Uncropped scans of Figures

Figure 1H

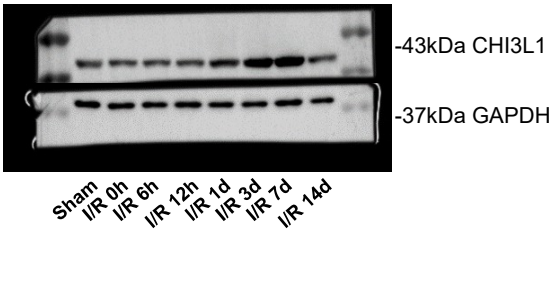


Figure 2B

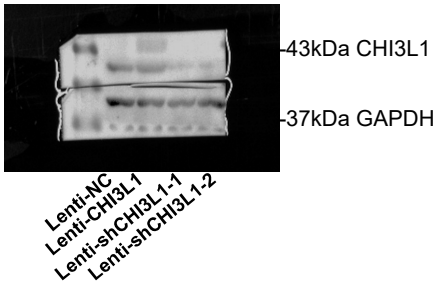


Figure 3B

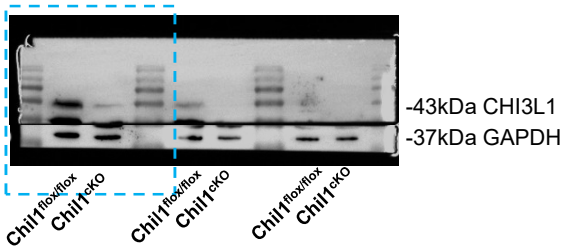


Figure 3K

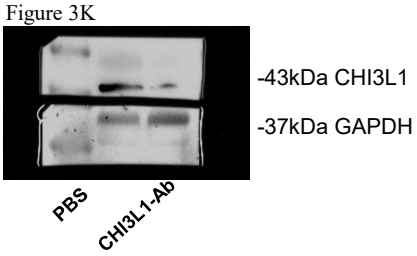


Figure 4H

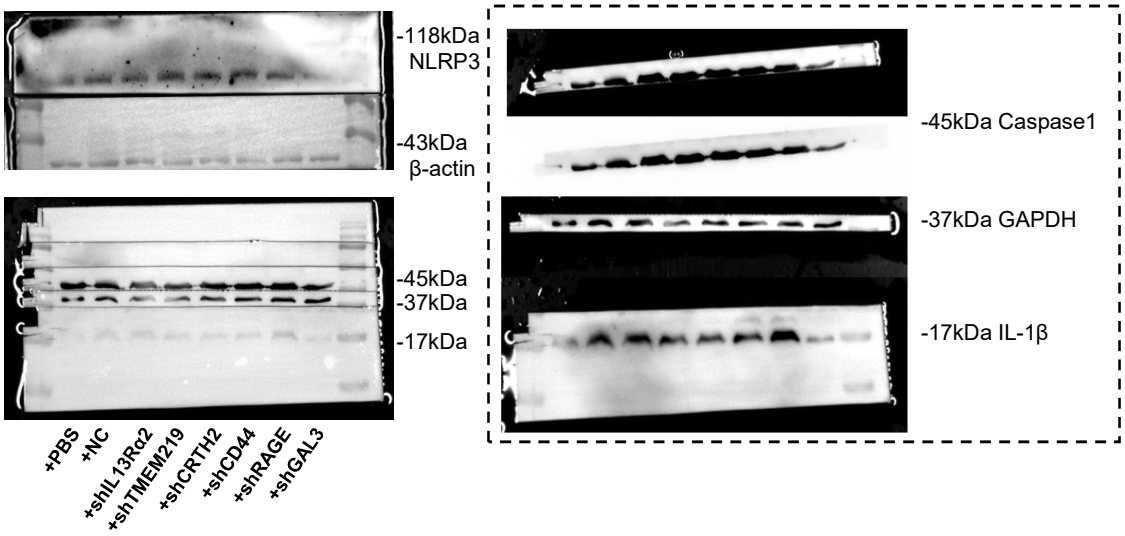


Figure 4B

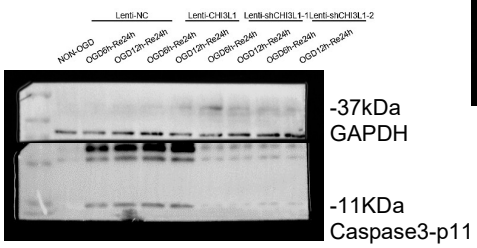
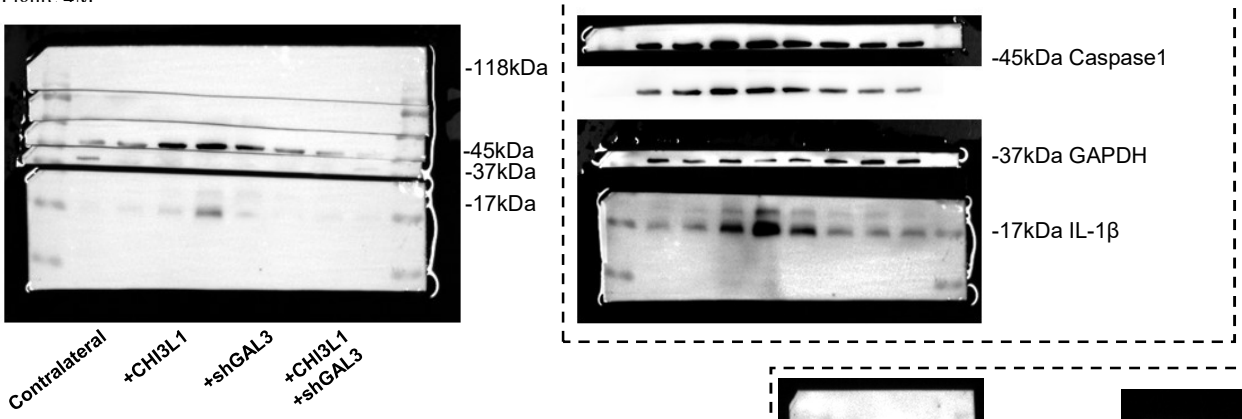


Figure 4M



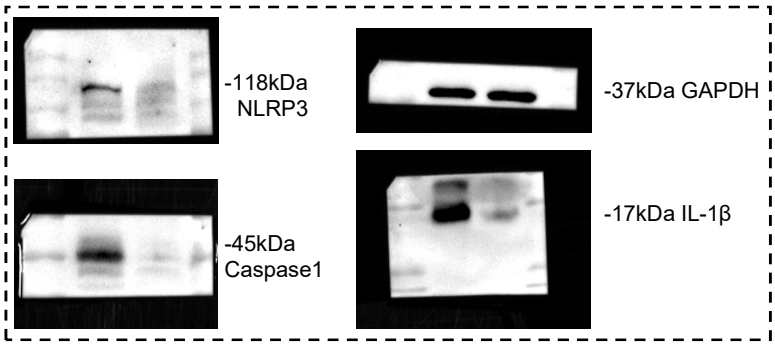
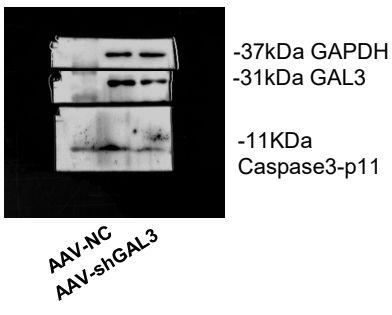


Figure 5L



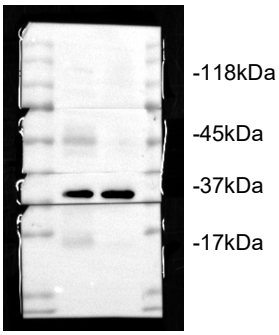
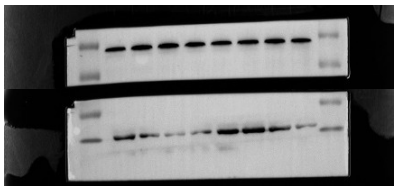


