## **Supporting Information**

## Probing enzyme-dependent pseudouridylation using direct RNA sequencing to assess neuronal transcriptome plasticity

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**Supplementary Figure 1.** a. Representative photomicrographs of a. TRUB1 knockdown (KD) and b. PUS7 KD vs Scrambled (control) cells show a substantial decrease in fluorescence in the KD library. c. Immunofluorescence (IF) of TRUB1 KD, PUS7 knockdown KD, and siRNA control show a decreased mean fluorescence intensity (MFI) in the KD cells compared to the control.



**Supplementary Figure 2.** Sequence identity matrix showing the sequence identity between each PUS enzyme nucleotide sequence in the multiple sequence alignment data (MSA).



**Supplementary Figure 3.** Differential mRNA expression of known differentiation markers supports a change in the SH-SY5Y cellular state after the retinoic acid-induced differentiation treatment.



**Supplementary Figure 4.** IF images of untreated and differentiated SH-SY5Y cells using a. PUS7 and b. TRUB1 antibodies. (right) Comparison of PUS7 and TRUB1 antibodies' fluorescence intensity in the nucleus and cytoplasm of untreated and differentiated SH-SY5Y cells.



**Supplementary Figure 5.** IF images of untreated and Lead exposed SH-SY5Y cells using PUS7 and TRUB1 antibodies. (right) Comparison of PUS7 and TRUB1 antibodies' fluorescence intensity in the nucleus and cytoplasm of untreated and lead treated SH-SY5Y cells.