

SUPPLEMENTAL MATERIALS

Tables S1. Primer sequences and conditions for RT-qPCR.

Target	Product length (bp)	Annealing temperature (°C)	Primer (5'-3')	Genbank accession no.
VEGFC-F	147	60	CTGCTGCACATTATAACACAGAGA	22341
VEGFC-R			ACGGACACACATGGAGGTTTAAA	
FOXC2-F	300	60	ACTCTTACGACTGCACCAAATACT	14234
FOXC2-R			TCAAACCTGAGCTGCGGATAAGT	
GAPDH-F	150	60	ATGGGAAGCTGGTCATCAAC	14433
GAPDH-R			TGCTGTAGCCAAATTCGTTG	

Table S2. Demographics and baseline data of Control and ICH/IVH

	Control(n=9)	ICH/IVH(n=9)	Fisher/t	P
Sex male, n (%)	5 (55.5)	4 (44.5)	-	0.999
Age ($\bar{X}\pm S$, years)	52.6±9.6	67±15	t=2.43	0.027
Education			-	0.999
Postgraduate, n (%)	1 (11.1)	2 (22.2)		
Undergraduate, n (%)	2 (22.2)	1 (11.1)		
Secondary school, n (%)	4 (44.4)	3 (33.3)		
Primary school, n (%)	2 (22.2)	3 (33.3)		
Psychological consultation, n (%)	0 (0)	0 (0)	-	—
Antidepressant, n (%)	0 (0)	0 (0)	-	0.999
Parkinson's disease, n (%)	0 (0)	0 (0)	-	0.999
Huntington, n (%)	0 (0)	0 (0)	-	0.999
Alzheimer's disease, n (%)	0 (0)	0 (0)	-	0.999
Hypertension, n (%)	1 (11.1)	8 (88.8)	-	<0.001
Hyperlipidemia, n (%)	0 (0)	1 (11.1)	-	0.999
Diabetes, n (%)	0 (0)	0 (0)	-	0.999
Warfarin treatment, n (%)	0 (0)	1 (11.1)	-	0.999
History of stroke, n (%)	0 (0)	0 (0)	-	0.999
Smoking, n (%)	2 (22.2)	4 (44.4)	-	0.620
Alcohol abuse, n (%)	1 (11.1)	3 (33.3)	-	0.576

Note:- stands for fisher exact probaliity method, t stands for Student's t test. ICH: intracerebral hemorrhage. IVH: intraventricular hemorrhage.

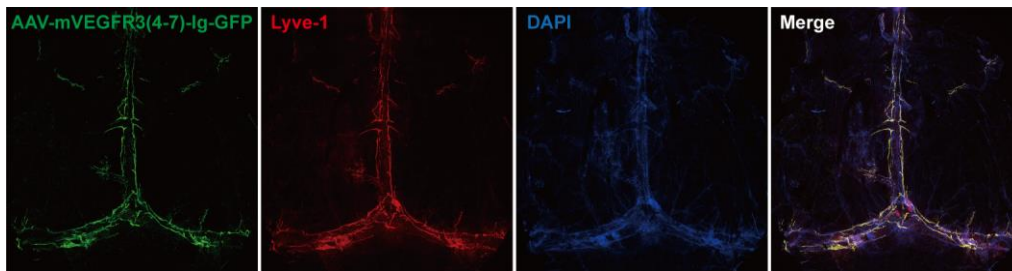


Figure S1. Representative photomicrographs of LYVE1-stained mLVs were obtained four weeks later, following the i.c.v. injection of the specified AAVs into six-week-old mice.

