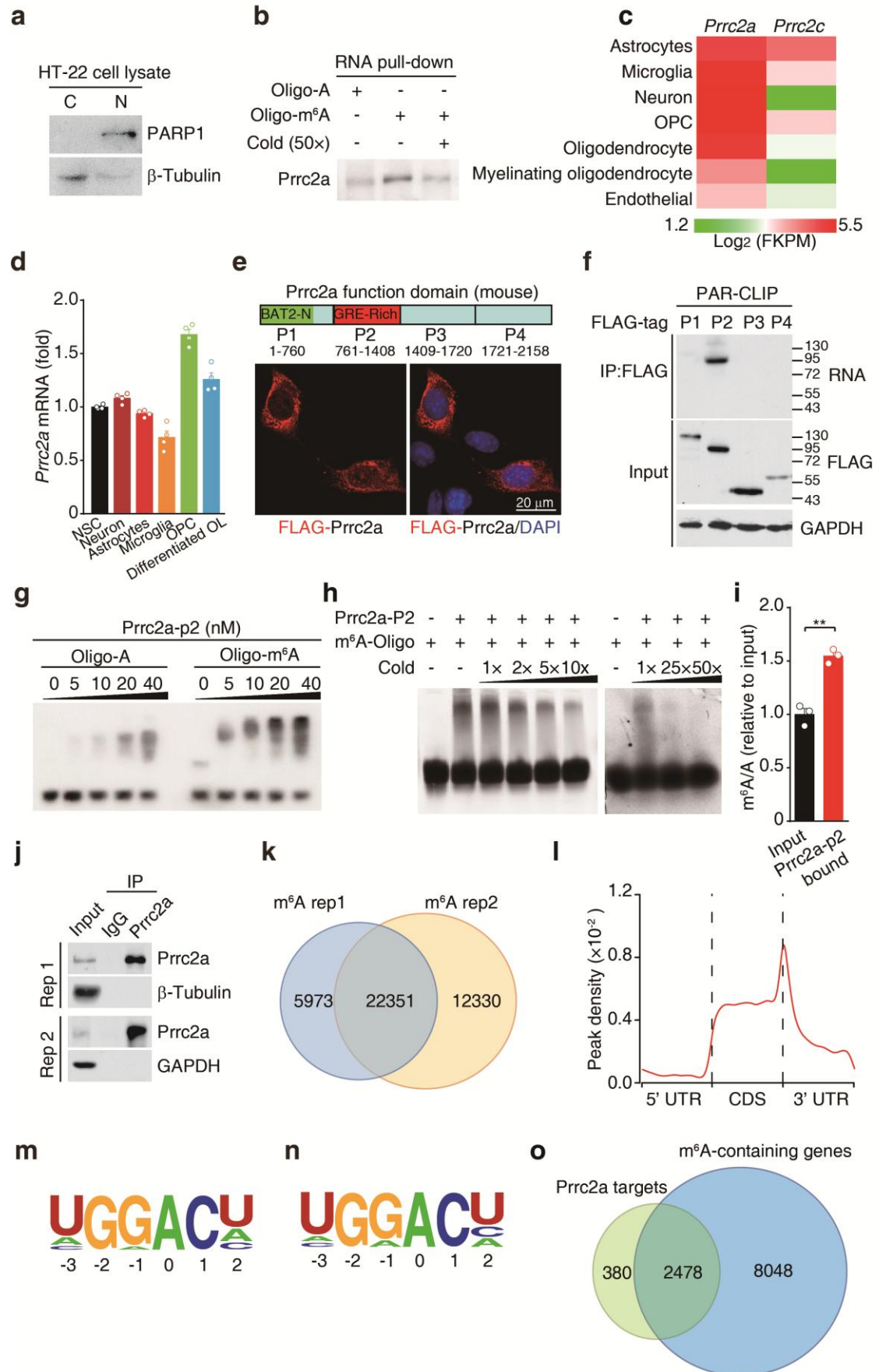


Figure S1



Supplementary Figure 1, related to Figure 1. Prrc2a is an m⁶A reader.

(a) The nuclear and cytoplasmic fractions of HT-22 cells were isolated and analyzed by western blotting using PARP1 and β -Tubulin as nuclear and cytoplasmic markers, respectively. The cytoplasmic fractions were used to identify the m⁶A binding proteins (refer to Fig.1a).

(b) Western blotting showing Prrc2a pulled down with an m⁶A-containing RNA probe, a 50 folded (50 $\times$) cold m⁶A competitor were used.

(c) The expression of Prrc2a and Prrc2c in different types of neural cell from Brain-seq Database (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html).

(d) RT-qPCR analyzed Prrc2a expression in different types of cultured neural cell.

(e) Upper panel shows schematic drawing of Prrc2a protein with predicted domains and deletion mutants and bottom panel shows immunofluorescence staining of FLAG-tagged full length Prrc2a location (red color).

(f) Mapping the RNA binding domains of Prrc2a. PAR-CLIP assay of RNA pulled down by Flag-tagged p1, p2, p3 and p4. RNA labeled with biotin at 3' end was visualized by the chemiluminescent nucleic acid detection module.

(g) Gel-shift assay measuring the dissociation constant (K_d , nM) of His₆-Prrc2a-p2-FLAG protein with methylated and unmethylated RNA probes. 300 nmol RNA probe was labelled with biotin and the protein concentration ranged from 0 nM to 40 nM. The dissociation constant $K_d(\text{Oligo-A})=22.8\pm2.3$ and $K_d(\text{Oligo-m}^6\text{A})=6.1\pm1.2$.

(h) Labeled RNA probe and Prrc2a-p2 protein were subjected to gel-shift assay, 1 to 50 fold cold m⁶A were used as a competitor.

24 (i) LC-MS/MS showing m⁶A enrichment in His₆-Prrc2a-p2-FLAG bound mRNA portion
25 (two-tailed unpaired student's *t*-test, ***P*<0.01, n=3, technical replicates).

26 (j) Western blotting detected Prrc2a immunoprecipitation efficiency of Prrc2a RIP assay in
27 whole brain tissue at P28.

28 (k) Overlap of two biological replicates for the m⁶A-seq data. Numbers are total m⁶A peaks
29 identified in each sample from whole brain at P28.

30 (l) Distribution of overlapped m⁶A peaks between two biological replicates across the length
31 of mRNA. Each region of 5' UTR, CDS, and 3' UTR were binned into 100 segments, and the
32 percentage of m⁶A-containing Prrc2a peaks that fall within each bin were determined.

33 (m) Enriched motif identified by HOMER with overlapped m⁶A peaks between two
34 biological replicates (*p* = 1e-200).

35 (n) Binding motif identified by HOMER with Prrc2a peaks overlapped with m⁶A peaks (*p* =
36 1e-51).

37 (o) Overlap of Prrc2a binding targets and m⁶A-containing genes.
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