Supple Table 1 Demographics and baseline data of Control and ICH

	Control（n=30）	ICH（n=91）	*P*
Sex male, n (%)	16（53.3）	63 (69.2)	0.113
Age ($\bar{X}$±S, years)	54.3±9.2	54.3±10.0	0.997
Education			
Postgraduate, n (%)	2（6.7）	4（4.4）	0.673
Undergraduate, n (%)	5（16.7）	8（8.8）	0.227
Secondary school, n (%)	19（63.3）	60（65.9）	0.795
Primary school, n (%)	4（13.3）	18（19.8）	0.427
Psychological consultation, n (%)	0（0）	0（0）	—
Antidepressant, n (%)	0（0）	0（0）	—
Parkinson's disease, n (%)	0（0）	0（0）	—
Huntington, n (%)	0（0）	0（0）	—
Alzheimer's disease, n (%)	0（0）	0（0）	—
Hypertension, n (%)	4（13.3）	84（92.3）	0.000
Hyperlipidemia, n (%)	3（10.0）	4（4.4）	0.254
Diabetes, n (%)	1（3.3）	6（6.6）	0.680*
Warfarin treatment, n (%)	0（0）	1（1.1）	1.000*
History of stroke, n (%)	0（0）	0（0）	—
Smoking, n (%)	7（23.3）	35（38.5）	0.131
Alcohol abuse, n (%)	10（33.3）	45（49.5）	0.124

*Fisher’s exact test. ICH: intracerebral hemorrhage

Supple Table 2 Demographics and baseline data of CI and no CI group in ICH

Spontaneous ICH (n=91)	CI（n=30）	No CI（n=61）	*P*
Sex male, n (%)	20（66.7）	43 (70.5)	0.710
Age ($\bar{X}$±S, years)	57.8±9.2	53.1±10.3	0.071
Education			
Postgraduate, n (%)	1（3.3）	3（4.9）	1.000*
Undergraduate, n (%)	2（6.7）	6（9.8）	1.000*
Secondary school, n (%)	19（63.3）	41（67.2）	0.714
Primary school, n (%)	8（26.7）	11（15.9）	0.213
Psychological consultation, n (%)	0（0）	0（0）	—
Antidepressant, n (%)	0（0）	0（0）	—
Parkinson's disease, n (%)	0（0）	0（0）	—
Huntington, n (%)	0（0）	0（0）	—
Alzheimer's disease, n (%)	0（0）	0（0）	—
Hypertension, n (%)	27（90.0）	55（90.2）	0.980
Hyperlipidemia, n (%)	1（3.3）	2（3.3）	0.989
Diabetes, n (%)	5（16.7）	1（1.6）	0.007
Warfarin treatment, n (%)	1（3.3）	0（0）	0.248*
History of stroke, n (%)	0（0）	0（0）	—
Smoking, n (%)	8（26.7）	27（44.3）	0.105
Alcohol abuse, n (%)	13（40.0）	31（50.8）	0.331
Admission GCS, median (IQR)			
Location			
Basal ganglia, n (%)	12（40.0）	35（57.4）	0.119
Intraventricular Extension, n (%)	13（43.3）	19（31.1）	0.252
Others, n (%)	5（26.7）	7（11.5）	0.521*
ICH volume, median (IQR), mL	34（27-50）	19（10-27）	0.000
GCS Admission, median (IQR)	10（8-14）	13（12-15）	0.000
Phase shift values, median (IQR)	-1.27（-2.31—-1.19）	-1.89（-2.06-0.17）	0.000
Surgery, n (%)	6（20.0）	6（9.8）	0.200*

*Fisher’s exact test. IQR: inter-quartile range, CI: cognitive impairment, ICH: intracerebral hemorrhage, GCS: Glasgow coma scale

Supple Table 3 multivariate analysis of CI in ICH

	OR (95%CI)	***P***
GCS (1 decrease)	1.50 (1.21-1.72)	0.000
ICH volume (10mL increase)	1.73 (1.14-2.69)	0.000
Phase shift values	1.40 (1.18-1.84)	0.006

