Supplementary Materials

1.Supplementary Methods

2.Supplementary Tables

3.Supplementary Figures

Supplementary Methods

Stroke cohort statistical analyses

Results were expressed as percentages for categorical variables and as mean (±SD) or median and range [25th and 75th percentiles] for continuous variables depending on whether their distribution was normal or not. The Kolmogorov-Smirnov test was used to assess normality. Proportions were compared using the chi-square or Fisher test, while the continuous variables between groups were compared with the Student´s t or the Mann-Whitney test depending on whether their distribution was normal or not. Bivariate correlations were performed using Pearson´s coefficient (normally distributed variables) or Spearman´s coefficient (variables without normal distribution).

The association between heavy drinking and early neurological deterioration (END) was assessed using logistic regression analysis; the influence on infarct volume was assessed by multiple linear regression models. Both logistic regression analysis and multivariate linear regression models were adjusted for those variables which showed biological relevance for each endpoint in order to avoid spurious associations. Results were expressed as adjusted odds ratios (ORs) or B estimate with the corresponding 95% confidence intervals (95% CI). Statistical significance was set at $P<0.05$. The statistical analysis was conducted in SPSS 20.0 (IBM, Chicago, IL, USA) for Mac.

Heavy drinking (HD) patients without stroke

HD patients were recruited in the Addiction Unit of the University Hospital of Caen (Normandy, France) while they were receiving withdrawal treatment as inpatients. They all met alcohol-dependence criteria according to the DSM-IV and AUD according to the DSM-V for at least 5 years. Control subjects were interviewed with the Alcohol Use Disorders Identification Test (AUDIT; (1)), in order to verify that they were not at risk for alcohol misuse or alcohol dependence (score <6 for women and <7 for men). Participants were matched for age, sex and body mass index (BMI).

Exclusion criteria for all the participants were: neurological diseases, active infectious disease, cardiovascular or psychiatric diseases, cancer, depression [Beck questionnaire, (2)], anxiety [State-Trait Anxiety Inventory scale, STAI A et B parts, (3)], or misuse of any other substance than alcohol (excepting tobacco) [misuse and/or dependence measured by the Fagerström score (4)].

The normality of the distribution of the laboratory measures for the HD and control groups was examined using the Shapiro Wilk test. *Student's t* tests were used to compare the two groups for measures of immune cell counts and CRP.

Thromboembolic Focal Cerebral Ischemia

Anesthetized mice were placed in a stereotaxic device, a small craniotomy was performed, the dura was excised, and the middle cerebral artery (MCA) was exposed. A pulled glass micropipette was introduced into the lumen of the MCA and 1 µL (1 UI/µL) of purified murine alpha-thrombin (Enzyme Research Labs, USA) was pneumatically injected to induce MCA occlusion (MCAo) by the *in situ* formation of a clot. Lesion volumes were quantified on Image J 24 hours after stroke onset by regular thionin staining or T2-w MRI scans.

***In vivo* microglial phagocytic capacity measurement**

One µl of nonionic latex beads (6 µm) (1:50, Molecular Probes, Life Technologies, USA) were gently injected in the brain cortex (2.2 mm lateral, -1.6 mm ventral to bregma) of anesthetized mice (N=4 mice/group) with a glass micropipette (angle 335°). The micropipette was left *in situ* for 2 min before slow withdrawal and then mice were sutured. Eight hours after the injection, mice were terminally anesthetized and prepared for immunohistochemical analyses. Alcohol-exposed mice kept free access to the alcohol solution after beads injection and until killing. The number of latex beads completely surrounded by $Iba1^+$ staining was counted in the ipsilateral cortex of control and alcohol-exposed mice. All beads forming clusters of more than 20 beads were omitted (tight clusters might prevent access of microglial cells to individual beads). The total number of beads phagocytosed was divided by the total not phagocytosed to calculate a ratio for each animal and these ratios were averaged for four animals in each treatment group.

Vascular adhesion molecular imaging

The quality of conjugated MPIOs was systematically checked in a naive mouse, by stereotaxic injection of LPS (1µl, 1mg/kg) in the striatum (0.5mm anterior, 2.0mm lateral, -3mm ventral to the Bregma).

Three-dimensional high resolution T2*- weighted gradient echo imaging with flow compensation (spatial resolution of 70 mm x 70 mm x 70 mm interpolated to an isotropic resolution of 70 mm), TE/TR 13.2ms/200 ms and a flip angle of 21° was performed to visualize MPIOs. MRI acquisitions started immediately after the intravenous injection of MPIOs (200 µl of 2 mg Fe/kg of conjugated MPIOs). All T2*-weighted images presented are minimum intensity projections of six consecutive slices. For the ischemic stroke study, signal void quantification on 3D T2*-weighted images was measured by using automatic triangle threshold in ImageJ software and results presented as MPIOs-induced signal void on the contralateral cortex divided by the signal void on the structure of interest (in percent). For the LPS study,

only cortical blood vessels (which were identified as straight lines in hyposignal on T2*-weighted images) were counted.

In vivo detection of blood-brain barrier (BBB) leakage

Analyses were performed on ImageJ by comparing the same brain area before and after gadolinium injection in control and alcohol-exposed mice (n=4/group). A positive control was performed on a stroke mouse (5 days after stroke) (Supplementary Figures 1G, H).

Near-infrared detection of BBB leakage

To allow the detection of Evan's blue (EB) at 650 nm, 4 mice per group were intravenously injected with 200 µL of 2% EB. EB was allowed to circulate for 60 minutes, and then deeply anesthetized mice were transcardially perfused with cold heparinized saline (15 mL/min; 75 mL) before organ removal. The brain was placed in a photon-imager (Biospace, France) and images were acquired with excitation filter set at 600 nm and emission filter at 700 nm. The photon count of the whole brain was measured ex vivo and normalized at 100% for control animal using M3 vision software (Biospace). A positive control was performed on a stroke mouse (24h after stroke) (Supplementary Figures 1C, D).

