Supplemental Figures

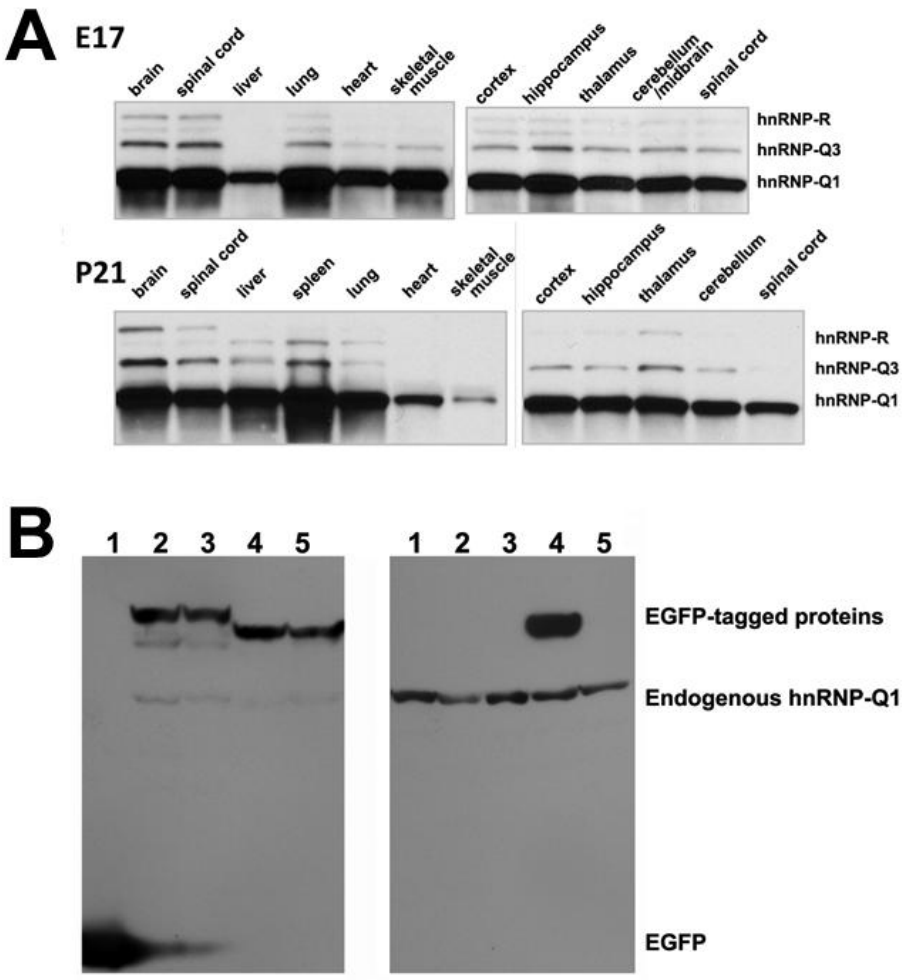

Figure S1. Expression of hnRNP-Q1 and Q3 in embryonic and postnatal tissues using a pan-hnRNP-Q antibody, and characterization of an hnRNP-Q1-specific antibody. (A) Western blot analysis of hnRNP-Q isoforms and hnRNP-R (an hnRNP-Q homolog) using a pan antibody in various tissues from E17 and P21 mouse tissues. Total protein lysates (10ug) were analyzed by western blot with antibodies recognizing all hnRNP-Q isoforms and hnRNP-R. hnRNP-Q1 was the major species in all tissues and at both ages examined. hnRNP-Q1 is ubiquitously expressed and abundant in several tissues, although relatively lower levels in heart and skeletal muscles at P21 mice. **(B)** hnRNP-Q1-specific antibodies detect only hnRNP-Q1, both endogenous and over-expressed, and do not detect hnRNP-Q3 or hnRNP-R. EGFP-tagged hnRNP-R, hnRNP-Q3, hnRNP-Q1 and hnRNP-Q1 with deletion of the C-terminus were expressed in 293 cells. The hnRNP-Q1-specific antibody is specific to the C-terminal sequence of hnRNP-Q1. Total protein extracted from transfected cells were resolved by SDS-PAGE and probed for western blot with mouse anti-GFP (left panel) or rabbit anti-hnRNP-Q1 (right panel) antibodies. Western blots with anti-GFP (left panel) or anti-hnRNP-Q1 (right panel) are shown for EGFP (lane1), EGFP-hnRNP-R (lane2), EGFP-hnRNP-Q3 (lane3), EGFP-hnRNP-Q1 (lane4) and EGFP-hnRNP-Q1ΔC (lane5, hnRNP-Q1 with C-terminal deletion).