Figure 6C



Uncropped scans of Supplemental Figures

Figure S3H

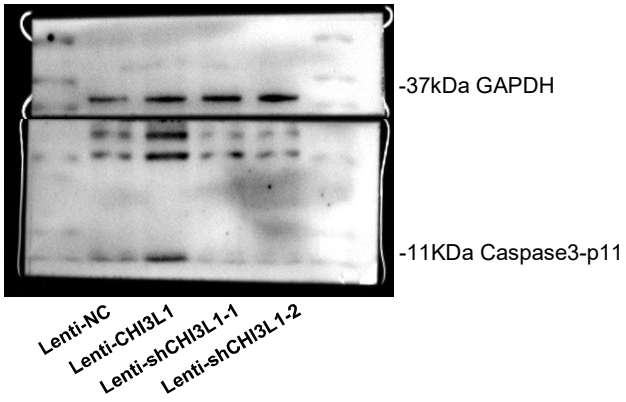


Figure S4E

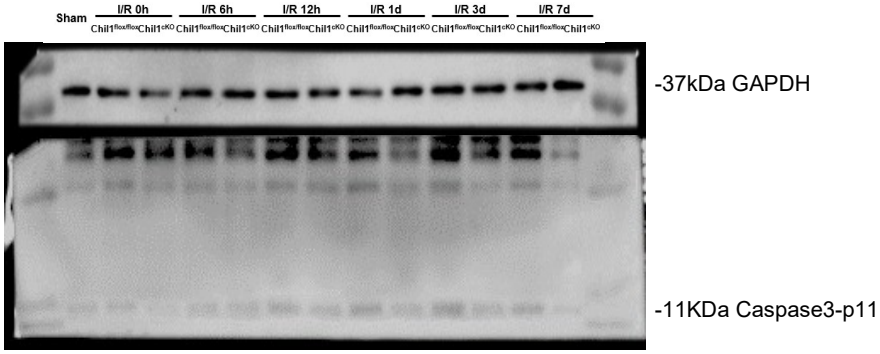


Figure S8D

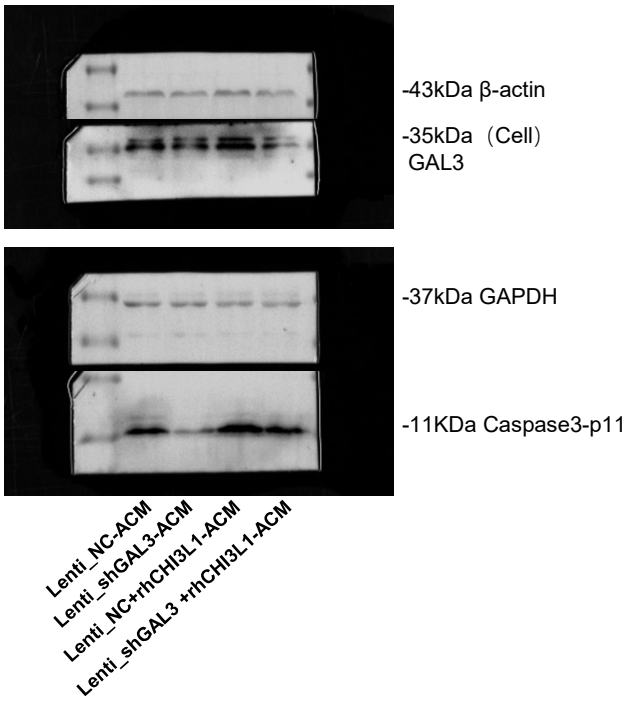


Figure S8G

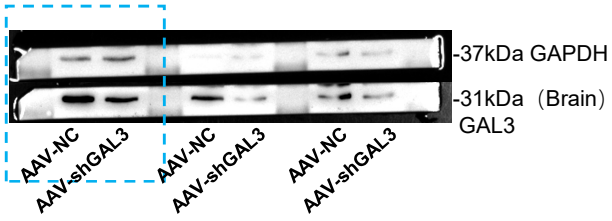
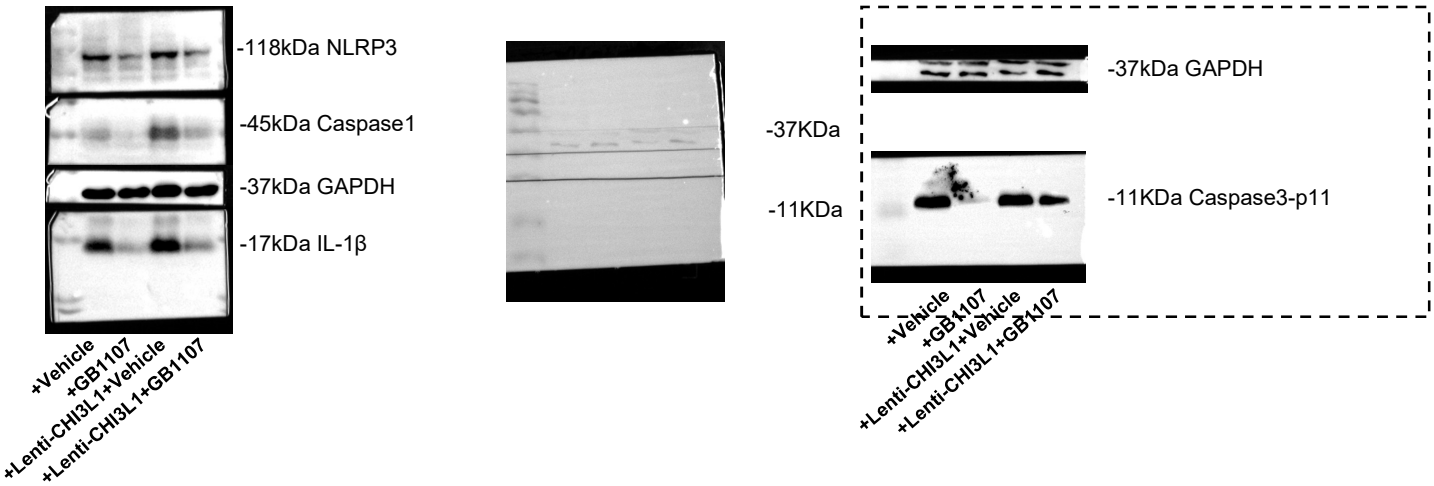


Figure S9F



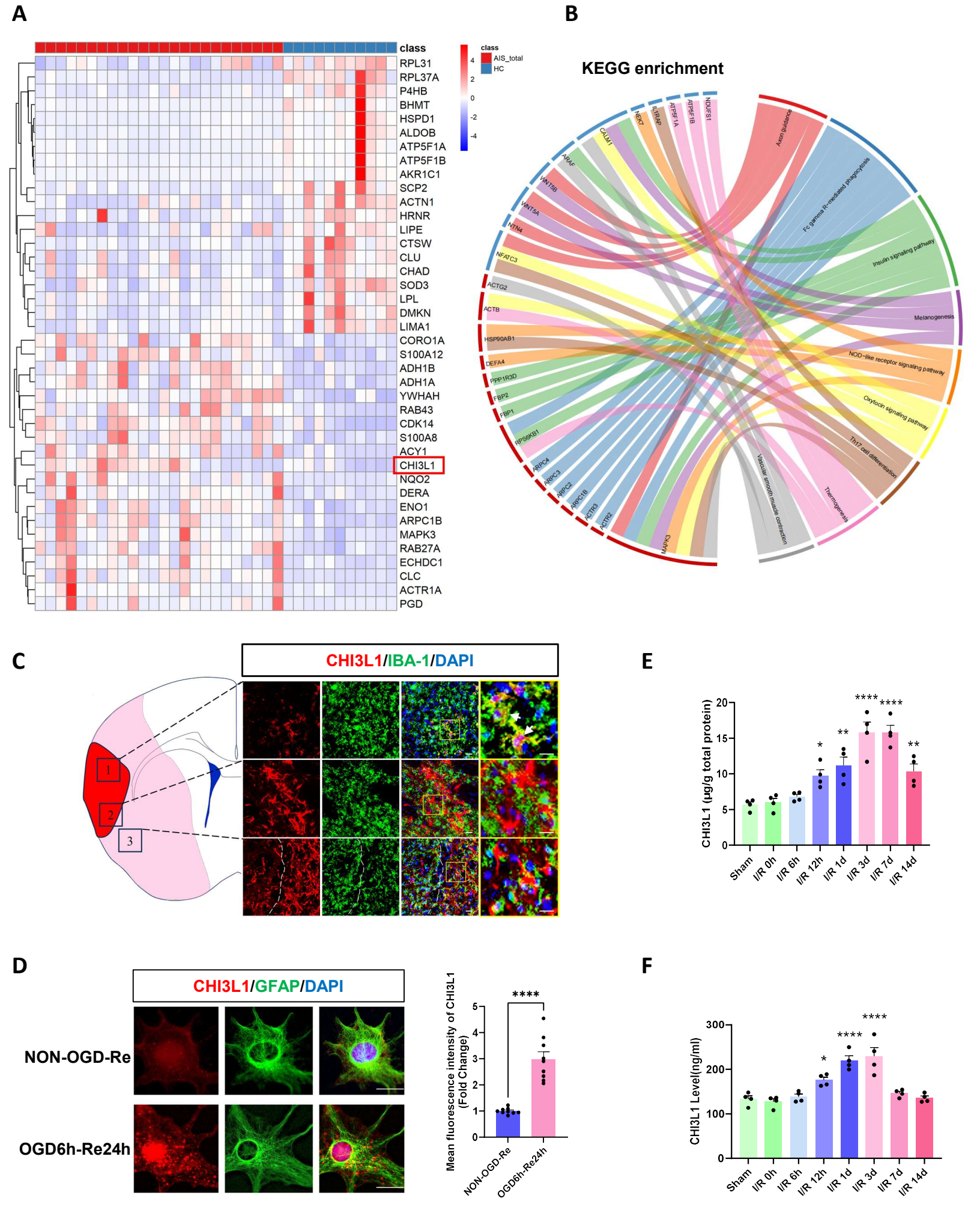

A
class
AIS_total
HC
RPL31
RPL37A
P4HB
BHMT
HSPD1
ALDOB
ATP5F1A
ATP5F1B
AKR1C1
SCP2
ACTN1
HRNR
LIPE
CTSW
CLU
CHAD
SOD3
LPL
DMKN
LIMA1
CORO1A
S100A12
ADH1B
ADH1A
YWHAH
RAB43
CDK14
S100A8
ACY1
CHI3L1
NQO2
DERA
ENO1
ARPC1B
MAPK3
RAB27A
ECHDC1
CLC
ACTR1A
PGD
B
KEGG enrichment
Axon guidance
Fc gamma R-mediated phagocytosis
Insulin signaling pathway
Melanogenesis
NOD-like receptor signaling pathway
Oxytocin signaling pathway
Th17 cell differentiation
Thermogenesis
Vascular smooth muscle contraction
C
CHI3L1/IBA-1/DAPI
1
2
3
D
CHI3L1/GFAP/DAPI
NON-OGD-Re
OGD6h-Re24h
Mean fluorescence intensity of CHI3L1 (Fold Change)
NON-OGD-Re
OGD6h-Re24h
E
CHI3L1 (μg/g total protein)
Sham
I/R 0h
I/R 6h
I/R 12h
I/R 1d
I/R 3d
I/R 7d
I/R 14d
F
CHI3L1 Level(ng/ml)
Sham
I/R 0h
I/R 6h
I/R 12h
I/R 1d
I/R 3d
I/R 7d
I/R 14d

Fig. S1 Molecular and Cellular Insights into CHI3L1 in Acute Ischemic Stroke

(A) Heatmap of differentially expressed serum proteins between acute ischemic stroke (AIS) patients (n = 24) and healthy controls (HC, n = 11), based on proteomic profiling. Red indicates upregulation; blue indicates downregulation.

(B) KEGG pathway enrichment analysis of the differentially expressed proteins. Enriched pathways are shown with lines denoting functional relationships.

(C) Representative immunofluorescence images of IBA-1 (green) and CHI3L1 (red) in various brain regions of tMCAO mice. A subset of microglia co-localized with CHI3L1 near the infarct core. Nuclei were stained with Hoechst (blue). Scale bar = 10 μm.

(D) Immunofluorescence images of primary astrocytes stained for GFAP (green) and CHI3L1 (red) following 6 h oxygen-glucose deprivation and 24 h reoxygenation (OGD6h–Re24h). CHI3L1 expression was significantly increased compared to control. n = 9 cells from three independent cultures. Scale bar = 10 μm.

(E) CHI3L1 protein levels in mouse brain tissue at various time points (Sham, IR 6h, IR 12h, IR 1d, IR 3d, IR 7d, IR 14d) after tMCAO, measured by ELISA.

(F) CHI3L1 protein levels in mouse serum at various time points after tMCAO, measured by ELISA.

Data analyzed by Student's t-test or one-way ANOVA, Statistical significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Data are presented as mean ± SEM.