OR：odds ratio, CI：cognitive impairment, GCS: Glasgow coma scale

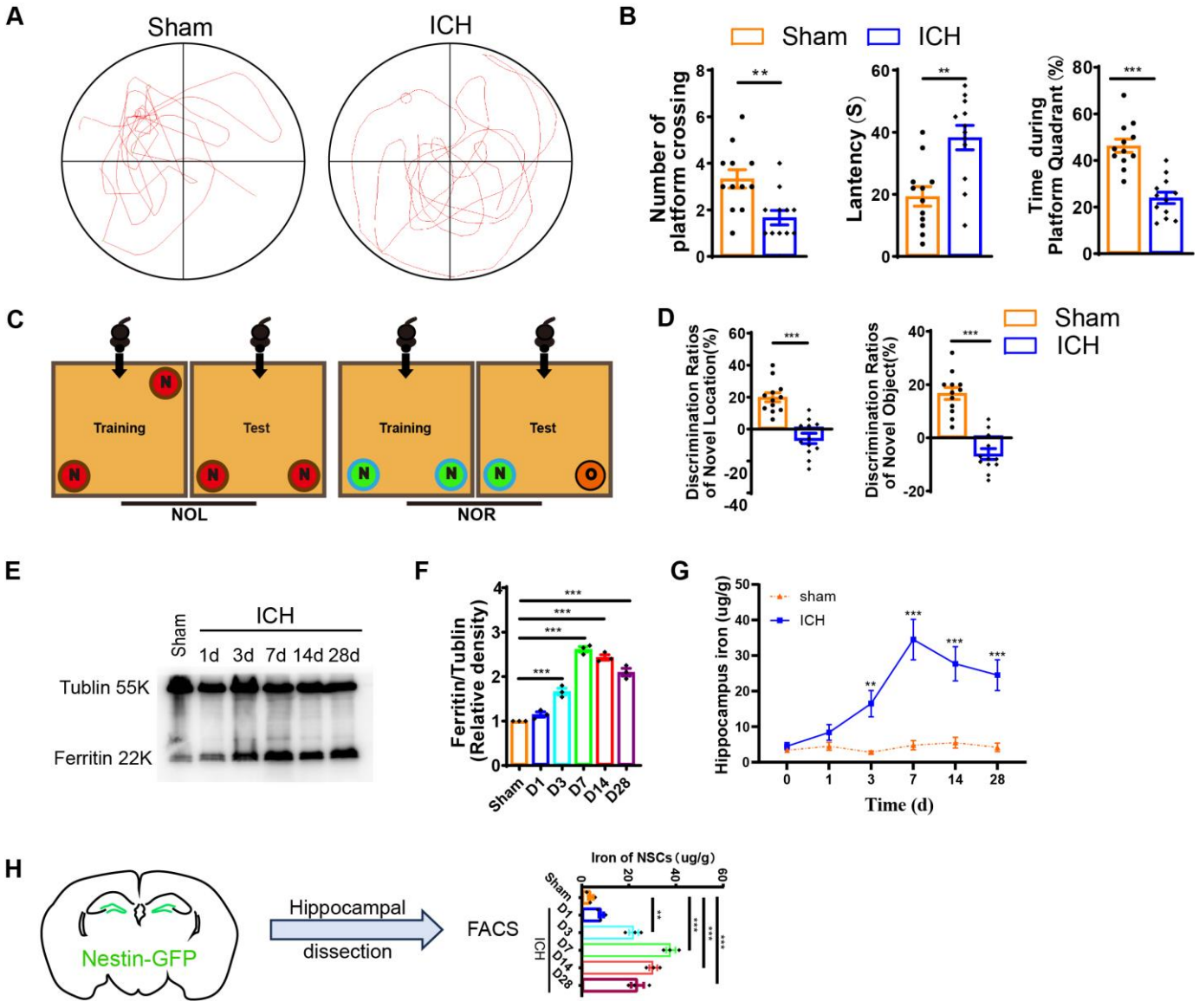

Figure S1. Iron deposition in hippocampus mediates the effect of ICH on cognitive impairments in mice

(A) Representative images of the swimming path of Sham and ICH mice during the probe trial test in Morris water maze test at 90 days after ICH.

(B) Platform-crossing times of Sham and ICH mice during probe trial test. Escape latency of probe trial test of Sham and ICH mice. Duration of stay in the platform quadrant. (n=12 for each group).

(C) Schematic diagram of novel object location and novel object recognition test.

(D) Quantification of the ratio of exploration time on novel objective location and novel object recognition in Sham and ICH mice at 90 days after ICH. (n=12 for each group).

(E-F) Bands showing temporal pattern of Ferritin expression in hippocampus after ICH.

Semi-quantitative analysis of WB results of Ferritin. Tublin was served as the internal control. (n=3 for each time point in each group).

(G) Time course of iron content in hippocampus between control and ICH mice. (n=6 for each time point in each group).

(H) Schematic diagram showing isolation of endogenous GFP^+ NSCs from the hippocampus using FACS. Quantified intracellular iron level in NSCs from Sham and ICH group at 1, 3, 7, 14 and 28 days after ICH. (n=3 for each time point).