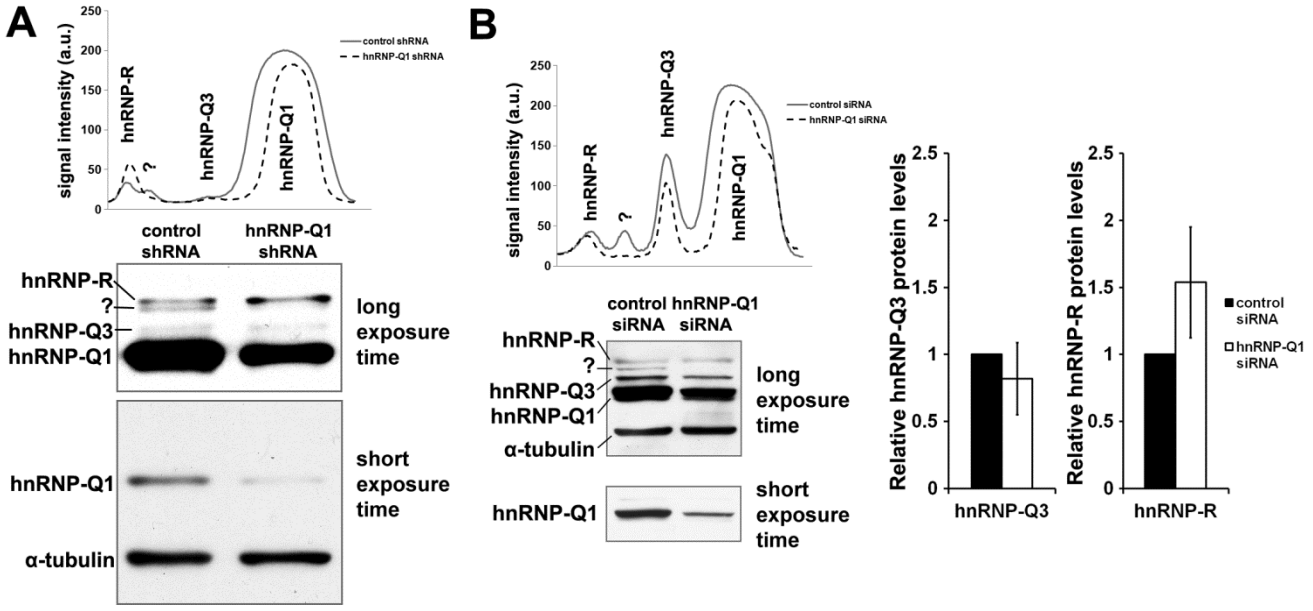

Figure S2. hnRNP-Q1 shRNA and siRNA specifically target hnRNP-Q1 but not hnRNP-Q3 and hnRNP-R in neurons and C2C12 cells. (A) Western blot analysis of endogenous hnRNP-Q/R expression in hippocampal neurons transduced with lentivirus expressing control or hnRNP-Q1 shRNA. hnRNP-Q isoforms and hnRNP-R were detected using pan-hnRNP-Q/R antibodies. A clear reduction of hnRNP-Q1 intensity can be observed with shorter exposure time **(A, bottom panel)**. The corresponding bands for the less abundant isoform hnRNP-Q3 and hnRNP-R could only be seen with extended exposure time as shown **(A, middle panel)**. Intensity profiling of this blot (long exposure time) **(A, upper panel)** revealed that only the intensity of hnRNP-Q1, but not hnRNP-Q3 or hnRNP-R, was greatly reduced by hnRNP-Q1 shRNA. **(B)** Western blot analysis of hnRNP-Qs/R proteins using pan-hnRNP-Qs/R antibodies on C2C12 cells transfected with control or hnRNP-Q1 siRNA. A clear reduction of hnRNP-Q1 intensity can be observed with shorter exposure time **(B, bottom panel)**. Quantitative analysis of protein levels of hnRNP-Q1 revealed a 90% reduction of hnRNP-Q1 (quantification of short exposure gel was shown in **Figure 2A**), yet no significant changes of hnRNP-Q3 and hnRNP-R between control and hnRNP-Q1 siRNA transfected cells were detected at longer exposure **(B, middle panel)**; three