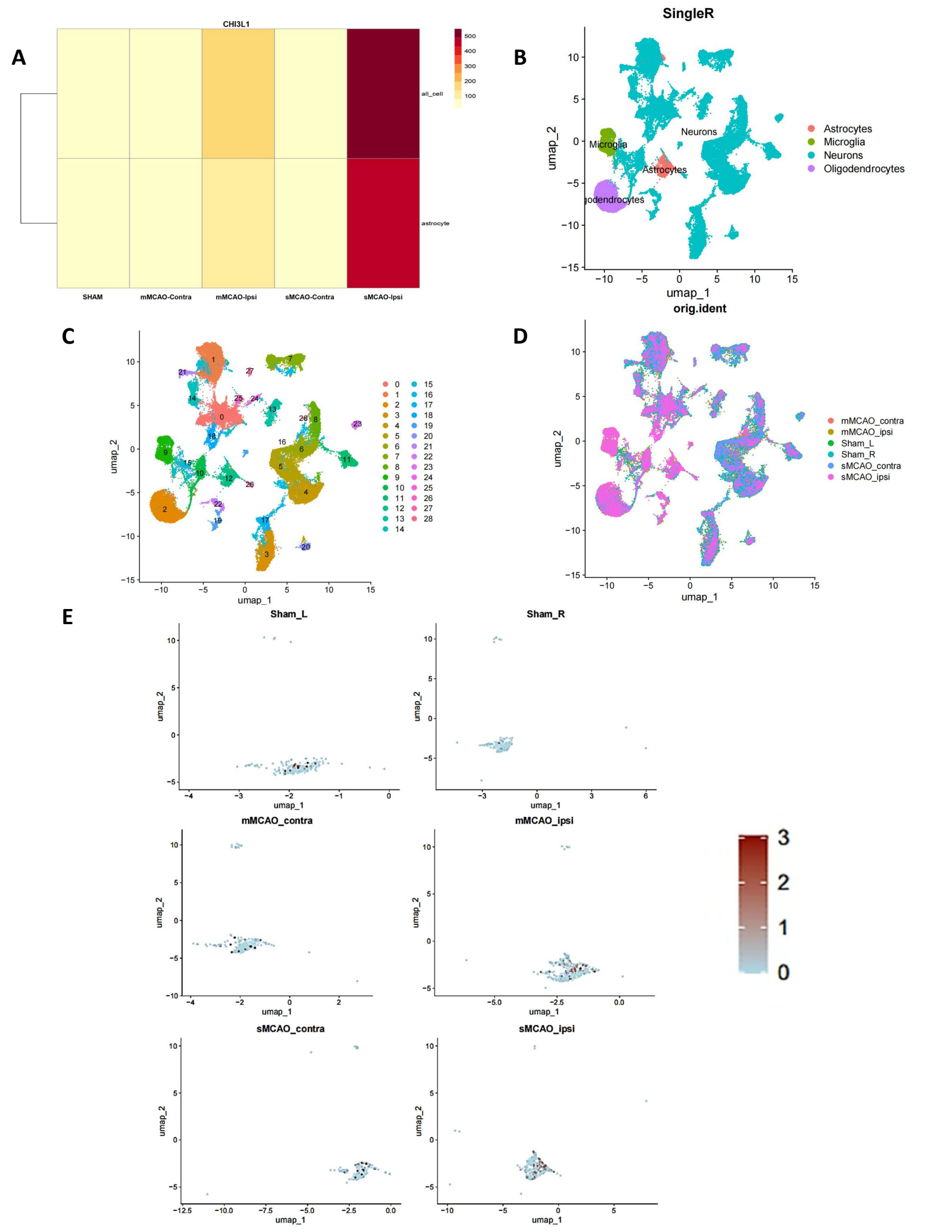

A
CHI3L1
500
400
300
200
100
all_cell
astrocyte
SHAM
mMCAO-Contra
mMCAO-Ipsi
sMCAO-Contra
sMCAO-Ipsi
B
SingleR
Neurons
Microglia
Astrocytes
odendrocytes
umap_2
umap_1
Astrocytes
Microglia
Neurons
Oligodendrocytes
C
umap_2
umap_1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
D
orig.ident
umap_2
umap_1
mMCAO_contra
mMCAO_ipsi
Sham_L
Sham_R
sMCAO_contra
sMCAO_ipsi
E
Sham_L
Sham_R
mMCAO_contra
mMCAO_ipsi
sMCAO_contra
sMCAO_ipsi
umap_2
umap_1
3
2
1
0

Fig. S2 Single-Cell RNA Sequencing Analysis of CHI3L1 Expression in Ischemic Brain

(A) Heatmap of CHI3L1 expression from single-cell RNA sequencing data (GEO). Experimental groups include SHAM, mMCAO-Contra, mMCAO-Ipsi, sMCAO-Contra, and sMCAO-Ipsi. CHI3L1 upregulation post-tMCAO is primarily attributed to astrocytes. Color scale represents expression intensity. （acc= GSE250245，PMID: 39043661）;Sham L = left brain hemispheres of Sham operated controls ; Sham R = right hemispheres of Sham operated controls; mMCAO contra: left brain hemispheres of MCAO group animals, with moderate infarction severity, hemispheres contralateral to infarction; mMCAO ipsi: right brain hemispheres of MCAO group animals, with moderate infarction severity, hemispheres ipsilateral to infarction, sMCAO contra: left brain hemispheres of MCAO group animals, with severe infarction severity, hemispheres contralateral to infarction; sMCAO ipsi: right brain hemispheres of MCAO group animals, with severe infarction severity, hemispheres ipsilateral to infarction.

(B) SingleR cell type annotation UMAP plot, identifying major cell populations: Astrocytes, Neurons, Microglia, and Oligodendrocytes.

(C) UMAP plot colored by cell clusters.

(D) UMAP plot colored by experimental groups (mMCAO_Contra, mMCAO_Ipsi, Sham_L, Sham_R, sMCAO_Contra, sMCAO_Ipsi).

(E) Feature plots showing CHI3L1 expression in Astrocytes distribution across groups: Sham_L, Sham_R, mMCAO_Contra, mMCAO_Ipsi, sMCAO_Contra, and sMCAO_Ipsi. The color scale represents CHI3L1 expression levels.

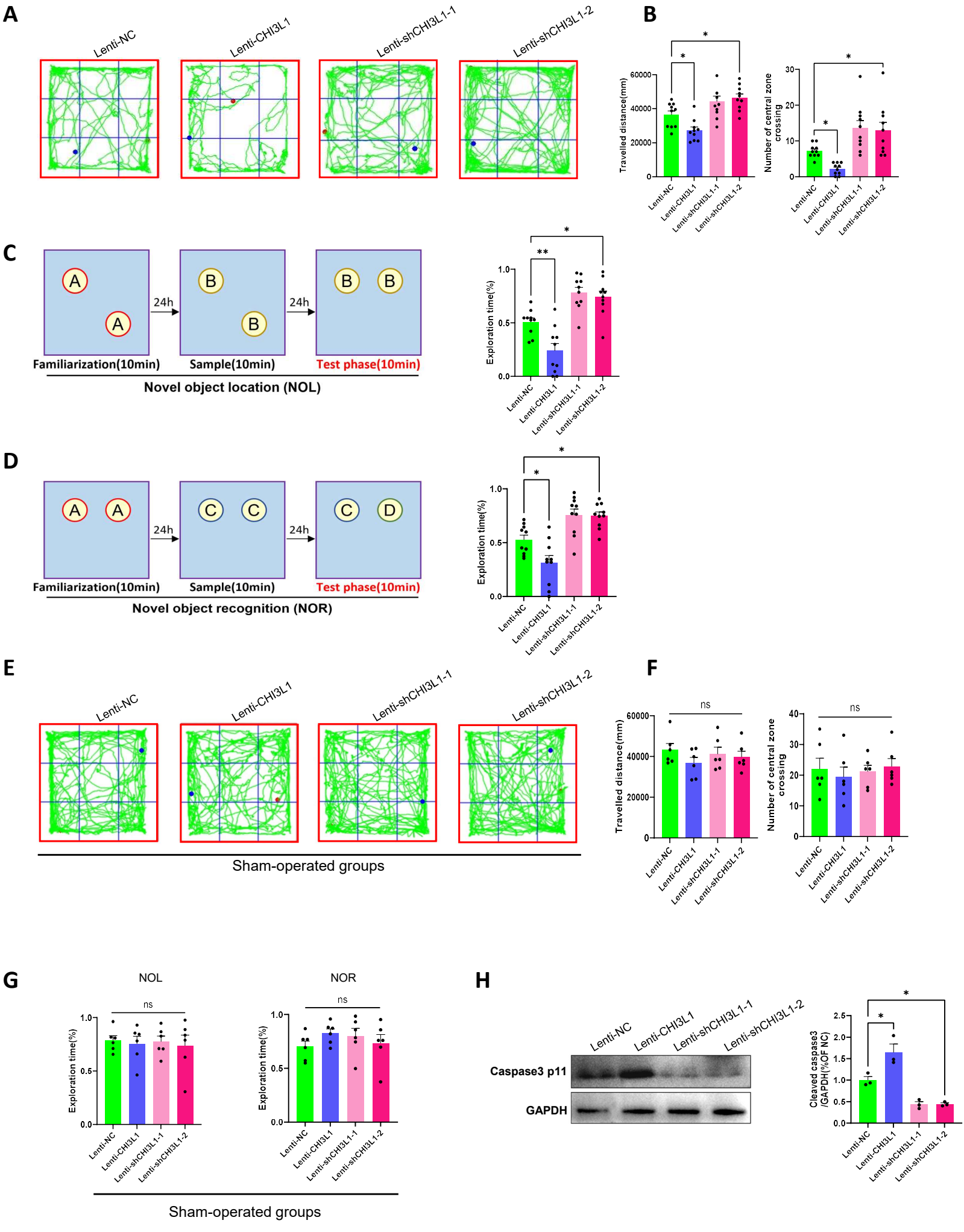

A
Lenti-NC
Lenti-CHI3L1
Lenti-shCHI3L1-1
Lenti-shCHI3L1-2
B
Travelled distance(mm)
Number of central zone crossing
C
A
B
24h
Familiarization(10min)
Sample(10min)
Test phase(10min)
Novel object location (NOL)
Exploration time(%)
D
C
D
Novel object recognition (NOR)
E
Sham-operated groups
F
ns
G
NOL
NOR
H
Caspase3 p11
GAPDH
Cleaved caspase3 /GAPDH(%OF NC)

Fig. S3 Behavioral and Apoptotic Effects of Lentivirus - mediated CHI3L1 Manipulation in a Mouse Model

(A-B) Open Field Test (OFT) in four experimental groups post tMCAO: Lent-NC, Lent-CHI3L1 (overexpression), Lent-shCHI3L1-1, and Lent-shCHI3L1-2 (knockdown). (A)Representative movement paths. (B)Quantification of total distance traveled and center area crossings. n = 10 mice per group.

(C) Novel Object Location (NOL) test post tMCAO. Schematic shows the familiarization, sample, and test phases (each 10 min). CHI3L1 knockdown mice showed increased exploratory preference for the novel location, while CHI3L1 overexpression reduced preference. n = 10 per group.

(D) Novel Object Recognition (NOR) test post tMCAO. Following the same trial structure as the NOL test, CHI3L1 knockdown enhanced, and overexpression reduced, preference for the novel object. n = 10 per group.

(E-F) OFT in four experimental groups (Sham-operated groups): Lent-NC, Lent-CHI3L1 (overexpression), Lent-shCHI3L1-1, and Lent-shCHI3L1-2 (knockdown). (E)Representative movement paths. (F)Quantification of total distance traveled and center area crossings. n = 6 mice per group.

(G) NOL and NOR test in Sham-operated groups. n = 6 mice per group.

(H) Western blot analysis of cleaved caspase-3 (Caspase3 p11) in brain tissue from lentivirus-injected groups (Lent-NC, Lent-CHI3L1, Lent-shCHI3L1-1, Lent-shCHI3L1-2) post tMCAO to assess neuronal apoptosis. n = 3 per group.

Data are presented as mean ± SEM. Statistical significance was evaluated using one-way ANOVA with Tukey's multiple comparisons test or Kruskal–Wallis test as appropriate. $p < 0.05$; $p < 0.01$; *$p < 0.001$; **$p < 0.0001$.