Depletion of perivascular macrophages (PVM)

Anesthetized mice were placed in a stereotaxic device. Then the skin was removed and a small craniotomy was performed (coordinates: -0.2mm anteroposterior; +1mm lateral; -2mm depth from the Bregma). A glass micropipette containing 10µl PBS-liposomes (PBS group) or clodronate-encapsulated liposomes (CLO group) (purchased at clodronateliposomes.com) was inserted and the product was gently injected in the left lateral ventricle during 20 minutes. CLO liposomes injected into the cerebral ventricles are phagocytized by PVM and, once in the cytosol, CLO acts as a cytotoxic ATP analog, which impairs mitochondrial oxygen consumption leading to cell death (11).

In vivo fluorescent PVM labelling for intravital microscopy

Three days after the injection of liposomes, a glass micropipette was inserted at the aforementioned coordinates and 3µl of TRITC-Dextran (4.4 kDa, Sigma Aldrich, France) were gently injected.

In vivo macrophage labelling for the follow-up study of macrophage accumulation in the brain

Two hundred microliters of 2 mg Fe/kg of nude Micro-sized Particles of Iron Oxide (MPIOs) (diameter 1.08 mm) (Invitrogen) were injected iv to naïve mice. The day after, a first 3D T2*-weighted (T2*-w) gradient echo imaging of flow compensation (spatial resolution 70mm^3 isotropic, TE/TR 13.2ms/200ms and a flip angle of 21°) acquisition was performed to control the absence of MPIOs signal. Mice were then exposed to alcohol during 6 weeks, and 3D T2*-w acquisitions were performed at 2 and 6 weeks after exposure. Control mice were subjected to the same experimental protocol but were given access only to tap water. A positive control was performed by intracortically injecting a single dose of LPS.

Thin-skull cranial window

Anesthetized mice used for two-photon experiments underwent thin-skull cranial window for the cortical *in vivo* detection of leukocyte rolling and adhesion. The head skin was opened to expose the skull and the right parietal bone was completely polished with a drill to leave only a thin layer of bone enabling the visualization of cortical cerebral blood vessels by transparency. The imaged area corresponds to the right somatosensory cortex (see Figure 6G).

Intravital two-photon microscopy

Anesthetized mice were placed in a stereotaxic device and aqueous medium was deposed between the thin-skull window and the X25 immersive objective. One hundred µl of

Rhodamine 6G (1mg/kg) and 100 µl of 70kDa FITC-Dextran (5mg/ml) (Sigma Aldrich, France) were injected in the tail vein to stain circulating leukocytes and to visualize the lumen of blood vessels, respectively. Acquisitions were performed using a Leica TCS SP5 MP microscope at 840 nm two-photon excitation wavelength (Coherent Chameleon, USA). Photomultiplier (PMT) 2 (recorded capacity: 500-550nm; gain 850V; offset 0) and PMT3 (recorded capacity: 565-605nm; gain 850V; offset 0) were used. The pulsing laser characteristics were: gain 23%; trans 17%; offset 50%.

Leukocyte rolling/adhesion counting

Leukocyte adhesion and rolling to venular endothelium were measured after the intravenous injection of 100 µl of Rhodamine 6G (1mg/kg) and 100 µl of 70kDa FITC-Dextran (5mg/ml) (Sigma Aldrich, France) to stain circulating leukocytes and visualize the lumen of blood vessels, respectively.

For adherent leukocyte quantification, the images obtained by time-lapse (2 minutes, 7.7 frames per second, 256x256 pixel resolution, 1000Hz frequency) were compiled and red spots, corresponding to adherent leukocytes, were counted for each group (N=4-5 mice per group). For rolling leukocytes, we used the Kymograph plugin on ImageJ software (developed by J. ***Rietdorf*** and A. ***Seitz***) (line width perpendicular to vessel lumen: 10; size pixel^2 after threshold: (2-5)-infinity). Leukocytes that were too fast were considered as background and not counted as rolling leukocytes.

Two-photon in vivo PVM visualization

In a separate subset of mice, TRITC$^+$ (PVM labelled) and GFP$^+$ (microglial cells from CX3CR1$^{GFP+/-}$) signals were evaluated two days after PBS/CLO-encapsulated liposomes (N=6 mice per group). Z stacks (60-80 µm depth) were acquired from sub-meningeal regions to avoid

any fluorescent signal coming from the meninges. PVM depletion was confirmed both *in vivo* (2-photon imaging) and by IHC ($CD206^+$ staining).

Quantitative PCR analyses

Terminally anesthetized mice (N=6/group) were transcardially perfused with cold heparinized saline (15 mL) and brains were excised. Cortex were dissected and maintained at −80 °C until messenger RNA (mRNA) extraction. Tissues were dissociated in TRI reagent (Sigma; Lyon, France), and RNA was isolated by the addition of chloroform. Total RNA was washed by ethanol and treated with TURBO DNase (Ambion; Saint Aubin, France). Total RNA was quantified by spectrophotometry (NanoDrop Technologies; Wilmington, USA). Reverse transcription from 1 µg of total RNA (or water as an internal control) was performed with the iScript Select cDNA Synthesis kit (Bio-Rad; Marnes-la-Coquette, France) in a total volume of 20 µL with the following cycle conditions: 42 °C (90 min); 85 °C (5 min). The cDNA products were then stored at −20 °C until their use.

Quantitative PCR was performed with 1 µL of 1:20 diluted cDNA, water and the RT-qPCR internal control), that were analyzed in 15 µL total of a 1× solution of iQ SYBR Green Supermix (Bio-Rad; Marnes-la-Coquette, France) containing 200 nM of each primer. Based on mRNA coding sequences (www.ensembl.org), mouse-specific primers were designed by using the Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Supplementary table 5). Two housekeeping genes (Hmbs and Ppib) were used. Assays were run in triplicate on the CFX96 real-time system c1000 thermal cycler (Bio-Rad; Marnes-la-Coquette, France), with the following cycle conditions: 95 °C (3 min); [95 °C (2 s), 60 °C (20 s)] × 39; 70 °C (30 s).

Immunohistochemistry

Terminally anesthetized mice were transcardially perfused with cold heparinized saline (15 mL) and fixed with 100 mL of 2% paraformaldehyde and 0.2% picric acid phosphate buffer (pH 7.4) (N=4 mice/group). Brains were post-fixed with 2% paraformaldehyde and 0.2% picric acid phosphate buffer (18 hours; 4°C) and cryoprotected (sucrose 20% in PBS; 24 hours; 4°C) before freezing in Tissue-Tek (Miles Scientific, Naperville, IL, USA). Cryostat-cut sections (10 µm excepting latex beads experiment, where 20 µm sections were used) were collected on poly-lysine slides and stored at – 80°C before processing.