independent experiments. Intensity profiling of the blot obtained with longer exposure time revealed no evident changes on the intensities of hnRNP-R and hnRNP-Q3 **(B, upper panel)**. Of note, knockdown of hnRNP-Q1 in both neurons **(A)** and C2C12 cells **(B)** show knockdown of a distinct species (indicated as ?) that is even less abundant than hnRNP-Q3 in C2C12 cells, which has not been previously reported, and may represent a modified form of hnRNP-Q1 or an alternatively spliced form.

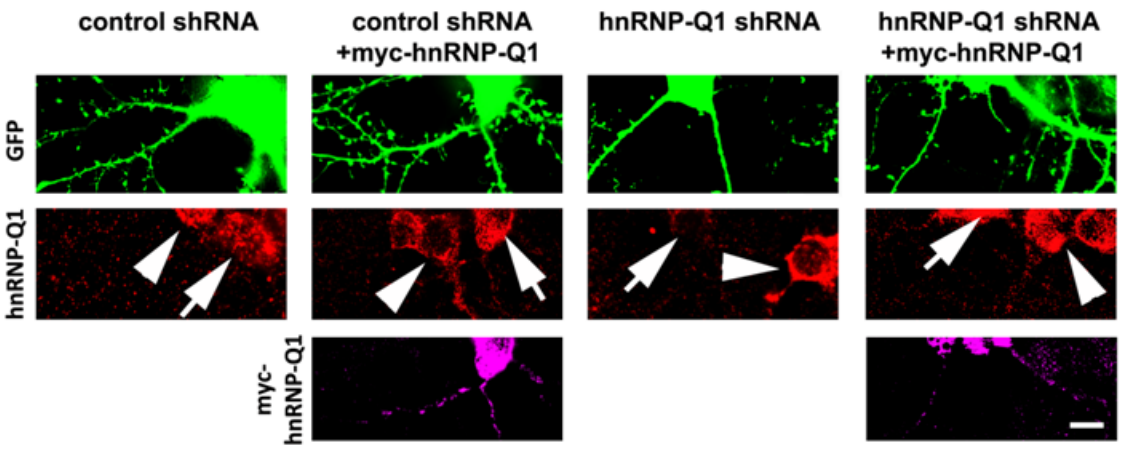

Figure S3. Single cell analysis of hnRNP-Q1 knockdown, overexpression and rescue in hippocampal neurons (associated with Figure 1C). Representative images showing hnRNP-Q1 knockdown and overexpression following neuronal transfection with plasmids encoding control or hnRNP-Q1 shRNA, GFP-Lifeact and myc-hnRNP-Q1. hnRNP-Q1 and myc-hnRNP-Q1 were detected by immunofluorescence staining with specific antibodies against hnRNP-Q1 or myc-tag, respectively. Transfected neurons (GFP-positive) are indicated by arrows and adjacent non-transfected neurons are indicated by arrowheads. GFP-positive neurons co-transfected with hnRNP-Q1 shRNA expressing plasmid show lower hnRNP-Q1 signals compared to adjacent GFP-negative cells. hnRNP-Q1 fluorescent signals in cells expressing hnRNP-Q1 shRNA can be restored by overexpression of myc-hnRNP-Q1 that is insensitive to shRNA. Scale bar, 10μm.