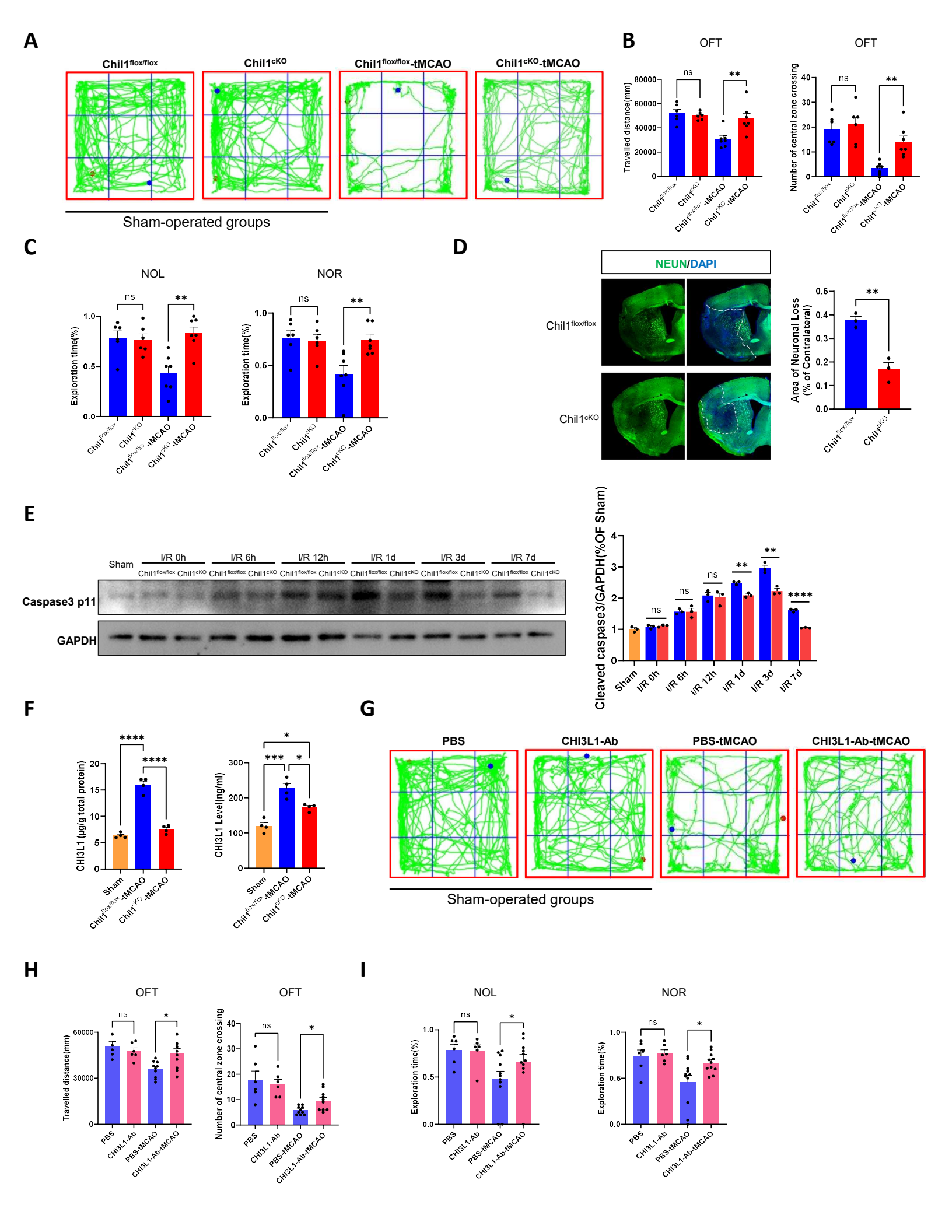

A
Chil1flox/flox
Chil1cKO
Chil1flox/flox-tMCAO
Chil1cKO-tMCAO
Sham-operated groups
B
OFT
Travelled distance(mm)
ns
**
Chil1flox/flox
Chil1cKO
Chil1flox/flox-tMCAO
Chil1cKO-tMCAO
OFT
Number of central zone crossing
C
NOL
NOR
Exploration time(%)
D
NEUN/DAPI
Chil1flox/flox
Chil1cKO
Area of Neuronal Loss (% of Contralateral)
E
Sham
I/R 0h
I/R 6h
I/R 12h
I/R 1d
I/R 3d
I/R 7d
Caspase3 p11
GAPDH
Cleaved caspase3/GAPDH(%OF Sham)
F
CHI3L1 (μg/g total protein)
CHI3L1 Level(ng/ml)
G
PBS
CHI3L1-Ab
PBS-tMCAO
CHI3L1-Ab-tMCAO
Sham-operated groups
H
OFT
Travelled distance(mm)
Number of central zone crossing
I
NOL
NOR
Exploration time(%)

Fig. S4 Behavioral and Neuronal Integrity Analysis in Genetically Altered and CHI3L1 - Antibody - Treated Mice

(A-B) OFT in Chil1$^{flox/flox}$ and Chil1^{cKO} Mice. (A) Representative movement paths. (B) Quantification of total distance traveled and center area crossings. Non-tMCAO groups: N = 6 animals per group; tMCAO groups: N = 7 animals per group.

(C) NOL and NOR Tests in Chil1$^{flox/flox}$ and Chil1^{cKO} Mice. Non-tMCAO groups: N = 6 animals per group; tMCAO groups: N = 7 animals per group.

(D) Immunofluorescence staining of NeuN (green) and DAPI (blue) in Chil1$^{flox/flox}$ and Chil1^{cKO} mice at day 7 post-tMCAO. N = 3 per group.

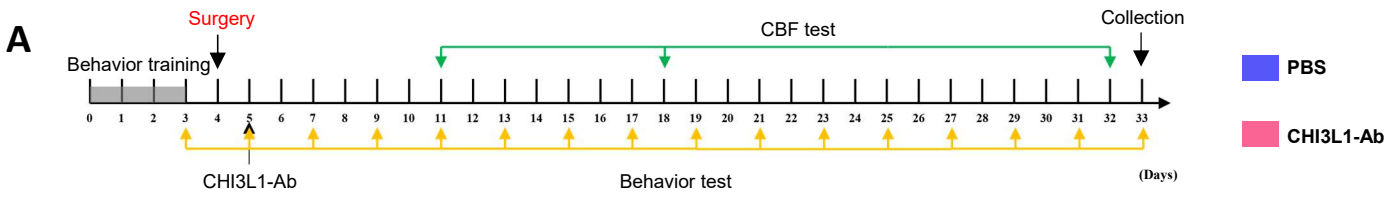
(E) Neuronal apoptosis evaluated by Western blotting for cleaved caspase-3 (Caspase3 p11) expression at different time points post-ischemia in genetically modified mouse groups (Chil1$^{flox/flox}$ and Chil1^{cKO} Mice). n = 3 per group.

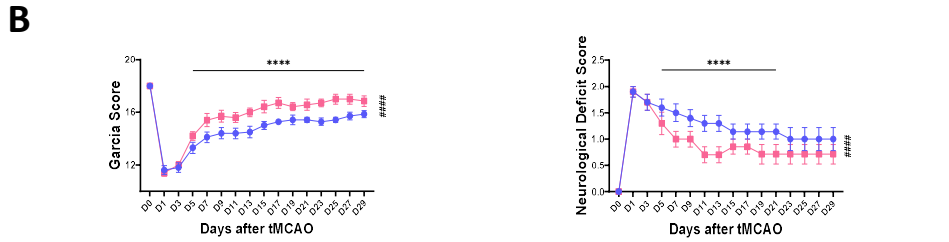
(F) Left: ELISA for CHI3L1 protein levels in brain tissue of Chil1$^{flox/flox}$ and Chil1^{cKO} mice at day 3 post-tMCAO. Right: ELISA for CHI3L1 levels in serum of Chil1$^{flox/flox}$ and Chil1^{cKO} mice at day 3 post-tMCAO. n = 4 per group.

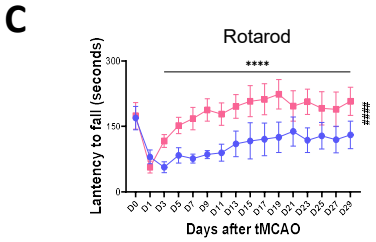
(G–H) OFT in PBS and anti-CHI3L1 treatment groups. (G) Representative movement paths. (H) Quantification of total distance traveled and center area crossings. Non-tMCAO groups: n = 6 animals per group; tMCAO groups: n = 11 animals per group.

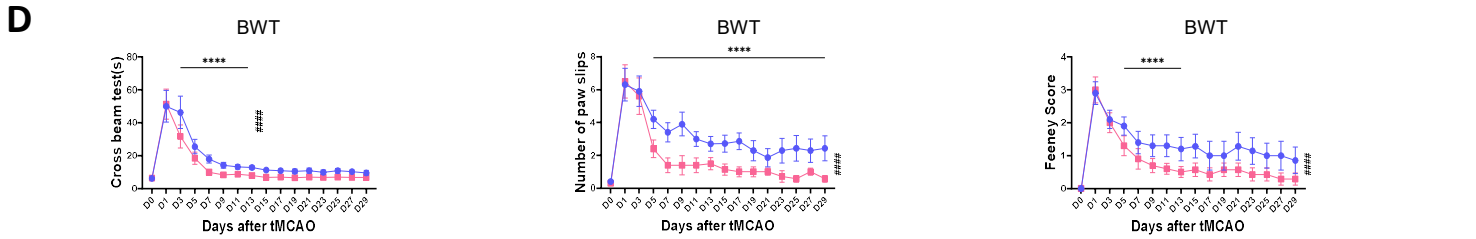
(I) NOL and NOR tests in PBS and anti-CHI3L1 treatment groups. Non-tMCAO groups: n = 6 animals per group; tMCAO groups: n = 11 animals per group.

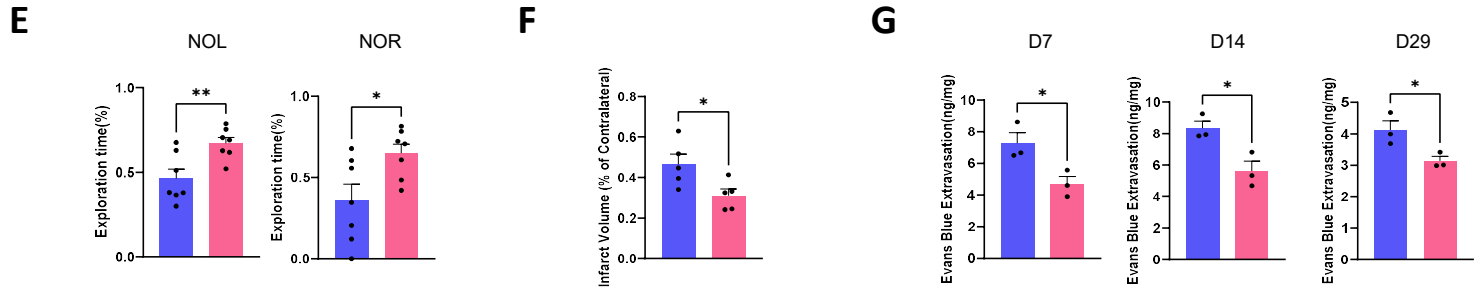
Data are presented as mean ± SEM. statistical significance was evaluated using the Mann - Whitney test or Student's t - test. Significance levels: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

