Materials and Methods

Middle cerebral artery occlusion procedure

Male and female mice, 8-12 weeks of age, underwent focal cerebral ischemia with silicone rubber-coated 6–0 nylon monofilaments (602256PK5Re; Doccol, Sharon, MA, USA). Briefly, mice were anesthetized by isoflurane of 3.5% isoflurane and maintained by inhalation of 1.0–2.0% isoflurane in 70% N_2O and 30% O_2 by a mask. A carotid incision was made at the left common carotid artery (CCA), the internal carotid artery (ICA) and external carotid artery (ECA) were separated. Once ligation of the ECA and CCA was achieved, the silicone tipped filament was inserted and passed through the ICA. Advancement of the filament was abated when resistance is encountered at 8-9 mm and the filament was secured. After 60 minutes of occlusion, the filament was withdrawn, allowing reperfusion. During the procedure, the body temperature was maintained at 37 °C. The mice were treated with meloxicam (5mg/kg) by injection every 24 h after operation. Of the 873 mice used in this study, 71 mice died after surgery and 45 mice had insufficient reperfusion and excluded from the experiment. After preliminary statistical analysis, the mortality of MCAO group was 8.1% (71 in 873). The success rate of the MCAO model was 86.7% (757/873).

Neurological assessment

The neural severity score, which covers the evaluation of such dimensions as motion, sensation and reflex, were also conducted. The maximum score, the sum of these dimensions, is 18 points. The evaluation is mainly conducted according to the following methods: 13-18 points, represents the severest degree of injury: 7-12 points, marks relatively lower degree of injury, and those lower than the above points are the least serious, that is, the score value is positive correlated with the injury severity. After surgery, each mouse was evaluated according to the above scoring method. Within one day after MCAO, if the final score is no less than 6 points and no more than 13 points, these mice will be excluded.

Zheng P, *et al. Stroke Vasc Neurol* 2023; 8:e001919. doi: 10.1136/svn-2022-001919

The method of corner turning test is as follows: the mice were all entered a corner at 30°. During this period, the animals included in the study could turn direction to leave the corner smoothly. The operation procedure was and recorded 10 times. At least half a minute between each test during operation, and the proportion of right turns to total turns is finally determined.

Flow cytometry

Single-cell suspensions were prepared from mouse blood, spleen, lung or brain tissues. Cells were incubated with FcγR blocker and the fluorochrome-labeled or their corresponding isotype controls antibodies were used to stain. The following antibodies were used to characterize ILC2s: CD45 (30-F11, 103116, BioLegend, San Diego, CA, USA), ST2 (DIH4, 146610, BioLegend, San Diego, CA, USA); CD90.2 (30-H12, 105315, BioLegend, San Diego, CA, USA), CD3e (17A2, 13-0032-82, Invitrogen, Carlsbad, CA, USA), CD45R (RA3-6B2, 13-0452-86, Invitrogen, Carlsbad, CA, USA), CD11b (M1/70,13-0112-82, Invitrogen, Carlsbad, CA, USA), Ter119 (TER-119, 13-5621-82, Invitrogen, Carlsbad, CA, USA), Ly-6G (RB6-8C5, 13-5931-82, Invitrogen, Carlsbad, CA, USA), CD11c (N418, 117303, BioLegend, San Diego, CA, USA), NK1.1 (PK136, 108703, BioLegend, San Diego, CA, USA), CD4 (GK1.5, 100403, BioLegend, San Diego, CA, USA), CD5 (53-7.3, 100603, BioLegend, San Diego, CA, USA), CD8a (53-6.7, 100703, BioLegend, San Diego, CA, USA), TCR-β (H57-597, 109203, BioLegend, San Diego, CA, USA) and TCR-γδ (GL3, 118103, BioLegend, San Diego, CA, USA). The cells were incubated for 4 h with phorbol-12-myristate-13-acetate, ionomycin and GolgiStop, then fixed and permeabilized based on relevant permeabilization kit (BD Biosciences, San Diego, CA, USA). The following intracellular staining antibodies were used: IL-4 (11B11, 504119, Biolegend, San Diego, CA, USA), IL-5 (TRFK5, 504311, Biolegend, San Diego, CA, USA), IL-9 (RM9A4, 514112, Biolegend, San Diego, CA, USA), IL-13 (W17010B, 159403, Biolegend, San Diego, CA, USA) and amphiregulin (AREG559, 12-5370-42, eBioscience, San Diego, CA, USA). During the whole experiment process, cells were stained by the live/dead fixable dye (Molecular Probes) to allow gating on

viable cells. Flow cytometric data were analyzed on a FACS Aria III flow cytometer (BD Bioscience). The gating was set using FMO controls and were carried out based on Flow Jo V10 (FlowJo.com) to analysis and are shown in **Figure S1**.