Sections were co-incubated overnight with rabbit anti-mouse Iba1 (1:1000, Wako 019-19741), rat anti-mouse CD68 (1:800, Abcam 53444), rat anti-mouse CD206 (1:200, Serotec, clone MR5D3), goat anti-mouse P-selectin (1:1000, RD System AF737), goat anti-mouse Collagen-IV (1:1000, SouthernBiotech 1340), rabbit anti-mouse Aquaporin 4 (sc 20812, Santa Cruz), rabbit anti-mouse Ki67 (1:1000, Abcam 15580). Primary antibodies were revealed by using Fab'2 fragments of Donkey anti-rabbit linked to FITC, anti-rat linked to Cy3, anti-goat IgG linked to Cy5 (1:600, Jackson ImmunoResearch, West Grove, USA). Washed sections (5 sections/mouse) were coverslipped with antifade medium containing DAPI. Epifluorescence images (~10 images/section) were digitally captured using a Leica DM6000 epifluorescence microscope coupled coolsnap camera, visualized with Leica MM AF 2.2.0 software (Molecular Devices, USA) and further processed using ImageJ 1.51k software. Studied regions were always located at the brain cortex [ipsi (peri-infarct and core) and contralateral cortices for stroke experiments]. Specificity controls were performed by not adding primary antibodies.

Confocal microscopy analysis of microglial activation

Microglial morphology and activation were analyzed on confocal images taken on a Leica TCS SP5 MP microscope. Using the X40 oil immersive objective, images were randomly taken using LAS AF Leica Software and analyses were performed using ImageJ 1.51k software. Iba1 analyses allowed the quantification of microglial cell numbers, soma area and number of main

processes starting from the soma. CD68 staining in Iba1$^+$ cells allowed the quantification of the activation of microglial cells. For this latter analysis, after thresholding CD68 and Iba1 channels, we used the Image Calculator ImageJ plugin to collect the mask of CD68 only in Iba1$^+$ cells. After that, we measured the area of Iba1 mask cell per cell, and the area of CD68 inside each cell in both groups (N=800-1000 cells/group; N=4 mice per group).

Microscopy of isolated brain vessels

Terminally anesthetized mice were transcardially perfused with cold heparinized saline (15 mL/min). Brain were then dissociated in a solution of HEPES and HBSS and then centrifuged at 2000g at 4°C during 10 min. Most of myelin is eliminated at this step, and vessels form a pellet. The pellet was then suspended in a solution of HEPES/HBSS and Dextran (Dextran from Leuconostoc spp. Mr ~70,000 kDa, Sigma-Aldrich) and centrifuged at 4400g at 4°C during 15 min. Remaining myelin is eliminated here. Vessels were then suspended in a solution of HEPES/HBSS and BSA 1% and filtrated on a 20µm mesh filter. After that, vessels stay on the filter. Vessels were then detached from the filter in a PBS solution. Vessels were then put on a poly-lysine coated slides, cryoprotected overnight in a 20% sucrose PBS solution and fixed during 15 min with PBS 0.1 M. pH 7.4 containing 2% paraformaldehyde and 0.2% picric acid. Slide were stored at -80°C before processing.

Supplementary Tables

Supplementary Table 1: Multivariate analysis of early neurological deterioration adjusted by heavy drinking (HD) (Model A) and by HD and inflammatory markers (Model B).

Supplementary Table 2: Multivariate analysis of early neurological deterioration adjusted by age or gender (Model A) and by age or gender and inflammatory markers (Model B).

Supplementary Table 3: Bivariate correlations: Infarct volume.

Supplementary Table 4: Linear regression analysis of infarct volume.

Supplementary Table 5: Detailed sequences of mouse primers used for qPCR analyses.

Supplementary Table 6: List of antibodies used for flow cytometry analyses.

Supplementary Table 1: Multivariate analysis of early neurological deterioration adjusted by heavy drinking (HD) (Model A) and by HD and inflammatory markers (Model B).

Model A

	OR	CI 95%	p
Hemorrhagic transformation	3.68	1.75 - 7.77	0.001
Infarct volume	1.01	1.00 - 1.01	< 0.0001
NIHSS on admission	0.93	0.89 - 0.96	< 0.0001
Heavy drinking	4.49	2.94 - 6.86	< 0.0001

Model B

	OR	CI 95%	p
Hemorrhagic transformation	4.65	2.08 - 10.41	< 0.0001
Infarct volume	1.00	1.00 - 1.00	< 0.0001
NIHSS on admission	0.91	0.87 - 0.95	< 0.0001
Heavy drinking	2.45	1.01 - 5.91	< 0.0001
Axillary temperature	1.05	0.74 - 1.50	0.756
Leukocytes	1.06	0.99 - 1.13	0.066
C-reactive protein	1.05	1.00 - 1.08	0.019

Supplementary Table 2: Multivariate analysis of early neurological deterioration adjusted by age or gender (Model A) and by age or gender and inflammatory markers (Model B)

Model A

	OR	CI 95%	p
Age	1.00	0.99 - 1.02	0.287
Women	1.56	0.98 - 2.12	0.075
Hemorrhagic transformation	3.53	1.46 - 8.13	0.001
Infarct volume	1.01	1.00 - 1.01	<0.0001
NIHSS on admission	0.96	0.85 - 0.99	<0.0001
Heavy drinking	5.22	2.18 - 7.35	<0.0001

Model B

	OR	CI 95%	p
Age	1.00	0.99 - 1.02	0.309
Women	1.35	0.95 - 2.93	0.092
Hemorrhagic transformation	4.19	1.97 - 12.13	0.001
Infarct volume	1.01	1.00 - 1.01	<0.0001
NIHSS on admission	0.94	0.82 - 0.99	0.001
Heavy drinking	3.19	1.86 - 8.13	<0.0001
Axillary temperature	1.00	0.53 - 2.18	0.804
Leukocytes	1.03	0.92 - 1.28	0.175
C-reactive protein	1.08	1.05 - 1.12	<0.0001