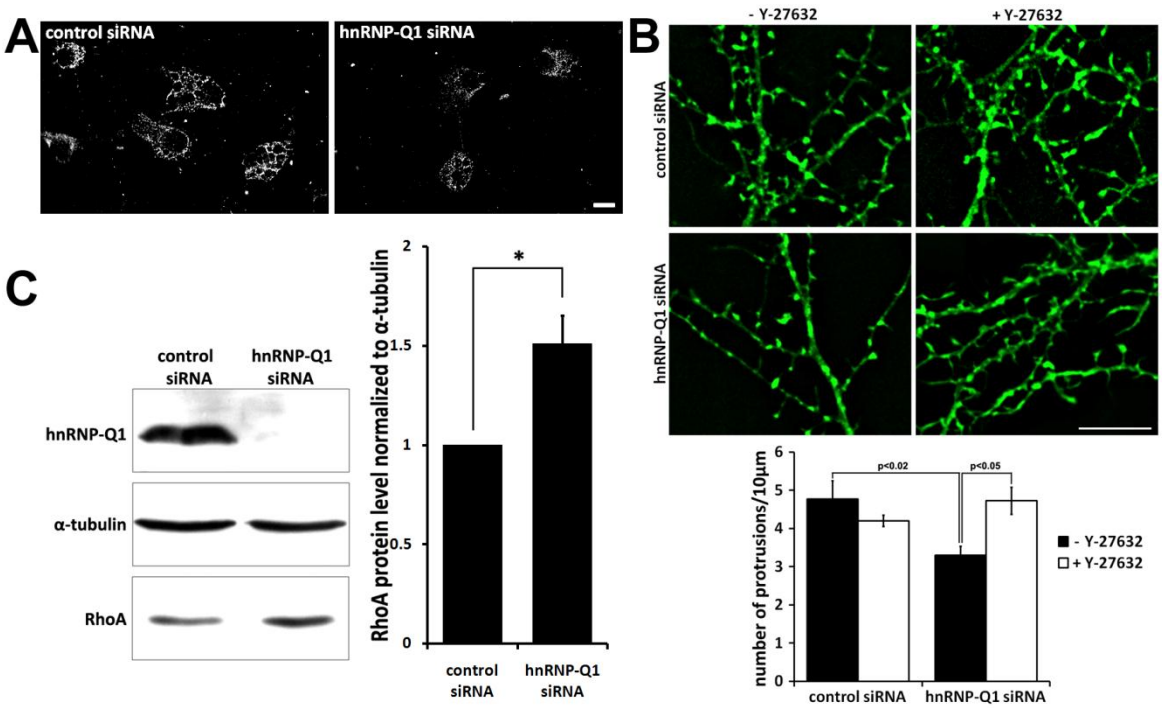

Figure S4. hnRNP-Q1 knockdown by siRNA reduced dendritic protrusions in hippocampal neurons and upregulated RhoA expression in fibroblasts. (A) Knockdown of hnRNP-Q1 in mature mouse hippocampal neurons by siRNA. Four days after siRNA transfection, neurons were fixed and stained with rabbit anti-hnRNP-Q1 antibodies. The majority of neurons transfected with hnRNP-Q1 siRNA exhibited reduced hnRNP-Q1 fluorescence signal compared with neurons transfected with control siRNA. Scale bar, 10μm. **(B)** hnRNP-Q1 knockdown by siRNA reduced the density of dendritic protrusions of mature mouse hippocampal neurons. To knockdown hnRNP-Q1, high density hippocampal neurons were transfected with siRNA at 9DIV and 12DIV for two rounds of siRNA delivery. In the second round of transfection, a GFP-Lifeact expressing plasmid was co-transfected to label F-actin-rich protrusions. Neurons were fixed at 13DIV. Representative images show control siRNA and hnRNP-Q1 siRNA transfected (GFP-positive) neurons, with or without treatment with a ROCK inhibitor, Y-27632. Histogram shows quantitative analysis of dendritic protrusion density of control and hnRNP-Q1 siRNA-transfected neurons with or without Y-27632 treatment. Neurons transfected with control siRNA have an average density of 4.8 protrusions/10μm (33 neurons, 3 experiments). Neurons transfected with hnRNP-Q1 siRNA exhibit reduced protrusion density, with 3.3 protrusions/10μm (28 neurons, three experiments). The reduction in protrusion density following hnRNP-Q1 knockdown was rescued by Y-27632 treatment to the level of control cells, with 4.7 protrusions/10μm (18 neurons, three experiments). The protrusion density of control neurons upon Y-27632 treatment was slightly reduced to 4.2 protrusions/10μm (33 neurons, three experiments), which was not statistically significant when compared to non-treated cells (p=0.26). Statistical analysis, one-way ANOVA followed by Tukey HSD. Scale bar, 10μm. All error bars represent standard error of the mean. **(C)** hnRNP-Q1 knockdown upregulated RhoA expression in primary mouse embryonic fibroblasts. Primary embryonic fibroblasts were harvested from E13.5 mouse embryos and passed two times before experiments. siRNA transfection was performed as for C2C12 cells (see methods section). 3 experiments; T-test, *p<0.05. Error bar represents standard error of the mean.

