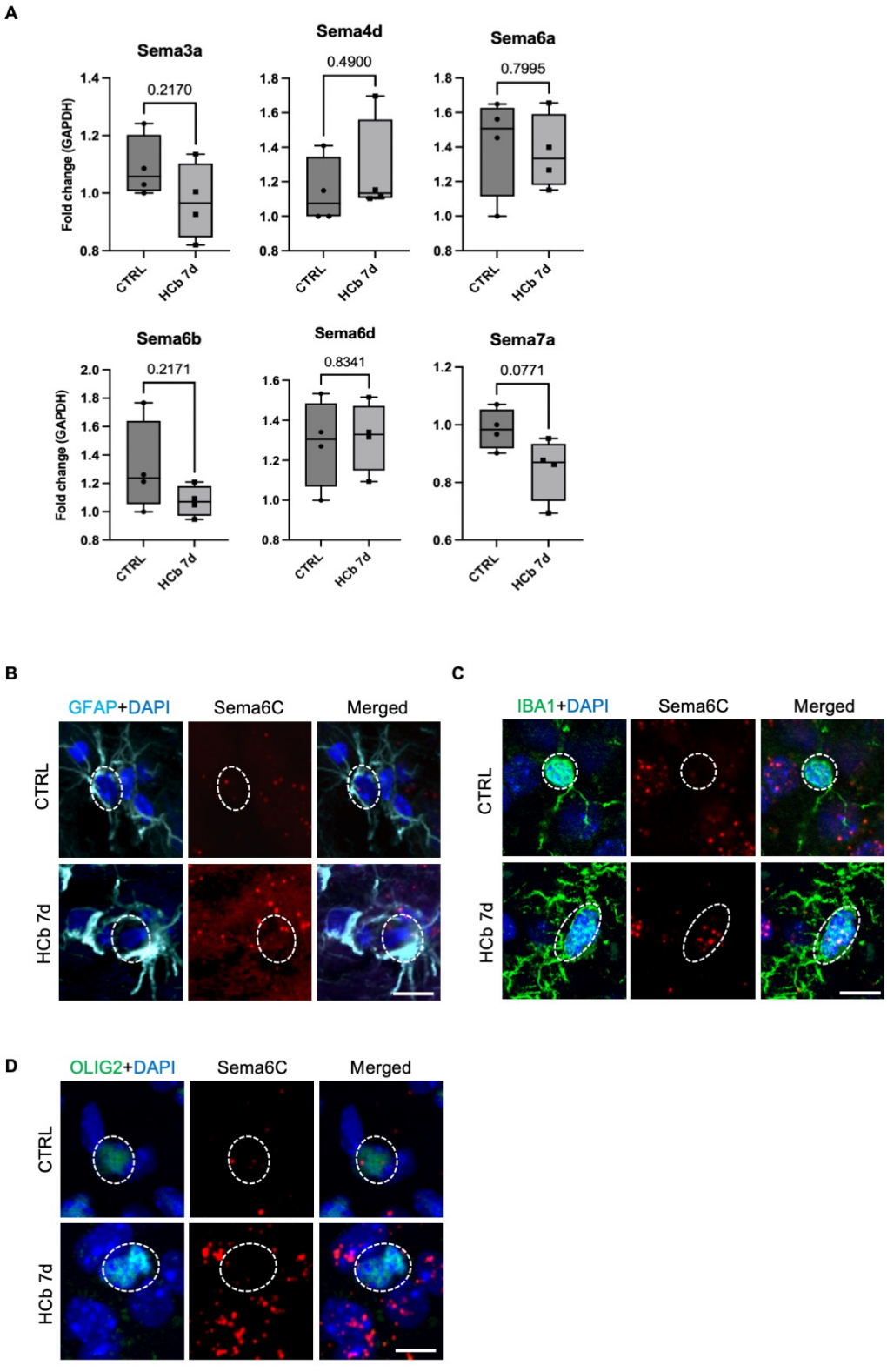
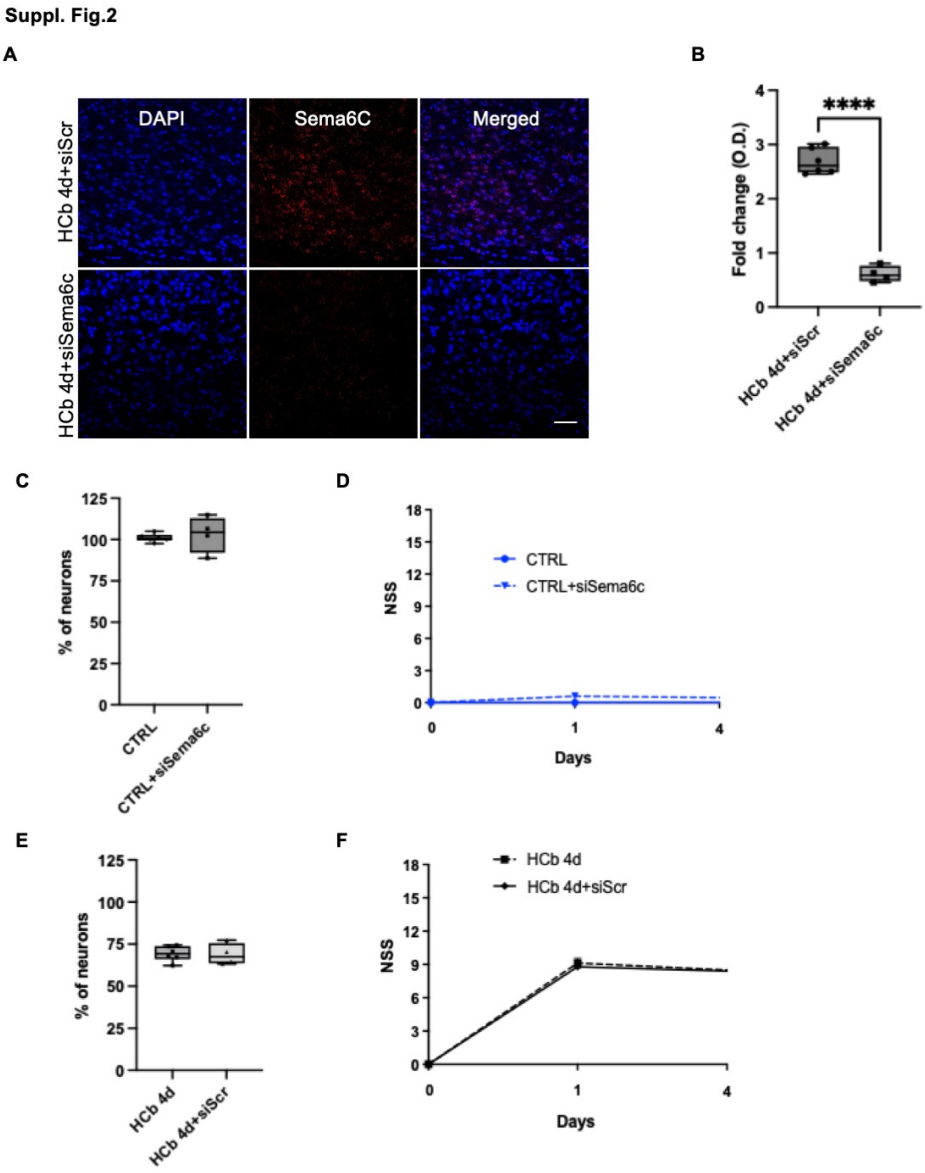
Supplemental Table 1. Primer sequences used for qPCR analysis.

