^{-/-} mice at 28 dpi.

Representative confocal images showing Collagen 1- and GFAP-immunoreactivity at the injury site in WT (**A**) and CCR2^{-/-} (**B**) mice at 28dpi. Higher magnification view of the boxed areas (**A'** in **A**, and **B'** in **B**) showing reduction in collagen I deposits in the center of the lesion (center of boxed areas) and fewer GFAP reactive cells and processes (arrowheads) at the collagen1⁺ structures around the injury site in the CCR2^{-/-} compared to WT mice. Scale bars: 100µm (A, B).

Appendix Figure S6: Reduced astrogliotic scar in CCR2^{-/-} mice at 90 dpi.

Confocal images displaying GFAP in the injured GM from WT (**A**) and CCR2^{-/-} (**B**) mice at 90dpi. Histogram in (**C**) depicts the total number of astrocytes (S100β⁺) and reactive (GFAP⁺ and S100β⁺) astrocytes in a comparable region of the cerebral cortex GM at the injury site (ipsilateral) or in the contralateral hemisphere. Note that the increased astrocyte proliferation in CCR2^{-/-} mice does not lead to a permanent increase in astrocyte numbers and rather results in a significant reduction of GFAP⁺ cells at 90 dpi. All data are represented as mean ± SEM per independent experiments (n=7 for intact 6 months old WT mice, n=4 for WT 90 dpi and n=5 for CCR2 KO mice). Marks depict individual data points (animals). Significance of differences between means was analyzed using one way ANOVA with Tukeys post-hoc test and is indicated based on the *p* value (***) (*p*<0.0001). Scale bars: 125µm.

Biological processes overrepresented with proteins more abundant in WT	GO-Term id	P-value	Fold enrichment	# Genes (observed)	# Genes (expected)	# Genes (total)	List of observed genes
Biological processes (GO-Term)							
one-carbon compound transport	GO:0019755	0.0001	175.0	2	0.0	14	Slc14a1, Car12
nicotinic acetylcholine receptor metabolic process	GO:1903570	0.0010	43.0	2	0.0	57	Itih3, B3gat2
kidney smooth muscle cell differentiation	GO:0072185	0.0016	612.8	1	0.0	2	Tahz3
chitosaminoglycan metabolic process	GO:0030203	0.0022	28.8	2	0.1	85	Itih3, B3gat2
kidney smooth muscle tissue development	GO:0072184	0.0024	408.4	1	0.0	3	Tahz3
negative regulation of neuron switching to IqE isotypes	GO:0048294	0.0024	40.4	1	0.0	3	Ndfip1
aminoacyl metabolic process	GO:006022	0.0029	24.8	2	0.1	99	Itih3, B3gat2
sensory perception of touch	GO:0050975	0.0033	306.3	1	0.0	4	Tahz3
negative regulation of isotype switching	GO:0048292	0.0033	30.3	1	0.0	4	Ndfip1
urea transmembrane transport	GO:0071918	0.0041	245.0	1	0.0	5	Slc14a1
urater smooth muscle cell differentiation	GO:0072183	0.0041	245.0	1	0.0	5	Tahz3
urater smooth muscle development	GO:0072191	0.0041	245.0	1	0.0	5	Tahz3
establishment of centrosome localization	GO:0051660	0.0041	245.0	1	0.0	5	Cep83
intermediate filament bundle assembly	GO:0045110	0.0049	204.2	1	0.0	6	Krt14
