Figure S1. Radial glial fibers are sustained after neonatal brain injury, Related to Figure 1. (A-D) Coronal sections of the cortex in WT mice after cryogenic injury stained for Nestin (red). (A) Cryogenic injury was performed in P2 mice, which were fixed at 7, 14, 21, and 28 days-post-injury (dpi). (B-D) Cryogenic injury was performed in P4 (B), P14 (C), and 8-week-old (8w, D) mice, which were fixed at 7 dpi. (E) Nestin+ fiber density after cryogenic injury. Neonatal (P2) injury increased the Nestin+ fiber density (7 dpi, n=3 mice; 14 dpi, n=4 mice; 21 dpi, n=4 mice; 28 dpi, n=4 mice; *p<0.05, paired t-test). Fiber density between the contralateral and ipsilateral cortex was also compared (###p<0.005 [vs P2, or 7 dpi of P2 injury in ipsilateral cortex], §§§p<0.005 [vs 7 dpi of P2 injury in contralateral cortex], One-way ANOVA followed by post-hoc Tukey multiple comparison test). Nestin+ fiber density was also increased by injury in the P4 (n=4 mice) but not in the P14 (n=3 mice) or 8w (n=4 mice) injury models (***p<0.005, paired t-test) (##p<0.01, ###p<0.005, One-way ANOVA followed by post-hoc Tukey multiple comparison test). Fiber density at P2 was analyzed in intact mice (n=3 mice). (F) Nestin+ fiber length in P14 intact mice, and in P14 and 8w injury mice at 7 dpi (P14, 495 fibers from 4 mice; contralateral in P14 injury mice at 7 dpi, 220 fibers from 4 mice; ipsilateral in P14 injury mice at 7 dpi, 261 fibers from 4 mice; contralateral in 8w injury mice at 7 dpi, 227 fibers from 3 mice; ipsilateral in 8w injury mice at 7 dpi, 264 fibers from 3 mice; ***p<0.005 [P14 vs 7dpi of P14, Kruskal-Wallis test followed by Steel test; contralateral vs ipsilateral, Wilcoxon signed-rank test]). (G) Coronal sections of the cortex and striatum in WT mice after neonatal hypoxia and ischemia stained for Nestin (red). Nestin+ fibers were observed from the V-SVZ toward the injured CC and striatum. (H) Nestin+ fiber density after hypoxia and ischemia at 7 dpi (n=3 mice; **p<0.01, unpaired t-test). (I and J) Morphological analysis of radial glial cells in the uninjured and injured cortex of P2 injury model mice. Traces of the radial glial morphology at P2, P9, and P16 of control and injured mice are shown (I). Fibers at P9 and P16 in the injury group were significantly longer than in the control group (J; P2, 14 cells from 3 mice; P9-control, 21 cells from 3 mice; P9-injury, 28 cells from 3 mice; P16-control, 19 cells from 3 mice; P16-injury, 12 cells from 3 mice). ***p<0.005, unpaired t-test. (K) Time-lapse images of the fiber extension of tdTomato-labeled cells after injury. Arrows and graph show the tip of the fiber and average extension speed of fibers, respectively (n=9 cells from 3 mice). Numbers indicate minutes from the first frame. Scale bars: 50 μm (A-D, G, I), 10 μm (K). Error bars indicate mean ± SEM.