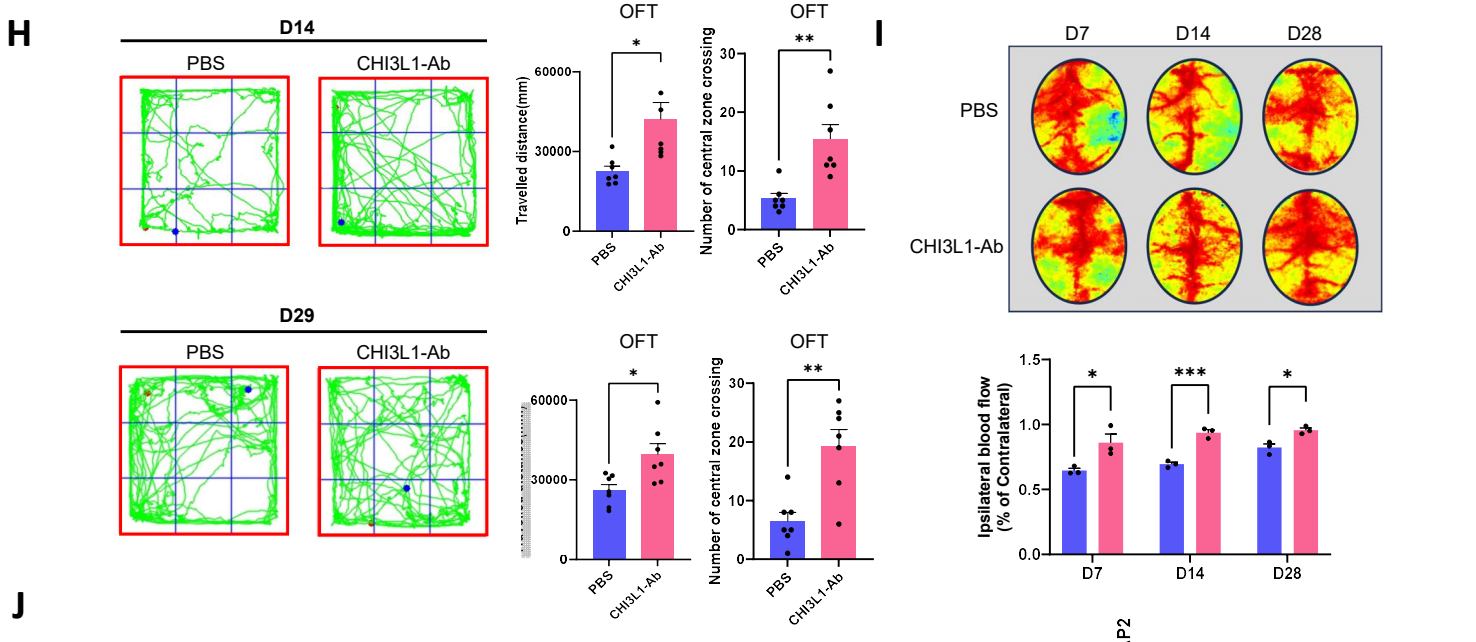

A
Surgery
Behavior training
CBF test
Collection
PBS
CHI3L1-Ab
CHI3L1-Ab
Behavior test
(Days)


B
Garcia Score
Days after tMCAO
Neurological Deficit Score
Days after tMCAO


C
Rotarod
Lantency to fall (seconds)
Days after tMCAO


D
BWT
Cross beam test(s)
Days after tMCAO
BWT
Number of paw slips
Days after tMCAO
BWT
Feeney Score
Days after tMCAO


E
NOL
NOR
Exploration time(%)
F
Infarct Volume (% of Contralateral)
G
D7
D14
D29
Evans Blue Extravasation(ng/mg)


H
D14
PBS
CHI3L1-Ab
OFT
Travelled distance(mm)
OFT
Number of central zone crossing
D29
I
D7
D14
D28
PBS
CHI3L1-Ab
Ipsilateral blood flow (% of Contralateral)

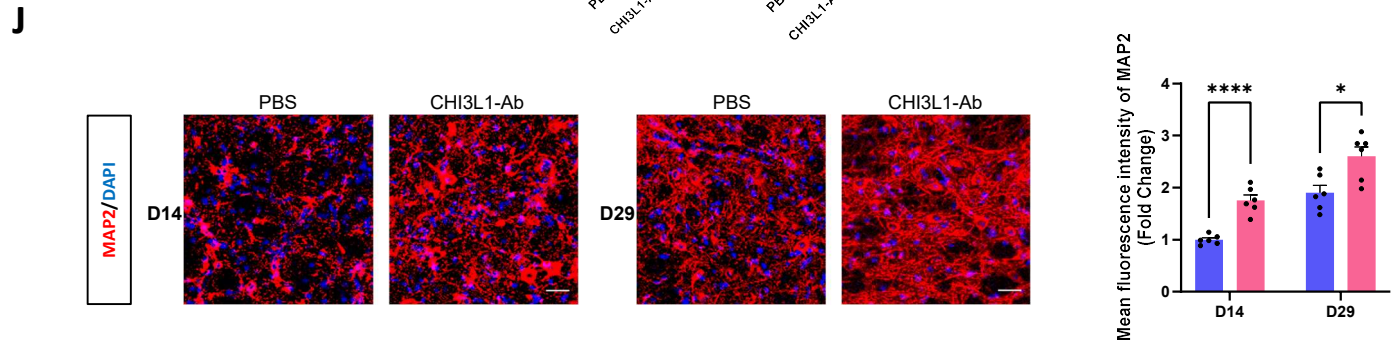

J
MAP2/DAPI
PBS
CHI3L1-Ab
D14
D29
Mean fluorescence intensity of MAP2 (Fold Change)

Fig. S5 Long-Term Prognostic Analysis of Post-tMCAO CHI3L1-Ab Treatment in Mice

(A) Mice received stereotaxic intracerebral injection of CHI3L1-Ab or PBS within 24 h post-tMCAO, followed by behavioral tests and sample collection up to 4 weeks.

(B-D) Motor function recovery assessed by Garcia scores, Neurological deficit scores (B), rotarod test (C), and beam-walking test (D) over 29 days post-tMCAO (n = 10 mice per group). Significance levels: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ compared to the control mice at indicated time points; #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$ compared to the control mice across the entire experiment.

(E) NOL and NOR tests assessing cognitive function at days 7 post-tMCAO. N = 7 animals per group.

(F) Infarct volume quantification by TTC staining at days 5 post-tMCAO. N = 5 animals per group.

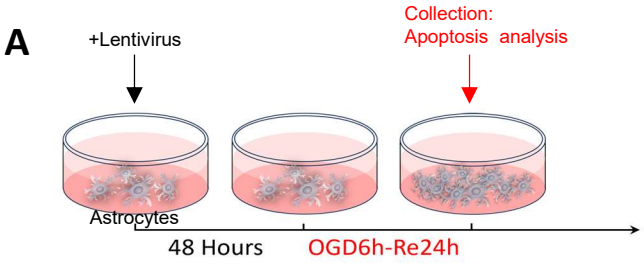
(G) Blood-brain barrier (BBB) permeability evaluated by Evans blue assay at days 7, 14, and 29 post-tMCAO (n = 3 per group at each time point).

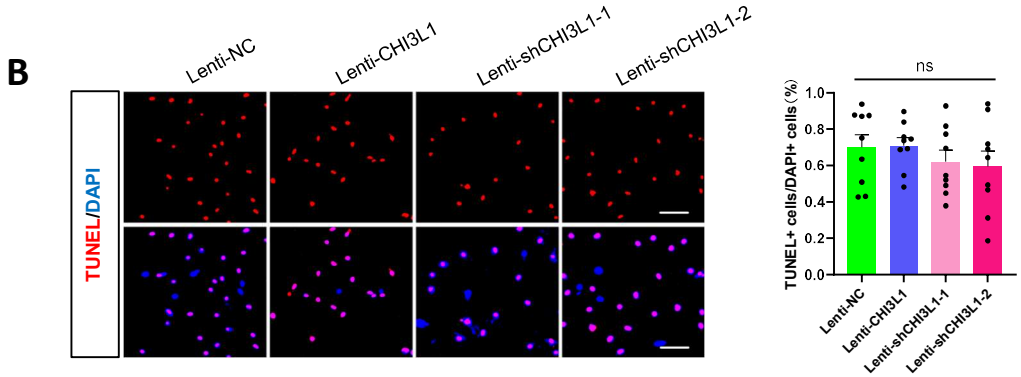
(H) OFT at days 14 and 29 post-tMCAO: (left) representative movement paths; (right) quantification of total distance traveled and center area crossings N = 7 animals per group.

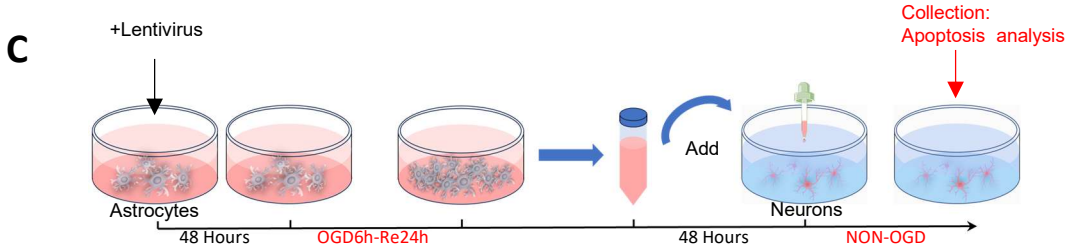
(I) Laser speckle imaging for CBF distribution and quantification of relative CBF at days 7, 14, and 28 post-tMCAO (n = 3 per group at each time point)

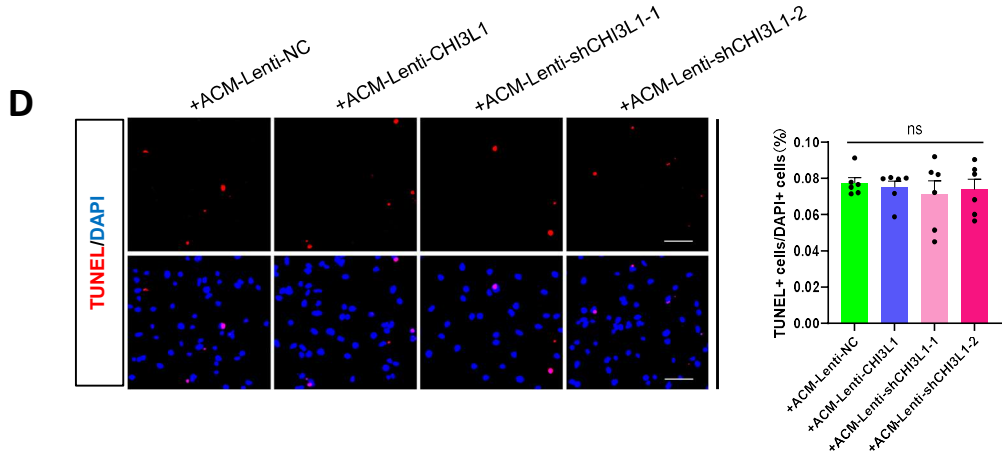
(J) Immunohistochemistry of MAP2 (red) and DAPI (blue) in peri-infarct penumbra at 14 and 29days post-stroke (n = 6).