urate transport	GO:0015840	0.0049	234.2	1	0.0	6	Slc14a1
isotype switching to IqE isotypes	GO:0048290	0.0057	175.0	1	0.0	7	Ndfip1
negative regulation of interleukin-4 production	GO:0032713	0.0057	175.0	1	0.0	7	Ndfip1
regulation of isotype switching to IqE isotypes	GO:0048293	0.0057	175.0	1	0.0	7	Ndfip1
miRNA loading onto RISC involved in gene silencing by miRNA	GO:0072590	0.0057	175.0	1	0.0	7	Agp4
negative regulation of B cell mediated immunity	GO:002713	0.0065	153.1	1	0.0	8	Ndfip1
negative regulation of immunoglobulin mediated immune response	GO:0026294	0.0065	153.1	1	0.0	8	Ndfip1
small RNA loading onto RISC	GO:0070922	0.0065	153.1	1	0.0	8	Agp4
carbon dioxide transport	GO:0015670	0.0065	153.1	1	0.0	8	Car12
negative regulation of autophagosome assembly	GO:1902902	0.0073	136.1	1	0.0	9	Fez1
ion homeostasis	GO:0050564	0.0081	122.5	1	0.0	10	Car12
osteolysis	GO:0030432	0.0081	122.5	1	0.0	10	Tahz3
negative regulation of immunoglobulin production	GO:0002638	0.0081	122.5	1	0.0	10	Ndfip1
SRP-dependent cotranslational protein targeting to membrane	GO:0006614	0.0089	111.4	1	0.0	11	Sr3
positive regulation of smooth muscle cell differentiation	GO:0051152	0.0089	111.4	1	0.0	11	Tahz3
monovalent inorganic anion homeostasis	GO:0050583	0.0089	111.4	1	0.0	11	Car12
negative regulation of 2'5' oligoadenylate synthetase gene expression	GO:0072829	0.0089	111.4	1	0.0	11	Ndfip1
regulation of isotype switching to IqG isotypes	GO:0048302	0.0089	102.1	1	0.0	12	Ndfip1
adenylate cyclase-activating dopamine receptor signaling pathway	GO:0071911	0.0098	102.1	1	0.0	12	Gnal
urinary tract smooth muscle contraction	GO:0014848	0.0098	102.1	1	0.0	12	Tahz3
fluid transport	GO:0002087	0.0106	94.2	1	0.0	13	Tahz3
isotype switching to IqG isotypes	GO:0048291	0.0108	94.2	1	0.0	13	Ndfip1
organelle assembly	GO:0009110	0.0108	2.4	8	3.3	4087	Bin2, Slc14a1, Fez1, Tmem63c, Cep83, Sr3, Ndfip1, Car12
urater development	GO:0072189	0.0114	87.5	1	0.0	14	Tahz3
mitochondrial tubulation	GO:0097320	0.0114	87.5	1	0.0	14	Bin2
establishment of mitochondrion localization	GO:0051654	0.0114	87.5	1	0.0	14	Fez1
one-mRNA processing	GO:0031054	0.0114	87.5	1	0.0	14	Agp4
ribosome biogenesis	GO:0031000	0.0114	87.5	1	0.0	14	Gnal
miRNA mediated inhibition of translation	GO:0038278	0.0122	81.7	1	0.0	15	Agp4
cotranslational protein targeting to membrane	GO:0006613	0.0122	81.7	1	0.0	15	Sr3
fluid transport	GO:0009110	0.0122	81.7	1	0.0	15	Ndfip1
regulation of translation, ncRNA-mediated	GO:0495974	0.0130	76.6	1	0.0	16	Agp4
regulation of respiratory system process	GO:0044065	0.0130	76.6	1	0.0	16	Tahz3
negative regulation of translation, ncRNA-mediated	GO:0040033	0.0130	76.6	1	0.0	16	Agp4
gas transport	GO:0015669	0.0130	76.6	1	0.0	16	Car12
establishment of localization	GO:0051234	0.0133	2.3	8	3.5	4232	Bin2, Slc14a1, Fez1, Tmem63c, Cep83, Sr3, Ndfip1, Car12
