Dysmyelination by oligodendrocyte-specific ablation of Ninj2 contributes to depressive-like behaviors

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Supporting Information

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Supplementary Figure 1. Oligodendrocytes play a role in depression. Related to Figure 1.

(A) The strategy of generating \( \text{Olig}^1 \text{cre/+}; \text{Gi-DREADD} \) mice, and the timeline of the behavioral tests. (B-D) Tail suspension test (TST), force swimming test (FST) and sucrose preference test (SPT) were performed in CNO-treated \( \text{Gi-DREADD} \) and \( \text{Olig}^1 \text{cre/+}; \text{Gi-DREADD} \) mice, \( n = 5 \) mice/genotype. All the quantification data are presented as mean ± SEM, \( p \)-values are calculated using two-tailed unpaired Student’s t-test. ** \( p < 0.01 \), *** \( p < 0.001 \).
Supplementary Figure 2. Confirmation of Ninj2 knockout in oligodendrocytes, corpus callosum and spinal cord. Related to Figure 1.

(A) The strategy of generating Olig1<sup>cre/+;Ninj2<sup>fl/fl</sup></sub> and Cnp<sup>cre/+;Ninj2<sup>fl/fl</sup></sub> mice. (B-D) Real-time PCR analysis on the mRNA level of Ninj2 in oligodendrocytes (B) (n = 3 independent experiments), corpus callosum (CC) (C) and spinal cord (SC) (D) from WT or Olig1<sup>cre/+;Ninj2<sup>fl/fl</sup></sub> (cKO) mice (n = 3 mice/genotype). All the quantification data are presented as mean ± SEM, p-values are calculated using two-tailed unpaired Student’s t-test, * p < 0.05, **** p < 0.0001.
Supplementary Figure 3. Loss of Ninj2 in oligodendrocytes has no effect on memory, recognition, or anxiety-like behaviors. Related to Figure 1.

WT and Olig1\textsuperscript{cre/+};Ninj2\textsuperscript{fl/fl} (cKO) mice at P60 were subjected to (A) Morris water maze test, (B) Y maze test, (C) Novel object recognition test, (D) Elevated plus maze test, (E) Open field test to evaluate their status on memory, recognition, or anxiety-like behaviors. \( n = 6 \) mice/genotype. All the quantification data are presented.
as mean ± SEM, p-values are calculated using two-tailed unpaired Student’s t-test.

Supplementary Figure 4. Loss of Ninj2 reduces dendritic complexity of pyramidal neurons in the hippocampus CA1 area. Related to Figure 1E.

Golgi staining of WT and Olig1\textsuperscript{cre/+};Ninj2\textsuperscript{0/0} (cKO) mice at P60. Scale bar, 50 μm.
Supplementary Figure 5. Loss of Ninj2 reduces the expression of myelin-related proteins in corpus callosum. Related to Figure 1.

(A) Immunofluorescent visualization and quantification of MBP expression in the corpus callosum from WT or Olig1cre/+;Ninj2flo/flo (cKO) mice at P14, P21 and P60, respectively. n = 3 mice/genotype. (B) Western blot analyses of MBP and CNP in the corpus callosum from WT or cKO mice at P14, P21, P30 and P60, respectively. n = 6 mice/genotype. All the quantification data are presented as mean ± SEM, p-values are calculated using two-tailed unpaired Student’s t-test, * p < 0.05, *** p < 0.001, **** p < 0.0001.
Supplementary Figure 6. Loss of Ninj2 has no effect on myelin thickness. Related to Figure 2.

(A-B) G-ratio of the optic nerve at P14 and P21, the corpus callosum at P21 and P60 from WT or Olig1cre/+;Ninj20/0 (cKO) mice. (C) G-ratio of the corpus callosum at P60 from WT or Cnpcre/+;Ninj20/0 (CcKO) mice. n = 3 mice/genotype, at least 50 axons/mouse had been analyzed. All the quantification data are presented as mean ± SEM, p-values are calculated using two-tailed unpaired Student’s t-test.
Supplementary Figure 7. Loss of Ninj2 leads to motor defect in mice. Related to Figure 2.

(A-B) Forelimb grip strength and rotarod test on WT or Olig1^{cre/+};Ninj2^{fl/fl} (cKO) mice at P60. (C-D) Forelimb grip strength and rotarod test on WT or Cnp^{cre/+};Ninj2^{fl/fl} (CcKO) mice at P60. n = 5 mice/genotype. All the quantification data are presented as mean ± SEM, p-values are calculated using two-tailed unpaired Student’s t-test. * p < 0.05, ** p < 0.01, *** p < 0.001.
Supplementary Figure 8. Loss of Ninj2 has no effect on oligodendrocyte proliferation or differentiation. Related to Figure 3.

(A-C) Immunofluorescent staining against CC1 (A), PDGFRα (B), and Ki67 (C), were performed in the corpus callosum sections from WT or Olig1cre+/Ninj2^/0 (cKO) mice at P7, P14 and P30, the percentages of the double-positive cells in total Olig2^+ cells were quantified and shown on the right panels. Scale bar, 50 µm. n = 4.
mice/genotype. All the quantification data are presented as mean ± SEM, p-values are calculated using two-tailed unpaired Student’s t-test.

**Supplementary Figure 9.** Nec-1s treatment restores dendritic complexity of pyramidal neurons in the hippocampus CA1 area of *Ninj2*-deficient mice.

**Related to Figure 7A.**

Golgi staining of WT or *Olig1*<sup>cre/+</sup>;*Ninj2*<sup>fl/fl</sup> (cKO) mice at P60, which received i.p. injection with vehicle or Nec-1s (10 mg/kg) from P60 to P90. Scale bar, 50 µm.