mGapdh (F:CTACATGGCCTCCAAGGAGTAAG and R:TGGAATTGTGAGGGAGATGCTC)
mPlxna1(F:GTTGAAAGGTACTATGCTGAC and R:CGATGTAGGAGTAGATCTCG)
mPlxna2 (F:GTATACAACTTGTGGAGAGTG and R:TGTGATCTGACACAGAGATG)
mSema3a (F: ATCAGTGGGTGCCTTACCAA and R: GCCAAATGTTTTACTGGGACA
mSema4c (F:CAGTTTGTGAGTACCAGTTG and R:GTTGTTGATTACACGAACCAG)
mSema6a (F:TTGCCACGCTCAATGTCCT and R:GCCTCCAGAGACCCGTATTG)
mSema6b (F:CCACGTCATTCTTGCACACA and R:TGGGGAAGCCACGTCTATTT)
mSema6c (F: AGCTATGGGATAACATCTCTG and R:GTAAACTACAGCATCACTGG)
mSema6d (F:TGAAGCTGGCGTGGTACTTA and R:CCACCTTTCTGTCCTCCTCA)
mSema7a (F:CGTGGCAAGGTCTACCACTT and R:GACAGGACCCCTTTGTGGAG)

Suppl. Fig.1


A
Sema3a
0.2170
Sema4d
0.4900
Sema6a
0.7995
Sema6b
0.2171
Sema6d
0.8341
Sema7a
0.0771
Fold change (GAPDH)
CTRL
HCb 7d
B
GFAP+DAPI
Sema6C
Merged
C
IBA1+DAPI
D
OLIG2+DAPI

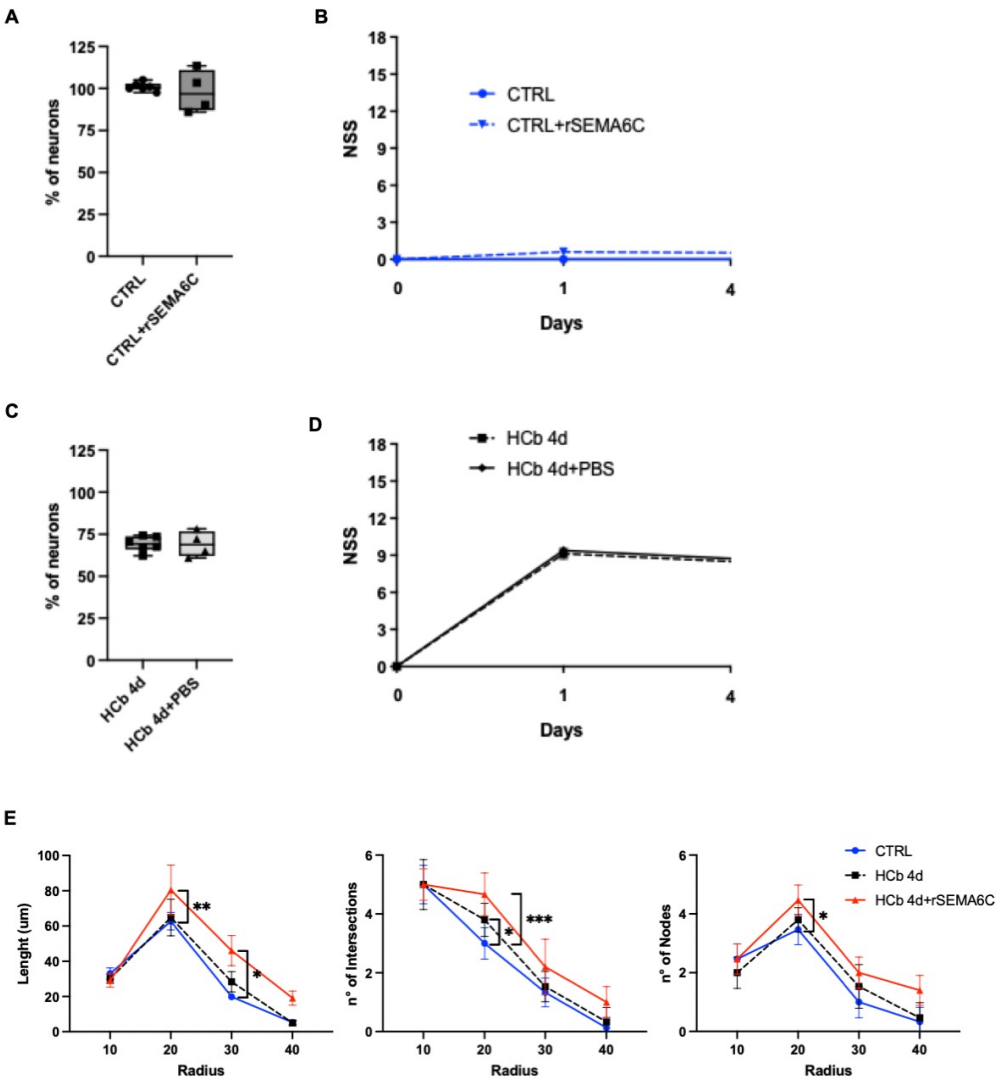
Supplementary Figure 1. Expression profiling of semaphorin family members after hemicerebellectomy. (A) Sema3a, 4d, 6a, 6b, 6d and 7a mRNA expression assessed by qPCR. Expression levels were normalized to GAPDH and expressed as fold change relative to the control group using the $2^{-\Delta\Delta Ct}$ method (N=4). Statistical analysis was performed using an unpaired two-tailed Student's t-test. Box plots indicate median, interquartile range, and min–max values. Each dot represents one animal. p values are shown on graphs. (B) Representative confocal images of RNAscope showing Sema6c mRNA expression (red) and marker of astrocytes GFAP (cyan) in PN of CTRL and HCb 7d. Nuclei were counterstained with DAPI (blue). (C) Representative confocal images of RNAscope showing Sema6c mRNA expression (red) and marker of microglia IBA1 (green) in PN of CTRL and HCb 7d. Nuclei were counterstained with DAPI (blue). (D) Representative confocal images of RNAscope showing Sema6c mRNA expression (red) and marker of oligodendrocytes OLIG2 (green) in PN of CTRL and HCb 7d. Nuclei were counterstained with DAPI (blue). Scale bar=25µm.



