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Supplemental information

Arhgef2 regulates neural differentiation in the cerebral cortex through mRNA m^6A-methylation of Npdc1 and Cend1

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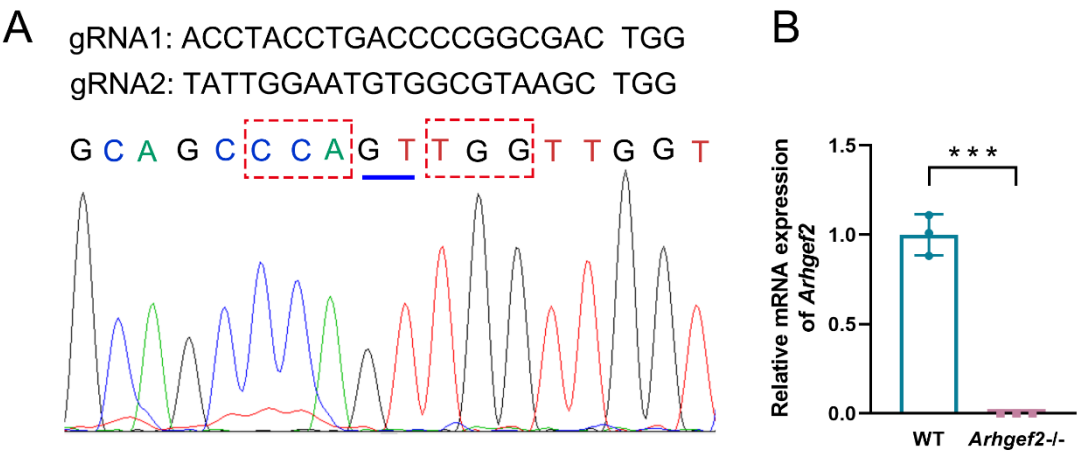

Figure S1. CRISPR/Cas9-meidated Arhgef2 knockout in mice. Related to Figure1. **(A)** Sanger-sequencing of genome editing locus in Arhgef2-/- mice (postnatal 6 weeks). The gRNA target site and PAM sequencing were labeled by blue and red, respectively. The most frequent editing event was deletion of 581 bp. **(B)** RT-qPCR quantification of *Arhgef2* mRNA level in wildtype (WT) and Arhgef2-/- mice. ACTB is used as internal control. The data are shown as mean ± S.D. n = 3, *** p < 0.001, unpaired t-test.

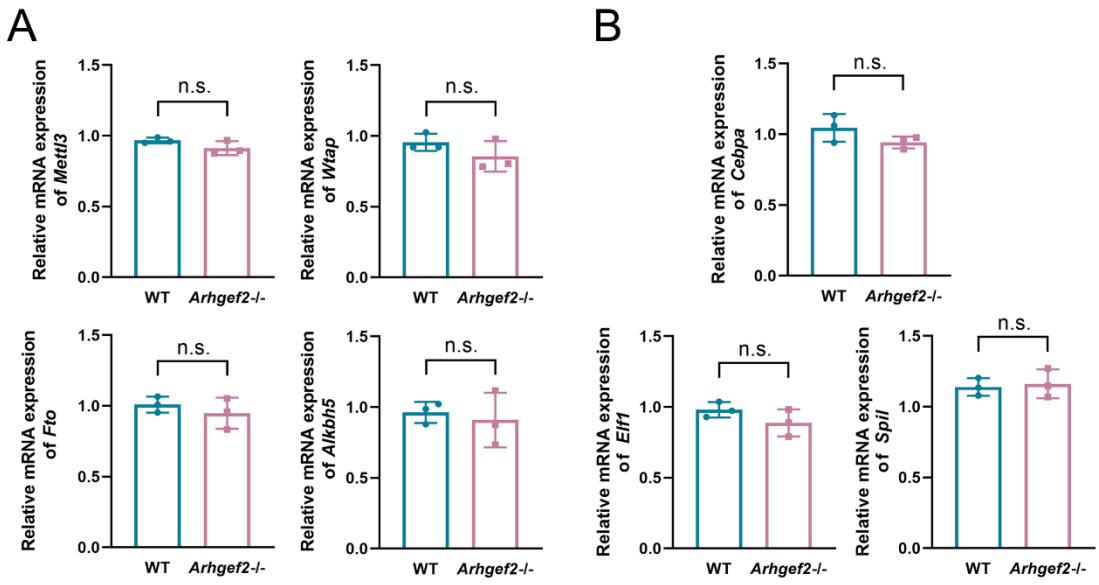

Figure S2. **RT-qPCR quantification of methylases, demethylases and transcription factors of Mettl14. Related to Figure 3. (A)** RT-qPCR quantification of methylases (*Mettl3*, *Wtap*) and demethylases (*Fto*, *Alkbh5)*. *Actb* is used as internal control. **(B)** RT-qPCR quantification of transcription factors of *Mettl14*. The data are shown as mean ± S.D. n = 3, n.s.: no significant difference, unpaired t-test.