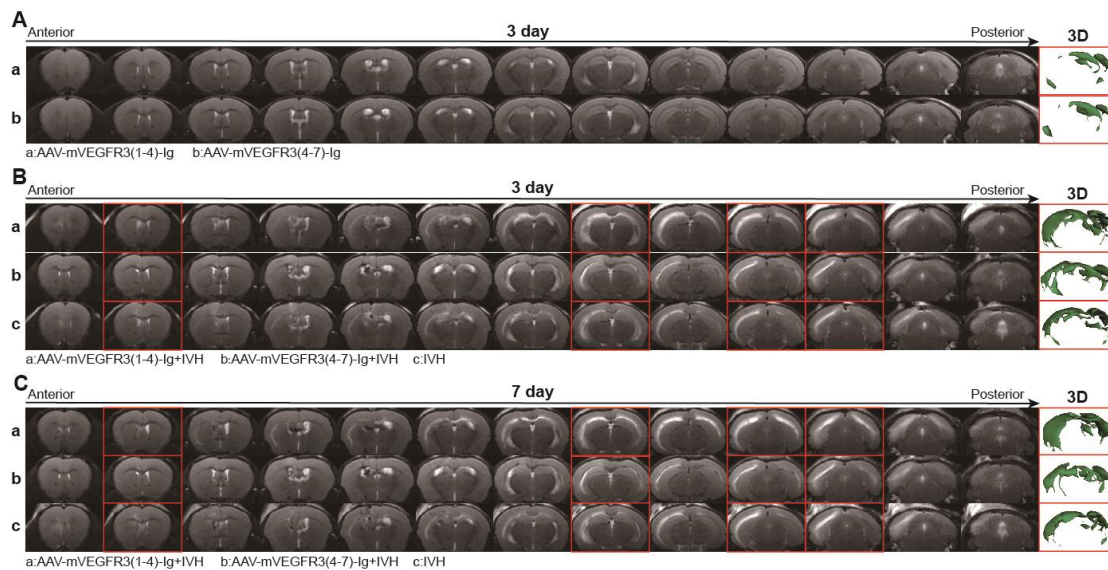


Figure S2. (A) All T2-weighted MRI images and 3D reconstructions of the lateral ventricles were acquired on 4 weeks after AAV-mVEGFR3(1-4)-Ig and AAV-mVEGFR3(4-7)-Ig injection. **(B,C)** Representative all T2-weighted images along with 3D reconstructions of lateral ventricles, captured on days 3 and 7 following IVH, from the AAV-mVEGFR3-Ig(1-4)+IVH group, AAV-mVEGFR3(4-7)-Ig+IVH group, and IVH group.

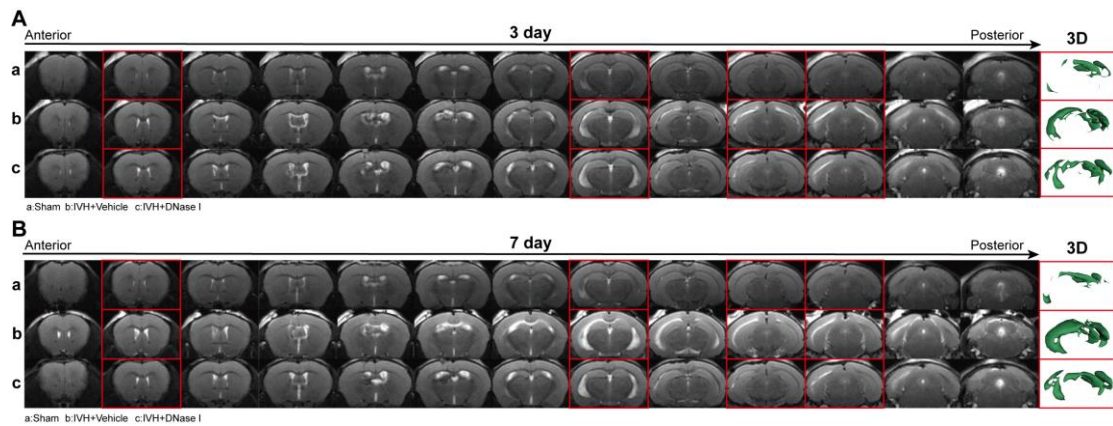


Figure S3. (A) All T2-weighted MRI images and 3D reconstructions of the lateral ventricles were acquired on three days for the Sham, IVH, and IVH+DNase I groups on days 3. **(B)** All T2-weighted MRI images and three-dimensional reconstructions of the lateral ventricles were acquired on seven days and for the Sham, IVH, and IVH+DNase I groups on days 7 after IVH.

