Supplementary Figures S1-S5

By Zhong-Wei Zhou *et al*.

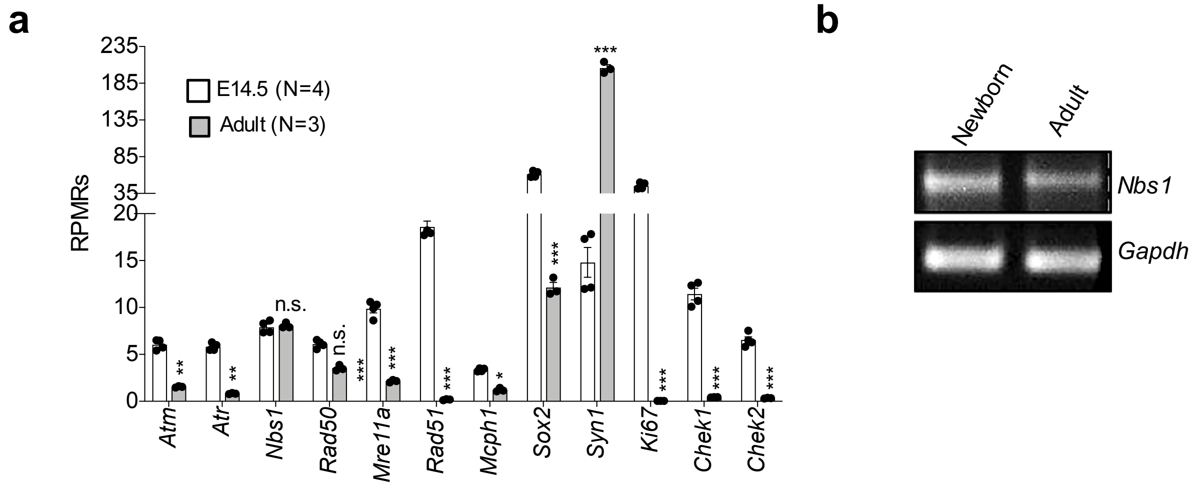
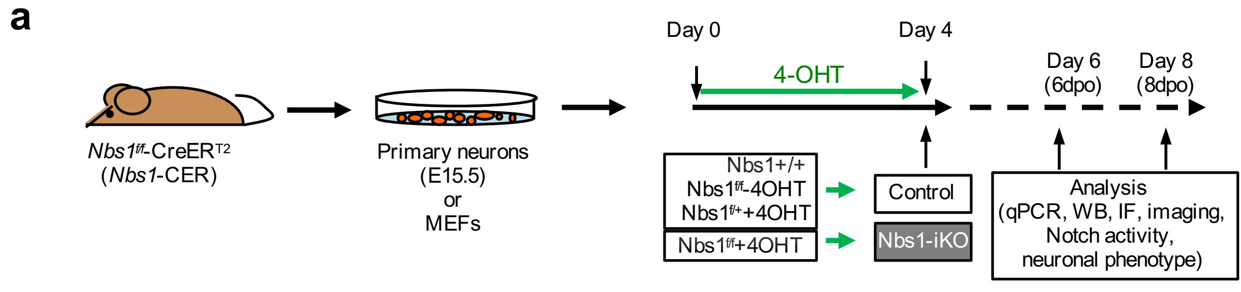
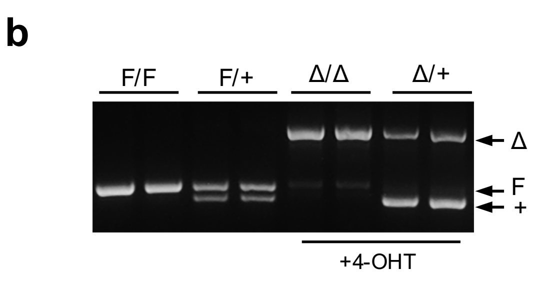


Fig. S1-Zhou et al.