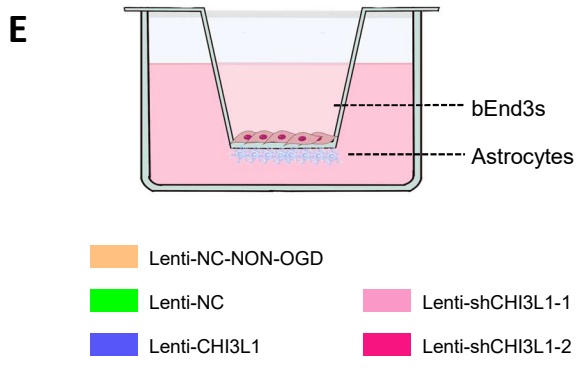
Data are presented as mean ± SEM. Data analyzed by two-way ANOVA or Mann - Whitney test or Student's t – test. Significance thresholds: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

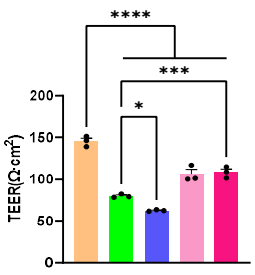

A
+Lentivirus
Collection:
Apoptosis analysis
Astrocytes
48 Hours
OGD6h-Re24h


B
Lenti-NC
Lenti-CHI3L1
Lenti-shCHI3L1-1
Lenti-shCHI3L1-2
TUNEL/DAPI
ns
TUNEL+ cells/DAPI+ cells (%)
1.0
0.8
0.6
0.4
0.2
0.0
Lenti-NC
Lenti-CHI3L1
Lenti-shCHI3L1-1
Lenti-shCHI3L1-2


C
+Lentivirus
Collection:
Apoptosis analysis
Add
Astrocytes
Neurons
48 Hours
OGD6h-Re24h
48 Hours
NON-OGD


D
+ACM-Lenti-NC
+ACM-Lenti-CHI3L1
+ACM-Lenti-shCHI3L1-1
+ACM-Lenti-shCHI3L1-2
TUNEL/DAPI
ns
TUNEL+ cells/DAPI+ cells (%)
0.10
0.08
0.06
0.04
0.02
0.00
+ACM-Lenti-NC
+ACM-Lenti-CHI3L1
+ACM-Lenti-shCHI3L1-1
+ACM-Lenti-shCHI3L1-2


E
bEnd3s
Astrocytes
Lenti-NC-NON-OGD
Lenti-NC
Lenti-CHI3L1
Lenti-shCHI3L1-1
Lenti-shCHI3L1-2


F
****
***
*
TEER(Ω·cm²)
200
150
100
50
0

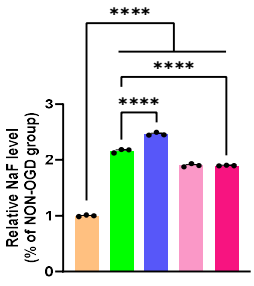

G
****
****
****
****
Relative NaF level
(% of NON-OGD group)
3
2
1
0

Fig. S6 Role of CHI3L1 in Astrocyte Apoptosis, Neuronal Apoptosis, and In Vitro Blood-Brain Barrier (BBB) Function Under OGD/Re Conditions

(A) Primary astrocytes were transfected with CHI3L1 - related lentiviruses (Lent - NC, Lent - CHI3L1, Lent - shCHI3L1 - 1, Lent - shCHI3L1 - 2). After 48 hours of culture, they were subjected to oxygen - glucose deprivation for 6 hours followed by reoxygenation for 24 hours (OGD6h - Re24h), and then apoptosis analysis was conducted.

(B) TUNEL assay was performed on the four groups of astrocytes. Representative images of TUNEL (red) and DAPI (blue) staining are shown. There were no significant differences in apoptosis among the four groups. N = 9 from three independent cultures per group.

(C) After the astrocytes were transfected with CHI3L1 - related lentiviruses and subjected to OGD6h - Re24h, the astrocyte - conditioned medium (ACM) was collected. This ACM was then added to primary - cultured neurons, which were not subjected to OGD treatment. Subsequently, apoptosis analysis was carried out on these neurons.

(D) TUNEL staining was performed on the neurons treated as described in (C). Representative images of TUNEL (red) and DAPI (blue) staining are presented.

(E-G) In Vitro Blood-Brain Barrier (BBB) Model Analysis of CHI3L1 Manipulation Under OGD/Re Conditions (E) Schematic of the Transwell co-culture system for the in vitro BBB model: Astrocytes were seeded on the basolateral side of Transwell inserts, subjected to lentiviral transfection (Lent-NC, Lent-CHI3L1, Lent-shCHI3L1-1, Lent-shCHI3L1-2), Mouse brain microvascular endothelial cells (bEnd.3) were subsequently seeded into the apical chamber. (F) Measurement of transendothelial electrical resistance (TEER) in the in vitro BBB model.(G) Sodium fluorescein (NAF) permeability assay to evaluate BBB permeability.

Data are presented as mean ± SEM; statistical significance by one-way ANOVA with Tukey's multiple comparisons test or Kruskal - Wallis test. Significance levels: $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

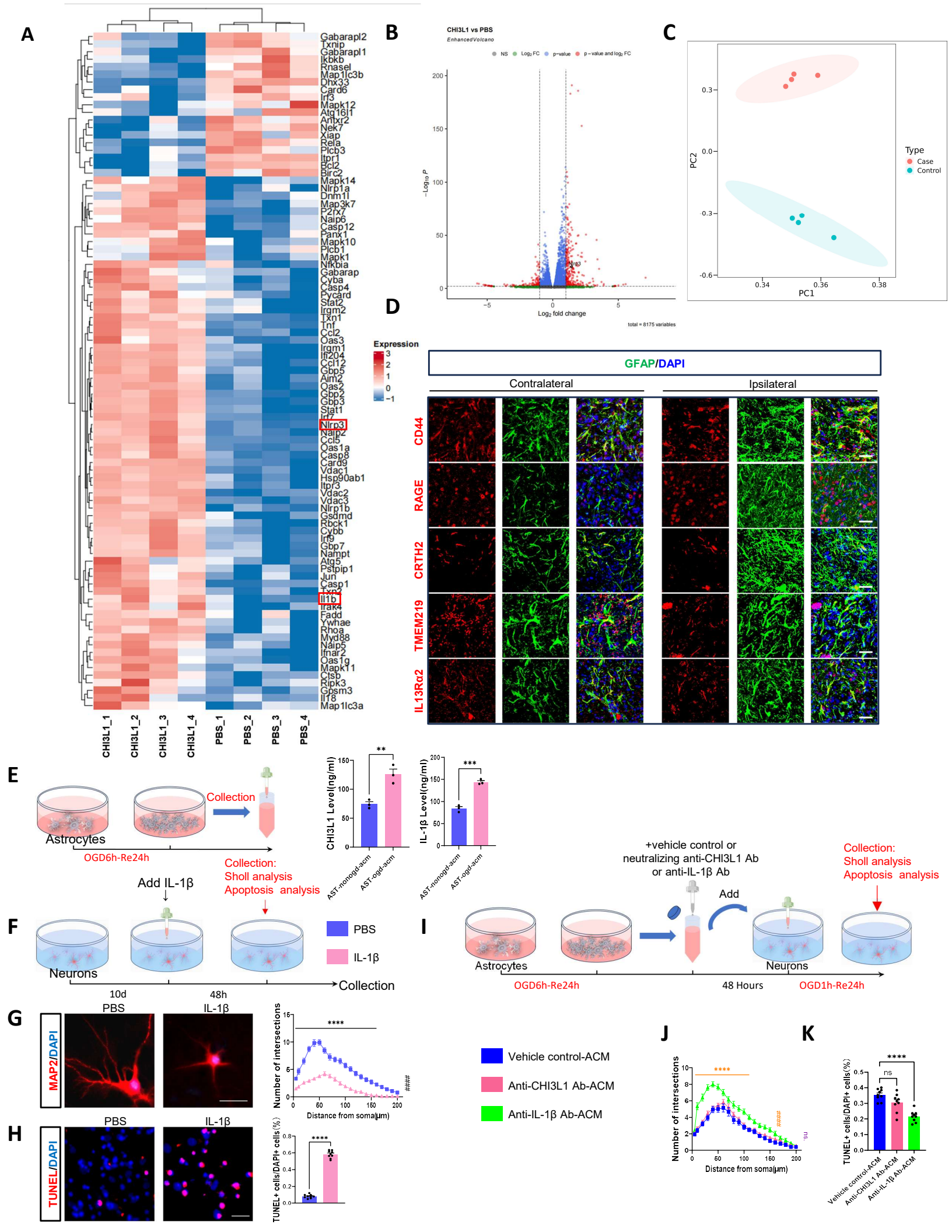

A
Gabarapl2
Txnip
Gabarapl1
Ikbkb
Rnasel
Map1lc3b
Dhx33
Card6
Irf3
Mapk12
Atg16l1
Antxr2
Nek7
Xiap
Rela
Plcb3
Itpr1
Bcl2
Birc2
Mapk14
Nlrp1a
Dnm1l
Map3k7
P2rx7
Naip6
Casp12
Panx1
Mapk10
Plcb1
Mapk1
Nfkbia
Gabarap
Cyba
Casp4
Pycard
Stat2
Irgm2
Txn1
Tnf
Ccl2
Oas3
Irgm1
Ifi204
Ccl12
Gbp5
Aim2
Oas2
Gbp2
Gbp3
Stat1
Irf7
Nlrp3
Naip2
Ccl5
Oas1a
Casp8
Card9
Vdac1
Hsp90ab1
Itpr3
Vdac2
Vdac3
Nlrp1b
Gsdmd
Rbck1
Cybb
Irf9
Gbp7
Nampt
Atg5
Pstpip1
Jun
Casp1
Xrn2
Il1b
Irak4
Fadd
Ywhae
Rhoa
Myd88
Naip5
Ifnar2
Oas1g
Mapk11
Ctsb
Ripk3
Gpsm3
Il18
Map1lc3a
CHI3L1_1
CHI3L1_2
CHI3L1_3
CHI3L1_4
PBS_1
PBS_2
PBS_3
PBS_4
B
CHI3L1 vs PBS
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
−Log10 P
Log2 fold change
total = 8175 variables
C
PC2
PC1
Type
Case
Control
D
Expression
GFAP/DAPI
Contralateral
Ipsilateral
CD44
RAGE
CRTH2
TMEM219
IL13Rα2
E
Astrocytes
OGD6h-Re24h
Collection
CHI3L1 Level(ng/ml)
IL-1β Level(ng/ml)
AST-nonogd-acm
AST-ogd-acm
F
Neurons
10d
Add IL-1β
48h
Collection:
Sholl analysis
Apoptosis analysis
Collection
PBS
IL-1β
I
Astrocytes
OGD6h-Re24h
+vehicle control or
neutralizing anti-CHI3L1 Ab
or anti-IL-1β Ab
Add
48 Hours
Neurons
OGD1h-Re24h
Collection:
Sholl analysis
Apoptosis analysis
G
MAP2/DAPI
PBS
IL-1β
Number of intersections
Distance from soma(μm)
H
TUNEL/DAPI
PBS
IL-1β
TUNEL+ cells/DAPI+ cells(%)
Vehicle control-ACM
Anti-CHI3L1 Ab-ACM
Anti-IL-1β Ab-ACM
J
Number of intersections
Distance from soma(μm)
K
TUNEL+ cells/DAPI+ cells(%)
Vehicle control-ACM
Anti-CHI3L1 Ab-ACM
Anti-IL-1β Ab-ACM

Fig. S7 Gene Expression Analysis in Astrocytes related to CHI3L1

(A) RNA - sequencing was performed on primary astrocytes treated with PBS or CHI3L1 - protein. N = 4 per group. Principal Component Analysis (PCA) was used to visualize the differences between the two groups. The PCA plot shows that the samples from the two groups (represented by different colors) are clearly separated, indicating distinct gene expression patterns.

(B) The volcano plot from the RNA - sequencing analysis of primary astrocytes treated with PBS or CHI3L1 - protein is shown. It illustrates the fold - change and statistical significance (p - value) of gene expression. Points above the horizontal dashed line represent genes with significant differential expression.

(C) Differential expression analysis of the RNA - sequencing data from primary astrocytes treated with PBS or CHI3L1 - protein identified significant changes in NLRP3 expression. The heatmap shows the expression levels of various genes, with NLRP3 highlighted. Different colors represent different expression levels, indicating the up - or down - regulation of genes in the two treatment groups.