(B, D, F, G, H) *P < 0.05; **P < 0.01; ***P < 0.001 vs corresponding Sham. Each experiment was repeated at least 3 times independently.

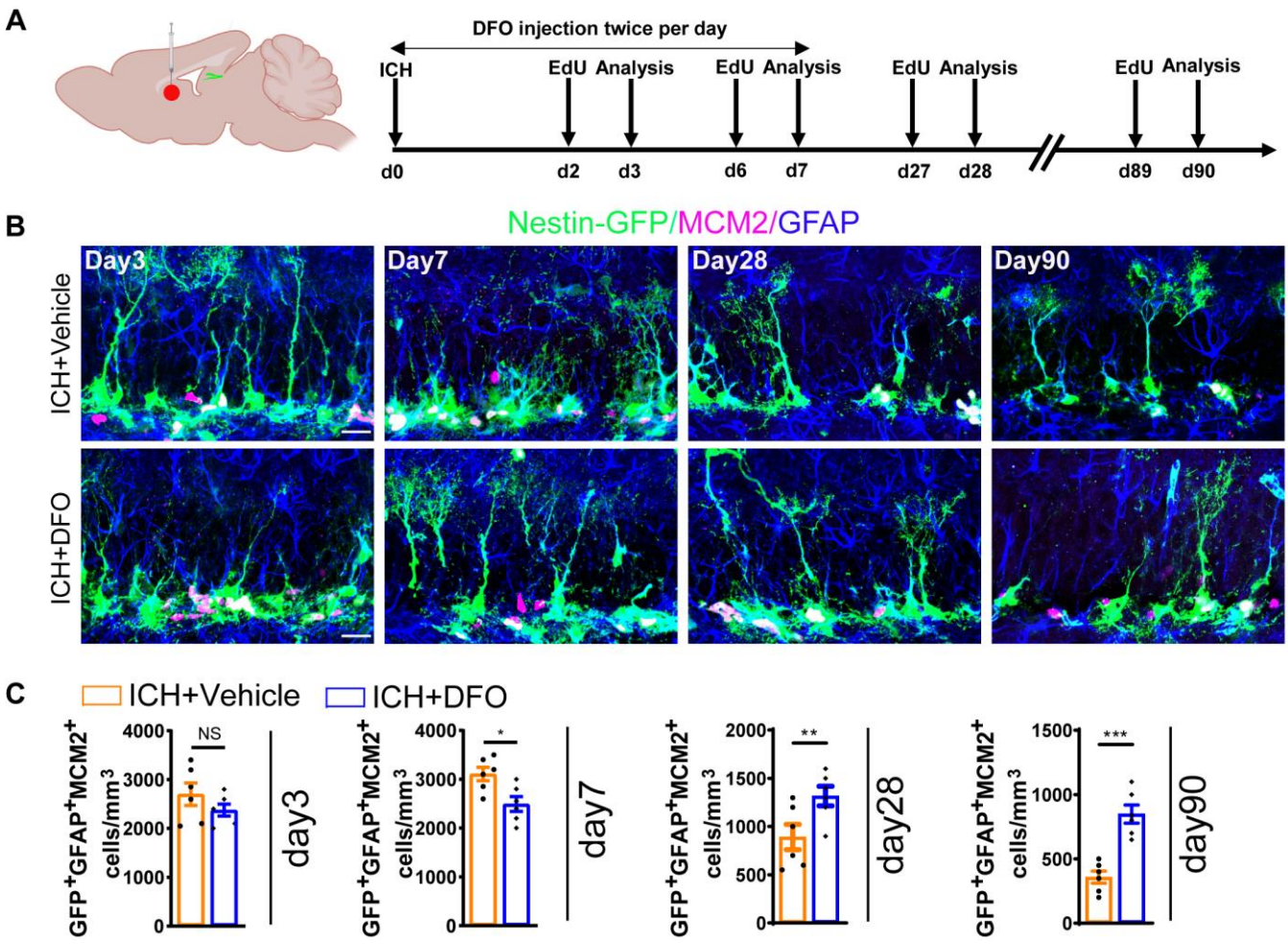

Figure S2. DFO can alleviate ICH-induced iron deposition, NSCs over-activation and exhaustion of NSCs pool

(A) Schematic diagram of the ICH model, as well as experimental timeline for cell proliferation analysis of ICH group with or without DFO treatment.

(B) Representative images of brain section stained with MCM2, GFAP, and genetically labeled GFP in the DG 3, 7, 28 and 90 days in ICH mice with or without DFO treatment. Scale bar = 20 μm.

(C) Quantification of numbers of proliferating rNSCs ($GFP^+GFAP^+MCM2^+$) cells from D. (n=6 for each group).