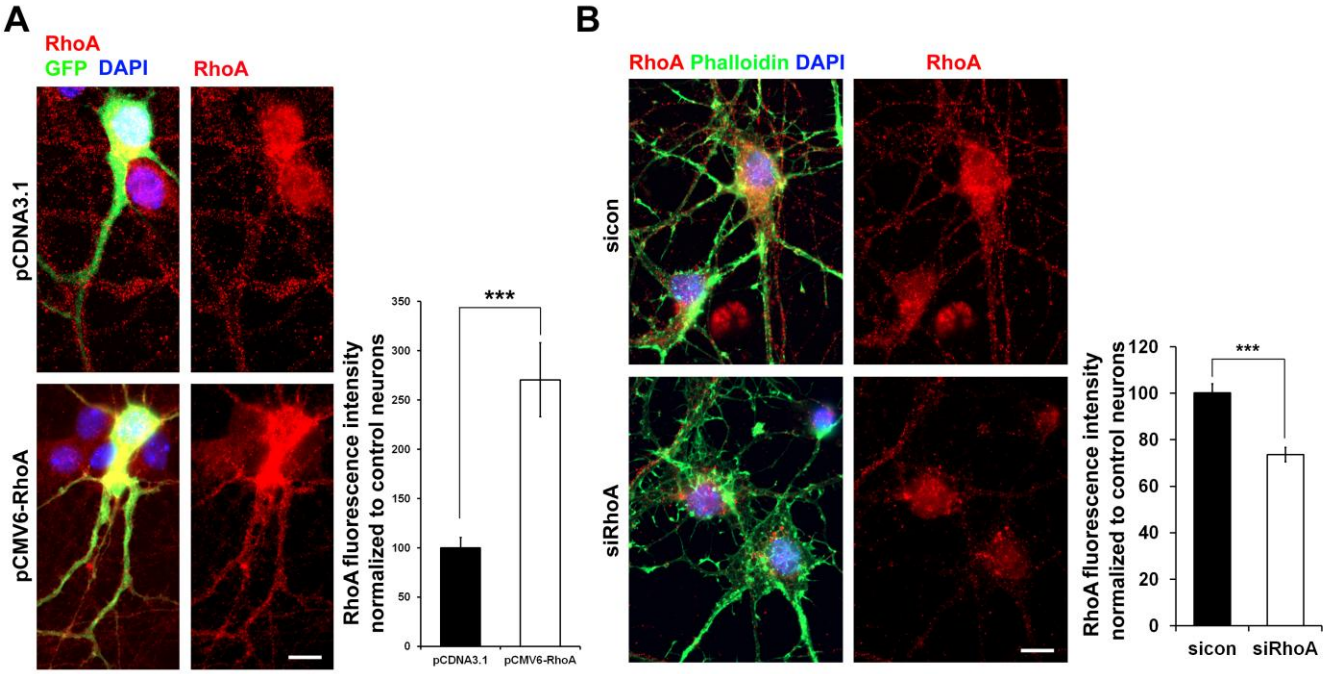

Figure S5. Specificity of RhoA antibody in neurons. **(A)** The mouse monoclonal antibody against RhoA detects overexpressed mouse RhoA protein in hippocampal neurons. Hippocampal neurons were co-transfected with a GFP control plasmid (pEGFPC1) and a plasmid encoding mouse RhoA protein (pCMV6-RhoA) or an empty vector (pCDNA3.1). Proteins were expressed (24 hours), fixed and processed for immonofluorescence (IF) staining with the antibody to RhoA. Quantitative IF analysis of RhoA signal revealed a significant increase in fluorescence intensity of neurons transfected with pCMV6-RhoA to overexpress RhoA. **(B)** Mouse hippocampal neurons transfected with siRhoA exhibited reduced RhoA fluorescence intensity as examined by IF staining with the same RhoA antibody. Hipocampal neurons transfected with siRhoA were cultured for additional 