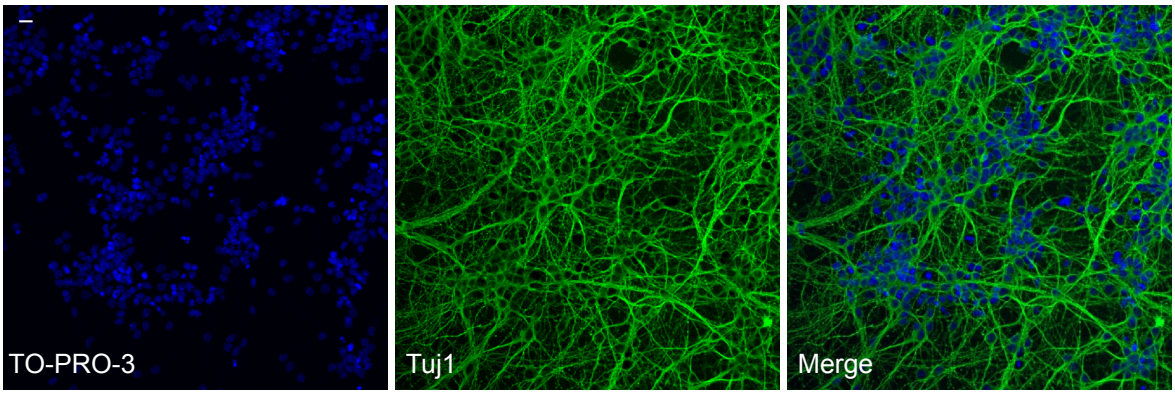
Psd-95 is post-transcriptionally repressed during early neural development by PTBP1 and PTBP2.

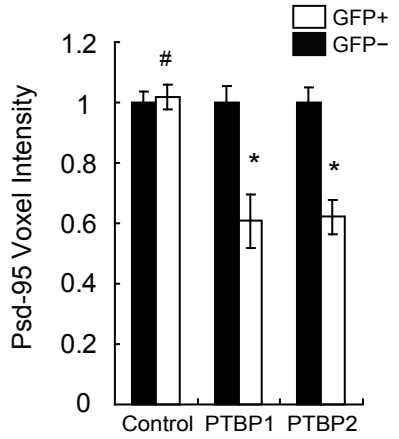
Sika Zheng, Erin E. Gray, Geetanjali Chawla, Bo Torben Porse, Thomas J. O'Dell, Douglas L. Black

Supplementary Figure 1



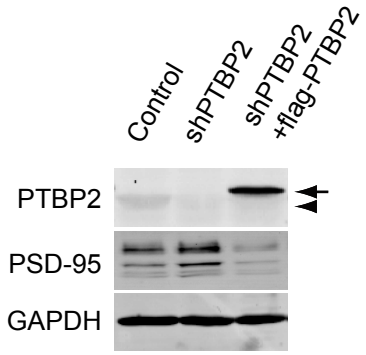
Supplementary Figure 1. Tuj1 (green) was expressed in most cells of the primary cortical cultures at 4 DIV. The cells were nuclear counterstained with TO-PRO-3. Scale bar: 10 μm.

Supplementary Figure 2



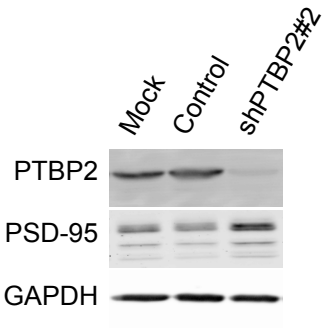
Supplementary Figure 2. PTBP1 decrease PSD-95 voxel intensity. The PSD-95 immunofluorescence signal in the soma of GFP+ or GFP− neurons was quantified for both PTBP1-transfected and pcDNA (control)-transfected primary cortical cultures. Total PSD-95 fluorescence intensity was normalized with cell volume to derive PSD-95 voxel intensity. The bar plot represents mean values±s.e.m. (N=23). * indicates $p=6.6x10^{-11}$. # indicates p=0.94. The p-values were determined by two-tailed pair-wise t-test comparing GFP+ and GFP− cells.