(C,) *P < 0.05; **P < 0.01; ***P < 0.001 vs corresponding ICH+Vehicle. NS= no significance. Each experiment was repeated at least 3 times independently.

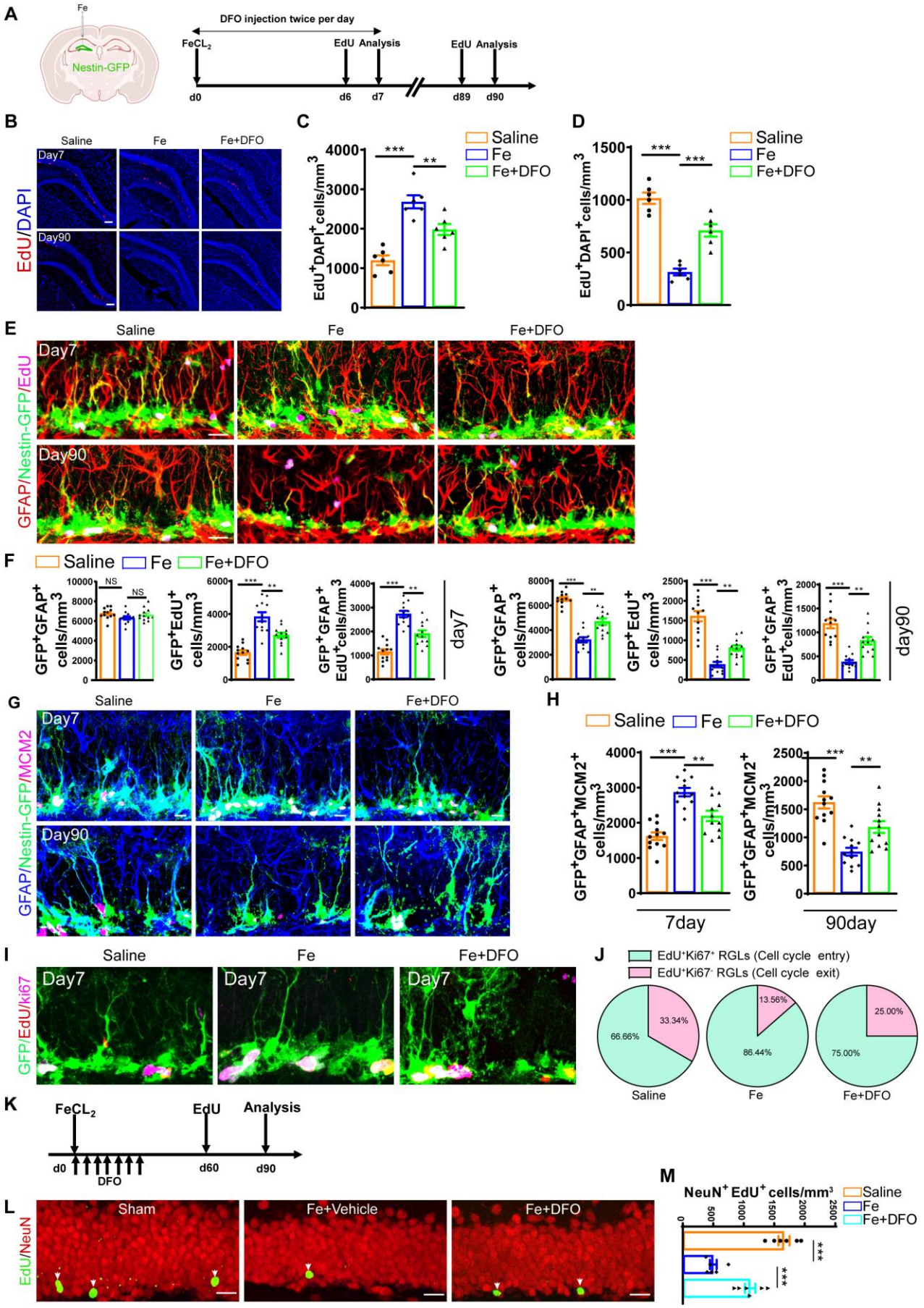
A
Fe
Nestin-GFP
DFO injection twice per day
FeCL2
EdU Analysis
EdU Analysis
d0
d6
d7
d89
d90
B
Saline
Fe
Fe+DFO
Day7
Day90
EdU/DAPI
C
Saline
Fe
Fe+DFO
EdU+DAPI+cells/mm3
D
Saline
Fe
Fe+DFO
EdU+DAPI+cells/mm3
E
Saline
Fe
Fe+DFO
Day7
Day90
GFAP/Nestin-GFP/EdU
F
Saline
Fe
Fe+DFO
GFP+GFAP+ cells/mm3
GFP+EdU+ cells/mm3
GFP+GFAP+EdU+cells/mm3
day7
day90
NS
G
Saline
Fe
Fe+DFO
Day7
Day90
GFAP/Nestin-GFP/MCM2
H
Saline
Fe
Fe+DFO
GFP+GFAP+MCM2+ cells/mm3
7day
90day
I
Saline
Fe
Fe+DFO
Day7
GFP/EdU/ki67
J
EdU+Ki67+ RGLs (Cell cycle entry)
EdU+Ki67- RGLs (Cell cycle exit)
33.34%
66.66%
13.56%
86.44%
25.00%
75.00%
Saline
Fe
Fe+DFO
K
FeCL2
EdU
Analysis
d0
DFO
d60
d90
L
Sham
Fe+Vehicle
Fe+DFO
EdU/NeuN
M
NeuN+ EdU+ cells/mm3
Saline
Fe
Fe+DFO