chondroitin sulfate proteoglycan biosynthetic process	GO:0050650	0.0138	72.1	1	0.0	17	B3gat2
protein transport	GO:0006853	0.0138	72.1	1	0.0	17	Slc14a1
activation of adenylate cyclase activity	GO:0007190	0.0138	72.1	1	0.0	17	Gnal
protein targeting to ER	GO:0046347	0.0146	66.1	1	0.0	18	Sr3
response to androgen	GO:0001975	0.0146	66.1	1	0.0	18	Gnal
response to amine	GO:0014075	0.0154	64.5	1	0.0	19	Gnal
phasic smooth muscle contraction	GO:0014821	0.0162	61.3	1	0.0	20	Tahz3
cytokine assembly	GO:0071801	0.0170	58.3	1	0.0	21	Bin2
synaptonemal complex assembly	GO:0007130	0.0170	58.3	1	0.0	21	Agp4
protein localization to centrosome	GO:0071538	0.0170	58.3	1	0.0	21	Cep83
mRNA metabolic process	GO:0014846	0.0170	58.3	1	0.0	21	Agp4
regulation of smooth muscle cell differentiation	GO:0051150	0.0170	58.3	1	0.0	21	Tahz3
negative regulation of organelle assembly	GO:1902116	0.0178	55.7	1	0.0	22	Fez1
establishment of protein localization to endoplasmic reticulum	GO:0072599	0.0178	55.7	1	0.0	22	Sr3
centrosome localization	GO:0051642	0.0178	55.7	1	0.0	22	Cep83
negative regulation of DNA recombination	GO:0045910	0.0178	55.7	1	0.0	22	Ndfip1
regulation of respiratory gaseous exchange	GO:0045376	0.0186	53.3	1	0.0	23	Tahz3
chondroitin sulfate metabolic process	GO:0030204	0.0186	53.3	1	0.0	23	B3gat2
fluid transport	GO:0042044	0.0184	51.0	1	0.0	24	Slc14a1
intermediate filament organization	GO:0045108	0.0184	51.0	1	0.0	24	Krt14
negative regulation of macroautophagy	GO:0016242	0.0184	51.0	1	0.0	24	Fez1
synaptonemal complex organization	GO:0070193	0.0184	51.0	1	0.0	24	Agp4
hyaluronan metabolic process	GO:0030212	0.0202	49.0	1	0.0	25	Itih3
regulation of type 2 immune response	GO:0050268	0.0210	47.1	1	0.0	26	Ndfip1
ion homeostasis	GO:0050581	0.0210	47.1	1	0.0	26	Car12
smooth muscle tissue development	GO:0048745	0.0218	45.4	1	0.0	27	Tahz3
regulation of isotype switching	GO:0045191	0.0226	43.8	1	0.0	28	Ndfip1
chondroitin sulfate proteoglycan metabolic process	GO:0050654	0.0226	43.8	1	0.0	28	B3gat2
production of miRNAs involved in gene silencing by miRNA	GO:0051396	0.0226	43.8	1	0.0	28	Agp4
regulation of interleukin-4 production	GO:0032713	0.0234	42.2	1	0.0	29	Ndfip1
negative regulation of production of molecular mediator of immune response	GO:0002701	0.0234	42.2	1	0.0	29	Ndfip1
production of small RNA involved in gene silencing by RNA	GO:0070918	0.0234	42.2	1	0.0	29	Agp4
mRNA transcription	GO:0031050	0.0234	42.2	1	0.0	29	Agp4
positive regulation of adenylate cyclase activity	GO:0045762	0.0242	40.8	1	0.0	30	Gnal
protein localization to microtubule cytoskeleton	GO:0072958	0.0250	39.5	1	0.0	31	Cep83
negative regulation of adaptive immune response based on somatic recombination of immunoglobulin genes	GO:0052623	0.0250	39.5	1	0.0	31	Ndfip1