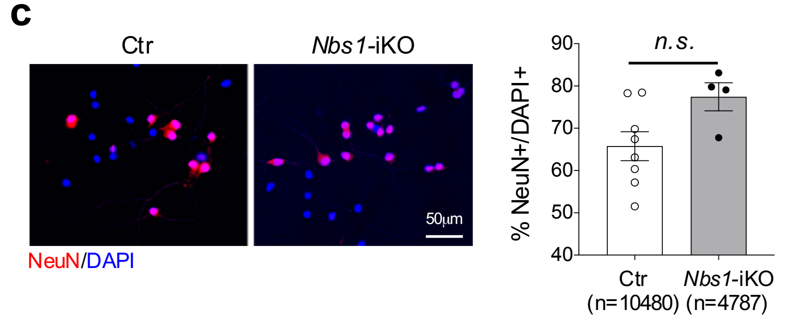
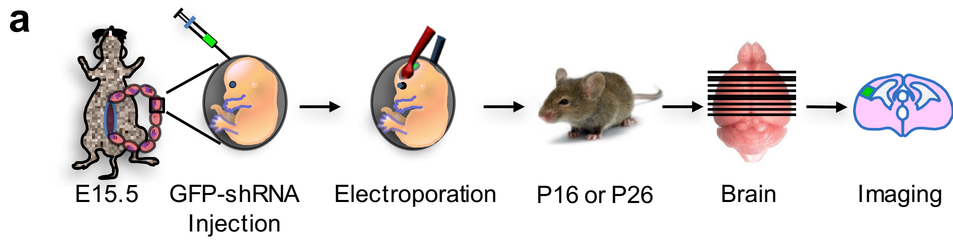


Fig. S2-Zhou et al.



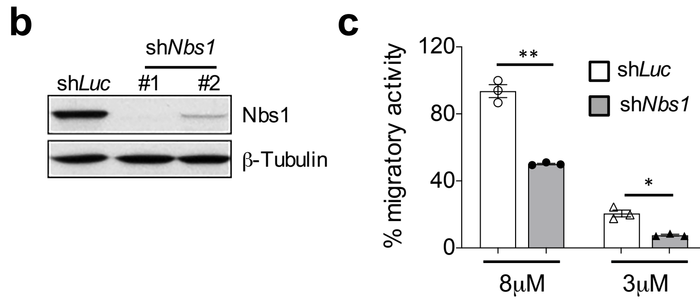


Fig. S3-Zhou et al.