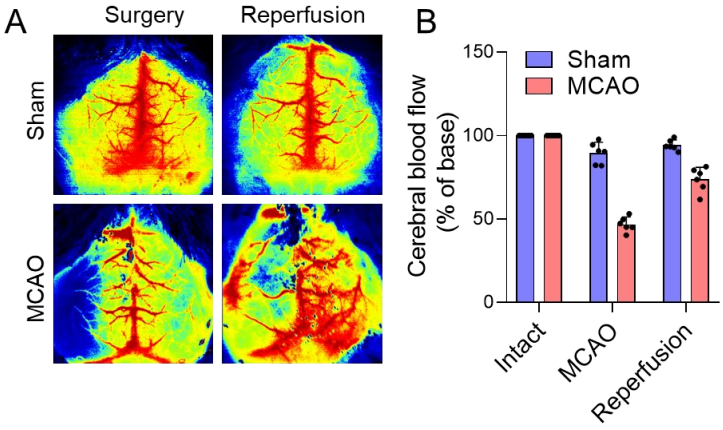

Figure S1. The blood perfusion monitoring in mice after MCAO surgery and reperfusion. The representative (A) and quantification (B) of cerebral blood flow before and right after MCAO surgery, as well as after 60 min reperfusion in C57/B6 mice. n = 6 mice per group. Error bars represent SD.