Figure S3. Injection of $FeCl_2$ recapitulates overactivation and exhaustion of the NSC pool

(A) Schematic diagram of $FeCl_2$ or saline injection to the hippocampus of mice and experimental timeline for cell proliferation analysis of $FeCL_2$ group with or without DFO treatment.

(B) Representative images of EdU^+ cells in the DG after 24hours EdU pulse labeling of Sham and $FeCl_2$, $FeCl_2$+DFO group. Scale bar = 100 μm.

(C) Quantification of numbers of EdU^+DAPI^+ cells from B at 7 days. (n=6 for each group).

(D) Quantification of numbers of EdU^+DAPI^+ cells from B at 90 days. (n=6 for each group).

(E) Representative images of brain section stained with EdU, GFAP, and genetically labeled GFP in the DG of $FeCl_2$ group at 7 and 90 days after surgical manipulations. Scale bar = 20 μm.

(F) Quantification of numbers of rNSCs (GFP^+GFAP^+), proliferating GFP^+ (GFP^+EdU^+) and proliferating rNSCs ($GFP^+GFAP^+EdU^+$) cells from E. (n=12 for each group).

(G) Representative images of brain section stained with GFAP, MCM2 and genetically labeled GFP in the DG in Sham, $FeCl_2$ and $FeCl_2$+DFO group at 7 and 90 days after surgical manipulations. Scale bar = 10 μm.

(H) Quantification of numbers of $GFP^+GFAP^+MCM2^+$ cells from G. (n=12 for each group).

(I) Representative images of brain section stained with EdU, Ki67, and genetically labeled GFP in the DG in Sham, $FeCl_2$ and $FeCl_2$+DFO group at 7 days after surgical manipulations. Scale bar = 10 μm.

(J) Quantified percentage of $GFP^+EdU^+Ki67^+$ (representing cells that entered cell cycle) and $GFP^+EdU^+Ki67^-$ (representing cells that exiteded cell cycle) cells from I. (n=12 for each group).

(K) Experiments timeline for cell differentiation analysis of Sham, Fe+Vehicle and Fe+DFO group.

(L) Representative images of brain section stained with EdU and NeuN in the DG of Saline, Fe and Fe+DFO group at 90 days. White arrows indicate $NeuN^+EdU^+$ (adult-born neurons) cells. Scale bar = 20 μm.

(M) Quantification of numbers of $NeuN^+EdU^+$ cells from L. (n=6 for each group).

(C, D, F, H, G, M) *P < 0.05; **P < 0.01; ***P < 0.001 vs corresponding Fe. Each experiment was repeated at least 3 times independently.