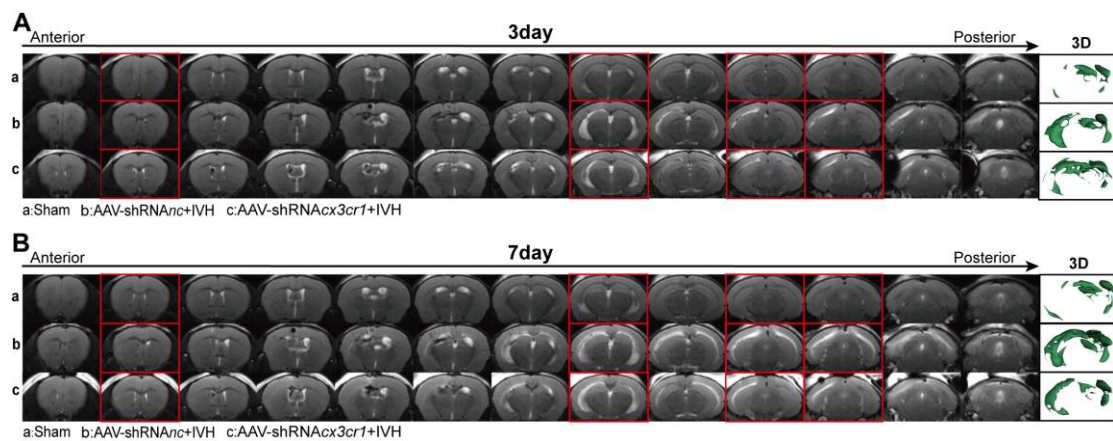


Figure S4. (A) All T2-weighted MRI images and 3D reconstructions of the lateral ventricles were acquired on three days for the Sham, AAV-shRNAnc+IVH, and AAV-shRNAcx3cr1+IVH groups on days 3 after IVH. **(B)** All T2-weighted MRI images and three-dimensional reconstructions of the lateral ventricles were acquired on seven days for the Sham, AAV-shRNAnc+IVH, and AAV-shRNAcx3cr1+IVH group on days 7 after IVH.

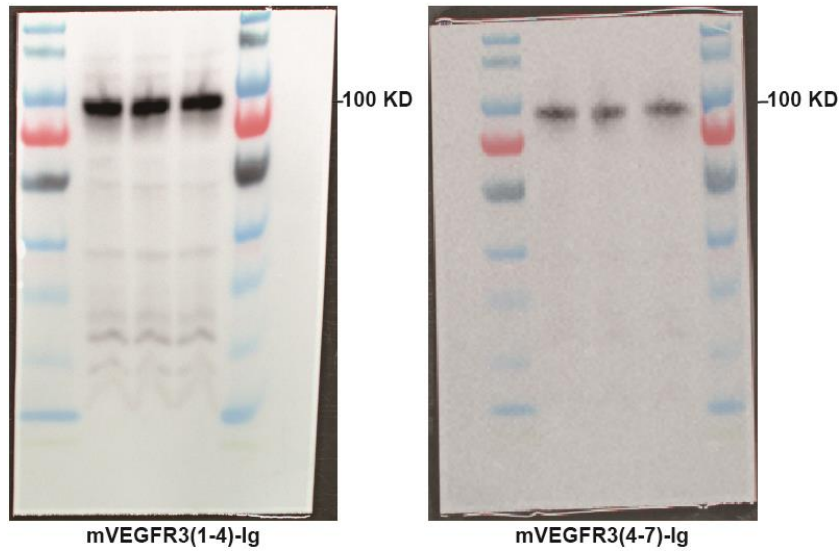


Figure S5. Western blot analysis revealing mVEGFR3-Ig protein in the serum one week following an i.p. AAV injection is shown.