Supplementary Figure-3 (Black)



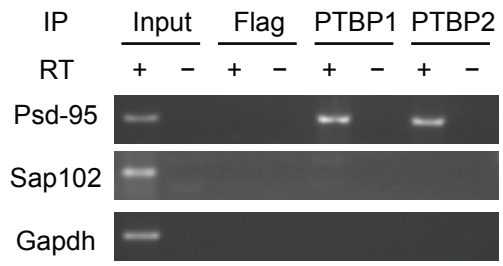
Supplementary Figure 3. Lentivirus expressing RNAi-resistant flag-PTBP2 reversed the effect of PTBP2 shRNA on PSD-95 expression in primary cortical neurons. The arrow points to the exogenous flag-PTBP2 and the arrowhead points to the endogenous PTBP2.

Supplementary Figure-4 (Black)



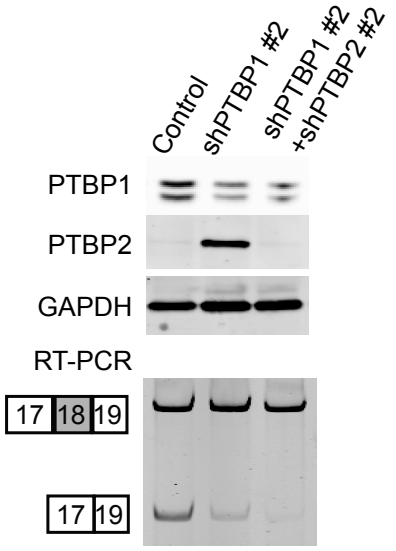
Supplementary Figure 4. A second PTBP2 shRNA increased PSD-95 expression in primary cortical neurons via lentivirus transduction.

Supplementary Figure-5 (Black)



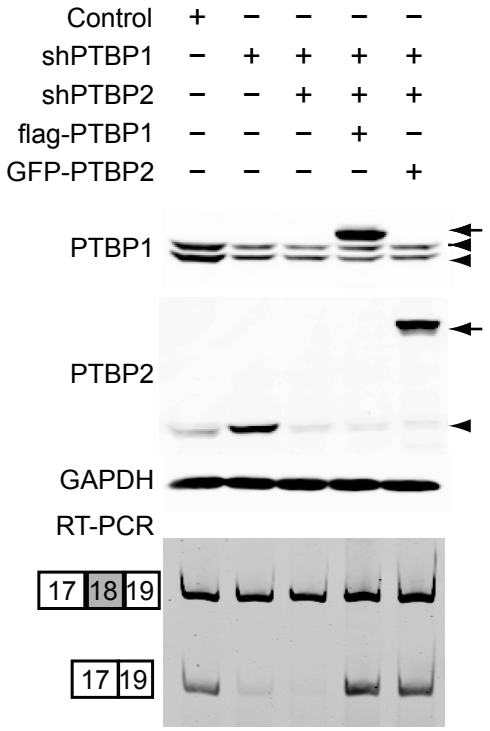
Supplementary Figure 5. Crosslinking and immunoprecipitation (CLIP) analysis of the PTB proteins binding to Psd-95 pre-mRNA in mouse embryonic (E16) brains. CLIP RNA was reversed transcribed (RT+) followed by PCR with primers detecting Psd-95 Element A, or Sap102 or Gapdh transcripts. The same RNA without reverse transcription (RT−) was used as controls. 5% of the input was loaded.

Supplementary Figure-6 (Black)



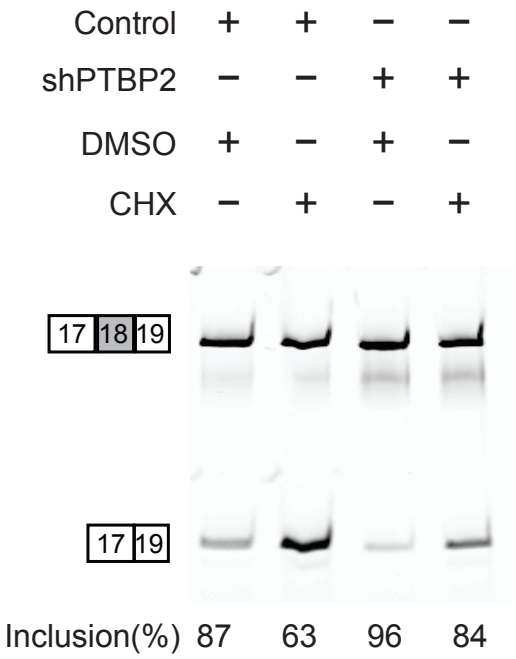
Supplementary Figure 6. A second set of shRNAs targeting PTBP1 and PTBP2 enhanced Psd-95 Exon 18 splicing.