negative regulation of B cell activation	GO:0050889	0.0250	39.5	1	0.0	31	Ndfip1
single-organism transport	GO:0044765	0.0258	2.5	6	2.4	2946	Bin2, Slc14a1, Tmem63c, Sr3, Ndfip1, Car12
regulation of autophagosome assembly	GO:2100726	0.0258	38.3	1	0.0	32	Fez1
respiratory system process	GO:0003016	0.0258	38.3	1	0.0	32	Tahz3
type 2 immune response	GO:0042092	0.0258	38.3	1	0.0	32	Ndfip1
regulation of developmental process	GO:0050793	0.0271	36.0	1	0.0	33	Itih3, Fez1, Tahz3, Ndfip1
negative regulation of adaptive immune response	GO:0002820	0.0274	36.0	1	0.0	34	Ndfip1
one-carbon metabolic process	GO:0006730	0.0274	36.0	1	0.0	34	Car12
mitochondrion localization	GO:0051646	0.0282	35.0	1	0.0	35	Fez1
protein localization to cytoskeleton	GO:0044380	0.0282	35.0	1	0.0	35	Cep83
dopamine receptor signaling pathway	GO:0007212	0.0290	34.0	1	0.0	36	Gnal
intermediate filament organization	GO:0045108	0.0290	34.0	1	0.0	36	Ndfip1
cellular component assembly	GO:0022907	0.0297	2.8	5	1.8	2227	Krt14, Bin2, Agp4, Fez1, Cep83
establishment of organelle localization	GO:0051656	0.0304	7.3	2	0.3	337	Fez1, Cep83
positive regulation of tyrosine activity	GO:0051349	0.0306	32.2	1	0.0	38	Gnal
protein localization to endoplasmic reticulum	GO:0070912	0.0306	32.2	1	0.0	38	Ndfip1
negative regulation of lymphocyte mediated immunity	GO:0002707	0.0306	32.2	1	0.0	38	Ndfip1
cellular iron homeostasis	GO:0050679	0.0306	32.2	1	0.0	38	Ndfip1
cell differentiation involved in kidney development	GO:0051025	0.0306	32.2	1	0.0	38	Tahz3
positive regulation of cyclase activity	GO:0031281	0.0306	32.2	1	0.0	38	Gnal
male meiosis	GO:0007140	0.0314	31.4	1	0.0	39	Agp4
regulation of vesicle organization	GO:0044088	0.0314	31.4	1	0.0	39	Fez1
intermediate filament cytoskeleton organization	GO:0045104	0.0330	29.9	1	0.0	41	Krt14
proteoglycan biosynthetic process	GO:0030166	0.0330	29.9	1	0.0	41	B3gat2
single-organism localization	GO:1902678	0.0335	2.3	6	2.6	3130	Bin2, Slc14a1, Tmem63c, Sr3, Ndfip1, Car12
intermediate filament-based process	GO:0045103	0.0337	29.2	1	0.0	42	Krt14
isotype switching	GO:0007128	0.0345	28.5	1	0.0	43	Agp4
somatic recombination of immunoglobulin genes involved in immune response	GO:0002204	0.0345	28.5	1	0.0	43	Ndfip1
somatic diversification of immunoglobulins involved in immune response	GO:0002208	0.0345	28.5	1	0.0	43	Ndfip1
positive regulation of neurite differentiation	GO:0046666	0.0359	6.6	2	0.3	369	Itih3, Fez1
multicellular organismal movement	GO:0050879	0.0361	27.2	1	0.0	45	Tahz3
RNA secondary structure unbinding	GO:0010501	0.0361	27.2	1	0.0	45	Agp4
cellular response to dsRNA	GO:0071359	0.0361	27.2	1	0.0	45	Agp4
musculoskeletal movement	GO:0050881	0.0361	27.2	1	0.0	45	Tahz3
cellular developmental process	GO:0048869	0.0363	2.1	7	3.3	4089	Krt14, Agp4, Itih3, Fez1, Tahz3, Cep83, Ndfip1