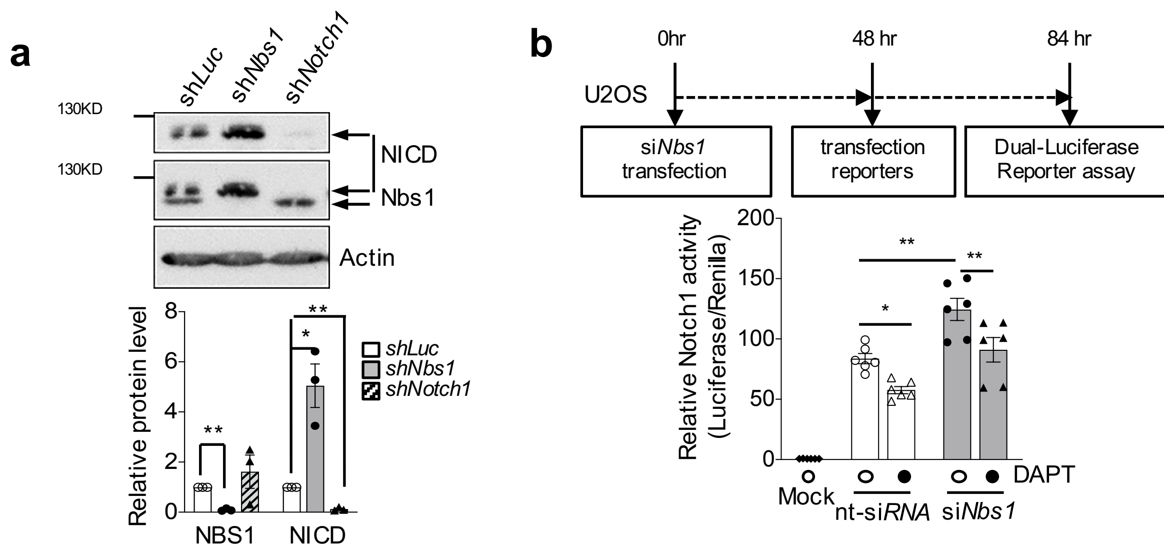
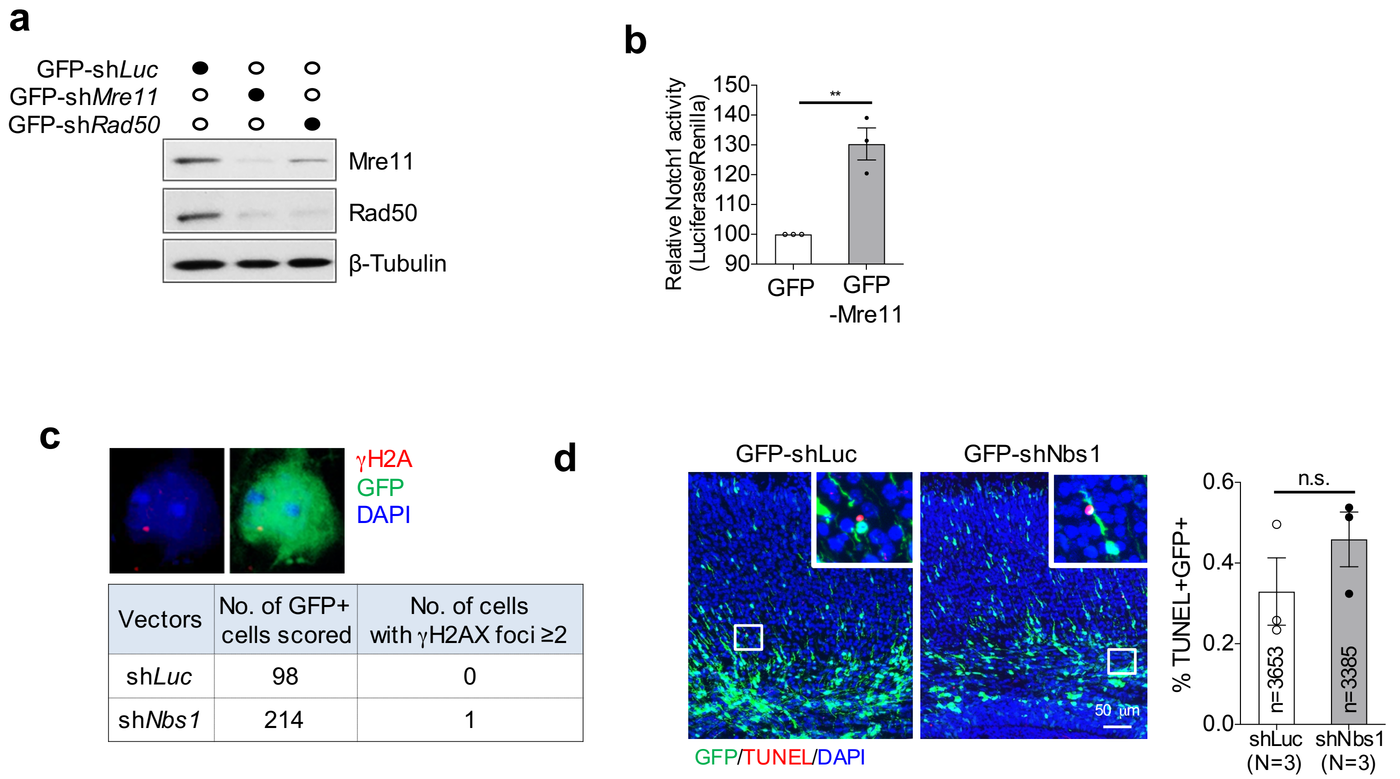


Fig. S4-Zhou et al.



Vectors	No. of GFP+ cells scored	No. of cells with γH2AX foci ≥2
sh*Luc*	98	0
sh*Nbs1*	214	1

Fig. S5-Zhou et al.

SUPPLEMENTARY FIGURES

Figure S1. Expression of *Nbs1* during neural development and in postmitotic cells.

(a) RNA-seq analysis of expression of MRN and other DDR molecules in the brain cortex from wildtype E14.5 embryos and 10-month old adult animals. The Mean ± SEM of reads per million reads (RMPs) of each gene is shown from the indicated number (N) of animals. Two-way ANOVA followed by uncorrected Fisher's LSD test was used for statistical analysis. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; n.s.: not significant. (b) Semi-quantitative RT-PCR analysis of *Nbs1* expression in the newborn mouse cortex, adult cerebrum (3 months).

Figure S2. Inducible deletion of *Nbs1* in differentiating neurons.