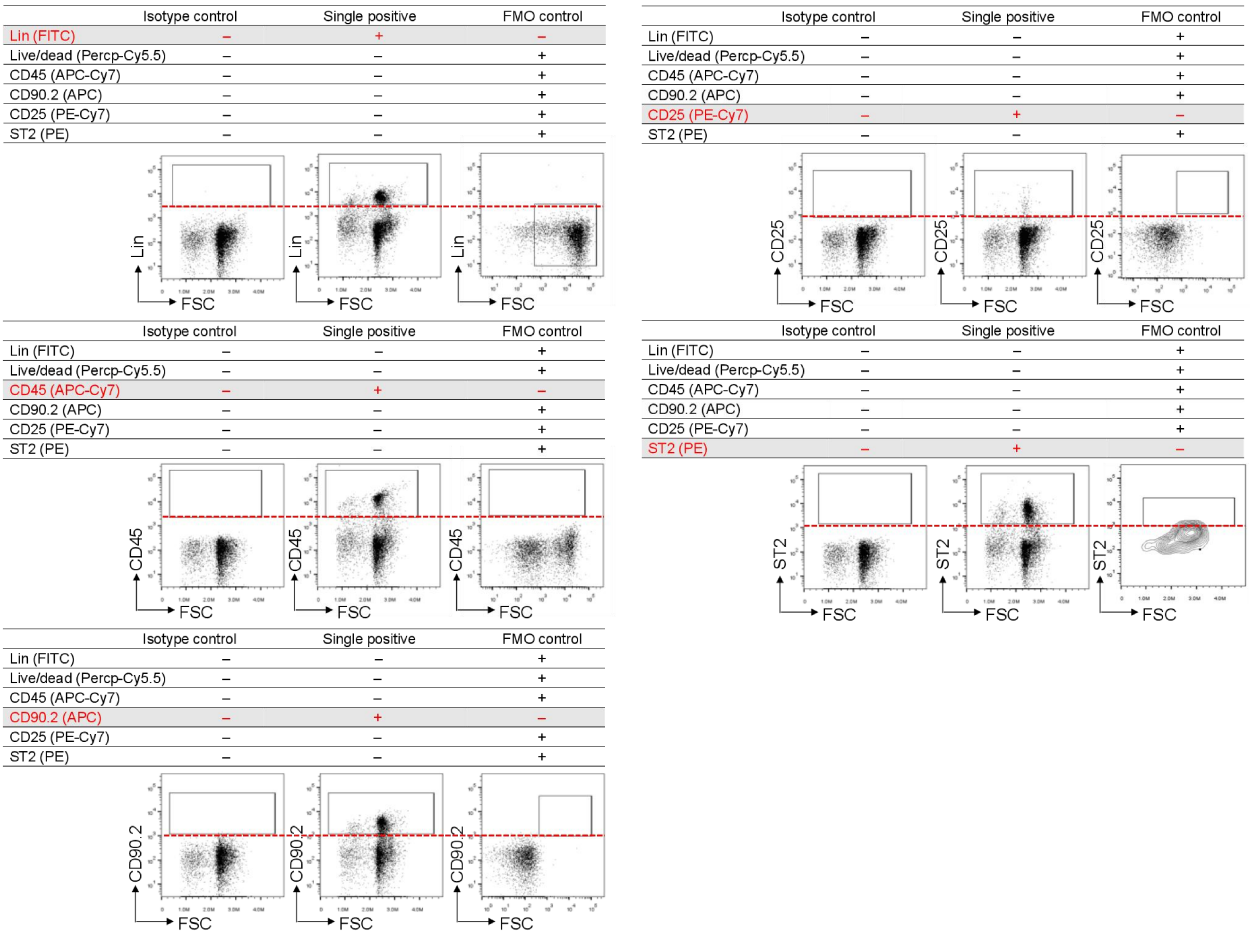

Figure S2. Gating strategy of isotype, single-positive and fluorescence minus one (FMO) controls for identification of splenic ILC2s. Single-cell suspensions were prepared from the spleens of *C57* mice at day 1 of MCAO and stained with fluorochrome-conjugated antibodies. ILC2s were defined as $CD45^{high}CD90.2^{+}$ $ST2^{+}$ lymphoid cells negative for lineage markers (CD3e, CD45R, CD11b, Ter119, Ly-6G, CD11c, NK1.1, CD4, CD5, CD8a, TCR-β and TCR-γδ). Representative flow cytometry dot plots show the identification of gating boundaries of Lin (FITC), CD45 (APC-Cy7), CD90.2 (APC), CD25 (PE-Cy7) and ST2 (PE) with corresponding FMO controls.

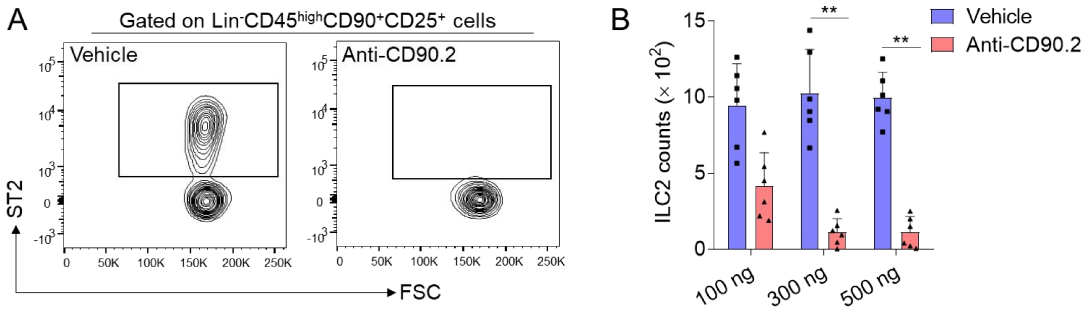

Figure S3. Antibody depletion of ILC2s in MCAO mice. For antibody depletion, anti-CD90.2 mAb was administered by i.v. injection every two days at a dose of 100, 300 and 500 ng/mouse starting two days before model induction. Mice were treated with rat IgG2b as an isotype control immunoglobulin. ILC2s were defined as $CD45^{high}CD90.2^{+}$ $ST2^{+}$ lymphoid cells negative for lineage markers (CD3e, CD45R, CD11b, Ter119, Ly-6G, CD11c, NK1.1, CD4, CD5, CD8a, TCR-β and TCR-γδ). **A-B.** Flow cytometry analysis of ILC2s counts in brain of mice at day 1 post-MCAO receiving IgG control **(A)** or anti-CD90.2 mAb **(B)**. n = 6 per group. **p < 0.01 by Mann-Whitney test. Error bars represent SD.