positive regulation of cell differentiation	GO:0045597	0.0370	4.0	3	0.7	313	Itih3, Fez1, Tahz3
chitosaminoglycan biosynthetic process	GO:0006024	0.0377	26.1	1	0.0	47	B3gat2
single-organism process	GO:0044689	0.0385	1.4	15	10.8	13200	Krt14, Bin2, Agp4, Gnal, Slc14a1, Itih3, Fez1, Tahz3, Tmem63c, Cep83, Sr3, Ndfip1, Fam129b, B3gat2, Car12
positive regulation of tyrosine activity	GO:0051349	0.0385	28.5	1	0.0	48	Gnal
protein localization to endoplasmic reticulum	GO:0070912	0.0385	28.5	1	0.0	48	Ndfip1
negative regulation of leukocyte mediated immunity	GO:0002704	0.0385	28.5	1	0.0	48	Ndfip1
somatic recombination of immunoglobulin gene segments	GO:0016447	0.0393	26.0	1	0.0	49	Ndfip1
regulation of cell differentiation	GO:0045595	0.0393	3.0	4	1.3	1624	Itih3, Fez1, Tahz3, Ndfip1
carbohydrate derivative metabolic process	GO:1901135	0.0400	3.9	3	0.8	947	Itih3, Gnal, B3gat2
immunoglobulin production involved in immunoglobulin mediated immune response	GO:0002381	0.0400	24.5	1	0.0	50	Ndfip1
interleukin biosynthetic process	GO:0006623	0.0408	24.0	1	0.0	51	B3gat2
cellular component biogenesis	GO:0044085	0.0412	2.5	5	2.0	243	

Appendix Table S4 Primary and secondary antibodies used for immunohistological analysis

Antibody	Provider	Catalog Nr	Dilution
<u>Primary antibody:</u>			
anti AhR rabbit polyclonal IgG	US Bio	031714.200	1:300
anti CD11b rat monoclonal IgG _{2b}	BIORAD	MCA74GA	1:250
anti CD31 rat monoclonal IgG _{2b}	BD Biosciences	550274	1:100
anti CD45 rat monoclonal IgG _{2b}	BD Biosciences	550539	1:500
anti Collagen Type I rabbit polyclonal IgG	Rockland	600-401-103-0.5	1:200
anti Collagen Type IV rabbit polyclonal IgG	Millipore	AB8201	1:40
anti CSPG (CS-56) mouse monoclonal IgM	Abcam	ab11570	1:250
anti GFAP mouse monoclonal IgG ₁ rabbit polyclonal IgG	Sigma Aldrich Dako	G3893 Z0334	1:500 1:500
anti Iba1 rabbit polyclonal IgG	Wako	019-19741	1:500
anti Ki67 rat monoclonal IgG _{2a} rabbit monoclonal IgG	eBioscience Thermo Fisher Scientific	14-5698-82 MA5-14520	1:300 1:100
anti NG2 (CSPG4) rabbit polyclonal	Millipore	AB5320	1:400
anti NeuN mouse monoclonal IgG ₁	Millipore	MAB377	1:100
anti RFP rabbit polyclonal IgG	Rockland	600-401-379	1:500
anti S100(β-Subunit) mouse monoclonal IgG ₁	Sigma Aldrich	S2532	1:250
anti TMEM119 rabbit monoclonal IgG	Abcam	ab209064	1:200
<u>Secondary antibody:</u>			
anti-mouse IgG ₁ Alexa Fluor 488	Thermo Fisher Scientific	A-21121	1:500
anti-mouse IgM Alexa Fluor 488	Thermo Fisher Scientific	A-21042	1:500
anti-rabbit IgG Alexa Fluor 488	Thermo Fisher Scientific	A-21206	1:500
anti-rabbit IgG Alexa Fluor 647	Thermo Fisher Scientific	A-31573	1:500
anti-rat IgG Alexa Fluor 647	Thermo Fisher Scientific	A-21247	1:500
anti-mouse IgG CY3	Dianova	115-165-166	1:500
anti-rabbit IgG CY3	Dianova	111-165-144	1:500
anti-rat IgG CY3	Dianova	112-165-167	1:500