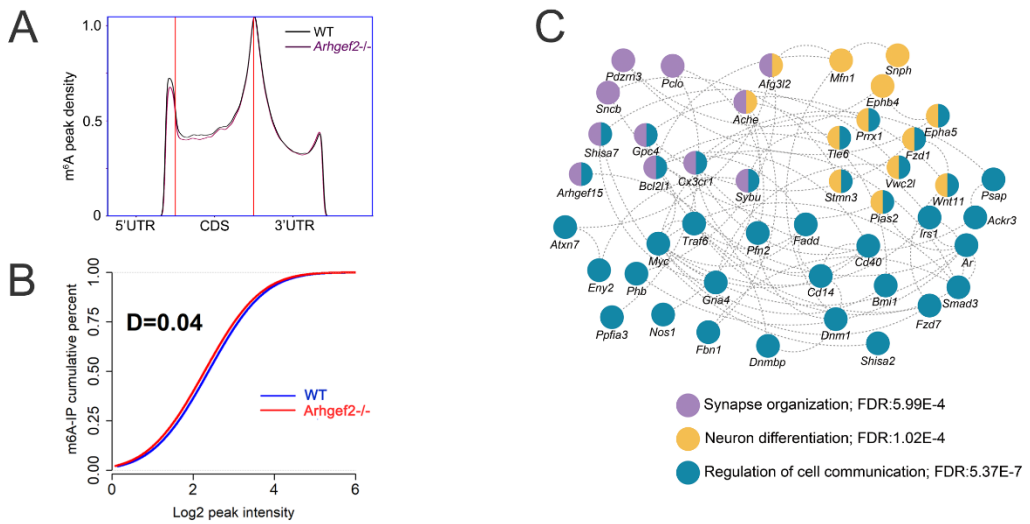

Figure S3. m^{6}A-seq analysis. Related to Figure 3. (A) Density distribution of m^{6}A peaks across mRNA transcripts. Regions of the 5′ untranslated region (5′UTR), coding region (CDS), and 3′ untranslated region (3′UTR) were split into 100 segments, and percentages of m^{6}A peaks that fall within each segment were determined. **(B)** Cumulative distribution of $\log_2$ (fold enrichment) for m^{6}A-tagged genes in wild-type and *Arhgef2-/-* mice. D represents the value of Kolmogorov-Smirnov test statistic corresponding to the maximum difference between wild-type and Arhgef2-/- mice. **(C)** Protein-protein interaction network of m^{6}A-tagged genes in GO enrichment related to cell communication, neuron differentiation and synapse organization. FDR represents false discovery rate.

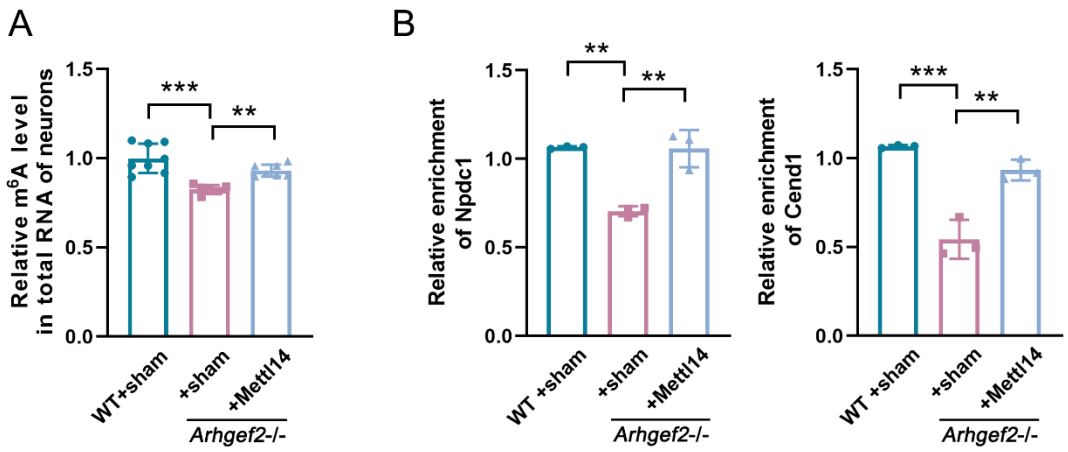

Figure S4. Rescue effect of Mettl14. Related to Figure 5. (A) Total m^{6}A level of primary neurons was significantly increased with overexpression of Mettl14. n = 8, **p < 0.01, ***p < 0.001, One-way ANOVA. (B) RNA immunoprecipitation (RIP) of m^{6}A-modified *Npdc1* and *Cend1* mRNA, followed by quantification using qRT-PCR. n = 3, **p < 0.01, ***p < 0.001, One-way ANOVA.