24 hours and fixed for immunofluorescence staining against RhoA. Quantitative IF analysis revealed a reduction of RhoA signal in neurons transfected with siRhoA. Scale bar, 10μm. T-test, ***p<0.001.

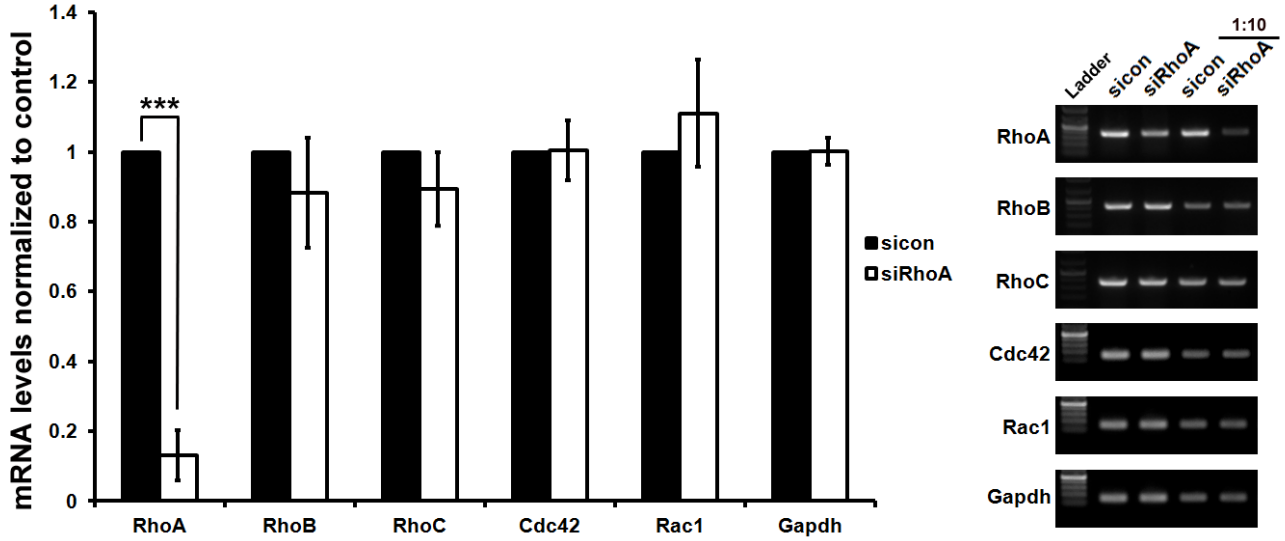

Figure S6. Specificity of siRhoA to target RhoA mRNA. C2C12 cells were transfected with siRhoA or siCon and cultured for additional 24 hours and subjected to total RNA extraction. cDNA synthesized from the same amount of total RNA and the same volume of cDNA at different dilution rates was used for semi-quantitative PCR analysis. Fluorescence intensity of each band from unsaturated PCR samples (further diluted by 1:10 as indicated in the figure) was measured using ImageJ. RhoA mRNA was efficiently depleted by siRhoA transfection; whereas RhoA homologs; RhoB, RhoC, Cdc42 and Rac1 were not affected. n=3; T-test, ***$p<0.001$.

