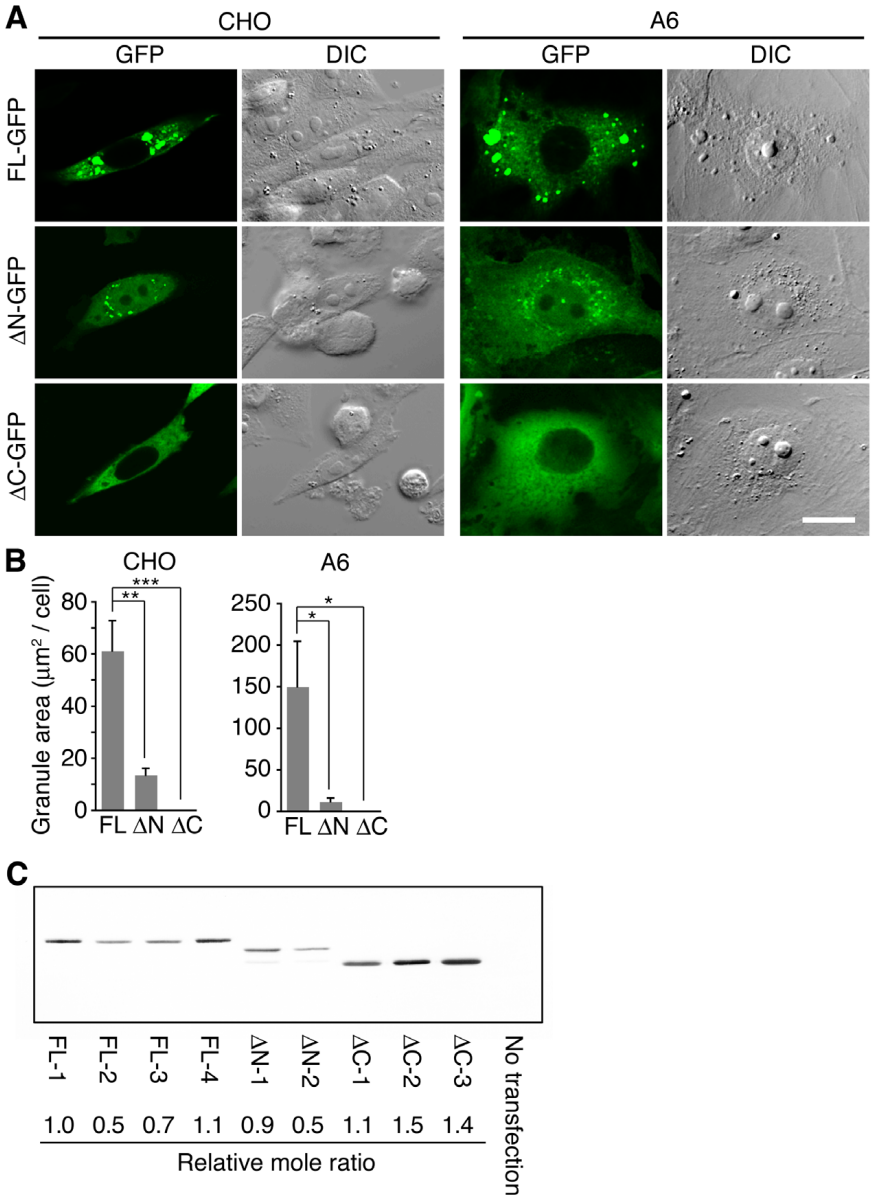
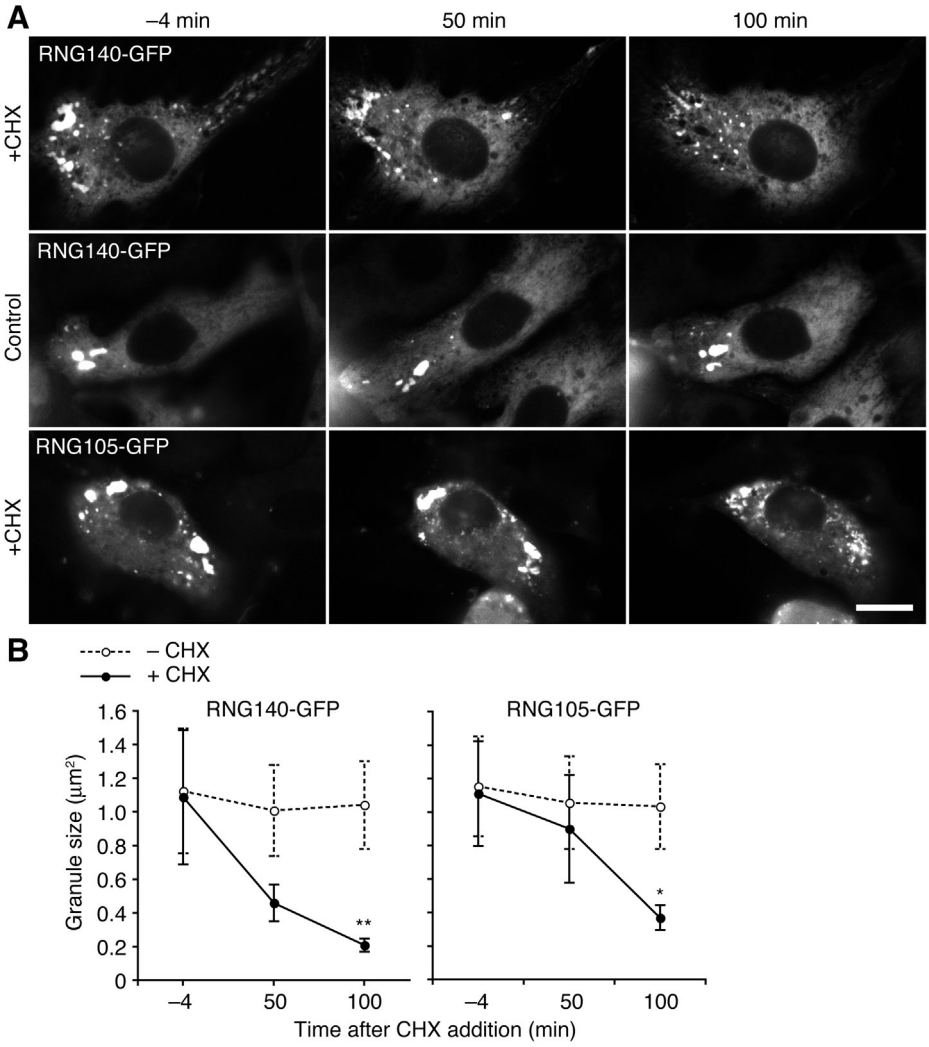
Supplemental Data