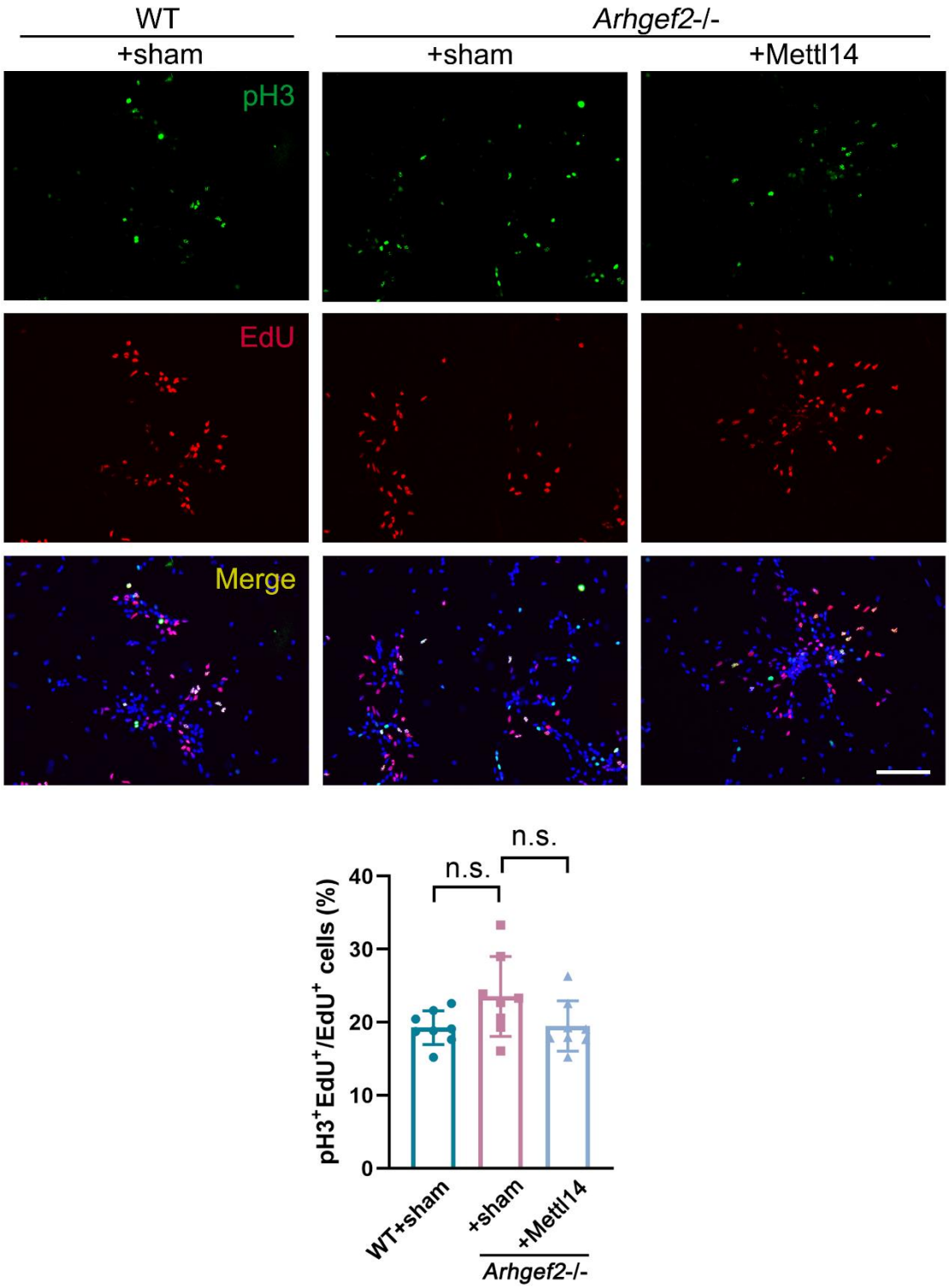

Figure S5. Immunostaining of phospho-histone H3 (pH3) in primary neurons exposed to EdU for 6 h. Related to Figure 5. pH3 (marker for cells entered M phase, green); EdU (incorporate into new DNA synthesis, red). Bar = 100 μm. n = 4 (2 measurement for each group), n.s.: no significant difference, One-way ANOVA.

