Supplementary Information

Synergistic regulation of Rgs4 mRNA by HuR and miR-26/RISC in neurons

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Supplementary Figure 1. Increase of Rgs4 mRNA upon HuR knock-down is HuR specific. (A) Quantification of HuB/C/D western blot signal from 14 DIV cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shHuR, normalized to shNTC. Paired Student’s t-test. (B) Quantification of endogenous HuR mRNA by qRT-PCR from 14 DIV cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shHuR, normalized to shNTC. Paired Student’s t-test. (C) Quantification of endogenous Rgs4 mRNA by qRT-PCR from 14 DIV cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shPum2, normalized to shNTC. Paired Student’s t-test. (D) Quantification of endogenous Pum2 mRNA by qRT-PCR from 14 DIV cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shPum2, normalized to shNTC. Paired Student’s t-test. (E) Analysis of HuR mRNA stability in 14 DIV cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shHuR and treated with DMSO or ActD for 90 min at 14 DIV. HuR mRNA levels were quantified by qRT-PCR and normalized to DMSO+shNTC. Paired Student’s t-test. (F) RT-PCR with cDNA from 14 DIV rat cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shHuR using primers detecting both annotated mmuRgs4 splice isoforms (Ensemble, mm10). All error bars are SEM from ≥ 3 independent biological replicates; asterisks represent p-values (∗p < 0.05). KD knock-down; NTC non-targeting control; ActD Actinomycin D; bp base pair; DIV days in vitro.
Supplementary Figure 2. HuB/C/D protein is less enriched in Rgs4 in vitro RNA purification. (A,B) Representative western blot against HuR (HuB/C/D) and Ago2 (A) and quantification (B) of HuB/C/D enrichment from adult rat cortex lysate in in vitro RNA affinity purification using 2xMS2 only, 2xMS2+Rgs4 CDS, 2xMS2+Rgs4 3’-UTR, and different 2xMS2+Rgs4 3’-UTR fragments as bait RNA, normalized to input. All error bars are SEM from ≥ 3 independent biological replicates. WT wild type.
Supplementary Figure 3. *HuR* mRNA is not affected by miR-26 overexpression or Ago2 KD. (A) qRT-PCR analysis of endogenous *HuR* mRNA in 14 DIV cortical neurons transduced at 11+3 DIV with lentiviruses expressing miR-scr or miR-26a, normalized to miR-scr. (B) qRT-PCR analysis of endogenous *HuR* mRNA in 14 DIV cortical neurons transduced at 9+5 DIV with lentiviruses expressing shNTC or shAgo2, normalized to shNTC. Paired Student’s *t*-test. All error bars are SEM from ≥3 independent biological replicates. KD knock-down; Scr scrambled; NTC non-targeting control; DIV *days in vitro*. 
Supplementary Figure 4. HuB/C/D binding in Rgs4 in vitro RNA purification is not affected by ARE6 or miR-26 mutations. (A) Minimal free energies of predicted folding (RNAfold) of the conserved region of WT Rgs4-3’-UTR and ARE6 and miR-26 binding site mutants. (B) Minimal free energy of bound miR-26a to Rgs4 conserved region calculated by IntaRNA for the Rgs4 WT conserved region, and ARE6 and miR-26 binding site mutants. (C) Quantification of HuB/C/D enrichment from adult rat cortex lysate in in vitro RNA affinity purification using 2xMS2 only, 2xMS2+Rgs4 3’-UTR WT, 2xMS2+Rgs4 3’-UTR ARE6 mut and 2xMS2+Rgs4 3’-UTR miR-26 mut as bait RNA, normalized to input. Paired Student’s t-test. (D) Quantification of eGFP fluorescence intensity in the cell body of hippocampal neurons at 15 DIV co-transfected at 14+1 DIV with eGFP-reporter and tagRFP or tagRFP-HuR and miR-scr or miR-26a. Ratio of eGFP reporter intensity between Rgs4 3’-UTR WT and Ctrl reporter is shown. Paired Student’s t-test. All error bars are SEM from ≥ 3 independent biological replicates; asterisks represent p-values (*p < 0.05). WT wild type; ARE AU-rich element; DIV days in vitro.
Supplementary Figure 5. Mutation of miR-26 and HuR binding sites does not change dendritic Rgs4 RNA distribution. (A) Density plot of the total number of MS2 particles over distance from cell body from hippocampal neurons transfected with tdMCP-GFP and 128xMS2+Ctrl, 128xMS2+Rgs4 3'-UTR WT, ARE6 mut or miR-26 mut reporter mRNA at 14+1 DIV. ARE AU-rich element; WT wild type; tdMCP-GFP tandem MS2 coat protein fused to GFP; DIV days in vitro.