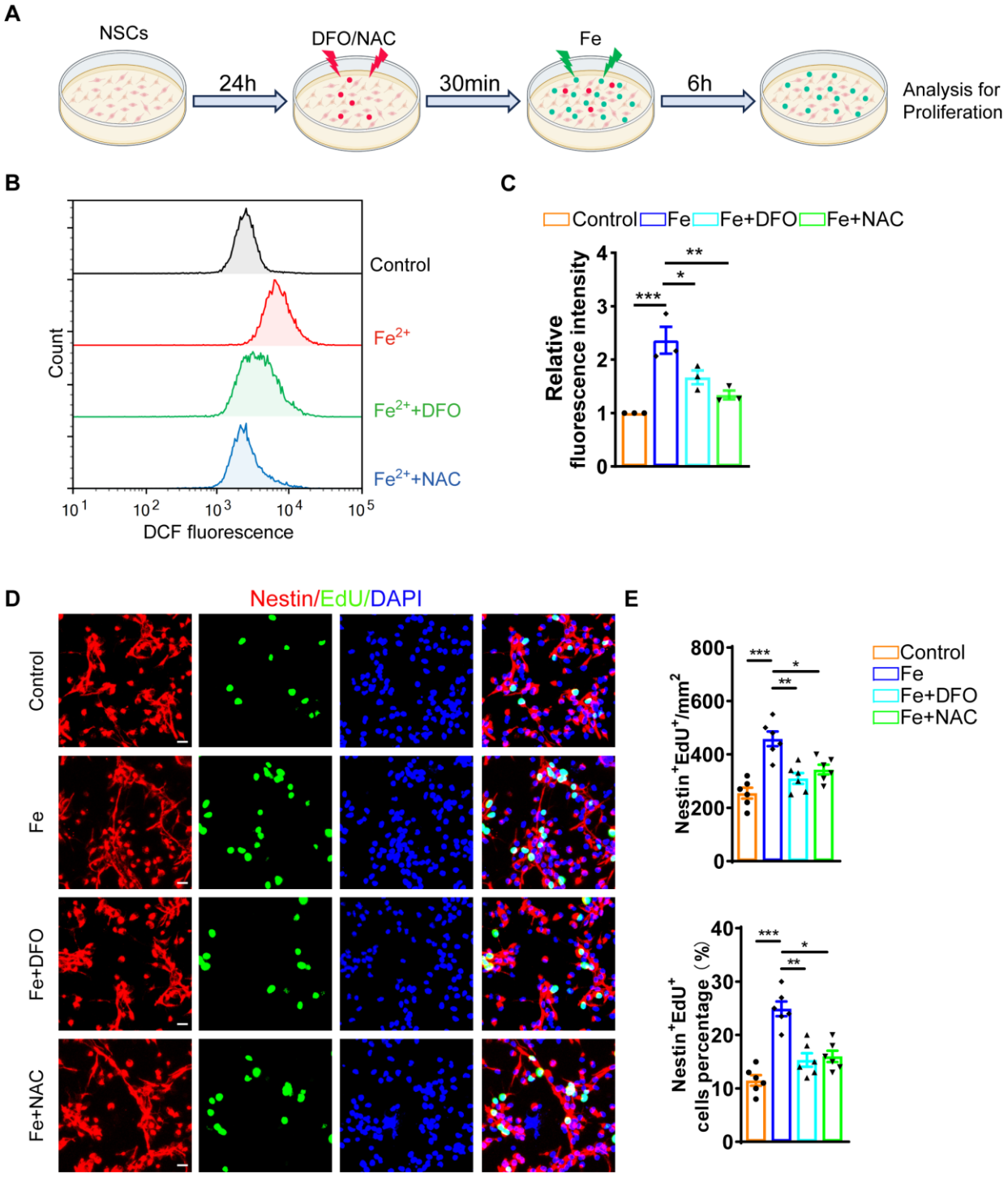
A
NSCs
24h
DFO/NAC
30min
Fe
6h
Analysis for Proliferation
B
Control
Fe2+
Fe2+ +DFO
Fe2+ +NAC
Count
DCF fluorescence
C
Control
Fe
Fe+DFO
Fe+NAC
Relative fluorescence intensity
D
Nestin/EdU/DAPI
Control
Fe
Fe+DFO
Fe+NAC
E
Nestin+EdU+/mm2
Nestin+EdU+ cells percentage (%)

Figure S4. ROS induced by iron activates proliferation of NSCs in vitro

(A) Schematic diagram of Fe^{2+} treated cultured NSCs group and experiments timeline for DFO, NAC treatment and cell proliferation analysis.

(B) ROS production assessment of Control, Fe^{2+}, Fe^{2+}+DFO and Fe^{2+}+NAC and ROS were assessed by flow cytometry using H2DCFDA.

(C) Quantification of fluorescence intensity of H2DCFDA from B. (n=3 for each group).

(D) Representative images of cultured NSCs stained with Nestin and EdU after Fe^{2+} treated for 6h. Scale bar = 20 μm.

(E) Quantification of numbers and percentage proliferating NSCs ($Nestin^{+}EdU^{+}$) cells from D. (n=6 for each group).

(C, E) *P < 0.05; **P < 0.01; ***P < 0.001 vs corresponding Fe. Each experiment was repeated at least 3 times independently.