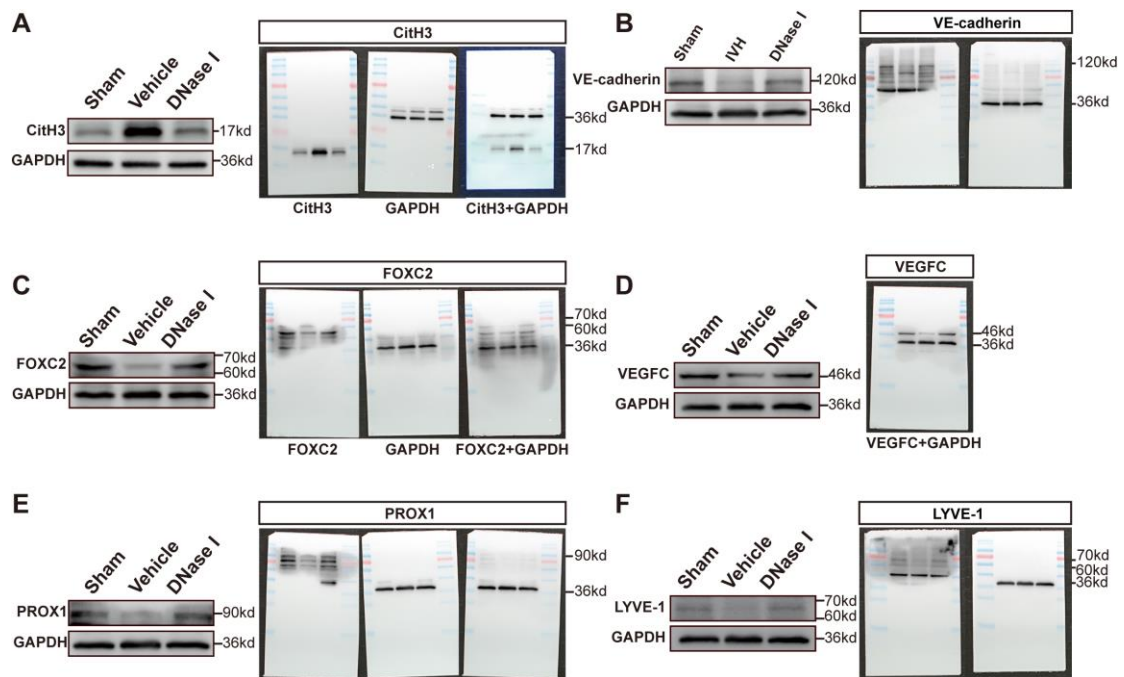
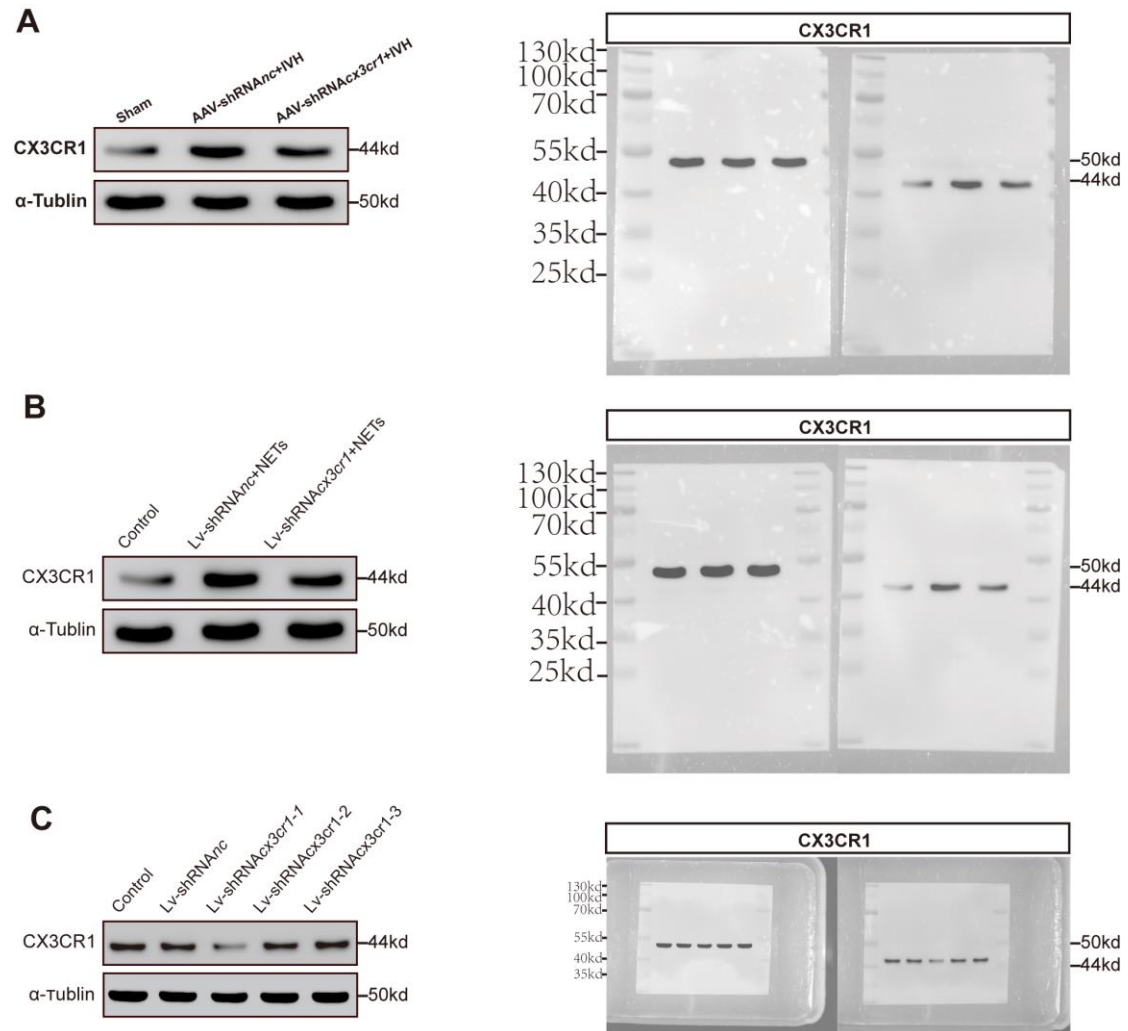