(D) Immunofluorescence staining was conducted to visualize the expression levels of six CHI3L1 downstream receptors (IL13Rα2、TMEM219、CRTH2、CD44、RAGE、GAL3) in the peri-infarct region of the contralateral and ipsilateral hemisphere after tMCAO. For each receptor, images show staining for the receptor in red, GFAP (a marker for astrocytes) in green, and nuclei counterstained with DAPI in blue.

(E) Experimental design: Primary astrocytes were subjected to OGD6h-Re24h, and the conditioned medium was collected. ELISA was used to detect CHI3L1 and IL-1β levels in the medium. N = 3 per group.

(F) Schematic of recombinant IL-1β treatment on primary neurons: Neurons were cultured for 10 days, then treated with IL-1β for 48 h, followed by Sholl analysis and apoptosis assessment.

(G) Immunofluorescence staining of MAP2 (red) in neurons treated with PBS or IL-1β, with Sholl analysis quantification of dendritic complexity. Scale bar=10 μm.

(H) TUNEL staining (red) of neurons treated with PBS or IL-1β, with quantification of TUNEL-positive cells. Scale bar=50 μm.

(I) Experimental design and outcomes of astrocyte-conditioned medium (ACM) pretreatment with CHI3L1-neutralizing antibody or IL-1β-neutralizing antibody, followed by neuronal OGD1h-Re24h: (I) Sholl analysis of neuronal dendritic complexity, In the analysis, orange bars represent the comparison between the Vehicle control-ACM and Anti-IL-1β Ab-ACM groups, while purple bars comparison between the Vehicle control-ACM and Anti-CHI3L1 Ab-ACM groups; (J) TUNEL staining quantification of neuronal apoptosis.

Data are presented as mean ± SEM. Statistical significance was evaluated using Student's t-test or two-way ANOVA. Significance levels: $p < 0.05$, *$p < 0.001$, **$p < 0.0001$; #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$ ns, not significant.

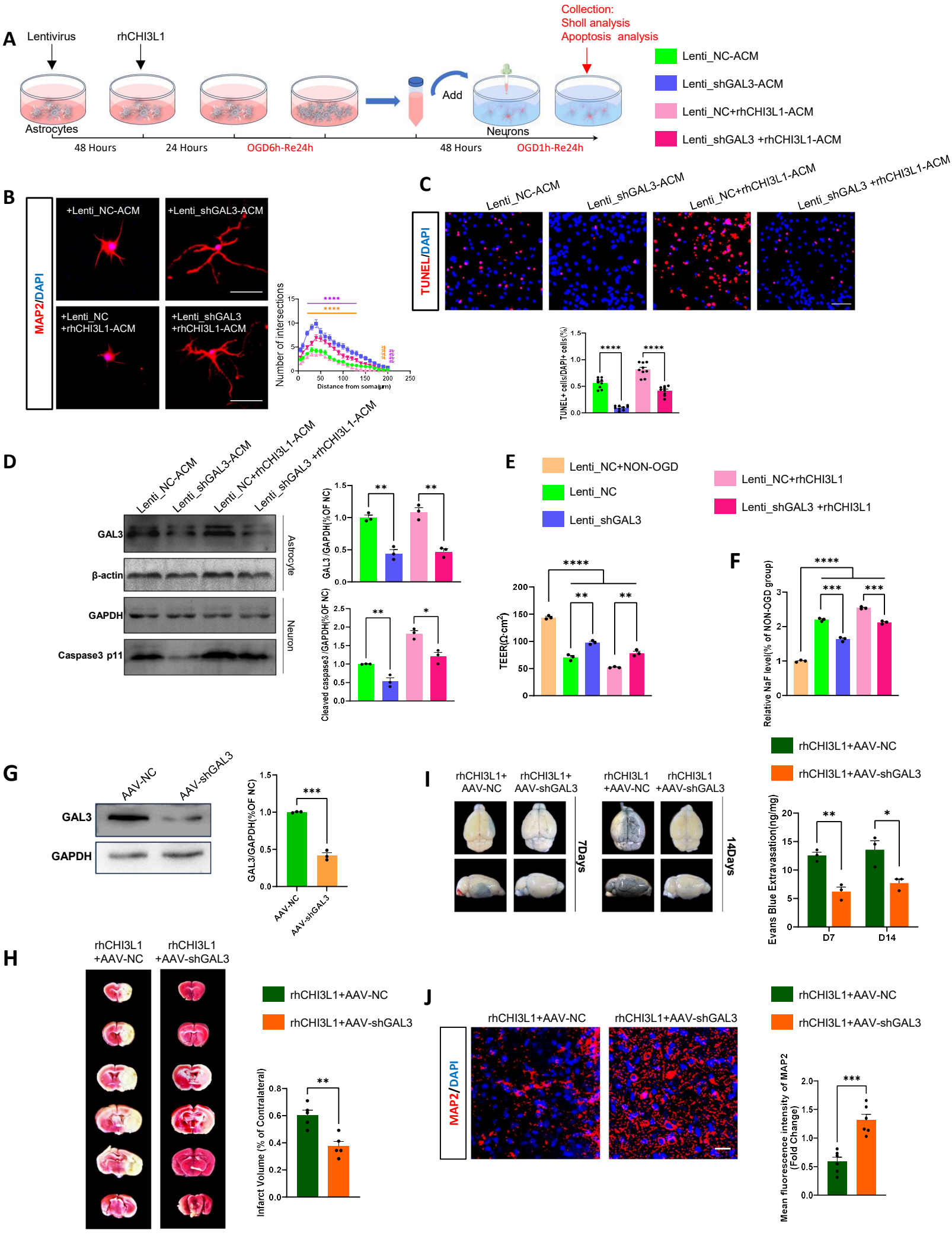

A
Lentivirus
rhCHI3L1
Collection:
Sholl analysis
Apoptosis analysis
Lenti_NC-ACM
Lenti_shGAL3-ACM
Lenti_NC+rhCHI3L1-ACM
Lenti_shGAL3 +rhCHI3L1-ACM
Astrocytes
Add
Neurons
48 Hours
24 Hours
OGD6h-Re24h
48 Hours
OGD1h-Re24h
B
MAP2/DAPI
+Lenti_NC-ACM
+Lenti_shGAL3-ACM
+Lenti_NC
+rhCHI3L1-ACM
+Lenti_shGAL3
+rhCHI3L1-ACM
Number of intersections
Distance from soma(μm)
C
Lenti_NC-ACM
Lenti_shGAL3-ACM
Lenti_NC+rhCHI3L1-ACM
Lenti_shGAL3 +rhCHI3L1-ACM
TUNEL/DAPI
TUNEL+ cells/DAPI+ cells(%)
D
Lenti_NC-ACM
Lenti_shGAL3-ACM
Lenti_NC+rhCHI3L1-ACM
Lenti_shGAL3 +rhCHI3L1-ACM
GAL3
β-actin
GAPDH
Caspase3 p11
Astrocyte
Neuron
GAL3 /GAPDH(%OF NC)
Cleaved caspase3 /GAPDH(%OF NC)
E
Lenti_NC+NON-OGD
Lenti_NC
Lenti_shGAL3
Lenti_NC+rhCHI3L1
Lenti_shGAL3 +rhCHI3L1
TEER(Ω·cm²)
F
Relative NaF level(% of NON-OGD group)
G
AAV-NC
AAV-shGAL3
GAL3
GAPDH
GAL3/GAPDH(%OF NC)
I
rhCHI3L1+ AAV-NC
rhCHI3L1+ AAV-shGAL3
rhCHI3L1 +AAV-NC
rhCHI3L1 +AAV-shGAL3
7Days
14Days
rhCHI3L1+AAV-NC
rhCHI3L1+AAV-shGAL3
Evans Blue Extravasation(ng/mg)
D7
D14
H
rhCHI3L1 +AAV-NC
rhCHI3L1 +AAV-shGAL3
rhCHI3L1+AAV-NC
rhCHI3L1+AAV-shGAL3
Infarct Volume (% of Contralateral)
J
rhCHI3L1+AAV-NC
rhCHI3L1+AAV-shGAL3
MAP2/DAPI
rhCHI3L1+AAV-NC
rhCHI3L1+AAV-shGAL3
Mean fluorescence intensity of MAP2 (Fold Change)

Fig. S8 GAL3 Mediates CHI3L1-Induced Neuronal Injury, BBB Dysfunction, and Infarct Expansion After Ischemic Stroke

(A) Primary astrocytes were transfected with lentivirus (Lenti - NC or Lenti - shGAL3). After 48 hours, recombinant human CHI3L1 protein (rhCHI3L1) was either added or omitted, and the astrocytes were subsequently subjected to oxygen - glucose deprivation followed by reoxygenation (OGD - Re). The conditioned medium (ACM) from these astrocytes was collected. Primary - cultured neurons were then treated with this ACM for 48 hours. Following this, the neurons were subjected to oxygen - glucose deprivation for 6 hours (OGD6h) and reoxygenation for 24 hours (Re24h).

(B) Morphology of neurons treated with ACM from different groups (Lenti - NC - ACM, Lenti - shGAL3 - ACM, Lenti - NC + rhCHI3L1 - ACM, Lenti - shGAL3 + rhCHI3L1 - ACM) was observed. Microscopic imaging was performed to visualize the neurons, with representative images of MAP2 (red) and DAPI (blue) staining shown. In the analysis, orange bars represent the comparison between the +Lenti_NC - ACM and +Lenti_shGAL3 - ACM groups, while purple bars represent the comparison between the +Lenti_NC + rhCHI3L1_ACM and +Lenti_shGAL3 + rhCHI3L1_ACM groups. N = 15 neurons from 3 independent experiments. Scale bar = 10 μm. Significance levels: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 compared to the 0 μm group at indicated distances; #p < 0.05; ##p < 0.01; ###p < 0.001; ####p < 0.0001 compared to the 0 μm group across the entire distance. Data were analyzed by two - way ANOVA and Sidak's multiple comparison tests (mean ± SEM).

(C) Neurons treated with ACM from CHI3L1 - protein - stimulated astrocytes showed increased apoptosis, as detected by TUNEL staining (red) and DAPI counterstaining (blue). ACM from shGAL3 - transfected astrocytes reduced this effect. N = 9 from 3 independent experiments. Scale bar = 100 μm.

(D) Western blotting was performed to confirm the transfection efficiency of shGAL3 in astrocytes and to assess cleaved caspase - 3 (Caspase3 p11) expression in neurons treated with ACM. The blots for GAL3, β - actin, GAPDH, and Caspase3 p11 are shown. N = 3 per group.

(E) Transendothelial electrical resistance (TEER) measurement in the in vitro BBB model across groups. N = 3 per group.

(F) Relative permeability of sodium fluorescein (NAF) in the in vitro BBB model across groups. N = 3 per group.

(G) Mice were stereotaxically injected with AAV (AAV - NC or AAV - shGAL3). Western blotting was used to verify the knockdown efficiency of GAL3 in the ischemic brain tissue. N = 3 per group.

(H) Infarct volume assessment by TTC staining in mice treated with rhCHI3L1 + AAV-NC or AAV-shGAL3 (n=5).

(I) Blood-brain barrier (BBB) permeability evaluated by Evans blue assay at days 7 and 14 post-tMCAO(n=3).

(J) Immunohistochemistry of MAP2 (red) and DAPI (blue) in peri-infarct penumbra at 14 days post-stroke (n = 6).