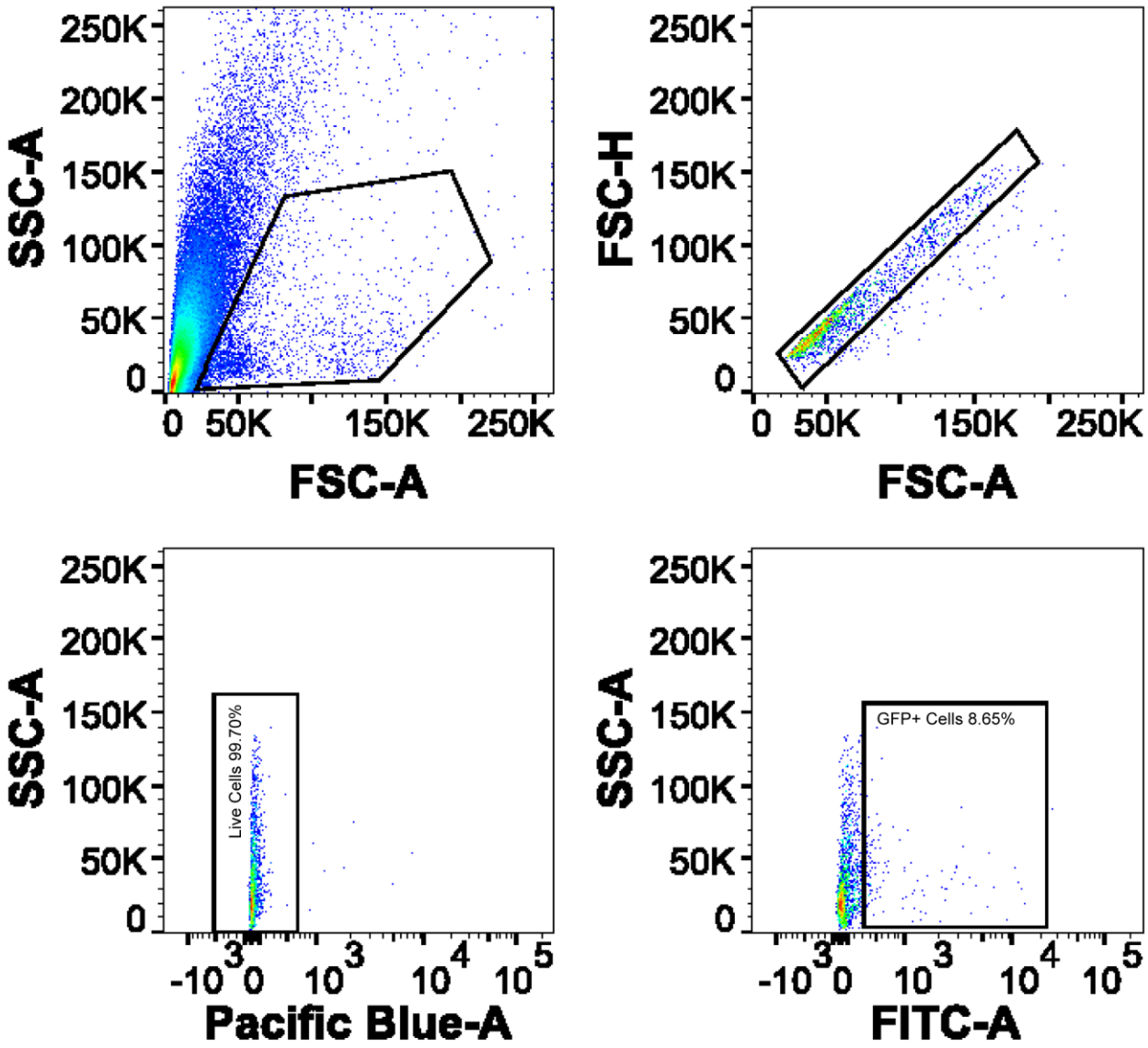

Figure S5. Gating strategy for detecting GFP$^+$ cells of Nestin-GFP mice sorting from Sham and ICH.

(A) Gating for intact cells according to SSC-A and FSC-A.

(B) Gating for single cells according to FSC-H and FSC-A.

(C) Gating for live according to DAPI exclusion.

(D) Gating for GFP$^+$ cells according to GFP reporter signal.