Figure S6. (A) A Western blot analysis revealing CitH3 protein in meninges at 24 h after IVH. GAPDH was used as an internal reference protein. (B) A Western blot analysis revealing VE-cadherin protein in meninges at 24 h after IVH. (C) Western blot analysis revealing FOXC2 protein in meninges at 24 h after IVH. (D) Western blot analysis revealing VEGFC protein in

meninges at 24 h after IVH. **(E)** Western blot analysis revealing PROX1 protein in meninges at 24 h after IVH. **(F)** Western blot analysis revealing LYVE-1 protein in meninges at 24 h after IVH.



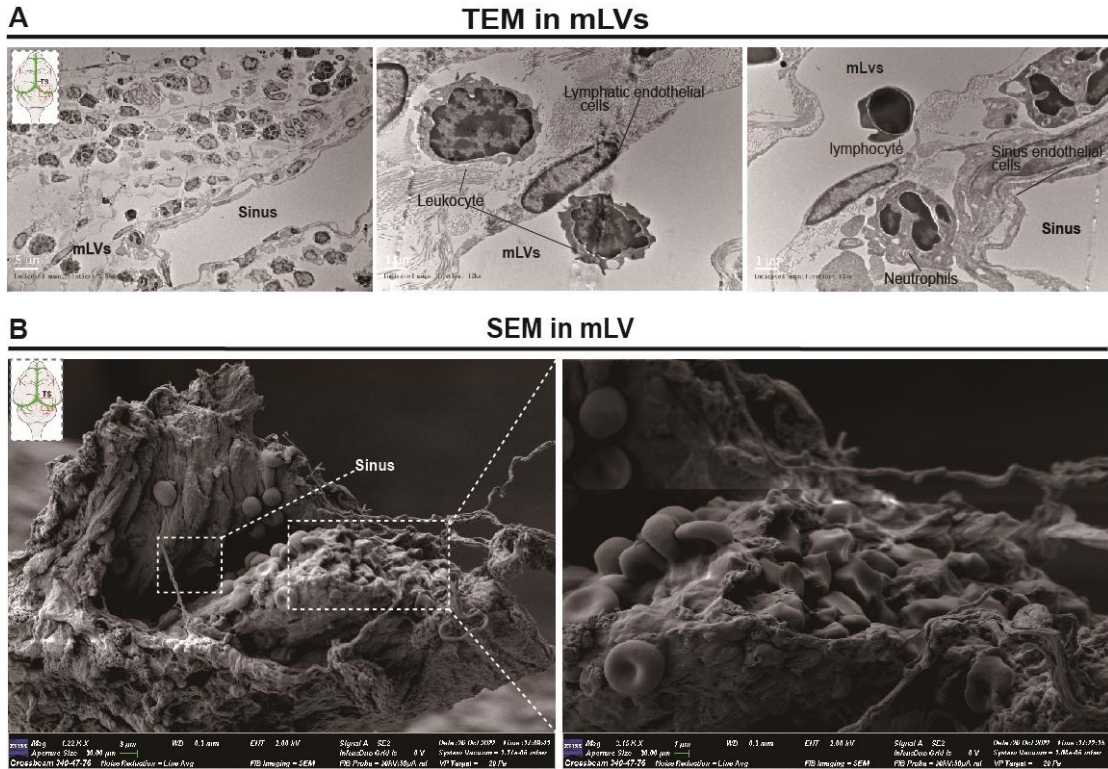


Figure S8. (A) Transmission electron microscopy (TEM) revealing the ultrastructural composition of the TS, including sinus endothelial cells, lymphatic endothelial cells, lymphocytes, neutrophils, and leukocyte interactions after 24 h of IVH. (B) Scanning electron microscopy (SEM) revealing the ultrastructural composition of the TS, including the sinus, mLVs, and entrapment of erythrocytes, polymorphonuclear neutrophils, and fibrin within the mLVs following post-IVH drainage.