Table S1. Characteristics of ischemic stroke patients and healthy controls

Subject	Sex Age (yr)	Etiology of stroke†	Location	Reason of death	Time of sample collection (hours after death)
AIS 1	M 76	Atheromatosis	Anterior circulation	Stroke	3
AIS 2	M 79	Atheromatosis	Anterior circulation	Stroke	4
AIS 3	M 66	Atheromatosis	Anterior and posterior circulation	Stroke	3.5
AIS 4	F 81	Atheromatosis	Anterior circulation	Stroke	3
AIS 5	F 64	Atheromatosis	Anterior circulation	Stroke	4
AIS 6	M 61	Atheromatosis	Anterior circulation	Stroke	3.5
AIS 7	F 72	Atheromatosis	Anterior and posterior circulation	Stroke	3
Control 1	M 55	NA	NA	Pancreatitis	2.5
Control 2	M 77	NA	NA	Heart failure	2.5
Control 3	M 72	NA	NA	Acute respiratory distress syndrome	4
Control 4	F 57	NA	NA	Respiratory failure	4
Control 5	F 72	NA	NA	Acute respiratory distress syndrome	2.5
Control 6	F 68	NA	NA	Respiratory failure	2.5
Control 7	M 81	NA	NA	Heart failure	3.5
Control 8	F 60	NA	NA	gastric cancer	4
Control 9	M 66	NA	NA	Colorectal cancer	4

† TrialHC of Org Acute Stroke Treatment (TOAST) classification.

AIS, acute ischemic stroke; NA, not applicable.

Table S2. Physiological parameters in sham and MCAO mice.

	Sham + vehicle	**Sham + CD90.2**		**MCAO + vehicle**	**MCAO + CD90.2**	
	n=4	**n=4**	***p value***	**n=4**	**n=4**	***p value***
	Before sham surgery			**Before MCAO surgery**		
Weight	23.28±2.15	21.85±0.66	*p* >0.05	22.5±1.02	21.55±1.61	*p* >0.05
Heart rate (bpm)	547.5±40.93	504.5±55.24	*p* >0.05	547.5±40.93	543.25±81.89	*p* >0.05
Arterial oxygen pressure (PaO)	98.25±22.91	97.5±16.46	*p* >0.05	117.25±23.43	106.25±11.95	*p* >0.05
Temperature (℃)	37.13±0.51	37.15±0.35	*p* >0.05	37.05±0.26	37.43±0.39	*p* >0.05
Systolic blood pressure (SBP)	109.48±5.81	109.1±5.79	*p* >0.05	108.25±6.99	112.3±1.63	*p* >0.05
Diastolic blood pressure (DBP)	75.75±3.17	64.25±4.04	*p* >0.05	70.25±19.47	71.73±6.32	*p* >0.05
Mid-blood pressure (MBP)	87.65±4.09	79.53±2.36	*p* >0.05	82.5±15.33	85.4±4.55	*p* >0.05
PH values	7.39±0.03	7.38±0.05	*p* >0.05	7.36±0.16	7.41±0.11	*p* >0.05
	After sham surgery			**After 60 min reperfusion**		
Weight	23±3.08	21.53±0.67	*p* >0.05	21.78±1.30	20.6±1.34	*p* >0.05
Heart rate (bpm)	566.5±81.38	577.75± 71.65	*p* >0.05	486.25±61.29	472.25±34.76	*p* >0.05
Arterial oxygen pressure (PaO)	115.5±43.56	126.75± 31.51	*p* >0.05	135±33.05	118.75±13.02	*p* >0.05
Temperature (℃)	36.38±0.79	36.15±0.76	*p* >0.05	33.6±2.31	34.1±0.81	*p* >0.05
Systolic blood pressure (SBP)	95.75±8.18	99.68±9.25	*p* >0.05	93.38±8.71	97.58±10.94	*p* >0.05
Diastolic blood pressure (DBP)	60±9.9	65.43±4.05	*p* >0.05	50.4±15.49	63.15±9.55	*p* >0.05
Mid-blood pressure (MBP)	71.93±9.17	76.75±5.33	*p* >0.05	65.08±12.81	74.75±0.17	*p* >0.05
PH values	7.23±0.05	7.18±0.15	*p* >0.05	7.03±0.05	7.12±0.04	*p* >0.05

Values are mean±SD.