Supplemental FIGURE 1



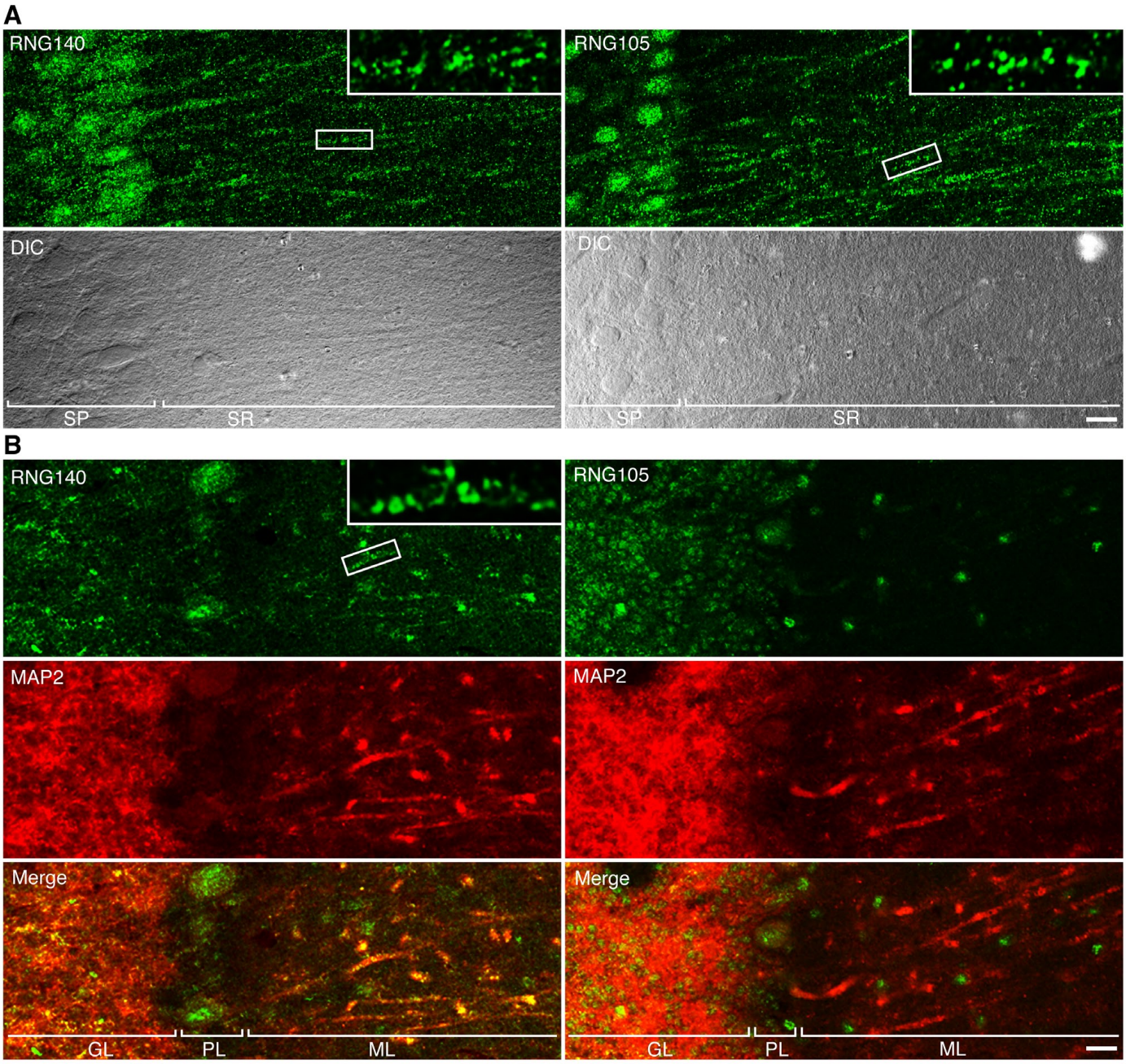
Supplemental FIGURE 1. **Expression of RNG140 induces cytoplasmic granules.** *A*, CHO cells and A6 cells were transfected with a plasmid that expresses RNG140-GFP (FL-GFP), RNG140ΔN-GFP or RNG140ΔC-GFP. DIC, differential interference contrast images. Scale bar, 10 μm. *B*, The area of the granules in *A* was measured. n=10, *p<0.05 **p<0.01, ***p<0.001, t-test. Error bars are SEM. *C*, Stable A6 transfectants used in *A* and *B* were immunoblotted with anti-GFP antibody. Each lane is loaded with 30 μg extracts from each stable line. Relative mole ratio of the GFP fusion proteins, estimated from the intensity of the bands, is indicated. It should be noted that ΔN and ΔC proteins induced less amount of granules compared to full-length RNG140 even if their expression levels were higher than full-length RNG140.