Supplementary Table 3: Bivariate correlations: Infarct volume

	Pearson or Spearman coefficient	P
Age	0.099	< 0.0001
Sex	0.072	0.001
History of hypertension	0.040	0.077
History of diabetes	0.009	0.688
History of smoking	- 0.039	0.095
History of dyslipemia	- 0.009	0.677
History of ischemic heart disease	0.040	0.074
Axillary temperature	0.117	< 0.0001
Leukocytes	0.139	< 0.0001
Fibrinogen	0.074	0.003
C-reactive protein	0.191	< 0.0001
tPA treatment	- 0.135	< 0.0001
Trombectomy	- 0.045	0.043
Hemorrhagic transformation	0.167	< 0.0001
NIHSS on admission	0.426	< 0.0001
Early neurological deterioration	0.176	< 0.0001
TOAST	- 0.090	< 0.0001
Heavy drinking	0.052	0.021

Supplementary Table 4: Linear regression analysis of infarct volume

	B	CI 95 %	p
Age	- 0.13	- 0.41 to 0.15	0.359
Sex	3.42	- 4.23 to 11.08	0.381
Axillary temperature	12.28	6.25 to 18.31	< 0.0001
Leukocytes	0.89	- 0.29 to 2.08	0.141
Fibrinogen	0.01	- 0.03 to 0.05	0.721
C-reactive protein	1.27	0.32 to 2.22	0.009
tPA treatment	- 8.07	- 16.11 to - 0.02	0.049
Trombectomy	- 10.87	- 31.68 to 9.94	0.306
Hemorrhagic transformation	5.03	- 0.26 to 10.32	0.062
NIHSS on admission	5.56	4.91 to 6.20	< 0.0001
Early neurological deterioration	40.40	25.17 to 55.63	< 0.0001
TOAST	- 2.25	- 5.25 to 0.75	0.142
Heavy drinking	19.66	8.03 to 31.30	0.001

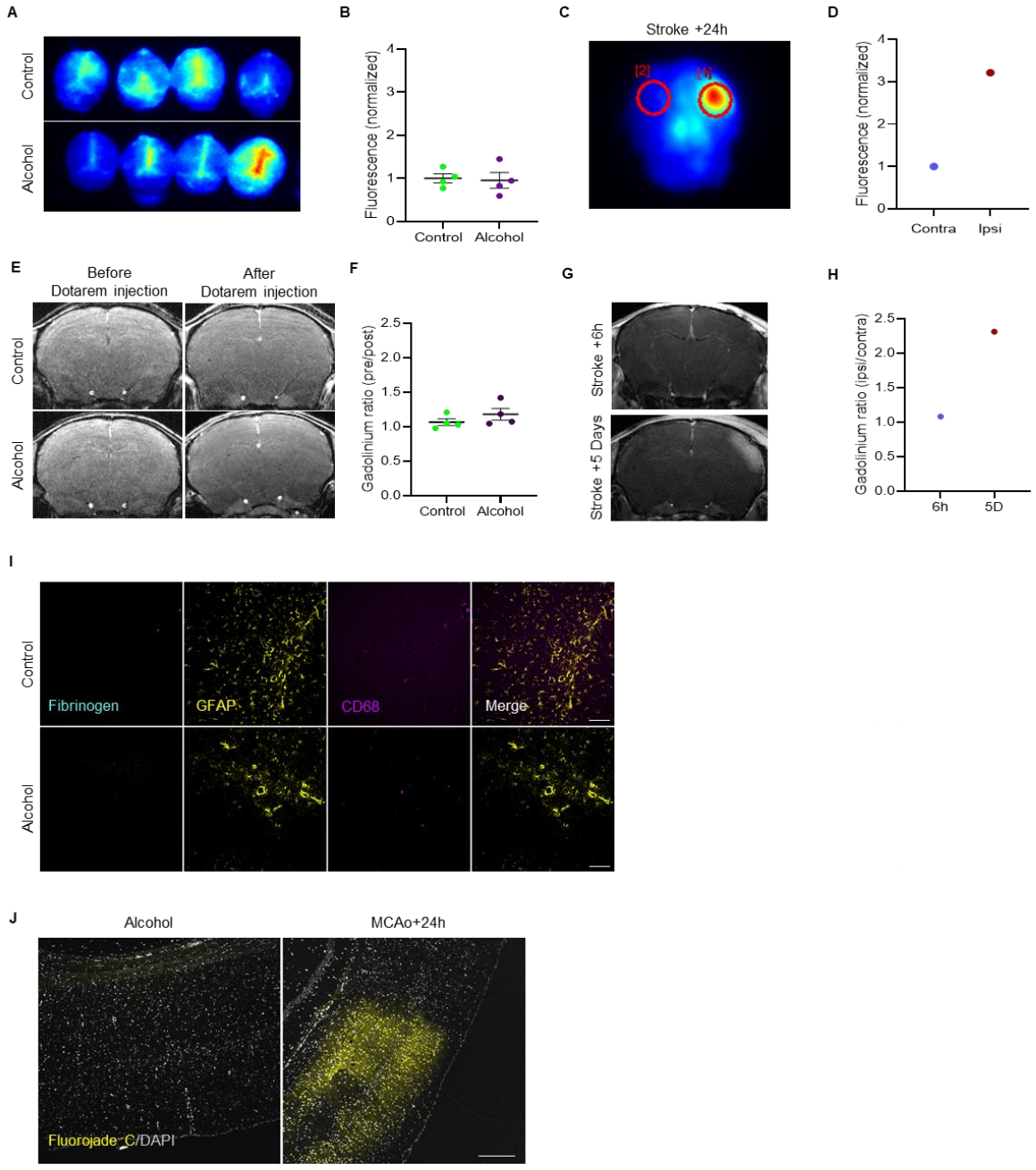
Supplementary Table 5: Detailed sequences of mouse primers used for qPCR analyses

Gene	Forward sequence	Reverse sequence
TNFα	TCTACTGAACTTCGGGGTGA	AGGGTCTGGGCCATAGAACT
IL-1β	GACCTTCCAGGATGAGGACA	AGGCCACAGGTATTTTGTCG
IL-6	GACAAAGCCAGAGTCCTTCAG	GCCACTCCTTCTGTGACTCC
TGFβ	GGCTACCATGCCAACTTCTG	TGGTTGTAGAGGGCAAGGAC
VCAM-1	GAACCCAAACAGAGGCAGAG	TGAGCAGGTCAGGTTCACAG
ICAM	AGGGCTGGCATTGTTCTCTA	CTTTGGGATGGTAGCTGGAA
P-selectin	GGCAAGTGGAATGATGAACC	CCATAGAAGCCTGGGTAGCA
Hmbs	GAAATCATTGCTATGTCCACCA	GGGTTTTCTAGCTCCTTGGTAA
Ppib	CAAGACCTCCTGGCTAGACG	TTCTCCACCTTCCGTACCAC

Supplementary Table 6: List of antibodies used for flow cytometry analyses.