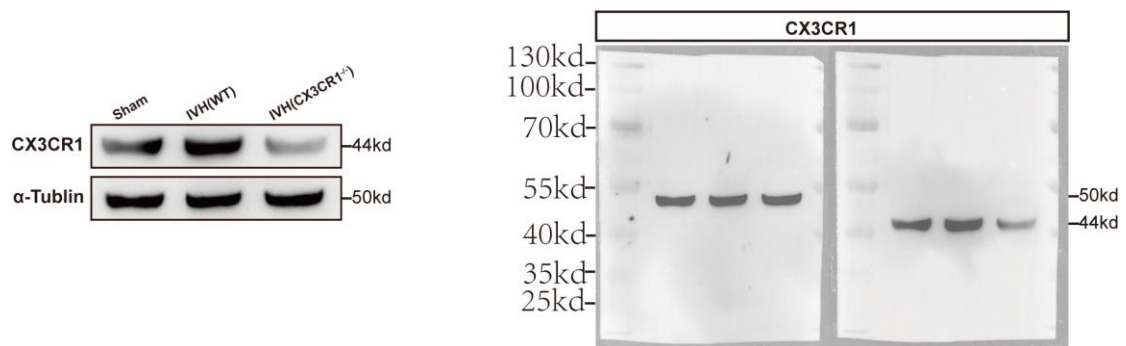


Figure S9. Western blot analysis revealing CX3CR1 protein in the meninges of mice in the Sham,

IVH(WT) and IVH(CX3CR1^{-/-}) groups at 24 h after IVH.

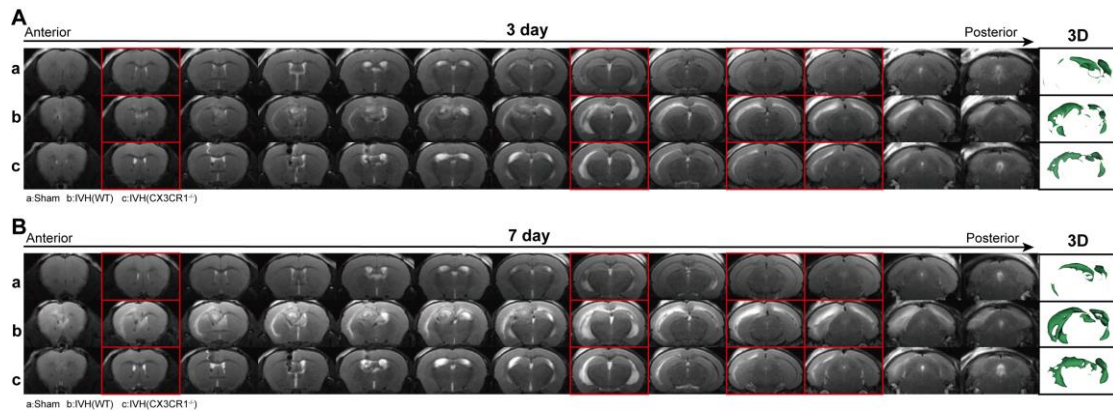


Figure S10. (A) All T2-weighted MRI images and 3D reconstructions of the lateral ventricles were acquired on three days for the Sham, IVH(WT), and IVH(CX3CR1^{-/-}) groups. (B) All T2-weighted MRI images and three-dimensional reconstructions of the lateral ventricles were acquired on seven days for the Sham, IVH(WT), and IVH(CX3CR1^{-/-}) groups.