Supplementary Figure-7 (Black)



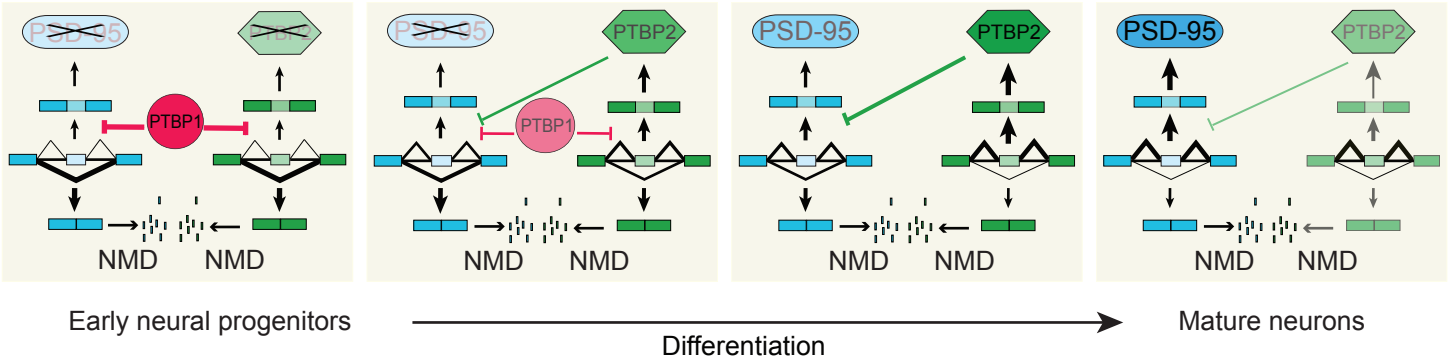
Supplementary Figure 7. The increases in Psd-95 Exon 18 splicing after knockdown of PTBP1 and PTBP2 were abolished by simultaneous overexpression of either flag-PTBP1 or GFP-PTBP2. The arrow points to the exogenous flag-PTBP1 or GFP-PTBP2 and the arrowhead points to the endogenous PTBP1 or PTBP2.

Supplementary Figure-8 (Black)



Supplementary Figure 8. Alternative splicing and nonsense-mediated mRNA decay regulation of Psd-95 mRNA in mouse primary cortical neurons. Neurons were infected with lentiviruses at DIV 0 and assayed at DIV 4. ΔExon 18 isoform was stabilized by cycloheximide (CHX) treatment (lane 2 vs lane 1). Knockdown of PTBP2 increased Exon 18 splicing (lane 3 vs lane 1) and thus produced less ΔExon 18 isoform to be degraded by the NMD pathway (lane 4 vs lane 2).

Supplementary figure-9 (Black)



Supplementary Figure 9. Psd-95 is post-transcriptional repressed by PTBP1, PTBP2 and nonsense mediated decay (NMD) during early neural development. PTBP1, PTBP2 and PSD-95 proteins are drawn in red circles, green hexagons and blue ovals. In early neural stem cells PTBP1 is expressed at a high level and represses inclusion of Psd-95 Exon 18 and PTBP2 Exon 10. These exon-skipped isoforms are subject to NMD. As cells differentiate, PTBP1 protein expression is repressed leading to expression of PTBP2 protein. PTBP2 maintains the repression of Psd-95 splicing. When PTBP2 expression is reduced in mature neurons, Psd-95 exon 18 splicing is further induced. Bar-headed solid lines mean direct repression. The size of an arrow indicates its magnitude.