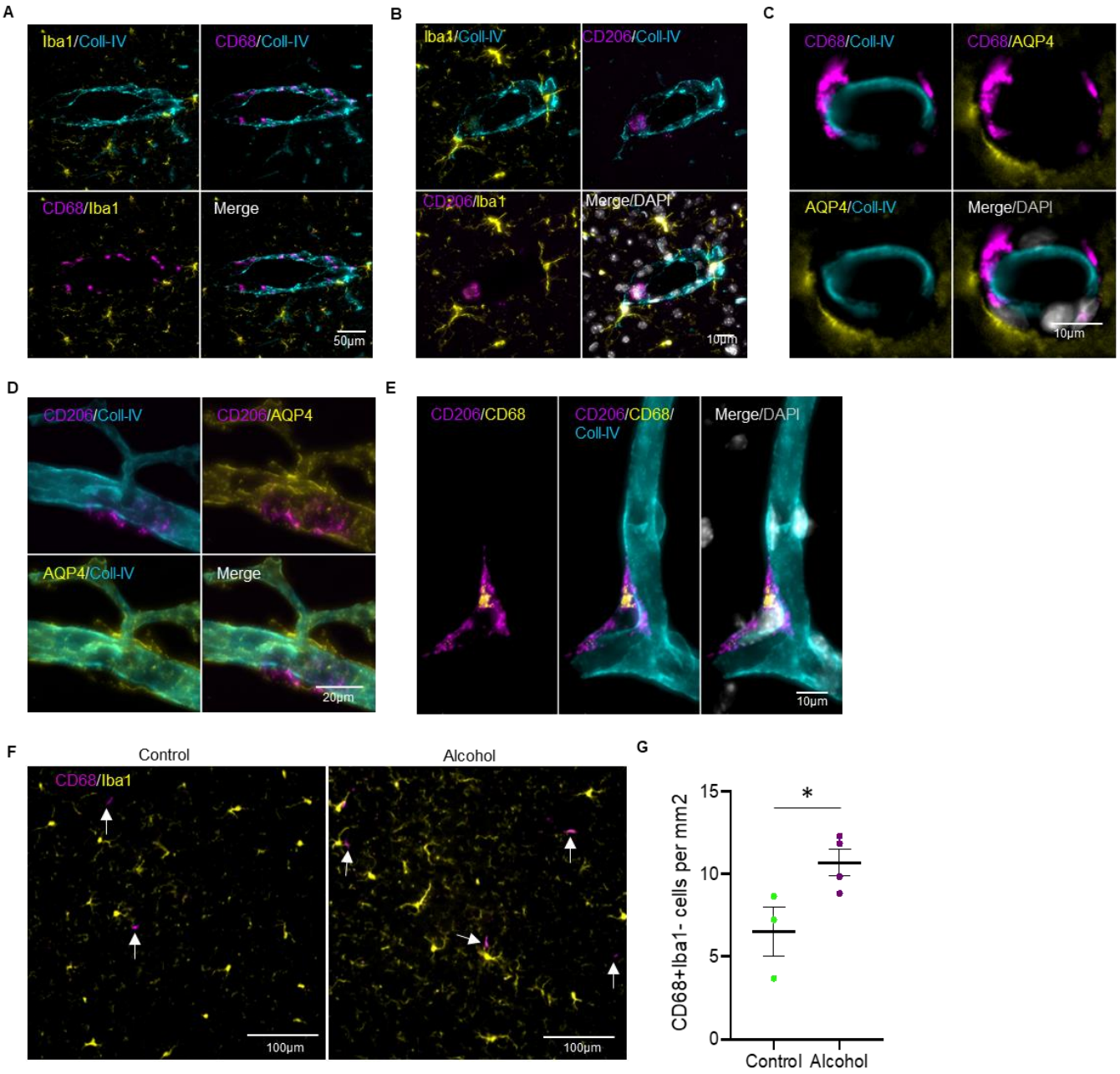
Antibody **-Lymphoid cell panel-**	**Clone**	**Isotype**	**Quantity per test**	**Supplier / Reference**
BV510 Hamster anti-mouse CD3e	145-2C11	Armenian hamster IgG1, κ	0.6 µg	BD Biosciences /563024
APC Rat anti mouse CD4	RM4-5	Rat (DA) IgG2a, κ	0.6 µg	BD Biosciences /553051
PE-Cy7 Rat anti-mouse CD8a	53-6.7	Rat (LOU) IgG2a, κ	0.6 µg	BD Biosciences /552877
Antibody **-Myeloid cell panel -**	**Clone**	**Isotype**	**Quantity per test**	**Supplier / Reference**
BV510 Hamster anti-mouse CD3e	145-2C11	Armenian hamster IgG1, κ	0.6 µg	BD Biosciences /563024
BV421 Rat anti-mouse CD11b	M1/70	Rat (DA) IgG2b, κ	0.6 µg	BD Biosciences /562605
APC Rat anti-mouse CD11c	HL3	Armenian Hamster IgG1, ʎ2	0.6 µg	BD Biosciences /550261
PE-Cy7 Rat anti-mouse CD45	30-F11	Rat (LOU) IgG2b, κ	0.6 µg	BD Biosciences /561868
FITC Rat anti-mouse Ly-6G	1A8	Rat (LEW) IgG2a, κ	1 µg	BD Biosciences /551460
Antibody **-Fc Blocking-**	**Clone**	**Isotype**	**Quantity per test**	**Supplier / Reference**
Purified Rat anti-mouse CD16/CD32 (Mouse BD Fc Block)	2.4G2	Rat IgG2b, κ	0.5 µg	BD Biosciences /553142

