

Supplementary information, Figure S9

Figure S9 Generation and verification of *Nestin-Cre;Prrt2^{-/-}* **mice.** (**A**) Conditional knockout of *Prrt2* in mouse CNS under the control of *Nestin-Cre*. Exon2 encoding the main part of PRRT2 was flanked by two *loxP* sequences. The *neo* cassette was ultimately deleted from the targeted alleles by *Flp* recombinase. (**B**) Representative genotyping results of the *Nestin-Cre;Prrt2^{-/-}* mice. (**C**) mRNA expression of *Prrt2* in *Nestin-Cre;Prrt2^{-/-}* brain was detected using probes targeting different exons as

indicated. (**D**) Western blot of expression levels of PRRT2 in brains of *Nestin-Cre;Prrt2^{-/-}* mice and their control littermates. None residual PRRT2 protein was detected in *Prrt2*-knockout (KO) mouse brains. *Nestin-Cre* and *Prrt2^{loxP/loxP}* mice expressed PRRT2 normally. (**E**, **F**) No obvious changes in gross morphology and brain size of the mutant mice. Error bars, mean \pm SEM. (**G**) Nissl staining of sections from the mouse brains (left panel, coronal; middle panel, sagittal) and spinal cord (right panel) with the indicated genotypes showed that the gross anatomic structure of central nervous system was not apparently altered in *Nestin-Cre;Prrt2^{-/-}* mice. Scale bars: 600 µm, for brain; 300 µm, for spinal cord. (**H**, **I**) Impaired performance of *Nestin-Cre;Prrt2^{-/-}* mice in both beam balance test (**H**) and *Rota rod* test (**I**). Ctrl, *n* = 10; *Nestin-Cre;Prrt2^{-/-}</sup>, <i>n* = 8. *Nestin-Cre* and *Prrt2^{loxP/loxP}* littermates expressing PRRT2 normally were pooled as controls (Ctrl). Error bars, mean \pm SEM. **P* < 0.05 and ***P* < 0.01, versus Ctrl; two-way ANOVA test.