Supplementary Figure 2. Validation of Sema6c silencing and assessment of the silencing on neuronal survival or neurological outcome. (A) Representative RNAscope confocal images showing Sema6c mRNA expression (red) and DAPI counterstaining (blue). Scale bar=50µm. (B) Graph of relative Sema6c mRNA-signal intensity quantification presented as optical density (O.D.) (N=6/group). (C) Graph of surviving neurons in the PN expressed as percentage of neurons relative to the CTRL group (N=4/group) in CTRL and CTRL+siSema6c mice. (D) Graph showing the Neurological Severity Score (NSS) of CTRL/ CTRL+siSema6c mice. (E) Graph of surviving neurons in the PN expressed as percentage of neurons relative to the CTRL group (N=4/group) in HCb 4d and

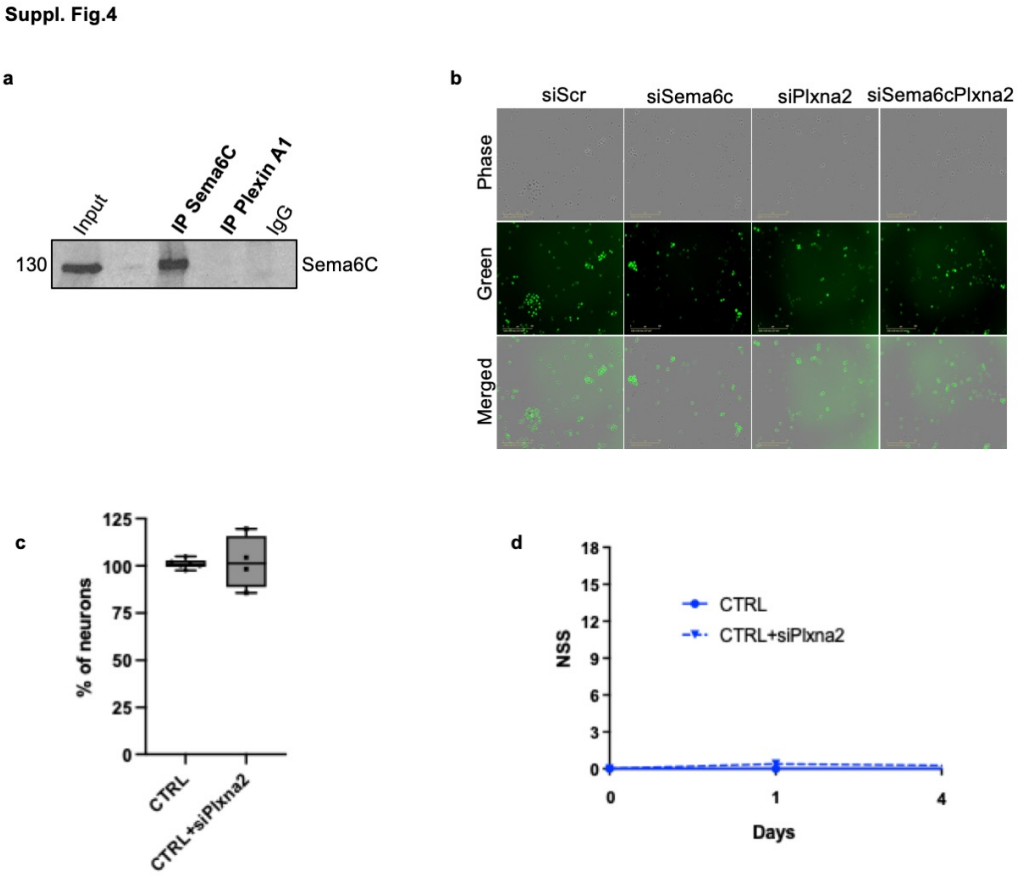
HCb 4d+siScr mice. (F) Graph showing the Neurological Severity Score (NSS) of HCb 4d and HCb 4d+siScr mice. Box plots indicate median, interquartile range, and min–max values. Each dot represents one mouse. *p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001.

Suppl. Fig.3



Supplementary Figure 3. Effects of rSema6Cand vehicle treatment on neuronal survival, neurological outcome, and microglial morphology after HCb. (A) Graph of the quantification of surviving neurons expressed as percentage of neurons relative to the CTRL group (N=4/group). (B) Graph showing the Neurological Severity Score (NSS) of CTRL and CTRL+rSEMA6C mice. (C) Graph of the quantification of surviving neurons expressed as percentage of neurons relative to the CTRL group (N=4/group) in HCb 4d and HCb 4d+PBS mice. (D) Graph showing the Neurological Severity Score (NSS) of HCb 4d and HCb 4d+PBS mice. (E) Graphs showing length of microglial process after Sholl's analysis in the three experimental groups. (F) Graph of the number of intersections obtained

from immunolabeled microglia and used to assess morphological changes across conditions. (G) Graph of and nodes measurements obtained from immunolabeled microglia. Box plots indicate median, interquartile range, and min–max values. Each dot represents one mouse. *p< 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001.



S**upplementary Figure 4. Sema6C/Plexin A1 binding assay and effects of Sema6c/Plxna2 silencing on apoptosis, neuronal survival and neurological outcome.** (A) Representative western blot with anti-Sema6C antibody on proteins obtained after Sema6C and Plexin A1 immunoprecipitation from COS overexpressing both Sema6C and Plexin A1. Input sample accounts for 10% of the extract. IgG represent the negative control. Representative experiment of three biological replicates. (B)

Representative images of green fluorescence cells by the Incucyte Dye for Apoptosis in previously H_2O_2 treated and silenced N2A cells for siScr, siSema6C, siPlxnA2 and both. (C) Graph of the quantification of surviving neurons in the PN expressed as the percentage of neurons relative to the CTRL group (N=4/group) in CTRL+sPlxna2 silencing. (D) Graph showing the Neurological Severity Score (NSS) of CTRL, CTRL+siPlxnA2 mice. Box plots indicate median, interquartile range, and min–max values. Each dot represents one mouse.