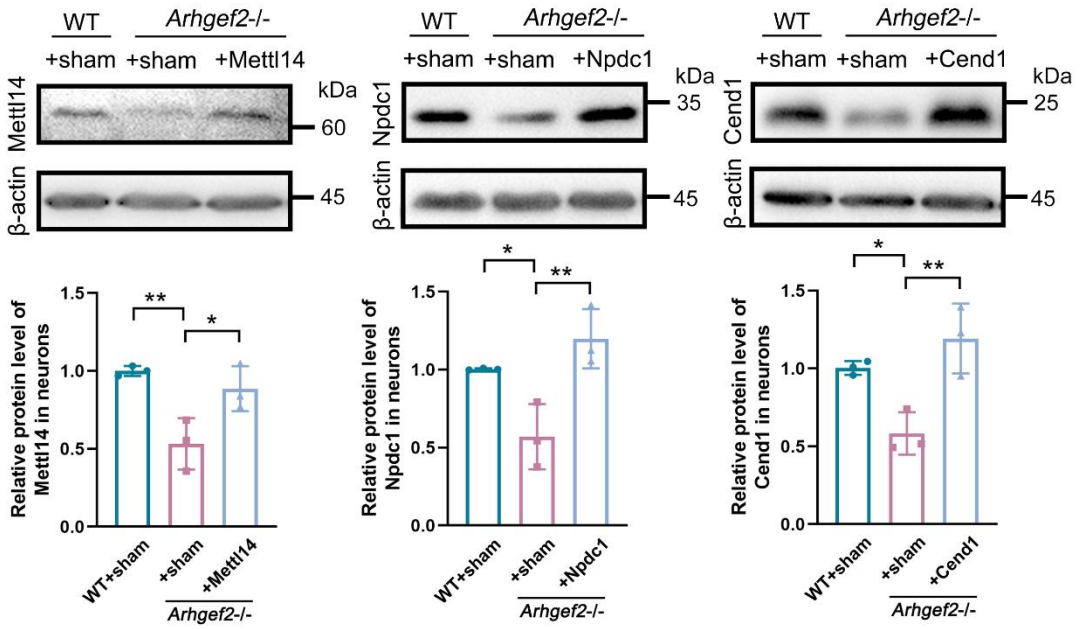

Figure S6. Protein level of Mettl14, Npdc1 and Cend1. Related to Figure 5, 6, 7. Protein expression of Mettl14, Npdc1 and Cend1 after overexpression in primary cells, respectively. n = 3, *p < 0.05, **p < 0.01, One-way ANOVA.

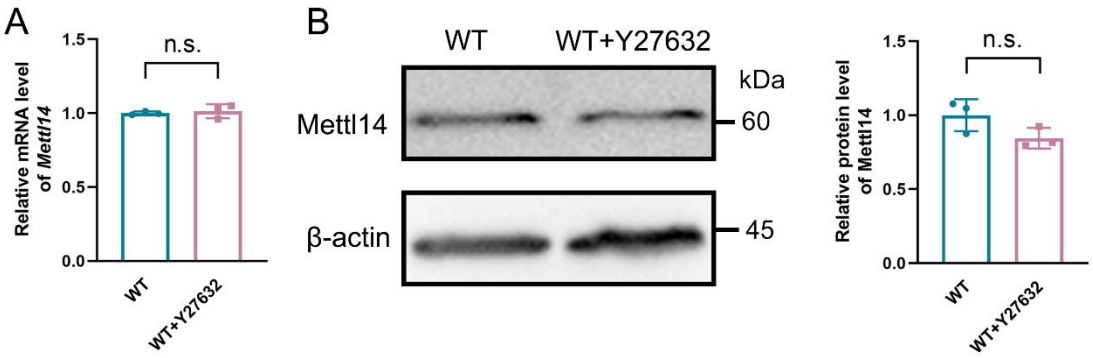

Figure S7. The mRNA and protein expression of Mettl14 with treatment of Rock inhibitor (Y27632, 5 μM) in wild-type primary cells. Related to Figure3. (A) RT-qPCR quantification of *Mettl14* mRNA. n = 3, unpaired t-test. (B) Protein level of Mettl14 was detected by western blot. n = 3, n.s.: no significant difference, unpaired t-test.