Supplemental FIGURE 2



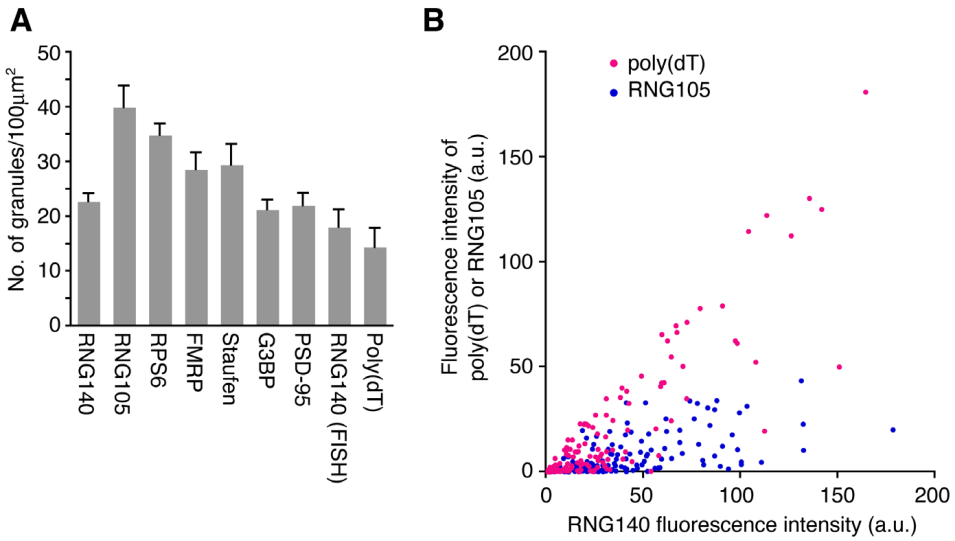
Supplemental FIGURE 2. **Cycloheximide disassembles RNG140- and RNG105-induced RNA granules.** *A*, A6 cells expressing RNG140-GFP or RNG105-GFP were treated with 20 μM cycloheximide (CHX) and subjected to time-lapse observation. Time after cycloheximide addition is indicated. RNG140 granules were disassembled 50-100 min after the addition of cycloheximide. RNG105 granules were also disassembled, but the disassembly of RNG105 granules was less extensive than that of RNG140 granules. Although both RNG140 and RNG105 granules were disassembled, small granules remained even after 100 min. This may be due to a population of mRNAs tightly associated with RNG140 and RNG105 granules, partial aggregation of RNG140 and RNG105, or other unknown reasons. Scale bar, 10 μm. *B*, Quantification of the size of the granules after the addition of CHX in *A*. Granules from 5 cells were measured. *p<0.05, **p<0.01, t-test at each time points.