Supplementary Figures

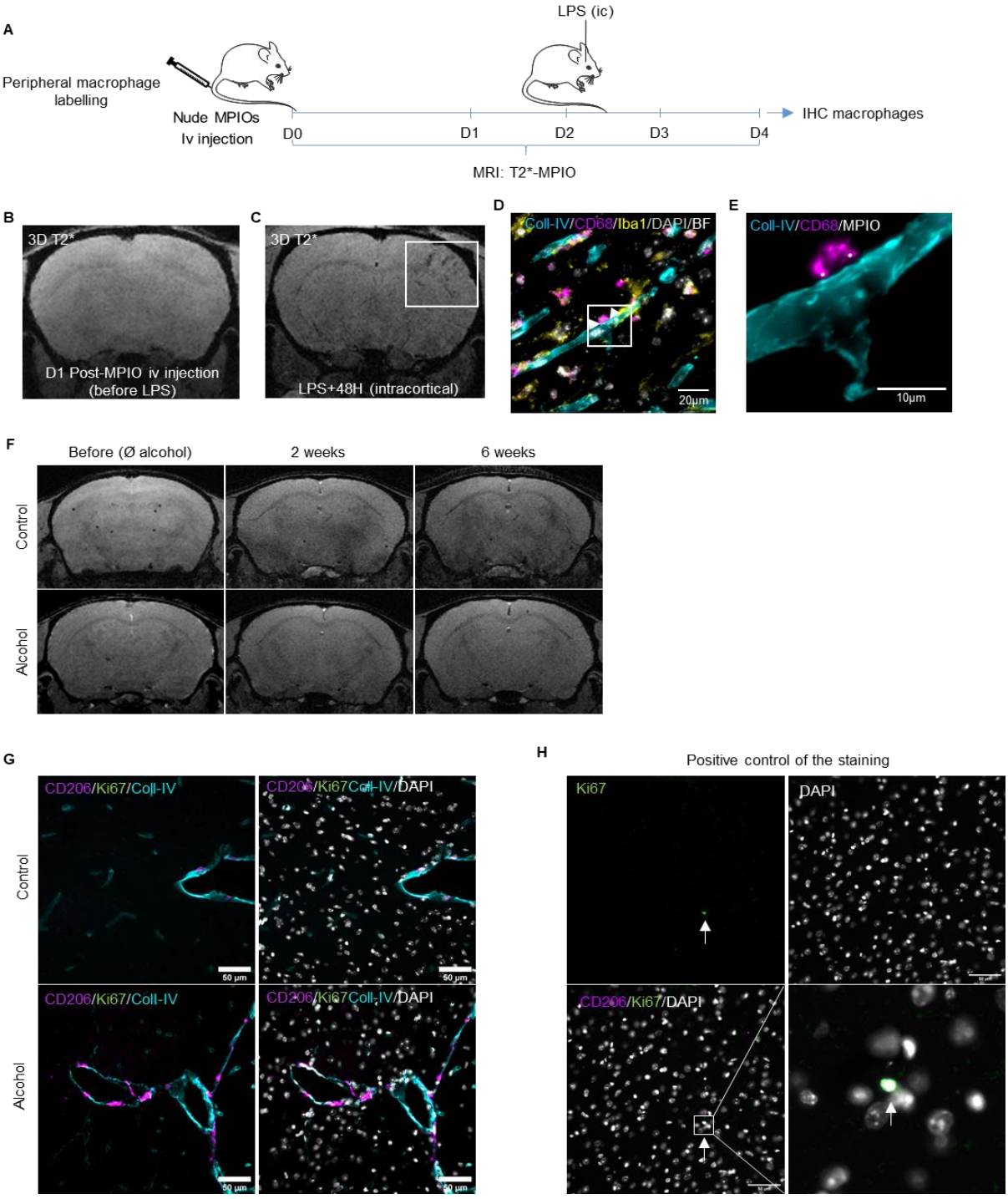


Supplementary figure 1: Lack of BBB leakage or neuronal degeneration after alcohol consumption. A) Photographs of Evan's blue (EB) extravasation measurement by near infrared imaging (NIRF) 60 min after EB iv injection in control and alcohol-exposed mice. B) Quantification of EB fluorescence in control and alcohol-exposed mice. C) Positive control of the technique: brain from mouse subjected to stroke 24h before the measurement of EB

extravasation. D) Quantification of the fluorescence detected in the contralateral and ipsilateral hemispheres shown in C. E) Representative T1-w images of control and alcohol-exposed mice before and after the injection of Dotarem (gadolinium chelate). F) Quantification of E. G) Positive control showing the parenchymal extravasation of Dotarem in the ipsilateral cortex of a mouse subjected to stroke (6h and 5 days after stroke onset). H) Quantification of G. I) Lack of fibrin(ogen) deposits in the brain parenchyma of control and alcohol-exposed mice. J) Lack of fluorojade C-positive staining after chronic alcohol exposure (right); right: positive control of fluorojade C staining performed 24h after MCAo by thrombin injection.