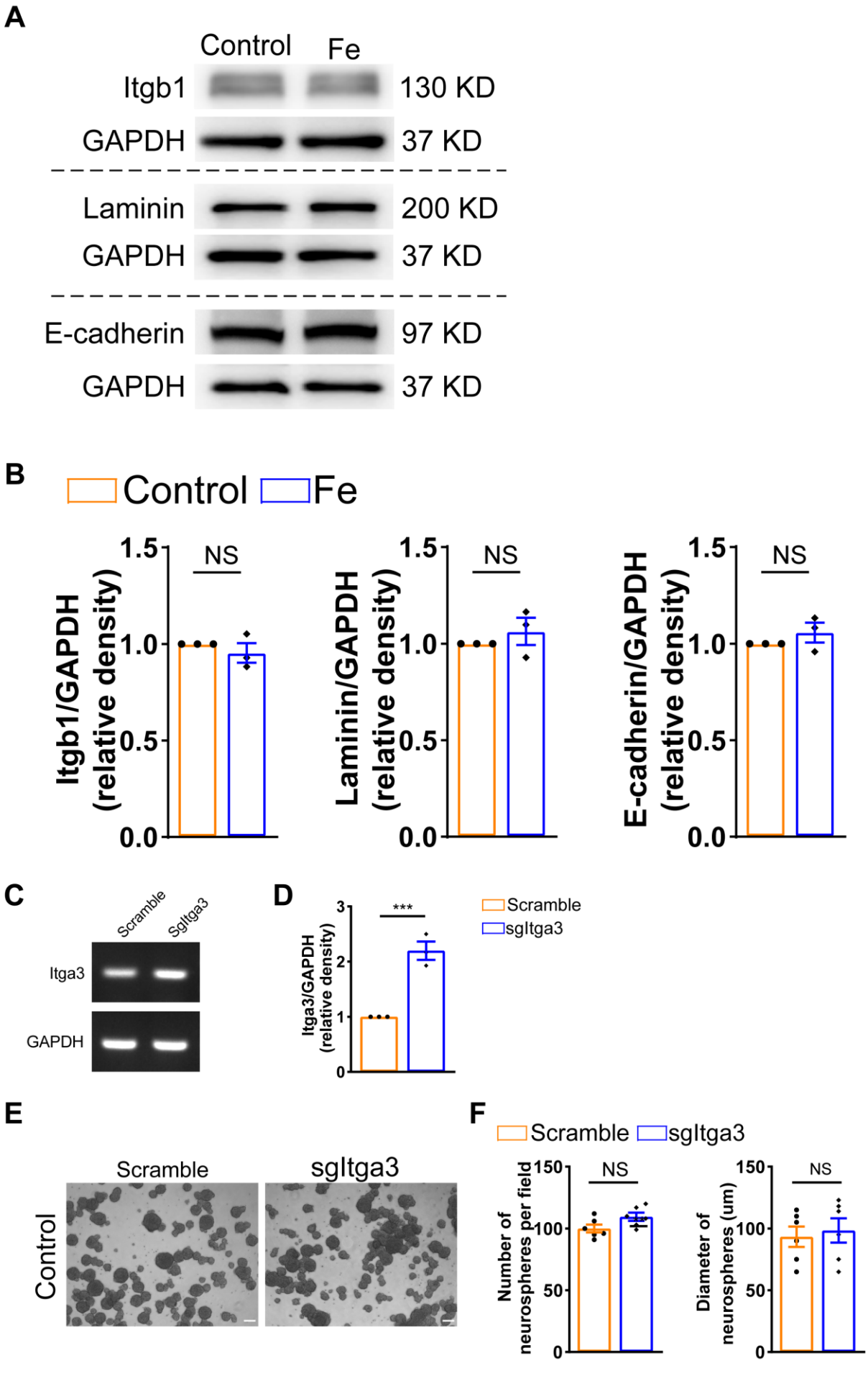
A
Control
Fe
Itgb1
130 KD
GAPDH
37 KD
Laminin
200 KD
GAPDH
37 KD
E-cadherin
97 KD
GAPDH
37 KD
B
Control
Fe
Itgb1/GAPDH (relative density)
Laminin/GAPDH (relative density)
E-cadherin/GAPDH (relative density)
NS
1.5
1.0
0.5
0.0
C
Scramble
SgItga3
Itga3
GAPDH
D
Itga3/GAPDH (relative density)
***
Scramble
sgItga3
E
Scramble
sgItga3
Control
F
Scramble
sgItga3
Number of neurospheres per field
Diameter of neurospheres (um)
NS
150
100
50
0

Figure S6. There was no significant difference in the expression of adhesion related molecules in NSCs exposed to iron

(A-B) The expression of Itgb1, Laminin, E-cadherin in cultured NSCs from Control and Fe^{2+} group. GAPDH was served as the internal control. Semi-quantitative analysis of WB results. (n=3 for each group).

(C) RT-PCR showing Itga3 mRNA expression with Itga3 sgRNA transfection.

(D) Quantification of Itga3 mRNA expression from C. GAPDH was served as the internal control. (n=3 for each group).

(E) The phase-contrasted images depicting NSCs condition in Itga3 overexpression and Scramble group. Scale bar: 100μm.

(F) The number and the diameter of neurospheres from C. (n=6 for each group).

(B, D, F) ***P < 0.001 vs corresponding Control or Scramble. NS= no significance. Each experiment was repeated at least 3 times independently.