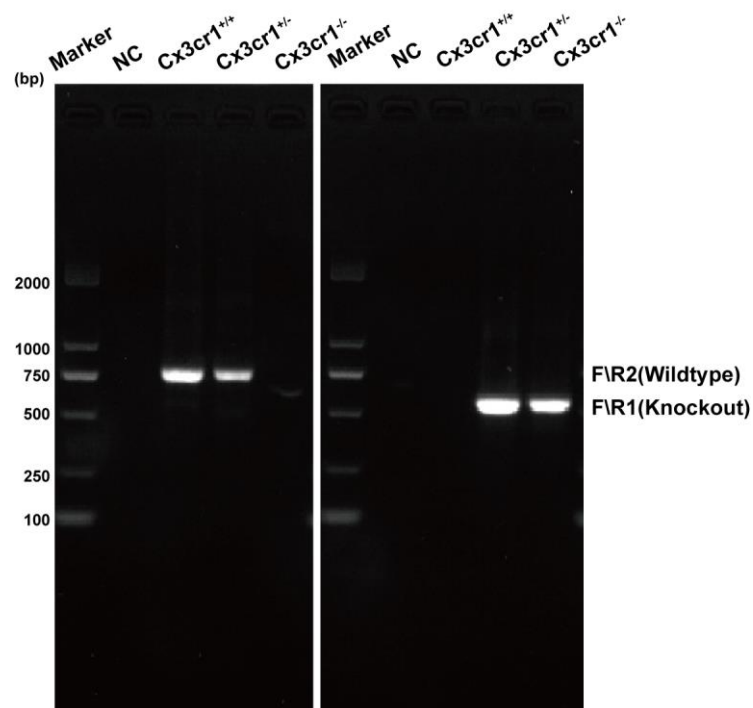


Figure S11. The PCR identification of mouse genotypes. F\R1 is used for knockout sequence and F\R2 for wildtype sequence.

Supplemental Methods

Experiments design and grouping:

The detail of the animals used was shown as follow: Total WT mice: 351 (0.8% mortality); Total Cx3cr1^{-/-} mice: 18 (0 mortality).

Experiment 1: Western blots (WB), Immunofluorescence staining (IF), Hydrocephalus and RNA sequencing (RNA-seq).

Tests	AAV-mVEGFR 3-Ig(1-4)(WT)	AAV-mVEGF R3-Ig(4-7)(W T)	AAV-mVEG FR3-Ig(1-4) +IVH(WT)	AAV-mVE GFR3(4-7) +IVH(WT)	IVH(WT)
WB	3	3	0	0	0
RNA-seq	0	0	3	3	0
IF	6	6	0	0	0
MRI	6	6	6	6	6

(Notes: Number of mice; Total number: 42.)

Experiment 2: Immunofluorescence staining (IF), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Tests	IVH(WT)
IF	12
SEM	3
TEM	3

(Notes: Number of mice; Total number: 18)

Experiment 3: Immunofluorescence staining (IF).

Tests	IVH(WT)
IF	3

(Notes: Number of mice; Total number: 3.)

Experiment 4: Immunofluorescence staining (IF), Western blots (WB), RT-qPCR, ELISA, NO analysis, hydrocephalus and neurofunction assessment.

Tests	Sham(WT)	IVH(WT)+Vehicle	IVH(WT)+DNase I
IF	6	6	6
WB	6	6	6
RT-qPCR	6	6	6
ELISA	6	6	6
NO	6	6	6
MRI	6	6	6
Open-field test	6	6	6
mNSS	6	6	6
Morris water maze test	6	6	6

(Notes: Number of mice; Total number: 90)

Experiment 5: Meningeal lymphatic vessel drainage function assessment.

Tests	Sham(WT)	IVH(WT)+Vehicle	IVH(WT)+DNase I
IF	18	18	18
Living image	6	6	6
Camera	6	6	6

(Notes: Number of mice; Total number: 90)

Experiment 6: Cell experiment

Tests	Sham(WT)
-------	----------

Neutrophil isolation	18
----------------------	----

(Notes: Number of mice; Total number: 18, 18 mice from experiment 4)

Experiment 7: Meningeal lymphatic vessel drainage function assessment

Tests	Sham(WT)	IVH(WT)+Vehicle	IVH(WT)+DNase I
IF	12	12	12
WB	6	6	6
MRI	6	6	6
Open-field test	6	6	6
mNSS	6	6	6

(Notes: Number of mice; Total number: 72)

Experiment 8: Meningeal lymphatic vessel drainage function assessment

Tests	Sham	IVH(WT)	IVH(Cx3cr1 ^{-/-})
WB	6	6	6
IF	6	6	6
MRI	6	6	6
Open-field test	6	6	6

(Notes: Number of mice; Total number: 54)

Experiment 9: Cell experiment

Tests	Sham(WT)
Neutrophil isolation	6

(Notes: Number of mice; Total number: 6, 6 mice from experiment 4.)