Data are presented as mean ± SEM. Statistical significance was evaluated using Student's t-test or one-way ANOVA. Significance levels: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

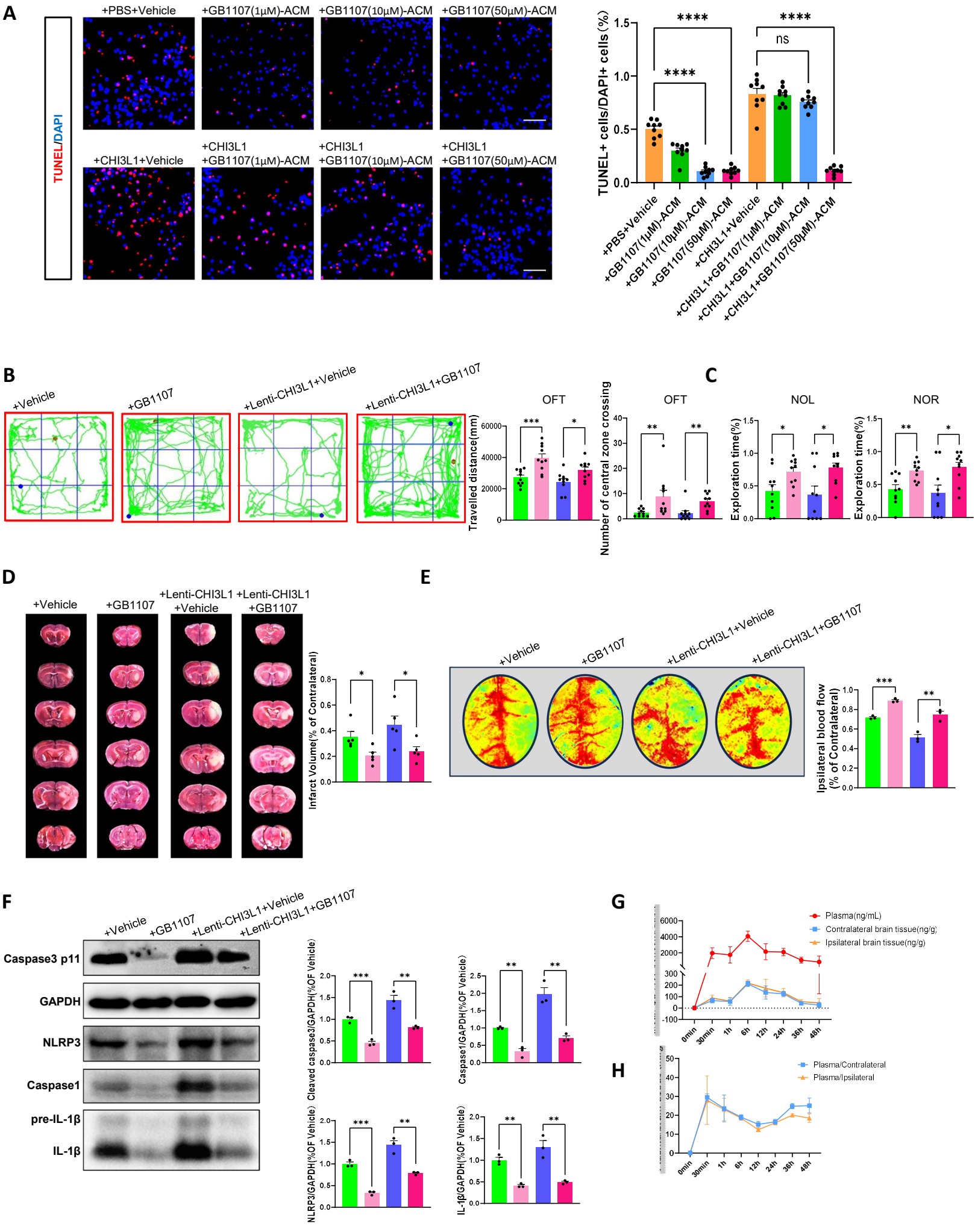

A
+PBS+Vehicle
+GB1107(1μM)-ACM
+GB1107(10μM)-ACM
+GB1107(50μM)-ACM
TUNEL/DAPI
+CHI3L1+Vehicle
+CHI3L1 +GB1107(1μM)-ACM
+CHI3L1 +GB1107(10μM)-ACM
+CHI3L1 +GB1107(50μM)-ACM
TUNEL+ cells/DAPI+ cells (%)
****
****
****
ns
B
+Vehicle
+GB1107
+Lenti-CHI3L1+Vehicle
+Lenti-CHI3L1+GB1107
OFT
Travelled distance(mm)
C
OFT
NOL
NOR
Number of central zone crossing
Exploration time(%)
D
+Vehicle
+GB1107
+Lenti-CHI3L1 +Vehicle
+Lenti-CHI3L1 +GB1107
Infarct Volume(% of Contralateral)
E
+Vehicle
+GB1107
+Lenti-CHI3L1+Vehicle
+Lenti-CHI3L1+GB1107
Ipsilateral blood flow (% of Contralateral)
F
+Vehicle
+GB1107
+Lenti-CHI3L1+Vehicle
+Lenti-CHI3L1+GB1107
Caspase3 p11
GAPDH
NLRP3
Caspase1
pre-IL-1β
IL-1β
Cleved caspase3/GAPDH(%OF Vehicle)
Caspase1/GAPDH(%OF Vehicle)
NLRP3/GAPDH(%OF Vehicle)
IL-1β/GAPDH(%OF Vehicle)
G
Plasma(ng/mL)
Contralateral brain tissue(ng/g)
Ipsilateral brain tissue(ng/g)
0min
30min
1h
6h
12h
24h
36h
48h
H
Plasma/Contralateral
Plasma/Ipsilateral

Fig. S9 GB1107: A Potential In Vitro Therapeutic Modulator of CHI3L1 - Induced Neuronal Stress

(A) TUNEL staining (red) and DAPI counterstaining (blue) were carried out on neurons treated with ACM from astrocytes that had been treated with different combinations of rhCHI3L1 and GB1107. N = 9 from 3 independent experiments. Scale bar = 100 μm.

(B) OFT showing improved locomotor activity in GB1107-treated mice.

(C) NOL and NOR tests evaluating cognitive outcomes (n = 10 per group).

(D) Infarct volume assessment by TTC staining (n = 5).

(E) CBF quantification on day 7 post-tMCAO (n = 3).

(F) Western blot analysis of cleaved caspase-3 levels and assessment of NLRP3 pathway (n = 3).

(G) Time-course of GB1107 concentration in mouse plasma, contralateral brain tissue, and ipsilateral (infarct-side) brain tissue at various time points post-administration.

(H) Ratio of GB1107 concentration in plasma to that in contralateral brain tissue, and plasma to ipsilateral brain tissue, at different time points post-administration.

Data are presented as mean ± SEM. Statistical significance was determined using one - way ANOVA followed by Tukey's multiple comparison test or Kruskal - Wallis test, with significance levels: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Studies of CH3L1 serum levels (by ELISA)	Controls ng/ml (n)	AIS ng/ml (n)	References
Park et al.,2012	41(34)	251-310(105)	PMID: 23272150 DOI: 10.1371/journal.pone.0051722.
Shao et al.,2016	76(85)	95(141)	PMID: 27650381 DOI: 10.1111/ane.12688.

Table S1 Comparison of CHI3L1/YKL - 40 Concentrations (Measured by ELISA) in Serum Samples between Healthy Control and Stroke Cohorts in Recent Studies
This table summarizes data from recent publications reporting serum concentrations of CHI3L1/YKL-40, measured by ELISA (in ng/mL), in healthy control and stroke cohorts. For each study, the mean CHI3L1/YKL-40 concentration and corresponding sample size (n) are provided for both groups.

	HCs (n=11)	AISs (n=24)	P-value (HCs vs AISs)
Age (years)	60.2±4.5	62.7±5.1	0.1727
Gender (Female/Male)	4/7	11/13	-
Mean admission NIHSS scores	-	4.2±4.6	-
Mean discharge NIHSS scores	-	3.1±4.0	-
Smoking	4/7	7/17	-
Alcohol Abuse	1/10	4/20	-
Diabetes	5/6	8/16	-
Other Cardiovascular and Cerebrovascular Diseases	5/6	14/10	-

Table S2 Comparison of Basic Information of Protein - Sequenced Serum Samples between Healthy Control Subjects and Acute Ischemic Stroke Patients
This table summarizes the baseline characteristics of 35 serum samples, including 11 healthy control (HC) subjects and 24 acute ischemic stroke (AIS) patients. Continuous variables are presented as mean ± standard deviation (SD), and categorical variables are reported as counts or proportions. The table **compares** demographic and clinical parameters such as age, sex, NIHSS scores (AIS group only), and the prevalence of risk factors including smoking, alcohol use, diabetes, and cardiovascular/cerebrovascular comorbidities. P-values indicate the statistical significance of differences between the two groups.

	HCs (n=15)	AISs (n=70)	P-value (HCs vs AISs)
Age (years)	60.7±9.8	62.9±14.3	0.5725
Gender (Female/Male)	8/7	45/25	-
Mean admission NIHSS scores	-	4.7±5.1	-
Mean discharge NIHSS scores	-	2.8±4.0	-
Mean infarct volume	-	45.0±106.0	-
Smoking	4/11	33/37	-
Alcohol Abuse	2/13	13/57	-
Diabetes	3/13	26/44	-
Other Cardiovascular and Cerebrovascular Diseases	10/5	41/29	-
CHI3L1 (ng/ml)	197.6±30.8	427.1±212.5	<0.0001

Table S3 Comparison of Basic Information and CHI3L1 ELISA Results of Serum Samples between Healthy Control Subjects and Acute Ischemic Stroke Patients
This table summarizes the characteristics of 85 serum samples, comprising 15 healthy control (HC) subjects and 70 acute ischemic stroke (AIS) patients. Continuous variables are expressed as mean ± standard deviation (SD), while categorical variables are shown as frequencies or proportions. The comparison includes age, sex, NIHSS scores at admission and discharge (AIS group), infarct volume, and the prevalence of vascular risk factors such as smoking, alcohol consumption, diabetes, and other cardiovascular or cerebrovascular diseases. CHI3L1 concentrations measured by ELISA in serum are also reported. P-values indicate the statistical significance of differences between the HC and AIS groups for each parameter.

Target sequences of the shRNAs and genotyping primers	
ALDH1L1::CreERT2-1	GCCTGCATTACCGGTCGATGC
ALDH1L1::CreERT2-2	CAGGGTGTTATAAGCAATCCC
Chil1-1	GCTACCCAACATGTCAATAGCTCA
Chil1-2	CATATGGTGGGCAATAATCTTGGA
shGal3-1	CTGGAGCTTATCCTGGCTCAA
shGal3-2	CCGCATGCTGATCACAATCAT
shIL-13Rα2-1	ATATTCTGATACCAACTATAC
shIL-13Rα2-2	TTGGACTCATCAGACTATAAA
shTMEM219-1	GTCGGCTTACTGACCACTTTG
shTMEM219-2	CATGGTAGTCAGATAGGATTG
shCRTH2-1	GCTCAACACAATACCATATTT
shCRTH2-2	CACTGCTCTATGTGTTCACAT
shCD44-1	GTGTAGTGCCTACGCCATTAA
shCD44-2	CCGAATTAGCTGGACACTCAA
shRAGE_1	GCACTTAGATGGGAAACTTCT
shRAGE_2	GGGCATTCAGCTGTTGGTTGA

Table S4 Target sequences of the shRNAs and genotyping primers