Figure S2. Association of neuroblasts with radial glial fibers after neonatal brain injury, Related to Figure 1 and 2. (A-D) Coronal section of the cortex in R26-tdTomato mice, into which Cre-expressing adenovirus had been injected into the cortical surface, stained for DsRed (red), a cell type-specific marker (A and B, Nestin; C, GFAP; D, Olig2) (white), ErbB4 (A, green), Pax6 (B, green), and Hoechst33342 (C, D, blue). (E) Classification of neuroblast directionality and association with fibers. (F) Proportion of neuroblasts of different fiber-association classes in the control and DN-N-cadherin group. DN-N-cadherin significantly decreased the proportions of neuroblasts in the whole-cell association and leading process (LP) association classes, and increased that in the non-association class. *$p<0.05$, ***$p<0.005$, Unpaired t-test or Mann-Whitney U-test (n=3 mice each). (G) Fiber density in control and DN-N-cadherin-expressing radial glia after injury. Mann-Whitney U-test (n=3 mice each). (H) Morphological classification of neonatal radial glia and their progenies after injury. The tdTomato+ cells were classified into 7 types based on their morphology and somal location: radial glia (RG) in the V-SVZ, branched RG in the V-SVZ, RG in the CC, branched RG in the CC, multipolar cells, ventricular cells with no radial process, and others with no radial process. Proportion of tdTomato+Nestin+ RG and their progenies in control (n=3 mice) and DN-N-cadherin (n=4 mice) expression groups are shown (graph). Unpaired t-test or Mann-Whitney U-test. (I-L) Evaluation of the N-cadherin-KD vector. (I, J) Suppression of exogenous N-cadherin in HEK293T cells by N-cadherin-KD. A plasmid expressing HA-N-cadherin was cotransfected with a control (lacZ) or N-cadherin-KD vector into HEK293T cells. Forty-eight hours after transfection, the cells were collected, lysed, and subjected to immunoblotting with an anti-HA or anti-actin antibody (I). Quantification of the N-cadherin KD (J, n=4 independent experiments; *$p<0.05$, paired t-test). (K, L) Suppression of endogenous N-cadherin in cultured neuroblasts by N-cadherin-KD. Representative images of cultured neuroblasts stained for N-cadherin (green), tdTomato (red, KD vectors), and PSA-NCAM (blue) (K). Quantification of N-cadherin KD (L; control, n=17 cells; N-cadherin-KD, n=14 cells; three independent experiments; ***$p<0.005$, unpaired t-test). (M) Expression patterns of FAK and L1-CAM in the radial glial fibers after neonatal brain injury. Coronal sections of the cortex in WT mice at 7 dpi stained for Nestin (red) and FAK or L1-CAM (green). (N, O) Evaluation of the FAK and L1-CAM-KD vectors. Suppression of exogenous FAK (N) and L1-CAM (O) in HEK293T cells by FAK- and L1-CAM-KD, respectively. (P) Density of fiber-associated Dcx+ cells in N-cadherin- (n=3 mice), FAK- (n=3 mice), and L1-CAM- (n=3 mice) KD groups. N-cadherin-KD significantly decreased the density of fiber-associated Dcx+ cells (***$p<0.005$, One-way ANOVA followed by post-hoc Dunnett test). (Q, R) Expression of neuregulin in the contralateral and ipsilateral cortex at 4 dpi. Neuregulin protein was detected in both the contralateral and ipsilateral cortex (Q). Quantification of neuregulin expression (R). Scale bars: 50 μm (A, B, M), 10 μm (C, D), 5 μm (K). Error bars indicate mean ± SEM.

Figure S3. V-SVZ-derived neurogenesis after neonatal brain injury, Related to Figure 3. (A-F) Numbers of glutamatergic and GABAergic neuronal progenitors at 4 (A, D) or 7 (B, C, E, F) days-post-injury (dpi). The numbers of Neurog2-d4Venus+ (A, n=4 mice) and Mash1+ (D, n=4 mice) progenitors were significantly increased by injury. Although the Tbr2+ (B, n=5 mice) and Tbr2+Dcx+ (C, n=5 mice) cell populations were not increased, the Dlx2+ (E, n=4 mice) and Dlx2+Dcx+ (F, n=4 mice) cell populations increased significantly in the cortex (CTX) after injury. Paired t-test or Wilcoxon signed-rank test. (G-M) Cortical interneurons generated from the V-SVZ. (G) Experimental scheme. EP, in vivo electroporation. (H, I) Number (H) and distribution (I) of V-SVZ-derived NeuN+ neurons at 28 dpi (control, n=8 mice; injury, n=7 mice; unpaired t-test). (J-L) Coronal section after brain injury of the cortex in WT mice, into which EmGFP-expressing plasmids had been electroporated into the V-SVZ. Sections were stained for GFP (green), GAD67 (J, red), Parvalbumin (K, red), Calretinin (L, red), and NeuN (white). Arrows indicate interneuron marker (GAD67, Parvalbumin, or Calretinin)-expressing NeuN+EmGFP+ neurons. (M) Proportion of marker-expressing EmGFP+NeuN+ neurons. Scale bars: 10 μm (J-L). *$p<0.05$, ***$p<0.005$. Error bars indicate mean ± SEM.

Figure S4. N-cadherin-fibers promote the migration of V-SVZ-derived neuroblasts in vitro but not in vivo, Related to Figure 3. (A) Time-lapse images of cultured neuroblasts migrating along control- (upper) and N-cadherin- (bottom) fibers. Arrows indicate cultured neuroblasts migrating along fibers. (B) Representative images of cultured neuroblasts (red) along fibers (green) stained for Dcx (red) and Hoechst 33342 (blue). (C) Speed of cultured neuroblasts migrating along the fibers (control-non-contact, n=19 cells; control-contact, n=34 cells; N-cadherin-non-contact, n=32 cells; N-cadherin-contact, n=124 cells; ***p<0.005, Mann-Whitney U-test). (D) Fold increase in migration speed by N-cadherin-fibers and -sponges. (E, F) Migration of Dcx+ neuroblasts within the N-cadherin-fibers-transplanted region at P9. (E) Coronal sections of the cortex in WT mice treated with control- or N-cadherin-fibers (DIC and dotted lines) stained for Dcx (red) and Hoechst 33342 (blue). Arrows indicate Dcx+ cells along the fibers. (F) The density of Dcx+ cells in N-cadherin-fibers (n=3 mice) was not statistically different from that in control-fibers (n=3 mice), and was significantly lower than that in N-cadherin-sponges (shown in Figure 3I) (***p<0.005, unpaired t-test). (G) Relative number of Dcx+ cells in the sponges in the P2, P14, and 8w injury models. The promotion of neuroblast migration by N-cadherin-sponge was most obvious in the 8w injury model. *p<0.05, **p<0.01, One way-ANOVA followed by post-hoc Tukey multiple comparison test. Scale bars: 10 μm (A, B), 20 μm (E). Error bars indicate mean ± SEM.