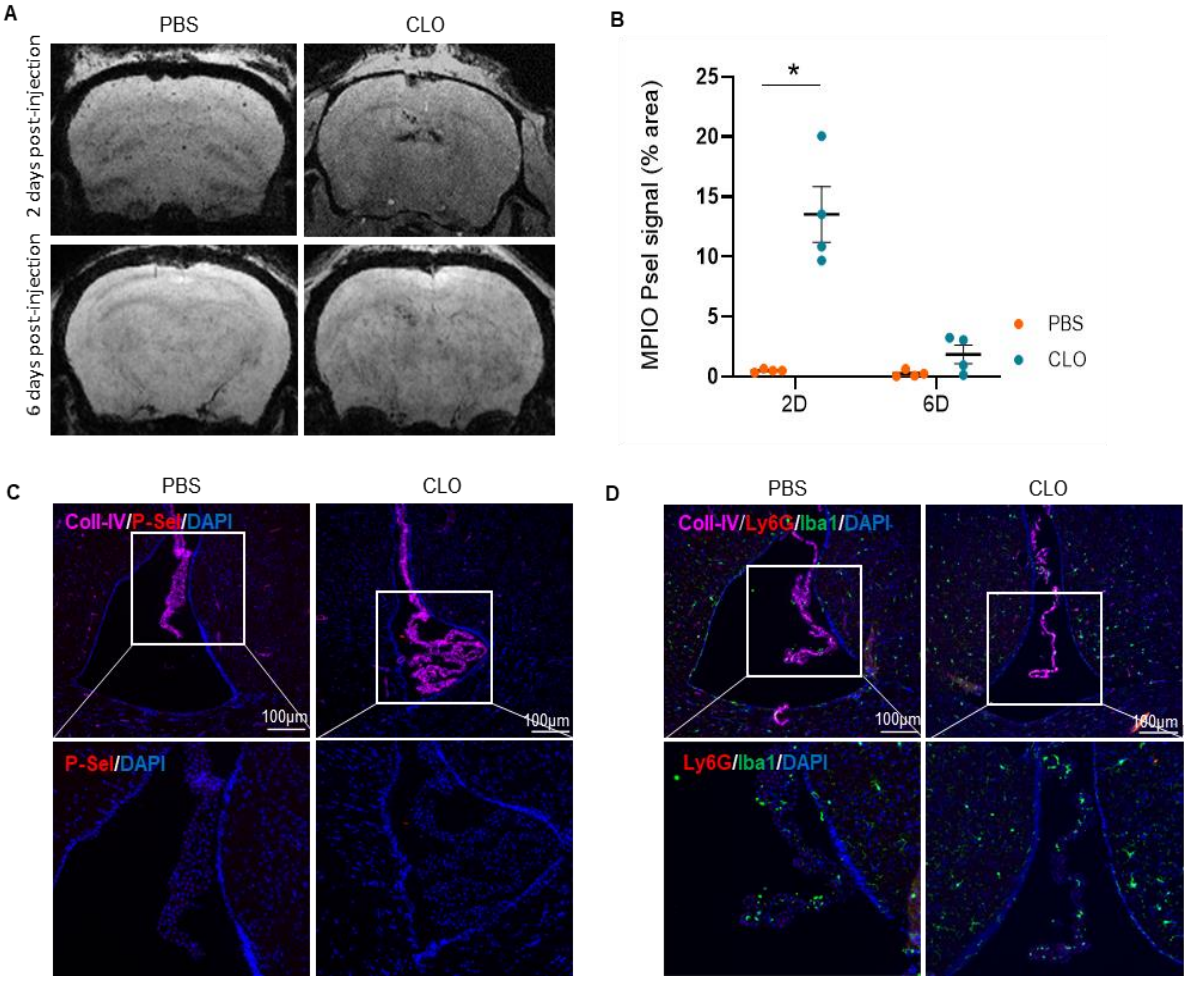


Supplementary figure 2: Immunohistological characterisation of PVM. PVM are positive for the lysosomal marker CD68 (A, C, E, F) and the membrane marker CD206 (B, D, E). PVM are negative for Iba1 (A, B, F). PVM are located surrounding brain blood vessels, between the blood vessel wall (Coll-IV) and astrocytic endfeet (AQP4) (C, D). Alcohol-induced increase in PVM numbers detected by CD68+/Iba1- counting (F, G).