(a) Scheme of isolation and generation of inducible *Nbs1*-deletion (*Nbs1*-iKO) of neurons and MEFs from $Nbs1^{f/f}$-CreER^{T2} (*Nbs1*-CER) mice. Cultures were treated with 4-OHT for 4 days and analyzed at indicated days after drug washout (days post 4-OHT treatment, dpo). *Nbs1*-iKO: *Nbs1*-CER with 4-OHT treatment; Controls: all genotypes without 4-OHT treatment, and other *Nbs1* genotypes ($Nb1^{+/+}$ or $Nbs1^{f/+}$) with 4-OHT treatment; (b) PCR genotyping analysis of genomic DNA from cultured neurons to confirm *Nbs1* knockout. +: wild-type allele; F: flox allele; Δ: deleted allele. The genotype of each cell population is indicated on the top of the gel. (c) Neuron survival. Cells prepared from (a) were stained with the neuron marker NeuN (red) and DAPI (blue). Neurons (n) were derived from the number of animals (N). The Mean ± SEM of the percentage of NeuN+DAPI+ cells from the indicated number of DAPI+ cells is shown. An unpaired Student *t*-test was used for statistical analysis. n.s.: not significant. *: $p<0.05$.

Figure S3. The MRN complex and neuronal migration.

(a) Scheme of *in utero* electroporation (*IUE*) at embryonic stage 15.5 (E15.5) and brain samples preparation from postnatal (P) day P16 or P26 for imaging analysis. (b) The efficiency of GFP-tagged shRNA expression vectors (GFP-sh*Nbs1*#1, #2) against *Nbs1* was tested in MEF cells. Proteins were extracted from sorted GFP positive cells 36 hr after transfection and subjected to immunoblotting against Nbs1 and β-Tubulin (loading control). (c) *Transwell* migration activity of Neuro2A cells after *Nbs1* knockdown. Neuro2A cells after transfection with indicated vectors were plated onto the membrane with different pore size (diameters of 8μm and 3μm) of the *transwell* after transfection. The migratory activity was analyzed 24 hr after seeding. Data are Mean ± SEM of the relative migration activity from three independent experiments. Two-way ANOVA followed by Tukey's post-hoc test was used for statistical analysis. *: $p<0.05$; **: $p<0.01$; n.s.: not significant.

Figure S4. *Nbs1* deficiency induces Notch activity and DNA damage modulates Nbs1-NICD-RBPJ interaction.

(a) Western blot analysis of Nbs1 and NICD in GFP+ MEF cells that were sorted out 36 hr after transfection with indicated GFP-shRNA vectors, using indicated antibodies. The Notch1 antibody was used to detect NICD. Actin is a loading control. Representative images from four experiments are shown. The Mean ± SEM of the relative protein level from three independent experiments is shown. Two-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis. *: $p<0.05$; **: $p<0.01$. (b) The scheme shows the protocol of the Notch1 activity assay in U2OS cells (upper panel). U2OS were co-transfected with non-targeting siRNA

(nt-siRNA) (D-001212-02; Thermo Scientific) or si*Nbs1* (D-009641-04; Thermo Scientific), together with the luciferase reporter. 24 hr after transfection, cells were treated with 5μM DAPT for 12 hr before measuring the Notch1 activity. Mock: cells without transfection. Data are Mean ± SEM of the relative Notch activity from six independent experiments. Two-way ANOVA followed by Holm-Sidak's multiple comparison test was used for statistical analysis. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

Figure S5. Apoptosis analysis of the brain cortex after knockdown of *Nbs1*.

(a) Western blot analysis of MRN in GFP positive (GFP+) MEF cells sorted out 36 hr after transfection with GFP-tagged shRNA, with indicated antibodies. β-Tubulin is a loading control. (b) Effect of overexpression of *Mre11* on Notch activity. Notch activity reporters were transiently co-transfected with empty vector or *Mre11* expression vectors into MEF cells. Dual-luciferase assay was carried out 24 hr after transfection. The Mean ± SEM of the relative Notch activity from three independent experiments is shown. Welch's *t-test* was used for statistical analysis. **: $p<0.01$. (c) Images depict γH2AX foci in the neurons of the P16 brain cortical section after *IUE*-mediated transfection of sh*Nbs1* knockdown at E15.5. The total number of GFP+ cells in layers IV-VI containing ≥ 2 γH2Ax foci is summarized in the table (below). (d) Cortical sections from P0 animals after electroporation at E15.5 were stained with TUNEL detection. Insets are enlarged areas indicated. GFP+ and TUNEL+ cells are shown. The quantification of TUNEL+GFP+ cells among the total GFP+ cells (n) is shown (right). Data are Mean ± SEM of the percentage of TUNEL+ cells from three animals (N) for each shRNA transfection. Welch's *t-test* was used for statistical analysis. n.s.: not significant.