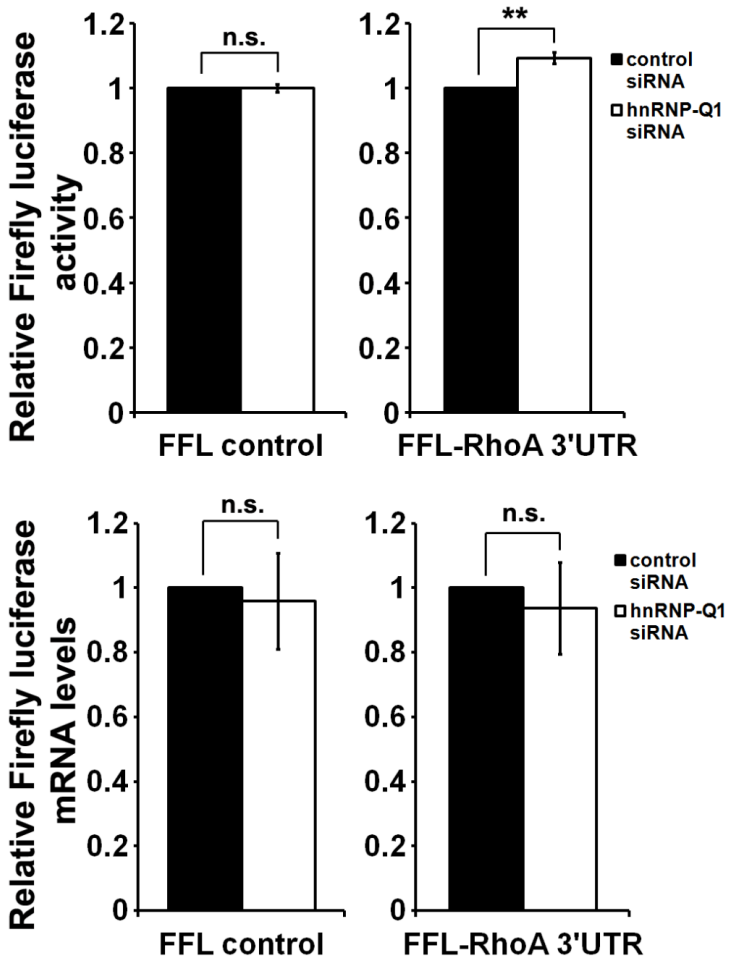

Figure S7. hnRNP-Q1 knockdown upregulated the expression of a RhoA 3'UTR luciferase reporter in primary cortical neurons. Firefly luciferase (FFL)-RhoA 3'UTR reporter and control vector were generated by replacing the GFP coding sequence (used in Neuro2a cells, **Figure 5D** and **5E**) with the FFL coding region. Cortical neurons (E17 mouse embryos) were transfected with hnRNP-Q1 or control siRNA and cultured for three days. Then, cortical neurons were transfected with mixed DNA containing carrier DNA (pCDNA3.1), FFL vector and the Renilla luciferase-expressing vector at a ratio of 20:4:1. Proteins were expressed for 12 hours and collected for Dual-luciferase assay (Promega). Relative FFL activity, which represents relative FFL protein levels, was calculated by normalizing FFL activity to the internal Renilla activity. FFL activity of hnRNP-Q1 siRNA transfected cells was then normalized to control cells. A 10% increase of FFL levels expressed from the FFL-RhoA 3'UTR reporter was consistently observed in hnRNP-Q1 siRNA transfected neurons relative to control levels **(upper panel)**. Such an increase was not detected for the FFL control vector. n=4; T-test, **$p<0.01$. Quantitative RT-PCR analysis revealed that FFL-RhoA 3'UTR mRNA levels were not affected by hnRNP-Q1 knockdown **(lower panel)**.