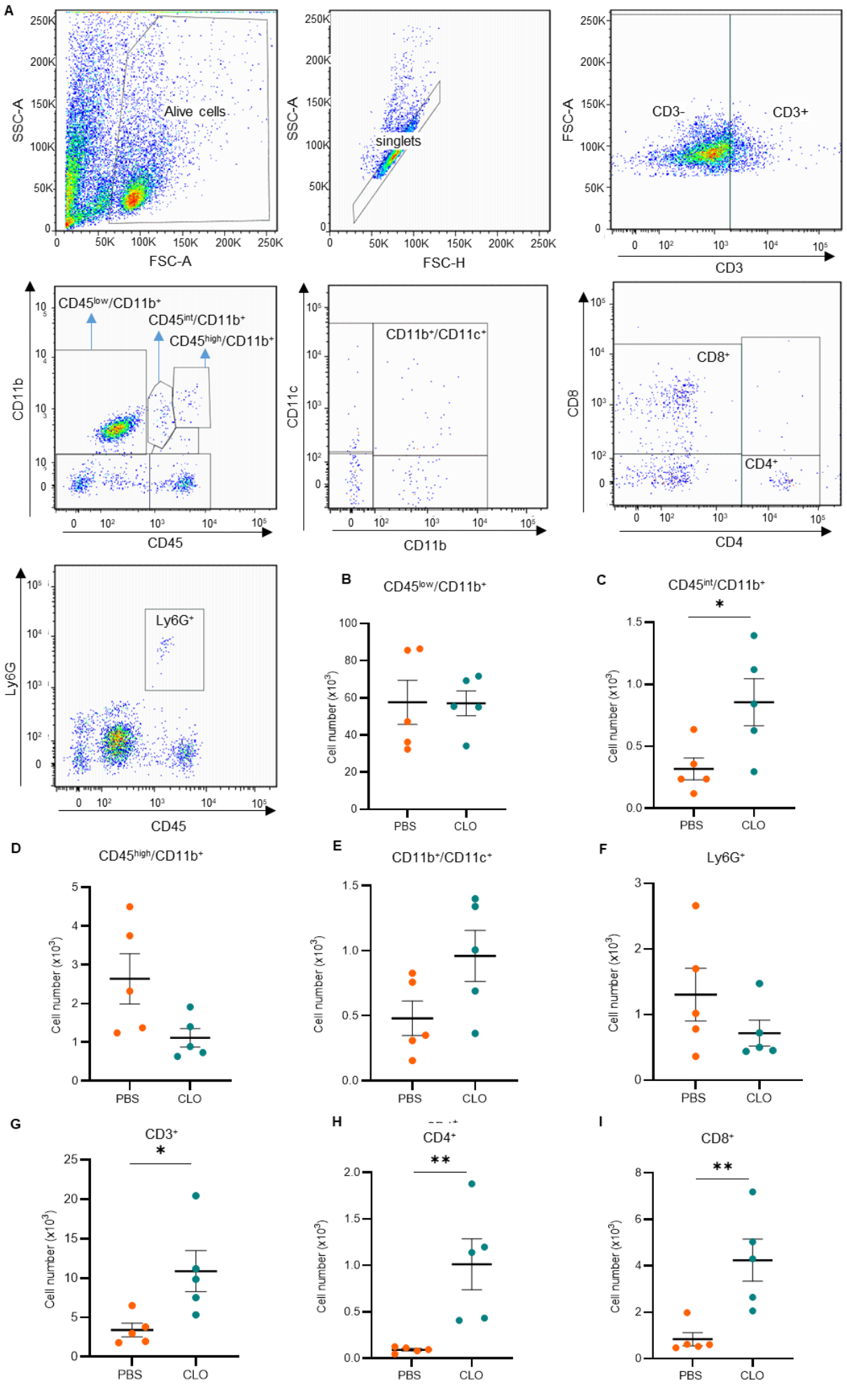


Supplementary figure 3: Lack of peripheral macrophage recruitment in mice chronically exposed to alcohol. A) Study design (positive control of the technique). Nude MPIOs were intravenously injected to naïve mice. B) The day after, a first 3D T2*-w acquisition was performed to control the absence of unspecific MPIOs signal. C) Two days after MPIOs IV injection, mice were subjected to a single intracortical LPS injection and, 48h later, MPIO+

macrophages were visible at the ipsilateral cortex. D) Representative photomicrographs of MPIO+ infiltrated macrophages (MPIO+/CD68+/Iba1-). E) Higher magnification of D, showing phagocyted MPIOs (white dots, arrows) in the cytoplasm of infiltrated macrophages. F) Representative T2*-w MRI scans of control and alcohol-exposed mice previously injected with MPIOs. T2*-w MRI acquisitions were performed before, after 2 weeks and after 6 weeks of alcohol exposure. No MPIO+ macrophages were detected in the brain at any time. G) Absence of Ki67 positive PVM in control and alcohol-exposed mice. H) Positive nucleus shown as a positive control of Ki67 staining.



Supplementary Figure 4: The intracerebroventricular (icv) injection of clodronate induces visible inflammatory responses in the brain ventricles 2 days after its injection, but they disappear at 6 days post-injection. (A) Representative T2*-GEFC acquisitions in PBS or CLO injected mice after 2 or 6 days post-injection. Note that MPIO-αP-selectin accumulation in the ventricles is detected exclusively 2 days after the injection of CLO. (B) Quantification of MPIO/αP-selectin positive area. (C, D) Representative photomicrographs of ventricles and choroid plexus 6 days after PVM depletion by CLO icv injection, stained for P-selectin (shown in panel C), microglia (Iba-1) and neutrophils (Ly6G) (shown in panel D). N=4 mice/group; *p<0.05 vs PBS, Mann-Whitney test.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SSC-A
FSC-A
Alive cells
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CD3-
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CD11b
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CD45int/CD11b+
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CD45
CD11c
CD11b+/CD11c+
CD11b
CD8
CD8+
CD4+
CD4
Ly6G
Ly6G+
CD45
B
CD45low/CD11b+
C
CD45int/CD11b+
D
CD45high/CD11b+
E
CD11b+/CD11c+
F
Ly6G+
G
CD3+
H
CD4+
I
CD8+
Cell number (x10³)
PBS
CLO
*
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Supplementary Figure 5: Flow cytometry analyses of the inflammatory responses in the brain 5 days after the icv injection of PBS or clodronate (CLO). A) Illustration of the gating strategy, taken from a representative CLO-injected mouse. B) Quantification of CD45low/CD11b+ cells (considered as resting microglia). C) Quantification of CD45int/CD11b+ cells (considered as activated microglia). D) Quantification of CD45high/CD11b+ cells (considered as macrophages). E) Quantification of CD11b+/CD11c+ cells (considered as dendritic cells). F) Quantification of Ly6G+ cells (considered as granulocytes). G) Quantification of CD3+ cells (total lymphocytes). H) Quantification of CD4+ lymphocytes. I) Quantification of CD8+ lymphocytes. N=5 mice/group; *$p<0.05$ vs PBS, Mann-Whitney test.

Supplementary materials and methods bibliography

1. Gache P et al. The Alcohol Use Disorders Identification Test (AUDIT) as a screening tool for excessive drinking in primary care: reliability and validity of a French version. *Alcohol Clin Exp Res*. 2005;29(11):2001-2007.

2. Beck AT, Steer RA. *BDI, Beck Depression Inventory: Manual*. San Antonio, Tex.; New York: Psychological Corp. ; Harcourt Brace Jovanovich; 1987.

3. Spielberger CD. *Manual for the State-Trait Anxiety Inventory (Form Y) ("self-Evaluation Questionnaire")*. Consulting Psychologists Press; 1983.

4. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. *Br J Addict*. 1991;86(9):1119-1127.

5. Orset C et al. Mouse Model of In Situ Thromboembolic Stroke and Reperfusion. *Stroke*. 2007;38(10):2771-2778. doi:10.1161/STROKEAHA.107.487520

6. Montagne A et al. Ultra-sensitive molecular MRI of cerebrovascular cell activation enables early detection of chronic central nervous system disorders. *NeuroImage*. 2012;63(2):760-770. doi:10.1016/j.neuroimage.2012.07.018

7. Hughes MM, Field RH, Perry VH, Murray CL, Cunningham C. Microglia in the degenerating brain are capable of phagocytosis of beads and of apoptotic cells, but do not efficiently remove PrPSc, even upon LPS stimulation. *Glia*. 2010;58(16):2017-2030. doi:10.1002/glia.21070

8. Quenault A et al. Molecular magnetic resonance imaging discloses endothelial activation after transient ischaemic attack. *Brain*. 2017;140(1):146-157. doi:10.1093/brain/aww260

9. Drieu A et al. Immune Responses and Anti-inflammatory Strategies in a Clinically Relevant Model of Thromboembolic Ischemic Stroke with Reperfusion. *Transl Stroke Res*. September 2019. doi:10.1007/s12975-019-00733-8

10. Marcos-Contreras OA et al. Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. *Blood*. 2016;128(20):2423-2434. doi:10.1182/blood-2016-03-705384

11. Lehenkari PP et al. Further insight into mechanism of action of clodronate: inhibition of mitochondrial ADP/ATP translocase by a nonhydrolyzable, adenine-containing metabolite. *Mol Pharmacol*. 2002;61(5):1255-1262.