Table S2. Oligonucleotide sequences, Related to Figure 1, 2, and S2

Primer	Sequence
targeting sequence: mouse N-cadherin gene 944_top	TGCTGTAAACATGTTGGGTGAAGGTGGTTTTGGCCACTGACTGACCACCTTCACAACATGTTTA
targeting sequence: mouse N-cadherin gene 944_bottom	CCTGTAAACATGTTGTGAAGGTGGTCAGTCAGTGGCCAAAACCACCTTCACCCAACATGTTTAC
targeting sequence: mouse FAK gene 229_top	TGCTGATAGCAGGCCACGTGCTTTACGTTTTGGCCACTGACTGACGTAAAGCATGGCCTGCTAT
targeting sequence: mouse FAK gene 229_bottom	CCTGATAGCAGGCCATGCTTTACGTCAGTCAGTGGCCAAAACGTAAAGCACGTGGCCTGCTATC
targeting sequence: mouse l1cam gene 408_top	TGCTGTTTACAGTCTCCTTCGGCCACGTTTTGGCCACTGACTGACGTGGCCGAGAGACTGTAAA
targeting sequence: mouse l1cam gene 408_bottom	CCTGTTTACAGTCTCTCGGCCACGTCAGTCAGTGGCCAAAACGTGGCCGAAGGAGACTGTAAAC
tdTomato Forward	TTTAAAATGGTGAGCAAGGGCGAGGA
tdTomato Reverse	TTTAAACTACTTGTACAGCTCGTCCA
mouse N-cadherin Forward	GGGCCCGTCGACATGTGCCGGATAGCGGGAGC
mouse N-cadherin Reverse	GGGCCCGTCGACTCAGTCGTCACCACCGCCGT
IRES-Cre Forward	GGGCCCAGATCTTCTCCCTCCCCCCCCCCTAA
IRES-Cre Reverse	GGGCCCAGATCTCTAATCGCCATCTTCCAGCA
Genotyping PCR of GFP #1	TTCTTCAAGTCCGCCATGCCCG
Genotyping PCR of GFP #2	TCCAGCAGGACCATGTGATCGC
Genotyping PCR of NSER-DTA: #1	AATTCTTAATTAAGGCGCGCCGG
Genotyping PCR of NSER-DTA: #2	GTCAGAATTGAGGAAGAGCTGGGG
Genotyping PCR of NSER-DTA: #3	CACTGAGGATTCTTCTGTGG
Genotyping PCR of tdTomato: wild type Forward	AAGGGAGCTGCAGTGGAGTA
Genotyping PCR of tdTomato: wild type Reverse	CCGAAAATCTGTGGGAAGTC
Genotyping PCR of tdTomato: mutant Forward	CTGTTCCTGTACGGCATGG
Genotyping PCR of tdTomato: mutant Reverse	GGCATTAAAGCAGCGTATCC

Table S1. Catwalk parameters, related to Figure 4.

Group1, spatial parameters related to individual paws; Group 2, relative spatial relationships between different paws; Group 3, interlimb coordination; Group 4, temporal parameters (Neumann et al., 2009). LF, left frontpaw; RF, right frontpaw; LH, left hindpaw; RH, right hindpaw. *p<0.05, **p<0.01, ***p<0.005 for Injury compared to Control; †p<0.05, ††p<0.01, †††p<0.005 for Injury + control-sponge compared to Control; ‡p<0.05, ‡‡p<0.01, ‡‡‡p<0.005 for Injury + N-cadherin-sponge compared to Control; §p<0.05 for Injury + control-sponge compared to Injury; ‖p<0.05, ‖‖p<0.01, ‖‖‖p<0.005 for Injury + N-cadherin-sponge compared to Injury; ¶p<0.05, ¶¶¶p<0.005 for Injury + N-cadherin-sponge compared to Injury + control-sponge.

Movie S1. Migratory behaviors of cultured neuroblasts on Fc- and N-cadherin-Fc stripes, related to Figure 2.

The behavior of migrating neuroblasts (red) was recorded at 5-min intervals. Green color shows N-cadherin-Fc stripes. Sequential images of these neuroblasts are shown in Figure 2K.

Movie S2. Time-lapse imaging of cultured neuroblasts migrating along control and N-cadherin-sponge, related to Figure 3.

The behavior of migrating neuroblasts (red) was recorded at 3-min intervals. Sequential images of these neuroblasts are shown in Figure 3D.