Supplemental FIGURE 3



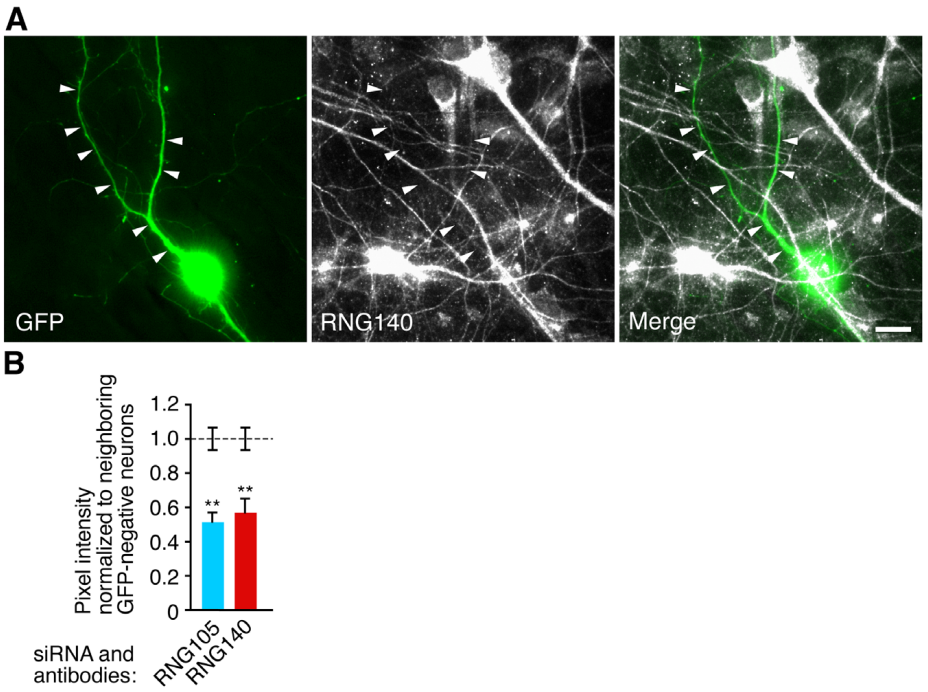
Supplemental FIGURE 3. **RNG140 is localized to dendritic granules of hippocampal and cerebellar neurons.** Slices from rat hippocampus (*A*) and cerebellum (*B*) were immunostained with anti-RNG140 and anti-RNG105 antibodies. Cerebellar slices were co-stained for a dendritic marker MAP2. Insets show magnification of boxed areas. SP, stratum pyramidale; SR, stratum radiatum; GL, granule cell layer; PL, Purkinje cell layer; ML, molecular layer. Scale bar, 10 μm.

Supplemental FIGURE 4



Supplemental FIGURE 4. **Quantification of poly(dT)-stained granules in hippocampal slices.** *A*, The density of granules stained with poly(dT) or the other markers in Fig. 6. The density of poly(dT)-stained granules was not higher, but rather lower than the other markers, suggesting that the higher co-localization of RNG140 with poly(dT) than with other markers (Fig. 6*H*) was not likely due to the high density of poly(dT)-stained granules. *B*, In Figs. 6, *A* and *G*, RNG140-positive granules were selected (= region of interest, ROI) and fluorescence intensity of RNG140 and RNG105 or RNG140 and poly(dT) in the ROI was measured. The fluorescence intensity of individual granules is plotted. Fluorescence intensity of poly(dT) shows good correlation with that of RNG140, which contrasts with the results between RNG105 and RNG140, supporting that mRNA is co-localized with RNG140 granules. a.u., arbitrary unit.

Supplemental FIGURE 5



Supplemental FIGURE 5. **Reduction of RNG105 and RNG140 by RNAi.** *A*, Primary cultured neurons from mouse cerebral cortex were co-transfected with RNG140 siRNA and a GFP reporter, and then immunostained for RNG140. Arrowheads denote dendrites of a GFP-positive neuron. RNG140 expression was lower in GFP-positive neurons than in neighboring GFP-negative neurons. Scale bar, 10 μm. *B*, Expression levels of RNG105 and RNG140 in GFP-positive neurons. Cultured neurons were co-transfected with siRNA for RNG105 or RNG140 and the GFP reporter, and then immunostained with anti-RNG105 or anti-RNG140 antibody, respectively. Pixel intensity in the GFP-positive neurons was normalized to that in neighboring GFP-negative neurons. n=8 for GFP-positive cells and n=35 for GFP-negative cells. **p<0.01, t-test. Error bars are SEM.